

**Figure S1. Schematic of simplified ferroptosis pathways with indicated proteins used in this proteomic study.** See Introduction for a detailed description.

**Figure S2. Proteomic analyses of the protein-protein interaction network for ferroptosis pathways.** **A**, Heat map showing the SAINT scores (Bayesian false discovery rate analyzed by SAINTexpress) for each of the baits and their HCIPs. **B**, Protein-protein interaction and function group of all the prey proteins that bind to the 14 ferroptosis bait proteins. **C**, Protein-protein interaction networks and function groups between 13 ferroptosis bait proteins (except LPCAT3) and their interacting proteins.

**Figure S3. Schematic of phospholipid synthesis pathways.** **A**, Schematic showing biosynthesis of phosphatidylethanolamine and phosphatidylcholine. **B**, Schematic showing the role of CEPT1 in synthesizing both phosphatidylethanolamine and phosphatidylcholine.

**Figure S4. Interaction with CEPT1 prevents LPCAT3 from undergoing lysosomal proteolysis.** **A**, Western blot analysis of indicated protein levels in 786-O and HT-1080 cells. **B**, Western blot analysis of indicated protein levels in control (sgControl) and *CEPT1*-knockout (sg1/2) HT-1080 cells. **C**, **D**, Statistical analysis of CEPT1 and LPCAT3 protein levels in control (sgControl) and *CEPT1*-knockout (sg1/2) HT-1080 (**C**) and 786-O (**D**) cells from three independent Western blot analyses. **E-H**, RT-PCR analyses of mRNA levels of the indicated genes in control (sgControl) and *CEPT1*-knockout (sg1/2) 786-O cells. **I-M**, RT-PCR analyses of mRNA levels of the indicated genes in control (sgControl) and *CEPT1*-knockout (sg1/2) HT-1080 cells. **N**, A pulldown assay was performed with S protein beads (SFB-tagged proteins were used as the baits), and the indicated proteins were detected by Western blot. **O**, Cystine uptake levels of control (sgControl) and *CEPT1*-knockout (sg1/2) HT-1080 cells. **P**, Western blot analysis of indicated protein levels in HT-1080 cells overexpressing CEPT1 or transfected with empty vector (EV). **Q**, RT-PCR analysis of the *LPCAT3* mRNA levels in HT-1080 cells overexpressing CEPT1 or transfected with EV. **R**, Western blot analysis of indicated protein levels in the control (Ctrl) and *CEPT1*-knockout (sg1/2) HT-1080 cells treated with DMSO or 5  $\mu\text{mol/L}$  MG-132 for 24 h. **S**, Western blot analysis of indicated protein levels in the control (Ctrl) and *CEPT1*-knockout (sg1/2) 786-O cells treated with DMSO or indicated concentrations of bafilomycin A1 for 24 h. **T**, Western blot analysis of indicated protein levels in the control (Ctrl) and *CEPT1*-knockout (sg1/2) 786-O cells treated with DMSO or 40  $\mu\text{mol/L}$  arachidonic acid (AA) for 24 h. **U**, Cell death was measured by PI staining in the control (sgControl) and *CEPT1*-knockout (sg1/2) 786-O cells treated with DMSO, 500 nM RSL3, 40  $\mu\text{mol/L}$  arachidonic acid (AA) or indicated combination for 24 h.

**Figure S5. CEPT1 inhibits ferroptosis.** **A**, Cell death was measured by propidium iodide (PI) staining in the control (sgControl) and *LPCAT3*-knockout (sg1/2) 786-O cells treated with DMSO or 50 nM RSL3 for 24 h. **B**, Relative lipid peroxidation levels in the control (sgControl) and *CEPT1*-knockout (sg1/2) 786-O cells cultured in cystine-containing (+ Cystine) or cystine-free (- Cystine) for 8 h. **C**, Relative lipid peroxidation levels in the control (sgControl) and *CEPT1*-knockout (sg1/2) 786-O cells treated with DMSO, 10  $\mu\text{mol/L}$  erastin, or 50 nM RSL3 for 8 h. **D**, Cell viability was measured in the control (sgControl) and *CEPT1*-knockout (sg1/2) HT-1080 cells treated with RSL3 for 24 h. **E**, Cell death was measured by PI staining in the control (sgControl) and *CEPT1*-knockout (sg1/2) HT-1080 cells treated with DMSO, 500 nM

RSL3, 100  $\mu\text{mol/L}$  deferoxamine (DFO), or 10  $\mu\text{mol/L}$  ferrostatin-1 (Fer-1) for 24 h. **F**, Cell death was measured by PI staining in the control (sgControl) and *CEPT1*-knockout (sg1/2) HT-1080 cells cultured in cystine-containing (+ Cystine) or cystine-free (- Cystine) medium with or without 100  $\mu\text{mol/L}$  deferoxamine (DFO) or 10  $\mu\text{mol/L}$  ferrostatin-1 (Fer-1) for 24 h. **G**, Cell death was measured by PI staining in the control (sgControl) and *CEPT1*-knockout (sg1/2) HT-1080 cells treated with DMSO, 2  $\mu\text{mol/L}$  erastin, 100  $\mu\text{mol/L}$  deferoxamine (DFO), or 10  $\mu\text{mol/L}$  ferrostatin-1 (Fer-1) for 24 h. **H**, Cell death was measured by PI staining in HT-1080 cells overexpressing *CEPT1* or transfected with EV treated with DMSO or 1  $\mu\text{mol/L}$  RSL3 for 24 h. **I**, Cell death was measured by PI staining in HT-1080 cells overexpressing *CEPT1* or transfected with EV cultured in cystine-containing (+ Cystine) or cystine-free (- Cystine) medium for 24 h. **J**, Cell death was measured by PI staining in the HT-1080 cells overexpressing *CEPT1* or transfected with EV treated with DMSO or 5  $\mu\text{mol/L}$  erastin for 24 h. **K**, Weight over time of mice with HT-1080 xenografts with the indicated treatments.

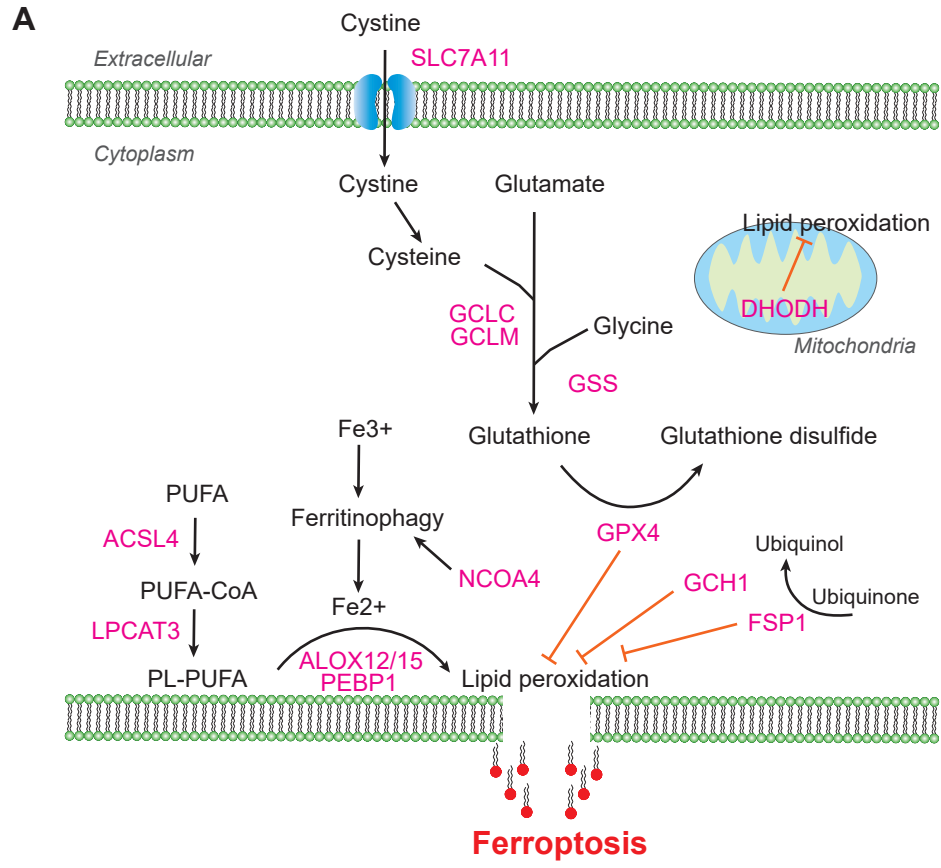
**Figure S6. Loss of EPT1 or CHPT1 renders cells resistant to ferroptosis.** **A**, Schematic showing phosphatidylethanolamine and phosphatidylcholine synthesis catalyzed by *CEPT1*, *EPT1*, and *CHPT1*. **B**, A pulldown assay was performed with S protein beads (SFB-tagged proteins were used as the baits), and the indicated proteins were detected by WB. **C**, **D**, RT-PCR analyses of *EPT1* mRNA levels (**C**) and western blotting analysis of indicated protein levels (**D**) in the control (shControl) and *EPT1*-knockdown (sh1/2) 786-O cells. **E**, **F**, Cell death was measured by propidium iodide (PI) staining in the control (shControl) and *EPT1*-knockdown (sh1/2) 786-O cells treated with DMSO, 50 nM RSL3, 10  $\mu\text{mol/L}$  erastin, or cystine starvation (- Cystine) for 24 h. **G**, Western blot analysis of indicated protein levels in the control (sgControl) and *CHPT1*-knockout (sg1/2) 786-O cells. Vinculin was used as a loading control. **H**, Cell death was measured by PI staining in the control (sgControl) and *CHPT1*-knockout (sg1/2) 786-O cells treated with DMSO, 50 nM RSL3, 10  $\mu\text{mol/L}$  erastin, or cystine starvation (- Cystine) for 24 h.

**Figure S7. CEPT1 suppresses ferroptosis partly through phospholipases.** **A**, Cell viability was measured in the control (sgCtrl) and *CEPT1-ACSL4* single or double knockout HT-1080 cells treated with RSL3 for 24 h. **B**, Schematic showing that PLA1 and PLA2 enzymes cleave fatty acyl ester bonds at the sn-1 and sn-2 sites of the glycerol backbone, respectively. **C**, **D**, Western blot analysis of indicated protein levels in the control (sgControl), *DDHD1*-knockout, or *DDHD2*-knockout (sg1/2) 786-O cells overexpressing *CEPT1* or transfected with empty vector (EV). **E**, RT-PCR analysis of *ABHD3* mRNA levels in the control (shControl) and *ABHD3*-knockdown (sh1/3) 786-O cells overexpressing *CEPT1* or transfected with EV. **F**, Western blot analysis of indicated protein levels in the control (sgControl) and *PLAA*-knockout (sg1/2) 786-O cells overexpressing *CEPT1* or transfected with EV. **G**, An immunoprecipitation assay was performed with Myc beads to analyze interaction between Myc-tagged *CEPT1* and *PLAA*, and the indicated proteins were detected by Western blot.

**Figure S8. CEPT1 downregulates PUFA-containing phospholipids and triacylglycerols.** **A**, Heat maps of significantly changed lipid species (One-way ANOVA; FDR-corrected p-value < 0.01; n = 4 repeats) in the control (NC) and *CEPT1*-knockout (KO) HT-1080 cells. **B**, Heat maps of significantly changed lipid species (One-way ANOVA; FDR-corrected p-value < 0.01; n = 4 repeats) in 786-O cells overexpressing *CEPT1* WT or K138M mutant (MU) or transfected with

an empty vector (EV). Each row represents z-score-normalized intensities of the detected lipid species. Each column represents a sample. The relative abundance of each lipid is color-coded, with red indicating high signal intensity and blue indicating low signal intensity.

Fig. S1



**Fig. S2**

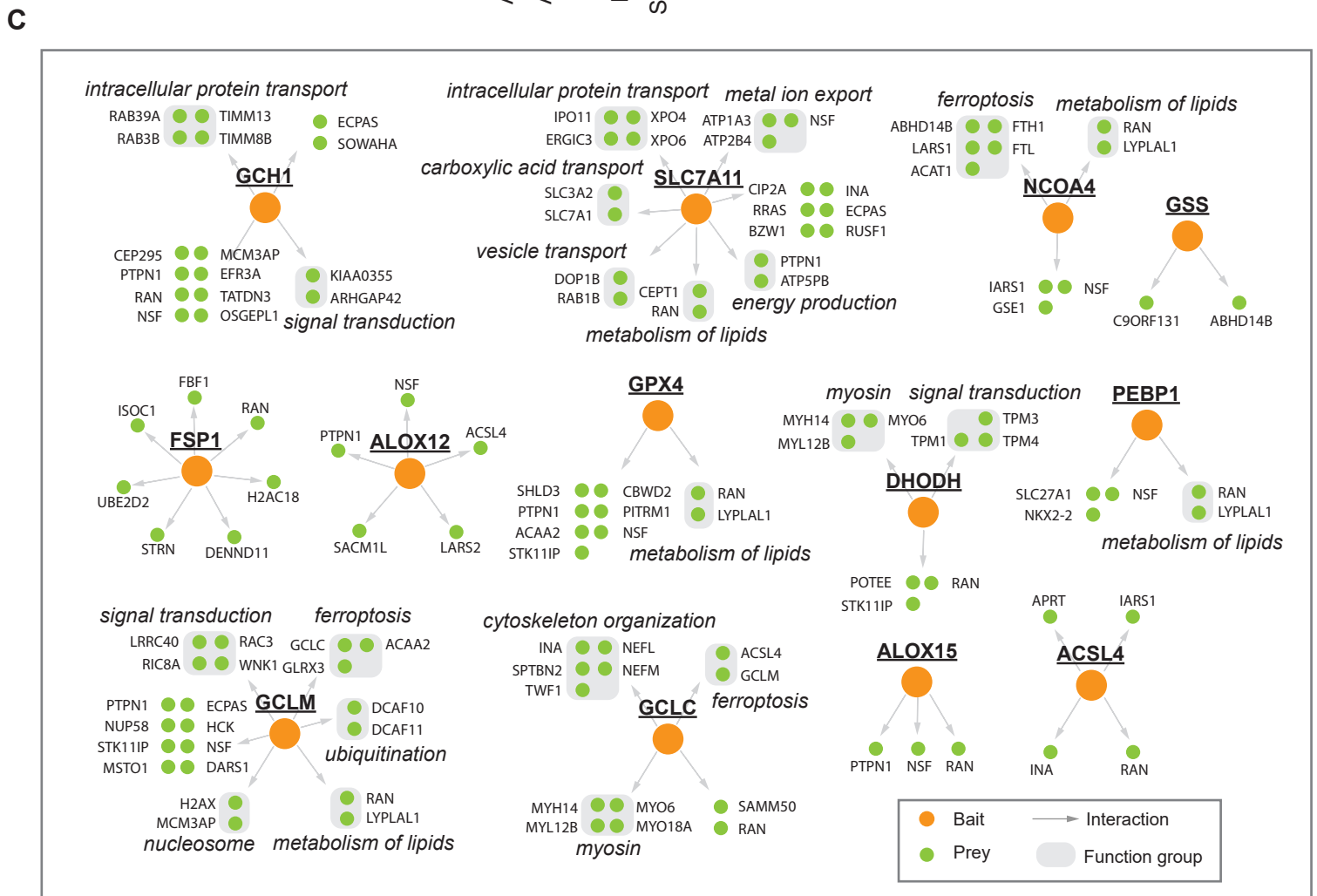
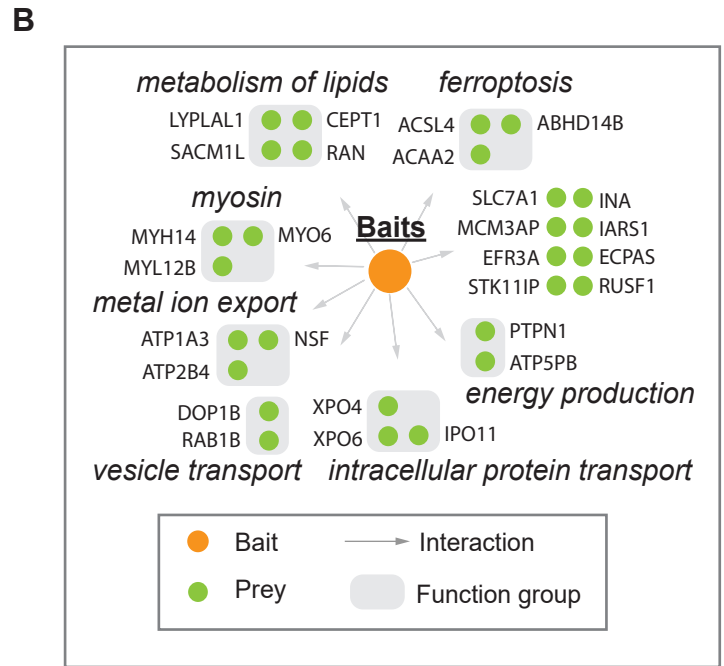
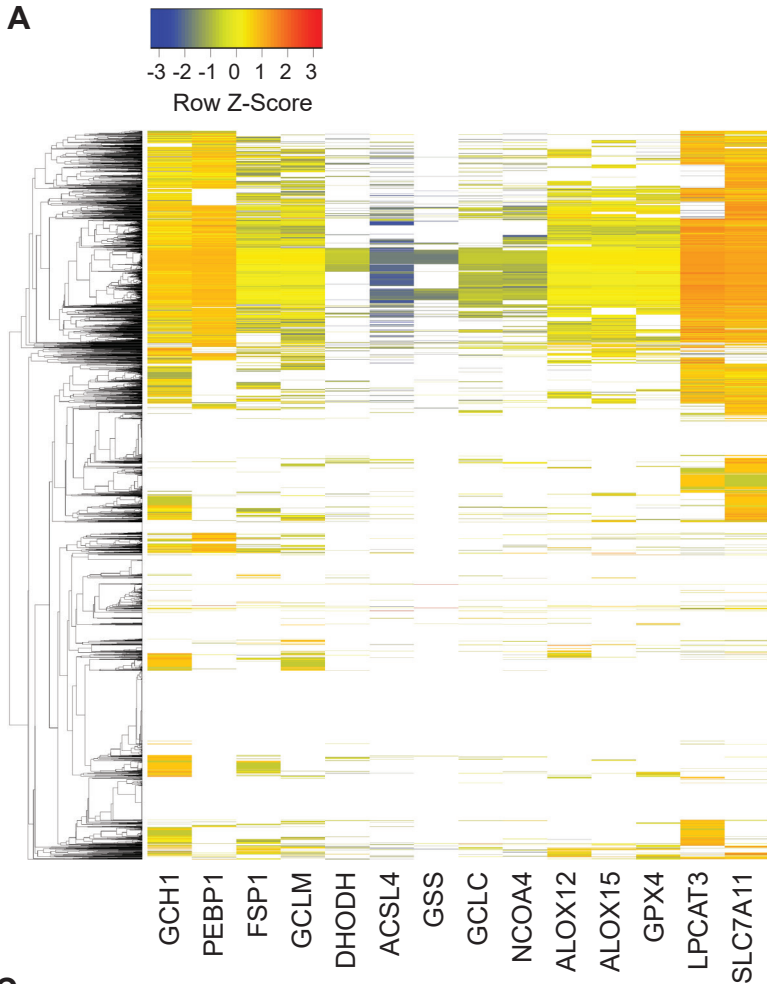
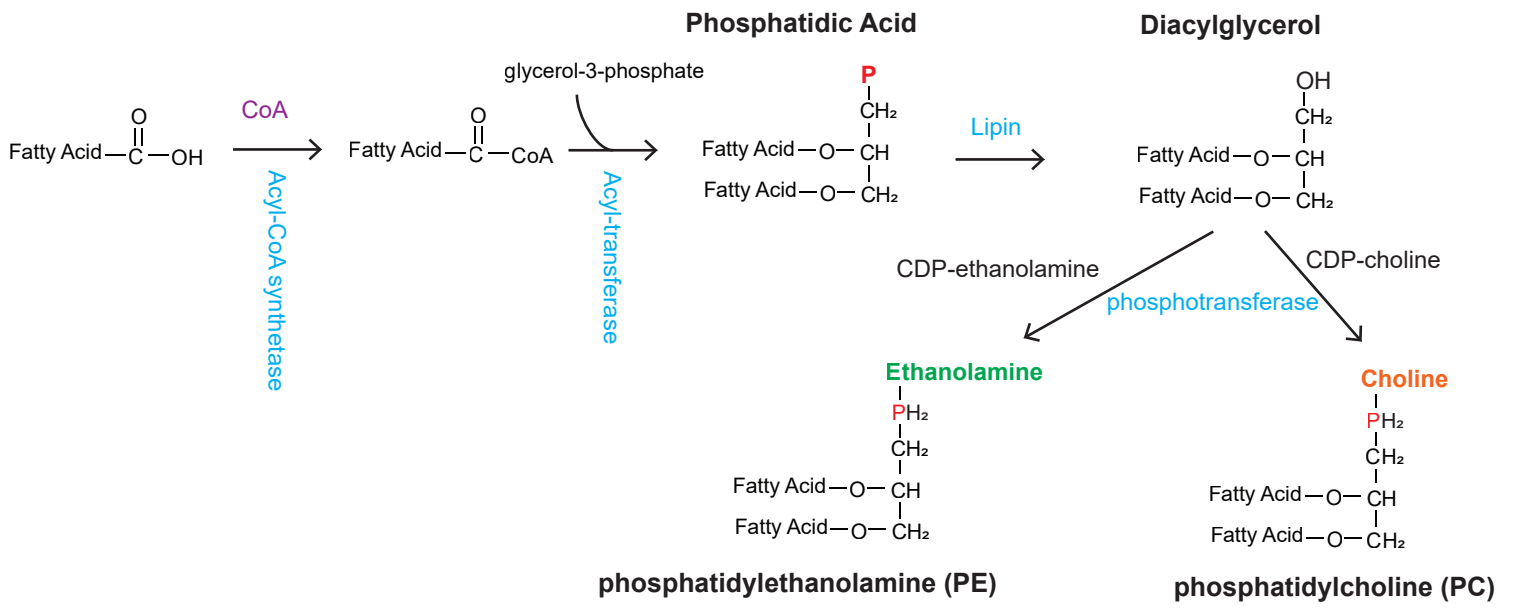
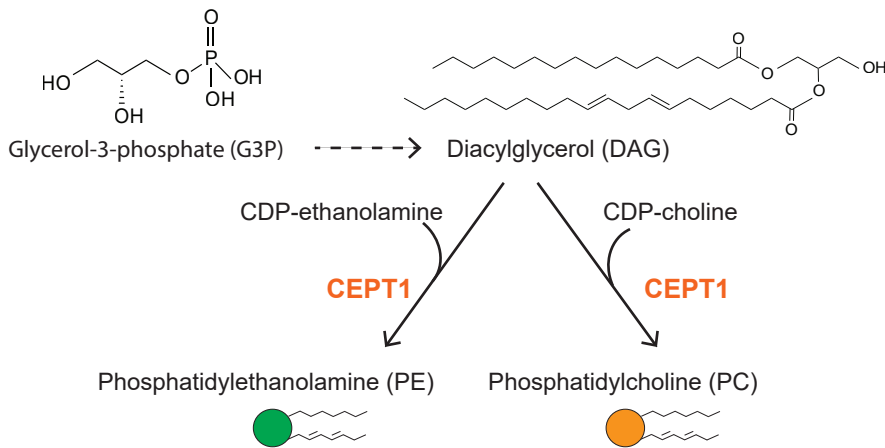


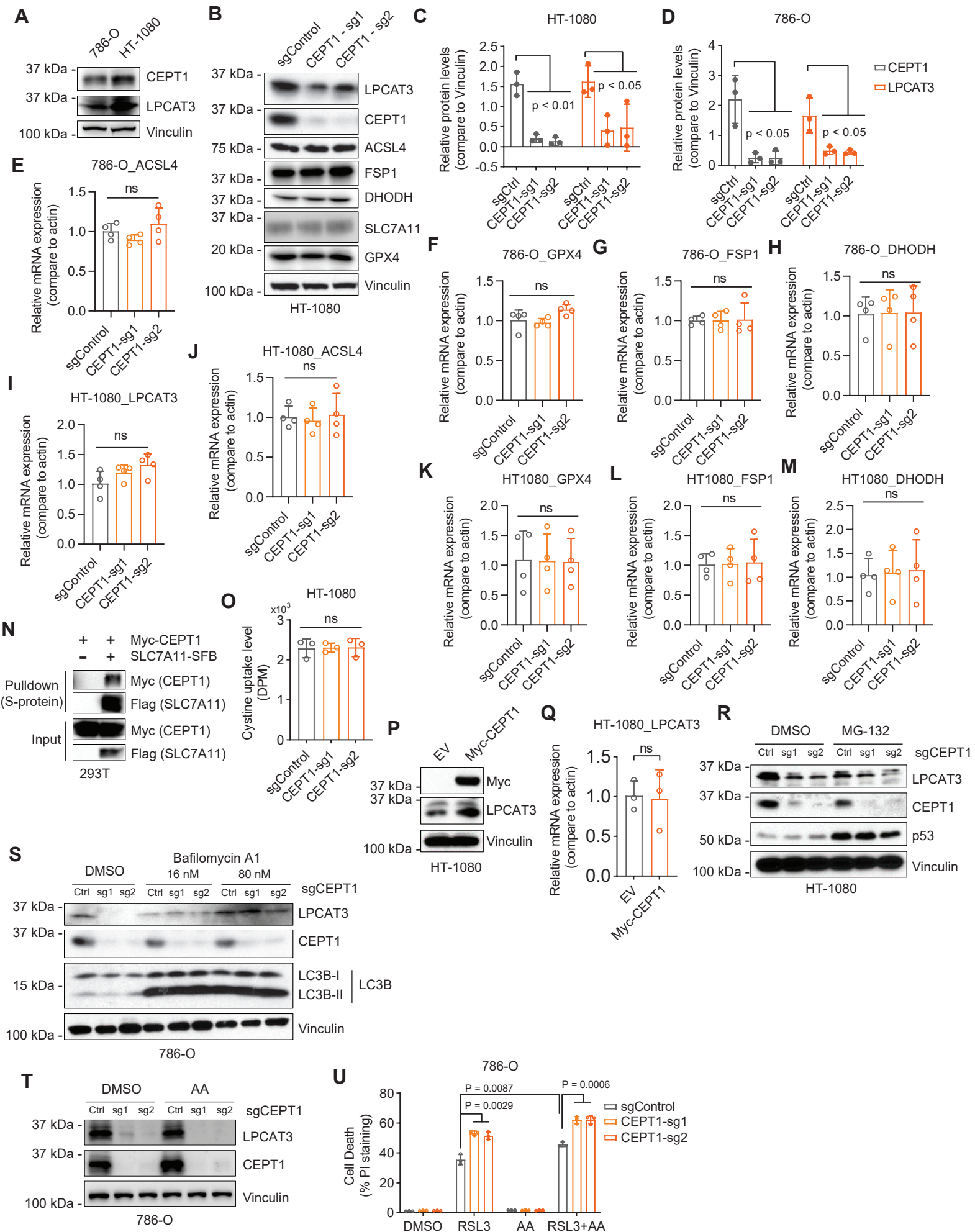
Fig. S3

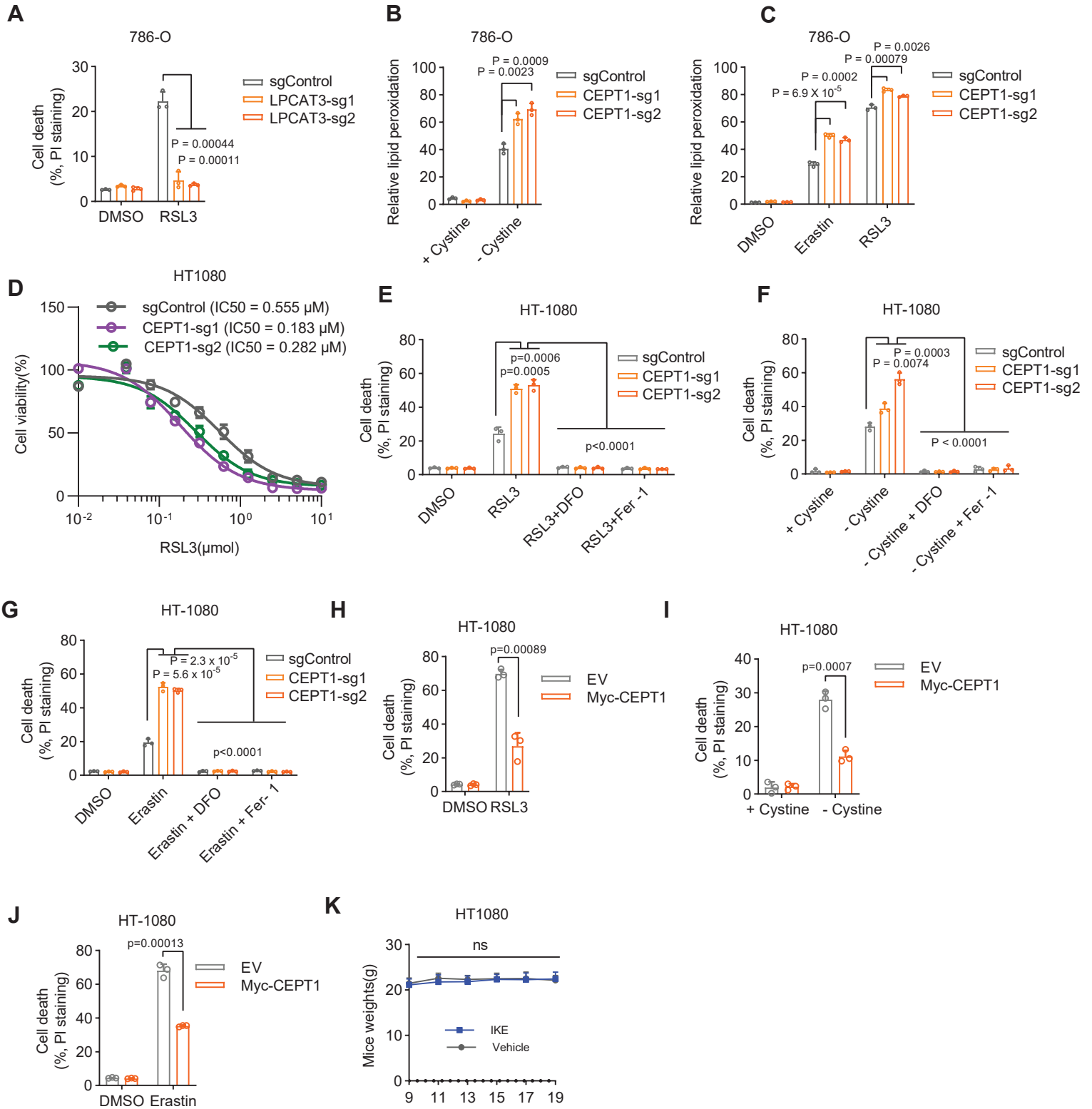
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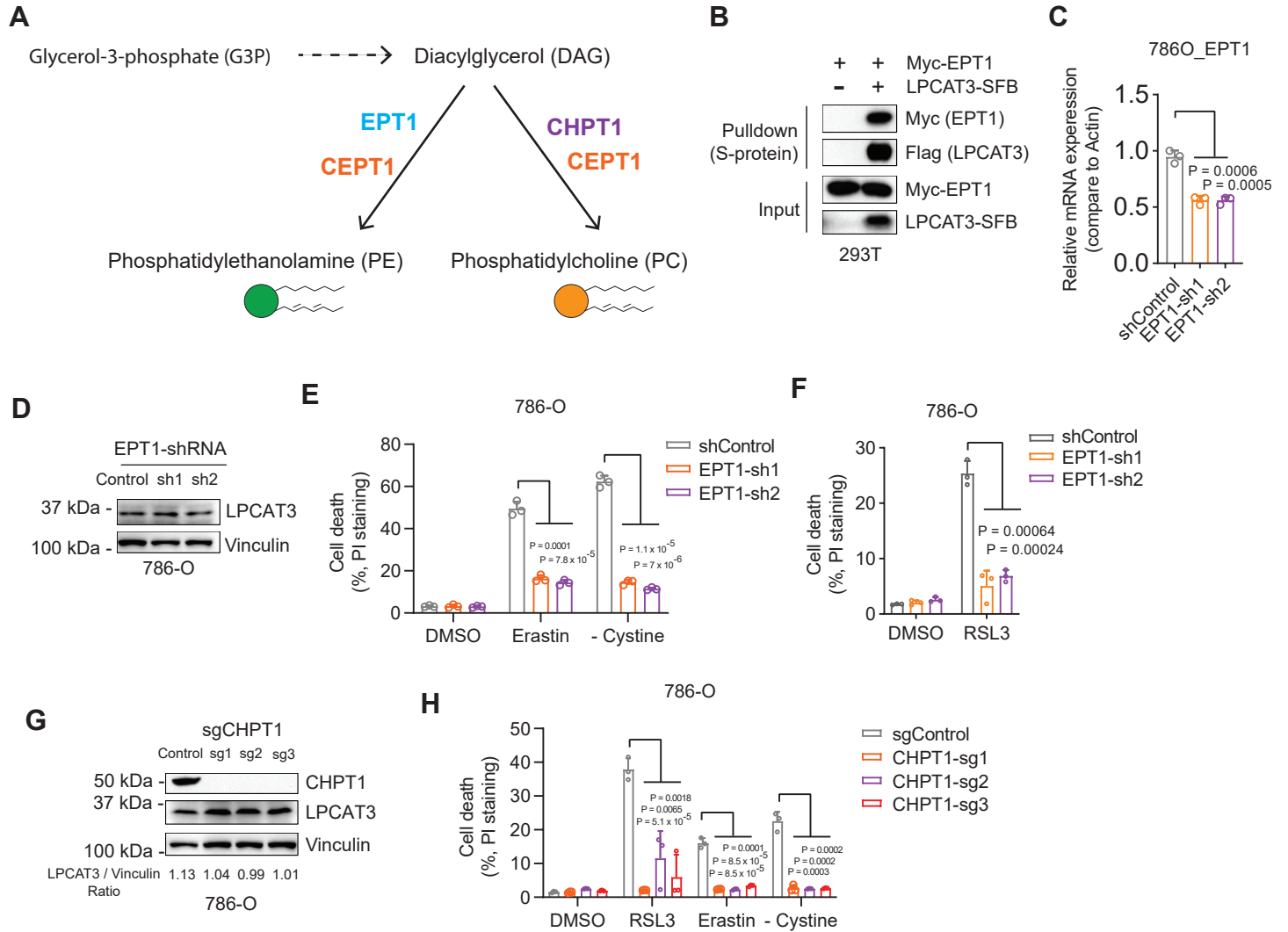
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**Fig. S4**

**Fig. S5**

**Fig. S6**



**Fig. S7**

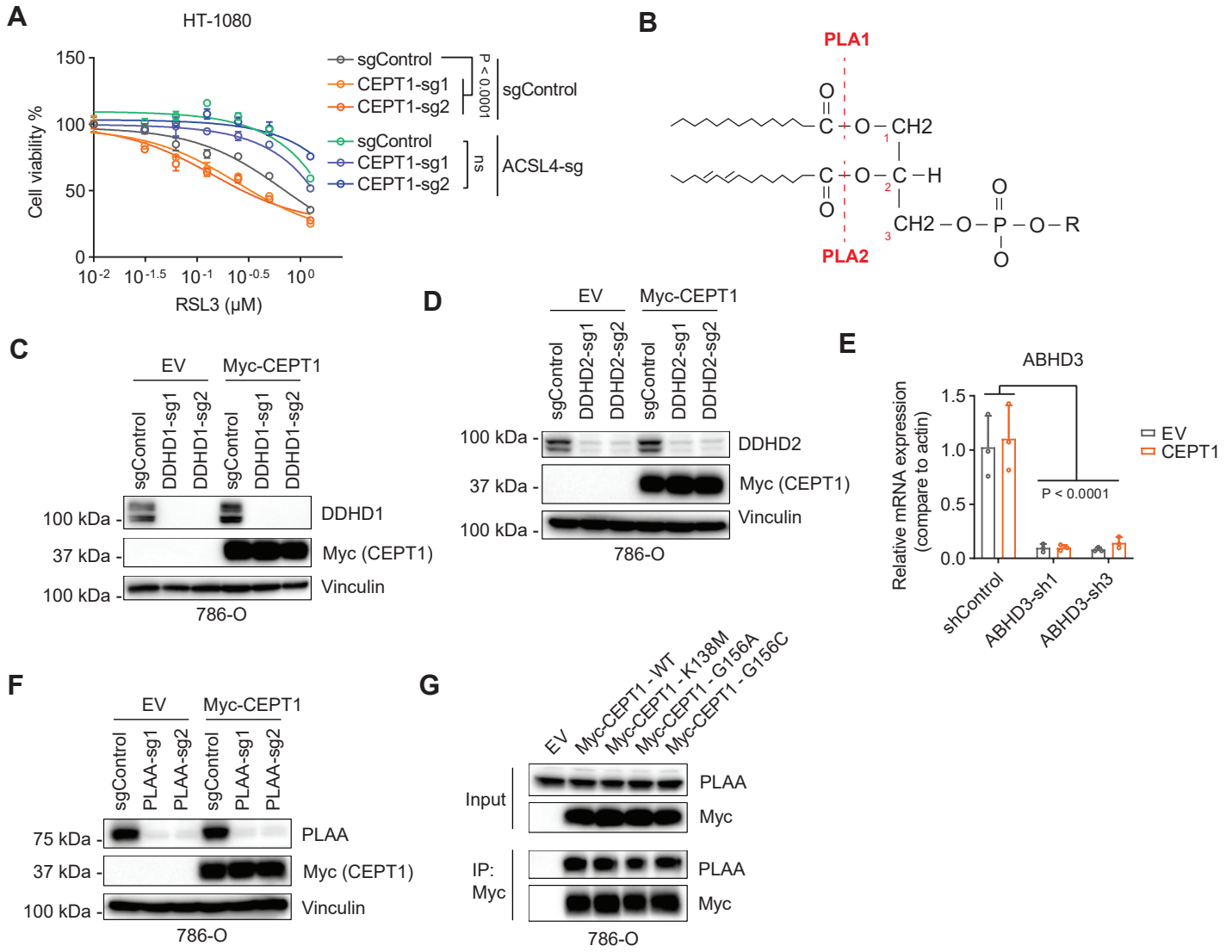
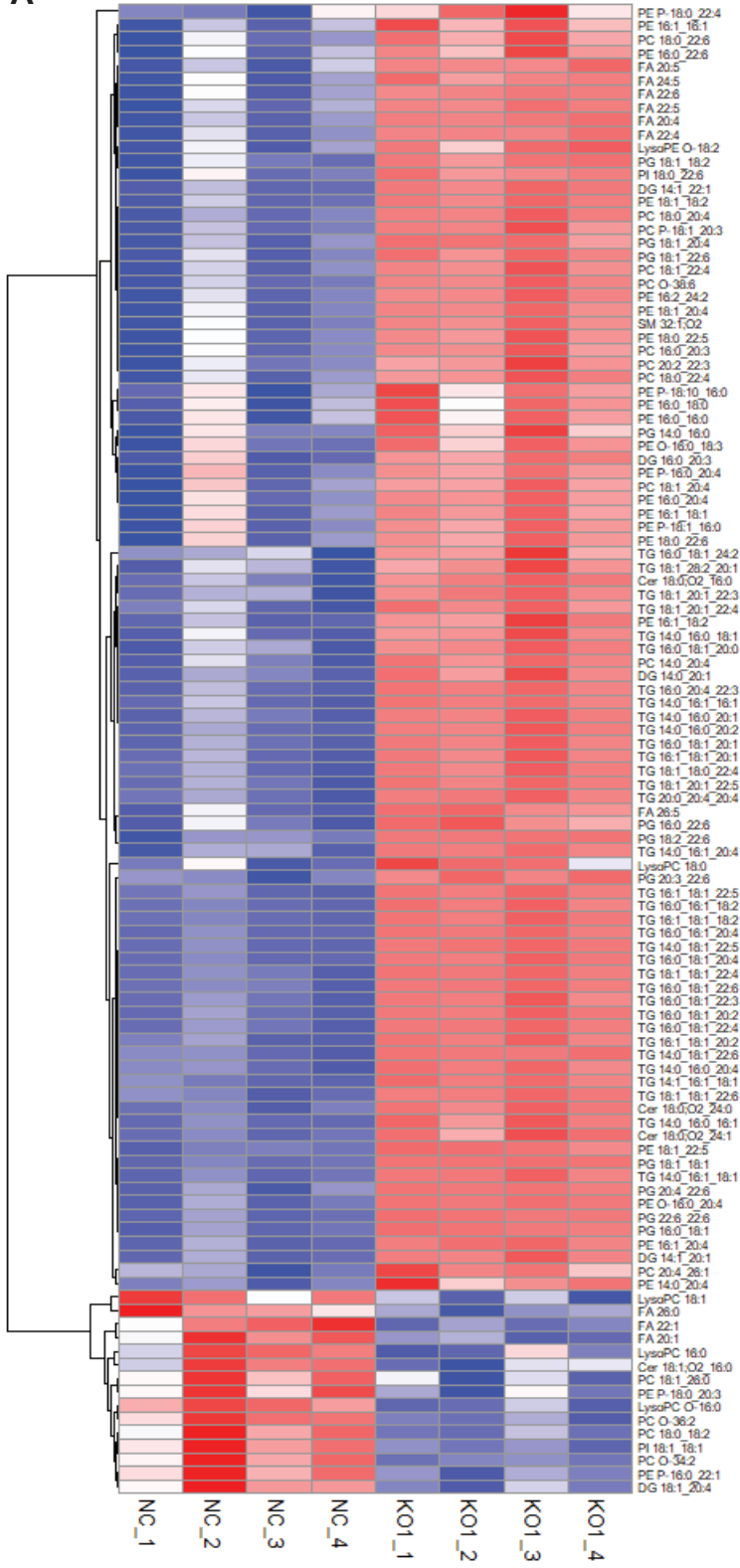


Fig. S8

A



B

