Supplementary Methods

CyTOF

Metal-labeled antibodies against cell surface markers were purchased from DVS Sciences and listed in Table S1. Prostate tumor single cells were isolated using the Mouse Tumor Dissociation kit (cat# 130-096-730, Miltenyl Biotec). Cells from spleen and lymph node were isolated by mincing with a 5mL syringe plunger against a 40μm cells strainer into a 60mm dish with RPMI medium containing 10% fetal bovine serum (FBS). The cells were depleted of erythrocytes by hypotonic lysis. Peripheral blood (100μL) was drawn using retroorbital bleeding and depleted of erythrocytes by hypotonic lysis. Next, tumor, spleen, lymph node, or blood cells were incubated with CD16/CD32 antibody (clone 2.4G2, BD Biosciences) to block FcγR binding for 10 minutes then with antibody mix for 30 minutes at room temperature. Cells were washed once and incubated with MAXPAR®Nucleic Acid Intercalator-^{103}Rh (Cat# 201103A, DVS Sciences) for 20 minutes for viability staining. Cells were fixed with 1.6% formaldehyde for 1 hour and incubated with MAXPAR®Nucleic Acid Intercalator-Ir (Cat# 201192A, DVS Sciences) at 4^{o}C overnight to stain the nuclei. The samples were analyzed with CyTOF instrument (DVS Sciences) in the Flow Cytometry and Cellular Imaging Core Facility at M.D. Anderson Cancer Center. The following markers were used to define different immune populations: T cells (CD3^{+} TCRβ^{+}, further classified as CD4^{+} and CD8^{+}), B cells (B220^{+} CD19^{+}), NK cells (NK1.1^{+}), dendritic cells (CD11c^{+}), MDSCs (CD11b^{+} Gr-1^{+}), and macrophages (CD11b^{+} Gr-1^{-}).

Flow cytometry

Fluorochrome-conjugated antibodies against CD45 (clone 30-F11), CD11b (M1/70), Gr-1 (RB6-8C5), Ly-6C (HK1.4) were purchased from eBiosciences. Antibody against Ly-6G (1A8) was

purchased from BioLegend. Antibody against Cxcr2 (242216) was purchased from R&D Systems. Primary cells from mice were isolated with the same methods as in CyTOF. To assess cell viability, cells were incubated with SYTOX Blue (Life Technologies) prior to FACS analysis. All samples were acquired with the LSRFortessa analyzer (Becton Dickinson) and analyzed with FlowJo software (Tree Star).

T cell suppression assays

Single cells were prepared from prostate tumors using the Tumor Dissociation Kit-Mouse (Miltenyi) and dead cells were removed using the Dead Cell Removal Kit (Miltenyi). MDSCs were isolated by FACS sorting. Purified MDSCs were spun down, counted, and plated into 96-well plates at an MDSC/T cell ratio of 0:1, 1:1, 1:2, 1:4, with 3.0×10^5 to 5.0×10^5 MDSCs used in each ratio. T cells were harvested from naive, wild-type littermate animals by mashing whole spleen through a 40-μm filter, followed by red blood cell lysis using ACK buffer. MACS-sorted (Miltenyi) $CD8^+$or $CD4^+$ T cells were then washed, counted, and stained with carboxyfluorescein diacetate succinimidyl ester (CFSE) (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. CFSE labeled T cells were plated onto 96-well plates in the presence of anti-CD3 (1 μg/ml, Clone 145-2C11, eBioscience) and soluble anti-CD28 (2 μg/ml, Clone 37.51, eBioscience) antibodies, with and without MDSCs, and allowed to proliferate for 72 hours. Wells without anti-CD3 and soluble anti-CD28 antibodies were used as negative controls. The suppression of T cells is calculated as described (1). The percentage of CFSE+ cells divided in the presence of MDSCs was compared to percentage of CFSE+ divided cells in the absence of any added MDSCs.

Cell culture and Lentivirus-mediated shRNA knockdown and overexpression

Pten$^{pc-/-}$*Smad4*$^{pc-/-}$ prostate cell lines have been described previously (2). All lentiviral shRNA clones targeting Yap1 and nontargeting shRNA control were obtained from Sigma Aldrich in the pLKO vector (Moffat et al., 2006) and prepared as described (3). The lentiviral constitutive active YAP1 S127A and inactive YAP1 S127A/S94A mutants have been described (3).

MDSC depletion with Gr-1 antibody

Purified neutralizing antibody against Gr-1 (clone RB6-8C5) (4) and isotype Rat IgG2b control (clone LTF-2) were purchased from BioXcell and dosed at 200 μg/mouse by intraperitoneal injection, every other day for 30 days.

MDSC depletion with peptibody

Plasmids for irrelevant control peptibody and MDSC-specific Pep-H6 peptibody were generously provided by Dr. Larry W Kwak. Plasmids used for in vivo administration were purified using EndoFree Plasmid Maxi Kit (Qiangen). 15 ug of plasmids were injected into mice through tail vein using the established protocol (5) in TransIT-EE Delivery Solution (Mirus Bio LLC) every 4 days.

Cxcr2 inhibitor *in vivo* treatment

SB225002 was obtained from Cayman Chemical (Cat# 13336) and dissolved in DMSO (10mg/ml) and diluted in vehicle (0.9%NaCl, 0.3%Tween 80) for in vivo administration. Mice are subjected to either SB225002 (5mg/kg) or vehicle treatment by daily intraperitonal injection for 14 days.

Microarray and computational analysis of mouse microarray data

Single cells from *Pten*$^{pc-/-}$*Smad4*$^{pc-/-}$*mTmG*$^{+}$ prostate were isolated by 1mg/ml collagenase digestion for 1 hour followed by DNase I treatment. Single cells were FACS-sorted for GFP^{+} and Tomato^{+} cells and RNA was purified with TRIzol (Life Technologies). RNA expression profiling was performed at the MD Anderson Microarray Core facility using the Mouse Genome 430 2.0 Array (Affymetrix) to generate a *Pten*$^{pc-/-}$*Smad4*$^{pc-/-}$ tumor/Stroma dataset. Genes with statistically significant differences between test and control conditions (*Pten*$^{pc-/-}$*Smad4*$^{pc-/-}$ tumors vs *Pten*$^{pc-/-}$ tumors or GFP+ vs. Tomato+) were generated using the following method: statistical analyses were performed on log scale data using parametric test and variances are not assumed equal (Welch t-test) with p-value cutoff 0.05. Additional fold change was applied to generate the final list of genes as indicated in the Figure legend. Gene filtering was performed by removing genes whose mean normalized test and control intensities are both less than the 20th percentile of the combined normalized signal intensities. The intensities reported below correspond to mean normalized test and control data rescaled to a median of 500. For GSEA analysis, the raw data of GSE25140 was processed and analyzed by GenePattern (6) using ExpressionFileCreator Module (version 11.14) and GSEA module (v14). For oPOSSUM (7) analysis (http://opossum.cisreg.ca/cgi-bin/oPOSSUM3/opossum_mouse_ssa), genes upregulated in the *Pten*$^{pc-/-}$*Smad4*$^{pc-/-}$ tumors as compared to *Pten*$^{pc-/-}$ tumors (≥1.5 Fold) were intersected with genes that are upregulated in GFP+ cells vs. Tomato+ cells (≥2 folds). The 70 overlapped genes were subjected to oPOSSUM Mouse Single Site Analysis.

MDSCs migration assay

Single cells were prepared from prostate tumors using the Tumor Dissociation Kit-Mouse (Miltenyi) and dead cells were removed as described above. Cells are labeled with Gr-1-FITC (Clone RB6-8C5, Biolegend) conjugated antibody and Sytox red for cell viability. FITC positive cells were sorted using BD FACSAria™ Fusion (BD Biosciences). $0.5x10^6$ of FACS sorted cells were placed on the upper chamber of a transwell system (6 μm, BD Falcon) and conditioned media (CM) with vehicle, CM with anti-Cxcl5 neutralizing antibody (10 mg/ml final concentration), CM with anti-Cxcr2 neutralizing antibody (10 mg/ml final concentration), or Cxcr2 specific inhibitor SB225002 (400 nM final concentration), CM from Pten/Smad4-deficient cells infected with control shRNA or shRNA for Yap1, CM from Pten/Smad4-deficient cells treated with vehicle or 1 μM Verteporfin (VP) were added to the bottom chamber of a 24-well plate. For Cxcr2 neutralizing antibody and Cxcr2 inhibitor SB225002 treatment, cells were pre-treated with 10 μg/ml antibody or 400 nM inhibitor before added to the upper chamber. Cells were allowed to migrate to the bottom well for 6 hours at 37 °C, 5% CO_2. Migrated cells were then analyzed by flow cytometry using BD Fortessa X20. Migrated FITC positive cells were gated to count the absolute number of cells migrated through the transwell.

Mouse histopathological analysis

The dorsolateral, anterior and ventral prostate from control, anti-Gr1 neutralizing antibody treatment, peptibody treatment, and SB225002 treatment groups were examined histologically and scored qualitatively for the following criterion: most advanced neoplastic classification, neoplastic classification of the predominant portion of the slide, stromal reaction and inflammation. Diagnoses were based on the Bar Harbor classification of mouse prostate pathology. Fibrosis and inflammation were graded qualitatively on a scale from 0 - 4.

Evaluation of histopathologic slides from the 3 components of the mouse prostate from animals either control or treated with Gr1 antibody, peptibody, and Cxcr2 inhibitor was performed.

Inducible Yap1 knockdown

Inducible Yap1 knockdown was constructed by cloning the two Yap1 shRNA used previously (3) from the pLKO.1 into a doxycycline inducible plasmid. Lentivirus was packaged in 293T and was used to infect PPS, a C57BL/6-syngeneic cell line isolated from prostate tumor of *Pten*$^{pc-/-}$ *Smad4*$^{pc-/-}$ *Tp53*$^{pc-/-}$mice. Stable sublines were selected with puromycine (2 μg/mL) and injected subcutaneously to the flank of 5-week old male C57BL/6 mice (Jackson Laboratory). Two weeks post-injection, mice were fed with doxycycline water (2 g/L), a method used to execute doxycycline inducible expression *in vivo* (8). Tumors were measured and extracted 6 days later to analyze for MDSC% in infiltrating immune cells.

TCGA analysis for MDSCs signature, YAP1 signature, and CXCL6 expression

Two different resources are collected to generate a short list of genes as putative signature genes for human MDSCs. The first resource is from a landmark review article about MDSCs (9). From Box 1 of this paper, 24 phenotypic markers of human MDSCs are adopted into our signature. The second resource is from automated literature mining. From PubMed database, we searched genes cited in literature related to three keywords: immunoscore, immune gene signature, and myeloid derived suppressor cell. After removing overlapping genes from the first method, 16 genes are left that were cited by at least two of the three keyword. We finally selected 39 MDSC genes (Supplementary Table S7). We clustered the 498 TCGA prostate samples or the prostate tumor samples from Wallace et al (10) (GSE6956) using the gene expression data of 39 MDSC

genes and identified subtypes: MDSC-high, MDSC-low, and MDSC-medium. We then compared the whole transcriptome profiles between MDSC-high and MDSC-low samples to identify differentially expressed genes. Gene set enrichment analysis (GSEA; Subramanian et al., 2005) was then used to identify significantly de-regulated pathways and biological processes between the two sample groups. The two YAP1 signatures were derived from two publications (11, 12). The expression of CXCL6 in MDSCs-high samples is compared to MDSCs-low samples using wilcoxon test.

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SUPPLEMENTARY FIGURE LEGENDS

Figure S1. CyTOF analysis of biological samples from *Pten*$^{pc-/-}$*Smad4*$^{pc-/-}$ mice (Related to Figure 2). **(A)** SPADE trees colored by the median intensity of individual markers. **(B)** CyTOF analysis of spleen and draining lymph node from 5, 8, and 14 weeks old *Pten*$^{pc-/-}$*Smad4*$^{pc-/-}$ mice and control 16 weeks old wild type mice (n=3). Percentages of various immune cell populations within CD45^{+} leukocytes are shown. Data were collected with CyTOF and analyzed with Flowjo. **(C)** Gating strategy of immune cell populations from CyTOF data. Splenocytes and prostate cells (containing both tumor and non-tumor cells) are shown as representatives. Blood and draining lymph nodes cells were gated following the same strategy.

Figure S2. Strategy used for MDSCs Isolation (Related to Figure 3). MDSCs were isolated through FACS of CD11b+Gr1+ cells from the tumor.

Figure S3. (A) Treatment scheme for Gr-1 antibody, peptibody, and Cxcr2 inhibitor SB225002. **(B)** Gr-1 antibody significantly reduced MDSCs in peripheral blood 2 days after 1 dose of injection, measured by Ly-6G and Ly-6C using flow cytometry (n=3). **(C)** Gr-1 antibody significantly reduced MDSCs in spleen and bone marrow (n=4). **(D)** Weight of anterior prostates (AP) (n=4) treated with IgG or Gr-1 antibody. No difference was observed due to excessive fluid accumulation in the Gr1 treated AP. In B, D ***$P<0.001$.

Figure S4. **(A)** IHC staining of Ki67, CD45, Vimentin, Smooth muscle actin (SMA) and Trichrome staining of mouse prostate tissues treated with IgG control or Gr1 antibody. Quantification was performed using ImageJ. **(B)** Pep-H6 peptibody treatment significantly

reduced MDSCs in peripheral blood 3 days after 1 dose of injection measured by flow cytometry (n=3). *$P<0.05$, **$P<0.01$.

Figure S5. (A) The top 10 differentially expressed genes in *Pten*$^{pc-/-}$*Smad4*$^{pc-/-}$ tumors as compared to *Pten*$^{pc-/-}$ tumors, identified by microarray analysis (n=5). **(B)** Prostate tumor enriches for CD11b+ Gr-1+ cells with higher Cxcr2 expression. From Pten/Smad4 mice with established prostate tumor, tissues including bone marrow, blood, spleen and prostate were harvested and stained with CD45, CD11b, Gr-1 and Cxcr2. From CD45+ cells in each tissue, both CD11b+ Gr-1+ cells and CD11b- Gr-1- cells were examined for Cxcr2 expression level. Cxcr2 expression is segmented to three levels: negative, positive and high, and CD11b+ Gr-1+ cells in each tissue are plotted for the three levels in pie charts (n=3). **(C-D)** SB225002 decreased MDSCs infiltration in the *Pten*$^{pc-/-}$*Smad4*$^{pc-/-}$ tumors as shown by flow cytometry analysis (n=4). **(E)** Weight of anterior prostates (AP) from mice treated with vehicle or Cxcr2 inhibitor SB225002 (n=4).

Figure S6. (A) Top 10 activated oncogenic signatures identified by GSEA analysis in *Pten*$^{pc-/-}$ *Smad4*$^{pc-/-}$ tumors as compared to *Pten*$^{pc-/-}$ tumors (n=5). **(B-C)** oPOSSUM analysis identified TEAD motifs as overrepresented in the upregulated tumor-specific genes in *Pten*$^{pc-/-}$*Smad4*$^{pc-/-}$ tumors as compared to *Pten*$^{pc-/-}$ tumors (n=5). **(D)** Putative Yap1/TEAD binding sites in the Cxcl5 promoter.

Figure S7. (A) Clustering of primary prostate tumors from Wallace et al into MDSC-high and MDSC-low subtypes. **(B)** GSEA analysis identified YAP1 signatures in MDSC-high as compared to MDSC-low samples.

Table S1. Markers used in CyTOF analysis.

Table S2: Detailed pathology description of the Gr1 treated mice and Cxcr2 inhibitor treated mice.

Table S3. Genes upregulated in *Pten*$^{pc-/-}$*Smad4*$^{pc-/-}$ tumors as compared to *Pten*$^{pc-/-}$ tumors (≥2 fold).

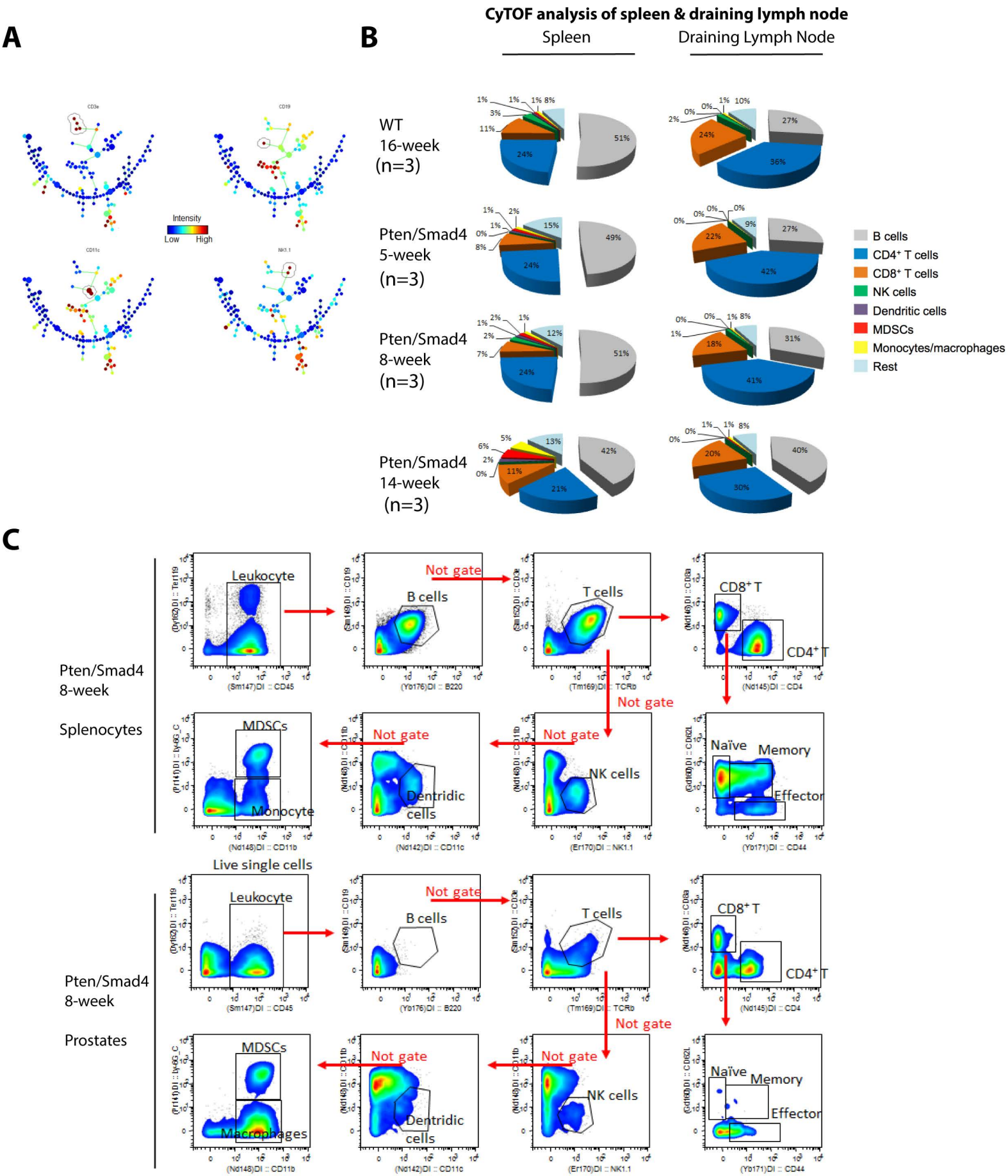
Table S4. Genes upregulated in GFP+ tumors cells from *Pten*$^{pc-/-}$*Smad4*$^{pc-/-}$ mice as compared to Tomato+ cells (≥4 fold)

Table S5. Overlapped genes between Genes upregulated in *Pten*$^{pc-/-}$*Smad4*$^{pc-/-}$ tumors as compared to *Pten*$^{pc-/-}$ tumors (≥2 fold) and genes upregulated in GFP+ tumors cells from *Pten*$^{pc-/-}$ *Smad4*$^{pc-/-}$ mice as compared to Tomato+ cells (≥4 fold).

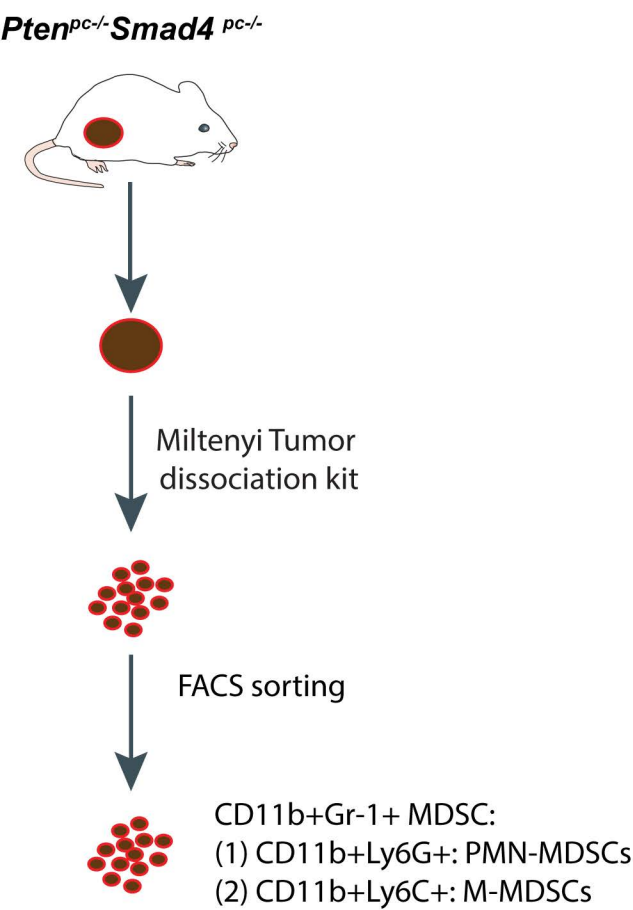
Table S6. Detailed information for the YAP1 IHC staining in human prostate cancers.

Table S7. The 39-gene signatures curated from literatures.

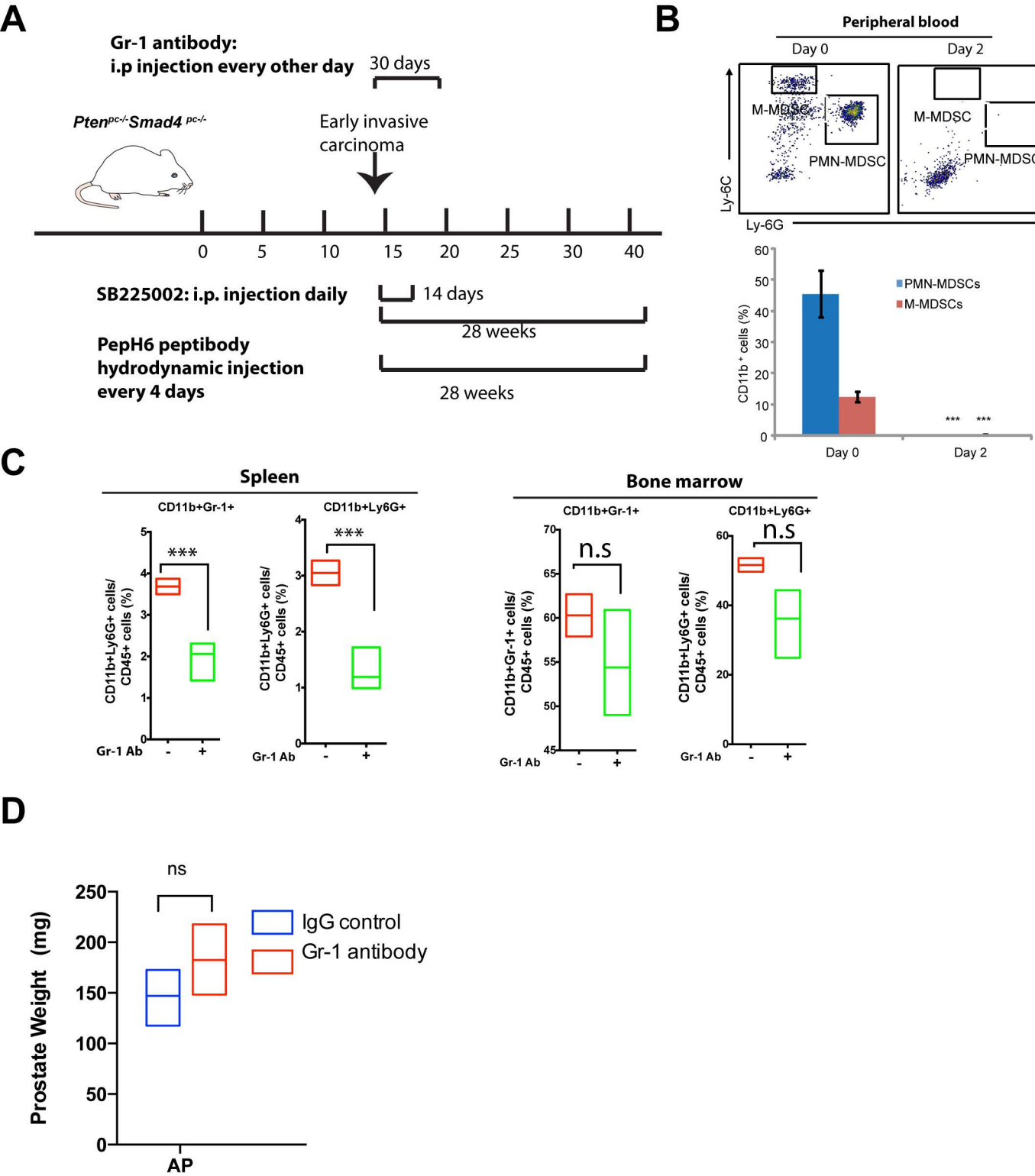
Supplementary Fig. S1



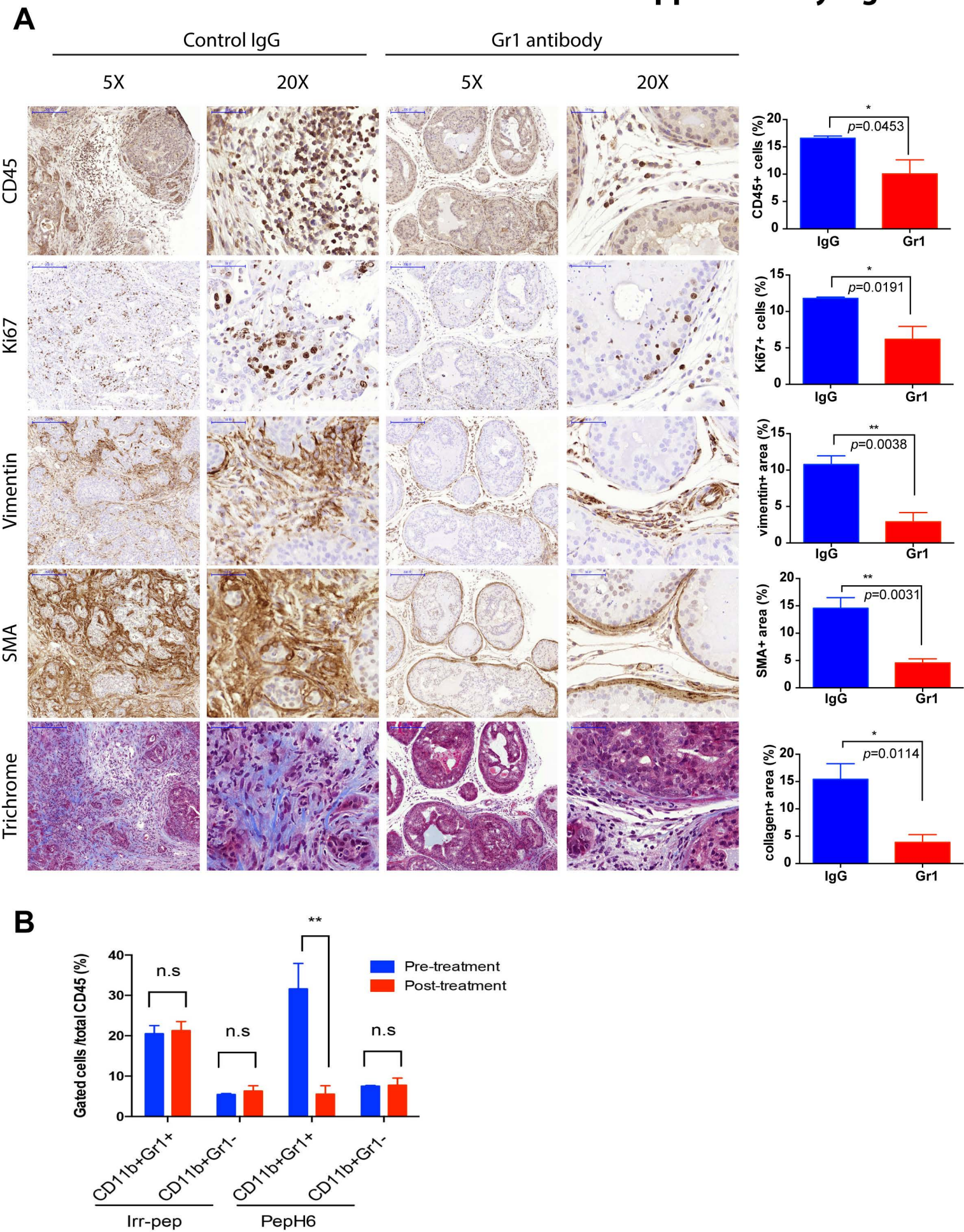
Supplementary Fig. S2



Supplementary Fig. S3



Supplementary Fig. S4

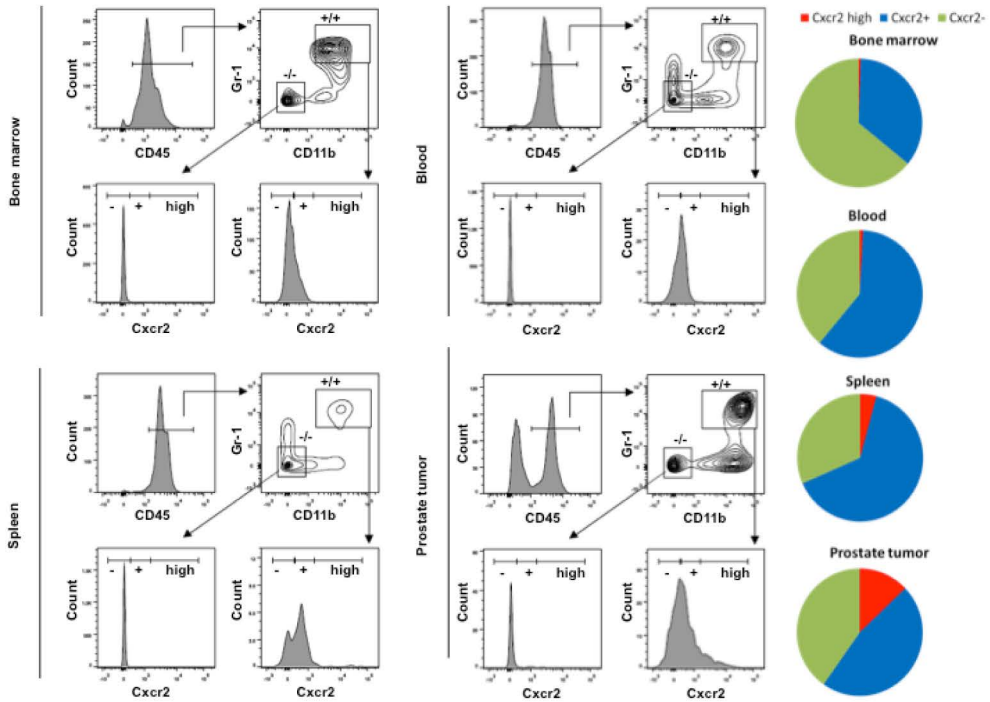


Supplementary Fig. S5

A

Symbol	Fold Change	P-Value	Rank	Up/down
Msmb	50.6	3.8E-5	1	down
Ttr	49.4	6.4E-5	2	down
A630095E13Rik	21.4	0.0246	3	down
Tgm4	14.1	0.0141	4	down
Krt17	12.3	0.004	5	up
Psca	12.1	0.0006	6	down
Cxcl5	**12**	**0.0004**	**7**	**up**
Pbsn	11.8	0.0341	8	down
Krt6a	11.6	0.0025	9	up
Reg3b	11.3	0.001	10	down

B



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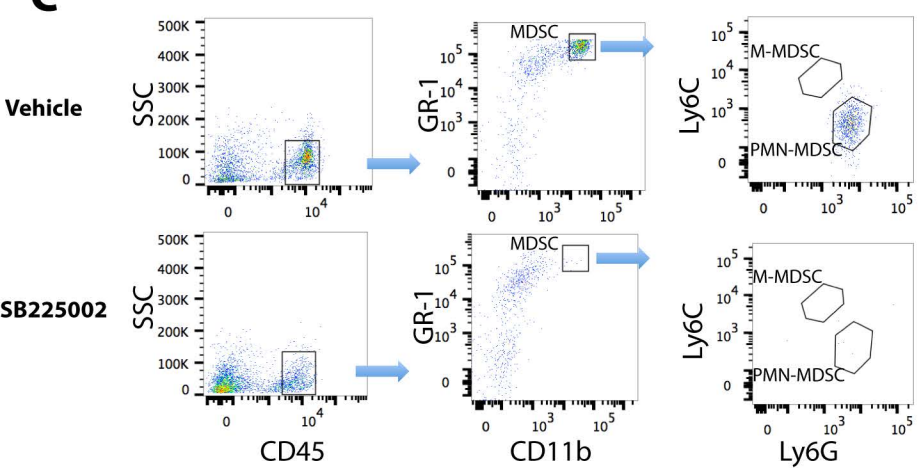

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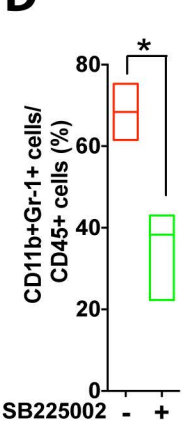

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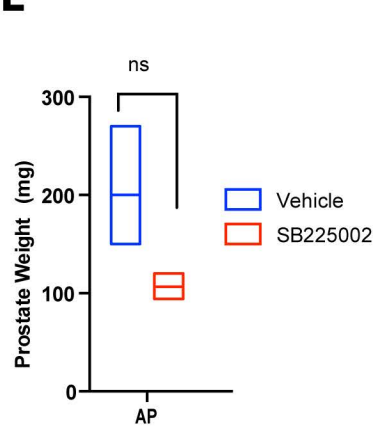
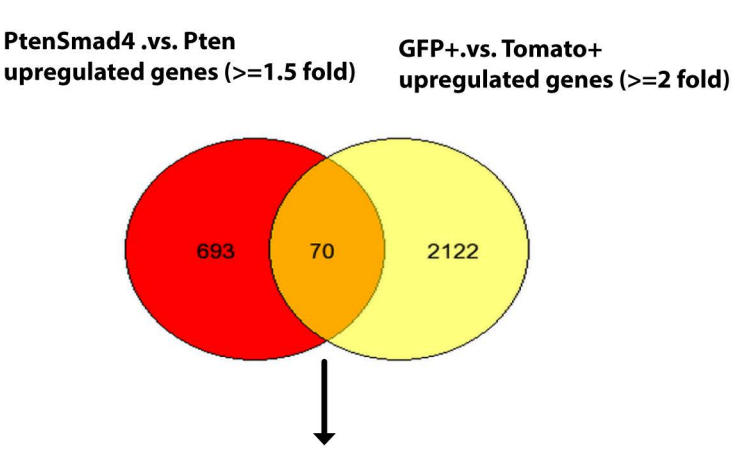


Supplementary Fig. 6

A

	Gene Signature	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX	LEADING EDGE
1	P53_DN.V2_UP	107	-0.51	-1.92	0.000	0.026	0.014	3074	tags=36%, list=14%, signal=42%
2	CORDENONSI_YAP _CONSERVED_SIGNATURE	52	-0.60	-1.83	0.020	0.047	0.035	3346	tags=56%, list=15%, signal=66%
3	RB_P107_DN.V1_UP	122	-0.59	-1.81	0.036	0.034	0.035	3321	tags=51%, list=15%, signal=60%
4	LEF1_UP.V1_UP	157	-0.45	-1.79	0.000	0.034	0.044	3086	tags=32%, list=14%, signal=38%
5	RPS14_DN.V1_UP	154	-0.49	-1.79	0.002	0.029	0.046	1685	tags=29%, list=8%, signal=31%
6	KRAS.LUNG .BREAST_UP.V1_UP	104	-0.45	-1.76	0.000	0.035	0.074	1718	tags=25%, list=8%, signal=27%
7	BMI1_DN.V1_UP	132	-0.49	-1.75	0.019	0.032	0.077	2272	tags=30%, list=10%, signal=34%
8	CSR_LATE_UP.V1_UP	135	-0.48	-1.74	0.020	0.033	0.081	3092	tags=31%, list=14%, signal=36%
9	MI1_DN_MEL18 _DN.V1_UP	121	-0.49	-1.73	0.002	0.033	0.095	1951	tags=30%, list=9%, signal=32%
10	VEGF_A_UP.V1_DN	161	-0.42	-1.72	0.000	0.034	0.103	3445	tags=32%, list=16%, signal=37%

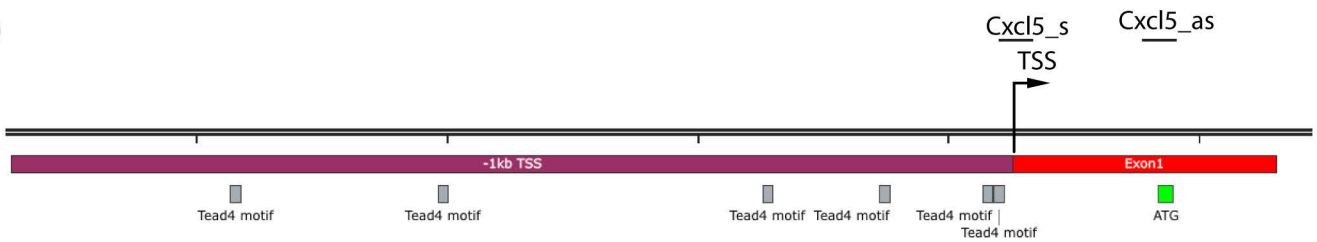
B



C

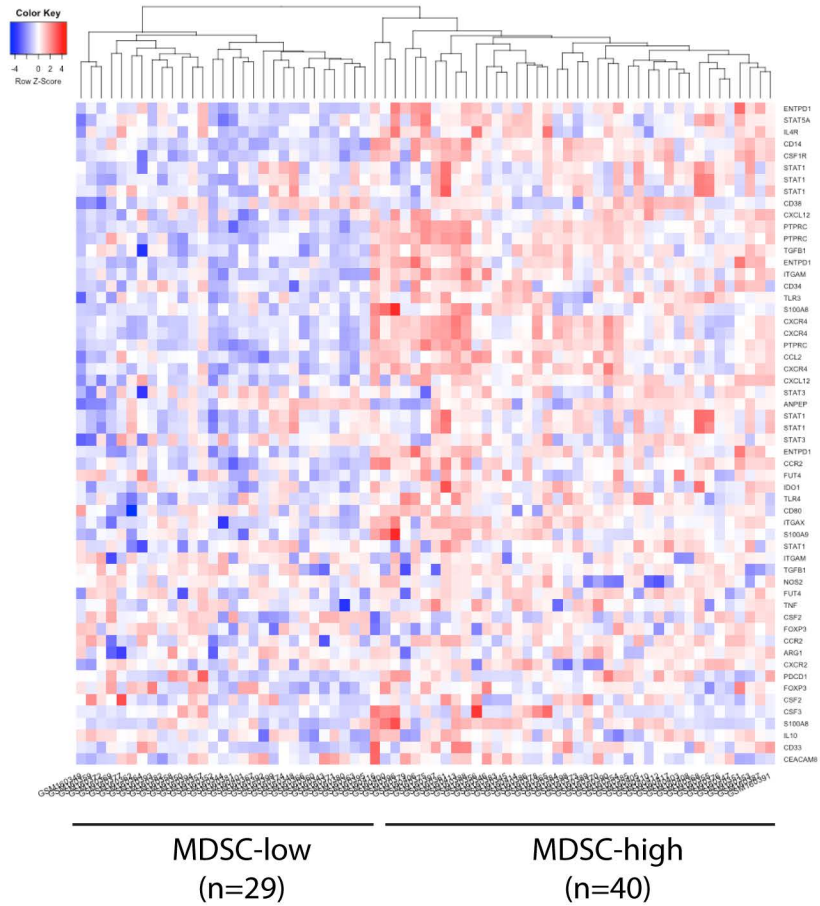
TF	JASPAR ID	Target gene hits	Target gene non-hits	Background gene hits	Background gene non-hits	Target TFBS hits	Target TFBS nucleotide rate	Background TFBS hits	Background TFBS nucleotide rate	Z-score	Fisher score
SRF	MA0083.1	5	64	16530	28340	9	0.00134	1077	0.000334	15.498	2.421
TEAD1	MA0090.1	24	45	6218	23129	36	0.00534	9111	0.00283	13.462	5.044
Pax4	MA0068.1	1	68	94	29253	1	0.000371	94	7.29e-05	9.729	1.608
ELF5	MA0136.1	49	20	21087	8260	316	0.0352	128774	0.0299	8.726	0.477
PPARG	MA0066.1	1	68	84	29263	1	0.000247	84	4.34e-05	8.537	1.708
FEV	MA0156.1	53	16	19307	10040	225	0.0223	88519	0.0183	8.409	3.413
Klf4	MA0039.2	44	25	16530	12817	220	0.0272	88626	0.0229	8.193	2.038
AP1	MA0099.2	54	15	21078	8269	316	0.0274	128539	0.0233	7.754	1.928
Myc	MA0147.1	22	47	9803	19544	57	0.00705	20133	0.0052	7.285	0.432
Nobox	MA0125.1	49	20	17499	11848	189	0.0187	75342	0.0156	7.176	3.386

D



Supplementary Fig. 7

A



B