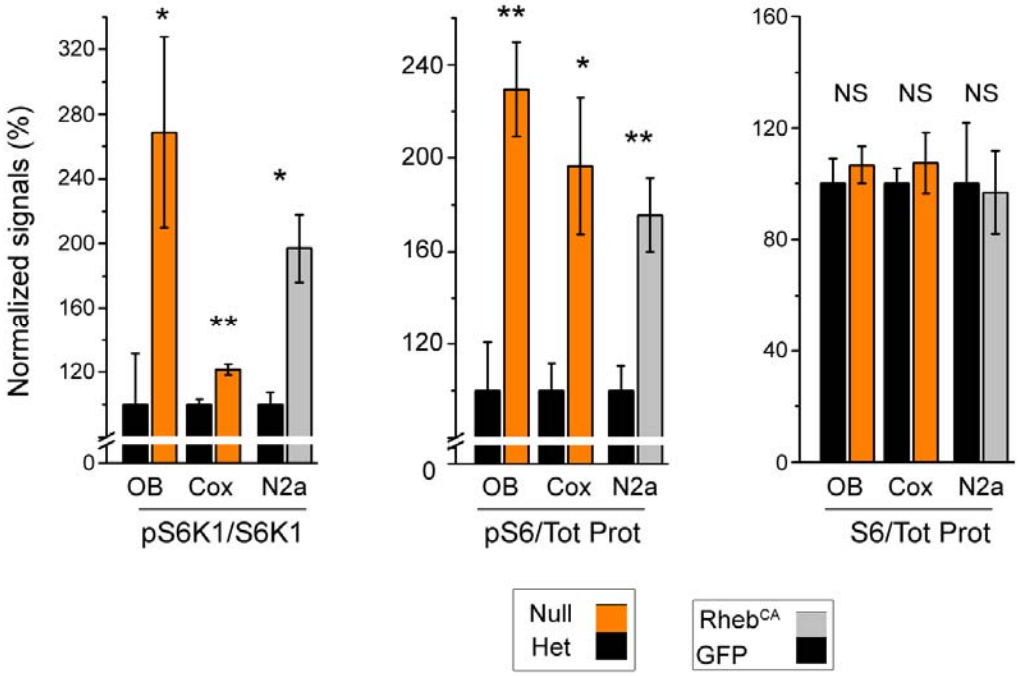
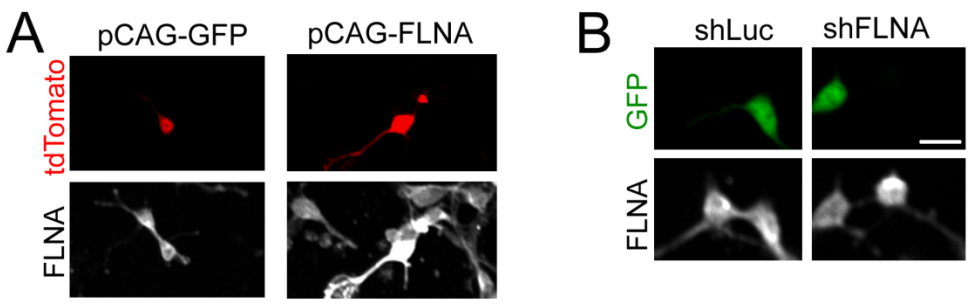
SUPPLEMENTAL MATERIAL

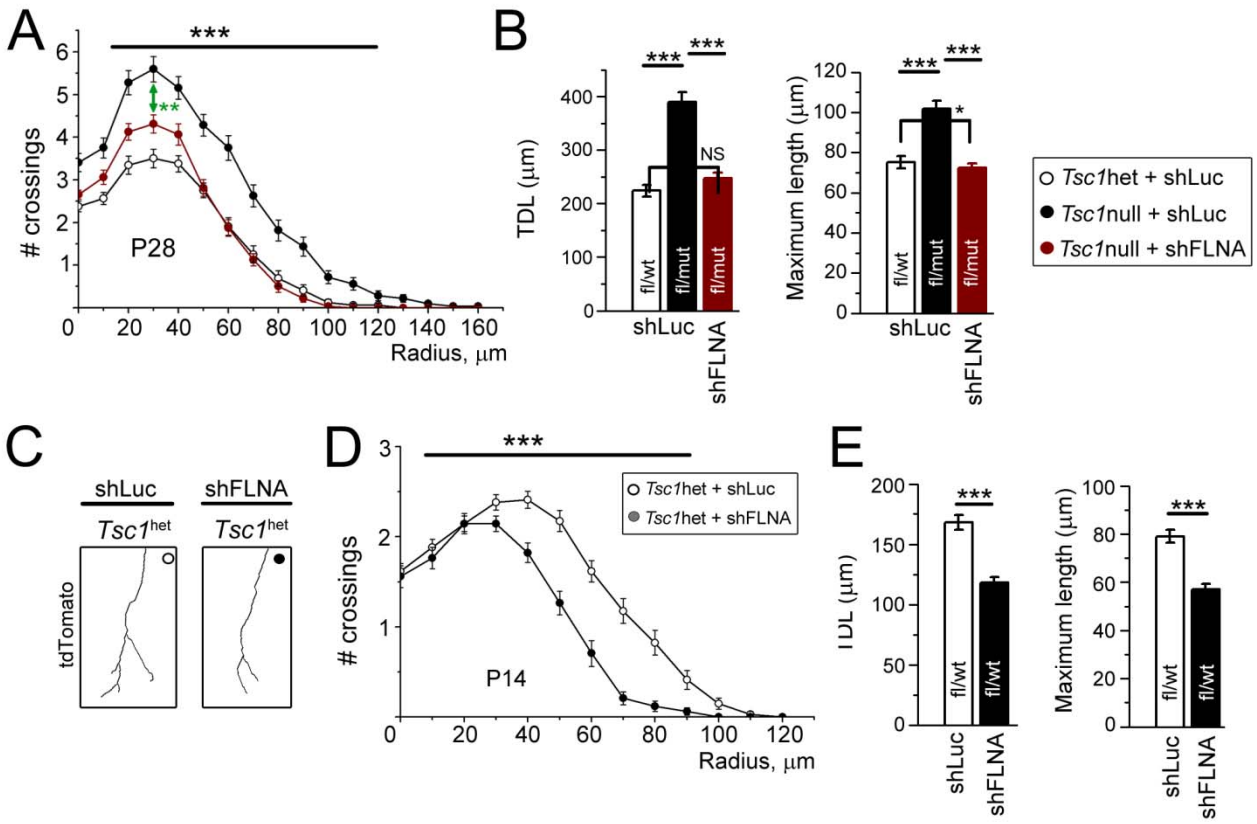
FIGURES AND FIGURE LEGENDS



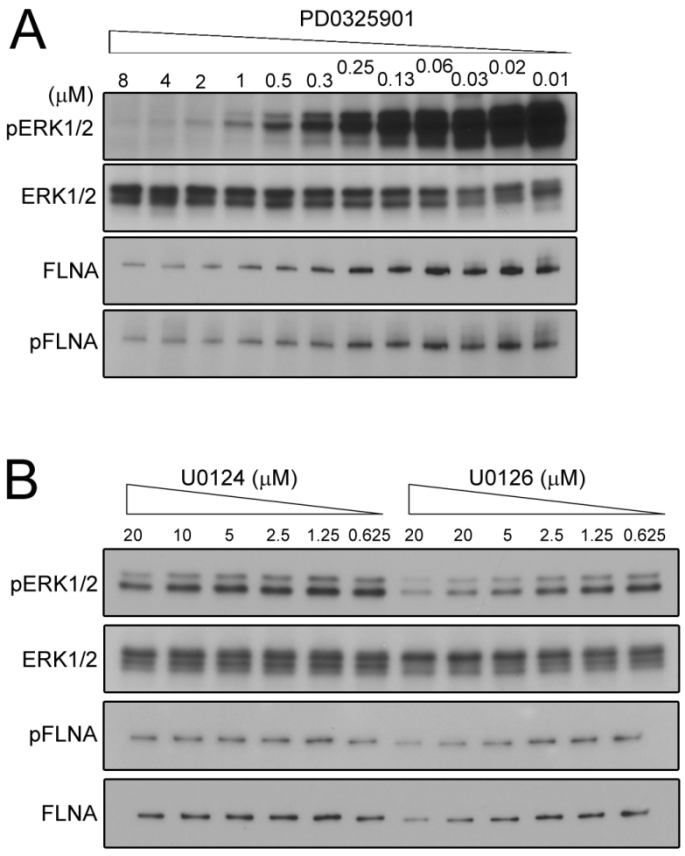
Supplemental Figure 1: **Additional quantifications related to Figure 2.** Bar graphs of the normalized % changes for pS6K1/S6K1, pS6/tot Prot, and S6/Tot Prot in the conditions and mice listed under the graphs. OB = olfactory bulb, Cox = cortex, N2a = Neuro2a cells. Statistical analysis was performed with One Way Anova. **, $p<0.01$, *,$p<0.05$, and NS, not significant.



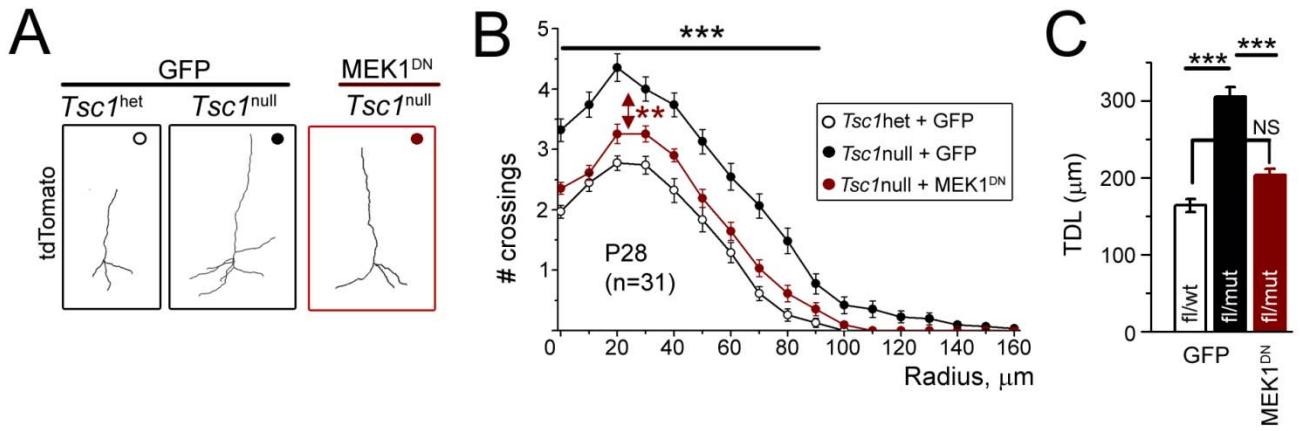
Supplemental Figure 2: **Related to Figure 3. (A)** Images of tdTomato fluorescence and FLNA immunostaining (white) in OB neurons transfected with pCAG-GFP + pCAG-tdTomato or pCAG-FLNA + pCAG-tdTomato. **(B)** Images of GFP fluorescence (from the shRNA vector) and FLNA immunostaining (white) in OB neurons transfected with shRNA against Luc (shLuc) or shFLNA.



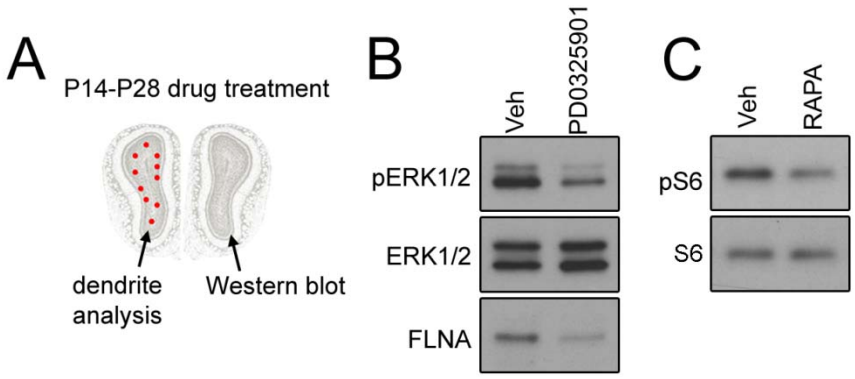
Supplemental Figure 3: **Related to Figure 4. (A)** Sholl analysis for P28 *Tsc1*^{het} neurons transfected with shLuc and *Tsc1*^{null} neurons transfected with either shLuc or shFLNA. **(B)** Bar graphs of the total dendritic length (TDL) and maximum length for neurons analyzed in (A). **(C)** Reconstructions of the basal dendrites of P14 *Tsc1*^{het} neurons transfected with shLuc (open circles) or shFLNA (black circles). **(D)** Sholl analysis for neurons whose examples are shown in (C). **(E)** Bar graphs of the (TDL) and maximum length of *Tsc1*^{het} neurons with shLuc or shFLNA. Statistical analysis of sholl data was performed with 2-way repeated measures ANOVA with *post hoc* Bonferroni's test. One way ANOVA was used for the comparison of data in the bar graphs. ***: $p<0.001$; **: $p<0.01$; * $p<0.05$.



Supplemental Figure 4: **Related to Figure 6. (A and B)** Western blot analysis for the molecules indicated on the left under different concentrations of the MEK1/2 blockers PD0325901 (A) and U0126 and the inactive MEK1 blocker U0124 (B). Neuro2a cells were cultured in DMEM in 5% serum and treated for 48 hours.



Supplemental Figure 5: **Related to Figure 7. (A)** Reconstructions of the basal dendrites of P28 *Tsc1*^{het} transfected with GFP and *Tsc1*^{null} neurons transfected with either GFP or MEK1^{DN} (red). **(B)** Sholl analysis for neurons whose examples are shown in A. The statistical analysis (** in red) refers to *Tsc1*^{het} + GFP compared to *Tsc1*^{null} + MEK1^{DN} and (***) refers to *Tsc1*^{null} + GFP compared to *Tsc1*^{het} + GFP.**(C)** Bar graphs of the total dendritic length (TDL) from sholl data in (B). Statistical analysis of sholl data was performed with 2-way repeated measures ANOVA with *post hoc* Bonferroni's test. One way ANOVA was used for the comparison of data with ***: $p<0.001$; **: $p<0.01$, NS: not significant.



Supplemental Figure 6: **Related to Figure 8. (A)** Diagram of paired olfactory bulbs used either for western blot or dendrite analysis from the same mice treated from P14 to P28 with either rapamycin or PD0325901. **(B)** Immunoblots for the molecules on the left from the OBs of mice treated with either PD0325901 (B) or rapamycin (C).

Supplemental Table 1: List of vector combinations used in study (related to Figures 1, 3-8)

#	Mice	Experiments/ Conditions	Control Vectors	FLNA or MEK vectors or *Tsc1* deletion	"rescue"
1	CD1	FLNA overexpression	pCAGIG-GFP + pCAG-tdTomato	pCAGIG-FLNA + pCAG- tdTomato	-
2	CD1	FLNA shRNA	pCGLH-shLuc + pCAG- tdTomato	pCGLH-shFLNA + pCAG- tdTomato	-
3	CD1	FLNA shRNA + FLNA rescue	pCGLH-shLuc + pCAG- tdTomato + pCEGFP-C1-GFP	pCGLH-shFLNA + pCAG- tdTomato + pEGFP-C1-GFP	pCGLH-shFLNA + pCAG- tdTomato + pEGFP-C1-hFLNA
4	CD1	$MEK1^{CA}$ overexpression + FLNA rescue	pCAG-GFP + pCGLH-shLuc + pCAG- tdTomato	pCAG-$MEK1^{CA}$ + pCGLH-shLuc + pCAG- tdTomato	pCAG-$MEK1^{CA}$ + pCGLH-shFLNA+ pCAG- tdTomato
5 (CTL for 6)	R*Tsc1*$^{fl/wt}$	*Tsc1*^{het} cells	pCAG-Cre + pCGLH-shLuc	pCAG-Cre + pCGLH-shFLNA	
6	R*Tsc1*$^{fl/mut}$	*Tsc1*^{null} cells	-	pCAG-Cre + pCGLH-shLuc	pCAG-Cre + pCGLH-shFLNA
7 (CTL for 8)	R*Tsc1*$^{fl/wt}$	*Tsc1*^{het} cells	pCAG-Cre + pCAG-GFP	-	
8	R*Tsc1*$^{fl/mut}$	*Tsc1*^{null} cells	-	pCAG-Cre + pCAG-GFP	pCAG-Cre + pCAG-$MEK1^{DN}$

CTL: control; shLuc: luciferase shRNA; shFLNA: FLNA shRNA; hFLNA: human FLNA
Notes:

- pCAGIG and pCGLH contain GFP
- pCAG-tdTomato was included to label dendrites similarly in all conditions.

Supplemental Table 2: Mean fold-change of genes (by alphabetic order) from qRT-PCR arrays comparing *Tsc1*$^{fl/mut}$ versus *Tsc1*$^{fl/wt}$ mice. Gene expression increased 1.5x or more shown in red (related to Figure 1).

Ache	1.10114
Adora1	0.6108479
Adora2a	0.7777225
Alk	4.166242
Apbb1	0.8148494
Apoe	0.4252194
Arnt2	0.9634512
Artn	1.02093
Ascl1	0.470261
Bai1	1.003442
Bdnf	0.6299626
Bmp15	0.6600086
Bmp2	1.485487
Bmp4	0.9011376
Bmp8b	1.95201
Cdk5r1	0.8928141
Cdk5rap1	0.7992517
Cdk5rap2	1.188832
Cdk5rap3	0.857231
Chrm2	0.7821316
Dlg4	0.7798033
Dll1	0.8047631
Drd2	0.5373427
Drd5	2.622925
Dvl3	0.8056233
Efnb1	0.8962556
Egf	1.222585
Ep300	0.7911294
Erbb2	0.7595387
Fez1	1.033782
Fgf13	0.9862834
Fgf2	0.6753014
Flna	2.332166
Gdnf	1.79681
Gnao1	1.124565
Gpi1	1.135795
Grin1	1.42026
Hdac4	1.100077
Hdac7	0.8721941
Hes1	0.9118276
Hey1	0.6244624
Hey2	0.2570637
Heyl	0.4790037
Il3	0.4368022
Inhba	0.729169
Mdk	0.8986309
Mef2c	0.7939952
Kmt2a	0.4938685
Ncoa6	1.033537
Ndn	1.137325
Ndp	0.7793611
Neurod1	0.5429814
Nog	1.179417
Notch2	0.67465
Nptx1	0.6059616
Nrcam	1.085413
Nrg1	0.787879
Nrp1	0.5523717
Nrp2	0.6500103
Ntn1	0.39646
Tenm1	0.9338449
Pafah1b1	1.300306
Pard3	1.249035
Pard6b	0.9254724
Pax5	0.5265465
Pax6	0.7207176
Pou3f3	0.9894819
Ptn	0.7807327
Rac1	0.9552641
Robo1	0.6019428
Rtn4	0.8106877
S100a6	0.9694514
S100b	0.7610806
Sema4d	1.14465
Shh	0.2394613
Slit2	0.6710171
Sox3	0.5739768
Stat3	1.168633
Tnr	1.358834
Vegfa	1.251607
Ywhah	1.265533
Gusb	0.9357073
Hprt	0.8961003
Hsp90ab1	1.229501
Gapdh	0.9995785
Actb	1.089489

Supplemental Table 3: Male patient information from NICHD tissue bank (related to Figure 2)

Patient #	Age	Type	PMI* (hrs)	Storage (yrs)	Medical notes
577	46	TSC	1	14	Intractable seizures at 10 yrs
1047	22	TSC	5	12	Death: ateriosclerotic cardiovascular disease. Drugs: amitryptiline for headaches, tegretol for unknown reasons. Also Wolff-Parkinson-White syndrome and kidney disease with 40% kidney function
1560	25	TSC	4	9	Complex partial and absence seizures, depression, cancer left temporal lobe amygdala, tubers, subependymal nodules, subacute hemorrhagic infarct right cerebral hemisphere, multiple microscopic infarcts in right brain
5226	28	TSC	7	2	Pyschomotor retardation with organized behavior disturbance, hallucinations, aggressive behavior and complex partial seizures. Sleep apnea, hypothyroidism, subungual fibroma, subcutaneous nodules on head and Shagreen spots. Generalized paroxysmal activity originating from left frontal area and cortical tubers.
5322	42	TSC	6	1	Seizures, mental retardation with developmental delay, hydrocephalus and surgical removal of tumors. Astrocytoma of left eye, renal hamartomas and hepatic cysts, tubers, subependymal nodules.
5243	41	Control	6	2	History of heart problems, unresponsive in bed, possible prescription drugs
4842	47	Control	6	15	Asphyxiation, food bolus. History of high blood pressure, nonsmoker
4786	22	Control	8	10	Motor vehicle accident, possibly drunk
1027	22	Control	7	9	Motor vehicle accident

*, PMI: post-mortem interval

Supplemental Table 4: List of antibodies (related to Figures 2-4, 6, and 7)

Primary antibody	Company	Catalog Number	Primary	Secondary
TSC1 (hamartin)	Abcam	ab32936	1:5000	1:5000
TSC2 (tuberin)	Santa Cruz	sc-893	1:5000	1:5000
Rheb	Cell Signaling Thermo Scientific	4935 PA5-20129	1:1000 1:5000	1:2000 1:5000
pS6K1 (Thr389)	Cell Signaling	9205	1:1000	1:5000
S6K1	Cell Signaling	9202	1:1000	1:5000
pS6 (Ser240/244)	Cell Signaling	5364	1:100,000	1:5000
S6	Cell Signaling	2217	1:5000	1:5000
pFLNA (Ser2152)	Abcam	ab75978	1:3000	1:5000
FLNA	Abcam	ab51217	1:5000	1:5000 1:1000 (IF)
FLNB	Abcam	ab97457	1:3000	1:5000
GAPDH	Santa Cruz	sc-25778	1:5000	1:5000
GFP	Abcam	ab13970	1:500	1:1000 (IF)
pERK1/2 (Thr202/Tyr204)	Cell Signaling	4370	1:5000	1:5000
ERK1/2	Santa Cruz	sc-94	1:250,000	1:5000
SRF	Cell Signaling	5147	1:5000	1:5000
MEK1	Cell Signaling	12671	1:5000	1:5000

IF: immunofluorescence

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Animals and genotyping

Research protocols were approved by the Yale University Institutional Animal Care and Use Committee. Experiments were performed on either gender wild-type CD1 and the following lines of transgenic mice: R26R^{tdTomato}/*Tsc1*$^{fl/mut}$ mice (abbreviated R*Tsc1*$^{fl/mut}$) and R*Tsc1*$^{fl/wt}$ mice; R26R^{tdTomato}/*Emx1*^{Cre}/*Tsc1*$^{fl/mut}$ mice (abbreviated R*Tsc1*^{cKO}) and R*Tsc1*^{cHet}; *Tsc1*$^{fl/mut}$ and *Tsc1*$^{fl/mut}$ mice. R*Tsc1*^{cKO} and R*Tsc1*^{cHet} mice were obtained by crossing R26R^{tdTomato}/*Tsc1*$^{fl/fl}$ (each individual line from Jackson Laboratories) and *Emx1*^{Cre}/*Tsc1*$^{wt/mut}$ (*Tsc1*$^{wt/mut}$ from NCI and *Emx1*^{Cre} provided by Dr. J. Cardin, originally obtained from Jackson labs). The *Tsc1*$^{fl/fl}$ and *Tsc1*$^{wt/mut}$ mouse lines were generated by David J. Kwiatkowski (Brigham and Women's Hospital, Harvard Medical School, Cambridge, Massachusetts, USA). Tail or toe samples were taken and were subjected to DNA isolation, PCR amplification. Amplicons were separated by standard electrophoresis methods.

For genotyping R*Tsc1*^{cKO} and R*Tsc1*^{cHet} mice, we used the following sets of primers: for *Tsc1* WT allele (295 bp): F: 5'- AGGAGGCCTCTTCTGCTACC, for *Tsc1* floxed allele (480 bp): R: 5'- CAGCTCCGACCATGAAGTG, and *Tsc1* mutant allele (370 bp): R: 5'- TGGGTCCTGACCTATCTCCTA; for Cre detection (307 bp): F: 5'- GCAACGAGTGATGAGGTTCGCAAG and R: 5'- TCCGCCGCATAACCAGTGAAACAG; and for tdTomato detection: wild type sequence F: 5'-AAGGGAGCTGCAGTGGAGTA and R: 5'- CCGAAAATCTGTGGGAAGTC, for tdTomato detection F: 5'- CTGTTCCTGTACGGCATGG and R:5'- GGCATTAAAGCAGCGTATCC.

Human samples

Human tissue was obtained from the NICHD brain and tissue bank for developmental disorders in accordance with Yale human ethical committee. Tissue was from the prefrontal cortex of male individuals. Information about the patients is provided in **Table S3**. We ran TSC and control samples on the same membrane in a randomized order. The last 4 lanes are shown in Figure 2.

RNA isolation, Mouse Neurogenesis RT^2 Profiler™ PCR Array, and qRT-PCR

We used OB tissue from R*Tsc1*$^{fl/mut}$ mice for the PCR array and qRT-PCR for *Flna* and *Gapdh* as well as cortical tissue from R*Tsc1*^{cKO} and R*Tsc1*^{cHet} mice for *Flna* and *Gapdh* qRT-PCR. Mice were anesthesized with pentobarbital (50 mg/kg) prior to tissue dissection. For RNA isolation, Trizol reagent and 21% chloroform were added to each electroporated olfactory bulb and the tissue was passed through a 22 gauge 1.5 inch needle and then vortexed. Following centrifugation for 15 minutes at 4°C and 12,000 *g*, the top aqueous phase was transferred to a fresh reaction tube. After adding isopropyl alcohol, the sample was incubated at room temperature for 20 minutes and centrifuged at 4ºC for 15 seconds. Pellets were rinsed three times with 75% ETOH and air-dried for 5-10 minutes. The RNA was eluted with RNase-free deionized H_2O prior to determining its concentration and purity using a spectrophotometer.

cDNAs were prepared using the RT^2 Nano PreAMP cDNA Synthesis Kit (SA Biosciences, Frederick, MD). cDNAs were combined with nuclease-free double-distilled water and FastStart Universal SYBR Green Master (Rox, Roche, #04913850001) and 12 µl per well was loaded onto a 384-well plate SA Biosciences Mouse Neurogenesis PCR Array. PCR was run for 40 cycles of 95 °C for 15 s, 60 °C for 30 s on an Applied Biosystems 7900HT Fast Real-Time PCR System. C_t values were put into Excel-based PCR Array Data Analysis Software from SA Biosciences to calculate relative gene expression, fold regulation, and *p*-values.

For qRT-PCR of a single gene, 2.12 µg of RNA was mixed with dNTPs, random primers (Invitrogen), and RNase/DNase-free deionized H_2O, heated for 5 minutes at 65°C, and then rapidly chilled on wet ice for 5 minutes, followed by brief centrifugation. DTT, RNase out, and SuperScript III were then added to each sample and reverse transcribed in a BioRad MyCycler. cDNA was then subjected to PCR using primers to mouse *Flna (*F, GCACCGCAAGCACAACCA; R, CACAGCTGTCTACCAGGGC) and *Gapdh* (F, ACCACCATGGAGAAGGC; R,

GGCATGGACTGTGGTCATGA). mRNA transcripts were quantified by the standard curve method of qRT-PCR. cDNA was amplified with the specified primers and detected with SYBR Green (Roche).

Vectors

We used the following vectors: pCAG-GFP and pCAG-Cre (Addgene, donated by Dr. C. Cepko), pCAG-tdTomato (Pathania et al., 2012), pCAG-Rheb^{CA} (from Dr. Hanada and Dr. Maehama (Lafourcade et al., 2013)), rat pCAG-FLNA (from Dr. Cardoso, INMED, (Carabalona et al., 2012), pEGFP-C1 encoding human FLNA or GFP (from Dr. F. Nakamura, Harvard (Nakamura et al., 2006) and Dr. Zenisek, Yale, respectively). The pCAG-MEK1^{CA} (ΔN; S22D) and pCAG-MEK1^{DN} (MEK1 kinase dead K97M acting as a dominant negative) were provided by Dr. Josh Breunig who obtained them from Dr. Anton Bennett (Yale) in a pMCL-1 construct (originally from Dr. Natalie Ahn (Mansour et al., 1994)). The shRNA sequence for FLNA was provided by Dr. Calderwood (5' CCTATGAAGCTGGAACCTATA 3'), and inserted into pCGLH (provided by Dr. N. Sestan, Yale). For control, we used the luciferase shRNA subcloned in the same vector (5' CGCTGAGTACTTCGAAATGTC 3', sequence from clone #TRCN0000072259, Broad Institute). Finally, we obtained a Gaussia luciferase reporter under the mouse FLNA promoter from GeneCopoeia (#MPRM20551-PG02) and a control Renilla luciferase under the SV40 promoter (addgene). The list of vector combination is provided in **Table S1**.

Cell culture and western blot

The Neuro2a mouse neuroblastoma cell line (American Type Culture Collection, CCL-131) was propagated in tissue culture-treated polystyrene multiwell plates or flasks (Falcon; BD Biosciences Discovery Labware). The medium consisted of DMEM (Invitrogen) supplemented with 10% or 5% heat-inactivated fetal calf serum and penicillin–streptomycin at 100 U/l and 100 µg/l each (Invitrogen). Cells were maintained at 37°C and 5% CO_2. Polyjet (SignaGen Laboratories) was used to transfect expression vectors according to the instructions of the manufacturer when cells reached 80% confluence. Protein was harvested 48 h after transfection.

For Western blots, samples were homogenized in RIPA buffer, 1x Halt Protease and phosphatase inhibitor cocktail (Thermo Fisher Scientific), and 8 U/ml DNase. Samples were boiled in 2× Laemmli's buffer. 10 or 20 μg protein/sample was loaded into a 4–15% polyacrylamide gel (Bio-Rad Mini Protean TGX gel). Proteins were transferred to PVDF and blocked in 5% milk or 5% BSA. When appropriate, the blots were probed for pS6 or other antibodies, stripped with Restore Western Stripping buffer from Pierce (catalog #21059), followed by probing for ERK1/2. All quantifications were performed using NIH Image J software. Primary antibodies for Western blots are listed in **Table S4**. HRP-conjugated donkey anti-rabbit antibody from Southern Biotech was used as secondary antibody (1:5,000). For western blot analysis, we determined the linear range for FLNA and pFLNA antibodies (data not shown). Experimental conditions were in alternating fashion into tris-glycine agarose gels. ERK or total protein obtained with Amido Black was used to normalize total protein immunoblot signals; normalization to either loading control gave similar results. For the phosphorylated form of specific proteins, the data were normalized to the total (unphosphorylated) levels from the same PVDF membrane (when possible), which was stripped and reprobed with the appropriate antibodies.

For western blot normalization across blots (see Figure 6C), but within a given experiment, a standard sample was run on each gel (not shown). One membrane was chosen as the reference membrane. For a given antibody, the raw optical density for the standard lane was normalized to the reference standard. This provided a ratio to which all raw experimental optical densities were first normalized. After cross-blot normalization, relative values were then calculated using standard methodologies (*i.e.*, phospho-protein signals were normalized to total protein signal and total protein signals were normalized to loading control signals).

Drug treatment

R*Tsc1*^{cKO} and R*Tsc1*^{cHet} mice received 4 intraperitoneal injections of 0.5 mg/kg rapamycin every other day from P7 to P14 (A.G. Scientific Inc., #R-1018). The brains were then dissected out, snap frozen on liquid nitrogen, and kept at -80^{o}C until lysate preparation. R*Tsc1*$^{fl/mut}$ and R*Tsc1*$^{fl/wt}$ mice underwent

neonatal électroporation after which they received intraperitoneal injections of 0.5 mg/kg rapamycin every other day or 1 mg/kg PD0325901 (Seleckbio, #PD0325901) daily from P14 to P28. For *in vivo* injections, rapamycin was dissolved in DMSO at a concentration of 12.5 mg/ml and resuspended in vehicle (0.25% PEG-400 and 0.25% Tween 80) at a final concentration of 0.1 mg/ml. PD was dissolved in DMSO at a concentration of 25 mg/ml and resuspended in vehicle (0.5% in hydroxypropyl methyl-cellulose and 0.2% Tween 80) to a concentration of 1 mg/ml. Neuro2a cells were treated with rapamycin, Torin 1 (Tocris Bioscience, #4247), and ERK blockers (U0126 and inactive form U0124, Tocris #1144 and #1868) and PD0325901 dissolved in DMSO. Vehicle treatment contained the same volume of DMSO.

Neonatal electroporation

Electroporations were performed as previously described (Feliciano et al., 2013b; Lacar et al., 2010; Lacar et al., 2012; Lafourcade et al., 2013; Platel et al., 2010). For postnatal electroporation, each plasmid was used at a final concentration of 1 μg/μl; plasmids were diluted in PBS containing 0.1% fast green as a tracer. 0.5-1 μl of plasmid solution was injected into the lateral ventricles of neonatal pups using a pulled glass pipette (diameter <50 μm). 5 square-pulses of 50 ms-duration with 950 ms-intervals at 100 V were applied using a pulse ECM830 BTX generator and tweezer-type electrodes (model 520, BTX) placed on the heads of P0 pups.

Culture of *in vivo* transfected neurons and FLNA immunostaining

P0 pups were electroporated as detailed above. 7 days following electroporation, pups were anesthetized on ice and decapitated, and the OB were placed in ice-cold dissection medium (HBSS with 10 mM HEPES). The tissue was chopped finely with scissors. After aspiration of the dissecting medium, the tissue was resuspended in 5 ml of HBSS containing 0.25% Trypsin (1X) and incubated for 10 minutes at 37°C while providing gentle agitation. After removing the trypsin-containing solution, the tissue was rinsed twice with 5 ml dissecting medium. The tissue was then dissociated by gentle trituration with a regular Pasteur pipette and followed by a flame-polished pipette until no chunks of tissue were visible.

Dissociated cells were spun down at 200 g for 5 minutes. After aspiration of the dissecting medium, cells were resuspended cells in neuronal plating medium (MEM supplemented with 0.6% glucose and containing 5% FBS), adjusted to a cell density of 1-1.5 million cells/ml, and then plated on poly-D-lysine (1mg/ml)-coated coverslips (BD Biosciences #354086). The medium was changed to neuronal maintenance media (neurobasal A medium with 1 X B27 and 1 X GlutaMAX-1) three hours after plating and every other day thereafter if necessary. 24 hours after plating, immunostaining for FLNA was performed as previously detailed for other antibodies in slices (Platel et al., 2009).

Culture of R*Tsc1*$^{fl/wt}$ and R*Tsc1*$^{fl/mut}$ P0 cortical neurons followed by nucleofaction

The cortex of P0 pups was dissected out to prepare primary neuron culture as described above. For the transfection, once in cell suspension, neurons were nucleofected using a Mouse Neuron Nucleofector kit VPG-1001 (Lonza) according to the manufacture's instructions. About 3 µg of plasmid DNA per $4x10^6$ cells were added to a total of 100 µl of the Nucleofector® Solution for each transfection reaction. The Nucleofection® was carried out with either of the following pre-defined programs and according to the manufacturer's instructions: AK-009 or AL-007 (included in Amaxa® Nucleofector® II Device, serial version "S"; software version S4-4 or higher, Lonza). Seven days from nucleofaction, western blot analysis was performed.

Morphometric analysis

P14 and P28 mice were deeply anesthetized with pentobarbital (50 mg/kg) and transcardially perfused with 4% paraformaldehyde and PBS. The brain was then quickly removed and placed in 4% paraformaldehyde overnight at 4°C, then washed in 1x PBS. The next day, 100-µm-thick slices were prepared using a vibratome (Leica VTS 1000). Images of tdTomato-expressing neuronal basal dendrites were acquired in the coronal plane using a Fluoview 1000 confocal microscope and 20X objective. Basal dendrites were traced with simple neurite tracer software (FIJI, GNU GPL v3). Sholl analyses were carried out using the number of intersections as a measure of morphological complexity. The number of intersections was measured in 10 µm-increment concentric circles and plotted as a function of

the radial distance from the soma. Confocal Z-stacks from 3 different square fields of view were taken from each olfactory bulb (OB) section. This was done for 3 different OB sections in a randomly selected series from each animal. Analysis was performed blindly from at least 4 animals per condition. The number of cells was > 20 per condition. For the dendritic analyses, we performed experiments at 2 time-points to validate that the dendrites did not recover by P28 due to dendritic pruning. In addition, we show the P14 data in the figures and P28 in the supplemental figures because by P28 the "rescue" effect is underestimated due to progressive dilution of the electroporated vectors. These explanations are included in the Methods under Morphometric Analysis.

Olfactory bulb acute slice preparation

Coronal OB slices (350 μm) were prepared from anesthetized (Isoflurane 5%, inhalation) P21–P24 mice using Vibratome 1000. Ice cold artificial cerebral spinal fluid (ACSF) containing (in mM): 124 NaCl, 3 KCl, 1.25 NaH_2PO_4, 1 $MgSO_4$, 26 $NaHCO_3$, 10 Dextrose, 2 $CaCl_2$, 0.4 ascorbate, 4 Na-Lactate, 2 Na-Pyruvate (290 ± 5 mOsm/kg, pH 7.2), equilibrated with 95% O_2/5% CO_2 was used during both brain dissection and slicing. Brain slices were kept at 28°C for the entire duration of the experiment. During experiments, slices were perfused with the same ACSF mentioned above at 28°C.

Whole-cell recording of olfactory bulb granule cells

Fluorescent cells were identified using Olympus BX51WI microscope, Olympus LUMPlanFL/IR objectives (40X water-immersion objective) and appropriate filters. Whole-cell recordings were performed with Axopatch 200B amplifier through glass pipettes (4-7 MΩ) filled with internal solution (in mM): 130 KCl, 10 HEPES, 10 di-tris-phosphocreatine, 1 EGTA, 0.1 $CaCl_2$, 1.5 $MgCl_2$, 4 Na2-ATP, and 0.3 Na-GTP. Liquid junction potential (~4 mV) was not corrected. Recordings were low-pass filtered at 5 kHz and digitized at 20 kHz using a Digidata 1320 digitizing board (Molecular Devices). The resting membrane potential was determined within 5 seconds upon break-in in current clamp mode without current injection (I=0). Input resistance was assessed under current clamp by injecting hyperpolarizing current pulses (-100 pA). Miniature synaptic currents were recorded for 5 minutes in

the presence of tetrodotoxin (TTX, 1 μM) with cells clamped at −70 mV. Capacitive and leak currents were not subtracted. All data were analyzed with Clampfit 10 (Molecular Devices).

Luciferase assay

1X Luciferase Assay Lysis Buffer was prepared immediately prior to performing assays. We removed the medium from the cultured cells, and gently added PBS to wash the surface of the culture vessel. The culture vessel was swirled briefly to remove detached cells and residual medium. After removing the solution and rinsing, 250 μl Luciferase Assay Lysis Buffer was added. The culture plates were then placed on a rocking platform for 15 min at room temperature (gentle rocking) to ensure complete and even coverage of the cell with the Luciferase Assay Lysis Buffer. The solution was then collected and centrifuged for 30 seconds at top speed in a refrigerated microcentrifuge. The cleared lysate was transferred to a fresh tube. In a luminometer tube, we added 100 μl of Luciferase Assay Reagent and 20 μl of cell lysate. The tube was vortexed for 1–2 seconds and then placed in a l Luminometer (Turner Designs). We performed duplicate assays for each sample