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SUPPLEMENTARY MATERIAL

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Overexpression of GPER1 suppressed esophageal carcinoma growth *via* activating cAMP pathway

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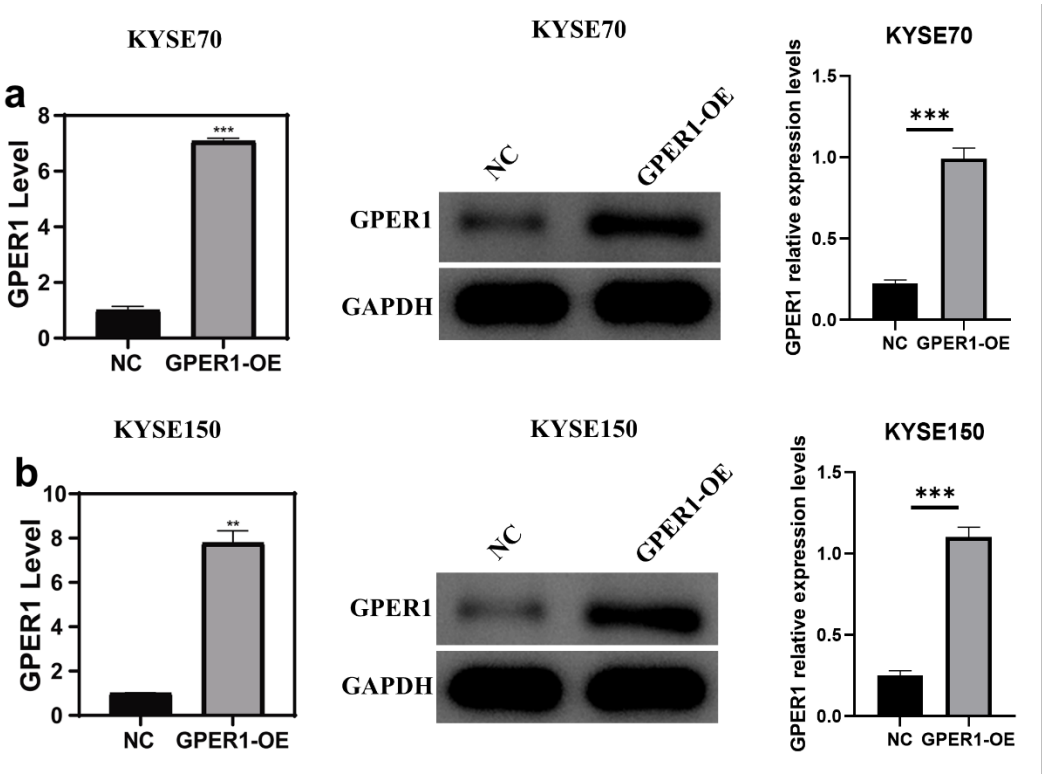
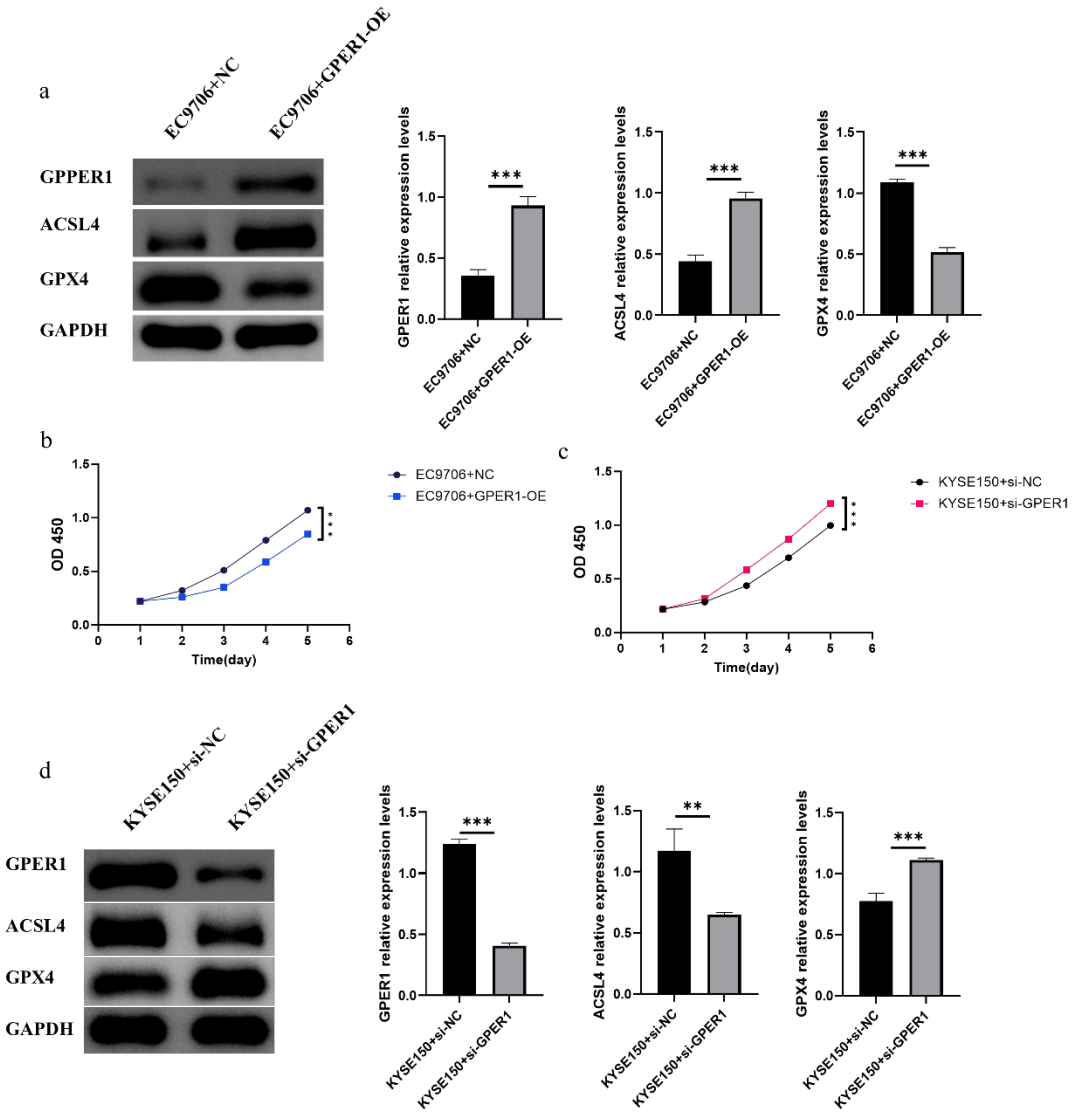
Supplementary Figure S1.

Efficiency of GPER1 over-expression in EC cells detected by qRT-PCR. QRT-PCR and Western blot were utilized to detect the expression of GPER1 in KYSE70 (a) and KYSE150 (b) cells. Data are displayed as mean ± SD. Unpaired t test, **p < 0.01 and ***p < 0.001.



Supplementary Figure 2.

Modulation of GPER1 expression bidirectionally regulates ferroptosis in EC cells.
(a, b) Overexpression of GPER1 in EC9706 cells:(a) Protein levels of GPER1, ACSL4, and GPX4 were determined by Western blotting (left panel) and quantitatively analyzed by densitometry (right panel). (b) Cell viability was measured by CCK-8 assay. (c, d) Knockdown of GPER1 in KYSE150 cells:(c) Cell viability was measured by CCK-8 assay. (d) Protein levels of GPER1, ACSL4, and GPX4 were determined by western blotting (left panel) and quantitatively analyzed by densitometry (right panel). Data are presented as mean ± SD from three independent experiments. Unpaired t test, *p < 0.05, **p < 0.01, ***p < 0.001.



Supplementary Figure 3.

Stable overexpression of GPER1 in tumor tissues at the endpoint of the *in vivo* study.

Protein levels of GPER1 in harvested tumor tissues from the negative control (NC) and GPER1-overexpression (GPER1-OE) groups were determined by western blotting. (a) Representative western blot images showing GPER1 expression in tumors from each group. GAPDH was used as a loading control. (b) Quantitative densitometric analysis of GPER1 protein levels normalized to GAPDH. Data are presented as the mean ± SD (n=6 tumors per group). Statistical significance was determined by an unpaired Student's t-test. ***$p < 0.001$.