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Supplementary information

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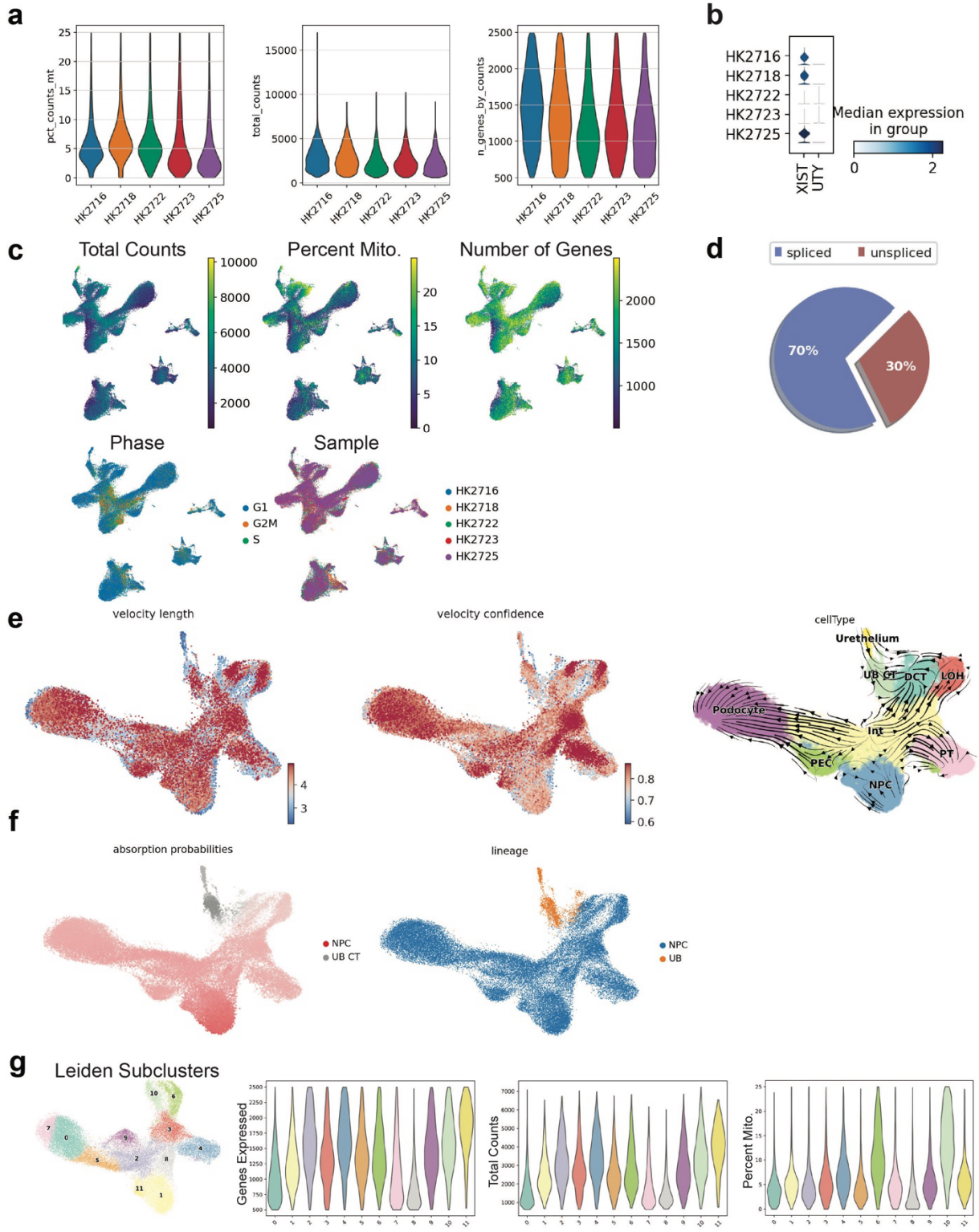
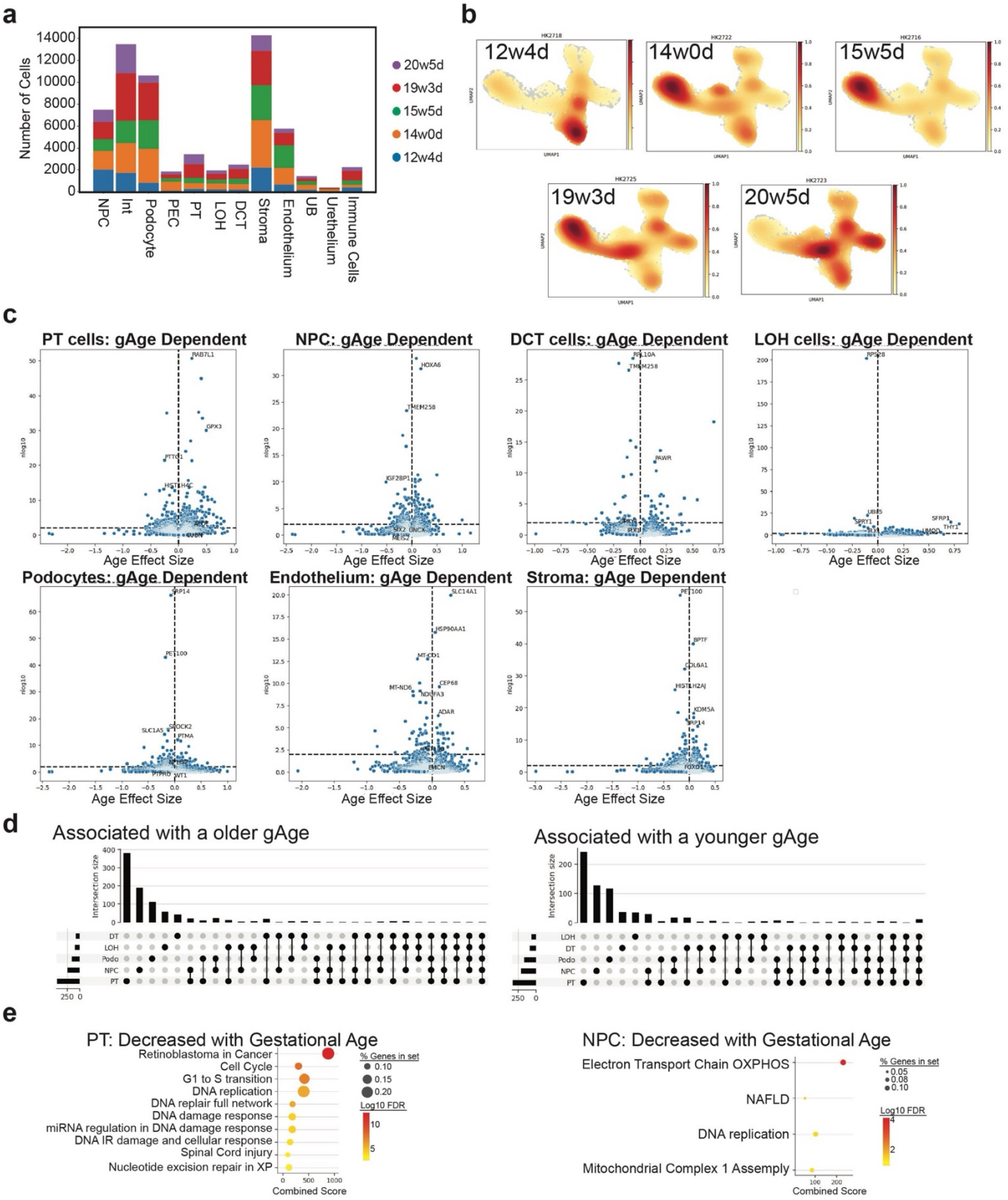
Single-cell spatial mapping of human kidney development implicates the microenvironment in guiding cell fate decisions

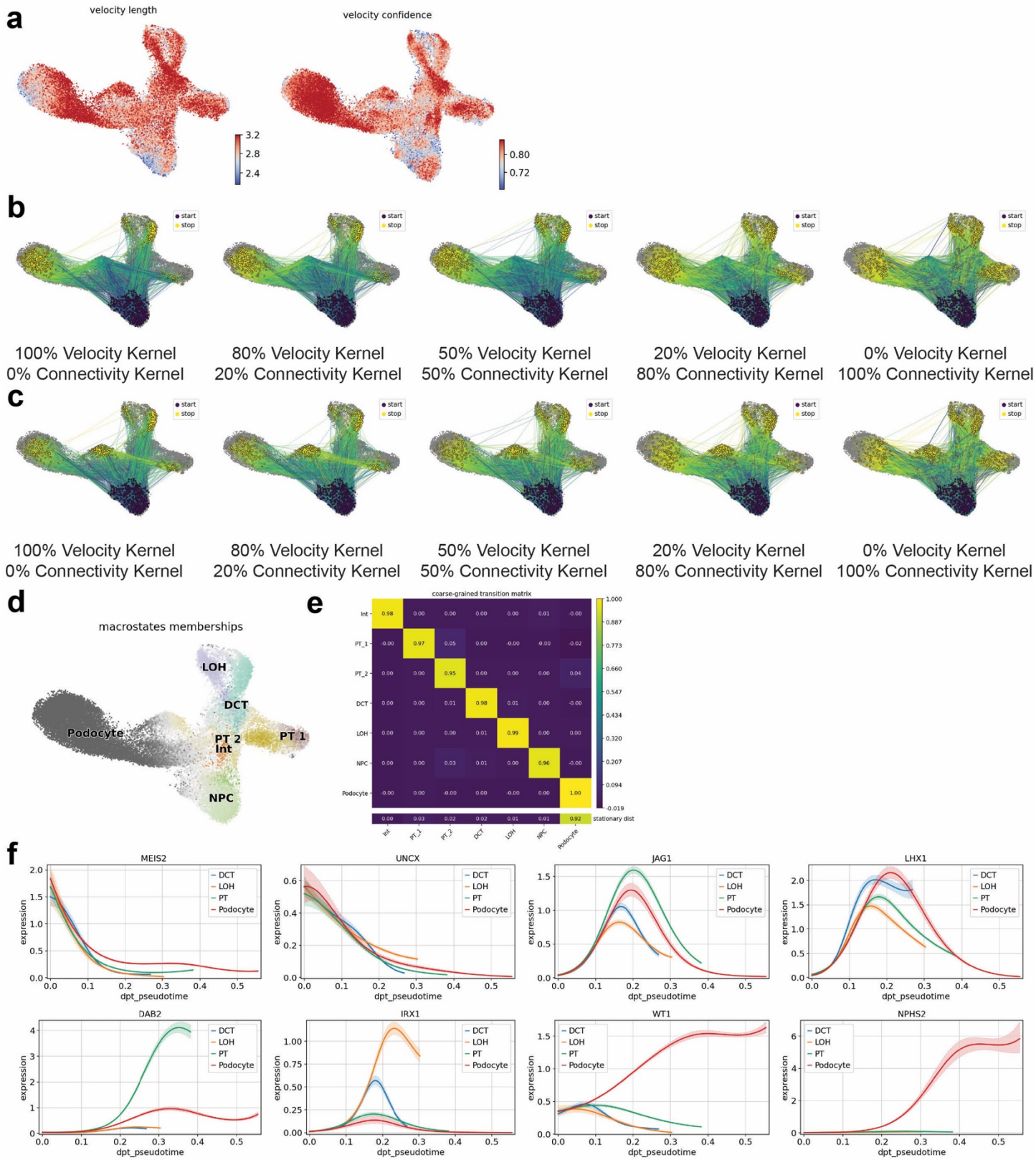
In the format provided by the authors and unedited



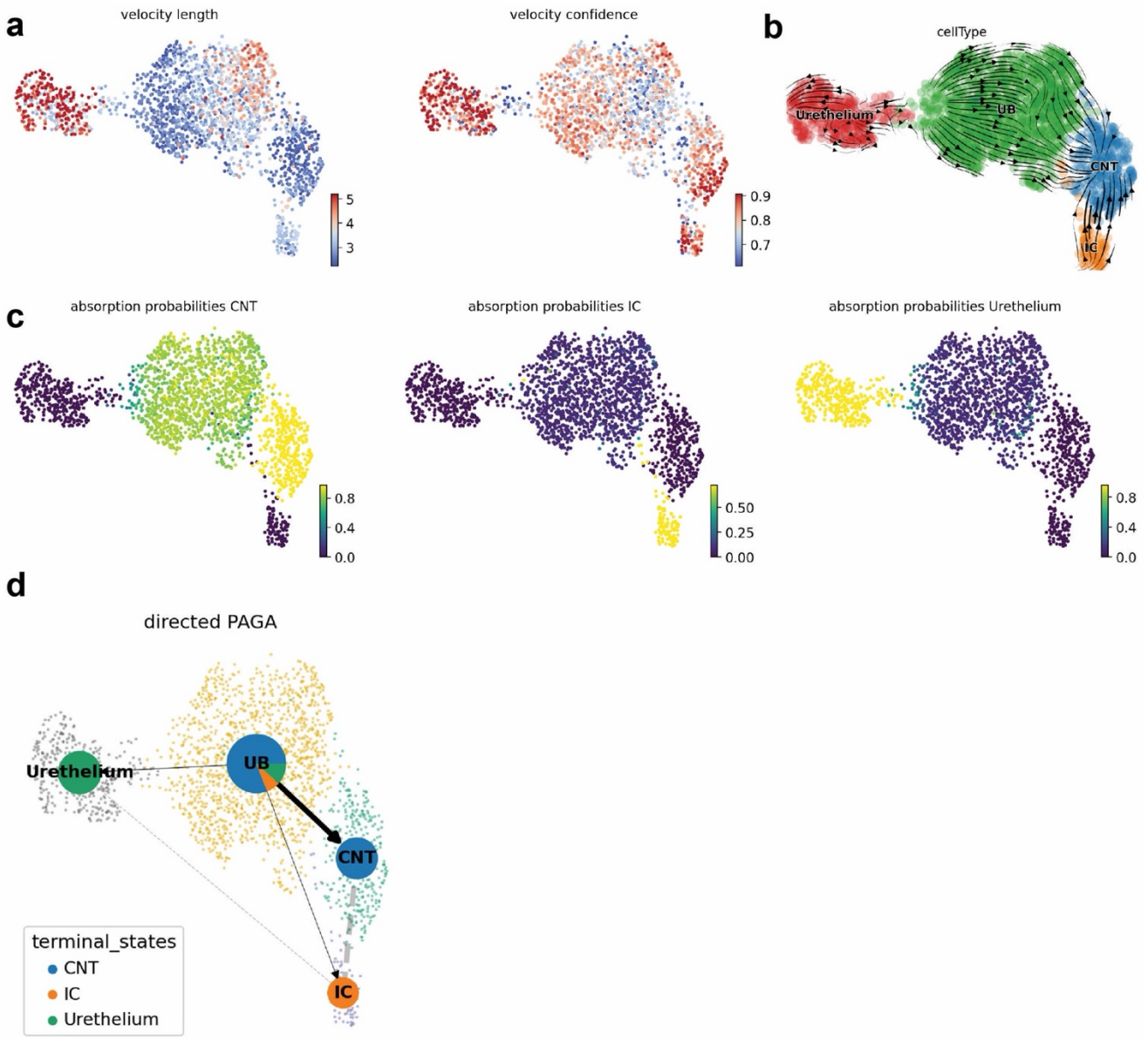
Supplementary Figure 1. Quality control of single cell RNA sequencing. (**a**) QC metrics of scRNAseq data parsed by sample (x-axis), showing percent mitochondrial, total number of RNA counts and number of genes identified (on x-axis). (**b**) Sex-specific gene expression for each sample, using *UTY* and *XIST*, as indicated by violin plot. (**c**) QC metrics from panel A projected on each cell on a UMAP, with color indicating value. (**d**) Pie chart showing the fraction of reads that are from spliced and un-spliced transcripts in aggregate all samples in aggregate. (**e**) RNA velocity length, confidence and RNA velocity projected on UMAP of NPC and UB lineages. Velocity length and confidence for each cell as indicated by color. RNA velocity is indicated by arrow length and direction. (**f**) UMAP of NPC and UB lineages showing reversed absorption probability from NPC and UB terminal states, as indicated by color with greater UB probability indicated by grey color and greater NPC probability indicated red color, and a discretized UMAP of these data showing cells with >50% absorption probability for NPC in blue, and those with <50% probability in orange. (**g**) Leiden subclustering and QC statistics of Leiden subclusters.



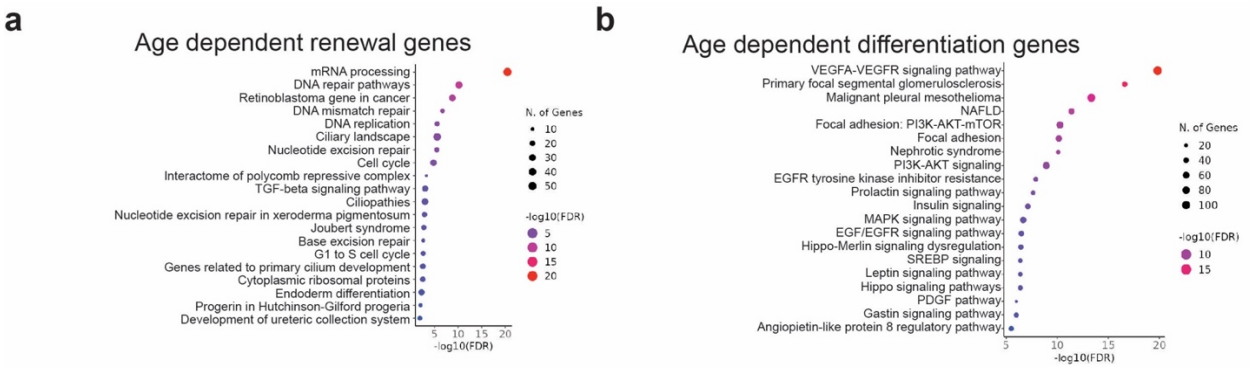
Supplementary Figure 2. Examination of genes associated with gestational age. (**a**) The number of cells (y-axis) and the type of cells (x-axis) for each analyzed sample (separated by color). (**b**) Density estimates on combined UMAP of nephron cell populations for each sample (**d**) Volcano plots of genes associated with gestational age for each cell type. Log2Fold change of expression is on the x-axis. Genes associated with increased expression at older gestational ages are represented by a positive log2Fold Change, and those associated with younger gestational ages are represented by a negative log2Fold Change. Negative log adjusted p-value is on the y-axis. Several example genes are labeled. (**d**) UpSet plot showing cell type populations and shared age dependent DEGs between cell types. (**e**) Pathway analysis of time dependent genes for NPC and PT cell types. Percent of genes within pathway is shown by dot size, with color indicating negative log FDR. Combined score is indicated by x-axis.



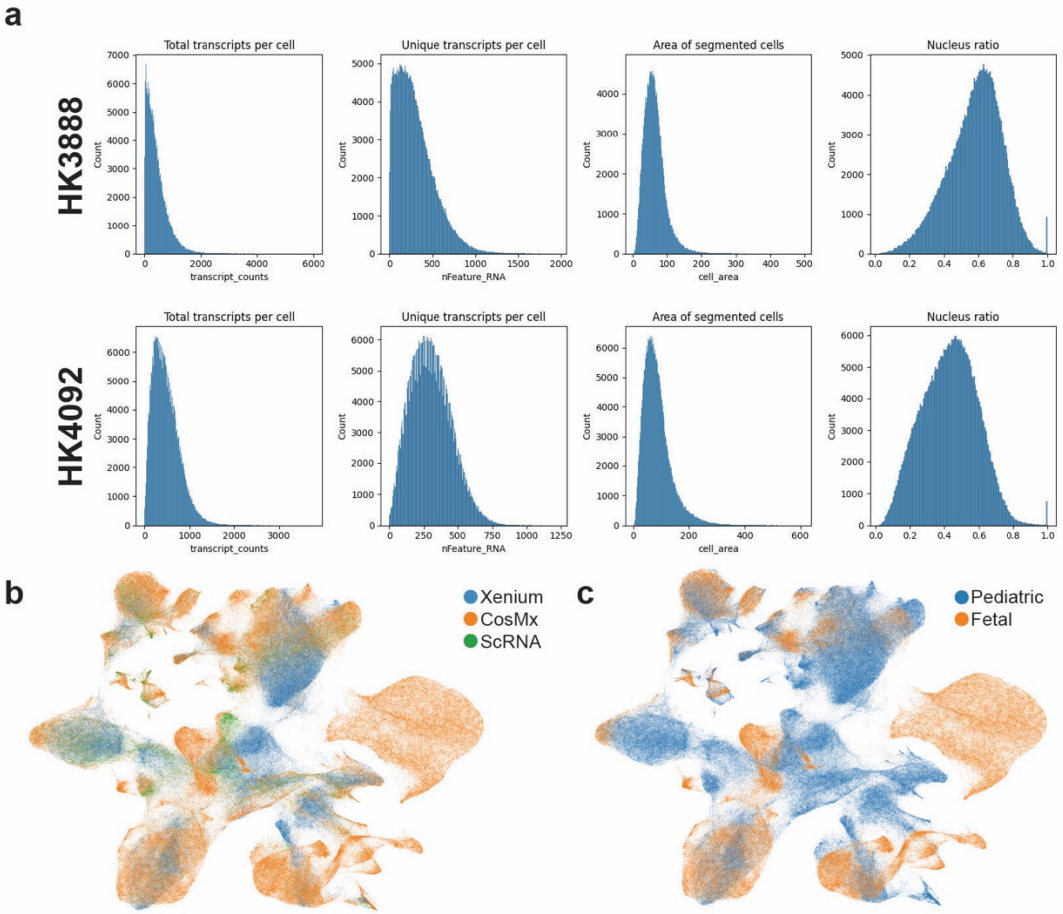
Supplementary Figure 3. CellRank absorption estimates of single cell data for the NPC lineage. (**a**) Velocity estimates from nephron specific UMAP, showing velocity length and confidence for each cell as indicated by color. (**b**) Random walks starting within the NPC population as determined by combined kernels using various combinations of velocity and connectivity kernels without counting PECs as a terminal state. (**c**) Random walks starting within the NPC population as determined by combined kernels using various combinations of velocity and connectivity kernels counting PECs as a terminal state. (**d**) Macrostate probability across the UMAP as indicated by coloring of each cell (**e**) Coarse grained transition matrix of each microstate, with color with transition probability indicated by color and numerically. (**f**) Gene expression (y-axis) along pseudotime (x-axis) for various marker genes, with each trajectory colored separately. PT: Proximal Tubule, LOH: Loop of Henle, DCT: Distal convoluted tubule, Int: Intermediate.



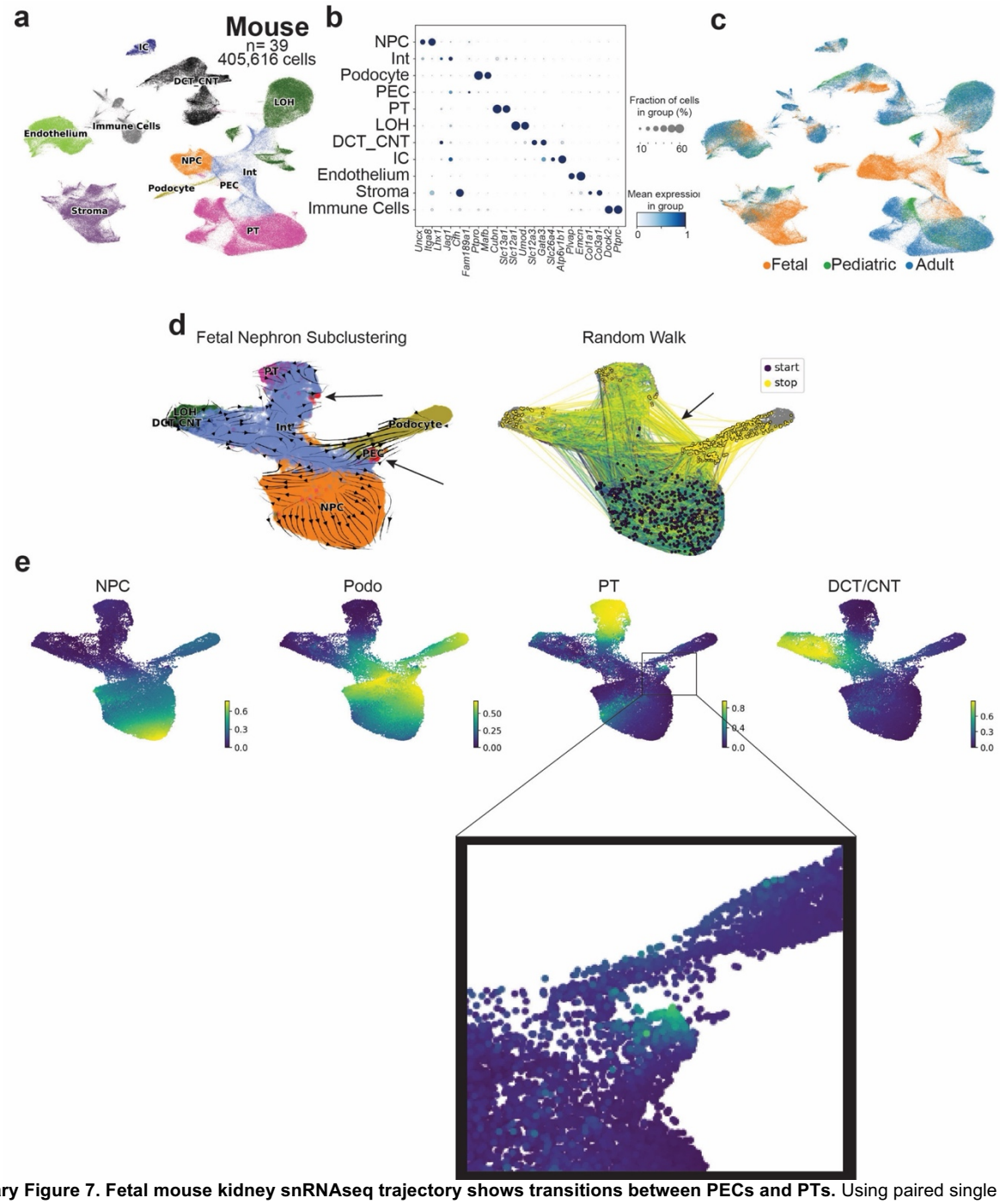
Supplementary Figure 4. CellRank absorption estimates of single cell data for the UB lineage. (**a**) Velocity length and velocity confidence for all cells within the UB lineage plotted on UMAP with value indicated by color. (**b**) RNA velocity plotted on UMAP of UB lineage. Arrow direction indicates velocity direction and length of arrow indicates velocity length. (**c**) Absorption probabilities of each cell for CNT, IC and Urothelium, starting from the UB. (**d**) Directed map of UB differentiation the showing direction and connectivity of cell type clusters. Cells are colored by Leiden clusters and each cluster has a pie chart showing an aggregate of cell fate probability of all cells within the Leiden cluster with each terminal state's likelihood indicated by color within pie chart. Arrows indicate likely transitions with darker arrows being more frequent than thin arrows, which more frequent than dotted lines. UB: ureteric bud, IC: intercalated cell, CNT: connecting tubule



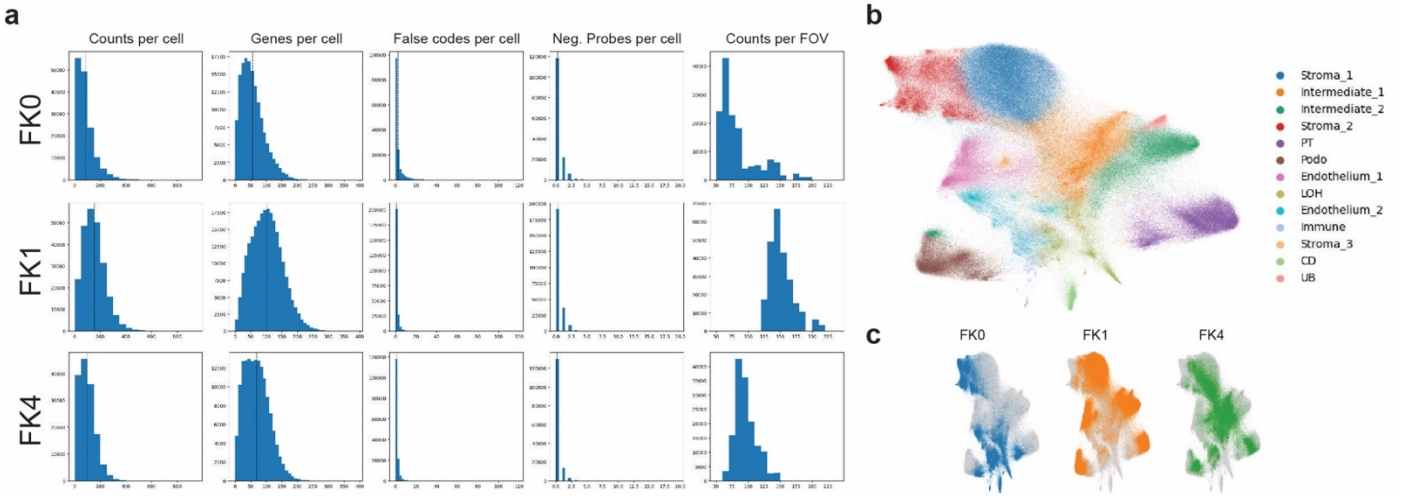
Supplementary Figure 5. Age dependent NPC genes are associated with renewal. (a) Pathway analysis of for gestational age dependent genes associated with renewal. **(b)** Pathway analysis of for age dependent genes associated with renewal.



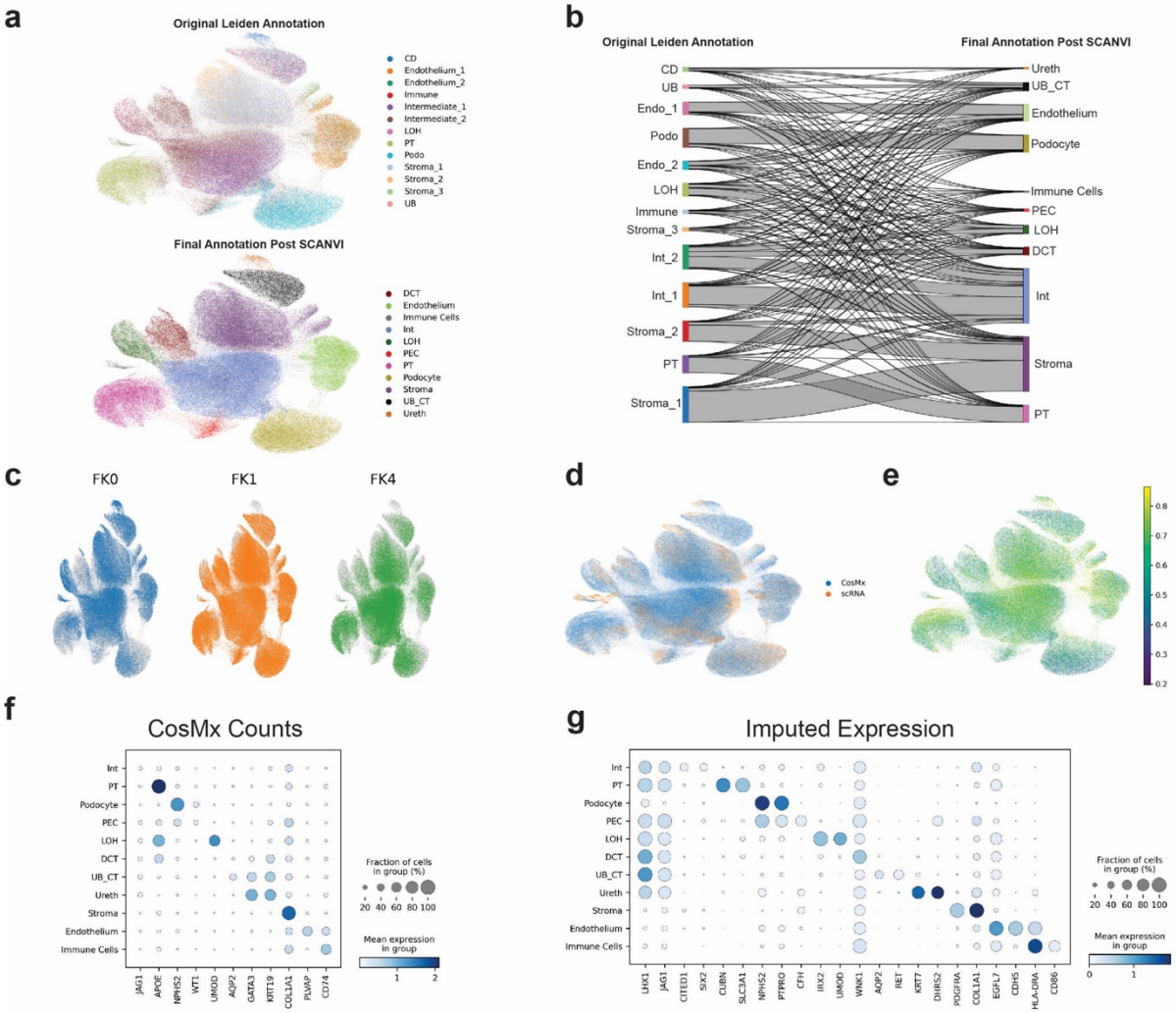
Supplementary Figure 6. Xenium QC and integration. (a) QC statistics for Xenium 5K data of human pediatric and fetal samples. (**b**) UMAP of integrated Xenium, CosMx and scRNAseq data showing modality of each generated cell. (**c**) UMAP of integrated Xenium, CosMx and scRNAseq data showing type (fetal or pediatric) of each generated cell.



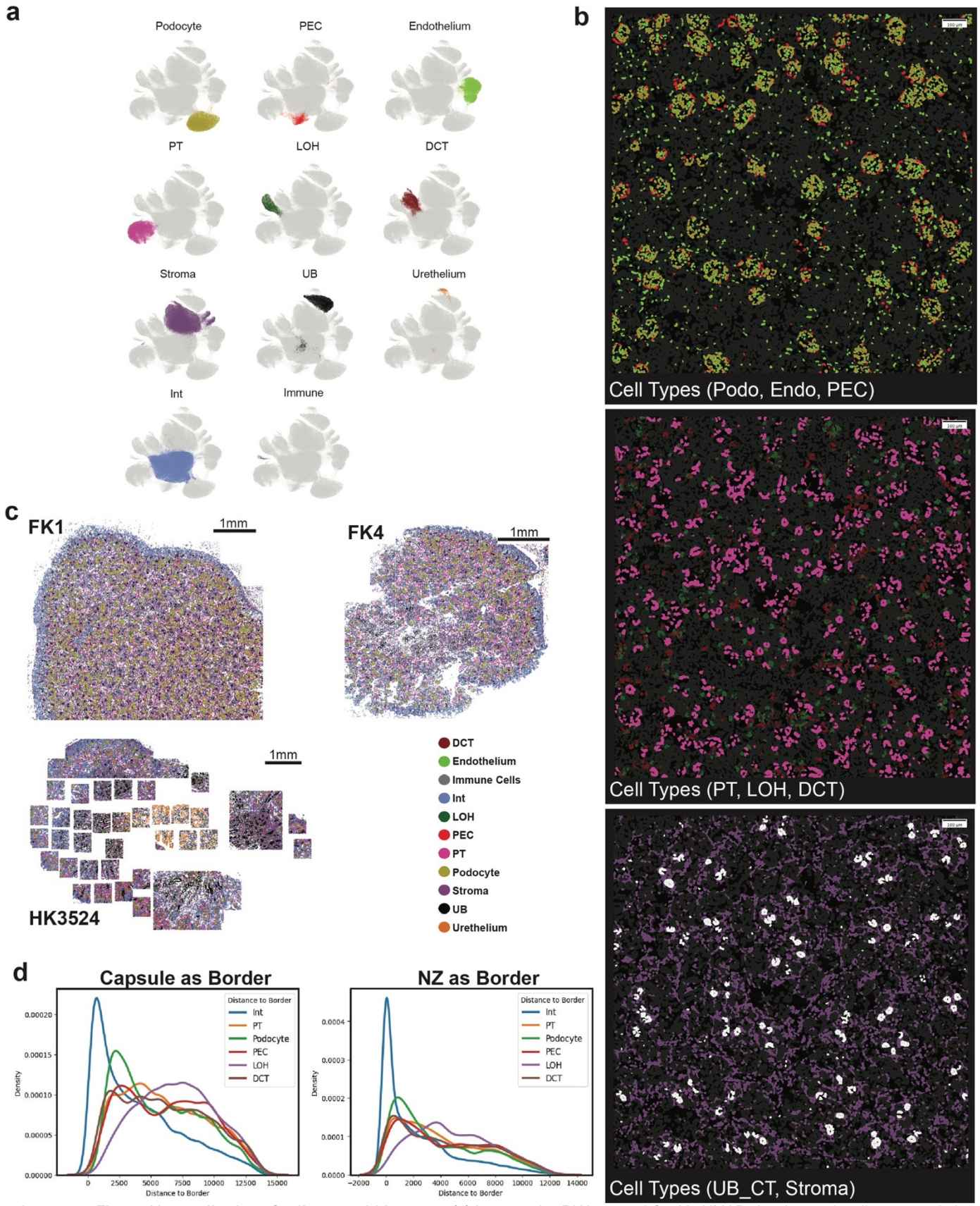
Supplementary Figure 7. Fetal mouse kidney snRNAseq trajectory shows transitions between PECs and PTs. Using paired single nuclei RNAseq and single nuclei ATAC seq from mouse kidneys across the lifespan, we performed an integration and celltyping as shown by UMAP (**a**) and dotplot (**b**). Cell types showed expected representation across the lifespan (**c**). Using the annotated fetal cells, we performed CellRank trajectory analysis, identifying that PEC annotations were split between a cluster near PTs and those near podocytes, with a high degree of expected interconversion as shown by random walk analysis (**d**). CellRank absorption probabilities also show that the PECs near the podocyte cluster have an increased PT absorption probability. Int: Intermediate nephron cells; NPC: nephron progenitor cells, PT: proximal tubule, PEC: parietal epithelial cell, LOH: loop of Henle, DCT: distal convoluted tubule, UB_CT: ureteric bud/collecting duct.



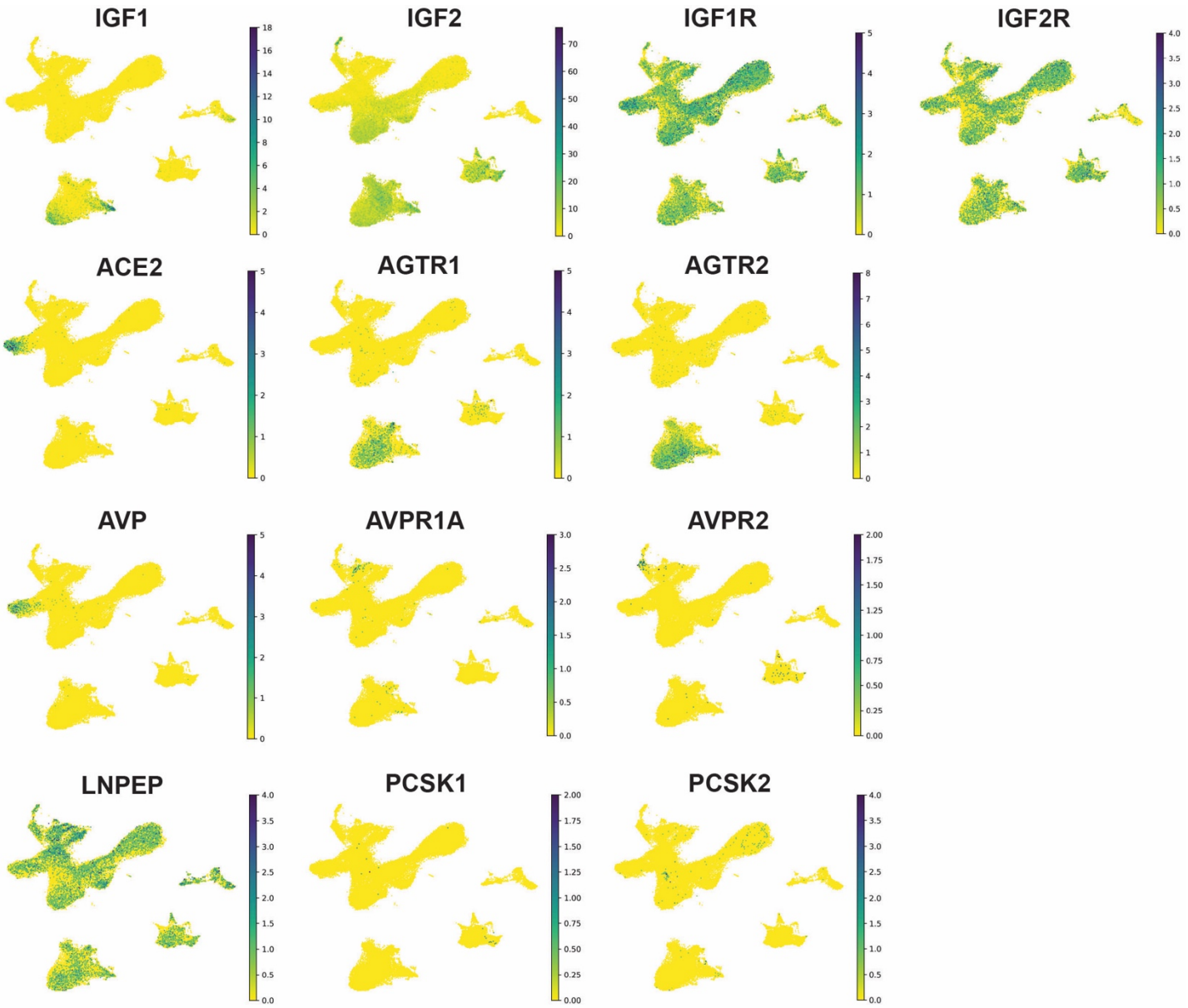
Supplementary Figure 8. CosMx single cell spatial imaging quality control. (**a**) Histograms demonstrating QC data for each of the 3 human fetal CosMx samples, showing counts per cell, genes per cell, false codes, negative probes and counts per FOV on the x-axis, with frequency on y-axis. (**b**) UMAP embedding of cells from all 3 CosMx samples (pre-integration) colored by cell type annotation. (**c**) Contribution of each sample to the shared UMAP embedding as indicated by color. PEC: parietal epithelial cell, PT: Proximal Tubule, LOH: Loop of Henle, DCT: Distal convoluted tubule, UB: ureteric bud, CD: collecting duct.



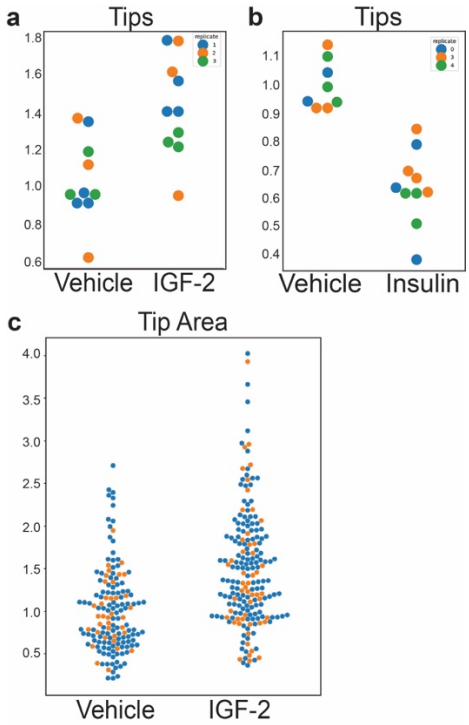
Supplementary Figure 9. Conservation of CosMx cell type annotations. Annotated original Leiden clusters from pre-integrated CosMx UMAP and post integration with scRNAseq annotations shown by (**a**) UMAP and (**b**) Sankey plot. (**c**) Contribution of each CosMx sample on integrated UMAP embedding, with cell color indicating sample. (**d**) Integrated UMAP colored by technology modality, with CosMx cells being colored blue and scRNAseq cells orange. (**e**) Intra-modality distance metric plotted on the UMAP—lower values indicate more similarity of CosMx and single cell within the shared embedding, values are indicated by cell color. (**f**) Marker genes for each cell population using original CosMx counts. Color indicated mean log expression, and dot size indicated percent of cells within the annotation expressing the marker gene. (**g**) Marker genes for each cell population using imputed CosMx expression. Color indicated mean log expression, and dot size indicated percent of cells within the annotation expressing the marker gene. PT: Proximal Tubule, LOH: Loop of Henle, DCT: Distal convoluted tubule, UB_CT: ureteric bud and collecting duct, Int: intermediate.



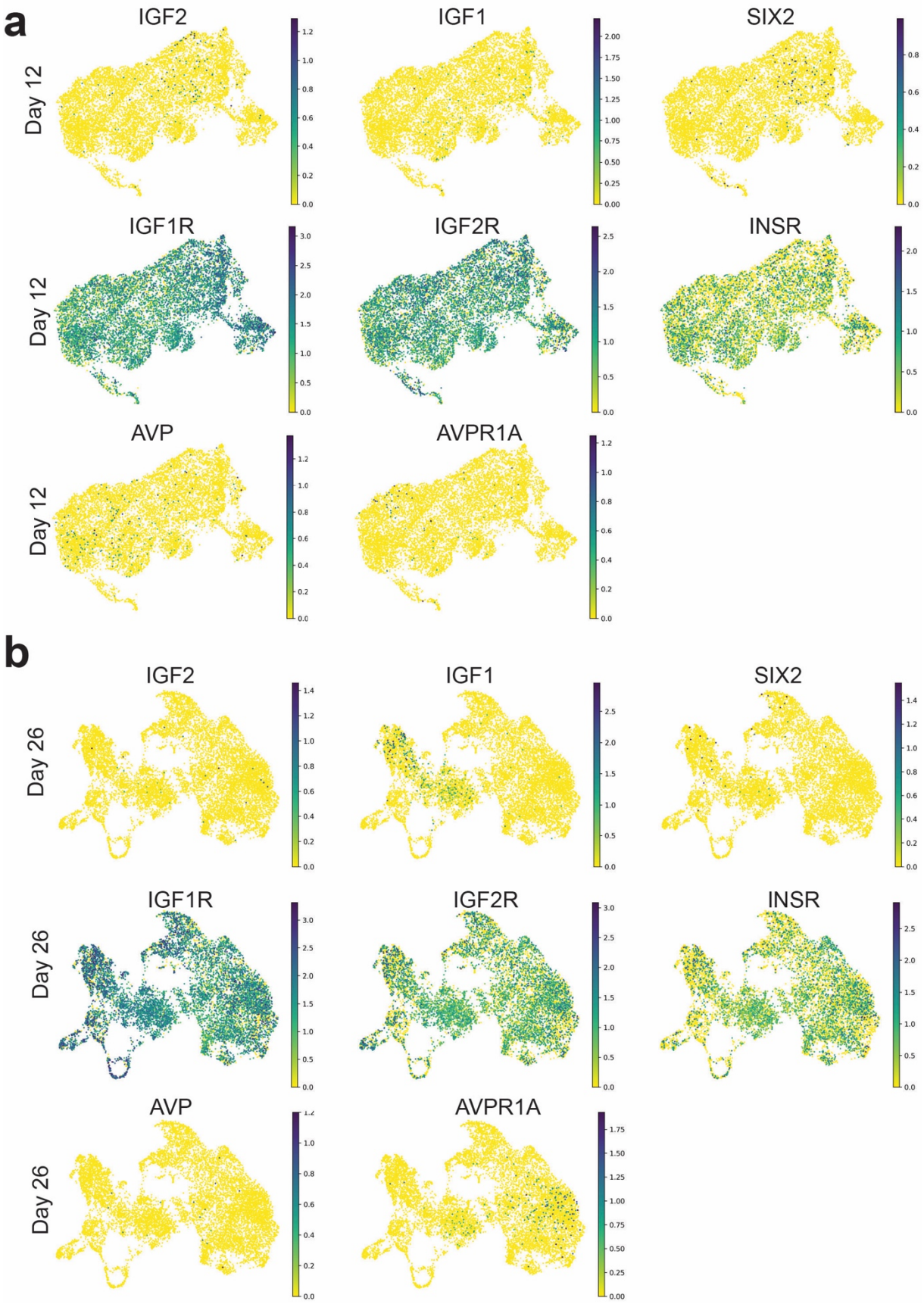
Supplementary Figure 10. Localization of cell types within space. (a) Integrated scRNAseq and CosMx UMAP showing each cell type population as indicated by cell color. (**b**) Cell annotations in space in a zoomed in region of tissue, and (**c**) Tissue-wide, with cell annotation indicated by cell color. (**d**) Frequency of cellular annotation (y-axis) plotted against distance from the edge of the tissue or from the deep boundary of the nephrogenic zone (x-axis) shown for each cell type for the FK1 sample (18 weeks, 5 days gestation). PT: Proximal Tubule, LOH: Loop of Henle, DCT: Distal convoluted tubule, UB_CT: ureteric bud and collecting duct, Int: intermediate.



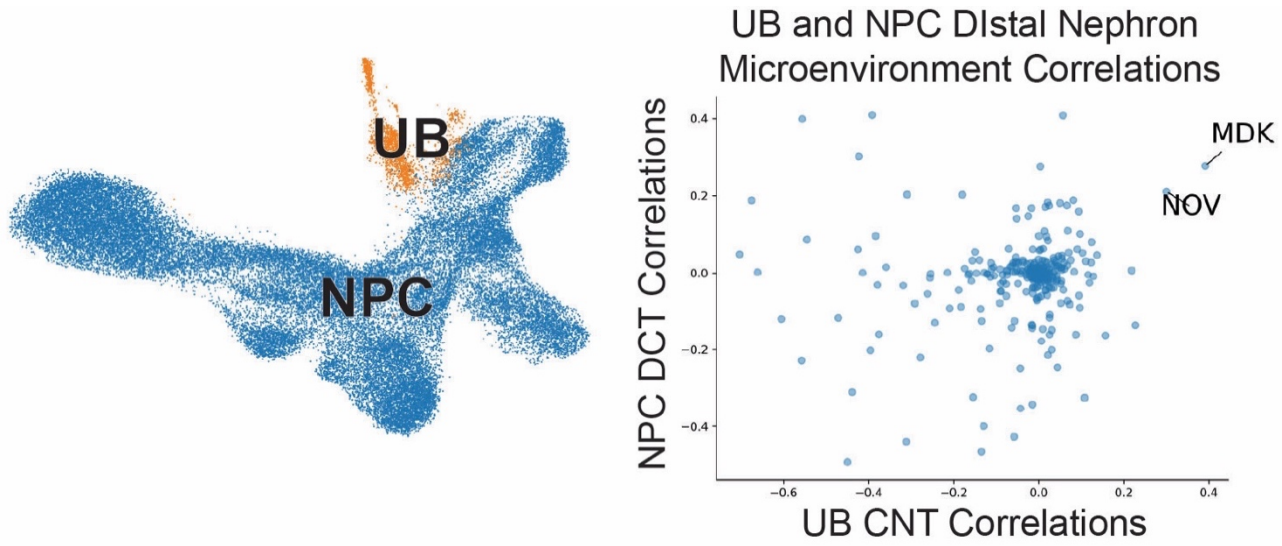
Supplementary Figure 11. Single cell expression feature plots of selected ligand and receptor genes across all cell type populations. UMAP of scRNAseq data, with raw counts of gene expression for *IGF1, IGF2, IGF1R, IGF2R, ACE2, AGTR1, AGTR2, AVP, AVPR1A, LNPEP, PCSK1, PCSK2* and *AVPR2* for each cell, as indicated by color with low expression being yellow and higher expression in purple.



Supplementary Figure 12. Mouse explants reproducible demonstrate changes in tip number and tip size when exposed to IGF2 and insulin. **(a)** Quantified number of UB tips (y-axis) for mouse fetal explants incubated with ectopic IGF2 ligand. Total number of tips was normalized for average number of tips in the control samples for each replicate. Each replicate is shown in a separate color. **(b)** Quantified number of UB tips (y-axis) for mouse fetal explants incubated with ectopic insulin. Total number of tips was normalized for average number of tips in the control samples for each replicate. Each replicate is shown in a separate color. (**c**) Normalized tip size (assessed using Six2 expression positivity) from explants treated with vehicle and IGF2. Each replicate is in a different color.



Supplementary Figure 13. NPC lineage organoids UMAP feature plots from Yoshimura et al., 2023. UMAP of NPC based organoid snRNAseq data showing expression of various genes at day 12 (**a**) and at day 26 (**b**).



Supplementary Figure 14. Correlations of ligands with UB and NPC distal lineages. UMAP of single cell data showing absorption probability of UB and NPC lineages (right), with cells predicted from the UB lineage in orange, and those from the NPC lineage in blue. Correlations of the NPC DCT lineage (y-axis) and the UB CNT lineage (x-axis) for each ligand are plotted (left).