Supplemental Figure 1. Knockdown of clathrin causes ATP7A mislocalization and inhibits Tf internalization.

(A) clathrin-depleted (CLTC) or control HeLa cells transfected with non-silencing siRNA (-ve) were treated with BCS and incubated at 37 °C with Alexa Fluor 633-Tf prior to fixation (e;f, g;h pink). Cells depleted of clathrin are identified by loss of clathrin heavy chain signal (c;d, g;h red). Cells are counterstained for ATP7A (a;b, g;h green). For quantification of ATP7A and Tf in clathrin-depleted cells see supplemental figure 2.

(B) HeLa cells silenced for clathrin expression were treated with BCS, $CuCl_2$ or $CuCl_2$ followed by washout. Cells silenced for clathrin are identified by accumulation of Alexa Fluor 594-Tf at the cell surface (a-c, g-i, red). Cells are counterstained for ATP7A (d-f, g-i, green). In merge panels examples of clathrin-depleted cells are marked *. Cells with no detectable clathrin knockdown act as internal negative controls and examples are marked with an arrow. Scale bar 10μm.

Supplemental Figure 2. Inhibition of ATP7A endocytosis in HeLa cells depleted of clathrin.

HeLa cells, subjected to clathrin heavy chain RNAi (CLTC) or cells transfected with non targeting siRNA (–ve) were allowed to uptake Alexa Fluor 633-Tf (red) prior to processing for IF and detection of ATP7A (green) or clathrin heavy chain (not shown). From the binary image the area occupied by fluorescence was measured in 3000-4000 depleted/control cells and adjusted so the read-out corresponds to area of fluorescence per cell. The chart represents data normalized against the control and therefore clathrin-depleted data is expressed as a fold increase. In control cells ATP7A (or Tf) staining is compact, whereas a significant increase in the area of fluorescence is observed in depleted cells due to the redistribution of ATP7A (or Tf). * $p<0.0001$ vs -ve control, ** $p<0.01$ vs -ve control.

Supplemental Figure 3. Silencing of CLTC and AP-2 inhibits endocytosis of the CD8/ATP7A C-terminal chimera.

(A) Hela cells treated with non-targeting siRNA (-ve, a, d, g, j) or siRNA against clathrin heavy chain (CLTC, b, e, h, k) or against AP-2 (c, f, i) are transiently expressing CD8-LL4, a construct in which the CD8 cytoplasmic domain is replaced by the C-terminal 24 amino acids of ATP7A. Internalization of CD8-LL4 was monitored by uptake of the CD8 specific antibody OKT8 (a-c, g-i green). Cells showing knockdown of clathrin or AP-2 were identified by accumulation of Alexa Fluor 633-Tf (d-f, g-i pink) and, for clathrin, by loss of clathrin heavy chain staining (j-k, g-h red). Scale bar 10μm.

(B) The area occupied by OKT8 (or Tf) fluorescence was measured in depleted/control cells from the binary image and adjusted so the read-out corresponds to area of fluorescence per cell. The chart represents data from clathrin or AP-2 depleted conditions, normalized against the control. In control cells OKT8 (or Tf) staining is compact, while in both CLTC and AP-2 depleted cells an increase in the area of fluorescence is observed due to the redistribution of OKT8 (or Tf) caused by the inhibition of internalization. * $p<0.001$ vs -ve control, ** $p<0.01$ vs -ve control.

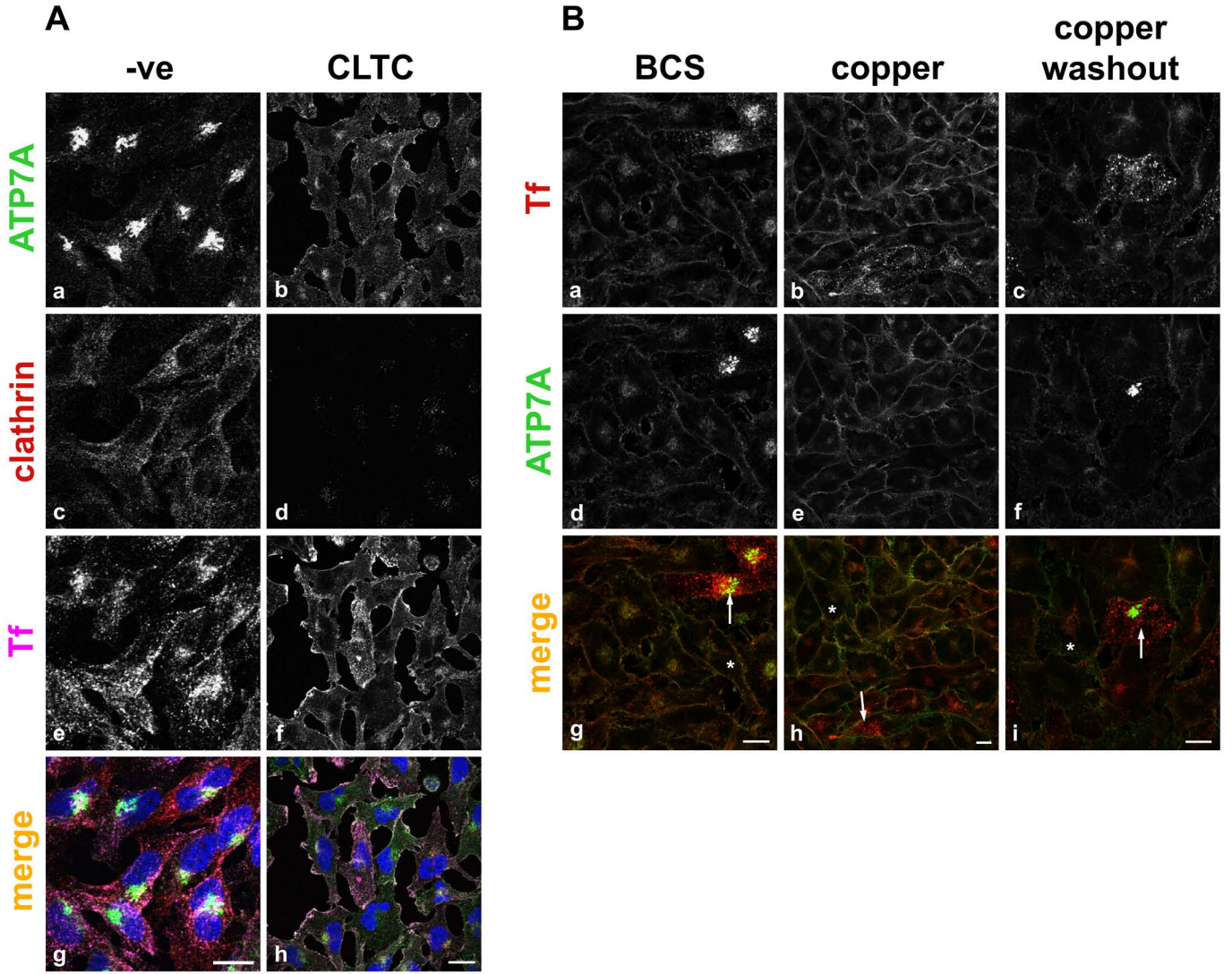
Supplemental Figure 4. Rab11 does not regulate ATP7A trafficking**.**

HeLa cells transiently expressing GFP tagged dominant negative $Rab11a_{S25N}$ or $Rab11b_{S25N}$ or constitutively active $Rab11b_{Q70L}$ were treated with BCS (a-c) or subjected to $CuCl_2$ treatment (d)

and labeled for endogenous ATP7A (red). No effect on ATP7A localization or trafficking is observed in cells expressing the Rab11 mutants. Importantly, ATP7A does not co-localize with the dispersed recycling endosomes containing $Rab11b_{Q70L}$,even after copper treatment. Scale bar 10μm.

Supplemental Figure 5. Expression of dynamin K44A mutants has no effect on ATP7A internalization.
HeLa cells transiently expressing dynamin 1 K44A (panel A) or dynamin 2 K44A (panel B) were treated with BCS, $CuCl_2$ or $CuCl_2$ followed by washout. Cells were incubated with Alexa Fluor 594-Tf (g-l, s-x red) at 37°C prior to washing and fixation. Detection of ATP7A (a-f, s-x green), HA-tagged dynamin 1 K444A (m-o, s-u pink) or myc-tagged dynamin 2 K44A (p-r, v-x pink) is shown. Arrows indicate examples of cells expressing the dynamin mutants. Scale bar 10μm.

Supplemental Figure 6. Dynasore inhibits ATP7A endocytosis following copper treatment and washout.
HeLa cells were treated with $CuCl_2$ followed by washout either in the presence of the dynamin inhibiting drug Dynasore or DMSO only (vehicle). ATP7A is detected using a specific antibody (green). In merge panels DAPI stains the nuclei (blue). Scale bar 10μm.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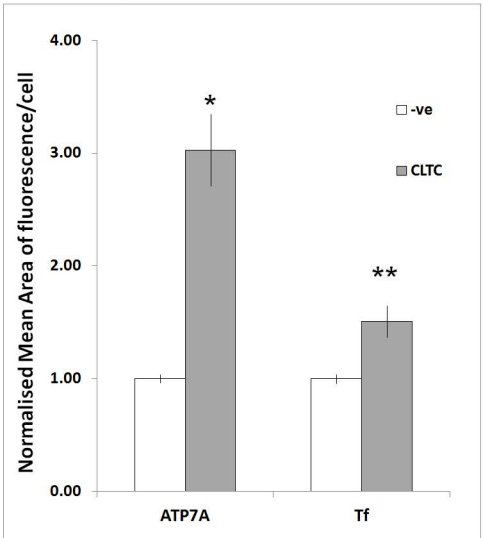
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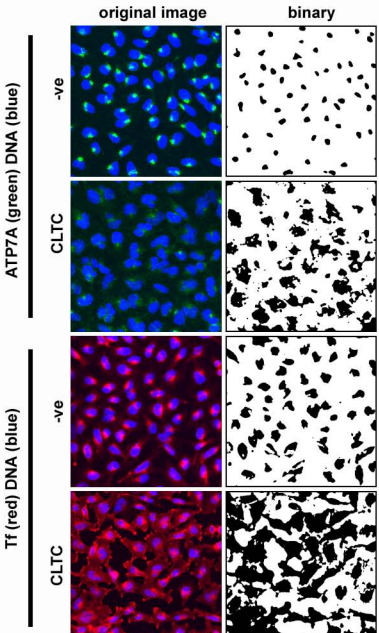
Tf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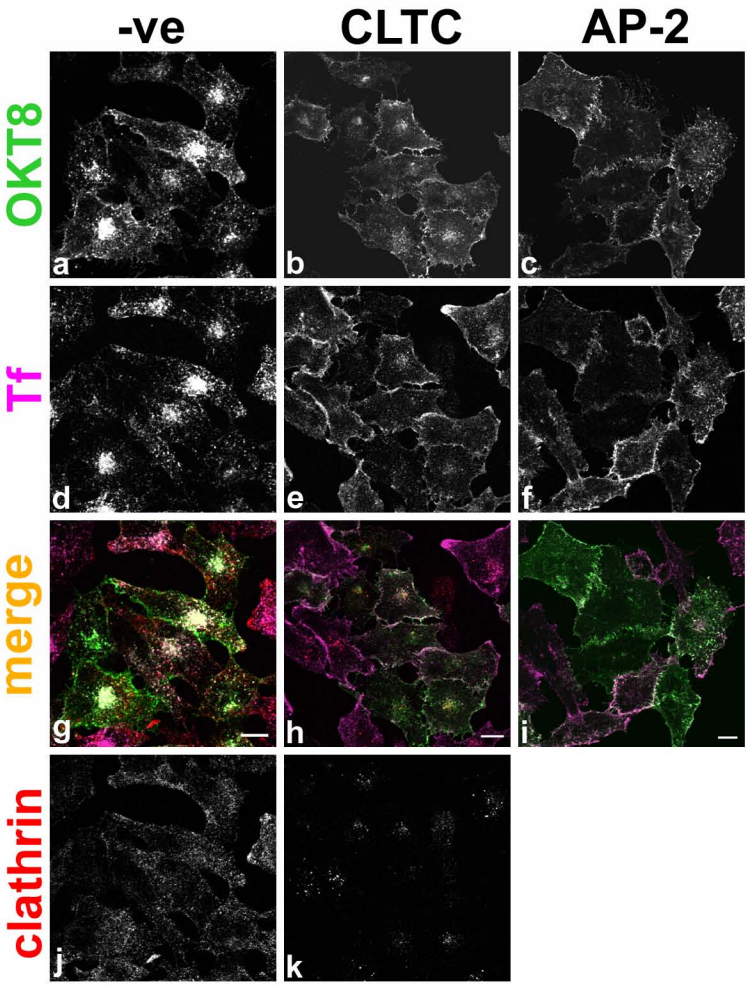
CLTC

A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OKT8
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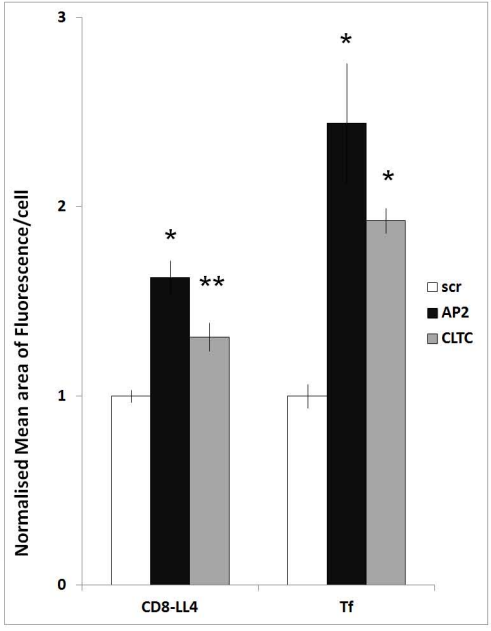
B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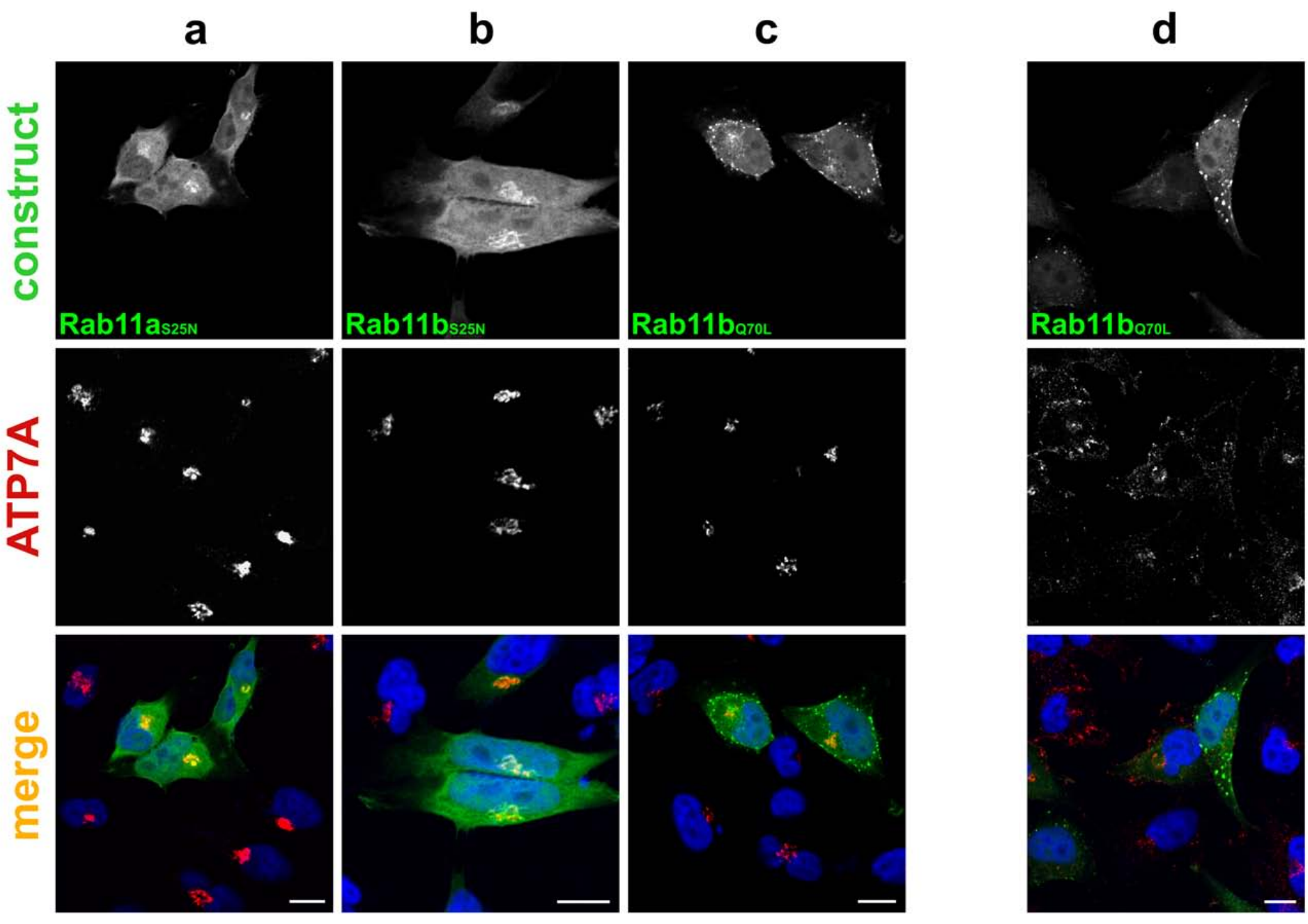
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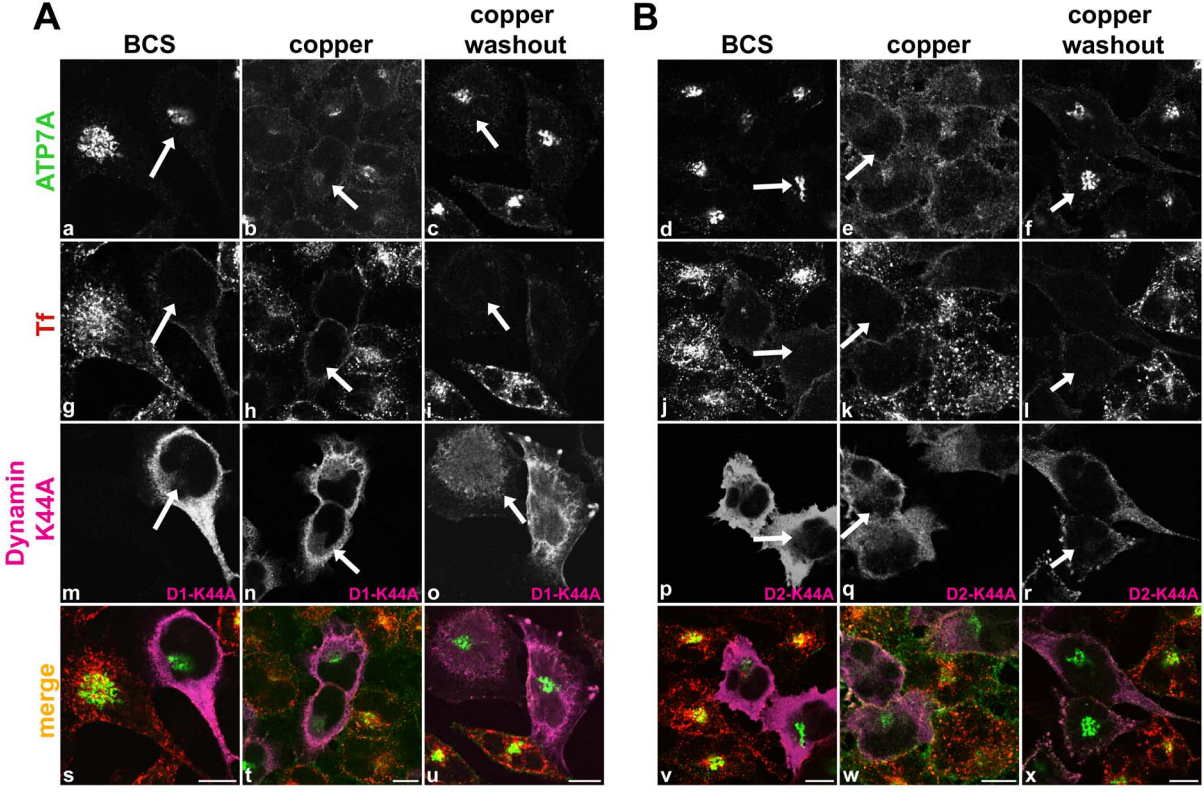
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ATP7A
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Dynamin K44A
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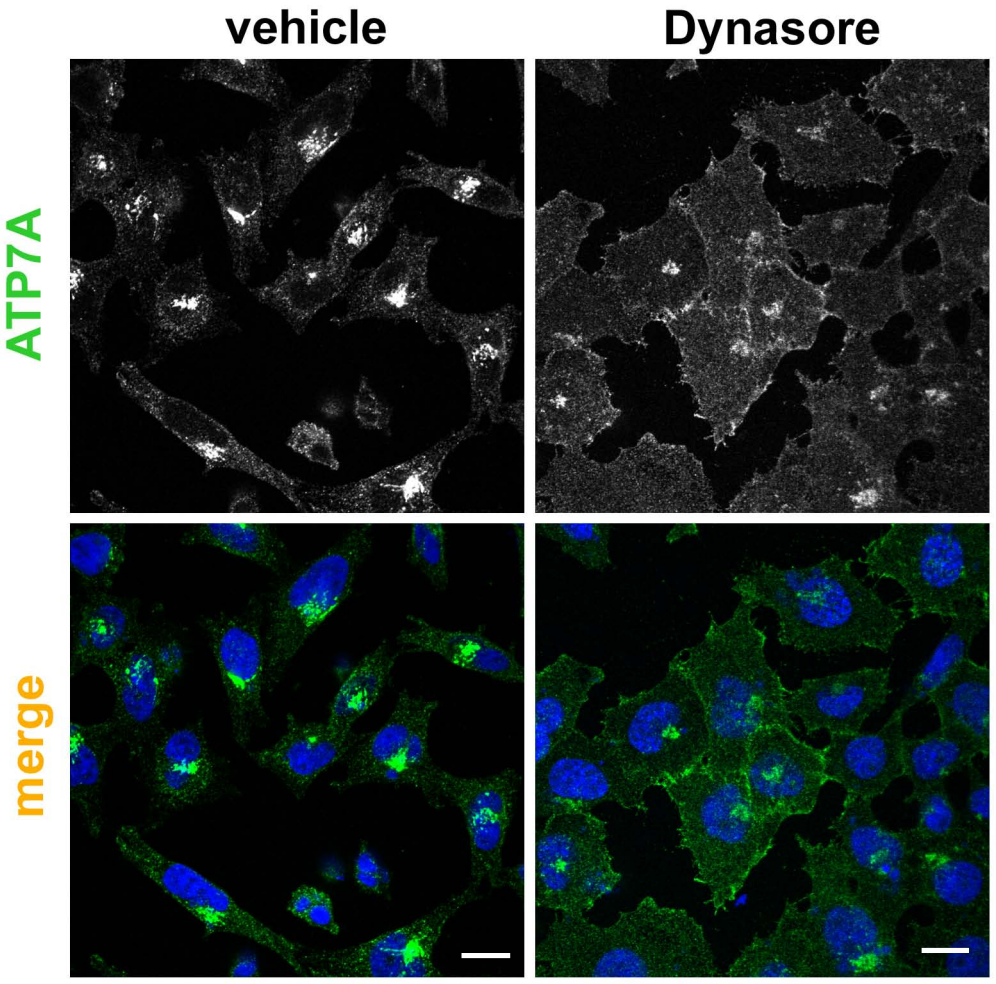
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Dynasore
ATP7A
merge