

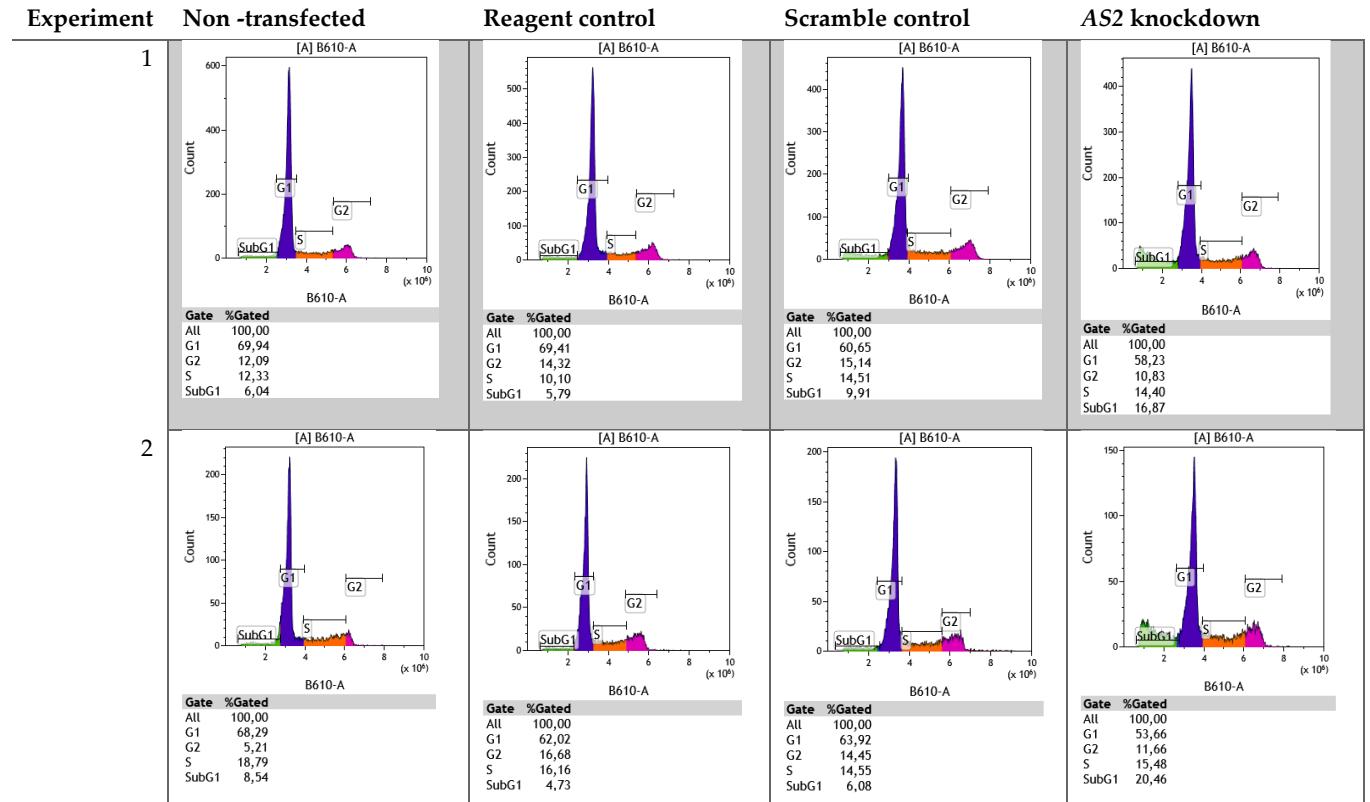
## Supplementary Data

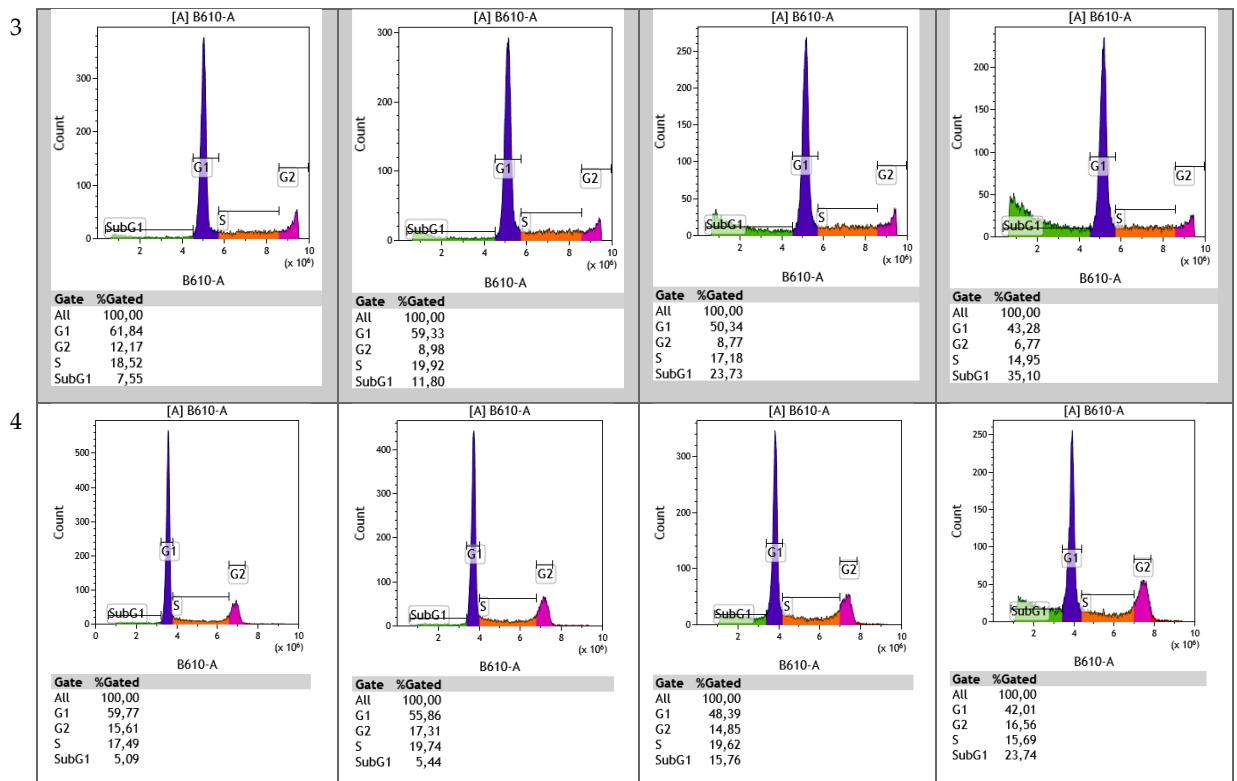
**Table S1:** The average expression of selected lncRNAs in normal prostate, benign prostate, high grade prostate cancer, and CRPC.

lncRNA	Normal prostate	Benign hyperplasia	High grade	CRPC
NAALADL2-AS2	0,00	0,00	0,00	9,92
RP11-20F24.2	0,013	0,00	0,50	8,47
CTD-2126E3.1	0,14	0,20	0,11	1,64
CTD-2126E3.3	0,41	0,21	0,27	3,03
SNORD57	108,99	39,82	35,98	168,22
SNORD3C	94,61	3,19	4,78	21,06
RP11-334J6.4	29,23	1,29	4,49	14,63
SNHG3	113,47	25,03	65,36	197,05
SNORA71A	30,15	8,71	27,71	74,33
RNU11	112,55	20,8	10,74	28,28
AC012531.25	0,091	0,03	0,68	1,74
RN7SL1	39335,03	58220,24	31405,61	61942,52
PCGEM1	0,25	3,37	0,43	0,70
AC132217.4	4,94	12,49	2,61	4,22
PCA3	0,15	0,05	21,99	2,03

Normal prostate (n=4); benign prostate (n=3); high grade prostate cancer (n=4); CRPC (n=4).

**Table S2:** Flow cytometry cell cycle histograms.





**Table S3:** Cell culture media.

Cell line	Media	Supplemented nutrients	
LNCaP	RPMI 1640*	2mM L-Glutamine*	Fetal Calf Serum (10%)**
22Rv1	RPMI 1640*	2mM L-Glutamine*	Fetal Calf Serum (10%)**
DuCaP	RPMI 1640*	2mM L-Glutamine*	Fetal Calf Serum (10%)**
LAPC-4	IMDM*	1nM R1881***	Fetal Calf Serum (7.5%)**

**Table S4:** Primer list.

<b>Gene</b>	<b>Forward primer</b>	<b>Reverse primer</b>
NAALADL2-AS2	5'-ACCAGCGGAAATTGAAAAGCAA-3'	5'-GCTCAGCTCATGTCTTCCT-3'
RP11-20F24.2	5'-TTCCAGTGTGTCACGTGGTT-3'	5'-TCTAGTCAGCACCAACCGT-3'
CTD-2126E3.1	5'-TGGCCAACGCCAAGACTTAT-3'	5'-AGACGCGGAGCCTAAACAG-3'
CTD-2126E3.3	5'-GCCTGACGCCAGAGTTCT-3'	5'-CAGGCAATTGCTAGGCTCG-3'
SNORD57	5'-GGAGGTGATGAACGTCT-3'	5'-GGATCAGGCTCATTAAATC-3'
SNORD3C	5'-CTTGGCATGTCGCGAGAAAG-3'	5'-AATAGGAGGTGCCACACAGC-3'
RP11-334J6.4	5'-CCAATAGATCCTCTGACCCCTCC-3'	5'-TCTCTCAGTCACTGTCCTCTCA-3'
SNHG3	5'-TGCACCTCGCATTGGCAT-3'	5'-GCACCTCAATCTTGCTCCA-3'
SNORA71A	5'-GCCTGTGCCTAGGTATTGAT-3'	5'-TAGGGTGGACCCTCCAAACA-3'
RNU11	5'-AAGGGCTCTGCGTGAGTG-3'	5'-CGGGACCAACGATCACCAAG-3'
AC012531.25	5'-CCCTGTGGTGACAGACTTC-3'	5'-GGGTGGGCTCAACTTCTCA-3'
RN7SL1	5'-TCCGCACTAACGTTCGGCATC-3'	5'-TCAGCACGGGAGTTTGACC-3'
PCGEM1	5'-AGATGCACTGGACTCAACG-3'	5'-CCCTAGGAGTAGGCCTGTGT-3
AC132217.4	5'-CTGCCCATCGACCAGGTTG-3'	5'-CACGGGGAGGAGACAAGATG-3'
PCA3	5'-AGGGGAGATTGTGTGGCTG-3'	5'-ATGTCCTCCCTCACAGCG-3'
AR	5'-AAGGAACTCGATCGTATCATTGC-3'	5'-TTGGGCACCTGCACAGAGAT-3'
NAALADL2	5'-CCTAACACGTCTTGCAAGGTAA-3'	5'-CTAAGTCAAGGGCAGTGGCT-3'
KLK3	5'-CGGTTGTCTTCCTCACCCCTG-3'	5'-CCTCCCACAATCCGAGACAG-3'
GATA2	5'-AAGAGTCCGCTGCTGTAGTC-3'	5'-AGGCCTAGCTACTATGGGCA-3'
FOXA1	5'-AGGGCATGAAACCAGCGAC-3'	5'-GAGTCATGTTGCTGACCGGG-3'
GAPDH	5'-TCAAGGCTGAGAACGGGAAG-3'	5'-TGGACTCCACGACGTACTCA-3'

**Table S5:** Probe set used to image NAALADL2-AS2.

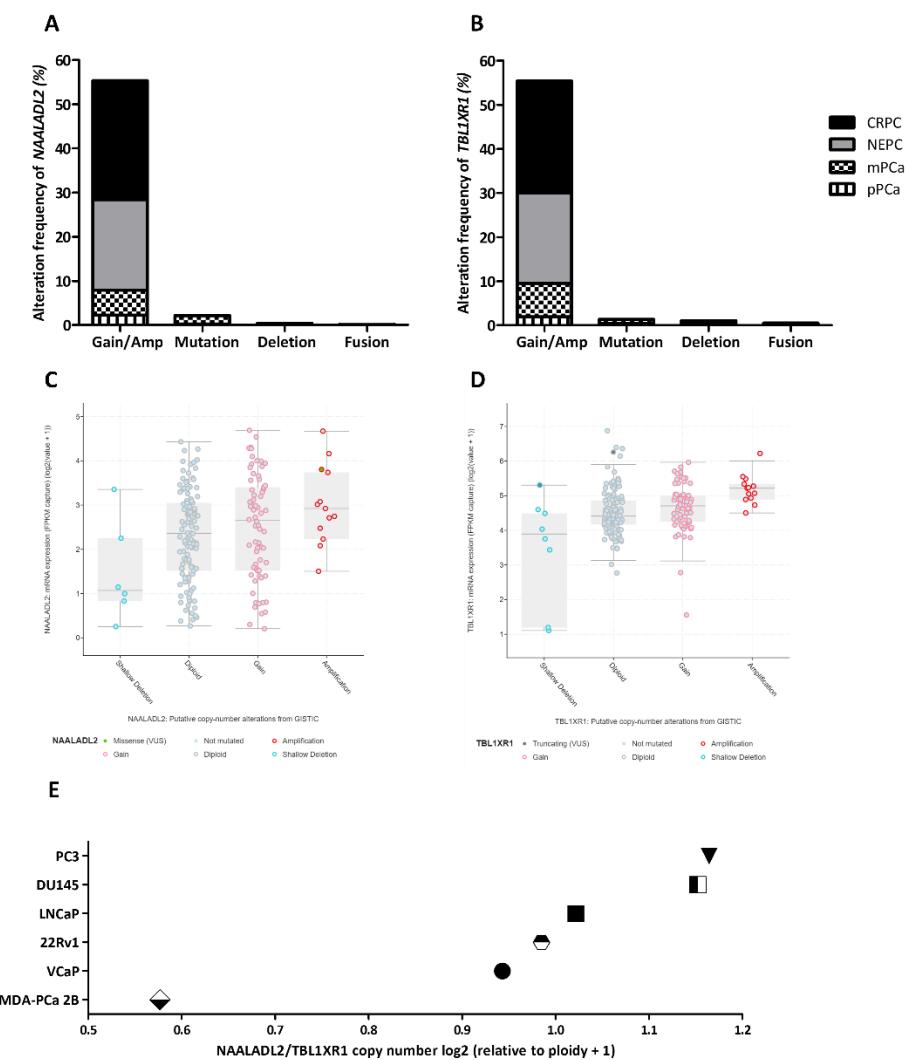
Probe #	Probe (5'-> 3')	Probe position	Percent GC
1	gagattgtttgatgtctca	26	40.0%
2	attcagtcttcagaacta	48	35.0%
3	ctcatttcagcagatgctgc	70	50.0%
4	ccgctggtaaacattgatta	93	45.0%
5	ccatcttttgctttcaat	115	35.0%
6	cttgagtggatccttggaa	140	45.0%
7	agctcatgtcttcctaaa	205	40.0%
8	tttcaacctcttcatacc	235	35.0%
9	tactctgtctaaggccatacg	258	45.0%
10	aagactctaaaaggtaaccc	299	40.0%
11	tcagaagcttggcctaag	321	45.0%
12	gaaggcaactgtcactctt	348	45.0%
13	tattatgctttctggcag	408	40.0%
14	atttctactgttaggactcat	446	35.0%
15	caccttgaatgtcttacca	503	40.0%
16	tttagccaaagagctgcatt	534	40.0%
17	cagcatcgttctattctgt	571	40.0%
18	tgctcgttcataatagatct	629	40.0%
19	ccttctatggactagttt	878	35.0%
20	acgtgtaaatggcaacagt	900	40.0%

Designed by Biosearchtech.

**Table S6:** GapmeR and siRNA oligos.

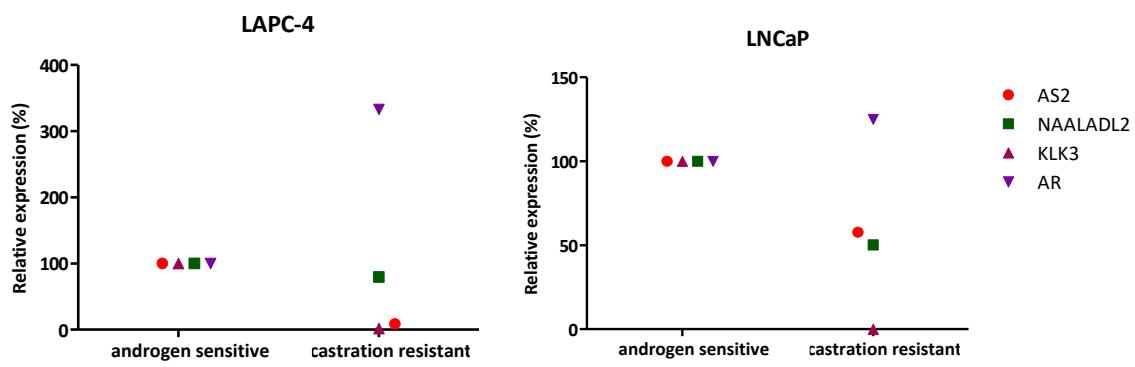
Name	siRNA/LNA	Sequence	Supplier
SCR (Control)	LNA	[C*C*U*U*C*]C*C*T*G*A*A*G*G*T*T*[C*C*U*C*C]	Eurogentec
AS2_IDT	LNA	A*U*A*-A*C*5*-7*8*5*-5*5*8*-7*6*8*-C*G*U*-U*C	Eurogentec
siFOXA1	siRNA	ACAUGACCAUGAACACCAUtt	Ambion, Silencer Select
siGATA2	siRNA	GGCCCACAAACUACAUUGGAAtt	Ambion, Silencer Select
SCR (Control)	siRNA	Ambion Silencer Select® Negative Control #2 (P/N AM4613)	Ambion, Silencer Select

**Figure S1:** Somatic alterations of NAALADL2 and TBL1XR1 in prostate cancer.



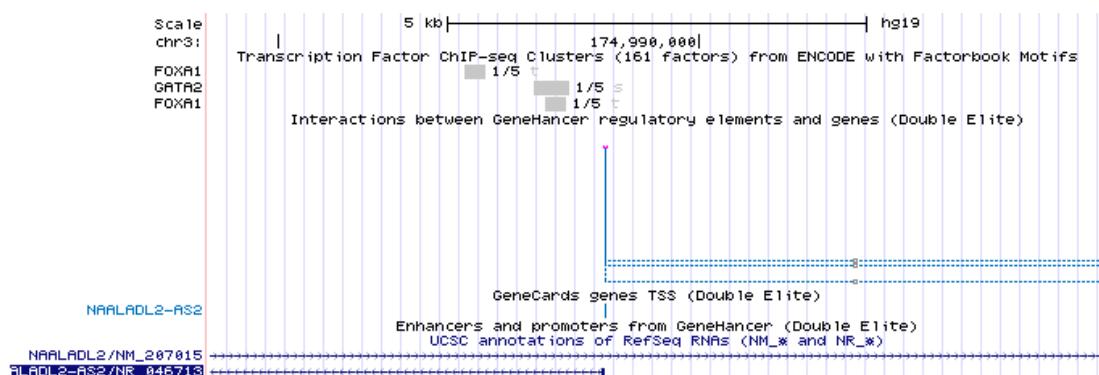
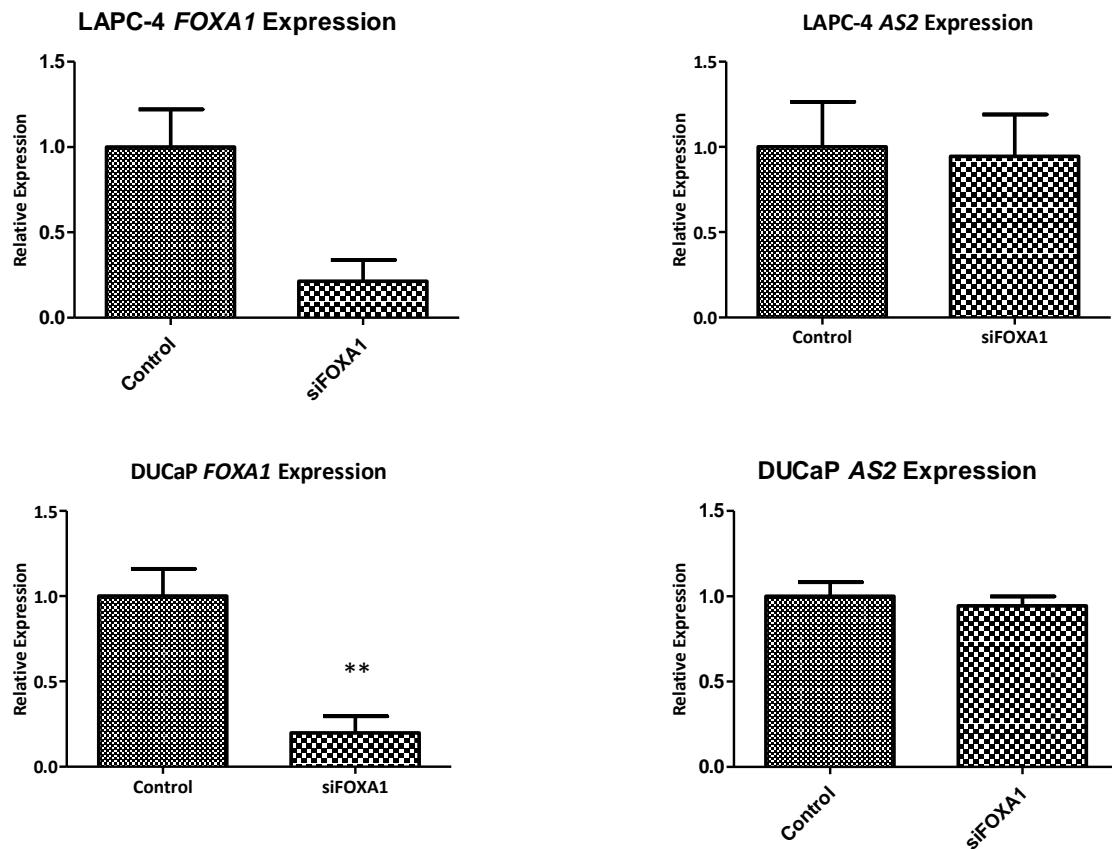
Alteration frequency of *NAALADL2* (A) and *TBL1XR1* (B) in prostate cancer subtypes. Copy number alterations and relative mRNA expression levels of *NAALADL2* (C) and *TBL1XR1* (D) in PCa. Copy numbers of *NAALADL2* and *TBL1XR1* in 6 common prostate cancer cell lines (E). Publicly available data containing 3804 patients in 16 non-overlapping studies consisting of 11 primary PCa (pPCA), 4 metastatic PCa (mPCA), 1 neuroendocrine PCa (NEPC) and castration resistant PCa (CRPC) (A/B) [1]. The copy number and mRNA plots were produced using cBioPortal including 2 studies (C/D) [2,3]. Copy number alterations in cell lines were obtained from the Broad Institute's depmap portal (<https://depmap.org/portal/cclc/>).

**Figure S2:** Relative expression of *NAALADL2-AS2*, *NAALADL2*, *KLK3*, and *AR* in LAPC-4 and LNCaP.



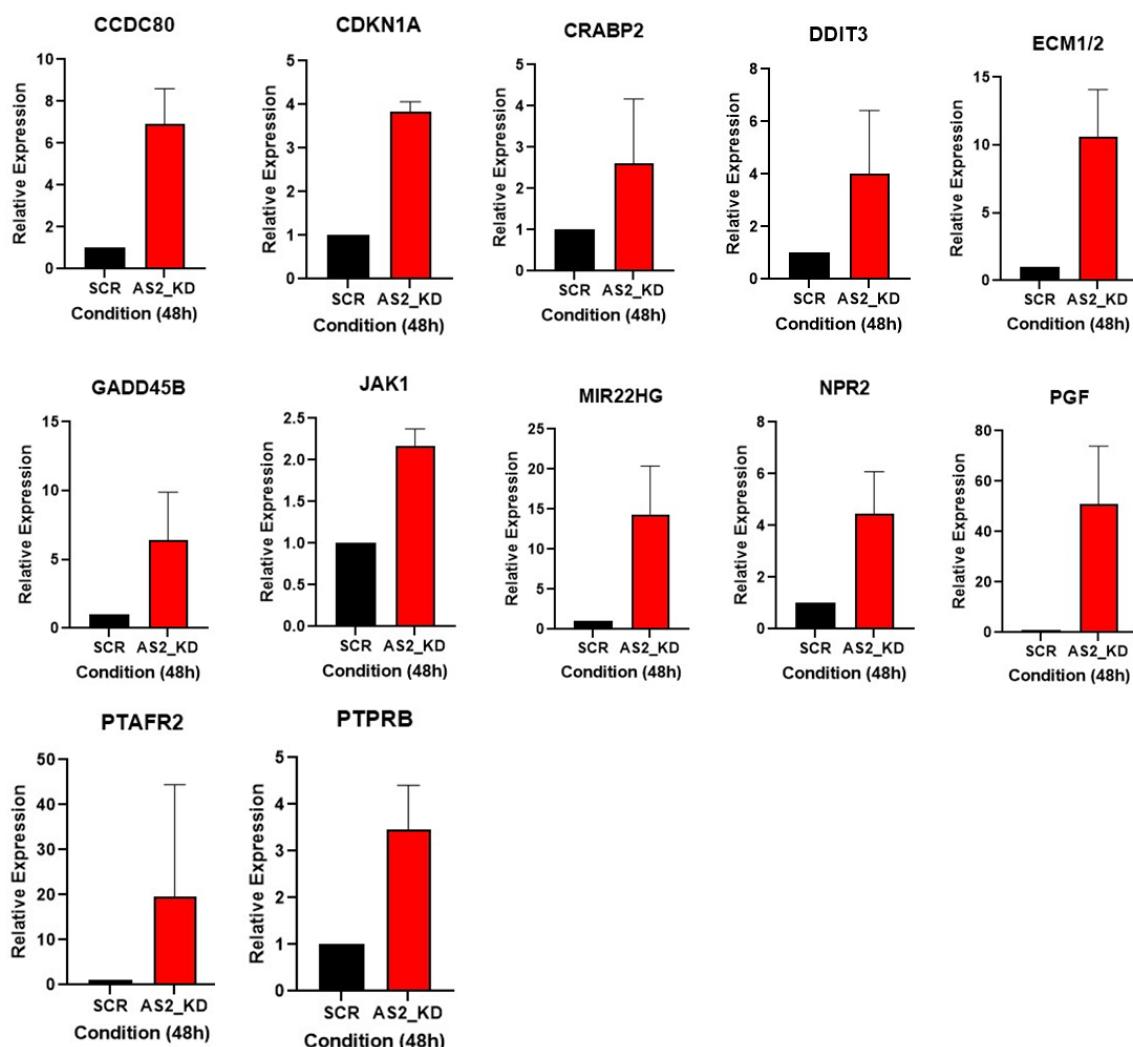
Data was downloaded from the Gene Expression Omnibus (GSE178963).

**Figure S3:** *FOXA1* has a transcriptional start site at the *NAALADL2-AS2* locus.



Genomebrowser was used to generate the bottom figure (<http://genome.ucsc.edu>).

**Figure S4:** Quantitative PCR validation of NAALADL2-AS2 regulated genes.



All data shown is from triplicate qPCR assays.

## References

1. Simpson, B.S.; Camacho, N.; Luxton, H.J.; Pye, H.; Finn, R.; Heavey, S.; Pitt, J.; Moore, C.M.; Whitaker, H.C. Genetic Alterations in the 3q26.31-32 Locus Confer an Aggressive Prostate Cancer Phenotype. *Commun Biol* **2020**, 3, doi:10.1038/s42003-020-01175-x.
2. Beltran, H.; Prandi, D.; Mosquera, J.M.; Benelli, M.; Puca, L.; Cyrta, J.; Marotz, C.; Giannopoulou, E.; Chakravarthi, B.V.S.K.; Varambally, S.; et al. Divergent Clonal Evolution of Castration-Resistant Neuroendocrine Prostate Cancer. *Nat Med* **2016**, 22, 298–305, doi:10.1038/nm.4045.
3. Weinstein, J.N.; Collisson, E.A.; Mills, G.B.; Shaw, K.R.M.; Ozenberger, B.A.; Ellrott, K.; Sander, C.; Stuart, J.M.; Chang, K.; Creighton, C.J.; et al. The Cancer Genome Atlas Pan-Cancer Analysis Project. *Nat Genet* **2013**, 45, 1113–1120, doi:10.1038/ng.2764.