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Supplemental Information

Non-ionotropic NMDA receptor signaling gates bidirectional structural plasticity of dendritic spines

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Figure S1

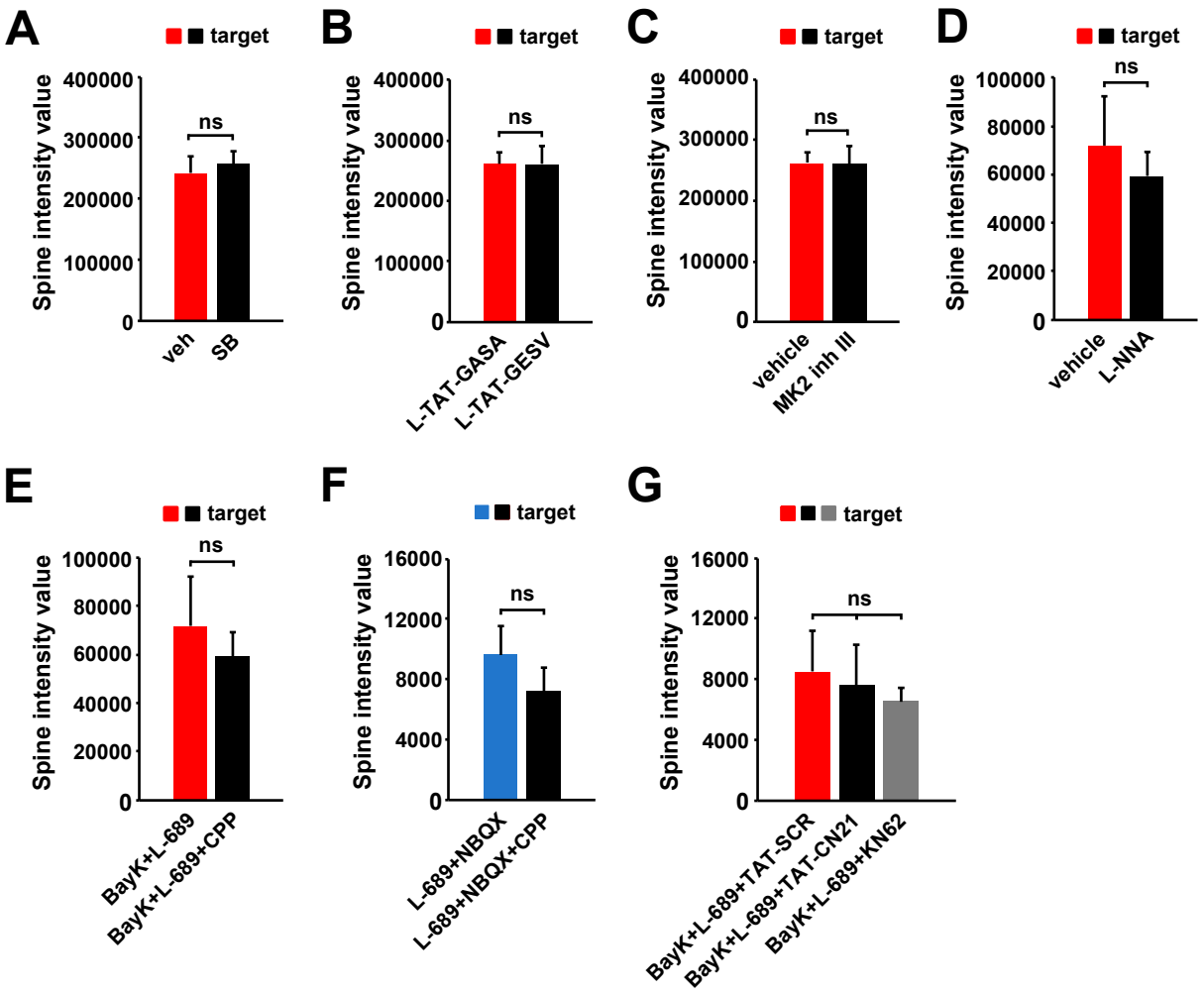

Figure S1, related to Figures 1-4: Drug incubation does not alter baseline spine volumes. **(A)** No difference in baseline volume of spines between vehicle (red bar; 11 spines/11 cells) and SB203580 (2 μM) (black bar;11 spines/11 cells) after incubation for 30 min. **(B)** No difference in baseline volume of spines between 1 μM L-TAT-GASA (red bar; 9 spines/9 cells) and 1 μM L-TAT-GESV (black bar; 9 spines/9 cells). **(C)** No difference in baseline volume of spines between vehicle (red bar; 12 spines/ 12 cells) and 10 μM MK2 inhib III (black bar;11 spines/11 cells). **(D)** No difference in baseline volume of spines between vehicle (red bar; 11 spines/11 cells) and 100 μM L-NNA (black bar;12 spines/12 cells). **(E)** No difference in baseline volume of spines between 10 μM Bay-K + 10 μM L-689 (black bar; 9 spines/9 cells) and those also with 50 μM CPP (black bar; 10 spines/10 cells). **(F)** No difference in baseline volume of spines between L-689 and 50 μM NBQX (blue bar; 7 spines/7 cells) and those also with 50 μM CPP (black bar; 6 spines/6 cells). **(G)** No difference in baseline volume of spines between 10 μM Bay-K + 10 μM L-689 + 5 μM TAT-SCR (red bar; 6 spines/6 cells), Bay-K + L-689 + 5 μM TAT-CN21 (black bar; 6 spines/6 cells), and Bay-K + L-689 + KN62 (10 μM) (grey bar; 6 spines/6 cells). Two-tailed t-test in (A-F), and one-way ANOVA with Tukey's test in (G). Data are represented as mean +/- SEM. *p < 0.05; **p < 0.01, ***p < 0.001.

Figure S2

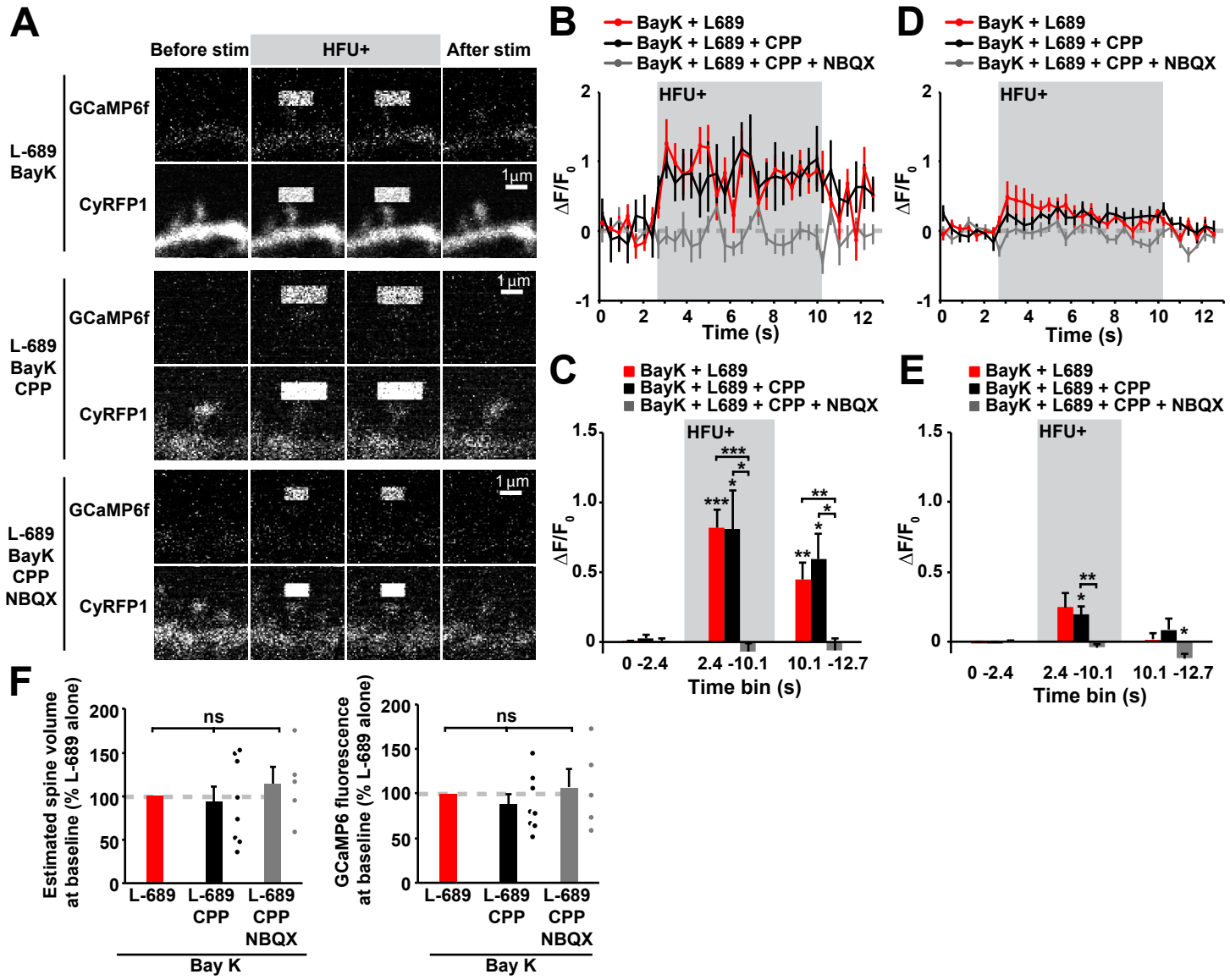

Figure S2, related to Figure 3: Blocking non-ionotropic NMDAR signaling with CPP does not affect the magnitude of Ca2+ influx during HFU+ stimulation.

(A) Images of dendrites from CA1 neurons of organotypic slices expressing both CyRFP and GCaMP6f at DIV13-18 before, during, and after HFU+ at individual spines in the presence of L-689 (10 µM) and Bay K (10 µM), in combination with CPP (50 µM) alone or with CPP (50 µM) and NBQX (50 µM). Middle images in each row show bleed through of uncaging laser stimulation. **(B, C)** HFU+ led to comparable levels of calcium influx into spines in the presence of L-689 and Bay K (red filled circles/bar; 11 spines/11 cells) and in combination with CPP (black filled circles/bar; 12 spines/11 cells). Calcium influx through VGCCs is blocked by inhibition of AMPARs with NBQX (gray filled circles/bar; 10 spines/9 cells). **(D, E)** Small, delayed calcium influx is observed in dendrite during HFU+ in the presence of L689 and Bay K (red filled circles/bar; 11 spines/11 cells) or in combination with CPP (black filled circles/bar; 12 spines/11 cells) or NBQX (gray filled circles/bar;10 spines/9 cells). **(F)** Left: No difference in baseline volume of spines in Bay K, L-689, and CPP (red; 8 spines/8 cells); and Bay K, L-689, CPP, and NBQX (gray; 5 spines/5 cells) relative to those exposed to Bay K and L-689 alone (black; 8 spines/8 cells). Right: No difference in baseline GCaMP6 fluorescence of spines in Bay K, L-689, and CPP (red; 8 spines/8 cells); and Bay K, L-689, CPP, and NBQX(gray; 5 spines/5 cells) relative to that for Bay K and L-689 alone (black; 8 spines/8 cells). Two-way RM ANOVA with Bonferroni test was used in (C) and (E), and one-way ANOVA with Dunnett's test was used in (F). Data are represented as mean +/- SEM. *p < 0.05; **p < 0.01, ***p < 0.001.

Figure S3

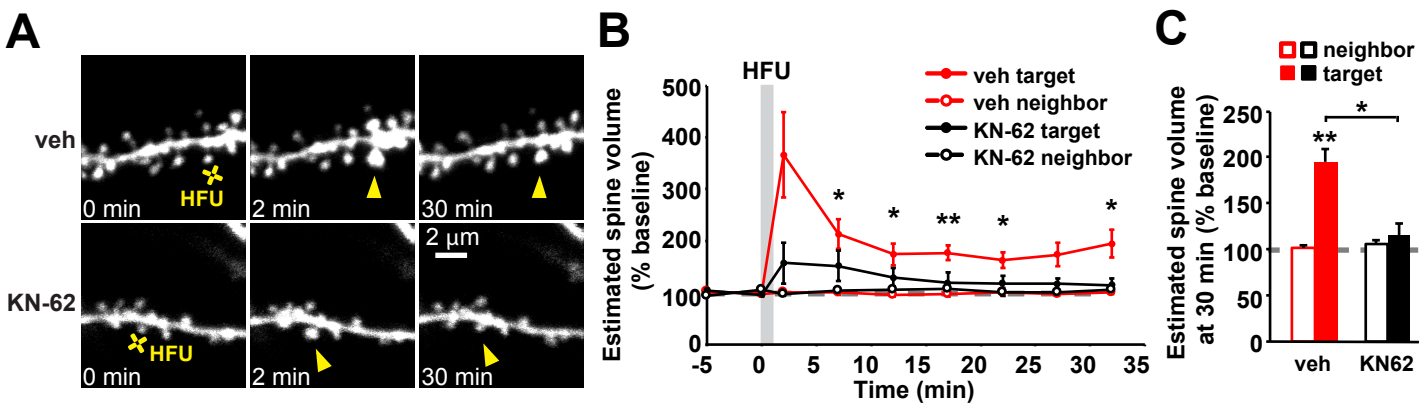

Figure S3, related to Figure 4: Inhibition of CaMKII blocks LTP-induced long-term spine growth **(A)** Images of dendrites from CA1 neurons of acute slices from P16-20 GFP-M mice before and after HFU stimulation (yellow cross) of individual spines during vehicle conditions and in the presence of the CaMKII inhibitor KN-62 (10 µM). **(B, C)** HFU-induced dendritic spine growth (vehicle, red filled circles/bar; 8 spines/8 cells) was prevented by KN-62 (black filled circles/bar; 6 spines/6 cells). Volume of the unstimulated neighbors did not change (open bars). Two-way repeated measure ANOVA with Dunnett's test used in (B) and two-way ANOVA with Tukey's test used in (C). Data are represented as mean +/- SEM. *p < 0.05; **p < 0.01.