

Supplementary

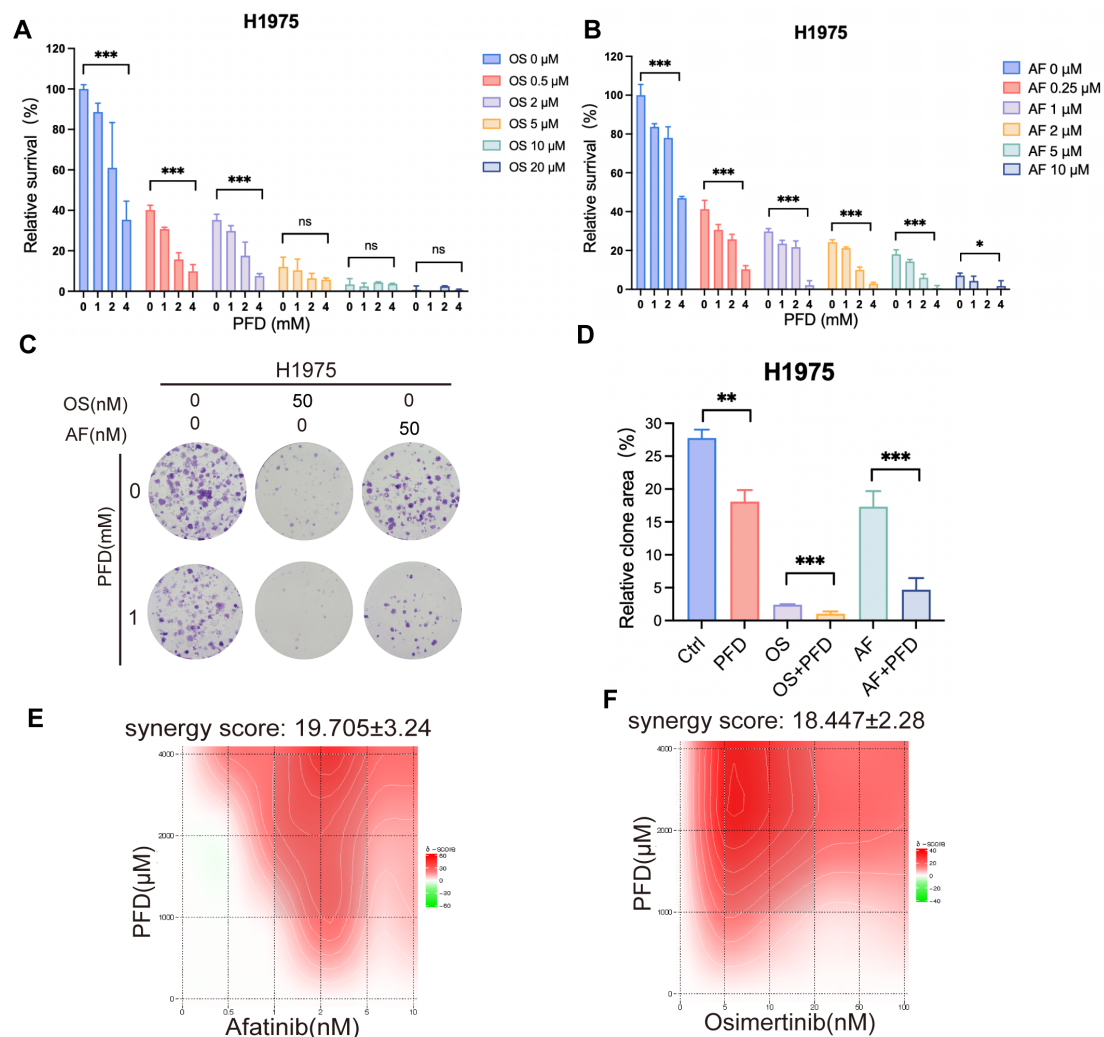


Figure S1 PFD synergizes with OS/AF against H1975 NSCLC cells in vitro. (A,B) Relative survival rate of H1975 cells treated with gradient concentrations of PFD combined with OS (A) or AF (B), detected by CCK-8 assay. (C,D) Colony formation capacity of H1975 cells treated with PFD alone, OS/AF alone, or their combination, with quantitative analysis of relative clone area shown in D. (E,F) Bliss synergy scores and dose-effect matrix heatmaps of PFD combined with AF (E) or OS (F) in H1975 cells, analyzed via SynergyFinder platform. ns, no significance; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. control or monotherapy group.

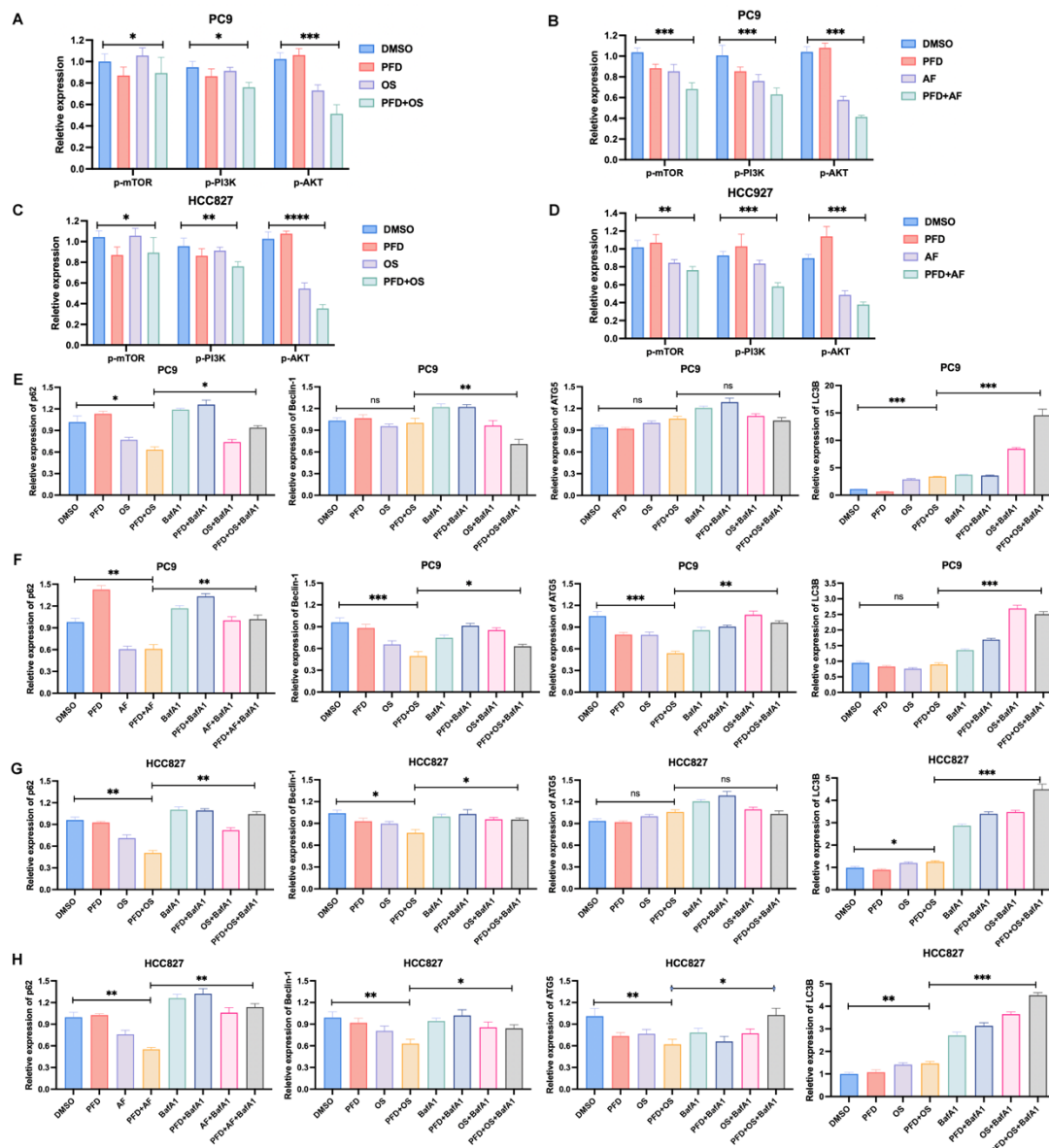


Figure S2 Pirfenidone combined with EGFR-TKIs suppresses PI3K-AKT-mTOR signaling and triggers autophagy in EGFR-mutant NSCLC cells. (A-D) Quantitative analysis of relative protein levels of phosphorylated mTOR, PI3K and AKT in PC9 and HCC827 cells treated with DMSO, PFD, OS, AF, and PFD combined with OS or AF, based on western blot grayscale quantification. (E-H) Western blot quantification of autophagy-associated proteins p62, Beclin-1, ATG5 and LC3B in PC9 and HCC827 cells under the indicated drug treatments. DMSO served as vehicle control. Data are presented as mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$; ns, not significant.

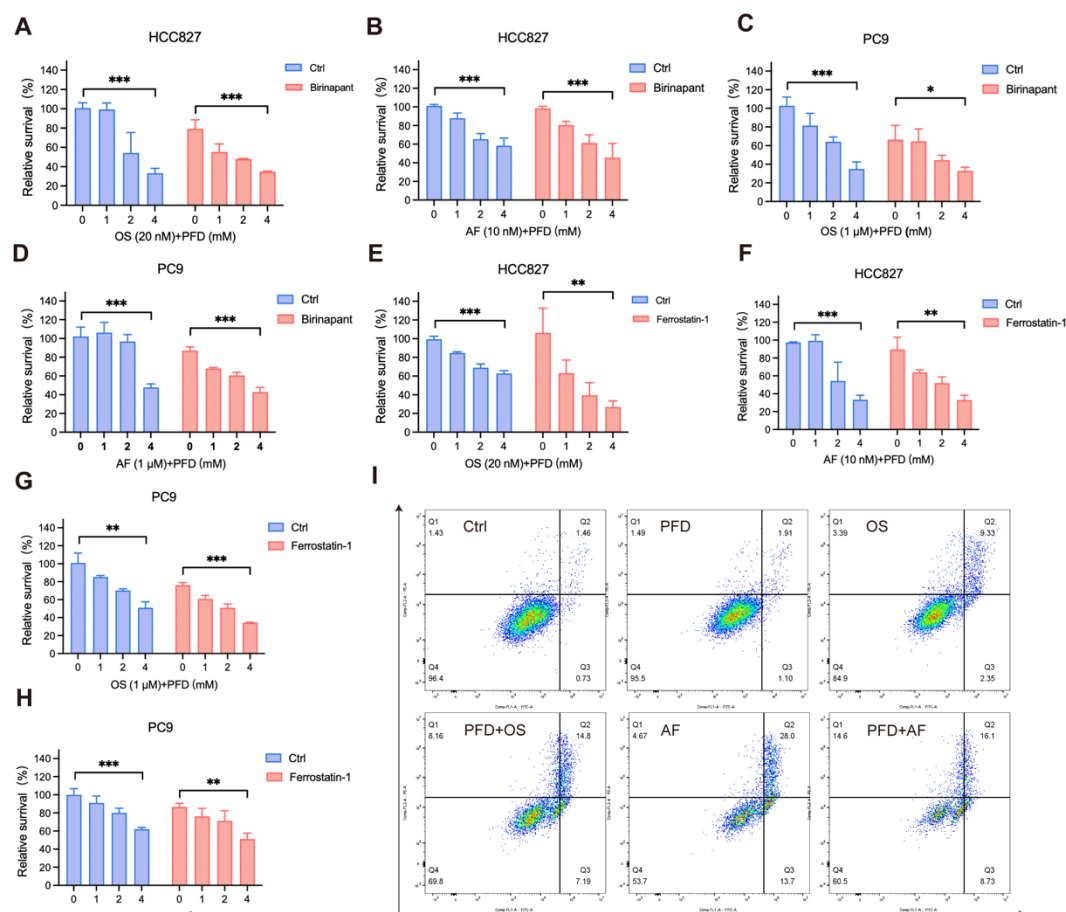


Figure S3 The synergistic cytotoxicity of PFD combined with OS/ AF is independent of pyroptosis and ferroptosis. (A-D) Relative survival rate of PC9 (A,C) and HCC827 (B, D) cells treated with gradient concentrations of PFD combined with AF (A,B) or OS (C, D), with or without pyroptosis inhibitor Birinapant, detected by CCK-8 assay. (E-H) Relative survival rate of PC9 (E,G) and HCC827 (F,H) cells treated with gradient concentrations of PFD combined with AF (E,F) or OS (G,H), with or without ferroptosis inhibitor Ferrostatin-1, detected by CCK-8 assay. (I) Flow cytometry analysis of cell apoptosis via Annexin V/PI double staining in HCC827 cells treated with control (Ctrl), PFD alone, OS alone, AF alone, PFD+OS combination, or PFD+AF combination.

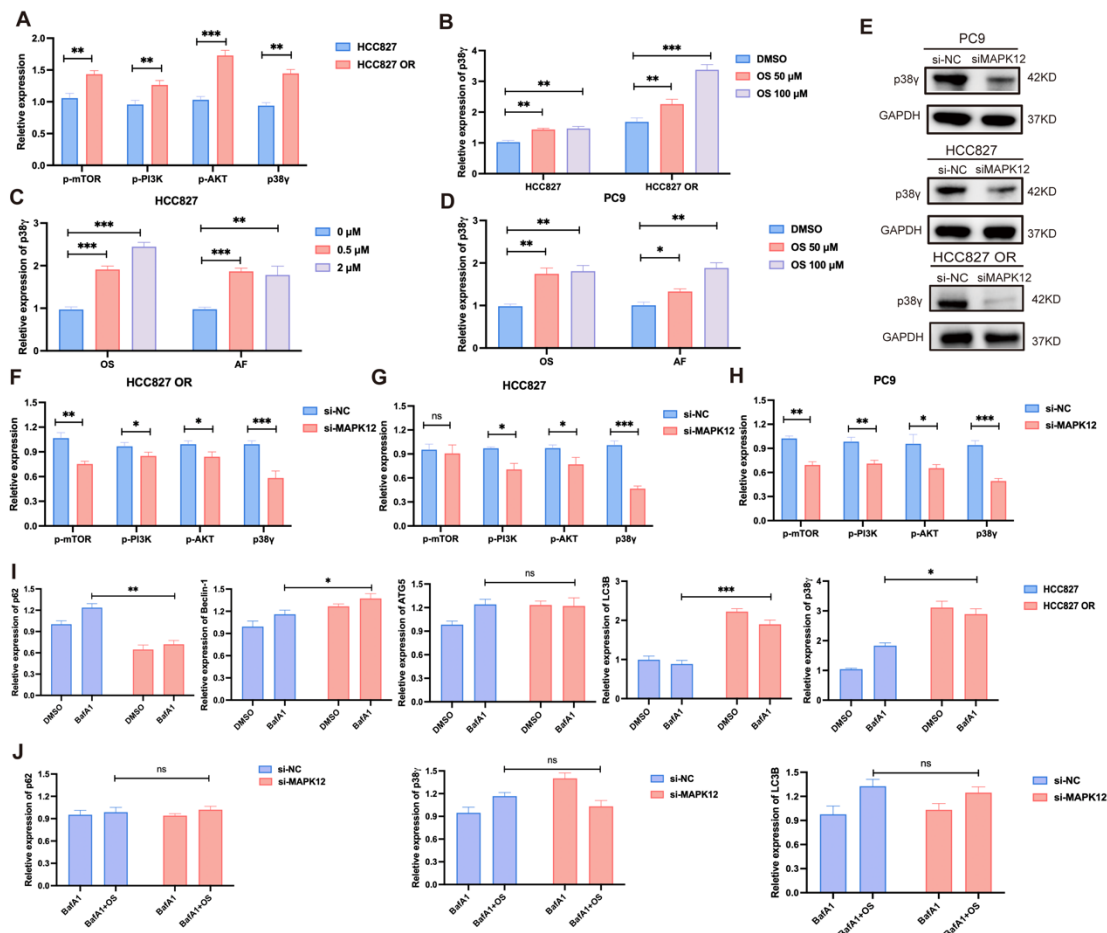


Figure S4 p38 γ mediates PI3K-AKT-mTOR activation and autophagy regulation in EGFR-TKI-resistant NSCLC cells. (A) Relative protein levels of p-mTOR, p-PI3K, p-AKT and p38 γ in parental HCC827 and OS-resistant HCC827-OR cells. (B) Quantification of p38 γ protein expression in HCC827 and HCC827-OR cells after treatment with gradient concentrations of OS. (C,D) Quantification of p38 γ in HCC827 and PC9 cells exposed to different concentrations of OS or AF. (E) Western blot showing the knockdown efficiency of p38 γ by si-MAPK12 in PC9, HCC827 and HCC827-OR cells. GAPDH was used as the loading control. (F-H) Western-blot-based quantification of p-mTOR, p-PI3K, p-AKT and p38 γ in HCC827-OR, HCC827 and PC9 cells transfected with si-NC or si-MAPK12. (I) Relative protein expression of p62, Beclin-1, ATG5, LC3B and p38 γ in HCC827 and HCC827-OR cells treated with autophagy inhibitor BafA1. (J) Expression levels of autophagy markers p62 and LC3B under MAPK12 knockdown combined with BafA1 intervention. Data are presented as mean $\pm$ SD. *P<0.05, **P<0.01, ***P<0.001; ns, not significant.