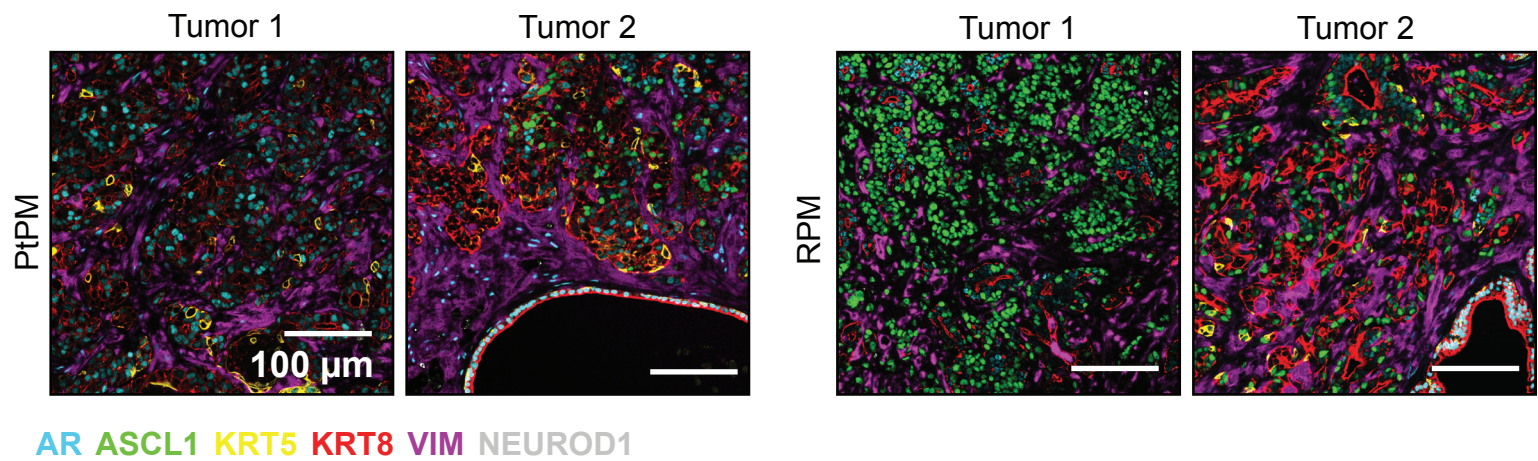


The neuroendocrine transition in prostate cancer is dynamic and dependent on ASCL1

In the format provided by the
authors and unedited

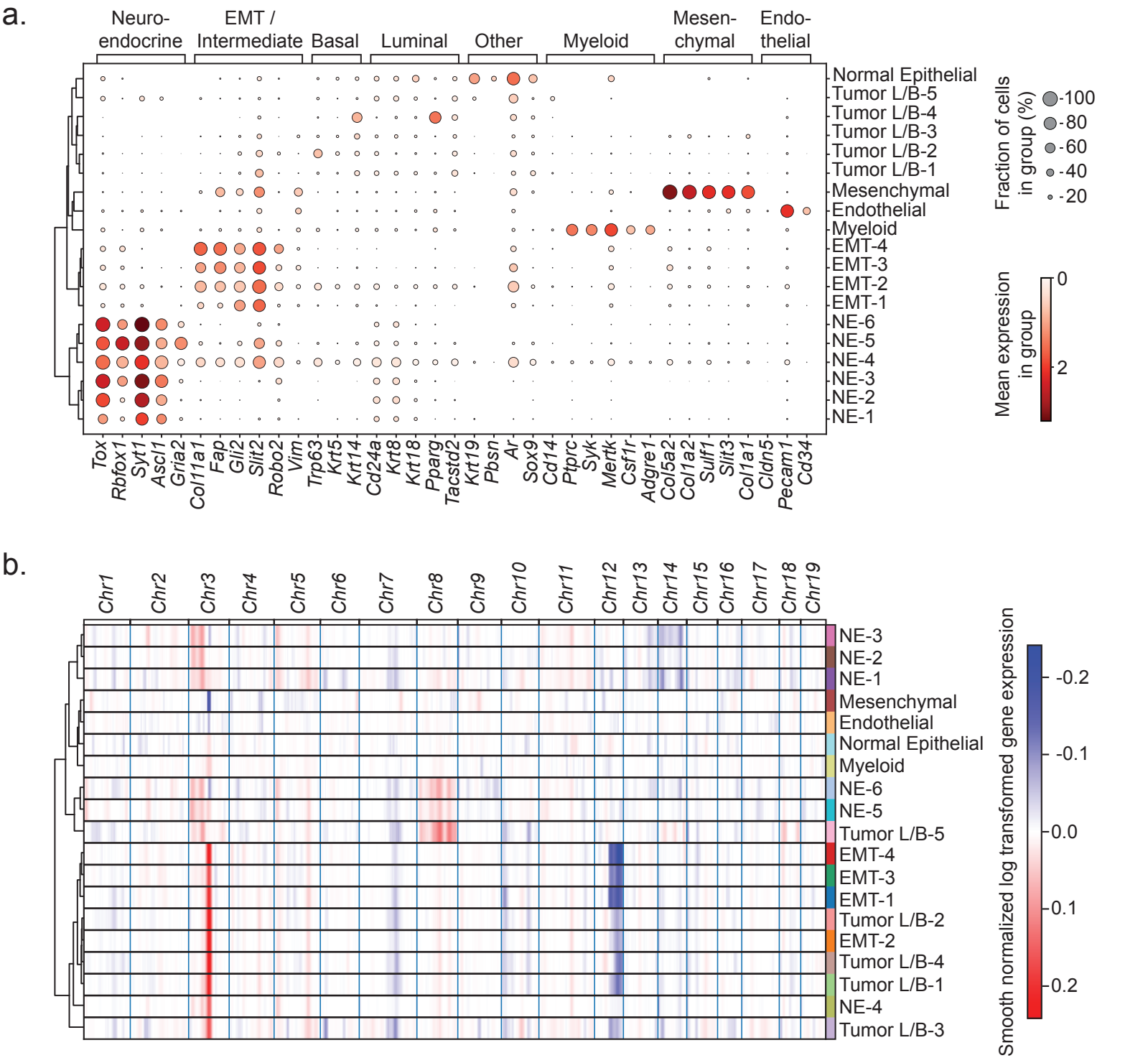
SUPPLEMENTARY FIGURE 1:



Supplementary Figure 1: *Rb1* loss sensitizes prostate tumors for the neuroendocrine transition

Representative multiplexed immunofluorescence images, $n = 2$ tumor samples (3-5 weeks post transplantation) for each genotype. Images related to Extended Data Fig. 2j-l. Data representative of $n = 4$ tumors (PtPM) and $n = 5$ tumors (RPM).

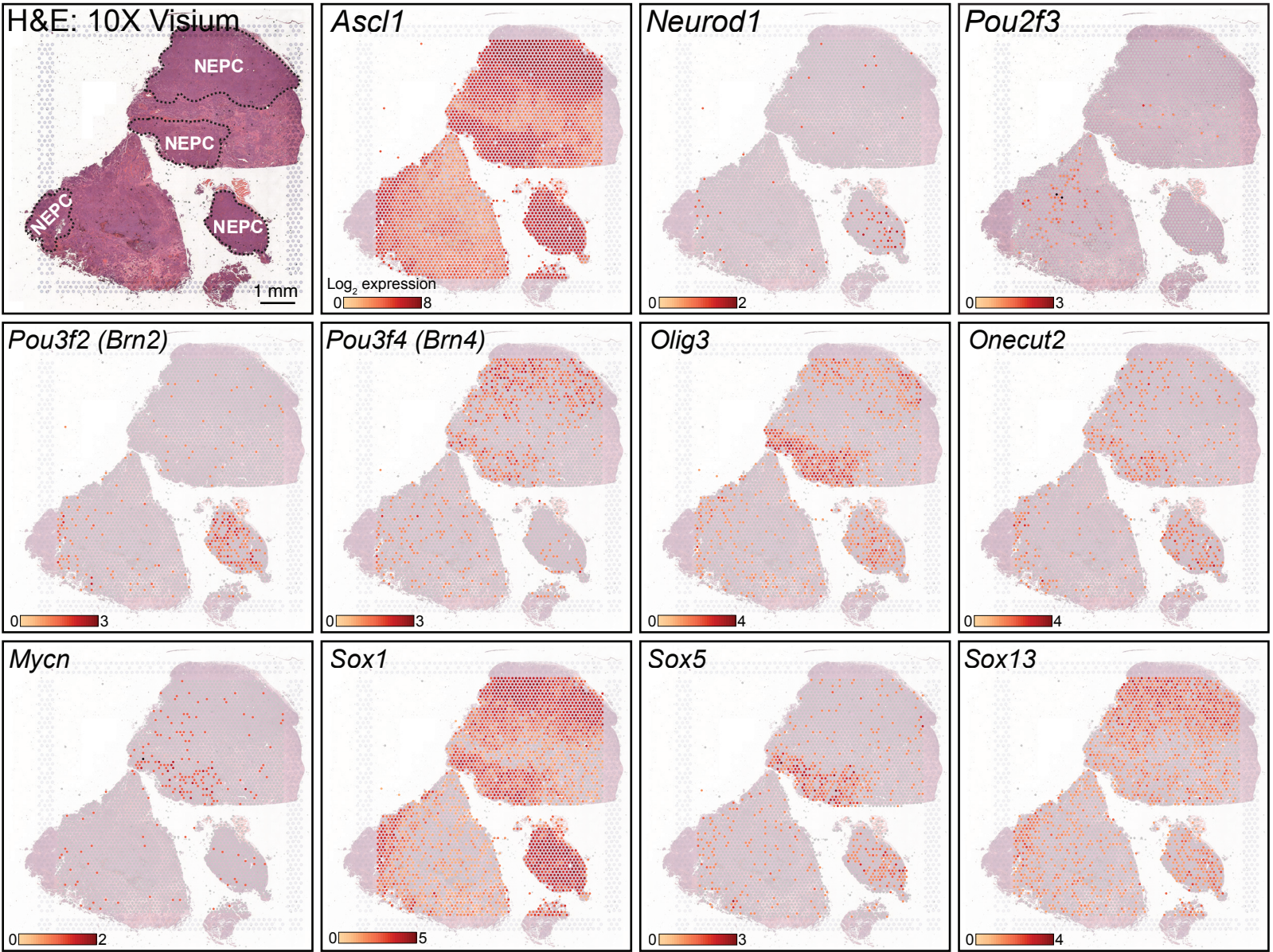
SUPPLEMENTARY FIGURE 2:



Supplementary Figure 2: Single nuclear RNAseq cell typing used as input for Visium analysis.

- a. Dot plot of selected marker genes (DEGs) associated with each of the indicated cell types from snRNA-seq profiled from RPM tumors. Data represent the mean normalized $\log_2(X+1)$ expression scaled from 0 to 1. Dot size represents percent of cells expressing a given gene.
- b. Inferred copy number variation (CNV) applying inferCNV to snRNAseq profiled from RPM tumors. Data represent the smooth normalized and log-transformed gene expression values across each murine chromosome. Inferred CNV for each cell type identified in panel a listed on the right.

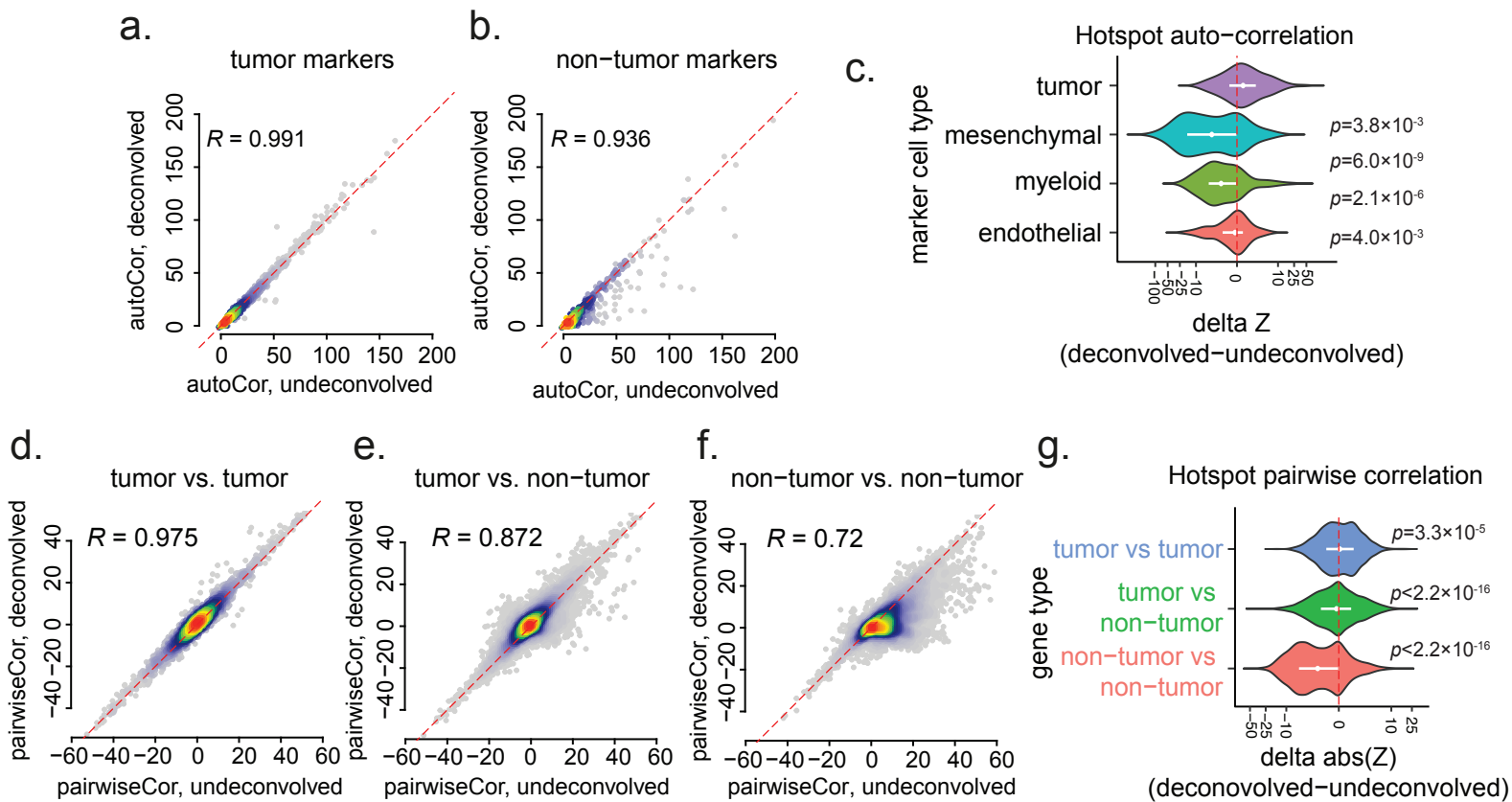
SUPPLEMENTARY FIGURE 3:



Supplementary Figure 3: Spatial heterogeneity in TF expression

Log₂ spatial expression (Visium) across the indicated genes. Top Left: H&E depicting NEPC tumor regions within two independent 10-week RPM tumors (related to Fig. 5c).

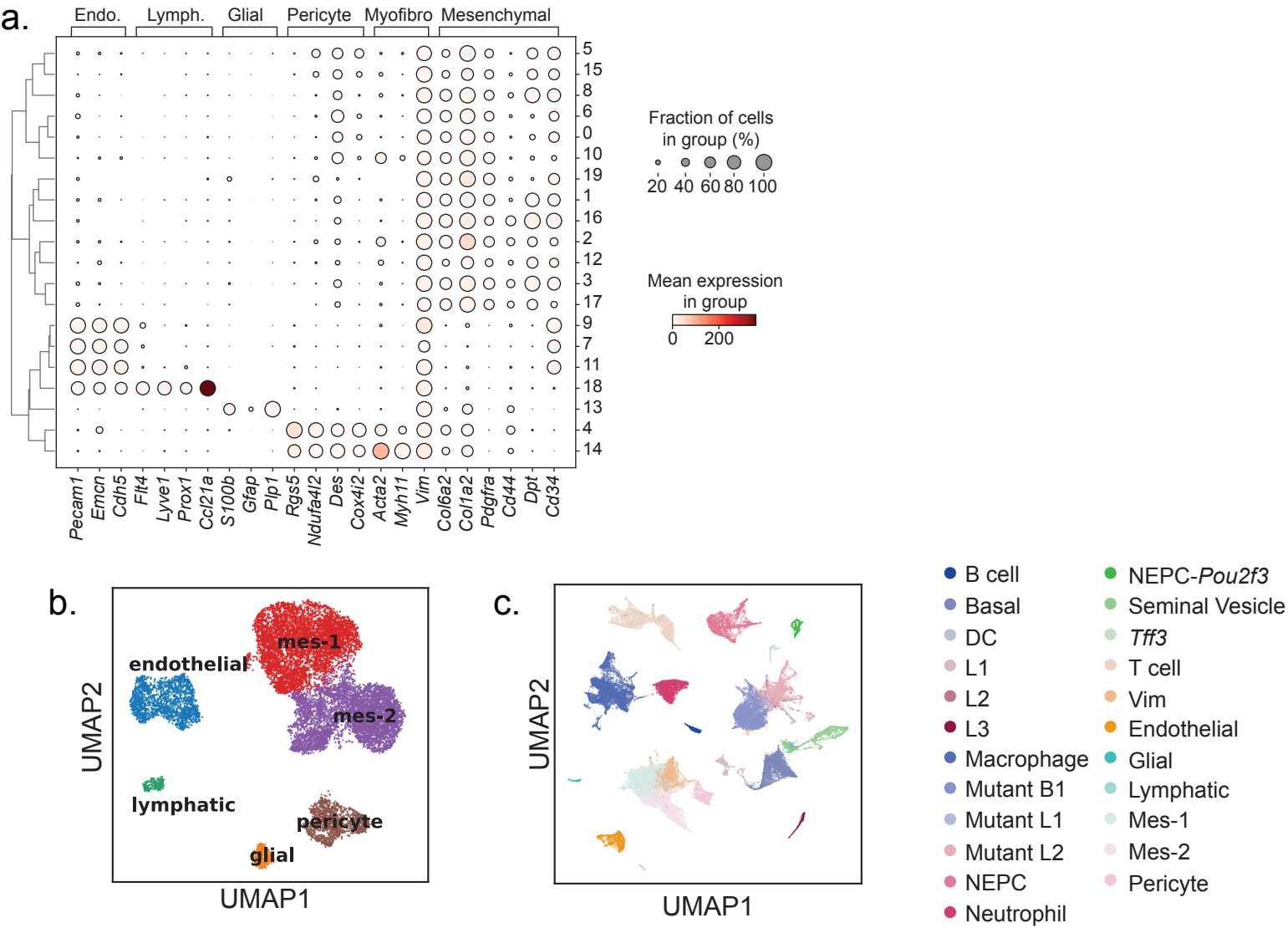
SUPPLEMENTARY FIGURE 4:



Supplementary Figure 4: PrismSpot demonstrates stronger signal to noise for tumor and non-tumor-specific gene modules

a. Density plot shows the Hotspot autocorrelation Z-scores for BayesPrism deconvolved (y-axis) vs undeconvolved expression (x-axis) across markers of tumor cells derived from GEMM scRNA-seq. Each dot in the density plot represents a tumor marker gene. Red dashed line marks $y=x$. **b.** Same as panel a. but across markers of non-tumor cell types derived from GEMM scRNA-seq, including endothelial, myeloid, and mesenchymal cells. Autocorrelation computed using the deconvolved expression shows smaller value than that from the undeconvolved expression. **c.** Violin plot shows the distribution of the difference between Z-scores of PrismSpot and un-deconvolved Hotspot for marker genes of each cell type. Red dashed line marks zero. **d.** Density plot shows the Hotspot pairwise local correlation Z-scores for BayesPrism deconvolved (y-axis) versus undeconvolved expression (x-axis) between pairs of tumor marker genes defined using GEMM scRNA-seq. Each dot in the density plot represents a pair of tumor marker genes. Red dashed line marks $y=x$. **e.** Same as panel d. but between a tumor marker gene and marker gene from any non-tumor cell types. Using deconvolved expressions shrinks local correlation Z-scores towards zero. **f.** Same as panel d. but between a pair of marker genes from any non-tumor cell types. Using deconvolved expressions shrinks local correlation Z-scores towards zero. **g.** Violin plot shows the distribution of the difference between the absolute value of Z-scores of PrismSpot and un-deconvolved Hotspot for genes of each category. Red dashed line marks zero.

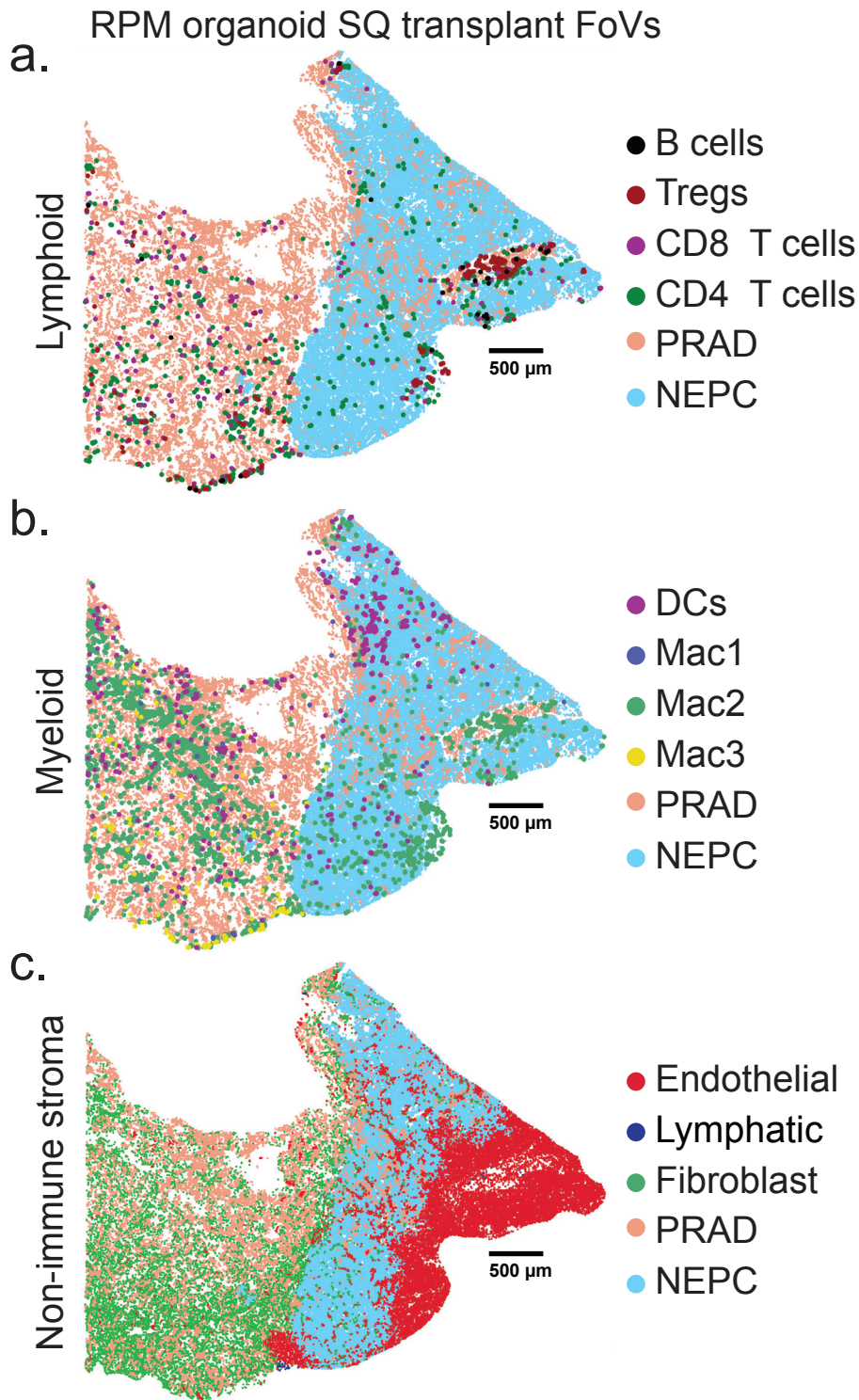
SUPPLEMENTARY FIGURE 5:



Supplementary Figure 5: Non-tumor cell typing from previously published datasets used as input for BayesPrism analysis of Visium dataset.

- a. Dot plot of marker genes associated with each of the indicated cell types across phenograph clusters. Data represent the mean normalized $\log_2(X+1)$ expression scaled from 0 to 1. Dot size represents percent of cells expressing a given gene.
- b. UMAP of non-tumor cell populations in GEMM scRNA-seq dataset, colored by cell types. Mes = Mesenchymal.
- c. UMAP of the original GEMM scRNA-seq dataset, colored by cell types with the GFP-negative stromal populations replaced with finer-grained cell types. Data derived from $n = 13$ “PtRP” mice.

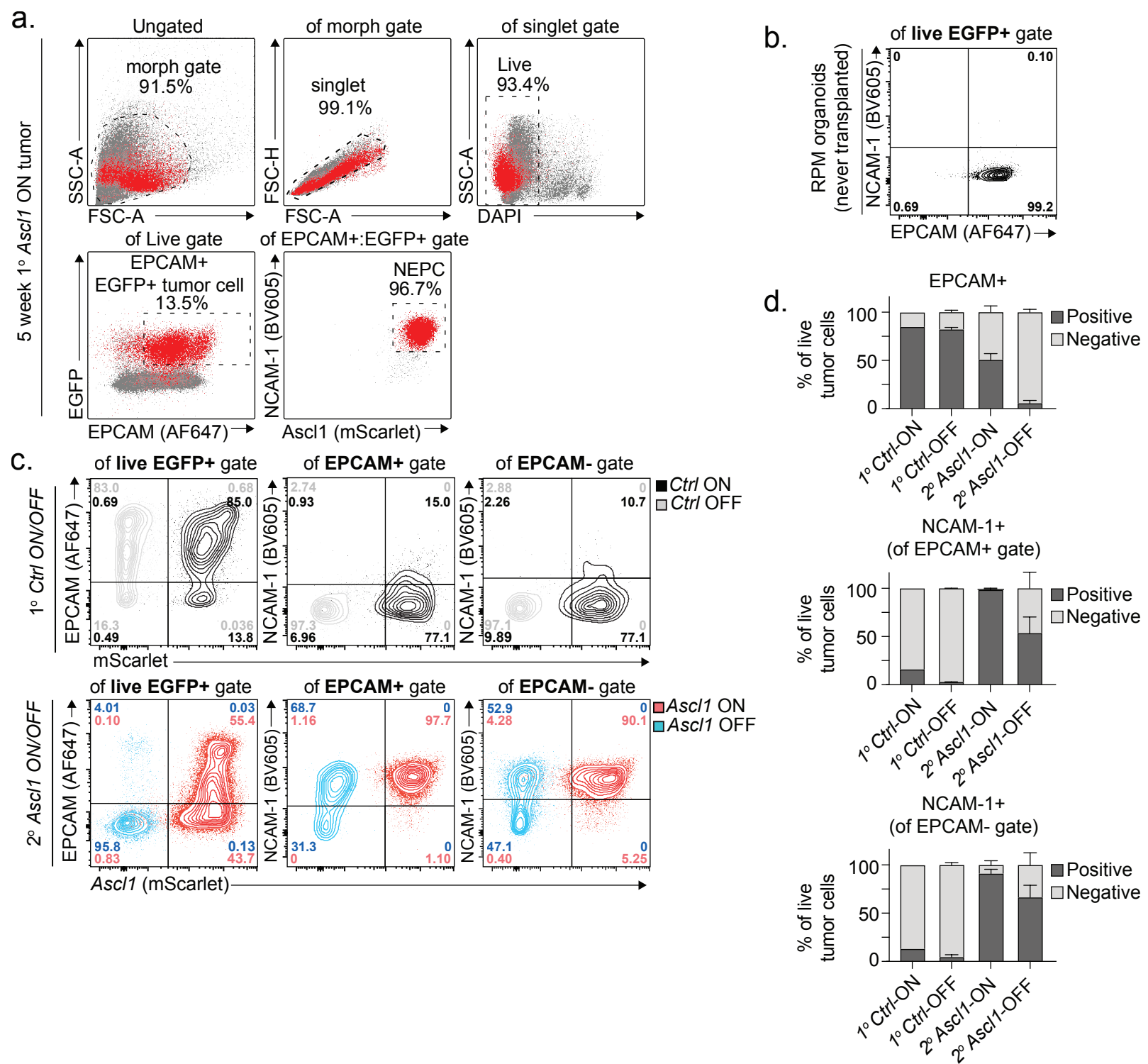
SUPPLEMENTARY FIGURE 6:



Supplementary Figure 6: TME in flank RPM tumors is similar to RPM orthotopic tumors.

Representative segmented field of view (FoV) for the indicated general **a.** lymphoid cell, **b.** Myeloid, and **c.** non-immune stromal types in 8-week SQ RPM tumor. Data are representative of $n = 5$ tumors. SQ = subcutaneous tumor.

SUPPLEMENTARY FIGURE 7:



Supplementary Figure 7: *Ascl1* loss in established NEPC results in loss of EPCAM.

- Gating strategy used to purify RPM-NEPC tumor fraction from primary (1°) *Ascl1* ON mice on dox for 5 weeks post transplantation. Data related to Fig. 8c. See methods for more information.
- Representative flow plot of control (DAPI-negative) RPM organoids (never transplanted, EGFP+) stained with NCAM-1 or EPCAM and used for positive and negative gating in panels c-d.
- (Top) Representative OT Ctrl ON or Ctrl OFF primary (1°) tumors (endogenous *Ascl1*^{KO}, can only initiate and progress as PRAD) stained for the depicted markers. Used as negative controls for NCAM-1 staining. (Bottom) Representative SQ *Ascl1* ON or *Ascl1* OFF secondary (2°) tumors stained for the depicted markers. Outliers marked as dots. Note 95.8% of *Ascl1* OFF tumors (dox withdrawal for 2 weeks) have lost EPCAM expression.
- Stacked bar charts for the indicated tumors staining positively or negatively for (top) EPCAM, (middle) NCAM-1 of EPCAM+ gate, or (bottom) NCAM-1 of EPCAM- gate. Related to panel c. Error bars represents mean and standard deviation and data from panels c-d are derived from $n = 5$ tumors per group.