

1 Bisphenol F exposure induces ovarian
2 ferroptosis via the ER β -ATM-ferritinophagy
3 axis

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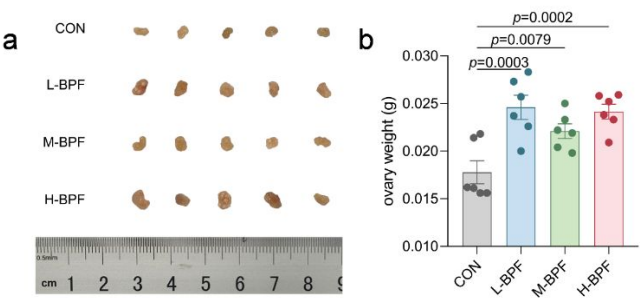
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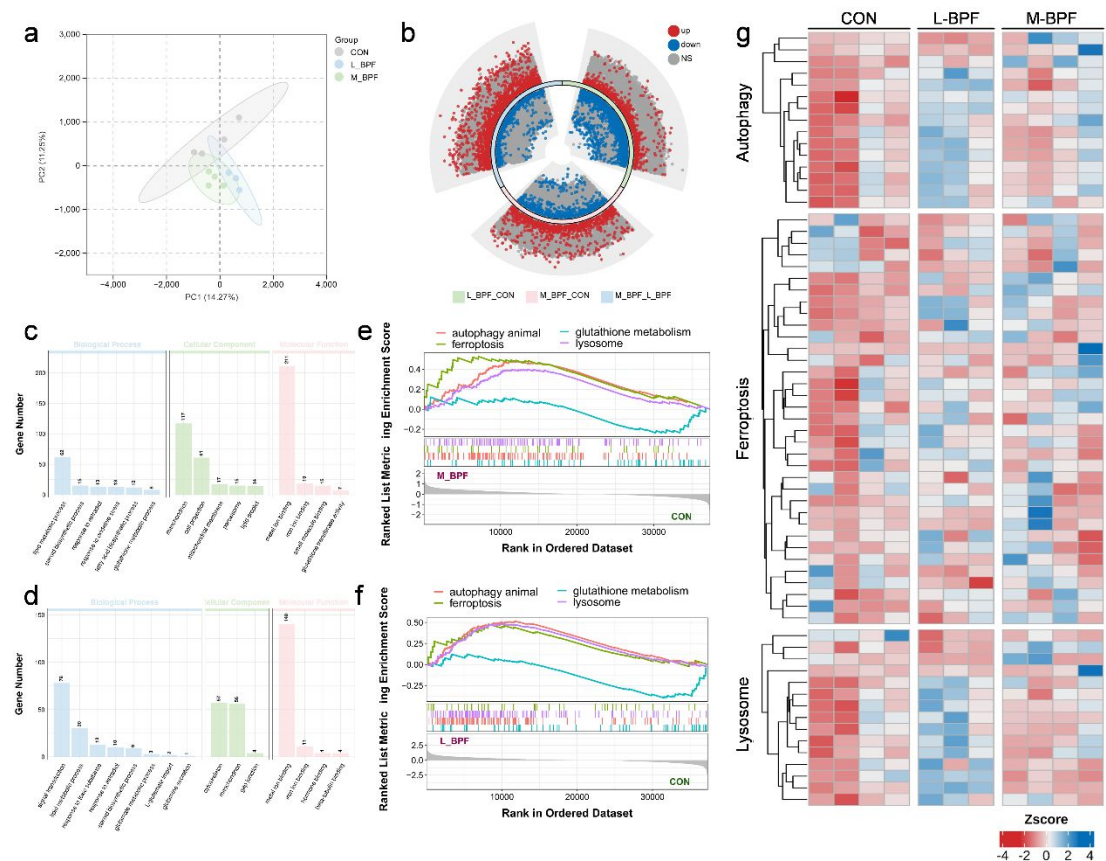
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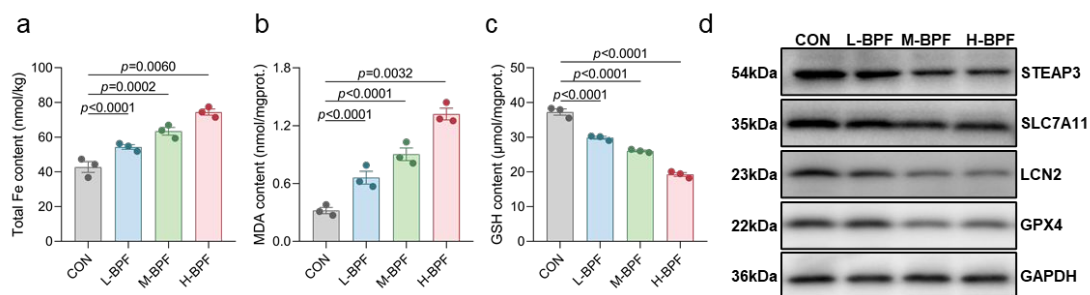
17 **Supplementary Figures**



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19 **Supplementary Fig. 1. BPF exposure impairs ovarian structure and reduces ovarian weight.**
20 **a** Representative images of ovaries isolated from mice following exposure to different doses of BPF (L-
21 BPF, M-BPF, H-BPF). **b** Ovarian weight from the indicated treatment groups (n = 6 biologically
22 independent mice). Data are presented as mean $\pm$ S.E.M.

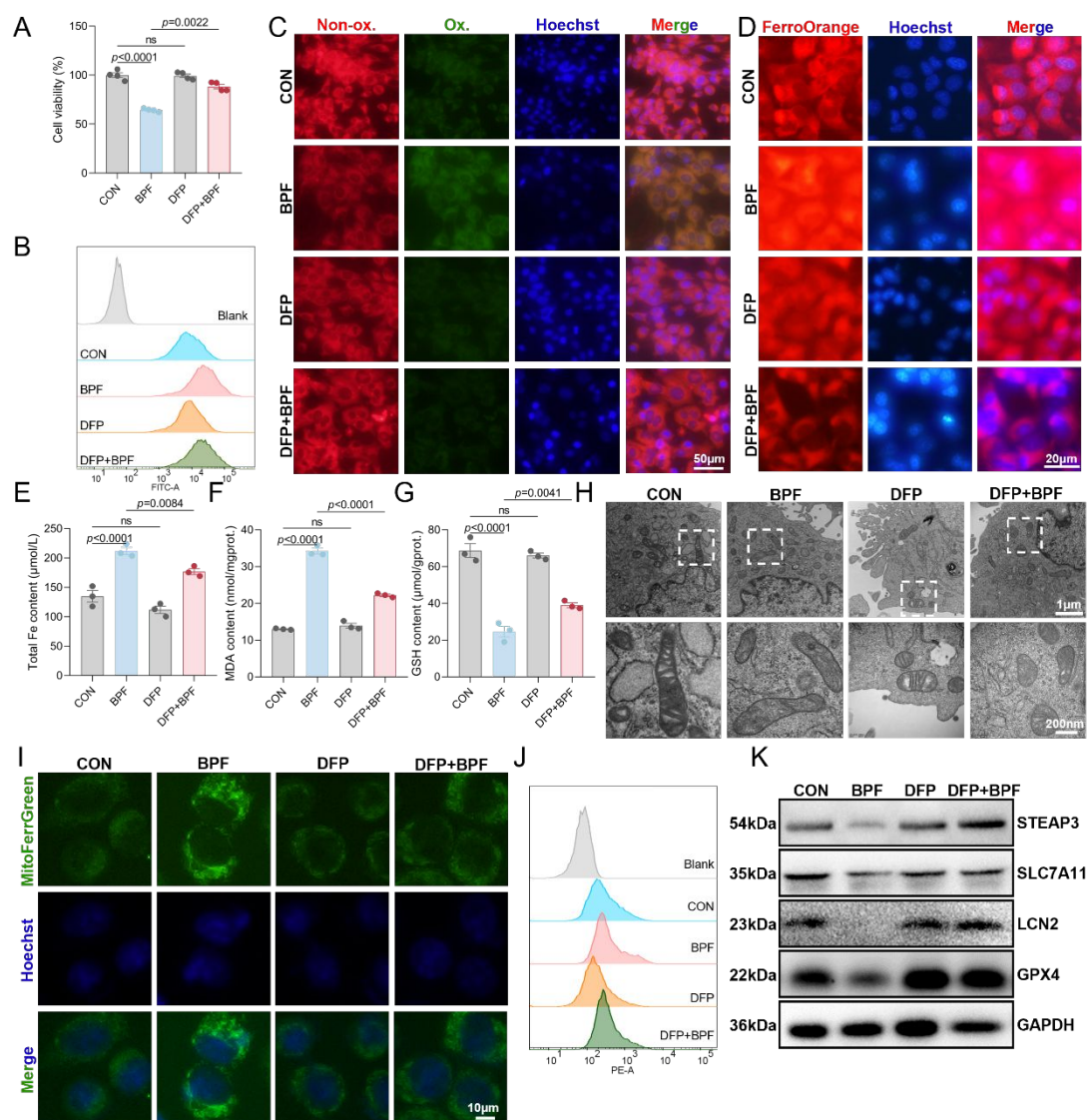


Supplementary Fig. 2. Transcriptome data indicates that BPF induces ovarian ferroptosis.
a PCA score plot of ovarian transcriptome data ($n = 3-4$ mice/group). **b** Circle volcano plot showing ovarian mRNA changes in BPF-treated mice versus CON mice. **c-d** GO enrichment bar plot showing ovarian differentially expressed mRNA function in BPF-treated mice versus CON mice. **e-f** GSEA analysis revealing the enrichment of autophagy, glutathione metabolism, ferroptosis and lysosome. **g** Cluster heatmap showing the effects of different concentrations of BPF on ferroptosis, autophagy, and lysosome related genes.



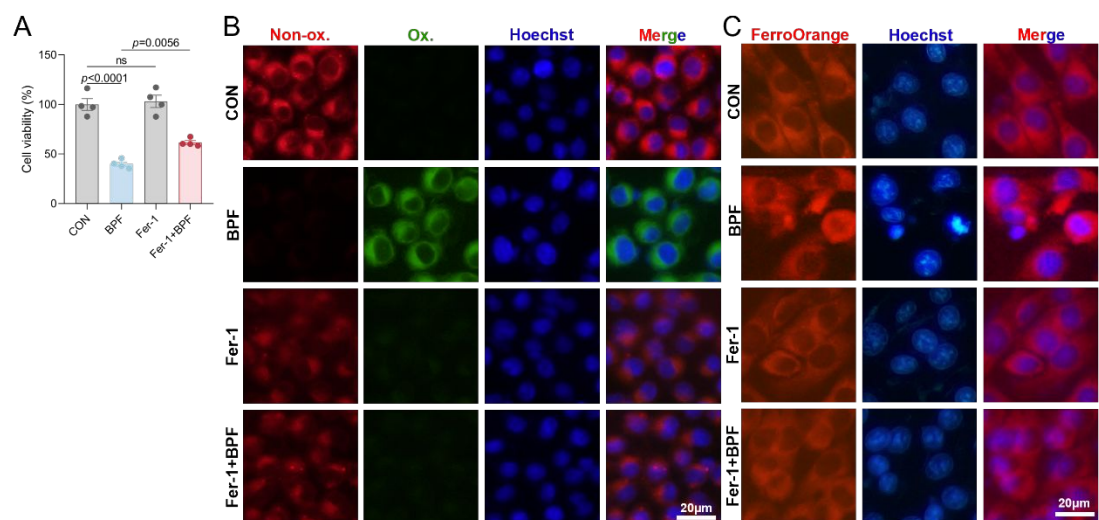
Supplementary Fig. 3. BPF disrupts ovarian iron metabolism and lipid metabolism, and regulates ferroptosis-related proteins.

a Different doses of BPF administration increased total Fe content ($n = 3/\text{group}$). **b** Exposure to BPF increased MDA content ($n = 3/\text{group}$). **c** Different doses of BPF administration decreased GSH content ($n = 3/\text{group}$). **d** Ovaries from different doses of BPF group and CON group mice were subjected to Western analysis. The expression of STEAP3, SLC7A11, LCN2, GPX4 are shown.

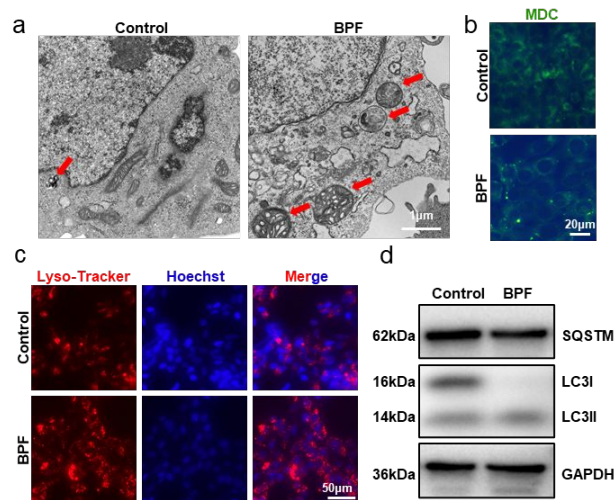


Supplementary Fig. 4. DFP alleviates BPF-induced ovarian granulosa cell ferroptosis.

a CCK8 assays of kk-1 cells treated with BPF or DFP for 24 h, and treated with BPF and DFP for 24 h ($n = 4$). **b** Flow cytometry analysis of ROS of kk-1 cells treated with BPF, DFP, DFP+BPF for 24 h. **c** Lipid ROS in granulosa cells after BPF and DFP treatment. **d** Intercellular ferrous ion in kk-1 cells treated with BPF or DFP for 24 h, and treated with BPF and DFP for 24 h. **e** DFP reversed BPF-increased total Fe content. **f** DFP reversed BPF-increased MDA content. **g** DFP reversed BPF-decreased GSH content. **h** kk-1 cells were harvested after BPF and DFP treatment and analyzed by TEM for mitochondrial morphology. **i** Representative images of MitoFerroGreen in kk-1 cells treated with BPF, DFP, DFP+BPF for 24 h. **j** Flow cytometry analysis of mitochondrial ROS of kk-1 cells treated with BPF, DFP, DFP+BPF for 24 h. **k** Immunoblotting analysis of ferroptosis-related proteins STEAP3, SLC7A11, LCN2, GPX4 in kk-1 cells treated with BPF or DFP or BPF+DFP for 24 h. Representative experiment ($n = 3$ technical replicates; 3 independent experiments).

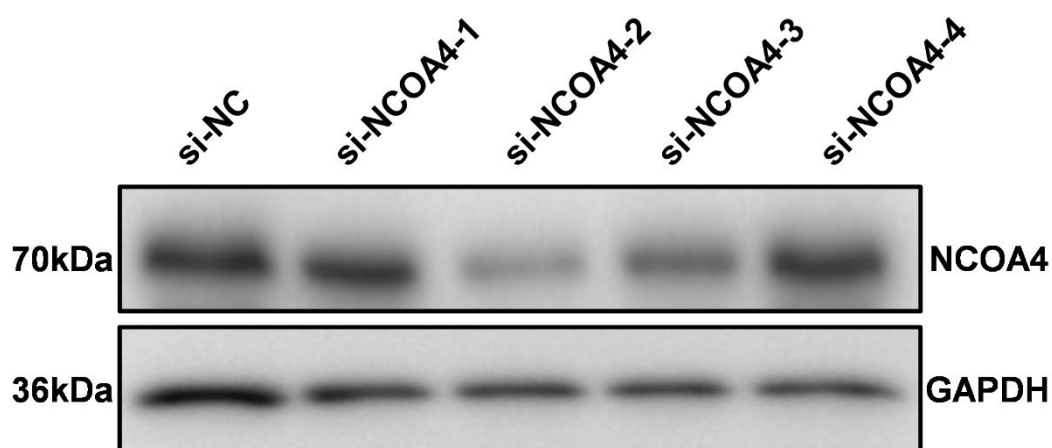


Supplementary Fig. 5. Fer-1 alleviates BPF-induced ovarian granulosa cell ferroptosis. a CCK8 assays of kk-1 cells treated with BPF and Fer-1 for 24 h (n = 4). **b** Lipid ROS in granulosa cells after BPF and Fer-1 treatment. **c** Intercellular ferrous ion in kk-1 cells treated with BPF and Fer-1 for 24 h.

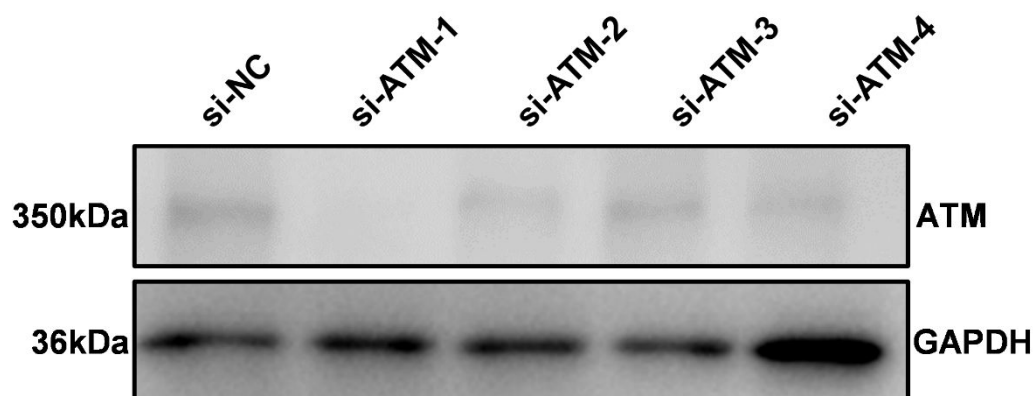


Supplementary Fig. 6. BPF induces ovarian granulosa cell autophagy.

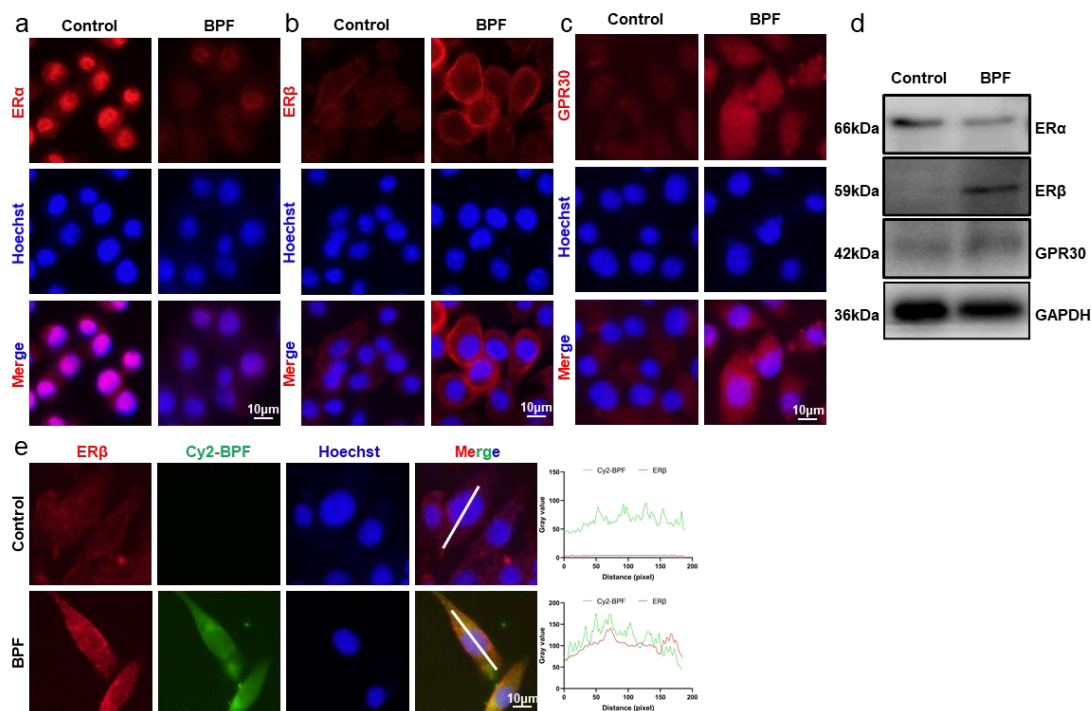
a kk-1 cells were harvested after BPF treatment for 24 h and analyzed by TEM for autophagosome morphology. Red arrows indicate the autophagosomes. **b** Autophagic vacuoles in granulosa cells marked in MDC after BPF and DFP treatment. **c** Lysosomes in kk-1 cells treated with BPF or DFP for 24 h, and treated with BPF and DFP for 24 h. **d** Immunoblotting analysis of ferroptosis-related proteins SQSTM1 and LC3 in kk-1 cells treated with BPF for 24 h. Representative experiment (n = 3 technical replicates; 3 independent experiments).



Supplementary Fig. 7. Immunoblotting analysis of NCOA4 in kk-1 cells transfected with different small interfering RNAs of NCOA4.

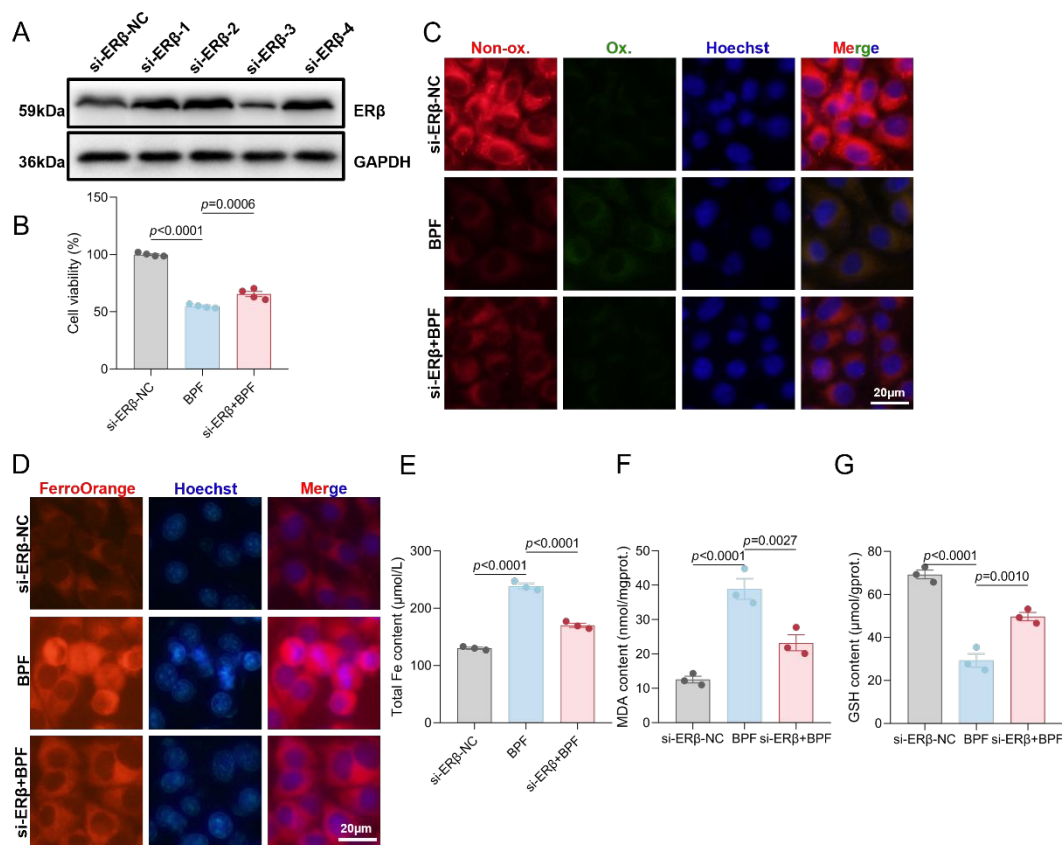


Supplementary Fig. 8. Immunoblotting analysis of ATM in kk-1 cells transfected with different small interfering RNAs of ATM.



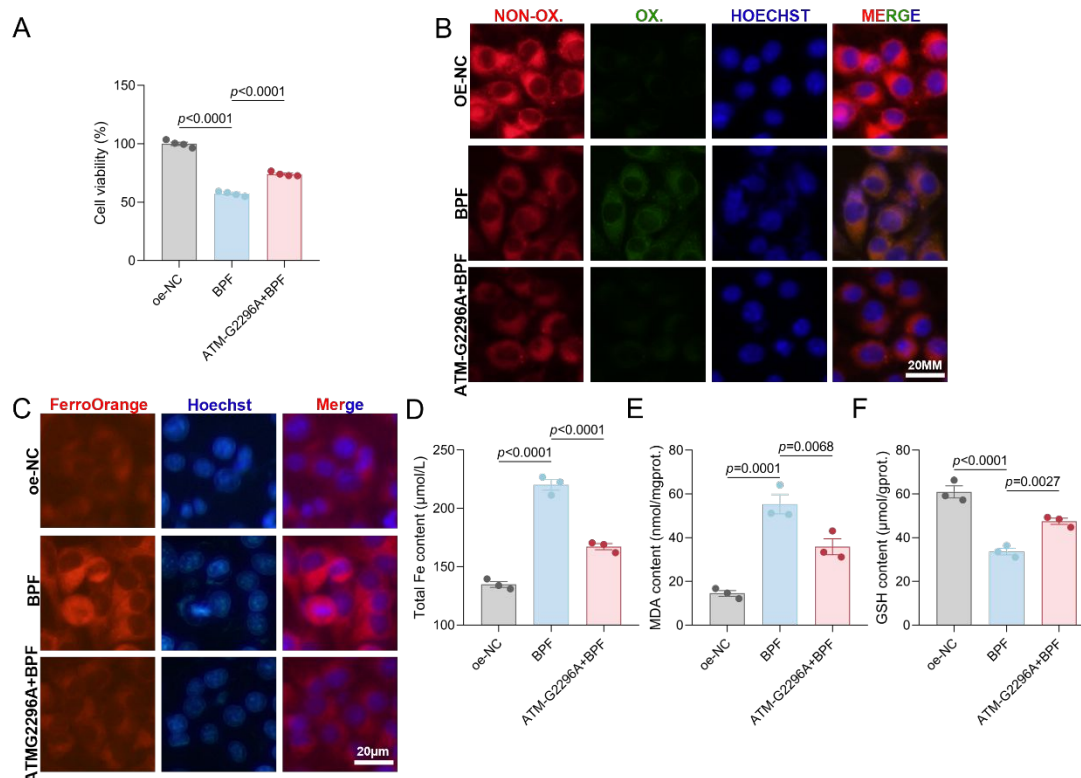
Supplementary Fig. 9. BPF directly binds to ERβ in ovarian granulosa cells.

a-c Immunofluorescence (IF) using anti-ERα antibody (**a**), anti-ERβ antibody (**b**) and anti-GPR30 antibody (**c**) was performed in kk-1 cells after BPF treatment for 24 h. **d** Immunoblotting analysis of ERα, ERβ and GPR30 in kk-1 cells treated with BPF for 24 h. **e** IF using anti-ERβ antibody (Red) was performed in kk-1 cells after Cy2-labeled BPF treatment. Co-localization analysis was conducted using ImageJ software.



Supplementary Fig. 10. ERβ knockdown reverses BPF-induced ovarian granulosa cell ferroptosis.

a Immunoblotting analysis of ERβ in kk-1 cells transfected with different small interfering RNAs of ERβ (si-ERβ). **b** CCK8 assays of kk-1 cells transfected with si-ERβ, and treated with BPF. **c** Lipid ROS in granulosa cells after si-ERβ transfection and BPF treatment. **d** Intercellular ferrous ion in kk-1 cells transfected with si-ERβ and treated with BPF. **e** ERβ knockdown reversed BPF-upregulated total Fe content. **f** ERβ knockdown alleviated BPF-upregulated MDA content. **g** ERβ knockdown alleviated BPF-downregulated GSH content. Representative experiment (n = 3 technical replicates; 3 independent experiments).



Supplementary Fig. 11. ATM point mutation (G2296A) alleviates BPF-induced ovarian granulosa cell ferroptosis.

a CCK8 assays of kk-1 cells transfected with ATM point mutation plasmid and treated with BPF. **b** Lipid ROS in granulosa cells after ATM point mutation plasmid transfection and BPF exposure. **c** Inter cellular ferrous ion in kk-1 cells transfected with ATM point mutation plasmid and treated with BPF. **d** ATM G2296A reversed BPF-upregulated total Fe content. **e** ATM G2296A alleviated BPF-upregulated MDA content. **f** ATM G2296A alleviated BPF-downregulated GSH content. Representative experiment (n = 3 technical replicates; 3 independent experiments).

94 Supplementary Tables

95 **Supplement Table 1 siRNA sequences used for transfection**

siRNA	Sense strand	Antisense strand	SOURCE
si-NC	5'-UUCUCCGAACGUGUCACGUTT-3'	5'-ACGUGACACGUUCGGAGAATT-3'	GenePharma
si-NCOA4-1	5'-GGGCUGAACAGCAAAUUAATT-3'	5'-UUAUUUGCUGUUCAGCCCTT-3'	GenePharma
si-NCOA4-2	5'-GGAGUAUACUCAGAACAAATT-3'	5'-UUUGUUCUGAGUAUACUCCTT-3'	GenePharma
si-NCOA4-3	5'-CCACGUCAUAAAGAAUAGUTT-3'	5'-ACUAUUCUUUAUGACGUGGTT-3'	GenePharma
si-NCOA4-4	5'-CCCUACAAGAAGAACGUAATT-3'	5'-UUACGUUCUUCUUGUAGGGTT-3'	GenePharma
si-ERβ-1	5'-GCUUCUCUAUGCAGAACCUTT-3'	5'-AGGUUCUGCAUAGAGAAGCTT-3'	GenePharma
si-ERβ-2	5'-CAAGGACAUAUAGACUAUATT-3'	5'-UAUAGUCAUUAUGUCCUUGTT-3'	GenePharma
si-ERβ-3	5'-CUGGGUGAUUUCGAAGAGUTT-3'	5'-ACUCUUCGAAAUCACCCAGTT-3'	GenePharma
si-ERβ-4	5'-GAGGGUACAAGUCCUCAAUTT-3'	5'-AUUGAGGACUUGUACCCUCTT-3'	GenePharma
si-ATM-1	5'-GAGCGAUGCAAAUGAUUAUTT-3'	5'-AUAUACAUUUGCAUCGCUCTT-3'	GenePharma
si-ATM-2	5'-GGGUCAGUCAACAGAUUAUTT-3'	5'-AUAUUCUGUUGACUGACCCTT-3'	GenePharma
si-ATM-3	5'-GAGGCAUAAACAGUGAAUUTT-3'	5'-AUUUCACUGUUUAUGCCUCTT-3'	GenePharma
si-ATM-4	5'-CUCCCUGAAAGAGCAAUAUTT-3'	5'-AUAUUGCUCUUUCAGGGAGTT-3'	GenePharma

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