Supplemental Materials

Molecular Biology of the Cell

Makino et al.

SUPPLEMENTAL FIGURE LEGENDS

Suppl. Figure 1. (A) Wild type Huh7 cells (Control), ACAT1 stable knockout cell line derived from Huh7 cells (ACAT1), ACAT2 stable knockout cell line derived from Huh7 cells (ACAT2) and Huh7 cells cultured for 24 h in the presence of 10 μg/ml F1394 (F1394) were labeled for 4 h with [1- ^{14}C] oleic acid as described in **METHODS** to examine cholesterol esterification. (**B**) LD fractions were prepared from ACAT2 stable knockout cell line and lipids analyzed by HPTLC as described in **METHODS**. (**C**) Wild type Huh7 cells (Control), ACAT1 stable knockout cell line derived from Huh7 cells (ACAT1), ACAT2 stable knockout cell line derived from Huh7 cells (ACAT2) and Huh7 cells cultured for 24 h in the presence of 10 μg/ml F1394 (F1394) were then labeled with 2 μg/ml BODIPY 493/503 for 30 min. Bar, 20 μm.

Suppl. Figure 2. (**A**) Huh7 cells were cultured for 24 h in the absence (Control) or the presence (F1394) of 10 μg/ml F1394. Cellular PLIN1 was measured by western blotting using tubulin as a control. (**B**) Cells were cultured as described in (**A**). Cells were then fixed, permeabilized with digitonin and doubly labeled with anti-PLIN1 and anti-PLIN2 antibodies. Bar, 20 μm.

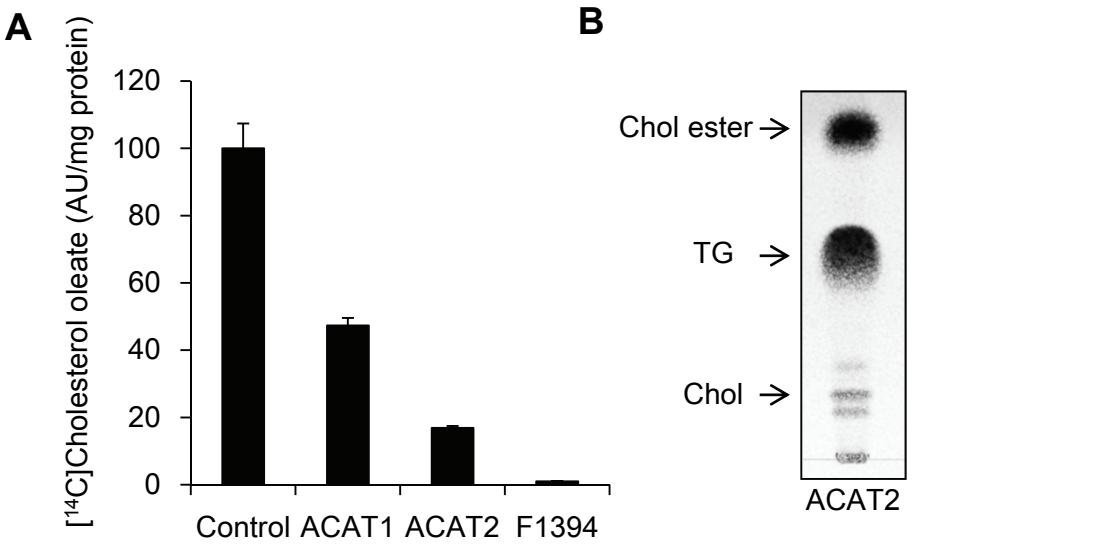
Suppl. Figure 3. (**A**) Huh7 cells were treated with 100 nM BAY13-9952, 10 μg/ml F1394 or both BAY13-9952 and F1394 as described in **METHODS**. Cells were then fixed, permeabilized with digitonin and labeled with anti-ApoB antibody. Bar, 20 μm. (**B**) Cells were treated as described in (**A**) and ApoB in LD fraction was measured by western blotting.

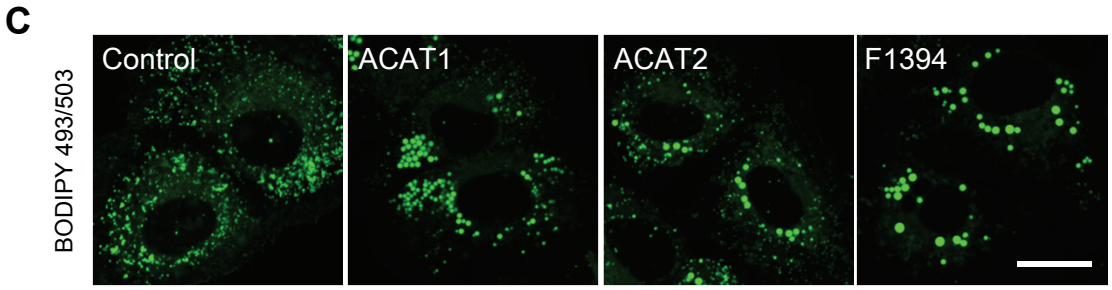
Suppl. Figure 4. Enlarged picture of Figure 7(A) control cell. The squared region was enlarged. Bar, 5 μm.

Suppl. Figure 5. (**A**)(**B**) Huh 7 cells overexpressing mCherry-Rab18 Q67L (**A**) and mCherry-Rab18 S22N (**B**) were treated with F1394 as described in **METHODS.** Cells were doubly labeled with BODIPY 493/503. (**C**) Control Huh 7 cells (lane 1, 2) and cells overexpressing GFP-PLIN2 (3, 4), GFP-Rab18 (5, 6), GFP-Rab18 S22N (7, 8), and GFP-Rab18 Q67L (9, 10) were treated with (2, 4, 6, 8, 10) and without (1, 3, 5, 7, 9) F1394 as described in **METHODS.** Cellular PLIN2 was measured by western blotting as described in **METHODS**. (**D**)(**E**) Huh 7 cells overexpressing mCherry-Rab18 Q67L (**D**) and mCherry-Rab18 S22N (**E**) were treated with F1394 as described above. Cells were then fixed, permeabilized and labeled with anti-PLIN2 antibody. Bar, 10 μm.

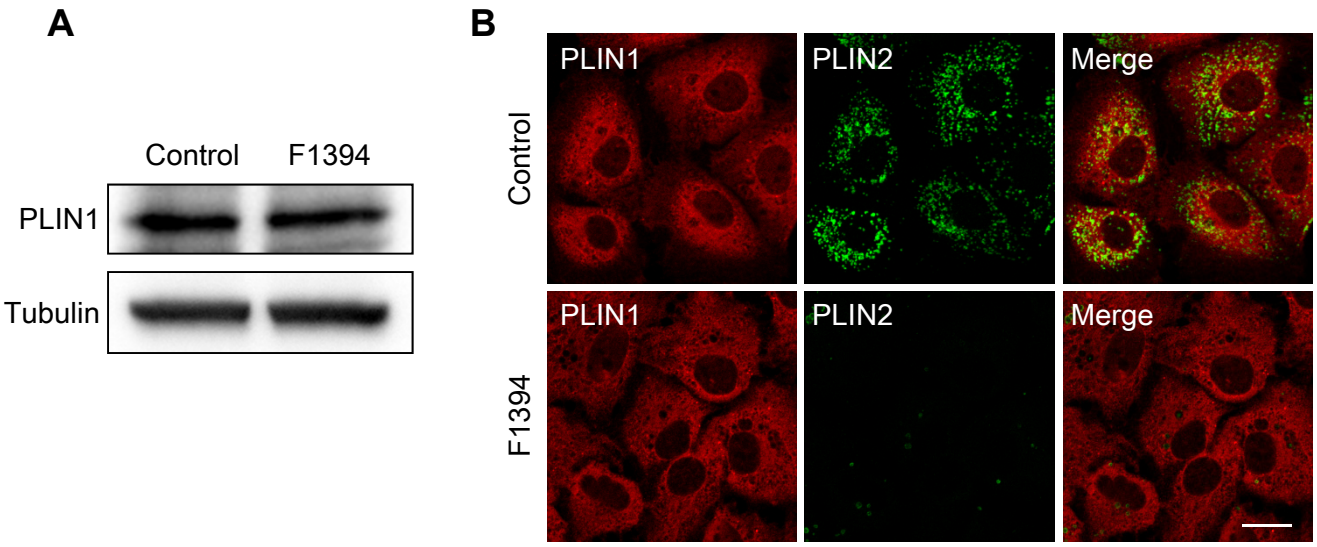
Suppl. Figure 6. Schematic presentation of the effect of free cholesterol on the morphology of LDs. Acute free cholesterol accumulation induces fusion of LDs followed by the degradation of PLIN2. PLIN2 degradation is inhibited by the inhibitors of autophagy, ubiquitination and protein synthesis. LDs then fuse with ER in Rab18 activity-dependent manner.

Suppl Fig. 1. Makino et al.





Suppl Fig. 2. Makino et al.



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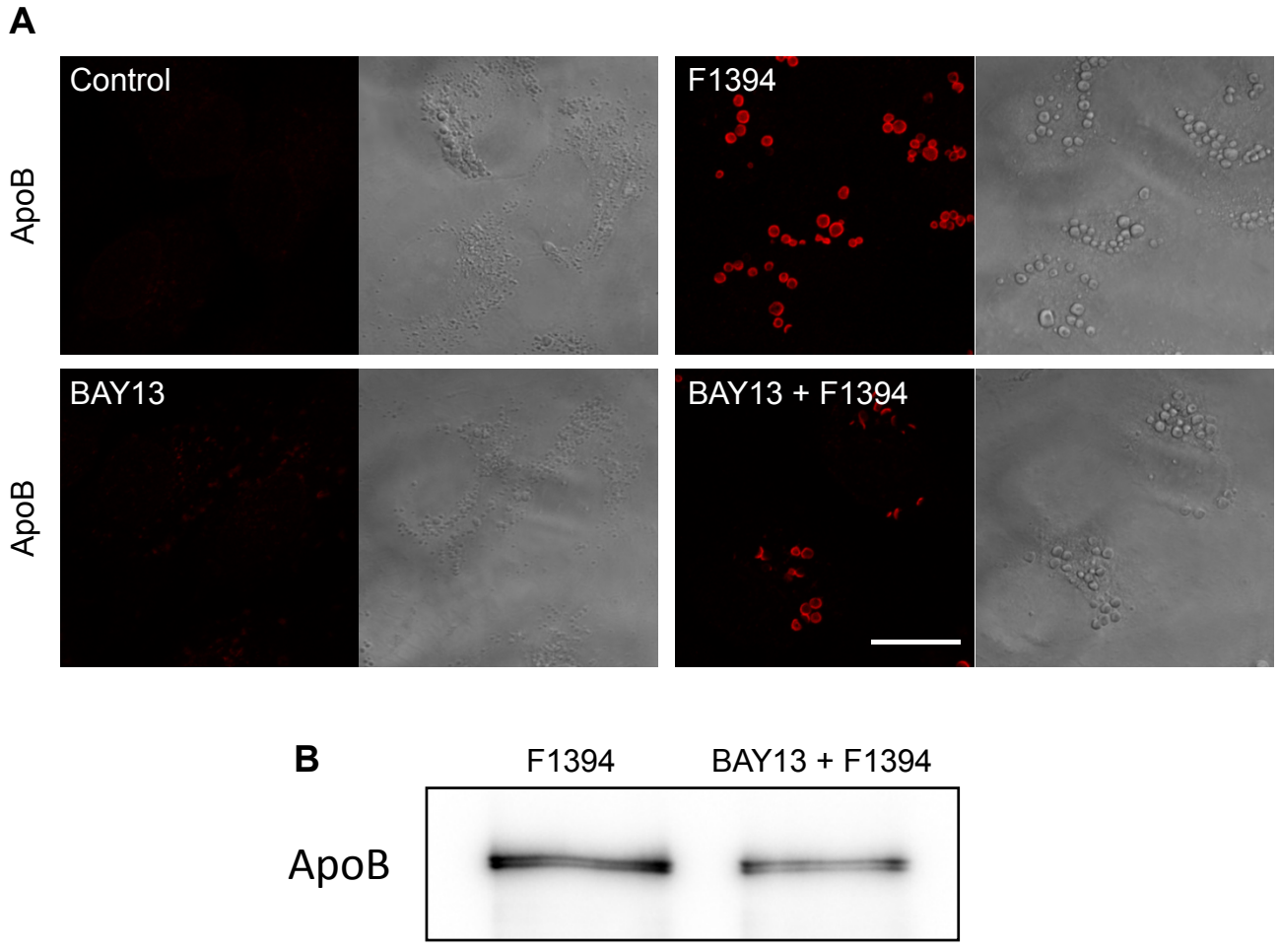

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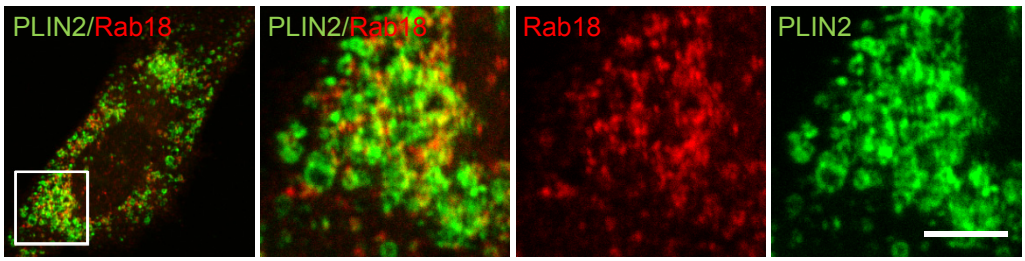
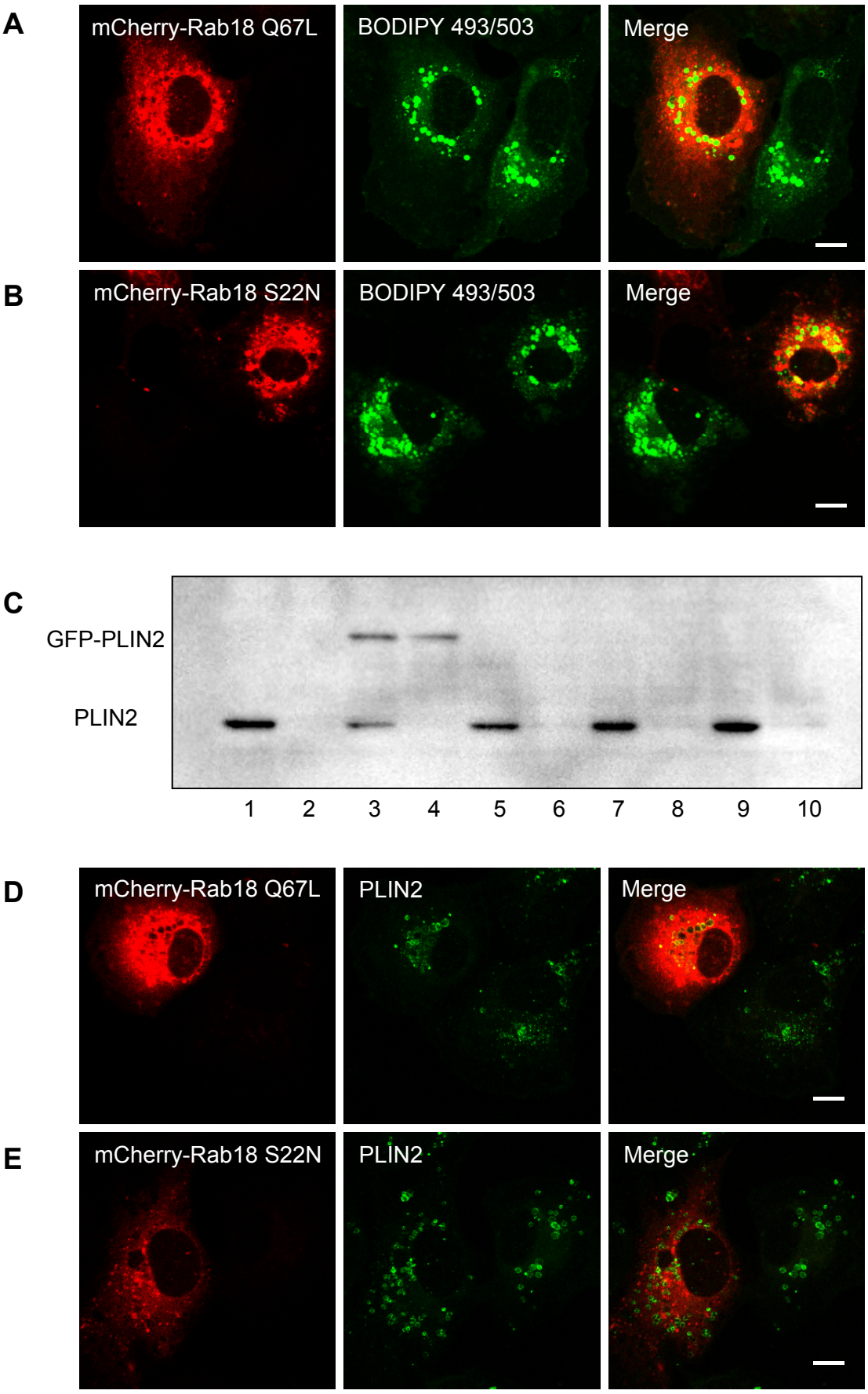


Suppl Fig. 5. Makino et al.



Suppl Fig. 6. Makino et al.