Supplementary Information for

Oncogenic activation of PI3K-AKT-mTOR signaling suppresses ferroptosis via SREBP-mediated lipogenesis

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Figures S1 to S7

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Fig. S1.

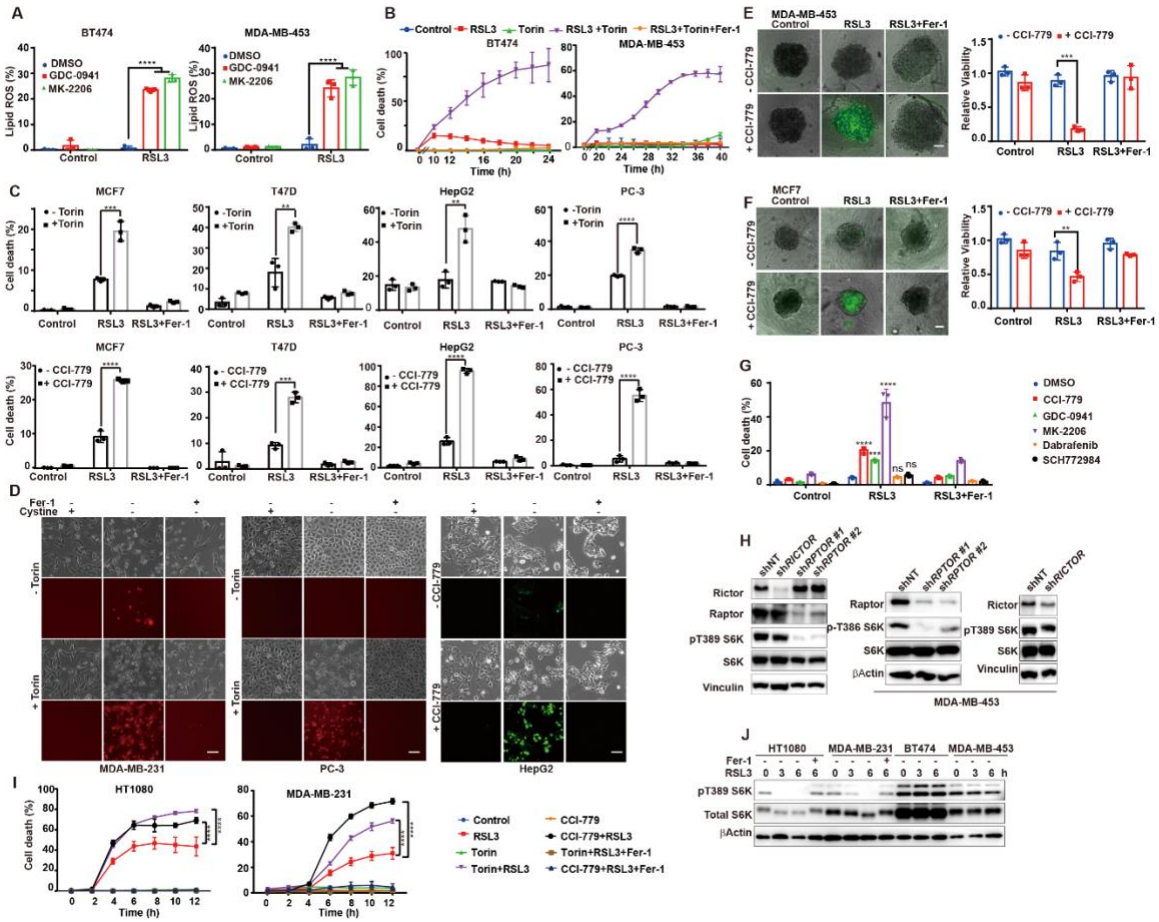

Fig. S1. PI3K-AKT-mTOR signaling regulates ferroptosis sensitivity. (**A**) Cells were treated as indicated. GDC-0941, 2 μM; MK-2206, 2 μM; RSL3, 1 μM; Fer-1, 1 μM. Lipid peroxidation was measured. **(B)** Cells were treated with indicated conditions. Torin, 1 μM; RSL3, 1 μM for MDA-MB-453 cells and 0.5 μM for BT474 cells; Fer-1, 1 μM. Cell death was measured. **(C)** Cells were treated with indicated conditions. RSL3, 10 μM for MCF cells and PC-3 cells, 5 μM for T47D cells, 1 μM for HepG2 cells; Torin, 1 μM; CCI-779, 0.5 μM. **(D)** Cells were treated as indicated. CCI-779, 0.5 μM; Torin, 1 μM; Fer-1, 1 μM. Cell death was staining by propidium iodide (PI) (red) or Sytox Green (green) (scale bar, 100 μm). **(E-F)** 3D spheroids for MDA-MB-453 cells and MCF7 cells were treated as indicated. CCI-779, 0.5 μM; RSL3, 1 μM for MDA-MB-453 cells and 5 μM for MCF7 cells; Fer-1, 1 μM. Top panels, cell death staining (scale bar, 100 μm). Bottom panels, cell viability. **(G)** MDA-MB-453 cells were treated as indicated for 24 h. RSL3, 1 μM; CCI-779, 0.5 μM; GDC-0941, 2 μM; MK-2206, 2 μM; Dabrafenib, 2 μM; SCH772984, 2 μM; Fer-1, 1 μM. (H) Western blot was performed to detect *RPTOR* and *RICTOR* knockdown efficiency. (**I**) HT1080 cells and MDA-MB-231 cells (both with wild-type PI3K-AKT-mTOR pathway) were treated as indicated. RSL3, 0.1 μM for HT1080 and 0.25 μM for MDA-MB-231; Torin, 1 μM; CCI-779, 0.5 μM; Fer-1, 1 μM. Cell death was measured. (**J**) Two lines of PI3K-AKT-mTOR pathway wild-type cells (HT1080 and MDA-MB-231) and two lines of cells harboring activating mutation of the pathway (BT474 and MDA-MB-453) were treated as indicated. RSL3, 0.25 μM; Fer-1, 1 μM. Western blot was performed to detect the level of pT389 S6K.

Fig. S2.

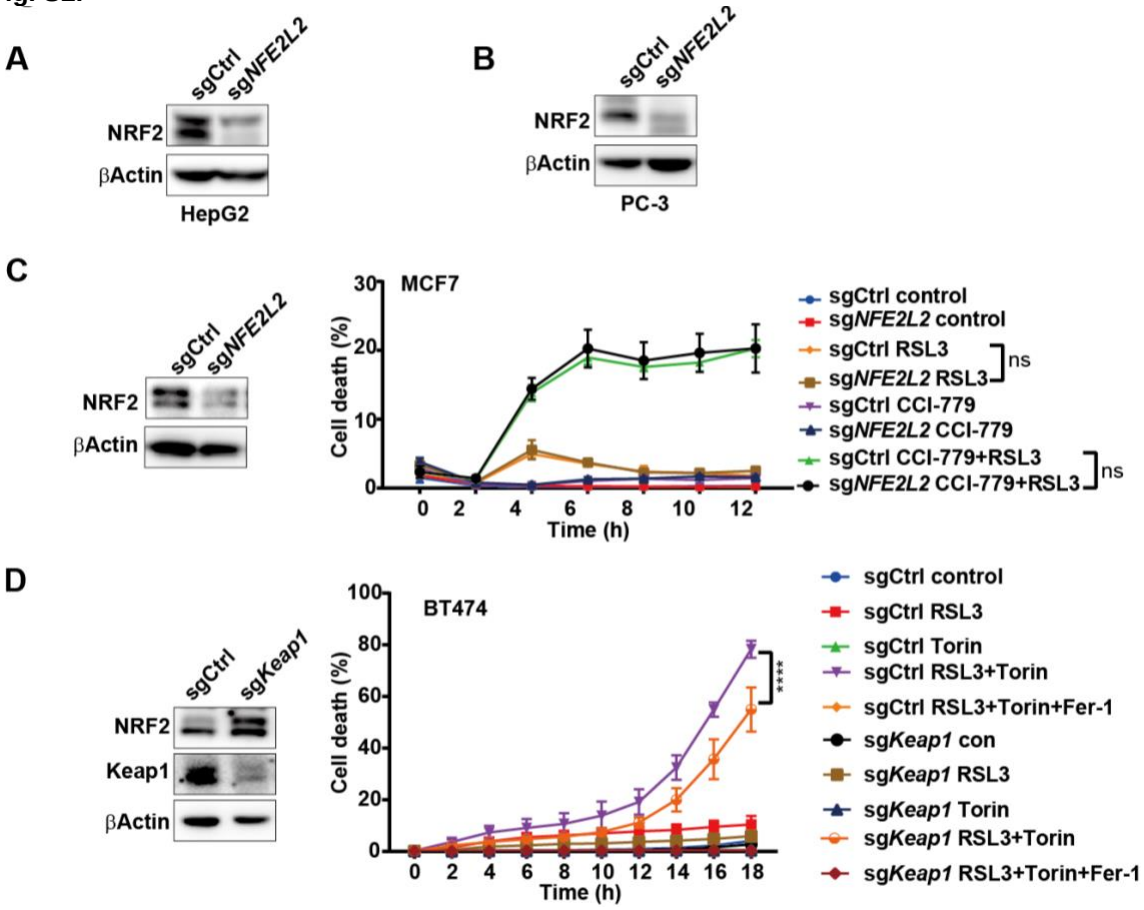

Fig. S2. NRF2 is not the main player mediating the ferroptosis-suppressing activity of mTORC1. (A) NRF2 was depleted by CRISPR/Cas9 technology in HepG2 cells. NRF2 level was measured by western blot. (B) NRF2 was depleted by CRISPR/Cas9 technology in PC-3 cells. NRF2 level was measured by western blot. (C) NRF2 was depleted by CRISPR/Cas9 technology in MCF7 cells. (Left) NRF2 level was measured by western blot. (Right) MCF7 cells with or without NRF2 depletion were treated as indicated. RSL3, 5 μM; CCI-779, 0.5 μM; Fer-1, 1 μM. Cell death was measured. (D) Keap1 was depleted by CRISPR/Cas9 technology in BT474 cells. (Left) NRF2 and Keap1 levels were measured by western blot. (Right) BT474 cells with or without Keap1 depletion were treated as indicated. RSL3, 0.5 μM; Torin, 1 μM; Fer-1, 1 μM.

Fig. S3.

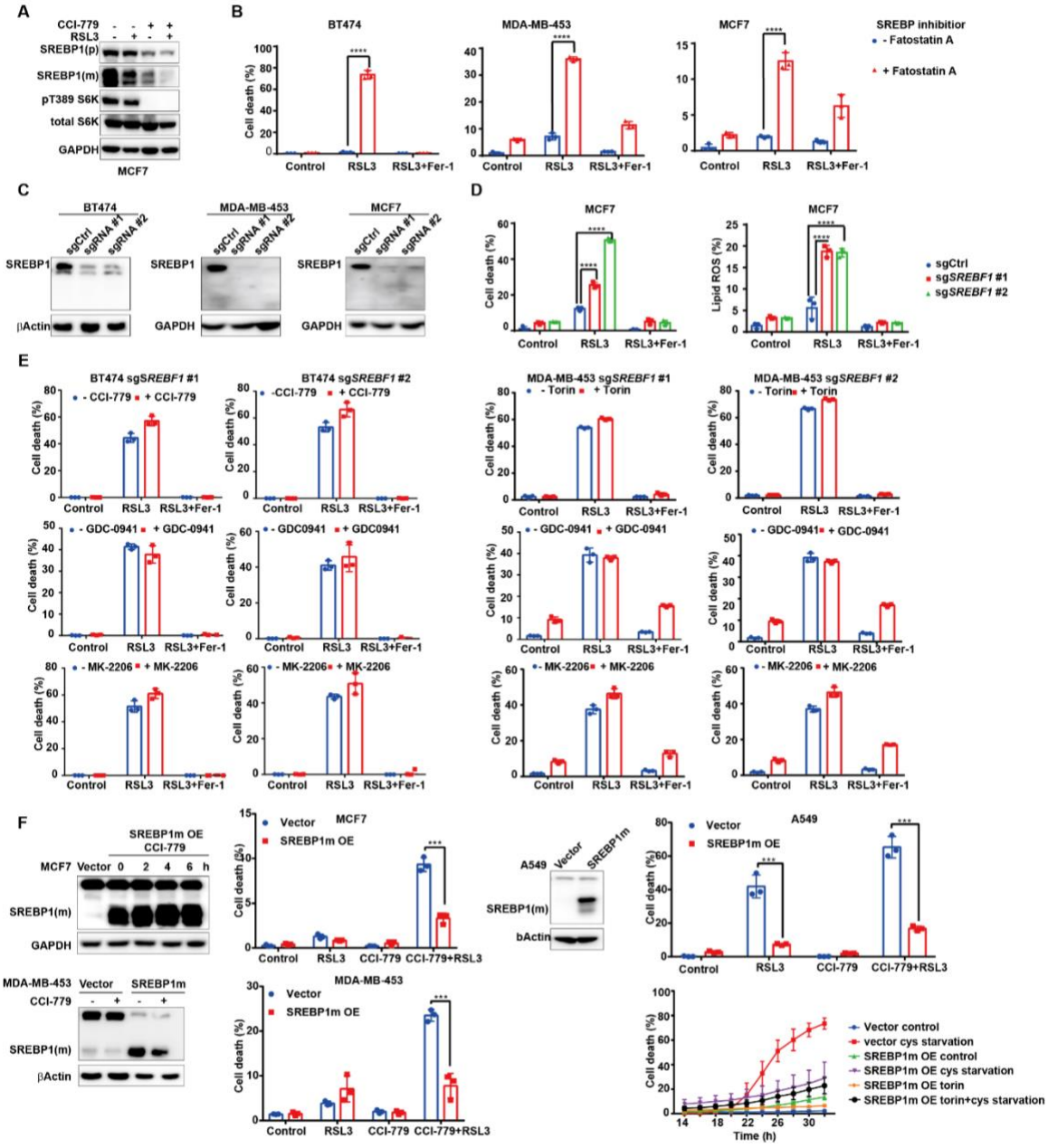

Fig. S3. SREBP1 protects cells from ferroptosis. (A) MCF7 cells were treated as indicated. RSL3, 5 μM; CCI-779, 0.5 μM. Cell lysates were collected 24 h after treatment for Western blot detecting p-T389 S6K, total S6K, SREBP1(P) and SREBP1(m). (B) Cells were pretreated with 5 μM Fatostatin A overnight and treated as indicated. RSL3, 0.5 μM for BT474 cells, 1 μM for MDA-MB-453 and 5 μM for MCF7 cells; Fer-1, 1 μM. (C) Efficiency of *SREBF1* Knockout in BT474, MDA-MB-453, and MCF7 cells was monitored by western blot. (D) MCF7 cells were treated as indicated. RSL3, 5 μM; Fer-1, 1 μM. (E) Cells were treated as indicated. RSL3, 1 μM for MDA-MB-453 cells and 0.5 μM for BT474 cells; Fer-1, 1 μM; Torin, 1 μM; GDC-0941, 2 μM; MK-2206, 2 μM; CCI-779, 0.5 μM. (F) SREBP1m was overexpressed in MCF7, MDA-MB-453 and A549 cells and determined

by western blot. Cells were treated as indicated. RSL3, 5 μM for MCF7 cells, 0.5 μM for MDA-MB-453 cells and 0.25 μM for A549 cells; CCI-779, 0.5 μM.

Fig. S4.

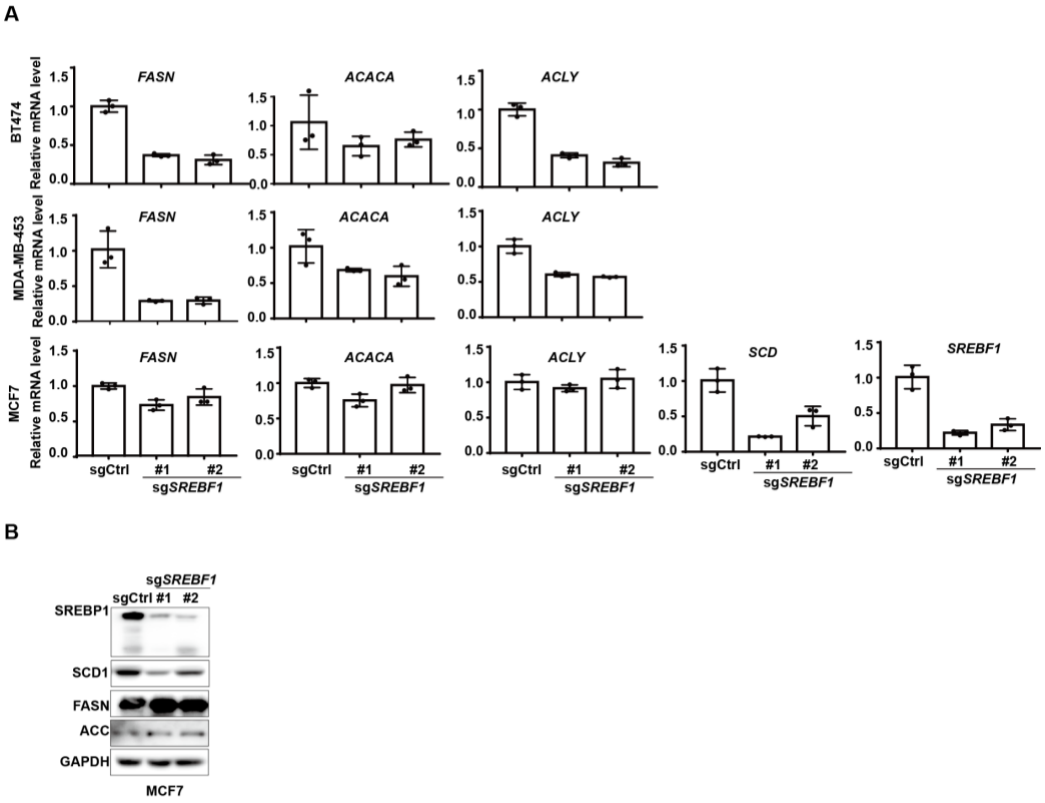

Fig. S4. SREBP1 knockout downregulates SCD1. (A) Indicated lines of cells harboring *SREBF1* knockout were collected. (A)The mRNA level of *SREPF1* and its targets genes (*ACACA, FASN, SCD, ACLY*) were measured by RT-PCR. (B) Determine FASN, ACC and SCD1 in MCF7 cells with *SREBF1* knockout by western blot.

Fig. S5.

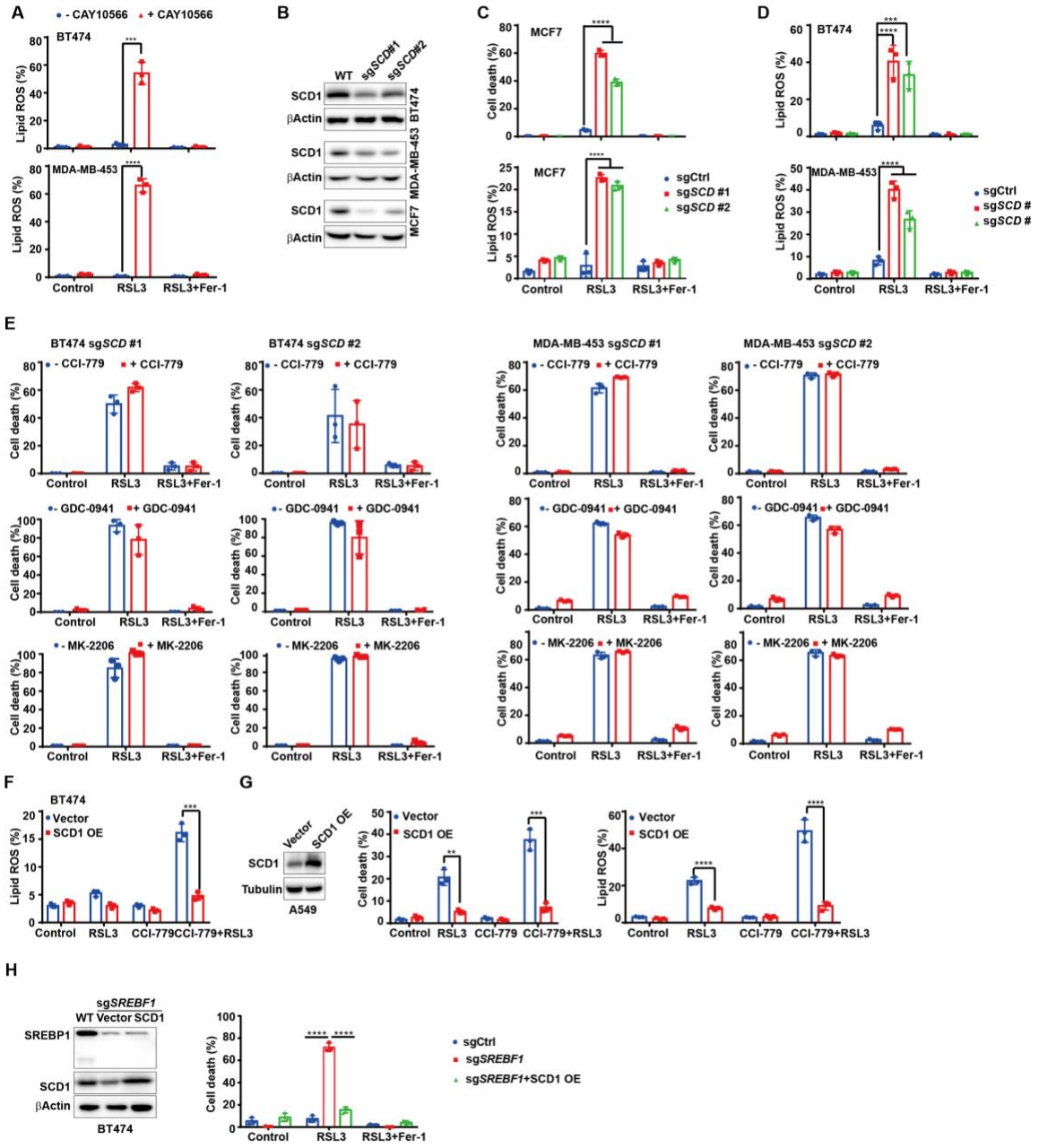

Fig. S5. SCD1 protects cells against ferroptosis. (**A**) Cells were pretreated cells with 5 μM CAY10566 overnight. Cells were treated as indicated. RSL3, 1 μM for MDA-MB-453 and 0.5 μM for BT474; Fer-1, 1 μM; CAY10566, 5 μM; Fer-1, 1 μM. (**B**) Western blot, measuring the *SCD* knockout efficiency in BT474, MDA-MB-453 and MCF7 cells. (**C**) MCF cells (sgCtrl, sg*SCD*#1 and sg*SCD*#2) were treated as indicated. RSL3, 5 μM; Fer-1, 1 μM. (**D**) Cells were treated as indicated. RSL3, 1 μM for MDA-MB-453 cells and 0.5 μM for BT474 cells; Fer-1, 1 μM. (**E**) Cells were treated as indicated. RSL3, 1 μM for MDA-MB-453 cells and 0.5 μM for BT474 cells; Fer-1, 1 μM; CCI-779, 0.5 μM; GDC-0941, 2 μM; MK-2206, 2 μM. (**F**) SCD1 was overexpressed in BT474 cells. Cells were treated as indicated. RSL3, 0.5 μM; CCI-779, 0.5 μM. Lipid peroxidation was measured. (**G**) SCD1 was overexpressed in A549 cells and determined by western blot. Cells were treated as indicated. RSL3, 0.25 μM; CCI-779, 0.5 μM. Lipid peroxidation and cell death were measured 6 h and 24 h after treatment, respectively. (**H**) SCD1 was overexpressed in BT474 cells harboring

SREBF1 knockout. SCD1 and SREBP1 level were determined by western blot. Cells were treated as indicated. RSL3, 0.5 μM; CCI-779, 0.5 μM; Fer-1, 1 μM.

Fig. S6.

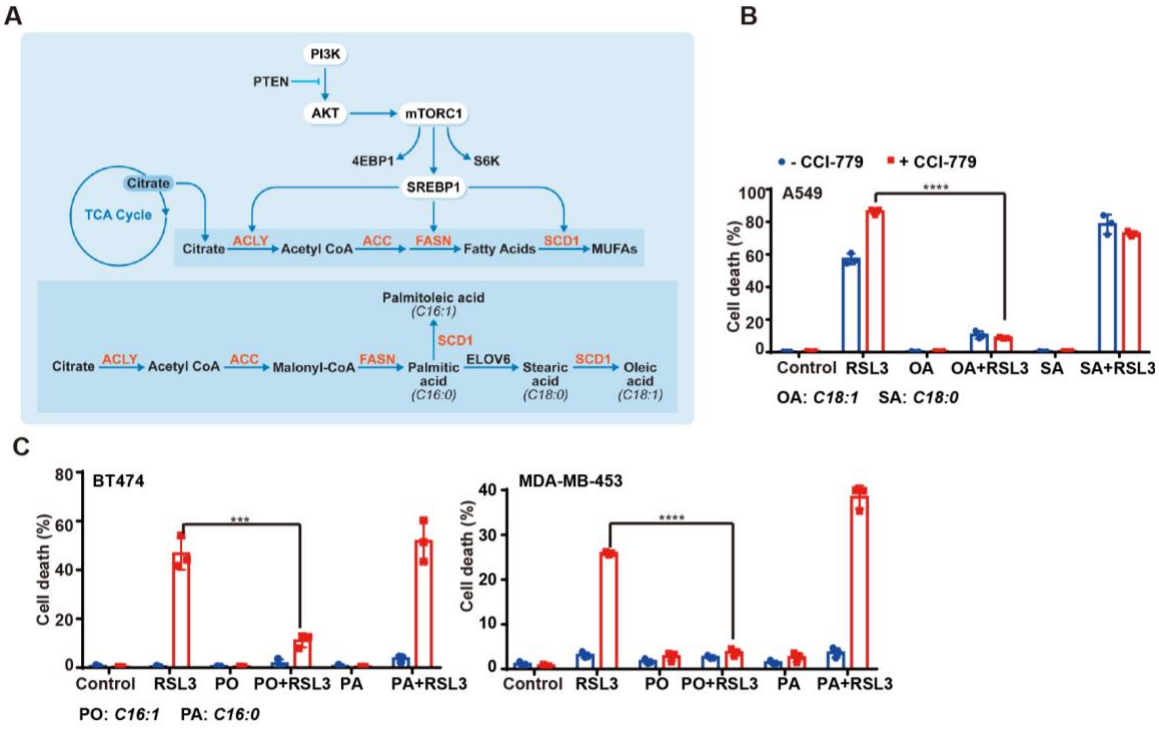

Fig. S6. Ferroptosis sensitization triggered by mTORC1 inhibition can be prevented by exogenous MUFAs. (A) An overview of lipogenesis regulated by SREBP1-driven transcription. (B) A549 cells were treated as indicated. Oleic acid (18:1, OA), 0.5 mM; stearic acid (18:0, SA), 0.5 mM; RSL3, 0.5 μM; CCI-779, 0.5 μM. (C) Cells were treated as indicated. Palmitoleic acid (16:1, PO), 0.5 mM; Palmitic acid (16:0, PA), 0.5 mM; RSL3, 1 μM for MDA-MB-453 cells and 0.5 μM for BT474 cells; CCI-779, 0.5 μM.

Fig. S7.

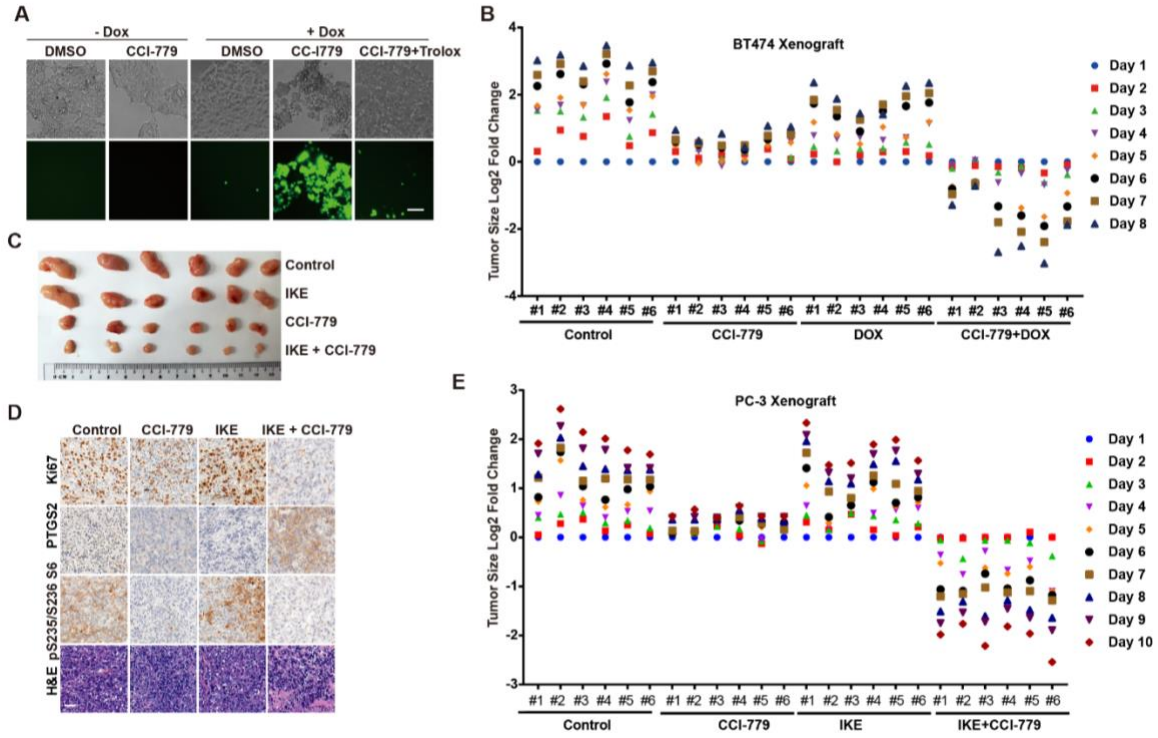

Fig. S7. Combination of mTORC1 inhibition with ferroptosis induction leads to tumor regression. (A) GPX4-iKO BT474 cells were treated as indicated for 30 h. CCI-779, 0.5 μM; DOX, 100 ng/ml; Trolox, 200 μM. Dead cells were stained with Sytox Green (scale bar, 100 μm). (B) BT474 tumor volume was measured every day for each mouse. The log2 fold change of tumor volume of each individual mouse was plotted. (C) Images of resected tumors from mice xenografted with PC-3 cells. Groups of mice were treated with CCI-779 and/or IKE as indicated (n = 6 per group). See Methods for detail. (D) Representative haematoxylin and eosin (H&E) and immunostaining images of Ki67, PTGS2 and pS235/236 S6, all counterstained with haematoxylin (blue), are shown from sections of PC-3 xenografted tumors. Scale bar, 50 μm. (E) PC-3 tumor volume was measured every day for each mouse. The log2 fold change of tumor volume of each individual mouse was plotted.