

Expanded View Figures

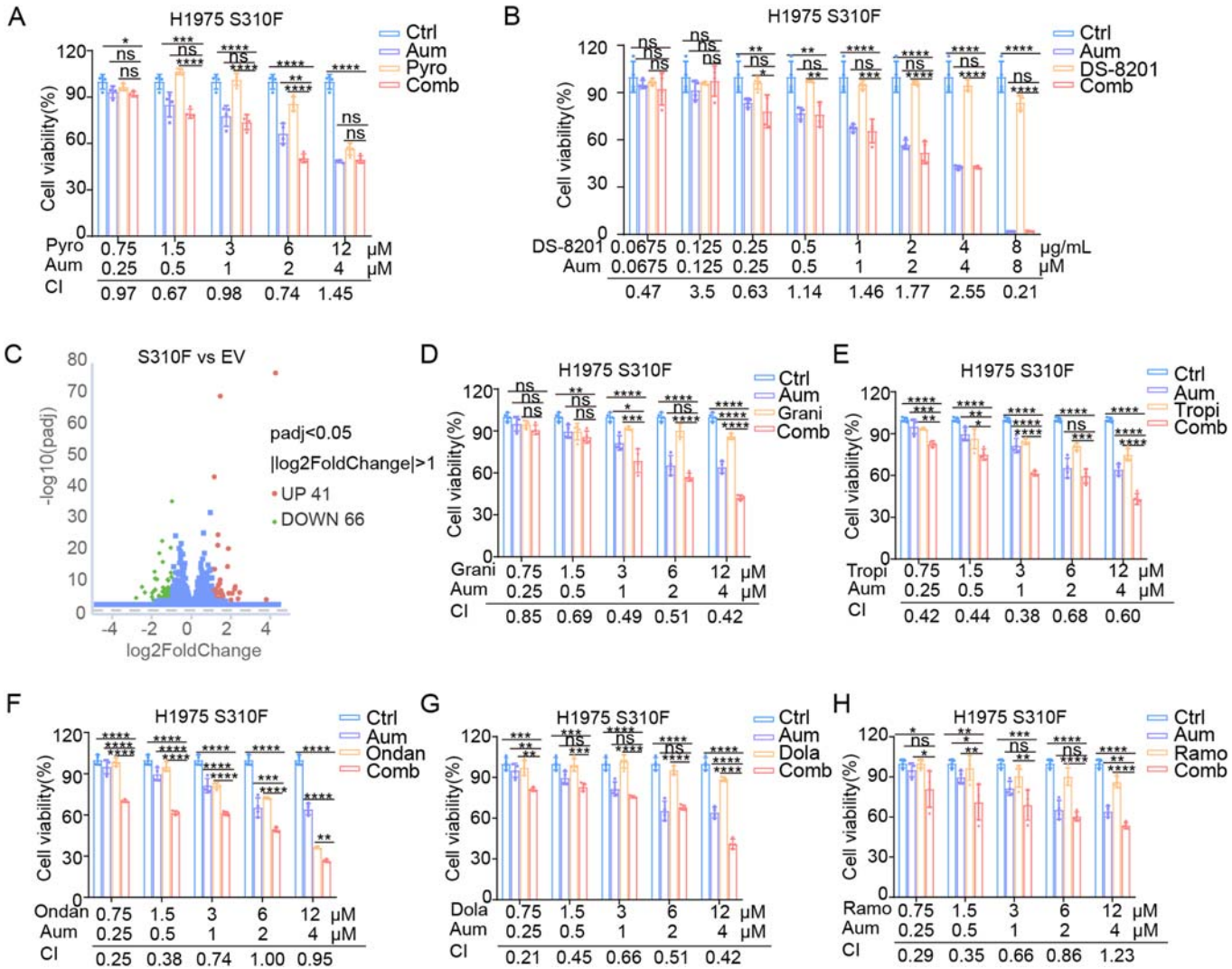


Figure EV1. Therapeutic efficacy of aumolertinib combined with HER2-targeted agents and HTR3 antagonists.

(A) Cell viability and CI analysis in H1975 S310F cells treated with indicated concentrations of aumolertinib and pyrotinib alone or in combination for 72 h ($n = 4$). P value (Aum 0.25 μ M, Pyro 0.75 μ M): Ctrl vs Comb, $P = 0.0204$; P value (Aum 0.5 μ M, Pyro 1.5 μ M): Ctrl vs Comb, $P = 0.0004$, Pyro vs Comb, $P < 0.0001$; P value (Aum 1 μ M, Pyro 3 μ M): Ctrl vs Comb, $P < 0.0001$, Pyro vs Comb, $P < 0.0001$; P value (Aum 2 μ M, Pyro 6 μ M): Ctrl vs Comb, $P < 0.0001$, Aum vs Comb, $P = 0.0037$, Pyro vs Comb, $P = 0.0001$; P value (Aum 4 μ M, Pyro 12 μ M): Ctrl vs Comb, $P < 0.0001$. (B) Cell viability and CI analysis in H1975 S310F cells treated with indicated concentrations of aumolertinib and DS-8201 alone or in combination for 72 h ($n = 4$). P value (Aum 0.25 μ M, DS-8201 0.25 μ g/ml): Ctrl vs Comb, $P = 0.007$, DS-8201 vs Comb, $P = 0.0239$; P value (Aum 0.5 μ M, DS-8201 0.5 μ g/ml): Ctrl vs Comb, $P = 0.0011$, DS-8201 vs Comb, $P = 0.003$; P value (Aum 1 μ M, DS-8201 1 μ g/ml): Ctrl vs Comb, $P < 0.0001$, DS-8201 vs Comb, $P = 0.0002$; P value (Aum 2 μ M, DS-8201 2 μ g/ml): Ctrl vs Comb, $P < 0.0001$, DS-8201 vs Comb, $P < 0.0001$; P value (Aum 4 μ M, DS-8201 4 μ g/ml): Ctrl vs Comb, $P < 0.0001$, DS-8201 vs Comb, $P < 0.0001$; P value (Aum 8 μ M, DS-8201 8 μ g/ml): Ctrl vs Comb, $P < 0.0001$, DS-8201 vs Comb, $P < 0.0001$. (C) Differential gene expression volcano plot of H1975 EV/S310F transcriptome sequencing. (D) Cell viability and CI analysis in H1975 S310F cells treated with indicated concentrations of aumolertinib and granisetron alone or in combination for 72 h ($n = 4$). P value (Aum 0.5 μ M, Grani 1.5 μ M): Ctrl vs Comb, $P = 0.0050$; P value (Aum 1 μ M, Grani 3 μ M): Ctrl vs Comb, $P < 0.0001$, Aum vs Comb, $P = 0.0208$, Grani vs Comb, $P = 0.0002$; P value (Aum 2 μ M, Grani 6 μ M): Ctrl vs Comb, $P < 0.0001$, Grani vs Comb, $P < 0.0001$; P value (Aum 4 μ M, Grani 12 μ M): Ctrl vs Comb, $P < 0.0001$, Aum vs Comb, $P < 0.0001$, Grani vs Comb, $P < 0.0001$. (E) Cell viability and CI analysis in H1975 S310F cells treated with indicated concentrations of aumolertinib and tropisetron alone or in combination for 72 h ($n = 4$). P value (Aum 0.25 μ M, Tropi 0.75 μ M): Ctrl vs Comb, $P < 0.0001$, Aum vs Comb, $P = 0.0003$, Tropi vs Comb, $P = 0.0012$; P value (Aum 0.5 μ M, Tropi 1.5 μ M): Ctrl vs Comb, $P < 0.0001$, Aum vs Comb, $P = 0.0050$, Tropi vs Comb, $P = 0.0270$; P value (Aum 1 μ M, Tropi 3 μ M): Ctrl vs Comb, $P < 0.0001$, Aum vs Comb, $P < 0.0001$, Tropi vs Comb, $P < 0.0001$; P value (Aum 2 μ M, Tropi 6 μ M): Ctrl vs Comb, $P < 0.0001$, Tropi vs Comb, $P = 0.0001$; P value (Aum 4 μ M, Tropi 12 μ M): Ctrl vs Comb, $P < 0.0001$, Aum vs Comb, $P < 0.0001$, Tropi vs Comb, $P < 0.0001$. (F) Cell viability and CI analysis in H1975 S310F cells treated with indicated concentrations of aumolertinib and ondansetron alone or in combination for 72 h ($n = 4$). P value (Aum 0.25 μ M, Ondan 0.75 μ M): Ctrl vs Comb, $P < 0.0001$, Aum vs Comb, $P < 0.0001$, Ondan vs Comb, $P < 0.0001$; P value (Aum 0.5 μ M, Ondan 1.5 μ M): Ctrl vs Comb, $P < 0.0001$, Aum vs Comb, $P < 0.0001$, Ondan vs Comb, $P < 0.0001$; P value (Aum 1 μ M, Ondan 3 μ M): Ctrl vs Comb, $P < 0.0001$, Aum vs Comb, $P < 0.0001$, Ondan vs Comb, $P < 0.0001$; P value (Aum 2 μ M, Ondan 6 μ M): Ctrl vs Comb, $P < 0.0001$, Aum vs Comb, $P = 0.0006$, Ondan vs Comb, $P < 0.0001$; P value (Aum 4 μ M, Ondan 12 μ M): Ctrl vs Comb, $P < 0.0001$, Aum vs Comb, $P < 0.0001$, Ondan vs Comb, $P = 0.0020$. (G) Cell viability and CI analysis in H1975 S310F cells treated with indicated concentrations of aumolertinib and dolasetron alone or in combination for 72 h ($n = 4$). P value (Aum 0.25 μ M, Dola 0.75 μ M): Ctrl vs Comb, $P = 0.0003$, Aum vs Comb, $P = 0.0039$, Dola vs Comb, $P = 0.0015$; P value (Aum 0.5 μ M, Dola 1.5 μ M): Ctrl vs Comb, $P = 0.0007$, Dola vs Comb, $P = 0.0009$; P value (Aum 1 μ M, Dola 3 μ M): Ctrl vs Comb, $P < 0.0001$, Dola vs Comb, $P < 0.0001$; P value (Aum 2 μ M, Dola 6 μ M): Ctrl vs Comb, $P < 0.0001$, Dola vs Comb, $P < 0.0001$; P value (Aum 4 μ M, Dola 12 μ M): Ctrl vs Comb, $P < 0.0001$, Aum vs Comb, $P < 0.0001$, Dola vs Comb, $P < 0.0001$. (H) Cell viability and CI analysis in H1975 S310F cells treated with indicated concentrations of aumolertinib and ramosetron alone or in combination for 72 h ($n = 4$). P value (Aum 0.25 μ M, Ramo 0.75 μ M): Ctrl vs Comb, $P = 0.0179$, Ramo vs Comb, $P = 0.0174$; P value (Aum 0.5 μ M, Ramo 1.5 μ M): Ctrl vs Comb, $P = 0.0023$, Aum vs Comb, $P = 0.0440$, Ramo vs Comb, $P = 0.0059$; P value (Aum 1 μ M, Ramo 3 μ M): Ctrl vs Comb, $P = 0.0004$, Ramo vs Comb, $P = 0.0082$; P value (Aum 2 μ M, Ramo 6 μ M): Ctrl vs Comb, $P < 0.0001$, Ramo vs Comb, $P < 0.0001$; P value (Aum 4 μ M, Ramo 12 μ M): Ctrl vs Comb, $P < 0.0001$, Aum vs Comb, $P = 0.0074$, Ramo vs Comb, $P < 0.0001$. Data are presented as mean $\pm$ SD. Statistical test: one-way ANOVA, ns: not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$.

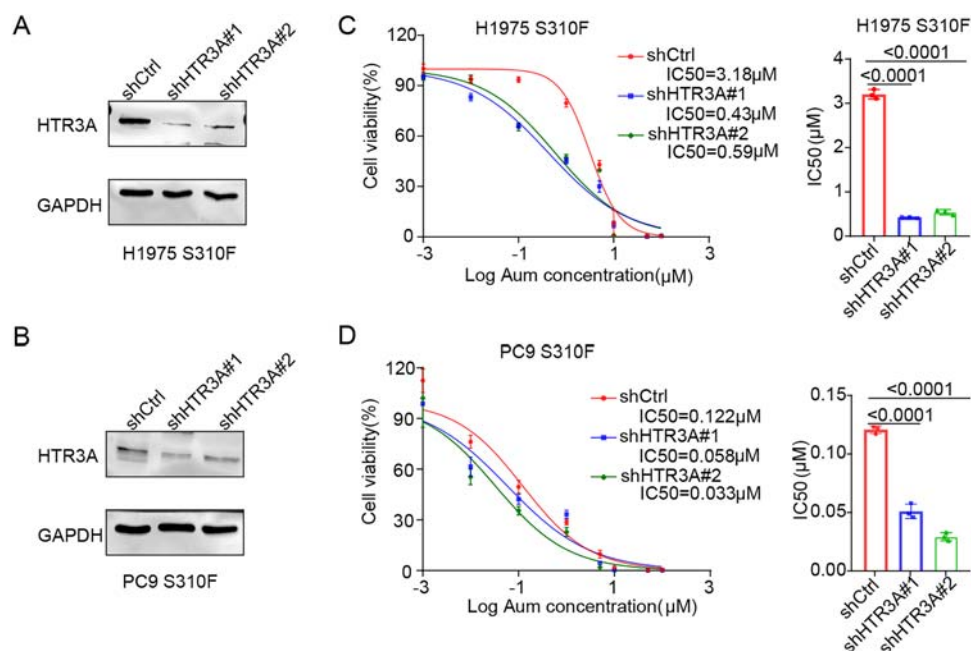
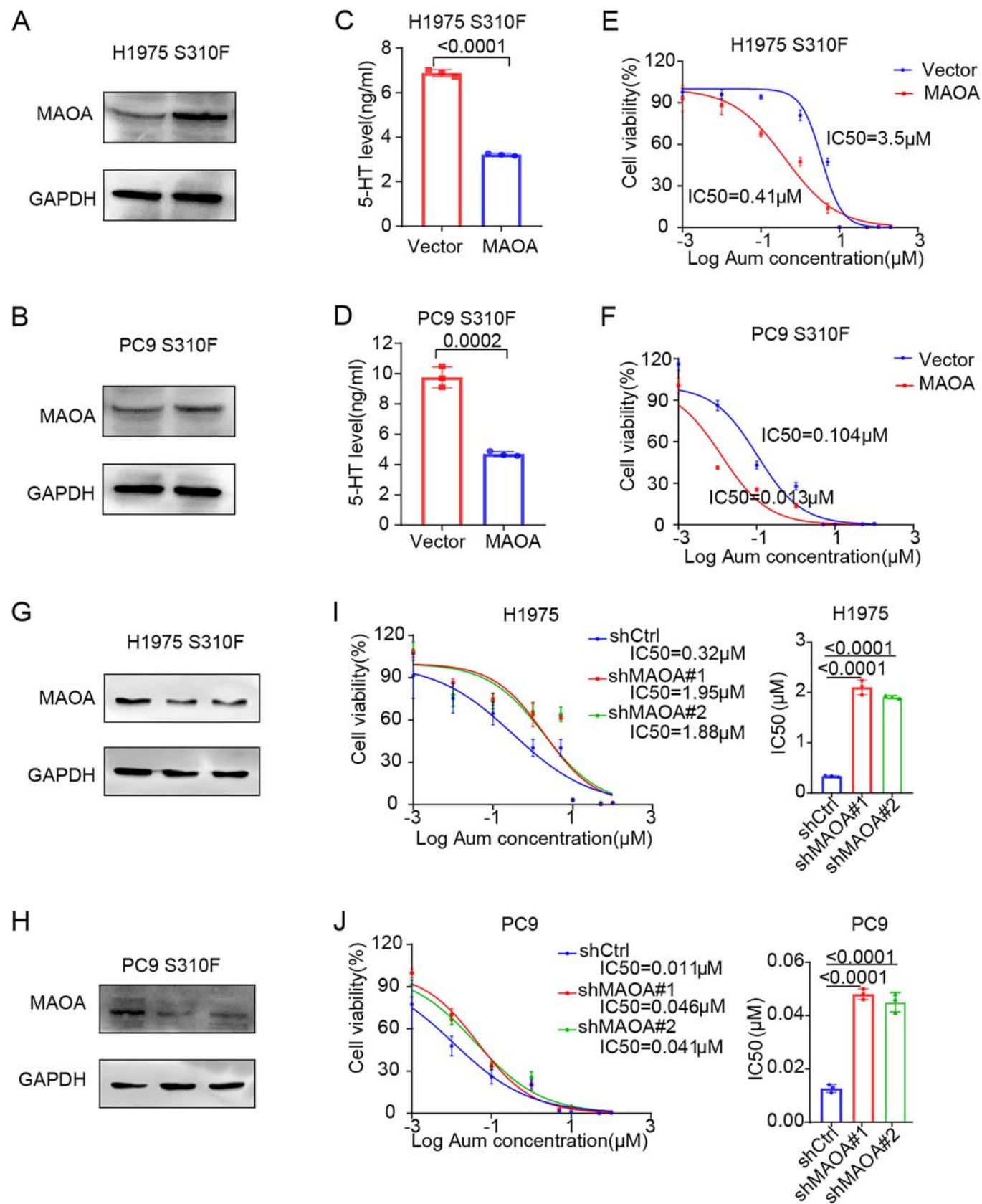


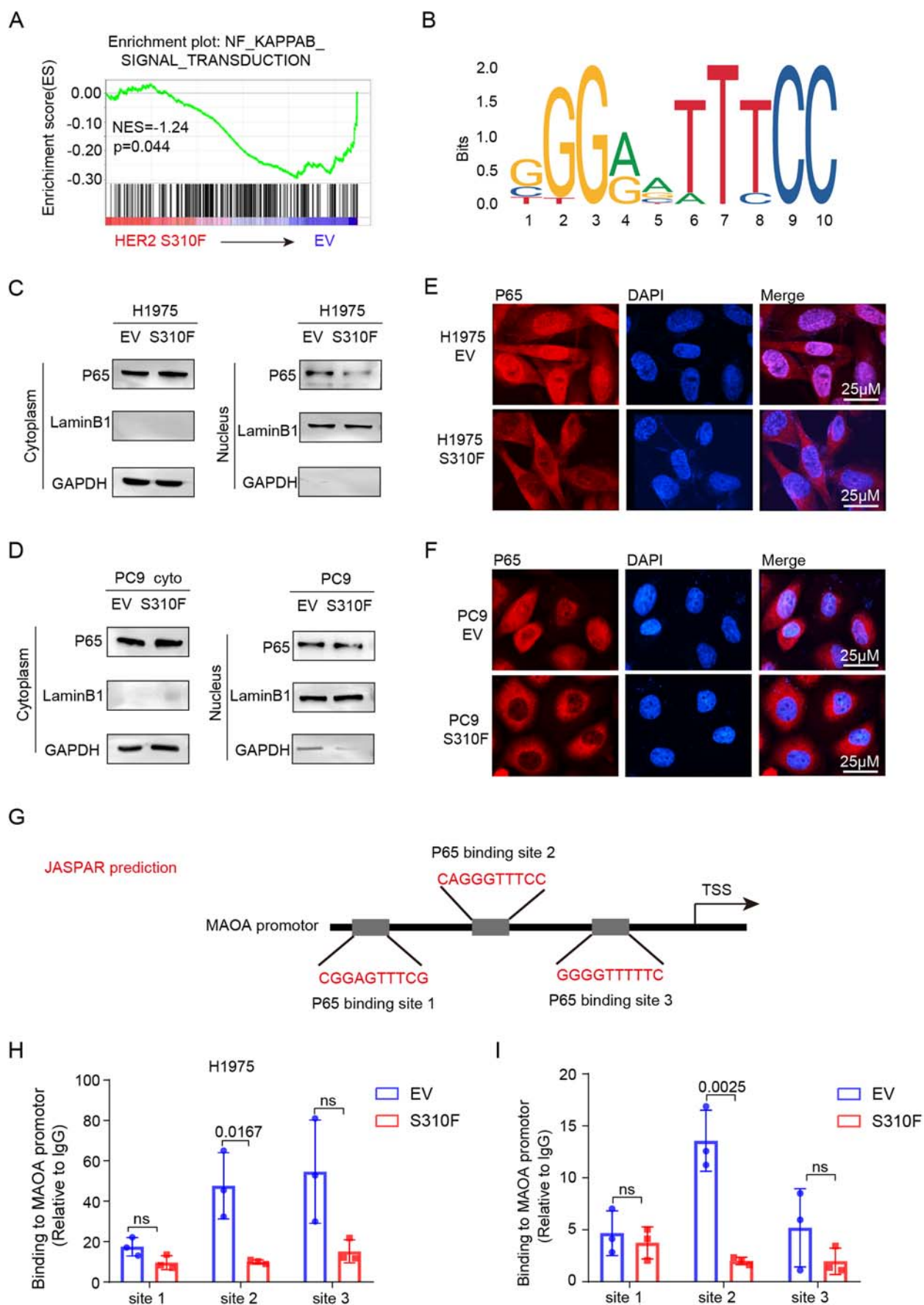
Figure EV2. HTR3A knockdown potentiates the sensitivity to aumolertinib in HER2 S310F-mutant cells.

(A, B) Western blot validation of stable HTR3A knockdown cell lines. (C, D) The aumolertinib IC₅₀ value of H1975 S310F and PC9 S310F cells were calculated between shCtrl and shHTR3A groups after treating with aumolertinib at the indicated concentrations for 5 days ($n = 3$). Data are presented as mean $\pm$ SD. Statistical test: two-tailed unpaired Student's t test.



**Figure EV3. MAOA expression regulates aumolertininib resistance.**

(A, B) H1975 S310F and PC9 S310F cells stably overexpressing MAOA were validated by western blot. (C, D) 5-HT levels in the supernatant ($n = 3$). (E, F) The aumolertininib IC_{50} value of H1975 S310F and PC9 S310F cells were calculated between vector and MAOA overexpression groups after treating with aumolertininib at the indicated concentrations for 5 days ($n = 4$). (G, H) Western blot validation of stable MAOA knockdown cell lines. (I, J) The aumolertininib IC_{50} value of H1975 and PC9 cells was calculated between shCtrl and shMAOA groups after treating with aumolertininib at the indicated concentrations for 5 days ($n = 3$). Data are presented as mean $\pm$ SD. Statistical test: Two-tailed unpaired Student's t test.



**Figure EV4. The mechanism of downregulation of MAOA expression.**

(A) Perform GSEA analysis on H1975 EV/S310F RNA seq data. (B) The JASPAR database forecasts the sequence of the P65 binding site within the promoter region of the MAOA gene. (C, D) The nuclear and cytoplasmic expression levels of P65 were assessed in H1975 EV/S310F and PC9 EV/S310F cells through nuclear-cytoplasmic separation experiments. (E, F) Immunofluorescence assays were conducted to determine the localization of P65 in H1975 EV/S310F and PC9 EV/S310F cells, with P65 appearing in red and the nuclei in blue. (G) The P65-binding motif was predicted by JASPAR, and schematic images of the potential P65 binding sites in the MAOA promoter region are shown. (H, I) ChIP-qPCR analysis of P65 binding on MAOA promoter in H1975 EV/S310F and PC9 EV/S310F cells ($n = 3$). Data are presented as mean $\pm$ SD. Statistical test: Two-tailed unpaired Student's t test.