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Supplemental Material to:

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Perilipin 1 moves between the fat droplet and the endoplasmic reticulum

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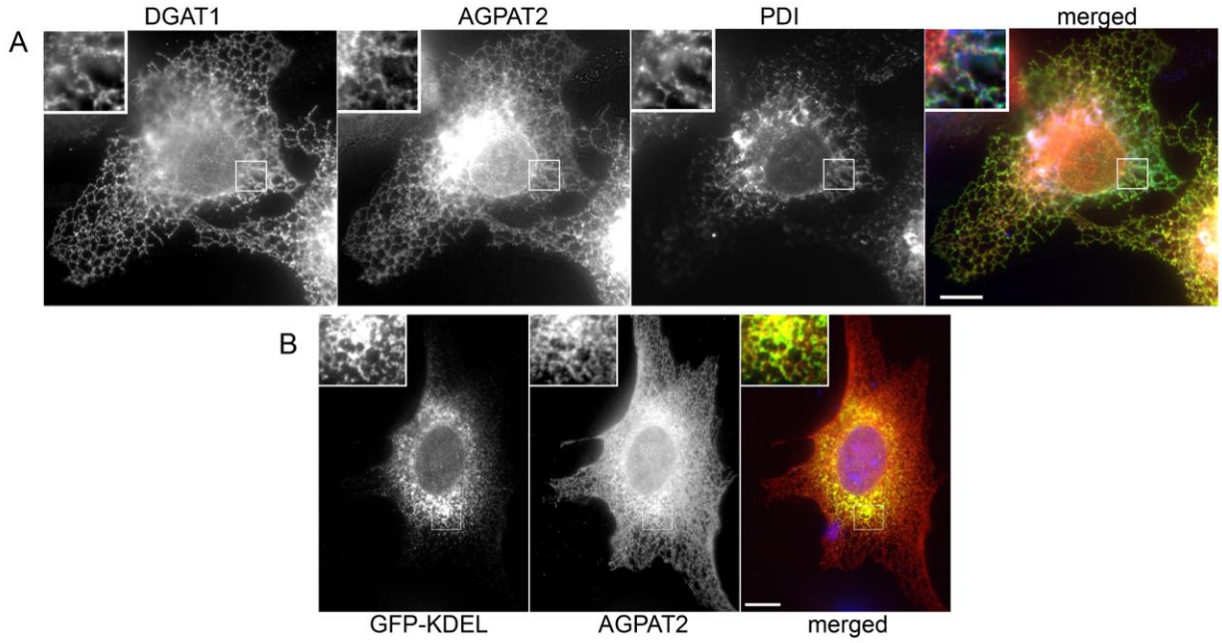
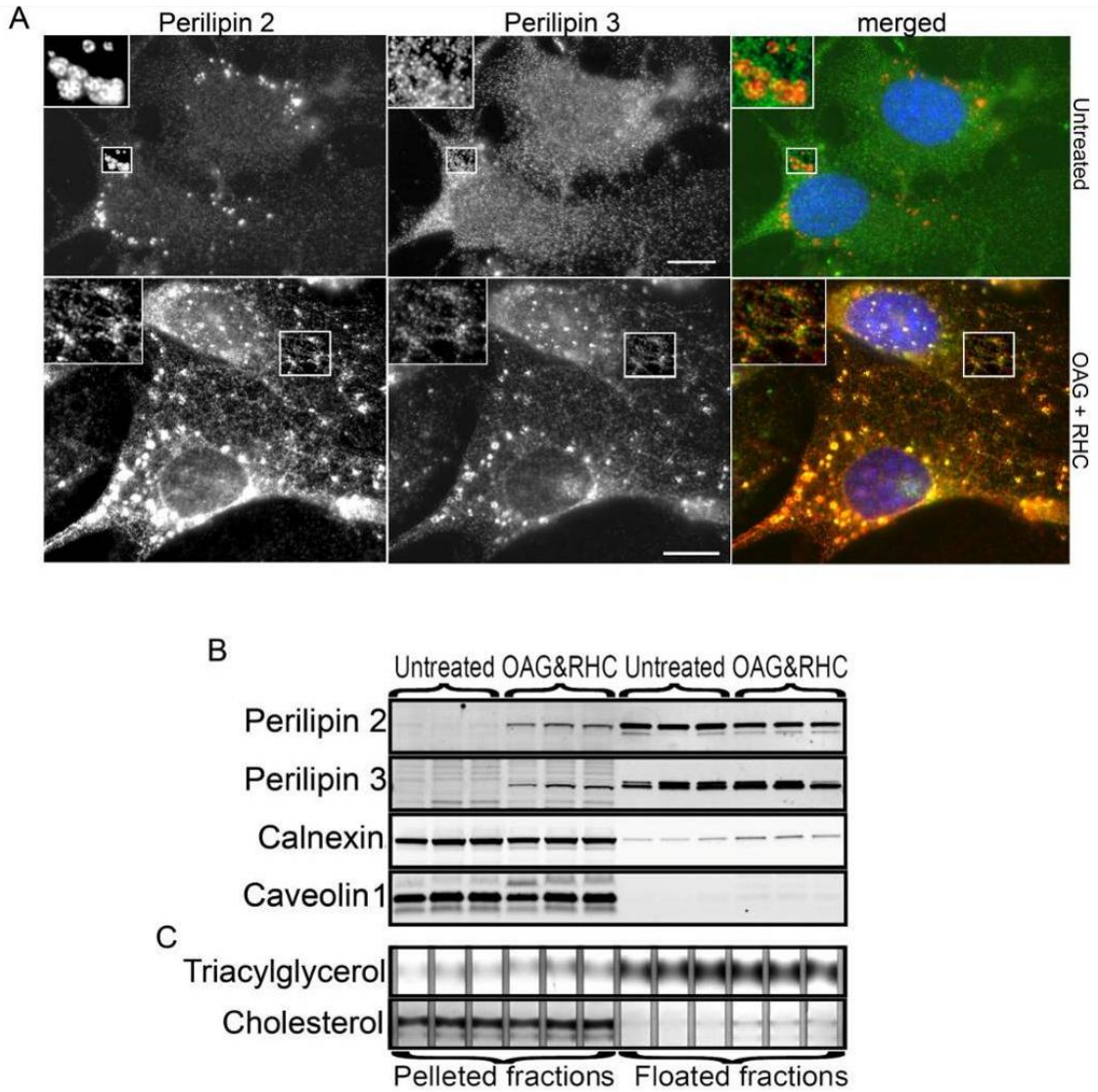
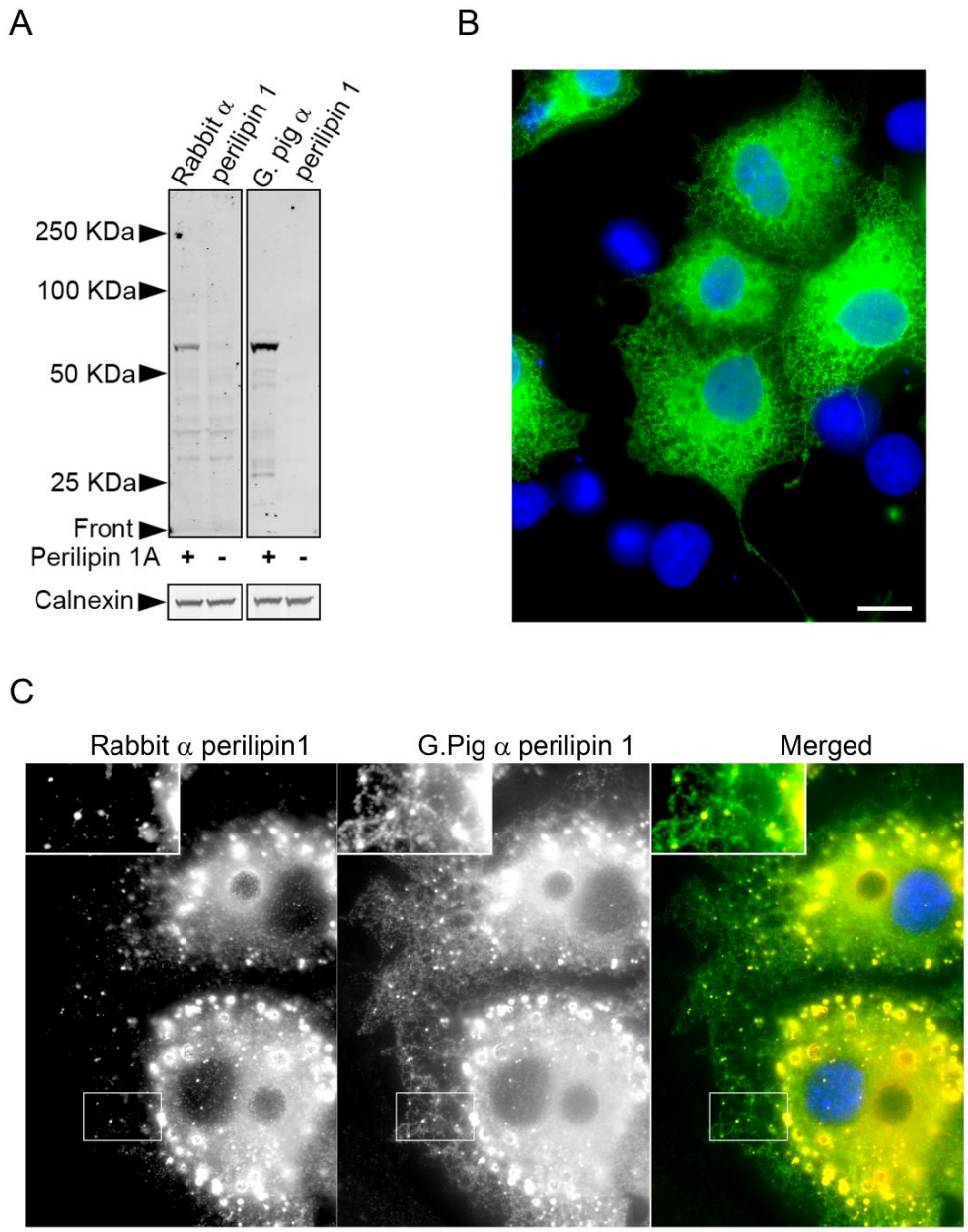


Supplemental Figure 1. ER enzymes vary greatly in distribution. A) Shows COS7 cells transfected with FLAG-DGAT1 and AGPAT2-HA and labeled with goat antibodies against FLAG (400 ng/ml), rabbit anti-HA (1:500), and mouse anti-protein disulfide isomerase, PDI (1.0 ug/ml) as indicated on panels. Merged panel shows DGAT staining as green, AGPAT2 as red and PDI as blue. Red-green overlap is shown as yellow, red-blue is shown as pink, green-blue overlap is shown as teal and red-green-blue overlap is shown as white. B) Shows OP9 cell transfected with GFP-KDEL and AGPAT2-HA and then labeled with mouse antibody against HA (2ug/ml). Merged panel shows GFP-KDEL staining as green, AGPAT2 as red. Bar = 10 μm. Inset is outlined area expanded three-fold.

Note that PDI, a luminal-rough ER protein almost exclusively labels the perinuclear ER while the lysozyme-GFP-KDEL, also a luminal protein, more evenly labels the ER. The lysozyme-GFP-KDEL staining fades at the cell periphery as the ER lumen narrows. In contrast, the integral-membrane acylating enzymes DGAT1 and AGPAT2 uniformly label the ER.

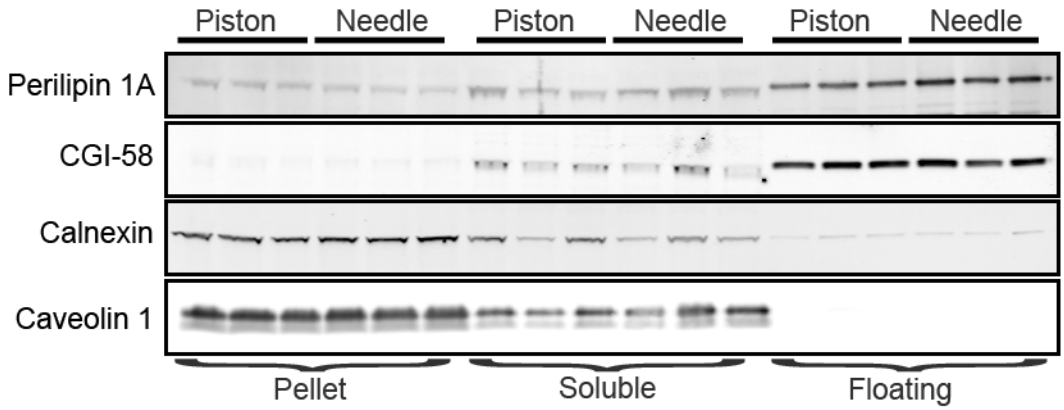


Supplemental Figure 2. Treating cells with DAG causes perilipin 2 to coat the ER. 60% confluent 100 mm plates of OP9 preadipocytes were grown in media with 100 μM oleate complexed to albumin (20 μM). Then OP9 preadipocytes were treated with 1 mM OAG and 40 μM RHC or left untreated as indicated. A) After 4 h of treatment cells were fixed and labeled with antibody against perilipin 2 (1:1000) and antiserum against perilipin 3 (800 ng/ml) as indicated in the panels. In the color panel perilipin 2 is shown as red and perilipin 3 is shown as green. Bar = 10 μm. B) After 4 h of treatment cells were fractionated into floated, soluble and pelleted fractions. The floated (5% of each) and pelleted fractions (50% of each) were Western blotted. The blot was probed with antisera against perilipin 2 (1:3000), antibodies against perilipin 3 (400 ng/ml), antibodies against calnexin (500 ng/ml), and antibodies against caveolin-1 (200 ng/ml) as indicated on the panel. C) Equal volumes of the fractions described above were solvent extracted and the lipids were resolved by TLC. Both treatments were done in triplicate and 6 separate dishes of cells are represented. Note that this demonstrates that perilipin 2 can be directed to the ER. Previous similar treatments have been shown to direct perilipins 3, 4 and 5 to the ER[12].



Supplemental Figure 3. Guinea pig perilipin 1 antibody is more specific, sensitive and recognizes perilipin 1 in more contexts than the previously described rabbit anti perilipin 1 antibody. A) shows immunoblotted lysates from HeLa cells either untransfected or transfected with pCAGGS-mouse perilipin 1A as indicated. Then identically loaded blots were probed with the antibody indicated. B) shows COS7 cells treated with transfection reagent and pCAGGS-mouse perilipin 1A DNA and then stained with Guinea pig perilipin 1 in green and DAPI in blue. Transfected cells are seen as nuclei surrounded by perinuclear and reticular staining while untransfected cells are seen as blue nuclei against the black background. C) OP9 adipocytes were stained as indicated with the two antibodies shown in A. In the merged image the rabbit anti perilipin 1 antibody is shown in red and Guinea pig anti perilipin 1 antibody is shown in green. Bar = 10 μm

Note that this shows that the rabbit and Guinea pig antibodies both recognize perilipin 1 on fat droplets. However, the Guinea pig anti-perilipin recognizes perilipin 1 on the ER much better than the rabbit anti-perilipin 1.



Supplemental Figure 4. Needle or Teflon pestle glass homogenizer are equally effective at disrupting adipocytes for fractionation. OP9 adipocytes were disrupted by being forced though a 25G needle or with a teflon pestle glass homogenizer. All cells were treated with fatty acid, glucose and insulin as in Figure 5. Cells were fractionated as described in the methods and previous figure legends.