Supporting Information

Wiegert and Oertner 10.1073/pnas.1315926110

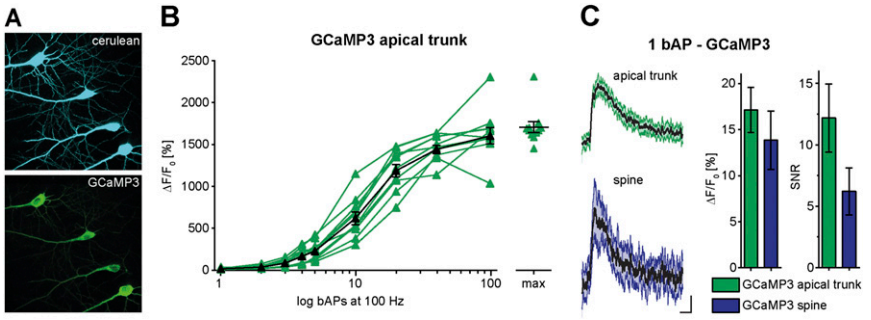

Fig. S1. Calibration of GCaMP3 performance using back-propagating action potentials (bAPs). (*A*) CA1 pyramidal cells expressing cerulean and GCaMP3 at 4 d after electroporation (21 days in vitro). (*B*) Calibrating GCaMP3 dynamic range with a series of bAPs at 100 Hz and two-photon line scanning at the proximal apical trunk. The dynamic range showed little variability between cultures as seen at the maximal response amplitude (n = 11 neurons from 11 slice cultures). (*C*) (*Left*) Mean traces of five GCaMP3 responses to one bAP measured in the proximal apical trunk (*Upper*; mean ± SD in green) and a spine head on a primary oblique dendrite (*Lower*; mean ± SD in blue). Calibration: 200 ms, 10% $\Delta F/F_0$. (*Right*) Mean Ca^{2+} transients and signal-to-noise ratios (SNRs) during one bAP measured with GCaMP3 in the proximal apical trunk (green) or a spine head on a primary oblique dendrite (blue; n = 10 neurons).

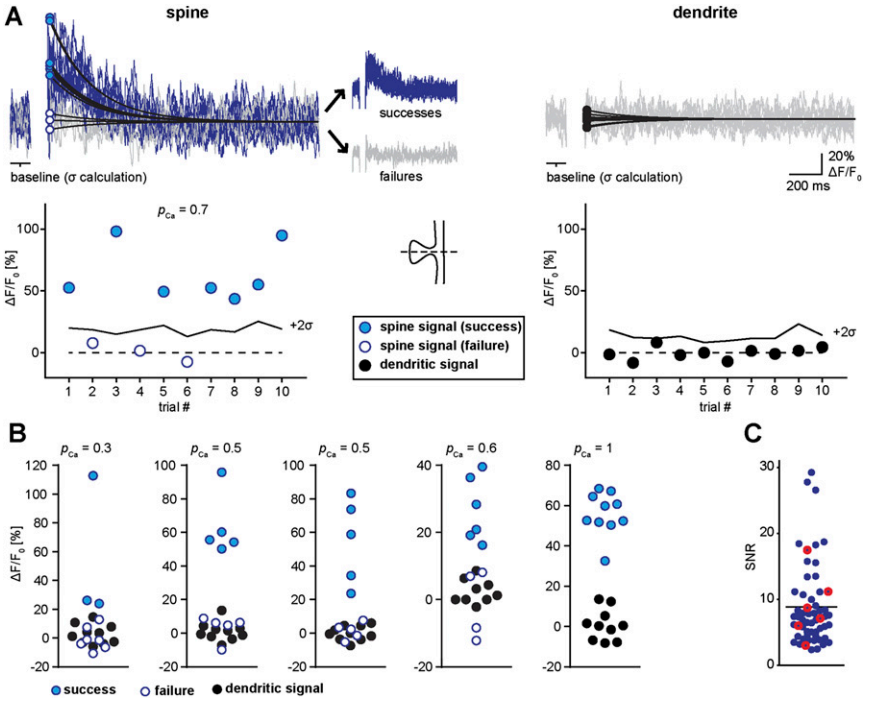

Fig. S2. Excitatory postsynaptic Ca^{2+} transient (EPSCaT) analysis. (*A*) GCaMP3 fluorescence transients were recorded simultaneously in the spine head (*Left*) and parent dendrite (*Right*). The amplitude of fluorescence transients was determined by fitting a template (single exponential function; *Materials and Methods*) to the fluorescence decay. Responses exceeding 2σ of baseline noise (calculated from the baseline signal of each trial) were classified as successes (blue traces and filled blue markers). Fluorescence fluctuations in the adjacent dendrite did not exceed 2σ of baseline noise (black markers). In each trial, CA3 pyramidal cells were stimulated by two light pulses at 40 ms interstimulus interval. (*B*) EPSCaT analysis in spines and their parent dendrites in five additional experiments, spanning low to high EPSCaT probability (p_{Ca}). Amplitudes and classification (filled/open markers) are shown for the first 10 trials. (*C*) SNR in spine Ca^{2+} measurements across all experiments (all stimulated spines). Markers with red outline indicate the six examples shown in *A* and *B*.

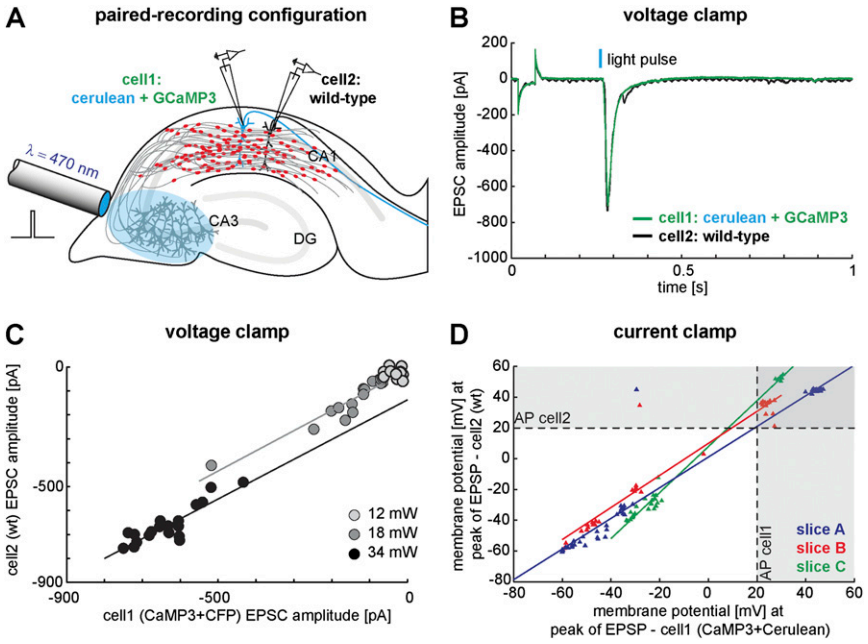

Fig. S3. Verification of "reporter neuron" strategy. (*A*) Schematic drawing of recording configuration. Paired recordings were made from two CA1 pyramidal neurons to compare light-evoked synaptic drive between the two. As only one of the two CA1 neurons was expressing GCaMP3/cerulean, these experiments were also used to rule out the possibility that overexpression of the two constructs affected synaptic transmission. (*B*) Representative light-evoked currents. For a given light pulse intensity, excitatory postsynaptic current (EPSC) amplitudes were similar in both neurons, indicating that a comparable number of synapses were activated. (*C*) Quantification of correlated synaptic input. EPSC amplitudes were highly correlated between pairs of CA1 neurons at different light intensities. EPSC amplitude in cell 2 is plotted against EPSC amplitude in cell 1 at three different light intensities. Each circle represents a single trial. Lines show linear regression function. (*D*) Postsynaptic membrane depolarization evoked by light-induced presynaptic APs is highly correlated between CA1 neurons. Peak membrane depolarization during the excitatory postsynaptic potential (EPSP)/AP of cell 2 vs. cell 1 is plotted for three independent experiments. In each experiment, the intensity of presynaptic light pulses was set to different values between 10 and 34 mW to recruit a variable number of CA3 cells. Colored triangles represent individual trials. Lines show linear regression function. Markers inside gray shaded area correspond to postsynaptic APs.

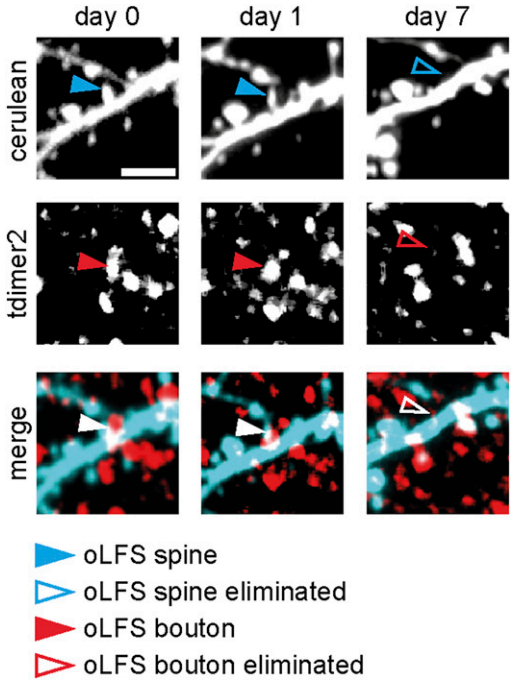

Fig. S4. Presynaptic vesicle cluster vanished with the elimination of optogenetic low-frequency stimulation (oLFS) spine. (*Top*) Maximum-intensity projection of cerulean signal before (day 0) and 1 and 7 d after oLFS. (Scale bar, 3 μm.) The oLFS spine is indicated by a cyan arrowhead. Open cyan arrowhead indicates dendritic location of eliminated spine at day 7. (*Middle*) Maximum-intensity projection of tdimer2 signal in the same volume. Red arrowhead indicates a bouton in contact with oLFS spine. Open red arrowhead indicates position of eliminated spine. Note absence of a red vesicle cluster at this position. (*Bottom*) Merged images.

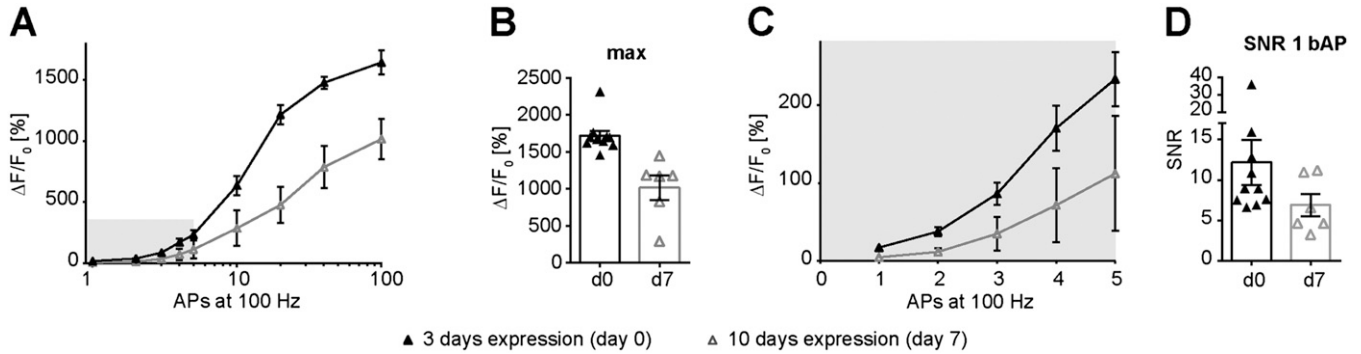

Fig. S5. Comparing GCaMP3 performance 3 d and 10 d after electroporation. (*A*) Average GCaMP3 responses in patch-clamped CA1 pyramidal cells using trains of bAPs (100 Hz) and two-photon line scanning at the proximal apical trunk. Black trace indicates neurons expressing GCaMP3 for 3 d [day 0 in long-term depression (LTD) experiment]. Gray trace indicates neurons expressing GCaMP3 for 10 d (day 7 in LTD experiment; $n = 6$ neurons in six slice cultures). (*B*) Maximum GCaMP3 response amplitude (200 bAPS at 200 Hz) is decreased at day 7 (10 d of expression) whereas variability is increased. (*C*) GCaMP3 responses to one to five bAPs are plotted on a linear *x* axis (shaded area from *A*). Note the smaller amplitude and the increased variability of GCaMP3 responses at day 7 (10 d of expression). (*D*) SNR for GCaMP3 responses evoked by one bAP is decreased at day 7 (10 d of expression).

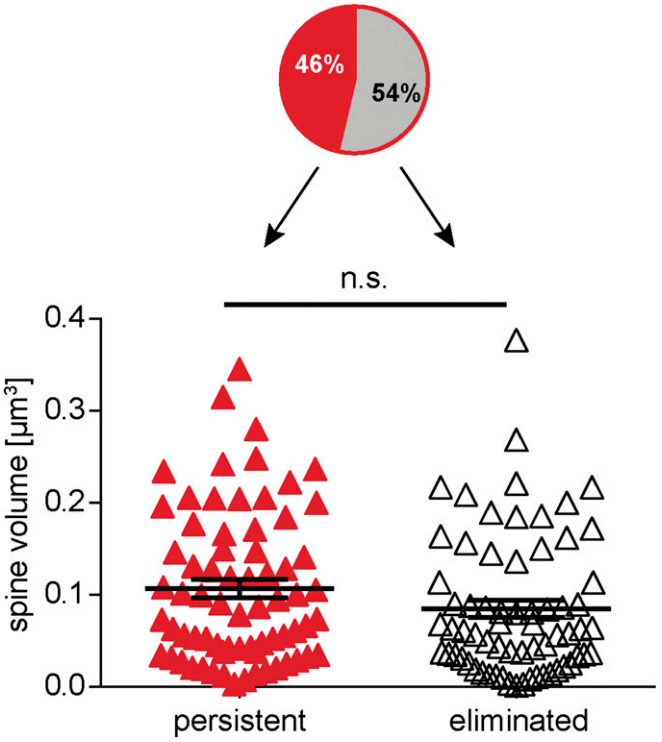

Fig. S6. Volume of neighboring spines does not predict persistence. The initial volume of spines next to oLFS spines that persisted for 7 d (red triangles; $n = 67$ spines) was not different from the initial volume of spines next to oLFS spines that were eliminated (open triangles; $n = 70$ spines). Black lines indicate mean ± SEM; $P = 0.09$, Mann–Whitney test, two-sided.

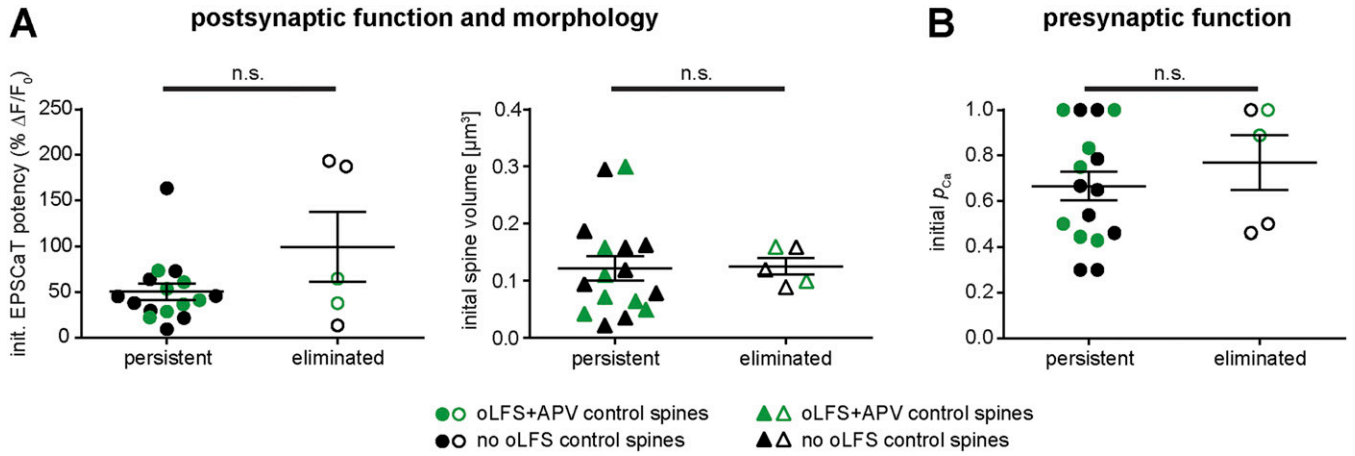

Fig. S7. Initial properties of persistent vs. eliminated control spines. (*A*) Initial EPSCaT potency ($P = 0.57$) and initial spine volume ($P = 0.93$) was not different for spines that persisted for 7 d ($n = 16$ spines in 16 slice cultures) or were eliminated ($n = 5$ of 5). Two control conditions in which no LTD was induced [no oLFS, oLFS plus D-2-amino-5-phosphonopentanoic acid (APV)] were pooled for statistical analysis, as spontaneous spine elimination was a rare event. (*B*) Under conditions in which no LTD was induced, initial p_{Ca} was not different in persistent and eliminated spines ($P = 0.98$, same spines as in *A*). Black symbols indicate control spines without oLFS. Green symbols indicate control spines with APV present during oLFS. Black horizontal lines indicate mean ± SEM (*B*, *Left*, and *C*, Mann–Whitney test, two-sided; *A*, *Right*, unpaired *t* test, two-sided).