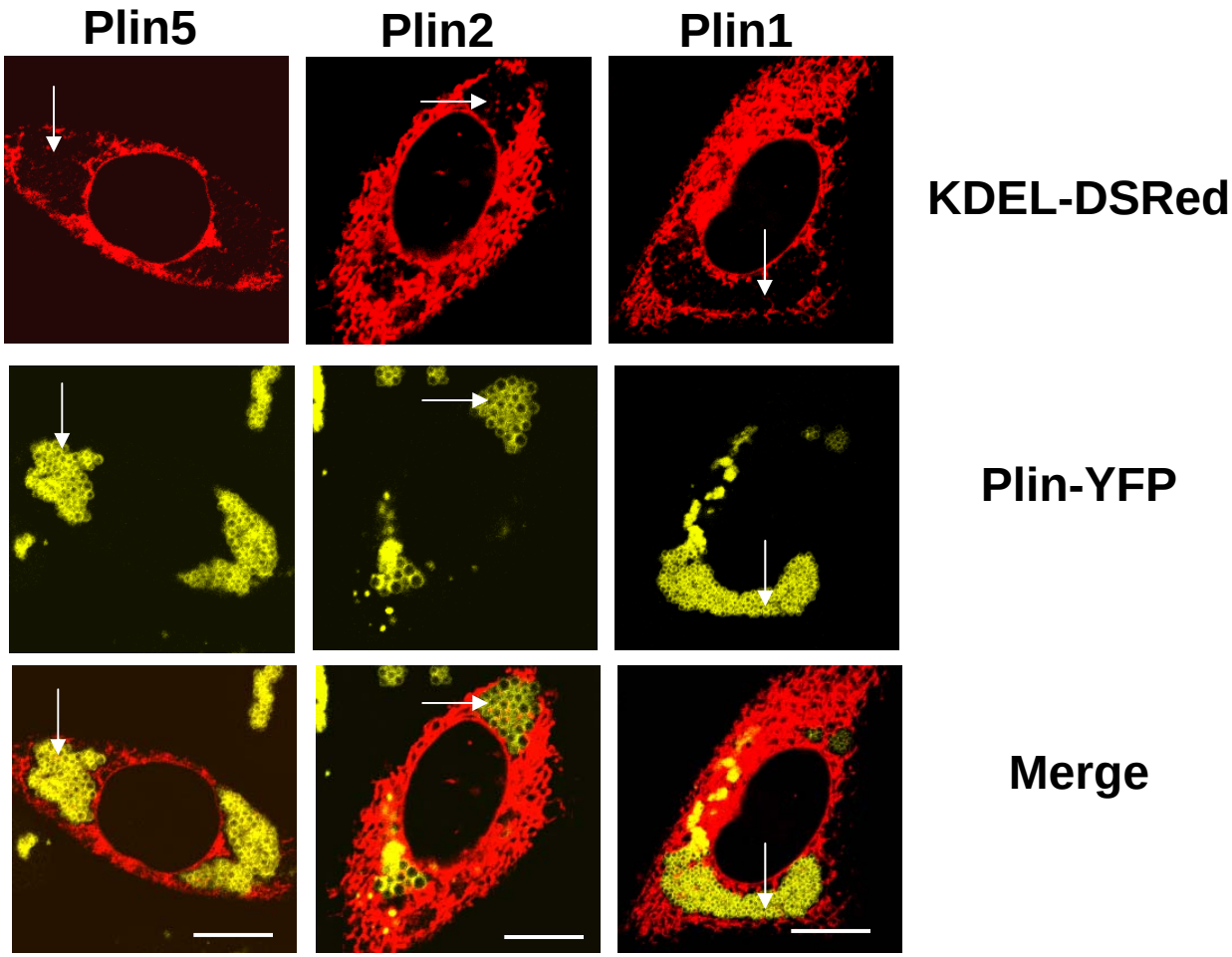
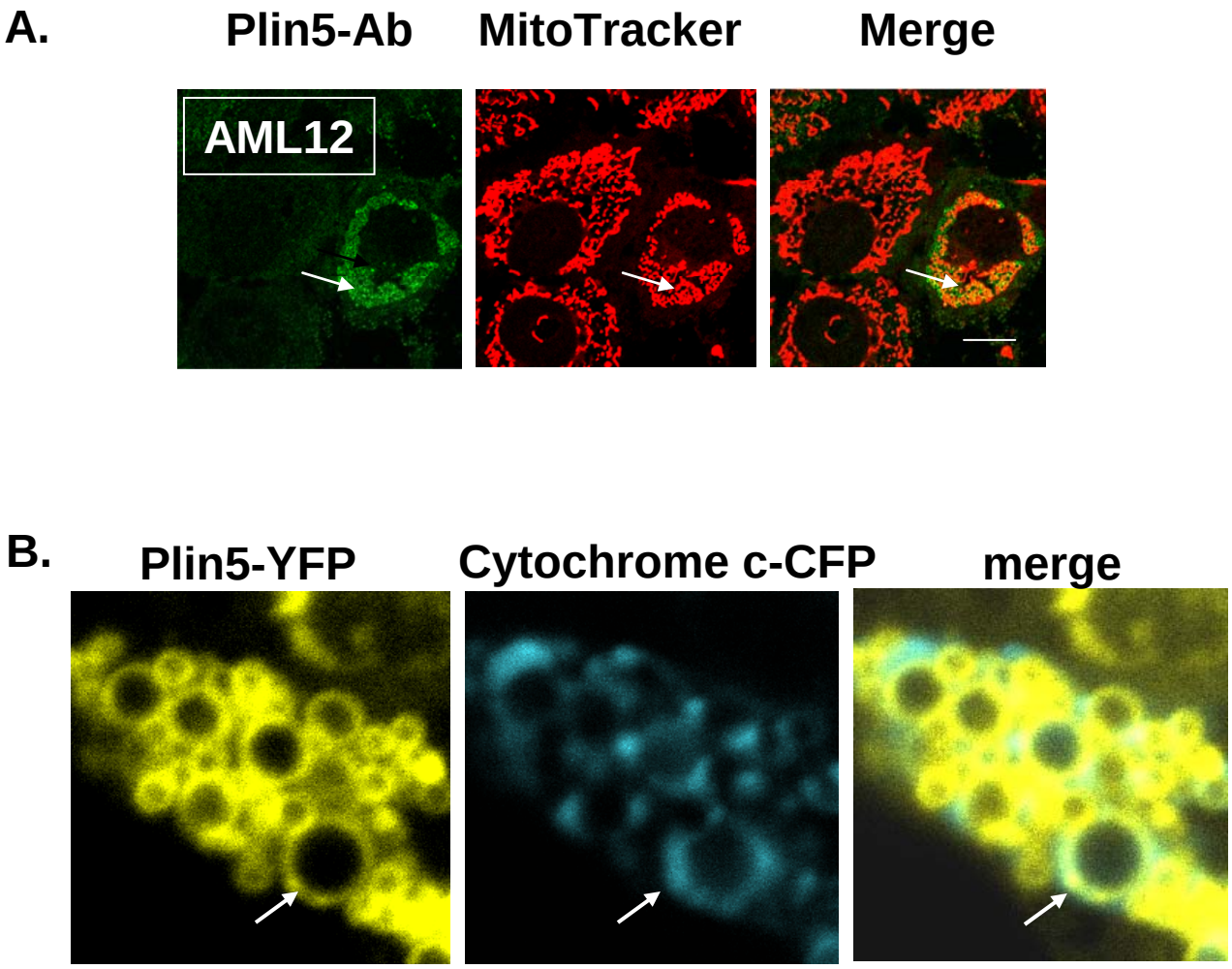
Supplement Figure 1

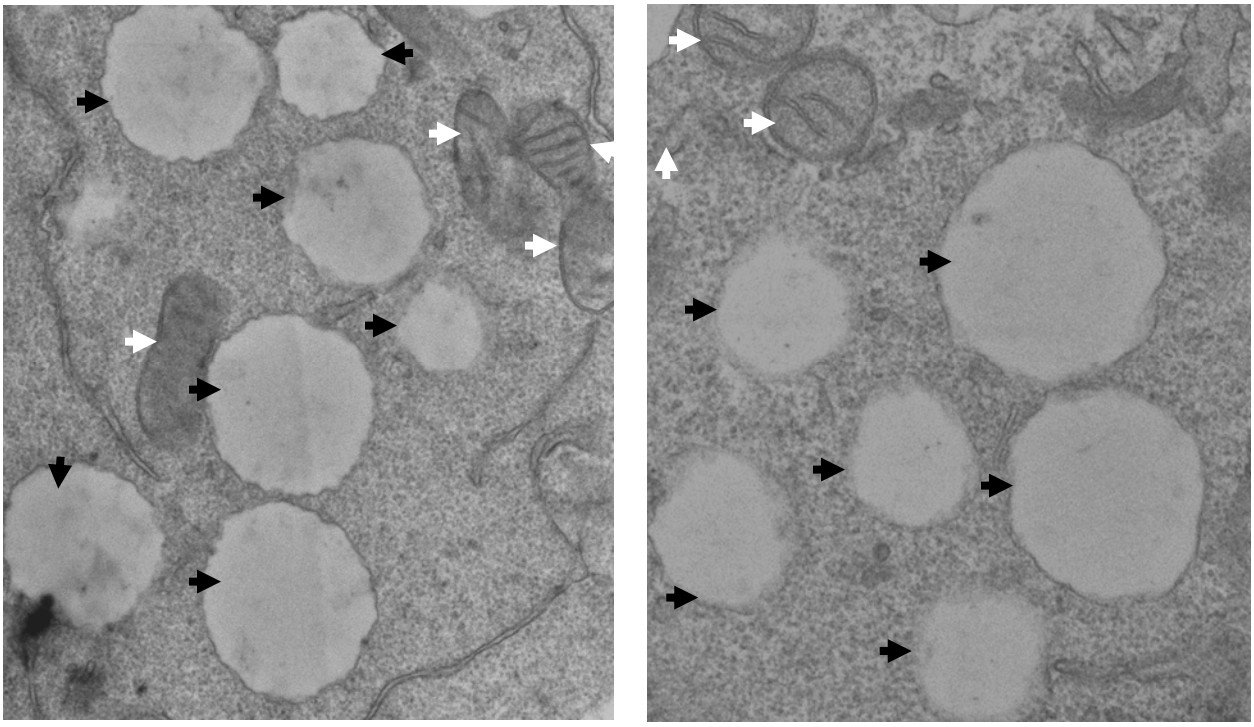


Supplement Figure 2

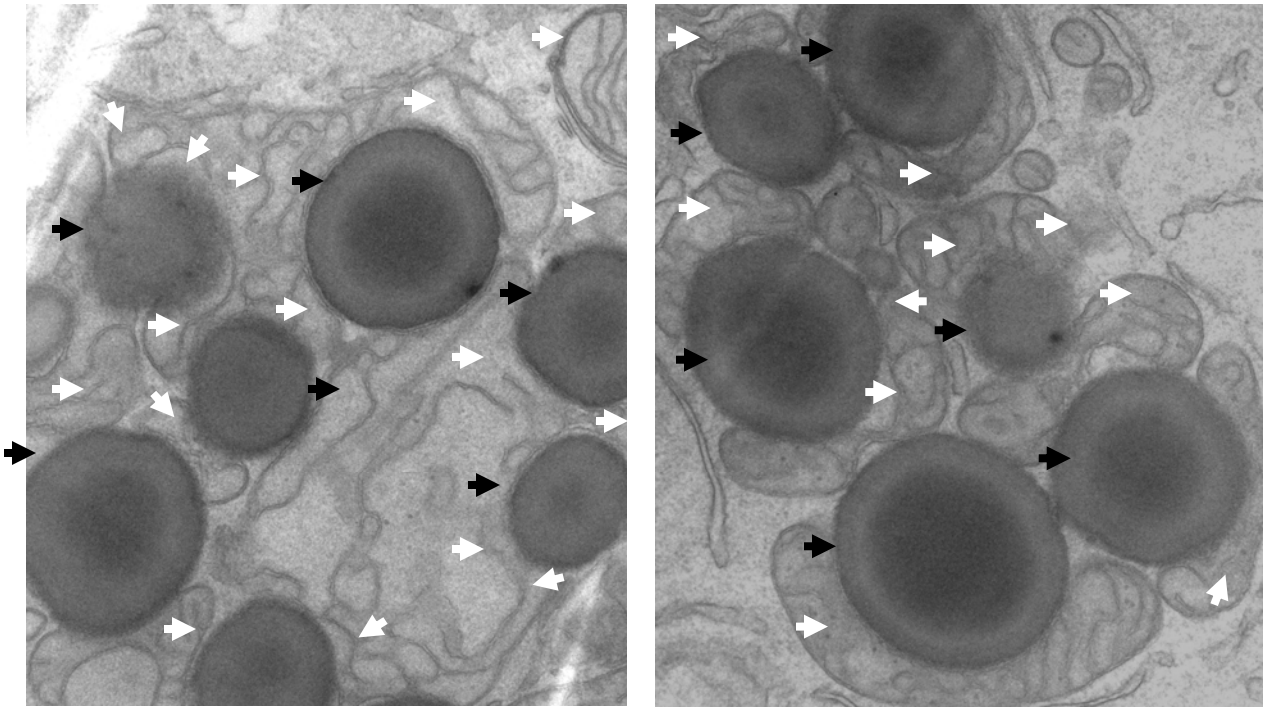


Supplement Figure 3

Ad.GFP

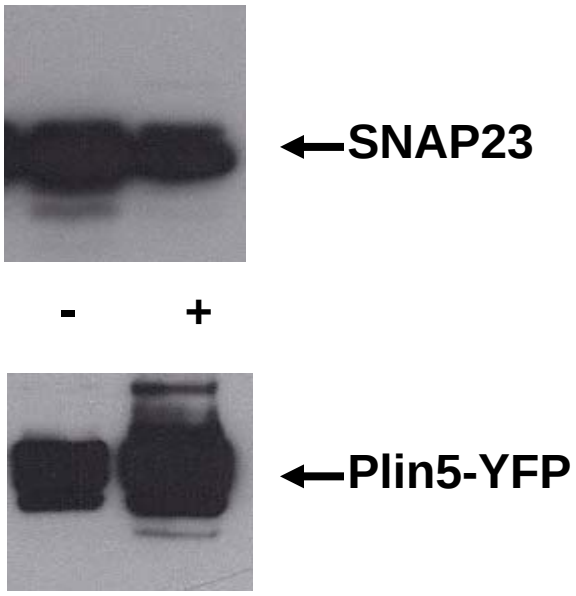


Ad.Plin5

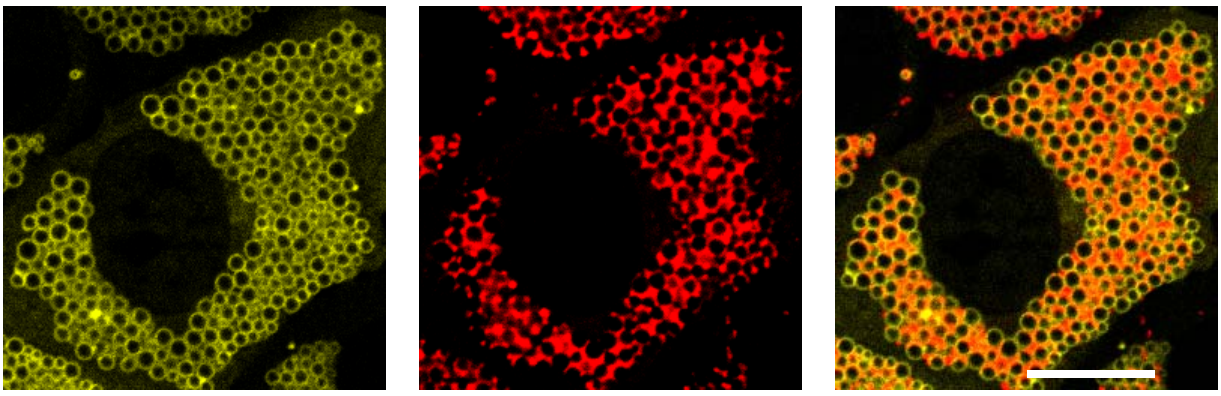


Supplement Figure 4

A.



B.



Supplemental figures

Supplemental Figure 1: Plin5 over-expression does not induce gross morphological re-structuration of ER. CHO-K1 cells were co-transfected with KDEL-DsRed (ER retention peptide) and one of the following perilipin fusion proteins: perilipin 5-YFP (Plin5-YFP), perilipin 1-YFP (Plin1-YFP), or perilipin 2-YFP (Plin2-YFP) and cells were incubated with 400 μM oleic acid overnight. The following day, cells viewed with confocal microscopy as in Figure 1. Micrographs depict one or two representative cells of hundreds observed in three experiments. Bar: 10 μm. White arrows indicate LDs.

Supplemental Figure 2: Plin5 recruits mitochondria at LD surface. (A) AML12 (liver) cells were transiently transfected with 6x histag Plin5 and incubated overnight with 400 μM oleic acid before being stained with MitoTracker (1μM) and being fixed with 3% paraformaldehyde. Cells were stained with antibody against Plin5 and viewed with confocal microscopy as in Figure 1. White arrows indicate lipid droplets. Micrographs depict one or two representative cells of hundreds observed in three experiments. Bar: 10 μm. (B) AML12 (liver) cells were transiently transfected with Plin5-YFP and cytochrome C-CFP. Cells were incubated with 400 μM oleic acid overnight. The following day, cells were viewed with a confocal microscope with a 63x oil objective as in Figure 1. Micrographs depict one or two representative cells of hundreds observed in three experiments. Magnification x 20. White arrows indicate LDs.

Supplemental Figure 3: Plin5 recruits mitochondria in AML12 cells

AML12 (liver) cells were transduced with GFP- adenovirus or Plin 5- YFP adenovirus and TEM performed as shown in Figure 5B. White arrows indicate mitochondria, Black arrows LD. Magnification x4400

<u>Supplemental Figure 4:</u> RNAi down regulation of SNAP23 does not alter mitochondria clustering at LD surface. (A**)** AML12 cells were treated for 4 days with either siRNA SNAP23 (42) or siRNA control (All Star negative, Qiagen). On day 2, cells were additionally transduced with an adenoviral construct for the expression of perilipin 5 YFP (Plin5-YFP) constructs for 48 hr prior to harvest the cells. Immunoblots of total cellular protein extract from AML12 cells treated with control siRNA (Qiagen) (lane 1) or with siRNA SNAP23 (lane 2). Antibodies against SNAP23 and GFP proteins were used. (**B**) AML12 cells were treated for 4 days with siRNA SNAP23. On day 2, cells were additionally transduced with an adenoviral construct for the expression of perilipin 5 YFP (Plin5-YFP) constructs for 48 hr prior to live imaging by confocal. Micrographs depict one or two representative cells of hundreds observed in two experiments. Bar: 10 μm.

SUPPLEMENTARY TABLE 1

Construct Name	**PCR Primer Sequence**	***Digestion Sites***
Perilipin 1-EYFP-C1	Forward 5'-TAGAGCTCGGATGTCAATGAACAAGGGCC-3' Reverse 5'-TAGGTACCGCAGTCTGCTCAGCTCTTCTTGC-3'	*SacI /KpnI*
Perilipin 2-EYFP-C1	Forward 5'-TAGGTACCAAAATGGGAGCAGCAGTAGTGGAT-3′ Reverse 5'-TAGGTACCAGGAGGGGTTTACTGAGCTTTGAC-3′	*KpnI /KpnI*
Perilipin 3-EYFP-C1	Forward 5'-TAGAGCTCCCATGTCTAGCAATGGTACAGAT-3′ Reverse 5'-TAGGTACCTCCCTACTTCCCTTCAGGGGTTT-3′	*SacI /KpnI*
Perilipin 5-EYFP-C1	Forward 5'-TAGAGCTCAGGAAATGGACCAGAGAGGTGAAG-3′ Reverse 5'-TAGGTACCCCTCGATAGTCAGAAGTCCAGCTC-3′	*SacI /KpnI*
Perilipin 5 (1-188) -EYFP-C1	Forward 5'-TAGAGCTCAGGAAATGGACCAGAGAGGTGAAG-3′ Reverse 5'-TAGCGGTACCTCCTATAGCTCAGCCTCAGTCAT-3'	*SacI /KpnI*
Perilipin 5 (1-391) *-EYFP-C1*	*Forward 5'-TAGAGCTCAGGAAATGGACCAGAGAGGTGAAG-3′* *Reverse 5'-TAGCGGTACCTCCTACCCCACAAGCCAGGGCAG-3'*	*SacI /KpnI*

Table 1 legend: List of PCR primer sequences and digestion sites used to create the different constructs used in the manuscript