**Supplementary Information**

**The lncRNA *DRAIC/PCAT29* locus constitutes a tumor suppressive nexus**

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 **[1] Supplementary material and methods**

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**[3] Supplementary References**

**[1] Supplementary material and methods**

**(1) Plasmid constructions**

For the construction of the *DRAIC* expression vector, the full length of *DRAIC* was amplified by PCR using the primer pair (Supplementary Table 1) from LNCaP cDNA and was inserted into pCR4-TOPO vector (Life Technology) to generate pCR4-*DRAIC*. The BamHI/XhoI fragment of pCR4-*DRAIC* was inserted into pcDNA3-FLAG (1) to generate pcDNA3-FLAG- *DRAIC*.

For the construction of the *FOXA1* expression vectors, the full length of *FOXA1* was amplified by PCR using the primer pair (Supplementary Table1) from LNCaP cDNA and was inserted into pCR4-TOPO vector to generate pCR4-*FOXA1*. The HindIII/XhoI fragment of pCR4-*FOXA1* was inserted into pcDNA3 to generate pcDNA3-*FOXA1*. For *NKX3-1* overexpression, we used pcDNA3-*NKX3-1* (2) (#42897, Addgene).

For the construction of the short hairpin RNAs (shRNAs) that targets *DRAIC*, the sense and antisense oligonucleotides (Supplementary Table 1) were annealed and inserted between the BbsI/EcoRI sites of pmU6 kindly gifted by Dr.Iba (3) to generate pmU6-shRNA. This product was digested with BamHI/EcoRI and inserted between these sites in pLSP kindly gifted by Dr.Iba (3) to generate the lentiviral vector, pLSP-mU6-shRNA.

**(2) Tissues**

De-identified mid-Gleason-grade prostate cancer tissues were obtained from the University of Virginia mid-Atlantic CHTN (Cooperative Human Tissue Network). A pathologist screened sections so that at least 70% of the cells in a cancer section were malignant.

**(3) Constructions of stable *DRAIC* overexpressing cells**

pcDNA-FLAG (*Empty*) and pcDNA-FLAG-*DRAIC* were digested with Bgl II and linearized vectors were purified from agarose gel by QIAquick Gel Extraction Kit (QIAGEN). PC3M-luc cells were seeded into 6 well plate and linearized vector (1µg) was transfected by Lipofectamine 2000 (Invitrogen). At 24h after transfection, the medium was changed to fresh medium and at 48 hours after transfection, cells were selected with 300µg/mL of G418. During G418 selection, the medium was replaced with fresh medium containing G418 every 3-4 days. The selections were completed at 20-30 days after the transfection. After the selection, the G418 concentration was reduced to 30µg/mL and maintained.

**(4) Virus production and transduction**

For virus production, vesicular stomatitis virus-G–pseudotyped lentiviral vectors (pLSP) kindly gifted by Dr.Iba (3) (3.0µg) were transfected into HEK293T cells (7x106 cells) in 10cm plate by the Lipofectamine 2000 (Invitrogen) with virapower packaging mix (9.0 ug) (pLP1, pLP2 and pLP-VSVG) (Invitrogen). Supernatants were collected at 24h and 48h after transfection and mixed, followed by filtering through 0.45µm filter (Corning) as virus solutions and stored the aliquots at -80ºC.

For virus infection, the virus solutions with 8.0µg/mL polybrene were added to the cells (1x105 cells) in 6 well plate. At 24h after infection, the medium was changed and at 48 hours after infection, the cells were selected with 1µg/mL puromycin (Sigma). During puromycin selection, the medium containing puromycin was replaced every 2 days. The selections were completed at 7-8 days after transduction.

At 12 days after sh*DRAIC* transduction in LNCaP cells, the expression of *DRAIC* was measured by RT-qPCR (Fig. 6C). At 17 days after sh*DRAIC* transduction in LNCaP cells, the cells were seeded to new 6 well plates and scratch wound healing assay were performed (Fig. 6E). At 12 days after sh*DRAIC* transduction in LNCaP cells, the cells were seeded to Matrigel invasion chamber and invasion assays were performed (Fig. 6F).

**(5) RT-PCR**

cDNA was synthesized with Random hexamer by the Superscript III First Strand Synthesis System (Invitrogen). Quantitative RT-PCR was performed by 7300 Real time PCR system (Applied Biosystems) with ABsolute Blue SYBR Green Mixes (Thermo SCIENTIFIC). Relative expressions for the target RNAs were determined by the comparative CT method after normalization to *GAPDH*. Semi-quantative RT-PCR was performed by LA-Taq (TaKaRa) with 2720 Thermal Cycler (Applied Biosystems). The primers sequences for RT-PCR analysis are shown in Supplementary Table 1.

**(6) Western blotting**

Total protein extracts were prepared by boiling the cells in SDS sample gel buffer for 10 min at 95°C. The proteins (10-20µg) were then separated by 6-8% SDS-PAGE and transferred into Immobilon-P PVDF membranes (Millipore). Immunoblotting was performed by incubating the membrane for 1-2 hours at RT with primary antibodies. After three washes with phosphate-buffered saline containing Tween, secondary antibodies were incubated with the membranes for 45 minutes at RT. After five washes with phosphate-buffered saline containing Tween, signals were detected using Immobilon Western Chemiluminescent HRP Substrate (Millipore). The primary antibodies used in western blotting are: anti-AR (H-280, Santa Cruz), anti-FOXA1 (ab23738, Abcam), anti-NKX3-1 (sc-15022, Santa Cruz) and anti-Actin (sc-47778, Santa Cruz). The secondary antibodies used in western blotting are: Rabbit (P0448, Dako), Mouse (P0161, Dako) and Goat (P0160, Dako).

**(7) 3’RACE (Rapid amplification of cDNA ends)**

3’RACE was performed with FirstChoice RLM-RACE Kit (Invitrogen) according to the manufacturer’s instruction. 70 ng of LNCap polyA RNA was reverse-transcribed with 3’RACE adaptor at 42ºC for 1 hour. Outer 3’RACE PCR using 1 µl of cDNA was performed with outer forward and reverse primer. PCR cycle : Initial denaturation (1 cycle) 94ºC for 2 minutes, Amplification (35 cycles) 94ºC for 30 seconds & 60ºC for 30 seconds & 72ºC 1 minutes, Final extension (1 cycle) 72ºC for 5 minutes. Nested PCRs using nested forward and reverse primers were performed because PCR bands were not seen at expected size in outer PCR (Supplementary Fig.2B). Nested PCR condition is same as outer PCR as mentioned above. cDNA generated by nested PCR were cut from 2% agarose gel by QIAquick Gel Extraction Kit (QIAGEN) and inserted into pCR4-TOPO vector (life technology). cDNA sequences were confirmed by sanger sequencing.

The primer sequences for 3’RACE are shown in Supplementary Table 1.

**(8) ChIP assay**

LNCaP cells were crosslinked with 1% formaldehyde at RT for 15 min and neutralized in the presence of 0.125 M Glycine at RT for 5 min. The cells were homogenized with ChIP lysis buffer (50 mM Tris-HCl (pH 8.0), 10 mM EDTA, 0.1%SDS, and a proteinase inhibitor cocktail (Complete Mini) (Roche). Each sample was immunoprecipitated with pre-mixed antibody (5µg) -dynabead protein G complex. The immunoprecipitant was incubated at 65°C overnight to de-crosslink and treated with RNaseA at 37°C for 30 min and proteinase K at 55°C for 60 min followed by phenol/chloroform treatment. Purified DNA was analyzed by qPCR using the primers shown in Supplementary Table 1. The antibodies for ChIP assay are: anti-AR (H-280, Santa Cruz), anti-FOXA1 (ab23738, Abcam), anti-NKX3-1 (sc-15022, Santa Cruz), normal rabbit IgG (12-370, Millipore) and normal goat IgG (I5256, SIGMA).

**(9) ChIP-seq analysis**

The supplemental alignment files for ChIP-Seq performed with LNCaP cells for AR without or with R1881, for FOXA1 in regular medium, for NKX3-1 with DHT treatment, and for AR after FOXA1 knockdown were downloaded from these GEO accessions: GSM353643, GSM353644, GSM353633, GSM699633, GSM916521 and GSM916522, respectively. The coverage at each nucleotide in the *DRAIC/PCAT29* locus was recorded and plotted at single nucleotide resolution.

**(10) RNA-seq analysis**

All sequencing reads were aligned to the hg19 reference genome with Tophat using the default settings (4). Novel transcripts were discovered by de novo assembly with Cufflinks and differentially expressed genes were identified using Cuffdiff (5). The datasets used in our analysis are as follows; LNCaP Vehicle and R1881 treated: GSM855072-GSM855077, C4-2B: GSM984368 and GSM984369, pre- and post-androgen deprivation therapy: GSM1177208-GSM1177214 and GSM1177215-GSM1177221, respectively (6–8). The expression of *DRAIC* for each of the pre- and post-Androgen Deprivation Therapy (ADT) samples was determined using the Tuxedo suite and plotted using R. The statistical significance of the changes in *DRAIC* expression was evaluated using a paired t-test. For the correlation curve, all tier-3 RNA-seq data for prostate adenocarcinoma (PRAD) was downloaded from the TCGA (<https://tcga-data.nci.nih.gov/tcga/>). The pre-determined RSEM expression values of NKX3-1, FOXA1 and *DRAIC* were collected for all tumors and the Spearman correlation coefficient for each comparison was calculated using R.

**[2] Supplementary Figures**

**Supplementary Figure 1**

**Cellular localization of *DRAIC* and 3’RACE of *DRAIC* gene**

(A) Semi-quantative RT-PCR was performed using LNCaP nuclear (Nuc) and cytoplasm (Cyt) RNA. *APTR*, which is a lncRNA identified by our group (1), is located mostly in the nuclear fraction. *DRAIC* and *GAPDH* mRNA are expressed in cytoplasm. (B) 3’RACE was performed using LNCaP poly A+ RNA. 3’ end of *DRAIC* is shown and polyA signal (AATAAA) is boxed. The products of the reaction are shown on the gel and were sequenced to confirm the 3' end.

**Supplementary Figure 2**

**Transcriptional variants of *DRAIC* gene**

(A) Gene structures of transcript variants (variant1, 2 and 3) and *DRAIC*. Forward and reverse RT-qPCR primers which can distinguish each transcripts are shown as arrows. (B) The expressions of variant 1, 2 and 3 in a panel of prostate cancer cells were measured by RT-qPCR and normalized to *GAPDH*. Rest as in Fig. 1C.

**Supplementary Figure 3**

**AR, FOXA1 and NKX3-1 binding sequences at *DRAIC* locus**

Putative binding sequences of AR and FOXA1 at *DRAIC* locus were searched by ALGGEN-PRIMO (<http://alggen.lsi.upc.es/cgi-bin/promo_v3/promo/promoinit.cgi?dirDB=TF_8.3>).

Half-ARE sequences are boxed under AR sites. Putative binding sequences of NKX3-1 identical to the consensus sequences (5’TAAGTA3’) are shown.

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