Kawabe et al., https://doi.org/10.1083/jcb.201606086

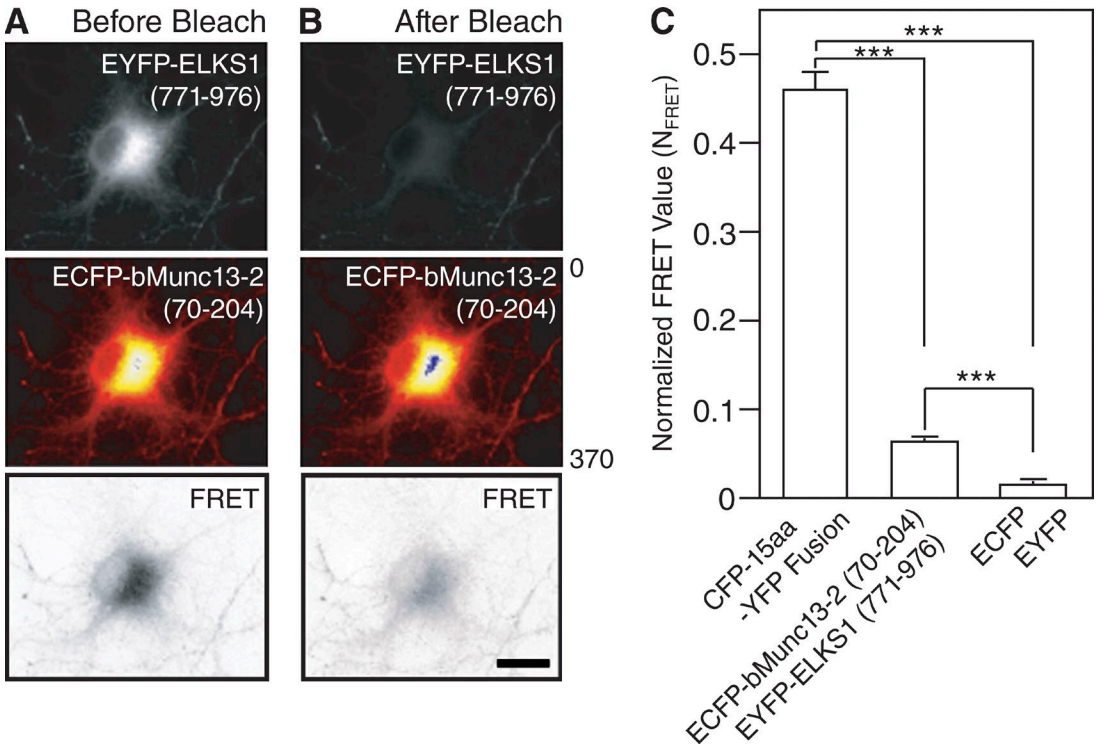

Figure S1. **FRET analysis of the bMunc13-2–ELKS1 interaction in neurons.** (A and B) FRET measurements in WT hippocampal neurons coexpressing ECFP–bMunc13-2(70–204) and EYFP-ELKS1(771–976). Images were acquired in the YFP channel (top), CFP channel (middle), and FRET channel (bottom) before (A) and after acceptor photobleaching (B). The donor fluorescence intensity, which after photobleaching exceeds the maximum intensity obtained before bleaching (false color scale on the right), is indicated in blue. Bar, 10 µm. (C) Graph showing the normalized FRET values. Data are means ± SEM and were analyzed by one-way analysis of variance with Tukey–Kramer multiple comparisons test. $n > 5$ for each group; ***, $P < 0.001$.

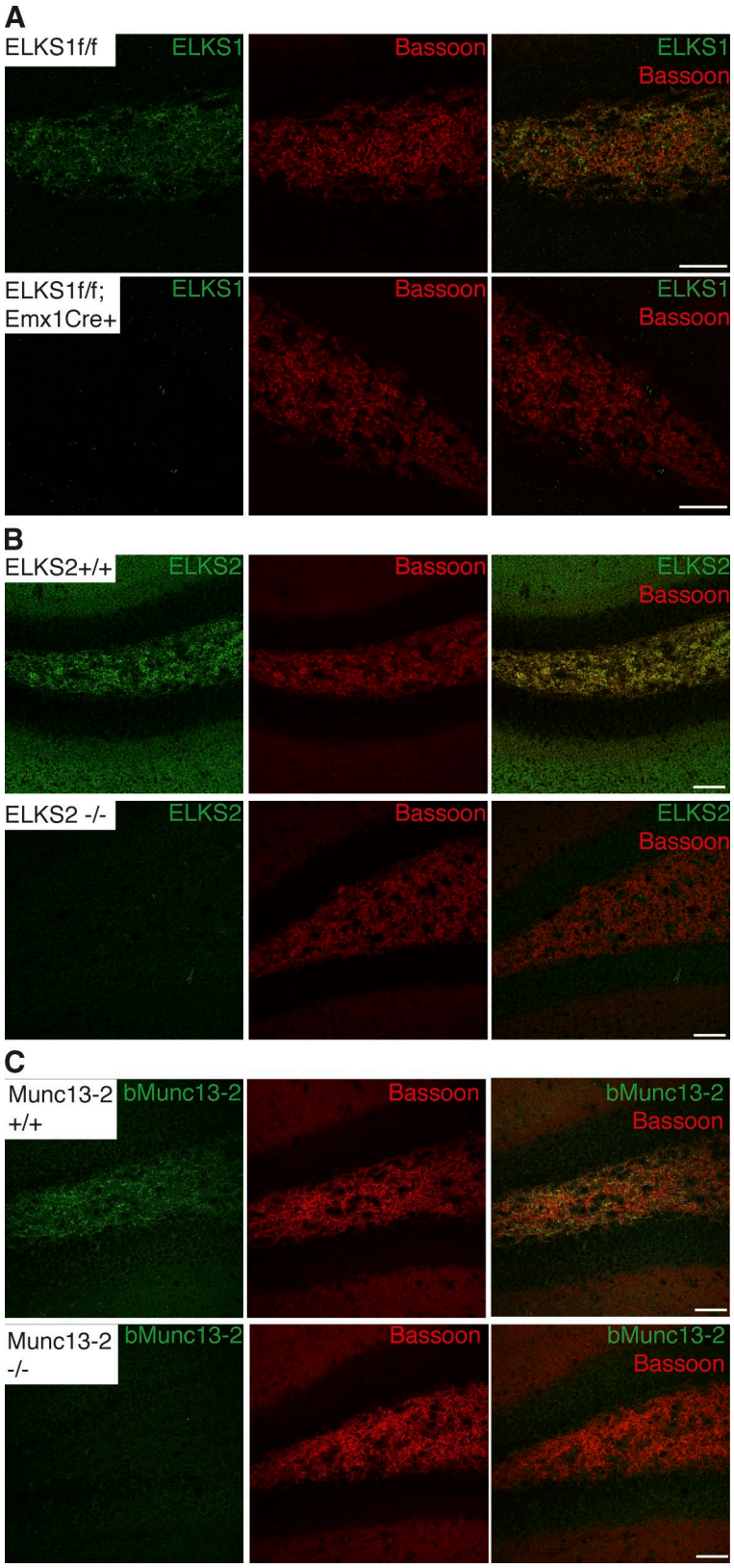

Figure S2. **Specificity of antibodies to ELKS1, ELKS2, and bMunc13-2.** (A–C) Immunohistochemical analyses of the dentate gyrus of control (top) and the indicated KO mice (bottom) with anti-ELKS1 (A), anti-ELKS2 (B), and anti–bMunc13-2 (C) antibodies. All sections were counterstained for Bassoon as a marker of AZs. Most ELKS1, ELKS2, and bMunc13-2 signals were eliminated in the corresponding KOs. Note that signals for ELKS1 and bMunc13-2 are enriched in the hilus and colocalize with a limited fraction of Bassoon-positive structures, whereas most ELKS2 signals colocalize with Bassoon-positive structures. Bars, 50 µm.

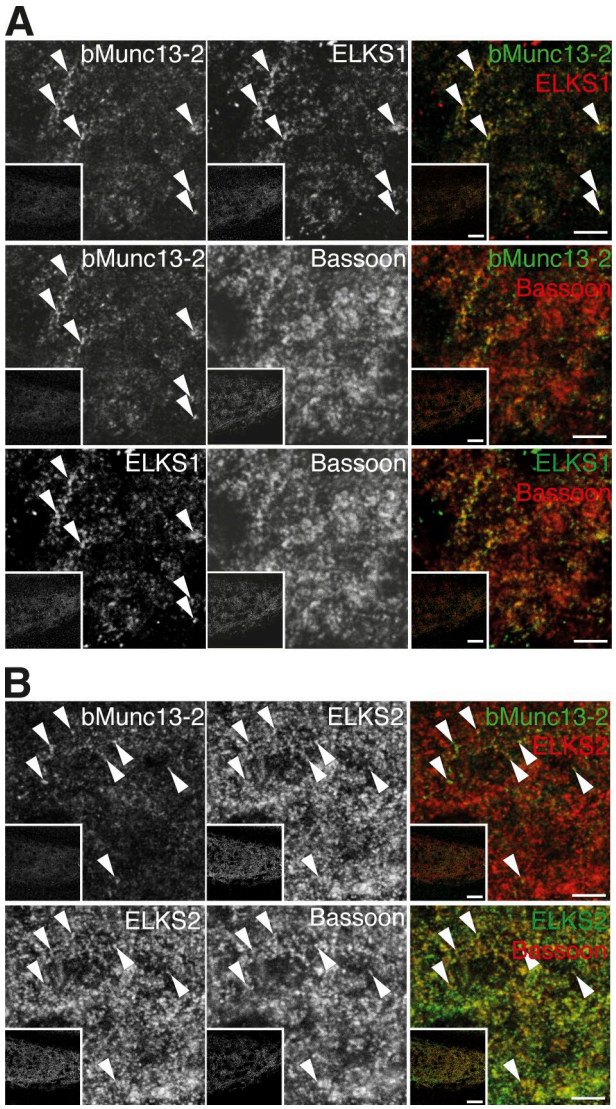

Figure S3. **Preferential recruitment of bMunc13-2 to ELKS1 enriched synapses in the hippocampus.** (A and B) Localization of endogenous bMunc13-2, ELKS1, and Bassoon (A) and of bMunc13-2, ELKS2, and Bassoon (B) in the WT mouse hippocampus (hilus of the dentate gyrus). Note that bMunc13-2 shows a distribution pattern similar to that of ELKS1 with a restriction to a limited number of synapses (arrowheads), whereas ELKS2 is accumulated in most Bassoon-positive AZs (B). Bars: (low-magnification insets) 50 µm; (high magnification) 5 µm.

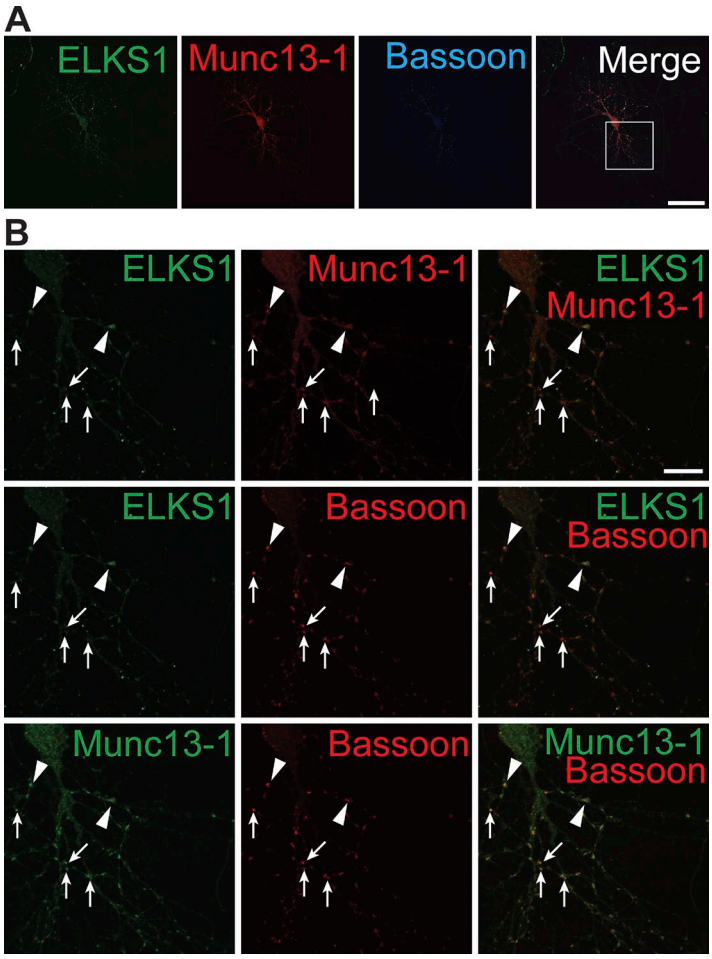

Figure S4. **Neuronal distribution of ELKS1, Munc13-1, and Bassoon.** Autaptic primary cultured neurons immunostained with antibodies to ELKS1, Munc13-1, and Bassoon. (A) Low-power image of an entire autaptic neuron showing that Munc13-1–containing synapses (red) are more abundant than ELKS1-containing synapses (green). Bar, 50 µm. (B) High-power image of boxed region in A. Some synapses show enriched ELKS1 levels (arrowheads), whereas most of them contain no or very little ELKS1 (arrows). Overall, ∼14% of Munc13-1 and Bassoon double-positive puncta appeared to accumulate ELKS1 (*n* = 960 synapses, *n* = 12 neurons). Bar, 10 µm.

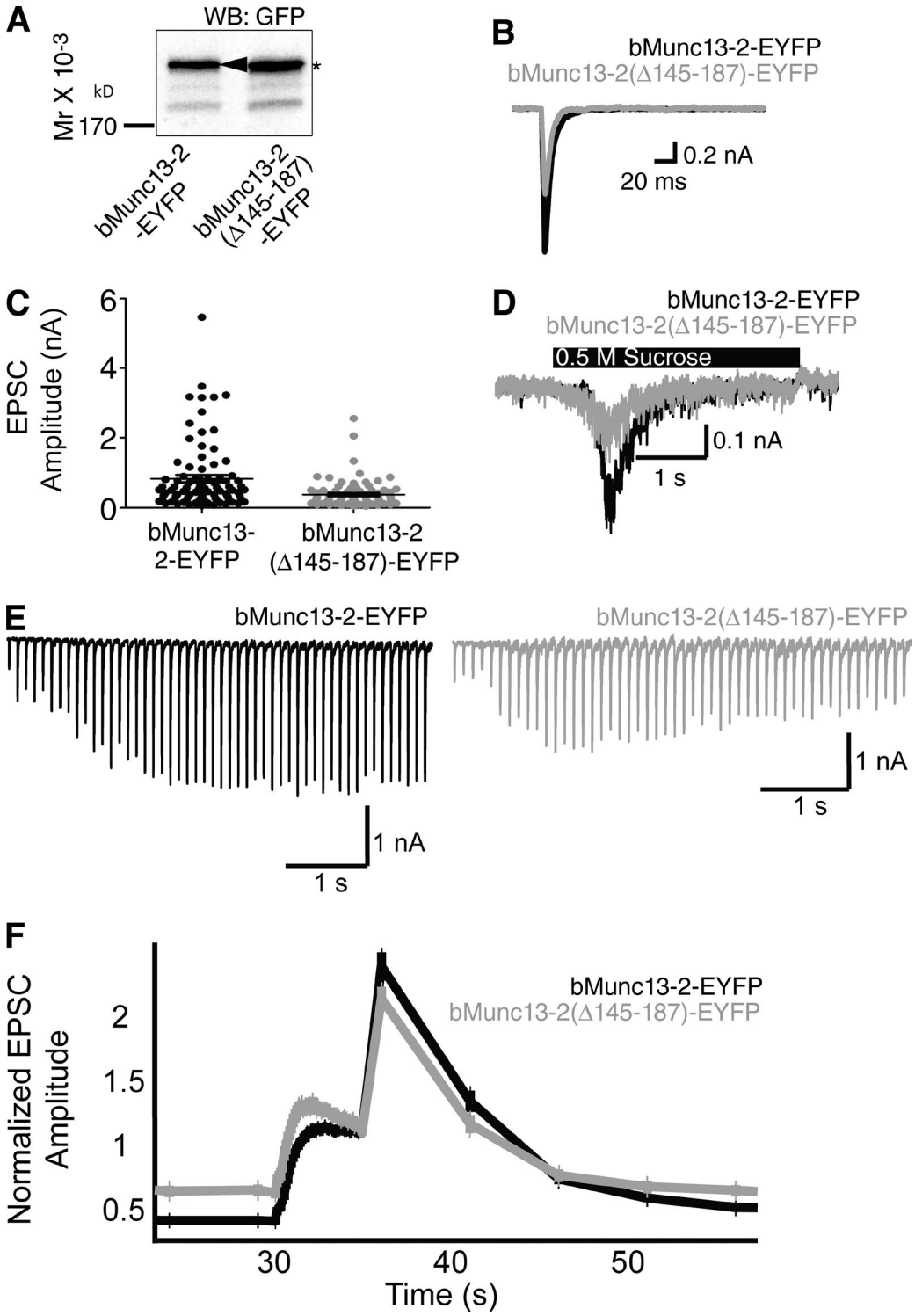

Figure S5. **Rescue of Munc13-1/2 DKO neurons by reexpression of WT of ELKS1-binding–deficient mutant of bMunc13-2.** (A) Expression levels of bMunc13-2–EYFP and bMunc13-2(Δ145–187)–EYFP proteins upon Semliki Forest virus infection. Relative protein levels of bMunc13-2–EYFP (arrowhead) versus bMunc13-2(Δ145–187)–EYFP (asterisk), as assessed by densitometric analysis of bands on films (ImageJ) and normalized to infection efficiencies, were 1:1.23. WB, Western blot. (B) Example traces of evoked EPSCs from neurons expressing bMunc13-2–EYFP (black) or bMunc13-2(Δ145–187)–EYFP (gray) in response to a single action potential. (C) Scatterplots of EPSC amplitudes from Munc13-1/2 DKO neurons expressing bMunc13-2–EYFP (black dots) or bMunc13-2(Δ145–187)–EYFP (gray dots). Data from neurons that did not show responses were not included. (D) Example traces of evoked EPSCs induced by 0.5 M sucrose from neurons expressing bMunc13-2–EYFP (black) or bMunc13-2(Δ145–187)–EYFP (gray). (E) Example traces of evoked EPSCs from neurons expressing bMunc13-2–EYFP (black) or bMunc13-2(Δ145–187)–EYFP (gray) during a 10-Hz action potential train. (F) Time course of short-term plasticity (10-Hz stimulus train at 30–35 s) of Munc13-1/2 DKO neurons expressing bMunc13-2–EYFP (black, *n* = 87) or bMunc13-2(Δ145–187)–EYFP (gray, *n* = 83). Evoked EPSCs were normalized to the levels at the end of the 10-Hz action potential trains. Note that the time courses of facilitation and augmentation of EPSCs are almost identical between Munc13-1/2 DKO neurons expressing bMunc13-2–EYFP (black) and bMunc13-2(Δ145–187)–EYFP (gray).