SUPPLEMENTAL INFORMATION

Figure S1 Optimizing dissociation and primary analysis metrics *Related to Figure 1*

A-B, Plot of qPCR results shown as dCt ([Ct_Gapdh]-[Ct_gene-of-interest], *Gapdh* set to zero) comparing cDNA yields for representative cell type enriched gene markers and stress response genes (*Egr1, Fos*) from cold active protease (CAP) dissociation varying temperature (A) and time (B) of dissociation. 'Undissociated' and 'No CAP' samples were directly extracted adult kidney samples. **C,** Sequencing metrics reported by Cell Ranger for all 12 primary tissue samples (4 kidneys x 3 zones). **D**, Primary analysis dataset graphically represented as violin plots of all 30 clusters (ordered as in Figure1C) showing per cluster levels for nGenes, nUMI and percent mitochondrial genes. Notably, distal tubule cells (clusters 6,12), have high percent mitochondrial genes while showing typical RNA complexity (nUMI), suggesting high metabolic rates in good quality cells. **E,** tSNE and stacked barplots of the primary dataset (as in Figure1B-D), illustrating the distribution (left) and composition (right) of the clusters with respect to the four replicates. F: female, M: male, UE: ureteric epithelium, Int: interstitial.

Figure S2 Nephron dataset metrics and sex bias validation for the proximal tubule *Related to Figure 2*

A, N dataset as violin plots of the 19 clusters in Figure 2B, showing per cluster levels for nGenes (top), nUMI (middle) and percent mitochondrial genes (bottom). **B**, tSNE and stacked barplots of N dataset illustrating the distribution (left) and composition (right) of the clusters by replicate. **C**, Expression of sex biased, PT S3+S2 enriched markers *Ascm3* (m) and *Prlr* (f) in adult kidney tissue by RNAscope *in situ* and antibody labelling; inset scale bar 20µm. **D**, Expression of segment restricted, sex biased PT S2 markers are non-overlapping and demarcate the boundary between PT S2 and S3 segments. Scales and probes indicated on each panel. **E-F**, Gene Ontology analyses showing the top five GO Terms returned for genes showing a sex specific enrichment in female or male S3 (E) or throughout all female and male PT segments (F. RC: renal corpuscle, f-PT: female proximal tubule, m-PT: male proximal tubule, tl-LoH, Loop of Henle-thin limbs, DT: distal tubule.

Figure S3 Localization of thin Loop of Henle markers in early vs late forming nephrons *Related to Figure 3*

A-B, Low power views of thin limbs of Loop of Henle (tl-LoH) markers in tdT-labelled adult kidney sections; boxes indicate region of inset below each panel. Boxes also show where high power images in Figure 3E, F were collected relative to medullary depth shown at left; text above field details genetic combinations and tamoxifen (TX) injections; scale bar and probes indicated on each panel; arrowheads: a1, *Fst*$^{+}$/Apq1^{+}/tdT^{+}; a2, *Slc39a8*$^{+}$/Apq1^{+}/tdT^{+}; a3, *Sptssb*$^{+}$/Clcnka^{-}/tdT^{+}; a4,

Sptssb^{+}/Clcnka^{+}/tdT^{+}; a5, *Crlf1*$^{+}$/Clcnka^{+}/tdT^{+}; a6, *Slc14a2*$^{+}$/Apq1^{-}/tdT^{-}; b1-2, *Slc14a2*$^{+}$/Apq1^{-}/tdT^{+}; b3-4 *Slc39a8*$^{+}$/Apq1^{-}/tdT^{+}; b5, *Fst*$^{+}$/Apq1^{-}/tdT^{-}.

Figure S4 Dataset metrics for ureteric epithelial cell groupings *Related to Figure 4*

A, UE dataset as violin plots of the 16 clusters in Figure 4B, showing per cluster levels for nGenes (top), nUMI (middle) and percent mitochondrial genes (bottom). **B**, tSNE and stacked barplots of UE dataset illustrating the distribution (left) and composition (right) of the clusters by replicate; IC, intercalated cells, type A and B; CNT, connecting tubule; CCD, cortical collecting duct; OMCD, IMCD, outer and inner medullary collecting duct, types1-3; dME, deep medullary epithelium.

Figure S5 Nephron lineage derived principal and intercalated cell types at connection to collecting system *Related to Figure 5*

A, General cell markers of intercalated cells (IC, Atp6v1b1) and principal cells (PC, Aqp2) in nephron (N) and ureteric epithelium (UE) around the junction with collecting system. **B-D**, Cell specific analysis of indicated markers in PC, IC and other epithelial cell types around the junction with collecting system. **E,F**, tSNE projections of re-clustered cortical epithelial cell types from N and UE datasets displayed as zone (E) or lineage (F) of origin. **G**, Differentially expressed genes for each cluster in Figure 5I. **H**, Feature plots showing expression of key marker genes defining cell types. **I**, Feature plot displaying *Hoxd10* expression across clusters in Figure 5I. **J**, Rare Ckb^{+} cells (arrowhead) within the Slc12a3^{+} distal convoluted tubule cluster 8 in Figure 5I. **K**, NonA-nonB IC identified by double label for Rhbg and Slc26a4; arrowheads: white, IC-B; orange, nonA-nonB IC. **L-M**, Immunofluorescent and RNAscope validation (arrowheads) of Ache in UE-PCs and *Sh2d4b* in UE-IC-B, respectively; merged panel on left.

Figure S6 Progressive shift of transcription factors from the ureteric lineage into the distal nephrogenic lineage in early nephrogenesis *Related to Figure 5*

A-D, Human (week 15) and mouse (day of birth) kidney sections immunostained to examine cellular distributions of transcription factors first expressed in the UE and subsequently in distal cell types of the developing nephron. Nephron development initiates with the epithelial renal vesicle (RV), proceeds though a Comma-shaped body (CSB) to an S-shaped body (SSB). A patent luminal interconnection is generated between the nephron and collecting system by the SSB stage; dashes follow proximal-distal axis of nephron precursors; arrowheads indicate transcription factors detected in distal nephron. Scale bar 10μm.

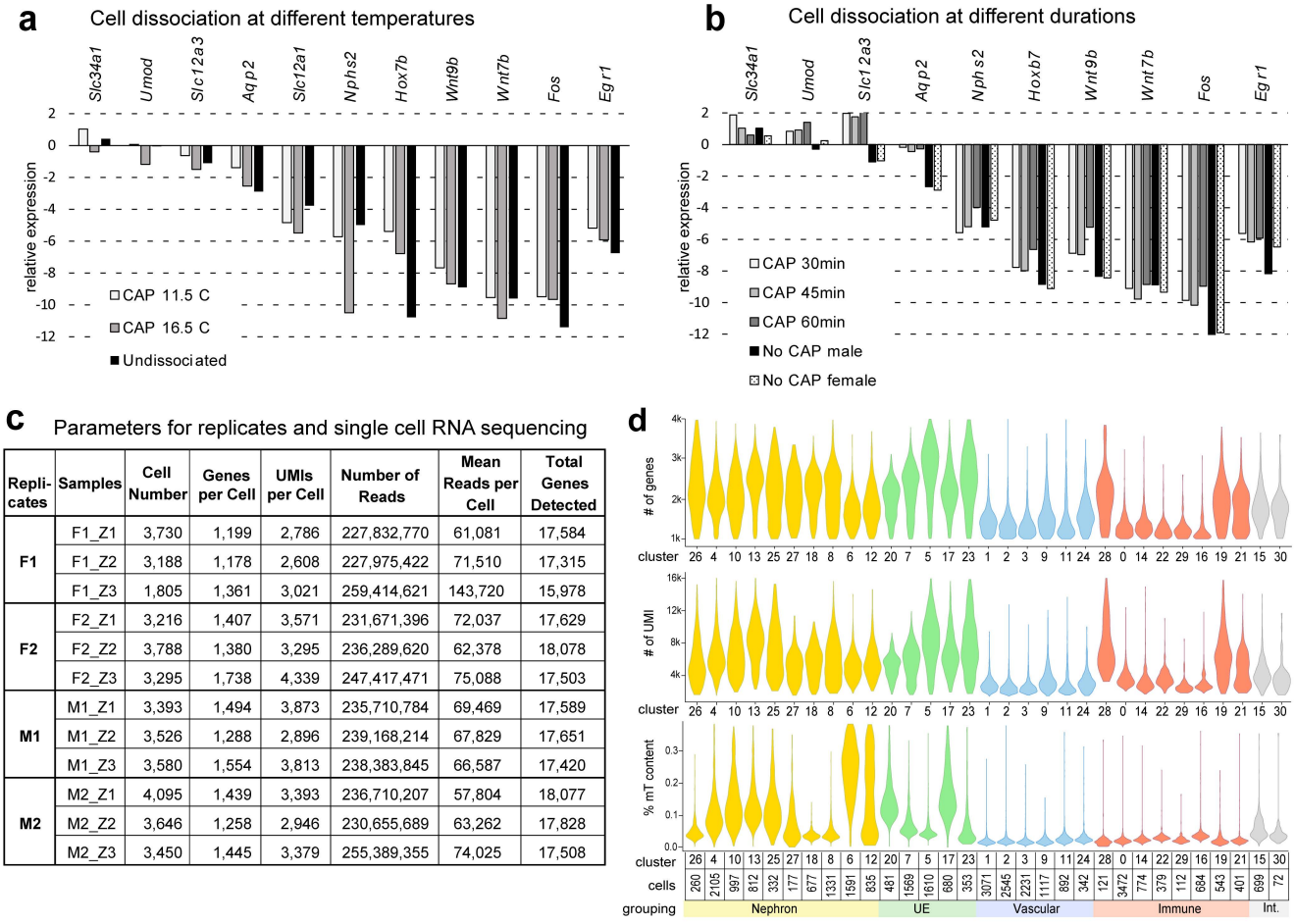
Figure S7 Enriched expression of transcription factors in clusters of nephron and ureteric epithelium datasets *Related to Figure 2 and Figure 4*

A-B, Dot plots showing differential expression of transcription factors along the proximal-distal axis of the mature nephron (A) and ureteric epithelium of the collecting system (B), with a schematic (below) indicating the region sampled: RC, renal corpuscle; f-PT, female proximal tubule; m-PT, male proximal tubule; tl-LoH, thin limbs of Loop of Henle; DT, distal tubule; IC, intercalated cell, type a and b; CNT, connecting tubule; CCD, cortical collecting duct; OMCD, outer medullary collecting duct; IMCD, inner medullary collecting duct; dME, deep medullary epithelium.

Figure S8 Kidney Cell Explorer batch search outputs heatmaps spanning all metacells *Related to Figure 6*

A-C, Heat maps of gene expression for multi-gene searches in metacells of nephron and collecting duct showing examples for batch searches with genes linked to hormone regulation (A), nephrotic syndrome (B) and specific physiological activities (C).

Figure S1



c Parameters for replicates and single cell RNA sequencing

Repli-cates	Samples	Cell Number	Genes per Cell	UMIs per Cell	Number of Reads	Mean Reads per Cell	Total Genes Detected
F1	F1_Z1	3,730	1,199	2,786	227,832,770	61,081	17,584
	F1_Z2	3,188	1,178	2,608	227,975,422	71,510	17,315
	F1_Z3	1,805	1,361	3,021	259,414,621	143,720	15,978
F2	F2_Z1	3,216	1,407	3,571	231,671,396	72,037	17,629
	F2_Z2	3,788	1,380	3,295	236,289,620	62,378	18,078
	F2_Z3	3,295	1,738	4,339	247,417,471	75,088	17,503
M1	M1_Z1	3,393	1,494	3,873	235,710,784	69,469	17,589
	M1_Z2	3,526	1,288	2,896	239,168,214	67,829	17,651
	M1_Z3	3,580	1,554	3,813	238,383,845	66,587	17,420
M2	M2_Z1	4,095	1,439	3,393	236,710,207	57,804	18,077
	M2_Z2	3,646	1,258	2,946	230,655,689	63,262	17,828
	M2_Z3	3,450	1,445	3,379	255,389,355	74,025	17,508

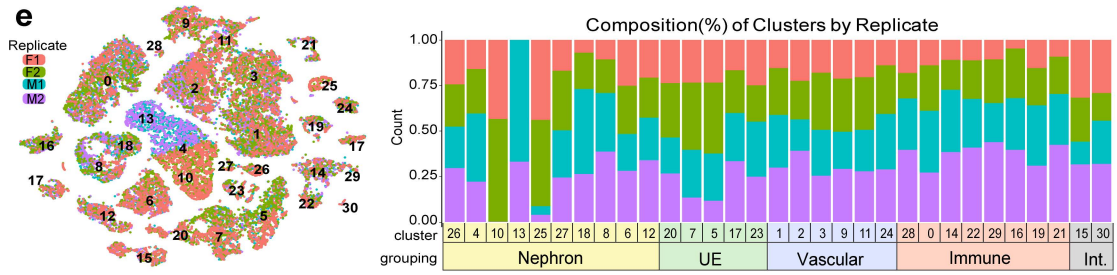


Figure S2

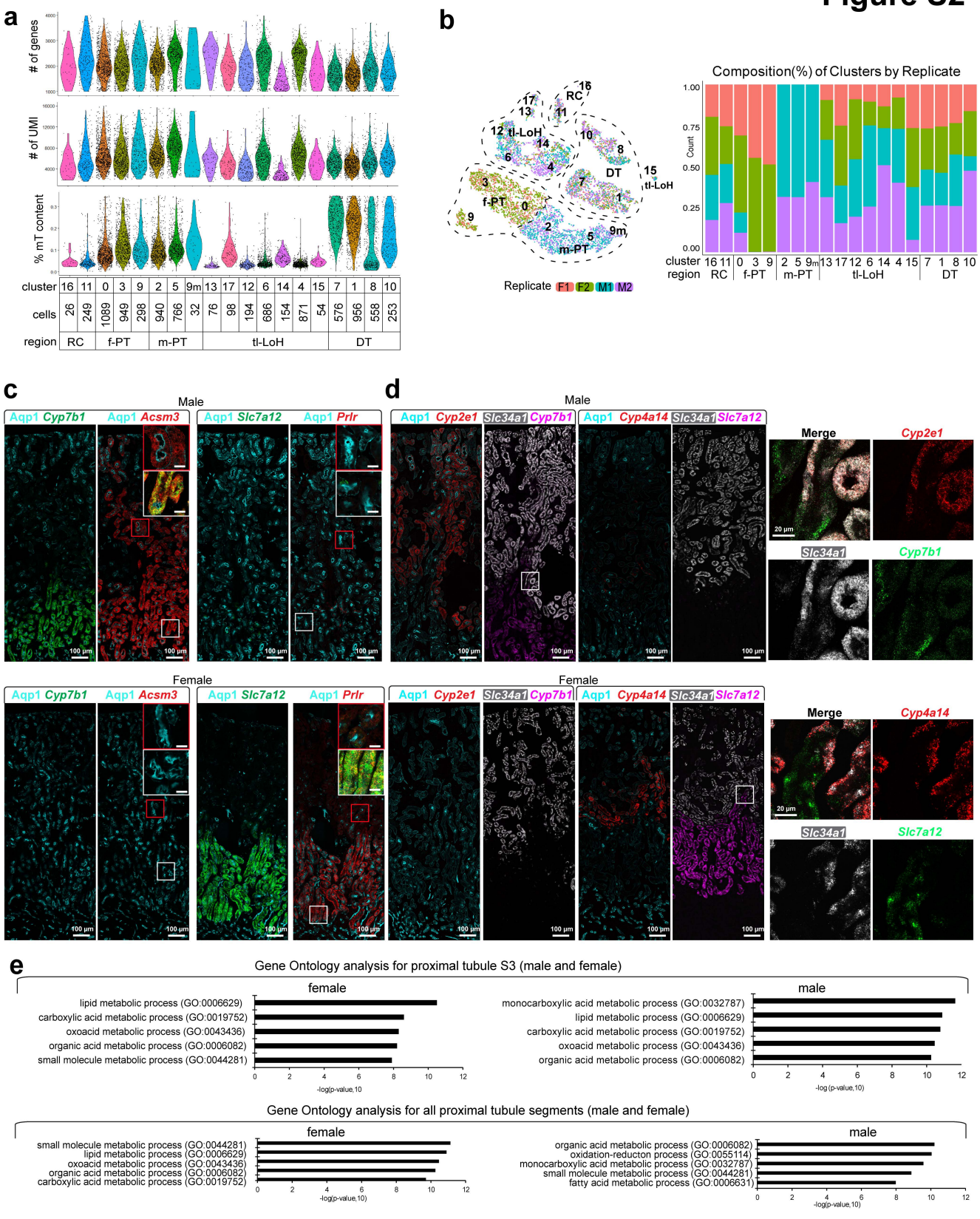

a
# of genes
# of UMI
% mT content
cluster 16 11 0 3 9 2 5 9m 13 17 12 6 14 4 15 7 1 8 10
cells 26 249 1089 949 298 940 766 32 76 98 194 686 154 871 54 576 956 558 253
region RC f-PT m-PT tl-LoH DT
b
RC
tl-LoH
f-PT
m-PT
DT
Replicate F1 F2 M1 M2
Composition(%) of Clusters by Replicate
Count
cluster 16 11 0 3 9 2 5 9m 13 17 12 6 14 4 15 7 1 8 10
region RC f-PT m-PT tl-LoH DT
c
Male
Aqp1 Cyp7b1
Aqp1 Acsm3
Aqp1 Slc7a12
Aqp1 Prlr
Female
d
Male
Aqp1 Cyp2e1 Slc34a1 Cyp7b1
Aqp1 Cyp4a14 Slc34a1 Slc7a12
Merge
Cyp2e1
Slc34a1
Cyp7b1
Female
Cyp4a14
Slc7a12
20 μm
100 μm
e
Gene Ontology analysis for proximal tubule S3 (male and female)
female
lipid metabolic process (GO:0006629)
carboxylic acid metabolic process (GO:0019752)
oxoacid metabolic process (GO:0043436)
organic acid metabolic process (GO:0006082)
small molecule metabolic process (GO:0044281)
-log(p-value,10)
male
monocarboxylic acid metabolic process (GO:0032787)
lipid metabolic process (GO:0006629)
carboxylic acid metabolic process (GO:0019752)
oxoacid metabolic process (GO:0043436)
organic acid metabolic process (GO:0006082)
-log (p-value,10)
Gene Ontology analysis for all proximal tubule segments (male and female)
female
small molecule metabolic process (GO:0044281)
lipid metabolic process (GO:0006629)
oxoacid metabolic process (GO:0043436)
organic acid metabolic process (GO:0006082)
carboxylic acid metabolic process (GO:0019752)
male
organic acid metabolic process (GO:0006082)
oxidation-reducton process (GO:0055114)
monocarboxylic acid metabolic process (GO:0032787)
small molecule metabolic process (GO:0044281)
fatty acid metabolic process (GO:0006631)

Figure S3

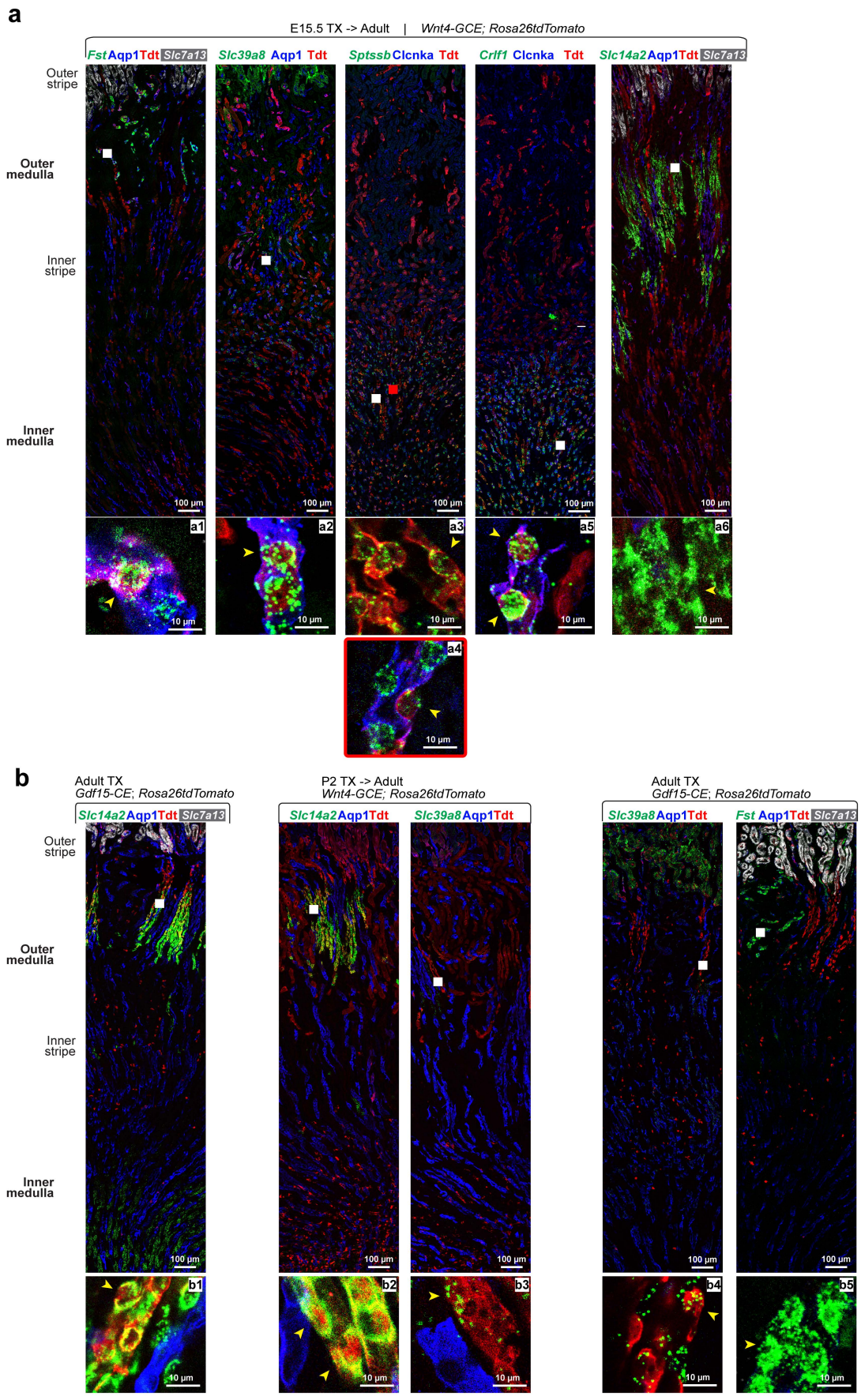

a
E15.5 TX -> Adult | Wnt4-GCE; Rosa26tdTomato
Fst Aqp1 Tdt Slc7a13
Slc39a8 Aqp1 Tdt
Sptssb Clcnka Tdt
Crlf1 Clcnka Tdt
Slc14a2 Aqp1 Tdt Slc7a13
Outer stripe
Outer medulla
Inner stripe
Inner medulla
100 µm
10 µm
a1
a2
a3
a4
a5
a6
b
Adult TX
Gdf15-CE; Rosa26tdTomato
Slc14a2 Aqp1 Tdt Slc7a13
P2 TX -> Adult
Wnt4-GCE; Rosa26tdTomato
Slc14a2 Aqp1 Tdt
Slc39a8 Aqp1 Tdt
Adult TX
Gdf15-CE; Rosa26tdTomato
Slc39a8 Aqp1 Tdt
Fst Aqp1 Tdt Slc7a13
b1
b2
b3
b4
b5

Figure S4

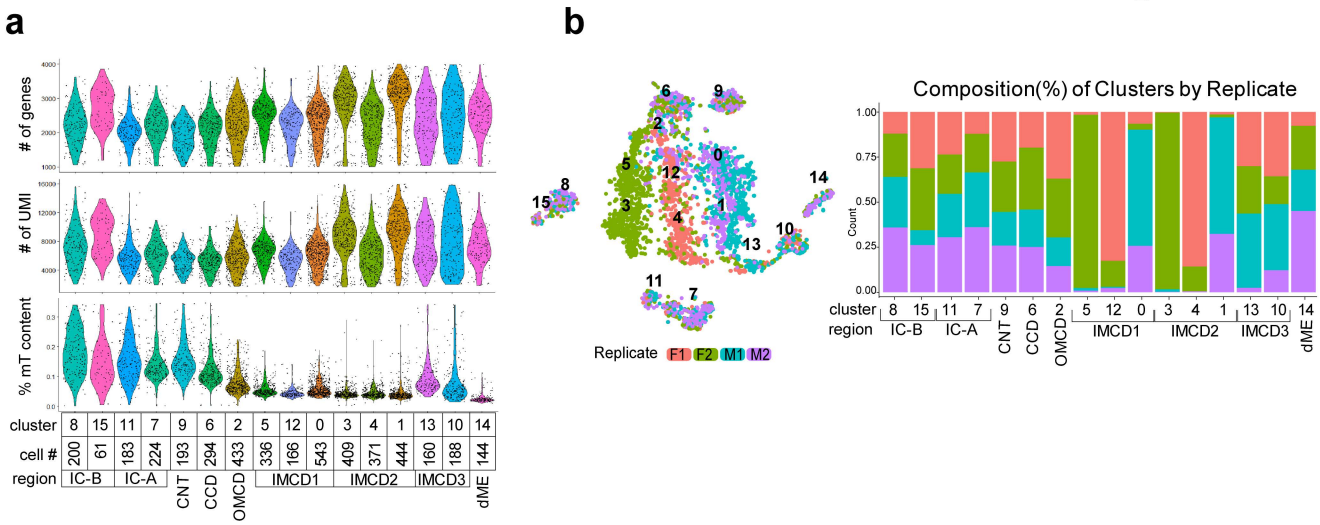

a
# of genes
# of UMI
% mT content
cluster 8 15 11 7 9 6 2 5 12 0 3 4 1 13 10 14
cell # 200 61 183 224 193 294 433 336 166 543 409 371 444 160 188 144
region IC-B IC-A CNT CCD OMCD IMCD1 IMCD2 IMCD3 dME
b
Replicate F1 F2 M1 M2
Composition(%) of Clusters by Replicate
Count
cluster 8 15 11 7 9 6 2 5 12 0 3 4 1 13 10 14
region IC-B IC-A CNT CCD OMCD IMCD1 IMCD2 IMCD3 dME

Figure S5

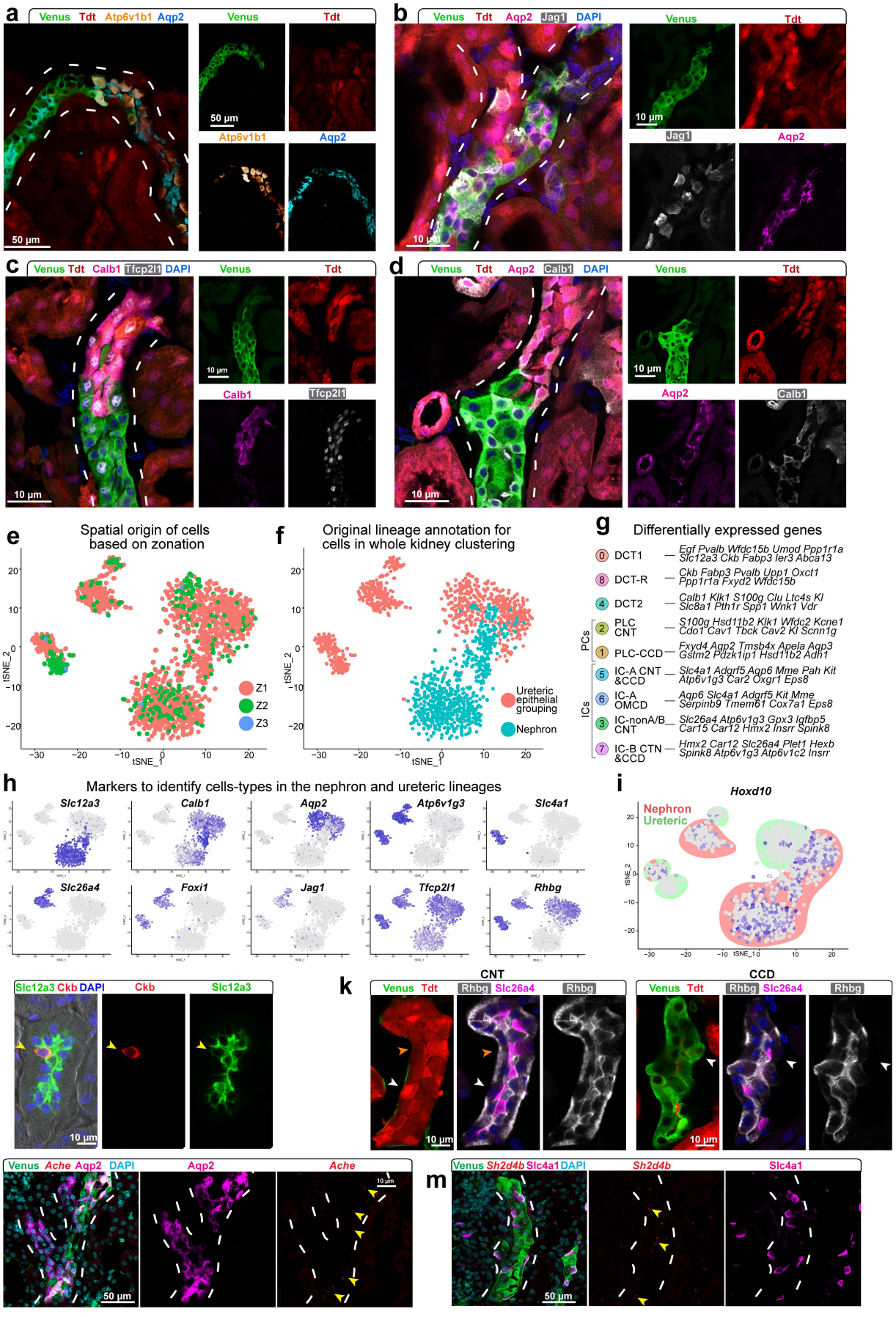

a
Venus Tdt Atp6v1b1 Aqp2
Venus
Tdt
Atp6v1b1
Aqp2
50 μm
b
Venus Tdt Aqp2 Jag1 DAPI
Venus
Tdt
Jag1
Aqp2
10 μm
c
Venus Tdt Calb1 Tfcp2l1 DAPI
Venus
Tdt
Calb1
Tfcp2l1
10 μm
d
Venus Tdt Aqp2 Calb1 DAPI
Venus
Tdt
Aqp2
Calb1
10 μm
e
Spatial origin of cells based on zonation
tSNE_1
tSNE_2
Z1
Z2
Z3
f
Original lineage annotation for cells in whole kidney clustering
Ureteric epithelial grouping
Nephron
g
Differentially expressed genes
0 DCT1 — Egf Pvalb Wfdc15b Umod Ppp1r1a Slc12a3 Ckb Fabp3 Ier3 Abca13
8 DCT-R — Ckb Fabp3 Pvalb Upp1 Oxct1 Ppp1r1a Fxyd2 Wfdc15b
4 DCT2 — Calb1 Klk1 S100g Clu Ltc4s Kl Slc8a1 Pth1r Spp1 Wnk1 Vdr
PCs
2 PLC CNT — S100g Hsd11b2 Klk1 Wfdc2 Kcne1 Cdo1 Cav1 Tbck Cav2 Kl Scnn1g
1 PLC-CCD — Fxyd4 Aqp2 Tmsb4x Apela Aqp3 Gstm2 Pdzk1ip1 Hsd11b2 Adh1
ICs
5 IC-A CNT &CCD — Slc4a1 Adgrf5 Aqp6 Mme Pah Kit Atp6v1g3 Car2 Oxgr1 Eps8
6 IC-A OMCD — Aqp6 Slc4a1 Adgrf5 Kit Mme Serpinb9 Tmem61 Cox7a1 Eps8
3 IC-nonA/B CNT — Slc26a4 Atp6v1g3 Gpx3 Igfbp5 Car15 Car12 Hmx2 Insrr Spink8
7 IC-B CTN &CCD — Hmx2 Car12 Slc26a4 Plet1 Hexb Spink8 Atp6v1g3 Atp6v1c2 Insrr
h
Markers to identify cells-types in the nephron and ureteric lineages
Slc12a3
Calb1
Aqp2
Atp6v1g3
Slc4a1
Slc26a4
Foxi1
Jag1
Tfcp2l1
Rhbg
i
Hoxd10
Nephron
Ureteric
tSNE_1
tSNE_2
j
Slc12a3 Ckb DAPI
Ckb
Slc12a3
10 μm
k
CNT
CCD
Venus Tdt Rhbg Slc26a4 Rhbg
10 μm
l
Venus Ache Aqp2 DAPI
Aqp2
Ache
50 μm
m
Venus Sh2d4b Slc4a1 DAPI
Sh2d4b
Slc4a1
50 μm

Figure S6

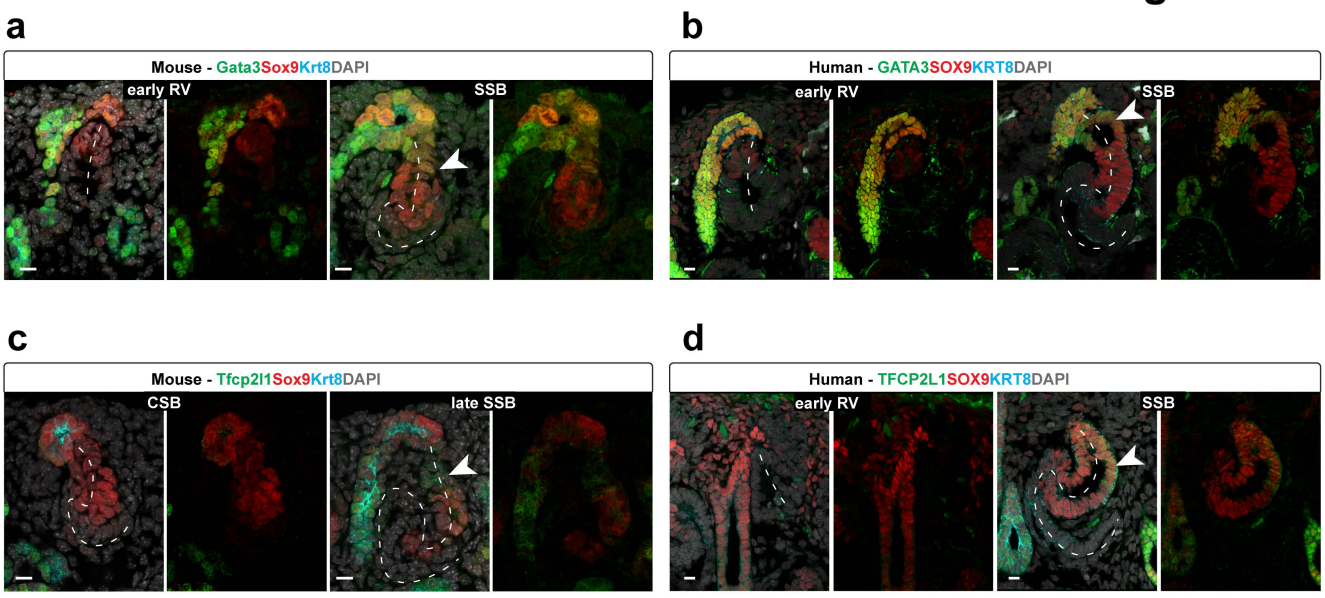

a
Mouse - Gata3Sox9Krt8DAPI
early RV
SSB
b
Human - GATA3SOX9KRT8DAPI
early RV
SSB
c
Mouse - Tfcp2l1Sox9Krt8DAPI
CSB
late SSB
d
Human - TFCP2L1SOX9KRT8DAPI
early RV
SSB

Figure S7

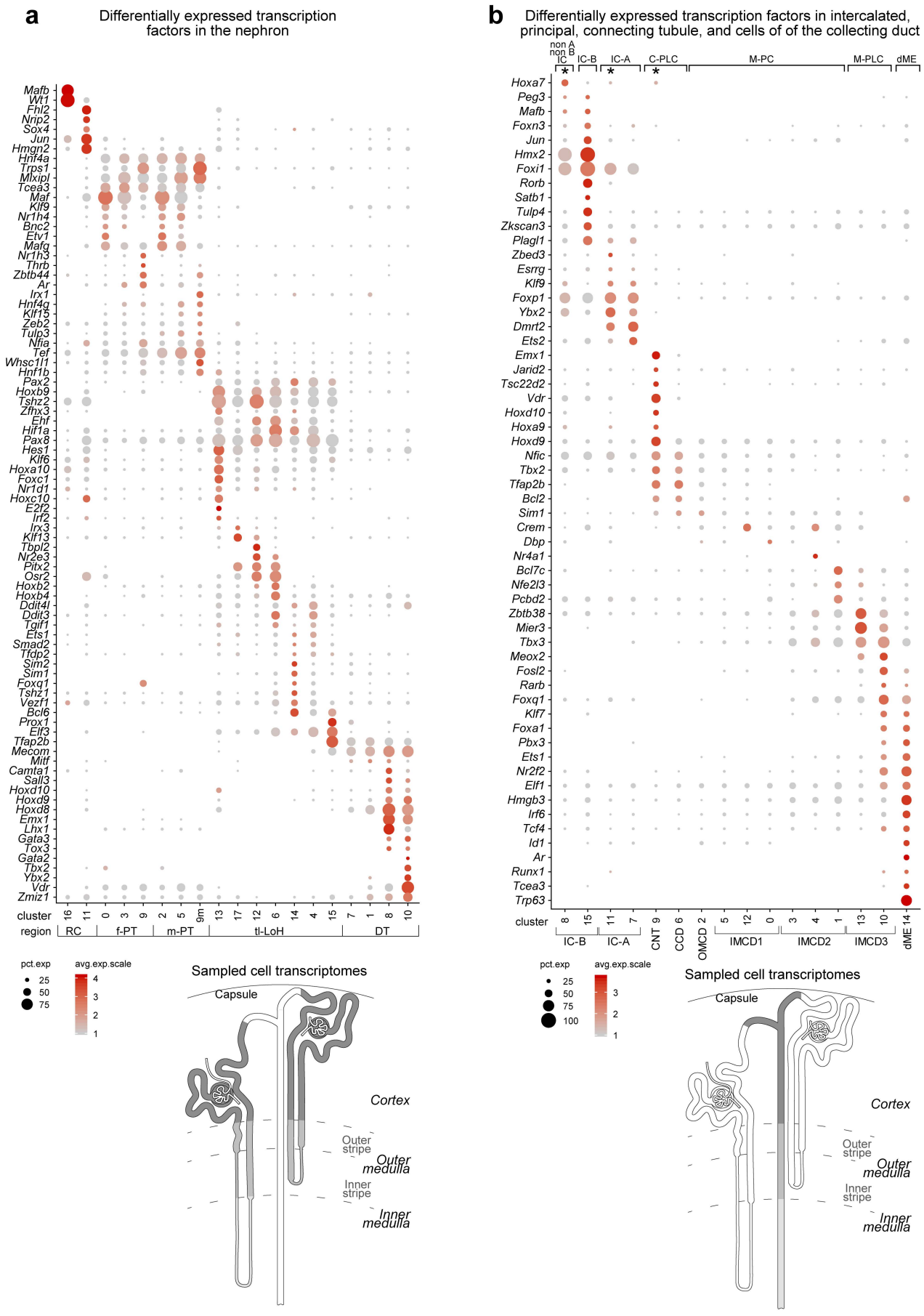
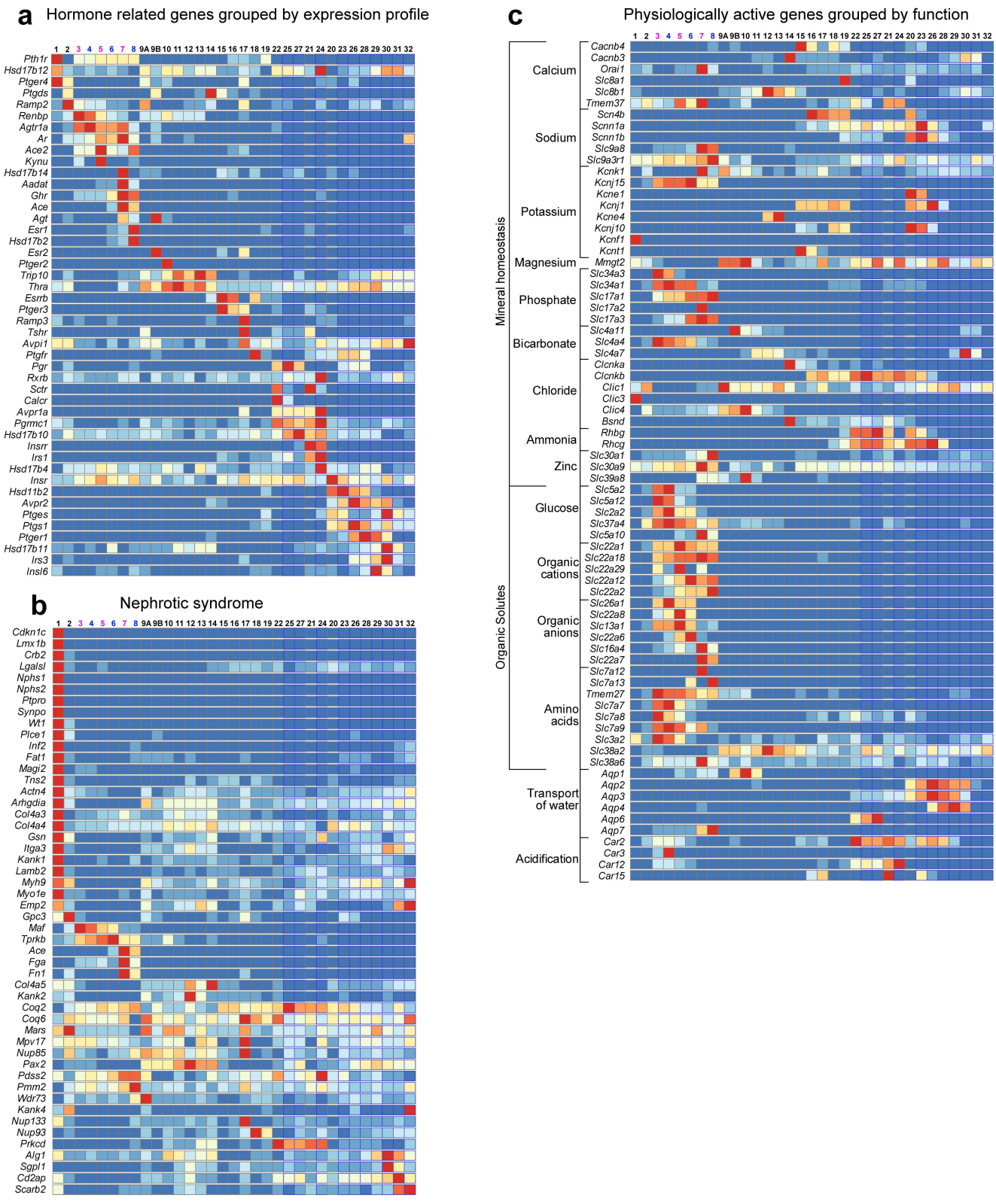

a
Differentially expressed transcription factors in the nephron
b
Differentially expressed transcription factors in intercalated, principal, connecting tubule, and cells of of the collecting duct
non A non B IC
IC-B
IC-A
C-PLC
M-PC
M-PLC
dME
cluster
region
RC
f-PT
m-PT
tl-LoH
DT
IC-B
IC-A
CNT
CCD
OMCD
IMCD1
IMCD2
IMCD3
dME
pct.exp
avg.exp.scale
Sampled cell transcriptomes
Capsule
Cortex
Outer stripe
Outer medulla
Inner stripe
Inner medulla

Figure S8


a
Hormone related genes grouped by expression profile
b
Nephrotic syndrome
c
Physiologically active genes grouped by function
Mineral homeostasis
Organic Solutes
Calcium
Sodium
Potassium
Magnesium
Phosphate
Bicarbonate
Chloride
Ammonia
Zinc
Glucose
Organic cations
Organic anions
Amino acids
Transport of water
Acidification