SUPPLEMENTARY INFORMATION

This supplementary material contains the following

1. Supplementary figures: Figure S1 to Figure S7
2. Supplementary figure and movie legends
3. Supplementary methods
4. Supplementary movies: Movies1-4

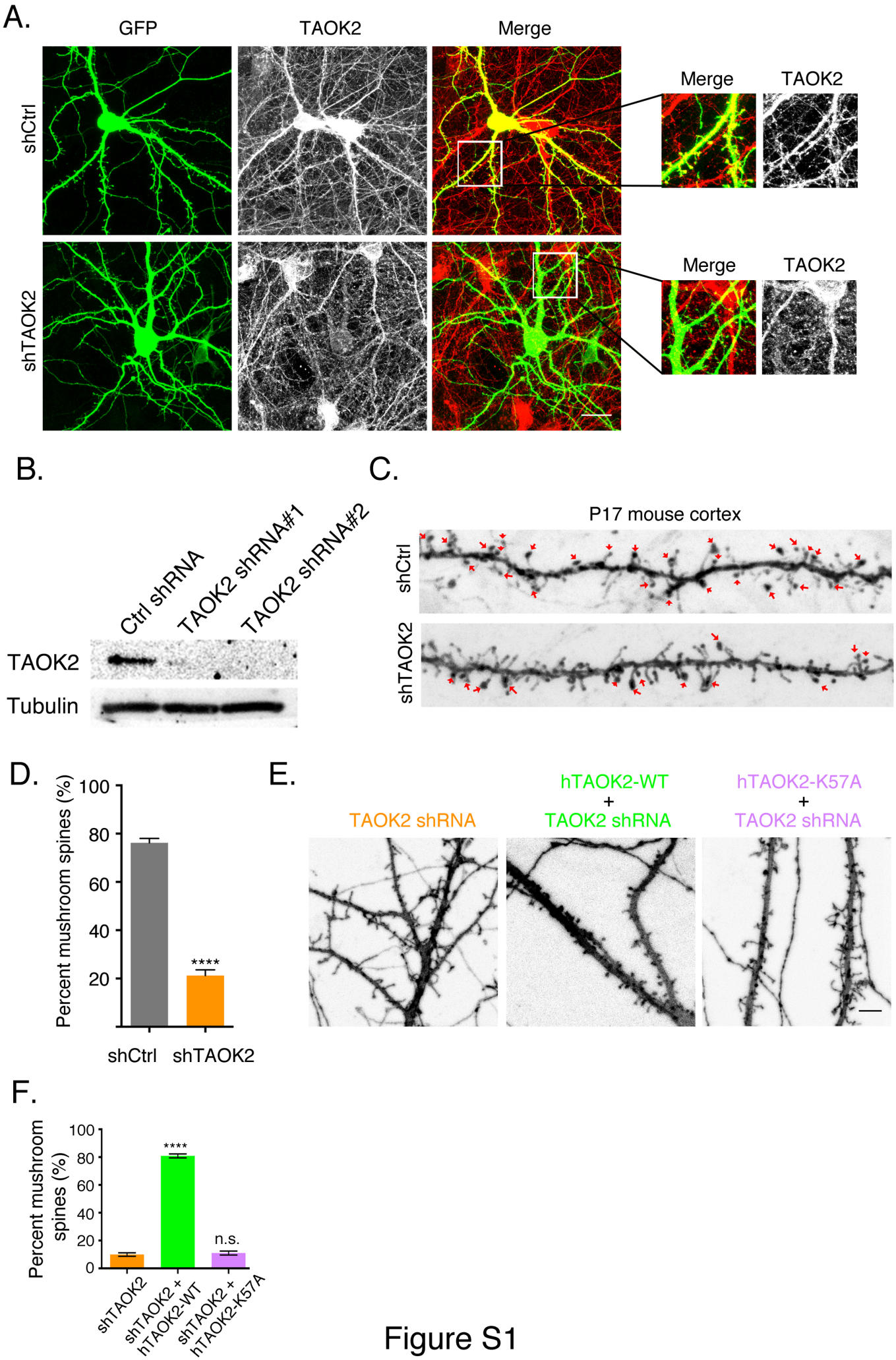

A.
GFP
TAOK2
Merge
shCtrl
shTAOK2
Merge
TAOK2
Merge
TAOK2
B.
Ctrl shRNA
TAOK2 shRNA#1
TAOK2 shRNA#2
TAOK2
Tubulin
C.
P17 mouse cortex
shCtrl
shTAOK2
D.
Percent mushroom spines (%)
100
80
60
40
20
****
shCtrl
shTAOK2
E.
TAOK2 shRNA
hTAOK2-WT
+
TAOK2 shRNA
hTAOK2-K57A
+
TAOK2 shRNA
F.
Percent mushroom spines (%)
100
80
60
40
20
0
****
n.s.
shTAOK2
shTAOK2 + hTAOK2-WT
shTAOK2 + hTAOK2-K57A

Figure S1

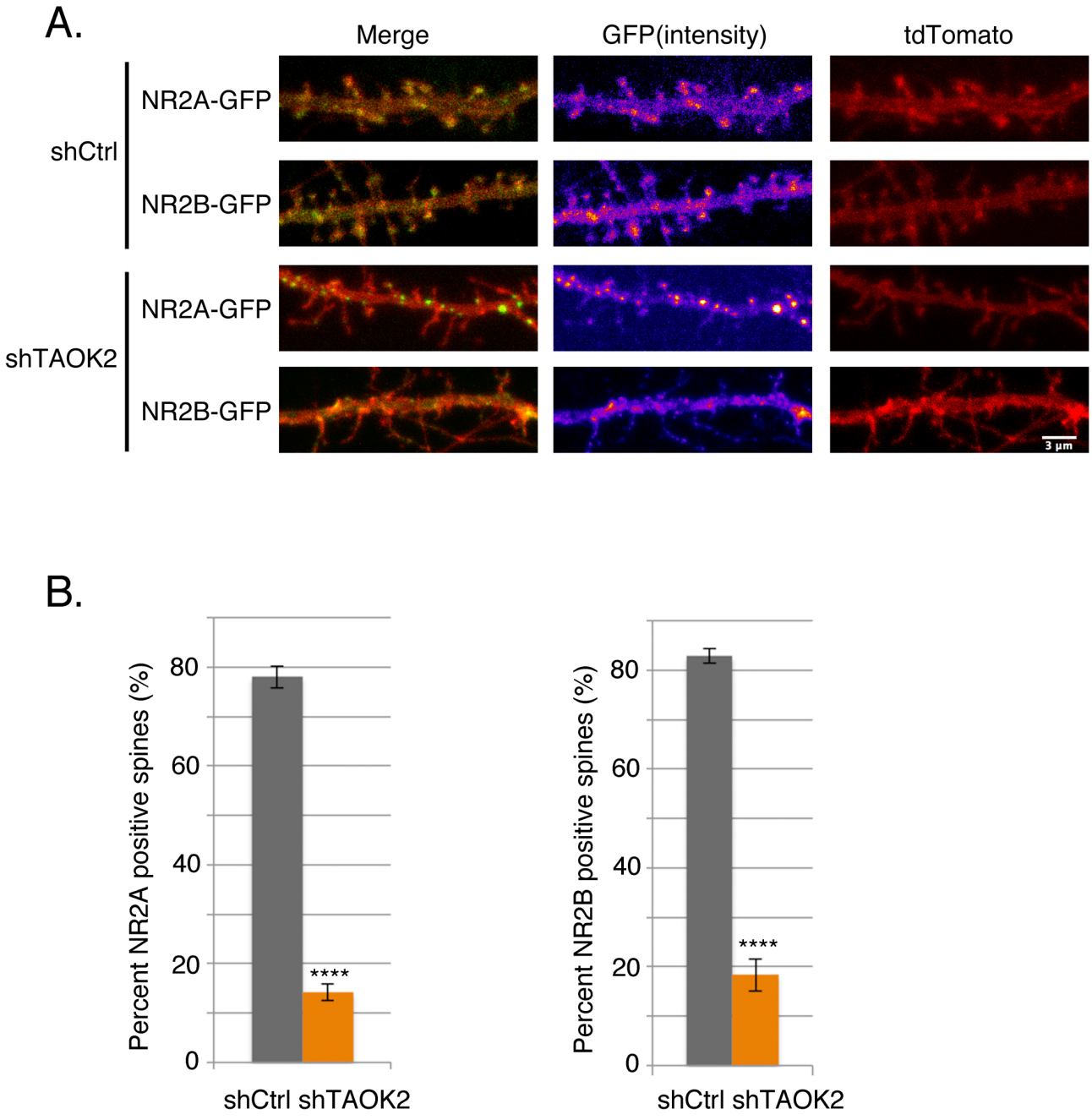

A.
Merge
GFP(intensity)
tdTomato
shCtrl
NR2A-GFP
NR2B-GFP
shTAOK2
NR2A-GFP
NR2B-GFP
3 µm
B.
Percent NR2A positive spines (%)
80
60
40
20
0
****
shCtrl shTAOK2
Percent NR2B positive spines (%)
80
60
40
20
0
****
shCtrl shTAOK2

Figure S2

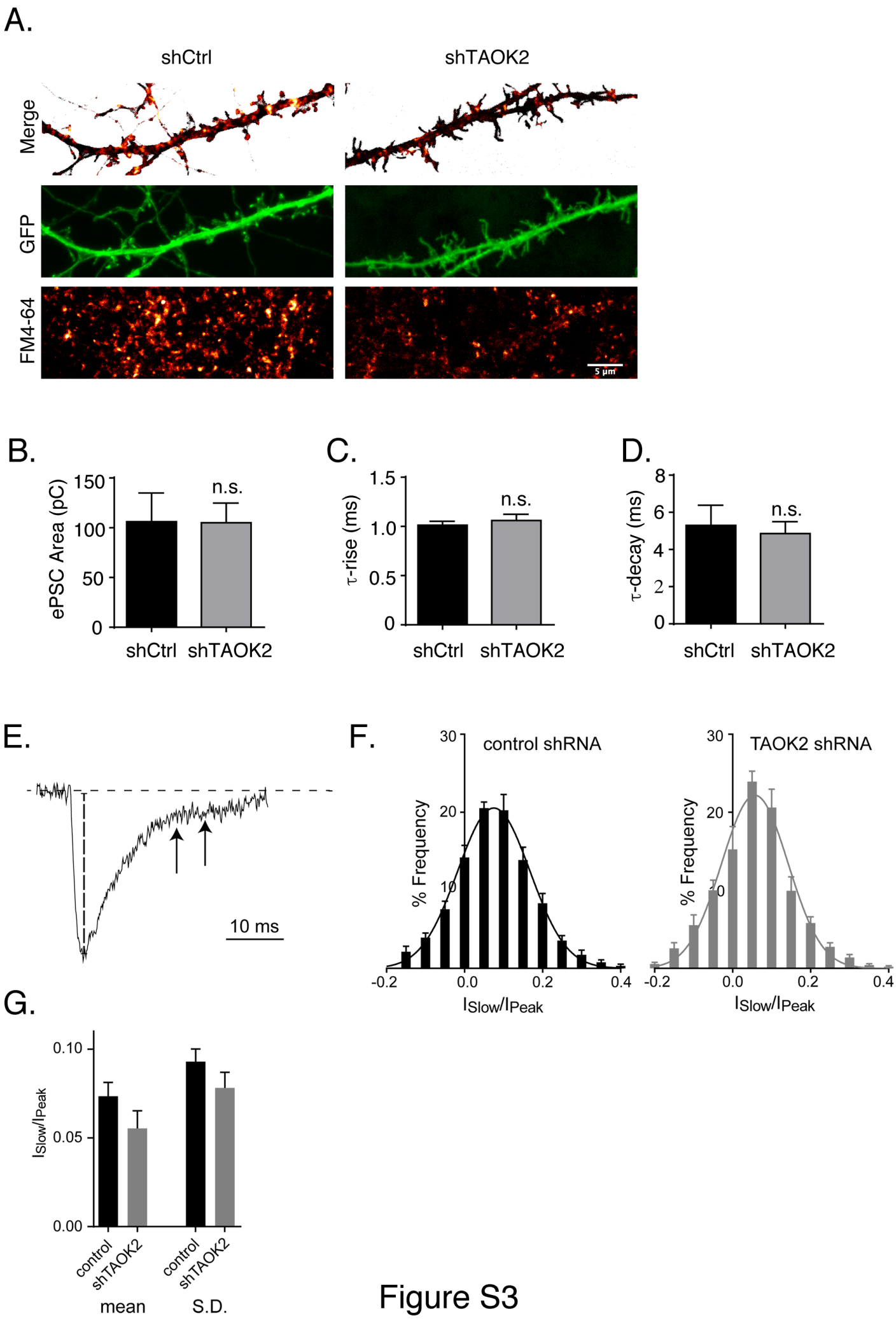

A.
shCtrl
shTAOK2
Merge
GFP
FM4-64
5 μm
B.
ePSC Area (pC)
150
100
50
0
n.s.
shCtrl
shTAOK2
C.
τ-rise (ms)
1.5
1.0
0.5
0
n.s.
shCtrl
shTAOK2
D.
τ-decay (ms)
8
6
4
2
0
n.s.
shCtrl
shTAOK2
E.
10 ms
F.
control shRNA
TAOK2 shRNA
% Frequency
30
20
10
0
-0.2
0.0
0.2
0.4
ISlow/IPeak
G.
ISlow/IPeak
0.10
0.05
0.00
control
shTAOK2
control
shTAOK2
mean
S.D.

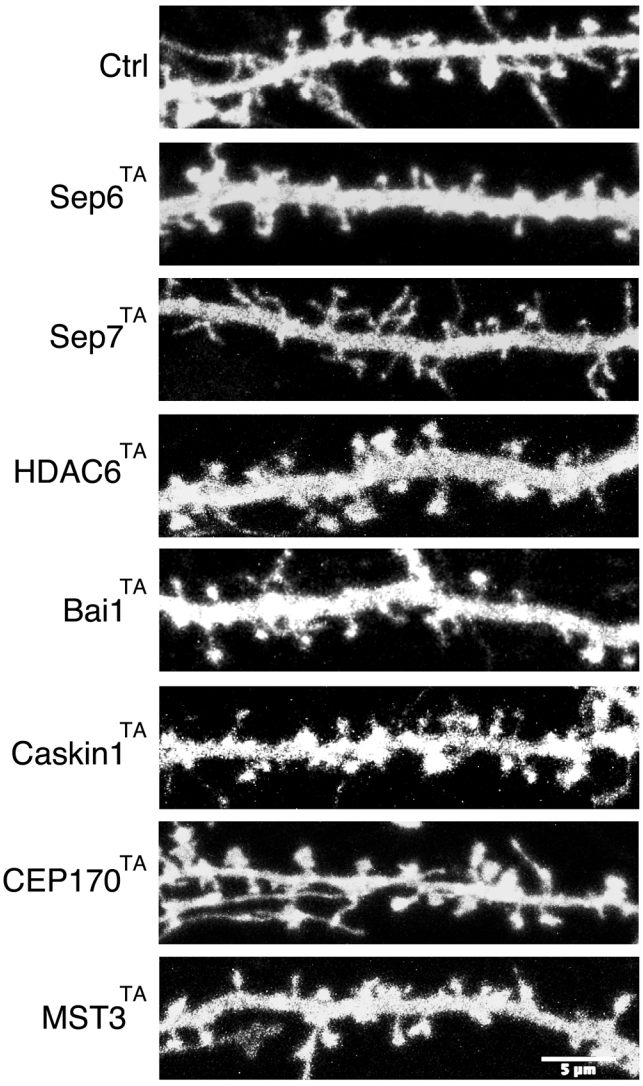
Figure S3

A.

Protein	Species	Sequence
Septin-6	Human	GGSQTLKR
	Mouse	GGSQTLKR
Septin-7	Human	NSSRTLEK
	Mouse	NSSRTLEK
Hdac6	Human	DSSVTSKR
	Mouse	ESSATLKR
Bai1	Human	NHSLTLKR
	Mouse	NHSLTLKK
Cep170	Human	SKSTTLPR
	Mouse	SKSTTLPR
Caskin1	Human	TPPQTPTK
	Mouse	TPPQTPTK
MST3	Human	QLTDTQIK
	Mouse	QLTDTQIK
Asap1	Human	RKTD***H
	Mouse	RKTETSHH

Figure S4

A.


Ctrl
Sep6TA
Sep7TA
HDAC6TA
Bai1TA
Caskin1TA
CEP170TA
MST3TA
5 µm

B.

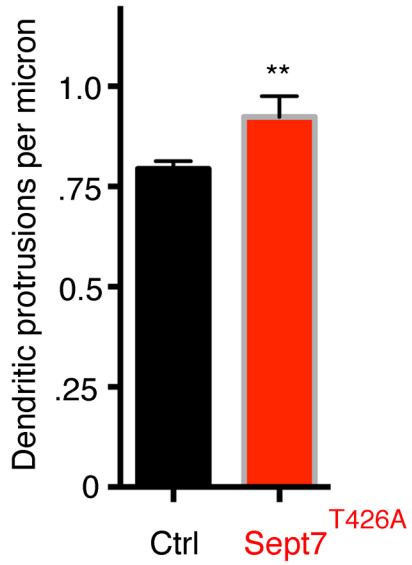

Dendritic protrusions per micron
1.0
.75
0.5
.25
0
**
Ctrl
Sept7T426A

Figure S5

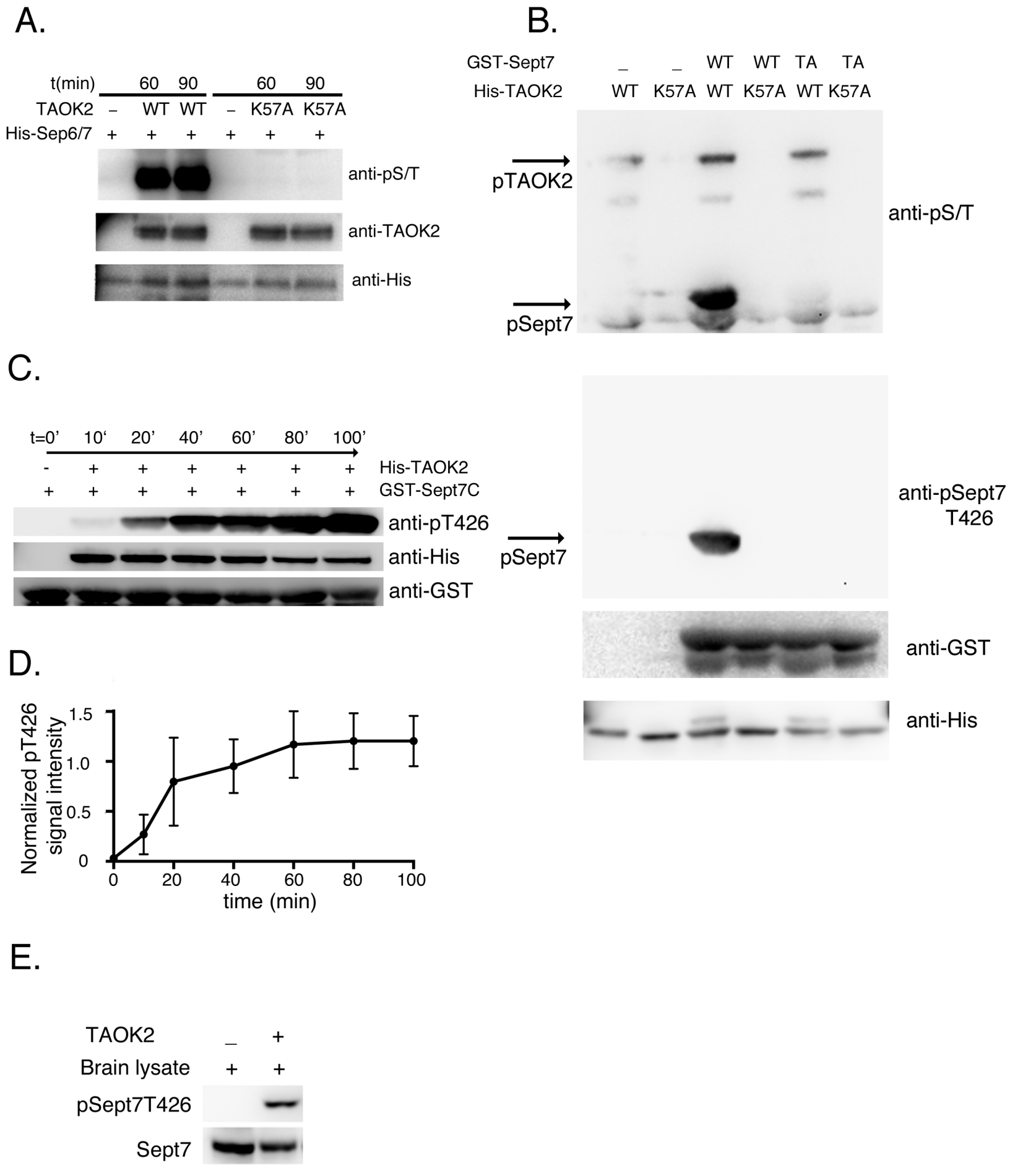

A.
t(min)
60
90
60
90
TAOK2
–
WT
WT
–
K57A
K57A
His-Sep6/7
+
+
+
+
+
+
anti-pS/T
anti-TAOK2
anti-His
B.
GST-Sept7
_
_
WT
WT
TA
TA
His-TAOK2
WT
K57A
WT
K57A
WT
K57A
pTAOK2
pSept7
anti-pS/T
C.
t=0’
10‘
20’
40’
60’
80’
100’
-
+
+
+
+
+
+
His-TAOK2
+
+
+
+
+
+
+
GST-Sept7C
anti-pT426
anti-His
anti-GST
pSept7
anti-pSept7
T426
anti-GST
anti-His
D.
Normalized pT426
signal intensity
1.5
1.0
0.5
0
0
20
40
60
80
100
time (min)
E.
TAOK2
_
+
Brain lysate
+
+
pSept7T426
Sept7

Figure S6

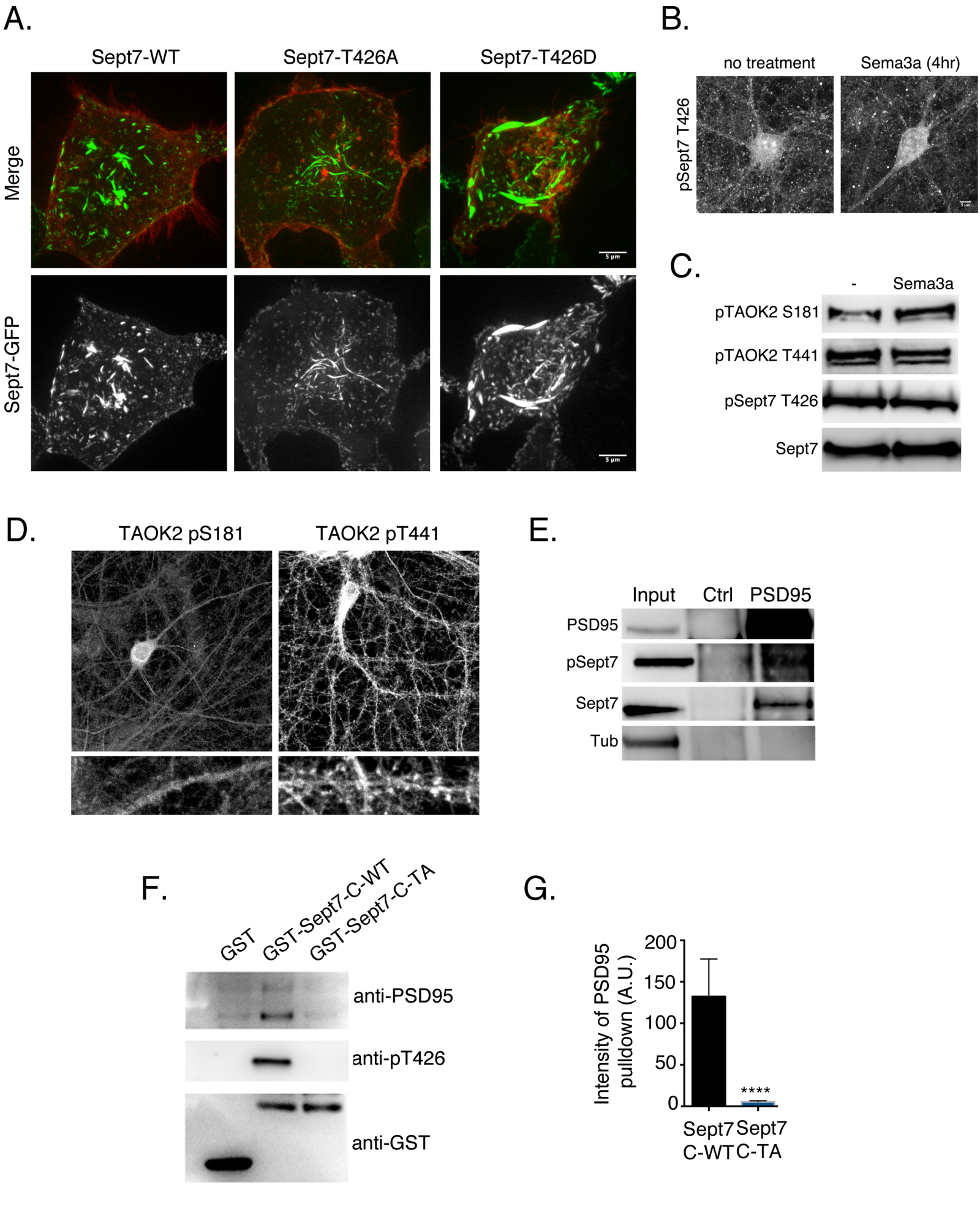

A.
Sept7-WT
Sept7-T426A
Sept7-T426D
Merge
Sept7-GFP
B.
no treatment
Sema3a (4hr)
pSept7 T426
C.
-
Sema3a
pTAOK2 S181
pTAOK2 T441
pSept7 T426
Sept7
D.
TAOK2 pS181
TAOK2 pT441
E.
Input
Ctrl
PSD95
PSD95
pSept7
Sept7
Tub
F.
GST
GST-Sept7-C-WT
GST-Sept7-C-TA
anti-PSD95
anti-pT426
anti-GST
G.
Intensity of PSD95 pulldown (A.U.)
200
150
100
50
0
****
Sept7 C-WT
Sept7 C-TA

Figure S7

SUPPLEMENTARY FIGURE LEGEND

Figure S1. TAOK2 is expressed in hippocampal neurons and its kinase activity is required for spine formation. Related to Figure 1.

(A) DIV16 hippocampal neurons transfected with plasmid encoding GFP to visualize the neuronal morphology along with either control or TAOK2 shRNA were immunostained using an antibody against TAOK2 that recognizes both α and β isoforms. Scale bar = 20μm. The region marked by the white boxes is shown enlarged in the panels on the right to enable visualization of dendritic protrusions. (B) Hippocampal neurons transfected with lentivirus expressing either control or two different shRNA targeting distinct sequences of TAOK2 were lysed and run on a gel, western blot was probed by TAOK2 antibodies to determine level of TAOK2. Tubulin antibody served as a loading control. (C) Dendritic spine morphology on layer 2/3 pyramidal neurons was visualized by the GFP fluorescence in brain slices obtained from P17 mice that were *in utero* electroporated at E14.5 with either control or TAOK2 shRNA. Red arrows point to mushroom spines. (D) The percent of protrusions that were mushroom spines in control and TAOK2 shRNA transfected neurons is plotted. Images were collected from three animals of each control and TAOK2 shRNA transfected pups (n=6 neurons per animal, ****=p<0.0001, Student's t-test). (E) Hippocampal neurons transfected with either TAOK2 shRNA alone or TAOK2 shRNA co-transfected with either wild type human TAOK2-WT or kinase dead TAOK2-K57A on DIV16, were fixed on DIV18, and dendritic spine morphology was analyzed. Scale bar = 5μm. (F) Quantification of dendritic morphology plotted as percent of protrusions that were mature spines (n=15 neurons per condition, ****=p<0.0001, one-way ANOVA).

Figure S2. NMDA-receptors are mislocalized to the dendritic shaft in TAOK2 knockdown neurons. Related to Figure 2.

(A) Hippocampal neurons transfected with either control or TAOK2 shRNA along with either GFP tagged NR2A or NR2B constructs were fixed and imaged at DIV 18. TdTomato expression was used to visualize neuronal morphology and was expressed under a different promoter in the shRNA plasmid. Scale bar = 3μm. (B) Bar graph

depicts the percent of NR2A and NR2B punctae in dendritic protrusions in control and TAOK2 shRNA transfected neurons (n=15 neurons per experimental condition, ****=$p<0.0001$, t-test).

Figure S3. Functional synapses are made directly on the dendritic shaft in absence of TAOK2. Related to Figure 3.

(A) Hippocampal neurons transfected with either control or TAOK2 shRNA (GFP) were incubated with media containing FM4-64 dye for 5 min, washed with media without dye and then fixed to observe the localization of presynaptic membrane contacts on the transfected neurons (red punctae). Scale bar = 5μm. (B-D) Electrophysiological measurement of mEPSC parameters such as area, rise time and decay time in control and TAOK2 knockdown neurons are depicted ($p>0.05$, t-test). (E) Spontaneous ePSCs were recorded from hippocampal neurons transfected with either control or TAOK2 shRNA at DIV14 in Mg+2 free media. The NMDA/AMPA ratio was determined after peaks were aligned and set as t=0ms, and the slower decaying NMDA-mediated currents were measured by averaging current amplitudes between 15-20ms following the peak and dividing them by peak amplitude for each event. (F-G) Event ratios for each cell plotted on a frequency histogram and fit with a Gaussian function is shown. Error bars represent SEM. Bar graph represents the mean and standard deviation of NMDA/AMPA ratios within each neuron transfected with control and TAOK2 shRNA.

Figure S4. TAOK2 phosphorylation sites on candidate substrates identified through mass spectrometry are conserved between mouse and human. Related to Figure 4.

(A) Candidate substrates of TAOK2 from mouse brain lysate and the site of phosphorylation (marked in red) as identified through mass spectrometry are listed along with the homologous sequence in humans. Seven out of the eight sequences are conserved; hence the conserved seven candidate substrates were analyzed further in the study.

Figure S5. Phosphomutants of TAOK2 substrates and their role in dendritic spine morphology. Related to Figure 5.

(A) Representative images of dendritic spines of DIV18 hippocampal neurons transfected with GFP along with the TAOK2 substrate phosphomutants (T>A). Scale bar = 5μm. (B) Mean density of protrusions per unit dendritic length in control and Sept7^{T426A} phosphomutant expressing neurons (n=10 per condition, **= 0.001>*p*<0.01, unpaired t-test with Welch correction). Error bars represent SEM.

Figure S6. TAOK2 phosphorylates Sept7 at residue T426. Related to Figure 6.

(A) TAOK2 phosphorylates full length Sept6/Sept7 complex. Bacterially purified His-Sept6/Sept7 complex was incubated in a kinase buffer with either wildtype TAOK2 kinase domain (1-320 amino acids) or the kinase-dead TAOK2 (K57A) mutant for 60min or 90min, after which, the reaction was stopped by adding sample buffer. Phosphorylation was detected using a phospho-serine/threonine (anti-pS/T) antibody. (B) Specificity of antibody against phospho(T426)Sept7. Purified TAOK2 kinase (WT) or kinase dead (K57A) was incubated with either GST alone, GST-Sept7-C^{WT} or GST-Sept7-C^{T426A} in a kinase buffer and phosphorylation was detected using the pT426(Sept7) antibody. The generated pT426 Sept7 antibody detected a signal only in the WT kinase reaction with GST-Sept7-C^{WT}, but not in presence of kinase dead TAOK2 nor when Sept7-C^{T426A} was incubated with either WT or kinase-dead TAOK2. In contrast, the pan pS/T antibody detected both phosphorylated Sept7 and TAOK2. (C) Purified GST-tagged Sept7 C terminal tail (330-438) and His-tagged TAOK2 kinase domain (1-320) were incubated together in an *in-vitro* kinase reaction for varying lengths of time. Western blot of the reactions were probed for phosphorylated Sept7 using the pT426 antibody, total Sept7 protein using GST antibody and total TAOK2 using His-tag specific antibody. (D) Phosphorylated Sept7 normalized to total Sept7 (ratio of intensity of pT426 antibody signal and GST antibody signal) as a function of time. Error bars represent SEM, n=3. (E) Brain lysate from P13 mice was incubated in a kinase assay either without or with purified TAOK2, and then probed for phosphorylation of Sept7 using the pT426 antibody. Total Sept7 levels were probed by antibody against Sept7.

Figure S7. Phosphorylation at T426 mediates interaction with PSD95 but does not affect septin filament formation. Related to Figure 7.

(A) Phosphorylation at T426 does not affect filament formation. HEK cells were transfected with TdTomato along with either GFP-tagged full length Sept7^{WT}, Sept7^{T426A} or Sept7^{T426D} and after 24hr were fixed and imaged to analyze filament formation. Scale bar = 5μm. (B) Immunofluorescence images of hippocampal neurons before and after 4 hr incubation with purified Sema3a using the antibody against pSept7 (T426). Scale bar= 5μm. (C) Western blot of neuronal lysates incubated with or without Sema3a for 4hr were probed for level of phosphorylated and total Sept7 using the pSept7(T426) and total Sept7 antibody. (D) Hippocampal neurons at DIV18 were fixed and immunostained using antibodies against pS181 and pT441 residues of TAOK2. Higher magnification images depict differential localization of TAOK2 when phosphorylated at S181 and T441. (E) Mouse brain lysate was immunoprecipitated with either control or PSD95 antibody and immunoprecipitates were probed on a western blot with antibodies against PSD95, pSept (T426), Sept7, and Tubulin. (F) Purified TAOK2 kinase was incubated with purified GST, GST-Sept7-C^{WT} or GST-Sept7-C^{T426A} in a kinase reaction, followed by incubation with mouse brain lysate. Glutathione beads were then used to pull down the GST-tagged proteins and the co-immunoprecipitates were probed in a western blot for presence of PSD95 and phosphorylated Sept7. Total GST protein was detected by GST antibody and TAOK2 by His-specific antibody. (G) Normalized signal intensities of PSD95 pulldown from brain lysate with phosphorylated Sept7 WT or the T426A mutant detected through western blot using PSD95 antibody (n=3, p<0.0001, t-test). Error bars represent SD.

Supplementary Movie 1. Related to Figure 1.

Combined time lapse image stacks show representative neurons transfected with control (left) and TAOK2 specific shRNA (right) along with GFP to visualize the dynamics of dendritic protrusions. Image stacks were acquired every 20 sec. Movie compiled at 8 frames per sec (fps). Scale bar = 5μm.

Supplementary Movie 2. Related to Figure 3.

Combined time-lapse image stacks show representative neuron transfected with the calcium sensor GCaMP6f and control shRNA (tdTomato), to simultaneously visualize calcium transients (green) and dendritic protrusions (red).

Dual color images were acquired at a single focal plain every 0.3s. Note enrichment of calcium signal in the dendritic spines. Movie compiled at 8fps.

Supplementary Movie 3. Related to Figure 3.

Combined time-lapse image stacks show representative neuron transfected with the calcium sensor GCaMP6f and TAOK2 specific shRNA (tdTomato), to simultaneously visualize calcium transients (green) and dendritic protrusions (red). Calcium signals are spread across large areas of the dendritic shaft. Dual color images were acquired at a single focal plain every 0.3s. Movie compiled at 8fps.

Supplementary Movie 4. Related to Figure 6.

Combined time-lapse image stacks show representative neurons transfected with tdTomato and either Sept7^{T426D} or Sept7^{T426A} to simultaneously visualize Sept7 localization (green), along with dynamics of dendritic protrusions (red). Dual color image stacks were acquired every 1min. Scale bar=2μm.

SUPPLEMENTARY METHODS

Reagents: DNA Constructs, shRNA, Lentivirus and Antibodies

Human TAOK2 cDNA in pCMV sport 6.0 (Ultanir et al., 2014) was a gift from lab of Dr. Li-Huei Tsai (MIT). The kinase domain of TAOK2 (1-320 amino acids) was cloned into the pRK5-HA vector using sites SalI and NotI. Rat and human Septin7 cDNA clones were purchased from Transomics, and cloned into pEGFP-C1 vector using sites HindIII and BamHI. Human HDAC6 (#30482), human CEP170 (#41150), rat pEGFP-NR2A (#17924), rat pEGFP-NR2B (#17295), rat pSEP-GluR1 (#24000), pGP-CMV-GCaMP6f (#40755) and pCMV-RGeco1.2 (#45494) plasmids were obtained from Addgene. Vector pRK5-HA-MST3 has been described previously (Ultanier et al., 2014). The mammalian expression vector pEGFP-C3-Sept6 and bacterial bicistronic expression vector His-Sept6/Sept7 were gifts from Dr. Spiliotis (Drexel). The expression vector pcDNA3.1-Bai1 and pCMV5-Caskin1 were gifts from laboratories of Dr. Hall (Emory) and Dr. Südhof (Stanford), respectively. Expression vector for rat RFP-PSD95 was gift from laboratory of Dr. Nicoll (UCSF). Site directed mutagenesis was used to create analog sensitive mutations in TAOK2 as well as phosphorylation site mutations of TAOK2 substrates where Threonine was mutated to Alanine or Aspartate. All clones were verified by sequencing. For knockdown experiments, shRNA with a scrambled sequence containing no matches to rat mRNA sequences targeting 5'AGACCCAAGGATTAGAAGG 3' was used as a control (shCtrl). TAOK2 shRNA#1 was generated using the sequence: 5'CACCCACAGTCATCATGGA3' and TAOK2 shRNA#2 using sequence: 5' GGGACAATATGATGGCAAA3'. All shRNA sequences were 19 base pairs long and were selected via http://katahdin.cshl.org/html/scripts/main.pl. Hairpins targeting these sequences were cloned in pLentiLox 3.7, which expresses either EGFP or tdTomato via a separate promoter in addition to expressing shRNA via a U6 promoter. Lentivirus expressing expressing control shRNA, TAOK2 shRNA #1, and TAOK2 shRNA #2 were generated at UCSF core facility (UCSF ViraCore). Purified recombinant Sema3a was purchased commercially (R&DSystems).

Antibodies used in the study are as follows: Thiophosphate ester (Epitomics, #2686-1, Rabbit), Phosphoserine/threonine (BD Transduction Lab, 612548, Mouse IgG1), Phosphothreonine (Cell Signalling, #9381, Rabbit), PSD95 (ThermoScientific, 6G6-1C9, Mouse IgG2a), Strep tag II (abcam, ab76949, Rabbit), Homer1a (SynapticSystems, Rabbit, 160003), Bassoon (Abcam, ab82958), GFP (chicken), GFP (NeuroMab, Mouse, 75-131), TAOK2 (ThermoScientific, Rabbit, PA5-29249), Sept7 (Sigma, Rabbit, HPA029524), pTAOK2 (T441) (Ultanir et. al., 2014), pTAOK2(S181) (Santa Cruz, Rabbit, sc135712), GST (NeuroMab, Mouse clone100/13), HA-tag (Roche, Mouse, 12CA5), tetra-His-tag (Qiagen, Mouse, 34670).

Immunofluorescence Assay

Hippocampal neurons were fixed with room temperature 4%PFA+4%sucrose for 20min followed by incubation with blocking buffer (10% normal donkey serum, 0.2M Glycine pH7.4, 1% TritonX-100 in phosphate buffer saline (PBS)) for an hour at room temperature. Coverslips were incubated with primary antibodies diluted in blocking buffer overnight, washed six times with phosphate buffered saline (PBS) followed by incubation with secondary antibodies for 3hr. After washes with PBS, the coverslips were mounted on slides using mounting media (FluoromontG, EMS).

Dendritic Spine Morphology Analysis

Confocal images of dendritic spines were acquired using the Leica SP5 LSM on a 63X objective with 6X zoom and 0.3µm z-step size. Maximum projection images obtained through ImageJ were analyzed to determine spine morphology. Spines were manually scored and binned in one of the following four categories: Spiny (mushroom shaped head with thin neck), filopodia (long, thin with no head), stubby (short with no neck) or atypical (none of the aforementioned morphology). A minimum of 15 spines per image, were scored and a minimum of 15 neurons from 3 independent experiments per condition, were analyzed. Dendritic lengths were measured in ImageJ using the Measure function.

Spine Motility Analysis

Hippocampal neurons grown on 35mm MaTek glass bottom dishes were imaged at DIV16-18 in live cell imaging media (125mM NaCl, 5mM KCl, 10mM HEPES pH7.2, 2mM $CaCl_2$, 1mM $MgCl_2$ and 10mM Glucose) on the Nikon Ti-E microscope using a 60X oil objective. Confocal z-stack 4D images were acquired every 20 sec for 10

minutes. Maximum projections of the z-stacks were created using ImageJ. At least ten spines per neuron were analyzed; where increase in spine length between successive frames was quantified as a spine extension, decrease in length as retraction, and change in spine shape as a morphing event. Five different neurons per condition were imaged in five independent experiments.

***In utero* Electroporation**

Timed pregnant CD-1 mice (Charles River) were used for in utero electroporation with control and TAOK2 shRNA. Surgery was performed at E14.5 when mice were anesthetized with isoflurane, which was delivered as a known percentage (4% for induction, 1.5% for maintenance) in oxygen from a precision vaporizer. A midline incision was made and the uterus was exposed. DNA solution including the plasmid and 0.04% trypan blue was injected into the medial region of the lateral ventricle of the brain with a glass micropipette. Embryos were injected with either 0.4 μg/μl pCAG-GFP + 1 μg/μl control shRNA or 0.4 μg/μl pCAG-GFP + 1 μg/μl TAOK2 shRNA. Electrical pulses then were delivered to embryos by electrodes connected to a square-pulse generator (ECM830, BTX). For each electroporation, five 35-V pulses of 50ms were applied at 1s intervals. After the electroporation, the uterus was returned to the abdominal cavity, followed by suturing of the abdominal wall and skin. Mice were perfused at P17 using 4% paraformaldehyde followed by post-fixed overnight incubation in 30% sucrose at 4°C. 100 μm thick coronal sections were obtained using cryostat sectioning. Sections were dried on glass slides, blocked by 10% Normal Donkey Serum and 0.5% Triton-X and immunostained by mouse GFP antibody (Roche) in blocking buffer, followed by goat anti-mouse conjugated with Alexa-488 (Invitrogen).

Synaptic Scoring

Three channel images of hippocampal neurons transfected with cytosolic GFP was immunostained with antibodies against GFP, Homer1a and Bassoon were acquired. GFP channel provided the neuronal morphology, Homer1a the postsynaptic marker and Bassoon was the presynaptic marker. To score for synaptic density as well synaptic localization, the GFP channel was converted to grayscale and then inverted. The Bassoon (red) and Homer1a (cyan) channel were made into a composite image using ImageJ and the colocalized punctae were annotated as a synapse. This annotated composite image was then combined with the inverted GFP image to determine whether the synapse was on the shaft or spine based on the GFP membrane marker.

FM4-64 Labeling

A stock solution of FM4-64 dye (Life Technologies) was prepared in water to 1mg/ml and then diluted in live cell imaging media to a working solution of 5μg/ml. Coverslips were immersed in depolarization staining solution (5μg/ml dye + live cell imaging media + 90mM KCl) for 30sec, washed for 10min in media, fixed with 4%PFA+4%sucrose and visualized under a microscope within the same day.

Electrophysiology

All electrophysiological recordings were performed from cultured rat hippocampal neurons plated on poly-D-Lysine coated coverslips and transfected with shRNA constructs using Lipofectamine2000 24hr prior to recording. For mEPSC recordings on DIV16/DIV18, coverslips were transferred to a recording chamber containing aCSF (artificial cerebrospinal fluid) bubbled with carbogen gas (95% O_2, 5% CO_2). ACSF contained in mM: 127 NaCl, 1.8 KCl, 1.2 KH_2PO_4, 26 $NaHCO_3$, 2.4 $CaCl_2$, 1.3 $MgSO_4$ and 15 glucose, and was adjusted to 305-310 mOsm with sucrose and then sterile filtered. 1μM tetrodotoxin and 100μM picrotoxin were added to block Nav channels and GABAA receptors, respectively. Recording pipettes were pulled from 1.5/0.86 mm borosilicate glass (Sutter Instrument, Novato CA), and then fire-polished to a resistance of 3-5 MΩ with a microforge. Pipettes were filled with a recording solution containing, in mM: 129.5 K-Gluconate, 6.5 KCl, 2 MgCl2, 4 Na-ATP, 0.2 EGTA, 10 HEPES, pH 7.2 (with KOH) and adjusted to 295-300 mOsm with sucrose. Membrane patches were established with gentle negative pressure, and were used only if the seal resistance exceeded 3 GΩ prior to break-in. After seal rupture, cells with a series resistance above 25 MΩ were discarded. Recordings were made using a Multiclamp 700 amplifier system (Axon Instruments), and were sampled at 10 kHz and low-pass filtered online at 4 kHz (Bessel). Cells were held at -65mV to record excitatory currents for >2 minute each, and only cells with a stable baseline were used for analysis. Spontaneous events were detected using the template detection algorithm in Clampfit10 and were simultaneously fit with products of two exponentials. Events were also examined manually to ensure accurate fits of exponential functions. Event parameters for individual neurons were averaged and an average of averages was calculated for comparison between transfection conditions. To quantify the presence of AMPA and NMDA receptors, we recorded spontaneous ePSCs in DIV 14-16 neurons using a Mg2+-free ACSF solution, containing 128 mM NaCl, 1.8 mM KCl, 1.2 mM KH_2PO_4, 26

mM $NaHCO_3$, 2.4 mM $CaCl_2$, 15 mM glucose, 0.1 mM picrotoxin and 1 μM TTX, instead of normal ACSF. Events were collected for analysis by a threshold-based detection method. To measure the NMDA/AMPA ratio, peaks were aligned and set as t=0ms, and the slower decaying NMDA-mediated currents were measured by averaging current amplitudes between 15-20ms following the peak and dividing them by peak amplitude for each event. Event ratios for each cell were plotted on a frequency histogram and fit with a Gaussian function to determine mean and standard deviation of NMDA/AMPA ratios within each cell. Statistical analyses (unpaired Student's t-tests) were performed in GraphPad Prism software and a threshold for statistical significance of p=0.05 was used.

Calcium Imaging

Hippocampal neurons were plated on 35mm MaTek glass bottom dishes and transfected at DIV16/18 with calcium sensor GCaMP6f along with control or shRNA against TAOK2 that co-expresses cytosolic tdTomato to visualize the neuronal morphology. In other experiments, the red genetically encoded calcium sensor RGeco1.2 was cotransfected with either phosphomimetic or phosphomutant GFP-tagged Sept7. Prior to imaging the neurons were incubated in ACSF media containing 1mM $MgCl_2$, 1μM TTX for 10 minutes followed by incubation in ACSF+TTX without $MgCl_2$. After 5 min, dishes were transferred onto a 37°C heated microscope stage and imaged using a 60X Nikon objective for 10 minutes, where a two-channel single focal frame image was acquired every 300ms.

TAOK2 Kinase assays

HA-tagged TAOK2 kinase (1-320 amino acids) was expressed in HEK293T cells grown in media containing 10% FBS, 1X penicillin/streptomycin in Dulbecco's Modified Eagle Medium. TAOK2 kinase was purified via HA epitope tag using Anti-HA Affinity matrix (Roche, clone 3F10). Briefly, cells were incubated on ice in lysis buffer (1% TritonX100, 10% glycerol, 1mM Na_3VO_4, 20mM β-glycerol phosphate, 50mM NaF, 1 X complete protease inhibitor cocktail (Roche), 1 X phosphatase inhibitor cocktail I or III (Sigma) in 20mM Tris-HCl pH 8.0 and 150mM NaCl) for 30 min and homogenized by passing through a 25gauge syringe. The supernatant was collected after centrifugation at 20,000g for 15 min at 4°C, pre-cleared using IgG-Sepharose (GE Healthcare) for 30 min at 4°C and then incubated with HA-affinity matrix for 3 hours at 4°C. Beads were washed twice with lysis

buffer, incubated with 1M NaCl for 10 min, followed by a 10 min wash with lysis buffer at 4°C. Following this beads were washed twice with the kinase reaction buffer not including ATP. Kinase assay was conducted in 20mM Tris-HCl pH7.5, 10mM $MgCl_2$, 1mM dithiothreitol (DTT), 100μM ATP, 1x phosphatase inhibitor cocktail and 0.5mM of one of the following three ATP analogs: ATPγS, 6-Bn-ATPγS (BN) or 6-Furfuryl-ATPγS (FF) (BioLog Life Science Institute) for 30 min at 30°C. Phosphorylation reaction was followed by alkylation reaction by addition of 2μl of 100mM p-nitrobenzyl mesylate (PNBM) per 30μl of kinase reaction for 1 hour at room temperature. Sample buffer was added to the reactions, incubated at 90°C for 10 min, run on 4-12 % Bis–Tris gel (Novex) and then analyzed by western blotting. TAOK2 auto-phosphorylation was detected by anti-thiophosphate ester antibody followed by HRP conjugated secondary antibody incubation. Western blots were imaged on a VersaDoc Imager after ECL incubation (Thermo).

Mouse Brain Extract

To prepare mouse brain extracts, P13-P15 mice pups were euthanized by CO_2 (5psi for 10min) followed by decapitation. The brains were dissected out and incubated in high detergent lysis buffer (20mM Tris pH7.5, 100mM NaCl, 10mM $MgCl_2$, 0.5mM DTT, 1% TritonX-100, 1% deoxycholic acid, 0.2% SDS and protease inhibitors) on ice for 30min. Homogenization was achieved by sonication. Supernatant was collected after centrifugation at 14,000g at 4°C for 10 min, aliquots were prepared by diluting with lysis buffer without detergent to 10-20μg/μl, flash frozen in liquid nitrogen and stored at -80°C.

TAOK2 Substrate Labeling and Covalent Capture

Covalent capture to identify TAOK2 kinase substrates using mass spectrometry was done as previously described (Ultanir et al., 2012; 2014). Briefly, HA-tagged TAOK2 kinase domain was purified from HEK293T cells by affinity purification using HA antibody-coated beads. Untransfected cells and cells expressing HA-tagged kinase-dead TAOK2-K57A were used as negative controls. Cells were lysed with 20mM Tris-HCL, 150mM NaCl, protease inhibitor cocktail (Roche), phosphatase inhibitors (Sigma), 10% glycerol and 1% NP40. Lysate was incubated on ice for 30 min, followed by centrifugation at 20,000g for 15 min. Supernatant was pre-cleared with IgG sepharose beads followed by incubation with 25μL anti-HA resin for 3hr. About 100μl brain lysate at 20μg/μl protein concentration was used for labeling by TAOK2-AS or TAOK2-KD each. A lysate only sample

with no HA beads was used as a second negative control for each experiment. Total 5 experiments were conducted with P15 brain lysates. Labeling reaction comprised of 1μM cAMP-dependent protein kinase inhibitor, 0.2μM protein kinase C inhibitor (BisindolylmaleimideI, Calbiochem), 3mM GTP, 100μM ATP, 1μM okadaic acid and 0.5mM Benzyl-ATP-γ-S. Labeling of the brain lysate was quenched by addition of EDTA to 20mM final concentration. The labeled reaction was denatured by adding solid urea (60% w/v) and TCEP (10mM final volume) and incubating at 55°C for an hour. Samples were then digested by Trypsin (Promega, sequencing grade) after diluting with two volumes of ammonium bicarbonate (Sigma) and the pH adjusted to 8.0, followed by overnight rotation at room temperature. Trypsin digestion was quenched by adding 0.5% TFA (Trifluoroacetic acid, Sigma). Peptides were ascertained to be at pH3.0, then desalted and cleaned using C18 cartridges (Waters Sep-Pak Classic C18 Cartridges), and finally eluted by 70% ACN (Acitonitrile) and 0.1% TFA. Peptide solution was reduced in volume by speedvac and final solution adjusted to 50% ACN and 50mM HEPES. Samples were evenly adjusted to pH7.0 using 5% NaOH, incubated with SulphoLink Coupling Resin (ThermoScientific, 20401) and mixed overnight on a nutator. Addition of 25μg Bovine Serum Albumin (BSA) ensured low background binding. The beads were washed over equilibrated fritted columns (Sigma) sequentially using 5M NaCl, 50% ACN, 5% Formic Acid and 1mM DTT. Bound phospho-peptides were eluted through hydrolysis reaction by addition of Oxone (1mg/ml Oxone in ddH2O freshly prepared, Sigma 228036) and then desalted and prepared for mass spectrometry through C18 resin ziptip (C18 resin Millipore ZTC18S096).

Reverse-phase LC-MS/MS Analysis

Peptides were resuspended in 10μl 0.1% formic acid, and analyzed by nanoflow-UPLC -MSMS. Samples were separated by nano-flow liquid chromatography using a 75-μm x 150-mm reverse phase C18 column PepMap® C18 EasySpray column (Thermo Scientific) packed with 3μm particles, at a flow rate of 300nL/min in a NanoAcquity UPLC system (Waters). Mobile phase A was 0.1% formic acid in water and mobile phase B was 0.1% formic acid in acetonitrile. Following equilibration of the column in 2% solvent B, an aliquot of each digest (5μl) was injected, then the organic content of the mobile phase was increased linearly to 30% over 60 min, and then to 50% in 2 min. The liquid chromatography eluate was coupled to a hybrid linear ion trap-Orbitrap mass spectrometer (LTQ-Orbitrap Velos, Thermo Scientific, San Jose, CA). Peptides were analyzed in positive ion

mode and in information-dependent acquisition mode to automatically switch between MS and MS/MS acquisition. MS spectra were acquired in profile mode using the Orbitrap analyzer in the m/z range between 350 and 1500. For each MS spectrum, the 3 most intense multiple charged ions over a threshold of 2000 counts were selected to perform HCD and ETD experiments. Product ions were analyzed on the Orbitrap (HCD) and in the linear ion trap (ETD) in centroid mode. HCD activation time was set to 100ms; normalized collision energy was set to 30. ETD reaction time was adjusted to 100ms for precursor ions with z= +2, and automatically adjusted in a ratio inversely proportional to the charge state of the precursor A dynamic exclusion window of 0.5Da was applied that prevented the same m/z from being selected for 60 s after its acquisition.

Peak lists were generated using PAVA in-house software (Guan et al., 2011), based on the RawExtract script from Xcalibur v2.4 (Thermo Fisher Scientific, San Jose, CA). The peak lists were searched against the murine subset of the UniProtKB database as of June 17, containing 73955 entries, using in-house ProteinProspector version 5.10.17 (public version is available on line) (Clauser et al., 1999). A randomized version of all entries was concatenated to the database for estimation of false discovery rates in the searches. Peptide tolerance in searches was 20 ppm for precursor and either 30 ppm (HCD data) or 0.6 Da (ETD data) for product ions, respectively. Peptides containing two miscleavages were allowed. Carboxymethylation of cysteine was allowed as constant modification; acetylation of the N terminus of the protein, pyroglutamate formation from N terminal glutamine, oxidation of methionine, and phosphorylation of serine, threonine and tyrosine were allowed as variable modifications. The number of modification was limited to two per peptide. A minimal ProteinProspector protein score of 20, a peptide score of 15, a maximum expectation value of 0.01 for protein and 0.05 for peptide were used. A minimal discriminant score threshold of 0.0 was used for identification criteria.

Protein Expression and Purification

TAOK2 kinase domain (1-320 amino acids) was cloned into the pET28a-StrepII-sfGFP bacterial expression vector using BamHI and NotI sites. The StrepII tag was used for protein purification using Strep-Tactin Superflow Plus beads (Qiagen), and the His-tag for protein detection on western blot. Full-length proteins His-Sept6/Sept7 heteromer encoding recombinant expression plasmid (Spiliotis lab) was transformed into BL21 bacterial strain. After bacterial cultures reached an OD600 of 0.8, protein expression was induced with 0.5mM

IPTG for 16 h at 18°C. Bacteria were centrifuged at 5,000 rpm for 5 min at 4°C. Pellets were resuspended in buffer containing 1% Triton X-100, 50mM Tris pH 8.0, 150mM NaCl, 10% glycerol, and 10mM imidazole, and lysed using sonication. Supernatants were clarified by centrifuging at 14,000g for 30 min at 4°C and then loaded on columns containing 500ul Ni-NTA beads (Qiagen). Columns were washed with 10 ml washing buffer (50mM Tris, pH 8.0, 300mM NaCl, 10% glycerol, and 10mM imidazole). Proteins were eluted with elution buffer (50mM Tris, pH 8.0, 300mM NaCl, 10% glycerol, and 250mM imidazole) and dialyzed overnight in buffer containing 50mM Tris, pH 8.0, 150mM NaCl, and 10% glycerol. Septin7 C-terminal coiled coil tail (321-438 amino acids) was cloned into the pGEX4T1 vector using restriction sites BamHI and XhoI, and site-directed mutagenesis was used to create the T426A mutant and then transformed into the BL21 bacterial strain to bacterially express the GST-tagged Sept7C-WT and Sept7C-T426A proteins. A 25ml starter culture grown from a single colony overnight was used to inoculate 1L culture, which was allowed to grow till OD of 0.6 at 37°C. Protein expression was induced by addition of 120mg IPTG and grown for another 5hr at 30°C. Bacteria was collected by a 15min spin at 5000rpm, washed with ice cold 150mM NaCl and the pellet was then resuspended in ice cold lysis buffer (50mM Tris pH8.0, 5mM EDTA, 150mM NaCl, 10% glycerol, 5mM DTT, protease inhibitors and PMSF). Further lysis was achieved by incubating the lysed bacteria for 30min on ice after addition of 4mg lysozyme (Sigma) followed by addition of 0.5% TritonX-100 and sonication on ice. The supernatant was collected after 30min spin on a SLA600 rotor at 13000rpm and then incubated with GST beads (GE Healthcare) overnight. Beads were washed with wash buffer (PBS + 1mMDTT + 0.1% TWEEN) followed by wash buffer without detergent. Bound protein was eluted and collected in fractions by glutathione elution buffer at pH8.0 (50mM Tris pH8.0, 250mM KCl, 1mM DTT and 25mM glutathione)

Purified Protein Kinase Assay

Kinase assay was performed with purified proteins by incubating in the Kinase Buffer (25mM Tris pH 7.5, 10mM $MgCl_2$, 1mM DTT, 0.5mM ATP or ATPγS, protease inhibitors (EDTA free Complete Protease inhibitor, Roche) and phosphatase inhibitors (Halt, ThermoFisher)) for 30 minutes at 30°C on a nutator. Time course to assay level of septin7 phosphorylation was performed by incubating purified GST-Sept7C^{WT} (330-438) with purified TAOK2 (1-320) in the presence of kinase buffer for the indicated time after which sample buffer was added. The samples

were run on an 4-12% Bis-Tris gel (Novex), transferred on a PVDF membrane (Millipore) and phosphorylation was measured by probing the western blots with either the generated anti-pT426 antibody, anti-thiophosphate ester, anti-pThreonine antibody or the anti-pS/T antibody. Level of phosphorylation was quantified by plotting the ratio of the pT426 and the GST signal intensity as a function of time.

Septin7 phospho-T426 Antibody Generation

Rabbit antibody against phosphorylated Sept7 (pT426), was generated commercially by YenZym (San Francisco, CA). Highly purified phosphorylated peptide pThr426-Septin7, C-ILEQQNSSR**pT**LEKN and its non-phosphorylated peptide counterpart C-ILEQQNSSRTLEKN were synthesized, and conjugated to a carrier protein to render it immunogenic. Two rabbits were immunized per peptide. The elicited antibody was affinity-purified against the same phospho-peptide used for immunization. The purified antibody was affinity-absorbed against the non-phosphorylated peptide counterpart, to separate the phospho-peptide-specific antibody from the cross-reactive population yielding a phospho-specific antibody.

Co-immunoprecipitation Assay

Brain lysate (0.5mg) was incubated with 10µg TAOK2-WT, 0.5mM ATP and protease and phosphatase inhibitors for 90min at 30°C to allow for phosphorylation of endogenous Sept7. The phosphorylated sample was divided into two parts, one incubated with a control mouse IgG antibody and the other with PSD95 mouse antibody. The lysate plus antibody mixture was then added to 10ul of washed ProteinA Sepahrose4B beads (Life Technologies) and incubated overnight at 4°C. The beads were washed with HKT buffer thrice followed by two times with buffer without detergent. Sample buffer was added and beads were incubated at 90°C for 10min. Western blot was probed with pSept7 antibody to detect interaction of endogenous pSept7 with PSD95.

Protein Binding Assay

Purified proteins (10µg) GST-Sept7C-WT or GST-Sept7C-T426A were bound to 10µl glutathione beads washed in a HKT binding buffer (10mM HEPES pH7.4, 100mM KCl, 1mM DTT, protease inhibitors) by incubating them together for 30min at 4°C. After two washes in the binding buffer followed by one wash in the kinase buffer, 2.5ug of either TAOK2 or kinase-dead TAOK2-K57A were added along with 0.5mM ATP for 90min at 30°C. After phosphorylation reaction, 50µl of 5µg/µl brain lysate was incubated with each reaction at 4°C to allow

interaction of the GST proteins with the brain lysate overnight. GST beads were washed with HKT buffer thrice followed by two washes with HKT buffer without the detergent. Sample buffer was added and beads were incubated at 90°C for 10min and samples were loaded on a 4-12% Bis-Tris gel. Proteins were transferred on PVDF membrane and interaction of pSept7 with PSD95 was probed using mouse PSD95 and generated pSept7 antibodies.

Fluorescence Recovery After Photobleaching (FRAP) Assay

The photobleaching experiments were performed on an inverted Leica Laser Scanning Confocal Microscope SP8 with 63X NA1.4 objective using an Argon laser line. The FRAP wizard in the Leica LSM software was used to define the region of interest (ROI) and perform localized photobleaching. Hippocampal neurons were transfected with GFP-PSD95 along with either the control/TAOK2 shRNA or with Sept7(T426A or T426D) on DIV14 and FRAP experiments were performed on DIV16. The FRAP wizard in the Leica LSM software was used to define the region of interest (ROI) and perform localized photobleaching. Images were acquired before photobleaching (pre) and then every 1 min for 20 min after photobleaching. ImageJ Measure function was used to determine the fluorescence intensity at the ROI, normalized against the initial fluorescence intensity ('pre') and then plotted as a function of time.