Supplementary Information

Materials and Methods

Animal husbandry

C57BL/6 mice were kept on a 12 hour light / 12 hour dark cycle and allowed free access to water. *Pten*$^{flox/flox}$ mice (C57BL/6; The Jackson Laboratory) (1) were crossed with probasin (*PB*)-*Cre4* transgenic mice (C57BL/6; NCI-Frederick MMHCC Repository) to obtain *PB-Cre4*$^{+/-}$; *Pten*$^{+/-}$ males; these mice were mated with *Pten*$^{flox/flox}$ females to generate males with heterozygous or homozygous *Pten* inactivation in the prostate epithelium (PE) (*Pten*$^{PE+/-}$ or *Pten*$^{PE-/-}$ respectively). *Ptpn1*$^{-/-}$ mice (C57BL/6) were generated as described (2) and were mated appropriately to generate *Ptpn1*$^{-/-}$; *Pten*$^{flox/flox}$ females and either *PB-Cre4*$^{+/-}$; *Ptpn1*$^{+/-}$; *Pten*$^{flox/WT}$ or *PB-Cre4*$^{+/-}$; *Ptpn1*$^{+/+}$; *Pten*$^{flox/WT}$ males; subsequent mating of these mice led to the generation of *Ptpn1*$^{-/-}$; *Pten*$^{PE-/-}$ or *Ptpn1*$^{+/+}$; *Pten*$^{PE-/-}$ males respectively, and also *Ptpn1*$^{+/-}$; *Pten*$^{PE-/-}$, *Ptpn1*$^{-/-}$; *Pten*$^{PE+/-}$ and *Ptpn1*$^{+/+}$; *Pten*$^{PE+/-}$ mice.

Generation of PTPN1 knock-in mice

Briefly, we have subcloned - in the anti-sense orientation - a 3X-FLAG-tagged (N-term) *PTPN1* into a *Rosa26*-targeted pCAGG-promoter-based construct. Between 5 and 7 x10^6 R1 (from Nagy Lab) embryonic stem (ES) cells were electroporated with the construct and selected for the insertion following 10 days of neomycin treatment. Clones that were positive for the 3X-FLAG-*PTPN1*-IRES-eGFP/luciferase transgene (*R26*$^{PTPN1/WT}$) were validated by quadruplex-PCR screening, to detect the *R26* wild-type allele and the *knocked-in* allele *R26*$^{PTPN1/WT}$ (by validating the *GFP* insertion) (Supplementary Table S1); these clones were injected into C57BL/6 recipient mouse embryos at the blastocyst stage (3.5 day). Chimeric males with 50% or higher ES cell contribution (according to coat color) were mated with C57BL/6 mice to test for germline transmission. The offspring that demonstrated germline transmission of the *Rosa26* transgene was backcrossed at least four times (total of 5 backcrosses) before being examined for phenotypic differences. Since a Cre-mediated excision of a STOP cassette downstream of the pCAGG promoter is necessary to drive expression of the *R26*$^{PTPN1/WT}$ transgene, mice were mated against *PB-Cre4* in order to obtain PE-specific expression of the transgene. Supplementary Table S1 has a list of the different oligonucleotides used for genotyping.

Analyses of protein expression

Mouse prostates were dissected, immediately flash frozen in liquid nitrogen, disrupted with the Tissue Lyser II (Qiagen) and then lysed [1% NP40, 0.5% sodium deoxycholate, 150 mmol/L NaCl, 50 mmol/L Tris (pH 7.4), 0.1% SDS, 50 mmol/L NaF, 1 mmol/L Na3VO4, complete protease inhibitors (Roche)]. Cells were rinsed on ice with PBS and lysed as for mouse prostates. For immunoprecipitation, equal amount of proteins extracted from prostatic tissues were pooled and incubated in lysis buffer [1% triton, 150 mmol/L NaCl, 20 mmol/L Tris (pH 7.5) 1 mmol/L EDTA, 5% glycerol, complete protease inhibitors (Roche)] overnight at 4 °C in the presence of anti-FLAG M2 affinity gel (Sigma) and a mixture of proteins A- and G-coupled Sepharose beads. Equal amounts of denatured protein were separated by SDS-PAGE and transferred to polyvinylidene difluoride membranes (Immobilon-P, Millipore). Membranes were probed with the following antibodies according to manufacturer's instructions: mouse monoclonal anti-FLAG M2 (Sigma), rabbit polyclonal anti-PTP1B (Millipore), rabbit monoclonal anti-pAkt^{Ser473}, rabbit polyclonal anti-Akt, rabbit monoclonal anti-PTEN and anti-calnexin (Cell Signaling

Technology). When required, blots were stripped [62.5 mM Tris, 2% SDS, 65 mM β-mercaptoethanol] at 50 °C for 10 minutes and re-probed. Densitometry analyses were done with ImageJ (U.S. NIH, Bethesda, MD; http://imagej.nih.gov/ij/).

Histopathological and immunohistochemical analyses

Mouse urogenital apparatus was fixed in 10% buffered formalin, processed for paraffin embedding, sectioned (4 μm) and stained with H&E. Tissues were sectioned such that all prostate lobes (dorsolateral prostate, DLP; anterior prostate, AP; ventral prostate, VP) were visible on the same slide, and slides were analyzed by an expert uropathologist (L.R.B.). The presence and extent of mouse prostatic intraepithelial neoplasia (mPIN) was estimated for each mouse by evaluating the percentage of the gland affected for each prostate lobe. Prostate-specific PTP1B-overexpressing mice and 48-week-old *Ptpn1*$^{-/-}$; *Pten*$^{PE+/-}$ mice were characterized by a board certified veterinary pathologist (M.P.), who also identified desmoplastic reactions, microinvasive and invasive adenocarcinomas. Desmoplasia was graded according to the extent of the reaction and the number of zones affected; the criteria used for classification of mPIN, microinvasive and invasive adenocarcinomas were in accordance with the consensus report from the Bar Harbor Meeting of the mouse models of human cancer consortium prostate pathology committee (3). Microinvasive adenocarcinomas were scored based on an approximation of the number of microinvasive foci (groups of malignant neoplastic cells crossing the basement membrane), and were defined as mild, moderate or severe. Invasive adenocarcinomas were defined as a large focus of neoplastic cells invading deeply into a severely desmoplastic stroma or into vessels. Invasive adenocarcinomas were recorded but not scored. All histopathological analyses were done by pathologists who were blind to the experimental conditions.

Immunostaining with the rabbit monoclonal Ki-67 antibody (clone SP6, Thermo Scientific) was performed using the biotin-streptavidin-peroxidase method. Formalin-fixed paraffin-embedded sections were deparaffinized in xylene and rehydrated through graded ethanol followed by distilled water. Antigen retrieval was done by heating slides in the microwave for 15 minutes in citrate buffer (10 mM citric acid, pH 6.0). Non-specific sites were blocked with 10% goat serum and slides were then incubated with the primary antibody at a 1/200 dilution in PBS (0.1% Tween 20, 5% goat serum) for 1 hour at room temperature. Endogenous peroxidase was quenched in 3% hydrogen peroxide. A biotinylated goat anti-rabbit secondary antibody (Abcam), followed by streptavidin-HRP (Lab Vision), were applied for 10 minutes each. All rinsing between antibodies were done with PBS-Tween 20 (0.1%), 3 times for 3 minutes. Chromogen reaction using a 3,3'-diaminobenzidine tetrahydrochloride (DAB) solution kit (Lab Vision) was carried out for 5 minutes. Slides were counterstained with Harris Hematoxylin for 90 seconds, dehydrated in ethanol and xylene and then mounted. Immunostaining with the rabbit monoclonal antibody against pAkt^{Ser473} (D9E XP®, Cell Signaling Technology) was done as above, except that antibodies were diluted in SignalStain® antibody diluent (Cell Signaling Technology) according to the manufacturer's recommendation. Sections were incubated overnight at 4 °C and subsequently incubated with SignalStain® Boost (Cell Signaling), a polymer-based detection reagent, for 30 minutes. Quantification of nuclear Ki-67 and membrane-bound pAkt^{Ser473} was completed using specialized Aperio® algorithms.

Cell culture and lentiviral infections

HEK 293T/17 for lentivirus production were obtained from the American Type Culture Collection (ATCC) as described by Abba *et al.* (4) and kindly provided by the laboratory of Jerry Pelletier (McGill University) in 2011. HEK 293T/17 were not further authenticated. Immortalized *Ptpn1*$^{+/+}$ and *Ptpn1*$^{-/-}$ mouse embryonic fibroblasts (MEFs) were generated by our laboratory in 2001 as described by Cheng *et al*. (5) and *Ptpn1* genotype was validated by western blot (Figure 4A). Cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM, Thermo Scientific) supplemented with 10% fetal bovine serum and 5 mg/mL penicillin/streptomycin. HEK 293T/17 cells were cultured and transfected (Lipofectamine 2000, Invitrogen) with a lentiviral packaging vector (PAX2), comprising an envelope protein (VSV-G) and pLKO.1-Puro lentiviral shRNA vector against *Pten* mRNA (5'-CCACAGCTAGAACTTATCAAAC -3'), or a scramble sequence. Medium was refreshed at 24 hours following transfection, supernatant was collected 24 and 48 hours thereafter, aliquoted, and frozen at -80 °C. *Ptpn1*$^{+/+}$ and *Ptpn1*$^{-/-}$ MEFs were plated and infected 6 hours later, with a mix of lentiviral supernatant (collected after 24 and 48 hours, 50/50 mix) and polybrene (5 μg/mL) and cultured for 72 hours before serum starvation (6 hours) and IGF-1 stimulation at 100 ng/mL for 30 minutes (Peprotech, Cat #250-19, Lot #0407170).

Supplementary Tables

Table S1: List of the different oligonucleotides used for mouse genotyping

Name	**Sequence (5' to 3')**
PTPN1^{WT} forward	GAGGTCAGGGACCTTCTGTCTGG
PTPN1^{KO} forward	ATTGCTGAAGAGCTTGGCGGC
PTPN1 reverse	GGTACCCCGTGGATGTCTTAGCG
PTEN$^{flox/flox}$ forward	CAAGCACTCTGCGAACTGAG
PTEN$^{flox/flox}$ reverse	AAGTTTTTGAAGGCAAGATGC
PB-Cre4 forward	ATCCGAAAAGAAAACGTTGA
PB-Cre4 reverse	ATCCAGGTTACGGATATAGT
R26 5' forward	GCAGGAAGCACTTGCTCTCCCAA
R26 3' reverse	CTGTGGGAAGTCTTGTCCCTCC
R26 GFP forward	CGAACACCACGGTAGGCTGC
R26 GFP reverse	GCACAAGCTGGAGTACAACTACAAC

Table S2: Phenotypic evaluation of prostate-specific PTP1B-overexpressing mice

Prostate lobe	**Lesion observed**	***R26*$^{WT/WT}$**	***R26*$^{PTPN1/WT}$**
DLP	Cystic dilatation	6/9 (67%)	10/14 (71%)
	Lymphocytic inflammation	2/9 (22%)	3/14 (21%)
	mPIN (≤ 5%)	0/9 (0%)	1/14 (7%)
AP	Epithelial hyperplasia	0/9 (0%)	7/15 (46%)
VP	Lymphocytic inflammation	4/7 (57%)	4/11 (36%)

Supplementary References

1. Wang S, Gao J, Lei Q, Rozengurt N, Pritchard C, Jiao J, et al. Prostate-specific deletion of the murine Pten tumor suppressor gene leads to metastatic prostate cancer. Cancer Cell 2003;4(3):209-21.
2. Elchebly M, Payette P, Michaliszyn E, Cromlish W, Collins S, Loy AL, et al. Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. Science 1999;283(5407):1544-8.
3. Shappell SB, Thomas GV, Roberts RL, Herbert R, Ittmann MM, Rubin MA, et al. Prostate pathology of genetically engineered mice: definitions and classification. The consensus report from the Bar Harbor meeting of the Mouse Models of Human Cancer Consortium Prostate Pathology Committee. Cancer Res 2004;64(6):2270-305.
4. Malina A, Mills JR, Cencic R, Yan Y, Fraser J, Schippers LM, et al. Repurposing CRISPR/Cas9 for in situ functional assays. Genes Dev 2013;27(23):2602-14.
5. Cheng A, Bal GS, Kennedy BP, Tremblay ML. Attenuation of adhesion-dependent signaling and cell spreading in transformed fibroblasts lacking protein tyrosine phosphatase-1B. J Biol Chem 2001;276(28):25848-55.

Supplementary Figures

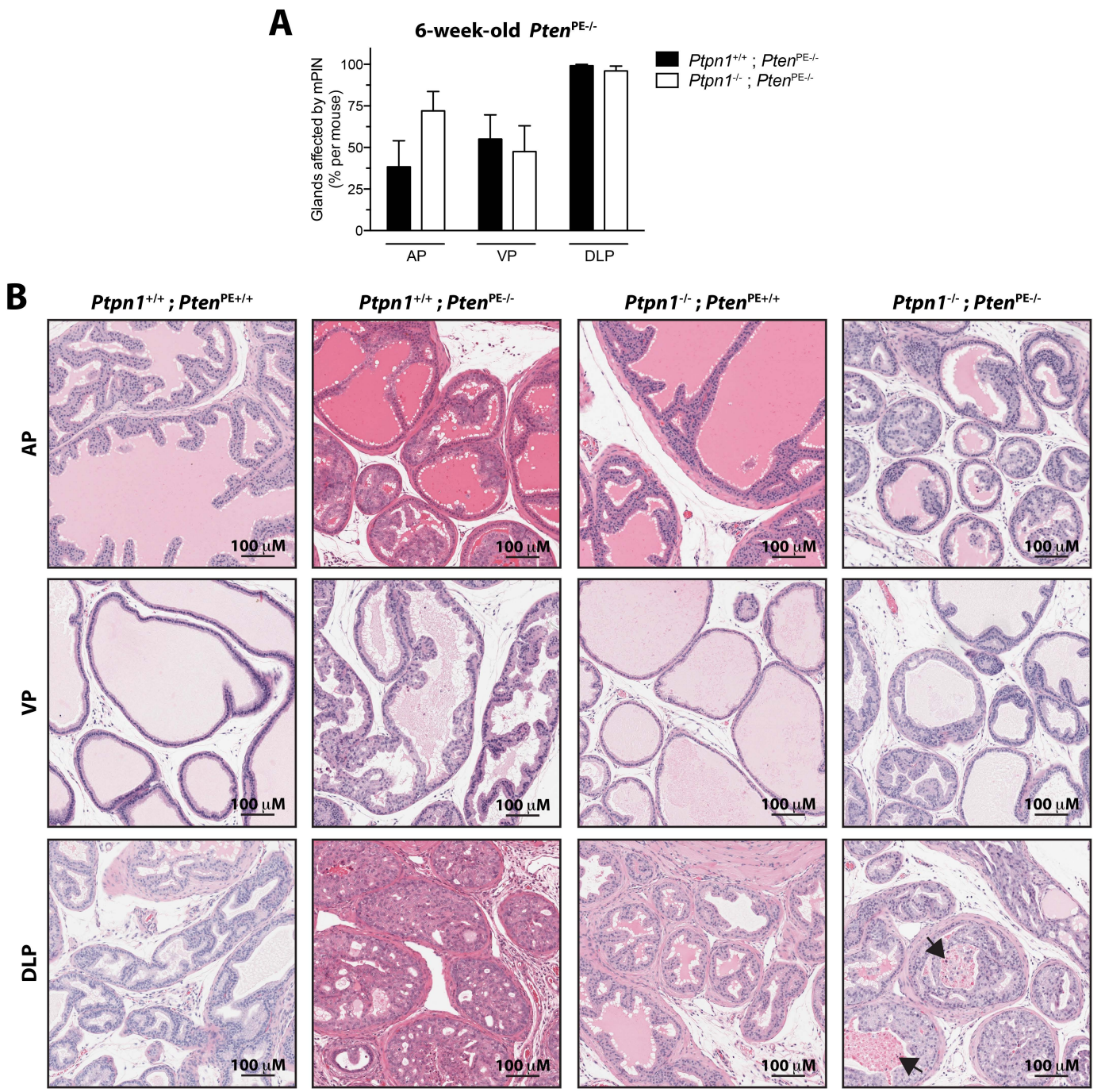

Figure S1: *Ptpn1 status does not alter the transition to mPIN in Pten$^{PE-/-}$ mice*
Slides were analyzed by an expert uropathologist (L.R.B.) blind to the experimental conditions. The presence and extent of mouse prostatic intraepithelial neoplasia (mPIN), which was estimated for each mouse by evaluating the percentage of the gland affected for each prostate lobe. **(A)** *Ptpn1*$^{-/-}$; *Pten*$^{PE-/-}$ mice fed a chow diet (N=5) develop mPIN to the same extend as *Ptpn1*$^{+/+}$; *Pten*$^{PE-/-}$ mice (N=6) at 6 weeks of age. **(B)** Representative hematoxylin-eosin staining of 6 weeks old prostate lobes 1) AP Showing focal mPIN with a papillary tufting and incipient cribriform pattern for both *Ptpn1*$^{+/+}$; *Pten*$^{PE-/-}$ and *Ptpn1*$^{-/-}$; *Pten*$^{PE-/-}$ mice; 2) VP showing focal mPIN with a cell stratification and papillary tufting pattern for both *Pten*$^{PE-/-}$ genotypes; 3) DLP showing diffuse mPIN with a cribriform and near solid pattern for *Ptpn1*$^{+/+}$; *Pten*$^{PE-/-}$ mice and diffuse mPIN with a cribriform, cell stratification and papillary tufting pattern for *Ptpn1*$^{-/-}$; *Pten*$^{PE-/-}$ mice; note the presence of intraluminal cellular necrotic debris (arrows). Control mice that had retained *Pten* expression have a normal glandular constituent without any cytoarchitectural alteration.

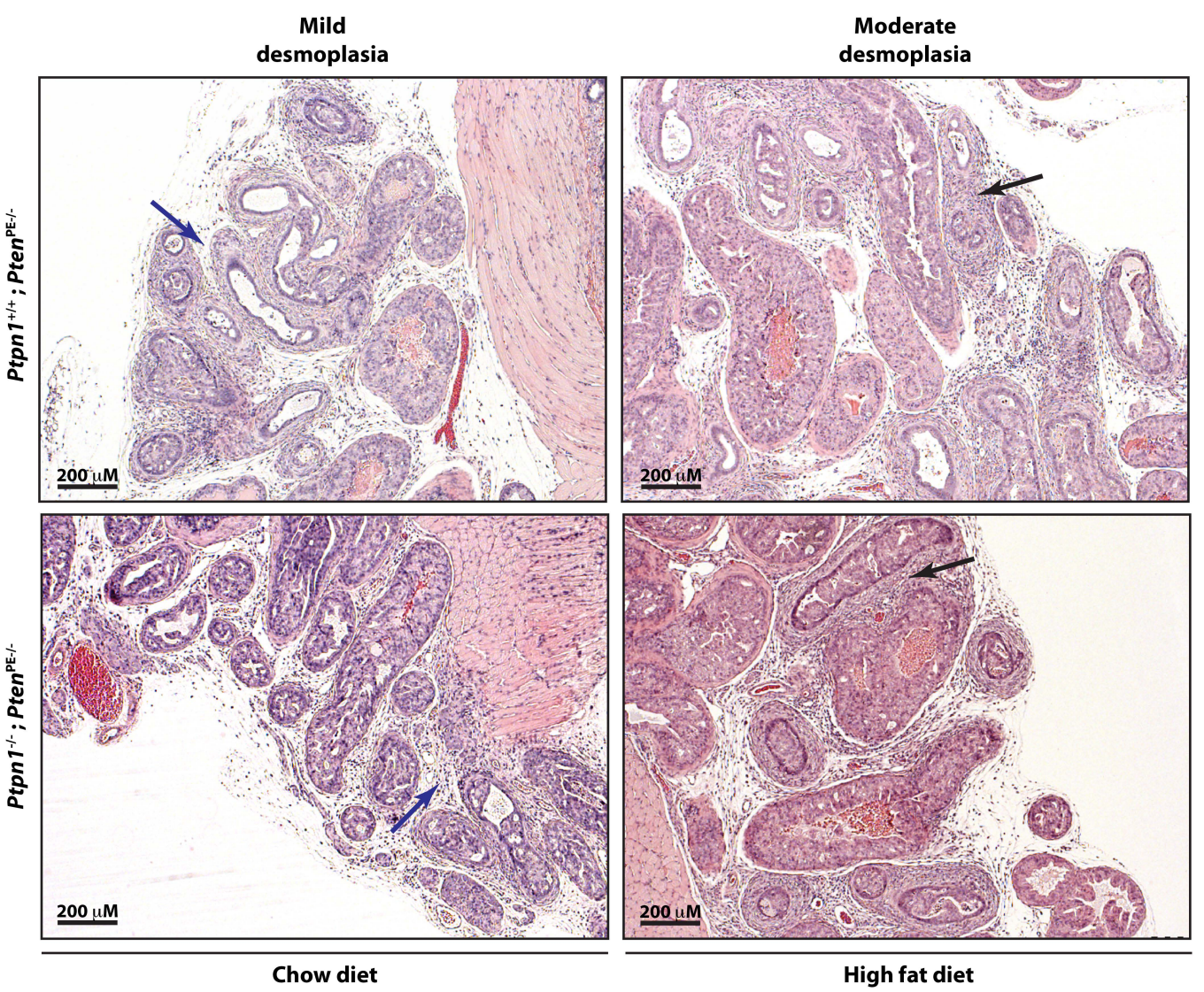

Figure S2: *Examples of mild and moderate desmoplasia*
Representative picture of desmoplastic reaction observed in the DLP of *Ptpn1*$^{-/-}$; *Pten*$^{PE-/-}$ mice and *Ptpn1*$^{+/+}$; *Pten*$^{PE-/-}$ mice fed either a chow or a HFD. The desmoplasia in the glandular stroma was graded semi-quantitatively. Moderate desmoplasia has a denser and wider stroma with higher amount of fibroblasts and collagen and more inflammatory cells compared to mild desmoplasia. Arrows indicate areas affected with desmoplastic reaction.

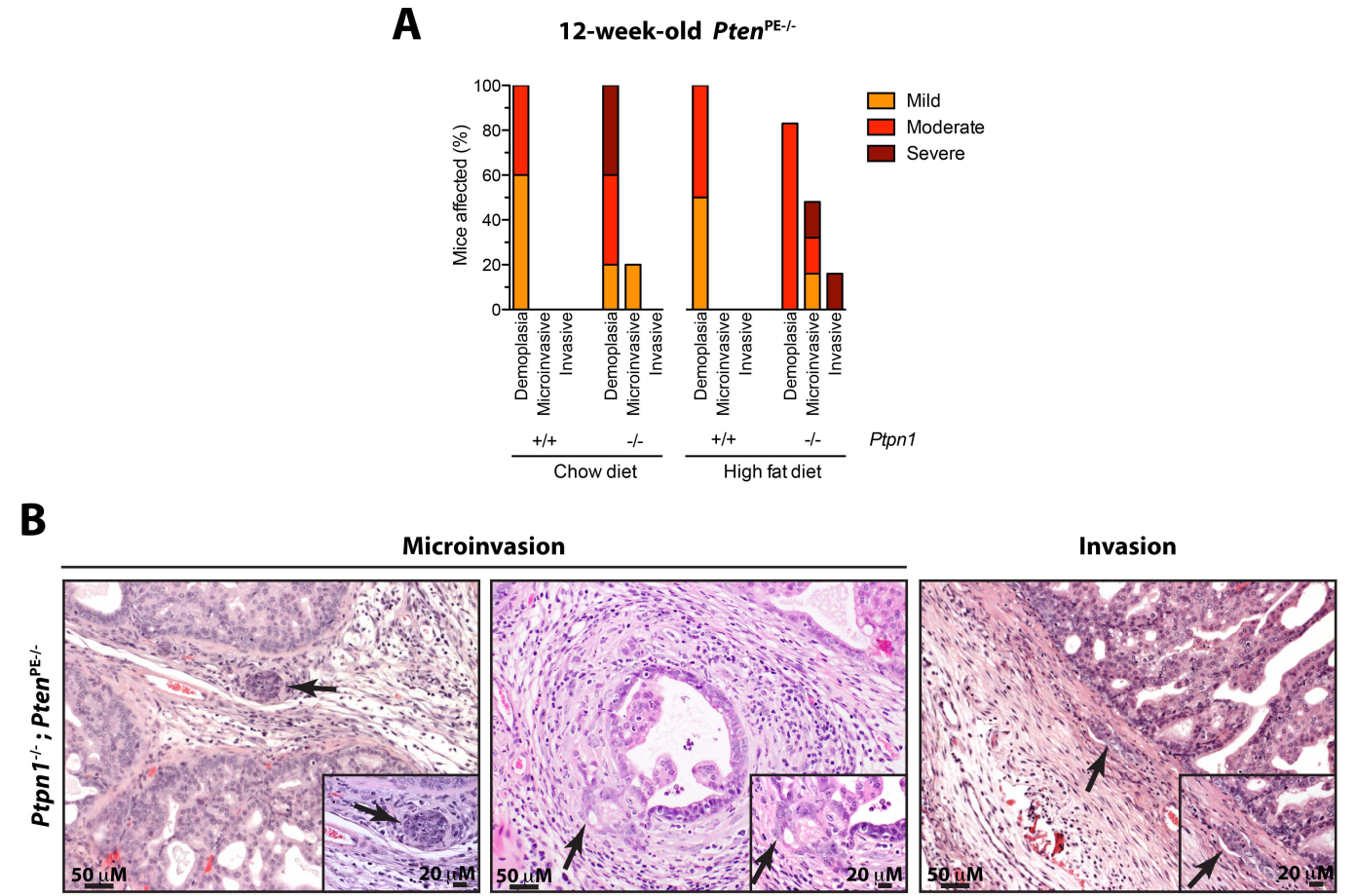

Figure S3: *HFD drives invasive prostate cancer in the AP only in absence of Ptpn1*
(A) Microinvasive adenocarcinomas were observed in the AP of *Ptpn1*$^{-/-}$; *Pten*$^{PE-/-}$ mice (N=5) fed on chow diet while none were found in *Ptpn1*$^{+/+}$; *Pten*$^{PE-/-}$ mice (N=5). Only *Ptpn1*$^{-/-}$; *Pten*$^{PE-/-}$ mice (N=6) displayed microinvasive and invasive adenocarcinomas in the anterior prostate when fed a HFD (*Pten*$^{PE-/-}$ N=4).
(B) H&E stained section showing representative microinvasive adonocarcinomas observed in a *Ptpn1*$^{-/-}$; *Pten*$^{PE-/-}$ mice fed a HFD (left and center panels; arrows indicate areas of microinvasion). Evidence of lymphovascular invasion identified in a *Ptpn1*$^{-/-}$; *Pten*$^{PE-/-}$ mouse fed a HFD (right panel; arrows).

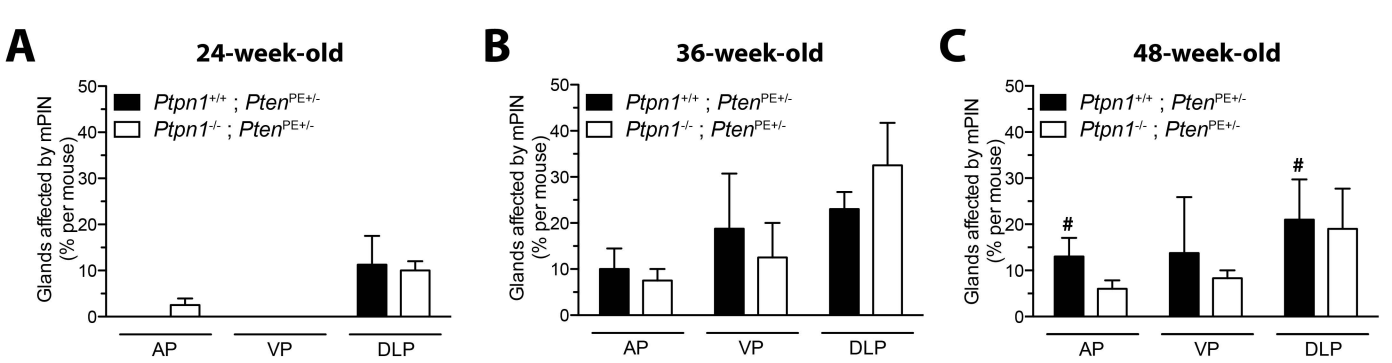

Figure S4: *Ptpn1 status does not modulate mPIN formation in Pten$^{PE+/-}$ mice*
(A) At 24 weeks, only few DLP ductules were affected by mPIN in *Ptpn1*$^{+/+}$; *Pten*$^{PE+/-}$ and *Ptpn1*$^{-/-}$; *Pten*$^{PE+/-}$ mice (N=4). **(B)** The different prostate lobes were increasingly affected by mPIN at 36 weeks of age in *Ptpn1*$^{+/+}$; *Pten*$^{PE+/-}$ (N=5) and *Ptpn1*$^{-/-}$; *Pten*$^{PE+/-}$ mice (N=4). **(C)** At 48 weeks of age, both genotypes were still demonstrating similar levels of mPIN. Symbol (#) denoted one mouse that developed microinvasive adenocarcinomas (beyond mPIN) and was thus excluded from the figure. *Ptpn1*$^{+/+}$; *Pten*$^{PE+/-}$ (N=6) and *Ptpn1*$^{-/-}$; *Pten*$^{PE+/-}$ mice (N=5).

	DLP 6-week-old						DLP 12-week-old						AP 12-week-old					
Diet	Chow			High fat			Chow			High fat			Chow			High fat		
Ptpn1	+/+	+/-	-/-	+/+	+/-	-/-	+/+	+/-	-/-	+/+	+/-	-/-	+/+	+/-	-/-	+/+	+/-	-/-
Desmoplasia - Mild	50	40	60	33	75	40			20	25	17	67	60	20	20	50		
Desmoplasia - Moderate		20	20	17		20	80	60	80	75	67	33	40	60	40	50	50	83
Desmoplasia - Severe							20	40			17			20	40		50	
Microinvasion - Mild			20			20	60	20	20	25	17				20		33	17
Microinvasion - Moderate						20		20	20		17							17
Microinvasion - Severe						40				25	33	50						17
Invasion											17	33						17
N	6	5	5	6	4	5	5	5	5	4	6	6	5	5	5	4	6	6

Figure S5: *Ptpn1 status influences Pten$^{PE-/-}$ phenotype in a dose-dependent manner*
Numbers indicate the proportion of mice (percentage) affected by the associated lesion following histopathological analysis. Number of mice analyzed for each conditions is reported in the last row. Color intensity is based on a different color cut for each lesion types (desmoplasia, microinvasion, invasion).