

Supplemental Information

Nkx3.1 and c-Myc Cross-regulate Shared Target Genes in Mouse and Human Prostate Tumorigenesis

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Supplemental Methods

ChIP Assays

Whole mouse prostates were removed and snap-frozen at -80°C. Prostates were pulverized under liquid nitrogen and fixed in PBS for 15 minutes at 4°C in 1% formaldehyde with protease inhibitors. Fixation was terminated with 0.5M glycine, subjected to centrifugation and washed in PBS to remove traces of fixative. The pellet was resuspended in NEBA buffer and homogenized on ice with a dounce pestle. The cells were maintained on ice for 10 mins, followed by centrifugation and resuspended in lysis buffer. Samples were divided in half and sonicated using a Bioruptor (Diagenode) in the Vanderbilt Functional Genomics Shared Resource laboratory. Agarose gel electrophoresis determined the average fragment size to be 250 nucleotides. For the IP, samples were diluted 1:10 in ChIP dilution buffer, then divided in half (each approx. 2 mL) for each sample. 5 µg of anti-goat IgG (Santa Cruz) or anti-Nkx3.1 (Santa Cruz) antibody was added and samples were rotated overnight at 4°C. 50 µL of Protein A/G agarose bead slurry (Santa Cruz) were added and samples rotated for 2 hours at 4°C. Samples were washed and DNA-protein complexes eluted twice in 250 µl Elution Buffer. 20µL of saturated NaCl was added to the combined fractions and incubated at 65°C overnight. Proteins were digested for 1h at 45°C with 10 mg/ml proteinase K, 0.5M EDTA, and 1 M Tris. DNA was purified using a PCR purification kit (Qiagen) and DNA was eluted in pure water. For qPCR, 2 µL of DNA was used per 25 µL reaction. Amplification was detected using SYBR-Green (Life Technologies Corporation)

ChIP-re-ChIP assays

ChIP-re-ChIP was performed as described (1) with the following modifications. Formaldehyde was added directly to the media for eight minutes with gentle agitation. Cells were washed with PBS and collected into 1.5 mL microcentrifuge tubes. Cells were lysed and chromatin was sonicated using the same settings for ChIP (above). Following the protocol, DNA was eluted in pure water using a PCR purification kit (Qiagen).

Microarray analysis

Control and c-MYC-expressing regenerated prostates has been described (2). RNA was isolated from these grafts and subjected to microarray expression analysis using Mouse Gene 1.0 ST microarrays (Affymetrix, Santa Clara CA) and will be presented in detail elsewhere (3). CEL files were pre-processed in Expression Console (Affymetrix, Santa Clara CA) using the RMA-Sketch normalization model. Log2-transformed data were analyzed using the Significance Analysis of Microarrays (SAM) algorithm (4) for statistical analysis.

Cell Culture and Quantitative RT-PCR

p53^{-/-};Arf^{-/-} double knockout (DKO) MEFs expressing MycER (gift from Dr. Steve Hann) were cultured in DMEM supplemented with 10% FBS and 100U/ml penicillin and

streptomycin. Cell transfection was performed using PEI, with GFP control or FUGW-Nkx3.1 plasmid (kind gift from Dr. Hong Wu). pBabe-Puro-MYC-ER plasmid (gift from Dr. Gerard Evan) was used to generate a retrovirus and infect LNCaP cells to develop LNCaP-MycER cells. LNCaP-MycER cells were maintained in RPMI supplemented with 10% FBS and 100U/ml penicillin and streptomycin. Two consecutive cell transfections were performed using Superfect (Invitrogen), with siNKX3.1 or GFP duplex (Thermo Fisher). Following transfection, cells were cultured in media containing 0.1% FBS for 36h followed by activation of MycER with 10 μ M 4-hydroxytamoxifen (4-OHT) for DKO and 100nM for LNCaP-MycER. At the indicated times total RNA was isolated, reverse transcribed and quantitative PCR was performed. LNCaP si471 cells with stable siRNA knockdown of NKX3.1 and LNCaP siLuc cells with Luciferase siRNA control (5) (kind gifts from Drs. Cai Bowen and Edward Gelmann) were cultured in RPMI supplemented with 10% FBS and 100U/ml penicillin and streptomycin.

Co-immunoprecipitation and Western blotting

Western blot analysis was performed as previously described (6) using the following antibodies: anti-Nkx3.1 (rabbit, 1:4000, gift from Dr. Charles Bieberich), anti-c-MYC (mouse, 1:400, Santa Cruz), anti-beta-actin (goat, 1:1000, Santa Cruz). For co-immunoprecipitations, HA tagged-NKX3.1 and FLAG tagged-MYC constructs (wild type, delta 20-48 MYC Box I mutant or delta 118-152 MYC BoxII mutant (7)) were co-transfected into 293T cells, and lysates used for immunoprecipitation experiment. Antibodies used were: anti-FLAG (Sigma) anti-HA affinity Matrix (Roche), Nkx3.1 antibody (Santa Cruz) and mouse IgG (Santa Cruz). For Ethidium bromide (EtBR) treatment, 50 mg/ml of EtBR was added to the lysates and incubated on ice for 30 min. The same concentration of EtBR was used during the washing steps. For input, 5-10 % of lysates was used.

Histology and Immunohistochemistry

Preparation of tissues for histological and immunohistochemical examination were performed using standard procedures as described (8, 9). MYC immunohistochemistry was performed following a previously described protocol (10). The following antibodies were used: anti-activated caspase 3 (rabbit 1:200, Cell Signaling), anti-phospho-histone H3 (rabbit 1:500, Upstate), anti-c-MYC (rabbit 1:5000 or 1:1500 with TSA, Santa Cruz), anti-Nkx3.1 (rabbit 1:1000, gift from Dr. Charles Bieberich), anti-smooth muscle actin (mouse 1:2000, Sigma), anti-Hexokinase II (rabbit 1:50, Cell Signaling), anti-Prdx6 (rabbit 1:200, gift from Dr. Aron Fisher (11)), anti-Nedd4-2 (rabbit 1:400, Abcam), anti-p63 (PIN cocktail, Biocare medical).

Oligonucleotides used in this study

ChIP-qPCR in LNCaP cells

| | |
|------------|------------------------|
| ASNS_Myc-F | CGCTGCTTAGTGAATCCCTGTA |
| ASNS_Myc-R | TGCCTCTTTGTTCTCCCTTTT |

| | |
|--------------|----------------------------|
| ASNS_Nkx-F | GCAAGGAGGAGCTCTTTTGT |
| ASNS_Nkx-R | TGTCAGAACAGCAGGTAGCC |
| CTSB_Myc-F | CCCATTCTGGAGTCAGTGT |
| CTSB_Myc-R | TCGGATGAGCTGGTCAACTA |
| CTSB_Nkx-F | TGGGCACTTAACACAAATGG |
| CTSB_Nkx-R | ATGGATCCTTTTTCACTCACG |
| HDLBP_Myc-F | CCACCATGAATAAAGGGACA |
| HDLBP_Myc-R | CCTGTGGCACCAGTTTCTTT |
| HDLBP_Nkx-F | AACAAAGGGTGGCAGAACAC |
| HDLBP_Nkx-R | TTTACTGTGGGGGTGGAGAC |
| IGF1R_Myc-F | GGGTCCCCAGTTCAGAGG |
| IGF1R_Myc-R | CCACCAACGCACACTCG |
| IGF1R_Nkx-F | TGGTCTCTTGAAGTGTTACAGATTTT |
| IGF1R_Nkx-R | GCTCAGCTGGAGACACACAA |
| UTRN_Myc-F | AAGACTGAAGCAGAGGATTTGG |
| UTRN_Myc-R | TTTTTGCTCCTTCTGAGTTTACA |
| UTRN_Nkx-F | CCTTTATGGGACCGATGAGA |
| UTRN_Nkx-R | CGCTCCAGGGAAGGTTAATA |
| NEDD4L_Myc-F | AAGGCAACATGGCTTCTCAC |
| NEDD4L_Myc-R | CCCAGCCGTAGTGCAGTATT |
| NEDD4L_Nkx-F | GCCCTGGACACCTGATGTTA |
| NEDD4L_Nkx-R | TCCCAGGACTGCCAAGTTAC |
| HK2_Myc-F | CTTCTGCAGCGCGAGTTC |
| HK2_Myc-R | GAACCGCTCGTCTCCTACAC |
| HK2_Nkx-F | GCCAGGGCCTTGTCTTTATT |
| HK2_Nkx-R | GCCCAAAGAGTCCTTAGCC |
| PRDX6_Myc-F | GTACGCCCTGCAGAGTCAA |
| PRDX6_Myc-R | AGGTGGCATCCTTCAGACAC |

| | |
|-------------|----------------------|
| PRDX6_Nkx-F | GCCGAAAGACTTTTTGTCCA |
| PRDX6_Nkx-R | CCCTCCAAGTGTTGCAAGTT |
| LNCaP_Neg_F | AGCAGGGTATCCACAACAGG |
| LNCaP_Neg_R | CTGTTCCCTGCCTAGCAAAG |

ChIP-re-ChIP in LNCaP cells

| | |
|-------------|----------------------|
| HK2_Myc-F | CTTCTGCAGCGCGAGTTC |
| HK2_Myc-R | GAACCGCTCGTCTCCTACAC |
| LNCaP_Neg_F | AGCAGGGTATCCACAACAGG |
| LNCaP_Neg_R | CTGTTCCCTGCCTAGCAAAG |

ChIP-qPCR in mouse prostate

| | |
|---------------|----------------------|
| Neg_control-F | CATGGAGCAACAAGTCAGGA |
| Neg_control-R | GACAGCACGAGACAGCTCAC |
| Nfkb1-F | TGCCCTGCTTCATCTTTTCT |
| Nfkb1-R | GAAGGACGGGTGAAAGGAAC |
| Cpeb3-F | GGGGCTGTAATGTGAATGGA |
| Cpeb3-R | GTACAGCGCTGGCATAGTGA |
| Cab39l-F | GTGCCCTTCTGCTCAGAGAC |
| Cab39l-R | AAGCCTCAGGGGAACAGAAG |
| Nucb2-F | GGCTTGGGCTTTTGAAAAC |
| Nucb2-R | GAATGGCTCCCTTAGGTTCA |
| Slc31a1-F | GAAGTCCAAGTGCAGATGA |
| Slc31a1-R | TTCAACATTCCGACCTTTCC |
| Sgef-F | GGACACAGGGAGTTTCTTCC |
| Sgef-R | GCAGACGCCCCTACAATAAA |
| Hk2-F | AGCCCTAATGTTCCCCAGAG |
| Hk2-R | TGACACGGAAGTGAAGTCAC |

ChIP-PCR in Myc-CaP

| | |
|---------|-------------------------|
| Hk2-F | CTCAGAGGAAGAGACAGCTG |
| Hk2-R | CTCCTTGACTGGGTCAGTG |
| Id1-F | TGGAGAGGTAGCTGAGAGTT |
| Id1-R | GCTCTGATGTCCCATATGCT |
| Mt2-F | CTGGCCATATCCCTTGAGC |
| Mt2-R | AGTTCTAGGAGCGTGATGGA |
| Mt3-F | TCTGCCCCAACCTAGAAACCT |
| Mt3-R | TAGCCTGGATTCTCCTCCAG |
| Prdx6-F | CCACTGACTTCCTATTTCTAAGC |
| Prdx6-R | CTCCTTTCTCCAGAGTAGTTGG |
| Sept9-F | GGCTGACAGAAGACCAAGATG |
| Sept9-R | TGTGCCAATCCAGAATCACG |
| Txnip-F | GGAATGGCTCCAACCAGATA |
| Txnip-R | CTCTCGGTGCTTTGACTTGT |
| Utrn-F | CCATACTAAGGATGCAGGCATG |
| Utrn-R | CAGTGTAAGCTCCAAGACAGC |

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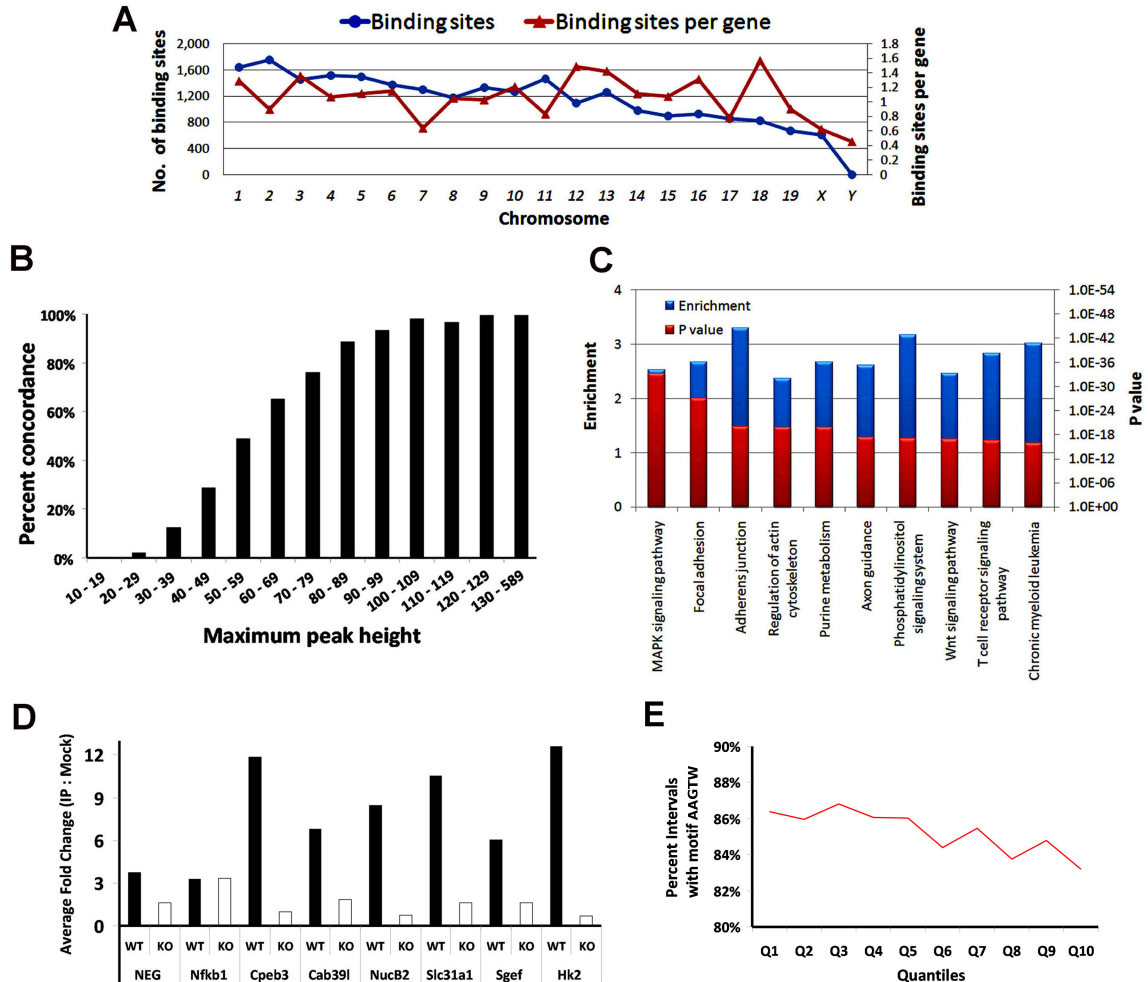


Figure S1 (related to Figures 1 and 2). ChIP-Seq Analysis of Nkx3.1 in Mouse Prostate

(A) Distribution of Nkx3.1 binding sites (intervals) across all mouse chromosomes shown as number of binding sites and as number of binding sites per gene.

(B) Graph showing that concordance among biological replicates (percent concordance) increases with increasing ChIP-seq peak height of the bound region.

(C) Gene ontology analysis of pathways enriched in Nkx3.1 bound genes by WebGestalt.

(D) Confirmation of selected Nkx3.1 binding sites by ChIP-qPCR analysis. Results are shown as fold enrichment relative to IgG control. (E) Percent of all intervals with the refined consensus Nkx3.1 binding motif AAGTW.

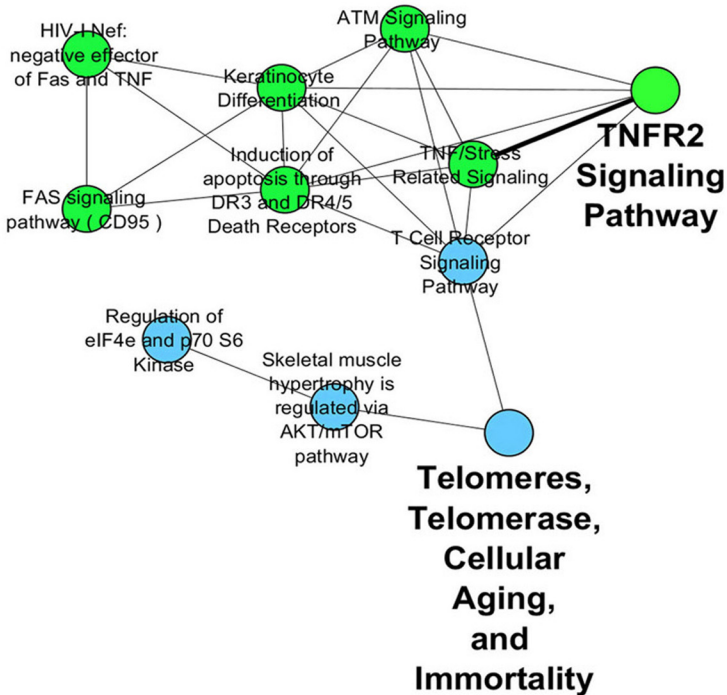
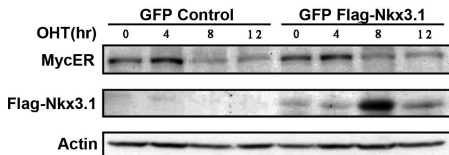


Figure S3 (related to Figure 4). Co-regulation of a Common Set of Target Genes by c-Myc and Nkx3.1

We used ClueGO to uncover functional associations among the 65 Nkx3.1/Myc target genes identified by GeneGO Network analysis. Sizes of the circles are proportional to the number of genes in each node.

A DKO MycER Cells



B LNCaP MycER Cells

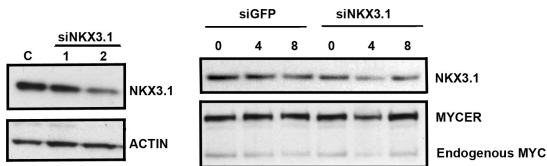


Figure S4 (related to Figure 5). Modulation of NKX3.1 Expression in Inducible MycER cells

(A) Expression of the indicated proteins determined by western blot analysis in DKO-MycER cells transfected with Flag-tagged Nkx3.1-expressing lentivirus co-expressing GFP and treated with 4-hydroxytamoxifen (4-OHT).

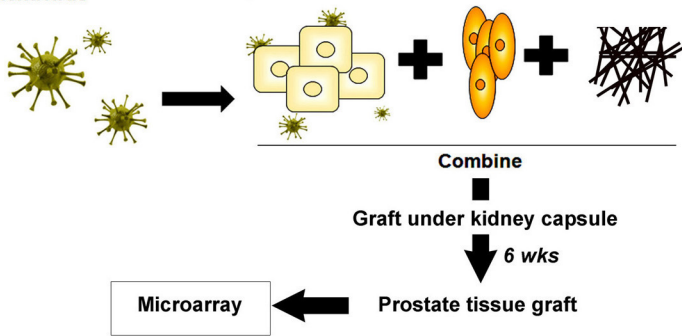
(B) Expression of NKX3.1 knockdown in LNCaP-MycER cells before tamoxifen induction. C denoted Control; 1 and 2 are different siRNA transfection conditions.

Expression of the indicated proteins determined by western blot analysis in LNCaP-MycER cells transfected with NKX3.1 or GFP siRNA and treated with 4-hydroxytamoxifen (4-OHT).

AControl or MYC
lentivirusInfected mouse
prostate cells

Rat UGM

Collagen

**B**

Control prostate graft

MYC prostate graft

Nkx3.1

Nkx3.1

MYC/pHH3

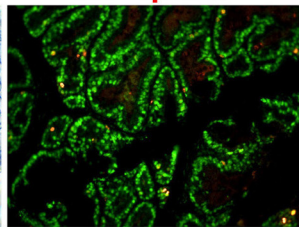
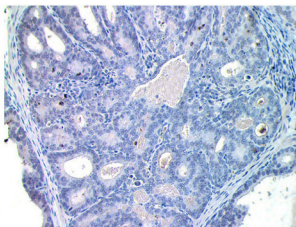
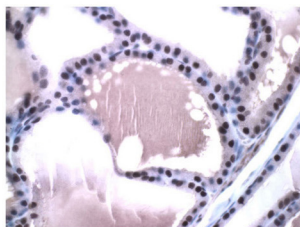


Figure S5 (related to Figure 6). Gene Expression Analysis of c-MYC-expressing regenerated prostate grafts

(A) Tissue recombination scheme used to generate prostate grafts overexpressing c-MYC (41).. Adult mouse prostate epithelial cells were isolated and infected with control lentivirus or MYC-expressing lentivirus. Cells were combined with fetal rat urogenital mesenchyme and collagen and grafted under the kidney capsule. Six weeks later, prostate grafts were taken and processed for microarray analysis.

(B) Immunohistochemical analysis of graft tissues used in microarray analysis shows loss of Nkx3.1 protein (brown) in c-MYC grafts compared to control grafts. c-MYC grafts show elevated expression of mitotic marker phospho-histone H3 (pHH3, red) and c-MYC protein (green).

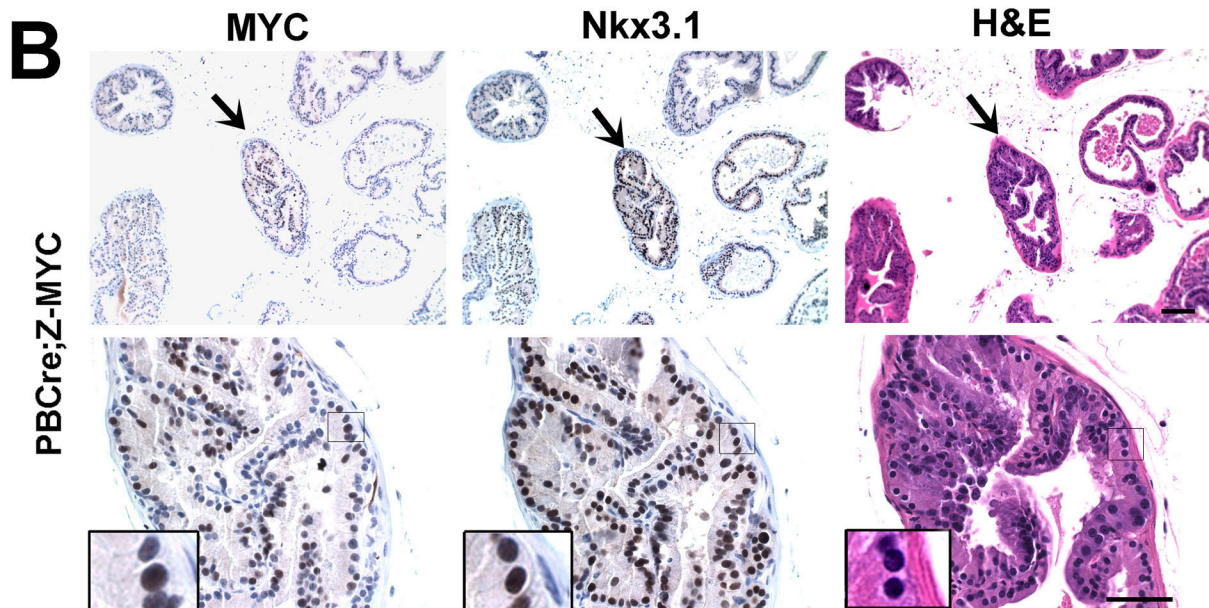
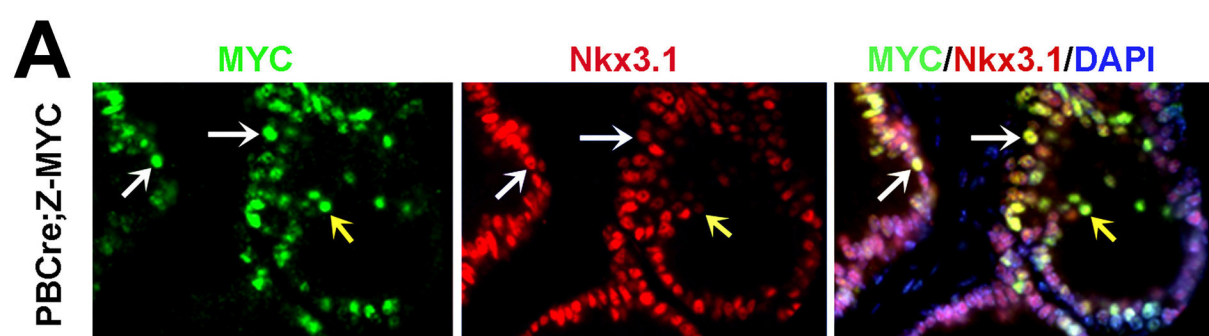


Figure S6 (related to Figures 7 and 8). c-MYC overexpression does not directly suppress Nkx3.1 expression in prostate cells.

(A) Double immunofluorescence for MYC (green) and Nkx3.1 performed on a 57-wk old *PBCre;Z-MYC* transgenic mouse prostate. Note cells co-expressing c-MYC and Nkx3.1 (white arrows) as well as other c-MYC-expressing cells in which Nkx3.1 is downregulated (yellow arrows). Sections were counter-stained for DNA with DAPI (blue).

(C) Adjacent prostate sections from a 37-wk old *PBCre;Z-MYC* transgenic mouse were stained for MYC (brown) or Nkx3.1 (brown) or by H&E. MYC and Nkx3.1-stained sections were counterstained with hematoxylin. Arrows point to a gland overexpressing MYC while retaining robust Nkx3.1 expression. Scale bars, 50 mm.

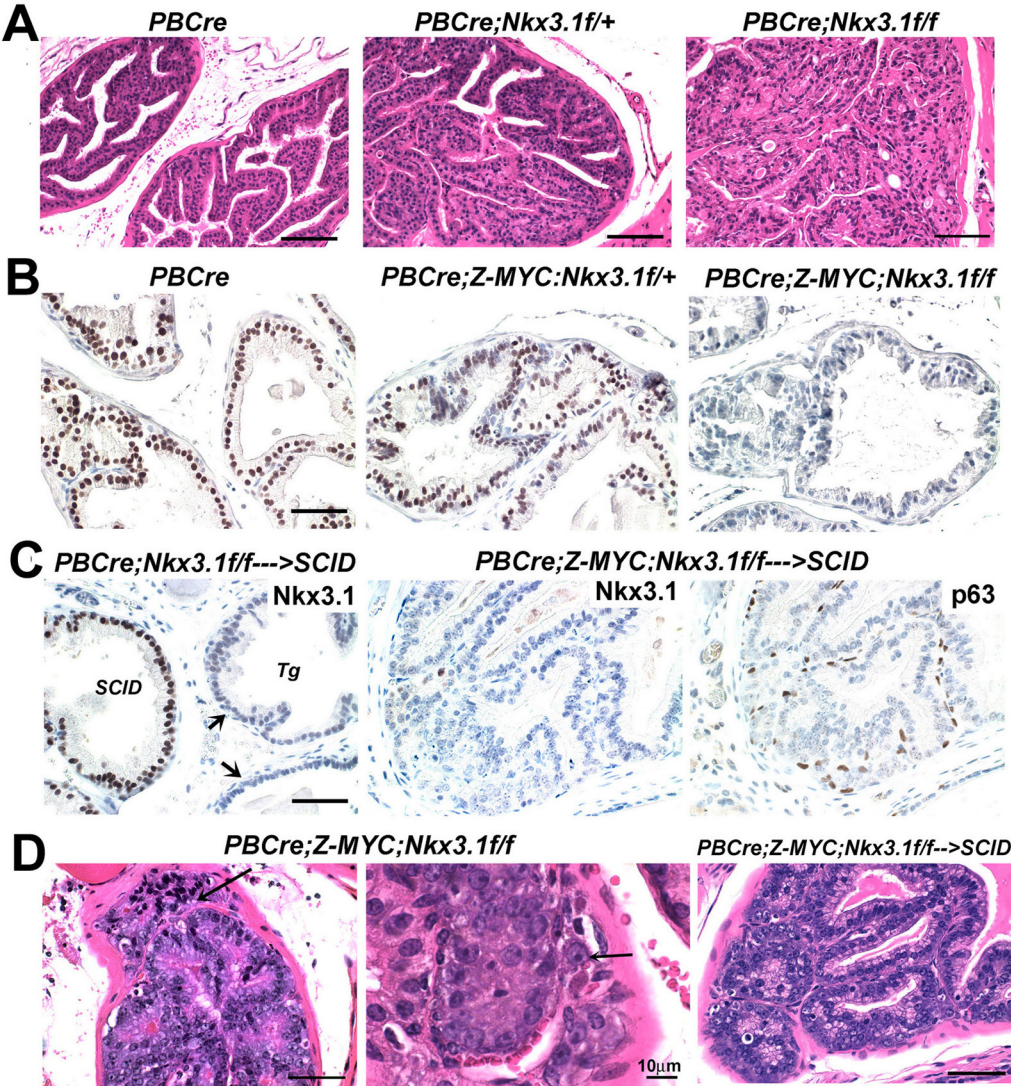


Figure S7 (related to Figures 7 and 8). Further characterization of conditional *MYC/Nkx3.1* mutant mice

(A) H&E staining showing prostate epithelial hyperplasia in conditional *Nkx3.1* mutant mice. Shown are sections from mice at 35 weeks of age. Scale bar, 100mm

(B) Immunohistochemical analysis shows loss of *Nkx3.1* expression (brown) in conditional homozygous *Nkx3.1*-deficient prostates.

(C) Loss of *Nkx3.1* (brown) in regenerated prostate orthotopic grafts of the indicated genotypes. Arrows indicate transgenic tissue (Tg) next to SCID host prostate gland. p63 staining marks basal cells in HGPIN lesion from *PBCre;Z-MYC;Nkx3.1f/f* regenerated prostate. Scale bars in "B" and "C", 50mm

(D) Additional examples of lesions in regular and regenerated *PBCre;Z-MYC;Nkx3.1f/f* mouse prostates with areas of possible microinvasion (arrows). Scale bars, 50mm except where indicated otherwise.

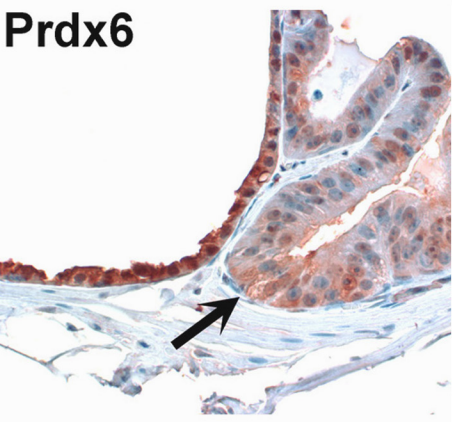
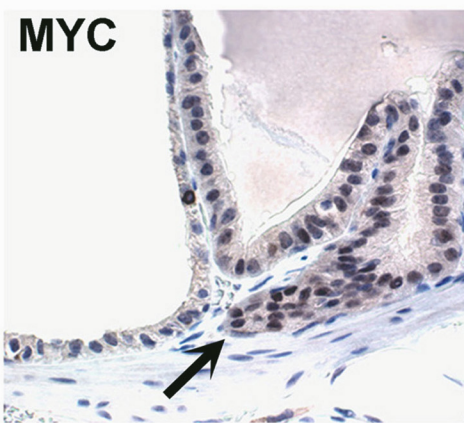
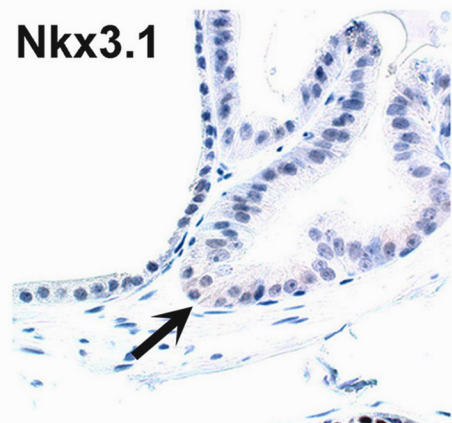
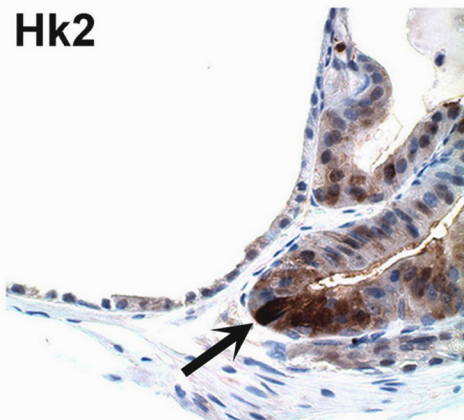
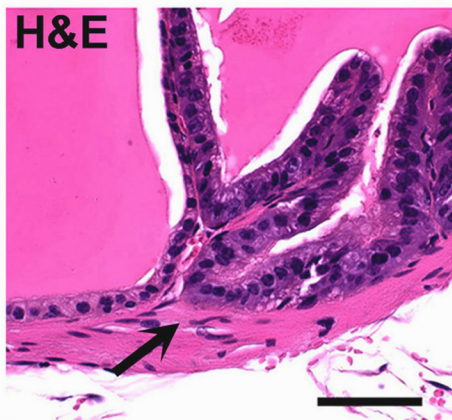


Figure S8 (related to Figure 8).

Expression analysis of Nkx3.1/MyC target genes in vivo

Adjacent sections from 10 week *PBCre;Z-MYC;Nkx3.1^{f/f}* prostate grafts show upregulation of Nedd4l and Hk2 and downregulation of Prdx6 expression in HGPIN lesion with MYC overexpression and loss of Nkx3.1 (arrows). H&E, Hematoxylin and eosin stain. Scale bars, 50 μ m.

| | <i>Binding</i> | | | | <i>Regulation</i> | | | |
|---------------|----------------|---------------|-------------|-------------|--------------------|------------------|------------------|--------------|
| Experiment | ChIP Nkx3.1 | ChIP Myc | ChIP NKX3.1 | ChIP MYC | Microarray-Mouse | Microarray-Mouse | Microarray-Human | IHC |
| Cell/tissue | Mouse prostate | Myc-CaP cells | LNCaP cells | LNCaP cells | Nkx3.1 KO prostate | MYC TR prostate | LNCaP MYC siRNA | MYC;Nk3.1 Tg |
| Genes: | | | | | | | | |
| <i>Hk2</i> | X | X | X | X | X | X | X | X |
| <i>Igf1r</i> | X | | X | X | X | X | X | |
| <i>Nedd4l</i> | X | | | | X | X | X | X |
| <i>Prdx6</i> | X | X | X | X | X | X | | X |
| <i>Txnip</i> | X | X | | | X | X | X | |
| <i>Mt2</i> | X | X | | | X | X | X | |
| <i>Mt3</i> | X | X | | | X | X | | |
| <i>Utrn</i> | X | X | X | X | X | X | X | |
| <i>Asns</i> | X | | X | X | X | | X | |
| <i>Ctsb</i> | X | | X | X | X | | | |
| <i>Hdlbp</i> | X | | X | X | X | | | |

Figure S9 (related to Figure 9). Summary of Nkx3.1/Myc target gene experimental validation studies.
 TR, tissue recombination. Tg, transgenic mouse prostate. IHC, immunohistochemistry.

Table S1: Nkx3.1 ChIP-seq tag statistics from wild type and Nkx3.1-mutant prostates

| | KO | WT1 | WT2 | HET1 | HET2 |
|-------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Total reads | 27587425 | 24425179 | 27404459 | 21012401 | 23660370 |
| Total quality-filtered | 18243796 | 18329586 | 18378926 | 16291886 | 17755926 |
| Non-matching | 489967 | 773393 | 459581 | 441689 | 494693 |
| QC culled (too many Ns) | 7730 | 7408 | 7652 | 3689 | 4341 |
| Non-unique | 2817702 | 2321921 | 2595661 | 2015287 | 2285825 |
| Contamination filter | 390249 | 399353 | 167513 | 223025 | 330220 |
| Uniquely mapped | 14538148 | 14827511 | 15148519 | 13608196 | 14640847 |

Table S2. Distribution of Nkx3.1 Binding Sites

| | WT1 | WT2 | HET1 | HET2 | ALL |
|-----------------------------------|-------------------|-------------------|--------------------|--------------------|--------------------|
| No. intervals. Total | 14608 | 11045 | 17720 | 17769 | 61142 |
| No. (%) intervals near genes | 9556 (65%) | 6972 (63%) | 11495 (65%) | 11802 (66%) | 39825 (65%) |
| No. (%) intervals NOT near genes | 5052 (35%) | 4073 (37%) | 6225 (35%) | 5967 (34%) | 21317 (35%) |
| No. intervals near genes | 9556 | 6972 | 11495 | 11802 | 39825 |
| No. (%) intervals -10 Kb to -2 Kb | 1075 (11%) | 795 (11%) | 1298 (11%) | 1356 (11%) | 4524 (11%) |
| No. (%) intervals -2 Kb to TSS | 476 (5%) | 243 (3%) | 589 (5%) | 785 (7%) | 2093 (5%) |
| No. (%) intervals TSS to +2 Kb | 272 (3%) | 177 (3%) | 331 (3%) | 375 (3%) | 1155 (3%) |
| No. (%) intervals +2 Kb to TES | 6547 (69%) | 4952 (71%) | 7826 (68%) | 7774 (66%) | 27099 (68%) |
| No. (%) intervals TES to +10 Kb | 1186 (12%) | 805 (12%) | 1451 (13%) | 1512 (13%) | 4954 (12%) |

Table S3: Known transcription factor motifs identified by Genomatix Software in Nkx3.1 intervals. TFs with at least one motif in >80% of intervals are shown.

All Targets

| Family | Description | % Seq w motif | No. Seq w motif | P value |
|---------|--|---------------|-----------------|----------|
| V\$FKHD | Forkhead domain factors | 94% | 940 | 1.58E-40 |
| V\$ABDB | Abdominal-B type homeodomain | 93% | 931 | 8.64E-44 |
| V\$HOMF | Homeodomain transcription factor | 93% | 930 | 1.31E-06 |
| V\$GATA | GATA binding factors | 93% | 926 | 4.76E-50 |
| V\$SORY | Sox/Sry box factors | 91% | 909 | 3.51E-10 |
| V\$OCT1 | Octamer binding protein | 91% | 908 | 2.90E-16 |
| O\$VTBP | TATA binding factors | 90% | 900 | 5.13E-41 |
| V\$EV1 | EV1 myeloid transforming proteins | 87% | 871 | 1.40E-10 |
| V\$NKXH | NKX homeodomain sites | 86% | 862 | 4.11E-15 |
| V\$HOXF | Factors with moderate activity to homedomain | 86% | 961 | 3.36E-13 |
| V\$BRNF | Brn POU-domain factors | 85% | 850 | 2.99E-22 |
| V\$HNF1 | Hepatic nuclear factor 1 | 82% | 819 | 4.80E-17 |
| V\$MYT1 | Myt1 zinc finger proteins | 82% | 818 | 9.76E-18 |
| V\$HAND | Twist subfamily of class B bHLH transcription factor | 81% | 811 | 1.63E-02 |

Direct Targets

| Family | Description | % Seq w motif | No. Seq w motif | P value |
|---------|--|---------------|-----------------|----------|
| V\$FKHD | Forkhead domain factors | 96% | 963 | 2.18E-51 |
| V\$HOMF | Homeodomain transcription factor | 94% | 941 | 1.08E-09 |
| V\$SORY | Sox/Sry box factors | 93% | 930 | 3.26E-17 |
| V\$GATA | GATA binding factors | 93% | 925 | 2.03E-49 |
| V\$ABDB | Abdominal-B type homeodomain | 92% | 920 | 1.91E-37 |
| V\$OCT1 | Octamer binding protein | 92% | 917 | 1.27E-19 |
| O\$VTBP | TATA binding factors | 91% | 911 | 4.36E-47 |
| V\$NKXH | NKX homeodomain sites | 89% | 892 | 1.48E-25 |
| V\$EV1 | EV1 myeloid transforming proteins | 89% | 891 | 2.58E-16 |
| V\$HOXF | Factors with moderate activity to homedomain | 87% | 870 | 8.87E-16 |
| V\$BRNF | Brn POU-domain factors | 86% | 864 | 2.18E-27 |
| V\$HNF1 | Hepatic nuclear factor 1 | 85% | 853 | 2.20E-28 |
| V\$HBOX | Homeobox transcription factors | 82% | 818 | 7.09E-22 |
| V\$MYT1 | Myt1 zinc finger proteins | 81% | 812 | 4.56E-16 |
| V\$LHXF | LIM homeodomain factors | 81% | 807 | 5.26E-27 |

Table S4: List of Direct Nkx3.1 Target Genes. (Activated genes are down in the KO prostate while repressed genes are up)

| <u>Gene Symbol</u> | <u>Regulation</u> | <u>Gene Title</u> |
|---------------------------|--------------------------|---|
| 1110003E01Rik | Activated | RIKEN cDNA 1110003E01 gene |
| 1600029D21Rik | Activated | RIKEN cDNA 1600029D21 gene |
| Aass | Activated | aminoadipate-semialdehyde synthase |
| Abcc3 | Activated | ATP-binding cassette, sub-family C (CFTR/MRP), member 3 |
| Acrbp | Activated | proacrosin binding protein |
| Acvr2b | Activated | activin receptor IIB |
| Adcy9 | Activated | adenylate cyclase 9 |
| Agr2 | Activated | anterior gradient 2 (Xenopus laevis) |
| Alcam | Activated | activated leukocyte cell adhesion molecule |
| Aldh2 | Activated | aldehyde dehydrogenase 2, mitochondrial |
| Aldh3a2 | Activated | aldehyde dehydrogenase family 3, subfamily A2 |
| Ang | Activated | angiogenin, ribonuclease, RNase A family, 5 |
| Ank3 | Activated | ankyrin 3, epithelial |
| Ankrd28 | Activated | ankyrin repeat domain 28 |
| Asl | Activated | argininosuccinate lyase |
| Asna1 | Activated | arsA arsenite transporter, ATP-binding, homolog 1 (bacterial) |
| Asns | Activated | asparagine synthetase |
| Atf3 | Activated | activating transcription factor 3 |
| Atf5 | Activated | activating transcription factor 5 |
| Atp13a3 | Activated | ATPase type 13A3 |
| Bcas3 | Activated | breast carcinoma amplified sequence 3 |
| Bckdhb | Activated | branched chain ketoacid dehydrogenase E1, beta polypeptide |
| Bsdcl | Activated | BSD domain containing 1 |
| Cacnb3 | Activated | calcium channel, voltage-dependent, beta 3 subunit |
| Car2 | Activated | carbonic anhydrase 2 |
| Casp6 | Activated | caspase 6 |
| Cdk5rap3 | Activated | CDK5 regulatory subunit associated protein 3 |
| Ceacam1 | Activated | carcinoembryonic antigen-related cell adhesion molecule 1 |
| Cflar | Activated | CASP8 and FADD-like apoptosis regulator |
| Chchd3 | Activated | coiled-coil-helix-coiled-coil-helix domain containing 3 |
| Commd1 | Activated | COMM domain containing 1 |
| Comt1 | Activated | catechol-O-methyltransferase 1 |

| | | |
|------------|------------------|---|
| Csrp2 | Activated | cysteine and glycine-rich protein 2 |
| Ctage5 | Activated | CTAGE family, member 5 |
| Cyp7b1 | Activated | cytochrome P450, family 7, subfamily b, polypeptide 1 |
| D17Wsu92e | Activated | DNA segment, Chr 17, Wayne State University 92, expressed |
| D2Ertd391e | Activated | DNA segment, Chr 2, ERATO Doi 391, expressed |
| Daxx | Activated | Fas death domain-associated protein |
| Dbt | Activated | dihydrolipoamide branched chain transacylase E2 |
| Ddit3 | Activated | DNA-damage inducible transcript 3 |
| Ddit4 | Activated | DNA-damage-inducible transcript 4 |
| Deb1 | Activated | differentially expressed in B16F10 1 |
| Degs2 | Activated | degenerative spermatocyte homolog 2 (Drosophila), lipid desaturase |
| Dhrs4 | Activated | dehydrogenase/reductase (SDR family) member 4 |
| Dnajc2 | Activated | DnaJ (Hsp40) homolog, subfamily C, member 2 |
| Dnajc3 | Activated | DnaJ (Hsp40) homolog, subfamily C, member 3 |
| Ebp | Activated | phenylalkylamine Ca ²⁺ antagonist (emopamil) binding protein |
| Edem2 | Activated | ER degradation enhancer, mannosidase alpha-like 2 |
| Emcn | Activated | endomucin |
| Exosc5 | Activated | exosome component 5 |
| Fam171a1 | Activated | family with sequence similarity 171, member A1 |
| Fam98a | Activated | family with sequence similarity 98, member A |
| Fgf1 | Activated | fibroblast growth factor 1 |
| Fut8 | Activated | fucosyltransferase 8 |
| Fut9 | Activated | fucosyltransferase 9 |
| Gars | Activated | glycyl-tRNA synthetase |
| Gpld1 | Activated | glycosylphosphatidylinositol specific phospholipase D1 |
| Gpr37l1 | Activated | G protein-coupled receptor 37-like 1 |
| Gpx2 | Activated | glutathione peroxidase 2 |
| Gramd3 | Activated | GRAM domain containing 3 |
| Grhpr | Activated | glyoxylate reductase/hydroxypyruvate reductase |
| Gtf2ird2 | Activated | GTF2I repeat domain containing 2 |
| Guk1 | Activated | guanylate kinase 1 |
| Hdlbp | Activated | high density lipoprotein (HDL) binding protein |
| Herpud1 | Activated | homocysteine-inducible, endoplasmic reticulum stress-inducible, ubiquitin-like domain 1 |
| Hsp90ab1 | Activated | heat shock protein 90 alpha (cytosolic), class B member 1 |

| | | |
|----------|------------------|---|
| Hspa5 | Activated | heat shock protein 5 |
| Iars | Activated | isoleucine-tRNA synthetase |
| Ica1 | Activated | islet cell autoantigen 1 |
| Il1r2 | Activated | interleukin 1 receptor, type II |
| Inpp5b | Activated | inositol polyphosphate-5-phosphatase B |
| Ints3 | Activated | integrator complex subunit 3 |
| Ipo7 | Activated | importin 7 |
| Itpk1 | Activated | inositol 1,3,4-triphosphate 5/6 kinase |
| Itpr2 | Activated | inositol 1,4,5-triphosphate receptor 2 |
| Jarid2 | Activated | jumonji, AT rich interactive domain 2 |
| Kalrn | Activated | kalirin, RhoGEF kinase |
| Krt8 | Activated | keratin 8 |
| Lars | Activated | leucyl-tRNA synthetase |
| Limch1 | Activated | LIM and calponin homology domains 1 |
| Lmo7 | Activated | LIM domain only 7 |
| Man1a | Activated | mannosidase 1, alpha |
| Mars | Activated | methionine-tRNA synthetase |
| Mcoln2 | Activated | mucolipin 2 |
| Mmp7 | Activated | matrix metalloproteinase 7 |
| Mrpl50 | Activated | mitochondrial ribosomal protein L50 |
| Mrpl9 | Activated | mitochondrial ribosomal protein L9 |
| Mt1 | Activated | metallothionein 1 |
| Mt2 | Activated | metallothionein 2 |
| Mt3 | Activated | metallothionein 3 |
| Mthfd2 | Activated | methylenetetrahydrofolate dehydrogenase methenyltetrahydrofolate cyclohydrolase |
| Mvd | Activated | mevalonate (diphospho) decarboxylase |
| Ndubfab1 | Activated | NADH dehydrogenase (ubiquinone) 1, alpha/beta subcomplex, 1 |
| Nkain1 | Activated | Na ⁺ /K ⁺ transporting ATPase interacting 1 |
| Nkx3-1 | | NK-3 transcription factor, locus 1 (Drosophila) |
| Pabpc1 | Activated | poly(A) binding protein, cytoplasmic 1 |
| Pbrm1 | Activated | polybromo 1 |
| Pbsn | Activated | probasin |
| Pcf11 | Activated | cleavage and polyadenylation factor subunit homolog (S. cerevisiae) |
| Pck2 | Activated | phosphoenolpyruvate carboxykinase 2 (mitochondrial) |

| | | |
|---------------|------------------|---|
| Pcmt1 | Activated | protein-L-isoaspartate (D-aspartate) O-methyltransferase 1 |
| Pctk3 | Activated | PCTAIRE-motif protein kinase 3 |
| Pdcd4 | Activated | programmed cell death 4 |
| Pde9a | Activated | phosphodiesterase 9A |
| Pepd | Activated | peptidase D |
| Pex14 | Activated | peroxisomal biogenesis factor 14 |
| Phyh | Activated | phytanoyl-CoA hydroxylase |
| Pias2 | Activated | protein inhibitor of activated STAT 2 |
| Piga | Activated | phosphatidylinositol glycan anchor biosynthesis, class A |
| Plk3 | Activated | polo-like kinase 3 (Drosophila) |
| Pnliprp1 | Activated | pancreatic lipase related protein 1 |
| Ppif | Activated | peptidylprolyl isomerase F (cyclophilin F) |
| Pppde2 | Activated | PPPDE peptidase domain containing 2 |
| Prdx6 | Activated | peroxiredoxin 6 |
| Prkca | Activated | protein kinase C, alpha |
| Psat1 | Activated | phosphoserine aminotransferase 1 |
| Psma4 | Activated | proteasome (prosome, macropain) subunit, alpha type 4 |
| Pten | Activated | phosphatase and tensin homolog |
| Ptger3 | Activated | prostaglandin E receptor 3 (subtype EP3) |
| Ptgs1 | Activated | prostaglandin-endoperoxide synthase 1 |
| Ptov1 | Activated | prostate tumor over expressed gene 1 |
| Rab3d | Activated | RAB3D, member RAS oncogene family |
| Rbm17 | Activated | RNA binding motif protein 17 |
| Ren1 /// Ren2 | Activated | renin 1 structural /// renin 2 tandem duplication of Ren1 |
| Rit1 | Activated | Ras-like without CAAX 1 |
| Rnase1 | Activated | ribonuclease, RNase A family, 1 (pancreatic) |
| Rnase4 | Activated | ribonuclease, RNase A family 4 |
| Rrbp1 | Activated | ribosome binding protein 1 |
| Sec14l2 | Activated | SEC14-like 2 (S. cerevisiae) |
| Slc16a2 | Activated | solute carrier family 16 (monocarboxylic acid transporters), member 2 |
| Slc1a5 | Activated | solute carrier family 1 (neutral amino acid transporter), member 5 |
| Slc25a28 | Activated | solute carrier family 25, member 28 |
| Slc38a10 | Activated | solute carrier family 38, member 10 |
| Slc39a4 | Activated | solute carrier family 39 (zinc transporter), member 4 |

| | | |
|---------------|------------------|--|
| Slc3a2 | Activated | solute carrier family 3 (activators of dibasic and neutral amino acid transport), member 2 |
| Spin1 | Activated | spindlin 1 |
| Strbp | Activated | spermatid perinuclear RNA binding protein |
| Tars | Activated | threonyl-tRNA synthetase |
| Thop1 | Activated | thimet oligopeptidase 1 |
| Tm9sf3 | Activated | transmembrane 9 superfamily member 3 |
| Tmprss2 | Activated | transmembrane protease, serine 2 |
| Tnks1bp1 | Activated | tankyrase 1 binding protein 1 |
| Traf1 | Activated | TRAF type zinc finger domain containing 1 |
| Tusc2 | Activated | tumor suppressor candidate 2 |
| Ufm1 | Activated | ubiquitin-fold modifier 1 |
| Ugcg | Activated | UDP-glucose ceramide glucosyltransferase |
| Unc13b | Activated | unc-13 homolog B (C. elegans) |
| Uso1 | Activated | USO1 homolog, vesicle docking protein (yeast) |
| Uty | Activated | ubiquitously transcribed tetratricopeptide repeat gene, Y chromosome |
| Wbp5 | Activated | WW domain binding protein 5 |
| Yars | Activated | tyrosyl-tRNA synthetase |
| Yes1 | Activated | Yamaguchi sarcoma viral (v-yes) oncogene homolog 1 |
| Zcchc3 | Activated | zinc finger, CCHC domain containing 3 |
| 4933426M11Rik | Repressed | RIKEN cDNA 4933426M11 gene |
| Ablim1 | Repressed | actin-binding LIM protein 1 |
| Ace | Repressed | angiotensin I converting enzyme (peptidyl-dipeptidase A) 1 |
| Actr3 | Repressed | ARP3 actin-related protein 3 homolog (yeast) |
| Adrb2 | Repressed | adrenergic receptor, beta 2 |
| Alas1 | Repressed | aminolevulinic acid synthase 1 |
| Angpt2 | Repressed | angiopoietin 2 |
| Anxa2 | Repressed | annexin A2 |
| Apaf1 | Repressed | apoptotic peptidase activating factor 1 |
| Apcdd1 | Repressed | adenomatosis polyposis coli down-regulated 1 |
| Arsa | Repressed | arylsulfatase A |
| Btg3 | Repressed | B-cell translocation gene 3 /// B-cell translocation gene 3 pseudogene |
| Cbx5 | Repressed | chromobox homolog 5 (Drosophila HP1a) |
| Ccnb2 | Repressed | cyclin B2 |
| Cd164 | Repressed | CD164 antigen |

| | | |
|----------|-----------|---|
| Cd52 | Repressed | CD52 antigen |
| Cenpv | Repressed | centromere protein V |
| Ces3 | Repressed | carboxylesterase 3 |
| Chpt1 | Repressed | choline phosphotransferase 1 |
| Cited2 | Repressed | Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2 |
| Clic4 | Repressed | chloride intracellular channel 4 (mitochondrial) |
| Cmas | Repressed | cytidine monophospho-N-acetylneuraminic acid synthetase |
| Col4a5 | Repressed | collagen, type IV, alpha 5 |
| Col6a3 | Repressed | collagen, type VI, alpha 3 |
| Cpe | Repressed | carboxypeptidase E /// similar to carboxypeptidase E |
| Ctsb | Repressed | cathepsin B |
| Cugbp2 | Repressed | CUG triplet repeat, RNA binding protein 2 |
| Cx3cl1 | Repressed | chemokine (C-X3-C motif) ligand 1 |
| Dek | Repressed | DEK oncogene (DNA binding) |
| Dnajc8 | Repressed | DnaJ (Hsp40) homolog, subfamily C, member 8 |
| Eid1 | Repressed | EP300 interacting inhibitor of differentiation 1 |
| Eif2b5 | Repressed | eukaryotic translation initiation factor 2B, subunit 5 epsilon |
| Enpp2 | Repressed | ectonucleotide pyrophosphatase/phosphodiesterase 2 |
| Fam105a | Repressed | family with sequence similarity 105, member A |
| Fam3b | Repressed | family with sequence similarity 3, member B |
| Frmd6 | Repressed | FERM domain containing 6 |
| Gad2 | Repressed | glutamic acid decarboxylase 2 |
| Gadd45a | Repressed | growth arrest and DNA-damage-inducible 45 alpha |
| Gfra2 | Repressed | glial cell line derived neurotrophic factor family receptor alpha 2 |
| Ggt1 | Repressed | gamma-glutamyltransferase 1 |
| Gm7035 | Repressed | predicted gene 7035 |
| Gstk1 | Repressed | glutathione S-transferase kappa 1 |
| H2-DMb1 | Repressed | histocompatibility 2, class II, locus Mb1 |
| H2-Q1 | Repressed | histocompatibility 2, Q region locus 1 |
| Hk2 | Repressed | hexokinase 2 |
| Hopx | Repressed | HOP homeobox |
| Hsd17b11 | Repressed | hydroxysteroid (17-beta) dehydrogenase 11 |
| Htra2 | Repressed | HtrA serine peptidase 2 |
| Id1 | Repressed | inhibitor of DNA binding 1 |

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|----------|-----------|---|
| Igf1r | Repressed | insulin-like growth factor I receptor |
| Igh | Repressed | Immunoglobulin heavy chain complex |
| Il17ra | Repressed | interleukin 17 receptor A |
| Inmt | Repressed | indolethylamine N-methyltransferase |
| Itm2b | Repressed | integral membrane protein 2B |
| Ivns1abp | Repressed | influenza virus NS1A binding protein |
| Kcnj15 | Repressed | potassium inwardly-rectifying channel, subfamily J, member 15 |
| Klf3 | Repressed | Kruppel-like factor 3 (basic) |
| Lamp2 | Repressed | lysosomal-associated membrane protein 2 |
| Lbr | Repressed | lamin B receptor |
| Lifr | Repressed | leukemia inhibitory factor receptor |
| Lmo2 | Repressed | LIM domain only 2 |
| Lpgat1 | Repressed | lysophosphatidylglycerol acyltransferase 1 |
| Mat2a | Repressed | methionine adenosyltransferase II, alpha |
| Mdfic | Repressed | MyoD family inhibitor domain containing |
| Med12 | Repressed | mediator of RNA polymerase II transcription, subunit 12 homolog (yeast) |
| Mll1 | Repressed | myeloid/lymphoid or mixed-lineage leukemia 1 |
| Mllt3 | Repressed | myeloid/lymphoid or mixed-lineage leukemia translocated to, 3 |
| Mrps18a | Repressed | mitochondrial ribosomal protein S18A |
| Mttnr9 | Repressed | myotubularin related protein 9 |
| Mx1 | Repressed | myxovirus (influenza virus) resistance 1 |
| Nampt | Repressed | nicotinamide phosphoribosyltransferase |
| Nedd4l | Repressed | neural precursor cell expressed, developmentally down-regulated gene 4-like |
| Nfkb1 | Repressed | nuclear factor of kappa light polypeptide gene enhancer in B-cells 1, p105 |
| Notch2 | Repressed | Notch gene homolog 2 (Drosophila) |
| Npc2 | Repressed | Niemann Pick type C2 |
| Nr1d2 | Repressed | nuclear receptor subfamily 1, group D, member 2 |
| Nrsn1 | Repressed | neurensin 1 |
| Nucb2 | Repressed | nucleobindin 2 |
| Oit1 | Repressed | oncoprotein induced transcript 1 |
| Osbpl1a | Repressed | oxysterol binding protein-like 1A |
| Pcolce | Repressed | procollagen C-endopeptidase enhancer protein |
| Plac8 | Repressed | placenta-specific 8 |
| Pnpt1 | Repressed | polyribonucleotide nucleotidyltransferase 1 |

| | | |
|---------|-----------|---|
| Pdpf | Repressed | pancreatic progenitor cell differentiation and proliferation factor homolog |
| Psmb9 | Repressed | proteasome subunit, beta type 9 (large multifunctional peptidase 2) |
| Ptgr1 | Repressed | prostaglandin reductase 1 |
| Ptpn22 | Repressed | protein tyrosine phosphatase, non-receptor type 22 (lymphoid) |
| Ptpre | Repressed | protein tyrosine phosphatase, receptor type, E |
| Qsox1 | Repressed | quiescin Q6 sulfhydryl oxidase 1 |
| Rab4a | Repressed | RAB4A, member RAS oncogene family |
| Rab7 | Repressed | RAB7, member RAS oncogene family |
| Ramp1 | Repressed | receptor (calcitonin) activity modifying protein 1 |
| Rel1 | Repressed | RELT-like 1 |
| Rock2 | Repressed | Rho-associated coiled-coil containing protein kinase 2 |
| Rplp1 | Repressed | ribosomal protein, large, P1 |
| S100a1 | Repressed | S100 calcium binding protein A1 |
| Sema5a | Repressed | Semaphorin 5A |
| Sept | Repressed | selenoprotein |
| Sept9 | Repressed | sepin 9 |
| Sgk1 | Repressed | serum/glucocorticoid regulated kinase 1 |
| Shisa5 | Repressed | shisa homolog 5 (Xenopus laevis) |
| Six4 | Repressed | sine oculis-related homeobox 4 homolog (Drosophila) |
| Slc30a1 | Repressed | solute carrier family 30 (zinc transporter), member 1 |
| Slc30a4 | Repressed | solute carrier family 30 (zinc transporter), member 4 |
| Slc31a1 | Repressed | solute carrier family 31, member 1 |
| Slc6a8 | Repressed | solute carrier family 6 (neurotransmitter transporter, creatine), member 8 |
| Snx2 | Repressed | sorting nexin 2 |
| Spink3 | Repressed | serine peptidase inhibitor, Kazal type 3 |
| Spred2 | Repressed | sprouty-related, EVH1 domain containing 2 |
| Suds3 | Repressed | suppressor of defective silencing 3 homolog (S. cerevisiae) |
| Tank | Repressed | TRAF family member-associated Nf-kappa B activator |
| Tbc1d14 | Repressed | TBC1 domain family, member 14 |
| Tbc1d24 | Repressed | TBC1 domain family, member 24 |
| Tbce | Repressed | tubulin-specific chaperone E |
| Tmem131 | Repressed | transmembrane protein 131 |
| Tmem56 | Repressed | transmembrane protein 56 |
| Tnfaip8 | Repressed | tumor necrosis factor, alpha-induced protein 8 |

| | | |
|----------|-----------|---|
| Tnfrsf21 | Repressed | tumor necrosis factor receptor superfamily, member 21 |
| Tspan8 | Repressed | tetraspanin 8 |
| Txnip | Repressed | thioredoxin interacting protein |
| Ube2z | Repressed | ubiquitin-conjugating enzyme E2Z (putative) |
| Uqcrc2 | Repressed | ubiquinol cytochrome c reductase core protein 2 |
| Usp24 | Repressed | ubiquitin specific peptidase 24 |
| Utrn | Repressed | utrophin |
| Vcam1 | Repressed | vascular cell adhesion molecule 1 |
| Wars | Repressed | tryptophanyl-tRNA synthetase |
| Wnk2 | Repressed | WNK lysine deficient protein kinase 2 |
| Zfp36 | Repressed | zinc finger protein 36 |
| Zfp36l1 | Repressed | zinc finger protein 36, C3H type-like 1 |

Table S5: GeneGO MetaCORE Network Analysis for Transcription Factor Networks of Direct Nkx3.1 Target Genes

| No | Network | GO Processes | Total nodes | Root nodes | p-Value | zScore | gScore |
|----|------------|--|-------------|------------|------------|--------|--------|
| 1 | SP1 | response to chemical stimulus (43.6%; 2.506e-10), response to copper ion (7.7%; 1.245e-08), negative regulation of interleukin-12 biosynthetic process (5.1%; 1.352e-08), negative regulation of vitamin D biosynthetic process (5.1%; 2.695e-08), negative regulation of vitamin metabolic process (5.1%; 2.695e-08) | 80 | 79 | 3.550E-186 | 125.06 | 125.06 |
| 2 | c-Myc | negative regulation of molecular function (21.1%; 5.612e-10), negative regulation of interleukin-12 biosynthetic process (5.6%; 9.218e-09), negative regulation of vitamin D biosynthetic process (5.6%; 1.838e-08), negative regulation of vitamin metabolic process (5.6%; 1.838e-08), negative regulation of calcidiol 1-monooxygenase activity (5.6%; 3.298e-08) | 73 | 72 | 3.940E-169 | 119.31 | 119.31 |
| 3 | HNF4-alpha | response to hormone stimulus (20.8%; 1.038e-05), response to endogenous stimulus (20.8%; 2.783e-05), response to organic substance (26.4%; 3.025e-05), response to steroid hormone stimulus (15.1%; 3.301e-05), cellular amino acid and derivative metabolic process (15.1%; 7.787e-05) | 55 | 54 | 8.690E-126 | 103.07 | 103.07 |

| | | | | | | | |
|---|-------------------|---|----|----|------------|-------|-------|
| 4 | p53 | apoptosis (38.3%; 7.256e-13), programmed cell death (38.3%; 9.795e-13), cell death (40.4%; 1.289e-12), death (40.4%; 1.498e-12), regulation of apoptosis (40.4%; 6.519e-11) | 48 | 47 | 4.080E-109 | 96.02 | 96.02 |
| 5 | Androgen receptor | negative regulation of interleukin-12 biosynthetic process (10.0%; 8.708e-10), negative regulation of vitamin D biosynthetic process (10.0%; 1.739e-09), negative regulation of vitamin metabolic process (10.0%; 1.739e-09), negative regulation of calcidiol 1-monooxygenase activity (10.0%; 3.124e-09), regulation of vitamin D biosynthetic process (10.0%; 5.199e-09) | 41 | 40 | 1.510E-92 | 88.41 | 88.41 |
| 6 | CREB1 | response to organic substance (39.5%; 4.652e-08), response to chemical stimulus (47.4%; 9.349e-07), regulation of apoptosis (31.6%; 4.839e-06), regulation of programmed cell death (31.6%; 5.470e-06), regulation of cell death (31.6%; 5.762e-06) | 38 | 37 | 1.780E-85 | 84.95 | 84.95 |

| | | | | | | | |
|----|--------------------------------|---|----|----|-----------|-------|-------|
| 7 | ESR1 (nuclear) | negative regulation of interleukin-12 biosynthetic process (12.5%; 3.430e-10), negative regulation of vitamin D biosynthetic process (12.5%; 6.852e-10), negative regulation of vitamin metabolic process (12.5%; 6.852e-10), negative regulation of calcidiol 1-monooxygenase activity (12.5%; 1.232e-09), regulation of vitamin D biosynthetic process (12.5%; 2.050e-09) | 33 | 32 | 9.810E-74 | 78.83 | 78.83 |
| 8 | c-Jun | negative regulation of interleukin-12 biosynthetic process (14.8%; 1.675e-10), negative regulation of vitamin D biosynthetic process (14.8%; 3.347e-10), negative regulation of vitamin metabolic process (14.8%; 3.347e-10), negative regulation of calcidiol 1-monooxygenase activity (14.8%; 6.018e-10), regulation of vitamin D biosynthetic process (14.8%; 1.002e-09) | 28 | 27 | 4.770E-62 | 72.2 | 72.2 |
| 9 | NF-kB | response to chemical stimulus (61.5%; 3.125e-08), response to stress (57.7%; 2.461e-07), response to stimulus (73.1%; 1.742e-06), negative regulation of biological process (53.8%; 2.795e-06), regulation of apoptosis (38.5%; 4.002e-06) | 26 | 26 | 3.800E-61 | 72.17 | 72.17 |
| 10 | RelA (p65 NF-kB subunit) | negative regulation of cellular process (61.5%; 1.031e-08), regulation of interleukin-12 biosynthetic process (15.4%; 1.561e-08), membrane protein intracellular domain proteolysis (15.4%; 3.552e-08), negative regulation of biological process (61.5%; 4.149e-08), negative regulation of apoptosis (34.6%; 6.068e-08) | 27 | 26 | 1.020E-59 | 70.8 | 70.8 |

| | | | | | | | |
|----|------------|---|----|----|-----------|-------|-------|
| 11 | C/EBPalpha | negative regulation of molecular function (42.3%; 2.731e-11), negative regulation of interleukin-12 biosynthetic process (15.4%; 1.427e-10), negative regulation of vitamin D biosynthetic process (15.4%; 2.852e-10), negative regulation of vitamin metabolic process (15.4%; 2.852e-10), negative regulation of calcidiol 1-monooxygenase activity (15.4%; 5.128e-10) | 26 | 25 | 2.170E-57 | 69.38 | 69.38 |
| 12 | HIF1A | negative regulation of interleukin-12 biosynthetic process (16.0%; 1.208e-10), negative regulation of vitamin D biosynthetic process (16.0%; 2.413e-10), negative regulation of vitamin metabolic process (16.0%; 2.413e-10), negative regulation of calcidiol 1-monooxygenase activity (16.0%; 4.340e-10), regulation of vitamin D biosynthetic process (16.0%; 7.226e-10) | 25 | 24 | 4.610E-55 | 67.92 | 67.92 |
| 13 | EGR1 | negative regulation of interleukin-12 biosynthetic process (17.4%; 8.458e-11), negative regulation of vitamin D biosynthetic process (17.4%; 1.690e-10), negative regulation of vitamin metabolic process (17.4%; 1.690e-10), negative regulation of calcidiol 1-monooxygenase activity (17.4%; 3.039e-10), regulation of vitamin D biosynthetic process (17.4%; 5.061e-10) | 23 | 22 | 2.030E-50 | 64.91 | 64.91 |

| | | | | | | | |
|----|-----------|---|----|----|-----------|-------|-------|
| 14 | C/EBPbeta | response to organic substance (50.0%; 1.583e-07), response to endogenous stimulus (40.9%; 2.972e-07), response to chemical stimulus (59.1%; 1.234e-06), response to organic nitrogen (22.7%; 1.334e-06), response to stimulus (77.3%; 1.706e-06) | 22 | 21 | 4.230E-48 | 63.35 | 63.35 |
| 15 | E2F1 | apoptosis (36.8%; 1.204e-05), programmed cell death (36.8%; 1.352e-05), cell death (36.8%; 3.892e-05), death (36.8%; 4.111e-05), negative regulation of cellular process (47.4%; 2.984e-04) | 20 | 19 | 1.800E-43 | 60.11 | 60.11 |
| 16 | GCR-alpha | intracellular signaling cascade (58.8%; 7.907e-07), response to copper ion (17.6%; 5.601e-06), regulation of signal transduction (47.1%; 1.017e-05), detoxification of copper ion (11.8%; 1.374e-05), regulation of steroid biosynthetic process (17.6%; 1.619e-05) | 18 | 17 | 7.480E-39 | 56.69 | 56.69 |

| | | | | | | | |
|----|--------------|---|----|----|-----------|-------|-------|
| 17 | YY1 | negative regulation of steroid metabolic process (27.8%; 1.046e-11), negative regulation of interleukin-12 biosynthetic process (22.2%; 2.925e-11), negative regulation of vitamin D biosynthetic process (22.2%; 5.846e-11), negative regulation of vitamin metabolic process (22.2%; 5.846e-11), negative regulation of calcidiol 1-monooxygenase activity (22.2%; 1.052e-10) | 18 | 17 | 7.480E-39 | 56.69 | 56.69 |
| 18 | ETS1 | regulation of apoptosis (52.9%; 4.506e-07), regulation of programmed cell death (52.9%; 4.985e-07), regulation of cell death (52.9%; 5.204e-07), negative regulation of biological process (64.7%; 2.903e-06), regulation of protein metabolic process (41.2%; 3.488e-06) | 17 | 16 | 1.510E-36 | 54.9 | 54.9 |
| 19 | AML1 (RUNX1) | embryonic organ development (25.0%; 1.638e-06), embryonic hemopoiesis (18.8%; 1.902e-06), response to reactive oxygen species (25.0%; 9.298e-06), response to oxidative stress (25.0%; 1.343e-04), response to hydrogen peroxide (18.8%; 1.527e-04) | 16 | 15 | 3.020E-34 | 53.05 | 53.05 |
| 20 | SP3 | regulation of angiotensin levels in blood (12.5%; 8.089e-06), regulation of angiotensin metabolic process (12.5%; 1.213e-05), urogenital system development (25.0%; 6.174e-05), regulation of blood volume by renin-angiotensin (12.5%; 6.283e-05), regulation of systemic arterial blood pressure by circulatory renin-angiotensin (12.5%; 6.283e-05) | 16 | 15 | 3.020E-34 | 53.05 | 53.05 |

| | | | | | | | |
|----|-------|---|----|----|-----------|-------|-------|
| 21 | PAX5 | negative regulation of interleukin-12 biosynthetic process (26.7%; 1.305e-11), negative regulation of vitamin D biosynthetic process (26.7%; 2.609e-11), negative regulation of vitamin metabolic process (26.7%; 2.609e-11), negative regulation of calcidiol 1-monooxygenase activity (26.7%; 4.694e-11), regulation of vitamin D biosynthetic process (26.7%; 7.819e-11) | 16 | 15 | 3.020E-34 | 53.05 | 53.05 |
| 22 | STAT3 | response to copper ion (18.8%; 4.619e-06), response to chemical stimulus (62.5%; 1.103e-05), detoxification of copper ion (12.5%; 1.213e-05), negative regulation of biological process (62.5%; 1.318e-05), response to steroid hormone stimulus (31.2%; 2.602e-05) | 16 | 15 | 3.020E-34 | 53.05 | 53.05 |
| 23 | p73 | apoptosis (66.7%; 8.546e-11), programmed cell death (66.7%; 1.018e-10), negative regulation of catalytic activity (53.3%; 2.771e-10), cell death (66.7%; 5.053e-10), death (66.7%; 5.492e-10) | 15 | 14 | 6.000E-32 | 51.14 | 51.14 |

| | | | | | | | |
|----|-------|---|----|----|-----------|-------|-------|
| 24 | c-Myb | negative regulation of interleukin-12 biosynthetic process (26.7%; 1.305e-11), negative regulation of vitamin D biosynthetic process (26.7%; 2.609e-11), negative regulation of vitamin metabolic process (26.7%; 2.609e-11), negative regulation of calcidiol 1-monooxygenase activity (26.7%; 4.694e-11), regulation of vitamin D biosynthetic process (26.7%; 7.819e-11) | 15 | 14 | 6.000E-32 | 51.14 | 51.14 |
| 25 | STAT1 | negative regulation of biological process (71.4%; 2.176e-06), response to organic substance (57.1%; 2.237e-06), positive regulation of biological process (71.4%; 6.877e-06), response to chemical stimulus (64.3%; 2.259e-05), positive regulation of cellular process (64.3%; 3.289e-05) | 14 | 13 | 1.180E-29 | 49.15 | 49.15 |
| 26 | IRF1 | regulation of interleukin-12 biosynthetic process (35.7%; 1.840e-12), negative regulation of interleukin-12 biosynthetic process (28.6%; 9.573e-12), negative regulation of vitamin D biosynthetic process (28.6%; 1.914e-11), negative regulation of vitamin metabolic process (28.6%; 1.914e-11), regulation of interleukin-12 production (35.7%; 2.244e-11) | 14 | 13 | 1.180E-29 | 49.15 | 49.15 |

| | | | | | | | |
|----|-------|---|----|----|-----------|-------|-------|
| 27 | HSF1 | regulation of cellular protein metabolic process (38.5%; 6.870e-05), response to mechanical stimulus (23.1%; 9.903e-05), induction of apoptosis by extracellular signals (23.1%; 1.192e-04), regulation of protein metabolic process (38.5%; 1.389e-04), negative regulation of insulin receptor signaling pathway (15.4%; 1.694e-04) | 13 | 12 | 2.310E-27 | 47.08 | 47.08 |
| 28 | E2A | leukocyte differentiation (33.3%; 1.463e-05), cellular developmental process (66.7%; 3.901e-05), regulation of immune system process (41.7%; 4.527e-05), positive regulation of cell proliferation (41.7%; 6.616e-05), regulation of cell proliferation (50.0%; 7.726e-05) | 13 | 12 | 2.310E-27 | 47.08 | 47.08 |
| 29 | SMAD4 | negative regulation of biological process (76.9%; 7.146e-07), positive regulation of cellular component organization (38.5%; 1.340e-06), positive regulation of cell development (30.8%; 1.531e-06), negative regulation of cellular process (69.2%; 4.853e-06), regulation of developmental process (53.8%; 5.309e-06) | 13 | 12 | 2.310E-27 | 47.08 | 47.08 |
| 30 | c-Fos | negative regulation of interleukin-12 biosynthetic process (30.8%; 6.839e-12), negative regulation of vitamin D biosynthetic process (30.8%; 1.367e-11), negative regulation of vitamin metabolic process (30.8%; 1.367e-11), negative regulation of calcidiol 1-monooxygenase activity (30.8%; 2.460e-11), response to copper ion (38.5%; 3.811e-11) | 13 | 12 | 2.310E-27 | 47.08 | 47.08 |

Table S6: List of Common Nkx3.1/Myc Target Genes

| Mouse EntrezID | GeneSymbol | GeneName |
|----------------|------------|---|
| 76408 | Abcc3 | Canalicular multispecific organic anion transporter 2 |
| 226251 | Ablim1 | Actin-binding LIM protein 1 |
| 11421 | Ace | Angiotensin-converting enzyme |
| 74117 | Actr3 | Actin-related protein 3 |
| 11515 | Adcy9 | Adenylate cyclase type 9 |
| 11669 | Aldh2 | Aldehyde dehydrogenase, mitochondrial |
| 27053 | Asns | Asparagine synthetase [glutamine-hydrolyzing] |
| 11910 | Atf3 | Cyclic AMP-dependent transcription factor ATF-3 |
| 12419 | Cbx5 | Chromobox protein homolog 5 |
| 53599 | Cd164 | Sialomucin core protein 24 |
| 26365 | Ceacam1 | Carcinoembryonic antigen-related cell adhesion molecule 1 |
| 12633 | Cflar | CASP8 and FADD-like apoptosis regulator |
| 29876 | Clic4 | Chloride intracellular channel protein 4 |
| 12764 | Cmas | N-acylneuraminate cytidyltransferase |
| 13030 | Ctsb | Cathepsin B |
| 13163 | Daxx | Death domain-associated protein 6 |
| 22791 | Dnajc2 | DnaJ homolog subfamily C member 2 |
| 27998 | Exosc5 | Exosome complex exonuclease RRP46 |
| 13197 | Gadd45a | Growth arrest and DNA-damage-inducible protein GADD45 alpha |
| 110611 | Hdlbp | Vigilin |
| 64209 | Herpud1 | Homocysteine-responsive endoplasmic reticulum-resident ubiquitin-like domain member 1 protein |
| 15277 | Hk2 | Hexokinase-2 |
| 15516 | Hsp90ab1 | Heat shock protein HSP 90-beta |
| 14828 | Hspa5 | 78 kDa glucose-regulated protein |
| 105148 | Iars | Isoleucyl-tRNA synthetase, cytoplasmic |
| 15901 | Id1 | DNA-binding protein inhibitor ID-1 |
| 16001 | Igf1r | Insulin-like growth factor 1 receptor |
| 233726 | Ipo7 | Importin-7 |
| 16439 | Itpr2 | Inositol 1,4,5-trisphosphate receptor type 2 |
| 117198 | Ivns1abp | Influenza virus NS1A-binding protein homolog |
| 16784 | Lamp2 | Lysosome-associated membrane glycoprotein 2 |
| 98386 | Lbr | Lamin-B receptor |

| | | |
|--------|---------|--|
| 16880 | Lifr | Leukemia inhibitory factor receptor |
| 216443 | Mars | Methionyl-tRNA synthetase, cytoplasmic |
| 232087 | Mat2a | S-adenosylmethionine synthetase isoform type-2 |
| 70122 | Mllt3 | Protein AF-9 |
| 17750 | Mt2 | Metallothionein-2 (NCBI calls this gene MT2A) |
| 17751 | Mt3 | Metallothionein-3 |
| 17869 | Myc | Myc proto-oncogene protein |
| 59027 | Nampt | Nicotinamide phosphoribosyltransferase |
| 83814 | Nedd4l | E3 ubiquitin-protein ligase NEDD4-like |
| 18033 | Nfkb1 | Nuclear factor NF-kappa-B p105 subunit |
| 18034 | Nfkb2 | Nuclear factor NF-kappa-B p100 subunit |
| 18095 | Nkx3-1 | Homeobox protein Nkx-3.1 |
| 18458 | Pabpc1 | Polyadenylate-binding protein 1 |
| 74737 | Pcf11 | cleavage and polyadenylation factor subunit homolog (S. cerevisiae) |
| 18537 | Pcmt1 | Protein-L-isoaspartate(D-aspartate) O-methyltransferase |
| 56273 | Pex14 | Peroxisomal membrane protein PEX14 |
| 71701 | Pnpt1 | Polyribonucleotide nucleotidyltransferase 1, mitochondrial |
| 28075 | Pppde2 | PPPDE peptidase domain-containing protein 2 |
| 11758 | Prdx6 | Peroxiredoxin-6 |
| 18750 | Prkca | Protein kinase C alpha type |
| 26441 | Psma4 | Proteasome subunit alpha type-4 |
| 19211 | Pten | Phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase PTEN |
| 84113 | Ptov1 | Prostate tumor overexpressed gene 1 protein homolog |
| 19696 | Rel | C-Rel proto-oncogene protein |
| 19697 | Rela | Transcription factor p65 |
| 19698 | Relb | Transcription factor RelB |
| 56040 | Rplp1 | 60S acidic ribosomal protein P1 |
| 53860 | Sept9 | Septin-9 |
| 20529 | Slc31a1 | High affinity copper uptake protein 1 |
| 17254 | Slc3a2 | 4F2 cell-surface antigen heavy chain |
| 114716 | Spred2 | Sprouty-related, EVH1 domain-containing protein 2 |
| 21353 | Tank | TRAF family member-associated NF-kappa-B activator |
| 110960 | Tars | Threonyl-tRNA synthetase, cytoplasmic |
| 70430 | Tbce | Tubulin-specific chaperone E |

| | | |
|-------|---------|--|
| 80385 | Tusc2 | Tumor suppressor candidate 2 |
| 56338 | Txnip | Thioredoxin-interacting protein |
| 22234 | Ugcg | Ceramide glucosyltransferase |
| 67003 | Uqcrc2 | Cytochrome b-c1 complex subunit 2, mitochondrial |
| 56041 | Uso1 | General vesicular transport factor p115 |
| 22288 | Utrn | utrophin |
| 22695 | Zfp36 | Tristetraproline |
| 12192 | Zfp36l1 | Butyrate response factor 1 |

Table S7: Leading Edge of NKX3.1/MYC Target Genes Associated With Human Prostate Cancer Relapse

| NAME | PROBE | RANK IN GENE LIST | RANK METRIC SCORE | RUNNING ES | CORE ENRICHMENT |
|--------|----------|-------------------|-------------------|------------|-----------------|
| row_0 | ZFP36 | 16 | 0.286424577 | 0.0625669 | Yes |
| row_1 | ATF3 | 72 | 0.219299048 | 0.10620268 | Yes |
| row_2 | MT2A | 123 | 0.196858987 | 0.14531067 | Yes |
| row_3 | ITPR2 | 149 | 0.184756219 | 0.18420343 | Yes |
| row_4 | UGCG | 239 | 0.154251173 | 0.20987274 | Yes |
| row_5 | CLIC4 | 268 | 0.149313346 | 0.24052612 | Yes |
| row_6 | CEACAM1 | 331 | 0.139526606 | 0.26559255 | Yes |
| row_7 | PRKCA | 354 | 0.135626271 | 0.2937788 | Yes |
| row_8 | ZFP36L1 | 370 | 0.13266547 | 0.3220007 | Yes |
| row_9 | HERPUD1 | 385 | 0.12818566 | 0.34931886 | Yes |
| row_10 | IVNS1ABP | 386 | 0.128029123 | 0.37799972 | Yes |
| row_11 | CFLAR | 393 | 0.12681143 | 0.40580878 | Yes |
| row_12 | ALDH2 | 402 | 0.126047686 | 0.43324703 | Yes |
| row_13 | NAMPT | 464 | 0.120009899 | 0.45404118 | Yes |
| row_14 | ACTR3 | 561 | 0.112028569 | 0.46955296 | Yes |
| row_15 | GADD45A | 580 | 0.110817462 | 0.49258098 | Yes |
| row_16 | LIFR | 582 | 0.110420525 | 0.51721734 | Yes |
| row_17 | MAT2A | 586 | 0.110093884 | 0.54158086 | Yes |
| row_18 | CTSB | 709 | 0.101412289 | 0.5521186 | Yes |
| row_19 | NFKB1 | 741 | 0.099147223 | 0.57123435 | Yes |
| row_20 | TANK | 1006 | 0.084168211 | 0.56373173 | Yes |
| row_21 | ID1 | 1063 | 0.081980832 | 0.57650584 | Yes |
| row_22 | IPO7 | 1107 | 0.080541305 | 0.59025544 | Yes |
| row_23 | USO1 | 1135 | 0.07961379 | 0.6053947 | Yes |
| row_24 | TXNIP | 1277 | 0.074165143 | 0.60793155 | Yes |
| row_25 | PNPT1 | 1369 | 0.071516708 | 0.61486715 | Yes |
| row_26 | PSMA4 | 1406 | 0.070452161 | 0.62705547 | Yes |