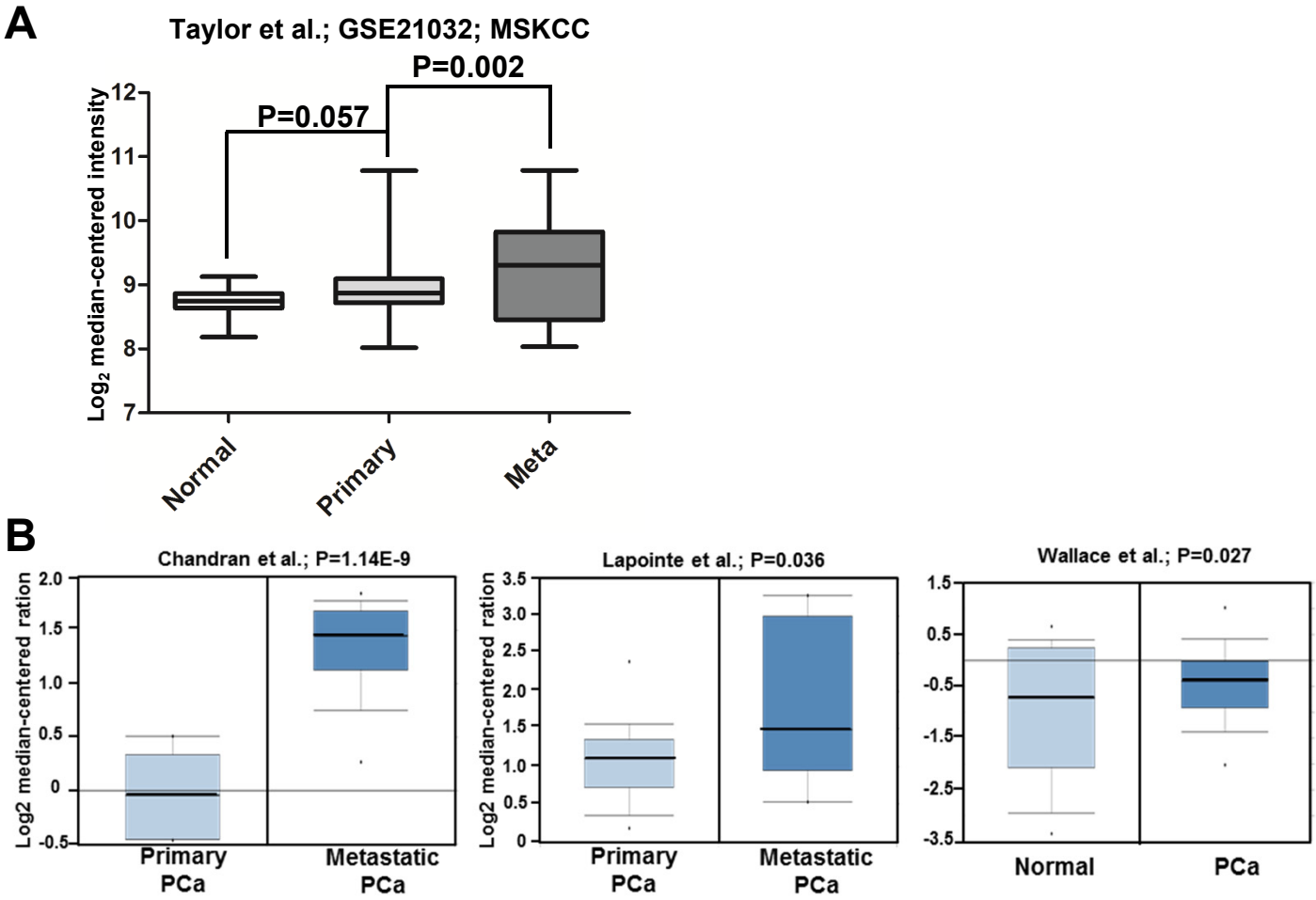
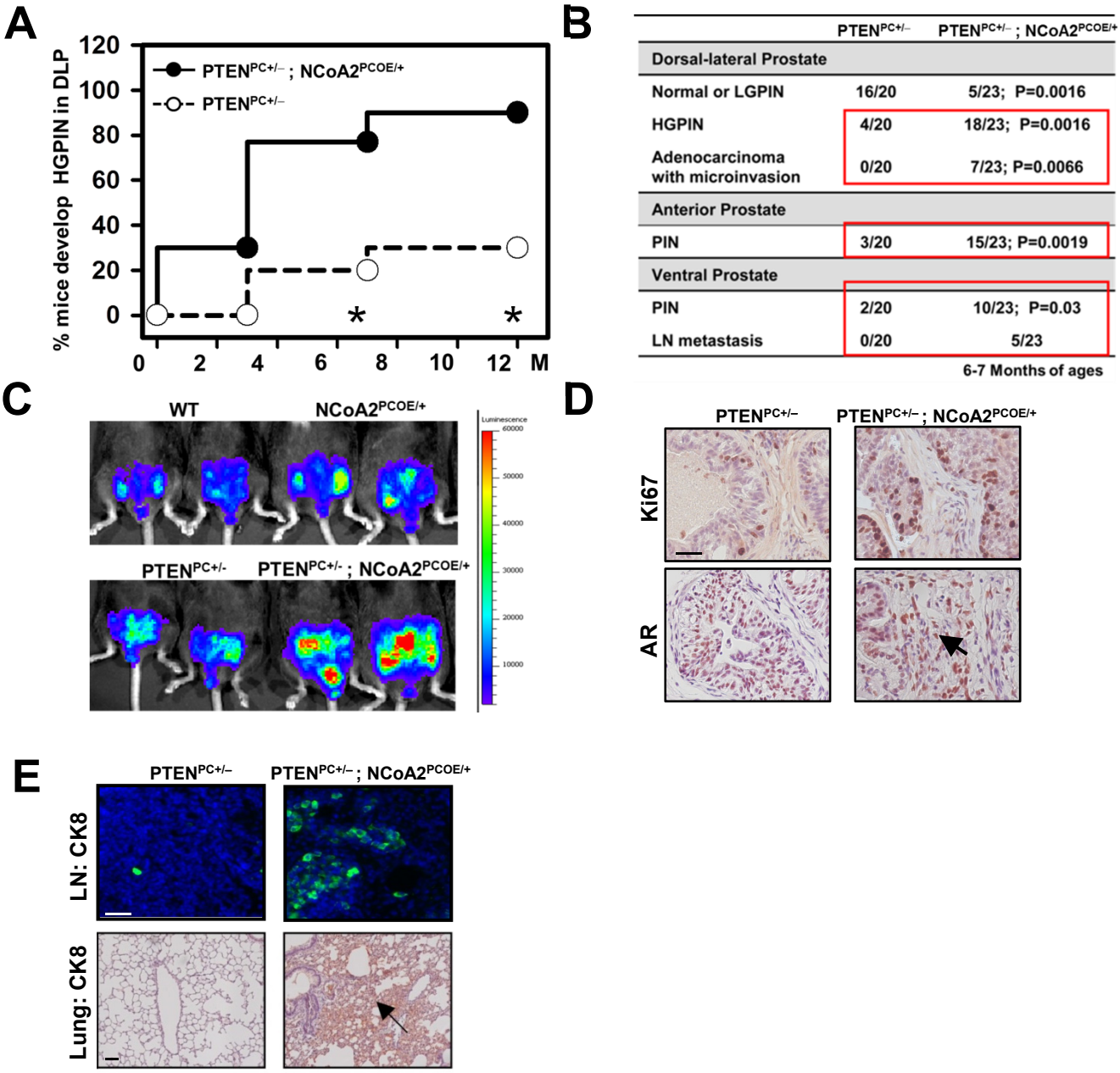


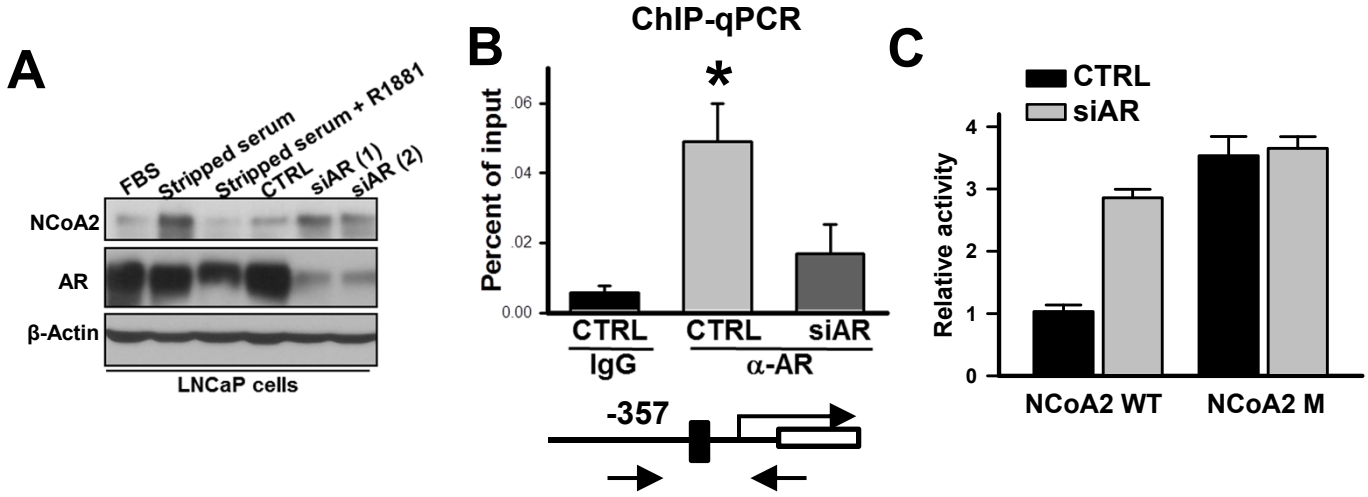
Supplementary Figure 1 NCoA2 expression in prostate cancer patients. (A) Boxed plot of NCoA2 expression levels in normal prostate (N=29), primary tumors (N=131) and metastasis tumors (N=19) by querying dataset: GSE21032 (Taylor et al. 2010). **(B)** Boxed plot of NOCA2 expression levels in prostate cancer patients using Oncomine expression analysis (Chandran et al., 2007; Lapointe et al., 2004; Wakkace et al., 2008).



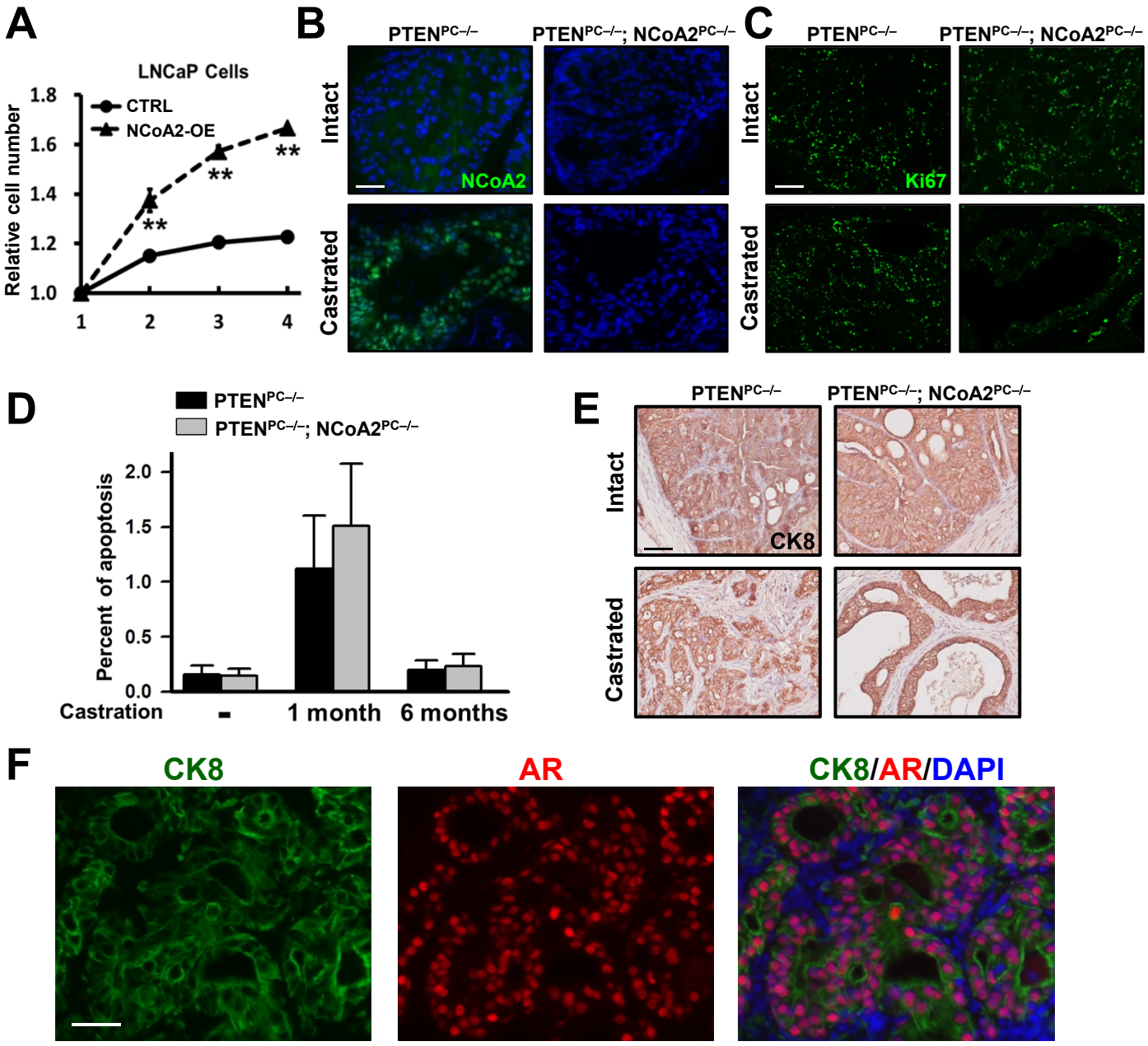
	PTEN PC+/–	PTEN PC+/– ; NCoA2 PCOE/+
Dorsal-lateral Prostate		
Normal or LGPIN	16/20	5/23; P=0.0016
HGPIN	4/20	18/23; P=0.0016
Adenocarcinoma with microinvasion	0/20	7/23; P=0.0066
Anterior Prostate		
PIN	3/20	15/23; P=0.0019
Ventral Prostate		
PIN	2/20	10/23; P=0.03
LN metastasis	0/20	5/23

6-7 Months of ages

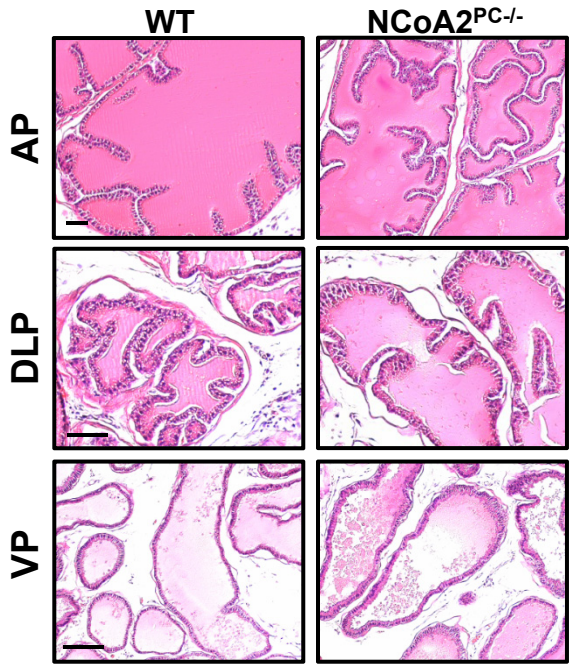
Supplementary Figure 2. Over-expression of NCoA2 renders prostate cancer cells more invasive. (A) Kaplan-Meier plot of the development of HGPIN in PTEN$^{PC+/-}$ and PTEN$^{PC+/-}$; NCoA2$^{PCOE/+}$ mice. 10 pairs of mice were sacrificed at 3 months, 7 months and 12 months of ages separately. Fisher exact test: * $P < 0.05$ **(B)** Summarize of tumor progression and metastasis in PTEN$^{PC+/-}$ and PTEN$^{PC+/-}$; NCoA2$^{PCOE/+}$ mice at 6-7months of age. LN metastasis: lymph node metastasis **(C)** Representative images of luciferase-reporter activity of WT, NCoA2$^{PCOE/+}$, PTEN$^{PC+/-}$ and PTEN$^{PC+/-}$; NCoA2$^{PCOE/+}$ mice at 12 months of age are displayed. **(D)** Immunostaining of Ki67 and CK8 expression in the tumors from PTEN$^{PC+/-}$ and PTEN$^{PC+/-}$; NCoA22$^{PCOE/+}$ mice at 12 months of age. **(E)** Immunostaining of CK8 expression in the lymph node and lung and from PTEN$^{PC+/-}$ and PTEN$^{PC+/-}$; NCoA2$^{PCOE/+}$ mice at 12 months of age. Scale bars: 50 μm **(D)** and **(E)**.



Supplementary Figure 3 AR transcriptionally inhibits NCoA2 expression. (A) Western blotting analysis of NCoA2 expression in LNCaP cells cultured in normal growth medium, stripped medium and stripped medium supplement with AR agonist R1881 as well as LNCaP cell treated with control siRNA or siRNA against AR. **(B)** ChIP analysis of AR recruitment to NCoA2 proximal promoter in control and AR depleted LNCaP cells. * $P<0.05$ **(C)** Luciferase reporter analysis of NCoA2 wild type promoter (NCoA2 WT) or the promoter bearing the mutation for AR binding site (NCoA2 M) in control and AR depleted LNCaP cells.

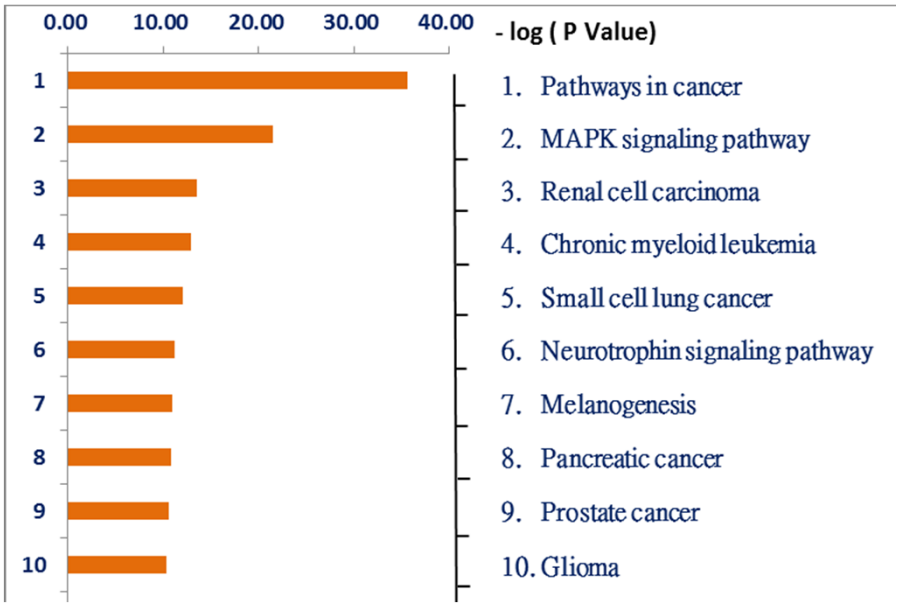


Supplementary Figure 4 NCoA2 is important for PTEN null tumors to develop into castrate resistant prostate cancer. (A) MTT analysis of cell growth alteration in CTRL and NCoA2 overexpressing LNCaP cells in charcoal-stripped media. N=3; ** P<0.01. Immunohistochemical analyses of the expression of NCoA2 **(B)**, Ki67 **(C)**, CK8 **(E)** and the quantitative results of cleavage Caspase 3 staining **(D)** in prostrate tumors from PTEN$^{PC-/-}$ and PTEN$^{PC-/-}$; NCoA2$^{PC-/-}$mice with or without Castration (6 months after castration). **(F)** Co-immunostaining of CK8 and AR expression in the lymph node from PTEN$^{PC-/-}$ mice. Scale bars: 50 μm **(B)**, **(C)** and **(F)**; 200 μm **(E)**.

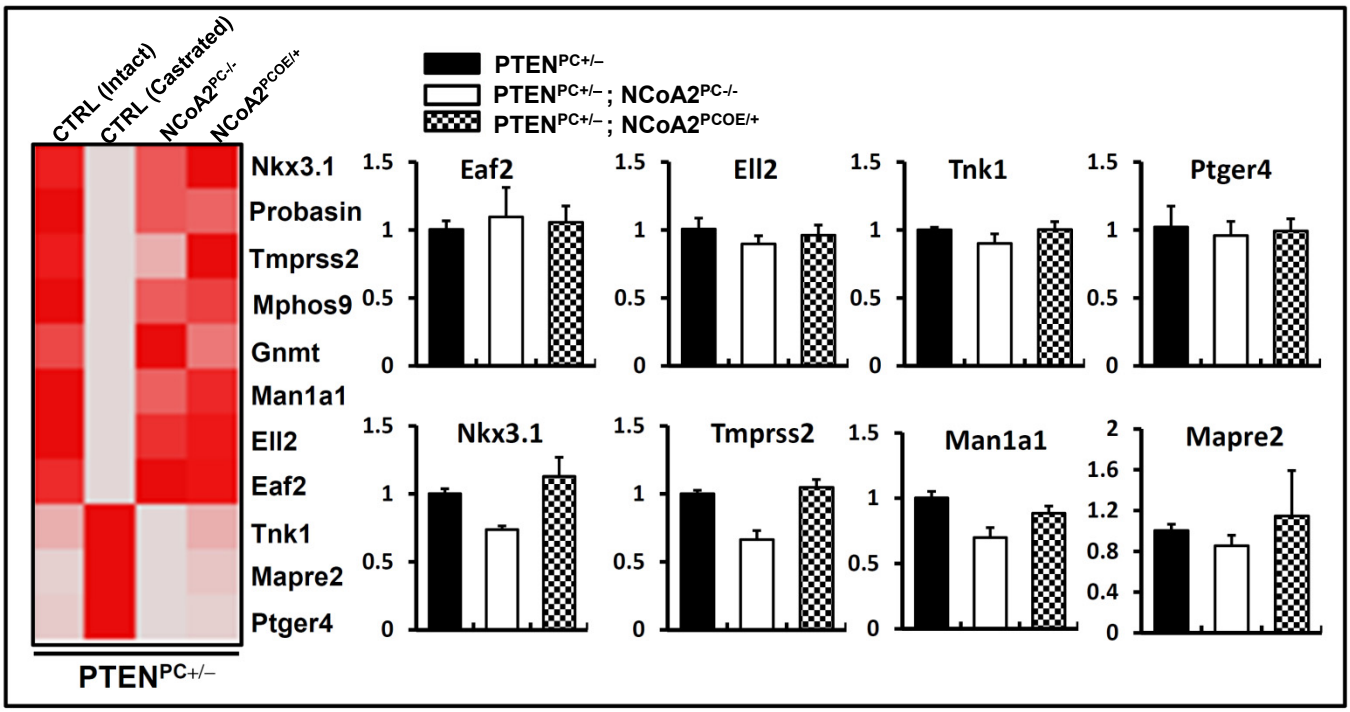


Supplementary Figure 5 NCoA2 ablation did not affect normal prostate histology. H&E stained sections of representative AP (anterior prostate), DLP (dorsal-lateral prostate) and VP (ventral prostate) of wild type and NCoA2$^{PC-/-}$ mice at 8 months of age. Scale bars: 50 μm.

A

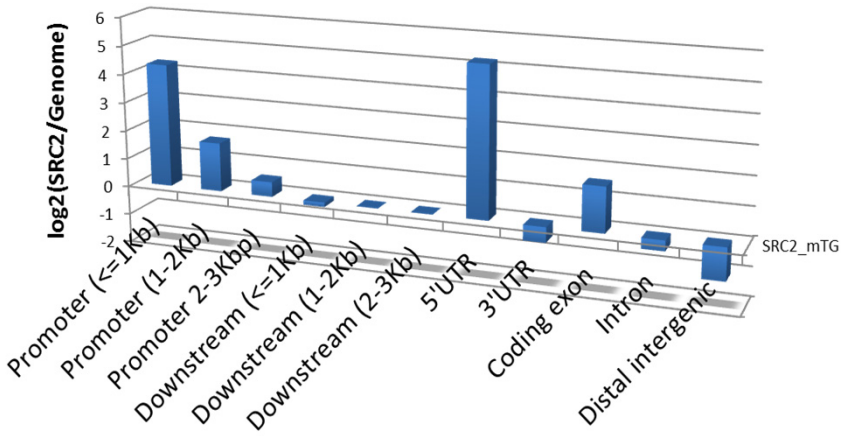


Supplementary Figure 6 NCoA2 regulates important signaling pathways involved in cancers. Biological interpretation of NCoA2 by KEGG signaling pathway analysis.

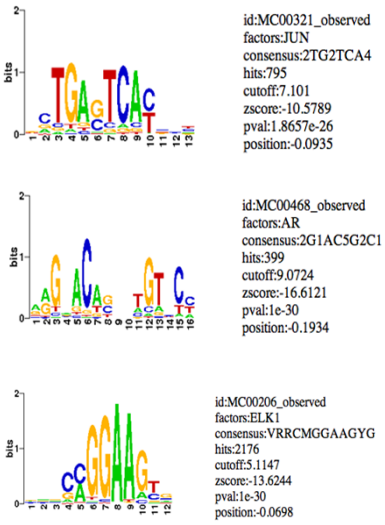


Supplementary Figure 7 Effects of NCoA2 in transcriptional regulation of AR target genes. qRT-PCR analysis of the mRNA expression levels of AR target genes. Heat map of gene expressions is indicated in left panel. CTRL (PTEN mutant prostate; Intact) versus CTRL (PTEN mutant prostate; Castrate) is used to indicate that examined genes are regulated by androgen signaling. Loss or overexpression of NCoA2 did not significantly regulate their expressions. Right panel shows the represented bar graphs of relative mRNA levels. Total RNA was extracted from the dorsal lateral prostate of 2 month old mice. Samples were collected three days after castration. Data are presented as mean ± SE (N = 4).

A



B

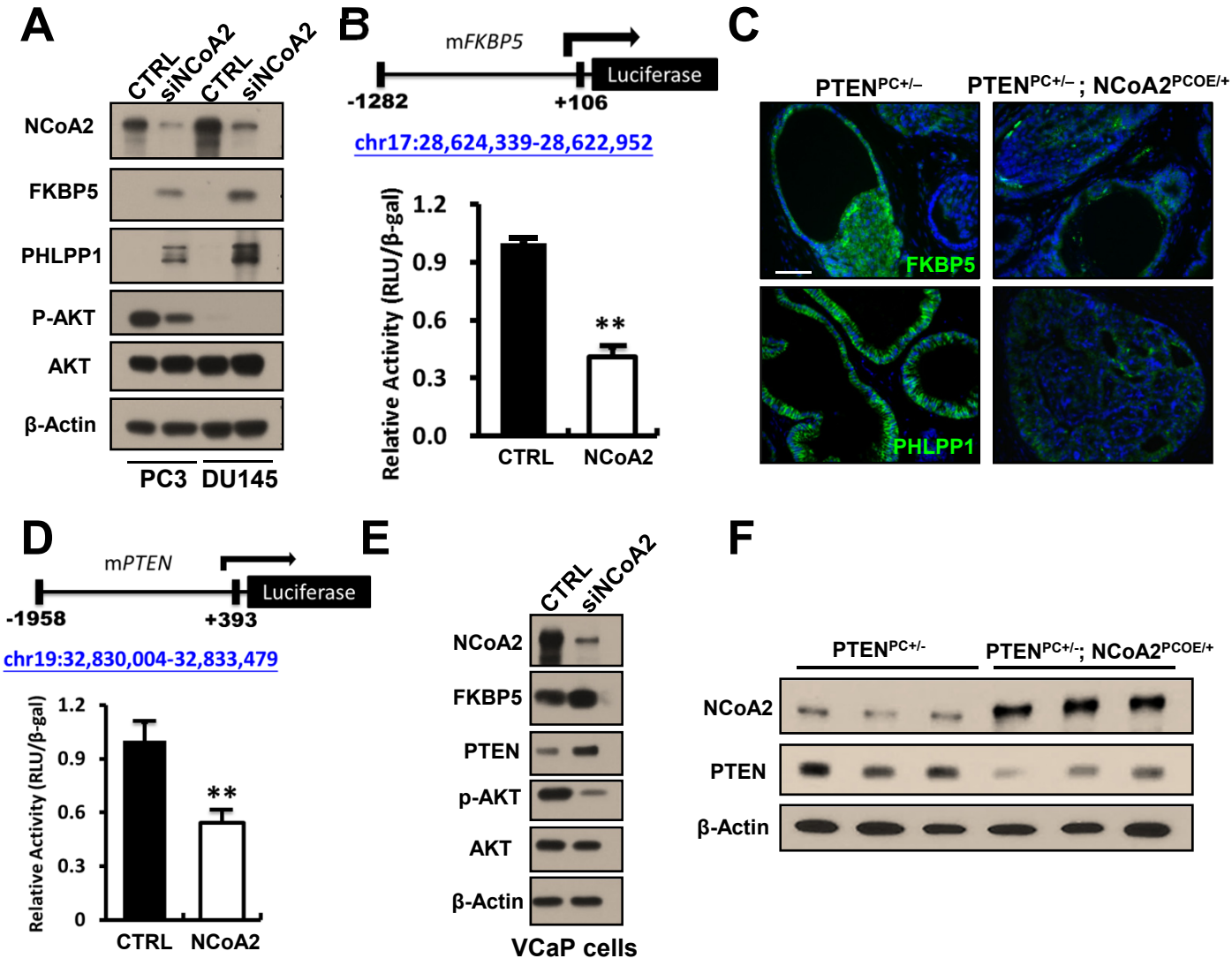


C

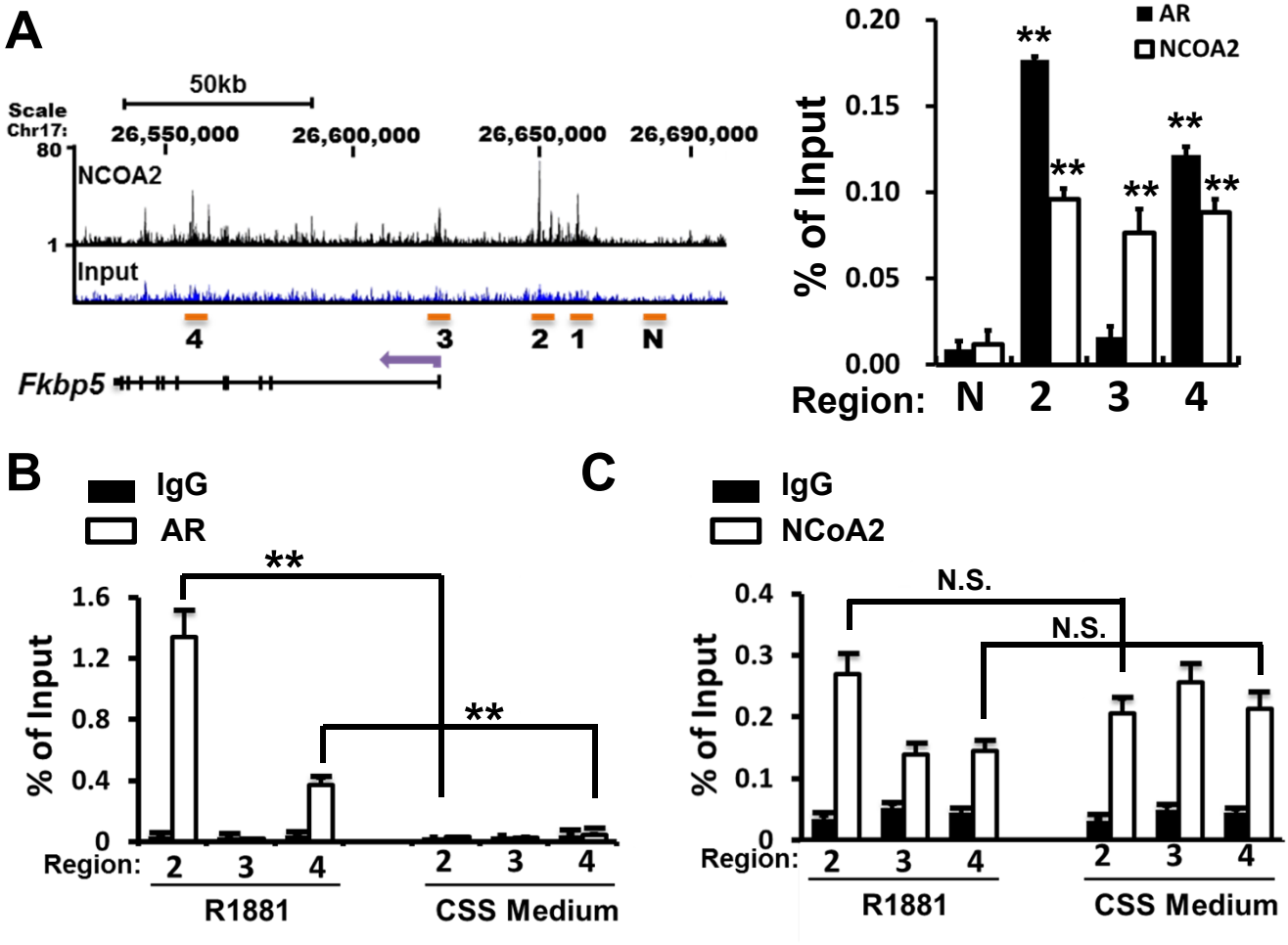
Category	Term	Count	P Value
KEGG_PATHWAY	mmu05215:Prostate cancer	53	4.18E-14
KEGG_PATHWAY	mmu05220:Chronic myeloid leukemia	47	1.18E-13
KEGG_PATHWAY	mmu05212:Pancreatic cancer	44	1.45E-12
KEGG_PATHWAY	mmu05213:Endometrial cancer	33	3.74E-10
KEGG_PATHWAY	mmu05223:Non-small cell lung cancer	33	1.47E-09
KEGG_PATHWAY	mmu05214:Glioma	32	1.33E-06
KEGG_PATHWAY	mmu05219:Bladder cancer	21	1.37E-04

Category	Term	Count	P Value
GOTERM_BP_FAT	GO:0043549~regulation of kinase activity	77	2.73E-12
GOTERM_BP_FAT	GO:0045859~regulation of protein kinase activity	75	3.94E-12
GOTERM_BP_FAT	GO:0000165~MAPKKK cascade	46	8.31E-08
GOTERM_BP_FAT	GO:0043405~regulation of MAP kinase activity	33	1.42E-05
GOTERM_BP_FAT	GO:0033674~positive regulation of kinase activity	45	1.44E-05
GOTERM_BP_FAT	GO:0043406~positive regulation of MAP kinase activity	21	0.00293715

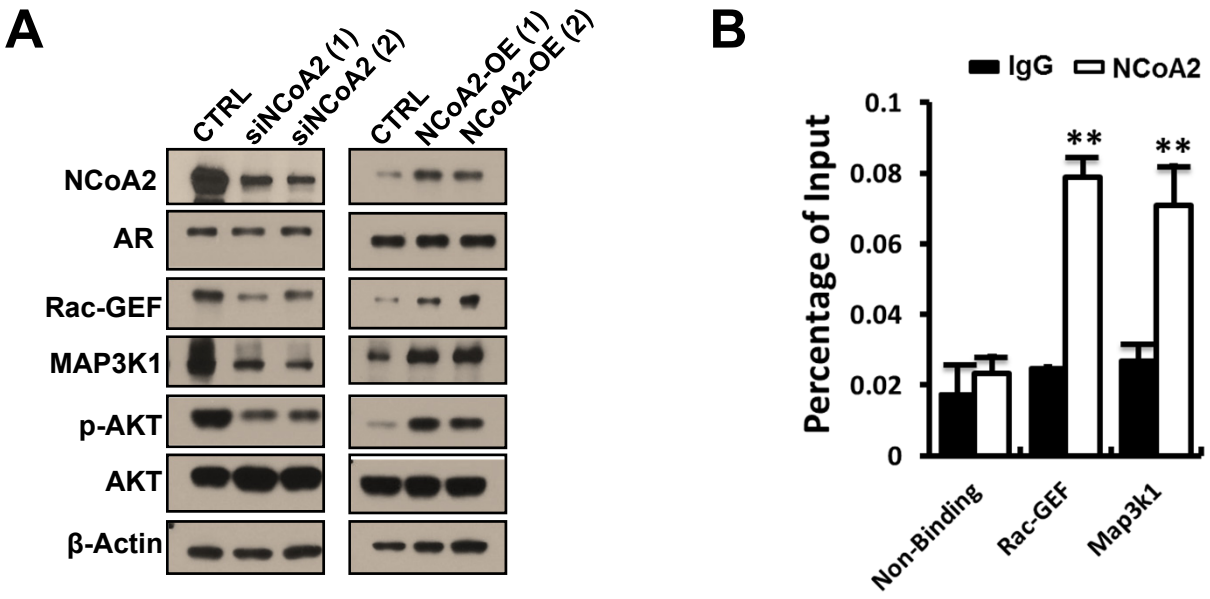
Supplementary Figure 8 Genomic annotations and functional annotation for NCoA2 ChIP binding intervals. **(A)** Enrichment distribution for genomic annotations for NCoA2 ChIP binding. **(B)** Sequence motif analysis of NCoA2 ChIP-Seq intervals. **(C)** Functional annotation of NCoA2 ChIP binding gene groups. DAVID v6.7 was used to identify enriched biological themes.



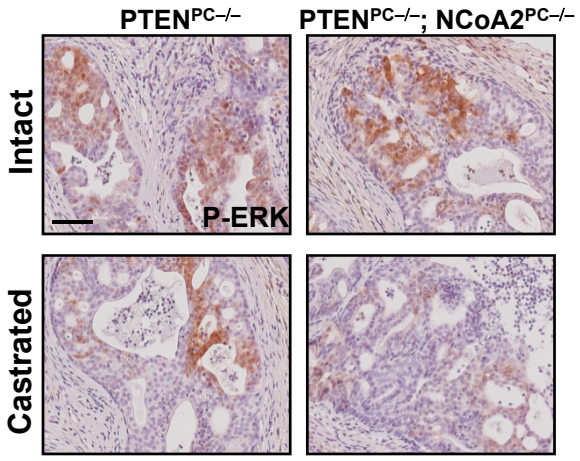
Supplementary Figure 9 NCoA2 represses FKBP5, PHLPP1 and PTEN expression to modulate PI3K/AKT signaling. (A) Western blot examination of FKBP5 and PHLPP1 protein expression in NCoA2 silencing PC3 and DU145 cells. **(B)** Illustration of the construct of mouse FKBP5 promoter driven luciferase reporter. Overexpression of NCoA2 in 293-T cells inhibits luciferase reporter activity driven by FKBP5 promoter. -1282 to +106 (ChIP Seq peak 3; transcription start site is designed as +1) corresponds to genomic region: chr17:28,624,339-28,622,952. Data are presented as mean ± SE (n = 3). Student's t test ** P<0.01 **(C)** Immunohistochemical analyses of FKBP5, PHLPP1 and Rac-GEF expression in $PTEN^{PC+/-}$ and $PTEN^{PC+/-}$; $NCoA2^{OE/+}$mice at 7 months of ages. Scale bars: 50 μm. **(D)** Luciferase report analysis of NCoA2 regulation for PTEN expression. -1985 to +395 (ChIP Seq positive region; transcription start site is designed as +1) corresponds to genomic region: chr19:32,830,004-32,833,479. **(E)** Western blot examination of FKBP5 and PTEN protein expression in NCoA2 silencing VCaP cells (PTEN wild type). **(F)** Western blot analysis of PTEN expression in anterior prostate from 24-week-old mice as indicated.



Supplementary Figure 10 NCoA2 binds to *Fkbp*5 locus in an AR independent manner. (A) ChIP-Seq results of NCoA2 binding on the *Fkbp5* locus. Numbers indicate NCoA2 binding sites. N indicates a region without NCoA2 binding sites that served as a negative control (Left panel). ChIP-qPCR assays for AR and NCoA2 binding to ~~genes~~ gene: *Fkbp5* in LNCaP cells using anti-NCoA2 or AR antibody. Bar graphs show an enrichment of DNA fragments pulled down by anti-AR or NCoA2 antibodies. ** P<0.01. ChIP-qPCR assays for AR **(B)** and NCoA2 **(C)** recruitment to gene: *Fkbp5* locus in LNCaP cells cultured in Charcoal Stripped Serum medium (CSS) with or without AR agonist, R1881. Bar graphs show an enrichment of DNA fragments pulled down by anti-AR or NCoA2 antibodies. Student's t test; ** P<0.01. N.S.: Not significant



Supplementary Figure 11 NCoA2 stimulates Rac-GEF and MAP3k1 expression. (A) Western blot examination of AR, Rac-GEF and MAP3K1 protein expression in NCoA2 silencing LNCaP-Abl or two individual NCoA2 overexpressing LNCaP cells. **(B)** ChIP-qPCR assays for NCoA2 binding to genes: *Rac-GEF* and *Map3k1* locus in LNCaP cells using anti-NCoA2 antibody or IgG. Bar graphs show an enrichment of DNA fragments pulled down by anti-NCoA2 antibodies. Student's t test; ** P<0.01.



Supplementary Figure 12 NCoA2 is important for MAPK activation in CRPC PTEN null tumors. Immunohistochemical analyses of phospho-ERK expression in prostrate tumors from PTEN$^{PC-/-}$ and PTEN$^{PC-/-}$; NCoA2$^{PC-/-}$mice with or without Castration (6 months after castration). Scale bars: 50 μm .

Supplementary Table 1. Public gene expression profile datasets used in this study

Pathway	Study	Data source	Model system	Upregulated genes	Downregulated genes
PTEN KO	Mulholland, et al. Cancer Cell 19, 792-804 (2011)	GSE24691	PTEN knockout mice	1262	1311
PTEN loss	Saal et al. Proc Natl Acad Sci U S A. 2007 May 1;104(18):7564-9.	Supplemental Materials	IHC of human breast tumors	111	62
AKT	Majumder et al. Nat Med. 2004 Jun;10(6):594-601.	GSE1413	Transgenic (Probasin driven Myr-AKT) mouse model	770	775
EGFR	Creighton et al. Cancer Res. 2006 Apr 1;66(7):3903-11.	GSE3542	Over-expression in MCF-7 breast cancer cells.	734	940
MEK	Creighton et al. Cancer Res. 2006 Apr 1;66(7):3903-11.	GSE3542	Over-expression in MCF-7 breast cancer cells.	1238	1182
MAPK	Creighton et al. Cancer Res. 2006 Apr 1;66(7):3903-11.	GSE3542	Over-expression in MCF-7 breast cancer cells.	124	271
Androgen	Deprimo et al. Genome Biol. 2002; 3(7)1:RESEARCH0032.	Stanford Microarray Database	R1881 treatment of LNCaP cells.	259	---

Supplementary Table 2. Enrichments of pathways in NCoA2 overexpressing prostate tumors

NCoA2 upregulated genes

Term	Count in Selected Genes	Count in Total Population	P-value
PTEN KO _GSE24691	268	1111	1.52E-64
Akt_up_p001_Majumder	125	717	6.34E-16
up_AKt_vs_WT	195	1292	2.01E-11
EGFR_up_p01_Creighton	99	650	2.02E-06
MEK_up_p01_Creighton	136	1120	0.002072
Mapk_up_Creighton	20	106	0.002484
Saal PTEN loss_UP	18	99	0.00597
R1881_up_p01_ave1.8_Deprimo	16	238	0.989

NCoA2 downregulated genes

Term	Count in Selected Genes	Count in Total Population	P-value
PTEN KO _GSE24691	199	1109	5.46E-42
down_Akt_p001	115	720	4.35E-12
down_Akt_vs_WT	181	1351	2.26E-11
R1881_up_p01_ave1.8_Deprimo	23	238	0.0474
R1881_down_p01_ave1.8_Deprimo	2	22	0.561351

Supplementary Table 3. Primer sequences in this study

RT-qPCR	Primer Sequences
mACTB	5′-GGCTGTATTCCCCTCCATCG-3′; 5′-CCAGTTGGTAACAATGCCATGT-3′
mNKX3.1	5′-GACTGTGAACATAATCCAGGGG-3′; 5′-TGATGGCTGAACTTCCTCTCC-3′
PROBASIN	5′-ATTGAGAACCTACTTCCGTCACA-3′; 5′-CAGTTGGCACTTAGTCCCTTTC-3′
mTMPRSS2	5′-ATGCTCCGAGGATTACAACGC-3′; 5′-CGAGGGCTAAACACAGCGATT-3′
mEAF2	5′-GAGGCTGATGCTACTTGTCAC-3′; 5′-CTCACTGTCGCTTTCTGACTC-3′
mELL2	5′-ACTCAAGACCTGCCTGTTGAC-3′; 5′-TTGGACACTTTAGCAGAGCTG-3′
mGNMT	5′-AAGAGGGCTTCAGCGTGATG-3′; 5′-CTGGCAAGTGAGCAAAACTGT-3′
mMAN1A1	5′-GGAGCTGGACTGGAAGACAAC-3′; 5′-GCAAGCCCCTAAACAGATCCT-3′
mMPHOS9	5′-TGCTAATAGAAGTAGTCCCCACC-3′; 5′-GGCCACTGTTCTGTAGGTCT-3′
mMAPRE2	5′-GATCAACGGGTAATCAGAAAGGG-3′; 5′-CCCAATTTGAACATCCAGGCAC-3′
mPTGER4	5′-ACCATTCCTAGATCGAACCGT-3′; 5′-CACCACCCCGAAGATGAACAT-3′
mTNK1	5′-CGCTCAGACAGTCCTCTGTG-3′; 5′-AGTGTCCATAACCCTCGATGT-3′
mFKBP5	5′-TGAGGGCACCAGTAACAATGG-3′; 5′-CAACATCCCTTTGTAGTGGACAT-3′
mMAP3K1	5′-CAGGAGTGAGACGGAAACGAG-3′; 5′-AGAGTTGGGTCCTATCTGCTG-3′
mPI3KAP1	5′-GTCCCGGATGCCTCTTTCTC-3′; 5′-CACAAGTCATTTCCTGCCAGT-3′
mPHLPP1	5′-CAGTTGCCAGTGAACCGATG-3′; 5′-GAGCTGAACGCTAAACAGTGC-3′
mRac-GEF	5′-CTGCGGGCAACATCCAAGA-3′; 5′-CCAGTGACCCTAGACTTCCG-3′
mPTEN	5′-ATGGATTCGACTTAGACTTGACC-3′; 5′-ATGTCTCTCAGCACATAGATTGTAT-3′

ChIP-qPCR Primer Sequences	
Fkbp5 N	5′-TGCAGCAGGTACACCAGAAG-3′; 5′-AGAGTGGGCTGGAATTGATG-3′
Fkbp5 #1	5′-CACCTTCTCTGAGCCAGTCC-3′; 5′-TAATCCCAGGAGTGTGAGGG-3′
Fkbp5 #2	5′-CACTAAGTGTTTGCTGGGCA-3′; 5′-CATTTACTGCCCTTTGGCTC-3′
Fkbp5 #3	5′-CTGCCTATGCAAATGAGGCT-3′; 5′-GGTGTCAGTCAGCTTCCTCC-3′
Fkbp5 #4	5′-AGGCCTCTGCACAGTAAGGA-3′; 5′-GAGTCAGAGCAGGGGAAGTG-3′
Phlpp1 N	5′-GAAATCACATTAAACAACCAACTG-3′; 5′-TAGTGAATAGGTGGTCATTG-3′
Phlpp1 #1	5′-GGTGGGCAGAATTTGATGAT-3′; 5′-CATAGAGGAGAGGTGGCGAG-3′
Phlpp1 #2	5′-TCCAGTCTCTCCCTGTGCTT-3′; 5′-GCCTCCAAGAGGAAGGAACT-3′
Phlpp1 #3	5′-GAAGTTTGGCACAAAGGAGG-3′; 5′-CCTGCAGAACTGGCTGTGTA-3′
Phlpp1 #4	5′-TTTGCAATAACCCTGCTTCC-3′; 5′-GGCTCTGTTGGAGAAACTGC-3′
PI3KAP1	5′-CTTCTCAGTGAACCGGAAGC-3′; 5′-AGTTCCCCACTCTGTGGTTG-3′
PTEN	5′-GGATGCTATTCTCTGCTCCG-3′; 5′-TCCCCTATCTGTGTGCTCC-3′
MAP3K1	5′-TTCTCCTGCCCCAGAGTCTA-3′; 5′-GGGTGAGATAGGGACGACAA-3′
Rac-GEF	5′-TTCAAACTATGCACAAGGCG-3′; 5′-TACTCAACGAGATCCTGGGC-3′