Expanded View Figures

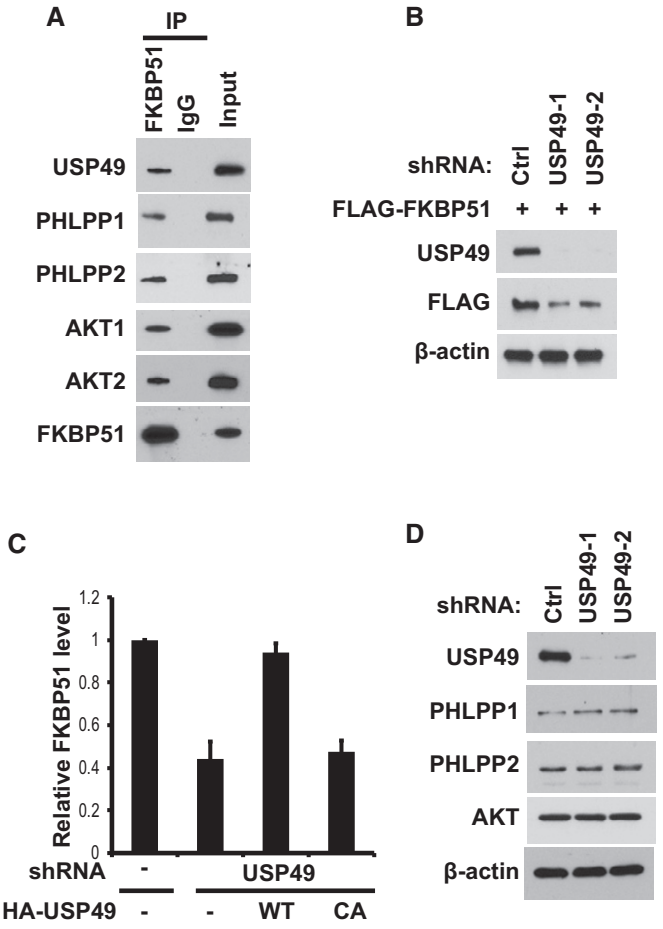


Figure EV1. USP49 interacts and stabilizes FKBP51.

A SU86 cell lysates were subjected to immunoprecipitation with control IgG or anti-FKBP51 antibody. The immunoprecipitates were then blotted with the indicated antibodies.

B SU86 cells stably expressing FLAG-FKBP51 were infected with lentivirus encoding shRNAs against control or USP49, the cells were lysed, and Western blot was performed with the indicated antibodies.

C Quantification of the relative FKBP51 protein levels for Fig 2B. Data are represented as mean ± SEM of three independent experiments.

D SU86 cells stably expressing control or USP49 shRNAs were lysed and Western blot was performed with the indicated antibodies.

Figure EV2. USP49 regulates AKT phosphorylation through FKBP51-PHLPP axis.

A, B SU86 cells stably expressing control or USP49 shRNAs were lysed and subjected to immunoprecipitation with (A) anti-AKT1 or (B) anti-AKT2 antibodies. The immunoprecipitates were then blotted with the indicated antibodies.

C CFPAC-1 cells stably expressing USP49 shRNA were transfected with the indicated constructs. After 48 h, cells were lysed and cell lysates were blotted with the indicated antibodies.

D Proliferation of the cells from (C) was assessed. Data are represented as mean ± SEM of three independent experiments.

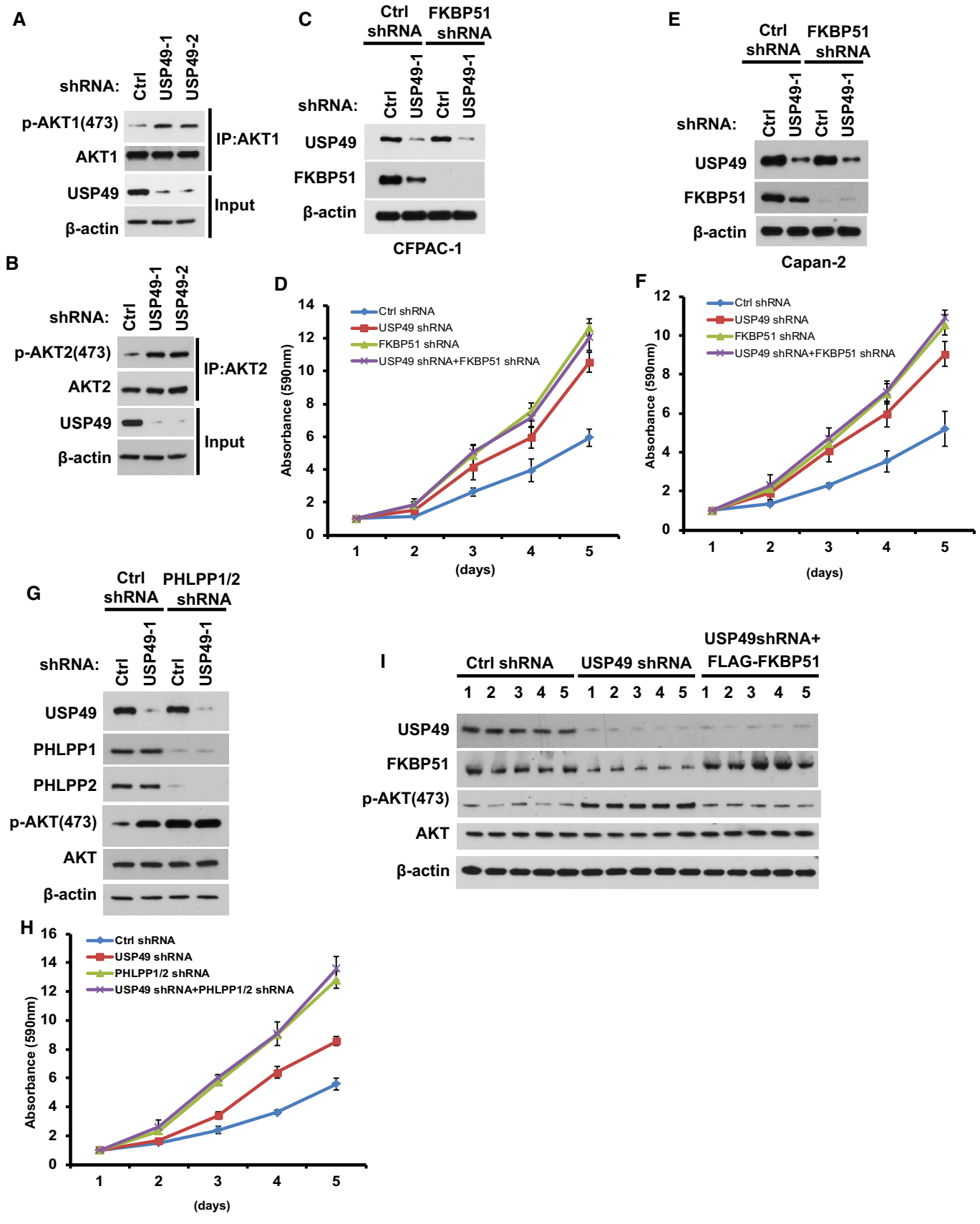
E Capan-2 cells stably expressing USP49 shRNA were transfected with the indicated constructs. After 48 h, cells were lysed and cell lysates were blotted with the indicated antibodies.

F Proliferation of the cells from (E) was assessed. Data are represented as mean ± SEM of three independent experiments.

G SU86 cells stably expressing the indicated shRNAs were lysed and Western blot was performed with the indicated antibodies.

H Proliferation of the cells from (G) was assessed. Data are represented as mean ± SEM of three independent experiments.

I The tumors obtained from *in vivo* tumorigenesis experiments (Fig 3H) were lysed and Western blot was performed with the indicated antibodies.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USP49-2
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AKT1
USP49
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AKT2
USP49
β-actin
IP:AKT2
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shRNA:
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Figure EV2.

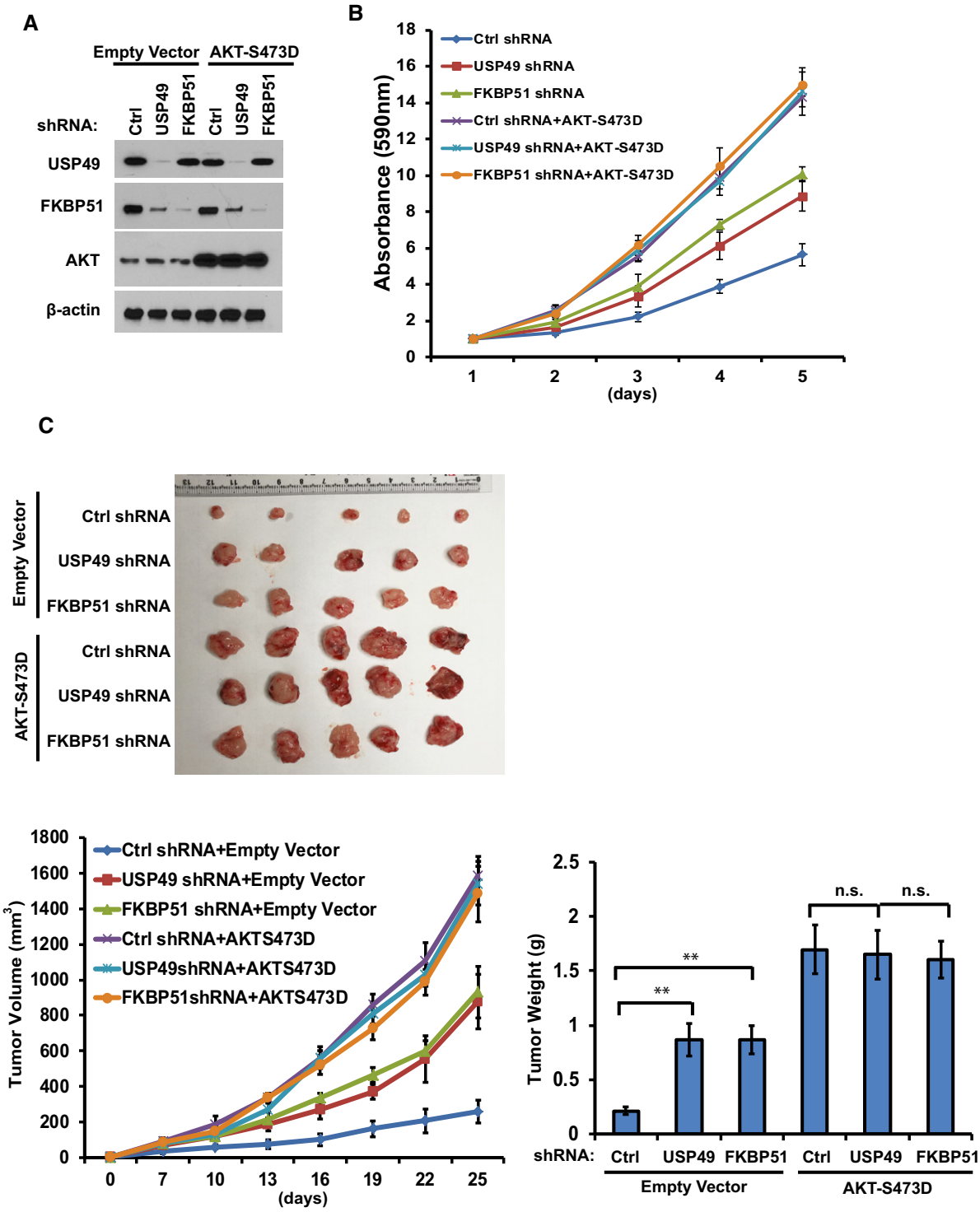

Figure EV3. USP49 regulates tumor growth in the FKBP51-AKT dependent manner.

A Su86 cells stably expressing empty vector or AKTS473D were infected with lentivirus encoding shRNAs against control, USP49, or FKBP51. The cells were lysed and Western blot was performed with the indicated antibodies.

B Proliferation of the cells from (A) was assessed. Data are represented as mean ± SEM of three independent experiments.

C 2×10^6 cells from (A) were subcutaneously injected into nude mice. Tumor volumes were measured at indicated days. Mice were sacrificed after 4 weeks. Tumor images were acquired and tumor weights were measured as shown in the lower panels. $n = 5$; data points in the lower left graph represent mean tumor volume ± SD. Data points in the lower right graph represent mean tumor weight ± SD. $^{**}P < 0.01$, statistical analyses were performed with ANOVA.

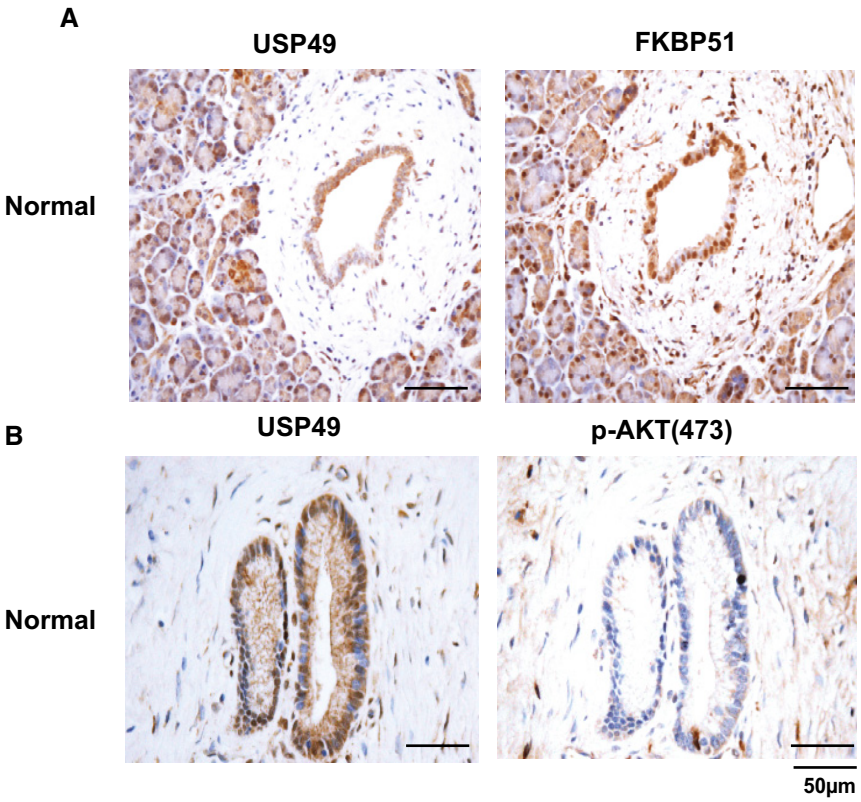

Figure EV4. USP49 is overexpressed in normal pancreatic tissues.

A, B Representative images of immunohistochemical staining of USP49, FKBP51, and p-AKT(S473) in normal pancreatic tissues.

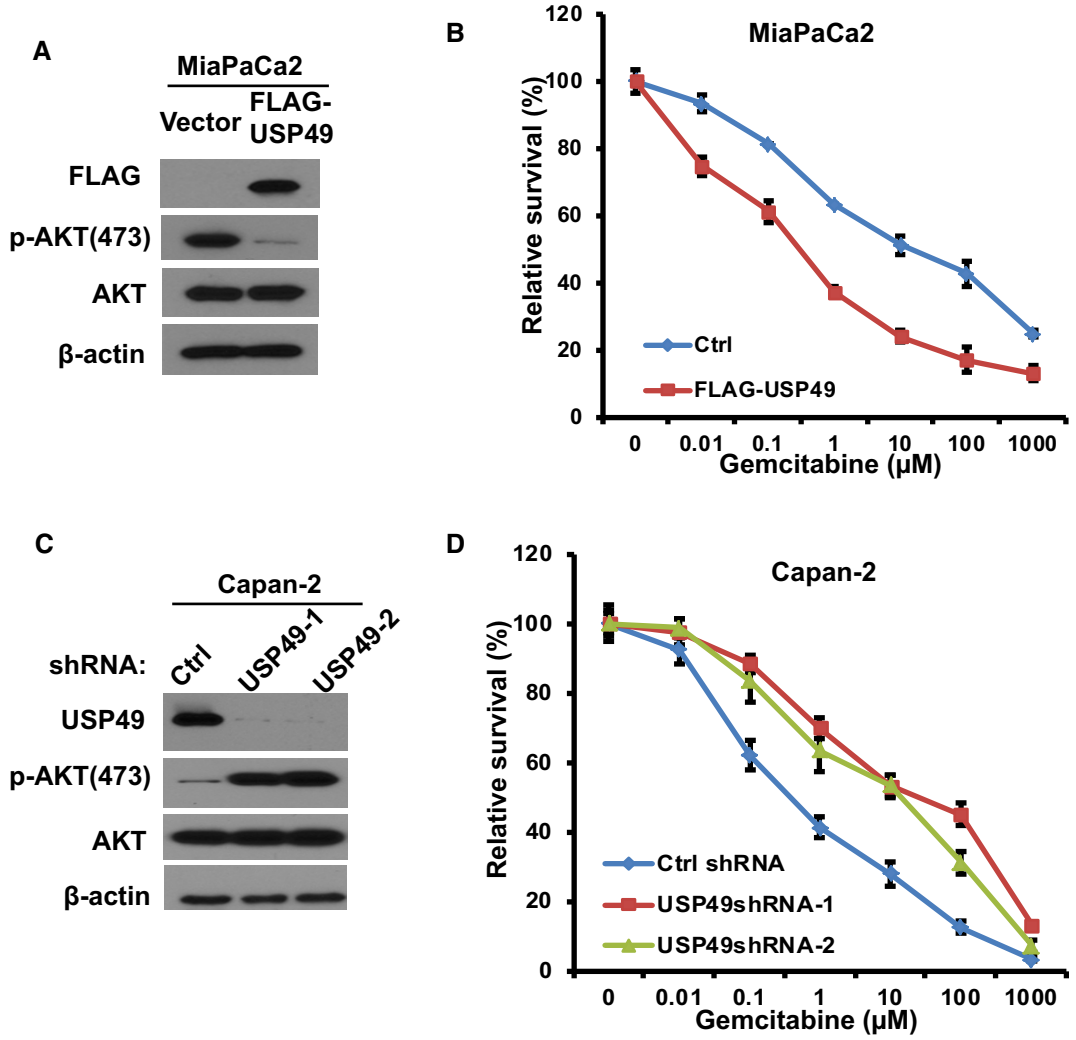

Figure EV5. USP49 regulates response of pancreatic cancer cells to gemcitabine.

A MiaPaCa2 cells were stably infected with retrovirus encoding control (Ctrl) or FLAG-USP49. Cells were lysed and cell lysates were blotted with indicated antibodies.

B Cells from (A) were treated with gemcitabine and cell survival was determined using MTS assay. The data presented are mean ± SD (n = 6).

C Capan-2 cells stably expressing control (Ctrl) or USP49 shRNAs were lysed and cell lysates were blotted with indicated antibodies.

D Cells from (C) were treated with gemcitabine and cell survival was determined using MTS assay. The data presented are mean ± SD (n = 6).