Lee et al., http://www.jcb.org/cgi/content/full/jcb.200807188/DC1

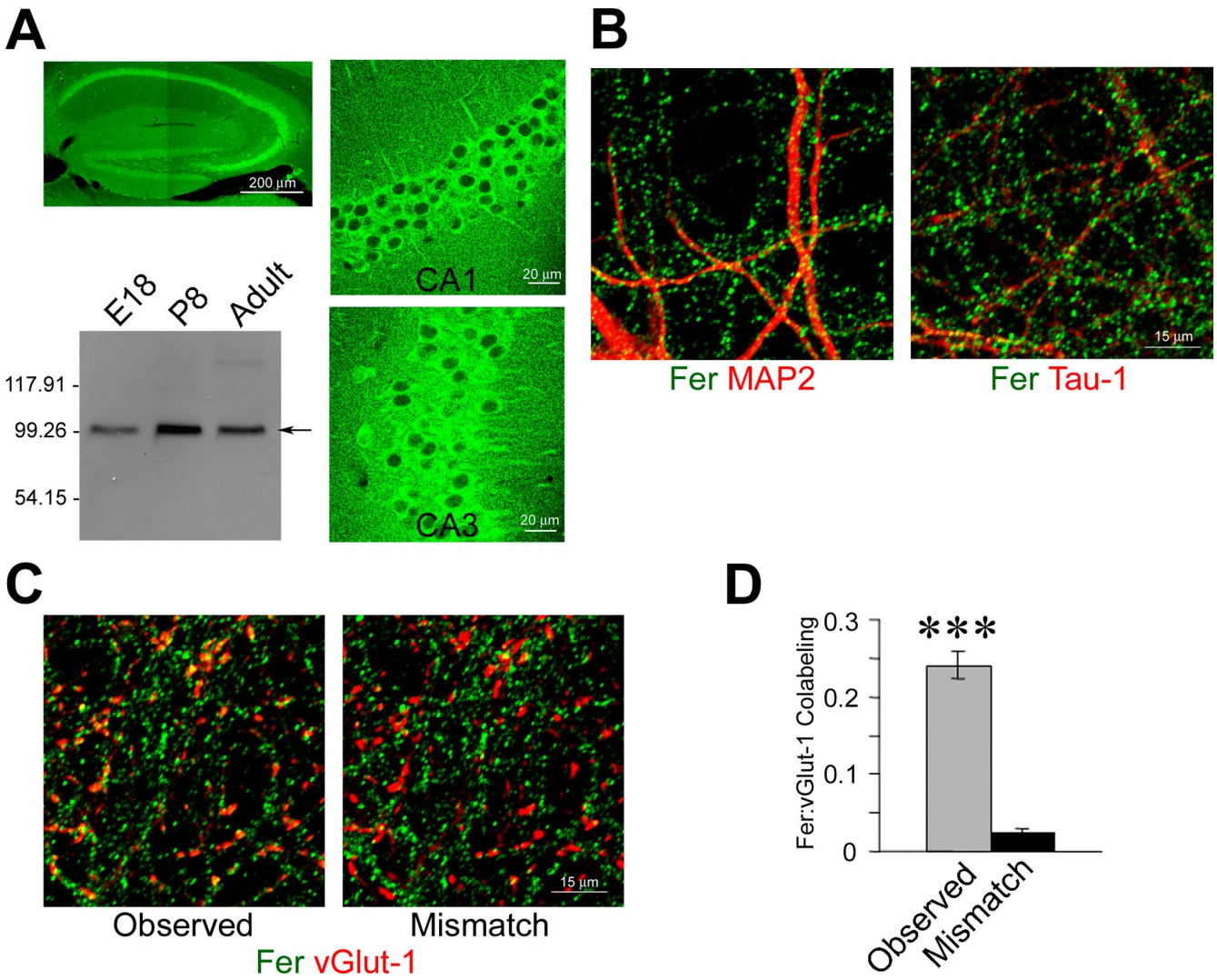


Figure S1. **Hippocampal localization of Fer and partial colocalization with vGlut1.** (A) Expression of Fer in a rodent hippocampus. 50 µm of a hippocampal coronal slice of a 9-wk-old mouse was stained with anti-Fer antibody (top left and right). Rat hippocampi of the indicated ages were lysed with PI buffer and subjected to immunoblotting with anti-Fer antibody (bottom left). E18, embryonic day 18. (B) 14 DIV rat hippocampal neurons were stained with anti-Fer antibody (green) together with anti-MAP2 or anti–Tau-1 antibodies (red). (C) An example of an observed (left) and mismatched (right) super positioning of Fer (green) and vGlut1 (red). The red channel image was shifted 10 µm along the x axis relative to the green channel image. (D) Pearson's coefficient to indicate colocalization of Fer (green) and vGlut1 (red) puncta in the observed and mismatched images. E18, embryonic day 18; P8, postnatal day 8. ***, $P < 0.001$. Error bars represent SEM.

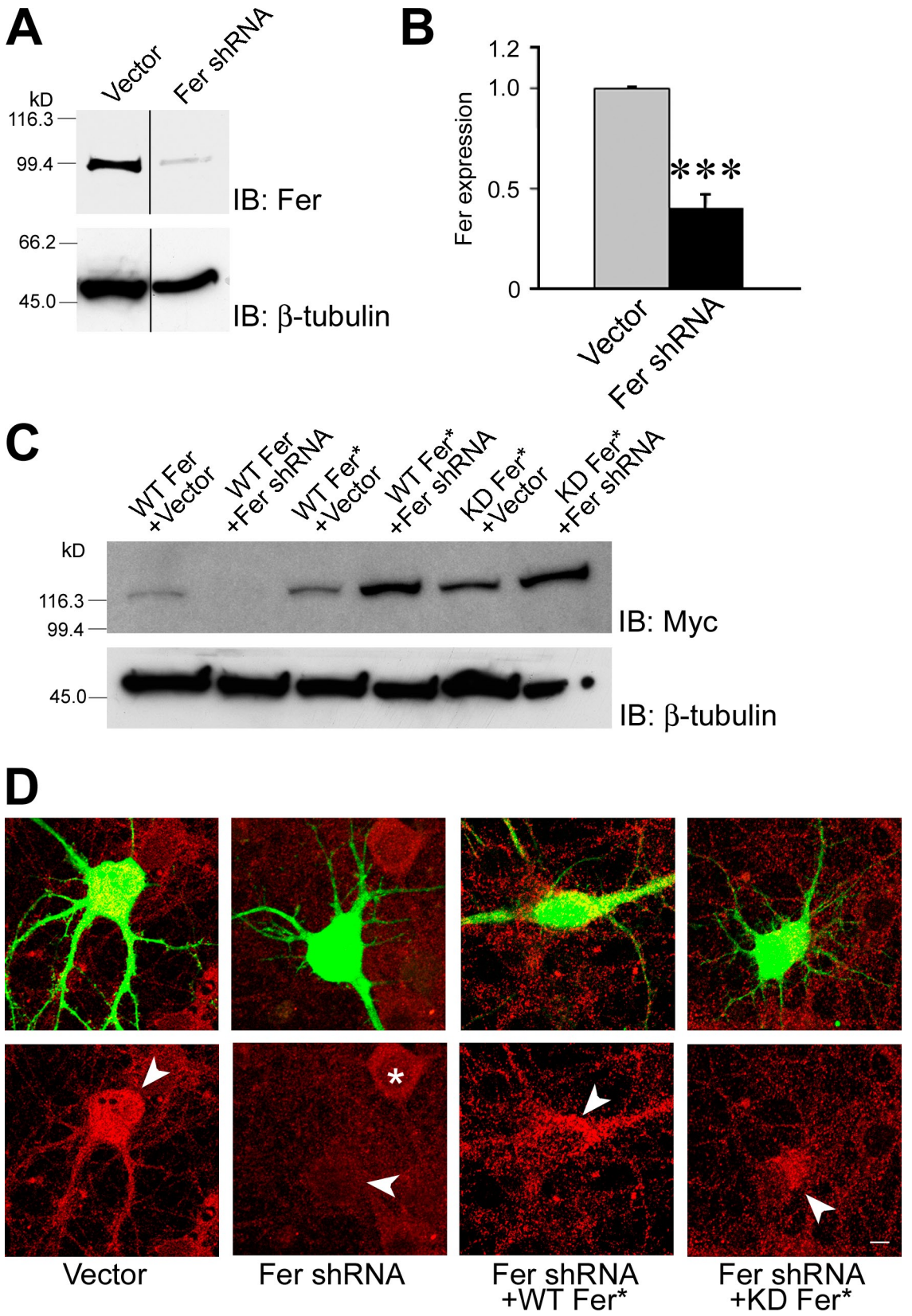


Figure S2. **Verification of Fer shRNA and shRNA refractory constructs.** (A) Rat cortical neurons were transfected with a Fer shRNA–expressing plasmid by electroporation, after which lysates were subjected to immunoblotting with anti-Fer or anti–β-tubulin. Black lines indicate that intervening lanes have been spliced out. (B) Quantification of Fer expression in A. $n = 6$; ***, $P < 0.005$. (C) Coexpression of shRNA-resistant Fer constructs restores Fer protein levels. 293T cells were transfected with the indicated constructs and were subjected to immunoblotting with anti-Fer and anti–β-tubulin. (D) 10 DIV hippocampal neurons were transfected with the indicated constructs containing GFP and were immunostained at 14 DIV with anti-Fer (red). Transfected cells express GFP (arrowheads). WT-Fer* and KDFer* are shRNA refractory mutant Fer expression constructs. KDFer, kinase-dead mutant (K591R) of Fer. *, untransfected cells. Bar, 5 µm.

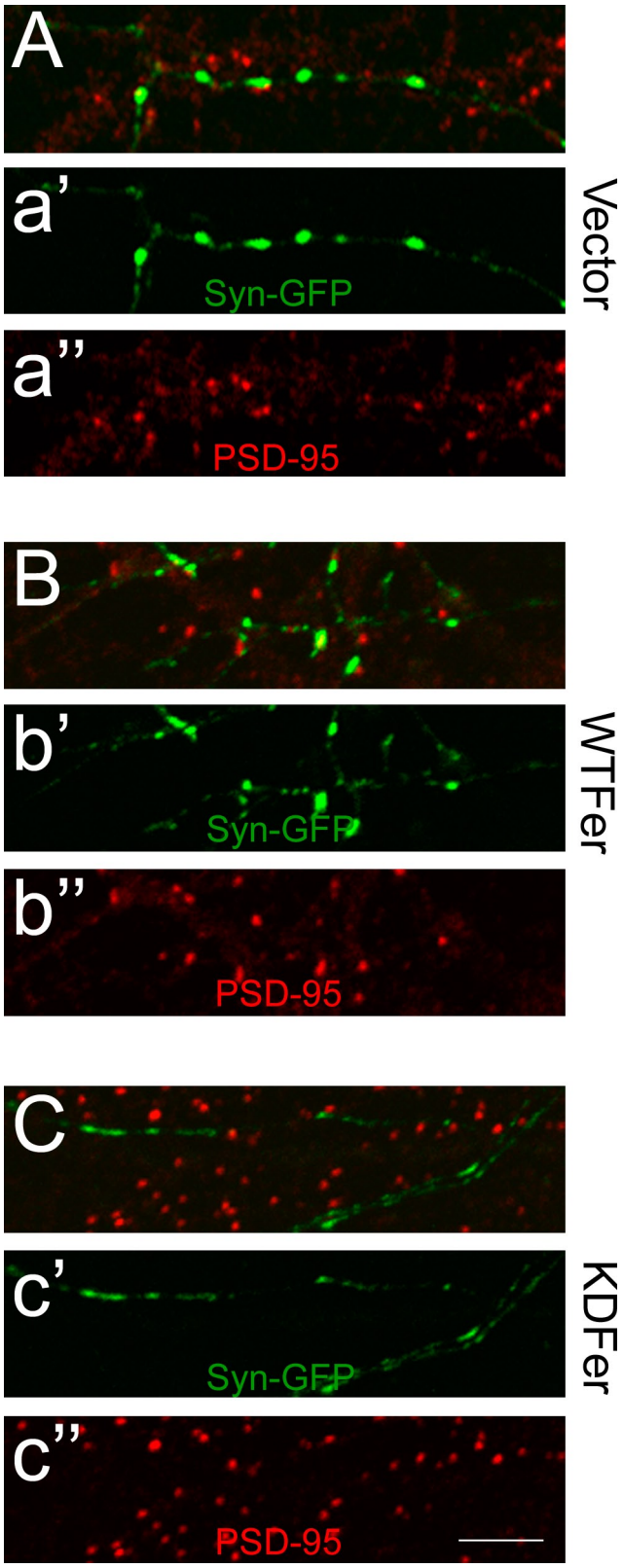


Figure S3. **Effect of kinase inhibition of Fer on the localization of pre- and postsynaptic markers for excitatory synapse formation.** Visualization at 14 DIV of colocalization of synaptophysin-GFP (Syn-GFP) and PSD-95 in rat hippocampal neurons transfected at 10 DIV with synaptophysin-GFP together with control vector (A), wild-type Fer (B), or kinase-dead (D743R) Fer (C). Cells were also fixed, permeabilized, and stained with anti–PSD-95. Bar, 5 μm.

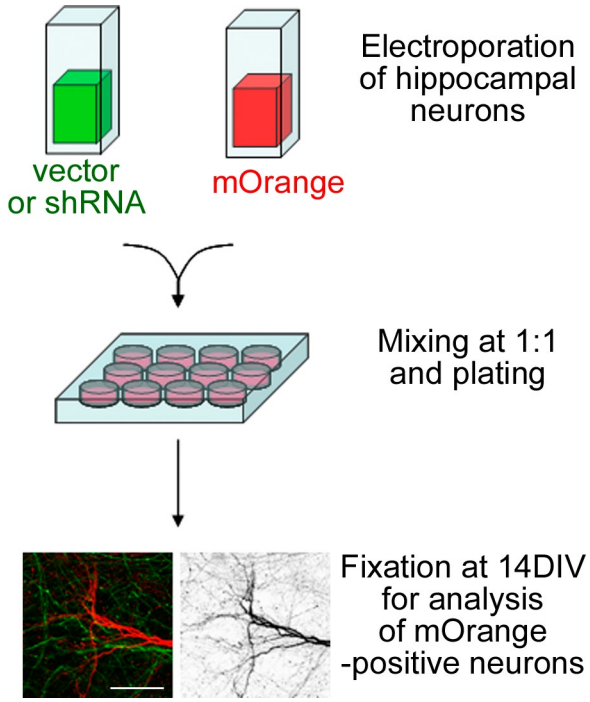


Figure S4. **Schematic of the experiment to analyze the transsynaptic effect of presynaptic Fer KD on postsynaptic dendritic spine morphogenesis.** See Materials and methods for details. Bar, 50 μm.

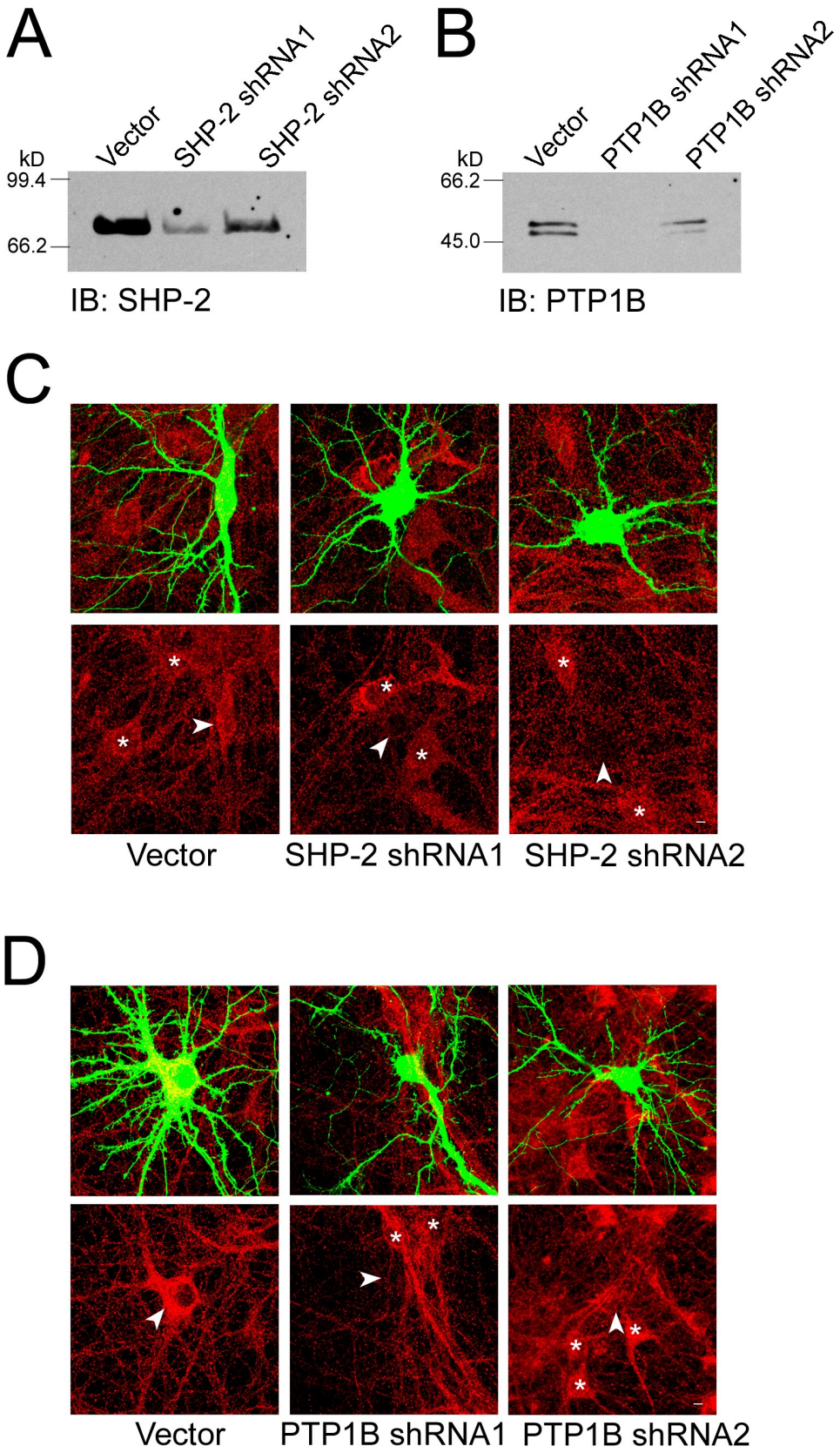
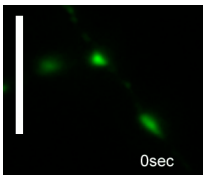
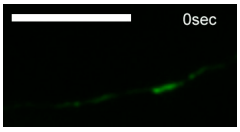


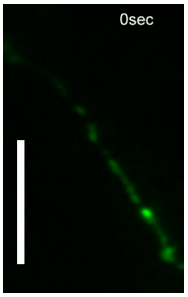
Figure S5. **Verification of SHP-2 and PTP1B shRNA constructs.** (A and B) Rat cortical neurons were transfected with the indicated constructs by electroporation, and at 5 DIV total lysates were subjected to immunoblotting (IB) with anti–SHP-2 (A) or anti-PTP1B (B). Note the strong reductions in SHP-2 (A) and PTP1B (B) protein levels. (C and D) 10 DIV hippocampal neurons were transfected with the indicated constructs, fixed at 14 DIV, and stained with anti–SHP-2 (C) or anti-PTP1B (D) antibodies. Green channels indicate transfected cells, and red channels show SHP-2 (C) or PTP1B (D) immunoreactivity. *, untransfected cells; arrowheads, transfected cells. Bars, 5 µm.



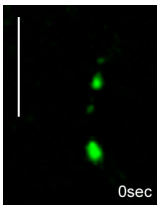
Video 1. **Video from Fig. 2 A (vector).** Live cell imaging of 14 DIV cultured hippocampal neurons that were transfected at 10 DIV with synaptophysin-GFP and control vector. Images were captured every 60 s. Bar, 5 µm.



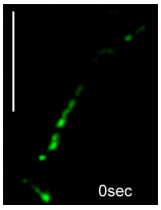
Video 2. **Video of Fig. 2 A (Fer shRNA1).** Live cell imaging of 14 DIV cultured hippocampal neurons that were transfected at 10 DIV with synaptophysin-GFP and Fer shRNA–expressing vector. Images were captured every 60 s. Bar, 5 µm.



Video 3. **Video of Fig. 2 A (Fer shRNA2).** Live cell imaging of 14 DIV cultured hippocampal neurons that were transfected at 10 DIV with synaptophysin-GFP and Fer shRNA–expressing vector. Images were captured every 60 s. Bar, 5 µm.



Video 4. **Video of Fig. 6 (G and H; FerCo).** Time-lapse analysis at 13 DIV of synaptophysin-GFP–expressing organelles in hippocampal neurons transfected at 10 DIV. Cultures were treated with FerCo for 2 h before time-lapse imaging. Bar, 5 µm.



Video 5. **Video of Fig. 6 (G and H; FerP).** Time-lapse analysis at 13 DIV of synaptophysin-GFP–expressing organelles in hippocampal neurons transfected at 10 DIV. Cultures were treated with FerP for 2 h before time-lapse imaging. Bar, 5 µm.