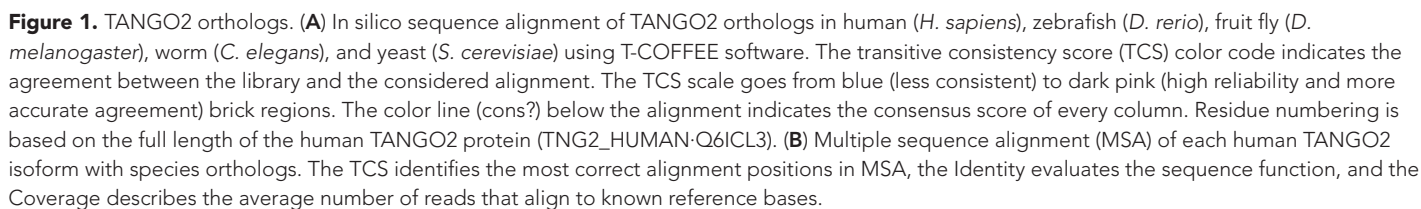


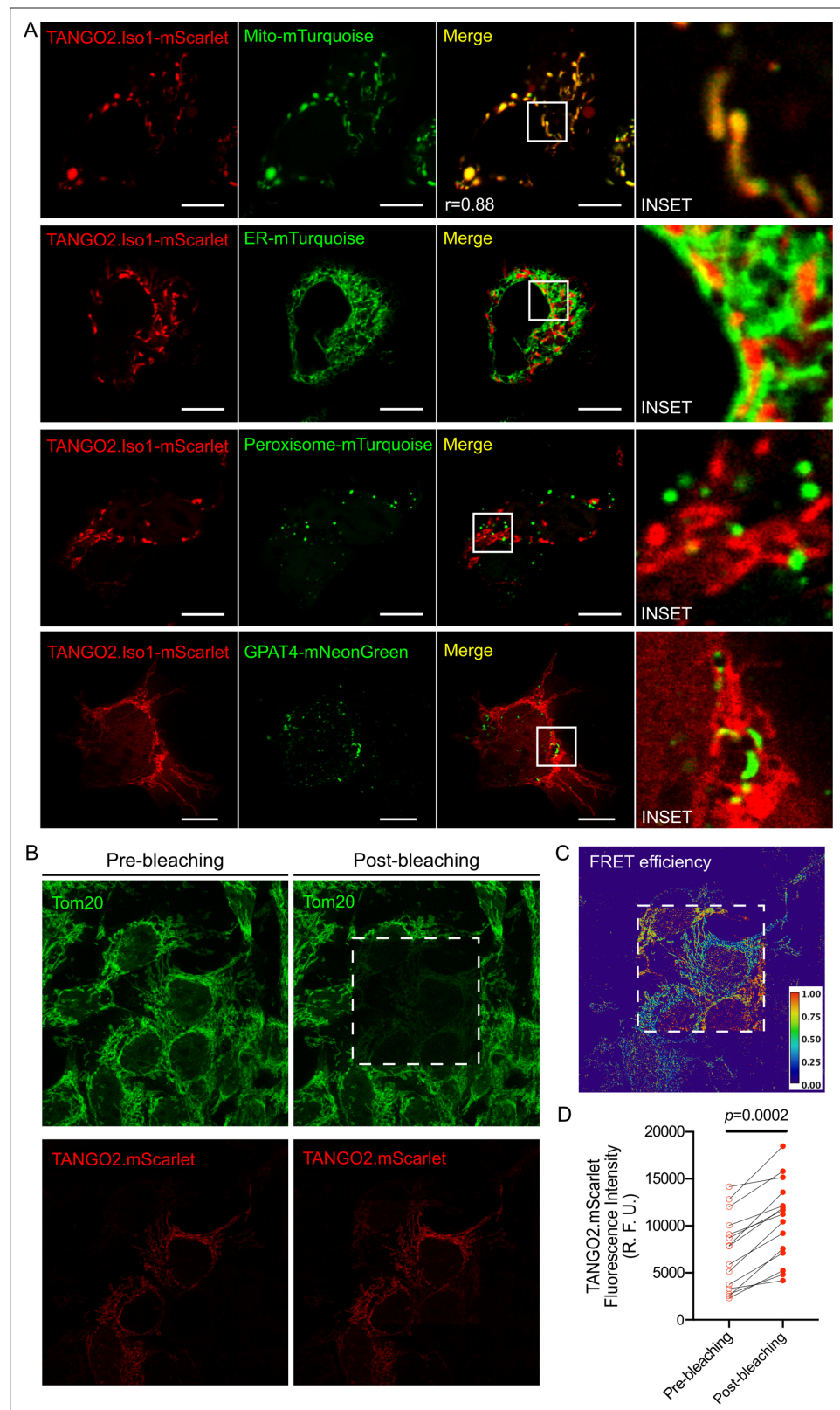
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## Figures and figure supplements

Defects in lipid homeostasis reflect the function of TANGO2 in phospholipid and neutral lipid metabolism

**Agustin Leonardo Lujan et al.**





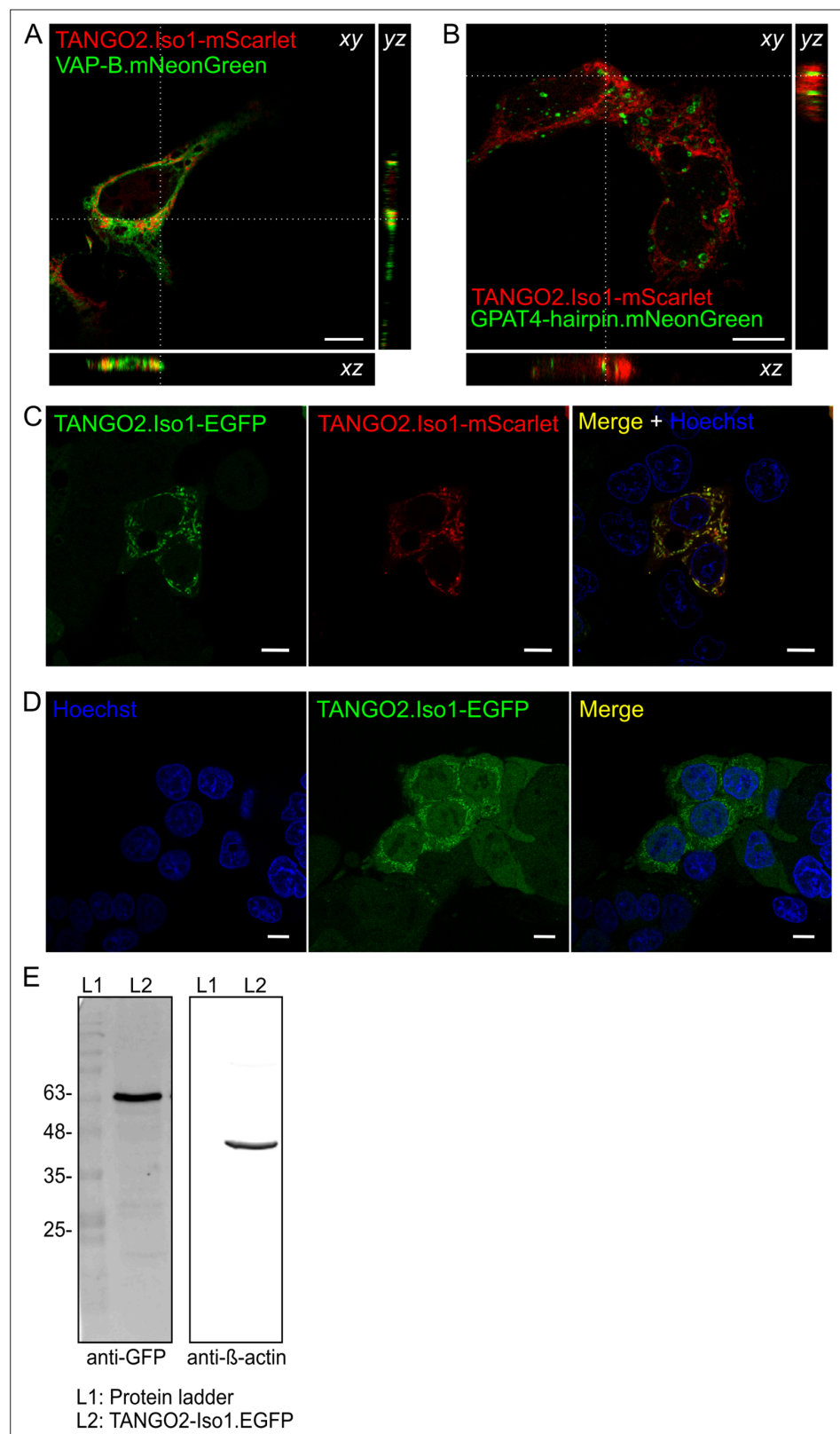
**Figure 2.** TANGO2 location in HepG2 cells. **(A)** Cells expressing TANGO2.Iso1-mScarlet co-transfected with Mitochondria-pmTurquoise2 (Mito-mTurquoise), ER-pmTurquoise2 (ER-mTurquoise), Peroxisome-SKL-mTurquoise2 (Peroxisome-mTurquoise), or GPAT4-hairpin-mNeonGreen (GPAT4-mNeonGreen) to detect lipid droplets. The yellow color indicates colocalization between both red and green labels. Pearson coefficient ( $r$ ) was calculated with

Figure 2 continued on next page

*Figure 2 continued*

the coloc2 plugin in ImageJ software. Images are representative of three independent experiments. Scale bars = 10  $\mu\text{m}$ . **(B–D)** HeLa cells were transfected with TANGO2.mScarlet, fixed, and incubated with anti-Tom20 followed by Alexa 633 secondary antibodies. **(B)** Representative image of the fluorescence intensity in the acceptor (upper panels) and donor (bottom panels) channels before (left panels) and after photobleaching (right panels) of the acceptor. **(C)** Representative map of Förster resonance energy transfer (FRET) efficiency calculated from the donor fluorescence intensity as described in Materials and methods. **(D)** Quantification of the increase in fluorescence intensity of the donor channel (TANGO2.mScarlet) after photobleaching ( $n = 15$  cells). R. F. U. means relative fluorescence units.



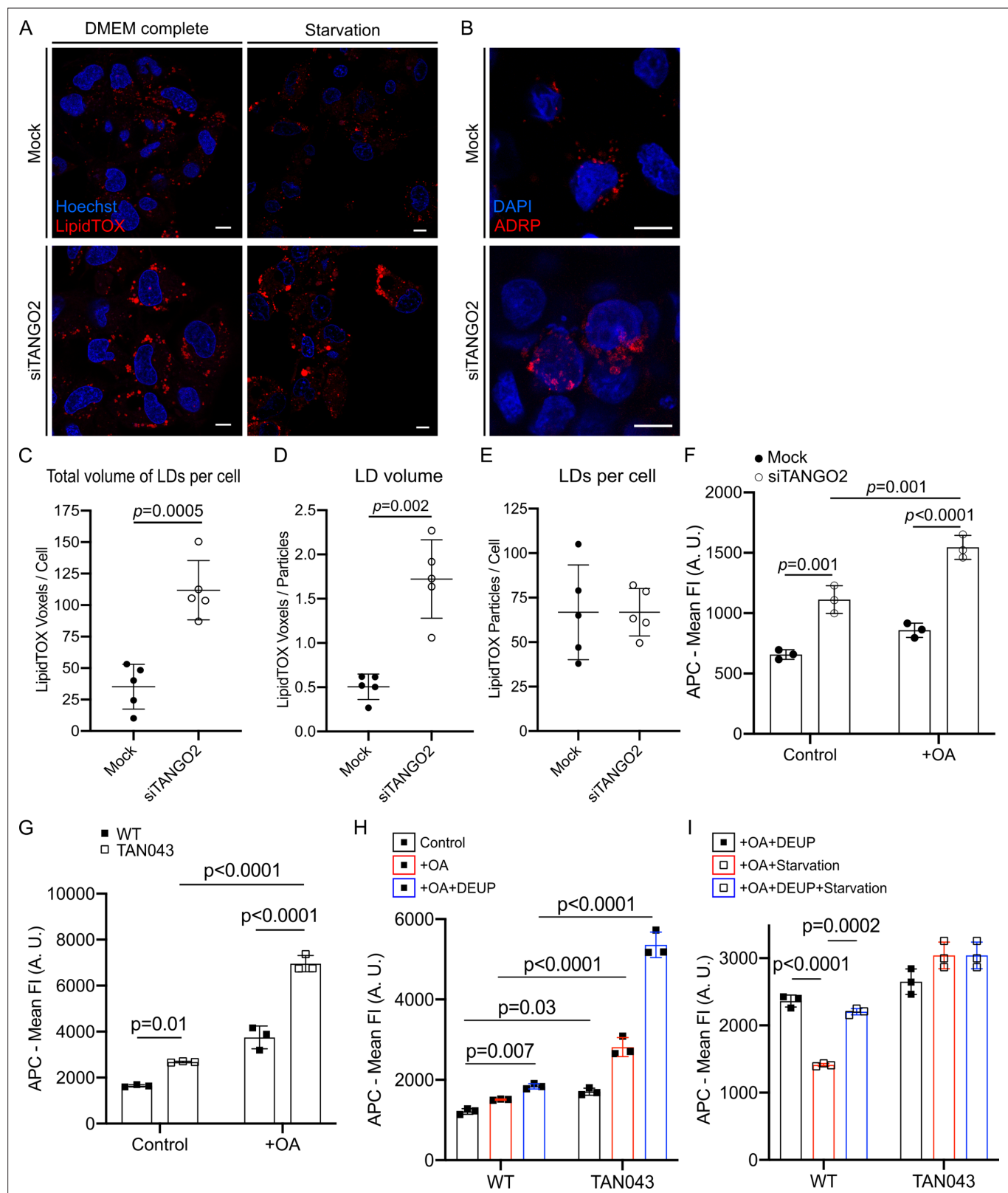


**Figure 2—figure supplement 1.** Intact expressed tagged TANGO2 is in close proximity to endoplasmic reticulum (ER) and lipid droplets (LDs). (**A**) HepG2 cells expressing TANGO2.Iso1-mScarlet and co-transfected with VAP-B.mNeonGreen to detect ER membranes. The dotted line in the XY plane shows the slice projected in the YZ and XZ axes. The yellow color indicates colocalization between red and green labels in the different projections.

Figure 2—figure supplement 1 continued on next page

*Figure 2—figure supplement 1 continued*

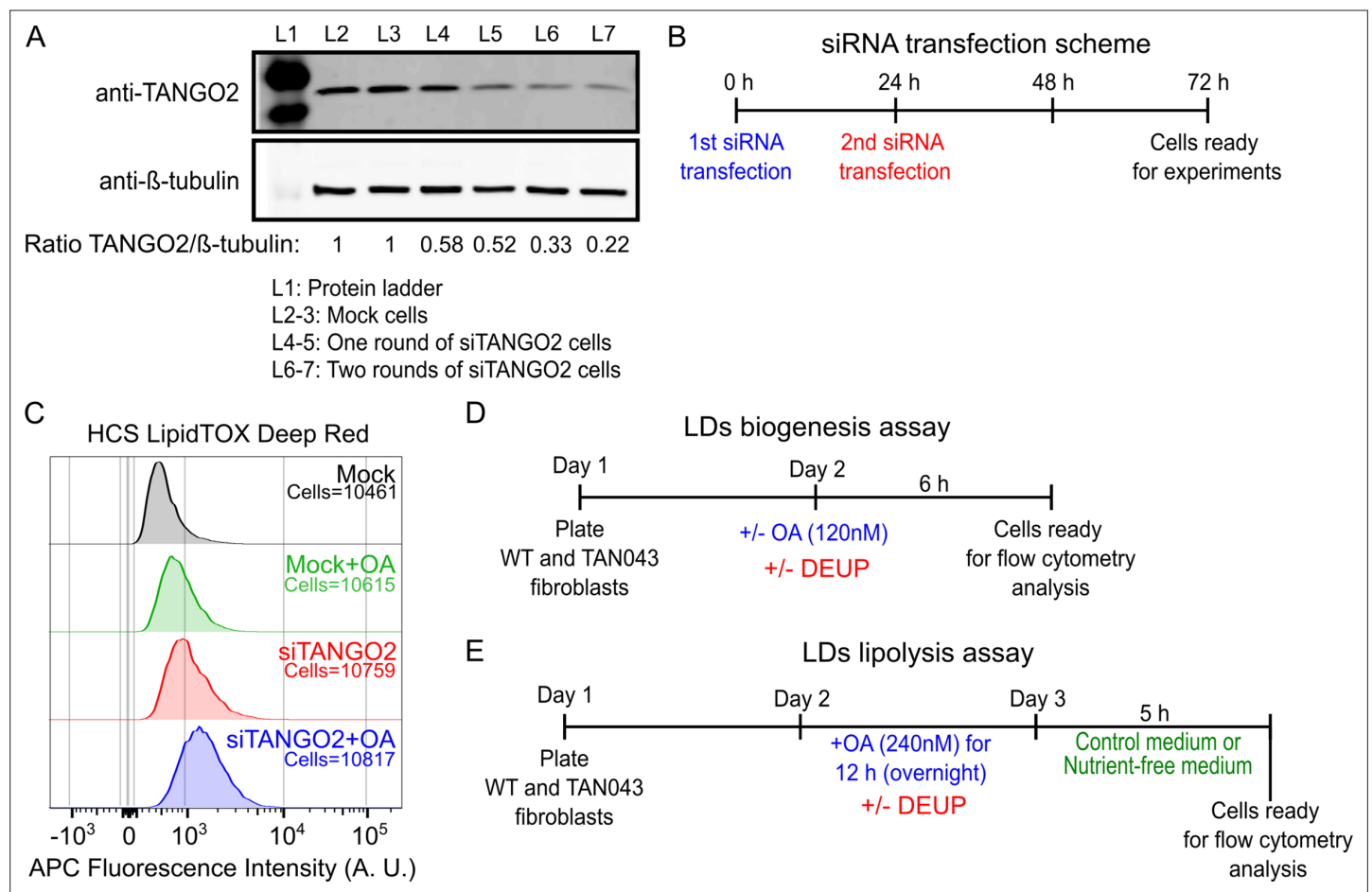
Scale bars = 10  $\mu\text{m}$ . **(B)** HepG2 cells expressing TANGO2.Iso1-mScarlet and co-transfected with GPAT4-hairpin. mNenonGreen to detect LDs (LDs do not have a membrane). The dotted line in the XY plane shows the slice projected in the ZY and XZ axes. The yellow color indicates colocalization between red and green labels in the different projections. Scale bars = 10  $\mu\text{m}$ . **(C)** TANGO2.Iso1-EGFP HepG2 stable cells were transiently transfected with TANGO2.Iso1-mScarlet (red), and labeled with Hoechst (blue). Merge shows all overlapping channels. **(D)** TANGO2.Iso1-EGFP (green) HepG2 stable cell was labeled with Hoechst (blue). **(E)** Immunoblot analysis of TANGO2.Iso1-EGFP HepG2 stable cell line incubated with anti-GFP and anti- $\beta$ -actin. Anti- $\beta$ -actin was used as a loading control.



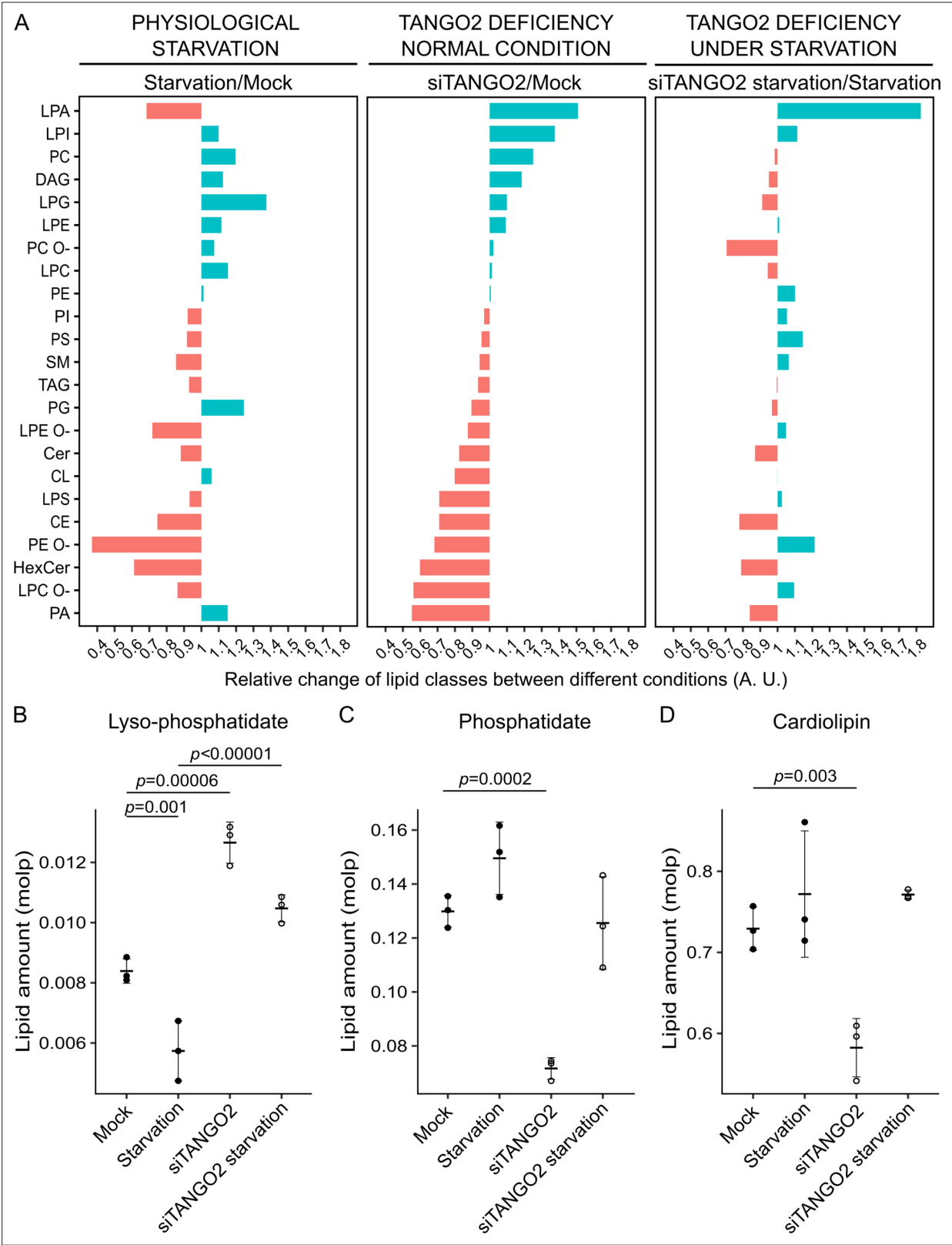
**Figure 3.** TANGO2 depletion affects lipid droplets' (LDs) size, biogenesis, and lipolysis. **(A)** Confocal images of HCS LipidTOX Deep Red marker (red) and Hoechst-33342 (blue) in mock (top) and TANGO2-depleted (bottom) HepG2 cells cultured in control conditions (Dulbecco's Modified Eagle's Medium [DMEM] complete; left panel) or starved of nutrients (starvation; right panel). Scale bars = 10  $\mu$ m. **(B)** Mock (top) and TANGO2-depleted (bottom) cells in control conditions were fixed and incubated with anti-ADRP (red) and DAPI (blue). Scale bars = 10  $\mu$ m. **(C–E)** Quantification of LDs

*Figure 3 continued*

in mock ( $n = 67$  cells) and TANGO2-depleted ( $n = 75$  cells) cells in five samples for each condition by an ImageJ macro developed in our laboratory (open code access in Materials and methods). **(C)** Total volume of LDs present per cell. **(D)** Volume of each LD in a cell. **(E)** Number of LDs per cell. **(F, G)** Quantification of total LD volume in DMEM complete medium (control) and supplemented with 120 nM Oleic acid (+OA) using HCS LipidTOX Deep Red marker by flow cytometry. **(F)** Mean fluorescence intensity (mean FI) of allophycocyanin (APC) detected in Mock and TANGO2-depleted HepG2 cells in three different samples of each condition. **(G)** Mean fluorescence intensity (mean FI) of APC detected in wild-type (WT) and TANGO2-deficient disease (TAN043) fibroblasts in three different samples of each condition. **(H)** Quantification of total LD's volume in DMEM complete medium (control), supplemented with 120 nM Oleic acid (+OA), or supplemented with 120 nM OA and 500  $\mu$ M diethylumbelliferyl phosphate (DEUP) in WT and TAN043 fibroblasts by flow cytometry. **(I)** Quantification of total LD volume after supplementation with 240 nM OA for 12 hr, washed and starved for 5 hr with DEUP in WT and TAN043 fibroblasts by flow cytometry. Images and graphs are representatives of three independent experiments. In graphs, boxes and bars are the mean  $\pm$  standard deviation (SD). A.U. means arbitrary units.



**Figure 3—figure supplement 1.** Depletion of TANGO2 in HepG2 cells. **(A)** Immunoblot analysis of HepG2 wild-type (L2–3) cells silenced after one (L4–5) or two (L6–7) rounds of TANGO2 siRNA transfection. **(B)** Double siRNA transfection scheme used to reduce endogenous TANGO2 levels. **(C)** Mock and TANGO2-depleted HepG2 cells maintained in control conditions or with 120 nM Oleic acid (OA), and incubated with HCS LipidTOX Deep Red for 30 min before flow cytometry analysis. Neutral lipids were detected by measuring the fluorescence intensity of allophycocyanin (APC). **(D)** Scheme of the method used for the neutral lipids biogenesis analysis in WT and TAN043 fibroblasts. **(E)** Scheme of the method used for the neutral lipids lipolysis analysis induced by nutrient fasting in WT and TAN043 fibroblasts.

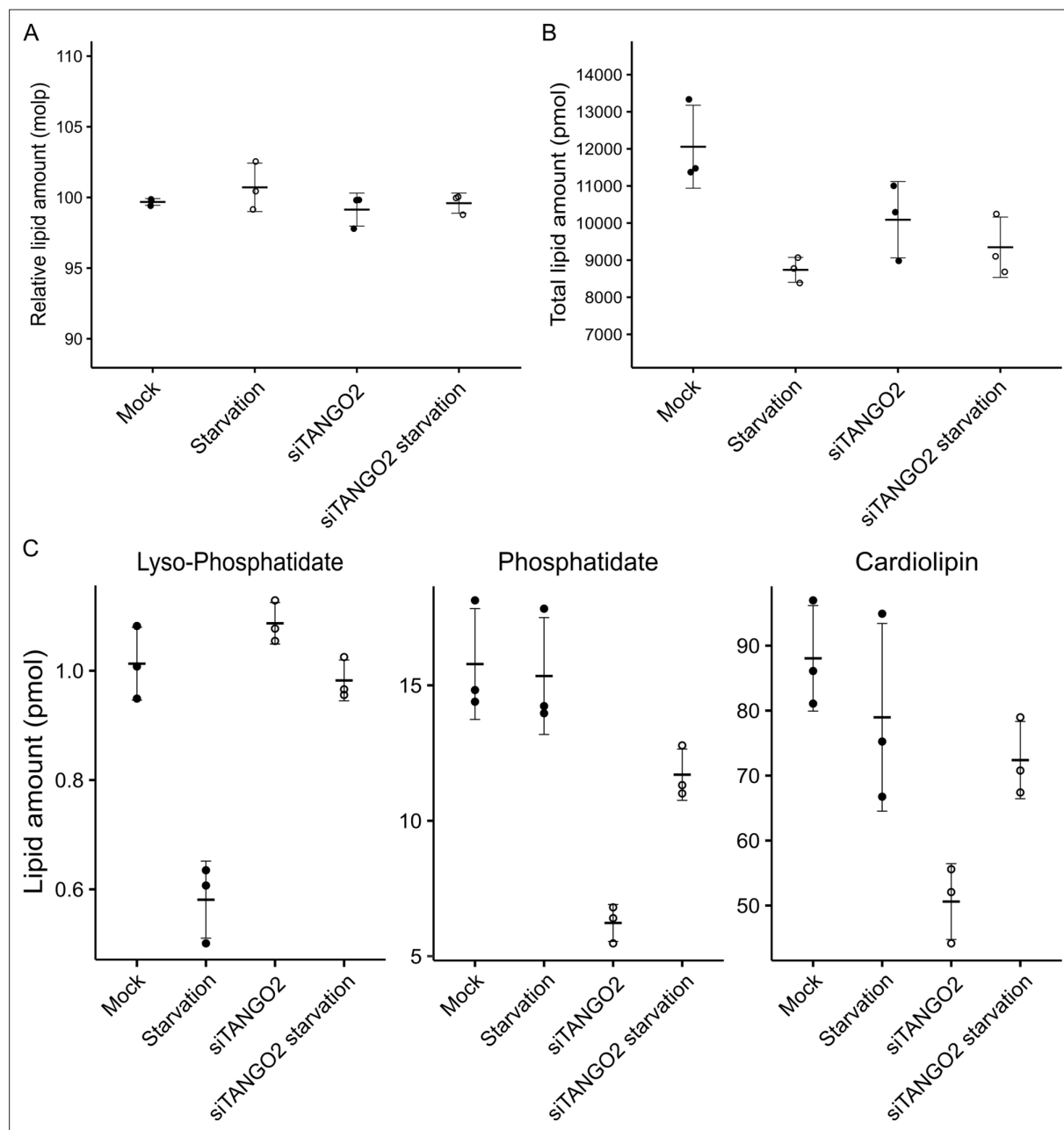


**Figure 4.** Lipidomics of TANGO2-depleted cells for comparison with control and nutrient-starved cells. **(A)** Relative change of lipid class according to different conditions: physiological starvation (left panel), TANGO2-depleted cells in control conditions (middle panel), and TANGO2-depleted cells upon nutrient starvation (right panel). **(B)** Quantification of lysophosphatidic acid (LPA) changes in mock and TANGO2-depleted cells expressed in mole percent (molp) fractions. **(C)** Quantification of phosphatidic acid (PA) changes in mock and TANGO2-depleted cells expressed in molp fractions. *Figure 4 continued on next page*

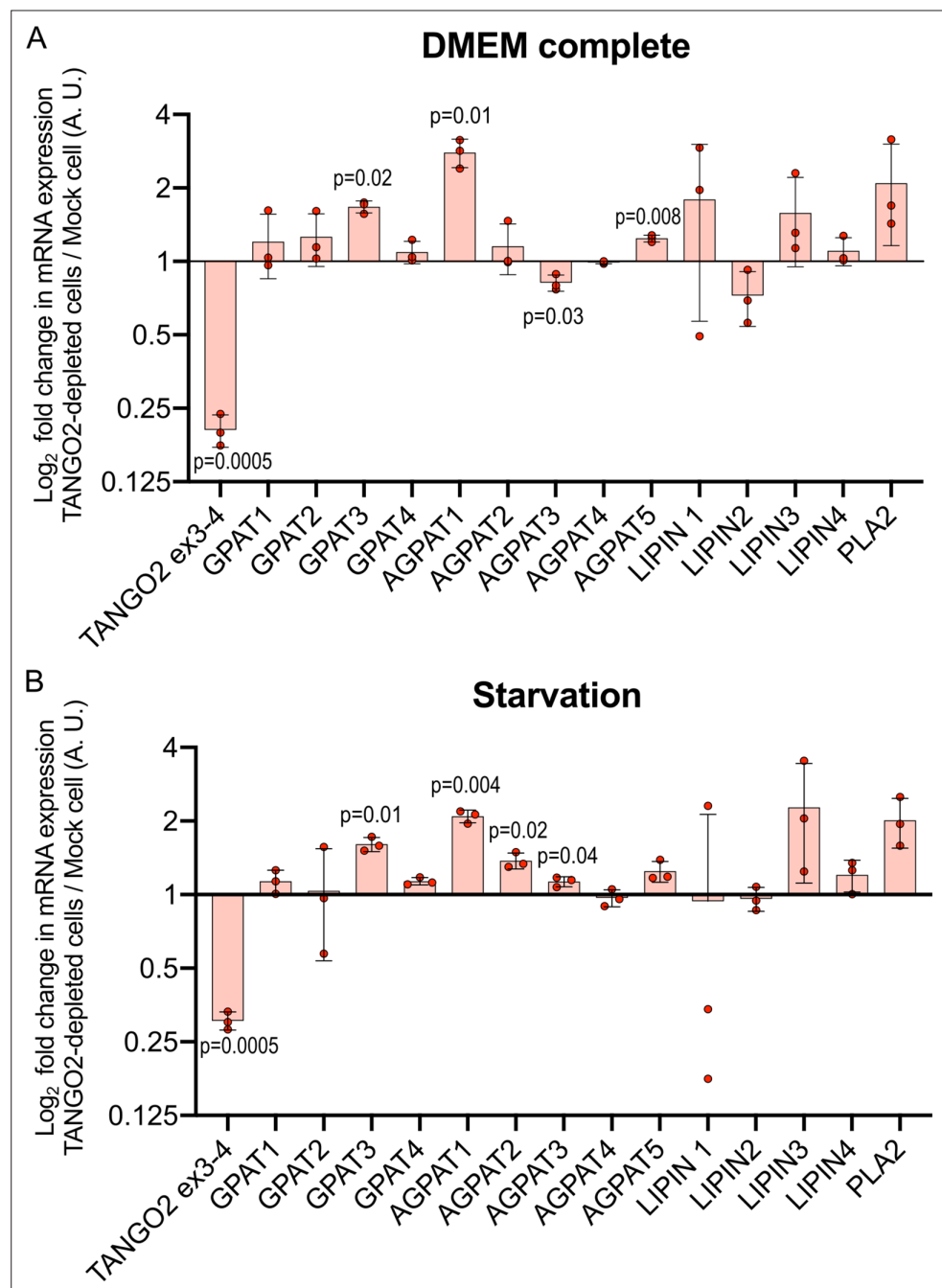


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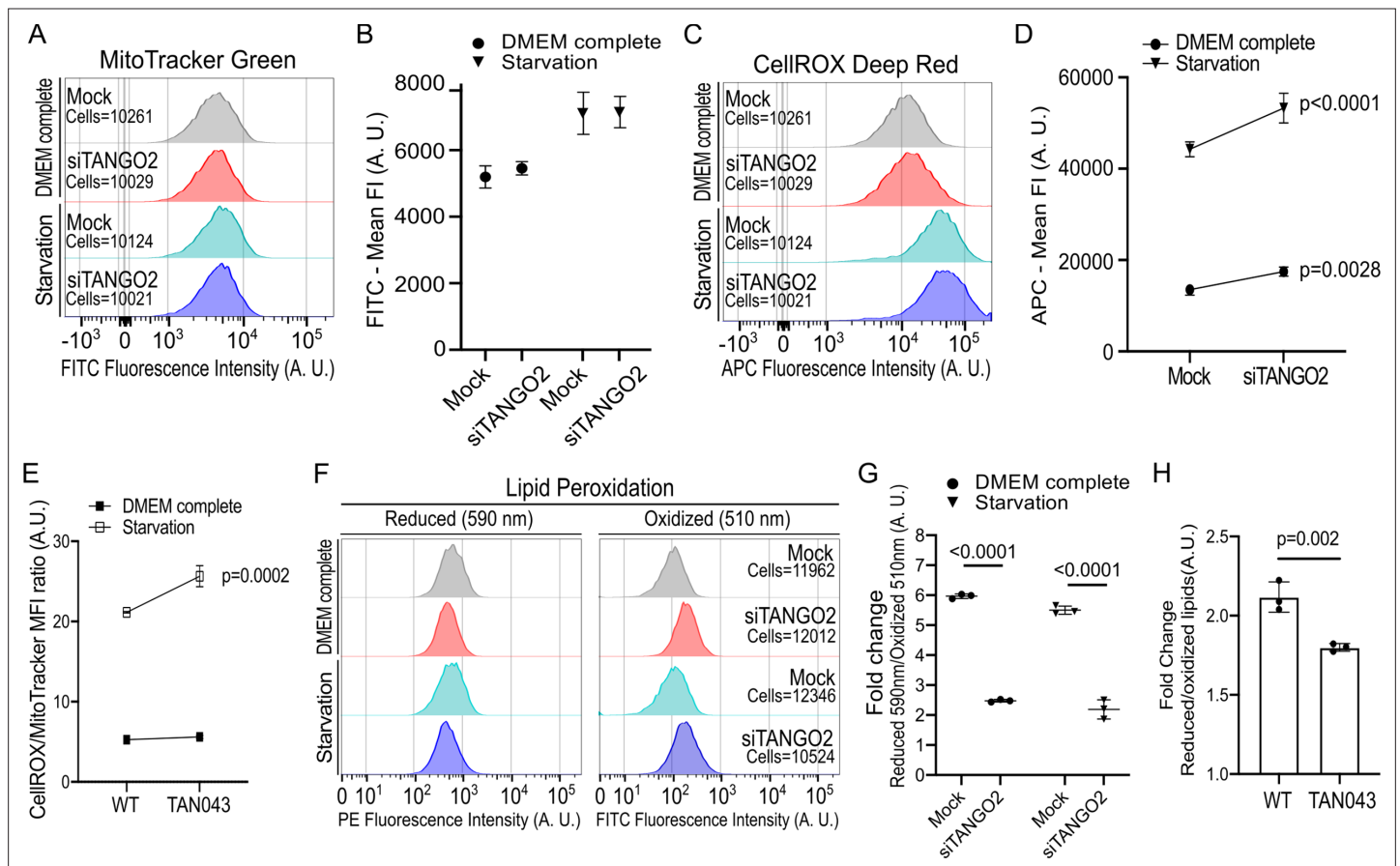
**(D)** Quantification of CL changes in mock and TANGO2-depleted cells expressed in molp fractions. Data are representative of three independent experiments. In graphs, boxes are the mean  $\pm$  standard deviation (SD).



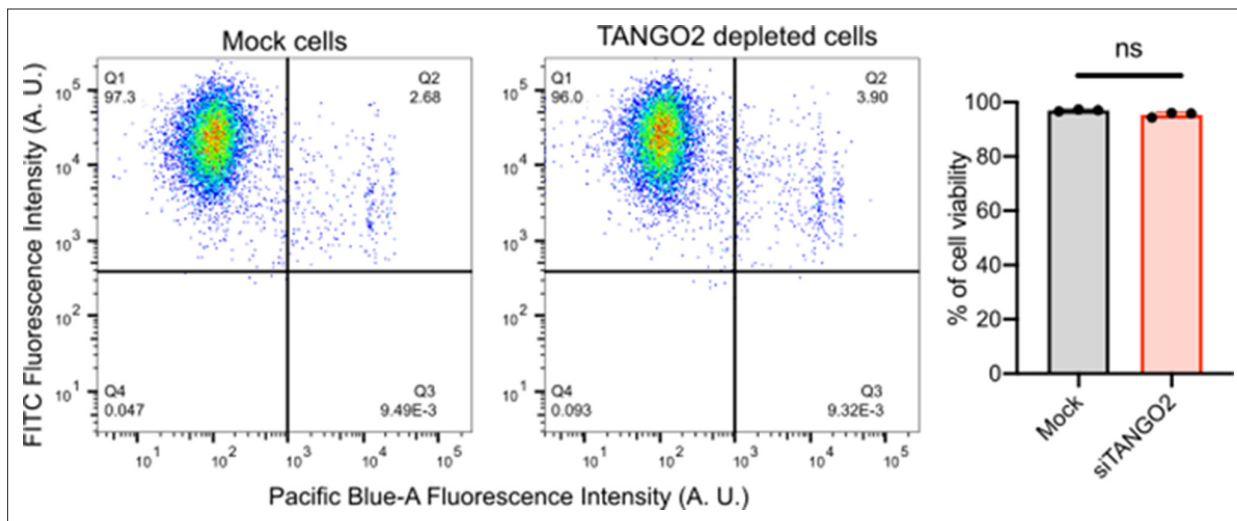
**Figure 4—figure supplement 1.** Absolute values comparison of lipids in HepG2 mock and TANGO2-depleted cells. **(A)** Relative levels of lipids in mock and TANGO2-depleted cells in control and nutrient-starved conditions in mole percent fraction (molp). **(B)** Quantification of the absolute amount of lipids in mock and TANGO2-depleted cells in control and nutrient-starved conditions in pico-mole (pmol). **(C)** Quantification of the absolute amount of lysophosphatidic acid (LPA), phosphatidic acid (PA), and cardiolipin (CL) in mock and TANGO2-depleted cells in control and nutrient-starved conditions in pmol. In graphs, boxes are the mean  $\pm$  standard deviation (SD).



**Figure 5.** TANGO2 depletion impacts enzymes of lipid metabolism. Reverse transcription quantitative polymerase chain reaction (RT-qPCR) analyses of TANGO2-depleted HepG2 cells compared to mock cells in normal (A) and starvation (B) conditions. Three biological repeats were performed for each condition. Values were normalized to GAPDH and graphed as log fold change relative to Mock cells of each condition. Statistical significance was determined using a simple unpaired Student's t-test with subsequent Welch's correction analysis between TANGO2-depleted and Mock cells. In graphs, boxes are the mean  $\pm$  standard deviation (SD). A.U. means arbitrary units.



**Figure 6.** TANGO2-depleted cells exhibit increased reactive oxygen species (ROS) levels and lipid peroxidation. (A–D) Mock and TANGO2-depleted cells were incubated in control conditions (Dulbecco's Modified Eagle's Medium [DMEM] complete) or in a low-nutrient medium (Starvation) for 4 hr. Cells were incubated with CellROX Deep Red to detect ROS levels and MitoTracker Green to monitor mitochondria mass. (A) Mitochondria mass was detected by measuring the fluorescence intensity of fluorescein isothiocyanate (FITC) by flow cytometry. (B) The mean fluorescence intensity (Mean FI) of FITC was measured in three different samples of each condition. (C) Cellular ROS levels were detected by measuring the fluorescence intensity of allophycocyanin (APC) by flow cytometry. (D) The mean fluorescence intensity of APC was measured in three different samples of each condition. (E) Ratio between ROS levels (CellROX) and mitochondria mass (MitoTracker) was measured in WT and TAN043 fibroblasts by flow cytometry in three independent experiments. (F) Lipid peroxidation in HepG2 and fibroblasts was measured by detecting the mean fluorescence intensity change between PE and FITC spectral emissions by flow cytometry. (G) The fold change shows the ratio between reduced and oxidized lipid species in Mock and TANGO2-depleted HepG2 cells in the control and nutrient-free medium. (H) The fold change shows the ratio between reduced and oxidized lipid species in WT and TAN043 fibroblasts. In graphs, bars and boxes are the mean  $\pm$  standard deviation (SD). A.U. means arbitrary units.



**Figure 6—figure supplement 1.** Cell viability of TANGO2-depleted cells. Mock and TANGO2-depleted cells were incubated with CellTrace Calcein Green AM (fluorescein isothiocyanate, FITC) and DAPI (Pacific Blue-A) to determine cell viability by flow cytometry. Each dot in the dot plot graph represents one cell. Each dot in the column graph represents one of three independent experiments. A.U. means arbitrary units.