Expanded View Figures

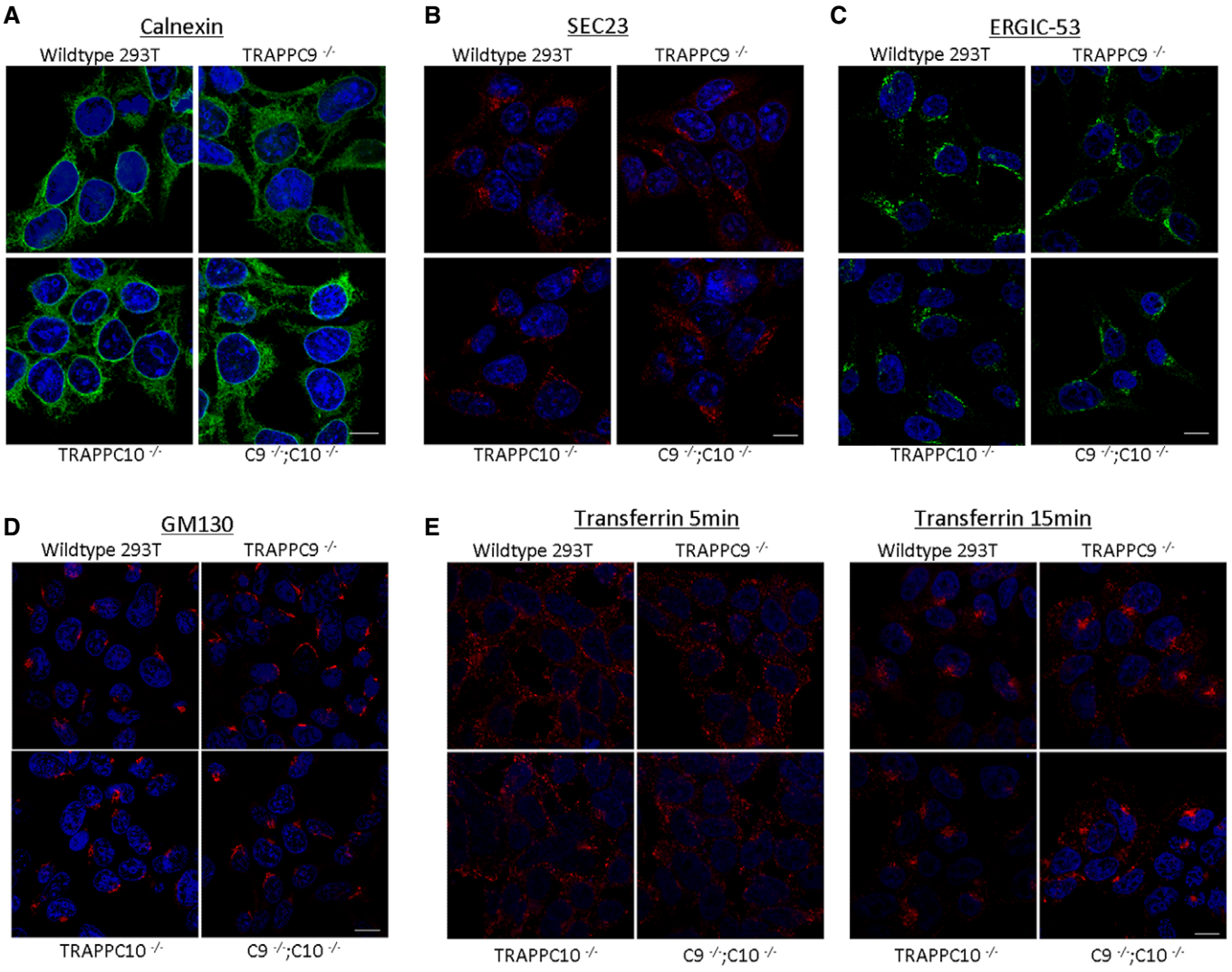

Figure EV1. Deletion of TRAPPII subunits does not affect the morphologies of the indicated cellular organelle markers or inhibit the internalization of transferrin.

Wild-type, TRAPPC9-deleted ($TRAPPC9^{-/-}$), TRAPPC10-deleted ($TRAPPC10^{-/-}$), or TRAPPC9 and TRAPPC10 doubly deleted ($C9^{-/-}$;$C10^{-/-}$) 293T cells were stained with antibodies for the labeled proteins. The cells were counter-stained with DAPI (blue). Scale bars = 10 μm.

A ER marker calnexin.
B ER exit site marker Sec23.
C ERGIC marker ERGIC-53.
D Golgi marker GM130.
E Internalization of rhodamine-transferrin (red) for 5 min (left panels) and 15 min (right panels).

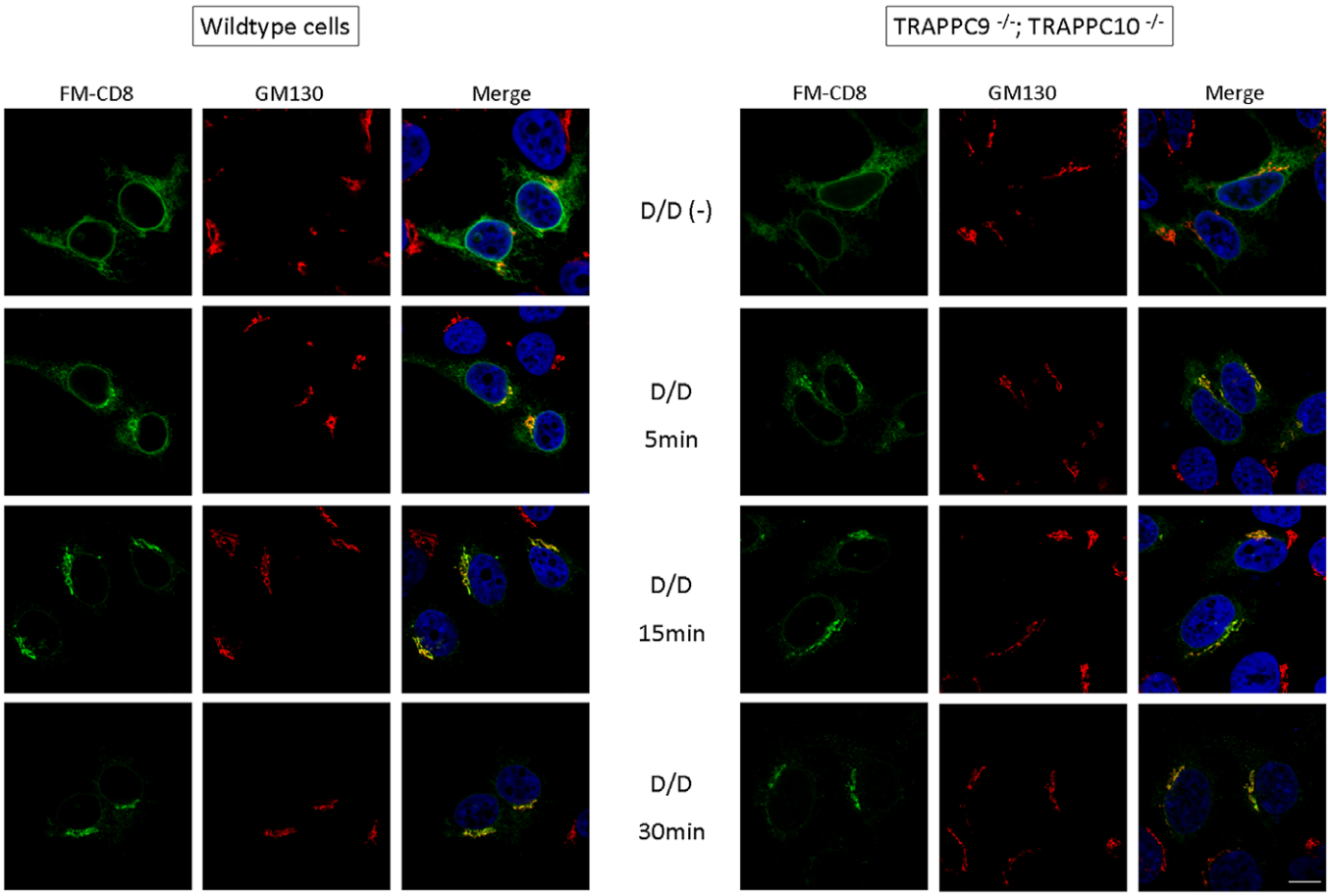

Figure EV2. Deletion of TRAPPII subunits does not affect ER-to-Golgi traffic as monitored by the trafficking of GFP-FM4-CD8 protein.
Transport of FM4-CD8 out of the ER was initiated by applying the disaggregating drug AP21998 (D/D) to a final concentration of 2 μM for the indicated time (Lavieu *et al* 2013). Transport of GFP-FM4-CD8 to the Golgi (GM130) was monitored for up to 30 min in wild-type and TRAPPII-deleted (TRAPPC9$^{-/-}$;TRAPPC10$^{-/-}$) HEK293T cells. Scale bar = 10 μm.

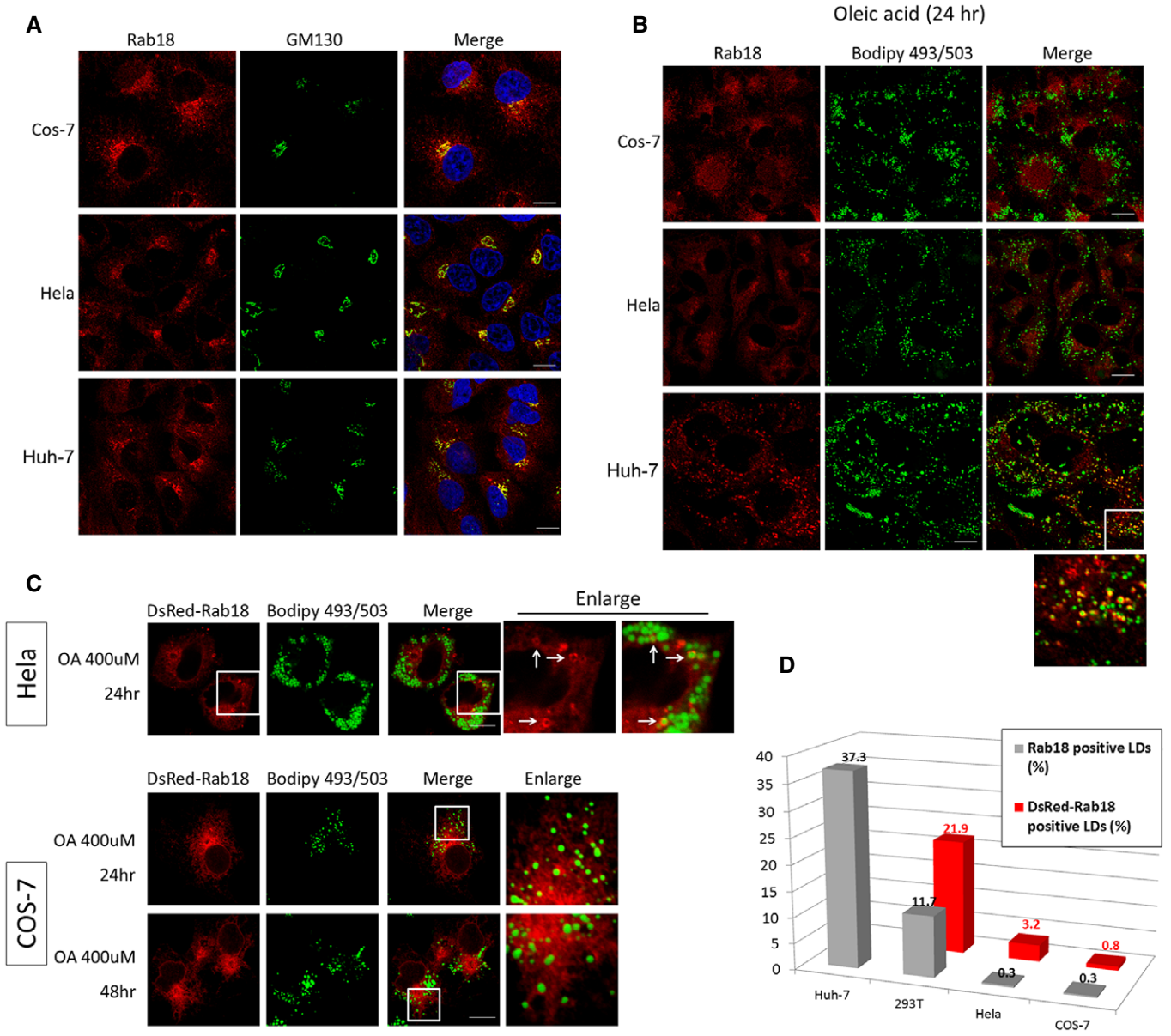

Figure EV3. The ability of Rab18 to be associated with LD surface varies among different cell lines.

A Endogenous Rab18 is enriched in the perinuclear region and colocalized largely with Golgi marker GM130 in cells grown in growth medium. Scale bars = 10 μm.

B The cells were incubated with 400 μM of oleic acid for 24 h before staining with Rab18. Rab18 signals on the Golgi were slightly reduced and became more dispersed throughout the cytoplasm in COS and HeLa cells, but were very poorly colocalized with LDs. In Huh-7 cells, Rab18 signals redistributed to highly punctate structures which partially decorated the LDs. Scale bars = 10 μm.

C Overexpression of DsRed-Rab18 slightly increased its association with LDs in HeLa but not in COS cells even after 48 h of oleic acid loading. Scale bars = 10 μm.

D Statistical quantification of the percentage of LDs decorated with Rab18 in each of the indicated cell lines. Gray bars = endogenous Rab18; red bars = overexpressed DsRed-Rab18. Total number of LD fluorescence dots counted: n = 834, 1041, 670, and 383, for Huh-7, COS, HeLa, and HEK293T, respectively, in the endogenous Rab18 group (gray bars). N = 860, 1119, and 469 for COS, HeLa, and HEK293T, respectively, in the DsRed-Rab18-transfected group (red bars). In each cell sample, the LD fluorescence dots were derived from at least 10 different cells.

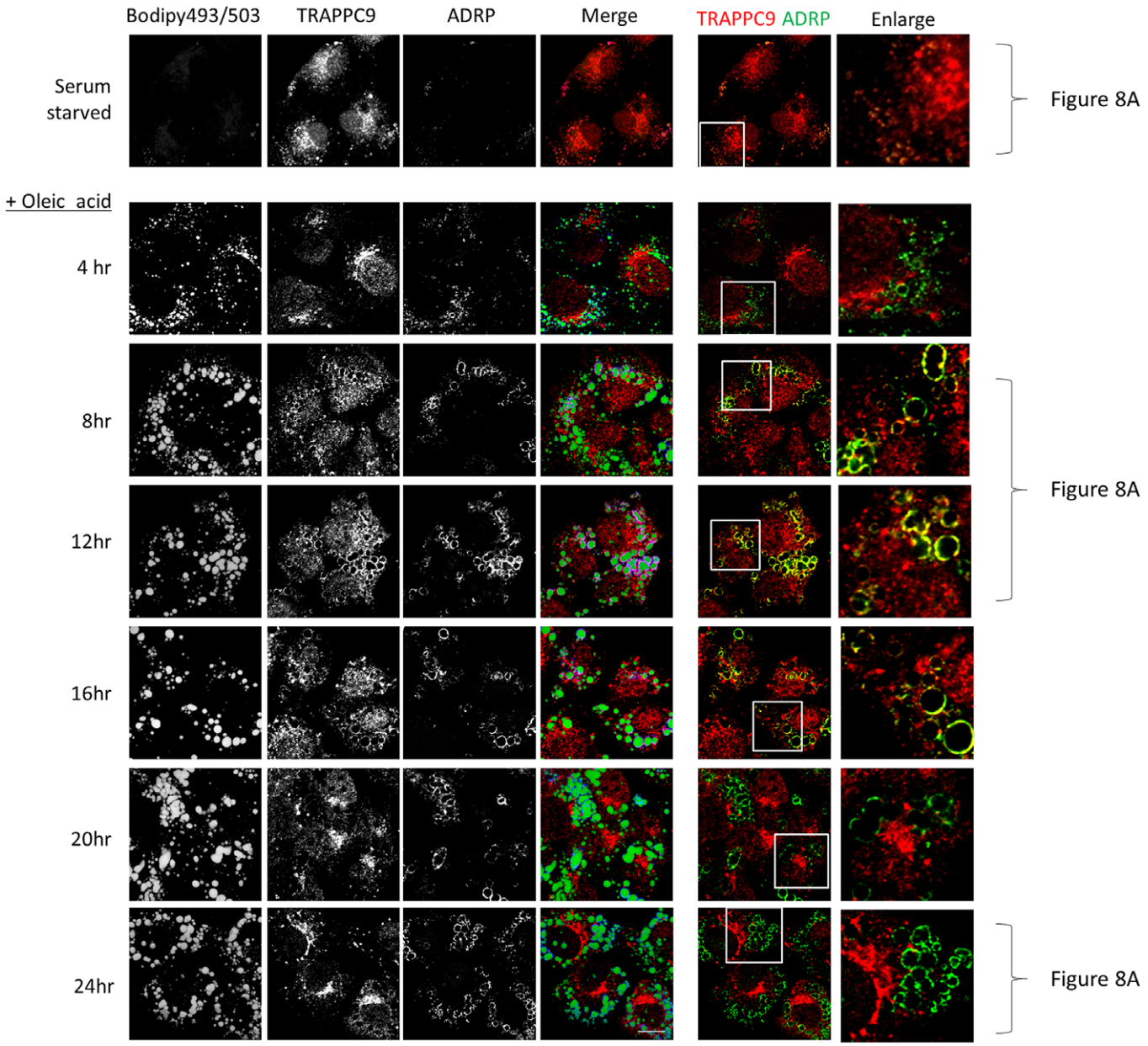

Figure EV4. TRAPPC9 localization on LD surface was investigated as a function of time after oleic acid incubation.
Huh-7 cells were first serum-starved and then incubated with oleic acid for the indicated time before staining with Bodipy 493/503, TRAPPC9, and ADRP. In the merge images, Bodipy 493/503 was pseudocolored in green, TRAPPC9 in red, and ADRP in blue. In the colocalization between TRAPPC9 and ADRP on the right side, the ADRP signal was re-colored to green for easy visualization and overlapped signals around the LDs became yellow. Scale bar = 10 μm.

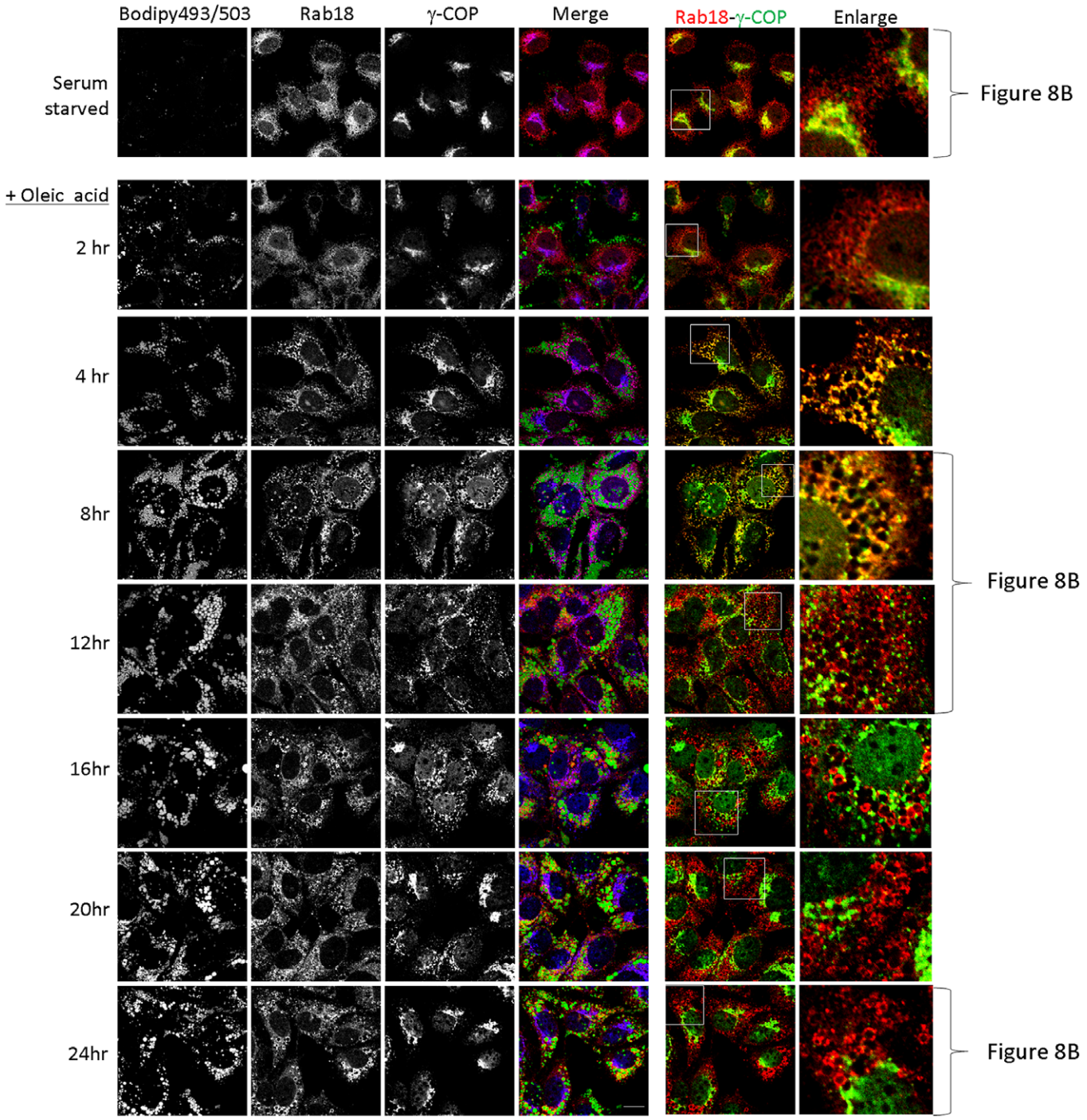

Figure EV5. COPI and Rab18 localizations were investigated as a function of time after oleic acid incubation.
Huh-7 cells were first serum-starved and then incubated with oleic acid for the indicated time before staining with Bodipy 493/503, Rab18, and γ-COP. In the merged images, Bodipy 493/503 was pseudocolored in green, Rab18 in red, and γ-COP in blue. In the colocalization between Rab18 and γ-COP on the right side, the γ-COP signal was re-colored to green for easy visualization. Scale bar = 10 μm.

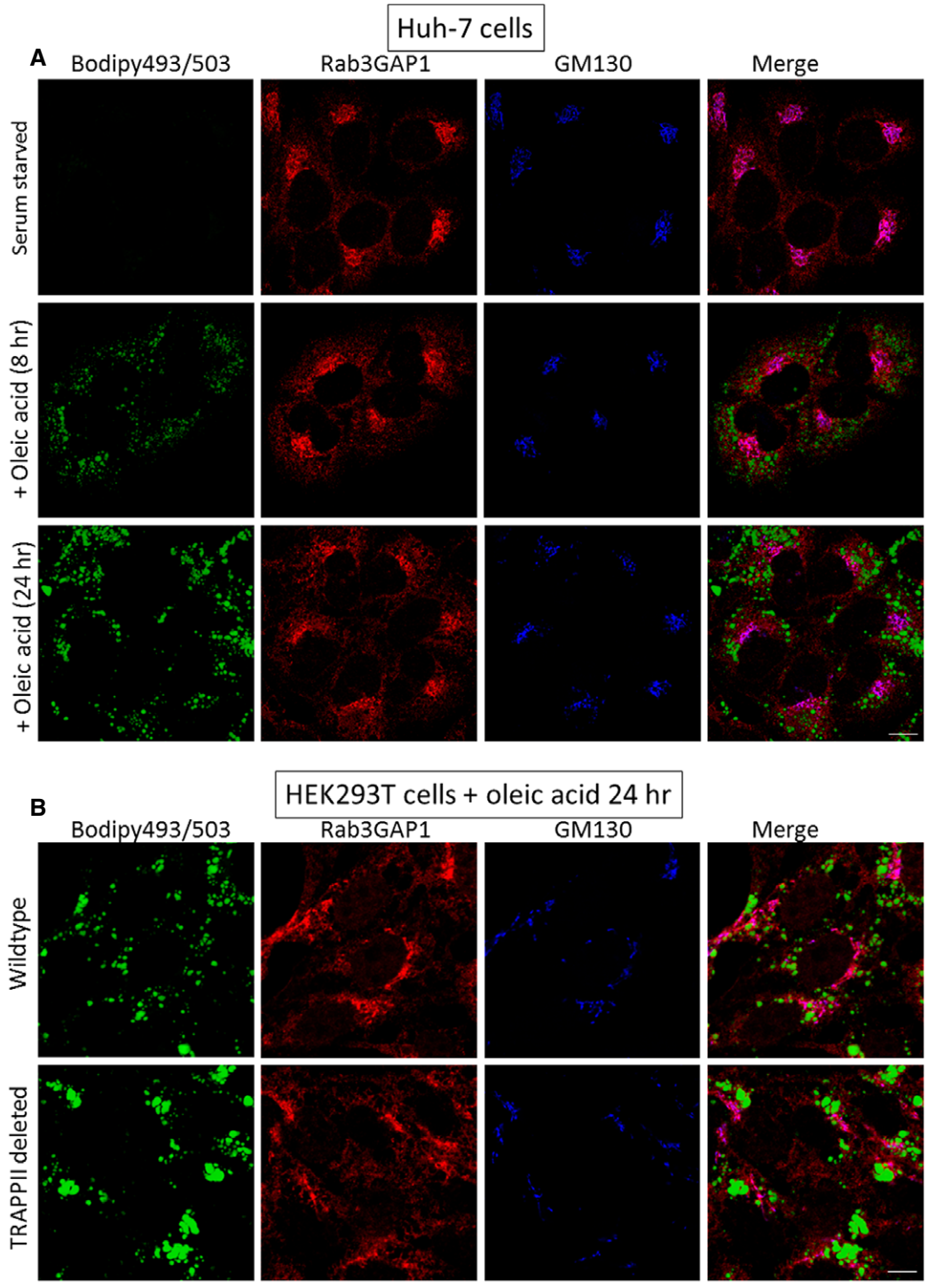

Figure EV6. Subcellular localization of Rab3GAP1 and GM130 in response to oleic acid incubation and TRAPPII deletion.

A Rab3GAP1 and GM130 were stained after Huh-7 cells were serum-starved (top panels), and then incubated with oleic acid for 8 and 24 h. Bodipy 493/503 was pseudocolored in green, Rab3GAP1 in red, and GM130 in blue. Rab3GAP1 signals remained perinuclear and significantly colocalized with Golgi marker GM130 regardless of oleic acid incubation. Scale bar = 10 μm.

B Rab3GAP1 and GM130 were stained after HEK293T cells of indicated genetic background were incubated with oleic acid for 24 h. TRAPPII deletion did not change the perinuclear signal of Rab3GAP1. Rab3GAP1 was not associated with LDs in any of the cells shown. Scale bar = 10 μm.