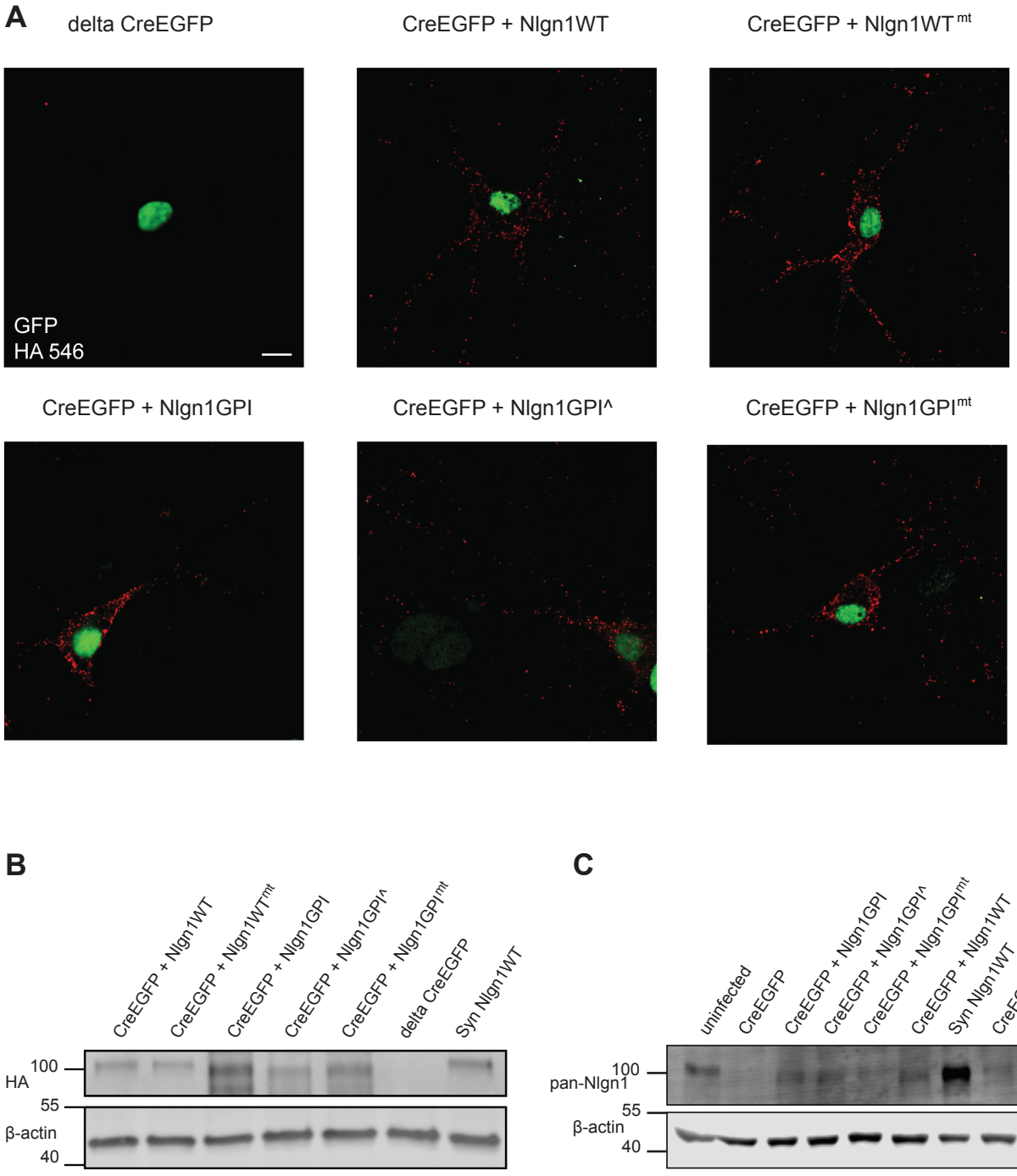

Figure S1. Nlgn1 Rescue Constructs Reach the Plasma Membrane in Cultured Neurons, related to Figure 1.

(A) Images of cultured hippocampal neurons from Nlgn1234 cKO mice infected at DIV 14 with lentiviruses expressing Cre recombinase and HA tagged Nlgn1 in which Nlgn1 sequences were translated via an IRES insert and stained 11 days later for surface HA. (Nlgn1-WT = wild-type Nlgn1, Nlgn1-WTmt = wild-type Nlgn1 with neurexin binding site mutations, Nlgn1-GPI = GPI-anchored Nlgn1, Nlgn1-GPI^ = GPI-anchored Nlgn1 with dimerization site mutations, Nlgn1-GPImt = GPI-anchored Nlgn1 with neurexin binding site mutations) Scale bar: 10 µm

(B) Western blot probed with HA antibody and β-actin as loading control from primary hippocampal neuron lysates, infected as in A. Lentiviruses expressing delta Cre-EGFP and Nlgn1-WT (without IRES) were used as controls.

(C) Expression level of Nlgn1 rescue constructs compared to endogenous Nlgn1. Western blot probed with pan-Nlgn1 antibody from Nlgn1234 cKO primary hippocampal neuron lysates, infected with Cre or Cre + replacements. Uninfected cells and Nlgn1-WT (without IRES) were used as controls. When infected with the same titer, Nlgn1-WT overexpression achieved a much greater expression level as compared to the replacements constructs.

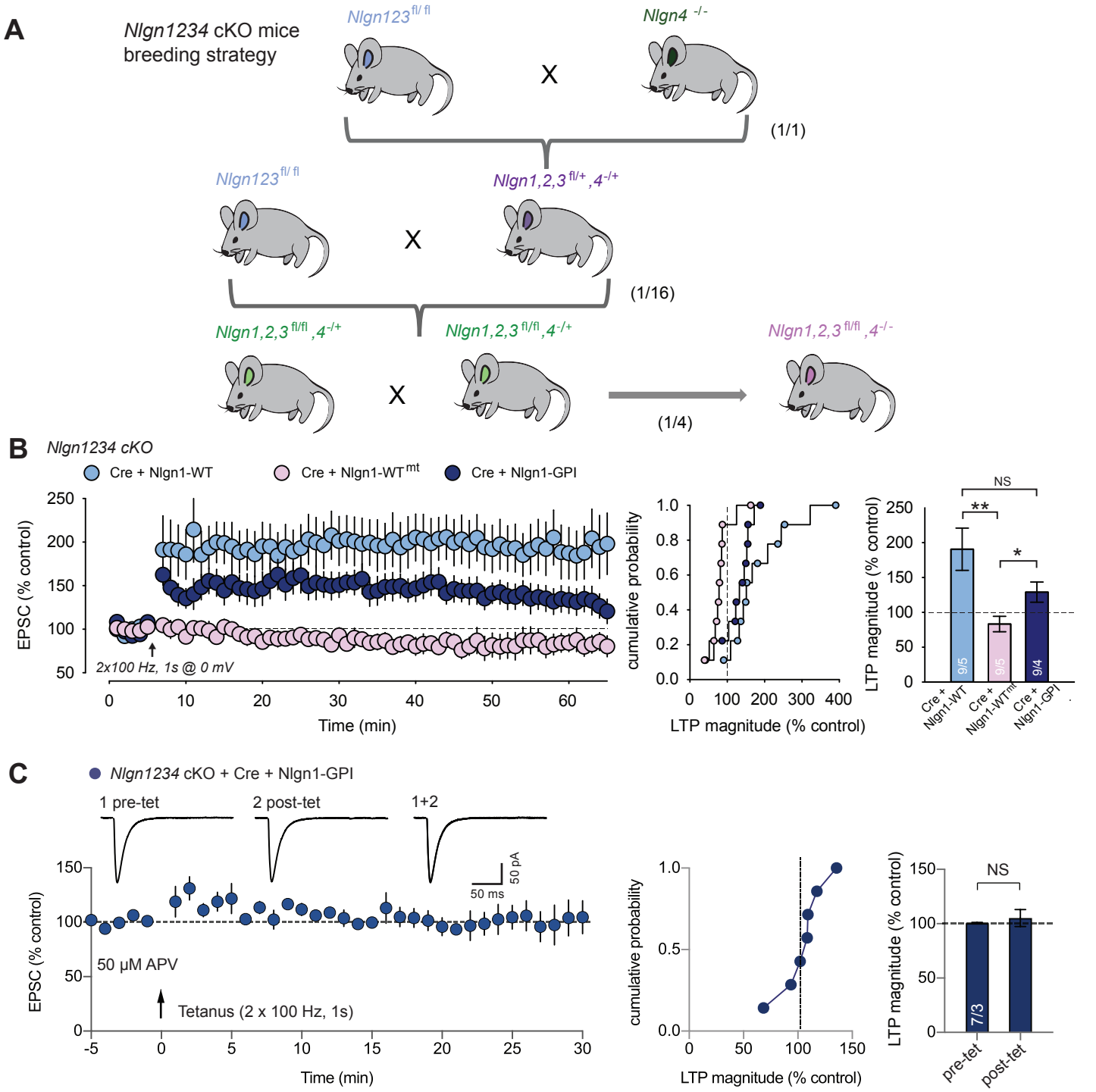

Figure S2. Independent Blinded Study Confirms Nlgn1 Domain Requirements for LTP, related to Figure 2.

(A) Nlgn123fl/fl (cKO) mice were crossed to Nlgn4-/- (KO). After two generations of crossings, Nlgn123fl/fl4-/- (cKO) mice were obtained.
(B) Summary of LTP time course (top panel) in lentiviral infected CA1 pyramidal neurons from Nlgn1234 cKO mice; cumulative frequency plots of LTP magnitudes from individual experiments (lower left); and quantification of LTP magnitude (lower right) from experiments examining the effects of molecular replacement with full-length wild-type Nlgn1 (Cre-EGFP + Nlgn1-WT), wild-type Nlgn1 with neurexin binding mutations (Cre-EGFP + Nlgn1-WTmt) or GPI-anchored Nlgn1 (Cre-EGFP + Nlgn1-GPI). All experiments were performed and analyzed without knowledge of the manipulation that had been performed.
(C) Summary of LTP time course (left panel) in lentiviral Cre + Nlgn1-GPI infected CA1 pyramidal neurons from Nlgn1234 cKO mice with representative traces; cumulative frequency plots of LTP magnitudes from individual experiments (middle panel); and quantification of LTP magnitude (right panel) from experiments examining the effects of molecular replacement with GPI-anchored Nlgn1 (Cre + Nlgn1-GPI) with bath applied APV to block NMDARs. Data in the LTP time course and bar graphs are means ± SEM; numbers in bars indicate cells/mice. Mann Whitney test, ,*p<0.05, **p<0.01, NS = not significant.

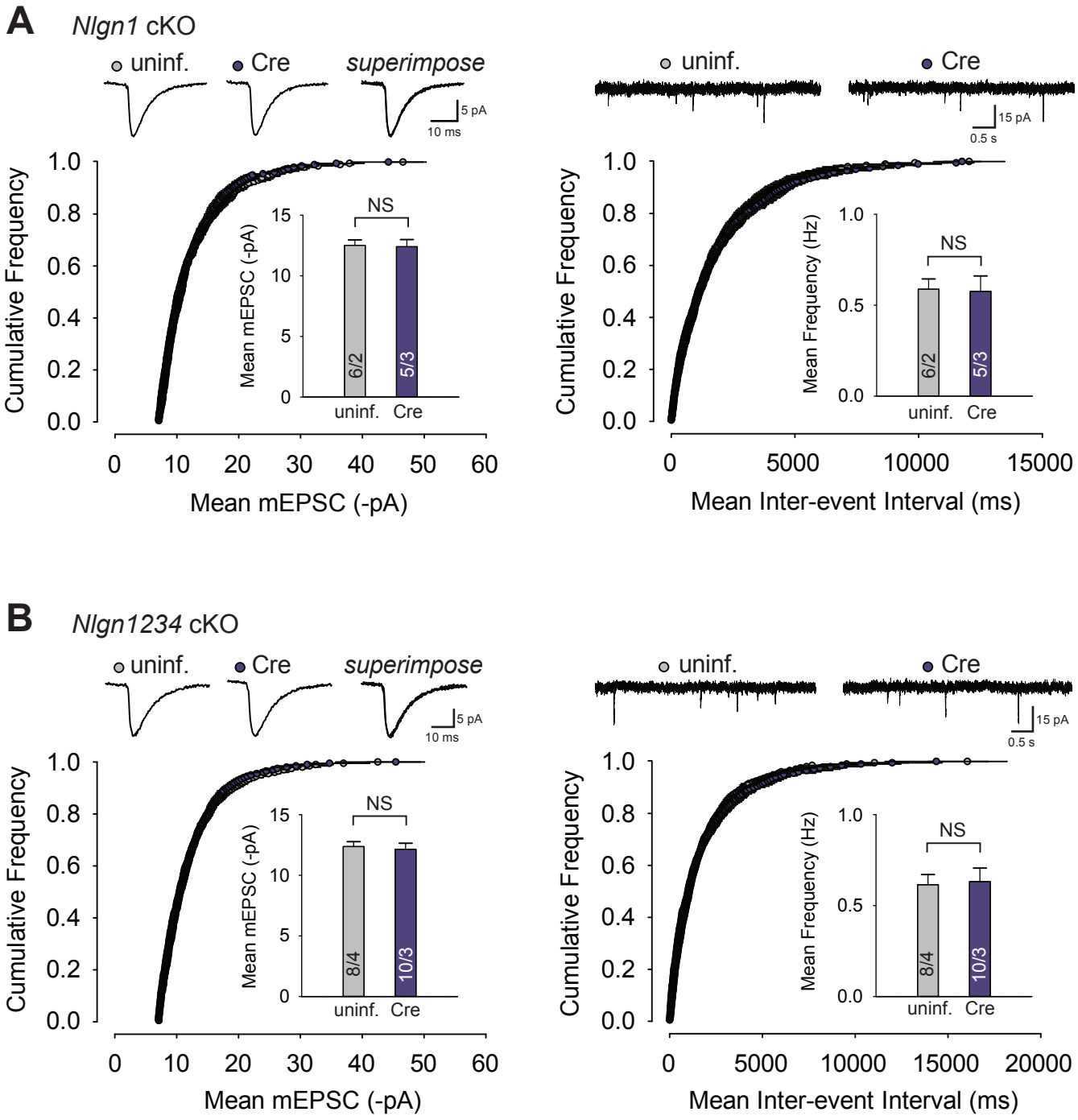

Figure S3. Nlgn1 or Nlgn1234 Conditional Deletion Do not Affect AMPAR Miniature EPSC (mEPSC) Amplitude and Frequency, related to Figure 3.

Conditional knock out of Nlgn1 (A) or Nlgn1234 (B) has no significant effect on the amplitude and frequency of mEPSCs. Cumulative plots and summary bar graphs (mean+SEM) are shown for mEPSC amplitude, inter-event interval and frequency (n=cells/mice). Representative averaged mEPSC traces and an epoch of sampled mEPSCs are shown at the top of each graph. 200 consecutive mEPSCs were collected from each cell. Statistical significance was determined by a t-test comparing Cre expressing cells with uninfected (uninf.) cells. Mann Whitney test, NS = not significant.

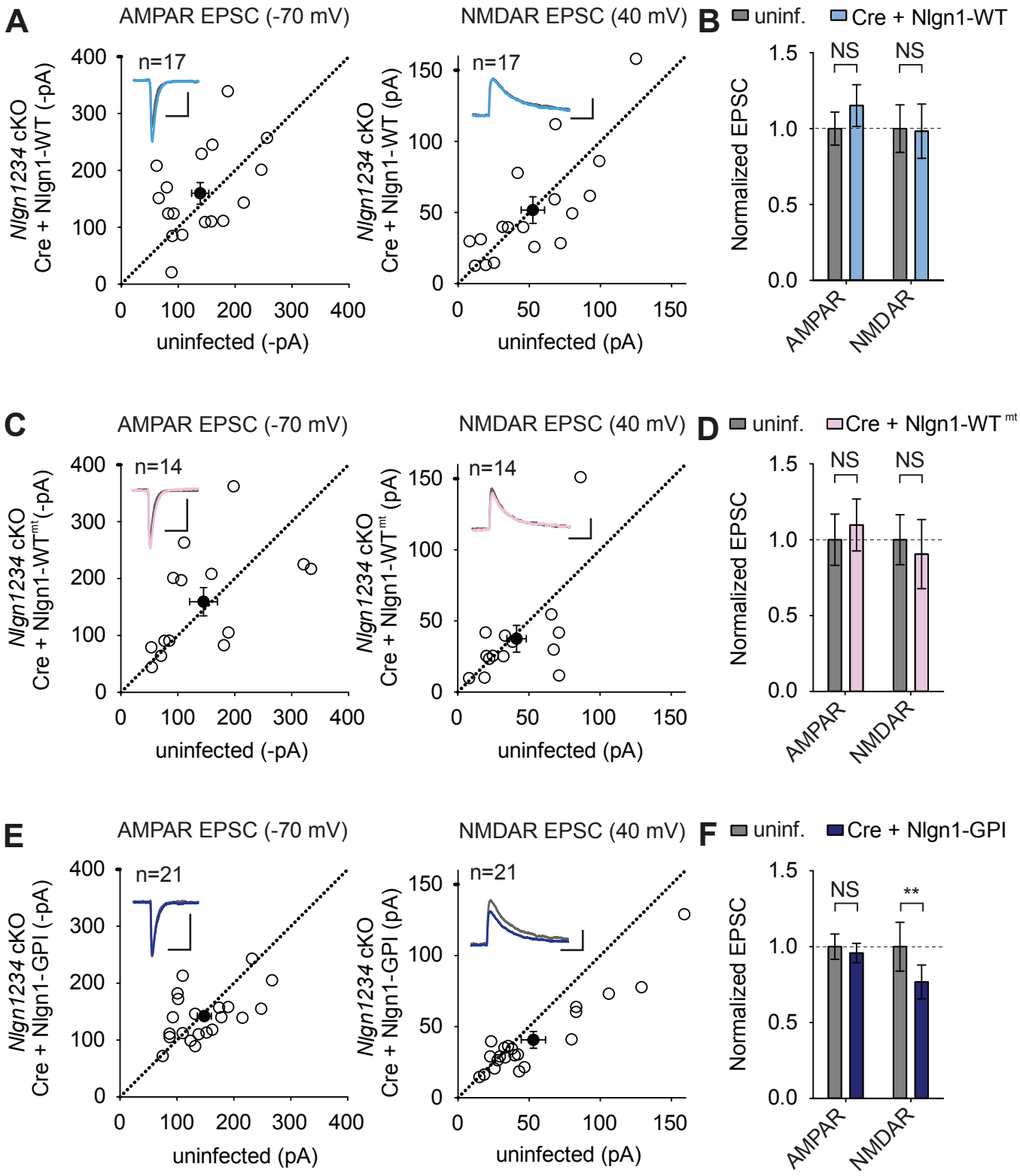

Figure S4. Blinded Paired Whole-cell Recordings Confirms Nlgn1 Domain Requirements for NMDAR-mediated EPSCs, related to Figure 3.

(A, B) Scatter plot (A) and summary (B) of AMPAR- and NMDAR EPSCs from control cells and cells expressing Cre + Nlgn1-WT. Insets show sample EPSCs (scale bars; 50 pA, 100 ms).
(C, D) Scatter plot (C) and summary (D) from control cells and cells expressing Cre + Nlgn1-WTmt. Insets show sample EPSCs (scale bars; 50 pA, 100 ms).
(E, F) Scatter plot (E) and summary (F) from control cells and cells expressing Cre + Nlgn1 GPI.
Insets show sample EPSCs (scale bars; 50 pA, 100 ms). Closed circles and bar graphs are means ± SEM; n = the number of paired recordings. Wilcoxon signed rank test; **p<0.01.

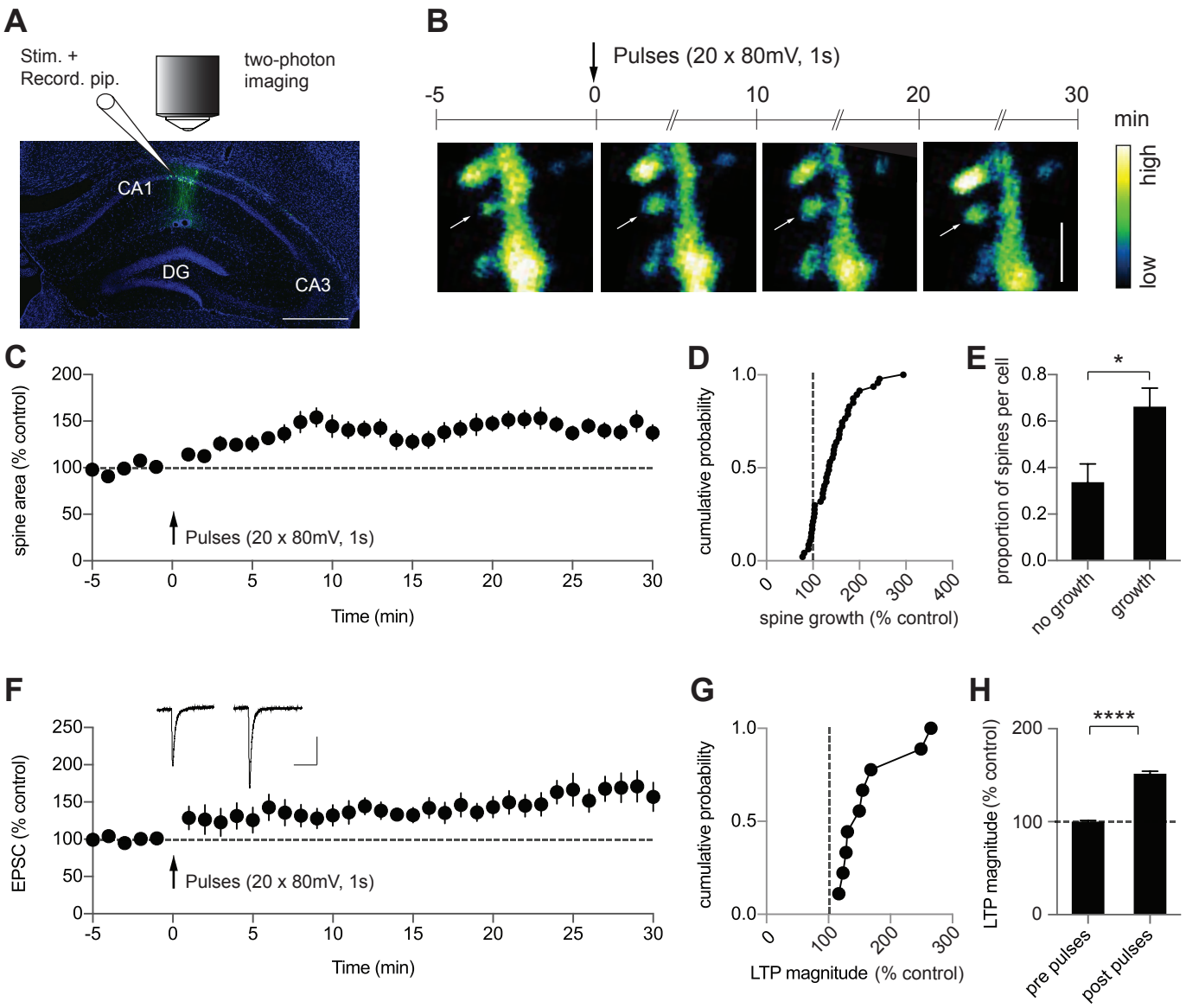

Figure S5. Voltage-pulse LTP Induces Spine Growth in BL6WT Mice, related to Figure 5.

(A) Annotated confocal image of hippocampal slice showing lentiviral Cre-EGFP + AAV-DJ-CMV-DIO-EGFP (cytoplasmic) infection of CA1 pyramidal neurons. The recording pipette (Recording pip.), sealed onto a CA1 pyramidal neuron, was used to elicit LTP. DAPI (blue), GFP (green). Scale bar: 500 µm.(Same as Figure 5A.)
(B) Time course shows representative two-photon images of dendritic segments from BL6WT slices infected with AAV-DJ-CMV-DIO-EGFP (cytoplasmic) and lentiviral Cre-EGFP. White arrow indicates example spine that grew relative to the baseline. Bar on the right displays the color coding reflecting the degree of fluorescence intensity. Scale bar: 2.5 µm
(C) Summary time course of spine growth during VGCC-dependent LTP.
(D) Cumulative frequency plot of changes in spine area for all imaged spines.
(E) Summary graph showing proportion of spines that grew versus those that did not per cell (mean ± SEM). Spines were classified “growth” if the post- to pre-LTP induction area exceeded 1.2. 54 spines from 4 cells were analyzed.
(F) Summary time course of EPSCs following induction of VGCC-dependent LTP. Insets show sample traces pre- and post-pulses (Scale bars: 50 pA, 50 ms).
(G) Cumulative frequency plot of LTP magnitude for individual cells.
(H) Quantification of LTP magnitude (mean ± SEM). Mann Whitney test,*p<0.05, ****p<0.000

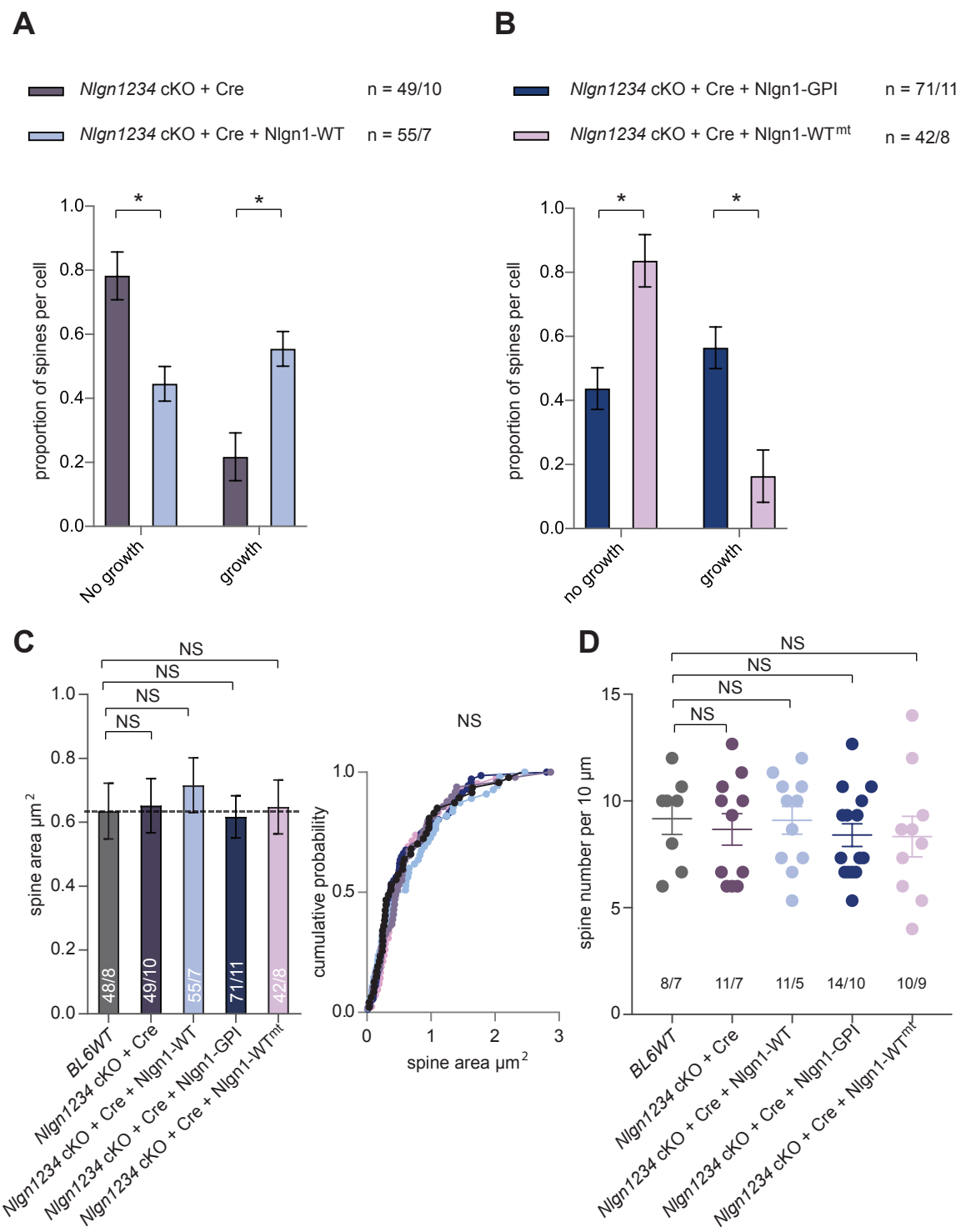

Figure S6. LTP-Induced Growth of Spines Requires Nlgn1 Extracellular Domain Binding to Presynaptic Neurexins, while Baseline Spine Sizes and Spine Numbers Are Not Affected by any Neuroligin Manipulations, related to Figure 5 and 6.

(A) Summary graph showing proportion of spines that grew versus those that did not per cell (mean ± SEM) in Nlgn1234 cKO mice expressing Cre versus Cre + Nlgn1-WT (n=spines/cells).
(B) Summary graph showing proportion of spines that grew versus those that did not per cell (mean ± SEM) in Nlgn1234 cKO mice expressing Cre + Nlgn1-GPI versus Cre + Nlgn1-WTmt. (n=spines/cells). Mann Whitney test,* $p<0.05$
(C) Summary graph (left) and cumulative frequency plot of average spine area (right) in BL6WT mice and Nlgn1234 cKO mice expressing Cre, Cre + Nlgn1-WT, Cre + Nlgn1-GPI and Cre + Nlgn1WTmt (n=spines/cells). Multiple comparison in one way-Anova, NS = not significant.
(D) Summary plot of spine number per 10 µm in BL6WT mice and Nlgn1234 cKO mice expressing Cre, Cre + Nlgn1-WT, Cre + Nlgn1-GPI and Cre + Nlgn1WTmt (n=cells/mice). Multiple comparison in one way-Anova, NS = not significant.

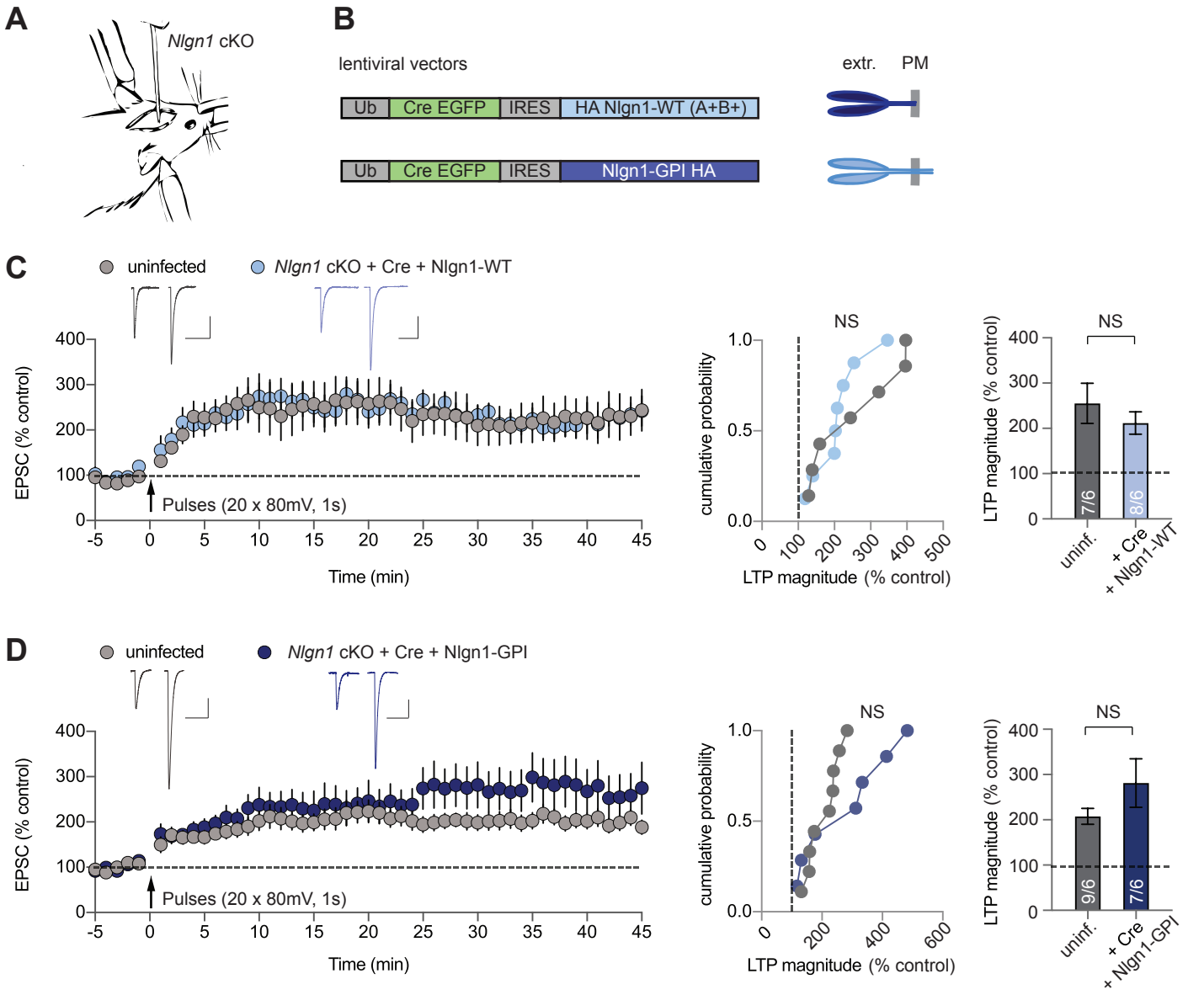

Figure S7. The Extracellular Domain of Nlgn1 Rescues VGCC-dependent LTP in Nlgn1 cKO Mice, related to Figure 5 and 6.

(A) Schematic of lentiviral stereotactic injection into the CA1 region of the hippocampus of Nlgn1 cKO mice.
(B) Schematics of lentiviral constructs (left) with diagrams of Nlgn1 rescue proteins (right).
(C, D) Summary of voltage pulse-induced LTP time course (left panels) in uninfected and lentiviral infected CA1 pyramidal neurons showing sample average EPSCs pre- and post-tetanus (35 – 40 min); cumulative frequency plots of LTP magnitudes from individual experiments (middle panels); and quantification of LTP magnitude (right panels). Effects of expressing Cre-EGFP and wild-type Nlgn1 are shown in C. Effects of expressing Cre-EGFP and Nlgn1-GPI are shown in D. Scale bars: 50 pA, 100 ms. Numbers in bars correspond to cells/animals. All values plotted with error bars are mean ± SEM. Mann-Whitney test, NS = not significant