

**Supplemental Figure legends:**

**Supplemental Figure 1: Dynamic co-localization of EHD1 with endocytosed NgCAM and transferrin**

Cells were transfected with cherry-EHD1 and NgCAM, and endocytosis assays carried out with Alexa488-anti NgCAM antibody (30 minutes uptake), and DyLight649-Tfn (1 hour uptake) 24 hours later. Live cells were imaged after washing. (A-B) Dynamic behavior of cherry-EHD1 (green), endocytosed NgCAM (blue) and endocytosed transferrin (red) in dendrites. The time dimension is shown on the y-axis, the retrograde direction is indicated above. Duration of time-lapse is 3.3 minutes. Scale is  $6\mu\text{m}$ . (A) Example of stationary EHD1-positive vesicle (arrow) colocalizing with both NgCAM (continuously throughout the time-lapse) and transferrin (transiently in time). Example of one NgCAM-positive motile vesicle (arrowhead) and two transferrin-positive motile vesicles (arrowheads). (B) Example of EHD1-NgCAM-transferrin-positive stationary vesicle (arrow). Example of one EHD1-NgCAM-positive motile vesicle (arrowhead), and one NgCAM-positive motile vesicle (arrowhead).

**Supplemental Figure 2: Dynamic co-localization of EHD1 with NEEP21-positive endosomes**

(A-C) First frame of time-lapse of part of dendrites of neurons co-expressing cherry-EHD1 (cyan) and NEEP21-GFP (red) is shown with corresponding kymograph underneath. The time dimension is shown on the y-axis, the retrograde direction is indicated above. Duration of time-lapse is 10 minutes (A,B), and 1 minute (C). Scale is  $6\mu\text{m}$ . (A) Example of two stationary EHD1-positive vesicles (arrows), one of them colocalizing with NEEP21, and motile NEEP21-positive vesicle (arrowhead). (B) Example of EHD1-NEEP21-positive motile vesicle (arrowhead). (C) Examples of EHD1- or NEEP21-positive stationary vesicles (arrows) and NEEP21-positive motile vesicle (arrowhead). (D) Quantification of colocalization of EHD1 with NEEP21 in stationary and motile vesicles (n=1054 endosomes). There are three classes of vesicles: EHD1-alone that are positive for only EHD1; EHD1+NEEP21 that are positive for both EHD1 and NEEP21, and “offset overlap” vesicles. The “offset overlap” vesicles constituted a subpopulation of compartments where the overlap of EHD1 and NEEP21 was partial in

space: the maximum fluorescent intensity of cherry-EHD1 did not completely overlap with that of NEEP21-GFP. Rather, they appeared to be laterally segregated. We already observed this “offset” effect with endocytosed NgCAM and Tfn (Yap et al., 2008b), suggesting lateral sorting of cargos within single early endosomal compartments. This is also the case in polarized non-neuronal cells for rabs (Miaczynska and Zerial, 2002) and was recently shown in neurons for GRASP1 and rab4 (Hoogenraad et al., 2010).

**Supplemental Figure 3: Co-localization of cherry-EHD1 and GFP-EEA1 in live fibroblast**

Fibroblasts expressing cherry EHD1 and GFP-EEA1 were imaged live. Single channels as well as overlaid channels are shown for the whole cell and the part of soma expressing cherry-EHD1 (cyan) and GFP-EEA1 (red). Scale bar is 10 $\mu$ m.

**Supplemental Movie 1: Motility of cherry-EHD1-positive endosomes**

Part of dendrite is shown. Interval between frames is 0.5 sec., duration of time-lapse is 30 sec. Movie is played 10 frames per second (see Fig. 2).

**Supplemental Movie 2: Motility of cherry-EHD1 and endocytosed NgCAM**

Part of dendrite is shown. Cherry-EHD1 is shown in cyan and endocytosed NgCAM in red. Interval between frames is 10 sec., duration of time-lapse is 7.5 minutes. Movie is played 7 frames per second (see Fig. 3).

**Supplemental Movie 3: Motility of cherry-EHD1 and endocytosed transferrin**

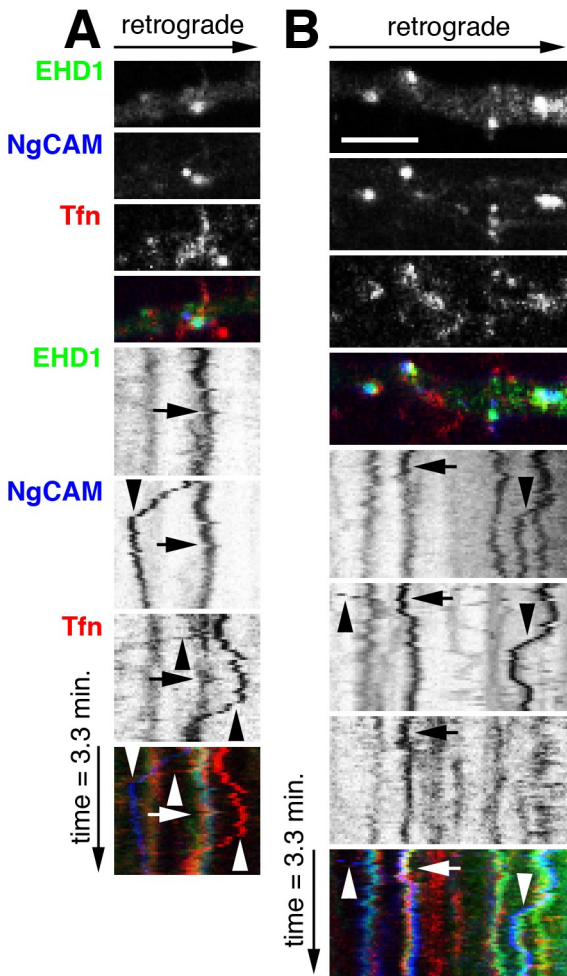
Part of dendrite is shown. Cherry-EHD1 is shown in cyan and endocytosed transferrin in red. Interval between frames is 0.5 sec., duration of time-lapse is 30 sec. Movie is played 10 frames per second (see Fig.3).

**Supplemental Movie 4: Motility of cherry-EHD1 and rab11-GFP**

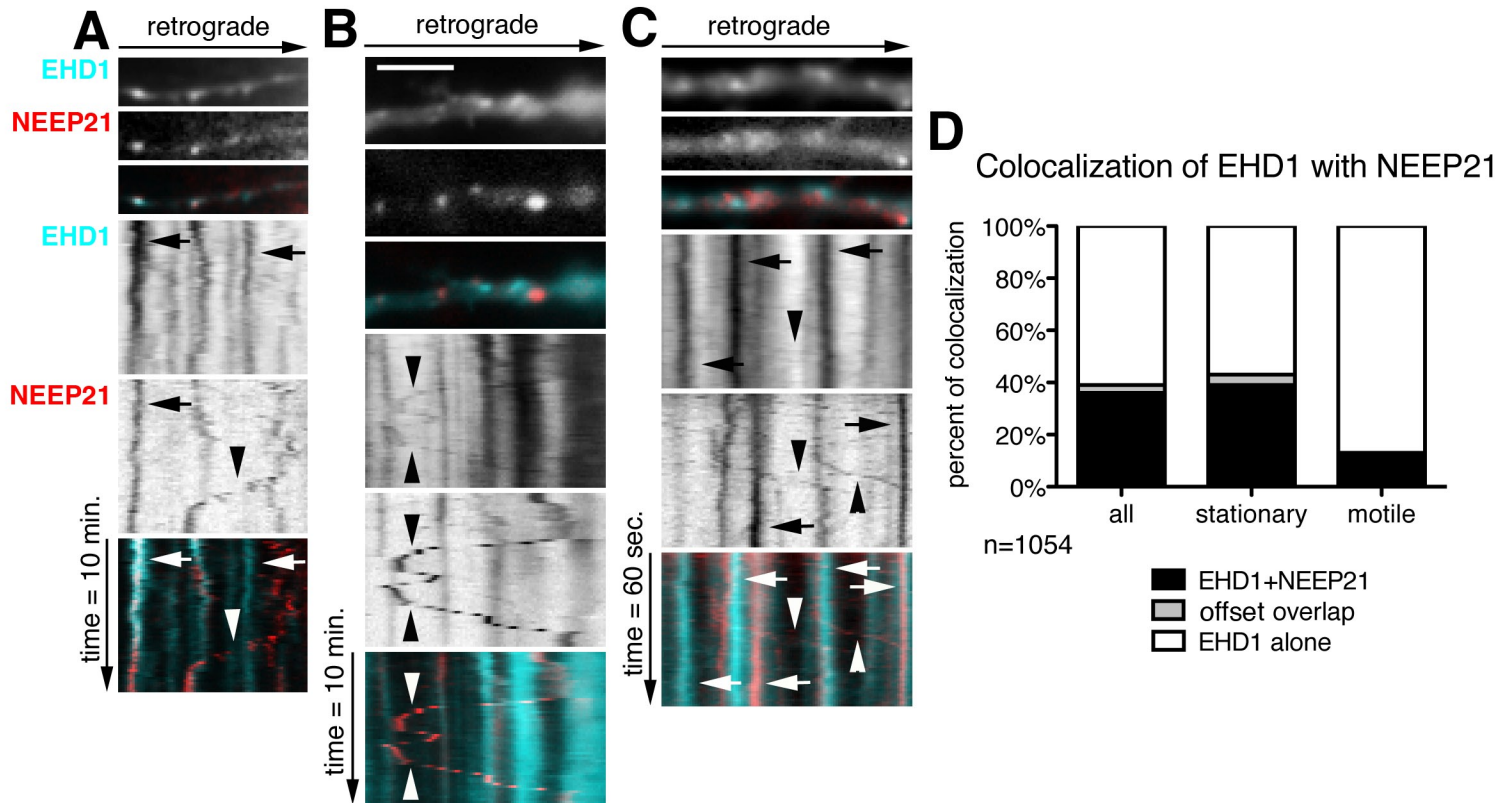
Part of dendrite is shown. Cherry-EHD1 is shown in cyan and rab11-GFP in red. Interval between frames is 0.5 sec., duration of time-lapse is 30 sec. Movie is played 10 frames per second (see Fig. 5).

**Supplemental Movie 5: Motility of cherry-EHD1 and GFP-EEA1**

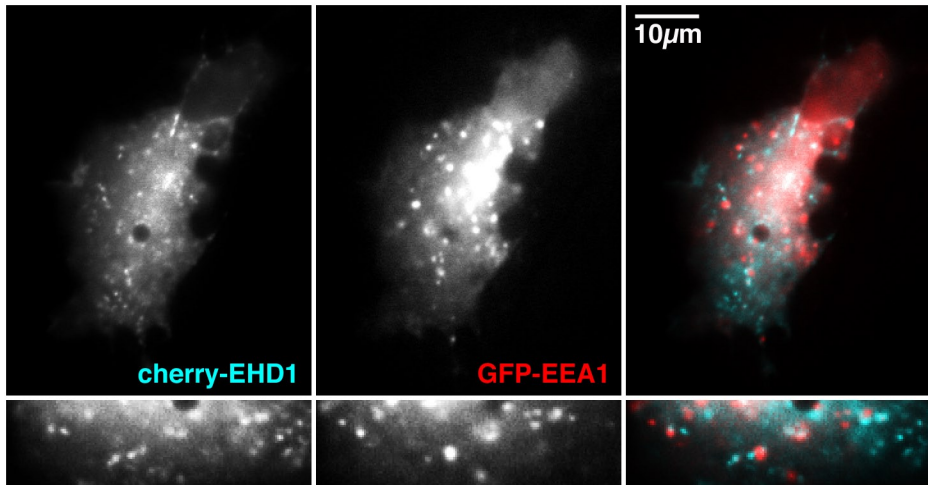
Part of dendrite is shown. Cherry-EHD1 is shown in cyan and GFP-EEA1 in red. Interval between frames is 10 sec., duration of timelapse is 10 minutes. Movie is played 7 frames per second (see Fig. 6).



**Lasiecka, Yap et al.  
Supplemental Figure 1**



**Lasiacka, Yap et al. Supplemental Figure 2**



**Lasiecka, Yap et al. Supplemental Figure 3**