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Supplemental information

Validating an artificial organelle: Studies of lipid droplet-specific proteins on adiposome platform

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Supplemental Information

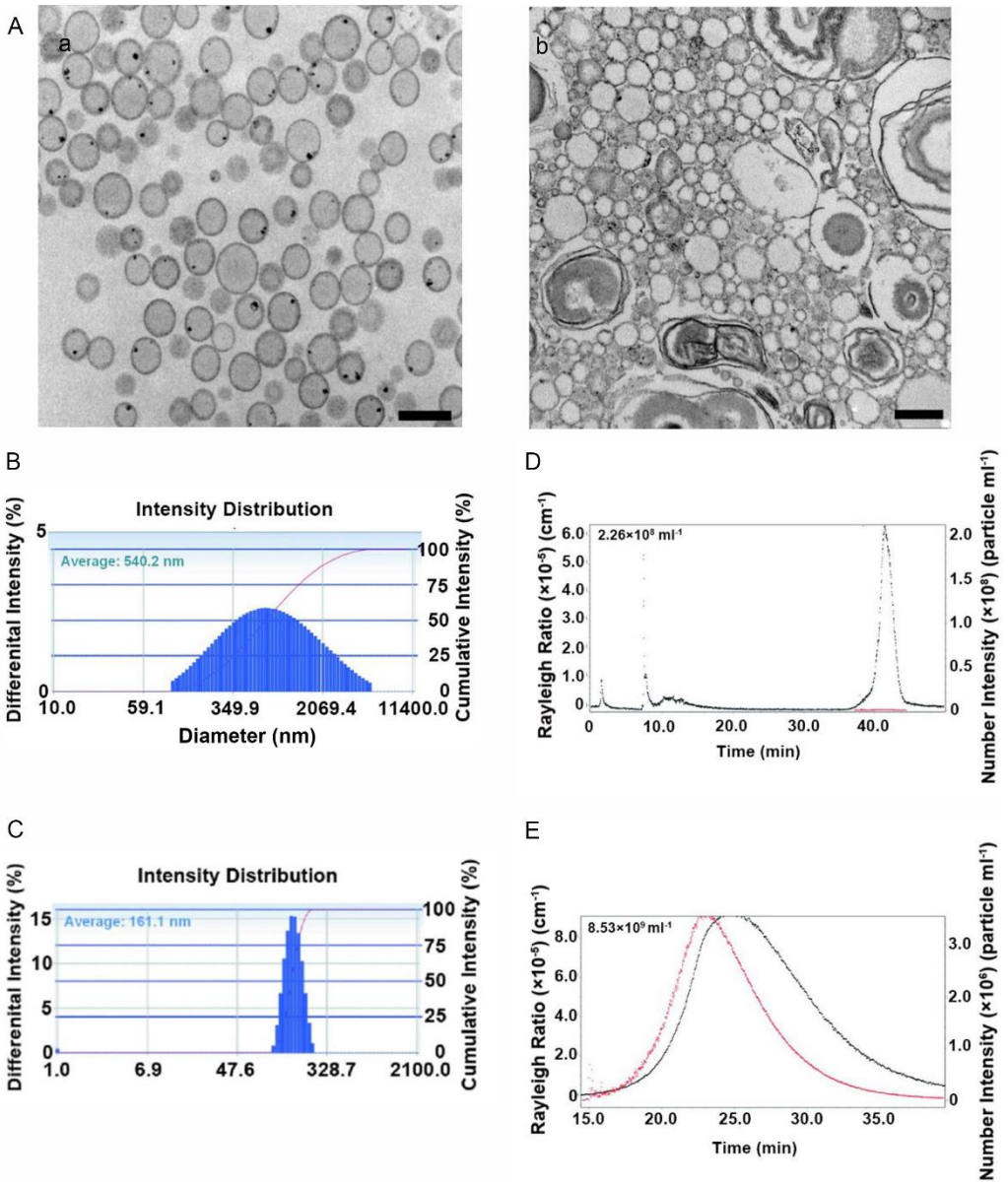


Figure S1. Structural validation of adiposomes as artificial lipid droplets, related to Figure 1.

Lipid emulsion was prepared using sonication in a water bath sonicator for 6 min and adiposomes were prepared using methods reported previously with DOPC and TAG (Wang et al., 2016) and analyzed by TEM. LDs were isolated from C2C12 cells using the method described in Transparent Methods. The diameter distribution of isolated LDs or adiposomes were determined using dynamic light scattering (DLS) (Delsa Nano C Particle Analyzer, Beckman Coulter). The number density of adiposomes were measured using a FFF-MALS system when OD600 of the adiposomes was adjusted to 20. The number density of 40 µl LDs was measured using the same machine. (A) Morphology of adiposomes and lipid emulsions. Scale bar = 500 nm. (B) Diameter distribution of intracellular LDs. (C) Diameter distribution of adiposomes. (D) The number density of isolated LDs. (E) The number density of adiposomes. The red scatters and black scatters in (D) and (E) represented the number density of peak

and Rayleigh ratio at light scatter angle 90.00º, respectively.

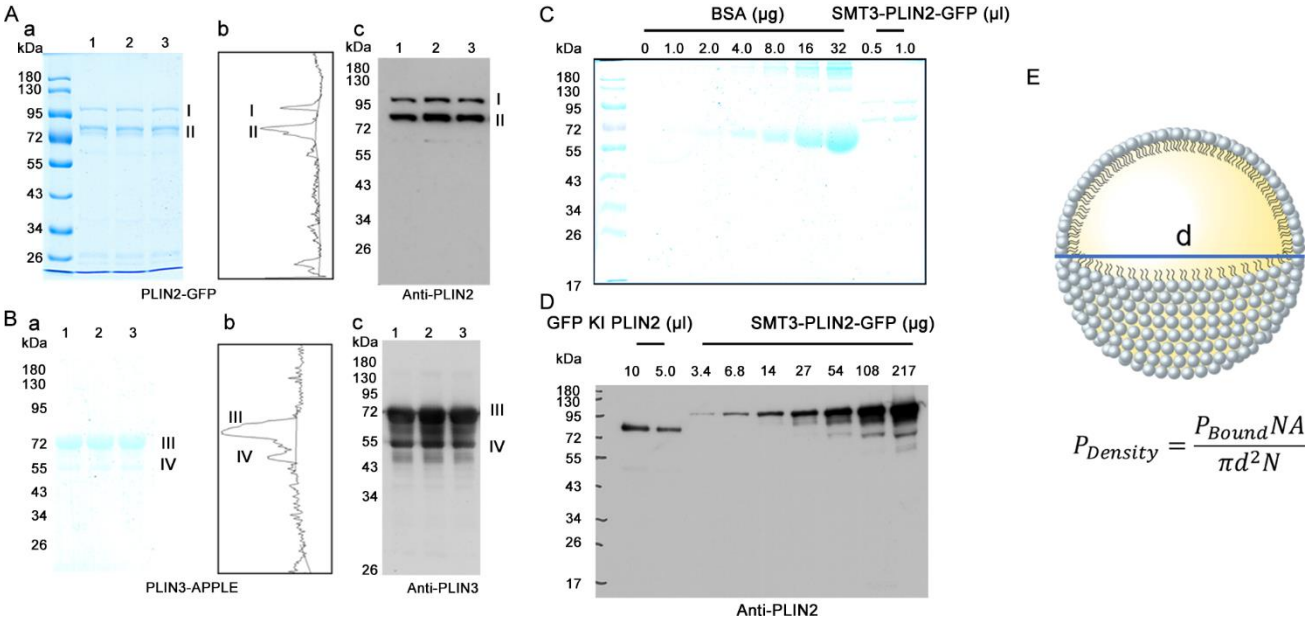


Figure S2. Purification of recombinant PLIN2 and PLIN3 and the quantification of PLIN2 on lipid droplets and adiposomes, related to Figure 1, 2, 3, 4.

PLIN2-GFP and PLIN3-APPLE were expressed and purified using the published method with minor modifications (Wang et al., 2016). Both purified proteins were analyzed using Colloidal Blue staining and Western blot with anti-PLIN2 or anti-PLIN3 antibody. (A) PLIN2-GFP was identified, (a) using Colloidal Blue staining, (b) using ImageJ to measure the purity, and (c) using Western blot to verify. (B) PLIN3-APPLE was identified, (a) using Colloidal Blue staining, (b) using ImageJ to measure the purity, and (c) using Western blot to verify. The numbers on the top of figures represent three repeats. Both Ab and Bb represent the intensity of protein band for lane 3 of A a and B a, scanned using ImageJ. All three repeats in A a and B a lanes 1-3 were scanned and calculated for the purity of proteins. An aliquot of the SMT3-PLIN2-GFP preparation was electrophoresed together with different amounts of BSA, and the gel was stained with Colloidal Blue Staining Kit overnight. ImageJ was used for quantification by densitometry to determine the original concentration of the SMT3-PLIN2-GFP preparation. Next, a dilution series of recombinant PLIN2 was compared to the PLIN2 content of lipid droplets that were purified from PLIN2–GFP knock-in C2C12 cells. They were detected by Western blot with anti-PLIN2 antibody. ImageJ was used for quantification. (C) Rough quantification of recombinant SMT3-PLIN2-GFP. (D) Rough quantification of endogenous PLIN2. (E) The binding density calculating model of protein on the adiposomes. $P_{Density}$ is the binding density of protein, d is the diameter of the LDs/adiposomes, P_{Bound} is the maximum amount of the protein on the surface of LDs/adiposomes, NA is Avogadro's constant, and N is the absolute number of LDs/adiposomes.

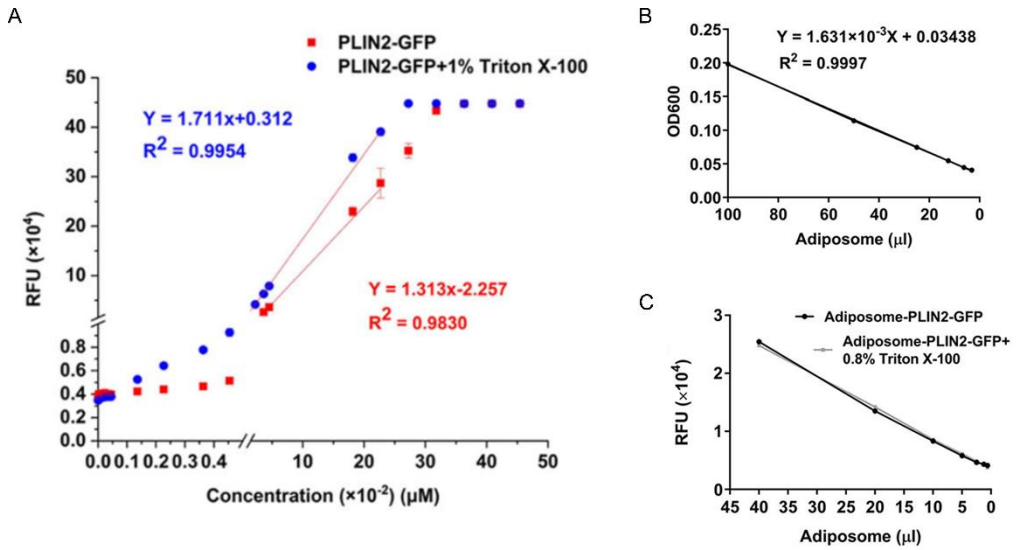


Figure S3. Quantification of multi-scan spectrum with protein samples in a series of concentrations, related to Figure 1, 4.

A series of doses of SMT3-PLIN2-GFP ranging 0 to 0.45 μM were scanned for their fluorescence intensity, measured in Relative Fluorescence Units (RFU). 1% Triton X-100 was mixed with SMT3-PLIN2-GFP to test whether the protein aggregates formed by hydrophobic PLIN2 sequences influenced the fluorescence intensity. This determination was performed using EnSpire Multimode Plate Reader (Perkin Elmer). Then, 100 μl of adiposomes (OD600 = 40) were diluted as: 1, 1/2, 1/4, 1/8, 1/16, and 1/32 to investigate the linear regression between OD600 and adiposome amount. After incubation with SMT3-PLIN2-GFP (at a final concentration of 0.3 μM), 40 μl of adiposomes (OD600 = 10) were also diluted as: 1, 1/2, 1/4, 1/8, 1/16, 1/32, and 1/64 to detect the linear regression between RFU and PLIN2-coated adiposomes. 0.8% Triton X-100 was also mixed with SMT3-PLIN2-GFP coated adiposomes to isolate protein from the surface of adiposomes. It was used to test the difference between the fluorescence intensity emitted by the PLIN2 coated on adiposomes or free PLIN2 striped by Triton X-100 (Cui et al., 2019). (A) The linear regression region of RFU to recombinant PLIN2 concentration. (B) The linear relationship between OD600 and adiposome amount. (C) The linear relationship between RFU and the amount of SMT3-PLIN2-GFP coated adiposome in the absence (black) or presence (gray) of Triton X-100, and the R^2 values for them were 0.9963 and 0.9993, respectively. Data represent mean ± s.e.m., n = 3.

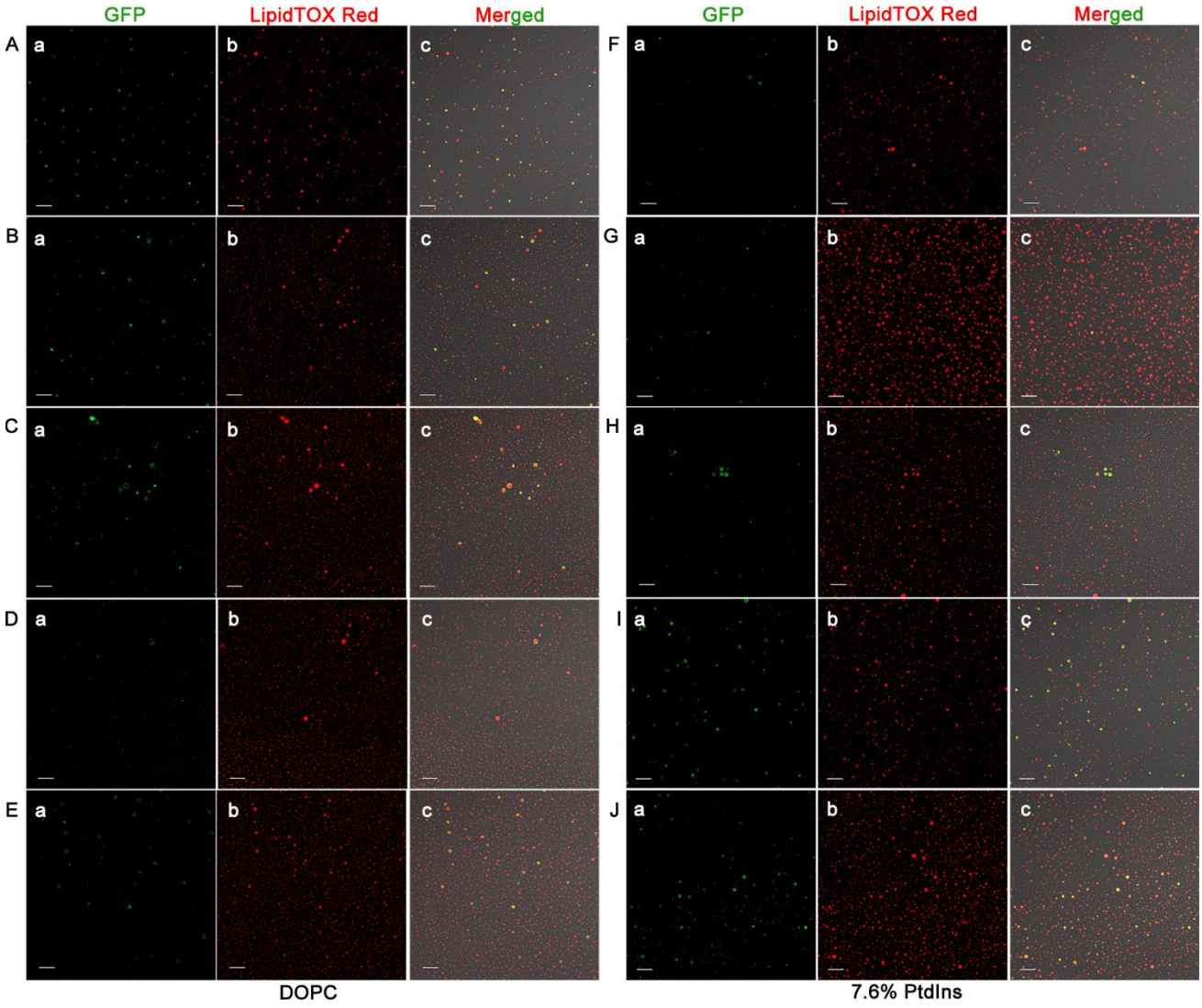


Figure S4. Increased binding of PLIN2 E73K mutant on adiposomes with PtdIns, related to Figure 5, 6.

Adiposomes were prepared with DOPC only or with 92.4% DOPC and 7.6% PtdIns in molar ratio. 30 µl adiposomes were incubated with 1 µg recombinant PLIN2 or its mutants per aliquot in 37ºC for 5 min. After reisolation and stained with LipidTOX Red (red), the PLIN2 targeting adiposomes were visualized using Olympus FV1200 confocal microscope. Wild type PLIN2 (WT) was set as the control for mutants, and the proteins binding on adiposomes with only DOPC were set as the control for adiposomes with PtdIns. The images were grouped as (a) SMT3-PLIN2-GFP wild type or its mutants, (b) adiposomes stained with LipidTOX Red, (c) merged signals. Adiposomes were stained with LipidTOX Red (red) and PLIN2 as well as its mutants were labelled by GFP (green). The proteins incubated with adiposomes with DOPC only were in the left panel (DOPC), while the proteins incubated with adiposomes with DOPC and PtdIns were in right panel (7.6% PtdIns). (A) WT. (B) E48K mutant. (C) E48Q mutant. (D) E73K mutant. (E) E73Q mutant. (F) WT. (G) E48K mutant. (H) E48Q mutant. (I) E73K mutant. (J) E73Q mutant. Scale bar = 5 µm.

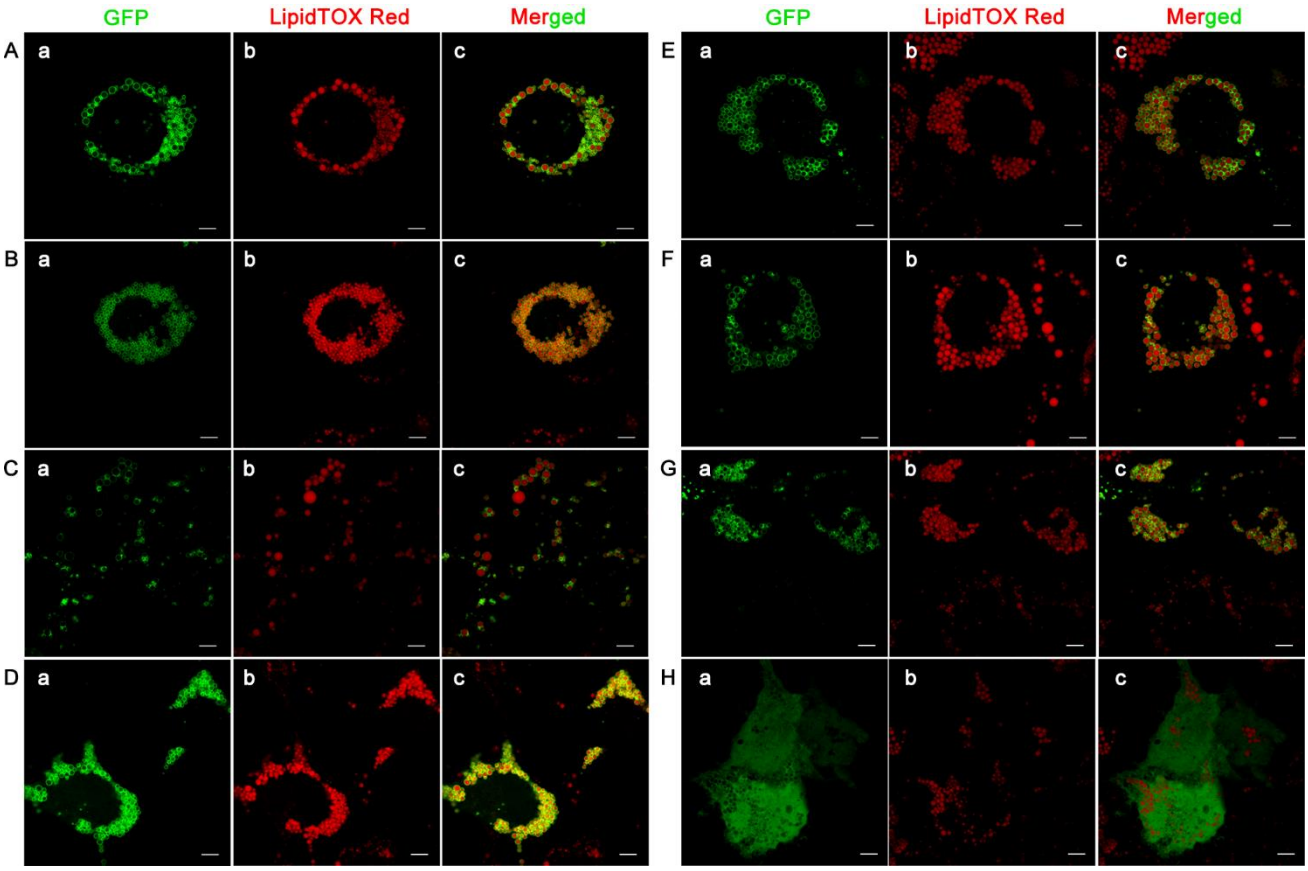


Figure S5. Targeting of GFP-tagged PLIN2 mutants on lipid droplets, related to Figure 5, 6.

PLIN2 mutants were expressed in Huh7 cells. Lipid droplets were stained with LipidTOX Red (1:1,000, v/v) and PLIN2 as well as its mutants were labelled by GFP (green). Fluorescence images were obtained using a confocal microscope Olympus FV1000. Wild type PLIN2 (WT) was set as the control for mutants and GFP was set as the negative control. (A) PLIN2 wild type, (B) E48K, (C) E48Q, (D) E73K, (E) E73Q, (F) D341K, (G) D341Q mutant, and (H) GFP. The images were grouped as (a) PLIN2-GFP wild type or its mutants, (b) LDs stained by LipidTOX Red, (c) merged signals. Scale bar = 5 μm.

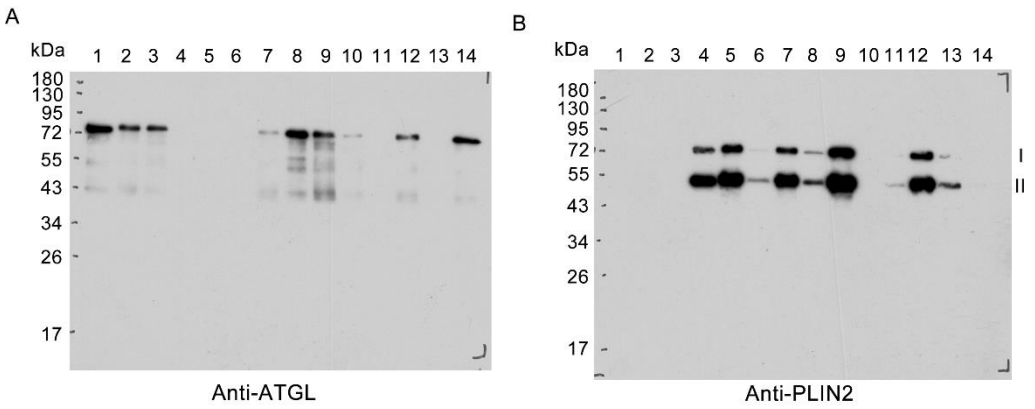


Figure S6. Colocalization of recombinant SMT3-ATGL and SMT3-PLIN2 on adiposomes, related to Figure 7. Adiposomes were prepared using TAG and DOPC. (A) Western blot of protein colocalization detected using anti-ATGL antibody. (B) Western blot of protein colocalization detected using anti-PLIN2 antibody. Lane 1: ATGL loading control; 2: ATGL binding on bare adiposomes; 3: ATGL in washing solution; 4: PLIN2 loading control; 5: PLIN2 binding on bare adiposomes; 6: PLIN2 in solution; 7: ATGL and PLIN2 colocalizing on adiposomes together; 8: unbound ATGL and PLIN2 in solution after colocalization with adiposomes; 9: PLIN2 binding on adiposomes coated by ATGL; 10: ATGL in solution; 11: PLIN2 in solution after incubation with ATGL-coated adiposomes; 12: ATGL binding on adiposomes coated by PLIN2; 13: PLIN2 in solution; and 14: ATGL in solution after incubation with PLIN2-coated adiposomes. In (B), band I was SMT3-PLIN2, and band II was PLIN2 without SMT3 tag.

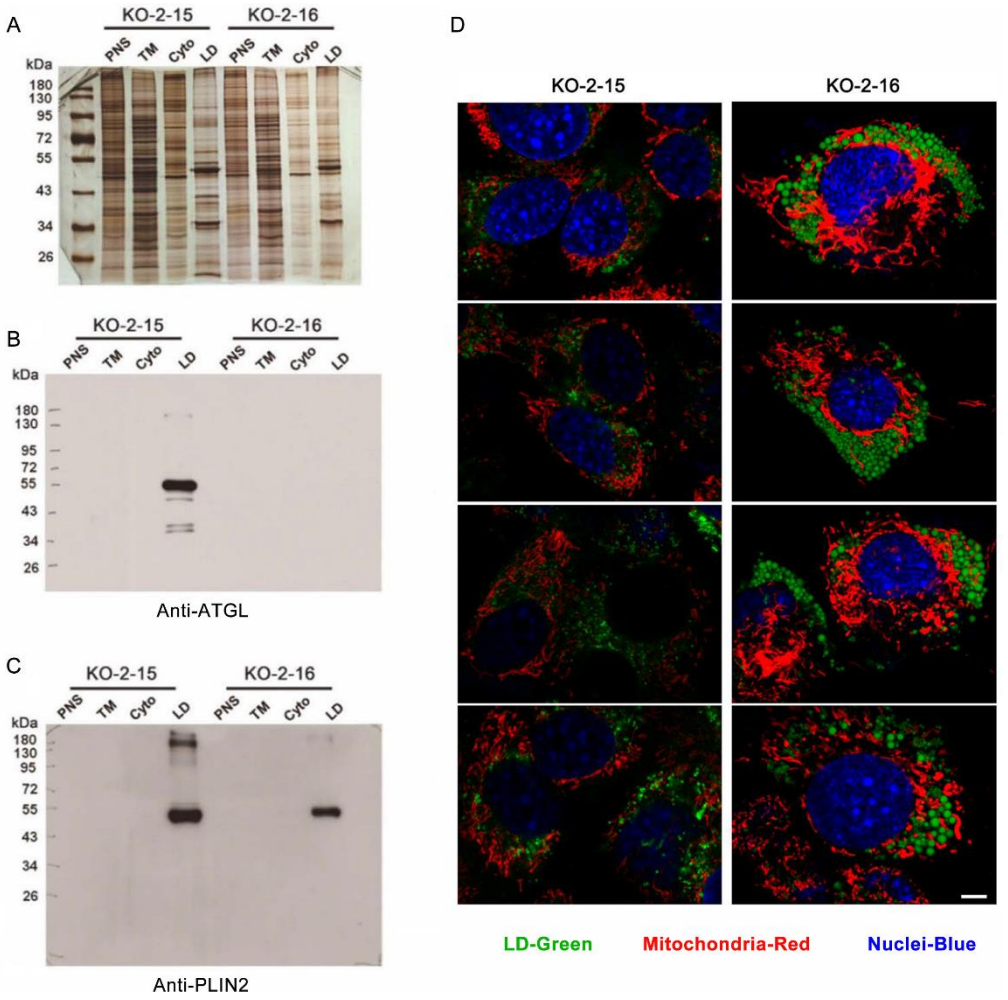


Figure S7. Knockout of ATGL inhibits the intracellular lipolysis, related to Figure 7.

The cell line with no deletion or insertion in the ATGL locus was constructed as KO-2-15, and the cell line with a single base deletion causing frameshift in the ATGL gene was constructed as KO-2-16. The cells were treated with 50 μM oleate for 24 h and imaged by Olympus FV1000 confocal microscope. (A) Protein profile of lipid droplets purified from these two clones. (B) Western blot analysis of cell fractions from those two cell lines with anti-ATGL antibody. (C) Western blot analysis of cell fractions with anti-PLIN2 antibody. PNS: post-nuclear supernatant, TM: total membrane, Cyto: cytosol, and LD: lipid droplet. (D) The variation of LDs in two cell lines. Cells grown overnight in confocal dishes were stained using LipidTOX Green (LDs, green) and MitoTracker Red (mitochondria, red) and Hoechst (nuclei, blue). The cells were examined using an Olympus FV1000 confocal microscope. Both images were obtained using the same exposure time and microscopy settings. Scale bar = 5 μm.

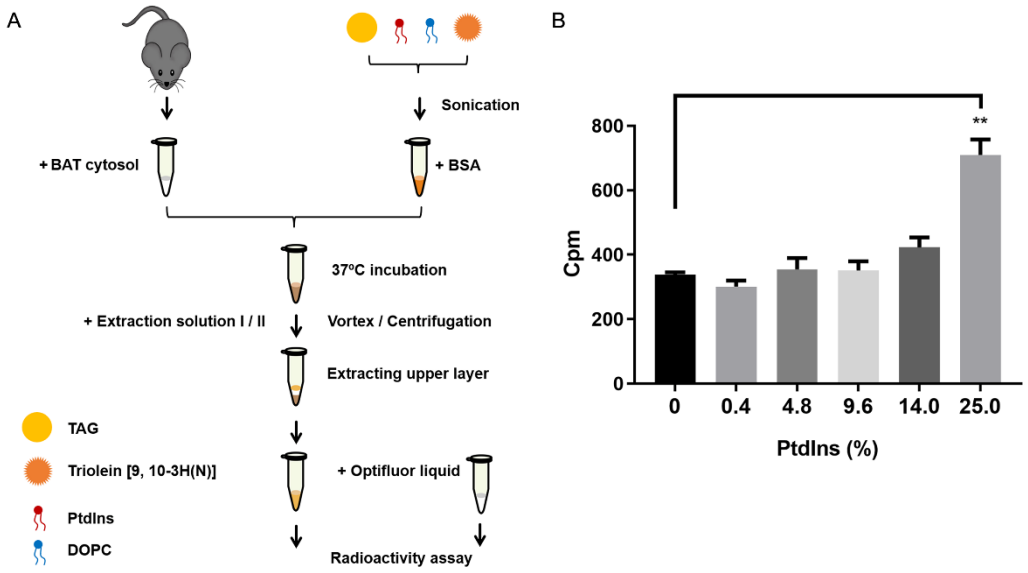
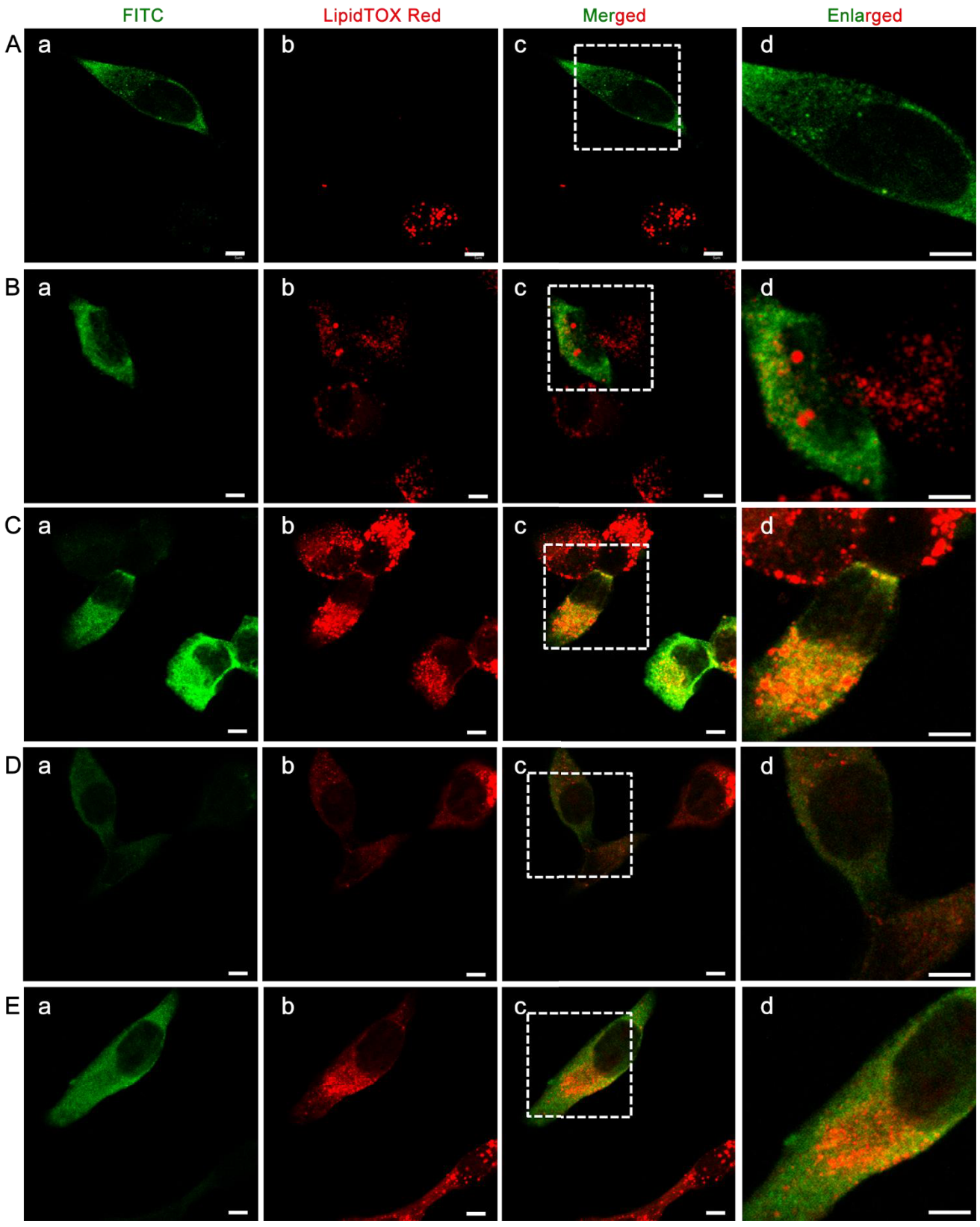


Figure S8. Increase of ATGL activity with PtdIns, related to Figure 7.

Triolein [9, 10-^{3}H(N)] (0.5 µCi/µl) was used as a radio labelled substrate to perform enzyme activity assays and the radio labelled triolein was mixed with label-free triolein at a ratio 28 µg triolein per µCi. The lipid emulsions were prepared using triolein and a mixture of DOPC and PtdIns by ice bath sonication. An increasing dose of PtdIns was applied, from 0 to 25%, in molar ratio. 150 µl lipid emulsions were mixed with 50 µl 20% BSA to prepare the substrate. The BAT cytosol containing endogenous ATGL was used as the source of ATGL. 25 µl BAT cytosol was incubated with 25 µl substrate in 37ºC for 1 h and then the specimen was isolated according to Transparent Method. The radioactivity was analyzed by gamma meter (PerkinElmer). (A) Schematic of the experimental design. (B) The increase of ATGL activity with PtdIns in lipid emulsion. Data represent mean ± s.e.m., n = 3. **P < 0.01, two-tailed t-test.


FITC
LipidTOX Red
Merged
Enlarged
A
B
C
D
E
a
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c
d

Figure S9. Reduction of ATGL activity with the mutations of S47 and S87, related to Figure 7.

Wild type ATGL and mutants were overexpressed in C2C12 cells without OA treatment. ATGL (green) was stained by immunofluorescence using mouse anti-Flag followed by anti-mouse FITC coupled antibody. LDs (red) were stained with LipidTOX Red. Fluorescence images were obtained using a confocal microscope Olympus FV1000. (A) ATGL wild type, (B) S47A, (C) S47D, (D) S87A, (E) S87D. The images were grouped as (a) ATGL wild type or its mutants, (b) LDs stained by LipidTOX Red, (c) merged signals and (d) enlarged area. Scale bar = 5 μm.