Supplemental Information:

Supplemental Videos

Video S1 (relates to Figure 1). Local control of molecular recruitment to individual synapses

Shown is a section of dendrite from a dissociated hippocampal neuron expressing CRY2-GFP-homer1c (channel not displayed) along with CIB-mCh (displayed). A single dendritic spine was photoexcited with a 1 ms pulse of diffraction-limited 488 nm excitation (timing of photoexcitation is given by the appearance of the white circle, upper left corner). The dimensions of the movie frame are 7 μm x 5.8 μm. The duration of the video is 6 min with an acquisition rate of 1 frame/10s.

Video S2 (Relates to Figure 2). Optical recruitment of AMPA receptors to postsynaptic sites

Shown is a hippocampal neuron expressing mOr2-GluA1-CIB (displayed) and CRY2-GFP-homer1c (channel not displayed) before and after full field 488 nm photostimulation (timing of photoexcitation is given by the appearance of the white circle, upper left corner). Note the robust accumulation of GluA1-CIB in dendritic spines following light exposure. The dimensions of the movie frame are 51 μm x 51 μm. The duration of the video is 18 min with an acquisition rate of 1 frame/30s.

Supplemental Data

Data S1 (Relates to Figure 3). Matlab script for defining PSD boundary and receptor occupancy within the PSD. Script takes spine-localization files, segmented in VividSTORM, and output from DBSCAN module in PALMsiever (see methods), uses MATLAB's boundary function to define the edge of the cluster (channel1 localizations, e.g. PSD95). The inploygon function is then used to determine the number and percent of total channel2 localizations (e.g. GluA1) that fall

within or on the boundary. Script then plots localizations, saves a .mat file, and saves the statistics to a .csv file. See internal comments in the downloadable file for more details.

Supplemental Figures:

Figure S1 (relates to figure 2) Expressed GluA1-CIB displays similar surface localization to endogenous GluA1, GluA2 and expressed GluA1 receptors lacking the CIB tag.

A. Shown is a cultured hippocampal neuron expressing mCh-GluA1-CIB that has been surface labeled with an antibody against mCh under non-permeabilizing conditions. The total mCh signal is shown in red (middle panel) with the surface (antibody labeled) fraction shown in grey scale (left panel). Yellow arrowheads highlight examples where robust spine surface signal is difficult to visualize in the total mCh channel. Scale bar=3μm

B. Shown are cultured hippocampal neurons labeled with an antibody that recognizes a surface epitope on either GluA1 (top) or GluA2 (bottom) (red). The green signal is a GFP cell fill used to visualize cellular morphology. Scale bar=3μm

C. Shown are dendrites from cultured hippocampal neurons expressing SEP-GluA1-CIB (top) or SEP-GluA1 (bottom). Arrowheads point to spines enriched with GluA1. Scale bar 2μm.

D. The ratio of AMPA receptor signal in spines compared to the adjacent dendritic shaft for endogenous and expressed AMPA receptors was quantified and is plotted for surface labeled endogenous GluA1, surface labeled endogenous GluA2, mCh-GluA1-CIB (surface labeled mCh signal), mCh-GluA1-CIB (total mCh signal), SEP-GluA1 and SEP-GluA1-CIB.

E. Receptors recruited to the PSD are at the cell surface. A version of mCh-GluA1-CIB with a thrombin cleavage site inserted between mCh and GluA1 was recruited to synaptic sites followed

by treatment with extracellular thrombin. Note the abrupt decrease in signal at dendritic spines following thrombin cleavage. The graph plots relative spine mCh signal (F/F_0) at baseline, following synaptic recruitment (blue dashed line above plot) and following thrombin treatment (green line above plot) (n=50 spines from 5 neurons).

Figure S2 (relates to Figure 5) Spine number and presynaptic properties remain unaltered following GluA1-CIB recruitment to the PSD.

A. Presynaptic neurotransmitter vesicle fusion was measured using FM4-64 in cultured hippocampal neurons expressing mCh-GluA1-CIB along with CRY2-GFP-homer1c. FM4-64 was loaded into presynaptic terminals with a brief (30 sec) exposure to isosmotic ACSF containing 50 mM KCl (red). Cells were either treated with blue light to recruit GluA1 to the PSD, or kept in darkness prior to unloading FM4-64 with 50 mM KCl. The green signal represents CRY2-GFP-homer1c at postsynaptic sites (arrowheads).

B. Kinetics of FM4-64 unloading in response to depolarization. Following FM4-64 loading, the rate of unloading was quantified in terminals apposed to cells expressing mCh-GluA1-CIB and CRY2-GFP-homer1c upon depolarization with 50 mM K^+. Cells were either treated with 10 min of blue light (1 pulse every 2 min, blue trace, 140 terminals from 7 cells from 3 independent cultures) to recruit GluA1-CIB to postsynaptic sites, or left in the dark (red trace, 120 terminals from 6 cells from 3 independent cultures) prior to FM4-64 unloading. Data represent the average±SEM.

C. Optical quantal analysis of presynaptic release using the red fluorescent Ca^{2+} indicator JRGECO1a. Spontaneous Ca^{2+} transients resulting from quantal release of neurotransmitter activating postsynaptic NMDA receptors can be measured before (top panel) and after (bottom

panel) AMPA receptor recruitment. We used superecliptic pHluroin (SEP)-tagged GluA1-CIB (green signal) and untagged CRY2-homer1c so we could image Ca^{2+} in the red channel. The kymographs (lower panels) were generated from the JRGECO1a (red) signal using the solid line displayed in the panels above and show a spontaneous spine Ca^{2+} transient before and after SEP-GluA1-CIB recruitment to CRY2-homer1c. The traces to the right show jRGECO1a Ca^{2+} transients measured at single spines recorded over a 60 sec interval before (red) and after (blue) GluA1 recruitment.

D. The plot displays the average change in frequency ($\Delta F/F_{dark}$) and amplitude ($\Delta A/A_{dark}$) pre- and post-GluA1 recruitment (n=72 synapses from 8 neurons). We observed no change in the frequency or amplitude of spontaneous quantal events following GluA1 recruitment to postsynaptic sites.

E. Spine density was measured on the same dendritic segments before and 8-10 min after recruitment of GluA1-CIB to CRY2-homer1c (n= 9 dendritic segments from 9 different neurons).

Figure S3 (relates to Figure 6) cLTP stimulus recruits GluA1-SEP and mCh-GluA1-CIB receptors to synapses and partially occludes light-recruitment.

A. Shown are dissociated hippocampal neurons expressing SEP-GluA1 (left) or mCh-GluA1-CIB (right) under baseline conditions (top panels, pre-cLTP) and 8 min. following a 5 min treatment with $0Mg^{2+}$/200μM glycine/30μM bicuculline (bottom panels, 8 min post). Note the accumulation of SEP and mCh signal in dendritic spines following cLTP. Zoomed images pre- and post-cLTP are shown to the right (zoomed image for mCh-GluA1-CIB has been rotated 90 degrees). Arrowheads denote spines with enriched GluA1 post-cLTP. Scale bar 10μm.

B. The plot displays the normalized spine fluorescence of mCh-GluA1-CIB and SEP-GluA1 before and 10 min following cLTP treatment. mCh-GluA1-CIB (49 spines from 7 neurons) undergoes activity-triggered recruitment to spines to a similar extent as SEP-GluA1 without a C-terminal CIB tag (60 spines from 5 neurons).

C. Dissociated hippocampal neurons expressing mCh-GluA1-CIB and CRY2-homer1c were imaged before and after cLTP stimulation as in (A). 5 min following washout of the cLTP solution, cells were exposed to blue light. The graph to the right reports the normalized mCh fluorescence (F/F_0) in spines following cLTP (cLTP dark, 49 spines from 7 neurons), then at the same spines 8 min following onset of blue light exposure (cLTP→light, $p<0.001$ paired Student's t-test). Synaptic accumulation of mCh-GluA1-CIB in separate cells exposed to blue light without prior cLTP stimulation is also plotted (light, 60 spines from 7 neurons, n.s. comparing cLTP→light to light treatment alone, Student's t-test). Scale bar=2μm.

Figure S4 (relates to Figure 7) Expressed GluA1-CIB receptors are functional and do not displace GluA2 receptors from spines.

A. Ca^{2+} entry through NMDA receptors was measured to confirm single spine activation when uncaging MNI-glutamate. Shown is a dendritic spine from a cultured hippocampal neuron expressing GCaMP6s before and after uncaging MNI-glutamate in low Mg^{2+} ACSF (containing 1μM TTX) with a 500μsec pulse of 405 nm excitation. The uncaging site is denoted by the magenta asterisk. A kymograph of GCaMP6s signal was generated using the yellow line in the panel above drawn through the uncaged spine, the dendritic shaft and a neighboring spine. Ca^{2+} responses were consistently restricted to the targeted spine. Scale bar=2μm.

B. AMPA receptor μEPSCs at individual spines were measured at +40mV (red) and -65mV (blue) following light recruitment of either mCh-GluA1-CIB (right traces) or CIB-mCh (left traces) in the presence of 100μM APV to block NMDA receptor currents. Traces represent the average of 3 sweeps. Rectification values were measured as the ratio of maximum μEPSC amplitudes at +40 and -65 mV and are plotted to the right (CIB-mCh, 22 spines from 5 neurons; mCh-GluA1-CIB, 40 spines from 8 neurons; $p<0.001$ Student's t-test). Scale bar=10 pA/20 msec.

C. Shown are peak-scaled μEPSCs from synapses with recruited mCh-GluA1-CIB (grey) and CIB-mCh (black). μEPSC decay kinetics were extracted from single exponential fits (red) of the averaged traces (at least 10 sweeps). Tau values for μEPSC decay are plotted to the right (GluA1-CIB, n= 27 spines from 7 neurons; CIB-mCh, 17 spines from 5 neurons, $p<0.001$ Student's t-test). Scale bar=10 msec.

D. Recruited GluA1-CIB does not displace endogenous GluA2 from spines. Endogenous surface GluA2 was labeled in live neurons expressing mCh-GluA1-CIB and CRY2-GFP-homer1c by antibody labeling. The plot to the right displays the normalized (F/F_0) fluorescence intensity of endogenous GluA2 at individual spines in neurons exposed to blue light (blue trace, timing of light exposure is shown by the blue bar above the data points) and in control cells also expressing mCh-GluA1-CIB and CRY2-GFP-homer1c but not exposed to blue light (red trace). In both cases we observed a steady decrease in signal (likely due to antibody dissociation over the time course of the experiment) that was not influenced by light exposure. Scale bar=2μm.

Figure S1 Sinnen et al.

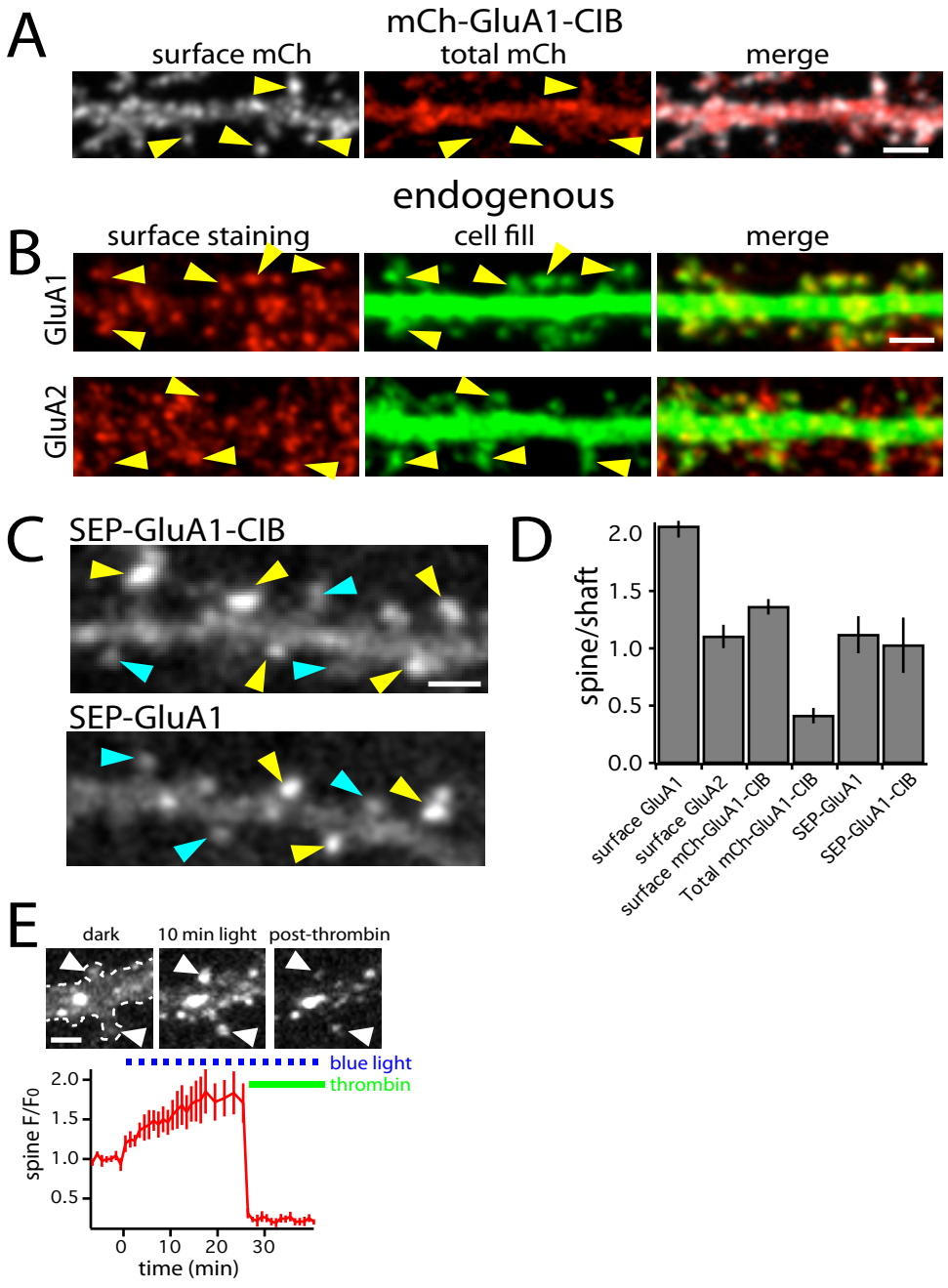

Figure S2 Sinnen et al.

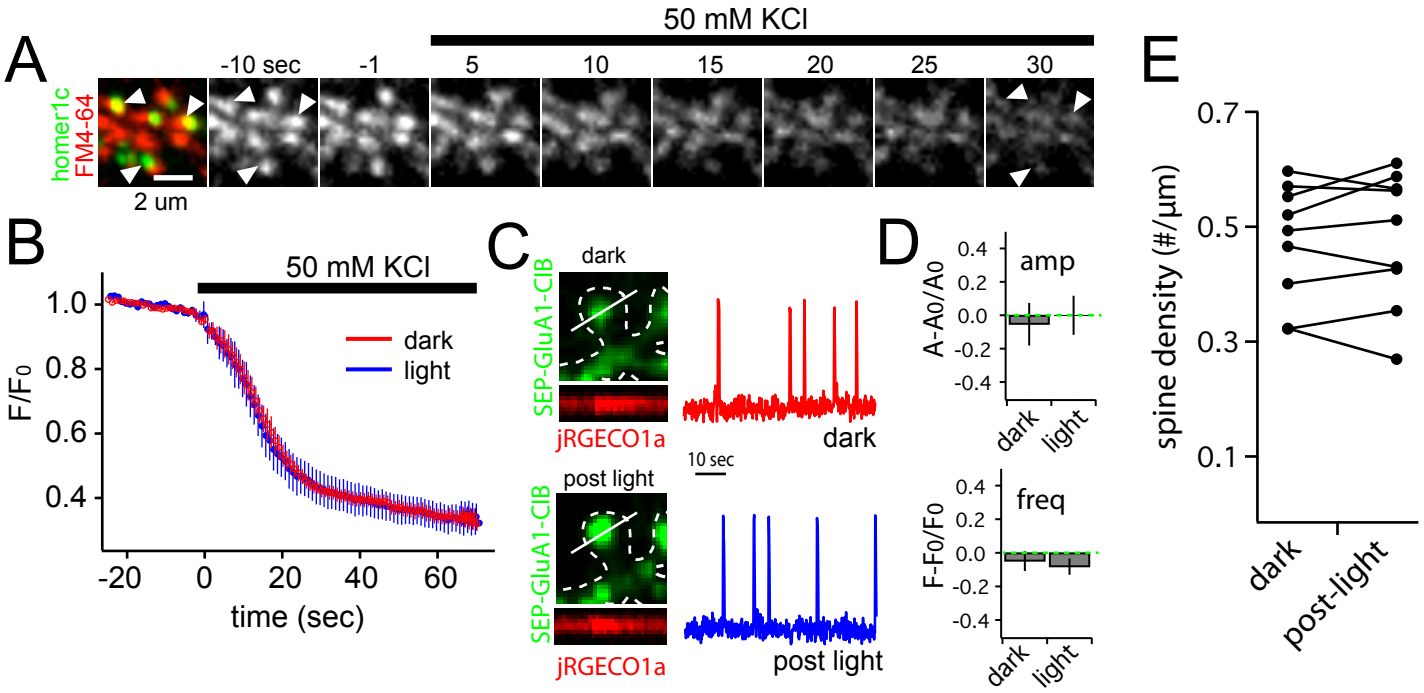

Figure S3 Sinnen et al.

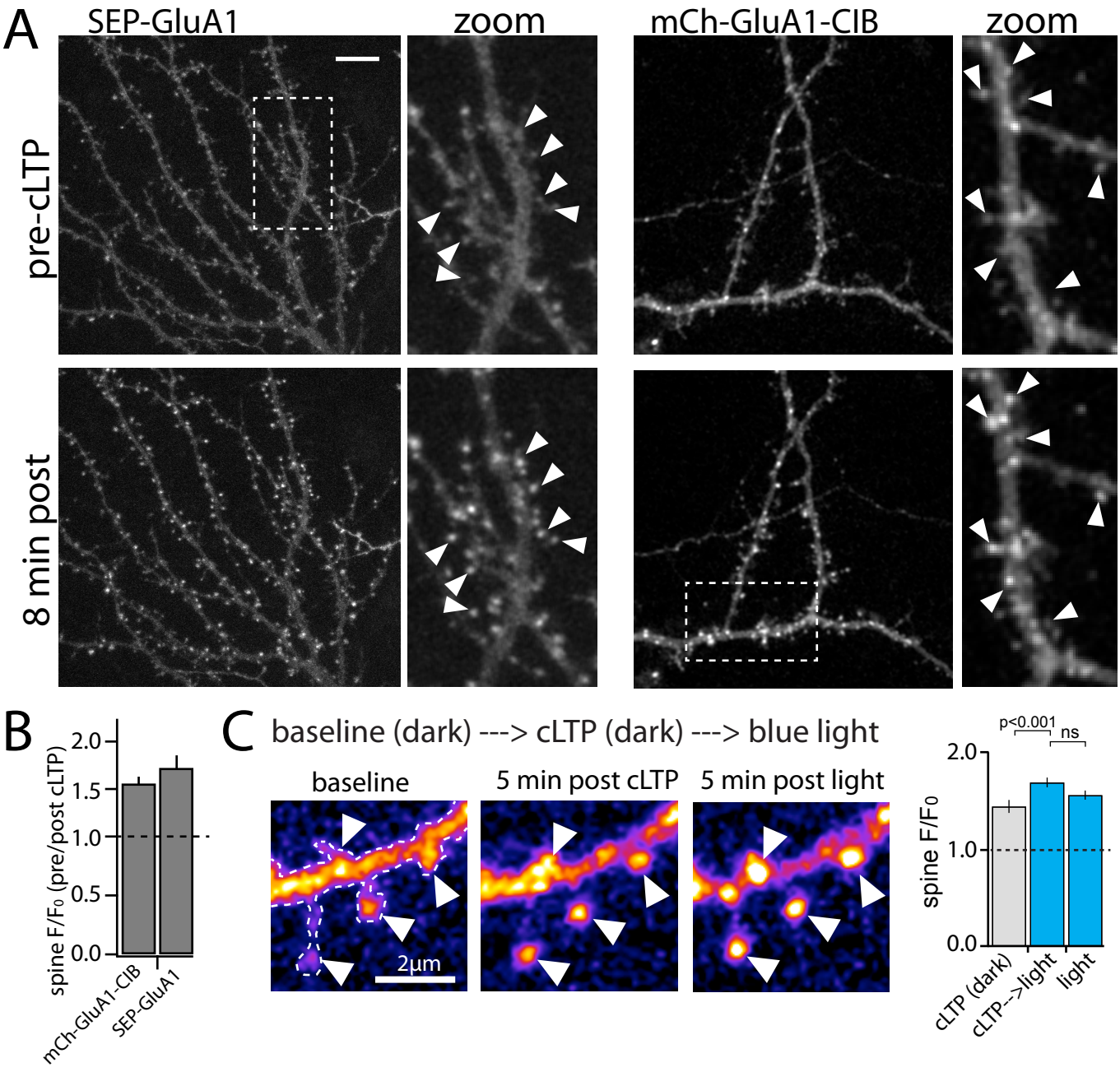

Figure S4 Sinnen et al.