Biomolecules and Biomedicine
ISSN: 2831-0896 (Print) | ISSN: 2831-090X (Online)
Journal Impact Factor® (2024): 2.2
CiteScore® (2024): 5.2
www.biomolbiomed.com | blog.bjbms.org

**SUPPLEMENTAL DATA**

# Regulatory B cells and *SIVA1-LAG3* immune suppression predict poor prognosis in prostate cancer

**Weicheng Tian[1,2#], Yize Li[4,5#], Jinchuang Li[1,6#], Chenyu Liang[1,5], Shanghua Cai[4,5], Biyan Wen[1,5], Zeheng Tan[1,6], Zhenjie Wu[1,6], Yulin Deng[7], Zige Liu[3], Yongding Wu[6], Haiyin Xiao[6], Weide Zhong[1,2], Huichan He[4,5], Chitin Hon[3*], Jianheng Ye[1,2*]**

[1]Department of Urology, Guangzhou First People's Hospital, Guangzhou Medical University, Guangzhou, Guangdong, China;

[2]State Key Laboratory of Quality Research in Chinese Medicine, Macau University of Science and Technology, Taipa, Macau, China;

[3]Respiratory Disease AI Laboratory in Epidemic Intelligence and Applications of Medical Big Data Instruments, Faculty of Innovation Engineering, Macau University of Science and Technology, Taipa, Macau SAR, China;

[4]Guangzhou Laboratory, Guangzhou International Bio Island, Guangzhou, Guangdong, China;

[5]Guangdong Provincial Key Laboratory of Urology, the First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University, Guangzhou, Guangdong, China;

[6]Guangdong Key Laboratory of Clinical Molecular Medicine and Diagnostics, Guangzhou First People's Hospital, School of Medicine, South China University of Technology, Guangzhou, Guangdong, China;

[7]Department of Urology, The First Dongguan Affiliated Hospital, Guangdong Medical University, Dongguan, Guangdong, China.

[#]Equally contributed to this work: Weicheng Tian, Yize Li, and Jinchuang Li.

*Correspondence to Jianheng Ye: yejianheng1989@hotmail.com and Chitin Hon: cthon@must.edu.mo.

**Full article is available at the following link:** Regulatory B cells and SIVA1-LAG3 immune suppression predict poor prognosis in prostate cancer

**Table S1. Representative ligands from patient samples**

| fname | Tumor | Adj-normal | Grade | Gleason | Grade group | Path stage | Margin | Age | PSA pre-op | Race | BMI |
|---|---|---|---|---|---|---|---|---|---|---|---|
| PCA3 | Y | Y | low_grade | 3+4=7 | 2 | pT2a | neg | 69 | 2.16 | White | 27 |
| PCA4 | Y | Y | high_grade | 4+5=9 | 5 | pT3a | neg | 71 | 4.86 | White | 33.2 |
| PCA5 | Y | Y | low_grade | 4+3=7 | 3 | pT3a | neg | 68 | 12.35 | White | 32.9 |
| PCA6 | Y | Y | high_grade | 3+5=8 | 4 | pT3a | neg | 57 | 8.37 | White | 28.6 |
| PCA7 | Y | N | high_grade | 5+5=10 | 5 | pT3b N1 | pos | 53 | 6.6 | White | 30 |
| PCA8 | Y | N | high_grade | 4+4=8 | 4 | pT2c | neg | 66 | 28.51 | White | 27.4 |
| PCA9 | Y | Y | low_grade | 4+3=7 | 3 | pT2c | neg | 65 | 3.2 | White | 33.4 |
| PCA10 | Y | N | low_grade | 3+3=6 | 1 | pT3a | neg | 46 | 3.14 | White | 28.1 |
| PCA11 | Y | Y | low_grade | 3+4=7 | 2 | pT2c | neg | 71 | 10.31 | White | 29.8 |
| PCA12 | Y | Y | low_grade | 3+3=6 | 1 | pT2 N0 | neg | 54 | 4.1 | White | 27.8 |
| PCA15 | Y | Y | low_grade | 3+4=7 | 2 | pT2 N0 | neg | 71 | 5.83 | White | 27.2 |
| PCA16 | Y | N | high_grade | 4+5=9 | 5 | pT3a N1 | neg | 61 | 4.88 | White | 25.8 |
| PCA17 | Y | Y | low_grade | 3+3=6 | 1 | pT2 N0 | neg | 65 | 3.01 | White | 26.5 |

EARLY ACCESS

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| PCA18 | Y | Y | low_grade | 3+3=6 | 1 | pT2 N0 | neg | 59 | 3.67 | White | 25.8 |
| PCA19 | Y | Y | high_grade | 5+3=8 | 4 | pT3b N1 | neg | 59 | 12.66 | White | 30.6 |
| PCA20 | Y | Y | low_grade | 3+4=7 | 2 | pT2 Nx | neg | 61 | 5.17 | White | 28 |
| PCA21 | Y | Y | low_grade | 3+4=7 | 2 | pT3a N0 | neg | 73 | 7.6 | White | 26.5 |
| PCA22 | Y | Y | high_grade | 4+5=9 | 5 | pT3b N0 | neg | 63 | 5.16 | Hispanic | 23.4 |
| PCA24 | N | Y | low_grade | 3+3=6 | 1 | pT2 N0 | neg | 61 | 9.77 | White | 30.9 |

Abbreviations: Adj-normal, adjacent normal tissue; BMI, body mass index; N, no; N0, no regional lymph node metastasis; N1, regional lymph node metastasis present; neg, negative surgical margin; Nx, regional lymph nodes cannot be assessed; pos, positive surgical margin; PSA pre-op, preoperative prostate-specific antigen; pT, pathological tumor stage.

**Table S2. Bregs-related ligands used for modeling**

| Negative regulation of immune system genes | B10 associated genes | Bregs associated genes |
|---|---|---|
| *CD68* | *APOE* | *CTLA4* |
| *CLEC2G* | *NRP2* | *PTPRJ* |
| *DHX58* | *TBC1D9* | *CD5* |
| *DUSP3* | *NTN1* | *FCRL5* |
| *FBXW7* | *RBM47* | *CD300LF* |
| *FGR* | *LIPC* | *CCR1* |

EARLY ACCESS

*FSTL3*
*GPR137*
*LDLR*
*LILRB4A*
*NBL1*
*NLRC3*
*NOD2*
*NRARP*
*PRDM1*
*PRDM3*
*RHBDD3*
*SERPINB9*
*SOCS1*
*SOCS6*
*SYT11*
*TIP2*
*TWSG1*
*TNFRSF4*
*TNFRSF14*
*TRAF3IP1*
*TLL12*
*SLC7A7*
*ZFP945*
*NEBL*
*CAPG*
*ADH1C*
*AHNAK*
*TNFRSF8*
*S100A11*
*CSF2RB*
*KRT222*
*CACTD1*
*ACTN1*
*LYSMD2*
*SERPINC1*
*CEBPD*
*C130026I21RIK*
*CLSTN1*
*ZBTB32*
*RGS13*
*ATXN1*
*PIK3CG*
*LGALS3*
*IL10*
*PDCD1*
*CDKN2A*
*GPR55*
*TIGIT*
*SH2D1B1*

EARLY ACCESS

*UBASH3B*
*AHR*
*ZC3H8*
*EGR1*
*ADORA2A*
*DLG1*
*DOCK2*
*SPN*
*TLR4*
*PRKCZ*
*ZC3H12D*
*NR2F6*
*PYCARD*
*HSPD1*
*PAWR*
*EBI3*
*VSIR*
*SIRPA*
*SLAMF1*
*PTPN22*
*GFER*
*MIC31*
*BCL2L15*
*RAB11FIP4*
*MLKL*
*RYK*
*TNFSF9*
*TNFSF8*
*STK39*
*PERP*
*TIMD2*
*FHIT*
*ZAP70*
*CRIM1*
*BAG3*
*STAT1*
*HDAC9*
*NT5E*
*ELK3*
*NEK6*
*CD70*
*CTLA4*

EARLY ACCESS

*PGLYRP2*
*FURIN*
*SIRT1*
*CUEDC2*
*RIN3*
*TLR6*
*NLRC5*
*GRN*
*CD274*
*CIQBPP*
*PRDX2*
*GIT1*
*CD80*
*CD86*
*CD44*
*CTLA4*
*PTPRJ*
*CD5*
*FCRL5*
*CD300LF*
*CCR1*
*PTPRJ*
*CD5*
*FCRL5*
*CD300LF*
*CCR1*
*LGALS3*
*IL10*
*PDCD1*
*CDKN2A*
*GPR55*
*TIGIT*
*SH2D1B1*

EARLY ACCESS

*LGALS3*

*IL10*

*PDCD1*

*CDKN2A*

*GPR55*

*TIGIT*

*SH2D1B1*

**Table S3. Cell-type-specific ligands used for annotation**

| Cell type | Marker genes |
|---|---|
| B cell | "MS4A1", "CD79B", "BANK1", "CD79A" |
| Basal cell | "KRT5","KRT18","KRT8","KRT19" |
| T cell | "CD3E","CD2","CD3D","CD3G" |
| Endothelial cell | "RAMP2", "EGFL7", "PLVAP", "AQP1" |
| Luminal cell | "KRT5","KRT18","KRT8","AR" |
| Macrophage | "C1QA", "C1QC", "C1QB", "GPR34" |
| Fibroblast | "DCN", "IGF1", "APOD", "FBLN1" |
| Pericytes | "RGS5", "ACTA2", "MYH11", "MT1M" |
| Monocytes | "S100A9", "FCN1", "S100A8", "EREG" |
| Mast cell | "TPSAB1", "CPA3", "HPGDS", "RGS13" |

EARLY ACCESS

**Table S4. Summary of datasets utilized for model training and validation**

| Dataset ID | Dataset name | Title | Alias | GEO/Array Express accession | SRA Accession | cBioPortal | Total # of Samples | Outcome | Platform | Technology | Data preprocessing |
|---|---|---|---|---|---|---|---|---|---|---|---|
| PCaDB 0001 | TCGA -PRAD | Prostate Adenocarcinoma (The Cancer Genome Atlas) | | | | Prostate Adenocarcinoma (TCGA, PanCancer Atlas) | 547 | Time to BCR Overall Survival | Illumina HiSeq 2000 | RNA sequencing | PCaDB |
| PCaDB 0002 | CPC-Gene | The Proteogenomic Landscape of Curable Prostate Cancer | | GSE107299 | | Prostate Adenocarcinoma (CPC-GENE, Nature 2017) | 213 | Time to BCR | Affymetrix Human Gene 2.0 ST Array (Batch 1,3,4,5) Affymetrix | Microarray | GEO |

EARLY ACCESS

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Human Transcriptome Array 2.0 (Batch 2) | | |
| PCaDB 0003 | Taylor | Whole-transcript and exon-level expression data for human primary and metastatic prostate cancer samples and control normal adjacent | MSKCC; MSKCC2010 | GSE21034 | | Prostate Adenocarcinoma (MSKCC, Cancer Cell 2010) | 185 | Time to BCR | Affymetrix Human Exon 1.0 ST Array | Microarray | PCaDB |

EARLY ACCESS

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | benign prostate | | | | | | | | | |
| PCaDB 0004 | DKFZ | Prostate Cancer (DKFZ, Cancer Cell 2018) | DKFZ2018 | | | Prostate Cancer (DKFZ, Cancer Cell 2018) | 118 | Time to BCR | Illumina HiSeq 2000 | RNA sequencing | cBioPortal |
| PCaDB 0005 | GSE54460 | RNAseq Analysis of Formalin-Fixed Paraffin-Embedded Prostate Cancer Tissues | | GSE54460 | SRP036848 | | 100 | Time to BCR | Illumina HiSeq 2000 | RNA sequencing | PCaDB |
| PCaDB 0007 | Stockholm | Prostate cancer stratification using molecular | CamCap:Stockolm | GSE70769 | | | 94 | Time to BCR | Illumina HumanHT-12 V4.0 Expressi | Microarray | GEO |

EARLY ACCESS

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | profiles [Stockholm ExpressionArray] | | | | | | | on Beadchip | | |
| PCaDB 0008 | Cancer Map | CancerMap project prostate cancer microarray dataset | | GSE94767 | | | 236 | Time to BCR | Affymetrix Human Exon 1.0 ST Array | Microarray | PCaDB |
| PCaDB 0009 | CIT | Expression array for Multi-omics molecular profiling of primary prostate adenocarcinoma | | E-MTAB-6128 | | | 141 | Time to BCR | Affymetrix Human Gene 2.0 ST Array | Microarray | PCaDB |

The original dataset annotation "Time to BCR" served as a proxy for BCRFS and was standardized as the primary endpoint in this study. Expression matrices for all bulk cohorts were obtained from PCaDB. RNA-seq datasets underwent TMM normalization, while

EARLY ACCESS

microarray datasets were normalized using the RMA method. Gene annotations were standardized to Ensembl IDs, and for genes represented by multiple probes, the probe with the largest interquartile range (IQR) was selected as the representative probe.

**Abbreviations**: BCR, biochemical recurrence; CPC-Gene, Canadian Prostate Cancer Genome Network; DKFZ, German Cancer Research Center; GEO, Gene Expression Omnibus; MSKCC, Memorial Sloan Kettering Cancer Center; PCaDB, Prostate Cancer Database; PRAD, prostate adenocarcinoma; RNA-seq, RNA sequencing; SRA, Sequence Read Archive; TCGA, The Cancer Genome Atlas.

**Table S5. Clinicopathological characteristics and follow-up information of the tissue microarray (TMA) cohort**

| Tissue ID | **Gleason score** | Age (years) | Date of hospitalization | Follow-up date | Survival status | Date of death | Survival time (as of November 2021) | Recurrence status | Paraffin block ID | BCRFS.time | BCRFS | BCRFS.time | BCRFS | OS.time | OS | Gleason score | Tumor | Normal |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| P1306707 | 3+4=7 | 74 | 2013/06 | 2021/11/5 | 0 | | 101 | Recurrence | 13067407-C | 101 | 1 | 101 | 1 | 101 | 0 | 7 | 2 | 1 |
| P1308580 | 3+4=7 | 58 | 2013/08 | 2021/11/5 | 0 | | 99 | No Recurrence | 13085807-C | 99 | 0 | 99 | 0 | 99 | 0 | 7 | 2 | 1 |
| P1311610 | 3+3=6 | 61 | 2013/11 | 2021/11/8 | 0 | | 96 | No Recurrence | 13116106-C | 96 | 0 | 96 | 0 | 96 | 0 | 6 | 3 | 0 |

EARLY ACCESS

| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | e | | | | | | | | | | | |
| P13117007 | 3+4=7 | 70 | 2013/11 | 2021/11/5 | 0 | | 96 | Recurrence | 1311700 7-C | 96 | 1 | 96 | 1 | 96 | 0 | 7 | 3 | 3 |
| P13125608 | 4+4=8 | 56 | 2013/12 | 2021/11/5 | 0 | | 95 | No Recurrence | 1312560 8-C | 95 | 0 | 95 | 0 | 95 | 0 | 8 | 3 | 1 |
| P14017207 | 4+3=7 | 72 | 2014/01 | 2021/11/17 | 0 | | 94 | Recurrence | 1401720 7-C | 94 | 1 | 94 | 1 | 94 | 0 | 7 | 3 | 1 |
| P14026107 | 3+4=7 | 61 | 2014/02 | 2021/11/8 | 0 | | 93 | No Recurrence | 1402610 7-C | 93 | 0 | 93 | 0 | 93 | 0 | 7 | 2 | 1 |
| P14046907 | 4+3=7 | 69 | 2014/04 | 2021/11/8 | 0 | | 91 | Recurrence | 1404690 7-C | 91 | 1 | 91 | 1 | 91 | 0 | 7 | 1 | 2 |
| P14047107 | 4+3=7 | 71 | 2014/04 | 2021/11/5 | 1 | 2020/6/24 | 74 | | 1404710 7-C | | | 74 | 1 | 74 | 1 | 7 | 1 | 1 |
| P14056407 | 3+4=7 | 64 | 2014/05 | 2021/11/17 | 0 | | 90 | No Recurrence | 1405640 7-C | 90 | 0 | 90 | 0 | 90 | 0 | 7 | 3 | 1 |
| P14066508 | 4+4=8 | 65 | 2014/06 | 2021/11/12 | 0 | | 89 | No Recurrence | 1406650 8-C | 89 | 0 | 89 | 0 | 89 | 0 | 8 | 3 | 1 |

EARLY ACCESS

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| P14065709 | 4+5=9 3+4=7, a small amount was scored as 5 | 57 | 2014/06 | 2021/11/5 | 0 | | 89 | Recurrence | 14065709-C | 89 | 1 | 89 | 1 | 89 | 0 | 9 | 2 | 1 |
| P14067005 | | 70 | 2014/06 | 2021/11/5 | 0 | | 89 | No Recurrence | 14067005-C | 89 | 0 | 89 | 0 | 89 | 0 | 7 | 3 | 1 |
| P14066507 | 4+3=7 | 65 | 2014/06 | 2021/11/5 | 0 | | 89 | No Recurrence | 14066507-C | 89 | 0 | 89 | 0 | 89 | 0 | 7 | 3 | 1 |
| P14077607 | 3+4=7 4+3=7, | 76 | 2014/07 | 2021/11/5 | 1 | 2021/10/10 | 87 | | 14077607-C | | | 87 | 1 | 87 | 1 | 7 | 2 | 1 |
| P14076805 | partially scored as 5 | 68 | 2014/07 | 2021/11/5 | 0 | | 88 | No Recurrence | 14076805-C | 88 | 0 | 88 | 0 | 88 | 0 | 7 | 2 | 1 |

EARLY ACCESS

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| P14087305 | 4+4=8, a small focal area was scored as 5 | 73 | 2014/08 | 2021/11/5 | 0 | 87 | No Recurrence | 14087305-C | 87 | 0 | 87 | 0 | 87 | 0 | 8 | 2 | 0 |
| P14096906 | 3+3=6 | 69 | 2014/09 | 2021/11/5 | 0 | 86 | No Recurrence | 14096906-C | 86 | 0 | 86 | 0 | 86 | 0 | 6 | 1 | 1 |
| P14096407 | 3+4=7 | 64 | 2014/09 | 2021/11/8 | 0 | 86 | No Recurrence | 14096407-C | 86 | 0 | 86 | 0 | 86 | 0 | 7 | 2 | 1 |
| P14096107 | 3+4=7 | 61 | 2014/09 | 2021/11/8 | 0 | 86 | No Recurrence | 14096107-C | 86 | 0 | 86 | 0 | 86 | 0 | 7 | 3 | 2 |
| P14096707 | 3+4=7 | 67 | 2014/09 | 2021/11/8 | 0 | 86 | No Recurrence | 14096707-C | 86 | 0 | 86 | 0 | 86 | 0 | 7 | 2 | 1 |
| P14105 | 3+4= | 57 | 2014/10 | 2021/11 | 0 | 85 | No | 14105 | 85 | 0 | 85 | 0 | 85 | 0 | 7 | 2 | 2 |

EARLY ACCESS

| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 707 | 7 | | | /8 | | | Recurrence | 7-C | | | | | | | | | | | |
| P14106407 | 3+4=7 | 64 | 2014/10 | 2021/11/5 | 0 | 85 | No Recurrence | 1410640 7-C | 85 | 0 | 85 | 0 | 85 | 0 | 7 | 2 | 2 | | |
| P14116307 | 3+4=7 | 63 | 2014/11 | 2021/11/12 | 0 | 84 | No Recurrence | 1411630 7-C | 84 | 0 | 84 | 0 | 84 | 0 | 7 | 2 | 2 | | |
| P14127406 | 3+3=6 | 74 | 2014/12 | 2021/11/5 | 0 | 83 | No Recurrence | 1412740 6-C | 83 | 0 | 83 | 0 | 83 | 0 | 6 | 2 | 1 | | |
| P14126906 | 3+3=6 | 69 | 2014/12 | 2021/11/5 | 0 | 83 | No Recurrence | 1412690 6-C | 83 | 0 | 83 | 0 | 83 | 0 | 6 | 2 | 2 | | |
| P14126807 | 4+3=7 | 68 | 2014/12 | 2021/11/8 | 0 | 83 | No Recurrence | 1412680 7-C | 83 | 0 | 83 | 0 | 83 | 0 | 7 | 3 | 1 | | |
| P14126007 | 3+4=7 | 60 | 2014/12 | 2021/11/5 | 0 | 83 | No Recurrence | 1412600 7-C | 83 | 0 | 83 | 0 | 83 | 0 | 7 | 2 | 2 | | |
| P11017 | 3+4= | 71 | 2011/01 | 2021/11 | 1 | 2021/5/ | 124 | | 1101710 | | | 124 | 1 | 124 | 1 | 7 | 2 | 0 | |

EARLY ACCESS

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 107 | 7 | | | /5 | 19 | | | 7-C | | | | | | | | | | |
| P14077206 | 3+3=6 | 72 | 2014/07 | 2021/11/12 | 0 | | 88 | Recurrence | 1407720 6-C | 88 | 1 | 88 | 1 | 88 | 0 | 6 | 1 | 1 |
| P13106007 | 3+4=7 | 60 | 2013/10 | 2021/11/8 | 1 | 2020/6/1 | 80 | | 1310600 7-C | | | 80 | 1 | 80 | 1 | 7 | 1 | 1 |
| P14106306 | 3+3=6 | 63 | 2014/10 | 2021/11/5 | 0 | | 85 | No Recurrence | 1410630 6-C | 85 | 0 | 85 | 0 | 85 | 0 | 6 | 2 | 2 |
| P13096707 | 4+3=7 | 67 | 2013/09 | 2021/11/5 | 0 | | 98 | No Recurrence | 1309670 7-C | 98 | 0 | 98 | 0 | 98 | 0 | 7 | 2 | 2 |
| P14016406 | 3+3=6 | 64 | 2014/01 | 2021/11/5 | 0 | | 94 | No Recurrence | 1401640 6-C | 94 | 0 | 94 | 0 | 94 | 0 | 6 | 2 | 2 |
| P14116307 | 4+3=7 | 63 | 2014/11 | 2021/11/17 | 0 | | 84 | No Recurrence | 1411630 7-C | 84 | 0 | 84 | 0 | 84 | 0 | 7 | 3 | 2 |
| P14057207 | 3+4=7 | 72 | 2014/05 | 2021/11/8 | 0 | | 90 | Recurrence | 1405720 7-C | 90 | 1 | 90 | 1 | 90 | 0 | 7 | 2 | 2 |
| P14057205 | 3+4=7 | 72 | 2014/05 | 2021/11/5 | 0 | | 90 | No Recurrenc | 1405720 5-C | 90 | 0 | 90 | 0 | 90 | 0 | 7 | 2 | 2 |

EARLY ACCESS

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | e | | | | | | | | | | | |
| P14077006 | 3+3=6 | 70 | 2014/07 | 2021/11/5 | 0 | 88 | No Recurrence | 14077006-C | 88 | 0 | 88 | 0 | 88 | 0 | 6 | 2 | 1 |
| P14106307 | 4+3=7 | 63 | 2014/10 | 2021/11/12 | 0 | 85 | No Recurrence | 14106307-C | 85 | 0 | 85 | 0 | 85 | 0 | 7 | 2 | 2 |
| P14127207 | 3+4=7 | 72 | 2014/12 | 2021/11/5 | 0 | 83 | No Recurrence | 14127207-C | 83 | 0 | 83 | 0 | 83 | 0 | 7 | 2 | 2 |
| P13126508 | 4+4=8 | 65 | 2013/12 | 2021/11/5 | 0 | 95 | No Recurrence | 13126508-C | 95 | 0 | 95 | 0 | 95 | 0 | 8 | 2 | 1 |
| P14057107 | 3+4=7 | 71 | 2014/05 | 2021/11/5 | 0 | 90 | No Recurrence | 14057107-C | 90 | 0 | 90 | 0 | 90 | 0 | 7 | 2 | 2 |
| P14087306 | 3+3=6 | 73 | 2014/08 | 2021/11/5 | 0 | 87 | No Recurrence | 14087306-C | 87 | 0 | 87 | 0 | 87 | 0 | 6 | 2 | 1 |
| P13026806 | 3+3=6 | 68 | 2013/02 | 2021/11/5 | 0 | 105 | No Recurrence | 13026806-C | 105 | 0 | 105 | 0 | 105 | 0 | 6 | 2 | 1 |

EARLY ACCESS

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | e | | | | | | | | | | | |
| P14067207 | 3+4=7 | 72 | 2014/06 | 2021/11/5 | 0 | 89 | No Recurrence | 14067207-C | 89 | 0 | 89 | 0 | 89 | 0 | 7 | 1 | 2 |
| P14076207 | 4+3=7 | 62 | 2014/07 | 2021/11/5 | 0 | 88 | No Recurrence | 14076207-C | 88 | 0 | 88 | 0 | 88 | 0 | 7 | 3 | 1 |
| P14097507 | 3+4=7 | 75 | 2014/09 | 2021/11/5 | 0 | 86 | No Recurrence | 14097507-C | 86 | 0 | 86 | 0 | 86 | 0 | 7 | 2 | 2 |
| P14116206 | 3+3=6 | 62 | 2014/11 | 2021/11/5 | 0 | 84 | No Recurrence | 14116206-C | 84 | 0 | 84 | 0 | 84 | 0 | 6 | 2 | 2 |
| P13037007 | 3+4=7 | 70 | 2013/03 | 2021/11/5 | 0 | 104 | No Recurrence | 13037007-C | 104 | 0 | 104 | 0 | 104 | 0 | 7 | 2 | 2 |
| P13097707 | 3+4=7 | 77 | 2013/09 | 2021/11/5 | 0 | 98 | No Recurrence | 13097707-C | 98 | 0 | 98 | 0 | 98 | 0 | 7 | 2 | 0 |
| P14035707 | 3+4=7 | 57 | 2014/03 | 2021/11/5 | 0 | 92 | No Recurrence | 14035707-C | 92 | 0 | 92 | 0 | 92 | 0 | 7 | 2 | 2 |

EARLY ACCESS

| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | e | | | | | | | | | | | |
| P14046807 | 3+4=7 | 68 | 2014/04 | 2021/11/12 | 0 | | 91 | No Recurrence | 14046807-C | 91 | 0 | 91 | 0 | 91 | 0 | 7 | 1 | 1 |
| P14055707 | 4+3=7 | 57 | 2014/05 | 2021/11/5 | 0 | | 90 | No Recurrence | 14055707-C | 90 | 0 | 90 | 0 | 90 | 0 | 7 | 2 | 2 |
| P14076907 | 4+3=7 | 69 | 2014/07 | 2021/11/5 | 1 | 2021/10/1 | 87 | | 14076907-C | | | 87 | 1 | 87 | 1 | 7 | 2 | 2 |
| P14106507 | 4+3=7 | 65 | 2014/10 | 2021/11/5 | 0 | | 85 | No Recurrence | 14106507-C | 85 | 0 | 85 | 0 | 85 | 0 | 7 | 2 | 2 |
| P14116507 | 4+3=7 | 65 | 2014/11 | 2021/11/5 | 0 | | 84 | No Recurrence | 14116507-C | 84 | 0 | 84 | 0 | 84 | 0 | 7 | 2 | 2 |
| P13016307 | 3+4=7 | 63 | 2013/01/17 | 2021/11/5 | 0 | | 105 | No Recurrence | 13016307-C | 105 | 0 | 105 | 0 | 105 | 0 | 7 | 2 | 2 |
| I05A0380 | 3+4=7 | 74 | 2011/3/11 | 2021/11/10 | 1 | 2016/1/1 | 57 | No Recurrence | I05A0380-C | 57 | 0 | 57 | 0 | 57 | 1 | 7 | 1 | 1 |

EARLY ACCESS

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I05A0410 | 4+3=7 | 64 | 2012/7/18 | 2021/11/10 | 1 | 2020/4/27 | 93 | No Recurrence | I05A0410-C | 93 | 0 | 93 | 0 | 93 | 1 | 7 | 3 | 2 |
| I05A0423 | 4+3=7 | 72 | 2012/12/7 | 2021/11/10 | 1 | 2020/2/1 | 85 | No Recurrence | I05A0423-C | 85 | 0 | 85 | 0 | 85 | 1 | 7 | 2 | 2 |

Abbreviations: BCRFS, biochemical recurrence-free survival; ID, identifier; OS, overall survival; TMA, tissue microarray.

**Table S6. siRNA sequences used for gene silencing in this study**

| | siRNA | |
|---|---|---|
| SIVA1 | | 5'-3' |
| si-1 | sense | CUUCGACAGAACAAAUGGATT |
| | antisense | UCCAUUUGUUCUGUCGAAGTT |
| si-2 | sense | CAAGCGUCUUAACUGGGAATT |
| | antisense | UUCCCAGUUAAGACGCUUGTT |
| si-3 | sense | CCUACAGGCUACACAUCUATT |
| | antisense | UAGAUGUGUAGCCUGUAGGTT |
| si-NC | sense | UUCUCCGAACGUGUCACGUTT |
| | antisense | ACGUGACACGUUCGGAGAATT |

Abbreviations: si-NC, small interfering RNA negative control; siRNA, small interfering RNA; SIVA1, SIVA1 apoptosis inducing factor.

EARLY ACCESS

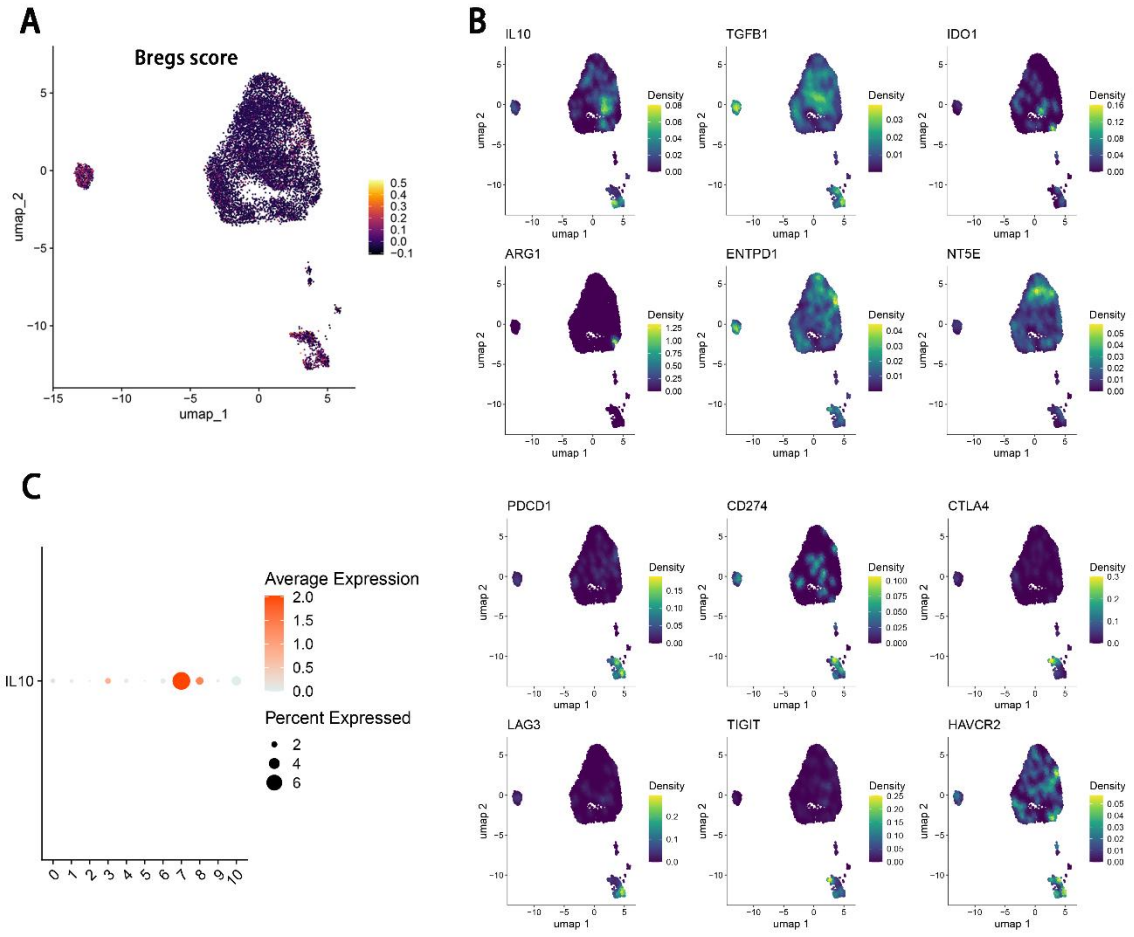



**Figure S1. Distribution and expression of Bregs-related ligands and immunosuppressive markers across B cell subpopulations. (A)** The UMAP distribution illustrates the presence of Bregs-related ligands. **(B)** Immunosuppressive ligands (IL10, TGFB1, LAG3, TIGIT, HAVCR2) expressed in Bregs are presented. **(C)** A bubble plot depicting IL10 expression across B cell subpopulations reveals the highest expression in cluster 7, identified as Bregs. **Abbreviations**: Bregs, regulatory B cells; HAVCR2, hepatitis A virus cellular receptor 2; IL10, interleukin 10; LAG3, lymphocyte activation gene 3; TGFB1, transforming growth factor beta 1; TIGIT, T cell immunoreceptor with Ig and ITIM domains; UMAP, Uniform Manifold Approximation and Projection.

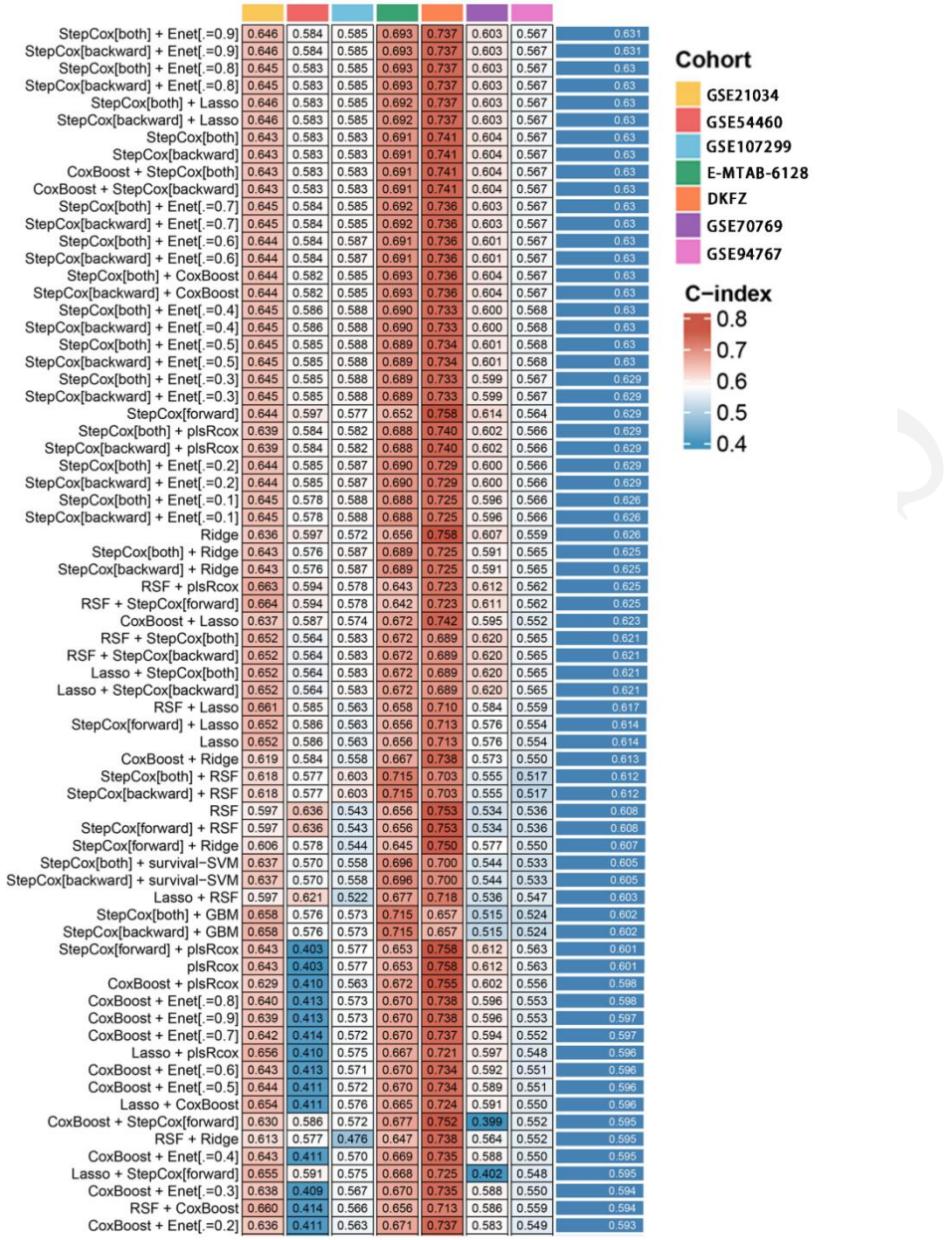



**Figure S2. Performance landscape of 117 machine-learning model combinations for construction of the BRPG prognostic signature across independent validation cohorts.** Heatmap showing the concordance index (**C-index**) of 117 candidate model combinations generated from 20 Bregs-related genes and multiple machine-learning algorithms. Each row represents one algorithm combination, and each column represents an independent validation cohort (GSE21034, GSE54460, GSE107299, E-MTAB-6128, DKFZ, GSE70769, and GSE94767). Warmer colors indicate better predictive performance, whereas cooler colors indicate lower performance. The

rightmost column summarizes the mean C-index across validation cohorts and was used to rank model performance. Among all tested strategies, StepCox (both) + Elastic Net (α = 0.9) achieved the best overall performance and was therefore selected for construction of the final BRPG model. **Abbreviations**: BRPG, Bregs-related prognostic gene; C-index, concordance index; DKFZ, German Cancer Research Center.

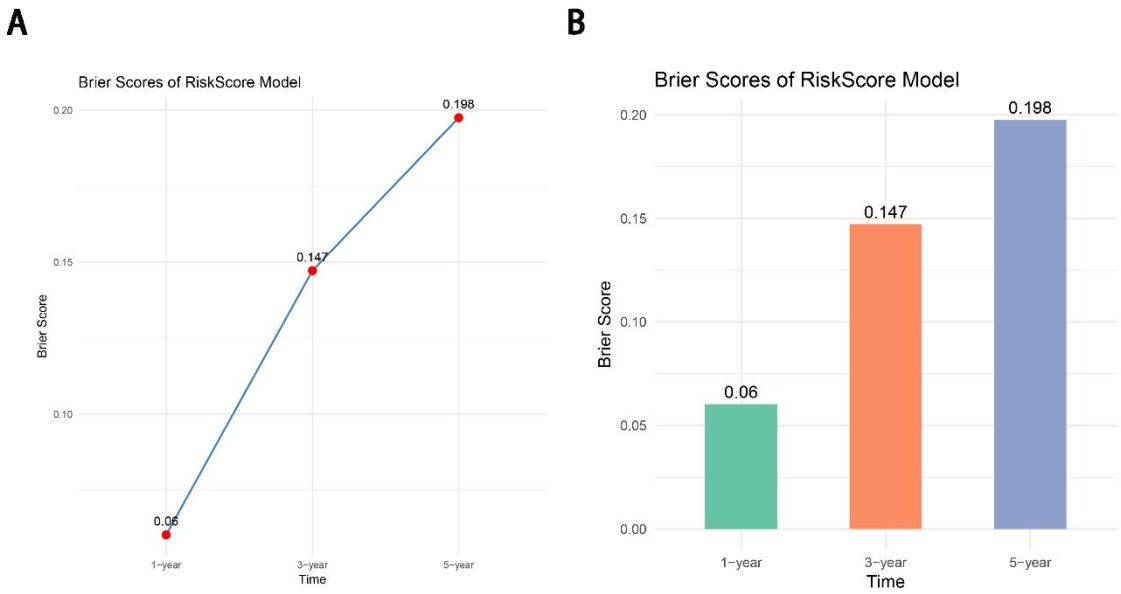



**Figure S3. Brier scores of the riskscore model at 1-year, 3-year, and 5-year follow-ups. (A)** A line plot illustrates the Brier scores of the RiskScore model at 1-year, 3-year, and 5-year follow-ups. **(B)** A bar plot displays the Brier scores at 1-year, 3-year, and 5-year follow-ups.

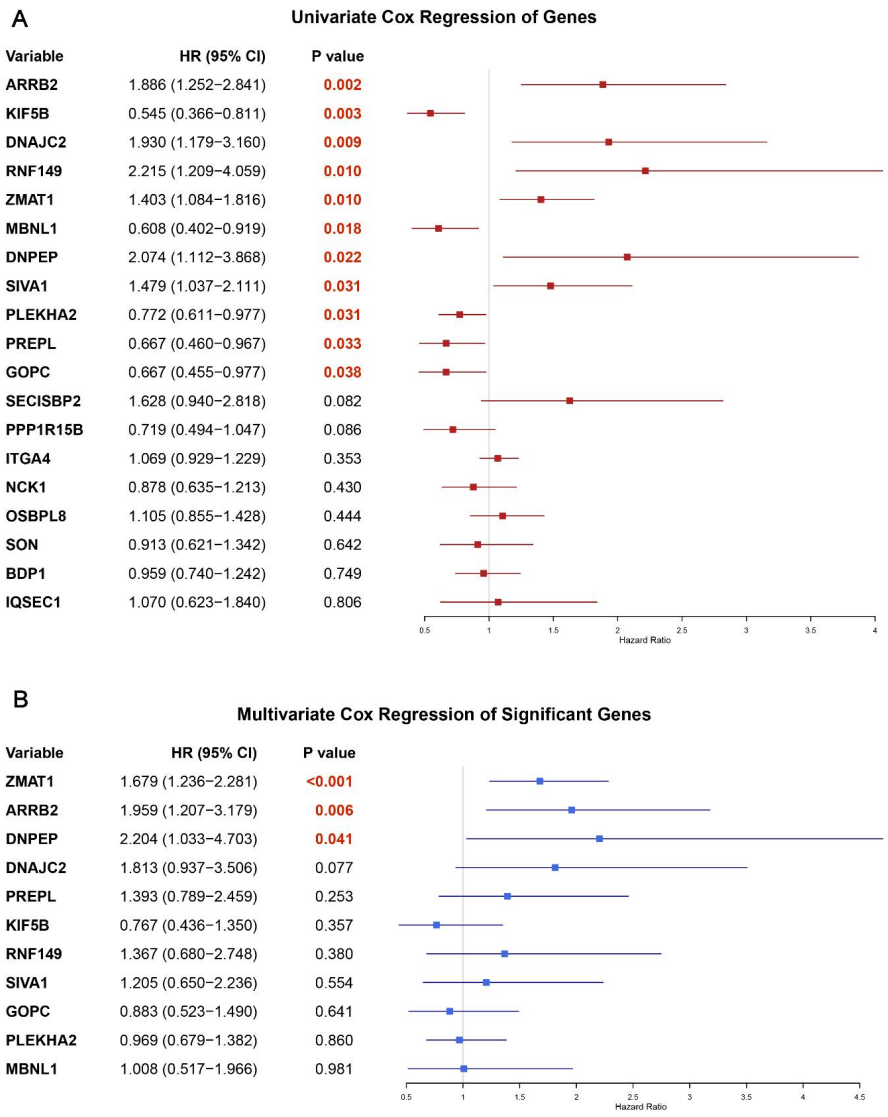



**Figure S4. Univariate and multivariate Cox regression analyses of functional core genes associated with BCRFS in prostate cancer. (A)** Univariate Cox regression analysis of 20 functional core genes. The HRs, 95% CIs, and p-values are provided for each gene. **(B)** Multivariate Cox regression analysis of genes identified in the univariate analysis. The HRs, 95% CIs, and p-values are reported for each variable. **Abbreviations**: BCRFS, biochemical recurrence-free survival; CI, confidence interval; HR, hazard ratio.

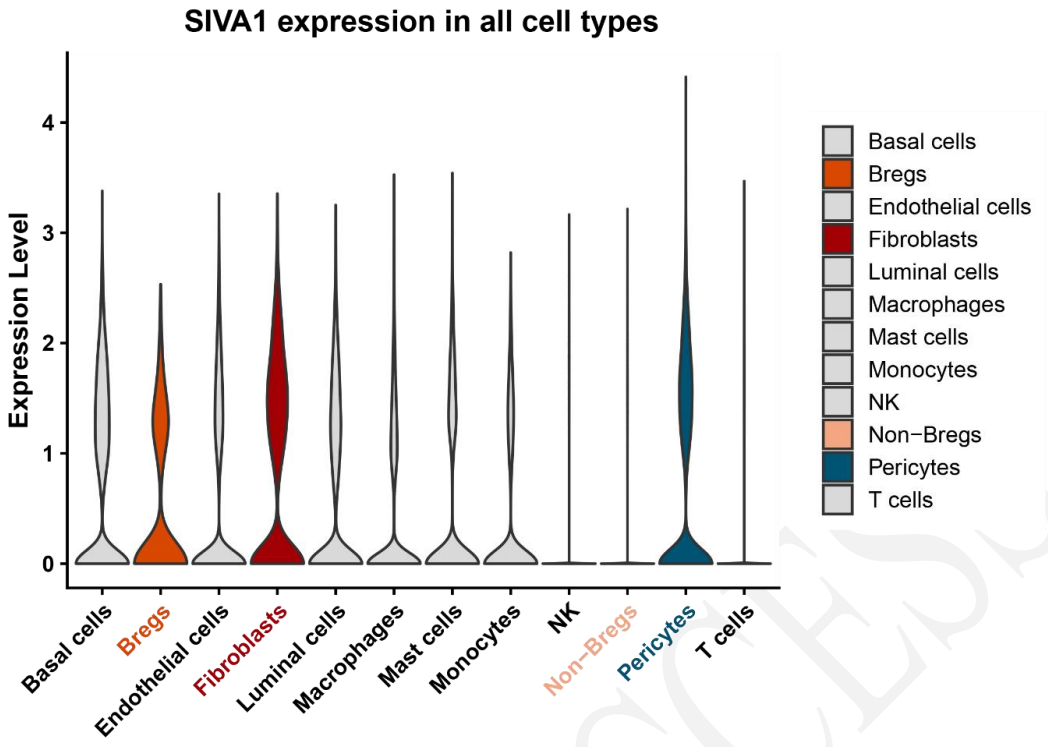



**Figure S5. Single-cell RNA-seq analysis shows relative enrichment of *SIVA1* expression in stromal compartments of the prostate tumor microenvironment.** Violin plots showing the distribution of SIVA1 expression across major cell populations identified in the single-cell dataset, including epithelial, stromal, and immune compartments. SIVA1 expression was relatively enriched in stromal cell populations, most prominently in fibroblasts and pericytes, while lower expression was observed in most other cell types. Detectable expression was also present in Bregs, supporting the association between SIVA1 and the Bregs-related program observed in downstream analyses. **Abbreviations**: Bregs, regulatory B cells; NK, natural killer; RNA-seq, RNA sequencing; SIVA1, SIVA1 apoptosis inducing factor; TME, tumor microenvironment.

EARLY ACCESS

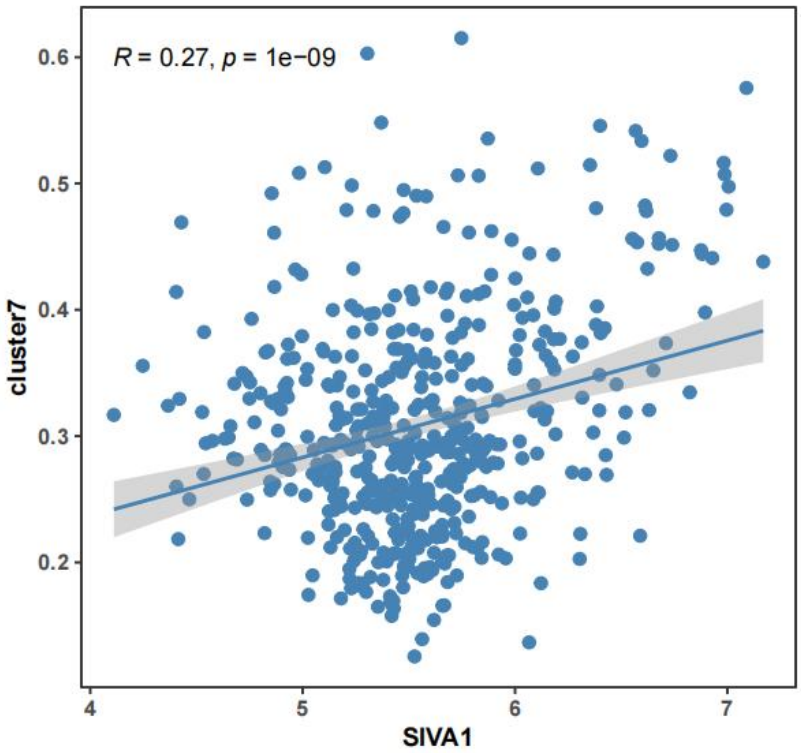



**Figure S6. Positive correlation between SIVA1 expression and the inferred abundance of the Bregs-associated cluster 7 in prostate cancer.** Scatter plot showing the relationship between SIVA1 expression and cluster 7 abundance across prostate cancer samples. Each dot represents one sample. The solid line indicates the fitted linear regression, and the shaded area represents the 95% confidence interval. Higher SIVA1 expression was significantly associated with greater abundance of cluster 7 ($R = 0.27$, $p = 1 \times 10^{-9}$), supporting the association of SIVA1 with the Bregs subpopulation. **Abbreviations**: Bregs, regulatory B cells; CI, confidence interval; SIVA1, SIVA1 apoptosis-inducing factor.

EARLY ACCESS

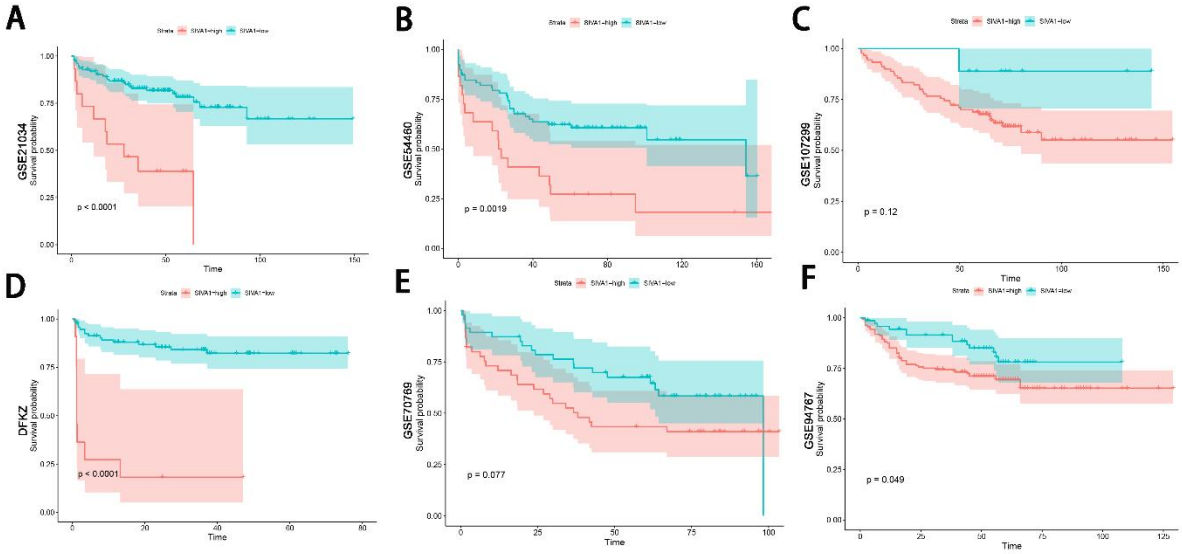



**Figure S7. Kaplan–Meier analyses across independent cohorts show that high SIVA1 expression is associated with shorter biochemical recurrence-free survival in prostate cancer.** Kaplan–Meier curves comparing patients with high and low **SIVA1** expression in six independent cohorts: **(A)** GSE21034, **(B)** GSE54460, **(C)** GSE107299, **(D)** DKFZ, **(E)** GSE70769, and **(F)** GSE94767. Across cohorts, higher SIVA1 expression was consistently associated with less favorable BCRFS; this association reached statistical significance in GSE21034, GSE54460, DKFZ, and GSE94767, while similar but non-significant trends were observed in GSE107299 and GSE70769. Shaded areas represent 95% confidence intervals, and *p* values were calculated using the log-rank test. **Abbreviations**: BCRFS, biochemical recurrence-free survival; CI, confidence interval; DKFZ, German Cancer Research Center; SIVA1, SIVA1 apoptosis inducing factor.

EARLY ACCESS

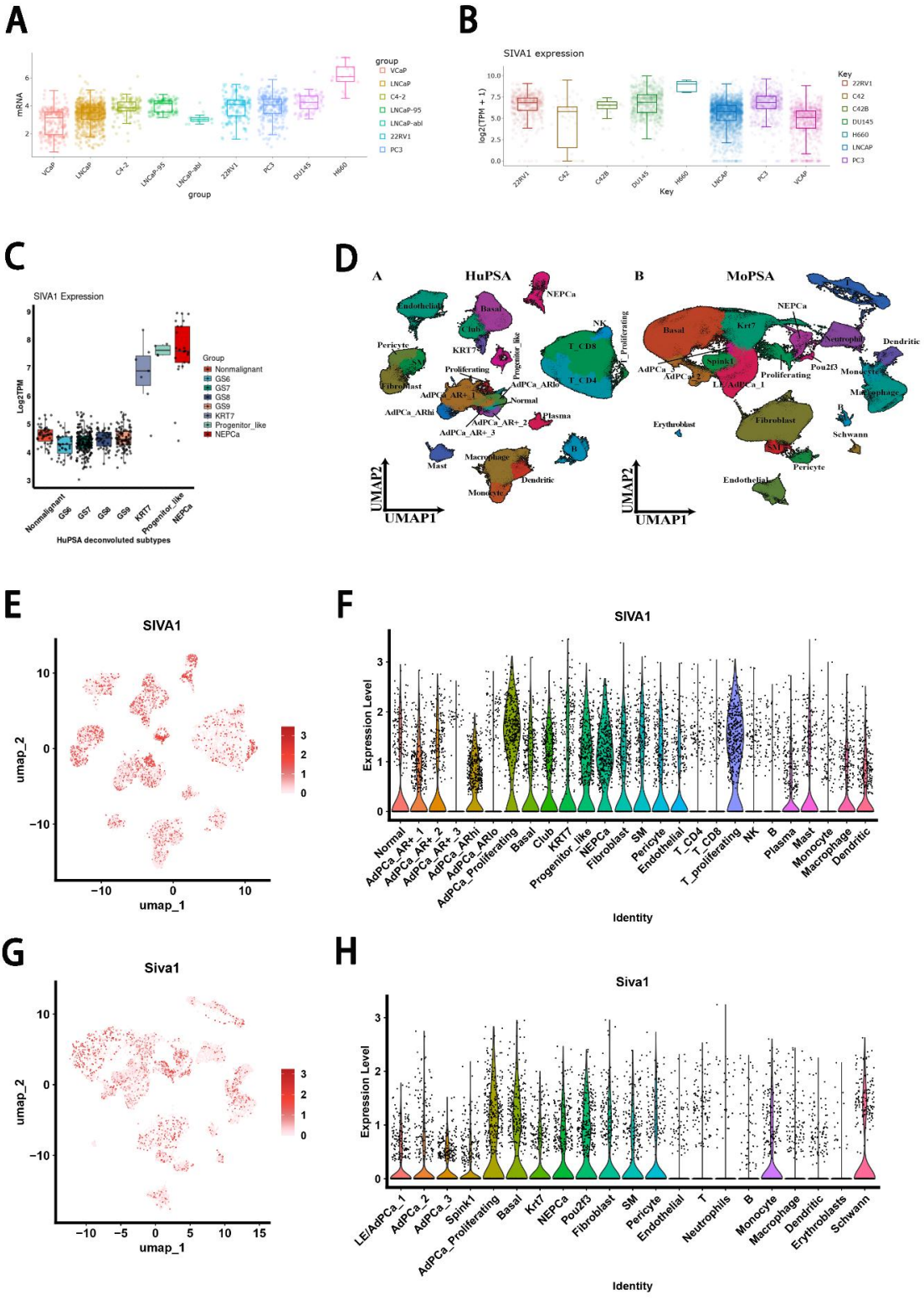



**Figure S8. SIVA1 expression is enriched in aggressive prostate cancer cell lines and subtype-defined cell populations across independent transcriptomic resources. (A)** Distribution of *SIVA1* mRNA expression across prostate cancer cell lines, showing heterogeneous expression with relatively high levels in aggressive

models, particularly H660. **(B)** Independent validation of *SIVA1* expression across prostate cancer cell lines in a second dataset, confirming elevated expression in aggressive cell lines, including H660, C4-2, and C4-2B. **(C)** Box plot of *SIVA1* expression across HuPSA-deconvoluted prostate cancer subtypes, showing marked enrichment in neuroendocrine prostate cancer, progenitor-like, and KRT7-positive subgroups compared with nonmalignant and lower-grade subtypes. **(D)** UMAP annotations of cell populations in the HuPSA (left) and MoPSA (right) single-cell datasets used for downstream visualization of *SIVA1/Siva1* expression. **(E)** UMAP feature plot showing the distribution of *SIVA1* expression in the HuPSA dataset. **(F)** Violin plots with overlaid points showing cell-type-specific *SIVA1* expression in HuPSA, with higher expression in aggressive epithelial populations, including progenitor-like, androgen receptor-positive adenocarcinoma-like, and neuroendocrine prostate cancer cells. **(G)** UMAP feature plot showing the distribution of *Siva1* expression in the MoPSA dataset. **(H)** Violin plots with overlaid points showing cell-type-specific *Siva1* expression in MoPSA, demonstrating differential expression across tumor, stromal, and immune compartments, with relatively higher levels in neuroendocrine and proliferative populations. **Abbreviations**: HuPSA, human prostate single-cell atlas; MoPSA, mouse prostate single-cell atlas; NEPCa, neuroendocrine prostate cancer; UMAP, Uniform Manifold Approximation and Projection.

EARLY ACCESS

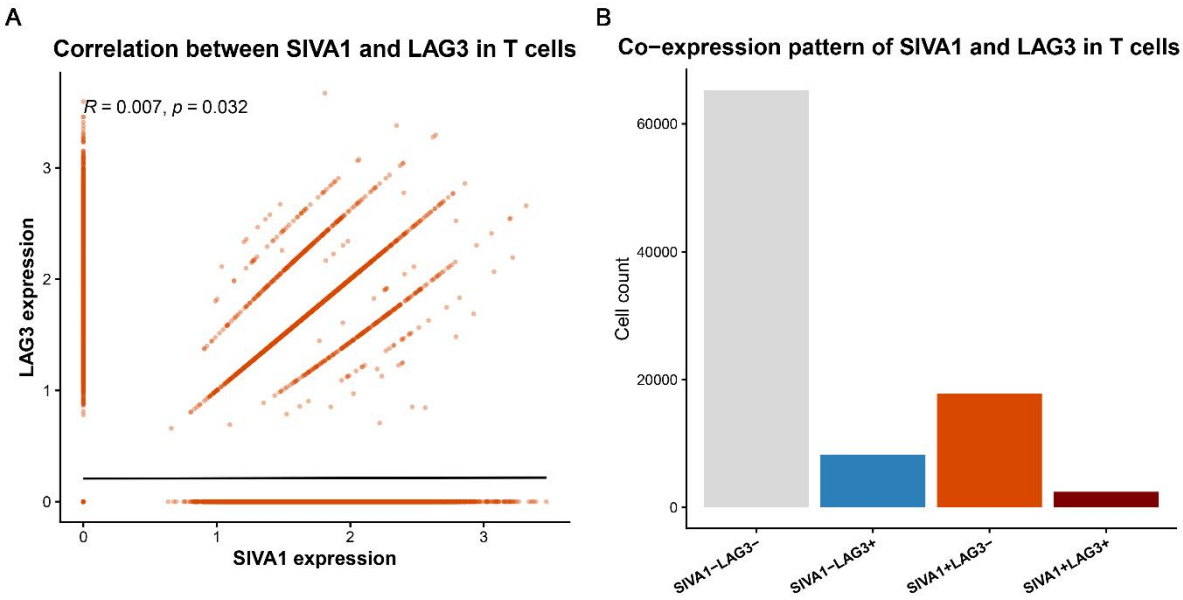



**Figure S9. Analysis of the relationship between *SIVA1* and *LAG3* expression in T cells based on single-cell RNA-seq data. (A)** A scatter plot illustrating the correlation between SIVA1 and LAG3 expression in T cells reveals a statistically significant yet negligible correlation (R = 0.007), suggesting a lack of meaningful association. **(B)** A bar plot depicting the co-expression patterns of SIVA1 and LAG3 in T cells indicates that only a small proportion of cells co-express both genes, while the majority display either single-gene expression or double-negative patterns.
**Abbreviations**: LAG3, lymphocyte activation gene 3; RNA-seq, RNA sequencing; SIVA1, SIVA1 apoptosis inducing factor.

EARLY ACCESS