

Supplementary Table

Table S1. The sequences and lengths of the PCR primers

Oligo Name	Sequence (5' to 3')	Length
POU5F1 Forward	cca ttt tgg tac ccc agg ct	20
POU5F1 Reverse	gag aca ggg gga aag gct tc	20
SOX2 Forward	ggc aat agc atg gcg agc	18
SOX2 Reverse	ttc atg tgc gcg taa ctg tc	20
NANOG Forward	atg gtg tga cgc agg gat g	19
NANOG Reverse	tgg ttg ctc cag gtt gaa ttg	21
GPX4 Forward	tga aga tcc aac cca agg gc	20
GPX4 Reverse	gtg gag aga cgg tgt cca aa	20
SMPD1 Forward	tcc tgg tta ccg tgt gta cc	20
SMPD1 Reverse	gcc tgg gtc aga ttc agg at	20
GAPDH Forward	cag gag gca ttg ctg atg at	20
GAPDH Reverse	gaa ggc tgg ggc tca ttt	18

Supplementary Figures

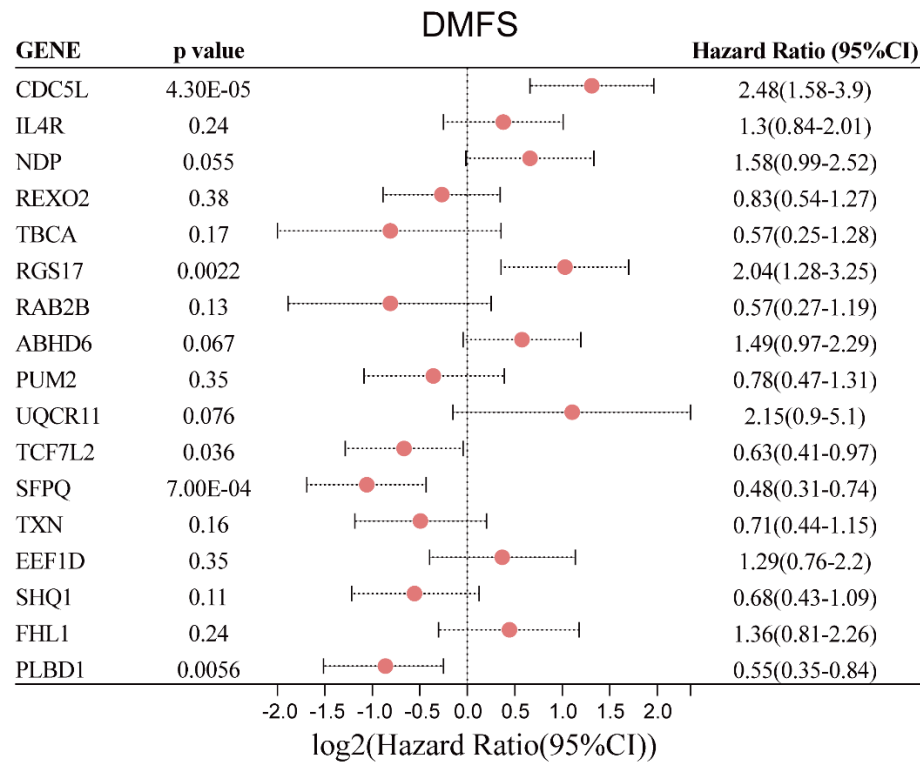


Figure S1. TNBC prognostic forest plot of the 17 stem-related genes

Distant metastasis free survival (DMFS) of 17 stem-related genes based on the TCGA database.

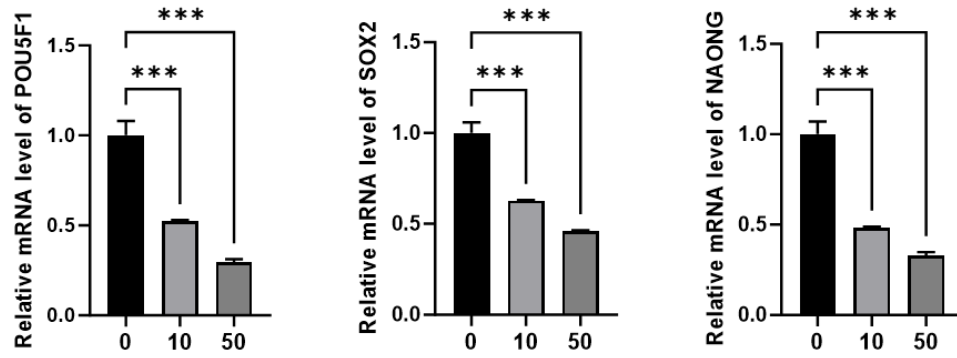


Figure S2. PS-T suppresses stemness markers in HCC1806 TNBC cells.

The mRNA levels of POU5F1, SOX2, and NANOG in HCC1806 cells treated with 0, 10, and 50 µg/mL PS-T for 24 hours were detected by quantitative PCR. Data are presented as mean $\pm$ standard deviation. One-way ANOVA with Bonferroni's post-hoc test was used for statistical analysis. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

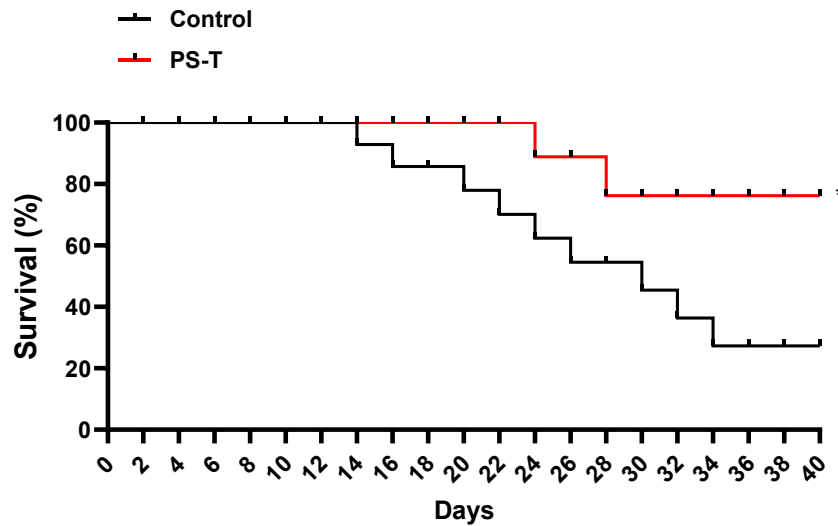


Figure S3. PS-T prolongs survival in mice bearing TNBC xenografts.

Female nude mice bearing orthotopic xenografts derived from ALDH⁺ MDA-MB-231 cells were randomly divided into control and PS-T-treated groups (n=10 per group). Survival was monitored, and the Kaplan-Meier survival curve shows that PS-T treatment significantly extended median survival. Statistical difference was analyzed using the Log-rank test, *P < 0.05.

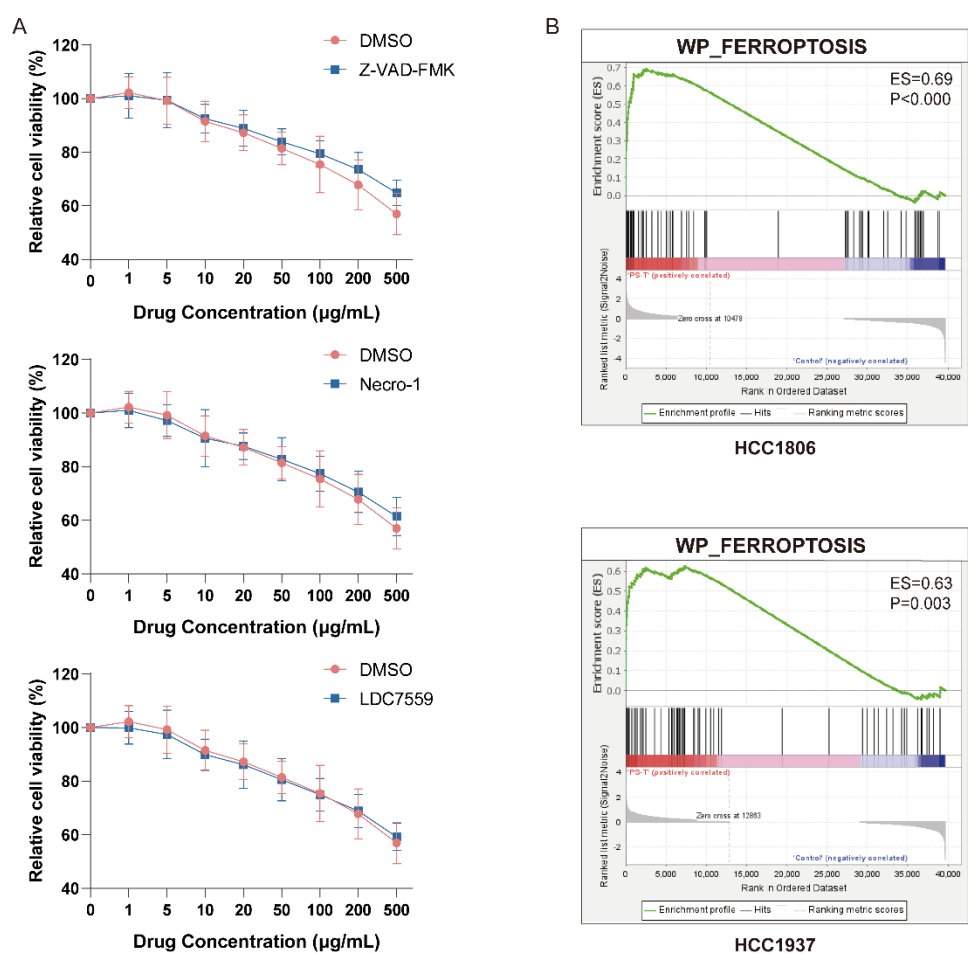


Figure S4. PS-T induces ferroptosis in TNBC cell lines

(A) ALDH⁺ MDA-MB-231 cells were flow-sorted and treated with different concentration of PS-T with or without Z-VAD-FMK (30 μM) (up), Necrostatin-1 (Necro-1, 20 μM) (middle), LDC7559 (5 μM) (down). Cell viability was measured by CCK-8 assays (n = 9). (B) GSEA result of HCC1806 (up) and HCC1937 cells (down) treated with or without PS-T using WP_FERROPTOSIS gene set.

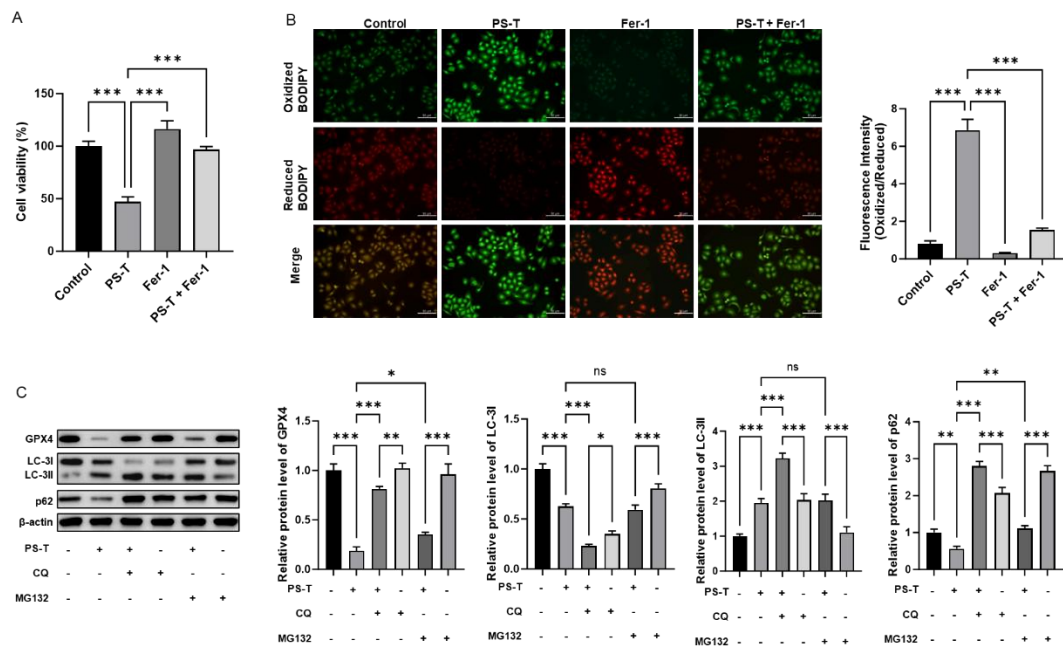


Figure S5. PS-T induces ferroptosis in HCC1806 TNBC cells.

(A) Cell viability of HCC1806 cells treated with PS-T (10 μ g/mL) and the ferroptosis inhibitor Ferrostatin-1 (Fer-1, 2 μ M) for 24 hours, measured by CCK-8 assays (n = 6 biological replicates). (B) Lipid peroxidation was assessed using the BODIPYTM 581/591 C11 probe in HCC1806 cells treated as in (A). (C) Western blot analysis of GPX4 and LC3 protein levels in HCC1806 cells treated as in (A). β -actin served as a loading control. Data are presented as mean $\pm$ standard deviation. (A, B) One-way ANOVA with Bonferroni's post-hoc test; (C) Student's t-test. ns, not significant; *P < 0.05; **P < 0.01; ***P < 0.001.

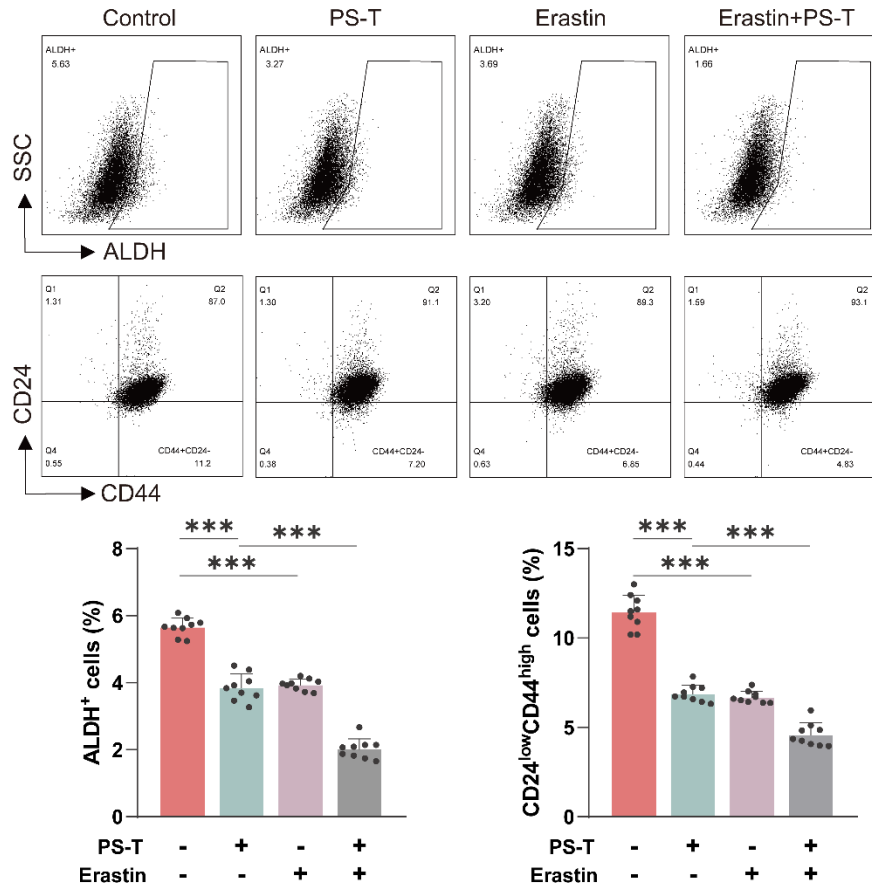


Figure S6. Ferroptosis inducers enhances the inhibitory effect of PS-T on BCSCs

The proportion of ALDH⁺ (up) and CD44^{high}CD24^{low} cells (down) in PS-T-treated ASM-overexpressing MDA-MB-231 cells, with or without co-treatment of Erastin (10 μ M) (n = 9). (mean $\pm$ standard deviation; ***P < 0.001). Experiment was repeated three times, and data was pooled. One-way analysis of variance and post-hoc Bonferroni tests were utilized.

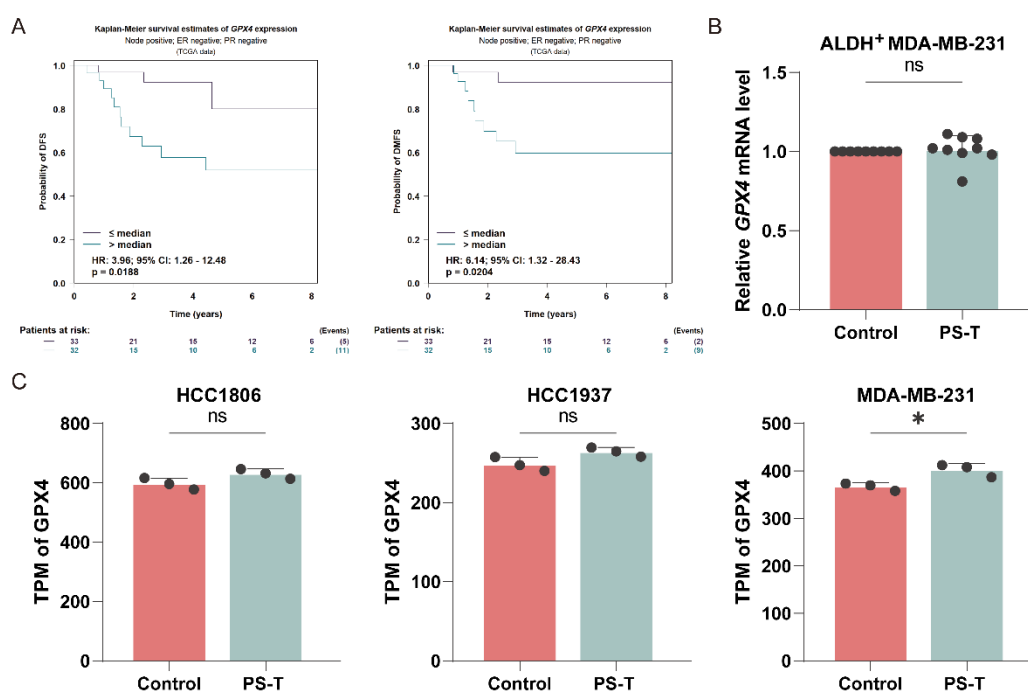


Figure S7. The effect of PS-T on GPX4 expression

(A) Correlation of GPX4 expression with DFS (left) and DMFS (right) in TNBC patients from TCGA database. (B) The GPX4 mRNA level in ALDH⁺ MDA-MB-231 cells was assessed by quantitative PCR. (C) Transcriptome sequencing was performed to measure the TPM of GPX4 mRNA levels in the HCC1937 (left), HCC1806 (middle), and MDA-MB-231 cells (right) treated with or without PS-T. (mean $\pm$ standard deviation; ns, not significant; *P < 0.05).

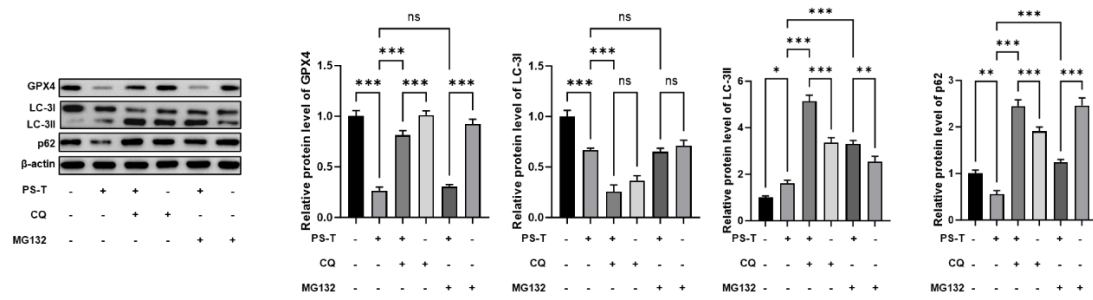


Figure S8. PS-T induces GPX4 degradation primarily through the autophagic-lysosomal pathway.

ALDH+ MDA-MB-231 cells were treated with PS-T (10 μ g/mL) for 24 hours in the presence or absence of the lysosomal inhibitor chloroquine (CQ, 20 μ M) or the proteasomal inhibitor MG132 (10 μ M). (Left) Western blot showing the accumulation of LC3-II upon CQ treatment, indicating enhanced autophagic flux by PS-T. GPX4 degradation induced by PS-T was reversed by CQ but not by MG132. (Right) Quantification of GPX4 protein levels relative to β -actin. Data are presented as mean $\pm$ standard deviation. One-way ANOVA with Bonferroni's post-hoc test was used. ns, not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

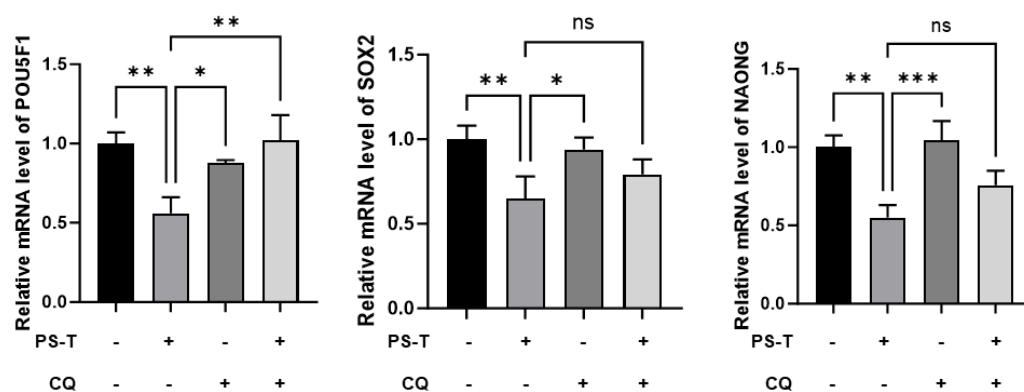


Figure S9. Inhibition of autophagic flux by chloroquine (CQ) reverses the PS-T-mediated downregulation of stemness markers.

The mRNA levels of POU5F1, SOX2, and NANOG in ALDH⁺ MDA-MB-231 cells were measured by quantitative PCR after treatment with DMSO, PS-T (10 μ g/mL), CQ (20 μ M), or their combination for 24 hours. This data corresponds to the results obtained with the autophagy inhibitor 3-MA presented in Figure 6J and confirms the role of autophagy in mediating PS-T's effects. Data represent mean $\pm$ standard deviation. Statistical analysis was performed using one-way ANOVA with Bonferroni's post-hoc test. ns, not significant; *P < 0.05; **P < 0.01; ***P < 0.001.

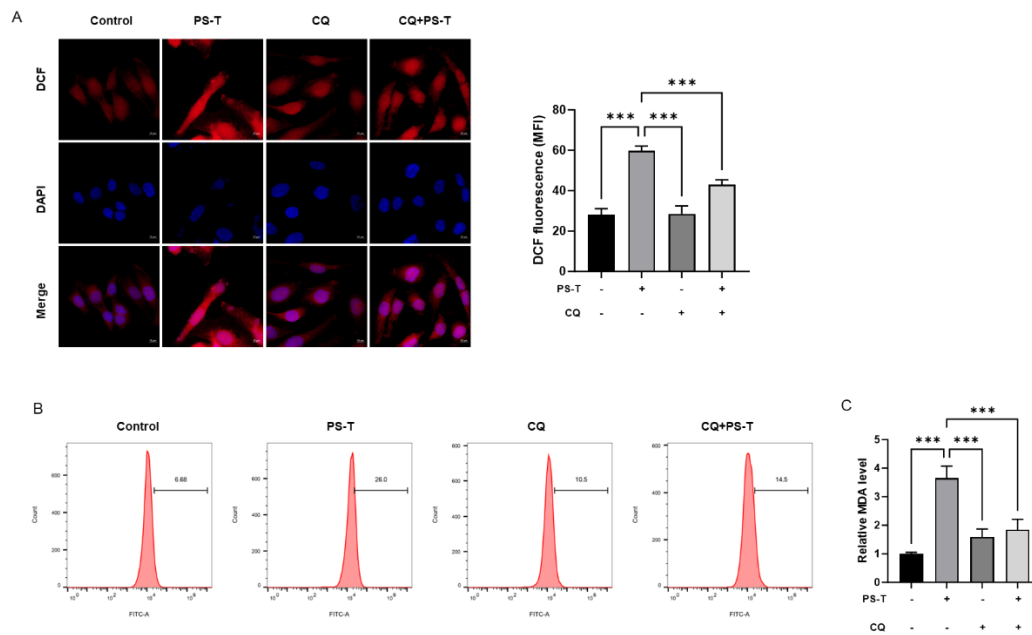


Figure S10. Inhibition of autophagic flux by chloroquine (CQ) reverses PS-T-induced ferroptotic events.

ALDH+ MDA-MB-231 cells were treated with DMSO, PS-T (10 μ g/mL), CQ (20 μ M), or their combination for 24 hours. (A) ROS fluorescence, (B) C11 BODIPY fluorescence (lipid peroxidation), and (C) relative MDA concentration were assessed. These panels correspond to and corroborate the findings with 3-MA shown in Figures 6G, 6H, and 6I, respectively, using a more specific lysosomal inhibitor. Scale bars = 20 μ m. Data represent mean $\pm$ standard deviation. Statistical analysis was performed using one-way ANOVA with Bonferroni's post-hoc test. ns, not significant; ***P < 0.001.

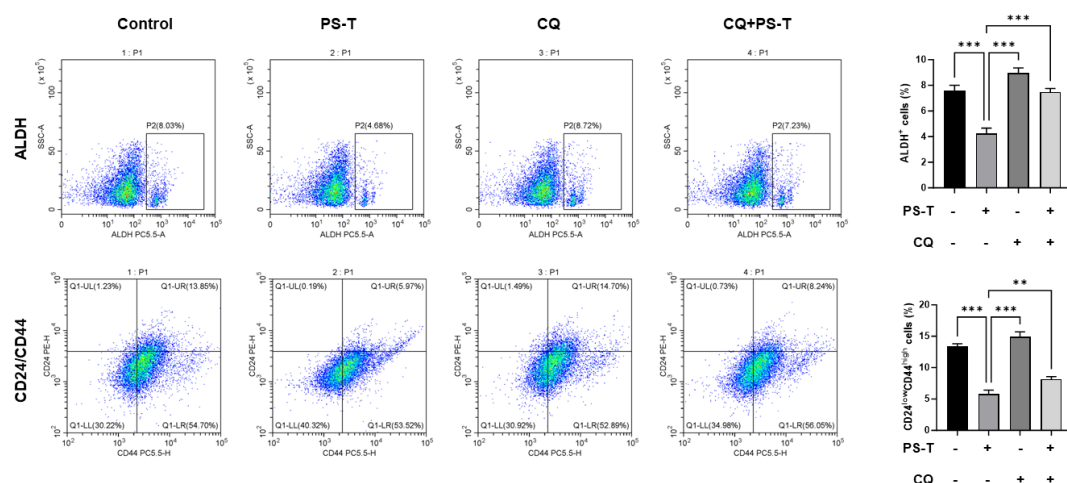
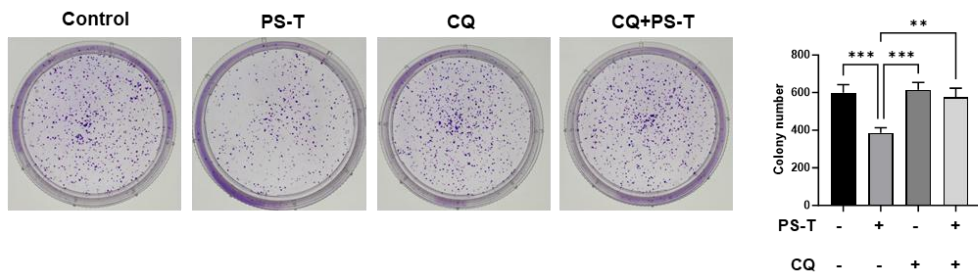


Figure S11. Inhibition of autophagic flux by chloroquine (CQ) reverses the PS-T-mediated reduction of BCSC populations.

The proportion of (Top) ALDH⁺ and (Bottom) CD44^{high}CD24^{low} MDA-MB-231 cells was determined by flow cytometry after treatment with DMSO, PS-T (10 μ g/mL), CQ (20 μ M), or their combination for 24 hours. This data corresponds to the results presented in Figure 6K and reinforces the role of autophagy in PS-T's suppression of BCSCs. Data represent mean $\pm$ standard deviation. Statistical analysis was performed using one-way ANOVA with Bonferroni's post-hoc test. ns, not significant; ***P < 0.001.

A



B

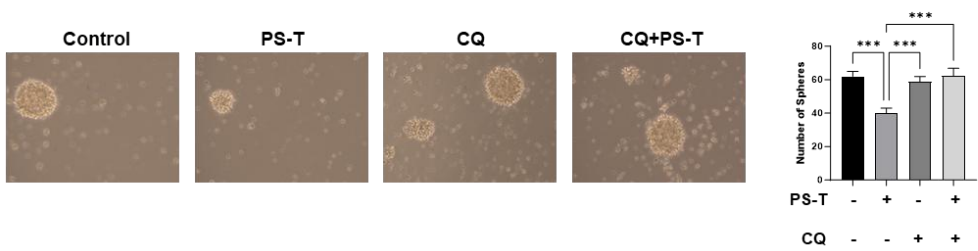


Figure S12. Inhibition of autophagic flux by chloroquine (CQ) reverses the PS-T-mediated suppression of self-renewal capacity.

ALDH+ MDA-MB-231 cells were treated with DMSO, PS-T (10 μ g/mL), CQ (20 μ M), or their combination. (A) Colony formation and (B) mammosphere formation assays were performed. Scale bars = 50 μ m. Data represent mean $\pm$ standard deviation. Statistical analysis was performed using one-way ANOVA with Bonferroni's post-hoc test. ns, not significant; **P < 0.01, ***P < 0.001.

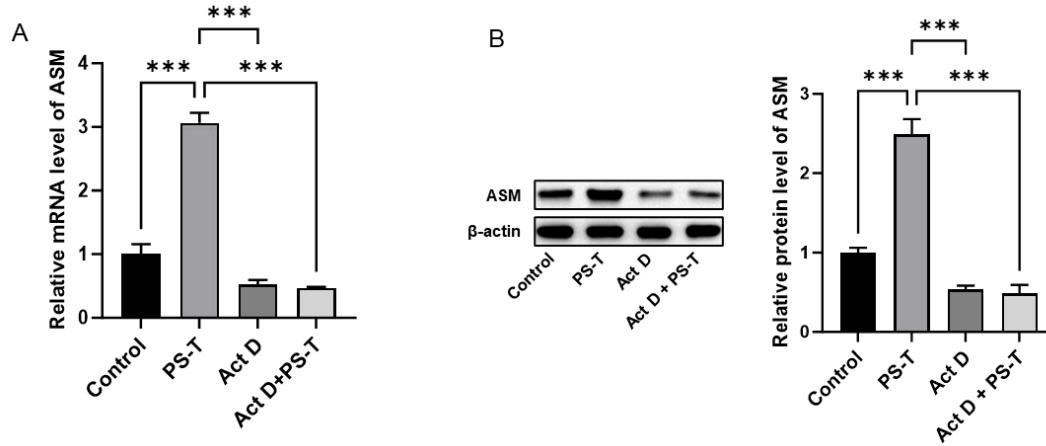


Figure S13. PS-T transcriptionally upregulates ASM expression.

(A-B) ALDH⁺ MDA-MB-231 cells were pre-treated with the transcriptional inhibitor actinomycin D (Act D, 5 μ g/mL) for 1 hour, followed by co-treatment with or without PS-T (10 μ g/mL) for an additional 24 hours. (A) SMPD1 mRNA levels were assessed by quantitative PCR. (B) ASM protein levels were assessed by western blotting. Data are presented as mean $\pm$ standard deviation. One-way ANOVA with Bonferroni's post-hoc test was used. ns, not significant; ***P < 0.001.

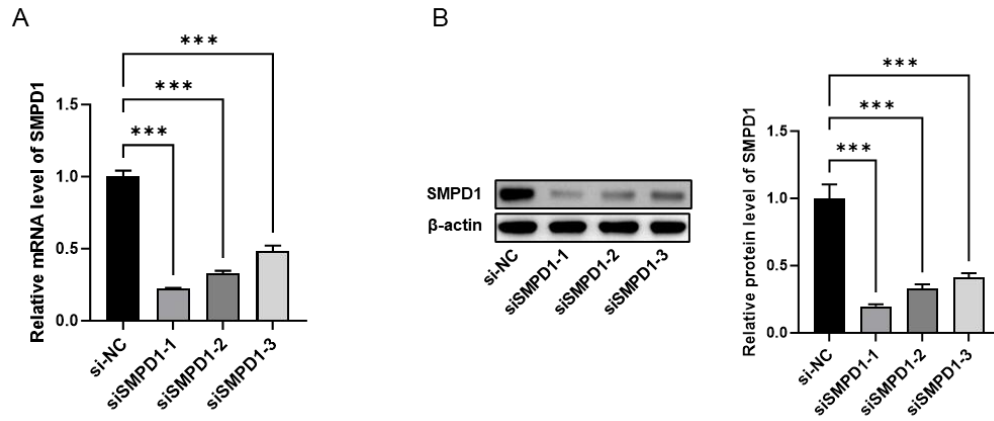


Figure S14. Efficient knockdown of ASM (SMPD1) in MDA-MB-231 cells.

(A-B) MDA-MB-231 cells were transfected with a non-targeting control siRNA (si-NC) or three independent siRNAs targeting SMPD1 (si-ASM#1, #2, #3). (A) SMPD1 mRNA levels were assessed by quantitative PCR 48 hours post-transfection. (B) ASM protein levels were assessed by western blotting 72 hours post-transfection. Data are presented as mean $\pm$ standard deviation. One-way ANOVA with Bonferroni's post-hoc test was used. *** $P < 0.001$.

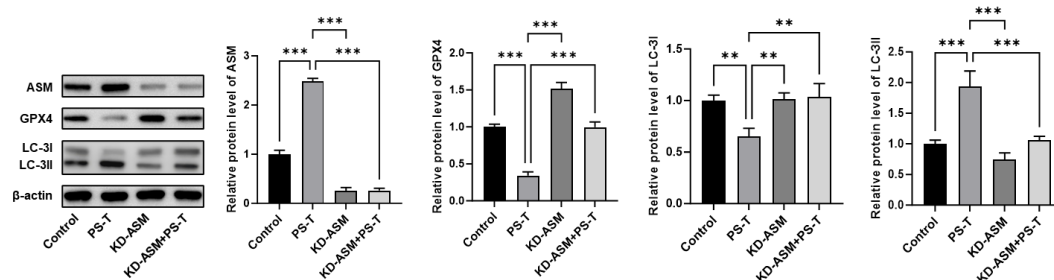


Figure S15. ASM is essential for PS-T-induced GPX4 degradation and LC3-II accumulation.

Control (si-NC) and ASM-knockdown (si-SMPD1#1) MDA-MB-231 cells were treated with or without PS-T (10 μ g/mL) for 24 hours. Western blot analysis of ASM, GPX4, and LC3 protein levels. β -actin served as a loading control. Quantification of GPX4 and LC3-II protein levels relative to β -actin. Data are presented as mean $\pm$ standard deviation. One-way ANOVA with Bonferroni's post-hoc test was used. ns, not significant; *P < 0.05; **P < 0.01, ***P < 0.001.