

SUPPLEMENTAL FIGURE LEGENDS

Figure S1: Single-Cell RNA-Seq Analyses of Week 15 and Week 17 Human Fetal Kidneys Showing Transcriptional Changes during Differentiation of human NPCs to Podocytes (Related to Figure 1A-E)

(A) Unsupervised clustering of Week 15 kidney cells from both Zone 1 and Zone 2 displayed in a tSNE plot with annotation of cluster identities. In parentheses are differentially expressed genes used for cluster identification).

(B) tSNE plot of Week 15 kidney cells colored by their original zonal identities.

(C) Violin plots of differentially expressed genes used to classify 21 clusters of Week 15 cells.

(D) and (E) Bar graphs presenting zonal contribution in Week 15 kidney (D) or Week 17 (E) clusters

(F) and (G) Volcano plots with annotations of genes that are differentially expressed between Zone 1 and Zone 2 of Week 15 (F) or Week 17 kidney (G).

Figure S2: Pseudotime Analyses Showing Transcriptional Changes during Human Podocyte Development (Related to Figure 1F-H)

(A) and (B) Pseudotime trajectories from NPCs to Podocytes with cells colored by their cluster identities (A) or zonal identities (B).

(C) Expression of *EYA1*, *MAFB*, and *TOP2A* along the pseudotime trajectory indicating B3 is mostly comprised of proliferative cells.

(D) Representative EP and LP genes that were used in gene expression correlation analysis to obtain a more complete list of EP and LP genes.

(E) and (F) GO term analyses of human EP gene list (E) or human LP gene list (F) using ToppFun.

(G) and (H) Venn diagrams displaying shared and species-specific EP genes (G) or LP genes (H).

(I) and (J) Fluorescent *in situ* hybridization (I) and quantification (J) showing expression of RPS21 in human developing nephrons. Scale bars denote 50 μ m.

(K) Dotplot showing expression of OMIM genes associated with glomerulosclerosis and glomerulopathy in the human glomerular cell types and organoid EP and LP. Genes detected in *in vitro* derived podocytes are highlighted red.

B: Branch, hu: human, EP: early podocyte, LP: late podocyte

Figure S3: Examination of *in vitro* Derived Kidney Organoids Containing Podocyte-like Cells (Related to Figure 3)

(A) Schematic diagram of CRISPR-Cas9 targeting of *MAFB* locus in H9 hESC.

(B) IF stains of dd16 and dd28 kidney organoids suggesting formation of nephron-like structures. Scale bars denote 50µm.

(C) Expression levels of NPC markers (*SIX1*, *SIX2*, and *EYA1*), developing nephron markers (*PAX8*, *JAG1*, and *HNF1B*), nephron segment markers (*SLC3A1* and *SLC12A1*), early and late podocyte markers (*OLFM3*, *MAFB*, and *SYNPO*, *PLA2R1*) along the kidney organoid differentiation protocol.

(D) IF stains showing co-expression of eGFP with *MAFB* and *WT1* in dd28 organoids. Scale bars denote 50µm.

(E) GO term analyses of genes enriched in dd28 *MAFB*-eGFP⁺ cells in comparison to *MAFB*-eGFP⁻ cells using ToppFun.

(F) Hierarchical clustering using the top 2000 most variably expressed genes of transcriptional profiles of eGFP⁺ hESC-derived podocytes, eGFP⁻ organoid cells, imPods, human fetal RCs and kidneys.

(G) tSNE plot of merged kidney organoid cells colored by their original “differentiation day” identities

(H) Pseudotime trajectories from organoid NPCs to podocytes with cells colored by their cluster identities (left) or “differentiation day” identities (right).

(I) Pseudotime trajectory from organoid NPCs to podocytes with cells colored by their original “differentiation day” identities after removal of proliferative cells.

(J) Expression of *EYA1*, *MAFB*, and *TOP2A* along the pseudotime trajectory indicating B3 is mostly comprised of proliferative cells.

Figure S4: Identification of Mesangial Cells, Glomerular Endothelial Cells and Early/Late Podocytes in Human Week 17, Merged Kidney Organoid and Czerniecki Organoid scRNA-Seq Datasets (Related to Figure 4 and 5)

(A) and (B) Feature plots showing the presence of pericytes (PDGFRB⁺ GATA3⁺), mesangial cells (GATA3⁺ TMEFF2⁺) and glomerular endothelial cells (PECAM1⁺ EHD3⁺) in Week 17 (A), and absence of these cell types in (B) Merged Kidney Organoids.

(C) and (D) Feature plots showing the presence of early podocytes (*MAFB*⁺ *OLFM3*⁺), late podocytes (*MAFB*⁺ *TGFBR3*⁺), and late podocytes expressing EP gene (*ANXA1*⁺ *GFRA3*⁺) in (C) Czerniecki Organoids (Czerniecki et al., 2018), and (D) Wu Organoids (Wu et al., 2018) using the Morizane protocol (M).

(E) and (F) Fluorescent *in situ* hybridization of *OLFM3*, *PLA2R1*, *COL4A3*, *TNNT2* and *MAFB* in human developing nephron. Scale bars denote 50µm.

Figure S5: Heatmaps Presenting Expression of Human Early and Late Podocyte Genes along the *in vivo* Differentiation Trajectory from NPCs to Podocytes (Related to Figure 1H)

NPC: nephron progenitor cell, EP: early podocyte, LP: late podocyte, hu: human.

Figure S6: Heatmaps Presenting Expression of Human Early and Late Podocyte Genes along the *in vitro* Differentiation Trajectory from NPCs to Podocytes (Related to Figure 3H)

NPC: nephron progenitor cell, EP: early podocyte, LP: late podocyte, hu: human, RC: renal corpuscle.

Figure S1

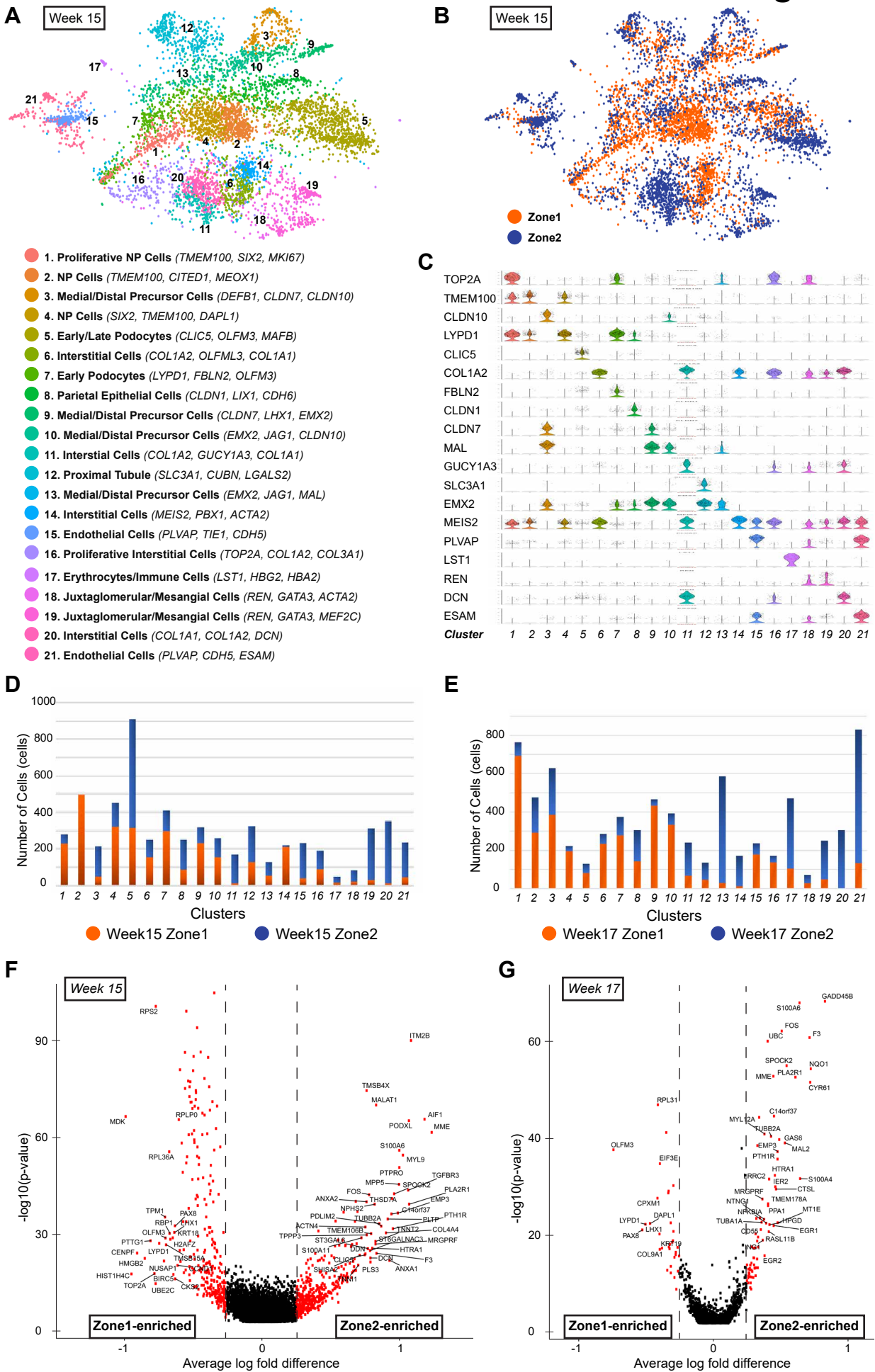


Figure S2

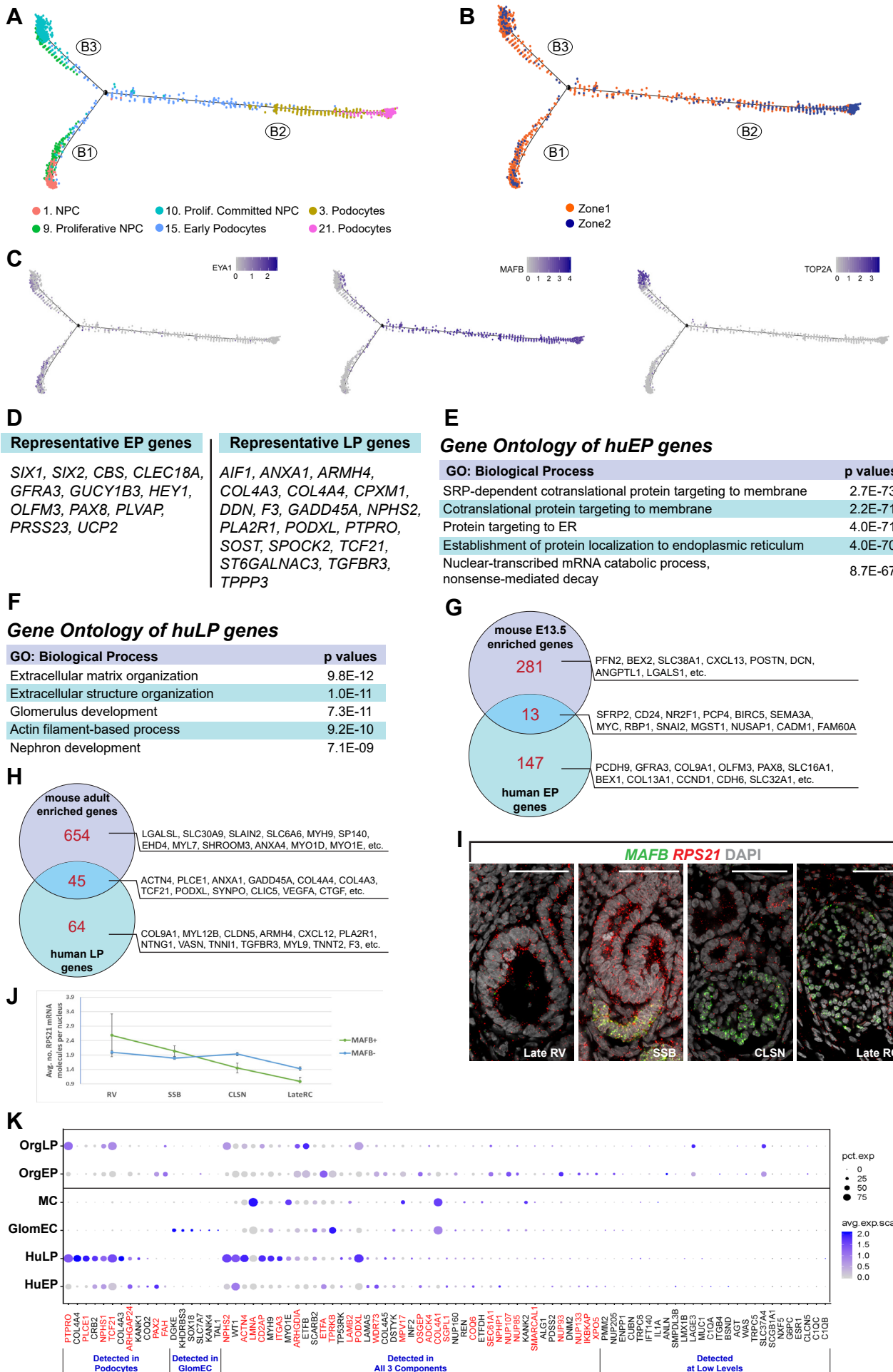


Figure S3

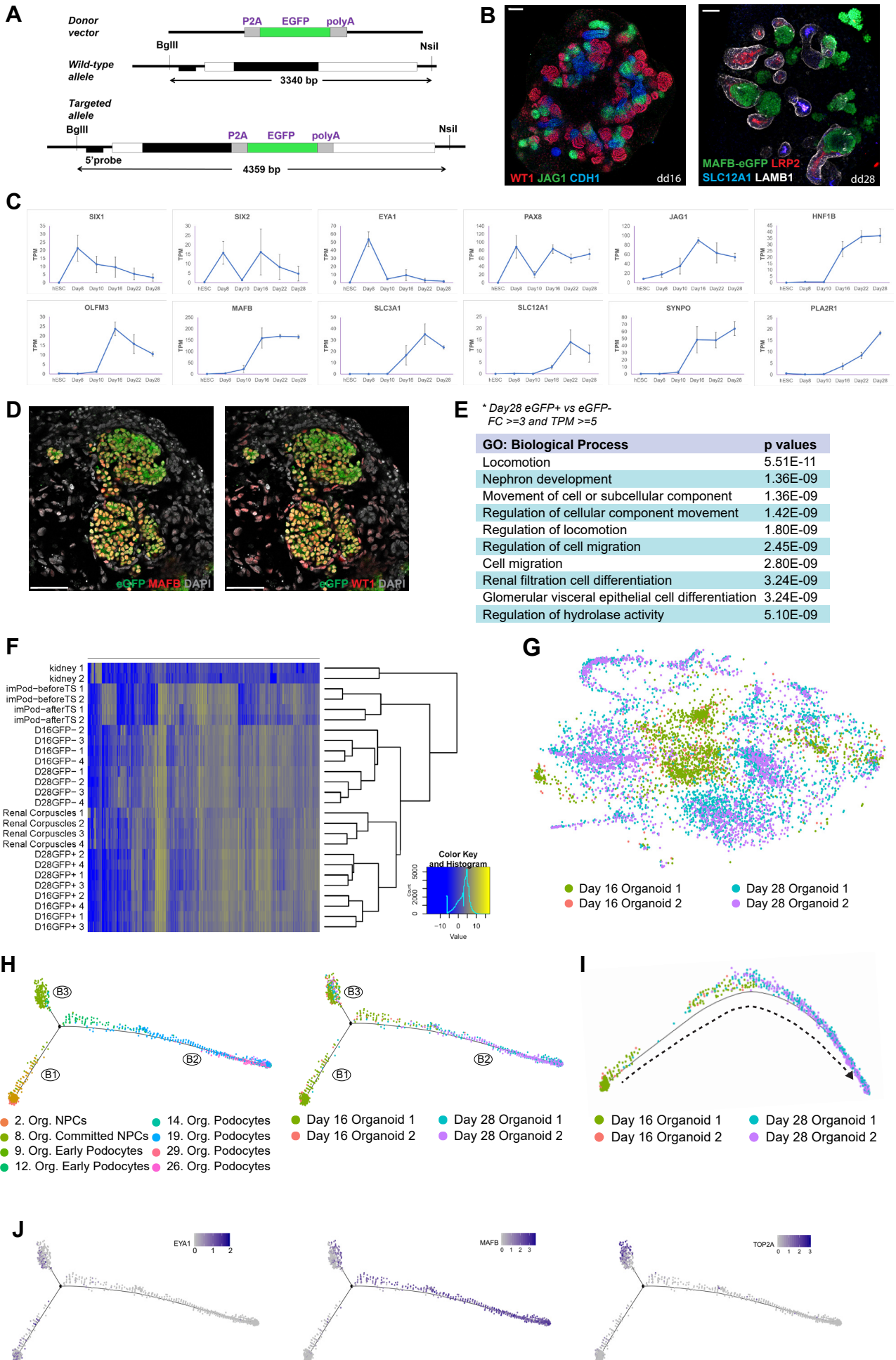


Figure S4

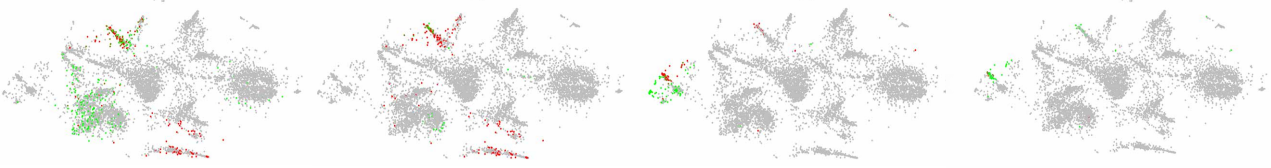
A Week 17

PDGFRB GATA3

TMEFF2 GATA3

PECAM1 EHD3

EHD3 GATA5

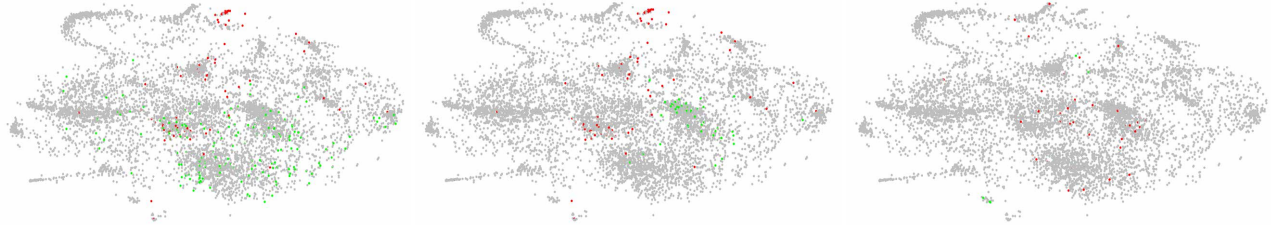


B Organoid Merged

PDGFRB GATA3

TMEFF2 GATA3

CDH5 EHD3



C Czerniecki Organoid

MAFB OLFM3

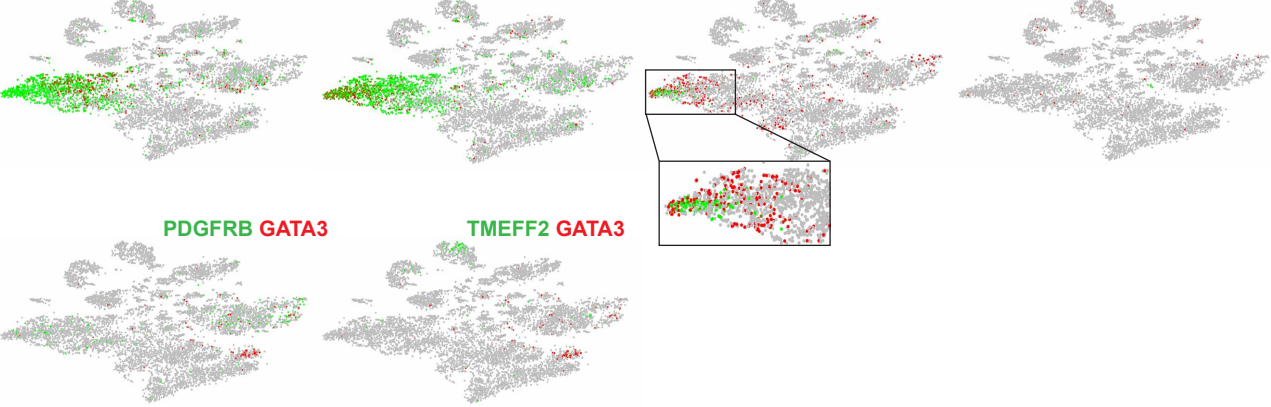
MAFB TGFB3

ANXA1 GFRA3

PECAM1 EHD3

PDGFRB GATA3

TMEFF2 GATA3



D Wu Organoid

MAFB OLFM3

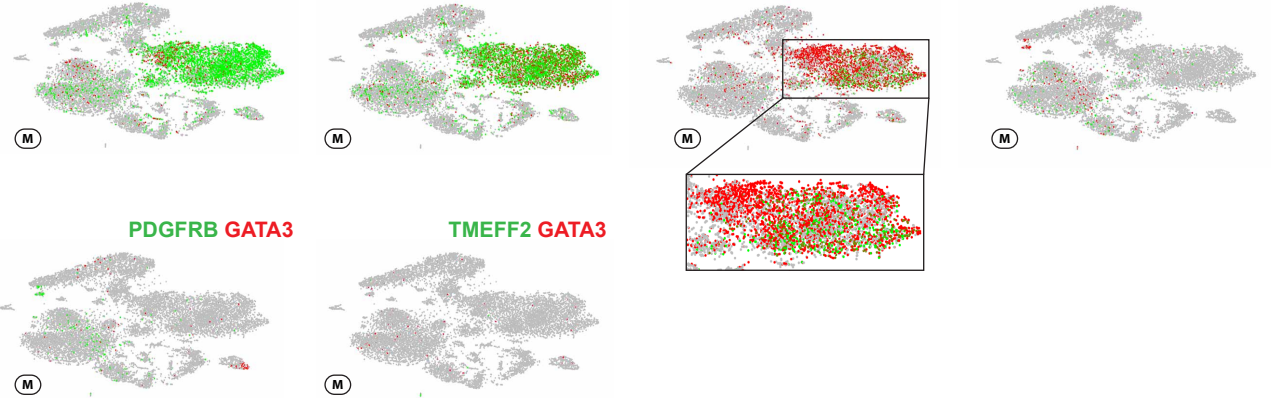
MAFB TGFB3

ANXA1 GFRA3

PECAM1 EHD3

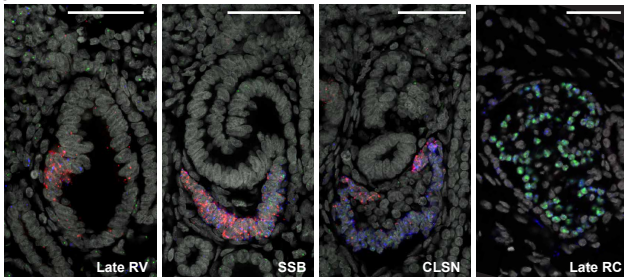
PDGFRB GATA3

TMEFF2 GATA3



E

OLFM3 PLA2R1 MAFB DAPI



F

COL4A3 TNNT2 MAFB DAPI

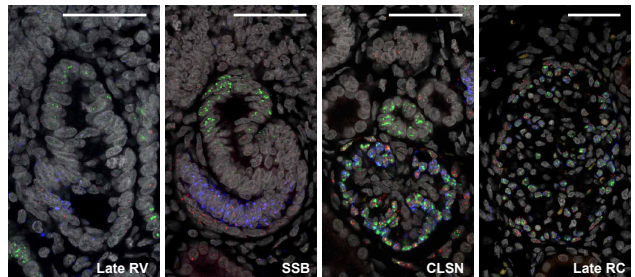


Figure S5

Human Early Podocyte Signature Genes

Human Late Podocyte Signature Genes

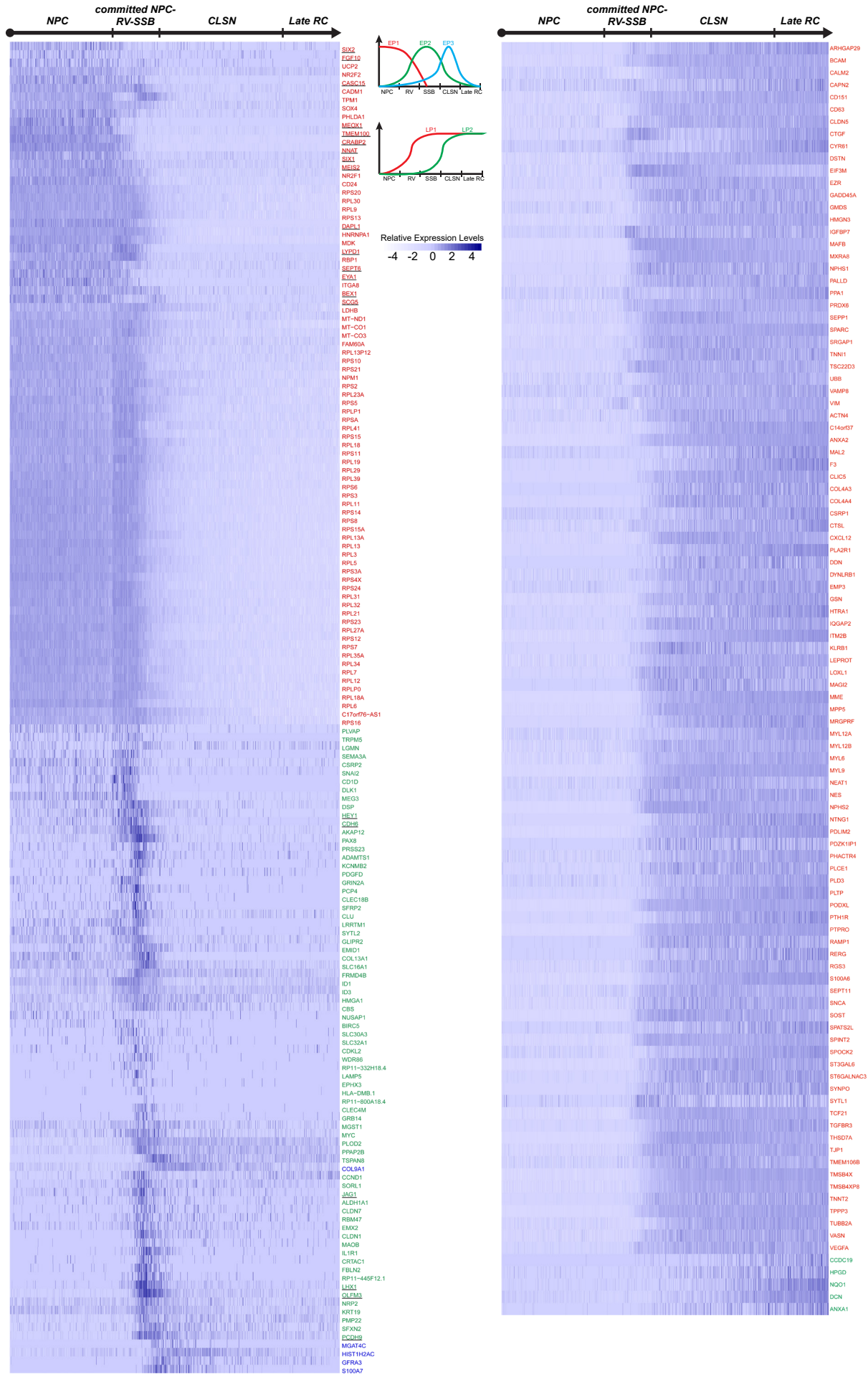
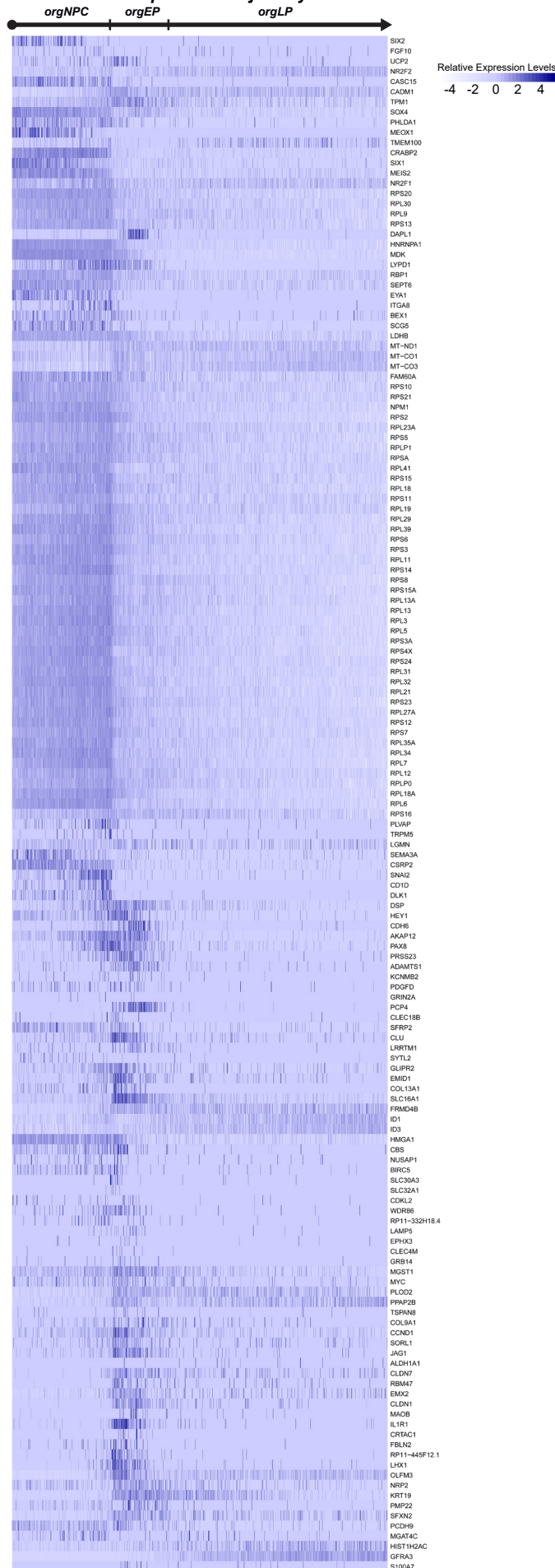


Figure S6

Human EP Signature Genes on in vitro Podocyte Developmental Trajectory



Human LP Signature Genes on in vitro Podocyte Developmental Trajectory

