

Figure Legends for Supplemental Figures

Supplemental Figure S1. Expression of Cre recombinase activity in Grik4-Cre mice during development. β -Galactosidase staining of whole brain slices (50 μ m thick) from reporter mice (*Grik4*^{Cre/+}; CAG-CAT-Z11/+) at P0, P1, P3, P8, P2W, P4W, and P10W. Scale bar, 3 mm.

Supplemental Figure S2. A, Expression of Cre recombinase activity in Grik4-Cre mice in hippocampus. β -Galactosidase staining in hippocampus slices (50 μ m thick) of reporter mice (*Grik4*^{Cre/+}; CAG-CAT-Z11/+) at P0, P1, P3, P8, P2W, P4W, and P10W. Scale bar, 100 μ m. **B,** β -Galactosidase staining in colonal sections (30 μ m thick) of reporter mice (*Grik4*^{Cre/+}; CAG-CAT-Z11/+) at P4W. Scale bar, 100 μ m. **C,** Immunostaining for β -galactosidase in hippocampal sagittal sections (50 μ m thick) of reporter (*Grik4*^{Cre/+}; CAG-CAT-Z11/+) and control (*Grik4*^{+/+}; CAG-CAT-Z11/+) mice at P10W. Scale bar, 100 μ m. Lower panels show high-power views of the CA3 area. Scale bar, 10 μ m. **D,** Immunostaining for Cre recombinase in the hippocampus of reporter and control mice at P4W and P10W. Similar patterns were obtained with use of rabbit Cre antibody (Novagen) and guinea pig Cre antibody (data not shown). Scale bar, 100 μ m.

Supplemental Figure S3. Immunohistochemical changes of postsynaptic molecules in CA3- GluN2B-KO mice. **A,** Immunostaining for GluN2A, GluN1, PSD-95, and GluA1 on pepsin-untreated paraffin sections. Due to very low or negative staining, changes in distribution of these molecules can not be properly evaluated. Compare these images with Fig. 3A. Scale bar, 0.1 mm. **B,** Immunostaining for GluN1 and GluA1 by free-floating incubation of frozen sections. By this method, low signals can be detected

for GluA1 without pepsin pre-treatment (right top panel), but the intensity is increased for both GluN1 and GluA1 after pepsin pre-treatment of frozen sections (middle panels). Note reduced GluN1 signals in the CA3 of CA3- GluN2B-KO mice (left bottom panel), but the signal-to-noise ratio of this image is apparently high when using pepsin pre-treated paraffin sections (compare Fig. S3B with Fig. 3A). Scale bar, 0.1 mm.

Supplemental Figure S4. Quantitative immunoblots using CA3 crude fraction. **A**, Expression level of GluN2B subunit protein in homozygous neo-containing Grin2b-flox mice (flox/flox) was comparable to that in wild-type mice. Left panel, immunoblots of three independent preparations with GluN2BN antibody; right panel, relative intensity of immunoreactivity for GluN2B ($100 \pm 4.88\%$ in wild-type, $n=3$; $92.2 \pm 4.41\%$ in homozygous neo, $n=3$, $p=0.31$). **B**, Left panel shows lack of truncated GluN2B bands as revealed by GluN2BN antibody (three independent preparations from control (flox/flox) and mutant). Right panel shows immunoblots with respective antibodies (GluN2A, GluN1, PSD-95 and NSE) for quantitative analysis (three independent preparations from control (flox/flox) and mutant).

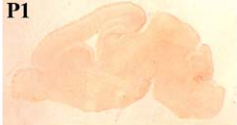
Supplemental Figure S5. Determination of protein ratio of GluN2B to GluN2A in hippocampal CA3 region using titer ratio of the GluN2AC and GluN2BN antibodies. **A**, Schematic representation of the structure of the GluN2A/GluN2B chimeric proteins. White and gray boxes indicate the GluN2A and GluN2B protein fragments, respectively. Numbers below the boxes indicate the amino acid numbers of each fragment. GluN2A–2B, GluN2A N-terminal fragment fused to GluN2B C-terminal fragment; GluN2B–2A, GluN2B N-terminal fragment fused to GluN2A C-terminal fragment. **B**,

Specificity of GluN2AC and GluN2BN antibodies. Immunoblot analysis of extracts of COS-7 cells (COS) transfected with expression vector only (Vector), full-length GluN2A (2A), full-length GluN2B (2B), chimeric GluN2A-2B (2A-2B), chimeric GluN2B-2A (2B-2A), and whole hippocampal crude fraction (Brain). Replicate blots were probed with GluN2AC or GluN2BN antibody. **C**, Left panel, titer ratio of the two antibodies was determined by immunoblotting of GluN2B-2A expressed in COS-7 cells; right panel, immunoblots of CA3 crude fraction with the two antibodies. Serial 1.5-fold dilutions starting at 30 µg protein (CA3 crude fraction) or at 5 µl of extracts (COS-7 cells). **D**, Left panel, relative titer of the GluN2AC and GluN2BN antibodies from three independent preparations; right panel, relative intensity of immunoreactivity of CA3 crude fraction from three independent preparations.

P0



P1



P3



P8



P2W

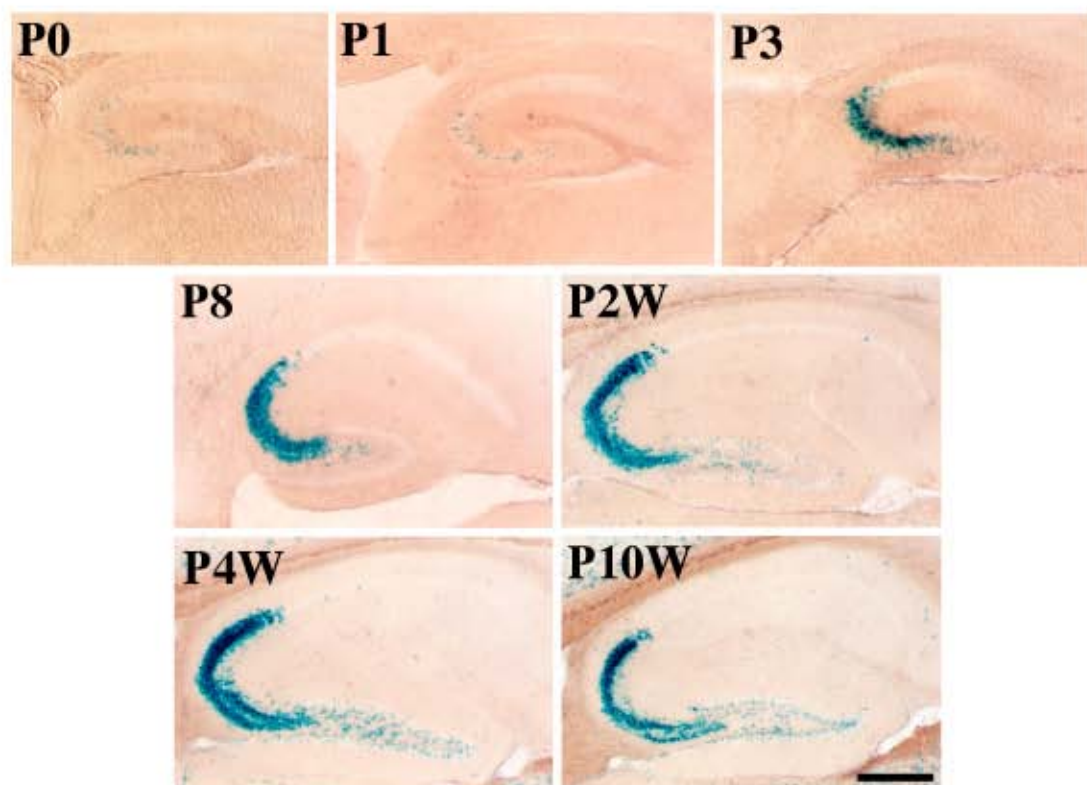
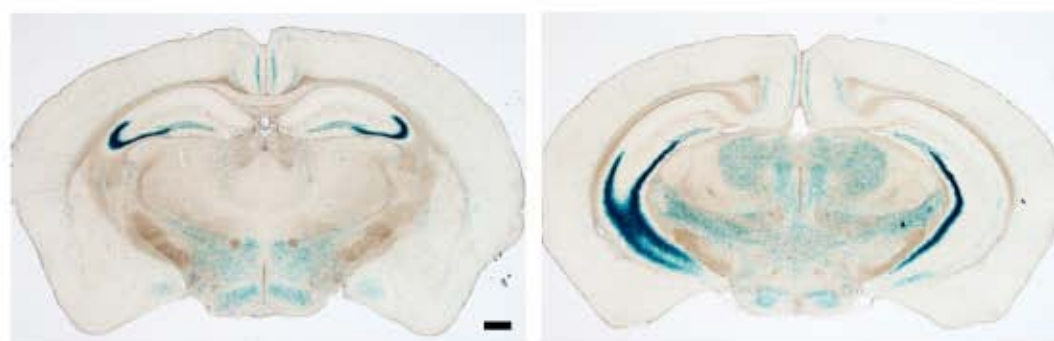


P4W



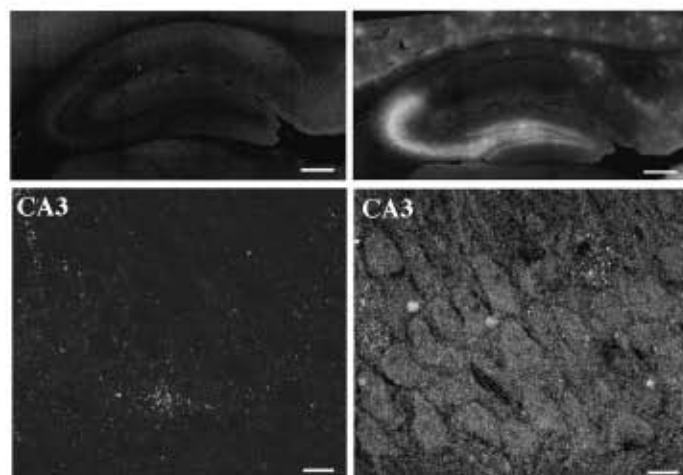
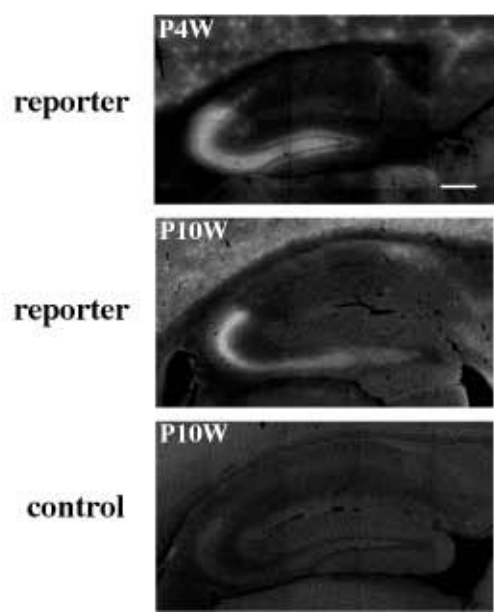
P10W



A**B****C** **β -galactosidase**

control

reporter

**D****Cre**

A

paraffin section (no pepsin)

GluN2A

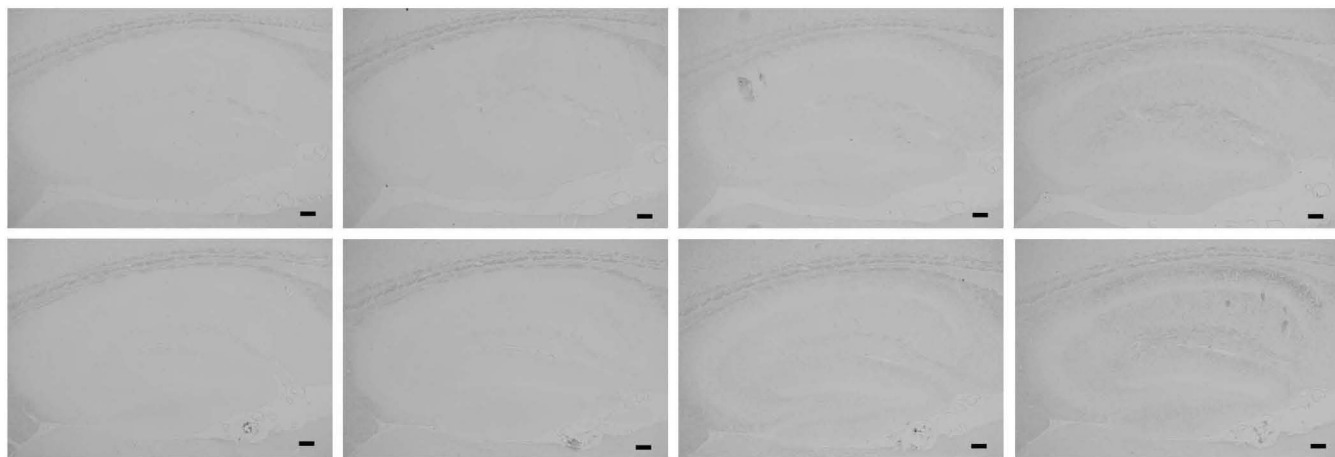
GluN1

PSD-95

GluA1

control

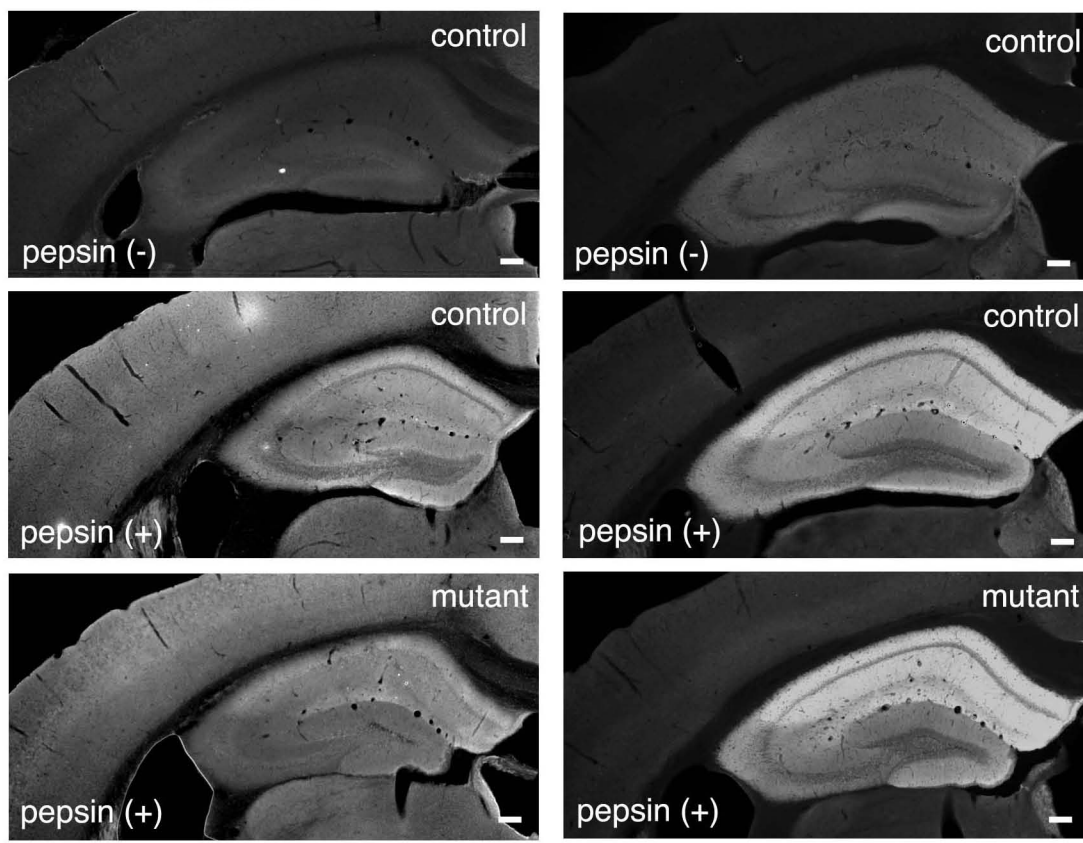
mutant

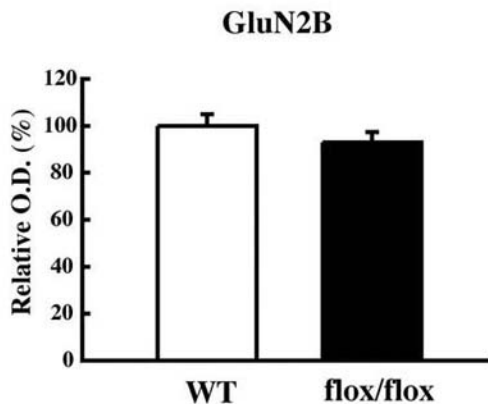
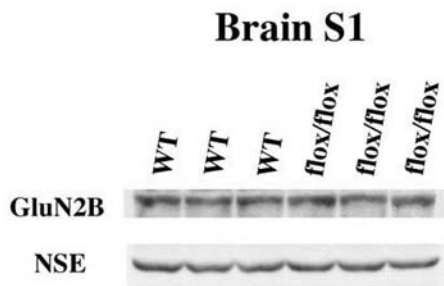
**B**

frozen section

GluN1

GluA1



A**B**