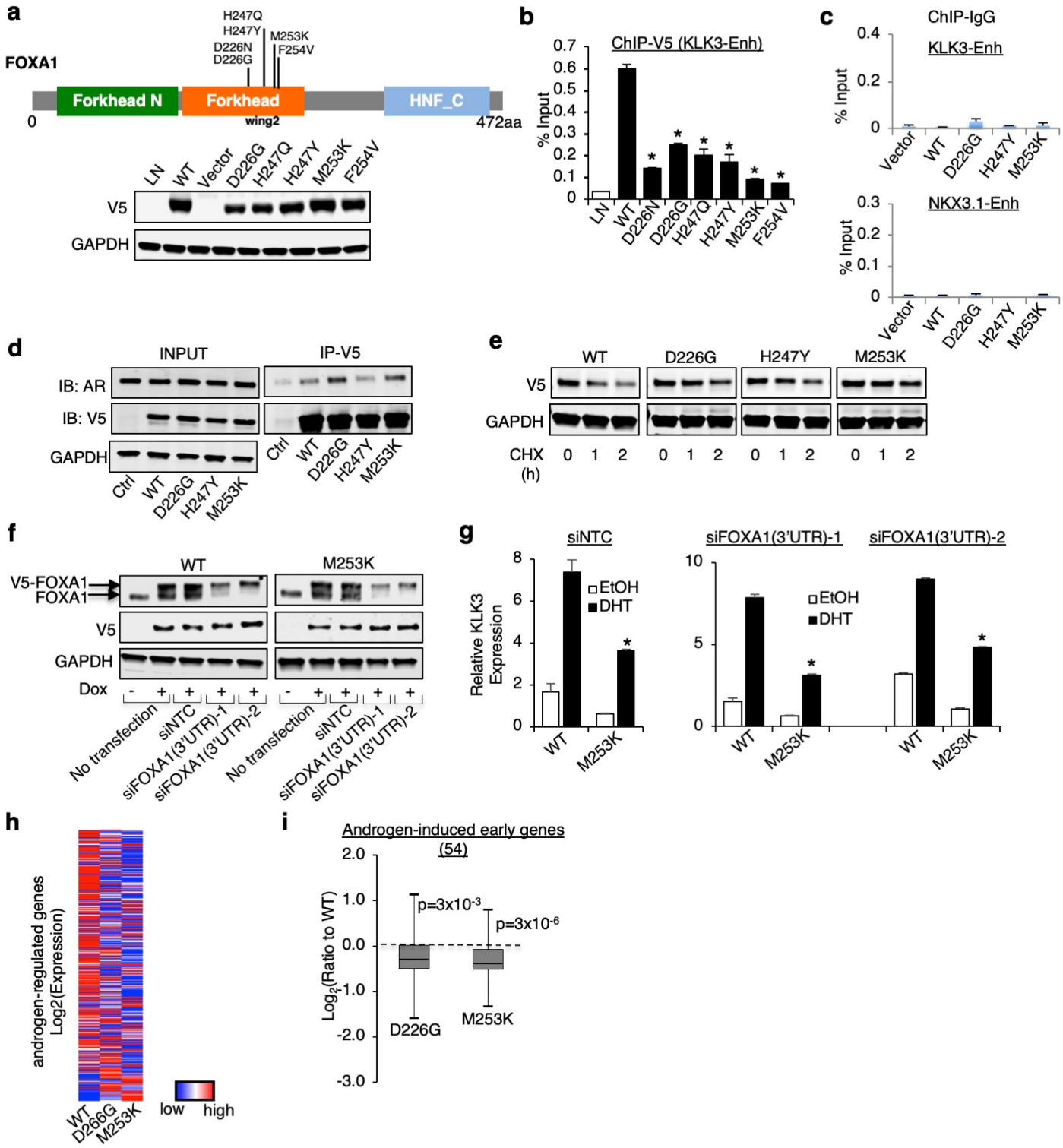
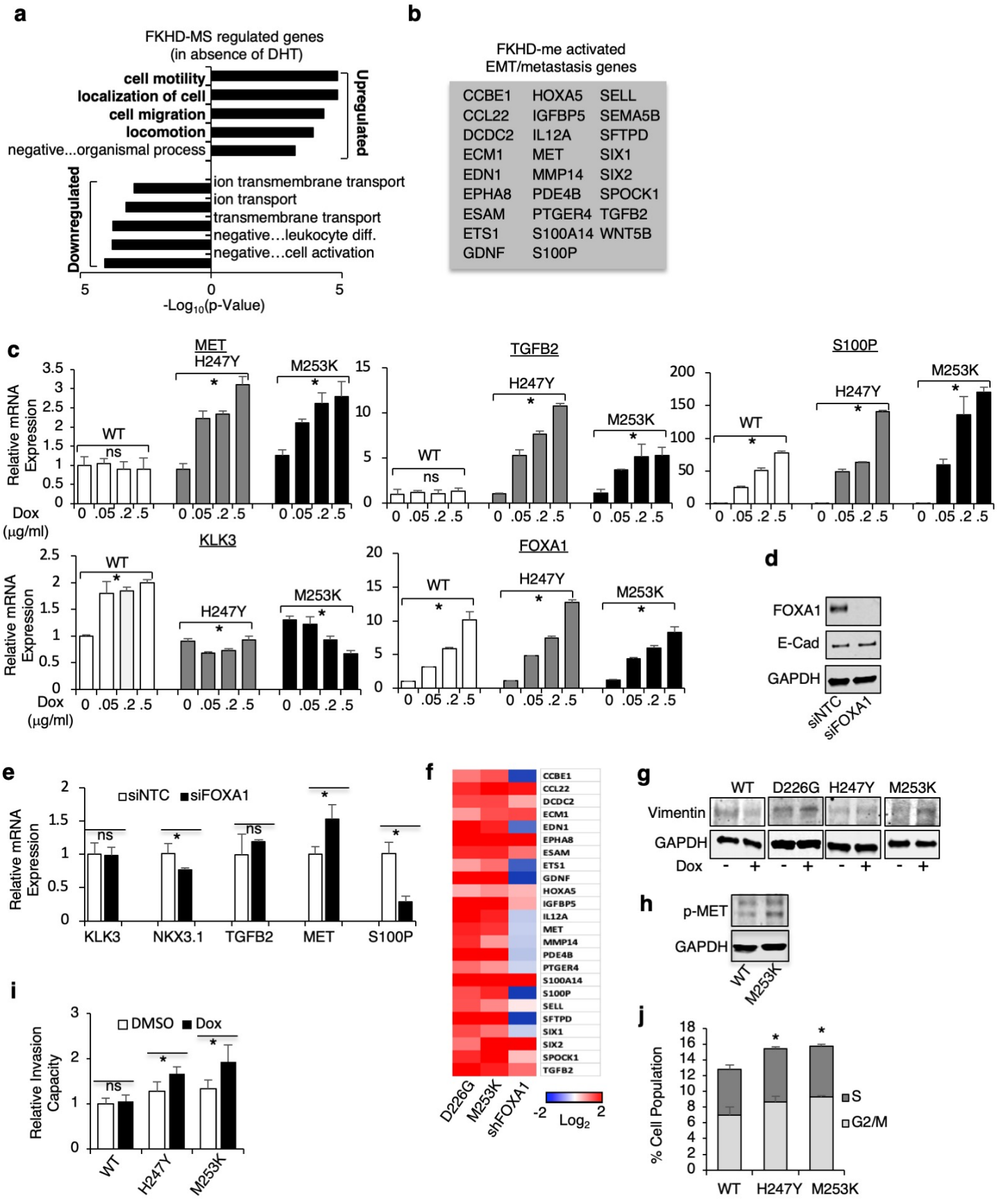
Supplementary information, Fig. S1



Supplementary information Fig. S1. FKHD-MSs impaired FOXA1 chromatin binding at AR-regulated enhancers and decreased AR activity.

a Establishment of LNCaP stable cell lines that constitutively express V5-tagged FKHD-MSs. **b** ChIP-V5 in those stable lines at KLK3 enhancers (examined by qRT-PCR). **c** ChIP-IgG in tetracycline-inducible LNCaP stable cell lines treated with doxycycline (2d) (days) at KLK3 and NKX3.1 enhancers (examined by qRT-PCR). **d** Immunoprecipitation of V5 in LNCaP stable cells treated with doxycycline for 2d and then DHT for 1d, followed by immunoblotting for AR and V5. **e** LNCaP stable cells were treated with cycloheximide for 0-2h (hours), followed by immunoblotting for V5. **f** LNCaP stable cells (in hormone-depleted condition) were treated with doxycycline for 2d and transfected with siNTC or siRNA against FOXA1 3'UTR, followed by immunoblotting for FOXA1 and V5. **g** WT or M253K-expressing LNCaP stable cells (in hormone-depleted condition) were treated with DHT for 24h and followed by qRT-PCR measuring KLK3 gene expression, **h** RNA-seq analyses on WT/D226G/M253K-expressing lines treated with/out DHT (24h) were performed. The effects on the expression of AR-regulated genes were shown in the heatmap. **i** The expression of previously identified 54 direct AR regulated genes were assessed using the RNA-seq results.

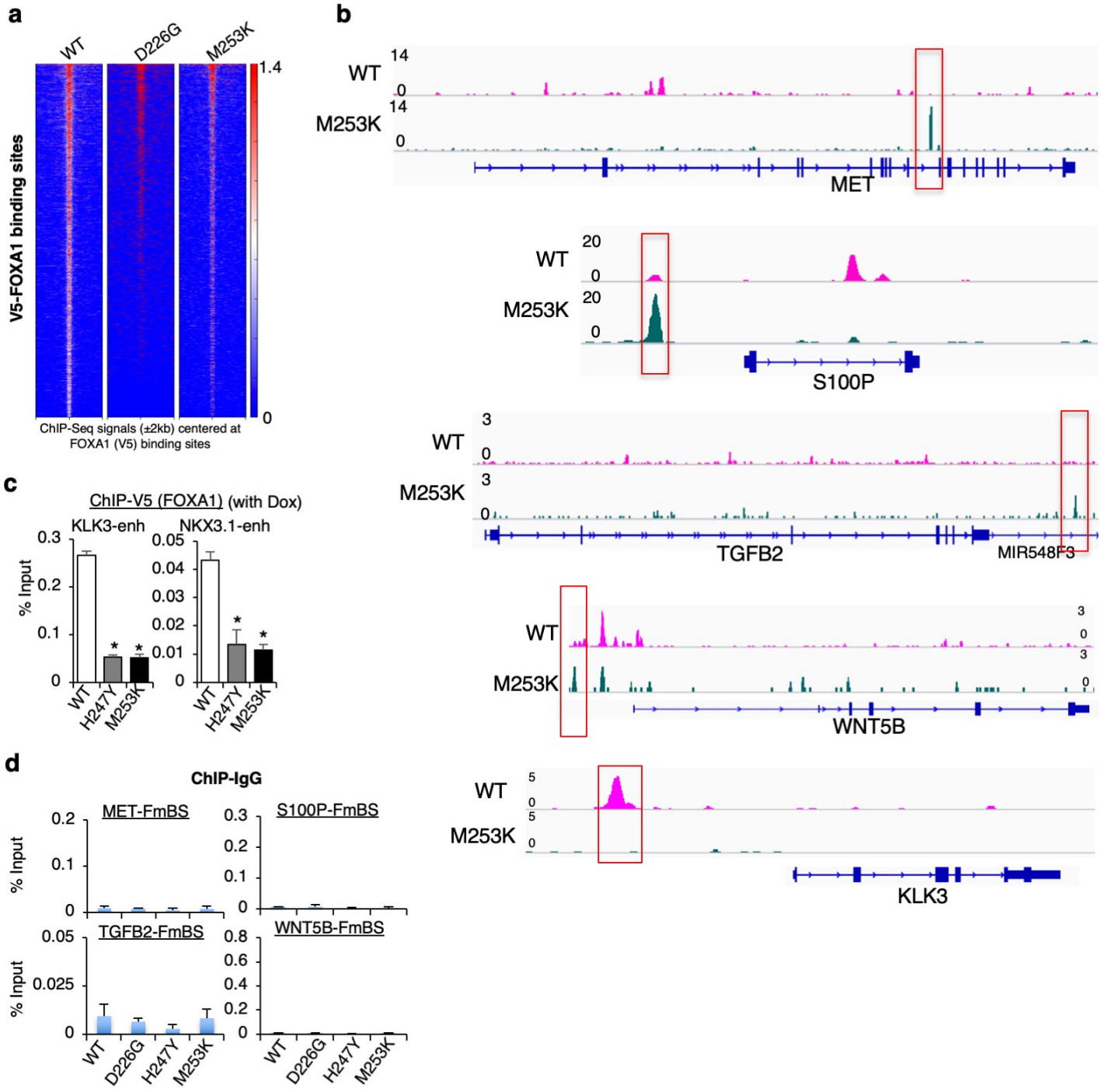
Supplementary information, Fig. S2



Supplementary information, Fig. S2. FKHD-MSs transcriptionally activated genes mediating EMT.

a Gene ontology analysis on FKHD-MS regulated genes (determined by RNA-seq). **b** FKHD mutants specifically increased the expression of a subset of genes mediating EMT/metastasis in absence of DHT treatment. **c** Four EMT/metastasis genes were selected for validation. The mRNA expression of these genes (examined by qRT-PCR) was rapidly increased by induction of FKHD-MSs in LNCaP stable lines expressing tetracycline-inducible FKHD-MSs (in hormone-depleted condition). **d** Immunoblotting for FOXA1 in LNCaP cells treated with siFOXA1 versus siNTC. **e** Silencing FOXA1 did not significantly increase the expression of EMT genes. **f** Knocking down FOXA1 (public RNA-seq dataset, GSE119759) acts differently from FKHD-MSs on regulating the subset of genes mediating EMT (identified from Supplementary information, Fig. S2b). **g** Immunoblotting for vimentin in LNCaP stable cells treated with doxycycline for 2d. **h** Immunoblotting for phosphorylated MET (T1234/1235) in LNCaP stable cells. **i** LNCaP stable cells invasion capacity was measure by Matrigel invasion chambers, with/out doxycycline for 4d. **j** LNCaP stable cells were treated with doxycycline for 2d and subjected to cell cycle analysis.

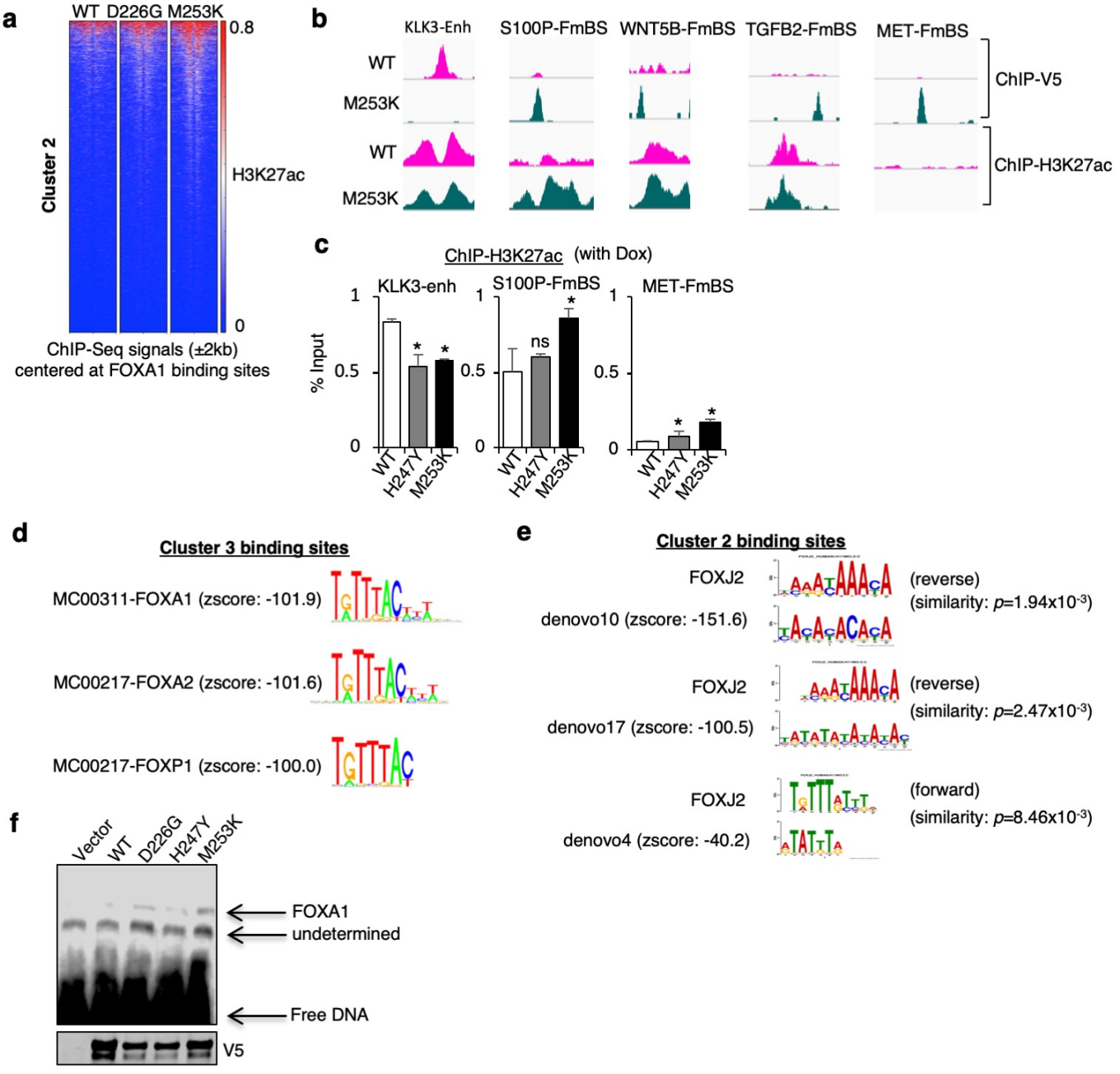
Supplementary information, Fig. S3



Supplementary information, Fig. S3. FKHD-MSs reprogrammed global FOXA1 binding and displayed increased binding affinity at the EMT gene loci.

a Heatmap view for global FOXA1 (V5) binding in LNCaP stable lines**. b** Identification of sites for FKHD-MS-preferred (M253K) chromatin binding at EMT gene loci. **c** ChIP-V5 at two AR-regulated enhancers (examined by qRT-PCR). **d** ChIP-IgG at sites with FKHD-MS-preferred binding (examined by qRT-PCR).

Supplementary information, Fig. S4

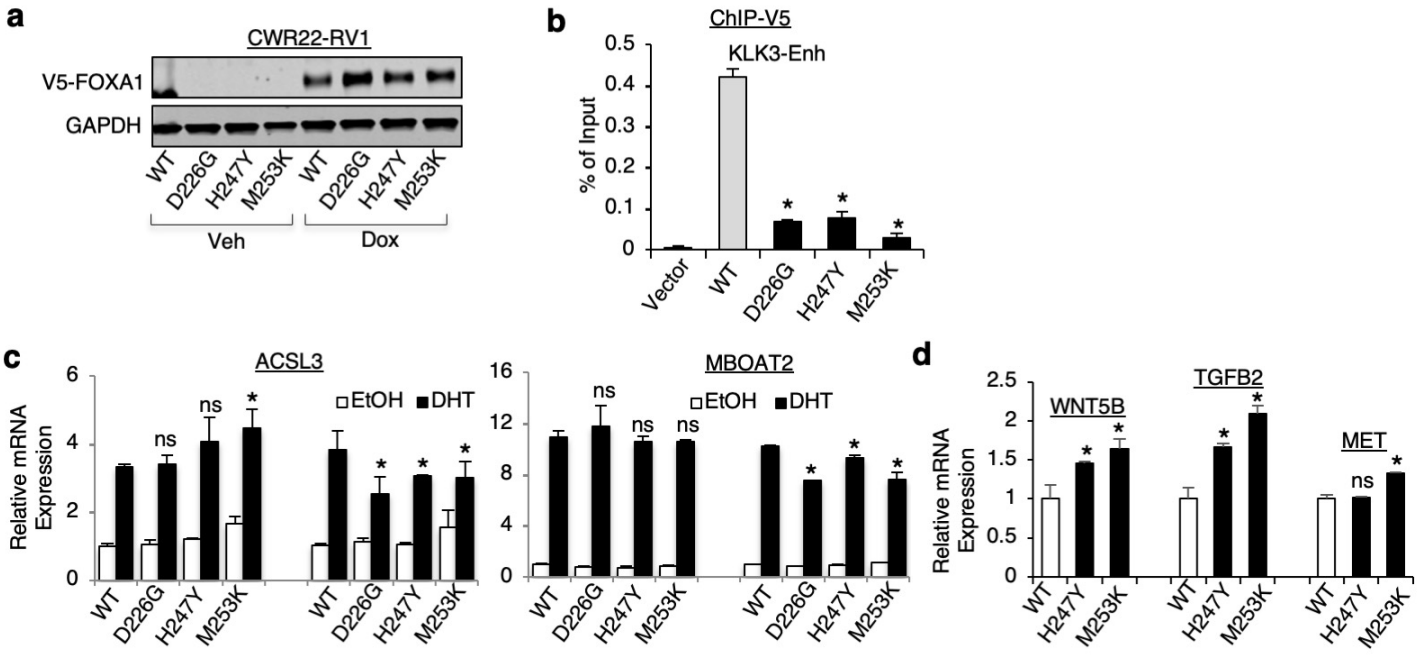


Supplementary information, Fig. S4. FKHD-MS-preferred bindings were associated with increased levels of H3K27ac.

a Heatmap for H3K27ac binding at cluster 2 sites (FKHD-MS-preferred binding sites identified in Fig 1k**). b** The H3K27ac levels at the identified FKHD-MS-preferred binding sites. **c** ChIP-qPCR for H3K27ac at sites with

FKHD-MS-preferred binding. **d** Top ranked motifs identified in the cluster 3 sites. **e** Top ranked motifs identified in the cluster 2 sites with comparison to the classic FOXJ2 binding motif. **f** FKHD-MSs or FOXA1 WT were overexpressed in PC3 cells and the cell lysate was incubated with a DNA probe containing denovo10 motif. FKHD-MSs showed stronger binding to DNA (examined by gel shift assay).

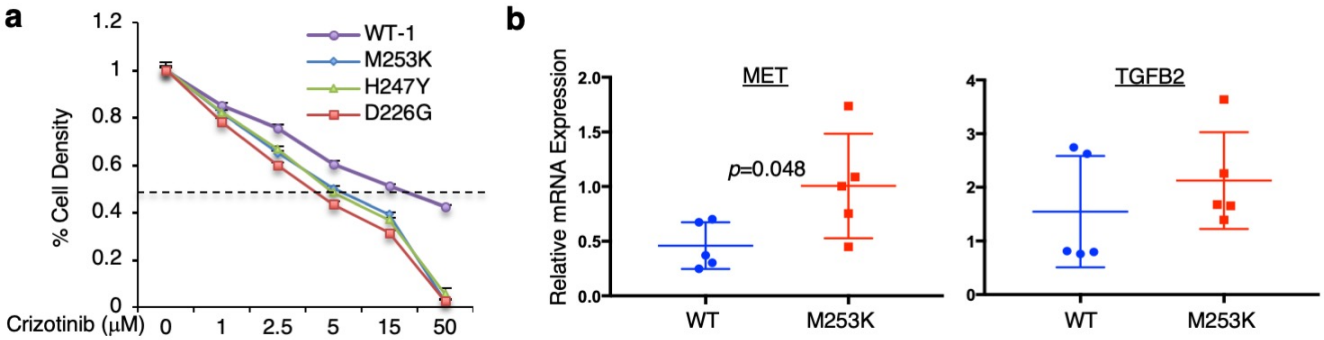
Supplementary information, Fig. S5



Supplementary information, Fig. S5. FKHD-MSs downregulated AR-regulated genes and upregulated EMT genes in CWR22-RV1 cells.

a Establishment of CWR22-RV1 derived stable cell lines that express tetracycline-regulated V5-tagged FKHD-MSs. **b** FOXA1 binding at KLK3 enhancer was impaired by FKHD-MSs (by ChIP-qPCR of V5). **c** The expression of two previously identified AR/AR-V7-regulated lipid synthesis genes was decreased by FKHD-MSs (examined by qRT-PCR). **d** The mRNA expression of the EMT genes was increased by FKHD-MSs (examined by qRT-PCR).

Supplementary information, Fig. S6



Supplementary information, Fig. S6. The expression of FKHD-MSs sensitized PCa cells to crizotinib.

a CWR22-RV1 derived stable lines were treated with different doses of crizotinib for 4d, followed by cell counting.

b The mRNA expression of MET and TGFB2 was examined by qRT-PCR in the xenograft tumor biopsies from CWR22-RV1 tumor expressing WT versus M253K.

MATERIALS and METHODS

Generation of FOXA1 mutant constructs and transduction: FOXA1 in pDONR was purchased from Harvard PlasmID database. The missense mutations were introduced using QuickChange Lightning Site-Directed Mutagenesis Kit (Agilent). The specific primers are listed as following: D226G: Forward, 5'-CACCTTGACGAAGCAGCCATTGAAGGACAGCGA-3', Reverse, 5'-TCGCTGTCCTTCAATGGCTGCTTCGT-CAAGGTG-3'; H247Y: Forward, 5'-CCGGAGTCCGGGTACAGCGTCCAGTAG-3', Reverse, 5'-CTACT-GGACGCTGTACCCGGACTCCGG-3'; H247Q: Forward, 5'-CTACTGGACGCTGCAACCGGACTCCGG-3', Reverse, 5'-CCGGAGTCCGGTTGCAGCGTCCAGTAG-3'; M253K: Forward, 5'-AGCCGTTCTCGAACT-TGTTGCCGGAGTCC-3', Reverse, 5'-GGACTCCGGCAACAAGTTCGAGAACGGCT-3'; F254V: Forward, 5'-GCAGCCGTTCTCGACCATGTTGCCGGAGT-3', Reverse, 5'-ACTCCGGCAACATGGTCGAGAACGGCTGC-3'. FOXA1 Mutants were subcloned into pLenti6 or pLIX_403 vector (Addgene) via using gateway cloning kit (Invitrogen). Lentiviruses were packaged in 293T cells by using third generation system. The 293T supernatant containing lentiviruses was mixed with polybrene (Millipore) and added to LNCaP or CWR-22RV1 cells followed by puromycin selection for 1-2 weeks to establish the stable cell lines.

Cell culture: The LNCaP and CWR22-RV1 cells were recently authenticated using short tandem repeat (STR) profiling by DDC Medical (Fairfield, OH, USA). LNCaP and CWR-22RV1 cells were cultured in RPMI-1640 with 10% FBS. LNCaP-FOXA1 or 22RV1-FOXA1-derived tetracycline-inducible FOXA1 overexpressing stable cells were maintained in RPMI-1640 with 10% tetracycline-free FBS.

Chromatin immunoprecipitation (ChIP) and ChIP-seq: For preparation of ChIP, the chromatin of fixed cells was sonicated into 500–800 bp fragments, followed by immunoprecipitation using anti-V5 antibody (Thermo Fisher Scientific), anti-AR antibody (Santa Cruz), anti-H3K27ac (abcam), anti-IgG (Millipore). The qPCR analysis was carried out using the SYBR Green method. The primers are listed as following: MET-enh: Forward, 5'-TGAGACACAGTGGATGTGTGA-3', Reverse, 5'-GATCTCCCTGGTTGTTGCAT-3'; S100P-enh: Forward, 5'-CAGTGAATGCCCTTCCTGAG-3', Reverse, 5'-ATACCACCCCAGCCAGAGA-3'; TGFB2-enh: Forward, 5'-TTGCACATGTCCTTCCTTGC-3', Reverse, 5'-CCGGGTCTGTTTTGGAATCC-3'; WNT5B-enh: Forward, 5'-

AGGTTGAACGCATCTCTCCT-3', Reverse, 5'-GACTGCAATCCTGAGTGTGC-3'; KLK3-enh: Forward, 5'-TCGATTGTCCTTGACAGTAAACA-3', Reverse, 5'-TCTCAGATCCAGGCTTGCTT-3'; NKX3.1-enh: Forward, 5'-CTGGCAAAGAGCATCTAGGG-3', Reverse, 5'-GGCACTTCCTGAGCAAACTT-3'.

For ChIP-seq analyses, the libraries were prepared using ThruPlex DNA-seq 48D Kit (Rubicon Genomics) and then sequenced using Illumina HiSeq 2500 at the Genomics Core of University of Massachusetts Boston. MACS2 (version 2.0.10.20131216) was used to call peak on the bam files. The R package ChIPpeakAnno (version 3.10.1) was used for analyzing peak intervals. deepTools (version 2.4.1) was used to analyze signal from bigwig files. To detect different binding patterns for WT-FOXA1 and mutant FOXA1, the unsupervised kmeans cluster was used, by setting the parameter '-- kmeans' in deeptools to 5. For Motif analysis,

RT-PCR and RNA-seq: RNA was extracted with TRIzol Reagent (Invitrogen) based on manufacturer's protocol. The gene expression was measured using real-time RT-PCR analyses with Taqman one-step RT-PCR reagents (Thermo Fisher Scientific) and results were normalized to co-amplified GAPDH. S100P (Hs00195584_m1), MET (Hs01565584_m1), TGFB2 (Hs00234244_m1), WNT5B (Hs01086864_m1). KLK3, NKX3-1, ACSL3, MBOAT2 were described previously [1].

For RNA-seq analysis, RNA was purified using RNeasy Mini Kit (Qiagen). TruSeq® Strnd Total RNA LT (Illumina) was used for library construction, and the sequencing was performed on HiSeq 2500 Illumina Genome Analyzer. Raw reads were analyzed through Tophat pipeline on Galaxy (https://usegalaxy.org) and subjected to KEGG-Pathway analysis (https://david.ncifcrf.gov). GSEA was performed using the hallmark gene sets from version 3.0 of the molecular signature database (MSigDB) which represent well-defined biological states or processes.

1 Han W, Gao S, Barrett D *et al.* Reactivation of androgen receptor-regulated lipid biosynthesis drives the progression of castration-resistant prostate cancer. *Oncogene* 2018; **37**:710-721.

Immunoblotting

Cells were lysed with RIPA buffer with protease inhibitors and the below antibodies were used: Anti-V5 (Abcam), anti-FOXA1 (Abcam), anti-E-Cadherin (Cell Signaling), anti-vimentin (Abcam), anti-phospho-Met (T1234/1235)

(Cell Signaling), anti-GAPDH (Abcam). Gels shown are representative of at least 3 independent experiments.

RNAi and small molecule treatments: Two siRNAs against FOXA1 3'-UTR and non-target control (NTC) were purchased from Dharmacon and transfected into cells using lipofectamine 2000 (Thermo Fisher Scientific). Cells were treated with Doxycycline (Tocris) as mentioned in the experiments.

Gel shift/Electrophoretic mobility shift assay (EMSA): PC-3 cells were transfected with pLenti6-FOXA1 vectors (FKHD-MSs or WT) and subjected to cellular fractionation according to manufacturer's protocol (Thermo Fisher Scientific). The nuclear fractions were then incubated with biotin labeled oligonucleotides at room temperature using LightShift Chemiluminescent EMSA kit from Thermo Fisher Scientific. The specific oligo sequences (identified at chr7: 55,270,913-55,270,944, based on hg19) are: Forward, 5'[Biotin]-TACATACACAC-CACACACACATACATGTATAC-3', Reverse, 5'-GTATACATGTATGTGTGTGTGGTGTGTATGTA-3'. The protein-DNA complex was run on 6% TBE gel, followed by electrophoretic transfer, crosslink, and detection by chemiluminescence, per manufacturer's protocol.

Xenograft study: 22RV1-FOXA1 xenograft was established in the flanks of castrated male SCID mice (Taconic) by injecting approximately 2 million cells in 50% Matri-gel (BD Biosciences). When tumors reach ~5mm, mice were feed with doxycycline-supplemented food (Envigo) and then received daily treatments [crizotinib (Selleck): 30mg/kg] via oral gavage. The tumor volume was measured by manual caliper. Frozen sections were examined to confirm that the samples used for RNA and protein extraction contained predominantly non-necrotic tissue.

Statistical analysis: Data in bar graphs represent mean±SD of at least 3 biological repeats. Statistical analysis was performed using Student's *t*-test by comparing FKHD-MSs versus WT FOXA1 or otherwise as indicated. p-Value<0.05 (*) was considered to be statistically significant. For the animal study, two-way ANOVA-test was performed for the tumor volume data measured across the treatments (M253K+PBS versus M253K+crizotinib).