**Supplementary Materials and Methods**

**Analysis of ANCCA and NSD2 expression association in a microarray expression data set**

Raw microarray data (CEL files) from record number GSE3325 (Varambally et al 2005) were downloaded from the NCBI GEO DataSets website (http://www.ncbi.nlm.nih.gov/gds) and analysis performed using GeneSpring GX (version 11) software (Agilent Technologies, Inc. Santa Clara, CA). Normalized probe set expression intensities were obtained using robust multi-array average (RMA) (Irizarry et al 2003) for probe summarization and normalization of background-adjusted and log-transformed perfect match (PM) probe intensity values. The data set was also filtered to retain only those probe sets having expression values that exceeded the 10% lower cut-off threshold in at least one of the samples. Comparison analysis was then performed in order to identify genes that were differentially expressed in primary or metastatic CaP relative to BPH. Criteria for the selection of genes exhibiting significant expression changes included an average fold change of $\geq 2.0$ between groups and p-values of $\leq 0.05$. Correlations of *ANCCA* and *NSD2* expression were assessed by computing the Pearson correlation coefficient ($r$) and a two-tailed $t$-test for significance.

**Antibodies for Western blotting and ChIP**

Antibodies against AR (N-20), Beta-Actin (C-4), Cyclin D1 (H-295), Cyclin D2 (C-17), Cyclin D3 (C-16), Cyclin E2 (N-20), c-Myc (N-262), Bcl-2 (clone 100), survivin/Birc5 (D-8) were from Santa Cruz Biotechnology. Antibodies against Cyclin A2, Cyclin B1 (clone 18) were from BD Biosciecnes. Antibodies against NF-kB1 (p105/p50), Bcl-xL, IL-6R, VEGFa, SGK-1 and GAPDH were from Cell Signaling Technology. Antibodies against human AR (Ab-1 AR441) and Cyclin D1 were from NeoMarkers. Anti-NSD2 monoclonal antibody (clone 29D1) was purchased from Abcam. For ChIP, anti-RelA/p65 antibody (C-20) and p300 (C-20) were from Santa Cruz. Anti-H3K36me2 antibodies were from Active Motif and Abcam. Anti-H3K36me3 antibody was from Abcam. Anti-H3K9K14ac (06-599) was from Millipore. Two NSD2 anti-sera were raised in rabbit (Covance) by injecting purified GST-NSD2 N-terminal (aa 1-261) or middle (aa 536-781) fragments and were used in ChIP.

**Primers used for qRT-PCR analysis:**

| | |
|---|---|
| NSD2 | 5’TCA AGC AGA AGA AGC TGC AA3’ |
| | 5’TCT GCC GTC TTT TGA GGA GT3’ |
| hGAPDH | 5’GAA ATC CCA TCA CCA TCT TCC AG3’ |
| | 5’ ATG AGT CCT TCC ACG ATA CCA AAG3’ |
| Cyclin A2 | 5’CCC CCA GAA GTA GCA GAG TTT GTG3’ |
| | 5’GCT TTG TCC CGT GAC TGT GTA GAG3’ |
| Cyclin D1 | 5’AAA TCG TGC GGG GTC ATT GC3’ |
| | 5’TCC TGT GCT GCG AAG TGG AAA C3’ |
| Cyclin D2 | 5’TCA CCA ACA CAG ACG TGG A3’ |
| | 5’TGT AGG GGT GCT GGC TTG3’ |
| Cyclin D3 | 5’CGT GGT CGG TGT AGA TGC3’ |
| | 5’TGG ATG CTG GAG GTA TGT G3’ |
| Cyclin E2 | 5’ACT GAC TGC TGC TGC CTT GTG C3’ |
| | 5’TCG GTG GTG TCA TAA TGC CTC C3’ |
| Myc | 5’TGA CAC TGT CCA ACT TGA CCC TCT T3’ |
| | 5’TCG CAA GAC TCC AGC GCC TTC TC3’ |
| Bcl2 | 5’GGT GCC ACC TGT GGT CCA3’ |
| | 5’ACT TGT GGC CCA GAT AGG3’ |
| Bcl-xl | 5’GGC AAC CCA TCC TGG CAC CT3’ |
| | 5’AGC GTT CCT GGC CCT TTC G3’ |
| Survivin | 5’CCC TGC CTG GCA GCC CTT TC3’ |
| | 5’CTG GCT CCC AGC CTT CCA3’ |
| SGK-1 | 5’GAA CCA CGG GCT CGT TTC TAT3’ |
| | 5’GCA GGC CAT ACA GCA TCT CAT3’ |
| IL-6 | 5’CAG CCC TGA GAA AGG AGA CAT3’ |
| | 5’AAT CTG AGG TGC CCA TGC TAC3’ |
| IL8 | 5’ATG ACT TCC AAG CTG GCC GTG GCT3’ |
| | 5’TCT CAG CCC TCT TCA AAA ACT TCT3’ |
| VEGFa | 5’ACC AAA CAA GGA GCT GGA TG3’ |

5’TGG TGG TGG AAC TTC TTT CC3’

CXCL1 5’ATG GCC CGC GCT GCT CTC TCC3’

5’GTT GGA TTT GTC ACT GTT CAG3’

**Primers used for ChIP analysis:**

IL-8-up-ChIP1F 5’ACCTCCAATGTGCCAGGTGCCAT3’

Il-8-up-ChIP1R 5’CTGAGCAAAGTTGGTTCGTGTTGCCA3’

Il-8-prom-ChIP3F 5’GAGGGGATGGGCCATCAGTTGCA3’

Il-8-prom-ChIP3R 5’ACAGAGCTGCAGAAATCAGGAAGGCT3’

IL-6-up-ChIP1F 5’CCA ACC GAT TAC CCA CAC AAT GTC AG3’

IL-6-up-ChIP1R 5’TCA GCT TGG CAA GTG ATC TGG ATG TG3’

IL6-prom- ChIP-U 5’AGC ACT GGC AGC ACA AGG CAA AC3’

IL6-prom- ChIP-L 5’CAA GCC TGG GAT TAT GAA GAA GG3’

Survivin-prom-ChIP2F 5’GTC CTT CAT GCC CGT CTG GAG TAG ATG3’

Survivin-prom-ChIP2R 5’CTG GCC ATC ACG GTG AAA CCT TGT CTC3’

Survivin-down-ChIP3F 5’CAG TTG TGG GTG AAG CAT GCT GTG AGA GA3’

Survivin-down-ChIP3R 5’GGT TCC AGG CAC ACA AGC TGC TCC TTG3’

**References**

Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U *et al* (2003). Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* **4:** 249-264.

Varambally S, Yu J, Laxman B, Rhodes DR, Mehra R, Tomlins SA et al (2005). Integrative genomic and proteomic analysis of prostate cancer reveals signatures of metastatic progression. Cancer Cell 8: 393-406.

**Supplementary Figure legends**

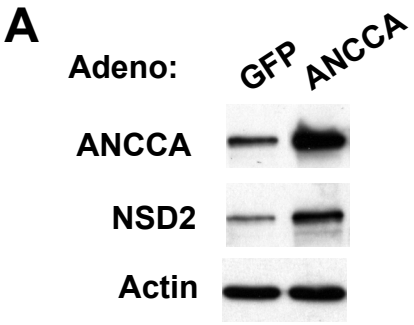
**Supplementary Figure 1** (A) ANCCA overexpression increased NSD2 expression. LNCaP cells were infected with adenovirus vectors that mediate full-length ANCCA expression or GFP as control and 72 hrs later harvested for Western blotting with indicated antibodies. (B) Comparison of ANCCA and NSD2 mRNA expression in a microarray dataset of BPH, localized primary prostate tumors, and metastatic lesions (described in Supplementary Methods) demonstrated a strong correlation ($r$ = 0.9160; two-tailed $t$-test $p$<0.001) and that positive, normalized expression values for both genes were found only in samples derived from metastatic lesions (upper right quadrant of scatter plot).

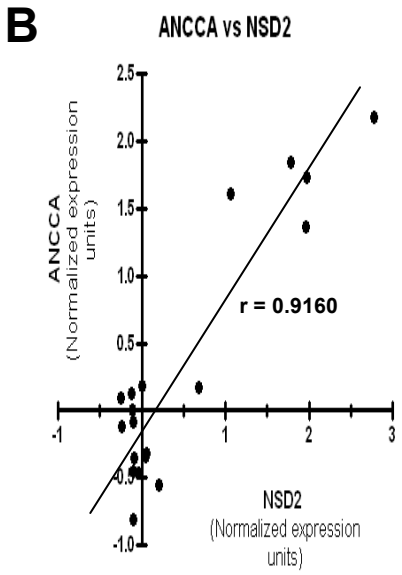
**Supplementary Figure 2** IHC analysis of NSD2 expression in normal, hyperplasia, or prostate cancer tissue with indicated Gleason scores. Human prostate tissue on tissue microarrays was subject to anti-NSD2 IHC analysis as described in Materials and Methods. Representative images at x200 magnification were shown for tissue with hyperplasia scored negative for anti-NSD2 (A), Gleason 2 tumor scored negative for anti-NSD2 (B), and Gleason 9 tumor scored positive for anti-NSD2 (C).

**Supplementary Figure 3** NSD2 expression is required for proliferation and survival of CWR22Rv1 cells. CWR22Rv1 cells were transfected with siRNA-NSD2 or siRNA-control. (A) Cells were harvested for enumeration (top panels) or Western analysis (bottom panels) at indicated times. (B) Three days after the transfection, cells were lysed and examined for apoptotic cell death by measuring DNA fragmentation using a Cell Death Detection ELISA Kit.

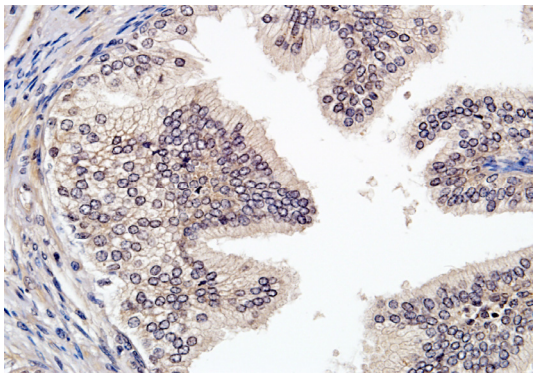
**Supplementary Figure 4** (A and B) The requirements of NSD2 for cell proliferation and survival is also

observed using si-NSD2-JL targeting a region of NSD2 mRNA different from si-NSD2 used in the other Figures. C4-2B and PC-3 cells were transfected with the siRNA control, si-NSD2 and the si-NSD2-JL targeting different NSD2 sequence (sequences shown in Materials and Methods) or control luciferase sequences. Cells were harvested for enumeration (top panels) or Western analysis (middle panels) or for apoptotic cell death by measuring DNA fragmentation using a Cell Death Detection ELISA Kit (bottom panels). Expression of both isoforms of NSD2/MMSET II (Full-length) and MMSET I (80 kD) were suppressed by si-NSD2 and si-NSD2-JL. (C) Western blotting analysis with indicated antibodies was performed with tumor cell lysates obtained from individual tumors dissected from mice injected with PC-3 cells treated with either adeno-shRNA-control or adeno-shRNA-NSD2. Similar results were obtained with several other pairs of tumors.


A
Adeno:
GFP
ANCCA
ANCCA
NSD2
Actin

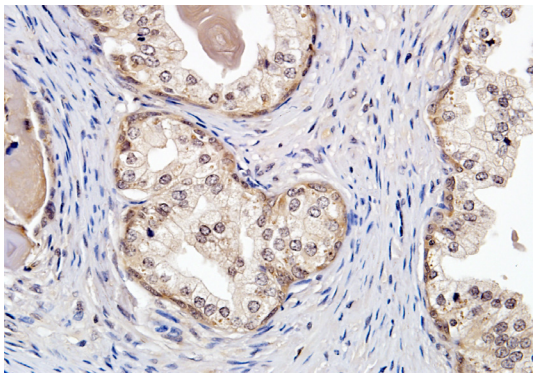

B
ANCCA vs NSD2
ANCCA
(Normalized expression
units)
2.5
2.0
1.5
1.0
0.5
-1
1
2
3
-1.0
r = 0.9160
NSD2
(Normalized expression
units)

A



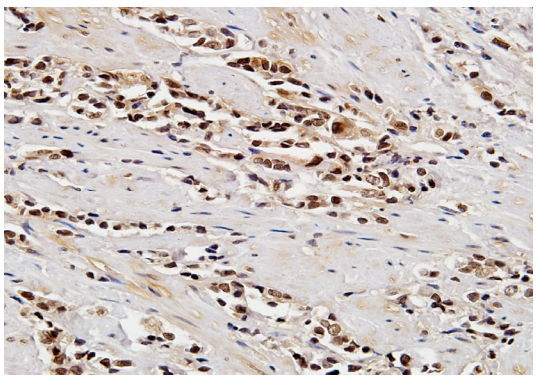
Hyperplasia

B

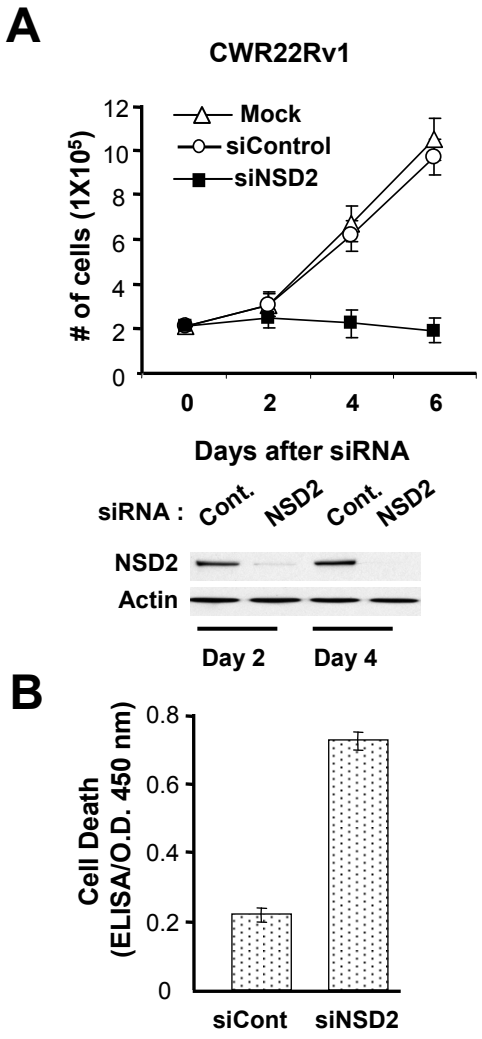


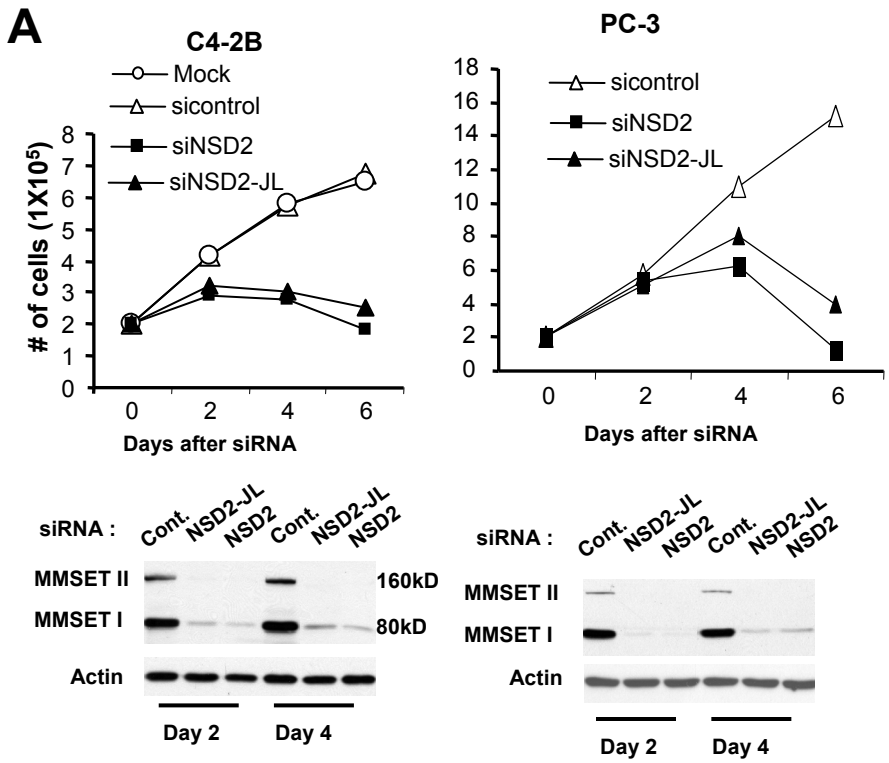
Gleason score 2

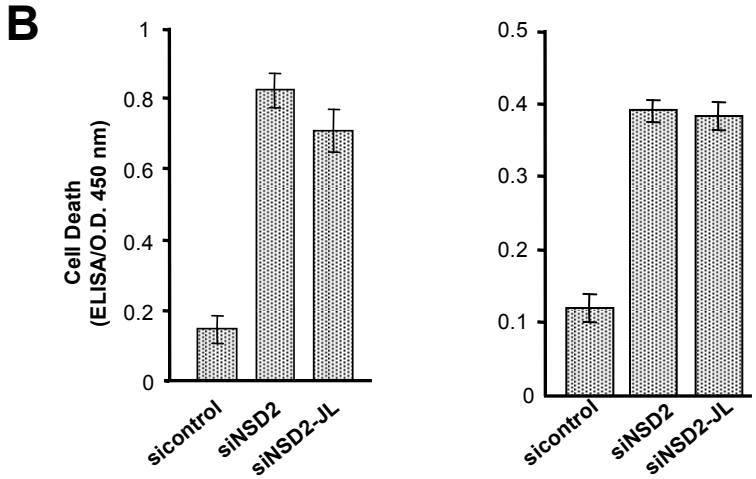
C

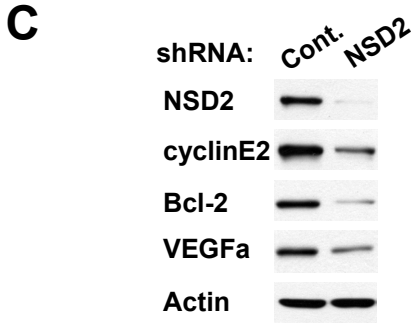


Gleason score 9


A
CWR22Rv1
Mock
siControl
siNSD2
# of cells (1X10^5)
12
10
8
6
4
2
0
0
2
4
6
Days after siRNA
siRNA :
Cont.
NSD2
Cont.
NSD2
NSD2
Actin
Day 2
Day 4
B
Cell Death
(ELISA/O.D. 450 nm)
0.8
0.6
0.4
0.2
0
siCont
siNSD2


A
C4-2B
Mock
sicontrol
siNSD2
siNSD2-JL
# of cells (1X10^5)
Days after siRNA
PC-3
sicontrol
siNSD2
siNSD2-JL
Days after siRNA
siRNA :
Cont.
NSD2-JL
NSD2
MMSET II
MMSET I
Actin
160kD
80kD
Day 2
Day 4


B
Cell Death
(ELISA/O.D. 450 nm)
sicontrol
siNSD2
siNSD2-JL


C
shRNA:
Cont.
NSD2
NSD2
cyclinE2
Bcl-2
VEGFa
Actin