Spatial transcriptomics reveals a transition from a prostate luminal to club-like cell state in 5-alpha reductase inhibitor treated BPH patients

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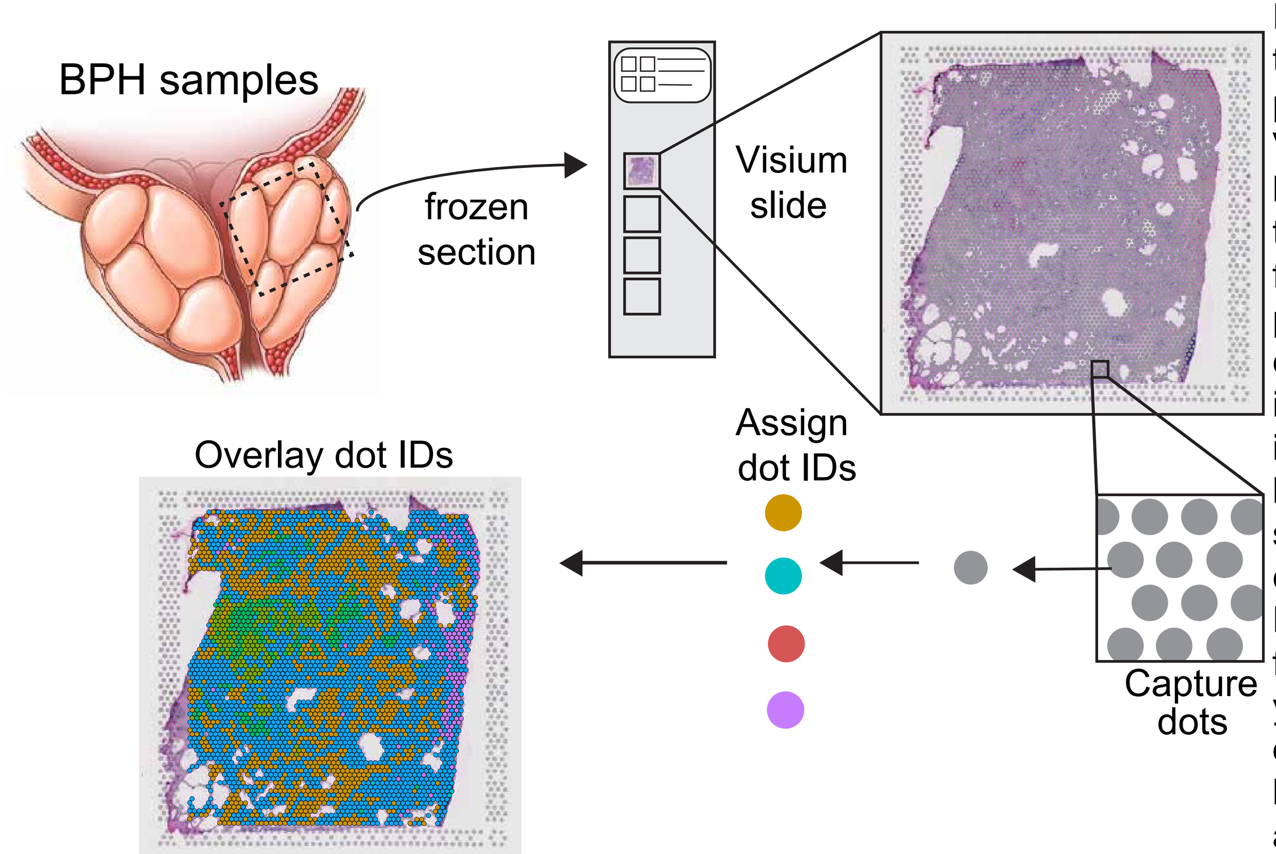
Introduction

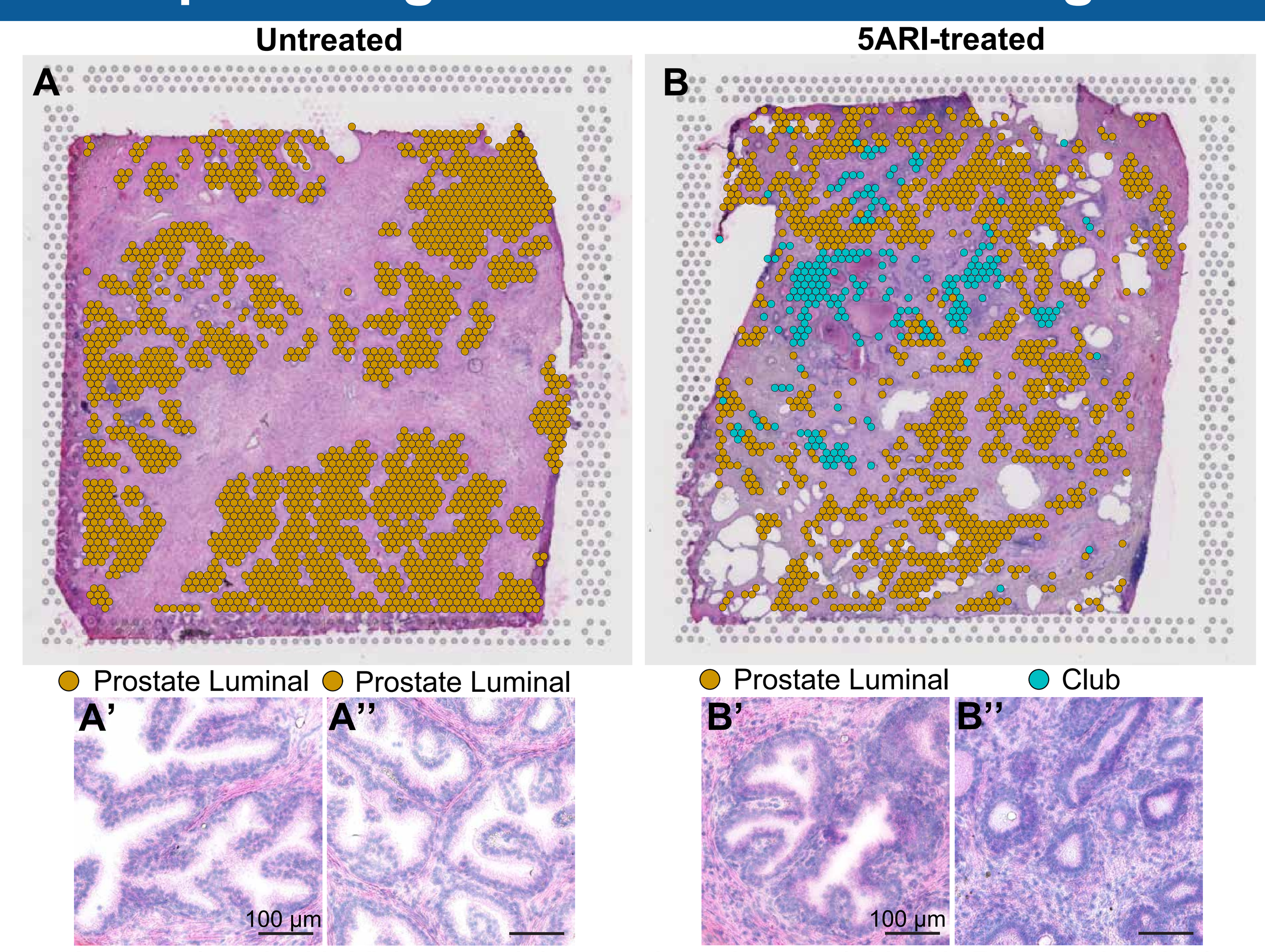
Understanding how prostate luminal cells adapt and survive in a low androgen environment is central to the clinical treatment of benign and malignant disease. 5-alpha reductase inhibitors (5ARIs) block the conversion of testosterone to dihydrotestosterone (DHT) and are used to treat Benign Prostatic Hyperplasia (BPH). 5ARI treatment reduces tissue DHT levels and causes involution of prostate glands. However, the histologic response to 5ARIs is heterogeneous and lower urinary tract symptoms in treated BPH patients often persist or worsen, leading to surgical intervention. We examined transcriptional changes accompanying 5ARI treatment to determine the cellular mechanisms of treatment resistance.

Approach



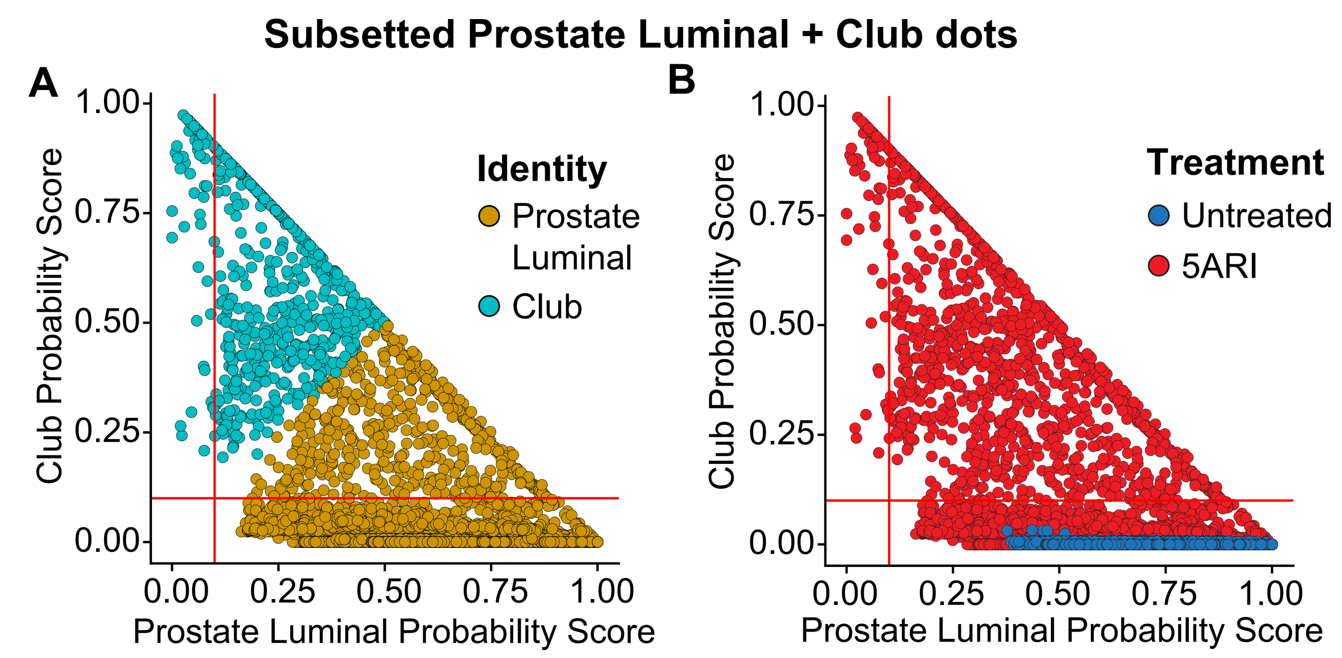
Frozen sections from untreated and 5ARI treated prostates were placed on Visium slides. The grid of barcoded capture dots on the slides binds to mRNA from the tissue. mRNA template is used to synthesise cDNA on the slide. Following the ligation of sequencing primers and indices, libraries are prepared and sequenced. Data is processed using Space Ranger from 10x genomics followed by secondary analysis using Seurat. Capture dots are assigned an ID based on their transcriptional similarity to our previous defined prostate cell types.

Atrophied regions have a club cell signature



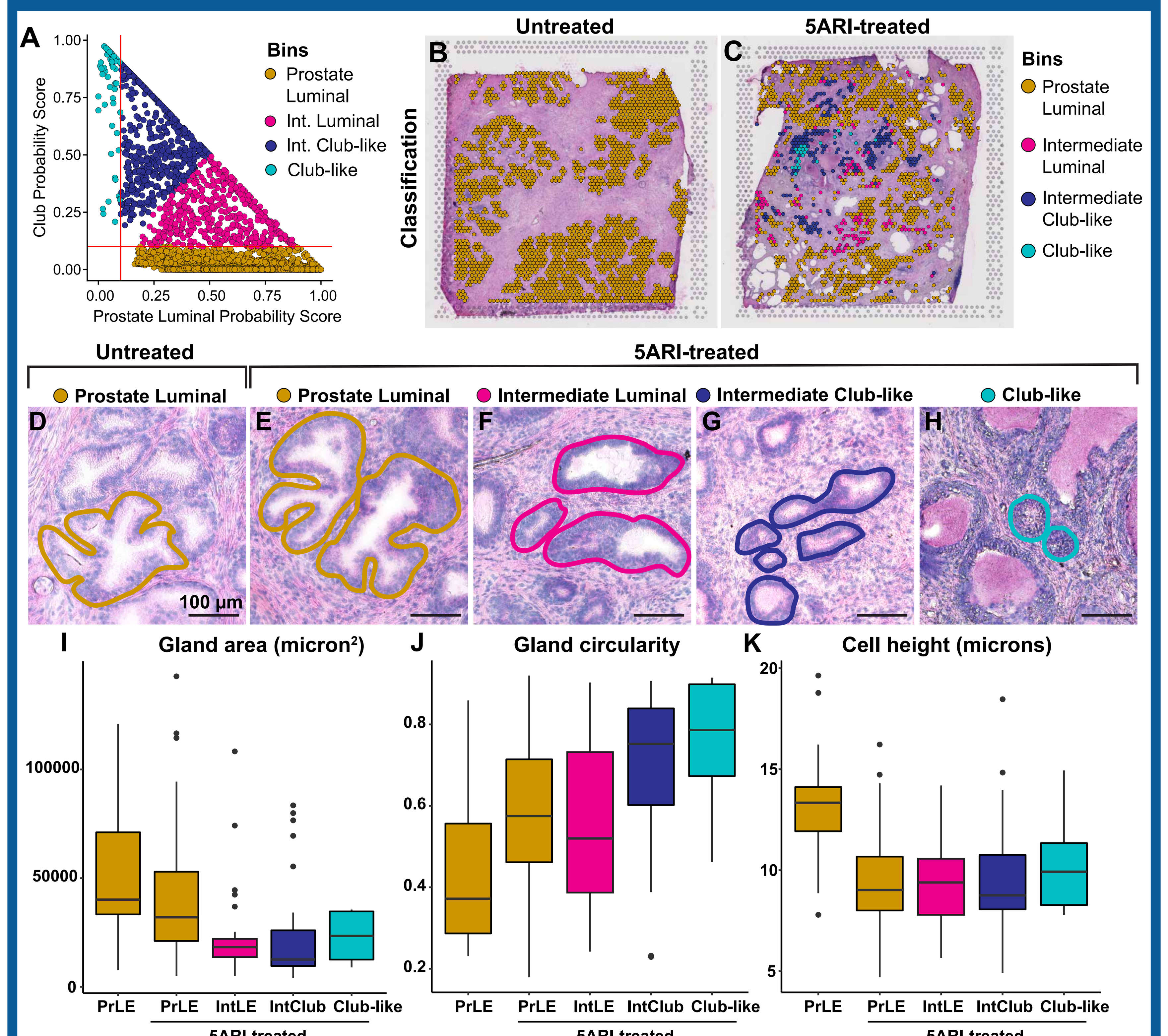
Tissue sections analyzed with Visium spatial transcriptomics overlaid with Prostate Luminal and Club cell identities. Club cells are normally found in the urethra and transition zone in the normal prostate. Club cells are absent in untreated prostates (A) but their transcriptional signature is increased in regions of 5ARI atrophy (B).

Prostate luminal to club-like adaptation



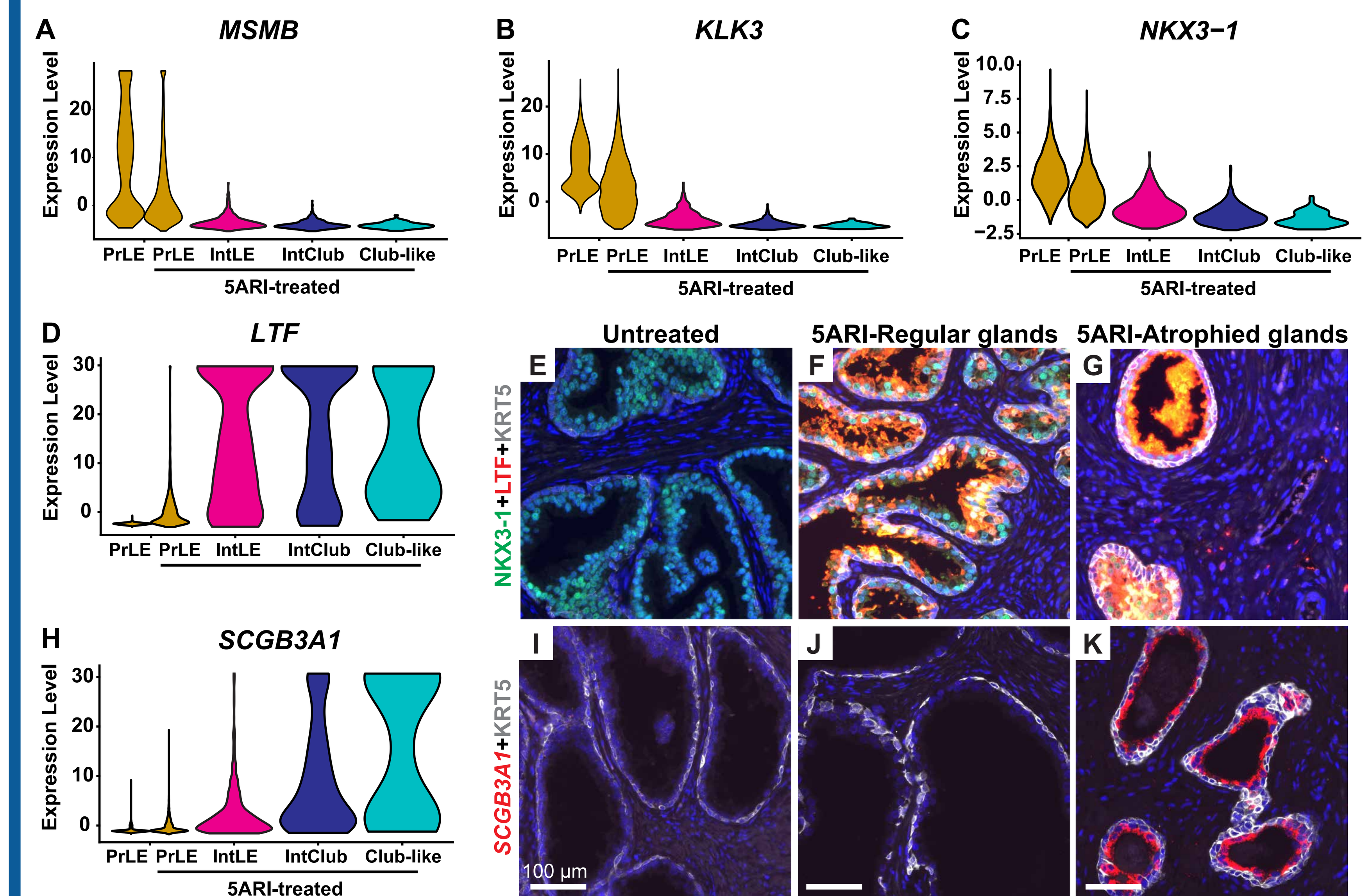
Prostate luminal epithelia undergo a transition to a club-like state in 5ARI treated prostates. Plotting dots that identify as prostate luminal and club against probability scores of being prostate luminal or club, we found that 5ARI treated samples displayed a prostate luminal to club spectrum (A). Notably, untreated samples were largely prostate luminal cells with a low club probability score (B).

Morphological changes in club-like adaptation



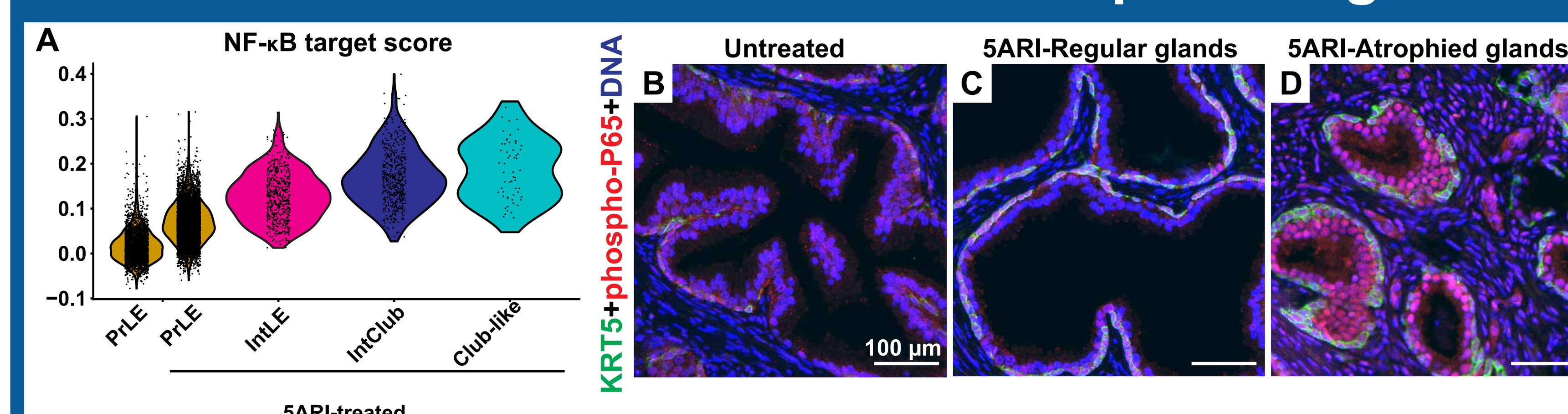
To study the transition between the prostate luminal and club cell state, we binned prostate luminal and club dots into 4 categories (A). The categories were overlaid on the tissue sections on which the Visium experiment had been performed (B-C). The glands underlying the different categories were manually outlined in ImageJ (D-H). Using in built functions in ImageJ, gland area (I) and gland circularity (J) were calculated. Gland area decreased after 5ARI treatment compared to untreated. Gland area decreased as glands transitioned from a luminal to a club-like phenotype. Similarly, circularity increased in 5ARI treated glands compared to untreated. Gland circularity was highest in club-like categories indicating a loss of epithelial infoldings. Prostate luminal cell heights (K) decreased after 5ARI treatment compared to the untreated condition.

Decrease in AR signaling and increase in club genes



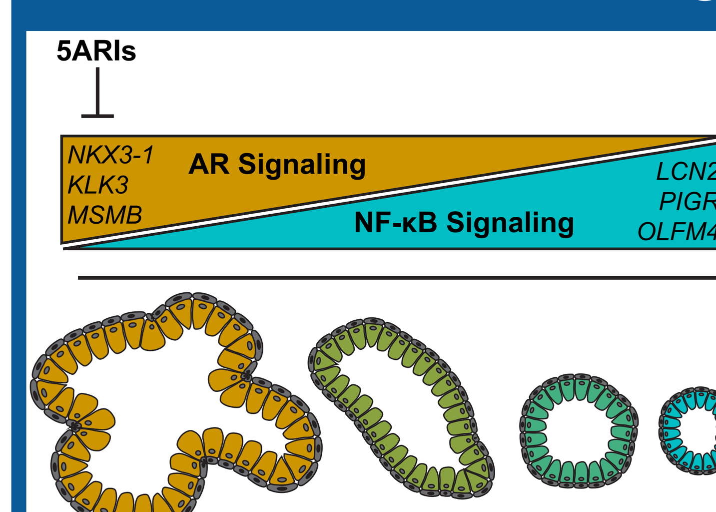
Expression of androgen regulated genes *MSMB* (A), *KLK3/PSA* (B) and the prostate specific transcription factor *NKX3-1* (C) decreased after treatment with 5ARIs. A reciprocal increase in the club cell markers LTF (D-G) and SCGB3A1 (H-K) was observed in 5ARI treated prostates compared to untreated prostates. Specifically, regions of atrophy displayed increased expression of club cell markers.

Increase in NF-κB activation in atrophied regions



A custom list of NF-κB target genes from *Pahl (1998), Oncogene* was used to compute a target score which showed increased NF-κB activation after 5ARI treatment (A). Nuclear localization of activated phospho-p65/RELA, a key NF-κB subunit was increased in regions of 5ARI induced atrophy (B-D).

Conclusions



1. 5ARI response is heterogeneous and results in localized atrophy.
2. Atrophied regions display a club-like signature and have loss of androgen receptor signaling.
3. Atrophied regions have increased NF-κB activation.
4. Epithelial cells in prostate glands undergo a survival adaptation from a prostate luminal to a club-like state in response to low androgen levels.

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