

Figure S1: A) Table showing the summary of the quality control (QC) parameters including number of genes/cell (nGene), number of unique molecular identifier (nUMI) and mitochondrial read content for the 5 fetal kidney scRNASeq datasets **B)** Violin plots of the QC parameters in the 5 datasets before and after filtering out cells with < 500 genes and > 25% mitochondrial read content. **C)** tSNE plot depicting the distribution of cells from the 5 fetal kidney datasets in the 11 clusters from the initial unsupervised clustering. **D)** Bar plots depicting the contribution of the cells from the 5 fetal kidney datasets in the 11 clusters from the initial clustering **E)** Violin plots of mitochondrial read content per cell in the 11 clusters from the initial clustering. **F)** tSNE plot showing the clusters from the unsupervised clustering of the postnatal day 1 mouse kidney samples (GSE94333). **G)** Top 5 Gene Ontology biological processes for the cluster-specific significantly differentially expressed (p < 0.05) genes of 11 clusters from the initial clustering. **H)** H&E staining of thin paraffin sections of human embryonic kidneys of 87 and 132 days post period.



cluster

0







Figure S2: A) tSNE plot depicting the distribution of cells from the 5 fetal kidney datasets in the 8 sub-clusters of initial clusters 0, 1 and 2 **B)** Bar plots depicting the contribution of the cells from the 5 fetal kidney datasets in the 8 sub-clusters from the initial clusters 0,1 and 2. **C)** Feature plot of proliferation-related genes including CCND1, UBE2C, TOP2A, MKI67, CDK4 and CDK6 in the sub-clustering from the initial unsupervised clustering of clusters 0,1 and 2. **D)** tSNE plot depicting the distribution of cells from the 5 fetal kidney datasets in the 3 sub-clusters of cluster 4 from the initial unsupervised clustering. **E)** Bar plots depicting the contribution of the cells from the 5 fetal kidney datasets in the 3 sub-clusters of NPHS1, CLDN1 expression in the three sub-clusters of original cluster 4. **F)** Feature plot of NPHS1 are in red and the cells with high expression of CLDN1 are in blue. Cells with high expression of both NPHS1 and CLDN1 are in green.



Figure S3: A) tSNE plot depicting the distribution of cells from the 5 fetal kidney datasets in the 3 sub-clusters of cluster 3 (Stromal) from the initial unsupervised clustering. **B)** Bar plots depicting the contribution of the cells from the 5 fetal kidney datasets in the 3 sub-clusters from the initial cluster 3 (Stromal) **C)** Heatmap with the expression of the top 10 cluster-specific genes in the 3 sub-clusters of the original cluster 3 (Stromal). **D)** tSNE plot showing the three sub-clusters of cluster 6 (UB) from the initial clustering. **E)** Heatmap with the expression of the top 10 cluster-specific genes in the 3 sub-clusters of the original cluster of the original cluster 6 (UB). **F)** tSNE plot depicting the distribution of cells from the 5 fetal kidney datasets in the 3 sub-clusters of cluster 6 (UB) from the initial unsupervised clustering. **G)** Bar plots depicting the contribution of the cells from the 3 sub-clusters from the initial cluster 6 (UB) **H)** Expression of known markers (RET, CALB1, SPINK1, GATA3) in cluster 6 (UB) sub-clusters. **(I)** Immunofluorescent staining of human embryonic sections with anti-Calbindin1 and anti-PAX2 antibodies. Calbindin staining is excluded from the tips of the ureteric buds (asterisks). Scale bar: 100um. Representative image of 3 independent stainings.

A

B

Significantly differentially expressed gene (ligand/receptor)	cluster	Corresponding interactor (ligand/receptor)	cluster (> mean expression across all clusters)
BMP7	4	ACVR1	0, 3
EFNB2	4, 6, 9	EPHB3	0, 2, 7, 8
EPHA7	5	EPHB3	0, 2, 7, 8
ERBB2	8	NRG1	4, 6, 7
ERBB4	2, 7, 8	BTC	4, 5, 6
FLT1	9	VEGFC	4, 8, 9
IGF2	0, 3	IGF2R	4, 7, 8, 9
IL6ST	9	CTF1	2, 4, 7, 8
JAG1	5	NOTCH4	9
JAG1	5	NOTCH2	0, 3, 4
KDR	9	VEGFC	4, 8, 9
LIFR	9	CTF1	2, 4, 7, 8
MDK	0, 3	PTPRB	9
NOTCH4	9	JAG1	0, 1, 2, 5, 8
NTRK2	0	BDNF	0, 3, 4, 5, 6
PTN	3	PTPRB	9
TGFBR2	9	TGFB3	0, 3, 6
TGFBR2	9	TGFB2	0, 3
TGFBR3	4	TGFB3	0, 3, 6, 9
TGFBR3	4	TGFB2	0, 3
TNFSF10	5	TNFRSF10B	2, 4, 5, 7, 8

Ligand- Receptor JAG1-NOTCH4 Cap mesenchyme 0 1 2 Renal vesicle / Pretubular aggregates 3 4 Endothelial (NOTCH4) 5 Proximal

Figure S4: A) Table showing the list of either ligand or receptor that was significantly differentially expressed in a cell cluster compared to all other clusters (adjusted p value < 0.001). The corresponding interacting partner expression is greater than the mean expression across all other clusters. **B)** The figure illustrates the possible interaction of ligand JAG1-NOTCH4 interaction. NOTCH4 expression was specifically seen in endothelial cell cluster.

Supplementary Tables

Table S1: The table provides list of significantly expressed genes for each of the 11 clusters compared to all other clusters. pct.1 is the proportion of cells expressing the gene in the cluster of interest and pct.2 is the proportion of cells expressing the gene in all other clusters. avg_logFC is the log fold-change of the average expression between the cluster of interest and all other clusters. Positive values indicate that the gene is more highly expressed in the first group.

Click here to Download Table S1

Table S2: The cells from the progenitor cell clusters (clusters 0, 1 and 2) in the first clustering were further sub-clustered. The table provides list of significantly expressed genes for each of the 8 sub-clusters compared to all other sub-clusters. pct.1 is the proportion of cells expressing the gene in the cluster of interest and pct.2 is the proportion of cells expressing the gene in all other clusters. avg_logFC is the log fold-change of the average expression between the cluster of interest and all other clusters. Positive values indicate that the gene is more highly expressed in the first group.

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Table S3: The cells from the podocyte-like cluster (cluster 4) in the first clustering were further subclustered. The table provides list of significantly expressed genes for each of the 3 sub-clusters compared to all other sub-clusters. pct.1 is the proportion of cells expressing the gene in the cluster of interest and pct.2 is the proportion of cells expressing the gene in all other clusters. avg_logFC is the log fold-change of the average expression between the cluster of interest and all other clusters. Positive values indicate that the gene is more highly expressed in the first group.

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Table S4: The cells from the stromal cluster (cluster 3) in the first clustering were further sub-clustered. The table provides list of significantly expressed genes for each of the 3 sub-clusters compared to all other sub-clusters. pct.1 is the proportion of cells expressing the gene in the cluster of interest and pct.2 is the proportion of cells expressing the gene in all other clusters. avg_logFC is the log fold-change of the average expression between the cluster of interest and all other clusters. Positive values indicate that the gene is more highly expressed in the first group.

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Table S5: The cells from the collecting duct-like cluster (cluster 6) in the first clustering were further sub-clustered. The table provides list of significantly expressed genes for each of the 3 sub-clusters compared to all other sub-clusters. pct.1 is the proportion of cells expressing the gene in the cluster of interest and pct.2 is the proportion of cells expressing the gene in all other clusters. avg_logFC is the log fold-change of the average expression between the cluster of interest and all other clusters. Positive values indicate that the gene is more highly expressed in the first group.

Click here to Download Table S5



Movie 1: 3-Dimensional view of the pseudo-temporal trajectory analysis of the fetal kidney single cell expression data. Single cell RNASeq data from 5 human fetal kidney datasets (87, 105, 110, 115, and 132 days old) were used in this analysis. Each developmental trajectory is color-coded and each dot represents a cell. Cells with > 1000 genes /cell and < 25% mitochondrial read content were used this cell-ordering analysis. 3 independent developmental trajectories were identified: the stroma, the collecting duct and the nephron lineage. The proximity between the stromal trajectory and the nephron progenitor trajectory reflects a significant degree of gene expression overlap. Similarly, the proximity between the collecting duct and the distal/connecting tubules trajectory reflects the expected proximity in gene expression, as well as function between these two closely located segments, despite originating from distinct renal compartments, the UB and the nephron progenitors respectively.