# Supplemental Materials

***Molecular Biology of the Cell***

Hirst et al.

# Supplemental Material

**Supplemental Figure S1.** The epsinR knocksideways does not cause rerouting of other proteins associated with CCVs. A mixed population of epsinR-FKBP-expressing cells, from which endogenous epsinR had been depleted using siRNA, and wild-type cells, were treated with rapamycin and labelled for either AP-1 γ, GGA2, or clathrin heavy chain (CHC17). None of these proteins shows colocalisation with Mitotrap. Scale bar: 20 μm.

**Supplemental Figure S2.** Depletion of hydrolases and their receptors (CDMPR, CIMPR, and sortilin) from the CCV fraction in the gadkin and epsinR knocksideways. The gadkin knocksideways depleted hydrolases and their receptors equally (~3-fold), whereas the epsinR knocksideways depleted the hydrolase receptors more strongly (*$p < 0.05$; ns, not significant).

**Supplemental Figure S3.** Colocalisation of CCV cargo and machinery with Mitotrap in the gadkin knocksideways. (A) Wild-type and gadkin-FKBP/Mitotrap-expressing cells were mixed, treated with rapamycin, and labelled with antibodies against endogenous ATP7A, ATP7B, or GGA2; alternatively, the cells were transfected with an mCherry-tagged transmembrane construct with a cytoplasmic tail derived from carboxypeptidase D (CPD). All of these proteins partially relocate to the cell periphery in the knocksideways cells. (B) Cells were pretreated with rapamycin, then allowed to endocytose labelled transferrin for the indicated length of time. Although at early time points, the endocytosed transferrin did not colocalise with Mitotrap, by 30 minutes there was substantial colocalisation at the cell periphery. Scale bars: 20 μm.

**Supplemental Figure S4.** Stills from Movies M5 and M6, showing rapamycin induced movement of mCherry-CIMPR and mCherry-CPD in cells coexpressing gadkin-FKBP and Mitotrap.

**Supplemental Figure S5.** Lack of effect of gadkin knocksideways on non-CCV cargo, clathrin, and other APs. (A) Cells expressing gadkin-FKBP and Mitotrap were mixed with non-expressing HeLa cells, treated with rapamycin, and labelled with antibodies against non-CCV cargo proteins localised to early endosomes (EEA1), late endosomes and lysosomes (LAMP1), the Golgi stack (GM130), and the TGN (myc-tagged sialyl transferase, a TGN resident protein), as well as with antibodies against clathrin heavy chain (CHC17), AP-2, and AP-3. Scale bar: 20 μm. (B) Cells expressing gadkin-FKBP and Mitotrap were transfected with a clathrin light chain (CLC construct tagged with mCherry, then treated with rapamycin for the indicated length of time. After 30 min, the Mitotrap can be seen to have accumulated at the cell periphery; however, the clathrin has not followed. Scale bar: 20 μm. (C) Quantification of fold increase in peripheral labelling of selected proteins using automated microscopy. p=0.876 for EEA1; p=0.447 for GM130; ns = not significant.

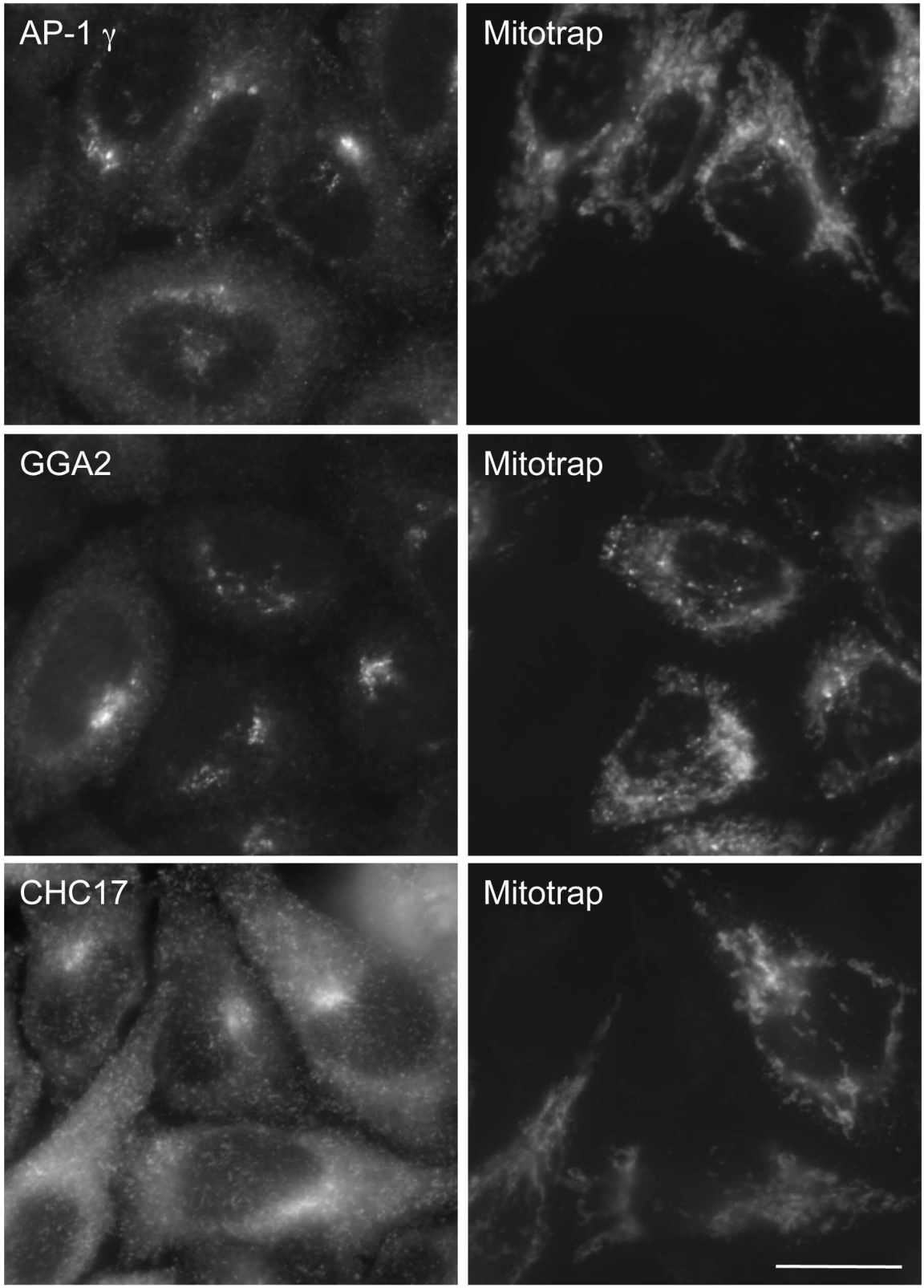
**Supplemental Table S1.** Mass spectrometry data processed using MaxQuant.

**Movies M1-M3.** Electron tomography of the HeLa cell CCVs shown in Figure 3d.

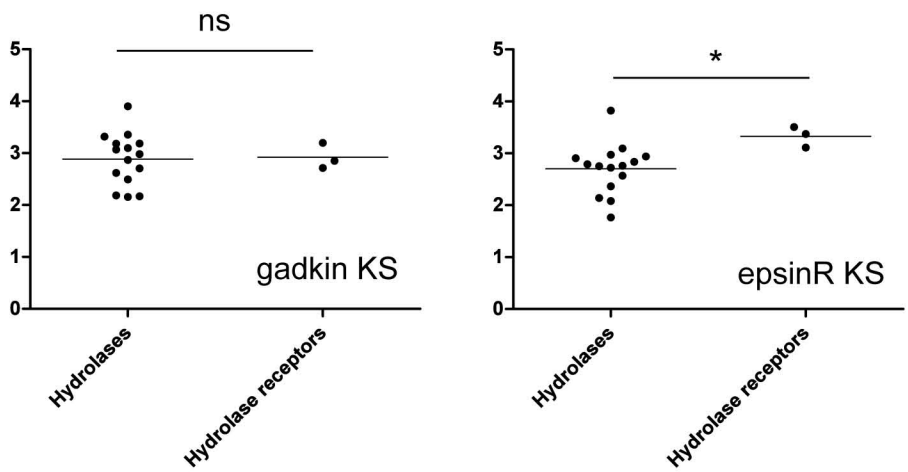
**Movie M4.** Movement of mitochondria out to the cell periphery in gadkin-FKBP-expressing cells upon addition of rapamycin. For movies M4-M6, images were captured every 10 seconds for a period of up to 30 minutes, and run at 10 frames per second.

**Movie M5.** Movement of mCherry-CIMPR out to the cell periphery in gadkin-FKBP-expressing cells upon addition of rapamycin.

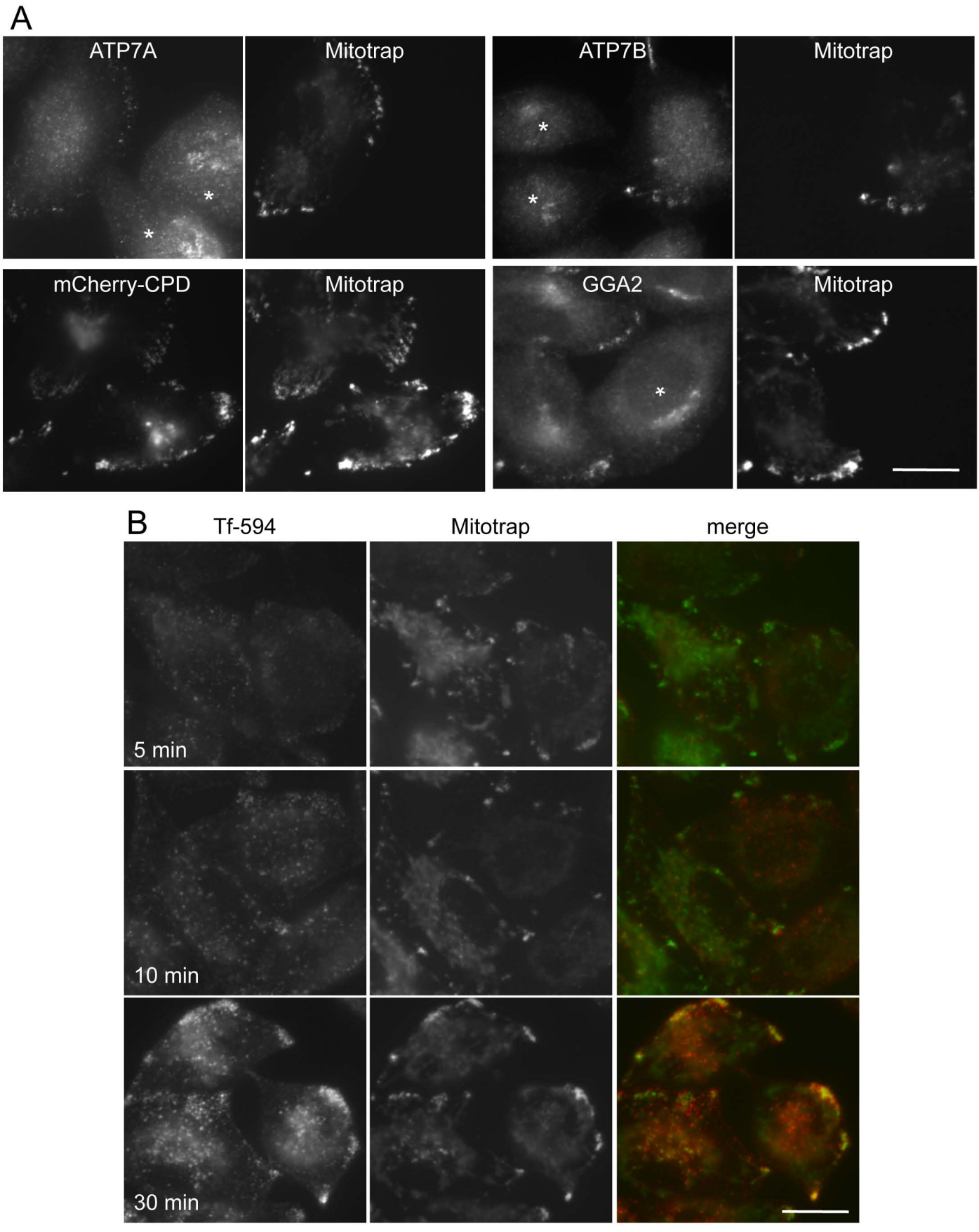
**Movie M6.** Movement of mCherry-CPD out to the cell periphery in gadkin-FKBP-expressing cells upon addition of rapamycin.



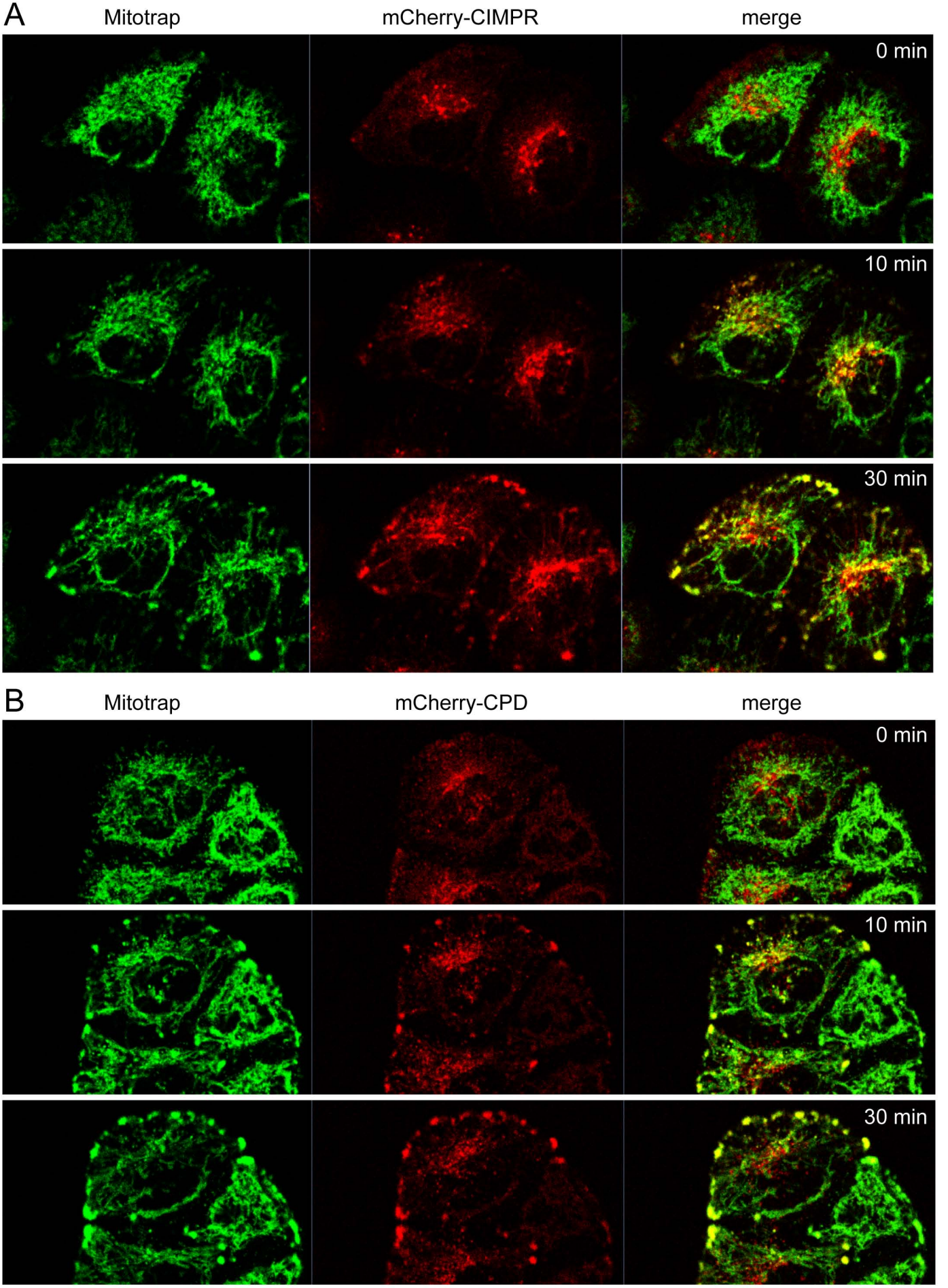

Supplemental Figure S1. Hirst et al.



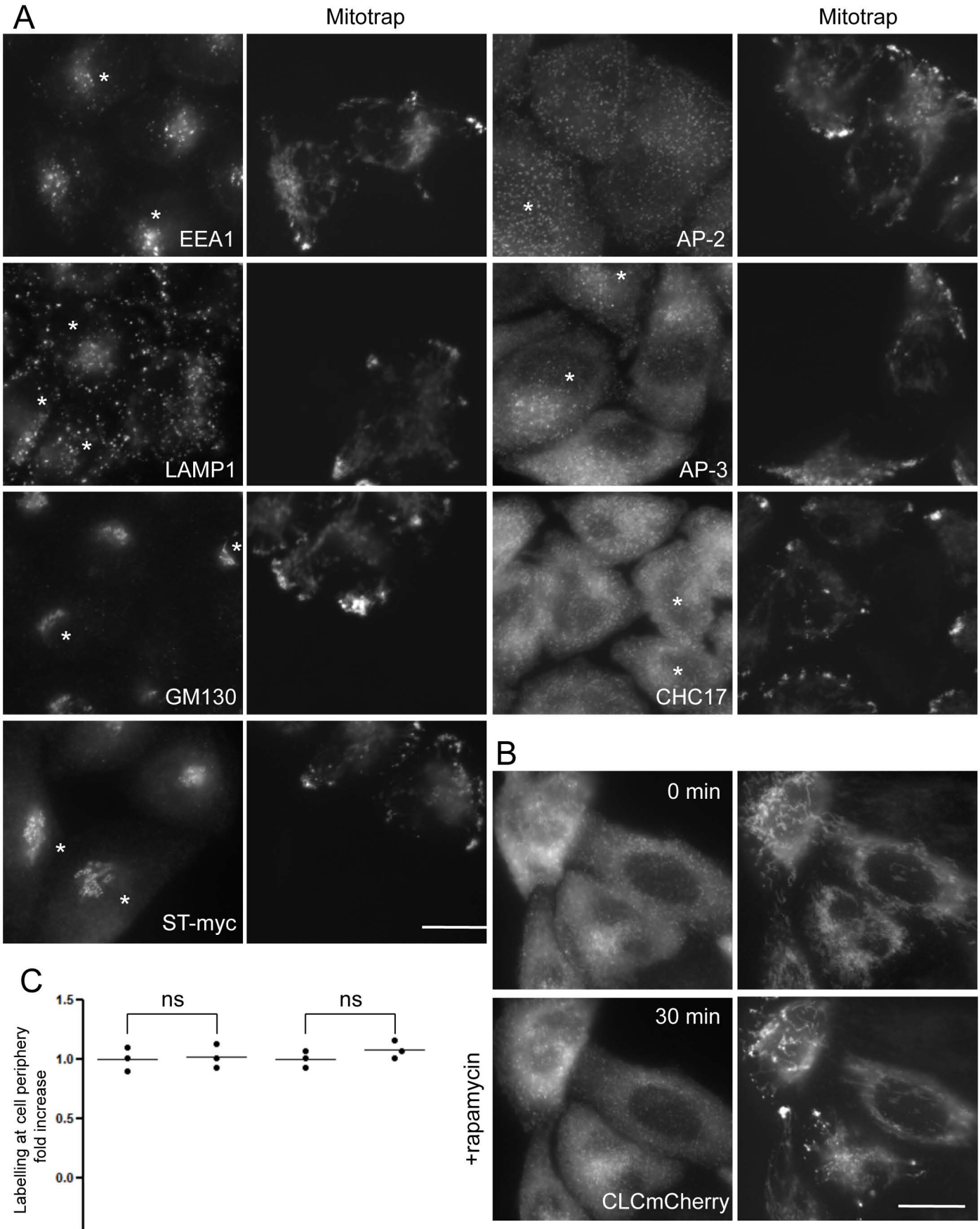

Supplemental Figure S2. Hirst et al.




Supplemental Figure S3. Hirst et al.




Supplemental Figure S4. Hirst et al.




Supplemental Figure S5. Hirst et al.