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Original Article

Distinct cell-types in the prostate share an aging signature suggestive of metabolic reprogramming

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Abstract: Age is a significant risk factor for disease of the prostate. However, the mechanisms by which age increases disease risk have not been well described. We previously reported age-related changes within the inflammatory and luminal compartments of the mouse prostate. Old mouse prostates exhibit an expansion of the population of Trop2+ luminal progenitor cells and a reduction in the frequency and functional capacity of Trop2- luminal cells, indicating that different cell-types have distinct responses to aging. Whether distinct cell-types in the prostate share a common signature of aging has not been established. We transcriptionally profiled four distinct cell-types in young adult and old mouse prostates: stromal, basal, Trop2+ luminal progenitor and Trop2- luminal cells. Motif analysis of genes upregulated in old prostate cell-types pointed to transcriptional regulators of inflammatory and hypoxia-related signaling. Glutathione metabolism and the antioxidant response emerged as a common signature of aging across prostatic lineages. Expression of genes implicated in mouse prostate aging, including the antioxidant response gene *Hmox1*, correlates with age of diagnosis in primary prostate tumors from the TCGA cohort. These findings reveal a common signature shared by distinct cell-types in the old prostate reflective of age-associated metabolic reprogramming.

Keywords: Prostate, stromal, basal, luminal, metabolism, inflammation, hypoxia

Introduction

Aging in the human prostate is strongly associated with histological changes including tissue enlargement, or benign prostatic hyperplasia (BPH), which occurs in 70% of men in their 70's [1]. Risk of prostate cancer also increases with age [2], and histological evidence of malignancy in the prostate is observed in 50% of men in their 70's [3]. As the percentage of men over 65 increases, understanding the fundamental changes that occur with age in the prostate will be essential to combat disease risk. However, the mechanisms by which age increases risk of BPH and prostate cancer is still poorly understood. Studies in rodents have begun to shed light on fundamental mechanisms of aging in the prostate.

The prostate is a cellular heterogeneous organ, with two epithelial layers consisting of basal and luminal cells, stromal cells, and an inflammatory component [4]. We and others have demonstrated a significant increase in prostate-infiltrating inflammatory cells in old mouse prostates, with a notable expansion in T and B-lymphocytes [5, 6]. In a recent study, we demonstrated a fundamental age-related shift within the luminal compartment, identifying Trop2+ luminal progenitor cells that are rare in young mouse prostate and expand in old prostates [5]. The age-related increase in inflammatory cells and luminal progenitor cells is observed not only in mouse prostate but also in non-cancerous human prostate, indicating that discoveries of prostatic aging in rodents can inform mechanisms of human prostate aging.

In several tissue systems, aging varies between cell-types. For example, an age-related increased frequency of megakaryocyte/erythrocyte progenitor cells and decreased frequency of common lymphoid progenitors indicates that not all cells in the blood system age equivalently [7]. This is true in the prostate, where basal cells and Trop2+ luminal cells maintain their organoid-forming activity with age, while Trop2- luminal cell-initiated organoid-forming capacity diminishes with age [5]. Despite functional differences between cell-types, we hypothesized that we might gain insight into prostatic aging by comparing age-related changes in gene expression profiles across distinct cell-types.

Inflammation is closely linked with tissue hypoxia, or low oxygen conditions, and oxidative stress [8]. Interestingly, hypoxia is associated with aging in the prostate [9], and increased glutathione, which protects cells against oxidative stress through scavenging of reactive oxygen species [10], has been observed in the old rat prostate [11]. Microdissection of stromal cells adjacent to prostatic glands in young and old mice revealed increased expression of inflammatory and oxidative stress-related genes [6], which may be caused by the inflammatory microenvironment in the aging prostate. We reasoned that shared signatures of aging across multiple distinct cell-types may reflect cellular responses to the local microenvironment.

Here we performed RNA-sequencing of stromal cells, basal cells, Trop2+ luminal progenitor cells and Trop2- luminal cells from 3-month-old and 24-month-old mouse prostates and evaluated age-related changes in gene expression profiles. Common signatures of aging were associated with inflammation and glutathione metabolism. Larger changes in metabolic gene expression were observed including alterations in genes that regulate glucose, lactate, glutamine, proline and lipid metabolism in old epithelial cell-types. Shared features of mouse prostate aging were associated with age of diagnosis in the TCGA primary prostate cancer cohort. These findings suggest that distinct prostate cell-types with distinct functional capacities share a common aging signature indicative of metabolic reprogramming as a response to the microenvironment.

Materials and methods

Animals

C57BL/6 mice were purchased from Jackson Laboratories and housed and maintained at UCLA by the Division of Laboratory Animal Medicine (DLAM) following approved protocols for animal care.

Mouse prostate dissociation and cell sorting

Prostates were collected from C57BL/6 mice aged to 3 or 24 months, dissociated to single cells, and stained with antibodies for Fluorescence Activated Cell Sorting as previously described [5, 12]. Stromal cells (Lin- EpCAM- CD49f-), basal cells (Lin- EpCAM+ CD49f^{hi}), Trop2+ luminal (Lin- EpCAM+ CD49f^{mid} Trop2+) and Trop2- luminal (Lin- EpCAM+ CD49f^{mid} Trop2-) were isolated on a FACSAria II cell sorter (BD Biosciences) as previously described [5].

RNA sequencing and analysis

RNA extraction from cells was carried out using the RNeasy Mini Kit (QIAGEN) following manufacturer's instructions. RNA-seq Libraries were prepared using KAPA Stranded mRNA-Seq Kit (Roche). The workflow was comprised of mRNA enrichment, cDNA generation, and end repair to generate blunt ends, A-tailing, adaptor ligation and PCR amplification. Different adaptors were used to multiplex samples in a single lane. Sequencing was performed on the Illumina HiSeq 3000 for 1x50 run. Data analysis was carried out as previously described [5] and sequencing data have been deposited in NCBI's Gene Expression Omnibus and can be accessed through GEO Series accession number GSE122367 and GSE128724. Principal component analysis was determined using iDEP [13] (http://bioinformatics.sdstate.edu/idep/). Gene set enrichment analysis (GSEA) was performed as described previously [14, 15] using GSEA 4.0.3 software and MSigDB hallmark gene sets. Motif analysis was performed using i-cisTarget [16, 17] (https://gbiomed.kuleuven.be/apps/lcb/i-cisTarget/). KEGG pathway and Gene Ontology analysis was performed using DAVID Bioinformatics [18, 19] (https://david.ncifcrf.gov/). TCGA primary prostate cancer data [20, 21] was accessed using the cBioPortal [22, 23] (https://www.cbioportal.org/). Tumors were stratified

based on age of diagnosis, and the bottom and top quartiles were compared for analysis of relative gene expression.

Results

Prostate stromal cells exhibit age-related transcriptional changes

In our previous analysis of aging prostates, we found an increase in the number of non-epithelial cells in 24-month-old prostates compared to 3-month-old young adult prostates, whereas the total number of basal and luminal cells did not significantly change with age [5]. Not only did we find a statistically significant increase in the number of Lin- EpCAM- CD49f- stromal cells in old mouse prostates, but we also found a significant decrease in stromal cell forward scatter, a measurement of Fluorescence Activated Cell Sorting (FACS) indicative of cell size. These findings suggest that aging is associated with multiple changes in the prostate stromal compartment. Here we set out to transcriptionally profile stromal cells from young adult and old mouse prostates using RNA-sequencing (RNA-seq) to gain further insight into age-related changes within the stromal compartment.

When we looked for genes that were increased or decreased at least 1.5-fold with a p-value < 0.05, we identified 1520 genes that changed significantly with age within the stromal compartment of the mouse prostate (**Figure 1A**). Out of 1520 genes, 1020 were elevated in old prostates, whereas only 500 were significantly downregulated with age. Gene set enrichment analysis showed a significant enrichment for hallmarks of inflammatory response and interferon signaling (**Figure 1B**). We performed gene ontology (GO) and pathway analysis (**Figures 1C**, **1D**, S1A, S1B) on significantly up- or downregulated genes. Immune-related GO terms and pathways were well represented in the aging signature (**Figures 1C**, S1A, S1B), consistent with age-related inflammation as we and others have previously described [5, 6]. We noted "response to drug" and "drug metabolism" as enriched pathways in old stromal cells (**Figures 1C**, S1A, S1B). In contrast, extracellular matrix (ECM) and adhesion-related terms and pathways were enriched in genes downregulated with age (**Figures 1D**, S1A, S1B), consistent with previous reports of reduced ECM stiffness in the aging prostate microenvironment [6, 24]. The most differentially expressed genes reflected these major pathways, including *Stc1*, an antioxidant response gene and inhibitor of inflammation [25], which was significantly elevated in old stromal cells (**Figure 1E**). The elastin gene (*Eln*), which encodes a protein critical for ECM elastic fibers [26], is the most significantly reduced gene in the old stromal compartment (**Figure 1E**).

We performed motif analysis on the 1020 genes elevated in old prostate stromal cells to identify candidate transcriptional regulators of the aging stromal signature. Motif analysis yielded the transcription factors Arnt, Irf3 and Egr2 (**Figure 1F**), suggesting that these transcription factors, or closely related family members with similar binding patterns, may be involved in promoting the aging stromal signature. We observed elevated mRNA expression of *Arnt2*, *Irf4*, *Egr1 and Egr2* in old prostate stromal cells (**Figures 1F**, S1C). Arnt2 forms a complex with Hif1a under conditions of low oxygen and activates hypoxia-regulated genes [27]. Irf4, Egr1 and Egr2 are associated with inflammation and immune function [28-30], while Egr family members may also be regulated by hypoxia [31]. These findings suggest that the aging stromal signature is likely to be influenced by the inflammatory microenvironment.

Prostate basal cells exhibit age-related transcriptional changes

We next looked into age-related changes in prostate basal cells, which sit adjacent to stromal cells in the gland. We previously demonstrated that the number and functional capacity of basal cells does not change significantly with age [5]. Using transcriptional profiling, we determined that 855 genes exhibited at least 1.5-fold differential gene expression between 3- and 24-month-old prostate basal cells. Similar numbers of genes were up (432) and downregulated (423) with age in the basal cell compartment (**Figure 2A**). Motif analysis on the 432 genes increased in old basal cells revealed enrichment for binding sites of Ets factors, Cebpb, and Egr1 (**Figure 2B**). Increased expression of *Egr1*, *Egr2* and *Elf3* in old basal cells (**Figures 2B**, **2C**, S2A) pointed to a potential role for these transcription factors in the aging basal cell signature.

Pathway analysis revealed enrichment for drug metabolism and glutathione metabolism in

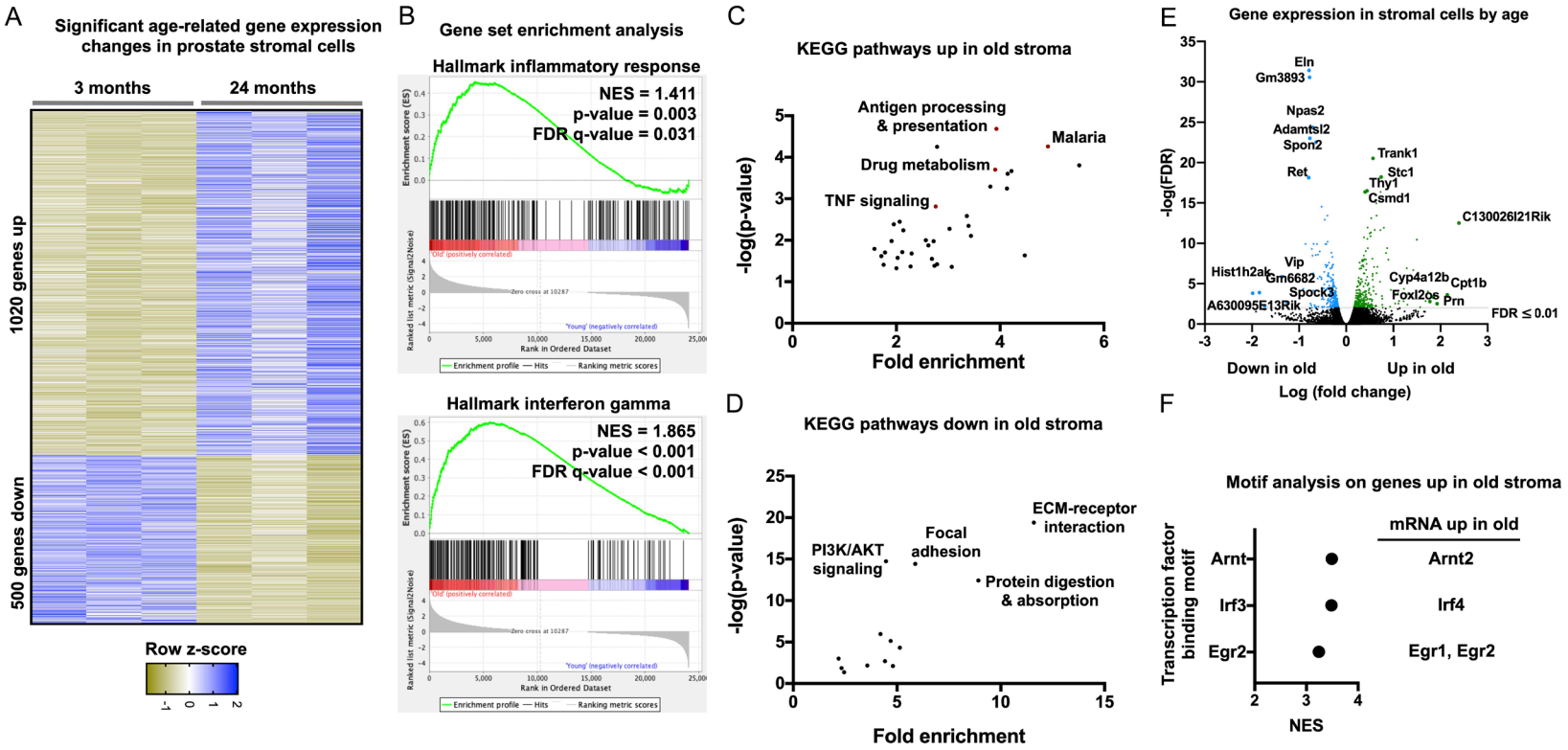

Figure 1. Age-related transcriptional changes in prostate stromal cells. (A) Heatmap of significantly differentially expressed genes in 3- and 24-month-old prostate stromal cells. Only genes greater than 1.5-fold enriched in either direction with a *p*-value < 0.05 are included. (B) Gene set enrichment analysis indicates significant enrichment for inflammatory response and interferon gamma hallmarks in the old prostate stromal signature. NES: normalized enrichment score. (C, D) KEGG pathway analysis reveals significantly enriched pathways in 24-month-old (C) or 3-month-old (D) stromal cell signatures. (E) Volcano plot reveals significantly altered genes based on -log (FDR) and log (fold change). FDR: false discovery rate. (F) Motif analysis reveals transcription factors with binding motif enrichment in the old prostate stromal signature. NES: normalized enrichment score.

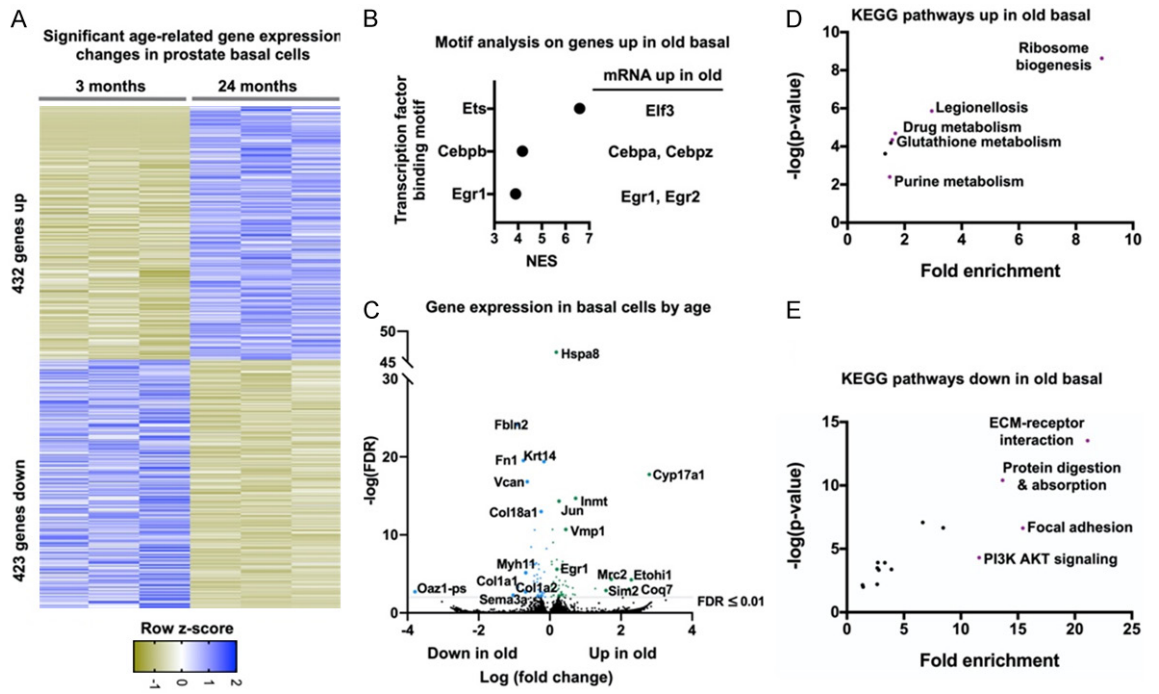

Figure 2. Age-related transcriptional changes in prostate basal cells. (A) Heatmap of significantly differentially expressed genes in 3- and 24-month-old prostate basal cells showing genes greater than 1.5-fold enriched in either direction with a *p*-value < 0.05. (B) Motif analysis reveals potential transcriptional regulators of the old prostate basal cell signature. NES: normalized enrichment score. (C) Volcano plot reveals significantly altered genes based on -log (FDR) and log (fold change). FDR: false discovery rate. (D, E) KEGG pathway analysis reveals significantly enriched pathways in 24-month-old (D) or 3-month-old (E) basal cell signatures.

aging basal cells (**Figures 2D**, S2B, S2C). Pathways downregulated in old basal cells included ECM interactions and adhesion (**Figures 2E**, S2B, S2C), with genes encoding ECM proteins including *Col1a1*, *Col1a2*, *Col18a1* and *Fn1* among the most significantly down with age (**Figure 2C**). Based on similarities in age-related pathway enrichment and potential transcriptional regulators in old stromal and basal cells, we set out to evaluate age-related signatures of additional cell subsets in the old mouse prostate.

Trop2+ luminal progenitor cells and Trop2- luminal cells share aging signatures

In our previous work, we identified Trop2 as a marker of luminal progenitor cells that generate large organoids and expand with age in the mouse prostate [5]. While Trop2+ luminal progenitor cells maintain their organoid-forming capacity with age, old Trop2- luminal cells exhibit reduced functional activity and reduced expression of cell cycle-related genes. We asked whether these two distinct luminal subsets with distinct functional responses to aging share common signatures of aging. Principal component analysis distinguished luminal subsets based on cell-type (PCA #1: Trop2+ vs Trop2-) and age (PCA #2: 3 months vs 24 months), suggesting that both luminal subsets share an aging signature (**Figure 3A**).

We evaluated genes that changed significantly with age in Trop2+ and Trop2- luminal cells (**Figure 3B**, **3C**) and performed GO analysis on significantly up and down-regulated genes (**Figures 3D**, **3E**, S3A-D). Both luminal populations in old prostates exhibited enrichment for terms associated with the immune response (**Figure 3D**, **3E**), consistent with increased inflammation in old prostates [5, 6]. GO analysis also revealed enrichment for glutathione metabolism and response to hypoxia in old luminal cells (**Figure 3A**, **3B**). ER unfolded protein response was among the common GO terms enriched for genes downregulated with age in the two luminal subsets (Figure S3C, S3D). Motif analysis of significantly upregulated genes in old Trop2+ and Trop2- luminal cells

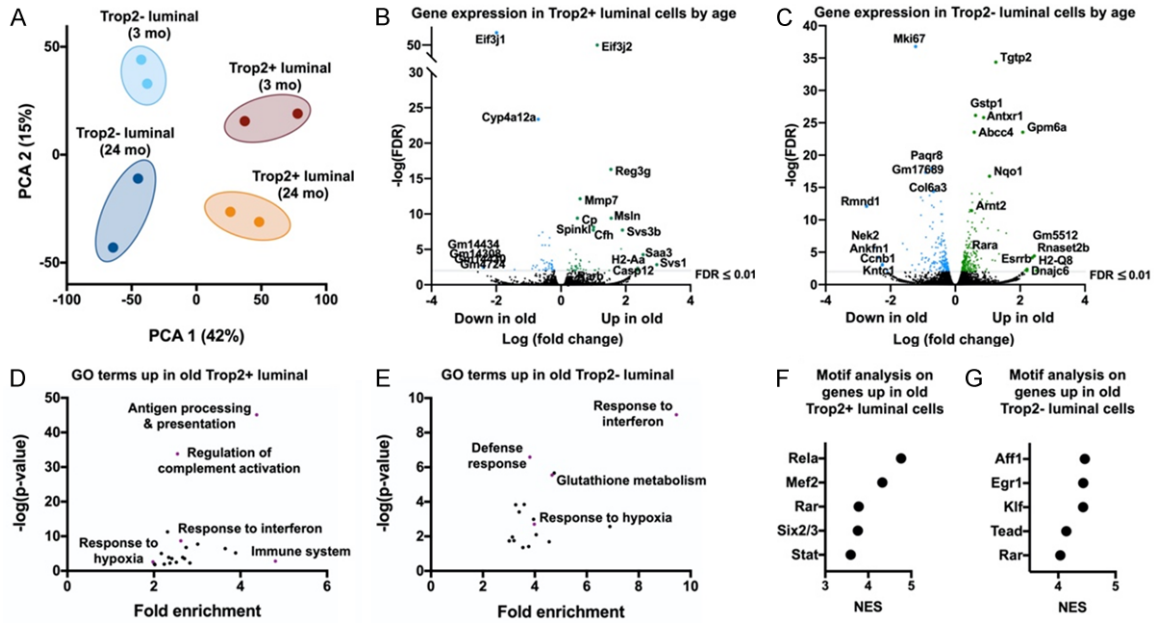

Figure 3. Age-related transcriptional changes in distinct luminal cell subsets. (A) Principal component analysis of gene signatures of Trop2+ and Trop2- luminal subsets from 3 and 24-month-old mouse prostates. (B, C) Volcano plots reveal significantly altered genes based on -log (FDR) and log (fold change) in Trop2+ (B) and Trop2- (C) luminal cells. FDR: false discovery rate. (D, E) Gene Ontology (GO) terms enriched in old Trop2+ (D) and Trop2- (E) luminal cell signatures. (F, G) Motif analysis reveals potential transcriptional regulators of the old Trop2+ (F) and Trop2- (G) luminal cell signatures. NES: normalized enrichment score.

revealed immune-related transcription factors and retinoic acid receptors (**Figure 3F**, **3G**). Elevated mRNA expression of retinoic acid receptor family members (*Rara*, *Rarb, Rarg)*, and the previously described gene *Arnt2*, was also observed in old luminal cells (**Figures 3B**, **3C**, S3E, S3F). Retinoic acid receptors are upregulated in response to all-trans retinoic acid (ATRA) and have been associated with inflammation [32, 33] as well as protection from oxidative stress caused by hypoxia [34, 35]. These findings suggest that distinct luminal subsets share common features of aging, despite differences in age-related functional capacity.

Glutathione metabolism is a common feature of aging across distinct epithelial cell-types

We compared the genes significantly upregulated in old basal cells, old Trop2+ luminal cells, and old Trop2- luminal cells to identify common age-related changes (**Figures 4A**, S4A). Interestingly, 45% (217/484) of the genes upregulated in old Trop2+ luminal cells were shared with old basal cells, old Trop2- luminal cells, or with both. In contrast, 23% (201/888) of genes upregulated in old Trop2- luminal cells, and 23% (101/432) of genes upregulated in old basal cells were shared by other old epithelial cell-types. A total of 11 genes were found to be significantly upregulated in all three epithelial subsets (**Figure 4A**). This list included *Hmox1*, an antioxidant response gene, and *Gclc*, a regulator of glutathione synthesis (Figure S4A). Motif analysis of these common genes revealed a significant enrichment for the Mef2 family of transcription factors (Figure S4B). Mef2d has been shown to activate Nrf2 [36], a master regulator of the antioxidant response [37].

We evaluated age-related mRNA expression of metabolic genes and found consistently increased expression of many genes involved in glutathione metabolism in old epithelial cell-types (**Figure 4B-D**). We also observed increased expression of the glycolytic gene *Pkm*, the lactate transporter *Slc16a1* (which encodes Mct1), and *Pdk4 (*pyruvate dehydrogenase kinase 4) in old basal and Trop2+ luminal progenitor cells. Old Trop2+ and Trop2- luminal cells exhibited increased expression of the glycolytic gene *Pfkp* and lactate dehydrogenase b (*Ldhb*) (**Figure 4B-D**). Metabolic enzymes

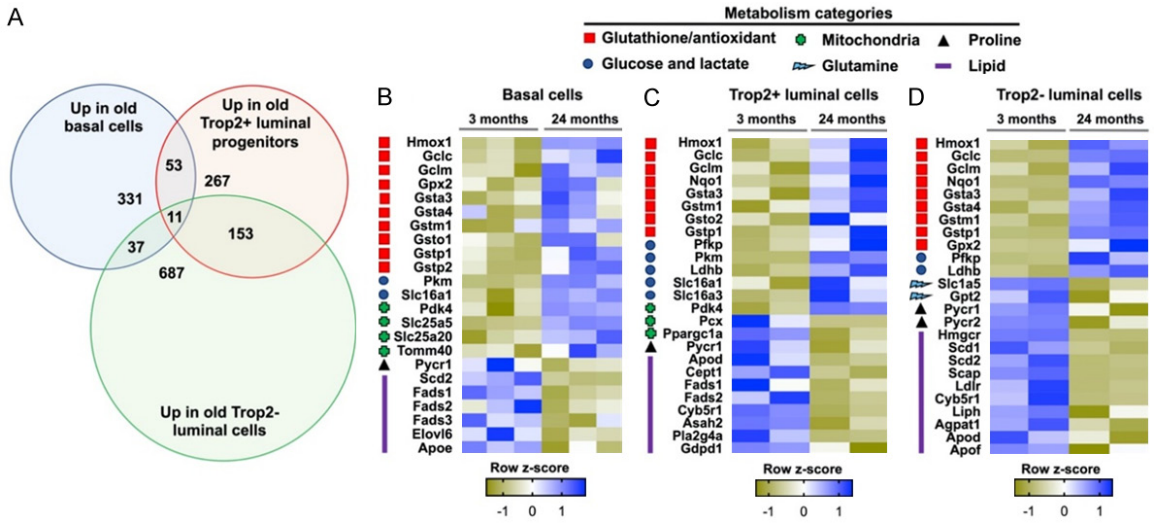

Figure 4. Shared aging signatures and metabolic genes across three distinct epithelial cell subsets. (A) Venn diagram comparing genes that are significantly upregulated in old epithelial cells. (B-D) Heatmaps reveal metabolic genes that are differentially expressed between 3- and 24-month-old basal cells (B), Trop2+ luminal cells (C) and Trop2- luminal cells (D).

involved in proline metabolism and lipid metabolism were consistently reduced in old epithelial cells (**Figure 4B-D**), consistent with GO analysis (Figure S3C). These results suggest that aging in prostate epithelial cells is likely associated with metabolic reprogramming beyond glutathione metabolism.

In contrast to old Trop2- luminal cells which exhibit reduced functional activity, both basal and Trop2+ luminal cells maintain their progenitor activity with age [5]. We further evaluated genes elevated in old basal and Trop2+ luminal cells to identify potential factors regulating the age-related maintenance of progenitor activity. Motif analysis revealed enrichment for Rela/Nfkb1 and Sox2 specifically for genes shared by basal and Trop2+ luminal cells, and these transcription factors were not significantly enriched in any other group (Figure S4B). Sox2 is an important regulator of self-renewal and stemness in many tissues [38, 39] and also plays a role in prostate cancer progression and antiandrogen-resistance [40, 41]. Importantly, *Sox2* and *Nfkb1* were both elevated in basal cells and in Trop2+ luminal cells at the mRNA level (Figure S4C, S4D).

Hmox1 and genes associated with aging in the mouse prostate correlate with age in human primary prostate cancer

We wondered whether transcriptional features of aging shared by distinct cell-types in the mouse prostate may also be observed in human prostate cancer. The Cancer Genome Atlas (TCGA) primary prostate cancer cohort contains nearly 500 cases of prostate cancer [20, 21], with age of diagnosis distributed over 4 decades (41 to 78 years). The antioxidant response gene [42] and Nrf2 target [43] *Hmox1* was correlated with age of diagnosis in a statistically significant manner (Figure S5). We evaluated mRNA expression of candidate genes in the lowest (41-56 years, 123 tumors) and highest (66-78 years, 124 tumors) quartiles from the TCGA cohort. In addition to *Hmox1*, we also observed increased mRNA expression of candidate regulators of the mouse prostate aging signature including *Rara*, *Egr2* and several interferon regulatory factors (*Irf1*, *Irf3*, *Irf4*, *Irf5*) in the upper quartile (**Figure 5A**). In contrast, lipid metabolism genes that are reduced in old mouse prostate cells (*Hmgcr*, *Scd*), and a regulator of the lipogenic program (*Srebf2*) [44], were also reduced in the upper quartile (**Figure 5A**). We evaluated the correlation between *Hmox1* mRNA and several age-related genes in the TCGA primary prostate cancer cohort and found positive correlations with *Egr2*, *Rara* and *Irf4* and negative correlations with *Hmgcr* and *Srebf2* (**Figure 5B**). These findings suggest an age-related signature in human primary prostate cancer associated with increased antioxidant response and inflammatory response genes and reduced lipid metabolism genes.

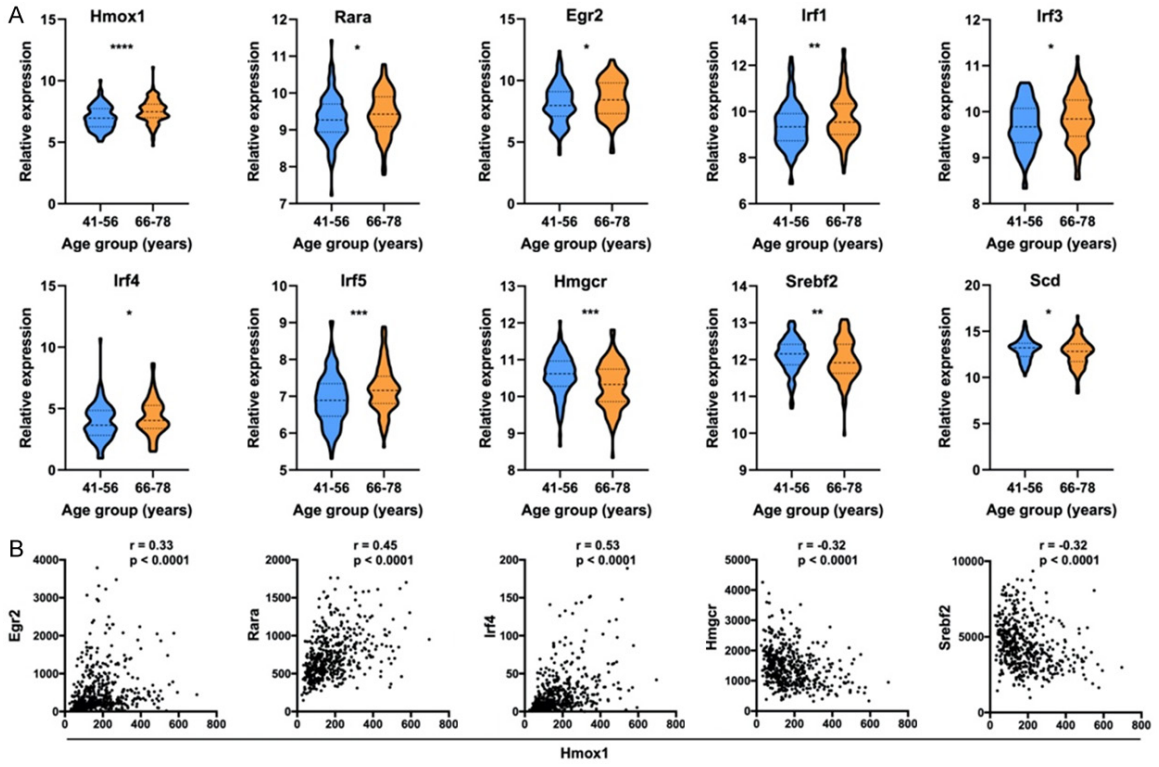

Figure 5. Genes associated with aging mouse prostate correlate with age in primary prostate cancer. A. Violin plots of mRNA expression in TCGA cohort of primary prostate cancer comparing the bottom and top quartiles by age of diagnosis. * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001. B. Plots revealing relative mRNA expression of Hmox1 with Egr2, Rar1, Irf4, Hmgcr and Srebf2 in TCGA primary prostate cancer specimens. Spearman correlation coefficients and *p*-values are shown. Note: 5-11 points with very high expression lie outside of the range of plots in order to better demonstrate correlations in B.

Discussion

Aging in the prostate is associated with an increase in inflammatory cell infiltration, most notably T and B lymphocytes which are relatively rare in the young adult prostate [5, 6]. How the old inflammatory microenvironment influences normal prostate stromal and epithelial cells has not been well described. We evaluated age-related changes to gene expression profiles in four distinct cell-types of the mouse prostate and identified a shared signature of aging. Stromal cells, basal cells, Trop2+ luminal progenitor cells, and Trop2- luminal cells exhibited age-related increases in inflammatory/immune-related and antioxidant/glutathione metabolism-related signatures. These results suggest that prostate stromal and epithelial cells are likely responding to a hypoxic inflammatory environment by increasing the antioxidant program. Some of the candidate transcriptional regulators of prostate aging signatures in multiple cell-types, including Arnt2, Egr1/2, Rara/b, may contribute to protecting old prostate cells from oxidative stress associated with an inflammatory and hypoxic environment. Future studies will be necessary to functionally evaluate the role of various transcriptional and metabolic regulators of prostate aging.

We previously reported age-related changes to the stromal compartment [5], based on an increase in the total number of stromal cells and a decrease in the forward scatter, a surrogate for cell size, in old mouse prostates. We now show significant age-related changes to the transcriptional profiles of isolated stromal cells. Transcriptional changes to the stromal compartment with age are consistent with a previous study that used laser capture microdissection to collect RNA from non-epithelial regions of the young and old mouse prostate [6]. The stromal subset used in our study is likely heterogeneous, containing fibroblasts and smooth muscle cells. One of the most sig-

nificant upregulated genes in old stromal cells is *Thy1* (CD90) (**Figure 1E**), which has recently been shown to mark a distinct subset of prostate stromal cells [45]. It is possible that a change in the proportion of subsets within the stromal compartment contributed to the age-related transcriptional changes observed here, similar to our previous report that age-related changes in the bulk luminal signature reflected an age-related increase in the proportion of Trop2+ luminal cells. By isolating out these distinct subsets and comparing their profiles from young and old mouse prostates, we can further understand signatures of aging.

In addition to increased glutathione metabolism in old prostate cells, we observed age-related changes in mRNA expression of metabolic genes encoding enzymes and transporters involved in glucose, lactate, glutamine and lipid metabolism. Age-related changes to the microenvironment, including inflammatory cytokines, hypoxia and nutrient availability, are capable of altering metabolic gene expression. Collectively, these results suggest a role for metabolic reprogramming in prostatic aging. Interestingly, gene expression patterns of aging in the mouse prostate were observed in primary prostate cancers from the TCGA cohort, with increased mRNA expression of antioxidant and inflammatory response genes and decreased expression of lipid metabolism genes in the oldest quartile by age. Age-related changes to the human prostate microenvironment may influence gene expression patterns of prostate cancer. Hypoxia is associated with increased genomic instability, mutations in p53, loss of Pten, and telomere shortening in localized prostate cancer [46]. This process is believed to occur through selective pressure on the tumor, whereby survival clones emerge when normal oxygen levels are restored. Whether hypoxia similarly exerts selective pressure on normal cells in the aging prostate has not been determined. Differential response to a hypoxic environment may provide one explanation for the increase in the proportion of Trop2+ luminal progenitor cells and the decrease in Trop2- luminal cells in the old mouse prostate.

In this study, we focused on age-related gene signatures shared by distinct cell-types, including basal and Trop2+ luminal cells that maintain progenitor activity with age as well as Trop2- luminal cells that exhibit functional decline with age. We reasoned that these analyses may also elucidate factors that enable basal and Trop2+ luminal cells to retain their functional capacity. Motif analysis of genes upregulated with age in basal and Trop2+ luminal cells revealed Sox2 and Nfkb1. Importantly, both *Sox2* and *Nfkb1* are expressed at higher levels (mRNA) in the populations that maintain progenitor activity with age as compared to Trop2- luminal cells. Whether these transcription factors contribute to the age-related maintenance of progenitor activity will need to be experimentally determined. Both basal and luminal progenitor cells are capable of responding to genetic alterations to initiate prostate cancer [47-51]. Therefore, understanding how they maintain their functional activity with age may yield new strategies to prevent prostate cancer initiation. In summary, we offer age-related signatures of distinct stromal and epithelial cell-types as a resource to gain new insight into mechanisms of aging and increased risk of disease in the prostate.

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Disclosure of conflict of interest

None.

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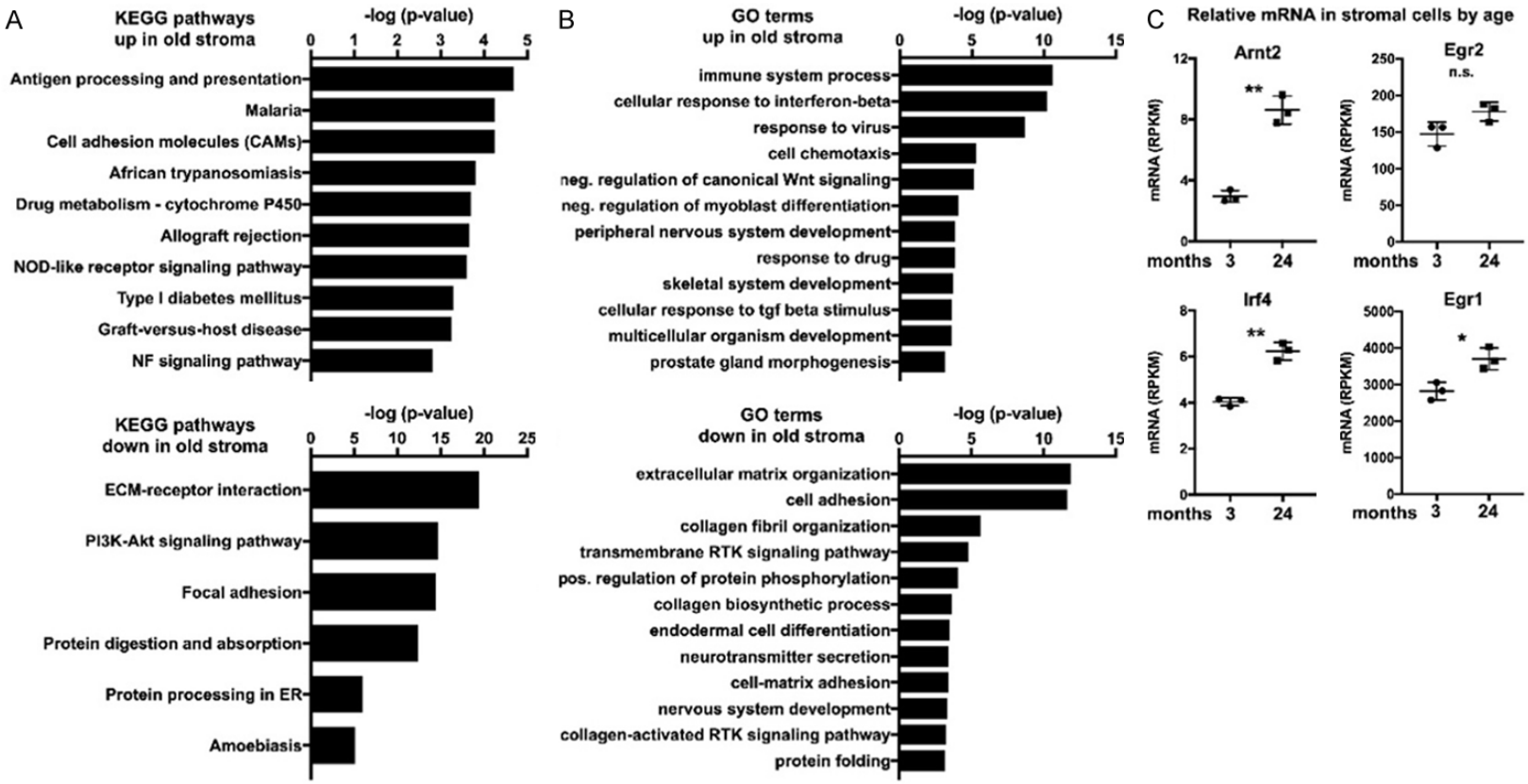

Figure S1. Related to **Figure 1**. A, B. KEGG pathways and Gene Ontology (GO) terms for genes significantly up or downregulated in old stromal cells. C. Relative mRNA expression (RPKM: reads per kilobase of transcript, per million mapped reads) in stromal cells from 3- and 24-month-old mouse prostate. * $P < 0.05$, ** $P < 0.01$, n.s. not significant.

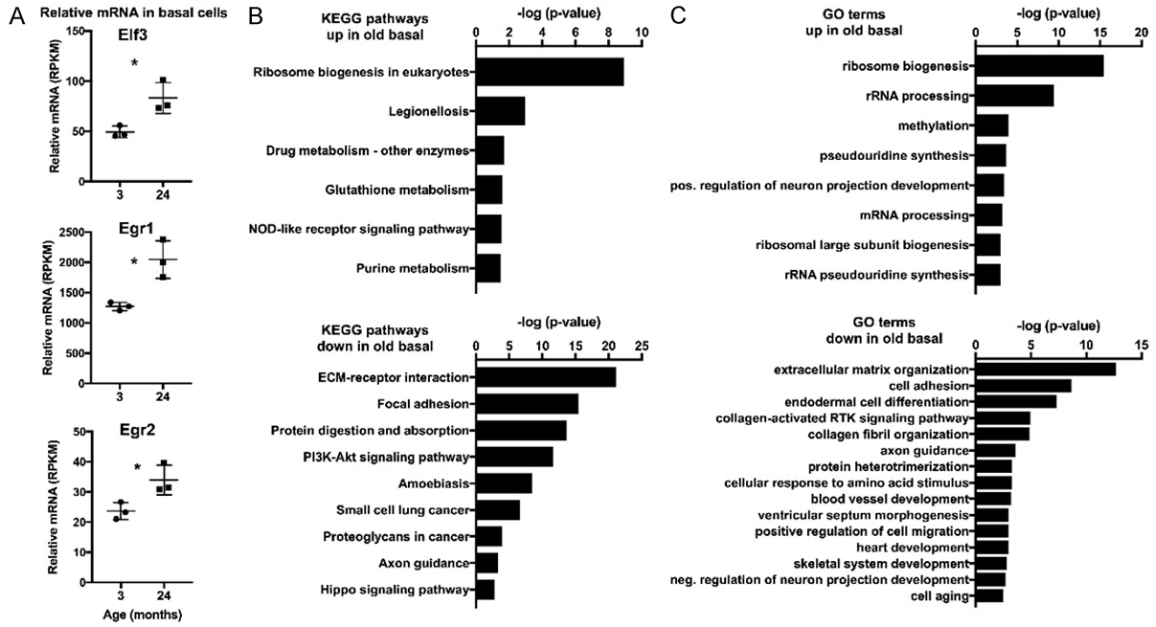

Figure S2. Related to **Figure 2**. A. Relative mRNA expression (RPKM: reads per kilobase of transcript, per million mapped reads) in basal cells from 3- and 24-month-old mouse prostate. * P < 0.05. B, C. KEGG pathways and Gene Ontology (GO) terms for genes significantly up or downregulated in old basal cells.

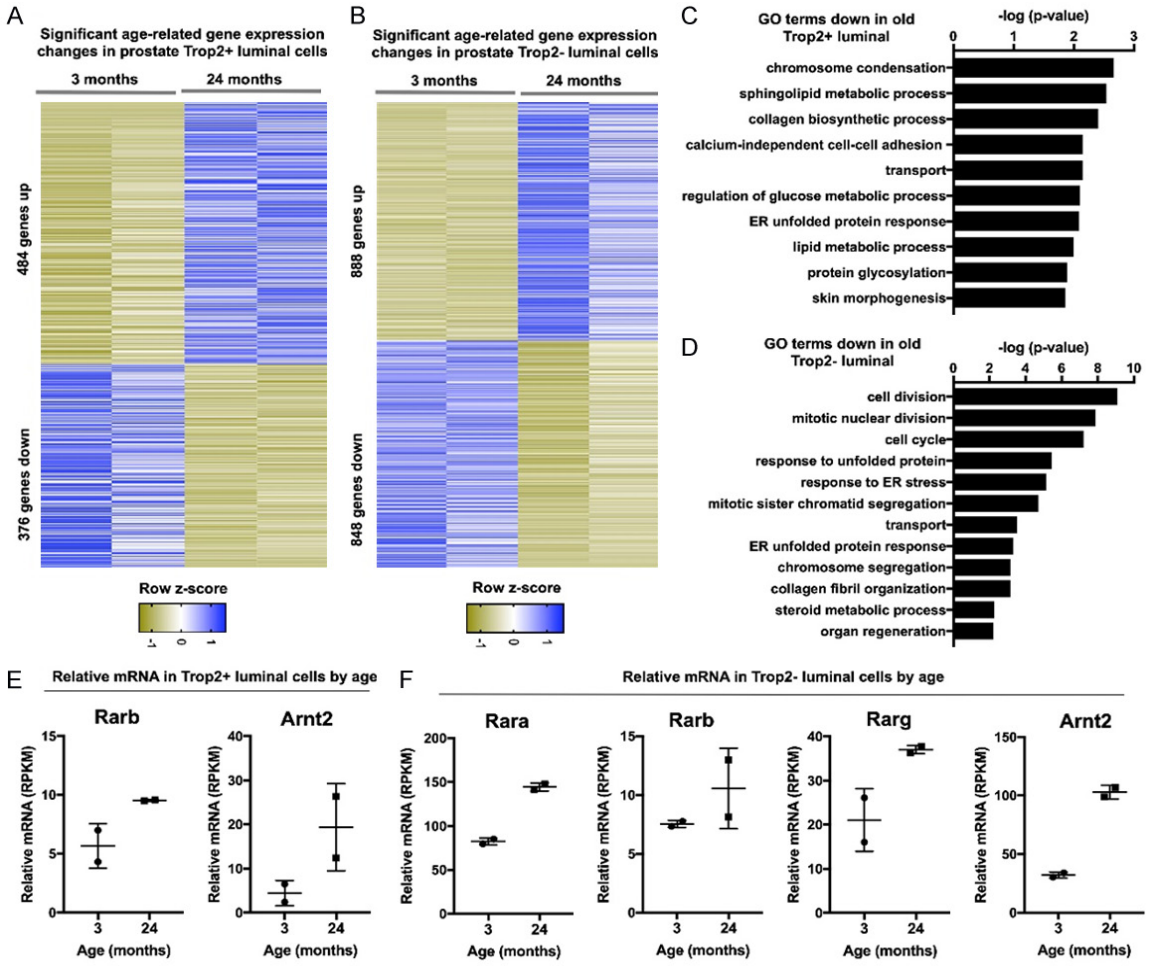

Figure S3. Related to **Figure 3**. (A, B) Heatmaps of significantly differentially expressed genes in 3- and 24-month-old Trop2+ luminal (A) and Trop2- luminal (B) cells. Only genes greater than 1.5-fold enriched in either direction with a *p*-value < 0.05 are included. (C, D) Gene Ontology (GO) terms for genes significantly downregulated in old Trop2+ luminal (C) and old Trop2- luminal (D) cells. (E, F) Relative mRNA expression (RPKM: reads per kilobase of transcript, per million mapped reads) in Trop2+ luminal (E) and Trop2- luminal (F) cells from 3- and 24-month-old mouse prostate.

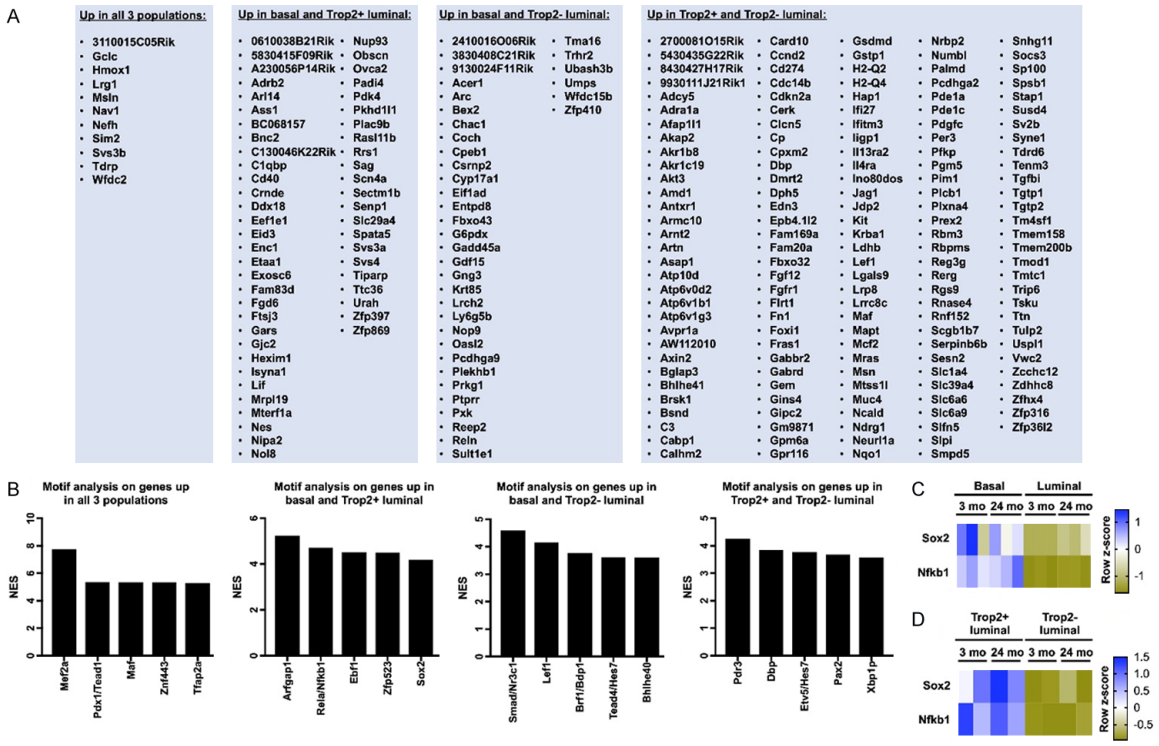

Figure S4. Related to **Figure 4**. (A) Lists of genes commonly upregulated in 2 or 3 cell-types in old mouse prostate. (B) Motif analysis of genes commonly upregulated in 2 or 3 cell-types in old mouse prostate. (C, D) Heatmaps reveal relative mRNA expression of Sox2 and Nfkb1. Regardless of mouse age, both genes are expressed at higher levels in basal cells compared to bulk luminal cells (C) and in Trop2+ luminal cells compared to Trop2- luminal cells (D).

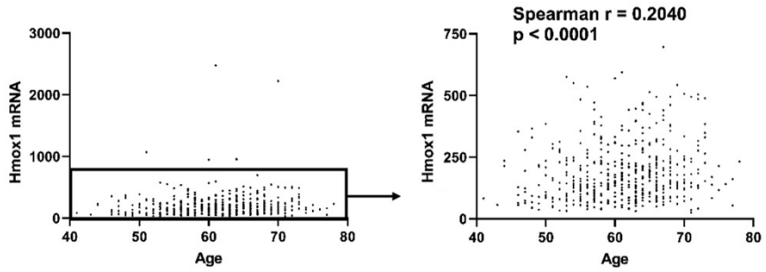

Figure S5. Related to **Figure 5**. Plot of relative Hmox1 mRNA expression and age of diagnosis in the TCGA primary prostate cancer cohort. Box on left is re-analyzed on the right to demonstrate correlation between Hmox1 mRNA and age. Spearman correlation coefficient and *p*-value are shown. 5 data points with Hmox1 mRNA > 750 are excluded from plot on the right.