SUPPORTING INFORMATION

Supporting Information Figure 1. *A*, The major ~61 kDa SNX27 isoform is not observed upon immunoprecipitation with EHD1 antibodies. HeLa lysates were incubated at 4°C overnight with either anti-SNX27, anti-EHD1, anti-EB3, or anti-GFP antibodies. Protein G beads were then added to the lysate-antibody mix at 4°C for 4 h. Bound proteins were eluted by boiling at 95°C in β-mercaptoethanol-containing loading buffer, separated by SDS PAGE, and immunoblotted with anti-SNX27. The input lysate (20%) is depicted. Data shown is representative of three independent experiments. ***B*, The SNX17 FERM residue tryptophan 321 is not required for interaction with EHD1.** Purified His-EHD1 bait was bound to Ni^{2+}-NTA-beads for 2 h at 4°C, as described in the Experimental Procedures. The bait and purified GST-fusion target proteins: GST alone, GST-FERM C, GST-FERM C W321A, and full length GST-SNX17 were treated with micrococcal nuclease at 30°C for 10 min. Target proteins were then incubated with bait for 2 h at 4°C. Samples were washed and subjected to SDS-PAGE. In the left panel, the membrane was blotted with anti-His-HRP antibody, showing equivalent concentrations of His-EHD1 used to incubate with GST-fusion proteins. In the right panel, the membrane was blotted with anti-GST antibody. Input refers to the concentrations of purified GST, GST-FERM C, GST-FERM C W321A, and GST-SNX17 used for incubation with His-EHD1-bound to beads. Data shown is representative of three independent experiments.

Supporting Information Figure 2. The EHD1 EH-domain is not required for interaction with SXN17. *A*, Schematic diagram of the domain architecture of full-length EHD1 and truncations used in this study. *B,* Purified His-EHD1, His-EH1, and His-EHD1DEH were bound to Ni^{2+}-NTA-beads for 2 h at 4°C and treated with micrococcal nuclease at 30°C for 10 min. The nuclease-treated purified proteins were then incubated for 2h at 4°C. Samples were washed, eluted and separated by SDS-PAGE followed by immunoblotting with anti-GST (left panel) or Coomassie blue staining (right panel). Input refers to the amounts of purified GST and GST-SNX17 used for incubation with His-tagged fusion proteins. *C,* Densitometric quantification of purified GST-SNX17 protein concentrations precipitated by purified His-EHD1, His-EH1, and His-EHD1ΔEH. Error bars denote standard deviation. *p* values were determined by the Student's two-tailed *t*-test. Data shown is representative of 3 independent experiments.

Supporting Information Figure 3. Dynamics of LRP1 internalization and recycling. CRISPR/Cas9 gene-edited NIH3T3 cells expressing EHD1-GFP were subjected to antibody-induced LRP1 uptake for 15 (*A*) or 30 (*B*) min. as indicated in the representative images, followed by washing with PBS and 1 min. antibody-stripping with buffer containing 0.5% acetic acid, 0.5 M NaCl at pH 3.0. Cells were then washed and transferred to complete media at 37°C for the indicated chase times (*C-F*). Cells were fixed and immunostained with Alexa fluor 568 conjugated anti-mouse antibody diluted in staining buffer lacking saponin to monitor LRP1 recycling to the plasma membrane. *A-F,* Representative images consisting of a field of cells are shown. Bar, 10 μm. *G,* Quantification of LRP1 signal in the cells. Error bars denote standard deviation. ANOVA analysis indicates that upon comparing all values, significance is $p < 0.0001$. Data shown is representative of 3 independent experiments.

Supporting Information Figure 4. EHD1 is recruited to endosomes upon LRP1 receptor uptake, but not by uptake of a control antibody. CRISPR/Cas9 gene-edited NIH3T3 cells expressing EHD1-GFP were either Mock-treated (no uptake) (*A*), incubated with anti-V5 (*B*), or incubated with anti-LRP1 antibodies (*C*), as described in Fig 3. Cells were fixed and imaged by confocal microscopy. *A-C*, Representative images consisting of a field of cells are shown. z-sections obtained from confocal microscopy were processed with IMARIS software to construct 3D surfaces for EHD1, as discussed in the Experimental Procedures, and total EHD1 surface area per cell was

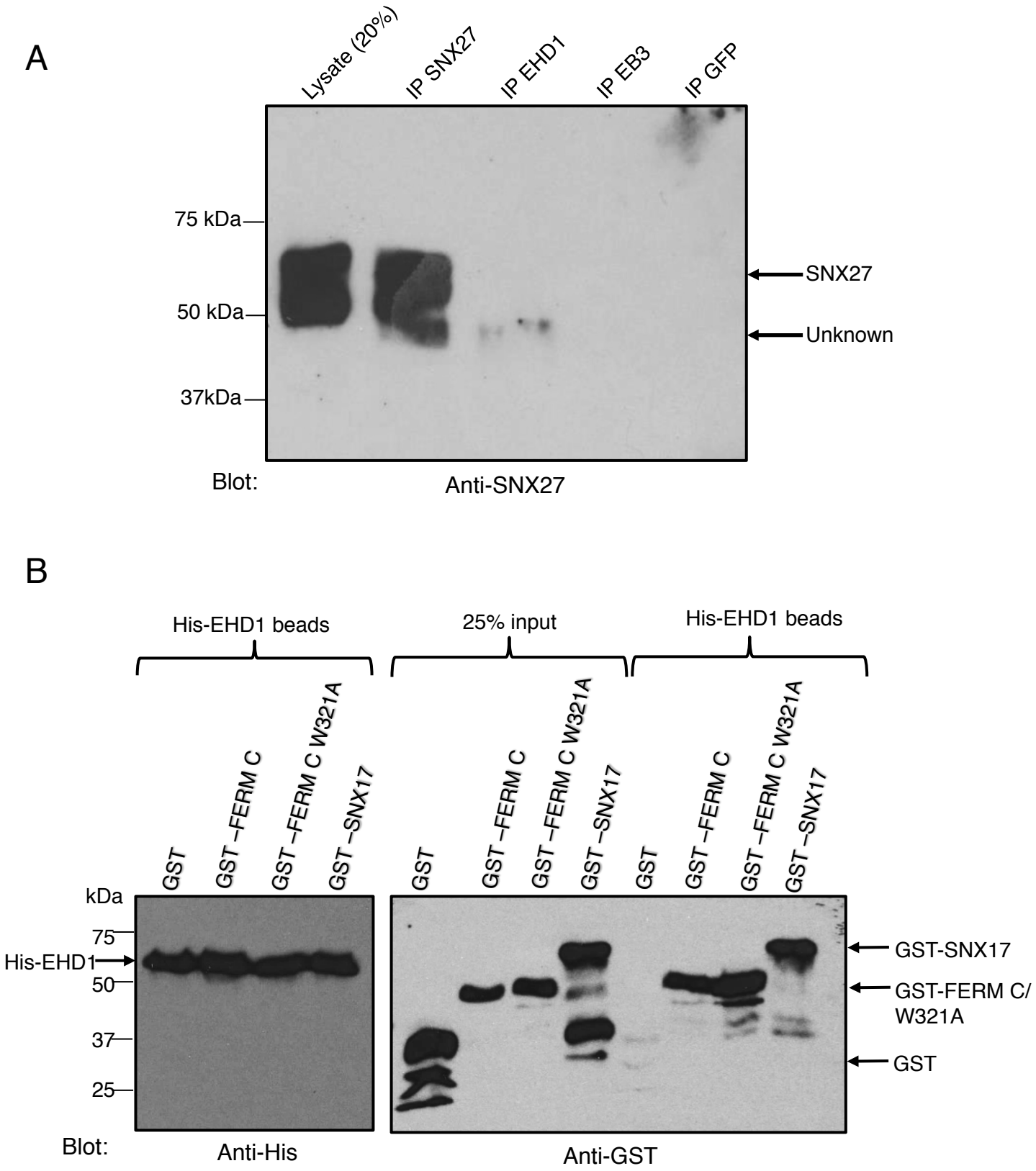
calculated. Regions of interest are shown in the inset a. Bar, 10 μm. *D,* The graph depicts the total surface area of EHD1-containing endosomes in cells with or without antibody uptake. Data shown is representative of three independent experiments.

Supporting Information Figure 5. Co-localization between EHD1 and SNX17 increases upon LRP1 uptake. CRISPR/Cas9 gene-edited NIH3T3 cells expressing EHD1-GFP (green) were either *A*-*C*, Mock-treated (no uptake) or *D*-*F*, incubated with anti-LRP1 antibody. The cells were fixed and stained with anti-SNX17 antibody (red) and imaged by confocal microscopy. *A*-*F*, Representative confocal micrographs display a field of cells. Zoomed merged insets are shown in *C* and *E*. Insets highlight the extent of co-localization (yellow) between EHD1 (green) and SNX17 (red). Data shown is representative of three independent experiments.

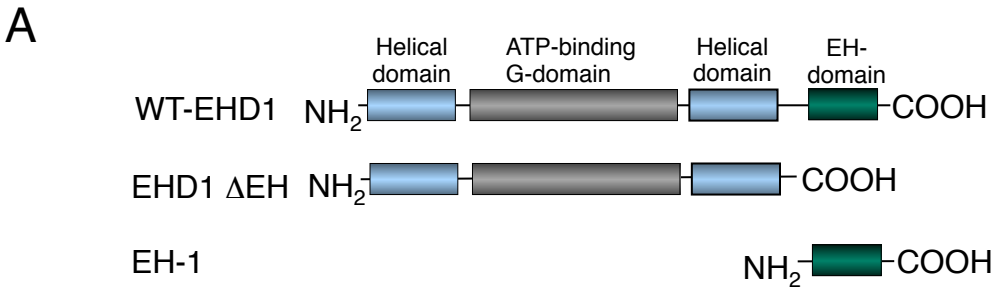
Supporting Information Video 1. Co-localization between EHD1 and SNX17 at steady-state. CRISPR/Cas9 gene-edited NIH3T3 cells expressing EHD1-GFP (green) were Mock-treated (no uptake). The cells were fixed and stained with anti-SNX17 antibody (red) and imaged by confocal microscopy with serial z-sections. IMARIS software was used to render surfaces and calculate the overlapping EHD1 and SNX17 3D surface overlap volumes (yellow).

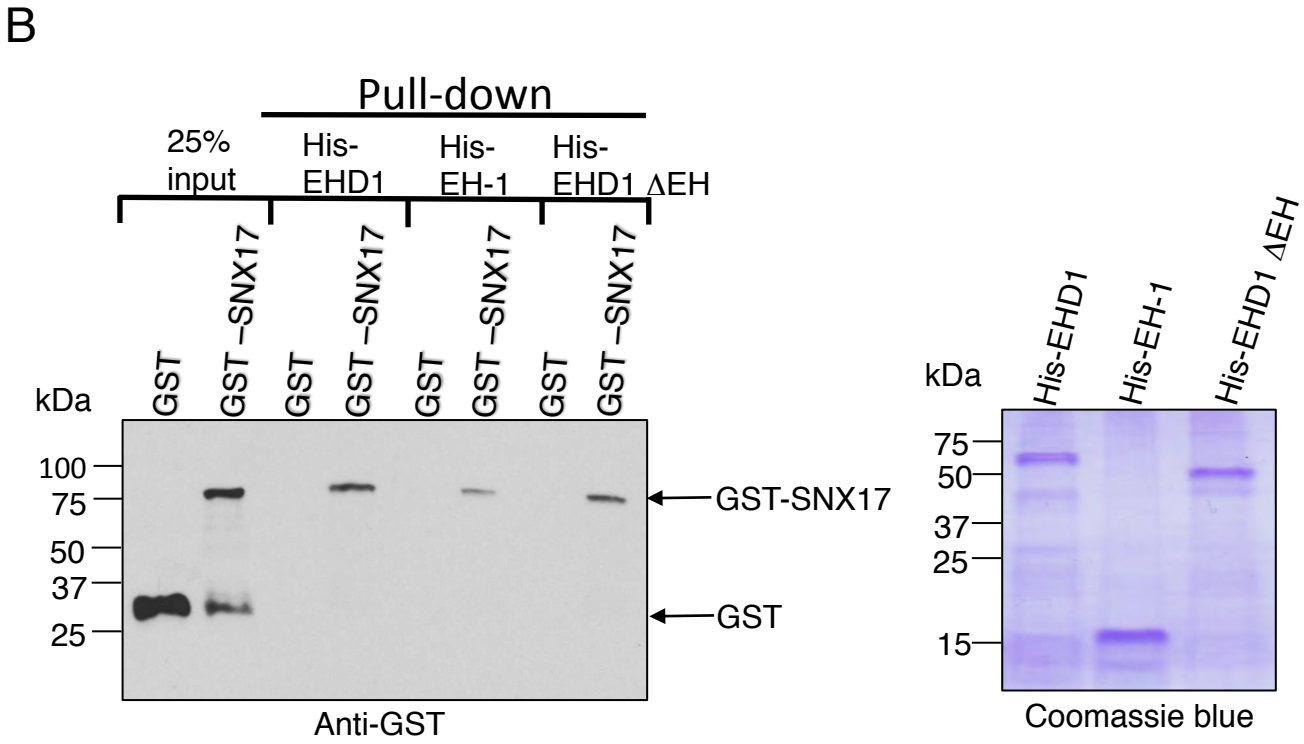
Supporting Information Video 2. Co-localization between EHD1 and SNX17 upon LRP1 uptake. CRISPR/Cas9 gene-edited NIH3T3 cells expressing EHD1-GFP (green) were incubated with anti-LRP1 antibody. The cells were fixed and stained with anti-SNX17 antibody (red) and imaged by confocal microscopy with serial z-sections. IMARIS software was used to render surfaces and calculate the overlapping EHD1 and SNX17 3D surface overlap volumes (yellow).

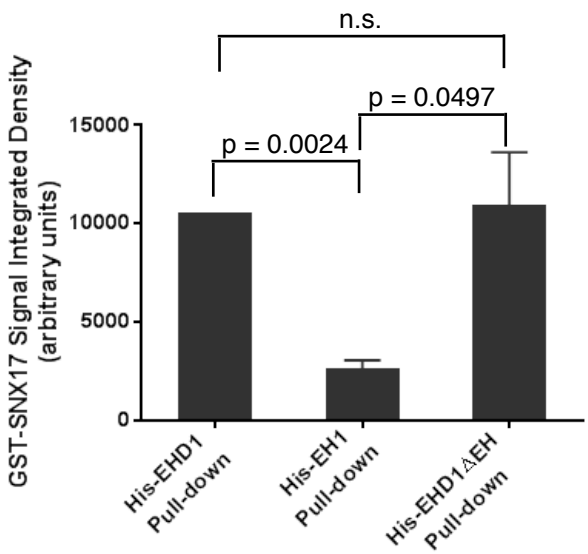
Supporting Information Fig. 1



Supporting Information Fig. 2







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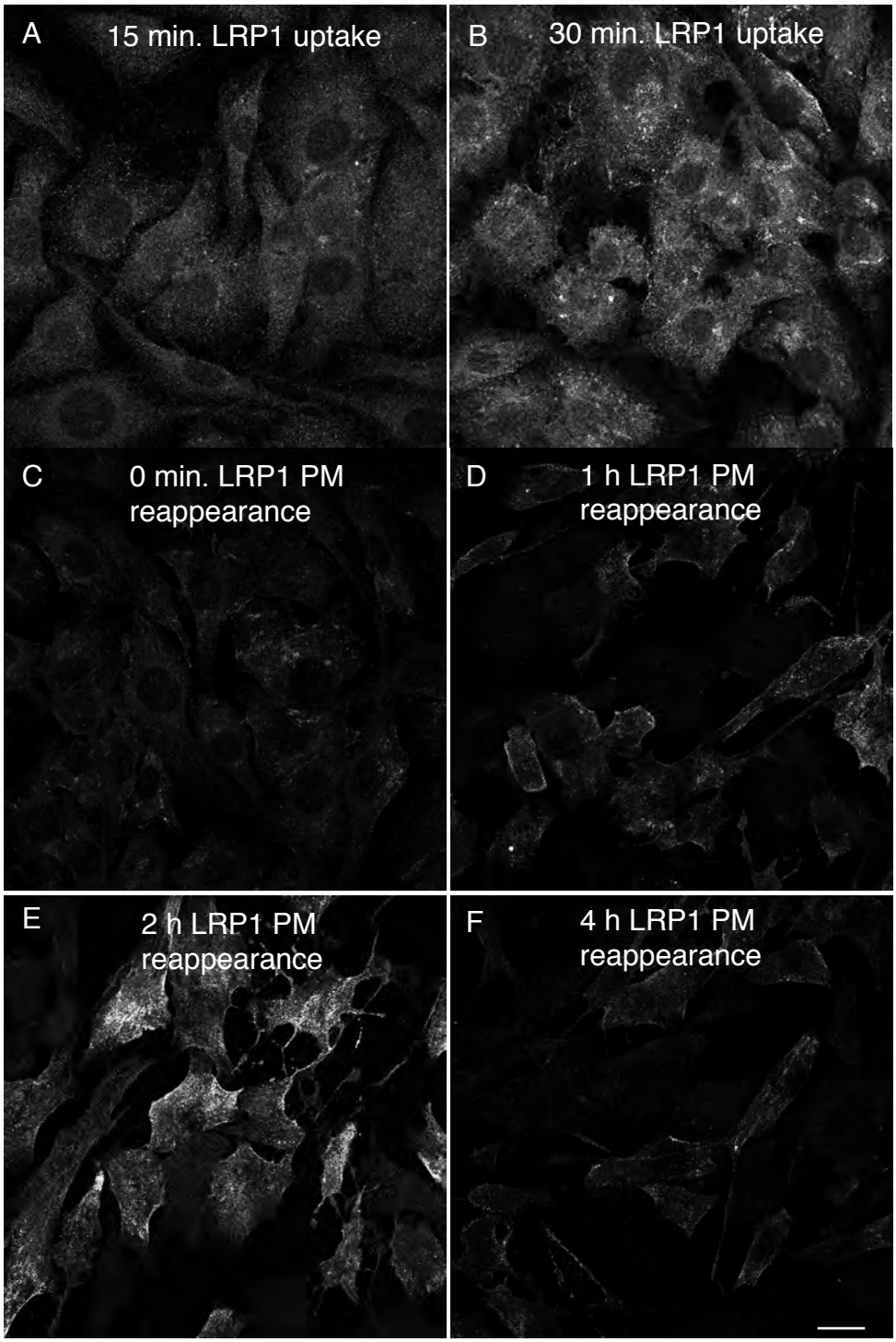

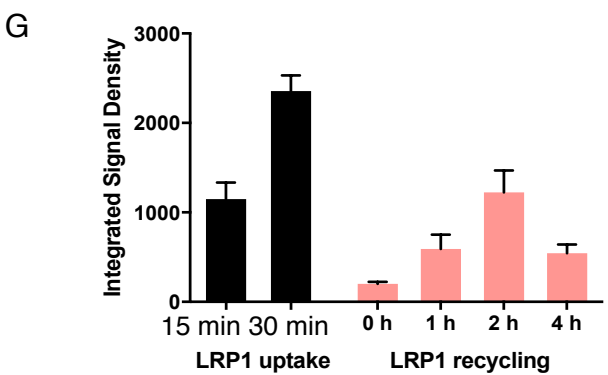

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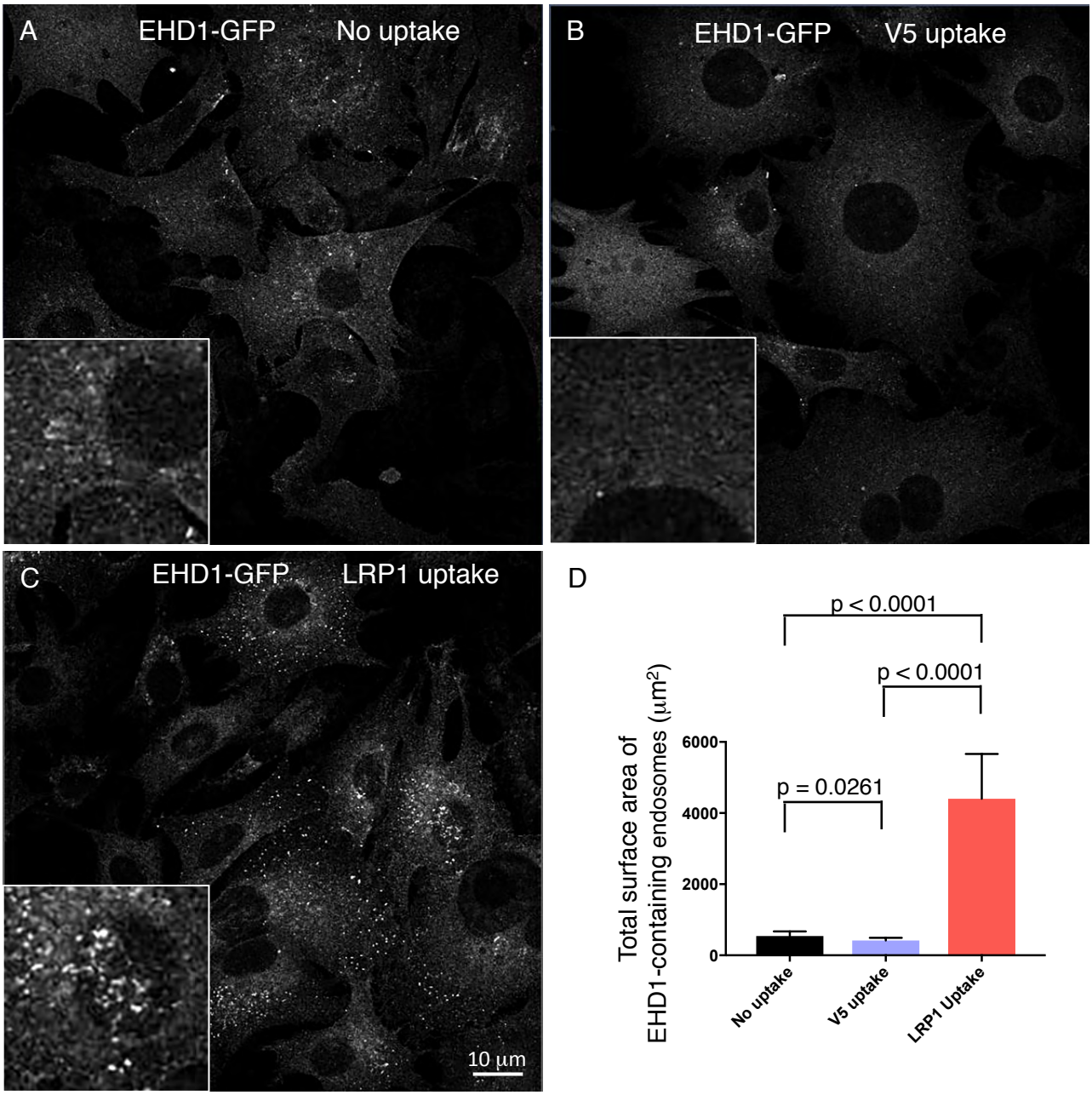

Supporting Information Fig. 5