

Spatiotemporal modeling reveals cellular regulatory dynamics driving tissue and organ development

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Abstract

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Understanding how cellular behaviors give rise to tissue- and organ-scale morphology is a central challenge in developmental biology. The multiscale nature of development makes it difficult to experimentally determine which interactions are responsible for observed developmental outcomes. This dissertation uses computational modeling to investigate these questions, with a primary focus on neural stem cell lineages in the developing *Drosophila* brain. Chapter 1 reviews recent spatiotemporal multiscale modeling studies across developmental biology, gynecological pathology, and cellular senescence, and introduces a framework for categorizing models according to the biological scales at which dynamics are imposed and outcomes emerge. Chapter 2 develops ordinary differential equation models of neural stem cell growth, division, and differentiation to evaluate candidate regulatory mechanisms capable of constraining mutant stem cell lineage expansion. Chapter 3 develops a spatially explicit Cellular Potts model to investigate how spatially regulated growth and division behaviors shape lineage morphology during brain development. Across these studies, computational models are used as tools for identifying the minimal cellular-scale mechanisms sufficient to generate observed developmental phenotypes. Together, this work demonstrates how computational modeling can be used to investigate multiscale developmental systems and generate hypotheses linking cellular interactions to tissue-scale outcomes.

TABLE OF CONTENTS

	Page
List of Figures	iii
List of Tables	xiii
Abbreviations	xv
Introduction	1
Chapter 1: Spatiotemporal multiscale models reveal how cross-scale coordination shapes human health and longevity	3
1.1 Introduction	4
1.2 Development: Shape and function emerge from multiscale coordination	10
1.3 Gynopathology: A high-impact opportunity for spatiotemporal modeling . . .	15
1.4 Senescence & Chronic Disease: Pathology Emerges from Multiscale Desyn- chronization	18
1.5 Conclusion	23
Chapter 2: Altering cell size asymmetry in Drosophila neural stem cells creates supernumerary stem cells with limited lineage expansion potential . .	25
2.1 Introduction	26
2.2 Results	28
2.3 Discussion	49
2.4 Methods	52
Chapter 3: A calibrated Cellular Potts Model quantifies how division geometry and size-dependent growth regulation impact Drosophila neuroblast lineages	86
3.1 Introduction	87
3.2 Results	90
3.3 Discussion	110
3.4 Conclusion	115

3.5 Methods	116
Conclusion	139
Bibliography	141

LIST OF FIGURES

Figure Number		Page
1.1	Imposed and emergent dynamics across reviewed multiscale models. For each reviewed study (rows), the matrix indicates the biological scale(s) of imposed dynamics (left) and measured emergent outcomes (right). Columns are colored by scale of biological organization: subcellular (purple), cellular (blue), tissue (green), and organ or organism (red).	9
1.2	Cross-scale structure of reviewed multiscale models. Sankey diagram relating the biological scale of imposed dynamics, the scale of emergent dynamics, and the modeling formalism across all reviewed studies. Band width is proportional to the number of studies in each category. A single study can appear in more than one category per axis.	22
2.1	Cell size and position determine the cell's proliferation potential, Representative image sequence of an (A) asymmetrically dividing wild-type, (B) nanobody-expressing NB dividing with inverted (C) or symmetric cell size asymmetry. All examples show Sqh::EGFP in green and mCherry::Jupiter in white. Division mode of wild-type (D) or nanobody-expressing (E) neuroblasts. Only inverted and symmetric divisions were counted in nanobody-expressing neuroblasts. (F) Quantification of apical and basal cell divisions, following an inverted asymmetric (F) or symmetric (G) neuroblast division. Quantification of cell cycle time and frequency of apical and basal cell division for (H) inverted or (I) symmetric neuroblast divisions. Numbers on bars indicate number of observed neuroblasts. Scale bar is 10 μ m; time in hours:minutes.	30
2.2	Dpn is inherited by the siblings of symmetrically or inverted asymmetrically dividing neuroblasts. Representative image sequence of a (A) wild type, (D) mud ⁴ mutant, or (G, J, M) nanobody-expressing neuroblasts. All shown examples express tdTomato::Dpn (white) and Sqh::EGFP (green). Dotted lines outline the parental and sibling cells. (B, E, H, K, N) Sum of intensity quantification of tdTomato::Dpn over time in the two sibling cells for the shown examples. Schematics in the upper right corner of the graph illustrate the division outcome and Dpn (green) inheritance. (C, F, I, L, O) Quantification of division outcomes. Numbers on bars indicate number of observed neuroblasts. Scale bar: 5 μ m. Time in hours:minutes:seconds	33

2.3	Altering neuroblast sibling cell size asymmetry changes cell numbers but not cell types. UMAP plots of the scRNAseq clusters for (A) wild type, (B) <i>mud</i> ⁴ and (C) nanobody-expressing neuroblasts. All 28 clusters (0-27) are present in all 3 conditions. (D) Annotations of all clusters (y-axis) with representative marker genes (x-axis). Dot size indicates the percent of cells per cluster expressing the shown gene. Dot color indicates the relative expression of the gene where zero represents average expression. Values below zero show up as gray, regardless of magnitude. Clusters are ordered vertically by developmental age and type. (E) Graph of cell numbers per cluster, normalized for library size, and normalized to control for each individual cluster.	35
2.4	Altering neuroblast sibling cell size asymmetry changes neuroblast size, lineage size and cell number. (A) UMAP plot of scRNAseq data for the neuroblast clusters 15 and 19 for each condition. (B) Quantification of the relative total neuroblast number, normalized for library size and control neuroblast numbers. Representative type-I (<i>AseGal4</i> > <i>UAS-mCherry::Jupiter</i> ; white) neuroblast lineages for (C) wild type, (D) nanobody-expressing and (E) <i>mud</i> ⁴ mutant neuroblasts stained with anti-Dpn (cyan) and anti-Prospero (magenta). Segmentation of <i>AseGal4</i> -expressing lineages and neuroblasts (not shown) was used to count (F) neuroblast number per lineage, (G) neuroblast (Dpn) nuclear size, (H) lineage size and (I) number of Prospero cells per lineage. Representative images (J) for wild type, <i>mud</i> ⁴ and nanobody-expressing type I (<i>AseGal4</i> > <i>UAS-mCherry::Jupiter</i> ; white) neuroblasts stained with anti-Dpn (cyan) and anti-PH3 (magenta). (K) Quantification of mitotic index. Scale bar is 10 μ m. <i>p</i> < 0.05 were considered significant; * <i>p</i> < 0.05, ** <i>p</i> < 0.01, *** <i>p</i> < 0.001, **** <i>p</i> < 0.0001. The following statistical tests were used: Anova and Tukey HSD for (G, H, I, K).	37

2.5	Altering neuroblast sibling cell size asymmetry reduces GMC, newborn neurons and overall prospero-positive cell numbers. (A) Plots of prospero expression for wild type, nanobody-expressing and <i>mud</i> ⁴ mutant brains overlaid on the UMAP. High Prospero-expressing cells are visualized with intense purple. (B) Fraction of cells per condition with at least 1 prospero transcript. UMAP plots for wild type, nanobody and <i>mud</i> ⁴ highlighting (C) GMC or (E) newborn neuron clusters. Quantification of relative (D) GMC numbers and (F) newborn neurons, respectively, normalized for library size and control. Representative type I neuroblast lineages (<i>AseGal4</i> ; <i>UAS-mCherry::Jupiter</i> ; white), stained for (G) anti-Dpn (cyan) and anti-Grh (GMCs; magenta) or (H) anti-Dpn (cyan) and anti-Hey (newborn neurons; magenta). Quantifications of the number of cells expressing prospero (pros+) determined by (I) RNAseq or (J) IHC. Quantifications of the number of cells expressing grainyhead, determined by (K) RNAseq or (L) IHC. Quantifications of the number of cells expressing Hey determined by (M) RNAseq or (N) IHC. Scale bars are 5 μ m. $p < 0.05$ were considered significant; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. The following statistical tests were used: One-way Anova and Dunnett's multiple comparisons test for (J, L, N).	39
2.6	ODE modeling shows volume-sensitive NB growth and NB self-inhibition are both sufficient mechanisms for constraining NB lineage expansion. (A) Cartoon showing the differentiation cascade of NBs following asymmetric (bottom) and symmetric (top) division. (B) Summary of dynamics included in each model. Bold numbers and green check marks denote models in which mutant proliferation was effectively constrained. (C-F) Cell counts for each model over the duration of the simulation for wild type (left), <i>mud</i> ⁴ mutant (center), and nanobody (right) simulations.	41

2.7	mud⁴ and nanobody-expressing neuroblasts differentially express genes associated with neuroblast fate, the cell cycle, cell differentiation, neurogenesis, and the cytoskeleton. Volcano plots of differentially expressed genes (DEGs) comparing (A) wild type with mud ⁴ , and (C) wild type with nanobody-expressing neuroblasts, respectively. DEGs with an adjusted p-value of 0.001 or lower and a log2FC of 0.5 or higher are plotted with colored dots. Annotated dots refer to genes of interest. Dotplots of the top 30 DEGs comparing (B) wild type with mud ⁴ , (D) wild type with nanobody-expressing neuroblasts, and (F) DEGs shared between mud ⁴ and nanobody-expressing NBs compared to wild type, respectively. Genes annotated in green and red correspond to genes expressed in NBs and neurons, respectively 94. Dotplots only show the top 30 DEGs by log2FC. (E) Frequency of selected keywords associated with the DEGs. (G-J) Summary and Model. NBs dividing with altered CSA (symmetric or inverted asymmetric) create shortened and asymmetric lineage trees, resulting in diminished lineage expansion (different cell types are indicated with colors, see legend). (H) The position and size of sibling NBs (A, B), generated with altered CSA define their (I) proliferation rates and (J) differentiation potential.	47
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2.S1	Nanobody-expressing neuroblasts divide asymmetrically, symmetrically or with inverted asymmetry. (A) Representative cartoon of the nanobody approach: the apically tethered, anti-GFP nanobody is equipped with a kinase domain from Rho-kinase, phosphorylating and constitutively activating Myosin's regulatory subunit Spaghetti Squash (Sqh) upon binding of GFP to the vhhGFP. Consequently, activated Myosin will be retained on the apical neuroblast cortex 29. (B) Representative image sequences of wild-type and nanobody-expressing neuroblasts. All neuroblasts co-express UAS-mCherry::Jupiter (white) with worGal4 and endogenously tagged Sqh::EGFP (green). Representative symmetric and inverted asymmetric divisions are shown for nanobody-expressing neuroblasts. (C) Quantification of division modes for wild type, control, and nanobody-expressing neuroblasts. Each dot represents one ratio measurement. Average and standard deviation are shown overlaid with a scatter plot. Significance was tested with Bonferroni's multiple comparisons test. Wild type vs control: $p > 0.9999$. Wild type vs nanobody: $p < 0.0001$. (D) Wild-type neuroblasts align the mitotic spindle in parallel to the intrinsic apical (blue) – basal (red) polarity axis, repeatedly generating large self-renewed neuroblasts (green) and small differentiating GMCs (red), dividing only once more. Trapping activated Myosin on the apical neuroblast cortex can induce (E) inverted asymmetric or (F) symmetric cell divisions with variable lineage trees. The resulting brains could contain neuroblasts of various sizes, many of which are smaller than normal wild-type neuroblasts. (G) Symmetric neuroblast divisions induced through spindle misalignment generate lineage trees with various outcomes, resulting in brains with potentially differently sized neuroblasts.	66
2.S2	Clusters 15 and 19 represent neuroblasts (A) Neuroblast clusters 15 and 19 highlighted on a UMAP plot. (B) DotPlot of marker genes used in the annotation of the neuroblast clusters.	68
2.S3	Cluster 11 represents INPs, and clusters 5, 7, 9, 12 represent GMCs, respectively. (A) INP cluster 11 highlighted on a UMAP plot. (B) DotPlot of marker genes used in the annotation of the INP cluster. (C) GMC clusters 5, 7, 9, and 12 are highlighted on a UMAP plot. (D) DotPlot of marker genes used in the annotation of the GMC clusters.	69
2.S4	Immature, developing, and mature neurons separate into specific clusters (A) UMAP plot showing Newborn neuron (clusters 3, 13, 14) and immature neuron clusters (clusters 1, 2, 4, 8, 10, 18). (B) DotPlot of marker genes used in the annotation of the newborn neuron and immature neuron clusters. (C) UMAP plot showing developing and mature neurons. (D) DotPlot of marker genes used to annotate developing and mature neurons. . .	70

2.S5	Cluster 21 and cluster 22 represent Iro-C and Kenyon cells, respectively. (A, C) UMAP plot highlighting cluster 21 containing mature Iro-C neurons and Kenyon cells, respectively. (B, D) DotPlot of marker genes used to annotate Iro-C neurons and Kenyon cells, respectively.	71
2.S6	New cluster markers and a comparison of immature and mature neuron numbers between genotypes. (A) The top three differentially expressed genes per cluster (by log2FC) are shown on a DotPlot (see methods). Validated markers are not shown here. Red boxes outline the dots corresponding to the three genes per cluster for easier viewing. These are genes that have not been validated in vivo as cell type specific but are among the most significant principal components that distinguish clusters from one another and could be used as markers for cell typing in future RNA sequencing experiments. Immature (B) and mature (E) neuron clusters highlighted on UMAP plots for each condition, respectively. The total number of cells belonging to (C) immature neuron and (F) mature neuron clusters, normalized for library size and control. Immature neurons (D) and mature neurons (G) per neuroblast. Cells within immature neuron clusters with at least 1 elav transcript were used for the quantification for each condition. Cells within mature neuron clusters with at least 1 nSyb or brp transcript were used for the quantification for each condition.	72
2.S7	Model dynamics details. Base model shown for (A) wild type and (B) mud mutant neuroblasts. The base model predicts that mud mutant NBs generate far more cells than wild-type NBs, inconsistent with experimental results.	73
2.S8	Mutant NB cell cycle durations continue increasing over time for models 3, 4, and 5. (A) NB Cell cycle duration is constant in the base model. (B) In model 1, volume activation increases cell-cycle duration to a new steady state in mud and nanobody-expressing NBs. (C) In model 2, a symmetric division threshold ultimately causes cell cycle duration to decrease to its minimum. Model 3 (D), model 4 (E), and model 5 (F) show the increase in cell cycle for mud mutant and nanodoby-expressing NBs. Models are only shown with an $\alpha = 3$ (D, E) and $\beta = 4$ (F).	74

2.S9	Symmetrically dividing mud4 mutant neuroblasts show asymmetric lineage behavior and extended interphase times (A) Schematic lineage tree of a symmetrically dividing neuroblast, illustrating the used nomenclature and lineage structure. Parental (P) neuroblasts generate an A and a B neuroblast after a symmetric division. A and B neuroblasts can either divide symmetrically or asymmetrically. Only dividing cells propagate the lineage and receive a lineage label. (B) Plot showing cell diameter for multiple generations in symmetrically dividing mud4 mutant neuroblasts. A and B neuroblasts are consistently smaller and do not regrow to parental NB size. A lineages are consistently longer than B lineages. (C) Cell cycle length comparison between wild type and mud4 mutant neuroblasts. Statistical test used: unpaired T-test. ** $p < 0.01$	75
2.S10	NB growth rate-sensitivity to NB volume (α) determines whether volume-sensitive growth effectively suppresses mutant NB proliferation when division threshold volume is constant. Cell counts shown for Model 3 run with different values of α	77
2.S11	NB growth rate-sensitivity to NB volume (α) determines whether volume-sensitive growth effectively suppresses mutant NB proliferation when division threshold volume is regulated by symmetric division. Cell counts shown for Model 4 run with different values of α	78
2.S12	NB growth rate sensitivity to NB count (β) determines whether NB self-inhibition effectively suppresses mutant NB proliferation. Cell counts shown for Model 5 run with different values of β	79
2.S13	GO-terms for mud mutant and nanobody-expressing neuroblasts of differentially expressed genes. GO term analysis for differentially expressed genes comparing (A) mud ⁴ versus control, (B) nanobody-expressing neuroblasts versus control, and (C) genes differentially expressed in both mud ⁴ and nanobody-expressing neuroblasts versus control. The combined score consists of a combination of the p-value and z-score. The z-score measures the deviation from the expected rank of the GO term. Dot size reflects number of associated genes and dot color the corresponding p-value. Genes are listed for each GO-term.	80

2.S14	Genes differentially expressed between wild type, <i>mud</i>⁴, and nanobody-expressing brains associate with neuroblast, differentiation, cell cycle, neurogenesis, and cytoskeleton functions. Genes differentially expressed in (A) <i>mud</i> ⁴ , (B) nanobody-expressing, or (C) both <i>mud</i> ⁴ and nanobody-expressing neuroblasts, compared to wild type, are associated with neuroblast, differentiation, cell cycle, neurogenesis, and cytoskeleton functions. Blue rectangles indicate keywords associated with the gene on the x-axis. Keywords are derived from GO terms. Pros 42 (D) and (E) Dpn 103 target genes that are differentially expressed in <i>mud</i> ⁴ and nanobody-expressing brains. Green and red boxes indicate whether the genes are up- or downregulated in <i>mud</i> ⁴ and nanobody-expressing neuroblasts compared to wild-type NBs.	82
2.S15	NB growth genes are not significantly differentially expressed in <i>mud</i>⁴ or nanobody-expressing neuroblasts. Volcano plots of DEGs between wild-type and <i>mud</i> ⁴ (left), and wild-type and nanobody (right), highlighting genes in the (A) Insulin pathway 95, (B) the Mediator complex 50, (C) the oxidative phosphorylation pathway 50, or (D) other genes regulating NB growth 96,97. We only considered genes that are expressed in at least 25% of neuroblasts, with a p-value of 0.001 or better, and a minimum log2-fold change of 0.5. Any gene appearing in one plot but not the other signifies that that gene was expressed by less than 25% of cells in that group.	84
3.1	Calibrated CPM of developing neuroblast lineage recapitulates expected WT lineage scale, composition, and organization. A. WT planes of division and differentiation cascades. B. Schematic of how the CPM implements WT asymmetric division and GMC division. C. Top: Three example simulated WT neuroblast lineages. Bottom: Three example experimentally imaged WT lineages. Example lineages reflect the 10th, 50th and 90th percentile of lineage area within each dataset. D. Distributions of five calibration metrics across experimental and simulated lineages. Box plots show median $\pm$ IQR; whiskers extend to the last data point within 1.5 x IQR.	91
3.2	Sensitivity of NB clustering metrics to apical axis reorientation and division orientation range Box plots show median $\pm$ IQR; whiskers extend to the last data point within 1.5 x IQR	95
3.3	Sensitivity of NB clustering metrics to offset shift and differentiation rules. Box plots show median $\pm$ IQR; whiskers extend to the last data point within 1.5 x IQR	98

3.4	The model predicts that unregulated <i>mud</i> mutant lineages should overgrow WT lineages. A. NB undergoing symmetric division and resulting differentiation cascade. B. Symmetric division as implemented in the model. C. Representative experimental and simulated <i>mud</i> mutant lineages at the 10th, 50th, and 90th percentiles of lineage area. D. Distributions of the five calibration metrics — lineage area, Pros ⁺ cell count, Dpn ⁺ cell count, total Dpn area, and mean Dpn area — across WT experimental, WT simulated, <i>mud</i> mutant experimental, and unregulated <i>mud</i> simulated lineages. Box plots show median $\pm$ IQR; whiskers extend to the last data point within 1.5 x IQR.	103
3.5	Volume-based regulation paired with birth-size-dependent division thresholds constrains <i>mud</i> mutant lineage overgrowth. A. Schematic contrasting the two division threshold rules. B. Endpoint distributions of the five calibration metrics across the unregulated baseline plus four candidate regulatory dynamics (NB-ABM, NB-PDE, Vol-ABM, Vol-PDE), under each threshold rule (light: VCV=0; dark: VCV=1). The red band marks the experimental <i>mud</i> interquartile range; the dashed red line marks the experimental median. Y-axes are log-scaled. C. Representative simulated <i>mud</i> mutant lineages at the 50th percentile of lineage area, organized by regulatory dynamic (columns) and threshold rule (rows).	105
3.6	NB connectivity in regulated <i>mud</i> mutant lineages is shaped by both NB-NB adhesion and post-inheritance rotation of the daughter-NB apical axis. A. Representative simulated <i>mud</i> lineages across selected (regulatory dynamic $\times$ adhesion state $\times$ post-inheritance rotation) combinations. B. NB connectivity (fraction of lineages with all NBs in a single connected component) across all twenty-four tested conditions.	109
3.S1	Median and spread of WT lineage metrics are consistent across 2D and 3D representations. Box-and-whisker plots of per-lineage fold-change from the WT mean for five calibration metrics, comparing the 2D max-projection rasterization (light gray) and the 3D mesh segmentation (dark gray) across all retained WT lineages ($n = 60$). Boxes show the interquartile range; whiskers extend to 1.5 $\times$ IQR; circles indicate outliers. The dashed line marks a fold-change of 1. Median and IQR are nearly identical between representations for all five metrics.	135

3.S2	Median fold-change of <i>mud</i> mutant metrics is preserved across 2D and 3D representations. Box-and-whisker plots of per-lineage fold-change from the WT mean for five calibration metrics in <i>mud</i> mutant lineages ($n = 59$), comparing the 2D max-projection rasterization (light gray) and the 3D mesh segmentation (dark gray). Boxes show the interquartile range; whiskers extend to $1.5 \times$ IQR; circles indicate outliers. The dashed line marks the WT reference. Both representations place the median below 1 for all area metrics and Pros count, and above 1 for NB count, confirming directional consistency. The 3D representation shows a modestly lower median for area metrics, reflecting the larger effect size captured by volumetric measurement relative to 2D projection.	136
3.S3	Calibration of division-plane rotation parameters from experimental time-lapse measurements. Division-to-division absolute rotation angles for WT (<i>left</i>) and <i>mud</i> mutant (<i>right</i>) neuroblasts, binned in 15° increments (see Methods). Curves show folded-normal $N(0, \sigma^2)$ fits with $\sigma = 26^\circ$ (WT) and $\sigma = 50^\circ$ (<i>mud</i>). The dashed line marks the 75° symmetric-division threshold; the shaded region contains 14% of observed <i>mud</i> divisions, consistent with the 13% predicted by $N(0, 50^\circ{}^2)$ and with the symmetric-division frequency reported by [26]. The 75° – 90° bin contains no observed <i>mud</i> divisions, motivating the symmetric-division threshold at 75°	136
3.S4	Endpoint metrics for experimental and simulated WT and <i>mud</i> mutant lineages. Mean $\pm$ SD of five lineage endpoint metrics across experimental WT (wt exp), WT simulation (wt sim), experimental <i>mud</i> mutant (mud exp), and unregulated <i>mud</i> mutant simulation (mud sim). Metrics shown are total lineage area, total neuroblast (NB) area, mean per-NB area, Prospero-positive (Pros) cell count, and NB count. Colored markers indicate experimental groups. Y axis is log-scaled.	137
3.S5	Regulated WT simulations remain in experimental WT IQR. Distribution of calibration metrics shown for last time point of regulated WT simulations. Each bar represents 50 simulations.	137
3.S6	Rate of NB connectivity in <i>mud</i> mutant experimental lineages. Bar chart showing the fraction of lineages in which all neuroblasts in a lineage are adjacent, grouped by the number of neuroblasts per lineage (n values shown on bars; 59 lineages total). The dotted red line indicates the overall mean fraction (91.5%). Neuroblasts remain adjacent in the large majority of <i>mud</i> mutant lineages regardless of lineage size.	138

LIST OF TABLES

Table Number		Page
2.1	Neuroblast lineage ODE base model parameters	60
2.2	Neuroblast lineage ODE model 1 volume-dependent division model parameters	62
2.3	Neuroblast lineage model 2 dynamic division threshold parameters	63
2.4	Neuroblast lineage model 3 volume-dependent growth rate parameters	64
2.5	Neuroblast lineage model 5 self-repression parameters	65
3.1	Core WT model parameters used in the calibrated baseline simulations. . . .	130
3.2	Parameters across the offset perturbation conditions. NB <code>SIZE_TARGET</code> and <code>CRITICAL_VOLUME</code> are set by the geometric constraints of the asymmetric-division rule (Section 3.5.8.1), subject to a $S \leq 2$ cap that is binding for offsets $\leq 50\%$. NB and GMC growth rates are calibrated at each offset to maintain an approximately constant cell-cycle duration across conditions (Section 3.5.8.2). GMC <code>CRITICAL_VOLUME</code> follows from $V_{c,GMC} = (1 - Y/100) \cdot S \cdot V_c$. Neuron <code>CRITICAL_VOLUME</code> equals $0.7 \times$ the GMC critical volume in each condition.	131
3.3	Additional parameters used in mutant growth-regulation simulations.	132
3.S1	Calibration metrics agree in direction across 2D and 3D representations. Group means and fold-change ($FC = \textit{mut}$ mean / WT mean) for five lineage metrics computed from the 2D max-projection Voronoi rasterization and from the original 3D mesh segmentations. All five metrics change in the same direction between representations, and the relative ranking of fold-change magnitudes is preserved: NB area metrics show the largest reduction, followed by lineage area and Pros count while NB count is elevated. NB count and Pros count are identical between 2D and 3D representations because cell counts do not depend on projection.	134

3.S2 3D mesh reports a modestly stronger *mud* mutant phenotype than 2D projection. Representations are indistinguishable within WT. Paired Wilcoxon signed-rank tests compare per-lineage 2D and 3D fold-change distributions within each genotype (each lineage contributes one value per representation; fold-changes are normalized by the WT mean of the respective representation). Within WT, no significant difference is detected between representations for any metric, indicating that 2D projection captures the same within-genotype variability as the full 3D mesh. Within *mud* mutant lineages, all three area metrics differ significantly between representations ($p < 0.001$): 3D consistently reports a larger reduction from WT than 2D. Notably, direction and statistical significance of the *mud* phenotype are preserved in both representations for all metrics (Table 3.S1). Cell-count metrics are omitted from this table as they are identical in 2D and 3D. . . . 135

ABBREVIATIONS

ABM: Agent-based model

CMP: Cellular Potts model

ODE: Ordinary Differential Equation

PDE: Partial Differential Equation

NB: Neuroblast

GMC: Ganglion Mother Cell

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DEDICATION

To my parents, who have supported me relentlessly, and who have never once doubted my calling to science.

INTRODUCTION

Biological systems are organized across multiple scales of space and time. Subcellular interactions influence cellular behaviors, cellular behaviors shape tissues, tissues form organs, and organs collectively give rise to organismal function. Interactions occurring at small scales accumulate to produce outcomes at larger scales, often giving rise to emergent behaviors that are not obvious from the properties of small-scale system components alone. During development, complex tissues and organs arise through the coordinated growth, division, and differentiation of individual cells. Understanding how local cellular interactions give rise to tissue- and organ-scale morphology is a fundamental question in developmental biology.

Emergent biological phenomena can be difficult to interrogate experimentally. Determining how processes at one level of biological organization influence outcomes at another often requires isolating perturbations to a single biological scale while simultaneously observing responses across multiple scales. Such experiments can be difficult or impossible to perform in biological systems. As a result, identifying the mechanisms responsible for observed developmental outcomes often becomes an inverse problem: given a tissue-scale phenotype, what cellular interactions are sufficient to produce it?

Computational models provide a complementary approach for investigating emergent biological phenomena. In computational models, all modeled entities and interactions are explicitly specified. This specification allows researchers to systematically manipulate individual model components and observe the consequences of those changes throughout the system. Computational models therefore provide a framework for moving between the forward problem of predicting tissue-level outcomes from hypothesized cellular rules and the inverse problem of inferring candidate cellular mechanisms from observed developmental behaviors.

The work presented in this thesis uses computational modeling to investigate the mechanisms underlying emergent developmental behaviors, with a primary focus on morphogenesis in the developing *Drosophila* brain. Chapter 1 reviews recent advances in multiscale computational modeling across developmental biology, gynecological pathology, and cellular

senescence. To facilitate comparisons across diverse model types, I introduce a framework for describing models according to the biological scales at which dynamics are imposed and the scales at which outcomes emerge. Chapter 2 develops an ordinary differential equation model of neural stem cell growth, division, and differentiation in the developing *Drosophila* brain. Together with our experimental collaborators we use this model to evaluate candidate regulatory mechanisms and generate hypotheses regarding the control of neural stem cell population dynamics. Chapter 3 presents a spatially explicit Cellular Potts agent-based model of the same developmental system. We use the model to investigate how division geometry and morphology-based cell growth regulation influences lineage morphology during brain development.

All modeling work presented in this thesis shares a commitment to model simplicity and interpretability. Models are most useful when their behavior can be understood, interrogated, and related back to biological mechanisms. Increasing model complexity beyond what is necessary to explain an observed phenomenon risks creating a second system (in addition to the biological system of interest) that is itself difficult to understand. The models developed here seek to identify the minimal set of assumptions and interactions sufficient to reproduce key experimental observations.

This philosophy also shapes how models are evaluated against experimental data. Rather than attempting to reproduce every experimental measurement exactly, the focus is on capturing the qualitative and quantitative trends that characterize system behavior. Biological systems are influenced by many factors that are difficult or impossible to observe directly, and computational models necessarily represent abstractions of that complexity. The goal is therefore not perfect prediction, but the identification of mechanisms sufficient to generate observed biological outcomes. By determining which assumptions are sufficient to reproduce specific behaviors, models can generate experimentally testable hypotheses and provide mechanistic insight into the processes that govern development.

Ultimately, this thesis endeavors to show how computational modeling can be used to further our understanding of how complex biological systems emerge from the interactions of their constituent parts.

Chapter 1

SPATIOTEMPORAL MULTISCALE MODELS REVEAL HOW CROSS-SCALE COORDINATION SHAPES HUMAN HEALTH AND LONGEVITY

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Author's note

This chapter is adapted from a piece submitted to Current Opinion in Systems Biology on April 26th 2026. The version here is extended with explanations of the model formalisms and the imposed and emergent dynamics for each model in the review.

In this work, I propose a framework for categorizing and discussing spatiotemporal multi-scale models intended to help biologists become acquainted with the modeling landscape. Computational modeling developed in parallel across many quantitative disciplines, each with its own terminology and conceptual conventions. As a result, the language surrounding these approaches can be unfamiliar to newcomers, particularly biologists who may have deep expertise in the systems being modeled but limited familiarity with mathematics or engineering vocabulary.

In this review, I aim to bridge that gap using two strategies. First, I describe model formalisms using a limited, clearly defined set of terms. In the introduction, I establish the modeling terminology used throughout the paper and apply it consistently, translating vocabulary from disparate fields into a common nomenclature. Second, I discuss and categorize models according to the biological scales of organization they investigate. For each model, I highlight the scales at which dynamics are imposed and the scales at which dynamics emerge.

I hope this perspective helps readers navigate the modeling landscape by tying the multi-scale nature of modeling approaches to the multi-scale biology with which they are familiar.

Abstract

Spatiotemporal multiscale models provide a powerful framework for investigating how coordinated biological dynamics across scales give rise to tissue organization and disease. Here, we review recent modeling studies in the fields of development, gynopathology, and senescence, emphasizing where dynamics are imposed, where emergent behavior is measured, and the different model formalisms that bridge these scales. Across the literature, dynamics are most often imposed at the cellular level and assessed at the tissue scale, with agent-based and cellular Potts models serving as common bridging frameworks. We discuss key limitations of these approaches, including challenges in establishing mechanistic necessity, parameter identifiability, and computational tractability. Finally, we highlight emerging opportunities to address these challenges through spatial omics, scientific machine learning, and AI-assisted model construction.

1.1 Introduction

Tissue form and function are controlled by the coordination of molecular pathways and cell behaviors across space, time, and biological scale. Subcellular signaling, cellular behaviors, and tissue structure continuously interact, shaping each other's dynamics through multilateral regulation. When this coordination becomes mistimed, mislocalized, or misintegrated, pathology emerges even when individual components remain intact. These principles highlight the importance of elucidating multilateral regulation to understand and maintain healthy tissue dynamics and prevent disease. Despite their central role in tissue physiology and disease, cross-scale coordination mechanisms remain poorly understood because they are difficult to observe and perturb systematically in living systems.

Experimental approaches face two fundamental barriers. First, *observability*: resolving

interactions across molecular, cellular, and tissue scales requires simultaneous, time-resolved measurements that remain technically infeasible in many systems. Second, *controllability*: selectively perturbing one aspect of coordination—such as timing, spatial localization, or signal integration—without unintentionally altering others is rarely possible *in vivo*. Spatiotemporal multiscale models directly address these limitations by encoding hypothesized coordination rules explicitly across scales, rendering all modeled dynamics observable and systematically perturbable. In doing so, they function as epistemic frameworks for causal inquiry, clarifying which assumptions are sufficient to generate observed tissue-scale behaviors, and which disruptions are capable of producing pathology. By specifying which molecular, cellular, and tissue-scale components are present and how processes at each scale interact, these models make all represented dynamics observable and systematically perturbable. In this way, models function as *in silico* testbeds; mechanistic hypotheses are formally encoded and evaluated against experimental or clinical observation. Iterative testing across candidate mechanisms provides a principled basis for identifying which are worth pursuing in human-relevant experimental systems. Importantly, these models do not replace *in vitro* or *in vivo* experiments, they augment them.

Here, we review recent spatiotemporal modeling studies and examine how explicit representation of cross-scale interactions advances mechanistic understanding of tissue organization and dysfunction, and enables systematic causal inquiry. We focus on case studies in development, gynopathology, and senescence—contexts in which tissue scale outcomes arise from tightly coupled spatial and temporal coordination across biology scales. Across these examples, models function as *in silico* testbeds that clarify which assumptions are necessary and sufficient to reproduce observed dynamics and how their disruption leads to pathological conditions. We argue that for systematic causal inquiry into cross-scale coordination, this model-guided approach is deeply impactful and arguably indispensable, especially in systems where experimental observability and controllability are fundamentally limited.

1.1.1 *Multiscale models incorporate cross-scale interactions*

We define spatiotemporal multiscale models as computational models that couple biological processes that evolve in time and across multiple levels of biological organization by explicitly representing their spatial structure, temporal dynamics, and reciprocal regulatory interactions. A central design choice in spatiotemporal model development is deciding which processes to prescribe explicitly (or impose) and which to allow to arise organically as emergent consequences of cross-scale interactions. Prescribed dynamics codify model assumptions while other features of the system emerge from interactions among these assumed dynamics. This distinction is critical for mechanistic interpretation. If a model is intended to demonstrate that a given tissue-level dynamic arises from a set of local rules, those rules must not directly impose the dynamics of interest. Instead, the dynamic must emerge from the modeled interactions across scales.

Figure 1.1 summarizes, for each model, the scales at which dynamics are imposed and the scales at which emergent outcomes are measured. A model appearing at the same scale on both sides of the figure is not measuring the dynamics it imposes; it is imposing one process at that scale and reporting a separate emergent process at the same scale.

1.1.2 *Multiscale models comprise diverse spatial representations*

Models in this review represent biological systems either as collections of discrete, rule-following entities or as continuous fields that evolve over space and time. Discrete approaches explicitly track individual components, while continuous approaches describe aggregate behavior through spatially distributed variables. Discrete models are often formulated as **agent-based models** (ABMs), in which agents represent entities that sense local signals, interact with neighbors, and modify their environment. In many models in this review, agents correspond to whole cells (cell agents), but they can represent biological entities at any organizational scale.

The agent-based paradigm does not prescribe a spatial representation; how agents occupy and move through space is a separate and explicit design choice. The cell-based models reviewed here primarily use one of three common spatial representations. In **cellular Potts**

models (CPMs) (also known as Glazier-Graner-Hogeweg models) cells occupy collections of lattice sites and behaviors emerge from energy minimization over adhesion, volume, and motility constraints [69, 64]. CPMs are effective for modeling development and morphological change over time [127, 77]. In **vertex models**, cells are represented as polygons or polyhedra defined by shared vertices, making them well-suited for epithelial mechanics and coordinated sheet deformation [56, 3]. In **center-based (off-lattice) models**, cells are treated as point-like or sphere-like objects that interact through contact forces, enabling simulation of growth and migration in three-dimensional tissues [143, 128]. In each of these representations, individual cells are explicit objects with their own geometry. These spatial models can also be used outside of an agent-based framework when system dynamics are fully specified by the governing equations rather than by explicit agent rules.

Some discrete models operate at the subcellular scale, representing molecular complexes or structural elements as stochastic, rule-following entities. These **stochastic molecular models**, often implemented using Monte Carlo or Gillespie algorithms, explicitly track molecular states and probabilistic events such as binding, unbinding, conformational change, and force-dependent deformation [62, 63, 33]. Like cell-scale ABMs, they represent individual components rather than population averages, but their agents are molecular entities rather than whole cells. Continuous-time dynamics at the subcellular scale are often represented using ordinary differential equations (ODEs), which describe the time evolution of system variables without explicitly resolving stochastic events or spatial structure. Discrete mechanical models can also represent deformable biological structures as interacting particles or mesh elements, allowing membrane deformation, rupture, vesiculation, and material remodeling to emerge from local mechanical interactions [55, 71].

Continuous models, typically formulated as **partial differential equations** (PDEs), extend ODE-like continuous descriptions to spatially distributed systems by representing space as a continuous field in which concentrations of biological system components evolve as smooth functions of position and time. In PDEs the same field equations govern the entire spatial domain. Subcellular and extracellular processes are typically resolved at the same spatial scale, and if incorporated, cells appear implicitly as regions or densities within the field rather than as discrete objects. Continuous fields often represent molecular

concentration distributions or mechanical quantities like displacement, strain, or stress.

Many spatiotemporal multiscale models couple discrete and continuous representations. In hybrid ABM-PDE models, entities, often cells, are represented as discrete agents at one spatial scale, while extracellular signals are characterized as continuous fields at another scale, allowing multiple levels of biological organization to coexist within the framework. Agent behaviors are shaped by local field values, and agents themselves modify those fields in return, creating tightly coupled cross-scale interactions. Analogous hybrid coupling can also occur below the cell scale, when stochastic molecular models are linked to continuum descriptions of the surrounding biochemical or mechanical environment. In these models, local molecular events can generate larger-scale outputs such as force transmission, material deformation, or cell-level signaling responses.

1.1.3 Open-source platforms support multiscale model construction

Several open-source platforms support spatiotemporal multiscale modeling, particularly models that couple discrete cell behaviors to continuous biochemical or mechanical fields. CompuCell3D [170], the most widely used platform in this review, is built around the CPM framework and supports coupled PDE fields, embedded gene regulatory networks, and flexible rule-based cell behaviors. Other studies reviewed here use custom code in Python, MATLAB, and C++, as well as specialized tools such as the CPM framework Tissue Simulation Toolkit [44], the red-blood cell specific modeling framework OpenRBC[171], and the ABM frameworks Morpheus [167], and Repast Simphony [138].

Several widely used frameworks were not featured in the reviewed studies but are relevant to spatiotemporal multiscale biology. PhysiCell [60] supports center-based agent-based modeling of multicellular systems. Mesa [94] provides a general-purpose Python framework for agent-based modeling. FLAME GPU 2 [151] enables large-scale ABMs to run on GPUs. ARCADE [196] is a Java-based ABM framework designed for multicellular tissue and tumor modeling.

Making the code for multiscale models open-source is essential for ensuring that models are verifiable, reproducible, and extensible by the broader community [190]. All models

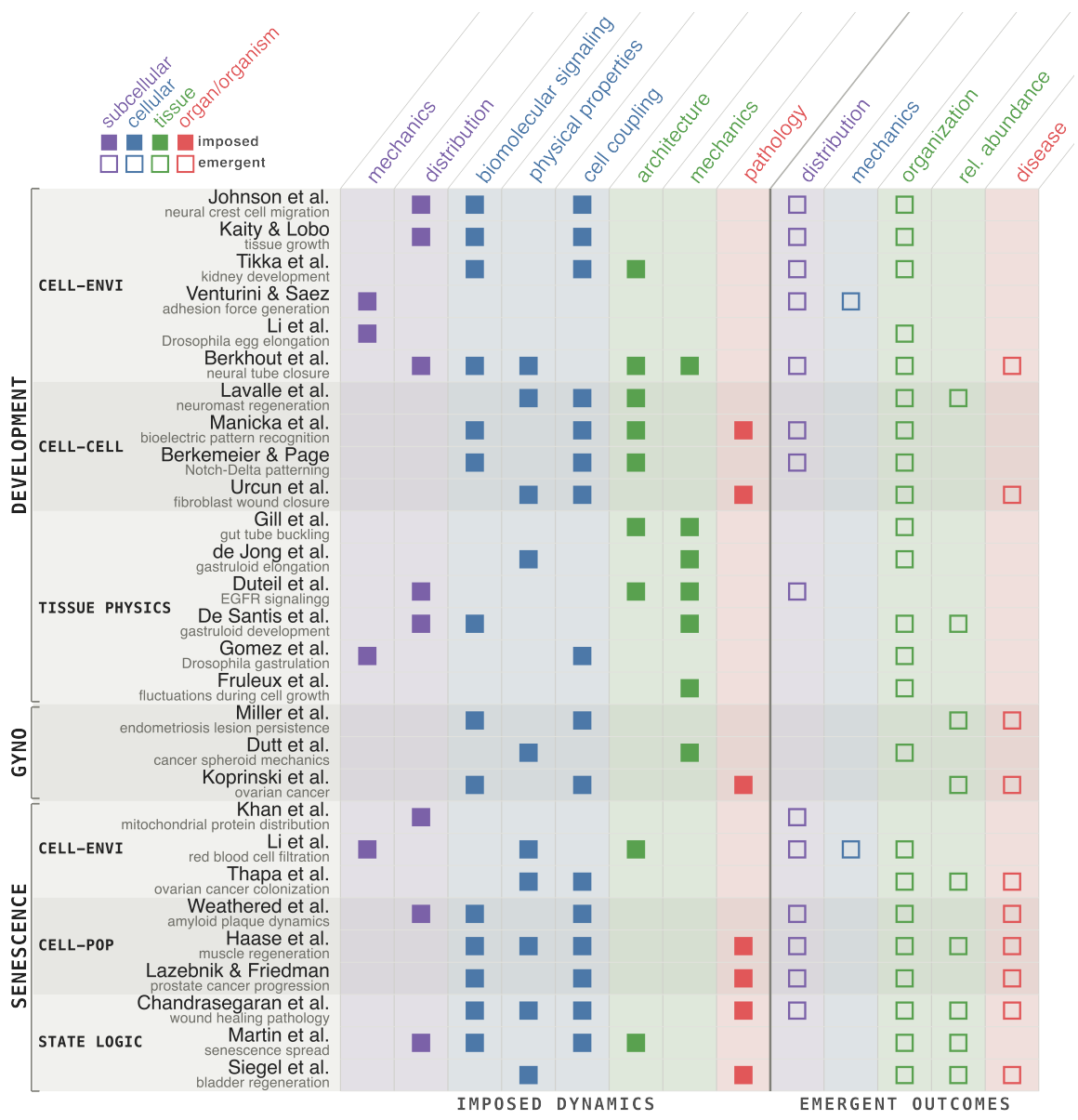


Figure 1.1: **Imposed and emergent dynamics across reviewed multiscale models.** For each reviewed study (rows), the matrix indicates the biological scale(s) of imposed dynamics (left) and measured emergent outcomes (right). Columns are colored by scale of biological organization: subcellular (purple), cellular (blue), tissue (green), and organ or organism (red).

reviewed here have their code or governing equations freely accessible.

1.2 *Development: Shape and function emerge from multiscale coordination*

Development is an ordered, cross-scale regulated process describing how biological organisms grow, change, and sustain healthy tissue in both structure and function over time, beginning from a single cell and progressing to a mature form. Central questions of development are precisely the questions spatiotemporal multiscale models are designed to answer: *which local rules are sufficient to produce global organization, and which disruptions cause it to fail?* In the subsections below, we introduce three classes of coordination in development and describe how spatiotemporal multiscale models enable the discovery of new biology. We highlight how tissue shape and spatial organization emerge as consequences of explicitly specified assumptions, whether encoded at the scale of cell-environment interactions, cell-cell coupling, or tissue-scale physical dynamics. By specifying rules at one scale and observing outcomes at another, these models reveal which assumptions are necessary and sufficient to produce observed tissue organization, and which perturbations are capable of disrupting it. For systematic causal inquiry of this kind, the *in silico* testbed is often the only tool available.

1.2.1 *Tissue shape is coordinated by physical and chemical interactions between cells and their molecular environments*

Multiscale models can represent molecular signaling fields and subcellular force-generation mechanisms alongside cells that sense, respond, and remodel their surroundings. By encoding hypothesized cell responses to molecular cues, these models can ask which local molecular and cellular dynamics are sufficient to reproduce experimentally observed tissue organization, generating experimentally testable hypotheses.

Johnson et al. used a center-based cellular agent-based model of chick cranial neural crest cell (CNCC) collective migration coupled to reaction-diffusion PDEs for extracellular signaling molecules to test whether proposed cell-signal interactions are sufficient to reproduce experimentally observed migratory pathways. The model encodes hypothesized CNCC responses to these molecules as rules for movement, adhesion, and speed, identifying rule sets that allow collective CNCC migration to emerge. In doing so, it generates experimentally

actionable hypotheses about how local signaling coordinates collective migration, including that CNCCs locally degrade Dan, a movement-inhibitory protein, making previously occupied regions more permissive for subsequent migration [92]. Kaity and Lobo similarly built a lattice-free center-based cellular ABM of tissue growth in which cell positions are determined by overdamped ODEs that impose spring-like mechanical forces between neighboring cells. Cells synthesize, carry, and exchange morphogens with neighboring cells. Local morphogen concentrations and mechanical crowding regulate cell growth and shrinkage, which trigger proliferation or apoptosis and mechanically reshape neighboring cells, reciprocally coupling molecular signals, cell behaviors, and tissue morphology. They show that different stable tissue shapes emerge under different morphogen source arrangements and regulatory rule-sets, highlighting candidate morphogen regulatory mechanisms for tissue shape control [93]. Tikka et al. used a CPM-based cellular ABM of early kidney organogenesis to test which chemotactic and adhesive interactions could drive nephron progenitor cells to aggregate near the ureteric bud as observed in vivo. In the model, nephron progenitor cells chemotax in response to a diffusing chemoattractant field governed by reaction-diffusion dynamics. By varying chemoattractant sources and cell-type-specific adhesion, the authors identified the conditions that best reproduced experimentally observed nephron progenitor movement. The best-matching simulations included chemoattractant secretion by both ureteric bud and progenitor cells, as well as distinct adhesion properties between cell types [174].

Multiscale models can also represent subcellular force-generation mechanisms and assess their impact on mechanical properties of cells and tissues. At the molecular to cell scale, Venturini and Sáez present a stochastic molecular model that links molecular adhesion mechanics to cell-scale traction force. The model represents individual adhesion chains composed of actomyosin, talin, and transmembrane integrins that stochastically bind to ligand sites on a continuous elastic extracellular matrix. By varying integrin dynamics, ECM stiffness, and ligand spacing, the model shows how molecular adhesion properties shape adhesion-complex behavior and the traction forces transmitted by cells [181]. At the cell to tissue scale, Li et al. combined extensive live imaging and perturbation experiments with a modified vertex model to study how the epithelial follicle cells surrounding the developing *Drosophila* egg expand and elongate during late oogenesis. Experimentally, they found that

actomyosin pulses on the basal, matrix-facing side of these cells, which might be expected to contract the tissue, instead accompany cell flattening and posterior-to-anterior expansion waves. The model encodes experimentally identified regulators of this process as forces acting on the vertices that define cell perimeters. This allowed the authors to test whether local protrusive, adhesive, actomyosin, and oocyte-growth-associated forces are sufficient to reproduce the observed expansion dynamics. Together, the experiments and simulations support the hypothesis that spatiotemporally coordinated basal actomyosin dynamics can drive epithelial expansion and tissue elongation without imposing elongation directly [118].

These models can also function as predictive failure detectors, showing which molecular perturbations are likely to disrupt a developmental system. Berkhout et al. implemented an ABM of mammalian neural tube closure, the morphogenetic process that forms the neural tube, which later gives rise to the brain and spinal cord. They used a CPM to represent changing cell positions and shapes as the tissue bends and closes, and reaction-diffusion equations on the CPM lattice to represent morphogen fields. Each cell contained a gene regulatory network that translated local morphogen signals into key behaviors required for closure. By simulating gene knockout and hyperactivation scenarios, the model predicted the probability and type of clinically relevant closure defects, including open, closed, and midline fusion phenotypes [17].

1.2.2 Tissue shape is coordinated by cell-cell coupling

Multiscale models can impose rules for local interactions between neighboring cells, revealing how tissue-scale pattern and size control emerge from cell-cell coupling. Lavalle et al. used a CPM to simulate regeneration of zebrafish neuromasts, small sensory organs that recover after severe laser ablation. In simulations with proliferation alone, surviving support cells regenerated the organ but continued dividing, producing unchecked overgrowth. The authors therefore tested local negative-feedback rules for stopping proliferation and found that regeneration was best reproduced by including two features: a delay of about 20 hours after ablation before proliferation begins, followed by a cell-type-specific contact rule in which some cells stop dividing once they encounter enough neighbors of their own

type. This local rule recovered neuromast size, cell-type proportions, and tissue organization, suggesting that proportional organ regeneration can emerge from neighborhood-level control of proliferation [106]. Manicka et al. similarly ask how local cell-cell coupling could allow cells to interpret tissue-scale patterning information during *Xenopus* development. They hypothesize that bioelectric prepatterning coordinate embryonic brain morphogenesis by coupling membrane voltage dynamics to gene-regulatory states across neighboring cells. Their model represents discrete cells on a lattice, each containing a generic gene-regulatory network coupled to membrane voltage and connected to neighboring cells through intercellular signaling links. Using a combination of the genetic algorithm and gradient descent, they identified model architectures in which the coupled cell network could distinguish an endogenous *Xenopus* neural-plate voltage pattern from abnormal patterns by activating genes in response to the endogenous pattern and repressing them in response to abnormal patterns. This supports the idea that bioelectric and intercellular signaling can allow cells to integrate nonlocal pattern information when coordinating developmental gene-expression states [122].

Multiscale models have also uncovered how the geometry of coupling determines which tissue-scale patterns are attainable, and how altered coupling rules can produce pathological outcomes. Berkemeier and Page use a lattice-based model of Notch-Delta signaling in which each cell is described by coupled ordinary differential equations describing their Notch and Delta activity. In the model cell-cell interactions are mediated through a weighted network that determines how Delta signals from connected cells contribute to Notch activation. By varying the geometry and weighting of this network, they show that different tissue-scale Notch-Delta patterns emerge from changes in the structure of cellular connectivity [16]. Urcun et al. used a CPM of fibroblast migration in scratch assays to test whether contact inhibition of locomotion distinguishes healthy from scar-like, or keloid fibroblast behavior. In the model, healthy fibroblast motility is limited by cell-cell contact, whereas keloid fibroblast motility is better reproduced when this contact inhibition rule is removed. The resulting simulations suggest that loss of contact inhibition of locomotion can contribute to excessive keloid fibroblast migration during pathological wound closure, providing an experimentally testable hypothesis [176].

1.2.3 *Tissue shape is coordinated by tissue-scale physical dynamics*

Multiscale models that impose physical rules at the tissue scale can reveal how organ-level morphology emerges from growth, geometry, and material properties. Gill et al. complemented their experimental characterization of folding patterns in the developing chick gut with continuum mechanical models of gut tube growth. In the model, the tissue is represented as growing endodermal and mesenchymal layers with region-specific geometry, stiffness, and growth properties. By imposing differential growth in these layers, they show that distinct regional buckling patterns can emerge when differences in thickness, stiffness, radius, and prestretch place gut compartments into different mechanical regimes [61]. de Jong et al. used a CPM to ask which cell-level physical mechanisms could explain the shapes of elongating mouse gastruloids. They first quantified experimental gastruloid shapes and then compared these shape distributions to simulations with different imposed mechanisms. They found that differential adhesion between cell types alone was insufficient to drive elongation, and that incorporating active cell crawling inward along the short axis produced more realistic shape distributions. The simulated shapes became still more realistic when active crawling was combined with differential adhesion between cell types. Time-lapse imaging showed coordinated cell movements as the gastruloid elongated, and perturbing a force-generating pathway required for cell crawling reduced elongation, supporting the hypothesis that active cell crawling and differential adhesion jointly drive gastruloid elongation [54].

Multiscale models have also revealed how tissue geometry and mechanical properties actively shape signaling outcomes. Duteil et al. develop a continuum transport-reaction-diffusion model of EGFR signaling during *Drosophila* oogenesis, in which ligand diffusion, receptor interactions, and downstream signaling are modeled on an imposed, growing spatial domain. By representing the egg chamber as a growing structure in which the morphogen source and signal-receiving follicle cells move relative to one another, they show that normal morphogen and signaling patterns depend on the changing physical arrangement of the tissue in addition to the biochemical rules governing signal production, diffusion, and reception [52]. De Santis et al. develop a continuum mechanochemical model of human stem-cell

gastruloids, in which diffusible developmental signals are represented by reaction-diffusion PDEs and tissue tension is imposed as a spatial field that changes how cells respond to those signals. The model asks why the same externally induced signal can produce different spatial patterns of cell identity depending on the mechanical state of the tissue. They show that patterned cell-fate organization is not reproduced by localized biochemical signaling alone, but requires coupling signaling dynamics to high-tension regions that enable downstream fate-specifying signals. Thus, the model reframes tissue tension as an instructive regulator of developmental patterning [45].

Multiscale models have further uncovered how time-dependent tissue mechanics and growth dynamics can generate spatial structure without explicit long-range coordination. Gomez et al. complement their experimental characterization of mechanical properties in the developing *Drosophila* embryo with a CPM-based model of gastrulation in which apical constriction forces are coupled to spatially and temporally varying tissue stiffness. By comparing static and dynamically regulated stiffness patterns, they show that robust tissue folding during gastrulation requires transient, spatially localized cellular stiffening in addition to contractile forces, identifying the timing of mechanical transitions as a key determinant of morphogenetic robustness [66]. At a more general level, Fruleux et al. use a minimal stochastic model of a growing tissue in which a generic tissue property varies across space and persists over time. In the model, tissue expansion transports and stretches local fluctuations in that property, converting temporally persistent variability into spatial correlations across distant tissue regions. They show that the spatial range of these correlations, meaning how far across the tissue local fluctuations remain statistically related, is determined by the persistence time of the fluctuations relative to the rate of tissue expansion [58].

1.3 Gynopathology: A high-impact opportunity for spatiotemporal modeling

Endometriosis and ovarian cancer involve failures of spatial and temporal coordination that are difficult to systematically dissect experimentally. Endometriosis is a chronic, estrogen-dependent inflammatory disease defined by the presence of endometrial-like tissue lesions at ectopic sites. Lesion establishment is thought to often originate from dissemination of

endometrial cells to ectopic sites, but under healthy conditions immune clearance, inflammatory regulation, and hormonally driven tissue remodeling usually prevent ectopic tissue from persisting [200, 5]. This implies that disease persistence requires both cell dissemination and a breakdown in the spatiotemporal coordination of these processes. Similarly, many epithelial ovarian cancers are thought to arise from transformed epithelial cells that shed into the peritoneal cavity, where their survival and spread depend on interactions with the local tissue and fluid microenvironment [159, 31]. This implies that disease progression requires both malignant transformation and disruption of the peritoneal microenvironmental conditions that normally limit disseminated cell survival, adhesion, and spread.

Although spatiotemporal modeling work remains sparse in gynopathology, the studies below show how mechanistic insight can be gained by modeling these diseases as dynamic, multiscale systems. Because the available mechanistic models in this area are still limited, we include two non-spatial compartment models that capture temporal and cross-compartment coordination but do not explicitly resolve tissue geometry.

Multiscale models can reveal when competing biological processes create threshold transitions between healthy and pathological states. Miller et al. build a compartmental ODE model of endometrioses that tracks interacting populations of endometrial cells, macrophages, and natural killer cells across activation and attachment states, including eutopic endometrial cells, endometrial cells in peritoneal fluid, and attached endometrial cells representing early lesion cells. Within this framework, retrograde influx (the entry of shed endometrial cells into the peritoneal cavity during menstruation), immune detection, and immune cytotoxicity govern whether disseminated endometrial cells are cleared or persist. Their simulations show that reduced immune detection or cytotoxic clearance can shift the system from a low/no-disease state to a sustained ectopic-attachment state. Because the model exhibits bistability, disease onset is predicted to behave as a threshold transition in immune control rather than as a gradual consequence of increasing retrograde influx alone [130].

Multiscale models can test how collective mechanical properties emerge from interactions among individual cells. Dutt et al. combine microfluidic deformation assays of ovarian cancer spheroids with a CPM of spheroid passage through a constricted channel to assess

which physical properties allow spheroids to remain intact while squeezed through narrow tissue spaces. Experimentally, they observed that lumen-containing blastuloid spheroids are more elastic, recover their shape more rapidly, and disintegrate less than lumenless moruloid spheroids when passing through the constricted channel. They then modeled this assay *in silico* using a CPM, representing spheroids as collections of adjacent cells with tunable differential adhesion. Blastuloids additionally have a deformable lumen that deflates and reinflates during passage through the narrow channel. The simulations predict that strong intercellular adhesion and a dynamic lumen are sufficient to produce blastuloid-like mechanical resilience [53].

Temporal multiscale models can reveal how intervention outcomes depend on delayed feedback between biological response and treatment timing. Koprinski et al. develop a two-compartment delay-differential equation model of high-grade serous ovarian cancer, representing interactions between the tumor site and the tumor-draining lymph node. The model links chemotherapy-induced tumor cell death to downstream immune activation, including dendritic cell maturation, T cell priming and proliferation, immune-cell trafficking, cytokine feedback, and tumor killing. Explicit time delays represent processes that unfold over hours to days, including dendritic cell migration and T cell clonal expansion. By optimizing chemotherapy administration schedules *in silico*, they find that lower, more frequent dosing can maintain tumor reduction while reducing peak toxicity, and that schedules with rest weeks may improve recovery from treatment-associated toxicity. Thus, the model shows that therapy response depends not only on drug dose, but also on how dosing schedules interact with delayed tumor-immune dynamics [101].

Together, these studies illustrate why gynopathology is fertile ground for multiscale modeling. Across endometriosis and ovarian cancer, clinically important outcomes emerge from threshold-like failures of clearance, mechanically mediated survival of displaced cells, and delayed feedback between tumor, immune, and treatment dynamics. These are precisely the kinds of coupled processes that are difficult to isolate experimentally but well suited to mechanistic *in silico* testbeds. Much remains to be gained in this field from additional multiscale spatiotemporal modeling.

1.4 Senescence & Chronic Disease: Pathology Emerges from Multiscale Desynchronization

Cellular senescence is a durable state in which cells permanently stop dividing and adopt characteristic metabolic and signaling changes, including secretion of pro-inflammatory factors (the senescence-associated secretory phenotype, or SASP) in response to stress or damage[184]. In senescence-associated diseases such as aging, fibrosis, and neurodegeneration, pathology emerges when the coordinating dynamics that sustain healthy tissue become disrupted[142, 125, 186]. As in the development section, the studies below are organized by the biological scale at which those disruptions are imposed. Across these subsections, senescence-associated pathology emerges as a failure of coordination at the scale of cell-molecular coupling, cell-state transition logic, and population renewal dynamics. In each case, spatiotemporal models are a powerful tool for identifying which coordinating rules must be intact for healthy tissue function and which disruptions are sufficient to produce pathology.

1.4.1 Pathology emerges when molecular and subcellular environments constrain cell behavior

At the molecular-to-cell scale, multiscale models can show how subcellular organization, cell-intrinsic mechanics, and local molecular fields constrain cell behavior and survival.

Khan et al. combine live-cell imaging of mitochondrial protein and mRNA localization with a stochastic model of protein delivery across fragmented mitochondrial networks. Experimentally, they show that aging is associated with mitochondrial fragmentation and increased heterogeneity in mitochondrial protein distribution. In the model, newly produced mRNAs associate with mitochondrial fragments in proportion to fragment size, deliver proteins to those fragments, and fusion events mix protein concentrations between fragments. Their simulations and perturbation experiments show that mitochondrial protein heterogeneity emerges from fragmented organelle architecture, stochastic mRNA-driven protein delivery, and limited fusion-mediated mixing [95]. This suggests that senescence-associated mitochondrial dysfunction can emerge from altered subcellular organization and protein

localization dynamics.

Li et al. use a particle-based mechanical model to simulate red blood cells at different life stages traversing narrow interendothelial slits in the spleen. The model explicitly represents the red blood cell membrane and cytoskeleton with coarse-grained particles and drives cells through geometrically defined slits using imposed pressure gradients. Young red blood cells are modeled with excess membrane surface area and weaker cytoskeletal connectivity, while senescent red blood cells are modeled primarily with reduced surface-area-to-volume ratios. Their simulations show that slit passage can mechanically promote young red blood cell maturation by removing excess membrane, expelling intracellular remnants, and promoting acquisition of the mature biconcave shape. They also show that senescent red blood cells are retained by the slits and can undergo mechanical lysis under elevated pressure gradients, suggesting a mechanism by which splenic geometry contributes to senescent red blood cell removal [114].

Weathered et al. build a spatial ABM of microglia-amyloid interactions in Alzheimer’s disease to test how microglial behaviors shape amyloid plaque formation and coverage. In the model, individual microglia are represented as discrete agents moving on a three-dimensional grid, while amyloid- β monomers and oligomers are represented as continuous fields that diffuse, react, and aggregate into immobile plaque objects once a threshold is reached. Microglia activate in response to local amyloid- β monomer concentration, chemotax up amyloid- β gradients, phagocytose nearby amyloid- β monomer, and proliferate. Their simulations show that plaque size and microglial plaque coverage depend on different components of the coordinated microglial response. Phagocytosis most strongly limits plaque size, chemotaxis most strongly promotes plaque coverage, and rapid microglial activation before plaque formation is associated with smaller plaques [187].

1.4.2 Pathology emerges when cellular interactions disrupt tissue coordination

At the cell-to-tissue scale, multiscale models can show how altered adhesion, extrusion, and cytokine-mediated communication reshape collective tissue outcomes.

Thapa et al. examine how senescence in mesothelial cells alters ovarian cancer coloniza-

tion of the peritoneal lining. Experimentally, they show that ovarian cancer cells adhere more efficiently to senescent mesothelial monolayers and preferentially expand in regions where they clear senescent mesothelial cells. Using a CPM, they show that reproducing this clearance requires both increased adhesion between cancer cells and senescent mesothelial cells and an active extrusion rule, in which senescent cells are removed once they share at least 55% of their perimeter with cancer cells. They link this modeled adhesion increase to senescence-driven changes in the extracellular matrix, which make senescent regions more adhesive to cancer cells through matrix-binding interactions. Together, these results suggest that ovarian cancer colonization can emerge from senescence-driven changes in local adhesive interactions that alter how cancer cells engage the peritoneal lining [173].

Haase et al. build a two-dimensional CPM-based ABM of acute muscle injury to ask how spatial cytokine dynamics coordinate tissue regeneration. The model represents muscle fibers, extracellular matrix, microvasculature, lymphatics, immune cells, fibroblasts, and satellite stem cells as interacting agents within a muscle cross-section. Diffusible cytokines and related signals are represented as spatial fields that diffuse through the tissue environment and are locally secreted, taken up, or degraded by cells. Local signal concentrations regulate cell recruitment, migration, proliferation, differentiation, apoptosis, matrix remodeling, and vascular repair, so cells both respond to and reshape their molecular environment. They use this model to show that regeneration improves most when multiple signal-transport properties are changed together, shifting the system toward stronger early stem-cell and vascular responses and later resolution of inflammation and matrix remodeling [74].

1.4.3 Pathology emerges when cell-state transition logic is mistimed or misregulated

Multiscale models that impose cell-state transition logic can reveal how outcomes depend on when cells switch states, what functions those states carry, and how state transitions are coordinated with local context.

Lazebnik and Friedman develop a spatiotemporal PDE model of metastatic prostate cancer to examine how treatment-driven transitions among tumor cell states shape combination-therapy outcomes. The model represents proliferating cancer cells, therapy-induced senes-

cent cancer cells, castration-resistant cancer cells, dendritic cells, T cells, endothelial cells, oxygen, VEGF, IL-12, and three treatments as continuous densities within a growing tumor domain. ADT and chemotherapy reduce proliferating cancer cells but also shift some cells into senescent or castration-resistant states, while the senolytic drug fisetin selectively removes senescent cells. This makes treatment outcome depend not only on tumor-cell killing, but also on the timing and consequences of therapy-induced cell-state redistribution. Because senescent cancer cells secrete VEGF, their persistence can promote angiogenesis and oxygen supply that support continued tumor growth. Simulations predict that adding a senolytic can improve the combined effects of ADT and chemotherapy, especially when it is administered shortly after chemotherapy, when senescent cancer cells have been generated but before their pro-tumor effects persist [108].

Chandrasegaran et al. build a CPM-based ABM of dermal wound healing to test how the timing and phenotype of senescent cells shape repair outcomes. The model represents fibroblasts, myofibroblasts, macrophages, senescent myofibroblasts, and extracellular matrix as spatial agents, coupled to reaction-diffusion fields for diffusible regulators including PDGF, CSF1, MMP, and inflammatory SASP factors.. Within this framework, fibroblasts, myofibroblasts, macrophages, and senescent cells transition between behaviors in response to local biochemical signals, ECM contact, mechanical stress, and stochastic senescence rules. Senescent myofibroblasts first produce a fibrogenic SASP that promotes matrix deposition and then switch to producing a fibrolytic SASP that promotes matrix breakdown and inflammatory clearance. Simulations show that healthy repair requires senescence to appear transiently at the right stage of healing. Excess or pre-existing inflammatory senescence produces chronic wound-like inflammation, whereas absent or delayed senescence produces fibrotic, matrix-rich repair [34].

Martin et al. combine a probabilistic spatial model of SASP ligand diffusion and receptor binding with a stochastic simulation of senescence spread in a two-dimensional cell layer. They use this framework to ask how senescence can remain locally contained rather than propagating indefinitely. In the model, cells can be primary senescent cells, become senescent through diffusible SASP signals, or become senescent through direct contact with neighboring senescent cells. SASP-induced senescent cells can continue secreting SASP,

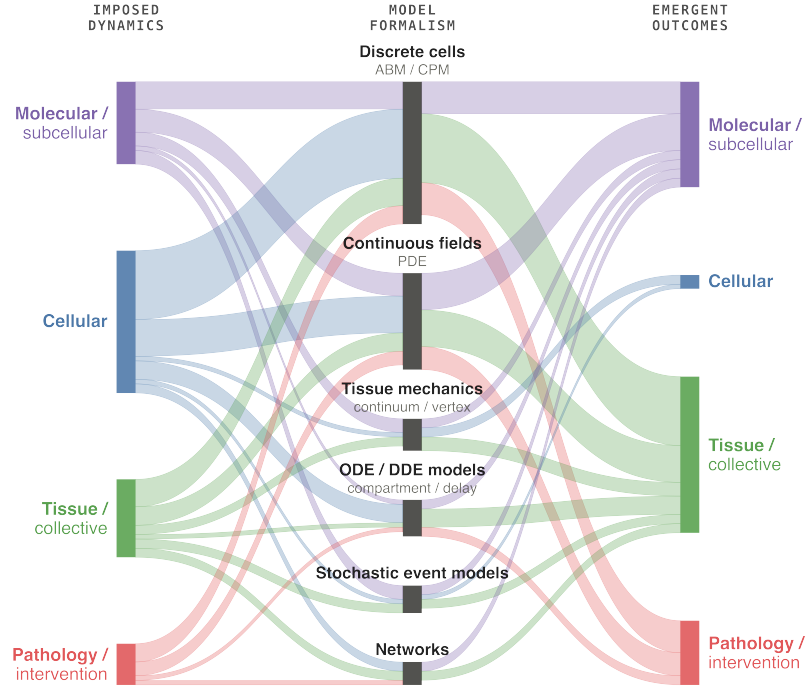


Figure 1.2: **Cross-scale structure of reviewed multiscale models.** Sankey diagram relating the biological scale of imposed dynamics, the scale of emergent dynamics, and the modeling formalism across all reviewed studies. Band width is proportional to the number of studies in each category. A single study can appear in more than one category per axis.

whereas contact-induced senescent cells are assumed to produce little or no SASP. Simulations show that contact-induced cells can limit senescence spread by forming a low-secretory buffer around primary senescent cells, especially when contact-mediated induction precedes delayed SASP-mediated induction. This generates the experimentally testable hypothesis that contact-induced senescent cells locally contain senescence spread by adopting a lower-SASP state than paracrine-induced cells [123].

Siegel et al. use a CPM-based ABM to test sixteen hypotheses for how cell division and differentiation are controlled in the human urothelium during homeostasis and regeneration after injury. Each hypothesis is encoded as a different set of rules governing how progenitor, basal, and intermediate cells proliferate, divide, and differentiate. These rules vary in whether cell-state transitions are driven by intrinsic division programs or by contact

with positional structures such as the basal membrane and tissue surface. By comparing simulated regeneration time and long-term tissue organization using an experimentally informed fitness function, they show that only a small subset of hypotheses can reproduce both rapid surface repair and stable tissue maintenance. The best-performing hypotheses use contact-dependent differentiation rules: basal cells differentiate when they lose basal-membrane contact, and intermediate cells differentiate when they reach the tissue surface. This suggests that healthy urothelial renewal depends on state-transition rules that let cells interpret their position within the tissue architecture [162].

1.5 Conclusion

Here we reviewed recent spatiotemporal modeling work in the fields of development, gynopathology, and senescence. We highlighted the spatial scales at which dynamics are imposed and measured, and the model formalisms used to span scales. Figure 1.2 summarizes the formalisms used to impose and measure spatial scales across the models. Across the reviewed papers, dynamics were most often imposed at the cellular level and emergent behavior was most often measured at the tissue scale, with ABMs and CPMs most commonly used to bridge those scales.

Spatiotemporal multiscale models face several limitations and challenges. The most fundamental limitation is that models can establish that a set of mechanisms is sufficient to produce an observed dynamic, but rarely that those mechanisms are necessary. For example, a model may reproduce tissue-level outputs while implementing incorrect or incomplete lower-scale mechanisms. A mechanism’s necessity can be established through experimental validation by experimentally perturbing the implicated scale and observing the system’s response. However, multiscale models are often applied to systems that are challenging to experimentally perturb or observe precisely because they are challenging to target experimentally. Instead of identifying causal interactions, multiscale mechanistic models generate plausible hypotheses for experimentalists to target. These are valuable contributions, but can be insufficient to drive discovery independently.

Parameter identifiability is a related challenge. These models encode interaction rules at multiple scales while the spatiotemporal data needed to constrain them remain sparse,

making it difficult to distinguish biologically correct mechanisms from mechanisms that are merely flexible enough to fit available data. The computational cost of running spatiotemporal models compounds this issue because broad exploration of parameter spaces and alternative mechanisms can be infeasible given hardware and time constraints. Two converging developments may help address this issue. Spatial omics, including spatial transcriptomics, proteomics, and multiplexed imaging, is generating datasets that simultaneously characterize cell states, signaling gradients, and tissue architecture, providing new constraints for model parameters and new targets for multiscale validation [179, 19, 107, 73, 117]. Scientific machine learning, integrating mechanistic model structure with data-driven components, offers paths forward for parameter inference and surrogate modeling in regimes where full mechanistic simulation is computationally prohibitive [137, 195].

Additionally, the automation of model construction itself is advancing. Natural language processing systems can now assemble molecular mechanisms from literature at scale [8, 88], and reinforcement learning agents have been shown to generate mechanistic models of spatially distributed systems from experimental data [105, 12]. Applied to spatiotemporal multiscale biology, AI-assisted model construction could expedite the model development process while preserving what distinguishes mechanistic frameworks from purely data-driven approaches: outputs remain interpretable as explicit causal hypotheses about which coordination rules are sufficient to produce observed outcomes [9, 4].

Across development, gynopathology, and senescence, the reviewed studies show that spatiotemporal multiscale models provide a powerful framework for studying multilateral regulation across biological scales. By making cross-scale assumptions explicit, these models enable mechanistic hypotheses about tissue organization, dysfunction, and disease to be formalized, tested, and iteratively refined *in silico*. The imposed-emergent framing developed throughout this review provides a transferable vocabulary for comparing modeling efforts across systems and a useful scaffold for future AI-assisted workflows in which mechanistic assumptions must be explicitly specified.

Chapter 2

ALTERING CELL SIZE ASYMMETRY IN DROSOPHILA NEURAL STEM CELLS CREATES SUPERNUMERARY STEM CELLS WITH LIMITED LINEAGE EXPANSION POTENTIAL

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Author's note

This work was posted on bioRxiv on September 29 2025 and can be found at this link <https://doi.org/10.1101/2025.09.26.678890>. We are currently working on addressing reviewer feedback before resubmitting the work to a peer-reviewed journal.

In this work, we present a combined experimental and computational study of growth and differentiation dynamics in the developing fly brain. I designed, implemented, and analyzed all ODE models used in this study, and developed an interactive web application that allows readers to explore the models and test their own hypotheses which can be viewed here: https://jannetty.github.io/Neurogenesis_ODE_Models_Webapp/. All experimental work and analysis were conducted by my fantastic collaborators.

Abstract

Asymmetrically dividing invertebrate and vertebrate stem cells can generate unequal sized sibling cells. However, the functional implications of cell size asymmetry (CSA) are under-

explored. Here, we use *Drosophila* neural stem cells (NSCs) to investigate how changes in CSA impact NSC proliferation and cell fate decisions. Using live cell imaging, NSC lineage analysis, and gene expression profiling, we find that altering CSA increases the NSC pool but decreases lineage size and the number of differentiating progeny cells. Modeling CSA *in silico* with a volume-sensitivity and NSC self-inhibition model can recapitulate these findings. Gene expression profiling further revealed that the NSC growth regulator Imp and the G1-S cell cycle regulator CycE are upregulated in NSCs with altered CSA, providing a potential molecular link to the volume-sensitivity model. We propose that cell size and position regulate NSC proliferation and differential potential, impacting lineage progression and progeny cell differentiation in the developing *Drosophila* brain.

2.1 Introduction

Asymmetric cell division (ACD) is an evolutionarily conserved division mode, generating two daughter cells that acquire different fates [84]. Binary cell fate outcomes can be achieved via asymmetric segregation of cell fate determinants, including RNA molecules, proteins, or organelles. Alternatively, extrinsic signaling cues can influence cell fate decisions (reviewed in [169, 99, 180, 116]). ACD is used by stem cells to self-renew while forming a differentiating sibling cell at the same time. The correct regulation of ACD is important to prevent tumor formation or neurodevelopmental disorders such as microcephaly [194, 110, 20].

One manifestation of ACD is the formation of unequally sized sibling cells during mitosis. Sibling cell size asymmetry, referred to as Cell Size Asymmetry (CSA) hereafter, is evolutionarily conserved and has been observed during embryogenesis in ascidians [37], leeches [189], the roundworm *Ascaris megalocephala* [22], sea urchins [79], *Ilyanassa obsoleta* (Eastern mudsnail) [67, 68], *C. elegans* [90] and *Drosophila melanogaster* [47]. CSA also occurs in vertebrate embryos, such as *Xenopus laevis*, where the second division creates an asymmetric embryo with two larger and two smaller blastomeres [172]. Recent observations further suggest that the first cleavage in the human embryo produces two unequally sized blastomeres [197]. Empirical evidence suggests that CSA contributes to cell fate decisions during development. For instance, equalizing the first division in *C. elegans* caused faster

cell cycle progression in P1 descendants, defects in cell positioning, division orientation, and cell fate [90]. In *Volvox carteri*, a multicellular green alga, CSA plays a central role in germ/soma specification [96]. Changing CSA in asymmetrically dividing *C. elegans* neuroblasts rescued the smaller sibling from apoptosis [141]. However, how CSA shapes cell fate decisions remain underexplored. Three main mechanisms have been identified to regulate and implement CSA: (1) spindle displacement through cortical pulling forces, positioning the cleavage furrow machinery off cell center [110, 65, 146], (2) forming and retracting cortical polar lobes into one of the two sibling cells [67, 68, 47] or (3) asymmetric localization of cortical proteins regulating unequal membrane expansion [169, 47, 141, 28, 38, 32, 42].

Drosophila neural stem cells, called neuroblasts (NBs), generate CSA by asymmetrically localizing, and dynamically displacing Actomyosin [28, 38, 32, 154, 175, 155, 145, 102, 11]. Neuroblasts divide asymmetrically by size and fate, self-renewing the larger neuroblast while either forming a small, differentiating Ganglion Mother Cell (GMC) or an immature Intermediate Progenitor cell (INP). Neuroblasts are intrinsically polarized, manifested in the apical localization of the Par complex, directing the formation of a basal cell fate determinant complex. The latter is composed of the cortical protein Miranda (Mira), the Notch inhibitor Numb (NUMB, NUMBL in humans), the translational repressor Brain Tumor (Brat; TRIM2,3 in humans) and the transcription factor Prospero (Pros; Prox1, 2 in humans). Basal cell fate determinants segregate into the small INP or GMC, while the apical Par complex and associated proteins remain in the self-renewed neuroblast [147, 144, 80].

In *Drosophila* larval neuroblasts, asymmetric Myosin localization is regulated via intrinsic polarity cues. For instance, in early mitosis, the polarity protein Partner of Inscuteable (Pins; LGN/AGS3 in vertebrates) enriches Myosin on the apical neuroblast cortex. In early anaphase, Pins induces a basally directed cortical flow by recruiting Protein Kinase N (Pkn) to the apical neuroblast cortex, relieving it from Actomyosin contractile tension [28, 175, 155, 11, 144, 140]. This release in Actomyosin-dependent cortical tension permits biased membrane expansion to form a large, self-renewed apically positioned neuroblast and a smaller differentiating Ganglion Mother Cell [38, 145, 103]. While the larger NB retains apically localized proteins, Mira, Pros, Brat and Numb segregate into the small GMC

promoting the expression of neural differentiation genes and represses neuroblast stem cell identity genes [165, 97, 78, 124, 166, 51, 59]. Although ample experimental evidence supports the model that GMC differentiation is induced through asymmetric segregation of basal cell fate determinants, it is unclear whether also CSA contributes to cell fate decisions and/or stem cell behavior in this system.

Here, we use asymmetrically dividing *Drosophila* neural stem cells to investigate how altered CSA impacts neurogenesis and brain development. We employ two complementary approaches to change CSA of dividing neuroblasts and find that cell positioning and cell size regulates the NBs proliferation and differentiation potential. Surprisingly, live cell imaging, detailed lineage analysis and gene expression profiling shows that changing CSA increases the neural stem cell pool, without increasing the number of differentiating siblings. Using iterative mathematical modeling, we show that volume-based growth regulation and neuroblast self-repression are possible mechanisms to constrain neuroblast lineage expansion. We conclude that the size and placement of the NB is necessary to maintain a proliferative stem cell pool to produce the correct number of differentiating siblings. This finding could inform our current understanding of neurodevelopmental disorders, such as primary microcephaly.

2.2 Results

2.2.1 Retaining activated Myosin on the apical neuroblast cortex is sufficient to alter CSA

Drosophila neuroblasts divide asymmetrically by size, producing a large apically positioned neuroblast and a small, basally located GMC [80, 59]. To understand the role of CSA in *Drosophila* neurodevelopment, we aimed to create developing larval fly brains in which some neuroblasts divide symmetrically by size and/or show inverted physical asymmetry. In the latter case, this should lead to an apically positioned cell that adopts the size of a GMC and a basally positioned cell the size of a neuroblast. To this end, we used a fly strain, harboring the regulatory subunit of Non-muscle Myosin II (Myosin hereafter), encoded by spaghetti squash (*sqh*), endogenously tagged with EGFP (*Sqh::EGFP*, hereafter) [83]. Next, we expressed UAS-ALD-RockCA::vhhGFP4 (called nanobody hereafter)

with the neuroblast-specific driver *worniu-Gal4* (*worGal4* [2]) in flies containing *Sqh::EGFP*. The nanobody construct contains *Inscuteable*'s apical localization domain (ALD) [98] and the constitutively active kinase domain from Rho kinase (RockCA). The apically localized nanobody binds to the EGFP moiety of *Sqh::EGFP*, tethering and activating it only on the apical neuroblast cortex (Figure 2.S1 A)[155, 113]. The apical neuroblast cortex can be identified by the increased concentration of *Sqh::EGFP* (Figure 2.S1 B). Additionally, retaining activated Myosin apically does not disrupt neuroblast polarity[155]. Consistent with our earlier findings, maintaining a pool of activated Myosin on the apical neuroblast cortex resulted in three division outcomes: (1) approximately 60% normal asymmetric, (2) approximately 27% symmetric, and (3) approximately 8% inverted asymmetric divisions. The inverted divisions produced a small apical and a larger basal cell. Some neuroblasts also displayed a more pronounced NB-GMC cell size ratio; however, these occur less frequently in wild-type and control conditions (Figure 2.S1 B, C). Here, we focus on the cellular and developmental consequences of symmetric and inverted asymmetric divisions.

2.2.2 Sibling cell size and position determines the cell's division potential

We next asked whether cell size correlates with proliferation potential. Wild-type *Drosophila* neuroblasts are at least twice as large as GMCs and divide every 1 - 4 hours. GMCs have a long cell cycle exceeding 8 hrs [82, 163]. If cell size predicts cell cycle progression, we would expect that small apical cells of inverted neuroblast divisions have a longer cell cycle than their larger basal siblings. Similarly, the siblings resulting from symmetric neuroblast divisions should display similar cell cycle lengths. To test these hypotheses, we performed long-term live cell imaging experiments, imaging control and nanobody-expressing larval brains for 8-14 hrs (see methods). We used the spindle marker *mCherry::Jupiter* 51, expressed with the neuroblast driver *worGal4* [2] to assess cell cycle stage and length. As expected, wild-type neuroblasts, expressing *Sqh::EGFP* in conjunction with *mCherry::Jupiter* showed cell cycle lengths between 1.5 - 4 hrs and always created a large apical neuroblast and a small basal GMC (Figure 2.1 A, D). Neuroblasts expressing the nanobody in the presence of *Sqh::EGFP* and *mCherry::Jupiter* generally divided less frequently (see below).

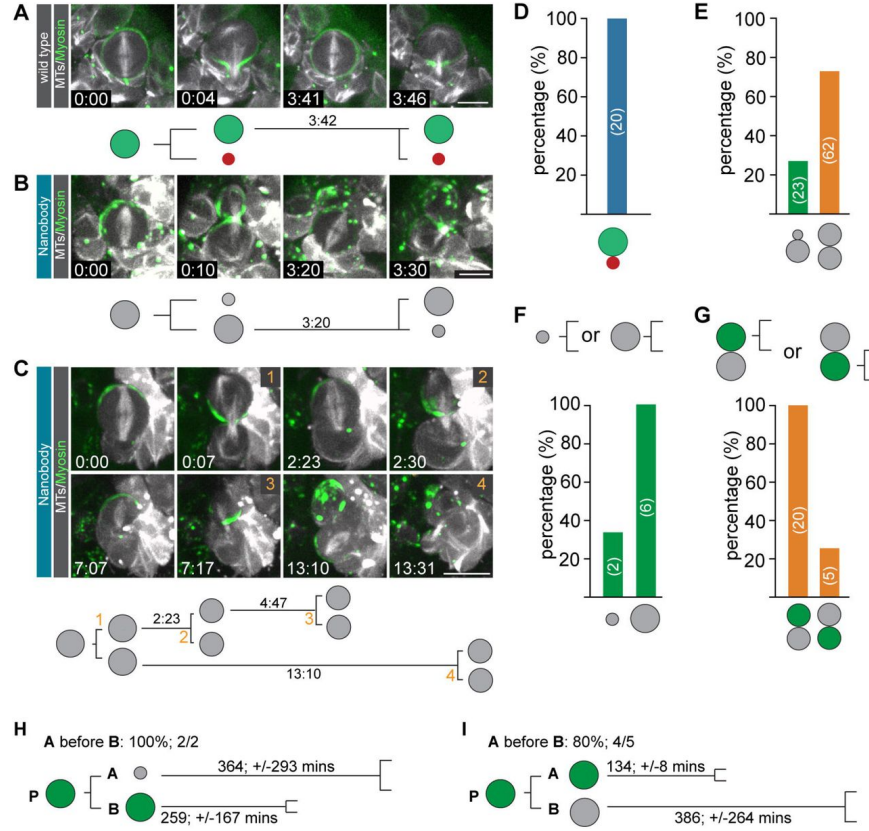


Figure 2.1: Cell size and position determine the cell's proliferation potential. Representative image sequence of an (A) asymmetrically dividing wild-type, (B) nanobody-expressing NB dividing with inverted (C) or symmetric cell size asymmetry. All examples show Sqh::EGFP in green and mCherry::Jupiter in white. Division mode of wild-type (D) or nanobody-expressing (E) neuroblasts. Only inverted and symmetric divisions were counted in nanobody-expressing neuroblasts. (F) Quantification of apical and basal cell divisions, following an inverted asymmetric (F) or symmetric (G) neuroblast division. Quantification of cell cycle time and frequency of apical and basal cell division for (H) inverted or (I) symmetric neuroblast divisions. Numbers on bars indicate number of observed neuroblasts. Scale bar is 10 μ m; time in hours:minutes.

Still, we found occasional inverted or symmetric neuroblast divisions, whereby symmetric divisions occurred more regularly than inverted asymmetric divisions (73% symmetric, 27% inverted asymmetric; n = 85; Figure 2.1 B, C, E). Symmetrically dividing neuroblasts, induced through spindle misalignment, divide orthogonal to the neuroblast's initial apical-basal polarity axis [163, 89, 23], whereas nanobody-induced symmetric or inverted

divisions divide along the apical–basal polarity axis (Figure 2.S1 D–G) [155]. Therefore, nanobody-induced symmetric or inverted divisions maintain the sibling cells’ original position while changing their cell size. We next asked whether the apical or basal cell is more proliferative after nanobody-induced inverted and symmetric divisions. To answer this question, we analyzed lineages of symmetric and inverted neuroblast divisions where either the apical, basal, or both siblings divided again. We found 6 inverted neuroblast divisions where all basal cells divided again. In these lineages, only 2 small apical cells were able to divide within the recorded time frame, but only after the basal cell divided first (Figure 2.1 F, H). Interestingly, in size-symmetric divisions, apical cells were more proliferative than basal cells. For instance, of 20 symmetrically dividing neuroblasts, all apical but only 5 basal cells divided again. Of these 5, 4 apical cells divided before its basal sibling (Figure 2.1 G, I). Our live cell imaging protocols allow to record multiple neuroblast divisions in larval brain explants [26, 27, 111, 160] but although we imaged 10–14 hours, we never detected more than 2 divisions of the apically-positioned cell following a symmetric NB division (Figure 2.1 C). Therefore, nanobody-expressing NBs created short, but asymmetric hemilineages. Based on these results, we conclude that if cell volume is equal between neuroblast siblings, the apically positioned cell is more proliferative. However, if cell volume is unequal, the larger cell is more proliferative.

2.2.3 Cell position and cell size determine cell fate outcomes in NB divisions with altered CSA

Next, we asked whether the sibling cells of symmetric or inverted asymmetric neuroblast divisions adopt neuroblast fate. To that end, we used CRISPR/Cas9 to generate a live neuroblast marker by knocking in the fluorescent protein tdTomato [161]58 at Deadpan’s (Dpn) endogenous locus [18] (see methods). We crossed tdTomato::Dpn to sqh::EGFP and the worGal4-driven nanobody construct. In wild-type neuroblasts, tdTomato::Dpn was localized in the nucleus during interphase and released into the cytoplasm in early mitosis. In telophase, cytoplasmic tdTomato::Dpn segregated into the self-renewed wild-type neuroblast and the differentiating GMC. Shortly after cytokinesis, tdTomato::Dpn was incorporated

into the neuroblast’s nucleus but was also visible in the differentiating GMC. However, the neuroblast Dpn signal was stronger compared to the GMC in wild-type neuroblasts (Figure 2.2 A-C;). Next, we assayed tdTomato::Dpn segregation and expression in *mud* mutants because symmetrically dividing *mud*⁴ null mutant neuroblasts give rise to two Dpn+ sibling cells 51. As expected, symmetrically dividing *mud*⁴ mutant neuroblasts inherited equal amounts of tdTomato::Dpn, entering the nucleus in both sibling cells. In both equal-sized siblings, tdTomato::Dpn signal was maintained at comparable levels (Figure 2.2 D – F). Similarly, the apical and basal siblings of symmetrically dividing nanobody-expressing neuroblasts inherited and maintained equal amounts of tdTomato::Dpn (Figure 2.2 G-I). Surprisingly, nanobody-expressing neuroblasts dividing with inverted cell-size asymmetry showed two different outcomes: (1) in one instance, we found a small apical cell that showed no detectable tdTomato::Dpn after mitosis (Figure 2.2 J-L). (2) A second event showed a smaller apical cell containing nuclear tdTomato::Dpn (Figure 2.2 M – O). Interestingly, in both instances, the large basal sibling contained tdTomato::Dpn. We conclude that for induced symmetric and inverted divisions, larger sibling cells always receive and maintain nuclear Dpn, while smaller siblings may also inherit and sustain nuclear Dpn.

2.2.4 Larval brains with altered CSA maintain the same cell populations, but with altered ratios

Wild type neuroblasts generate all the cell types in the developing larval brain [80]. Since changes in CSA alter the number, size, and proliferation potential of larval neuroblasts (see 51 and above), we hypothesized that changes in NB CSA could impact the number and type of neuroblast progeny cells. To define the molecular changes and cell fate outcomes of CSA, we sought to investigate gene expression profiles in single cells. To this end, we performed single-cell RNA sequencing on control, *mud*⁴, and nanobody-expressing brains at 72 - 96 hours after egg laying (see methods). While control brains only contain asymmetrically dividing neuroblasts, *mud*⁴ mutant brains are composed of a mix of asymmetric and symmetrically dividing neuroblasts. Nanobody-expressing brains develop with normal asymmetric, symmetric, and inverted asymmetric divisions (Figure 2.S1 D-G). For each

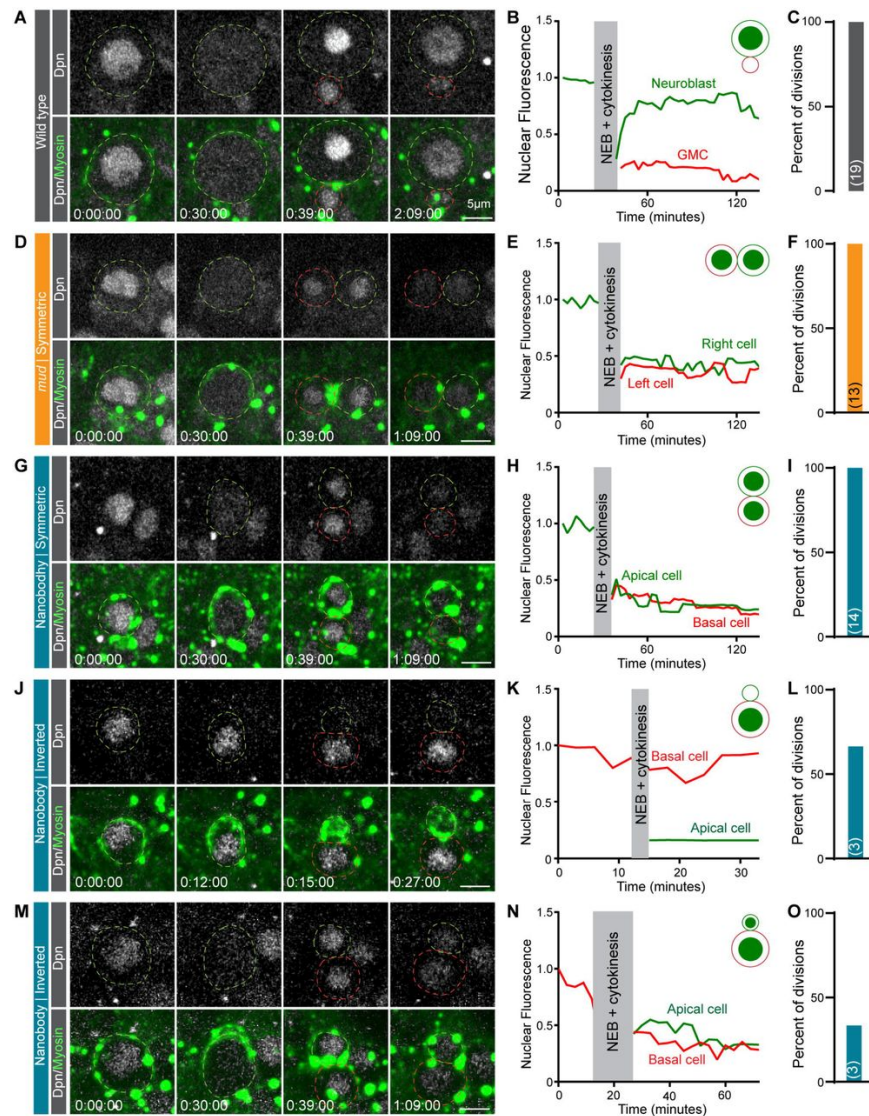


Figure 2.2: Dpn is inherited by the siblings of symmetrically or inverted asymmetrically dividing neuroblasts. Representative image sequence of a (A) wild type, (D) *mud*⁴ mutant, or (G, J, M) nanobody-expressing neuroblasts. All shown examples express tdTomato::Dpn (white) and Sqh::EGFP (green). Dotted lines outline the parental and sibling cells. (B, E, H, K, N) Sum of intensity quantification of tdTomato::Dpn over time in the two sibling cells for the shown examples. Schematics in the upper right corner of the graph illustrate the division outcome and Dpn (green) inheritance. (C, F, I, L, O) Quantification of division outcomes. Numbers on bars indicate number of observed neuroblasts. Scale bar: 5μm. Time in hours:minutes:seconds

condition, we dissected and dissociated approximately 50 larval brains into single-cell suspensions, sorted for cells of interest, and prepared cDNA libraries. We used the standard 10x Genomics Cellranger pipeline for initial alignment, and a Seurat pipeline for quality control, integration, and further analysis (detailed protocols in methods). After quality control, we retained approximately 13,500 cells for each condition. We identified 28 individual clusters and annotated them based on the presence and abundance of validated marker genes, as well as by comparing differentially expressed genes (DEGs) from annotated clusters in published *Drosophila* scRNAseq datasets [25, 49, 135, 39, 185]. We annotated clusters 15 and 19 as neuroblasts based on the expression of *dpn*, *mira*, and *CycE* among others [18, 29, 87, 6, 24, 7]. Cells in cluster 11 expressed the validated INP genes *ham* and *opa* [1, 119]. Clusters 5, 7, 9, 12 expressed the GMC genes *grh*, *tap*, *dap* [104, 129], clusters 3, 13, 14 the early neuronal marker *Hey* [131], clusters 1, 2, 4, 8, 10, 18 the immature neuron markers *elav* and *fne*, but no *nSyb* or *brp* [156, 153]. Several clusters contained cells expressing mature neuronal markers such as *nSyb*, *brp*, and various neurotransmitter marker genes [46, 183]. We also found more specialized, terminal cell types in our atlas, such as Kenyon cells (*Rgk1*) [132], glia (*repo*, *wrapper*) [192, 136], and a neuron type with high expression of all three Iroquois gene complex members (*ara*, *caup*, and *mirr*) [39]. (Figure 2.3 A, D, Figure 2.S2, Figure 2.S3, Figure 2.S4, Figure 2.S5, Figure 2.S6).

We hypothesized that symmetric and/or inverted asymmetric cell divisions will either (1) create new or hybrid cell types with unique expression profiles, (2) eliminate characterized cell types or, (3) create differences in the number of specific brain cells. Surprisingly, cluster analysis revealed that control, *mud*, and nanobody-expressing brains all contained the same 28 clusters. While the expression profiles of all three genotypes varied (see below), standard clustering methods neither revealed a new, nor prevented the formation of a known cell type compared to the wild-type cell atlas (Figure 2.3 A-D). However, more detailed analysis of cluster sizes showed that certain cell types appeared in increased or decreased numbers (Figure 2.3 E). Most noteworthy, the neuroblast cluster 19 showed the highest difference in neuroblast numbers between the three conditions. These findings suggest that altering CSA in neuroblasts neither creates new, nor eliminates existing brain cell types but changes the number of specific brain cell populations.

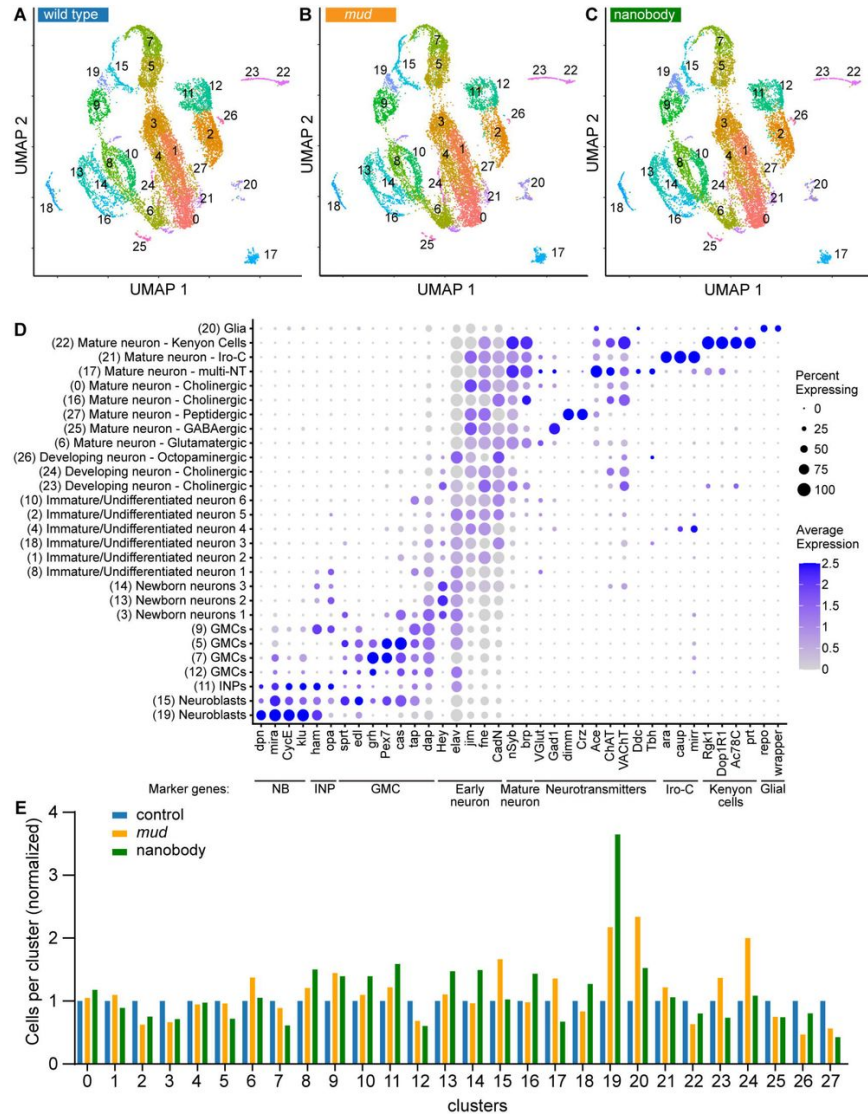


Figure 2.3: Altering neuroblast sibling cell size asymmetry changes cell numbers but not cell types. UMAP plots of the scRNAseq clusters for (A) wild type, (B) *mud*⁴ and (C) nanobody-expressing neuroblasts. All 28 clusters (0-27) are present in all 3 conditions. (D) Annotations of all clusters (y-axis) with representative marker genes (x-axis). Dot size indicates the percent of cells per cluster expressing the shown gene. Dot color indicates the relative expression of the gene where zero represents average expression. Values below zero show up as gray, regardless of magnitude. Clusters are ordered vertically by developmental age and type. (E) Graph of cell numbers per cluster, normalized for library size, and normalized to control for each individual cluster.

2.2.5 *Changes in neuroblast sibling cell size increase the neuroblast pool*

In wild-type third instar brains, the neural stem cell pool remains constant, but *mud*⁴ mutant brains contain ~50% more neuroblasts, due to symmetric, neuroblast-amplifying divisions [26, 112]. Consistent with this result, the neuroblast clusters 15 and 19 showed more cells in both *mud*⁴ mutant and nanobody-expressing brains (Figure 2.4 A, B). To verify this result, and to assay how increasing the neuroblast pool affects neurogenesis, we labelled type I neuroblast lineages in wild type, *mud*⁴, and nanobody-expressing brains with AseGal4 [198], driving the expression of mCherry::Jupiter. Type I wild-type lineages only contain 1 Dpn+ neuroblast [21, 24, 13] and multiple differentiating Pros+ cells, providing a clear binary readout. We stained third instar larval control, *mud*⁴ mutant, and nanobody expressing brains with anti-Dpn and anti-Prospero. We segmented type I neuroblasts, Pros+ cells, and identifiable Ase-Gal4 expressing lineages to quantify cell type, cell size, cell number, and lineage size. While all wild-type type I lineages contained a single Dpn+ neuroblast surrounded by multiple smaller Pros+ differentiating cells, nanobody-expressing brains often contained two, and *mud*⁴ mutant brains up to four Dpn+ neuroblasts (Figure 2.4 C-F). Notably, we did not find any nanobody or *mud*⁴ mutant lineages with more than four Dpn+ neuroblasts. In addition, both *mud*⁴ and nanobody-expressing brains contained significantly smaller Dpn+ nuclei, indicating that *mud*⁴ and nanobody-expressing neuroblasts are smaller than wild-type neuroblasts (Figure 2.4 G). *mud* mutant brains have been reported to contain enlarged mushroom bodies, containing more neuroblasts and differentiated Kenyon cells [54]. Surprisingly, our segmentation analysis revealed that type I neuroblast lineages are smaller and contain fewer Pros+ cells in *mud*⁴ and nanobody-expressing brains (Figure 2.4 H, I). While in nanobody-expressing brains the neuroblast's mitotic index was significantly reduced compared to wild-type brains, *mud*⁴ mutant neuroblasts surprisingly showed only a small, albeit significant, decrease in their mitotic index (Figure 2.4 J, K). From these data, we conclude that altering neuroblast CSA increases the neuroblast pool while decreasing neuroblast size. We further conclude that a larger neuroblast pool fails to generate more differentiating cells, thereby restricting lineage expansion.

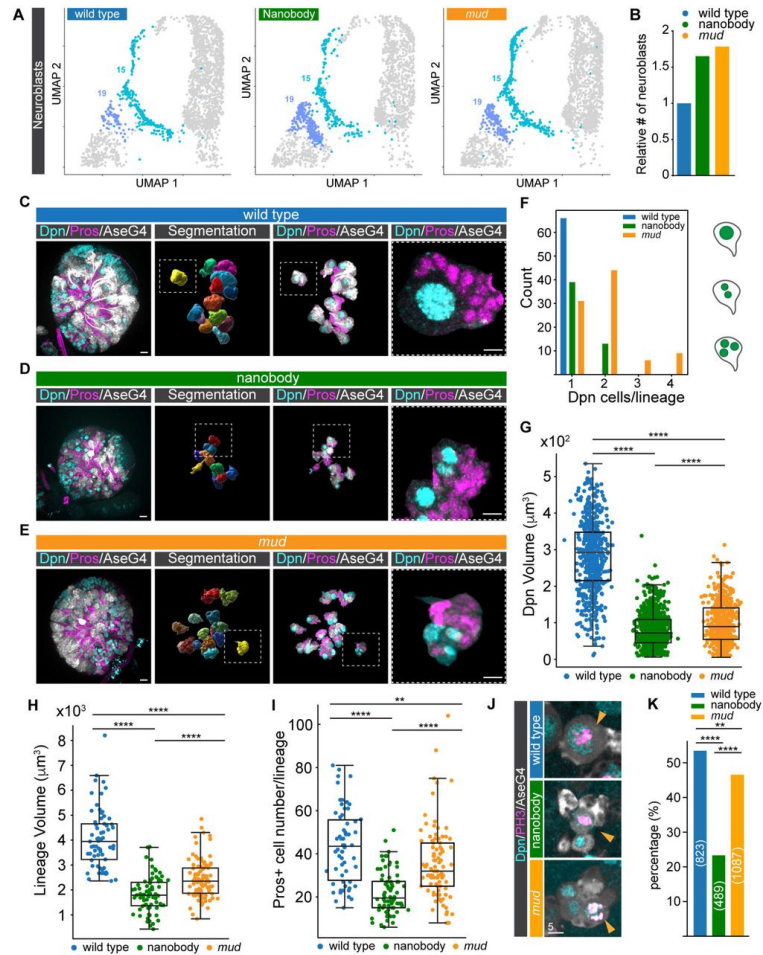


Figure 2.4: Altering neuroblast sibling cell size asymmetry changes neuroblast size, lineage size and cell number. (A) UMAP plot of scRNAseq data for the neuroblast clusters 15 and 19 for each condition. (B) Quantification of the relative total neuroblast number, normalized for library size and control neuroblast numbers. Representative type-I (AseGal4 > UAS-mCherry::Jupiter; white) neuroblast lineages for (C) wild type, (D) nanobody-expressing and (E) *mud*⁴ mutant neuroblasts stained with anti-Dpn (cyan) and anti-Prospero (magenta). Segmentation of AseGal4-expressing lineages and neuroblasts (not shown) was used to count (F) neuroblast number per lineage, (G) neuroblast (Dpn) nuclear size, (H) lineage size and (I) number of Prospero cells per lineage. Representative images (J) for wild type, *mud*⁴ and nanobody-expressing type I (AseGal4 > UAS-mCherry::Jupiter; white) neuroblasts stained with anti-Dpn (cyan) and anti-PH3 (magenta). (K) Quantification of mitotic index. Scale bar is 10 μm . $p < 0.05$ were considered significant; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. The following statistical tests were used: Anova and Tukey HSD for (G, H, I, K).

2.2.6 *An increased neuroblast pool does not increase the number of GMCs, early-born, immature, or mature neurons*

We next wondered whether our RNAseq dataset could be used to determine how changes in CSA affect cell differentiation by assessing the relative numbers of brain cell populations. As a proof of principle, we first compared the number of cells expressing prospero mRNA (pros+ cells) with cells immunoreactive for the anti-Prospero antibody (Pros+ cells). prospero is expressed ubiquitously in wild-type, mud⁴, and nanobody-expressing cells, providing a generic marker for various types of neuroblast progeny (Figure 2.5 A, B). Based on the IHC data, we expected to see a proportional reduction in pros+ cells in our cell atlas in mud⁴ and nanobody-expressing brains. Indeed, while the fraction of pros+ cells (excluding clusters 15 and 19) is similar across the different conditions (Figure 5A, B), we calculated that one wildtype neuroblast generates approximately 31 pros+ cells based on the RNAseq dataset. This number was reduced in mud⁴ and nanobody-expressing brains (mud⁴: 18; nanobody: 20). We next calculated the number of Pros+ cells per neuroblast based on our IHC dataset. Consistent with the RNAseq data, we found that wild type neuroblasts generate on average 42 Pros+ cells (SD+/-17.7; n = 62 lineages), mud⁴ mutant neuroblasts produce 22 Pros+ cells (SD+/-14; n = 93 lineages) and nanobody-expressing neuroblasts generate 20 Pros+ cells (SD+/-11; n = 72 lineages) (Figure Figure 2.5 I, J). Thus, the RNAseq and IHC datasets show comparable numbers of Pros+ cells per neuroblast.

Each asymmetric type I neuroblast division generates a GMC, and we wondered whether an increase in the neuroblast pool also increases the number of GMCs. The transcription factor Grainyhead (grh) [177] is a specific marker for the GMC clusters [116, 110, 79] (Figure 3D, Figure S3C, D). Per RNAseq data, a wild-type neuroblast should generate 5 grh+ GMCs, whereas mud⁴ mutant and nanobody-expressing NBs are expected to produce approximately 3 and 2, respectively (Figure 2.5 K). To validate these numbers, we stained wild-type, mud⁴, and nanobody-expressing brains with anti-Dpn and anti-Grh antibodies and counted the number of Grh+ cells per neuroblast. Consistent with the RNAseq results, we calculated 5.2 Grh+ cells per neuroblast in wild type (SD+/-1.9; n = 59 lineages), 3.9 Grh+ cells in mud⁴ (SD+/-1.4; n = 28 lineages) and 1.8 Grh+ cells in nanobody-expressing

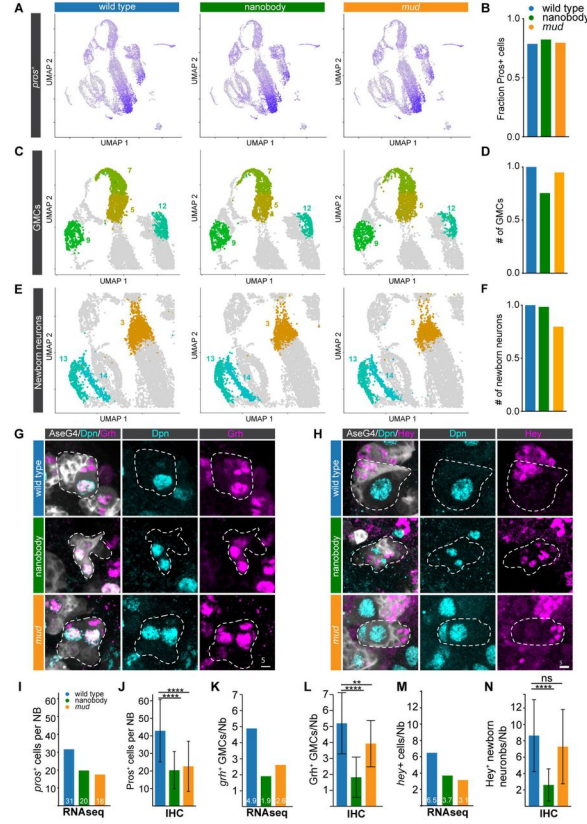


Figure 2.5: Altering neuroblast sibling cell size asymmetry reduces GMC, newborn neurons and overall prospero-positive cell numbers. (A) Plots of prospero expression for wild type, nanobody-expressing and *mud*⁴ mutant brains overlaid on the UMAP. High Prospero-expressing cells are visualized with intense purple. (B) Fraction of cells per condition with at least 1 prospero transcript. UMAP plots for wild type, nanobody and *mud*⁴ highlighting (C) GMC or (E) newborn neuron clusters. Quantification of relative (D) GMC numbers and (F) newborn neurons, respectively, normalized for library size and control. Representative type I neuroblast lineages (AseGal4 λ UAS-mCherry::Jupiter; white), stained for (G) anti-Dpn (cyan) and anti-Grh (GMCs; magenta) or (H) anti-Dpn (cyan) and anti-Hey (newborn neurons; magenta). Quantifications of the number of cells expressing prospero (pros+) determined by (I) RNAseq or (J) IHC. Quantifications of the number of cells expressing grainyhead, determined by (K) RNAseq or (L) IHC. Quantifications of the number of cells expressing Hey determined by (M) RNAseq or (N) IHC. Scale bars are 5 μ m. $p < 0.05$ were considered significant; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. The following statistical tests were used: One-way Anova and Dunnett's multiple comparisons test for (J, L, N).

neuroblasts ($SD \pm 1.3$; $n = 70$ lineages) (Figure 2.5 G, K, L). We also assessed the number of newborn neurons, using Hey as a marker (Figure 2.3 D and Figure 2.S4 A, B). We again found similar numbers of Hey+ cells per wild-type neuroblast comparing RNAseq and IHC data, and both mud^4 and nanobody-expressing brains had a significant reduction in Hey+ cells (Figure 2.5 H, M, N). Finally, our RNAseq dataset also highlighted a decrease in $elav+$ immature and $nSyb/brp+$ mature neurons in mud^4 and nanobody-expressing brains (Figure 2.6 B-G). We conclude that despite an increase in the neuroblast pool, the number of GMCs, early-born, immature, or mature neurons decreases in larval type I NB lineages with altered CSA.

2.2.7 Modeling predicts that unregulated CSA would exceed NB, GMC and neuron numbers

Symmetrically dividing mud^4 mutant neuroblasts have a reduced mitotic index (Figure 2.4K) but still divide frequently compared to wild type NBs[26]. Thus, it is surprising that the increase in the neural stem cell pool does not exceed 4 NBs per lineage, which fail to generate more differentiating progeny than observed in wild type lineages. We used an ordinary differential equation (ODE) model to better understand the consequences of neuroblast division mode on cell differentiation and cell numbers. We first developed a base model that describes three core processes of NB lineage progression: NB division, GMC division, and neuron maturation. The cell types in the model include NBs, GMCs, immature neurons, and mature neurons (Figure 2.6 A). In the base model, NBs divide either symmetrically, yielding two NBs, or asymmetrically, yielding one NB and one GMC. Model parameters include NB and GMC division rates, neuronal maturation rate, and the fraction of NB divisions that are symmetric. To simulate wild type lineage development, we initialized the model with one neuroblast, set the fraction of symmetric divisions to 0%, and parameterized the division and maturation rates from experimental data (see methods). The wild-type simulation produced 1 NB, 6 GMCs, and 35 immature neurons, comparable to what we observe in vivo (Figure 2.S7 A).

To simulate mutant NB lineage development, we increased the fraction of symmetric divisions to 15%, consistent with the rate of symmetric divisions experimentally observed

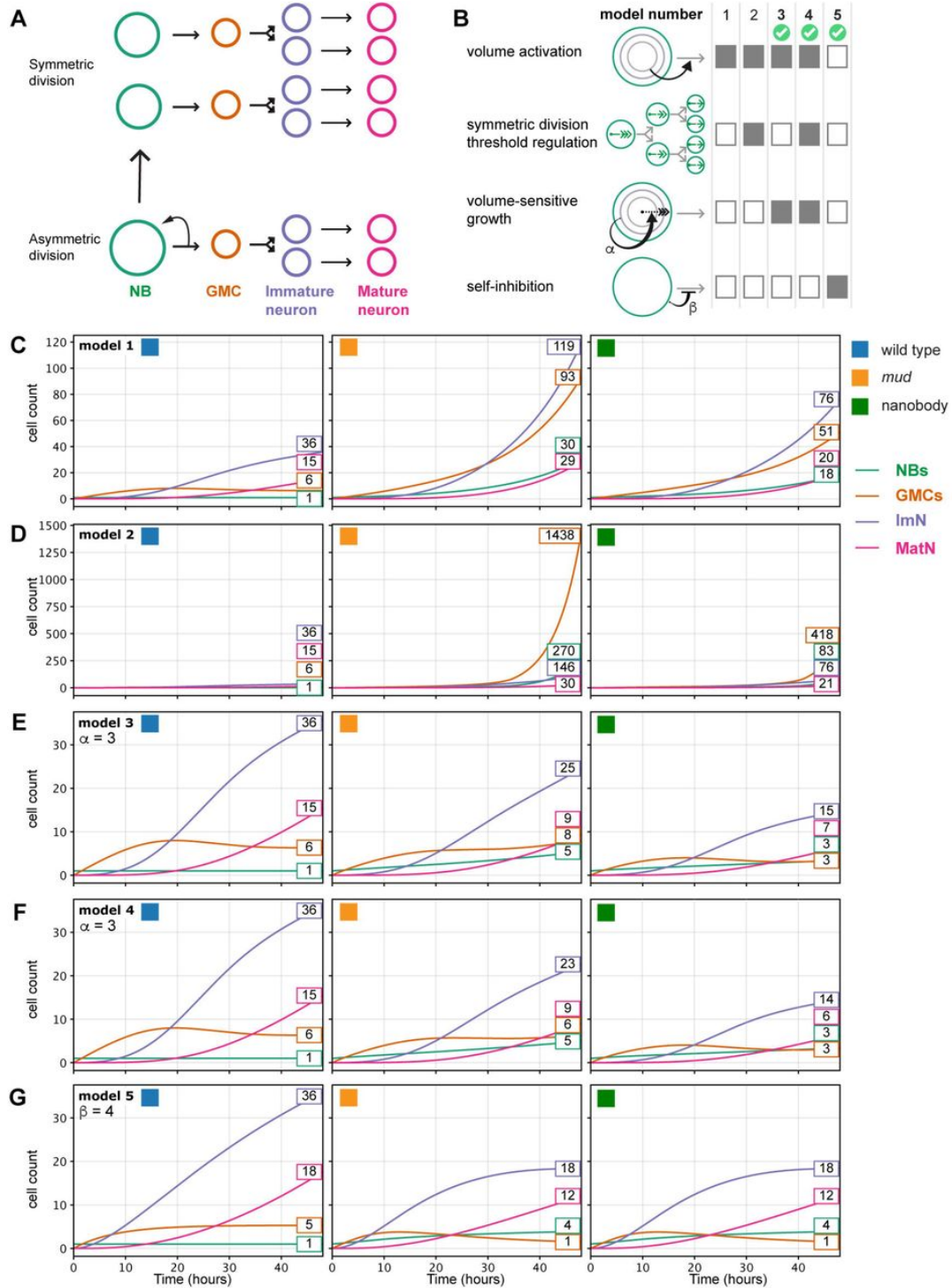


Figure 2.6: **ODE modeling shows volume-sensitive NB growth and NB self-inhibition are both sufficient mechanisms for constraining NB lineage expansion.** (A) Cartoon showing the differentiation cascade of NBs following asymmetric (bottom) and symmetric (top) division. (B) Summary of dynamics included in each model. Bold numbers and green check marks denote models in which mutant proliferation was effectively constrained. (C-F) Cell counts for each model over the duration of the simulation for wild type (left), *mud*⁴ mutant (center), and nanobody (right) simulations.

in *mud*⁴ mutant NBs [26]. This simulation yielded a lineage with a substantially higher cell count than the wild-type simulation (122 NBs, 326 GMCs, 596 immature neurons; Figure 2.S7 B). This mismatch between observed cell counts in vivo and in silico model output suggests that additional regulatory mechanisms restrict the NB pool size and the number of differentiating siblings after symmetric divisions.

2.2.8 Volume-dependent division dynamics are insufficient to explain mutant lineage behavior

Our base model does not account for cell growth or volume, but it is known that NBs regrow after each division during in third instar larval brains [80, 81]. To evaluate whether growth- and volume-dependent regulatory mechanisms could explain the reduced size of mutant lineages, we extended the base model to explicitly track both cell counts and cell volume (Figure 2.6 A, B). In this framework, there is one volume term for each cell type that tracks the total volume of all cells for the given cell type. Simulations are initialized with one NB with an initial volume of $905\mu\text{M}^3$. NBs grow at a constant parameterized rate and divide either asymmetrically, or symmetrically. In this model, when NBs divide asymmetrically, the NB volume term retain 80% of the dividing NB volume and pass 20% to the GMC volume term. Asymmetric divisions increase the GMC count by one, whereas the NB count remains unchanged (1 NB total). When NBs divide symmetrically, the NB volume term retains the full dividing NB volume and the NB count increases by one. GMCs also grow and divide in our simulations, passing their volume to neurons; neurons do not grow or divide.

To regulate NB division, we implemented a volume-activation mechanism (Figure 2.6 B) in which division rates follow a power-law dependence on average NB size. In this model, the NB's growth rate is constant, but their division rates are very low until their volume approaches a threshold, set to 125% of their initial volume. At that threshold the division rate increases sharply. A lower bound is imposed so NBs with volumes less than 25% their initial size are unable to divide.

We first asked whether this volume-activation mechanism was sufficient on its own to

constrain mutant proliferation. Because NBs produced by symmetric divisions begin smaller and NB growth rate is constant between mutant and wild type NBs, we expected the mutant NBs to take longer to reach the division threshold, prolonging their cell cycles and potentially suppressing mutant NB lineage proliferation. To test this theory, we simulated mud mutant NB colonies that had 15% symmetric divisions, and nanobody-expressing NB colonies that had both 15% symmetric divisions and an NB growth rate reduced to 80% of wild type. As expected, mud and nanobody-expressing NBs showed prolonged cell-cycle durations relative to wild type (Figure 2.S8 A, B). However, this delay was insufficient to offset the expansion of the NB pool, and mutant simulations still predicted substantial lineage overgrowth (Figure 2.6 C; Model 1).

Experimental evidence indicates that mud⁴ mutant NBs generated through symmetric division do not regrow to their full initial volume but divide at smaller volumes than wild type NBs (Figure S9A, B). To capture this observation, we introduced an additional dynamic in which the NB division volume threshold decreased incrementally with each symmetric division (Figure 2.6 B; Model 2). As expected, this modification accelerated proliferation in mutant simulations, ultimately driving mud mutant and nanobody models to the maximum NB division rate, causing an increase in cell counts that is inconsistent with experimental observations (Figure 2.6 D; Figure S8C).

Together these results show that despite the smaller volumes of NBs resulting from symmetric divisions, volume-based division alone is insufficient to constrain mutant NB lineages. Thus, additional negative feedback must act on NB growth and/or division to suppress symmetrically dividing NB overgrowth.

2.2.9 Volume-sensitive growth could suppress excessive lineage expansion of symmetrically dividing NBs if volume sensitivity is high

The preceding sections established that some form of negative regulation must act on symmetrically dividing neuroblasts (NBs) to prevent excessive lineage expansion and NB pool amplification. A salient phenotypic difference between NBs produced by symmetric versus asymmetric divisions is starting size. As noted above, symmetrically produced NBs are

smaller than the pre-symmetric division parental NB (Figure 2.S9 A, B). In contrast to model 1 and 2, where NB growth rate was constant across all NBs, we decided to evaluate whether volume-sensitive growth would effectively suppress mutant NB proliferation. In this model, smaller NBs grow at a slower rate than larger NBs: when the average NB volume was smaller than a reference value (set to equal the initial NB volume), the growth rate was reduced below the base rate. As the average NB volume exceeded the reference, the growth rate increased proportionally. We introduced a sensitivity parameter, α , to control how sharply the growth rate responds to volume. Low α values cause the NB growth rate to rise gradually as the average NB volume increases; high α values strongly suppress growth when average volume is below the reference and accelerate it once the reference is exceeded.

We implemented this volume-sensitive growth in two settings: (i) with a static volume threshold for division (Model 3), and (ii) with a threshold that decreases with each symmetric division (Model 4) (Figure 2.6B). Our simulations show that volume-dependent growth can suppress mutant proliferation if growth-volume coupling is sufficiently strong. For $\alpha \leq 2$ both models predicted that symmetrically dividing mutant NBs outgrew wild-type lineages. For $\alpha \geq 3$, the slowing of growth in smaller NBs was sufficient to reduce lineage cell counts in *mud* and nanobody-expressing NBs compared to wild type. Notably, Model 4 consistently predicted fewer cells than Model 3 at a given α (Figure 2.6 E, F; Figure 2.S8 D, E; Figure 2.S10, Figure 2.S11). Although lowering the division threshold with each symmetric in Model 4 decreases the amount the NBs must grow to divide, by allowing cells to divide at smaller volumes it also allows NB starting size to decrease. At high alpha values, where NB growth is strongly volume-sensitive, this reduction feeds back to further suppress NB growth, ultimately extending the cell cycle duration and constraining proliferation more strongly than in Model 3 (Figure 2.S8 D, E). Consistent with these modeling results, we measured cell cycle durations in *mud*⁴ mutant neuroblasts that are similar to what model 3 and 4 predicted (Figure 2.S9 C). Similarly, nanobody-expressing NBs, dividing symmetrically or inverted asymmetrically generate siblings with extended cell cycle times and reduced proliferation rates (Figure 2.1C, H, I).

Taken together, Models 3 and 4 suggest that strong coupling of NB growth rate to NB volume is sufficient to restrict NB numbers and lineage expansion after symmetric NB

divisions. We conclude that volume-sensitive growth is a possible regulatory mechanism if NB growth is highly sensitive to cell size.

2.2.10 Neuroblast self-repression can suppress lineage expansion of symmetrically dividing NBs

We next asked whether the size of the neuroblast pool itself could act as a regulatory signal. Specifically, we wondered whether a negative feedback mechanism, in which neuroblasts repress their own division and/or growth could effectively constrain NB lineage expansion (Figure 2.6 B; model 5). To test this, we extended the base model to include a Hill-type negative feedback mechanism, repressing NB division rates. Hill-type feedback mechanisms represent regulatory processes in which the strength of the (in this case, inhibitory) regulation increases as the regulator abundance increases. In our system, an increase in NB number per lineage would increase the inhibition on the NB's division rate. The inhibition strength is regulated by the Hill coefficient: a low coefficient increases repression gradually as a function of NB number, while higher coefficients (here, $\beta \geq 2$) strongly inhibit division once the NB count passes 2. Our simulations showed that for $\beta \geq 3$, NB self-repression was sufficient to reduce mud and nanobody-expressing NB lineage sizes below wild-type cell counts (Figure 2.6G; Figure 2.S8 F; Figure 2.S12). Together these results show that NB self-repression can effectively suppress lineage expansion in mud and nanobody-expressing NBs.

2.2.11 mud^4 and nanobody-expressing neuroblasts differentially express neuroblast and differentiation genes

Both our experimental and modeling data highlight that lineage expansion is mainly driven by NBs proliferation behavior. mud^4 and nanobody-expressing neuroblasts are sufficiently similar to wild type NBs to form the same clusters. Nevertheless, we aimed to use gene expression to better define their molecular identity. We performed a differential expression analysis of cluster 15 and 19, separately comparing gene expression profiles of mud^4 and nanobody-expressing neuroblasts with wild-type neuroblasts. To ensure we retained the

most significant Differentially Expressed Genes (DEGs), we only considered genes that are expressed in at least 25% of neuroblasts, had a p-value of 0.001 or better, and a minimum log2-fold change of 0.5. This left us with 161 DEGs between wild type and *mud*⁴, and 104 between wild type and nanobody (Figure 2.7 A-D). In our initial analysis, we noticed several genes associated with neuroblast or neuronal cell fate that were differentially expressed in *mud*⁴ or nanobody-expressing neuroblasts compared to wild-type NBs (highlighted in Figure 2.7 A, C). For instance, the neuroblast markers *miranda* (*Mira*) was upregulated in *mud*⁴ and nanobody-expressing neuroblasts. Nervous finger 1 (*nerfin-1*), a transcription factor required for the maintenance of larval neuron differentiation [57, 182], or *ham*, a PRDM family transcription factor controlling intermediate precursor cell maturation in the type II larval neuroblast lineages [152], were also differentially expressed in both *mud*⁴ mutant and nanobody-expressing neuroblasts (Figure 2.7 F). To group our DEGs into ‘NB’ and ‘differentiation’ genes, we checked whether the identified DEGs are preferentially expressed in NBs or neurons, using a published dataset as a reference [14]. This analysis revealed that genes differentially expressed in both *mud*⁴ and nanobody-expressing NBs were slightly enriched for genes that are normally expressed in wild type NBs (Figure 2.7 F).

We next performed a GO term analysis and searched FlyBase for specific keywords to get functional insight into the DEGs. Interestingly, while several GO terms highlighted neuroblast fate commitment, cell proliferation, or neurogenesis processes, we found many genes associated with the keywords ‘cell cycle’, ‘cytoskeleton’, ‘differentiation’, ‘neuroblast’ or ‘neurogenesis’ (Figure 2.7 E and Figure 2.S13 and Figure 2.S14). In conclusion, *mud*⁴ mutant and nanobody-expressing neuroblasts differentially express genes that are associated with neuroblast expression or function, as well as genes associated with differentiation, cell cycle or cytoskeleton.

2.2.12 The NB size regulator Imp and cell cycle regulator CycE are both upregulated in mud and nanobody-expressing neuroblasts

After this initial characterization, we screened our DEGs for genes and pathways involved in NB growth to find a potential mechanism for the proposed volume-based coupling to cell

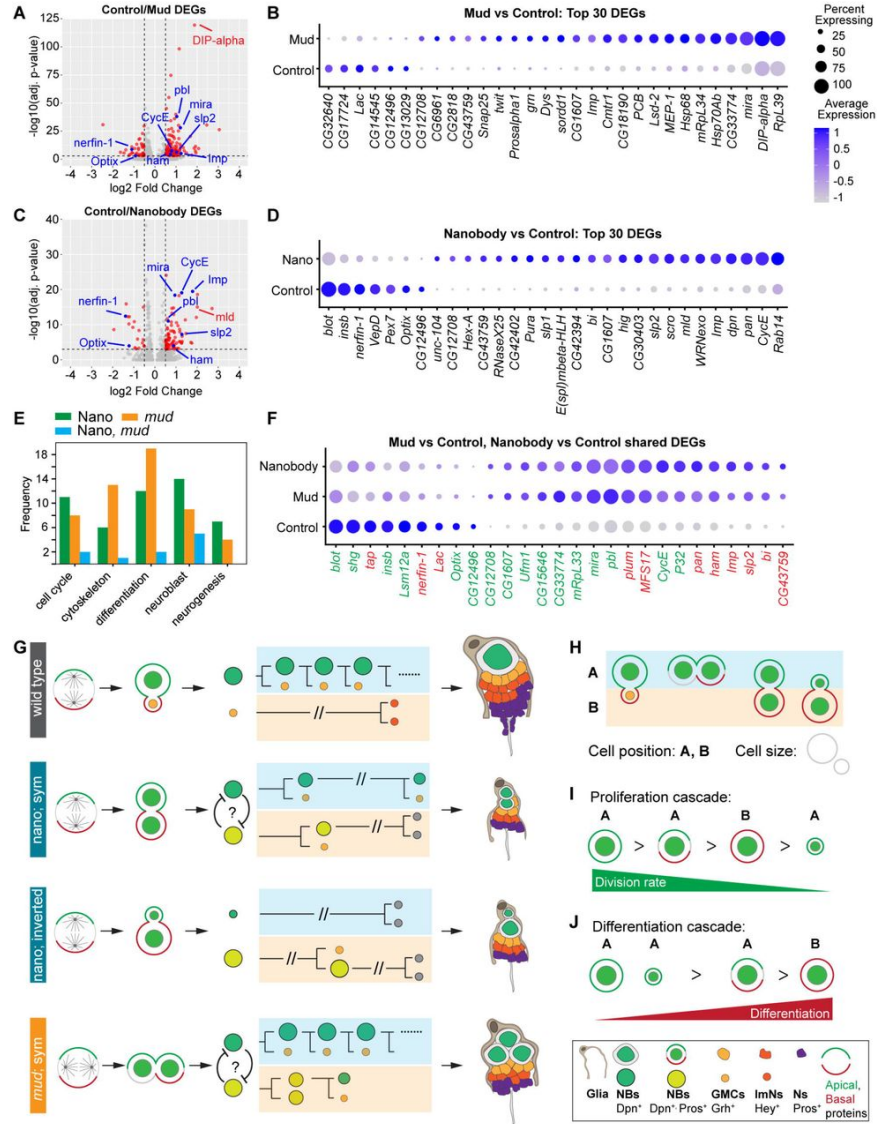


Figure 2.7: *mud*⁴ and nanobody-expressing neuroblasts differentially express genes associated with neuroblast fate, the cell cycle, cell differentiation, neurogenesis, and the cytoskeleton. Volcano plots of differentially expressed genes (DEGs) comparing (A) wild type with *mud*⁴, and (C) wild type with nanobody-expressing neuroblasts, respectively. DEGs with an adjusted p-value of 0.001 or lower and a log₂FC of 0.5 or higher are plotted with colored dots. Annotated dots refer to genes of interest. Dotplots of the top 30 DEGs comparing (B) wild type with *mud*⁴, (D) wild type with nanobody-expressing neuroblasts, and (F) DEGs shared between *mud*⁴ and nanobody-expressing NBs compared to wild type, respectively. Genes annotated in green and red correspond to genes expressed in NBs and neurons, respectively 94. Dotplots only show the top 30 DEGs by log₂FC. (E) Frequency of selected keywords associated with the DEGs. (G-J) Summary and Model. NBs dividing with altered CSA (symmetric or inverted asymmetric) create shortened and asymmetric lineage trees, resulting in diminished lineage expansion (different cell types are indicated with colors, see legend). (H) The position and size of sibling NBs (A, B), generated with altered CSA define their (I) proliferation rates and (J) differentiation potential.

cycle progression. Wild type NBs exiting quiescence activate cell growth and cell cycle entry via the Pi3K/Akt pathway in response to secreted Insulin-like peptides via the Insulin Receptor (InR) [35]. Similarly, the progressive reduction in neuroblast cell size in pupal stages is mediated by Ecdysone signaling, the mediator complex, and oxidative phosphorylation [82]. The p21-activated kinase Mushroom bodies tiny (Mbt; PAK4 in vertebrates) and the Casein Kinase 2 target Mushroom body miniature (Mbm) are also implicated in NB growth and proliferation control [126, 85]. Except for Mld, a transcription factor required for production of the steroid hormone ecdysone [133], none of these pathways showed differentially expressed genes in either *mud*⁴ or nanobody-expressing brains (Figure 2.S15A-D).

However, the mRNA-binding protein Imp (IGF2BP1 in vertebrates), regulating NB size and division rates [157], is upregulated in *mud*⁴ and nanobody-expressing NBs (Figure 2.7A - D, F). Interestingly, *mud*⁴ mutant neuroblasts also upregulated DIP-alpha, a member of the immunoglobulin superfamily (IgSF) cell adhesion molecules. DIP-alpha has been shown to localize to NB membranes, interacting with Dpr10 and Dpr6, which are expressed in cortex glia. This pathway has been proposed to regulate NB proliferation via mechanical signaling between NBs and cortex glia [40]. Strikingly, both *mud*⁴ and nanobody-expressing brains upregulated the cell cycle regulator CycE. CycE regulates entry into S-phase and overexpression of CycE accelerates cell proliferation without stimulating cell growth [134]. CycE also regulates cell fate in embryonic NBs, a function that is independent of its role in the cell cycle [15]. We conclude that while most genes from the Insulin, mediator complex and oxidative phosphorylation pathway are not differentially expressed, the neuroblast growth gene Imp and the cell cycle regulator and neuroblast marker CycE are both upregulated in *mud*⁴ and nanobody-expressing neuroblasts.

2.2.13 Dpn and Pros targets are differentially regulated in mud and nanobody-expressing NBs

We finally searched our DEGs for clues of how NB self-inhibition could be implemented molecularly. In wild type NBs, GMC differentiation and thus NB inhibition is induced via asymmetric segregation of basal cell fate determinants, such as Prospero, Numb or

Brat [80]. Miranda serves as a shuttle for the transcriptional repressor Prospero [80, 59]. The basal cell fate determinant Mira can still segregate asymmetrically in symmetrically dividing *mud*⁴ or nanobody-expressing NBs [28, 155, 26]. This is consistent with our live cell imaging data: equal-sized *mud*⁴ mutant, originating from symmetric divisions, show different proliferation behavior, reflected in the fact that one sibling divides more frequently than the other. Similarly, basal cells of symmetrically dividing nanobody-expressing NBs are not as proliferative as their apical siblings. These differences in sibling NB proliferation rates gives rise to asymmetric hemilineages (Figure 2.1F-I; Figure 2.S9B).

We wondered whether *mud*⁴ and nanobody-expressing NBs would differentially express Pros targets. We found several DEGs shared between *mud*⁴ and nanobody-expressing brains that are differentially expressed in *pros* mutants [36]. For instance, the Pros targets *CycE*, *bi*, *mRpL33*, *mira* and *pbl*, were upregulated, whereas *Lac* and *blot* were downregulated in *mud*⁴ and nanobody-expressing neuroblasts (Figure 2.S15D).

We also considered the fact that *Dpn*, a repressor of cell differentiation in the nervous system [121], segregates symmetrically in *mud*⁴ and nanobody-expressing neuroblasts (Figure 2.2D-I). Thus, we hypothesized whether *Dpn* could induce neuroblast-fate by repressing cell differentiation genes. We screened our DEGs, shared between *mud*⁴ and nanobody-expressing brains for *Dpn*-target genes [121] and found that *CG15646*, *ham*, *imp*, *mira*, *slp2* are up-, whereas *nerfin-1* is downregulated in *mud*⁴ and nanobody-expressing genes (Figure 2.S15E). In conclusion, *mud*⁴ and nanobody-expressing neuroblasts differentially express genes downstream of Pros and *Dpn* transcriptional regulators. This suggests that Pros and *Dpn* segregation in symmetrically, or inverted asymmetrically dividing neuroblasts impacts their gene expression profile.

2.3 Discussion

Cell Size Asymmetry is an evolutionary conserved form of Asymmetric cell division. However, how sibling cell size differences relate to cellular function and cell fate decisions is mostly unknown. Here, we used *mud* mutants and an apically localized nanobody to alter sibling cell size asymmetry in *Drosophila* neural stem cells. *mud* mutant neuroblasts can divide symmetrically by size due to spindle orientation defects [26]. In contrast,

nanobody-mediated maintenance of activated Myosin on the apical neuroblast cortex in mitosis induces either inverted asymmetric or symmetric neuroblast divisions [155]. Importantly, neither *mud* nor nanobody-expressing neuroblasts compromise neuroblast polarization [155, 26, 163]. However, *mud* mutants divide orthogonal to the apical-basal polarity axis while nanobody-expressing neuroblasts divide in parallel to the intrinsic polarity axis [155, 163, 89, 23].

Here, while both *mud* and nanobody-expressing brains increase the neuroblast pool in the developing brain, neuroblasts are smaller under these conditions compared to wild type. Live cell imaging data also showed that symmetrically and inverted asymmetrically dividing neural stem cells segregate Dpn into both siblings, giving rise to two equally sized Dpn+, or one smaller and one larger Dpn+ cell. Our live cell imaging data further revealed that neuroblasts created from inverted or symmetric divisions show strong size and position-dependent proliferation behavior. For instance, we only found two instances where the small, apically positioned neuroblast siblings divided again. And in these instances, larger basal siblings divided before the apical cell. In contrast, apically positioned neuroblast siblings originating from symmetric divisions are more proliferative than their equal sized basal siblings. Siblings of symmetrically dividing *mud* mutant neuroblasts also showed asymmetric proliferation behavior, whereby one sibling divided more frequently than the other (Figure 2.7 G).

Surprisingly, we discovered that despite the increase in neural stem cells, differentiating cell types do not increase in number. Both *mud* and nanobody-expressing brains have smaller neuroblast lineages, with fewer GMCs, immature and mature neurons. We used mathematical modeling to find mechanisms that could recapitulate the observed cell numbers and found that either a volume-sensitive mechanism, restricting neuroblast proliferation when neuroblast volumes are reduced, or a direct NB self-inhibition mechanism could recapitulate the observed cell type numbers.

Gene expression profiling further shows that *mud* and nanobody-expressing neuroblasts upregulate genes associated with neuroblast fate or neuronal differentiation, but also genes implicated in cytoskeletal functions, the cell cycle, or neurogenesis.

Based on these data, we propose that neuroblast size and position regulates cell cycle

progression and lineage expansion (Figure 2.7 G-J). Larger, apically positioned neuroblasts are more proliferative, producing more differentiating siblings than smaller apical or equally sized basal siblings. These differences in proliferation create hemilineage asymmetry, whereby one sibling generates more differentiating progeny than its sibling. In both instances, the neuroblasts' proliferation ability could be regulated by asymmetrically segregating cell fate determinants such as Miranda or Pros. In fact, we previously showed that symmetrically dividing *mud* mutants still segregate Miranda asymmetrically [28, 26]. Since apical Myosin sequestration does not change the NB's intrinsic polarity, basal siblings most likely still inherit Miranda and Prospero in neuroblasts expressing the nanobody construct. Wild-type GMCs inherit Prospero, which induces the expression of differentiation genes, while downregulating neural stem cell and cell cycle genes[36]. However, since Dpn segregates symmetrically, entering the nucleus in symmetrically, or inverted asymmetrically dividing *mud* and nanobody-expressing neuroblasts, Prospero-induced differentiation could be offset by Dpn, which is known to repress differentiation genes[121]. Neuroblasts in *mud* and nanobody-expressing brains also upregulate genes associated with neuroblast fate, such as *mira* or *CycE*. *CycE* has a dual role in cell cycle progression and neuroblast fate decisions[15]. Overall, symmetrically, and inverted asymmetrically dividing neuroblasts generate siblings that retain neural stem cell fate, but with reduced proliferation potential, which creates asymmetric neuroblast lineages, preventing normal lineage expansion.

All the cells in the developing wild type *Drosophila* brain originate from asymmetrically dividing neuroblasts[80]. *mud* mutants contain supernumerary mushroom body neuroblasts and increased numbers of Kenyon cells[23, 148, 72]. This suggests that in contrast to the type I neuroblasts we investigated here, mushroom body neuroblasts lack certain regulatory mechanisms to rein in the production of extra neurons. Based on our modeling, type I neuroblasts could use volume-sensitivity or NB self-inhibition as extra levels of regulation. While we ultimately do not know the molecular nature of the volume-sensitive and NB self-inhibition mechanisms, it is noteworthy that we find *Imp* and *CycE* upregulated in both *mud* and nanobody-expressing brains. While *Imp* is required for neuroblast growth, *CycE* drives cell cycle progression. Similarly, changes in CSA create NBs in direct contact with each other, and cell-cell adhesion-based mechanisms could regulate their cell proliferation behav-

ior. Several genes upregulated in either mud or nanobody-expressing neuroblasts encode for cell adhesion molecules (e.g plum, lac, shg) or members of the Notch signaling pathway, such as E(spl)mgamma-HLH, or E(spl)beta-HLH. Interestingly, we found the Immunoglobulin superfamily member DIP-alpha upregulated in mud mutant neuroblasts. DIP-alpha has been proposed to regulate NB proliferation in Drosophila brains via NB-cortex glia interactions. Thus, it is possible that increasing the neural stem cell pool in this cellular niche could provide a regulatory mechanism to regulate stem cell proliferation potential. It is noteworthy that equal sized siblings in mud4 and the apical sibling in nanobody-expressing NBs would be in contact with cortex glia, possibly regulating their proliferation behavior (Figure 2.7G).

In vertebrate systems, stem cells often undergo an amplification step to increase the size of the stem cell pool, which is necessary to produce differentiating progeny in sufficient numbers[109, 191, 86, 178]. Primary microcephaly, a neurodevelopmental disorder manifested in small brains and reduced cognitive functions, has been proposed to be a consequence of altered neural stem cell divisions: a premature shift towards asymmetric neural stem cell divisions could result in an insufficiently expanded neural stem cell pool and thus a reduction in the number of neurons [164, 91]. However, here we show that an increase in the neural stem cell pool leads to a reduction in neuronal numbers, suggesting that additional mechanisms need to be considered to understand the developmental consequences of altered CSA under physiological normal and disease conditions.

2.4 Methods

2.4.1 Experimental model and subject details

The following alleles and deficiencies were used: mud4 104. Transgenes and fluorescent markers: worGal4, UAS-Cherry::Jupiter, Sqh::GFP (2nd chromosome) 23, worGal4, UAS-Cherry::Jupiter, Sqh::GFP (3rd chromosome) 28, worGal4, UAS-mCherry::Jupiter 51, Sqh::EGFP 46, tdTomato::Dpn (this work). pUAST-attB-ALD-Rock CA::VhhGFP4::HA 29, Ase-Gal4 112. The following recombinant chromosomes were generated using standard genetic procedures: mud4, sqh::EGFP.

2.4.2 Immunohistochemistry

Primary antibodies used in this study were rat anti-dpn (1:100, Abcam 11D1BC7), mouse anti-pros (1:500, gift from Chris Doe), rabbit anti-phospho-Histone-H3 (1:3000, Sigma Aldrich, H0412), rabbit anti-Hey (1:100, gift from Chris Doe), rabbit anti-grh (1:500, gift from Melissa Harrison).

Secondary antibodies used were goat anti-rat (AlexaFluor 647, Invitrogen A21247), goat anti-mouse (AlexaFluor 405, Invitrogen A31553), goat anti-rabbit (AlexaFluor 405, Invitrogen A31556), goat anti-rabbit (AlexaFluor 647, Invitrogen A21245). All secondary antibodies were used at 1:1000. All experiments were conducted with at least 1 overnight in primary and 1 overnight in secondary antibody.

Larvae were dissected by removing the posterior half and inverting, then removing organs, and severing the connections between the back end of the brain and the mouth parts, to minimize stretching of the brain during fixation. Dissected larvae were put in schneiders insect media until fixation. Fixation was performed in 4% PFA in schneiders medium on a nutator for 20 minutes. PFA was then washed off with PBSBT (PBS, 1% BSA, 0.1% Triton-X) 3 times for 20 minutes on a nutator at room temperature. Antibodies were diluted in PBSBT and samples were incubated on a nutator at 4C. After primary staining, samples were washed with PBSBT 3 times for 20 minutes on a nutator at room temperature. Secondary antibody, also diluted in PBSBT, was then applied and incubated on a nutator at 4C. After secondary staining, samples were again washed 3 times for 20 minutes on a nutator at room temperature. Brains were then dissected off of the cuticles and put into vectashield. Brains were mounted on glass slides in a small channel of vectashield between two spacers (glass coverslips), with a third coverslip on top. All samples were imaged immediately after mounting on slides.

2.4.3 Generation of *tdTomato::Dpn* CRISPR line

First, *tdTomato* was PCR amplified from pTIGER_*tdTomato::3xFLAG::dCrk* (Plasmid #131138, Addgene) with the following primers:

5' GCGGCCGCGGACATATGATGGTGAGCAAGGGCGAGG 3'

5' TATACGAAGTTATAGGTACCCTTGTACAGCTCGTCCATGCC 3'

The destination vector, pHDR-SSPB-eGFP-DsRed 46[83], was cut with KpnI and NdeI to remove the SSPB-eGFP, and tdTomato was inserted in its place upstream of LoxP sites (which flank a DsRed for visual confirmation of successful transformation). We then generated a genomic prep of BDSC#58492 (injection stock) and sequenced across the deadpan gene region to confirm left and right homology arm (LHA and RHA) sequences with primers:

5' GTCCGATTCCGATCCATTGC 3'

5' AGATGGTCGGGCTAATTGTTTG 3'

We used FlyCRISPR target finder tool (<http://targetfinder.flycrispr.neuro.brown.edu/>) to find CRISPR/Cas9 cut sites. LHA and RHA were amplified by PCR from genomic DNA extracted from BDSC#58492 using the following primers:

LHA: 5' GAAGCAGGTGGAATTCCAAGTAAAAAGTAAAAAAAAGT 3'

5' TTGCTCACCATCATATGTTTGTAACGATTTTAATTTGTATATGCAAGTG 3'

RHA:

5' AGTTATACAGGCGCGCCATGGATTACAAAAACGAT 3'

5' ACGGAAGAGCCTCGAGGTCGCTCGCCAGCCCCGC 3'

The tdTomato destination vector was digested with EcoRI, NdeI, AscI, XhoI, to excise tdTomato and open the vector backbone. Homology arms with 15bp sequence homology were subsequently inserted into the linearized backbone together with the recovered tdTomato DNA using In-Fusion Cloning (Takara BioSciences).

For the guide RNA, we digested pU6-BbsI-chiRNA [70] (#45946, Addgene) with BbsI, and inserted the guides by annealing the two overhanging oligos:

5' GTAATACGACTCACTATAGGGC 3'

5' AAACAGTGGGAGATAATCCAAGATC 3'

The finished plasmids were sent to Bestgene Inc (Chino Hills, CA) for injection into embryos. Transformants were delivered and screened for eye DsRed expression. The DsRed cassette was floxed out by crossing to hsCre.

2.4.4 *Live cell imaging*

Brains were dissected from animals with fine forceps (Dumont #5, Electron Microscopy Sciences, item number 0103-5-PO) in Schneiders insect media with 10% bovine growth serum (HyClone, item number SH30541.03) and placed in 8-well slides (Ibidi, catalogue no. 80826). Brains were imaged with an Intelligent Imaging Innovations (3i) spinning disk confocal system, with a Yokogawa CSU-W1 spinning disk unit and two Prime 95B Scientific CMOS cameras, and a 60x/1.4NA objective. Imaging was done for 3 -16 hours with the lowest possible laser power and exposure time to minimize bleaching.

2.4.5 *Cell dissociation*

10mg/ml, 50ul aliquots of collagenase and papain were made upon receipt of the enzyme with Hank's Balanced Salt Solution as the solvent, and kept at -20C. Rinaldini solution (100ml milliQ water, 800 mg NaCl, 20 mg KCl, 5 mg NaH₂PO₄, 100 mg NaHCO₃, 100 mg Glucose) was prepared and kept at room temperature. Dissociation solution (400μl Rinaldini buffer, 50μl collagenase, 50μl papain) as well as PBS+1%BSA were prepared on ice immediately before dissection, and kept on ice for the duration of the experiment. All tubes used in this experiment were DNA Lo-Bind tubes. PBS+1%BSA was made fresh and sterile-filtered twice before use. First, larvae were washed briefly in room-temperature PBS before dissection to remove fly media particles. We dissected 50-60 brains for each condition in PBS+1%BSA. Approximately 10 brains were dissected before removing the media and replacing with fresh media. All brains were immediately put in an Eppendorf tube on ice containing 500μl PBS+1%BSA. We dissected for one hour before moving on to the dissociation procedure. As much media as possible was removed from the tubes, and then the brains were washed once with cold Rinaldini buffer. Then, 500μl of dissociation solution was added to each tube and tubes were placed at 30C for 30 minutes on a thermomixer with 1000rpm of agitation. Tubes were removed every ten minutes (3 total times) and brains were manually triturated 100x with a P1000 pipette. This cell solution was then filtered through a 40um filter into ice cold FACS tubes. Finally, 0.5μl of far red live/dead cell stain was added (Thermo Fisher L34973). These cell solutions were transported to the FACS

sorter on ice. Besides the 30 minutes in the thermomixer, samples and tubes were kept on ice for the full duration of the experiment.

2.4.6 FACS sorting

Cells were sorted on a BD FACSAria 3 sorter with a 100um nozzle and a flow rate of 1.2. The threshold rate was around 1000-1300 events/second. We targeted 60,000 sorted events per condition, and it took approximately 10-15 minutes of sorting to meet or exceed this number. Cells were sorted for GFP+, mCherry+, and Live/Dead-fluorescence. All cells were sorted into fresh DNA LoBind tubes containing cold 500µl PBS+1%BSA. After sorting all three genotypes, tubes were centrifuged at 300g for 10 minutes at 4C. Excess supernatant was removed, leaving about 60µl of liquid in which to resuspend cells. Cell concentration was noted to be around 600-700 cells per microliter.

2.4.7 Single cell RNA sequencing

To prepare our cDNA library, we used the 10x Genomics Chromium GEM-X 3' V4 kit, targeting 20,000 cells per condition. The protocol we followed for this kit can be found at (https://cdn.10xgenomics.com/image/upload/v1710230393/support-documents/CG000731_ChromiumGEM-X_SingleCell3_ReagentKits_v4_UserGuide_RevA.pdf). We performed GEM generation and RT incubation right after dissection and sorting, put the samples at 4C overnight, and finished the cleanup, cDNA amplification, and library construction and sent the samples for sequencing the following day. Sequencing was done by MedGenome Inc (now Signios Biosciences, Foster City, CA) on a NovaSeq X+ with 1 10B lane, and 1.25B paired (2.5B total) reads split across libraries. Samples were demultiplexed by MedGenome Inc and fastq files were delivered.

2.4.8 scRNAseq analysis

Samples were initially processed with the CellRanger (10x Genomics) Count function with a custom genome. The base *Drosophila Melanogaster* genome was downloaded from Ensembl (release 112), and sequences for GFP and mCherry were added to the.gtf and.fa files. All

subsequent analysis was done in R. Initial QC was performed for each genotype separately: empty droplets were filtered out with DropletUtils, and low quality cells were removed ($<0.8 \log_{10}(\text{Genes}/\text{Counts})$, total UMIs <1000 , total genes <500 , mitochondrial DNA content $<18\%$, ribosomal DNA content $<5\%$ or $<40\%$, heat shock genes $<5\%$). Doublets were predicted and removed with the DoubletFinder package. All three genotypes were then integrated into a single Seurat object. PCA analysis was run and the first 30 PCs were used. Clustering was done at a resolution of 1.0, which yielded 28 clusters. Annotation was performed by comparing expression level and abundance of validated marker genes, as well as other DEGs for annotated clusters from other datasets. Dotplots, DEGs, and other expression analyses were performed in R with Seurat.

2.4.9 Cluster annotation

Clusters were annotated based on validated marker expression. Neuroblasts had high expression of validated markers *dpn*, *mira*, and *CycE*, and non-validated but consistent markers *Mcm7*, *Mcm3*, *PCNA*, and *klu*. INPs had high expression of validated markers *ham* and *opa*, though little of validated marker *erm 72*. GMCs, while all slightly different, shared expression of validated markers *tap* and *dap*, and non-validated but consistent markers *edl*, *grh*, *Pex7*, and *insb*. Newborn neurons were characterized by high expression of *Hey 75*. Immature/undifferentiated neurons lacked *Hey*, *nSyb*, and *brp*, but contained varying levels of neuronal genes *elav*, *fne*, and *CadN 76,77*. Mature neurons were characterized by expression of *nSyb* and *brp*, as well as a neurotransmitter marker, such as *Gad1* and *VGAT* for GABA-ergic cells 114, *VGlut* for glutamatergic cells 115, *VACHT* and *CHAT* for cholinergic cells 116, *dimm* and *Crz* for peptidergic cells 117, and *Tbh* for octopaminergic cells 118. Our Kenyon cell cluster had high expression of many consistent markers, such as *Rgk1*, *jdp*, *prt*, *Dop1R1*, *Ac78C*, and *PKA-R1 80,119*. The glial cluster was the only one that contained any *repo 81*. Therefore, our cell atlas represents cell types from neuroblast to differentiated neuron (Figure 2.5 A, B and Figures 2.S2 - 2.S5).

2.4.10 Image analysis and quantification

tdTomato::Dpn

To quantify tdTomato::Dpn fluorescence over time, we used ImageJ to make a max intensity projection for the range that the nucleus in each neuroblast and GMC occupies, then quantified sum of the pixel intensities (called rawintden in ImageJ) within the nuclei over the duration of the video. We used background subtraction to correct for microscope minimum values. After background subtraction, numbers were normalized to 1 (value of nucleus of first frame shown in representative image sequence).

2.4.11 IHC image segmentation and analysis

To quantify Dpn volume, lineage volume, Pros+ cell number and Dpn number/lineage, we used Imaris 9.6 (and higher) to segment Dpn+ and Pros+ nuclei. Similarly, lineages were segmented and manually corrected if necessary. For lineage volume quantification, axonal projections were manually cut to account for image depth variations in our datasets. Segmentation was done by training Imaris' pixel classifier or by using threshold segmentation. Grh+ and Hey+ cells were counted in Imaris using spot detection and counting.

Cell diameter and lineage analysis was performed by manually measuring NB diameters and averaging over three measurements. Cell cycle length was extracted from Imaris. All lineage, diameter, cell cycle, volume and cell count measurements were analyzed in Jupyter notebooks with custom Python scripts.

2.4.12 GO term network and keyword analysis

For the GO term network and keyword analysis, we only considered DEGs that were expressed in at least 25% of neuroblasts, had a p-value of 0.001 or better, and a minimum log2-fold change of 0.5. DEGs were then converted to dataframes in Jupyter notebooks and analyzed with GSEApys's Enrichr (GO_Biological_Process_2018).

Keyword analysis was performed with custom Python code in Jupyter notebooks.

2.4.13 Statistical analysis

Statistical analysis was performed in either Prism or in Jupyter notebooks.

2.4.14 Code

Code used for RNAseq analysis was written in R. Code used for image, GO-term, and keyword analysis was written in Jupyter notebooks/Python. All the code used in the manuscript is available on github: <https://github.com/Jannetty/Neuroblast-Lineage-ODE-Model>

2.4.15 Software and figures

Images and figure panels were assembled and annotated in Adobe Illustrator 2023 (and higher). Image contrast and brightness was adjusted for better visibility, without changing the underlying data.

2.4.16 RNA sequencing data availability

RNA sequencing data has been submitted to NCBI GEO. Accession number: GSE308236.

Seurat files and code can be found on github.

2.4.17 ODE Modeling

All models were written and executed in Python. Code is publicly available at this URL: https://github.com/Jannetty/Neuroblast-Lineage-ODE-Model/blob/main/ode_model_manuscript_figures.ipynb. All model equations and parameters can be found in jupyter notebook and all simulations can be regenerated accordingly. Readers interested in running the model with different parameters can do so at our web application: https://jannetty.github.io/Neurogenesis_ODE_Models_Webapp/.

All models are initialized with one NB. In models that track volume the initial volume of the NB is set to $905\mu\text{M}^3$, a value based on our experimental observations.

2.4.17.1 Base Model

The Base Model tracks cell counts of each cell type in a developing NB lineage.

$$\begin{aligned}\frac{dN_{NB}}{dt} &= sym_frac \cdot k_{NB} \cdot N_{NB}(t) \\ \frac{dN_{GMC}}{dt} &= (1 - sym_frac) \cdot k_{NB} \cdot N_{NB}(t) - k_{GMC} \cdot N_{GMC}(t) \\ \frac{dN_{ImNeuron}}{dt} &= 2 \cdot k_{GMC} \cdot N_{GMC}(t) - k_{Neuron} \cdot N_{ImNeuron}(t) \\ \frac{dN_{MatNeuron}}{dt} &= k_{Neuron} \cdot N_{ImNeuron}(t)\end{aligned}$$

Where N_{NB} represents the number of cells in the NB population, N_{GMC} represents the number of cells in the GMC population, $N_{ImNeuron}$ represents the number of cells in the neuron population, and $N_{MatNeuron}$ represents the number of cells in the mature neuron lineage.

The parameter values are as described in Table 2.1.

Symbol	Parameter	Value	Units	Justification
sym_frac	Fraction of NB divisions that are symmetric (all others are asymmetric)	0 for WT .15 for mutants	Unitless	Experimental observation
k_{NB}	Rate of NB division	.67	$\frac{1}{hr}$	Experimental observation
k_{GMC}	Rate of GMC division	.11	$\frac{1}{hr}$	Experimental observation
k_{Neuron}	Rate of neuron maturation	.021	$\frac{1}{hr}$	Experimental observation

Table 2.1: Neuroblast lineage ODE base model parameters

2.4.17.2 Model 1

Model 1 includes the equations and parameters from the Base Model and adds the following:

$$\begin{aligned}
\frac{dV_{NB}}{dt} &= g_{NB} \cdot N_{NB}(t) - .02 \cdot asym_divs \cdot \bar{V}_{NB}(t) \\
\frac{dV_{GMC}}{dt} &= g_{GMC} \cdot N_{GMC}(t) + .02 \cdot asym_divs \cdot \bar{V}_{NB}(t) - k_{GMC} \cdot N_{GMC}(t) \cdot \bar{V}_{GMC}(t) \\
\frac{dV_{ImNeuron}}{dt} &= k_{GMC} \cdot N_{GMC}(t) \cdot \bar{V}_{GMC}(t) - k_{neuron} \cdot N_{Im}(t) \cdot \bar{V}_{Imneuron}(t) \\
\frac{dV_{MatNeuron}}{dt} &= k_{neuron} \cdot N_{Im}(t) \cdot \bar{V}_{Imneuron}(t)
\end{aligned}$$

Where V_* is the total volume of all cells of that cell type in the simulation, $\bar{V}_*$ is the average volume of that cell type's cells ($\frac{V_*}{N_*}$), g_* is the per-cell growth rate of the cell type, and $asym_divs$ is the number of asymmetric divisions, equal to $(1 - sym_frac)k_{NB}N_{NB}$. The NB and GMC division rates are now defined by:

$$\begin{aligned}
k_{NB} &= \begin{cases} k_{max_{NB}} \cdot N_{NB}(t) \cdot \min\left(1, \left(\frac{\bar{V}_{NB}(t)}{V_{thresh_{NB}}}\right)^n\right), & \text{if } \bar{V}_{NB}(t) \geq V_{floor_{NB}} \\ 0 & \text{otherwise} \end{cases} \\
k_{GMC} &= \begin{cases} k_{max_{NB}} \cdot N_{GMC}(t) \cdot \min\left(1, \left(\frac{\bar{V}_{GMC}(t)}{V_{thresh_{GMC}}}\right)^n\right), & \text{if } \bar{V}_{GMC}(t) \geq V_{floor_{GMC}} \\ 0 & \text{otherwise} \end{cases}
\end{aligned}$$

Where k_{max_*} is the maximum growth rate of the cell type, V_{thresh_*} is the division volume threshold for the cell type, V_{floor_*} is the minimum volume a cell of the cell type must reach before dividing, and n is the sensitivity coefficient.

The new parameters are as described Table 2.2.

Symbol	Parameter	Value	Units	Justification
g_{NB}	Growth rate of NBs	120.67 for WT and mudmut 30.4 for nanobody	$\frac{\mu M^3}{hr}$	Set so V_{NB} is constant in WT simulation. Nanobody rate decreased to 80% WT growth rate based on experimental observation
g_{GMC}	Growth rate of GMCs	25.14	$\frac{\mu M^3}{hr}$	Set so GMCs double in volume in 9 hours (literature)
k_{maxNB}	Maximum rate of NB division	1.31	$\frac{1}{hr}$	Set so constant rate of division in WT simulation is .67/hr (NB division rate used in base model)
k_{maxGMC}	Maximum rate of GMC division	.125	$\frac{1}{hr}$	Estimated based on experimental observation
$V_{floorNB}$	Minimum volume NB must reach before dividing	226.25	μM^3	$\frac{1}{4}$ of initial NB volume
$V_{floorGMC}$	Minimum volume GMC must reach before dividing	56.56	μM^3	$\frac{1}{4}$ of initial GMC volume
$V_{threshNB}$	The volume threshold at which NB division rate steeply increases	1131.25	μM^3	NBs grow to 125% their initial size, based on experimental observation
$V_{threshGMC}$	The volume threshold at which GMC division rate steeply increases	452.5	μM^3	GMCs grow to 200% their initial size in WT, based on experimental observation
n	Coefficient determining how steeply proximity to cell volume division threshold affects division rate	3	unitless	Set to make division rate increase steeply at division threshold

Table 2.2: Neuroblast lineage ODE model 1 volume-dependent division model parameters

2.4.17.3 Model 2

Model 2 retains all of the ODEs from Model 1 and adds an additional state variable to track the changes in NB division threshold over time.

$$\frac{dV_{thresh_{NB}}}{dt} = -\Delta_{thresh} \cdot sym_divs$$

Where Δ_{thresh} is the constant amount the division threshold decreases with each symmetric division, and sym_divs is the number of symmetric divisions that have occurred in the simulation, equal to $(sym_frac) \cdot k_{NB} \cdot N_{NB}(t)$. $V_{thresh_{NB}}$ is initialized to $1131.25 \mu M^3$.

The new parameters are as described in Table 2.3.

Symbol	Parameter	Value	Units	Justification
Δ_{thresh}	Constant amount $V_{thresh_{NB}}$ is decreased with each symmetric division	33.94	μM^3	3% the initial division threshold. Calibrated so NBs do not reach their minimum volumes by the end of 48 hours in the simulation.

Table 2.3: Neuroblast lineage model 2 dynamic division threshold parameters

2.4.17.4 Model 3

Model 3 takes Model 1 but determines g_{NB} using the following formula:

$$g_{NB} = g_{NB_{base}} \cdot \left(\frac{\bar{V}_{NB}(t)}{V_{ref}} \right)^\alpha$$

Where $g_{NB_{base}}$ is the base NB growth rate, V_{ref} is a reference NB volume, and α is the sensitivity of the growth rate to the NB volume.

The new parameter values are as described in Table 2.4.

Symbol	Parameter	Value	Units	Justification
$g_{NB_{base}}$	Base NB growth rate	120.67 for WT and mudmut 96.53 for nanobody	μM^3	Set to the growth rate in previous models
V_{ref}	Volume at which $g_{NB} = g_{NB_{base}}$	905	μM^3	Set to the initial NB volume
α	Sensitivity of growth rate to NB volume	[1,5]	unitless	Capability of model to constrain mutant growth depends on this value

Table 2.4: Neuroblast lineage model 3 volume-dependent growth rate parameters

2.4.17.5 Model 4

Model 4 takes Model 2 and makes g_{NB} dynamic as described in Model 3. All parameter values are as defined in Model 2 and Model 3.

2.4.17.6 Model 5

Model 5 builds on the Base Model by using the equation below to calculate k_{NB} :

$$k_{NB} = k_{NB_{max}} \cdot \frac{K^\beta}{K^\beta + N_{NB}(t)}$$

Where $k_{NB_{max}}$ is the maximum NB division rate, K is the quantity of NBs at which the NB self-repression is half-maximal, and β is the Hill coefficient.

Parameters for this model are as described in Table 2.5.

Symbol	Parameter	Value	Units	Justification
$k_{NB_{max}}$	Maximum NB division rate	1.30	$\frac{1}{hr}$	Set so the rate of division in WT simulations is constant
K	The quantity of NBs at which NB self-repression is half-maximal	2	NBs	Set so repression is strong following the first symmetric division
β	Sensitivity of growth rate to NB quantity	[1,5]	unitless	Capability of model to constrain mutant growth depends on this value

Table 2.5: Neuroblast lineage model 5 self-repression parameters

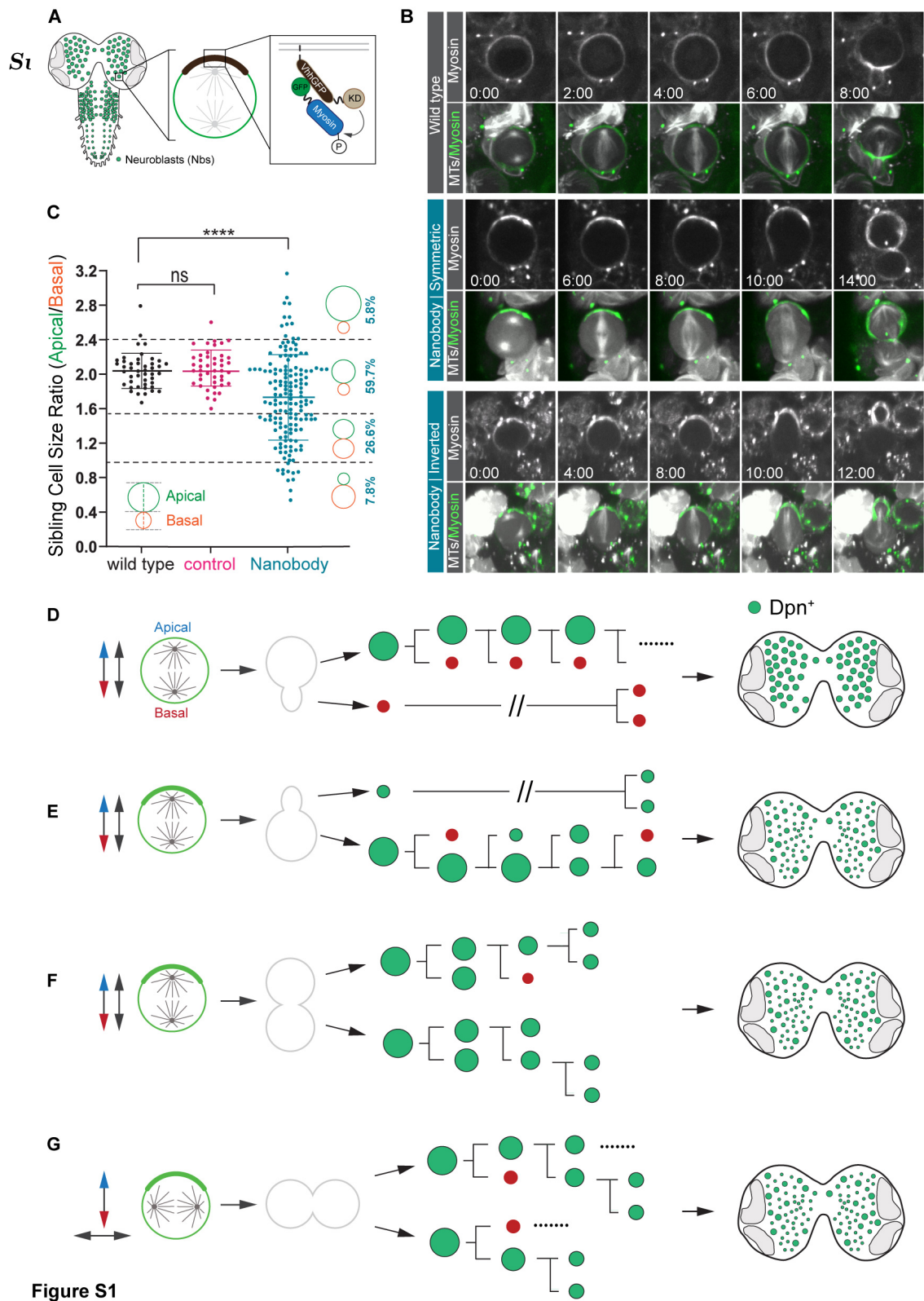


Figure 2.S1: Nanobody-expressing neuroblasts divide asymmetrically, symmetrically or with inverted asymmetry. (A) Representative cartoon of the nanobody approach: the apically tethered, anti-GFP nanobody is equipped with a kinase domain from Rho-kinase, phosphorylating and constitutively activating Myosin's regulatory subunit Spaghetti Squash (Sqh) upon binding of GFP to the vhhGFP. Consequently, activated Myosin will be retained on the apical neuroblast cortex 29. (B) Representative image sequences of wild-type and nanobody-expressing neuroblasts. All neuroblasts co-express UAS-mCherry::Jupiter (white) with worGal4 and endogenously tagged Sqh::EGFP (green). Representative symmetric and inverted asymmetric divisions are shown for nanobody-expressing neuroblasts. (C) Quantification of division modes for wild type, control, and nanobody-expressing neuroblasts. Each dot represents one ratio measurement. Average and standard deviation are shown overlaid with a scatter plot. Significance was tested with Bonferroni's multiple comparisons test. Wild type vs control: $p > 0.9999$. Wild type vs nanobody: $p < 0.0001$. (D) Wild-type neuroblasts align the mitotic spindle in parallel to the intrinsic apical (blue) – basal (red) polarity axis, repeatedly generating large self-renewed neuroblasts (green) and small differentiating GMCs (red), dividing only once more. Trapping activated Myosin on the apical neuroblast cortex can induce (E) inverted asymmetric or (F) symmetric cell divisions with variable lineage trees. The resulting brains could contain neuroblasts of various sizes, many of which are smaller than normal wild-type neuroblasts. (G) Symmetric neuroblast divisions induced through spindle misalignment generate lineage trees with various outcomes, resulting in brains with potentially differently sized neuroblasts.

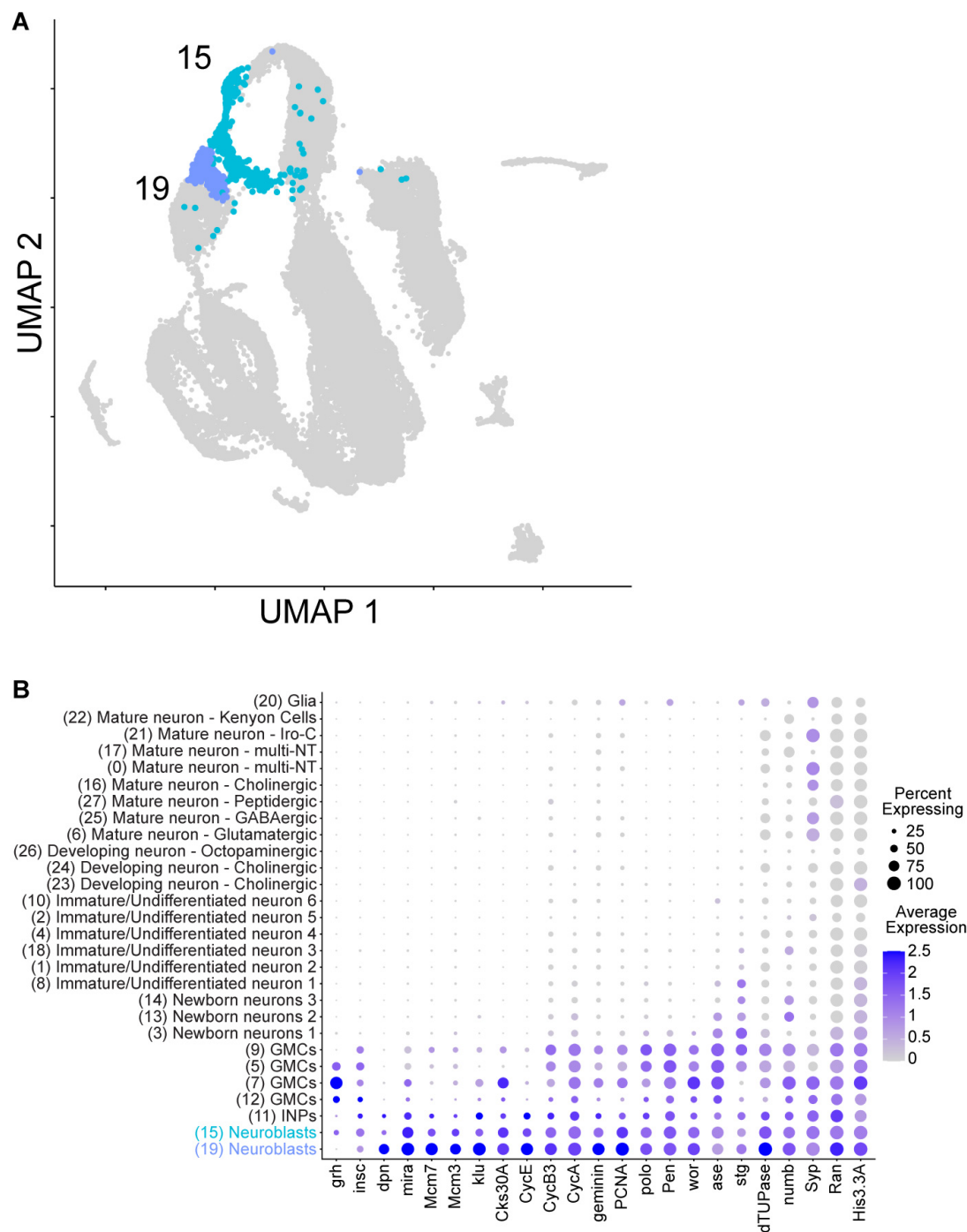


Figure S2

Figure 2.S2: **Clusters 15 and 19 represent neuroblasts** (A) Neuroblast clusters 15 and 19

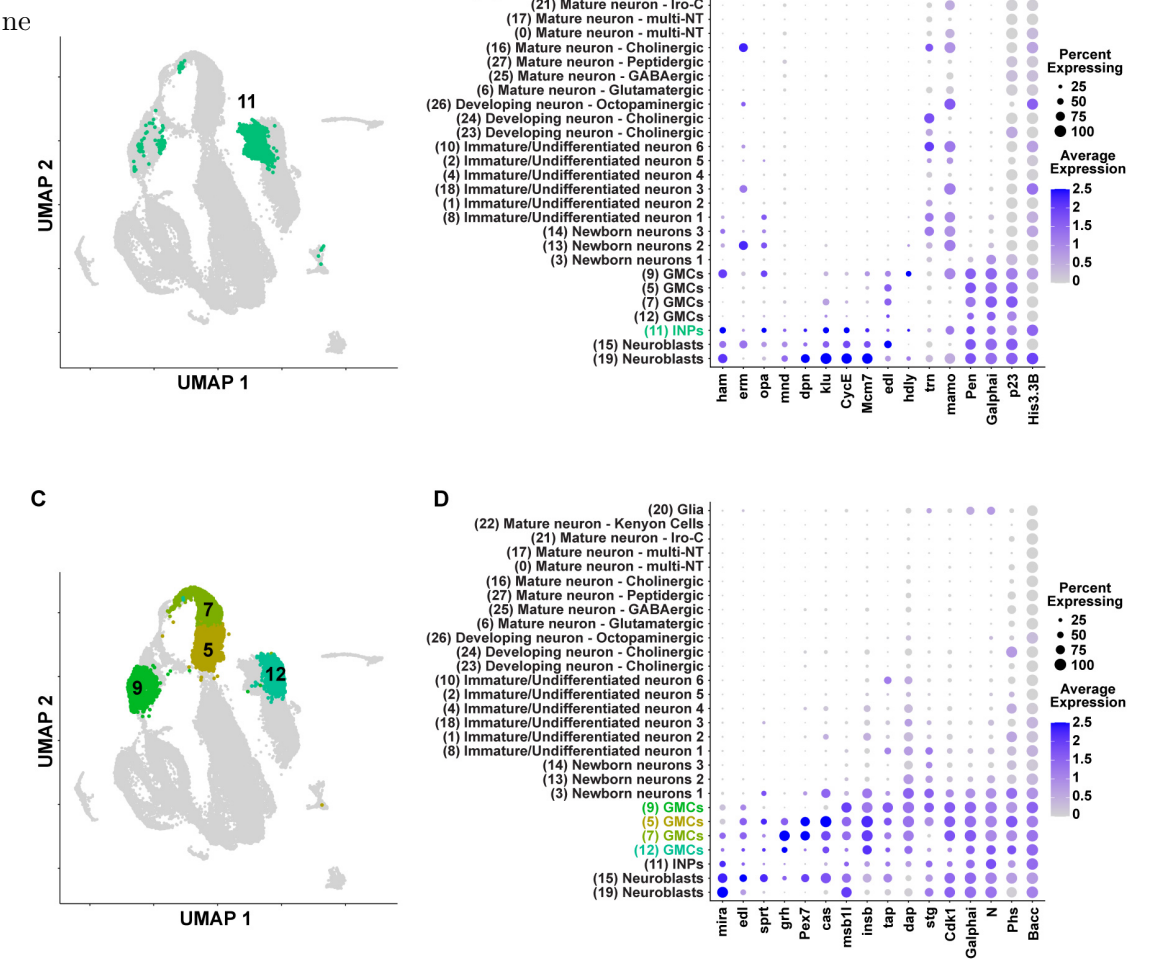


Figure S3

Figure 2.S3: **Cluster 11 represents INPs, and clusters 5, 7, 9, 12 represent GMCs, respectively.** (A) INP cluster 11 highlighted on a UMAP plot. (B) DotPlot of marker genes used in the annotation of the INP cluster. (C) GMC clusters 5, 7, 9, and 12 are highlighted on a UMAP plot. (D) DotPlot of marker genes used in the annotation of the GMC clusters.

Figure 2.S4: **Immature, developing, and mature neurons separate into specific clusters** (A) UMAP plot showing Newborn neuron (clusters 3, 13, 14) and immature neuron clusters (clusters 1, 2, 4, 8, 10, 18). (B) DotPlot of marker genes used in the annotation of the newborn neuron and immature neuron clusters. (C) UMAP plot showing developing and mature neurons. (D) DotPlot of marker genes used to annotate developing and mature neurons.

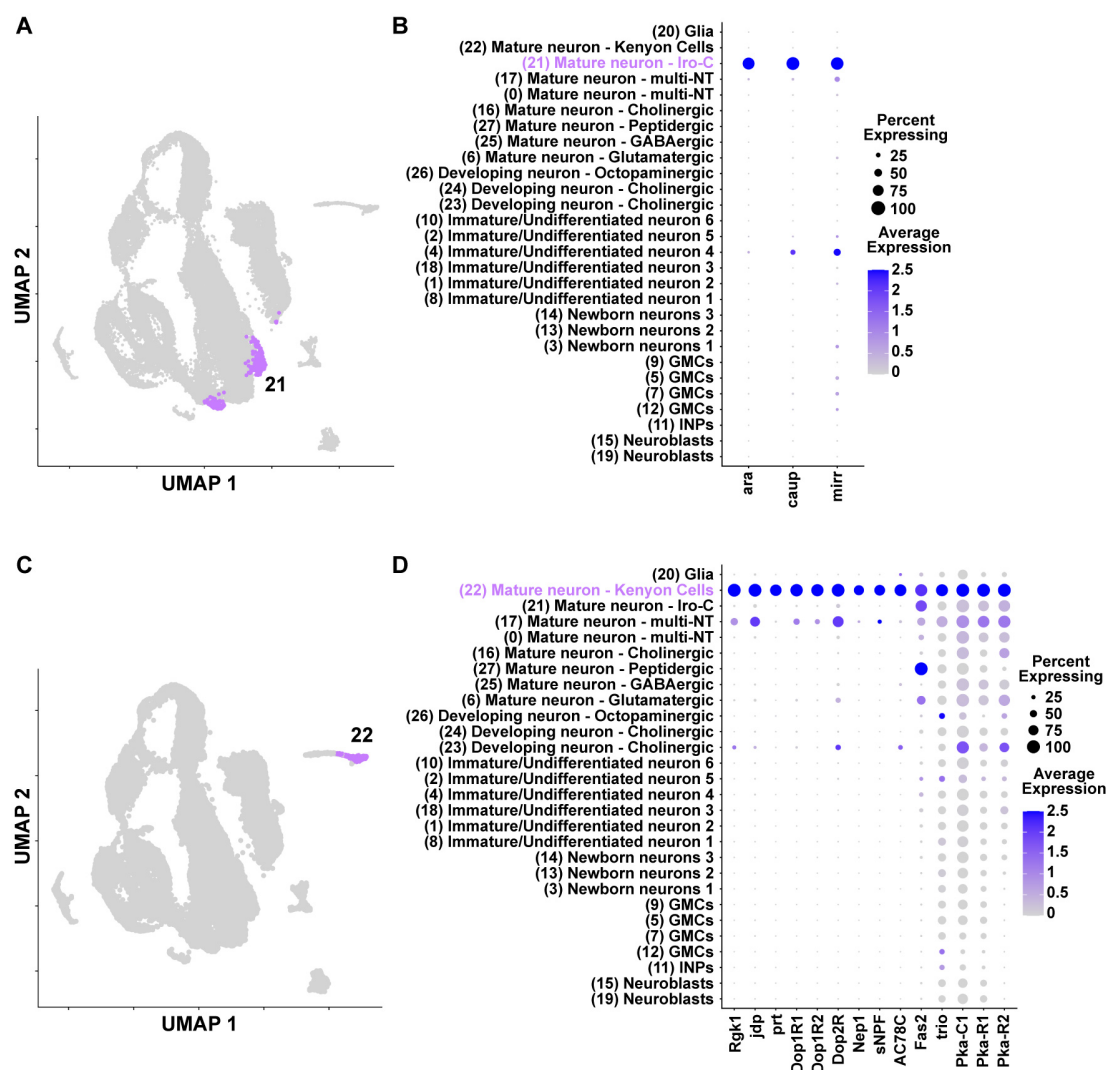


Figure S5

Figure 2.S5: **Cluster 21 and cluster 22 represent Iro-C and Kenyon cells, respectively.** (A, C) UMAP plot highlighting cluster 21 containing mature Iro-C neurons and Kenyon cells, respectively. (B, D) DotPlot of marker genes used to annotate Iro-C neurons and Kenyon cells, respectively.

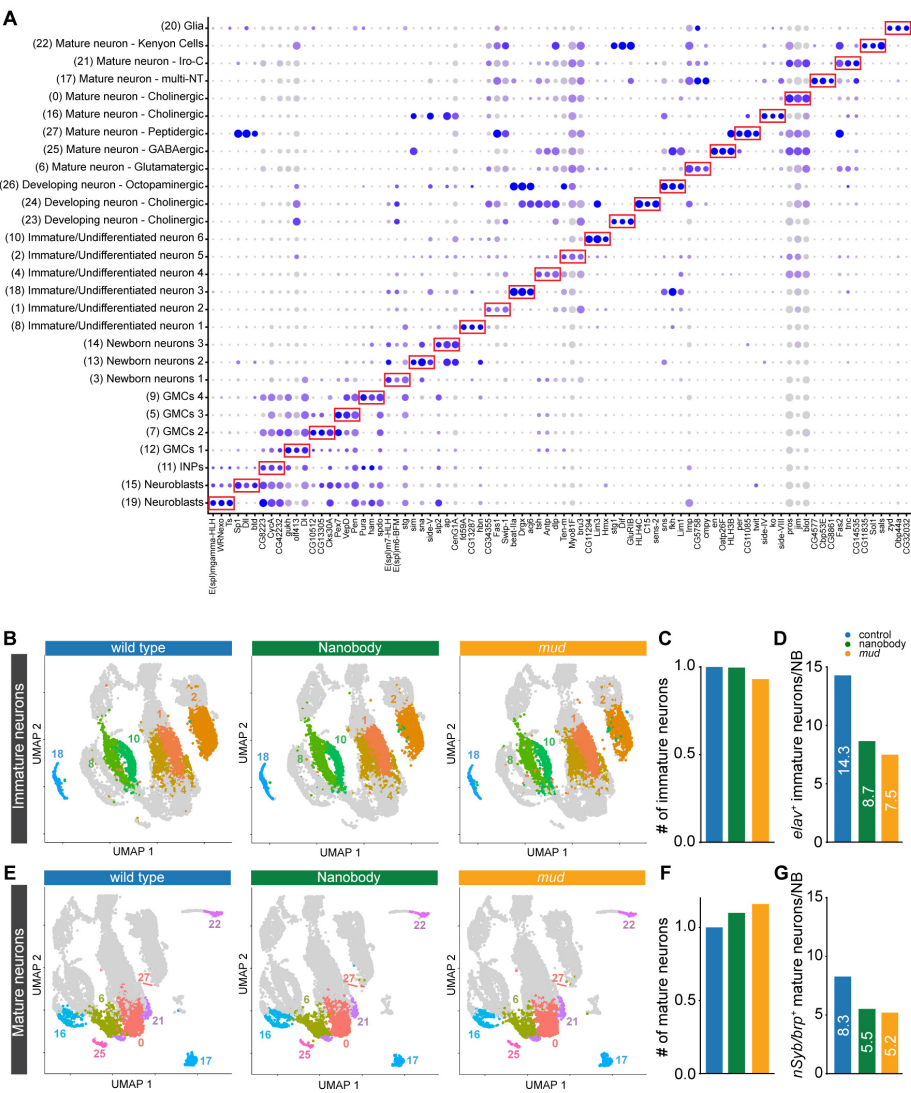


Figure S6

Figure 2.S6: **New cluster markers and a comparison of immature and mature neuron numbers between genotypes.** (A) The top three differentially expressed genes per cluster (by log2FC) are shown on a DotPlot (see methods). Validated markers are not shown here. Red boxes outline the dots corresponding to the three genes per cluster for easier viewing. These are genes that have not been validated in vivo as cell type specific but are among the most significant principal components that distinguish clusters from one another and could be used as markers for cell typing in future RNA sequencing experiments. Immature (B) and mature (E) neuron clusters highlighted on UMAP plots for each condition, respectively. The total number of cells belonging to (C) immature neuron and (F) mature neuron clusters, normalized for library size and control. Immature neurons (D) and mature neurons (G) per neuroblast. Cells within immature neuron clusters with at least 1 elav transcript were used for the quantification for each condition. Cells within mature neuron clusters with at least 1 nSyb or brp transcript were used for the quantification for each condition.

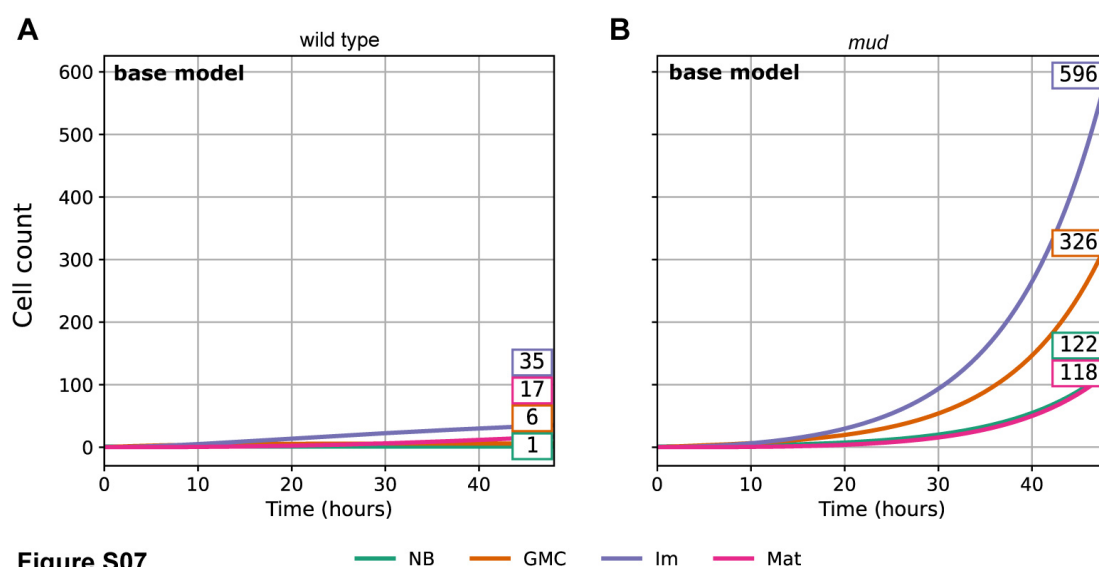


Figure 2.S7: **Model dynamics details.** Base model shown for (A) wild type and (B) *mud* mutant neuroblasts. The base model predicts that *mud* mutant NBs generate far more cells than wild-type NBs, inconsistent with experimental results.

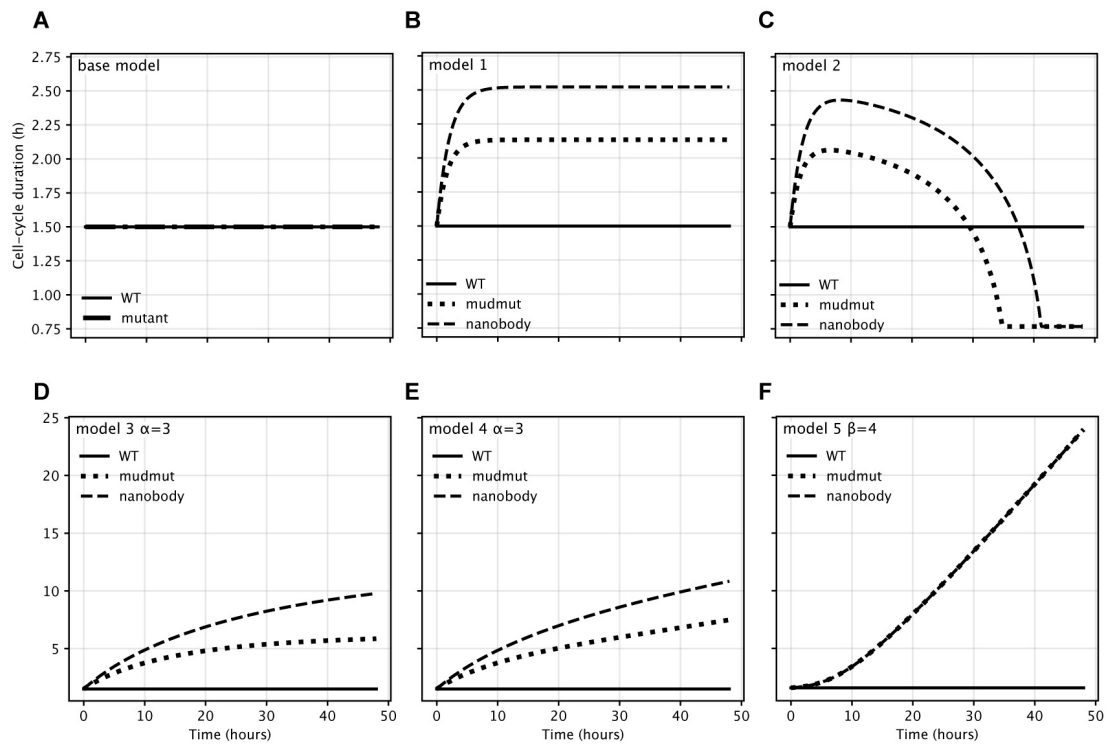


Figure S08

Figure 2.S8: **Mutant NB cell cycle durations continue increasing over time for models 3, 4, and 5.** (A) NB Cell cycle duration is constant in the base model. (B) In model 1, volume activation increases cell-cycle duration to a new steady state in mud and nanobody-expressing NBs. (C) In model 2, a symmetric division threshold ultimately causes cell cycle duration to decrease to its minimum. Model 3 (D), model 4 (E), and model 5 (F) show the increase in cell cycle for mud mutant and nanodoby-expressing NBs. Models are only shown with an $\alpha = 3$ (D, E) and $\beta = 4$ (F).

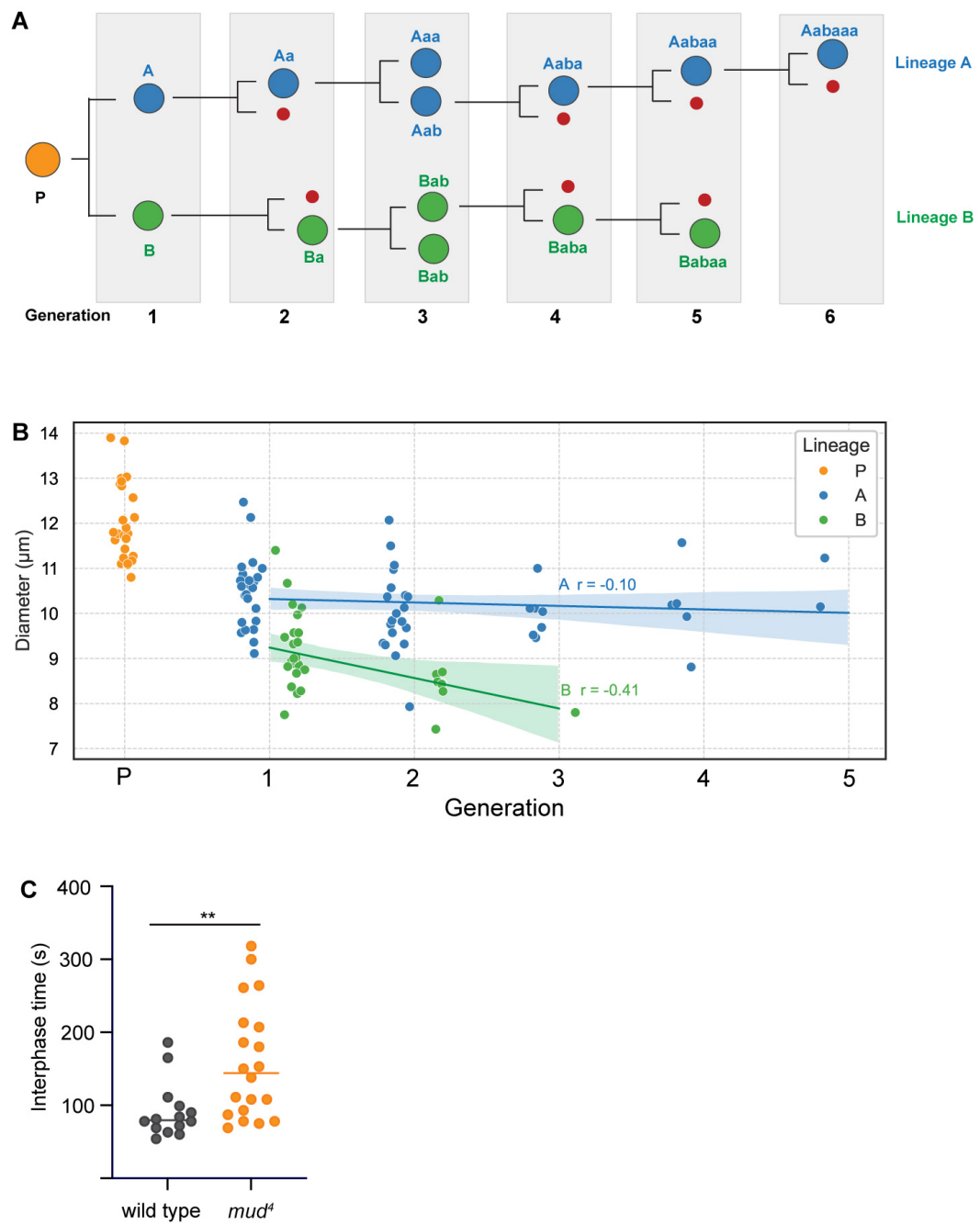


Figure S09

Figure 2.S9: Symmetrically dividing mud4 mutant neuroblasts show asymmetric lineage behavior and extended interphase times (A) Schematic lineage tree of a symmetrically dividing neuroblast, illustrating the used nomenclature and lineage structure. Parental (P) neuroblasts generate an A and a B neuroblast after a symmetric division. A and B neuroblasts can either divide symmetrically or asymmetrically. Only dividing cells propagate the lineage and receive a lineage label. (B) Plot showing cell diameter for multiple generations in symmetrically dividing mud4 mutant neuroblasts. A and B neuroblasts are consistently smaller and do not regrow to parental NB size. A lineages are consistently longer than B lineages. (C) Cell cycle length comparison between wild type and mud4 mutant neuroblasts. Statistical test used: unpaired T-test. ** $p < 0.01$

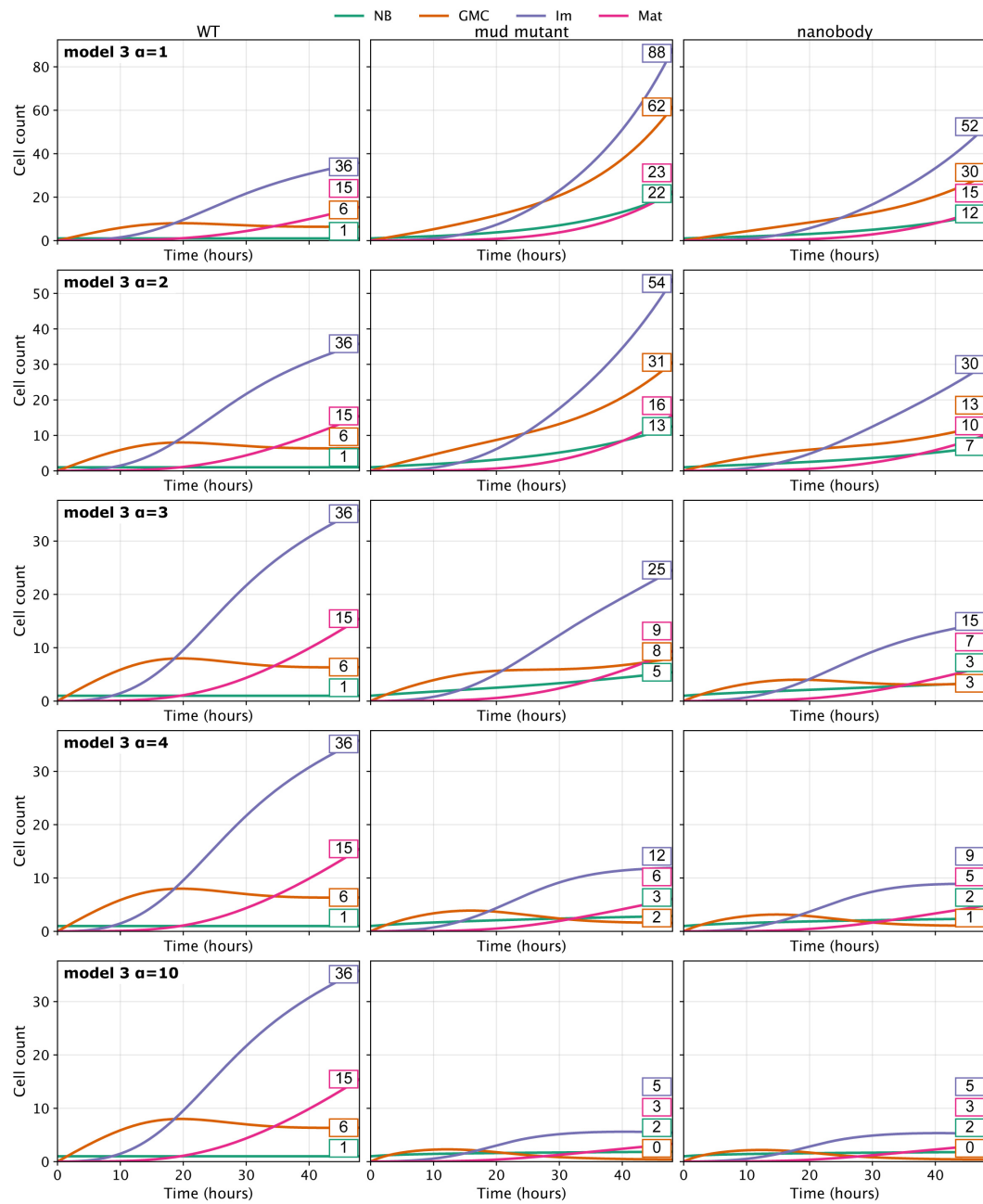


Figure S10

Figure 2.S10: NB growth rate-sensitivity to NB volume (.) determines whether volume-sensitive growth effectively suppresses mutant NB proliferation when division threshold volume is constant. Cell counts shown for Model 3 run with different values of α .

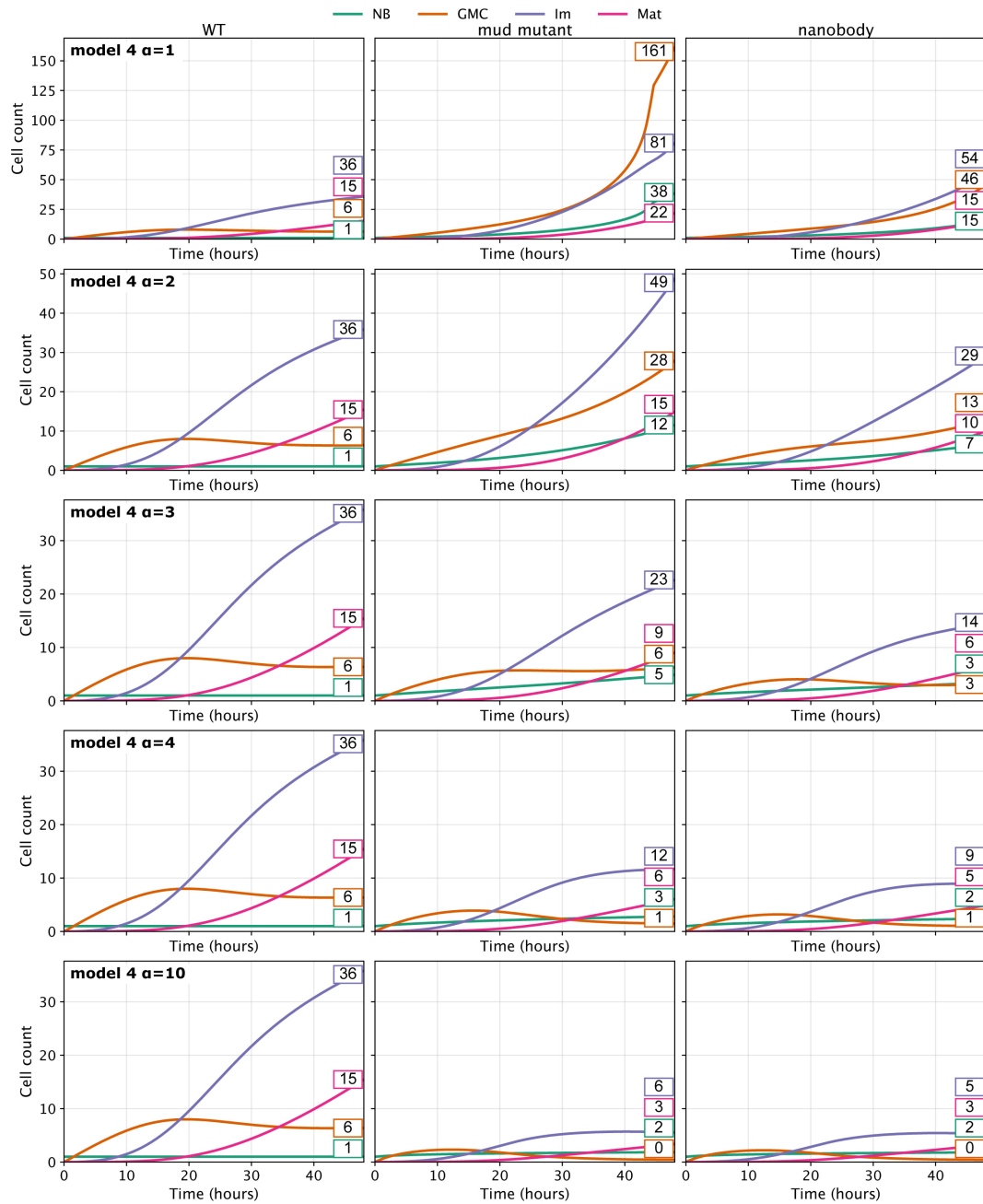


Figure S11

Figure 2.S11: NB growth rate-sensitivity to NB volume (α) determines whether volume-sensitive growth effectively suppresses mutant NB proliferation when division threshold volume is regulated by symmetric division. Cell counts shown for Model 4 run with different values of α .

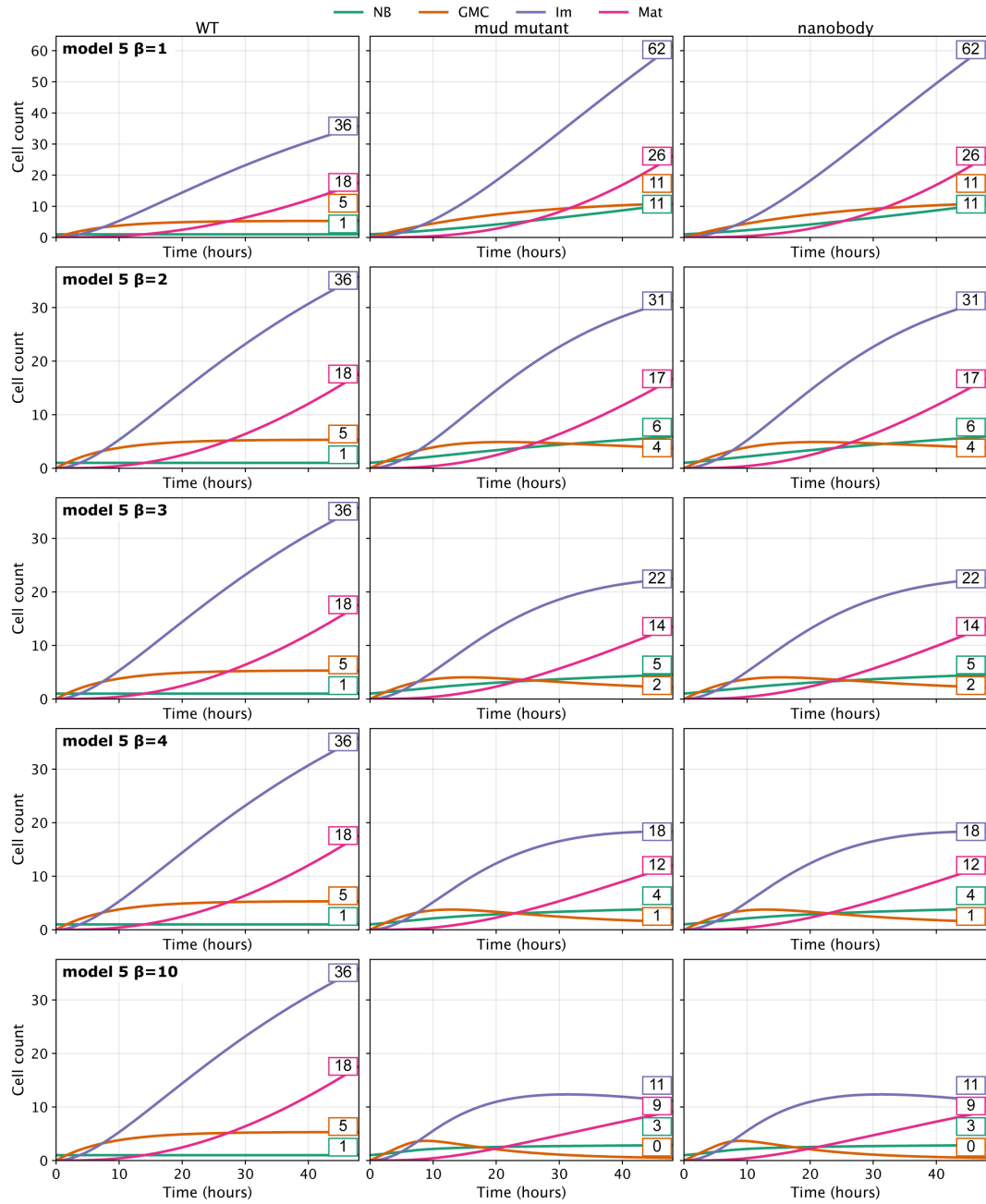


Figure S12

Figure 2.S12: NB growth rate sensitivity to NB count (β) determines whether NB self-inhibition effectively suppresses mutant NB proliferation. Cell counts shown for Model 5 run with different values of β .

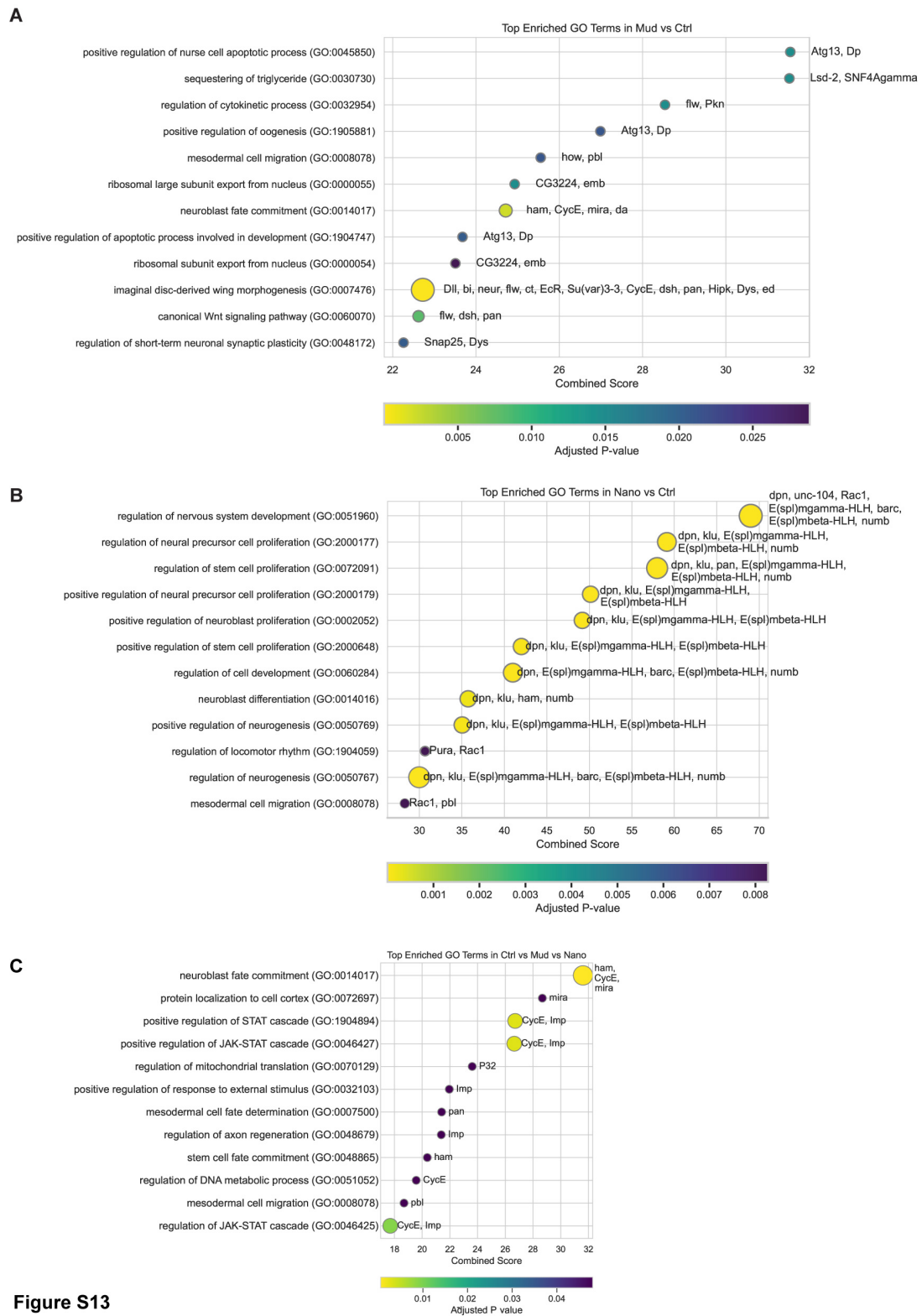


Figure S13

Figure 2.S13: **GO-terms for mud mutant and nanobody-expressing neuroblasts of differentially expressed genes.** GO term analysis for differentially expressed genes comparing (A) *mud*⁴ versus control, (B) nanobody-expressing neuroblasts versus control, and (C) genes differentially expressed in both *mud*⁴ and nanobody-expressing neuroblasts versus control. The combined score consists of a combination of the p-value and z-score. The z-score measures the deviation from the expected rank of the GO term. Dot size reflects number of associated genes and dot color the corresponding p-value. Genes are listed for each GO-term.

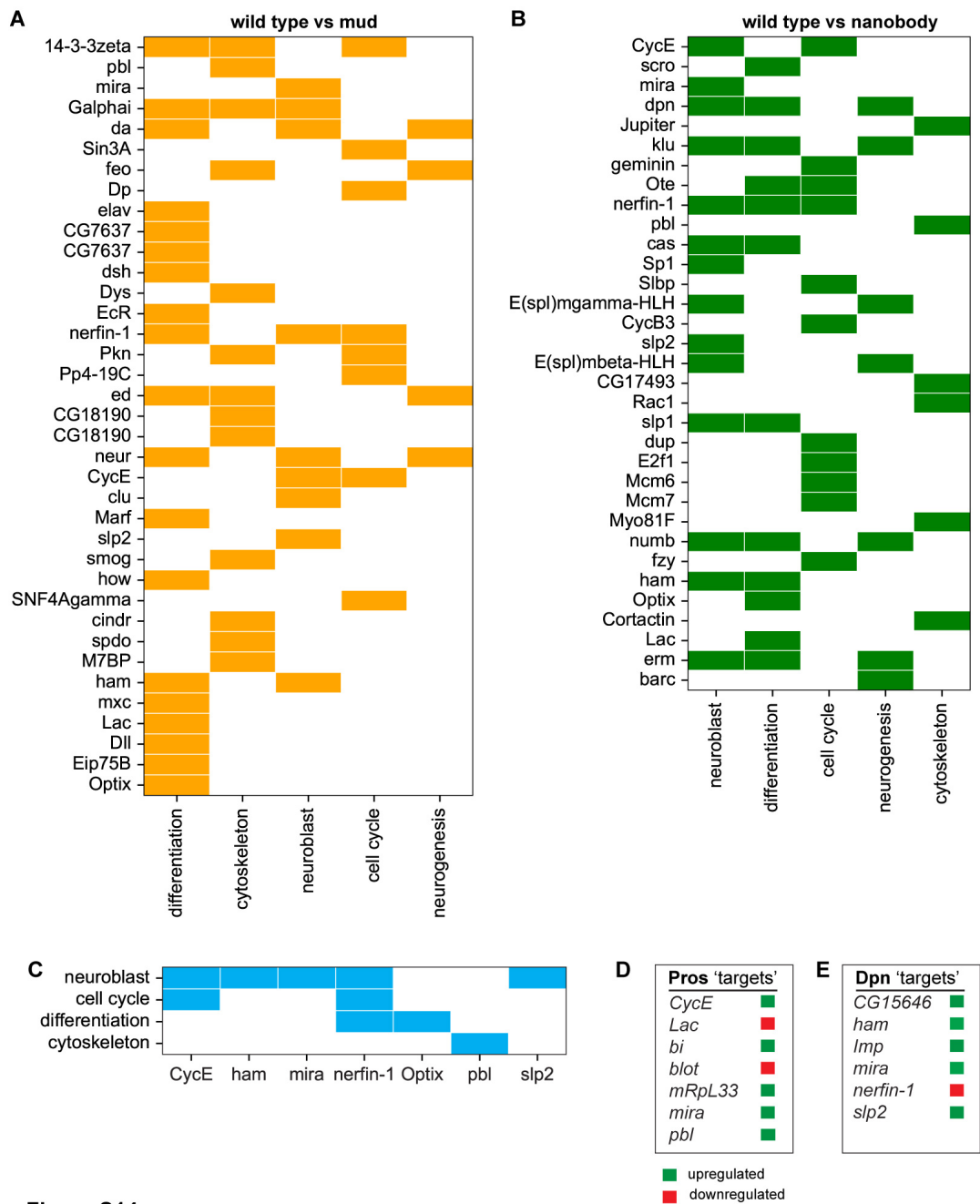


Figure S14

Figure 2.S14: **Genes differentially expressed between wild type, mud^4 , and nanobody-expressing brains associate with neuroblast, differentiation, cell cycle, neurogenesis, and cytoskeleton functions.** Genes differentially expressed in (A) mud^4 , (B) nanobody-expressing, or (C) both mud^4 and nanobody-expressing neuroblasts, compared to wild type, are associated with neuroblast, differentiation, cell cycle, neurogenesis, and cytoskeleton functions. Blue rectangles indicate keywords associated with the gene on the x-axis. Keywords are derived from GO terms. Pros 42 (D) and (E) Dpn 103 target genes that are differentially expressed in mud^4 and nanobody-expressing brains. Green and red boxes indicate whether the genes are up- or downregulated in mud^4 and nanobody-expressing neuroblasts compared to wild-type NBs.

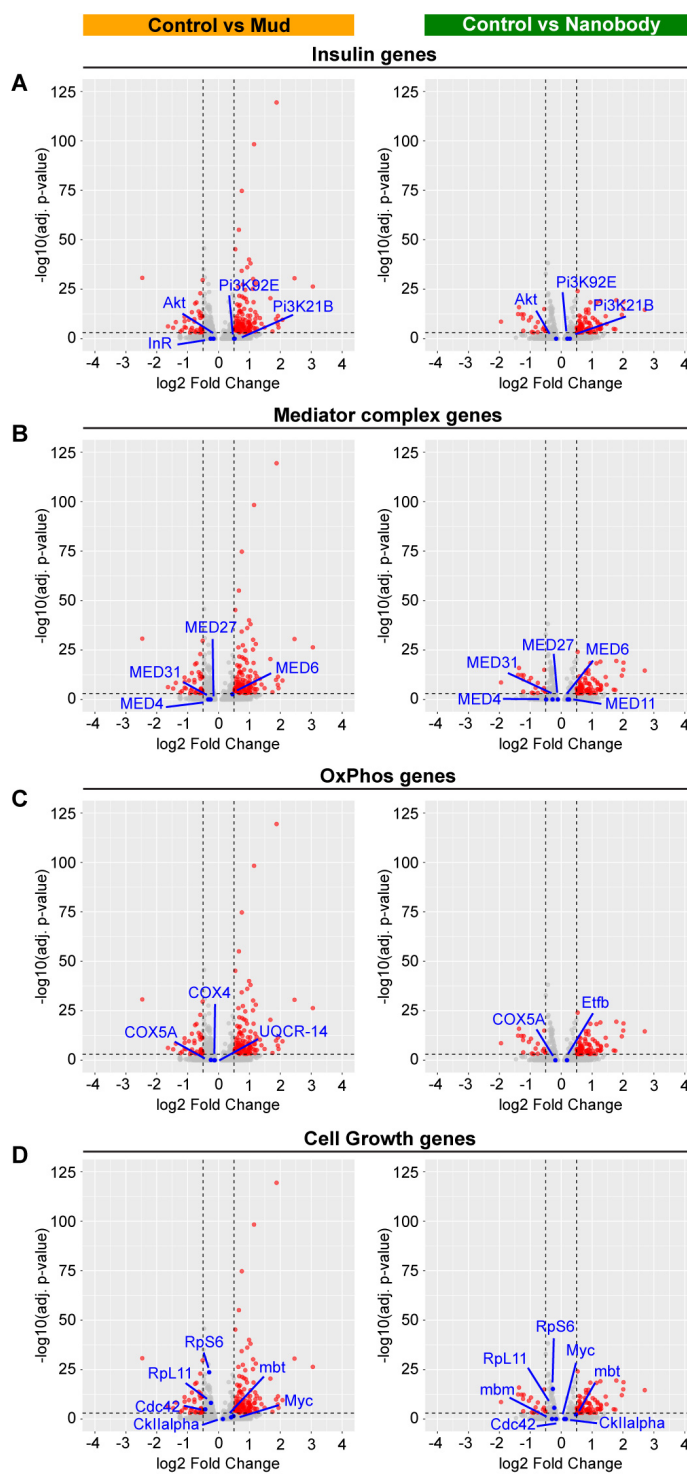


Figure S15

Figure 2.S15: **NB growth genes are not significantly differentially expressed in mud4 or nanobody-expressing neuroblasts.** Volcano plots of DEGs between wild-type and mud⁴ (left), and wild-type and nanobody (right), highlighting genes in the (A) Insulin pathway 95, (B) the Mediator complex 50, (C) the oxidative phosphorylation pathway 50, or (D) other genes regulating NB growth 96,97. We only considered genes that are expressed in at least 25% of neuroblasts, with a p-value of 0.001 or better, and a minimum log2-fold change of 0.5. Any gene appearing in one plot but not the other signifies that that gene was expressed by less than 25% of cells in that group.

Chapter 3

A CALIBRATED CELLULAR POTTS MODEL QUANTIFIES HOW DIVISION GEOMETRY AND SIZE-DEPENDENT GROWTH REGULATION IMPACT DROSOPHILA NEUROBLAST LINEAGES

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Author's note

We are currently in the process of preparing this work for submission.

Abstract

Asymmetric cell division (ACD) is essential for neural stem cell self-renewal, yet the impact of cell morphology and division geometry on lineage architecture remain difficult to isolate experimentally. We developed and calibrated a two-dimensional cellular Potts model (CPM) of developing *Drosophila* neuroblast (NB) lineages and used it to test how division geometry and growth regulation shape lineage morphology in wild-type and *mud* mutant conditions. The calibrated wild-type computational model recapitulated experimental lineage scale and composition, and the characteristic peripheral NB organization emerged without being used as a calibration target. Systematic perturbation of three separable geometric components revealed that intergenerational stability of the apical/basal axis is the dominant determinant of peripheral NB positioning: persistent axis rotation strongly disrupted lineage sorting, whereas spindle-orientation variance and division-plane offset were tolerated across a wide range. Consistent assignment of NB identity to either pole of the division was sufficient to maintain peripheral NB positioning regardless of offset or daughter-size asymmetry, whereas randomizing identity assignment

only partially disrupted the phenotype, indicating that NBs retain an intrinsic tendency to expand into extracellular space due to the energetic favorability of growth into open lattice sites combined with the model’s constrained division geometry. We also used this framework to assess the sufficiency of hypothetical regulatory dynamics acting on *mud* mutant lineages. Of our assessed regulatory mechanisms, only volume-based growth regulation paired with birth-size-dependent division thresholds brought all five calibration metrics within the experimental *mud* range, suggesting that cell-size-sensitive growth machinery and birth-size memory acting together is sufficient constrain mutant lineage scale *in vivo*. Finally, we assessed contributors of NB clustering in regulated *mud* lineages and found clustering was shaped primarily by post-inheritance apical-axis rotation and secondarily by preferential NB–NB adhesion. Together, these results identify apical-axis stability, fate-position coupling consistency, and size-sensitive growth regulation as the key determinants of NB lineage architecture in WT and *mud* mutant lineages.

3.1 Introduction

Asymmetric cell division (ACD) is the process by which cells divide into two daughter cells that differ in fate, size, or both [84, 169, 99, 180, 116]. ACD determines the number and identity of cells in developing tissues and organs; its regulation is crucial for ensuring consistent developmental outcomes [194, 110, 20]. In the nervous system, disruption of ACD regulation has been linked to abnormal brain growth and neurodevelopmental disorders, including microcephaly [194, 164, 178].

Drosophila larval neuroblasts provide a tractable system for studying the regulation of ACD during brain development [59, 169]. In wild-type (WT) flies, a neural stem cell called a neuroblast (NB) divides asymmetrically along a plane perpendicular to its apical/basal axis to produce a larger apical daughter that retains NB identity, and a smaller basal daughter called a ganglion mother cell (GMC) (Figure 3.1 A) [59, 26, 80]. The GMC then divides once more to generate two terminally differentiated neurons (Figure 3.1 A) [59, 26]. These successive divisions produce a consistent lineage morphology in which the NB remains at one peripheral end of the lineage (Figure 3.1 C bottom).

Division geometry is important in determining the identity of the daughter cells [26]. In *mud* mutant flies, spindle orientation is disrupted and approximately 15% of NB divisions become symmetric, producing two neuroblast daughters side-by-side instead of a single neuroblast and a GMC [163, 26] (Figure 3.4 A). This change in the plane of division alters the differential sequestration of cell identity determinant proteins in the daughter cells, causing both daughter cells of symmetric divisions to retain their NB identity [26] (Figure 3.4 A). Paradoxically, despite having more neuroblasts and therefore greater apparent proliferative potential, *mud* mutant lineages are smaller overall and contain fewer total cells than WT lineages (Figure 3.4D) [48]. Experimental observations further suggest that when multiple neuroblasts are present in a *mud* mutant lineage, they often remain adjacent to each other rather than becoming separated by differentiated progeny.

Understanding the regulatory mechanisms ensuring the development of morphologically consistent NB lineages in WT flies can advance our understanding of how healthy brain development progresses in humans. Similarly, understanding how this regulation goes awry in mutants can shed light on the causes of human developmental abnormalities. Despite its relevance to basic science and human health, many questions about this system remain unresolved and are challenging to investigate experimentally.

Several coupled features of asymmetric NB division act simultaneously *in vivo* : the orientation of the apical/basal axis across generations, stochastic variation in spindle orientation relative to that axis, and the position of the division plane along the axis. These features are biologically meaningful to separate because they are governed by partially distinct cellular machinery: cortical polarity proteins establish the apical/basal axis, Pins/Mud-dependent machinery aligns the spindle relative to that axis, and polarized actomyosin dynamics together with cleavage-furrow positioning contribute to daughter-size asymmetry and asymmetric furrow (or plane of division) placement [147, 144, 163, 28, 38, 175, 155, 145]. Because these features normally change together, it is difficult to experimentally determine which features are responsible for maintaining morphologically consistent WT lineages, and how. In *mud mutants* is unclear which regulatory mechanisms are sufficient to prevent lineages from outgrowing WT lineages, and what mechanisms drive neuroblast adjacency.

Agent-based models (ABMs) are well suited to address these questions. ABMs represent individual cells as interacting entities with explicit spatial representations and rules for growth, division, differentiation, and neighbor-dependent behavior. ABMs enable the modeling and analysis of how cell behaviors are influenced by internal and external factors. For representing cells in space, cellular Potts models (CPMs) are effective at capturing cell shape, adhesion, packing, and rearrangement, allowing lineage morphology to emerge from local cell-scale interactions[69, 64, 127, 77]. These capabilities make CPM-based agent-based models particularly useful for cell/organism developmental questions in which tissue organization is an output of interest. CPM-based ABMs enable *in silico* interrogation of the evolution of neurogenesis after imposing perturbations that are difficult or impossible to execute and observe *in vivo*.

Here, we develop a calibrated CPM-based ABM of developing *Drosophila* NB lineages and use it to test how division geometry and growth regulation shape lineage morphology in WT and *mud* mutant conditions. We first calibrate the model to experimental WT lineage scale and composition, then ask which separable components of asymmetric division are most important for preserving the WT peripheral NB organization. Notably, we implement cell division modes that yield daughter cells with very different morphologies by shifting the plane of division along a cell-intrinsic apical axis. To our knowledge, this study is the first time a CPM has been used to model asymmetric cell division in which daughter cells differ in morphology due to asymmetric cleavage.

We next use the model to evaluate candidate *mud* regulatory mechanisms by testing whether altered division geometry alone explains mutant behavior, whether growth regulation linked to birth-size-dependent division thresholds or other salient differences between mutant and NB lineages are sufficient to constrain mutant overgrowth, and whether preferential NB-NB adhesion is necessary to maintain NB proximity. Our results show that stable intergenerational apical axis orientation is more important than moderate spindle-angle variance or consistent division-plane offset for maintaining WT lineage sorting, that altered division geometry alone predicts *mud* lineage overgrowth, that volume-based regulation constrains mutant lineages only when division thresholds scale with daughter birth size, and that preferential NB-NB adhesion and consistent

intergenerational apical axis orientation both contribute to NB adjacency in *mud* mutant lineages. Together, these findings identify division geometry and size-dependent growth regulation as coupled determinants of reproducible NB lineage architecture.

3.2 Results

We built and calibrated a spatiotemporal two-dimensional CPM-based ABM of the developing *Drosophila* NB lineage. The model contains three cell types with distinct growth and division dynamics: neuroblasts (NBs), ganglion mother cells (GMCs), and neurons. WT NBs divide asymmetrically, producing a larger apical daughter that remains a NB and a smaller basal daughter that becomes a GMC (Fig. 3.1B, left). GMCs then divide once more symmetrically to produce two neurons (Fig. 3.1B, right). Neurons neither grow nor divide.

NBs and GMCs grow until they reach a division threshold defined as their initial area multiplied by a size-target parameter. For NBs, the model additionally enforces a minimum viable critical volume: a daughter NB's critical volume is held at no less than 20% of the calibrated WT NB critical volume (see Section 3.5.7.2). This floor reflects the biological constraint that a cell cannot shrink indefinitely while remaining viable and is relevant in perturbation conditions and regulatory regimes that produce small daughter NBs. Each model was initialized with one NB centered in the simulation space and ran for 36 hours of simulation time.

3.2.1 *Spatial organization of the WT neuroblast lineage emerges from calibrated growth and division dynamics.*

To implement WT asymmetric NB division, we assigned each NB an apical axis (Fig. 3.1B, left, dashed line) and a default division plane that passes through a point displaced toward the basal end of the cell (Fig. 3.1B, left, dotted line). This offset from the apical edge of the cell is a calibrated parameter. When calibrating the apical axis offset, the size target for the neuroblast was set such that the asymmetric division would yield a cell that was approximately the same area as the initial NB (see Section 3.5.8).

We used experimental WT lineage data to define the target metrics for the 36-hour

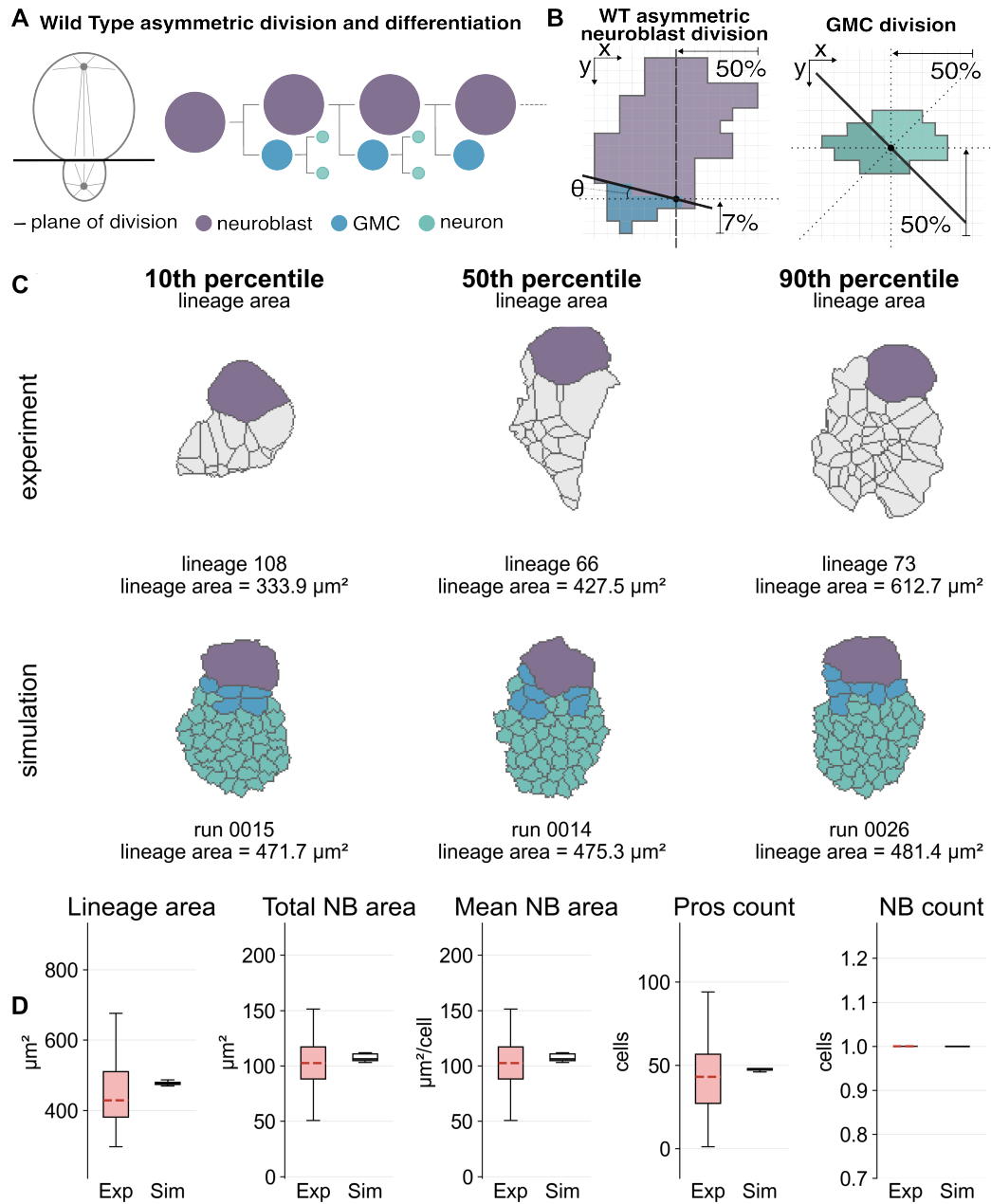


Figure 3.1: Calibrated CPM of developing neuroblast lineage recapitulates expected WT lineage scale, composition, and organization. A. WT planes of division and differentiation cascades. B. Schematic of how the CPM implements WT asymmetric division and GMC division. C. Top: Three example simulated WT neuroblast lineages. Bottom: Three example experimentally imaged WT lineages. Example lineages reflect the 10th, 50th and 90th percentile of lineage area within each dataset. D. Distributions of five calibration metrics across experimental and simulated lineages. Box plots show median $\pm$ IQR; whiskers extend to the last data point within 1.5 x IQR.

simulation window. Our reference dataset consisted of immunohistochemistry-stained WT and *mud* mutant larval brains collected 72–96 hours after egg laying, which corresponds to the end of the developmental period represented in the model. These samples were stained for the nuclear NB marker Deadpan (Dpn), the nuclear differentiation marker Prospero (Pros), which marks GMCs and neurons [199, 115], allowing us to identify NB and non-NB nuclei within each lineage. The experimental data we used were 3D. To enable direct comparison with the 2D simulations, segmented experimental lineages were projected into two dimensions and converted into standardized maps of Dpn and Pros cell territories. Territory boundaries were then assigned based on the relative position and size of each cell within the projected lineage 3.5.3. From the processed WT dataset, we extracted five endpoint summary metrics for calibration: lineage area, Pros⁺ cell count, Dpn⁺ cell count, total Dpn area, and mean Dpn area (see Section 3.5.9.1). Comparison of the projected 2D representations with the original 3D segmentations showed that our calibration metrics were highly consistent between representations, indicating that the projection preserved the relative distribution and variability of the calibration measurements used in the model (Table 3.S1, Table 3.S2, Figure 3.S1).

WT lineages contained a mean of 44.93 ± 21.86 Pros⁺ cells and exactly one Dpn⁺ NB at the terminal time point. WT lineage area averaged $501.16 \pm 126.87 \mu\text{m}^2$, and because WT lineages end with one NB, total and mean NB area were both $107.83 \pm 41.02 \mu\text{m}^2$. We additionally constrained the parameter search to biologically reasonable cell-cycle durations, requiring NB cell cycles to be under 90 minutes and GMC cell cycles to exceed 8 hours [82, 163].

Using these experimental metrics as targets, we calibrated the NB growth rate, NB size target, and NB division plane offset first, with the GMC size target held at an initial default value of 2 (dividing after doubling in size). Calibration of the NB parameters was completed jointly through multiple rounds of estimation and iterative testing, stopping once NB area, NB cell-cycle duration, and Pros⁺ cell count were each within the experimental distribution. At that point simulated lineage area remained above the experimental range. We treated the remaining lineage area error as a signal that average Pros⁺ size was too large and calibrated the GMC size target and growth rate jointly to

bring the total lineage area within the experimental inter-quartile range (see Table 3.1 for parameter values).

After calibration, the NB division plane offset was identified as 93% down the apical axis from the apical end of the cell (Fig. 3.1B, left, dotted line). The NB divides after growing to $1.075\times$ its initial size, generating an apical NB daughter that is approximately the same size as the initial NB. The GMC divides after growing to $1.4\times$ its initial size. By default, the NB division plane is perpendicular to the apical axis (Fig. 3.1B left, dotted line). Division-to-division variation in NB division orientation is introduced by rotating that default plane by a value θ drawn from a normal distribution centered at 0° with a standard deviation of 26° , matching the scale of the experimental WT angle distribution (Fig. 3.1B, left, solid line; Fig. 3.S3 left, [26]). Full parameter values and the calibration workflow are described in the Methods section. Across simulated WT runs, the model produces lineages with a mean area of $500.4 \mu\text{m}^2$, 47.6 Pros⁺ cells, one Dpn⁺ neuroblast, and a mean neuroblast area of $107.2 \mu\text{m}^2$, placing the simulated distribution within the central inter-quartile range (and within one standard deviation) of the experimental WT mean for all calibration targets (Fig.3.1D, Fig. 3.S4).

The distribution of cells in the simulated baseline lineages resembled experimental WT lineages (Figure3.1C). In both simulation and experiment, the neuroblast typically remains peripheral to the lineage (Figure3.1C). Importantly, this peripheral organization was not itself a calibration target; it emerged from the model dynamics.

Together, these results indicate that the calibrated WT CPM captures the expected scale and composition of the lineage.

3.2.2 Distinct components of WT division geometry differentially affect lineage organization.

The peripheral NB organization of the lineage emerged in the calibrated WT model without being used as a fitting target; thus, we concluded that the growth and division dynamics implemented in the model were sufficient to establish and maintain this organization. We next sought to identify which aspects of the imposed division geometry

were most important for establishing and maintaining that organization.

In the model, WT division dynamics contain three separable geometric components. The first is *apical axis reorientation*, which defines how much the NB's internal apical axis changes from one division to the next (Fig. 3.2A). *in vivo*, the apical/basal axis is re-established at each cell cycle by polarity proteins that localize asymmetrically to the cortex: the Par complex (Bazooka/Par-3, Par-6, aPKC), Inscuteable, and Pins/Gai mark the apical pole, while basal determinants such as Miranda, Numb, Prospero, and Brat are excluded to the opposing cortex [147, 144, 102, 140]. This polarity axis is rebuilt *de novo* each cycle, its orientation can drift from one generation to the next [147, 26]. The second component is *division orientation range*, which defines how much the division plane can deviate from its default orientation at each division. It is implemented as the standard deviation of the distribution from which the division-plane rotation is sampled (Fig. 3.2B). *in vivo*, the mitotic spindle and the division axis are aligned with the apical/basal axis through Pins/Mud-dependent capture of astral microtubules at the apical cortex [163, 23, 26]. Importantly, work in *mud* mutants has shown that the division orientation can be divorced from the underlying apical/basal cortical polarity [163, 26]. The third component is *offset shift*, which defines how far the division plane is displaced from the apical end of the NB along the apical/basal axis (Fig. 3.3 A). *in vivo*, the position of the cleavage furrow along this axis is set by a spindle-independent pathway in which polarized actomyosin flows bias the furrow basally to produce the characteristic size asymmetry between daughters [28, 38, 155, 145, 175]. The relative timing of myosin flow from the apical and basal cortices can shift this furrow apically or basally along the axis, providing a means of tuning division-plane position [155, 145]. We systematically perturbed each of these three components of asymmetric cell division while holding the other two constant to assess the individual impact of each on lineage organization.

We used two spatial readouts to quantify the effects of these perturbations (Fig. 3.2 C, D, Fig. 3.3). First, we measured the normalized heterotypic contact fraction, which reports the extent to which neuroblast and non-neuroblast cell territories mix relative to the mixing expected from the lineage composition alone (see Section 3.5.9.3). Higher values of this metric indicate greater mixing of cell types within the lineage, whereas lower

values indicate greater spatial separation. Second, we measured the fraction of the neuroblast perimeter exposed to extracellular space, which reports how peripherally the neuroblast remains positioned within the lineage. Together, these metrics distinguish lineages in which the neuroblast remains spatially segregated at the edge from lineages in which it becomes internalized and intermixed with differentiated progeny.

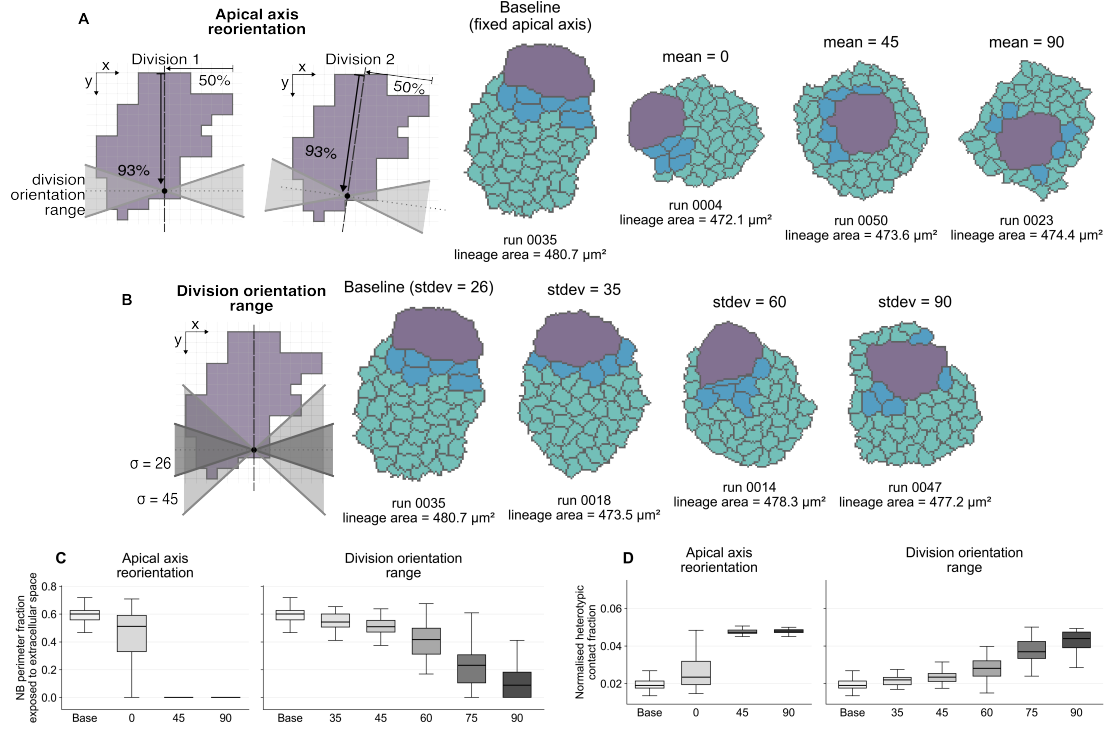


Figure 3.2: **Sensitivity of NB clustering metrics to apical axis reorientation and division orientation range** Box plots show median $\pm$ IQR; whiskers extend to the last data point within 1.5 x IQR

3.2.2.1 Persistent apical axis rotation disrupts WT lineage sorting.

To test the contribution of persistent apical-axis rotation, we ran simulations across a range of imposed inter-division apical-axis rotations (Fig. 3.2 A). In these simulations, fixed-axis variance was held at the biologically grounded WT value of 26° and the division offset was held at the calibrated WT value of 93%. At each division, the apical axis was

rotated by an angle drawn from a normal distribution: the baseline WT condition sampled a distribution with mean 0° (corresponding to no persistent intergenerational drift), while the perturbed conditions sampled distributions with means of 45° or 90° . All three distributions shared a standard deviation of 30° .

The standard deviation of intergenerational apical-axis variation is not directly constrained by the available data. Published measurements of NB division-axis variability track the orientation of the division plane, which reflects the pairwise combination of division orientation and apical-axis orientation [147, 26]. The polarity axis is known to be re-established *de novo* at each division, not inherited from the parent NB [147, 26]. Thus, generation-to-generation variation in axis orientation is expected, but the magnitude of this variation has not been measured. We therefore treat the standard deviation of intergenerational apical-axis variation as a free parameter and set it to 30° , which is both comparable in scale to the calibrated WT division orientation standard deviation (26°) and large enough to introduce non-trivial division-to-division variability without dominating the imposed mean shift. Because the mean-zero condition carries this 30° stochastic spread, it serves as the within-sweep reference: differences between it and the mean-shifted conditions reflect the imposed mean shift rather than the introduction of stochastic spread.

Introducing persistent intergenerational rotation caused a marked loss of the WT peripheral NB phenotype (Fig. 3.2 C). Relative to the baseline condition, lineages with imposed apical axis rotation showed substantially higher normalized heterotypic contact and reduced neuroblast exposure to extracellular space, consistent with neuroblast internalization and increased mixing among cell types (Fig. 3.2 D)). This effect was clear at 45° and remained strong at 90° . Thus, within the model, maintaining a stable relative orientation across generations appears to be an important requirement for preserving the peripheral NB organization of the developing WT lineage.

3.2.2.2 *Increasing division orientation range has limited effects until extreme values.*

We next asked whether increasing the stochastic variation of the division orientation relative to a stable WT apical axis was sufficient to disrupt lineage organization. To isolate this effect, we set both the intergenerational apical-axis rotation mean and standard deviation to 0° , matching the calibrated WT baseline, and held the division offset at 93%. We increased the standard deviation of the division orientation range distribution from the biologically grounded WT value of 26° to 35° , 60° , and 90° (Fig. 3.2 B). Here successive divisions fluctuate around the same underlying apical/basal orientation and do not persistently drift away from it.

Normalized heterotypic contact rose gradually with increasing division orientation range, but the effect remained substantially smaller than the disruption caused by persistent apical axis rotation (Fig. 3.2D). Likewise, neuroblast exposure to extracellular space declined progressively rather than collapsing immediately (Fig. 3.2 C). Qualitatively, these lineages largely retained their peripheral NB phenotype. Thus, the calibrated WT lineage tolerated appreciable stochastic variation around a stable axis while largely preserving the peripheral NB phenotype.

At the largest tested standard deviation (90°), this tolerance began to break down. Large rotations of the division plane produced more mixing and reduced neuroblast exposure (Fig. 3.2 C, D). Even so, the resulting disorganization remained weaker than that produced by persistent intergenerational rotation. These results indicate that division orientation variability can disrupt lineage organization, but only when it becomes very large. *In silico* analyses suggest that preserving a consistent central orientation across generations is more important for maintaining the WT peripheral NB organization than tightly minimizing every division-to-division fluctuation around that orientation.

3.2.2.3 *The peripheral NB phenotype persists across the full range of tested division offsets.*

We next asked how strongly lineage organization depends on maintaining the WT asymmetric division offset. To isolate this effect, we held the intergenerational apical-axis

rotation mean and standard deviation both at 0° and the division orientation range at 26° (the calibrated WT value), and shifted the division plane from the WT offset of 93% to 50%, 22%, and 7%, spanning the range from strongly asymmetric (WT) to symmetric (50%) to inversely asymmetric (Fig. 3.3 A).

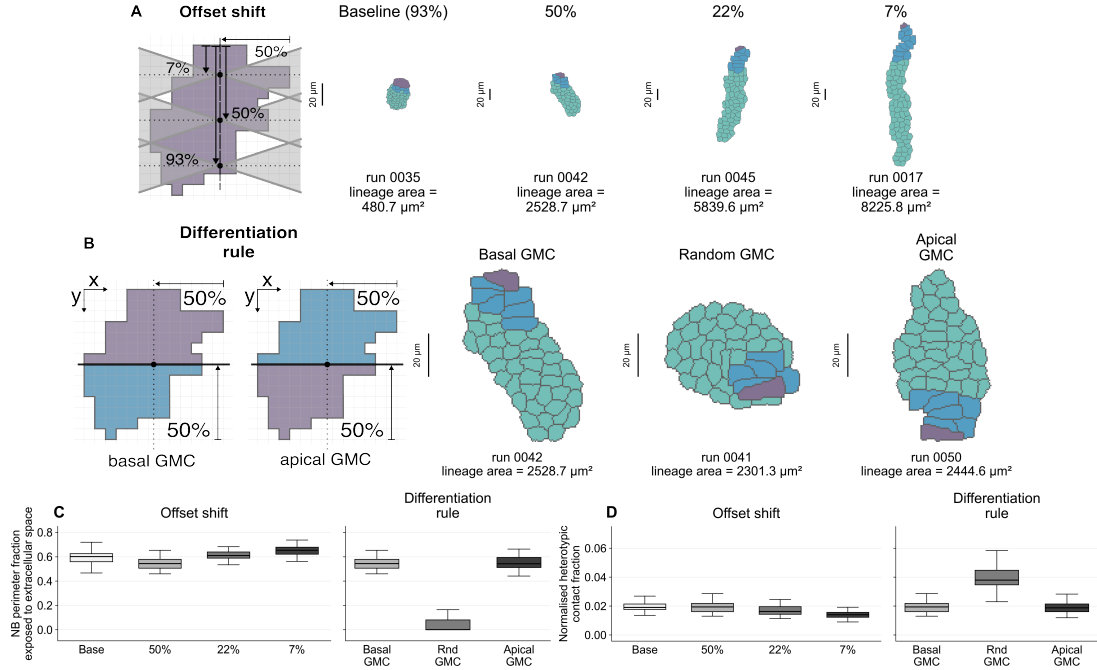


Figure 3.3: Sensitivity of NB clustering metrics to offset shift and differentiation rules. Box plots show median $\pm$ IQR; whiskers extend to the last data point within 1.5 x IQR

The /insilico offset parameter also defines the birth volumes of both daughters, since the apical and basal daughters roughly inherit fractions offset/100 and (100 – offset)/100 of the parent volume at each division (see Section 3.5.8.1). Lower offsets therefore produce smaller apical NBs and larger basal GMCs. Because GMCs grow to $1.4\times$ their birth size before dividing, the larger basal-daughter (GMC) birth volume at lower offsets results in larger non-NB cells throughout the lineage.

For each tested offset we recalibrated the NB growth rate to keep the NB cell-cycle duration approximately constant across conditions, ensuring that lineages reach comparable cell counts by the simulation endpoint (see Section 3.5.8.2). The NB critical

volume and size target were chosen so that, at the $\geq 50\%$ offset, the apical daughter is born at the NB critical volume and the cell divides at the same volume threshold as the WT baseline. We held the NB critical volume and size target values at these recalibrated values across all offset simulations and scaled the GMC growth rate to maintain a comparable GMC cell-cycle duration (and therefore similar cell counts across simulations) despite the larger GMC starting volume (see Table 3.2).

Across the parameter sweep, normalized heterotypic contact and NB exposure to extracellular space remained close to the calibrated WT baseline (Fig. 3.3 C, D). Qualitatively, the offset-perturbed lineages remained recognizably organized, with the NB occupying a peripheral position even at fully symmetric placement and at offsets that produced an apical daughter substantially smaller than its basal sibling (Fig. 3.3 A). At the inversely asymmetric offsets, lineages adopted an elongated, narrow morphology, which we attribute to the larger basal daughters produced under these conditions. These daughter cells expand the lineage along the apical/basal axis which affects the overall shape of the lineage without disrupting the peripheral location of the NB. We conclude that the spatial position of the division plane is not a major determinant of where the NB is positioned within the lineage.

3.2.2.4 Randomizing daughter identity assignment partially disrupts NB peripheral positioning.

Neither the spatial position of the division plane nor the resulting daughter-size asymmetry is the primary determinant of the peripheral NB phenotype in the model. We reasoned that a more likely candidate, held constant across all of these perturbations, is the consistent assignment of NB identity to the apical daughter at each division. Because the apical daughter is assigned NB identity and successive divisions add new daughters basally, this identity rule could position the NB consistently at the apical surface of the growing lineage.

To test whether the consistent assignment of NB identity to the apical daughter is responsible for the peripheral NB phenotype, we implemented three differentiation rulesets

that vary inherited NB daughter identity at each division (see Section 3.5.8.3). In the **basal GMC** WT ruleset, the apical daughter remains a NB and the basal daughter becomes a GMC (Fig. 3.3 B). In the inverted (**apical GMC**) ruleset, the apical daughter becomes a GMC and the basal daughter remains a NB. In the **random GMC** ruleset, GMC identity is assigned to one of the two daughters at random at each division. We ran all three conditions at a 50% division-plane offset rather than at the WT 93% offset, because the minimum-NB-critical-volume floor means that a basal-born NB at the WT offset would be driven into a rapid post-division expansion that would confound the identity perturbation with size-asymmetric cell-cell interactions (see Section 3.5.8.3). All other parameters were held at their WT-baseline values.

Under both the **basal GMC** and **apical GMC** conditions, the NB occupied a peripheral position in the resulting lineage (Fig. 3.3 B). In the **basal GMC** condition the NB was positioned at the apical surface as observed in the WT baseline, and in the **apical GMC** condition the NB was positioned at the basal surface. Normalized heterotypic contact and NB exposure to extracellular space were comparable between these two conditions and to the WT baseline (Fig. 3.3 C, D). Consistent assignment of NB identity to one pole is therefore sufficient to produce a peripheral NB.

Under the **random GMC** ruleset, lineage sorting was partially disrupted but it did not collapse. In the randomized-identity lineages the NB usually occupied an intermediate position, often near the lineage periphery (Fig. 3.3 B). Normalized heterotypic contact rose modestly and NB exposure to extracellular space declined in comparison to the non-random differentiation rules, but these changes were smaller than those produced by persistent intergenerational apical-axis rotation (Fig. 3.3 C, D). We interpret this partial morphological disruption as evidence that the identity rule contributes substantially to, but it is not the sole mechanism underlying, peripheral NB positioning. *in silico* observations suggest that the NB preferentially grows into adjacent extracellular space when available instead of displacing clusters of neighboring cells. This bias toward outward expansion is enabled by the constrained division geometry of our model: each NB division places its daughter on one of two faces of the parent cell (the apical or basal face) rather than across a wider range of possible orientations because the apical axis is fixed

and the division orientation range is narrow. The remaining faces of the NB therefore tend to retain contact with extracellular space, providing the openings into which the NB can expand, sustaining its lineage periphery.

Together, the offset sweep and the identity-randomization experiment indicate that the peripheral NB phenotype is shaped by at least two contributing factors: a consistent assignment of NB identity to one geometric pole of the parent cell, and an NB-intrinsic tendency to expand into open space at the lineage surface. The spatial position of the division plane and the daughter-size asymmetry it produces, by contrast, contribute little to where the NB ends up within the lineage.

3.2.3 NB starting size effects are insufficient to constrain mud mutant lineage overgrowth.

Next, we used our model to explore which regulatory mechanisms are sufficient to constrain lineage overgrowth in *mud* mutants by implementing a *mud* mutant cell type. We added a rule for symmetric division: whenever the sampled division orientation rotation exceeds 75° in magnitude from the apical/basal axis, the cell instead divides symmetrically along that axis, producing two neuroblast daughters (Fig. 3.4A, B). We set the parameters of the normal distribution that determines division orientation rotations in *mud* mutants to a mean of 0° and a standard deviation of 50° , calibrated from time-lapse measurements of NB division-axis angles across successive divisions in *mud* mutant and WT animals (see Section 3.5.7.1, Fig 3.S3). Together, this broader rotation distribution and the 75° threshold cause approximately 13% of *mud* mutant divisions to be symmetric, matching both the experimentally observed symmetric-division rate and the rotation-based switch between asymmetric and symmetric divisions [26, 163]. We strengthened the NB–NB adhesion term so that neuroblasts adhere more strongly to one another than to other cell types, reflecting the experimental observation that neuroblasts in *mud* mutant lineages tend to remain adjacent to one another (Fig. 3.S6). We also added a rule for how daughter NBs inherit an apical axis after a symmetric division: each daughter NB inherits its parent’s apical axis rotated by an angle drawn from a normal distribution with mean 0° and standard deviation 30° . This rule approximates the

qualitative experimental observation that sibling NBs born from a symmetric division have similar division-plane orientations in their first subsequent division.

Using this model, we asked whether the smaller starting volume of mutant NBs born from symmetric divisions is sufficient to constrain lineage overgrowth. Symmetric division splits the parent NB volume roughly in half, so mutant NBs born from symmetric divisions begin at approximately half the WT NB volume. Fixing the division threshold at the WT division volume would force these smaller daughters to spend additional time growing before dividing again, slowing lineage expansion in proportion to the number of symmetric divisions a lineage has undergone. We simulated *mud* mutant lineages with a constant division threshold calibrated to the expected WT division volume (see Section 3.5.7.2) and held all other parameters at WT values.

The unregulated simulated *mud* mutant lineages substantially overgrew the experimental *mud* mutant range on all five calibration metrics, producing larger lineages with more Pros⁺ progeny and more Dpn⁺ neuroblasts than observed experimentally (Fig. 3.4C, D). Within the model, then, the smaller starting size of NBs born from symmetric divisions combined with a fixed WT-calibrated division threshold is not on its own sufficient to constrain mutant lineage overgrowth; an additional regulatory mechanism is required.

3.2.4 Volume-regulated NB growth combined with birth-size-dependent thresholds constrains mud mutant lineages within the experimental range.

Two salient differences between *mud* mutant and WT neuroblast lineages are that *mud* mutant lineages have more NBs and that those NBs are, on average, smaller than WT NBs [48] (Fig. 3.S4 red data). We interrogated whether regulatory feedback responsive to either of these features could constrain mutant lineage overgrowth.

We implemented four candidate regulatory regimes that varied along two axes: the cellular feature being sensed (NB volume or NB–NB contact) and the nature of the feedback mechanism (cell-autonomous or population-averaged). Under volume-based regulation, NB growth rate is suppressed when NB volume falls below the expected WT

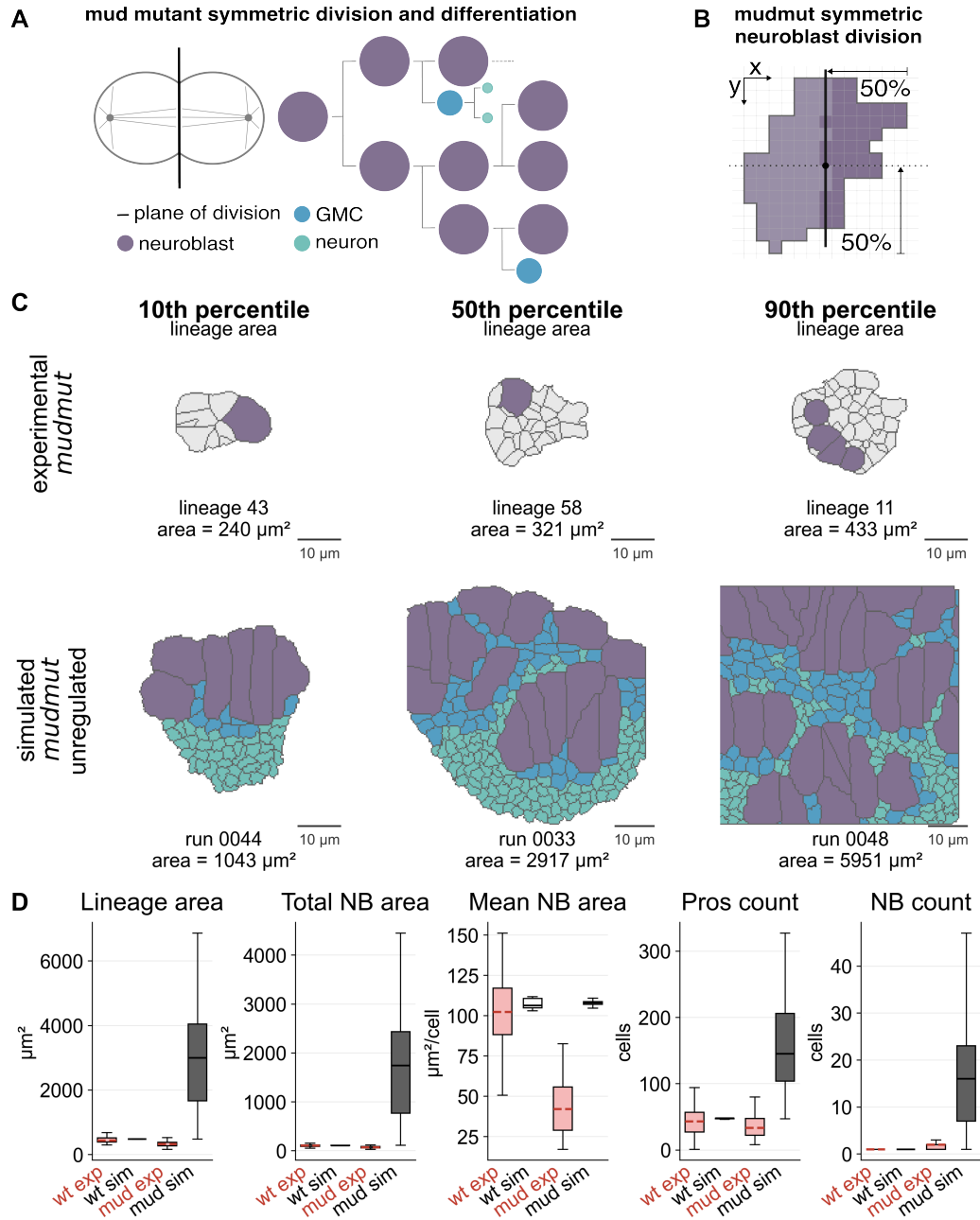


Figure 3.4: The model predicts that unregulated *mud* mutant lineages should overgrow WT lineages. **A.** NB undergoing symmetric division and resulting differentiation cascade. **B.** Symmetric division as implemented in the model. **C.** Representative experimental and simulated *mud* mutant lineages at the 10th, 50th, and 90th percentiles of lineage area. **D.** Distributions of the five calibration metrics — lineage area, Pros⁺ cell count, Dpn⁺ cell count, total Dpn area, and mean Dpn area — across WT experimental, WT simulated, *mud* mutant experimental, and unregulated *mud* simulated lineages. Box plots show median $\pm$ IQR; whiskers extend to the last data point within 1.5 x IQR.

average volume, and it is accelerated when NB volume exceeds this reference. In the cell-autonomous (ABM-like) implementation each NB responds to its current volume; in the population-averaged (PDE-like) implementation, all NBs respond to the mean NB volume across the simulation. The reference volume was set to the volume halfway between a WT NB's initial critical volume and its division threshold. Under NB-contact regulation, NB growth rate is suppressed via a Hill function of the number of NB neighbors (ABM-like) or the total number of NBs in the simulation (PDE-like). Biologically, volume-based regulation could represent cell-size-sensitive growth machinery, and NB-contact regulation could represent a contact-mediated or diffusible repressive signal exchanged between neuroblasts (see Section 3.5.7.3).

We applied each of the four regulatory regimes under two threshold rules as defined in Section 3.5.7.2: the constant division threshold ($\text{VCV}=0$) in the unregulated *mud* simulations, and a birth-size-dependent threshold ($\text{VCV}=1$) where a NB division-volume threshold is a multiple of the NB's birth volume (Figure 3.5A). The biological intuition for $\text{VCV}=1$ is that the smaller birth size produced by a symmetric division propagates forward across generations: a smaller-born mutant NB has a smaller division threshold, so it divides at a smaller volume, producing daughters that are themselves smaller still, keeping descendants of symmetric divisions persistently smaller across successive cell cycles. We first confirmed that each of the four candidate regulatory dynamics reproduced the five calibration metrics within the experimental ranges for WT cell types (Fig. 3.S5).

Under the constant division threshold, NBs do not shrink in size across generations (Fig. 3.5 A, left; Fig. 3.5 B, light boxes; Fig. 3.5 C, top row). This regulation produced mean NB area that was consistent with the WT value across all four regulated conditions and surpassed the smaller average NB size observed in experimental *mud* lineages (Fig. 3.5 B). Because each NB occupies WT-scale volume, total NB area and lineage area at $\text{VCV}=0$ also remained above the experimental range across all four regulators (Fig. 3.5 B). The NB-PDE regulator was the only $\text{VCV}=0$ mechanism that produced an Pros count consistent with the experimental *mud* mutant range (Fig. 3.5 B). All other regulation regimes overgrew the experimental *mud* lineages on all five calibration metrics.

Under the division threshold that scales with the NB's birth volume, the smaller

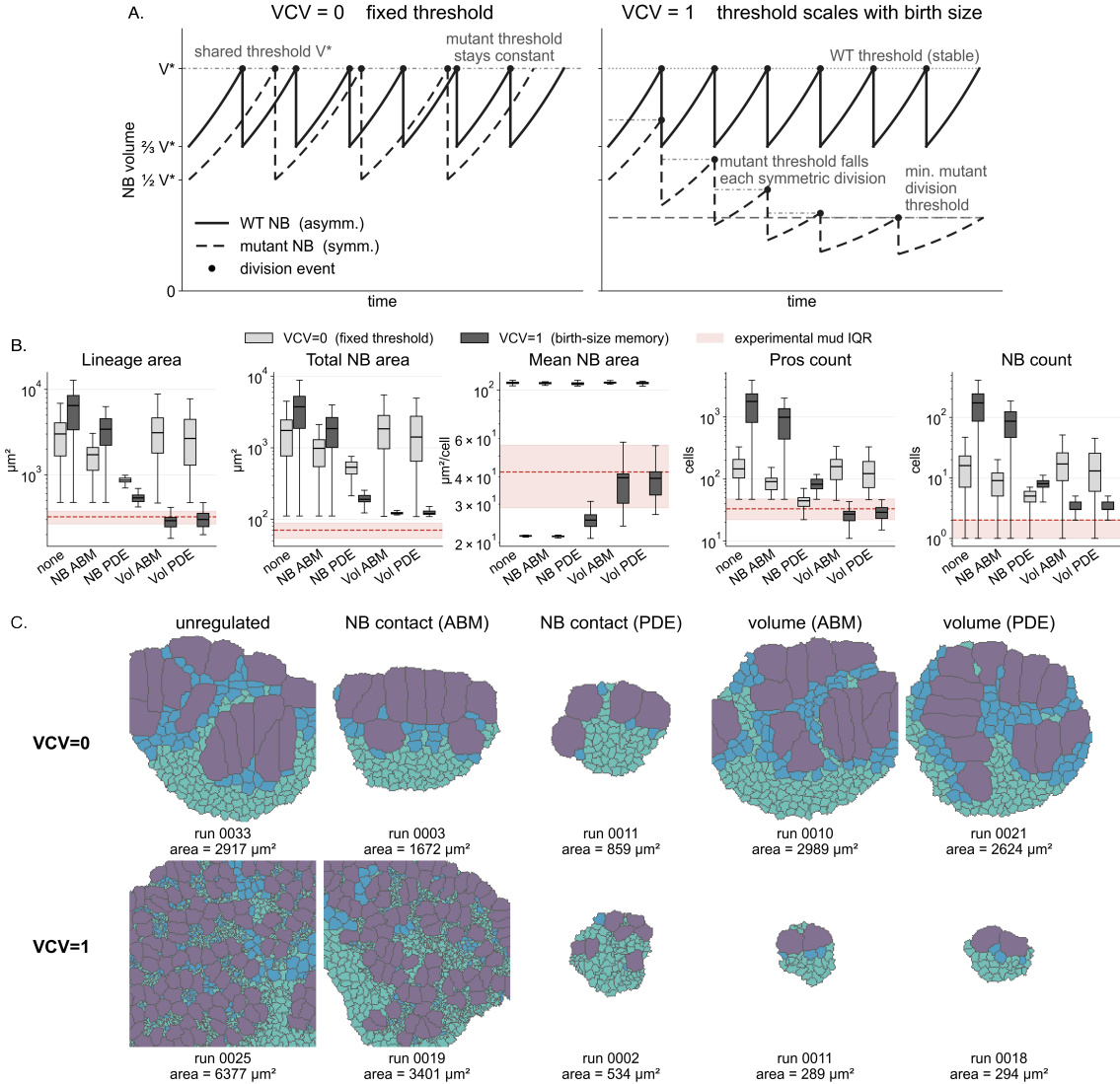


Figure 3.5: Volume-based regulation paired with birth-size-dependent division thresholds constrains *mud* mutant lineage overgrowth. **A.** Schematic contrasting the two division threshold rules. **B.** Endpoint distributions of the five calibration metrics across the unregulated baseline plus four candidate regulatory dynamics (NB-ABM, NB-PDE, Vol-ABM, Vol-PDE), under each threshold rule (light: $\text{VCV}=0$; dark: $\text{VCV}=1$). The red band marks the experimental *mud* interquartile range; the dashed red line marks the experimental median. Y-axes are log-scaled. **C.** Representative simulated *mud* mutant lineages at the 50th percentile of lineage area, organized by regulatory dynamic (columns) and threshold rule (rows).

starting sizes of symmetric-division descendants to propagate forward (Fig. 3.5 A, right; Fig. 3.5 B, dark boxes; Fig. 3.5 C, bottom row). This division leads to progressively smaller NB volumes until they reach steady state or hit the enforced minimum (Fig. 3.5 A right). Without regulation, mean NB area at $\text{VCV}=1$ collapsed onto the imposed minimum critical-volume floor and the lineages contained many small, rapidly-dividing NBs (Fig. 3.5 C, bottom left). NB count climbed to several-fold above its $\text{VCV}=0$ unregulated value. The unregulated lineages therefore overshoot the experimental *mud* range under either threshold rule, but in different ways: under $\text{VCV}=0$ by maintaining many WT-sized NBs, and under $\text{VCV}=1$ by producing even more numerous, smaller NBs (Fig. 3.5 C).

Among the regulated conditions under $\text{VCV}=1$, Vol-ABM and Vol-PDE were the only combinations that constrained lineage area. They also did this without driving mean NB area below the experimental range. NB-ABM regulation with $\text{VCV}=1$ produced lineage area and NB count that were well above the experimental range as mean NB area collapsed to the floor. NB-PDE regulation with $\text{VCV}=1$ produced lineage area and total NB area closer to the experimental range (when compared to the $\text{VCV}=0$ counterpart), but undershot mean NB area and left Pros count and NB count above the experimental range. Vol-ABM and Vol-PDE under $\text{VCV}=1$, by contrast, produced experimentally consistent calibration metrics. Among the eight regulator-threshold combinations tested, Vol-ABM and Vol-PDE with $\text{VCV}=1$ produced the closest match to the experimental *mud* phenotype across all five calibration metrics simultaneously.

Recapitulating the experimental *mud* phenotype in our model therefore requires both a regulatory feedback that responds to NB volume, and a threshold rule that lets the smaller birth size of symmetric-division descendants accumulate across generations. Neither alone is sufficient. When $\text{VCV}=0$, no regulator we tested sustained mean NB area below the WT value, because the absolute division threshold prevents NBs from shrinking generation-to-generation. When $\text{VCV}=1$, the threshold rule that would allow NBs to remain smaller amplifies proliferation in the absence of a compensating regulator, producing lineages with many sub-experimental-sized NBs. Only when volume-based regulation is paired with the birth-size-dependent threshold do simulated *mud* lineages come close to the experimental phenotype on all five calibration metrics.

3.2.5 NB clustering in regulated mud mutant lineages is shaped by both NB-NB adhesion and post-inheritance rotation of the daughter-NB apical axis.

A distinctive spatial feature of experimental *mud* mutant lineages is that NBs tend to remain adjacent to one another. Quantitatively, 91.5% of the experimental *mud* lineages we analyzed had all of their NBs in a single connected component (Fig. 3.S6). The regulated baseline carried forward from the previous section (*off* post-inheritance rotation with preferential NB-NB adhesion) reproduced this clustering closely, reaching 96% connectivity in both the Vol-ABM and Vol-PDE formulations under $\text{VCV}=1$ (Figure 3.6B). We wanted to know which features of the model are responsible for producing this connectivity.

Two candidate features could plausibly affect NB clustering: the preferential NB-NB adhesion term in the Cellular Potts Hamiltonian (Section 3.5.4), and the amount the apical axis is allowed to drift after the inheritance step at each symmetric division (Figure 3.2, top). The inheritance step itself was held constant across all conditions tested in this section: each daughter NB inherits its parent’s apical axis rotated by $\mathcal{N}(0^\circ, 30^\circ)$, as in the regulated simulations of the previous section. We tested four post-inheritance rotation conditions, spanning from the original baseline (no additional rotation) to progressively larger imposed rotations. The baseline condition (*off*) is the apical-axis rule used in the regulated simulations of the previous section: each successive division plane is sampled relative to the daughter NB’s inherited apical axis ($\mathcal{N}(0^\circ, 50^\circ)$ rotation), and the apical axis itself does not rotate further after the inheritance step. The remaining three conditions ($\mu=0$, $\mu=45$, and $\mu=90$) impose additional post-inheritance rotation of the apical axis, with means of 0° , 45° , and 90° respectively (each with 30° standard deviation). This range parallels the apical-axis reorientation sweep we performed in the WT model (Figure 3.2A, top).

We crossed these four post-inheritance rotation conditions with three adhesion states: NB-NB adhesion set equal to all other cell–cell adhesions ($J=50$), preferential NB-NB adhesion as in the baseline regulated simulations ($J=40$), and significantly stronger NB-NB adhesion compared to other cell types ($J=20$). We did this for each of the two

volume-based regulators (Vol-ABM and Vol-PDE) under $V_{CV}=1$, yielding 24 conditions in total.

Permitting post-inheritance rotation of the apical axis was the single largest determinant of NB connectivity (Figure 3.6B). In every adhesion state and both volume regulators, the *off* condition gave the highest connectivity (0.88–0.96), and introducing any post-inheritance rotation reduced it: switching from *off* to $\mu=0$ lowered connectivity by 14–22 percentage points. Beyond this initial drop, however, connectivity did not decline monotonically with further rotation. The lowest values typically occurred at $\mu=45$ (as low as 0.60), while $\mu=90$ frequently recovered relative to $\mu=45$ —in some conditions substantially (e.g. 0.84 for Vol-ABM and 0.88 for Vol-PDE under the strongest adhesion), and in Vol-PDE under strong adhesion connectivity in fact rose with rotation past $\mu=0$ ($0.80 \rightarrow 0.90 \rightarrow 0.88$).

NB-NB adhesion modulated connectivity in a direction- and rotation-dependent manner, rather than as a uniformly smaller effect. In the Vol-PDE regulator, connectivity increased monotonically with adhesion strength (equal $\rightarrow$ preferential $\rightarrow$ strong) across all four rotation conditions. In Vol-ABM the same ordering held at low rotation (*off*, $\mu=0$) but broke down at $\mu=90$, where the preferential baseline (0.62) was below both the equal-adhesion (0.70) and strong-adhesion (0.84) conditions. Either strengthening or weakening adhesion raised connectivity there. The magnitude of the adhesion effect grew with rotation: at *off* the full equal-to-strong swing was only 6–8 percentage points, whereas at $\mu=45$ – $\mu=90$ it reached 10–30 percentage points, rivaling or exceeding the rotation effect (the largest single swing, 30 percentage points, occurred at Vol-PDE $\mu=45$). Stronger NB-NB adhesion thus partially rescued the connectivity lost to post-inheritance rotation.

Among the 24 conditions tested, the *off* configurations with preferential or strong NB-NB adhesion were the only ones to match the experimental *mud* connectivity, reaching 96% against the experimental 91.5% in both regulators (Figure 3.6B); all conditions with any post-inheritance rotation fell short. NB clustering in our simulated *mud* lineages is therefore governed primarily by whether post-inheritance rotation of the daughter-NB apical axis is permitted with differential NB-NB adhesion acting as a secondary modulator

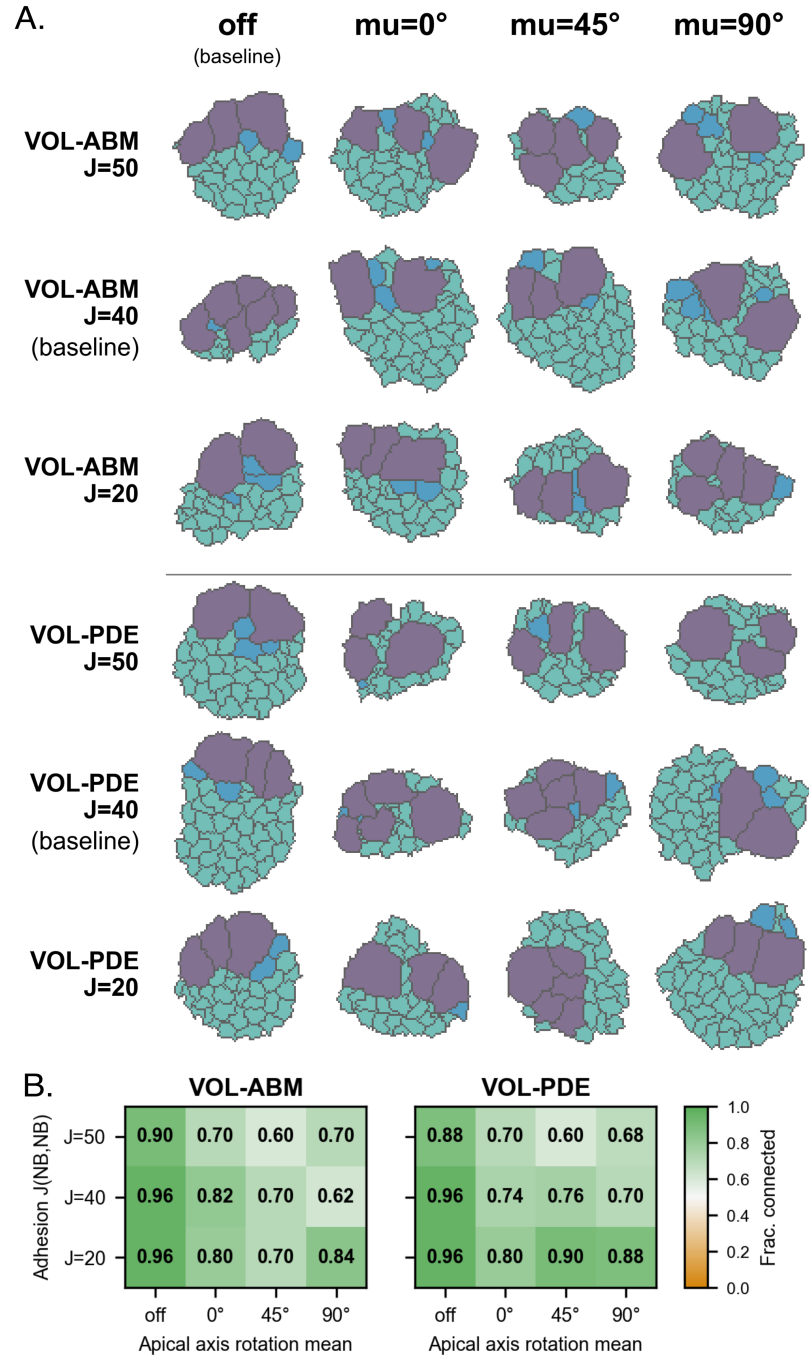


Figure 3.6: NB connectivity in regulated *mud* mutant lineages is shaped by both NB-NB adhesion and post-inheritance rotation of the daughter-NB apical axis. **A.** Representative simulated *mud* lineages across selected (regulatory dynamic $\times$ adhesion state $\times$ post-inheritance rotation) combinations. **B.** NB connectivity (fraction of lineages with all NBs in a single connected component) across all twenty-four tested conditions.

whose influence increases at higher rotation, where stronger adhesion can substantially restore connectivity. The adhesion response was monotonic in the Vol-PDE regulator but non-monotonic in Vol-ABM at high rotation, suggesting a spatial-formulation-specific interaction that the mean-field model does not reproduce.

3.3 Discussion

We developed and calibrated a CPM-based agent-based model of the developing *Drosophila* NB lineage that recapitulates the experimentally observed WT lineage scale and composition. To our knowledge, this is the first cellular Potts model of asymmetric cell division in which the daughter cells differ in morphology as a consequence of asymmetric cleavage. The peripheral NB organization of the WT lineage emerged from the calibrated model without being used as a fitting target, and we used the model to test which components of the WT division geometry and differentiation rulesets contribute most to that organization. We then extended the model with *mud* mutant division dynamics to investigate which regulatory mechanisms are sufficient to constrain *mud* mutant lineage overgrowth and which features of the model produce the NB clustering observed in experimental *mud* lineages.

3.3.1 Consistent apical-axis orientation and fate-position coupling drive peripheral NB localization

Our model allowed us to perturb in isolation components of asymmetric cell division that are mechanistically distinct but difficult to separate experimentally [147, 26, 28]. Across these perturbations, intergenerational rotation of the apical/basal axis dramatically disrupted the peripheral NB phenotype, whereas spindle-orientation variance and division-plane offset were tolerated across a wide range. That phenotype was preserved even when division became fully symmetric or inversely asymmetric. Randomizing daughter identity assignment partially disrupted sorting, but consistent assignment of NB identity to either pole was sufficient to maintain a peripheral NB, indicating that what matters is the consistency of fate-position coupling, not its specific direction. Even under randomized identity, NBs retained a tendency to expand into adjacent extracellular space,

which we attribute to the energetic favorability of growth into empty space combined with the model’s constrained division geometry: because daughters are placed on only two of the NB’s faces at each division, the remaining faces retain access to open space. Together, these results indicate that the peripheral NB phenotype emerges from a fate-position coupling rule combined with a constrained division geometry, while the spatial position of the cleavage plane and the daughter-size asymmetry it produces are downstream effects rather than primary drivers.

Cortical polarity in WT NBs is reestablished *de novo* each cell cycle rather than being inherited from the parent NB [147, 144, 102, 140]. Our work identifies the consistency with which the apical/basal axis is restored in the same direction at each successive division as the most influential geometric component of WT NB peripheral positioning. The tight consistency of WT NB lineage morphology presumably reflects tight regulation of NB cortical polarity *in vivo*. In the model, our apical-axis rotation perturbation is a phenomenological proxy for a polarity-machinery defect that fails to re-establish polarity in a consistent orientation across cell cycles. Crucially, within each division the model assumes that basal determinants are still correctly localized along the (rotated) axis and that the spindle is still aligned to that axis via Inscuteable-mediated coupling. Under such a defect, NBs would retain their stem identity at each division but could end up positioned inside the lineage rather than at the periphery. *mud* mutants do not test this prediction: in *mud*, the spindle rotates relative to a still-intact apical/basal cortex [147, 163, 26, 23], so heavily rotated divisions yield two NBs through misallocation of fate determinants (Miranda, Numb, Prospero, Brat) rather than through a re-oriented polarity axis. The model’s prediction that rotating the polarity axis itself between generations would disrupt the peripheral NB phenotype would be most cleanly tested in mutants of polarity-establishment machinery (e.g., Par-complex), not spindle-orientation machinery.

In the model, the basal GMC differentiation rule (apical daughter retains NB identity) was sufficient to maintain a peripheral NB regardless of the offset of the division plane or the resulting size difference between NB and GMC daughters. Biologically, this rule corresponds to polar segregation of fate determinants: aPKC at the apical pole phosphorylates and excludes basal determinants (Miranda, Numb, Prospero, Brat),

partitioning them into the basal daughter at division [147, 99, 59, 144]. The model's **basal=GMC** rule is the morphological consequence of this molecular segregation. The **apical=GMC** rule corresponds to inverted determinant localization (basal determinants localized apically) and also yielded peripheral NBs in the model. Identity randomization corresponds to determinant inversion at 50% of divisions. The partial disruption of the peripheral NB phenotype under random assignment indicates that the consistency of fate-position coupling matters more than its specific direction.

Even under randomized fate assignment, the peripheral NB phenotype was not completely lost. We attribute this residual peripheral tendency to two NB-intrinsic mechanisms operating in the model. First, NBs preferentially expand into adjacent extracellular space rather than displacing clusters of neighboring cells. In the model this bias arises from the Cellular Potts volume constraint (growth into open lattice sites is energetically favored over rearranging neighbors), capturing the relative energetic cost *in vivo* of pushing through tightly packed cells versus extracellular space. Second, NB division geometry is constrained: a fixed apical/basal axis and a WT-calibrated spindle-orientation range place each daughter on only one of two faces of the NB (apical or basal), leaving the remaining faces in contact with extracellular space at the lineage surface. Together, the constrained geometry typically leaves the NB with open faces available, and the energetic bias drives NB growth through those faces. The resulting outward expansion persists regardless of which daughter inherits NB identity, contributing to peripheral NB localization alongside, and partially independently of, the consistency of fate-position coupling.

Peripheral localization of stem cells within tissue compartments is a recurring feature of stem-cell-driven systems, occurring in basal epidermal stem cells in mammalian skin and intestinal stem cells at the crypt base [110, 109]. Our results suggest that consistent fate-positioning coupled to a constrained division geometry offers a simple mechanism by which peripheral stem-cell localization can emerge from local cell-scale rules without requiring long-range patterning signals or dedicated adhesion-based architectures. Our model's prediction that perturbations of polarity establishment should disrupt lineage architecture more strongly than perturbations of spindle orientation or division plane offset

alone may apply beyond *Drosophila* NBs, in any stem-cell system where polarized fate-determinant segregation maintains tissue architecture.

3.3.2 *Constraining mud mutant lineage scale requires both responsive regulation and birth-size memory*

No single regulator–threshold combination we tested matched the experimental *mud* range across all five calibration metrics. Cell-size memory ($\text{VCV}=1$) without a responsive regulator produced runaway proliferation of small NBs. Regulation (volume-based or contact-based) without $\text{VCV}=1$ could not bring NBs to the smaller size observed in experimental *mud* lineages. Only volume-based regulation paired with the birth-size-dependent threshold rule brought all five calibration metrics close to the experimental *mud* range. Division thresholds being a function of birth size is observed across many systems, for example “adder” mechanisms require that a certain amount of volume be added to a cell before it can divide, with that amount being a constant regardless of initial size, causing smaller cells to divide at smaller volumes [150]. Although *in vivo* cell growth rate is unlikely to be a strict function of cell volume alone [50], a simple volume-sensitive growth response, combined with birth-size memory, suffices in our model to predict the *mud* phenotype without requiring *mud*-specific machinery beyond the symmetric-division rule.

3.3.3 *NB clustering in regulated mud mutant lineages reflects both adhesion and apical-axis dynamics*

In our regulated *mud* lineages, NB connectivity depends primarily on whether post-inheritance apical-axis rotation is permitted at all: the *off*-to-rotation switch is the dominant determinant, rather than a graded decline with rotation magnitude. Preferential NB-NB adhesion acts as a secondary modulator whose influence grows at higher rotation, where stronger adhesion can partially restore the connectivity that rotation disperses. The same mechanisms that produce peripheral NB localization in WT — stable apical orientation and constrained division geometry — also produce NB clustering in regulated *mud* mutants, suggesting the machinery regulating these mechanisms is active in both

genotypes. A third factor emerges from the simulations themselves: NB adjacency was never fully lost in any run, indicating that rotation and adhesion alone do not account for the connected morphology. This residual adjacency likely reflects our criterion for “sufficiently constrained” lineages: their cell counts closely matched experimental *mud* lineages, so they underwent very few NB divisions. Because sibling NBs from symmetric divisions are born adjacent, lineages with suppressed growth lack the later divisions that would insert intervening cells between them. Baseline NB adjacency is therefore likely a structural consequence of the constraint criterion itself, independent of any explicit adhesion or rotation effect.¹

3.3.4 Limitations

Our model is two-dimensional, while real lineages develop in 3D. Because we calibrated cell counts against measurements from 3D lineages, our simulated lineages are likely more spatially clustered than their *in vivo* counterparts. This choice was driven by data availability: the angle measurements characterizing how the division plane rotates between divisions were collected in 2D, precluding accurate parameterization of a 3D model. With additional angle measurements, future work could extend this framework to 3D and examine how clustering in three dimensions shapes growth dynamics.

Our *mud* mutant regulatory regimes (volume-based and contact-based) are phenomenological abstractions; the model does not commit to specific molecular implementations. These regimes were chosen because the most apparent quantitative differences between WT and *mud* mutant lineages are in NB size and NB number. Whether those differences drive the morphological distinctions between genotypes or are themselves downstream of other regulatory mechanisms remains unknown. Here we claim only that these regimes are sufficient to reproduce the observed morphologies, not that they are necessary nor that they reflect the regulation actually occurring *in vivo*.

Our results establish sufficiency rather than presence. We show that the proposed mechanisms can produce the observed lineage morphologies, but cannot show that they are the ones actually operating *in vivo*. Bridging this gap will require experimental

validation. The most direct tests would combine live imaging of NB divisions that measure apical-axis dynamics in real lineages with targeted perturbations to adhesion or division geometry. Comparing the resulting clustering phenotypes against the model's predictions would identify which proposed mechanisms are operating *in vivo*.

3.3.5 Future directions

The most immediate next steps are experimental tests of two of the model's central predictions. The first concerns cortical polarity: the model predicts that mutants disrupting cortical polarity (e.g., *bazooka*, *Inscuteable*, *Pins*) should produce qualitatively different lineage architectures than mutants that primarily disrupt spindle orientation (e.g., *mud*), since the two classes of perturbation break different components of the division-geometry machinery. Comparing lineage morphologies across these mutant classes would test this prediction directly. The second concerns size-based regulation: if NB growth is regulated by a volume-sensing mechanism, symmetric-division descendants in *mud* lineages should carry size-memory signatures in their NB volumes and cell-cycle durations. Measuring these quantities across known symmetric-division descendants would either support or rule out the volume-based regime explored here.

The model itself can also be extended to address questions beyond the scope of this study. Extending the framework to 3D (which is feasible with additional measurements of division-plane rotation) would enable direct comparison to *in vivo* lineage architecture and address the spatial-clustering bias introduced by the 2D simplification. Incorporating sub-cellular cell-fate determinants such as Prospero and Deadpan would let the model assess differentiation hypotheses.

3.4 Conclusion

We used a calibrated CPM-based agent-based model to assess the contributions of separable division geometry components and growth regulation mechanisms to NB lineage architecture in wild-type and *mud* mutant *Drosophila*. Several findings emerge from this work.

First, the peripheral NB phenotype is primarily shaped by the intergenerational

stability of the apical/basal axis and by the consistency of fate-position coupling, while the range of spindle rotations around that axis and the offset of the cleavage plane from the apical cell surface are comparatively minor contributors. This points to cortical polarity establishment as the geometric feature most critical for normal lineage architecture, a prediction that could be tested in polarity-establishment mutants such as *bazooka* or *Inscuteable*.

Second, a regulator that responds to NB volume combined with a division threshold that propagates birth size forward across generations is sufficient to constrain *mud* mutant lineage overgrowth. Neither mechanism alone produces this constraint. This is consistent with a sizer-like framework in which birth-size memory and volume-sensitive growth together keep descendant lineages of symmetric divisions compact — a result the model achieves without invoking any *mud*-specific regulatory circuitry beyond the symmetric division itself.

Third, NB clustering in *mud* lineages reflects a similar geometric logic as peripheral NB positioning in wild-type: the apical-axis inheritance rule that keeps daughter NBs adjacent is the same class of mechanism that keeps the wild-type NB at the lineage surface. The geometric constraints of the division machinery therefore shape both wild-type and mutant spatial phenotypes.

The model makes testable predictions: that polarity-machinery mutants should disrupt lineage sorting more severely than spindle-orientation mutants, and that NB-volume measurements in *mud* lineages should reveal a birth-size-dependent scaling of cell-cycle duration across symmetric-division generations.

3.5 Methods

3.5.1 Immunohistochemistry

Primary antibodies used in this study were rat anti-dpn (1:100, Abcam 11D1BC7), mouse anti-pros (1:500, gift from Chris Doe)

Secondary antibodies used were goat anti-rat (AlexaFluor 647, Invitrogen A21247), goat anti-mouse (AlexaFluor 405, Invitrogen A31553), goat anti-rabbit (AlexaFluor 405,

Invitrogen A31556), goat anti-rabbit (AlexaFluor 647, Invitrogen A21245). All secondary antibodies were used at 1:1000. All experiments were conducted with at least 1 overnight in primary and 1 overnight in secondary antibody.

Larvae were dissected by removing the posterior half and inverting, then removing organs, and severing the connections between the back end of the brain and the mouth parts, to minimize stretching of the brain during fixation. Dissected larvae were put in schneiders insect media until fixation. Fixation was performed in 4% PFA in schneiders medium on a nutator for 20 minutes. PFA was then washed off with PBSBT (PBS, 1% BSA, 0.1% Triton-X) 3 times for 20 minutes on a nutator at room temperature. Antibodies were diluted in PBSBT and samples were incubated on a nutator at 4C. After primary staining, samples were washed with PBSBT 3 times for 20 minutes on a nutator at room temperature. Secondary antibody, also diluted in PBSBT, was then applied and incubated on a nutator at 4C. After secondary staining, samples were again washed 3 times for 20 minutes on a nutator at room temperature. Brains were then dissected off of the cuticles and put into vectashield. Brains were mounted on glass slides in a small channel of vectashield between two spacers (glass coverslips), with a third coverslip on top. All samples were imaged immediately after mounting on slides.

3.5.2 Immunohistochemistry image segmentation and analysis

To quantify Dpn volume, lineage volume, Pros+ cell number and Dpn number/lineage, we used Imaris 9.6 (and higher) to segment Dpn+ and Pros+ nuclei. Similarly, lineages were segmented and manually corrected if necessary. For lineage volume quantification, axonal projections were manually cut to account for image depth variations in our datasets. Segmentation was done by training Imaris' pixel classifier or by using threshold segmentation.

3.5.3 Experimental lineage dataset and preprocessing

Experimental lineage measurements were derived from three-dimensional microscopy segmentations of *Drosophila* larval brain lobes. For each lobe, three VRML (.wrl) files

were available: lineage hull segmentations, Dpn-positive nuclei, and Pros-positive nuclei. The analyzed dataset included WT control lobes (lobe1, lobe2, lobe7, lobe8, lobe13, and lobe15), *mud* mutant lobes (lobe110, lobe113, lobe116, and lobe119), and nanobody perturbation lobes (lobe16, lobe17, lobe19, lobe20, lobe21, and lobe22).

Experimental preprocessing uses the pipeline implemented in <https://github.com/Jannetty/drosophila-neuroblast-CPM-analysis> scripts/preprocess_exp.py and src/npa/exp_preprocessing/. WRL files are parsed into triangle meshes by extracting vertex and face blocks from the VRML text and fan-triangulating polygonal faces. Pros-positive cells are assigned to lineage hulls by bounding-box overlap, with assignment requiring at least 95% of vertices to fall within a lineage bounding box. Dpn-positive cells are assigned by sampled mesh containment, using a bounding-box prefilter followed by containment testing of 20 sampled vertices and requiring at least 60% of sampled vertices to lie within a lineage hull.

Lineages are excluded before analysis if they fail either of two quality-control criteria. First, lineages are rejected as disconnected if they contain a secondary connected component with at least 50 faces and volume at least 5.0 in the native WRL coordinate system. This removes all lineages that are not fully connected, presumably due to imaging error. Second, lineages are rejected if no Dpn-positive cell can be assigned to the lineage. Rejected lineages are preserved in a separate index but are excluded from all summary analyses. Among WT lineages, 60 of 143 total segmented lineages were retained after filtering (2 lineages were rejected as disconnected and 81 rejected for lacking an assigned Dpn-positive cell). Among *mud* mutant lineages, 59 of 63 total segmented lineages were retained after filtering, and the retained lineages included between 1 and 5 Dpn-positive cells.

For each retained lineage, the mesh vertices are projected into two dimensions using the first two principal components of their 3D coordinates. The projected lineage outline is constructed as the union of the projected mesh triangles, buffered by $0.5 \times ds$ with $ds = 0.3 \mu\text{m}$; this yields a possibly non-convex polygon that follows the surface of the segmented lineage. Each projected lineage is rasterized onto a (200×200) canvas at $(ds = 0.3 \mu\text{m}, \text{px}^{-1})$, matching the voxel resolution of the simulation data so that experimental

and simulated tensors share a common physical scale. Each Dpn and Pros centroid is assigned a circle radius equal to that of a sphere with the same 3D segmented volume, and pixels inside the lineage outline are partitioned by an additively weighted Voronoi tessellation: pixels inside any Dpn circle are assigned to the nearest Dpn centroid, pixels inside only Pros circles are assigned to the nearest Pros centroid, and remaining pixels are assigned by minimizing the additively weighted distance $\|p - c_i\| - r_i$ across all cells. This yields a two-channel tensor in which channel 0 represents Dpn territory and channel 1 represents Pros territory. Per-lineage mesh files and stacked genotype-level analysis tensors are written to `data/exp/processed/`.

Experimental endpoint summaries are then computed from the processed lineage dataset. For each lineage we recorded the number of Dpn-positive cells (`n_dpn`), the number of Pros-positive cells (`n_pros`), lineage area in occupied pixels (`lin_area_vox`), total Dpn area (`dpn_area_vox`), and mean Dpn area per lineage (`avg_dpn_area_vox`). Genotype-level means and standard deviations are written to `data/exp/processed/exp_summary.csv` and used as the experimental reference for model calibration and comparison.

3.5.4 Cellular Potts model of WT and *mud* mutant lineages

All code for this model is publicly available at this url: [ARCADE-URL](#). All code for the analysis run in this manuscript is available at this url: [REPO-URL](#).

We model neuroblast lineage development with a two-dimensional spatiotemporal Cellular Potts model implemented in the ABM framework ARCADE. Although the simulations are visualized and analyzed in two dimensions, ARCADE uses a three-dimensional volume formalism, so growth and division thresholds are specified in μm^3 and represented on a lattice of height one voxel. Simulations run for 2161 ticks with $dt = 0.01667$ h per tick, corresponding to 36 h of total biological time with 1 minute per tick. The default lattice size is $200 \times 200 \times 1$ voxels, with $ds = 0.3 \mu\text{m}$ per voxel edge. 50 simulations were run per condition in this paper with random seeds 1-50.

3.5.5 Cellular Potts Hamiltonian

In a Cellular Potts model, each lattice site i is assigned a cell identity σ_i , and each identity belongs to a cell type $\tau(\sigma_i)$. Cell morphology and movement emerge from stochastic updates that consider whether a given lattice site should take its neighbor's identity. Whether a proposed copy is accepted depends on the resulting change in the Hamiltonian energy.

The full Hamiltonian can be written generically as

$$H = H_{\text{adhesion}} + H_{\text{volume}} + H_{\text{surface}}.$$

The adhesion term is

$$H_{\text{adhesion}} = \sum_{\langle i,j \rangle} J(\tau(\sigma_i), \tau(\sigma_j)) (1 - \delta_{\sigma_i, \sigma_j}),$$

where $\langle i, j \rangle$ indexes neighboring lattice sites, σ is the occupying cell identity or extracellular medium, τ is the corresponding cell type, J is the contact-energy coefficient, and $\delta_{\sigma_i, \sigma_j}$ is the Kronecker delta. This term contributes only at interfaces between distinct cells or between a cell and extracellular space.

The volume constraint term is

$$H_{\text{volume}} = \sum_{\sigma} \lambda_v (v(\sigma) - V(\sigma))^2,$$

where $v(\sigma)$ is the current cell volume, $V(\sigma)$ is the target cell volume, and λ_v sets the strength of the volume penalty. A cell's initial target volume is set to its critical volume and is updated each tick by adding volume according to its growth rate. The surface constraint term is

$$H_{\text{surface}} = \sum_{\sigma} \lambda_s (s(\sigma) - S(\sigma))^2,$$

where $s(\sigma)$ is the current cell perimeter, $S(\sigma)$ is the perimeter of a voxelized circle that is equal to the current cell's target volume, and λ_s sets the strength of the surface penalty.

Proposed lattice updates are accepted according to the standard Metropolis rule

$$P(\text{accept}) = \begin{cases} 1, & \Delta H < 0, \\ e^{-\Delta H/T}, & \Delta H \geq 0, \end{cases}$$

where ΔH is the change in Hamiltonian associated with the proposed copy event and T is the simulation temperature.

In all simulations analyzed here, the contact-energy coefficient for cell–extracellular-space interfaces is 80, whereas the default cell–cell coefficient is 50. Because lower contact-energy values correspond to stronger effective adhesion in this formalism, this choice ensured cohesion within each lineage, and prevented cells from dispersing into extracellular space. For mutant simulations that included preferential NB adhesion, the NB–NB contact-energy coefficient was reduced further to 40. Surface lambda values are 0.5 for neuroblasts, 1 for GMCs, and 2 for neurons. Because the contribution of the surface term increases with cell size, these values are chosen so that cells of different types nevertheless maintain comparably smooth and rounded morphologies. The Boltzmann temperature is visually calibrated to 18 so that WT neuroblasts can relax toward rounder shapes between successive asymmetric divisions. Apoptosis is not included.

3.5.6 *WT growth and division rules*

In the WT rule set, the neuroblast grows until it reaches a division threshold equal to $1.075\times$ its initial volume and then divides asymmetrically. The default WT division plane is perpendicular to the apical axis and passes through a point located 93% of the cell length from the apical edge and 50% across the lateral axis. Default angular variation (spindle variation) around the default division plane is drawn from a normal distribution centered at 0° with standard deviation 26° . This was based on the evaluation of experimental data that tracked the angle of cell divisions of WT NBs after each successive division (supplemental figure). After WT division, the larger apical daughter remains a neuroblast and the smaller basal daughter becomes a GMC. The GMC grows until it reaches $1.4\times$ its initial volume and then divides symmetrically to produce two neurons. Neurons neither grow nor divide.

3.5.7 *mud* mutant growth and division rules

To model *mud* mutant lineages, division-plane rotation at each division is drawn from a normal distribution centered at 0° with standard deviation 50° , compared with 26° for WT. We impose a threshold at $\pm 75^\circ$ such that any sampled rotation exceeding 75° in magnitude is converted into a deterministic symmetric division along the neuroblast apical axis. In the mutant rule set used here, this yields approximately 13% symmetric divisions, consistent with experimental observation [26]. Mutant daughter neuroblasts inherit apical axes rotated relative to the parent axis by an amount drawn from a normal distribution with mean 0° and standard deviation 30° . This inheritance rule is chosen to approximate the qualitative loss of stable spindle orientation observed experimentally in *mud* mutant lineages.

3.5.7.1 Calibration of division-plane rotation parameters

The division-plane rotation standard deviation and symmetric-division threshold for *mud* mutant simulations were calibrated from time-lapse measurements of NB division-axis angles tracked across successive divisions in *mud* mutant and WT animals (Supplemental Figure X; see also [26]). At each time point, the 3D angle between the current spindle-axis vector and the first division axis was computed. Successive differences of this cumulative angle were binned in (15°) increments spanning (0°) – (180°) (Fig. 3.S3). The *mud* mutant distribution contained no observed divisions in the 75° – 90° bin, whereas the WT distribution showed no analogous gap. A similar discontinuity in *mud* mutant division-axis rotation was reported by [26]. We therefore set the symmetric-division threshold at 75° : any rotation exceeding this value is classified as a symmetric division.

Under this threshold, 13% of observed *mud* mutant division steps exceeded 90° and were classified as symmetric divisions, consistent with the symmetric-division frequency reported by [26]. We calibrated the rotation standard deviation by finding the σ of a $N(0, \sigma^2)$ distribution satisfying $P(|X| > 75^\circ) \approx 13\%$, which yields $\sigma = 50^\circ$. As a cross-check, the empirical standard deviation of the observed step-to-step rotations computed after excluding values greater than 90° was 44° , which we considered sufficiently

consistent with the model value of 50° .

3.5.7.2 *Imposed and volume-sensitive division thresholds*

The division threshold for *mud* mutants is implemented in two different ways. Under the fixed-threshold ruleset ($\text{VCV}=0$), all neuroblasts of a given type share the same critical volume V_c , and division occurs when the cell reaches

$$V \geq \text{SIZE_TARGET} \times V_c.$$

In practice, this means *mud* mutant cells divide when they surpass the same absolute threshold, which is set to $1.075\times$ the expected WT neuroblast initial volume.

Under the birth-volume-dependent ruleset ($\text{VCV}=1$), daughter critical volume is reset from daughter birth volume. For daughter neuroblasts, the new critical volume is

$$V_{c,\text{daughter}} = \max(V_{\text{birth}}, 0.2V_{c,\text{WT}}),$$

where $V_{c,\text{WT}}$ is the baseline WT neuroblast critical volume. For GMC daughters, the analogous rule is

$$V_{c,\text{GMC}} = \max(V_{\text{birth}}, 0.1V_{\text{NB,birth}}).$$

Under this ruleset, the division threshold decreases as mutant daughter cells are born progressively smaller after successive symmetric divisions, but it cannot fall below a fixed minimum of 20% of the WT neuroblast critical volume (or 10% the initial GMC critical volume for the GMCs). Biologically, this corresponds to the assumption that a cell can only divide if it remains above a minimum size. Thus, $\text{VCV}=0$ tests a fixed-threshold size hypothesis, whereas $\text{VCV}=1$ allows daughter birth size to propagate into the next division threshold.

3.5.7.3 *NB growth regulatory mechanisms*

To test candidate mechanisms constraining mutant lineage growth, we vary whether neuroblast growth rate is regulated by cell volume or by NB–NB contact. Each candidate repression mechanism can operate either in a cell-autonomous ABM-like mode or in a population-averaged PDE-like mode.

3.5.7.4 Volume-regulated growth

When volume-sensitive growth is enabled, the effective growth rate is scaled as a power law of cell volume relative to a reference volume. In the ABM-like implementation, each cell updates its own growth rate according to

$$r_i = r_0 \left(\frac{V_i}{V_{\text{ref}}} \right)^s,$$

where r_0 is the baseline growth rate, V_i is the current cell volume, V_{ref} is the expected equilibrium volume for that cell type, and s is the volume-sensitivity exponent. In all volume-regulated simulations analyzed here, $s = 3$. For neuroblasts, the reference volume is computed from the midpoint of the WT-like asymmetric division cycle,

$$V_{\text{ref,NB}} = \frac{V_{\text{div}}(1 + f_{\text{retain}})}{2},$$

where $V_{\text{div}} = \text{SIZE_TARGET} \times V_{c,\text{WT}}$ and f_{retain} is the fraction of pre-division volume retained by the NB after division. For the wild-type condition ($f_{\text{retain}} = 0.93$), $V_{\text{ref,NB}} \approx 41.6 \mu\text{m}^3$. For GMCs, the reference volume is

$$V_{\text{ref,GMC}} = \frac{V_c + \text{SIZE_TARGET} \times V_c}{2} = V_c \frac{1 + \text{SIZE_TARGET}}{2}.$$

In the PDE-like implementation, all cells of a given type share a common growth rate based on the population-average volume rather than their own individual volume. This is implemented by setting `PDELIKE=1`; the ABM-like implementation uses `PDELIKE=0`. For neuroblasts this becomes

$$r_{\text{NB}} = r_0 \left(\frac{\bar{V}_{\text{NB}}}{V_{\text{ref,NB}}} \right)^s,$$

where $\bar{V}_{\text{NB}}$ is the mean neuroblast volume across the simulation. For GMCs the analogous update is

$$r_{\text{GMC}} = r_0 \left(\frac{\bar{V}_{\text{GMC}}}{V_{\text{ref,GMC}}} \right)^s,$$

with $\bar{V}_{\text{ref,GMC}} = \bar{V}_c(1 + \text{SIZE_TARGET})/2$. Biologically, this PDE-like formulation can be interpreted as a population-wide signal that causes all cells of a given type to respond to the mean state of that population.

3.5.7.5 NB-contact-regulated growth

When NB self-repression is enabled, growth rate is instead downregulated by a Hill-type function of neuroblast contact. In the ABM-like implementation, each neuroblast responds to its own number of unique NB neighbors:

$$r_i = r_0 \frac{K^n}{K^n + N_i^n},$$

where N_i is the number of NB neighbors for cell i , K is the half-maximal neighbor count, and n is the Hill exponent. In all NB-contact-regulated simulations analyzed here, $K = 2$ neighbors and $n = 3$. This gives maximal growth in the absence of neighboring neuroblasts and progressively stronger repression as local NB crowding increases.

In the PDE-like implementation, all neuroblasts share a common growth rate determined by a population-level NB signal. As above, this is implemented by setting PDELIKE=1; the ABM-like NB-contact implementation uses PDELIKE=0. In the implementation used here, that signal is the number of neuroblasts in the simulation rather than each cell's local neighbor count, yielding

$$r_{\text{NB}} = r_0 \frac{K^n}{K^n + N_{\text{pop}}^n},$$

where N_{pop} is the population-level NB signal. This PDE-like variant therefore acts as a global repression regime in which all neuroblasts slow together as the mutant NB population expands.

3.5.8 Model calibration and simulation subsets

The WT baseline is calibrated against experimental endpoint summaries. Calibration targets are lineage area, number of Pros-positive cells, number of Dpn-positive cells, total Dpn area, and mean Dpn area per lineage at the terminal time point. These quantities are compared to the averages calculated from the WT experimental data. Additional calibration constraints require WT neuroblast cycle times below 90 min and GMC cycle times of at least 8 h, both also experimentally observed metrics. When calibrating the apical axis offset, the size target for the neuroblast was set such that, assuming the

neuroblast was a circle, the asymmetric division would yield a cell that was the same area as the initial NB.

Offset-perturbed WT simulations require partial recalibration because shifting the division plane toward a more symmetric split changes daughter birth sizes and therefore alters lineage-scale outcomes even when the cell identity rules are unchanged. For these offset-perturbed conditions, NB and GMC growth rates are recalibrated to maintain approximately comparable terminal cell counts across conditions, rather than to preserve the WT volume-based metrics or cell-cycle-duration targets. This choice is made so that differences in lineage mixing can be interpreted more fairly, since the opportunity for mixing depends in part on how many cells a lineage contains.

3.5.8.1 *Coupling between division offset and daughter-size asymmetry*

A structural feature of the offset perturbation is that the spatial position of the division plane and the resulting daughter-size asymmetry are not independent within the model. At each NB division, the apical daughter inherits approximately a fraction $Y/100$ of the parental volume and the GMC inherits $(100 - Y)/100$, where Y is the offset parameter (% of apical-axis length from the apical end). In principle, the NB size target S can be set so that the apical daughter returns to its resting volume V_c after each division ($S = 100/Y$); we impose a practical cap $S \leq 2$, however, which is binding for all offsets $Y \leq 50\%$ (see Section 3.5.8.2 for the calibration scheme used at each offset). The apical NB is therefore born at volume $V_c \cdot Y/100 \cdot S$ — equal to V_c for $Y > 50\%$ but less than V_c for $Y \leq 50\%$ — and divides at volume SV_c . The basal daughter (GMC) is born at $(1 - Y/100) \cdot SV_c$ and, with a size target of 1.4, grows to $1.4(1 - Y/100) \cdot SV_c$ before dividing symmetrically into two neurons each of volume $0.7(1 - Y/100) \cdot SV_c$. Averaging the NB volume over its cycle gives a steady-state NB-to-neuron volume ratio of

$$\frac{\bar{V}_{\text{NB}}}{V_{\text{neuron}}} = \frac{Y + 100}{1.4(100 - Y)},$$

which evaluates to approximately $19.7\times$ at the calibrated WT offset of $Y = 93\%$, $5.8\times$ at $Y = 78\%$, $3.3\times$ at $Y = 64\%$, $2.1\times$ at $Y = 50\%$, $1.1\times$ at $Y = 22\%$, and $0.8\times$ at $Y = 7\%$. Because this ratio is independent of V_c , the coupling between offset and daughter-size

asymmetry cannot be removed by tuning growth rates or critical volumes within the existing model framework while keeping the offset as the only degree of freedom: a more central division plane inherently produces more similarly sized daughters.

3.5.8.2 Calibration of offset perturbation parameters

At each tested offset Y , we recalibrated the NB growth rate to maintain an approximately constant NB cell-cycle duration ($\tau_{\text{NB}} \approx 1.1$ h), and scaled the GMC growth rate proportionally (with GMC size target $S_{\text{GMC}} = 1.4$) to maintain a constant GMC cell-cycle duration ($\tau_{\text{GMC}} \approx 8.4$ h) across all conditions. This choice keeps the number of cells generated over the simulation period comparable across conditions, so that differences in spatial readouts can be interpreted independently of differences in lineage scale.

The NB critical volume V_c and size target S at each offset are not independently calibrated; they are set by the geometric constraints of the asymmetric-division rule described above (Section 3.5.8.1). The ideal choice $S = 100/Y$ would ensure the apical daughter returns to V_c after each division, but we impose a cap of $S \leq 2$ for biological plausibility (a cell that more than doubles between divisions is unrealistic). At offsets $Y > 50\%$, $S = 100/Y$ and V_c is chosen so that the mean NB area over the cell cycle is close to the calibrated WT NB area. At offsets $Y \leq 50\%$, the cap is binding: both S and V_c are held at their $Y = 50\%$ values ($S = 2$ and $V_c = 21.57 \mu\text{m}^3$). A consequence of the cap is that for $Y < 50\%$, the apical NB is born at less than its critical volume ($V_{\text{birth}} = V_c \cdot Y/100 \cdot S$ — approximately $9.5 \mu\text{m}^3$ at $Y = 22\%$ and $3 \mu\text{m}^3$ at $Y = 7\%$), and the cell cycle begins with a rapid expansion toward V_c before the slower growth-rate-driven approach to the division threshold.

The NB growth rate at each offset is then chosen to satisfy the cycle-duration target:

$$\tau_{\text{NB}} = \frac{(1 - Y/100) \cdot S \cdot V_c}{r_{\text{NB}}} \approx 1.1 \text{ h},$$

where $(1 - Y/100) \cdot S \cdot V_c$ is the total volume the NB must add between birth and the division threshold. The GMC critical volume follows from the same geometric constraint, $V_{c,\text{GMC}} = (1 - Y/100) \cdot S \cdot V_c$, since the basal daughter inherits a fraction $(1 - Y/100)$ of the parent's division volume. Calibrated parameter values for all tested offsets are listed in

Table 3.2.

3.5.8.3 Daughter identity-assignment perturbations

To test the contribution of consistent NB-identity assignment to the peripheral NB phenotype, we implemented three differentiation rulesets that vary which daughter inherits NB identity at each division. In the calibrated WT (*basal GMC*) ruleset, the basal daughter becomes a GMC and the apical daughter remains a NB. In the inverted (*apical GMC*) ruleset, the apical daughter becomes a GMC and the basal daughter remains a NB. In the *random GMC* ruleset, GMC identity is assigned to one of the two daughters at random at each division, with equal probability for the apical and basal daughter.

We ran all three rulesets at a 50% division-plane offset rather than at the WT 93% offset. At the WT offset, the basal daughter is born at only $\sim 7\%$ of the parent NB volume, so under the inverted or random rulesets this small daughter would inherit NB identity in some or all divisions. Because the model imposes a minimum NB critical volume of 20% of the calibrated WT NB critical volume (Section 3.5.7.2), such a basal-derived NB would inherit a critical volume well above its actual birth volume. The Cellular Potts volume-constraint term would then drive the cell to rapidly expand toward this critical volume, independent of the cell-cycle growth-rate parameter (which controls only the slower volume increase from the critical volume to the division threshold, Section 3.5.6). This rapid expansion would aggressively displace neighboring cells, confounding the identity perturbation with size-asymmetric cell-cell interactions. At a 50% offset, both daughters are born at comparable volumes, so identity randomization or inversion does not trigger this rapid-expansion regime. All other model parameters were held at their WT-baseline values for these perturbations.

3.5.9 Quantitative measures for simulation–experiment comparison

All quantitative analyses are performed on terminal snapshots. For both experimental and simulated lineages, occupied pixel area is converted to projected area in μm^2 by multiplying by $ds^2 = 0.09 \mu\text{m}^2$ per pixel whenever areas are reported in physical units.

3.5.9.1 Endpoint composition and scale metrics

The primary non-spatial comparison metrics are: `lin_area_vox`, the total occupied lineage area in pixels; `n_pros`, the number of Pros-positive progeny; `n_dpn`, the number of Dpn-positive neuroblasts; `dpn_area_vox`, the total Dpn area in the lineage; and `avg_dpn_area_vox`, the mean Dpn area per lineage. For experimental lineages, Dpn and Pros areas are computed from the Voronoi territories of the processed lineage tensors. For simulations, these metrics are computed directly from raw `CELLS.json` and `LOCATIONS.json` voxel lists at the last timepoint. Simulation outputs are summarized either as per-run endpoint values or as condition-level means and standard deviations, depending on the figure.

3.5.9.2 Parameters

Core parameters for the calibrated WT baseline, the offset-perturbed WT simulations, and the mutant growth-regulation simulations are summarized in Tables 3.1, 3.2, and 3.3. The full parameterization logic, calibration sweeps, and offset-specific recalibration details are documented in `ARCADE/PARAMETERS_EXPLAINED.md` and `ARCADE/PARAMETERS_y50_EXPLAINED.md`.

3.5.9.3 Normalised heterotypic contact fraction

To quantify lineage mixing, we compute a normalised heterotypic contact fraction from each simulated lineage snapshot. This choice is motivated by the Cellular Potts formalism itself, in which heterotypic interfaces are directly related to the contact-energy term in the Hamiltonian and mismatched cell contacts are a standard readout of cell sorting behavior (DOI: 10.1103/PhysRevE.47.2128; DOI: 10.1371/journal.pcbi.1008576). Pixels are classified as neuroblast (NB), non-NB progeny, or empty. All horizontally and vertically adjacent occupied pixel pairs are then enumerated using 4-connectivity. A contact is considered heterotypic if one pixel is NB and the other is non-NB, and homotypic otherwise. The raw heterotypic contact fraction is defined as

$$\text{het_frac} = \frac{\text{heterotypic contacts}}{\text{heterotypic contacts} + \text{homotypic contacts}}.$$

Table 3.1: Core WT model parameters used in the calibrated baseline simulations.

Parameter	Value	Units	Role
<code>ds</code>	0.3	$\mu\text{m}/\text{voxel}$	Spatial scale of the lattice
<code>dt</code>	0.01667	h/tick	Temporal scale (≈ 1 min/tick)
<code>ticks</code>	2161	ticks	Total simulated duration (≈ 36 h)
<code>length, width, height</code>	600, 600, 1	voxels	Two-dimensional simulation domain
<code>NB CRITICAL_VOLUME</code>	31.2	μm^3	Neuroblast initial volume
<code>NB SIZE_TARGET</code>	1.075	fold	Neuroblast divides at $1.075\times$ initial size
<code>GMC SIZE_TARGET</code>	1.4	fold	GMC divides at $1.4\times$ initial size
<code>NB CELL_GROWTH_RATE</code>	1.80	$\mu\text{m}^3/\text{h}$	Sets WT neuroblast cycle length
<code>GMC CELL_GROWTH_RATE</code>	0.155	$\mu\text{m}^3/\text{h}$	Sets GMC cycle length
<code>Neuron CELL_GROWTH_RATE</code>	0	$\mu\text{m}^3/\text{h}$	Neurons do not grow
<code>WT division offset</code>	93	% of apical axis length	Asymmetric NB/GMC split position
<code>WT division-angle variation</code>	$N(0, 26)$	degrees	Stochastic variation around the default division axis
<code>ADHESION</code>	80	arbitrary units	Generic cell-cell adhesion
<code>TEMPERATURE</code>	18	arbitrary units	Potts acceptance temperature

Table 3.2: Parameters across the offset perturbation conditions. NB `SIZE_TARGET` and `CRITICAL_VOLUME` are set by the geometric constraints of the asymmetric-division rule (Section 3.5.8.1), subject to a $S \leq 2$ cap that is binding for offsets $\leq 50\%$. NB and GMC growth rates are calibrated at each offset to maintain an approximately constant cell-cycle duration across conditions (Section 3.5.8.2). GMC `CRITICAL_VOLUME` follows from $V_{c,GMC} = (1 - Y/100) \cdot S \cdot V_c$. Neuron `CRITICAL_VOLUME` equals $0.7 \times$ the GMC critical volume in each condition.

Y (%)	NB SIZE_TARGET	GMC SIZE_TARGET	NB cv (μm^3)	NB r ($\mu\text{m}^3/\text{h}$)	GMC cv (μm^3)	GMC r ($\mu\text{m}^3/\text{h}$)
93 (WT)	1.075	1.4	31.20	1.80	2.35	0.155
50	2.000	1.4	21.57	16.59	21.57	1.03
22	2.000	1.4	21.57	25.89	33.65	1.60
7	2.000	1.4	21.57	30.86	40.12	1.91

Because this metric is computed from occupied pixel-pixel contacts, we normalize the raw heterotypic contact fraction by the value expected under random pixel-label mixing across occupied lineage territory, while preserving the observed NB and non-NB area fractions. If p_{NB} is the fraction of occupied pixels belonging to the neuroblast and p_{nonNB} is the fraction belonging to non-neuroblast cells, then the expected heterotypic fraction under this voxel-scale random-mixing null is $2p_{\text{NB}}p_{\text{nonNB}}$. The normalized metric is therefore

$$\text{norm_het_frac} = \frac{\text{het_frac}}{2p_{\text{NB}}p_{\text{nonNB}}}.$$

Values less than 1 indicate greater segregation than expected from voxel-scale random label shuffling, values near 1 indicate approximately random interfacial mixing, and values greater than 1 indicate excess intermixing.

3.5.9.4 Neuroblast exposure to extracellular space

To quantify how peripherally the neuroblast is positioned, we measure the fraction of the neuroblast perimeter exposed to extracellular space. Using the NB mask from each simulated lineage, we enumerate all horizontal and vertical NB boundary contacts. A

Table 3.3: Additional parameters used in mutant growth-regulation simulations.

Parameter	Value	Units	Role
VOLUME_BASED_ CRITICAL_VOLUME	0 or 1	—	Selects imposed (VCV=0) or birth-volume-dependent (VCV=1) division thresholds
PDELIKE	0 or 1	—	Selects ABM-like (0) or population-averaged PDE-like (1) regulation
DYNAMIC_GROWTH_RATE_ VOLUME	0 or 1	—	Enables or disables volume-regulated growth
GROWTH_RATE_VOLUME_ SENSITIVITY	3.0	—	Exponent s controlling the strength of volume-dependent growth repression
DYNAMIC_GROWTH_RATE_NB_ SELF_REPRESSION	0 or 1	—	Enables or disables NB-contact-regulated growth
NB_CONTACT_ HALF_MAX	2.0	neighbors	Half-maximal repression constant K for NB-contact regulation
NB_CONTACT_HILL_N	3.0	—	Hill exponent n controlling NB-contact repression steepness

boundary segment is counted as exposed if it separates NB from empty space and as internal contact if it separates NB from a non-NB cell. The neuroblast exposure metric is defined as

$$\text{exposed_frac} = \frac{\text{NB-empty boundary contacts}}{\text{NB-empty boundary contacts} + \text{NB-nonNB boundary contacts}}.$$

Higher values indicate a more peripheral neuroblast, whereas lower values indicate increasing neuroblast internalization within the lineage.

3.5.9.5 Representative lineage selection

For the representative WT lineages shown in Figure 1, simulated runs and experimental lineages are selected by lineage-area percentile. We identify examples closest to the 10th, 50th, and 90th percentiles of the WT lineage-area distributions. For the WT geometry perturbation figures, representative simulated runs are chosen from the corresponding condition-specific endpoint distributions, with manual overrides applied only when necessary to avoid a misleading representative example for a given spatial phenotype.

Supplementary Material

Metric	2D projection (μm^2)			3D mesh (μm^3)		
	WT mean	<i>mud</i> mean	FC	WT mean	<i>mud</i> mean	FC
NB count	1.0	1.8	1.80×	1.0	1.8	1.80×
Total NB area/vol.	107.8	74.7	0.69×	353.9	210.0	0.59×
Mean NB area/vol.	107.8	48.2	0.45×	353.9	131.5	0.37×
Lineage area/vol.	501.2	366.7	0.73×	4084.7	2434.6	0.60×
Pros cell count	44.9	36.9	0.82×	44.9	36.9	0.82×

Table 3.S1: Calibration metrics agree in direction across 2D and 3D representations. Group means and fold-change ($\text{FC} = \textit{mud} \text{ mean} / \text{WT mean}$) for five lineage metrics computed from the 2D max-projection Voronoi rasterization and from the original 3D mesh segmentations. All five metrics change in the same direction between representations, and the relative ranking of fold-change magnitudes is preserved: NB area metrics show the largest reduction, followed by lineage area and Pros count while NB count is elevated. NB count and Pros count are identical between 2D and 3D representations because cell counts do not depend on projection.

Metric	Fold-change (<i>mud</i> /WT)		<i>p</i> (Wilcoxon, 2D vs. 3D)	
	2D	3D	WT	<i>mud</i>
Total NB area/vol.	0.69×	0.59×	0.22	< 0.001
Mean NB area/vol.	0.45×	0.37×	0.22	< 0.001
Lineage area/vol.	0.73×	0.60×	0.84	< 0.001

Table 3.S2: 3D mesh reports a modestly stronger *mud* mutant phenotype than 2D projection. Representations are indistinguishable within WT. Paired Wilcoxon signed-rank tests compare per-lineage 2D and 3D fold-change distributions within each genotype (each lineage contributes one value per representation; fold-changes are normalized by the WT mean of the respective representation). Within WT, no significant difference is detected between representations for any metric, indicating that 2D projection captures the same within-genotype variability as the full 3D mesh. Within *mud* mutant lineages, all three area metrics differ significantly between representations ($p < 0.001$): 3D consistently reports a larger reduction from WT than 2D. Notably, direction and statistical significance of the *mud* phenotype are preserved in both representations for all metrics (Table 3.S1). Cell-count metrics are omitted from this table as they are identical in 2D and 3D.

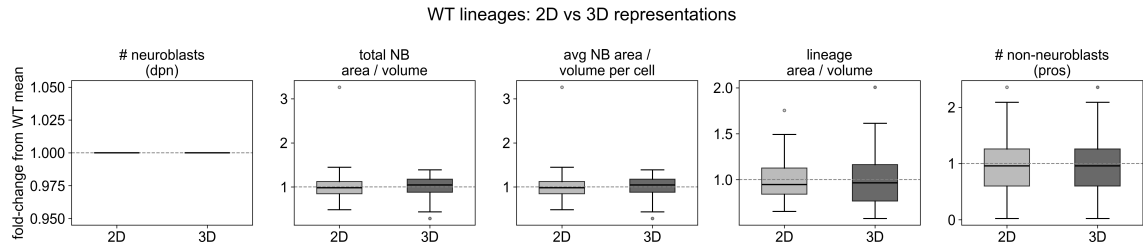


Figure 3.S1: Median and spread of WT lineage metrics are consistent across 2D and 3D representations. Box-and-whisker plots of per-lineage fold-change from the WT mean for five calibration metrics, comparing the 2D max-projection rasterization (light gray) and the 3D mesh segmentation (dark gray) across all retained WT lineages ($n = 60$). Boxes show the interquartile range; whiskers extend to $1.5 \times$ IQR; circles indicate outliers. The dashed line marks a fold-change of 1. Median and IQR are nearly identical between representations for all five metrics.

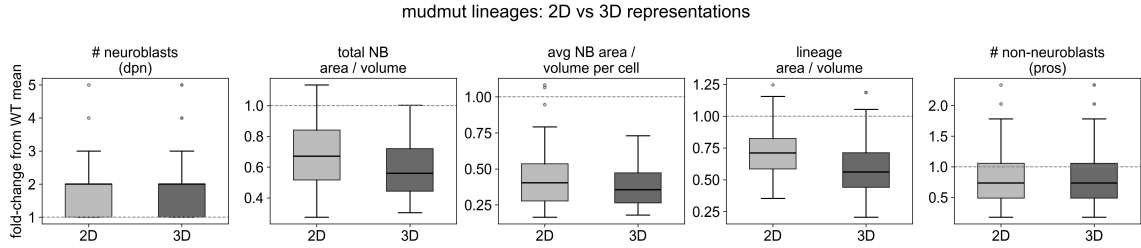


Figure 3.S2: Median fold-change of *mud* mutant metrics is preserved across 2D and 3D representations. Box-and-whisker plots of per-lineage fold-change from the WT mean for five calibration metrics in *mud* mutant lineages ($n = 59$), comparing the 2D max-projection rasterization (light gray) and the 3D mesh segmentation (dark gray). Boxes show the interquartile range; whiskers extend to $1.5 \times$ IQR; circles indicate outliers. The dashed line marks the WT reference. Both representations place the median below 1 for all area metrics and Pros count, and above 1 for NB count, confirming directional consistency. The 3D representation shows a modestly lower median for area metrics, reflecting the larger effect size captured by volumetric measurement relative to 2D projection.

Experimentally measured distributions of division-to-division rotation angles

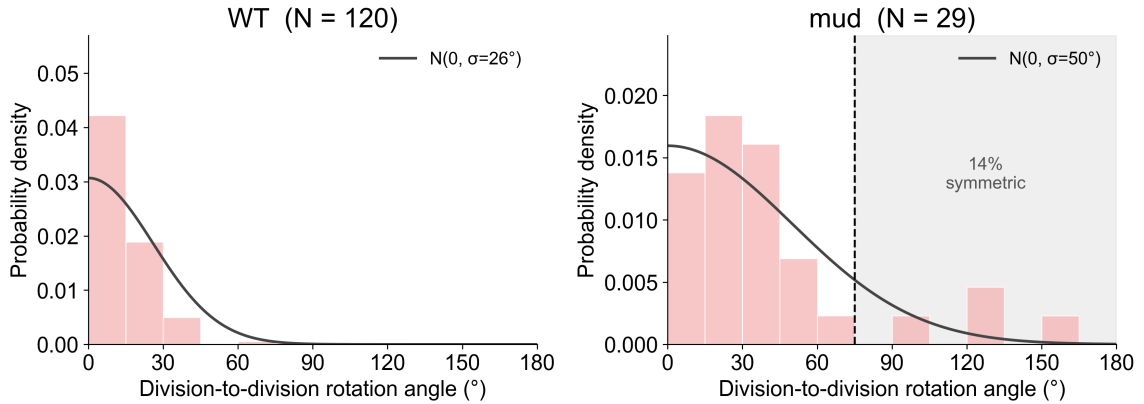


Figure 3.S3: Calibration of division-plane rotation parameters from experimental time-lapse measurements. Division-to-division absolute rotation angles for WT (*left*) and *mud* mutant (*right*) neuroblasts, binned in 15° increments (see Methods). Curves show folded-normal $N(0, \sigma^2)$ fits with $\sigma = 26^\circ$ (WT) and $\sigma = 50^\circ$ (*mud*). The dashed line marks the 75° symmetric-division threshold; the shaded region contains 14% of observed *mud* divisions, consistent with the 13% predicted by $N(0, 50^{\circ 2})$ and with the symmetric-division frequency reported by [26]. The 75° – 90° bin contains no observed *mud* divisions, motivating the symmetric-division threshold at 75° .

Endpoint metrics: mean $\pm$ SD across all simulation runs and experimental lineages

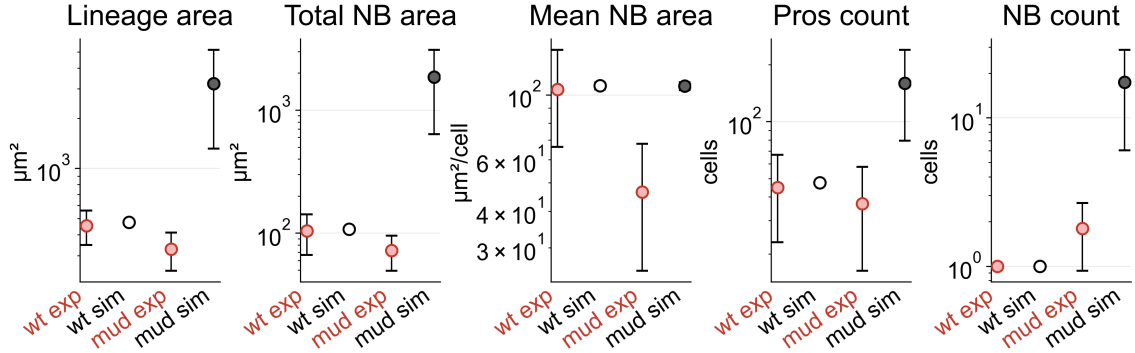


Figure 3.S4: **Endpoint metrics for experimental and simulated WT and *mud* mutant lineages.** Mean $\pm$ SD of five lineage endpoint metrics across experimental WT (wt exp), WT simulation (wt sim), experimental *mud* mutant (mud exp), and unregulated *mud* mutant simulation (mud sim). Metrics shown are total lineage area, total neuroblast (NB) area, mean per-NB area, Prospero-positive (Pros) cell count, and NB count. Colored markers indicate experimental groups. Y axis is log-scaled.

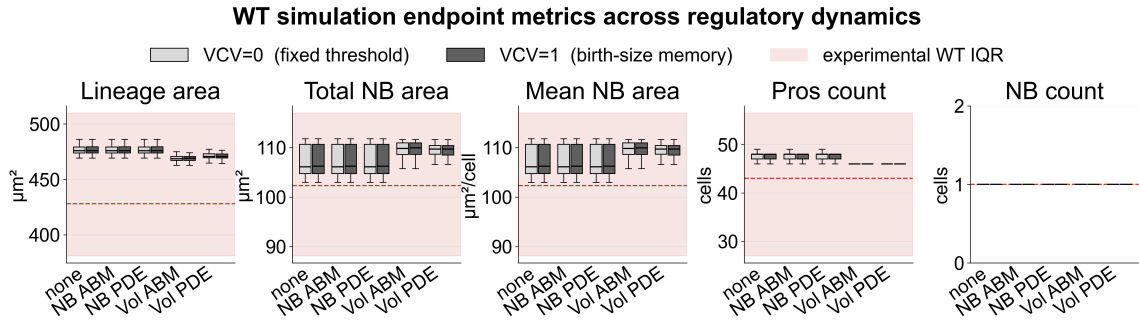


Figure 3.S5: **Regulated WT simulations remain in experimental WT IQR.** Distribution of calibration metrics shown for last time point of regulated WT simulations. Each bar represents 50 simulations.

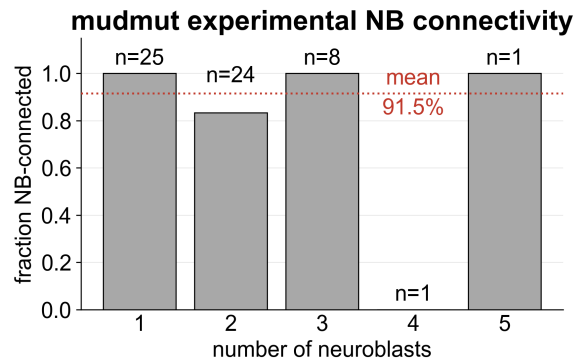


Figure 3.S6: **Rate of NB connectivity in *mud* mutant experimental lineages.** Bar chart showing the fraction of lineages in which all neuroblasts in a lineage are adjacent, grouped by the number of neuroblasts per lineage (n values shown on bars; 59 lineages total). The dotted red line indicates the overall mean fraction (91.5%). Neuroblasts remain adjacent in the large majority of *mud* mutant lineages regardless of lineage size.

CONCLUSION

A central challenge in developmental biology is understanding how local cellular interactions give rise to large-scale tissue and organ morphology. This challenge can be viewed through two complementary questions. The forward problem asks how a specified set of cellular rules gives rise to tissue-scale outcomes. The inverse problem asks the reverse: given an observed developmental outcome, what cellular interactions and regulatory mechanisms are capable of producing it? Throughout this thesis, I used computational models to address this inverse problem. By constructing mechanistic models, simulating their forward behavior, and comparing model predictions to experimental observations, I evaluated which mechanisms are sufficient to reproduce observed developmental phenotypes.

In Chapter 1, I reviewed recent spatiotemporal multiscale modeling studies across development, gynopathology, and senescence. Across these diverse systems, computational models provided a framework for systematically linking processes operating at different scales of biological organization. The reviewed studies demonstrate how computational models can be used to investigate how complex biological phenomena emerge from interactions occurring across scales.

In Chapters 2 and 3, I applied this philosophy to the developing *Drosophila* brain. In Chapter 2, we used ordinary differential equation models alongside experimental data to evaluate candidate mechanisms capable of constraining mutant neuroblast proliferation. These studies demonstrated that multiple regulatory mechanisms are sufficient to explain the observed limitation of lineage expansion, generating experimentally testable hypotheses regarding the regulation of neural stem cell growth and differentiation. In Chapter 3, a spatially explicit Cellular Potts model was used to investigate how division geometry and growth regulation influence lineage morphology. Together, these studies illustrate how computational models can be used to constrain inverse problems in developmental biology by identifying cellular-scale assumptions that are sufficient to generate observed tissue-level outcomes.

A recurring theme throughout this work is that useful biological insight does not

necessarily require increasingly complex models. Rather, computational models are most informative when they identify the minimal set of interactions required to explain an observed phenomenon. The models presented here were intentionally designed to balance biological realism with interpretability, allowing their assumptions to be directly connected to experimentally testable hypotheses. In this way, modeling serves not as an attempt to recreate biology, but as a tool for identifying which biological interactions are sufficient to generate a particular behavior.

The work presented here also raises several opportunities for future investigation. An immediate next step is the experimental evaluation of the candidate neuroblast regulatory mechanisms identified in Chapters 2 and 3. Within the modeling framework developed in Chapter 3, many additional questions regarding neuroblast growth, differentiation, and lineage organization can also be explored. For example, spatially localized cell-fate determinants could be incorporated explicitly, enabling investigation of how asymmetric inheritance influences daughter-cell identity. Similarly, the framework could be used to further examine how lineage geometry, cell-cell interactions, and exposure to the extracellular environment influence growth, division, and differentiation decisions. By combining biologically interpretable rules with an experimentally grounded spatial representation, this framework provides a foundation for investigating a broad range of questions in *Drosophila* brain development.

Looking further ahead, this work also motivates broader questions regarding how developing systems achieve robust morphological outcomes despite noisy environments and continual perturbation. Developmental systems frequently converge on reliable tissue architectures even when the trajectories leading to those outcomes vary substantially. Understanding how cellular populations sense, integrate, and respond to deviations from expected developmental trajectories remains an important open question in biology. Although the models presented here do not directly address this problem, they provide a framework for asking how local interactions can generate robust large-scale outcomes. Ultimately, understanding how tissues regulate their own development and recovery may help guide future efforts in regenerative medicine, tissue engineering, and developmental reprogramming.

BIBLIOGRAPHY

- [1] Merve Deniz Abdusselamoglu, Elif Eroglu, Thomas R Burkard, and Jürgen A Knoblich. The transcription factor odd-paired regulates temporal identity in transit-amplifying neural progenitors via an incoherent feed-forward loop. 8:e46566.
- [2] Roger Albertson, Chiswili Chabu, Amy Sheehan, and Chris Q. Doe. Scribble protein domain mapping reveals a multistep localization mechanism and domains necessary for establishing cortical polarity. 117:6061–6070.
- [3] Silvanus Alt, Poulami Ganguly, and Guillaume Salbreux. Vertex models: From cell mechanics to tissue morphogenesis. 372(1720):20150520.
- [4] Gary An. Specialty Grand Challenge: What it Will Take to Cross the Valley of Death: Translational Systems Biology, “True” Precision Medicine, Medical Digital Twins, Artificial Intelligence and In Silico Clinical Trials. 2:901159.
- [5] Sawsan As-Sanie, Scott C. Mackenzie, Leigh Morrison, Andrew Schrepf, Krina T. Zondervan, Andrew W. Horne, and Stacey A. Missmer. Endometriosis: A Review. 334(1):64.
- [6] S. I. Ashraf and Y. T. Ip. The Snail protein family regulates neuroblast expression of inscuteable and string, genes involved in asymmetry and cell division in *Drosophila*. 128(23):4757–4767.
- [7] Shovon I. Ashraf, Atish Ganguly, John Roote, and Y. Tony Ip. Worniu, a Snail family zinc-finger protein, is required for brain development in *Drosophila*. 231(2):379–386.
- [8] John A Bachman, Benjamin M Gyori, and Peter K Sorger. Automated assembly of molecular mechanisms at scale from text mining and curated databases. 19(5):e11325.
- [9] Julio R. Banga and Alejandro F. Villaverde. Mechanistic dynamic modelling of biological systems: The road ahead. 42:100553.
- [10] Rehan Baqri, Rakshita Charan, Kristina Schimmelpfeng, Swati Chavan, and Krishanu Ray. Kinesin-2 differentially regulates the anterograde axonal transports of acetylcholinesterase and choline acetyltransferase in *Drosophila*. 66(4):378–392.

- [11] Claudia S. Barros, Chris B. Phelps, and Andrea H. Brand. Drosophila nonmuscle myosin II promotes the asymmetric segregation of cell fate determinants by cortical exclusion rather than active transport. 5(6):829–840.
- [12] Maxime Bassenne and Adrián Lozano-Durán. Computational model discovery with reinforcement learning.
- [13] Bruno C. Bello, Natalya Izergina, Emmanuel Caussinus, and Heinrich Reichert. Amplification of neural stem cell proliferation by intermediate progenitor cells in Drosophila brain development. 3:5.
- [14] Christian Berger, Heike Harzer, Thomas R. Burkard, Jonas Steinmann, prefix=van der useprefix=true family=Horst, given=Suzanne, Anne-Sophie Laurenson, Maria Novatchkova, Heinrich Reichert, and Juergen A. Knoblich. FACS purification and transcriptome analysis of drosophila neural stem cells reveals a role for Klumpfuss in self-renewal. 2(2):407–418.
- [15] Christian Berger, S. K. Pallavi, Mohit Prasad, L. S. Shashidhara, and Gerhard M. Technau. A critical role for cyclin E in cell fate determination in the central nervous system of Drosophila melanogaster. 7(1):56–62.
- [16] Francisco Berkemeier and Karen M. Page. Coupling dynamics of 2D Notch-Delta signalling. 360:109012.
- [17] Job H. Berkhout, James A. Glazier, Aldert H. Piersma, Julio M. Belmonte, Juliette Legler, Richard M. Spencer, Thomas B. Knudsen, and Harm J. Heusinkveld. A computational dynamic systems model for in silico prediction of neural tube closure defects. 8:100210.
- [18] E. Bier, H. Vaessin, S. Younger-Shepherd, L. Y. Jan, and Y. N. Jan. Deadpan, an essential pan-neural gene in Drosophila, encodes a helix-loop-helix protein similar to the hairy gene product. 6(11):2137–2151.
- [19] Sandra Boldu-Fernández, Carolina Lliberos, Carlos Simon, and Aymara Mas. Mapping Human Uterine Disorders Through Single-Cell Transcriptomics. *Cells*, 14(3):156, January 2025.
- [20] Sema Bolkent. Cellular and molecular mechanisms of asymmetric stem cell division in tissue homeostasis. 29(12):1099–1110.
- [21] Jason Q. Boone and Chris Q. Doe. Identification of Drosophila type II neuroblast lineages containing transit amplifying ganglion mother cells. 68(9):1185–1195.
- [22] Theodor Boveri and Theodor Boveri. *Die Entwicklung von Ascaris Megalocephala Mit Besonderer Rücksicht Auf Die Kernverhältnisse*. G. Fischer.

- [23] Sarah K. Bowman, Ralph A. Neumüller, Maria Novatchkova, Quansheng Du, and Juergen A. Knoblich. The *Drosophila* NuMA Homolog Mud regulates spindle orientation in asymmetric cell division. 10(6):731–742.
- [24] Sarah K. Bowman, Vivien Rolland, Joerg Betschinger, Kaolin A. Kinsey, Gregory Emery, and Juergen A. Knoblich. The Tumor Suppressors Brat and Numb Regulate Transit-Amplifying Neuroblast Lineages in *Drosophila*. 14(4):535–546.
- [25] Clarisse Brunet Avalos, G Larisa Maier, Rémy Bruggmann, and Simon G Sprecher. Single cell transcriptome atlas of the *Drosophila* larval brain. 8:e50354.
- [26] Clemens Cabernard and Chris Q. Doe. Apical/basal spindle orientation is required for neuroblast homeostasis and neuronal differentiation in *Drosophila*. 17(1):134–141.
- [27] Clemens Cabernard and Chris Q. Doe. Live imaging of neuroblast lineages within intact larval brains in *Drosophila*. 2013(10):970–977.
- [28] Clemens Cabernard, Kenneth E. Prehoda, and Chris Q. Doe. A spindle-independent cleavage furrow positioning pathway. 467(7311):91–94.
- [29] M. C. Caldwell and S. Datta. Expression of cyclin E or DP/E2F rescues the G1 arrest of *trol* mutant neuroblasts in the *Drosophila* larval central nervous system. 79(1–2):121–130.
- [30] P Capris, G Spinelli, and M Zingirian. Comparing continuous and stepwise luminance variation in static campimetry using the Grignolo-Tagliasco-Zingirian projection campimeter. 8(1):55–58.
- [31] Giuseppe Caruso, S. John Weroha, and William Cliby. Ovarian Cancer: A Review. 334(14):1278.
- [32] Giulia Cazzagon, Chantal Roubinet, and Buzz Baum. Polarized SCAR and the Arp2/3 complex regulate apical cortical remodeling in asymmetrically dividing neuroblasts. 26(7):107129.
- [33] Clarence E. Chan and David J. Odde. Traction Dynamics of Filopodia on Compliant Substrates. *Science*, 322(5908):1687–1691, December 2008.
- [34] Sharmilla Chandrasegaran, James P. Sluka, and Daryl Shanley. Modelling the spatiotemporal dynamics of senescent cells in wound healing, chronic wounds, and fibrosis. *PLOS Computational Biology*, 21(4):e1012298, April 2025.
- [35] James M. Chell and Andrea H. Brand. Nutrition-responsive glia control exit of neural stem cells from quiescence. 143(7):1161–1173.

- [36] Semil P. Choksi, Tony D. Southall, Torsten Bossing, Karin Edoff, prefix=de useprefix=true family=Wit, given=Elzo, Bettina E. Fischer, prefix=van useprefix=true family=Steensel, given=Bas, Gos Micklem, and Andrea H. Brand. Prospero Acts as a Binary Switch between Self-Renewal and Differentiation in *Drosophila* Neural Stem Cells. 11(6):775–789.
- [37] Edwin G. Conklin. Mosaic development in ascidian eggs. 2(2):145–223.
- [38] Marisa Connell, Clemens Cabernard, Derek Ricketson, Chris Q. Doe, and Kenneth E. Prehoda. Asymmetric cortical extension shifts cleavage furrow position in *Drosophila* neuroblasts. 22(22):4220–4226.
- [39] Marc Corrales, Benjamin T. Cocanougher, Andrea B. Kohn, Jason D. Wittenbach, Xi S. Long, Andrew Lemire, Albert Cardona, Robert H. Singer, Leonid L. Moroz, and Marta Zlatic. A single-cell transcriptomic atlas of complete insect nervous systems across multiple life stages. 17(1):8.
- [40] Anna Segú Cristina, David Briand, Aman Kukde, Emeline Perthame, Stéphane Rigaud, Jean-Yves Tinevez, Agata Banach-Latapy, Nick D. L. Owens, Léo Valon, Yohanns Bellaïche, and Pauline Spéder. Adhesion-Controlled Mechanics of the Glial Niche Regulate Neural Stem Cell Proliferative Potential.
- [41] Jill R. Crittenden, Efthimios M.C. Skoulakis, Kyung-An Han, Daniel Kalderon, and Ronald L. Davis. Tripartite Mushroom Body Architecture Revealed by Antigenic Markers. 5(1):38–51.
- [42] Alicia Daeden, Alexander Mietke, Emmanuel Derivery, Carole Seum, Frank Jülicher, and Marcos Gonzalez-Gaitan. Polarized branched Actin modulates cortical mechanics to produce unequal-size daughters during asymmetric division. 25(2):235–245.
- [43] Richard W. Daniels, Maria V. Gelfand, Catherine A. Collins, and Aaron DiAntonio. Visualizing glutamatergic cell bodies and synapses in *Drosophila* larval and adult CNS. 508(1):131–152.
- [44] Josephine T. Daub and Roeland M. H. Merks. Cell-Based Computational Modeling of Vascular Morphogenesis Using Tissue Simulation Toolkit. In Domenico Ribatti, editor, *Vascular Morphogenesis: Methods and Protocols*, pages 67–127. Springer.
- [45] Riccardo De Santis, Laurent Jutras-Dubé, Sophia Bourdrel, Eleni Rice, Francesco M. Piccolo, and Ali H. Brivanlou. Crosstalk between tissue mechanics and BMP4 signaling regulates symmetry breaking in human gastrula models. 32(11):1691–1704.e6.

- [46] David L. Deitcher, Atsushi Ueda, Bryan A. Stewart, Robert W. Burgess, Yoshi Kidokoro, and Thomas L. Schwarz. Distinct Requirements for Evoked and Spontaneous Release of Neurotransmitter Are Revealed by Mutations in the *Drosophila* Gene neuronal-synaptobrevin. 18(6):2028–2039.
- [47] Melissa K. Delgado and Clemens Cabernard. Mechanical regulation of cell size, fate, and behavior during asymmetric cell division. 67:9–16.
- [48] Melissa K. Delgado, J. Ian Hertzler, Sophia Jannetty, Mia Hoover, Chang Yin, Ilara Yilmaz, Danielle Vahdat, Adam von Barnau Sythoff, Jay Z. Parrish, Neda Bagheri, and Clemens Cabernard. Altering cell size asymmetry in *Drosophila* neural stem cells creates supernumerary stem cells with limited lineage expansion potential.
- [49] Noah Dillon, Ben Cocanougher, Chhavi Sood, Xin Yuan, Andrea B. Kohn, Leonid L. Moroz, Sarah E. Siegrist, Marta Zlatic, and Chris Q. Doe. Single cell RNA-seq analysis reveals temporally-regulated and quiescence-regulated gene expression in *Drosophila* larval neuroblasts. 17(1):7.
- [50] Julie C. Dixon, Christopher L. Frick, Chantelle L. Leveille, Philip Garrison, Peyton A. Lee, Saurabh S. Mogre, Benjamin Morris, Nivedita Nivedita, Ritvik Vasan, Jianxu Chen, Cameron L. Fraser, Clare R. Gamlin, Leigh K. Harris, Melissa C. Hendershott, Graham T. Johnson, Kyle N. Klein, Sandra A. Oluoch, Derek J. Thirstrup, M. Filip Sluzewski, Lyndsay Wilhelm, Ruian Yang, Daniel M. Toloudis, Matheus P. Viana, Julie A. Theriot, and Susanne M. Rafelski. Colony context and size-dependent compensation mechanisms give rise to variations in nuclear growth trajectories. 16(5).
- [51] C. Q. Doe, Q. Chu-LaGraff, D. M. Wright, and M. P. Scott. The prospero gene specifies cell fates in the *Drosophila* central nervous system. 65(3):451–464.
- [52] Nastassia Pouradier Duteil, Nicole T. Revaitis, Mathew G. Niepielko, Eric A. Klein, Nir Yakoby, and Benedetto Piccoli. A computational framework to study EGFR signaling distribution in egg chambers during dynamic interactions between soma and germline. 21(12):e1013802.
- [53] Tavishi Dutt, Jimpi Langthasa, Monica Umesh, Satyarthi Mishra, Siddharth Bothra, Kottipalli Vidhipriya, Annapurna Vadaparty, Prosenjit Sen, and Ramray Bhat. Rheological transition driven by matrix makes cancer spheroids resilient under confinement. 8(6).
- [54] prefix=de useprefix=false family=Jong, given=Martijn A., Esmée Adegeest, Noémie M. L. P. Bérenger-Currias, Maria Mircea, Roeland M. H. Merks, and Stefan Semrau. The shapes of elongating gastruloids are consistent with convergent extension driven by a combination of active cell crawling and differential adhesion. 20(2):e1011825.

- [55] Dmitry A. Fedosov, Bruce Caswell, and George Em Karniadakis. A Multiscale Red Blood Cell Model with Accurate Mechanics, Rheology, and Dynamics. 98(10):2215–2225.
- [56] Alexander G. Fletcher, Miriam Osterfield, Ruth E. Baker, and Stanislav Y. Shvartsman. Vertex Models of Epithelial Morphogenesis. 106(11):2291–2304.
- [57] Francesca Froldi, Milan Szuperak, Chen-Fang Weng, Wei Shi, Anthony T. Papenfuss, and Louise Y. Cheng. The transcription factor Nerfin-1 prevents reversion of neurons into neural stem cells. 29(2):129–143.
- [58] Antoine Fruleux, Lilan Hong, Adrienne H. K. Roeder, Chun-Biu Li, and Arezki Boudaoud. Growth couples temporal and spatial fluctuations of tissue properties during morphogenesis. 121(23):e2318481121.
- [59] Emmanuel Gallaud, Tri Pham, and Clemens Cabernard. *Drosophila melanogaster* Neuroblasts: A Model for Asymmetric Stem Cell Divisions. In Jean-Pierre Tassan and Jacek Z. Kubiak, editors, *Asymmetric Cell Division in Development, Differentiation and Cancer*, volume 61, pages 183–210. Springer International Publishing.
- [60] Ahmadreza Ghaffarizadeh, Randy Heiland, Samuel H. Friedman, Shannon M. Mumenthaler, and Paul Macklin. PhysiCell: An open source physics-based cell simulator for 3-D multicellular systems. 14(2):e1005991.
- [61] Hasreet K. Gill, Sifan Yin, John C. Lawlor, Tyler R. Huycke, Nandan L. Nerurkar, Clifford J. Tabin, and L. Mahadevan. The developmental mechanics of divergent buckling patterns in the chick gut. 121(28):e2310992121.
- [62] Daniel T. Gillespie. Exact stochastic simulation of coupled chemical reactions. *The Journal of Physical Chemistry*, 81(25):2340–2361, December 1977.
- [63] Daniel T. Gillespie. Stochastic Simulation of Chemical Kinetics. *Annual Review of Physical Chemistry*, 58(1):35–55, May 2007.
- [64] James A. Glazier and François Graner. Simulation of the differential adhesion driven rearrangement of biological cells. 47(3):2128–2154.
- [65] Michael Glotzer. Cytokinesis in Metazoa and Fungi. 9(10):a022343.
- [66] Juan Manuel Gomez, Carlo Bevilacqua, Abhisha Thayambath, Jean-Karim Hériché, Maria Leptin, Julio M. Belmonte, and Robert Prevedel. Highly dynamic mechanical transitions in embryonic cell populations during *Drosophila* gastrulation. 16(1):6473.

- [67] Morgan Q. Goulding. Cell Lineage of the Ilyanassa Embryo: Evolutionary Acceleration of Regional Differentiation during Early Development. 4(5):e5506.
- [68] Morgan Q. Goulding and J. David Lambert. Mollusc models I. The snail Ilyanassa. 39:168–174.
- [69] François Graner and James A. Glazier. Simulation of biological cell sorting using a two-dimensional extended Potts model. 69(13):2013–2016.
- [70] Scott J. Gratz, Alexander M. Cummings, Jennifer N. Nguyen, Danielle C. Hamm, Laura K. Donohue, Melissa M. Harrison, Jill Wildonger, and Kate M. O’Connor-Giles. Genome engineering of Drosophila with the CRISPR RNA-guided Cas9 nuclease. 194(4):1029–1035.
- [71] Rob Groot and Patrick Warren. Dissipative particle dynamics: Bridging the gap between atomistic and mesoscopic simulation. 107.
- [72] Z. Guan, A. Prado, J. Melzig, M. Heisenberg, H. A. Nash, and T. Raabe. Mushroom body defect, a gene involved in the control of neuroblast proliferation in Drosophila, encodes a coiled-coil protein. 97(14):8122–8127.
- [73] Gunsagar S. Gulati, Jeremy Philip D’Silva, Yunhe Liu, Linghua Wang, and Aaron M. Newman. Profiling cell identity and tissue architecture with single-cell and spatial transcriptomics. *Nature Reviews Molecular Cell Biology*, 26(1):11–31, January 2025.
- [74] Megan Haase, Tien Comlekoglu, Alexa Petrucciani, Shayn M Peirce, and Silvia S Blemker. Agent-based model demonstrates the impact of nonlinear, complex interactions between cytokines on muscle regeneration. *eLife*, 13:RP91924, June 2024.
- [75] Randall S. Hewes, Dongkook Park, Sebastien A. Gauthier, Anneliese M. Schaefer, and Paul H. Taghert. The bHLH protein Dimmed controls neuroendocrine cell differentiation in Drosophila. 130(9):1771–1781.
- [76] Mathis Heyer, Jiyizhe Zhang, Naoto Sugisawa, Jan-Frederic Laub, and Alexei A. Lapkin. Automated generation of mechanistic models for chemical process digital twins using reinforcement learning part I: Conceptual framework and equation generation. *Computers & Chemical Engineering*, 202:109281, November 2025.
- [77] Tsuyoshi Hirashima, Elisabeth G. Rens, and Roeland M. H. Merks. Cellular Potts modeling of complex multicellular behaviors in tissue morphogenesis. 59(5):329–339.

- [78] J. Hirata, H. Nakagoshi, Y. Nabeshima, and F. Matsuzaki. Asymmetric segregation of the homeodomain protein Prospero during *Drosophila* development. 377(6550):627–630.
- [79] Jon Holy and Gerald Schatten. Differential behavior of centrosomes in unequally dividing blastomeres during fourth cleavage of sea urchin embryos. 98(3):423–431.
- [80] Catarina C. F. Homem and Juergen A. Knoblich. *Drosophila* neuroblasts: A model for stem cell biology. 139(23):4297–4310.
- [81] Catarina C. F. Homem, Ilka Reichardt, Christian Berger, Thomas Lendl, and Juergen A. Knoblich. Long-term live cell imaging and automated 4D analysis of *drosophila* neuroblast lineages. 8(11):e79588.
- [82] Catarina C. F. Homem, Victoria Steinmann, Thomas R. Burkard, Alexander Jais, Harald Esterbauer, and Juergen A. Knoblich. Ecdysone and mediator change energy metabolism to terminate proliferation in *Drosophila* neural stem cells. 158(4):874–888.
- [83] Nicole Horsley, Adam von Barnau Sythoff, Mark Delgado, Selina Liu, and Clemens Cabernard. CrisprBuildr: An open-source application for CRISPR-mediated genome engineering in *Drosophila melanogaster*. 16(1):jkaf251.
- [84] H.Robert Horvitz and Ira Herskowitz. Mechanisms of asymmetric cell division: Two Bs or not two Bs, that is the question. 68(2):237–255.
- [85] Anna Hovhanyan, Eva K. Herter, Jens Pfannstiel, Peter Gallant, and Thomas Raabe. *Drosophila* mbm is a nucleolar myc and casein kinase 2 target required for ribosome biogenesis and cell growth of central brain neuroblasts. *Molecular and Cellular Biology*, 34(10):1878–1891, May 2014.
- [86] Wieland B Huttner and Yoichi Kosodo. Symmetric versus asymmetric cell division during neurogenesis in the developing vertebrate central nervous system. 17(6):648–657.
- [87] H. Ikeshima-Kataoka, J. B. Skeath, Y. Nabeshima, C. Q. Doe, and F. Matsuzaki. Miranda directs Prospero to a daughter cell during *Drosophila* asymmetric divisions. 390(6660):625–629.
- [88] Hiroaki Imoto, Sawa Yamashiro, and Mariko Okada. A text-based computational framework for patient -specific modeling for classification of cancers. 25(3):103944.
- [89] Yasushi Izumi, Nao Ohta, Kanako Hisata, Thomas Raabe, and Fumio Matsuzaki. *Drosophila* Pins-binding protein Mud regulates spindle-polarity coupling and centrosome organization. 8(6):586–593.

- [90] Radek Jankele, Rob Jelier, and Pierre Gönczy. Physically asymmetric division of the *C. elegans* zygote ensures invariably successful embryogenesis. 10:e61714.
- [91] Divya Jayaraman, Byoung-Il Bae, and Christopher A. Walsh. The Genetics of Primary Microcephaly. 19:177–200.
- [92] Samuel W. S. Johnson, Paul M. Kulesa, Ruth E. Baker, and Philip K. Maini. Mathematical modeling predicts novel mechanisms of stream confinement from Trail/Colec12/Dan in the collective migration of cranial neural crest cells. n/a(n/a).
- [93] Bivash Kaity and Daniel Lobo. Emergent stable tissue shapes from the regulatory feedback between morphogens and cell growth. 620:112354.
- [94] Jackie Kazil, David Masad, and Andrew Crooks. Utilizing Python for Agent-Based Modeling: The Mesa Framework. In Robert Thomson, Halil Bisgin, Christopher Dancy, Ayaz Hyder, and Muhammad Hussain, editors, *Social, Cultural, and Behavioral Modeling*, pages 308–317. Springer International Publishing.
- [95] Abdul Haseeb Khan, Xuefang Gu, Rutvik J. Patel, Prabha Chuphal, Matheus P. Viana, Aidan I. Brown, Brian M. Zid, and Tatsuhisa Tsuboi. Mitochondrial protein heterogeneity stems from the stochastic nature of co-translational protein targeting in cell senescence. *Nature Communications*, 15(1):8274, September 2024.
- [96] M. M. Kirk, A. Ransick, S. E. McRae, and D. L. Kirk. The relationship between cell size and cell fate in *Volvox carteri*. *The Journal of Cell Biology*, 123(1):191–208, October 1993.
- [97] J. A. Knoblich, L. Y. Jan, and Y. N. Jan. Asymmetric segregation of Numb and Prospero during cell division. 377(6550):624–627.
- [98] J. A. Knoblich, L. Y. Jan, and Y. N. Jan. Deletion analysis of the *Drosophila* Inscuteable protein reveals domains for cortical localization and asymmetric localization. 9(3):155–158.
- [99] Juergen A. Knoblich. Asymmetric cell division: Recent developments and their implications for tumour biology. 11(12):849–860.
- [100] Alex C. Koon, James Ashley, Romina Barria, Shamik DasGupta, Ruth Brain, Scott Waddell, Mark J. Alkema, and Vivian Budnik. Autoregulatory and paracrine control of synaptic and behavioral plasticity by octopaminergic signaling. 14(2):190–199.
- [101] Cristina Koprinski, Georgio Hawi, and Peter S. Kim. Optimising Chemotherapy for Advanced High-Grade Serous Ovarian Cancer via Delay-Differential Equations. page 112396.

- [102] Bryce LaFoya and Kenneth E. Prehoda. Actin-dependent membrane polarization reveals the mechanical nature of the neuroblast polarity cycle. 35(7):109146.
- [103] Bryce LaFoya and Kenneth E. Prehoda. Consumption of a polarized membrane reservoir drives asymmetric membrane expansion during the unequal divisions of neural stem cells. 58(11):993–1003.e3.
- [104] M. E. Lane, K. Sauer, K. Wallace, Y. N. Jan, C. F. Lehner, and H. Vaessin. Dacapo, a cyclin-dependent kinase inhibitor, stops cell proliferation during *Drosophila* development. 87(7):1225–1235.
- [105] Jan-Frederic Laub, Jiyizhe Zhang, Mathis Heyer, and Alexei A. Lapkin. Automated generation of mechanistic models for chemical process digital twins using reinforcement learning part II: Compartmentalization and learning-based recalibration. 204:109384.
- [106] Natalia G. Lavalle, Jerónimo Miranda-Rodríguez, Emanuel Cura Costa, Augusto Borges, Oriol Viader-Llargués, Hernán López-Schier, and Osvaldo Chara. Local control of cellular proliferation underlies neuromast regeneration in zebrafish. page 112389.
- [107] Enikő Lázár and Joakim Lundeberg. Spatial architecture of development and disease. *Nature Reviews Genetics*, 27(2):118–136, February 2026.
- [108] Teddy Lazebnik and Avner Friedman. Spatio-temporal model of combining ADT and chemotherapy with senolytic treatment in metastatic prostate cancer. *Journal of Theoretical Biology*, 602–603:112069, April 2025.
- [109] Terry Lechler and Elaine Fuchs. Asymmetric cell divisions promote stratification and differentiation of mammalian skin. 437(7056):275–280.
- [110] Terry Lechler and Marina Mapelli. Spindle positioning and its impact on vertebrate tissue architecture and cell fate. 22(10):691–708.
- [111] Cheng-Yu Lee, Ryan O. Andersen, Clemens Cabernard, Laurina Manning, Khoa D. Tran, Marcus J. Lanskey, Arash Bashirullah, and Chris Q. Doe. *Drosophila* Aurora-A kinase inhibits neuroblast self-renewal by regulating aPKC/Numb cortical polarity and spindle orientation. 20(24):3464–3474.
- [112] Cheng-Yu Lee, Kristin J. Robinson, and Chris Q. Doe. Lgl, Pins and aPKC regulate neuroblast self-renewal versus differentiation. 439(7076):594–598.

- [113] Katarzyna Lepeta, Chantal Roubinet, Milena Bauer, M. Alessandra Vigano, Gustavo Aguilar, Oguz Kanca, Amanda Ochoa-Espinosa, Dimitri Bieli, Clemens Cabernard, Emmanuel Caussinus, and Markus Affolter. Engineered kinases as a tool for phosphorylation of selected targets in vivo. 221(10):e202106179.
- [114] He Li, Zixiang Leonardo Liu, Lu Lu, Pierre Buffet, and George Em Karniadakis. How the spleen reshapes and retains young and old red blood cells: A computational investigation. *PLOS Computational Biology*, 17(11):e1009516, November 2021.
- [115] Ling Li and Harald Vaessin. Pan-neural Prospero terminates cell proliferation during *Drosophila* neurogenesis. 14(2):147–151.
- [116] Rong Li. The Art of Choreographing Asymmetric Cell Division. 25(5):439–450.
- [117] Sheng Li, Paula A. Agudelo Garcia, Constantin Aliferis, Michael J. Becich, Jazmin Calyeca, Benjamin D. Cosgrove, Jennifer Elisseeff, Negin Farzad, Elana J. Fertig, Carolyn Glass, Liangcai Gu, Qianjiang Hu, Zhicheng Ji, Melanie Königshoff, Nathan K. LeBrasseur, Dongmei Li, Anjun Ma, Qin Ma, Vilas Menon, Jacob T. Mitchell, Ana L. Mora, Sushma Nagaraj, Andrew C. Nelson, Laura J. Niedernhofer, Mauricio Rojas, Hash Brown Taha, Jinhua Wang, Siyuan Wang, Pei-Hsun Wu, Jichun Xie, Ming Xu, Miao Yu, Xu Zhang, Yue Zhao, Peter D. Adams, Cristina Aguayo-Mazzucato, Darren J. Baker, Christopher Benz, David A. Bernlohr, Marta Bueno, Jin Chen, Bennett G. Childs, Jeffrey H. Chuang, Dongjun Chung, Mythili Dileepan, Li Ding, Mingze Dong, Francesca Duncan, Archibald Enninfu, William F. Flynn, Ana Catarina Franco, David Furman, Vesna Garovic, Stephanie Halene, Allison B. Herman, Ann V. Hertz, Kanako Iwasaki, Hyeonseon Jeon, Jeon Woong Kang, Shilpita Karmakar, James L. Kirkland, Ron Korstanje, Erich Kummerfeld, Jun Hee Lee, Yang Liu, Yao Lu, Jose Lugo-Martinez, Helene Martini, Simon Melov, Nicolas Musi, João F. Passos, Samuel T. Peters, Irfan Rahman, Ramalakshmi Ramasamy, Alexandra N. Rindone, Paul D. Robbins, Paul Robson, Jhonny Rodriguez-Lopez, Lorena Rosas, Nadia Rosenthal, Marissa J. Schafer, Birgit Schilling, Elizabeth L. Schmidt, Kevin Schneider, Kaustav Sengupta, Jian Shu, Peter T. C. So, Li Sun, Tamar Tchkonja, Marcos G. Teneche, Natalia Vanegas, Julia Wang, Juan Xie, Shanshan Yin, Ke Zhang, Quan Zhu, and Rong Fan. Advancing biological understanding of cellular senescence with computational multiomics. *Nature Genetics*, 57(10):2381–2394, October 2025.
- [118] Shun Li, Zong-Yuan Liu, Hao Li, Sijia Zhou, Jiaying Liu, Ningwei Sun, Kai-Fu Yang, Vanessa Dougados, Thomas Mangeat, Karine Belguise, Xi-Qiao Feng, Yiyao Liu, and Xiaobo Wang. Basal actomyosin pulses expand epithelium coordinating cell flattening and tissue elongation. 15(1):3000.
- [119] Xiaosu Li, Yonggang Xie, and Sijun Zhu. Notch maintains *Drosophila* type II neuroblasts by suppressing expression of the Fez transcription factor Earmuff. 143(14):2511–2521.

- [120] Yang Liu, Yang Wang, Leilei Zhang, Yedong Tang, Yetao Hong, Meng Liu, Mengyuan Wang, Ya Sun, Xiaoyue Shen, Jie Mei, Min Wu, Na Kong, Shuangbo Kong, Haili Bao, Wenbo Deng, Guijun Yan, Haibin Wang, and Haixiang Sun. Decrypting spatiotemporal code of human endometrial receptivity. 17(1):801.
- [121] Srivathsa S. Magadi, Chrysanthi Voutyraki, Gerasimos Anagnostopoulos, Evanthia Zacharioudaki, Ioanna K. Poutakidou, Christina Efraimoglou, Margarita Stapountzi, Vasiliki Theodorou, Christoforos Nikolaou, Konstantinos A. Koumbanakis, John F. Fullard, and Christos Delidakis. Dissecting Hes-centred transcriptional networks in neural stem cell maintenance and tumorigenesis in *Drosophila*. 147(22):dev191544.
- [122] Santosh Manicka, Vaibhav P. Pai, and Michael Levin. Information integration during bioelectric regulation of morphogenesis of the embryonic frog brain. 26(12):108398.
- [123] Lucy Martin, Linus Schumacher, and Tamir Chandra. Modelling the dynamics of senescence spread. 22(8):e13892.
- [124] F. Matsuzaki, K. Koizumi, C. Hama, T. Yoshioka, and Y. Nabeshima. Cloning of the *Drosophila* prospero gene and its expression in ganglion mother cells. 182(3):1326–1332.
- [125] Mate Maus, Vanessa López-Polo, Lidia Mateo, Miguel Lafarga, Mònica Aguilera, Eugenia De Lama, Kathleen Meyer, Anna Sola, Cecilia Lopez-Martinez, Ines López-Alonso, Marc Guasch-Piqueras, Fernanda Hernandez-Gonzalez, Selim Chaib, Miguel Rovira, Mayka Sanchez, Rosa Faner, Alvar Agusti, Rodrigo Diéguez-Hurtado, Sagrario Ortega, Anna Manonelles, Stefan Engelhardt, Freddy Monteiro, Camille Stephan-Otto Attolini, Neus Prats, Guillermo Albaiceta, Josep M. Cruzado, and Manuel Serrano. Iron accumulation drives fibrosis, senescence and the senescence-associated secretory phenotype. 5(12):2111–2130.
- [126] Juliane Melzer, Karoline F. Kraft, Rolf Urbach, and Thomas Raabe. The p21-activated kinase Mbt is a component of the apical protein complex in central brain neuroblasts and controls cell proliferation. 140(9):1871–1881.
- [127] Roeland M. H. Merks and James A. Glazier. A cell-centered approach to developmental biology. 352(1):113–130.
- [128] John Metzcar, Yafei Wang, Randy Heiland, and Paul Macklin. A Review of Cell-Based Computational Modeling in Cancer Biology. *JCO Clinical Cancer Informatics*, (3):1–13, December 2019.
- [129] Sylvia N. Michki, Ye Li, Kayvon Sanjasaz, Yimeng Zhao, Fred Y. Shen, Logan A. Walker, Wenjia Cao, Cheng-Yu Lee, and Dawen Cai. The molecular landscape of neural differentiation in the developing *Drosophila* brain revealed by targeted scRNA-seq and multi-informatic analysis. 35(4):109039.

- [130] Claire M. Miller, Domenic P. J. Germano, Alicia M. Chenoweth, and Sarah Holdsworth-Carson. Mathematical modelling of macrophage and natural killer cell immune response during early stages of peritoneal endometriosis lesion onset. 22(229):20250076.
- [131] Maria Monastirioti, Nikolaos Giagtzoglou, Konstantinos A. Koumbanakis, Evanthia Zacharioudaki, Myrto Deligiannaki, Irmgard Wech, Mara Almeida, Anette Preiss, Sarah Bray, and Christos Delidakis. Drosophila Hey is a target of Notch in asymmetric divisions during embryonic and larval neurogenesis. 137(2):191–201.
- [132] Satoshi Murakami, Maki Minami-Ohtsubo, Ryuichiro Nakato, Katsuhiko Shirahige, and Tetsuya Tabata. Two Components of Aversive Memory in Drosophila, Anesthesia-Sensitive and Anesthesia-Resistant Memory, Require Distinct Domains Within the Rgk1 Small GTPase. 37(22):5496–5510.
- [133] Dagmar Neubueser, James T. Warren, Lawrence I. Gilbert, and Stephen M. Cohen. Molting Defective is required for ecdysone biosynthesis. 280(2):362–372.
- [134] Thomas P Neufeld, prefix=de la useprefix=true family=Cruz, given=Aida Flor A, Laura A Johnston, and Bruce A Edgar. Coordination of Growth and Cell Division in the Drosophila Wing. 93(7):1183–1193.
- [135] Tho Huu Nguyen, Rosario Vicidomini, Saumitra Dey Choudhury, Tae Hee Han, Dragan Maric, Thomas Brody, and Mihaela Serpe. scRNA-seq data from the larval Drosophila ventral cord provides a resource for studying motor systems function and development. 59(9):1210–1230.e9.
- [136] J. N. Noordermeer, C. C. Kopczynski, R. D. Fetter, K. S. Bland, W. Y. Chen, and C. S. Goodman. Wrapper, a novel member of the Ig superfamily, is expressed by midline glia and is required for them to ensheath commissural axons in Drosophila. 21(5):991–1001.
- [137] Ben Noordijk, Monica L. Garcia Gomez, prefix=ten useprefix=true family=Tusscher, given=Kirsten H. W. J., prefix=de useprefix=true family=Ridder, given=Dick, prefix=van useprefix=true family=Dijk, given=Aalt D. J., and Robert W. Smith. The rise of scientific machine learning: A perspective on combining mechanistic modelling with machine learning for systems biology. 4.
- [138] Michael J. North, Nicholson T. Collier, Jonathan Ozik, Eric R. Tatara, Charles M. Macal, Mark Bragen, and Pam Sydelko. Complex adaptive systems modeling with Repast Simphony. 1(1):3.
- [139] Dick R Nässel, Lina E Enell, Jonathan G Santos, Christian Wegener, and Helena AD Johard. A large population of diverse neurons in the Drosophila central

nervous system expresses short neuropeptide F, suggesting multiple distributed peptide functions. 9:90.

- [140] Chet Huan Oon and Kenneth E. Prehoda. Phases of cortical actomyosin dynamics coupled to the neuroblast polarity cycle. 10:e66574.
- [141] Guangshuo Ou, Nico Stuurman, Michael D'Ambrosio, and Ronald D. Vale. Polarized Myosin Produces Unequal-Size Daughters During Asymmetric Cell Division. 330(6004):677–680.
- [142] Steven O'Reilly, Pei-Suen Tsou, and John Varga. Senescence and tissue fibrosis: Opportunities for therapeutic targeting. 30(12):1113–1125.
- [143] P Pathmanathan, J Cooper, A Fletcher, G Mirams, P Murray, J Osborne, J Pitt-Francis, A Walter, and S J Chapman. A computational study of discrete mechanical tissue models. 6(3):036001.
- [144] Rhiannon R. Penkert, Bryce LaFoya, Lucille Moholt-Siebert, Elizabeth Vargas, Sarah E. Welch, and Kenneth E. Prehoda. The *Drosophila* neuroblast polarity cycle at a glance. 137(5):jcs261789.
- [145] Tri Thanh Pham, Arnaud Monnard, Jonne Helenius, Erik Lund, Nicole Lee, Daniel J. Müller, and Clemens Cabernard. Spatiotemporally Controlled Myosin Relocalization and Internal Pressure Generate Sibling Cell Size Asymmetry. 13:9–19.
- [146] Jessica Poon, Annaliese Fries, Gary M. Wessel, and Mamiko Yajima. Evolutionary modification of AGS protein contributes to formation of micromeres in sea urchins. 10(1):3779.
- [147] K. E. Prehoda. Polarization of *Drosophila* Neuroblasts During Asymmetric Division. 1(2):a001388–a001388.
- [148] A. Prokop and G. M. Technau. Normal function of the mushroom body defect gene of *Drosophila* is required for the regulation of the number and proliferation of neuroblasts. 161(2):321–337.
- [149] Anaïs Rat, Veronica Martinez Fernandez, Marie Doumic, Maria Teresa Teixeira, and Zhou Xu. Mathematical model linking telomeres to senescence in *Saccharomyces cerevisiae* reveals cell lineage versus population dynamics. *Nature Communications*, 16(1):1024, January 2025.
- [150] Nicholas Rhind. Cell-size control. 31(21):R1414–R1420.

- [151] Paul Richmond, Robert Chisholm, Peter Heywood, Mozhgan Kabiri Chimeh, and Matthew Leach. FLAME GPU 2: A framework for flexible and performant agent based simulation on GPUs. 53(8):1659–1680.
- [152] Noemi Rives-Quinto, Hideyuki Komori, Cyrina M. Ostgaard, Derek H. Janssens, Shu Kondo, Qi Dai, Adrian W. Moore, and Cheng-Yu Lee. Sequential activation of transcriptional repressors promotes progenitor commitment by silencing stem cell identity genes. 9:e56187.
- [153] S. Robinow and K. White. Characterization and spatial distribution of the ELAV protein during *Drosophila melanogaster* development. 22(5):443–461.
- [154] Michaela Roth, Chantal Roubinet, Niklas Iffländer, Alexia Ferrand, and Clemens Cabernard. Asymmetrically dividing *Drosophila* neuroblasts utilize two spatially and temporally independent cytokinesis pathways. 6(1):6551.
- [155] Chantal Roubinet, Anna Tsankova, Tri Thanh Pham, Arnaud Monnard, Emmanuel Caussinus, Markus Affolter, and Clemens Cabernard. Spatio-temporally separated cortical flows and spindle geometry establish physical asymmetry in fly neural stem cells. 8(1):1383.
- [156] Marie-Laure Samson and Fabienne Chalvet. Found in neurons, a third member of the *Drosophila* elav gene family, encodes a neuronal protein and interacts with elav. 120(3):373–383.
- [157] Tamsin J Samuels, Aino I Järvelin, David Ish-Horowicz, and Ilan Davis. Imp/IGF2BP levels modulate individual neural stem cell growth and division through myc mRNA stability. 9:e51529.
- [158] Beatriz P. San-Juán and Antonio Baonza. The bHLH factor deadpan is a direct target of Notch signaling and regulates neuroblast self-renewal in *Drosophila*. 352(1):70–82.
- [159] Clare L. Scott, Susana Banerjee, Florence Joly, Jung-Min Lee, Asima Mukhopadhyay, David S. Tan, and Elise C. Kohn. Ovarian cancer. 12(1):10.
- [160] Roberto Carlos Segura and Clemens Cabernard. Live-Cell Imaging of *Drosophila melanogaster* Third Instar Larval Brains. (196):e65538.
- [161] Nathan C. Shaner, Robert E. Campbell, Paul A. Steinbach, Ben N. G. Giepmans, Amy E. Palmer, and Roger Y. Tsien. Improved monomeric red, orange and yellow fluorescent proteins derived from *Discosoma* sp. red fluorescent protein. 22(12):1567–1572.

- [162] Fabian Siegel, Angelo Torelli, Minca Mattis, Julian Debatin, Philipp Erben, and Markus Gumbel. Proliferation and regeneration of the healthy human urothelium: A multi-scale simulation approach with 16 hypotheses of cell differentiation. *PLOS One*, 20(6):e0325132, June 2025.
- [163] Karsten H. Siller, Clemens Cabernard, and Chris Q. Doe. The NuMA-related Mud protein binds Pins and regulates spindle orientation in *Drosophila* neuroblasts. 8(6):594–600.
- [164] Nikistratos Siskos, Electra Stylianopoulou, Georgios Skavdis, and Maria E. Grigoriou. Molecular Genetics of Microcephaly Primary Hereditary: An Overview. 11(5):581.
- [165] Cathy Slack, Paul M Overton, Richard I Tuxworth, and William Chia. Asymmetric localisation of Miranda and its cargo proteins during neuroblast division requires the anaphase-promoting complex/cyclosome. 134(21):3781–3787.
- [166] E. P. Spana and C. Q. Doe. The prospero transcription factor is asymmetrically localized to the cell cortex during neuroblast mitosis in *Drosophila*. 121(10):3187–3195.
- [167] Jörn Starrauß, prefix=de useprefix=true family=Back, given=Walter, Lutz Brusch, and Andreas Deutsch. Morpheus: A user-friendly modeling environment for multiscale and multicellular systems biology. 30(9):1331–1332.
- [168] Hetian Su and Nan Hao. Computational systems biology approaches to cellular aging—Integrating network maps and dynamical models. *Quantitative Biology*, 13(4):e70007, 2025.
- [169] Bharath Sunchu and Clemens Cabernard. Principles and mechanisms of asymmetric cell division. 147(13):dev167650.
- [170] Maciej H. Swat, Gilberto L. Thomas, Julio M. Belmonte, Abbas Shirinifard, Dimitrij Hmeljak, and James A. Glazier. Multi-Scale Modeling of Tissues Using CompuCell3D. 110:325–366.
- [171] Yu-Hang Tang, Lu Lu, He Li, Constantinos Evangelinos, Leopold Grinberg, Vipin Sachdeva, and George Em Karniadakis. OpenRBC: A Fast Simulator of Red Blood Cells at Protein Resolution. 112(10):2030–2037.
- [172] Jean-Pierre Tassan and Jacek Z. Kubiak, editors. *Asymmetric Cell Division in Development, Differentiation and Cancer*. Number 61 in Results and Problems in Cell Differentiation. Springer.

- [173] Bharat Vivan Thapa, Mallar Banerjee, Tilmann Glimm, Deepak K. Saini, and Ramray Bhat. The senescent mesothelial matrix accentuates colonization by ovarian cancer cells. *Cellular and Molecular Life Sciences*, 81(1):2, December 2024.
- [174] Pauli Tikka, Moritz Mercker, Ilya Skovorodkin, Ulla Saarela, Seppo Vainio, Veli-Pekka Ronkainen, James P. Sluka, James A. Glazier, Anna Marciniak-Czochra, and Franz Schaefer. Computational modelling of nephron progenitor cell movement and aggregation during kidney organogenesis. 344:108759.
- [175] Anna Tsankova, Tri Thanh Pham, David Salvador Garcia, Fabian Otte, and Clemens Cabernard. Cell Polarity Regulates Biased Myosin Activity and Dynamics during Asymmetric Cell Division via *Drosophila* Rho Kinase and Protein Kinase N. 42(2):143–155.e5.
- [176] Stéphane Urcun, Yasmin El Mahi, Raluca Eftimie, Zélie Dirand, Gwenaél Rolin, and Stéphane P. A. Bordas. Simple cellular Potts model of scratch assays on healthy and keloid fibroblasts driven by contact inhibition of locomotion. page 112365.
- [177] A. E. Uv, E. J. Harrison, and S. J. Bray. Tissue-specific splicing and functions of the *Drosophila* transcription factor Grainyhead. 17(11):6727–6735.
- [178] Samir Vaid and Wieland B. Huttner. Progenitor-Based Cell Biological Aspects of Neocortex Development and Evolution. 10:892922.
- [179] Abhijeet Venkataraman, Ivan Kordic, JiaXun Li, Nicholas Zhang, Nivik Sanjay Bharadwaj, Zhou Fang, Sandip Das, and Ahmet F. Coskun. Decoding senescence of aging single cells at the nexus of biomaterials, microfluidics, and spatial omics. *npj Aging*, 10(1):57, November 2024.
- [180] Zsolt G. Venkei and Yukiko M. Yamashita. Emerging mechanisms of asymmetric stem cell division. 217(11):3785–3795.
- [181] Chiara Venturini and Pablo Sáez. A multi-scale clutch model for adhesion complex mechanics. 19(7):e1011250.
- [182] Joseph H. A. Vissers, Francesca Froldi, Jan Schroder, Anthony T. Papenfuss, Louise Y. Cheng, and Kieran F. Harvey. The Scalloped and Nerfin-1 Transcription Factors Cooperate to Maintain Neuronal Cell Fate. 25(6):1561–1576.
- [183] Dhananjay A. Wagh, Tobias M. Rasse, Esther Asan, Alois Hofbauer, Isabell Schwenkert, Heike Dürrbeck, Sigrid Buchner, Marie-Christine Dabauvalle, Manuela Schmidt, Gang Qin, Carolin Wichmann, Robert Kittel, Stephan J. Sigrist, and Erich Buchner. Bruchpilot, a protein with homology to ELKS/CAST, is required for structural integrity and function of synaptic active zones in *Drosophila*. 49(6):833–844.

- [184] Boshi Wang, Jin Han, Jennifer H. Elisseeff, and Marco Demaria. The senescence-associated secretory phenotype and its physiological and pathological implications. 25(12):958–978.
- [185] Mingyue Wang, Qinan Hu, Zhencheng Tu, Lingshi Kong, Jiajun Yao, Rong Xiang, Zhan Chen, Yan Zhao, Yanfei Zhou, Tengxiang Yu, Yuetian Wang, Zihan Jia, Kang Ouyang, Xianzhe Wang, Yinqi Bai, Mingwei Lian, Zhenyu Yang, Tao Yang, Jing Chen, Yunting Huang, Ni Yin, Wenyuan Mo, Wenfu Liang, Chang Liu, Xiumei Lin, Chuanyu Liu, Ying Gu, Wei Chen, Longqi Liu, Xun Xu, and Yuhui Hu. A single-cell 3D spatiotemporal multi-omics atlas from *Drosophila* embryogenesis to metamorphosis.
- [186] Yating Wang, Kamil Kuca, Li You, Eugenie Nepovimova, Zbynek Heger, Marian Valko, Vojtech Adam, Qinghua Wu, and Klaudia Jomova. The role of cellular senescence in neurodegenerative diseases. 98(8):2393–2408.
- [187] Catherine Weathered, Sophia Bardehle, Choya Yoon, Niyanta Kumar, Cheryl E. G. Leyns, Matthew E. Kennedy, Peter Bloomingdale, and Elsje Pienaar. Microglial roles in Alzheimer’s disease: An agent-based model to elucidate microglial spatiotemporal response to beta-amyloid. *CPT: Pharmacometrics & Systems Pharmacology*, 13(3):449–463, 2024.
- [188] Georgia R. Weatherley, Robyn P. Araujo, Samantha J. Dando, and Adrianne L. Jenner. Therapeutic targeting of oligodendrocytes in an agent-based model of multiple sclerosis. *PLOS Computational Biology*, 22(1):e1013273, January 2026.
- [189] Charles Otis Whitman. The Embryology of *Clepsine*1. s2-18(71):215–315.
- [190] Mark D. Wilkinson, Michel Dumontier, given-i=IJJ family=Aalbersberg, given=IJsbrand Jan, Gabrielle Appleton, Myles Axton, Arie Baak, Niklas Blomberg, Jan-Willem Boiten, Luiz Bonino Da Silva Santos, Philip E. Bourne, Jildau Bouwman, Anthony J. Brookes, Tim Clark, Mercè Crosas, Ingrid Dillo, Olivier Dumon, Scott Edmunds, Chris T. Evelo, Richard Finkers, Alejandra Gonzalez-Beltran, Alasdair J.G. Gray, Paul Groth, Carole Goble, Jeffrey S. Grethe, Jaap Heringa, Peter A.C ’T Hoen, Rob Hooft, Tobias Kuhn, Ruben Kok, Joost Kok, Scott J. Lusher, Maryann E. Martone, Albert Mons, Abel L. Packer, Bengt Persson, Philippe Rocca-Serra, Marco Roos, Rene Van Schaik, Susanna-Assunta Sansone, Erik Schultes, Thierry Sengstag, Ted Slater, George Strawn, Morris A. Swertz, Mark Thompson, Johan Van Der Lei, Erik Van Mulligen, Jan Velterop, Andra Waagmeester, Peter Wittenburg, Katherine Wolstencroft, Jun Zhao, and Barend Mons. The FAIR Guiding Principles for scientific data management and stewardship. 3(1):160018.

- [191] Scott E. Williams, Slobodan Beronja, H. Amalia Pasolli, and Elaine Fuchs. Asymmetric cell divisions promote Notch-dependent epidermal differentiation. 470(7334):353–358.
- [192] W. C. Xiong, H. Okano, N. H. Patel, J. A. Blendy, and C. Montell. Repo encodes a glial-specific homeo domain protein required in the *Drosophila* nervous system. 8(8):981–994.
- [193] Manako Yamaguchi, Hirofumi Nakaoka, Kazuaki Suda, Kosuke Yoshihara, Tatsuya Ishiguro, Nozomi Yachida, Kyota Saito, Haruka Ueda, Kentaro Sugino, Yutaro Mori, Kaoru Yamawaki, Ryo Tamura, Sundaramoorthy Revathidevi, Teiichi Motoyama, Kazuki Tainaka, Roel G. W. Verhaak, Ituro Inoue, and Takayuki Enomoto. Spatiotemporal dynamics of clonal selection and diversification in normal endometrial epithelium. 13(1):943.
- [194] Jiajun Yang, Xiaoqing Hu, Jian Ma, and Song-Hai Shi. Centrosome regulation and function in mammalian cortical neurogenesis. 69:256–266.
- [195] Yang Ye, Abhishek Pandey, Carolyn Bawden, Dewan Md Sumsuzzman, Rimpi Rajput, Affan Shoukat, Burton H. Singer, Seyed M. Moghadas, and Alison P. Galvani. Integrating artificial intelligence with mechanistic epidemiological modeling: A scoping review of opportunities and challenges. 16(1):581.
- [196] Jessica S. Yu and Neda Bagheri. Agent-Based Models Predict Emergent Behavior of Heterogeneous Cell Populations in Dynamic Microenvironments. 8.
- [197] Simon Zernicki-Glover, Nicola Stanislawski, Ekta M. Patel, Yu Hua Kavanagh, and Maciej Meglicki. Blastomere size in the human 2-cell embryo predicts the division order that leads to imbalanced lineage contribution to the future body. 2024:10.17912/micropub.biology.001181.
- [198] Sijun Zhu, Suewei Lin, Chih-Fei Kao, Takeshi Awasaki, Ann-Shyn Chiang, and Tzumin Lee. Gradients of the *Drosophila* Chinmo BTB-zinc finger protein govern neuronal temporal identity. 127(2):409–422.
- [199] Sijun Zhu, Jill Wildonger, Suzanne Barshow, Susan Younger, Yaling Huang, and Tzumin Lee. The bHLH Repressor Deadpan Regulates the Self-renewal and Specification of *Drosophila* Larval Neural Stem Cells Independently of Notch. 7(10):e46724.
- [200] Krina T. Zondervan, Christian M. Becker, Kaori Koga, Stacey A. Missmer, Robert N. Taylor, and Paola Viganò. Endometriosis. 4(1):9.

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