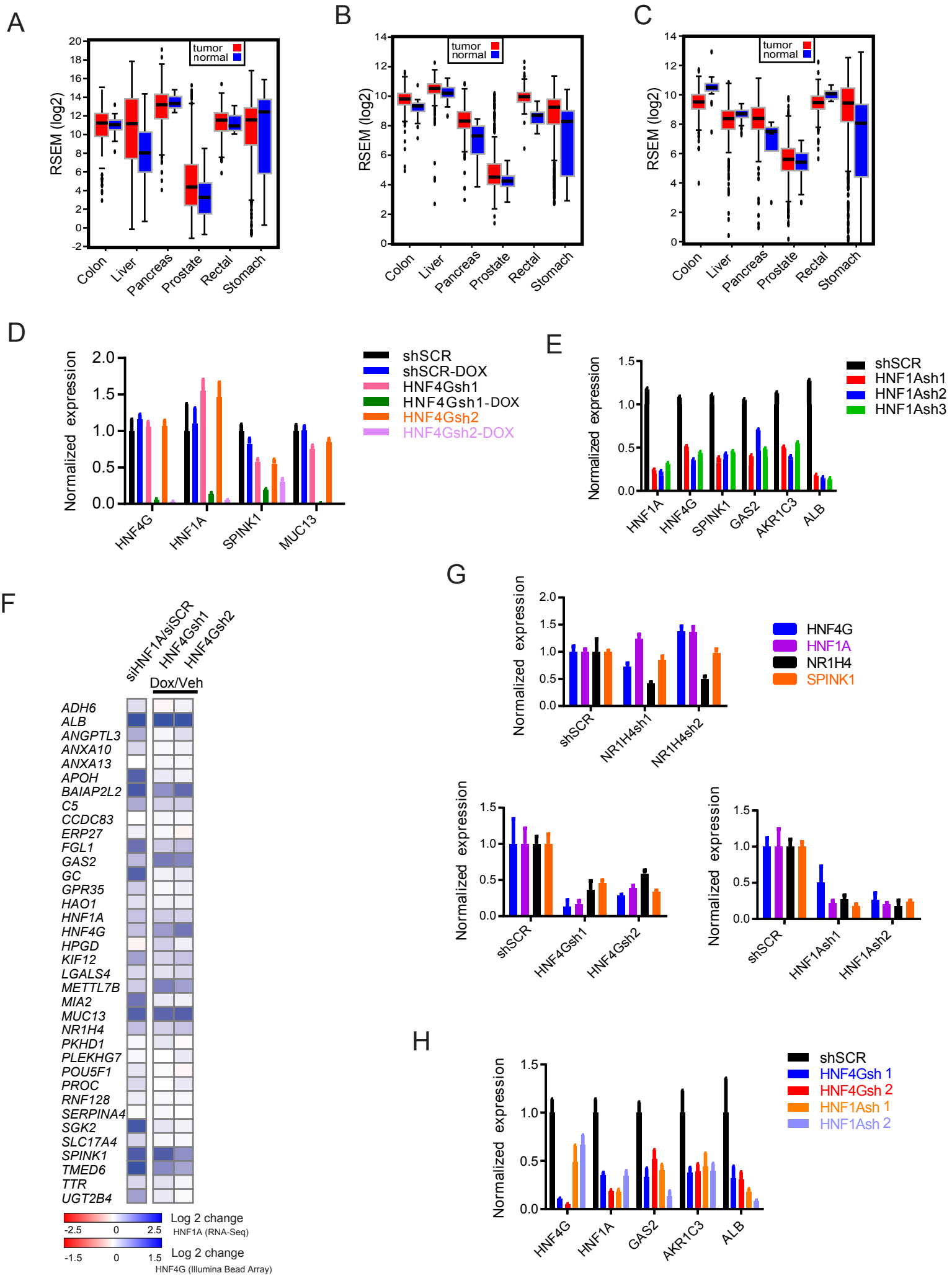
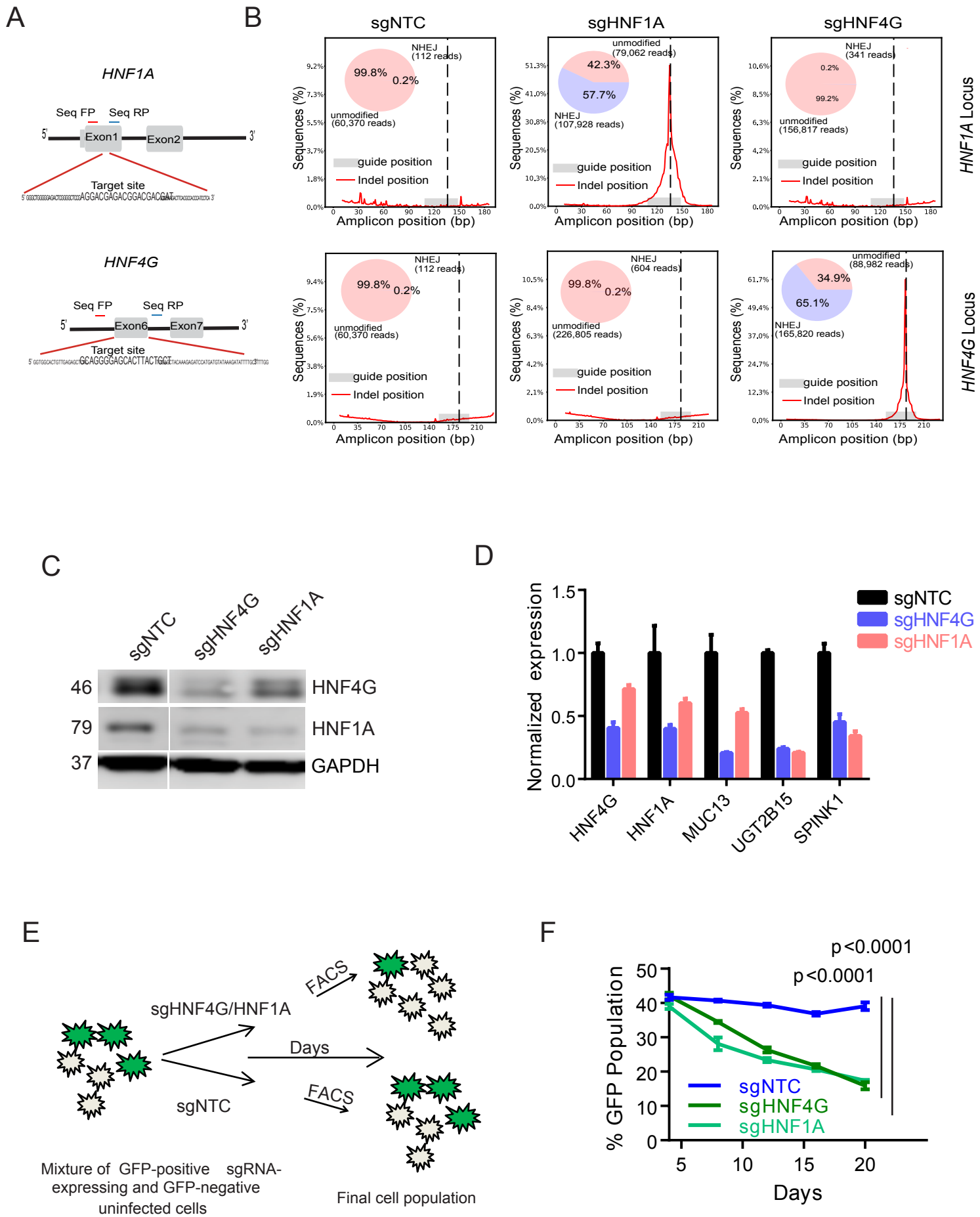

A
B
C
tumor
normal
RSEM (log2)
Colon
Liver
Pancreas
Prostate
Rectal
Stomach
D
Normalized expression
shSCR
shSCR-DOX
HNF4Gsh1
HNF4Gsh1-DOX
HNF4Gsh2
HNF4Gsh2-DOX
HNF4G
HNF1A
SPINK1
MUC13
E
shSCR
HNF1Ash1
HNF1Ash2
HNF1Ash3
HNF1A
HNF4G
SPINK1
GAS2
AKR1C3
ALB
F
siHNF1A/siSCR
HNF4Gsh1
HNF4Gsh2
Dox/Veh
ADH6
ALB
ANGPTL3
ANXA10
ANXA13
APOH
BAIAP2L2
C5
CCDC83
ERP27
FGL1
GAS2
GC
GPR35
HAO1
HNF1A
HNF4G
HPGD
KIF12
LGALS4
METTL7B
MIA2
MUC13
NR1H4
PKHD1
PLEKHG7
POU5F1
PROC
RNF128
SERPINA4
SGK2
SLC17A4
SPINK1
TMED6
TTR
UGT2B4
Log 2 change
HNF1A (RNA-Seq)
Log 2 change
HNF4G (Illumina Bead Array)
G
HNF4G
HNF1A
NR1H4
SPINK1
shSCR
NR1H4sh1
NR1H4sh2
HNF4Gsh1
HNF4Gsh2
HNF1Ash1
HNF1Ash2
H
shSCR
HNF4Gsh 1
HNF4Gsh 2
HNF1Ash 1
HNF1Ash 2
HNF4G
HNF1A
GAS2
AKR1C3
ALB

Figure S1, related to Figure 1: HNF4G and HNF1A upregulate PCa-GI signature in prostate cancer.

(A-C) Tukey box-and-whisker plot of expression of *SPINK1* (A), *HNF1A* (B) and *HNF4G* (C) in benign and cancer tissues of the prostate and of gastrointestinal organs (colon, liver, pancreas) from TCGA RNA-seq gene expression data. RSEM: RNA-Seq by Expectation Maximization. Horizontal line inside the box is the median. Lines at the bottom and top of the box represent, respectively, the 25th and the 75th quartile, and lines above and below the box show the minimum and maximum. Individual points plotted represent outliers. **(D)** qRT-PCR of selected PCa-GI signature genes upon doxycycline mediated HNF4G knockdown in 22Rv1. **(E)** qRT-PCR of selected PCa-GI signature genes upon shRNA mediated HNF1A knockdown using three different hairpins in 22Rv1 cells. **(F)** Heatmap of core PCa-GI signature genes (Genes from all 3 datasets in **Figure 1A**) in 22Rv1 cells after knockdown of HNF1A using siRNA and knockdown of HNF4G using two different doxycycline induced shRNAs. **(G)** qRT-PCR of selected PCa-GI signature genes upon shRNA mediated NR1H4 knockdown in 22Rv1 cells. **(H)** qRT-PCR of selected PCa-GI signature genes upon shRNA mediated HNF4G and HNF1A knockdown in MSK-PCa10 organoids. Data are presented as mean ± SD, n=3.


A
HNF1A
Seq FP
Seq RP
Exon1
Exon2
Target site
HNF4G
Exon6
Exon7
B
sgNTC
sgHNF1A
sgHNF4G
HNF1A Locus
HNF4G Locus
NHEJ (112 reads)
99.8%
0.2%
unmodified (60,370 reads)
NHEJ (107,928 reads)
42.3%
57.7%
unmodified (79,062 reads)
NHEJ (341 reads)
0.2%
99.2%
unmodified (156,817 reads)
NHEJ (604 reads)
unmodified (226,805 reads)
NHEJ (165,820 reads)
34.9%
65.1%
unmodified (88,982 reads)
guide position
Indel position
Sequences (%)
Amplicon position (bp)
C
sgNTC
sgHNF4G
sgHNF1A
46
79
37
HNF4G
HNF1A
GAPDH
D
Normalized expression
sgNTC
sgHNF4G
sgHNF1A
HNF4G
HNF1A
MUC13
UGT2B15
SPINK1
E
sgHNF4G/HNF1A
sgNTC
FACS
Days
Mixture of GFP-positive sgRNA-expressing and GFP-negative uninfected cells
Final cell population
F
p<0.0001
p<0.0001
% GFP Population
sgNTC
sgHNF4G
sgHNF1A
Days

G

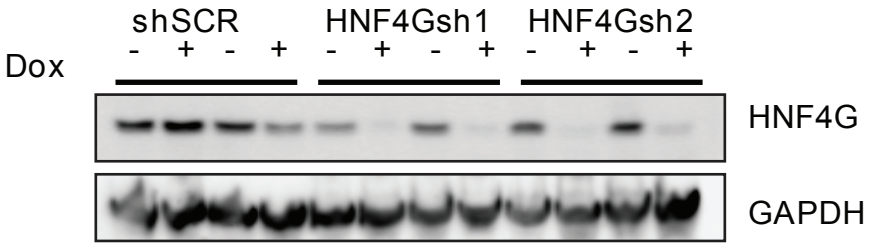
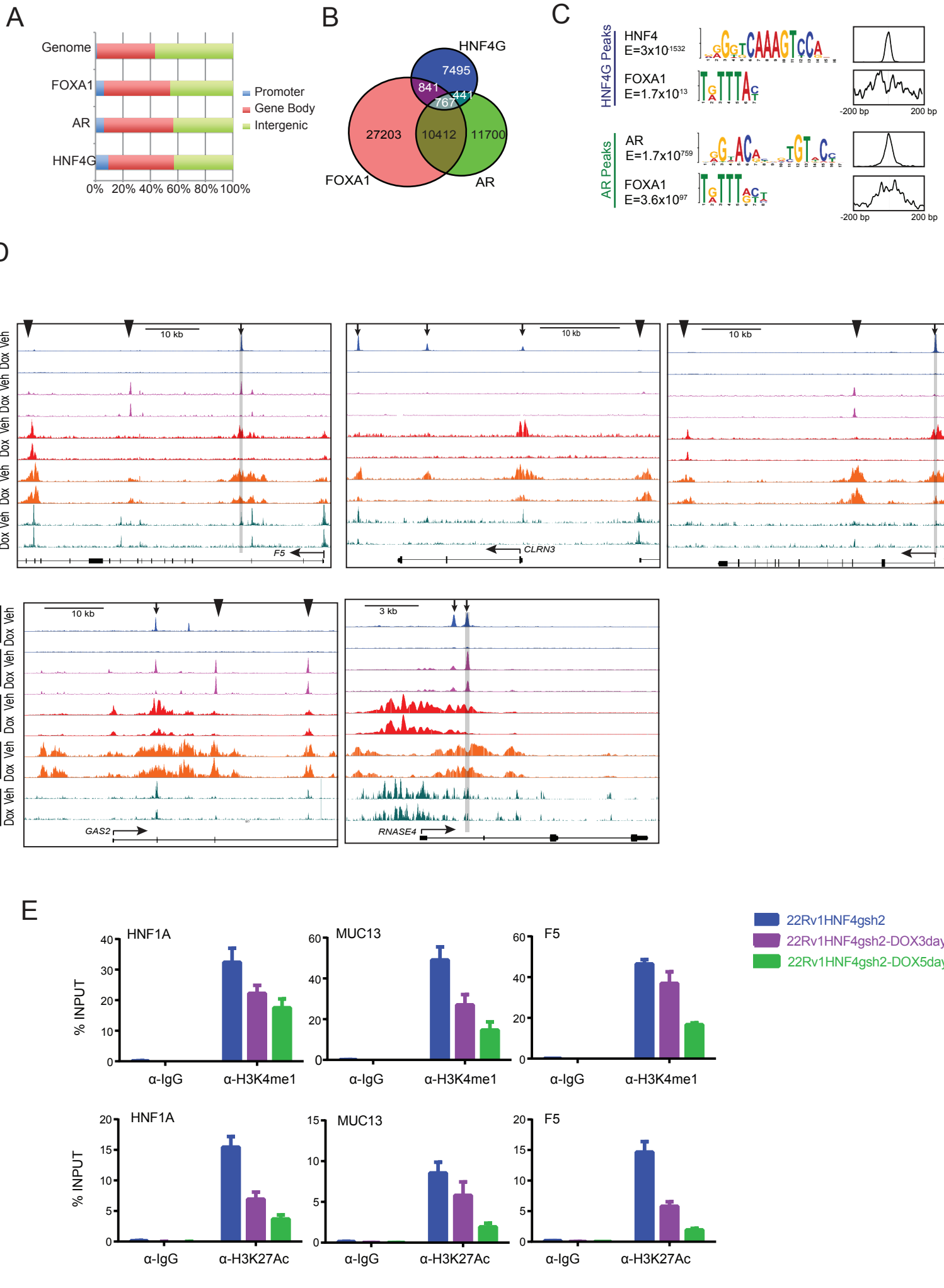


Figure S2, related to Figure 2: CRISPR-Cas9 mediated knockout of HNF4G and HNF1A in 22Rv1 cells.
(A) Schematic of *HNF1A* and *HNF4G* CRISPR/Cas9 guide RNA location and PCR primer location for amplicon-Seq. **(B)** Result of HNF1A and HNF4G amplicon sequencing of 22Rv1 cells infected with non-targeting (NTC), HNF1A, and HNF4G guide RNA. Graph shows percent of mutations relative to amplicon position and inset pie chart shows percent of amplicons with mutation. **(C)** Immunoblots against indicated proteins of 22RV-Cas9 lysates transduced for expression of CRISPR guides against HNF4G, HNF1A and non-target control (NTC). Lysates were prepared after 7 days of transduction. The image is digitally cropped to depict relevant lanes. **(D)** qRT-PCR analysis of selected HNF4G and HNF1A downstream targets at 7 days after transduction of 22Rv1-Cas9 cells with CRISPR guides against HNF4G, HNF1A and NTC control. The mRNA of the targeted gene itself is also depleted, likely through non-sense mediated RNA decay. Mean ± SD, n=3. **(E)** Schematic showing experimental design for CRISPR competition assays. 22Rv1 cells expressing Cas9 were transduced with dual expression vector of GFP and CRISPR sgRNAs against HNF4G, HNF1A or non-target control (NTC). Cells were infected with a MOI~0.4 so that initial population was a mixture of GFP-positive and GFP-negative cells. FACS analysis was performed to determine GFP-positive cells over the course of the experiment. **(F)** Percentage of GFP positive 22Rv1 cells expressing CRISPR guides against HNF4G and HNF1A or NTC over twenty days. Mean ± SD. Two-tailed unpaired t-test, n=3. **(G)** Immunoblots of two representative 22Rv1 explants obtained after two days of doxycycline or sucrose water administration in SCID mice from shSCR, HNF4Gsh1 and HNF4Gsh2 expressing xenograft tumors.


A
Genome
FOXA1
AR
HNF4G
0% 20% 40% 60% 80% 100%
Promoter
Gene Body
Intergenic
B
HNF4G
7495
841
441
767
27203
10412
11700
FOXA1
AR
C
HNF4G Peaks
HNF4
E=3x10-1532
FOXA1
E=1.7x10-13
-200 bp
200 bp
AR Peaks
AR
E=1.7x10-759
FOXA1
E=3.6x10-97
-200 bp
200 bp
D
HNF4G
FOXA1
H3K27Ac
H3K4me1
ATAC
Dox Veh
10 kb
F5
CLRN3
MUC13
GAS2
3 kb
RNASE4
E
22Rv1HNF4gsh2
22Rv1HNF4gsh2-DOX3days
22Rv1HNF4gsh2-DOX5days
HNF1A
MUC13
F5
% INPUT
α-IgG
α-H3K4me1
α-H3K27Ac

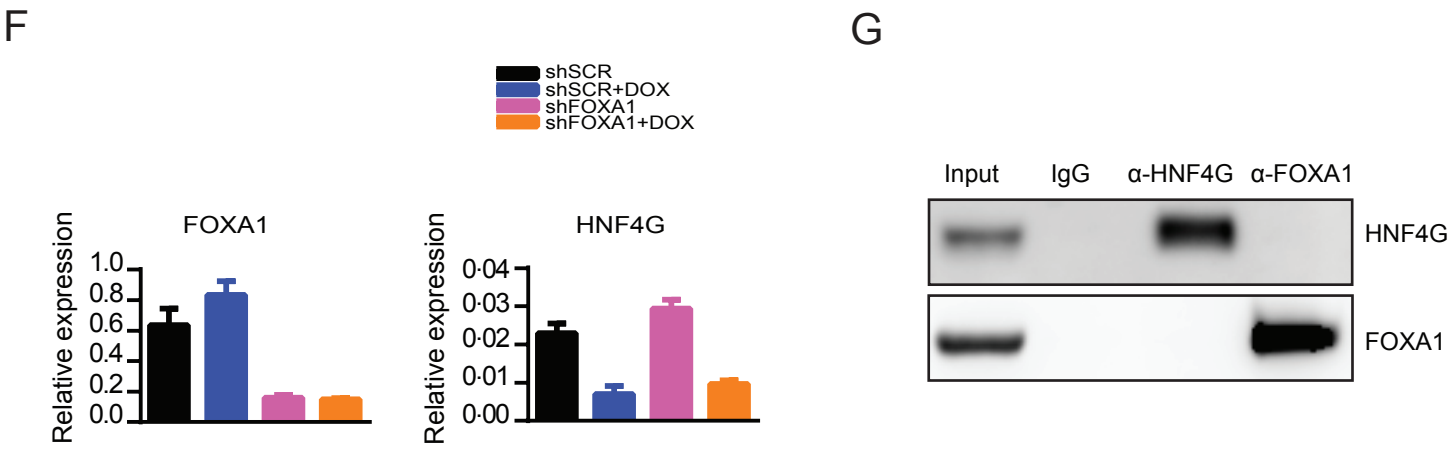


Figure S3, related to Figure 3: HNF4G suppression reduces enhancer chromatin at target genes.

(A) Genomic distribution of HNF4G, AR, and FOXA1 peaks with whole genome control mapped to promoter (-1 kb to +100 bp of TSS), gene body (+100 bp TSS to +1 kb TES), and intergenic regions. **(B)** Venn diagram depicting overlap of HNF4G, FOXA1 and AR binding sites identified by ChIP-seq analysis in vehicle treated 22Rv1-HNF4Gsh2-Dox cells. **(C)** *De novo* motif analysis of top 1,000 HNF4G and top 1,000 AR peaks by significance showing top 2 most enriched motifs. Motif, percentage of peaks, and significance is shown on left, the sequence logo is shown in the middle, and the histogram of motif around peak summit is shown on the right. **(D)** Representative ChIP-seq and ATAC-Seq profiles of vehicle or doxycycline treated 22Rv1- HNF4Gsh2 cells at *F5, CLRN3, MUC13,GAS2 and RNASE4* loci. Arrows indicate enhancers with HNF4G peaks and arrowheads indicate control enhancers without HNF4G peaks. Region assayed by ChIP-PCR in **Figure 3** are highlighted. **(E)** ChIP-qRT-PCR showing time dependent decrease of H3K4 monomethylation and H3K27 acetylation upon HNF4G knockdown at select HNF4G target PCa-GI genes. **(F)** qRT-PCR showing the mRNA levels of HNF4G and FOXA1 upon respective knockdowns in 22Rv1-HNF4Gsh2 cells. **(G)** Western blot depicting co-immunoprecipation of HNF4G and FOXA1 performed using either antibody or IgG as a control. Data are presented as mean ± SD, n=3.

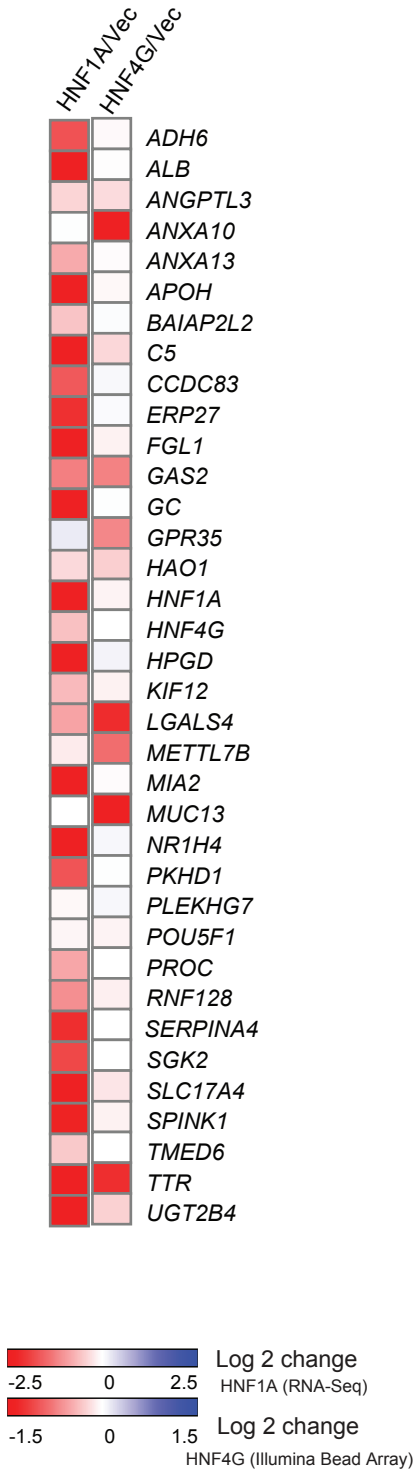
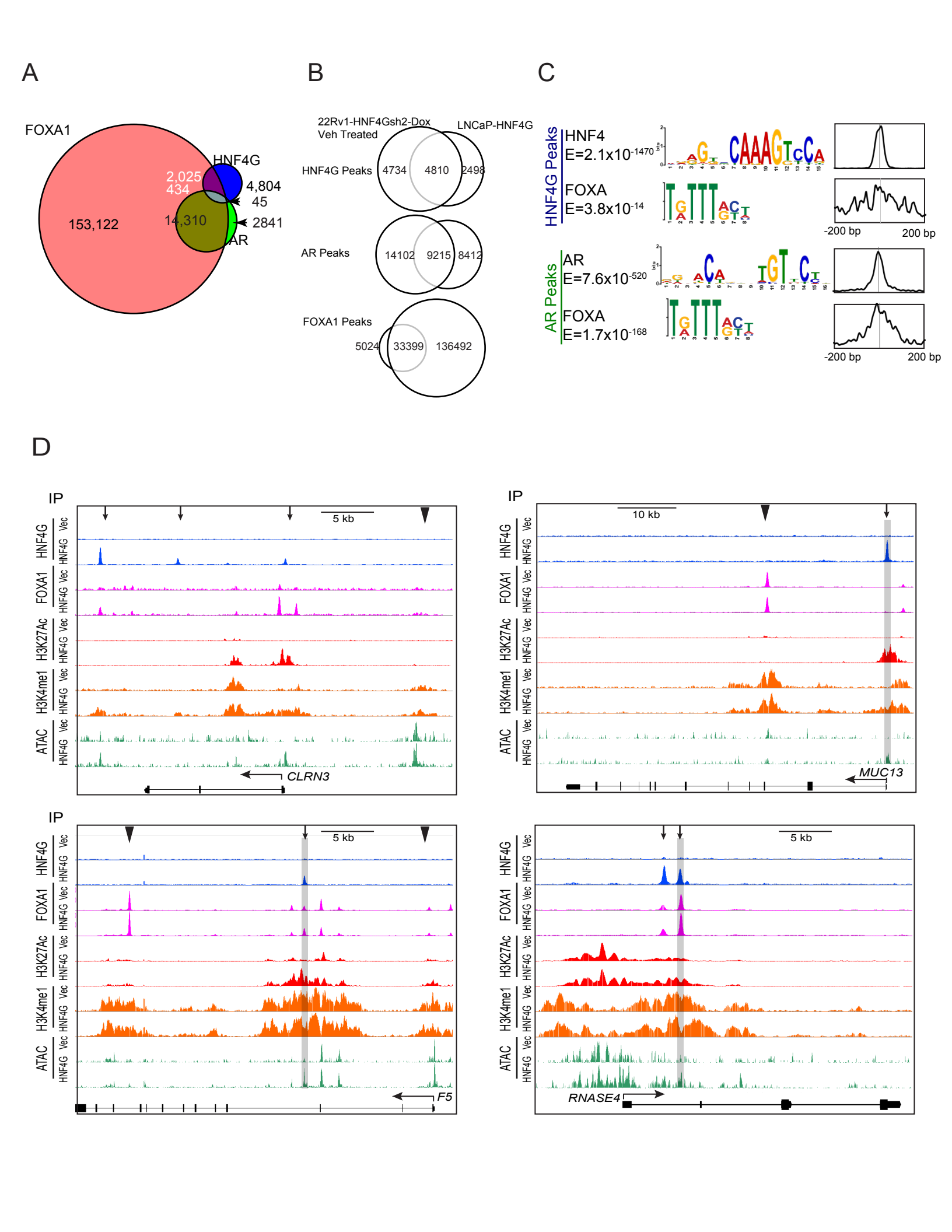


Figure S4, related to Figure 4: HNF4G and HNF1A upregulate PCa-GI signature in LNCaP. Heatmap shows difference in expression of core PCa-GI signature genes common to all three datasets (see Figure 1A) after exogenous expression of HNF4G and HNF1A and empty vector control in LNCaP cells.


A
FOXA1
HNF4G
AR
153,122
2,025
434
4,804
45
14,310
2841
B
22Rv1-HNF4Gsh2-Dox
Veh Treated
LNCaP-HNF4G
HNF4G Peaks
4734
4810
2498
AR Peaks
14102
9215
8412
FOXA1 Peaks
5024
33399
136492
C
HNF4G Peaks
HNF4
E=2.1x10-1470
FOXA
E=3.8x10-14
AR Peaks
AR
E=7.6x10-520
FOXA
E=1.7x10-168
-200 bp
200 bp
-200 bp
200 bp
D
IP
HNF4G
FOXA1
H3K27Ac
H3K4me1
ATAC
Vec
HNF4G
5 kb
CLRN3
IP
10 kb
MUC13
IP
5 kb
F5
IP
5 kb
RNASE4

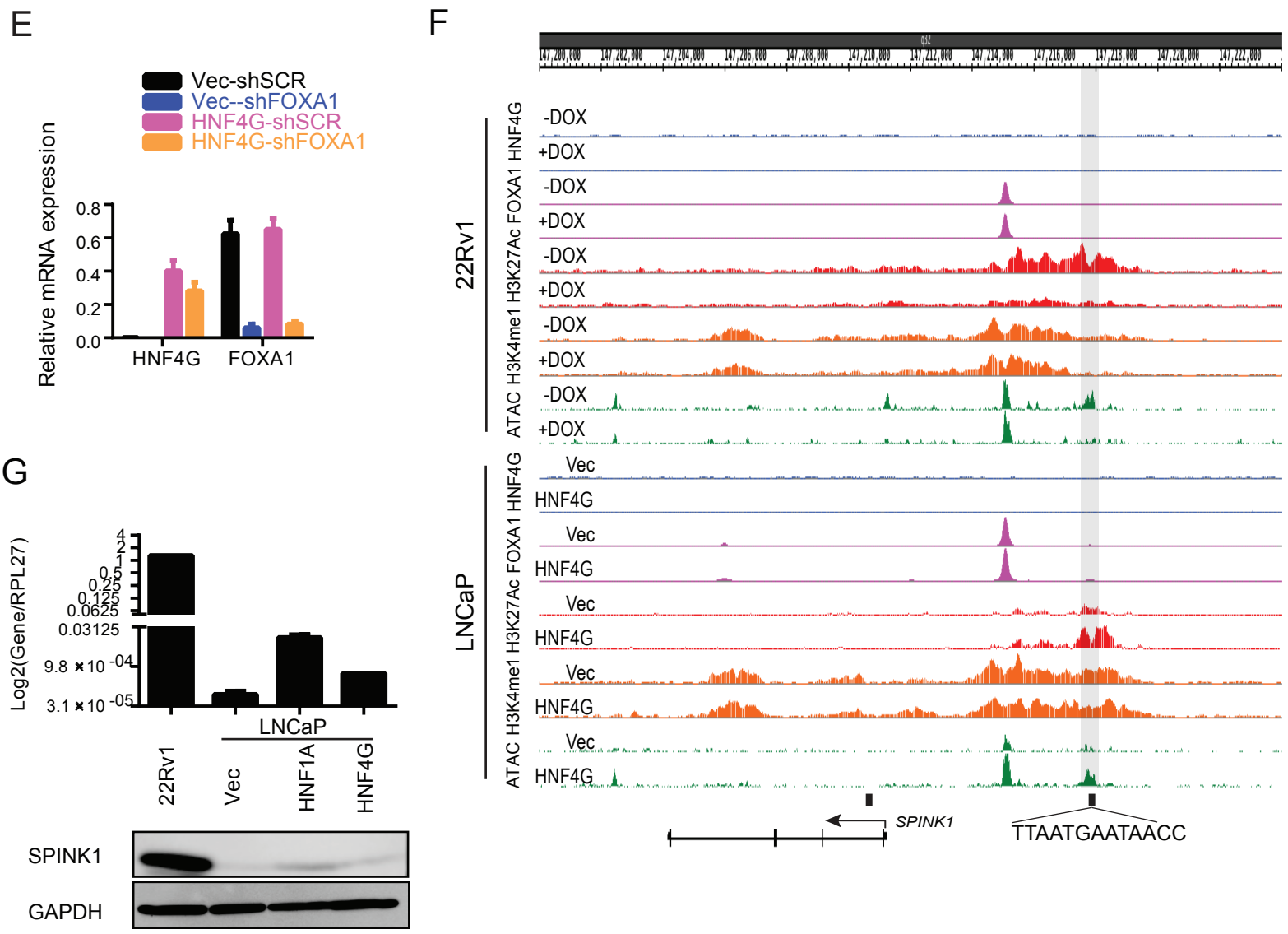


Figure S5, related to Figure 5: HNF4G expression increases active enhancer chromatin at PCa-GI signature genes.

(A) Venn diagram depicting overlap of HNF4G, FOXA1 and AR binding sites identified by ChIP-seq analysis in LNCaP expressing HNF4G. **(B)** Venn diagram depicting overlap of HNF4G, AR, and FOXA1 binding sites between vehicle treated 22Rv1-HNF4Gsh2-Dox cells with endogenous HNF4G and LNCaP-HNF4G cells with exogenous HNF4G. **(C)** De novo motif analysis of top 1,000 HNF4G and top 1,000 AR peaks by significance showing top 2 most enriched motifs. Motif, percentage of peaks, and significance is shown on left, the sequence logo is shown in the middle, and the histogram of motif around peak summit is shown on the right. **(D)** Representative ChIP-seq and ATAC-Seq profiles of LNCaP cells exogenously expressing HNF4G or empty vector control at *CLRN3*, MUC13, *F5* and *RNASE4* loci using indicated antibodies for ChIP. Arrows indicate enhancers with HNF4G peaks and arrowheads indicate control enhancers without HNF4G peaks. Region assayed by ChIP-PCR in Figure 5 are highlighted. **(E)** mRNA levels of HNF4G and FOXA1 upon HNF4G overexpression and FOXA1 knockdown in LNCaP-Vec and LNCaP-HNF4G cells. **(F)** ChIP-Seq and ATAC-Seq profiles of the *SPINK1* locus of 22Rv1 cells (top) with doxycycline mediated HNF4G knockdown and LNCaP cells (bottom) with vector control and HNF4G overexpression. There is an upstream HNF1A motif (shaded) that showed decreased H3K27Ac and ATAC signal after HNF4G knockdown and increased H3K27Ac and ATAC signal after HNF4G overexpression respectively. **(G)** qRT-PCR (top) and Western blot (bottom) showing increase in SPINK1 transcript and protein level upon exogenous expression of HNF4G and HNF1A in LNCaP. Data are presented as mean ± SD, n=3.

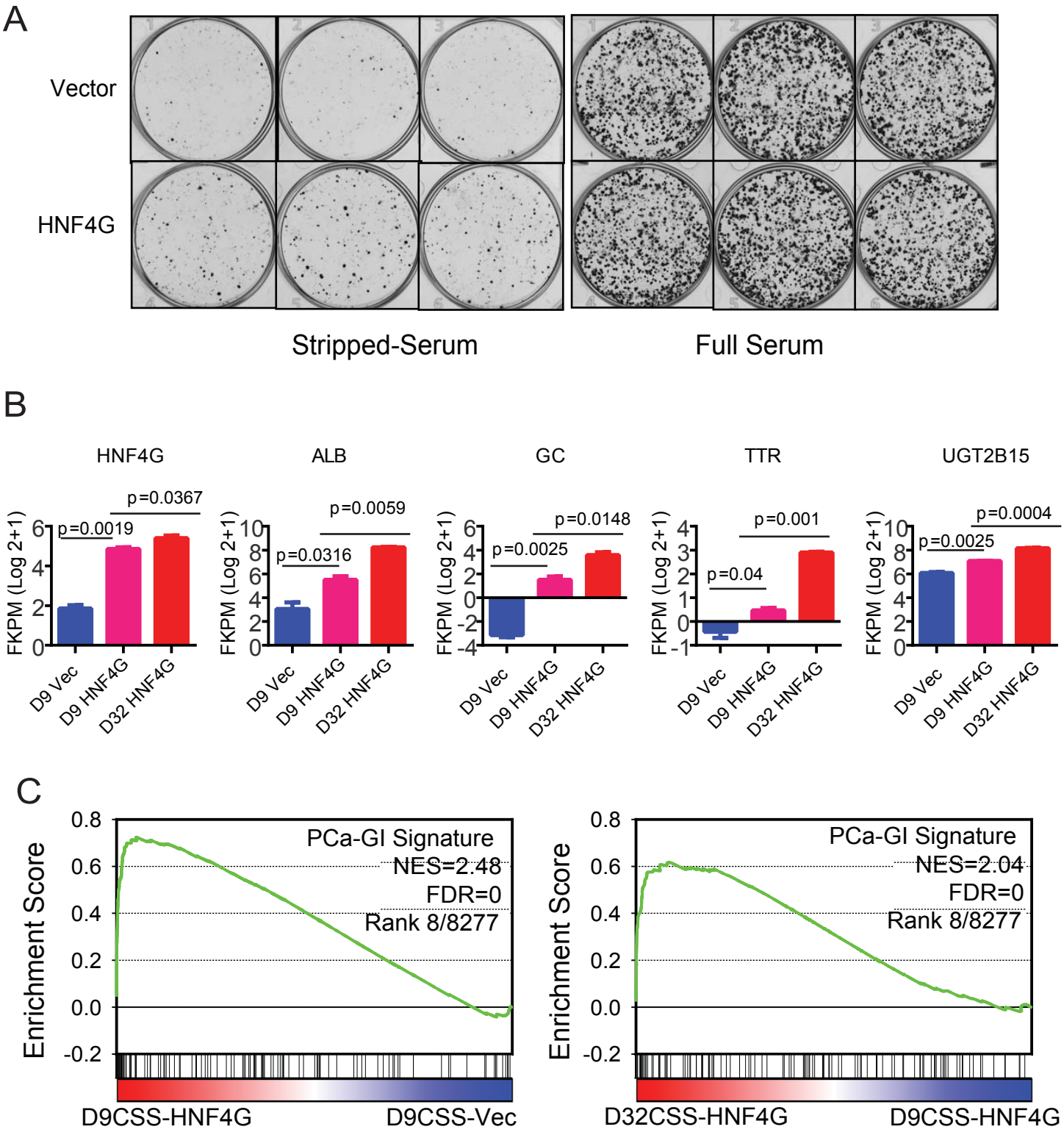


Figure S6, related to Figure 6: Enzalutamide reistance and androgen ablated growth of LNCaP/AR cells by HNF4G expression.

(A) Photograph of crystal violet stained colonies of LNCaP/AR cells expressing HNF4G or vector grown in CSS. **(B)** RNA-Seq gene expression levels of stripped-serum grown LNCaP/AR cells with vector expression at day 9 of growth or HNF4G expression at day 9 and day 32 of growth. n=2, Mean ± SD. **(C)** GSEA plots of PCa-GI signature in LNCaP/AR cells expressing HNF4G vs vector when grown for 9 days in CSS (left) and in LNCaP/AR cells expressing HNF4G at day 32 of growth in CSS as compared with day 9 in CSS (right).

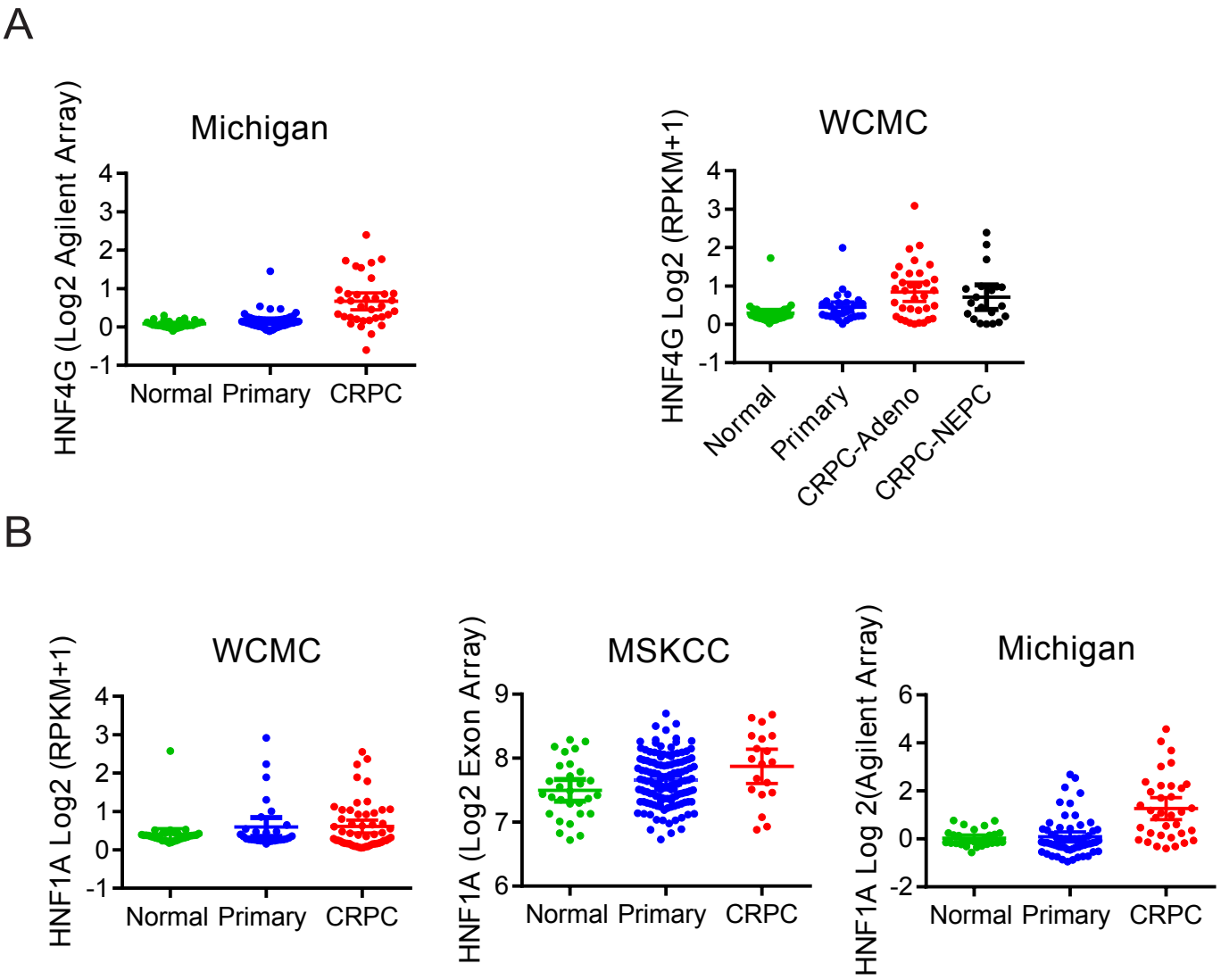


Figure S7, related to Figure 7: HNF4G and HNF1A expression in CRPC.
(A) HNF4G expression in normal prostate, primary and CRPC from the Michigan and WCMC dataset . CRPC cases from WCMC are further characterized as adenocarcinoma (CRPC-Adeno) or neuroendocrinal prostate cancer (CRPC-NEPC). **(B)** HNF1A expression in normal prostate, primary and CRPC from the WCMC, MSKCC and Michigan dataset. Data are presented as mean ± 95% CI.