Cell Reports Methods, Volume *2*

Supplemental information

Multimodal imaging of synaptic vesicles with a single probe

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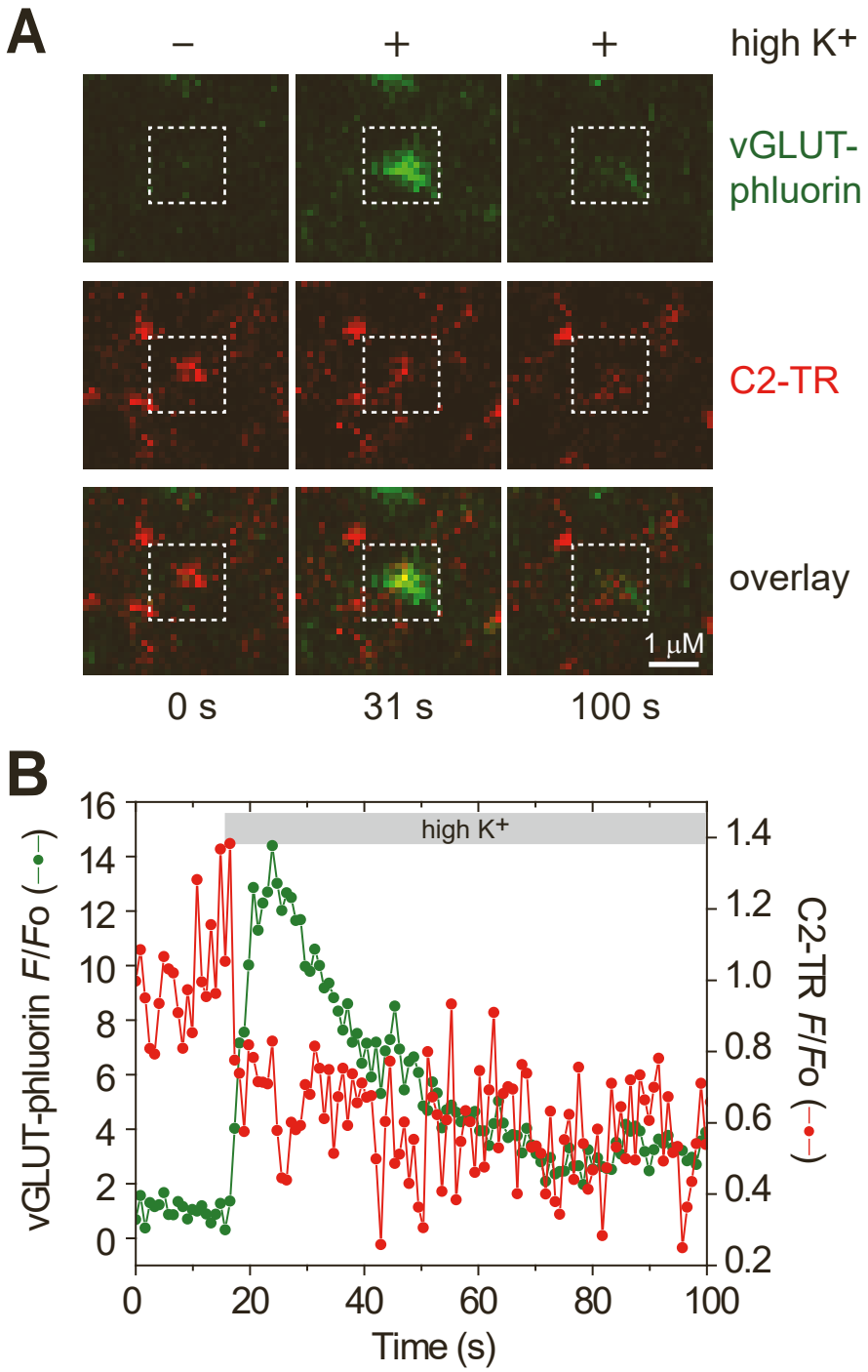

Figure S1. Stimulation-dependent changes in C2-TR and vGLUT-pHluorin fluorescence of an individual presynaptic terminal, related to Figure 2.

(A) Confocal images of hippocampal neurons expressing vGLUT-pHluorin and labeled with 1 mM C2-TR, before and during high K^+ stimulation. (B) Time course of fluorescence intensity (*F*) normalized to initial fluorescence (*Fo*) of the presynaptic terminal in the white dashed box in (A).

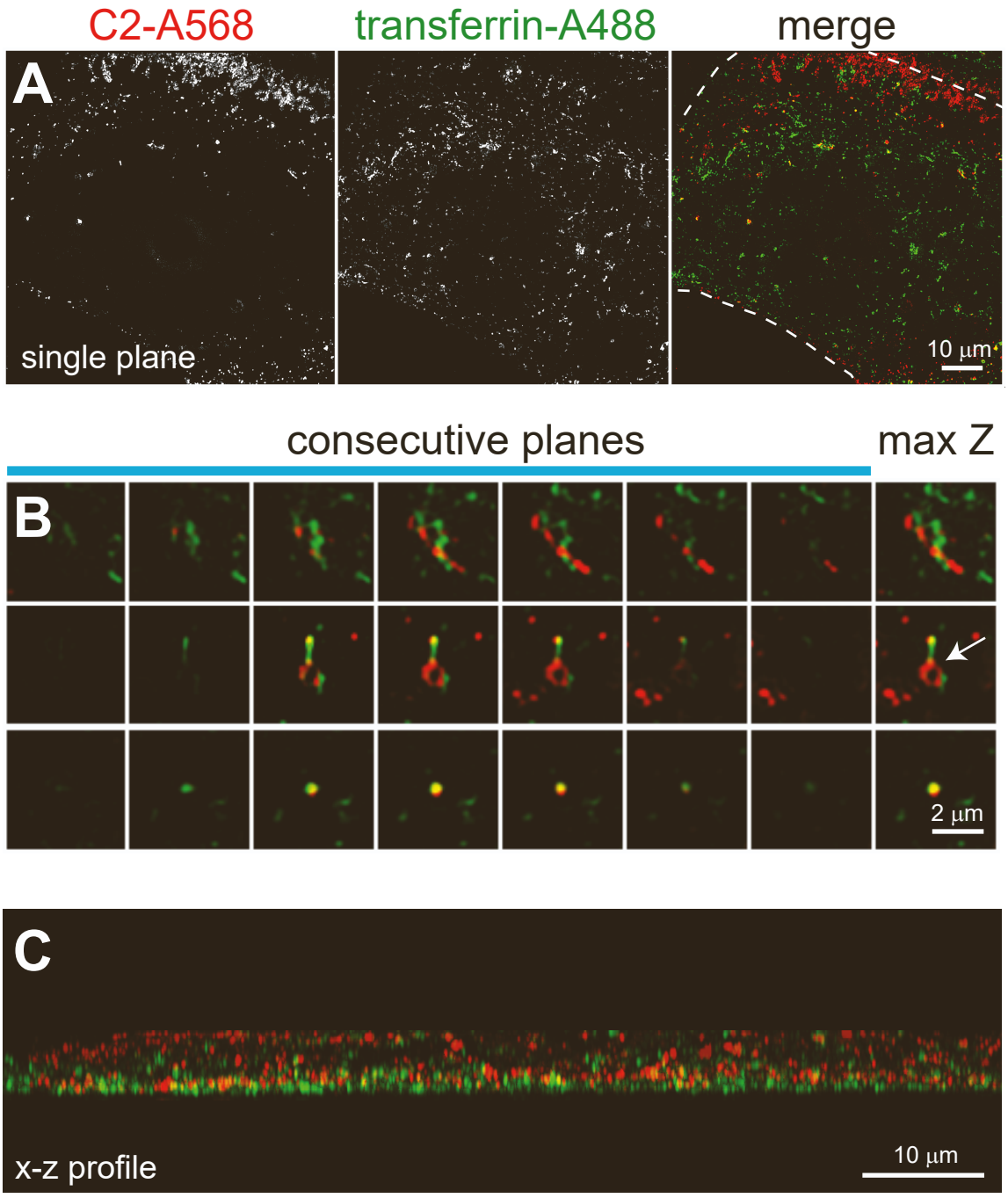

Figure S2. Endocytic internalization of C2-Alexa568 and transferrin-Alexa488 in HeLa cells, related to Figure 1.

(A) HeLa cells were starved for 2 h and incubated with 1 mM C2-Alexa568 and 10 mg/mL transferrin-Alexa488 for 30 min in serum-free media, washed and fixed with 4% paraformaldehyde. Cells were then imaged by structured-illumination microscopy. Dashed white line, cell outline. (B) Consecutive planes and maximum intensity projection of selected individual structures within the cell at a higher magnification. Note partial overlap of C2-Alexa-568 and transferrin-Alexa488 labeling in one structure (white arrow). (C) x-z profile section of the cell.

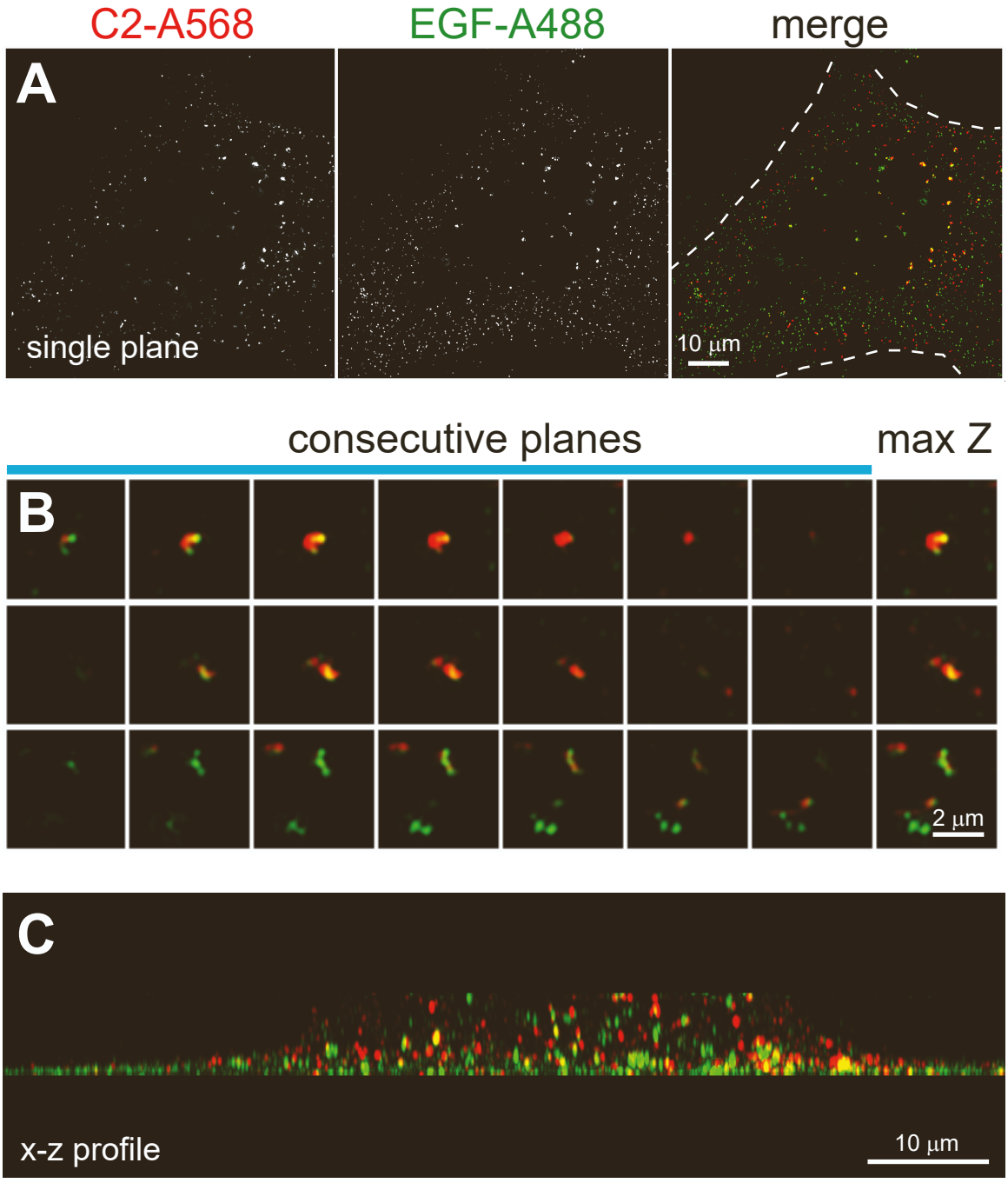

Figure S3. Endocytic internalization of C2-Alexa568 and EGF-Alexa488 in HeLa cells, related to Figure 1. (A) HeLa cells were starved for 2 h and incubated with 1 mM C2-Alexa568 and 100 ng/mL EGF-Alexa488 for 30 min in serum-free media, washed and fixed with 4% paraformaldehyde. Cells were then imaged by structured-illumination microscopy. Dashed white line, cell outline. (B) Consecutive planes and maximum intensity projection of selected individual structures within the cell at a higher magnification. (C) x-z profile section of the cell.