Supplementary Materials

Cryptotanshinone differentially induces cell death in *ATP6V0D1*-deficient pancreatic cancer cells

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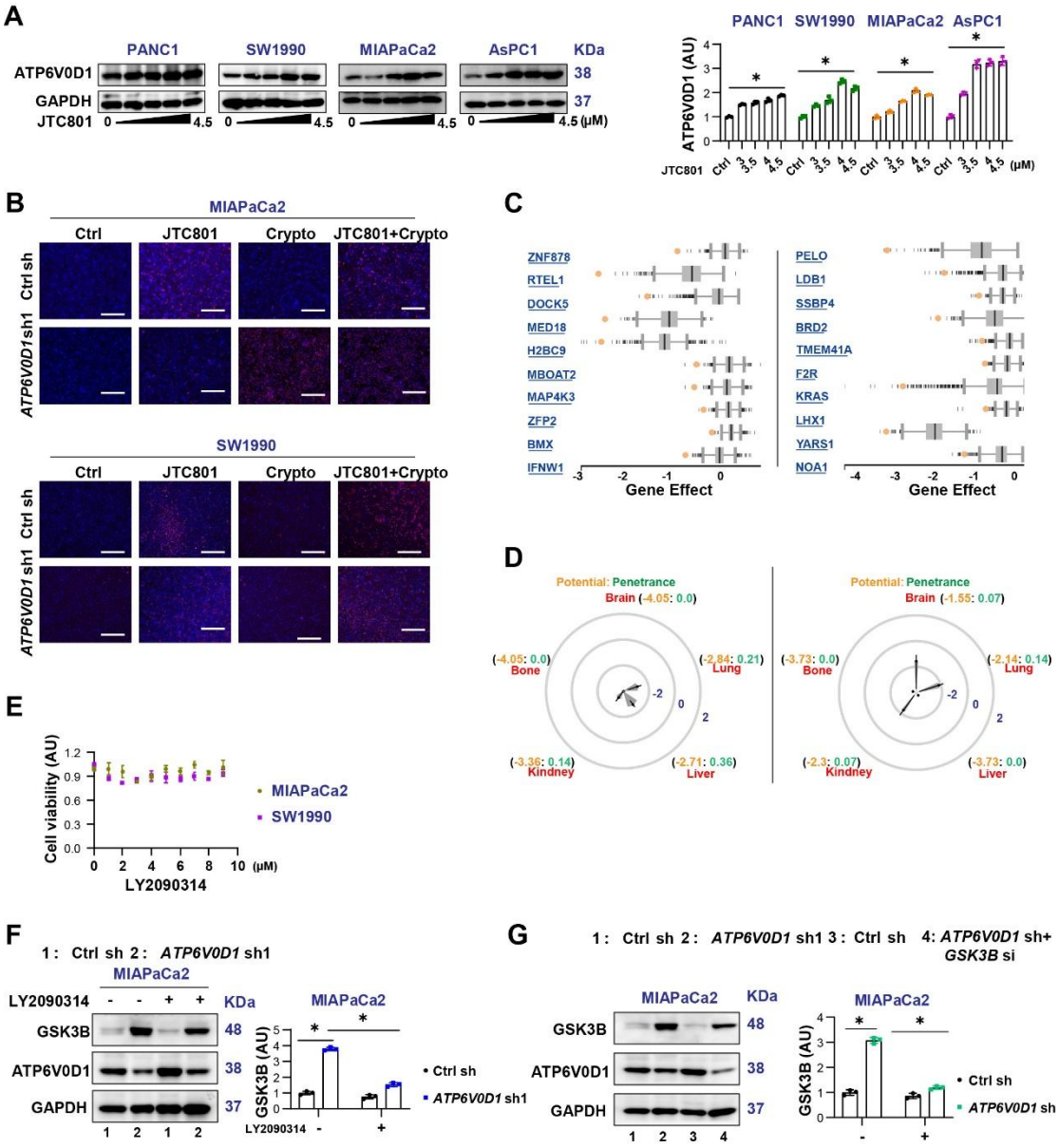
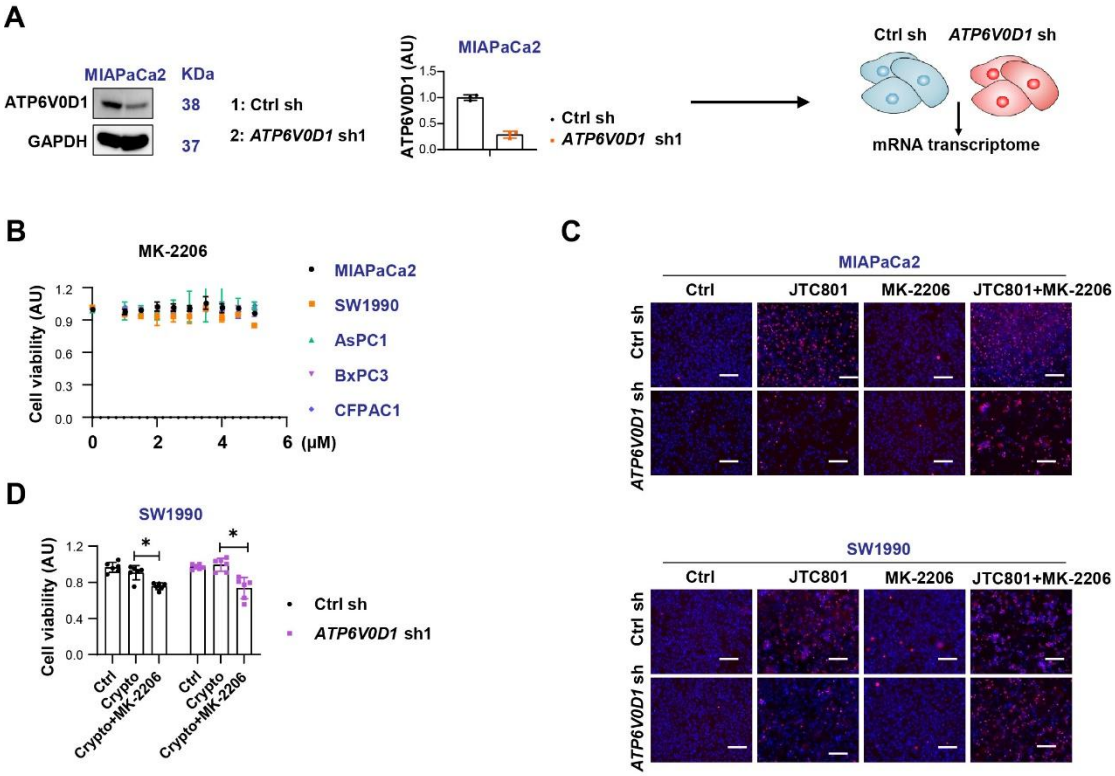
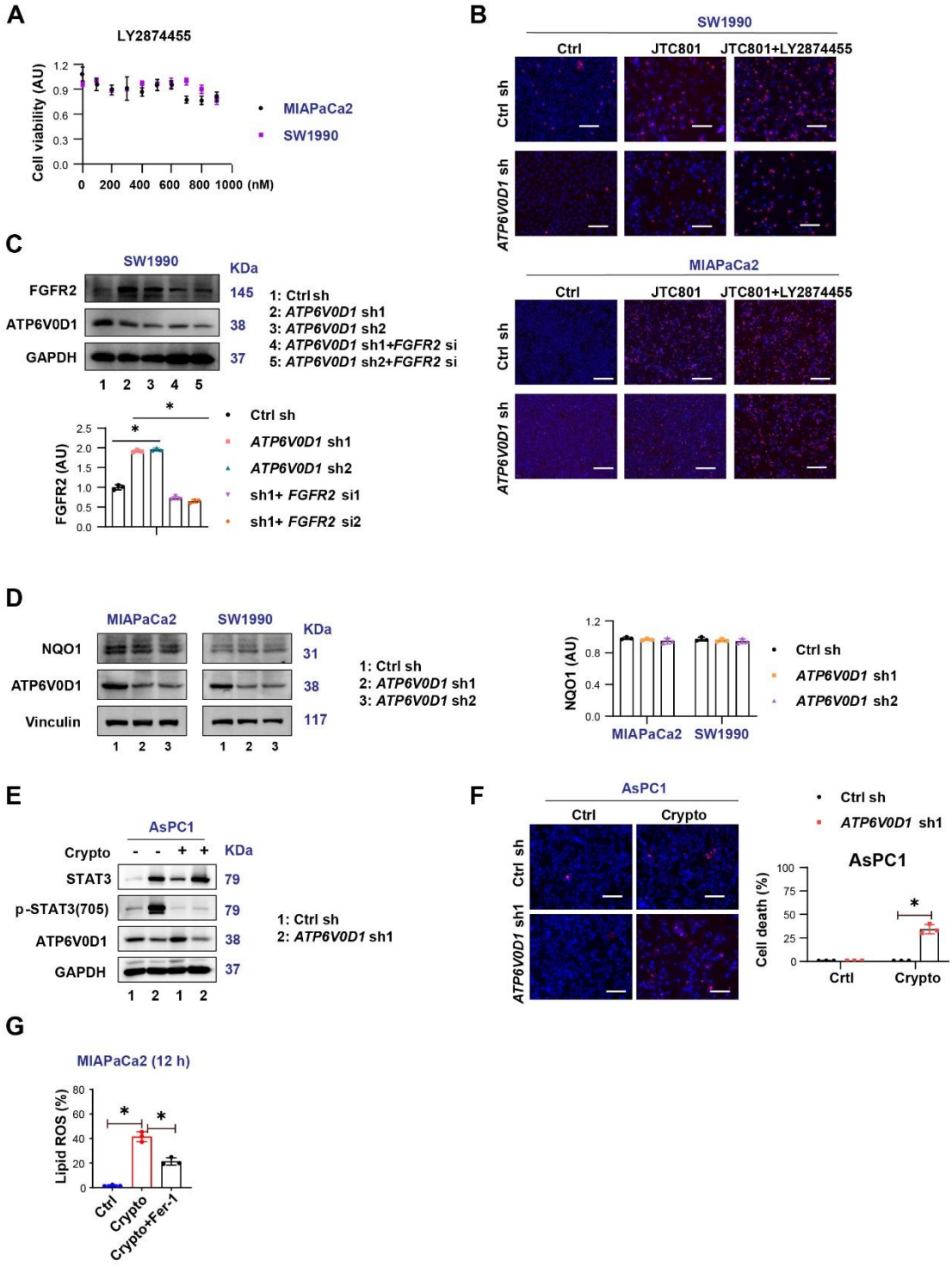


Supplementary Figure 1. Biological differences between SW1990 and MIAPaCa2 cells. (A) Western blot analysis of ATP6V0D1 protein expression under the indicated JTC801 treatments (JTC801 concentration: 3, 3.5, 4 and 4.5 μM; treatment time: 24 h). The bar chart on the right shows the semi-quantitative levels of ATP6V0D1 under JTC801 treatment. The untreated group was set to a relative value of 1; (B) Representative image corresponding to Figure 1B. Scale bar = 200 μm; (C) DepMap database analysis of the top 10 genes essential for the survival of different PDAC cell lines after genome-wide CRISPR knockout; (D) Analysis of the metastatic potential of SW1990 and MIAPaCa2 cells to different organs. Data are presented on a $\log_{10}$ scale (range: -4 to 4). ≤ -4: non-metastatic; -4 to -2: weakly metastatic, low confidence; ≥ -2: metastatic, higher confidence; (E) CCK-8 assay to assess the

cytotoxicity of LY2090314 at concentrations of 0-9 μM; (F and G) Western blot analysis of protein expression in the indicated groups (LY2090314, 5 μM; treatment time 24 h). The bar chart shows the semi-quantitative levels of the detected proteins. (A, F and G) Western blot data are representative of three independent experiments. Data are presented as mean ± SD. Statistical significance was determined using one-way ANOVA with Tukey's multiple comparisons test. Source data are provided. $^{*}P < 0.05$ was considered statistically significant; ns: not significant.



Supplementary Figure 2. AKT inhibition restores sensitivity to alkaliptosis in *ATP6V0D1*-deficient PDAC cells. (A) Western blot analysis of protein expression in the indicated groups and corresponding mRNA transcriptomic profiles. The bar chart on the right shows semi-quantitative levels of ATP6V0D1. The untreated group was set to a relative value of 1; (B) CCK-8 assay assessing the cytotoxicity of MK-2206 at various concentrations (μM); (C) Representative image corresponding to Figure 2D. Scale bar = 200 μm; (D) CCK-8 assay assessing cell viability following treatment with Crypto alone or Crypto combined with MK-2206 (Drug concentration: Crypto, 5 μM; MK-2206, 2 μM; treatment time: 24 h). (D) Data represent six biologically independent samples and are presented as mean ± SD. Statistical significance was determined using two-way ANOVA with Tukey's multiple comparisons test. (A) Western blot data are representative of three independent experiments. Data are presented as mean ± SD, and statistical significance was determined using a two-sided unpaired *t*-test. Source data are provided. $^{*}P < 0.05$ was considered statistically significant; ns: not significant.



Supplementary Figure 3. FGFR2 inhibition restores sensitivity to alkaliptosis in *ATP6V0D1*-deficient PDAC cells. (A) CCK-8 assay assessing the cytotoxicity of LY2874455 at different concentrations (nM); (B) Representative image corresponding to Figure 3D. Scale bar = 200 μm; (C and D) Western blot analysis of protein expression in the indicated groups. Bar charts show semi-quantitative levels of the detected proteins. Grouping: (1) control; (2-3) *ATP6V0D1* shRNA; (4-5) *ATP6V0D1* + *FGFR2* knockdown; (E) Western blot analysis of protein expression in the indicated

groups (Drug concentration: Crypto, 5 μM; treatment time: 24 h); (F) Cell death assay assessing the proportion of dead cells following Crypto treatment (Drug concentration: 5 μM; treatment time: 24 h); (G) Lipid ROS levels in the indicated groups measured using the BODIPY 581/591 C11 probe (Drug concentration: Crypto, 5 μM; treatment time: 12 h). (C and D) Western blot data are representative of three independent experiments. Data are presented as mean ± SD. Statistical significance was determined using one-way ANOVA with Tukey's multiple comparisons test (C) or two-way ANOVA with Tukey's test (D and F). Source data are provided. $^{*}P < 0.05$ was considered statistically significant; ns: not significant.