

Pleiotropic functions of TAOK2 and its dysregulation in neurodevelopmental disorders

Sujin Byeon

A dissertation

submitted in partial fulfillment of the

requirements for the degree of

Doctor of Philosophy

University of Washington

2025

Reading Committee:

Smita Yadav, Chair

Jessica Young

Larry Zweifel

Program Authorized to Offer Degree:

Neuroscience

©Copyright 2025

Sujin Byeon

University of Washington

Abstract

Pleiotropic functions of TAOK2 and its dysregulation in neurodevelopmental disorders

Sujin Byeon

Chair of the Supervisory Committee:

Smita Yadav

Department of Pharmacology

Thousand and one amino acid kinases (TAOKs) are relatively understudied and functionally pleiotropic protein kinases that have emerged as important regulators of neurodevelopment. Through their conserved amino-terminal catalytic domain, TAOKs mediate phosphorylation at serine/threonine residues in their substrates, but it is their divergent regulatory carboxyl-terminal domains that confer both exquisite functional specification and cellular localization. In this work, we focus specifically on diverse functions of TAOK2 in the context of neurodevelopment and neurodevelopmental disorders. TAOK2 is the only member of the TAOK family with two coiled coiled domains instead of three, and the canonical isoform, TAOK2 α , has a unique endoplasmic reticulum (ER) targeting domain and microtubule binding domain functioning to tether ER to microtubules. Its spliced isoform, TAOK2 β , is less understood. The precise mechanisms underlying the role of TAOK2 in neurodevelopment and neurodevelopmental disorders are not clearly understood. Following up on previous work showing that TAOK2 phosphorylation of

cytoskeletal protein Septin 7 is critical for dendritic spine maturation, in Chapter 2, we identify 14-3-3 proteins as downstream interacting proteins important for maintaining this phosphorylation. We have also identified changes in primary cilium length, trafficking, and signaling as potential mechanisms underlying copy number variation of 16p11.2, a genomic locus in which TAOK2 resides, in Chapter 3. Further, in Chapter 4, we lay preliminary work showing that autism associated TAOK2 mutations differentially affect the catalytic activity and localization. In summary, this work elucidates how TAOK2 contributes to neurodevelopment and associated disorders through its pleiotropic functions.

Acknowledgements

Thank you to everyone who believed in me and made my PhD possible.

I would like to thank my advisor, Dr. Smita Yadav, for all the support, guidance, and mentorship she provided throughout my PhD. You always pushed me to do more, and I have learned so much. I have grown as a scientist, stepping out of my comfort zone and expanding my knowledge and skill sets during my time in your lab. I had the privilege of presenting my work every year at different conferences and symposiums, even giving a talk at Gordon Conference, thanks to you. You always believed in my ability, and I wholeheartedly thank you for believing in me.

Thank you to my thesis committee members for their support and guidance. Thank you, Dr. Hannele Ruohola-Baker for always asking thought-provoking questions, Dr. Jane Sullivan for helping me improve my presentation, Dr. Jessica Young and Dr. Larry Zweifel for writing all my recommendation letters. I would especially like to thank Dr. Jessica Young for all the help and guidance with stem cell related questions and protocols. Additionally, I am grateful to my undergraduate students, Alison and Siri, for all of their hard work and help with the experiments. Thank you to all the Yadav Lab members who provided support and helpful insights throughout my PhD. The work seen here and beyond would not have been possible without these people.

I am also grateful to my friends, Pralaksha, Aria, Alyssa, Michelle, and Emily, as well as Chi who have always been there for me and kept me sane throughout my PhD. Cheers to more café runs and trips!

Lastly, I am immensely grateful to my parents who have supported me in every way. I am here writing my thesis thanks to their unwavering support.

Table of Contents

CHAPTER 1: INTRODUCTION.....	1
TAOK SIGNALING AND REGULATION.....	3
Identification of TAOs, their expression, and their structure	3
TAOs regulate MAPK signaling.....	8
TAOs regulate Hippo signaling	11
TAOs regulate the organization of the cytoskeleton.....	12
TAOs regulate mitosis	16
TAOs regulate apoptosis	17
TAOs regulate the host immune response.....	18
THE TAO FAMILY OF KINASES IN NEUROLOGICAL DISORDERS.....	22
TAOs in neurodevelopment	22
TAOs in neurodevelopmental disorders	27
TAOs in addiction, pain, and depression.....	34
TAOs in neurodegenerative disorders	35
CONCLUSIONS AND PERSPECTIVES	36
References	39
CHAPTER 2: PROTEOMIC IDENTIFICATION OF PHOSPHORYLATION- DEPENDENT SEPTIN 7 INTERACTORS THAT DRIVE DENDRITIC SPINE FORMATION	51
Abstract.....	52
Introduction.....	52

Materials and Methods	55
Results	64
References	85

CHAPTER 3: TAOK2 DRIVES OPPOSING CILIA LENGTH DEFICITS IN 16P11.2

DELETION AND DUPLICATION CARRIERS	89
Summary	90
Introduction	90
Results	93
Discussion	118
Limitations of the study	121
Methods	122
Resource availability	124
Lead contact	124
Materials availability	125
Data and code availability	125
Acknowledgments	125
Author contributions	125
Declaration of interests	126
References	127

CHAPTER 4: TAOK2 ASD-ASSOCIATED MUTATIONS DIFFERENTIALLY AFFECT CATALYTIC ACTIVITY AND LOCALIZATION OF TAOK2

Abstract	134
-----------------------	-----

Introduction.....	134
Materials and Methods.....	135
Results	139
Discussion.....	143
References	145
CHAPTER 5: DISCUSSION AND FUTURE DIRECTIONS.....	147
References	150

List of Figures and Tables

Figure 1.1. TAOs are evolutionarily conserved kinases.	3
Figure 1.2. TAOs are highly expressed in the human brain.	5
Figure 1.3. TAOs regulate the MAPK and Hippo signaling pathways.....	10
Figure 1.4. TAOs regulate cytoskeletal organization, mitosis, and apoptosis.	14
Figure 1.5. TAOs mediate various modes of immune signaling.	19
Figure 1.6. TAOs are important for neurodevelopment.	24
Figure 1.7. Dysfunction in TAOs leads to neurological diseases.....	29
Table 1.1. Neurodevelopmental disorder–associated missense and truncation mutations within the TAO family.	31
Figure 2.1. TAO kinases phosphorylate the C-terminal tail of Sept7 during neuronal development.	66
Figure 2. 2. Phosphorylation of Sept7 alters its localization and dynamics.	70
Figure 2.3. Proteomic identification of Septin 7 phosphorylation-dependent binding partners. ..	73
Figure 2.4. 14-3-3 γ associates with phosphorylated Sept7 and enhances dendritic spine maturation.	76
Figure 2.5. Association with 14-3-3 protects phosphorylation state of Sept7.	79
Table 2.1. List of proteins identified by Mass Spectrometry that associate with Septin 7 when phosphorylated at residue T426.	74

Figure 2S.1. TAO kinases phosphorylate the C-terminal tail of Sept7 during neuronal development (related to main Figure 1).....	67
Figure 3.1. Quantitative Phosphoproteomics of Neural Progenitors from 16p11.2 CNV Carriers Identifies Ciliary Proteins	96
Figure 3.2. Opposing Cilia Length Alterations in Neural Progenitors Derived from 16p11.2 Deletion and Duplication Carriers	101
Figure 3.3. Cellular Screens Identify Genetic Drivers of Altered Ciliary Length in 16p11.2 CNV	103
Figure 3.4. TAOK2 Kinase Localizes to the Base of the Primary Cilium and Restricts Cilium Elongation	107
Figure 3.5. TAOK2 KO and 16p11.2 deletion NPCs show aberrant IFT88 Accumulation at Ciliary Tip.....	114
Figure 3.6. TAOK2 is Important for SHH Signaling.....	116
Figure 3.7. TAOK2 Catalytic Activity is Important for its Role in Ciliary Growth Restriction	118
Figure 3S.1. Human Neuronal Model of 16p11.2 Deletion and Duplication (related to main.....	97
Figure 3S.2. Generation of TAOK2 Knockout Human iPSC and NPCs Using Genome Editing (related to main Figure 4)	108
Figure 3S.3. Characterization of TAOK2 Knockout NPCs (related to main Figure 4)	110
Figure 4. 1. Characterization of ASD associated TAOK2 mutations.	142

Preface

Chapters 1-3 are reprinted from the following works with minimal formatting adjustments.

Chapter 1: **Byeon S**, Yadav S. (2024). Pleiotropic functions of TAO kinases and their dysregulation in neurological disorders. *Sci. Signal.* <https://doi.org/10.1126/scisignal.adg0876>

Chapter 2: **Byeon S***, Werner B*, Falter R, Davidsen K, Snyder C, Ong S, Yadav S. (2022). Proteomic identification of phosphorylation-dependent Septin 7 interactors that drive dendritic spine formation. *Front. Cell Dev. Biol.* 10:836746. <https://doi.org/10.3389/fcell.2022.836746>

Chapter 3: **Byeon S***, Ferreccio A*, Cornell M, Oses-Prieto J, Deshpande A, Manvi S, Blencowe A, Weiss L, Burlingame A, Yadav S. (2025, accepted). TAOK2 drives opposing cilia length deficits in 16p11.2 deletion and duplication carriers. *Stem Cell Reports.* <https://doi.org/10.1016/j.stemcr.2025.102608>

*Co-first authors

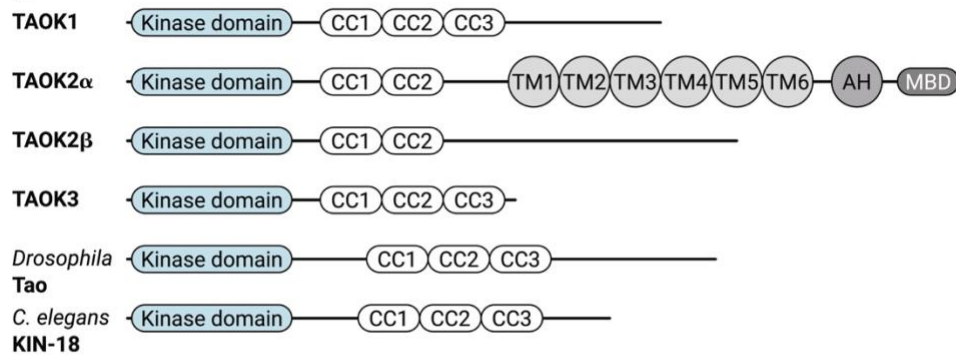
Chapter 1: Introduction

The thousand and one amino acid kinase (TAOK) family of protein kinases belong to the subfamily of germinal center kinases (GCK) within the sterile-20-like (Ste20) kinase family (1). Understudied for the most part, interest in TAOKs has increased with the growing evidence for their association with severe neurodevelopmental disorders (2-22). The TAOK family of kinases have been established functionally as MAPKKKs (mitogen-activated protein kinase kinase kinases), which phosphorylate MEKs [mitogen-activated protein/extracellular signal-regulated kinase kinases (MAPKKs)] that activate MAPK pathways, notably those mediated by p38 and c-Jun N-terminal kinase (JNK) (23-34). TAOKs regulate multiple cellular processes, including cell proliferation (35-38), cytoskeleton organization (39-48), mitosis (35-38, 42, 49-52), apoptosis (27, 33, 34), and immune responses (53-66).

The TAOK family is evolutionarily conserved, with three paralogous TAOKs in vertebrates (*TAOK1/PSK2/MARKK*, *TAOK2/PSK1*, and *TAOK3/JIK*) (23, 24, 67) and one TAOK ortholog in invertebrates (*Drosophila Tao* and *Caenorhabditis elegans Kin-18*) (Fig. 1A) (46, 68, 69). There has been much focus on the catalytic function of these kinases, and the crystal structure of the isolated kinase domain of TAOK2 has been solved (26). In vitro kinase assays using purified kinase domains of TAOK1 or TAOK2 show very similar substrate phosphorylation, consistent with the high homology in the protein sequence of the kinase domain (23, 24). Because of the indistinguishable catalytic function of the TAOKs, it was only recently that major differences between the functions of TAOK1 and TAOK2 have been revealed. This brings to light the importance of investigating kinase functions in the context of their often more protein-specific and structurally diverse regulatory domains (Fig. 1B). Relatively little is currently known about the

direct downstream phosphorylation targets of TAOs, their interaction partners and upstream activating kinases, or the signaling molecules that impinge upon TAOs. Furthermore, how mutations in TAOs affect their function at the molecular, cellular, and physiological level has been difficult to assess, given that the full-length structure of these kinases has not been solved. The main goal of this review is to highlight the functional pleiotropy that is intrinsic to TAOs and the evolutionary conservation as well as divergence in their functions, underscore the importance of studying the TAOs to understand neurodevelopment and disease, and define some of the main outstanding questions in the field.

A



B

Model confidence:

- Very high (pLDDT > 90)
- Confident (90 > pLDDT > 70)
- Low (70 > pLDDT > 50)
- Very low (pLDDT < 50)

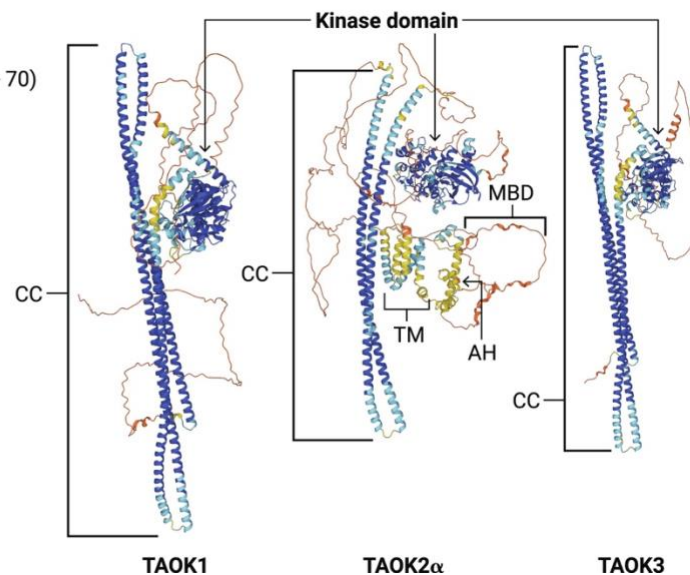


Figure 1.1. TAOs are evolutionarily conserved kinases.

(A) Schematic representations of secondary structures of human TAO1, alternatively spliced isoforms of TAO2, α and β , TAO3, *Drosophila* Tao, and *C. elegans* KIN-18 with highly conserved N-terminal kinase domain and variable C-terminal regulatory domains. CC, coiled-coil domain; TM, transmembrane domain; AH, amphipathic helix; MBD, microtubule binding domain.

(B) AlphaFold2.0-predicted protein structures of human TAO1, TAO2 α , and TAO3. Structures are color coded on the basis of the confidence score of prediction. AlphaFold produces a per-residue confidence score (pLDDT) between 0 and 100. Some regions below 50 pLDDT may be unstructured in isolation. CREDIT: A. FISHER/SCIENCE SIGNALING

TAO SIGNALING AND REGULATION

Identification of TAOs, their expression, and their structure

The first mammalian TAO, TAO1, was identified from rat in search of a mammalian ortholog of the *Saccharomyces cerevisiae* (yeast) protein kinase Ste20p (24). It was named *Tao1* for the thousand and one amino acids that encoded it. The same study also isolated partial complementary DNAs (cDNAs) encoding a closely related gene *Tao2* (24). Northern blotting showed that both *Tao1* and *Tao2* transcripts are enriched in the brain and testis (24). The complete sequence of rat *Tao2* was identified shortly after (23), followed by the subsequent identification of the human ortholog, PSK1 (prostate-derived STE20-like kinase 1)—called TAO2 hereafter—in a screen for kinases expressed in human prostate carcinoma (43). Human TAO3 was subsequently identified as JIK [JNK/stress-activated protein kinase (SAPK)—inhibitory kinase] in a screen for proteins that associate with a substrate of the epidermal growth factor receptor (EGFR) called EPS8 (67). The sequence of the human ortholog of TAO1—TAO1 hereafter—was reported shortly afterward

(70). Tissue-based mapping of the human proteome has shown that, in humans, TAOK1 is ubiquitously present, with the highest expression of TAOK1 in the brain, parathyroid gland, liver, prostate, cervix, adipose tissue, and skin (71-73). The highest tissue expression of TAOK2 is in the brain, parathyroid gland, liver, testis, pancreas, stomach, and colon (71, 72, 74). For TAOK3, the highest tissue expression in humans is in the stomach and kidney followed by the brain (71, 72, 75). Human brain transcriptome data show that within the brain, *TAOK1* is most highly expressed, followed by *TAOK2* and *TAOK3* during development and across the life span in humans (Fig. 2), consistent with the association of all three TAOKs with neurological disorders (76-78). Single-cell transcriptomics mapping of human tissues shows that within the brain, all members of the TAOK family are expressed in both neuronal and nonneuronal cells (72, 79-82). At the mRNA level, TAOK1 is most highly expressed in oligodendrocytes, TAOK2 in astrocytes, and TAOK3 in oligodendrocyte precursor cells, suggesting unique roles that each may play in different nonneuronal cells, in addition to neuronal cells, during development and in disease (72, 79-82).

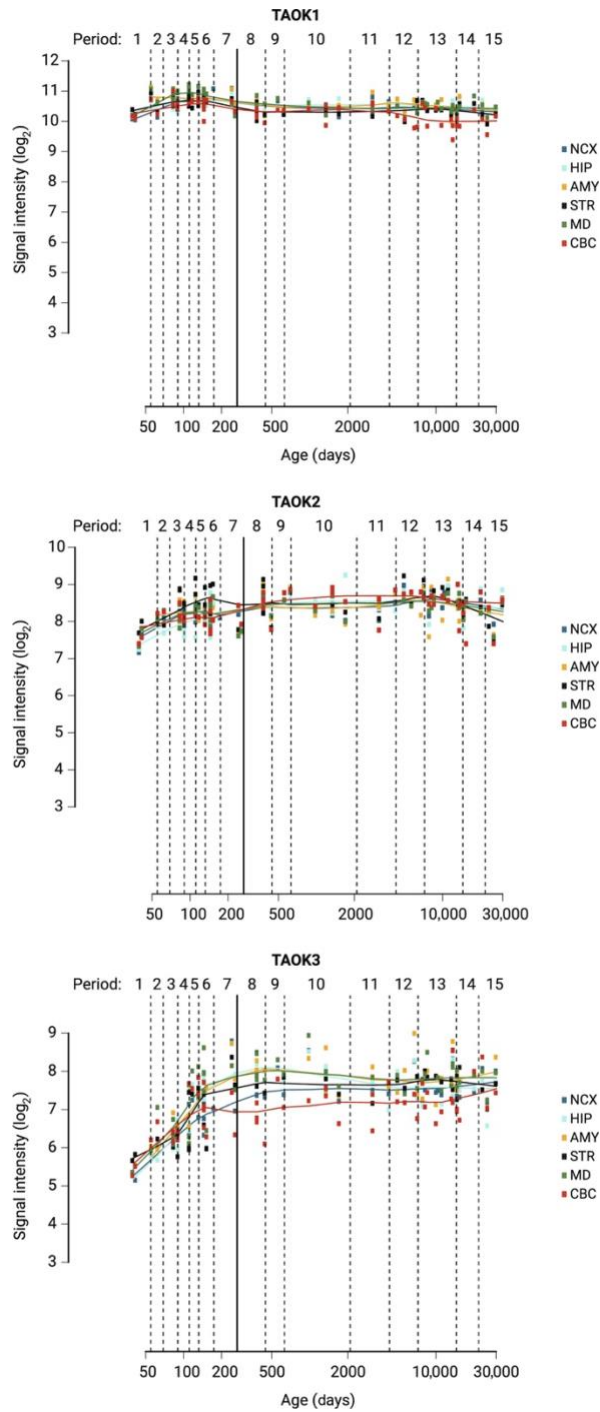


Figure 1.2. TAOKs are highly expressed in the human brain.

Gene expression level of human TAOK1, TAOK2, and TAOK3 in different brain regions from early human development to late adulthood. Images are from the Human Brain Transcriptome.

NCX, neocortex; STR, striatum; HIP, hippocampus; MD, mediodorsal nucleus of the thalamus; AMY, amygdala; CBC: cerebellar cortex. CREDIT: A. FISHER/*SCIENCE SIGNALING*

At the structural level, all three mammalian TAOK paralogs share a highly conserved N-terminal kinase domain, followed by regions predicted to form coiled coil domains (Fig. 1, A and B) (83, 84). In TAOK1, these coiled coil domains likely fold into a triple helix (85). This triple helix has a crescent shape and shows surface amino acid charge separation similar to inverse bin/amphiphysin/rvs (BAR) domain proteins, where the convex side is positively charged and can thus bind the plasma membrane through electrostatic interactions with the negatively charged plasma membrane (85). The purified TAOK1 triple helix directly interacts with negatively charged phosphoinositides, including PI(4)P, PI(4,5)P₂, and PI(3,4,5)P₃, that are enriched in the plasma membrane (85). Interestingly, membrane binding by the TAOK1 triple helix is negatively regulated by its kinase domain through phosphorylation at residues Thr440 and Thr443 that sit at the interface of the kinase domain and the triple-helical bundle (85). The helical bundle of TAOK1 is evolutionarily conserved and is found in both *Drosophila* Tao and *C. elegans* KIN-18, implying that the plasma membrane-binding ability might be a shared feature among vertebrate TAOK1 and invertebrate Tao proteins (85). This evolutionarily conserved triple-helical architecture is shared by TAOK3, but whether it binds membranes has not been tested (Fig. 1, A and B). In vertebrates, TAOK2 has evolved a unique hydrophobic domain not shared by TAOK1 or TAOK3 kinases. TAOK2 has at least two alternatively spliced isoforms, TAOK2 α and TAOK2 β (34, 43). Instead of three distinct coiled-coiled domains that form a triple helix, TAOK2 α contains two coiled coils followed by a region of very high hydrophobicity predicted to form six transmembrane domains followed by an amphipathic helix (Fig. 1, A and B) (42). Through its six transmembrane

domains, TAOK2 α localizes to the endoplasmic reticulum (ER), and the amphipathic helix was shown to be important for localizing TAOK2 α to specific subdomains within the ER membrane (42). After the ER targeting domain, TAOK2 α harbors an SXIP motif that allows its association with a microtubule plus-end binding protein, EB1, and a microtubule binding domain for direct interaction with tubulin (Fig. 1, A and B) (42). Through these distinct domains, TAOK2 α functions to tether the ER to the microtubule cytoskeleton (42). It is important to note that none of the mammalian TAOK1/2 β /3, the fly Tao, or *C. elegans* KIN-18 have domains that have been shown to directly bind microtubules or contain the ER targeting domain. Hence, it appears that the function of TAOK2 α as an ER-microtubule tether is unique in the TAOK family and has been acquired in vertebrates (42).

Expression analyses of TAOKs in *Xenopus laevis* revealed that they are expressed during early development before gastrulation (86). Taok1 and Taok3 are highly expressed in pre-gastrula and gastrula stage embryos, whereas Taok2 expression is lower in the early embryo (86). *C. elegans* TAOK ortholog, KIN-18, was shown to regulate cortex contractility through interaction with Ras-like GTP binding protein (RHO-1) in the early embryo, contributing to establishment of polarity (69). KIN-18 is highly expressed in pharynx, gut, anus, and excretory cell and was shown to regulate feeding (68). This suggests that the TAOK family of kinases may be critical during very early embryonic development in addition to neurodevelopment and immunity, with isoform-specific differences in their distinct roles that may be consistent with their expression patterns.

TAOKs regulate MAPK signaling

MAPK pathways are signaling cascades that mediate responses to a variety of extracellular stimuli. The TAOK family of kinases mediate MAPK signaling by regulating the activity of MAPKKs (Fig. 3A). TAOK1 and TAOK2 selectively activate MEK3, MEK4, and MEK6, but not MEK1 or MEK2 of the classical MAPK cascades (23-26). TAOK1/2 activate the JNK/SAPK pathway through phosphorylation of MEK4, although direct binding has not been observed (23-25). TAOK1/2 can bind and phosphorylate MEK3, thereby activating the stress-sensitive p38 MAPK cascade (23-25). In addition to MEK3, TAOK2 also associates with and activates MEK6 on residues Ser207 and Thr211 (23, 25, 26). TAOK2 activity on MEK3 and MEK6 is enhanced by stress stimuli that activate JNK/SAPK or p38, such as sorbitol and, to a lesser extent, NaCl, Taxol, and nocodazole (25). Sorbitol, NaCl, and staurosporine also increase TAOK1 activity and downstream JNK activity (27). Another study showed that TAOK2 associates with TAK1 (transforming growth factor- β -activated kinase 1), an essential MAPKKK for the activation of JNK and p38 in signaling pathways mediated by tumor necrosis factor (TNF), interleukin 1 (IL-1), and Toll-like receptors (TLRs), and together may mediate osmotic stress-induced activation of JNK (32). Interestingly, this association was shown to be independent of kinase activity, as a catalytically deficient TAOK2 mutant (D169A) can still bind TAK1 (32). TAOK1 was also shown to bind TAK1, but it is unclear whether osmotic stress-induced JNK activation by TAOK1 is mediated through its interaction with TAK1 (32). TAOK1/2-mediated activation of p38 is increased by the muscarinic receptor agonist carbachol, which then activates transcription factors (specifically ternary complex factors) in the nucleus (28). The role of TAOK3 in MAPK signaling, on the other hand, has been perplexing. TAOK3 was shown to inhibit both basal JNK activity and epidermal growth factor (EGF)-induced activation of JNK when transfected in CV-1 in Origin

with SV40 genes (COS7) cells (67). TAOK3 also inhibits ethanol-elicited JNK activation in vivo in mice (87). In contrast, when transfected in NIH3T3 cells, TAOK3 was shown to activate ERK1/2 and JNK/SAPK pathway (29). TAOK3 also increases JNK phosphorylation in response to ER stress induced by protein misfolding, by binding to and driving interaction between inositol-requiring transmembrane kinase/endoribonuclease 1 α (IRE1 α), an ER stress sensor, and TNF receptor-associated factor (TRAF2) (30). Intriguingly, all three TAOKs were shown to mediate activation of p38 by genotoxic stimuli, such as ionizing radiation and ultraviolet radiation, thereby regulating DNA damage-induced G2-M cell cycle checkpoint (31). DNA damage can occur as a result of other cellular stressors mentioned earlier, such as NaCl (88, 89); therefore, TAOKs may be an important component of cell cycle checkpoints in response to DNA damage, with each member exhibiting differential responsivity to distinct stressors. These studies suggest that the TAOK family of kinases may mediate MAPK signaling in a context- and stimuli-dependent manner.

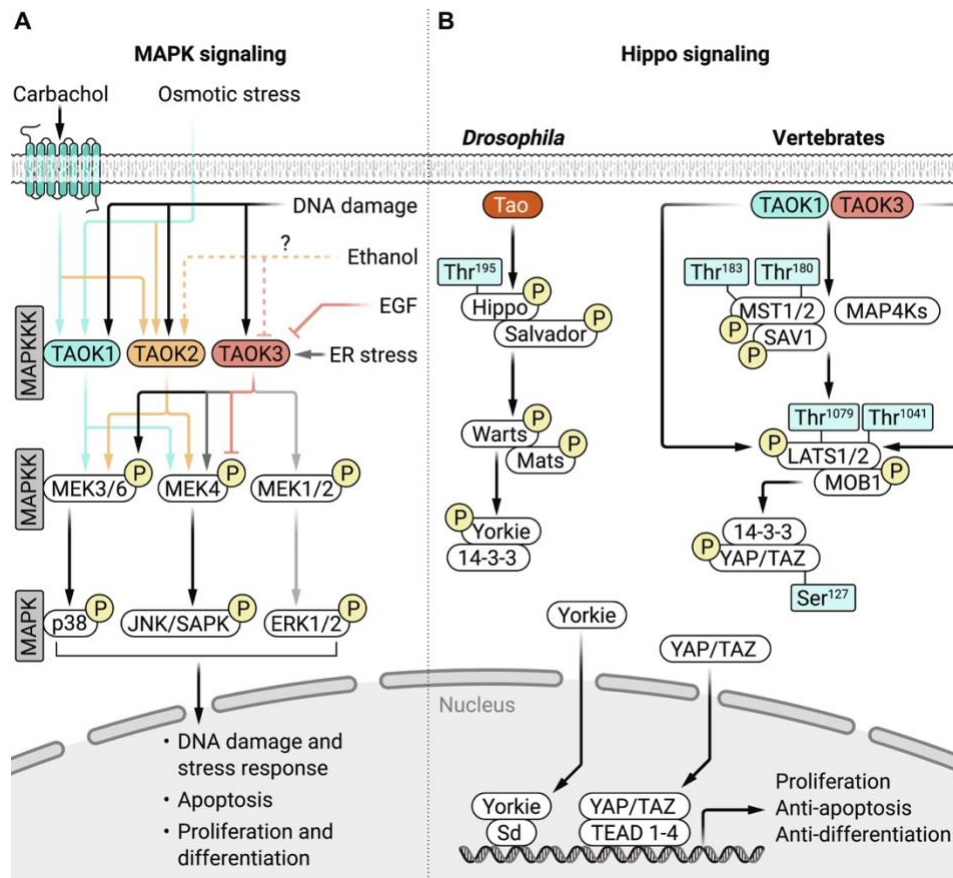


Figure 1.3. TAOKs regulate the MAPK and Hippo signaling pathways.

(A) All three members of TAOK family are involved in MAPK signaling pathways. TAOK1/2/3 are activated by DNA damage, leading to MEK3/6-mediated p38 activation. Muscarinic receptor agonist carbachol increases TAOK1/2 activity on p38. Osmotic stress increases TAOK2 activity on MEK3/6 and TAOK1 activity on JNK. TAOK1/2 activate JNK through MEK4 activation, whereas TAOK3 can both negatively and positively regulate JNK in a context-dependent manner. Ethanol induces JNK activation through its effect on TAOK2 and TAOK3 through unclear mechanisms. TAOK3 inhibits both ethanol- and EGF-induced JNK activation. EGF also inhibits TAOK3 activity. TAOK3 activates JNK pathway in response to ER stress. TAOK3 can uniquely activate ERK1/2 through MEK1/2 activation. **(B)** *Drosophila* Tao and mammalian TAOK1 and TAOK3 activate Hippo signaling pathways. Tao phosphorylates Hippo kinase, which, in turn,

phosphorylates Warts and Yorkie. Yorkie associates with 14-3-3 upon phosphorylation, preventing Yorkie-mediated transcription in the nucleus. TAOK1 and TAOK3, but not TAOK2, similarly activate Hippo pathway either through phosphorylation of MST1/2 (mammalian homolog of Hippo) and MAP4K kinases, or direct phosphorylation of downstream LATS1/2 (mammalian homolog of Warts), inhibiting YAP/TAZ (mammalian homolog of Yorkie)–mediated transcription in the nucleus. Phosphorylation sites are labeled in teal. CREDIT: A. FISHER/*SCIENCE SIGNALING*

TAOKs regulate Hippo signaling

TAOKs are important regulators of Hippo signaling (Fig. 3B) (35–38). *Drosophila* Tao negatively regulates cell proliferation through phosphorylation of Hippo kinase at the critical activating residue (Thr195) that leads to down-regulation of Hippo-Salvador-Warts (HSW) pathway target genes including Yorkie, a transcriptional coactivator that promotes cell growth (35, 36, 38). Mammalian TAOK1 and TAOK3, but not TAOK2, can either phosphorylate mammalian sterile-20–like kinase 1/2 (MST1/2) (mammalian homolog of Hippo) directly at residues Thr183 on MST1 and Thr180 on MST2 (35–37), or bypass MST1/2 and phosphorylate large tumor suppressor kinase 1/2 (LATS1/2) directly at residue Thr1079 on LATS1 and Thr1041 on LATS2 (37), which leads to phosphorylation of yes-associated protein (YAP) (at Ser127) and transcriptional coactivator with PDZ-binding motif (TAZ) (mammalian homolog of Yorkie), thereby inhibiting YAP/TAZ-mediated transcription in the nucleus and in turn limiting cell proliferation.

TAOKs regulate the organization of the cytoskeleton

The TAOK family of kinases can induce substantial changes in the cytoskeleton through their ability to regulate three major cytoskeletal components: microtubules, actin, and septins (Fig. 4A). In mammals, TAOK1 regulates microtubule dynamics through phosphorylation of downstream kinase MARK2 (at Thr208) or polarity kinase 1 (PAR-1) (at Thr408), which then phosphorylates the microtubule-associated protein, tau, at KXGS motifs causing its detachment from microtubules and microtubule disassembly (39). This function of TAOK1 was shown to be regulated by TESK1 (testis-specific protein kinase), an actin-regulating kinase (40). TESK1 inhibits TAOK1 independently of its kinase activity, preventing microtubule disassembly while increasing actin stress fibers through phosphorylation and inactivation of cofilin (40). TESK1 activity is inhibited by Spred1 [Sprouty-related protein with *Drosophila* enabled/vasodilator-stimulated phosphoprotein homology 1 (EVH-1) domain1], a membrane-associated protein that negatively regulates ERK/MAPK pathway and also associates with TAOK1 (40). Although TAOK1 does not affect Spred1 inhibition of TESK1 and Spred1 does not affect TESK1 inhibition of TAOK1, the interaction of TAOK1 with TESK1 and Spred1 could potentially coordinate a cross-talk between actin and the microtubule cytoskeleton (40). In neuronal PC12 cells, increased microtubule dynamics induced by TAOK1 activity promotes the development of neurites (39). TAOK1 colocalizes with MARK and phosphorylated tau at the plasma membrane and at neurite growth cones (39). TAOK2 α is the only member of the TAOK family that has been reported to directly bind microtubules via its C-terminal tail region that is not present in TAOK1 or TAOK3 (41, 42). Interestingly, microtubule binding by TAOK2 α is negatively regulated by its kinase activity; a kinase-deficient (K57A) mutant TAOK2 α exhibits increased association with microtubules and enhanced ER-microtubule tethering (42). Through the SXIP motif, TAOK2 α also directly binds to

the microtubule plus-end binding protein EB1, regulating ER movement along growing microtubule plus tips (42). Moreover, TAOK2 α can directly phosphorylate tubulin (41), but whether TAOK1 or TAOK3 can directly phosphorylate tubulin remains to be determined. TAOK signaling also regulates the actin cytoskeleton. TAOK2 expression disrupts actin filament organization and reduces actin stress fibers, although it is not clear whether this is a direct role or is mediated indirectly through other effectors (43). TAOK2 β associates with RhoA, a guanosine triphosphatase (GTPase) involved in modulating stability of F-actin, leading to actin stabilization (18). Both TAOK1 and TAOK2 also directly phosphorylate Septin 7, a member of the septin family, at the Thr426 residue, regulating its localization (44, 45). The role of TAOK3 in cytoskeleton organization awaits further investigation.

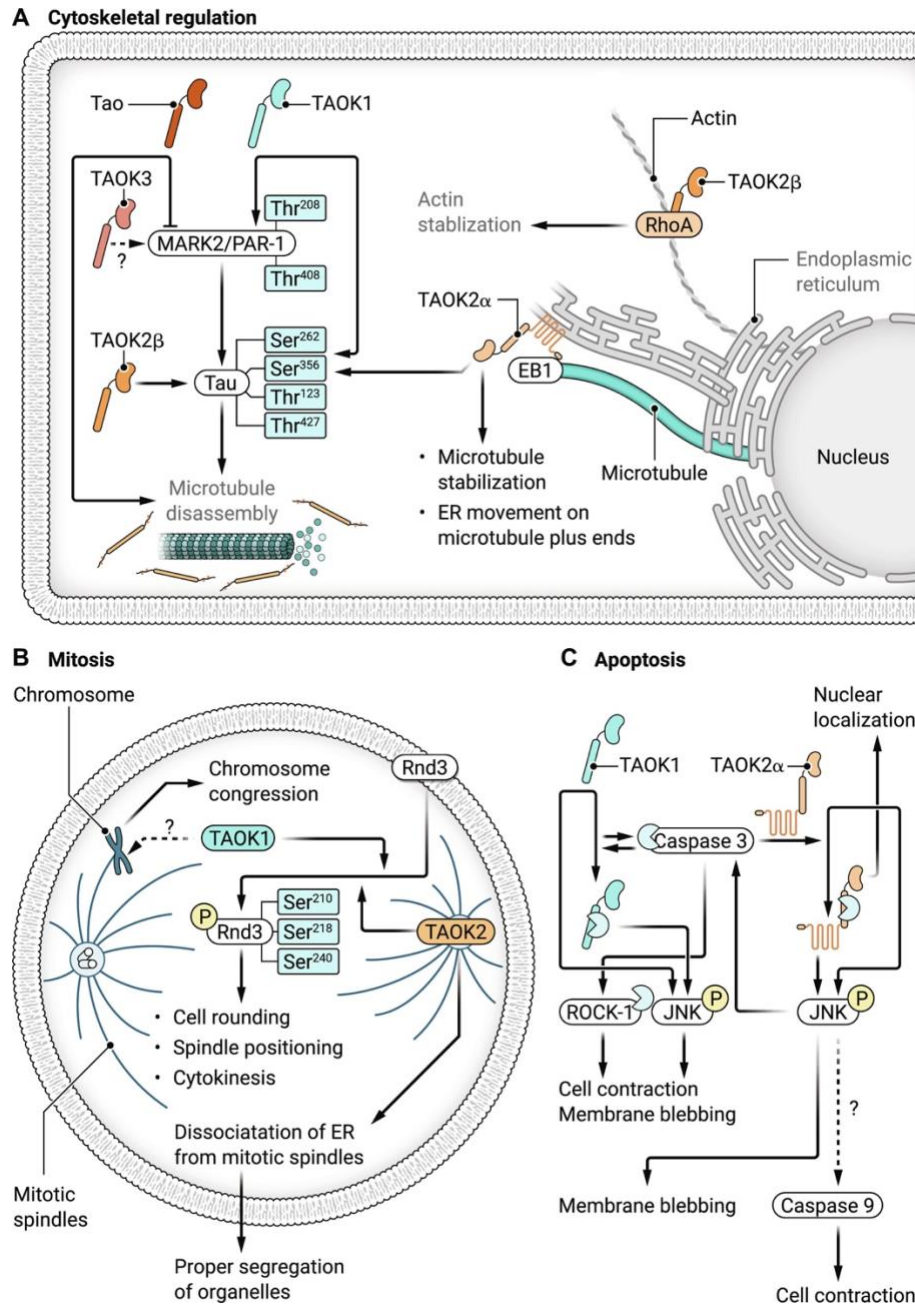


Figure 1.4. TAOKs regulate cytoskeletal organization, mitosis, and apoptosis.

(A) TAOK1 phosphorylates MARK2/PAR-1, which then phosphorylates tau, leading to its dissociation from microtubules and microtubule disassembly. *Drosophila* Tao has been reported to not only down-regulate MARK2/PAR-1 phosphorylation and increase microtubule stability but also negatively regulate microtubule stability similarly to mammalian TAOK1. TAOK1/2 can also

directly phosphorylate Tau. TAOK2 α , an integral ER kinase, can directly bind both tubulin and the microtubule plus tip binding protein, EB1, regulating microtubule dynamics and ER-microtubule tethering. TAOK2 β activates RhoA GTPase, which leads to actin stabilization. TAOK3 function in cytoskeletal control is unknown. **(B)** TAOK1/2 activity is increased in mitosis. TAOK1/2 phosphorylate GTPase Rnd3, leading to its dissociation from the plasma membrane, which is required for proper spindle positioning and cell rounding. TAOK1 is cytoplasmic, whereas TAOK2 α is enriched at the spindle poles, where it plays an important role in tethering of ER membranes to the spindle pole ensuring proper mitotic segregation. TAOK1 depletion leads to defects in chromosomal congression, likely through an effect on spindle microtubules. **(C)** TAOK1 and TAOK2 α are both substrates of caspase-3 and regulate apoptosis. TAOK1 induces cell contraction and membrane blebbing through activation of JNK and caspase-mediated cleavage of ROCK-1. TAOK2 α is translocated to nucleus once cleaved by caspase-3 and induces membrane blebbing through JNK activation and cell contraction potentially through activation of downstream caspase-9. Phosphorylation sites are labeled in teal. CREDIT: A. FISHER/*SCIENCE SIGNALING*

Studies in *Drosophila* showed contrasting roles of Tao in regulation of microtubule dynamics. One study showed that *Drosophila* Tao, similar to mammalian TAOK1, negatively regulates microtubule stability (46). Loss of Tao activity prevents depolymerization of microtubule plus ends, which then continue to grow and push against the actin cortex, ultimately causing microtubule-based protrusions (46). Formation of abnormal membrane protrusions were also observed in mammalian cells expressing neurodevelopmental disorder-associated mutations in *TAOK1* that render it catalytically inactive (85). In contrast to mammalian TAOK1, *Drosophila* Tao was also shown to be a negative regulator of PAR-1, leading to reduced phosphorylated tau and, thereby, increased microtubule stability (47, 48).

TAOKs regulate mitosis

TAOK1 and TAOK2 are activated during mitosis with increased levels of phosphorylation at the regulatory TAOK1/2 site Ser¹⁸¹; however, the upstream kinase or trigger that leads to its mitotic activation is unknown. Mitotically activated TAOK1 and TAOK2 localize to the cytoplasm and the spindle poles, respectively, and regulate mitotic cell rounding and spindle positioning (Fig. 4B) (49). Rnd3, a constitutively guanosine 5'-triphosphate (GTP)-bound member of the Rho family, has been proposed to be one of the potential downstream substrates of TAOK1/2 in their role in mitosis, specifically mitotic cell rounding, spindle centralization, and cytokinesis (50). TAOK1 and TAOK2 interact with and phosphorylate Rnd3 at residues Ser²¹⁰, Ser²¹⁸, and Ser²⁴⁰ on the C terminus, causing Rnd3 to translocate from the active site at the plasma membrane to the cytosol (50). An initial study in which TAOK1 was identified as a spindle checkpoint protein that interacted with the checkpoint kinase BubR1 and recruited checkpoint protein Mad2 to sites of defective kinetochore attachment has been refuted, and the results were likely off-target effects of

small-interfering RNA (siRNA) (51). Further studies have demonstrated that down-regulation of TAOK1 with a more specific siRNA did not affect the spindle checkpoint and that TAOK1 did not interact with BubR1, questioning its role in the spindle checkpoint (90, 91). Nevertheless, the initial study also implicated the role of TAOK1 in chromosome congression (51), which was further corroborated in a subsequent study using a different siRNA (52). Down-regulation of TAOK1 was shown to increase missegregated lagging chromosomes and concurrently led to a delay in anaphase, potentially through impaired microtubule stability and kinetochore-microtubule attachment, although the exact molecular mechanism is not yet known (52). Further studies in both fly and mammalian system have shown that knockdown of Tao/TAOK1 has a proliferative effect on cells through its role in Hippo signaling, as discussed above (35–38). Last, as an ER-microtubule tether, TAOK2 α kinase activity is required for the dissociation of ER from mitotic spindles, failure of which leads to mitotic defects (42). The downstream substrates or upstream kinases mediating these roles of TAOs in mitosis remain to be identified.

TAOs regulate apoptosis

TAOK1 regulates apoptotic morphological alterations, specifically cell contraction and plasma membrane blebbing, through activation of JNK and ROCK-1 (Rho kinase-1), which, in turn, leads to reorganization of cortical actin (Fig. 4C) (27). TAOK1 is cleaved by caspase-3 that is activated during apoptosis, and caspase 3 is required for TAOK1-mediated membrane blebbing (27). Active TAOK1 further enhances caspase-3 activation (27). However, the kinase activity of TAOK1 has not been universally required to induce apoptosis despite the involvement of both caspase-3 and JNK activity (33). Similar to TAOK1, the α isoform of TAOK2 also induces apoptosis-associated morphological changes, including cell contraction, membrane blebbing, and apoptotic body

formation, whereas the β isoform does not (34). TAOK2 α -mediated apoptosis is dependent on its catalytic activity and JNK activation, which, in turn, may potentially lead to activation of caspase-3 and caspase-9, causing C-terminal cleavage of TAOK2 α and its translocation to the nucleus (Fig. 4C) (34).

TAOKs regulate the host immune response

There is emerging evidence that the TAOK family of kinases play a critical role in immune response against viral infection (Fig. 5A). Screens to identify evolutionarily conserved proteins that interact with viral genetic material—double-stranded DNA or RNA—revealed TAOKs as the strongest hit with evolutionarily conserved antiviral activity in humans, mice, and flies (53). In human cells, each TAOK exhibited specificity in the response to distinct viruses: All three TAOKs mediate the response to influenza A virus (IAV), whereas TAOK2 also mediates that against herpes simplex virus 1 (HSV1) and Semliki Forest virus (SFV) (53). *Drosophila* Tao promotes the antiviral response against *Drosophila C* virus (DCV) and vesicular stomatitis virus (VSV) (53). The TAOKs directly bind viral double-stranded RNA; however, the domain responsible for nucleotide binding is yet unidentified. Furthermore, each TAOK differentially modulates interferon induction in a cell type– and stimulus-specific manner.

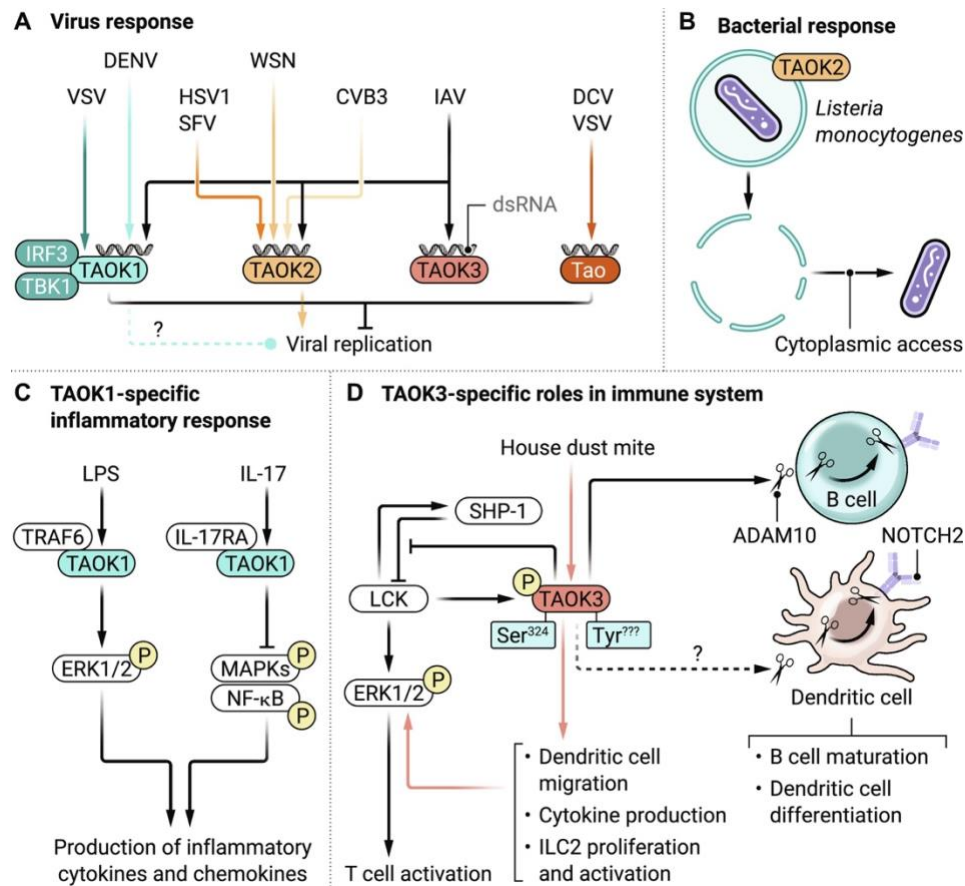


Figure 1.5. TAOKs mediate various modes of immune signaling.

(A) All members of TAOK family can directly bind viral double-stranded RNA and either inhibit or promote viral replication in a stimulus-dependent manner. TAOK1/2/3 inhibit the replication of IAV, whereas TAOK2 also inhibits that of HSV1, SFV, and CVB3. TAOK1 interaction with TBK1 and IRF3 inhibits replication of VSV. TAOK1 interacts with a component of DENV but whether it regulates viral replication is unclear. TAOK2 promotes viral replication in response to influenza A strain WSN. *Drosophila* Tao is antiviral against DCV and VSV. (B) TAOK2 can bind and rupture *L. monocytogenes*-containing vacuoles, enabling their cytoplasmic access. (C) TAOK1 promotes inflammatory responses to LPS through interaction with TRAF6 and activation of ERK1/2, whereas it inhibits IL-17-mediated responses through interaction with IL-17RA and inhibition of MAPKs and NF-κB. (D) Upon TCR signaling, LCK induces phosphorylation of

TAOK3, and TAOK3 promotes T cell activation by inhibiting interaction of LCK with Src homology region 2 domain-containing phosphatase-1 (SHP-1) and activating ERK1/2. TAOK3 regulates house dust mite-induced asthma by affecting dendritic cell migration and innate ILC2 function, which also mediates ERK1/2 phosphorylation. Last, TAOK3 mediates B cell and potentially dendritic cell maturation by regulating the surface expression of ADAM10 and the signaling activity of NOTCH2. CREDIT: A. FISHER/*SCIENCE SIGNALING*

TAOK2 was unexpectedly shown to localize to nuclear speckles, regulating splicing and nuclear export of M mRNA of IAV strain WSN (56). Accordingly, depletion of TAOK2 in host cells decreases influenza viral protein levels and influenza viral replication (Fig. 5A) (56). In contrast, TAOK2 inhibits replication of Coxsackie virus B3 (CVB3) (57), consistent with its antiviral activity against HSV1, SFV, and IAV (Fig. 5A) (53). An initial study linking the TAOK2 homozygous variant R700C with generalized verrucosis caused by human papillomavirus (HPV) has since been retracted; therefore, no conclusions can yet be made on the role of TAOK2 in HPV infection or on the functional consequence of the R700C TAOK2 variant. In addition, TAOK2 appears to directly function in response to internalized bacterial pathogens, specifically *Listeria monocytogenes*, aiding in their escape from host-cell vacuoles (Fig. 5B) (58).

TAOK1 negatively regulates IL-17-mediated proinflammatory cytokine production in a kinase activity-independent manner via interaction with IL-17RA through its C-terminal domain and inhibition of MAPKs and nuclear factor κ B (NF- κ B) (Fig. 5C) (59). In contrast, TLR4-mediated LPS (lipopolysaccharide)-induced proinflammatory cytokine production is positively regulated by TAOK1, also in a kinase activity-independent manner via interaction with TRAF6 through its

C-terminal domain and activation of ERK1/2 (Fig. 5C) (60). Moreover, TAOK1 positively regulates VSV-induced interferon production but in a kinase activity–dependent manner through its interaction with TBK1 kinase and IRF3 (Fig. 5A) (61). TAOK1 may also be potentially involved in immune response to dengue virus (DENV) infection, as it was shown to interact with DENV nonstructural protein 1; however, further studies are required to determine the role of TAOK1 in DENV infection (Fig. 5A) (62).

In the spleen, TAOK3 regulates differentiation of neurogenic locus notch homolog protein 2 (NOTCH2)–dependent transitional B cells into MZB (marginal zone B) cells by inducing surface expression of a metalloprotease ADAM10 (a disintegrin and metalloprotease 10) (Fig. 5D) (63). TAOK3 also regulates differentiation of conventional dendritic cells in the spleen and, in turn, CD4⁺ T cell priming in response to allogeneic red blood cells (Fig. 5D) (64). Moreover, TAOK3 mediates canonical TCR (T cell receptor) signaling in T cells by regulating interaction between an Src family tyrosine kinase, LCK (lymphocyte-specific protein tyrosine kinase), and SHP-1, a phosphatase that terminates TCR signaling (Fig. 5D) (65). TAOK3 was also shown to modulate LCK-dependent ERK1/2 activation (65). In addition, TAOK3 was shown to mediate development of house dust mite–induced asthma in mice through both adaptive and innate type 2 immunity in the lungs, by promoting CD4⁺ T_H2 (T helper cell 2) lymphocyte–mediated cytokine production and epithelium-derived alarmin IL-33–dependent type 2 innate lymphoid cells (ILC2) proliferation and function, respectively (Fig. 5D) (66). Interestingly, loss of Taok3 in hematopoietic cells, but not in lung epithelial cells, failed to develop asthma features, suggesting that TAOK3 may play a critical role in immune cells (66). The immune function of TAOK3 in asthma is dependent on its kinase activity, as an inactivating K53A mutation also leads to reduced asthma and ILC2 activation

(66). Furthermore, TAOK3 modulated IL-33–induced ERK1/2 activation (66). These studies suggest that the role of TAOK3 in immune response may potentially be mediated through its role as an MAPKKK, specifically regulating ERK1/2 signaling, in a stimuli-dependent manner; however, further studies are required to test this hypothesis and elucidate the exact molecular mechanism underlying the immune function of TAOK3. Mechanistically, at which step distinct TAOKs regulate viral entry, replication, signaling, and host gene expression in response to infection remains to be determined. Given the distinct structure and cellular localization of TAOKs and their splice isoforms, it is highly probable that each member of the TAOK family plays very specific and distinct roles.

THE TAOK FAMILY OF KINASES IN NEUROLOGICAL DISORDERS

TAOKs in neurodevelopment

The TAOKs play an important role in neuronal development. *Drosophila* Tao was shown to affect brain size as well as branching of axon and dendrites and synapse formation (6, 8, 38, 48, 92, 93). Knockdown of *Drosophila* Tao, an ortholog of human TAOK, in larval neuroblasts results in increased brain volume by modulating neuroblast division and proliferative and differentiating capacity via downstream transcription coactivator Yorkie (38). Tao controls axon guidance within the mushroom body, one of the major neuropil structures of the *Drosophila* central brain, during metamorphosis via negative regulation of the downstream Par-1 kinase, which, in turn, regulates phosphorylation of tau (Fig. 6A, top left) (48). Depletion of *Tao* also leads to a decrease in ventral nerve cord size, number of axons originating in the ventral nerve cord, and number of larval neuromuscular junctions (6). Tao negatively regulates dendritic arborization in a kinase activity–dependent manner by regulating microtubule dynamics and F-actin levels and distribution, both

during development and in adults (93). Moreover, Tao negatively regulates synapse formation (92, 93). Loss of *Tao* leads to increased postsynaptic connectivity and, further, ectopic synapse formation in a kinase activity–dependent manner, altering behavioral responses during larval development (92). Similar to *Drosophila* Tao, TAOK1 in mouse primary hippocampal neurons was also shown to negatively regulate dendritic branching (8). In addition, changes in levels of TAOK1 expression, either up-regulation or down-regulation, leads to impaired neuronal migration both in vitro and in vivo in mice (8).

dendritic spine development (top right), phosphorylation of TAOK1 at Thr⁴⁴⁰ and of TAOK2 at Thr⁴⁶⁸ by MST3 kinase induces their interaction with myosin Va and its recruitment to dendrites, promoting spine and synapse formation. TAOK1 can also autophosphorylate at both Thr⁴⁴⁰ and Thr⁴⁴³ residues. Myosin Va recruitment to ER is likely mediated by Rab proteins. Spine density and synaptic activity (bottom right) are regulated by TAOK1 and TAOK2 through various mechanisms. Phosphorylation of septin 7 (gray), a cytoskeletal GTPase, at Thr⁴²⁶ by TAOK1/2 translocates septin 7 from the base of dendritic protrusions to dendritic spine heads, promoting spine synapse maturation through stabilization of PSD95 and interaction with 14-3-3. TAOK2 β also regulates spine number through association with RhoA (orange) and subsequent stabilization of F-actin. TAOK2 β can negatively regulate spine number in response to synaptic activity. Synaptic activity increases expression of arcadlin in excitatory synapses. Arcadlin dimerizes and activates TAOK2 β , which constitutively associates with the intracellular domain of arcadlin. TAOK2 β phosphorylates downstream MEK3 and in turn p38 (purple), which then phosphorylates TAOK2 β at Ser¹⁰³⁸, leading to endocytosis of N-cadherin and arcadlin at the synapse. CREDIT: A. FISHER/*SCIENCE SIGNALING*

Depletion of TAOK2 leads to an overall increase in total brain volume in mice (18). TAOK2 activation by neuropilin 1 in complex with semaphorin 3A (Sema3A), a secreted guidance cue, is important for the downstream activation of JNK through dual phosphorylation at Thr¹⁸³ and Tyr¹⁸⁵ residues that mediates axon projection and basal dendrite arborization in mouse cortical neurons both in vitro and in vivo (Fig. 6, bottom left) (94). Phosphorylation of TAOK1 and

TAOK2 at the conserved residue (Thr⁴⁴⁰ and Thr⁴⁶⁸, respectively) by MST3 mediates dendritic spine and synapse formation through association with and recruitment of myosin Va, an actin-based motor protein, to dendrites in rat hippocampal neurons (Fig. 6, top right) (95). Myosin Va was shown to colocalize with Rab11, which is associated with recycling endosomes (95). In a chemical-genetic screen of direct TAOK2 substrates, the cytoskeletal GTPase, septin 7, was identified (44). TAOK2 phosphorylated septin 7 at the residue Thr⁴²⁶, which causes septin 7 to translocate to the dendritic spine head and stabilize the postsynaptic scaffolding protein, PSD95, facilitating spine and synapse maturation in rat hippocampal neurons (Fig. 6, bottom right) (44). Using a phosphopeptide pulldown and biochemical assays, septin 7 function in dendritic spine development was shown to be mediated through its association with 14-3-3 γ protein upon phosphorylation at the Thr⁴²⁶ residue by TAOK1/2 (Fig. 6, bottom right) (45). In contrast, another study using *Taok2* heterozygous (Het) and complete knockout (KO) mice did not find defects in dendritic branching and synapse formation in hippocampal neurons but only in cortical neurons, predominantly of the prefrontal cortex (18). *Taok2* Het and KO mice exhibit dosage-dependent changes in brain development and behavioral changes related to cognition, anxiety, and social interaction similar to behaviors observed in autism spectrum disorder (ASD) (18). A kinase dead de novo ASD-associated mutation identified in this study, A135P variant of both TAOK2 α and TAOK2 β , could not rescue the defects in dendritic branching and dendritic spine maturation in *Taok2* Het mouse cortical neurons, whereas the wild-type TAOK2 α/β could, suggesting that TAOK2-mediated regulation of dendritic and spine development is dependent on its catalytic activity (18). The same study also revealed that TAOK2 function in dendritic spine formation is mediated through activation of RhoA (Fig. 6, bottom right) (18). Interestingly, the RhoA-mediated effect may be specifically through its interaction with the TAOK2 β isoform, because it was shown

to preferentially bind the β compared with the α isoform by immunoprecipitation (Fig. 6, bottom right) (18). In both cortical neurons from *Taok2* KO mice and stem cell–derived human neurons with disruption of TAOK2, neurons exhibited altered cellular respiration and mitochondrial activity, size, and number (96). The isoform-specific contribution of TAOK2 in mitochondrial function and its underlying mechanism would be an important area of future investigation. Loss of TAOK2 function, either through knockdown or expression of catalytically inactive variant, also led to delayed neuronal migration in mice (97). Intriguingly, impaired neuronal migration was observed only in the α isoform harboring the kinase dead ASD–associated variant, A135P, but not in the β isoform, suggesting an isoform-specific role in migration (97). Furthermore, another study has identified a β isoform–specific contribution of TAOK2 in neurodevelopment. TAOK2 β isoform regulates activity-induced dendritic spine formation in rat and mouse hippocampal neurons through endocytosis of cell adhesion protein complex of N-cadherin and arcadlin (Fig. 6, bottom right) (98). Upon synaptic activity, homophilic interactions of arcadlin on the cell surface activate TAOK2 β , which then activates downstream MEK3 and p38 MAPK (98). Activated p38 phosphorylates TAOK2 β at Ser¹⁰³⁸ residue, causing internalization of N-cadherin and arcadlin, which then leads to decreased spine density (98). Whereas the importance of TAOK1 and TAOK2 in neurodevelopment has been clearly demonstrated through these studies, the role of TAOK3 in neurodevelopment is yet to be determined.

TAOKs in neurodevelopmental disorders

The initial identification of a de novo codon deletion variant in *TAOK1* in a single patient from the Autism Sequencing Consortium suggested TAOK1 as a potential autism-associated gene (Fig. 7 and Table 1) (2). Subsequent identification of a novel de novo microdeletion at chromosome

17q11.2, which encompasses *TAOK1*, in a patient with a developmental delay and microcephaly further posited *TAOK1* as a potential candidate gene for neurodevelopmental disorders (NDDs) (3). Two microdeletions at this chromosomal region were previously reported in the morbidity map of developmental delay (nsv1062993) (4) and in the Decipher database (decipher no. 250045) (5). A patient with *TAOK1* deletion (case no. 250045) exhibited microcephaly and seizures (3, 5). Both deletions included part of *TAOK1* gene without affecting other genes, suggesting the importance of *TAOK1* in NDDs (3). Further support came from analysis of the diagnostic exome sequencing data and GeneMatcher, identifying eight de novo variants in *TAOK1* in patients with NDDs (Table 1) (6). When compared between NDD and non-NDD trios, the difference in the frequency of de novo changes was the highest in *TAOK1* among the genes with high intolerance to loss-of-function variants (6). The eight variant carriers exhibited developmental delay with varying phenotypes, including seizures, intellectual disability, muscular hypotonia, attention deficit hyperactivity disorder (ADHD), autism, macrocephaly, and facial abnormalities (Fig. 7) (6). A variant of *TAOK1* that causes a frameshift in the protein at Leu⁷⁹⁰ (L790Ffs*3) led to reduced *TAOK1* expression at the cDNA, mRNA, and protein levels as well as reduced phosphorylation of a downstream substrate, tau (6). A large-scale, whole-exome sequencing (WES) analysis further identified *TAOK1* as one of the 102 ASD-associated genes (7). Nineteen additional NDD-associated variants in *TAOK1*, including two inherited mutations, have been identified through a collaboration with GeneMatcher (Table 1) (8). These included five missense variants, seven premature stop variants, four indels, and three canonical splice site variants (8). The individuals with these variants exhibited overlapping facial features, varying degrees of developmental delay and intellectual disability, neonatal feeding difficulties, muscular hypotonia, joint hypermobility, and recurrent ear and airway infections (8). Two individuals were diagnosed with autism, and

seven of them exhibited macrocephaly (Fig. 7) (8). The same study also identified three additional individuals with a chromosomal microdeletion (8). One of them was diagnosed with autism (8). Functional assessment of the five missense variants revealed changes in TAOK1 protein expression, neurite length, dendrite arborization, and neuronal migration (8). Five additional novel variants in TAOK1 were identified in recent case studies (Table 1) (9–11). A study identified one maternally inherited mutation using whole-genome sequencing (WGS) in a sibling pair and two de novo mutations, one missense, and one splice site variant using WES in unrelated individuals (9). Consistent with previously reported phenotypes in TAOK1-associated neurodevelopmental disorders, common features included global developmental delay, hypotonia, joint hypermobility, motor delay, speech delay, macrocephaly, autistic behavior, and neonatal feeding difficulties (Fig. 7) (9). The study reported additional consistent features including ventriculomegaly, increased body mass index, and GERD (gastroesophageal reflux disease) (9). A separate case report of a fetus with an expanded left lateral ventricle identified a de novo variant (E76G) through trio WES (Table 1) (10). Another de novo frameshift variant of TAOK1 (P422Lfs*50) was identified as the candidate gene associated with childhood-onset tremor through trio WES (Fig. 7 and Table 1) (11).

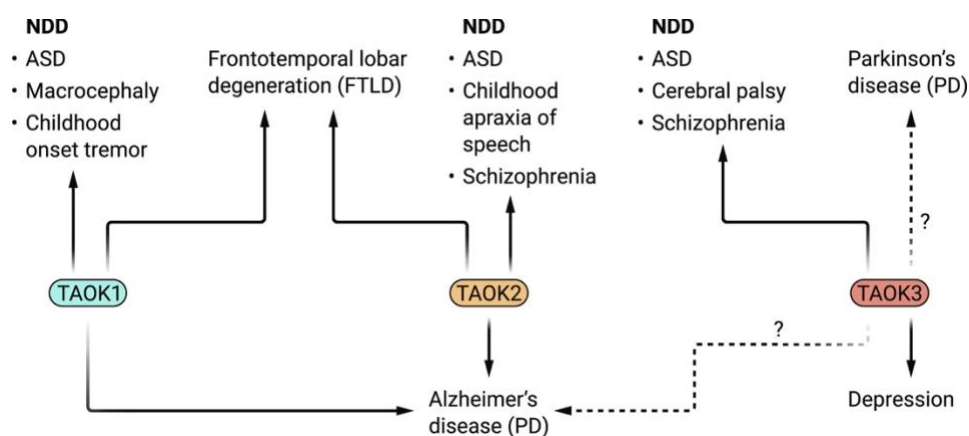


Figure 1.7. Dysfunction in TAOKs leads to neurological diseases.

Neurological disorders associated with the TAOK family are shown. The dotted line indicates connections that are not yet defined or tested and need further exploration. CREDIT: A. FISHER/*SCIENCE SIGNALING*

Table 1.1. Neurodevelopmental disorder–associated missense and truncation mutations within the TAOK family.

The table shows a list of the missense and truncation mutations in TAOK1, TAOK2, and TAOK3 that are associated with NDDs, noting their mode of inheritance.

Gene	Amino acid change	Inheritance	Reference
TAOK1	R82del	De novo	(2)
	P24T	De novo	(118)
	L167R	De novo	(118)
	A219V	De novo	(118)
	V289M	De novo	(118)
	E17G	De novo	(6)
	K298E	De novo	(6)
	E781*	De novo	(6)
	D305A	De novo	(6)
	Q544*	De novo	(6)
	S111F	De novo	(6)
	L790Ffs*3	De novo	(6)
	L790Ffs*3, L642Ffs*3	De novo	(119)
	E830*	De novo	(6)
	Y815Ifs*31	De novo	(8)
	L548P	De novo	(8)
	Q607*	De novo	(8)
	K277*	De novo	(8)
	E220*	Maternal, familial NDD	(8)
	R709*	Paternal	(8)
	R150I	De novo	(8)
	R695*	De novo	(8)
	T132Yfs*19	De novo	(8)
	R702*	Unknown	(8)
	L315F	De novo	(8)
	K429Nfs*42	De novo	(8, 119)
	K78Vfs*20	De novo	(8)
	M231V	Unknown	(8)
	R605*	Unknown	(8)
	R735Dfs*6	Maternal	(9)
	R442W	De novo	(9)
	E76G	De novo	(10)
	I157S	De novo	(119)
	R46*	De novo	(119)
	K166T	De novo	(119)
	P186L	De novo	(119)
	Y235C	De novo	(119)
	R269Q	De novo	(119)
	I294Rfs*7	De novo	(119)
	K314_F317delinsMC	De novo	(119)
	E558Rfs*3	De novo	(119)
	Q201R	De novo	(119)
	p.Arg472*	De novo	(119)
	F280Lfs*11	De novo	(119)
	I89M	De novo	(119)
	H143Y	De novo	(119)
	W209*	De novo	(119)
	P422Lfs*50	De novo	(11)

continued on next page

Gene	Amino acid change	Inheritance	Reference
TAOK2	A522T	Undetermined	(120)
	P935S	De novo	(118)
	R1126C	De novo	(118)
	E388*	De novo	(17)
	A135P	De novo	(18)
	T604Sfs*45	Unknown	(18)
	Q622*	Unknown	(18)
	L1030Wfs*3 (α)	Paternal, present in unaffected and affected sibling	(18)
	A335V	Paternal	(18)
	R489Q	Paternal from paternal grandmother, not present in affected sibling	(18)
	P826S (α)	Paternal	(18)
	D836N (α)	Paternal	(18)
	C938Y (α)	Paternal	(18)
	L951I (α)	Paternal, not present in unaffected sibling	(18)
	R1109Q (α)	Maternal	(18)
	L1114V (α)	Maternal, present in affected sibling	(18)
	V1129I (α)	Maternal, present in affected sibling	(18)
	R1226C (α)	Maternal, present in affected sibling	(18)
	P1232T (α)	Maternal, present in affected sibling	(18)
	H781R (β)	Maternal	(18)
	L833H (β)	Unknown	(18)
	A867V (β)	Paternal	(18)
	R870Q (β)	Maternal	(18)
	S889A (β)	Maternal	(18)
	A908E (β)	Not maternal, no paternal DNA	(18)
TAOK3	N980Y (β)	Maternal	(18)
	R499Q	De novo	(19)
	N229H, N56H	De novo	(119)
	T499A	De novo	(21)
	R632W	De novo	(21)
	H348Y	De novo	(119)
	M101Gfs*1	De novo	(119)
	Y16*	De novo	(119)
	P893L,P88L,P433L	De novo	(119)
	T240M	De novo	(119)

TAOK2 has been extensively studied in the context of neurodevelopment and associated disorders because of its location within the 16p11.2 genomic region, the duplication or deletion of which are associated with neurodevelopmental disorders, including ASD (12, 13) and schizophrenia (Fig. 7) (14). Among the genes within this locus, *TAOK2* gene expression has the highest correlation with head circumference in ASD patients with 16p11.2 copy number variations (15). WGS analyses of families with ASD identified a de novo loss-of-function, frameshift deletion variant (P1022*) in *TAOK2* (16), followed by identification of another de novo nonsense variant (E388*) (Table 1) (17). Another 23 variants, both de novo and inherited, were revealed through WGS and WES of more than 2600 families with ASD (Table 1) (18). Six individuals were diagnosed with autism (18). Two de novo mutations (A135P and P1022*) and two rare inherited variants (A335V and H781R) were further characterized (18). The A135P mutation, located in the kinase domain, reduces both TAOK2 autophosphorylation and the protein expression (18). The P1022* variant also reduces TAOK2 protein expression but does not affect autophosphorylation, whereas the two rare inherited mutations affect neither (18). Moreover, the A135P mutation significantly decreases phosphorylation of downstream substrate JNK1, whereas the opposite is seen with the P1022* mutation (18). Interestingly, the effect of the A135P variant on JNK1 phosphorylation was specific to the α isoform (18). These variants also impair dendrite and synaptic development (18). More recently, another variant in *TAOK2* (R499Q) was identified in WGS analyses of families with childhood apraxia of speech, a rare neurodevelopmental speech disorder (Fig. 7 and Table 1) (19).

Although TAOK3 has not been extensively studied as TAOK1 and TAOK2, several studies have implicated TAOK3 in neurodevelopmental disorders (20–22). WGS of families with schizophrenia identified a de novo deletion at chromosome 12q24.23, which contains TAOK3,

potentially suggesting its role in schizophrenia (Fig. 7) (20). Moreover, WES of more than 2500 families with ASD revealed two de novo variants in *TAOK3* (T499A and R632W) (Fig. 7 and Table 1) (21). Recently, a de novo mutation in *TAOK3* (c.1357-11A>G) was identified through WES and chromosomal microarray in a patient diagnosed with cerebral palsy from infancy who also exhibits spastic paraplegia and microcephaly (Fig. 7) (22).

TAOKs in addiction, pain, and depression

Both *Drosophila* Tao and mammalian TAOKs regulate ethanol-induced behaviors in a species-specific yet overlapping manner, suggesting an evolutionarily conserved role of the TAOK family (48, 87, 99, 100). *Drosophila* Tao mediates the ethanol-induced hyperactivity, potentially through its role in the development of the mushroom body via negative regulation of the downstream Par-1 kinase and tau phosphorylation (48). In contrast, in mice, Taok2 and Taok3 are important for ethanol-induced sedative effects but not hyperactivity (87, 99). Down-regulation of JNK pathway signaling was also shown to confer ethanol-induced locomotor and sedative effects mediated by *Drosophila* Tao and mouse Taok3, respectively, independent of the role of Tao in mushroom body structure (87). Suppression of EGFR signaling upstream of JNK pathway may contribute to these behaviors as well (100). In addition, Taok2 mediates ethanol-induced conditional place preference in mice (99). Last, the study on the mushroom body development suggested that *Drosophila* Tao may also potentially regulate behavioral responses to other types of drugs of abuse, such as cocaine and nicotine, the mechanisms for which are currently unclear (48).

TAOK3 is also associated with responses to the opioid morphine, an analgesic drug of abuse (101–104). A genome-wide association approach to identify genetic variants linked with opioid

analgesic response variability demonstrated that TAOK3 is associated with total morphine requirement and postoperative pain in a retrospective pediatric population who had undergone day surgery of tonsillectomy and adenoidectomy (101, 102). Interestingly, whereas the association with pain sensitivity was present in children of both European Caucasian and African American ancestry, the association with morphine dose was observed only in the former (101, 102). Moreover, TAOK3 was reported to be associated with opioid requirement in a retrospective adult patient population with advanced cancer pain (103). In contrast, no significant association was found between TAOK3 and thermal pain sensitivity in a pediatric population (104). These studies suggest that TAOK3 may modulate pain sensitivity in a modality-specific manner or rather regulate opioid responsiveness similarly to the way it regulates ethanol sensitivity, or it may be involved in both processes. Further studies are required to test these and understand the exact function of TAOK3 in pain sensation. Last, a recent study identified TAOK3 as a potential druggable protein candidate for depression through Mendelian randomization, which showed that the altered TAOK3 protein expression potentially contributes to depression (Fig. 7) (105).

TAOKs in neurodegenerative disorders

Although there is no genetic evidence for a direct involvement of TAOKs in neurodegeneration, they may play indirect roles (106–109). One of the major pathological hallmarks of Alzheimer's disease (AD) and frontotemporal lobar degeneration (FTLD) is the formation of neurofibrillary tangles resulting from aggregates of hyperphosphorylated tau that can no longer bind to microtubules (110, 111). TAOK1 and both isoforms of TAOK2 phosphorylate tau on multiple sites in the tau microtubule-binding domain and in the regions flanking this domain, including several disease-associated sites (Fig. 4A) (106). Moreover, TAOK1/2 activity was enhanced in the

brain regions susceptible to AD, and activated TAOK1/2 (phosphorylated Ser¹⁸¹ as a readout) was found in pretangles and tangles in human postmortem brain tissues of AD and FTLN patients (Fig. 7) (106, 107). A study using a machine learning approach also identified association between TAOK3 methylation and AD (Fig. 7) (108). TAOK3 was found to be differentially methylated in AD (108). In addition, TAOK3 is a downstream substrate for the kinase leucine rich repeat kinase 2 (LRRK2), mutations in which are the most common genetic cause of Parkinson's disease (PD) (Fig. 7) (109). Tau hyperphosphorylation has been observed in PD patients with LRRK2 mutations, and LRRK2 has been reported to promote tau phosphorylation and aggregation (112, 113). Consistent with TAOK1/2, TAOK3 may also regulate tau phosphorylation. These findings suggest that TAOKs may contribute to neurodegenerative disorders through phosphorylation-dependent regulation of microtubule dynamics and potentially through epigenetic modifications.

CONCLUSIONS AND PERSPECTIVES

The TAOK family of kinases are relatively understudied serine/threonine kinases that play important roles in broad cellular functions, immunity, and particularly neuronal development. Strong evidence associating TAOK1/2 mutations with NDDs has increased the focus toward understanding the basic molecular functions and precise roles of TAOKs in brain development. Much still remains to be uncovered, including the cell-type expression profiles of TAOK1, 2, and 3 and their alternatively spliced isoforms. Because of the conserved nature of their kinase domains, identifying specific direct substrates of each TAOK will be extremely challenging. Nevertheless, recent advances in the field will guide future efforts in identifying direct substrates. An analysis of the entire human Ser/Thr kinome suggests that TAOKs have a preference to phosphorylate threonine compared with serine in substrates, and there is a clear selection of basic residues at the

+2 and +3 amino acid to the phospho-threonine (pT) site (114). This is consistent with a previous study that used a chemical-genetics approach to find the minimum consensus sequence for TAOK2 kinase targets, pT-X-X-R/H/K (44), and another study that used peptide arrays to define the phosphorylation site specificity for Thr followed by basic residues at the +2 and +3 positions (115). Amino acid selectivity in the phosphorylation consensus sequence for TAOK1, TAOK2, and TAOK3, identified using peptide arrays, shows no identifiable differences in the motifs (114), indicating that physiological substrates of each are likely determined by differential localization and expression of each kinase in distinct cell types. Mass spectrometry–based phosphoproteomic analysis of *TAOK1/2/3*-KO cells or immunoprecipitation–mass spectrometry strategies in distinct cell types will aid future discovery of new physiologically relevant substrates. Relevant to the point above, the extent of redundancy versus distinct functions among the TAOKs might be directly explored using small-molecule inhibitors, although current compounds may be hampered by selectivity issues (55, 116). The association of TAOKs with disease—neurological, immune, and cancer—may spur renewed interest in identifying and developing TAOK1–, TAOK2–, and TAOK3–specific allosteric modulators that could advance this field in leaps and bounds.

Furthermore, the molecular functions of the TAOKs in neurodevelopment versus those in the adult brain are not yet known. Is the contribution of TAOK mutations in NDDs due to their known roles—including Hippo/MAPK signaling, cytoskeletal organization, mitosis, and apoptosis—or due to those that are yet unknown? Functional binning of TAOK1 and TAOK2 variants associated with disease could shed light on whether loss-of-function or gain-of-function mechanisms (or both) underlie the pathology. Emerging data on the role of the TAOK family of kinases in immunity are fascinating for understanding both the immune system and neurophysiology, given their emerging

interconnectedness (117), but how distinct TAOs sense viral nucleotides and mediate signaling in response to various pathogens are key questions to be answered. In addition to their pleiotropic cellular functions, the regulatory C-terminal regions make TAOs molecularly pleiotropic, endowing distinct functional domains that include ER-targeting, microtubule binding, nucleotide binding, or plasma membrane–tubulating properties. Modern structural analysis tools, such as cryo–electron microscopy, will yield further molecular insights into their autoregulation and the conformational changes regulating TAO activity during neurological development, aging, and states in between. Integration of comprehensive structural, molecular, and functional investigations of the TAOs will reveal much-needed insight into how these fascinating kinases contribute to development and disease in the brain and beyond.

Funding

This work was funded by NIH grants MH149343 and MH107205 to S.Y.

Author contributions: S.B. wrote a complete manuscript draft and revised the draft based on discussions with S.Y. S.Y. edited the manuscript.

Competing interests: The authors declare that they have no competing interests.

References

1. Dan I, Watanabe NM, Kusumi A, The Ste20 group kinases as regulators of MAP kinase cascades. *Trends Cell Biol.* 11, 220–230 (2001).
2. De Rubeis S, He X, Goldberg AP, Poultney CS, Samocha K, Cicek AE, Kou Y, Liu L, Fromer M, Walker S, Singh T, Klei L, Kosmicki J, Fu S-C, Aleksic B, Biscaldi M, Bolton PF, Brownfeld JM, Cai J, Campbell NJ, Carracedo A, Chahrour MH, Chiocchetti AG, Coon H, Crawford EL, Crooks L, Curran SR, Dawson G, Duketis E, Fernandez BA, Gallagher L, Geller E, Guter SJ, Hill RS, Ionita-Laza I, Gonzalez PJ, Kilpinen H, Klauck SM, Klevzon A, Lee I, Lei J, Lehtimäki T, Lin C-F, Ma'ayan A, Marshall CR, McInnes AL, Neale B, Owen MJ, Ozaki N, Parellada M, Parr JR, Purcell S, Puura K, Rajagopalan D, Rehnström K, Reichenberg A, Sabo A, Sachse M, Sanders SJ, Schafer C, Schulte-Rüther M, Skuse D, Stevens C, Szatmari P, Tammimies K, Valladares O, Voran A, Wang L-S, Weiss LA, Willsey AJ, Yu TW, Yuen RKC; the DDD Study, Homozygosity Mapping Collaborative for Autism, UK10K Consortium, the Autism Sequencing Consortium, Cook EH, Freitag CM, Gill M, Hultman CM, Lehner T, Palotie A, Schellenberg GD, Sklar P, State MW, Sutcliffe JS, Walsh CA, Scherer SW, Zwick ME, Barrett JC, Cutler DJ, Roeder K, Devlin B, Daly MJ, Buxbaum JD, Synaptic, transcriptional and chromatin genes disrupted in autism. *Nature* 515, 209–215 (2014).
3. Xie B, Fan X, Lei Y, Chen R, Wang J, Fu C, Yi S, Luo J, Zhang S, Yang Q, Chen S, Shen Y, A novel de novo microdeletion at 17q11.2 adjacent to NF1 gene associated with developmental delay, short stature, microcephaly and dysmorphic features. *Mol. Cytogenet* 9, 41 (2016).
4. Cooper GM, Coe BP, Girirajan S, Rosenfeld JA, Vu TH, Baker C, Williams C, Stalker H, Hamid R, Hannig V, Abdel-Hamid H, Bader P, McCracken E, Niyazov D, Leppig K, Thiese H, Hummel M, Alexander N, Gorski J, Kussmann J, Shashi V, Johnson K, Rehder C, Ballif BC, Shaffer LG, Eichler EE, A copy number variation morbidity map of developmental delay. *Nat. Genet* 43, 838–846 (2011).
5. Firth HV, Richards SM, Bevan AP, Clayton S, Corpas M, Rajan D, Vooren SV, Moreau Y, Pettett RM, Carter NP, DECIPHER: Database of chromosomal imbalance and phenotype in humans using ensembl resources. *Am. J. Hum. Genet* 84, 524–533 (2009).
6. Dulovic-Mahlow M, Trinh J, Kandaswamy KK, Braathen GJ, Di Donato N, Rahikkala E, Beblo S, Werber M, Krajka V, Busk ØL, Baumann H, Al-Sannaa NA, Hinrichs F, Affan R, Navot N, Al Balwi MA, Oprea G, Holla ØL, Weiss MER, Jamra RA, Kahlert A-K, Kishore S, Tveten K, Vos M, Rolfs A, Lohmann K, De novo variants in TAOK1 cause neurodevelopmental disorders. *Am. J. Hum. Genet* 105, 213–220 (2019).
7. Satterstrom FK, Kosmicki JA, Wang J, Breen MS, De Rubeis S, An J-Y, Peng M, Collins R, Grove J, Klei L, Stevens C, Reichert J, Mulhern MS, Artomov M, Gerges S, Sheppard B, Xu X, Bhaduri A, Norman U, Brand H, Schwartz G, Nguyen R, Guerrero EE, Dias C, Aleksic B, Anney R, Barbosa M, Bishop S, Brusco A, Bybjerg-Grauholm J, Carracedo A, Chan MCY, Chiocchetti AG, Chung BHY, Coon H, Cuccaro ML, Curró A, Bernardina BD, Doan R, Domenici E, Dong S, Fallerini C, Fernández-Prieto M, Ferrero GB, Freitag CM, Fromer M, Gargus JJ, Geschwind D, Giorgio E, González-Peñas J, Guter S, Halpern D, Hansen-Kiss E, He X, Herman GE, Hertz-Picciotto I, Hougaard DM, Hultman CM, Ionita-Laza I, Jacob S, Jamison J, Jugessur A,

Kaartinen M, Knudsen GP, Kolevzon A, Kushima I, Lee SL, Lehtimäki T, Lim ET, Lintas C, Lipkin WI, Lopergolo D, Lopes F, Ludena Y, Maciel P, Magnus P, Mahjani B, Maltman N, Manoach DS, Meiri G, Menashe I, Miller J, Minshew N, Montenegro EMS, Moreira D, Morrow EM, Mors O, Mortensen PB, Mosconi M, Muglia P, Neale BM, Nordentoft M, Ozaki N, Palotie A, Parellada M, Passos-Bueno MR, Pericak-Vance M, Persico AM, Pessah I, Puura K, Reichenberg A, Renieri A, Riberi E, Robinson EB, Samocha KE, Sandin S, Santangelo SL, Schellenberg G, Scherer SW, Schlitt S, Schmidt R, Schmitt L, Silva IMW, Singh T, Siper PM, Smith M, Soares G, Stoltenberg C, Suren P, Susser E, Sweeney J, Szatmari P, Tang L, Tassone F, Teufel K, Trabetti E, del Pilar Trelles M, Walsh CA, Weiss LA, Werge T, Werling DM, Wigdor EM, Wilkinson E, Willsey AJ, Yu TW, Yu MHC, Yuen R, Zachary E, Agerbo E, Als TD, Appadurai V, Bækvad-Hansen M, Belliveau R, Buil A, Carey CE, Cerrato F, Chambert K, Churchhouse C, Dalsgaard S, Demontis D, Dumont A, Goldstein J, Hansen CS, Hauberg ME, Hollegaard MV, Howrigan DP, Huang H, Maller J, Martin AR, Martin J, Mattheisen M, Moran J, Pallesen J, Palmer DS, Pedersen CB, Pedersen MG, Poterba T, Poulsen JB, Ripke S, Schork AJ, Thompson WK, Turley P, Walters RK, Betancur C, Cook EH, Gallagher L, Gill M, Sutcliffe JS, Thurm A, Zwick ME, Børglum AD, State MW, Cicek AE, Talkowski ME, Cutler DJ, Devlin B, Sanders SJ, Roeder K, Daly MJ, Buxbaum JD, Large-scale exome sequencing study implicates both developmental and functional changes in the neurobiology of autism. *Cell* 180, 568–584.e23 (2020).

8. Woerden GM, Bos M, Konink C, Distel B, Avagliano Trezza R, Shur NE, Barañano K, Mahida S, Chassevent A, Schreiber A, Erwin AL, Gripp KW, Rehman F, Brullemann S, McCormack R, Geus G, Kalsner L, Sorlin A, Bruel A, Koolen DA, Gabriel MK, Rossi M, Fitzpatrick DR, Wilkie AOM, Calpena E, Johnson D, Brooks A, Slegtenhorst M, Fleischer J, Groepper D, Lindstrom K, Innes AM, Goodwin A, Humberson J, Noyes A, Langley KG, Telegrafi A, Blevins A, Hoffman J, Guillen Sacoto MJ, Juusola J, Monaghan KG, Punj S, Simon M, Pfundt R, Elgersma Y, Kleefstra T, TAOK1 is associated with neurodevelopmental disorder and essential for neuronal maturation and cortical development. *Hum. Mutat* 42, 445–459 (2021).

9. Hunter JM, Massingham LJ, Manickam K, Bartholomew D, Williamson RK, Schwab JL, Marhabaie M, Siemon A, Reyes E. d. L., Reshmi SC, Cottrell CE, Wilson RK, Koboldt DC, Inherited and de novo variants extend the etiology of TAOK1-associated neurodevelopmental disorder. *Cold Spring Harb. Mol. Case Stud* 8, a006180 (2022).

10. Yu L, Yang C, Shang N, Ding H, Zhu J, Zhu Y, Tan H, Zhang Y, Paternal de novo variant of TAOK1 in a fetus with structural brain abnormalities. *Front. Genet* 13, 836853 (2022).

11. Blaschek A, Sitzberger A, Brugger M, Graf E, Berutti R, Zech M, Vill K, TAOK1-related neurodevelopmental disorder: A new differential diagnosis for childhood-onset tremor? *Parkinsonism Relat. Disord* 109, 105320 (2023).

12. Weiss LA, Shen Y, Korn JM, Arking DE, Miller DT, Fossdal R, Saemundsen E, Stefansson H, Ferreira MAR, Green T, Platt OS, Ruderfer DM, Walsh CA, Altshuler D, Chakravarti A, Tanzi RE, Stefansson K, Santangelo SL, Gusella JF, Sklar P, Wu B-L, Daly MJ, Association between microdeletion and microduplication at 16p11.2 and autism. *N. Engl. J. Med* 358, 667–675 (2008).

13. Kumar RA, KaraMohamed S, Sudi J, Conrad DF, Brune C, Badner JA, Gilliam TC, Nowak NJ, Cook EH, Dobyns WB, Christian SL, Recurrent 16p11.2 microdeletions in autism. *Hum. Mol. Genet* 17, 628–638 (2008).

14. McCarthy SE, Consortium WTCC, Makarov V, Kirov G, Addington AM, McClellan J, Yoon S, Perkins DO, Dickel DE, Kusenda M, Krastoshevsky O, Krause V, Kumar RA, Grozeva D, Malhotra D, Walsh T, Zackai EH, Kaplan P, Ganesh J, Krantz ID, Spinner NB, Roccanova P, Bhandari A, Pavon K, Lakshmi B, Leotta A, Kendall J, Lee Y, Vacic V, Gary S, Iakoucheva LM, Crow TJ, Christian SL, Lieberman JA, Stroup TS, Lehtimäki T, Puura K, Haldeman-Englert C, Pearl J, Goodell M, Willour VL, DeRosse P, Steele J, Kassem L, Wolff J, Chitkara N, McMahon FJ, Malhotra AK, Potash JB, Schulze TG, Nöthen MM, Cichon S, Rietschel M, Leibenluft E, Kustanovich V, Lajonchere CM, Sutcliffe JS, Skuse D, Gill M, Gallagher L, Mendell NR, Craddock N, Owen MJ, O'Donovan MC, Shaikh TH, Susser E, DeLisi LE, Sullivan PF, Deutsch CK, Rapoport J, Levy DL, King M-C, Sebat J, Microduplications of 16p11.2 are associated with schizophrenia. *Nat. Genet* 41, 1223–1227 (2009).
15. Luo R, Sanders SJ, Tian Y, Voineagu I, Huang N, Chu SH, Klei L, Cai C, Ou J, Lowe JK, Hurles ME, Devlin B, State MW, Geschwind DH, Genome-wide transcriptome profiling reveals the functional impact of rare de novo and recurrent CNVs in autism spectrum disorders. *Am. J. Hum. Genet* 91, 38–55 (2012).
16. Yuen RK, Merico D, Bookman M, Howe JL, Thiruvahindrapuram B, Patel RV, Whitney J, Deflaux N, Bingham J, Wang Z, Pellecchia G, Buchanan JA, Walker S, Marshall CR, Uddin M, Zarrei M, Deneault E, D'Abate L, Chan AJS, Koyanagi S, Paton T, Pereira SL, Hoang N, Engchuan W, Higginbotham EJ, Ho K, Lamoureux S, Li W, MacDonald JR, Nalpathamkalam T, Sung WWL, Tsoi FJ, Wei J, Xu L, Tasse A-M, Kirby E, Van Etten W, Twigger S, Roberts W, Drmic I, Jilderda S, Modi BM, Kellam B, Szego M, Cytrynbaum C, Weksberg R, Zwaigenbaum L, Woodbury-Smith M, Brian J, Senman L, Iaboni A, Doyle-Thomas K, Thompson A, Chrysler C, Leef J, Savion-Lemieux T, Smith IM, Liu X, Nicolson R, Seifer V, Fedeale A, Cook EH, Dager S, Estes A, Gallagher L, Malow BA, Parr JR, Spence SJ, Vorstman J, Frey BJ, Robinson JT, Strug LJ, Fernandez BA, Elsabbagh M, Carter MT, Hallmayer J, Knoppers BM, Anagnostou E, Szatmari P, Ring RH, Glazer D, Pletcher MT, Scherer SW, Whole genome sequencing resource identifies 18 new candidate genes for autism spectrum disorder. *Nat. Neurosci* 20, 602–611 (2017).
17. Lim ET, Uddin M, De Rubeis S, Chan Y, Kamumbu AS, Zhang X, D'Gama AM, Kim SN, Hill RS, Goldberg AP, Poultney C, Minshew NJ, Kushima I, Aleksic B, Ozaki N, Parellada M, Arango C, Penzol MJ, Carracedo A, Klevzon A, Hultman CM, Weiss LA, Fromer M, Chiocchetti AG, Freitag CM, Consortium AS, Church GM, Scherer SW, Buxbaum JD, Walsh CA, Rates, distribution and implications of postzygotic mosaic mutations in autism spectrum disorder. *Nat. Neurosci.* 20, 1217–1224 (2017).
18. Richter M, Murtaza N, Scharrenberg R, White SH, Johanns O, Walker S, Yuen RKC, Schwanke B, Bedürftig B, Henis M, Scharf S, Kraus V, Dörk R, Hellmann J, Lindenmaier Z, Ellegood J, Hartung H, Kwan V, Sedlacik J, Fiehler J, Schweizer M, Lerch JP, Hanganu-Opatz IL, Morellini F, Scherer SW, Singh KK, Calderon de Anda F, Altered TAOK2 activity causes autism-related neurodevelopmental and cognitive abnormalities through RhoA signaling. *Mol. Psychiatry* 24, 1329–1350 (2019).
19. Kaspi A, Hildebrand MS, Jackson VE, Braden R, van Reyk O, Howell T, Debono S, Lauretta M, Morison L, Coleman MJ, Webster R, Coman D, Goel H, Wallis M, Dabscheck G, Downie L, Baker EK, Parry-Fielder B, Ballard K, Harrold E, Ziegenfusz S, Bennett MF, Robertson

E, Wang L, Boys A, Fisher SE, Amor DJ, Scheffer IE, Bahlo M, Morgan AT, Genetic aetiologies for childhood speech disorder: Novel pathways co-expressed during brain development. *Mol. Psychiatry* 28, 1647–1663 (2023).

20. Malhotra D, McCarthy S, Michaelson JJ, Vacic V, Burdick KE, Yoon S, Cichon S, Corvin A, Gary S, Gershon ES, Gill M, Karayiorgou M, Kelsoe JR, Krastoshevsky O, Krause V, Leibenluft E, Levy DL, Makarov V, Bhandari A, Malhotra AK, McMahon FJ, Nöthen MM, Potash JB, Rietschel M, Schulze TG, Sebat J, High frequencies of de novo CNVs in bipolar disorder and schizophrenia. *Neuron* 72, 951–963 (2011).

21. Iossifov I, O’Roak BJ, Sanders SJ, Ronemus M, Krumm N, Levy D, Stessman HA, Witherspoon KT, Vives L, Patterson KE, Smith JD, Paepers B, Nickerson DA, Dea J, Dong S, Gonzalez LE, Mandell JD, Mane SM, Murtha MT, Sullivan CA, Walker MF, Waqar Z, Wei L, Willsey AJ, Yamrom B, Lee Y, Grabowska E, Dalkic E, Wang Z, Marks S, Andrews P, Leotta A, Kendall J, Hakker I, Rosenbaum J, Ma B, Rodgers L, Troge J, Narzisi G, Yoon S, Schatz MC, Ye K, McCombie WR, Shendure J, Eichler EE, State MW, Wigler M, The contribution of de novo coding mutations to autism spectrum disorder. *Nature* 515, 216–221 (2014).

22. Yechieli M, Gulsuner S, Ben-Pazi H, Fattal A, Aran A, Kuzminsky A, Sagi L, Guttman D, Schneebaum Sender N, Gross-Tsur V, Klopstock T, Walsh T, Renbaum P, Zeligson S, Shemer Meiri L, Lev D, Shmueli D, Blumkin L, Lahad A, King M-C, Levy EL, Segel R, Diagnostic yield of chromosomal microarray and trio whole exome sequencing in cryptogenic cerebral palsy. *J. Med. Genet* 59, 759–767 (2022).

23. Chen Z, Hutchison M, Cobb MH, Isolation of the protein kinase TAO2 and identification of its mitogen-activated protein kinase/extracellular signal-regulated kinase binding domain. *J. Biol. Chem* 274, 28803–28807 (1999).

24. Hutchison M, Berman KS, Cobb MH, Isolation of TAO1, a protein kinase that activates MEKs in stress-activated protein kinase cascades. *J. Biol. Chem* 273, 28625–28632 (1998).

25. Chen Z, Cobb MH, Regulation of stress-responsive mitogen-activated protein (MAP) kinase pathways by TAO2. *J. Biol. Chem* 276, 16070–16075 (2001).

26. Zhou T, Raman M, Gao Y, Earnest S, Chen Z, Machius M, Cobb MH, Goldsmith EJ, Crystal structure of the TAO2 kinase domain: Activation and specificity of a Ste20p MAP3K. *Structure* 12, 1891–1900 (2004).

27. Zihni C, Mitsopoulos C, Tavares IA, Ridley AJ, Morris JDH, Prostate-derived sterile 20-like kinase 2 (PSK2) regulates apoptotic morphology via C-Jun N-terminal kinase and Rho kinase-1. *J. Biol. Chem* 281, 7317–7323 (2006).

28. Chen Z, Raman M, Chen L, Lee SF, Gilman AG, Cobb MH, TAO (thousand-and-one amino acid) protein kinases mediate signaling from carbachol to p38 mitogen-activated protein kinase and ternary complex factors. *J. Biol. Chem* 278, 22278–22283 (2003).

29. Zhang W, Chen T, Wan T, He L, Li N, Yuan Z, Cao X, Cloning of DPK, a novel dendritic cell-derived protein kinase activating the ERK1/ERK2 and JNK/SAPK pathways. *Biochem. Biophys. Res. Commun* 274, 872–879 (2000).
30. Yoneda T, Imaizumi K, Oono K, Yui D, Gomi F, Katayama T, Tohyama M, Activation of Caspase-12, an endoplasmic reticulum (ER) resident Caspase, through tumor necrosis factor receptor-associated factor 2-dependent mechanism in response to the ER stress. *J. Biol. Chem* 276, 13935–13940 (2001).
31. Raman M, Earnest S, Zhang K, Zhao Y, Cobb MH, TAO kinases mediate activation of p38 in response to DNA damage. *EMBO J.* 26, 2005–2014 (2007).
32. HuangFu W-C, Omori E, Akira S, Matsumoto K, Ninomiya-Tsuji J, Osmotic stress activates the TAK1-JNK pathway while blocking TAK1-mediated NF- κ B activation. *J. Biol. Chem* 281, 28802–28810 (2006).
33. Wu M, Wang S, Human TAO kinase 1 induces apoptosis in SH-SY5Y cells. *Cell Biol. Int* 32, 151–156 (2008).
34. Zihni C, Mitsopoulos C, Tavares IA, Baum B, Ridley AJ, Morris JDH, Prostate-derived sterile 20-like kinase 1- α induces apoptosis. *J. Biol. Chem* 282, 6484–6493 (2007).
35. Boggiano JC, Vanderzalm PJ, Fehon RG, Tao-1 phosphorylates Hippo/MST kinases to regulate the Hippo-Salvador-Warts tumor suppressor pathway. *Dev. Cell* 21, 888–895 (2011).
36. Poon CLC, Lin JJ, Zhang X, Harvey KF, The sterile 20-like kinase tao-1 controls tissue growth by regulating the Salvador-Warts-Hippo pathway. *Dev. Cell* 21, 896–906 (2011).
37. Plouffe SW, Meng Z, Lin KC, Lin B, Hong AW, Chun JV, Guan K-L, Characterization of Hippo pathway components by gene inactivation. *Mol. Cell* 64, 993–1008 (2016).
38. Poon CLC, Mitchell KA, Kondo S, Cheng LY, Harvey KF, The Hippo pathway regulates neuroblasts and brain size in *Drosophila melanogaster*. *Curr. Biol* 26, 1034–1042 (2016).
39. Timm T, MARKK, a Ste20-like kinase, activates the polarity-inducing kinase MARK/PAK-1. *EMBO J.* 22, 5090–5101 (2003).
40. Johne C, Matenia D, Li X, Timm T, Balusamy K, Mandelkow E-M, Spred1 and TESK1—Two new interaction partners of the kinase MARKK/TAO1 That link the microtubule and actin cytoskeleton. *Mol. Biol. Cell* 19, 1391–1403 (2008).
41. Mitsopoulos C, Zihni C, Garg R, Ridley AJ, Morris JDH, The prostate-derived sterile 20-like kinase (PSK) regulates microtubule organization and stability. *J. Biol. Chem* 278, 18085–18091 (2003).
42. Nourbakhsh K, Ferreccio AA, Bernard MJ, Yadav S, TAOK2 is an ER-localized kinase that catalyzes the dynamic tethering of ER to microtubules. *Dev. Cell* 56, 3321–3333.e5 (2021).

43. Moore TM, Garg R, Johnson C, Coptcoat MJ, Ridley AJ, Morris JDH, PSK, a novel STE20-like kinase derived from prostatic carcinoma that activates the c-Jun N-terminal kinase mitogen-activated protein kinase pathway and regulates actin cytoskeletal organization. *J. Biol. Chem* 275, 4311–4322 (2000).
44. Yadav S, Oses-Prieto JA, Peters CJ, Zhou J, Pleasure SJ, Burlingame AL, Jan LY, Jan Y-N, TAOK2 kinase mediates PSD95 stability and dendritic spine maturation through Septin7 phosphorylation. *Neuron* 93, 379–393 (2017).
45. Byeon S, Werner B, Falter R, Davidsen K, Snyder C, Ong S-E, Yadav S, Proteomic identification of phosphorylation-dependent septin 7 interactors that drive dendritic spine formation. *Front. Cell Dev. Biol* 10, 836746 (2022).
46. Liu T, Rohn JL, Picone R, Kunda P, Baum B, Tao-1 is a negative regulator of microtubule plus-end growth. *J. Cell Sci* 123, 2708–2716 (2010).
47. Wang J-W, Imai Y, Lu B, Activation of PAR-1 kinase and stimulation of tau phosphorylation by diverse signals require the tumor suppressor protein LKB1. *J. Neurosci* 27, 574–581 (2007).
48. King I, Tsai LT-Y, Pflanz R, Voigt A, Lee S, Jackle H, Lu B, Heberlein U, *Drosophila tao* controls mushroom body development and ethanol-stimulated behavior through par-1. *J. Neurosci* 31, 1139–1148 (2011).
49. Wojtala RL, Tavares IA, Morton PE, Valderrama F, Thomas NSB, Morris JDH, Prostate-derived sterile 20-like kinases (PSKs/TAOKs) are activated in mitosis and contribute to mitotic cell rounding and spindle positioning. *J. Biol. Chem* 286, 30161–30170 (2011).
50. Garg R, Koo C-Y, Infante E, Giacomini C, Ridley AJ, Morris JDH, Rnd3 interacts with TAO kinases and contributes to mitotic cell rounding and spindle positioning. *J. Cell Sci* 133, jcs235895 (2020).
51. Draviam VM, Stegmeier F, Nalepa G, Sowa ME, Chen J, Liang A, Hannon GJ, Sorger PK, Harper JW, Elledge SJ, A functional genomic screen identifies a role for TAO1 kinase in spindle-checkpoint signalling. *Nat. Cell Biol* 9, 556–564 (2007).
52. Shrestha RL, Tamura N, Fries A, Levin N, Clark J, Draviam VM, TAO1 kinase maintains chromosomal stability by facilitating proper congression of chromosomes. *Open Biol.* 4, (2014).
53. Pennemann FL, Mussabekova A, Urban C, Stukalov A, Andersen LL, Grass V, Lavacca TM, Holze C, Oubraham L, Benamrouche Y, Girardi E, Boulos RE, Hartmann R, Superti-Furga G, Habjan M, Immler J-L, Meignin C, Pichlmair A, Cross-species analysis of viral nucleic acid interacting proteins identifies TAOs as innate immune regulators. *Nat. Commun* 12, 7009 (2021).
54. Esparza M, Mor A, Niederstrasser H, White K, White A, Zhang K, Gao S, Wang J, Liang J, Sho S, Sakthivel R, Sathe AA, Xing C, Muñoz-Moreno R, Shay JW, García-Sastre A, Ready J,

Posner B, Fontoura BMA, Chemical intervention of influenza virus mRNA nuclear export. *PLOS Pathog.* 16, e1008407 (2020).

55. Piala AT, Akella R, Potts MB, Dudics-Giagnocavo SA, He H, Wei S, White MA, Posner BA, Goldsmith EJ, Discovery of novel TAO2 inhibitor scaffolds from high-throughput screening. *Bioorg. Med. Chem. Lett* 26, 3923–3927 (2016).

56. Gao S, Esparza M, Dehghan I, Aksenova V, Zhang K, Batten K, Ferretti MB, Begg BE, Cagatay T, Shay JW, García-Sastre A, Goldsmith EJ, Chen ZJ, Dasso M, Lynch KW, Cobb MH, Fontoura BMA, Nuclear speckle integrity and function require TAO2 kinase. *Proc. Natl. Acad. Sci* 119, e2206046119 (2022).

57. Yang Q, Li Y, Wang Y, Qiao X, Liu T, Wang H, Shen H, The circRNA circSIAE inhibits replication of coxsackie virus B3 by targeting miR-331-3p and thousand and one amino-acid kinase 2. *Front. Cell. Infect. Microbiol* 11, 779919 (2022).

58. Quereda JJ, Morel C, Lopez-Montero N, Ziveri J, Rolland S, Grenier T, Aulner N, Danckaert A, Charbit A, Enninga J, Cossart P, Pizarro-Cerdá J, A role for Taok2 in *Listeria monocytogenes* vacuolar escape. *J. Infect Dis* 225, 1005–1010 (2022).

59. Zhang Z, Tang Z, Ma X, Sun K, Fan L, Fang J, Pan J, Wang X, An H, Zhou J, TAO2 negatively regulates IL-17-mediated signaling and inflammation. *Cell. Mol. Immunol.* 15, 794–802 (2018).

60. Zhu L, Yu Q, Gao P, Liu Q, Luo X, Jiang G, Ji R, Yang R, Ma X, Xu J, Yuan H, Zhou J, An H, TAO2 positively regulates TLR4-induced inflammatory responses by promoting ERK1/2 activation in macrophages. *Mol. Immunol* 122, 124–131 (2020).

61. Luo X, Ji R, Liu Q, Xiao X, Song W, An H, Li Y, Zhou J, Ste20-like kinase TAO2 positively regulates antiviral responses by controlling the TBK1-IRF3 signaling axis. *J. Innate Immun* 15, 1–17 (2023).

62. Dechtawewat T, Paemanee A, Roytrakul S, Songprakhon P, Limjindaporn T, Yenchitsomanus P, Saitornuang S, Puttikhunt C, Kasinrerk W, Malasit P, Noisakran S, Mass spectrometric analysis of host cell proteins interacting with dengue virus nonstructural protein 1 in dengue virus-infected HepG2 cells. *Biochim. Biophys. Acta* 1864, 1270–1280 (2016).

63. Hammad H, Vanderkerken M, Pouliot P, Deswarte K, Toussaint W, Vergote K, Vandersarren L, Janssens S, Ramou I, Savvides SN, Haigh JJ, Hendriks R, Kopf M, Craessaerts K, de Strooper B, Kearney JF, Conrad DH, Lambrecht BN, Transitional B cells commit to marginal zone B cell fate by TAO3-mediated surface expression of ADAM10. *Nat. Immunol* 18, 313–320 (2017).

64. Vanderkerken M, Maes B, Vandersarren L, Toussaint W, Deswarte K, Vanheerswynghe M, Pouliot P, Martens L, Van Gassen S, Arthur CM, Kirkling ME, Reizis B, Conrad D, Stowell S, Hammad H, Lambrecht BN, TAO-kinase 3 governs the terminal differentiation of NOTCH2-dependent splenic conventional dendritic cells. *Proc. Natl. Acad. Sci* 117, 31331–31342 (2020).

65. Ormonde JVS, Li Z, Stegen C, Madrenas J, taok3 regulates canonical TCR Signaling by preventing early SHP-1-mediated inactivation of LCK. *J. Immunol* 201, 3431–3442 (2018).
66. Maes B, Smole U, Vanderkerken M, Deswarte K, Van Moorleghem J, Vergote K, Vanheerswynghe M, De Wolf C, De Prijck S, Debeuf N, Pavie B, Toussaint W, Janssens S, Savvides S, Lambrecht BN, Hammad H, The STE20 kinase TAOK3 controls the development of house dust mite-induced asthma in mice. *J. Allergy Clin. Immunol* 149, 1413–1427.e2 (2022).
67. Tassi E, Biesova Z, Di Fiore PP, Gutkind JS, Wong WT, Human JIK, a novel member of the STE20 kinase family that inhibits JNK and is negatively regulated by epidermal growth factor. *J. Biol. Chem* 274, 33287–33295 (1999).
68. Berman KS, Hutchison M, Avery L, Cobb MH, *kin-18*, a *C. elegans* protein kinase involved in feeding. *Gene* 279, 137–147 (2001).
69. Spiga FM, Prouteau M, Gotta M, The TAO kinase KIN-18 regulates contractility and establishment of polarity in the *C. elegans* embryo. *Dev. Biol.* 373, 26–38 (2013).
70. Yustein JT, Xia L, Kahlenburg JM, Robinson D, Templeton D, Kung H-J, Comparative studies of a new subfamily of human Ste20-like kinases: Homodimerization, subcellular localization, and selective activation of MKK3 and p38. *Oncogene* 22, 6129–6141 (2003).
71. Uhlén M, Fagerberg L, Hallström BM, Lindskog C, Oksvold P, Mardinoglu A, Sivertsson Å, Kampf C, Sjöstedt E, Asplund A, Olsson I, Edlund K, Lundberg E, Navani S, Szigyiarto CA-K, Odeberg J, Djureinovic D, Takanen JO, Hober S, Alm T, Edqvist P-H, Berling H, Tegel H, Mulder J, Rockberg J, Nilsson P, Schwenk JM, Hamsten M, von Feilitzen K, Forsberg M, Persson L, Johansson F, Zwahlen M, von Heijne G, Nielsen J, Pontén F, Tissue-based map of the human proteome. *Science* 347, 1260419 (2015).
72. The Human Protein Atlas. www.proteinatlas.org/.
73. Tissue expression of TAOK1 - Summary - The Human Protein Atlas. www.proteinatlas.org/ENSG00000160551-TAOK1/tissue.
74. Tissue expression of TAOK2 - Summary - The Human Protein Atlas. www.proteinatlas.org/ENSG00000149930-TAOK2/tissue.
75. Tissue expression of TAOK3 - Summary - The Human Protein Atlas. www.proteinatlas.org/ENSG00000135090-TAOK3/tissue.
76. Kang HJ, Kawasawa YI, Cheng F, Zhu Y, Xu X, Li M, Sousa AMM, Pletikos M, Meyer KA, Sedmak G, Guennel T, Shin Y, Johnson MB, Krsnik Z, Mayer S, Fertuzinhos S, Umlauf S, Lisgo SN, Vortmeyer A, Weinberger DR, Mane S, Hyde TM, Huttner A, Reimers M, Kleinman JE, Sestan N, Spatio-temporal transcriptome of the human brain. *Nature* 478, 483–489 (2011).
77. Johnson MB, Kawasawa YI, Mason CE, Krsnik Ž, Coppola G, Bogdanović D, Geschwind DH, Mane SM, State MW, Sestan N, Functional and evolutionary insights into human brain development through global transcriptome analysis. *Neuron* 62, 494–509 (2009).

78. Pletikos M, Sousa AMM, Sedmak G, Meyer KA, Zhu Y, Cheng F, Li M, Kawasaki YI, Šestan N, Temporal specification and bilaterality of human neocortical topographic gene expression. *Neuron* 81, 321–332 (2014).
79. Karlsson M, Zhang C, Méar L, Zhong W, Digre A, Katona B, Sjöstedt E, Butler L, Odeberg J, Dusart P, Edfors F, Oksvold P, von Feilitzen K, Zwahlen M, Arif M, Altay O, Li X, Ozcan M, Mardinoglu A, Fagerberg L, Mulder J, Luo Y, Ponten F, Uhlén M, Lindskog C, A single-cell type transcriptomics map of human tissues. *Sci. Adv* 7, eabh2169, (2021).
80. Single cell type - TAOK1 - The Human Protein Atlas. www.proteinatlas.org/ENSG00000160551-TAOK1/single+cell+type.
81. Single cell type - TAOK2 - The Human Protein Atlas. www.proteinatlas.org/ENSG00000149930-TAOK2/single+cell+type.
82. Single cell type - TAOK3 - The Human Protein Atlas. www.proteinatlas.org/ENSG00000135090-TAOK3/single+cell+type.
83. Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, Tunyasuvunakool K, Bates R, Židek A, Potapenko A, Bridgland A, Meyer C, Kohl SAA, Ballard AJ, Cowie A, Romera-Paredes B, Nikolov S, Jain R, Adler J, Back T, Petersen S, Reiman D, Clancy E, Zielinski M, Steinegger M, Pacholska M, Berghammer T, Bodenstein S, Silver D, Vinyals O, Senior AW, Kavukcuoglu K, Kohli P, Hassabis D, Highly accurate protein structure prediction with AlphaFold. *Nature* 596, 583–589 (2021).
84. Varadi M, Anyango S, Deshpande M, Nair S, Natassia C, Yordanova G, Yuan D, Stroe O, Wood G, Laydon A, Židek A, Green T, Tunyasuvunakool K, Petersen S, Jumper J, Clancy E, Green R, Vora A, Lutfi M, Figurnov M, Cowie A, Hobbs N, Kohli P, Kleywegt G, Birney E, Hassabis D, Velankar S, AlphaFold Protein Structure Database: Massively expanding the structural coverage of protein-sequence space with high-accuracy models. *Nucleic Acids Res.* 50, D439–D444 (2022).
85. Beeman N, Sapre T, Ong S-E, Yadav S, Neurodevelopmental disorder-associated mutations in TAOK1 reveal its function as a plasma membrane remodeling kinase. *Sci. Signal* 16, eadd3269 (2023).
86. Yoder MD, Van Osten S, Weber GF, Gene expression analysis of the Tao kinase family of Ste20p-like map kinase kinases during early embryonic development in *Xenopus laevis*. *Gene Expr. Patterns* 48, 119318 (2023).
87. Kapfhamer D, King I, Zou ME, Lim JP, Heberlein U, Wolf FW, JNK pathway activation is controlled by Tao/TAOK3 to modulate ethanol sensitivity. *PLOS One* 7, e50594 (2012).
88. Kültz D, Chakravarty D, Hyperosmolality in the form of elevated NaCl but not urea causes DNA damage in murine kidney cells. *Proc. Natl. Acad. Sci. U.S.A* 98, 1999–2004 (2001).

89. Dmitrieva NI, Bulavin DV, Burg MB, High NaCl causes Mre11 to leave the nucleus, disrupting DNA damage signaling and repair. *Am. J. Physiol. Renal Physiol* 285, F266–F274 (2003).
90. Westhorpe FG, Diez MA, Gurden MDJ, Tighe A, Taylor SS, Re-evaluating the role of Tao1 in the spindle checkpoint. *Chromosoma* 119, 371–379 (2010).
91. Hübner NC, Wang LH-C, Kaulich M, Descombes P, Poser I, Nigg EA, Re-examination of siRNA specificity questions role of PICH and Tao1 in the spindle checkpoint and identifies Mad2 as a sensitive target for small RNAs. *Chromosoma* 119, 149–165 (2010).
92. Tenedini FM, Sáez González M, Hu C, Pedersen LH, Petrucci MM, Spitzweck B, Wang D, Richter M, Petersen M, Szpotowicz E, Schweizer M, Sigrist SJ, Calderon de Anda F, Soba P, Maintenance of cell type-specific connectivity and circuit function requires Tao kinase. *Nat. Commun* 10, 3506 (2019).
93. Hu C, Kanellopoulos AK, Richter M, Petersen M, Konietzny A, Tenedini FM, Hoyer N, Cheng L, Poon CLC, Harvey KF, Windhorst S, Parrish JZ, Mikhaylova M, Bagni C, Calderon de Anda F, Soba P, Conserved tao kinase activity regulates dendritic arborization, cytoskeletal dynamics, and sensory function in *Drosophila*. *J. Neurosci* 40, 1819–1833 (2020).
94. de Anda FC, Rosario AL, Durak O, Tran T, Gräff J, Meletis K, Rei D, Soda T, Madabhushi R, Ginty DD, Kolodkin AL, Tsai L-H, Autism spectrum disorder susceptibility gene TAOK2 affects basal dendrite formation in the neocortex. *Nat. Neurosci* 15, 1022–1031 (2012).
95. Ultanir SK, Yadav S, Hertz NT, Osés-Prieto JA, Claxton S, Burlingame AL, Shokat KM, Jan LY, Jan Y-N, MST3 kinase phosphorylates TAO1/2 to enable myosin va function in promoting spine synapse development. *Neuron* 84, 968–982 (2014).
96. Murtaza N, Cheng AA, Brown CO, Meka DP, Hong S, Uy JA, El-Hajjar J, Pipko N, Unda BK, Schwanke B, Xing S, Thiruvahindrapuram B, Engchuan W, Trost B, Deneault E, Calderon de Anda F, Doble BW, Ellis J, Anagnostou E, Bader GD, Scherer SW, Lu Y, Singh KK, Neuron-specific protein network mapping of autism risk genes identifies shared biological mechanisms and disease-relevant pathologies. *Cell Rep.* 41, 111678 (2022).
97. Scharrenberg R, Richter M, Johanns O, Meka DP, Rücker T, Murtaza N, Lindenmaier Z, Ellegood J, Naumann A, Zhao B, Schwanke B, Sedlacik J, Fiehler J, Hanganu-Opatz IL, Lerch JP, Singh KK, de Anda FC, TAOK2 rescues autism-linked developmental deficits in a 16p11.2 microdeletion mouse model. *Mol. Psychiatry* 27, 4707–4721 (2022).
98. Yasuda S, Tanaka H, Sugiura H, Okamura K, Sakaguchi T, Tran U, Takemiya T, Mizoguchi A, Yagita Y, Sakurai T, De Robertis EM, Yamagata K, Activity-induced protocadherin arcadlin regulates dendritic spine number by triggering n-cadherin endocytosis via TAO2 β and p38 MAP kinases. *Neuron* 56, 456–471 (2007).
99. Kapfhamer D, Taylor S, Zou ME, Lim JP, Kharazia V, Heberlein U, Taok2 controls behavioral response to ethanol in mice. *Genes Brain Behav.* 12, 87–97 (2013).

100. King IFG, Eddison M, Kaun KR, Heberlein U, EGFR and FGFR pathways have distinct roles in *Drosophila* mushroom body development and ethanol-induced behavior. *PLOS One* 9, e87714 (2014).
101. Cook-Sather SD, Li J, Goebel TK, Sussman EM, Rehman MA, Hakonarson H, TAOK3, a novel genome-wide association study locus associated with morphine requirement and postoperative pain in a retrospective pediatric day surgery population. *Pain* 155, 1773–1783 (2014).
102. Li J, Wei Z, Zhang J, Hakonarson H, Cook-Sather SD, Candidate gene analyses for acute pain and morphine analgesia after pediatric day surgery: African American versus European Caucasian ancestry and dose prediction limits. *Pharmacogenomics J.* 19, 570–581 (2019).
103. Gutteridge T, Kumaran M, Ghosh S, Fainsinger R, Klepstad P, Tarumi Y, Damaraju S, Baracos VE, Single-nucleotide polymorphisms in TAOK3 are associated with high opioid requirement for pain management in patients with advanced cancer admitted to a tertiary palliative care unit. *J. Pain Symptom Manage.* 56, 560–566 (2018).
104. Matic M, Van Den Bosch GE, De Wildt SN, Tibboel D, Van Schaik RHN, Genetic variants associated with thermal pain sensitivity in a paediatric population. *Pain* 157, 2476–2482 (2016).
105. Liu J, Cheng Y, Li M, Zhang Z, Li T, Luo X-J, Genome-wide Mendelian randomization identifies actionable novel drug targets for psychiatric disorders. *Neuropsychopharmacology* 48, 270–280 (2023).
106. Tavares IA, Touma D, Lynham S, Troakes C, Schober M, Causevic M, Garg R, Noble W, Killick R, Bodi I, Hanger DP, Morris JDH, Prostate-derived sterile 20-like kinases (PSKs/TAOKs) phosphorylate tau protein and are activated in tangle-bearing neurons in Alzheimer disease. *J. Biol. Chem* 288, 15418–15429 (2013).
107. Giacomini C, Koo C-Y, Yankova N, Tavares IA, Wray S, Noble W, Hanger DP, Morris JDH, A new TAO kinase inhibitor reduces tau phosphorylation at sites associated with neurodegeneration in human tauopathies. *Acta Neuropathol. Commun* 6, 37 (2018).
108. Huang Y, Sun X, Jiang H, Yu S, Robins C, Armstrong MJ, Li R, Mei Z, Shi X, Gerasimov ES, De Jager PL, Bennett DA, Wingo AP, Jin P, Wingo TS, Qin ZS, A machine learning approach to brain epigenetic analysis reveals kinases associated with Alzheimer’s disease. *Nat. Commun* 12, 4472 (2021).
109. Zach S, Felk S, Gillardon F, Signal transduction protein array analysis links LRRK2 to Ste20 kinases and PKC zeta that modulate neuronal plasticity. *PLOS One* 5, e13191 (2010).
110. Buée L, Bussi re T, Bu e-Scherrer V, Delacourte A, Hof PR, Tau protein isoforms, phosphorylation and role in neurodegenerative disorders. *Brain Res. Rev* 33, 95–130 (2000).
111. Iqbal K, Alonso A. d. C., Chen S, Chohan MO, El-Akkad E, Gong C-X, Khatoon S, Li B, Liu F, Rahman A, Tanimukai H, Grundke-Iqbal I, Tau pathology in Alzheimer disease and other tauopathies. *Biochim. Biophys. Acta.* 1739, 198–210 (2005).

112. Henderson MX, Sengupta M, Trojanowski JQ, Lee VMY, Alzheimer's disease tau is a prominent pathology in LRRK2 Parkinson's disease. *Acta Neuropathol. Commun* 7, 183 (2019).
113. Esteves AR, Cardoso SM, LRRK2 at the crossroad between autophagy and microtubule trafficking: Insights into Parkinson's disease. *Neuroscientist* 23, 16–26 (2017).
114. Johnson JL, Yaron TM, Huntsman EM, Kerelsky A, Song J, Regev A, Lin T-Y, Liberatore K, Cizin DM, Cohen BM, Vasan N, Ma Y, Krismer K, Robles JT, van de Kooij B, van Vlimmeren AE, Andrée-Busch N, Käufer NF, Dorovkov MV, Ryazanov AG, Takagi Y, Kastenhuber ER, Goncalves MD, Hopkins BD, Elemento O, Taatjes DJ, Maucuer A, Yamashita A, Degterev A, Uduman M, Lu J, Landry SD, Zhang B, Cossentino I, Linding R, Blenis J, Hornbeck PV, Turk BE, Yaffe MB, Cantley LC, An atlas of substrate specificities for the human serine/threonine kinome. *Nature* 613, 759–766 (2023).
115. Miller CJ, Lou HJ, Simpson C, van de Kooij B, Ha BH, Fisher OS, Pirman NL, Boggon TJ, Rinehart J, Yaffe MB, Linding R, Turk BE, Comprehensive profiling of the STE20 kinase family defines features essential for selective substrate targeting and signaling output. *PLOS Biol.* 17, e2006540 (2019).
116. Koo C-Y, Giacomini C, Reyes-Corral M, Olmos Y, Tavares IA, Marson CM, Linardopoulos S, Tutt AN, Morris JDH, Targeting TAO kinases using a new inhibitor compound delays mitosis and induces mitotic cell death in centrosome amplified breast cancer cells. *Mol. Cancer Ther.* 16, 2410–2421 (2017).
117. Salvador AF, de Lima KA, Kipnis J, Neuromodulation by the immune system: A focus on cytokines. *Nat. Rev. Immunol* 21, 526–541 (2021).
118. Deciphering Developmental Disorders Study, Prevalence and architecture of de novo mutations in developmental disorders. *Nature* 542, 433–438 (2017).
119. Zhou X, Feliciano P, Shu C, Wang T, Astrovskaia I, Hall JB, Obiajulu JU, Wright JR, Murali SC, Xu SX, Brueggeman L, Thomas TR, Marchenko O, Fleisch C, Barns SD, Snyder LG, Han B, Chang TS, Turner TN, Harvey WT, Nishida A, O'Roak BJ, Geschwind DH, Michaelson JJ, Volfovsky N, Eichler EE, Shen Y, Chung WK, Integrating de novo and inherited variants in 42,607 autism cases identifies mutations in new moderate-risk genes. *Nat. Genet* 54, 1305–1319 (2022).
120. Kumar RA, Marshall CR, Badner JA, Babatz TD, Mukamel Z, Aldinger KA, Sudi J, Brune CW, Goh G, KaraMohamed S, Sutcliffe JS, Cook EH, Geschwind DH, Dobyns WB, Scherer SW, Christian SL, Association and mutation analyses of 16p11.2 autism candidate genes. *PLOS ONE* 4, e4582 (2009).

Chapter 2: Proteomic Identification of Phosphorylation-Dependent Septin 7 Interactors that Drive Dendritic Spine Formation

Sujin Byeon^{1†}, Bailey Werner^{2†}, Reilly Falter², Kristian Davidsen³, Calvin Snyder², Shao-En Ong²
and Smita Yadav^{2*}

¹Graduate Program in Neuroscience, University of Washington, Seattle, WA, United States

²Department of Pharmacology, University of Washington, Seattle, WA, United States

³Human Biology Division, Fred Hutchinson Cancer Research Center, Seattle, WA, United States

[†]Equal contribution

*Corresponding author

Abstract

Septins are a family of cytoskeletal proteins that regulate several important aspects of neuronal development. Septin 7 (Sept7) is enriched at the base of dendritic spines in excitatory neurons and mediates both spine formation and spine and synapse maturation. Phosphorylation at a conserved C-terminal tail residue of Sept7 mediates its translocation into the dendritic spine head to allow spine and synapse maturation. The mechanistic basis for postsynaptic stability and compartmentalization conferred by phosphorylated Sept7, however, is unclear. We report herein the proteomic identification of Sept7 phosphorylation-dependent neuronal interactors. Using Sept7 C-terminal phosphopeptide pulldown and biochemical assays, we show that the 14-3-3 family of proteins specifically interacts with Sept7 when phosphorylated at the T426 residue. Biochemically, we validate the interaction between Sept7 and 14-3-3 isoform gamma and show that 14-3-3 gamma is also enriched in the mature dendritic spine head. Furthermore, we demonstrate that interaction of phosphorylated Sept7 with 14-3-3 protects it from dephosphorylation, as expression of a 14-3-3 antagonist significantly decreases phosphorylated Sept7 in neurons. This study identifies 14-3-3 proteins as an important physiological regulator of Sept7 function in neuronal development.

Introduction

Septins are evolutionarily conserved cytoskeletal proteins important for diverse cellular processes including cell division, regulation of actin and microtubule dynamics, localization of scaffolding proteins, and membrane trafficking (Mostowy and Cossart, 2012). Septins structurally contain a GTP-binding domain and a variable N- and C-terminal domain, which oligomerize with each other to form symmetric filaments and higher order structures such as rings (Sirajuddin et al., 2007; Mendonça et al., 2021). In humans, there are 13 septin genes within four homology

groups: *SEPT2* (*SEPT1*, *SEPT2*, *SEPT4*, and *SEPT5*), *SEPT3* (*SEPT3*, *SEPT9*, and *SEPT12*), *SEPT6* (*SEPT6*, *SEPT8*, *SEPT10*, *SEPT11*, and *SEPT14*), and *SEPT7* (*SEPT7*) (Kinoshita, 2003a). Within these septin oligomers, septins from the same group are interchangeable, suggesting potential redundancy in their function. Notably, forming its own group, Septin7 (Sept7) is a unique and non-redundant core component of the septin complexes (Kinoshita, 2003b).

Several members of the septin family were found to be enriched in postsynaptic density (PSD) fractions in the mouse brain, by a mass spectrometry analysis, with Sept7 suggested to be the most abundant (Walikonis et al., 2000). Sept7 is expressed throughout all stages of neuronal differentiation and localizes at axonal and dendritic branching points as well as at the base of dendritic protrusions (Tada et al., 2007; Xie et al., 2007). Depletion of Sept7 leads to decreased branching of axon and dendrites both *in vitro* and *in vivo* (Tada et al., 2007; Xie et al., 2007; Hu et al., 2012; Ageta-Ishihara et al., 2013). Moreover, an increase in the number of immature dendritic filopodia was observed when Sept7 was knocked down (Tada et al., 2007). Sept7 localizes at the base of dendritic spines to create an important diffusion barrier for membrane protein entry into the dendritic spine head (Ewers et al., 2014). Furthermore, Sept7 was found to be a phosphorylation target of thousand-and-one amino acid kinase 2 (TAOK2), a serine/threonine kinase encoded by the autism risk gene *TAOK2* (de Anda et al., 2012; Yadav et al., 2017; Richter et al., 2018; Nourbakhsh et al., 2021). Phosphorylation of Sept7 by *TAOK2* at an evolutionarily conserved C-terminal tail residue T426 was shown to be essential for spine maturation. While in its unphosphorylated state Sept7 localizes to the base of dendritic spines and filopodia, when phosphorylated at T426, Sept7 relocates to the dendritic spine head (Yadav et al., 2017). Although

mechanisms through which phosphorylation induces Sept7 translocation are unclear, it was found that preventing this phosphorylation leads to increased dendritic filopodia as well as mislocalization of synaptic scaffold proteins to the dendritic shaft instead of the spine. This eventually led to formation of mislocalized synapses on the dendritic shafts (Yadav et al., 2017). How phosphorylation at the conserved T426 residue on the C-terminal tail of Sept7 regulates its function is unknown. The C-terminal tail extends perpendicularly out to the axis of the oligomeric septin filament (Sirajuddin et al., 2007). This is thought to facilitate protein–protein interactions in addition to lateral associations between septin filaments (Marques et al., 2012; Finnigan et al., 2015; Finnigan et al., 2016).

Herein, we show that phosphorylation of Sept7 C-terminal tail at the T426 residue can be mediated by several members of the TAO kinase family, including TAOK1 and two distinct isoforms of TAOK2 kinase. Furthermore, Sept7 phosphorylation at T426 was found to increase during development in rat embryonic hippocampal neurons. Sept7 phosphorylation was found to be important for spine maturation, as expression of phosphomimetic Sept7 T426D in early stages of neuronal development (DIV9) leads to precocious maturation of dendritic spines. Using live confocal imaging, we show that phosphorylated Sept7 is stably associated with the dendritic spine head over time in contrast with the wild-type and phosphomutant Sept7. To identify potential mechanisms through which phosphorylated Sept7 contributes to dendritic spine maturation, we performed an unbiased proteomic screening to identify phosphorylation-dependent binding partners of Sept7. Among the identified candidate interactors were several isoforms of 14-3-3 proteins and actin-binding proteins. Since 14-3-3 proteins bind and modulate functions of phosphorylated proteins, we tested whether phosphorylated Sept7 associates with 14-3-3 proteins.

Using biochemical assays, we demonstrate that phosphorylated Sept7 interacts with the 14-3-3 gamma (14-3-3 γ) isoform. Furthermore, we found that 14-3-3 γ is enriched in dendritic spines compared to other 14-3-3 isoforms. Finally, we found that disrupting 14-3-3 and Sept7 interaction perturbs the level of phosphorylated Sept7 and maturation of dendritic spines in neurons, revealing an important role of this interaction in regulating neuronal development.

Materials and Methods

Antibodies and Plasmids

Antibodies used for these experiments include: anti-HA-tag (Mouse, ProteinTech, Thermo Fisher Scientific, 50–173–6,449), phospho-TAOK2 (S181) (Rabbit, R&D Systems, PPS037), GFP (Mouse, Roche, 11,814,460,001), GST (Mouse, Invitrogen, MA4-004), 14-3-3 pan isoform (Rabbit, CST, #8312), and 14-3-3 gamma (Mouse, Sigma, MA1-16587). Rabbit phospho-Septin7 (pT426) antibody was generated, as described previously (Yadav et al., 2017). Human TAOK2 α was PCR amplified from pCMV-Sp6-TAOK2 plasmid (Ultanir et al., 2014) and cloned into sfGFP-C1 vector (Addgene #54579) using restriction sites HindIII and MfeI. Human TAOK2 β cDNA was obtained from Transomics (#BC152413) and cloned into the sfGFP-C1 vector using the same restriction sites. Similarly, TAOK1 cDNA from Transomics (#BC144067) was inserted into the sfGFP-C1 vector using restriction enzymes. Addgene plasmids used for these experiments include: HA-14-3-3 zeta (#116888) and pcDNA3-HA-14-3-3 gamma (#13274). EYFP-Difopein and EYFP-Control were gifts from Dr. Yi Zhou (Florida State University).

Immunoprecipitation Kinase Assays

HEK293T cells were grown in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin (Thermo Fisher Scientific). Expression constructs sfGFP-tagged TAOK1, TAOK2 α , and TAOK2 β (3 μ g) were transfected with JetOptimus, following the manufacturer's instructions. After 24 h post-transfection, cells were collected and incubated in HKT lysis buffer (25 mM HEPES pH 7.2, 150mM KCl, 1% Triton X-100, 2 mM DTT, and 1X protease inhibitor (Roche, cOmplete™ EDTA free)) for 30 min on ice prior to homogenization with a 25-gauge syringe needle. Pierce™ Protein G Agarose beads (Thermo Fisher Scientific) were washed with HKT buffer thrice. The supernatant was collected from cell lysates through centrifugation at 800g for 5 min, precleared with Protein G Agarose beads for 30 min, and immunoprecipitated with the GFP antibody overnight at 4°C. Beads were washed with HKT buffer twice, incubated with high salt HKT buffer (25 mM HEPES pH 7.2, 1M NaCl, 1% Triton X-100, 2 mM DTT, and 1X protease inhibitor (Roche, cOmplete™ EDTA free)) for 10 min, and washed with HK buffer (25 mM HEPES, 150mM KCl, 2 mM DTT, and 1X protease inhibitor (Roche, cOmplete™ EDTA free)) twice and then with kinase buffer (20 mM Tris HCl pH 7.5, 10 mM MgCl₂, 1 mM DTT, 1X protease inhibitor (Roche, cOmplete™ EDTA free)) twice at 4°C. Kinase assay was performed by incubating the beads with the kinase buffer, 1 mM ATP, and 10X phosphatase inhibitor (Thermo Fisher Scientific, Halt) at 30°C for 45 min at 920 rpm. To assay for phosphorylation of Septin7 by TAO kinases, purified GST-tagged Sept7 C-terminal tail (321–438 amino acids) was also added to the reaction. Samples were prepared by adding NuPAGE™ LDS sample buffer (Thermo Fisher Scientific) containing 125 mM DTT, heating for 10 min at 95°C, and centrifuging at 5,000g for 5 min. Samples were run on NuPAGE™ 4–12% bis-tris polyacrylamide gels (Thermo Fisher Scientific) with NuPAGE™ MOPS running buffer (Thermo Fisher Scientific) at 165 V for 20 min and then at 175 V for 50 min. Gels were

transferred to Immobilon-P membrane at 100 V for 1 h. The blots were blocked with 5% BSA blocking buffer and probed with phospho-TAO2 (S181) and phospho-Sept7 (T426) antibodies at 1:500 dilution overnight at 4°C followed by 3 h incubation with HRP-conjugated secondary antibody at 1:5,000 dilution at room temperature. The kinase activity was quantified by normalizing phospho-TAOK2 signal intensity to that of the GFP signal and phospho-Sept7 signal intensity to that of the GST signal.

Protein Purification

Septin 7 C-terminal tail (321–438 amino acids) was cloned into pGEX4T1 vector using restriction sites BamHI and XhoI and transformed into the BL21 *E. coli* bacterial strain to bacterially express GST-tagged Sept7C-WT (Yadav et al., 2017). A 25 ml starter culture grown from a single colony overnight was used to inoculate 1 L culture, which was allowed to grow at 37°C until OD600 reached 0.6. Protein expression was induced by adding IPTG at the final concentration of 0.3 mM and growing the culture for additional 5 h at 30°C. Cells were harvested by centrifugation at 4500 g for 15 min at 4°C, washed with ice-cold PBS, and then resuspended in lysis buffer (50 mM Tris pH 8.0, 5 mM EDTA, 150 mM NaCl, 10% glycerol, 5 mM DTT, 1X protease inhibitor (Roche, cOmplete™ EDTA free), and 2 mM PMSF). To further lyse the cells, they were incubated with 4 mg of lysozyme on ice for 30 min followed by addition of 0.5% Triton X-100 and sonication. The supernatant was collected after a 30-min spin at 25,000 g and incubated with prewashed GST beads (Thermo Fisher Scientific) for 2 h. Beads were washed with wash buffer (PBS + 1 mM DTT +0.1% Tween 20) and then with wash buffer without detergent. Bound protein were eluted and collected in fractions by glutathione elution buffer at pH 8.0 (50 mM Tris pH 8.0, 250 mM KCl, 1 mM DTT, and 25 mM glutathione).

Co-Immunoprecipitation

Co-immunoprecipitation assays were performed in HEK293T cells grown in DMEM media (Thermo Fisher Scientific, Gibco) with 10% fetal bovine serum (Axenia) and 1% penicillin–streptomycin (Invitrogen). Cells were grown to confluence in a flat-bottom plate with 35-mm deep wells at 5% CO₂ and 37°C and transfected with 2.0 µg of HA-14-3-3 gamma, HA-14-3-3 zeta, or 1.5 µg of both gamma and zeta using JetOptimus, following the manufacturer's instructions. Cells were co-transfected with 1.0 µg of GFP-tagged Sept7-WT. Approximately 36 h post-transfection, cells were treated with 0.1 µM okadaic acid for 10 min and lysed with 1 ml HKT buffer (25 mM HEPES pH7.2, 100 mM KCl, 1% Triton X-100, 1 mM DTT, 1 mM EDTA, phosphatase inhibitor (Thermo Fisher Scientific, Halt), and protease inhibitor (Roche, cOmplete™ EDTA free)) per 2-well construct. Lysate was incubated on ice for 20 min prior to homogenization with a 25-gauge syringe needle. Homogenate was pelleted via centrifugation at 6,000 g and 4°C for 5 min. Pierce™ Protein G Agarose (Thermo Fisher Scientific) beads and EZview™ Red Anti-HA Affinity Gel (Millipore-Sigma) beads were washed twice for 5 min at 4°C and re-suspended in 150 µL HKT per sample tube. Pellet supernatant was pre-cleared with 20 µL Pierce™ Protein G Agarose (Thermo Fisher Scientific) beads, and pre-cleared supernatant was collected for input samples. Remaining volume of pre-cleared supernatant was immunoprecipitated with 20 µL EZview™ Red Anti-HA Affinity Gel (Millipore-Sigma) beads. Beads were washed three times with HKT and twice with HK buffer (25 mM HEPES pH 7.2, 100mM KCl, 1 mM DTT, and 1 mM EDTA). Both immunoprecipitation and input samples were prepared with 4X LDS sample buffer (Thermo Fisher Scientific) and 125 mM DTT followed by heat treatment at 95°C for 10 min. Samples were centrifuged at 14,000g for 5 min at 4°C and electrophoresed on NuPAGE™ 4–12% bis-tris

polyacrylamide gels (Thermo Fisher Scientific) with NuPAGE™ MOPS running buffer (Thermo Fisher Scientific) for 40 min at 160 V.

Neuronal lysates were prepared at DIV18 with HKT buffer (25 mM HEPES pH7.2, 100 mM KCl, 1% Triton X-100, 1 mM DTT, 1 mM EDTA, and protease inhibitor (Roche, cOmplete). Lysates were precleared with agarose beads and then incubated with 14-3-3 gamma (mouse) or 14-3-3 pan (Rabbit)–bound sepharose beads overnight. Beads were washed thrice with HKT buffer and twice with buffer without detergent. In experiments where phosphospecific interaction was probed, Protein G beads with immunoprecipitated 14-3-3γ were incubated overnight with neuronal lysates that were prepared in 500 μL of lysis buffer (50 mM HEPES, 100 mM NaCl, 2 mM DTT, 1% TritonX100, and 1 mM MnCl₂) and pretreated with or without 10 μL of lambda protein phosphatase (NEB, Cat#P0753) at 30°C for an hour. Beads were then washed thrice with lysis buffer and twice with buffer without detergent before running on SDS-PAGE gels.

Western Blot

For Western blot analysis, gels were transferred to Immobilon-P PVDF membrane (Millipore-Sigma) with transfer buffer (25 mM Tris, 192 mM glycine, and 20% (v/v) Methanol) for 60 min at 100 V. Blots were cut below the 50 kDa mark to produce one half-membrane consisting of high molecular weights to detect phosphorylated Septin 7 and one half-membrane consisting of low molecular weights to detect 14-3-3 isoforms (endogenous or HA-tagged). Blots were blocked in 5% milk (5% milk powder (Carnation), 50 mM Tris-Cl, 150 mM NaCl, and 0.1% Tween™ 20 (Thermo Fisher Scientific)) or 2% BSA (2% bovine serum albumin (VWR), 50 mM Tris-Cl, 150 mM NaCl, and 0.1% Tween™ 20 (Thermo Fisher Scientific)) blocking buffer for 1 hour at

room temperature and incubated in primary antibody overnight at 4°C. Primary rabbit antibody to detect phosphorylated Septin 7 (T426) was diluted to 1:250 and applied to high molecular weight half-membrane, and primary mouse antibody to detect HA-tag or endogenous 14-3-3 was diluted to 1:1,000 and applied to low molecular weight half-membrane. Blots were washed with blocking buffer at room temperature before secondary antibody incubation. Blots were incubated in HRP-conjugated secondary antibody at 1:1,000 dilution for 3 hours at room temperature. Blots were washed successively with blocking buffer, TBST (50 mM Tris-Cl, 150 mM NaCl, and 0.1% Tween™ 20 (Thermo Fisher Scientific)), and TBS (50 mM and 150 mM NaCl). Western blot images were visualized with Pierce™ ECL Western Blotting Substrate (Thermo Fisher Scientific) and captured with the ChemiDoc Imager (Bio-Rad).

Peptide Pulldown and Mass Spectrometry

Synthetic peptide corresponding to Sept7 C-terminal tail residues 416–438 were commercially synthesized with an N-terminal cysteine residue and T426 in either phosphorylated or unphosphorylated forms (Elim Biopharmaceuticals). Peptides were coupled to SulfoLink beads (Invitrogen), according to manufacturer's protocol. In brief, 0.5 mg of peptide was dissolved in 1 ml of coupling buffer (50 mM Tris and 5 mM EDTA, pH8.5) and then TCEP was added to a final concentration of 25 mM. Beads (50 ul for each peptide) were washed four times in the coupling buffer, quenched with l-cysteine-HCl and then incubated with 100 ul of 0.5 mg/ml P15 mouse brain lysate. Beads were washed four times with lysis buffer, containing 20 mM Tris pH 7.5, 100 mM NaCl, 10 mM MgCl₂, 0.5 mM DTT, 1% Triton X-100, 0.1% deoxycholic acid, and protease inhibitors (Roche), and thrice with lysis buffer without detergent. Proteins bound to beads were then denatured by adding 3x bead volumes of 8M urea/10 mM Tris pH 8.0 to resuspend

beads. TCEP was added to a final concentration of 1 mM and incubated at room temperature for 30 min with thermomixer/slow vortex. Chloroacetamide (CAM) was added to final concentration of 3 mM and incubated at room temperature for 10 min with thermomixer/slow vortex. TCEP was added to quench excess CAM after alkylation is complete. The pH was then adjusted to 8. For protein digestion, LysC (Mass Spec Grade) at 1:100 enzyme:substrate ratio was added and incubated at 37°C for 2 h on a thermomixer with gentle agitation. Then 3x reaction volumes of TEAB was added to dilute urea to less than or equal to 2M urea. Then pH was adjusted to 8.0. Trypsin (MS grade, Promega) was added at 1:100 enzyme:substrate ratio and incubated at 37°C for 12–16 h with gentle agitation. Digestion was stopped by adding TFA to a final concentration of 1%. Peptide samples were desalted on C18 StageTips. Peptide samples were separated on an Thermo Dionex UltiMate 3000 RSLCnano System (Thermo Fisher Scientific) using 20-cm long fused silica capillary columns (100 µm ID, laser pulled in-house with Sutter P-2000, Novato CA) packed with 3 µm 120 Å reversed phase C18 beads (Dr. Maisch, Ammerbuch, DE). Liquid chromatography (LC) solvent A was 0.1% (v/v) aq. Acetic acid and LC solvent B was 20% of 0.1% (v/v) acetic acid and 80% acetonitrile. The LC gradient was 100-min long with 5–35% B at 300 nL/min. MS data was collected using an Orbitrap Elite mass spectrometer (Thermo Fisher Scientific).

Mass Spectrometry Data Analyses

Data-dependent analysis was applied using top15 selection with CID fragmentation. Data raw files were analyzed by MaxQuant/Andromeda (Cox et al., 2011) version 1.6.2.8 using protein, peptide, and site FDRs of 0.01, a score minimum of 40 for modified peptides and 0 for unmodified peptides, and a delta score minimum of 17 for modified peptides and 0 for unmodified peptides. MS/MS

spectra were searched against the UniProt mouse database (updated July 2016). MaxQuant search parameters are as follows: variable modifications included Oxidation (M) and Phospho (S/T/Y). Carbamidomethyl (C) was a fixed modification. Maximum missed cleavages were 2, enzyme was trypsin/P, and maximum charge was 7. The initial search tolerance for FTMS scans was 20 ppm and 0.5 Da for ITMS MS/MS scans. Label-free quantification (LFQ) of MS intensities from five process replicates/condition was processed with Perseus. Protein groups were filtered to remove proteins 'only identified by site' as well as reverse and potential contaminants. MS intensities were log2 transformed, and missing values were imputed by randomly selecting from a distribution downshifted by 1.8 and a width of 0.3. Significance was calculated by a two-sample Student's t-test.

MS raw data (ID MSV000088699) is publicly available at repository UCSD Massive database and can be accessed freely at <https://massive.ucsd.edu>.

Immunofluorescence

Rat embryonic hippocampal neurons were grown on coverslips coated with 0.1M borate buffer (50 mM boric acid and 12.5 mM sodium tetraborate in water, pH 8.5), 60 ug/mL poly-d-Lysine (Sigma), and 2.5 ug/mL laminin (Sigma) in 12-well plates. For immunofluorescence, neurons were fixed with warm 4% paraformaldehyde + 4% sucrose in PBS, followed by 60-min incubation in blocking buffer (0.2% Triton X-100, 10% normal goat serum (Jackson Labs), and 0.2M glycine pH 7.2) at room temperature. Primary antibody was incubated overnight at 4°C followed by secondary antibody for 3 hours at room temperature. Coverslips were mounted on slides using Fluoromount-G.

Neuronal Culture

Hippocampi were obtained from E17–E18 Sprague Dawley rat embryos (Envigo), trypsin dissociated, and plated at a density of 150,000 cells per 18 mm coverslip (Fisher Scientific) and 50,000–500,000 cells per 35 mm glass bottom dish (MatTek). Dishes were coated as previously described. Neurons were seeded in plating media (10% fetal bovine serum heat inactivated, 20% dextrose, 1x Glutamax (Invitrogen), 1x penicillin/streptomycin (Thermo Fisher Scientific), Eagle's MEM with Earle's BBS (Lonza)) for 4 hours. Media was changed to maintenance media (B27 (Invitrogen), 1x penicillin/streptomycin, 1x Glutamax, and neurobasal media (Invitrogen)). Half of the media was replaced with new maintenance media every 3–4 days. Transfection of mammalian expression constructs in neurons was performed using Lipofectamine 2000, following the manufacturer's instructions. Neurons were either fixed for 48 h after transfection using 4% PFA+ 4% sucrose, or imaged live 48 h after transfection.

Microscopy and Image Analyses

All live and fixed cell imaging was performed using a Nikon Ti2 Eclipse-CSU-X1 confocal spinning disk microscope equipped with four laser lines 405, 488, 561, and 670 nm and a sCMOS Andor camera for imaging. The microscope was caged within the OkoLab environmental control setup enabling temperature and CO₂ control during live imaging. Imaging was performed using Nikon 1.49 ×100 Apo, ×60, or ×40 oil objectives. All image analyses were done using the open access Fiji software.

Statistics

All statistics except for the mass spectrometry data were performed in GraphPad software Prism 9.0. Two group comparisons were made using unpaired t-test, unless otherwise stated. Statistically, p-value less than 0.05 was considered significant. All experiments were done in triplicate, unless stated otherwise, and experimental sample size and p-values are indicated with the corresponding figures.

Results

Conserved C-Terminal Tail in Septin 7 is Phosphorylated by TAO Kinases During Neuronal Development

Sept7 was identified through an unbiased chemical and genetic screening as a phosphorylation target of TAOK2 (Yadav et al., 2017). The site of phosphorylation residue T426 lies in the extended C-terminal coiled coil tail of Sept7. This threonine residue which harbors the consensus site for TAO kinases p[S/T]-X-X[R/H/K] is evolutionarily highly conserved (Figure 1A). Members of thousand-and-one amino acid kinase (TAOK) family contain a highly conserved N-terminal kinase domain, while their C-terminal domains considerably vary (Supplementary Figure S1A). TAO kinases, TAOK1 and spliced isoforms of TAOK2, α and β , are highly expressed in the brain (de Anda et al., 2012 and Allen Brain Atlas), and both TAOK1 and TAOK2 have been associated with neurodevelopmental disorders (Richter et al., 2018; Dulovic-Mahlow et al., 2019; van Woerden et al., 2021). TAOK2, both α and β , as well as TAOK1 have been shown to be important for several aspects of neuronal development including dendritic spine formation (Yasuda et al., 2007; Ultanir et al., 2014; Yadav et al., 2017; van Woerden et al., 2021). Recently, we discovered that TAOK2 α is an endoplasmic reticulum-associated kinase with a transmembrane C-terminal region (Nourbakhsh et al., 2021). Given the structural conservation among the kinase

domain of TAO kinases and their high expression in the brain (Hu et al., 2021), we tested whether closely related kinase TAOK1 and the alternatively spliced isoforms of TAOK2, TAOK2 α , and TAOK2 β could also phosphorylate Sept7 at T426. GFP-tagged TAOK1, TAOK2 α , and TAOK2 β were expressed independently in HEK293T cells, immuno-precipitated using GFP antibody and then incubated with purified GST-Septin7 protein (Supplementary Figure S1B) in an in vitro kinase reaction. Using a phosphospecific antibody (pT426) against the Sept7 T426 residue, we found that TAOK1, TAOK2 α , and TAOK2 β could each phosphorylate purified GST-Sept7 (Figures 1B,C). All of the immunoprecipitated TAO kinases were active as they could autophosphorylate themselves on the conserved residue S181 (TAOK1-2) in the kinase domain. Level of Sept7 phosphorylation by TAOK2 α was significantly higher than that by TAOK2 β , while phosphorylation by TAOK1 was not statistically different from TAOK2 α . Furthermore, by immunostaining cultured hippocampal neurons at different stages of development, we found that phosphorylated Sept7 (T426) levels increased in neurons as it matured from DIV3 and DIV10 to DIV16 (Figures 1D,E). This is consistent with the increase in TAOK1, TAOK2 α , and β expression in the mouse cortex from embryonic stages to the perinatal stage which then persists throughout adulthood (de Anda et al., 2012 and Allen Brain Atlas). These data suggest that the conserved family of TAO kinases show redundancy in Septin7 phosphorylation, where depending on the cellular context potentially multiple kinases of the TAO family can phosphorylate Sept7 C-terminal tail.

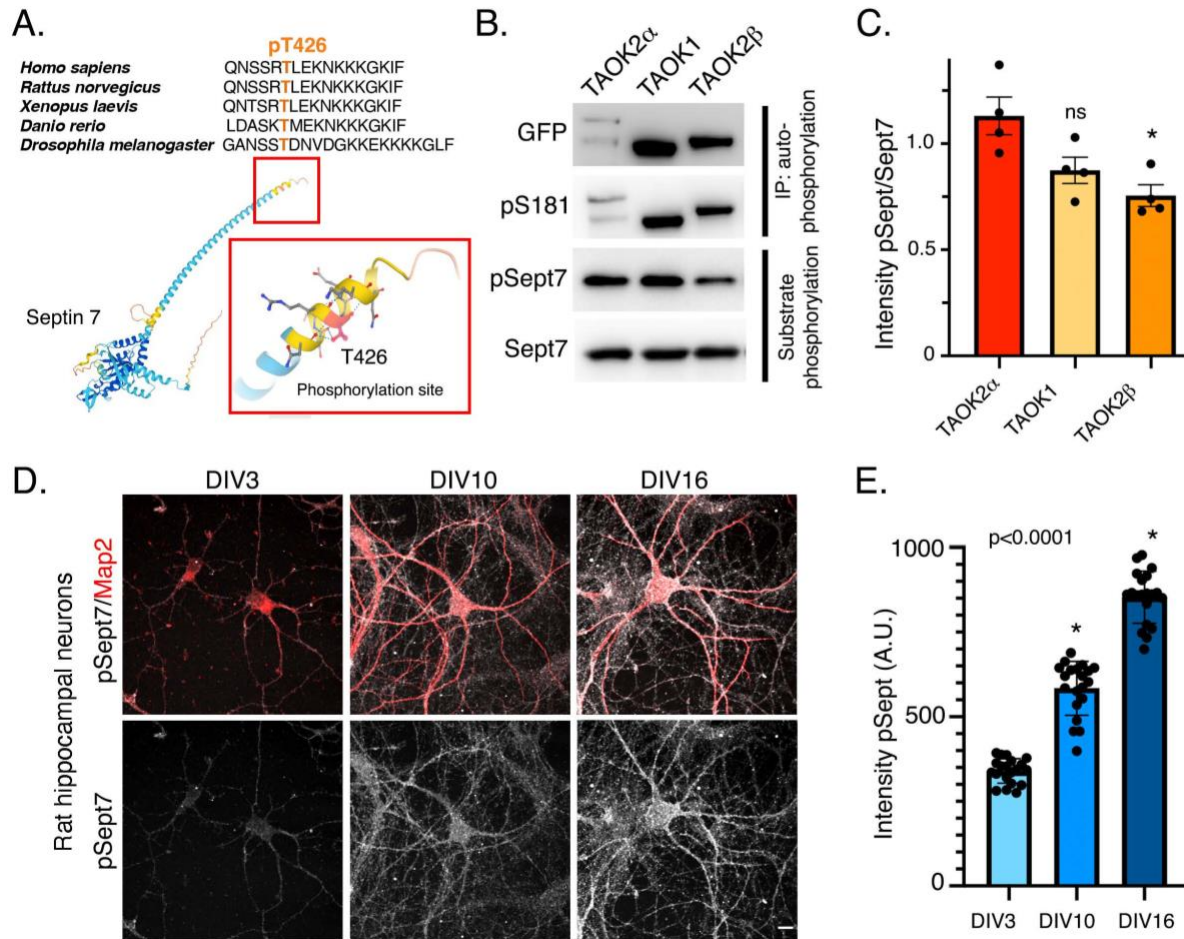


Figure 2.1. TAO kinases phosphorylate the C-terminal tail of Sept7 during neuronal development.

(A) Top: multiple sequence alignment of the C-terminal tail of Sept7 demonstrating its evolutionarily conserved sequence including the phosphorylation site. The phosphorylated residue T426 is shown in orange. Bottom: AlphaFold2.0 predicted protein structure of Sept7. Magnified inset depicts the T426 phosphorylation site (red) in the C terminal tail of Sept7. (B) Western blot shows kinase activity of sfGFP-TAOK2 α , sfGFP-TAOK1, or sfGFP-TAOK2 β expressed in HEK293T cells, immunoprecipitated with GFP antibody, and incubated with purified GST-Sept7 C-terminal tail (321–438 amino acids) for an *in vitro* kinase reaction. Substrate phosphorylation was determined by probing for phosphorylated Sept7 using the pT426 antibody. Total Sept7 was

detected by antibody against GST tag. TAO kinase autophosphorylation was determined by probing for phosphorylated S181 residue. **(C)** Quantification of protein band intensity of Western blot in **(B)**. Bar graph displays the ratio of phosphorylated Sept7 band intensity to GST band intensity for each TAO kinase construct in which no significant difference was observed between TAOK2 α and TAOK1 kinases. Error bars represent standard error of mean from $n = 4$ experiments. **(D)** Rat hippocampal neurons at DIV3, 10, and 16 were fixed and immunostained for endogenous phosphorylated Sept7 T426 and dendritic protein Map2. Scale bar represents 10 μ m. **(E)** Mean fluorescence intensity of phosphorylated Sept7 T426 at distinct stages of neuronal development DIV3, 10, and 16 are plotted. Error bars represent standard error of mean for $n = 20$ neurons from three different experiments. Dunnett's multiple comparison shows significant changes in pSept7 levels between each stage.

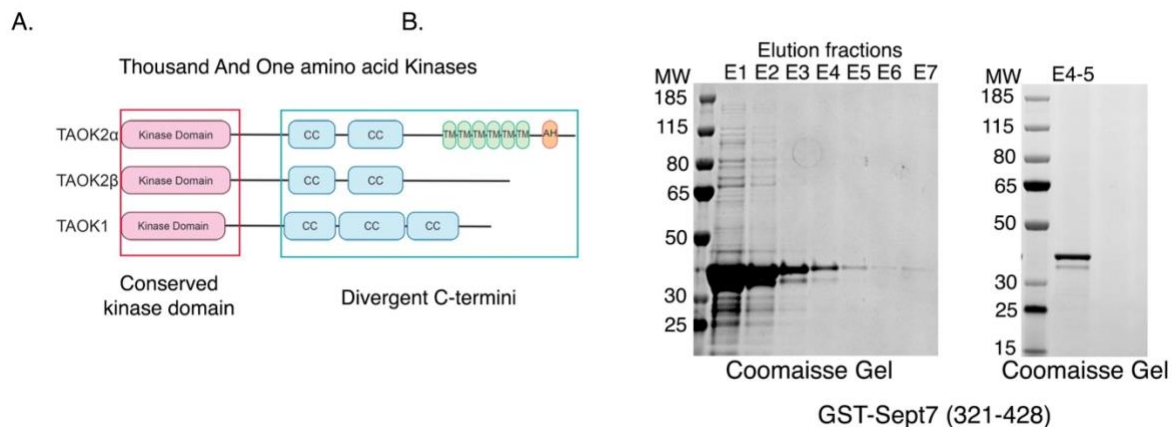


Figure 2S.1. TAO kinases phosphorylate the C-terminal tail of Sept7 during neuronal development (related to main Figure 1)

(A) Members of the TAO family of kinases comprise a conserved N-terminal kinase domain and variable C-terminal region. TAOK2 has two isoforms where the longer alpha isoform has

hydrophobic transmembrane domains that anchor it to the endoplasmic reticulum. TAO2 and TAO1 are cytosolic kinases.

(B) Coomassie gels show the purified GST tagged Septin 7 protein (amino acid 321-428). Elution fractions E1-E7 are shown on left. Fractions E4 and 5 were combined and further purified for use in in vitro kinase assay in Figure 1.

Altered Dynamics of Phosphorylated Septin 7

We had previously reported that phosphorylated Sept7 is enriched in the dendritic spine head and is important for dendritic spine formation and stability of PSD95 (Yadav et al., 2017). Expression of Sept7 phosphomutant T426A leads to failure of dendritic spine maturation, resulting in exuberant dendritic filopodia and shaft synapses (Yadav et al., 2017). To test the role of Sept7 phosphorylation during neuronal maturation, we expressed wild-type (WT), phosphomimetic (T426D), and phosphomutant (T426A) Sept7 in DIV9 neurons and imaged them at DIV11, a developmental stage where filopodial protrusions have not yet developed into mature mushroom dendritic spines. We found that expression of GFP-tagged phosphomimetic Sept7 (T426D) as opposed to WT Sept7 led to early maturation of dendritic spines in hippocampal neurons (Figure 2A). In contrast, expression of phosphomutant Sept7 T426A as expected resulted in extensive filopodial protrusions (Figure 2A). We found that even at this early stage of development at DIV11, there was accumulation of GFP-Sept7 in dendritic spines in neurons expressing phosphomimetic Sept7 T426D. We performed confocal live imaging of neurons expressing either the wild-type (WT), phosphomimetic (T426D), or phosphomutant (T426A) GFP-tagged Sept7. Live imaging of dendritic protrusions at DIV11 revealed that wild-type GFP-Sept7 exhibited dynamic changes in its accumulation within the filopodial protrusions within 10 s time period. In contrast,

phosphomimetic Sept7 (T426D) remained stably localized within the dendritic spine head, while the phosphomutant Sept7 (T426A) predominantly was localized at the base of the dendritic spine (Figure 2B, montage over 10 s). Quantification of Sept7 intensity in dendritic protrusions in DIV11 neurons revealed a significant increase in Sept7 T426D expressing neurons compared to WT, while there was a dramatic decrease in intensity of Sept7 T426A compared to WT ($n = 50$ protrusions, 12 neurons from three experiments, and one way ANOVA). Based on these data showing distinct localization and dynamics of Sept7 in its phosphorylated and unphosphorylated states at residue T426, we hypothesized that there must be unique interaction partners of phosphorylated Sept7 that modulate its discrete properties.

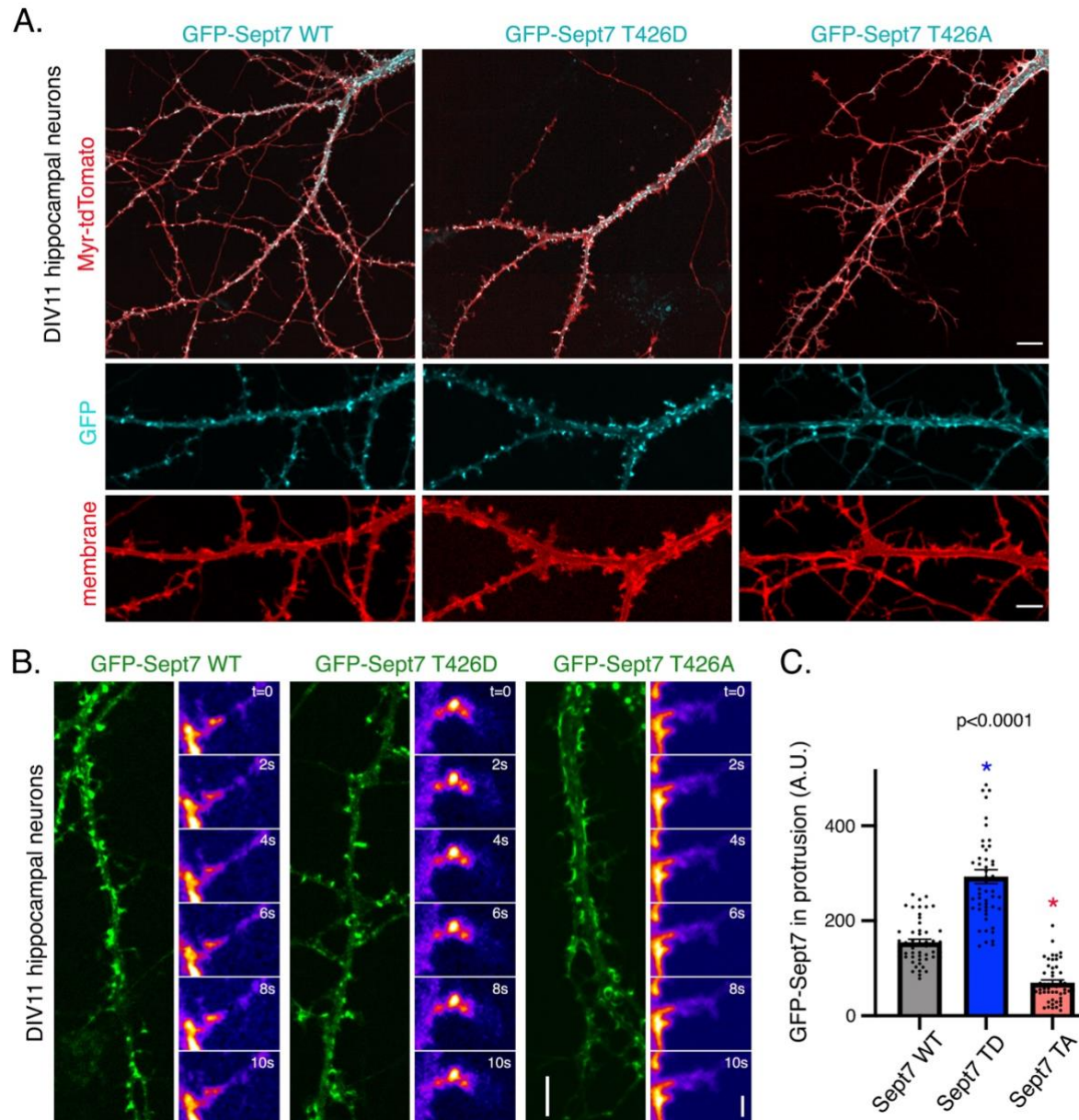


Figure 2. 2. Phosphorylation of Sept7 alters its localization and dynamics.

(A) Hippocampal neurons were transfected with GFP-tagged Sept7-WT, phosphomimetic Sept7-T426D, or phosphomutant Sept7-T426A along with membrane marker myristoylated-tdTomato at DIV9 and then fixed at DIV11. Phosphomimetic GFP-Sept7 (T426D) expression leads to early maturation of dendritic spines compared to Sept7-WT, while phosphomutant Sept7-T426A expressing neurons exhibited only filopodial protrusions. (B) Montage of time lapse images showing DIV11 hippocampal neurons transfected at DIV9 with GFP-tagged wild-type Sept7-WT,

phosphomimetic Sept7-T426D, or phosphomutant Sept7-T426A. Phosphomimetic Sept7-T426D is stably localized within the dendritic spine head, phosphomutant Sept7-T426A localizes to the base of the dendritic spine, whereas the wild-type Sept7-WT shows dynamic changes in its localization within protrusions. Scale bar represents 5 μm (green) and 1 μm (montage), respectively. (C) Quantification of GFP intensity in protrusions of DIV11 neurons transfected with GFP-tagged wild-type Sept7-WT, phosphomimetic Sept7-T426D, and phosphomutant Sept7-T426A. Values represent mean intensity, error bars represent SEM and $n = 50$ protrusions from 10 neurons in each condition. Dunnett's multiple comparison test, $p < 0.0001$.

Proteomic Identification of Septin 7 Phosphorylation-Dependent Binding Partners

We set out to identify proteins that specifically interact with Sept7 following phosphorylation at residue T426 in its C-terminal tail. This was achieved using an unbiased proteomic strategy to pull down proteins from P15 mouse brain lysate with a synthetic peptide corresponding to Sept7 C-terminal tail residues 416–438 (Figure 3A). This peptide of 22 amino acids was unique to Sept7, as BLAST analyses did not identify any other protein harboring this sequence. Next, the phosphorylated (pSept7) and non-phosphorylated (Sept7) peptides were coupled to beads. Agarose beads activated with iodoacetamide were used for covalent immobilization of peptides corresponding to pSept7 and Sept7 peptides. Peptide coupled beads were then incubated with mouse brain lysate. Proteins that were bound to peptide beads were enzymatically digested and then subjected to mass spectrometry-based identification. Label free quantification was used to measure the relative abundance of proteins bound to Sept7 and pT426-Sept7 (Figure 3A). In our analyses of five replicates of peptide pulldowns, we found 33 proteins (Table 1) that were significantly associated with phosphorylated Septin 7 peptide over unphosphorylated peptide

(Figure 3B). These proteins were analyzed by STRING11.2 and the most significant biological process was identified as regulation of cytoskeletal organization including actin-binding proteins as well as the phosphoprotein regulating proteins of the 14-3-3 family (Figures 3C,D). The 14-3-3 protein family is a group of highly conserved acidic proteins highly expressed in the mammalian brain (Boston et al., 1982; Ferl et al., 2002). In humans, there are seven known isoforms of 14-3-3: β , γ , ϵ , η , δ , τ , and ζ (Gardino et al., 2006). 14-3-3 proteins are ubiquitous regulatory proteins that form both homo- and heterodimers, and their dimerization provides binding sites for association with one or two phosphorylated ‘client’ proteins (Gardino et al., 2006; Uhart and Bustos, 2013). Among the candidate pSept7-binding partners identified by our mass spectrometry experiment (Figure 3C), we were specifically interested in 14-3-3 proteins as 1) binding of 14-3-3 proteins is largely determined by phosphorylation of the client, with the isoforms typically binding phosphorylated serine/threonine motifs (Aitken, 1996; Fu et al., 2000); 2) 14-3-3 are expressed in the brain and important for synapse development; and 3) binding of phosphoproteins to 14-3-3 can serve diverse purposes, including preventing protein–protein interactions, scaffolding, inducing conformational changes, protecting phosphorylation sites, and promoting or preventing ubiquitination.

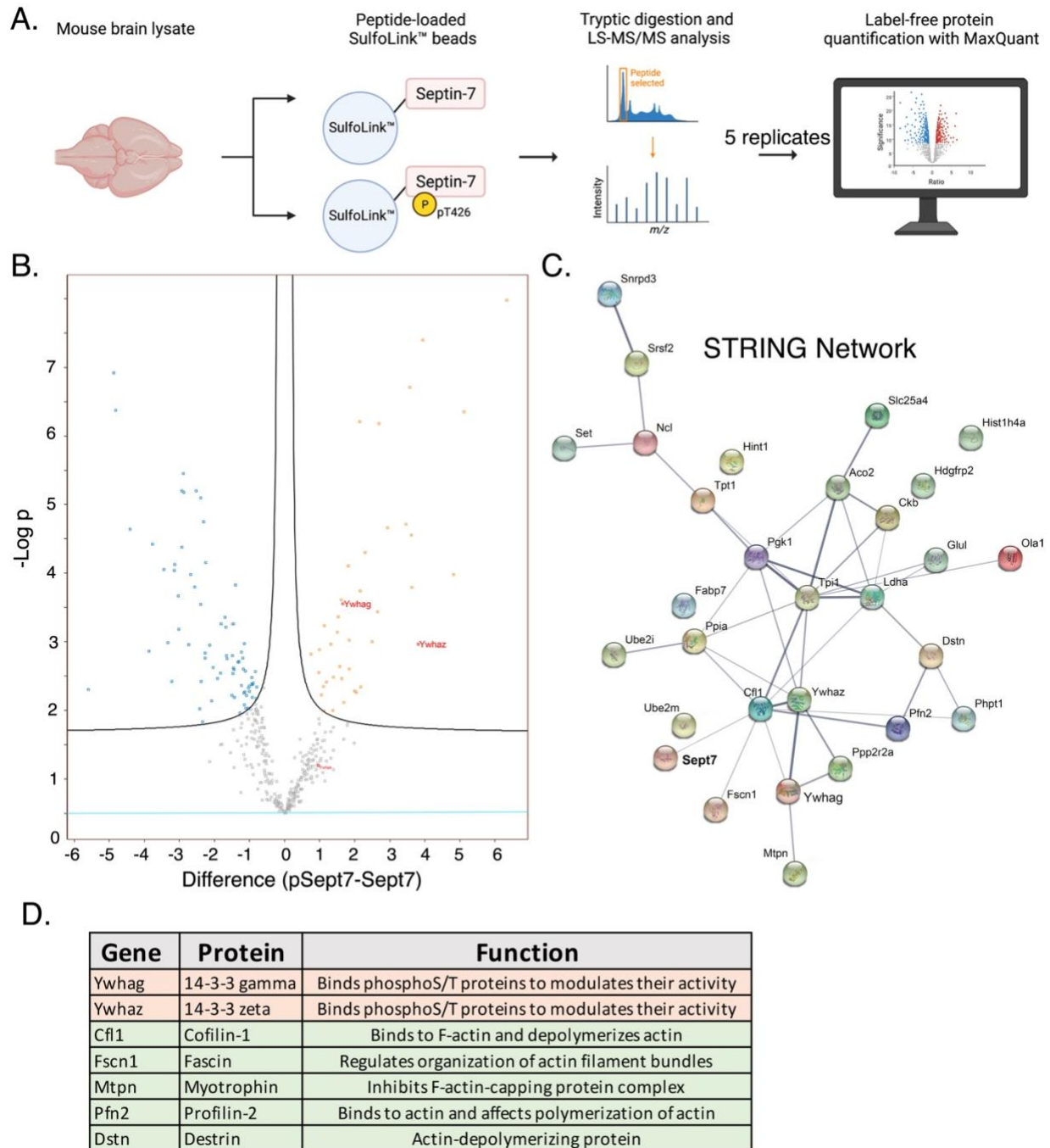


Figure 2.3. Proteomic identification of Septin 7 phosphorylation-dependent binding partners.

(A) Workflow shows the proteomic strategy for identifying Sept7 phosphorylation-dependent binding partners. (B) Volcano plot displays the difference in association of proteins with phosphorylated (pT246) Sept7 versus unphosphorylated Sept7 based on five replicate pulldowns. 14-3-3 protein isoforms are labeled in red. (C) The protein-protein association network of

candidate pSept7 interaction partners. The STRING network of identified proteins that significantly associate with phosphorylated Sept7 tail (pT426) versus non-phosphorylated Sept7 tail. Network nodes represent interacting proteins, and network edges indicate both functional and physical protein associations. Strength of the association is denoted by the thickness of the edges. **(D)** 14-3-3 family of proteins as well as several actin binding and depolymerizing proteins were found to significantly associate with Sept7 when phosphorylated at T426.

Table 2.1. List of proteins identified by Mass Spectrometry that associate with Septin 7 when phosphorylated at residue T426.

Protein name	Gene	Function
14-3-3 protein gamma	Ywhag	Binds phospho S/T proteins and modulates their of activity
14-3-3 protein zeta/delta	Ywhaz	Binds phospho S/T proteins and modulates their of activity
Myotrophin	Mtpn	Regulates growth of actin filaments. Inhibits the activity of the F-actin-capping protein complex
Destrin	Dstn	Actin-depolymerizing protein. Severs actin filaments (F-actin) and binds to actin monomers (G-actin)
Fascin	Fscn1	Organization of actin filament bundles and the formation of microspikes and stress fibers
Profilin-2	Pfn2	Binds to actin and affects the structure of the cytoskeleton
Cofilin-1	Cfl1	Binds to F-actin and exhibits F-actin depolymerizing activity
Glutamine synthetase	Glul	Catalyzes the production of glutamine and 4-aminobutanoate (gamma-aminobutyric acid, GABA)
Histidine triad nucleotide-binding protein 1	Hint1	Hydrolyzes purine nucleotide phosphoramidates
Triosephosphate isomerase	Tpi1	Triosephosphate isomerase 1
Eukaryotic translation initiation factor 5A-1; 5A-2	Eif5a; Eif5a2	mRNA-binding protein involved in translation elongation
Fatty acid-binding protein, brain	Fabp7	Belongs to the calycin superfamily
Translationally-controlled tumor protein	Tpt1	Involved in calcium binding and microtubule stabilization
Heterogenous nuclear ribonucleo protein A3	Gm6793; Gm9242; Hnmpa3	Plays a role in cytoplasmic trafficking of RNA
SUMO-conjugating enzyme UBC9	Ube2i	Necessary for sumoylation
Protein SET	Set	Multitasking protein, involved in apoptosis, transcription, and histone chaperoning
Guanine nucleotide-binding protein G(i)(G(s)/G(t)) subunit beta-1; beta-2; beta-4	Gnb1; Gnb2; Gnb4	Guanine nucleotide-binding proteins act as a modulator in various transmembrane signaling events
NEDD8-conjugating enzyme Ubc12	Ube2m	Ubiquitin conjugating enzyme
Ubiquitin-conjugating enzyme E2 D2; Ubiquitin-conjugating enzyme E2 D3; D2B	Ube2d2; Ube2d3; Ube2d2b	Ubiquitin conjugating enzyme
Serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B alpha isoform	Ppp2r2a	Protein Phosphatase
Serine/arginine-rich splicing factor 2	Srsf2	Necessary for the splicing of pre-mRNA
ADP/ATP translocase 1	Sic25a4	Involved in mitochondrial ADP/ATP transport
Peptidyl-prolyl cis-trans isomerase A; Peptidyl-prolyl cis-trans isomerase A	Ppia	PPases accelerate the folding of proteins
14 kDa phosphohistidine phosphatase	Phpt1	Exhibits phosphohistidine phosphatase activity
Hepatoma-derived growth factor-related protein 2	Hdgfrp2	Associates with chromatin
Histone H4	Histh4a	Core component of nucleosome
Obg-like ATPase 1	Oial	Obg-like ATPase 1; Hydrolyzes ATP, and can also hydrolyze GTP with lower efficiency
Small nuclear ribonucleoprotein Sm D3	Snrpd3	plays an important role in the splicing of cellular pre-mRNAs
L-lactate dehydrogenase A chain	Ldha	Lactate dehydrogenase A
Phosphoglycerate kinase 1	Pgkl	Catalyzes one of the two ATP producing reactions in the glycolytic pathway
Aconitate hydratase, mitochondrial	Aco2	Catalyzes the isomerization of citrate to isocitrate via cis-aconitate
Creatine kinase B-type	Ckb	Reversibly catalyzes the transfer of phosphate between ATP and various phosphogens
Nucleolin	Ncl	Induces chromatin decondensation by binding to histone H1

14-3-3 Proteins Associate with Septin 7

We next validated whether 14-3-3 proteins were *bona fide* interacting proteins of Sept7 using co-immunoprecipitation assays. Using an antibody that recognizes all isoforms of 14-3-3 (pan 14-3-3 polyclonal), we immunoprecipitated 14-3-3 proteins from neuronal lysate obtained from DIV18 cultured embryonic rat hippocampal neurons and then probed for phosphorylated Sept7 using the pT426 Sept7 antibody. We found that indeed pSept7 coimmunoprecipitated with 14-3-3 proteins from neuronal lysates (Figure 4A, $n = 3$ experiments). Since 14-3-3 zeta (ζ) and gamma (γ) were the isoforms most enriched in our proteomic data (Figure 3D), and are also highly expressed in the brain (Berg et al., 2003), we next tested if there was an interaction of these isoforms with phosphorylated Sept7. We co-expressed HEK293T cells with GFP-Sept7 along with either HA-tagged 14-3-3 zeta, 14-3-3 γ , or both isoforms together. Using the HA antibody, we pulled down the 14-3-3 isoforms and then probed for phosphorylated Sept7 using the pT426 antibody. We found that pSept7 co-immunoprecipitated strongly with 14-3-3 γ and 14-3-3($\zeta + \gamma$) but not 14-3-3 ζ (Figure 4B, $n = 4$ experiments). Furthermore, using a gamma isoform specific antibody, we found that endogenous 14-3-3 γ from neuronal lysates co-immunoprecipitated pSept7 (Figure 4C, $n = 3$). Next, we tested whether the interaction between Sept7 and 14-3-3 γ was dependent on phosphorylation. Neuronal lysates at DIV18 were either treated with lambda protein phosphatase or buffer and then incubated with immunoprecipitated 14-3-3 γ . When immunoprecipitates were then probed for the presence of total Sept7, we found that while in untreated condition Sept7 bound 14-3-3 γ , this interaction was not detected when lysate was treated with protein phosphatase (Figure 4D, $n = 3$). These data suggest that the interaction between Sept7 and 14-3-3 γ is phosphorylation dependent. Next, to determine the effect of expression of different 14-3-3 isoforms on dendritic spine maturation, we co-expressed HA-tagged 14-3-3 γ and 14-3-3 ζ along with membrane marker

myr-tdTomato in cultured DIV12 hippocampal neurons. Neurons were fixed at DIV14 and stained with anti-HA antibody to visualize the localization of overexpressed 14-3-3 proteins (Figure 4E). In neurons overexpressing HA-14-3-3 γ , about 52% of protrusions were spiny mushroom mature spines, while 42% of protrusions were spiny in neurons expressing HA-14-3-3 ζ (Figure 4F). Interestingly, HA-14-3-3 γ was enriched in dendritic spines with a spine/dendrite ratio of 1.2, whereas HA-14-3-3 ζ primarily localized to the dendritic shaft and was not enriched in the spine head (Figure 4G). In summary, we found that Sept7 interacts with 14-3-3 γ in a phosphorylation-dependent manner. Furthermore, overexpression of 14-3-3 γ but not the zeta isoform leads to its enrichment in the dendritic spine head and enhances spine maturation.

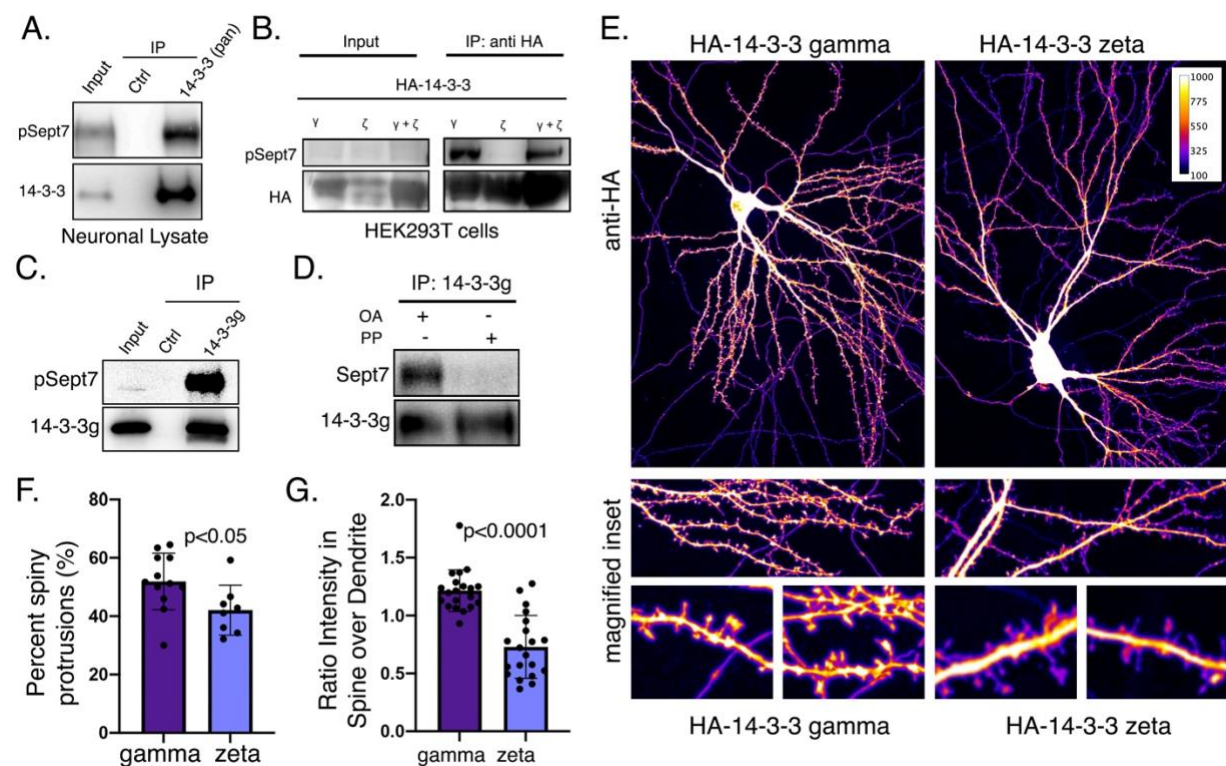


Figure 2.4. 14-3-3 γ associates with phosphorylated Sept7 and enhances dendritic spine maturation.

(A) Western blot of endogenous 14-3-3 protein in DIV18 hippocampal neurons immunoprecipitated with 14-3-3 (pan) antibody and probed for 14-3-3 and phosphorylated Sept7

(pT426) ($n = 3$ experiments). **(B)** Western blot of HA-14-3-3 γ , HA-14-3-3 ζ , or both HA-tagged isoforms co-expressed along with GFP-Sept7 in HEK293T cells immunoprecipitated with HA antibody and probed for HA and phosphorylated Sept7 (pT426). Phosphorylated Sept7 co-immunoprecipitated with HA-14-3-3 γ and HA-14-3-3($\zeta + \gamma$) but not HA-14-3-3 ζ ($n = 3$ experiments). **(C)** Western blot shows coimmunoprecipitation of phosphorylated Sept7 with endogenous 14-3-3 γ . Lysate from DIV18 hippocampal neurons immunoprecipitated with 14-3-3 (gamma) antibody and probed for 14-3-3 γ and phosphorylated T426 Sept7 ($n = 3$ experiments). **(D)** Western blot shows phosphorylation dependent interaction of Sept7 with endogenous 14-3-3 γ . DIV18 hippocampal neurons were treated with okadaic acid (OA) or DMSO, and lysate treated with or without lambda protein phosphatase (PP) were then immunoprecipitated with 14-3-3 (gamma) antibody and probed for 14-3-3 γ and total Sept7 ($n = 3$ experiments). **(E)** Fixed images of DIV14 hippocampal neurons co-transfected with membrane marker myristoylated tdTomato and HA-14-3-3 γ or HA-14-3-3 ζ were immunostained for HA tag. Scale bar is 10 μ m, and image pseudocolored to represent fluorescence intensity. **(F)** Bar graph depicts percent of protrusions with mature, spiny morphology in DIV14 hippocampal neurons transfected with HA-14-3-3 γ or HA-14-3-3 ζ . HA-14-3-3 γ expression results in significantly more spiny, mature protrusions ($p < 0.05$). Values indicate mean, error bars represent SEM and $n > 8$ neurons each. **(G)** Bar graph shows ratio of 14-3-3 fluorescent intensity in spines to intensity in corresponding dendritic shaft in DIV14 hippocampal neurons transfected with HA-14-3-3 γ or HA-14-3-3 ζ . Values indicate mean percent, error bars represent standard error of mean, and $n = 20$ spines from five neurons each.

Sept7 Association With 14-3-3 Protects its Phosphorylation at T426

To test the functional consequence of 14-3-3 association with phosphorylated Sept7, we utilized a genetically expressed inhibitor of 14-3-3, EYFP-Difoepin (dimeric 14-3-3 peptide inhibitor). This construct EYFP-Difoepin is based on R18, a Raf-1–derived phosphopeptide, which is a high-affinity peptide antagonist of 14-3-3 proteins (Wang et al., 1999). Difoepin is composed of two R18 coding sequences separated by a sequence coding for a short peptide linker in an EYFP fusion mammalian vector. Expressed Difoepin is capable of disrupting 14-3-3/ligand binding (Masters and Fu, 2001). Difoepin binds 14-3-3 without any isoform selectivity and thereby inhibits the interaction of all 14-3-3 isoforms with their physiological binding partners (Qiao et al., 2014). We transfected DIV14 hippocampal neurons with either control EYFP construct or EYFP-Difoepin construct and then measured the levels of pSept7 in neurons fixed at DIV16 (Figure 5A). Quantification of pSept7 intensity in the soma of neurons expressing Difoepin showed a significant decrease in the level of phosphorylated Sept7 compared to those expressing EYFP control, as detected by immunostaining using the pT426 Sept7 antibody (Figure 5A, yellow asterisk and 5B). Furthermore, neurons expressing Difoepin had a dramatic decrease in density of mature mushroom spines compared to EYFP-Control transfected neurons (Figure 5A, middle row and 5C). These data suggest that interaction of the C-terminal tail of Sept7 with 14-3-3 proteins is protective of the phosphorylation status of Sept7 in neurons, revealing a mechanism for modulation of Sept7 function by association with 14-3-3 proteins (Figure 5D).

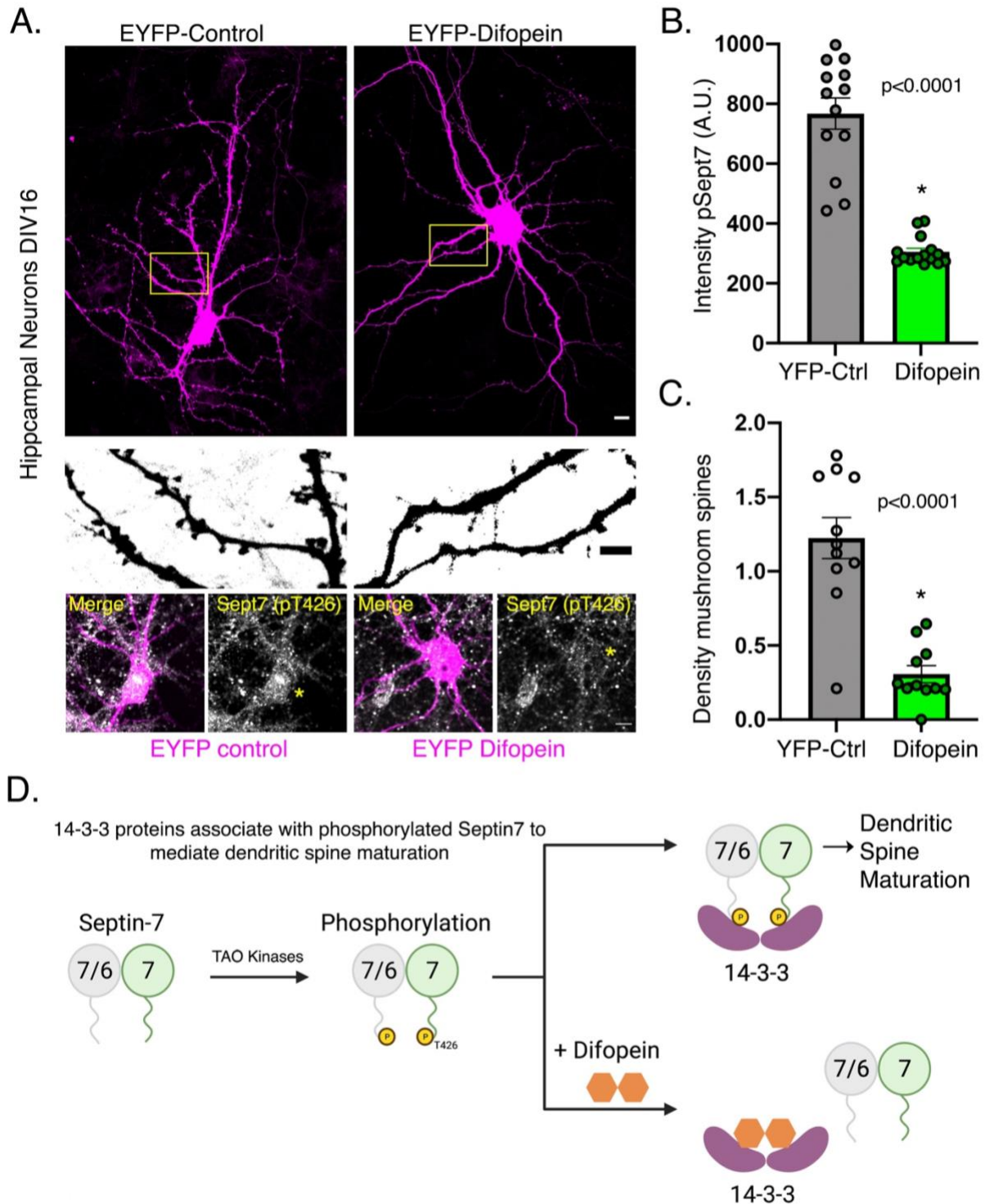


Figure 2.5. Association with 14-3-3 protects phosphorylation state of Sept7.

(A) Fixed images of DIV16 hippocampal neurons transfected at DIV14 with EYFP-control or EYFP-Difopein (magenta), scale bar is 10 μ m. Yellow inset is magnified to highlight dendritic

protrusions shown in inverted grayscale, scale bar is 5 μm . Bottom row shows levels of pSept7 immunostaining (gray) in the neuronal soma of control and difopein-transfected (magenta) neurons. Transfected neurons are depicted by yellow asterisk, and scale bar is 5 μm . **(B)** Quantification of phosphorylated Sept7 levels in the soma of DIV16 hippocampal neurons expressing YFP-control or YFP-Difopein. YFP-Difopein expression results in significantly decreased levels of phosphorylated Sept7 ($p < 0.0001$). Values indicate mean; error bars represent SEM; and $n = 13$ and 15 neurons, respectively, from three distinct experiments. **(C)** Density of mushroom spiny protrusions in DIV16 hippocampal neurons expressing YFP-control or YFP-Difopein. Values indicate mean, error bars represent SEM, and $n = 11$ neurons each from three distinct experiments. **(D)** Schematic summarizes findings of this study showing 14-3-3 protein association modulates Sept7 function in neurons through protection of its phosphorylation state.

Discussion

In this study, we utilize mass-spectrometry based discovery proteomics to identify binding partners that interact specifically with phosphorylated Sept7 in order to understand its role in dendritic spine development. Among the proteins we identified, we focus on the 14-3-3 family which are highly conserved phosphoprotein binding proteins that are important for brain development. We identify a specific interaction of pSept7 with 14-3-3 γ (gamma) encoded by *YWHA*G. 14-3-3 gamma is highly expressed during brain development, and mainly in neurons. Our findings show that 14-3-3 γ is specifically enriched in the dendritic spines, and that its expression in neurons leads to increased dendritic spine numbers.

14-3-3 proteins carry out a diverse array of functions in cellular processes, including in apoptosis (Masters and Fu, 2001), cell cycle progression (Peng et al., 1997), cytoskeletal rearrangements (Gohla and Bokoch, 2002), and neuronal growth (Cornell and Toyo-oka, 2017) that are mediated through the multitude of their phosphorylation dependent protein interactors. Accumulating evidence supports a profound role of 14-3-3 proteins in brain development. 14-3-3 proteins are important for neurogenesis, neuronal differentiation, and neuronal migration during cortical development (Cornell and Toyo-oka, 2017). Functional knockout of 14-3-3 proteins in mice brains with the dimeric 14-3-3 inhibitor Difopein results in reduced dendritic complexity and spine density accompanied by schizophrenia-related behaviors (Foote et al., 2015). 14-3-3 proteins are further required for hippocampal long term potentiation (LTP) and associative learning (Qiao et al., 2014), however, contribution of different 14-3-3 proteins in LTP was not ascertained. 14-3-3 γ plays an important role in neuronal migration as either knockdown or overexpression of 14-3-3 γ results in neuronal migration defects *in vivo* in mice (Wachi et al., 2015). Notably, 14-3-3 γ expression is significantly reduced in human brain from Down Syndrome patients (Peyrl et al., 2002), indicating that perturbation of 14-3-3 γ levels could contribute to disorders in human brain development. Furthermore, deletion of the *YWHAG* gene encoding 14-3-3 γ is also associated with epilepsy and autistic traits in patients with atypical Williams Beuren syndrome due to deletions in 7q11.23 locus (Fusco et al., 2014).

Septins are important regulator of several aspects of neuronal development including dendrite growth, axon development and dendritic spine maturation. Our data show that phosphorylated Sept7 associates with 14-3-3 proteins. While our mass spectrometry data indicates that isoforms gamma and zeta bind specifically with phosphorylated Sept7 C-terminal tail, we only were able to

biochemically validate the interaction with 14-3-3 gamma. It remains unknown whether different isoforms can heterodimerize to associate with phosphorylated Sept7. Using peptide inhibitor Difopein, we found that blocking interaction of Sept7 with 14-3-3 led to a decrease in the level of phosphorylated Sept7, highlighting the importance of the interaction. Since Difopein quenches the blocking site for phosphoproteins in all 14-3-3 proteins, our study did not directly test isoform specific effect of blocking Sept7 interaction with 14-3-3. Contribution of phosphorylated Sept7 and hence its protection by 14-3-3 proteins in these diverse neuronal contexts will be an important area of study. Dysfunction in both 14-3-3 and septins have been associated with various diseases including neurodegenerative diseases (Ide and Lewis, 2010) (Foote and Zhou, 2012) (Marttinen et al., 2015). In addition to the 14-3-3 proteins, several actin binding proteins were also identified in our proteomic study. Cofilin, an actin binding protein important for dendritic spine maturation was identified as a phosphorylation dependent Sept7 interacting protein (Gu et al., 2010; Pyronneau et al., 2017). Notably, phosphorylated cofilin also associates with 14-3-3 proteins (Gohla and Bokoch, 2002). Further understanding the interplay of septins, actin binding proteins, and 14-3-3 proteins in dendritic spine development may provide important insights into neurodevelopment and pathophysiology underlying neurological disorders.

Data Availability Statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics Statement

The animal study was reviewed and approved by IACUC UW.

Author Contributions

SB and BW contributed equally to the work including neuronal studies, biochemical assays, data analyses, and manuscript preparation. RF performed neuronal experiments, biochemical assays, and molecular biology experiments. KD and CS performed peptide pulldown and mass spectrometry experiments. SEO supervised the mass spectrometry and MS data analyses. SY designed and supervised all experiments, obtained funding, and wrote the manuscript.

Funding

We are grateful for research funding provided by the National Institute of Mental Health, R00MH108648 and R01MH121674 to SY. Mass spectrometry was supported by instrumentation and funding to SEO by R01GM129090.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors, and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Acknowledgments

We thank Yi Zhou (Florida State University) for generously sharing the EYFP-Difopein and its negative control expression constructs.

References

- Ageta-Ishihara, N., Miyata, T., Ohshima, C., Watanabe, M., Sato, Y., Hamamura, Y., et al. (2013). Septins Promote Dendrite and Axon Development by Negatively Regulating Microtubule Stability via HDAC6-Mediated Deacetylation. *Nat. Commun.* 4, 2532. doi:10.1038/ncomms3532
- Aitken, A. (1996). 14-3-3 and Its Possible Role in Co-Ordinating Multiple Signalling Pathways. *Trends Cell Biol.* 6 (9), 341–347. doi:10.1016/0962-8924(96)10029-5
- Almeida Marques, I., Valadares, N. F., Garcia, W., Damalio, J. C. P., Macedo, J. N. A., Araújo, A. P. U., et al. (2012). Septin C-Terminal Domain Interactions: Implications for Filament Stability and Assembly. *Cell Biochem. Biophys.* 62, 317–328. doi:10.1007/s12013-011-9307-0
- Berg, D., Holzmann, C., and Riess, O. (2003). 14-3-3 Proteins in the Nervous System. *Nat. Rev. Neurosci.* 4, 752–762. doi:10.1038/nrn1197
- Boston, P. F., Jackson, P., and Thompson, R. J. (1982). Human 14-3-3 Protein: Radioimmunoassay, Tissue Distribution, and Cerebrospinal Fluid Levels in Patients with Neurological Disorders. *J. Neurochem.* 38, 1475–1482. doi:10.1111/j.1471-4159.1982.tb07928.x
- Cornell, B., and Toyo-oka, K. (2017). 14-3-3 Proteins in Brain Development: Neurogenesis, Neuronal Migration and Neuromorphogenesis. *Front. Mol. Neurosci.* 10, 318. doi:10.3389/fnmol.2017.00318
- Cox, J., Neuhauser, N., Michalski, A., Scheltema, R. A., Olsen, J. V., and Mann, M. (2011). Andromeda: A Peptide Search Engine Integrated into the MaxQuant Environment. *J. Proteome Res.* 10 (4), 1794–1805. doi:10.1021/pr101065j
- de Anda, F. C., Rosario, A. L., Durak, O., Tran, T., Gräff, J., Meletis, K., et al. (2012). Autism Spectrum Disorder Susceptibility Gene TAOK2 Affects Basal Dendrite Formation in the Neocortex. *Nat. Neurosci.* 15, 1022–1031. doi:10.1038/nn.3141
- Dulovic-Mahlow, M., Trinh, J., Kandaswamy, K. K., Braathen, G. J., Di Donato, N., Rahikkala, E., et al. (2019). De Novo Variants in TAOK1 Cause Neurodevelopmental Disorders. *Am. J. Hum. Genet.* 105, 213–220. doi:10.1016/j.ajhg.2019.05.005
- Ewers, H., Tada, T., Petersen, J. D., Racz, B., Sheng, M., and Choquet, D. (2014). A Septin-dependent Diffusion Barrier at Dendritic Spine Necks. *PLoS ONE* 9, e113916. doi:10.1371/journal.pone.0113916
- Ferl, R. J., Manak, M. S., and Reyes, M. F. (2002). The 14-3-3s. *Genome Biol.* 3 (7), REVIEWS3010. doi:10.1186/gb-2002-3-7-reviews3010
- Finnigan, G. C., Booth, E. A., Duvalyan, A., Liao, E. N., and Thorner, J. (2015). The Carboxy-Terminal Tails of Septins Cdc11 and Shs1 Recruit Myosin-II Binding Factor Bni5 to the Bud Neck in *Saccharomyces cerevisiae*. *Genetics* 200, 843–862. doi:10.1534/genetics.115.17650

- Finnigan, G. C., Duvalyan, A., Liao, E. N., Sargsyan, A., and Thorner, J. (2016). Detection of Protein-Protein Interactions at the Septin Collar in *Saccharomyces Cerevisiae* using a Tripartite Split-GFP System. *MBoC* 27, 2708–2725. doi:10.1091/mbc.e16-05-0337
- Foote, M., and Zhou, Y. (2012). 14-3-3 Proteins in Neurological Disorders. *Int. J. Biochem. Mol. Biol.* 3 (2), 152–164.
- Foote, M., Qiao, H., Graham, K., Wu, Y., and Zhou, Y. (2015). Inhibition of 14-3-3 Proteins Leads to Schizophrenia-Related Behavioral Phenotypes and Synaptic Defects in Mice. *Biol. Psychiatry* 78, 386–395. doi:10.1016/j.biopsych.2015.02.015
- Fu, H., Subramanian, R. R., and Masters, S. C. (2000). 14-3-3 Proteins: Structure, Function, and Regulation. *Ann. Rev. Pharmacol. Toxicol.* 40, 617–647. doi:10.1146/annurev.pharmtox.40.1.617
- Fusco, C., Micale, L., Augello, B., Teresa Pellico, M., Menghini, D., Alfieri, P., et al. (2014). Smaller and Larger Deletions of the Williams Beuren Syndrome Region Implicate Genes Involved in Mild Facial Phenotype, Epilepsy and Autistic Traits. *Eur. J. Hum. Genet.* 22, 64–70. doi:10.1038/ejhg.2013.101
- Gardino, A. K., Smerdon, S. J., and Yaffe, M. B. (2006). Structural Determinants of 14-3-3 Binding Specificities and Regulation of Subcellular Localization of 14-3-3-Ligand Complexes: A Comparison of the X-Ray Crystal Structures of All Human 14-3-3 Isoforms. *Semin. Cancer Biol.* 16 (3), 173–182. doi:10.1016/j.semcancer.2006.03.007
- Gohla, A., and Bokoch, G. M. (2002). 14-3-3 Regulates Actin Dynamics by Stabilizing Phosphorylated Cofilin. *Curr. Biol.* 12, 1704–1710. doi:10.1016/s0960-9822(02)01184-3
- Gu, J., Lee, C. W., Fan, Y., Komlos, D., Tang, X., Sun, C., et al. (2010). ADF/Cofilin-Mediated Actin Dynamics Regulate AMPA Receptor Trafficking during Synaptic Plasticity. *Nat. Neurosci.* 13, 1208–1215. doi:10.1038/nn.2634
- Hu, C., Feng, P., Yang, Q., and Xiao, L. (2021). Clinical and Neurobiological Aspects of TAO Kinase Family in Neurodevelopmental Disorders. *Front. Mol. Neurosci.* 14, 655037. doi:10.3389/fnmol.2021.655037
- Hu, J., Bai, X., Bowen, J. R., Dolat, L., Korobova, F., Yu, W., et al. (2012). Septin-driven Coordination of Actin and Microtubule Remodeling Regulates the Collateral Branching of Axons. *Curr. Biol.* 22, 1109–1115. doi:10.1016/j.cub.2012.04.019
- Ide, M., and Lewis, D. A. (2010). Altered Cortical CDC42 Signaling Pathways in Schizophrenia: Implications for Dendritic Spine Deficits. *Biol. Psychiatry* 68, 25–32. doi:10.1016/j.biopsych.2010.02.016
- Kinoshita, M. (2003b). Assembly of Mammalian Septins. *J. Biochem.* 134, 491–496. doi:10.1093/jb/mvg182
- Kinoshita, M. (2003a). The Septins. *Genome Biol.* 4, 236. doi:10.1186/gb-2003-4-11-236

- Marttinen, M., Kurkinen, K. M., Soininen, H., Haapasalo, A., and Hiltunen, M. (2015). Synaptic Dysfunction and Septin Protein Family Members in Neurodegenerative Diseases. *Mol. Neurodegeneration* 10, 16. doi:10.1186/s13024-015-0013-z
- Masters, S. C., and Fu, H. (2001). 14-3-3 Proteins Mediate an Essential Anti-apoptotic Signal. *J. Biol. Chem.* 276, 45193–45200. doi:10.1074/jbc.m105971200
- Mendonça, D. C., Guimarães, S. L., Pereira, H. D., Pinto, A. A., de Farias, M. A., de Godoy, A. S., et al. (2021). An Atomic Model for the Human Septin Hexamer by Cryo-EM. *J. Mol. Biol.* 433, 167096. doi:10.1016/j.jmb.2021.167096
- Mostowy, S., and Cossart, P. (2012). Septins: the Fourth Component of the Cytoskeleton. *Nat. Rev. Mol. Cel Biol.* 13, 183–194. doi:10.1038/nrm3284
- Nourbakhsh, K., Ferreccio, A. A., Bernard, M. J., and Yadav, S. (2021). TAOK2 Is an ER-Localized Kinase that Catalyzes the Dynamic Tethering of ER to Microtubules. *Dev. Cell* 56 (24), 3321–3333.e5. doi:10.1016/j.devcel.2021.11.015
- Peng, C.-Y., Graves, P. R., Thoma, R. S., Wu, Z., Shaw, A. S., and Piwnicka-Worms, H. (1997). Mitotic and G 2 Checkpoint Control: Regulation of 14-3-3 Protein Binding by Phosphorylation of Cdc25C on Serine-216. *Science* 277, 1501–1505. doi:10.1126/science.277.5331.1501
- Peyrl, A., Weitzdoerfer, R., Gulesserian, T., Fountoulakis, M., and Lubec, G. (2002). Aberrant Expression of Signaling-Related Proteins 14-3-3 Gamma and RACK1 in Fetal Down Syndrome Brain (Trisomy 21). *Electrophoresis* 23, 152–157. doi:10.1002/1522-2683(200201)23:1<152::aid-elps152>3.0.co;2-t
- Pyronneau, A., He, Q., Hwang, J. Y., Porch, M., Contractor, A., and Zukin, R. S. (2017). Aberrant Rac1-Cofilin Signaling Mediates Defects in Dendritic Spines, Synaptic Function, and Sensory Perception in Fragile X Syndrome. *Sci. Signal.* 10, ean0852. doi:10.1126/scisignal.aan0852
- Qiao, H., Foote, M., Graham, K., Wu, Y., and Zhou, Y. (2014). 14-3-3 Proteins Are Required for Hippocampal Long-Term Potentiation and Associative Learning and Memory. *J. Neurosci.* 34, 4801–4808. doi:10.1523/jneurosci.4393-13.2014
- Richter, M., Murtaza, N., Scharrenberg, R., White, S. H., Johannis, O., Walker, S., et al. (2018). Altered TAOK2 Activity Causes Autism-Related Neurodevelopmental and Cognitive Abnormalities through RhoA Signaling. *Mol. Psychiatry* 20, 1237. doi:10.1038/s41380-018-0025-5
- Sirajuddin, M., Farkasovsky, M., Hauer, F., Kühlmann, D., Macara, I. G., Weyand, M., et al. (2007). Structural Insight into Filament Formation by Mammalian Septins. *Nature* 449, 311–315. doi:10.1038/nature06052
- Tada, T., Simonetta, A., Batterton, M., Kinoshita, M., Edbauer, D., and Sheng, M. (2007). Role of Septin Cytoskeleton in Spine Morphogenesis and Dendrite Development in Neurons. *Curr. Biol.* 17, 1752–1758. doi:10.1016/j.cub.2007.09.039

Uhart, M., and Bustos, D. M. (2013). Human 14-3-3 Paralogs Differences Uncovered by Cross-Talk of Phosphorylation and Lysine Acetylation. *PLoS One* 8 (2), e55703. doi:10.1371/journal.pone.0055703

Ultanir, S. K., Yadav, S., Hertz, N. T., Oses-Prieto, J. A., Claxton, S., Burlingame, A. L., et al. (2014). MST3 Kinase Phosphorylates TAO1/2 to Enable Myosin Va Function in Promoting Spine Synapse Development. *Neuron* 84, 968–982. doi:10.1016/j.neuron.2014.10.025

van Woerden, G. M., Bos, M., de Konink, C., Distel, B., Trezza, R. A., Shur, N. E., et al. (2021). TAOK1 Is Associated with Neurodevelopmental Disorder and Essential for Neuronal Maturation and Cortical Development. *Hum. Mutat.* 42 (4), 445–459. doi:10.1002/humu.24176

Wachi, T., Cornell, B., Marshall, C., Zhukarev, V., Baas, P. W., and Toyo-oka, K. (2015). Ablation of the 14-3-3gamma Protein Results in Neuronal Migration Delay and Morphological Defects in the Developing Cerebral Cortex. *Devel Neurobio* 76, 600–614. doi:10.1002/dneu.22335

Walikonis, R. S., Jensen, O. N., Mann, M., Provance, D. W., Mercer, J. A., and Kennedy, M. B. (2000). Identification of Proteins in the Postsynaptic Density Fraction by Mass Spectrometry. *J. Neurosci.* 20, 4069–4080. doi:10.1523/jneurosci.20-11-04069.2000

Wang, B., Yang, H., Liu, Y.-C., Jelinek, T., Zhang, L., Ruoslahti, E., et al. (1999). Isolation of High-Affinity Peptide Antagonists of 14-3-3 Proteins by Phage Display. *Biochemistry* 38, 12499–12504. doi:10.1021/bi991353h

Xie, Y., Vessey, J. P., Konecna, A., Dahm, R., Macchi, P., and Kiebler, M. A. (2007). The GTP-Binding Protein Septin 7 Is Critical for Dendrite Branching and Dendritic-Spine Morphology. *Curr. Biol.* 17, 1746–1751. doi:10.1016/j.cub.2007.08.042

Yadav, S., Oses-Prieto, J. A., Peters, C. J., Zhou, J., Pleasure, S. J., Burlingame, A. L., et al. (2017). TAOK2 Kinase Mediates PSD95 Stability and Dendritic Spine Maturation through Septin7 Phosphorylation. *Neuron* 93, 379–393. doi:10.1016/j.neuron.2016.12.006

Yasuda, S., Tanaka, H., Sugiura, H., Okamura, K., Sakaguchi, T., Tran, U., et al. (2007). Activity-Induced Protocadherin Arcadlin Regulates Dendritic Spine Number by Triggering N-Cadherin Endocytosis via TAO2 β and P38 MAP Kinases. *Neuron* 56, 456–471. doi:10.1016/j.neuron.2007.08.020

Chapter 3: TAOK2 drives opposing cilia length deficits in 16p11.2 deletion and duplication carriers

Sujin Byeon^{1,2,6}, Amy Ferreccio^{1,6}, Moira Cornell¹, Juan A. Oses-Prieto³, Aditi Deshpande⁴, Siri Manvi¹, Alison Blencowe¹, Lauren A. Weiss⁴, Alma L. Burlingame³, and Smita Yadav^{1,5,7,*},
smitay@uw.edu

¹Department of Pharmacology, University of Washington, Seattle, WA 98195, USA

²Graduate Program in Neuroscience, University of Washington, Seattle, WA 98195, USA

³Department of Pharmaceutical Chemistry, University of California, San Francisco, San Francisco, CA 94195, USA

⁴Institute for Human Genetics, Department of Psychiatry and Behavioral Sciences, Weill Institute for Neurosciences, University of California, San Francisco, San Francisco, CA 94195, USA

⁵Institute of Stem Cell and Regenerative Medicine, University of Washington, Seattle, WA 98106, USA

*Corresponding author

⁶Co-first authors

⁷Lead contact

Summary

Deletion and duplication of the 16p11.2 genomic locus are associated with opposing changes in brain size. To determine cellular mechanisms that underlie these opposing phenotypes, we performed quantitative phosphoproteomic analyses of induced pluripotent stem cells (iPSCs)-derived neural progenitor cells (NPCs) obtained from unaffected individuals, 16p11.2 deletion, and 16p11.2 duplication carriers. Differentially phosphorylated proteins were enriched in centrosomal and cilia proteins. Deletion NPCs showed longer primary cilium compared to unaffected individuals, while stunted cilia were observed in duplication NPCs. Through cellular screens in NPCs, we determined the contribution of genes within the 16p11.2 locus to cilium length. Protein kinase TAOK2 and phosphatase PPP4C were found to regulate primary cilia length. NPCs lacking TAOK2 exhibited elongated cilia, aberrant IFT88 and pericentrin (PCNT) accumulation, and were impaired in sonic hedgehog (SHH) signaling. These findings implicate aberrant cilia length in the pathophysiology of 16p11.2 copy number variation and establish TAOK2 kinase as a regulator of primary cilium length.

Introduction

The 16p11.2 genomic region between breakpoint 4 and 5 contains 30 annotated genes and is flanked by segmental duplications, making it prone to copy number variation (CNV) such as deletion and duplication (Weiss et al., 2008; Zufferey et al., 2012). 16p11.2 CNVs are associated with autism spectrum disorder (Weiss et al., 2008), schizophrenia (McCarthy et al., 2009), motor and speech impairment (Steinman et al., 2016), body weight disturbances (Jacquemont et al., 2011; Maillard et al., 2015; Walters et al., 2010; Zufferey et al., 2012), and structural abnormalities of the brain (Maillard et al., 2015; Martin-Brevet et al., 2018; Qureshi et al., 2014). Carriers of

16p11.2 deletion and duplication exhibit common developmental, behavioral, and social impairments implying convergent mechanisms caused by reciprocal genetic changes. On the other hand, certain opposing clinical manifestations in 16p11.2 deletion and duplication carriers, such as alteration in brain size and body mass index (BMI), provide a unique opportunity to determine the individual contributions of genes in this locus to pathophysiology of 16p11.2 CNV. Carriers of 16p11.2 deletion present with increased brain size or macrocephaly, including larger cortex and thicker corpus callosum; in contrast, duplication is associated with microcephaly, thinner corpus callosum, and smaller cortex (Martin-Brevet et al., 2018; Owen et al., 2014; Qureshi et al., 2014; Steinman et al., 2016). Also, 16p11.2 deletion carriers are prone to obesity (defined as $\text{BMI} \geq 30 \text{ kg/m}^2$) while duplication carriers have low BMI (defined as $\leq 18.5 \text{ kg/m}^2$) (Arbogast et al., 2016; Jacquemont et al., 2011; Walters et al., 2010; Zufferey et al., 2012). Mechanisms underlying these gene dosage-dependent phenotypes are unknown, and systematic efforts to map the genetic basis of these opposing effects are lacking. Even though the syntenic locus in mice offers an excellent genetic model to study this chromosomal disorder, phenotypes of deletion and duplication mice are opposing to the human condition (Arbogast et al., 2016; Portmann et al., 2014). However, human iPSC-based neuronal and organoid models derived from 16p11.2 deletion and duplication carriers have provided important insights into the pathophysiology of these disorders (Connacher et al., 2022; Deshpande et al., 2017; Fetit et al., 2023; Roth et al., 2020; Urresti et al., 2021). For instance, excitatory neurons derived from 16p11.2 deletion carriers show hypertrophy, implicating increased neuronal soma and arbor size as contributors to the macrocephaly phenotype (Deshpande et al., 2017).

To identify mechanisms underlying opposing phenotypes in 16p11.2 CNV, we utilized an unbiased proteomic approach. Our phosphoproteomic analyses of neural progenitor cells (NPCs) differentiated from 16p11.2 deletion, duplication, and control iPSCs revealed differentially phosphorylated proteins enriched in cilia and centrosomal proteins. Primary cilia are microtubule-based membrane protrusions rich in receptors for signaling molecules, and are present in most cell types including neuronal progenitors, neurons, and astrocytes (Anvarian et al., 2019; Lepanto et al., 2016; Singla and Reiter, 2006). Defects in ciliogenesis, ciliary length, and trafficking cause several human diseases including neurodevelopmental disorders (Bettencourt-Dias et al., 2011; Ferkol and Leigh, 2012; Guemez-Gamboa et al., 2014; Han and Alvarez-Buylla, 2010; Reiter and Leroux, 2017). Our study demonstrates that primary cilia are impacted in a gene dosage-dependent manner in 16p11.2 CNV, with longer primary cilium in 16p11.2 deletion carrier iPSC-derived NPCs and shorter primary cilium in duplication carrier iPSC-derived NPCs. Further, we identify the genetic drivers of opposing changes in cilia length in 16p11.2 CNV, and find Thousand And One amino acid Kinase 2 (*TAOK2*) to be the most significant and dosage-dependent regulator of cilia length.

TAOK2 is a protein kinase important for neuronal development, including dendritic spine development and synaptic function (Anda et al., 2012; Byeon and Yadav, 2024; Nourbakhsh and Yadav, 2021; Yadav et al., 2017). Mutations in TAOK2 are associated with autism spectrum disorder, and mutations impact both the catalytic and regulatory domains (Richter et al., 2018). The canonical isoform of TAOK2 encodes a transmembrane protein kinase localized to the endoplasmic reticulum (ER) and centrosomes, with ER-microtubule tethering property (Nourbakhsh et al., 2021). In this study, we find that TAOK2 knockout (KO) human iPSC-derived NPCs exhibit longer primary cilia, while TAOK2 overexpression leads to stunted cilia.

Centrosomal accumulation of pericentrin (PCNT) and ciliary tip distribution of IFT88 are significantly increased in TAOK2 KO NPCs. Functionally, TAOK2 KO neuroprogenitors show deficits in sonic hedgehog (SHH) signaling. Finally, using pharmacological inhibition, we show that the catalytic activity of TAOK2 is essential for cilia length elongation. Our study reveals reciprocal alterations of cilia length in neural progenitors derived from 16p11.2 deletion and duplication carriers, elucidates the genetic basis for opposing changes in ciliary length, and reveals a hitherto unknown function of TAOK2 kinase in primary cilium regulation.

Results

Centrosomal and ciliary proteins are differentially phosphorylated in 16p11.2 CNV carrier-derived neural progenitors

Three of the 30 genes in the 16p11.2 genetic locus encode for proteins that impact protein phosphorylation: MAPK3 and TAOK2 are serine threonine kinases, and PPP4C is the catalytic subunit of a protein phosphatase (Figure 1A). Therefore, we hypothesized that the phosphoproteome in patients with duplication and deletion would be altered, and that identification of differentially phosphorylated proteins could reveal insight into the neuropathology of 16p11.2 CNV. iPSCs from fibroblasts of three deletion carriers, three duplication carriers, and three unaffected individuals were obtained (Figure S1A). Differentiation of iPSCs into dorsal forebrain NPCs was induced by dual SMAD inhibition using our previously published protocol (Figure S1B) (Deshpande et al., 2017). All NPCs expressed forebrain progenitor markers PAX6, NESTIN, SOX1, and SOX2 (Figures S1C and S1D). NPCs were grown to confluence, cell lysates were prepared, and equal amounts of protein were utilized for downstream proteomics. Each sample was chemically labeled using the 10plex tandem mass tag (TMT) reagent. Phosphopeptides were

enriched using TiO₂ column, eluted and analyzed by mass spectrometry (Clauser et al., 1999; Guan et al., 2011) (Figure 1B). Mass spectrometry analyses detected 18,894 distinct phosphopeptides from 1,927 proteins, of which 43 phosphopeptides from 40 proteins were significantly increased (greater than 2-fold increase) in duplication NPCs, and 304 phosphopeptides from 213 proteins were significantly decreased (greater than 2-fold decrease) in deletion NPCs compared to control (Figures 1C and 1D; Table S1). Of the phosphoproteins downregulated in deletion carriers, 13.6% (29 out of 213) proteins had a known cilia and centrosomal function based on the Centrosome and Cilium Database (CCDB) (Gupta et al., 2015) (Figure 1D; Table S2).

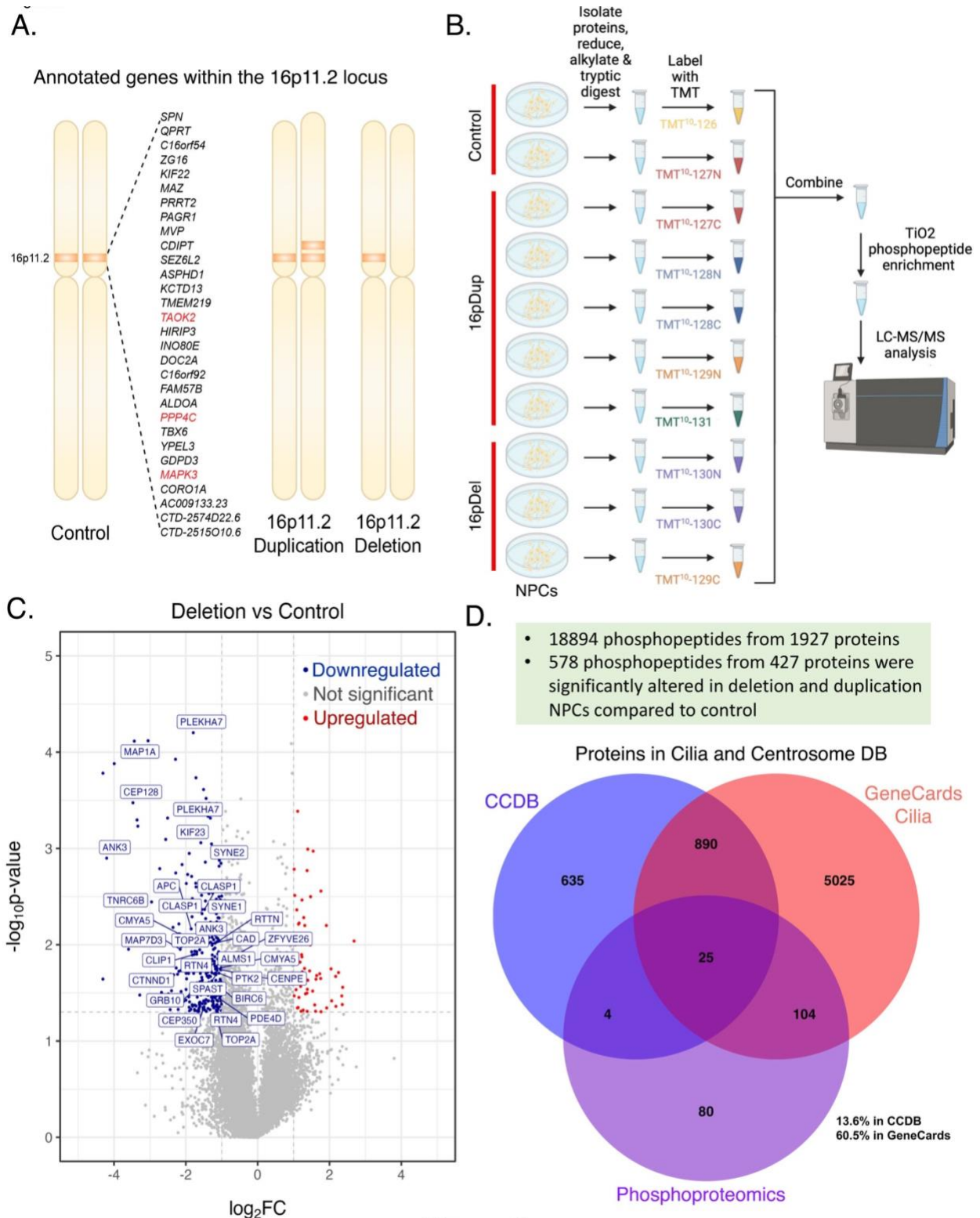


Figure 1

Figure 3.1. Quantitative phosphoproteomics of neural progenitors from 16p11.2 CNV carriers identifies ciliary proteins

(A) Schematic representations of annotated genes within the human 16p11.2 genomic locus (orange) that undergo heterozygous deletion or duplication. Genes encoding kinases MAPK3 and TAOK2 and phosphatase PPP4C are highlighted in red.

(B) Workflow for tandem mass tag (TMT) labeling of protein lysates isolated from dorsal neural progenitor cells (NPCs) differentiated from iPSCs derived from patients with 16p11.2 deletion and duplication as well as unaffected control individuals. Labeled peptides were enriched for phosphopeptides using TiO₂ affinity beads followed by identification through mass spectrometry. For proteomics experiments, one clone per patient sample with two to three patient samples per genotype were analyzed. Internal controls with clonal repeats were included. Created in BioRender. Byeon, S. (2025) <https://BioRender.com/6qww2wz>.

(C) Volcano plot shows phosphopeptides significantly downregulated (blue) in 16p11.2 deletion samples compared to controls.

(D) Phosphopeptides significantly downregulated in 16p11.2 deletion NPCs were enriched in centrosomal and ciliary proteins with 13.6% of proteins present in the Centrosome and Cilium DataBase (CCDB) and 60.5% proteins classified as cilia proteins in GeneCards.

(A) Table shows iPSC lines used in the study. Three lines were derived from unaffected individuals, 16p11.2 duplication and deletion carriers each, and their age, sex and source are shown. (B) Workflow and timeline for derivation of dorsal forebrain neural progenitors from iPSCs using dual SMAD inhibition by LDN and SB. Created in BioRender. Byeon, S. (2025) <https://BioRender.com/dbfc7lw>. (C) Representative confocal images of NPCs derived from control and 16p11.2 CNV carriers immunostained with NPC markers SOX2 (magenta) and SOX1 (green). Nuclei were stained with DAPI (blue). Scale bar is 10 μ m. (D) Representative confocal images of NPCs derived from control and 16p11.2 CNV carriers immunostained with NPC markers PAX6 (magenta) and NESTIN (green) and co-stained with DNA dye DAPI (blue). Scale bar is 10 μ m.

Primary cilia in 16p11.2 deletion and duplication patient-derived neural progenitor cells exhibit opposing defects in length

To test whether cilia were perturbed in 16p11.2 deletion and duplication carriers, we immunostained NPCs with antibodies against the cilia membrane protein ARL13B and NPC marker PAX6. We found that compared to NPCs from unaffected individuals (mean = 2.36–2.55 μ m, n = 60 each line), cilia in NPCs from patients with deletion (mean = 3.65–4.79 μ m, n = 60 each line) were significantly longer, while cilia in NPCs from patients with duplication were shorter (mean = 1.34–1.83 μ m, n = 60 each line) (Figures 2A and 2B). Cilia length is coupled to the cell cycle (Kasahara and Inagaki, 2021). Cilia elongate throughout the G1-S phase, disassemble in M phase, and regrow in G1. NPCs were grown in serum free media for 24 h to synchronize the cell cycle. We found that the reciprocal change in cilia length in NPCs from deletion and duplication carriers persisted, even when NPCs were grown in serum free media for 24 h (

Figures 2A–2C). NPCs responded to serum starvation by increasing their ciliary length, with cilia in deletion NPC (mean = 4.16–4.97 μm , $n > 33$ each line) significantly longer, and cilia in duplication NPCs (mean = 2.01–2.39 μm , $n > 45$ each line) shorter compared to control (mean = 2.96–3.40 μm , $n > 38$ each line). This suggests that cilia length deficits observed in 16p11.2 CNV carriers arise through a cell intrinsic mechanism independent of cell cycle phase.

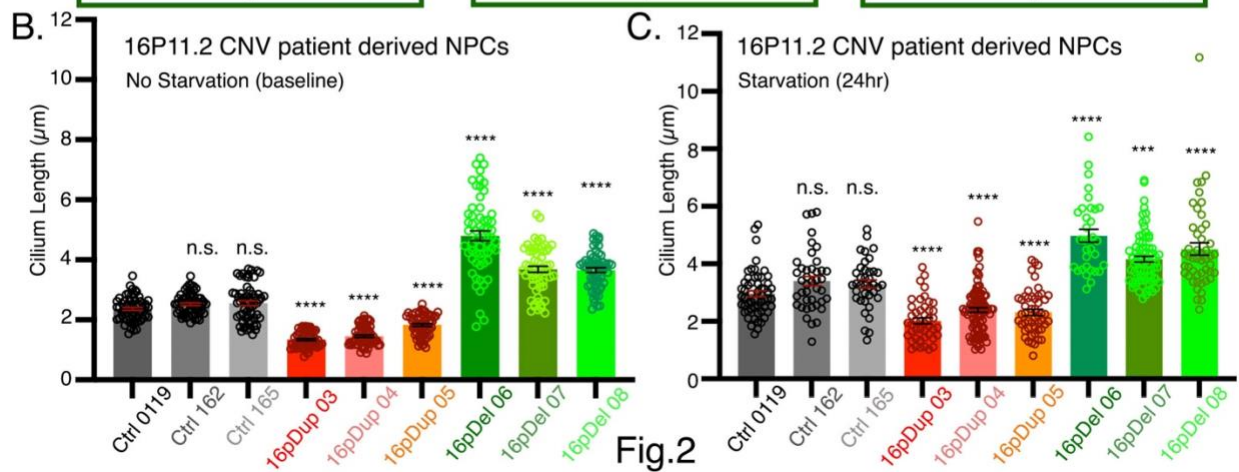
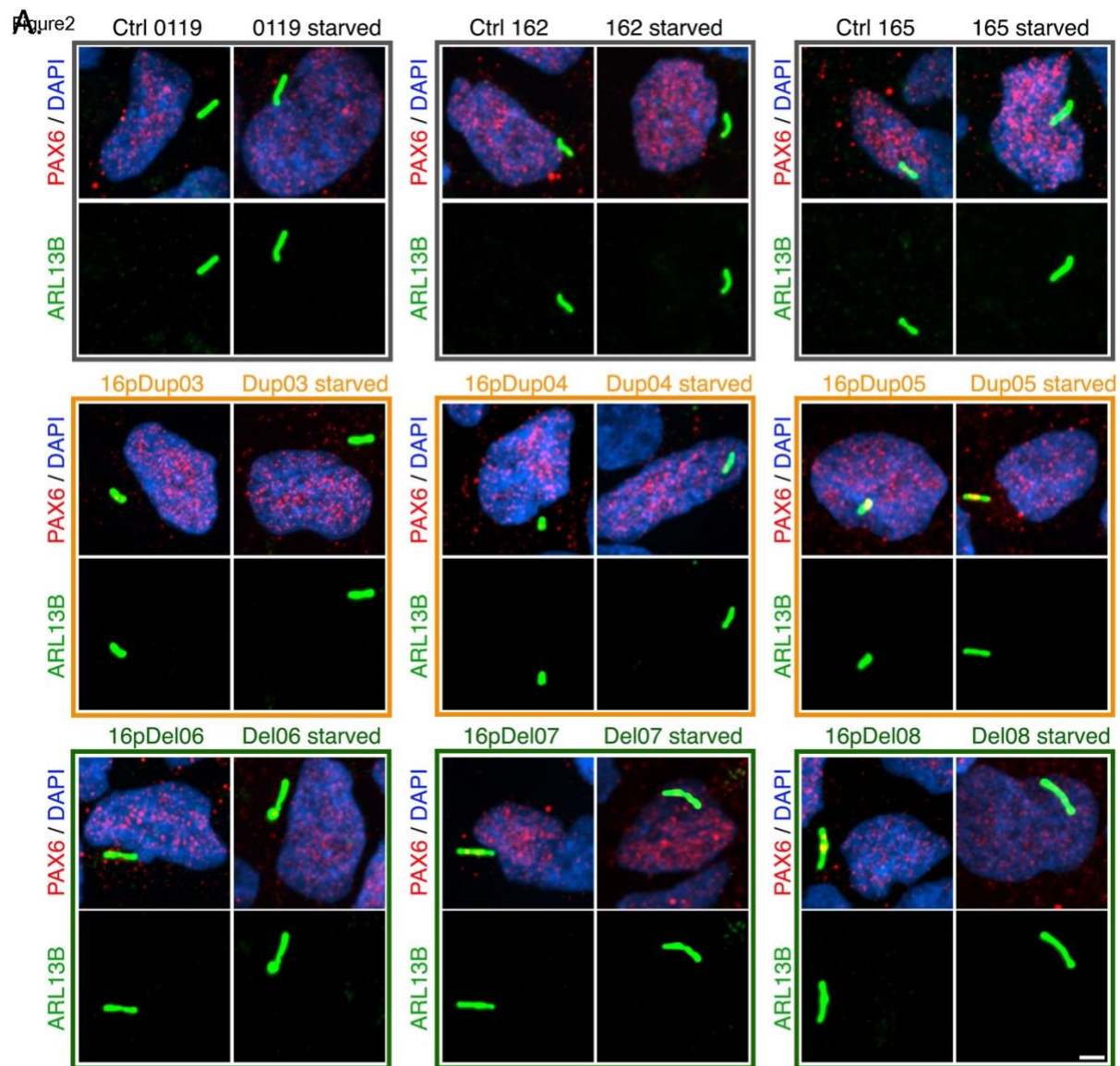


Figure 3.2. Opposing cilia length alterations in neural progenitors derived from 16p11.2 deletion and duplication carriers

(A) Representative confocal images of human iPSC-derived NPCs from unaffected individuals (RMK0119, RMK0162, and RMK0165), 16p11.2 duplication carriers (16pDup03, 16pDup04, and 16pDup05), and 16p11.2 deletion carriers (16pDel06, 16pDel07, and 16pDel08) immunostained for dorsal forebrain progenitor marker PAX6 (red) and primary cilium marker ARL13B (green). Nuclei were stained with DAPI. Scale bar represent 3 μ m.

(B) Length of primary cilia in human iPSC-derived NPCs from unaffected control, and 16p11.2 deletion and duplication carriers, growing in nutrient rich conditions is plotted (n = 60 cilia from 3 experiments for each NPC line, ****p < 0.0001 and n.s. not significant, by ordinary one-way ANOVA with Dunnett's multiple comparisons).

(C) Length of primary cilia in NPCs from unaffected control, and 16p11.2 deletion and duplication carriers, grown in starvation conditions for 24 h before fixation is plotted (n > 33 cilia from 3 experiments for each NPC line, ****p < 0.0001, *** p < 0.001, and n.s. not significant, by ordinary one-way ANOVA with Dunnett's multiple comparisons). For primary cilia length, two clones per line were used and data were pooled from three experimental replicates. Three patient samples per genotype were used, and each genotype is plotted separately. All error bars are standard error of the mean.

Cellular screens identify genetic basis of altered cilium length

To uncover mechanisms underlying altered ciliary length caused by opposing gene dosage changes in the 16p11.2 locus, we performed complementary shRNA and overexpression cellular screens. First, we employed an shRNA screen in NPCs differentiated from the human iPSC line WTC11

(Coriell Institute) and RMK0119b line (UCSF, Weiss Lab) using commercially validated shRNA targeting 23 annotated genes in the 16p11.2 locus. NPCs were transfected with GFP-tagged shRNA, starved for 24 h to induce ciliogenesis, fixed and then immunostained with ARL13B antibody 48 h after transfection (Figure 3A). We found that shRNA against TAOK2 (mean = 5.43 μ m, n = 26 NPCs, SEM = 0.32) and PPP4C (mean = 4.35 μ m, n = 27 NPCs, SEM = 0.27) significantly increased cilia length compared to control shRNA (mean = 3.43 μ m, n = 29 NPCs, SEM = 0.15) in WTC11-derived NPCs (Figures 3B and 3C). Similarly, in the RMK0119b-derived NPCs, shRNA against TAOK2 (mean = 4.82 μ m, n = 24 NPCs, SEM = 0.25) and PPP4C (mean = 4.60 μ m, n = 16 NPCs, SEM = 0.43) significantly increased the cilia length compared to control shRNA (mean = 3.21 μ m, n = 19 NPCs, SEM = 0.19) (Figure 3D). Next, we tested in an overexpression screen, the impact of increased gene expression on cilia length. Briefly, we generated mammalian expression vector with a BFP-tag inserted at the N-terminal of TAOK2 and PPP4C along with six other genes in the 16p11.2 locus. NPCs derived from WTC11 line were transfected with either control-BFP construct or constructs expressing the 16p11.2 genes, starved for 24 h, and immunostained with ARL13B antibody 48 h after transfection (Figure 3E). Overexpression of TAOK2 had the strongest effect in decreasing cilia length (mean = 1.66 μ m, n = 25 NPCs, SEM = 0.08), followed by PPP4C (mean = 2.39 μ m, n = 19 NPCs, SEM = 0.15), while control (mean = 3.19 μ m, n = 22 NPCs, SEM = 0.16) and none of the other six 16p11.2 genes had a significant effect on cilia length (Figure 3F). Therefore, gene dosage of TAOK2, and to a lesser extent PPP4C, drives opposing cilia length changes in NPCs derived from 16p11.2 CNV carriers.

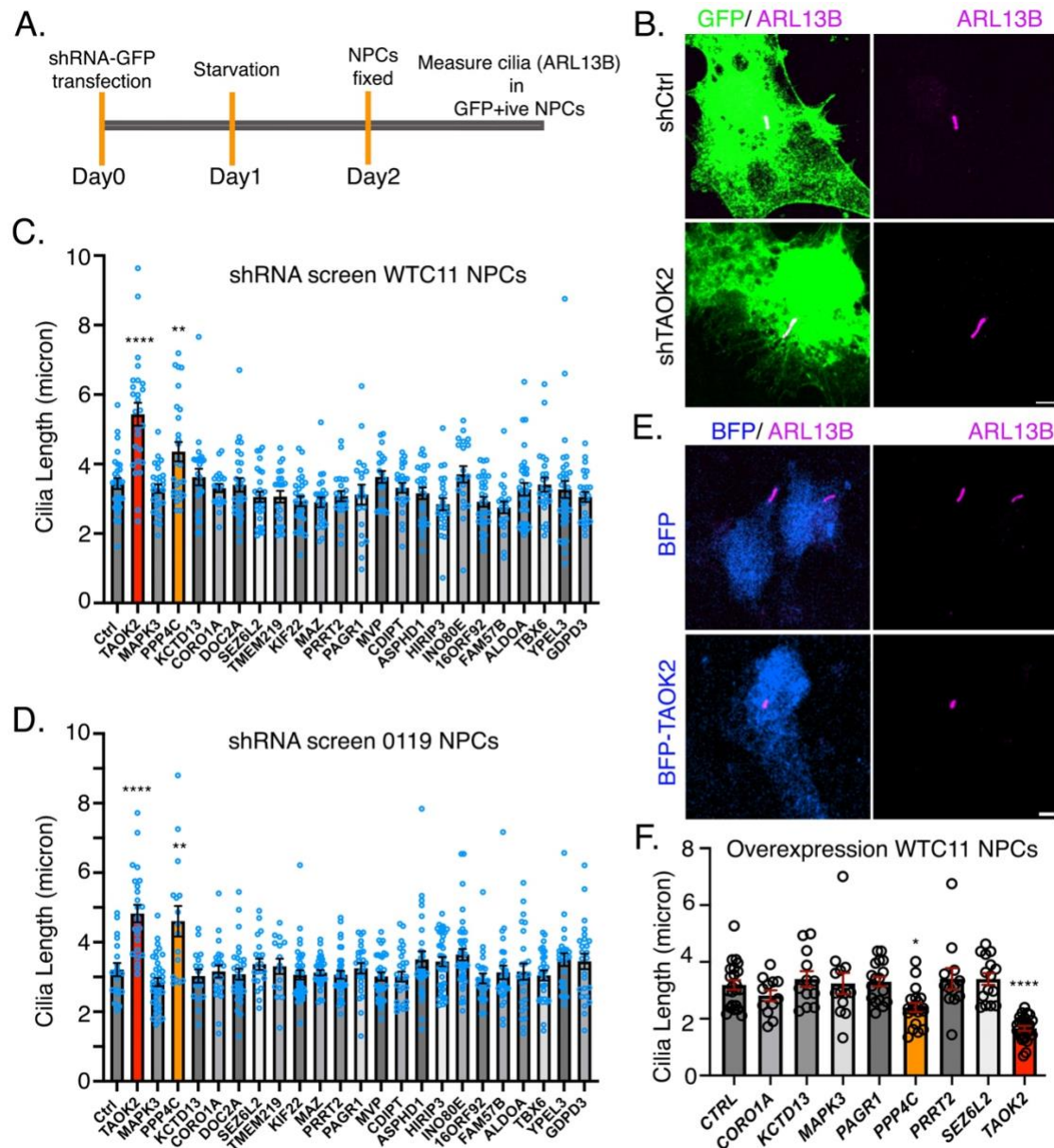


Figure 3

Figure 3.3. Cellular screens identify genetic drivers of altered ciliary length in 16p11.2 CNV

(A) Workflow for shRNA screen involves transfection of WTC11 or RMK0119 iPSC-derived NPCs with shRNA expression constructs (GFP), followed by growth under starvation conditions for 24 h before fixation. NPCs are immunostained for ARL13B to mark cilia and 60× images were acquired through confocal microscopy.

(B) Representative images of WTC11-derived NPCs transfected with control shRNA (green) and TAOK2-shRNA (green) stained for cilium marker ARL13B (magenta). Scale bar represents 2 μ m.

(C) Quantification of cilium length in WTC11-derived NPCs transfected with the indicated shRNA against genes in the 16p11.2 genomic locus ($n > 20$ NPCs from 3 experiments for each shRNA, **** $p < 0.0001$, ** $p < 0.005$, and ordinary one-way ANOVA with Dunnett's multiple comparisons).

(D) Quantification of cilium length in RMK0119-derived NPCs transfected with the indicated shRNA against genes in the 16p11.2 genomic locus ($n > 15$ NPCs from 3 experiments for each shRNA, **** $p < 0.0001$, ** $p < 0.005$, and ordinary one-way ANOVA with Dunnett's multiple comparisons).

(E) Representative images of WTC11-derived NPCs transfected with BFP empty vector (blue) and BFP-TAOK2 (blue) stained for cilium marker ARL13B (magenta). Scale bar represents 3 μ m.

(F) Quantification of cilium length in WTC11-derived NPCs transfected with BFP-tagged protein expression constructs for the indicated genes in the 16p11.2 locus ($n > 12$ NPCs from 3 experiments for each indicated expression construct, **** $p < 0.0001$, * $p < 0.05$, and ordinary one-way ANOVA with Dunnett's multiple comparisons). All error bars are standard error of the mean.

Cilium length deficits in TAOK2 knockout neural progenitors

TAOK2 is a serine threonine kinase localized to the ER (Nourbakhsh et al., 2021) that is important for neuronal development (Anda et al., 2012; Byeon and Yadav, 2024; Yadav et al., 2017). In mitotic cells, TAOK2 localizes strongly to spindle poles (Koo et al., 2017; Nourbakhsh et al., 2021). We used an antibody against TAOK2 to determine its distribution in ciliated NPCs. TAOK2 was present on the ER membrane in a punctate manner as expected, but was also strongly concentrated

at the centrosomes and the base of cilia marked by ARL13B antibody (Figure 4A). To further test the role of TAOK2 in regulating primary cilium length, we generated TAOK2 KO iPSC lines using gene editing. We used the AICS0032 stem cell line (Allen Institute), where the centrosomal protein centrin-2 (CETN2) is tagged with RFP at its endogenous locus, allowing exquisite visualization of centrosomes (Figures S2A and S2B). AICS0032 iPSCs were genetically edited using Cas9 along with guide RNA targeting exon4 to generate TAOK2 KO cell lines 4.4 and 4.9 (Figure S2C). Successful editing was confirmed by sequencing and KO was validated by western blot (Figures S2D and S2E). Unedited wild-type (WT) AICS0032 iPSCs were used as a control. All three lines were karyotypically normal (Figure S2F), and iPSCs retained pluripotency as evident by expression of NANOG and OCT4 (Figure S2G). Dorsal forebrain NPCs were obtained from iPSCs through dual SMAD inhibition using our published protocol (Deshpande et al., 2017). TAOK2 KO and WT control NPCs expressed neuronal progenitor markers PAX6, NESTIN (Figure S3A), as well as FOXG1 and SOX2 (Figure S3B). Loss of TAOK2 protein in NPCs was confirmed by western blot (Figure 4B).

Neural progenitors from control and TAOK2 KO iPSCs self-assembled into rosettes and maintained cell polarity, with enrichment of ZO1 and N-cadherin on the apical end of the rosettes (Figures 4C and S3C), and primary cilia projecting toward the apical side of the rosette (Figure 4D). To test whether primary cilium length is altered in TAOK2 KO NPCs, we immunostained NPCs with ARL13B (Figures 4D and 4E). We found a significant increase in cilia length in TAOK2 KO4.4 (mean = 3.52 μ m, n = 151 NPCs, SEM = 0.08) and TAOK2 KO4.9 NPCs (mean = 3.35 μ m, n = 170 NPCs, SEM = 0.06), compared to WT (mean = 2.14 μ m, n = 260 NPCs, SEM = 0.04) (Figure 4E).

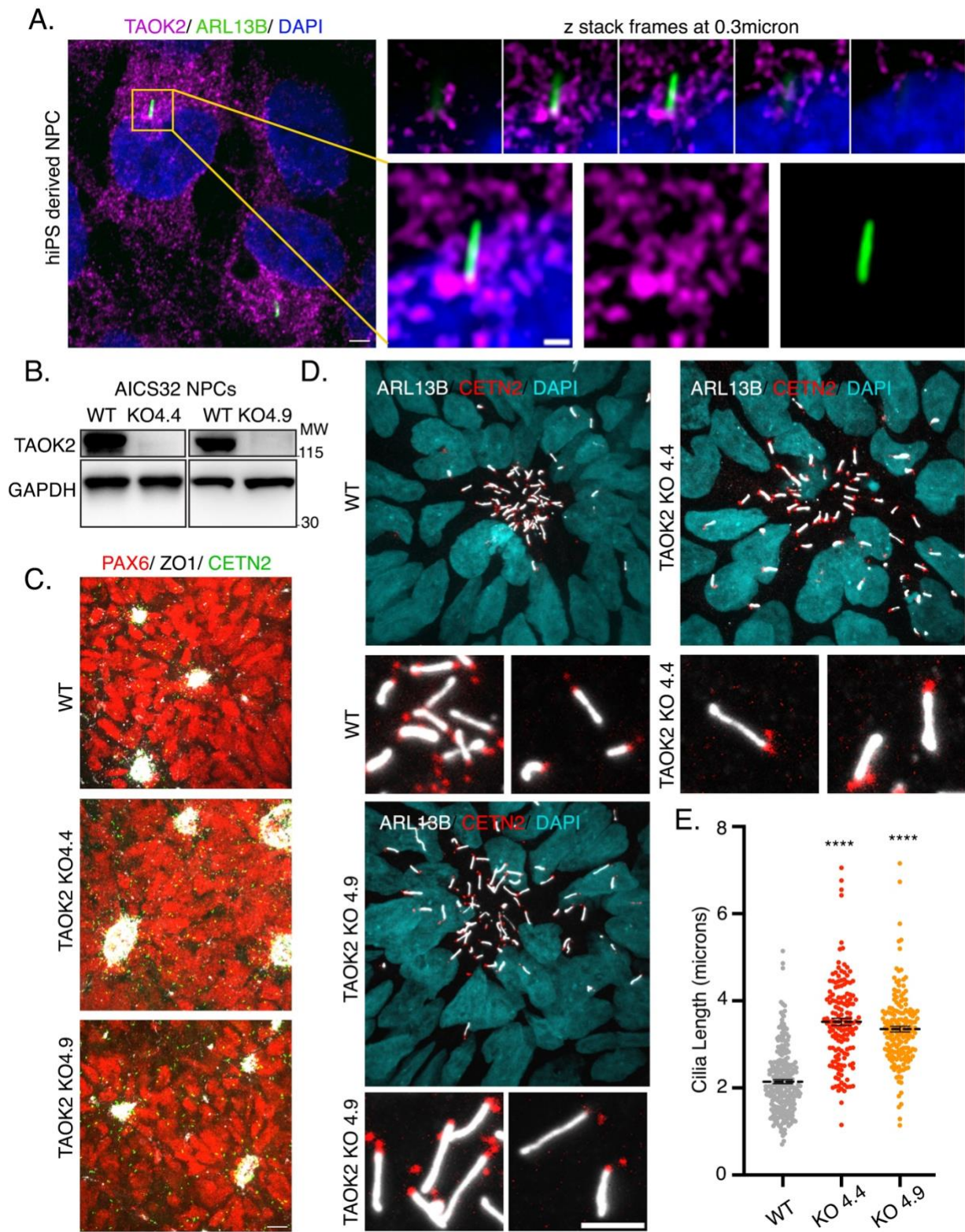


Figure 4

Figure 3.4. TAOK2 kinase localizes to the base of the primary cilium and restricts cilium elongation

(A) Human iPSC-derived NPCs stained for endogenous TAOK2 (magenta) and cilia marker ARL13B (green) show ER localization of TAOK2 as well as its localization at the base of the primary cilia. Nuclei were stained with DAPI. Scale bar represents 5 μm . Magnified inset is shown on the right with individual frames of the z stack acquired at 0.3 μm . Scale bar represents 1 μm .

(B) Validation of TAOK2 KO in NPCs by western blot analyses.

(C) Neural rosettes from control WT and TAOK2 KO NPCs expressing RFP tagged centrin-2 (green) were stained for apical polarity marker ZO1 (white) and NPC marker PAX6 (red). Scale bar represents 10 μm .

(D) NPCs from control WT and TAOK2 KO NPCs expressing RFP-centrin-2 (red) were stained for ARL13B (white). Nuclei were stained with DAPI (cyan). Scale bar represents 3 μm .

(E) Quantification of cilium length in control WT ($n = 260$ NPCs from 3 experiments), TAOK2 KO 4.4 ($n = 151$ NPCs from 3 experiments, **** $p < 0.0001$) and KO 4.9 ($n = 170$ NPCs from 3 experiments, **** $p < 0.0001$, and one-way ANOVA with Dunnett's multiple comparison) NPCs immunostained with ARL13B. All error bars are standard error of the mean.

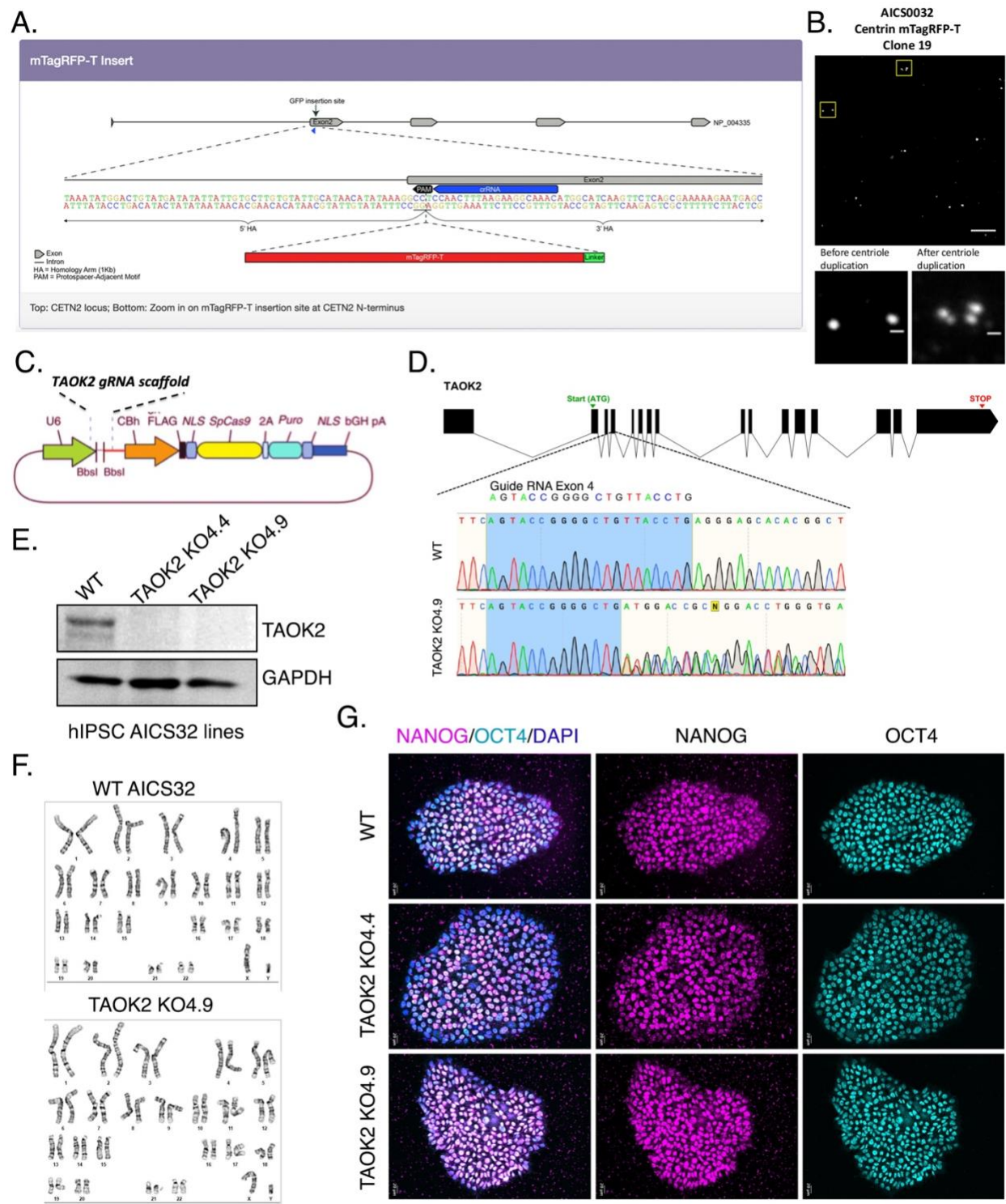


Figure S2

Figure 3S.2. Generation of TAOK2 Knockout Human iPSC and NPCs Using Genome Editing (related to main Figure 4)

(A) Schematic depicts the CETN2 genomic locus and position where mTagRFP-T was inserted using the indicated guideRNA to generate the AICS0032 stem cell line derived from WTC11. Data from Allen Institute. **(B)** Images show AICS0032 stem cell clone 19 expressing mTagRFPT-Centrin-2. Both mother and daughter centrioles are visible under the microscope. Data from Allen Institute website. **(C)** Expression plasmid backbone and site of guideRNA used to generate TAOK2 KO iPSC lines are shown. **(D)** Genomic sequencing of TAOK2 KO stem cells where the sequence within exon 4 that was targeted is indicated. **(E)** Western blot shows cell lysate from WT and TAOK2 KO clones 4.4 and 4.9 iPSCs immunoblotted with antibodies against TAOK2 and GAPDH. **(F)** Karyotype analyses of WT and TAOK2 KO 4.9. **(G)** WT and TAOK2 KO 4.4 and 4.9 iPSCs immunostained for pluripotency markers NANOG (magenta) and OCT4(cyan), and nuclear dye DAPI (blue). Scale bar is 20µm.

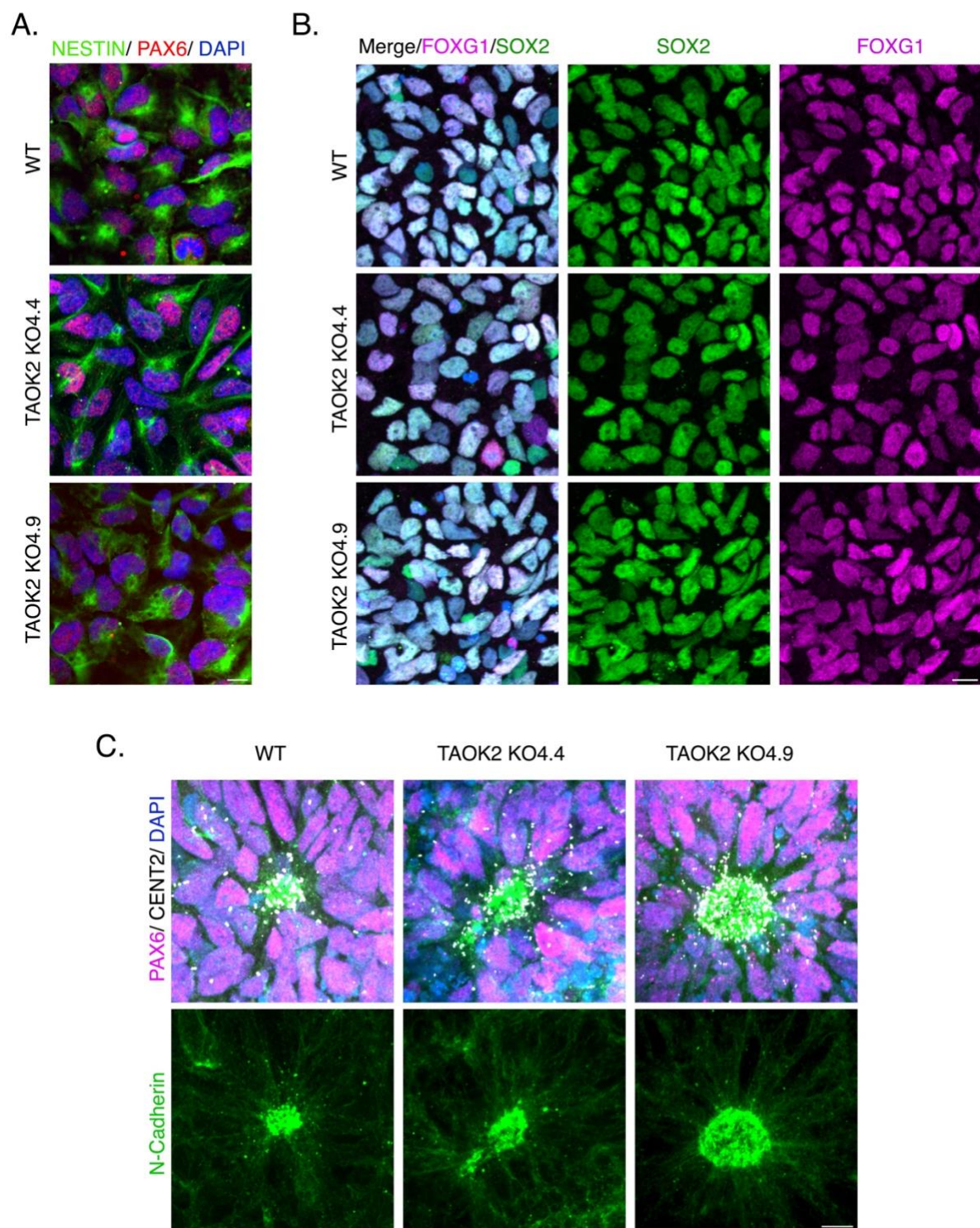


Figure S3

Figure 3S.3. Characterization of TAOK2 Knockout NPCs (related to main Figure 4)

(A) Representative images of WT and TAOK2 KO 4.4 and 4.9 NPCs immunostained with NPC

markers NESTIN (green) and PAX6 (red). Nuclei were stained with DAPI (blue). Scale bar is 10µm. **(B)** Representative images of WT and TAOK2 KO NPCs immunostained with NPC markers FOXG1 (magenta) and SOX2 (green). Nuclei were stained with DAPI (blue). Scale bar is 10µm. **(C)** Representative images of WT, TAOK2 KO4.4 and TAOK2 KO4.9 rosettes expressing RFP-Centrin-2 (white) immunostained with NPC marker PAX6 (magenta), apical polarity marker N-Cadherin (green), and nuclear dye DAPI (blue). Scale bar is 10µm.

TAOK2 knockout neural progenitors exhibit ciliary trafficking deficits

Dedicated trafficking machinery comprising intraflagellar transport (IFT) proteins mediates anterograde and retrograde motor-based transport of cargo in and out of the primary cilium (Ishikawa and Marshall, 2011; Pan and Snell, 2014). We first tested whether the distribution of the centrosomal scaffold protein PCNT, important for trafficking of IFT bound cargo (Jurczyk et al., 2004; Salisbury, 2004) and cilium elongation (Nakazato et al., 2023), was disrupted in TAOK2 KO NPCs. WT and TAOK2 KO NPCs were fixed and immunostained for endogenous PCNT and ciliary membrane marker ARL13B (Figure 5A). Mean fluorescence intensity of PCNT within a 5 µm radius of the CETN2-RFP tagged centrosomes was significantly increased in TAOK2 KO4.4 (mean = 336.4, 220 NPCs from n = 3 experiments, SEM = 23.22) and TAOK2 KO4.9 NPCs (mean = 354.4, 220 NPCs from n = 3 experiments, SEM = 29.60) compared to WT (mean = 204, 220 NPCs from n = 3 experiments, SEM = 8.10) cells (Figure 5B). Next, we tested whether IFT is maintained in TAOK2 KO cells by assessing the distribution of anterograde IFT-B component IFT88. NPCs were fixed and immunostained for endogenous IFT88 and ciliary membrane marker ARL13B (Figure 5C). IFT88 particles are present at the base of the cilia and distributed along the length of the cilia, indicating normal transport. Accumulation at the cilia tip

typically occurs in IFT-A mutants and indicates defective balance between retrograde and anterograde transport (Qin et al., 2011; Weijman et al., 2024). In WT condition, 9.76% and 16.37% of NPCs had IFT88 enriched at the ciliary base and tip, respectively, while in a majority (73.87%) of NPCs, IFT88 was uniformly distributed along the ciliary length (482 NPCs from n = 3 experiments). In contrast, IFT88 was enriched in the cilia tip in 61.95% of TAOK2 KO4.4 and 52.8% of KO4.9 NPCs, while it was uniformly distributed in 33.44% of TAOK2 KO4.4 and 35.43% of KO4.9 NPCs (460 and 595 NPCs, respectively from n = 3 experiments) (Figure 5D). These data show that TAOK2 regulates PCNT and IFT88 trafficking in the primary cilium, with loss of TAOK2 resulting in increased centrosomal PCNT and IFT88 accumulation at the primary cilium tip. We also tested whether IFT88 trafficking is impacted in 16p11.2 deletion carriers by immunostaining control and deletion NPCs for IFT88 and ARL13B (Figure 5E). We found IFT88 was enriched in the cilia tip in 57.90% of deletion and 27.57% of control NPCs, while it was uniformly distributed in 28.03% of deletion and 58.36% of control NPCs (679 and 639 NPCs, respectively from n = 3 experiments) (Figure 5F).

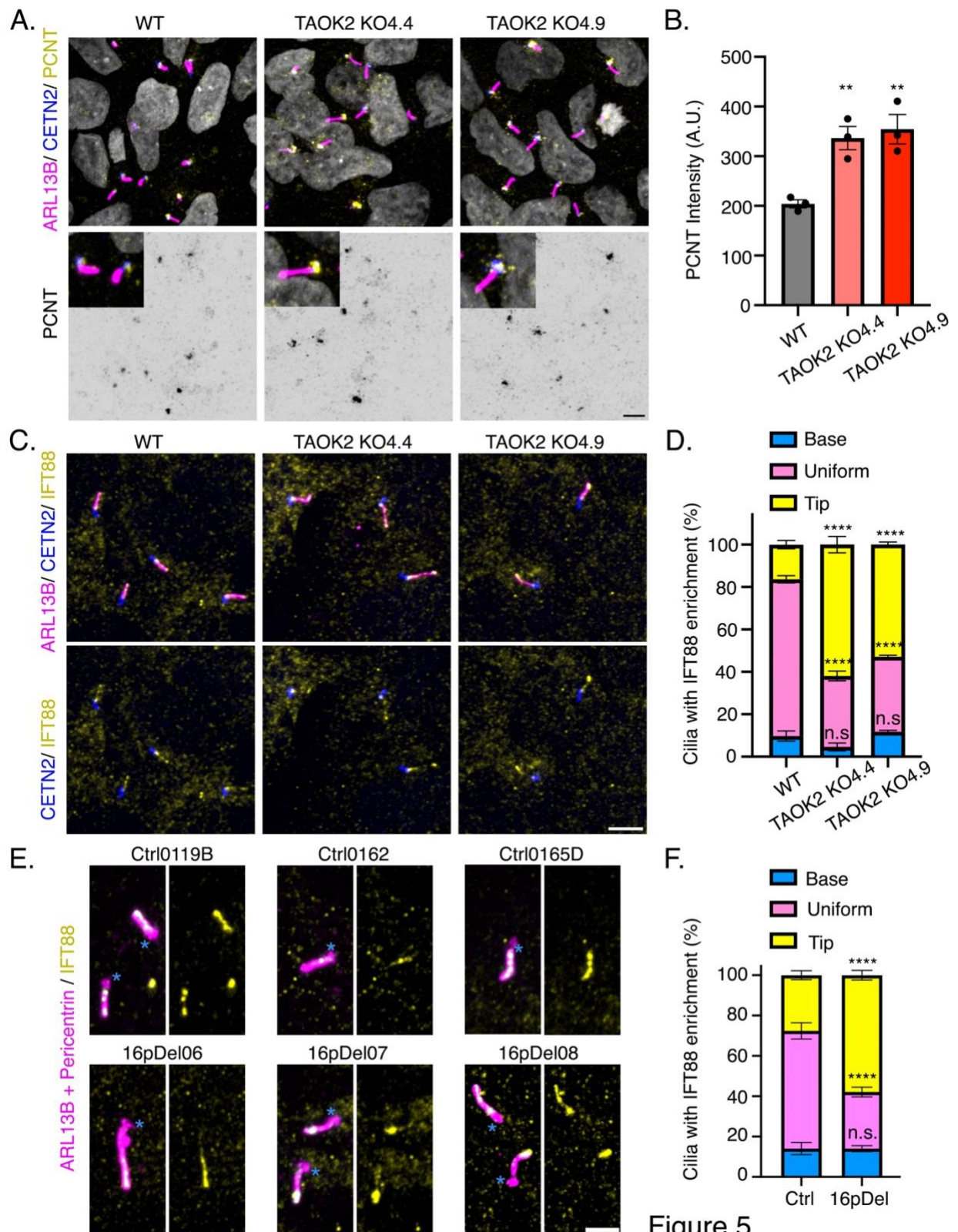


Figure 5

Figure 3.5. TAOK2 KO and 16p11.2 deletion NPCs show aberrant IFT88 accumulation at ciliary tip

(A) NPCs derived from control WT, TAOK2 KO 4.4, and 4.9 iPSC tagged with RFP-centrin-2 (blue) were immunostained for pericentrin (PCNT) (yellow) and cilia marker ARL13B (magenta). Bottom row shows PCNT immunostaining in inverted grayscale. Scale bar represents 5 μ m.

(B) Fluorescence intensity of PCNT staining within a 5 μ m radius around the centrosome was measured in control WT and TAOK2 KO 4.4 and 4.9 NPCs (220 NPCs each genotype from n = 3 experiments, $^{**}p < 0.005$, and repeated measures one-way ANOVA with Dunnett's multiple comparisons).

(C) Control and TAOK2 KO4.4 and 4.9 NPCs tagged with RFP-centrin-2 (blue) were immunostained for IFT88 (yellow) and cilia marker ARL13B (magenta). Bottom row shows merged images of IFT88 (yellow) with endogenous centrin-2 (blue) that marks the base of the primary cilium. Scale bar represents 5 μ m.

(D) Percentage of control WT NPCs (482 NPCs from n = 3 experiments), TAOK2 KO 4.4 NPCs (460 NPCs from n = 3 experiments), and TAOK2 KO4.9 NPCs (595 NPCs from n = 3 experiments, $^{****}p < 0.0001$, and two-way ANOVA with Tukey's multiple comparisons) with IFT88 enrichment at the cilium base, cilium tip, or uniform distribution along the length of the cilia are plotted.

(E) Control NPCs (RMK0119, RMK0162, and RMK0165) and 16p11.2 deletion carrier NPCs (16pDel06, 16pDel07, and 16pDel08) were immunostained for IFT88 (yellow), cilia marker ARL13B (magenta), and PCNT (magenta).

(F) Percentage of control NPCs (639 NPCs from n = 3 experiments) and deletion carrier NPCs (679 NPCs from n = 3 experiments, $^{****}p < 0.0001$, and two-way ANOVA with Sidak's multiple

comparisons) with IFT88 enrichment at the cilium base, cilium tip, or uniform distribution along the length of the cilia are plotted. Error bars are standard error of the mean.

Dysregulated SHH signaling in TAOK2 knockout neuroprogenitor cells

IFT dysfunction is associated with impaired SHH signaling as IFT proteins play key roles in cilia structure and ciliary trafficking (Huangfu et al., 2003; Liem et al., 2012; Tran et al., 2008). SHH is a key signaling pathway important for brain development including the forebrain (Altaba et al., 2002). Upon SHH ligand binding, the repressor protein patched1 (PTCH1) is removed from cilia, resulting in ciliary activation of smoothened (SMO), which turns on transcription of SHH target genes such as glioma-associated oncogene (*GLI*) (Rohatgi et al., 2007). First, we tested if ciliary accumulation of SMO was affected by absence of TAOK2. WT and TAOK2 KO NPCs were fixed and immunostained with antibody against SMO and ARL13B, and proportion of NPCs that had SMO present in the primary cilia was quantified (Figure 6A). We found a significant increase in percentage of NPCs with ciliary SMO distribution, with 34.69% of TAOK2 KO 4.4 NPCs (1,033 NPCs from n = 3 experiments, SEM = 0.77) and 39.22% of TAOK2 KO 4.9 NPCs (1,202 NPCs from n = 3 experiments, SEM = 3.39) showing ciliary SMO compared to 17.58% (1,147 NPCs from n = 3 experiments, SEM = 0.83) WT NPCs (Figure 6B). Since presence of SMO within the cilia impacts downstream SHH signaling, we next measured the transcriptomic outcome of SHH activation by small molecule SMO agonist SAG that activates SHH signaling independently of PTCH protein. We performed quantitative PCR to measure mRNA levels of SHH target genes in WT and TAOK2 KO NPCs upon 24 h treatment with 0.5 μ M SMO agonist SAG. We found a significant upregulation of *GLII* expression in TAOK2 KO4.4 and KO4.9 NPCs compared to WT NPCs (n = 7–9), whereas there was no significant change in *PTCHI* mRNA level (Figures 6C and

6D). Therefore, consistent with the upregulation of SMO within the cilia in TAOK2 KO NPCs, there was increased activation of SHH dependent *GLI1* transcription compared to control NPCs.

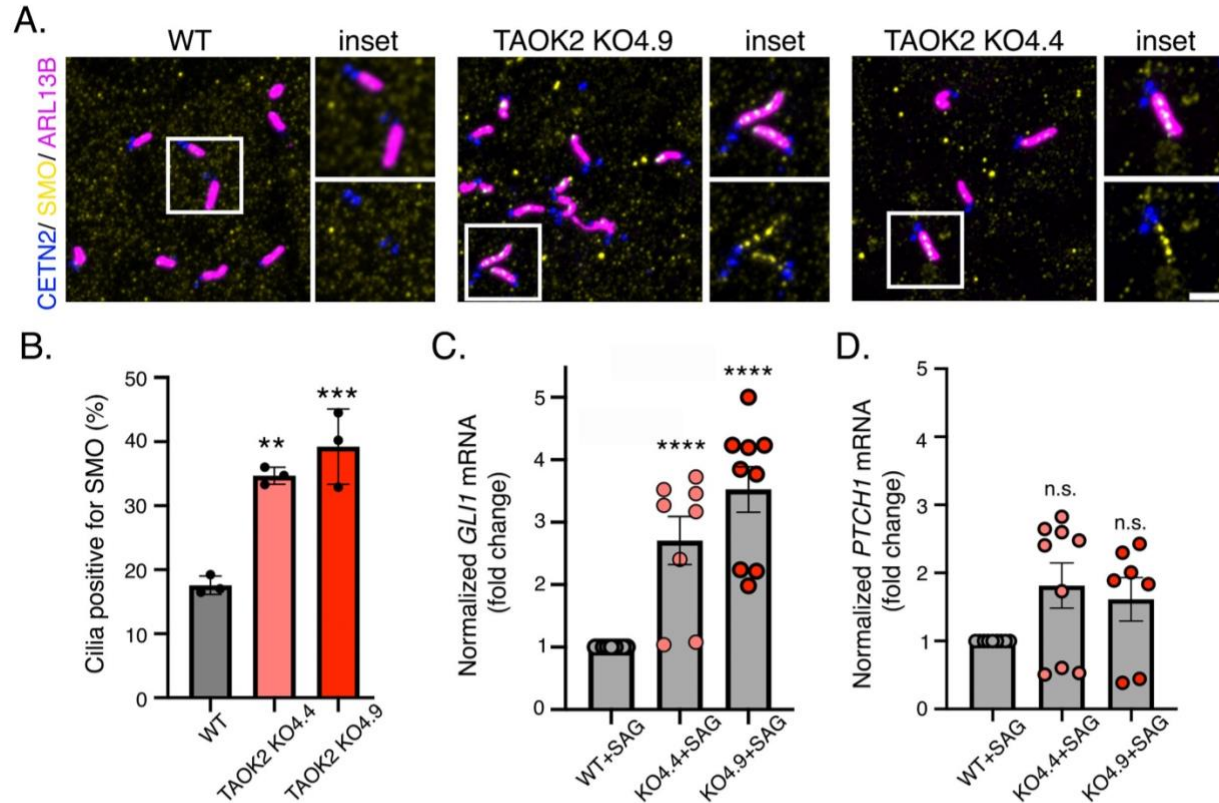


Figure 6

Figure 3.6. TAOK2 is important for SHH signaling

(A) Control WT and TAOK2 KO 4.4 and 4.9 NPCs expressing RFP-centrin-2 (blue) were immunostained for SMO (yellow) and ARL13B (magenta). Scale bar represents 2 μ m.

(B) Quantification of primary cilia positive for SMO in control WT NPCs (1,147 NPCs from n = 3 experiments), TAOK2 KO 4.4 (1,033 NPCs from n = 3 experiments, **p = 0.002), and TAOK2 KO 4.9 (1,202 NPCs from n = 3 experiments, ***p = 0.0006, one-way ANOVA with Dunnett's multiple comparisons) NPCs.

(C and D) Bar graph plots of individual values and geometric means of mRNA levels of GLI1 and PTCH1 in SAG treated TAOK2 KO 4.4 and 4.9 NPCs normalized to WT NPCs (**** $p < 0.0001$, mixed effects analyses with Geisser-Greenhouse correction). Error bars are standard error of the mean.

TAOK2 catalytic activity is required for its role in cilium length control

To determine whether TAOK2 controls cilium length through phosphorylation, we utilized a small molecule TAO kinase inhibitor compound 43 (CP43) with an IC₅₀ of 15 nM (Giacomini et al., 2018; Koo et al., 2017). WT NPCs were treated with control vehicle DMSO or CP43 at 10 μ M for 3 h. After drug treatment, cells were fixed and immunostained for cilia marker ARL13B. We found that NPCs treated with CP43 showed a robust increase in cilia length (mean = 3.13 μ m, $n = 100$, SEM = 0.06) compared to DMSO treated controls (mean = 2.29 μ m, $n = 100$, SEM = 0.07) indicating that TAOK2 kinase activity is important for its role in cilia length control (Figures 7A and 7B). These data reveal that TAOK2 catalytic activity controls cilium length, and its acute inhibition leads to increased cilia length, phenocopying the effect of TAOK2 KO.

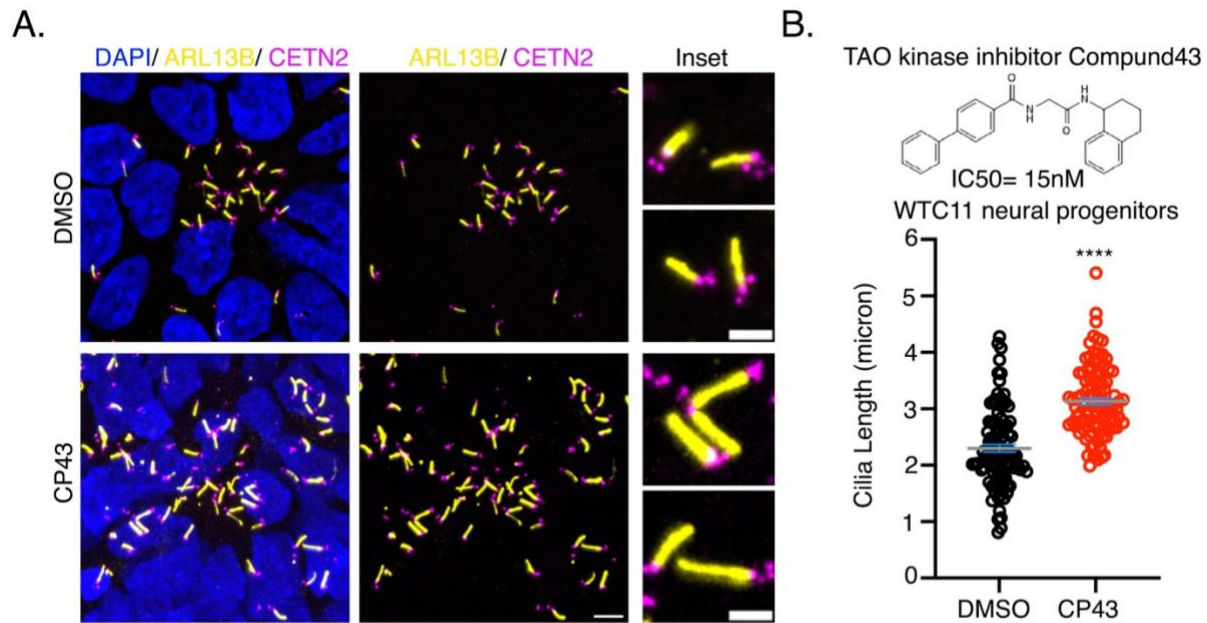


Figure 7

Figure 3.7. TAOK2 catalytic activity is important for its role in ciliary growth restriction

(A) Control WT NPCs endogenously tagged with RFP-centrin-2 (magenta) were treated with DMSO or TAO kinase inhibitor CP43 (10 μ M for 3 h), fixed and immunostained for ARL13B (yellow) and nuclear dye DAPI (blue). Scale bars represent 5 μ m. Inset on right shows magnified representative cilia treated with DMSO or CP43. Scale bar represents 2 μ m.

(B) Schematic shows the chemical structure and IC₅₀ of CP43. Cilia length in NPCs treated with vehicle DMSO or CP43 is plotted (n = 100 NPCs each condition from 3 experiments, ****p < 0.0001, and Student's t test with Welch correction). Error bars are standard error of the mean.

Discussion

Human diseases caused by mutations in genes affecting centrosomes and primary cilia, called ciliopathies, are commonly associated with brain development deficits (Guemez-Gamboa et al.,

2014; Han and Alvarez-Buylla, 2010). Also, disruption in cilia length has been observed in several neurological disorders. Cells derived from patients with Down syndrome exhibit shorter cilia, aberrant centrosomal accumulation of PCNT and impaired trafficking to the cilium (Galati et al., 2018). Aberrant ciliogenesis is seen in patients with focal malformations of cortical development caused by somatic activating mutations in mTOR (Park et al., 2018). Seckel syndrome, caused by mutations in the centrosomal-P4.1-associated protein (CPAP) is associated with microcephaly. NPCs derived from patients with Seckel syndrome exhibit extremely long cilia and undergo premature neuronal differentiation (Gabriel et al., 2016). At the outset, several lines of evidence pointed to a potential role of ciliary dysfunction in 16p11.2 CNV. First, transcriptomic profiles of individuals carrying reciprocal 16p11.2 CNV were enriched for genes associated with ciliopathies (Migliavacca et al., 2015). Second, radial glial cells in cerebral organoids generated from 16p11.2 CNV carriers show differentially expressed cilium assembly genes (Urresti et al., 2021), and lastly, patients with 16p11.2 CNV exhibit BMI and metabolic imbalances (Jacquemont et al., 2011; Maillard et al., 2016; Walters et al., 2010; Zufferey et al., 2012), phenotypes often found in patients with ciliopathy (Oh et al., 2015; Reiter and Leroux, 2017; Ritter et al., 2018). Reciprocal changes in cilia length in NPCs derived from 16p11.2 deletion and duplication carriers were identified in our study through unbiased proteomics. Given the incomplete penetrance of 16p11.2 clinical symptoms (Arbogast et al., 2016; Weiss et al., 2008), if such an association of cilia length is made with brain size, cilium length alteration could serve as a valuable biomarker for 16p11.2 CNV.

During development of the mammalian neocortex, polarized radial glial cells are organized with their apical plasma membranes forming the luminal side of the neural tube, such that the primary

cilia extend into the ventricular cerebrospinal fluid rich in signaling molecules (Willaredt et al., 2008). Enriched within the primary cilium are numerous signaling receptors that sense growth factors and ligands important for brain development such as insulin-like growth factors, bone morphogenic proteins, SHH, retinoic acid, and lysophosphatic acid (Han and Alvarez-Buylla, 2010 ; Reiter and Leroux, 2017). Cilia length dynamics are observed during neuronal differentiation, with the circadian rhythm and in response to growth factors. Through its critical signaling functions and dynamic regulation of length that dictates receptor availability, the primary cilium serves as key regulator of neural stem cell proliferation, neuronal differentiation, migration as well as site of specialized axon-cilium synapse (Ding et al., 2019; Han and Alvarez-Buylla, 2010; Han et al., 2009; Phua et al., 2017). Changes in cilia length are often associated with microcephaly. CPAP mutations lead to elongated cilia and microcephaly through premature neurogenesis (Gabriel et al., 2016). Mutations in CENPF, a centrosomal protein, also lead to microcephaly but result in shortened cilia (Cappuccio et al., 2022). Interestingly, mutations in EML1, a microtubule-associated protein, lead to shorter cilia and are associated with macrocephaly (Bizzotto et al., 2017 ; Oegema et al., 2019; Uzquiano et al., 2019). Whether and how primary cilium length deficits contribute to opposing brain size changes observed in 16p11.2 CNV remain to be investigated. Multiple studies have focused on the impact on NPC proliferation in 16p11.2 CNV using stem cell models with variable results. Studies have reported no differences in NPC proliferation in 16p11.2 CNV carriers using 2D cultured NPCs (Deshpande et al., 2017; Roth et al., 2020), hyperproliferation in deletion NPCs (Connacher et al., 2022), prolonged cell cycle in deletion 3D organoid models (Fetit et al., 2023), or alterations in the ratio of neurons to progenitors in 3D organoids during early neurogenesis (Urresti et al., 2021). Heterogeneity in the model systems and methods of assessing proliferation could contribute to these disparate findings.

Our results show that TAOK2 gene dosage regulates cilia length and that NPCs lacking TAOK2 have elongated cilia, increased centrosomal PCNT, and accumulation of IFT88 at distal cilia tip. Moreover, SHH signaling is dysregulated in the TAOK2 KO NPCs. A previous study found that in neuroepithelial cells SHH signaling upregulates PCNT expression, which accumulates at the centrosome and recruits the protein kinase A (PKA) to centrosomes, thereby exerting a positive-feedback onto SHH signaling (Saade et al., 2017). Therefore, it is possible that increased PCNT that we observe in TAOK2 KO NPCs is contributing to the increase in *GLII* activity downstream of SMO. Upregulation of SHH signaling has been noted in Nestin-Gpr161 conditional KO mice that leads to increased progenitor proliferation causing ventriculomegaly (Shimada et al., 2019). Other outcomes of high SHH signaling include dysregulated neuroepithelial cell to radial glia transition and aberrant cell cycle exit in neuroprogenitors, each of which could lead to an increase in brain size (Chenn and Walsh, 2002; Sahara and O’Leary, 2009; Shimada et al., 2019). Notably, TAOK2 is the only gene within the 16p11.2 locus, single nucleotide missense mutations in which have been associated with both obesity (Agrawal et al., 2021; Elkhateeb et al., 2024; Loid et al., 2022) and macrocephaly (Elkhateeb et al., 2024). Whether and how the ciliary role of TAOK2 contributes to obesity and macrocephaly is an exciting direction for future investigation.

Limitations of the study

Whether ciliary deficits exist broadly in other cell types within the brain and in other tissues in 16p11.2 CNV carriers needs to be examined. This study was based on samples from three distinct 16p11.2 deletion and three duplication carriers, with heterogeneous age and sex distribution, and distinct genetic background. Future studies that employ cilia length and function analyses in larger

number of 16p11.2 CNV carriers or genetically edited iPSC lines harboring 16p11.2 BP4-5 CNV would be insightful.

Methods

Induced pluripotent stem cell culture and neuronal differentiation

All iPSC lines were differentiated into NPCs as reported previously (Deshpande et al., 2017). Stem cells were treated with 1 U/mL dispase, lifted using a cell scraper, resuspended in mTeSR media (STEMCELL Technologies) containing 10 μ M ROCK Inhibitor (Selleck Chemical), and then transferred to uncoated T25 flasks for embryoid body formation. After 24–48 h (day 0), EBs were gravity settled and transferred to new uncoated T25 flasks in neural media (DMEM/F12 (Gibco), 1% N-2 supplement (Gibco), 1% non-essential amino acids (Gibco), 2 μ g/mL Heparin, and 1% penicillin/streptomycin [Gibco]) containing small molecule TGF- β /SMAD inhibitors, SB431542 (5 μ M; PeproTech), and LDN193189 (0.25 μ M; PeproTech). On day 3, EBs were transferred to Matrigel (1:50 dilution; Corning)-coated 6-well plates in fresh neural media with SB and LDN. Media was changed with neural media without SB and LDN every other day or as needed until day 11 for formation of neural rosettes. On day 11, neuroepithelia were lifted using cell scraper and transferred to uncoated T25 flasks in neural media. Neurospheres were fed with neural media every other day, or as needed, until day 25. On day 25, neurospheres were plated onto Matrigel-coated plates in STEMDiff Neural Progenitor Media and NPCs were obtained.

Proteomic sample preparation

Confluent 75-mL flasks of NPCs were washed with PBS and then homogenized in 1 mL 8 M urea, using a probe sonicator (Thermo Fisher Scientific). Protein content in extracts was determined

using Micro BCA Protein Assay Kit (Thermo Fisher Scientific). Aliquots containing 500 µg protein in 590 µL 8 M urea were added with 236 µL of 200 mM ammonium bicarbonate buffer and phosphatase inhibitors (Sigma Phosphatase Inhibitor Cocktails 1 and 3), then treated with 5 mM DTT at 56°C for 15 min, followed by a 30 min incubation at room temperature in dark with 7.5 mM iodoacetamide. For tryptic digestion, the samples were then diluted 4-fold with 100 mM ammonium bicarbonate to reduce urea to 2 M and then 5% (W/W) trypsin (Promega) was added. The pH was adjusted to 8.0 with 250 mM ammonium bicarbonate, and samples were incubated 12 h at 37°C. Another aliquot of trypsin was added (2% W/W) and digested for additional 6 h. The digests were desalted using a MAX-RP SepPak C18 cartridge (Waters) following manufacturer's protocol. Sep Pak eluates were dried-evaporated in preparation for labeling with TMT10plex reagents. TMT labeling, phosphopeptide enrichment, mass spectrometry, and peptide identification are described in the supplemental information.

Generation of TAOK2 knockout stem cell line

Two independent TAOK2 KO lines were generated using CRISPR-Cas9 genome editing in an AICS0032 stem cell line. Guide RNA was designed using Synthego design tool (<https://www.synthego.com>) to target coding exon4. Guide was cloned into CrisprV2pSpCas9(BB)-2A-Puro (PX459) V2.0 (Addgene no. 62988). Cells were passaged in single cell suspension and plated at 50% confluence. Cultures were then transfected Lipofectamine 2000 Transfection reagent (Invitrogen) and 2 mg of guide RNA used per KO line. Cells were then selected with Puromycin for 48 h to select for transfected cells. Edited cells were expanded into single cell colonies. Genomic DNA was extracted and the region around the cutting site was PCR amplified to send for sequencing. Successful TAOK2 KO clones 4.4 and 4.9 were confirmed by

Sanger sequencing analysis, and absence of encoded protein was validated by western blot (described in supplemental information). Absence of large chromosomal alterations was confirmed by karyotyping.

Confocal microscopy

Imaging was performed on a Nikon Ti2 Eclipse-CSU-X1 confocal spinning disk microscope equipped with four laser lines (405, 488, 561, and 670 nm) and an sCMOS Andor camera for image acquisition. The microscope was caged within the OkoLab environmental control setup enabling temperature and CO₂ control during live imaging. Imaging was performed using Nikon 1.49 100× Apo 60× or 40× oil objective lenses. All image analyses and quantification of fluorescence intensity was performed using Fiji ImageJ.

Statistics

Statistics were performed in GraphPad software Prism 10.0. Multiple groups were analyzed using ANOVA, while two group comparisons were made using unpaired t test unless otherwise stated. Statistically p value less than 0.05 was considered significant. All experiments were done in triplicate, and experimental sample size and p values are indicated with the corresponding figures.

Resource availability

Lead contact

Requests for further information and resources should be directed to the lead contact, Smita Yadav (smitay@uw.edu).

Materials availability

Stem cell lines used are available from SFARI iPSC collection. Stable reagents generated in this study are available from the lead contact with a completed materials transfer agreement. Key resources are listed in Table S3.

Data and code availability

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD066614.

Acknowledgments

We are grateful for funding provided by the National Institutes of Health (R01MH121674) to S.Y. and the Jaconette Tietze Award to S.Y. We extend our gratitude to the patients and families of 16p11.2 CNV carriers who have shared their skin samples to support research and funding from SFARI (345471) to L.A.W. for generation of the iPSC lines. Mass spectrometry was provided by the MS Resource at UCSF (A.L.B., Director) supported by the Dr. Miriam and Sheldon G. Adelson Medical Research Foundation and the UCSF Program for Breakthrough Biomedical Research.

Author contributions

S.B. characterized the TAOK2 KO iPSC lines, differentiated them into NPCs and performed all experiments on the TAOK2 KO lines with the help of S.M. and A.B. A.F. designed and performed shRNA and overexpression screening experiments and generated the TAOK2 KO iPSC lines. Cilia database analyses of proteomic data and molecular biology experiments were performed by M.C.

Proteomics experiments were performed and analyzed by J.A.O-P. under the supervision of A.L.B. Neuronal differentiation of 16p11.2 CNV and control iPSC lines were performed by A.D. under the supervision of L.A.W. The manuscript was written by S.B. and S.Y. All aspects of work, with exception of the proteomics, were supervised by S.Y.

Declaration of interests

The authors declare no competing interests.

References

- Agrawal, N., Lawler, K., Davidson, C.M., Keogh, J.M., Legg, R., Brand, A.H., Barroso, I., Barroso, I., Farooqi, I.S., 2021. Predicting novel candidate human obesity genes and their site of action by systematic functional screening in *Drosophila*. *PLoS Biol.* 19, e3001255. doi:10.1371/journal.pbio.3001255.
- Altaba, A.R. i, Palma, V., Dahmane, N., 2002. Hedgehog–GLI signaling and the growth of the brain. *Nat. Rev. Neurosci.* 3, 24–33. doi:10.1038/nrn704.
- de Anda, F.C., Rosario, A.L., Durak, O., Tran, T., Gräff, J., Meletis, K., Rei, D., Soda, T., Madabhushi, R., Ginty, D.D., et al., 2012. Autism spectrum disorder susceptibility gene TAOK2 affects basal dendrite formation in the neocortex. *Nat. Neurosci.* 15, 1022–1031. doi:10.1038/nn.3141.
- Anvarian, Z., Mykityn, K., Mukhopadhyay, S., Pedersen, L.B., Christensen, S.T., 2019. Cellular signalling by primary cilia in development, organ function and disease. *Nat. Rev. Nephrol.* 15, 199–219. doi:10.1038/s41581-019-0116-9.
- Arbogast, T., Ouagazzal, A.-M., Chevalier, C., Kopanitsa, M., Afinowi, N., Migliavacca, E., Cowling, B.S., Birling, M.-C., Champy, M.-F., Reymond, A., Herault, Y., 2016. Reciprocal Effects on Neurocognitive and Metabolic Phenotypes in Mouse Models of 16p11.2 Deletion and Duplication Syndromes. *PLoS Genet.* 12, e1005709. doi:10.1371/journal.pgen.1005709.
- Bettencourt-Dias, M., Hildebrandt, F., Pellman, D., Woods, G., Godinho, S.A., 2011. Centrosomes and cilia in human disease. *Trends Genet.* 27, 307–315. doi:10.1016/j.tig.2011.05.004.
- Bizzotto, S., Uzquiano, A., Dingli, F., Ershov, D., Houllier, A., Arras, G., Richards, M., Loew, D., Minc, N., Croquelois, A., et al., 2017. *Eml1* loss impairs apical progenitor spindle length and soma shape in the developing cerebral cortex. *Sci. Rep.* 7, 17308. doi:10.1038/s41598-017-15253-4.
- Byeon, S., Yadav, S., 2024. Pleiotropic functions of TAO kinases and their dysregulation in neurological disorders. *Sci. Signal.* 17, eadg0876. doi:10.1126/scisignal.adg0876.
- Cappuccio, G., Brillante, S., Tammara, R., Pinelli, M., De Bernardi, M.L., Gensini, M.G., Bijlsma, E.K., Koopmann, T.T., Hoffer, M.J.V., McDonald, K., et al., 2022. Biallelic variants in CENPF causing a phenotype distinct from Strømme syndrome. *Am. J. Med. Genet. C Semin. Med. Genet.* 190, 102–108. doi:10.1002/ajmg.c.31973.
- Chenn, A., Walsh, C.A., 2002. Regulation of Cerebral Cortical Size by Control of Cell Cycle Exit in Neural Precursors. *Science* 297, 365–369. doi:10.1126/science.1074192.
- Clauser, K.R., Baker, P., Burlingame, A.L., 1999. Role of accurate mass measurement (+/- 10 ppm) in protein identification strategies employing MS or MS/MS and database searching. *Anal.*

Chem. 71, 2871–2882.

Connacher, R., Williams, M., Prem, S., Yeung, P.L., Matteson, P., Mehta, M., Markov, A., Peng, C., Zhou, X., McDermott, C.R., et al., 2022. Autism NPCs from both idiopathic and CNV 16p11.2 deletion patients exhibit dysregulation of proliferation and mitogenic responses. *Stem Cell Rep.* 17, 1380–1394. doi:10.1016/j.stemcr.2022.04.019.

Deshpande, A., Yadav, S., Dao, D.Q., Wu, Z.-Y., Hokanson, K.C., Cahill, M.K., Wiita, A.P., Jan, Y.-N., Ullian, E.M., Weiss, L.A., 2017. Cellular Phenotypes in Human iPSC-Derived Neurons from a Genetic Model of Autism Spectrum Disorder. *Cell Rep.* 21, 2678–2687. doi:10.1016/j.celrep.2017.11.037.

Ding, W., Wu, Q., Sun, L., Pan, N.C., Wang, X., 2019. Cenpj Regulates Cilia Disassembly and Neurogenesis in the Developing Mouse Cortex. *J. Neurosci.* 39, 1994–2010. doi:10.1523/jneurosci.1849-18.2018.

Elkhateeb, N., Crookes, R., Spiller, M., Pavinato, L., Palermo, F., Brusco, A., Parker, M., Park, S.-M., Mendes, A.C., Saraiva, J.M., et al., 2025. Expanding the phenotype and genotype spectrum of TAOK1 neurodevelopmental disorder and delineating TAOK2 neurodevelopmental disorder. *Genet. Med.* 27, 101348. doi:10.1016/j.gim.2024.101348.

Ferkol, T.W., Leigh, M.W., 2012. Ciliopathies: the central role of cilia in a spectrum of pediatric disorders. *J. Pediatr.* 160, 366–371. doi:10.1016/j.jpeds.2011.11.024.

Fetit, R., Barbato, M.I., Theil, T., Pratt, T., Price, D.J., 2023. 16p11.2 deletion accelerates subpallial maturation and increases variability in human iPSC-derived ventral telencephalic organoids. *Dev. (Camb., Engl.)* 150, dev201227. doi:10.1242/dev.201227.

Gabriel, E., Wason, A., Ramani, A., Gooi, L.M., Keller, P., Pozniakovsky, A., Poser, I., Noack, F., Telugu, N.S., Calegari, F., et al., 2016. CPAP promotes timely cilium disassembly to maintain neural progenitor pool. *EMBO J.* 35, 803–819. doi:10.15252/embj.201593679.

Galati, D.F., Sullivan, K.D., Pham, A.T., Espinosa, J.M., Pearson, C.G., 2018. Trisomy 21 Represses Cilia Formation and Function. *Dev. Cell* 46, 641–650. doi:10.1016/j.devcel.2018.07.008.

Giacomini, C., Koo, C.-Y., Yankova, N., Tavares, I.A., Wray, S., Noble, W., Hanger, D.P., Morris, J.D.H., 2018. A new TAO kinase inhibitor reduces tau phosphorylation at sites associated with neurodegeneration in human tauopathies. *Acta Neuropathol. Commun.* 6, 37. doi:10.1186/s40478-018-0539-8.

Guan, S., Price, J.C., Prusiner, S.B., Ghaemmaghami, S., Burlingame, A.L., 2011. A data processing pipeline for mammalian proteome dynamics studies using stable isotope metabolic labeling. *Mol. Cell. Proteomics* 10, 010728. doi:10.1074/mcp.m111.010728.

Guemez-Gamboa, A., Coufal, N.G., Gleeson, J.G., 2014. Primary cilia in the developing and

mature brain. *Neuron* 82, 511–521. doi:10.1016/j.neuron.2014.04.024.

Gupta, G.D., Coyaude, É., Gonçalves, J., Mojarad, B.A., Liu, Y., Wu, Q., Gheiratmand, L., Comartin, D., Tkach, J.M., Cheung, S.W.T., et al., 2015. A Dynamic Protein Interaction Landscape of the Human Centrosome-Cilium Interface. *Cell* 163, 1484–1499. doi:10.1016/j.cell.2015.10.065.

Han, Y.-G., Alvarez-Buylla, A., 2010. Role of primary cilia in brain development and cancer. *Curr. Opin. Neurobiol.* 20, 58–67. doi:10.1016/j.conb.2009.12.002.

Han, Y.-G., Kim, H.J., Dlugosz, A.A., Ellison, D.W., Gilbertson, R.J., Alvarez-Buylla, A., 2009. Dual and opposing roles of primary cilia in medulloblastoma development. *Nat. Med.* 15, 1062–1065. doi:10.1038/nm.2020.

Huangfu, D., Liu, A., Rakeman, A.S., Murcia, N.S., Niswander, L., Anderson, K.V., 2003. Hedgehog signalling in the mouse requires intraflagellar transport proteins. *Nature* 426, 83–87. doi:10.1038/nature02061.

Ishikawa, H., Marshall, W.F., 2011. Ciliogenesis: building the cell's antenna. *Nat. Rev. Mol. Cell Biol.* 12, 222–234. doi:10.1038/nrm3085.

Jacquemont, S., Reymond, A., Zufferey, F., Harewood, L., Walters, R.G., Kutalik, Z., Martinet, D., Shen, Y., Valsesia, A., Beckmann, N.D., et al., 2011. Mirror extreme BMI phenotypes associated with gene dosage at the chromosome 16p11.2 locus. *Nature* 478, 97–102. doi:10.1038/nature10406.

Jurczyk, A., Gromley, A., Redick, S., San Agustin, J., Witman, G., Pazour, G.J., Peters, D.J.M., Doxsey, S., 2004. Pericentrin forms a complex with intraflagellar transport proteins and polycystin-2 and is required for primary cilia assembly. *J. Cell Biol.* 166, 637–643. doi:10.1083/jcb.200405023.

Kasahara, K., Inagaki, M., 2021. Primary ciliary signaling: links with the cell cycle. *Trends Cell Biol.* 31, 954–964. doi:10.1016/j.tcb.2021.07.009.

Koo, C.-Y., Giacomini, C., Reyes-Corral, M., Olmos, Y., Tavares, I.A., Marson, C.M., Linardopoulos, S., Tutt, A.N., Morris, J.D.H., 2017. Targeting TAO Kinases Using a New Inhibitor Compound Delays Mitosis and Induces Mitotic Cell Death in Centrosome Amplified Breast Cancer Cells. *Mol. Cancer Ther.* 16, 2410–2421. doi:10.1158/1535-7163.mct-17-0077.

Lepanto, P., Badano, J.L., Zolessi, F.R., 2016. Neuron's little helper: The role of primary cilia in neurogenesis. *Neurogenesis (Austin)* 3, e1253363. doi:10.1080/23262133.2016.1253363.

Liem, K.F., Ashe, A., He, M., Satir, P., Moran, J., Beier, D., Wicking, C., Anderson, K.V., 2012. The IFT-A complex regulates Shh signaling through cilia structure and membrane protein trafficking. *J. Cell Biol.* 197, 789–800. doi:10.1083/jcb.201110049.

- Loid, P., Pekkinen, M., Mustila, T., Tossavainen, P., Viljakainen, H., Lindstrand, A., Mäkitie, O., 2022. Targeted Exome Sequencing of Genes Involved in Rare CNVs in Early-Onset Severe Obesity. *Front. Genet.* 13, 839349. doi:10.3389/fgene.2022.839349.
- Maillard, A.M., Ruef, A., Pizzagalli, F., Migliavacca, E., Hippolyte, L., Adaszewski, S., Dukart, J., Ferrari, C., Conus, P., Männik, K., et al., 2015. The 16p11.2 locus modulates brain structures common to autism, schizophrenia and obesity. *Mol. Psychiatry* 20, 140–147. doi:10.1038/mp.2014.145.
- Maillard, A.M., Hippolyte, L., Rodriguez-Herreros, B., Chawner, S.J.R.A., Dremmel, D., Agüera, Z., Fagundo, A.B., Pain, A., Martin-Brevet, S., Hilbert, A., et al., 2016. 16p11.2 Locus modulates response to satiety before the onset of obesity. *Int. J. Obes.* 40, 870–876. doi:10.1038/ijo.2015.247.
- Martin-Brevet, S., Rodríguez-Herreros, B., Nielsen, J.A., Moreau, C., Modenato, C., Maillard, A.M., Pain, A., Richetin, S., Jøneh, A.E., Qureshi, A.Y., et al., 2018. Quantifying the Effects of 16p11.2 Copy Number Variants on Brain Structure: A Multisite Genetic-First Study. *Biol. Psychiatry* 84, 253–264. doi:10.1016/j.biopsych.2018.02.1176.
- McCarthy, S.E., Makarov, V., Kirov, G., Addington, A.M., McClellan, J., Yoon, S., Perkins, D.O., Dickel, D.E., Kusenda, M., Krastoshevsky, O., et al., 2009. Microduplications of 16p11.2 are associated with schizophrenia. *Nat. Genet.* 41, 1223–1227. doi:10.1038/ng.474.
- Migliavacca, E., Golzio, C., Männik, K., Blumenthal, I., Oh, E.C., Harewood, L., Kosmicki, J.A., Loviglio, M.N., Giannuzzi, G., Hippolyte, L., et al., 2015. A Potential Contributory Role for Ciliary Dysfunction in the 16p11.2 600 kb BP4-BP5 Pathology. *Am. J. Hum. Genet.* 96, 784–796. doi:10.1016/j.ajhg.2015.04.002.
- Nakazato, R., Matsuda, Y., Ijaz, F., Ikegami, K., 2023. Circadian oscillation in primary cilium length by clock genes regulates fibroblast cell migration. *EMBO Rep.* 24, e56870. doi:10.15252/embr.202356870.
- Nourbakhsh, K., Yadav, S., 2021. Kinase Signaling in Dendritic Development and Disease. *Front. Cell. Neurosci.* 15, 624648. doi:10.3389/fncel.2021.624648.
- Nourbakhsh, K., Ferreccio, A.A., Bernard, M.J., Yadav, S., 2021. TAOK2 is an ER-localized kinase that catalyzes the dynamic tethering of ER to microtubules. *Dev. Cell* 56, 3321–3333.e5. doi:10.1016/j.devcel.2021.11.015.
- Oegema, R., McGillivray, G., Leventer, R., Le Moing, A.G., Bahi-Buisson, N., Barnicoat, A., Mandelstam, S., Francis, D., Francis, F., Mancini, G.M.S., et al., 2019. EML1-associated brain overgrowth syndrome with ribbon-like heterotopia. *Am. J. Med. Genet. C Semin. Med. Genet.* 181, 627–637. doi:10.1002/ajmg.c.31751.
- Oh, E.C., Vasanth, S., Katsanis, N., 2015. Metabolic regulation and energy homeostasis through the primary Cilium. *Cell Metab.* 21, 21–31. doi:10.1016/j.cmet.2014.11.019.

Owen, J.P., Chang, Y.S., Pojman, N.J., Bukshpun, P., Wakahiro, M.L.J., Marco, E.J., Berman, J.I., Spiro, J.E., Chung, W.K., Buckner, R.L., et al., 2014. Aberrant white matter microstructure in children with 16p11.2 deletions. *J. Neurosci.* 34, 6214–6223. doi:10.1523/jneurosci.4495-13.2014.

Pan, J., Snell, W.J., 2014. Organelle Size: A Cilium Length Signal Regulates IFT Cargo Loading. *Curr. Biol.* 24, R75–R78. doi:10.1016/j.cub.2013.11.043.

Park, S.M., Lim, J.S., Ramakrishna, S., Kim, S.H., Kim, W.K., Lee, J., Kang, H.-C., Reiter, J.F., Kim, D.S., Kim, H.H., Lee, J.H., 2018. Brain Somatic Mutations in MTOR Disrupt Neuronal Ciliogenesis. *Neuron* 99, 83–97. doi:10.1016/j.neuron.2018.05.039.

Phua, S.C., Chiba, S., Suzuki, M., Su, E., Roberson, E.C., Pusapati, G.V., Schurmans, S., Setou, M., Rohatgi, R., Reiter, J.F., et al., 2017. Dynamic Remodeling of Membrane Composition Drives Cell Cycle through Primary Cilia Excision. *Cell* 168, 264–279.e15. doi:10.1016/j.cell.2016.12.032.

Portmann, T., Yang, M., Mao, R., Panagiotakos, G., Ellegood, J., Dolen, G., Bader, P.L., Grueter, B.A., Goold, C., Fisher, E., et al., 2014. Behavioral abnormalities and circuit defects in the basal ganglia of a mouse model of 16p11.2 deletion syndrome. *Cell Rep.* 7, 1077–1092. doi:10.1016/j.celrep.2014.03.036.

Qin, J., Lin, Y., Norman, R.X., Ko, H.W., Eggenschwiler, J.T., 2011. Intraflagellar transport protein 122 antagonizes Sonic Hedgehog signaling and controls ciliary localization of pathway components. *Proc. Natl. Acad. Sci.* 108, 1456–1461. doi:10.1073/pnas.1011410108.

Qureshi, A.Y., Mueller, S., Snyder, A.Z., Mukherjee, P., Berman, J.I., Roberts, T.P.L., Nagarajan, S.S., Spiro, J.E., Chung, W.K., Sherr, E.H., et al., 2014. Opposing brain differences in 16p11.2 deletion and duplication carriers. *J. Neurosci.* 34, 11199–11211. doi:10.1523/jneurosci.1366-14.2014.

Reiter, J.F., Leroux, M.R., 2017. Genes and molecular pathways underpinning ciliopathies. *Nat. Rev. Mol. Cell Bio.* 18, 533–547. doi:10.1038/nrm.2017.60.

Richter, M., Murtaza, N., Scharrenberg, R., White, S.H., Johanns, O., Walker, S., Yuen, R.K.C., Schwanke, B., Bedürftig, B., Henis, M., et al., 2019. Altered TAOX2 activity causes autism-related neurodevelopmental and cognitive abnormalities through RhoA signaling. *Mol. Psychiatry* 24, 1329–1350. doi:10.1038/s41380-018-0025-5.

Ritter, A., Friemel, A., Kreis, N.-N., Hooch, S.C., Roth, S., Kielland-Kaisen, U., Brüggmann, D., Solbach, C., Louwen, F., Yuan, J., 2018. Primary Cilia Are Dysfunctional in Obese Adipose-Derived Mesenchymal Stem Cells. *Stem Cell Rep.* 10, 583–599. doi:10.1016/j.stemcr.2017.12.022.

Rohatgi, R., Milenkovic, L., Scott, M.P., 2007. Patched1 Regulates Hedgehog Signaling at the

Primary Cilium. *Science* 317, 372–376. doi:10.1126/science.1139740.

Roth, J.G., Muench, K.L., Asokan, A., Mallett, V.M., Gai, H., Verma, Y., Weber, S., Charlton, C., Fowler, J.L., Loh, K.M., et al., 2020. 16p11.2 microdeletion imparts transcriptional alterations in human iPSC-derived models of early neural development. *eLife* 9, e58178. doi:10.7554/elife.58178.

Saade, M., Gonzalez-Gobartt, E., Escalona, R., Usieto, S., Martí, E., 2017. Shh-mediated centrosomal recruitment of PKA promotes symmetric proliferative neuroepithelial cell division. *Nat. Cell Biol.* 19, 493–503. doi:10.1038/ncb3512.

Sahara, S., O’Leary, D.D.M., 2009. Fgf10 Regulates Transition Period of Cortical Stem Cell Differentiation to Radial Glia Controlling Generation of Neurons and Basal Progenitors. *Neuron* 63, 48–62. doi:10.1016/j.neuron.2009.06.006.

Salisbury, J.L., 2004. Primary Cilia: Putting Sensors Together. *Curr. Biol.* 14, R765–R767. doi:10.1016/j.cub.2004.09.016.

Shimada, I.S., Somatilaka, B.N., Hwang, S.-H., Anderson, A.G., Shelton, J.M., Rajaram, V., Konopka, G., Mukhopadhyay, S., 2019. Derepression of sonic hedgehog signaling upon Gpr161 deletion unravels forebrain and ventricular abnormalities. *Dev. Biol.* 450, 47–62. doi:10.1016/j.ydbio.2019.03.011.

Singla, V., Reiter, J.F., 2006. The primary cilium as the cell’s antenna: signaling at a sensory organelle. *Science* 313, 629–633. doi:10.1126/science.1124534.

Steinman, K.J., Spence, S.J., Ramocki, M.B., Proud, M.B., Kessler, S.K., Marco, E.J., Green Snyder, L., D’Angelo, D., Chen, Q., Chung, W.K., et al., 2016. 16p11.2 deletion and duplication: Characterizing neurologic phenotypes in a large clinically ascertained cohort. *Am. J. Med. Genet.* 170, 2943–2955. doi:10.1002/ajmg.a.37820.

Tran, P.V., Haycraft, C.J., Besschetnova, T.Y., Turbe-Doan, A., Stottmann, R.W., Herron, B.J., Chesebro, A.L., Qiu, H., Scherz, P.J., Shah, J.V., et al., 2008. THM1 negatively modulates mouse sonic hedgehog signal transduction and affects retrograde intraflagellar transport in cilia. *Nat. Genet.* 40, 403–410. doi:10.1038/ng.105.

Urresti, J., Zhang, P., Moran-Losada, P., Yu, N.-K., Negraes, P.D., Trujillo, C.A., Antaki, D., Amar, M., Chau, K., Pramod, A.B., et al., 2021. Cortical organoids model early brain development disrupted by 16p11.2 copy number variants in autism. *Mol. Psychiatr.* 26, 7560–7580. doi:10.1038/s41380-021-01243-6.

Uzquiano, A., Cifuentes-Diaz, C., Jabali, A., Romero, D.M., Houllier, A., Dingli, F., Maillard, C., Boland, A., Deleuze, J.-F., Loew, D., et al., 2019. Mutations in the Heterotopia Gene *Eml1/EML1* Severely Disrupt the Formation of Primary Cilia. *Cell Rep.* 28, 1596–1611.e10. doi:10.1016/j.celrep.2019.06.096.

Walters, R.G., Jacquemont, S., Valsesia, A., de Smith, A.J., Martinet, D., Andersson, J., Falchi, M., Chen, F., Andrieux, J., Lobbens, S., et al., 2010. A new highly penetrant form of obesity due to deletions on chromosome 16p11.2. *Nature* 463, 671–675. doi:10.1038/nature08727.

Weijman, J.F., Vuolo, L., Shak, C., Pugnetti, A., Mukhopadhyay, A.G., Hodgson, L.R., Heesom, K.J., Roberts, A.J., Stephens, D.J., 2024. Roles for CEP170 in cilia function and dynein-2 assembly. *J. Cell Sci.* 137, jcs261816. doi:10.1242/jcs.261816.

Weiss, L.A., Shen, Y., Korn, J.M., Arking, D.E., Miller, D.T., Fossdal, R., Saemundsen, E., Stefansson, H., Ferreira, M.A.R., Green, T., et al., 2008. Association between microdeletion and microduplication at 16p11.2 and autism. *N. Engl. J. Med.* 358, 667–675. doi:10.1056/nejmoa075974.

Willaredt, M.A., Hasenpusch-Theil, K., Gardner, H.A.R., Kitanovic, I., Hirschfeld-Warneken, V.C., Gojak, C.P., Gorgas, K., Bradford, C.L., Spatz, J., Wölfl, S., et al., 2008. A crucial role for primary cilia in cortical morphogenesis. *J. Neurosci.* 28, 12887–12900. doi:10.1523/jneurosci.2084-08.2008.

Yadav, S., Oses-Prieto, J.A., Peters, C.J., Zhou, J., Pleasure, S.J., Burlingame, A.L., Jan, L.Y., Jan, Y.-N., 2017. TAOK2 Kinase Mediates PSD95 Stability and Dendritic Spine Maturation through Septin7 Phosphorylation. *Neuron* 93, 379–393. doi:10.1016/j.neuron.2016.12.006.

Zufferey, F., Sherr, E.H., Beckmann, N.D., Hanson, E., Maillard, A.M., Hippolyte, L., Macé, A., Ferrari, C., Kutalik, Z., Andrieux, J., et al., 2012. A 600 kb deletion syndrome at 16p11.2 leads to energy imbalance and neuropsychiatric disorders. *J. Med. Genet.* 49, 660–668. doi:10.1136/jmedgenet-2012-101203.

Chapter 4: TAOK2 ASD-associated mutations differentially affect catalytic activity and localization of TAOK2

Abstract

The autism risk gene thousand and one amino acid kinase 2 (TAOK2) encodes a serine/threonine kinase important for several aspects of neurodevelopment including axon elongation, dendritic branching, and spine/synapse formation. We recently discovered that TAOK2 is an endoplasmic reticulum (ER)-microtubule (MT) tether. Despite its importance in neuronal development and strong disease association, the molecular mechanisms underlying the role of TAOK2 in neurodevelopment and its biological function as an ER-MT tether are not understood. Here, we discovered that autism spectrum disorder (ASD)-associated TAOK2 mutations differentially affect its subcellular localization and catalytic activity in TAOK2 knockout (KO) HEK293T cells. Further, we generated a human TAOK2 KO induced pluripotent stem cell (hiPSC) line, which was differentiated into neural progenitor cells (NPCs). We found similar effects on subcellular localization in TAOK2 KO NPCs. Our results suggest that the role of TAOK2 in neurodevelopment and autism may be mediated through several different molecular mechanisms.

Introduction

Thousand and one amino acid kinase 2 (TAOK2) is an autism susceptibility gene (1–3) and regulates various neurodevelopmental processes implicated in autism spectrum disorder (ASD) (3–10). Reduced expression or absence of TAOK2 perturbs axogenesis (4) and decreases dendritic branching (3, 4) while increasing filopodia and shaft synapses (3–6).

TAOK2 consists of two spliced isoforms, α and β , differing in their C-terminal regulatory domains. Mutations in *TAOK2* have been identified in ASD patients through genetic sequencing (3). These mutations are not localized to specific domains of the protein, with some shared between the two isoforms and the others specific to each isoform. The two isoforms share the first 744 amino acids which comprise a kinase domain and two coiled coiled domains. We have previously identified that the C-terminal end of the canonical isoform TAOK2 α also contains six transmembrane domains, an amphipathic helix domain, and a microtubule binding domain (11). With six transmembrane domains along with amphipathic helix, TAOK2 α localizes to endoplasmic reticulum (ER) in a punctate pattern rather than throughout the entire ER membrane (11). Further, together with the MT binding domain, TAOK2 α functions to tether ER to MTs (11). Loss of TAOK2 disrupts the interaction between ER and MTs (11), which is essential for regulation of ER dynamics and neurodevelopment (12, 13). Given the importance of TAOK2 in neurodevelopment and strong association of *TAOK2* mutations with neurodevelopmental disorders, characterization of *TAOK2* mutations in its role as an ER-MT tether and of its kinase function is necessary to understand the molecular mechanism through which TAOK2 contributes to neurodevelopment and disease. Here, we utilized an immunoprecipitation kinase assay and confocal microscopy to assess changes in catalytic activity of the protein and localization.

Materials and Methods

Generation of TAOK2 knockout cell line

TAOK2 knockout HEK293T and AICS0032 derived NPC lines were generated using CRISPR/Cas9 genome editing as described previously (11, 14). Guides were designed using Synthego guide design tool (<https://www.synthego.com>) to target coding exon 2 for HEK293T

cells and exon 4 for NPCs. Guides were cloned into plasmid CrisprV2pSpCas9(BB)-2A-Puro (PX459) V2.0 (Addgene Plasmid #62988) with Puromycin resistance. Cells were passaged in single cell suspension and plated at 50% confluence. Cultures were then transfected Lipofectamine™ 2000 Transfection reagent (Invitrogen) and 2mg of guide RNA used per KO line. Cells were then selected with Puromycin for 48hr to select for transfected cells. Edited cells were expanded into single cell colonies. Genomic DNA was extracted and the region around the cutting site was PCR amplified to send for sequencing. Successful TAOK2 knockout clones were confirmed by Sanger sequencing analysis, and absence of encoded protein was validated using western blot. TAOK2 KO lines were confirmed by karyotyping for absence of large chromosomal alterations.

Immunoprecipitation kinase assay

HEK293T cells were grown in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin (Thermo Fisher Scientific). Expression constructs of sfGFP-tagged TAOK2 α ASD mutations (2 μ g) were transfected with JetOptimus, following the manufacturer's instructions. After 24 h post-transfection, cells were collected and incubated in HKT lysis buffer (25 mM HEPES pH 7.2, 150mM KCl, 1% Triton X-100, 2 mM DTT, and 1X protease inhibitor (Roche, cOmplete™ EDTA free)) for 30 min on ice prior to homogenization with a 25-gauge syringe needle. Pierce™ Protein G Agarose beads (Thermo Fisher Scientific) were washed with HKT buffer thrice. The supernatant was collected from cell lysates through centrifugation at 800g for 5 min, precleared with Protein G Agarose beads for 30 min, and immunoprecipitated with the GFP antibody overnight at 4°C. Beads were washed with HKT buffer twice, incubated with high salt HKT buffer (25 mM HEPES pH 7.2, 1M NaCl, 1%

Triton X-100, 2 mM DTT, and 1X protease inhibitor (Roche, cOmplete™ EDTA free)) for 10 min, and washed with HK buffer (25 mM HEPES, 150mM KCl, 2 mM DTT, and 1X protease inhibitor (Roche, cOmplete™ EDTA free)) twice and then with kinase buffer (20 mM Tris HCl pH 7.5, 10 mM MgCl₂, 1 mM DTT, 1X protease inhibitor (Roche, cOmplete™ EDTA free)) twice at 4°C. Kinase assay was performed by incubating the beads with the kinase buffer, 1 mM ATP, and 10X phosphatase inhibitor (Thermo Fisher Scientific, Halt) at 30°C for 45 min at 920 rpm. To assay for phosphorylation of Septin7 by TAO kinases, purified GST-tagged Sept7 C-terminal tail (321–438 amino acids) was also added to the reaction. Samples were prepared by adding NuPAGE™ LDS sample buffer (Thermo Fisher Scientific) containing 125 mM DTT, heating for 10 min at 95°C, and centrifuging at 5,000g for 5 min. Samples were run on NuPAGE™ 4–12% bis-tris polyacrylamide gels (Thermo Fisher Scientific) with NuPAGE™ MOPS running buffer (Thermo Fisher Scientific) at 165 V for 20 min and then at 175 V for 50 min. Gels were transferred to Immobilon-P membrane at 100 V for 1 h. The blots were blocked with 5% BSA blocking buffer and probed with phospho-TAO2 (S181) and phospho-Sept7 (T426) antibodies at 1:500 dilution overnight at 4°C followed by 3 h incubation with HRP-conjugated secondary antibody at 1:5,000 dilution at room temperature. The kinase activity was quantified by normalizing phospho-TAOK2 signal intensity to that of the GFP signal and phospho-Sept7 signal intensity to that of the GST signal.

Induced pluripotent stem cell culture and neuronal differentiation

TAOK2 KO iPSC line was differentiated into NPCs as reported previously (15). Stem cells were treated with 1U/mL Dispase, lifted using a cell scraper, resuspended in mTeSR media (STEMCELL Technologies) containing 10μM ROCK Inhibitor (Selleck Chemical), and then

transferred to uncoated T25 flasks for embryoid body (EB) formation. After 24-48 hours (Day 0), EBs were gravity settled and transferred to new uncoated T25 flasks in neural media (DMEM/F12 (Gibco), 1% N-2 supplement (Gibco), 1% Non-essential amino acids (Gibco), 2 μ g/mL Heparin, 1% Penicillin/Streptomycin [Gibco]) containing small molecule TGF- β /SMAD inhibitors, SB431542 (5 μ M; Peprotech) and LDN193189 (0.25 μ M; Peprotech). On Day 3, EBs were transferred to Matrigel (1:50 dilution; Corning)-coated 6 well plates in fresh neural media with SB and LDN. Media was changed with neural media without SB and LDN every other day or as needed until Day 11 for formation of neural rosettes. On Day 11, neuroepithelia were mechanically lifted using cell scraper and transferred to uncoated T25 flasks in neural media. Neurospheres were fed with neural media every other day, or as needed, until Day 25. On Day 25, neurospheres were plated onto Matrigel-coated plates in STEMDiff™ Neural Progenitor Media (STEMCELL Technologies) and NPCs were obtained.

Immunocytochemistry and transfection

HEK293T cells and NPCs grown on coverslips were washed with phosphate buffer saline (PBS) once, fixed with 4% paraformaldehyde (PFA) and 4% sucrose solution for 20 minutes at room temperature, and then washed thrice with PBS. Fixed HEK293T cells and NPCs were then incubated with blocking buffer (10% Normal Goat Serum, 200mM Glycine pH 7.4, 0.25% Triton X-100 in PBS) for one hour at room temperature. Cells were incubated with primary antibodies in blocking buffer overnight at 4°C, followed by six 5-minute PBS washes and incubation with secondary antibodies at 1:1000 dilution in blocking buffer overnight at 4°C. DAPI was included in the secondary antibody mixture at 1:2000 dilution. Coverslips were washed six to ten times with PBS and mounted on slides with Fluoromount-G (Electron Microscopy Sciences).

For transfection, HEK293T cells and NPCs were transfected using Lipofectamine™ Stem or 2000 Transfection Reagent (Invitrogen) according to the manufacturer's protocol.

Microscopy and image analyses

Imaging was performed on a Nikon Ti2 Eclipse-CSU-X1 confocal spinning disk microscope equipped with four laser lines (405nm, 488nm, 561nm, 670nm) and an sCMOS Andor camera for image acquisition. The microscope was caged within the OkoLab environmental control setup enabling temperature and CO₂ control during live imaging. Imaging was performed using Nikon 1.49 100x Apo 60X or 40X oil objective lenses. All image analyses and quantification of fluorescence intensity was performed using Fiji ImageJ2 version2.3.

Results

ASD-associated TAOK2 α mutations, except A135P, do not affect kinase activity

ASD-associated TAOK2 α mutations span throughout different domains within the entire protein, some of which are shared between both isoforms (Figure 1A, B). A135P mutation is the only mutation within the kinase domain and was previously shown to render TAOK2 α catalytically dead (3). However, it is unclear whether other ASD-associated mutations also disrupt the kinase activity of the protein. We therefore performed in vitro kinase assay in HEK293T cells to test whether the ASD-associated TAOK2 α mutations affect the catalytic activity. TAOK2 KO HEK293T cells were generated using CRISPR gene editing system to assess the impact of the ASD mutations without the potential rescue effect from endogenous TAOK2 α present in the wildtype cells. Briefly, TAOK2KO HEK293T cells expressing GFP tagged TAOK2 α WT or

indicated ASD mutant were pulled down using a GFP antibody and incubated with purified GST-Septin 7, a known substrate of TAOK2 (6), along with ATP. The kinase activity was assessed by measuring levels of phosphorylated S181 residue (pS181) normalized to GFP as a readout for autophosphorylation and phosphorylated Septin 7 (pSept7) normalized to GST (Sept7) as a readout for substrate phosphorylation. Consistent with the previous report, we found A135P to be kinase dead (Figure 1C-E). Interestingly, no other ASD mutations significantly impacted the catalytic activity of TAOK2 α (Figure 1C-E).

TAOK2 α truncated mutations change the localization of the protein

Next, we wondered whether the localization of TAOK2 α on ER is disrupted by the ASD mutations. We have previously shown that a kinase-dead mutation K57A leads to increased ER-MT tethering, with TAOK2 α exhibiting more of a tubule like localization pattern (11). Similarly, in TAOK2 KO HEK293T cells, a kinase-dead ASD associated mutation, A135P also led to increased localization of TAOK2 α on microtubule bundles (Figure 1F). Two truncation mutations, Q622* and L1030Wfs*3, are missing key domains of the protein. Q622* mutation completely lacks transmembrane domains and thereafter, whereas L1030Wfs*3 maintains four out of the six transmembrane domains but lacks the amphipathic helix that allows localization to specific subdomains within the ER membranes (Figure 1A, B). As expected, these two mutations drastically changed the distribution of TAOK2 α , to completely cytoplasmic localization and distribution across entire ER membranes rather than specific subdomains respectively (Figure 1F). Other missense mutations did not affect the TAOK2 α localization. We then tested whether the similar changes persist in a more relevant system for studying ASD. TAOK2 KO induced pluripotent stem cell (hiPSC) line was generated from AICS0032 iPSCs using Cas9 and guide

RNA targeting exon 4 and differentiated into dorsal forebrain neural progenitor cells (NPCs) as previously described (14). Consistent with the findings from TAOK2 KO HEK293T cells, WT TAOK2 α was present on the ER membrane in a punctate manner, while A135P mutation led to microtubule like localization of TAOK2 α (Figure 1G). Q622* was completely cytoplasmic as expected, and L1030Wfs*3 was present on the entire ER membrane (Figure 1G).

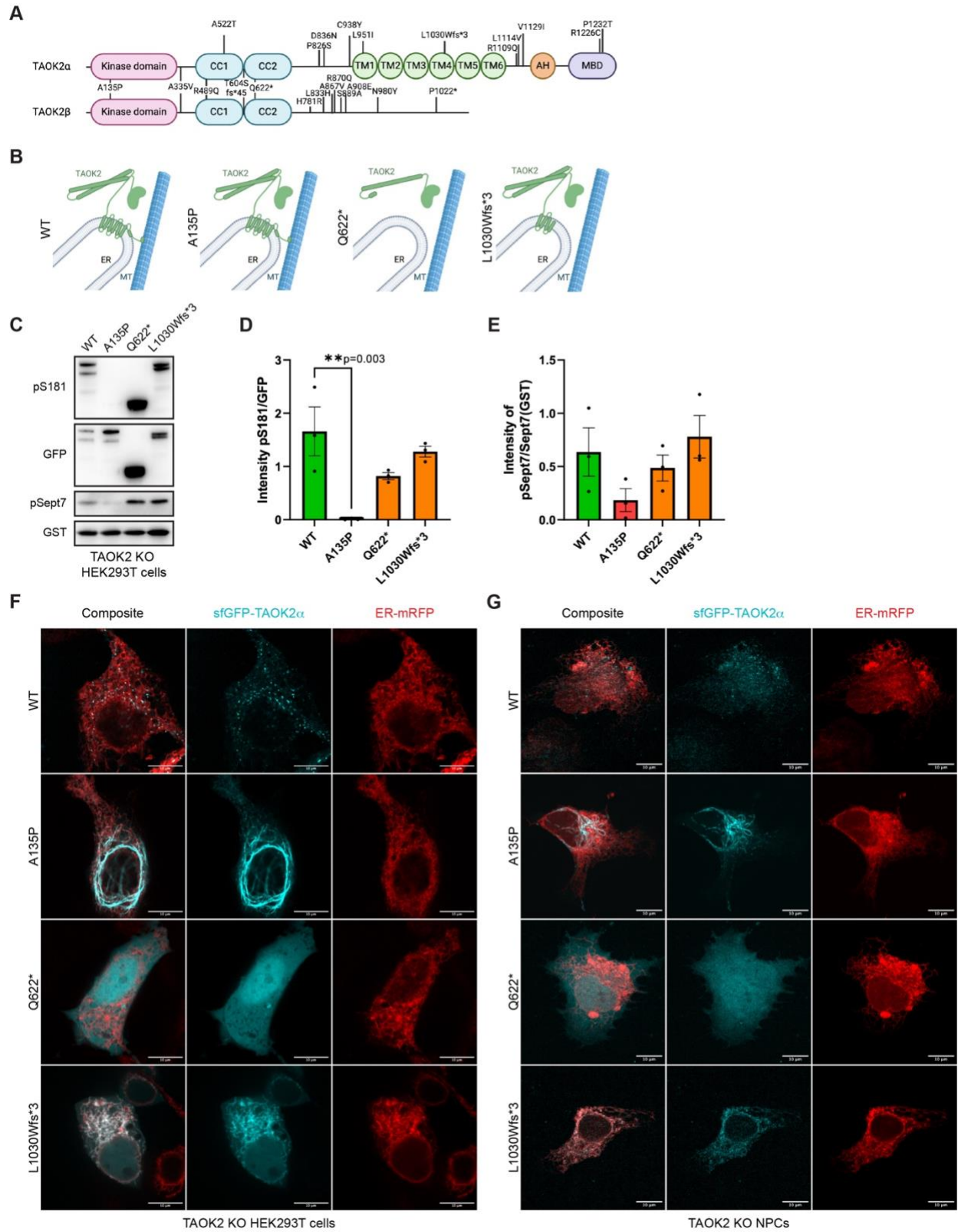


Figure 4. 1. Characterization of ASD associated TAOK2 mutations.

(A) Schematic of ASD associated mutations found in TAOK2 α and TAOK2 β . CC: Coiled coiled domain. TM: Transmembrane domain. AH: Amphipathic helix domain. MBD: Microtubule binding domain. **(B)** Schematic of TAOK2 α WT, A135P, Q622*, and L1030Wfs*3 mutant. **(C)** Western blot of kinase activity of sfGFP-TAOK2 α WT, A135P, Q622*, or L1030Wfs*3 expressed in TAOK2 KO HEK293T cells, immunoprecipitated with GFP antibody, and incubated with purified GST-Sept7 C-terminal tail (321-438 amino acids) for an in vitro kinase reaction. TAOK2 autophosphorylation was determined by probing for phosphorylated S181 residue (pS181), and substrate phosphorylation was determined by probing for phosphorylated Sept7 (pSept7). **(D)** Quantification of pS181 band intensity normalized to GFP band intensity. Error bars: SEM. **(E)** Quantification of pSept7 band intensity normalized to GST. Error bars: SEM. **(F, G)** Representative confocal images of TAOK2 KO HEK293T cells **(F)** and NPCs **(G)** transfected with sfGFP-TAOK2 α WT or indicated ASD-associated mutant constructs (cyan) along with ER-mRFP (red). Scale bar is 10 μ m.

Discussion

We found that different ASD-associated TAOK2 α mutations differentially affect the kinase activity and subcellular localization of TAOK2. Only A135P mutation within the kinase domain altered the catalytic activity of TAOK2 α , rendering it catalytically dead. A135P mutation also affected the localization of TAOK2 α in both HEK293T cells and NPCs, presumably increasing ER-MT tethering consistent with the previous finding with a kinase dead mutation, K57A (11). Additionally, two truncation mutations, Q622* and L1030Wfs*3, altered the localization. ER localization was completely abolished in cells expressing the Q622* mutation, whereas

L1030Wfs*3 expressing cells still maintained ER localization but punctate localization within the specific subdomains was lost. However, the other missense mutations did not significantly impact the kinase activity or the localization. This suggests that changes in kinase activity or cellular localization are not the sole mechanisms underlying pathophysiology of TAOK2-associated ASD. Further studies investigating what other molecular pathways or cellular processes the other ASD mutations affect will be an important future direction in understanding how TAOK2 contributes to ASD. Importantly, this study only focused on TAOK2 α mutations, and therefore, future research should also investigate the impact of the TAOK2 β mutations on kinase function, subcellular localization, as well as other functions and processes.

References

1. R. K. Yuen, D. Merico, M. Bookman, J. L. Howe, B. Thiruvahindrapuram, R. V. Patel, J. Whitney, N. Deflaux, J. Bingham, Z. Wang, G. Pellecchia, J. A. Buchanan, S. Walker, C. R. Marshall, M. Uddin, M. Zarrei, E. Deneault, L. D'Abate, A. J. S. Chan, S. Koyanagi, T. Paton, S. L. Pereira, N. Hoang, W. Engchuan, E. J. Higginbotham, K. Ho, S. Lamoureux, W. Li, J. R. MacDonald, T. Nalpathamkalam, W. W. L. Sung, F. J. Tsoi, J. Wei, L. Xu, A.-M. Tasse, E. Kirby, W. Van Etten, S. Twigger, W. Roberts, I. Drmic, S. Jilderda, B. M. Modi, B. Kellam, M. Szego, C. Cytrynbaum, R. Weksberg, L. Zwaigenbaum, M. Woodbury-Smith, J. Brian, L. Senman, A. Iaboni, K. Doyle-Thomas, A. Thompson, C. Chrysler, J. Leef, T. Savion-Lemieux, I. M. Smith, X. Liu, R. Nicolson, V. Seifer, A. Fedele, E. H. Cook, S. Dager, A. Estes, L. Gallagher, B. A. Malow, J. R. Parr, S. J. Spence, J. Vorstman, B. J. Frey, J. T. Robinson, L. J. Strug, B. A. Fernandez, M. Elsabbagh, M. T. Carter, J. Hallmayer, B. M. Knoppers, E. Anagnostou, P. Szatmari, R. H. Ring, D. Glazer, M. T. Pletcher, S. W. Scherer, Whole genome sequencing resource identifies 18 new candidate genes for autism spectrum disorder. *Nat Neurosci* **20**, 602–611 (2017).
2. E. T. Lim, M. Uddin, S. De Rubeis, Y. Chan, A. S. Kamumbu, X. Zhang, A. M. D'Gama, S. N. Kim, R. S. Hill, A. P. Goldberg, C. Poultney, N. J. Minshew, I. Kushima, B. Aleksic, N. Ozaki, M. Parellada, C. Arango, M. J. Penzol, A. Carracedo, A. Kolevzon, C. M. Hultman, L. A. Weiss, M. Fromer, A. G. Chiocchetti, C. M. Freitag, Autism Sequencing Consortium, G. M. Church, S. W. Scherer, J. D. Buxbaum, C. A. Walsh, Rates, distribution and implications of postzygotic mosaic mutations in autism spectrum disorder. *Nat Neurosci* **20**, 1217–1224 (2017).
3. M. Richter, N. Murtaza, R. Scharrenberg, S. H. White, O. Johanns, S. Walker, R. K. C. Yuen, B. Schwanke, B. Bedürftig, M. Henis, S. Scharf, V. Kraus, R. Dörk, J. Hellmann, Z. Lindenmaier, J. Ellegood, H. Hartung, V. Kwan, J. Sedlacik, J. Fiehler, M. Schweizer, J. P. Lerch, I. L. Hanganu-Opatz, F. Morellini, S. W. Scherer, K. K. Singh, F. Calderon de Anda, Altered TAOK2 activity causes autism-related neurodevelopmental and cognitive abnormalities through RhoA signaling. *Mol Psychiatry* **24**, 1329–1350 (2019).
4. F. C. de Anda, A. L. Rosario, O. Durak, T. Tran, J. Gräff, K. Meletis, D. Rei, T. Soda, R. Madabhushi, D. D. Ginty, A. L. Kolodkin, L.-H. Tsai, Autism spectrum disorder susceptibility gene TAOK2 affects basal dendrite formation in the neocortex. *Nat Neurosci* **15**, 1022–1031 (2012).
5. S. K. Ultanir, S. Yadav, N. T. Hertz, J. A. Oses-Prieto, S. Claxton, A. L. Burlingame, K. M. Shokat, L. Y. Jan, Y.-N. Jan, MST3 Kinase Phosphorylates TAO1/2 to Enable Myosin Va Function in Promoting Spine Synapse Development. *Neuron* **84**, 968–982 (2014).
6. S. Yadav, J. A. Oses-Prieto, C. J. Peters, J. Zhou, S. J. Pleasure, A. L. Burlingame, L. Y. Jan, Y.-N. Jan, TAOK2 Kinase Mediates PSD95 Stability and Dendritic Spine Maturation through Septin7 Phosphorylation. *Neuron* **93**, 379–393 (2017).
7. S. Byeon, B. Werner, R. Falter, K. Davidsen, C. Snyder, S.-E. Ong, S. Yadav, Proteomic Identification of Phosphorylation-Dependent Septin 7 Interactors that Drive Dendritic Spine

Formation. *Front. Cell Dev. Biol.* **10**, 836746 (2022).

8. N. Murtaza, A. A. Cheng, C. O. Brown, D. P. Meka, S. Hong, J. A. Uy, J. El-Hajjar, N. Pipko, B. K. Unda, B. Schwanke, S. Xing, B. Thiruvahindrapuram, W. Engchuan, B. Trost, E. Deneault, F. Calderon de Anda, B. W. Doble, J. Ellis, E. Anagnostou, G. D. Bader, S. W. Scherer, Y. Lu, K. K. Singh, Neuron-specific protein network mapping of autism risk genes identifies shared biological mechanisms and disease-relevant pathologies. *Cell Reports* **41**, 111678 (2022).
9. R. Scharrenberg, M. Richter, O. Johanns, D. P. Meka, T. Rücker, N. Murtaza, Z. Lindenmaier, J. Ellegood, A. Naumann, B. Zhao, B. Schwanke, J. Sedlacik, J. Fiehler, I. L. Hanganu-Opatz, J. P. Lerch, K. K. Singh, F. C. de Anda, TAOK2 rescues autism-linked developmental deficits in a 16p11.2 microdeletion mouse model. *Mol Psychiatry* **27**, 4707–4721 (2022).
10. S. Yasuda, H. Tanaka, H. Sugiura, K. Okamura, T. Sakaguchi, U. Tran, T. Takemiya, A. Mizoguchi, Y. Yagita, T. Sakurai, E. M. De Robertis, K. Yamagata, Activity-Induced Protocadherin Arcadlin Regulates Dendritic Spine Number by Triggering N-Cadherin Endocytosis via TAO2 β and p38 MAP Kinases. *Neuron* **56**, 456–471 (2007).
11. K. Nourbakhsh, A. A. Ferreccio, M. J. Bernard, S. Yadav, TAOK2 is an ER-localized kinase that catalyzes the dynamic tethering of ER to microtubules. *Developmental Cell* **56**, 3321–3333.e5 (2021).
12. T. Cui-Wang, C. Hanus, T. Cui, T. Helton, J. Bourne, D. Watson, K. M. Harris, M. D. Ehlers, Local Zones of Endoplasmic Reticulum Complexity Confine Cargo in Neuronal Dendrites. *Cell* **148**, 309–321 (2012).
13. G. G. Fariás, A. Fréal, E. Tortosa, R. Stucchi, X. Pan, S. Portegies, L. Will, M. Altelaar, C. C. Hoogenraad, Feedback-Driven Mechanisms between Microtubules and the Endoplasmic Reticulum Instruct Neuronal Polarity. *Neuron* **102**, 184–201.e8 (2019).
14. A. Ferreccio, S. Byeon, M. Cornell, J. Osés-Prieto, A. Deshpande, L. A. Weiss, A. Burlingame, S. Yadav, TAOK2 Drives Opposing Cilia Length Deficits in 16p11.2 Deletion and Duplication Carriers. bioRxiv [Preprint] (2024). <https://doi.org/10.1101/2024.10.07.617069>.
15. A. Deshpande, S. Yadav, D. Q. Dao, Z.-Y. Wu, K. C. Hokanson, M. K. Cahill, A. P. Wiita, Y.-N. Jan, E. M. Ullian, L. A. Weiss, Cellular Phenotypes in Human iPSC-Derived Neurons from a Genetic Model of Autism Spectrum Disorder. *Cell Reports* **21**, 2678–2687 (2017).

Chapter 5: Discussion and future directions

This body of work has demonstrated multifaceted roles of TAOK2 during neurodevelopment. TAOK2 is important for various stages of neurodevelopment, regulating axon elongation, dendritic branching, neuronal migration, and spine and synapse formation (1–8). TAOK2 is located within the 16p11.2 genomic region, duplication and deletion of which are associated with neurodevelopmental disorders (9, 10). Mutations in TAOK2 are also associated with ASD (2). Previous work identified TAOK2 α as an ER-localized kinase that tethers ER to microtubules (11). Within the ER, TAOK2 α localizes to distinct subdomains; however, the precise identity of these subdomains is unclear. Its spliced variant, TAOK2 β was previously shown to be important for protein translation (12) and synaptic function (6, 8); however, its precise location within the cell is unknown.

Chapter 1 provides a comprehensive summary of the current literature on TAOK2 functions and its dysfunction in neurodevelopmental disorders (13). Chapter 2 further elucidates molecular mechanisms underlying spine maturation mediated by TAOK2, showing that interaction with 14-3-3 proteins stabilizes the phosphorylation of Septin 7 by TAOK2 thereby maintaining spine maturation (5). Chapter 3 identifies a new function of TAOK2 in regulating the primary cilia length, trafficking, and SHH signaling and attributes this as a potential mechanism underlying pathophysiology of 16p11.2 CNV (14). Chapter 4 reveals that while several ASD-associated TAOK2 α mutations lead to impaired kinase activity and/or subcellular localization, the remaining missense mutations do not affect either, suggesting TAOK2 may contribute to ASD via other molecular or cellular mechanisms. These mutations could potentially impact the microtubule

dynamics and/or proteins that interact with TAOK2, ultimately altering their functions. Further studies are required to dissect the underlying mechanism of TAOK2-associated ASD.

While this work greatly advances our understanding of pleiotropic functions of TAOK2, the question remains as to how these functions work together to regulate different stages of neurodevelopment and how dysfunction in these processes contribute to neurodevelopmental disorders. How exactly TAOK2 α , an ER-microtubule tether, contributes to a plethora of functions requires further investigation. For instance, it is unclear whether and how TAOK2 α directly associates with Septin 7 and translocates Septin 7 from the dendritic shaft to the dendritic spine along the microtubule network. TAOK2 β , on the other hand, may be important for actin dynamics through its association with RhoA (1, 2). Whether the two isoforms interact and form a link between microtubules and actin cytoskeleton remains an open question. A major limitation in the current work on TAOK2 is the use of TAOK2 KO 2D cell lines or mouse cell lines where distinguishing isoform-specific contribution is difficult. Future studies utilizing cortical organoids either derived from patient cell lines or genetically modified to harbor ASD-associated mutations will provide further insights in a more physiologically relevant system. Whether and how TAOK2 regulates cellular processes such as NPC proliferation, neuronal differentiation, and cell cycle remain to be investigated. The use of cortical organoids will also allow better understanding of temporal dynamics of the isoform specific function as previous studies have suggested that TAOK2 α may be important for overall neurodevelopment from early progenitor stage to adulthood whereas TAOK2 β may be more critical for the later stage of the development (1–8). Cell-type expression profiles of TAOK2 isoforms remain to be uncovered. It is unknown whether TAOK2

is expressed in non-neuronal cell types and how its role in these cell types contribute to neurodevelopment.

References

1. F. C. de Anda, A. L. Rosario, O. Durak, T. Tran, J. Gräff, K. Meletis, D. Rei, T. Soda, R. Madabhushi, D. D. Ginty, A. L. Kolodkin, L.-H. Tsai, Autism spectrum disorder susceptibility gene TAOK2 affects basal dendrite formation in the neocortex. *Nat Neurosci* **15**, 1022–1031 (2012).
2. M. Richter, N. Murtaza, R. Scharrenberg, S. H. White, O. Johanns, S. Walker, R. K. C. Yuen, B. Schwanke, B. Bedürftig, M. Henis, S. Scharf, V. Kraus, R. Dörk, J. Hellmann, Z. Lindenmaier, J. Ellegood, H. Hartung, V. Kwan, J. Sedlacik, J. Fiehler, M. Schweizer, J. P. Lerch, I. L. Hanganu-Opatz, F. Morellini, S. W. Scherer, K. K. Singh, F. Calderon de Anda, Altered TAOK2 activity causes autism-related neurodevelopmental and cognitive abnormalities through RhoA signaling. *Mol Psychiatry* **24**, 1329–1350 (2019).
3. S. K. Ultanir, S. Yadav, N. T. Hertz, J. A. Oses-Prieto, S. Claxton, A. L. Burlingame, K. M. Shokat, L. Y. Jan, Y.-N. Jan, MST3 Kinase Phosphorylates TAO1/2 to Enable Myosin Va Function in Promoting Spine Synapse Development. *Neuron* **84**, 968–982 (2014).
4. S. Yadav, J. A. Oses-Prieto, C. J. Peters, J. Zhou, S. J. Pleasure, A. L. Burlingame, L. Y. Jan, Y.-N. Jan, TAOK2 Kinase Mediates PSD95 Stability and Dendritic Spine Maturation through Septin7 Phosphorylation. *Neuron* **93**, 379–393 (2017).
5. S. Byeon, B. Werner, R. Falter, K. Davidsen, C. Snyder, S.-E. Ong, S. Yadav, Proteomic Identification of Phosphorylation-Dependent Septin 7 Interactors that Drive Dendritic Spine Formation. *Front. Cell Dev. Biol.* **10**, 836746 (2022).
6. N. Murtaza, A. A. Cheng, C. O. Brown, D. P. Meka, S. Hong, J. A. Uy, J. El-Hajjar, N. Pipko, B. K. Unda, B. Schwanke, S. Xing, B. Thiruvahindrapuram, W. Engchuan, B. Trost, E. Deneault, F. Calderon de Anda, B. W. Doble, J. Ellis, E. Anagnostou, G. D. Bader, S. W. Scherer, Y. Lu, K. K. Singh, Neuron-specific protein network mapping of autism risk genes identifies shared biological mechanisms and disease-relevant pathologies. *Cell Reports* **41**, 111678 (2022).
7. R. Scharrenberg, M. Richter, O. Johanns, D. P. Meka, T. Rücker, N. Murtaza, Z. Lindenmaier, J. Ellegood, A. Naumann, B. Zhao, B. Schwanke, J. Sedlacik, J. Fiehler, I. L. Hanganu-Opatz, J. P. Lerch, K. K. Singh, F. C. de Anda, TAOK2 rescues autism-linked developmental deficits in a 16p11.2 microdeletion mouse model. *Mol Psychiatry* **27**, 4707–4721 (2022).
8. S. Yasuda, H. Tanaka, H. Sugiura, K. Okamura, T. Sakaguchi, U. Tran, T. Takemiya, A. Mizoguchi, Y. Yagita, T. Sakurai, E. M. De Robertis, K. Yamagata, Activity-Induced Protocadherin Arcadlin Regulates Dendritic Spine Number by Triggering N-Cadherin Endocytosis via TAO2 β and p38 MAP Kinases. *Neuron* **56**, 456–471 (2007).
9. L. A. Weiss, Y. Shen, J. M. Korn, D. E. Arking, D. T. Miller, R. Fossdal, E. Saemundsen, H. Stefansson, M. A. R. Ferreira, T. Green, O. S. Platt, D. M. Ruderfer, C. A. Walsh, D. Altshuler, A. Chakravarti, R. E. Tanzi, K. Stefansson, S. L. Santangelo, J. F. Gusella, P. Sklar, B.-L. Wu, M.

J. Daly, Association between Microdeletion and Microduplication at 16p11.2 and Autism. *N Engl J Med* **358**, 667–675 (2008).

10. R. A. Kumar, S. KaraMohamed, J. Sudi, D. F. Conrad, C. Brune, J. A. Badner, T. C. Gilliam, N. J. Nowak, E. H. Cook, W. B. Dobyns, S. L. Christian, Recurrent 16p11.2 microdeletions in autism. *Human Molecular Genetics* **17**, 628–638 (2007).

11. K. Nourbakhsh, A. A. Ferreccio, M. J. Bernard, S. Yadav, TAO2 is an ER-localized kinase that catalyzes the dynamic tethering of ER to microtubules. *Developmental Cell* **56**, 3321–3333.e5 (2021).

12. M. Henis, T. Rücker, R. Scharrenberg, M. Richter, L. Baltussen, S. Hong, D. P. Meka, B. Schwanke, N. Neelagandan, D. Daaboul, N. Murtaza, C. Krisp, S. Harder, H. Schlüter, M. Kneussel, I. Hermans-Borgmeyer, J. de Wit, K. K. Singh, K. E. Duncan, F. C. de Anda, The autism susceptibility kinase, TAO2, phosphorylates eEF2 and modulates translation. *Science Advances* **10**, eadf7001 (2024).

13. S. Byeon, S. Yadav, Pleiotropic functions of TAO kinases and their dysregulation in neurological disorders. *Science Signaling* **17**, eadg0876 (2024).

14. A. Ferreccio, S. Byeon, M. Cornell, J. Oses-Prieto, A. Deshpande, L. A. Weiss, A. Burlingame, S. Yadav, TAO2 Drives Opposing Cilia Length Deficits in 16p11.2 Deletion and Duplication Carriers. bioRxiv [Preprint] (2024). <https://doi.org/10.1101/2024.10.07.617069>.