Supplementary Material and Methods

Antibodies and Cell Lines

FKBP51 antibodies were raised against GST fusion proteins containing amino-terminal residues 1-100 of FKBP51. Antibodies against Akt, phospho-Akt (Ser473), phospho-Akt (Thr308), Foxo1, phospho-Foxo1(Thr 24), Gsk3® and phosphor-Gsk3® were purchased from Cell Signaling Inc. PHLPP and PHLPP2 antibodies were obtained from Bethyl Laboratories. SU86 and 293T cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum. MDA-MB-231 cell line were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% FBS. Mouse embryonic fibroblasts (MEFs) were cultured in DMEM containing 10% FBS.

Plasmids and Short Interfering RNA

S/FLAG/SBP-tagged FKBP51 was cloned into the pIRES2-EGFP vector. HA-tagged PHLPP1 and PHLPP2 were cloned into pCMV/Myc/nuc vector purchased from Invitrogen. Deletion mutants were generated by site-directed mutagenesis. Full length and deletion mutants of FKBP51 were cloned into retrovirus expression vector by use of the GATEWAY cloning system for the expression of these proteins in MEFs. Transfection was performed twice 24 hr apart with 200 nM of siRNA using Oligofectamine reagent (Invitrogen) or Lipofectamine™ RNAiMAX reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions.

Sequences for siRNA against FKBP51 were:

Sense strand: GGG UAA ACA GAU UGA GCA UdTdT

Antisense strand: AUG CUC AAU CUG UUU ACC CdGdT

Sequences for negative control siRNA without target gene were:

Sense strand: UUC UCC GAA CGU GUC ACG UdTdT

Antisense strand: ACG UGA CAC GUU CGG AGA AdTdT

Second FKBP51 siRNA

Sense strand: AAU AUC CCU CUC CUU UCC GdTdT

Antisense strand: CGG AAA GGA GAG GGA UAU UdGdT

Sequences for siRNA against PHLPP1 were:

Sense strand: GGA AUC AAC UGG UCA CAU UdTdT

Antisense strand: AAU GUG ACC AGU UGA UUC CdGdT

Co-Immunoprecipitation Assay

Cells were lysed with NETN buffer (20 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1 mM EDTA, 0.5% Nonidet P-40) containing 50 mM glycerophosphate, 10 mM NaF, and a protease inhibitor cocktail on ice for 20 min. The whole cell lysates obtained by centrifugation were incubated with 2 μg of antibody and Protein A Sepharose beads (Amersham Biosciences) for 2-3 hr at 4¡C. The immunocomplexes were then washed with NETN three times and applied to SDS-PAGE. Immunoblotting was performed following standard procedures.

Recombinant Protein Expression and Purification

To create bacterial plasmids expressing recombinant GST-tagged FKBP51 and AU1-Akt, full length FKBP51 and AU1-Akt were cloned into the pGEX4T-1 vector (Amersham). These plasmids were expressed in BL21 (DE)3 cells and proteins were purified with glutathione (GSH) beads (Amersham) according to the manufacturer's instruction. To

express the GST-tagged PHLPP in insect cells, full length PHLPP was cloned into the pDEST20 vector by use of the GATEWAY cloning system, and was then used to generate a bacmid using DH10Bac cells. Bacmid encoding PHLPP was then transfected into sf9 cell using Lipofectamine 2000 reagent according to manufacturer's instruction (Invitrogen). The recombinant baculovirus were harvested from cell culture at 72 hr post-transfection. A 50-ml culture of SF9 cells infected with the virus supernatant provided sufficient material for GST-tagged PHLPP purification.

***In Vitro* Binding Assay**

To assay the binding between FKBP51, Akt and PHLPP *in vitro*, purified Au1-Akt in binding buffer (0.5% Triton X-100 in PBS), at a final concentration of 0.2 μM, was incubated on ice with purified GST- PHLPP, with or without GST-tagged recombinant FKBP51. Akt was then immunoprecipitated with Akt antibody and Protein A Sepharose beads (Amersham Biosciences) for 2-3 hr at 4¡C. The immunocomplexes were then washed with NETN three times and applied to SDS-PAGE and immunoblotting analysis.

Quantitative RT-PCR QRT-PCR was performed with the 1-step, Brilliant SYBR Green QRT-PCR master mix kit (Stratagene, La Jolla, CA). Specifically, primers purchased from Qiagen (No.QT00056714) were used to perform QRT-PCR using the Stratagene Mx3005P™ Real-Time PCR detection system (Stratagene). All experiments were performed in triplicate with β-actin as an internal control. Reverse transcribed Universal Human reference RNA (Stratagene) was used to generate a standard curve. Control reactions lacked RNA template.

Supplementary Figure Legends:

Supplementary Figure 1. (A) SU86 cells were transfected with control or FKBP51 siRNA2. Transfected cells were then treated with indicated drugs, and cell survival was determined as described in the Methods. (B-C) SU86 cells were transfected with AktS473D mutant (B) or PHLPP siRNA (C). Transfected cells were then treated with gemcitabine, and cell survival was determined as described in the Methods. *Points*, mean values for three independent experiments; *Error bars*, +/- SEM. (D) The relative phosphorylation of pS473 Akt/total Akt were determined in Figure 2H from three independent experiments. *Error bars*, +/- SEM.

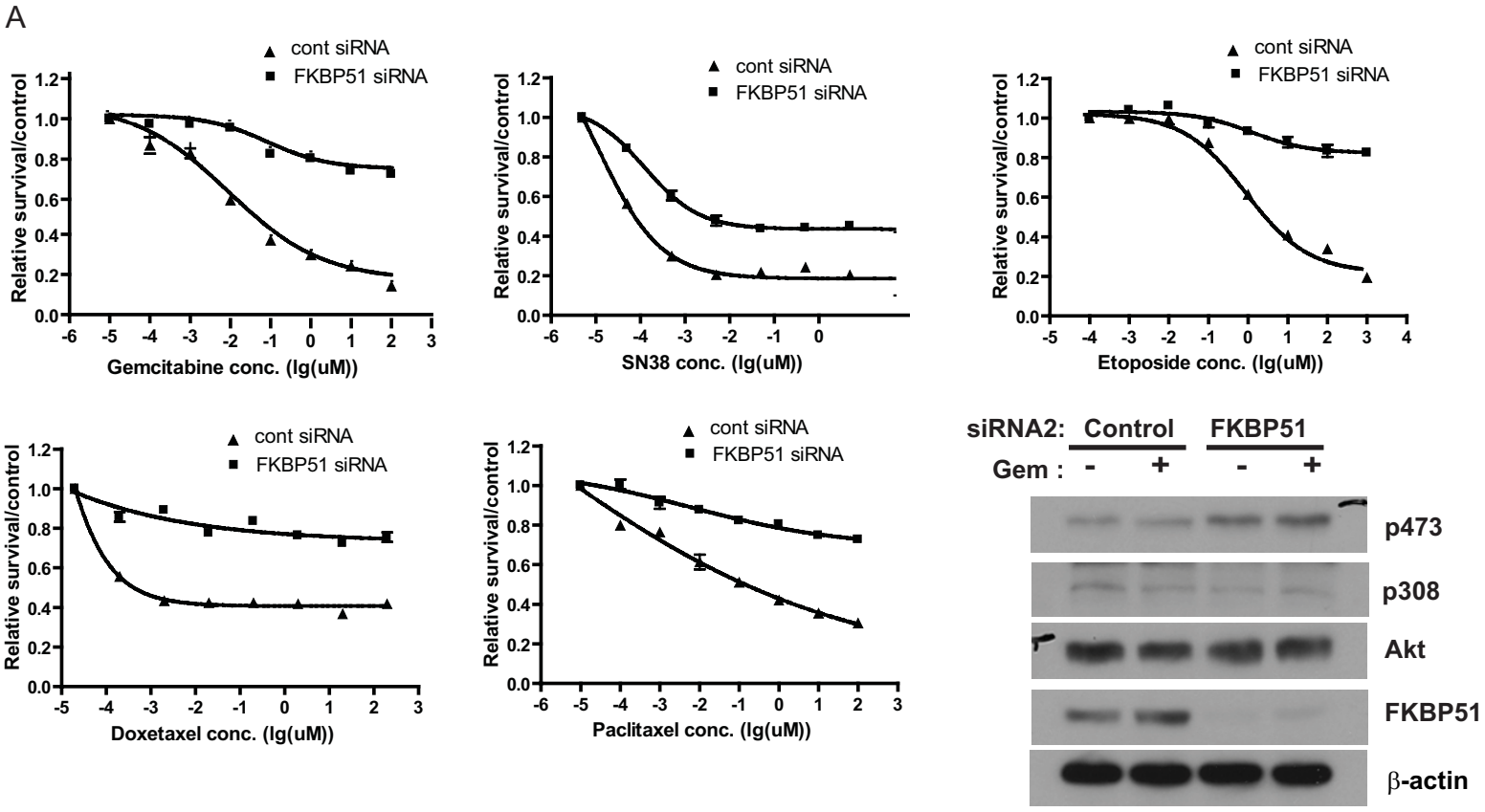
Supplementary Figure 2. (A) A549 cells or (B) MDA-MB-231 cells were transfected with control or FKBP51 siRNA1. Transfected cells were then treated with indicated drugs, and cell survival was determined as described in the Methods. *Points*, mean values for three independent experiments; *Error bars*, +/- SEM.

Supplementary Figure 3. (A) SU86 cells were transfected with indicated constructs, and AktSer473 phosphorylation was examined. (B) PHLPP was immunoprecipitated from FKBP51+/+ or FKBP51-/- cells and used for *in vitro* phosphatase assay using Akt as the substrate. Right panel: quantification of the phosphatase assay from three independent experiments. *Error bars*, +/- SEM. (C) FKBP51 was overexpressed in SU86 cells, and PHLPP was immunoprecipitated for *in vitro* phosphatase assay as (B). (D) FKBP51 was immunoprecipitated for SU86 cells to examine the interaction between

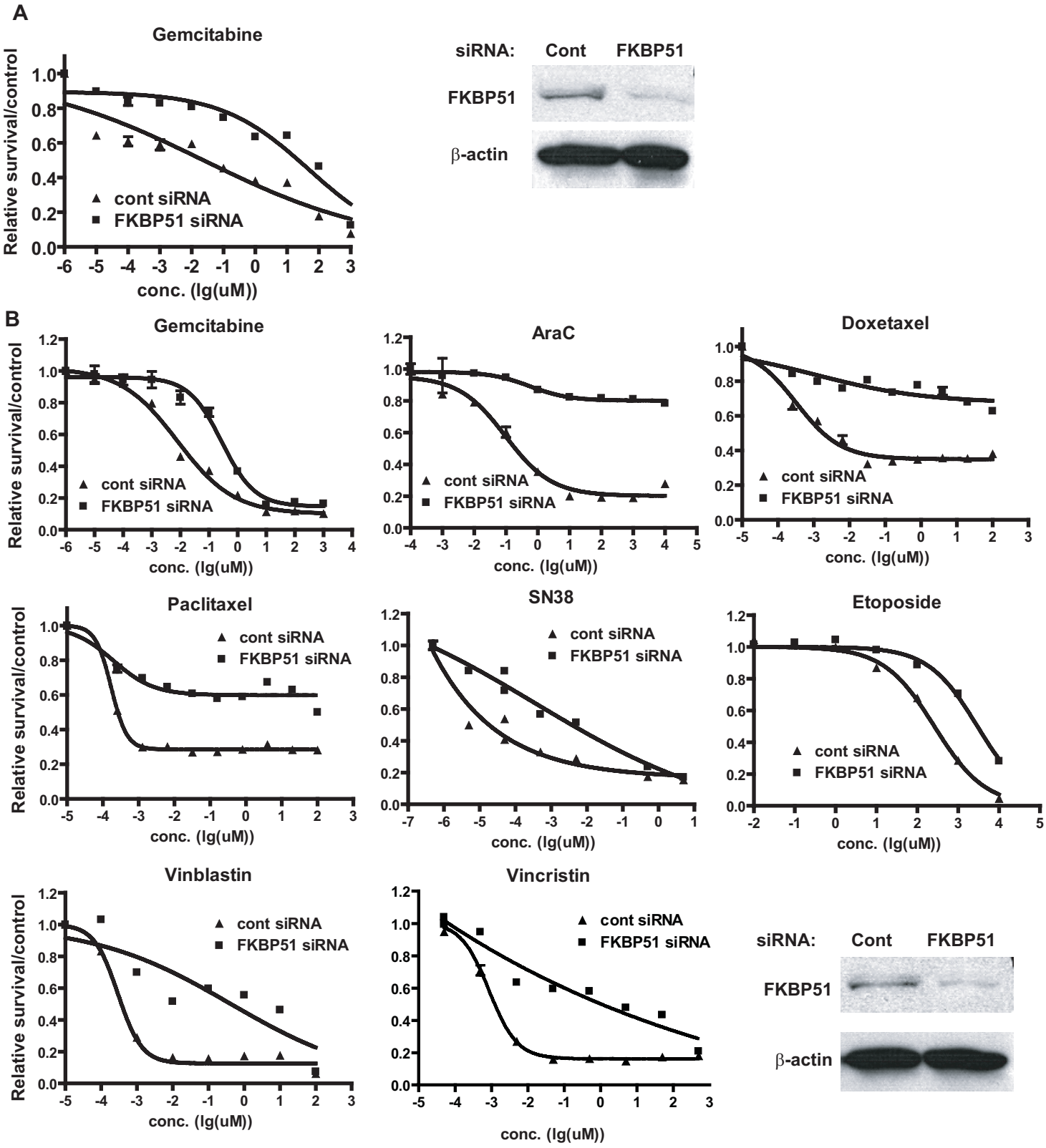
FKBP51 and PKCβII. (E). SU86 cells were transfected with control or FKBP51 siRNA. PKCβII phosphorylation was then examined.

Supplementary Figure 4. (A). Miapaca2 or Panc0403 cells were treated with gemcitabine alone or gemcitabine with wortmannin. Cell survival was then examined by the MTS assay. *Points*, mean values for three independent experiments; *Error bars*, +/- SEM. (B). Gene signature between normal and tumor pancreatic tissues. Gene expression were classified between normal and tumor with red representing higher expression and blue representing lower expression level for each probe sets. Top differentiate expressed genes between tumor and normal with $p<10^{-6}$ were listed in the figure. (C). Network analysis with top differentiate expressed genes between tumor and normal pancreatic tissues. Solid line indicates direct interaction and dash line indicates indirect interaction. Genes highlighted are part of the genes among the most differentiate expressed genes between the tumor and normal pancreatic tissues. (D-E) The relative density of pS473 Akt/total Akt and pGSK3β /total GSK3β were determined for pancreatic cancer samples (n=26) or normal pancreatic tissues (n=10). *Error bars*, +/- SEM.

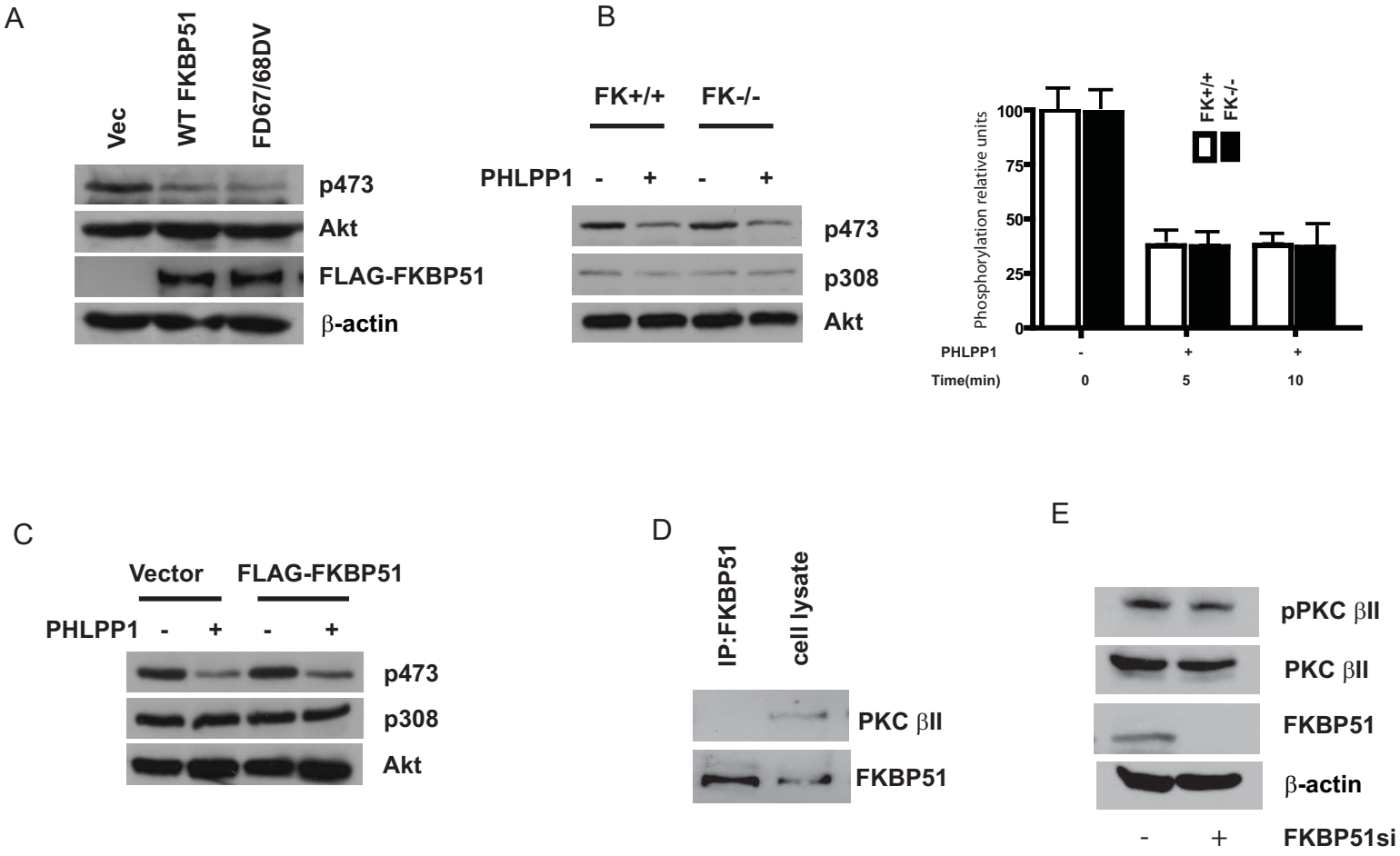
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4