

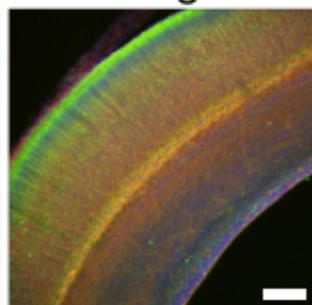
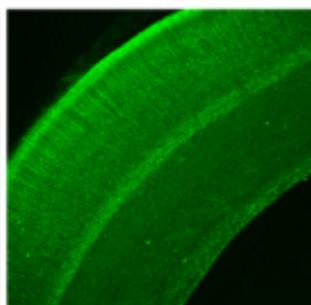
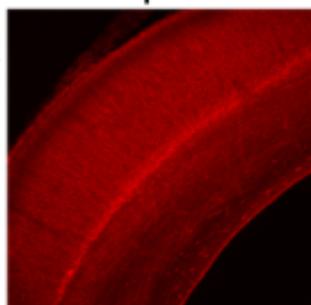
Supplementary figure 1

all spectrin

MAP2

Merge

a

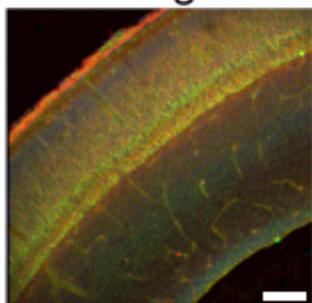
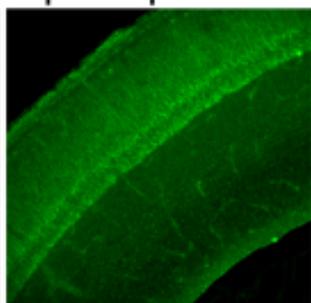
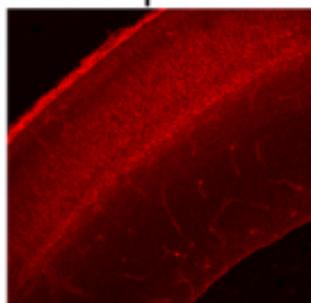


all spectrin

β IV spectrin

Merge

b

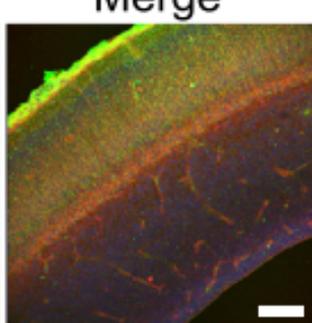
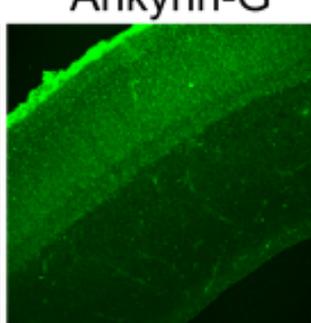
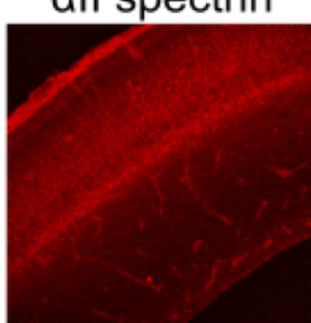


all spectrin

Ankyrin-G

Merge

d

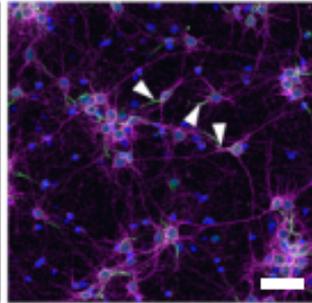
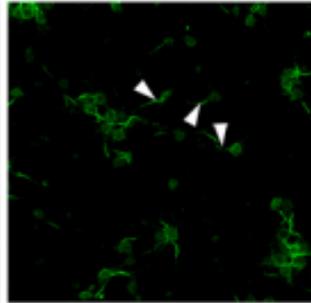
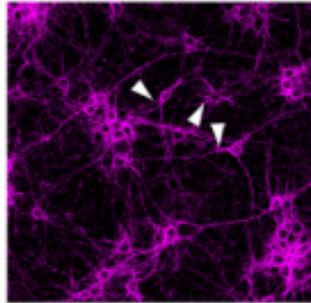


all spectrin

Ankyrin-G

Merge

e



Supplementary Figure 1. Expression of α II spectrin in embryonic rat forebrain. Immunostaining of E18 rat brain sections shows that α II spectrin expression overlaps with MAP2 (a), β IV spectrin (b) and ankryin G (d). Immunoreactivity is enriched in the cortical plate but low in the intermediate zone and ventricular/subventricular zones. The lateral ventricle is in the lower right corner of each panel. Co-immunostaining of α II spectrin and β IV (c) or Ankyrin-G (e) on dissociated rat cortical neurons is shown at higher magnification to highlight co-labeling at the axon initial segment (arrows). The image in panel e is the same as that shown in Figure 2e but with the red channel (RFP) replaced with the green channel (Ankyrin-G). Scale bar: 100 μ m for a, b and d; 50 μ m for c and e.

Supplementary figure 2



b

Sptan1 CRISPR A: 63,139 reads

Wild type: 25,527 pairs of reads, 40.43%
ctggatgggtaccacoggttcaaggagctctctaccttgogggogcagaactggaggattcctatogcttccagttttttcagagagatgctgaggagctggagaagtggattcaggag
L D R Y H R F K E L S T L R R Q K L E D S Y R F Q F F Q R D A E E L E K W I Q E

Mutation 1: 30,671 pairs of reads, 48.58%
ctggatgggtaccacoggttcaaggagctctctaccttgogggogcagaactggaggattcctatogcttccagttttttcagagagatgctgaggagctggagaagtggattcaggag
L D R Y H R F Q G A L Y L A A A E T G G F L S L P V F S E R C -

Mutation 2: 2,164 pairs of reads, 3.42%
ctggatgggtaccacoggttcaaggagctctctaccttgogggogcagaactggaggattcctatogcttccagttttttcagagagatgctgaggagctggagaagtggattcaggaga
L D R Y H R S R S S L P C G G R N W R I P I A S S F F R E M L R S W R S G F R R
agcttcaagttgctctgatgagaactcaaaagaccccaaccacttgagggaagaagctccagaacacacagccttgaagctgaaagtacagggcaactcaggagccattgtgaaagctgg
S F K L P L M R T T K T P P T C R E S S R N T K P L K L K Y R P T Q E P L -

Mutation 3: 1,229 pairs of reads, 1.94%
ctggatgggtaccacoggttcaaggagctctctaccttgogggogcagaactggaggattcctatogcttccagttttttcagagagatgctgaggagctggagaagtggattcaggaga
L D R Y H R S R S S L P C G G R N W R I P I A S S F F R E M L R S W R S G F R R
agcttcaagttgctctgatgagaactcaaaagaccccaaccacttgagggaagaagctccagaacacacagccttgaagctgaaagtacagggcaactcaggagccattgtgaaagctgg
S F K L P L M R T T K T P P T C R E S S R N T K P L K L K Y R P T Q E P L -

Sptan1 CRISPR B, 76,000 reads

Wild type: 39,725 pairs of reads, 52.27%
goccttgggagggatgacccgctgaacagtcacacccctctgagtgccaaaccagatc
A L C A E A D R L Q Q S H P L S A N Q I

Mutation 1: 20,736 pairs of reads, 27.28%
goccttgggagggatgacccgctgaacagtcacacccctctgagtgccaaaccagatc
A L C C R G -

Mutation 2: 6,137 pairs of reads, 8.07%
goccttgggagggatgacccgctgaacagtcacacccctctgagtgccaaaccagatc
A L C R G -

Mutation 3: 1,879 pairs of reads, 2.47%
goccttgggagggatgacccgctgaacagtcacacccctctgagtgccaaaccagatc
A E A D R L Q Q S H P L S A N Q I

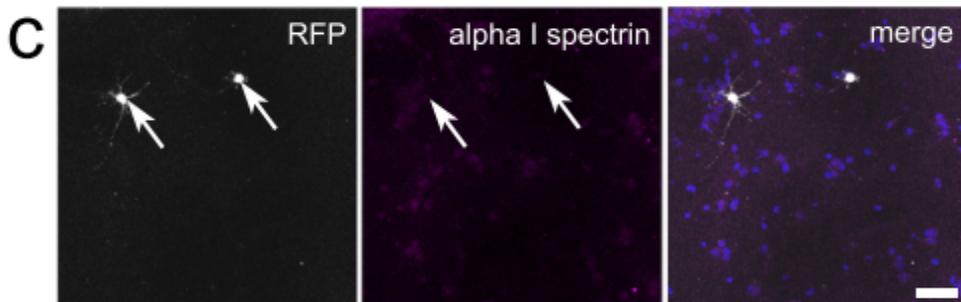
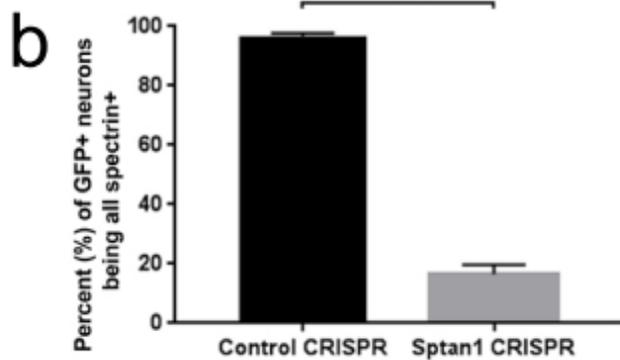
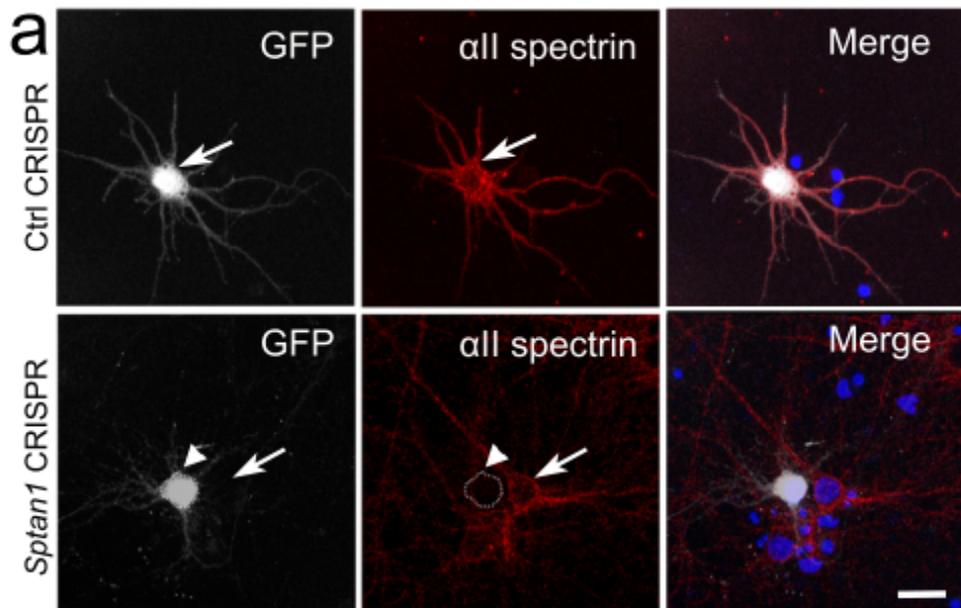
Mutation 4: 1,509 pairs of reads, 1.98%
goccttgggagggatgacccgctgaacagtcacacccctctgagtgccaaaccagatc
A L Q Q S H P L S A N Q I

Mutation 5: 1,211 pairs of reads, 1.59%
goccttgggagggatgacccgctgaacagtcacacccctctgagtgccaaaccagatc
A L T A C N S H T L -

Mutation 6: 766 pairs of reads, 1.98%
goccttgggagggatgacccgctgaacagtcacacccctctgagtgccaaaccagatc
A L P R L T A C N S H T L -

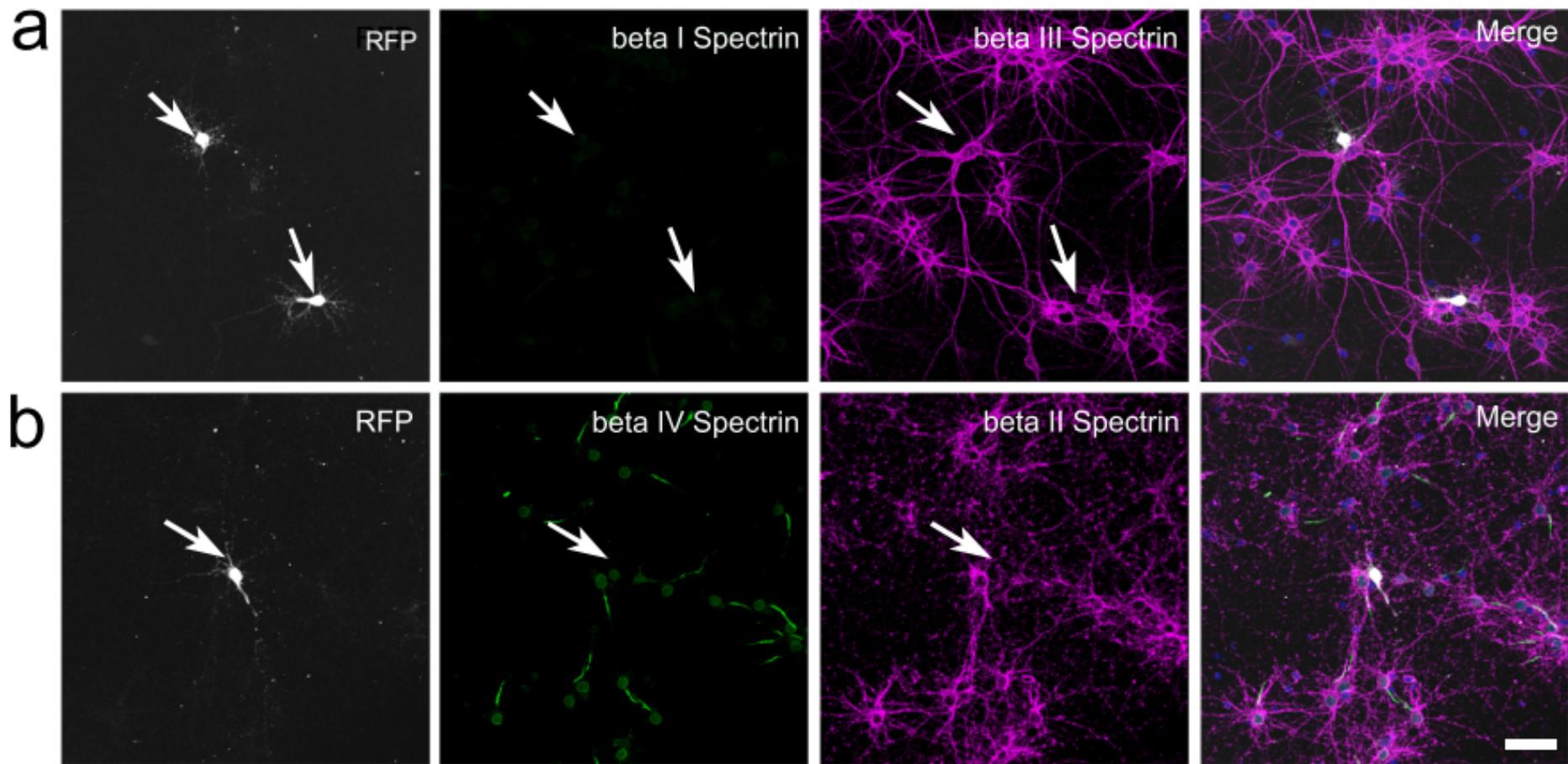
Supplementary Figure 2. a) Two short guide RNAs (sgRNAs) are used to target rat *Sptan1* either at exon 2 or exon 8. b) Next generation sequencing (NGS) shows that 59.6% of CRISPR A and 47.8% of CRISPR B transfected cells exhibit out-of-frame indels (mutations occurring at a frequency of <1% are not shown). This is an underestimate of the actual *Sptan1* knockout frequency for technical reasons (see text).

Supplementary figure 3



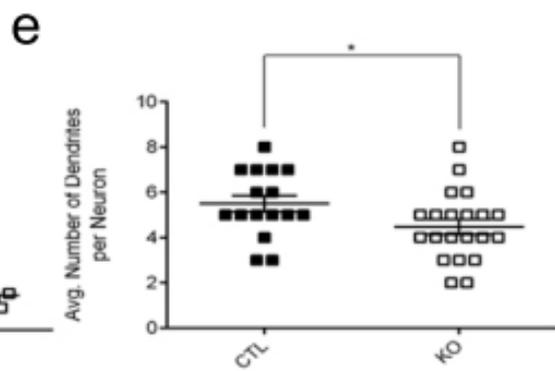
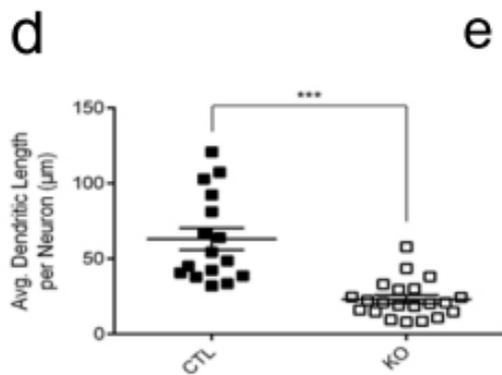
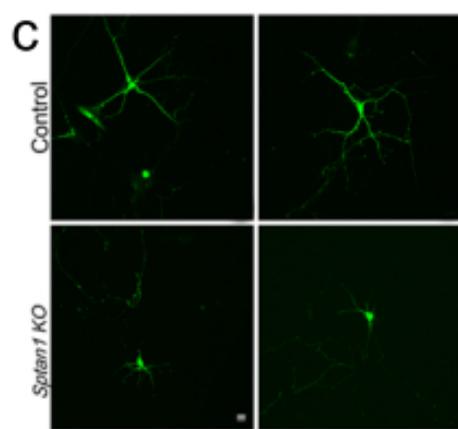
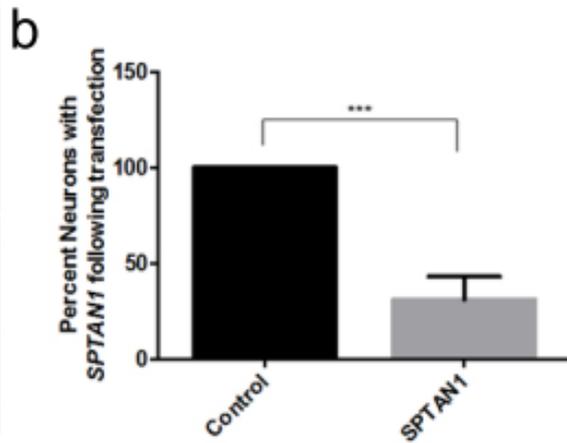
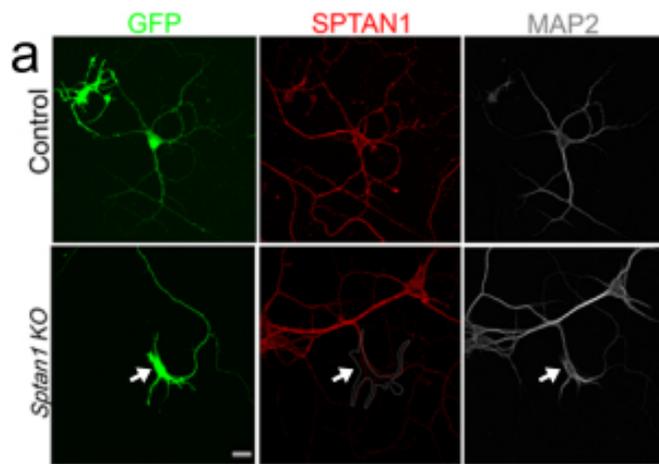
Supplementary Figure 3. a) Representative confocal images of α II spectrin (red) and GFP expression (white) in rat cortical neurons dissociated from brains 4 days after IUE at E14-15 and cultured for 10 days. Upper row: in control CRISPR experiments, the GFP+ cell (top panels, arrow) is α II spectrin+, whereas after *Sptan1* CRISPR knockout, the GFP+ CRISPR sgRNA-transfected cell lacks α II spectrin (bottom panels, arrowhead; outlined in the lower middle panel). Note that an adjacent non-transfected cell (bottom panels, arrow) is α II spectrin+. b) Nearly all (~96.6%) of control CRISPR and a small fraction (~16.5%) of *Sptan1* CRISPR-transfected neurons display α II spectrin immunoreactivity. Percentage data are transformed to arcsin value for statistical analysis. n=107 cells for *Sptan1* CRISPR A and n=118 cells for control CRISPR transfection from 3 experimental replicates. One tailed t test: ****, p<0.00001. c) Unlike α II spectrin, α I spectrin immunostaining (red) shows only slight, likely non-specific labeling of cultured rat cortical neurons, with no change in the RFP+ (white), *Sptan1* CRISPR-transfected neurons (arrows). Bisbenzimidazole nuclear stain is blue in panels a and c. Scale bar: 10 μ m in a, 50 μ m in c.

Supplementary figure 4



Supplementary Figure 4. a) Confocal image showing triple labeling for RFP (white), β I (green) and β III (magenta) spectrins in cultured neurons after co-transfection with *Sptan1* CRISPR sgRNA and RFP. β I spectrin immunoreactivity is absent in all neurons, even the RFP-labeled neurons with *Sptan1* deletion (arrows), while β III expression is strong in all neurons except those with *Sptan1* deletion (arrows). b) Confocal image showing triple labeling for RFP, β IV (green) and β II (magenta) spectrins in cultured neurons after co-transfection with *Sptan1* CRISPR sgRNA and RFP. β IV and β II spectrin immunoreactivity is present in all cells except the RFP-labeled neuron (arrow) with *Sptan1* deletion. Note the expected intense labeling of β IV immunoreactivity at the axon initial segments except for the RFP+ cell (arrow). Bisbenzimidazole nuclear stain is blue. Scale bar: 50 μ m.

Supplementary figure 5



Supplementary Figure 5. Deletion of *Sptan1* in cultured mouse hippocampal neurons. a)

Confocal image showing that control CRISPR-transfected mouse hippocampal neurons are positive for all spectrin and MAP2, while *Sptan1* CRISPR A-transfected neurons express MAP2 but not all spectrin (the all spectrin-negative cell is indicated by an arrow and outlined in light gray in the middle panel). b)

Quantification shows ~75% of *Sptan1* CRISPR A-transfected neurons lack all spectrin

immunoreactivity. c) Upper row: 2 representative hippocampal neurons transfected with control

CRISPR plasmid; lower row: 2 representative hippocampal neurons transfected with *Sptan1* CRISPR A that show fewer and much shorter processes. d) Average dendritic length and e) number of dendrites

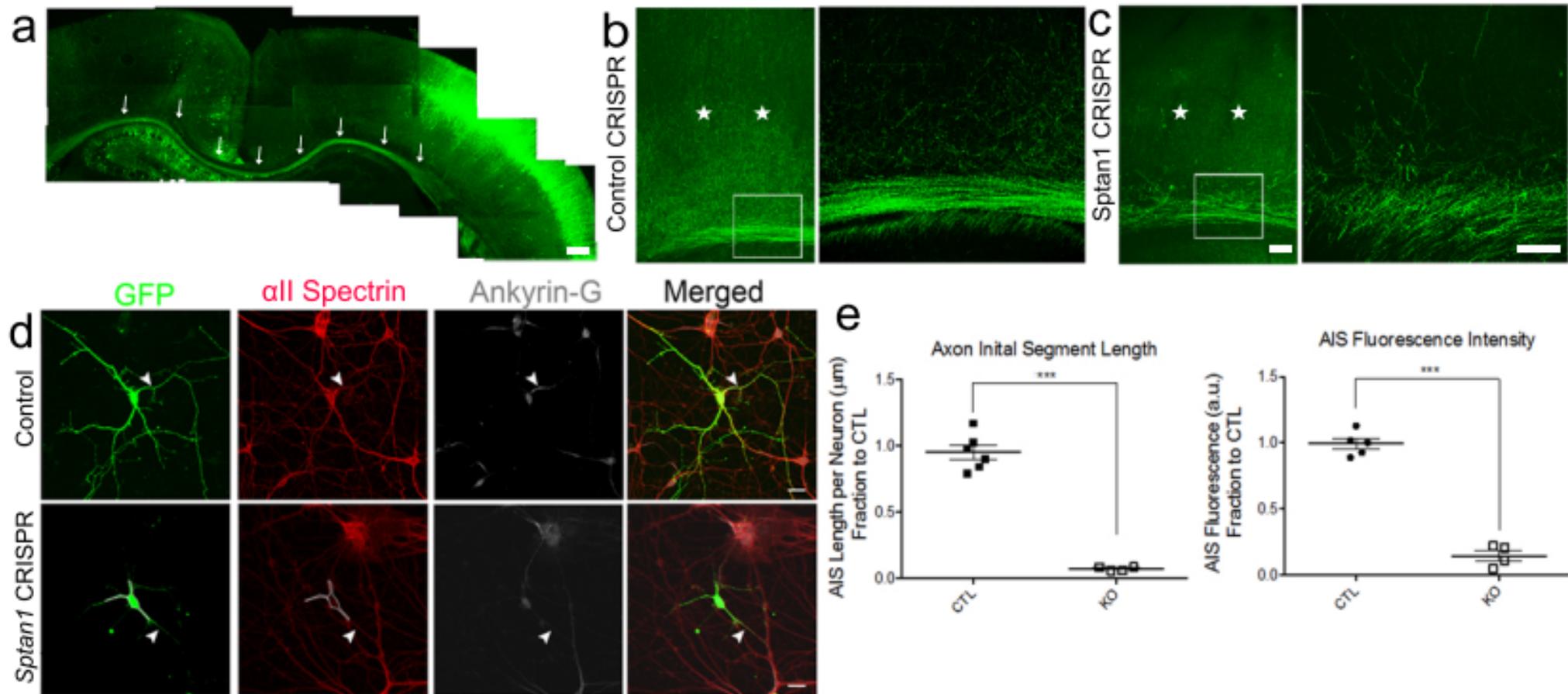
are significantly decreased in the *Sptan1* CRISPR A group. In b) n=17 neurons in *Sptan1* CRISPR A

and n=21 neurons in control CRISPR groups. In d) n=17 neurons in *sptan1* CRISPR A and n=22 in

control CRISPR groups. N=3 replicates in both control and *Sptan1* CRISPR transfection group. *t* test: *,

p<0.05; ***, p<0.001.

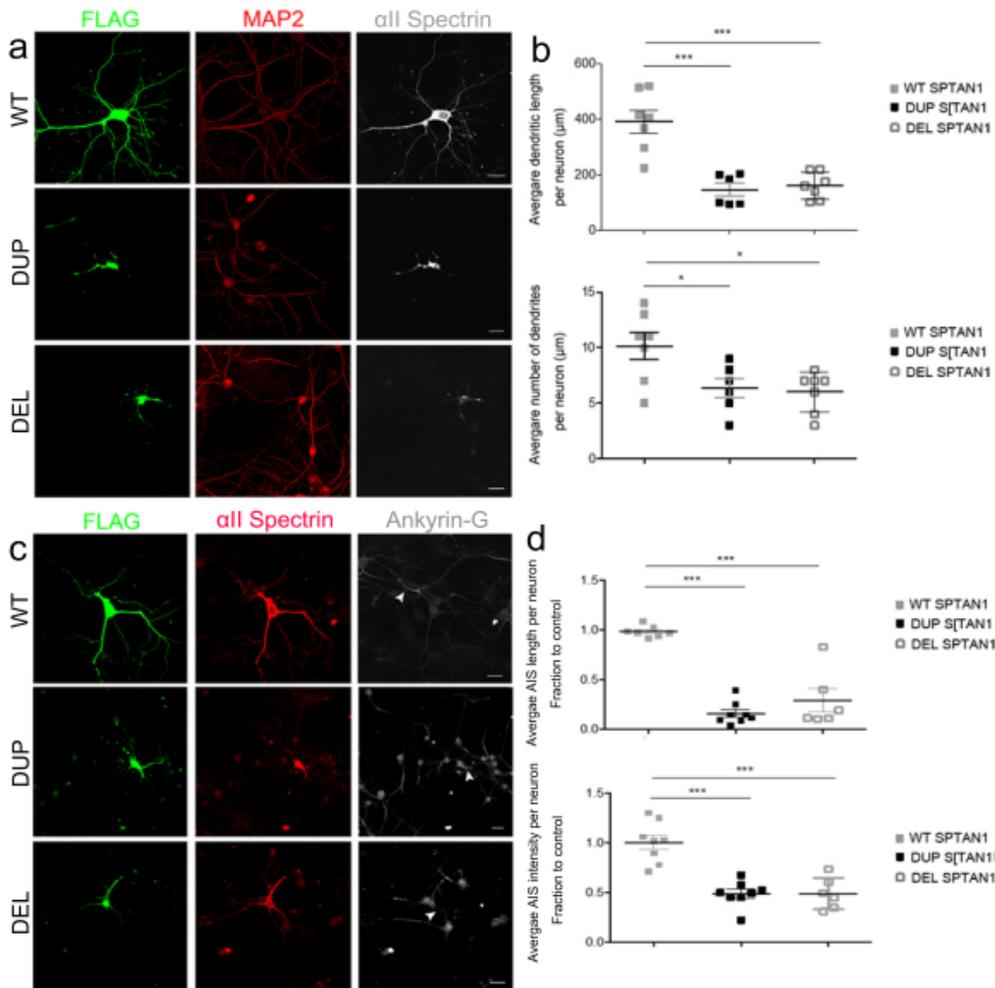
Supplementary figure 6



Supplementary Figure 6. Loss of all spectrin alters axon outgrowth and AIS formation. a)

Stitched confocal images from a control at P21 (previously transfected at E14) showing GFP-labeled axons originating from transfected cells (at right) running ventrally toward the white matter with extensive arborization. Some of the axons extend laterally, whereas others turn toward the midline and fasciculate to form the corpus callosum (arrows). b, c) Compared to control axons b), a representative *Sptan1* CRISPR-transfected brain c) shows decreased and disorganized callosal axons (boxed areas magnified at right in b, c) and arborization in the contralateral hemisphere (asterisks in both panels). d) Upper row: a representative mouse hippocampal neuron transfected with control CRISPR plasmid shows immunopositivity for all spectrin and ankyrin-G (arrowheads); lower row: a representative neuron transfected with *Sptan1* CRISPR A plasmid is negative for all spectrin staining and has very faint ankyrin-G staining. e) The length of the AIS labeled by ankyrin-G and ankyrin-G immunostaining intensity are significantly decreased by *Sptan1* CRISPR A. Scale bar: 500 μ m in a, 50 μ m in b and c, 10 μ m in d. n=4 in the *Sptan1* CRISPR A group and n=6 in the control CRISPR group. *t* test: ***
p<0.001.

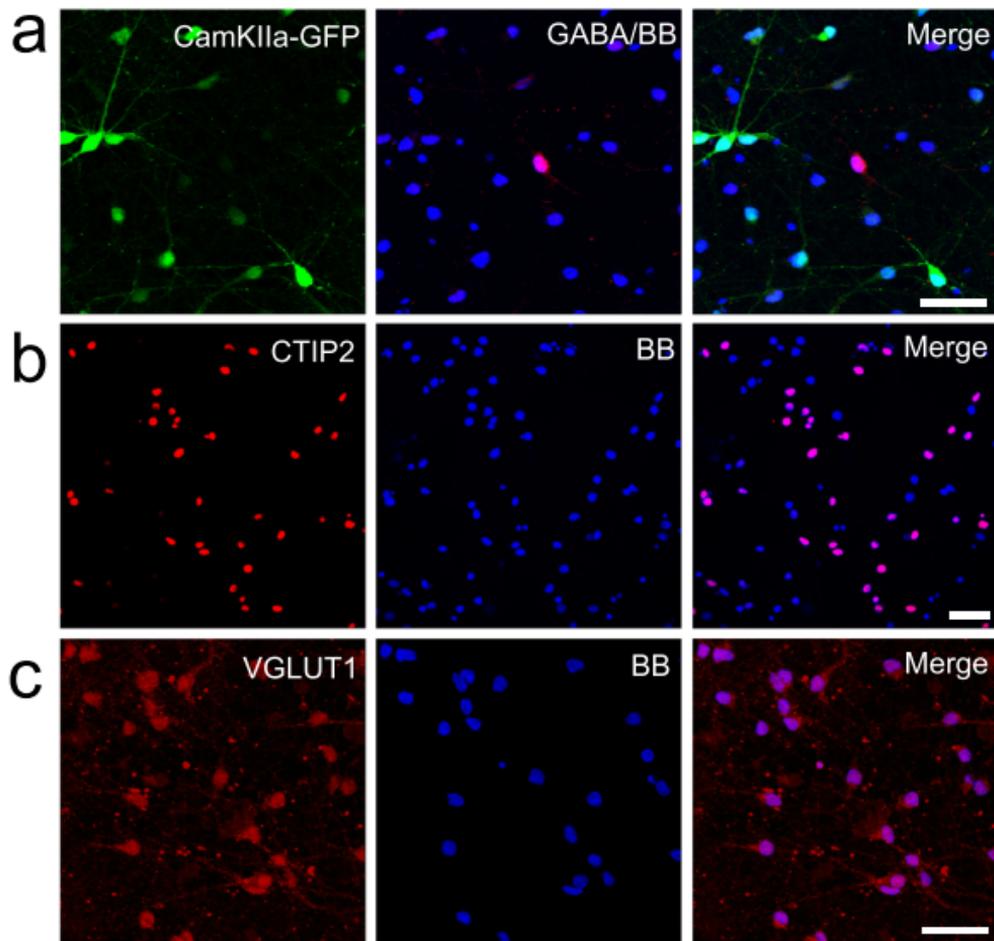
Supplementary figure 7



Supplementary Figure 7. Overexpression of pathogenic *SPTAN1* mutations in *vitro*. a, b)

Confocal images after overexpression of wild type *SPTAN1* (WT), in-frame *SPTAN1* duplication (DUP) and in-frame *SPTAN1* deletion (DEL) in mouse hippocampal neurons reveals that expression of pathogenic mutations significantly decreased dendritic complexity. Also note that the soma size is qualitatively decreased in cells with mutant overexpression (a). c, d) Overexpression of pathogenic mutations also shortened the AIS and impaired ankyrin-G clustering at the AIS. The *SPTAN1* mutants are the same as shown in Fig. 6. Scale bar: 10 μ m. In b) n=7 for the WT *SPTAN1* group, n=6 for the *SPTAN1* Dup group and n=6 for the *SPTAN1* Del group. In d) n=8 in for WT *SPTAN1*, n=6 for *SPTAN1* Dup, and n=6 for the *SPTAN1* Del group. ANOVA test: *, p<0.05; ***, p<0.001.

Supplementary figure 8



Supplementary Figure 8. iPSC-derived neurons are excitatory glutamatergic neurons. a)

iPSC-derived neurons infected with lentivirus expressing GFP under a CamKII α promoter express GFP, indicating they are excitatory neurons. In the middle panel, only one GFP-

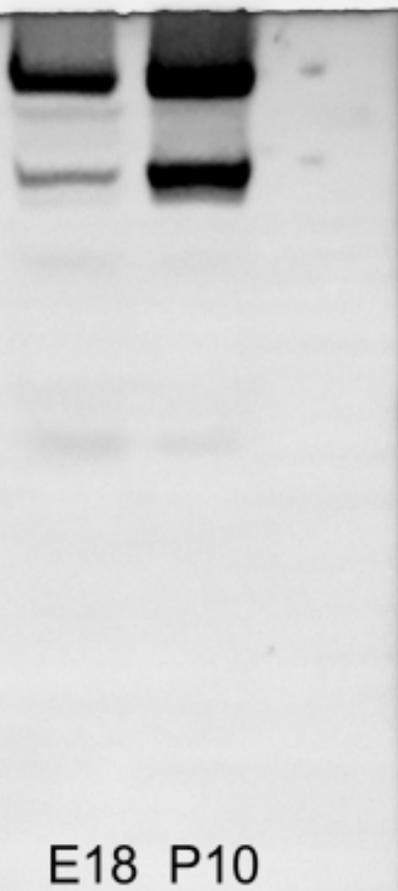
negative cell is positive for the inhibitory neuronal marker, GABA. b) Most iPSC-derived

neurons are CTIP2 positive, a cortical deep layer excitatory projection neuron marker. c) The

neurons also VGLUT1, a vesicular glutamate transporter expressed by excitatory neurons.

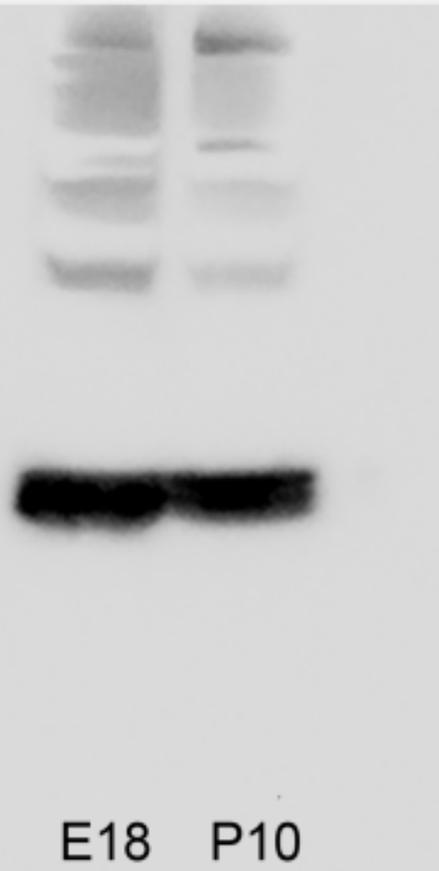
Bisbenzimidazole (BB) in blue. Scale bar: 50 μ m.

250 kD
150 kD
100 kD
75 kD
50 kD
37 kD
25 kD
20 kD



E18 P10

Anti-alpha II spectrin Ab



E18 P10

Anti-beta actin Ab