Supporting Information

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SI Materials and Methods

Reagents. NRG1 was used as a recombinant polypeptide containing the entire EGF domain of the β-type NRG1 (rHRG β177–244) (1). The concentration of NRG1 was 1 nM unless otherwise indicated. Ecto-ErbB4 was prepared from stable HEK293 cells that were previously described (2). Rabbit polyclonal anti-ErbB4 antibody 0618 has been described previously (3). Mouse monoclonal anti-PV antibody (PV235) was from Swant, anti–β-actin antibody (NB600-503) was from Novus, goat anti-rabbit IgG conjugated with Alexa Fluor 488 (A11034) and goat anti-mouse IgG conjugated with Alexa Fluor 594 (A11005) were from Invitrogen, HRP-conjugated goat anti-rabbit antibody was from Bio-Rad, antiphospho-ErK (no. 9101) and antiphospho-Smad1 (no. 9416S) antibodies were from Cell Signaling Technology, and recombinant human bone morphogenetic protein 2 (355-EBC-010) was from R&D Systems. D(−)-2-amino-5-phosphonovaleric acid (AP5), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), and BMI were purchased from Tocris Bioscience. AG1478 was from Calbiochem. Other chemicals were from Sigma–Aldrich. The stock solution of chemicals was diluted with artificial cerebrospinal fluid (ACSF) freshly before application. When DMSO (Sigma) was used to prepare solutions, its final concentration was 0.05% or less.

Slice Preparation. Hippocampal slices were prepared as described previously (2). In brief, mice (5–8 wk old, male) were decapitated, and transverse hippocampal slices (400 μm) were prepared using a Vibroslice (VT 1000S; Leica) in ice-cold ACSF. For field potential recording, the same ACSF was used for slice cutting and recording: 120 mM NaCl, 2.5 mM KCl, 1.2 mM NaH_2PO_4, 2.0 mM $CaCl_2$, 2.0 mM $MgSO_4$, 26 mM $NaHCO_3$, and 10 mM glucose. For whole-cell recording, the slice-cutting solution contained 220 mM sucrose, 2.5 mM KCl, 1.3 mM $CaCl_2$, 2.5 mM $MgSO_4$, 1 mM NaH_2PO_4, 26 mM $NaHCO_3$, and 10 mM glucose, whereas the recording ACSF contained 126 mM NaCl, 26 mM $NaHCO_3$, 3.0 mM KCl, 1.2 mM NaH_2PO_4, 2.0 mM $CaCl_2$, 1.0 mM $MgSO_4$, and 10 mM glucose. After cutting, hippocampal slices were left for "recovery" in the chamber for 30 min at 34 °C and then at room temperature (25 ± 1 °C) for an additional 2–8 h. All solutions were saturated with 95% O_2/5% CO_2 (vol/vol). A cut was made between CA1 and CA3 in hippocampal slices to prevent the propagation of epileptiform activity in the recording of LTP.

Electrophysiological Recordings. Slices were placed in the recording chamber, which was superfused (3 mL/min) with ACSF at 32–34 °C. fEPSPs were evoked in the CA1 stratum radiatum by stimulating SC with a two-concentrical bipolar stimulating electrode (FHC) and recorded in current-clamp by the Axon MultiClamp 700B (Molecular Devices) amplifier with ACSF-filled glass pipettes (1–5 MΩ). Test stimuli consisted of monophasic 100-μs pulses of constant currents (with intensity adjusted to produce 25% of the maximum response) at a frequency of 0.033 Hz. The strength of synaptic transmission was determined by measuring the initial (10–60% rising phase) slope of fEPSPs. LTP was induced by two trains of 100-Hz stimuli, each having 50 pulses, at 100 Hz separated by 10 s to the same intensity of the test stimulus. The level of LTP was determined at an average of 0–60 min after tetanus stimulation.

Whole-cell patch-clamp recordings from CA1 neurons were visualized with infrared optics using an upright microscope equipped with a 40× water-immersion lens (BX51WI; Olympus) and infrared-sensitive CCD camera. EPSCs were evoked by electrical stimulation of axons in the stratum radiatum (0.033 Hz) in the presence of the $GABA_A$ receptor antagonist BMI (20 μM) and were verified by adding AP5 (100 μM) and CNQX (20 μM). The pipette (input resistance: 4–6 MΩ) solution contained 105 mM K-gluconate, 30 mM KCl, 10 mM Hepes, 10 mM phosphocreatine, 4 mM ATP-Mg, 0.3 mM GTP-Na, 0.3 mM EGTA, and 5 mM QX314 (pH 7.35, 285 mOsm). For pharmacologically isolated AMPAR- or NMDAR-mediated EPSCs, we blocked NMDAR with 100 μM AP5 and AMPAR with 20 μM CNQX, respectively. To record mEPSCs, 1 μM TTX was added in bath solution. $GABA_A$ receptor-mediated IPSCs were pharmacologically isolated in the presence of AP5 (50 μM) and CNQX (20 μM). To record eIPSCs, the pipettes (input resistance: 2–5 MΩ) were filled with the solution: 100 mM $CsCH_3SO_3$, 60 mM CsCl, 10 mM Hepes, 0.2 mM EGTA, 1 mM $MgCl_2$, 4 mM Mg-ATP, 0.3 mM Na-GTP, and 5 mM QX314 (pH 7.25, 285 mOsm). To record mIPSCs, the concentration of CsCl was increased to 140 mM, $CsCH_3SO_3$ was omitted to enhance the driving force of Cl^-, and 1 μM TTX was added in the bath solution. Data were collected when series resistance fluctuated within 20% of initial values (20–40 MΩ), filtered at 1 kHz, and sampled at 10 kHz.

Neuron Culture. Hippocampal explants were isolated from embryo day 18 rat embryos and digested with 0.125% trypsin for 30 min at 37 °C, followed by trituration with a pipette in plating medium [DMEM with 10% (vol/vol) FBS]. Dissociated neurons were plated onto 60-mm dishes coated with poly-D-lysine (Sigma–Aldrich) at a density of 1×10^6 cells per dish. After culturing for 4 h, media were changed to neurobasal medium supplemented with 2% (vol/vol) B27 and 0.5 mM L-glutamate (Invitrogen). Twice a week, half of the medium was replaced by freshly prepared medium.

Western Blot Analysis. Hippocampus was dissected from PV-Cre; $ErbB4^{-/-}$ or control mice, whereas the CA1 region was dissected from CaMKII-Cre;$ErbB4^{-/-}$ or control hippocampal slices. Tissues were homogenized in the extraction buffer [150 mM NaCl, 10 mM Tris·HCl (pH 7.4), 1 mM $MgCl_2$, 0.5% Triton X-100, 0.1 mM PMSF, and a mixture of protease inhibitors] for 30 min. Homogenates (40 μg of protein) or neuronal lysates (DIV11, hippocampal) were resolved by 8% or 10% SDS/PAGE and analyzed by Western blotting using respective antibodies as described previously (2).

Immunohistochemistry. Immunofluorescence staining was carried out as described previously (4). Briefly, mice were perfused transcardially with 4% (4 g/100 mL) paraformaldehyde and 4% (4 g/100 mL) sucrose in 0.1 M PBS; brain tissues were then postfixed at 4 °C for 24 h, and coronal slices (40 μm) were prepared using a Vibroslice (VT 1000S). Brain sections were treated with 3% (vol/vol) normal goat serum in 0.1 M PBS and incubated with anti-ErbB4 antibody 0618 (1:400) and mouse monoclonal anti-PV (1:1,000) at 4 °C for 36–48 h. Immunoreactivity was imaged by Alexa 488- and Alexa 594-conjugated goat anti-mouse IgG (1:400).

Contextual Fear Conditioning. Behavioral analysis was carried out with 8- to 12-wk-old mice by investigators unaware of their genotypes. Mice were first habituated to the behavioral room and were then allowed to freely explore the apparatus (MED-VFC-NIR-M; Med Associates) for 3 min. During training, mice were placed in the conditioning chamber and exposed to two tone-footshock pairings (tone, 30 s, 80 dB; footshock, 2 s, 0.5 mA) with an interval of 60 s at 24 h after training. Mice were returned to the chamber to evaluate

contextual fear learning. Freezing during training and testing was scored using Med Associates Video-Tracking and scoring software. Data are expressed as percent freezing in 60-s epochs, with each epoch divided into 6 or 12 5-s bins.

1. Holmes WE, et al. (1992) Identification of heregulin, a specific activator of p185erbB2. *Science* 256:1205–1210.
2. Woo RS, et al. (2007) Neuregulin-1 enhances depolarization-induced GABA release. *Neuron* 54:599–610.
3. Zhu XJ, Lai C, Thomas S, Burden SJ (1995) Neuregulin receptors, erbB3 and erbB4, are localized at neuromuscular synapses. *EMBO J* 14:5842–5848.
4. Wen L, et al. (2010) Neuregulin 1 regulates pyramidal neuron activity via ErbB4 in parvalbumin-positive interneurons. *Proc Natl Acad Sci USA* 107:1211–1216.

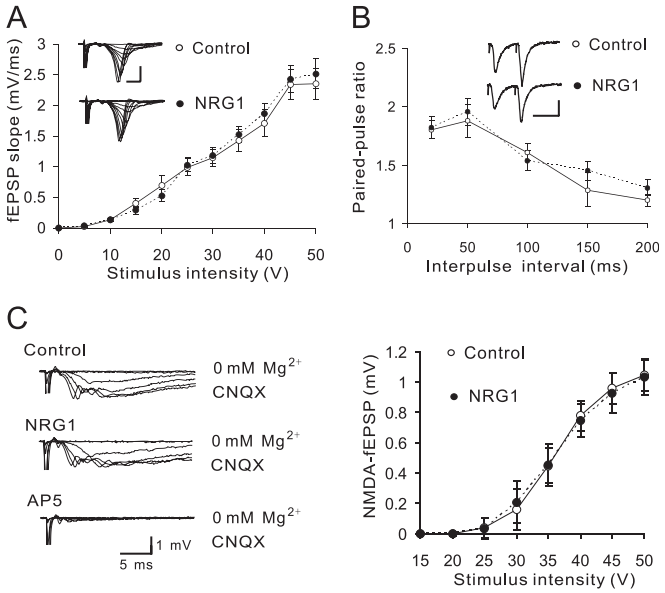

Fig. S1. No effect of NRG1 on AMPAR- or NMDAR-mediated fEPSPs in hippocampal slices. (*A*) No change in fEPSP slopes at different stimulus intensities (n = 11 slices; $P > 0.05$). (*Inset*) Representative recordings. (*B*) PPRs of fEPSPs without or with 1 nM NRG1 (n = 9 slices; $P > 0.05$). (*C*) No change in NMDAR fEPSPs slopes in the presence of 20 μM CNQX (n = 5 slices; $P > 0.05$). fEPSPs were recorded in the absence of Mg^{2+}. (*Left*) Representative traces. (*Right*) Input-output of NMDA fEPSPs.

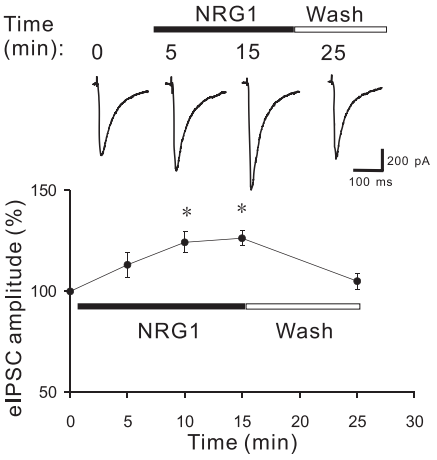

Fig. S2. Time-dependent effects of NRG1 on eIPSCs in CA1 neurons. NRG1 (1 nM) was applied during the period indicated by the bar. The holding potential was −70 mV. (*Upper*) Representative eIPSC traces obtained at indicated times. (*Lower*) Normalized eIPSC amplitudes. Data are presented as the mean ± SEM (n = 8). *$P < 0.01$.

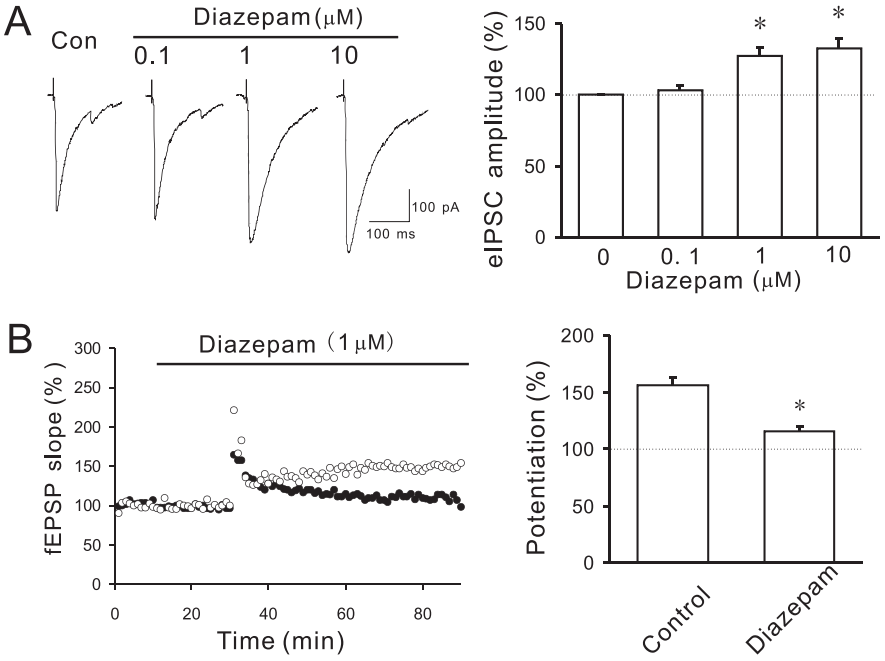

Fig. S3. Dose-dependent effects of diazepam on eIPSCs and LTP. (*A*) Effects of diazepam on eIPSCs. (*Left*) Representative eIPSC traces treated with vehicle or diazepam at indicated concentrations (Con). (*Right*) Summary of data on the left (n = 10). (*B*) Effects of diazepam on LTP. (*Left*) Normalized fEPSP slopes were plotted every 1 min for control (○), or slices were treated with 1 μM diazepam (●) that was applied during the period indicated by the bar. (*Right*) Summary of data on the left (n = 5 slices for control, n = 6 slices for 1 μM diazepam). Data are presented as the mean ± SEM. *$P < 0.01$.

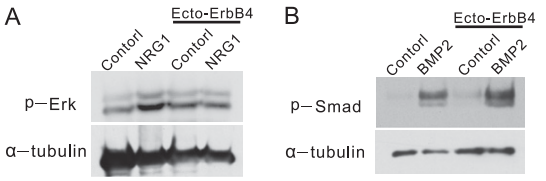

Fig. S4. Ecto-ErbB4 blocked NRG1 but not bone morphogenetic protein 2 (BMP2) signaling in hippocampal neurons. Hippocampal neurons (DIV 11) were treated with or without ecto-ErbB4 for 30 min before stimulation with 3 nM NRG1 (*A*) or 100 ng/mL BMP2 (*B*) for 20 min. Neuronal lysates were subjected to Western blot analysis with antibodies against phospho-Erk (*A*) or phospho-Smad (*B*) and for α-tubulin to indicate equal amounts of samples.

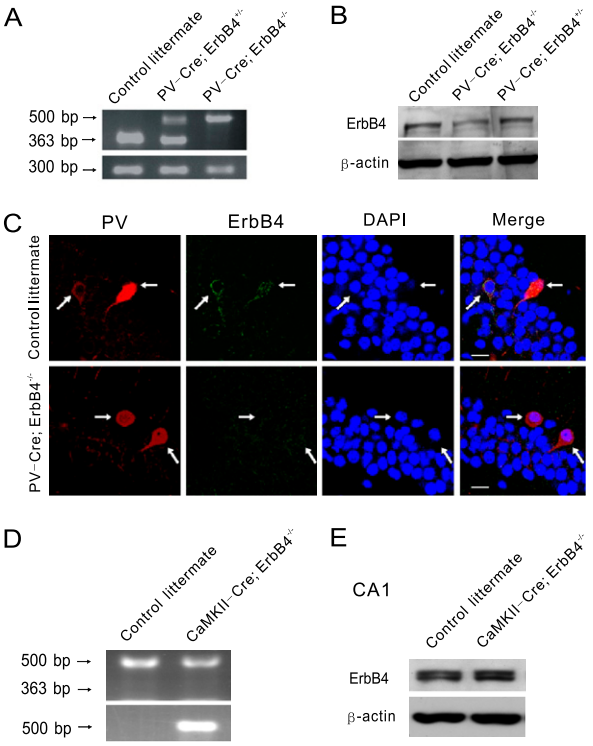

Fig. S5. Reduced levels of ErbB4 in PV-Cre;ErbB4$^{-/-}$ but not in CaMKII-Cre;ErbB4$^{-/-}$ hippocampus. (*A*) Generation of PV-Cre;ErbB4$^{-/-}$ mice. PV-Cre mice were crossed with loxP-flanked ErbB4 mice to generate PV-Cre;ErbB4$^{-/-}$ (PV-Cre;ErbB4$^{loxP/loxP}$) mice. Control littermates were PV-Cre;ErbB4$^{+/+}$ mice. PCR products were 300 bp for PV-Cre allele, 363 bp for WT ErbB4 allele, and 500 bp for loxP-flanked ErbB4 allele. (*B*) Reduced levels of ErbB4 in PV-Cre;ErbB4$^{-/-}$ hippocampus. Hippocampus was isolated from indicated mice, homogenized, and blotted for ErbB4 and β-actin (to indicate equal loading). (*C*) Specific ablation of ErbB4 in PV-positive neurons in hippocampus. Slices were stained with DAPI (to indicate nuclei) and antibodies against ErbB4 and PV, which were visualized by Alexa 488- and Alexa 594-conjugated secondary antibodies, respectively. Arrows indicate PV-positive neurons. (Scale bar: 15 μm.) (*D*) Characterization of CaMKII-Cre;ErbB4$^{-/-}$ mice. CaMKII-Cre mice were crossed with loxP-flanked ErbB4 mice to generate CaMKII-Cre;ErbB4$^{-/-}$ (CaMKII-Cre;ErbB4$^{loxP/loxP}$) mice. Control littermates were CaMKII-Cre$^{-/-}$;ErbB4$^{loxP/loxP}$ mice. The PCR product was 500 bp for Cre in CaMKII-Cre mice. (*E*) Similar levels of ErbB4 in CaMKII-Cre; ErbB4$^{-/-}$ hippocampus. Western blotting was done as in *B*.

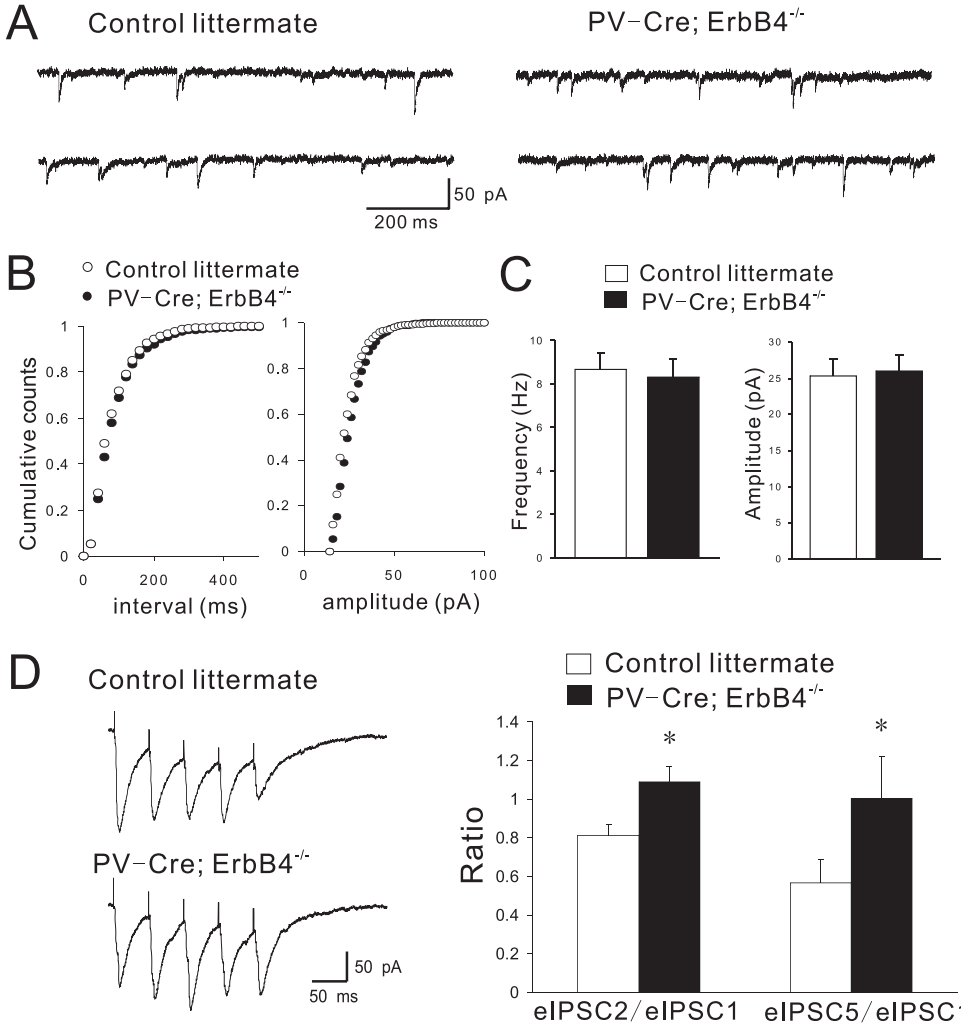

Fig. S6. Characterization of inhibitory transmission of PV-Cre;ErbB4$^{-/-}$ mice. (*A*) Representative traces of mIPSCs in CA1 pyramidal cells in control and PV-Cre;ErbB4$^{-/-}$ mice. (*B*) Cumulative plots of mIPSC frequencies and mIPSC amplitudes in *A*. (*C*) Summary of respective data ($n = 6$ for control and $n = 8$ for PV-Cre;ErbB4$^{-/-}$ mice). (*D*) Increased PPRs of eIPSCs in PV-Cre;ErbB4$^{-/-}$ hippocampus. (*Left*) Representative eIPSC traces by successive stimuli from control and PV-Cre;ErbB4$^{-/-}$ mice. (*Right*) eIPSC PPRs in PV-Cre;ErbB4$^{-/-}$ mice and control hippocampus ($n = 8$ for both groups). Data are presented as the mean ± SEM. *$P < 0.05$.