Supplemental material

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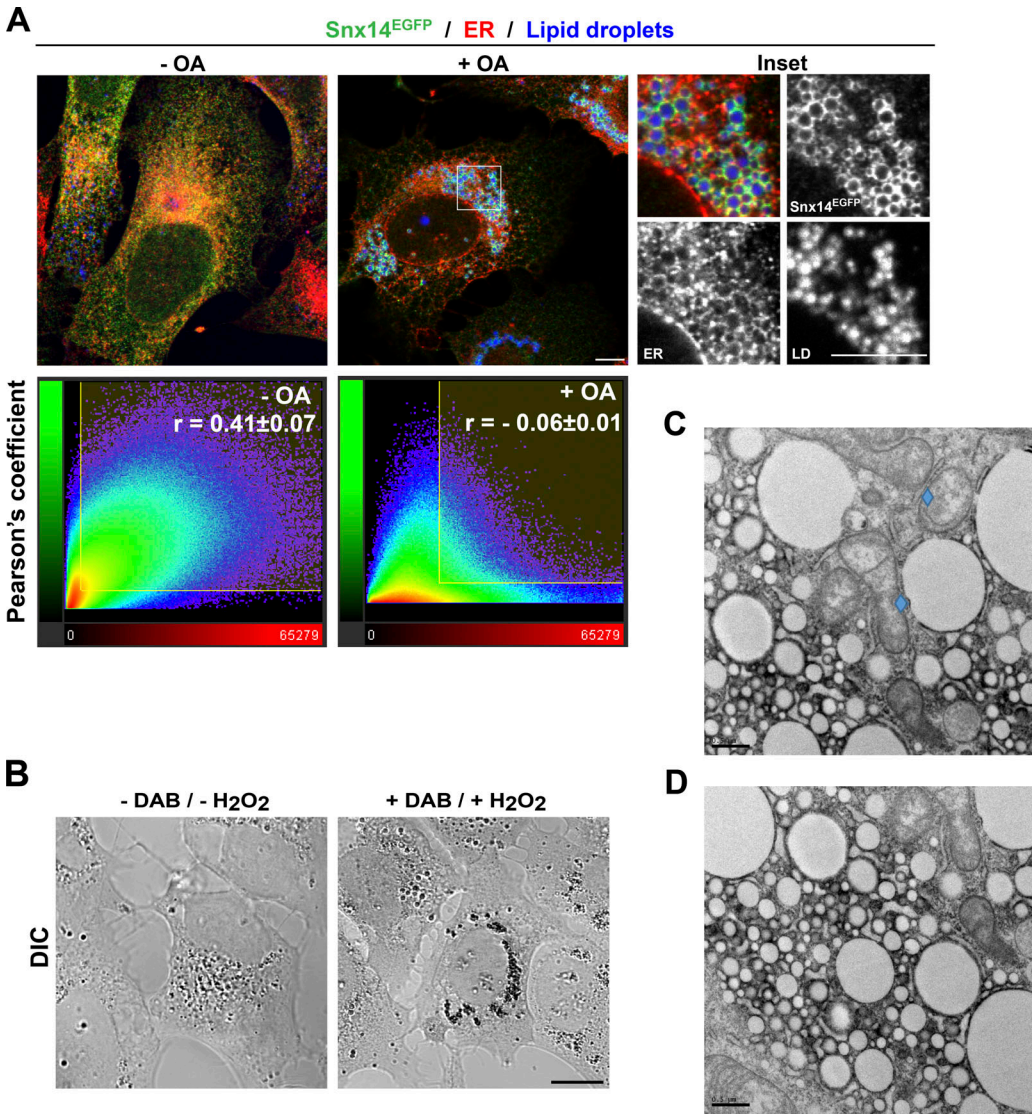

Figure S1. **Snx14 localizes to the ER–LD interface. (A)** CoIF staining of U2OS cells stably expressing Snx14^{EGFP}, in the absence and presence of OA, were performed with α-EGFP (green) and α-HSP90B1 (ER marker, red) antibodies, and LDs were stained with MDH (blue) and imaged by confocal microscopy. Scale bar = 10 µm. Representative cytofluorogram of Snx14 (green) and ER (red) showing Snx14 and ER colocalization (r = 0.41 ± 0.07) in untreated conditions, and following OA treatment (r = −0.06 ± 0.01). Approximately 20 cells were quantified for the average r values. **(B)** Differential interference contrast (DIC) images of U2OS cells expressing Snx14$^{EGFP\text{-}APEX2}$ and treated with OA overnight without (left) and following DAB + H_2O_2 staining (right). Dark regions indicate presence of DAB precipitate. Scale bar = 14 µm. **(C)** TEM image of cellular region showing mitochondria, LDs, and ER. Blue diamonds denote close junctions between ER–mitochondria and LD–mitochondria devoid of detectable DAB stain. Scale bar = 0.5 µm. **(D)** Zoom-in of small LD clusters showing grape-like clustering of LDs with associated ER membranes and DAB stain in between them. Scale bar = 0.5 µm.

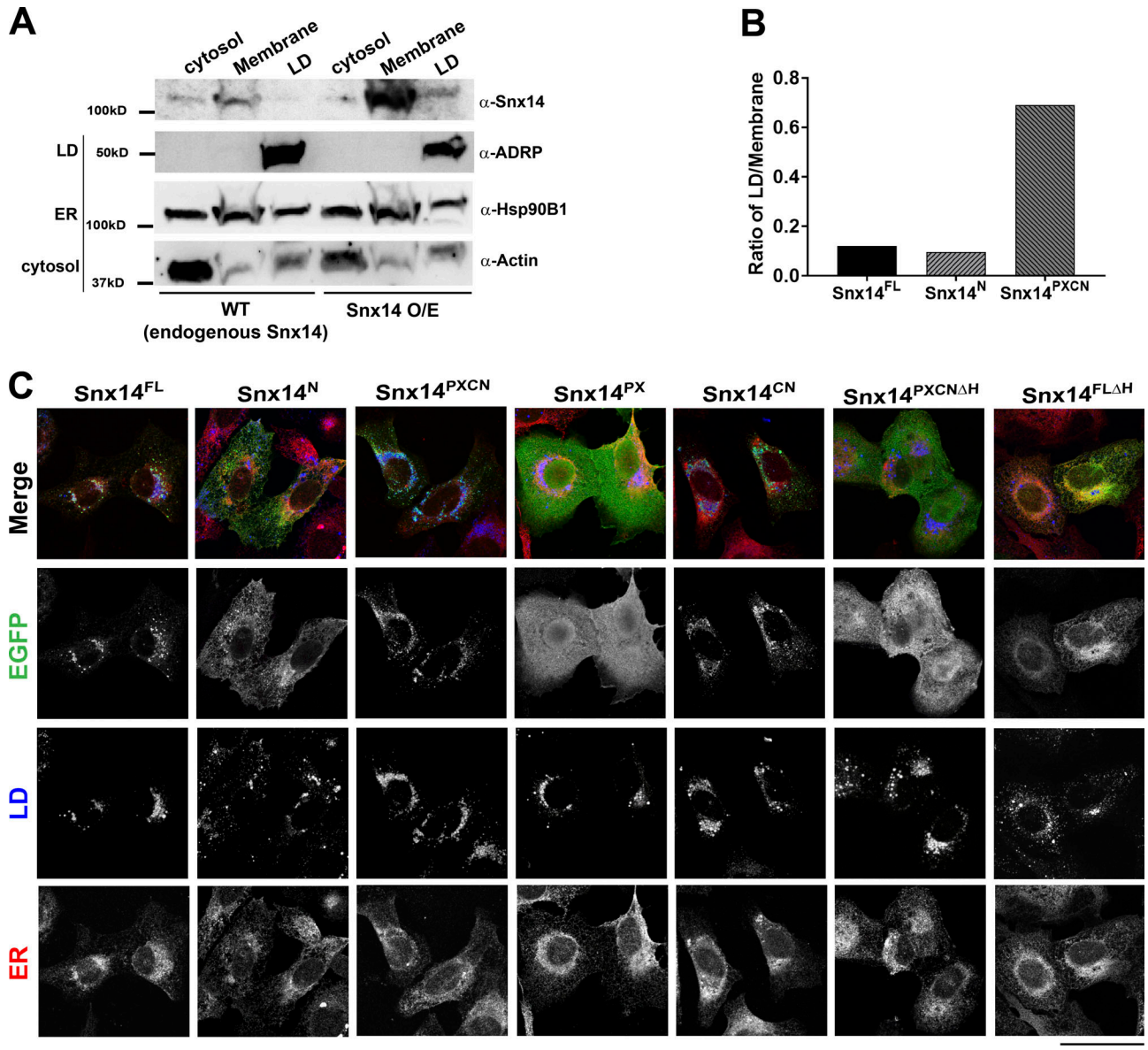

Figure S2. **Topological dissection of Snx14 LD association. (A)** Western blot with α-Snx14 antibody showing the enrichment of endogenous Snx14 in the membrane fraction similar to that in Snx14 O/E cells following OA treatment. **(B)** Quantified densitometry of bands in Fig. 2 C analyzed and plotted in the form of ratio of LD fraction over membrane fraction. **(C)** Larger field of view of Fig. 2 D showing localization of different Snx14 fragments. Scale bar = 50 µm.

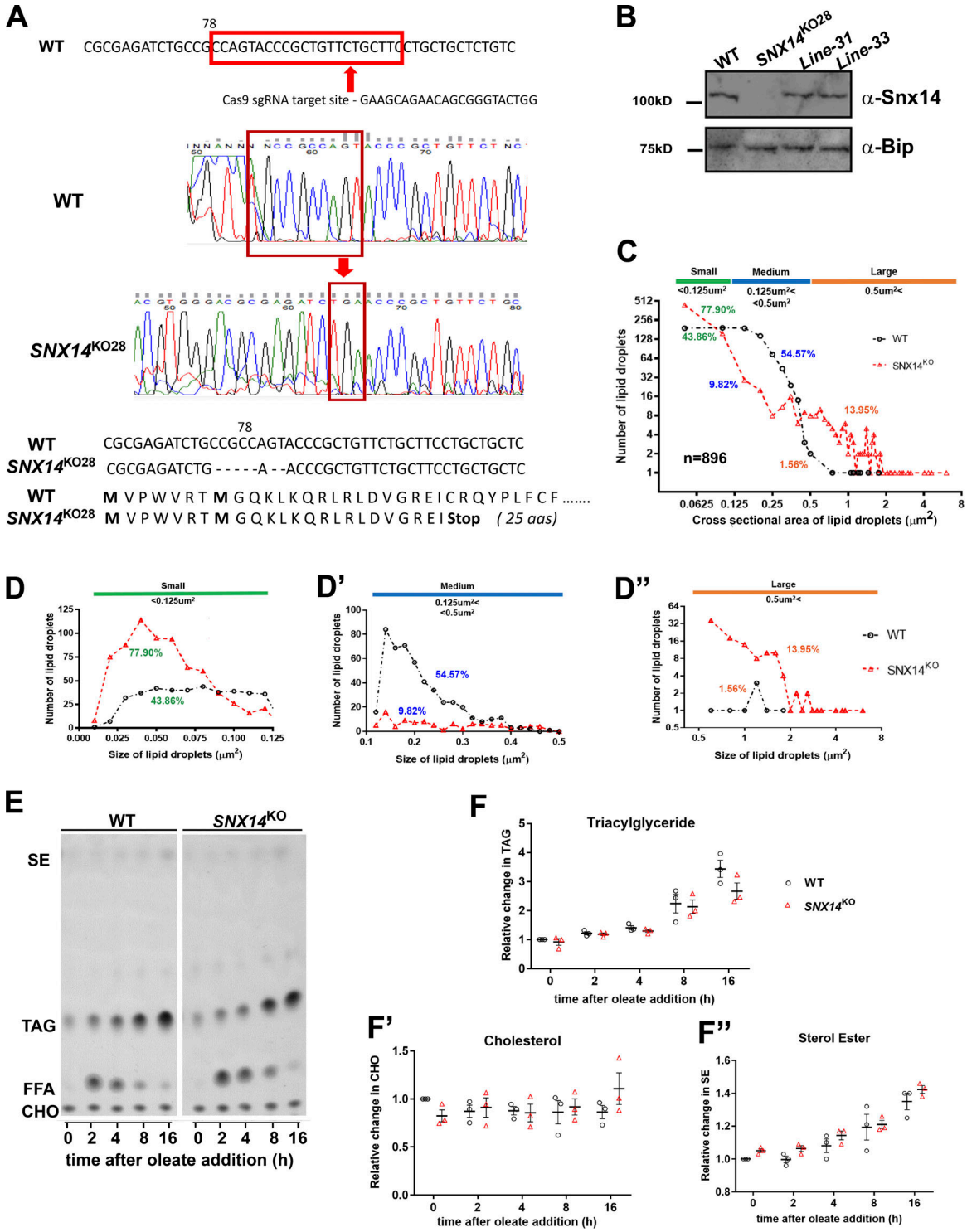

Figure S3. **Loss of SNX14 affects LD morphology but not TAG synthesis. (A)** Schematic diagram of generation of *SNX14* homozygous knockout ($SNX14^{KO}$) U2OS cells using CRISPR/Cas9 technology. The single guide RNA (sgRNA) was designed to target the first exon of the *SNX14* ORF, resulting in deletion of several bases and an inserting base "A" that led to the formation of a stop codon after 25 amino acids. **(B)** Western blot with α-Snx14 of WT and $SNX14^{KO}$ clonal line 28. Two other candidate lines (lines 31 and 33) are also shown, where Snx14 was not targeted. **(C)** The frequency of LD distributions by their cross-sectional areas in WT and $SNX14^{KO}$ cells as quantified from C (n = 896). The LD size ranges were grouped into small (size < 0.125 μm²; green bar), medium (0.125 μm² < size < 0.5 μm²; blue bar) and large (size > 0.5 μm²; orange bar) LDs. **(D)** The frequency distribution of the area of small LDs <0.125 μm² in WT and $SNX14^{KO}$ cells. **(D')** The frequency distribution of the area of medium-sized LDs >0.125 μm² and area <0.5 μm² in WT and $SNX14^{KO}$ cells. **(D'')** The frequency distribution of the area of large LDs of WT and $SNX14^{KO}$ of area >0.5 μm². **(E)** TLC plate of neutral lipids extracted from WT and $SNX14^{KO}$ cells treated with OA for 0, 2, 4, 8, and 16 h. **(F)** Quantification of relative fold-changes in TAG (F), cholesterol (CHO; F') and sterol-esters (SE; F''; normalized to cell pellet weight) of TLC from E. Values represent mean ± SEM.

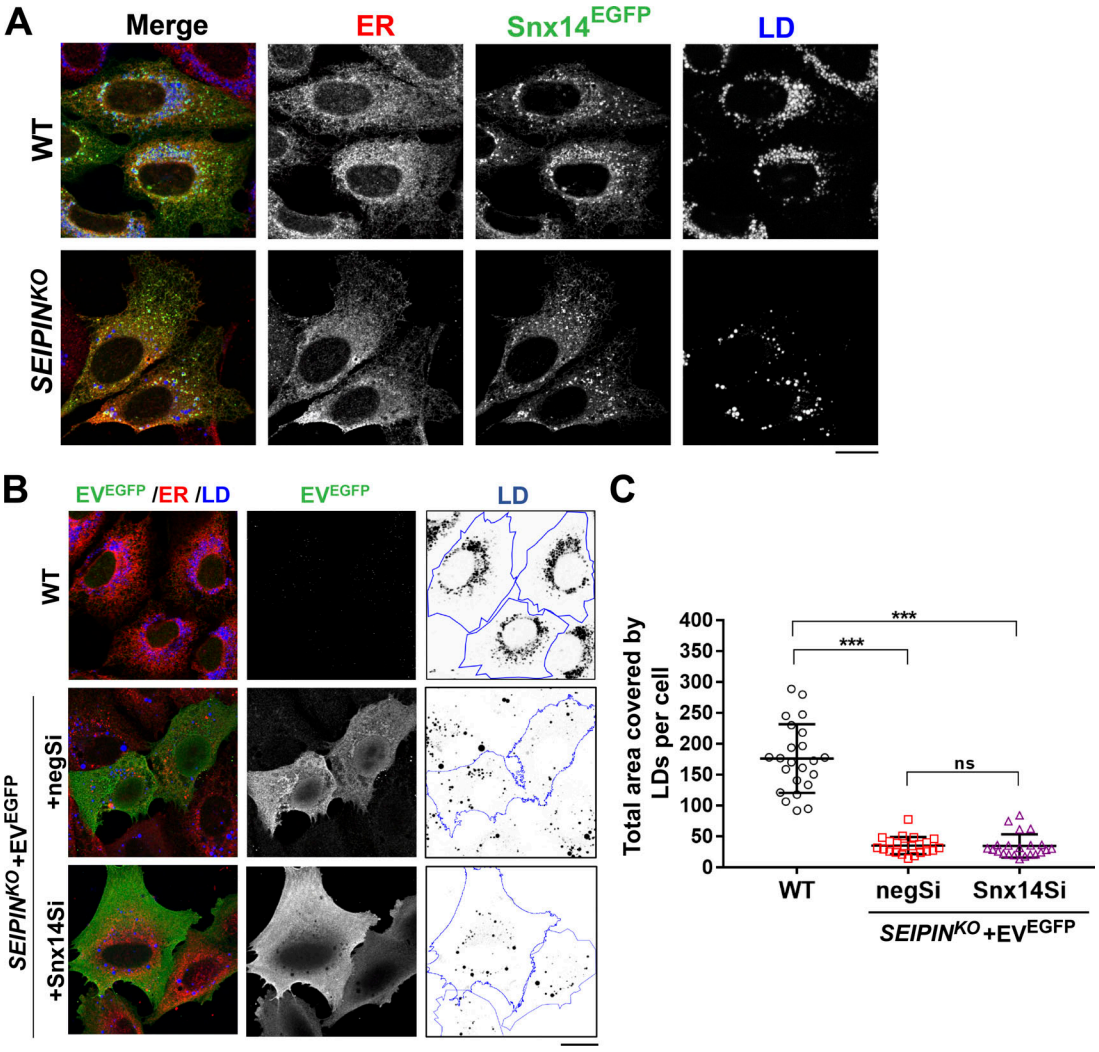

Figure S4. **Snx14 localizes to ER–LD contacts independently of Seipin. (A)** Larger field of view of Fig. 5 A showing localization of Snx14 in WT and *SEIPIN*^{KO} cells following OA treatment. Scale bar = 20 µm. **(B)** LD morphology of *SEIPIN*^{KO} transfected by negSi and Snx14Si, respectively, and comparing to that of WT in SUM159 cells. Labels were EV^{EGFP} (α-EGFP), ER (α-HSP90B1), and LDs (MDH stained, converted to grayscale, and inverted by ImageJ). Scale bar = 20 µm. **(C)** Area covered by LDs in each cell from B analyzed and plotted. Total no. of cells quantified is 23 from two different sets of experiments (***, $P < 0.0001$, one-way ANOVA with $\alpha = 0.05$). Line bars indicate mean ± SD.

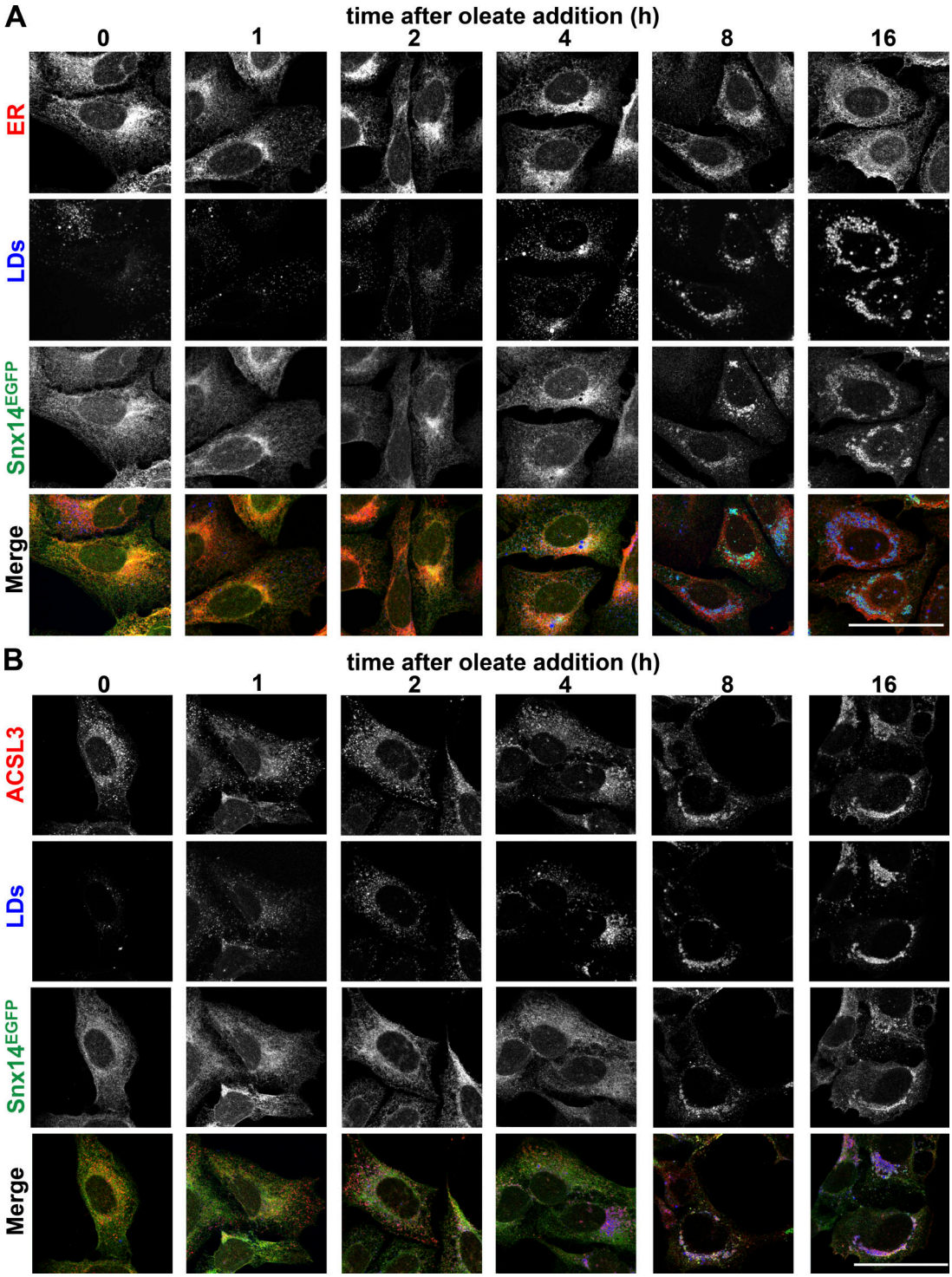

Figure S5. **Snx14 localizes to ACSL3-positive preLDs. (A)** IF staining of U2OS cell lines stably expressing Snx14^{EGFP} at set time points (t = 0, 1, 2, 4, 8, and 16 h) following OA addition. Labels were Snx14^{EGFP} (α-EGFP), the ER (α-HSP90B1), and LDs (MDH) and imaged by confocal microscopy. Scale bar = 10 µm. **(B)** IF staining of U2OS cell lines stably expressing Snx14^{EGFP} at set time points (t = 0, 1, 2, 4, 8, and 16 h) following OA addition. Labels were Snx14^{EGFP} (α-EGFP), the ACSL3 (α-ACSL3), and LDs (MDH) and imaged by confocal microscopy. Scale bar = 10 µm.

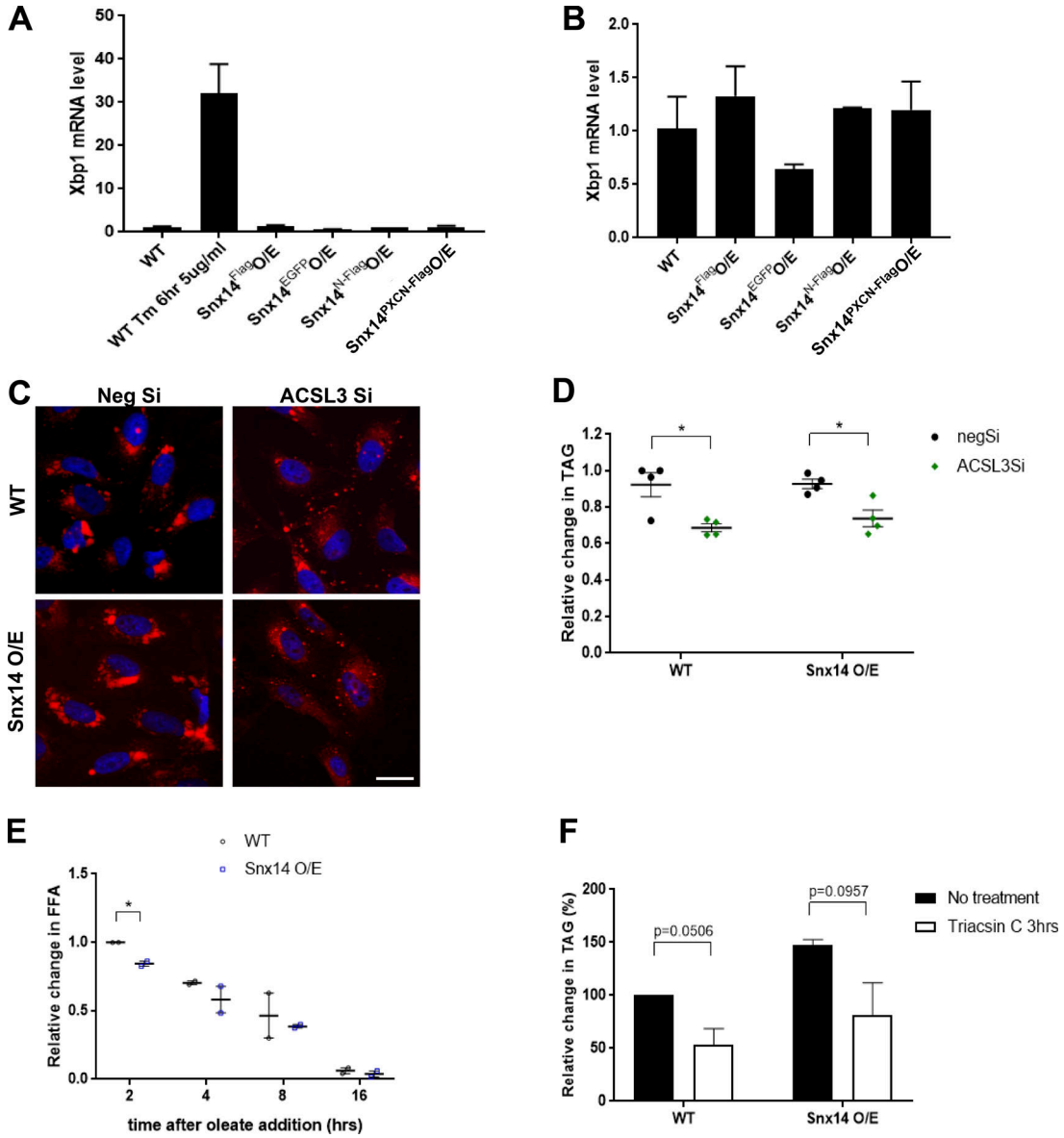

Figure S6. **Snx14 promotes LD biogenesis in an ACSL3-dependent manner. (A)** RT-PCR data monitoring mRNA levels of spliced Xbp1, an indicator of active UPR. WT Tm 6hr (5 µg/ml) indicates U2OS cells treated with tunicamycin (Tm) to induce UPR. Snx14^{Flag} O/E and Snx14^{EGFP} O/E represent stable cell lines overexpressing full-length Snx14 tagged with 3XFlag and EGFP, respectively. Snx14$^{N\text{-}Flag}$ O/E and Snx14$^{PXCN\text{-}Flag}$ O/E are the respective N-terminal and C-terminal fragments of Snx14 as depicted in Fig. 2 B which are now 3xFlag tagged and overexpressed stably in the cells. **(B)** Zoom-in of same RT-PCR data as in A but omitted the tunicamycin-treated sample. **(C)** U2OS cells treated with OA overnight receiving either scrambled (negative) siRNA or siRNA to reduce ACSL3 expression. Cells were stained with Nile Red (red) to label LDs and DAPI nuclear stain. Scale bar = 30 µm. **(D)** Quantification of relative fold change in TAG (normalized to cell pellet weight) from TLC in OA-treated WT cells and cells overexpressing Snx14^{Flag} and treated with neg siRNAs and ACSL3 siRNAs respectively. Values represent mean ± SEM (n = 4; *, P < 0.01, multiple t test by Holm–Sidak method with α = 0.05). **(E)** Quantification of relative fold-change in FFA (normalized to cell pellet weight) of TLC from Fig. 7 E. Values represent mean ± SEM (n = 3; *, P < 0.01, multiple t test by Holm–Sidak method with α = 0.05). **(F)** Quantification of percent change in TAG in WT and Snx14^{Flag} overexpressed cells when treated with triascin C for 3 h (n = 2; t test by Holm–Sidak method with α = 0.05).