Supplementary Material and Methods

Cell lines, mAbs and reagents: Capan 1 was cultured in Isocove's Modified Dulbecco's Eagle Medium (IMDM) supplemented with 1.5g/L Sodium Bicarbonate and 20% fetal bovine serum (FBS). PL 45 and Panc 1 were cultured in high glucose Dulbecco's Modified Eagle Medium (DMEM) supplemented with 1% Sodium pyruvate and 10% FBS. Panc 02.03 and Panc 10.05 were culture in RPMI 1640 supplemented with 1.5g/L Sodium Bicarbonate, 15% FBS, 1 % Sodium Pyruvate, 1 mM Hepes, 20 U insulin, and 4.5 g/L Glucose. BxPC 3 and CFPAC 1 were cultured in RPMI1640 supplemented with 1.5g/L Sodium Bicarbonate, 10% FBS, 1 % Sodium Pyruvate, 1 mM Hepes, and 4.5 g/L Glucose. WV was cultured in RPMI 1640 supplemented with 10% FBS, 1 % Sodium Pyruvate, 1 mM Hepes.

Anti-PrP mAbs 8H4, 7A12, 11G5, 8F9 and 8B4 were generated in our laboratory and have been characterized extensively (1). Anti-CD55 mAb was purchased from BD Bioscience. Anti-FLNa A mAb, horseradish peroxidase (HRP) conjugated goat anti-mouse IgG Fc specific antibody and mouse anti-actin mAb were purchased from Chemicon (Temecula Corporation.). Anti-tyrosine-phosphorylated protein, anti-LIMK1, anti-LIMK2, anti-cofilin, anti-phosphorylated Fyn, anti-Rac1, anti-phosphorylated Rac1, anti-MEK^{1} and anti-phosphorylated MEK^{1} and anti-phosphorylated cofilin antibodies were purchased from Cell Signaling Technology. Anti-Fyn, anti-ERK^{1,2} and anti-phosphorylated ERK^{1,2} were purchased from Santa Cruz Biotechnology. Fluorescein isothiocyanate (FITC)-labeled goat anti-mouse IgG antibody was purchased from Southern Biotech Co. Texas red-conjugated phalloidin and 4', 6-diamidino-2-phenylidole, dialactate, (DAPI), goat anti-mouse Alex Flour 488 secondary antibody, donkey anti-rabbit Alex Flour 555 secondary antibody, and Alex Flour 548 conjugated strepavidin were purchased from Invitrogen Corporation. Protein G-agarose beads were purchased from Roche. PNGase F

was purchased from New England BioLabs. Profound Co-Ip™ kit, EDTA-free protease inhibitor cocktail, dimethyl suberimidaet.2HCL (DMS), EZ-Link™ Sulfo-NHS-Biotin kit and SuperSignal®West Femto kit were purchased from Pierce Corporation. Bio-Rad protein assay kit and silver stain plus kit were purchased from Bio-Rad Corporation. Phenylmethanesulfonyl fluoride (PMSF), Triton x-100, Tween-20, and phospholipase C (PI-PLC) were purchased from Sigma. Carboxypeptidases B were purchased from Worthington Biochemical Corporation. Streptavidin-Agarose beads were purchased from MP Biomedicals Coporation.

Flow cytometry and confocal microscopy: To detect cell surface PrP in live tumor cell lines, cells were seeded in 25 cm^2 flask 12 hours before experiment, rinsed with ice cold DPBS once, and then released by treatment with Trypsin/EDTA. mAbs 8H4, 11G5, 8F9, 3F4 or D7C7 (0.01 μg/μl) were then added to the cell suspensions at 4°C. After washing, bound antibody was detected by an Alexa Fluor 488nm-conjugated goat anti-mouse Ig specific antibody and then analyzed in a BD FACS™ flow cytometer. To detect PrP or FLNa expression by confocal microscopy, tumor cell lines were cultured in poly-D-lysine-coated glass bottom Petri dish (MatTek Coporation) overnight. Cells were then rinsed 3 X with ice cold DPBS and fixed in 4 % paraformaldehyde for 15 minutes at 20 °C. PrP or FLNa was detected with anti-PrP or anti-FLNa mAbs (0.01 μg/μl). Bound antibody was further detected with an Alexa Fluor 488nm-conjugated goat anti-mouse Ig specific antibody. Nuclei were stained with DAPI. To detect FLNa in PrP down-regulated tumor cells, cells were fixed and then permeablized with 0.3 % Triton X-100 in PBS for 10 minutes at 20 °C. The other steps were carried out as described earlier. To detect change in tyrosine phosphorylated protein (p-tyr), tumor cells were prepared and treated as described in above, an anti–p-tyrosine antibody was added to the cells, and incubated overnight at 4 °C as suggested by the provider of the antibody. Bound antibody was detected with an Alexa Fluor 488nm-conjugated goat an anti-

mouse Ig antibody. Nuclei were stained with DAPI. F-actin was detected with a Texas Red-conjugated Phalloidin. All cytometry and confocal microscopy analysis were carried out at least twice with comparable results.

PI-PLC treatment and flow cytometry analysis of live cells: Tumor cells were seeded overnight as described. The next day, cells were first washed 3 times with ice-cold DPBS, and then treated with trypsin/EDTA to prepare a single cell suspension of the tumor cells. After washing twice with DPBS, cells were incubated with PI-PLC (500 X dilution of 1U) at 37^0C for one hour. At the end of the incubation, cells were washed twice with DPBS and then stained with control antibody or 8H4 as described (2).

For staining of live BxPC3 and Panc 02.03 cells with rabbit anti-PrP-GPI-PSS serum, single cell suspensions of the tumor cells were prepared as described and then incubated with either a rabbit non-immune serum (1:100) or affinity purified anti-PrP-GPI-PSS serum. An Alexa Fluor 488 nm conjugated donkey anti-rabbit antibody (Invitrogen Corporation) was used to detect bound rabbit antibody.

For staining of tumor cells with rabbit anti-PrP-GPI-PSS serum for confocal microscopy analysis, tumor cells were seeded overnight, washed 3 times with ice-cold DPBS, then fixed with 4% PFA for 15 minutes at 20^0C. Subsequently, tumor cells were washed 3 X with PBST, and then incubated with either the rabbit non-immune serum (1:100) or the affinity purified anti-PrP-GPI-PSS serum for 1 hour at 20^0 C. Bound primary antibody was detected with an Alexa Fluor 488 nm conjugated donkey anti-rabbit antibody.

All the above experiments were carried out at least twice with comparable results.

Immunoblotting and enzymatic treatment of PrP from various tumor cell lines: Cell lysates were prepared in lysis buffer containing 20 mM Tris (pH7.5), 150 mM NaCl, 1mM EDTA, 1mM EGTA, 1% Triton X-100, 2.5 mM sodium

pyrophosphate, 1mM b-glycerolphosphate, 1 mM Na_3VO_4. 1mM PMSF, and EDTA-free protease inhibitor cocktail was added just before cell lysis. PrP was affinity purified by mAb 8B4-conjugated beads, eluted and neutralized to pH 7.5 as described (3). Purified PrP was subjected to carboxypeptidases or PNGase-F treatment followed by PI-PLC treatment according to the protocols provided by the providers. After treatment, samples were separated on SDS-PAGE and immunoblotted with an anti-PrP antibody. Briefly, 2U PNGase F was added to 20 µl of eluted and neutralized PrP. 0.375 U PI-PLC was added to 20 ul PNGase F treated PrP. 1 U carboxypeptidase B was added to 20 ul of eluted and neutralized PrP at 20 °C for different periods of time.
All the above experiments were repeated at least twice with comparable results.

Sucrose gradient fractionation: Cell lysates prepared as described were mixed with an equal volume of ice-cold 80 % sucrose in MES buffer [25 mM 2-(4-Morpholino) ethane sulfonic acid pH 6.5, 150 mM NaCl, 5 mM EDTA]. Two ml of 40 % sucrose/cell lysate was transferred to a 5 ml Ultra-Clear centrifuge tubes (Beckman, Fullerton, CA) on ice. 2 ml ice-cold 30 % sucrose in MES buffer was placed on top. 1 ml ice-cold 5 % sucrose in MES buffer was loaded on the top of the gradient. Samples were centrifuged at 200,000 X g for 16 hours at 4 °C. 12 X 400 µl fractions were collected from top to bottom. 21 µl of each fraction was applied to 12 % SDS-PAGE and then immunoblotted with anti-PrP, anti-flottlin-1 or anti-FLNa mAbs. This experiment was repeated twice with comparable results.

Immunoprecipitation, immunoblotting, and identification of co-purified proteins by Mass Spectrometry: To identify proteins that are normally bound to PrP in various tumor cell lines, cell lysates were prepared in Cell Signaling Co-I.P. Buffer (Cell Signaling Technology). Immunoprecipitation was performed with mAbs 8B4, 8H4, D7C7 or anti-CD55 monoclonal antibody that were conjugated to Sepharose beads (3). Beads were collected by centrifugation and

washed extensively (X6). Bound proteins were eluted using IgG-Elution Buffer (Pierce Corporation). The eluted proteins were then separated by SDS-PAGE (12% gel) and silver stained (Bio-Rad Corporation). One of the unique bands at 280 kDa was cut out, washed, reduced/alkylated, and digested with trypsin. The digested products were then analyzed by mass spectrometry at the Case Center for Proteomics, Mass Spectrometry Core Facility using a LC-MS system (Finnigan LTQ linear ion trap mass spectrometer). Identification of the protein was based on peptide fragment sequence homology with FLNa in the NCBI database (4), using the search program, Mascot. All matching spectra were further verified by manual interpretation. The interpretation process was aided by additional searches using the programs Sequest and Blast.

To confirm that the protein bound to PrP^C was FLNa, immunoprecipitated proteins were separated by 12 % SDS-PAGE, electro-transferred to nitrocellulose membrane, and blotted with an anti-FLNa mAb. Bound antibody was detected with a goat anti-mouse-HRP antibody using the chemiluminescence blotting system (Pierce Corporation). All the immunoprecipitation and immunoblotting experiments have been repeated at least twice with comparable results.

Binding of GST-PrP23-253 to FLNa: 2μg of Flag-FLNa dimer or Flag-FLNa1-23 were mixed with 3μg of GST-PrP231-253 in 400ul binding buffer (20mM Tris.HCl, pH7.4, 150mM NaCl, 1mM EGTA and 0.1% Tween 20), respectively. GST was used as control. The tubes were rocked slowly and incubated at RT for 1hr. 10ul of GST binding beads (Novagen, pre-equilibrated with binding buffer) was added and further incubated for 30 min. The beads were then washed with binding buffer X 5 (5min/time). The beads were resuspended in 15μl of 2xSDS loading buffer and boiled at 95^0C for 10min. The proteins were separated on 4-20% Tris-glycine gel and then transferred to NC membrane. FLNa was detected with anti-Flag mAb (Sigma, 1:1000 dilution, 4^0C overnight). After second antibody incubation and washing, the membrane was developed by the addition

of Supersignal West Femito Maximum sensitive substrate (Pierce Corporation, 1:20 dilution). This experiment was repeated twice with comparable results.

Binding of FLNa to pro-PrP: 250ng of Flag-FLNa was mixed with 1.2μg of rPrP23-253 or rPrP23-230 in 400ul binding buffer (same as above). The tubes were rocked slowly and incubated at RT for 1hr. Then 3μg of anti-PrP mAb 8H4 was added and incubated for another hour with gentle rocking. 10μl of protein G agarose beads (pre-equilibrated with binding buffer) was then added for 30min. The beads were washed with binding buffer for 5 min. X 5. The beads were then resuspended in 15μl of 2xSDS loading buffer and boiled at 95^0C for 10min. The proteins were separated on a 4-20% Tris-glycine gel and then transferred to NC membrane. FLNa was detected with anti-Flag mAb (Sigma, 1:1000 dilution, 4^0C overnight). After second antibody incubation and washing, the membrane was developed as described above. On the same membrane, input rPrPs were detected with anti-PrP mAb 8B4. These experiments were repeated >three times with comparable results.

Competition of Co-I.P. with synthetic peptide: BxPC 3 and Panc 02.03 cell lysates were prepared as described in the co-i.p. experiment. mAb 8B4 conjugated beads were made as described by the provider (Pierce Corporation). Prior to the co-i.p. experiment, the efficiency of the beads was determined by direct immuno-precipitation of the cell lysate. For competition experiments, 400 μl of cell lysate from each cell type was loaded into the mAb 8B4 column. Synthetic peptides in the indicated amount were also added, as well as 4 μl of PMSF and 1μl/column of DMSO. The columns were placed in the 4°C cold room overnight with gentle rocking. Each column was then washed 6X with cell lysate buffer and eluted in 2X 100 μl of Immunepure-IgG elution buffer (Pierce Corporation) in the cold room. Eluted proteins were separated in a 4-20% Tris-glycine gel, transferred to NC membrane and then immunoblotted with anti-

FLNa mAb as described. These experiments were repeated twice with comparable results.

shRNA down regulation of PrP expression: We first identified 3 *PRNP* sequences (shRNA-10, shRNA-2 and shRNA-4) as potential targets of siRNA using OligoEngene™ software.

shRNA-10: 5′-gag,cag,gcc,cat,cat,aca,t-3′;
shRNA-2: 5′-tgg,ggg,cag,cct,cat,ggt,g-3′;
shRNA-4: 5′-cag,aac,aac,ttt,gtg,cac,g-3′.
A scrambled sequence, shRNA-s: 5′-aca,tgt,ata,cgc,acg,cac,g-3′ was used as a control.

The oligo sequences were first annealed and then ligated into linearized pSUPER RNAi vector (OligoEngene Corporation) (10:1) overnight at 4°C. The ligation product was further treated with Bgl II to reduce background and transformed into DH5α cells. Positive clones were selected after EcoRI and Hind III digestion showing the 281 bp band. Plasmid was then transfected into the 293T cell line (A.T.C.C.) by calcium-phosphate precipitation. Retroviral supernatant was collected 48 hrs later by filtering through a 0.45 μm filter. A siRNA with a "scrambled" sequence was generated identically and used as a control. The viral supernatant was used to infect PDAC cell lines for 6 hrs in the presence of 4 μg/ml polybrene. Culture mediums were then removed and replenished with fresh medium, and cells were allowed to recover for 24 hrs. Infected cells were selected with puromycin (2 μg/ml) for 48 hrs. Viable clones were expanded, and the levels of PrP^C expression then quantified by immunofluorescence staining with anti-PrP mAbs followed by analysis by flow cytometry or by observation by a confocal microscopy or immunoblotting.

Cell surface biotinylation: PDAC tumor cells were surface incubated with sulfosuccinomidobiotin (Pierce Corporation) (0.1mg/ml in labeling buffer

(150μM NaCl, 0.1 M Hepes, pH.8) for 30 min as described by us (5). After biotinylation cells were washed, lysed and immunoprecipitated with avdin conjugated beads in the co-immunoprecipitation buffer. Bound proteins were then eluted and immunoblotted with anti-PrP, anti-FLNa or anti-HSP90 mAbs. The flow-through from the avidin-bead column, which contains the non-biotinylated cytosolic protein was also collected and then immunoblotted with the same mAbs. This experiment was repeated twice with comparable results.

SiRNA knockdown of FLNa in Panc 02.03 cells: Filamin A (human) "knock-down" and control scramble reagents were purchased from Santa Cruz Biotechnology and used as suggested by the provider. This experiment was repeated twice with comparable results.

Co-localization of PrP and FLNa in different tumor cells: Seeded tumor cells were first assayed for filamin A expression as described earlier. The cells were then blocked with normal mouse serum (1mg/ml) for 1 hour at 20 °C. PrP was then detected with biotinylated 8H4 (0.01 μg/μl) or biotinylated anti-CD55 (BD Biosciences) as control. Streptavidin Alexa Fluor 555 (Invitrogen Corporation) was applied to detect bound biotinylated antibodies. Nuclei were detected with DAPI. These experiments were repeated twice with comparable results.

Sandwich ELISA for quantifying the level of soluble PrP in the culture supernatant of the PDAC cell lines: 1 $X10^5$ of each PDAC cell line in 200 μl of culture medium was cultured in 96 well tissue culture plate (Corning Corporation) in triplicate. Twenty fours after culture, 100 μl of the culture medium were carefully removed. The level of soluble PrP present in the culture medium was then assayed using a sandwich EILSA as described by us (6). In this sandwich ELISA, mAb 8B4 was used as a capture-antibody and a biotinylated mAb 7A12 as used as a detecting antibody. The results presented represent the average of the triplicate well +/- S.D. These experiments were repeated twice with comparable results.

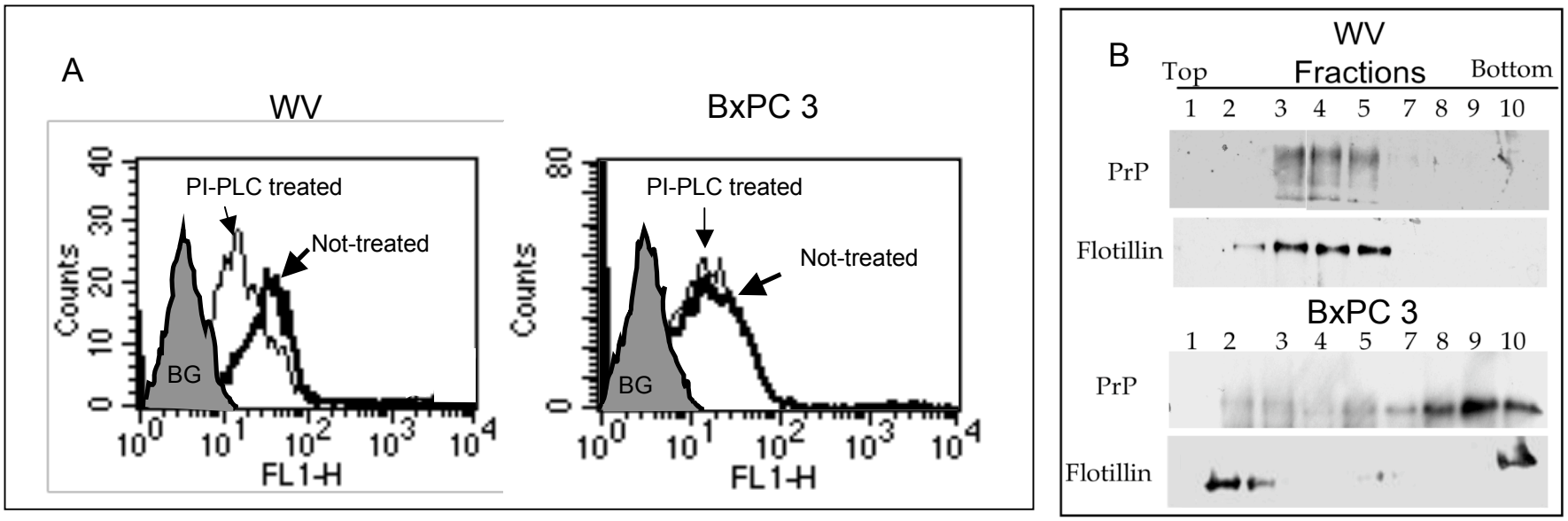
Immunohistochemical staining: Unstained, 5μ sections were cut from paraffin blocks of selected cases and de-paraffinized using standard techniques. Slides were treated with 1X sodium citrate buffer (diluted from 10X heat-induced epitope retrieval buffer; Ventana-Bio Tek) before heating for 20 min. in a microwave oven. Slides were then cooled at room temperature for 20 min., and incubated with 3% w/v H_2O_2 for 10 min. Mouse anti-human PrP mAb, 8H4, was then added and incubated at room temperature for 1 hr. An isotype control mAb D7C7 was included in all experiments as a negative control. After serial washing, bound primary antibody was detected by adding a secondary antibody followed by avidin-biotin complex and 3,3'-diaminobenzidine (DAB) (Dako Inc,). Sections were counterstained with hematoxylin. Each slide was coded and evaluated by two pathologists (W.X and A.A. P.). The cytoplasmic and membrane staining intensity of each sample was graded as diffuse (>50% neoplastic cells stained positive), focal positive (5-50% neoplastic cells stained positive) or negative (<5% neoplastic cells stained). The identity of the case was revealed only after a score had been given. The process to detect GPI-SS of pro-prion protein in tumor sample was described above. Instead of 8H4, rabbit polyclonal antibody specific for the PrP GPI-PSS or non-immune serum was added and the second step antibody was a goat anti-rabbit Ig antibody.

Supplementary Reference:

1. Zanusso, G., Liu, D., Ferrari, S., Hegyi, I., Yin, X., Aguzzi, A., Hornemann, S., Liemann, S., Glockshuber, R., Manson, J.C., et al. 1998. Prion protein expression in different species: analysis with a panel of new mAbs. *Proc. Natl. Acad. Sci. U. S. A.* **95**:8812-8816.
2. Liu, T., Li, R., Pan, T., Liu, D., Petersen, R.B., Wong, B.S., Gambetti, P., and Sy, M.S. 2002. Intercellular transfer of the cellular prion protein. *J. Biol. Chem.* **277**:47671-47678.

3. Pan, T., Li, R., Wong, B.S., Kang, S.C., Ironside, J., and Sy, M.S. 2005. Novel antibody-lectin enzyme-linked immunosorbent assay that distinguishes prion proteins in sporadic and variant cases of Creutzfeldt-Jakob disease. *J. Clin. Microbiol.* **43**:1118-1126.
4. Stossel, T.P., Condeelis, J., Cooley, L., Hartwig, J.H., Noegel, A., Schleicher, M., and Shapiro, S.S. 2001. Filamins as integrators of cell mechanics and signalling. *Nat. Rev. Mol. Cell Biol.* **2**:138-145.
5. Liu, D., and Sy, M.S. 1996. A cysteine residue located in the transmembrane domain of CD44 is important in binding of CD44 to hyaluronic acid. *J. Exp. Med.* **183**:1987-1994.
6. Pan, T., Li, R., Kang, S.C., Wong, B.S., Wisniewski, T., and Sy, M.S. 2004. Epitope scanning reveals gain and loss of strain specific antibody binding epitopes associated with the conversion of normal cellular prion to scrapie prion. *J. Neurochem.* **90**:1205-1217.

Figure S1


A
WV
BxPC 3
PI-PLC treated
Not-treated
BG
Counts
FL1-H
B
WV
Fractions
Top
Bottom
1 2 3 4 5 7 8 9 10
PrP
Flotillin
BxPC 3

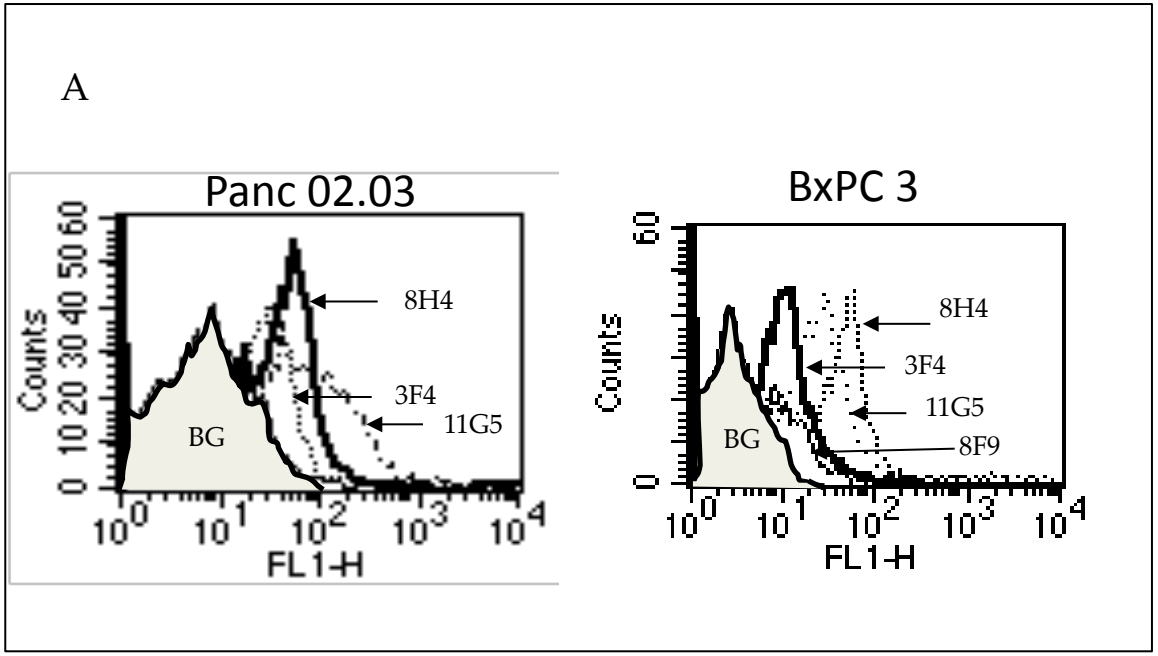
Supplementary legends for supplementary figures

Figure S1. Cell surface PrP on PDAC is not GPI-anchored

A. PrP on the cell surface of PDAC is resistant to PI-PLC: Histograms show that about 30-40% GPI-anchored PrP on the surface of WV cells is sensitive to PI-PLC. This is consistent with our earlier finding (2), as well as immunoblotting results described in Figure 2C. On the other hand, identical treatment of BxPC 3 cells with PI-PLC did not reduce the level of cell surface PrP.

B. PrP in BxPC 3 cells is not present in lipid rafts: Immunoblots show PrP in BxPC 3 cells is not longer present in lipid rafts but significant amount of flotillin-1 from BxPC 3 cells is still in lipid rafts. Cell lysates were prepared and fractionated in a sucrose gradient as described. Each fraction was immunoblotted with either mAb 8B4 or a mAb specific for flotillin-1, a lipid raft residential protein.

Figure S2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BxPC 3
Counts
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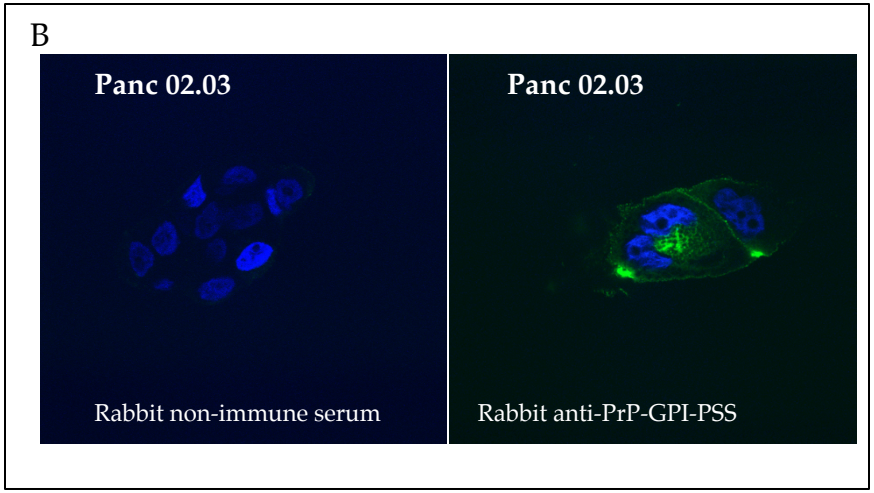
BG
8H4
3F4
11G5
8F9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Panc 02.03
Rabbit non-immune serum
Rabbit anti-PrP-GPI-PSS

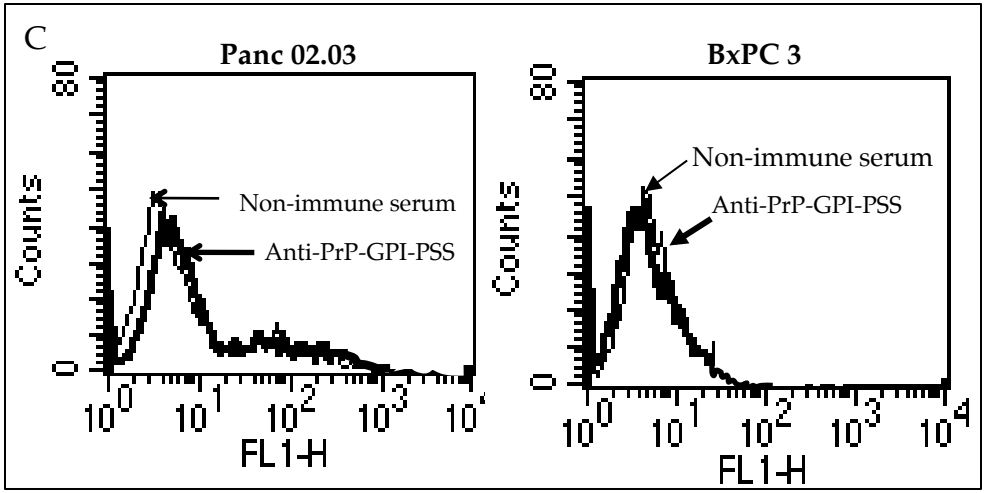

C
Panc 02.03
BxPC 3
Counts
FL1-H
Non-immune serum
Anti-PrP-GPI-PSS

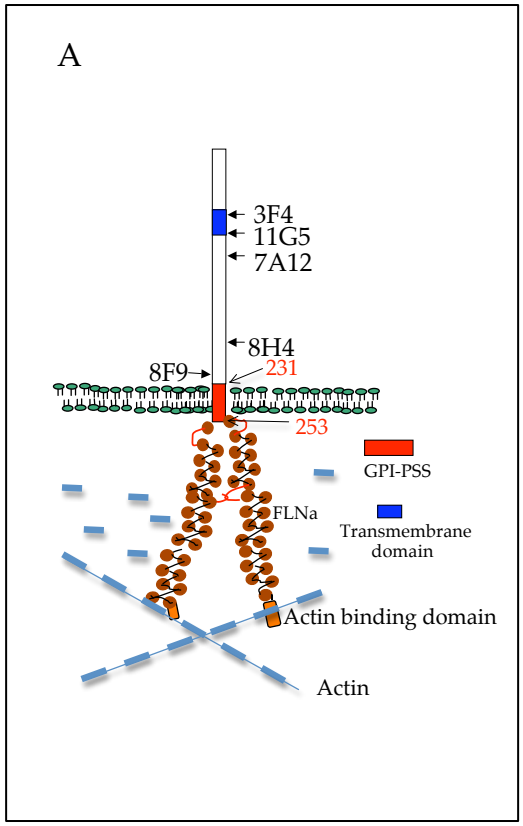
Figure S2. The topology of pro-PrP on the cell surface

A. The ectodomain of PrP is exposed on the cell surface: Histograms show that cell surface PrP on PDAC cell lines reacts with anti-PrP mAbs, 3F4, 11G5, 8H4 and 8F9. The difference in the intensity of the staining is likely due to differences in the affinity of the mAbs.

B. The anti-PrP-GPI-PSS antiserum reacts with fixed PDAC cells. Confocal microscopy images show that the anti-PrP-GPI-PSS antiserum reacts with fixed PDAC cells but the control non-immune serum does not.

C. The anti-PrP-GPI-PSS antiserum does not react with live PDAC cells. Histograms show that the anti-PrP-GPI-PSS does not react with live PDAC cells.

Figure S3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11G5
7A12
8H4
8F9
231
253
GPI-PSS
FLNa
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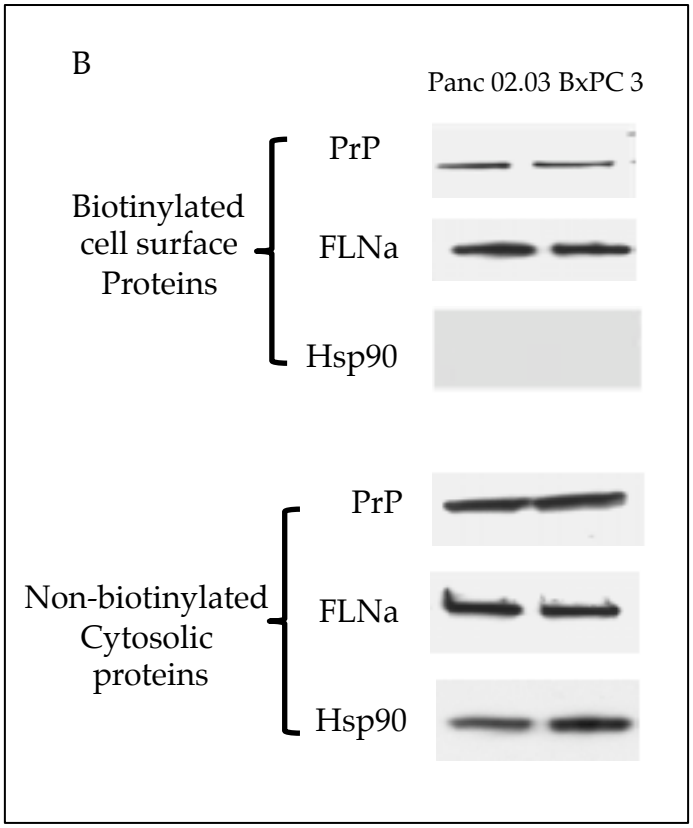
Actin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Biotinylated cell surface Proteins
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FLNa
Hsp90
Non-biotinylated Cytosolic proteins
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FLNa
Hsp90

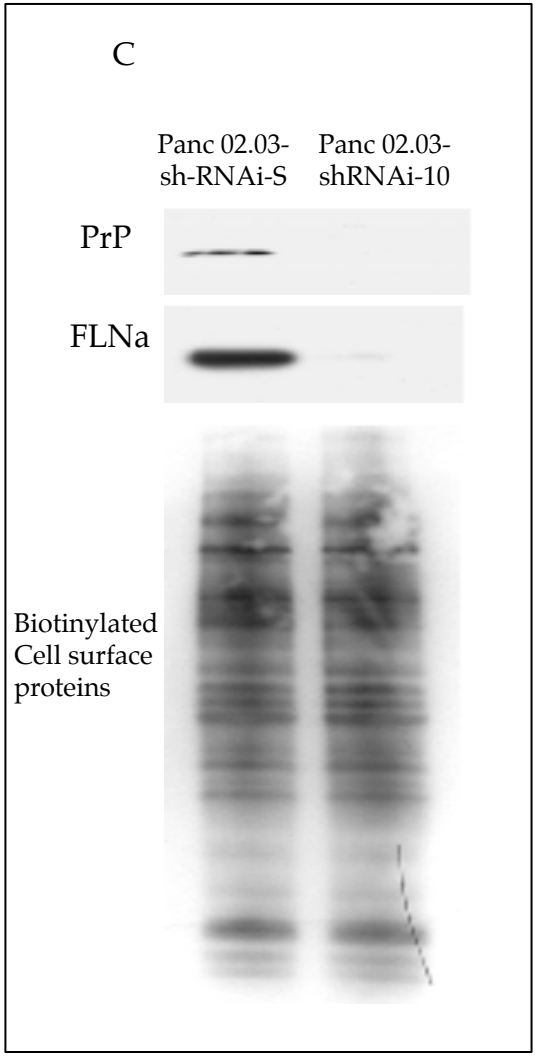

C
Panc 02.03-sh-RNAi-S
Panc 02.03-shRNAi-10
PrP
FLNa
Biotinylated Cell surface proteins

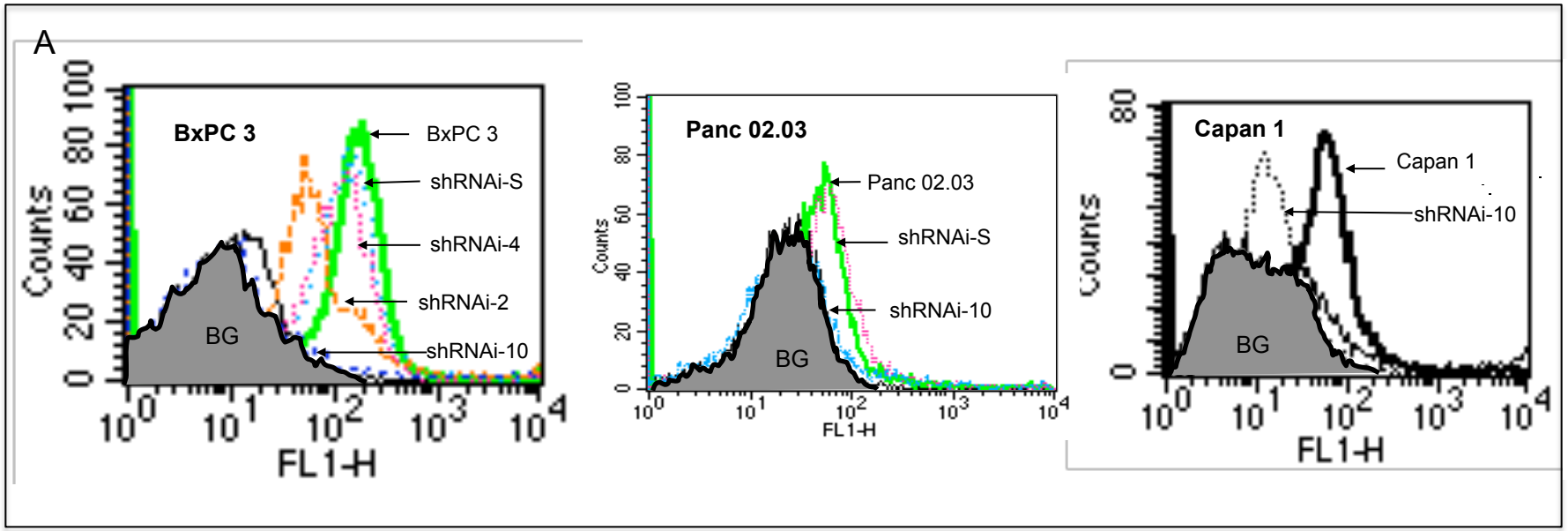
Figure S3. FLNa interacts with cell surface PrP

A. A hypothetic model of some of the pro-PrP on the cell surface of PDAC: The placement of FLNa is adopted from the model of Stossel T. P. et al. (4). A portion of the N-terminus of the PrP-GPI-PSS may be exposed, facing the cytoplasm, we found that the last 5 amino acids from 249 to 253 of the PrP-GPI-PSS is essential for binding FLNa. (Chaoyang, Li, Shuliang Yu, et. al., in preparation). The arrows indicate the epitope of the mAbs.

B. Immunoblot shows cell surface PrP co-purifies with FLNa but not Hsp90. Cell surface proteins from the PDAC cell lines were biotinylated and immunoprecipitated with avdin conjugated beads. Bound proteins were then separated by SDS-PAGE and then immunoblotted with mAbs specific for PrP, FLNa or Hsp90. It is clear that the affinity-purified proteins contain PrP and FLNa but not Hsp90. On the other hand, all three proteins are detected in the non-biotinylated cytosolic fraction.

C. Immunoblot shows that less FLNa is associated with membrane proteins in PrP "down-regulated" Panc 02.03 shRNA-10 cells. Experiments were carried out as described in (B). Separated proteins were also immunoblotted with avidin-conjugated enzyme and were used as a protein loading control.

Figure S4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BxPC 3
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shRNAi-4
shRNAi-2
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BG
Counts
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Panc 02.03
shRNAi-S
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BG
Counts
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Capan 1
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BG
Counts
FL1-H

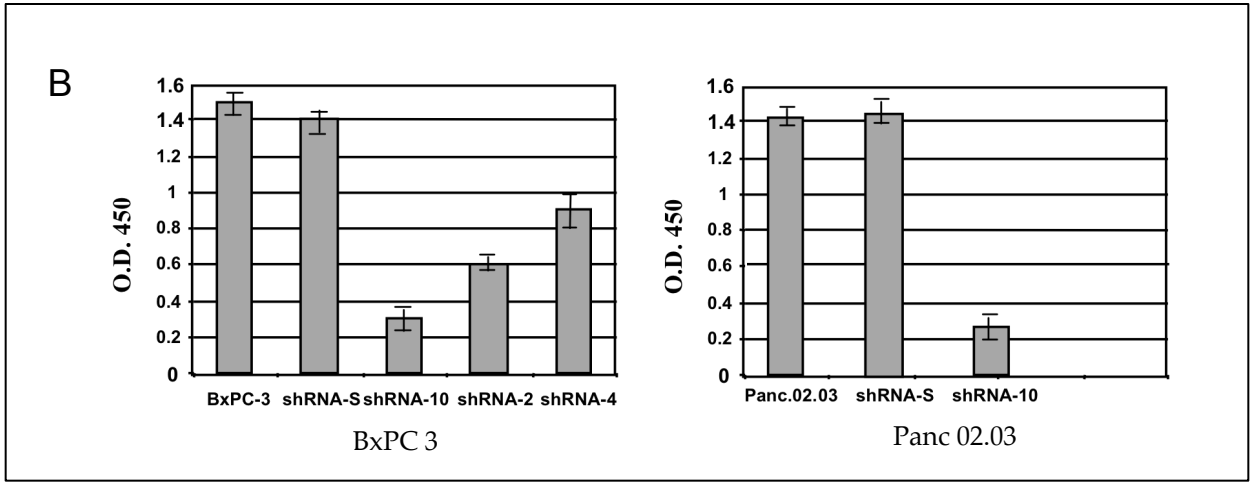
B
O.D. 450
BxPC-3
shRNA-S
shRNA-10
shRNA-2
shRNA-4
BxPC 3
O.D. 450
Panc.02.03
shRNA-S
shRNA-10
Panc 02.03

Figure S4. Down-regulation of PrP expression in the PDAC cell lines by shRNA

A. Histograms show PrP down-regulated BxPC 3, Panc 02.03 and Capan 1 cells have reduced levels of cell surface PrP: Single cell suspensions of tumor cells were prepared, stained with an anti-PrP mAb 8H4 and analyzed as described in Figure S1. The levels of PrP on BxPC 3-shRNA-10 cells and Panc 02.03-shRNA-10 cells were reduced by >90%. The level on BxPC 3-shRNA-2 cells was reduced by about 50%. The level on BxPC 3-shRNA-4 cells was reduced by about 20%. The levels of PrP in shRNA-10 down-regulated Capan 1 cells are reduced by >90%.

B. Graphs show that PrP down-regulated BxPC 3 and Panc 02.03 cells produce less soluble PrP into the culture medium: Similar number of tumor cells in 200 μl, were cultured in 96 wells in triplicate. 24 hrs after culture, 100 μl of the culture medium was carefully removed. A sandwich ELISA using anti-PrP mAb 8B4 as the capture mAb and a biotinylated anti-PrP mAb 7A12 as a detecting mAb was used to quantify the amount of soluble PrP present in the culture medium. PrP "down-regulated" PDAC cell lines produce less soluble PrP into the culture medium. These results concur with our earlier cell surface staining results.

Figure S5

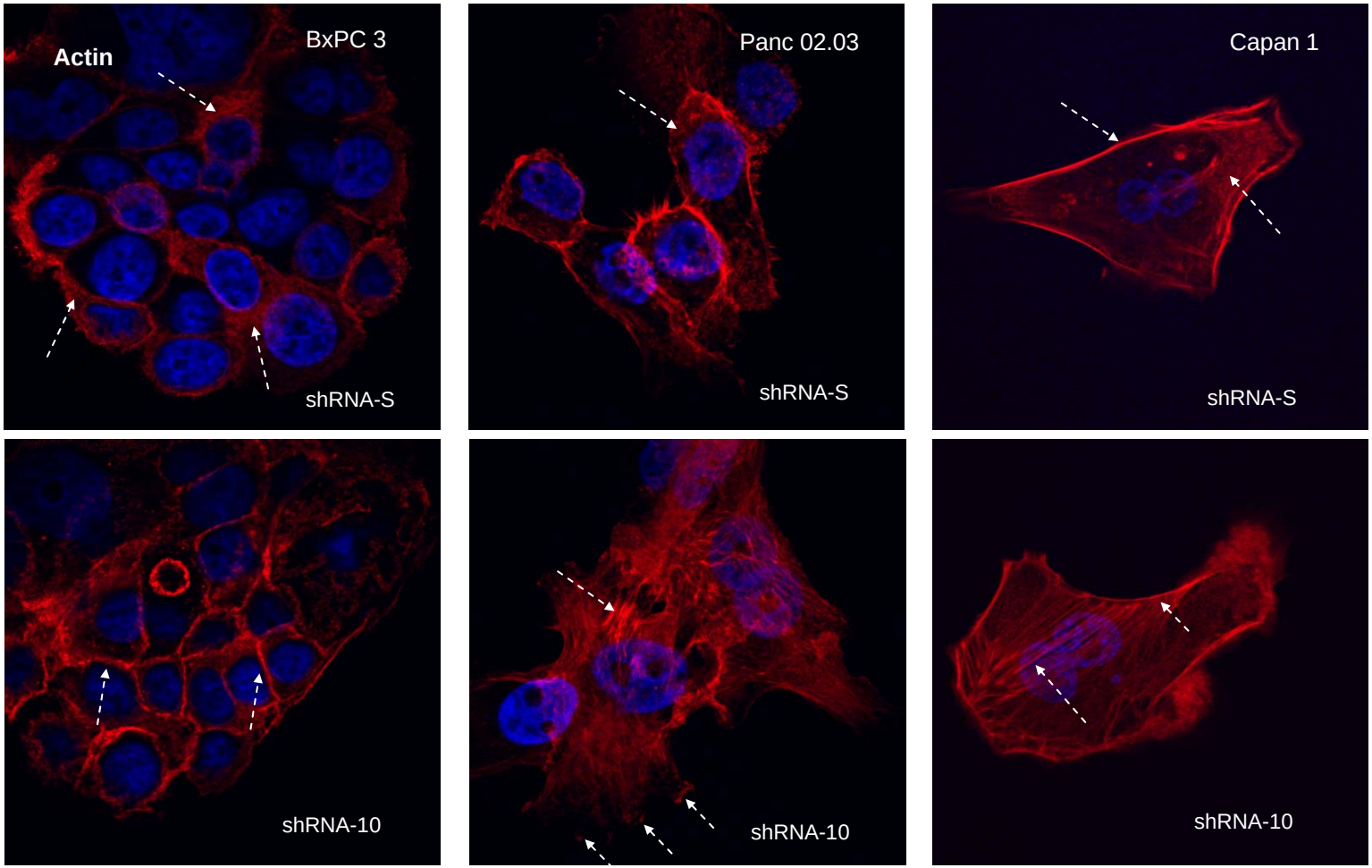
Actin
BxPC 3
shRNA-S
Panc 02.03
shRNA-S
Capan 1
shRNA-S
shRNA-10
shRNA-10
shRNA-10

Figure S5. Down-regulation of PrP expression alters the organization of actin filaments in the PDAC cell lines

Confocal microscopic images show down-regulation of PrP expression drastically alters the organization of actin filaments in the PDAC cell lines. The PDAC cell lines were cultured in poly-D-lysine-coated glass bottom Petri dish. overnight. Cells were then rinsed 3 X with ice cold DPBS and fixed in 4 % paraformaldehyde for 15 minutes at 20 °C. F-actin was detected with Texas Red-conjugated phalloidin. Nuclei were stained with DAPI. Total original magnification 1,000X.

Figure S6

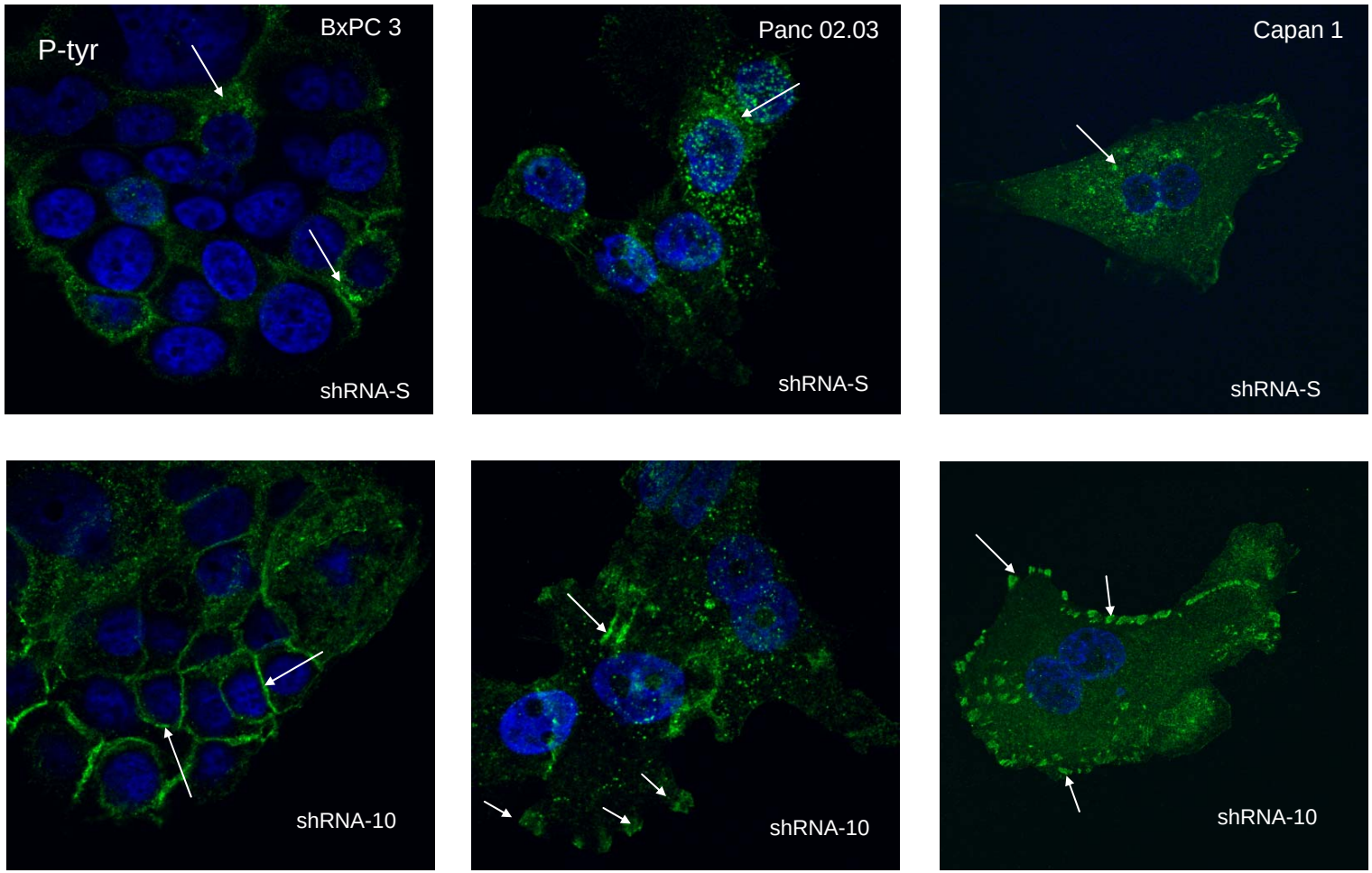
P-tyr
BxPC 3
shRNA-S
Panc 02.03
shRNA-S
Capan 1
shRNA-S
shRNA-10
shRNA-10
shRNA-10

Figure S6. Down-regulation of PrP expression alters the distribution of p-tyr immunoreactivity in the PDAC cell lines

Confocal microscopic images show down-regulation of PrP expression drastically alters the staining patterns of p-tyr in the PDAC cell lines. The PDAC cell lines were prepared as described earlier. An anti–p-tyrosine antibody was then added to the cells, and incubated overnight at 4 °C as suggested by the provider of the antibody. Bound antibody was detected with an Alexa Fluor 488nm-conjugated goat anti-mouse Ig antibody. Nuclei were stained with DAPI. Total original magnification 1,000X.

Figure S7

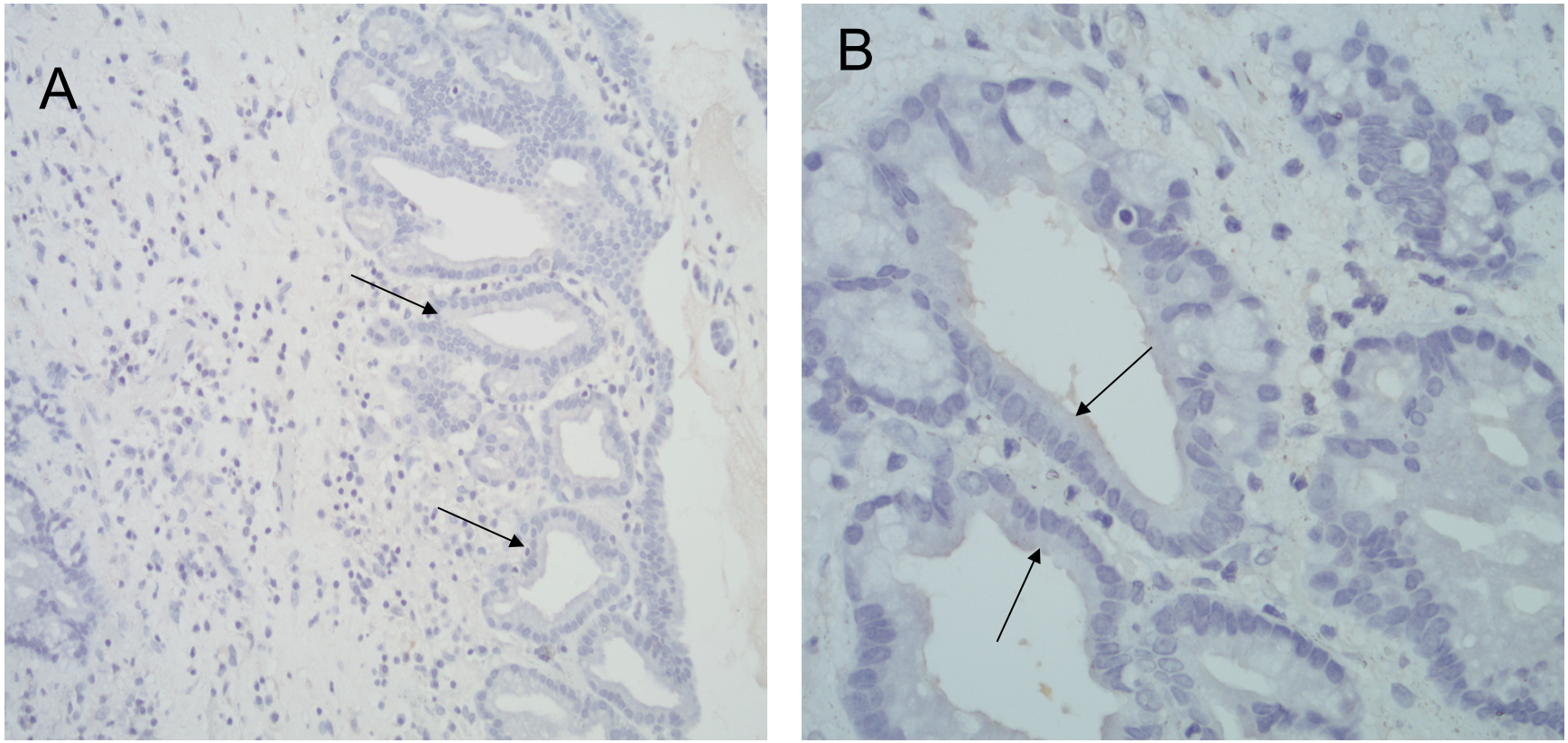

Figure 7S. The anti-PrP GPI-PSS antibody does not react with normal ductal cells in the pancreas of patients with PDAC

Microscopic pictures show that the anti-PrP-GPI-PSS antibody while reacted strongly with the PDAC, does not react with normal ducts in the same tissue sections. (A) Two arrows identify two ducts x200. (B) Two arrows identify two ducts X400.