Supplemental material

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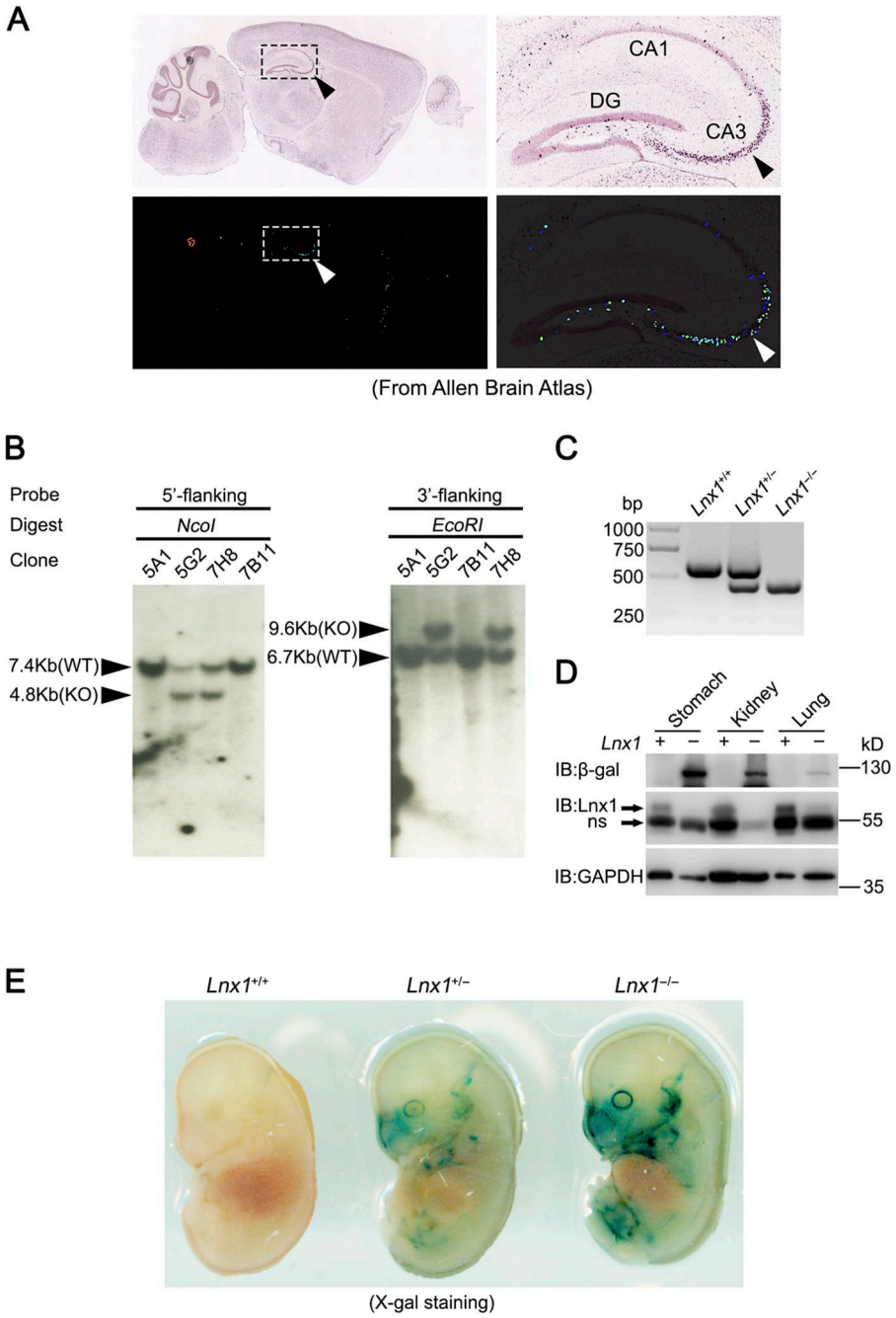


Figure S1. **Generation and characterization of *Lnx1* gene targeting mutant mice. (A)** Shown are data from the Allen Mouse Brain Atlas mRNA expression database. Typical images show that *Lnx1* gene is expressed especially in CA3 pyramidal neurons (arrowheads). **(B)** Southern blot analysis of colonies. Genomic DNA of four colonies was screened by Southern blot using the external probe shown in Fig. 1 A. KO, knockout. **(C)** PCR genotyping shows WT (565 bp) and mutant (421 bp) as indicated. **(D)** Protein lysates of adult $Lnx1^{+/+}$ and $Lnx1^{-/-}$ mice were prepared from tissues as indicated and immunoblotted (IB) with anti–β-gal and Lnx1 antibodies. +, $Lnx1^{+/+}$ mice; –, $Lnx1^{-/-}$ mice; ns, nonspecific band. **(E)** Expression of Lnx1 during embryo development visualized with X-gal staining.

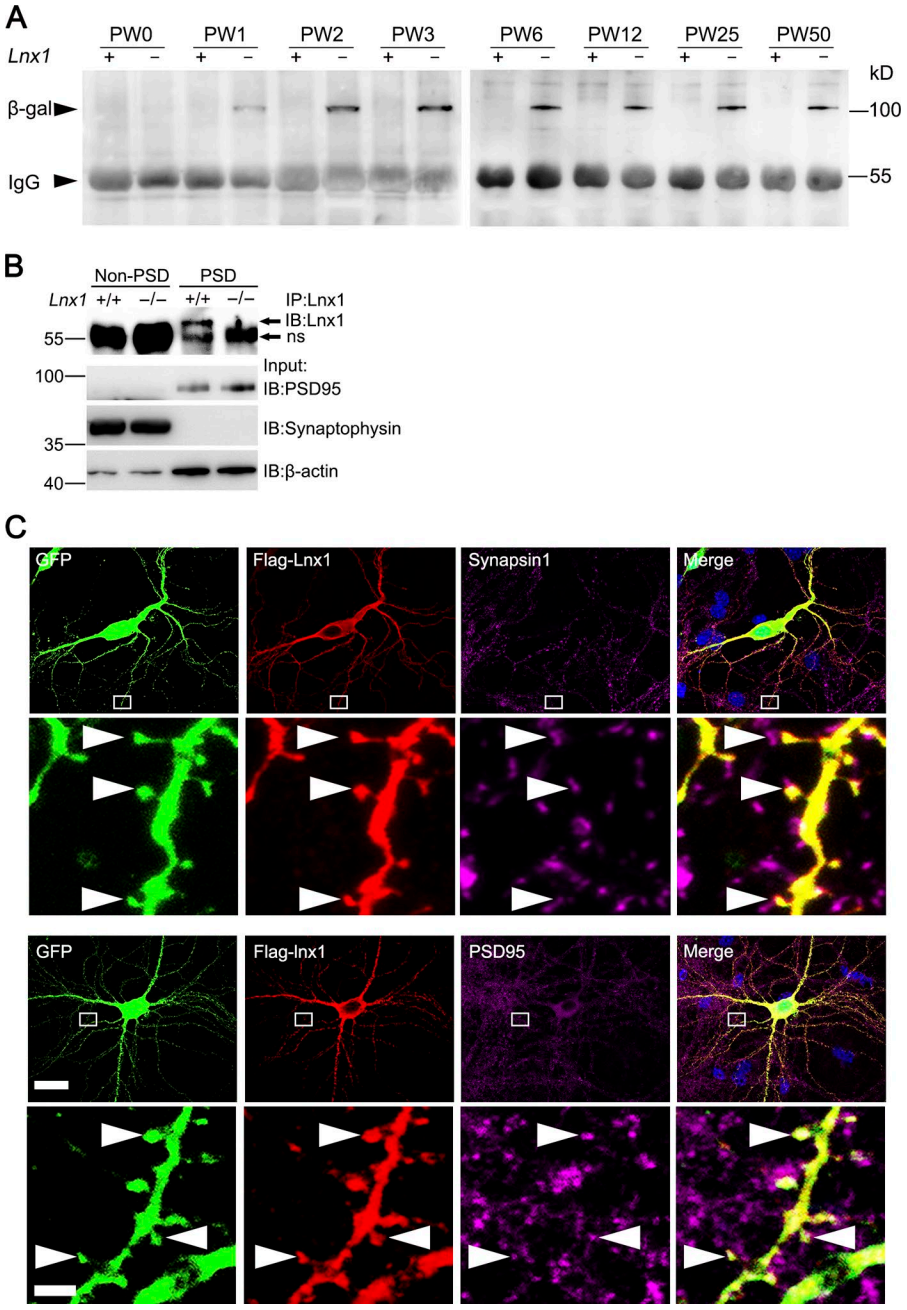


Figure S2. **Lnx1 is localized in postsynaptic compartment of CA3 neurons. (A)** Specific postnatal expression of Lnx1 in hippocampus was identified with quantitatively immunoprecipitated (IP) β-gal protein in hippocampal lysates from *Lnx1*$^{+/+}$ or *Lnx1*$^{-/-}$ mice during development. **(B)** Tissue immunoprecipitation showed expression of Lnx1 in PSD fraction from hippocampal lysates of *Lnx1*$^{+/+}$ mice. ns, nonspecific band; IB, immunoblot. **(C)** Cultured hippocampal neurons transfected with Flag-Lnx1 plasmid were immunostained with Flag antibody and presynaptic (synapsin1)/postsynaptic (PSD95) marker at day 16 in vitro. Lnx1 was observed in postsynaptic area but not presynaptic area. Arrowheads indicate synapses formed by Lnx1-expressed spines and presynaptic terminals. Bars, 25 µm (top); 2 µm (bottom).

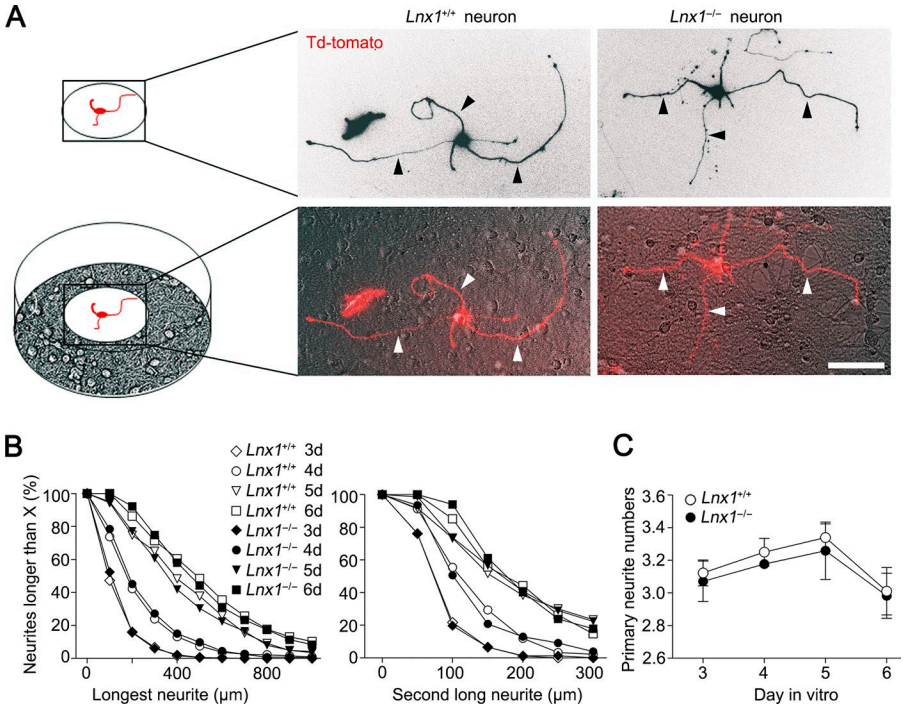


Figure S3. **Axon pruning primed by Lnx1 is independent of extracellular environmental factors. (A)** Schematic diagrams present the procedure for coculture of coverslip-loaded TdT+ neurons and neurons from *Lnx1*$^{-/-}$ or WT mice. Axons (arrowheads) of TdT+ primary hippocampal neurons on coverslips showed no difference in coculture with *Lnx1*$^{-/-}$ neurons or WT neurons. Bar, 100 μm. **(B)** Comparison for neurite lengths in coverslip-segregated neuron coculture with WT or *Lnx1*$^{-/-}$ hippocampal neurons. *n* = 60–317 neurons per group. **(C)** Comparison for primary neurite numbers in coverslip-segregated neuron coculture with WT or *Lnx1*$^{-/-}$ hippocampal neurons. *n* = 60–317 neurons per group. Means ± SEM. Student's *t* test (C).

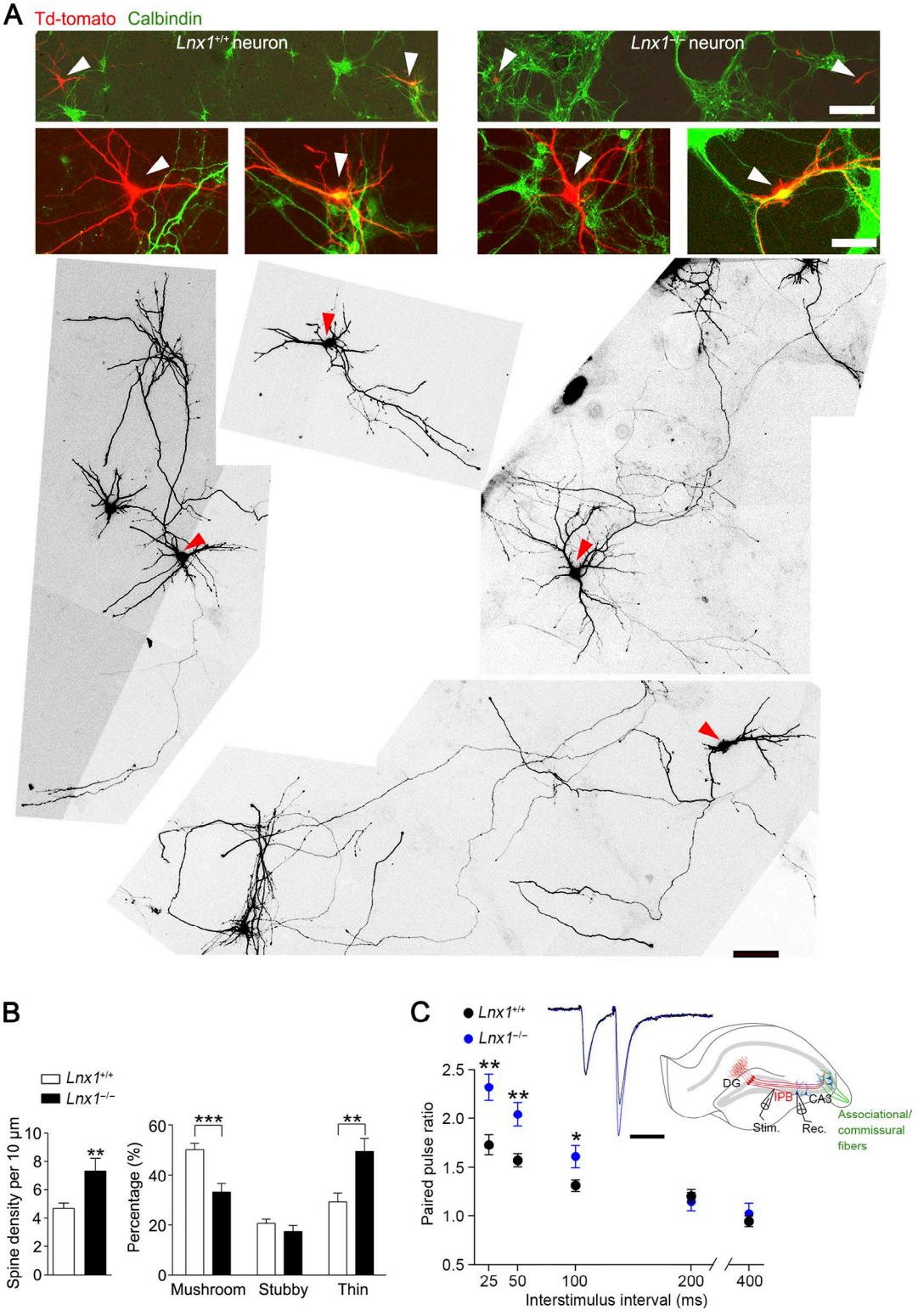


Figure S4. **Roles of Lnx1 for DG cell axon pruning, postsynaptic spine morphogenesis, and presynaptic release function. (A)** Calbindin staining to label the DG cells from TdT+ primary hippocampal neuron coculture with WT or *Lnx1*$^{-/-}$ hippocampal neurons. Top: TdT+ neurons (arrowheads) cocultured with WT or *Lnx1*$^{-/-}$ hippocampal neurons were stained with anti-calbindin. Middle: Magnified images of calbindin-negative or calbindin-positive TdT+ neurons. Bottom: Silhouettes of the calbindin-negative or calbindin-positive TdT+ neurons. Bars: 200 µm (top); 50 µm (middle); 100 µm (bottom). **(B)** Quantification of spine density and percentages of mushroom, stubby, and thin spine types in CA3 neurons of PW3 WT or *Lnx1*$^{-/-}$ mice. *n* = 39–43 neurons from four to five mice per group. **(C)** Schematic diagram shows EPSC recordings of the MF-CA3 synapse. Stimulating electrode was placed in the IPB layer and recording pipette was placed in the CA3 area. Stim, stimulating electrode; Rec, recording pipette. Representative average traces at interstimulus intervals of 50 ms (top) and summary graph (bottom) show PPRs at interstimulus intervals of 25, 50, 100, 200, and 400 ms in PW3 mice. Bar, 50 ms. *n* = 18–21 neurons from three mice per group. Means ± SEM; Student's *t* test (B and C); *, P < 0.05; **, P < 0.01; ***, P < 0.001.

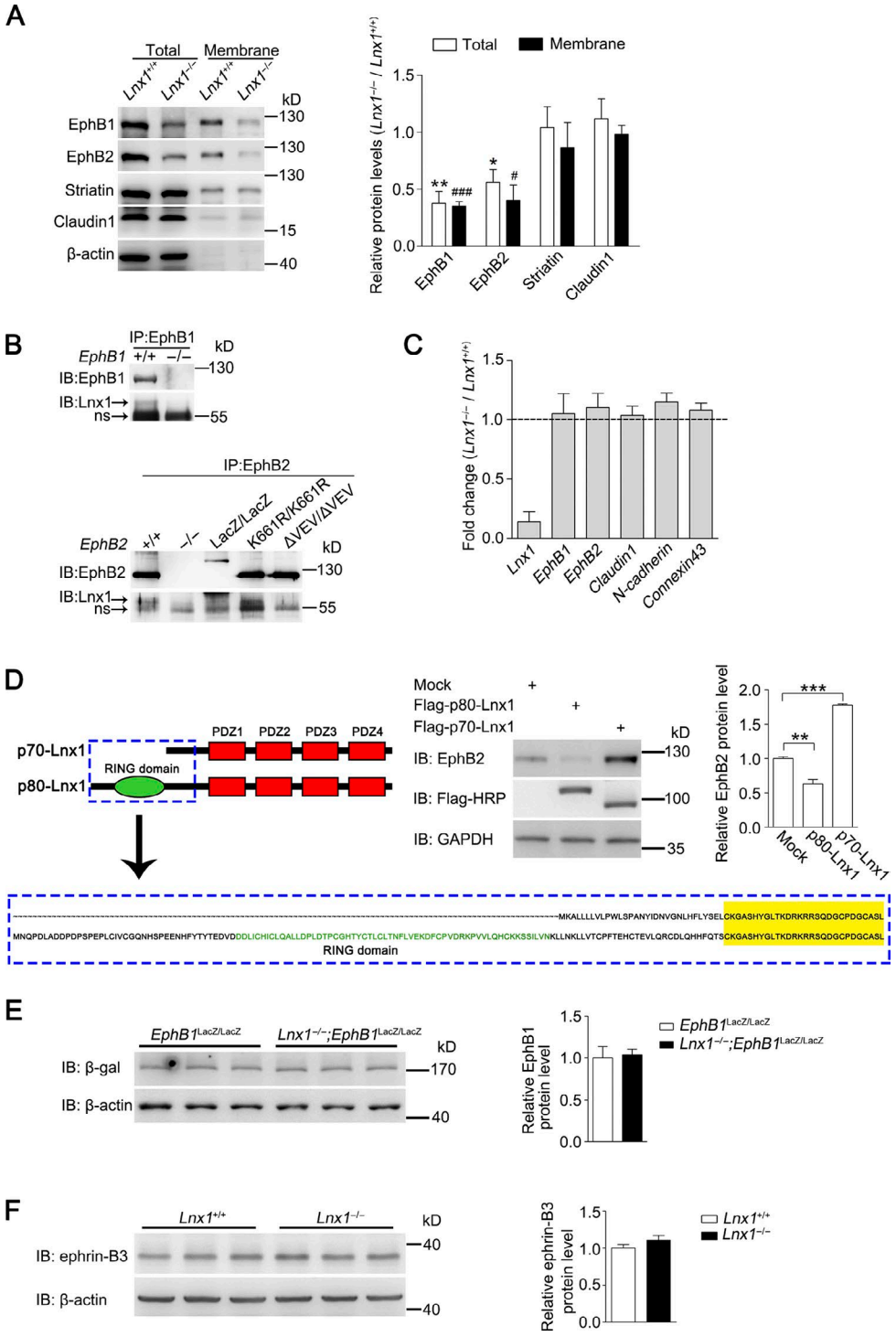
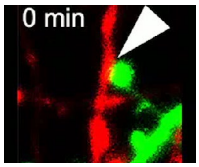
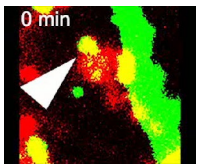


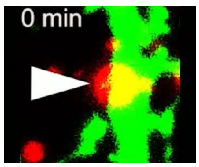
Figure S5. **Ablation of Lnx1 decreases the membrane levels of EphB receptors. (A)** Total levels or membrane levels of membrane proteins were analyzed by Western blotting of primary hippocampal neurons from WT or *Lnx1*$^{-/-}$ mice. *, total; #, membrane. Quantitative results of four biological replicates are shown. **(B)** Coimmunoprecipitation of Lnx1 and EphB1/2 in tissue lysates of hippocampus. Lnx1 was pulled down by EphB1 antibody from the lysates of WT (top) or by EphB2 antibody from the lysates of either WT or *EphB2*$^{K661R/K661R}$ mice, but not from that of *EphB2*$^{-/-}$, *EphB2*$^{LacZ/LacZ}$, or *EphB2*$^{\Delta VEV/\Delta VEV}$ mice (bottom). ns, nonspecific band; IP, immunoprecipitation; IB, immunoblot. **(C)** Quantitative real-time PCR analysis shows mRNA expression of membrane receptors in *Lnx1*$^{-/-}$ mice. *n* = 3 mice per group. **(D)** Schematic representation of p70-Lnx1 and p80-Lnx1. Dotted line shows sequence alignment of N terminus of p70-Lnx1 and p80-Lnx1. The sequences (yellow) to the end are identical between p70-Lnx1 and p80-Lnx1. Sequence of RING domain in p80-Lnx1 is labeled (green). Analysis of endogenous EphB2 protein level in MEF cells transfected with plasmids containing Mock, Flag-p70-Lnx1, or Flag-p80-Lnx1. Quantitative results of three biological replicates are shown. **(E)** The expression of EphB1 (β-gal) protein in hippocampus from PW3 *Lnx1*$^{-/-}$; *EphB1*$^{LacZ/LacZ}$ and *EphB1*$^{LacZ/LacZ}$ mice was detected by Western blot and quantified. *n* = 3 mice per group. **(F)** The expression of ephrin-B3 protein in hippocampus from PW3 *Lnx1*$^{-/-}$ and WT mice was detected by Western blot and quantified. *n* = 3 mice per group. Means ± SEM; one-way ANOVA with Tukey's post hoc test (D) or Student's *t* test (A, C, E, and F); *, P < 0.05; **, P < 0.01; ***, P < 0.001; #, P < 0.05; ###, P < 0.001.



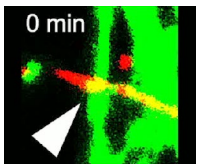
Video 1. **Example of newborn bouton in presynaptic alteration type in a dual color–labeled coculture system.** Time-lapse imaging was performed to observe the temporal dynamics of the axon terminals (red) as indicated by arrowhead. Time-lapse images were taken at 10-min intervals for 60 min.



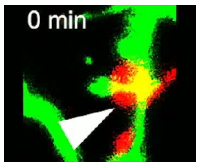
Video 2. **Example of refined bouton in presynaptic alteration type in a dual color–labeled coculture system.** Time-lapse imaging was performed to observe the temporal dynamics of the axon terminals (red) as indicated by arrowhead. Time-lapse images were taken at 10-min intervals for 60 min.



Video 3. **Example of postsynaptic alteration type in a dual color–labeled coculture system.** Time-lapse imaging was performed to observe the temporal dynamics of the spines (green) as indicated by arrowhead. Time-lapse images were taken at 10-min intervals for 60 min.



Video 4. **Example of both alteration type in a dual color–labeled coculture system.** Time-lapse imaging was performed to observe the temporal dynamics of the axon terminals (red) and matched spines (green) as indicated by arrowhead. Time-lapse images were taken at 10-min intervals for 60 min.



Video 5. **Example of none alteration type in a dual color–labeled coculture system.** Time-lapse imaging was performed to observe the temporal dynamics of the axon terminals (red) and matched spines (green) as indicated by arrowhead. Time-lapse images were taken at 10-min intervals for 60 min.

Table S1. **Genes and their primer sequences used for real-time PCR analysis**

Gene name	Gene name and primer sequence	Amplicon length (bp)
Lnx1	Forward: 5′-GGACTCCTATGGACCTCG-3′	220
	Reverse: 5′-CTCTGGACTGCCAAATCG-3′	
EphB1	Forward: 5′-GCCTACCGCAAGTTTA-3′	248
	Reverse: 5′-TCCAGGGTGTTGACGA-3′	
EphB2	Forward: 5′-TCCCGCAATGGTTTCTAC-3′	202
	Reverse: 5′-TGGGCACATCCACTTCTT-3′	
Claudin1	Forward: 5′-TTGATGATGGTTATCGGAACTG-3′	103
	Reverse: 5′-GCTCAGGGAAGATGGTAAGGTA-3′	
N-cadherin	Forward: 5′-AATGCTACCTTCCTTGCTTCTG-3′	146
	Reverse: 5′-GAGTTGGGTTCTGGAGTTTCAC-3′	
Connexin43	Forward: 5′-AAACCTTCCCTCCCTTCCTACT-3′	105
	Reverse: 5′-AATAACCTTGAGCCCTGTTGTA-3′	
β-actin	Forward: 5′-GCTCTTTTCCAGCCTTCCTT-3′	256
	Reverse: 5′-TGATCCACATCTGCTGGAAG-3′	