Supplementary Materials for

The castration-resistant prostate cancer-associated SNP rs11067228 facilitates neuroendocrine differentiation through an enhancer-mediated chromatin interaction with SRRM4

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This file includes:

Supplementary Figure 1

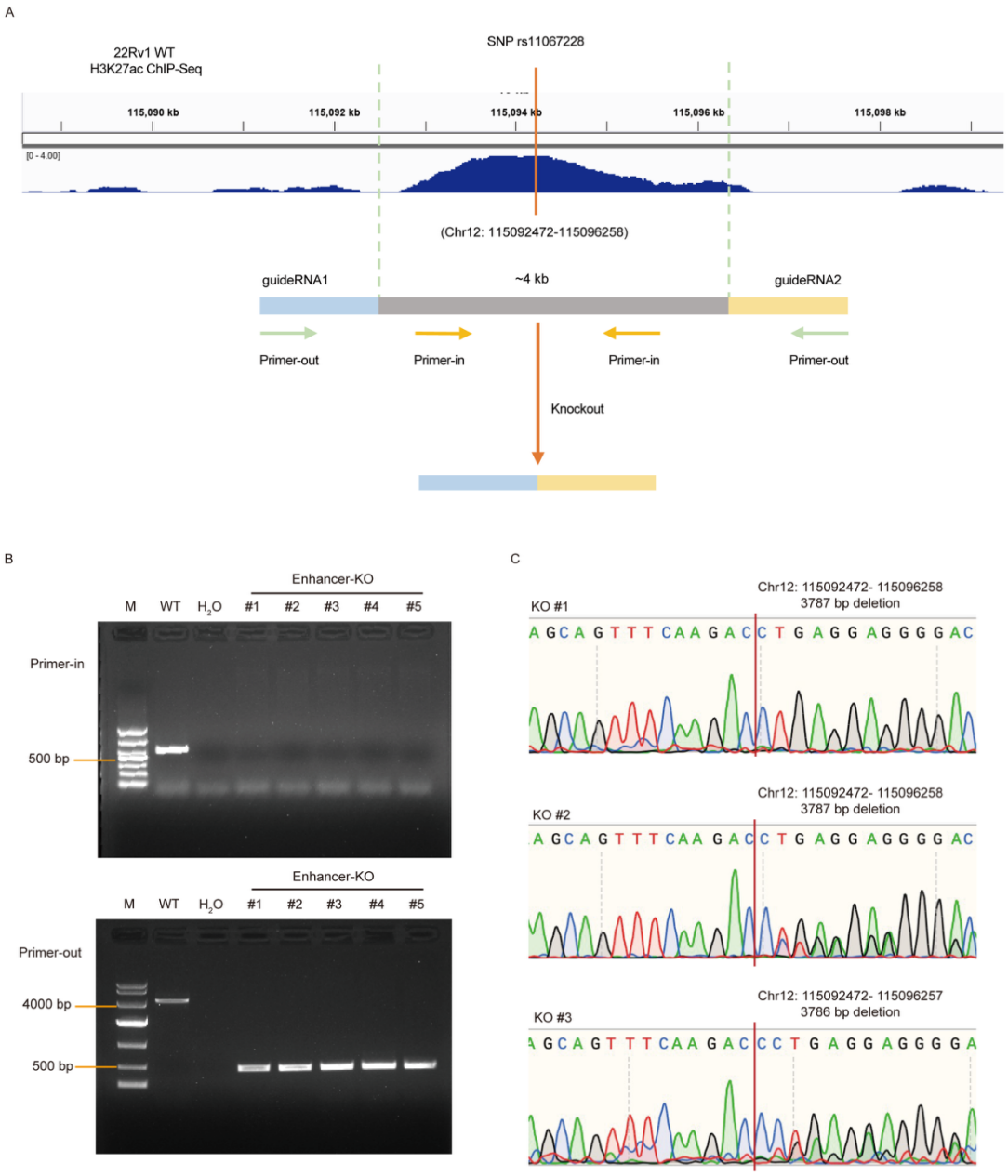

Figure S1. CRISPR/Cas9-mediated deletion of the rs11067228-associated enhancer. (A) Schematic showing the deleted 4 kb region and positions of knockout screening primers. (B) PCR analysis used to identify knockout cell

clones. Note the absence of a PCR product with “primer-in” (upper) and the appearance of 500bp PCR products with “primer-out” (lower), confirming deletion of the enhancer region. M: marker; H_2O: Negative control. (C) Sequencing of the deletion breakpoint described in (A) in relevant knockout clones generated by CRISPR/Cas9 editing. KO-#1: 3787 bp deletion (hg19, Chr12:115092472-115096258); KO-#2: 3787 bp deletion (hg19, Chr12:115092472-115096258); KO-#3: 3786 bp deletion (hg19, Chr12:115092472-115096257).

Supplementary Figure 2

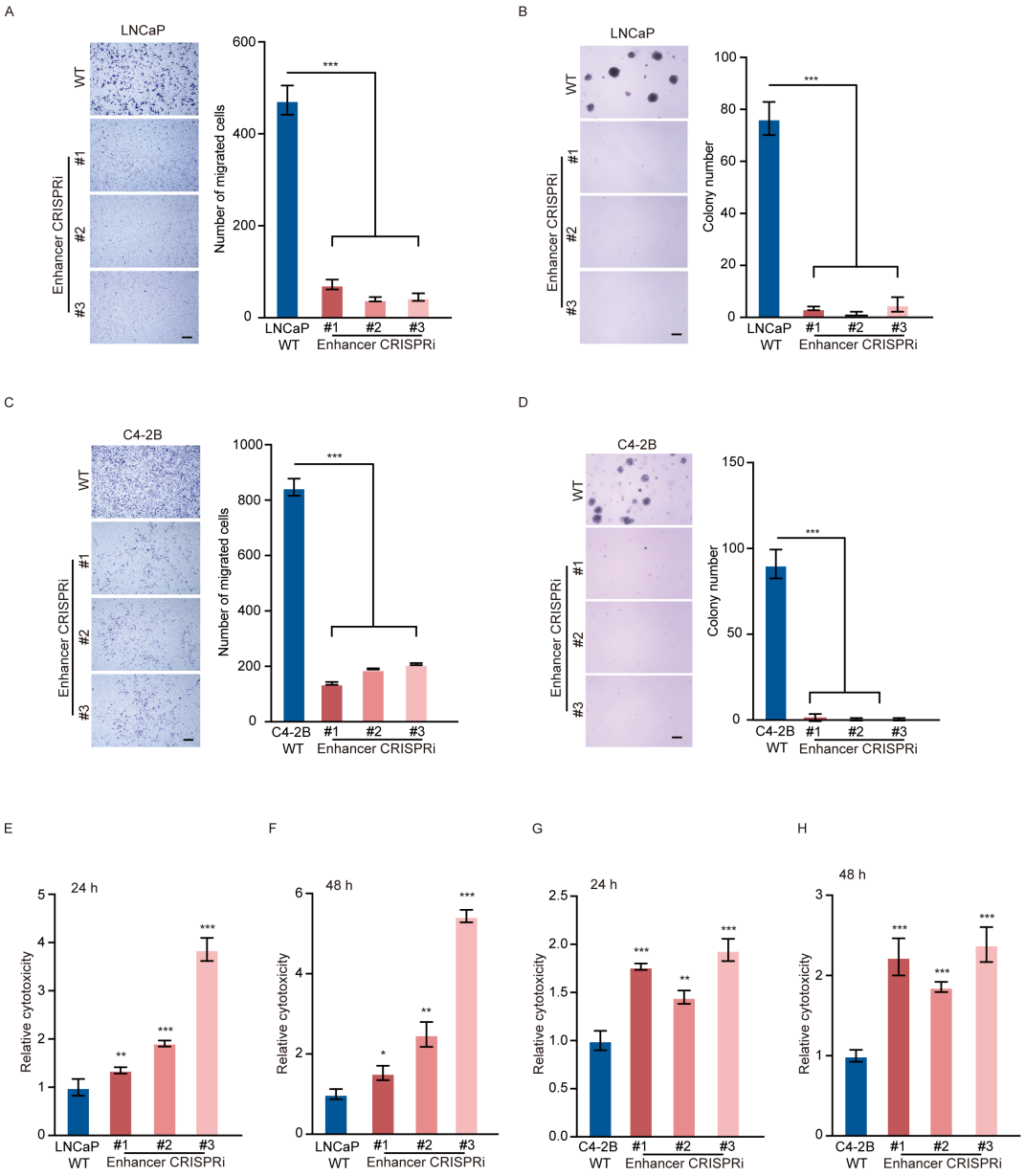

Figure S2. Deletion of the rs11067228-related enhancer decreases malignant phenotypes in LNCaP and C4-2B cells. (A) Transwell assays of LNCaP WT and enhancer-deleted cells generated by CRISPRi. Cells migrating to lower

chambers were stained with 0.1% crystal violet (left). Scale bar =100 μm. Quantification of migrated cells in corresponding cells (right). Data represent means ± S.E.M. of two independent experiments. ***P < 0.001. (B) Analysis of colony formation in soft agar of cells corresponding to those described in A (left). Scale bar =100 μm. Quantification is at right. Data represent means ± S.E.M. of two independent experiments. ***P < 0.001. (C) Transwell assays of C4-2B WT and enhancer-deleted cells generated using CRISPRi. Cells migrating to lower chambers were stained with 0.1% crystal violet (left). Scale bar =100 μm. Quantification of migrated cells is shown at right. Data represent means ± S.E.M. of two independent experiments. ***P < 0.001. (D) Analysis of colony formation in soft agar of cells corresponding to those described in C (left). Scale bar =100 μm. Quantification is at right. Data represent means ± S.E.M. of two independent experiments. ***P < 0.001. (E, F) Results of LDH release assays performed to assess cytotoxicity in cells described in A. After enzalutamide (50 nM) treatment, LDH release was assayed at 24 (E) and 48 (F) h. Data represent means ± S.E.M. of three independent experiments. ***P < 0.001, **P < 0.01, *P < 0.05. (G, H) Results of an LDH release assay in cells described in C. After enzalutamide (80 nM) treatment, LDH release was assayed at 24 (G) and 48 (H) h. Data represent means ± S.E.M. of three independent experiments. ***P < 0.001, **P < 0.01.

Supplementary Figure 3

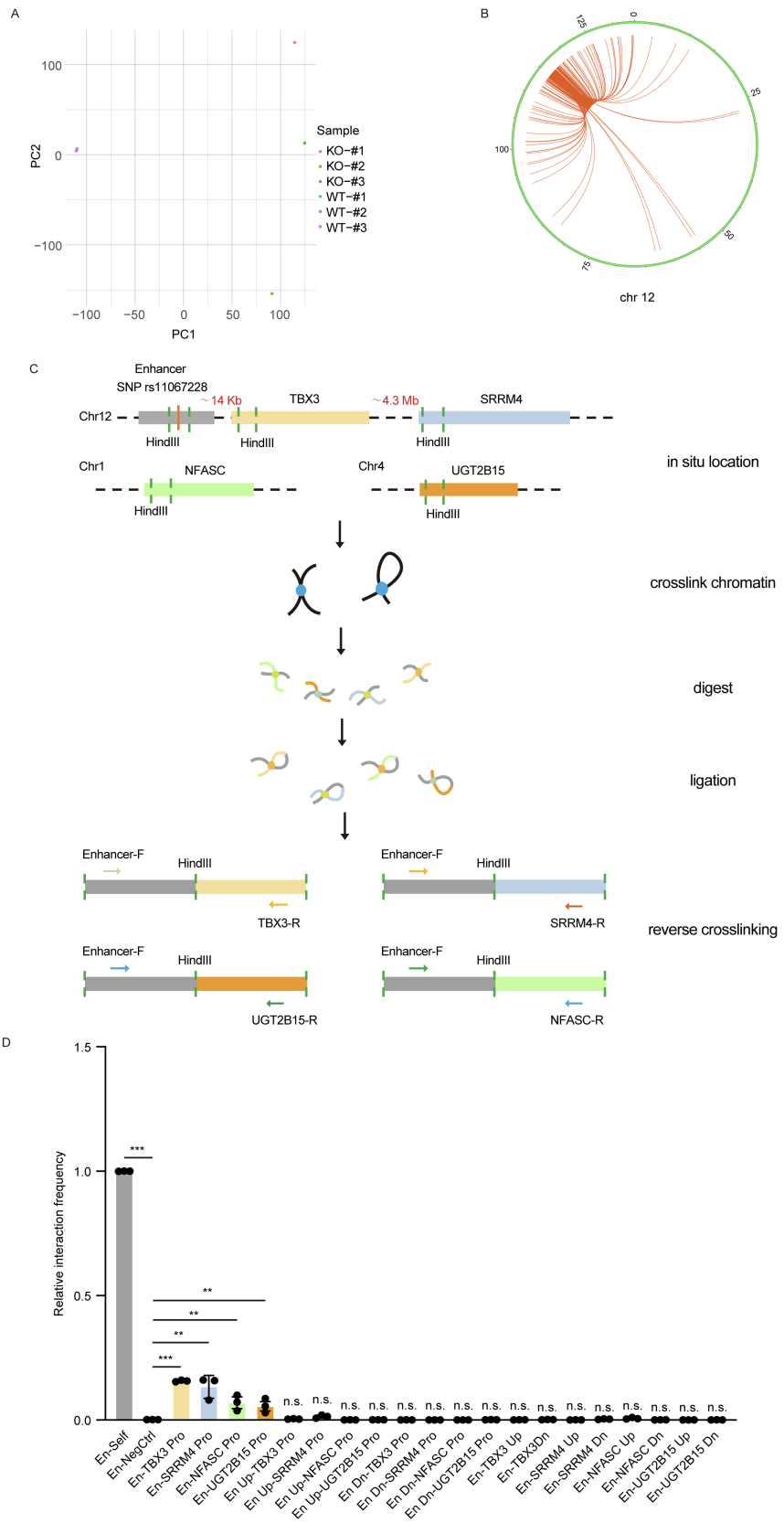

Figure S3. Integrated analysis of gene expression and chromatin architecture. (A) Principal component analysis of RNA-seq results. Triplicates of KO samples and triplicates of WT samples were analyzed. (B) The circus plot visualizing cis-interactions indicated by curves extending from the enhancer bait locus. (C) Schematic representation of the 3C experimental strategy for detecting chromatin interactions between the rs11067228-containing enhancer and its target genes. (D) Quantitative 3C-qPCR analysis of enhancer-promoter interactions. Data represent efficiency-corrected interaction frequencies normalized to anchor self-ligation. Data represent means ± S.E.M. of three independent experiments. ***P < 0.001, **P < 0.01, n.s., not significant.

Supplementary Figure 4

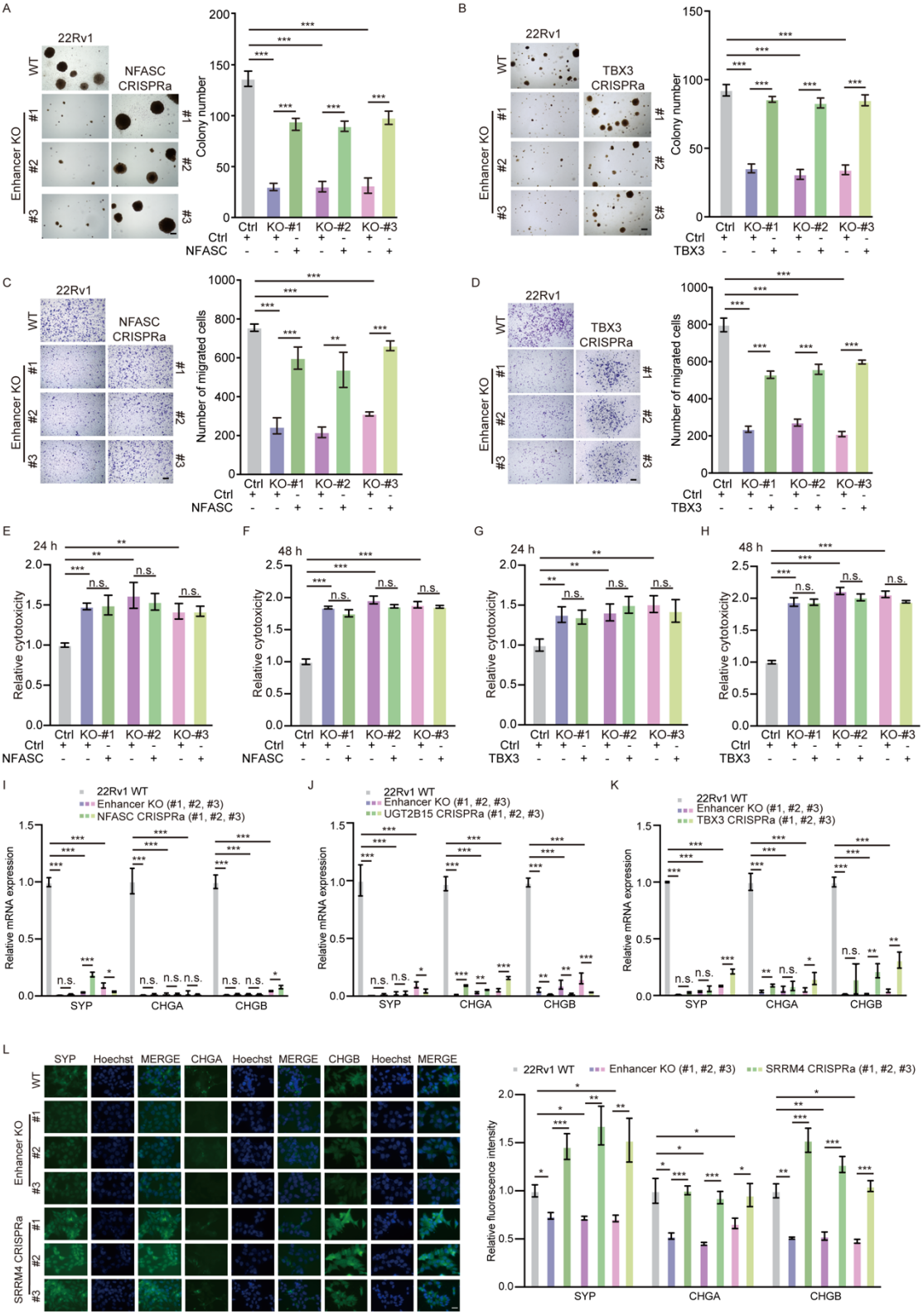

Figure S4. Rescue by candidate target genes of malignant phenotypes seen in

22Rv1 cells after deletion of the rs11067228 risk enhancer. Target genes were overexpressed using CRISPRa. (A, B) Soft agar colony formation assays in WT 22Rv1 cells and in three lines each of enhancer-deleted lines plus 3 enhancer-deleted cells overexpressing the indicated (*NFASC* and *TBX3*) genes (left). Scale bar =100 μm. Quantification is at right. Data represents means ± S.E.M. of three independent experiments. ***P < 0.001. (C, D) Transwell assays of enhancer-deleted and corresponding rescued cells described above (left). Scale bar =100 μm. Cells migrated to lower chambers were stained with 0.1% crystal violet. Quantification is at right. Data represents means ± S.E.M. of three independent experiments. ***P < 0.001, **P < 0.01. (E-H) LDH assays in cells corresponding to those described above and treated with enzalutamide (100 μM) at 24 (E, G) and 48 (F, H) h. Data represent means ± S.E.M. of three independent experiments. ***P < 0.001, **P < 0.01, n.s., not significant. (I-K) Real-time qPCR validation of transcript levels of NE-related genes (*SYP*, *CHGA* and *CHGB*) in WT 22Rv1 cells plus 3 enhancer-deleted cells overexpressing the indicated (*NFASC*, *UGT2B15* and *TBX3*) genes. Data represent means ± S.E.M. of three independent experiments. ***P < 0.001, **P < 0.01, *P < 0.05, n.s., not significant. (L) Representative immunofluorescence staining of NE-related genes (*SYP*, *CHGA* and *CHGB*) in wild-type 22Rv1 cells, as well as three lines each of enhancer-deleted lines plus enhancer-deleted cells re-expressing (by CRISPRa) *SRRM4* (left). Scale bar =10 μm. Relative fluorescence intensities were quantified in ~100 nuclei for each condition and

plotted (right). ***P<0.001, **P<0.01, *P<0.05.

Supplementary Figure 5

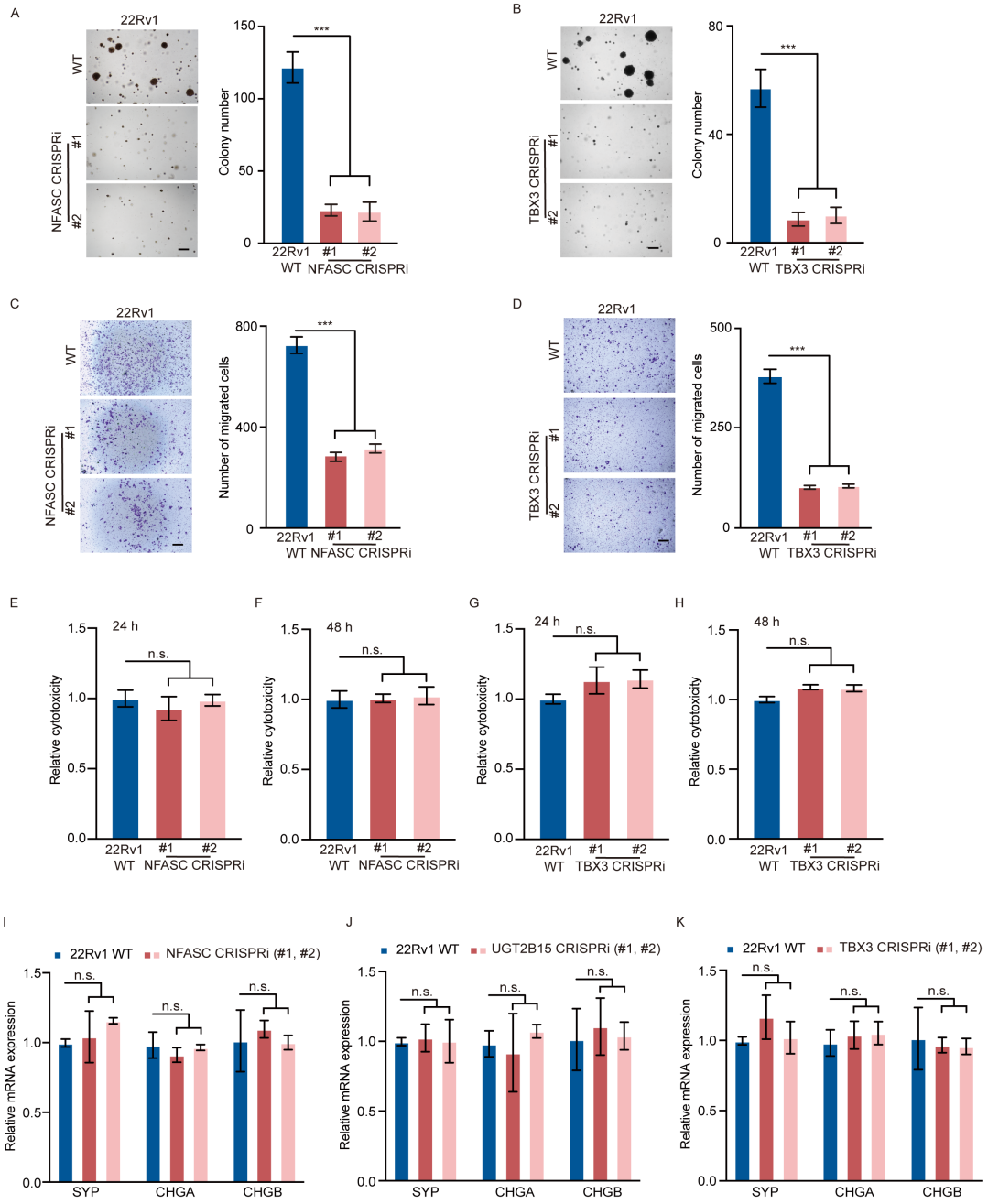

Figure S5. Knockdown of target genes partially suppresses malignant phenotypes of 22Rv1 PCa cells. (A, B) Soft agar colony formation assays in control WT cells and in cells made deficient in either *NFASC* or *TBX3* by

CRISPRi (left). Scale bar =100 μm. Quantification is at right. Data represents means ± S.E.M. of three independent experiments. ***P < 0.001. (C, D) Transwell assays in cells described above (left). Scale bar =100 μm. Cells that had migrated to lower chambers were stained with 0.1% crystal violet. Quantification is at right. Data represents means ± S.E.M. of three independent experiments. ***P < 0.001. (E-H) LDH assays in cells described above. After enzalutamide (100 μM) treatment, LDH release, was assayed as an indicator of cytotoxicity at 24 (E, G) and 48 (F, H) h. Data represent means ± S.E.M. of three independent experiments. n.s., not significant. (I-K) Real-time qPCR validation of transcript levels of NE-related genes (*SYP*, *CHGA* and *CHGB*) in WT control 22Rv1 cells and in cells made deficient in *NFASC*, *UGT2B15* and *TBX3* using CRISPRi. Data represent means ± SEM of three independent experiments. n.s., not significant.

Supplementary Figure 6

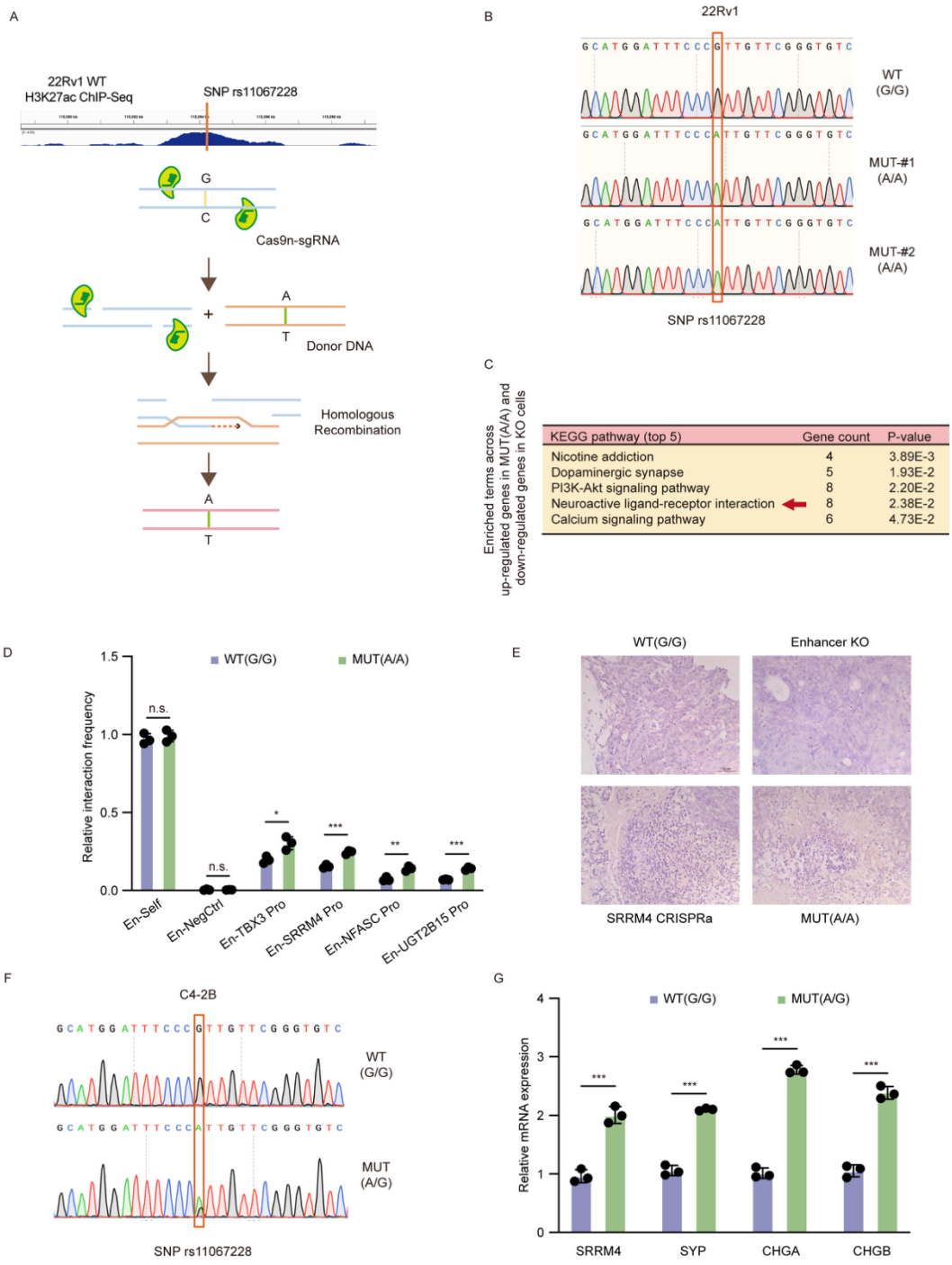

Figure S6. Functional characterization of the rs11067228 A allele across prostate cancer models. (A) Schematic showing CRISPR-Cas9-mediated mutation of SNP rs11067228 in 22Rv1 cells. (B) Sequencing analysis of

resultant SNP rs11067228 non-risk (G) and risk (A) alleles in 22Rv1 cells. (C) KEGG pathway analysis showing biological processes associated with genes overlapped between “KO vs WT” and “A vs G” in Figure 6I. (D) Quantitative 3C-qPCR analysis of enhancer-promoter interactions in WT (G/G) and MUT (A/A) cells. Data represent efficiency-corrected interaction frequencies normalized to anchor self-ligation. Data represent means ± S.E.M. of three independent experiments. ***P < 0.001, **P < 0.01, *P<0.05, n.s., not significant. (E) Representative H&E staining of indicated 22Rv1 xenografts in Figure 6M. Scale bar =50 μm. (F) Sequencing analysis of resultant SNP rs11067228 non-risk (G) and risk (A) alleles in C4-2B cells. (G) Real-time qPCR validation of transcript levels of *SRRM4* and NE-related genes (*SYP*, *CHGA* and *CHGB*) in WT (G/G) and MUT (A/G) C4-2B cells. Data represent means ± S.E.M. of three independent experiments. ***P < 0.001.

Supplementary Figure 7

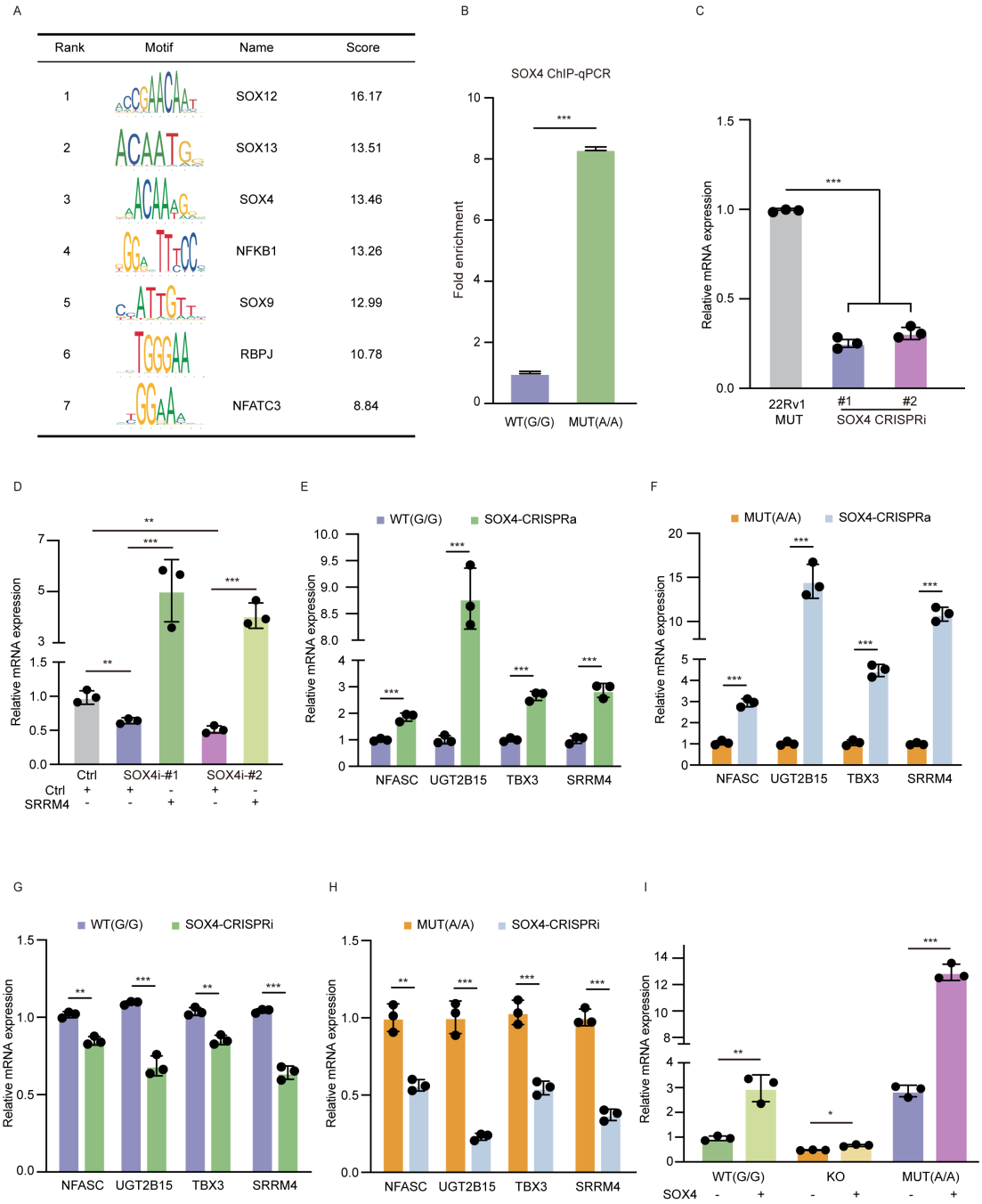

Figure S7. Identification and functional validation of SOX4 as the key transcription factor mediating rs11067228 enhancer activity. (A) Proteins binding to rs11067228 in 22Rv1 cells, based on JASPAR (an open-access

database of TF binding profiles (9th release)). (B) Results of SOX4 ChIP-qPCR performed in 22Rv1 cells homozygous for either SNP rs11067228 non-risk (G/G) or risk (A/A) alleles. Data represents means ± S.E.M. of three independent experiments. ***P < 0.001. (C) Validation of *SOX4* knockdown in 22Rv1 cells, as measured by real-time qPCR. sgRNAs targeting gene promoter regions were used in CRISPR-interference (CRISPRi) assays. Data represents means ± S.E.M. of three independent experiments. ****P* < 0.001. (D) RT-qPCR validation of mRNA level of *SRRM4* in MUT 22Rv1 cells, 2 *SOX4* knockdown lines and *SOX4*-KD cells subjected to CRISPRa to overexpress *SRRM4*. Data represents means ± S.E.M. of three independent experiments. ****P* < 0.001, **P < 0.01. (E) Transcript levels of indicated target genes before and after application of CRISPRa to overexpress SOX4 in WT(G/G) cells. Data represents means ± S.E.M. of three independent experiments. ***P < 0.001. (F) Transcript levels of indicated target genes before and after application of CRISPRa to overexpress SOX4 in MUT(A/A) cells. Data represents means ± S.E.M. of three independent experiments. ***P < 0.001. (G) Transcript levels of indicated target genes before and after application of CRISPRi to knockdown SOX4 in WT(G/G) cells. Data represents means ± S.E.M. of three independent experiments. ***P < 0.001, **P < 0.01. (H) Transcript levels of indicated target genes before and after application of CRISPRi to knockdown SOX4 in MUT(A/A) cells. Data represents means ± S.E.M. of three independent experiments. ***P < 0.001,

193 **P < 0.01. (I) Transcript levels of SRRM4 following CRISPRa-mediated

194 SOX4 overexpression in 22Rv1 WT (G/G), enhancer KO, or MUT (A/A) cells.

195 Data represents means ± S.E.M. of three independent experiments. ***P <

196 0.001, **P < 0.01, *P<0.05.

Supplementary Figure 8

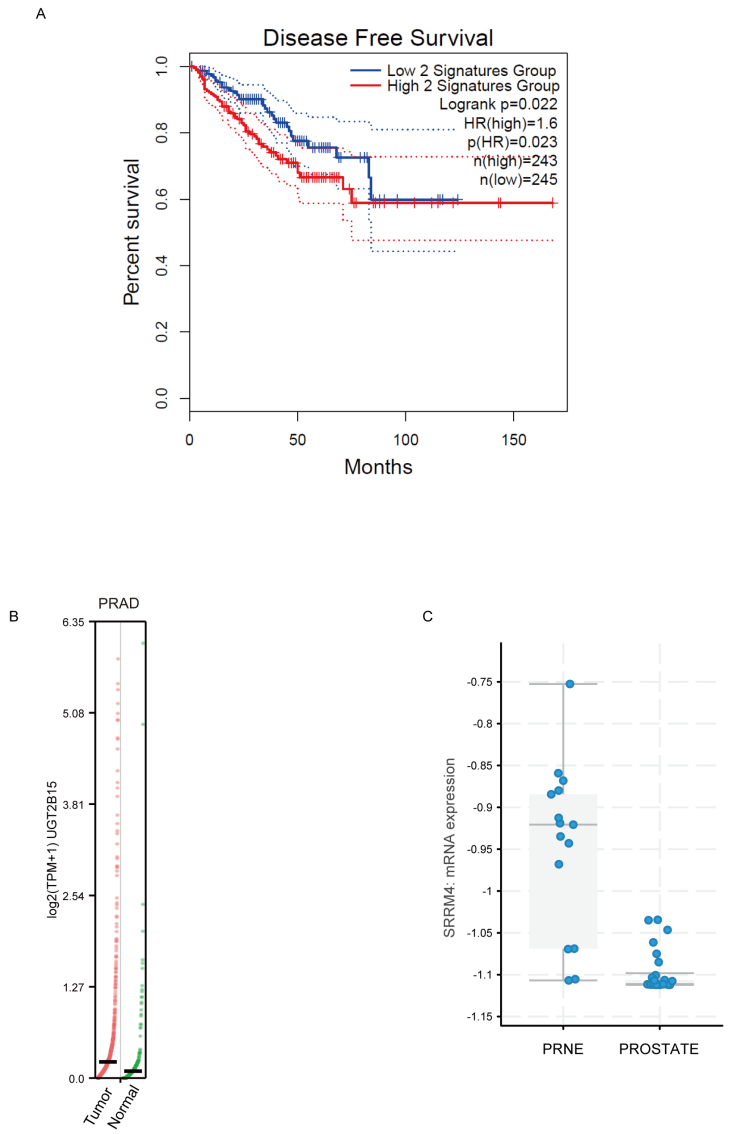

Figure S8. Combined prognostic value of *SRRM4*/*UGT2B15* and their subtype-specific expression patterns in prostate cancer. (A) Kaplan-Meier analysis of disease-free survival (DFS) in the TCGA-PRAD cohort, stratified by a combined expression score of *SRRM4* and *UGT2B15*. Patients with a high combined score exhibited significantly shorter DFS (Logrank $p < 0.05$). (B) Expression of

UGT2B15 is significantly elevated in primary prostate adenocarcinoma compared to matched normal prostate tissue in the TCGA-PRAD dataset. (C) Expression of *SRRM4* is significantly higher in neuroendocrine prostate cancer compared to conventional prostate adenocarcinoma in a combined cohort.

Table S1. Guide RNAs and primers used to assess CRISPR/Cas9-mediated deletions.

gRNA	Sequence
gRNA-1	GAAAGCAGTTTCAAGACACA
gRNA-2	CTCTCTAAGACCCTGCCCTG
Test primer-in forward	TTGGAGCTCCTGGTTCTATTTGA
Test primer-in reverse	GAGTCAGATATGTCTGTCCCCCT
Test primer-out forward	TACCTGTGCATTGGGTAGGAAG
Test primer-out reverse	GAAAAATCTTGCCGACTCCTGG

Table S2. Primers used in the qRT-PCR experiment.

Gene name	Primer	Sequence
TBX3	Forward	GGACACTGGAAATGGCCGAA
	Reverse	TCACCCTCGCTGGGACATAA
NFASC	Forward	GCACACGATCTCGGTGAGAG
	Reverse	TCACGGTTTGGGTTAGGTGG
HSPA1B	Forward	CTTCAACATGAAGAGCGCCG
	Reverse	GAGTCCCAACAGTCCACCTC
HSPA1A	Forward	AGCTGGAGCAGGTGTGTAAC
	Reverse	CAGCAATCTTGGAAAGGCCC

SRRM4	Forward	GTTCTGGCGAGGAACCTTCA
	Reverse	TGCGTCGCTTGTGTTTCTTG
UGT2B15	Forward	TGAGCTACTGGCTGAACTATTTAAC
	Reverse	TGTAGTGGGTCTTCCTAGAACT
MPHV2	Forward	GTGACAGTGGCTCCTTCTAAT
	Reverse	GGATAGTGAGCTCCATGTTCAG
HPRT1	Forward	CCTGGCGTCGTGATTAGTGAT
	Reverse	AGACGTTCAGTCCTGTCCATAA
SOX4	Forward	CAGCAAACCAACAATGCCGA
	Reverse	GATCTGCGACCACACCATGA
SYP	Forward	ACTATGGGCAGCAAGGCTAC
	Reverse	AGACTGGGCACCTAGTGGAT
CHGB	Forward	CACGCCATTCTGAGAAGAGC
	Reverse	TCTCCTGGCTCTTCAAGGTG
CHGA	Forward	TCCAAGGCGCCAAGGA
	Reverse	CATCTTCAAAACCGCTGTGTTTC

Table S3. Primers used for the 3C-PCR assay.

Primer	Sequence
Enhancer-3C-Forward	ATGTACTTTGGAGCTCCTG
UGT2B15-3C-Reverse	AGGAAAAATTTCAAGTGGCAGTGT
NFASC-3C-Reverse	TCTGTCTGCGCAGCATTAGT

TBX3-3C-Reverse	CACAGAGGCCTTTCTCCCAG
SRRM4-3C-Reverse	ACATCCGTATAGCTGAGCCC

Table S4. Guide RNAs and primers used to assess CRISPR/Cas9-mediated SNP editing.

gRNA	Sequence
gRNA-1 Forward	CACCGTGGACACGCAAGGATTGGTG
gRNA-1 Reverse	AAACCACCAATCCTTGCGTGTCCAC
gRNA-2 Forward	CACCGAAACTTACTGTCGGAGTCCC
gRNA-2 Reverse	AAACGGGACTCCGACAGTAAGTTTC
SNP-Forward	AGGAGAGGGGCATAGTTATCCA
SNP-Reverse	ACACTCGACGCCCTAGCAAT

Table S5. Primers used to construct the luciferase reporter plasmid.

Primer	Sequence
Luc-enhancer-F	ATGGAGTGCAGCGAGGTTTAT
Luc-enhancer-R	GGTTGCAGGGTGTCTCATCTT

Table S6. Primers used for 4C analysis.

Primer	Sequence
4C-Forward	GGGTAGTTACCTTGTTTCTGGATA
4C-Reverse	CTGGTGTCCAATGACATCAAATAG

Table S7. sgRNA sequences used in CRISPRa and CRISPRi assays.

Primer	Sequence
sgLacZ Forward	CACCGCCCGAATCTCTATCGTGCGG
sgLacZ Reverse	AAACCCGCACGATAGAGATTCGGGC
sgUGT2B15-1a Forward	CACCGTGGCATGCACCTATTCAGAC
sgUGT2B15-1a Reverse	AAACGTCTGAATAGGTGCATGCCAC
sgUGT2B15-2a Forward	CACCGATTCAGACTGTTAGTATTAT
sgUGT2B15-2a Reverse	AAACATAATACTAACAGTCTGAATC
sgUGT2B15-3a Forward	CACCGCAGATATAAGTATGAGAAAT
sgUGT2B15-3a Reverse	AAACATTTCTCATACTTATATCTGC
sgNFASC-1a Forward	CACCGCTGTCCTGGTCCCCGCTCAG
sgNFASC-1a Reverse	AAACCTGAGCGGGGACCAGGACAGC
sgNFASC-2a Forward	CACCGTCCGCCTCTGTCCTGGTCCC
sgNFASC-2a Reverse	AAACGGGACCAGGACAGAGGCGGAC
sgNFASC-3a Forward	CACCGGAGCATCCTTCCCTCCGCCT
sgNFASC-3a Reverse	AAACAGGCGGAGGGAAGGATGCTCC
sgTBX3-1a Forward	CACCGCGCCTATGCAGCAACACAAT
sgTBX3-1a Reverse	AAACATTGTGTTGCTGCATAGGCGC
sgTBX3-2a Forward	CACCGCCAGCACTCGACCTGTGAAA
sgTBX3-2a Reverse	AAACTTTCACAGGTCGAGTGCTGGC
sgTBX3-3a Forward	CACCGATGCAGCAACACAATTGGTC

sgTBX3-3a Reverse	AAACGACCAATTGTGTTGCTGCATC
sgSRRM4-1a Forward	CACCGATTGTGCGAGAGACAAAACC
sgSRRM4-1a Reverse	AAACGGTTTTGTCTCTCGCACAATC
sgSRRM4-2a Forward	CACCGGCGGGCTTTTGTTATGCAGA
sgSRRM4-2a Reverse	AAACTCTGCATAACAAAAGCCCGCC
sgSRRM4-3a Forward	CACCGCCCAAGACCTGCGGGCTTTT
sgSRRM4-3a Reverse	AAACAAAAGCCCGCAGGTCTTGGGC
sgHSPA1A-1a Forward	CACCGAGGACGGGAGGCGAAAACCC
sgHSPA1A-1a Reverse	AAACGGGTTTTCGCCTCCCGTCCTC
sgHSPA1A-2a Forward	CACCGTCTGGCCTCTGATTGGTCCA
sgHSPA1A-2a Reverse	AAACTGGACCAATCAGAGGCCAGAC
sgHSPA1A-3a Forward	CACCGCATCGAGCTCGGTGATTGGC
sgHSPA1A-3a Reverse	AAACGCCAATCACCGAGCTCGATGC
sgUGT2B15-1i Forward	CACCGAAGAAGCATTGCATAAGACC
sgUGT2B15-1i Reverse	AAACGGTCTTATGCAATGCTTCTTC
sgUGT2B15-2i Forward	CACCGGGTGACTGTGTTGACATCTT
sgUGT2B15-2i Reverse	AAAC AAGATGTCAACACAGTCACCC
sgUGT2B15-3i Forward	CACCGCTGGAAGAGCTTGTTCAGAG
sgUGT2B15-3i Reverse	AAACCTCTGAACAAGCTCTTCCAGC
sgNFASC-1i Forward	CACCGTGGTCTCTGCCCTAATGCGG
sgNFASC-1i Reverse	AAACCCGCATTAGGGCAGAGACCAC

sgNFASC-2i Forward	CACCGGCAGCGGACAGCTCGGACAG
sgNFASC-2i Reverse	AAACCTGTCCGAGCTGTCCGCTGCC
sgNFASC-3i Forward	CACCGTAATGCGGCGGCTGGCGGCG
sgNFASC-3i Reverse	AAACCGCCGCCAGCCGCCGCATTAC
sgTBX3-1i Forward	CACCGACCTTCTAGAGCCGCCGAGC
sgTBX3-1i Reverse	AAACGCTCGGCGGCTCTAGAAGGTC
sgTBX3-2i Forward	CACCGGAGAAGAGCCCAGCAAGATT
sgTBX3-2i Reverse	AAACAATCTTGCTGGGCTCTTCTCC
sgTBX3-3i Forward	CACCGGAAACCGAGACACCCTCCGG
sgTBX3-3i Reverse	AAACCCGGAGGGTGTCTCGGTTTCC
sgSRRM4-1i Forward	CACCGCCGCCCTGAACTCCGATCTC
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sgSRRM4-2i Forward	CACCGTCTCTGGGTTTCACCCGGAC
sgSRRM4-2i Reverse	AAACGTCCGGGTGAAACCCAGAGAC
sgSRRM4-3i Forward	CACCGTGAACTCCGATCTCTCCCAC
sgSRRM4-3i Reverse	AAACGTGGGAGAGATCGGAGTTCAC
sgSOX4-1a Forward	CACCGTGAAAGGATAAAGAGGCGCG
sgSOX4-1a Reverse	AAACCGCGCCTCTTTATCCTTTCAC
sgSOX4-2a Forward	CACCGGGTTTGGCATGAGGAAGCGT
sgSOX4-2a Reverse	AAACACGCTTCCTCATGCCAAACCC
sgSOX4-3a Forward	CACCGGCATCGGGTTCCAAGCCAAT

sgSOX4-3a Reverse	AAACATTGGCTTGGAACCCGATGCC
sgSOX4-1i Forward	CACCGCGCTCTTTAAGAGTCTGCAC
sgSOX4-1i Reverse	AAACGTGCAGACTCTTAAAGAGCGC
sgSOX4-2i Forward	CACCGGCAAGAGAAACTGTGTGTGA
sgSOX4-2i Reverse	AAACTCACACACAGTTTCTCTTGCC

Table S8. Sequences of biotinylated double-strand oligonucleotides used in pull-down assays.

Primer	Sequence
Biotin SNP-A Forward	GGATTTCCCATTGTTCGGGTG
SNP-A Reverse	CACCCGAACAATGGGAAATCC
Biotin SNP-G Forward	GATTTCCCGTTGTTCGGGTGT
SNP-G Reverse	ACACCCGAACAACGGGAAATC

Table S9. 3C-qPCR primer sequences and amplification efficiencies

Primer Pair	Sequence (5’-3’)	Efficiency (%)	Slope
Enhancer-F/ TBX3-Pro-R	TCCCATTGTTCGGGTGTCTG/ GAGGCTCCAACTGCTGACTT	98.3	-3.36
Enhancer-F/ SRRM4-Pro-R	GGGAGGTCTGGGGGTAGTTA/ ACAACTCTGTTGGGGGTCTC	96	-3.42

Enhancer-F/ NFASC-Pro-R	TTGGAGCTCCTGGTTCTATTTGA/ GCAGGGGACTCACCAAATGTT	102.6	-3.26
Enhancer-F/ UGT2B15-Pro-R	TGGGCTGCCGCAGTCTATAA/ ACCAAGGATCAAGGGACTAGC	106.4	-3.18
Enhancer-F/ TBX3-Up-R	TGTGCGGGATCTGCCTTATC/ GTGATCCCACCACGGATTCA	108.7	-3.13
Enhancer-F/ TBX3-Dn-R	GCTTAAGCTTTGGGACCCTG/ AGTGCCCTGGTCCAAACAAA	95.5	-3.44
Enhancer-F/ SRRM4-Up-R	TGTGCGGGATCTGCCTTATC/ TACCAAGCTGGTCGTATGCC	106.7	-3.17
Enhancer-F/ SRRM4-Dn-R	GCGGGATCTGCCTTATCTCC/ AACATCTGGCTGCTCCTTCC	100.5	-3.31
Enhancer-F/ NFASC-Up-R	CATGGATTTCCCGTTGTTCG/ AGACTGGAGTGATTTGAGCCC	92	-3.53
Enhancer-F/ NFASC-Dn-R	CTGGGCTGCCGCAGTCTATAA/ AGGAGGGTGTCGAGTCTGG	109.3	-3.12
Enhancer-F/ UGT2B15-Up-R	CCGTTGTTCGGGTGTCTGTG/ AAGGAGGGTGTCGAGTCTGG	104.1	-3.23
Enhancer-F/ UGT2B15-Dn-R	ATTTCCCGTTGTTCGGGTGT/ GGAGATAAGGCAGATCCCGC	100.4	-3.31

Enhancer-Up-F/ TBX3-R	GGGGATAGGAAGAAAGCTTATGT A/ CAGACACCCGAACAATGGGA	98	-3.37
Enhancer-Dn-F/ TBX3-R	GGTGATTCTCAGGCCCCAAA/ TAGTAGGTGCCTCCCACCTC	105.5	-3.2
Enhancer-Up-F/ SRRM4-R	GGGGATAGGAAGAAAGCTTGGA TT/ TGCTGTAGCGTGGGCTTTAG	91.1	-3.56
Enhancer-Dn-F/ SRRM4-R	GGACTCCCTTGTAGCTCGTT/ AGGAATCCAAGCTTCCTACAAT	100.5	-3.31
Enhancer-Up-F/ NFASC-R	GAGCTACTGTGAGGCTGATTTC/ TGGTGGCCCAGTTACCCATT	110.6	-3.09
Enhancer-Dn-F/ NFASC-R	AAGGGGACTCCCTTGTAGCTC/ CAAGTCTGAGATGGCTGGATGC	102.8	-3.26
Enhancer-Up-F/ UGT2B15-R	GTTAGCCAACAGGTAGGAGCTA /GGCGGGATATGAAATTCTGG	98.2	-3.37
Enhancer-Dn-F/ UGT2B15-R	GGTGATTCTCAGGCCCCAAA/ TAGTAGGTGCCTCCCACCTC	92.7	-3.51
Enhancer-F/ Self-R	ATGGACACGCAAGGATTGGT/ CAGACACCCGAACAATGGGA	97.6	-3.38
Enhancer-F/ NegCtrl-R	TGTGCCGACCAGGAAAGAAG/ TTTGTGCGCCACAACCAAGTCC	100.8	-3.3s

Table S10. Key resources table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit anti-H3K27ac	Abcam	Cat# ab4729 RRID: AB_2118291
Rabbit anti-UGT2B15	Abcam	Cat# ab154864 RRID: AB_2722651
Rabbit anti-Neurofascin	Abcam	Cat# ab31457 RRID: AB_881185
Rabbit anti-TBX3	Abcam	Cat# ab99302 RRID: AB_10861059
Rabbit anti-SRRM4	ThermoFisher	Cat# PA5-112589 RRID: AB_2867324
Rabbit anti-HSPA1A	ProteinTech	Cat# 10995-1-AP RRID: AB_2264230
Rabbit anti-Synaptophysin	Abcam	Cat# ab32127 RRID: AB_2286949
Rabbit anti-Chromogranin A	Abcam	Cat# ab283265
Rabbit anti-Chromogranin B	Abcam	Cat# ab12242 RRID: AB_298965

Rabbit anti-beta Tubulin	Biyotime	Cat# AF1216 RRID: AB_2924787
Goat anti-rabbit IgG H&L	Abcam	Cat# ab6702 RRID: AB_956012
Rabbit anti-SOX4	Abcam	Cat# ab86809 RRID: AB_10714562
Chemicals, Peptides, and Recombinant Proteins		
Fetal Bovine Serum (FBS)	VivaCell	Cat# C04001-500
Penicillin and Streptomycin	Gibco	Cat# 15140-122
Triton X-100	Sigma-Aldrich	Cat# T9284
HEPES	Solarbio	Cat# H1095
EDTA, FREE ACID	Solarbio	Cat# E8040
EDTA Na_2	Solarbio	Cat# E8030
EGTA	Solarbio	Cat# E8050
Deoxycholic acidsodium salt	Solarbio	Cat# D8330
Nonidet P-40	Solarbio	Cat# N8030
Tween-20	Solarbio	Cat# T8220
SDS	Solarbio	Cat# S8010

Protein A Magnetic Beads	ThermoFisher	Cat# 10002D
Protein G Magnetic Beads	ThermoFisher	Cat# 10004D
Puromycin	MedChemExpress	Cat# HY-B1743A
Blasticidin	Solarbio	Cat# B9300
Hygromycin	Solarbio	Cat# H8080
DpnII	New England Biolabs	Cat# R0543L
CviqI	New England Biolabs	Cat# R0639L
HindIII-HF	New England Biolabs	Cat# R3014L
BsmbI-v2	New England Biolabs	Cat# R0739L
Ampicillin	Solarbio	Cat# A8180
Formaldehyde solution	Sigma-Aldrich	Cat# F8775
Glycine	Solarbio	Cat# G8200
Streptavidin magnetic C1 beads	ThermoFisher	Cat# 65002

Crystal violet	Solarbio	Cat# G1063
RNase A	Solarbio	Cat# R1030
Matrigel	Corning	Cat# 356234
SeaPlaque^{TM}Agarose	Lonza	Cat# 50101
TRIzol	ThermoFisher	Cat# 15596018
PMSF	Sigma-Aldrich	Cat# P7626-25G
Complete EDTA-free Protease Inhibitor Cocktail	Roche	Cat# 11836170001
T4 DNA Ligase	Takara	Cat# 2011A
Proteinase K	CMBio	Cat# CW2298M
Dual-Luciferase Reporter Assay System kit	Promega	Cat# E1910
CytoTox 96® Non-Radioactive Cytotoxicity Assay	Promega	Cat# G1780
PrimeScript RT reagent with a gDNA eraser Kit	Takara	Cat# RR047B
qPCR SYBR Green Master Mix	YEASEN	Cat# 11201ES03

Premix Ex-Taq HS	Takara	Cat# RR030
Lipofectamine 3000	ThermoFisher	Cat# L3000-015
Falcon Cell Culture Inserts	Corning	Cat# 353097
Deposited Data		
H3K27ac ChIP-seq	This paper	GSE274154
4C-seq	This paper	GSE274153
RNA-seq	This paper	GSE274155
Mass spectrometry proteomics	This paper	PXD055628
Experimental Models: Cell Lines		
22Rv1	ATCC	Cat# CRL-2505
LNCaP	ATCC	Cat# CRL-1740
C4-2B	ATCC	Cat# CRL-3315
HEK-293T	ATCC	Cat# CRL-3216
Recombinant DNA		
pGL3 Promoter vector	Promega	Cat# E1761
pGL3 Basic vector	Promega	Cat# E1751
pRL-TK Renilla luciferase vector	Promega	Cat# E2241

pSpcas9n(sgRNAs)	Addgene	Cat# 62987
pMD2.G	Addgene	Cat# 12259
psPAX2	Addgene	Cat# 12260
lenti-dCas9-KRAB-blast	Addgene	Cat# 89567
lentiGuide-Puro	Addgene	Cat# 52963
lentiMPHv2	Addgene	Cat# 89308
lentiSAMv2	Addgene	Cat# 75112
lentiCRISPR v2	Addgene	Cat# 52961
Software and Algorithms		
GraphPad Prism9	GraphPad software	https://www.graphpad.com
ImageJ	NIH	https://imagej.nih.gov/ij
BWA	Li and Durbin, 2009 (58)	https://github.com/lh3/bwa
HTseq (v0.11.3)	Anders et al., 2015 (61)	https://github.com/simon-anders/htseq
DEseq2 (v1.38.3)	Love et al., 2014 (62)	https://bioconductor.org/packages/release/bioc/html/DESeq2.html
DAVID	Huang da et al., 2009 (63)	https://david-d.ncifcrf.gov/

HISAT2	Kim et al., 2019 (55)	https://github.com/DaehwanKimLab/hisat2
R (version 4.0.3)	R Core Team, 2017	https://www.R-project.org/
RStudio (version 2021.09.1)	RStudio Team, 2016	https://www.rstudio.com/
GPP Web Portal – sgRNA design	Broad institute	https://portals.broadinstitute.org/gpp/ public/analysis-tools/sgrna-design