SUPPLEMENTAL FIGURES

Probing the Global Cellular Responses to Lipotoxicity Caused by Saturated Fatty Acids

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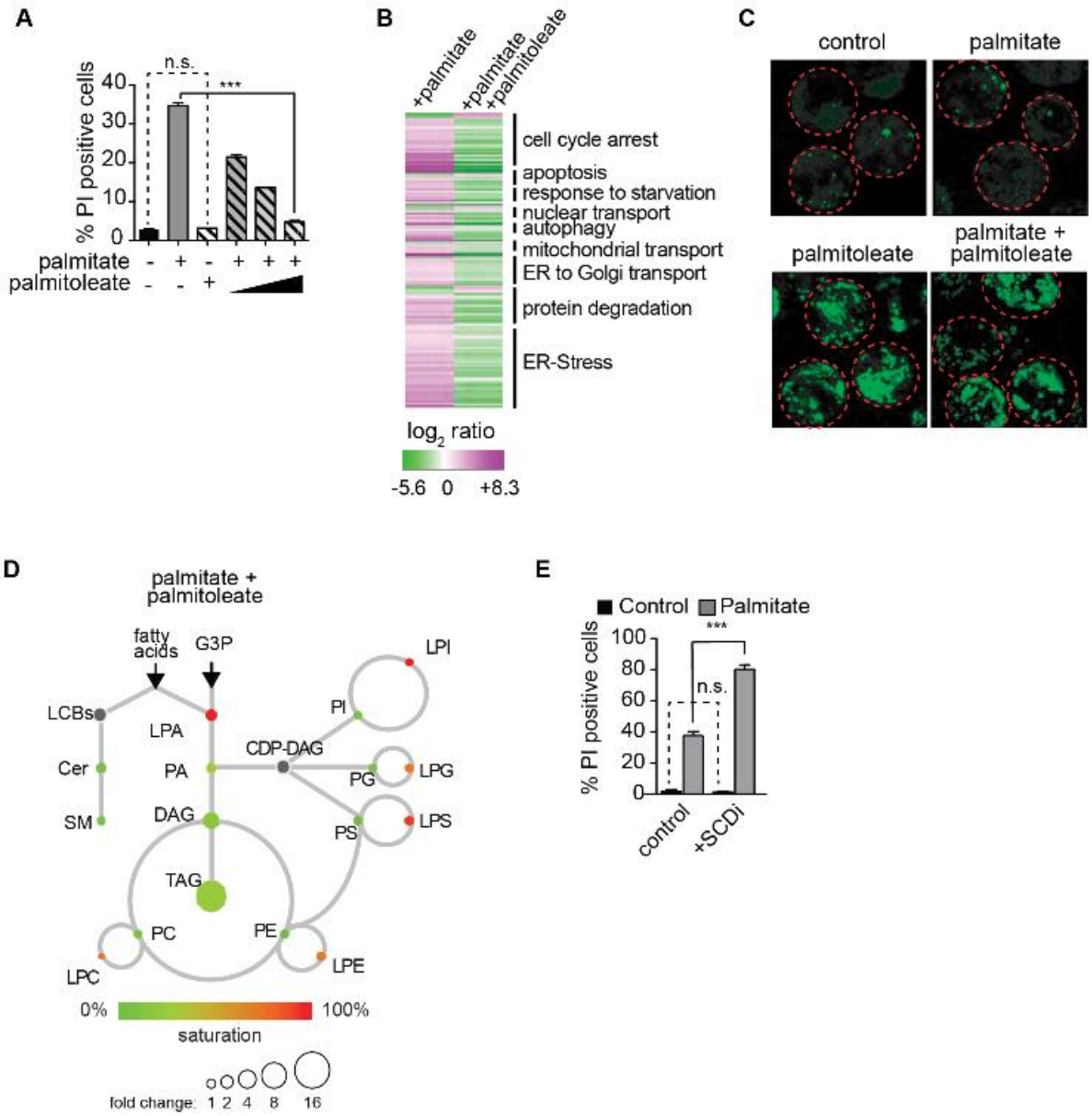

Figure S1. Lipidome of Palmitoleate-treated Cells, Related to Figure 1.

(A) Palmitoleate treatment protects K562 cells from palmitate-induced cell death. Propidium-iodide staining of K562 cells treated with palmitate (0.25 mM), palmitoleate (0.25 mM), or a mixture of palmitate (0.25 mM) and palmitoleate (0.06, 0.12 and 0.25 mM) for 24 h. n=3 for each treatment. ***$p < 0.001$; n.s., non-significant.

(B) Palmitoleate treatment protects against palmitate-induced activation of the UPR. RNAseq data of wild-type K562 cells treated with 0.2 mM palmitate for 20 h (left lane). Genes are shown as $\log_2$ ratio compared to the control and designated as being upregulated (violet) and downregulated (green) by palmitate ($p < 0.01$). Rigth lane, genes are shown as $\log_2$ ratio compared to the palmitate treated sample and divided in upregulated (violet) and downregulated (green) by the

palmitate-palmitoleate mix ($p < 0.01$). The annotation was performed using the app ClueGO of Cytoscape.
(C) Palmitoleate increases flux of palmitate into TG and lipid droplets. K562 cells were treated with palmitate (0.25 mM), palmitoleate (0.25 mM), or a mixture of palmitate (0.25 mM) and palmitoleate (0.25 mM) for 24 h. Lipid droplets were stained live with Bodipy.
(D) As in Figures 1B and 1C, the lipidome of K562 cells treated with a mixture of 0.2 mM palmitate and 0.2 mM palmitoleate. The scheme shows the relative levels of incorporation of exogenous fatty acids into sphingolipid and glycerophospholipid. Lipid classes identified by LC-MS^2 analysis are presented as color-coded circles. The lipid species were designated as saturated if all of its fatty acid chains were saturated, or unsaturated if it had at least one unsaturated fatty acid chain. The percentage of saturated lipid species is shown for each class from green (low saturation) to red (high saturation). Lipid classes not identified are shown in grey. The size of the circles is set to the arbitrary unit of 1 for the control cells. G3P: glycerol-3-phosphate; LPA: lyso-phosphatidic acids; PA: phosphatidic acids; DAG: diacylglycerol; TAG: triacylglycerol; PC: phosphatidylcholine: PE: phosphatidylethanolamine; LPE: lyso-phosphatidylethanolamine; LPC: lyso-phosphatidylcholine; PS: phosphatidylserine; LPS: lyso-phosphatidylserine; PI: phosphatidylinositol; LPI: lyso-phosphatidylinositol; PG: phosphatidylglycerol; LPG: lyso-phosphatidylglycerol.
(E) Inhibition of SCD1 aggravates palmitate-induced cell death. Propidium-iodide staining of K562 cells treated with palmitate (0.25 mM) for 24 h. Cells were pretreated with control vehicle or SCD inhibitor (4 μM) for 1-2 hours prior to palmitate treatment. n=3 for each treatment. ***$p < 0.001$; n.s., non-significant.

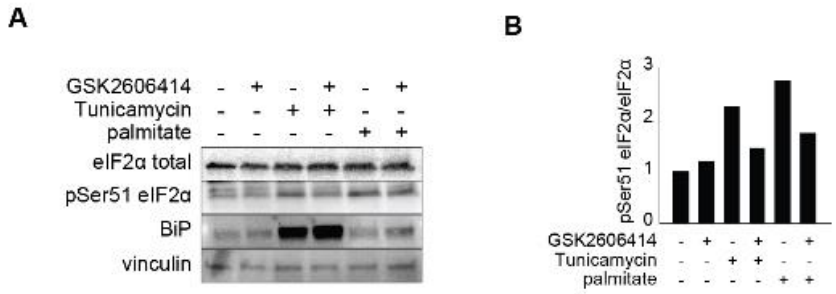

Figure S2. Palmitate-Induced Lipotoxicity in K562 Human Leukemia Cells, Related to Figure 2.

(A) GSK2606414 reduces palmitate- and tunicamycin-induced phosphorylation of eIF1α. Western blot of K562 whole cell lysates after treatment with tunicamycin (1.5ug/ml) or palmitate (0.2mM) for 20 h.

(B) Quantification of western blot in Figure S2A.

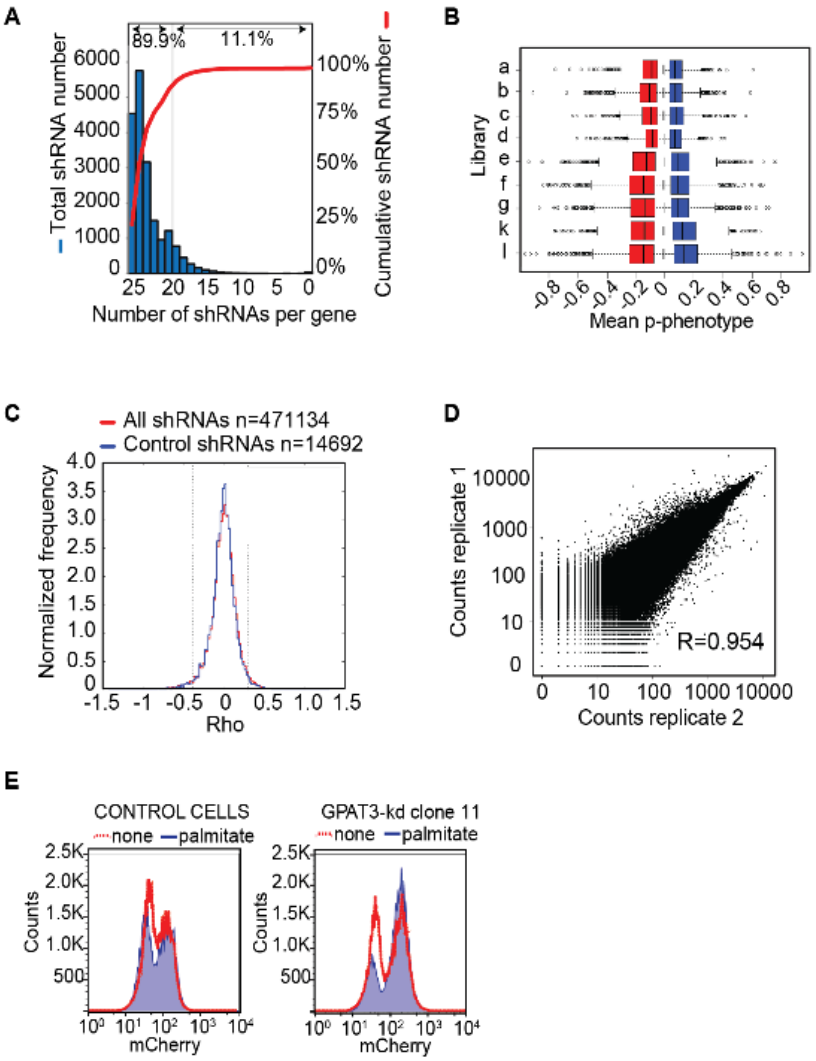

Figure S3. shRNA Screen Reveals Genes Involved in Response to Palmitate, Related to Figure 3.

(A) The shRNA library has high genome coverage. The graph shows the number of shRNAs per gene. The library covers ~19,000 ORFs. ~90% of the ORFs are targeted by at least 20 different shRNAs.

(B) The nine libraries (from “a” to “l”) have different performances. The performances of each library were evaluated by analyzing each library individually. Box plot of the 50 shRNAs having the strongest phenotype in each library.

(C) The control shRNAs and the targeting shRNAs show similar distribution.

(D) The screen biological replicates highly correlate. As example, the correlation among the shRNA counts (from sequencing) of two biological replicate samples, the palmitate treated samples, is shown.

(E) Competition assay is used to validate selected candidates in Figure 2C. Left panel, distribution of uninfected (cherry negative) and cells infected with control shRNAs (cherry positive), before (red line) and after palmitate treatment (blue line). Right panel, distribution of uninfected (cherry negative) and cells infected with GPAT3 shRNAs (cherry positive), before (red line) and after palmitate treatment (blue line).

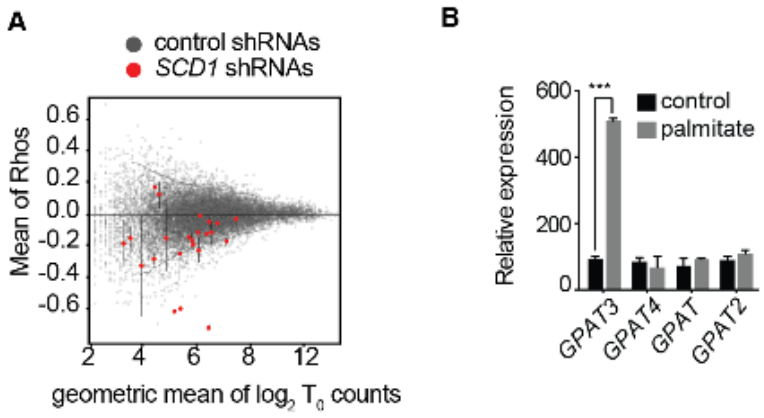

Figure S4. Palmitate Activates Expression of *GPAT3*, Related to Figure 4.

(A) An example of shRNA distribution on a sensitizing candidate is shown. SCD1 targeting shRNAs are shown in red. The phenotype (y-axis) is the mean of the two biological replicate screens. Control shRNAs are shown in grey.

(B) Palmitate treatment increases transcription of *GPAT3*. Relative expression for *GPAT3*, *GPAT4*, *GPAT* and *GPAT2* in K562 cells treated with palmitate (0.2 mM) for 20 h performed by qPCR. n=3 for each treatment; ***$p < 0.001$.

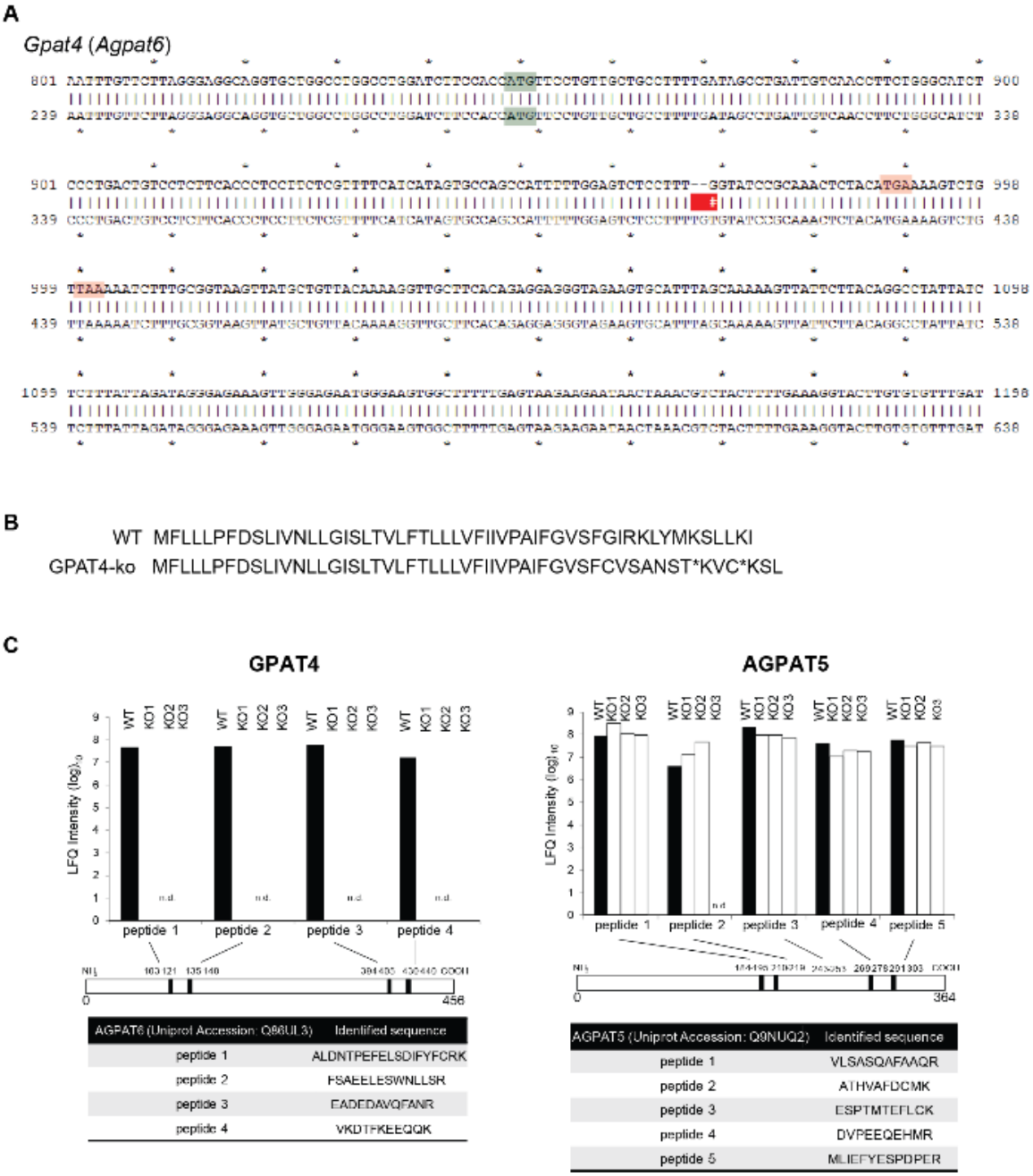

Figure S5. Generation of GPAT4KO Cell Line, Related to Figure 5.

(A) *Gpat4* nucleotide sequence of GPAT4-ko (top) and wild-type (WT) (bottom). CRISPR/Cas9-mediated genome editing resulted in deletion of two nucleotides (in red). Transcription start site is shown in green. The frame-shift mutation generated two premature stop codons (shown in orange).

(B) Amino acid sequence of WT and GPAT4-ko cells. The frame-shift mutation generated a two premature stop codons (*).

(C) GPAT4 peptides fragments were not detected with LC-MS/MS. For comparison, AGPAT5 levels were unaffected.

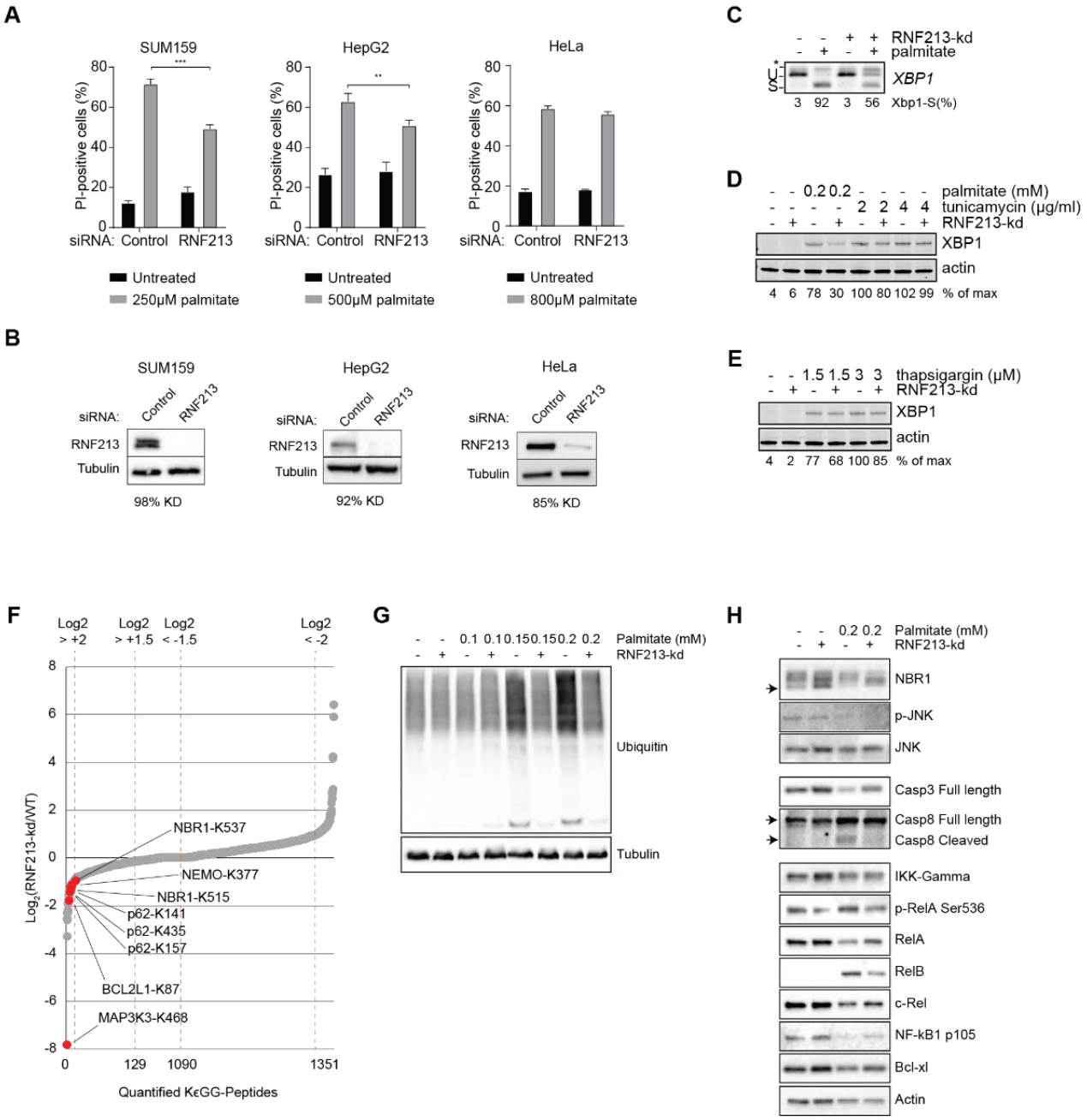

Figure S6. RNF213-kd Protects against Palmitate-Induced UPR and Caspase Activation, Related to Figure 6.

(A) RNF213 or control siRNA treatment in SUM159, HepG2, and HeLa cells. Cells were treated with palmitate 48h after siRNA treatment.

(B) RNF213 knockdown efficiency of RNF213 siRNA compared to control siRNA as measured by immunoblotting and quantification.

(C) Knockdown of RNF213 prevents palmitate induced cleavage of *XBP1* mRNA. U, unspliced; S, spliced; * unspecific band. *XBP1* splicing is quantified as percentage of the spliced form over the total detected (*XBP1*-S(%)).
(D) RNF213-kd does not protect cells against tunicamycin-induced UPR. Western blot of total cell lysate from control and RNF213-kd cells treated with vehicle, palmitate (0.2 mM) or tunicamycin (2 or 4 µg/µl) for 16 h. XBP1 protein is quantified as in Figure 6D.
(E) RNF213-kd does not protect cells against thapsigargin-induced UPR. Western blot of total cell lysate from control and RNF213-kd cells treated with vehicle or thapsigargin (1.5 or 4 µM) for 16 h. XBP1 protein is quantified as in Figure 6D.
F) Ubiquitylation changes in WT vs. RNF213-kd cells as measured by diGly proteomics. Proteins involved in NF-κB signaling pathway are labeled in red.
(G) Palmitate increases total cellular protein ubiquitylation, and knockdown of RNF213 abolishes the response.
(H) RNF213-kd protects cells from Caspase-3 and -8 cleavage and activation of NF-κB pathway. Western blot of total cell lysate from control and RNF213-kd cells untreated or treated with palmitate (0.2 mM) for 16 h.