
Supplementary information

**tRNA dosage regulates lineage dependency
and resistance in prostate cancer**

In the format provided by the
authors and unedited

Supplementary Information
tRNA dosage regulates lineage dependency and resistance in prostate cancer

Yeon Soo Kim¹, Sonali Arora¹, Dave Young¹, Ava Tsou¹, Amy Shiuan¹, Cynthia L. Wladyka¹, Dmytro Rudoy¹, Jin Yeong Kim¹, Jennifer A. Waters¹, Samantha L. Schuster^{1,2}, Ilsa M. Coleman¹, Mridu Kapur^{3,4}, Marek Sobczyk⁵, Christopher D. Katanski^{5,6}, Amin M. Bayat Tork⁵, Wen Zhang⁵, Peter S. Nelson^{1,7,8,9,10}, Gavin Ha^{1,2}, Michael C. Haffner^{1,10}, Eva Corey¹¹, Colm Morrissey¹¹, Yuzhuo Wang^{12,13}, Arvind R. Subramaniam^{2,9,14}, John K. Lee^{15,16}, Susan L. Ackerman^{3,4,17}, Tao Pan^{5*}, Andrew C. Hsieh^{1,7,8,9*}

Affiliations:

¹Human Biology Division, Fred Hutchinson Cancer Center, Seattle, WA 98109, USA

²Computational Biology Section of the Public Health Sciences Division, Fred Hutchinson Cancer Center, Seattle, WA 98109, USA

³Department of Cellular and Molecular Medicine, University of California, San Diego, School of Medicine, La Jolla, CA 92093, USA

⁴The Howard Hughes Medical Institute, La Jolla, CA 92093, USA

⁵Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, IL 60637, USA

⁶MesoRNA LLC, Chicago, IL 60607, USA

⁷Clinical Research Division, Fred Hutchinson Cancer Center, Seattle, WA 98109, USA

⁸Department of Medicine, University of Washington, Seattle, WA 98195, USA

⁹Department of Genome Sciences, University of Washington, Seattle, WA 98195, USA

¹⁰Department of Pathology and Laboratory Medicine, University of Washington, Seattle, WA 98195, USA

¹¹Department of Urology, University of Washington, Seattle, WA 98195, USA

¹²Prostate Centre at Vancouver General Hospital and University of British Columbia, Vancouver, British Columbia, Canada

¹³BC Cancer Research Institute, Vancouver, BC, Canada

¹⁴Basic Sciences Division, Fred Hutchinson Cancer Center, Seattle, WA 98109, USA

¹⁵Division of Hematology/Oncology, Department of Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA, 90095, USA

¹⁶Department of Molecular and Medical Pharmacology, David Geffen School of Medicine at UCLA, Los Angeles, CA, 90095, USA

¹⁷Department of Neurobiology, University of California San Diego, La Jolla, CA 92093, USA

Co-correspondence:

Andrew C. Hsieh, M.D.
Division of Human Biology
Fred Hutchinson Cancer Center
1100 Fairview Ave N
Seattle, WA 98109
Email: ahsieh@fredhutch.org

Tao Pan, Ph.D.
Department of Biochemistry & Molecular Biology
University of Chicago
924 E. 57th Street, BSLC R013A
Chicago, IL 60637
Email: taopan@uchicago.edu

Supplementary Figures

Figure S1. Original blots

Supplementary Tables

Table S1. MSR-seq data analysis

MSR-Seq Isoacceptor DESeq2 – related to Fig. 1a

MSR-Seq Isodecoder DESeq2 – related to Fig. 1b

MSR-Seq Isoacceptor edgeR – related to Extended Data Fig. 1c

MSR-Seq Isodecoder edgeR – related to Extended Data Fig. 1e

Table S2. Patient tissue MSR-seq analysis

Patient tissue isodecoder - related to Extended Data Fig. 2h

Table S3. UCU-1 KD RNA-seq data analysis

LNCaP RNA-seq (shScr vs shUCU) - related to Fig. 2b,d & Extended Data Fig. 3e

C4-2B RNA-seq (shScr vs shUCU) - related to Fig. 2b,d & Extended Data Fig. 3f

Table S4. RNA-seq analysis from LNCaP OE cell lines

CRPC-AR genes - related to Fig. 3b

UCU-1 - related to Extended Data Fig. 5d

UCU-2 - related to Extended Data Fig. 5f

UCU-3 - related to Extended Data Fig. 5f

UCU-4 - related to Extended Data Fig. 5f

UCU-5 - related to Extended Data Fig. 5f

ACG-1 - related to Extended Data Fig. 5h

CCG-2 - related to Extended Data Fig. 5h

CCU-4 - related to Extended Data Fig. 5h

Table S5. RNA-seq analysis from LNCaP AD and LP

LNCaP RNA-seq_AD_LP - related to Fig. 4b

Table S6. CUT&RUN POLR3A, TARDBP, ZSCAN29 differential binding analysis

TARDBP - related to Fig. 4f & Extended Data Fig. 8a

ZSCAN29 - related to Fig. 4f & Extended Data Fig. 8a

tRNA genes - related to Extended Data Fig. 8b

Table S7. LNCaP codon frequency calculation

Codon frequency summary - related to Fig. 5b-d

Table S8. Polysome RNA-seq from LNCaP LP and UCU

Polysome_RNA-seq_UCU_LP - related to Fig. 5e

Table S9. Resources

Antibodies - List of antibodies used in the study

Cloning - List of gBlocks and oligonucleotides used for cloning in the study

Plasmids - List of backbone plasmid used in the study

Primers - List of primers used in the study

Probes - List of northern blot probes used in the study

siRNAs - List of siRNA pools used in the study

Table S10. High confidence tRNA list for MSR-seq analysis**Table S11. GSVA gene list**

AR score - ARG.10 and ARG.6 genes (PMID: 29017058)

NE score - NE.10 and NE.6 genes (PMID: 29017058)

EMT score - GAVISH_3CA_MALIGNANT_METAPROGRAM_13_EMT_2 genes (PMID: 37258682)

ASCL1 score - NOURUZI_NEPC_ASCL1_TARGETS genes (PMID: 35477723)

SNAI1 score - SNAI1_TARGET_GENES genes (PMID: 30445619)

SOX2 score - SOX2 genes (PMID: 35067686)

Metastasis score - prostate cancer metastasis-associated genes (PMID: 17430594, 17173048)

Table S12. Statistical analyses of Extended Data Fig. 1f, 1g, 2g

Supplementary Figures

S1. Original blots

Extended Data Figure 1i (red box)
C4-2B CRISPR KO northern blot

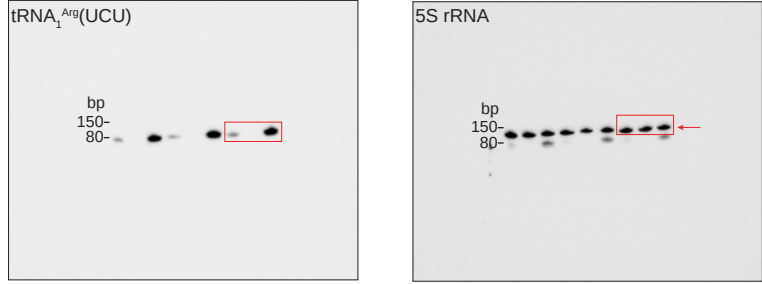


Figure 1c (red box)

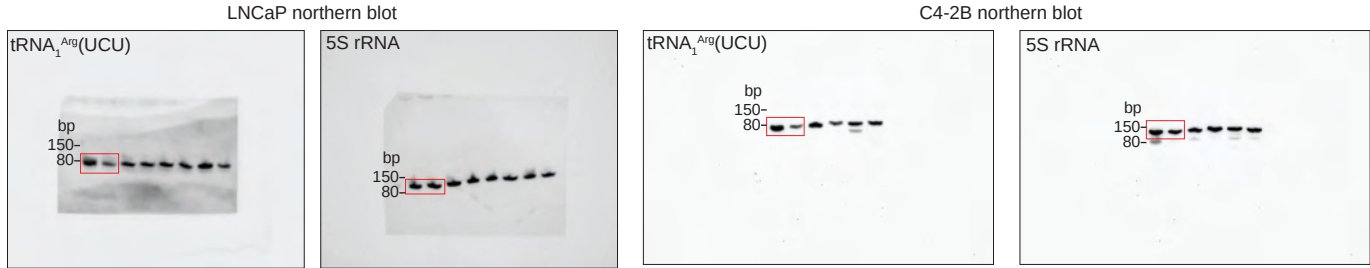


Figure 1f (red box) and Extended Data Figure 2a
LTL331 PDX northern blot

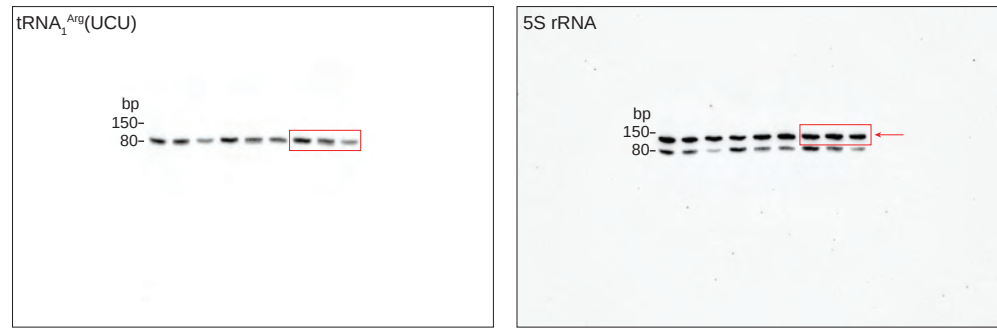


Figure 2a (red box)

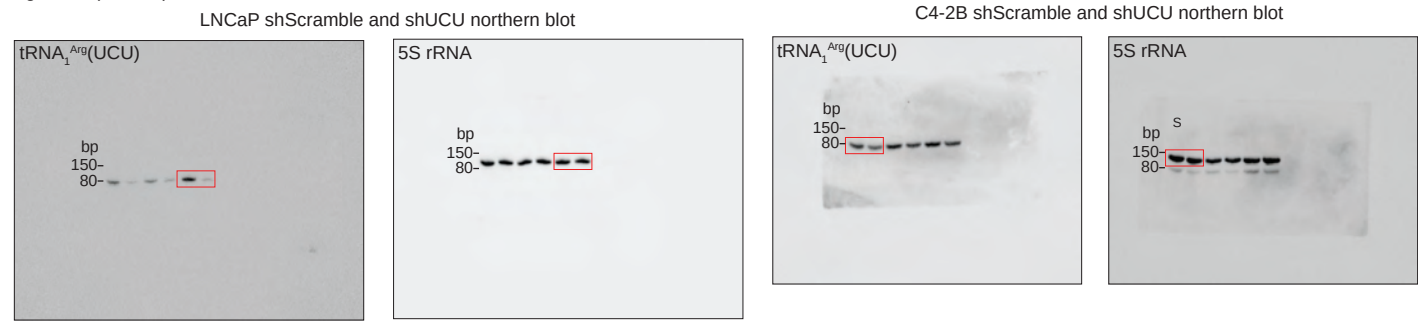
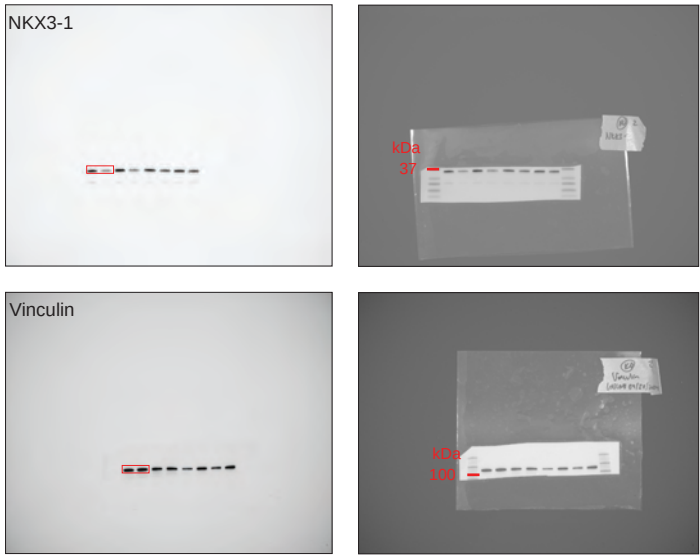
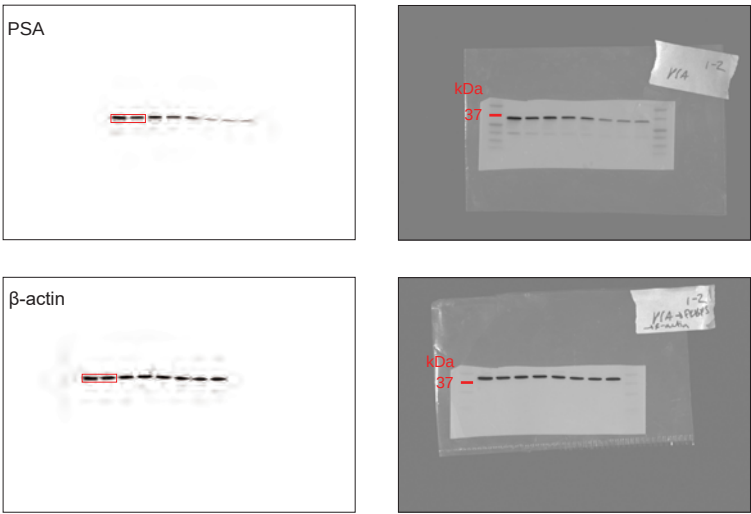


Figure 2c (red box)

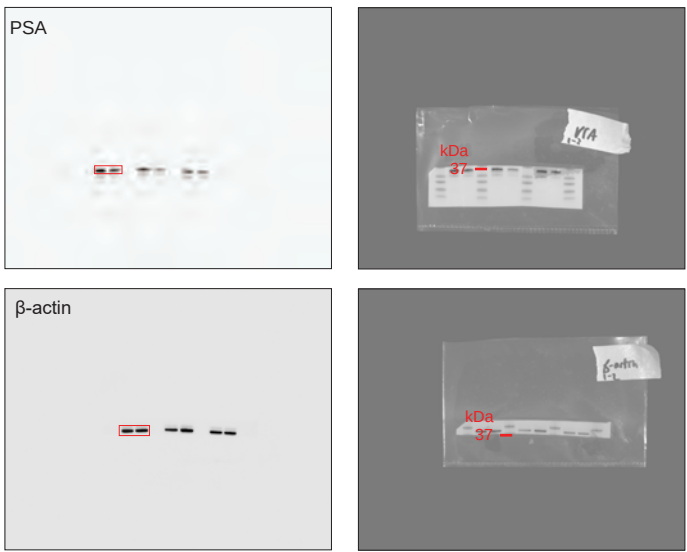
LNCaP shScramble and shUCU NKX3-1, Vinculin western blot



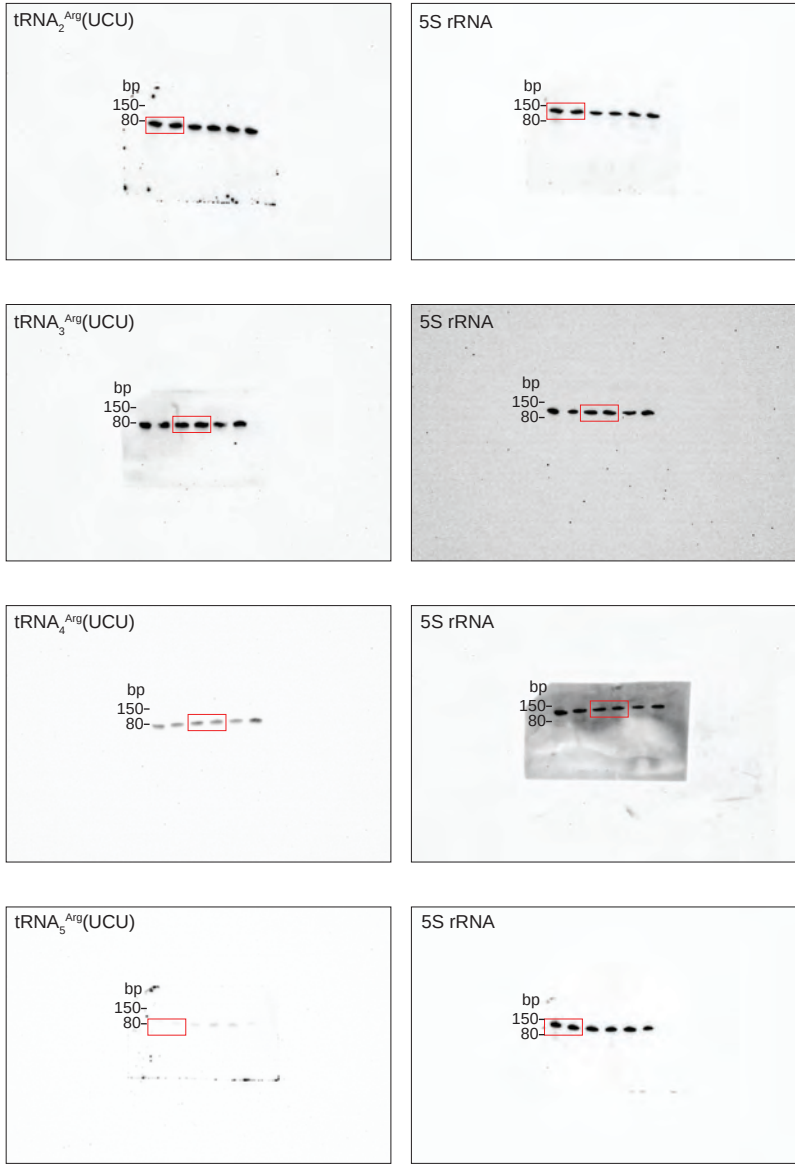
LNCaP shScramble and shUCU PSA, β -actin western blot



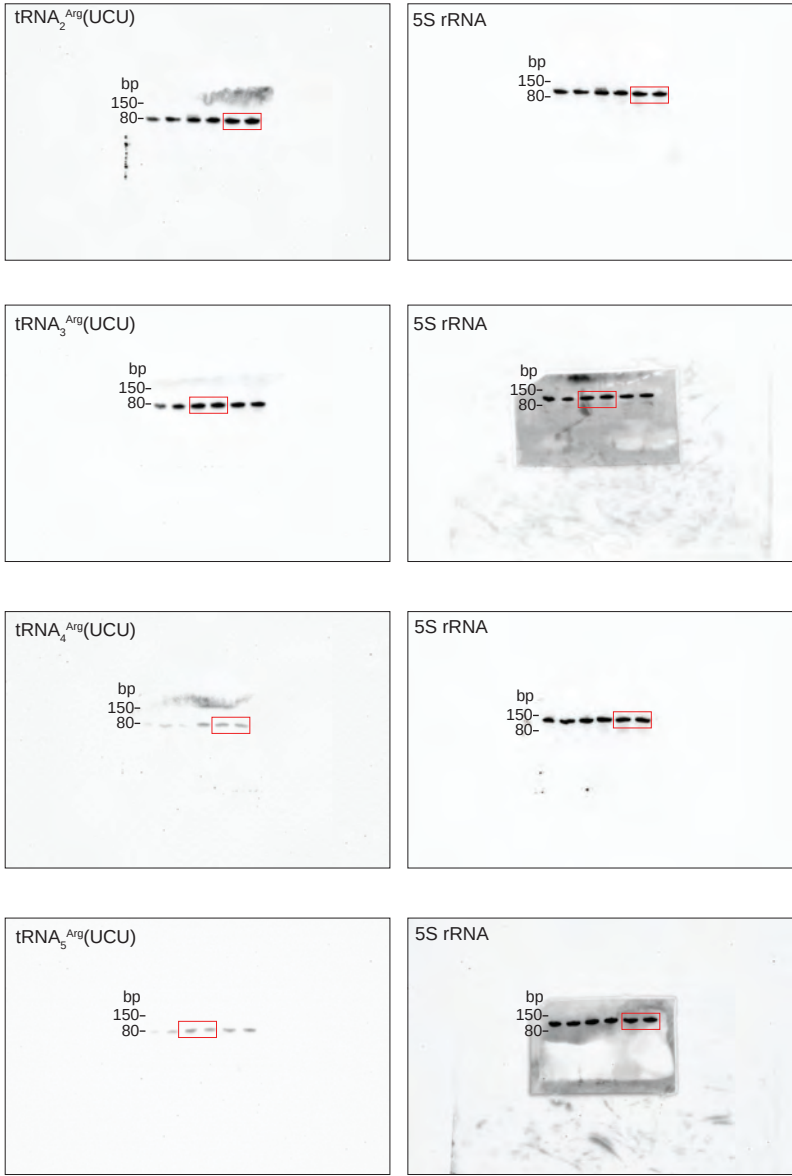
C4-2B shScramble and shUCU PSA, β -actin western blot



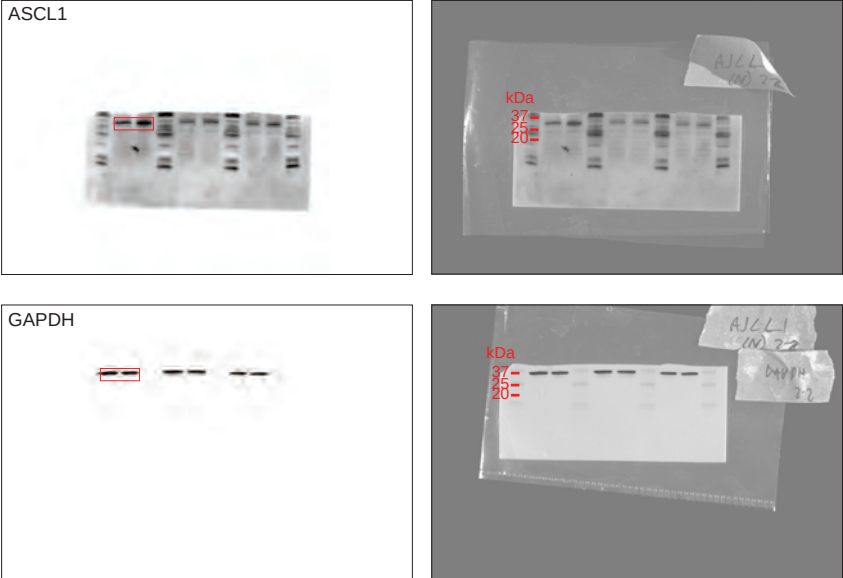
Extended Data Figure 3c (red box)
LNCaP shScramble and shUCU northern blot



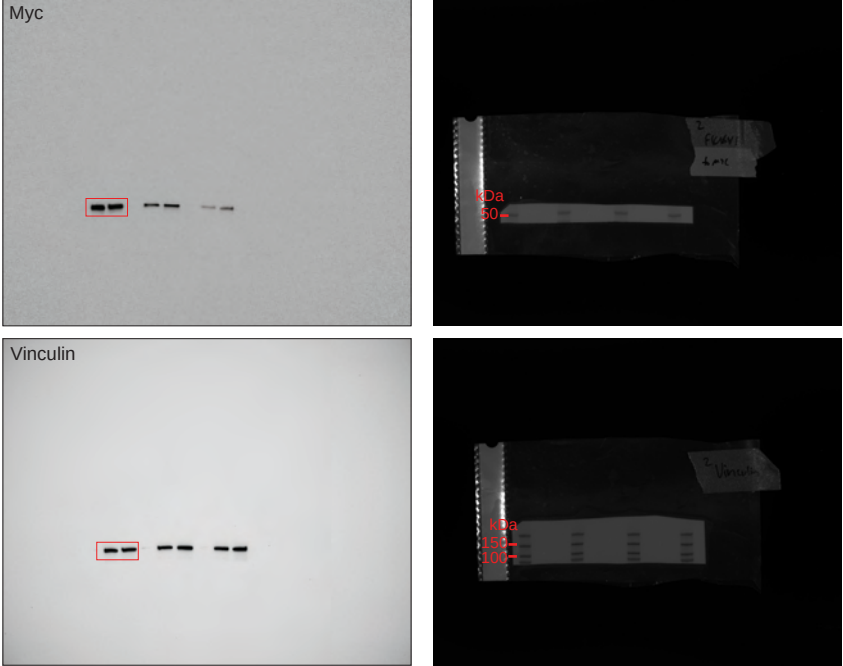
Extended Data Figure 3c (red box)
C4-2B shScramble and shUCU northern blot



Extended Data Figure 3g (red box)
C4-2B shScramble and shUCU ASCL1, GAPDH western blot



Extended Data Figure 4b (red box)
Mouse prostate organoid Myc, Vinculin western blot



Extended Data Figure 4c (red box)
Mouse prostate organoid northern blot

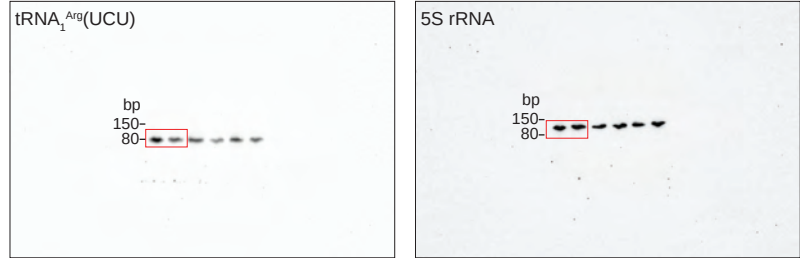


Figure 3a (red box)

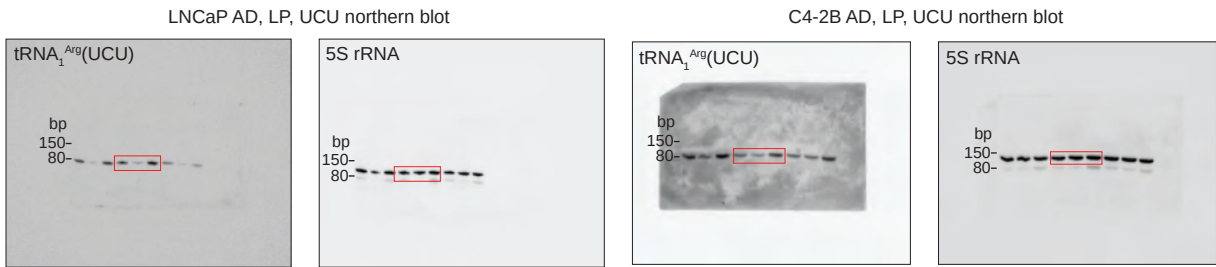


Figure 3d (red box)

LNCaP AD, LP, UCU western blot

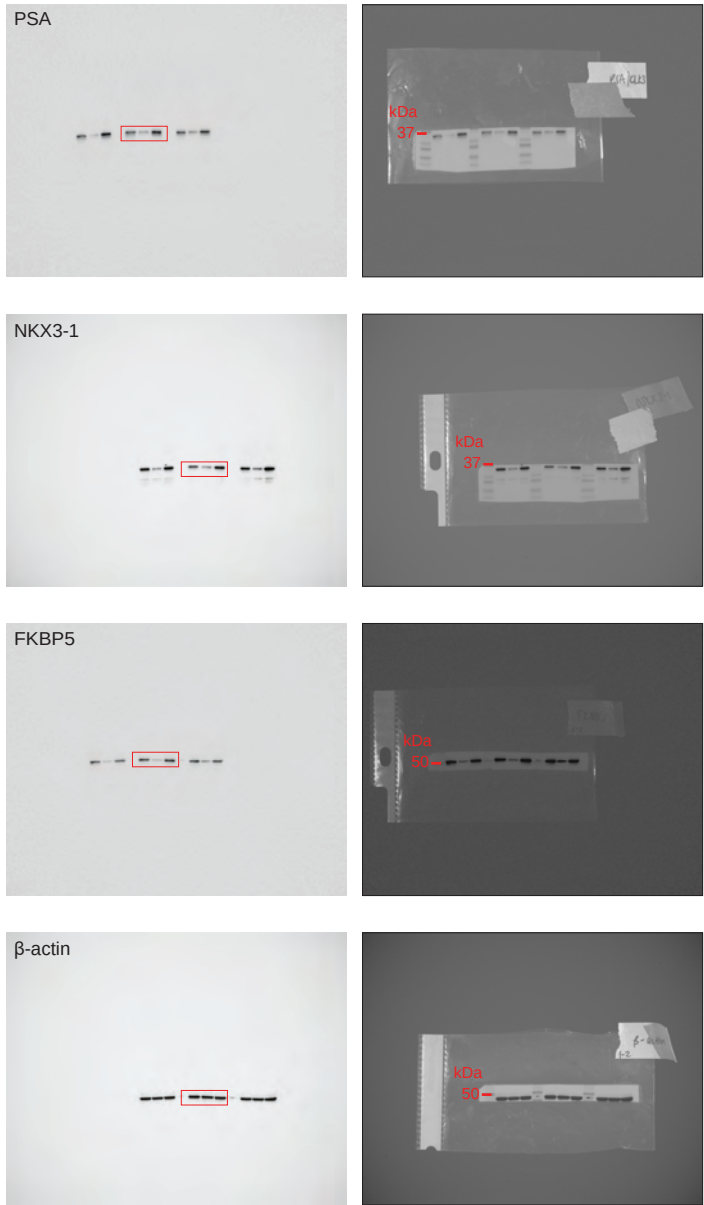


Figure 3d (red box)
C4-2B AD, LP, UCU western blot

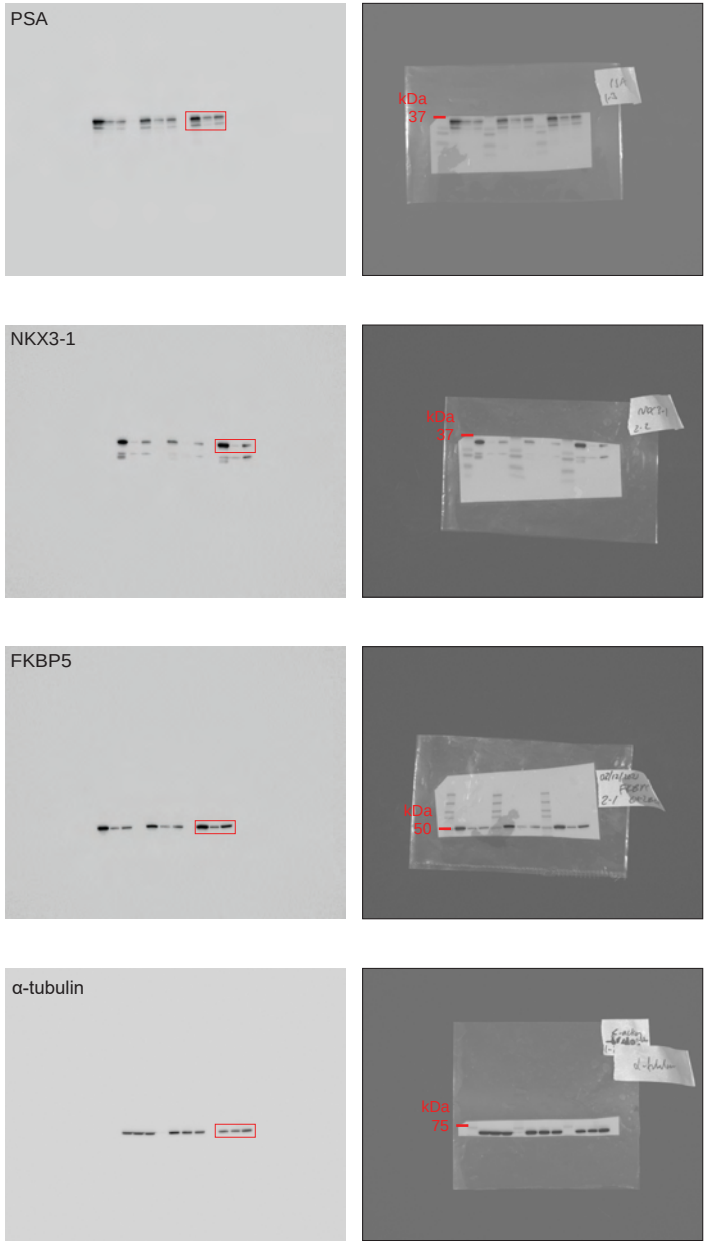
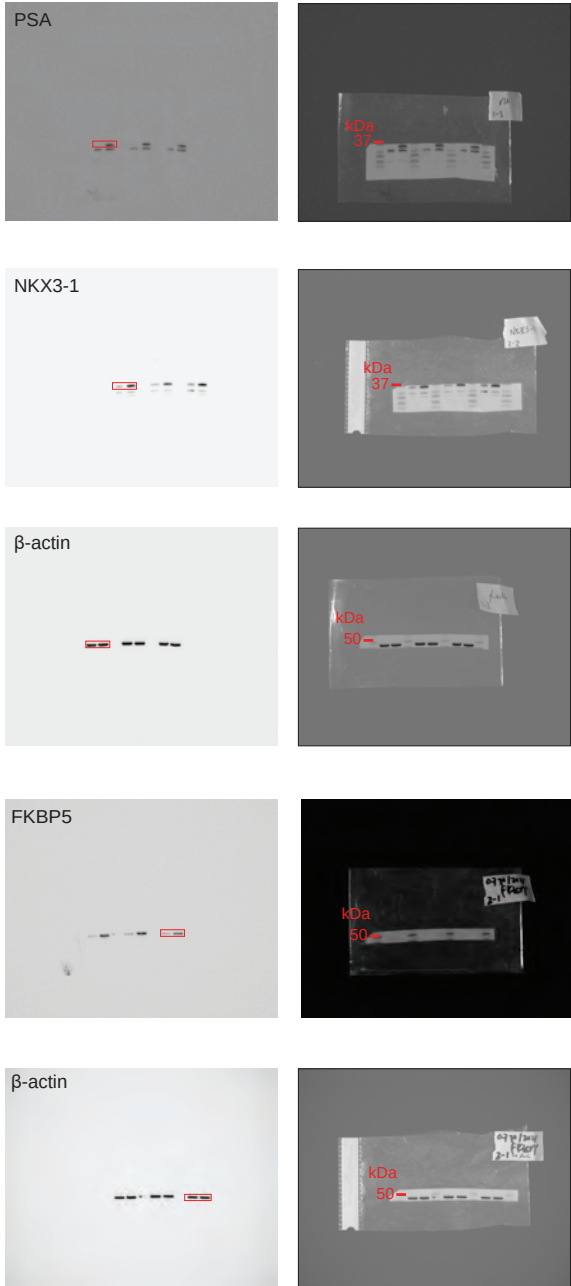
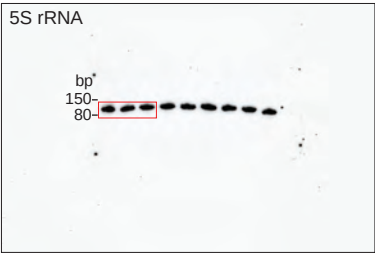
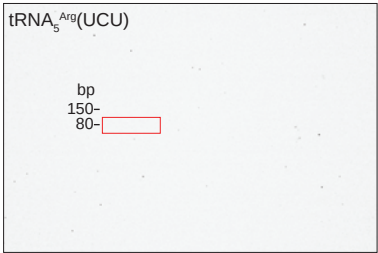
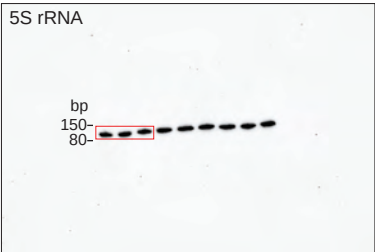
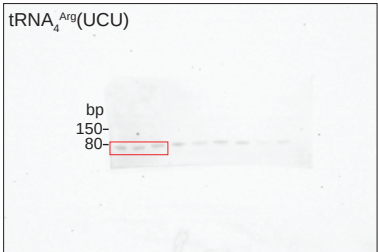
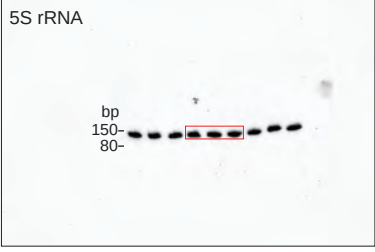
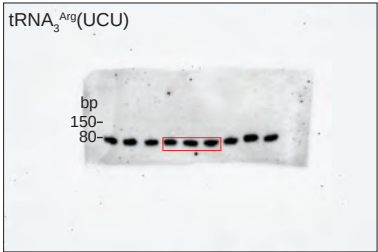
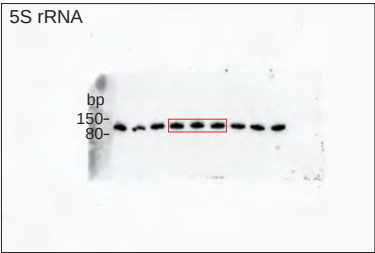
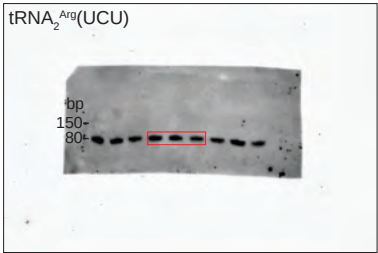


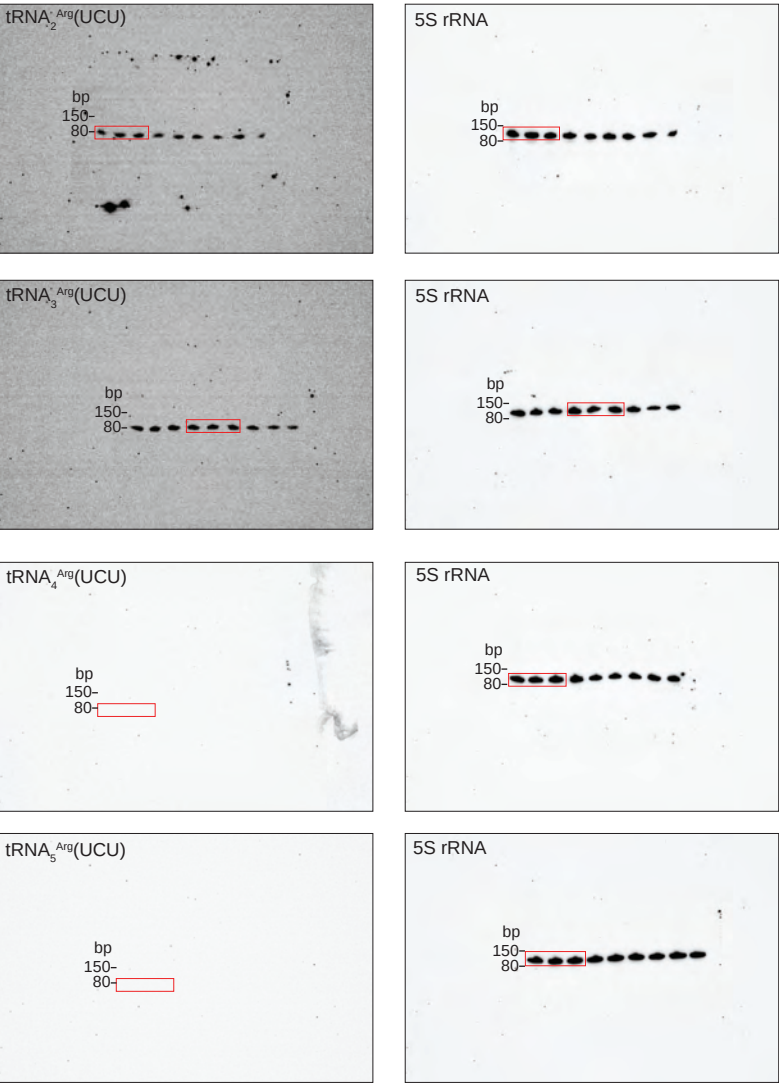
Figure 3g (red box)
LNCaP-abl WT and UCU western blot



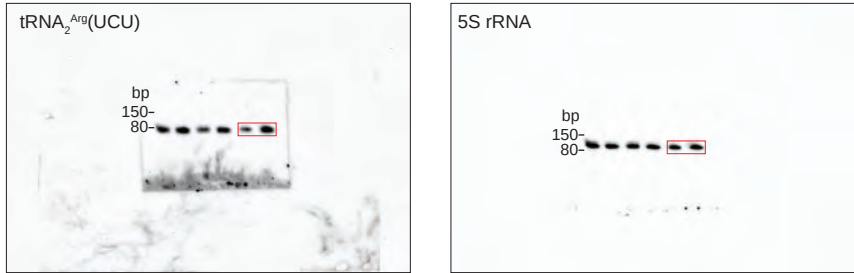
Extended Data Figure 5b (red box)
LNCaP AD, LP, UCU northern blot



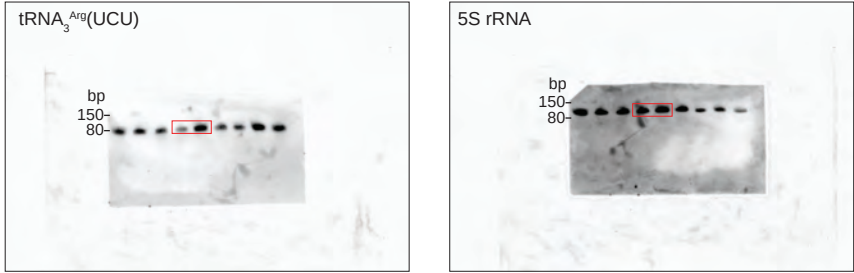
Extended Data Figure 5b (red box)
C4-2B AD, LP, UCU northern blot



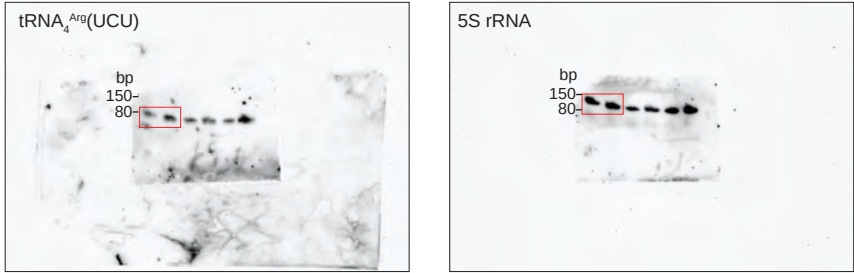
Extended Data Figure 5e (red box)
LNCaP LP, tRNA₂^{Arg}(UCU) northern blot



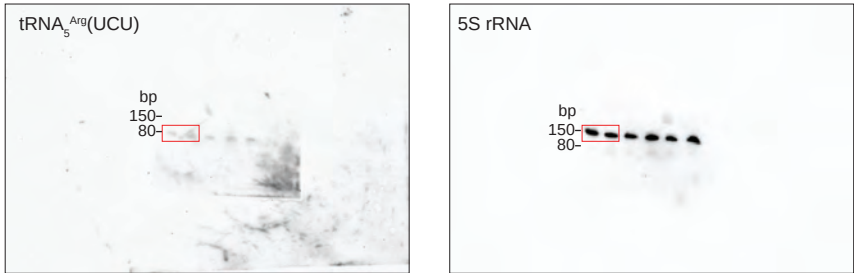
LNCaP LP, tRNA₃^{Arg}(UCU) northern blot



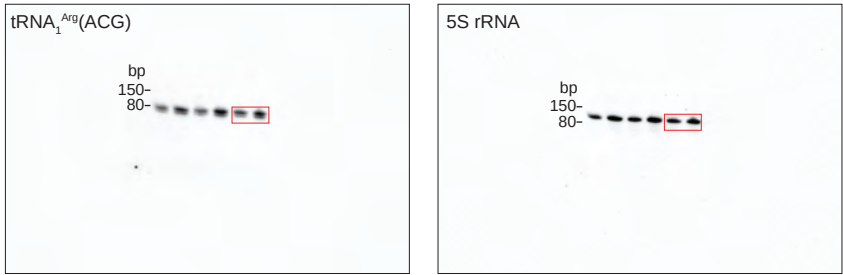
LNCaP LP, tRNA₄^{Arg}(UCU) northern blot



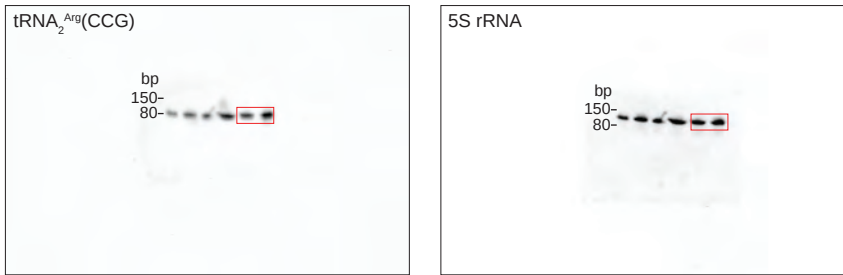
LNCaP LP, tRNA₅^{Arg}(UCU) northern blot



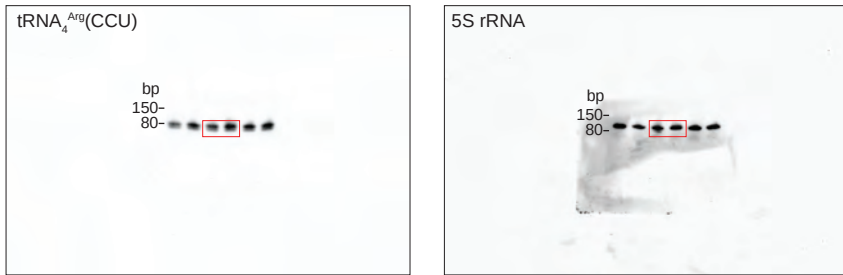
Extended Data Figure 5g (red box)
LNCaP LP, tRNA^{Arg}₁(ACG) OE northern blot



LNCaP LP, tRNA^{Arg}₂(CCG) OE northern blot



LNCaP LP, tRNA^{Arg}₄(CCU) OE northern blot



Extended Data Figure 6d (red box)
LNCaP-abl WT, UCU northern blot

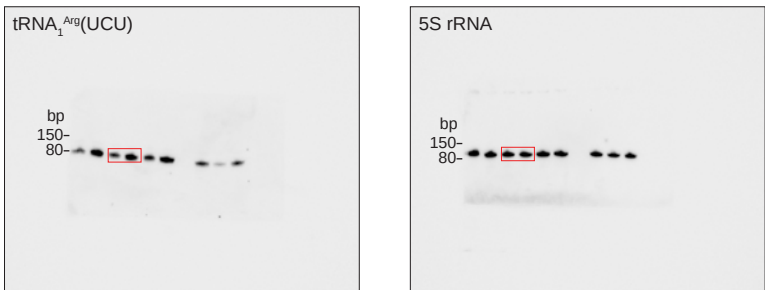


Figure 4d (red box) and Extended Data Figure 8f
LNCaP *TARDBP* and *ZSCAN29* KD northern blot

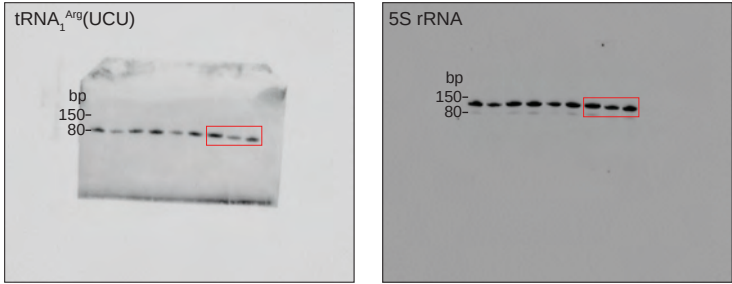
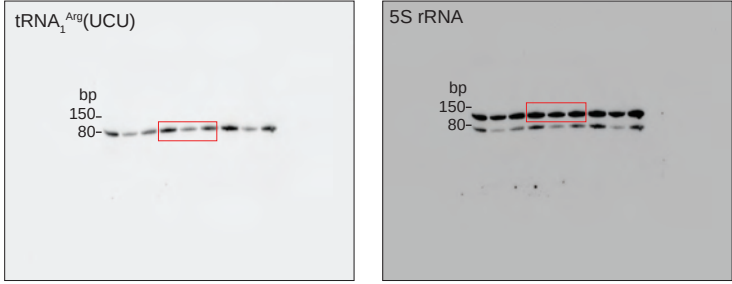
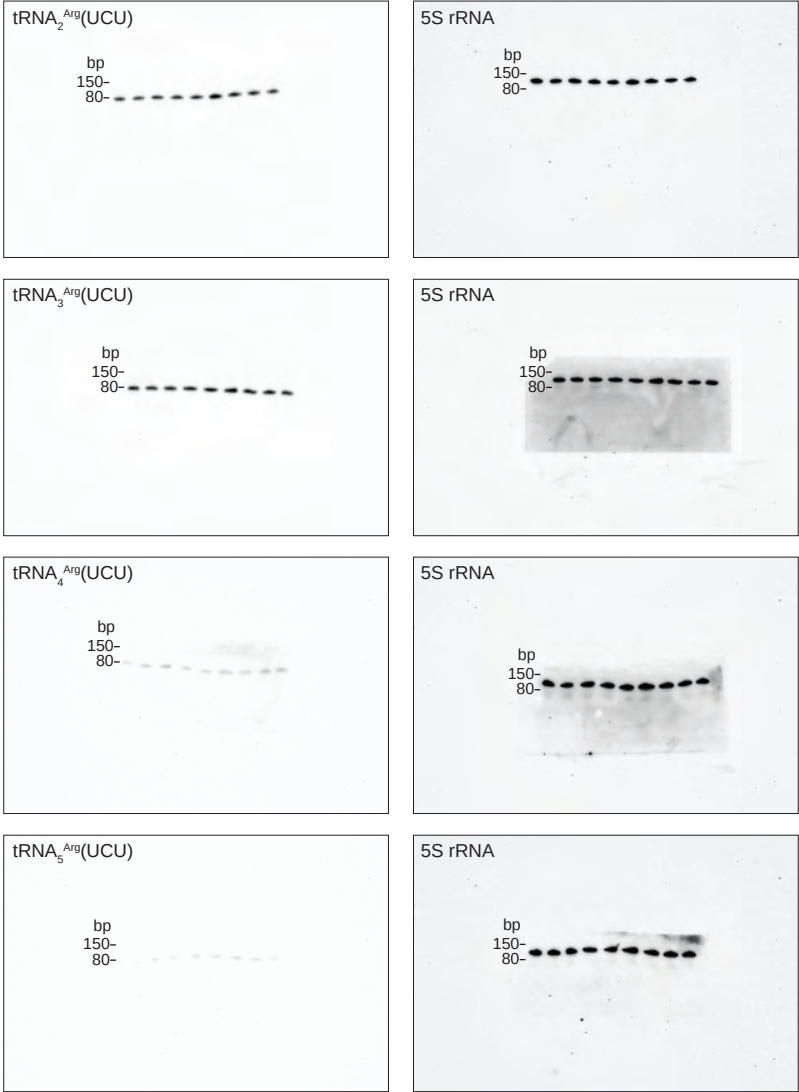


Figure 4d (red box) and Extended Data Figure 8g
C4-2B *TARDBP* and *ZSCAN29* KD northern blot



Extended Data Figure 8f
LNCaP *TARDBP* and *ZSCAN29* KD northern blot



Extended Data Figure 8g
C4-2B *TARDBP* and *ZSCAN29* KD northern blot

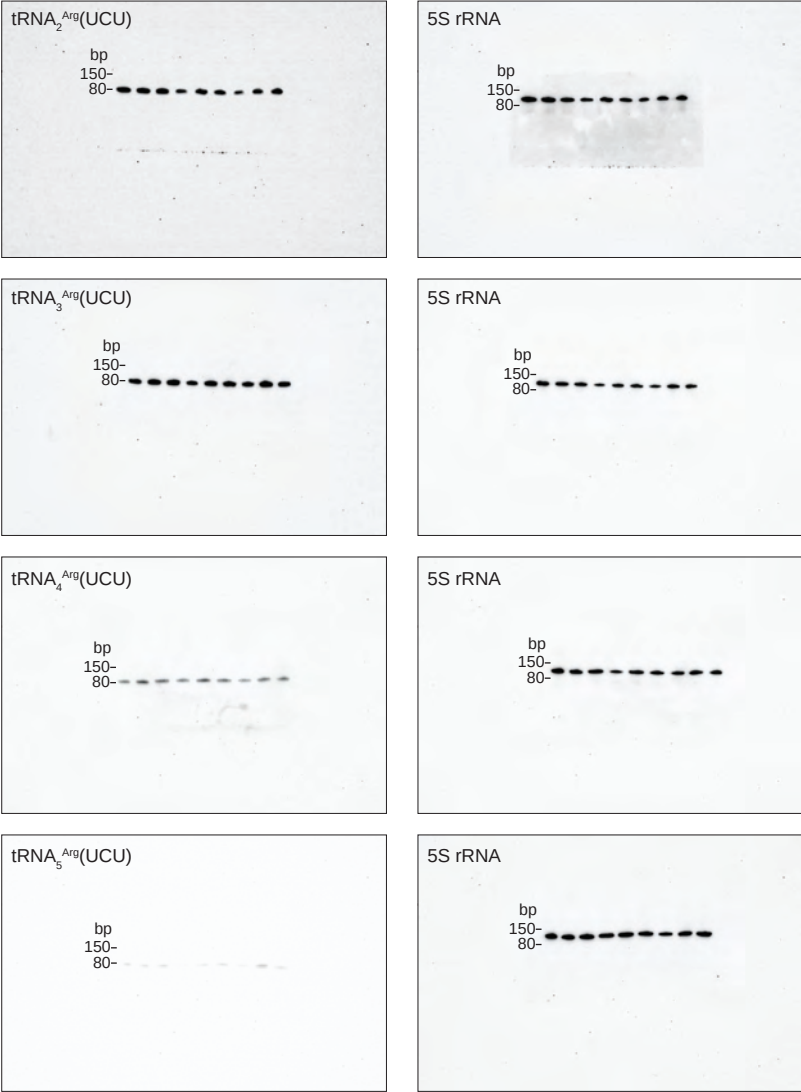
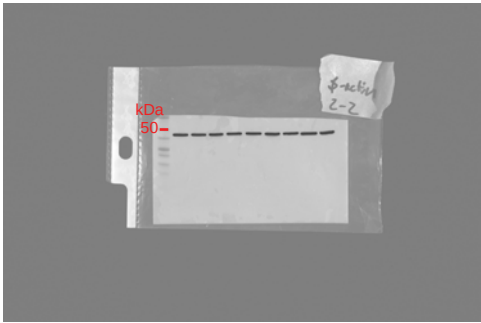
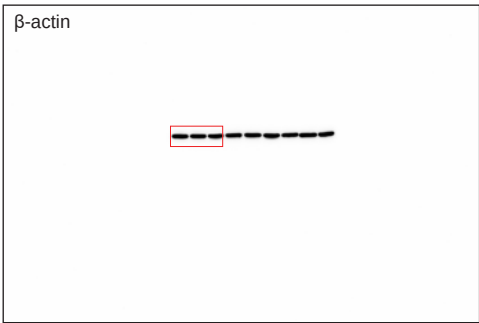
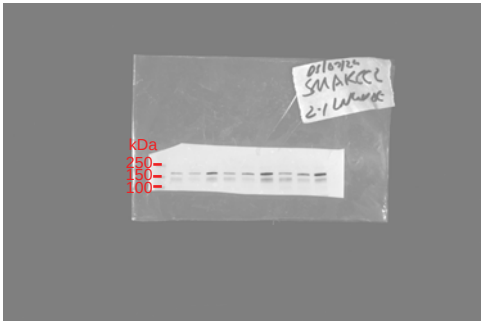


Figure 5f (red box)
LNCaP AD, LP, UCU western blot



Extended Data Figure 9d (red box)
LNCaP AD, LP, UCU western blot

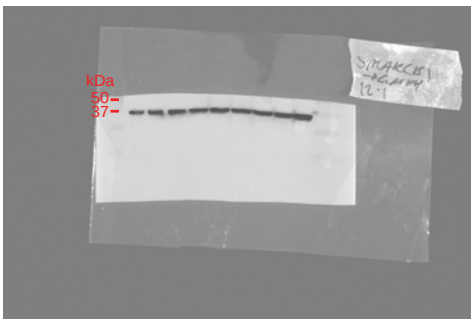
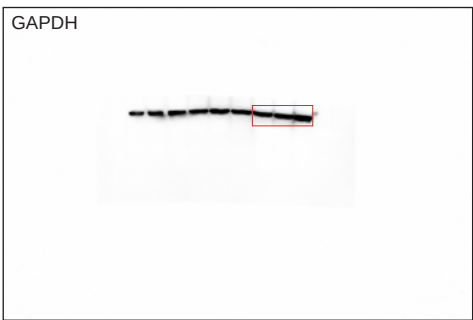
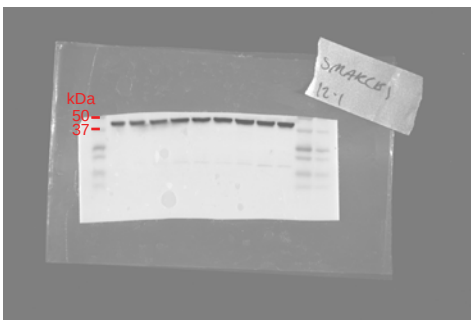
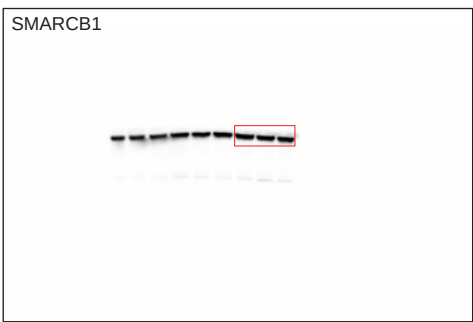
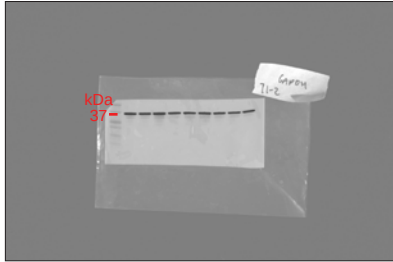
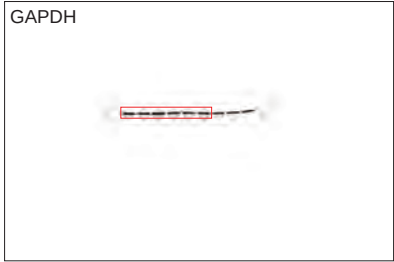
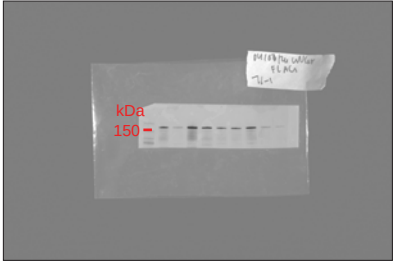
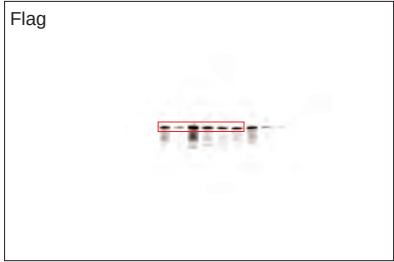


Figure 5h (red box)
LNCaP AD, LP, UCU western blot



Extended Data Figure 9f (red box)
LNCaP UCU shScramble and shSMARCC2 western blot

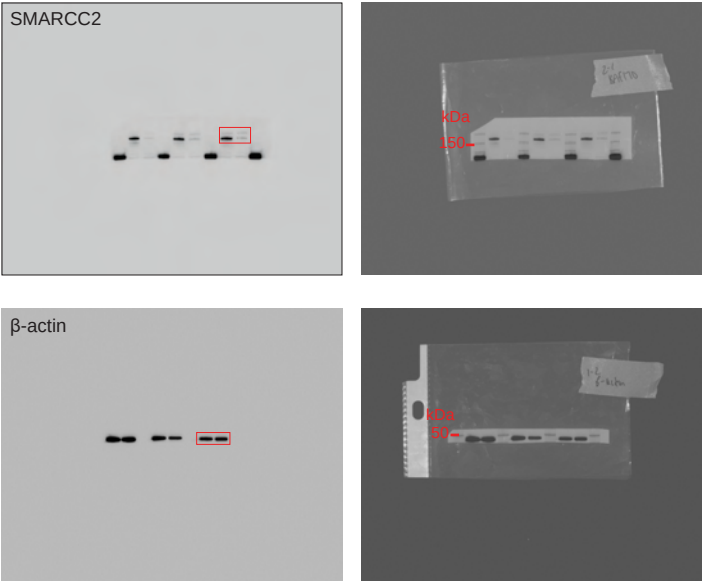


Figure 5j (red box)
LNCaP UCU shScramble and shSMARCC2 western blot

