Supplementary Materials for

A reference tissue atlas for the human kidney

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The PDF file includes:

Figs. S1 to S20
Legends for tables S1 to S13
Supplemental Information
References

Other Supplementary Material for this manuscript includes the following:

Tables S1 to S13
Supplemental Figure S1: Quality control of the integrated cluster analysis. (A) Violin plot showing the mitochondrial read percentage per cell in the single cell/nuclei integrated object. (B) Violin plot showing the number of genes per cell in the single cell/nuclei integrated object. (C) Violin plot showing the number of UMIs per cell in the single cell/nuclei integrated object. To exclude potential doublets from the analysis, cells with greater than 5000 genes per cell were filtered out. (D) Feature plot showing the expression of UMOD in all cells from the two single cell and one single nucleus data from the three tissue interrogation sites. UMOD is one of the highly abundant mRNA expressed in loop of Henle that is usually present as ambient mRNA due to cell breakage while dissociation. The feature plots show specific high expression of UMOD in loop of Henle cells. The ambient mRNA expression of UMOD in other cell types is relatively low. (E) Plot showing the proportion of cells in the cell types identified in the two single cell and the single nuclei data from the three tissue interrogation sites. (F) Plot showing the proportion of cells in the cell types identified in each of the sample studied for single /single nuclei assays. (G) Dot plot of cell type specific marker genes.
Absolute gene/protein expression for each cell type or segment of each subject

Keep only glomerulus/podocyte and proximal tubule/tubulointerstitium cell types/segments to compare only datasets that were generated by all technologies.

Absolute expression for each (cell type/segment + subject + technology) combination

Pairwise correlation + hierarchical clustering of absolute expression values

Calculate log₂(fold changes) between glomerular and proximal tubule/tubulointerstitium segments (and vice versa) and between podocytes and proximal tubule cell types (and vice versa) for each subject

Log₂(fc) for each (cell type/segment + subject + technology) combination

Pairwise correlation + hierarchical clustering of log₂(fold changes)

Average log₂(fold changes) across subjects within each cell type or segment and technology

Log₂(fc) for each (cell type/segment + technology) combination

Pairwise correlation between technologies

Average log₂(fold changes) across RNASeq and proteomics technologies

Log₂(fc) for each (cell type/segment + RNASeq/proteomics technology set) combination

Correlation between RNASeq and proteomics

Supplemental Figure S2
Dendrogram heights

1. 0.684
2. 0.484
3. 0.461
4. 0.446
5. 0.392
6. 0.387
7. 0.363
8. 0.349

Supplemental Figure S2
Supplemental Figure S2: Cross-platform comparison of gene and protein expression. (A) Pipeline for correlation analysis across different omics technologies. See methods for details. (B) Clustering dendrogram that was obtained after hierarchical clustering of pairwise correlations between all samples based on the log₂(fold changes) after removal of those genes and proteins that are not consistently detected. Numbers document heights of branches at indicated positions in the dendrogram. See figure 2C for associated heatmap. (C) Hierarchical clustering of pairwise correlation coefficients between all samples based on the log₂(fold changes) without removal of those genes and proteins that are not consistently detected across all assays also groups the samples by anatomical region and not technology. In contrast, pairwise correlation and hierarchical clustering based on logarithmized absolute expression values groups samples by technology, (D) with or (E) without removal of the not consistently detected genes and proteins.
Supplemental Figure S3

| Sample | Genes | Expression |
|--------|-------|------------|
| C12    | CD64  | 0.8        |
| C25    | CD31  | 0.5        |
| C27    | CD13  | 0.2        |
| C28    | CD20  | 0.1        |
| C4     | CD45  | 0.0        |
| C5     | CD47  | 0.1        |
| C6     | CD48  | 0.2        |
| C14    | CD19  | 0.3        |
| C19    | CD10  | 0.4        |
| C23    | CD11  | 0.5        |
| C31    | CD15  | 0.6        |
| C3    | CD10  | 0.7        |
| C4    | CD45  | 0.8        |
| C5    | CD47  | 0.9        |
| C6    | CD48  | 1.0        |

- **Mapped to EC-PTC**
- **Mapped to EC-AFA**
- **Mapped to POD**
- **Mapped to PT-PEC**
- **Mapped to PT**
- **Mapped to DTIL**
Supplemental Figure S3
Supplemental Figure S3: Comparison of expression across CODEX clusters and mapped cell-type specific transcriptomic profiles. For each CODEX cluster, we show its scaled average expression profile together with the scaled average expression profile of the mapped cell-type specific transcriptomic profile.
Supplemental Figure S4

A  Single cell RNAseq

B  Single nucleus RNAseq
Supplemental Figure S4
Supplemental Figure 4
Supplemental Figure S4: Separated and integrated analysis of sc and sn RNAseq datasets generate consistent cell type mapping. Cell types and subtypes identified by the separated analyses of the (A) sn and (B) sc RNAseq datasets. Bars indicate the percentage of all cells that mapped to a particular cell type or subtype, colors indicate the tissue collection method each particular cell was obtained by. Cell type assignments of separate clusters from (C) sn and (D) sc RNAseq datasets were compared to those obtained by the integrated analysis. Numbers indicate nuclei/cell counts; fields are colored by the percentage of cells within each field compared to the row margins. Note that in separated analyses of the sc RNAseq dataset, the applied cutoff for mitochondrial gene expression was higher (≤50% instead of ≤20%); consequently, some of the cells that were removed in the combined analysis were assigned to cell types in the separated analysis. Expression of cell type selective marker genes in cell subtypes identified (E) by single nucleus RNAseq and (F) single cell RNAseq. Mapping of the (G) nuclei and (H) cells to LMD segments documents that the annotations obtained from the separated analyses map to their correct anatomical origin, as observed for the integrated analysis. All heatmaps are colored according to the number of cells assigned to each LMD subsegment, scaled so each row has mean of 0 and standard deviation of 1. See figure 2A for cell type abbreviations.
A

Feature counts for each cell (complete or downsampled dataset)

Keep only cells/nuclei with >= 400/500 (sn/sc) features <= 5000 features and <= 50% mitochondrial genes

Normalize and scale data ('SCTransform') (based on the top 2,000 features)

Principal component analysis

Keep first 30 principal components

Identify adjacent cells/nuclei ('FindNeighbors')

Cluster cells based on an initial resolution of 0.5

Identify cluster-specific marker genes ('FindMarkers')

Cluster marker genes

Essential genes of kidney cell types

Fisher’s exact test:

1st most sig. p-value ≤ 0.05  
2nd most sig. p-value ≤ 0.05

Yes  
No

Assign 1st cell type to cluster  
Assign 1st/2nd cell type to cluster

All cell types identified or resolution = 2.0

Yes  
No

Merge cluster of same cell type

Identify upregulated genes for each cell type ('FindMarkers')

DEGs of cell type

Supplemental Figure S5
Complete single cell sequencing dataset

Iteratively progressively remove libraries from full dataset

Down sampled single cell sequencing dataset

Single cell sequencing analysis pipeline

DEGs of cell type

Single cells assigned to cell type

Quality control
- Total cell count
- For each cell type:
  - Average UMI counts
  - Average feature counts
  - Average % mitochondrial genes

Overlap of cell type assignments

Cells correlating with corresponding LMD segments

SCPs of cell type

DEGs of cell type in complete dataset

Compare

SCP sets of cell type in complete dataset

Compare

Pearson correlation for DEGs

Compare ranks for SCPs

Overlap of cell type assignments

Overlap with reference and other segments

Average results over all downsampled datasets with the same number of libraries
Supplemental Figure S5
Supplemental Figure S5
**Supplemental Figure S5. Complete results of single-cell/nucleus transcriptomic *post hoc* power analysis.** Subject libraries or samples were randomly and progressively removed from the sc (24 libraries) and sn (47 libraries) RNAseq to generate at max 100 non-overlapping random groups for each number of remaining libraries. (A) Sc and sn datasets were subjected to an automated sc/sn data analysis pipeline. (B) Results were averaged for each number of subject libraries and compared between the downsampled and complete datasets as indicated. *Post hoc* power results of the (C) sc and (D) sn RNAseq datasets. ‘**Cell type detected**’: This plot documents how often (in percent) a particular cell type was detected in dependence of the number of analyzed libraries. ‘**Significance of cell type**’: To assign cell types to each cluster we subjected cluster specific marker genes to enrichment analysis using Fisher’s Exact test and a list of literature curated cell-type specific essential genes. For each cluster predicted cell types were ranked by significance and the top ranked cell type was assigned to that cluster. The plot shows the $-\log_{10}(p\text{-values})$ of the first (i.e. the selected) and the second ranked cell type. Comparison of both $p$-values allows an estimation of the reliability of a particular cell type assignment. The larger the difference between both $-\log_{10}(p\text{-values})$, the more certain is that particular cell type assignment. ‘**element/not element of Reference cluster**’: Cells/nuclei that were assigned to the same (above abscise, positive values, full circles) or to a different cell type (below abscise, negative values, open circles) as in the full dataset were counted in each downsampled dataset. ‘**element/not element of indicated LMD subsegment**’: Using cell and nuclei mappings presented in Suppl. Figure 4E/F we counted how many cells/nuclei of a particular cell type mapped to the indicated LMD subsegment (above abscise, positive values, full circles) or to a different LMD subsegment (below abscise, negative values, open circles). ‘**DEGs log(fc)**’: Correlation between the log fold changes of cell type specific markers obtained for the downsampled and complete dataset. Notify that all comparisons were only done, if a particular cell type was detected (as indicated in the first diagram). See figure 2A for cell type abbreviations.
**Non-glomerular metabolite pathways**

Galactose metabolism  
Glycerophospholipid metabolism  
Purine metabolism  
Glycolysis / Gluconeogenesis  
D-Arginine and D-ornithine metabolism  
Phenylalanine, tyrosine and tryptophan biosynthesis  
Linoleic acid metabolism  
Fructose and mannose metabolism

Added after curation of metabolites: Carnitine shuttle  
Carnitine biosynthesis and transport

**Glomerular metabolite pathways**

Glycerophospholipid metabolism  
Linoleic acid metabolism  
alpha-Linolenic acid metabolism  
Glycosylphosphatidylinositol (GPI)-anchor biosynthesis  
Sphingolipid metabolism  
Arachidonic acid metabolism

Supplemental Figure S6
Supplemental Figure S6: Pathway enrichment analysis of spatial metabolomics data. All (A) Non-glomerular and (B) glomerular metabolites obtained from the three nephrectomy samples were subjected to pathway enrichment analysis using MetaboAnalyst. Some pathways were predicted from metabolites that are general precursors for the synthesis of multiple products and participate in multiple pathways. To exclude such unspecific and consequently uncertain pathway predictions, we focused only on those pathways that were predicted from a pathway specific metabolite (see methods for details). To merge the metabolic pathways with the MBCO SCP-networks, we mapped the MetaboAnalyst pathways ‘Glycolysis/Gluconeogenesis’ and ‘Glycerophospholipid metabolism’ to the MBCP SCPs ‘Glycolysis and Gluconeogenesis’ and to ‘Phosphoglyceride biosynthesis’, respectively. Based on identified metabolites, we added the MBCO SCPs “Carnitine shuttle” and “Carnitine biosynthesis and transport” to the predicted MetaboAnalyst pathways (see methods for details).
### Proximal tubule

- Galactose metabolism
- Fructose and mannose metabolism
- Aspartate and arginine metabolism
- D-Arginine and D-Ornithin metabolism
- Transamination pathways
- Urea cycle
- Beta-oxidation
- Carnitine biosynthesis and transport
- Carnitine shuttle

### Collecting duct/Principal cell

- Glycolysis and Gluconeogenesis
- Glutamate and glutamine metabolism
- Citric acid cycle
- Electron transport chain
- Purine metabolism
- Phosphoglyceride biosynthesis

### Thick ascending limb

| RNA seq PT-1 | RNA seq PT-2 | RNA seq PT-3 | RNA seq PT-4 | RNA seq PT-5 | RNA seq PT-6 | RNA seq TAL | RNA seq HDX | RNA proteomics PT | RNA proteomics HDX | RNA proteomics TAL | RNA proteomics HDX |
|--------------|--------------|--------------|--------------|--------------|--------------|-------------|-------------|-------------------|--------------------|--------------------|--------------------|
| 6            | 3            | 5            | 2            | 1            |              |             |             |                   |                    |                    |                    |
| 6            | 3            | 5            | 2            | 1            |              |             |             |                   |                    |                    |                    |
| 3            | 4            | 4            | 1            | 1            |              |             |             |                   |                    |                    |                    |
| 5            | 5            | 6            | 6            | 2            |              |             |             |                   |                    |                    |                    |
| 3            | 4            | 4            | 1            | 1            |              |             |             |                   |                    |                    |                    |
| 7            | 1            |              |              |              |              |             |             |                   |                    |                    |                    |
| 1            |              |              |              |              |              |             |             |                   |                    |                    |                    |
| 6            | 3            | 5            | 2            | 1            |              |             |             |                   |                    |                    |                    |
| 1            |              |              |              |              |              |             |             |                   |                    |                    |                    |
| 3            | 4            | 4            | 1            | 1            |              |             |             |                   |                    |                    |                    |
| 2            | 7            | 1            |              |              |              |             |             |                   |                    |                    |                    |

### Distal convoluted tubule

- Macrophages
- Tubulointerstitium

Supplemental figure S7
Supplemental Figure S7: Mapping of tubulointerstitial SCPs to cell types. SCPs predicted by dynamic enrichment analysis for the tubulointerstitial segment by the LMD Proteomics and spatial metabolomics assays were mapped to one of three detected glomerular cell types, because they were either detected in that cell type as well or related to SCPs detected for that cell type. Numbers indicate at which rank a particular SCP was detected. Notify that dynamic enrichment analysis can predict single SCPs or combinations of up to three SCPs, and consequently the same rank can be given to multiple SCPs. When an SCP was predicted by multiple cell subtypes, the highest rank is visualized in this figure.
Supplemental Figure S8: Enrichment analysis of differentially expressed genes and proteins in proximal tubule cells and subsegments. See Figure 5 for details. SCPs that were among the top seven predictions based on dynamic enrichment analysis of PT DEGs and DEPs and were removed from the main figure for space reasons.
Supplemental Figure S9

A

- **Podocytes**
- **Mesangial cells**
- **Endothelial cells**
- **Parietal epithelial cell**

**SCPs predicted based on bulk RNASeq/proteomics**

- **LMD RNASeq**
- **LMD Proteomics**
- **NSC Proteomics**
- **Spatial metabolomics**

B

- **Gap junction organization**
- **Hydrogen transmembrane transport**
- **Tight junction organization**
- **Natriuretic peptide receptor signaling**
- **Basement membrane assembly and organization**
- **Focal adhesion organization**
- **Semaphorin signaling**
- **Lamellipodium organization**
- **Actin filament bundling and crosslinking**
- **Actin filament nucleation and branching**
- **Inhibition of amyloid aggregation, amyloid degradation and uptake**
- **Thin myofilament organization**
- **Connection of muscle sarcomere to plasma membrane**
- **Connection of muscle sarcomere to extracellular matrix**
- **Myofibril formation**

*PEC = Parietal epithelial cell*
Amyloid
- Amyloid precursor protein cleavage
- Amyloid plaque organization
- Notch receptor signaling
- Discoidin domain receptor signaling

Cell-matrix adhesion
- Fibrillar collagen core structure organization
- Inhibition of amyloid aggregation, amyloid degradation and uptake
- Basement membrane attachment to cell surface
- Focal adhesion organization
- Semaphorin signaling
- Sphingolipid metabolism

Actin dynamics
- Lamellipodium organization
- Filopodium organization
- Actin filament severing and depolymerization
- Equatorial RhoA activation
- Actin filament nucleation and branching
- Myofibril formation
- Thin myofilament organization

Amyloid plaque organization
- Macrophage migration inhibitory factor signaling
- Recycling endosome dynamics
- Caveolin-mediated endocytosis

Cytoskeleton
- Connection of muscle sarcomere to extracellular matrix
- Connection of muscle sarcomere to plasma membrane
- Thin myofilament organization
- Myofibril formation

Vascular endothelial growth factor receptor signaling

BMs dynamics
- Basement membrane assembly and organization
- Integrin receptor signaling
- Integrin-mediated leukocyte rolling
- Leukocyte transmigration through endothelium
- Hydrogen transmembrane transport
- Gap junction organization
- Tight junction organization

Phosphoglyceride biosynthesis

Antigen presentation
- Antigen presentation via MHC class II molecules
- Antigen presentation via MHC class I molecules
- Immunoproteasome organization
- Protein polyubiquitination

Spatial metabolomics

Spatial metabolomics

E

MESANGIAL CELL/GLOMERULUS

Supplemental Figure S9
Cell-cell adhesion
- Hydrogen transmembrane transport
- Gap junction organization
- Tight junction organization

Cell-matrix adhesion
- Desmosome organization
- Adherens junction organization
- Hemidesmosome organization
- Basement membrane attachment to cell surface

Intermediate filament dynamics
- Vimentin-like intermediate filament dynamics
- Epithelial intermediate filament dynamics
- Nuclear intermediate filaments

Glutamate-mediated control of postsynaptic potential
U2 snRNP assembly
- Metaphase to anaphase checkpoint

SN RNASeq
SC RNASeq
LMD RNASeq
Supplemental Figure S9: Enrichment analysis for glomerular datasets. (A) Marker genes and proteins identified by LMD RNAseq and Proteomics and NSC Proteomics were subjected to dynamic enrichment analysis. (B) Predicted glomerular SCPs were mapped to one of four detected glomerular cell types, because they were either detected in that cell type as well or related to SCPs detected for that cell type. Numbers indicate at which rank a particular SCP was detected. Notify that dynamic enrichment analysis can predict single SCPs or combinations of up to three SCPs, and consequently the same rank can be given to multiple SCPs. (C) SCP networks predicted for podocytes based on the sn and sc RNAseq datasets were merged with the podocyte mapped SCPs identified in B. (D) Podocyte specific modules were generated by combined analysis of the podocyte sc and sn marker genes and all glomerular marker genes and proteins. SCP networks predicted for (E) mesangial cells, (F) glomerular endothelial cells and (G) parietal epithelial cells based on sn and sc RNAseq datasets were merged with the SCPs that were mapped to these cell types in (B).
Cell adhesion
- Desmosome organization
- Hemidesmosome organization
- Basement membrane attachment to cell surface
- Parathyroid hormone receptor signaling

Cytoskeleton
- Epithelial intermediate filament dynamics
- Nuclear intermediate filaments
- Actin filament nucleation and branching
- Semaphorin signaling
- Lamellipodium organization
- Filopodium organization
- Recycling endosome dynamics

Translation
- SN RNASeq (3 subtypes)
- SC RNASeq
- Chaperone mediated protein folding in ER
- ER unfolded protein response pathway
- Small ribosomal subunit organization
- Translation initiation
- Large ribosomal subunit organization
- Translation elongation

Number of sources supporting marker
- 1
- 2

- regulation of cell migration
- regulation of endopeptidase activity
- regulation of lymphocyte activation
- cell-cell adhesion

- response to endoplasmic reticulum stress
- intracellular protein transport
- Golgi calcium ion transport

- proteasomal protein catabolic process

- kidney morphogenesis
- regulation of cellular ketone metabolic process

- mRNA metabolic process
- telomere maintenance

M1 translation peptide metabolic process
M2 M3 cellular oxidant detoxification
M4 M5 M6 M7

humanbase

Supplemental Figure S10
Supplemental Figure S10: Enrichment analysis for the Loop of Henle. Descending limb cell specific marker genes were subjected to (A) dynamic enrichment and (B) humanbase module analysis. (C) Dynamic enrichment and (D) module analysis results of thin ascending limb cell marker genes. (E) Dynamic enrichment and (F) module analysis results of thick ascending limb cell marker genes.
Ion reabsorption

- Sodium transmembrane transport
- Chloride transmembrane transport
- Calcium transmembrane transport
- Potassium transmembrane transport
- Phosphate transmembrane transport
- Vesicle fusion with plasma membrane
- Caveolin-mediated endocytosis

ECM

- Extracellular matrix breakdown by serine proteases
- Extracellular matrix breakdown and membrane shedding by adamalysins
- Basement membrane assembly and organization

Vesicle traffic

- Osteopontin receptor signaling
- Apoptosis caspase pathway
- Electron transport chain
- Nucleosome assembly
- Nuclear pore complex organization
- Endoplasmic reticulum quality control system

Supplemental Figure S11
Supplemental Figure S11: Enrichment analysis for the distal convoluted tubule. Distal convoluted tubule cell and segment specific DEGs were subjected to (A) dynamic enrichment and (B) module analysis.
Ion reabsorption

- Sodium transmembrane transport
- Mineralocorticoid receptor signaling
- Potassium transmembrane transport
- Calcium transmembrane transport
- Cardiomyocyte repolarization during action potential and hyperpolarization

Adenyl cyclase signaling pathway
Nerve growth factor receptor signaling
Endoplasmic reticulum tubulus maintenance
Sarcoplasmic reticulum organization
Electron transport chain
Basement membrane assembly and organization
G-protein coupled receptor signaling pathway

SN RNASEq
SC RNASEq

Number of sources supporting marker
1
2

M1
mitochondrion organization
ribonucleotide biosynthetic process

M2
monovalent inorganic cation transport
sodium ion transport

M3
receptor-mediated endocytosis
Supplemental Figure S12: Enrichment analysis for the collecting duct. Connecting tubule cell specific DEGs were subjected to (A) dynamic enrichment and (B) module analysis. Principal cell and collecting duct specific DEGs were subjected to (C) dynamic enrichment and (D) module analysis. Intercalated cell and collecting duct specific DEGs were subjected to (E) dynamic enrichment and (F) module analysis. (G) Enrichment analysis of the marker genes for 4 different intercalated cell subtypes from sn and sc RNAseq using Gene Ontology Biological Processes identifies the pathways ‘Phagosome acidification’ and ‘Phagosome maturation’.
Supplemental Figure S13: Enrichment analysis for vascular cells. (A) Endothelial cell specific DEGs were subjected to dynamic enrichment. (B) Similarly, vascular smooth muscle cell specific DEGs were subjected to dynamic enrichment analysis.
NON-IMMUNE INTERSTITIAL CELLS

ECM dynamics
- Fibrillar collagen core structure organization
- Basement membrane assembly and organization
- Collagen fiber crosslinking
- Discoidin domain receptor signaling
- Tropoelastin synthesis
- Tenascin receptor signaling
- Fibronectin synthesis and extracellular assembly

Complement
- Classical complement pathway
- Endogenous control of complement activity
- Alternative complement pathway

Cytoskeleton
- Baseline membrane attachment to cell surface
- Connection of muscle sarcomere to plasma membrane
- Myofibril formation
- Thin myofilament organization
- Actin filament capping
- Actin polymerization
- Actin filament bundling and crosslinking
- Zinc transmembrane transport
- Cellular iron uptake and export

Supplemental Figure S14
Supplemental Figure S14: Enrichment analysis for interstitial cells. Interstitial fibroblast cell and segment specific DEGs were subjected to dynamic enrichment analysis.
Supplemental Figure S15: Enrichment analysis for immune cells.  (A) Macrophage/Monocyte, (B) Natural Killer cell, (C) B-cell, and (D) T-cell specific DEGs were subjected to dynamic enrichment analysis.
Supplemental Figure S16: Expression of immune related genes in the kidney cell types. Using all genes that are assigned to the Gene Ontology Biological Process “immune system process” or any of its children processes based on the “is_a” or “part_of” relationships, we documented the percentage of immune system related genes (orange) in all cell type, subtype and segment-specific marker genes and proteins. See figure 2A for cell type abbreviations.
Supplemental Figure S17

A
Proximal tubule cells
- Beta-oxidation
- Aspartate and arginine metabolism
- Proline metabolism
- Glutathione conjugation
- Sodium transmembrane transport
- Drug and toxin export via membrane transport proteins
- Urea cycle
- Glycolysis and gluconeogenesis
- Citric acid cycle

B
Podocytes
- Tight junction organization
- Fibronectin synthesis and extracellular assembly
- Basement membrane attachment to cell surface
- Hemidesmosome organization
- Adherens junction organization
- Basement membrane assembly and organization
- Collagen fiber crosslinking
- Gap junction organization
- Inhibition of apoptosis
- Hydrogen transmembrane transport
- Retinol metabolism

Glomerular endothelial cells
- Antigen presentation via MHC class I molecules
- Antigen presentation via MHC class II molecules
- Vascular endothelial growth factor receptor signaling
- Basement membrane assembly and organization
- Collagen fiber crosslinking
- Immunoproteasome organization
- Exosome secretion
- Protein polyubiquitination
C

Descending limb cells

Epithelial intermediate filament dynamics
Hemidesmosome organization
Basement membrane attachment to cell surface
Desmosome organization
Vimentin-like intermediate filament dynamics
Adherens junction organization

Thin ascending limb cells

Tight junction organization
Epithelial intermediate filament dynamics
Basement membrane attachment to cell surface
Desmosome organization
Hemidesmosome organization
Nuclear intermediate filaments
Adherens junction organization
Discoidin domain receptor signaling

Thick ascending limb cells

Tight junction organization
Citric acid cycle
Glycolysis and Gluconeogenesis
Retinol metabolism
Transmembrane glucose transport
Basement membrane attachment to cell surface
Chaperone mediated protein folding in ER
ER unfolded protein response pathway
Discoidin domain receptor signaling

SC RNASeq

Supplemental Figure S17
**Endothelial cells – Afferent/Efferent Arteriole**

- Antigen presentation via MHC class II molecules
- Fibronectin synthesis and extracellular assembly
- Antigen presentation via MHC class I molecules
- Basement membrane assembly and organization
- Hydrogen transmembrane transport
- Exosome secretion
- Collagen fiber crosslinking
- Natriuretic peptide receptor signaling
- Immunoproteasome organization

**F**

**Endothelial cells – descending vasa recta/afferent/efferent arteriole**

- Tight junction organization
- Leukocyte transmigration through endothelium
- Adherens junction organization
- Gap junction organization
- Basement membrane assembly and organization
- Inhibition of matrix metalloproteinase and adamalysin activity
- Extracellular matrix breakdown and membrane shedding by adamalysins
- Actin filament severing and depolymerization
- Actin filament bundling and crosslinking
- Actin polymerization
- Focal adhesion organization
- Lamellipodium organization

**G**

**Interstitial cells**

- Alternative complement pathway
- Fibrillar collagen core structure organization
- Classical complement pathway
- Fibulin receptor signaling
- Fibronectin synthesis and extracellular assembly
- Discoidin domain receptor signaling
- Collagen fiber crosslinking
- Tenascin receptor signaling
- Microfibril scaffold organization
- Fibrillin synthesis
- Basement membrane attachment to cell surface

**Supplemental Figure S17**
Supplemental Figure S17

Connecting tubule cells

Supernumerary RNASeq

Principal cells

Intercalated cells
Endothelial cells - arteriolar

Supplemental Figure S17
Supplemental Figure S17: Cellular key functions are most consistently predicted by downsampled sc and sn RNAseq datasets. To analyze the reliability of predicted cell type-specific biology we subjected the top 300 cell-type specific marker genes that were obtained from the full or down-sampled sc and sn RNAseq datasets (Suppl. Figure 5C and 5D, respectively) to dynamic enrichment analysis. SCPs that were among the top seven predictions for the complete sc and sn RNAseq were identified. We identified the dynamic enrichment ranks of these SCPs in the down-sampled datasets and averaged them across all datasets with the same number of libraries. Color scale ranges from 1 (dark green/orange/purple) to 21 or higher (white). Notify that SCPs predicted for the complete datasets are not necessarily the same as the one documented in figure 6. The 2124, 4447 and 721 individual complete and downsampled datasets were analyzed using our automated pipeline that did not allow manual ad hoc optimization and merged all clusters annotated to the same cell type instead of annotating each cluster to a cell subtype. The first set of subfigures shows the predicted SCPs identified from the sc RNAseq dataset for (A) proximal tubule cells, (B) glomerular cell types, (C) cell types of the Loop of Henle, (D) of the distal convoluted tubule, (E) of the collecting duct, (F) vascular cells and (G) non-immune and immune interstitial cells. The second set of subfigures shows the predicted SCPs identified from the sn RNAseq dataset for (H) proximal tubule cells, (I) glomerular cell types, (J) proximal tubule/descending limb cell, (K) cell types of the Loop of Henle, (L) of the distal convoluted tubule, (M) of the collecting duct and (N) vascular cells.
Only Mitochondrial

Only Peroxisomal

Shared enzymes

Ketogenesis

Gluconeogenesis only

Glycolysis only

PDH

L DH

Citric acid cycle

Only Ketone body catabolism

Only Ketogenesis

Beta oxidation

Supplemental Figure S18
Supplemental Figure S18: Prediction of cellular dependencies on aerobic and anaerobic metabolic pathway activities. (A) We designed a small ontology that allows distinguishing between aerobic and anaerobic as well as catabolic and anabolic reactions. Shown is the annotated pathway hierarchy. Colored pathways indicate parent and child pathway pairs, where the child contains only enzymes that are specifically involved in the function of its parent and of any other parent. Pathways were populated with genes by literature curation. Parents are populated with all genes of the child pathways. (B) Top 500 cell type, cell subtype and subsegment specific marker genes and proteins were subjected to enrichment analysis using the leaf pathways shown in A. Initial enrichment results determined with pathways were used for the analysis shown in figure 6. For each cell type, subtype and subsegment we only considered a higher level pathway, if the child pathway that contains the enzymes specifically involved in the higher level pathway activity was also predicted (as indicated by the colored pathway pairs in A). Cell types that contain many cells obtained from medullary samples are marked. See figure 2A for cell type abbreviations.
Screen

Gene Ontology, Molecular Biology of the Cell Ontology, Wikipedia, selected reviews
for genes involved in sodium or glucose transmembrane transport

Expert validate to confirm that gene products are transporters
(add references: NCBI/UniProt gene info, PubMed IDs)

Generate pathways that describe:
  a) Type of transporter:
      Symporter, antiporter, unimolecular transporter
  a) All involved molecules

Generate pathway hierarchies for each molecule:

**Final parents:**
  a) Sodium or Glucose transport in direction *lumen to blood (L2B)*
  b) Sodium transport in direction *blood to lumen (B2L)*

230 SCPs in parent child relationships
113 genes
670 pathway gene associations
Supplemental Figure S19

E

mRNA counts [%]

Experimental determined sodium reabsorption [%]

Proximal Tubule Loop Of Henle Distal Convoluted Tubule Collecting Duct Excretion

mRNA counts [%]

mRNA levels [%] Blood-to-lumen mRNA levels [%] Lumen-to-blood

F

Sodium L2B
Sodium L2B by symporter
Sodium sulfate symporter
NaS1 (SLC13A1)
Sodium potassium symporter
Sodium potassium chloride symporter
NKCC2 (SLC12A1)
Sodium phosphate symporter
Sodium phosphate symporter, Type III
PIT-2 (SLC20A2)
PIT-1 (SLC20A1)
Sodium phosphate symporter, Type II
NPT2a (SLC34A1)
NPT4 (SLC17A3)
NPT1 (SLC17A1)
Sodium nucleotide symporter
CNT1 (SLC28A1)
Sodium mono- d- or tricarboxylate symporter
Sodium monocarboxylate symporter
Sodium lactate symporter
SMCT2 (SLC5A12)
Sodium glucose symporter
SGLT2 (SLC5A2)
Sodium chloride symporter
Sodium chloride creatine symporter
CT1 (SLC6A8)
NCC (SLC12A3)
Sodium carnitine symporter
High affinity carnitine symporter
OCTN2 (SLC22A5)
Sodium bicarbonate symporter
NBCn1 (SLC4A7)
NBCe1 (SLC4A4)
Sodium amino acid symporter
Sodium aspartate symporter
Sodium hydrogen aspartate vs potassium antiporter
EAAT3 (SLC1A1)
EAAT1 (SLC1A3)
Sodium L2B by antiporter
Sodium vs hydrogen antiporter
NHE3 (SLC9A3)

Sodium B2L
Sodium B2L by symporter
Sodium mono- d- or tricarboxylate symporter B2L
Sodium dicarboxylate symporter B2L
NaBc3 (SLC13A3)
Sodium chloride symporter B2L
Sodium chloride GABA symporter B2L
GAT2 (SLC6A13)
Sodium amino acid symporter B2L
Sodium large neutral amino acid symporter B2L
Cationic amino acid vs large neutral amino acid sodium antiporter
gamma+LAT1 (SLC7A7)
Sodium glutamine symporter B2L
SNAT1 (SLC38A1)
Sodium B2L by antiporter
Hydrogen vs sodium antiporter
Hydrogen vs sodium amino acid antiporter
Hydrogen vs sodium alanine antiporter
SNAT3 (SLC38A3)
Calcium vs sodium antiporter
NCX1 (SLC8A1)
**G**

Exponentially determined glucose reabsorption [%]

- mRNA counts [%]
- Total Glucose reab.

| Experimentally determined glucose reabsorption [%] |
|---------------------------------------------------|
| Proximal Tubule | Loop Of Henle | Distal Convoluted Tubule | Collecting Duct | Excretion |
| 100             | 80            | 60                        | 40             | 20        |

**H**

Glucose transporter

- mRNA levels [%]
- Glucose L2B
- Glucose L2B by symporter
- Sodium glucose symporter
- SGLT2 (SLC5A2)
- SGLT1 (SLC5A1)

| Glucose transporter | PT cell | Loop of Henle | DCT cell | Collecting duct |
|---------------------|---------|---------------|----------|-----------------|
|                     | PT      | DTL cell      | ATL cell | TAL cell        |
|                     | DCT     | CNT cell      | PC       | IC              |

Supplemental Figure S19
Supplemental figure S19: Prediction of transmembrane ion and molecule movements. (A) Flow chart documenting the steps involved in the generation of the ontology for transmembrane sodium and glucose transport. Shown are example transporters (gray) involved in (B) sodium and glucose lumen-to-blood (L2B) and (C) sodium blood to lumen (B2L) transport and how they integrate into the hierarchy to finally converge on sodium and glucose L2B and B2L transport. Symporter mechanisms are colored in orange, antiporter mechanisms in blue. (D) Net reabsorption capacities for sodium (colored bars) were determined as describe in figure legend 7 and compared to experimentally determined total sodium reabsorption. (E) Comparison between experimentally measured total and transcellular sodium reabsorption profiles and reabsorption capacities that were predicted from the three sn RNAseq datasets used in figure 7 and the KPMP sc RNAseq dataset. See figure 7 for details. (F) Sodium reabsorption mechanisms were predicted based on all four datasets (three single nucleus and one single cell RNAseq datasets). See figure 2A for cell type abbreviations. (G) Reabsorption capacities for glucose transmembrane transport were calculated using the three sn RNAseq datasets as described in figure 7 and compared to experimentally determined glucose reabsorption profiles. Since only one physiology textbook documented the glucose reabsorption profiles, there is no standard error for the experimental values. Facilitated glucose transporters were excluded. (H) As for sodium, we analyzed the transport mechanisms involved in glucose reabsorption. (I) We compared the reabsorption capacities that were calculated using the three sn and the sc RNAseq datasets with the experimental reabsorption profiles, (J) followed by visualization of the individual transport mechanisms for glucose.
1) Serine incorporation into ER
2) Ceramide synthesis
3) (Stimulation of) Ceramide transport to SM-synthesis sites at Golgi
4) Sphingosin metabolism

Supplemental Figure S20
Supplemental Figure S20: Expression of genes involved in sphingomyelin synthesis and sphingosine metabolism in all kidney cell types and segments. Expression of curated enzymes was detected in the indicated cell types/segments. Genes were ranked by significance and ranks were added to the figure.
**Supplementary Table Captions**

**Supplemental Table S1.** Samples used for different analytical pipelines.

**Supplemental Table S2.** Laser microdissected (LMD) RNAseq gene expression

**Supplemental Table S3.** Laser microdissected (LMD) Proteomics protein expression

**Supplemental Table S4.** Near Single Cell (NSC) Proteomics protein expression

**Supplemental Table S5.** Top 2,000 marker genes and proteins predicted by each assay for each analyzed cell subtype, cell type and tissue subsegment. Marker genes and proteins are differentially expressed genes (DEGs) and proteins (DEPs) that were obtained by comparing each cell type, cell subtype or subsegment to all other cell subtypes, types or subsegments. Initially, we duplicated all subsegmental datasets and added them to each data integration term that describes a cell type localized in that particular segment. For cell type specific assignments of the subsegmental data see results section, Figure 5C and Supplementary Figure 8.

**Supplemental Table S6.** # of significant marker genes that were subjected to dynamic enrichment analysis.

**Supplemental Table S7.** Dynamic enrichment analysis results of the top 300 marker genes and proteins. We duplicated all predictions based on the subsegment specific LMD RNAseq and Proteomics and the NSC Proteomics and added them to each integration term that describes a cell type localized in that particular segment. From these results we assigned cell type specificity to the predicted pathways as described in the results sections and documented in Figure 5C and Supplementary Figure 8. Notify that the columns “Experimental_symbols_count” and “Scp_symbols_count” contain the experimental and scp genes after removal of all those genes that are not part of the background list of genes (See methods for details). Hence, they can be smaller than the gene counts documented in supplementary table 6.

**Supplemental Table S8.** Spatial metabolomics metabolite correlations for subjects 18-139 (A), 18-142 (B) and 18-342 (C).

**Supplemental Table S9.** Gene Ontology enrichments for modules in the kidney-specific functional network of top DEGs and DEPs in PT, podocytes, and principal cells.

**Supplemental Table S10.** Literature curated cell-type specific essential genes used for cell type identification.

**Supplemental Table S11.** Enrichment analysis of the top 500 significant marker genes and proteins using the generated metabolic ontology.

**Supplemental Table S12.** Sodium transporters identified in the sc and sn RNAseq datasets and their function in kidney sodium reabsorption.

**Supplemental Table S13.** Overview of cell type specific marker genes.
SUPPLEMENTAL INFORMATION

Cells of the kidney

Proximal tubular cells

Merged proximal tubule SCP networks predict a high level of metabolic activity dependent on β-oxidation of lipids, ammonium metabolism as well as absorption of ions, ion-dependent glucose reabsorption and detoxification mechanisms (Fig. 4A). These SCPs, as shown by the different colors, are inferred from multiple technologies. The size of the SCP circle reflects the number of technology types that support the prediction of the SCP, while pie slices represent the individual technologies. In some physiology functions, cases of multiple pie slices are shown for the same technology indicating that this technology predicts the same SCP for multiple subtypes of the PT cells. The solid lines indicate connections between SCPs predicted by MBCO relationships and the dashed lines indicate additional well-known relationships between SCPs. Typically, these edges can represent functional relationships such as enzyme-substrate relationships or cotransport of molecules by symporters. It should be noted that most SCPs consist of multiple gene/gene products/metabolites of which only some are experimentally determined. Both the LMD proteomics and spatial metabolomics assays only distinguish between glomerular and tubulointerstitial regions in the kidney. SCPs that were predicted by these two assays either overlapped with or described similar functions as the SCPs that were identified by the proximal tubule cell or segment-specific datasets (fig. S7). This agrees with the observation that most tubulointerstitial cells were proximal tubule cells (fig. S4A/B). Consequently, we added all SCPs identified by LMD proteomics and spatial metabolomics to the proximal tubule network as well. The identified predictions are in agreement with the well-established physiological functions of PT cells that include ATP-dependent reabsorption of ions, glucose and other small molecules like amino acids and mono- and dicarboxylates (e.g., lactate or oxalate) (69). The pathways also highlight the important role of PT cells in ammonium excretion, drug clearance (70) and iron homeostasis pathways (71). The latter - among other functions - mitigate kidney damage during AKI (72). The prediction of glucose, fructose and glutamine metabolism from integration of transcriptomic, proteomic and metabolomics assays is in agreement with the high levels of PT gluconeogenesis activity (73, 74). Beta-oxidation, which is the central pathway for energy generation in the PT cells (75, 76), is predicted by four out of six technologies. The identified genes and proteins document involvement of both mitochondrial and peroxisomal beta-oxidation (table S7). These findings support the notion that peroxisomes could be a target in kidney injury.

Both proteomic datasets of the PT subsegments highlight mitochondrial carnitine shuttle pathway that describes a central transport mechanism involved in both peroxisomal (77) and mitochondrial (78) beta-oxidation. We identify by spatial metabolomics the central carrier molecule carnitine, as well as acetyl-carnitine and palmitoyl-carnitine that are involved in transport processes during peroxisomal and mitochondrial beta-oxidation, respectively. The identification of carnitine biosynthesis and the carnitine precursor 3-Dehydroxycarnitine predicts that adult kidney - besides apical reabsorption of carnitine - also has the biosynthetic capacity for local carnitine production. Loss of beta-oxidation and consequently ATP synthesis is a significant contributor to tubulointerstitial fibrosis (79). Hence mapping of the variations in these pathways in different patient populations can provide a basis for molecular stratification of kidney fibrosis. Our data indicate the importance of beta-oxidation for proximal tubule function, since the prediction of local carnitine synthesis suggests an alternative carnitine source to dietary carnitine intake that might gain importance under a strictly vegetarian diet (80). Prediction of high levels of ATP generation and turnover rate is supported by the spatial metabolites that enrich for
a pathway involved in the biosynthesis and degradation of adenine nucleotides. The ability of proximal tubule cells to significantly contribute to gluconeogenesis, especially in states of starvation is documented by the identification of many enzymes involved in gluconeogenesis in our datasets. Glycolysis-specific enzymes were not detected, as described by others and in agreement with the low potential for glycolysis in the proximal tubule (74). Only a few pathways describing general cell biological functions (such as ECM dynamics, cell adhesion and translation) were predicted by one technology (fig. S8).

Consequently, our analyses show that the different technologies describe the same biology, even though they might detect different genes or proteins and analyzed samples from the overlapping and non-overlapping participants (table S1).

Community clustering of PT marker genes in a kidney-specific functional network (Fig. 4B) identifies four modules enriched for functions including translation (M2), cellular response to metal ion (M4), mitochondrial organization (M1), brush border assembly (M3), and anion transport (M3). The marker genes were identified across five distinct technologies (sc/sn/LMD transcriptomics, and two independent proteomics datasets), and include genes with a corrected p-value of less than 0.01 in each technology. Genes are shaded per number of technologies identifying each marker. Five genes (ALDH2, ANPEP, LRP2, PDZK1, and SHMT1) were identified as PT markers across all five technologies. Fifty-four genes were identified as PT markers by four of the five technologies, and 106 genes were identified as PT markers by three of the five technologies. Functional enrichments in module clustering provide a picture consistent with the SCP enrichments: key processes enriched in network modules and also identified in SCP enrichments include fatty acid beta-oxidation (M1, M4), ammonium ion metabolic process (M3), glucose metabolic process (M3), detoxification (M1), anion transport (M3), and cellular response to metal ion (M4). While we did not separate between male and female samples in this study, sex specific differences in proximal tubule cells have been described recently (5).

Glomerular cells

In agreement with a previous study focusing on human and mouse glomerular cells (4) we detected all four different glomerular cell types, podocytes, mesangial cells, endothelial cells and parietal epithelial cells. The sc and sn transcriptomic datasets (Fig. 2) lead to four glomerular cell type specific SCP-networks. We separately analyzed the LMD transcriptomic and LMD and NSC proteomics and spatial metabolomics datasets (that were obtained from the whole glomerulus thus lacking cell type specificity) and identified glomerular SCP networks (fig. S9A). Analyzing the overlap between the glomerular SCP networks with each of the three cell-type specific SCP-networks allows us to assign glomerular SCPs to podocytes, mesangial cells or glomerular endothelial cells (fig. S9B). Ten of the 19 glomerular SCPs are also predicted for at least one glomerular cell-type based on the sc/sn transcriptomic datasets. Seven other SCPs we identified map to particular cell types per functional relationships predicted from the sc/sn RNAseq datasets. These SCPs were added to each of the individual cell type specific SCP-networks. Podocyte SCPs (fig. S9C) focus on cell-cell/cell-matrix adhesion, glomerular basement membrane (GBM) and extracellular matrix (ECM) dynamics as well as actin dynamics. All these pathways are required for foot process maintenance and formation of the glomerular filtration barrier (81). Metabolomics data identify sphingolipid metabolism that could be involved in cell-cell adhesions as shown in other cell types (52, 82). LMD segmental proteomics and transcriptomics identified key pathways involved in actin dynamics as well as cell-cell and cell-matrix adhesion. Multiple technologies identify tight junction organization, focal adhesion
organization and lamellipodia organization. The glomerular slit diaphragm between mature podocytes develops from epithelial tight and adherens junctions. It contains many of these junctional protein components and was suggested to be a specialized form of either tight junctions or adherens junctions. This explains the prediction of these two structures from our data, thought they are not morphologically observed in healthy podocytes. We show WNT signaling as a central modulator of podocyte function. The pathway “Retinol metabolism” was predicted for both sc and sn RNAseq dataset as a regulator of tight junction similar structures. In agreement, retinoic acid has a regulatory effect on tight junctions in the epidermis and plays a significant role in mitigating podocyte apoptosis and dedifferentiation during podocyte injury.

Community clustering of podocyte marker genes in a kidney-specific functional network identifies six modules (fig. S9D). Functional enrichments in these modules included glomerulus development, vasculature development, cell-substrate adhesion, cell-cell adhesion, and actin cytoskeleton organization. Sixteen genes (AHNAK, CLIC5, FERMIT2, GOLIM4, IQGAP2, NES, NPHS2, PDLIM5, PODXL, PTPRO, SLK, SYNPO, and TJP1) were identified as podocyte markers by all five technologies surveyed. Forty-one genes were identified by four of the five technologies and 108 genes were identified by three of the five technologies.

Our datasets identify one mesangial and one transitional mesangial/VSMC cell type from the sn and sc RNASeq assays, respectively (Fig. 2). LMD transcriptomics and proteomics and NSC proteomics along with sc and sn transcriptomics data identify SCPs involved in actin cytoskeleton dynamics, ECM dynamics, cell adhesion and amyloid plaque generation in these mesangial cells (fig. S9E). Our results are in agreement with their well-known function in blood vessel contraction and ECM support. In addition, one glomerular endothelial cell type was identified by the sc RNAseq data (Fig. 2). Its SCP-network derived from integration of LMD proteomics and transcriptomics and NSC transcriptomics along with sc transcriptomic data identify cytoskeletal, trans-endothelial immune cell migration and antigen presentation pathways (fig. S9F). The assignment of “integrin-mediated leukocyte rolling” to endothelial cells is supported by the presence of the related “leukocyte transmigration through endothelium” SCP by sc and LMD RNA transcriptomics. Sn and sc RNAseq assays identified one parietal epithelial and one parietal epithelial cell type that also shows characteristics of loop of Henle cells, respectively (Fig. 2). Parietal epithelial SCP networks contain pathways involved in cell-cell and cell-matrix adhesion and intermediate filament dynamics (fig. S9G).

Loop of Henle

We identified one descending limb cell subtype by each sc and sn RNAseq assay (Fig. 2A). SCP networks from sc and sn RNAseq data for the descending limb cells identify cell adhesion functions and cytoskeleton dynamics (fig. S10A). The presence of “tight junction organization” is in agreement with barrier formation in the descending limb that can allow for paracellular water reabsorption but not for reabsorption of ions such as sodium or chloride. Community clustering of descending limb marker genes in a kidney-specific functional network identifies six modules enriched in functions including cell-cell adhesion, epithelium development, tube development, response to endoplasmic reticulum stress, and water homeostasis (fig. S10B).
Three thin ascending limb (ATL) cell subtypes are identified by sn RNAseq although only one type was identified by sc RNAseq (fig. 2A). SCP-networks obtained for ATL cells from these two technologies describe functions such as cell adhesion, cytoskeleton dynamics and translation (fig. S10C). Overall, these SCP networks agree with the known functions of these cells that initiate the formation of dilute urine by the establishment of a water impermeable barrier that is permeable to low levels of ions (88). Community clustering of ATL marker genes in a kidney-specific functional network identifies seven modules enriched in functions including translation (M1), kidney morphogenesis (M6), and cell-cell adhesion (M4) (fig. S10D).

Sc and sn transcriptomics identified one and two thick ascending limb (TAL) cell subtypes, respectively (Fig. 2A). TAL cell SCPs indicate sodium, potassium and chloride transport capabilities as detected by sc, sn and LMD transcriptomic technologies (fig. S10E). Tubulointerstitial SCPs identified by the LMD Proteomics and Spatial Metabolomics assays provide evidence for functional capabilities of the SCPs networks (fig. S7). These findings are in agreement with the known transcellular reabsorption of sodium and chloride that is initiated by the furosemide sensitive sodium chloride potassium symporter NKCC2 and supported by apical potassium recycling (36). The “tight junction organization” SCP is involved in the establishment of a physical barrier that makes this region impermeable to water and thus allows the dilution of urine (32). Among the tight junction associated genes are CLDN10 and CLDN16 that are involved in the paracellular reabsorption of sodium or calcium/magnesium (36, 40), respectively, which supports the well-known physiology of this nephron segment. Involvement of “retinol metabolism” suggests that retinol regulated transcription can play an important role in TAL tight junction maintenance, similarly to its contribution to podocyte integrity. SCPs involved in the late secretory and early endocytic pathway support the known morphologic observation of vesicles below the plasma membrane that contain the furosemide sensitive NKCC2 (36, 89) allowing its mobilization and retrieval on demand (37).

The high energy demand of the TAL cells is reflected by the identification of SCPs involved in mitochondrial energy generation from LMD transcriptomics and proteomics. Spatial metabolomics that identify purine metabolites in the tubulointerstitium also support this conclusion. Community clustering of TAL marker genes in a kidney-specific functional network (fig. S10F) identifies six modules enriched in functions including regulation of ion transport (M6), calcium ion import (M6), sodium ion transport (M6), translation (M1), and mitochondrion organization (M2).

**Distal convoluted tubules**

One distal convoluted (DCT) cell subtype was identified based on each of sc and sn RNAseq assays (Fig. 2A). Predicted SCPs for the DCT cells from sc, sn and LMD transcriptomics converge on sodium and chloride transmembrane transport (fig. S11A). Our results agree with the well-known sodium and chloride reabsorption by this cell type via the thiazide sensitive sodium chloride symporter NCC (90). Additionally, sc/sn transcriptomics highlight reabsorption of calcium, potassium, bicarbonate and phosphate. Community clustering of DCT marker genes in a kidney-specific functional network (fig. S11B) identifies three modules enriched in functions including regulation of ion transport (M3) and metal ion homeostasis (M2). A recent study focusing on the cells in the distal nephron purified by FACS-enrichment of mouse kidney cells further classifies the DCT cells into multiple subtypes (2).
Connecting tubules

Each sn and sc assay identified one connecting tubule (CNT) subtype (Fig. 2A). Both sn and sc transcriptomic datasets for CNT cells indicate that SCPs for sodium, potassium and calcium transmembrane transport activities are enriched (Fig. S12A), supporting its function in fine tuning electrolyte balances (91). Other SCPs indicate signaling, endoplasmic reticulum and energy functions in this cell type. Community clustering of CNT marker genes in a kidney-specific functional network (fig. S12B) identifies three modules enriched in functions including ion transport (M2), receptor-mediated endocytosis (M3), and mitochondrion organization (M1).

Collecting duct

Sc and sn RNAseq show two and three principal cell subtypes, respectively (Fig. 2A). The principal cell SCP networks were obtained by merging the principal cell specific SCPs predicted from sc and sn transcriptomics with the collecting duct (CD) specific SCPs predicted from LMD transcriptomics (fig. S12C). Overlapping or functionally related SCPs identified by LMD Proteomics and Spatial Metabolomics were added as well (fig. S7). Both sc and sn technologies identified "Potassium-" as well as "Sodium-transmembrane transport" SCPs for the principal cells. The SCP "Water transmembrane transport" was identified by both sn and sc RNAseq assays as well, though with a lower rank for sn RNASeq assays that did not pass our applied cutoff. The LMD transcriptomics and proteomics data identified the energy generation SCPs required for the various transport SCPs identified by the sc and sn transcriptomic data. The spatial metabolomics data sets provided support for energy generation pathways identified by the LMD technologies.

Principal cells play an important role in fine tuning ion and water reabsorption and thereby regulate systemic electrolyte and water balance (91). The anti-diuretic hormone working with prostaglandins regulates the levels of AQP2 on the apical plasma membrane (92) stimulating water reabsorption by the principal cell. Apically reabsorbed water is exported by basal water transporters AQP3 and AQP4. We detect both AQP2 and AQP3 in our datasets. Sodium reabsorption is regulated by the amiloride-sensitive sodium channel EnaC whose expression and protein turnover is regulated by aldosterone (93). The aldosterone-stimulated reabsorption of sodium is coupled with secretion of potassium, as highlighted by our data. Additionally, we show calcium transmembrane transport for one cell subtype by both sn and sc RNAseq assays. Both sc and sn technologies identify SCPs involved in drug and toxin transmembrane movement in one of the subtypes of the principal cell, although drug excretion is generally described to occur in the proximal tubule (70). Furthermore, community clustering of PC marker genes in a kidney-specific functional network (fig. S12D) identifies seven modules enriched in functions including ion transport and homeostasis (M7), regulation of vesicle-mediated transport (M4), and water homeostasis (M6).

We identified multiple subclusters of intercalated cells that could be assigned to IC-A, IC-B and one transitionary subtype, TPC-IC, as well as IC-A1, IC-A2 and IC-B in the sc and sn transcriptomic datasets, respectively (Fig. 2A). SCPs networks were identified by merging sc and sn transcriptomic data with LMD transcriptomic data obtained from the collecting duct (fig. S12E). Additionally we added overlapping or functionally related SCPs predicted by LMD Proteomics and Spatial Metabolomics (fig. S7). We find the SCP "Bicarbonate transmembrane transport" in all three sc subtypes and one sn subtype (fig. S12E), documenting the importance of the intercalated cells in the regulation of systemic acid-base homeostasis (94). Apical and basolateral bicarbonate transport is driven by exchange for chloride (94), as indicated by the
“Chloride transmembrane transport” SCP identified for one subtype in both sn and sc RNAseq datasets. Community clustering of IC marker genes in a kidney-specific functional network (fig. S12F) identifies six modules enriched in functions including regulation of body fluid levels (M3), translation (M1), mitochondrion organization (M2), bicarbonate transport (M5), and cell-cell adhesion (M4). Enrichment analysis using Gene Ontology predicts phagocytic activity (phagosome maturation and acidification) based on subunits of the vacuolar H⁺ATPase (94) (fig. S12G). In combination with the prediction of SCP involved in actin cytoskeleton our data supports the recent observation of phagocytic activity of the intercalated cells (95).

**Interstitium and the vasculature**

**Endothelial Cells:** We find four types of endothelial cells by sn transcriptomics and two by sc transcriptomics, in addition to glomerular endothelial cell identified sc transcriptomics (Fig. 2A). SCP networks for endothelial cells identified from sc and sn transcriptomic data sets contain pathways involved in cellular adhesion, trans-endothelial migration, actin cytoskeleton dynamics, caveolin-mediated endocytosis, signaling and antigen presentation (fig. S13A).

**Vascular smooth muscle cells:** We identified a single type of VSMC by sn RNAseq assay (Fig. 2A). The sc transcriptomic technology identified a variant of mesangial cells that has VSMC markers. We classified this subtype as a glomerular cell subtype. SCP networks from sn technology highlight cell contraction capabilities for the VSMC (fig. S13B).

**Fibroblasts:** We identified a single type of fibroblast from sc and sn RNAseq assays (Fig. 2). SCPs in fibroblasts identified from sc, sn and LMD transcriptomics data describe pathways related to ECM dynamics, cell adhesion, cytoskeleton dynamics and the complement pathways (fig. S14). The proteomic assays did not detect ECM components related SCPS among the highly ranked pathways.

**Immune cells:** Four types of immune cells are detected by sc or sn RNAseq technologies. These include natural killer cells, three types of T-cells, B-Cells and three types of macrophages and monocytes (Fig. 2A). SCP-networks for macrophages contains pathways involved in antigen presentation, actin cytoskeleton dynamics and translation (fig. S15A). Connection of the SCPs involved in actin cytoskeleton dynamics to the SCP ‘Macrophage migration inhibitory factor (MIF) signaling pathway indicates the potential for chemotactic activity. Macrophage migration is driven by rearrangements in the actin cytoskeleton that are activated by stimulation of the MIF receptor proteins CD74 and CXCR4 (96, 97) as identified in our data.

The SCP ‘Cellular iron uptake and export’ documents the central role of macrophages in iron homeostasis . It is predicted based on SLC39A8, a transmembrane transporter involved in transport of multiple divalent metal ions including iron (98) and the scavenger receptor CD163 that is also involved in removing hemoglobin or haptoglobin-hemoglobin complexes by splenic red pulp macrophages and Kupffer cells (99). This SCP and the SCPs involved in actin dynamics are also identified by LMD transcriptomics of the interstitium. SCPs in the natural killer cells identify antigen presentation, cell migration and actin cytoskeleton dynamics (fig. S15B). Similarly, SCP-networks predicted for B-cells and T-cells contain pathways involved in antigen presentation and the immunoproteasome and translation (fig. S15C and S15D, respectively). A detailed study of immune cell zonation of the human kidney has been published (8), while another single cell sequencing study characterized twelve myeloid cell subtypes associated with progression and regression of kidney disease in an animal injury model (3).
Since immune activity was documented for all cell types along the nephron (8), we analyzed the fraction of cell type and subtype specific marker genes and proteins that were annotated to immune pathways in Gene Ontology. In agreement with the indicated study, about 5-15% of all marker genes participate in immune cell functions (fig. S16). We want to emphasize that in the immune zonation study (14) the highest immune activity was predicted for epithelial cells of the pelvis, while our samples do not contain tissue from the pelvis.
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