Supplementary Materials for

Elfn1 Regulates Target-Specific Release Probability at CA1-Interneuron Synapses

Emily L. Sylwestrak and Anirvan Ghosh*

*To whom correspondence should be addressed. E-mail: aghosh@ucsd.edu

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Figs. S1 to S8

Supplementary Figure Legends

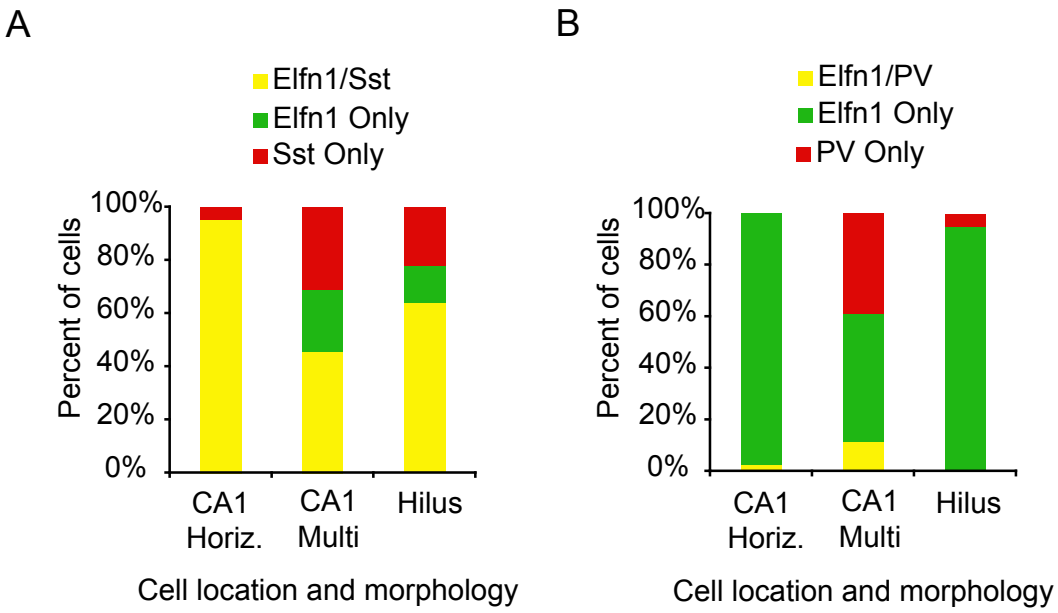

Fig. S1.

Elfn1 mRNA colocalizes with horizontal Sst (OLM) interneurons. **(A)** Colocalization of Elfn1 mRNA and tdTomato protein in Sst::tdTomato mice. Stained cells were classified by location (CA1 or hilus) and morphology (horizontal, oval cell soma with horizontal proximal dendrites; multipolar, circular cell soma with radial dendrites). Colors indicate the percentage of all stained cells that contain the corresponding mRNA or protein. **(B)** Colocalization of Elfn1 mRNA and tdTomato protein in PV::tdTomato mice. "Horizontal CA1" data points in (A) and (B) are reproduced from Figure 1D for ease of comparison.

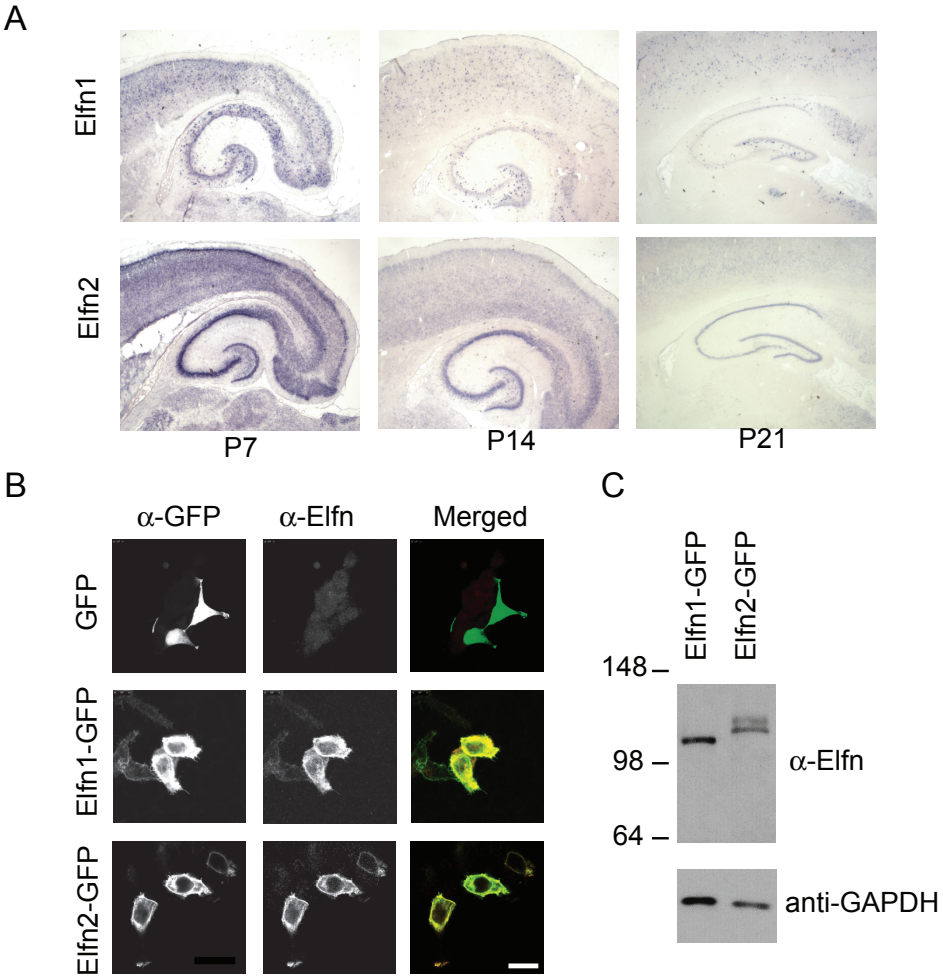

Fig. S2

Characterization of Elfn expression and antibody characterization. **(A)** In situ hybridization of Elfn1 and Elfn2 probes on rat hippocampal sections at P7, P14, and P21. **(B)** HEK cells transfected with GFP, Elfn1-GFP, or Elfn2-GFP and immunostained using an antibody that recognizes Elfn1 and Elfn2. **(C)** Western Blot of HEK cell lysates from cells transfected with GFP, Elfn1-GFP, or Elfn2-GFP and blotted using an antibody that recognizes Elfn1 and Elfn2. For (B), scale bar = 20μm.

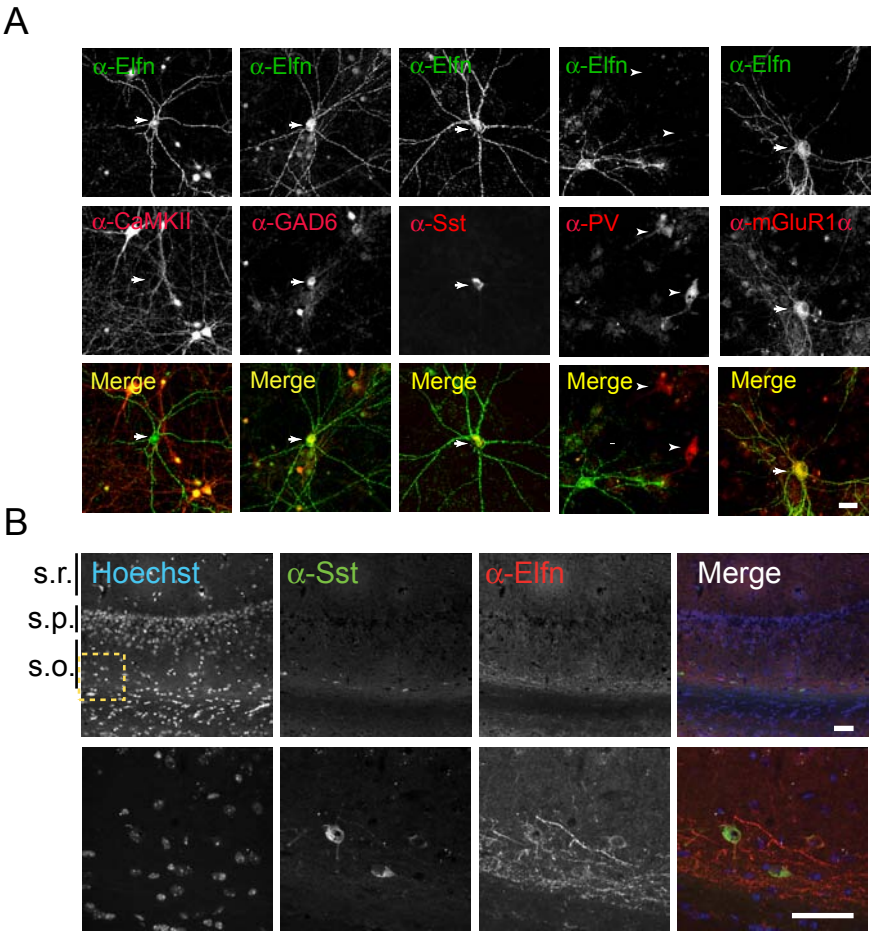

Fig. S3

(A) Dissociated hippocampal cultures immunostained for Elfn1 and CamKII, GAD6, somatostatin, parvalbumin or mGluR1a. 69% of Sst cells contain Elfn1, which is comparable to the 75% by in situ/immuno in Fig 1C,D. PV colocalization is much lower than with the in situ (4%), which may be due to lower levels of Elfn1 protein that fall below the detection threshold of the antibody. CamKII, n=350 cells; GAD6, n=104 cells; Sst, n=42 cells; PV, n=108 cells; mGluR1a, n=42 cells. **(B)** P14 rat hippocampal sections stained for Elfn, somatostatin, and Hoechst. Upper panels, low magnification images of CA1. Lower panels, high magnification images of CA1 stratum oriens area from dotted box above. For (A), scale bar = 10μm. For (B), scale bar = 20μm.

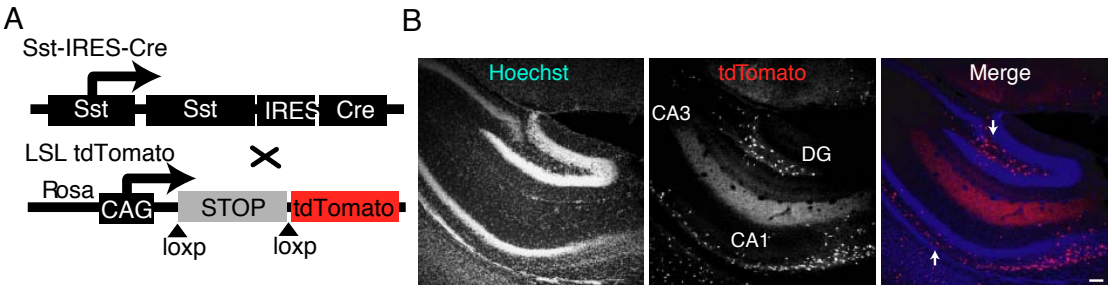

Fig. S4

Strategy to target OLM cells for electrophysiological recording. **(A)** Genetic cross labeling somatostatin-containing interneurons *in vivo*. **(B)** Hoechst staining of hippocampal slices from Sst::tdTomato mice. Sst interneurons can be seen in the stratum oriens and hilus (arrows). Sst cells are well labeled and electrophysiological recording was targeted to tdTomato cells adjacent to the alveus, corresponding the OLM cell type. The axons of these OLM cells can be seen as the band of tdTomato fluorescence in the stratum lacunosum moleculare.

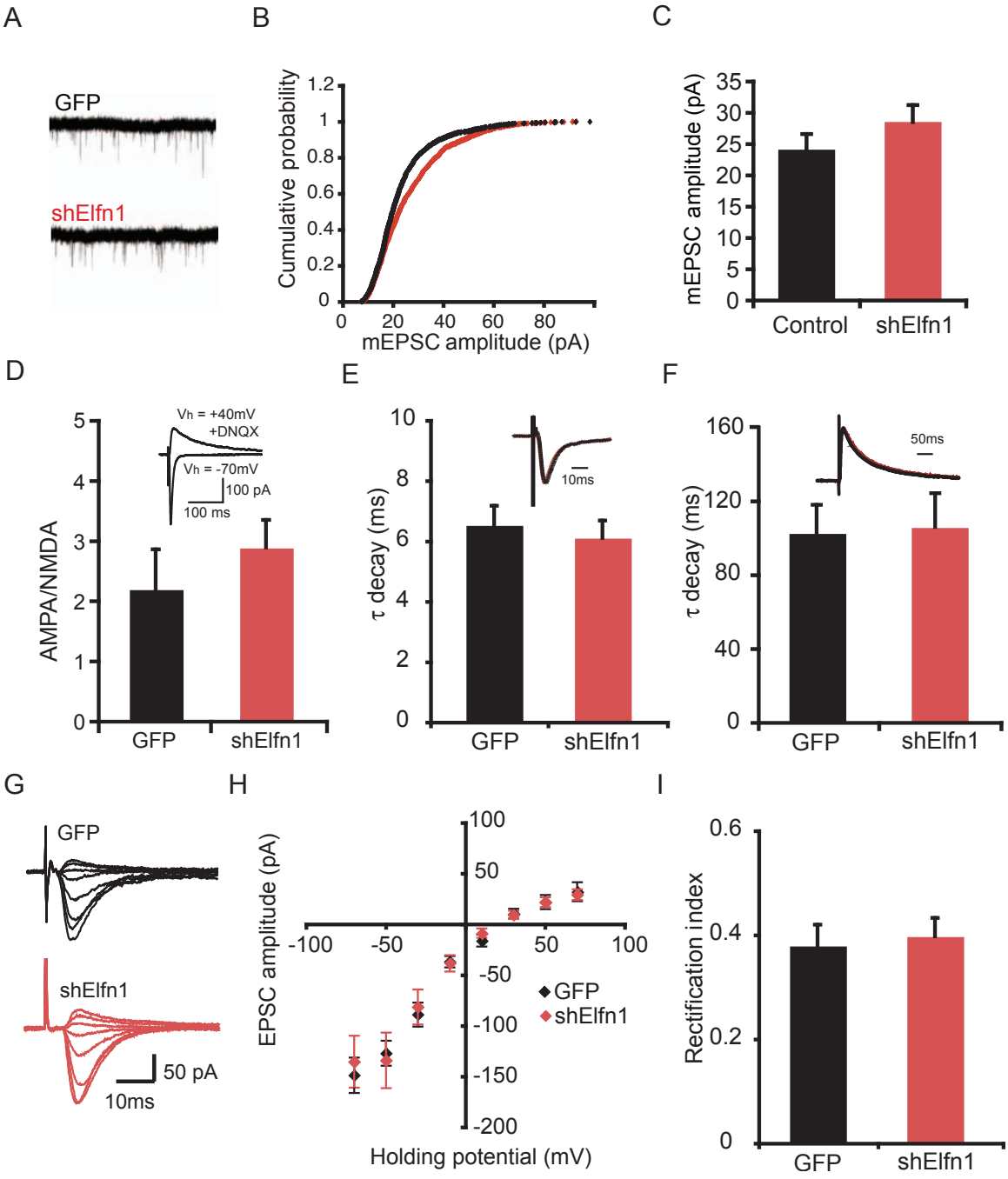

Fig. S5

Elfn1 knockdown does not affect postsynaptic properties **(A)** Example traces from OLM interneurons in acute slices infected with GFP or shElfn1 in the presence of TTX, APV and Gabazine to isolate AMPA mediated EPSCs. **(B)** Cumulative distribution of mEPSC amplitudes. **(C)** Quantification of average mEPSC amplitude for GFP control and shElfn1 infected neurons. GFP, n=8; shElfn1, n=9. p=.11, t-test. **(D)** Peak AMPA- and NMDA-mediated currents following a stimulus to the alveus. For AMPA EPSC, cells are voltage clamped at -70mV. For NMDA EPSCs, cells are held at +40mV in 20 μM DNQX. GFP,

n=8; shElfn1, n=10. p=.36, t-test. **(E)** Decay kinetics of AMPA EPSC. Tau calculated from a fit to a single exponential curve. GFP, n=22; shElfn1, n=15; p=.45, Mann-Whitney U test. **(F)** Decay kinetics of NMDA EPSC. Tau calculated from a fit to a single exponential curve. GFP, n=7; shElfn1, n=7. p=.89, t-test. **(G)** Examples traces and IV curve for AMPA-mediated currents. **(H)** The proportion of GluR2-lacking AMPA receptors controls rectification at CA1-OLM synapses and has been suggested to produce short-term facilitation (*33,34*). To test this, the holding potential was varied between -70mV and +70 mV and the evoked response from alvear stimulation was recorded in control and shElfn1-expressing cells. Spermine is included in the patch pipette. **(I)** Rectification index in control and shElfn1-expressing cells was calculated by the ratio of the slope of a line fit from +10mV to +70 mV to the slope of a line fit between -70 mV to -10mV. GFP, n=6; shElfn1, n=7. p=.96, t-test.

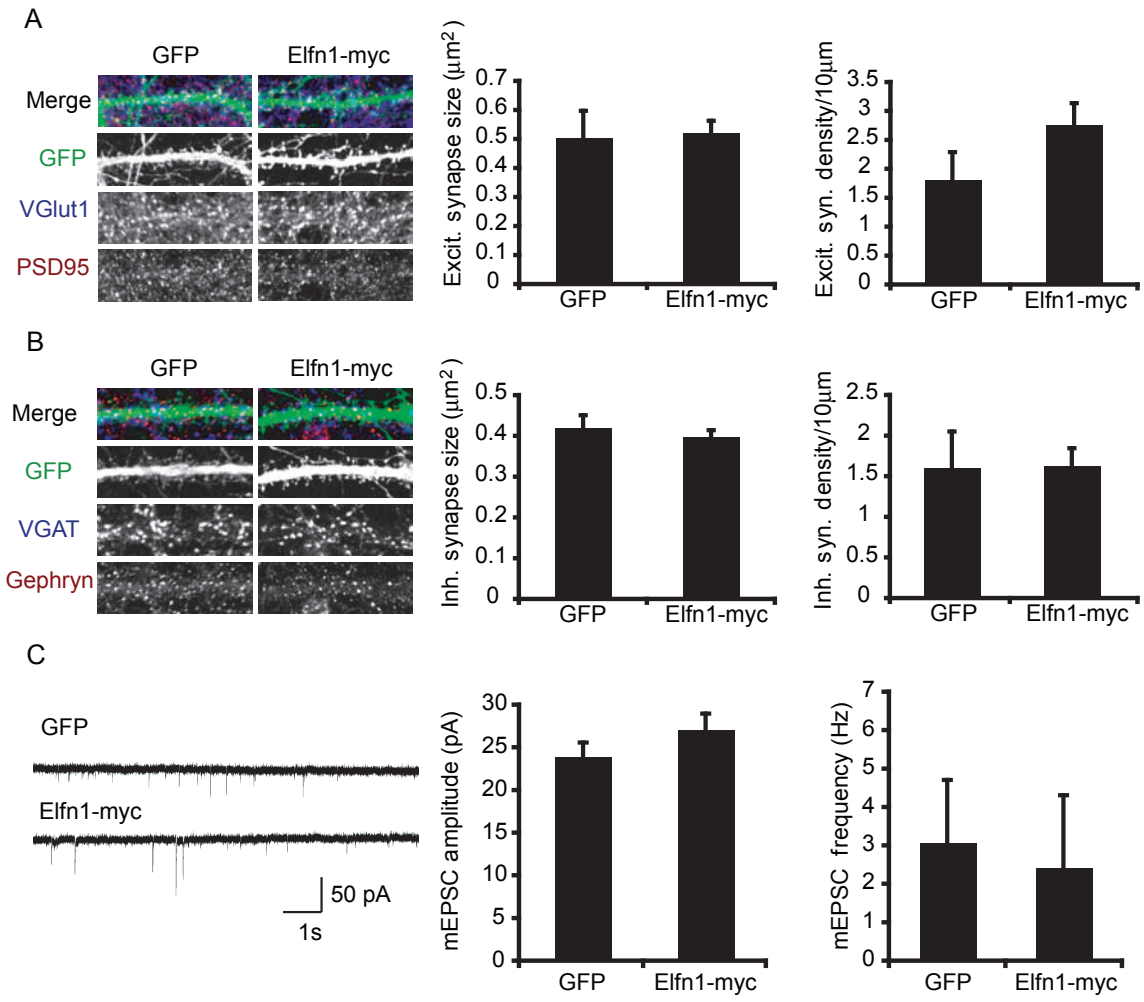

Fig. S6

Elfn1 overexpression does not alter synapse density. Neurons were electroporated at plating with GFP and Elfn1-myc and fixed at 14DIV. **(A)** Left, neurons were stained for Vglut and PSD95 to visualize excitatory synapses at 14DIV. Right, quantification of the size and density of excitatory synapses. GFP, n= 3372 synapses on 21 cells; Elfn1, n= 4006 synapses on 21 cells. Synapse density, p=.22 by t-test; synapse size, p=.88, Mann-Whitney U-test. **(B)** Left, neurons were stained for VGAT and gephyrin to visualize inhibitory synapses. Right, quantification of the size and density of inhibitory synapses. GFP, n= 902 synapses on 15 cells; Elfn1, n= 1508 synapses on 15 cells. Synapse density, p=.29 by Mann-Whitney U-test; synapse size, p=.45 by t-test. **(C)** Left, example traces of mEPSCs recorded at 14DIV in the presence of Gabazine, APV and TTX to isolate AMPA-mediated currents. Right, quantification of mEPSC amplitude and frequency. Error bars represent S.E.M.

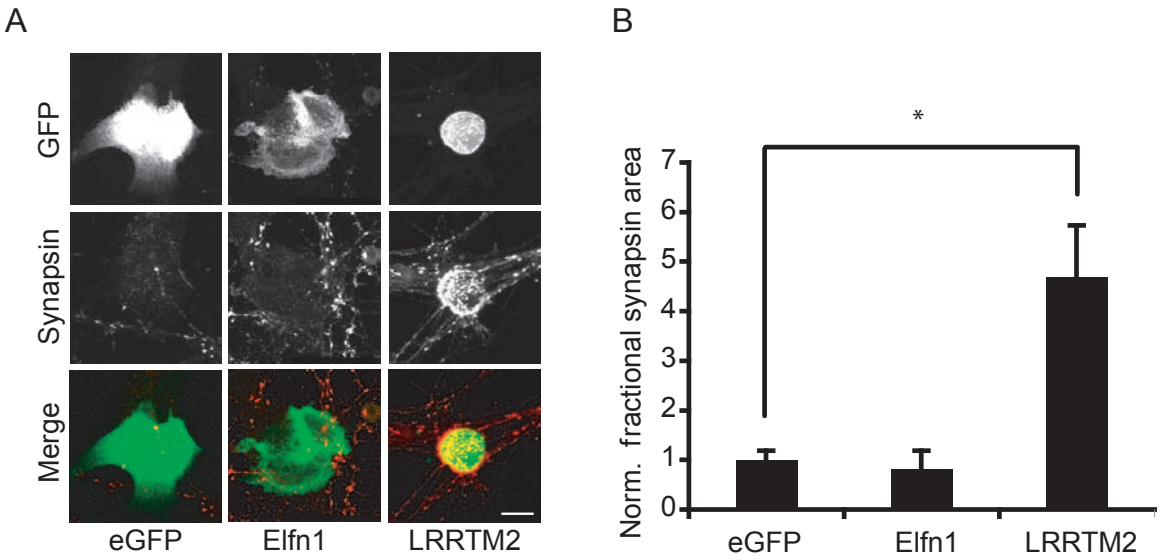

Fig. S7

Elfn1 is not a synapse-inducing molecule. **(A)** HEK cells expressing GFP, Elfn1, or LRRTM2 were co-cultured with dissociated hippocampal neurons from 7-9 DIV, fixed and stained for synapsin. **(B)** Fractional area of the HEK cell stained with synapsin, normalized to GFP transfected cells. Asterisk indicates $p<.01$; ANOVA. Scale bar is 20μm. Error bars represent S.E.M.

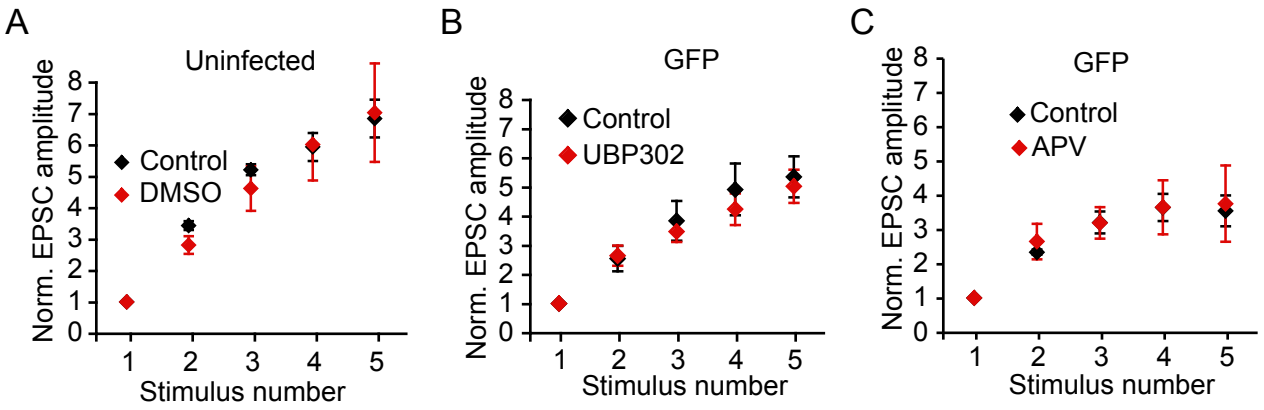

Fig. S8

CA1-OLM synapses do not have GluR5- or NMDAR-dependent mechanisms of short-term facilitation. **(A)** Average postsynaptic response of control uninfected OLM neurons to 20Hz stimulation of the alveus, normalized to the amplitude of the first response. Black and red points signify before and after application of the NS102 drug vehicle DMSO, respectively. **(B)** Average postsynaptic response of GFP infected OLM neurons to 20Hz stimulation of the alveus, normalized to the amplitude of the first response. Black, baseline response; Red, after application the GluR5 containing kainate receptor antagonist of UBP302 (15μM). **(C)** Average postsynaptic response of GFP infected OLM neurons to 20Hz stimulation of the alveus, normalized to the amplitude of the first response. Black, baseline response; Red, after application of 50 μM APV.