SUPPLEMENTARY INFORMATION
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The C-terminal tails of endogenous GluA1 and GluA2 differentially contribute to hippocampal synaptic plasticity and learning

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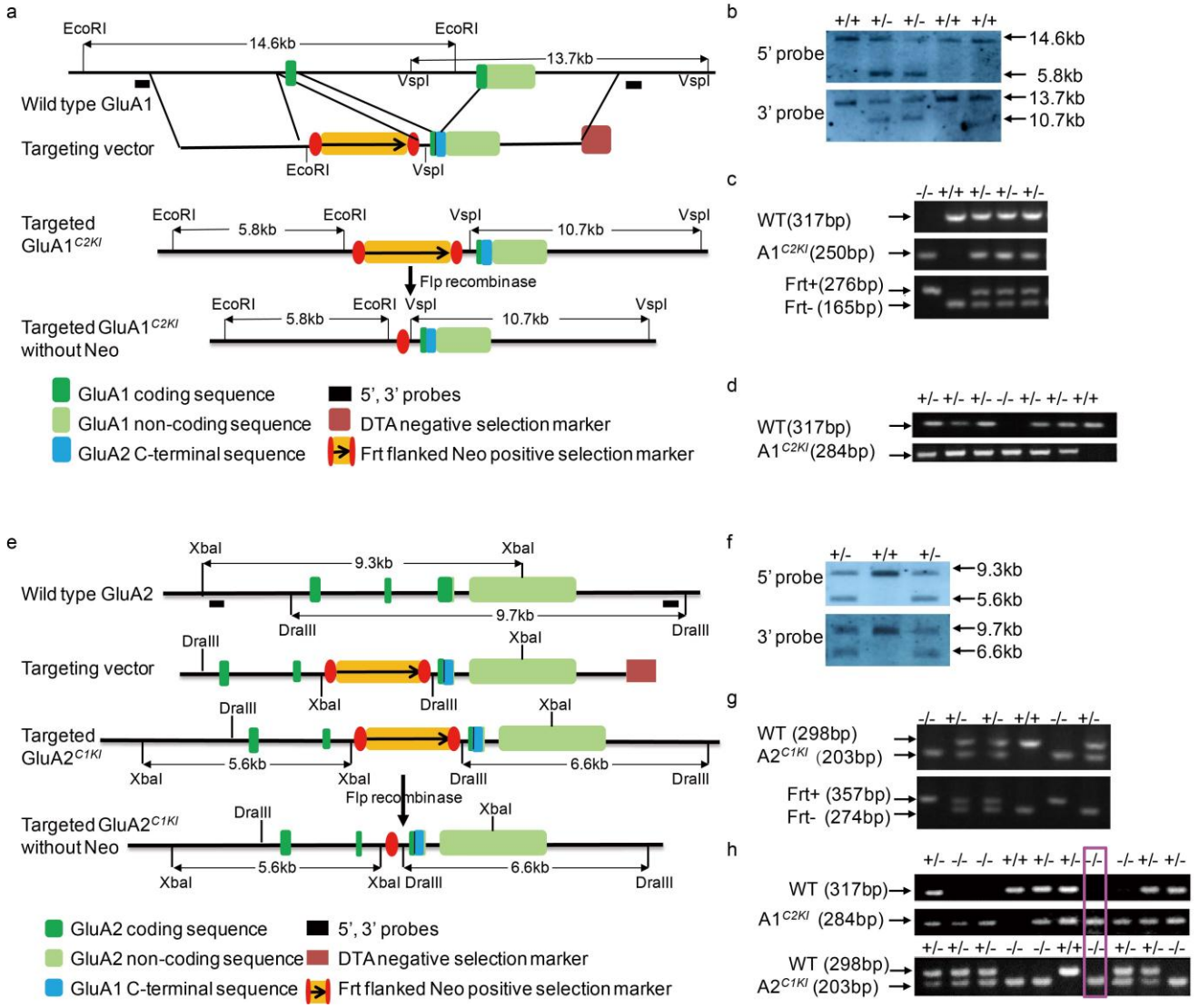
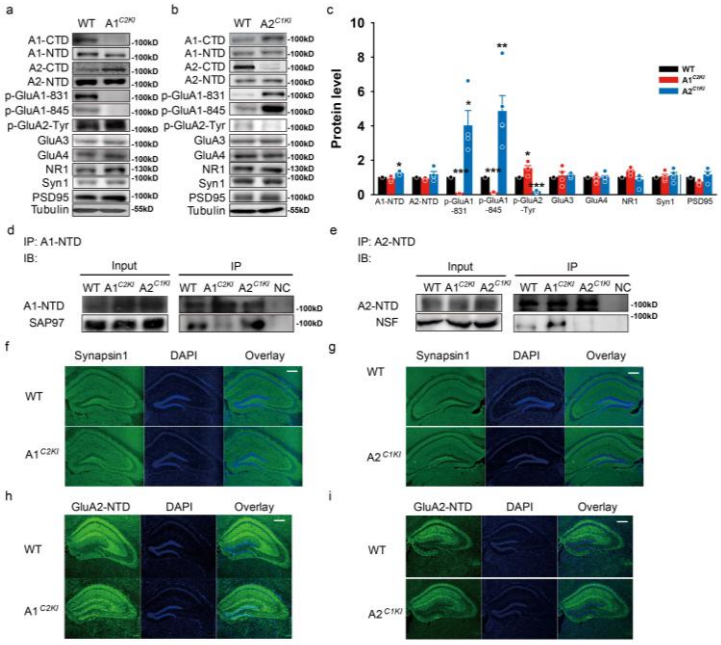


Supplementary Figure 1

Supplementary Fig. S1. Creation of GluA1^{C2KI}, GluA2^{C1KI} and GluA1^{C2KI}/GluA2^{C1KI} mice

(**a**) Schematic graph of the WT GluA1 gene locus, CTD replacement targeting vector and targeted GluA1 locus in GluA1^{C2KI} mice. (**b**) Southern blot analysis of ES DNA using 5' and 3' probes showing the successful replacement of the DNA region containing the CTD of the GluA1 with that of the GluA2 in GluA1^{C2KI} +/- clones. This experiment was repeated at least two times on independent clones with identical results. (**c**) PCR analysis of tail DNA of the offspring littermates from the heterozygous breeders showing wild type (+/+), heterozygous (+/-) and homozygous (-/-) for the CTD replacement. All the mice used in this study were homozygous (-/- = GluA1^{C2KI} and their wild type littermates (+/+ = WT). This experiment was repeated for all the mice used for the experiments. (**d**) PCR analysis of tail DNA of the offspring littermates from the heterozygous breeders using additional pairs of oligo-primers showing wild type (+/+), heterozygous (+/-) and homozygous (-/-) for the CTD replacement. This experiment was repeated for every mouse used for the present study. (**e**) Schematic graph of the WT GluA2 gene locus, CTD replacement targeting vector and targeted GluA2 locus in GluA2^{C1KI} mice. (**f**) Southern blot analysis of ES DNA showing the successful replacement of the DNA region containing the CTD of the GluA2 with that of the GluA1 in GluA2^{C1KI} mice, using the 5' and

3’ probes. This experiment was repeated at least two times for each of the independent ES clones with identical results. (**g**) PCR analysis of tail DNA of the offspring littermates from the heterozygous breeders showing wild type (+/+), heterozygous (+/-) and homozygous (-/-) for the CTD replacement. All the mice used in this study were homozygous (-/- = GluA2^{CIKI} and their wild type littermates (+/+ = WT). This experiments was repeated for every mouse used for the present study. (**h**) PCR analysis of tail DNA of the offspring littermates from the heterozygous breeders for both CTD replacements showing various genotypes, including wild type (+/+ = WT) and double homozygous (-/-, -/-, indicated by purple box) for the CTD replacements (GluA1C2KI/GluA2C1KI, i.e. CTD swapped mice). See **Supplementary Fig. S9** for full length blot/DNA gel scans.

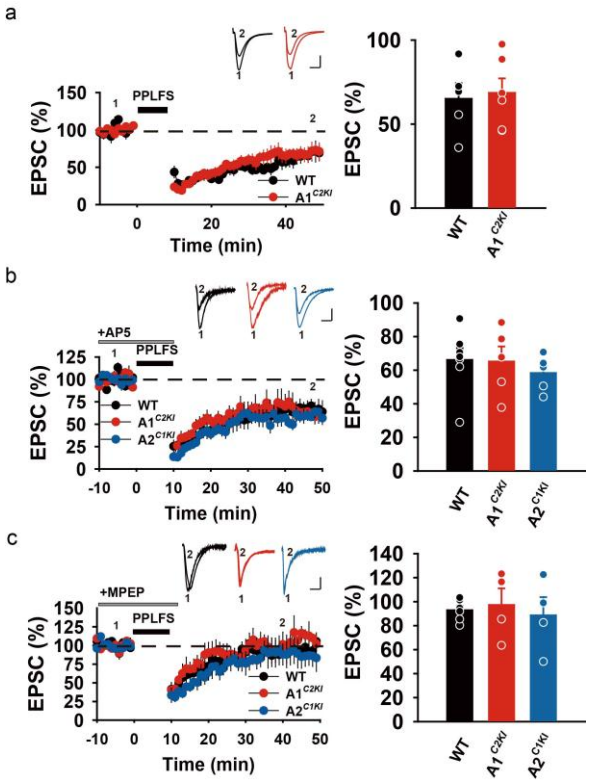


Supplementary Figure 2

Supplementary Fig. S2. Characterization of GluA1^{C2KI} and GluA2^{C1KI} mice

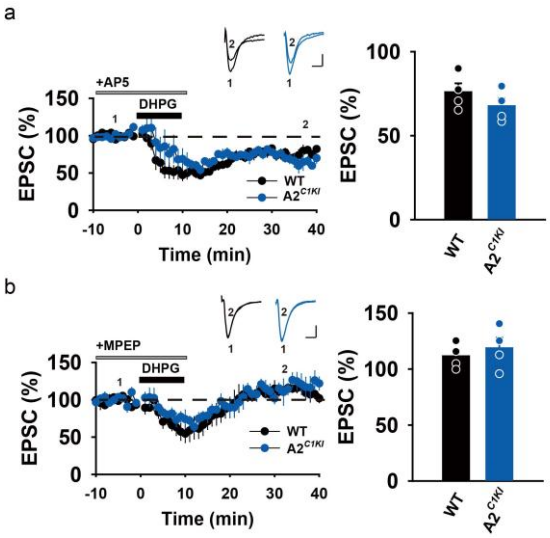
(**a**) Western blot analysis of total brain protein lysate from GluA1^{C2KI} and WT control littermates showing the successful replacement of the CTD of GluA1 with that of GluA2. Note in GluA1^{C2KI} mice, no GluA1 band was detected with a GluA1-CTD antibody, but a normal GluA1 band was seen with a GluA1-NTD antibody. (**b**) Western blot analysis of total brain protein lysate from GluA2^{C1KI} and WT control mice showing the successful replacement of the CTD of GluA2 with that GluA1. Note in GluA2^{C1KI} mice, no GluA2 band was detected with a GluA2-CTD antibody, but normal GluA2 level with a GluA2-NTD antibody. (**c**) Quantifications of various proteins tested in (**a,b**) showing that phosphorylated GluA1 (Ser831 and Ser 845) was absent in GluA1^{C2KI}, but increased in GluA2^{C1KI} compared to WT mice, and that phosphorylated GluA2 (Tyr869/873/876) was absent in GluA2^{C1KI}, but increased in GluA1^{C2KI} compared to WT mice. Other proteins were not altered except that there was a small increase in GluA1NTD in GluA2^{C1KI} mice (A1-NTD: WT = 1.00 ± 0.00, n = 4 mice; A1^{C2KI} = 0.86 ± 0.07, n = 4 mice, p = 0.085; GluA2^{C1KI} = 1.20 ± 0.05, n = 4 mice, *p = 0.023; $F_{(2, 9)}$ =10.993, p = 0.004. A2-NTD: WT = 1.00 ± 0.00, n = 4 mice; A1^{C2KI} = 0.88 ± 0.04, n =4 mice; GluA2^{C1KI} = 1.16 ± 0.18, n = 4 mice; $F_{(2, 9)}$ =1.743, p = 0.229. p-GluA1-831: WT = 1.00 ± 0.00, n = 4 mice; A1^{C2KI} = 0.08 ± 0.02, n = 3 mice, ***p = 0.00000002; GluA2^{C1KI} = 4.00 ± 0.89, n = 4 mice, *p = 0.015; $F_{(2, 8)}$ =12.786, p = 0.003. p-GluA1-845: WT = 1.00 ± 0.00, n = 5 mice; A1^{C2KI} = 0.10 ± 0.02, n = 4 mice, ***p = 0.0000006; GluA2^{C1KI} = 4.84 ± 0.92, n = 5 mice, **p = 0.003; $F_{(2, 11)}$ =19.077, p = 0.000265. p-GluA2-Tyr: WT = 1.00 ± 0.00, n = 4 mice; A1^{C2KI} = 1.51 ± 0.18, n = 4 mice, **p = 0.007; GluA2^{C1KI} = 0.15 ± 0.03, n = 4 mice, ***p = 0.000268; $F_{(2, 9)}$ = 43.860, p = 0.000022. GluA3: WT = 1.00 ± 0.00, n = 4 mice; A1^{C2KI} = 1.08 ± 0.26, n = 4 mice; GluA2^{C1KI} = 1.09 ± 0.08, n = 3 mice; $F_{(2, 8)}$ = 0.085, p = 0.919. GluA4: WT = 1.00 ± 0.00, n = 5 mice; A1^{C2KI} = 0.93 ± 0.11, n = 5 mice; GluA2^{C1KI} = 1.00 ± 0.10, n = 5 mice; $F_{(2, 12)}$ = 0.621, p = 0.555. NR1: WT = 1.00 ± 0.00, n = 4 mice; A1^{C2KI} = 1.34 ± 0.09, n = 4 mice; GluA2^{C1KI} = 0.85 ± 0.19, n = 4 mice; $F_{(2, 9)}$ = 4.180, p = 0.052. Syn1: WT = 1.00 ± 0.00, n = 4 mice; A1^{C2KI} = 1.04 ± 0.14, n = 4 mice; GluA2^{C1KI} = 1.10 ± 0.19, n = 4 mice; $F_{(2, 9)}$ = 0.145, p = 0.867. PSD95: WT = 1.00 ± 0.00, n = 4 mice; A1^{C2KI} = 0.69 ± 0.06, n = 4 mice; GluA2^{C1KI} = 1.14 ± 0.19, n = 4 mice;

$F_{(2, 9)} = 3.945$, $p = 0.059$.). (**d**) Whole brain protein lysates immunoprecipitated with anti-GluA1NTD an antibody and probed with anti-GluA1NTD and anti-SAP97 antibodies showing reduced or absence of SAP97 in the immunocomplex in GluA1^{C2KI} mice. (**e**) Whole brain protein lysates immunoprecipitated with an anti-GluA2NTD antibody and probed with anti-GluA2NTD and anti-NSF antibodies showing reduced or absence of NSF in the immunocomplex in GluA2^{C1KI} mice. (**f,g**) Fixed brain sections immunostained with anti-synapsin 1 (green) and the nuclear marker DAPI (blue) showing normal hippocampal anatomy and synapse distribution in GluA1^{C2KI} (**f**) and GluA2^{C1KI} (**g**) mice compared to WT littermates. (**h,i**) Fixed brain sections immunostained with anti-GluA2-NTD (green) and the nuclear marker DAPI (blue) showing normal distribution of AMPARs in the hippocampus of GluA1^{C2KI} (**h**) and GluA2^{C1KI} (**i**) mice compared to WT littermates. Scale bar: 200 μm. Experiments were repeated at least 3 times for a,b,d and i. One-way ANOVA test was used for Fig. S2c followed by post-hoc Fisher's LSD multiple comparison test for A1-NTD and p-GluA2-Tyr analysis, and by two tailed t-test at 95% confidence interval for p-GluA1-831 and p-GluA1-845 analysis. Data were presented as mean ± SEM. *$p<0.05$, **$p<0.01$, ***$p<0.001$. See **Supplementary Fig. S10** for full length Western blot scans.



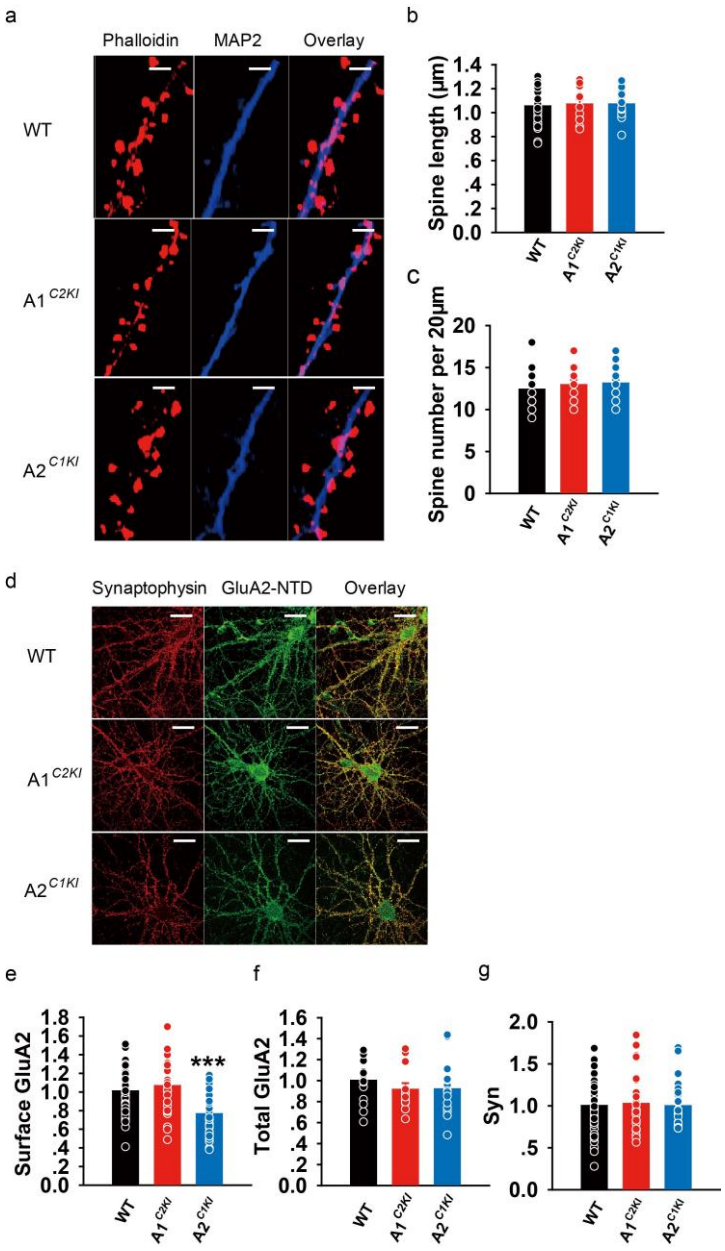
Supplementary Figure 3

Supplementary Fig. S3. Intact mGluR-LTD in GluA1^{C2KI} and GluA2^{C1KI} mice
(**a**) Intact PP-LFS-induced LTD in GluA1^{C2KI} mice (WT = 65.05 ± 9.27%, n = 5 slices from 5 mice; A1^{C2KI} = 68.58 ± 8.64%, n = 6 slices from 6 mice, two tailed t-test at 95% confidence interval, t(9) = -0.278, p = 0.787). (**b**) NMDAR independence of PP-LFS-LTD (WT = 66.06 ± 7.09%, n = 7 slices from 7 mice; A1^{C2KI} = 65.13 ± 9.00%, n = 5 slices from 5 mice; A2^{C1KI} = 58.28 ± 4.81%, n = 5 slices from 5 mice, $F_{(2, 14)}$ = 0.326, p = 0.737). **(c**) Dependence of PP-LFS-LTD on mGluR (WT = 92.84 ± 3.65%, n = 6 slices from 6 mice; A1^{C2KI} = 97.17 ± 13.89%, n = 4 slices from 4 mice; A2^{C1KI} = 88.56 ± 15.20%, n = 4 slices from 4 mice, $F_{(2, 11)}$ = 0.148, p = 0.864). One-way ANOVA test was used for b, c. Scale bar: 50 pA/25 ms. Data were presented as mean ± SEM.



Supplementary Figure 4

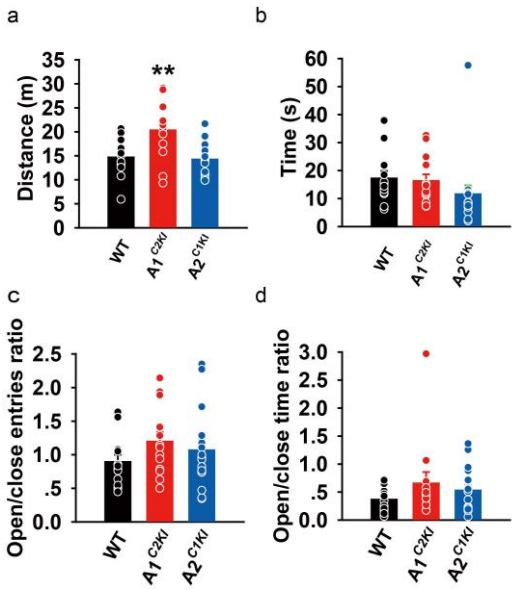
Supplementary Fig. S4. Intact DHPG-induced LTD in GluA2^{C1KI} mice
(**a**) Lack of effect of AP5 on DHPG-LTD (WT = 75.81 ± 5.33%, n = 4 slices from 4 mice; A2^{C1KI} = 67.52 ± 4.75%, n = 4 slices from 4 mice, t(6) = 1.161, p = 0.290). (**b**) Blockade of DHPG-LTD by MPEP (WT = 111.37 ± 5.70%, n = 4 slices from 4 mice; A2^{C1KI} = 118.55 ± 9.49%, n = 4 slices from 4 mice, t(6) = -0.648, p = 0.541). Two-tailed t-test at 95% confidence interval. Scale bar: 50 pA/25 ms. Data were presented as mean ± SEM.



Supplementary Figure 5

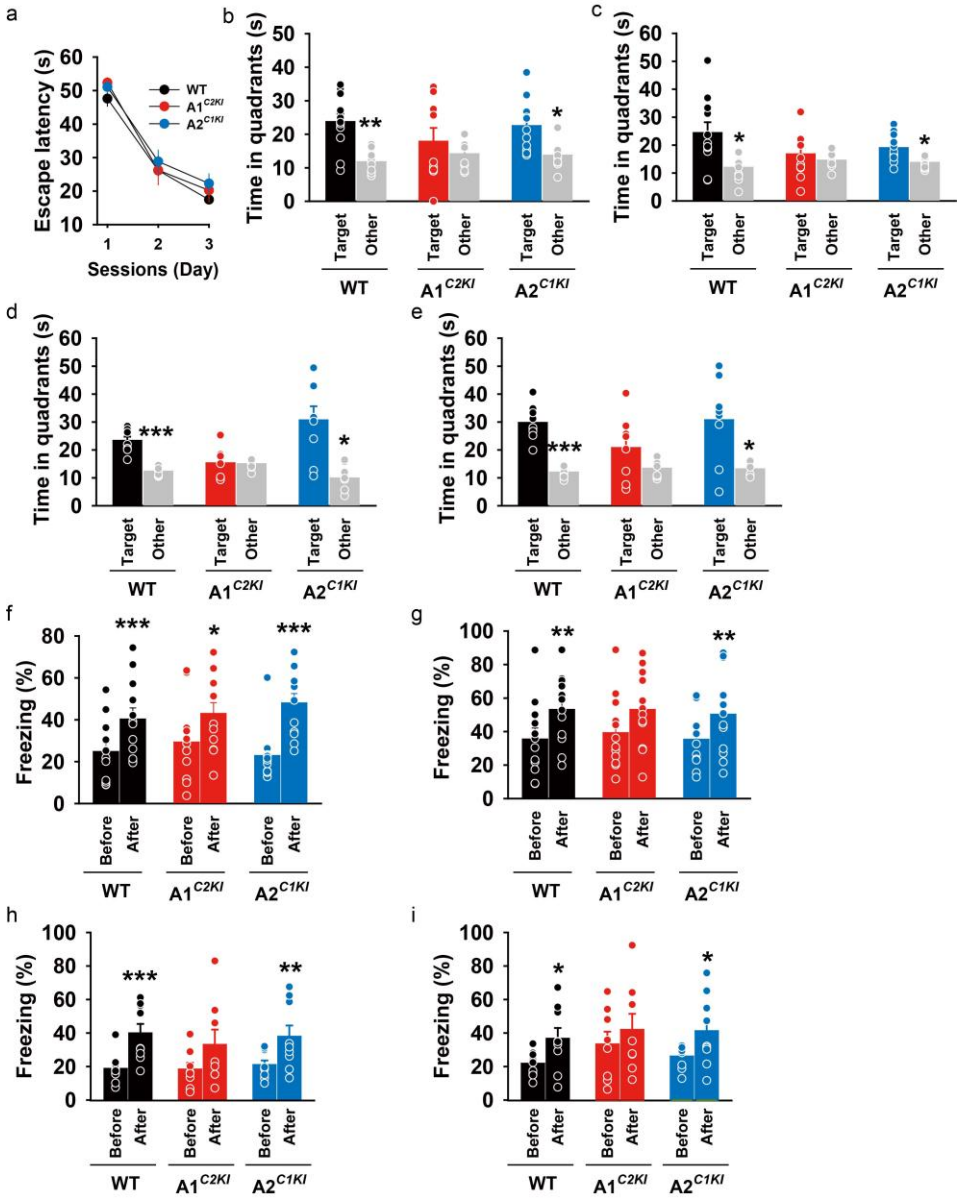
Supplementary Fig. S5. Dendritic spines and basal AMPARs in GluA1^{C2KI} and GluA2^{C1KI} mice
(**a**) Sample dendritic segments of cultured hippocampal neurons stained for MAP2 and F-actin (phalloidin) showing dendritic spines. Scale bar: 2 µm. (**b**) Summary graph of spine length (µm) (WT = 1.05 ± 0.02, n = 32 neurons from 3 independent cultures; A1^{C2KI} = 1.07 ± 0.04, n = 12 neurons from 3 independent cultures, p = 0.701 compared to WT; A2^{C1KI} = 1.07 ± 0.02, n = 19 neurons from 3 independent cultures, p = 0.647 compared to WT; $F_{(2, 60)}$ = 0.138, p = 0.872). (**c**) Summary graph of spine density (spines/20 µm) (WT = 12.33 ± 0.41, n = 24 neurons from 3 independent cultures; A1^{C2KI} = 12.92 ± 0.63, n = 12 neurons from 3 independent cultures, p = 0.418 compared to WT; A2^{C1KI} = 13.11 ± 0.45, n = 19 neurons from 3 independent cultures, p = 0.219 compared to WT; $F_{(2, 52)}$ = 0.842, p = 0.436). (**d**) Cultured hippocampal neurons stained for basal surface GluA2 (GluA2-NTD) and synaptophysin. Scale bar: 20 µm. (**e**) Normalized basal surface (non-permeabilized) GluA2 fluorescence intensity (WT = 1.01 ± 0.03, n = 55 neurons from 3 independent cultures; A1^{C2KI} = 1.01 ±

0.05, n =29 neurons from 3 independent cultures, p = 0.293 compared to WT; $A2^{C1KI}$ = 0.76 ± 0.04, n = 35 neurons from 3 independent cultures, ***p = 0.000003 compared to WT; $F_{(2, 116)}$ = 16.797, p = 0.0000004). (**f**) Normalized basal total (permeabilized) GluA2 fluorescence intensity (WT = 1.00 ± 0.05, n = 16 neurons from 3 independent cultures; $A1^{C2KI}$ = 0.91 ± 0.06, n = 12 neurons from 3 independent cultures, p = 0.319 compared to WT; $A2^{C1KI}$ = 0.92 ± 0.06, n = 16 neurons from 3 independent cultures, p = 0.051 compared to WT; $F_{(2,41)}$ = 0.718, p = 0.494). (**g**) Normalized basal total synaptophysin fluorescence intensity (WT = 1.00 ± 0.05, n = 42 neurons from 3 independent cultures; $A1^{C2KI}$ = 1.02 ± 0.05, n = 34 neurons from 3 independent cultures, p = 0.728 compared to WT; $A2^{C1KI}$ = 1.00 ± 0.05, n = 30 neurons from 3 independent cultures, p = 0.969 compared to WT; $F_{(2,103)}$ = 0.083, p = 0.921). One-way ANOVA followed by post-hoc Fisher's LSD multiple comparison test was used for b, c ,e-g. Data were presented as mean ± SEM. ***p < 0.001.



Supplementary Figure 6

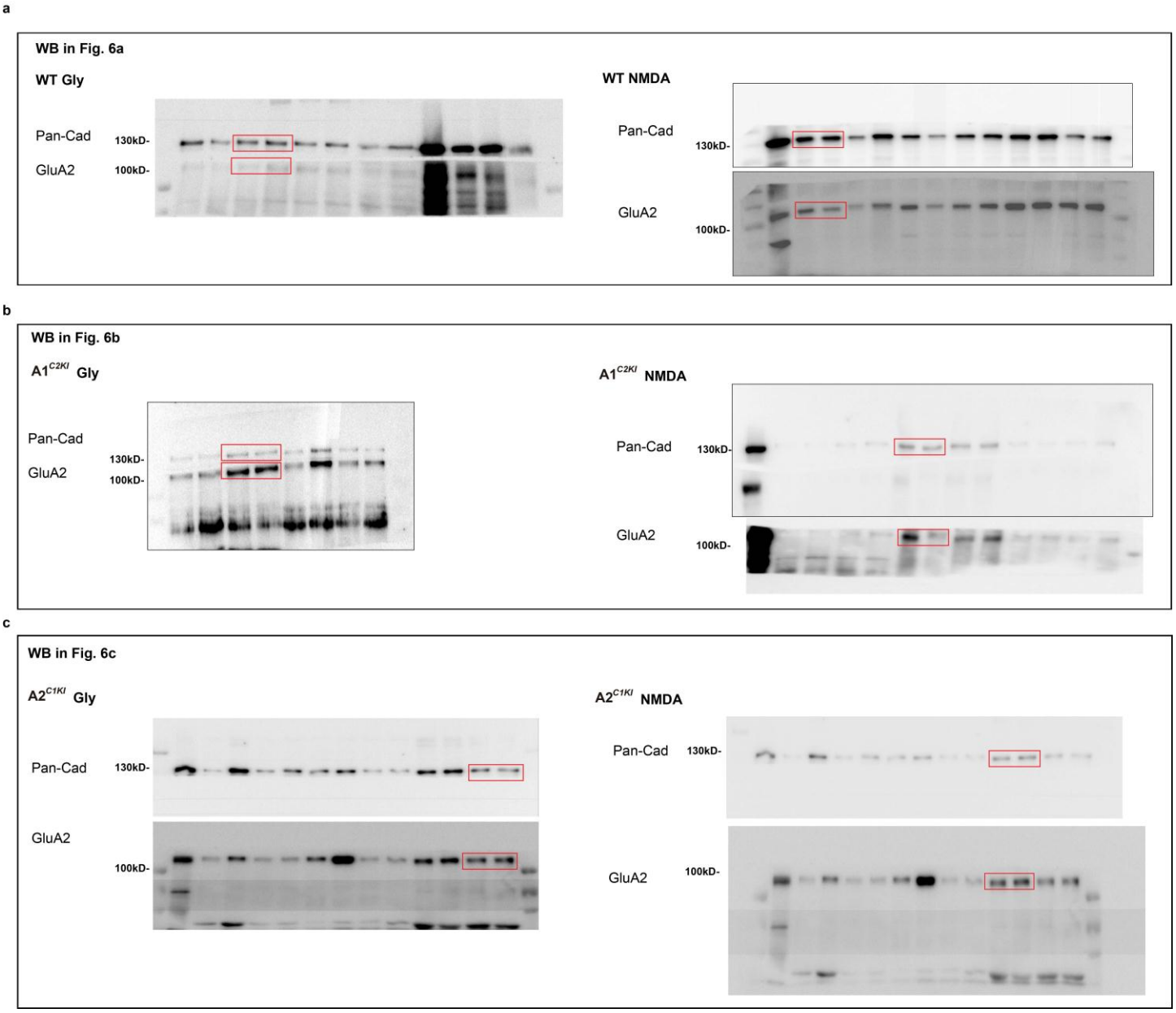
Supplementary Fig. S6. Locomotor activity and anxiety in GluA1^{C2KI} and GluA2^{C1KI} mice
(**a**) Total travel distance (meters) in the open field test (WT = 14.66 ± 1.17, n = 12 mice; A1^{C2KI} = 20.29 ± 1.65, n = 13 mice, **p = 0.003 compared to WT; A2^{C1KI} = 14.21 ± 0.83, n = 16 mice, p = 0.873 compared to WT; $F_{(2,38)}$ = 7.508, p = 0.002). (**b**) Total time (seconds) spent in the center arena of the open field (WT = 17.17 ± 2.71, n = 12 mice; A1^{C2KI} = 16.25 ± 2.41, n =13 mice; A2^{C1KI} = 11.53 ± 3.18, n = 16 mice; $F_{(2, 38)}$ = 1.172, p = 0.321). (**c**) Ratios of closed/open arm entries in the elevated plus maze test (WT = 0.89 ± 0.11, n = 12 mice; A1^{C2KI} = 1.19 ± 0.14, n = 13 mice; A2^{C1KI} = 1.07 ± 0.15, n = 16 mice; $F_{(2, 38)}$ = 1.075, p = 0.352). (**d**) Ratios of time spent in closed/open arms (WT = 0.36 ± 0.06, n = 12 mice; A1^{C2KI} = 0.65 ± 0.20, n =13 mice; A2^{C1KI} = 0.53 ± 0.10, n = 16 mice; $F_{(2, 38)}$ = 1.079, p = 0.350). One-way ANOVA followed by post-hoc Fisher's LSD multiple comparison test was used for a-d. Data were presented as mean ± SEM. **p<0.01.



Supplementary Figure 7

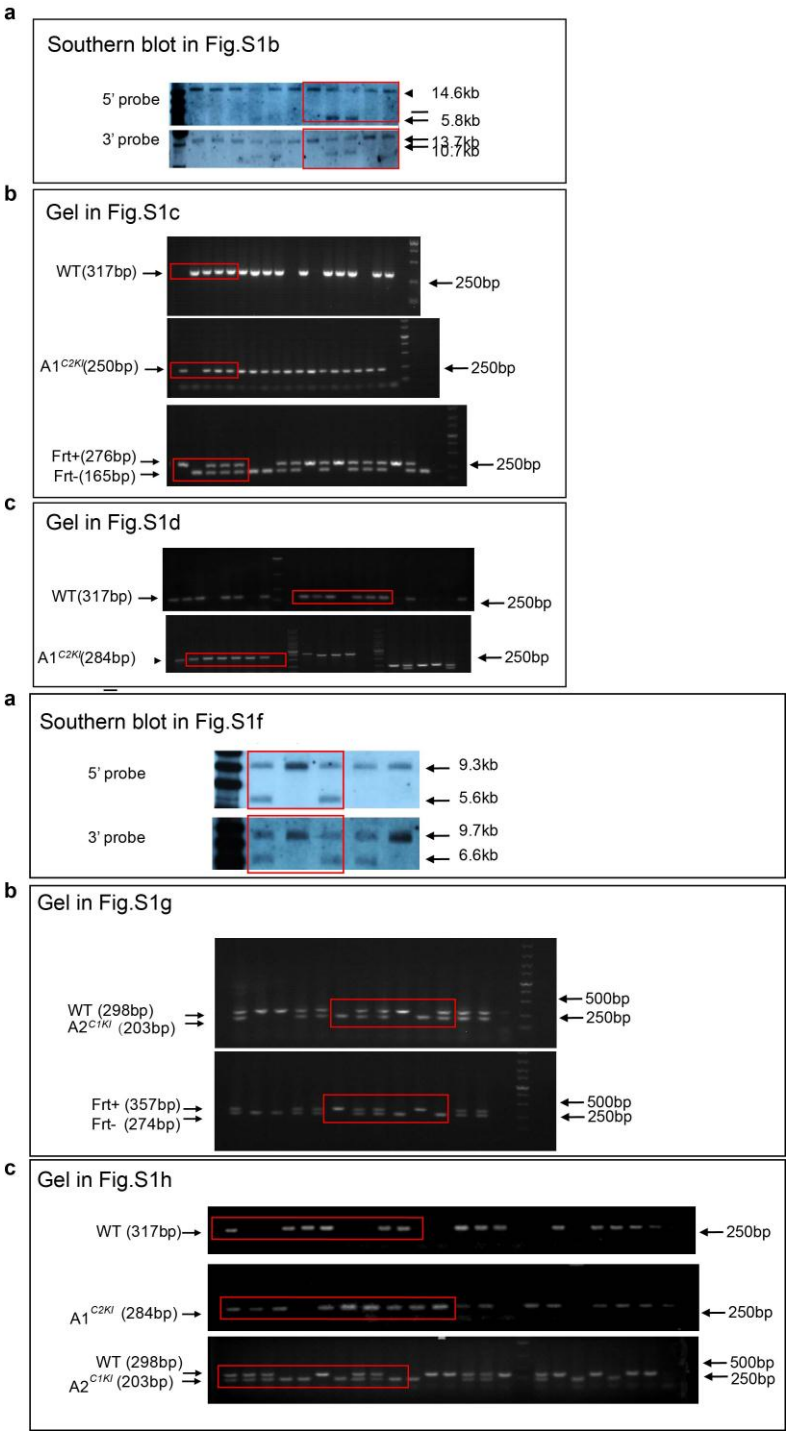
Supplementary Fig. S7. Spatial and fear memory deficits in GluA1^{C2KI} and GluA2^{C1KI} mice
(**a**) Visible platform water maze test showing all three genotypes (WT, n = 11 mice; GluA1^{C2KI}, n = 9 mice; GluA2^{C1KI}, n = 11 mice) reached the platform equally well (between genotypes: $F_{(2, 28)} = 0.981$, p = 0.388; repeated two way ANOVA). (**b**) Probe test at 2 h after the last training session showing that both WT and GluA2^{C1K}, but not GluA1^{C2KI} mice, spent significantly more time swimming in the target quadrant (WT: target = 23.68 ± 2.52, other = 11.70 ± 0.94, n = 11 mice, $t_{(10)} = 3.537$, **p = 0.005; A1^{C2KI}: target = 15.28 ± 3.76, other = 14.91 ± 1.25, n = 9 mice, $t_{(8)} = 0.689$, p = 0.510; A2^{C1KI}: target = 22.44 ± 2.87, other = 12.52 ± 0.96, n = 11 mice, $t_{(10)} = 3.028$, *p = 0.012) compared other quadrants. (**c**) Probe test at 24 h after the last training session showing that both WT and GluA2^{C1K}, but not GluA1^{C2KI} mice, spent significantly more time swimming in the target quadrant (WT: target = 24.34 ± 3.85, other = 11.89 ± 1.29, n = 11 mice, $t_{(10)} = 2.424$, *p = 0.036; A1^{C2KI}: target = 15.36 ± 2.77, other = 14.94 ± 0.941, n = 9 mice, $t_{(8)} = 0.113$, p = 0.913; A2^{C1KI}: target = 18.96 ± 1.49, other = 13.69 ± 0.50, n = 11 mice, $t_{(10)} = 2.654$, *p = 0.024) compared other quadrants. (**d**) Probe test at 2 h

after the enhanced training protocol showing that both WT and GluA2^{C1KI}, but not GluA1^{C2KI} mice, spent significantly more time swimming in the target quadrant (WT: target = 23.31 ± 1.46, other = 12.24 ± 0.49, n = 9 mice, $t_{(8)}$ = 6.78, ***p = 0.00002; A1^{C2KI}: target = 15.23 ± 1.91, other = 14.93 ± 0.64, n = 8 mice, $t_{(7)}$ = 0.116, p = 0.911; A2^{C1KI}: target = 30.60 ± 5.04, other = 9.81 ± 1.68, n = 8 mice, $t_{(7)}$ = 3.095, *p = 0.017). (**e**) Probe test 24 h after the enhanced training protocol showing that both WT and GluA2^{C1KI}, but not GluA1^{C2KI} mice, spent significantly more time swimming in the target quadrant (WT: target = 29.71 ± 2.02, other = 11.96 ± 0.63, n = 9 mice, $t_{(8)}$ = 7.08, ***p = 0.00005; A1^{C2KI}: target = 20.70 ± 4.11, other = 13.32 ± 0.97, n = 8 mice, $t_{(7)}$ = 1.679, p = 0.137; A2^{C1KI}: target = 30.66 ± 5.42, other = 13.10 ± 0.73, n = 8 mice, $t_{(7)}$ = 3.475, *p = 0.010). (**f**) Cued memory test 2 h after the three shock conditioning (WT: before = 21.56 ± 2.68%, after = 40.08 ± 3.52%, n = 12 mice, $t_{(11)}$ = -9.071, ***p = 0.000002; A1^{C2KI}: before = 20.43 ± 3.36%, after = 33.67 ± 3.74%, n = 12 mice, $t_{(11)}$ = -2.995, **p = 0.012; A2^{C1KI}: before = 23.79 ± 2.21%, after = 38.00 ± 3.11%, n = 12 mice, $t_{(11)}$ = -7.310, ***p = 0.00002). (**g**) Cued memory test 24 h after conditioning (WT: before = 32.91 ± 4.49%, after = 46.18 ± 4.55%, n = 12 mice, $t_{(11)}$ = -3.719, **p = 0.003; A1^{C2KI}: before = 34.41 ± 4.94%, after = 50.83 ± 4.88%, n = 12 mice, $t_{(11)}$ = -1.600, p = 0.138; A2^{C1KI}: before = 33.23 ± 3.49%, after = 50.07 ± 4.90%, n = 12 mice, $t_{(11)}$ = -4.043, **p = 0.002). (**h**) Cued memory test 2 h after the two shock conditioning (WT: before = 18.68 ± 2.99%, after = 39.87 ± 5.55%, n = 9 mice, $t_{(8)}$ = -5.432, ***p = 0.0006; A1^{C2KI}: before = 18.22 ± 4.01%, after = 32.92 ± 9.12%, n = 8 mice,$t_{(7)}$ = -1.682 , p = 0.137; A2^{C1KI}: before = 20.89 ± 2.71%, after = 37.81 ± 6.65%, n = 9, mice,$t_{(8)}$ = -3.899, **p = 0.005). (**i**) Cued memory test 24 h after the two shock conditioning (WT: before = 21.71 ± 2.52%, after = 36.61 ± 6.40%, n = 9 mice, $t_{(8)}$ = -2.549, *p = 0.034; A1^{C2KI}: before = 33.24 ± 7.57%, after = 41.87 ± 9.58%, n = 8 mice, $t_{(7)}$ = -0.688, p = 0.514; A2^{C1KI}: before = 25.94 ± 2.26%, after = 41.11 ± 7.03%, n = 9 mice, $t_{(8)}$ = -2.594, *p = 0.032). Paired t-test at 95% confidence interval was used for b-i. Data were presented as mean ± SEM. *p<0.05, **p<0.01,***p<0.001.



Supplementary Figure 8

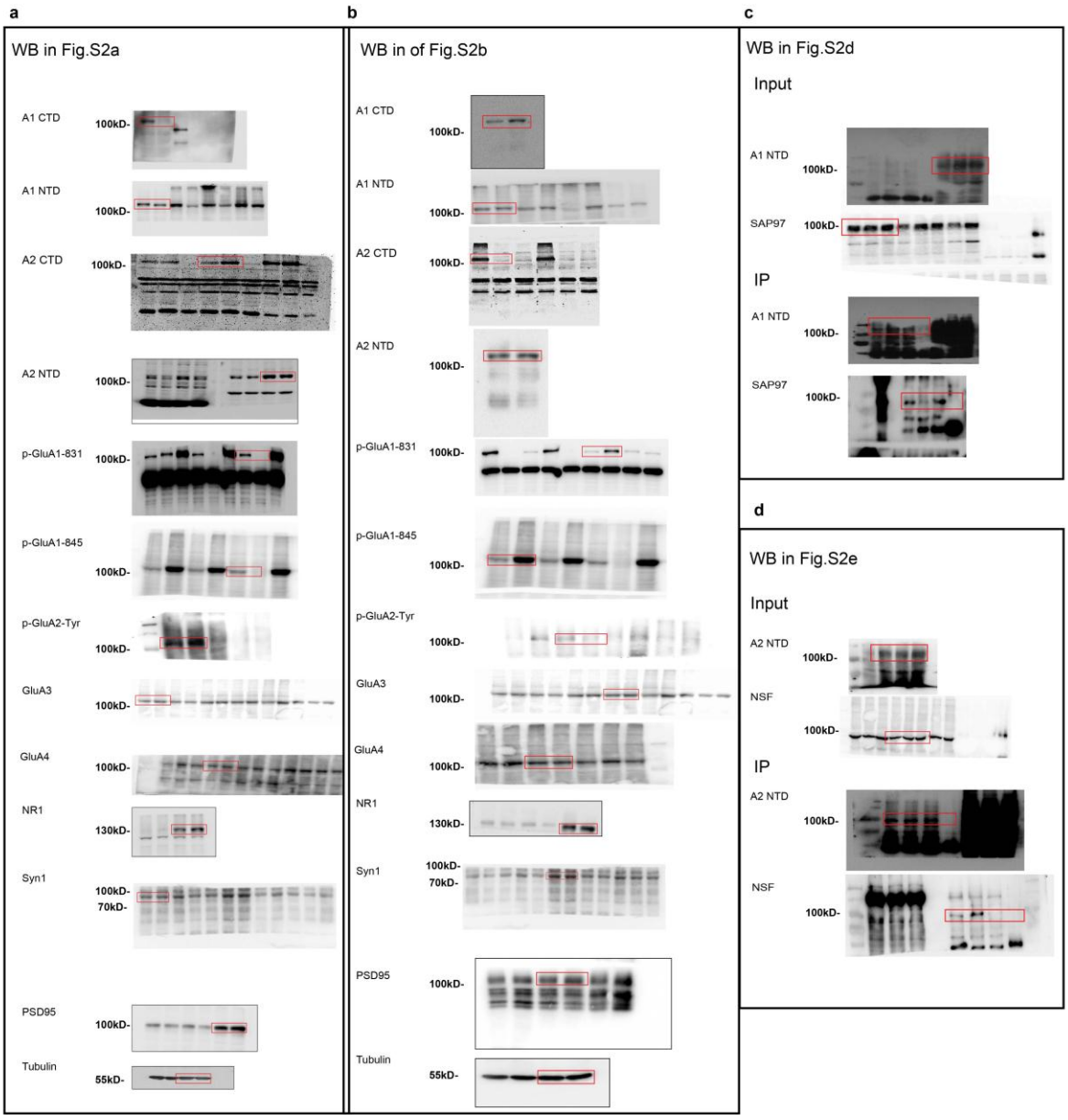
Supplementary Fig. S8. Full length Western blot scans for the cropped images presented in Fig. 6.
Full length Western blot scans for the cropped images presented in Fig. 6.



Supplementary Figure 9

Supplementary Fig. S9. Full length Southern blot and DNA gel scans for the cropped images presented in Supplementary Fig. S1.

Full length Southern blot and DNA gel scans for the cropped images presented in Supplementary Fig. S1.



Supplementary Figure 10

Supplementary Fig. S10. Full length Western blot scans for the cropped images presented in Supplementary Fig. S2.

Full length Western blot scans for the cropped images presented in Supplementary Fig. S2.