Supplemental Methods:

Cell culture and treatment: Both androgen dependent (LNCaP, VCaP, LAPC4) and castration resistant (PC3 and C4-2) cancer cell lines were cultured and maintained as previously described(1, 2). To determine transcriptional programs regulated by Cyclin D1 isoforms, LNCaP cells were transduced with the indicated virus as previous described(3). To assess AR activity, cells were plated on poly-L-lysine coated plates in androgen proficient (5% Fetal Bovine Serum (FBS) in IMEM) or androgen depleted (5% Charcoal Dextran Treated (CDT)) media for 48 or 72 hours respectively. Androgen proficient cells were treated with the AR antagonist Casodex (AstraZeneca Pharmaceuticals; Wilmington, DE)(10uM) or EtOH control (0.1%) for 24 hours, after which cells were harvested for RNA using the Trizol method. Cells maintained in androgen-depleted conditions were stimulated with 1nM DHT or control EtOH (0.1%) for 18 hours and harvested as above.

Generation of constructs: *SNAI2* cDNA was generated via PCR from RNA isolated from LNCaP cells. Total DNA was run on a 2% agarose gel, purified using Qiagen gel extraction kit (28074), and ligated into a TOPO TA PCDNA3.1 vector (Invitrogen 45-0641) overnight at room temperature. *SNAI2* cDNA was cut out of the TOPO TA vector and ligated into PCDNA3.1 (+) using the enzymes KpnI and XhoI. Insertion was validated via restriction digest, and sequence fidelity was validated via sequencing using at least two distinct primer sets targeting the 5' and 3' termini. 3x-Flag Cyclin D1b cDNA was excised from a

plasmid generated previously(2), using the enzymes BamHI and EcoRI and ligated, in frame, into an N-terminal 3x-Flag PCDNA 3.1 (+) vector. Insertion and sequence fidelity was confirmed by both restriction digest and 5' and 3' sequencing.

Microarray preprocessing, normalization, and analysis: Total RNA samples (0.5 µg) for each treatment condition (n = 3) were and hybridized to HG-U133plus2 GeneChips (Affymetrix). GeneChips were quantified with an Affymetrix Gene Array Scanner (software version 1.4, default settings), and then "CEL" files were generated using Affymetrix Microarray Suite 5.0. Preprocessing of the Affymetrix HG-U133 plus 2 arrays, including background correction, quantile normalization, and summarization, was performed on the microarray .CEL files using the Robust Multichip Average (RMA) [Irizarry 2003; PMID 12582260] algorithm implemented in *RMAExpress* software version 1.0 [http://rmaexpress.bmbolstad.com/]. Raw.CEL files and RMA processed expression data have been deposited in GEO [http://www.ncbi.nlm.nih.gov/geo/] with accession number GSE40794.. Log2 RMA expression data and Affymetrix probeset annotations (release 32, dated July 2011) were imported into MATLAB software (The Mathworks, Inc., Natick, MA), where subsequent statistical analysis was performed. Only probesets with gene annotations were retained for statistical analysis, representing 20639 unique genes.

Gene set enrichment analysis (GSEA)(4) was performed on ranked gene lists, ordered by their SAM standardized distance metric, to identify significant enrichment among Gene Ontology Biological Process terms. When ranking

genes represented on the HG-U133 plus 2 microarray with multiple probesets, only the probeset with the maximum absolute standardized distance metric was used.

Transfection and generation of stable cell lines: Indicated cell lines were plated at 80% confluency for 24 hours in complete media and transfected with the following DNA/lipid based complexes in serum-free Optimem media (Invitrogen 31985070) for 6 hours: Lipofectin (Invitrogen 18292011) (32μL) was used to transfect 10μg of DNA into both LNCaP and C4-2 cell lines, FuGENE (Roche 11-814-443-001) (manufacturers specifications) was used to transfect PC3 cells, and Lipofectamine 2000 (Invitrogen 52887) was used to transfect VCaP cells (according to manufacturers specifications). For lines that were stably generated, either 0.4μg/mL of Puromycin (Fisher Scientific 22742-0500) or 4μg/mL of G418 (GIBCO 10131) were added to transfected plates 48 hours post transfection, and individual clones picked for construct insertion validation by Q-PCR and immunoblot.

Immunoblotting: Cell lysates (30-40μg) were generated from cells treated as above, separated by gel electrophoresis on SDS-page polyacrylamide gels, transferred to PVDF membranes, and immunoblotted for the indicated proteins: Cyclin D1b(5), Cyclin D1 (Neomarkers RB-212-P), CDK4 (Santa Cruz sc-601), Slug (Cell Signaling -9585S), GFP (Santa Cruz sc-9996), E-cadherin (Abcam ab11512), GAPDH (Santa Cruz sc-166574), AR (Santa Cruz sc-816), Histone H4

(Millipore 07-108), and Vimentin (Cell Signaling 3932S) according to the manufacturer's specifications.

Gene expression analysis: Cells were treated as above and RNA was isolated using the Trizol method. cDNA was then generated with 2µg of total RNA using the VILO enzyme system (Invitrogen 11754050), and quantitative-pcr (QPCR) carried out with Power SYBR Green (Applied Biosystems 4367659) and a StepOne Machine (Applied Biosystems). Relative transcript changes are represented as the mean -fold change ± S.E. of 3–4 individual experiments where each condition within an experiment is the average of two technical replicates. Statistics were determined by ANOVA analysis, and significance ($p \leq 0.05$) was calculated using the Kriskal-Wallis and Dunn's test or students T-Test using GraphPad Prism version 4.

Morpholino analysis: To alter the Cyclin D1 splicing event to favor Cyclin D1b production, Morpholino oligo nucleotides were designed against the exon 4/intron 4 (5'GGACATCACCCTCACTTACCGG3') and intron 4/exon 5 (5'CCCCGGAGGCAGTCCTGAGAGAGAGA3') sequence of the CCND1 pre-mRNA. LNCaP cells were plated on poly-L-lysine coated 6cm plates (5×10^5) and allowed to adhere for 24 hours. Media was replenished following adhesion (IMEM 5% FBS) and control (5'AAACCCGGGTTTACG3') or *CCND1* morpholinos transfected into the cells at the indicated concentrations using the Endo-Porter system (Gene Tools**)** according to the manufactures specifications.

Cells were maintained in the transfection media for 48 hours, after which gene expression and protein analyses were conducted. Semi-quantitative PCR conditions used for *transcript b*, *GAPDH*, and *SNAI2* transcript levels are as follows: 95°C 2 min, [95°C – 30s, 54°C – 30s, 72°C 50s] x 26 cycles, 72°C 5 min.

RNA interference: RNA interference was preformed using LNCaP (3×10^5) cells plated on poly-L lysine in complete androgen proficient media for 24 hours. Cells were then transfected overnight (16 hours) in serum-free conditions with either control, *CCND1*, or *SNAI2* siRNA pools (D-001810-10-20, L-003210-00-0020, L-017386-00-0005 respectively; Thermo Scientific) according to the manufacturer's specifications. Cells were maintained in complete media for an additional 48 hours then subjected to either gene expression or matrigel-invasion analyses.

CAM Assay:

The chorioallantoic membrane (CAM) assay was performed as described previously (6). Fertilized special pathogen free eggs were purchased from Charles River and incubated for 10 days at 38°C in a rotary humidified incubator. After 10 days of incubation, small holes were drilled over the air sac and near the allantoic vein. The CAM was lowered by applying gentle pressure to the hole over the air sac, a $1cm^2$ window was cut over the hole near the allantoic vein, and 2×10^6 cultured human prostate adenocarcinoma cells (stable LNCaP-Vector, and LNCaP-Slug cell lines) were implanted onto the membrane in each egg. After sealing the windows, the eggs were incubated in a stationary incubator

for seven days and the embryos were sacrificed after 17 total days of incubation. The embryonic livers and lungs were harvested and analyzed for the presence of tumor cells using quantitative human *Alu*- specific PCR(6). The lungs and livers were processed using a Puregene Cell and Tissue DNA extraction kit from Qiagen. Subsequent quantification of human cells in the extracted DNA was performed as previously described(7).

Immunohistochemistry: Slug and Cyclin D1b expression was analyzed in 4 serial sectional tissue microarrays (described above), using the same antibodies described for immunoblotting. Each antibody was validated to be specific in human tissue for their respective targets prior to staining(5). Staining for both proteins was conducted as previously described(9) with dilutions 1:50 (Slug) and 1:1500 (Cyclin D1b) incubated overnight at 4°C. TMAs were then washed and processed using the Vectastain ABC Kit (Vector Laboratories Inc. PK-6101) and developed using DAB (Invitrogen 002014). Analysis of AR, Slug, and BrdU expression in human xenografts was conducted as described above from paraffin embedded, formalin fixed tissues using the following dilutions overnight at 4°C: AR (1:2500), Slug (1:50), BrdU (1:1000, Accurate Chemical & Scientific Corporation- OBT0030). BrdU counts were taken from at least 3 random fields from each of 3 slides/tumor, and are presented as %positive/total cell number.

Supplemental Table 1: Primers Used in the study

Primer	Sequence (5'-3')	Reference
GAPDH F	CCA GGT GGT CTC CTC TGA CTT	
GAPDH R	TCA TAC CAG GAA ATG AGC TTG ACA	
transcript b F	GTG CCA CAG ATG TGA AGT TC	
transcript b R	GGA CAT CAC CCT CAC TTA C	
SNAI2 F SET 1	GAG CAT ACA GCC CCA TCA CT	
SNAI2 R SET 1	CCA GCC CAG AAA AAG TTG AA	
SNAI2 F SET 2	GGC AAG GCG TTT TCC AGA C	
SNAI2 R SET 2	TTT GGA GCA GTT TTT GCA CTG G	
CDH1 F	AAG AAG GAG GCG GAG AAG AG	
CDH1 R	CAT AGT CAA ACA CGA GCA GAG A	
SNAI2 AROR1 F	CAT TTC CCT TTG GGT GGA TA	
SNAI2 AROR1 R	CCC TGA GCT GTC GCT TAA AA	
SNAI2 AROR 2 F	GGC CAG CCT CTG GTG TTA AT	
SNAI2 AROR 2 R	CCT TTG TCT TCC CGC TTC C	
KLK3 ARE III (G)	ACA GAC CTA CT TGG AGG AAC	(10)
KLK3 ARE III (H)	AAG ACA GCA ACA CCT TTT T	
KLK3 ARE II	CCT AGA TGA AGT CTC CAT GAG CTA CA	(10)
KLK3 ARE II	GGG AGG GAG AGC TAG CAC TTG	
KLK3 F	GTT GTC TTC CTC ACC CTG TCC	
KLK3 R	GGT TGG GAA TGC TTC TCG	

SNAI2 Cloning 5'	CTC CCG CCG GAC CGT TAT	
SNAI2 Cloning 3'	TTG CGT CAC TCA GTG TGC TAC A	
3C Site 1 digestion F	AGG CAA CTG CAC CAG ATT TT	
3C Site 1 digestion R	GCA CCA ATT TCT CAC CAA CC	
3C Site 2 digestion F	TCA GCT TCA ATG GCA TGG GGG T	
3C Site 2 digestion R	TGA CAC CTC CTC CAA GGA CCA CA	
3C Site 3 digestion F	ACG TAG TTC TTG GAC TTT CAC TTC T	
3C Site 3 digestion R	TGG GAA ATG TTG GGA GGT GCT	
3C Site 4 digestion F	GCG ACT GAA GGC GAT ACC T	
3C Site 4 digestion R	TCC AGC TTC TCT GCC CAT AC	
3C Test Site 1 F	AAA CTT TTC AGC TTC AAT GG	
3C Test Site 2 F	TAC TGC TGC CAA TCA TAC AG	
3C Test Site 3 F	TAG CTT GGT GTG GCT ACT TG	
3C Test Site 4 F	CCT TCT ATT ATC GCC TTT TCT G	
3C Constant R	TCA GCC TAA TTA TGG AGC AC	
3C Probe	6FAM-AAG GAT TCA GTC CTA GGT TGG TGA G-TAMRA	
3C Control Region F	GTG CCA CAG ATG TGA AGT TC	
3C Control Region R	GGA CAT CAC CCT CAC TTA C	
3C Control Region Probe	6FAM-CCA ACA ACT TCC TGT CCT ACT ACC-TAMRA	
KRT18 F	GAG GAC CTG AGG GCT CAG ATC TTC	

KRT18 R	TCT CTG TCT CCA GCT GCA GTC G	
KRT8 F	TGC TGA GCC CCC TTG TCC TG	
KRT8 R	TGC TCC AGG AAC CGT ACC TTG TC	
MMP2 F	ATG CCA AAG ACC CTG ATG CTG CC	
MMP2 R	CTT GGT GTA GGT GTA AAT GGG TGC C	
NANOG F	GCA ATG GTG TGA CGC AGA AGG C	
NANOG R	TGG GTC TGG TTG CTC CAG GTT G	
BMI1 F	TGG TTG CCC ATT GAC AGC GG	
BMI1 R	AAA AAT CC GGA AAG AGC AGC CG	
FN1 F	ATT CCA TCA CCC TCA CCA AC	
FN1 R	TTT CCT CCT GTC TCT CCG TAA	
CXCR4 F	TGG CCT TAT CCT GCC TGG TAT	
CXCR4 R	AGG AGT CGA TGC TGA TCC CAA	
CLDN1 F	ACC CCA GTC AAT GCC AGG TAC G	
CLDN1 R	AAG GCA GAG AGA AGC AGC AGC C	
SIN3A F	TGA GCA CAG AAT GAA GCG GCG	
SIN3A R	CTG TGG CAT GGC TGA AAC CTG G	
HDAC1 F	TCG CCC TCA CAA AGC CAA TGC	
HDAC1 R	GCA CTT GCC ACA GAA CCA CCA G	

Supplemental References:

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2. Burd CJ, Petre CE, Morey LM, et al. Cyclin D1b variant influences prostate cancer growth through aberrant androgen receptor regulation. Proc Natl Acad Sci U S A 2006; 103: 2190-5.
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7. van der Horst EH, Leupold JH, Schubbert R, Ullrich A, Allgayer H. TaqMan-based quantification of invasive cells in the chick embryo metastasis assay. Biotechniques 2004; 37: 940-2, 4, 6.
8. Sharma A, Yeow W-S, Ertel A, et al. The retinoblastoma tumor suppressor controls androgen signaling and human prostate cancer progression. J Clin Invest 2010.
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10. Shang Y, Myers M, Brown M. Formation of the androgen receptor transcription complex. Mol Cell 2002; 9: 601-10.

Supplemental Figure Legends:

Supplemental Figure S1: Cyclin D1b regulated gene networks are distinct from Cyclin D1a and are enriched for metastasis-associated genes. **(A)** Control and Cyclin D1b clones expressing low (D1b-L) and high (D1b-H) were starved of hormone for 72 hours. Expression of *transcript b* was analyzed in the presence (left panel) or absence (right panel) of 1nM DHT 18 hours post treatment. **(B)** Schematic of infection strategy used for Cyclin D1a or Cyclin D1b microarrays. **(C)** Heat maps are shown for Cyclin D1a-regulated genes (left) and Cyclin D1b-regulated genes (right), with horizontal bars delineating significant genes with FDR ≤ 1%. Heat maps are labeled along the y-axis at locations of peak enrichment for the topmost enriched Gene Ontology Biological Process terms identified in GSEA, at FDR ≤ 5%. **(D)** Venn Diagram comparing clusters 2 and 3 with a multi cancer invasion signature[5]. Error represents +/-SEM ***p<0.001

Supplemental Figure S2: Manipulation of Cyclin D1 isoforms results in deregulation of the *SNAI2 transcript*. **(A)** A pool of validated siRNA's targeting the Cyclin D1 transcript were introduced into LN-D1b cells for 72 hours in androgen proficient conditions. Cells were then harvested for RNA, and relative transcript levels of *SNAI2*, D-cyclins, and *GAPDH* determined. **(B)** LNCaP cells were infected with adeno-cyclin D1a virus from Supplemental Figure S1 for 24 hours then stimulated with either control EtOH (0.1%) or 1nM DHT for 24 hours. Cells were then harvested, RNA collected, and relative levels of *SNAI2* transcript

determined. **(C)** VCaP cells generated to stably express low levels of Cyclin D1b (left and middle), were cultured in androgen proficient conditions for 48 hours, harvested for RNA, and relative levels of *SNAI2* transcript determined. **(D)** Cells treated with increasing doses of Morpholino were harvested for RNA and the ratio of *transcript b* to *transcript a* is reported as a % of total *CCND1* transcript. **(E)**. Total *CCND1* levels from cells treated with Morpholino is reported as a function of control conditions. Error is reported as +/-SEM *<0.05, **<0.01 *** p<0.001

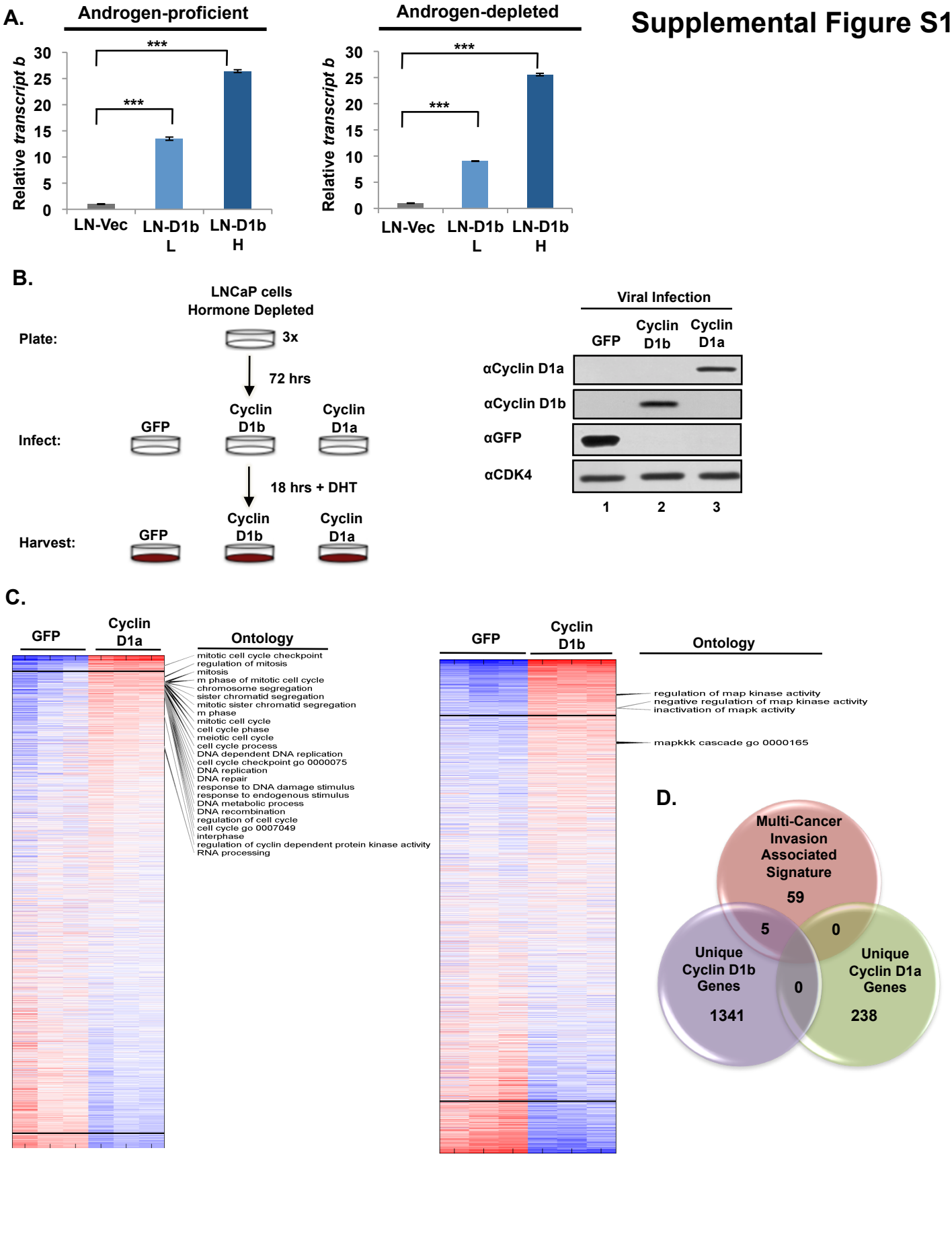
Supplemental Figure S3: Cyclin D1b associates with AR on chromatin and promotes assembly of transcriptional machinery at *SNAI2* regulatory loci. **(A)** LNCaP and VCaP cells expressing 3x-flag Cyclin D1b were cultured in androgen proficient conditions then subjected to chromatin tethering fractionation. GAPDH and histone H4 serve as soluble and chromatin tethered controls, respectably. **(B)** C4-2 cells expressing low (D1b-L) and High (D1b-H) levels of Cyclin D1b were cultured and harvested as in A (left), and subjected to immuno precipitation of AR (right). Precipitated lysates were probed for the presence of Cyclin D1b, AR, and the controls GAPDH and histone H4. 1% input and IgG serve as positive and negative controls (respectably). Using publically available genome wide databases, AR was mined for occupancy at *SNAI2* **(C)** and *KLK3* **(D)** regulatory loci. Two distinct AR occupied regions were found at ~600 and ~3500 bp from the transcriptional start sites of *SNAI2* and *KLK3* loci. [6-9] **(E)** Vector and Cyclin D1b expressing cells were subjected to ChIP analysis to probe for RNA Polymerase II occupancy after 3 hours stimulation with control

EtOH (0.1%) or DHT (10nM), at both the *SNAI2* and *KLK3* promoters with occupancy reported as % input. **(F).** Vector or Cyclin D1a expressing cells were hormone starved for 72 hours and treated as above. Samples were immunoprecipitated for acetylated histone H4 and occupancy at the *SNAI2* enhancer is reported as % input. Error represents +/-SEM *p<0.05, **p<0.01, ***p<0.001

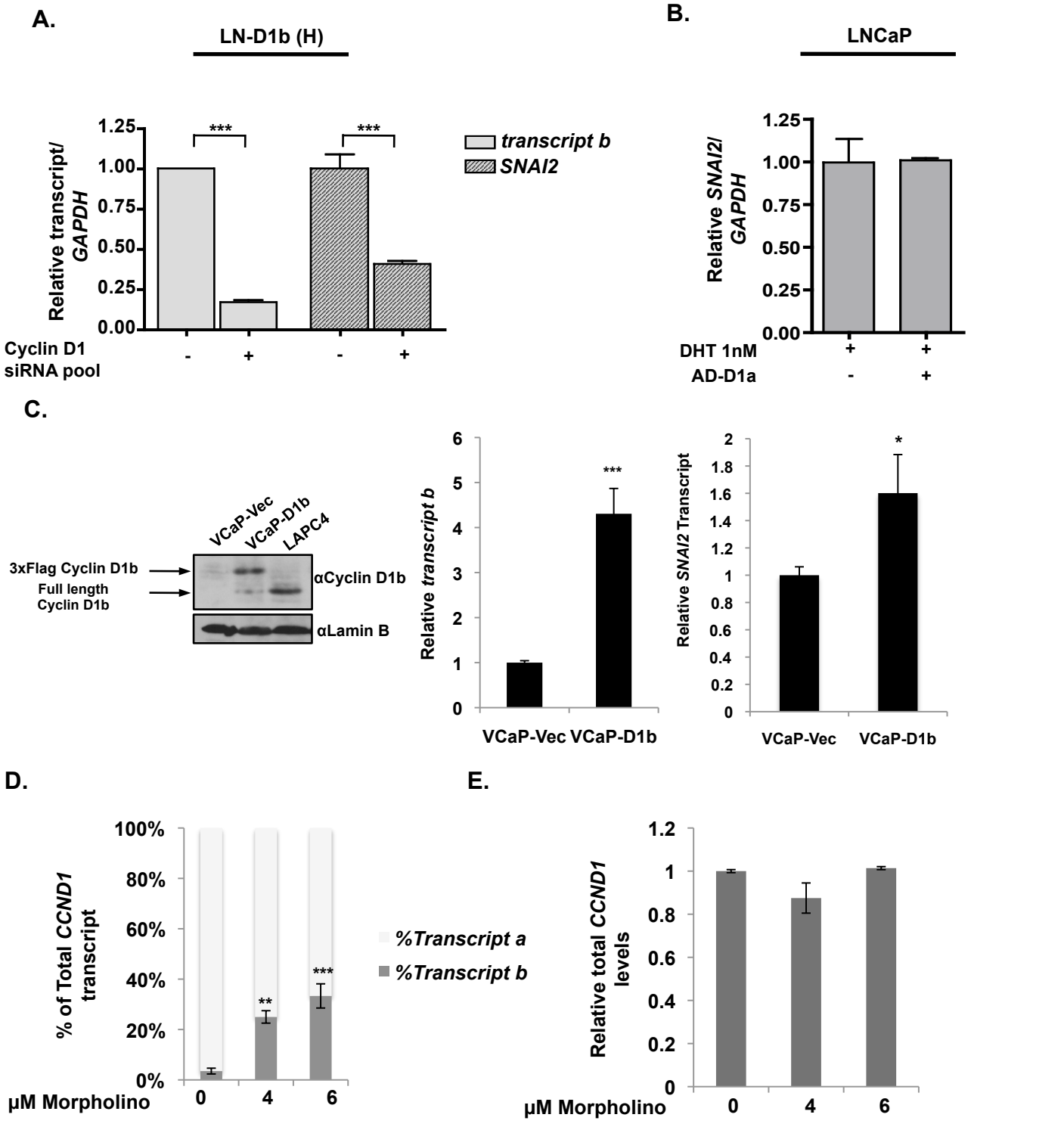
Supplemental Figure S4: Slug expression is not sufficient to induce markers of EMT. (A) LN-Vec, LN-SLUG, and LN-SLUG polyclonal (Figure 4C and 4D) were cultured in androgen proficient conditions for 24 hours and relative *CDH1* levels determined (*GAPDH* serves as a control). **(B)** LN-SLUG and LN-Vec samples from A were tested for expression of other EMT associated genes and analyzed as in A. Error represents +/-SEM

Supplemental Figure S5: Slug does not regulate expression of E-cadherin in vivo. **(A)** Protein lystates from Figure 6 were immuno-blotted for the presence of Cyclin D1a and E-cadherin **(B)** in both control and LN-SLUG tumors. Quantification of E-cadherin levels was determined using Li-cor software and represents the average expression of each sample +/-SEM **(C)** The presence of Slug and E-cadherin was determined by immuno-flourescence in paraffin embedded xenografts from Figure 6 in both control and LN-SLUG tumors.

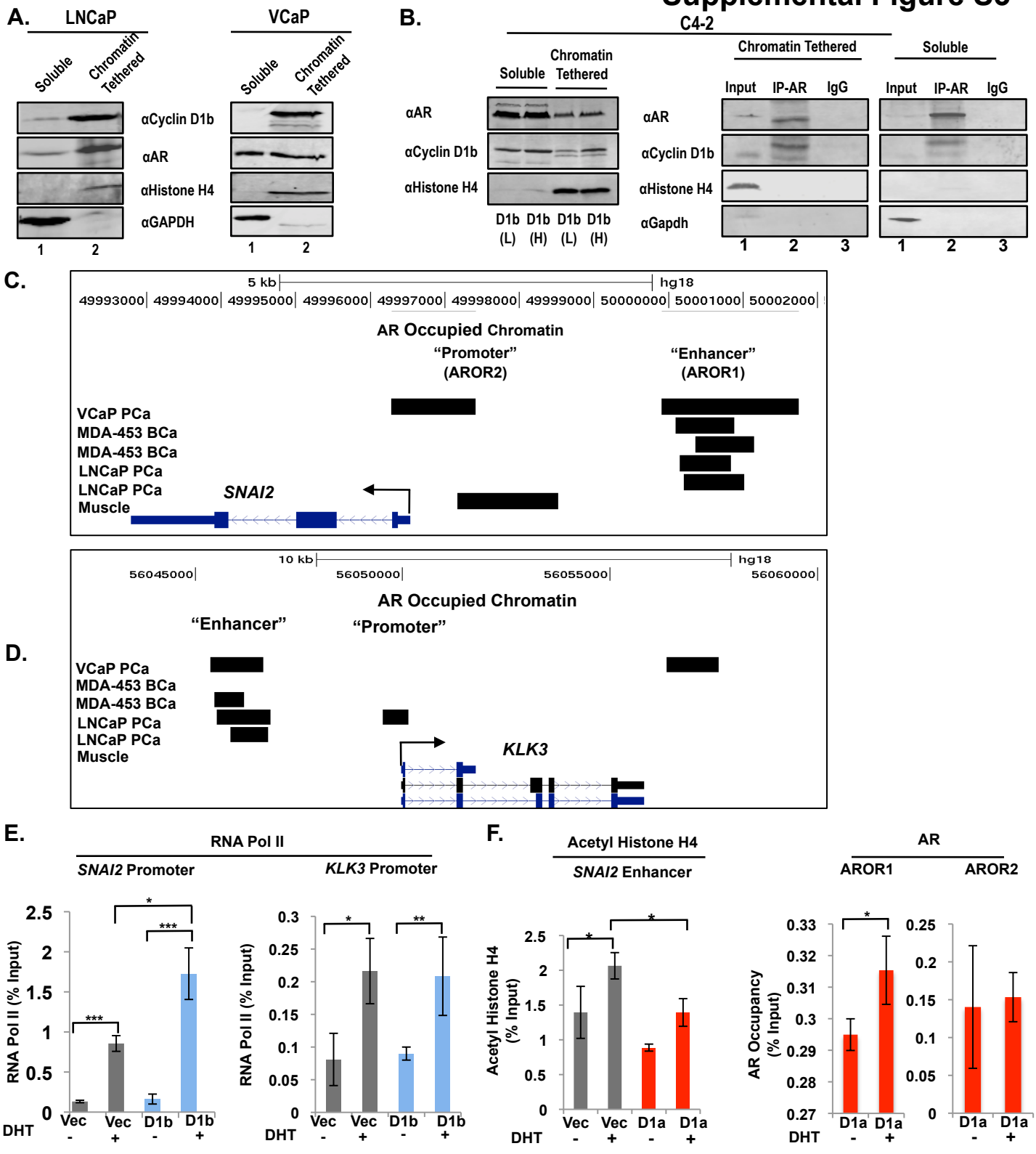
Supplemental Figure S1



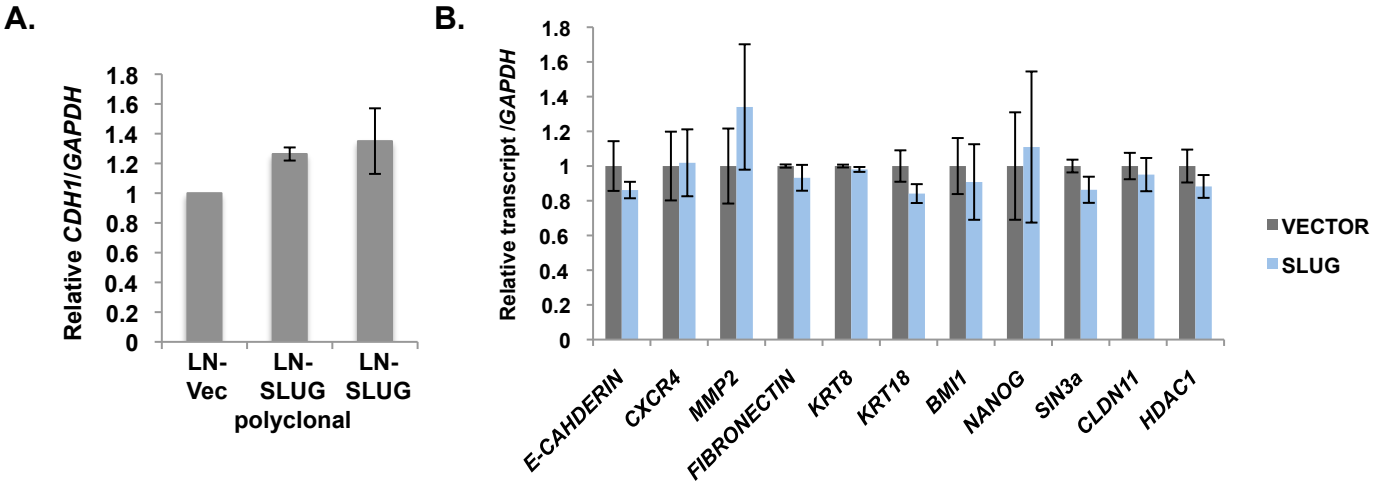
Supplemental Figure S2



Supplemental Figure S3



Supplemental Figure S4



Supplemental Figure S5