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Supplemental Information

Simultaneous Live Imaging of Multiple Endogenous Proteins Reveals a Mechanism for Alzheimer's-Related Plasticity Impairment

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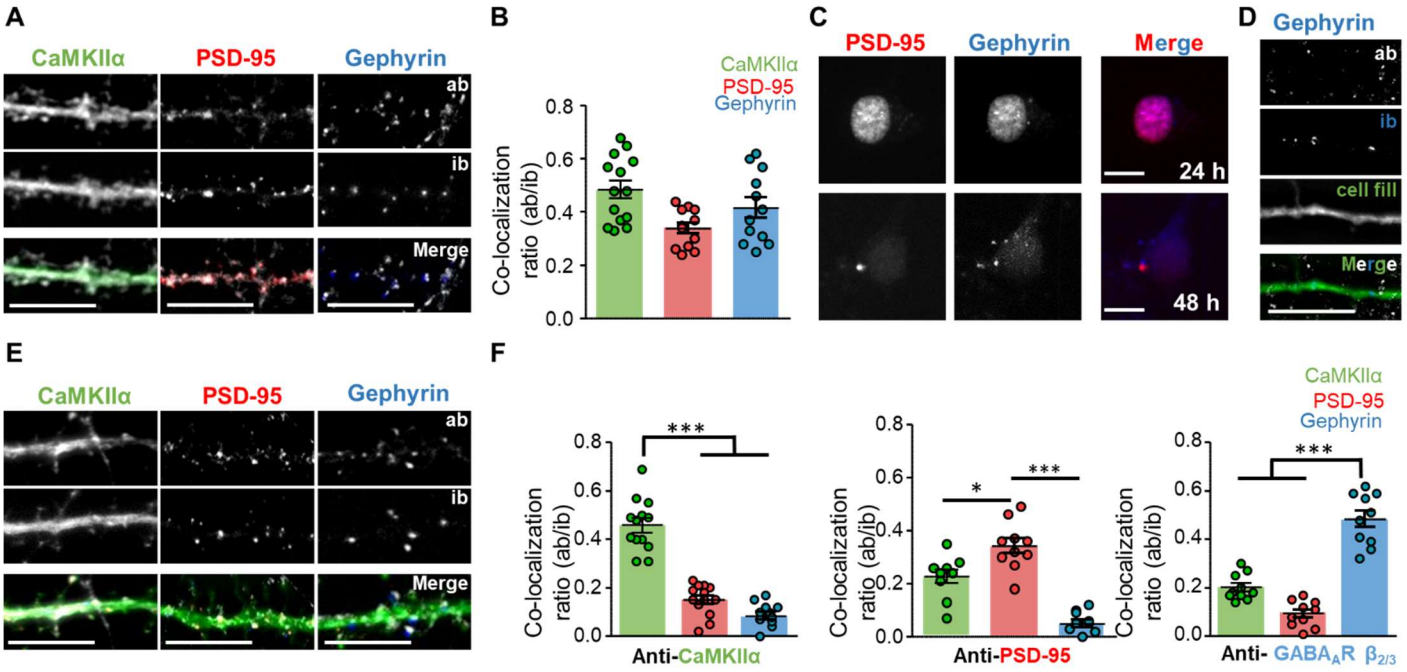

Figure S1. Simultaneous live imaging of endogenous CaMKII, PSD95 and gephyrin using FingR intrabodies. Related to Figure 1. Error bars indicate SEM.

(A) Our modified intrabodies against CaMKII, PSD95 and gephyrin were first individually expressed in hippocampal neurons, and then co-stained with the respective antibodies. Co-localization of both stains is shown for each protein in example images. Note that for the gephyrin intrabody, the co-staining was with an anti-GABA$_A$R antibody. This was necessitated by the fact that our gephyrin intrabody and antibody have overlapping epitopes, which effectively prevents gephyrin antibody staining in neurons transfected with the gephyrin intrabody (see panel D). However, the co-localization with GABA$_A$R provides even better evidence for faithful labeling of inhibitory synapses by the intrabody. Scale bars indicate 5 μm.

(B) Pearsons correlation of antibody (ab) positive intrabody (ib) punctae in cultures transfected with individual modified intrabodies. (n=14, 12, 12 neurons).

(C) Significant nuclear localization of the PSD-95 and gephyrin intrabodies is observed after 1 day of expression in hippocampal neurons; after 2 days of expression, this nuclear localization is largely eliminated. This is consistent with negative feedback mechanism controlling intrabody localization and expression (see Figure 1A). Scale bar indicates 10 μm.

(D) Gephyrin staining with our antibody was prevented in neurons expressing the gephyrin intrabody, but not in neighboring non-transfected neurons. This is consistent with their overlapping epitope on gephyrin and indicates that the intrabody binds to most of the gephyrin protein within the neuron. Scale bar indicates 5 μm.

(E) Example images for co-localization of intrabody and antibody staining as in panel A, but in hippocampal neurons expressing all three intrabodies simultaneously. Scale bar indicate 5 μm.

(F) Pearsons correlation of the intra- and anti-body staining in neurons expressing all three intrabodies. As expected, co-localization of each antibody stain was high for the matching intrabody, and significantly lower for the other intrabodies. As also expected, co-localization of each synaptic marker was better with CaMKII than with the other synaptic marker. (n=13, 10, 10 neurons, one-way ANOVA, Tukey's post-hoc test, * $p<0.05$, *** $p<0.001$).

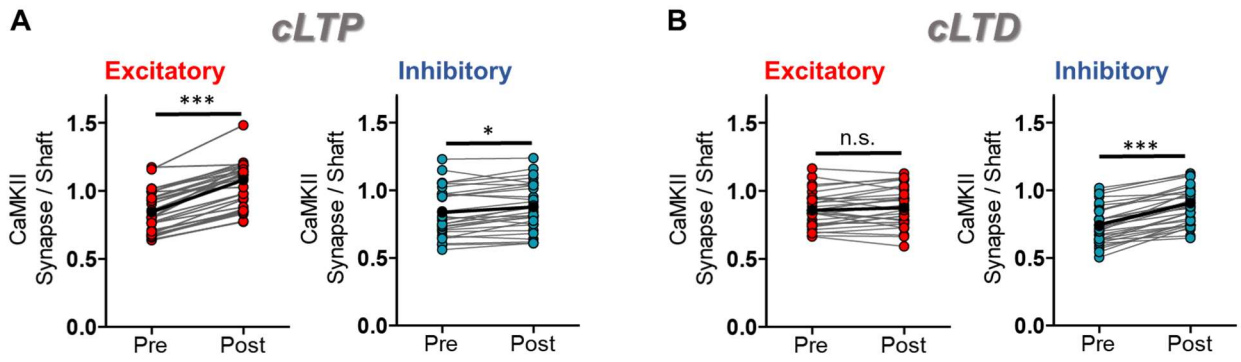

Figure S2. CaMKII movement to excitatory versus inhibitory synapses during LTP versus LTD in dissociated hippocampal neurons, as determined by live imaging of endogenous CaMKII and synaptic markers using the FingR intrabodies. Related to Figure 2; specifically the same data are shown in different representation (here showing the CaMKII synapse to shaft ratio in individual neuron pre and post stimuli).
(A) Chemical LTP (cLTP; left panels) stimuli caused a greater increase in CaMKII localization at excitatory versus inhibitory synapses. However, the smaller increase a inhibitory synapse was also significant when assessed by paired t-test (* $p<0.05$; *** $p<0.001$; n.s. $p>0.05$), even though it was not significant when all conditions were compared by two-way ANOVA (see Figure 2).
(B) Chemical LTD (cLTD; right panels) stimuli resulted in a significant increases CaMKII synapse to shaft ratio only at inhibitory synapses, but not at excitatory synapses, both when assessed by paired t-test (shown here) or when all conditions were compared by two-way ANOVA (see Figure 2).

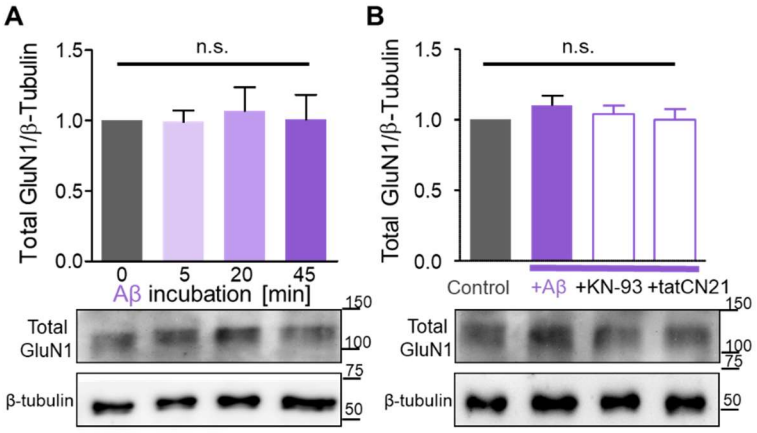

Figure S3. Aβ incubation does not affect the total GluN1 levels in surface biotinylation assays.
Related to Figure 4F and G.
(A) Aβ application (500 nM for 5, 20, or 45 mins) or
(B) CaMKII inhibition (using KN93 or tatCN21) during Aβ exposure (500 nM for 45 mins) does not impact overall GluN1 levels as verified by β-tubulin loading control (n=9 experiments in three different neuronal culture preparations; one-way ANOVA, Tukey's post-hoc test, n.s.: not significant).