Supplementary Material (ESI) for Analyst

Single particle tracking as a method to resolve differences in highly colocalized proteins

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Electronic Supplementary Information

Fig. S1. The use of ECFP and EYFP, in combination with the appropriate microscope parameters, does not lead to cross-talk with the opposing channel. This was confirmed using BS-C-1 cells transfected with a single fluorescent protein. A. LAMP1-EYFP B. ECFP-Rab7 C. EYFP-Rab9

Fig. S2. Colocalization of Rab7-LAMP1 and Rab7-Rab9 is also high in HeLa cells. A. Colocalization of ECFP-Rab7 (green) and LAMP1-EYFP (red). B. Colocalization of ECFP-Rab7 (green) and EYFP-Rab9 (red). Colocalized proteins appear yellow in the overlaid confocal images. Cells were fixed in 4% formaldehyde for 30 minutes then rinsed and imaged in PBS.

Fig. S3. The radial distance traveled by Rab7- and LAMP1-vesicles is greater than that of Rab7-LAMP1 hybrid vesicles (Fig. 3). A. Average radial distance from the centroid of the trajectory for 50 Rab7-vesicles. B. Average radial distance from the centroid of the trajectory for 50 LAMP1-vesicles. The lines are fits to a single exponential function.

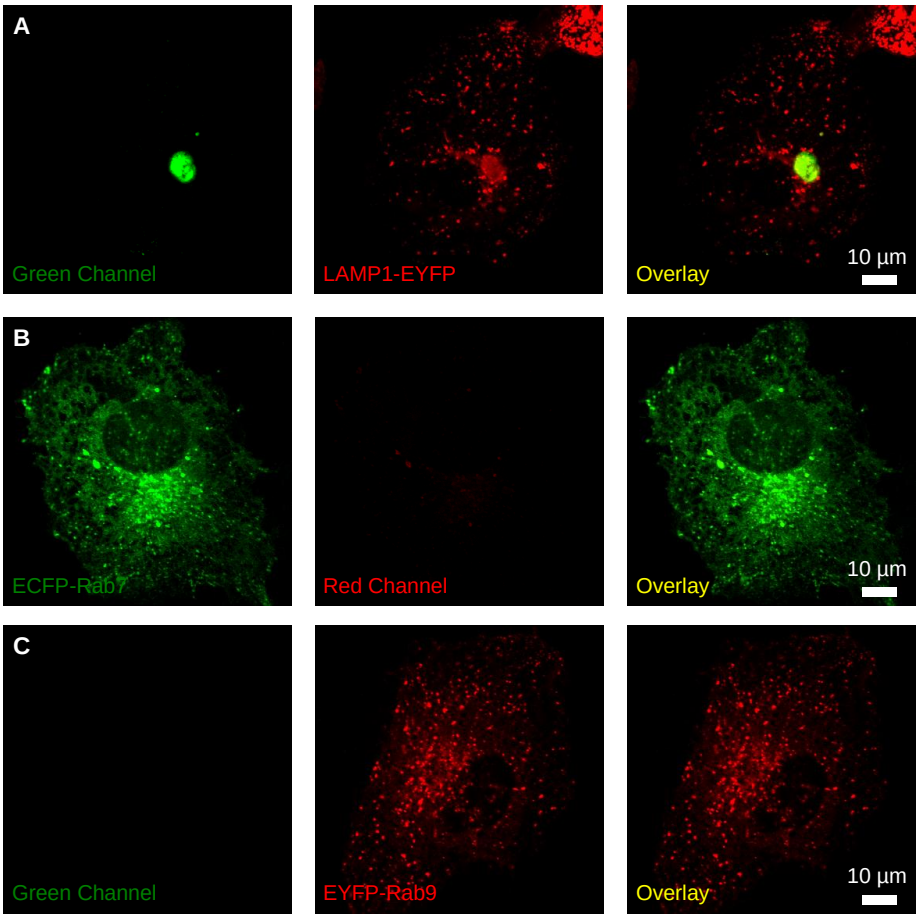
A
Green Channel
LAMP1-EYFP
Overlay
10 µm
B
ECFP-Rab7
Red Channel
Overlay
10 µm
C
Green Channel
EYFP-Rab9
Overlay
10 µm

Fig. S1

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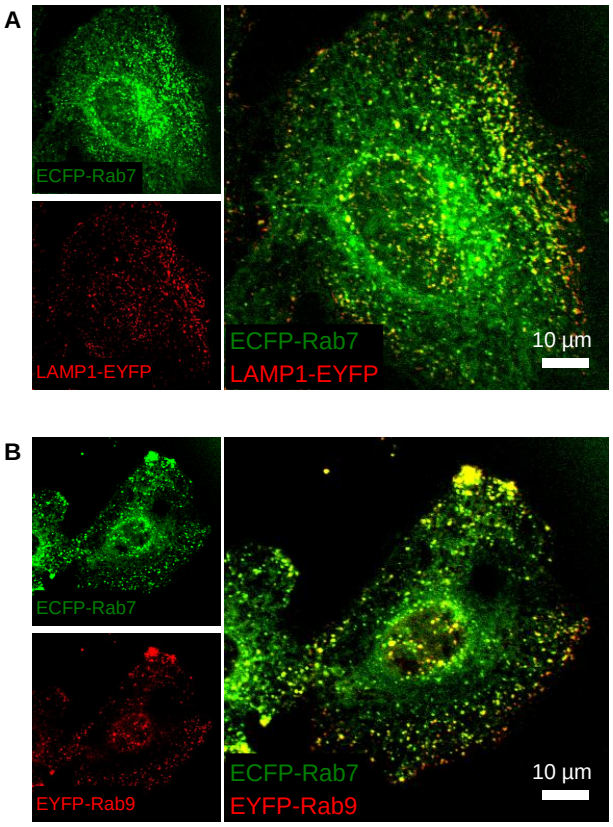

Fig. S2

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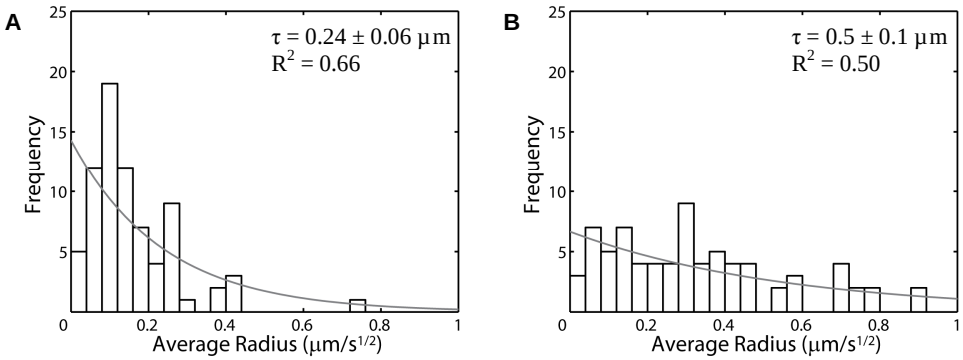

Fig. S3