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14. ABSTRACT

The emergence of cerebral cortex occurs as a result of coordinated unfolding of neural progenitor formation, neurogenesis, neuronal migration, post-migratory neuronal differentiation and connectivity. Mutations in TSC1 or TSC2 lead to tuberous sclerosis complex (TSC) characterized by aberrant cortical development. Efficient intracellular response to extracellular signaling proteins is fundamental to coordinate the multitude of cellular events underlying the normal formation of cerebral cortex. Primary cilia, the microtubule-based, slender projections found on virtually every eukaryotic cell, including cortical progenitors and neurons are emerging as essential conveyors of signal transduction underlying major cell functions. Disrupted cilia function in humans results in profound cortical abnormalities and intellectual disabilities. However, the essential roles of primary cilia dysfunction in tuberous sclerosis complex (TSC) related cerebral cortical development are mostly unknown. Thus, a critical question to be answered is what role cilia function or dysfunction plays in the development TSC pathology in cerebral cortex. Here, we will explore (1) how primary cilia signaling in cortical progenitors and newborn cortical neurons regulates TSC1/2 expression and function, (2) how disruptions in this process may contribute to the emergence of TSC phenotype, and (3) how manipulation of cilia signaling could be used as a therapeutic avenue to ameliorate TSC deficits. Towards this goal, we aim to systematically define the functions of primary cilia in TSC models of the cerebral cortex using cilia-specific Arl13b signaling as a molecular model, delineate the cilia-dependent molecular mechanisms that regulate TSC1/2 functions, and identify the developmental disruptions underlying brain abnormalities in TSC. The goals of this project, delineating the role of cilia in the emergence of brain abnormalities underlying TSC, will provide transformative insights into the biological basis of TSC. These studies will help us decipher the cilia- related molecular cascades and neurodevelopmental pathways, whose disruptions are integrally related to the development of TSC, and will contribute towards devising optimal therapeutic strategies for this brain disorder.

15. SUBJECT TERMS None listed

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a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
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TABLE OF CONTENTS

1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

The aim of this proposal is to elucidate the mechanisms that underlie disrupted brain formation in tuberous sclerosis complex (TSC). Little is known about the cellular dysfunctions that lead to the emergence of tuberous sclerosis complex (TSC) brain phenotype. However, the spectrum of neurobehavioral defects associated with primary cilia dysfunction in humans, suggest that primary cilia in neurons play critical and specific roles in the formation and maturation of cerebral cortex. Therefore, defining the TSC1/2 related functions of primary cilia in neurons and progenitors during the construction of cerebral cortex will be essential to understand the pathogenic mechanisms that underlie tuberous sclerosis complex. This knowledge could help identify and rationalize novel targets for therapeutic interventions for TSC related brain disorders. Towards this goal, we used mice in which TSC1/2 genes are inhibited in distinct populations of neural cells under the control of a variety of cell- type specific promoters. These mice were used to understand how cortical progenitors and neurons and progenitors malform and differentiate during the emergence of TSC.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

TSC (Tuberous sclerosis complex), brain development, progenitors, interneurons.

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.

What were the major goals of the project?

The following are the three main aims of this work. Aim 1: How do primary cilia signaling in cortical progenitors and newborn cortical neurons regulate TSC1/2 expression and function? Aim 2: How do disruptions in cilia signaling contribute to the emergence of TSC phenotype?, and Aim 3: Determine if manipulation of cilia signaling can used be used as a therapeutic avenue to ameliorate TSC deficits.

What was accomplished under these goals?

The three main goals of this work are (1) How do primary cilia signaling in cortical progenitors and newborn cortical neurons regulate TSC1/2 expression and function? (2) How do disruptions in cilia signaling contribute to the emergence of TSC phenotype? and (3) Determine if manipulation of cilia signaling can used be used as a therapeutic avenue to ameliorate TSC deficits. Findings related to each of these major tasks are described below.

Aim 1: How do primary cilia signaling in cortical progenitors and newborn cortical neurons regulate TSC1/2 expression and function?

To examine if primary cilia affects TSC expression and function, we examined TSC expression in embryonic, Arl13b deficient brains with disrupted primary cilia functions. We noticed that neuronal laminar formation was disrupted and neurons formed ectopic tuber-like clusters in the cerebral wall of cilia mutants.

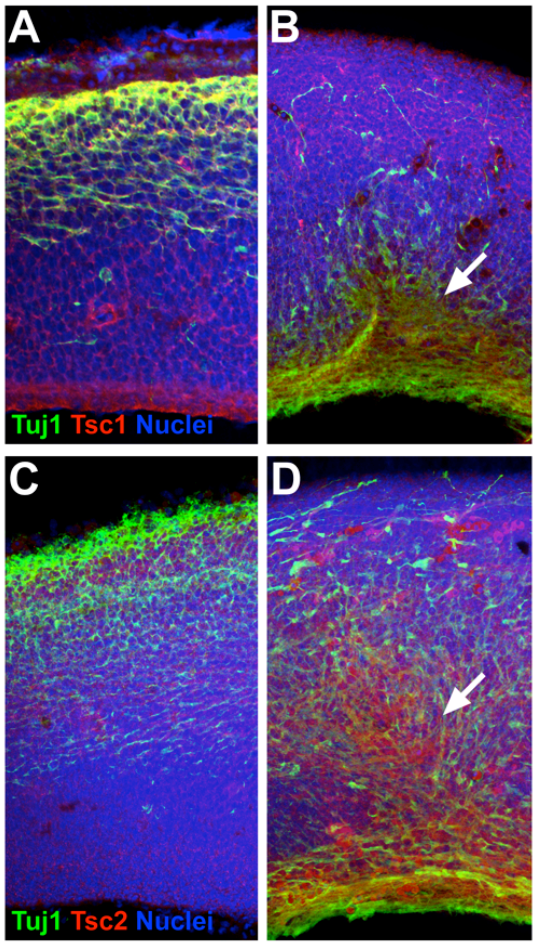


Figure 1. Expression of TSC1 and 2 in cortical tubers of Arl13b deficient (cilia mutant) brains. Control (A, C) and conditionally deleted, Arl13b deficient brains (B, D) were immunolabeled with anti-TSC1 (A, B) and TSC2 (C, D) antibodies. Both Tsc1 and 2 are prominently expressed in cilia mutant cortical tubers (arrow, C-D).

To examine patterns of TSC1 and 2 expression in these tubers, we immunolabeled control and mutant brain sections with anti-TSC1 and 2 antibodies. Both TSC1 and 2 were prominently expressed in the cilia mutant cortical tubers containing progenitors and neurons (Figure 1). These tubers in cilia mutants were marked by the differential, aberrant expression of the TSC proteins. These observations from studies outlined in Major Task 1 suggest that disruption of primary cilia functions affect appropriate TSC expression in the progenitors and neurons of developing brain.

Aim 2: How do disruptions in cilia signaling contribute to the emergence of TSC phenotype?

To examine cilia and TSC1/2's role in progenitor development, we generated and analyzed control ($TSC1^{Lox/+}$, $TSC2^{Lox/+}$, Emx1-Cre [Control]) and conditional null ($TSC1^{Lox/Lox}$, $TSC2^{Lox/Lox}$, Emx1-Cre [TSC1/2 cKO]) mice. Emx1-Cre enables TSC1/2 inactivation in cortical progenitors. We found that, deletion of TSC1 and 2 in cortical progenitors lead to increased mTORC1 signaling as indicated by enhanced S6 phosphorylation in the developing cerebral cortex (Figure 2. A-B). Further, TSC1/2 deletion lead to an increase in $Pax6^{+}$ basal progenitors (Figure 2. C-D), and increased length of $Arl13b^{+}$ progenitor cilia (Figure 2. E-F). Further, the organization of hippocampus is perturbed in TSC1/2 cKO. An increase in $Tbr2^{+}$ intermediate progenitors (Figure 2. G-H)

and disrupted layering was evident in TSC1/2 cKO. Importantly, TSC1/2 deletion led to altered basal/ apical progenitor balance in the cerebral cortex (Figure 3).

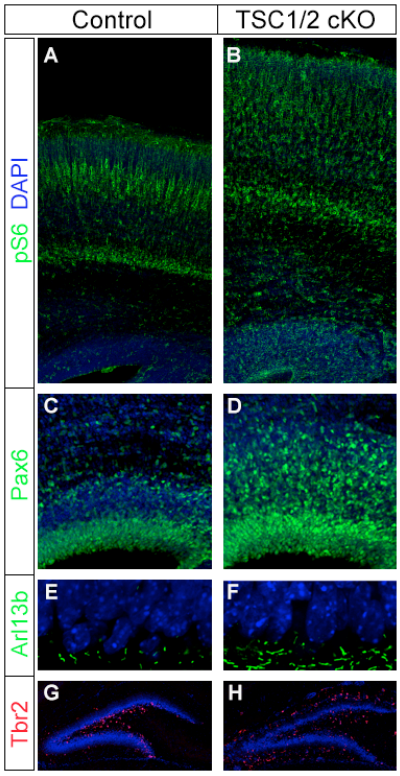


Figure 2. Altered mTORC1 signaling, progenitor population, and cilia development in TSC1/2 mutants.

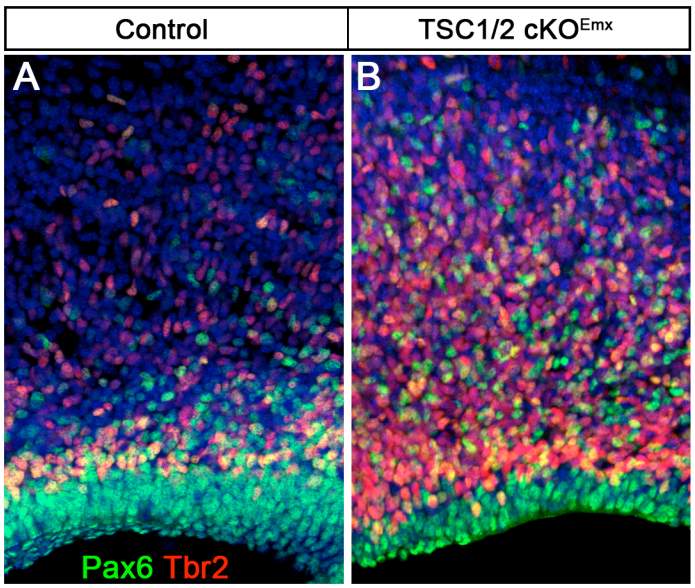


Figure 3. Altered apical/basal progenitor balance in TSC1/2 cKO cortex.

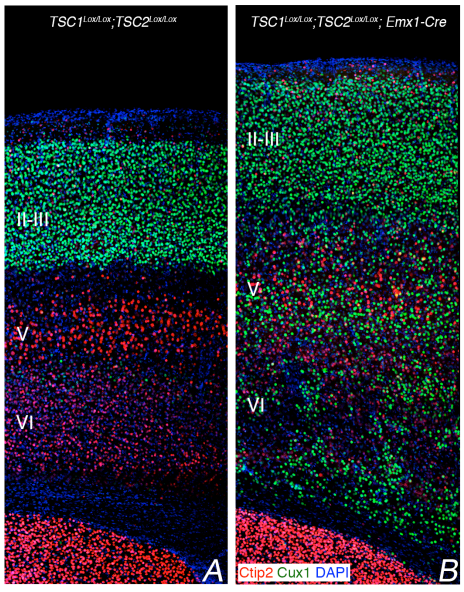


Figure 4. Increase in upper layer neurons in TSC1/2 cKO.

Further, deletion of TSC1 and 2 in cortical progenitors leads to disrupted neuronal placement and laminar organization of neurons in the developing cerebral cortex (Figure 4). In particular, an increase in Cux1+ upper layer neurons was evident. Upper layer neurons are thought to be essential for normal cognitive functions in humans. An increase in these neurons and the resultant changes in circuitry may contribute to the autism spectrum disorder phenotypes noticed in TSC patients.

Neuronal dysfunction, particularly that of interneurons is thought to be an important contributor to TSC pathology. To examine TSC1/2's role in interneuronal development and function, we generated control ($TSC1^{Lox/+}$, $TSC2^{Lox/+}$, Nkx2.1-Cre) and conditional null ($TSC1^{Lox/Lox}$, $TSC2^{Lox/Lox}$, Nkx2.1-Cre) mice. Nkx2.1-Cre inactivates in developing interneurons.

Interneuron specific deletion of TSC1 and 2 complex reveals a disruption in the characteristic architecture of majority of cortical interneurons (Parvalbumin$^+$[PV$^+$] and somatostatin$^+$ [SST$^+$]; Figures 5 and 6). Axo-dendritic architecture of TSC1/2 cKO interneurons is altered, with increased soma size (control: 183±4μm^2; TSC1/2 cKO:333±10μm^2), excessive dendritic branching and aberrant axon projection patterns (Figures 5 and 6). These defects in the TSC1/2 cKO interneurons suggest that TSC1/2 complex signaling is essential for emergence of appropriate interneuronal architecture and morphology.

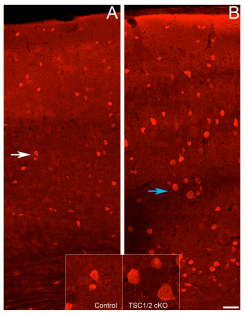


Figure 5. Interneuron specific deletion of TSC1 and 2 leads to an increase in the size of interneuronal soma. Interneurons in (A) control and (B) TSC1/2 cKO (Nkx2.1Cre; $TSC1^{Lox/Lox}$; $TSC2^{Lox/Lox}$) cortices (P30) were labeled with anti-parvalbumin antibodies. Interneuronal size is significantly increased in TSC1/2 cKO (arrows).

The morphological defects observed in TSC1/2 deficient PV^+ and SST^+ INs prompted us to examine their synaptic connections. Since PV^+ INs preferentially form perisomatic synapses, we examined the density of $tdTomato^+$ boutons of PV^+ INs around the soma of $NeuN^+$ cortical projection neurons (PNs) in control and TSC1/2 cKO cortex (P30). Compared to controls, density of $tdTomato^+$ or $parvalbumin^+$ perisomatic boutons (arrowheads, Figure 7) was significantly increased in TSC1/2 cKO INs (Figure 7). These results demonstrate that TSC1/2 deletion disrupts synaptic connectivity of INs.

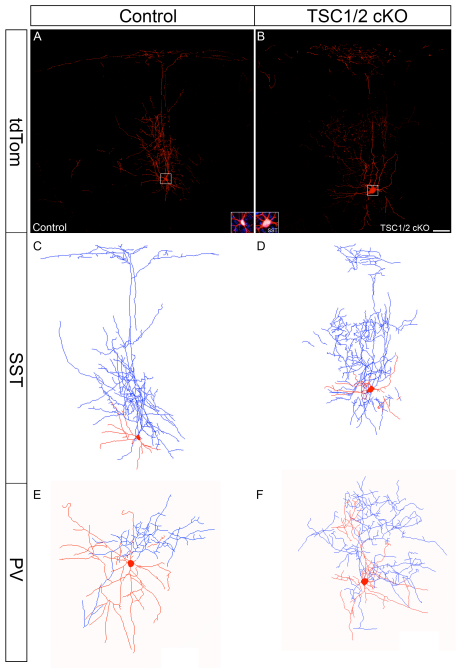


Figure 6. Altered interneuronal morphology in TSC1/2 cKO. Somatostatin positive Martinotti cells in layer 5 in control (A) and TSC1/2 cKO (B) cortex. TSC1/2 deletion leads to significant changes in cell soma size (outlined area) and drastically altered axo-dendritic architecture. Insets show somatostatin (white) expression. Reconstructed SST+ (C, D) and Parv+ (E, F) interneurons from control (C, E) and TSC1/2 cKO (D, F) cortices.

To assess the electrophysiological consequences of TSC1/2 deletion on interneuronal intrinsic excitability and synaptic innervation of downstream projection neurons, we performed patch-clamp recordings in the cortical slices from control and TSC1/2 cKO mice. The intrinsic excitability of TSC1/2 cKO GABA INs was greatly reduced as compared with control neurons and this was likely related to a reduction in input resistance (Figure 8A-D). Furthermore, compared to control littermates, recordings from projection neurons revealed an increase in mIPSC frequency and amplitude in TSC1/2 cKO mice, suggesting greater GABAergic synaptic innervation to these projection neurons (Figure 8E-G). The specific increase in the inhibitory synaptic input onto projection neurons of TSC1/2 cKO mice may lead to excitatory/inhibitory synaptic imbalance. Thus, consistent with the cellular characterization of TSC1/2 cKO IN morphology and synaptic connectivity, these electrophysiological data reveal a functional deficit in the excitability of TSC1/2 INs and altered synaptic innervation of downstream projection neurons.

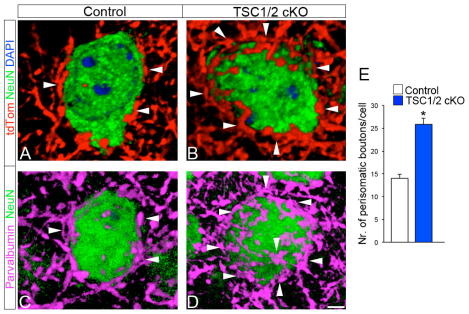


Figure 7. Increased $tdTom^+$ or $Parv^+$ perisomatic bouton density in control and TSC1/2 cKO cortices. Data shown are mean ± SEM. $^*P<0.05$ (Student's *t*-test).

Together, these studies (*as outlined in Major Task 2*) indicate altered cilia in TSC deficient developing neural cells. TSC deficiency leads to altered progenitor balance, upper layer neuronal organization, and interneuronal dysfunction during the assembly of cerebral cortex.

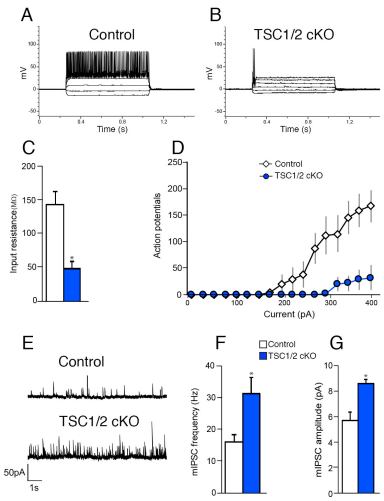


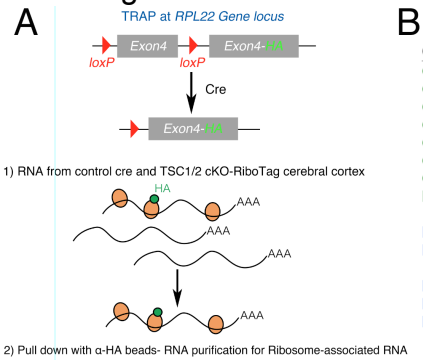
Figure 8. The functional impact of TSC1/2 deletion in interneurons. (A-B) Reduced excitability of TSC1/2 ckO INs. Representative voltage responses during both hyperpolarizing (minimum -100pA step) and depolarizing (maximum +400pA step) pulses, allowing quantification of basic membrane and action potential properties. (C) Reduced input resistance in TSC1/2 cKO interneurons versus control interneurons ($^{*}P$=0.0007 (Student's *t*-test). (D) Representative recordings showing action potentials in control and TSC1/2 INs. Reduced action potential frequency is evident in TSC1/2 cKO interneurons ($F_{16,176}$ = 10.72, $^{*}P$ <0.0001; Two-way ANOVA). (E) Representative patch-clamp electrophysiological recordings showing miniature inhibitory postsynaptic currents (mIPSCs) in PNs of control and TSC1/2 cKO mice. (D, E) Quantification of mIPSC frequency (D) and amplitude (E) in PNs (mIPSC frequency: t_{10} = 3.60, p=0.005; mIPSC amplitude: t_{10} = 3.01, p=0.013). Data shown are mean ± SEM.

Collectively, these defects may contribute to autism spectrum disorder and epilepsy phenotypes noticed in TSC patients

Aim 3. Determine if manipulation of cilia signaling can be used as a therapeutic avenue to ameliorate TSC deficits.

To evaluate gene pathways disrupted in neurons as a result of TSC1/2 deletion and to detect potential targets to ameliorate associated TSC phenotypes, we generated $TSC1^{Lox/Lox}$; $TSC2^{Lox/Lox}$; Nkx2.1-Cre; RiboTag mouse, in which large ribosomal subunit protein RPL22 is tagged with HA in a Cre-dependent manner (Figure 9). We purified tagged ribosomes from interneurons (control and TSC1/2 cKO) as they elaborate their morphology (P15) and performed RNAseq analyses on associated RNA samples to identify genes that are actively being translated in normal and TSC1/2 cKO cortical interneurons. To evaluate specific TSC1/2-dependent developmental pathways and clusters of coregulated genes modules, we performed GO and Weighted Gene Coexpression Network Analysis (WGCNA) on target genes. TSC-target genes in GO term and networks correlate with TSC phenotypes (e.g., autism, epilepsy). We identified gene pathways associated with autism, epilepsy, and neuronal projection morphogenesis, and interneurons as significantly altered in TSC1/2 cKO INs (Figure 9), indicating that this approach can lead us to the right neurodevelopmental disease relevant gene networks critical for TSC1/2 associated functions in neurons.

A



B

Go Term		*p* value
GO:0043005	Neuron projection	1.174e-11
GSE60361	Interneurons	1.78e-12
GO:0043025	Neuronal cell body	1.666e-7
GO:0061564	Axon development	2.52e-6
GO:0099536	Synaptic signaling	3.48e-6
GO:00367477	Somatodendritic compartment	6.66e-5
GO:0048812	Neuron projection morphogenesis	7.26e-5
MP:0002882	Abnormal neuron morphology	5.32e-4
HP:0000717	Autism	5.972e-5
HP:0000750	Delayed speech and language development	7.813e-6
MP:0000745	Tremors	3.679e-8
MP:0002064	Seizures	4.00e-5
MP:0003633	Abnormal nervous system physiology	1.052e-9

C

Gene	Fold Change	*p* value
Tspan10	6.59	1.52E-23
Gys2	4.325	6.54E-07
Cd44	2.00	1.85E-12
Myh4	1.73	3.44E-17
Klk6	-4.51	8.99E-06
Enpp6	-3.90	4.59E-17
Ermn	-3.54	3.70E-28
Mal	-3.32	3.70E-28
Sox10	-3.10	6.38E-29
Gpr37	-2.09	3.76E-22
Gsn	-2.05	1.01E-28
Gatm	-1.84	2.12E-12
Kit	-1.70	2.48E-08
Megf10	-1.50	2.39E-3
Magel2	-1.50	6.45E-07

Figure 9. RNAseq analysis of TSC1/2 cKO interneurons. (A) We will use RiboTag mice, in which large ribosomal subunit protein RPL22 is tagged with HA in a Cre-dependent manner, in our RNAseq analysis to identify genes that are actively being translated in interneurons (control and TSC1/2 cKO) as they elaborate their morphology. (B) GO analysis of RNAseq data reveals genes associated with neuronal projection, morphology, autism, and epilepsy are significantly altered in TSC1/2 cKO interneurons. TSC1/2 mutations in humans cause autism and epilepsy. (C) Sample of genes deregulated in TSC1/2 cKO. Several autism linked genes (purple) are deregulated in TSC1/2 cKO interneurons

Further, we have created a continuously updated toolbox of chemogenetic and optogentic reagents that can be used to activate or silence ciliary receptor or second messenger signaling (Figure 10). For example, we have Cre inducible, cilia targeted DREADD (Designer Receptors Exclusively Activated by Designer Drugs) AAVs to activate (Cilia-hM3D$_{q)}$ or silence (Cilia-hM3Di) GPCR signaling, optogenetic probes to selectively activate effector pathways (e.g., ACIII [Cilia-bPAC], PI3 kinase [mCh-CRY2-iSH2], Akt [mCh-CRY2-Akt], INPP5E [mCh-CRY2-5pase$_{INPP5E}$) that are down stream of diverse ciliary signaling receptors, and cilia targeted activity indicators (Ca^{2+} [Cilia-GECO], PIP3 biosensors [Cilia-RFP-PH$_{GRPI}$]) to monitor ciliary signaling. We will use these probes to selectively activate or silence ciliary signaling in progenitors and neurons with light or small molecules in TSC1/2 deficient brains to examine if such approaches can ameliorate the functional deficits.

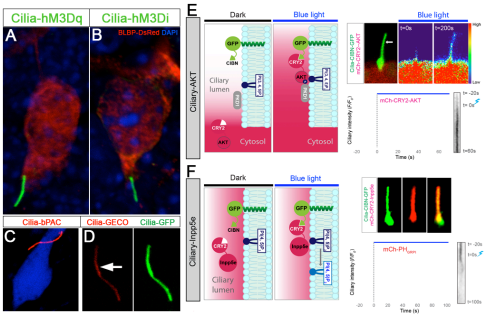


Figure 10. Chemogenetic and optogentic tool box to selectively manipulate primary cilia signaling. (**A-B**) cilia targeted DREADDS. (**C**) Blue light activatable cilia targeted bPAC. (**D**) Cilia targeted Ca^{2+} indicator(Cilia-GECO; arrow) after activation. (**E-F**) Blue light activated, Cilia-CIBN/CRY2 dimerization opto-system to selectively recruit AKT or Inpp5e to cilia-membrane. Kymographs indicate recruitment of AKT (E) or expected ciliary reduction of PIP3 biosensor (PH$_{GRPI}$) after Inpp5e recruitment (F).

Collectively, these studies (*as outlined in Major Task 3*) will help us characterize if and how cilia signaling can be manipulated to promote rescue of TSC deficits.

What opportunities for training and professional development has the project provided?

Training on TSC related neurodevelopmental brain research was provided to a post-doctoral fellow, Keiko Nakagawa, through this research. The PI, Eva Anton, mentored this post-doctoral fellow on the design and evaluation of studies related to this project and the general significance of this work. In addition, training in cell biological techniques and mouse genetics related to this work, was provided to two research technicians, Gary Wilkins and Janice Lee, and three undergraduate students, Tavian Mapp, Tia Andrade and Joshua Coy. This will help their competitiveness for further advanced academic training in medicine, biomedical sciences and neurobiology.

How were the results disseminated to communities of interest?

Some of the findings were presented to visiting investigators from other U.S. universities and in small group meetings at UNC School of Medicine.

What do you plan to do during the next reporting period to accomplish the goals?

N/A

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?

Understanding TSC protein's functions in the developing brain will help understand how TSC brain pathology emerges. This will be necessary to devise efficient therapeutic interventions for TSC.

What was the impact on other disciplines?

Understanding TSC effect on neural size and differentiation will help understand the mechanisms regulating cell morphology in general and how morphology impacts cell function and neural circuit formation.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Defining the TSC1/2 related functions during the construction of cerebral cortex will be essential to understand the pathogenic mechanisms that underlie tuberous sclerosis complex. This knowledge could help society understand why patients with neurodevelopmental disorders suffer and what could be done through scientific research to help them lead productive lives.

5. **CHANGES/PROBLEMS:** The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to report.

Changes that had a significant impact on expenditures

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Not applicable.

Significant changes in use or care of vertebrate animals

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. **PRODUCTS:** List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications.

Loo, L., Simon, J. M., Xing, L., McCoy, E. S., Niehaus, J. K., Guo, J., Anton, E. S., and Zylka, M. J. (2018). Single-cell transcriptomic catalog of mouse cortical development. Pp. 1-43. (*In press*, Nature Communications).

Nakagawa N, Li J, Yabuno-Nakagawa K, Eom TY, Cowles M, Mapp T, Taylor R, Anton ES. APC sets the Wnt tone necessary for cerebral cortical progenitor development. Genes Dev. 2017 Aug 15;31(16):1679-1692. doi: 10.1101/gad.302679.117. Epub 2017 Sep 15. PubMed PMID: 28916710; PubMed Central PMCID: PMC5647938.

Guo J, Otis JM, Higginbotham H, Monckton C, Cheng J, Asokan A, Mykytyn K, Caspary T, Stuber GD, Anton ES. Primary Cilia Signaling Shapes the Development of Interneuronal Connectivity. Dev Cell. 2017 Aug 7;42(3):286-300.e4. doi: 10.1016/j.devcel.2017.07.010. PubMed PMID: 28787594; PubMed Central PMCID: PMC5571900.

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Submitted manuscripts

Nakagawa, N., Yabuno-Nakagawa. K, and Anton, E. S. (2018). Memo1 Mediated tiling of radial glial cells drives cerebral cortical development. Pp. 1-52. (In revision, Neuron).

Otis, J.O., Zhu, M., Namboodiri, V. M. K., , Cook, C. A., Kosyk, O., Matan, A. M., Ying, R., Hashikawa, K., Trujillo-Pisanty, I., Guo, J., Ung, R. D., Rodriguez-Romaguera, J., Anton, E. S., and Stuber, G. D. (2018). Paraventricular thalamus projection neurons integrate cortical and hypothalamic signals for cue-reward processing. Pp. 1-61. (In revision, Neuron).

Guo, J., Otis, J. O., Gupton, S., Stuber, G., Caspary, T., and Anton, E. S. (2018). Effect of neuronal primary cilia on axonal tract development. Pp. 1-58. (Submitted).

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers and presentations.

Presented seminars on this work at:
Department of Biology, Brandeis University, 2018
Anatomical Society,UK Conference (Human Cerebral Cortex Development), 2018
Department of Physiology, Anatomy and Genetics, University of Oxford, 2018
Department of Neuroscience, University of Florida, 2017
FASEB Conference (Biology of Cilia and Flagella), Scottsdale, AZ, 2017,
Department of Neuroscience, Emory University, 2017
Department of Neuroscience, University of West Virginia, 2016
Volga Neuroscience Conference, St. Petersburg, Russia, 2016
Department of Neuroscience, Case Western Reserve University, 2016
Department of Biology, Dartmouth College, 2016
Department of Biology, University of South Carolina, 2015
Department of Cell Biology, Pontificia Universidad Católica de Chile, 2015

- **Website(s) or other Internet site(s)**

Nothing to report.

- **Technologies or techniques**

Nothing to report.

- **Inventions, patent applications, and/or licenses**

Nothing to report.

- **Other Products**

Submitted an NIH RO1 research proposal on TSC and interneuronal development and malfunction in 2018.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	*Eva Anton*
Project Role:	*PI*
Researcher Identifier	*Not Applicable*
Nearest person month worked:	*1.2*
Contribution to Project:	*Principal Investigator. Supervised this project.*
Funding support	*Not Applicable*
Name:	*Jiami Guo*
Project Role:	*Post- doctoral fellow*
Researcher Identifier	*Not Applicable*
Nearest person month worked:	*8.0*
Contribution to Project:	*Dr. Guo has performed work in the area of analysis of TSC in progenitor and neuronal development.*
Funding support	*Not Applicable*
Name:	*Keiko Nakagawa*
Project Role:	*Post doctoral fellow*
Researcher Identifier	*Not Applicable*
Nearest person month worked:	*6.0*
Contribution to Project:	*Dr. Nakgawa has performed work in the area of analysis of TSC in progenitor and neuronal development*
Funding support	*Not Applicable*

Name:	*Janice Lee*
Project Role:	*Technician*
Researcher Identifier	*Not Applicable*
Nearest person month worked:	*1.0*
Contribution to Project:	*Janice helped generate and maintain mouse genetic models necessary for this work.*
Funding support	*Not Applicable*
Name:	*Gary Wilkins*
Project Role:	*Technician*
Researcher Identifier	*Not Applicable*
Nearest person month worked:	*1.0*
Contribution to Project:	*Gary helped generate and maintain mouse genetic models necessary for this work.*
Funding support	*Not Applicable*
Name:	*Joshua Coy*
Project Role:	*Undergraduate Research Assistant*
Researcher Identifier	*Not Applicable*
Nearest person month worked:	*1.0*
Contribution to Project:	*Joshua helped analyze tissue from mouse genetic models necessary for this work.*
Funding support	*Not Applicable*
Name:	*Tia Andrade*
Project Role:	*Undergraduate Research Assistant*
Researcher Identifier	*Not Applicable*
Nearest person month worked:	*1.0*
Contribution to Project:	*Tia helped analyze tissue from mouse genetic models necessary for this work.*
Funding support	*Not Applicable*
Name:	*Tavien Mapp*
Project Role:	*Tavien helped analyze tissue from mouse genetic models necessary for this work.*
Researcher Identifier	*Not Applicable*
Nearest person month worked:	*1.0*
Contribution to Project:	
Funding support	*Not Applicable*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report.

What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

9. **APPENDICES:** Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.