Supplemental Information

Single Cell Profiling of Acute Kidney Injury Reveals Novel Transcriptional Signatures, Pro-Fibrotic Phenotype and Epithelial-to-Stromal Crosstalk

Valeria Rudman-Melnick, Mike Adam, Andrew Potter, Saagar M. Chokshi, Qing Ma, Keri A. Drake, Meredith P. Schuh, J. Matthew Kofron, Prasad Devarajan and S. Steven Potter

Supplemental Figures

Supplemental Figure 1. UIR induced proximal tubule dedifferentiation, tubular damage and gene expression changes.

Supplemental Figure 2. AKI results in formation of the Mixed Identity Cells in the adult kidney.

Supplemental Figure 3. Doublet removal does not affect the Mixed Identity Cells presence.

Supplemental Figure 4. UIR induces quantitative changes in mixed identity and proximal tubular dedifferentiation.

Supplemental Figure 5. AKI persists through UIR Day 4 and 7 and resolves by UIR Day 14.

Supplemental Figure 6. AKI induced unique gene expression signatures in the Injured Proximal tubules and Mixed Identity Cells.

Supplemental Figure 7. The integrated analysis reveals changes in transcription factor expression over AKI response.

Supplemental Figure 8. Injury induced gene expression analysis reveals enrichment of apoptotic, pro-fibrotic and developmental factors in the UIR induced proximal tubules and mixed identity cells.

Supplemental Figure 9. *Sox4* labels proximal tubules dedifferentiation throughout the AKI course.

Supplemental Figure 10. *Cd24a* marks distal nephron tubule segment injury and correlates with *Lcn2*.

Supplemental Figure 11. UIR Day 1 exhibits marked Cd24a protein elevation in the cortical and medullary tubules.

Supplemental Figure 12. Validation of AKI induced genes in the human kidney samples.

Supplemental Figure 13. Pro-fibrotic signaling in the injured kidney.

Supplemental Figure 14. Epithelial-to-Stromal crosstalk in the normal kidney.

Supplemental Figure 15. Epithelial-to-Stromal crosstalk in the UIR Day 4.

Supplemental Figure 16. Negative controls for *Sox4, Cd24a, Lcn2, Kim1, Slc34a1, Vim, Col18a1* RNAscope probes.

Supplemental Figure 1. UIR induced proximal tubule dedifferentiation, tubular damage and gene expression changes. Related to Figure 1.

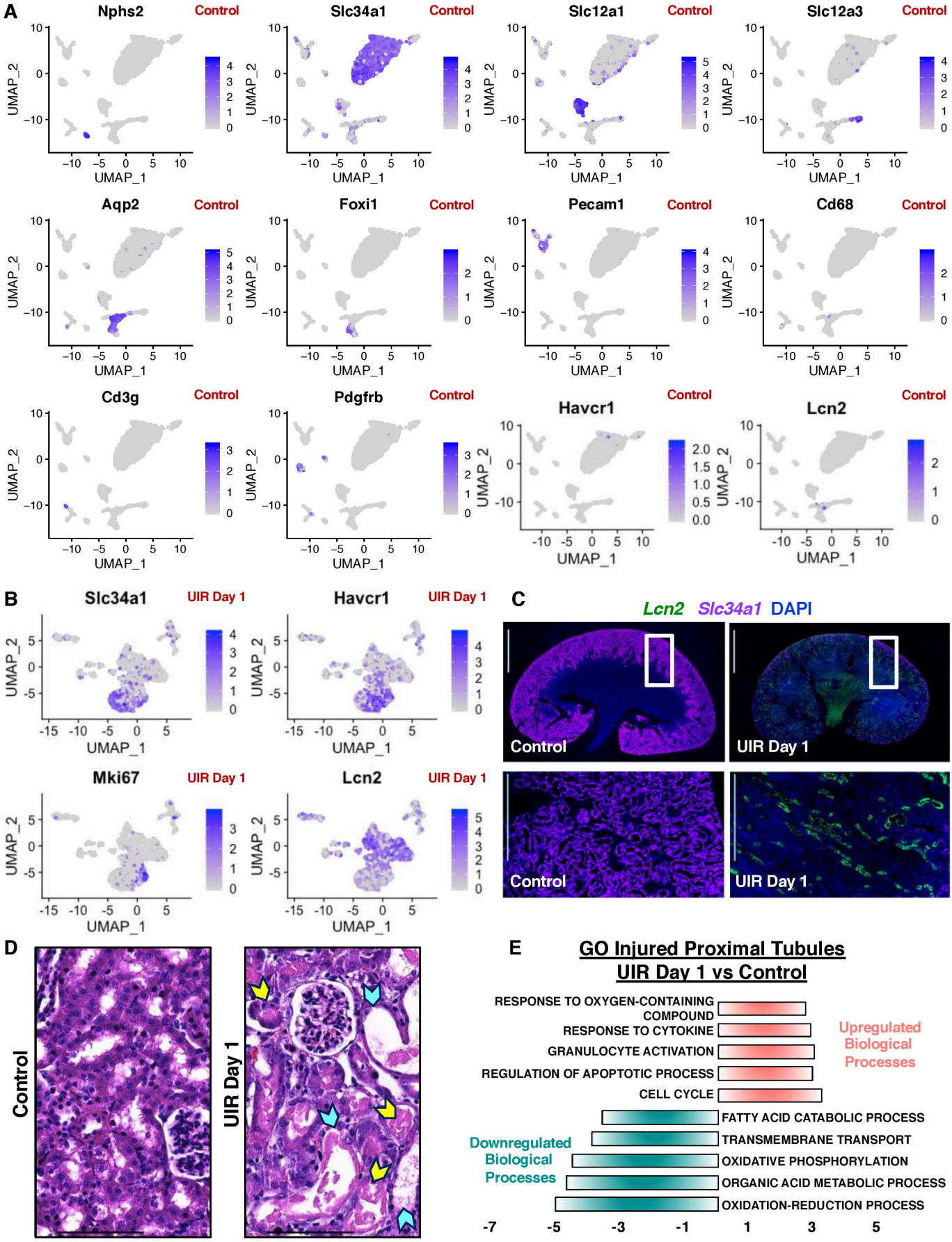
(A) Feature Plots for renal cell populations identified in the Control kidney by the scRNA-seq. Podocytes (*Nphs2*), proximal tubules (*Slc34a1*), loop of Henle (*Slc12a1*), distal tubules (*Slc12a3*), collecting duct principal (*Aqp2*) and intercalated (*Foxi1*) cells, endothelial (*Pecam1*), macrophages (*Cd68*), T cells (*Cd3g*), stromal/pericyte (*Pdgfrb*). Both injury markers *Havcr1* and *Lcn2* are nearly absent.

(B) Feature Plots identify UIR Day 1 cell populations, including sub-clusters of injured (high *Kim1, a.k.a. Havcr1,* and low *Slc34a1*) and cycling (*Mki67* positive) proximal tubules, the injured *Lcn2*-positive distal tubule, loop of Henle and collecting duct. The “Mixed Identity Cells” are positioned between the proximal tubules, distal tubules, loop of Henle and collecting duct.

(C) RNAscope showing *Lcn2* elevation in the distal nephron tubule and collecting duct of UIR Day 1. *Lcn2* (green), *Slc34a1* (purple), 4x (2500µm scale) and 10x (500µm scale).

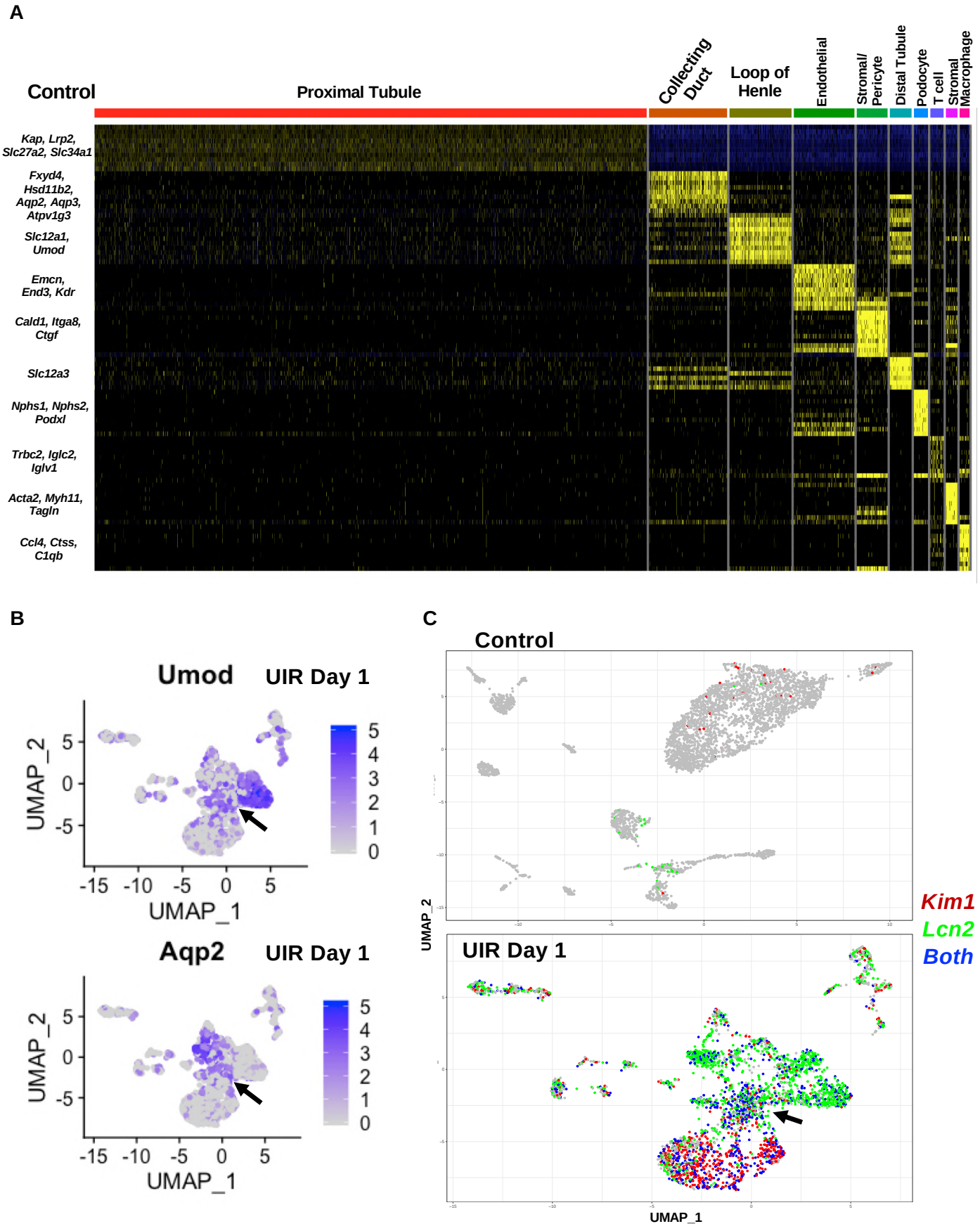
(D) H&E staining reveals AKI induced tubular dilation (blue pointers) and cast formation (yellow pointers) not detectable in the Control. 40x, 100µm scale.

(E) GO Biological Process of “Injured Prox” UIR Day 1 vs Control, –log2(pValue).


A
Nphs2
Control
Slc34a1
Control
Slc12a1
Control
Slc12a3
Control
Aqp2
Control
Foxi1
Control
Pecam1
Control
Cd68
Control
Cd3g
Control
Pdgfrb
Control
Havcr1
Control
Lcn2
Control
UMAP_1
UMAP_2
B
Slc34a1
UIR Day 1
Havcr1
UIR Day 1
Mki67
UIR Day 1
Lcn2
UIR Day 1
C
Lcn2 Slc34a1 DAPI
Control
UIR Day 1
Control
UIR Day 1
D
Control
UIR Day 1
E
GO Injured Proximal Tubules
UIR Day 1 vs Control
RESPONSE TO OXYGEN-CONTAINING COMPOUND
RESPONSE TO CYTOKINE
GRANULOCYTE ACTIVATION
REGULATION OF APOPTOTIC PROCESS
CELL CYCLE
Upregulated Biological Processes
FATTY ACID CATABOLIC PROCESS
TRANSMEMBRANE TRANSPORT
OXIDATIVE PHOSPHORYLATION
ORGANIC ACID METABOLIC PROCESS
OXIDATION-REDUCTION PROCESS
Downregulated Biological Processes
-7
-5
-3
-1
1
3
5

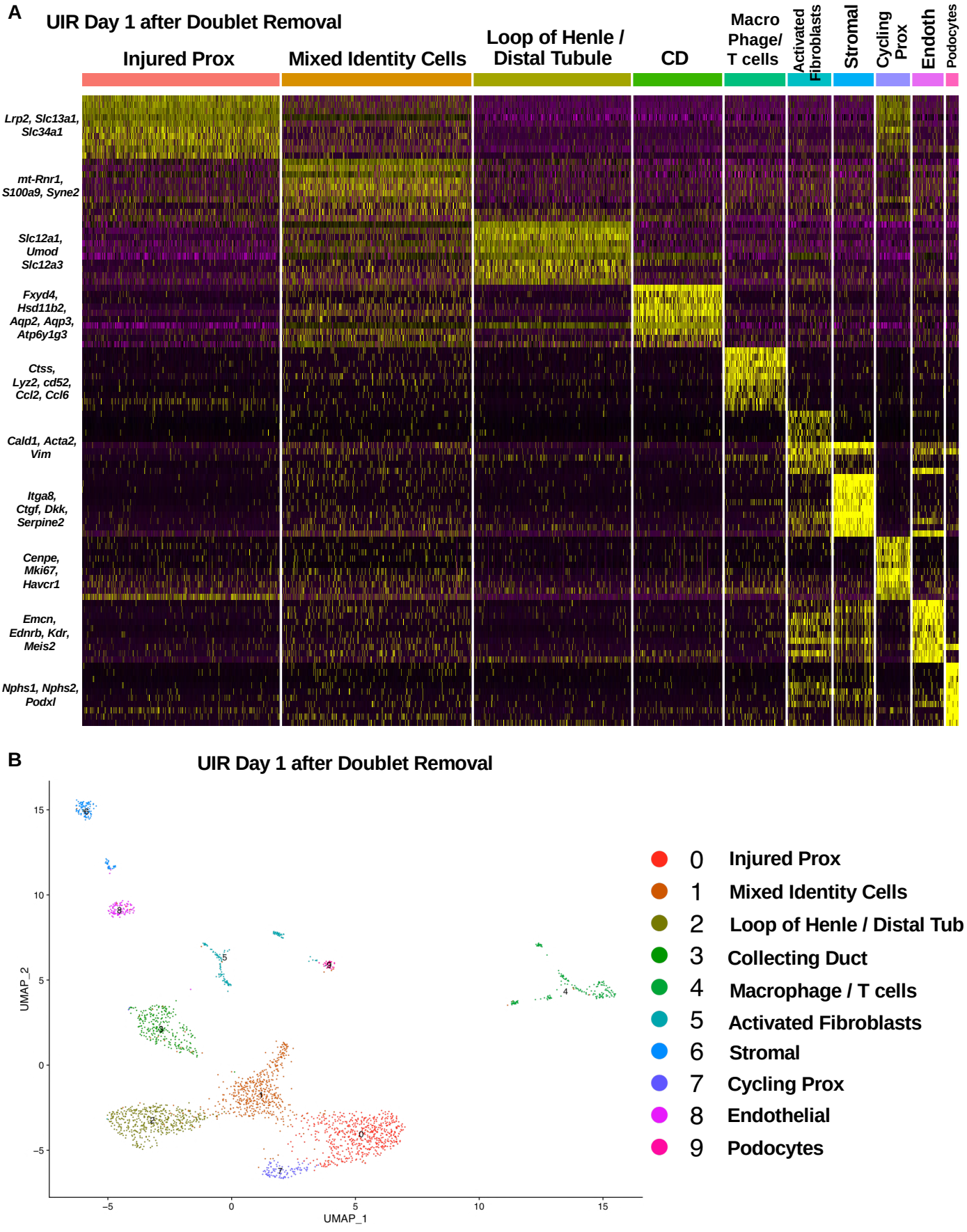
Supplemental Figure 2. AKI results in formation of the Mixed Identity Cells in the adult kidney. Related to Figures 1 and 2.

(A) Heatmap shows the relative marker gene expression in the Control renal cell populations. The “Mixed Identity Cells” with ectopic expression of multiple compartments markers are not identified.
(B) Feature plots show the expression of the loop of Henle marker *Umod* and the collecting duct marker *Aqp2* in the UIR Day 1. The intermediately located “Mixed Identity Cells” pointed with the arrow show overlapping expression of both markers.
(C) Combined feature plot shows the overlapping expression of the proximal and distal nephron tubule injury markers *Kim1* and *Lcn2* in the UIR Day 1 “Mixed Identity Cells” (shown with the arrow).


A
Control
Proximal Tubule
Collecting Duct
Loop of Henle
Endothelial
Stromal/ Pericyte
Distal Tubule
Podocyte
T cell
Stromal
Macrophage
Kap, Lrp2, Slc27a2, Slc34a1
Fxyd4, Hsd11b2, Aqp2, Aqp3, Atpv1g3
Slc12a1, Umod
Emcn, End3, Kdr
Cald1, Itga8, Ctgf
Slc12a3
Nphs1, Nphs2, Podxl
Trbc2, Iglc2, Iglv1
Acta2, Myh11, Tagln
Ccl4, Ctss, C1qb
B
Umod
UIR Day 1
UMAP_2
UMAP_1
Aqp2
UIR Day 1
UMAP_2
UMAP_1
C
Control
UIR Day 1
UMAP_2
UMAP_1
Kim1
Lcn2
Both

Supplemental Figure 3. Doublet removal does not affect the Mixed Identity Cells presence. Related to Figures 1 and 2.

(A) Heatmap shows the relative marker gene expression in UIR Day 1 renal cell types after doublet removal using Doublet Decon. The Mixed Identity cluster remains present and shows stochastic expression of markers of many different cell types. Please note that the heatmap shows genes elevated in renal cell populations relative to each other, based on the z-score. The yellow is above the mean, black is the mean, and purple/blue is below the mean, which represents the lowest expression levels. While other injured renal cell types exhibit some minor degree of “inappropriate” gene expression, the Mixed Identity Cells cluster is defined by remarkably elevated levels of many renal cell types markers. Endoth, Endothelial; Prox, Proximal Tubules; Distal Tub, Distal Tubule.
(B) UMAP shows UIR Day 1 renal cell populations after the doublet removal. The figure shows that presence of Mixed Identity cells (Cluster 1) is not affected by the doublet removal.


A
UIR Day 1 after Doublet Removal
Injured Prox
Mixed Identity Cells
Loop of Henle / Distal Tubule
CD
Macro Phage/ T cells
Activated Fibroblasts
Stromal
Cycling Prox
Endoth
Podocytes
Lrp2, Slc13a1, Slc34a1
mt-Rnr1, S100a9, Syne2
Slc12a1, Umod Slc12a3
Fxyd4, Hsd11b2, Aqp2, Aqp3, Atp6y1g3
Ctss, Lyz2, cd52, Ccl2, Ccl6
Cald1, Acta2, Vim
Itga8, Ctgf, Dkk, Serpine2
Cenpe, Mki67, Havcr1
Emcn, Ednrb, Kdr, Meis2
Nphs1, Nphs2, Podxl
B
UIR Day 1 after Doublet Removal
UMAP_1
UMAP_2
0 Injured Prox
1 Mixed Identity Cells
2 Loop of Henle / Distal Tub
3 Collecting Duct
4 Macrophage / T cells
5 Activated Fibroblasts
6 Stromal
7 Cycling Prox
8 Endothelial
9 Podocytes

Supplemental Figure 4. UIR induces quantitative changes in mixed identity and proximal tubular dedifferentiation. Related to Figures 1 and 2.

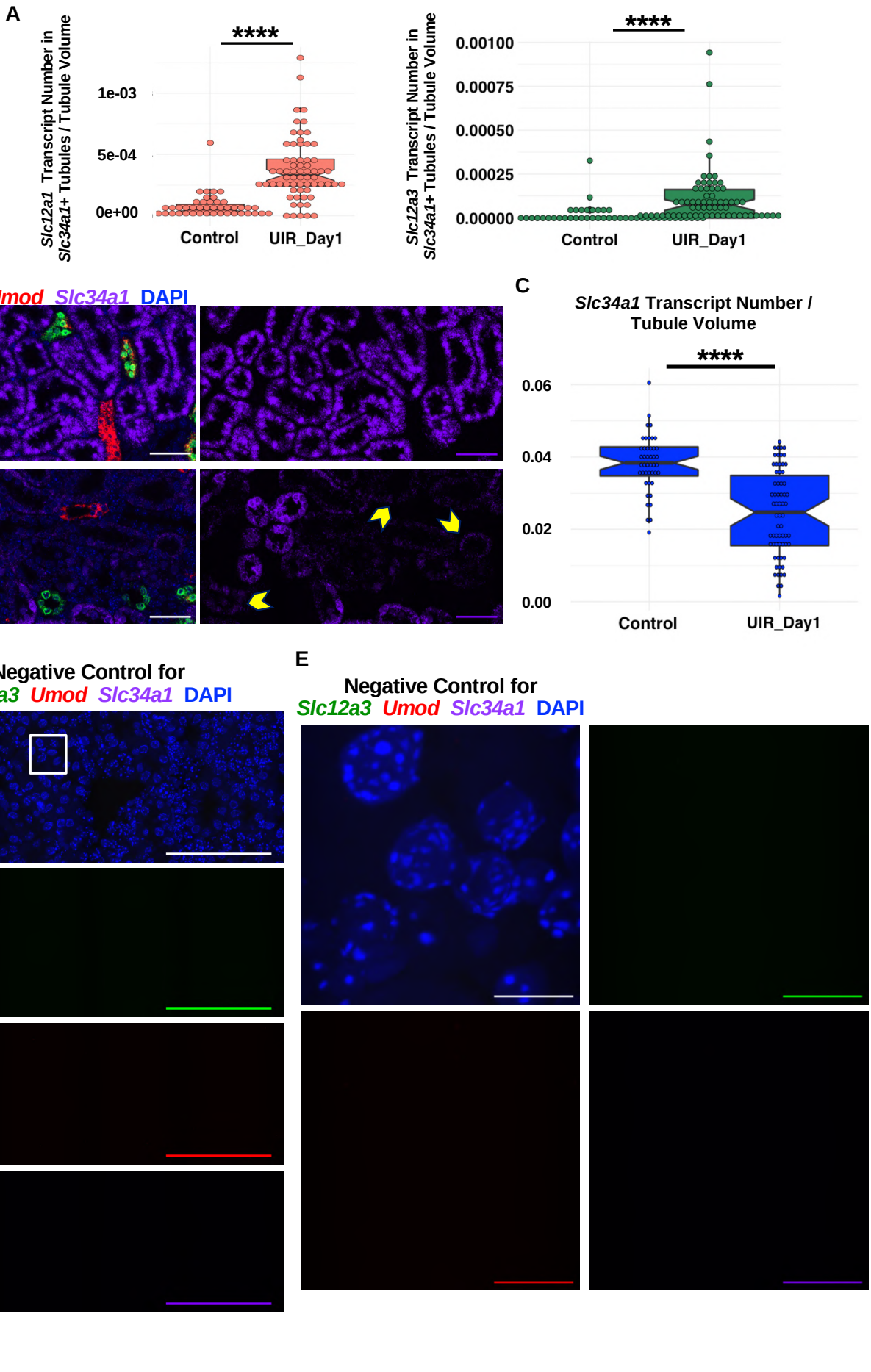
(A) IMARIS quantification of *Slc12a1* and *Slc12a3* transcripts in the *Slc34a1*-positive UIR Day 1 vs Control tubules, 12 Z-stacks (50-70 tubules) per group, Student's *t* test, **** p<0.0001. Data is presented as the transcript number normalized to the tubule volume.

(B) RNAscope with *Slc34a1* (purple), *Umod* (red) and *Slc12a3* (green) probes, DAPI (blue), Control and UIR Day 1. 60x 2x Nyquist zoom, 0.10 µm/px, Maximal Intensity Projection (MaxIP) from ~6µm Z-stack, 12 stitched images, scale 50 µm. Tubules with reduced *Slc34a1* expression are shown with the yellow pointers.

(C) IMARIS quantification of *Slc34a1* in the UIR Day 1 vs Control *Slc34a1*, n=12 Z-stacks (50-70 tubules) per group, Student's *t* test, **** p<0.0001. Data is shown as *Slc34a1* transcript number normalized to the tubule volume.

(D) Negative control for RNAscope with *Slc34a1* (purple), *Umod* (red) and *Slc12a3* (green) probes, DAPI (blue). 60x 2x Nyquist zoom, 0.10 µm/px, Maximal Intensity Projection (MaxIP) from ~6µm Z-stack, 12 stitched images, scale 50 µm. Negative control was processed in the same batch with the experimental samples. Higher magnification from panel E is highlighted with the white frame.

(E) Negative control for RNAscope with *Slc34a1* (purple), *Umod* (red) and *Slc12a3* (green) probes, DAPI (blue). 60x 6x Nyquist zoom, 0.03 µm/px, Maximal Intensity Projection (MaxIP) from ~6µm Z-stack, scale 10 µm. Negative control was processed in the same batch with the experimental samples.


A
Slc12a1 Transcript Number in Slc34a1+ Tubules / Tubule Volume
****
1e-03
5e-04
0e+00
Control
UIR_Day1
Slc12a3 Transcript Number in Slc34a1+ Tubules / Tubule Volume
****
0.00100
0.00075
0.00050
0.00025
0.00000
Control
UIR_Day1
B
Slc12a3 Umod Slc34a1 DAPI
Control
UIR Day 1
C
Slc34a1 Transcript Number / Tubule Volume
****
0.06
0.04
0.02
0.00
Control
UIR_Day1
D
Negative Control for
Slc12a3 Umod Slc34a1 DAPI
E
Negative Control for
Slc12a3 Umod Slc34a1 DAPI

Supplemental Figure 5. AKI persists through UIR Day 4 and 7 and resolves by UIR Day 14. Related to Figure 3.

(A) H&E shows the pronounced renal tubular injury at Day 2, which starts resolving at Day 4 and 7. UIR Day 11 and 14 exhibit normal renal histology. 40x. Tubular dilation (blue pointers), cast formation (yellow pointers).

(B) UMAP shows renal cell populations in the UIR Day 11.

(C) *Slc34a1* CISH, UIR Day 11, 4x, 40x.

(D) *Kim1* (green), *Aqp2* (red), *Slc34a1* (purple), DAPI (blue) RNAscope, UIR Day 11, 4x, 60x.

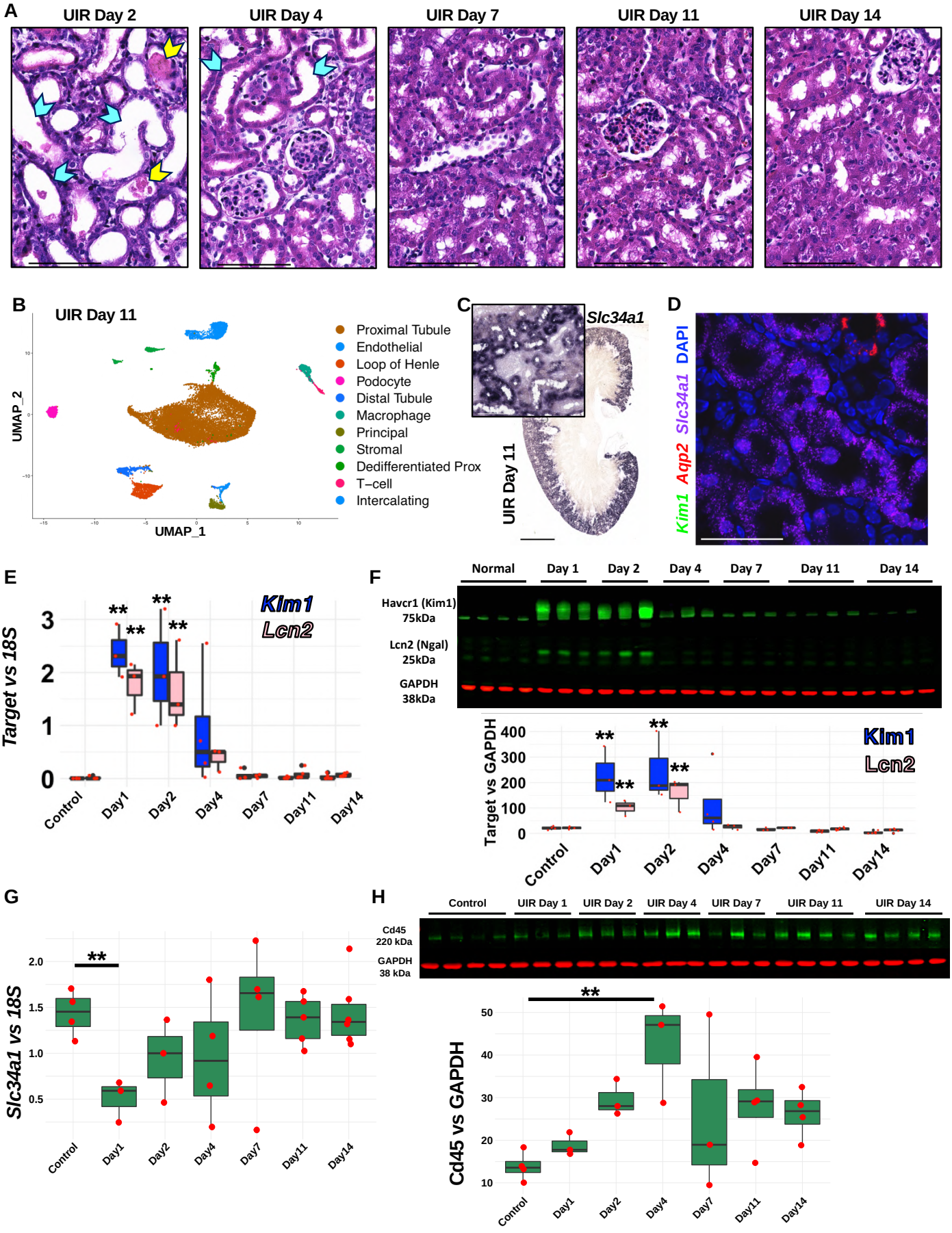
(E) qPCR shows *Kim1* and *Lcn2* expression over the AKI course, one-way ANOVA with Bonferroni and Holm, n=3-6 per group, ** pValue<0.01 compared to Control.

(F) Western blots image and quantification show Kim1 and Lcn2 expression over the AKI course, one-way ANOVA with Bonferroni and Holm, n=3-4 per group, ** pValue<0.01 compared to Control.

(G) qPCR shows *Slc34a1* expression over the AKI course, Student's *t* test, n=3-6 per group, ** pValue=0.0040 compared to Control.

(H) Western blot image and quantification shows Cd45 expression over the AKI course, one-way ANOVA with Bonferroni and Holm, n=3-4 per group, ** pValue<0.01 compared to Control.

Scale 4x, 2500µm, 40x, 100µm, 60x, 25µm.

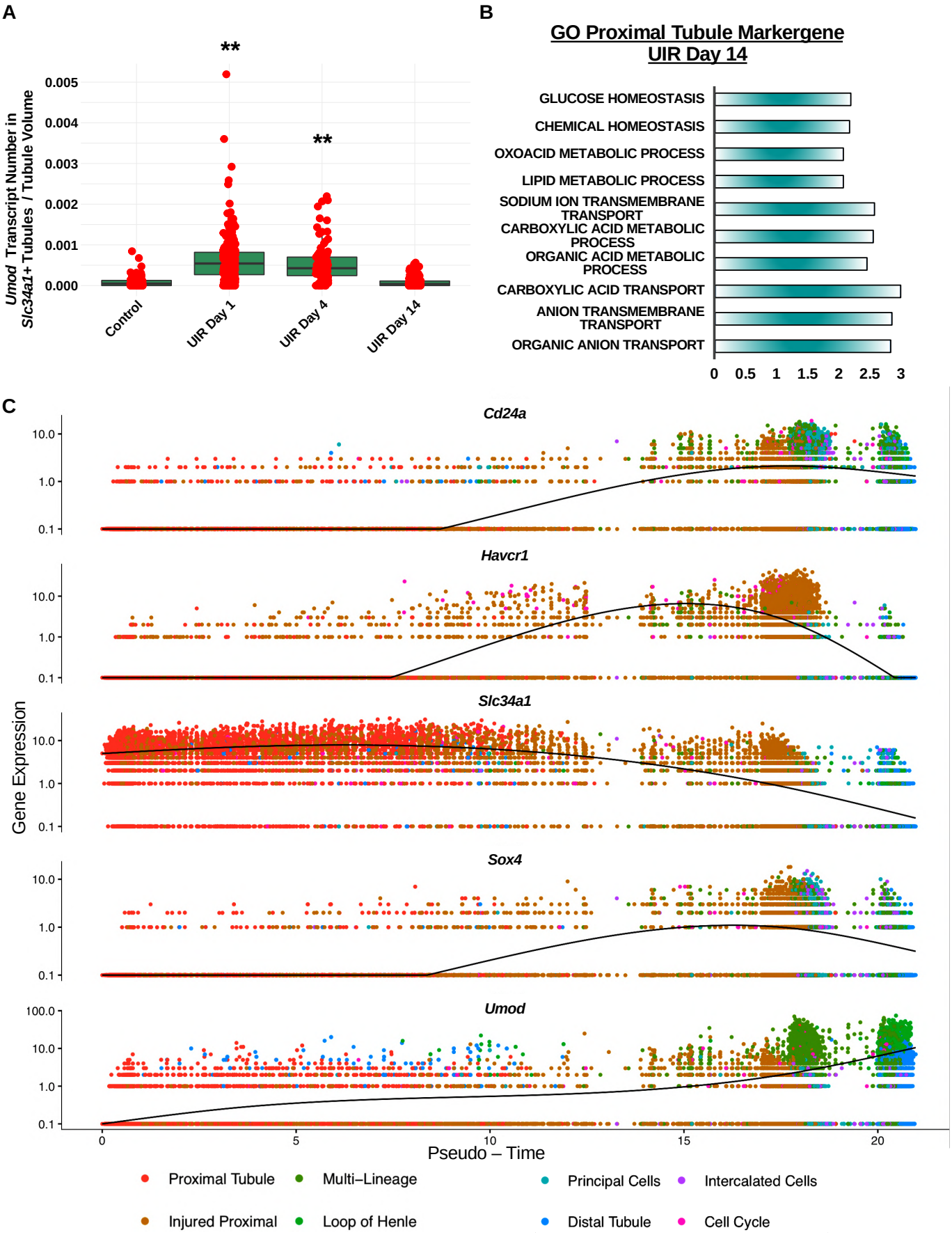

A
UIR Day 2
UIR Day 4
UIR Day 7
UIR Day 11
UIR Day 14
B
UIR Day 11
UMAP_2
UMAP_1
Proximal Tubule
Endothelial
Loop of Henle
Podocyte
Distal Tubule
Macrophage
Principal
Stromal
Dedifferentiated Prox
T–cell
Intercalating
C
Slc34a1
UIR Day 11
D
Kim1 Aqp2 Slc34a1 DAPI
E
Kim1
Lcn2
Target vs 18S
Control
Day1
Day2
Day4
Day7
Day11
Day14
F
Normal
Day 1
Day 2
Day 4
Day 7
Day 11
Day 14
Havcr1 (Kim1)
75kDa
Lcn2 (Ngal)
25kDa
GAPDH
38kDa
Target vs GAPDH
Kim1
Lcn2
G
Slc34a1 vs 18S
H
Control
UIR Day 1
UIR Day 2
UIR Day 4
UIR Day 7
UIR Day 11
UIR Day 14
Cd45
220 kDa
GAPDH
38 kDa
Cd45 vs GAPDH

Supplemental Figure 6. AKI induced unique gene expression signatures in the Injured Proximal tubules and Mixed Identity Cells. Related to Figure 3.

(A) IMARIS quantification of *Umod* transcripts in the UIR Day 1 (n=4 animals), 4 (n=2 animals) and 14 (n=2 animals) vs Control (n=3 animals) tubules, 12 ~6 µm Z-stack images per group, one-way ANOVA with Bonferroni and Holm, ** pValue<0.01 compared to Control. Data is presented as the transcript number normalized to the tubule volume.

(B) GO Biological Process analysis of the UIR Day 14 marker genes, -log(2) pValue.

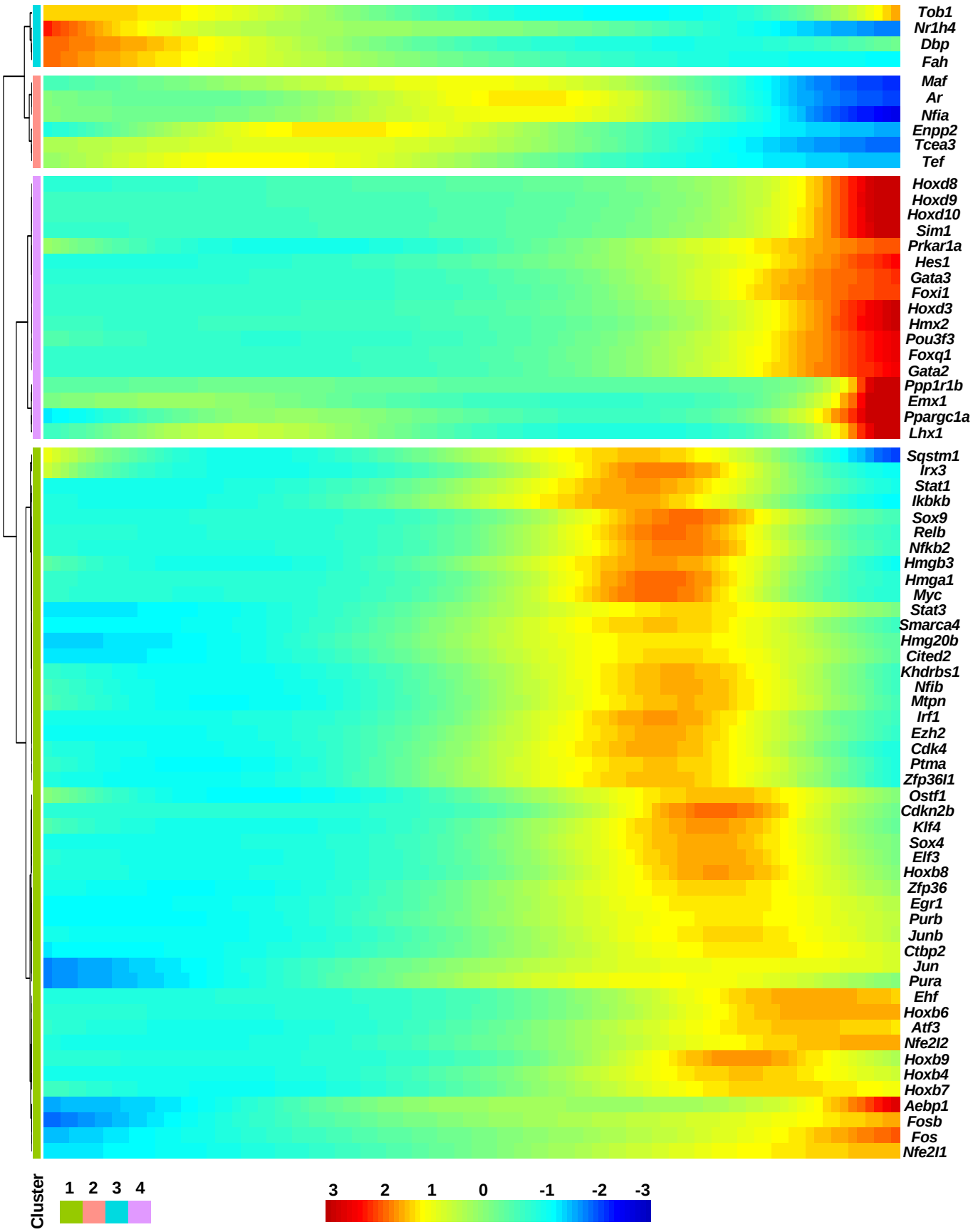
(C) Trajectory analysis of the UIR Day 1, 4, 7, 14 and the Control demonstrates the changes of kidney injury marker (*Kim1*), proximal tubular (*Slc34a1*) and loop of Henle (*Umod*) markers and renal developmental genes (*Cd24a, Sox4*) in the renal cell populations over the Pseudo – Time.


A
**
**
0.005
0.004
0.003
0.002
0.001
0.000
Umod Transcript Number in Slc34a1+ Tubules / Tubule Volume
Control
UIR Day 1
UIR Day 4
UIR Day 14
B
GO Proximal Tubule Markergene
UIR Day 14
GLUCOSE HOMEOSTASIS
CHEMICAL HOMEOSTASIS
OXOACID METABOLIC PROCESS
LIPID METABOLIC PROCESS
SODIUM ION TRANSMEMBRANE TRANSPORT
CARBOXYLIC ACID METABOLIC PROCESS
ORGANIC ACID METABOLIC PROCESS
CARBOXYLIC ACID TRANSPORT
ANION TRANSMEMBRANE TRANSPORT
ORGANIC ANION TRANSPORT
0 0.5 1 1.5 2 2.5 3
C
Cd24a
Havcr1
Slc34a1
Sox4
Umod
Gene Expression
10.0
1.0
0.1
100.0
0 5 10 15 20
Pseudo – Time
Proximal Tubule
Multi–Lineage
Principal Cells
Intercalated Cells
Injured Proximal
Loop of Henle
Distal Tubule
Cell Cycle

Supplemental Figure 7. The trajectory analysis reveals changes in transcription factor expression over AKI response. Related to Figure 3.

(A) The linear heatmap shows transcription factors expression changes in the Control, UIR Day 1, 4, 7 and 14 renal tubular cell clusters (proximal tubules, injured proximal tubules, cell cycle proximal tubules, mixed identity cells, distal tubules, loop of Henle, collecting duct) over a Pseudo – Time. The cells are clustered based upon similarity of transcription factors expression. Note the significant presence of renal developmental genes, including *Sox4* and *Hox* genes.

A

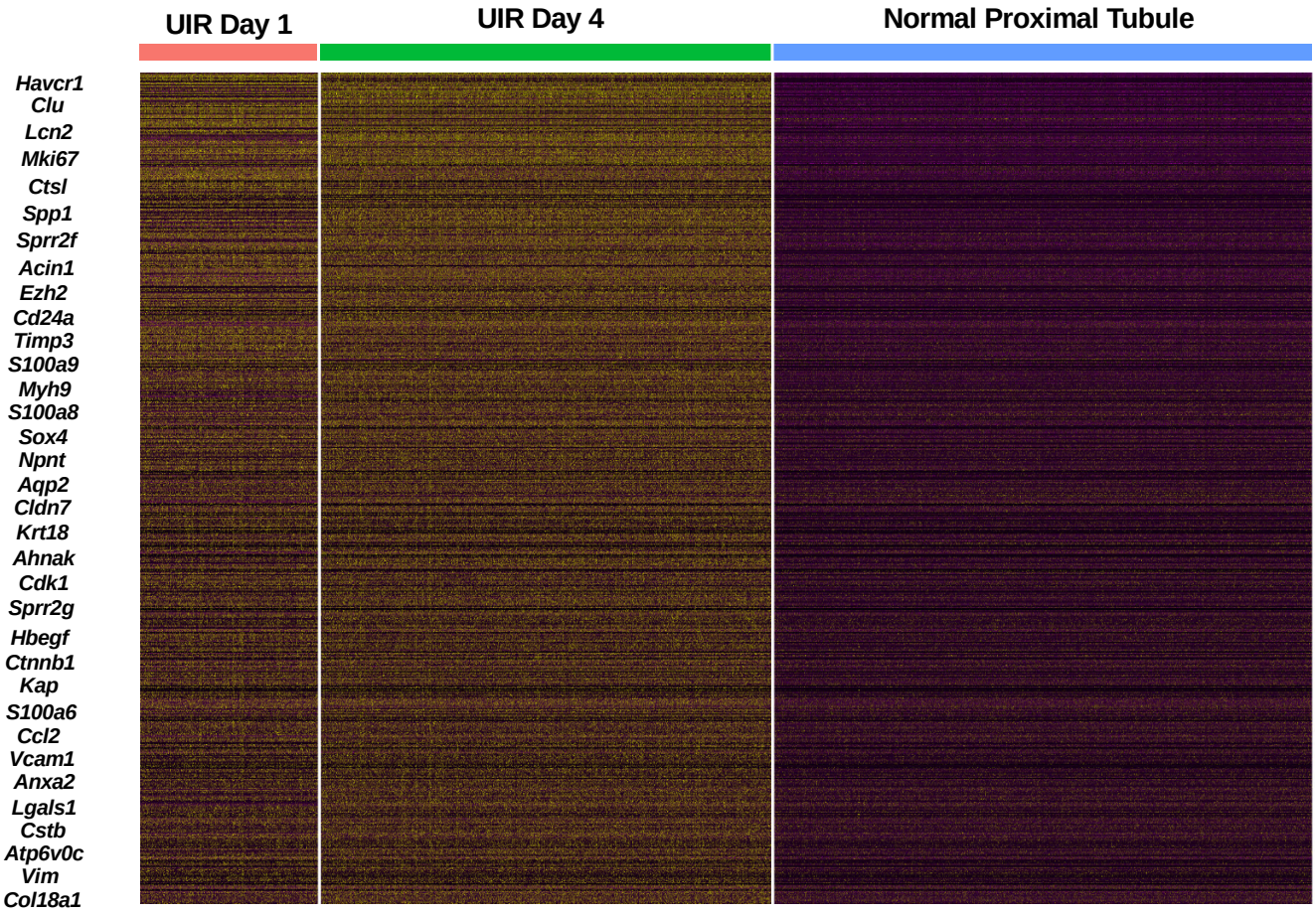

Tob1
Nr1h4
Dbp
Fah
Maf
Ar
Nfia
Enpp2
Tcea3
Tef
Hoxd8
Hoxd9
Hoxd10
Sim1
Prkar1a
Hes1
Gata3
Foxi1
Hoxd3
Hmx2
Pou3f3
Foxq1
Gata2
Ppp1r1b
Emx1
Ppargc1a
Lhx1
Sqstm1
Irx3
Stat1
Ikbkb
Sox9
Relb
Nfkb2
Hmgb3
Hmga1
Myc
Stat3
Smarca4
Hmg20b
Cited2
Khdrbs1
Nfib
Mtpn
Irf1
Ezh2
Cdk4
Ptma
Zfp36l1
Ostf1
Cdkn2b
Klf4
Sox4
Elf3
Hoxb8
Zfp36
Egr1
Purb
Junb
Ctbp2
Jun
Pura
Ehf
Hoxb6
Atf3
Nfe2l2
Hoxb9
Hoxb4
Hoxb7
Aebp1
Fosb
Fos
Nfe2l1
Cluster
1 2 3 4
3 2 1 0 -1 -2 -3

Supplemental Figure 8. Injury induced gene expression analysis reveals enrichment of apoptotic, pro-fibrotic and developmental factors in the UIR induced proximal tubules and mixed identity cells. Related to Figure 3.

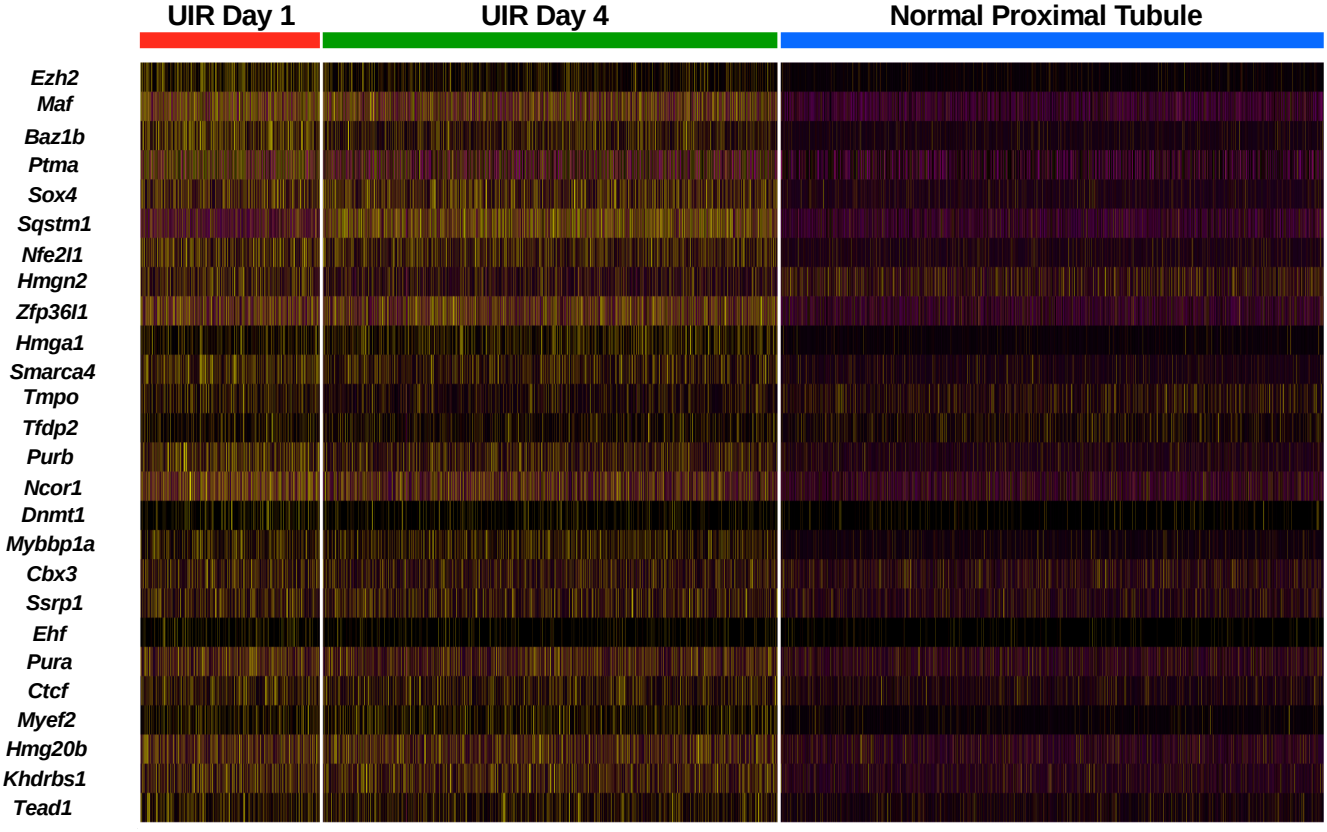
(A) Heatmap shows the relative gene expression in the UIR Day 1 and 4 renal clusters (proximal tubules, injured proximal tubules, mixed identity cells) compared to adult normal murine proximal tubules. Please note that the heatmap shows genes elevated in renal cell populations relative to each other, based on the z-score. The yellow is above the mean, black is the mean, and purple/blue is below the mean, which represents the lowest expression levels. Highlighted genes are labeled on the left side of the heatmap; the complete list of genes elevated in the UIR Day 1 and 4 compared to the normal is shown in the Supplemental Table 4.

(B) Heatmap shows the relative transcription factor expression in the UIR Day 1 and 4 renal clusters (proximal tubules, injured proximal tubules, mixed identity cells) compared to adult normal murine proximal tubules. Individual transcription factors elevated in the UIR Day 1 and 4 clusters compared to the normal are labeled on the left side of the heatmap.

A


UIR Day 1
UIR Day 4
Normal Proximal Tubule
Havcr1
Clu
Lcn2
Mki67
Ctsl
Spp1
Sprr2f
Acin1
Ezh2
Cd24a
Timp3
S100a9
Myh9
S100a8
Sox4
Npnt
Aqp2
Cldn7
Krt18
Ahnak
Cdk1
Sprr2g
Hbegf
Ctnnb1
Kap
S100a6
Ccl2
Vcam1
Anxa2
Lgals1
Cstb
Atp6v0c
Vim
Col18a1

B


UIR Day 1
UIR Day 4
Normal Proximal Tubule
Ezh2
Maf
Baz1b
Ptma
Sox4
Sqstm1
Nfe2l1
Hmgn2
Zfp36l1
Hmga1
Smarca4
Tmpo
Tfdp2
Purb
Ncor1
Dnmt1
Mybbp1a
Cbx3
Ssrp1
Ehf
Pura
Ctcf
Myef2
Hmg20b
Khdrbs1
Tead1

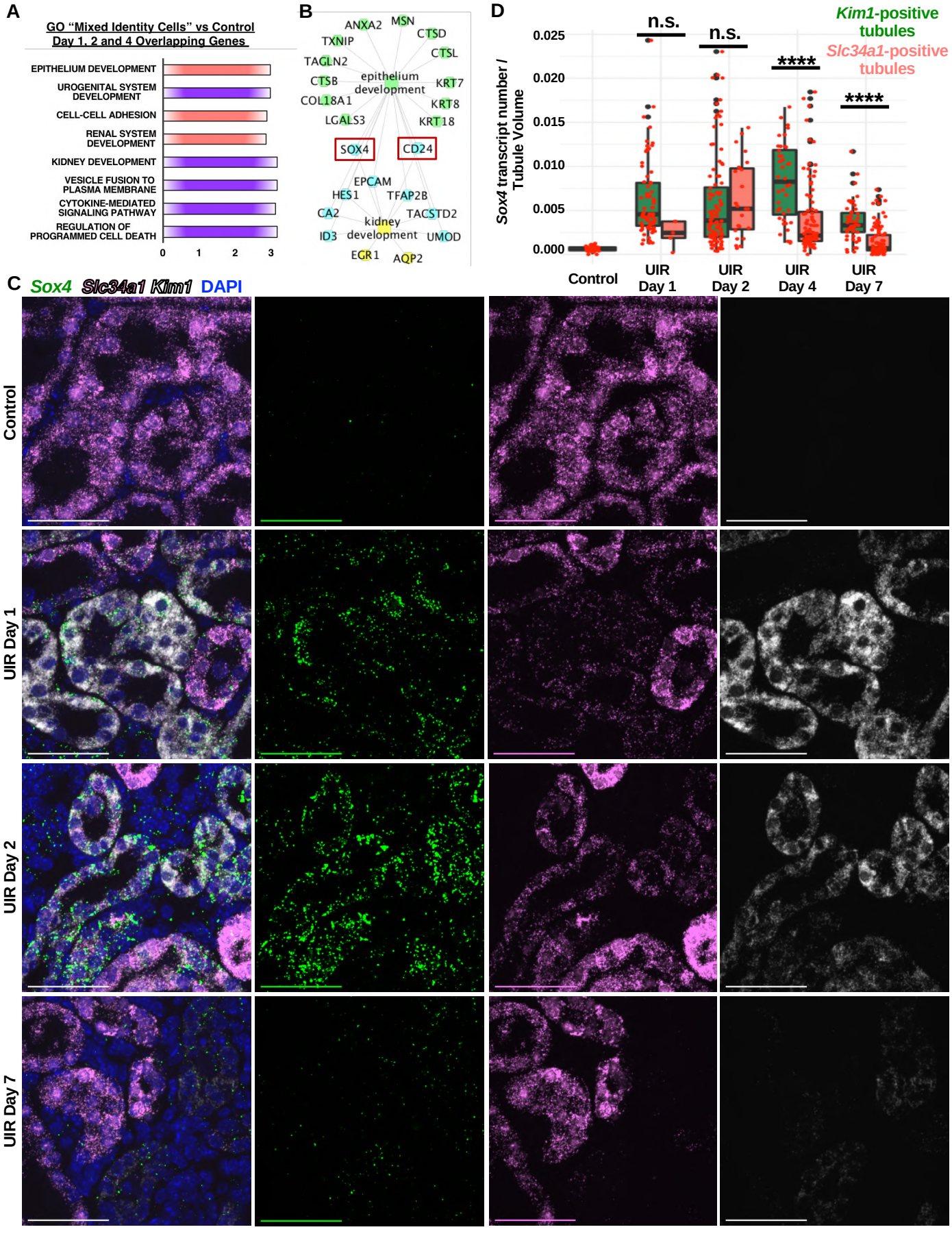
Supplemental Figure 9. *Sox4* labels proximal tubules dedifferentiation throughout the AKI course. Related to Figure 4.

(A) GO Biological processes of genes overlapping in the “Mixed Identity Cells” at Day 1, 2 and 4, -log(2) pValue. Renal developmental pathways highlighted in salmon color.

(B) ToppCluster analysis of the 99 genes overlapping between the UIR Day 1, 2 and 4 in the “Mixed Identity Cells” shows the enrichment of kidney (green) and epithelium development (yellow) biological processes. Genes involved in both processes are labeled with blue polygons. *Sox4* and *Cd24a* are highlighted in red. The analysis is done with 0.05 pValue cutoff and Bonferroni correction, the graph was made using the Fruchterman-Reingold algorithm.

(C) RNAscope with *Slc34a1* (pink), *Sox4* (green) and *Kim1* (white) probes, DAPI (blue), Control and UIR Day 1, 2, 7. 60x Nyquist zoom, 0.14 µm/px, Maximal Intensity Projection (MaxIP) from ~6 µm Z-stack, scale 25 µm.

(D) IMARIS quantification of *Sox4* in the UIR Day 1, 2, 4 and 7 vs Control *Slc34a1* vs *Kim1*-positive renal tubules, n=12 Z-stacks (50-70 tubules) per group, Student’s *t* test, **** $p<0.0001$, n.s. – not significant. Data is shown as *Sox4* transcript number normalized to the tubule volume.


A
GO "Mixed Identity Cells" vs Control
Day 1, 2 and 4 Overlapping Genes
EPITHELIUM DEVELOPMENT
UROGENITAL SYSTEM DEVELOPMENT
CELL-CELL ADHESION
RENAL SYSTEM DEVELOPMENT
KIDNEY DEVELOPMENT
VESICLE FUSION TO PLASMA MEMBRANE
CYTOKINE-MEDIATED SIGNALING PATHWAY
REGULATION OF PROGRAMMED CELL DEATH
0
1
2
3
B
ANXA2
MSN
CTSD
TXNIP
CTSL
TAGLN2
CTSB
epithelium development
KRT7
COL18A1
KRT8
LGALS3
KRT18
SOX4
CD24
EPCAM
HES1
TFAP2B
CA2
TACSTD2
kidney development
ID3
UMOD
EGR1
AQP2
D
Kim1-positive tubules
Slc34a1-positive tubules
Sox4 transcript number / Tubule Volume
0.025
0.020
0.015
0.010
0.005
0.000
n.s.
n.s.
****
****
Control
UIR Day 1
UIR Day 2
UIR Day 4
UIR Day 7
C
Sox4 Slc34a1 Kim1 DAPI
Control
UIR Day 1
UIR Day 2
UIR Day 7

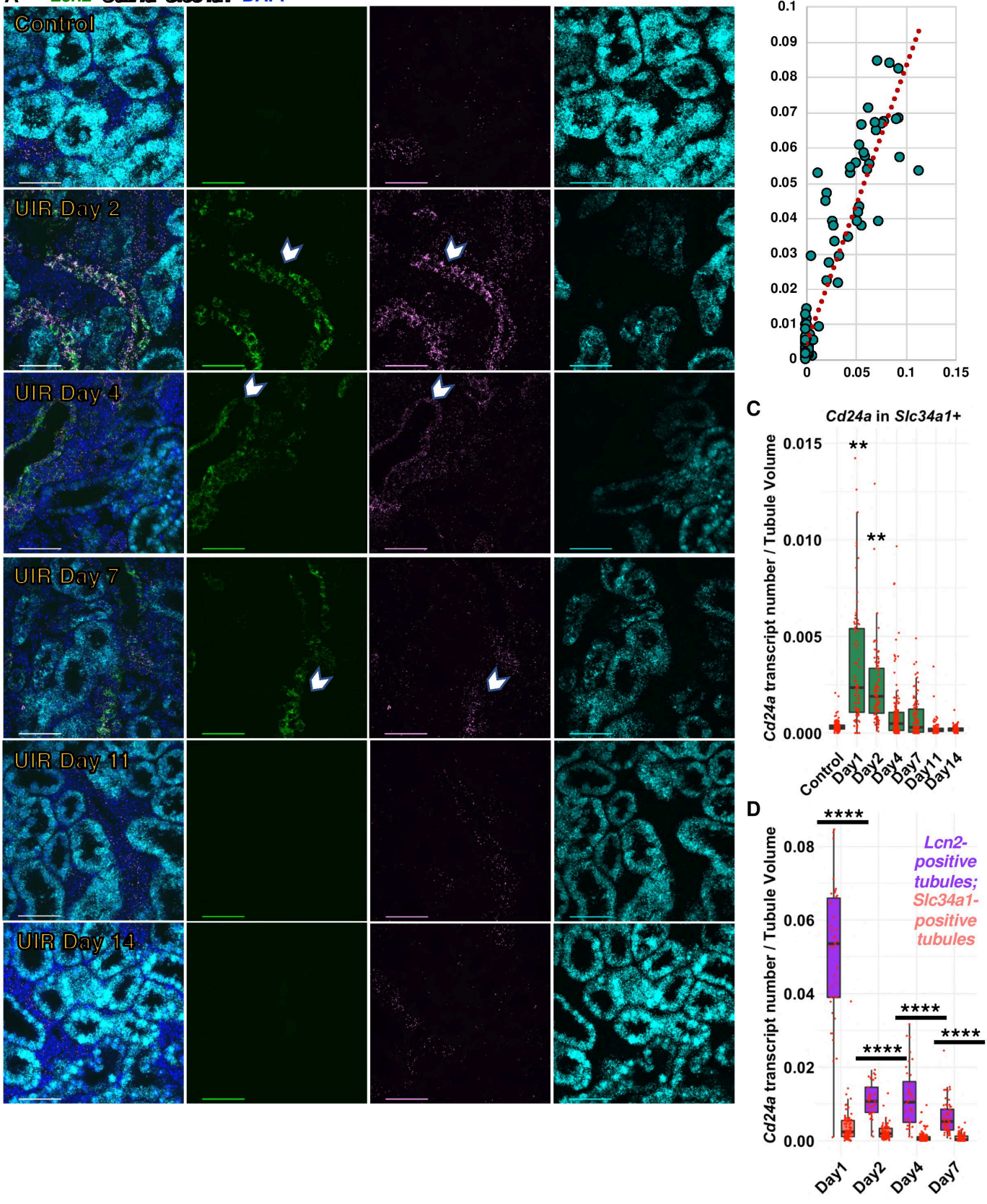
Supplemental Figure 10. *Cd24a* marks distal nephron tubule segment injury and correlates with *Lcn2*. Related to Figure 4.

(A) 60x RNAscope images show *Lcn2* (green) and *Cd24a* (pink) colocalization at UIR Day 2, 4 and 7 highlighted with pointers, 60x Nyquist zoom, 0.21 µm/px, Maximal Intensity Projection (MaxIP) from Z-stack, scale 50µm.

(B) Pearson's correlation analysis of *Cd24a* and *Lcn2* transcript numbers in UIR Day 1, n=9 Z-stacks (~60 tubules).

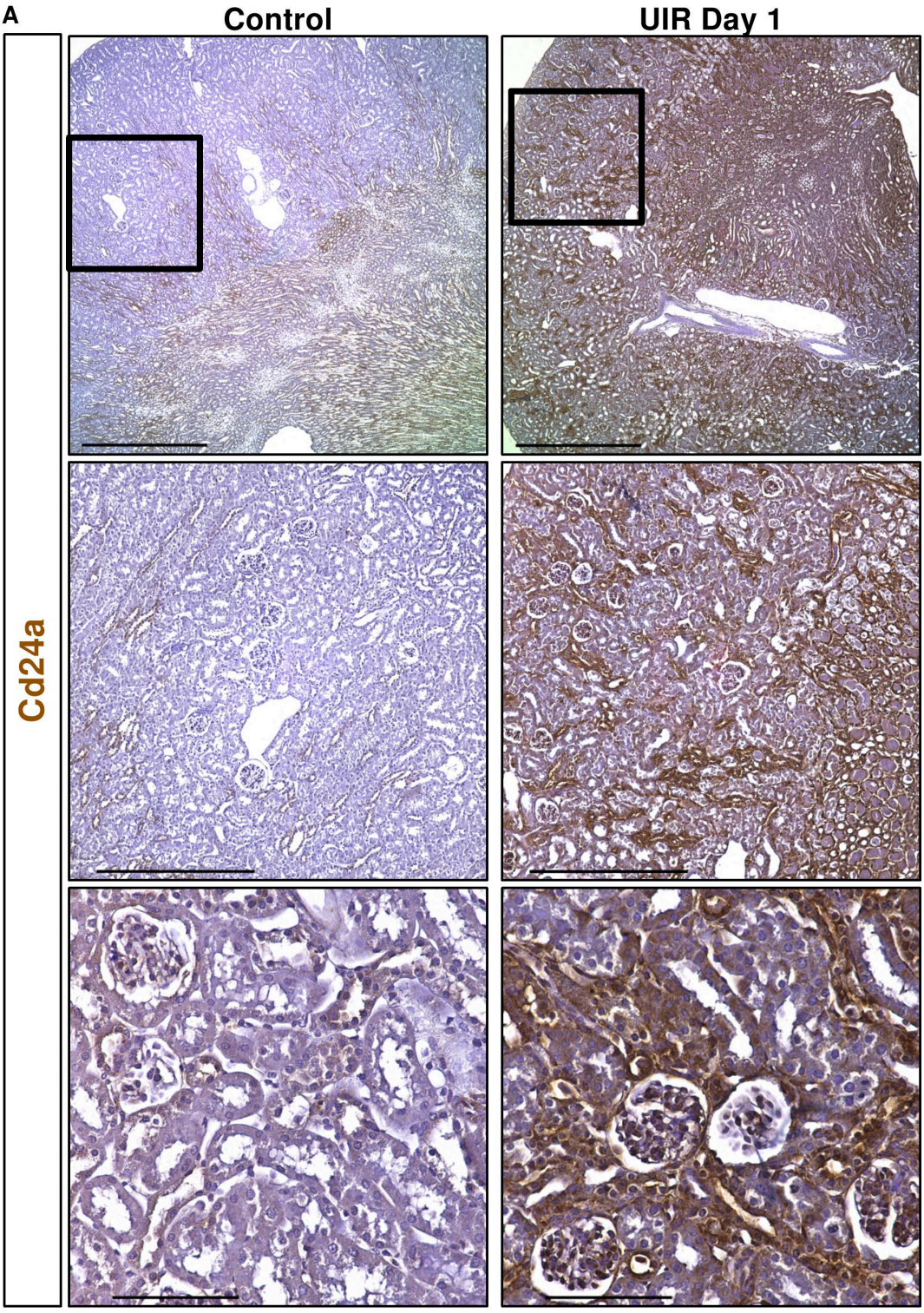
(C) IMARIS quantification of *Cd24a* in the UIR Day 1, 2, 4 and 7 and Control *Slc34a1*-positive renal tubules, n=9 Z-stacks (~60 tubules) per group, analyzed with one-way ANOVA with Bonferroni and Holm, ** $p<0.01$ compared to the Control.

(D) IMARIS quantification of *Cd24a* in the *Lcn2* vs *Slc34a1*-positive renal tubules at UIR Day 1, 2, 4 and 7, n=9 Z-stacks (~60 tubules) per group, Student's *t* test, **** $p<0.0001$.


A
Lcn2 Cd24a Slc34a1 DAPI
Control
UIR Day 2
UIR Day 4
UIR Day 7
UIR Day 11
UIR Day 14
B
Lcn2 and Cd24a Correlation
RSQ = 0.861; pValue < 0.0001
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0
0.05
0.1
0.15
C
Cd24a in Slc34a1+
Cd24a transcript number / Tubule Volume
0.015
0.010
0.005
0.000
**
**
Control
Day1
Day2
Day4
Day7
Day11
Day14
D
Cd24a transcript number / Tubule Volume
0.08
0.06
0.04
0.02
0.00
****
****
****
****
Lcn2-positive tubules;
Slc34a1-positive tubules
Day1
Day2
Day4
Day7

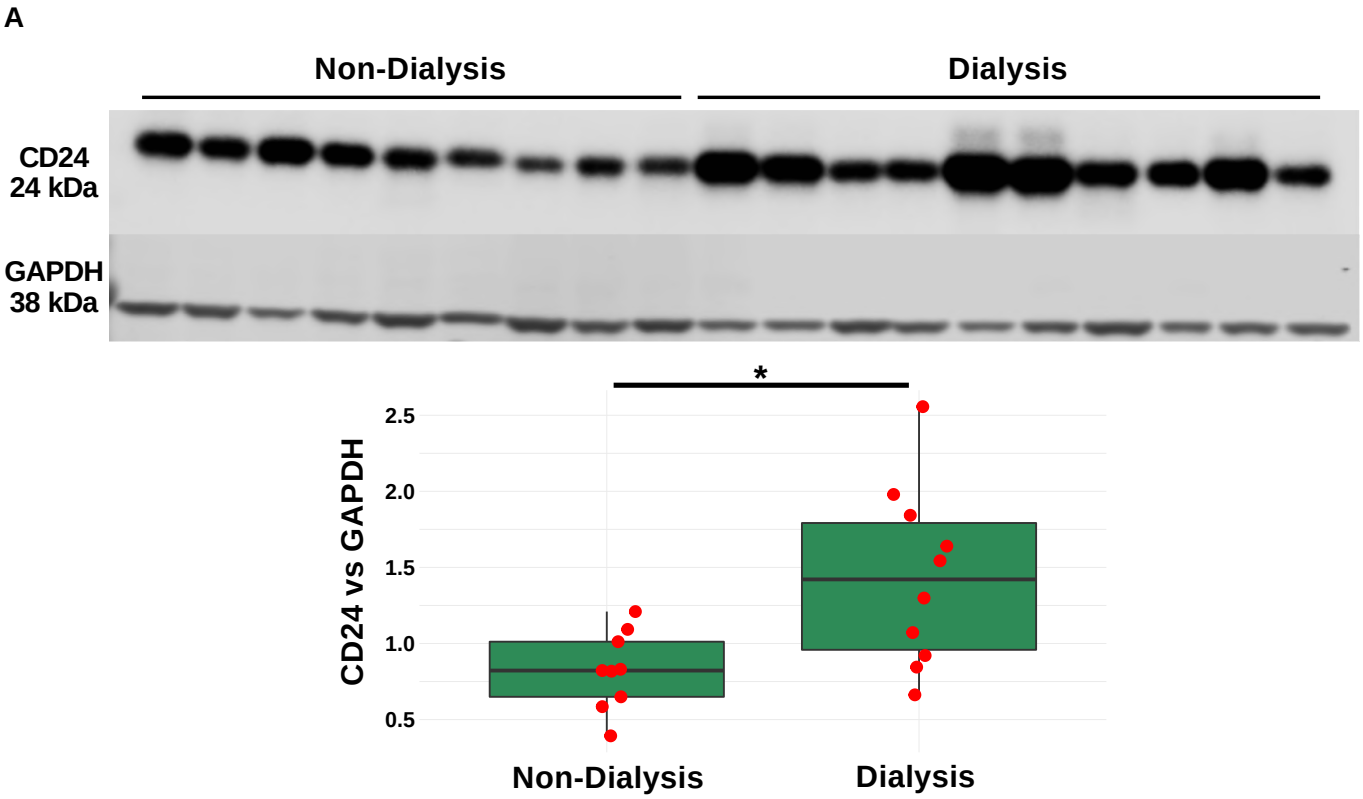
Supplemental Figure 11. UIR Day 1 exhibits marked Cd24a elevation in the cortical and medullary tubules. Related to Figure 4.

(A) Cd24a immunohistochemistry at UIR Day 1 vs Control. Black boxed show the 10x zoom into the cortex. 4x, 1000µm, 10x, 500µm, 40x, 100µm scale.



Supplemental Figure 12. Validation of AKI induced genes in the human kidney samples. Related to Figure 4.

(A) Western blot showing CD24 expression in human kidney biopsies from non-dialysis and dialysis patients, n=9-10 per group, Student's *t* test, * pValue<0.05, Fold Change 1.753 compared to Control.



Supplemental Figure 13. Pro-fibrotic signaling in the injured kidney. Related to Figures 5, 6 and 7.

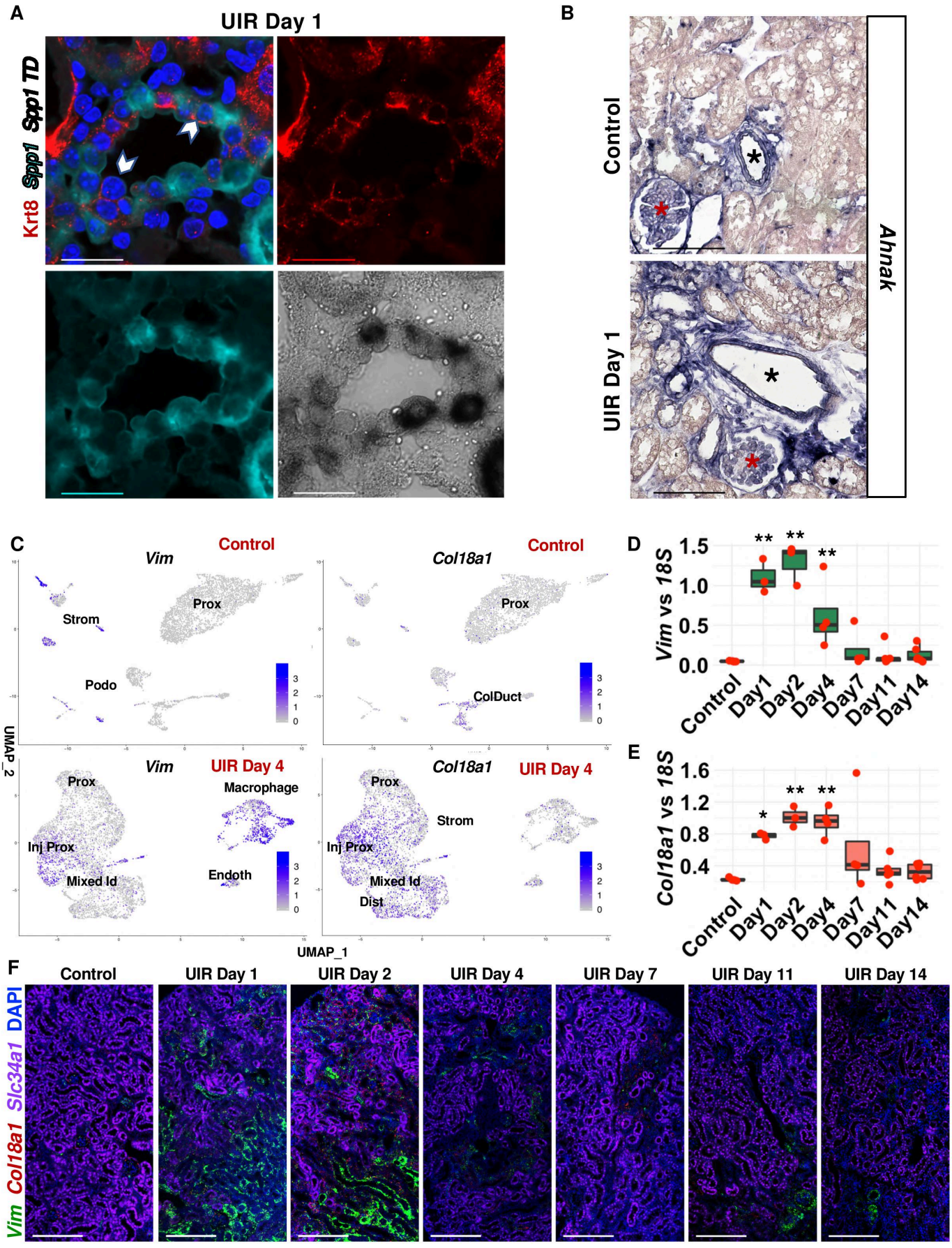
(A) *Spp1* CISH (cyan), Krt8 IF (red), DAPI (blue), UIR Day 1, 60x 0.09 px/µm Nyquist zoom, scale 25 µm. Pointers show *Spp1* and Krt8 overlap. The combined scRNA-seq and CISH results define the precise cell types with elevated *Spp1* and *Krt8* elevated expression following UIR. TD, transmitted detector, showing the chromogenic *Spp1* CISH signal.

(B) *Ahnak* CISH shows perivascular (black stars) and glomerular (red starts) expression in the Control and UIR Day 1. 40x, 100µm scale.

(C) Feature Plots show *Vim* and *Col18a1* in the UIR Day 4 vs Control renal cell populations. Prox, proximal tubules, Podo, podocytes, Strom, stromal, Endoth, endothelial, Inj Prox, Injured Proximal tubules, Mixed Id, mixed identity cells, Dist, distal tubules, ColDuct, collecting duct.

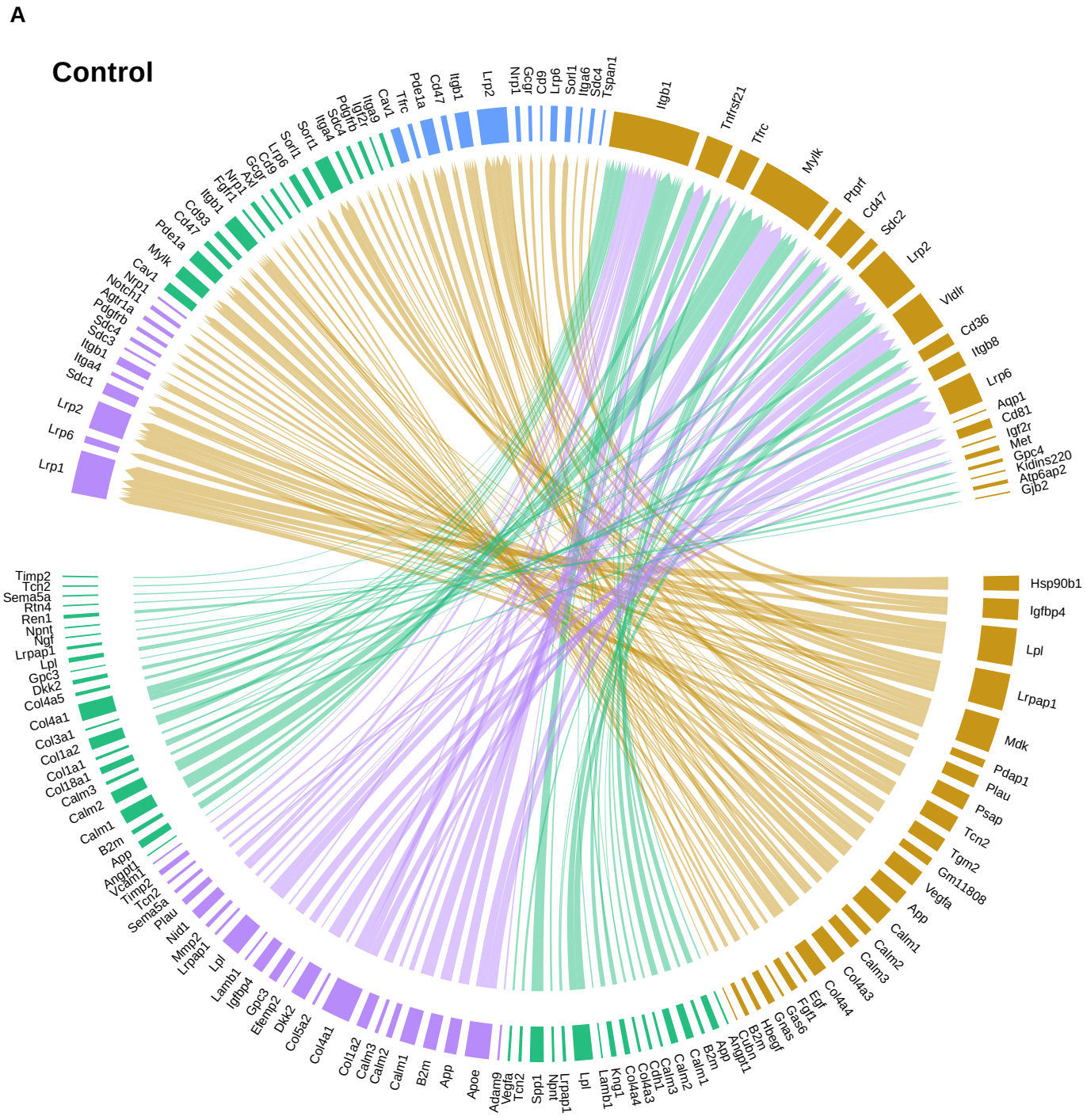
(D and E) qPCR shows Vim and Col18a1 gene expression changes over the AKI course, analyzed with one-way ANOVA with Bonferroni and Holm, * $p<0.05$, ** $p<0.01$ compared to the Control.

(F) RNAscope images show strong *Slc34a1* (purple) proximal tubule marker and low *Vim* (green) and *Col18a1* (red) expression in the interstitial and periglomerular spaces of the Control kidney. UIR causes significant *Slc34a1* decline and *Vim* and *Col18a1* elevation in the proximal tubules and in the stroma, which resolves at Day 11 and 14, with some remaining expression around the glomeruli. 10x, 500 µm scale.


A
UIR Day 1
Krt8
Spp1
Spp1 TD
B
Control
UIR Day 1
Ahnak
C
Vim
Control
Col18a1
Control
Strom
Prox
Podo
ColDuct
Vim
UIR Day 4
Col18a1
UIR Day 4
Macrophage
Endoth
Inj Prox
Mixed Id
Dist
UMAP_1
UMAP_2
D
Vim vs 18S
Control
Day1
Day2
Day4
Day7
Day11
Day14
E
Col18a1 vs 18S
F
Control
UIR Day 1
UIR Day 2
UIR Day 4
UIR Day 7
UIR Day 11
UIR Day 14
Vim
Col18a1
Slc34a1
DAPI

Supplemental Figure 14. Epithelial-to-Stromal crosstalk in the normal kidney. Related to Figure 7.

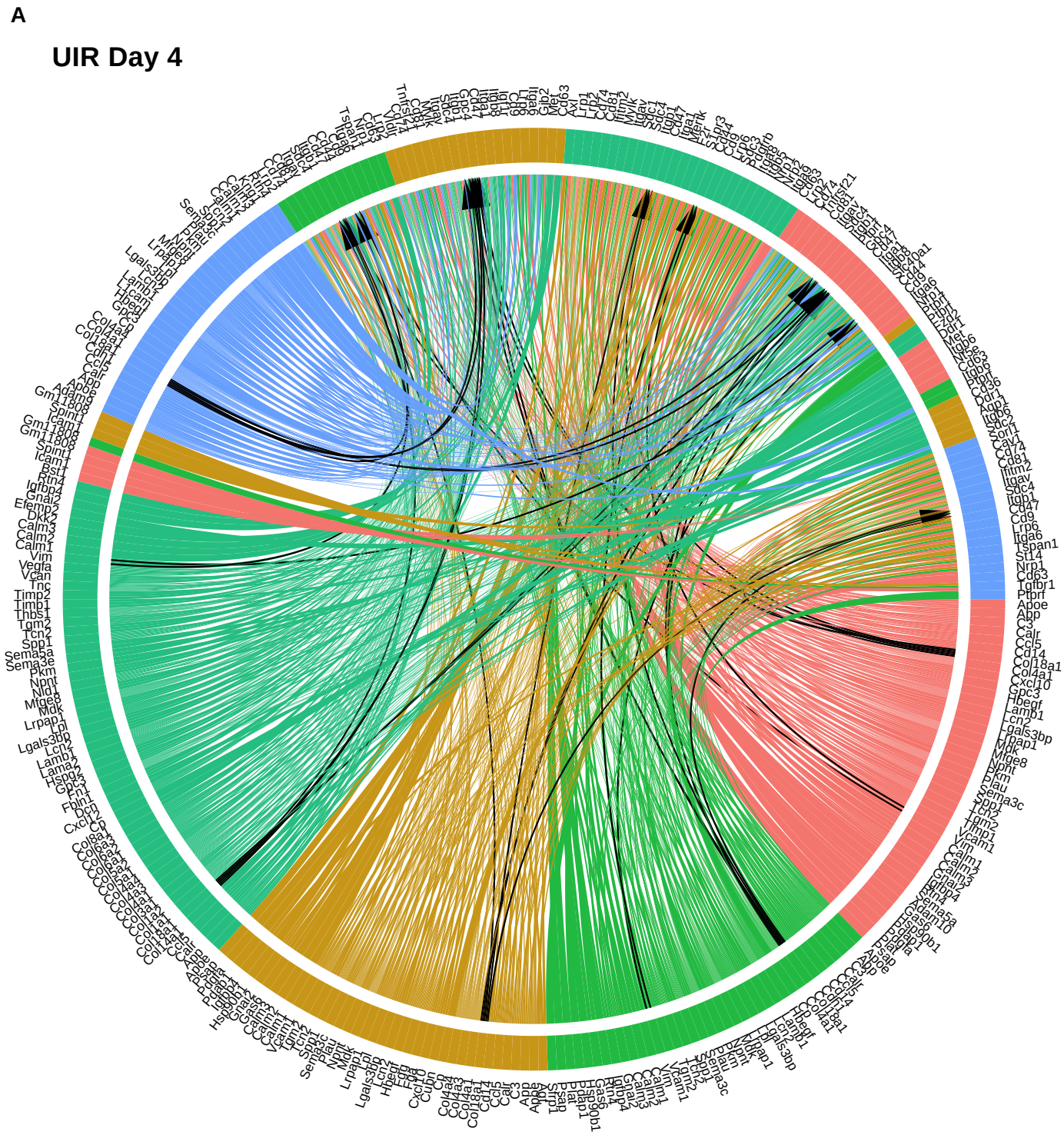
(A) Circos Plot of predicted ligand-receptor interactions between the proximal (brown) and distal (blue) tubules, the stromal cells (teal), and the stromal/pericyte cells (purple) in the normal kidney.



Supplemental Figure 15. Epithelial-to-Stromal crosstalk in the UIR Day 4.

(A) Circos Plot of ligand-receptor interactions between the proximal tubules (brown), injured proximal tubules (red), mixed identity cells (green), distal tubules (blue) and the stromal cells (teal) in the normal kidney. Black arrows show *Vim-Cd44*, *Col18a1-Gpc4* and *Col18a1-Itgb1* Ligand-Receptor pairs.

A



Supplemental Figure 16. Negative controls for *Sox4, Cd24a, Lcn2, Kim1, Slc34a1, Vim, Col18a1* RNAscope probes.

(A) Negative control for *Slc34a1* (pink), *Sox4* (green) and *Kim1* (white) RNAscope probes, DAPI (blue), 0.14 µm/px, Maximal Intensity Projection (MaxIP) from Z-stack, scale 50 µm.

(B) Negative control for *Cd24a* (pink), *Lcn2* (green) and *Slc34a1* (cyan) RNAscope probes, DAPI (blue), 60x Nyquist zoom, 0.21 µm/px, MaxIP from Z-stack, scale 50 µm.

(C) Negative control for *Col18a1* (red), *Vim* (green) and *Slc34a1* (purple) RNAscope probes, DAPI (blue), 60x Nyquist zoom, 0.21 µm/px, MaxIP from Z-stack, scale 50 µm.