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# Single-cell transcriptome-wide Mendelian randomization and colocalization analyses reveal immune-cell-specific mechanisms and actionable drug targets in prostate cancer

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## eLife Assessment

This study presents a **useful** compendium of triangulated single-cell eQTLs, Mendelian randomisation and colocalization of genetic signals in prostate cancer datasets. Biological interpretation in the context of the aging prostate gland, the tumour microenvironment and immune cell specificity is **incomplete**, so this study is a starting point for further study, and would require validation of the resulting putative causal genes.

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## Abstract

Prostate cancer remains a major health burden with limited success in immune-targeted therapies. To identify immune-cell-specific therapeutic targets, we integrated single-cell cis-eQTL data across 14 immune cell types, bulk eQTLs, GWAS summary statistics from PRACTICAL and FinnGen, and single-cell RNA-seq data from prostate tumors. Using Mendelian randomization and Bayesian colocalization, we prioritized 80 causal eGenes with shared genetic signals, especially in CD4 and CD8 T cells. Functional analyses revealed enrichment in immune-related pathways such as antigen processing and cytokine signaling. Meta-analysis validated 52 robust eGenes across cohorts. Single-cell transcriptomics confirmed cell-type-specific expression of key genes including *HLA-DQA2*, *TXN*, and *COX6B1* within the tumor microenvironment. Drug repurposing analysis identified potential therapeutic targets such as *IGF1R* and *FAAH*, with known drug interactions mapped via DrugBank and STRING. Our integrative framework highlights immune-cell-specific genetic drivers and actionable targets in prostate cancer, offering a high-resolution resource for precision immunotherapy development.

## 1. Introduction

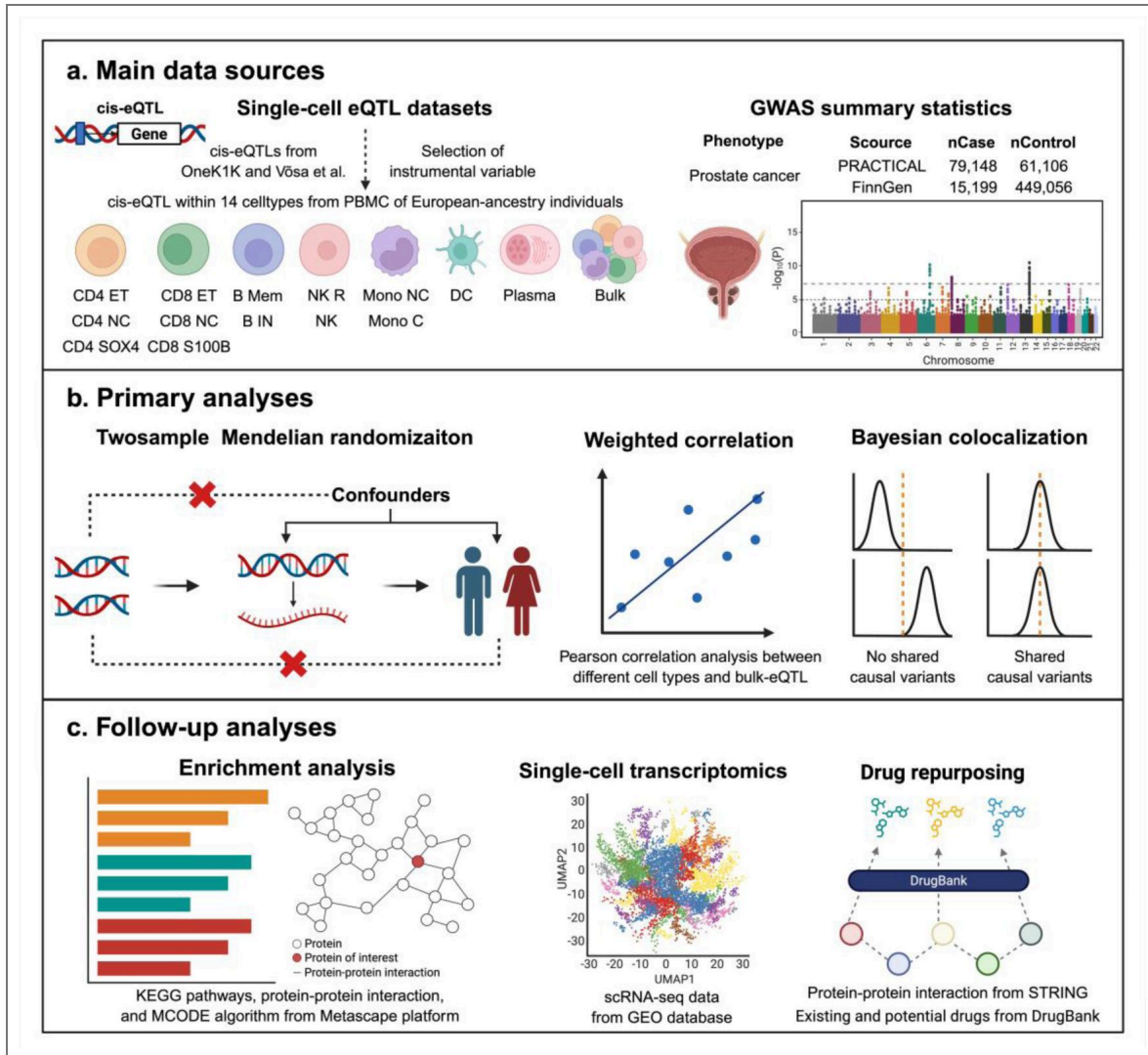
Prostate cancer is one of the most common malignancies among men worldwide and a leading cause of cancer-related mortality (Schafer et al., 2025 [↗](#)). Its incidence increases significantly with age, particularly in individuals over 50 years old (Kratzer et al., 2025 [↗](#)). Despite advances in early detection and treatment, a subset of patients still progress to aggressive or treatment-resistant disease, underscoring the need for deeper understanding of its molecular underpinnings and novel therapeutic approaches.

Numerous studies have sought to uncover the molecular drivers of prostate cancer, identifying alterations in androgen receptor signaling, DNA repair pathways, and cell cycle regulators as critical factors (Burdak-Rothkamm et al., 2020 [↗](#); Dai et al., 2023 [↗](#); He et al., 2022 [↗](#)). These insights have informed the development of targeted therapies, including androgen deprivation therapy (ADT) and next-generation antiandrogens (Chakrabarti et al., 2025 [↗](#); Gillissen et al., 2025 [↗](#)). However, resistance often emerges, and the effectiveness of current drugs remains limited, prompting continued efforts to identify new biomarkers and therapeutic targets (Cai et al., 2023 [↗](#); Swami et al., 2020 [↗](#)).

Emerging evidence suggests that the immune system plays a complex and context-dependent role in prostate cancer (Chen et al., 2024 [↗](#); Jiang et al., 2025 [↗](#)). Although traditionally considered a poorly immunogenic tumor, recent studies have revealed immune infiltration in the prostate tumor microenvironment (Chen et al., 2024 [↗](#); Hage Chehade et al., 2025 [↗](#); Xia et al., 2025 [↗](#)). These immune components may contribute to tumor progression, immune evasion, or therapeutic resistance. Immunotherapies such as immune checkpoint inhibitors have shown limited efficacy in prostate cancer, highlighting a crucial gap in understanding the immune regulatory mechanisms specific to this disease (Sridaran et al., 2023 [↗](#)). Therefore, there is an urgent need to systematically investigate immune-related genetic drivers of prostate cancer and their downstream pathways. Identifying causal immune-cell-specific genes could reveal novel targets for therapy and provide mechanistic insights into the interplay between host immunity and prostate tumorigenesis.

Human genetic studies provide robust, unbiased evidence to support drug target prioritization and reduce the failure rate in drug development (Carss et al., 2023 [↗](#); Reay and Cairns, 2021 [↗](#)). In prostate cancer, genome-wide association studies (GWAS) have identified hundreds of susceptibility loci, shedding light on inherited genetic variation contributing to disease risk (Akamatsu et al., 2012 [↗](#); Farashi et al., 2019 [↗](#); Schumacher et al., 2018 [↗](#)). However, most GWAS signals reside in non-coding regions, limiting biological interpretation. To bridge this gap, transcriptome-wide association studies (TWAS), particularly those using Mendelian randomization (MR) frameworks, have been employed to infer causal relationships between gene expression and disease risk (Reay and Cairns, 2021 [↗](#)). These approaches often integrate GWAS data with expression quantitative trait loci (eQTL) from bulk tissues to identify putative disease-associated genes (Porcu et al., 2019 [↗](#); Richardson et al., 2020 [↗](#)). Several TWAS-based studies have successfully uncovered genes with potential functional relevance to prostate cancer (Desai et al., 2024 [↗](#); Zheng et al., 2024 [↗](#)). Nevertheless, bulk eQTLs may mask cell-type-specific regulatory effects, especially in heterogeneous tissues like blood or tumors. Recently, single-cell eQTL (sc-eQTL) approaches have emerged, enabling the resolution of eQTL effects at individual immune cell types (Nathan et al., 2022 [↗](#); van der Wijst et al., 2018 [↗](#); Yazar et al., 2022 [↗](#)). Integrating sc-eQTL data with GWAS using a single-cell-informed TWAS (sc-TWAS) design can improve causal inference resolution and uncover cell-specific regulatory mechanisms that are otherwise undetectable in bulk data (Mai et al., 2025 [↗](#)). This strategy holds promise for identifying immune-cell-specific causal eGenes in prostate cancer and advancing precision oncology.

In this study, we systematically integrated single-cell and bulk eQTL data with large-scale prostate cancer GWAS using a two-sample Mendelian randomization framework (Fig. 1 [↗](#)). We aimed to identify immune-cell-specific causal eGenes for prostate cancer risk, validate findings across independent cohorts, explore biological functions through pathway analysis, and predict druggable targets. Our approach provides novel insights into the immune-related genetic architecture of prostate cancer and offers a resource for prioritizing therapeutic candidates for future translational research.



**Fig. 1. Overview of study design and workflow.**

(A) Main data sources: Single-cell and bulk cis-eQTL datasets from European-ancestry individuals were used to define immune-cell-specific gene expression. GWAS summary statistics for prostate cancer were obtained from the PRACTICAL and FinnGen consortia. (B) Primary analyses: Two-sample Mendelian randomization (MR) was used to infer causal effects, followed by weighted correlation analysis across immune cell types and Bayesian colocalization to assess shared genetic variants. (C) Follow-up analyses: Functional enrichment, protein-protein interaction (PPI) network analysis, single-cell RNA sequencing (scRNA-seq) validation, and drug repurposing via STRING and DrugBank were conducted to explore biological relevance and therapeutic potential.

## 2. Methods

### 2.1 Data sources

This study was conducted in accordance with the STROBE-MR guidelines to ensure methodological transparency and reproducibility (Skrivankova et al., 2021 [↗](#)). To define genetic instruments specific to immune cell gene expression, we utilized cis-eQTL data from the OneK1K sc-eQTL dataset (<https://onek1k.org> [↗](#)), which profiles 14 distinct human immune cell types (Yazar et al., 2022 [↗](#)). In addition, bulk cis-eQTL data were obtained from the eQTLGen Consortium (<https://eqtlgen.org> [↗](#)), which provides genetic and transcriptomic data from 31,684 individuals of European ancestry, covering a total of 16,987 genes (Võsa et al., 2021 [↗](#)). Genome-wide association summary statistics for prostate cancer were obtained from the PRACTICAL consortium (Prostate Cancer Association Group to Investigate Cancer Associated Alterations in the Genome) (Schumacher et al., 2018 [↗](#)). For replication purposes, we also used outcome data from the FinnGen consortium, which integrates genetic data from Finnish biobanks with nationwide health registry records (<https://www.finnngen.fi/en> [↗](#)) (Kurki et al., 2023 [↗](#)). Detailed information on the GWAS datasets used in this study were included in [Supplementary Table S1](#) [↗](#).

### 2.2 Instrument selection

To ensure robust instrument validity, only genome-wide significant cis-eQTLs ( $p < 1.0 \times 10^{-5}$ ) were included (Ray et al., 2025 [↗](#)). We performed linkage disequilibrium (LD) clumping using the 1000 Genomes Project European reference panel, applying a threshold of  $r^2 < 0.001$  within a 10 Mb window to retain independent single nucleotide polymorphisms (SNPs) (Gkatzionis et al., 2023 [↗](#)). For harmonization between exposure and outcome datasets, we aligned effect alleles and excluded ambiguous or strand-incompatible SNPs. Palindromic variants were retained only when allele frequency permitted unambiguous interpretation. To prevent weak instrument bias, SNPs with  $F$ -statistics  $< 10$  were excluded from the analysis (Pierce et al., 2011 [↗](#)).

### 2.3 MR analysis

We conducted Mendelian randomization (MR) analyses to estimate the causal effects of immune-cell-specific gene expression on prostate cancer risk. All analyses were performed using the TwoSampleMR R package (v0.6.6) (Hemani et al., 2018 [↗](#)). For each eGene, the MR method was selected based on the number of available independent SNP instruments. When only a single SNP was available, the Wald ratio method was applied; for eGenes with two or more independent SNPs, the inverse-variance weighted (IVW) method was used instead (Burgess et al., 2019 [↗](#)). To address the issue of multiple testing across thousands of eGenes, we applied a false discovery rate (FDR) correction. Only associations meeting the threshold of  $FDR < 0.05$  were considered statistically significant. Due to limited number of SNP, sensitivity analyses, including heterogeneity and pleiotropy tests, were not conducted. For each outcome, we computed the pairwise weighted Pearson correlation of MR results across different immune cell types and from the bulk-eQTL MR analyses, and visualized these as a correlation matrix. To further assess the robustness of causal estimates, we conducted random-effects meta-analyses by integrating MR results from both the discovery and replication datasets, using the metafor R package (v4.8). Genes that remained significantly associated in the meta-analysis ( $p < 0.05$ ) were considered robust causal candidates for prostate cancer susceptibility.

### 2.4 Bayesian colocalization analysis

To assess whether the same genetic variants influence both gene expression and prostate cancer susceptibility, we performed a Bayesian colocalization analysis using the coloc R package (v5.2.3) (Giambartolomei et al., 2014 [↗](#)). For each eGene, we computed the posterior probabilities for five distinct hypotheses, with particular focus on PP.H4, which estimates the probability that a shared causal variant underlies both the eQTL and the disease association. eGenes with PP.H4  $> 50\%$  were considered to exhibit evidence of colocalization, with values exceeding 70% interpreted as strong evidence, and were prioritized for further analysis. This approach allowed us to distinguish true

shared genetic signals from associations driven by LD or independent effects. For eGenes showing colocalization, we retrieved corresponding protein-protein interaction (PPI) data from the STRING database (<https://string-db.org>), using a medium confidence interaction score ( $\geq 0.40$ ). The resulting PPI networks were visualized in Cytoscape (v3.10.2).

## 2.5 Functional annotation and pathway analysis

To gain deeper insight into the biological functions and pathways associated with prostate cancer-associated causal eGenes, we performed Gene Ontology (GO) and pathway enrichment analyses using Metascape (<http://metascape.org>). Enrichment was assessed across multiple GO categories, including biological process, molecular function, and cellular component, as well as across Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. To further explore functional connectivity, PPI networks were analyzed using the MCODE algorithm, which identifies highly interconnected clusters of proteins that may represent key mechanistic modules. All visualizations were generated using ggplot2 (v3.5.2) in R and Cytoscape (v3.10.2).

## 2.6 The scRNA-seq data pre-processing and annotation

We analyzed single-cell RNA sequencing (scRNA-seq) data from the GEO database (accession number: GSE137829) (<https://www.ncbi.nlm.nih.gov/geo/>), which includes transcriptomic profiles of 21,292 cells derived from needle biopsies of six prostate cancer samples. Raw count data were processed using the Seurat R package (v5.1.0). Quality control procedures excluded cells with  $>25\%$  mitochondrial gene content or an abnormal number of detected genes ( $<200$  or  $>10,000$ ). Genes were retained if expressed in at least 3 cells, with total counts ranging from 250 to 50,000. Normalization and scaling were performed using the SCTransform method with linear regression, followed by principal component analysis (PCA) for dimensionality reduction. To correct for batch effects, we applied the Harmony algorithm, and unsupervised clustering was performed using the FindClusters function (resolution = 0.4). Cell type annotation was based on canonical markers and the unique expression profiles of cluster-specific genes. Subsequently, we extracted T cells, B cells, and plasma cells, the cell types overlapping with those in the sc-eQTL analysis, for subclustering. This was conducted using FindClusters with a higher resolution (resolution = 1) to allow for more refined clustering.

## 2.7 Drug repurposing

To identify clinically available compounds that target prostate cancer-associated causal eGenes identified through MR and colocalization analyses, we conducted a systematic drug search using the DrugBank database (<https://go.drugbank.com>). Gene symbols of prioritized causal eGenes were used as input queries to retrieve associated drugs with approved medications. In addition, we examined currently approved clinical drugs for prostate cancer and their known target proteins. To explore potential interactions, we used the STRING database (<https://string-db.org/>) to assess protein interactions between these known drug targets and the prostate cancer-associated causal eGenes identified in our study.

# 3. Results

## 3.1 MR reveals causal eGenes for prostate cancer

We performed two-sample MR analyses using cis-eQTL data from 14 immune cell types and bulk samples to identify eGenes causally associated with prostate cancer risk (Supplementary Tables S2–3). Notably, the greatest number of significant associations was observed in CD4 NC, CD8 ET, NK, and CD8 NC, indicating a prominent role for specific immune subtypes in prostate cancer susceptibility. To evaluate the consistency of MR effects across cell types, we calculated pairwise weighted Pearson correlations of MR results (Fig. 2A, Supplementary Tables S4–6). High correlations were observed between closely related T cell subsets, such as CD4 NC and CD8 NC ( $r =$

0.78), suggesting shared regulatory patterns. In contrast, correlations between sc-eQTL and bulk-eQTL data derived MR results were generally weaker, highlighting that sc-eQTL data may better capture cell-type-specific genetic regulatory effects that are diluted in bulk tissue analyses.

### 3.2 Colocalization identifies shared causal variants for gene expression and prostate cancer risk

Among the eGenes showing significant MR associations, 80 eGenes exhibited evidence of colocalization ( $PP.H4 > 0.50$ ), with 33 eGenes reaching strong support ( $PP.H4 > 0.70$ ) (Supplementary Table S7 [↗](#)). The number of colocalized eGenes varied by cell type, with the highest counts observed in CD4 NC, CD8 ET, CD8 NC, and NK, suggesting cell-type-specific regulatory architecture (Fig. 2B [↗](#)). These colocalized eGenes were further mapped into the PPI network to explore functional interconnectivity. Network topology analysis revealed key hub proteins such as H3C12, *TXN*, *LMNA*, and H4BC6, which showed high degree centrality and strong interconnectivity within the network (Fig. 2C [↗](#), Supplementary Table S8 [↗](#)).

### 3.3 Functional enrichment and PPI network analysis

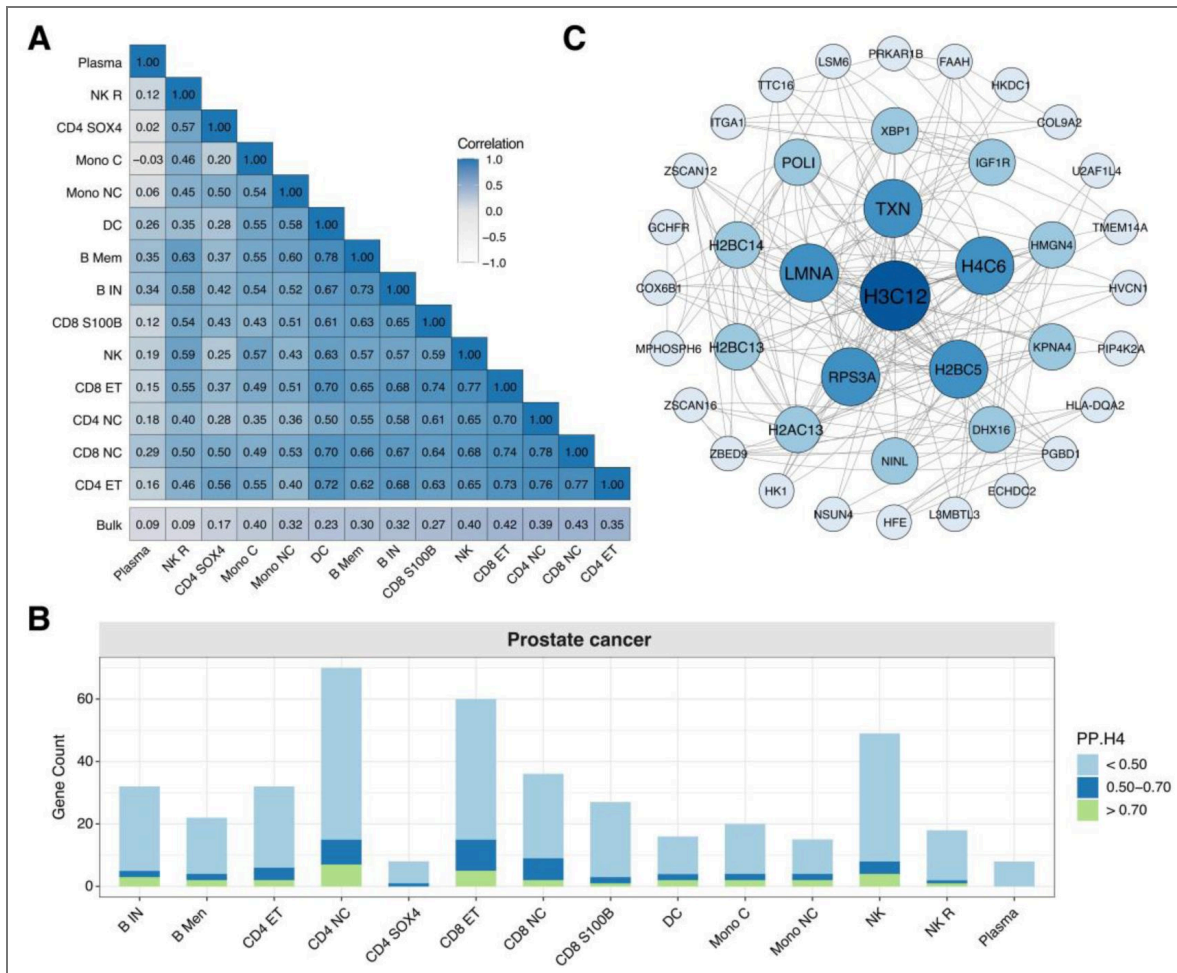
Metascape analysis showed that colocalized eGenes were significantly enriched in immune-related and inflammation-associated pathways, including antigen processing and presentation, Th1/Th2 cell differentiation, and systemic lupus erythematosus (Fig. 3A [↗](#), Supplementary Tables S9–10 [↗](#)). Additional enrichment was observed in pathways involved in cellular stress, necroptosis, and viral infections, highlighting the intersection of immune dysregulation and prostate cancer susceptibility. To explore the network architecture of these eGenes, we constructed a PPI network and applied the MCODE algorithm to identify densely connected modules. Four major modules (MCODE1–4) were detected (Fig. 3B [↗](#), Supplementary Table S11 [↗](#)), with MCODE1 comprising predominantly histone-related genes (e.g., H2BC5, H3C12, H2AC13) that showed high colocalization signals and centrality, suggesting epigenetic regulation may underlie risk pathways. MCODE2 contained HLA class I and II genes, further implicating immune presentation mechanisms.

### 3.4 Cell-type-specific expression of causal eGenes in the prostate tumor microenvironment

To assess the expression landscape of prostate cancer-associated causal eGenes in the tumor microenvironment, we integrated scRNA-seq data from prostate cancer cells. A total of 80 colocalized eGenes were mapped to distinct immune cell types, highlighting cell-type-specific regulatory patterns (Supplementary Fig. S1 [↗](#), Table S12 [↗](#)). Using Seurat clustering and canonical markers, we identified 10 major cell types, including epithelial, T cells, B cells, myeloid cells, fibroblasts, and endothelial cells (Fig. 4A–C [↗](#); Supplementary Table S13 [↗](#)). Marker gene expression and dot plot analysis confirmed the specificity and annotation of each cluster (Fig. 4D [↗](#)). Cell composition analysis showed epithelial cells as the predominant population, with varying contributions from immune and stromal compartments across patients (Fig. 4E–F [↗](#); Supplementary Table S14 [↗](#)). To further explore the potential roles of eGenes beyond immune cells, we analyzed bulk-prioritized eGenes that lacked consistent prioritization across single-cell immune cell types (Fig. 4G [↗](#)). Notably, several of these eGenes, including *MYO6*, *MRPS34*, *VAMP8*, and *MMAB*, exhibited predominant expression in epithelial and stromal cell populations, suggesting their functional relevance in non-immune components of the prostate tumor microenvironment (Fig. 4H–K [↗](#)).

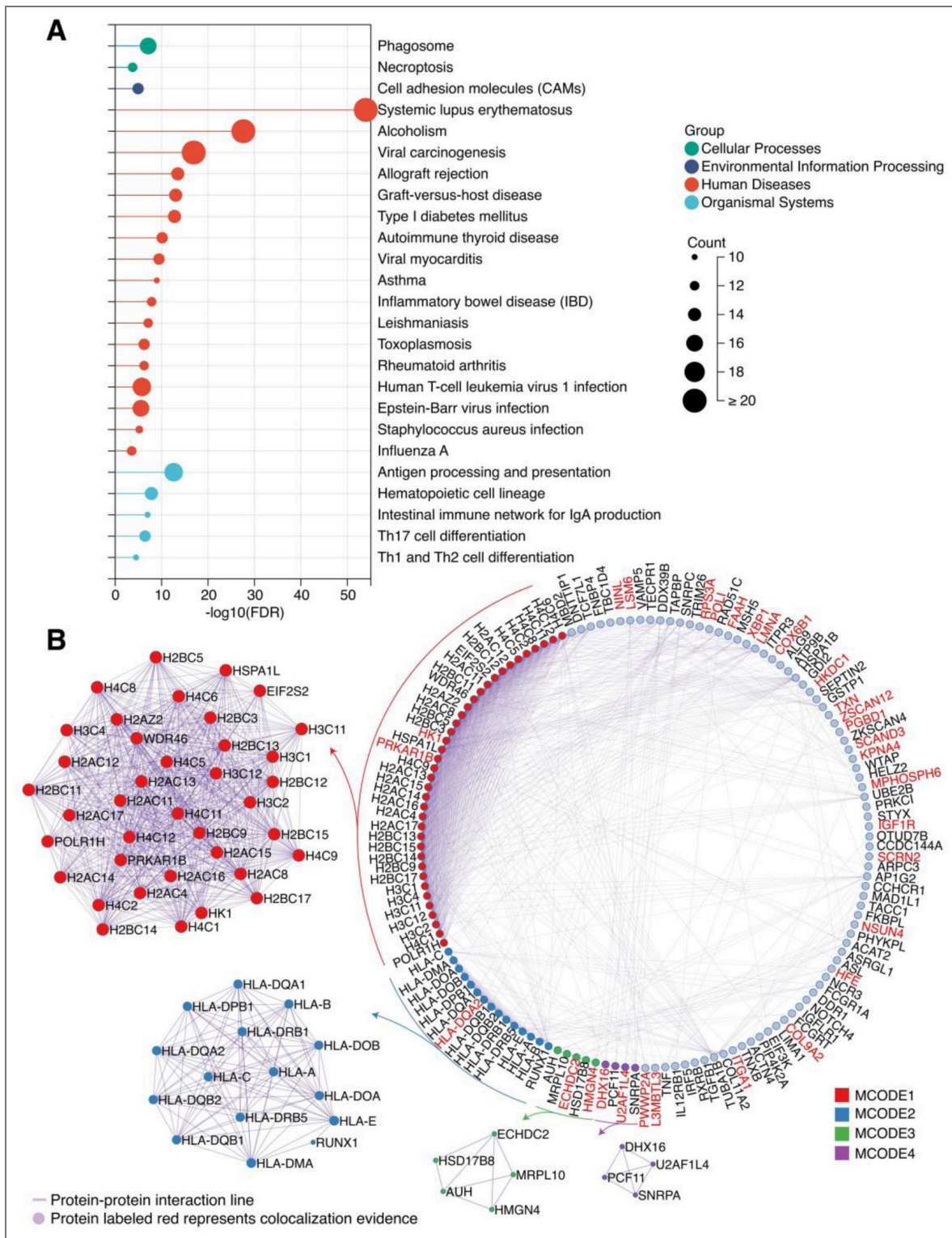
### 3.5 Refined clustering of immune subtypes reveals expression of key causal eGenes

To better resolve the expression patterns of causal eGenes within immune subsets, we performed reclustering of Plasma, T, and B cells from the prostate tumor microenvironment (Supplementary Fig. S2A–C [↗](#)). Based on canonical marker expression, we identified four main immune subtypes: Plasma, CD4 T, CD8 T, and B cells (Supplementary Fig. S2D [↗](#), Table S15 [↗](#)). Expression analysis



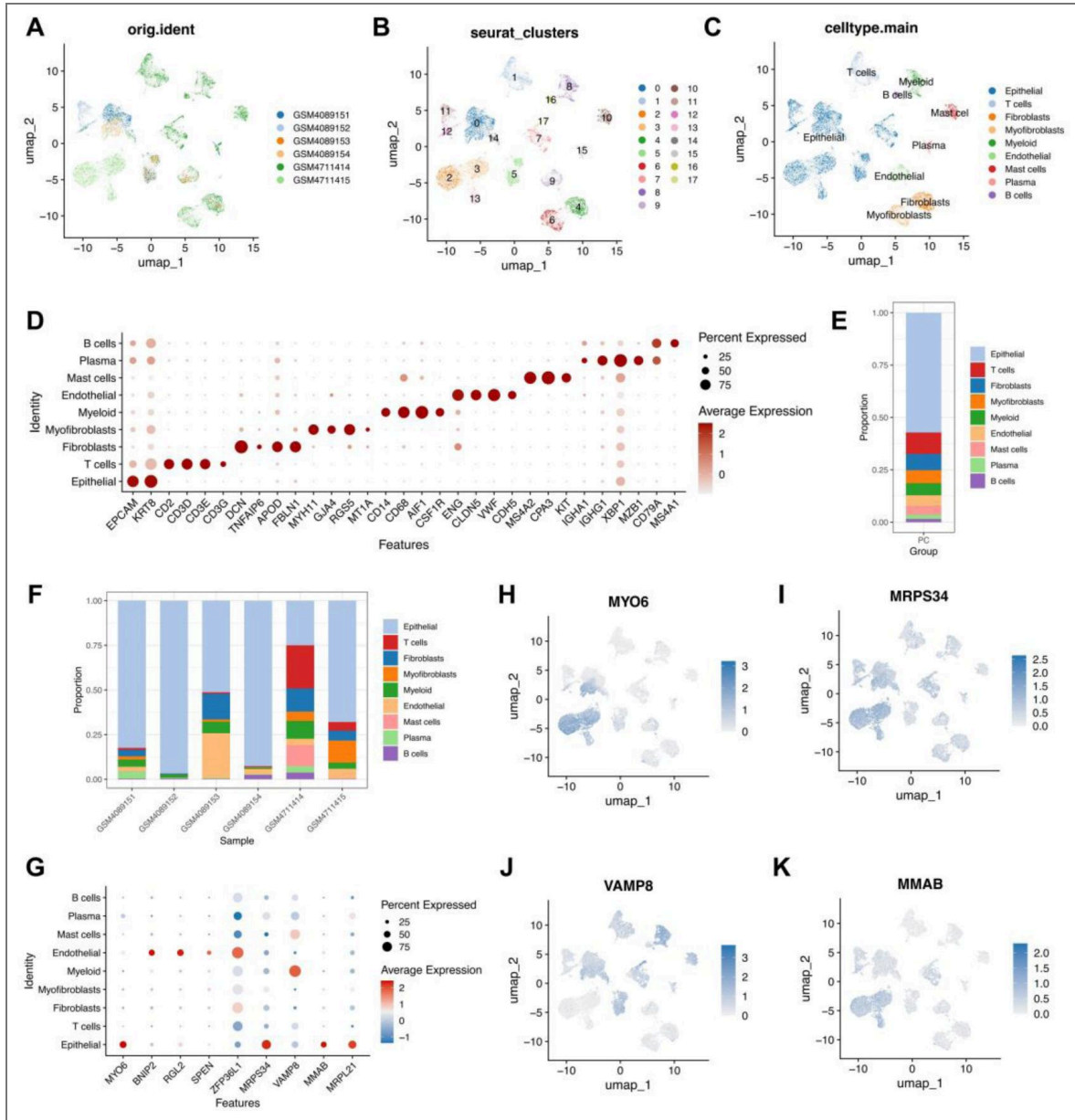
**Fig. 2. Correlation, colocalization, and protein interaction network in prostate cancer.**

(A) Pearson correlation matrix of MR results across immune cell types and bulk-eQTL, showing shared genetic influence. (B) Number of prostate cancer-associated eGenes in each immune cell type with varying levels of colocalization evidence. (C) PPI network of colocalized eGenes prioritized by Bayesian colocalization (PP.H4 > 0.50), with node size representing connectivity.



**Fig. 3. Pathway and network analyses of prostate cancer-associated eGenes.**

(A) KEGG pathway enrichment analysis of colocalized eGenes using Metascape. Dot size indicates count number; color represents pathway category. (B) MCODE clustering of PPI networks highlights tightly connected modules (MCODE1–4). Red-labeled proteins show colocalization with prostate cancer risk loci.



**Fig. 4. The scRNA-seq profiling of prostate tumor microenvironment.**

(A–C) UMAP plots showing sample identity, clustering, and major cell types identified. (D) Dot plot of canonical markers used for cell type annotation. (E–F) Cell composition proportions by cluster and by patient sample. (G) Expression patterns of prioritized bulk prostate cancer-associated eGenes across cell types. (H–K) Feature plots of representative eGenes (*MYO6*, *MRPS34*, *VAMP8*, *MMAB*) across cell types.

revealed that multiple prostate cancer-associated eGenes exhibited distinct and cell-type-specific transcriptional patterns (Supplementary Fig. S2E [↗](#)). For example, *HLA-DQA2* and *TXN* showed strong expression in B cells, whereas *TXN* was also expressed in both CD4 and CD8 T cells (Supplementary Fig. S2F–G [↗](#)). Additional eGenes such as *RPS3A*, *COX6B1*, and *XBP1* also displayed enriched expression in specific immune cell populations, suggesting their potential immunoregulatory roles in the tumor microenvironment.

### 3.6 Meta-analysis confirms robust associations across cohorts

To validate the robustness of prostate cancer-associated eGenes identified in the discovery MR analysis, we performed replication using summary statistics from the FinnGen consortium and conducted meta-analyses across the PRACTICAL and FinnGen datasets. Five eGenes were excluded from the replication due to insufficient overlapping SNPs in the FinnGen data (Supplementary Table S16 [↗](#)). Meta-analysis revealed 52 eGenes with significant associations ( $p < 0.05$ ), highlighting consistent genetic effects across cohorts (Supplementary Table S17 [↗](#)). Notably, several eGenes, including *NSUN4*, *TXN*, *HK1*, *LMNA*, and *L3MBTL3*, exhibited strong and concordant associations in both datasets, reinforcing their putative causal roles in prostate cancer susceptibility. The combined effect estimates remained directionally consistent and statistically significant, with many showing moderate to high colocalization probabilities ( $PPH4 > 0.70$ ), supporting shared causal variants (Fig. 5 [↗](#)). These results underscore the reproducibility and robustness of immune-cell-specific gene expression effects on prostate cancer risk and validate key candidates for further mechanistic and therapeutic exploration.

### 3.7 Drug repurposing identifies potential drugs

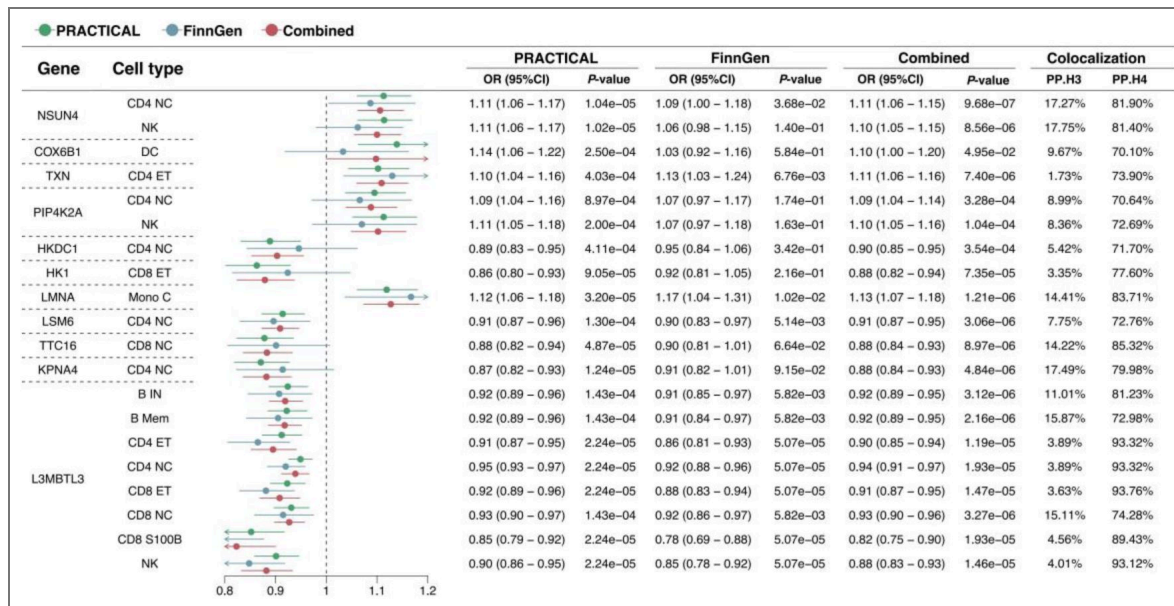
To explore therapeutic opportunities, we conducted drug repurposing analyses by integrating three key layers of interaction. First, we examined predicted associations between proteins encoded by prioritized prostate cancer-associated causal eGenes and their potential targeted drugs, identifying druggable candidates such as *COX6B1*, *IGF1R*, and *FAAH*, which are linked to approved or investigational agents including cholic acid, brigatinib, mecasermin, and acetaminophen (Supplementary Tables S18–19 [↗](#)). Second, we mapped known connections between existing prostate cancer drugs and their validated protein targets, revealing therapeutics such as estradiol, etoposide, and uracil that are already in clinical use (Supplementary Table S20 [↗](#)). Third, protein interaction analysis showed that proteins encoded by prioritized causal eGenes interact with proteins targeted by approved prostate cancer drugs, indicating additional indirect therapeutic avenues. Functional annotations of both the causal gene products and drug-targeted proteins confirmed their involvement in critical biological pathways associated with tumorigenesis and treatment response (Supplementary Tables S21–22 [↗](#)). Together, this integrative network highlights promising candidates for drug repurposing and provides a comprehensive resource for developing novel treatment strategies in prostate cancer (Fig. 6 [↗](#)).

## 4. Discussion

Prostate cancer remains a complex and heterogeneous disease with limited insight into the immune-related mechanisms driving its initiation and progression (Haffner et al., 2021 [↗](#)). In this study, we employed a comprehensive analytical framework integrating sc-TWAS, Bayesian colocalization, scRNA-seq, and drug repurposing approaches to systematically identify cell-type-specific causal eGenes associated with prostate cancer risk. By leveraging cis-eQTL data from 14 immune cell types and large-scale GWAS summary statistics, we revealed dozens of immune-cell-specific eGenes with significant causal effects on prostate cancer susceptibility. Notably, many of these associations were absent in traditional bulk eQTL analyses, highlighting the added resolution of single-cell-informed approaches. Meta-analysis using an independent replication cohort further confirmed the robustness of 52 prioritized causal eGenes. These findings provide high-confidence genetic evidence for immune-cell-mediated pathways in prostate cancer and offer a foundation for dissecting tumor–immune interactions at cell-type resolution, with broad implications for biomarker discovery and therapeutic development.

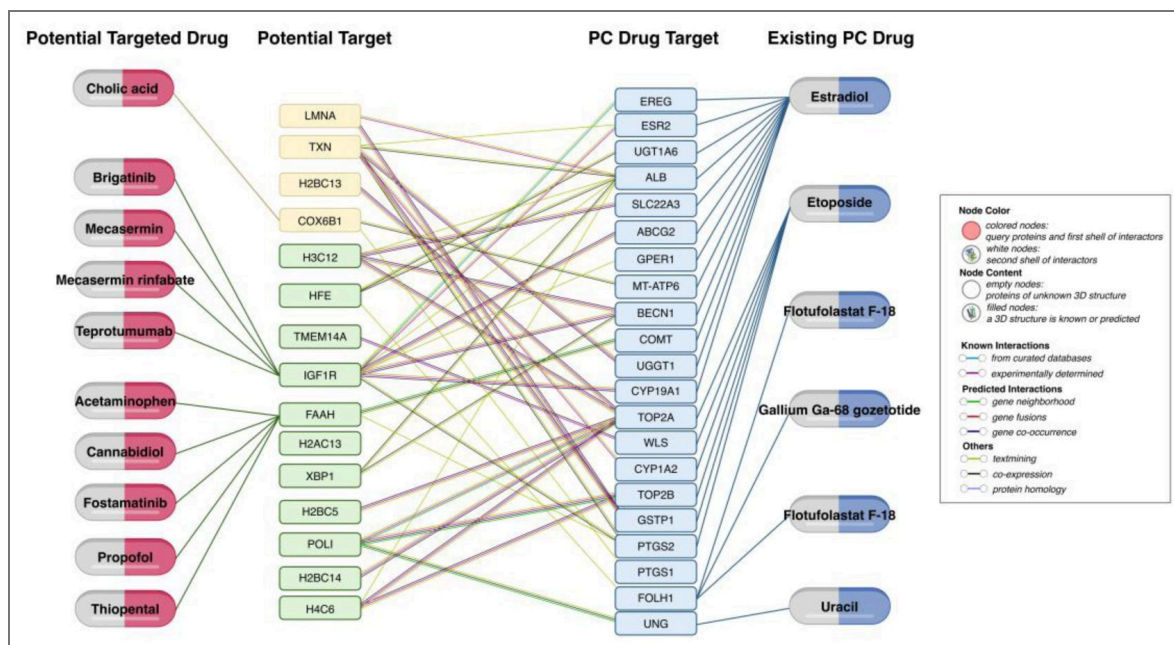
**Fig. 5. Causal effect estimates of prioritized eGenes across PRACTICAL, FinnGen, and meta-analysis.**

Only eGenes remained significant ( $p < 0.05$ ) in meta-analysis are shown.



**Fig. 6. Drug repurposing network of prostate cancer-associated targets.**

This network diagram illustrates three key relationships: (1) links between proteins encoded by prioritized prostate cancer-associated causal eGenes and their potential targeted drugs; (2) connections between existing prostate cancer drugs and their known protein targets; and (3) protein-protein interactions between proteins encoded by prioritized prostate cancer-associated causal eGenes and proteins targeted by approved prostate cancer drugs.



Several representative eGenes identified in our analysis demonstrate both cell-type-specific expression patterns and biologically plausible roles in prostate cancer pathogenesis. *HLA-DQA2*, a key component of MHC class II, was highly expressed in B cells according to scRNA-seq data and showed a protective effect in B Mem from sc-TWAS results. This aligns with its known role in antigen presentation and modulation of humoral immune responses, suggesting that efficient antigen processing by B cells may contribute to protective immunity in the prostate tumor microenvironment. Enrichment results supporting negative regulation of antigen presentation and humoral immune responses further reinforce this interpretation. In contrast, *TXN* (Thioredoxin), a regulator of redox homeostasis and immune signaling, was expressed in CD4, CD8 T cells, and B cells (Bradford et al., 2024). It showed a risk effect in CD4 NC, CD4 ET, and CD8 ET, implying that overexpression of *TXN* may promote T cell activation and survival, potentially aiding tumor immune evasion. Functionally, *TXN* is linked to redox control in lymphocyte activation and interferon regulation, which is consistent with our enrichment findings in regulation of lymphocyte-mediated immunity, regulation of inflammatory responses, and positive regulation of type II interferon production (Gonzalez et al., 2015; Matsuzawa, 2017). *RPS3A*, a ribosomal protein, was highly expressed in CD4, CD8 T cells, and B cells, and showed a protective effect in CD4 SOX4. Beyond its traditional role in translation, *RPS3A* has been implicated in apoptosis regulation and immune cell proliferation, potentially acting as a checkpoint in T cell-mediated immune surveillance (Tang et al., 2018; Zhou et al., 2020). Enriched pathways like regulation of cell population proliferation and proteolysis may reflect its broader role in maintaining immune cell homeostasis within the tumor microenvironment. *COX6B1*, a subunit of cytochrome c oxidase involved in mitochondrial respiration, exhibited the broadest pro-oncogenic profile, with risk associations across CD4 NC, CD4 ET, CD8 NC, CD8 ET, and B Mem (Abdulhag et al., 2015). Its elevated expression, especially in CD4 T cells, suggests that metabolic reprogramming of immune cells, particularly T cell subsets, may facilitate tumor-promoting inflammation or immune dysfunction. This is supported by enrichment in pathways such as intracellular glucose homeostasis, negative regulation of lymphocyte activation, and mitochondria-related immune modulation. Together, these genes illustrate distinct yet interconnected roles in immune regulation, energy metabolism, and antigen response, reflecting a multi-layered immune landscape in prostate cancer shaped by cell-type-specific transcriptional programs.

The identification of druggable prostate cancer-associated causal eGenes through integrative drug repurposing analysis offers promising translational opportunities. Among the prioritized targets, *IGF1R* stands out as a well-established oncogenic driver with existing inhibitors such as brigatinib and teprotumumab, which are already approved for other indications (Elemam et al., 2024). Its confirmed causal role in prostate cancer, combined with known pharmacological modulators, highlights its potential for repositioning or combinatorial therapy in selected patient subsets. Similarly, *FAAH* (fatty acid amide hydrolase), a key enzyme involved in endocannabinoid metabolism, emerged as another causal eGene with approved drugs like cannabidiol and acetaminophen predicted to interact with it (Dalman et al., 2015; Zhu et al., 2023). These findings suggest possible immunometabolic axes amenable to pharmacological intervention, expanding therapeutic strategies beyond traditional androgen receptor pathways. Furthermore, by mapping protein interactions between new causal eGenes and existing drug targets, our network analysis revealed extensive interconnectivity, pointing to mechanistic convergence and potential for synergistic drug combinations. For instance, several newly identified genes interact with proteins targeted by current prostate cancer drugs such as estradiol and etoposide, indicating shared pathways that may enhance treatment efficacy when co-targeted (Kovacs et al., 2020; Wang et al., 2025). This network-informed strategy can facilitate rational drug design and accelerate the development of novel therapeutics tailored to immune-related molecular profiles. Our study provides a valuable target resource for precision immunotherapy in prostate cancer. By integrating genetic evidence with single-cell resolution and pharmacological databases, we not only uncover immune-cell-specific vulnerabilities but also prioritize actionable eGenes with translational potential. These findings pave the way for biomarker-guided therapies and repurposed drug applications, offering a cost-effective and biologically grounded framework to expand treatment options for patients with prostate cancer.

Compared to traditional bulk TWAS and GWAS-only strategies, our study offers significant improvements in cellular resolution, functional relevance, and clinical translatability. While previous prostate cancer TWAS studies leveraging bulk eQTL data have successfully nominated candidate eGenes, they are limited by tissue heterogeneity and inability to resolve cell-type-specific regulatory effects (Liu et al., 2022 [↗](#); Wu et al., 2019 [↗](#)). In contrast, our integration of sc-eQTL, Bayesian colocalization, and scRNA-seq data enables precise mapping of causal gene expression to distinct immune cell subsets within the tumor microenvironment. To our knowledge, this is the first systematic study to combine single-cell genetic regulation and transcriptomics in the context of prostate cancer. This approach not only reveals novel mechanisms that would be obscured in bulk analyses but also provides a high-confidence set of functionally and clinically relevant targets for further exploration.

Despite its strengths, this study has several limitations that should be acknowledged. First, our genetic analyses were conducted primarily in European ancestry populations, which may limit the generalizability of findings to other ethnic groups. Further validation in diverse populations is essential to ensure broad clinical applicability. Second, the scRNA-seq data used for transcriptomic profiling were derived from prostate tumor tissues of a limited number of patients, potentially reflecting sample-specific features that may not fully capture the heterogeneity of prostate cancer across stages or subtypes. Additionally, the sc-eQTL datasets had relatively modest sample sizes, which may affect the power and stability of association signals, particularly in rare or less abundant immune cell types. As such, some findings should be interpreted with caution and warrant replication in larger, independently derived single-cell datasets. Moreover, while sc-TWAS and colocalization provide strong statistical evidence for causality, functional validation in experimental models will be critical to confirm the biological relevance of prioritized eGenes. Future work could also incorporate protein-level QTL (pQTL) analyses or spatial transcriptomics, enabling finer dissection of gene regulation, cell-cell interactions, and spatial context in prostate cancer. These directions hold promise for further refining mechanistic insights and therapeutic opportunities.

## Conclusion

This study presents a novel integrative framework to identify cell-type-specific causal eGenes and prioritize therapeutic targets for prostate cancer by combining sc-TWAS, colocalization, single-cell transcriptomics, and drug repurposing. By leveraging immune cell-specific genetic regulation and high-resolution expression data, we uncovered biologically and clinically relevant eGenes that were largely undetectable by conventional bulk analyses. These findings underscore the power of multi-omics integration in unraveling disease mechanisms and highlight the potential of genetic-guided approaches in precision oncology. As immunotherapy continues to transform cancer treatment, our results offer a robust foundation for immune-targeted drug development, repurposing, and biomarker discovery in prostate cancer. Looking forward, the incorporation of additional layers such as proteomics, spatial transcriptomics, and single-cell epigenomics will further enhance the resolution and impact of such frameworks, accelerating the translation of omics-driven insights into personalized therapeutic strategies.

## Data availability

The datasets analyzed in this study were obtained from publicly accessible repositories. Single-cell eQTL data were retrieved from the OneK1K project (<https://onek1k.org> [↗](#)), and bulk eQTL data were sourced from the eQTLGen Consortium (<https://www.eqtlgen.org> [↗](#)). Prostate cancer GWAS summary statistics were obtained from the PRACTICAL consortium and the FinnGen consortium (<https://www.finnngen.fi/en> [↗](#)). Single-cell RNA sequencing data were downloaded from the GEO database under accession number GSE137829. All other relevant data are included in the article and its supplementary files.

All analyses were performed using R (v4.3.1). The following R packages were used: TwoSampleMR (v0.6.6) for Mendelian randomization, coloc (v5.2.3) for Bayesian colocalization, Seurat (v5.1.0) for single-cell RNA-seq processing, Harmony (v1.0) for batch correction, metafor (v4.8) for meta-

analysis, and ggplot2 (v3.5.2) for data visualization. Custom R scripts used in this study are available from the corresponding author Yanggang Hong ([sun160414@icloud.com](mailto:sun160414@icloud.com)) upon reasonable request.

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## Additional information

### Authors' contributions

Yanggang Hong: Conceptualization, Investigation, Formal analysis, Data curation, Validation, Visualization, Writing - original draft, Writing - review & editing, Supervision, Project administration.

Yi Wang: Writing - original draft.

Yirong Wang: Writing - original draft.

Feng Chen: Validation.

Jiajun Li: Writing - review & editing.

### Abbreviations

GWAS: Genome-wide association study

MR: Mendelian randomization

eQTL: Expression quantitative trait loci

sc-eQTL: Single-cell eQTL

IV: Instrumental variable

LD: Linkage disequilibrium

SNP: Single nucleotide polymorphism

eGene: Gene with an eQTL

IVW: Inverse-variance weighted

CD4 NC: CD4<sup>+</sup> naïve and central memory T cells

CD4 ET: CD4<sup>+</sup> T cells with an effector memory or central memory phenotype

CD4 SOX4: CD4<sup>+</sup> T cells expressing SOX4

CD8 NC: CD8<sup>+</sup> naïve and central memory T cells

CD8 ET: CD8<sup>+</sup> T cells with an effector memory or central memory phenotype

CD8 S100B: CD8<sup>+</sup> T cells with expression of S100B

B IN: Immature and naïve B cells

B Mem: Memory B cells

Mono C: Classical Monocytes

Mono NC: Non-classical Monocytes

NK: Natural killer cells

NK R: NK recruiting cells

DC: Dendritic cells

Plasma: Plasma cells

FDR: False discovery rate

PPI: Protein-protein interaction

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## Additional files

**Supplementary File 1.**  Figure supplements.

**Supplementary File 2.**  Supplementary tables. Table S1. Summary of GWAS and eQTL datasets used in this study. Table S2. Mendelian randomization (MR) results for prostate cancer-associated eGenes from single-cell eQTL data (PRACTICAL cohort). Table S3. MR results for prostate cancer-associated eGenes from bulk eQTL data (eQTLGen cohort). Table S4. Pearson correlation of MR results between sc-eQTL and bulk eQTL datasets. Table S5. Overlap counts of prostate cancer-associated eGenes across immune cell types. Table S6. Pairwise weighted Pearson correlation coefficients of MR results across immune cell types. Table S7. Bayesian colocalization results of eGenes with prostate cancer GWAS signals (PRACTICAL cohort). Table S8. Network topology metrics of colocalized genes in the protein–protein interaction (PPI) network. Table S9. Pathway enrichment summary for colocalized genes (Metascape). Table S10. KEGG pathway enrichment results for colocalized eGenes. Table S11. MCODE network module analysis and enriched pathways in the PPI network. Table S12. Number of prostate cancer-associated eGenes detected in each immune cell type. Table S13. Marker genes of various cell types in the prostate tumor microenvironment. Table S14. Proportional distribution of major cell types in the prostate tumor microenvironment. Table S15. Marker genes of CD4+, CD8+ T cells, B cells, and plasma cells in the prostate tumor microenvironment. Table S16. MR results of prostate cancer-associated eGenes from the FinnGen replication cohort. Table S17. Meta-analysis results of prostate cancer-associated eGenes across PRACTICAL and FinnGen. Table S18. Predicted drug-gene interactions from DrugBank for prioritized causal eGenes. Table S19. DrugBank entries linking causal eGene targets with approved drugs. Table S20. Existing approved drugs for prostate cancer and their known targets. Table S21. Proteins encoded by prioritized prostate cancer-associated causal genes and their functional annotations. Table S22. Proteins targeted by approved prostate cancer drugs and their functional annotations.

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## Peer reviews

### Reviewer #1 (Public review):

#### Summary:

Using Mendelian randomisation on available GWAS data, the investigators identified eGenes associated with prostate cancer and applied the data to define relevant immune cell types involved. Additional analysis was performed to explore potential candidate targets and agents from licensed medicines.

This is an interesting approach as the investigators have expertise in other research fields, applied here to prostate cancers. The use of three different datasets is significant, and the approach to further analyse implicated eGenes in drug target analysis is relevant and timely.

A particular strength is taking putative genes from Mendelian randomisation analysis to target and potential drug agents.

Some aspects of the study would need to be clarified to enable interpretation of the findings in the context of the prostate gland and prostate cancers: expanding the descriptions of the

supporting Supplementary Data and Tables, explanations of the analysis for the general reader, and clarification of the selection of eGenes (Figure 5).

<https://doi.org/10.7554/eLife.110928.1.sa1>

## Reviewer #2 (Public review):

Summary:

This study integrates bulk and single-cell transcriptomic-derived eQTLs from two separate consortia (PRACTICAL and Finngen) to identify immune-cell-specific therapeutic targets in prostate cancer. Mendelian randomization and Bayesian colocalization have been used to produce druggable eGene modules through STRING and DrugBank.

This is an interesting study that is attempting to address risk-associated, immune-specific transcriptomic repertoires in prostate cancer. It is knitting together concepts of drug repurposing and prostate cancer immunogenicity. This is an entirely computational study, which would benefit from some wet lab experimental validation.

It is very tricky to attribute cell-type-specific responses, especially when the majority of genes involved represent cytoskeletal or stress responses, which are ubiquitous throughout the prostate microenvironment. This point is relevant for the drug repurposing section: if these drugs are targeting immune cell-specific repertoires, what would the response be of the entire environment? It would be useful to contextualize the validity of each proposed therapy in a specific prostate cancer context and the involvement of AR antagonism or radiotherapy.

Strengths and limitations of this study:

Strengths:

This is a scientifically interesting and potentially impactful study, particularly in its attempt to integrate immune-cell-specific transcriptomics, causal inference, and drug repurposing in prostate cancer. The methodology is well described, and the data (albeit limited) are well analyzed.

Limitations:

The central weakness is the overstatement of the conclusions regarding immune-cell-specific causality, without sufficiently contextualizing the biological meaning of the findings.

Highlighted genes, such as LMNA, XBP1, histone-related genes, and stress-response markers, are ubiquitous regulators involved in fundamental cellular processes, including ageing, unfolded protein response (UPR), integrated stress response (ISR), chromatin remodeling, proliferation, and metabolism. It is unclear whether these signatures truly represent immune mechanisms, or instead reflect broader inflammatory and age-associated biology expected within an ageing glandular organ such as the prostate.

Immune cell identity alone may not be sufficient to infer biological relevance because immune state characterization (e.g., exhausted versus functional T cells, or distinct macrophage/myeloid phenotypes) is largely absent from the current analysis. The assertion that specific immune populations are correlated with prostate cancer susceptibility is probably an overstatement unless the nature of these cells can also be characterized.

The interpretation of "causal variants" is not always specified, i.e., what phenotype is being associated: prostate cancer susceptibility, recurrence, progression, or treatment response (e.g. is there direct causality from immune-cell variants to prostate cancer?).

Overall, there is a need for stronger biological and translational contextualization: how do the identified pathways relate to ageing-associated inflammation, PIN, microbiome-driven inflammatory changes, and stress-response biology in the prostate gland? While the manuscript identifies network hubs and enriched pathways, it often stops short of explaining what these modules biologically represent or how they may influence prostate cancer development, progression, treatment resistance, or immune evasion.

There are additional publicly available spatial transcriptomic or single-cell datasets which could be used to validate whether the purported immune-cell-specific genes are genuinely enriched in immune populations adjacent to tumour cells. In the drug repurposing analyses, the current study does not explicitly handle prostate cancer subtypes such as HSPC, CRPC, NEPC, or DNPC and co-treatment with androgen receptor antagonism or radiotherapy.

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