Appendix

Li et al "COPI-TRAPPII activates Rab18 and regulates its lipid droplet association"

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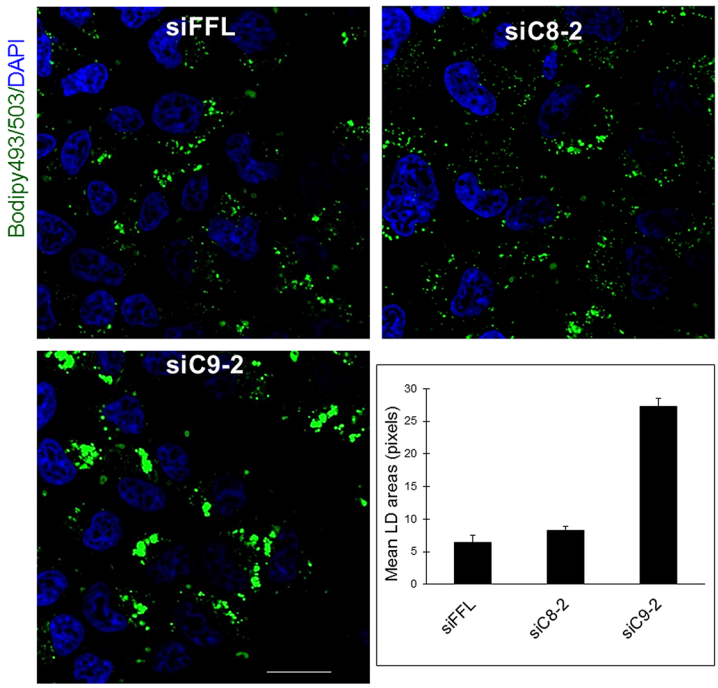
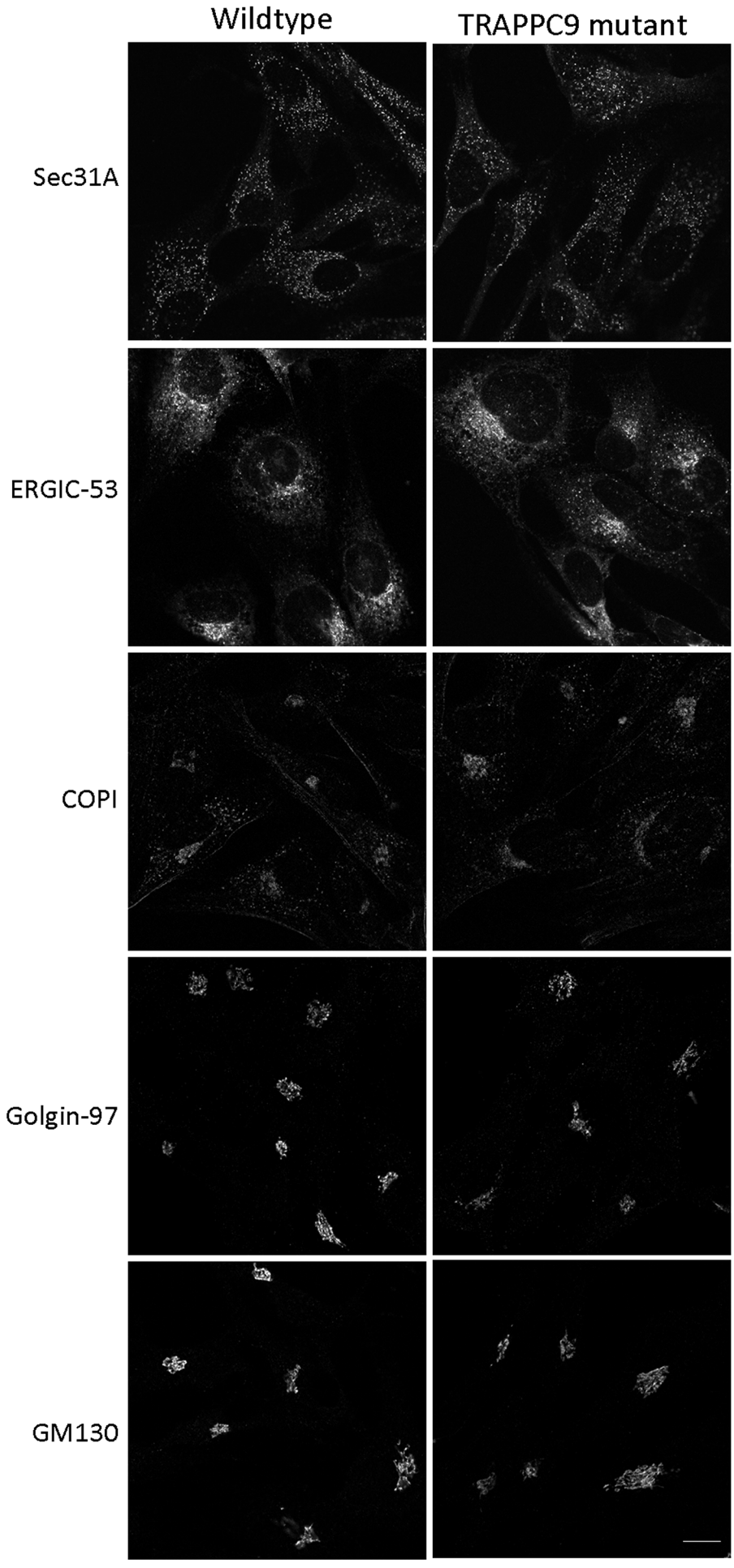
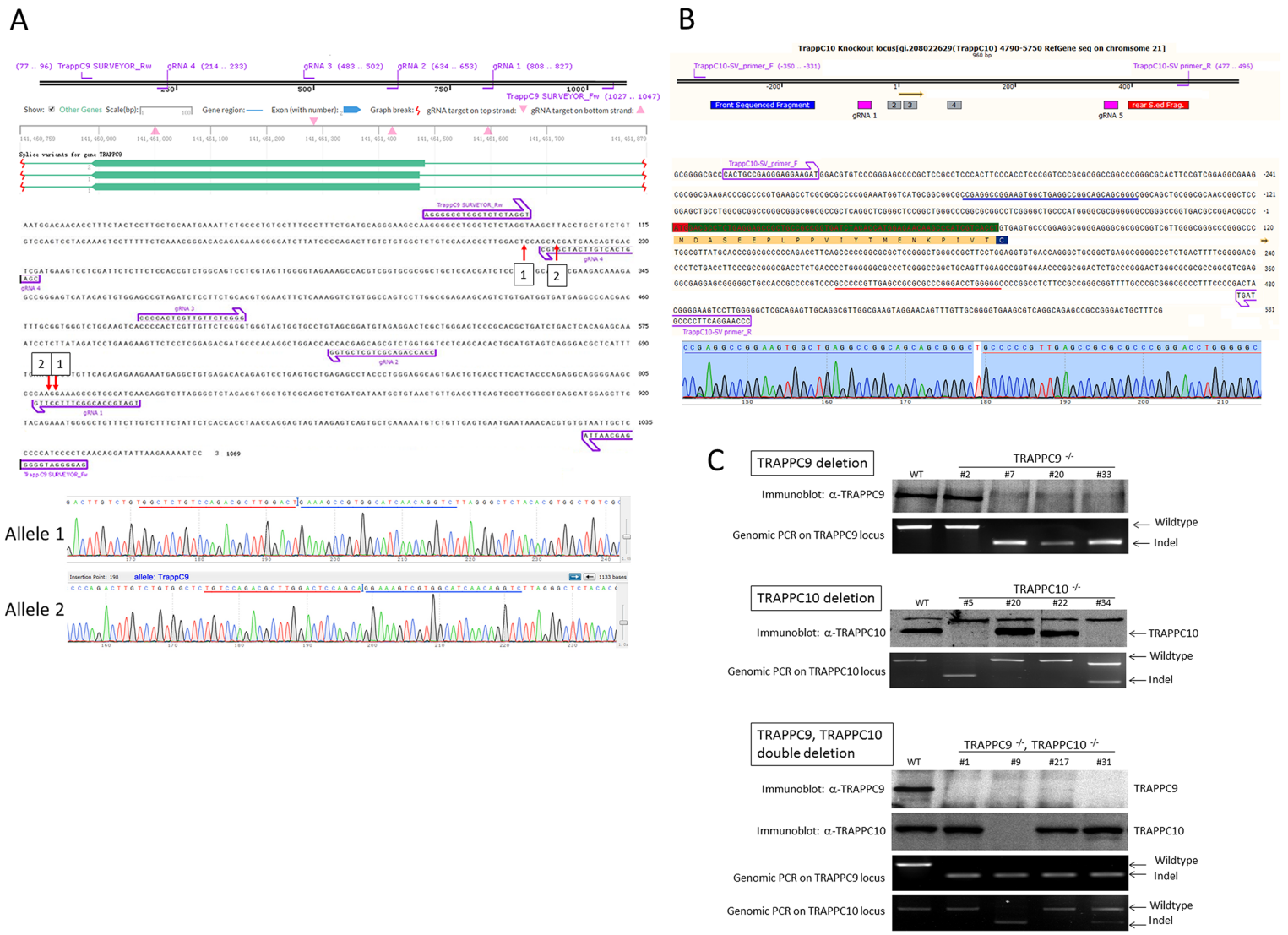


Appendix figure S1

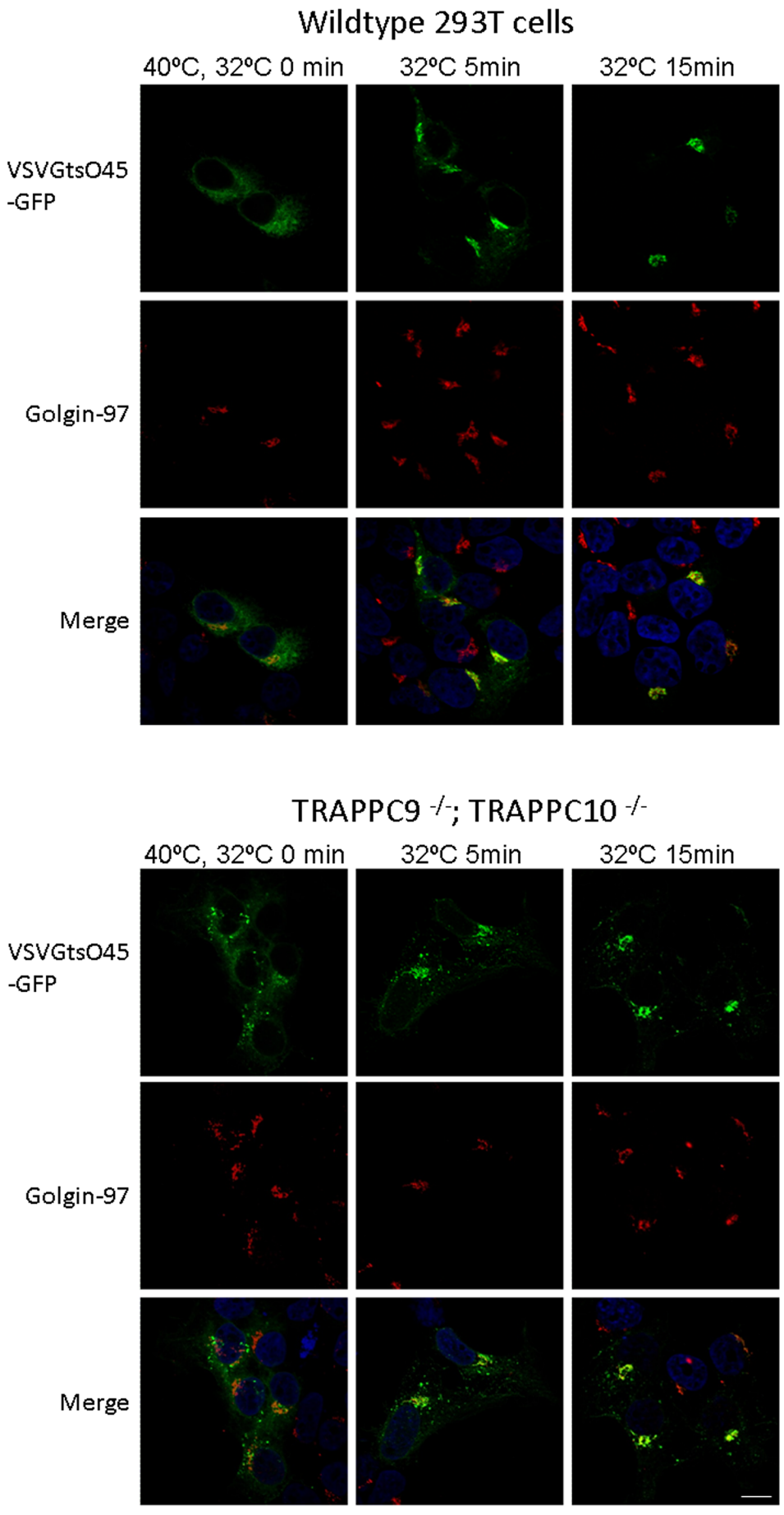


Appendix figure S2

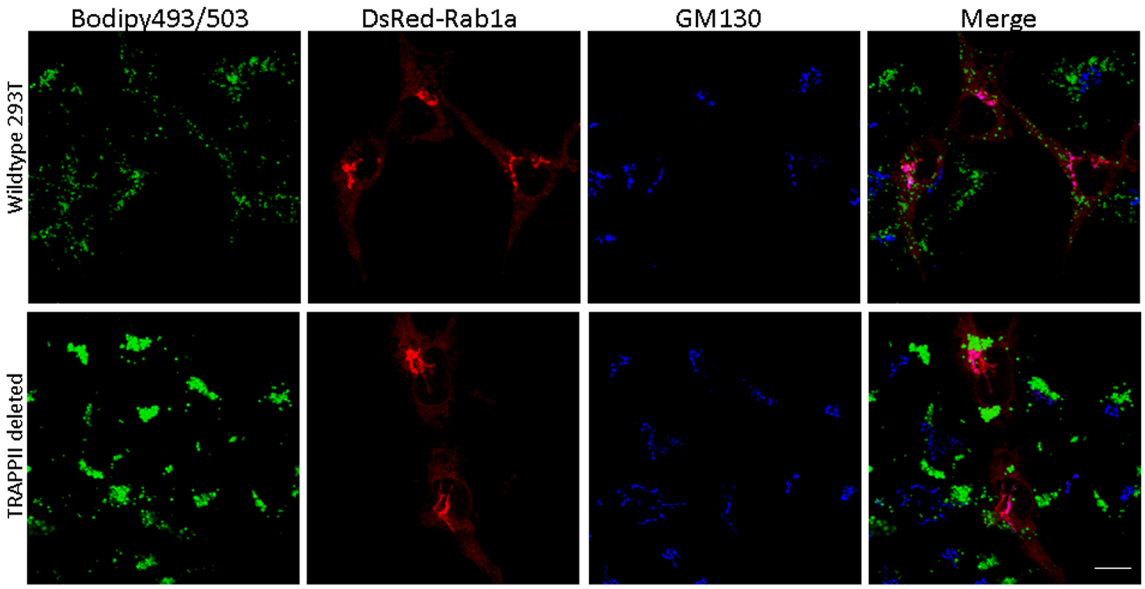
CRISPR-cas9 mediated deletion of TRAPPC9 and TRAPPC10



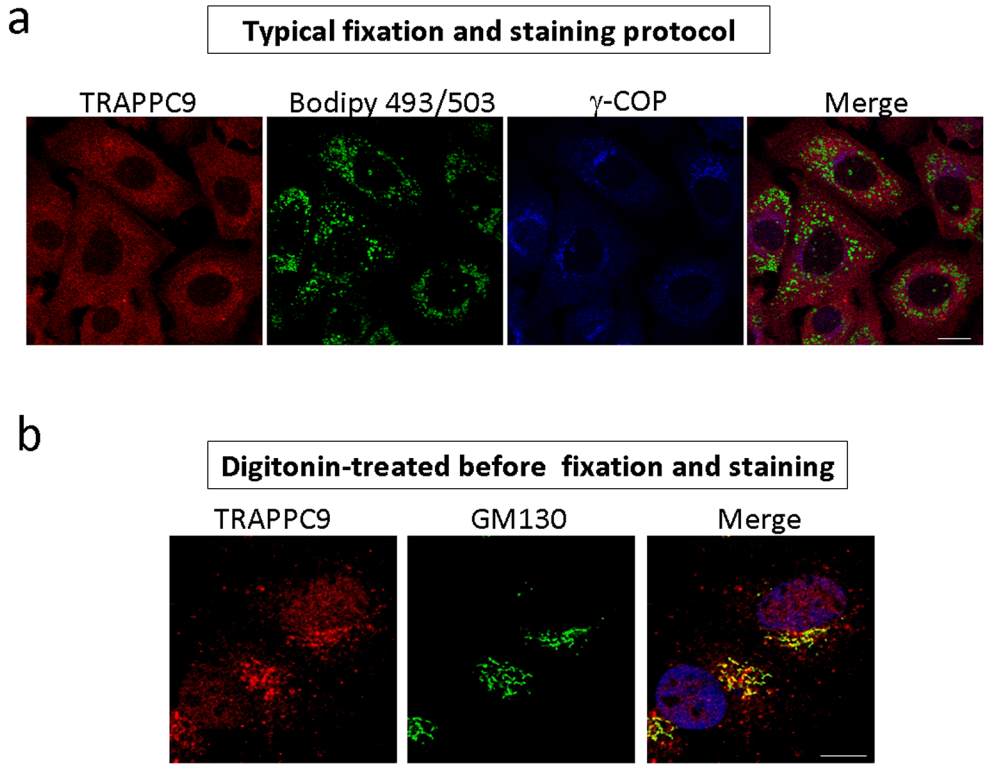
Appendix figure S3



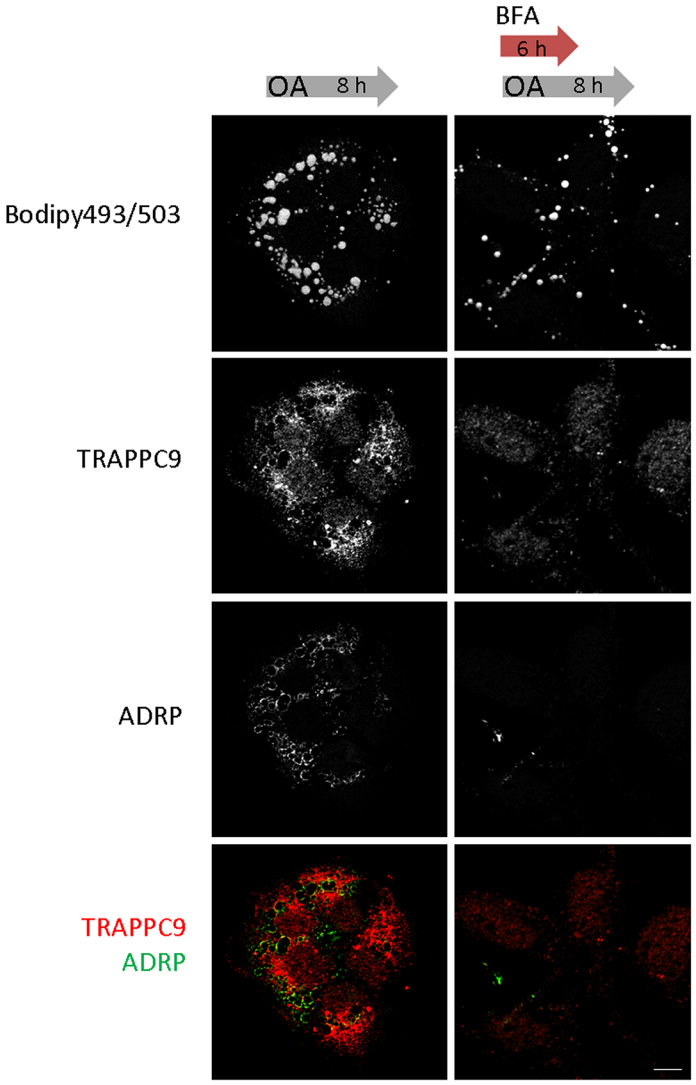
Appendix figure S4



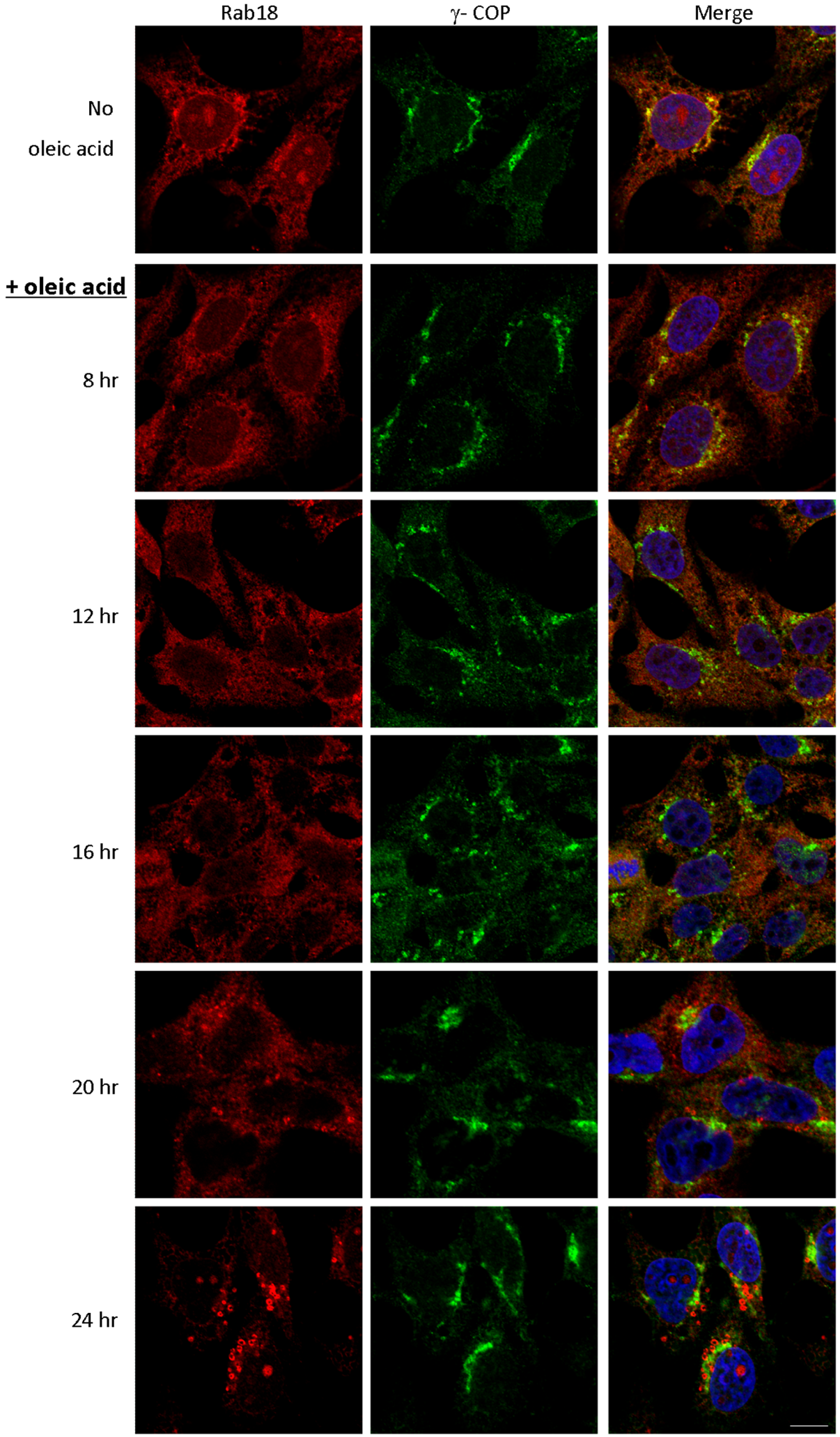
Appendix figure S5



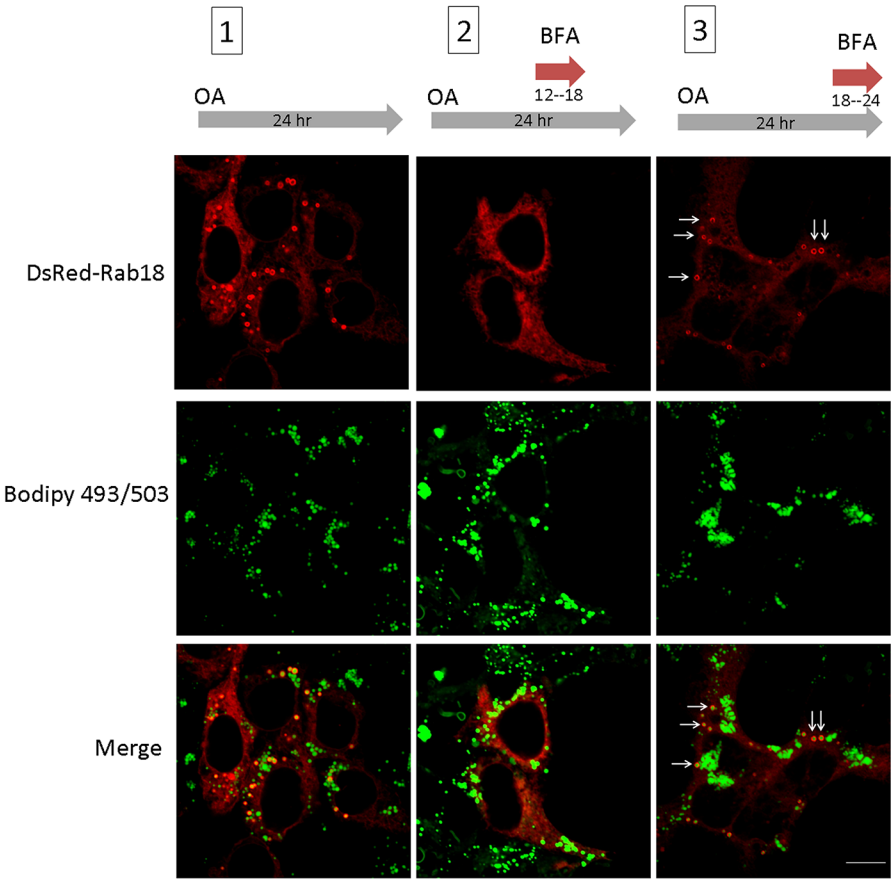
Appendix figure S6



Appendix figure S7



Appendix figure S8



Appendix figure S9

Legends for Appendix figures

Appendix figure S1 - siRNA depletion of TRAPPC9, but not TRAPPC8, caused formation of aberrantly large LDs in Hela cells.
Hela cells were transfected with the indicated siRNA duplexes to deplete TRAPPC8 (si-C8-2) and TRAPPC9 (si-C9-2) or control for firefly luciferase (si-FFL). The cells were loaded with oleic acid for 24 hours before staining with Bodipy493/503. Bar = 10 µm. The average sizes of lipid droplets in each experimental group were quantified (lower right), using the Analyze particles function in ImageJ software. Detailed method follows Ryan et al (Am. J. Hum. Genet. 93(6): 1001-1014 (2013)). For each statistic sample, more than 50 cells from five confocal images were analyzed. Error Bar = S.D.

Appendix figure S2 - The early secretory pathway in TRAPPC9 mutant human skin fibroblasts is indistiguishable from that in wildtype fibroblasts.
TRAPPC9 mutant or wildtype human skin fibroblasts were stained with Sec31A for ER exit sites, ERGIC-53 for intermediate compartments, COPI coatomer, Golgin-97 for trans Golgi, and GM130 for cis Golgi. Bar = 10 µm.

Appendix figure S3 - Deletions of TRAPPC9 and TRAPPC10 in HEK293T cells were carried out by CRISPR-Cas9 method.
A Genomic sequence of the TRAPPC9 and TRAPPC10 gene locus and the guide primers used for deletion and subsequent detection are shown. DNA sequencing of two alleles of the TRAPPC9 gene loci with indels are shown and the sites of deletion for allele 1 and 2 from the sequencing data are indicated by the red arrows in the genomic sequence above.
B TRAPPC10 gene locus and the guide primers used for deletion and subsequent DNA sequencing of a TRAPPC10 knockout clone.
C Candidate cell clones containing the desired deletion were confirmed by genomic PCR and immunoblotting using antibodies specific to TRAPPC9 and TRAPPC10.

Appendix figure S4 - Deletion of TRAPPII subunits does not affect ER to Golgi traffic as monitored by the trafficking of a temperature sensitive mutant VSV-G protein.
Wildtype (top panels) or TRAPPC9 and TRAPPC10 doubly deleted 293T cells (bottom panels) were transfected with VSV-GtsO45-GFP and the transfected cells were incubated at 40°C overnight. ER to Golgi transport of VSV-GtsO45-GFP was initiated when the cells are shifted to 32 °C incubation temperature. At 5 and 15 minutes after the temperature shift, the transport of VSV-GtsO45 to the Golgi was monitored by GFP signal (green) that was colocalized with Golgi marker Golgin-97 (red). Bar = 10 µm.

Appendix figure S5 - Subcellular localization of DsRed-Rab1a in wildtype and TRAPPII deleted HEK293T cells.
DsRed-Rab1a was transfected into wildtype or TRAPPII deleted HEK293T cells. The cells were incubated with oleic acid for 24 hours before fixation and staining with Bodipy493/503 for LD and GM130 for Golgi. Bar = 10 µm.

Appendix figure S6 – Immunofluorescence staining of TRAPPC9.
A Endogenous TRAPPC9 was stained in Huh-7 cells with typical fixation and staining protocol. The cells were first fixed in 3.7 % paraformaldehyde (PFA) and then permeabilized with 0.1 % digitonin before applying antibodies. From this method, TRAPPC9 was observed to be largely cytosolic. Bar = 10 µm.
B The cytosolic pool of TRAPPC9 was depleted with digitonin before PFA fixation. TRAPPC9 was found to be on the Golgi and colocalized with cis-Golgi marker GM130, consistent with its previously reported cis-Golgi localization in CHO cells. Bar = 10 µm.

Appendix figure S7 – LD localization of TRAPPC9 during oleic acid incubation was lost by BFA treatment.
Huh-7 cells were serum starved and then subjected to oleic acid incubation with (right) or without (left) brefeldin A (BFA) for 6 hours. Then BFA was removed and the cells were further incubated with oleic acid for a total of 8 hours, a time frame at which TRAPPC9 signal on LD surface was the strongest. The cells were stained with TRAPPC9 and ADRP (pseudocolored in red and green in the merge images, respectively) using the method described in Appendix figure S6 (i.e. Permeabilized with digitonin before fixation). Bar = 10 µm.

Appendix figure S8 - Subcellular localizations of endogenous Rab18 and γ-COP were investigated as a function of time after oleic acid incubation in HEK293T cells.
HEK293T cells were incubated with 400 µM oleic acid for the indicated time before fixation and staining with Rab18 (red) and γ-COP (green). The nuclei of the cells were counter-stained with DAPI (blue in Merge panels). The perinuclear pattern of Rab18 was reduced and became more cytosolic starting at 12 h after oleic acid incubation. Intense circular structures that were likely encircling LDs appeared after 20 and 24 h. In the 12 and 16 h time points, the perinuclear signal of γ-COP became slightly fragmented but returned to typical perinuclear pattern at 20 and 24 h. The intense Rab18 signals decorating LD surface shown in 24 h time point did not colocalize with the perinuclear γ-COP signals. Bar = 10 µm.

Appendix figure S9 - Recruitment of Rab18 onto LD is dependent on COPI subunit γ-COP at 12 hours after oleic acid incubation.
HEK293T cells were first transfected with DsRed-Rab18. The cells were incubated with oleic acid for a total of 24 hours. Condition 1, the

cells were not treated with BFA. Condition 2, the cells were treated with BFA starting at 12 hours after oleic acid incubation. BFA was removed after 6 hours of incubation. Condition 3, the cells were treated with BFA 18 hours after oleic acid. BFA treatment at 12 h inhibited Rab18 from being recruited onto LD surface. BFA treatment at 18 h could no longer inhibit. Arrows indicate DsRed-Rab18 signals encircling LDs in Condition 3. Bar = 10 μm.