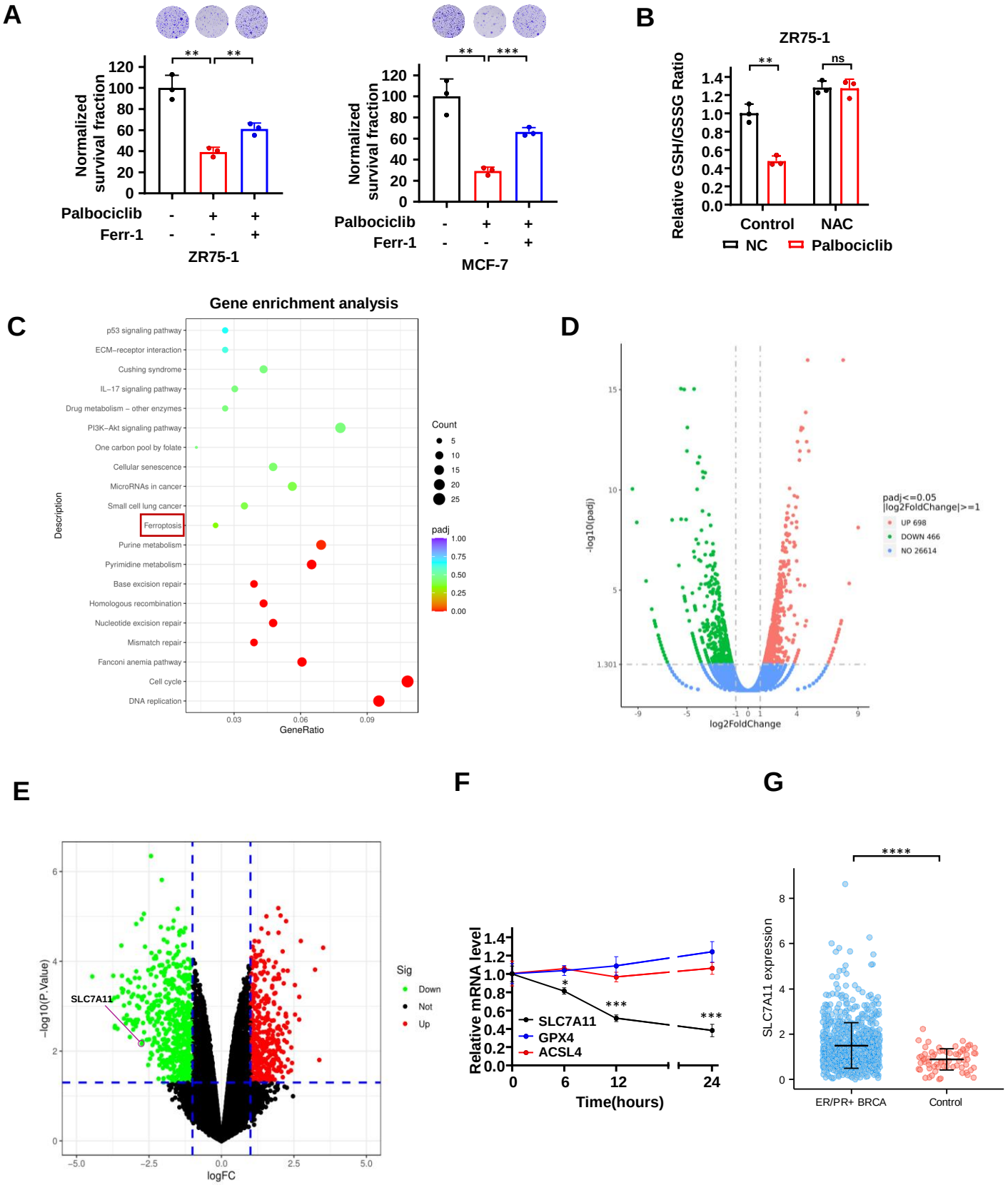
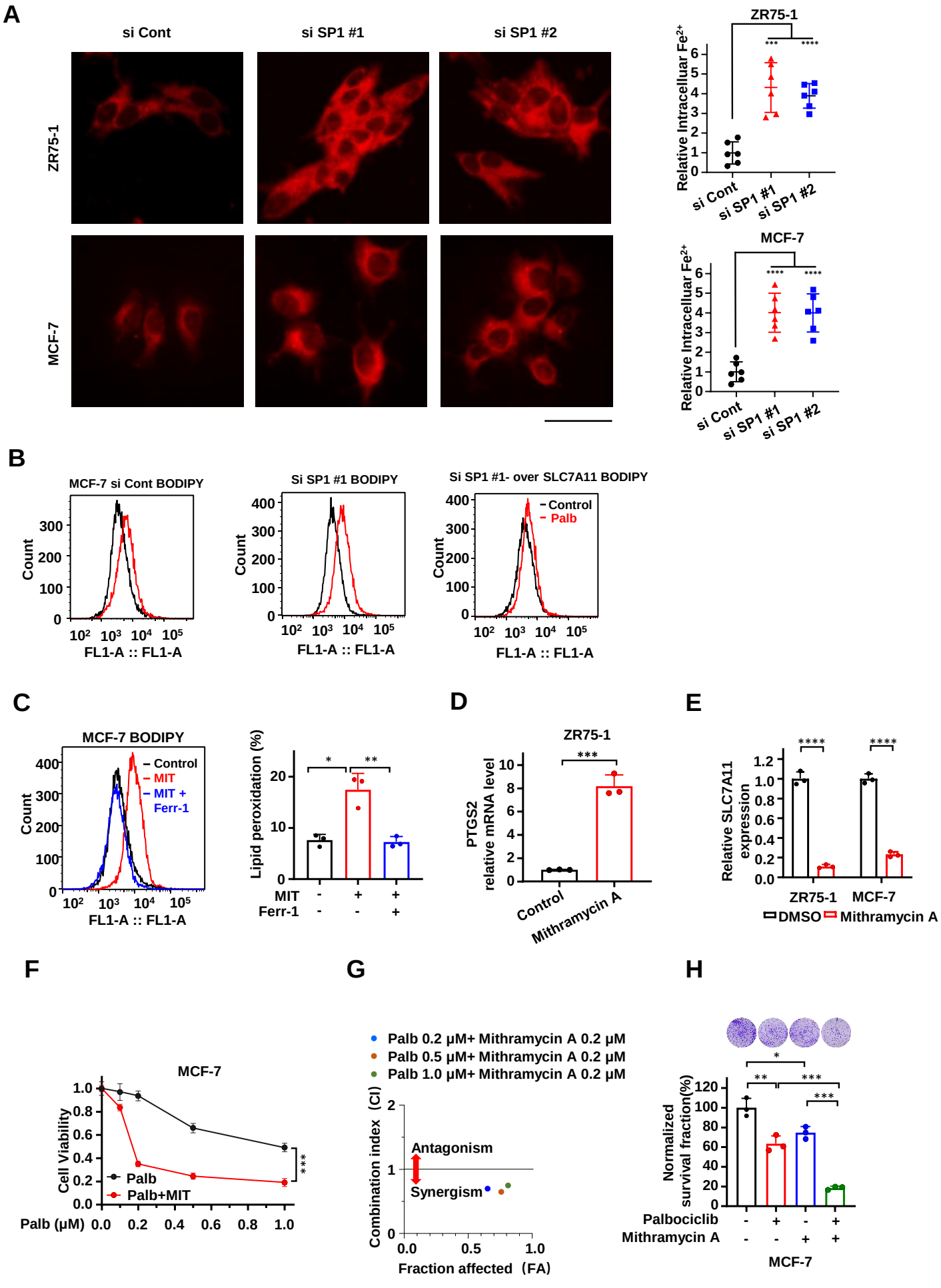
Supplement Figure. S1



Supplement Figure. S2



Supplement Fig. S1 Palbociclib promotes ferroptosis partially through suppressing SLC7A11-mediated GSH synthesis.

A, Clonogenic survival assay and representative images in ZR75-1 and MCF-7 cell lines treated with ddH2O or palbociclib (1 μM) in the absence or presence of ferrostatin-1(5 μM) for 24 h. The survival data were normalized to those of control cells treated with DMSO.

B, Relative GSH/GSSG ratio were assayed in ZR75-1 cells treated with ddH_2O or palbociclib (1 μM) in the absence or presence of NAC (5 mM) for 24h. The data were normalized to those of control cells treated with ddH_2O in respective cell lines.

C, Bubble chart displaying the gene enrichment analyses, as detected by RNA-seq in MCF-7 cells treated with palbociclib (1 μM) for 24 h, as compared to control (ddH2O).

D, Volcano plot showing genome-wide mRNA expression in MCF-7 cells treated with palbociclib (1 μM) for 24 h, as compared to control (ddH_2O) (Padj ≤0.05; log2 (Fold Change)| ≥1).

E, Volcano plot showing genome-wide mRNA expression in MCF-7 cells treated with different concentration of palbociclib according to GSE140758 from GEO datasets (Padj ≤0.05; log2 (Fold Change)| ≥1).

F, qRT-PCR analysis of SLC7A11, GPX4 and ACSL4 expression in ZR75-1 cells after treated with palbociclib (1 μM) at indicated time.

G, Gene expression level of SLC7A11 in TCGA ER+ breast tumor and matched TCGA normal breast tissues along with GTEx data.

H, Lipid peroxidation levels in EV- and SLC7A11-expressing A2780 cells treated with ddH_2O or palbociclib (1 μM) or palbociclib in combination with ferrostatin-1 (5 μ M) for 24 h.

Data represent mean ± SD, with n = 3 independent repeats. *P* values were determined using two-tailed unpaired Student's *t*-test (A, B and F-H) or Wilcox test (G) (ns, $P>0.05$; *, $P < 0.05$; **, $P \leq 0.01$; ***, $P < 0.001$; ****, $P \leq 0.0001$).

Supplement Fig. S2 SP1 inhibition represses SLC7A11 expression to promote ferroptosis.

A, ZR75-1 and MCF-7 cells were transfected with siRNAs against SP1 (si SP1#1 and si SP1#2) or control siRNA (si Cont) for 48 h, and then measured with FerroOrange staining for intracellular Fe^{2+}. Shown is one of six representative fields illustrating fluorescence intensity taken at identical exposures for each condition. Scale bars = 50 μm.

B, C11-BODIPY 581/591 probe was used to detected lipid peroxidation level in si Cont, si SP1#1 and si SP1#1- SLC7A11 over ZR75-1 cells after treated with ddH_2O or palbociclib (1 μM) for 24h.

C, C11-BODIPY 581/591 probe was used to detected lipid peroxidation level in in MCF-7 cells treated with DMSO or Mithramycin A (0.5 μM) in the absence or presence of ferrostatin-1(5 μM) for 24 h by flow cytometry. Quantification of C11-BODIPY 581/591 (FL1) fluorescence was shown. Quantification of C11-BODIPY 581/591 (FL1) fluorescence was shown.

D, qRT-PCR analysis of PTGS2 expression in ZR75-1 cells treated with DMSO or Mithramycin A (0.5 μM) for 24h.

E, qRT-PCR analysis of SLC7A11 expression in ZR75-1 and MCF-7 cell lines treated with ddH_2O or Mithramycin A (0.5 μM) for 24h.

F, Cell viability in MCF-7 cells treated with palbociclib and/or Mithramycin A at indicated concentrations.

G, The Chou-Talalay plot showing the combination effect of indicated treatments. The blue or orange dots in the plot represent the combination of palbociclib and Mithramycin A at indicated concentrations. CI values less than, equal to, or greater than 1 indicate synergistic, additive, or antagonistic effects, respectively.

H, Clonogenic survival assay and representative images in MCF-7 cell line treated with DMSO, palbociclib (0.2 μM), Mithramycin A (0.1 μM) or palbociclib in combination with Mithramycin A. The survival data were normalized to those of control cells treated with DMSO.

Data represent mean ± SD, with n = 3 independent repeats. *P* values were determined using two-tailed unpaired Student's *t*-test (ns, $P>0.05$; *, $P < 0.05$; **, $P \leq 0.01$; ***, $P < 0.001$; ****, $P \leq 0.0001$).

Supplement Table

MCF-7

CI Data for Non-Constant Combo: Palbociclib+Erastin)

Dose Palb	Dose Era	Effect	CI
0.1	4.0	0.141	1.43744
0.2	4.0	0.679	0.55266
0.5	4.0	0.751	0.6582
1.0	4.0	0.906	0.51144

ZR751

CI Data for Non-Constant Combo: Palbociclib+Erastin)

Dose Palb	Dose Era	Effect	CI
0.1	4.0	0.411	0.77466
0.2	4.0	0.693	0.47105
0.5	4.0	0.792	0.46419
1.0	4.0	0.89	0.39164

ZR751

CI Data for Non-Constant Combo: Palbociclib+SAS)

Dose Palb	Dose SAS	Effect	CI
0.1	1.0	0.184	1.35344
0.2	1.0	0.379	0.73592
0.5	1.0	0.725	0.42532
1.0	1.0	0.819	0.51838

ZR751

CI Data for Non-Constant Combo: Palbociclib+RSL3)

Dose Palb	Dose RSL3	Effect	CI
0.1	1.0	0.301	0.8718
0.2	1.0	0.49	0.76761
0.5	1.0	0.757	0.62538
1.0	1.0	0.866	0.59198

ZR751

CI Data for Non-Constant Combo: Palbociclib+Mithramycin A

Dose Palb	Dose MIT	Effect	CI
0.1	0.2	0.282	0.81775
0.2	0.2	0.452	0.56233
0.5	0.2	0.703	0.38695
1.0	0.2	0.821	0.3709

MCF-7

CI Data for Non-Constant Combo: Palbociclib+Mithramycin A

Dose Palb	Dose MIT	Effect	CI
0.1	0.2	0.164	3.36495
0.2	0.2	0.649	0.69582
0.5	0.2	0.755	0.64303
1.0	0.2	0.808	0.74343