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Supplemental information

Early developmental deletion of forebrain *Ank2* causes seizure-related phenotypes by reshaping the synaptic proteome

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Early developmental deletion of forebrain *Ank2* causes seizure-related phenotypes by reshaping the synaptic proteome

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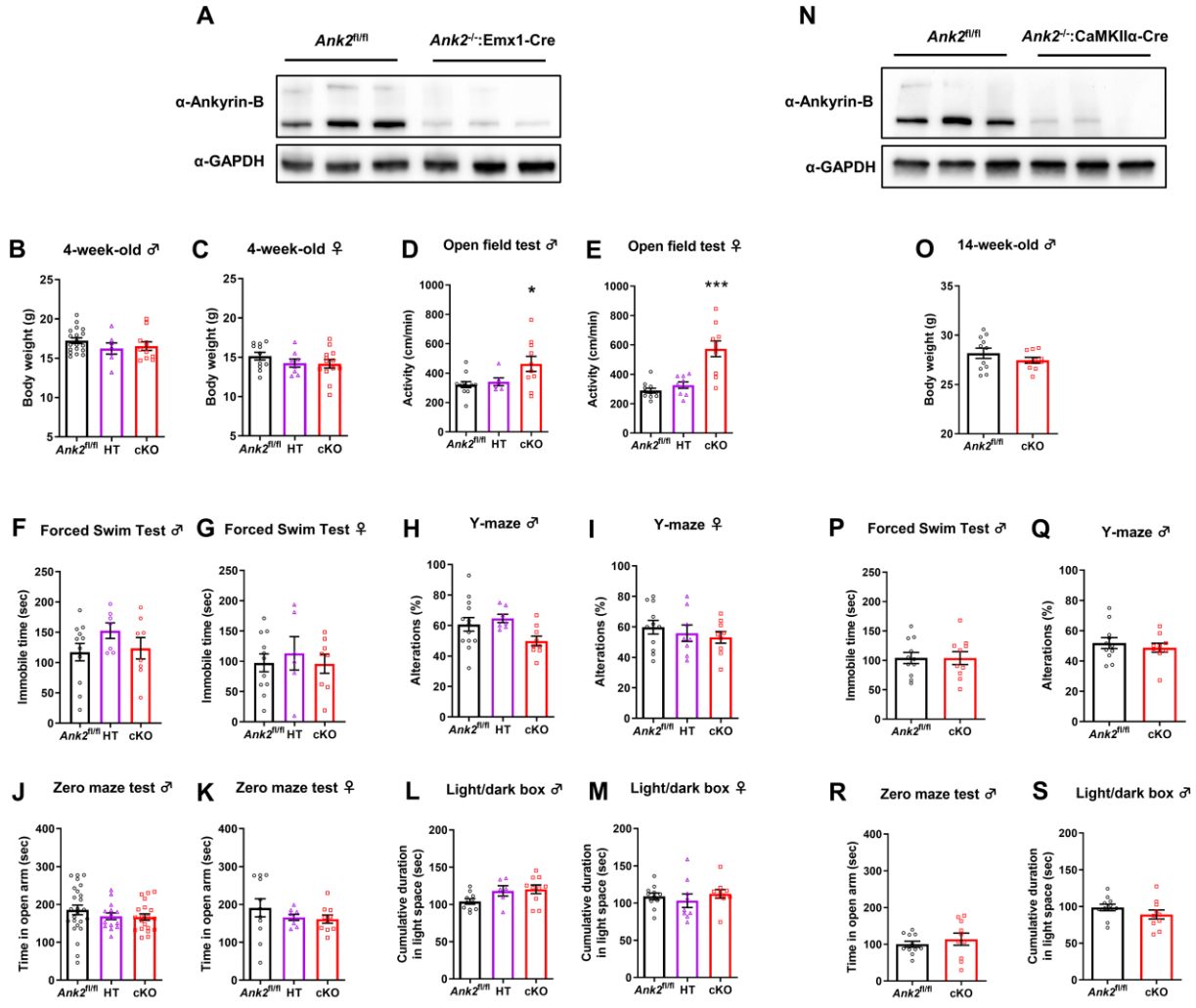
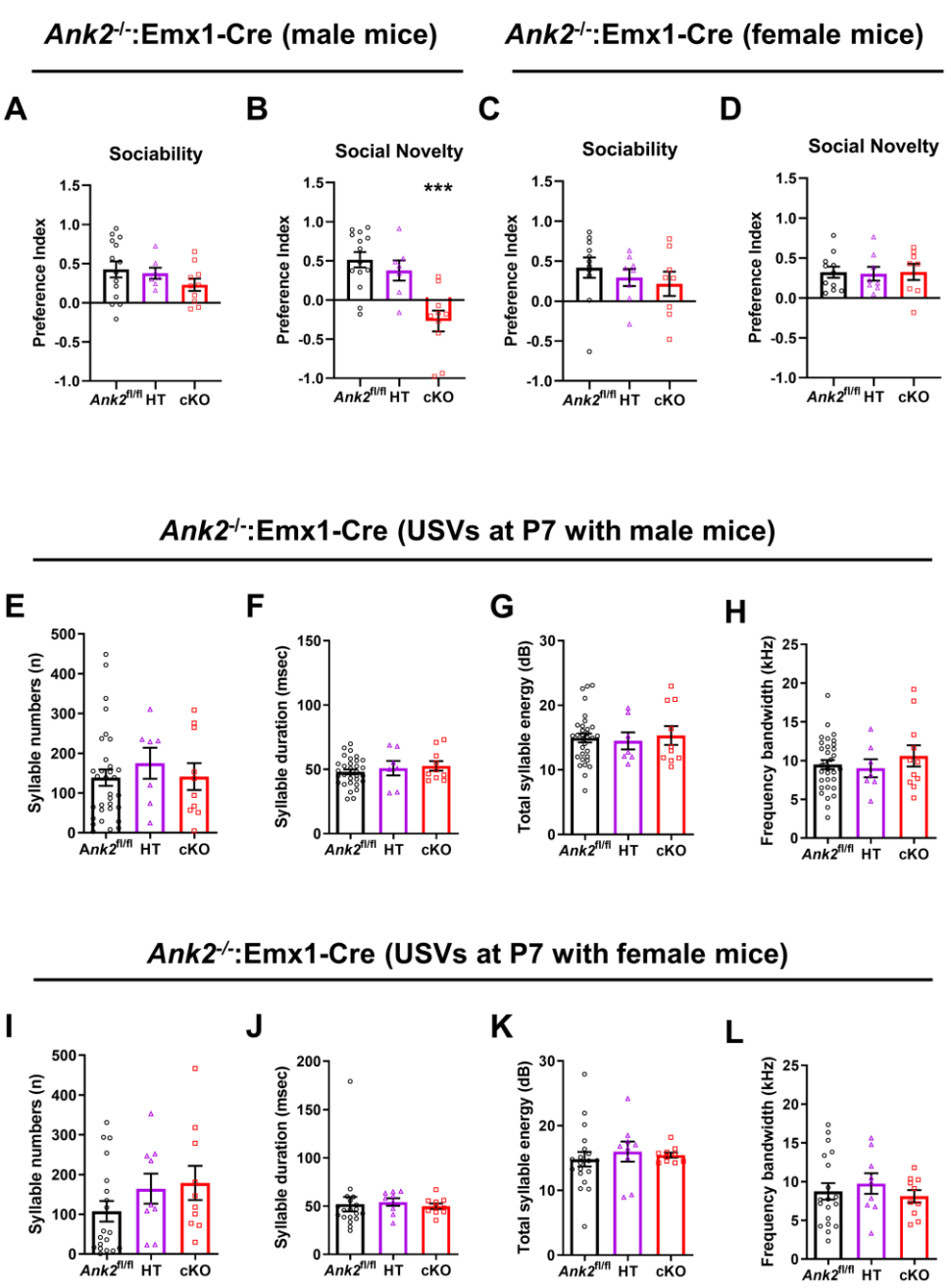
Phenotypes	Ank2 KO mice (Scotland et al., 1998) (Yang et al., 2019)	Ank2 fs mice (Yang et al., 2019)	Ank2 cKO mice (Yang et al., 2019)	Ank2 cKO mice (in this paper)	Ank2 cKO mice (in this paper)
Deletion target organ/cell types	Whole body	Whole body (frameshift R2608)	Neuronal and glial precursors (Nestin-Cre)	Neuronal and glial in the forebrain (Emx1-Cre)	Pyramidal cell in the forebrain (CamkIIα-Cre)
Deleted isoforms	220 and 440 kDa	440 kDa (truncated to 290 kDa)	440 kDa	220 and 440 kDa	220 and 440 kDa
Body weight	↓	Normal	Normal	Normal	Normal
Survival	Postnatal day 21	Normal	Normal	P40 (less then 50%)	Normal
Locomotion	↓	↓	↓	↑	Normal
Motor coordination	↓	Normal	Normal	N/A	N/A
Social interaction	N/A	Normal	N/A	social novelty ↓ (only male)	Normal
Ultrasonic vocalization	↓ (Ank2 HT mice)	↓	↓	↓ (5 weeks old) Normal (1 week old)	N/A
Urine territory marking	↓ (Ank2 HT mice)	↓	↓	N/A	N/A
Repetitive behavior	N/A	↑	N/A	N/A	N/A
Seizure	N/A	Normal	N/A	↑	Normal
Water maze	Normal (Ank2 HT mice)	Normal (but, reversal phase ↑)	Normal (but, reversal phase ↑)	N/A	N/A
Y-maze	N/A	N/A	N/A	Normal	Normal
Novel object recognition	N/A	Normal	N/A	N/A	N/A
Light/dark box	N/A	Normal	N/A	Normal	Normal
Elevated plus maze	N/A	N/A	N/A	Normal	Normal
Forced swim	N/A	N/A	N/A	Normal	Normal

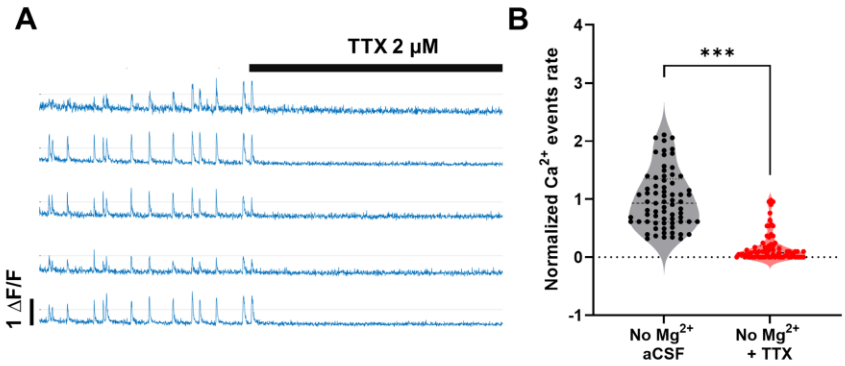
Supplementary Fig. S1, related to Fig. 1. Behavioral phenotypes based on different *Ank2* deletion mice models. cKO: conditional knockout; fs: frameshift



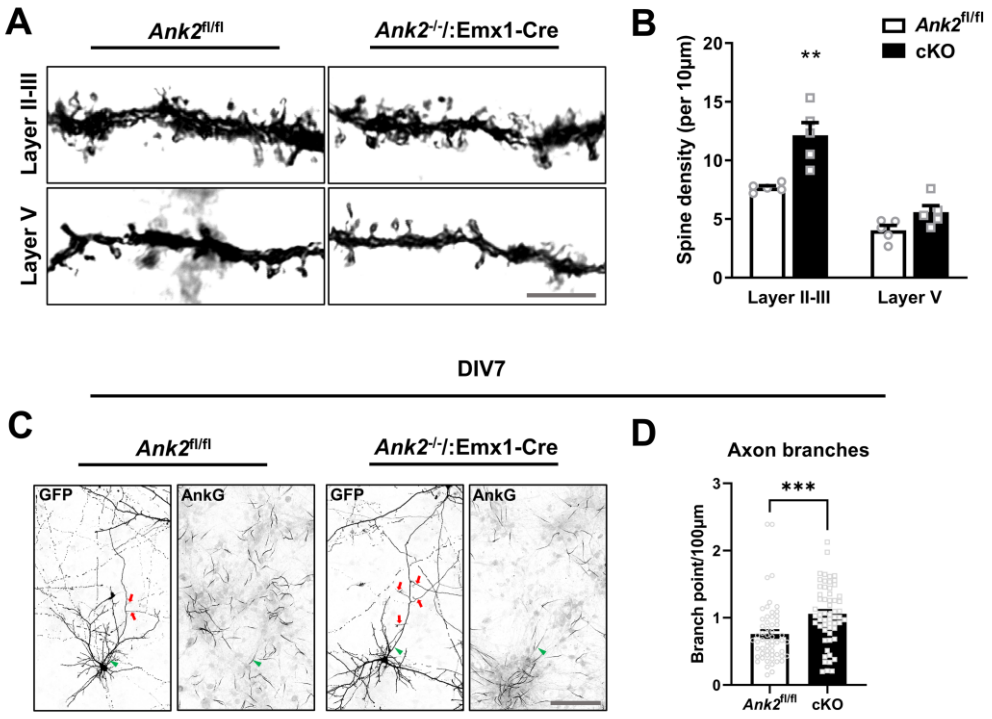
Supplementary Fig. 2, related to Fig. 1. Deletion of *Ank2* in the forebrain from early development or after an adolescent stage shows normal behavioral phenotypes in body weight, forced swim test, Y maze, zero maze, light/dark box test. (**A, N)** Representative Western blots from mouse cortex of different genotypes (n = 3 for each group). (**B, C, H**) The measurement of body weight. (**D, E**) Locomotor activity from an open field test. (**F, G, P**) Immobility was assessed in adult mice in the forced swim test for 4 min. (**H, I, Q**) Y maze test for 5 min. (**J, K, R**) Zero maze test for 5 min. (**L, M, S**) Light/dark box test for 5 min. The group of mice : *Ank2*$^{fl/fl}$, n = 10-14 (male), n = 11 (female); *Ank2*$^{+/-}$:Emx1-Cre, n = 6-7 (male), n = 6-9 (female); *Ank2*$^{-/-}$:Emx1-Cre, n = 8-11 (male), n = 8-13 (female); one-way ANOVA followed by a Bonferroni test was performed. *: $p < 0.05$, ***: $p < 0.001$. The group of male mice: *Ank2*$^{fl/fl}$, n = 11; *Ank2*$^{-/-}$:CaMKIIα-Cre, n = 10. All data are represented as mean ± SEM.


Ank2-/-:Emx1-Cre (male mice)
Ank2-/-:Emx1-Cre (female mice)
A
Sociability
Preference Index
B
Social Novelty
***
C
Sociability
D
Social Novelty
Ank2fl/fl HT cKO
Ank2-/-:Emx1-Cre (USVs at P7 with male mice)
E
Syllable numbers (n)
F
Syllable duration (msec)
G
Total syllable energy (dB)
H
Frequency bandwidth (kHz)
Ank2-/-:Emx1-Cre (USVs at P7 with female mice)
I
J
K
L

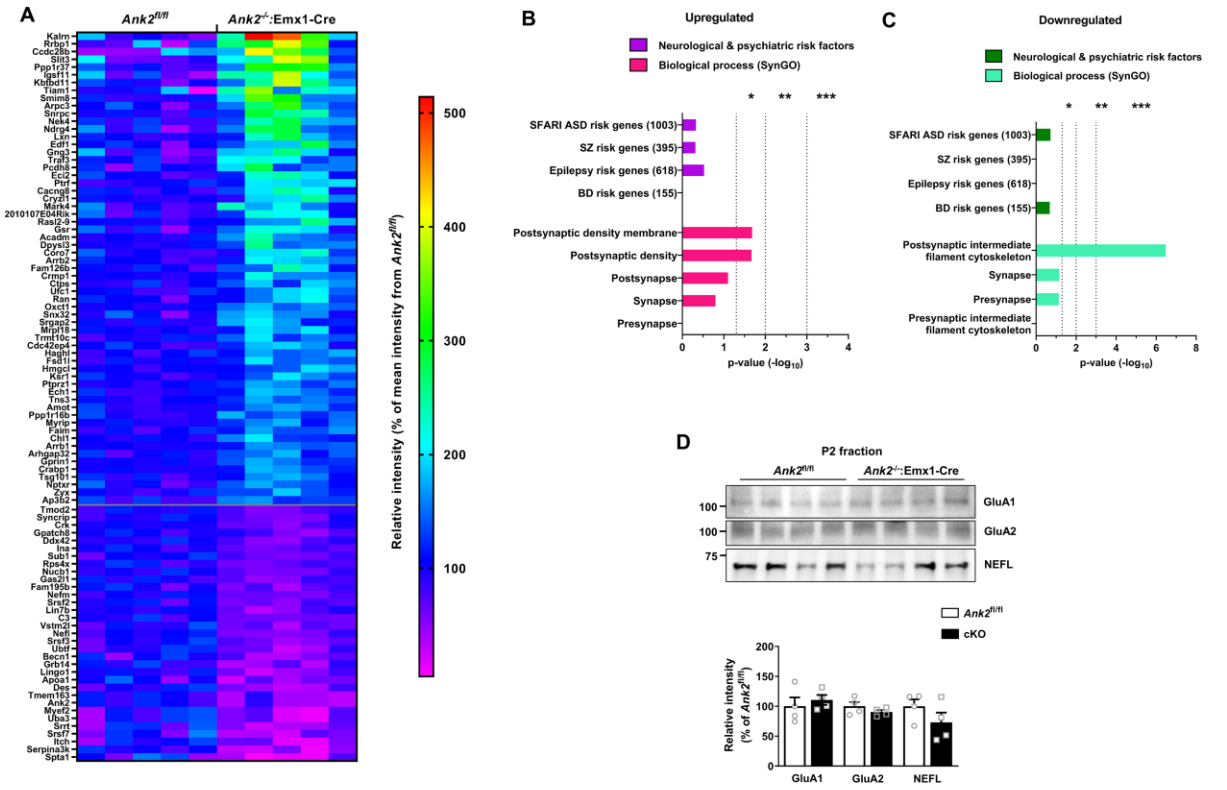
Supplementary Fig. 3, related to Fig. 1. *Ank2*$^{-/-}$:Emx1-Cre male mice show impaired social novelty recognition, but not female mice. (**A-D)** Quantification of time spent on social side (novel) or non-social side (empty) of social approach apparatus (A, C). Time spent with familiar subject or novel subject (B, D). *Ank2*$^{fl/fl}$, n = 14 (male), n = 11 (female); *Ank2*$^{+/-}$:Emx1-Cre (HT), n = 7 (male), n = 8 (female); *Ank2*$^{-/-}$:Emx1-Cre (cKO), n = 10 (male), n = 8 (female); ***p < 0.001; one-way ANOVA followed by a Bonferroni test. (**E-L**) 7 days old mice from *Ank2*$^{fl/fl}$, *Ank2*$^{+/-}$:Emx1-Cre (HT), and *Ank2*$^{-/-}$:Emx1-Cre (cKO) mice were recorded over 5kHz bandwidth of vocal sound. Syllable numbers (E, I), syllable duration (F, J), and syllable energy (G, K), and frequency bandwidth (H, L) were measured and analyzed by MUPET with MATLAB software. The group of *Ank2*$^{fl/fl}$, n = 32 (male), n =19 (female); *Ank2*$^{+/-}$:Emx1-Cre (HT), n = 7 (male), n = 9 (female); *Ank2*$^{-/-}$:Emx1-Cre (cKO), n = 10 (male), n = 10 (female).



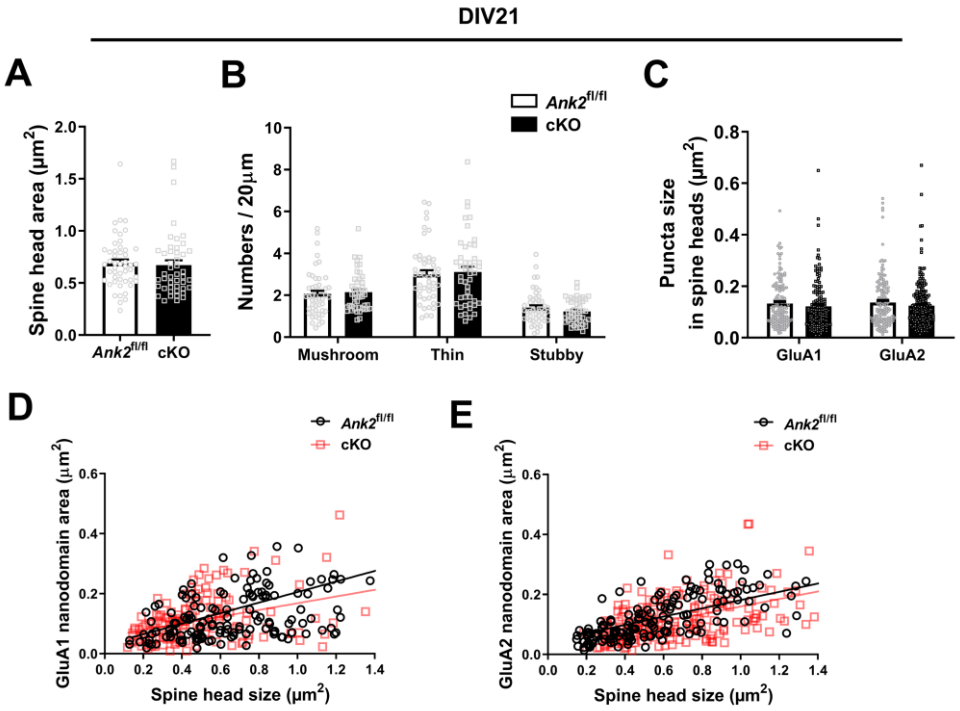
Supplementary Fig. 4, related to Fig. 2. Ca^{2+} events in no magnesium standard aCSF are neuronal activity-dependent. (**A**) Ca^{2+} events from the Cal520AM probe were recorded under a 2 photon microscope from acute brain slices of S1 cortex on 0 Mg^{2+} aCSF followed by bath application of TTX at 2 µM. (**B**) Quantification of the Ca^{2+} rate showed that TTX significantly reduced neuronal Ca^{2+} activity compared to the No Mg^{2+} aCSF. (paired t-test, ***: $p<0.001$, n = 73 neurons)



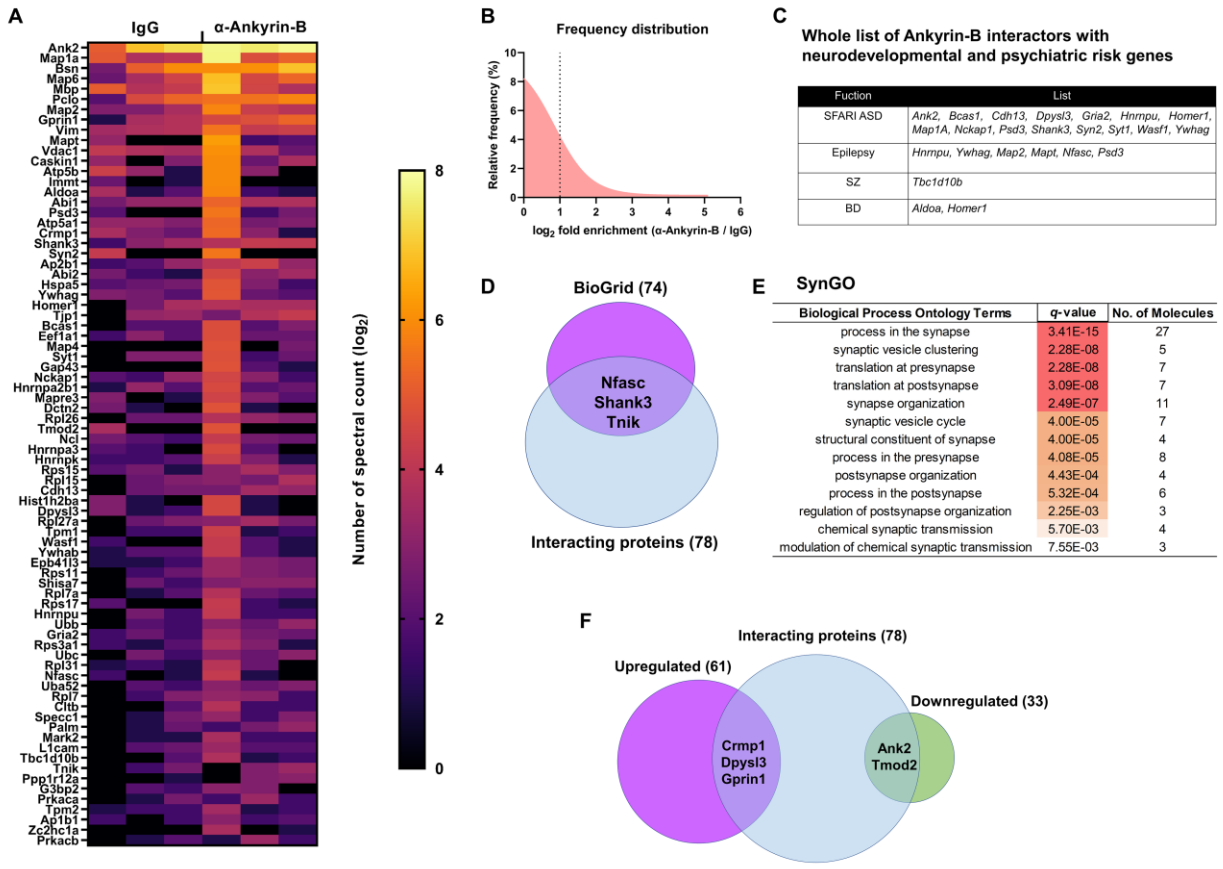
Supplementary Fig. 5, related to Fig. 2. Cortical spine development and axonal branching in cortical neurons of *Ank2*$^{-/-}$:Emx1-Cre mice. (**A**) Golgi-Cox staining of layer II-III pyramidal apical dendrites in 3-week-old *Ank2*$^{fl/fl}$ and *Ank2*$^{-/-}$:Emx1-Cre mice. Scale bar, 5 μm. (**B**) Dendritic spines were analyzed from 5 brains per each group (male and female; 5-7 neurons per each brain). **p < 0.01; two-tailed unpaired t test was performed. (**C**) Fluorescence images from eGFP-transfected cortical cultured neurons at DIV7 from *Ank2*$^{fl/fl}$ and *Ank2*$^{-/-}$:Emx1-Cre embryos. The cultured neurons were stained with ankyrin-G antibody. Scale bar, 100 μm. (**D**) Quantification of axon branching from *Ank2*$^{fl/fl}$ (n = 58 from four embryos) and *Ank2*$^{-/-}$:Emx1-Cre (n = 58 from four embryos) cortical neurons. Two independent experiments were performed. ***p < 0.001; two-tailed unpaired t test was performed.



Supplementary Fig. 6, related to Fig. 3. Upregulated proteins in cortical synapse of *Ank2*$^{-/-}$:Emx1-Cre mice. (**A**) Heatmap of identified up or downregulated proteins. (**B, C**) Gene ontology (GO) analysis of statistically overrepresented biological processes among the differentially upregulated (**B**) or downregulated (**C**) proteins. The analysis was performed from data in figure 3C by SynGO. Enrichment of ASD, schizophrenia, epilepsy, and bipolar disorder risk factors (identified through SFARI gene archive, GWAS, and de novo studies) was analyzed by hypergeometry test; *p < 0.05, **p < 0.01, ***p < 0.001. (**D**) Representative images of western blot from P2 fractionated samples. The graph shows relative abundance of proteins in cortex of 3-week-old *Ank2*$^{fl/fl}$ and *Ank2*$^{-/-}$:Emx1-Cre (cKO) mice (n = 4 per each group). WB images were normalized by Na+/K+ ATPase bands from figure 3E.

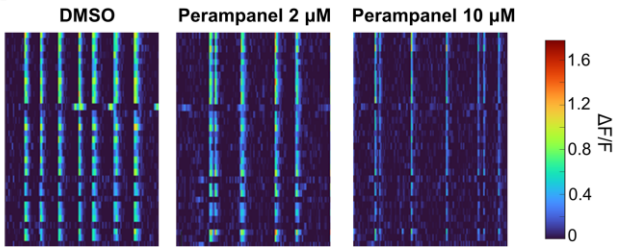


Supplementary Fig. 7, related to Fig. 3. The expression of GluA1 and GluA2 in primary cortical neurons from *Ank2*$^{-/-}$:Emx1-Cre mice. (**A**) Spine head size from mushroom spines was analyzed from eGFP transfected cortical neurons which were taken by structured illumination microscopy (SIM) at DIV21. (**B**) Mushroom, thin, and stubby types of spine morphology were analyzed (*Ank2*$^{fl/fl}$: n = 49 neurons; *Ank2*$^{-/-}$:Emx1-Cre : n = 46 neurons). (**C**) Puncta sizes of GluA1 (*Ank2*$^{fl/fl}$: n = 193 spines from 27 neurons; *Ank2*$^{-/-}$:Emx1-Cre : n = 187 spines from 21 neurons) or GluA2 (*Ank2*$^{fl/fl}$: n = 200 spines from 22 neurons; *Ank2*$^{-/-}$:Emx1-Cre : n = 230 spines from 25 neurons) were analyzed within mushroom spine heads. (**D-E**) Correlation plots of the puncta area of GluA1 or GluA2 versus spine head size.



Supplementary Fig. S8, related to Fig. 4. Ankyrin-B interactome in the synapse. (**A**) Heatmap of immunoprecipitated proteins with an anti-ankyrin-B antibody. (**B**) A graph shows the frequency distribution of a number of proteins (% of total proteins) that interact with anti-ankyrin-B based on the spectral count. (**C**) The list of ankyrin-B interactome which is associated with the neurodevelopmental and neuropsychiatric risk genes. This table is related to figure 4C. (**D**) Venn diagram comparing the ankyrin-B interacting proteins reported in BioGRID database and our results of the list of ankyrin-B interactome. (**E**) GO analysis of the biological process by SynGO. (**F**) Venn diagram comparing the ankyrin-B interacting proteins and up or downregulated proteins in the cortex of *Ank2*$^{-/-}$:Emx1-Cre mice from figure 4B.

A

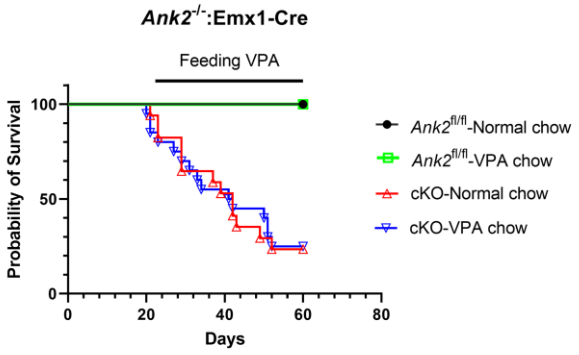


B

4th days of perampanel injection (3 weeks-old mice)

Drug dose	Phenotypes
4.0 mg/kg	Within 5 minutes of injecting the drug, 4/5 mice were paralyzed and lying on the floor and, 1/5 mouse was crawling on the floor. These behaviors lasted 30-60 minutes and then recovered.
0.8 mg/kg	Within 5 minutes of injecting the drug, 5/5 mice were alert, but they toddled and walked slowly. These behaviors lasted 30-60 minutes and then recovered.
0.2 mg/kg	4/4 mice were active and behaving normally.
0.1 mg/kg	5/5 mice were active and behaving normally.

C



Supplementary Fig. S9, related to Fig. 5. The effect of perampanel in *in vitro* and *in vivo*. (**A**) Zoom of the heatmap from figure 5A illustrating the effects of perampanel at 2 or 10 µM in brain slices. (**B**) Description of the different doses of *in vivo* injection of perampanel effects. Reactions were investigated after injection of perampanel by i.p. once a day for 4 days. (**C**) The rescue effect of valproic acid (feeding with chow containing 1.7%) on *Ank2*$^{-/-}$:Emx1-Cre (cKO) mice. This Kaplan-Meier survival curve is related to figure 5F.