Supplementary Figures

Fig. S1

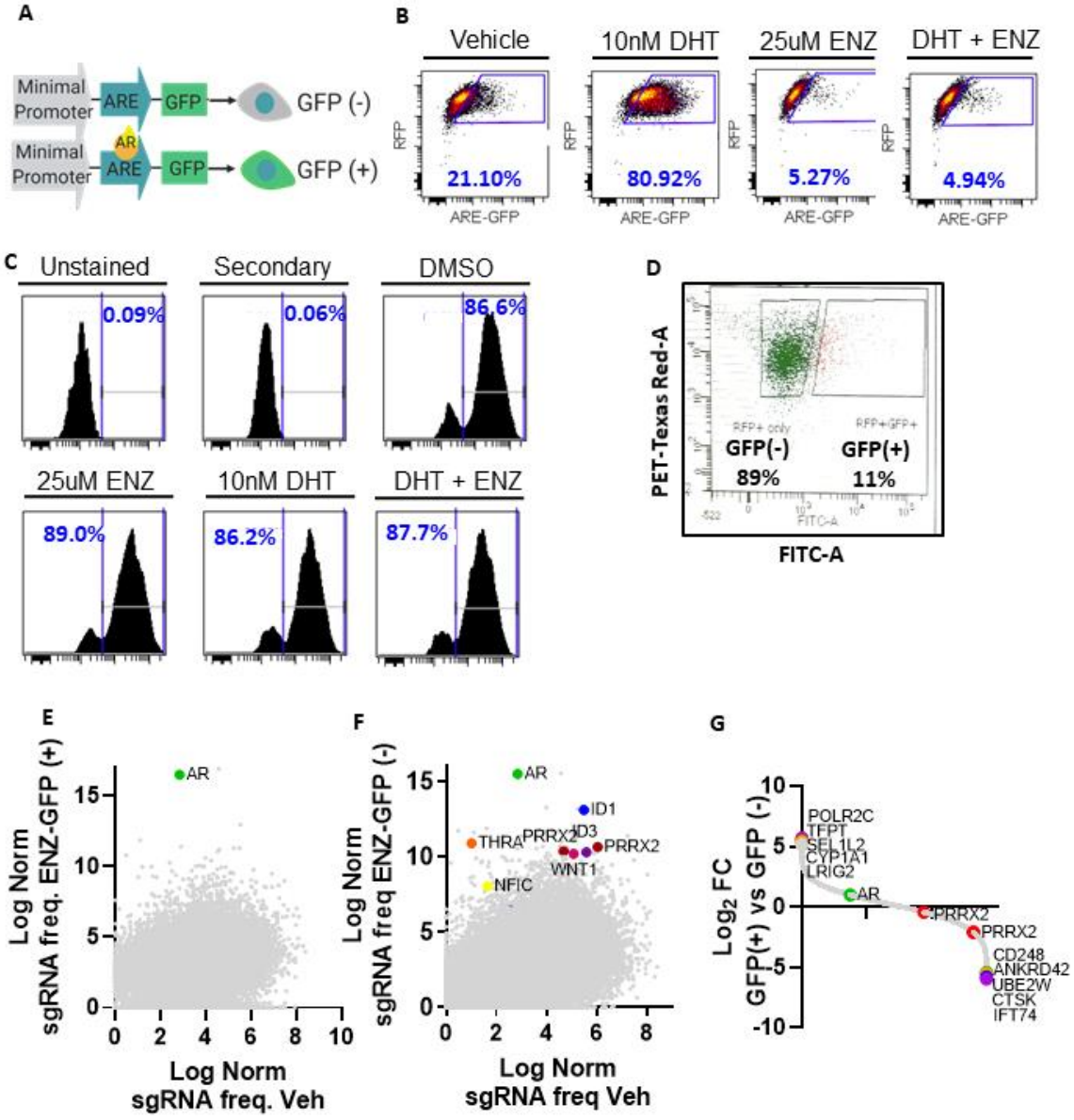

Fig. S1. CRISPRa reporter cell line validation and sgRNA scatter plots. **A)** AR activity reporter scheme. **B)** Characterization of the reporter cell line by FACS. Cells were treated for 48h. Percentage of GFP(+) cells after different treatments shown. **C)** Cells were treated for 48h. dCas9 protein expression measured by FACS. Percentage of positive cells shown. **D)** FACS sorting of the ENZ treated population at 7.5 weeks. **E, F)** Scatter plot of sgRNA count distribution. **G)** Ranked-order plot of the Log_2 fold change (FC) of GFP (+) vs GFP (-) cells sgRNA counts.

Fig. S2

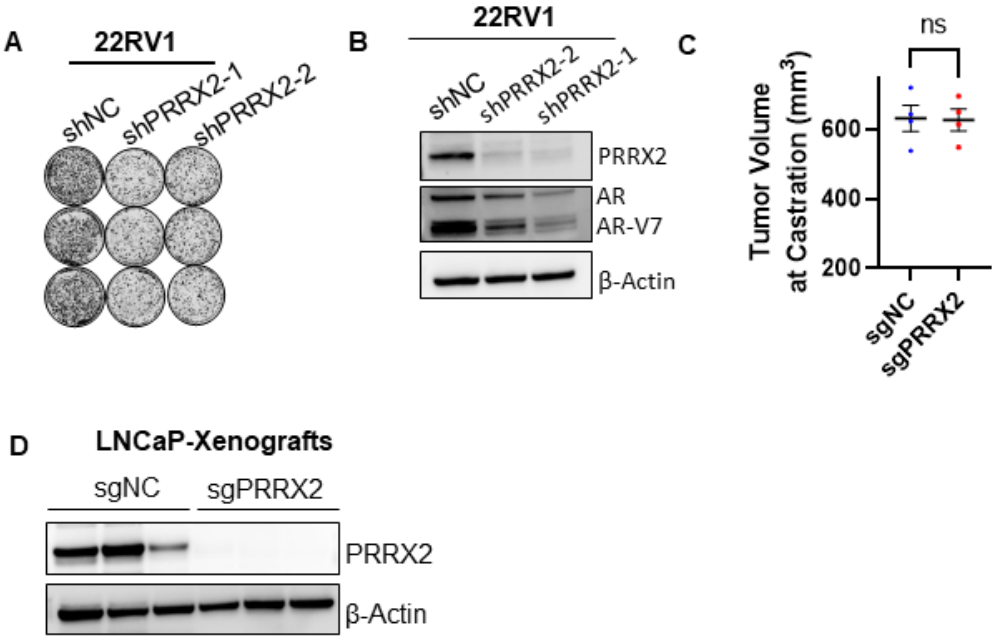

Fig. S2. PRRX2 mediates ENZ resistance in vitro and in vivo. **A)** Colony formation assay of 22RV1 cells after PRRX2 knock-down using two different shRNAs. **B)** Western blot of 22RV1 cells after PRRX2 knock-down. **C)** Tumor volume at castration. Mean with S.E.M shown. Unpaired t-test used for statistics. **D)** Western Blot of LNCaP-sgNC or LNCaP-sgPRRX2 xenografts.

Fig. S3

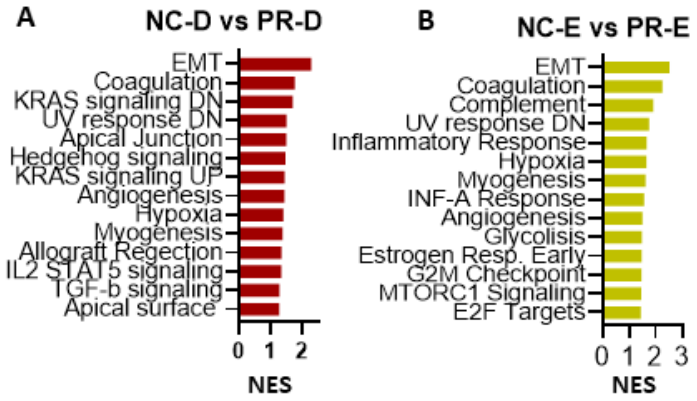

Fig. S3. GSEA pathway analysis of LNCaP-sgNC and LNCaP-sgPRRX2 cells. A) Top pathways enriched in LNCaP-sgPRRX2 cells in DMSO conditions compared to sgNC cells. **B)** Top pathways enriched in LNCaP-sgPRRX2 compared to LNCaP-sgNC cells after ENZ treatment. GSEA analysis used for enrichment in A,B. NES = Normalized enrichment score. FDR < 0.1

Fig.S4

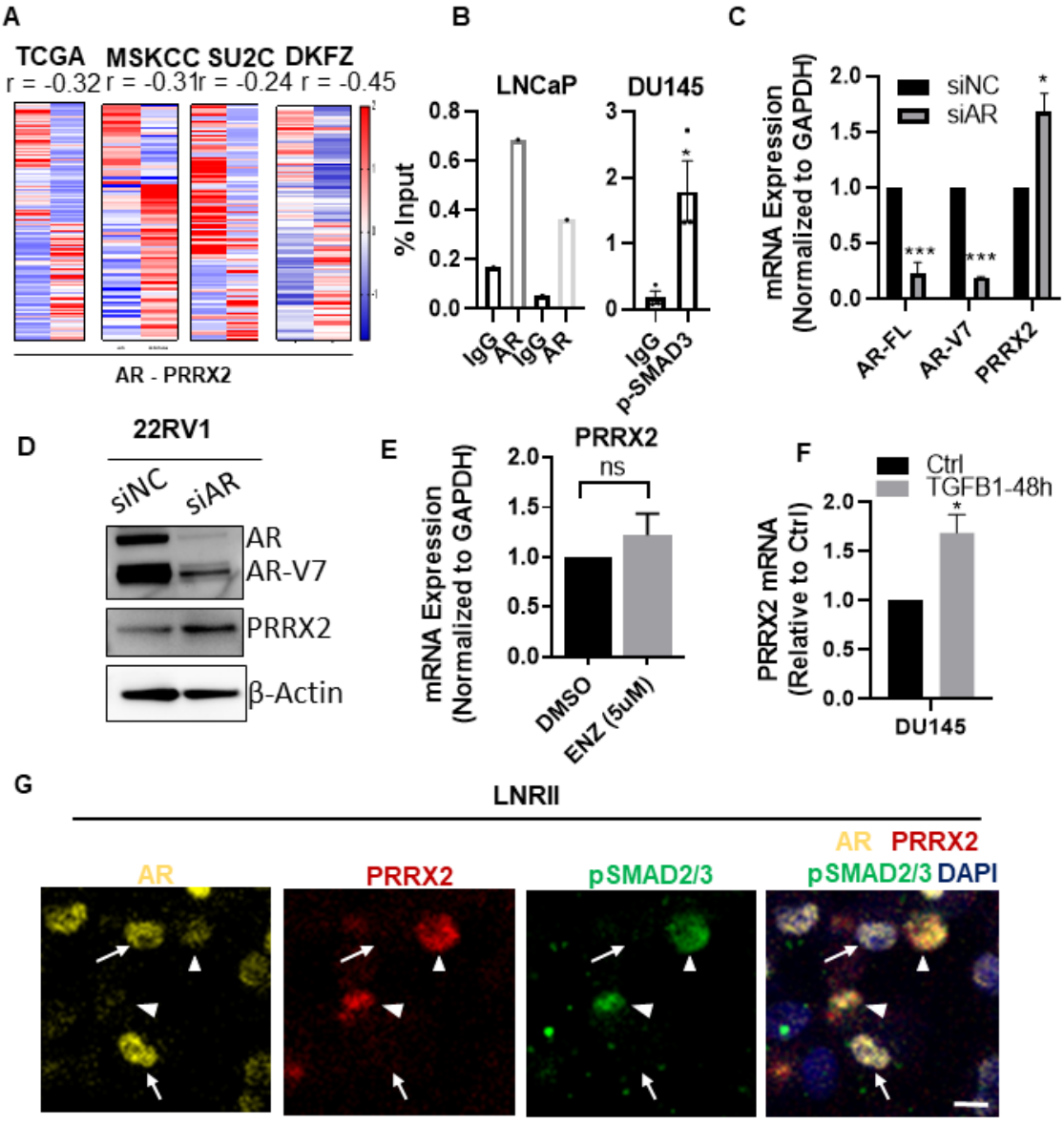

Fig. S4. AR and the TGF-β pathway co-regulate PRRX2 expression. A) Spearman correlation r between AR and PRRX2 mRNA expression. Data obtained from cBioPortal from datasets mention in figure. **B)** ChIP-qPCR experiment of LNCaP cells treated with DHT (10nM) during 3h and DU145 stimulated with TGF-β1 treatment (5ng/ml) during 48h. **C)** qRT-PCR of 22RV1 cells

after AR/ARV7 Knock-down using siRNA. Experiment performed in 3 independent replicates. Error bars represent S.E.M. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. **D)** Western blot of 22RV1 cells after AR/ARV7 Knock-down using siRNA. **E)** qRT-PCR of LNCaP cells treated with ENZ(5µM) during 2 days. Data represents 3 independent biological replicates. **F)** PRRX2 expression measured by qRT-PCR in DU145 cells after TGF-β1 treatment (5ng/ml) during 48h. **G)** Immunofluorescence staining of LNRII cells after stimulation with TGF-β1 (5ng/ml) during 48h. Arrows pointing at AR-high cells, arrow heads pointing at AR-low cells. (Scale bar 20µm)**.** ChIP experiments were performed in n=2 for AR binding and n=3 for p-SMAD3 binding. Statistics: unpaired t-test. Error bars represent S.E.M. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

Fig. S5

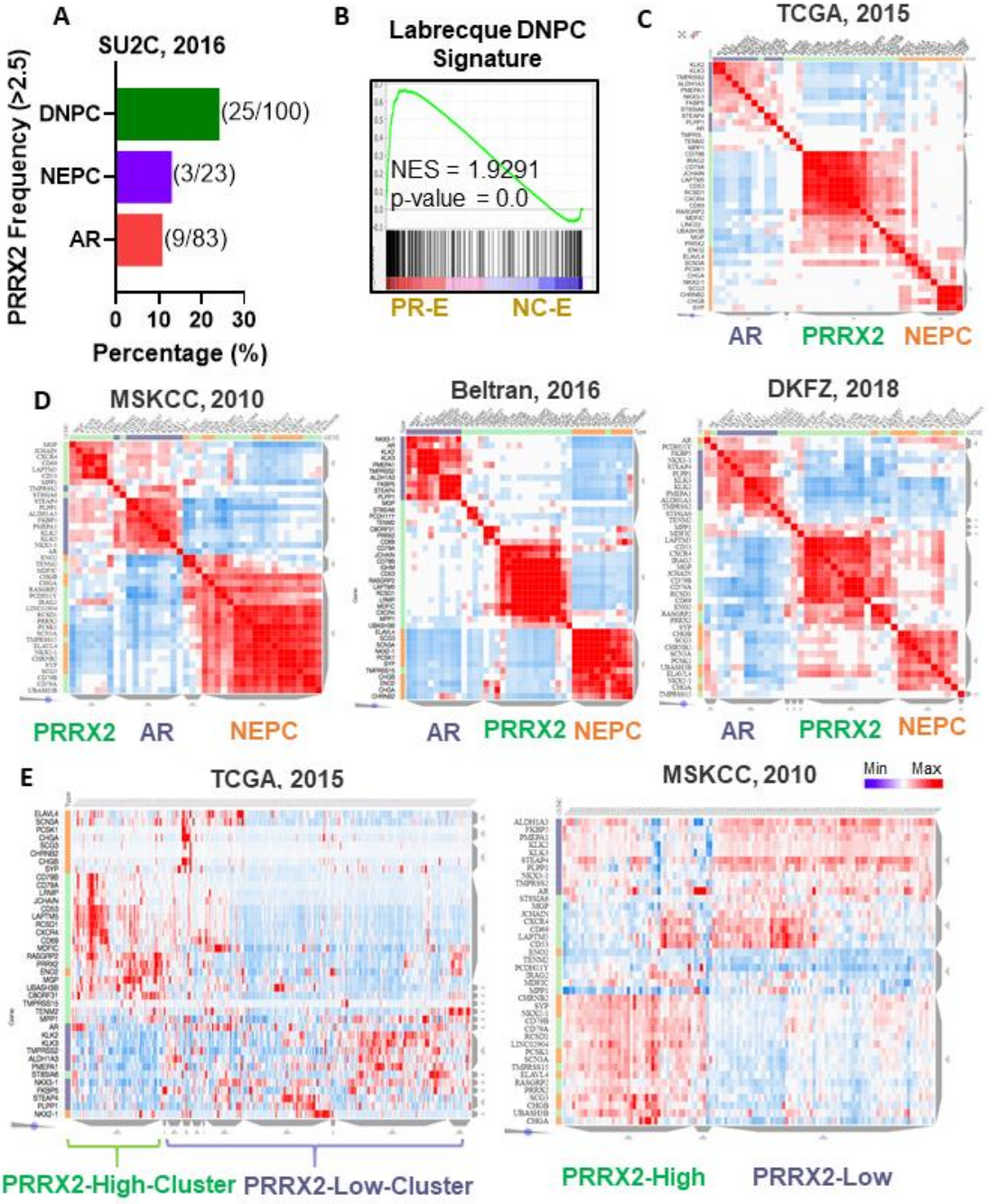
A
SU2C, 2016
PRRX2 Frequency (>2.5)
DNPC
NEPC
AR
(25/100)
(3/23)
(9/83)
0 10 20 30
Percentage (%)
B
Labrecque DNPC Signature
NES = 1.9291
p-value = 0.0
PR-E
NC-E
C
TCGA, 2015
AR
PRRX2
NEPC
D
MSKCC, 2010
PRRX2 AR NEPC
Beltran, 2016
AR PRRX2 NEPC
DKFZ, 2018
AR PRRX2 NEPC
E
TCGA, 2015
PRRX2-High-Cluster
PRRX2-Low-Cluster
MSKCC, 2010
Min
Max
PRRX2-High
PRRX2-Low

Fig. S5 (Continued)

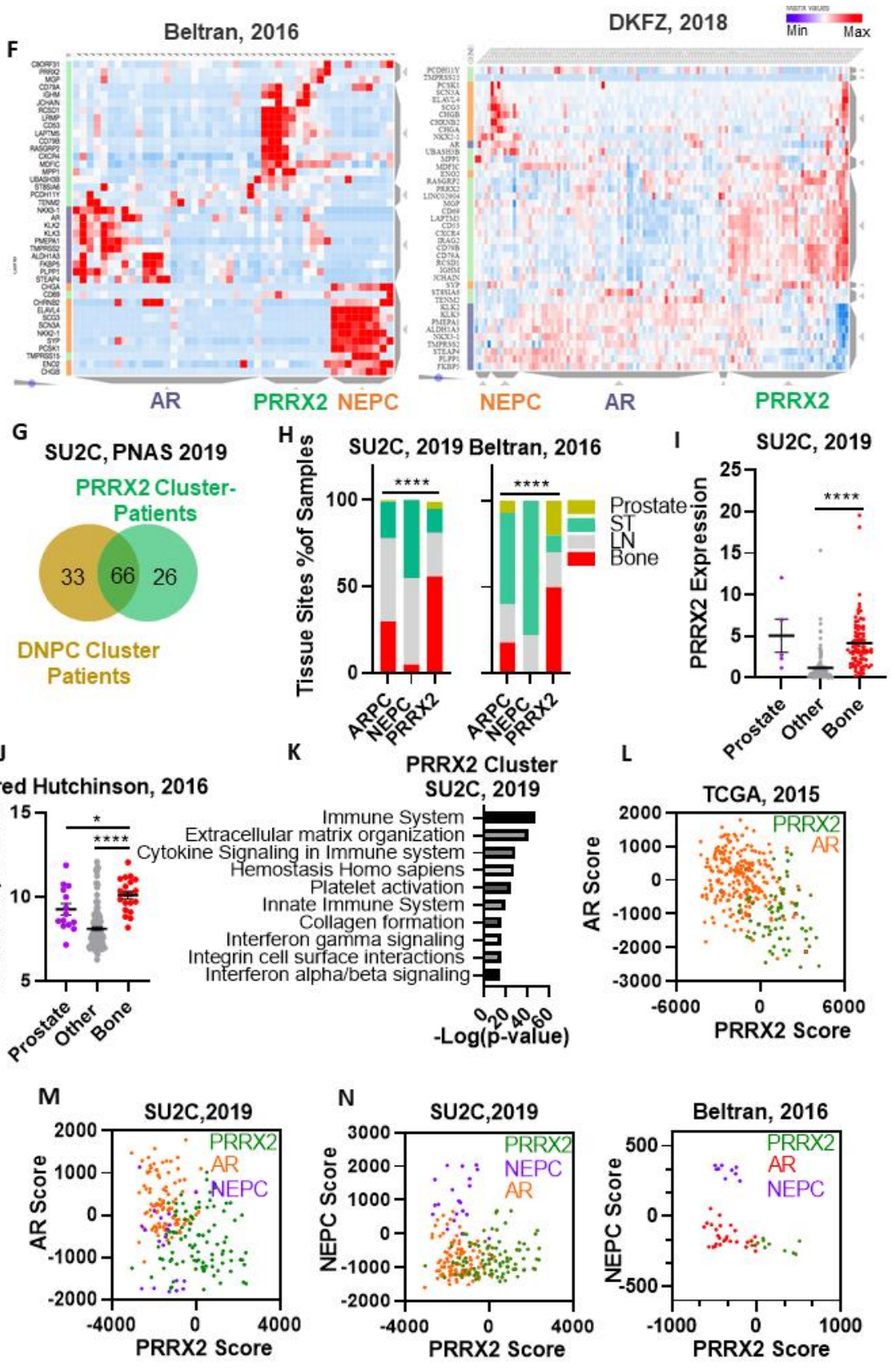

Fig. S5 (Continued)

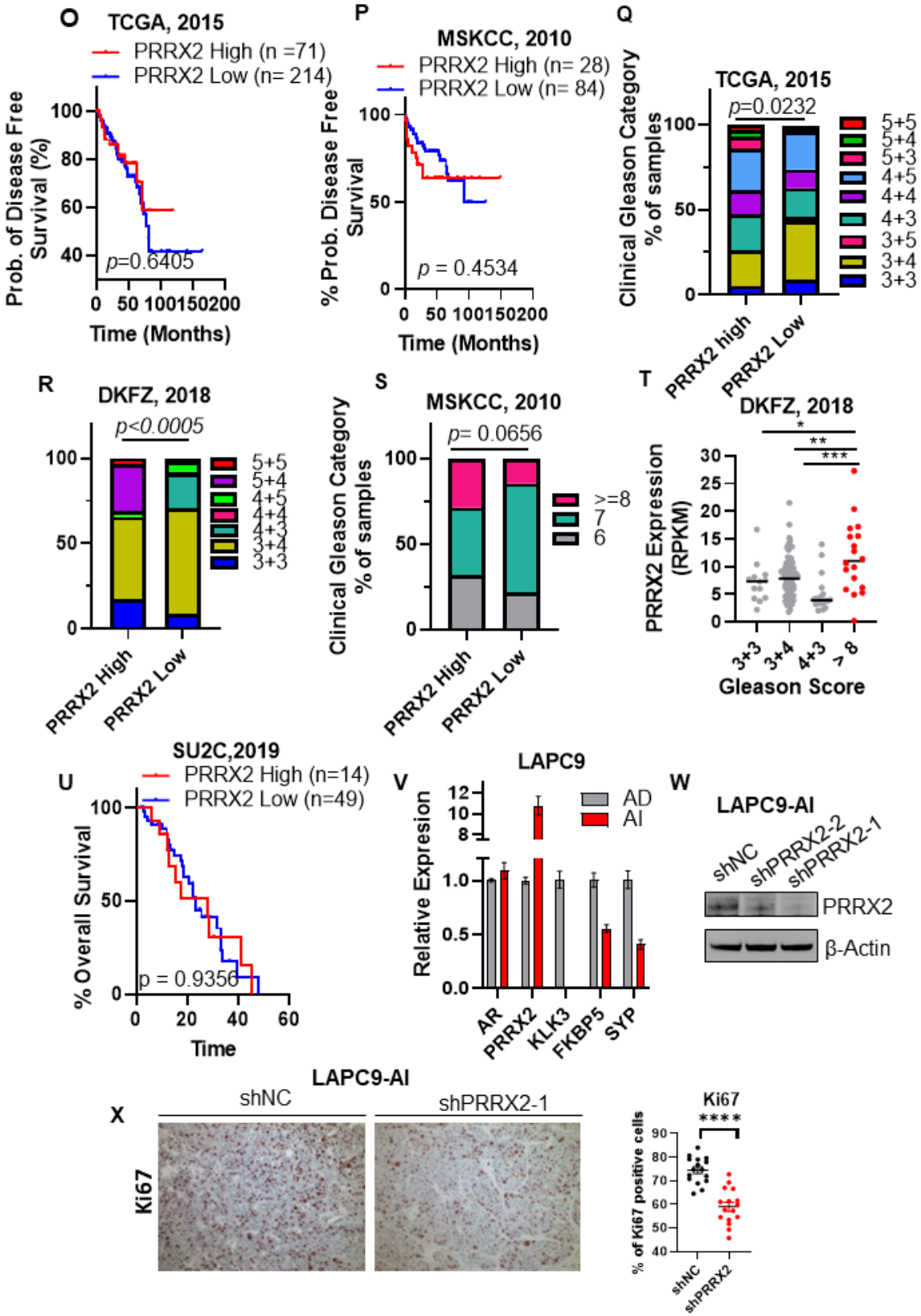

Fig. S5. PRRX2 signature stratifies PC patients within the DNPC cluster and is an oncogene in DNPC. A) PRRX2 expression frequency >2.5 FPKM Log_2. **B)** GSEA analysis comparing LNCaP-sgPRRX2-ENZ versus LNCaP-sgNC-ENZ cells with the Labreque, 2019, DNPC signature. **C, D.** Similarity Matrix of expression of AR, NEPC and PRRX2 target genes using different datasets. **E, F)** Hierarchical clustering of patients within different datasets using AR, NEPC and PRRX2 signature genes. **G)** Venn diagram showing the overlap between PRRX2 cluster patients and DNPC patients from the SU2C, 2019 dataset. **H)** Percentage of samples in different metastatic sites distributed within different clusters. **I, J)** PRRX2 mRNA expression in different tissues. **K)** Top 10 significant ($q < 0.05$) over-represented pathways in the PRRX2 patient cluster within the SU2C dataset**. L, M, N)** Scatterplot of AR, PRRX2 and NEPC scores. Colors represent the cluster to which the patient belongs. **O, P)** Probability of disease free survival. PRRX2 high represents the 25^{th} percentile of patients. TCGA, 2015 and MSKCC datasets, respectively. **Q, R, S)** Percentage of patients with different Clinical Gleason category in within PRRX2 score high (25^{th} percentile) vs low (rest of the patients) groups. Chi-square test used to calculate p-value. **T)** PRRX2 expression in patients with different Gleason Scores. DKFZ data obtained from cBioPortal. **U)** Probability of overall survival. PRRX2 high represents the 25^{th} percentile of patients. SU2C, 2019 dataset used **V)** Gene expression analysis using RT-qPCR in LAPC9-AD versus LAPC9-AI cells. **W)** Western blot of LAPC9-AI organoids after shPRRX2 knockdown. **X)** Immunostaining **(**IHC) images of Ki67 of LAPC9-AI-shNC and LAPC9-AI-shPRRX2-1 tumors. Quantification of data from B. Four tumors were stained per condition and 3 images/slide were analyzed. Statistical tests: Unpaired two tail t-test for IHC. Statistics: Log-rank test used for survival analysis in O,P. Chi-square exact test used in H,Q,R,S. Unpaired t-test used in I,J,T. Error bars represent S.E.M. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

Fig. S6

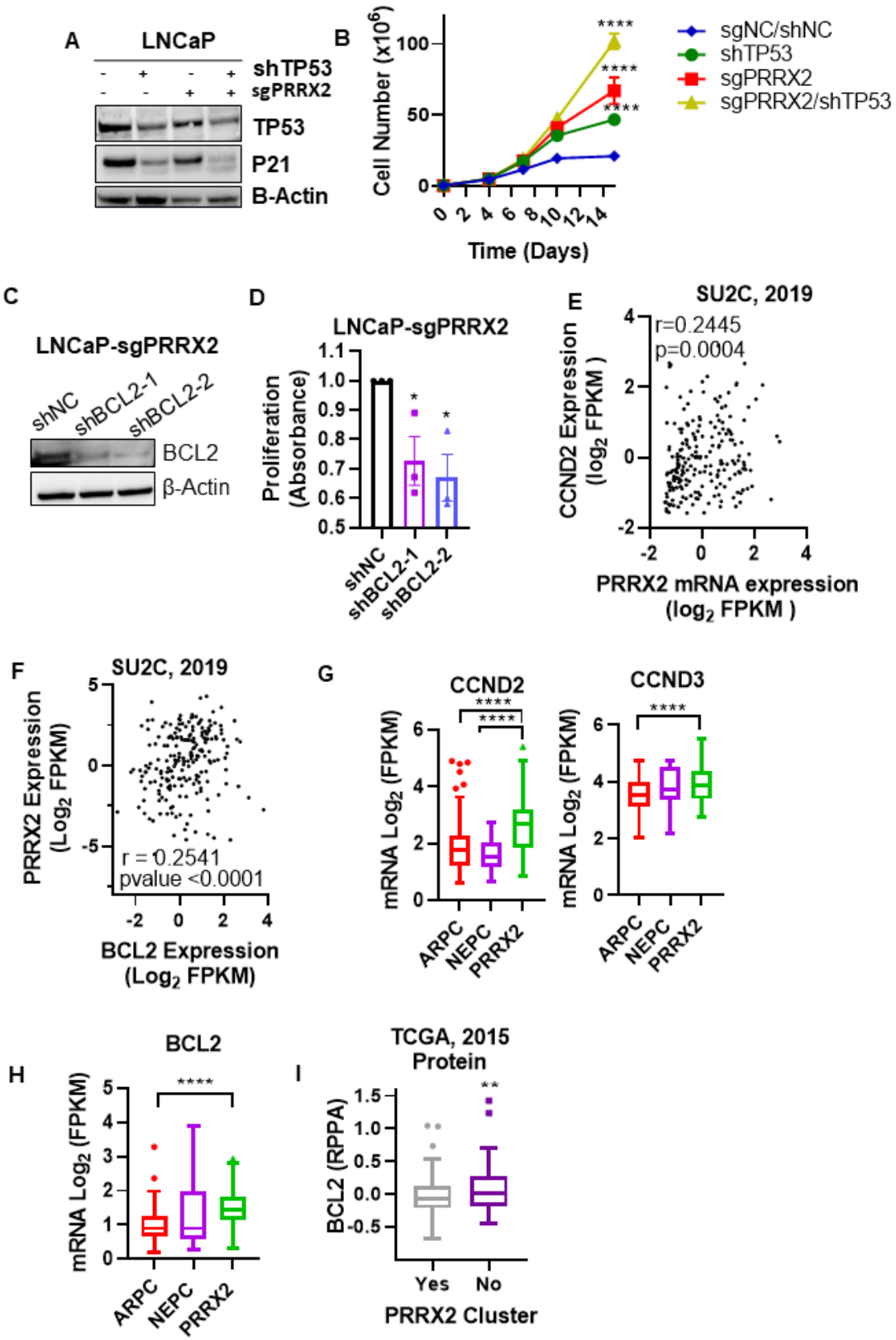
A
LNCaP
shTP53
sgPRRX2
TP53
P21
B-Actin
B
Cell Number (x10^6)
Time (Days)
sgNC/shNC
shTP53
sgPRRX2
sgPRRX2/shTP53
C
LNCaP-sgPRRX2
shNC
shBCL2-1
shBCL2-2
BCL2
β-Actin
D
LNCaP-sgPRRX2
Proliferation (Absorbance)
shNC
shBCL2-1
shBCL2-2
E
SU2C, 2019
r=0.2445
p=0.0004
CCND2 Expression (log2 FPKM)
PRRX2 mRNA expression (log2 FPKM)
F
SU2C, 2019
PRRX2 Expression (Log2 FPKM)
r = 0.2541
pvalue <0.0001
BCL2 Expression (Log2 FPKM)
G
CCND2
CCND3
mRNA Log2 (FPKM)
ARPC
NEPC
PRRX2
H
BCL2
mRNA Log2 (FPKM)
ARPC
NEPC
PRRX2
I
TCGA, 2015
Protein
BCL2 (RPPA)
Yes
No
PRRX2 Cluster

Fig. S6. Cell cycle and BCL2 pathway genes correlate with PRRX2 expression. A) Western blot of LNCaP-sgNC, LNCaP-sgPRRX2, LNCaP-shTP53 and LNCaP-sgPRRX2/shTP53 cells. B-actin used as loading control. **B)** Cell proliferation measured by manual cell count of cells treated with ENZ (5μM) in CSS media during 4,7,10 and 15 days. **C)** Western blot for LNCaP-sgPRRX2 cells after BCL2 knockdown. **D)** Proliferation assay of LNCaP-sgPRRX2 cells after BCL2 knockdown. Cells were treated with ENZ (5μM) during 3 days. **E, F)** Spearman correlation of expression between PRRX2, BCL2 and CCND2. **G, H)** CCND2, CCND3 and BCL2 mRNA expression levels in the ARPC, NEPC and PRRX2 clusters. Data obtained from the SU2C 2019. **I)** BCL2 expression in the TCGA PRRX2 cluster. Statistics: Unpaired t-test for B,D,G,H,I. Error bars represent S.E.M. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

Fig. S7

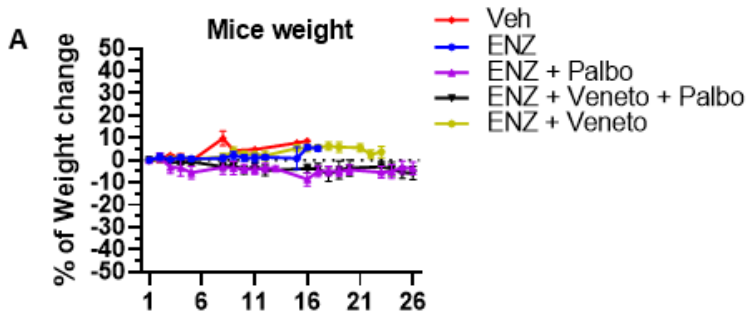

Fig. S7. A) Mouse body weight from in vivo experiment.