Supporting Information

Holbro et al. 10.1073/pnas.0905110106

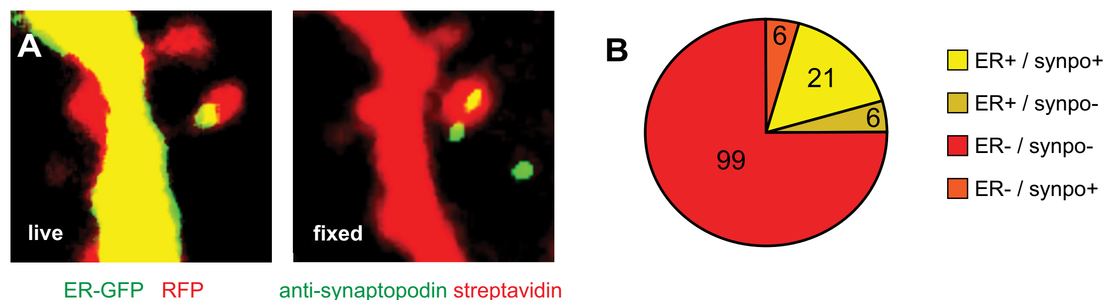

Fig. S1. Synaptopodin immunohistochemistry. (*A*) Posthoc immunostaining against the spine-apparatus-associated protein synaptopodin. Transfected cells were imaged and subsequently patched with an intracellular solution containing neurobiotin (1 mg/mL). Slices were then fixed in 4% paraformaldehyde and incubated with PBS containing 0.3% Triton X-100, 10 μg/mL Alexa Fluor 594 conjugated to streptavidin, and 4% horse serum for 3 h at room temperature. The fixed slices were incubated in blocking solution (20% horse serum in PBS) at room temperature for 4 h, then incubated with antisynaptopodin antibody (1:2,000; Sigma-Aldrich) in PBS overnight at 4 °C, then incubated in Alexa Fluor 488 goat anti-rat secondary antibody (1:200; Invitrogen) for 3 h at room temperature. (*B*) Correspondence between ER identification in live tissue (ER+) and antisynaptopodin immunoreactivity (synpo). Number of spines indicated in diagram.

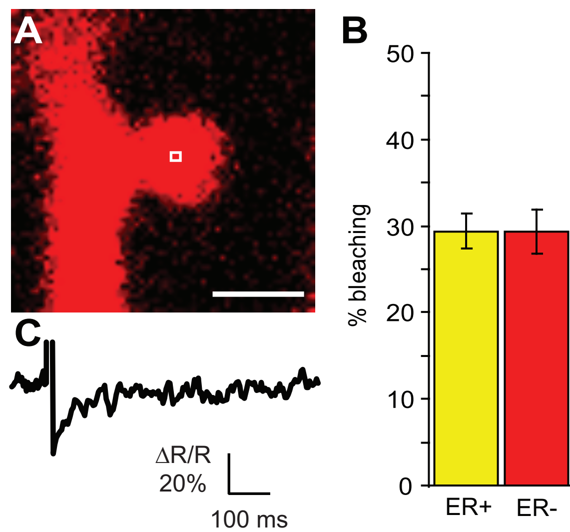

Fig. S2. Two-photon glutamate uncaging. (*A*) To test for the intensity of the uncaging laser beam, after the experiment, we directed a laser pulse to the center of the spine and monitored the amount of bleaching of the red fluorophore (Alexa Fluor 594). (Scale bar: 1 μm.) (*B*) FRAP of Alexa Fluor 594 in spine shown in *A*. (*C*) Alexa Fluor 594 bleaching was similar in ER+ and ER− spines, indicating identical uncaging laser intensities.

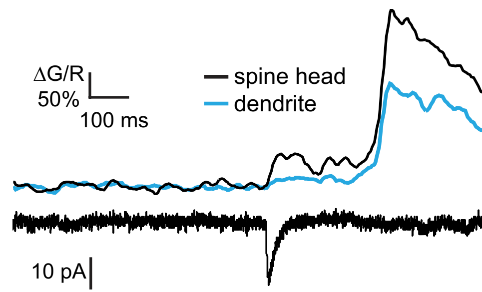

Fig. S3. Properties of delayed calcium transients. Delayed calcium transients had higher amplitudes in the spine compared with the dendrite, had no electrical correlate, and were never observed spontaneously (before the uncaging pulse).

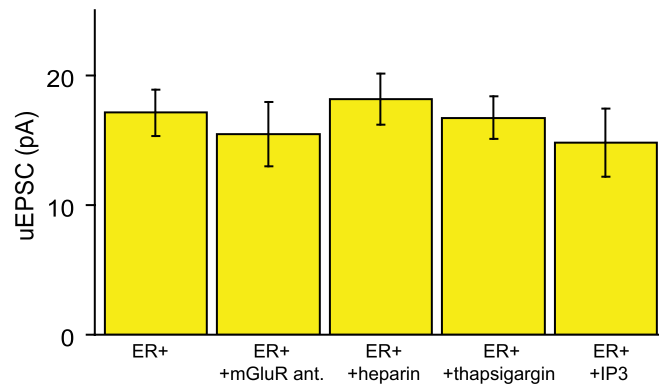

Fig. S4. Metabotropic pathway did not affect uEPSCs. Pharmacological blockade of group I mGluRs, IP_3 receptors (heparin), depletion of calcium stores (thapsigargin), or addition of intracellular IP_3 had no effect on the amplitude of uEPSCs in ER+ spines (control, n = 30; mGluR block, n = 9; heparin, n = 12; thapsigargin, n = 9; IP_3, n = 5).

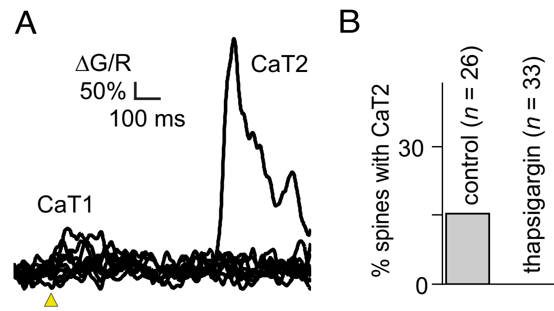

Fig. S5. Spine calcium transients in response to stimulation of Schaffer collaterals. (*A*) Delayed spine calcium transients (CaT2) in CA1 pyramidal cells from an acute hippocampal slice. Unreliability of the NMDAR-mediated transient (CaT1) reflects failures and successes of glutamate release from the presynaptic terminal. Cells were filled with K^+-based intracellular solution containing 600 μM Fluo5F and 30 μM Alexa Fluor 594. Yellow arrow indicates time of stimulation. (*B*) Delayed calcium transients in response to electrical stimulation were observed in four of 26 spines (acute slice, days 16–20). Thapsigargin (2 μM) completely blocked the occurrence of delayed calcium transients ($n = 33$).