

Title: NFIB licenses androgen receptor cistrome reprogramming during the transition from normal prostate epithelium to prostate cancer

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Background. We previously showed that NFI-family transcription factors support AR activity by enabling ARV7 binding at weak AR binding sites that lack canonical androgen response elements (AREs) during the transition to an enzalutamide-resistant state in VCaP16 cells. Clinical samples show a related pattern. NFI motifs are significantly more enriched at AR binding sites in primary prostate cancer (PCa) than in normal epithelium, and those tumor sites are correspondingly depleted of canonical AREs. We therefore hypothesized that NFI-family transcription factors also drive AR cistrome reprogramming during the transition from normal epithelium to PCa.

Methods. NFIA, NFIB, NFIC, and NFIX were individually depleted by siRNA in VCaP, VCaP16, 22Rv1, 22Rv1-E, and LNCaP cells, with immunoblot, qPCR, and RNA-seq readouts. ChIP-seq for AR, NFIB, and FOXA1 was performed on 17 clinical specimens (10 benign, 7 PCa), and for H3K27ac on 13 (6 benign, 7 PCa). Consensus peaks were partitioned into 7 exclusive occupancy classes. NFIB isoform usage was assessed by rMATS in ultra-deep RNA-seq from 274 patient biopsies (benign, localized, mCRPC; Zhang et al., Nat Cell Biol 2024) and in TCGA-PRAD.

Results. We first asked which NFI member supports AR activity across cell models. By GSEA of HALLMARK_ANDROGEN_RESPONSE, only NFIB depletion consistently decreased the signature, ranking among the most significant perturbations in every line (NES -1.72 to -2.15; FDR ≤ 0.0021). Other members were context-dependent. We therefore tested whether NFIB drives reprogramming in patients. The AR cistrome expanded ~24-fold in clinical tissue (1,346 sites in normal prostate versus 32,102 in PCa), and this gain was directly associated with NFIB binding that mirrored AR in intensity and topology. Profiling H3K27ac across the 7 occupancy classes, we found the active-enhancer mark deposited only at NFIB-occupied classes and absent at NFIB-negative classes. This pattern was reproduced in VCaP/VCaP16 and an independent cohort (Baca et al., Nat Genet 2022). This acquired chromatin binding was not explained by increased NFI expression. However, inclusion of NFIB variant chr9:14,116,180–14,116,346 rose progressively with stage in Zhang's et al. cohort (14.8% benign, 25.0% localized, 34.3% mCRPC, FDR $< 1e-9$). In TCGA-PRAD the same NFIB variant rose from 39.0% to 51.8% in localized PCa versus normal (FDR $5e-6$). Alternative explanations include NFIB post-translational modification or changes in cofactors such as FOXA1.

Conclusions. NFIB is required to sustain AR activity across PCa models. Its increased chromatin binding in primary PCa is directly associated with expanded AR occupancy and tightly linked to H3K27ac deposition. A stage-dependent switch in NFIB isoform usage is a candidate driver of this reprogramming, making NFIB an attractive therapeutic target.

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Conflicts of Interest Disclosure Statement. No conflicts of interest.