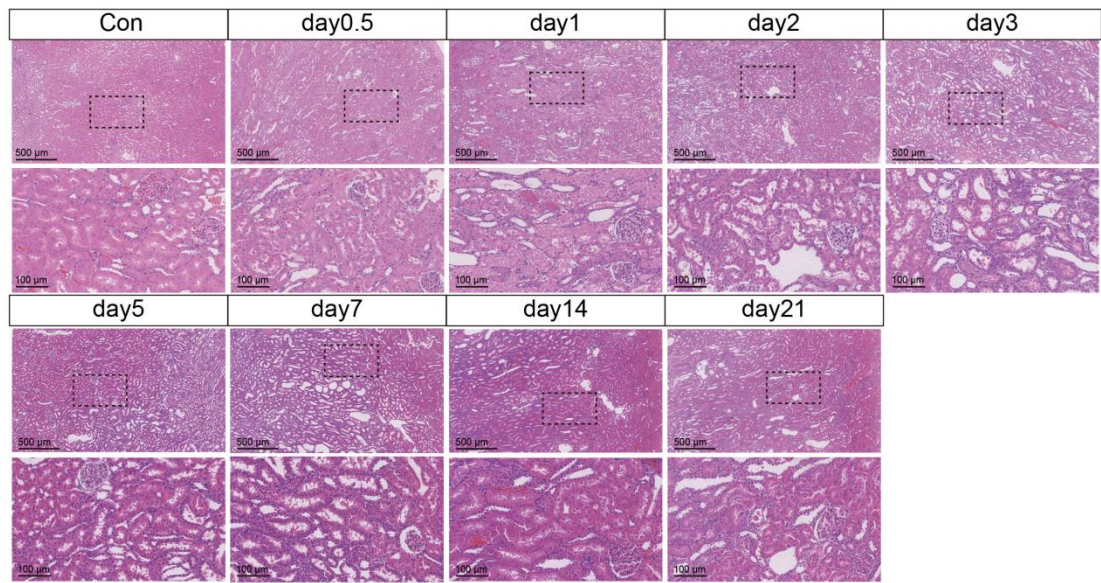
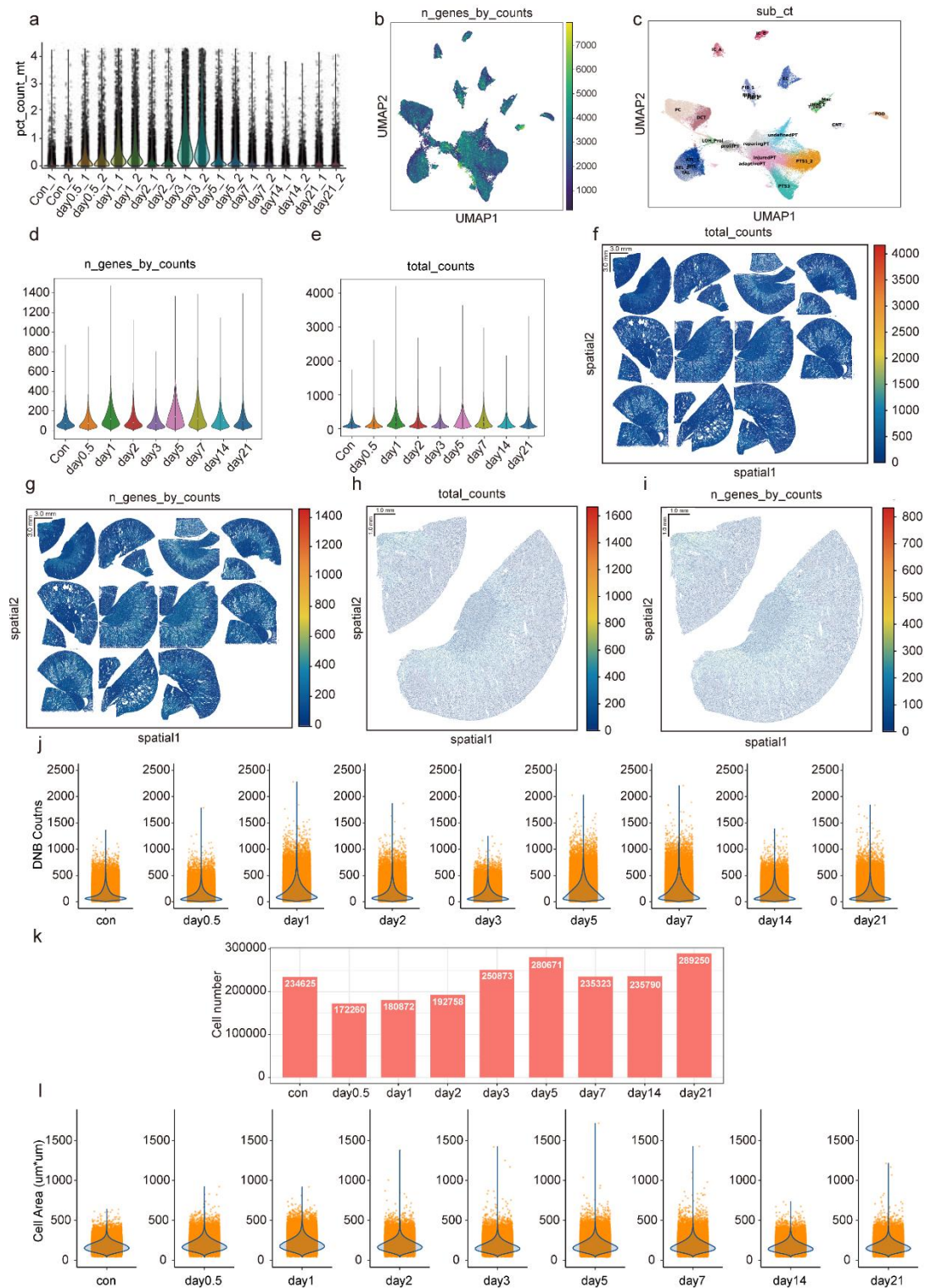


Supplementary Figure and Figure Legends



Supplementary Figure 1 | Histopathological analysis of renal tissues in rats following ischemia-reperfusion injury (IRI) surgery.

Representative Periodic Acid-Schiff (PAS) staining of kidney sections at key regeneration stages.



Supplementary Figure 2 | Sample Quality Control for Single-nucleus RNA-seq and Stereo-seq spatial transcriptomics.

a Violin plots showing the distribution of the number of mitochondrial gene counts per cell across different cell samples in single-nucleus RNA sequencing.

b UMAP visualization of single-nucleus RNA sequencing data, annotated by major

kidney cell types. Each point represents a single cell, and the color of the point corresponds to the number of genes detected in that cell, as indicated by the color bar on the right.

c UMAP visualization of single-nucleus RNA sequencing data, annotated by detailed kidney cell types.

d Violin plots showing the distribution of the number of genes detected per cell across different groups by Stereo-seq spatial transcriptomics.

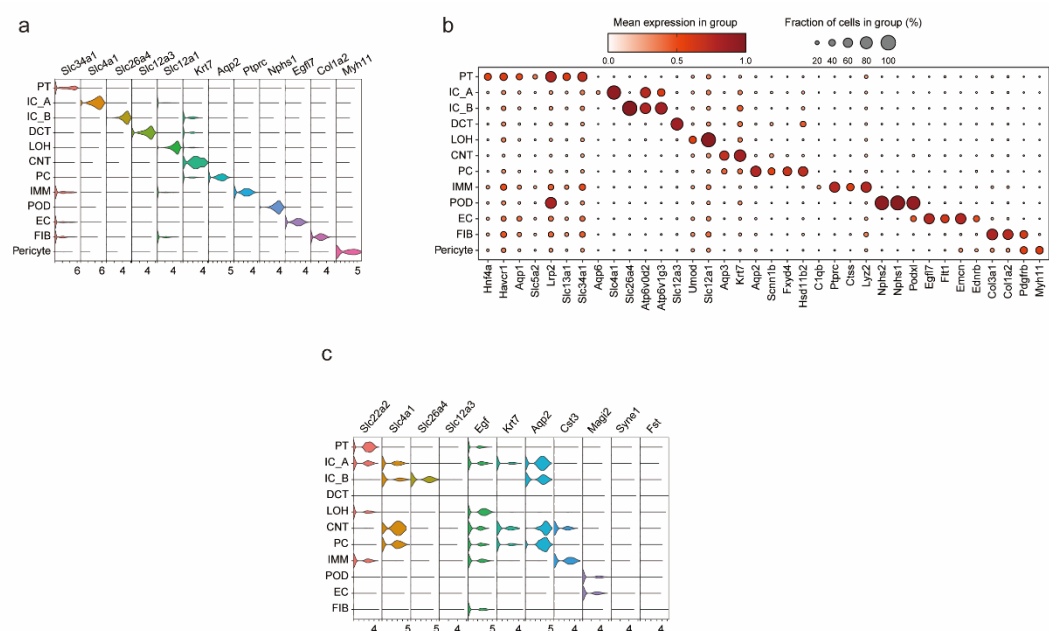
e Violin plots showing the distribution of total unique molecular identifiers (UMIs) detected per cell across different groups by Stereo-seq spatial transcriptomics.

f-i Heatmap showing the spatial distribution of gene expression counts within the kidney section.

j Distribution of DNB (Digital - Normalized Barcode) counts across different time post-IRI in Stereo-seq spatial transcriptomics.

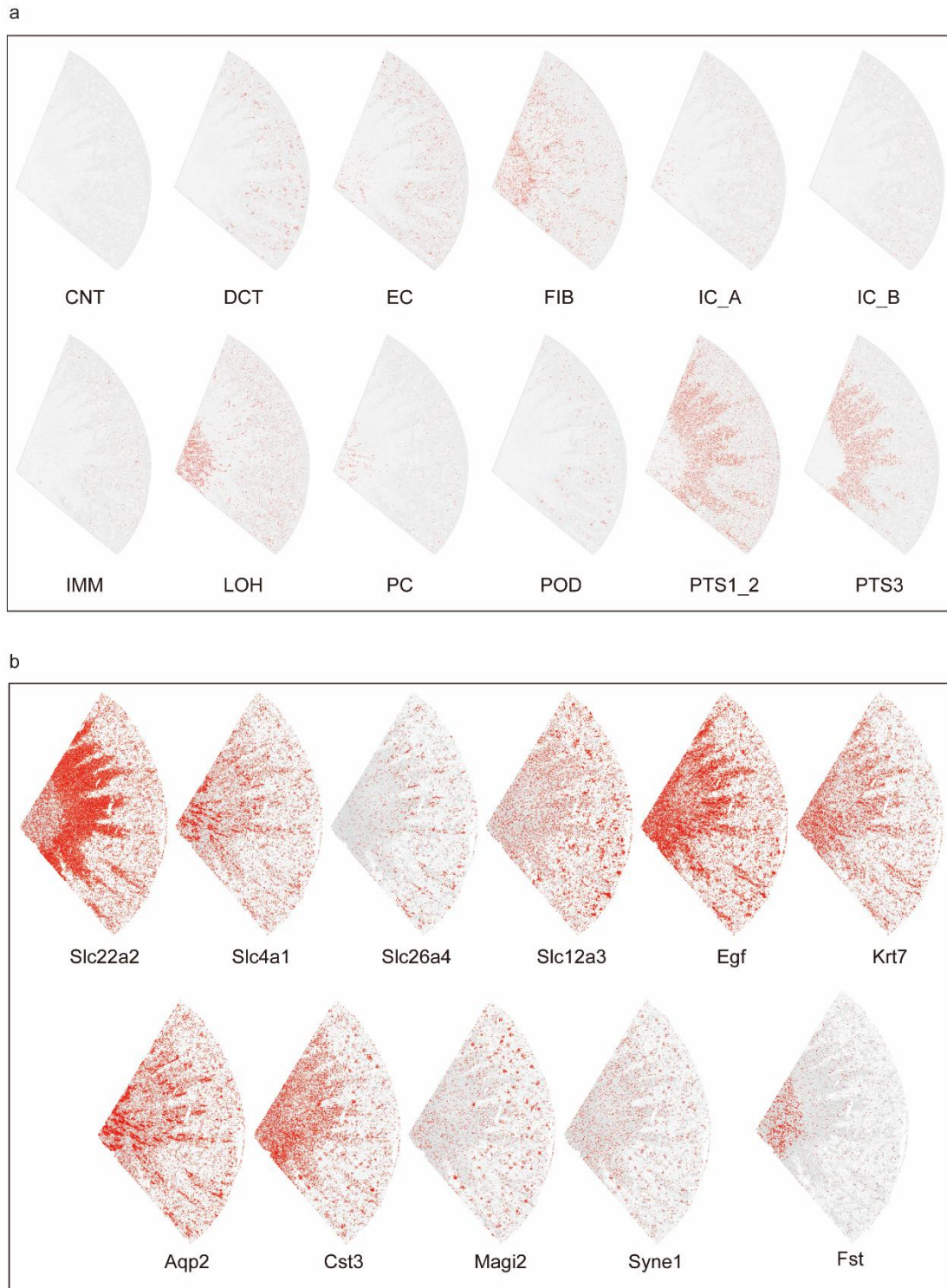
k Bar chart showing the number of cells detected in different groups in Stereo-seq spatial transcriptomics.

l Distribution of cell area across different time post-IRI in Stereo-seq spatial transcriptomics.



Supplementary Figure 3 |Feature genes for Single-nucleus RNA-seq and Stereo-seq spatial transcriptomics.

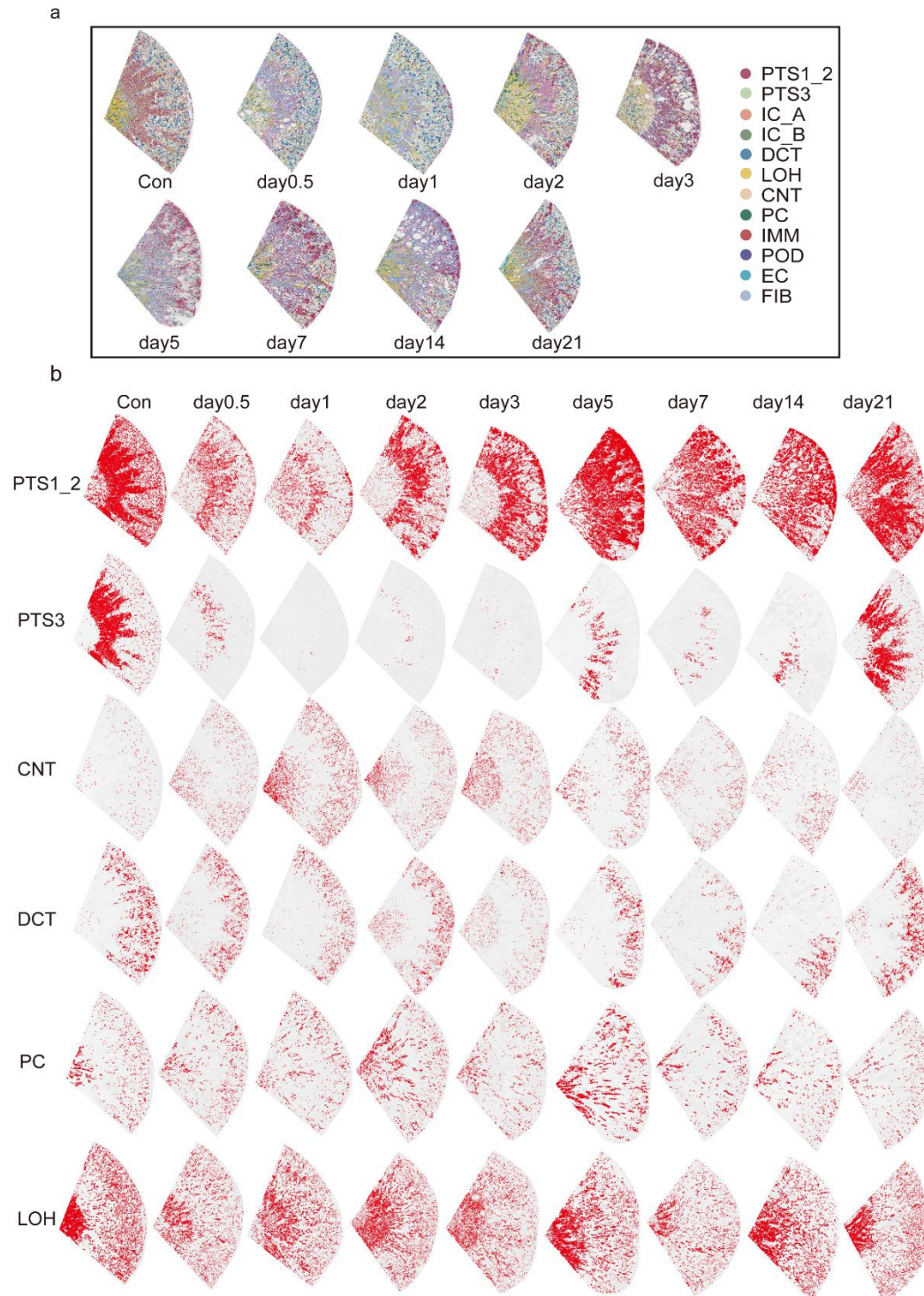
- a** Violin plot showing the expression levels of feature genes across various cell types in single-nucleus RNA sequencing.
- b** Dot plots of conserved marker genes for identified various cell types in single-nucleus RNA sequencing.
- c** Violin plot showing the expression levels of feature genes across various cell types in Stereo-seq spatial transcriptomics.



Supplementary Figure 4| snRNA-seq - Stereo-seq integration reveals the cellular atlas of normal rat kidneys.

a Spatial distribution of key cell types (e.g. proximal tubules, podocytes) via snRNA-seq - Stereo-seq integration in control group.

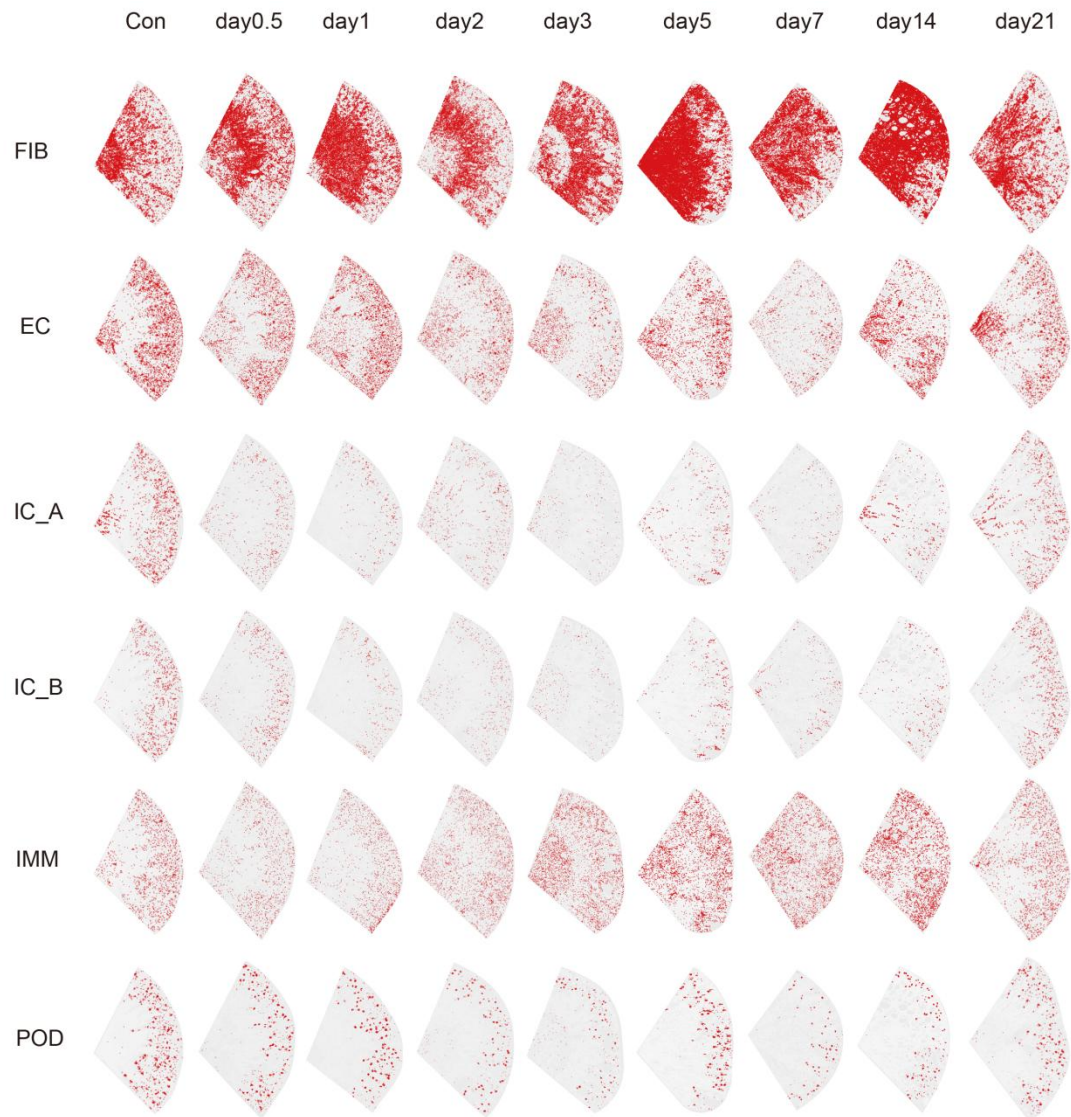
b Spatial distribution of kidney key marker genes (e.g. *slc22a2*, *slc4a1*) via snRNA-seq - Stereo-seq integration in control group.



Supplementary Figure 5 | snRNA-seq - Stereo-seq integration reveals the changes of each major kidney cell type.

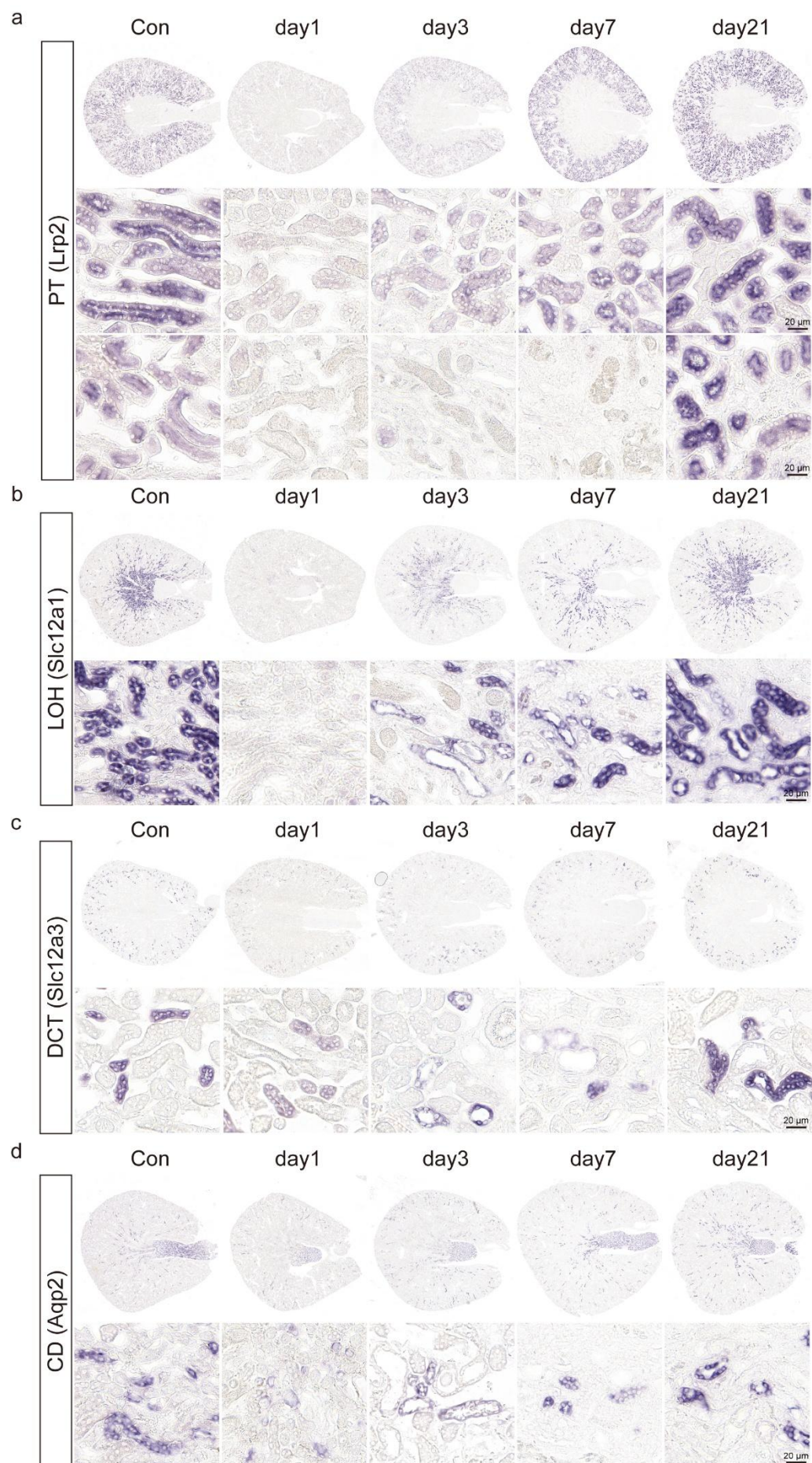
a Spatial transcriptomics maps showing the spatial distribution of major kidney cell types across control and different timepoints (day0.5, 1, 2, 3, 5, 7, 14, and 21) post IRI.

b Highlighted spatial expression patterns of kidney cell types at each timepoint.



Supplementary Figure 6 | Spatial expression patterns of other major kidney cell types.

Highlighted spatial expression patterns of other major kidney cell types at each timepoint.



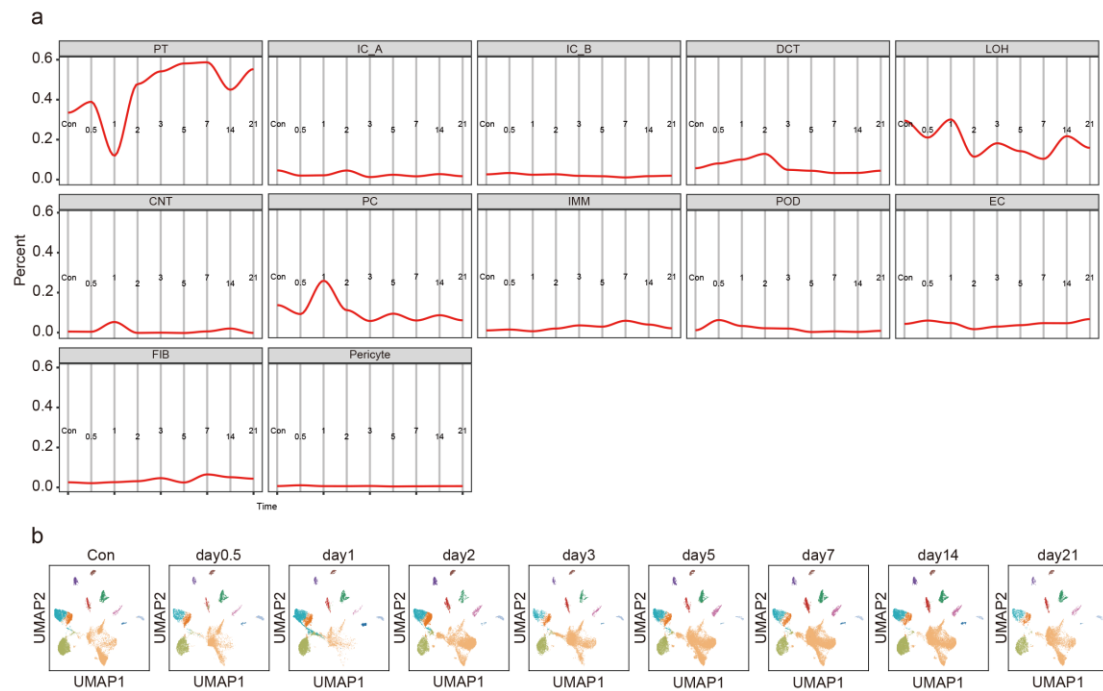
Supplementary Figure 7 | In Situ Hybridization (ISH) showing the expression of kidney major cell type markers across different time points after ischemia-reperfusion injury (IRI).

a Expression changes of *Lrp2* RNA (a marker for PT) at different timepoints (Con, day1, day3, day7, day21) post IRI.

b Expression changes of *Slc12a1* RNA (a marker for LOH) at different timepoints (Con, day1, day3, day7, day21) post IRI.

c Expression changes of *Slc12a3* RNA (a marker for DCT) at different timepoints (Con, day1, day3, day7, day21) post IRI.

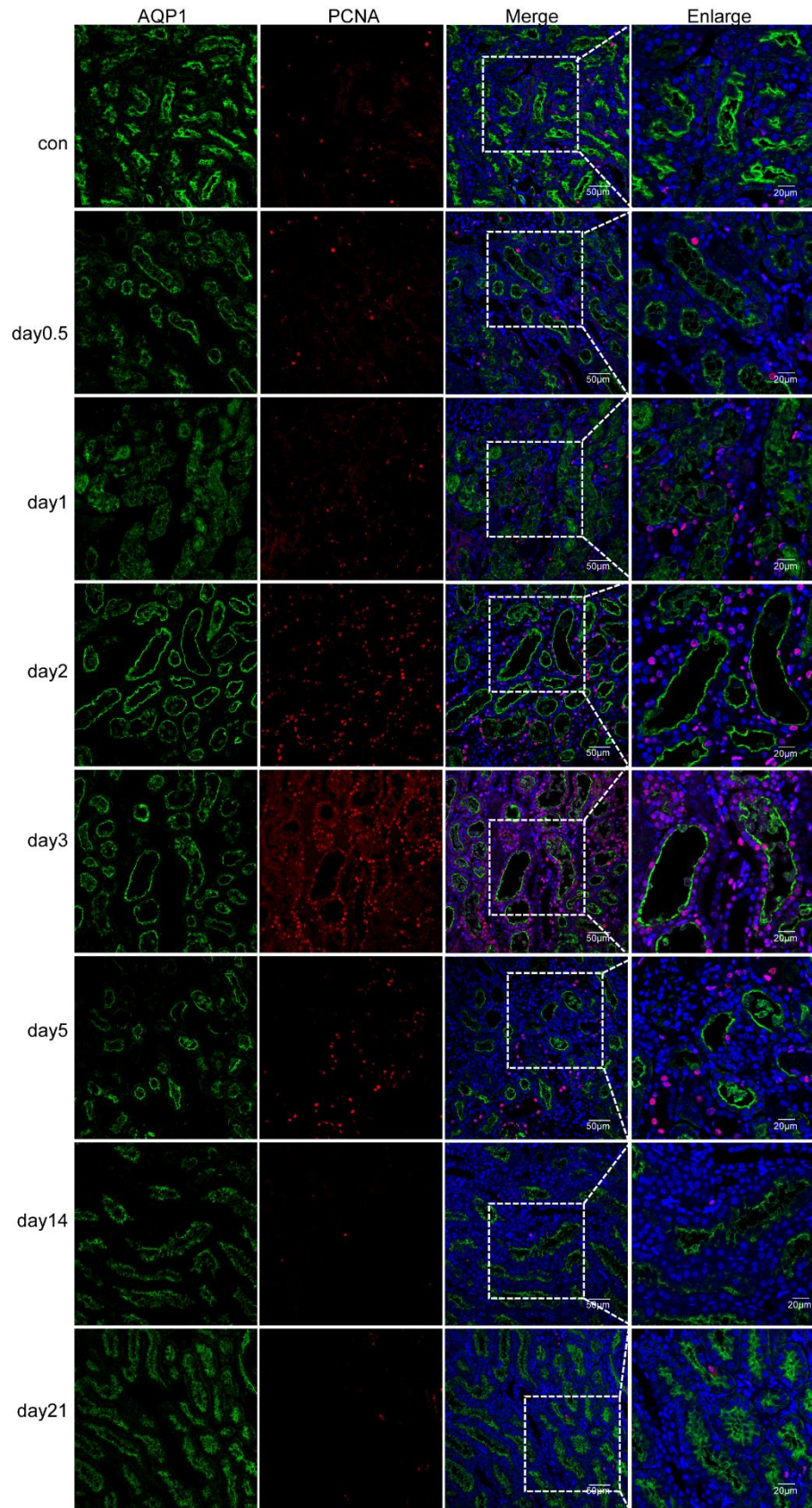
d Expression changes of *Aqp2* RNA (a marker for CD) at different timepoints (Con, day1, day3, day7, day21) post IRI.



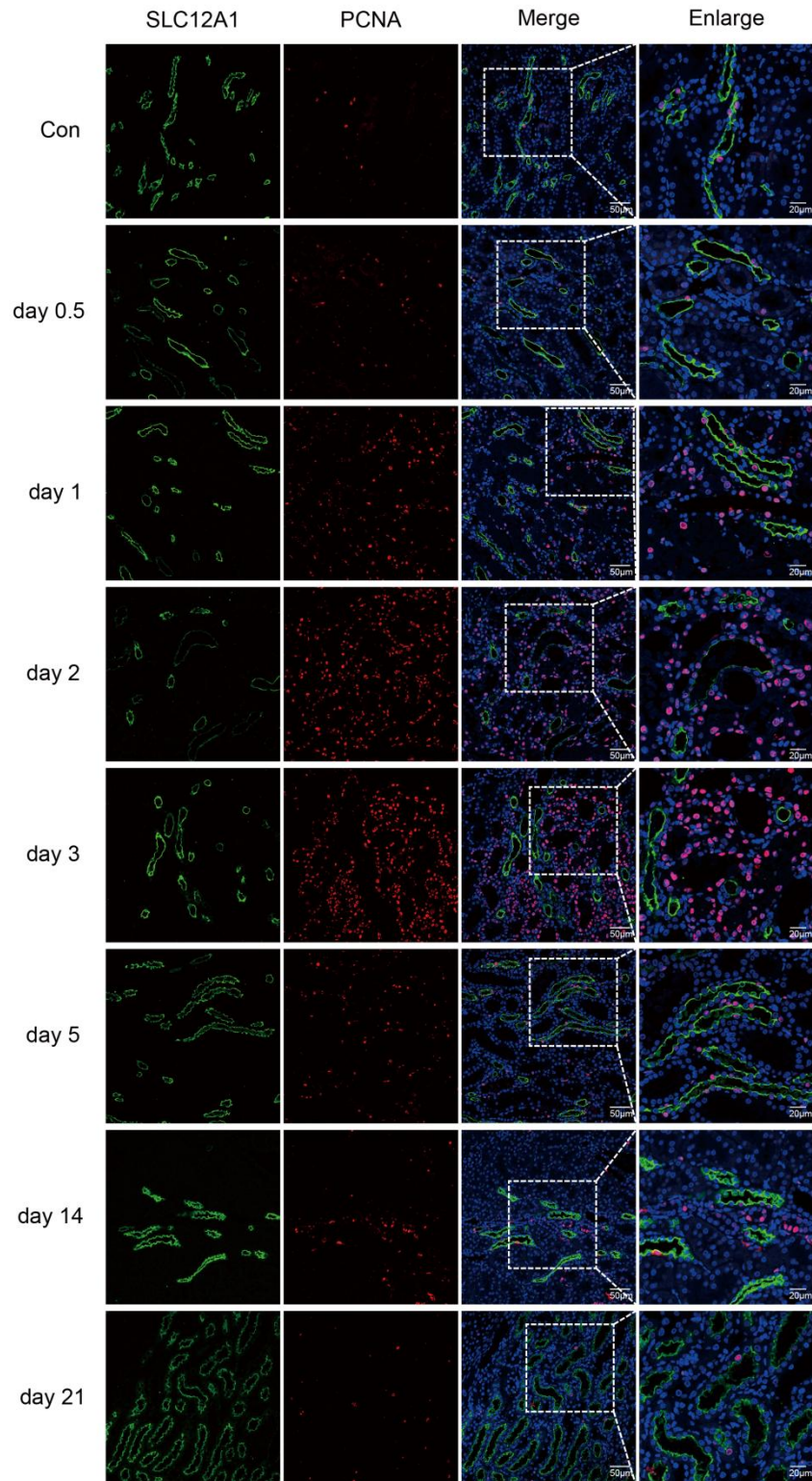
Supplementary Figure 8 | snRNA-seq - Stereo-seq integration reveals the changes of each major kidney cell type.

a Proportion Curve illustrating the changes of each major kidney cell types proportional composition post IRI.

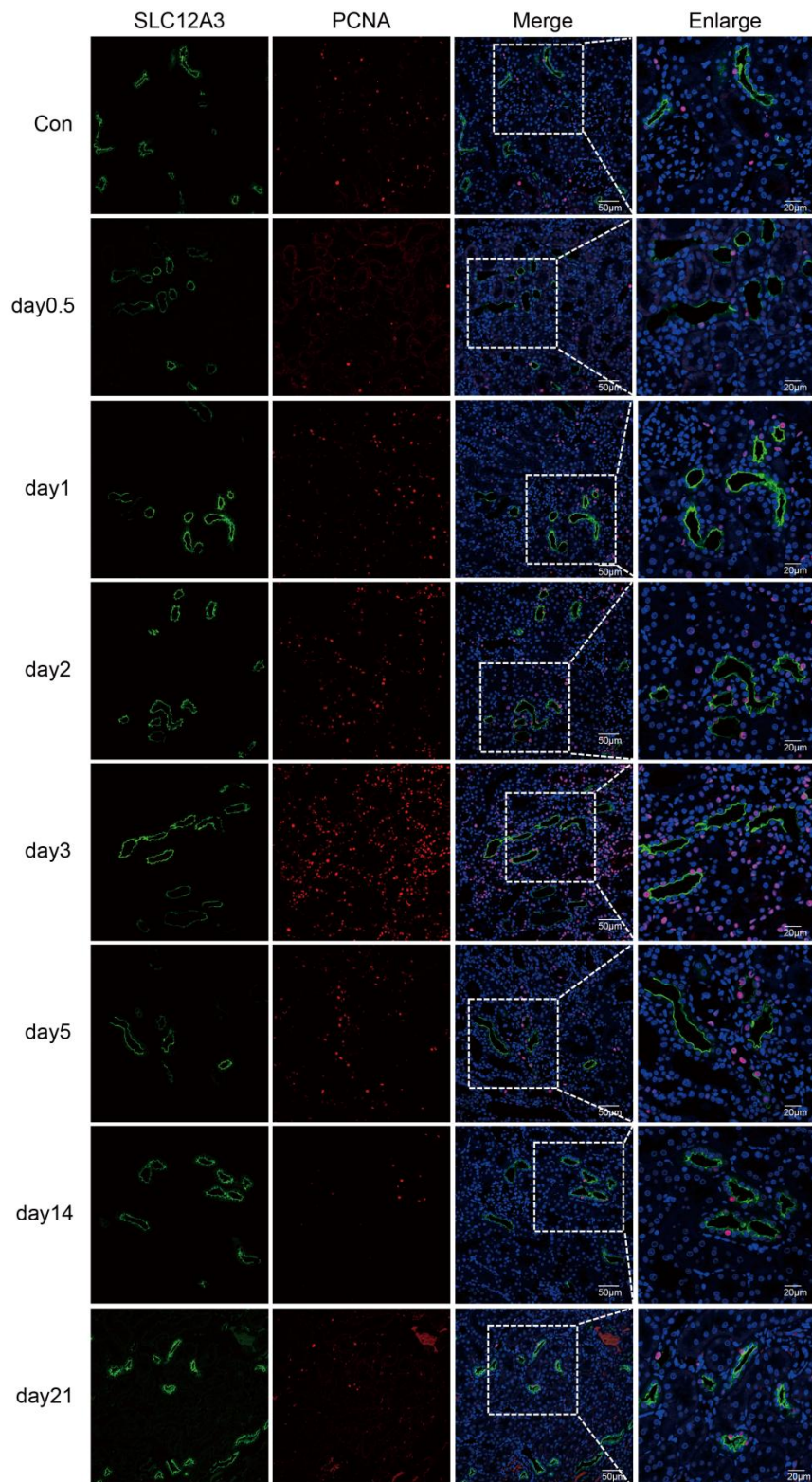
b UMAP visualizations depicting temporal dynamics of distinct kidney cell types at various time points post IRI.



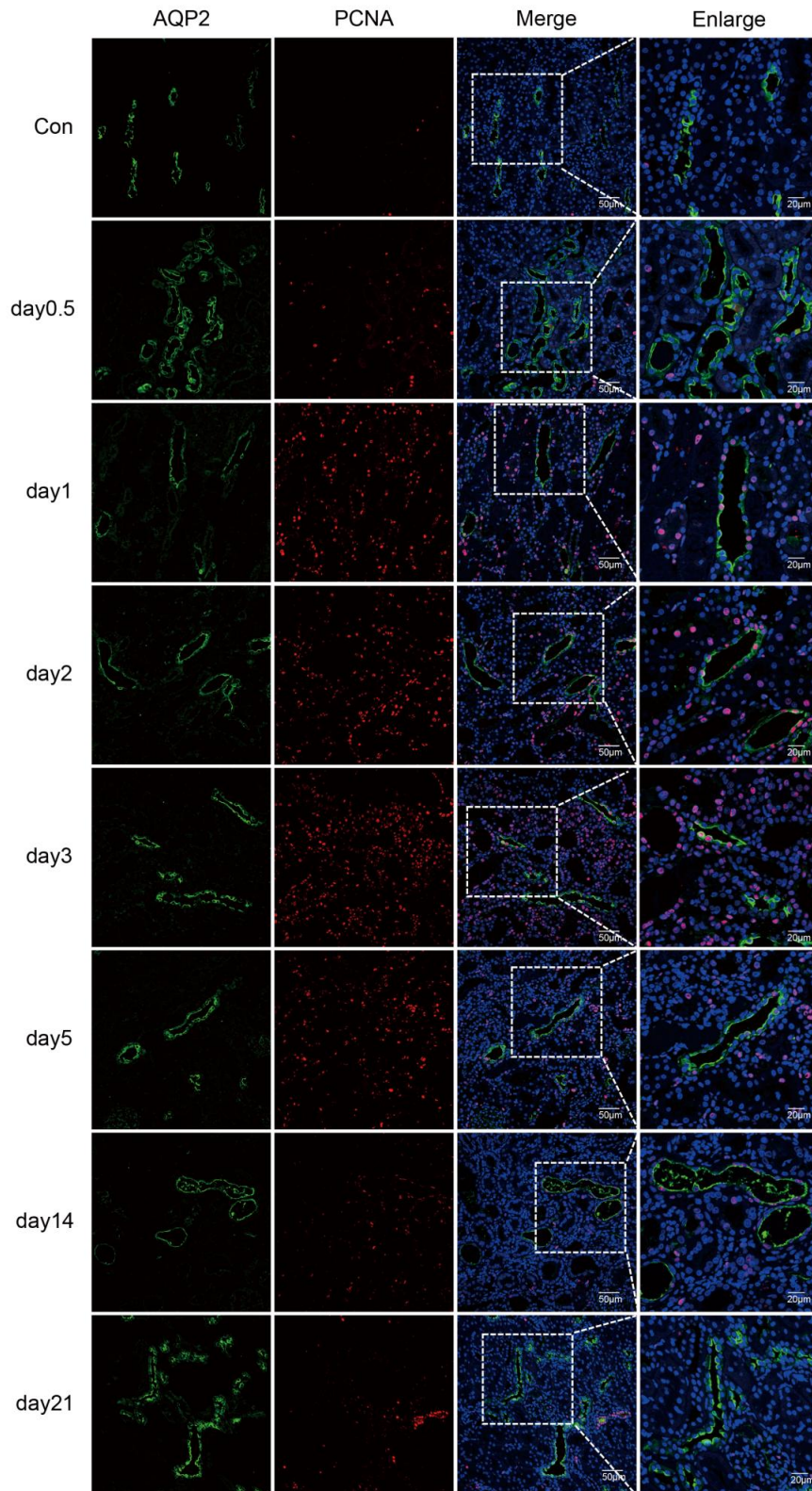
Supplementary Figure 9 |Immunofluorescence detection of co-localization between proximal tubule marker AQP1 and the cell proliferation marker PCNA.



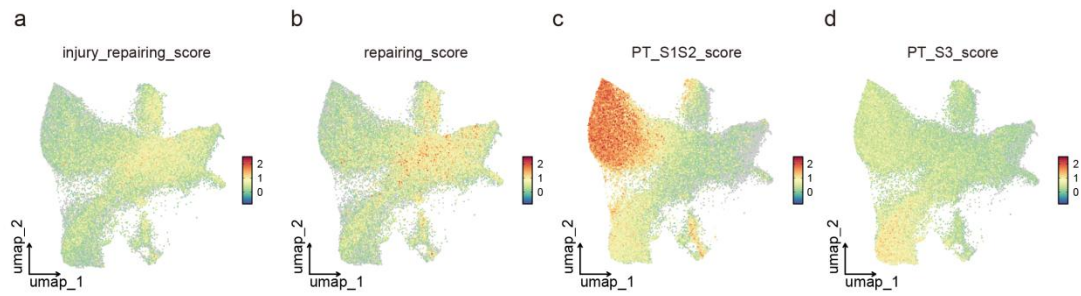
Supplementary Figure 10 |Immunofluorescence detection of co-localization between thick ascending limb (LOH) marker SLC12A1 and the cell proliferation marker PCNA.



Supplementary Figure 11 |Immunofluorescence detection of co-localization between Distal Convolved Tubule (DCT) marker SLC12A3 and the cell proliferation marker PCNA.



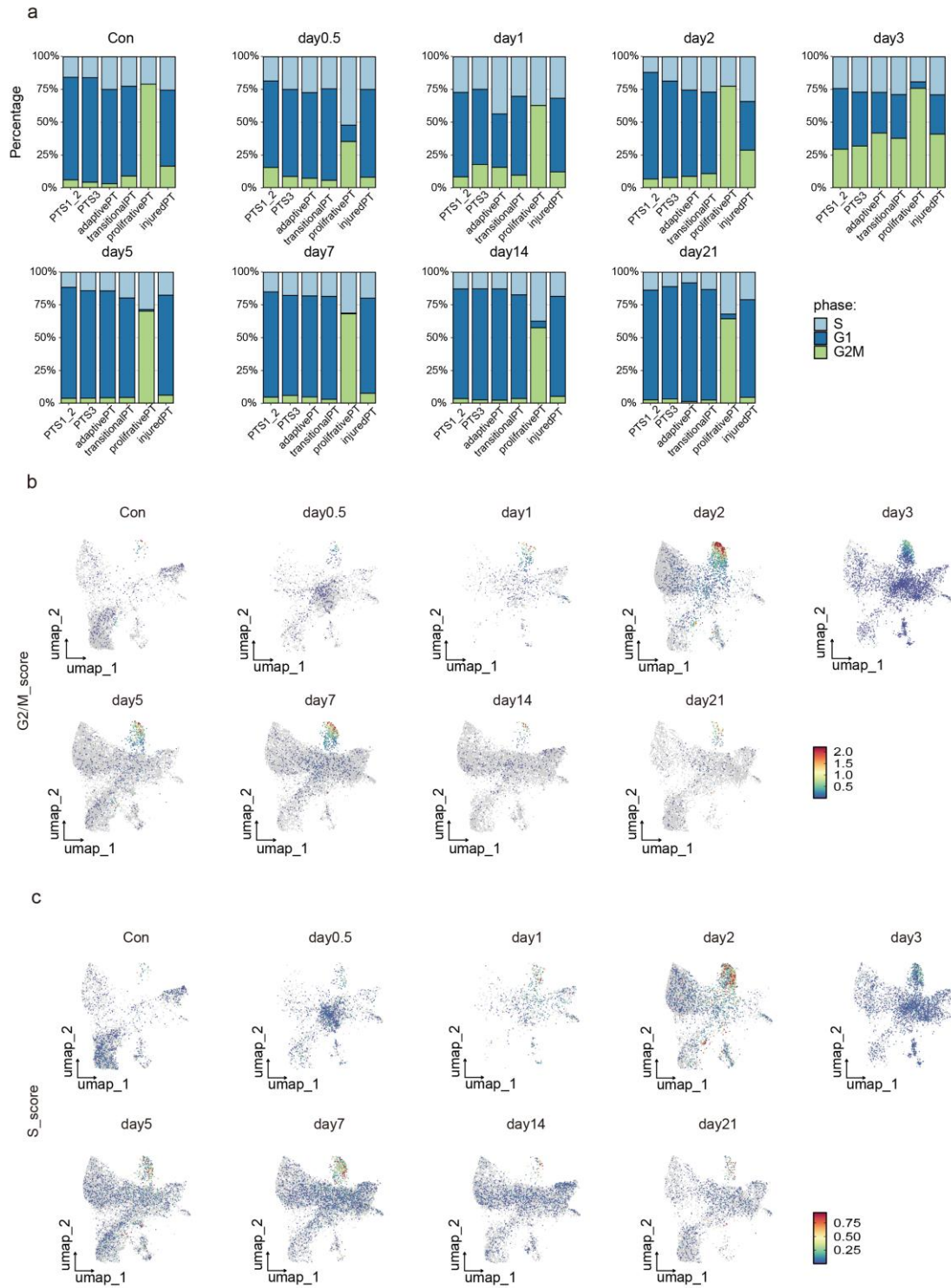
Supplementary Figure 12 | Immunofluorescence detection of co-localization between collecting duct cell marker AQP2 and the cell proliferation marker PCNA.



Supplementary Figure 13 | UMAP visualization illustrating injury-repairing, repairing, PT S1_S2 and PTS3 scores across six identified clusters.

UMAP plot showing an injury_repairing score, **b** repairing score, **c** PT_S1S2 score, and **d** PT_S3 score for six cell clusters.

Marker genes for injury_repairing_score: *Cd24*, *Cst3*, *Kim-1*, *Itga3*, *Krt20*, *Nupr1*, *Prom1* and *Vcam1*. Marker genes for repairing_score: *Cd24*, *Cst3*, *Kim-1*, *Itga3*, *Prom1*, *Vcam1*. Marker genes for PT_S1S2 Score: *Slc5a12*, *Slc13a3*, *Slc22a6*, *Prodh2*, *Slc5a2*, *Slc22a8*, *Slc34a1*, *Slc22a7*, *Lrp2*, *Cubn*. Marker genes for PT_S3 score: *Abcc3*, *Mogat1*, *Satb2*, *Slc22a24*, *Slc22a7*, *Slc5a11*, *Slc5a8*, *Slc7a13*.



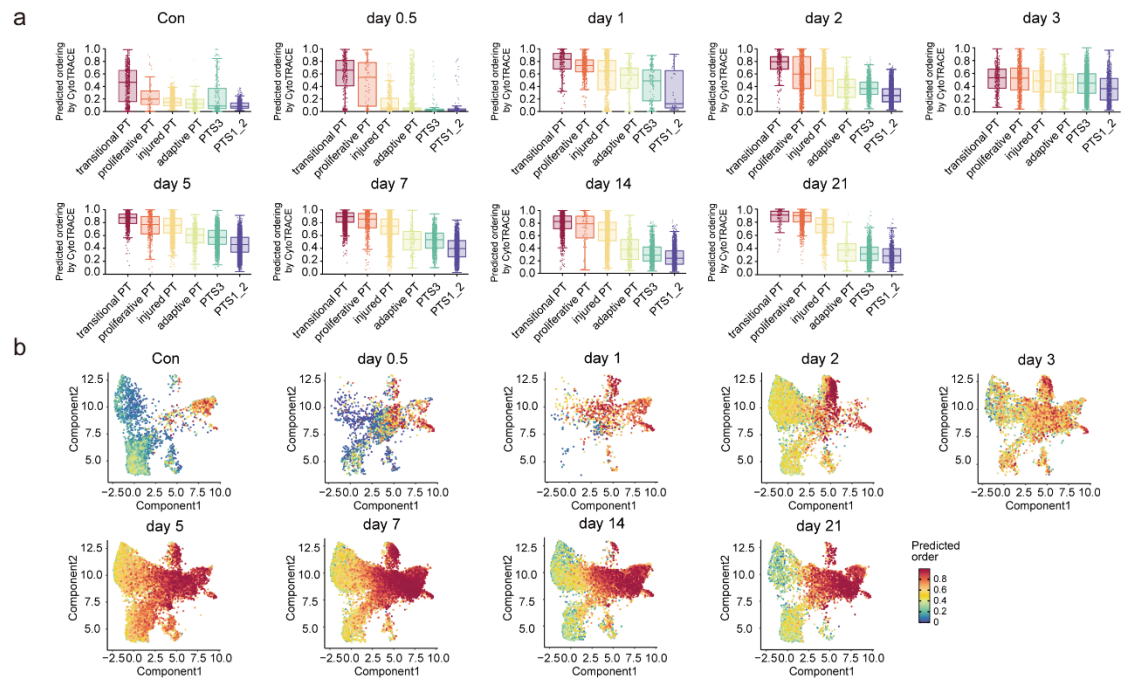
Supplementary Figure 14 | Cell cycle dynamics across distinct PT cell subtypes over different timepoints following IRI.

a Bar plots showing the distribution of cell cycle phases (G1, S, and G2/M) across distinct PT cell subtypes at different timepoints (Con, day0.5, 1, 2, 3, 5, 7, 14, 21) post IRI.

b UMAP plots showing the G2/M score across distinct PT cell subtypes at different

timepoints (Con, day0.5, 1, 2, 3, 5, 7, 14, 21) post IRI.

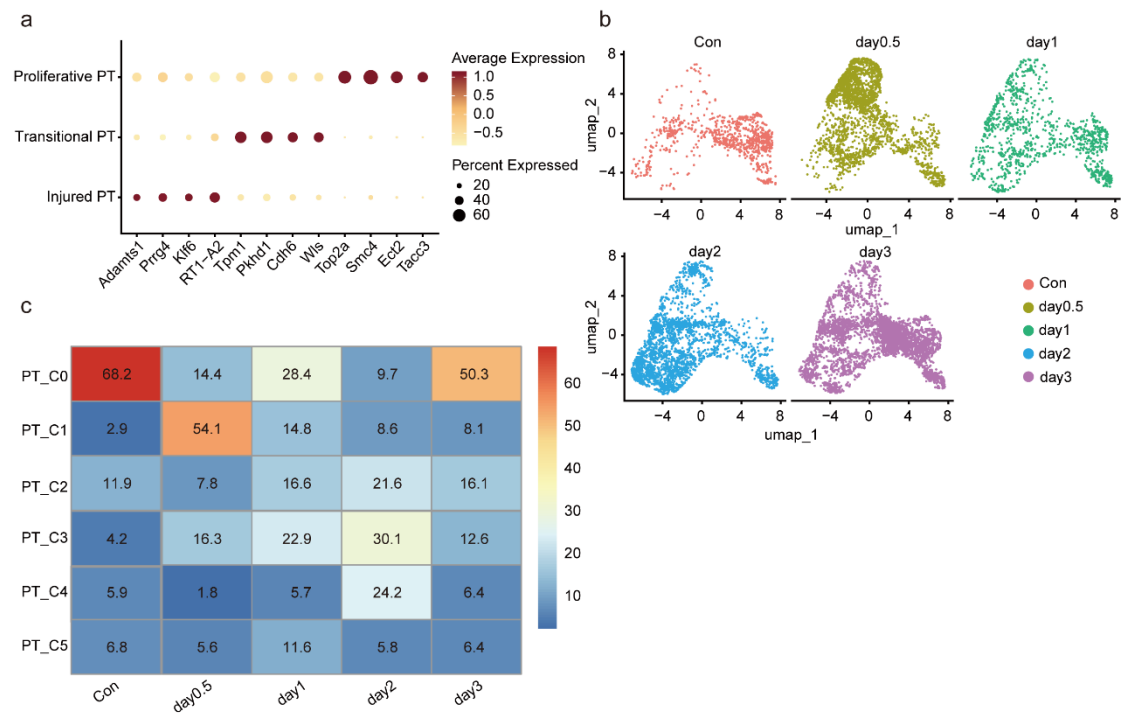
c UMAP plots showing the S score across distinct PT cell subtypes at different timepoints (Con, day0.5, 1, 2, 3, 5, 7, 14, 21) post IRI.



Supplementary Figure 15 | Differentiation status of renal tubule cell clusters at distinct timepoints post IRI.

a Violin plots showing the CytoTRACE scores of different PT subtypes. (Con: n=621, 57, 3056, 219, 218, 622; day 0.5: n=329, 88, 438, 109, 2186, 161; day 1: n=382, 150, 28, 64, 532, 95; day 2: 276, 1197, 815, 320, 1059, 3968; day 3: n=1034, 652, 342, 326, 2212, 805; day 5: n=876, 402, 2373, 497, 2621, 2964; day 7: n=2794, 511, 1021, 164, 3640, 2329; day 14: n=2116, 80, 1028, 332, 2513, 2291). In box plots, the center line denotes the median (50th percentile), box bounds indicate the 25th (Q1) and 75th (Q3) percentiles, and whiskers extend to the minimum and maximum values within $1.5 \times$ IQR (Q3–Q1).

b UMAP plot colored by CytoTRACE score, which infers cellular differentiation states based on transcriptional entropy. Cells with higher scores (red) are predicted to be less differentiated, while lower scores (blue) indicate more differentiated states.

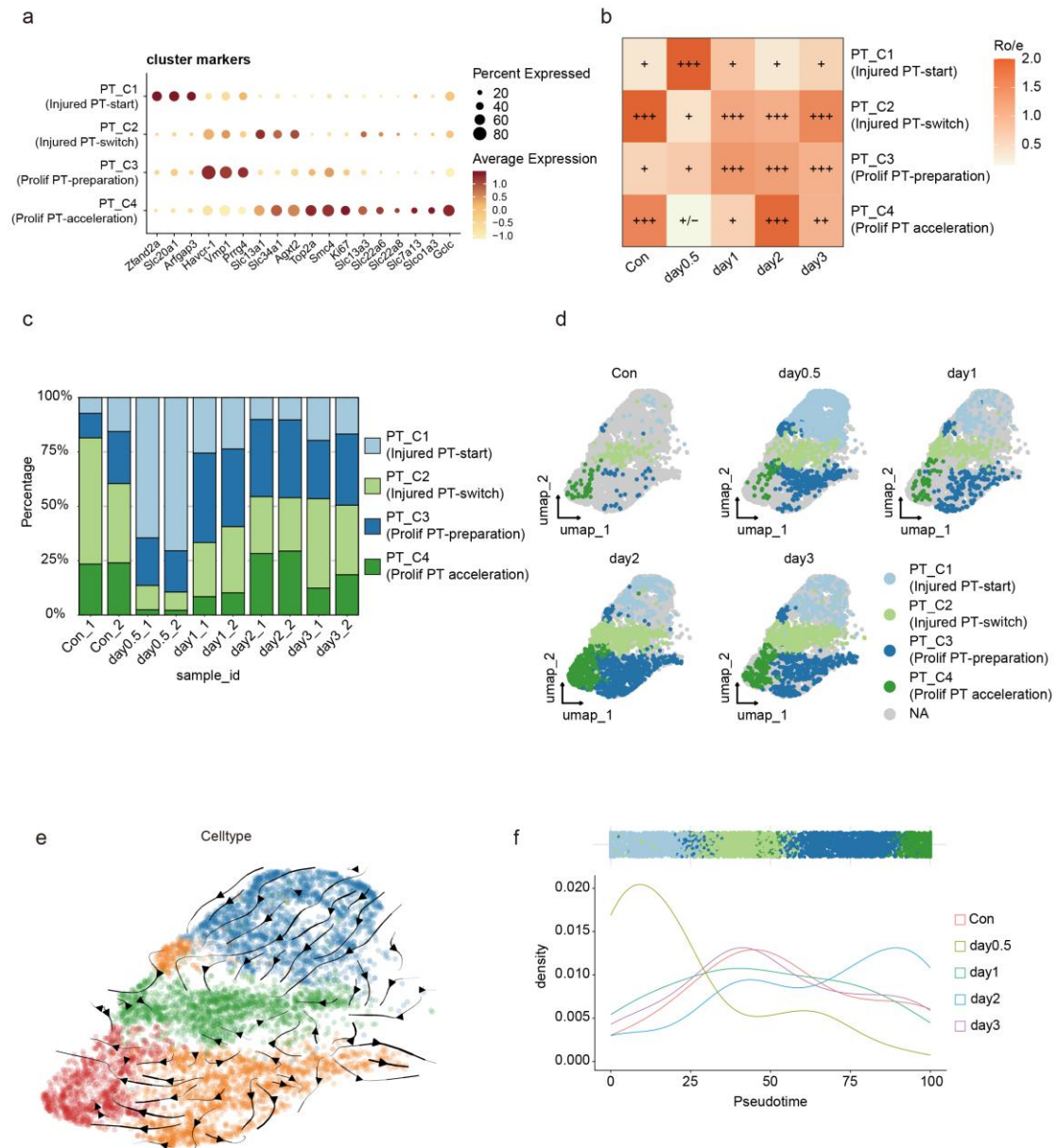


Supplementary Figure 16 | Clustering characteristics of PT subclusters.

a Bubble chart showing the expression of representative marker genes across 3 PT subclusters.

b UMAP plots showing re-clustering of PT cells across all timepoints post IRI.

c Compositional concordance between each timepoint (Con, day0.5, day1, day2, day3) of PT subtype states. Bars indicating relative abundance (%) of major cell populations.



Supplementary Figure 17 | Characterization of four distinct PT cell clusters.

a Bubble chart showing the expression of representative marker genes across 4 PT subclusters.

b Heatmap showing relative levels of Ro/e at different timepoints (Con, day0.5, day1, day2, day3) post IRI. Ro/e: First, create a contingency table for celltype and condition, where rows indicate whether cells belong to the target cell type, and columns indicate the conditions, then perform a chi-square test of independence on the contingency table using R's `chisq.test` function, which automatically calculates frequencies of actual values and theoretical expected values, for each condition in the target cell type, compute the ratio of observed to expected frequencies. "+++" value>1. "++" value>0.8

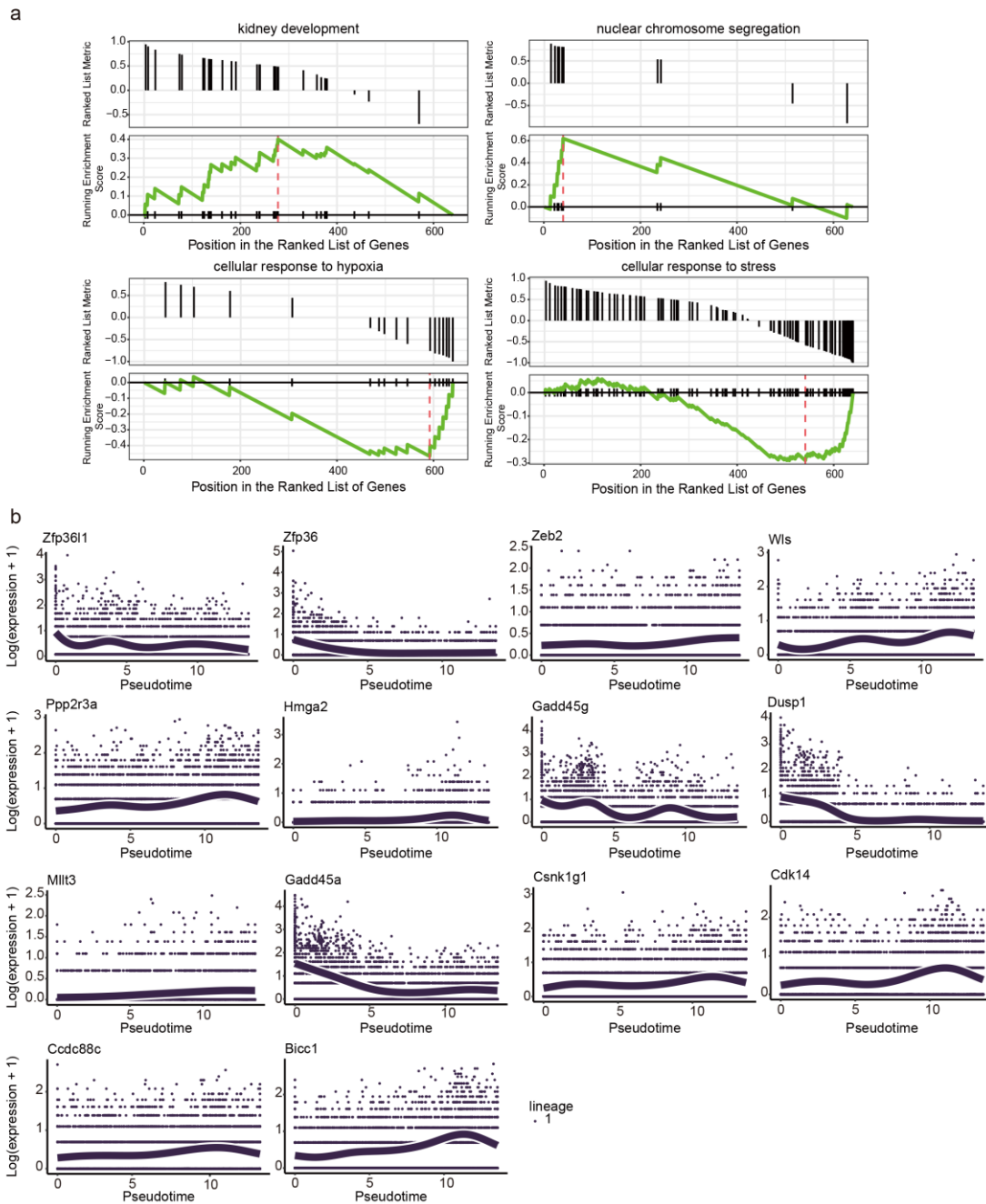
& value<1. "+" value>=0.2 & value<=0.8. "+/-" value>0 & value<0.2. "-" value==0.

c Stacked bar plot depicting the changes in the proportion of each PT subclusters across different time points following IRI.

d UMAP plot showing the distribution of four PT subclusters across distinct time phases following IRI.

e Cell type dynamic trajectory plots.

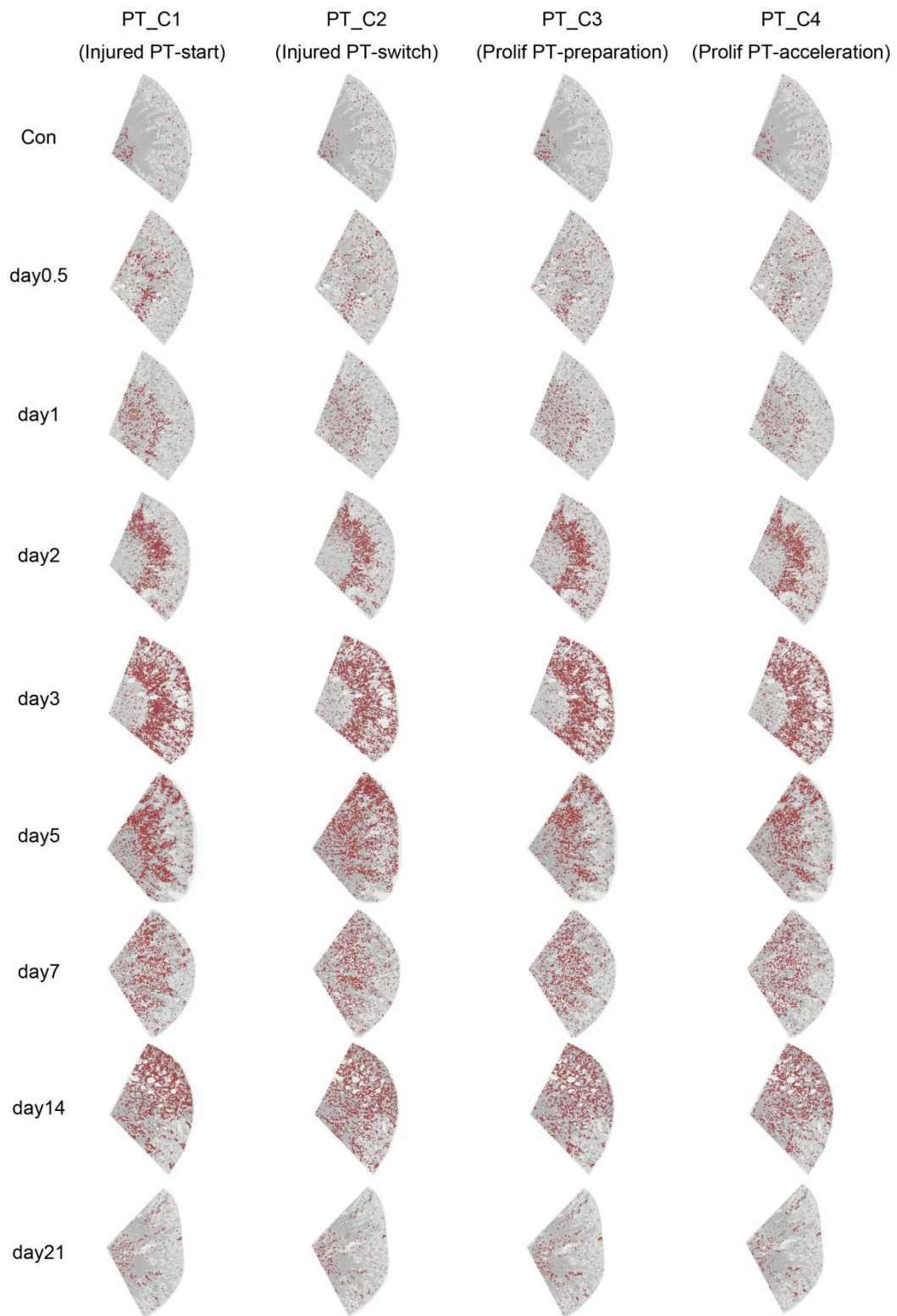
f Density plot showing distribution of cells from different timepoints (Con, day0.5, day1, day2, day3) along pseudotime, illustrating dynamic transitions during injury and repair.



Supplementary Figure 18 | Differentially expressed genes and enriched pathways among PT processes.

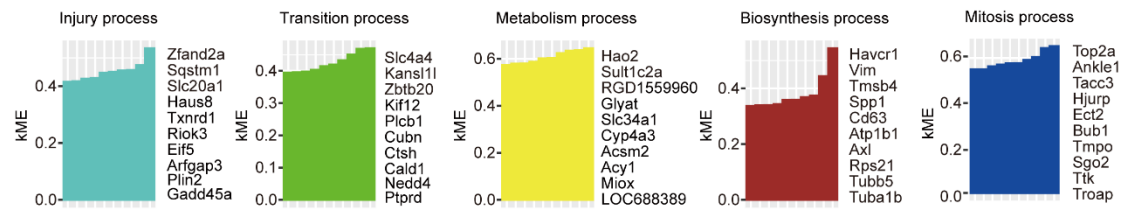
a Gene Set Enrichment Analysis (GSEA) indicating significant enrichment of the kidney development, nuclear chromosome segregation pathway, cellular response to hypoxia pathway and cellular response to stress pathway among pseudotime-associated gene programs.

b Scatter plots showing the expression levels of early (e.g., *Gadd45a*, *Zfp36*) and late (e.g., *Zeb2*, *Hmga2*) expression patterns gene over pseudotime.



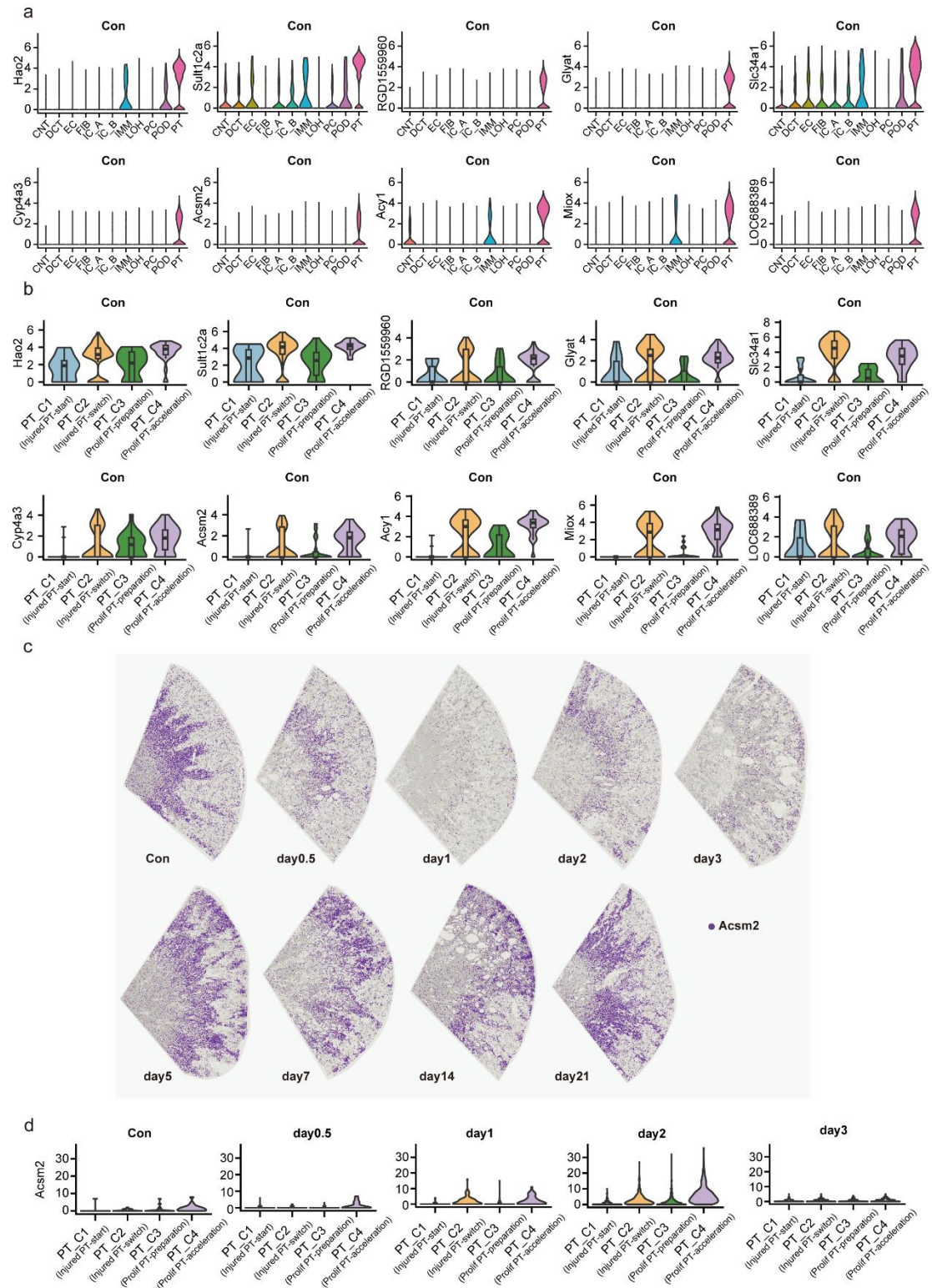
Supplementary Figure 19 | Spatial transcriptomic mapping of different PT states.

Spatial transcriptomic mapping of different PT states at different timepoints (Con, day0.5, day1, day2, day3, day5, day7, day14, day21) post IRI.



Supplementary Figure 20 | 10 top hub genes of each process.

Bar plots displaying top hub genes ranked by module eigengene-based connectivity (kME) for 5 PT states.



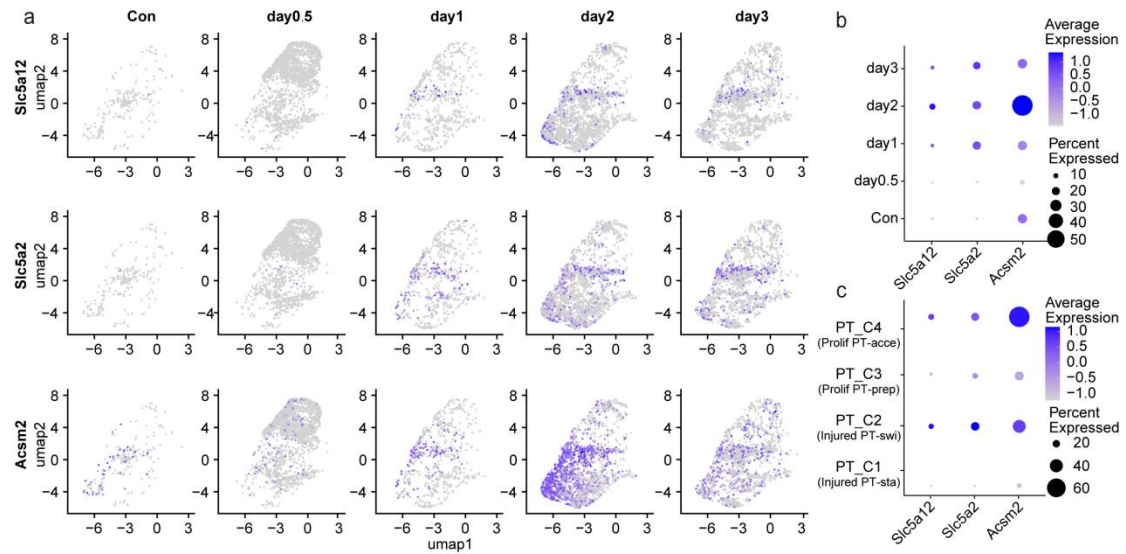
Supplementary Figure 21 | Expression of 10 top hub genes from Metabolism process.

a Violin plots depicting the expression levels of 10 top hub genes from Metabolism process across identified cell types in the kidney.

b Violin plots depicting the expression levels of 10 top hub genes from Metabolism process in Control group across PT_C1 to PT_C4 clusters.

c Highlighted spatial expression patterns of *Acsm2* at control and IRI timepoints (Con, day0.5, 1, 2, 3, 5, 7, 14, and 21).

d Violin plots depicting the expression levels of *Acsm2* across control and day0.5 to day3 post IRI in PT_C1 to PT_C4 cluster.

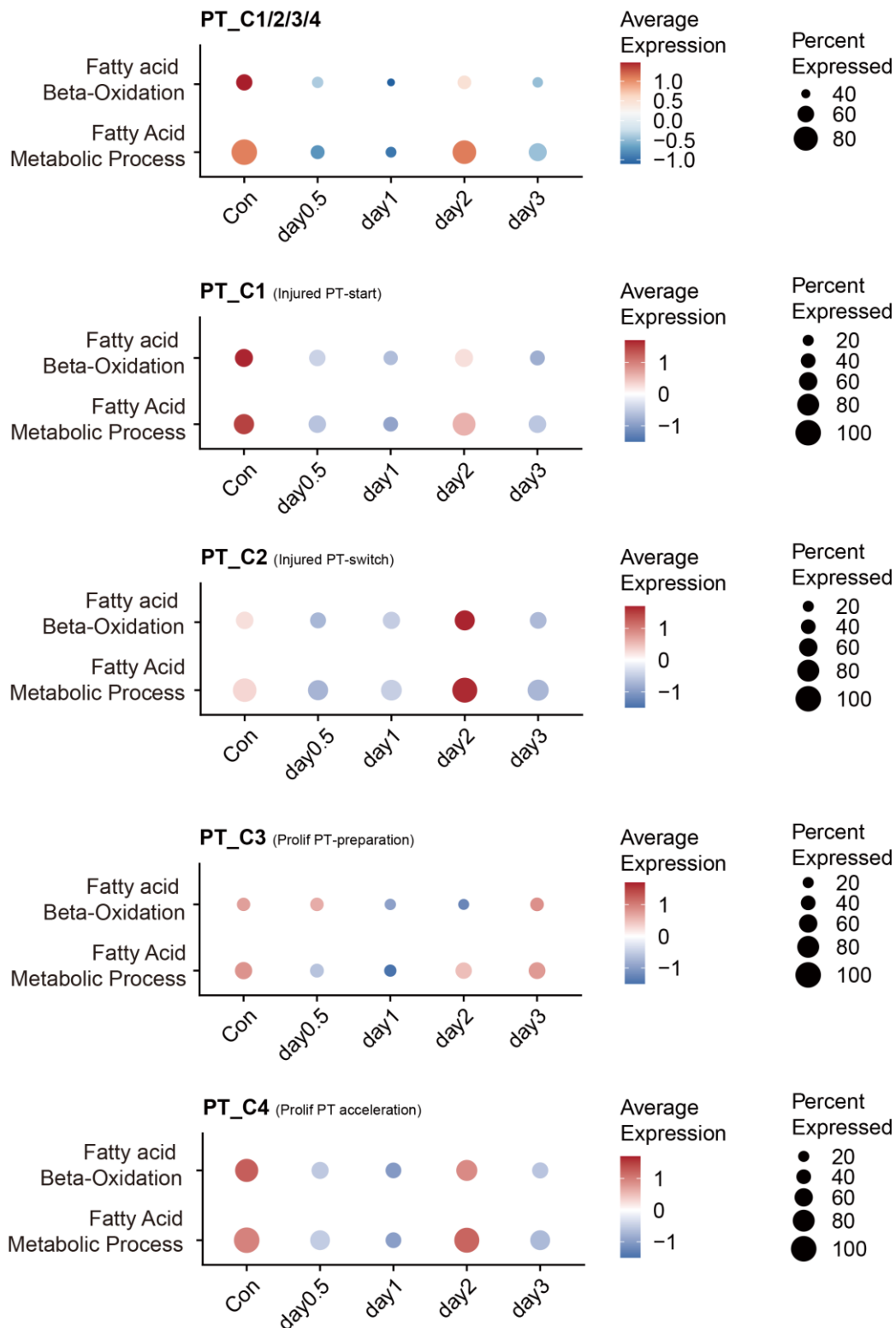


Supplementary Figure 22 | Expression of metabolism related genes in PT_C1 to PT_C4 across control and day0.5 to day3 after IRI.

a UMAP plots showing the expression of *Slc5a12*, *Slc5a2* and *Acsm2* and its distribution among different cell clusters across con and day0.5 to day3.

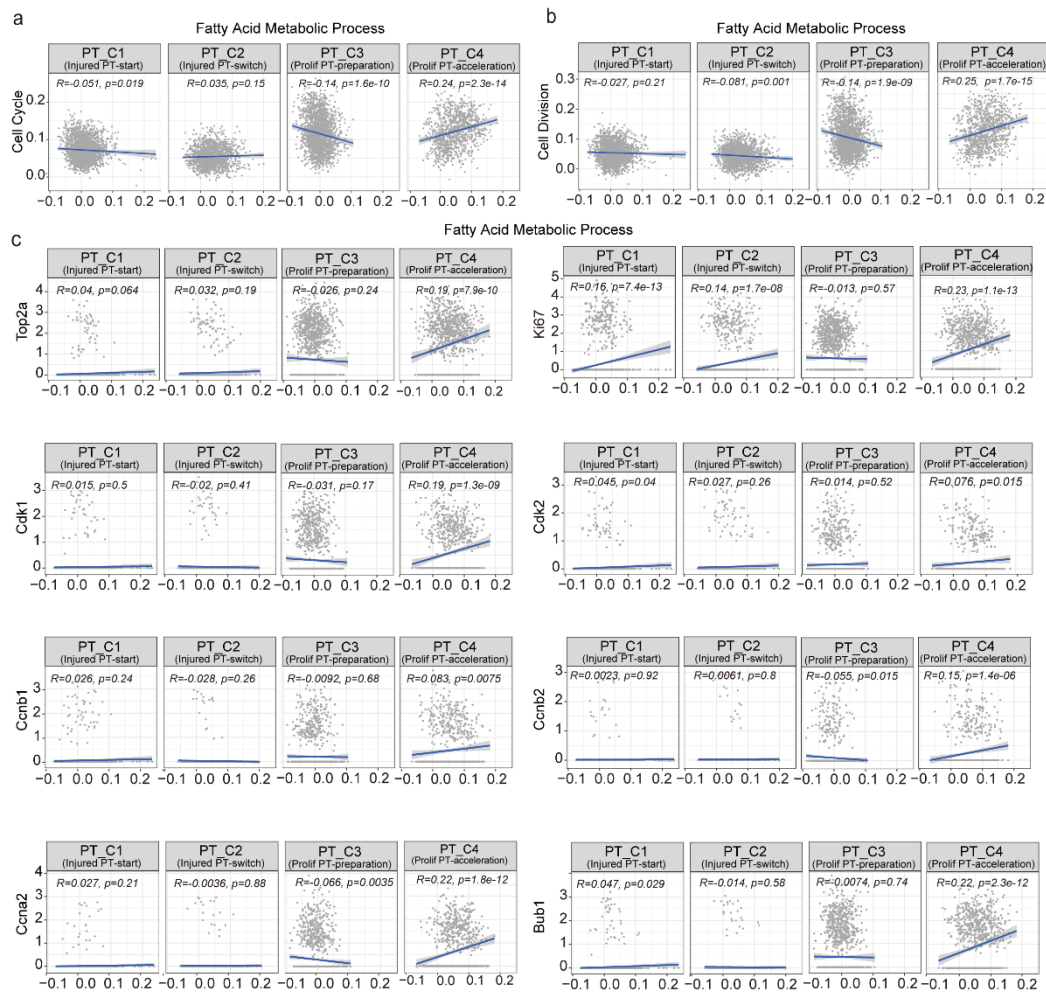
b Dot plot showing the expression levels of *Slc5a12*, *Slc5a2*, and *Acsm2* across Con and day 0.5 to day 3 post IRI.

c Dot plot showing the expression levels of *Slc5a12*, *Slc5a2*, and *Acsm2* across PT_C1 to PT_C4 post IRI.



Supplementary Figure 23 | Expression of fatty acid metabolism associated pathways in PT_C1 to PT_C4 across control and day0.5 to day3 after IRI.

Dot plots displaying the expression levels of pathways associated with fatty acid metabolism across control and day0.5 to day3 after IRI in PT_C4 cluster.

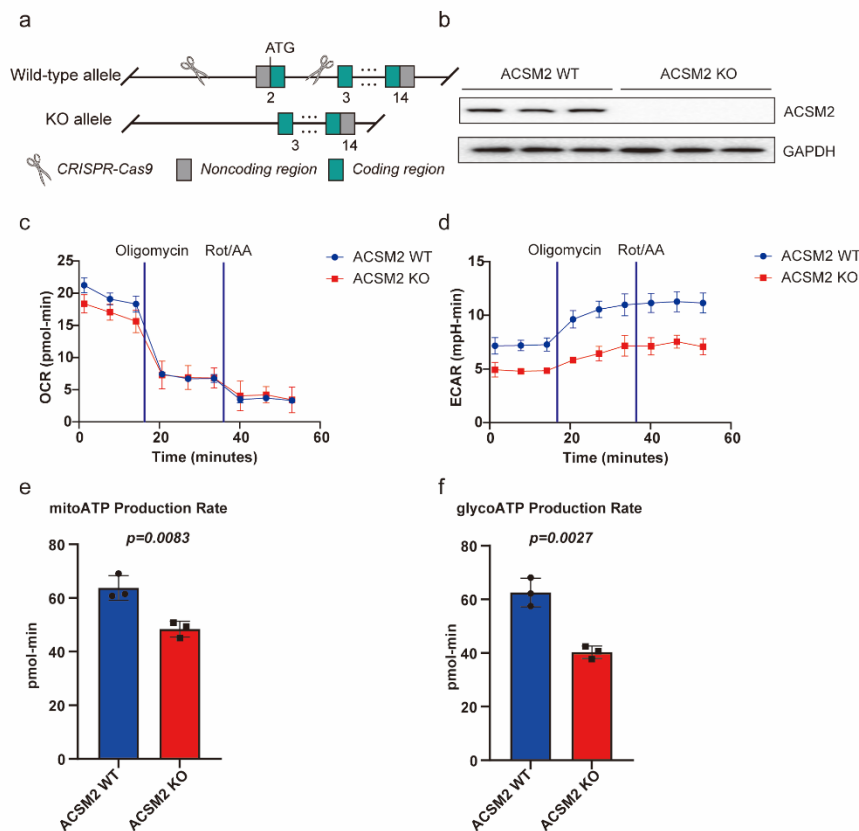


Supplementary Figure 24 | correlation between the fatty acid metabolic process pathway and cell cycle as well as regulatory related genes across PT_C1 to PT_C4 clusters.

a-b Scatter Plots showing the correlation between Cell Cycle pathway and Cell Division pathway across four distinct PT_C1 to PT_C4 clusters. (Cell cycle: PT_C1: 99% CI: -0.106-0.005; PT_C2: 99% CI: -0.027-0.099; PT_C3: 99% CI: -0.2- -0.086; PT_C4: 99%CI: 0.159-0.311; Cell Division: PT_C1: 99% CI: -0.083-0.029; PT_C2: 99% CI: -0.143- -0.018; PT_C3: 99% CI: -0.192- -0.078; PT_C4: 99% CI: 0.168-0.319;) The p values were determined using two-side t-test.

c Scatter plots showing the correlation between the fatty acid metabolic process pathway and cell cycle regulatory related genes (*Top2a*, *Ki67*, *Cdk1*, *Cdk2*, *Ccnb1*, *Ccnb2*, *Ccna2*, *Bub1*) across PT_C1 to PT_C4 clusters. (Top2a: PT_C1: 99%CI: -0.016-0.096; PT_C2: 99%CI: -0.031-0.095; PT_C3: 99%CI: -0.084-0.032; PT_C4:

99%CI: 0.112-0.267; ki67: PT_C1: 99% CI: 0.1-0.209; PT_C2: 99% CI: 0.076-0.2,; PT_C3: 99% CI: -0.071-0.045; PT_C4: 99% CI: 0.152-0.304; Cdk1: PT_C1: 99% CI: -0.041-0.071; PT_C2: 99% CI: -0.084-0.043; PT_C3: 99% CI: -0.089-0.027; PT_C4: 99% CI: 0.109-0.265; Cdk2: PT_C1: 99% CI: -0.011-0.1; PT_C2: 99% CI: -0.036-0.091; PT_C3: 99% CI: -0.044-0.073; PT_C4: 99% CI: -0.004-0.156; Ccnb1: PT_C1: 99% CI: -0.03-0.082; PT_C2: 99% CI: -0.091-0.036; PT_C3: 99% CI: -0.067-0.049; PT_C4: 99% CI: 0.003-0.163; Ccnb2: 99% CI: -0.054-0.058; PT_C2: 99% CI: -0.057-0.069; PT_C3: 99% CI: -0.113-0.003; PT_C4: 99% CI: 0.071-0.228; Ccna2: PT_C1: 99% CI: -0.029-0.083; PT_C2: 99% CI: -0.067-0.06; PT_C3: 99% CI: -0.124- -0.008; PT_C4: 99% CI: -0.14-0.293; Bub1: PT_C1: 99% CI: -0.009-0.103; PT_C2: 99% CI: -0.077-0.05; PT_C3: 99% CI: -0.066-0.051; PT_C4: 99% CI: 0.139-0.292). The p values were determined using two-side t-test.



Supplementary Figure 25 | The Impact of *Acsm2* Knockout on Mitochondrial Function and ATP Production Rates

a Schematic of *Acsm2* knockout mouse generation via CRISPR-Cas9.

b Western blot confirming the successful construction of *Acsm2* KO mice.

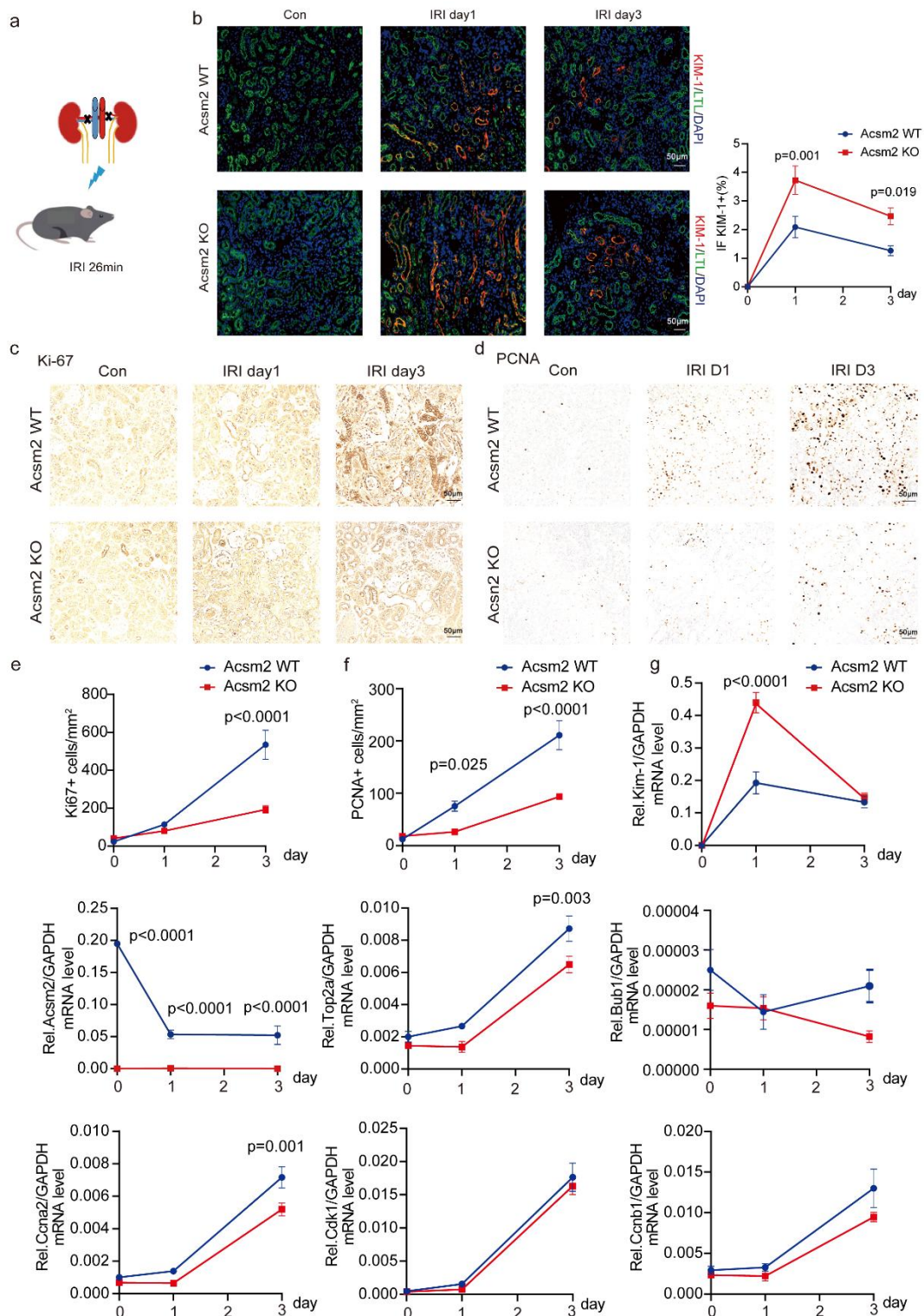
c Oxygen consumption rate (OCR) over time in *Acsm2* wild-type (WT, blue) and *Acsm2* knockout (KO, red) primary renal tubule epithelial cells. Oligomycin and Rotenone/Antimycin A (Rot/AA) were added at indicated time points to inhibit mitochondrial ATP synthase and complex I/III respectively, showing the impact on OCR dynamics.

d Extracellular acidification rate (ECAR) over time in *Acsm2* WT (blue) and *Acsm2* KO (red) primary renal tubule epithelial cells.

e The bar graph showing Mitochondrial ATP (mitoATP) production rate in *Acsm2* WT and KO primary renal tubule epithelial cells.

f The bar graph showing GlycoATP production rate comparison between *Acsm2* WT and KO groups in *Acsm2* WT and KO primary renal tubule epithelial cells.

Data were expressed as the mean $\pm$ SEM ($n=3$). The p values were determined using two-side t-test. Source data are provided as a Source Data file.



Supplementary Figure 26 | *Acsm2* KO delays the recovery of mouse kidneys after IRI.

a Schematic overview of the 26-minute IRI procedure in mice.

b Immunofluorescence staining of Kim-1 with LTL and DAPI in kidney sections of WT

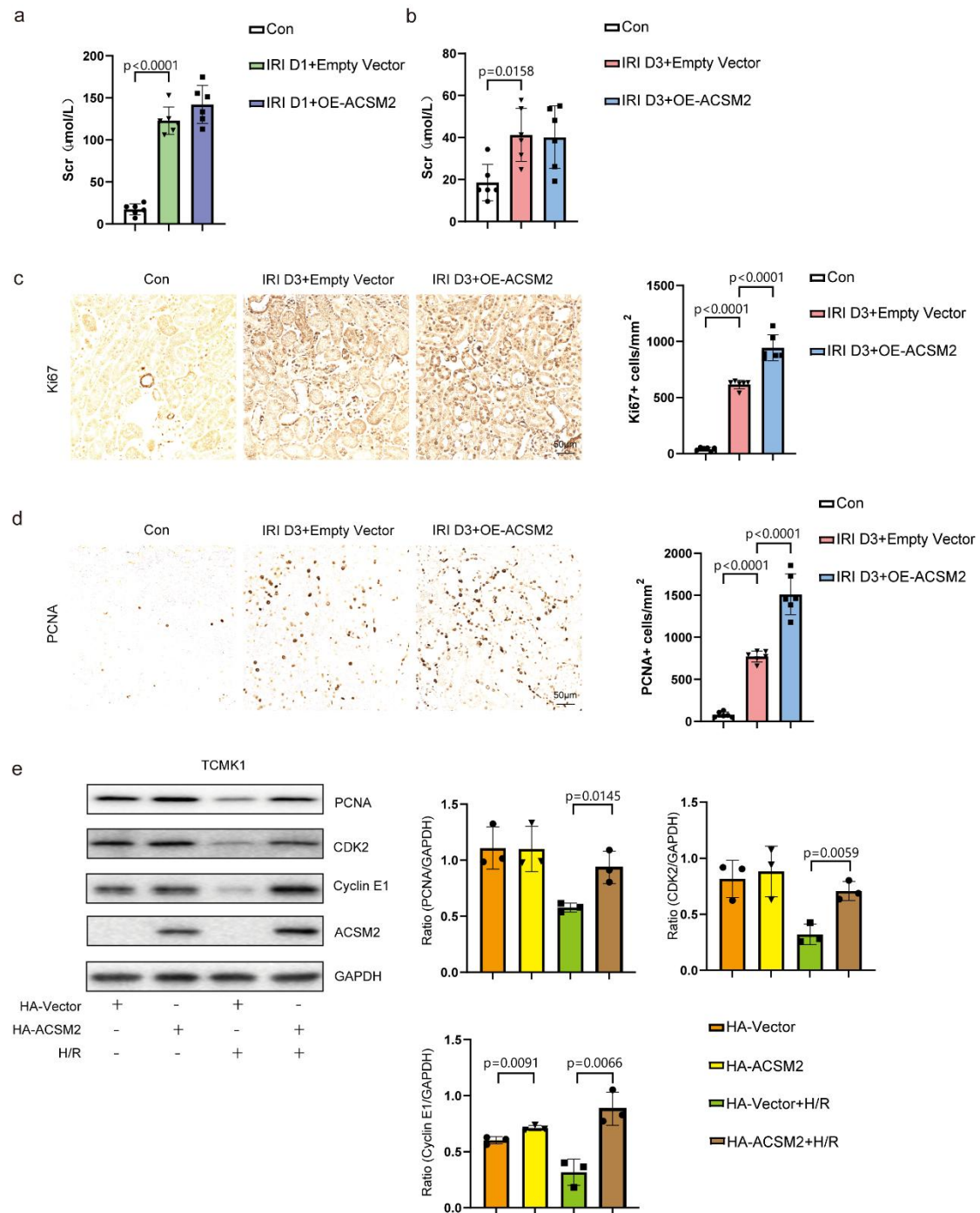
and *Acsm2* KO mice at day1 and day3 post-IRI.

c,e IHC staining of Ki-67 and the count of Ki67-positive cells.

d,f IHC staining of PCNA and the count of PCNA-positive cells.

g Line graph showing relative transcriptional levels of *Kim-1*, *Acsm2*, *Top2a*, *Bub1*, *Ccna2*, *Cdk1* and *Ccnb1* normalized to Gapdh in WT and *Acsm2* KO mouse kidneys post-IRI.

Data were expressed as the mean $\pm$ SEM (n=6). The p values were determined using a one-way ANOVA with two-side Tukey's test. Source data are provided as a Source Data file.



Supplementary Figure 27 | *Acsm2* overexpression promotes kidney repair after IRI.

a-b Bar chart depicting Serum creatinine (Scr) levels of Control, 1 or 3 days after 26-minute IRI procedure with empty vector or OE-Acsm2 plasmid intervention mice (n=6)

c IHC staining of Ki67 and the count of Ki67-positive cells. (n=6)

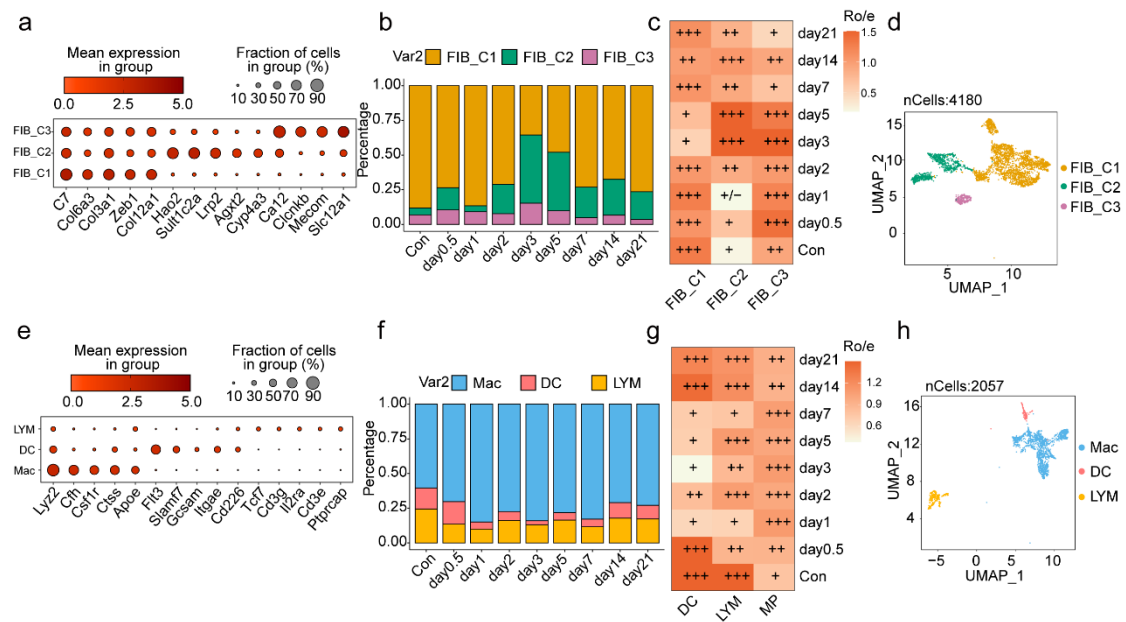
d IHC staining of PCNA and the count of PCNA-positive cells. (n=6)

e Western blot showing expression levels of PCNA, CDK2, Cyclin E1 and ACSM2 in

mouse renal epithelial cell-TCMK1 with hypoxia/reoxygenation procedure with or without ACSM2 overexpression (GAPDH as loading control). (n=3)

The p values were determined using a one-way ANOVA with two-side Tukey's test.

Source data are provided as a Source Data file.



Supplementary Figure 28 | Fibroblast and immune cell dynamics in renal tissue after IRI.

a Dot plots of conserved marker genes for identified three FIB cell types across snRNA-seq datasets. Dot size: gene fraction; color: mean expression.

b Stacked bar plots showing temporal distribution of FIB subtypes across control and IRI groups (day0.5 to day21).

c Heatmap showing relative levels of Ro/e in FIB clusters at different timepoints (Con and day0.5 to day21) post IRI.

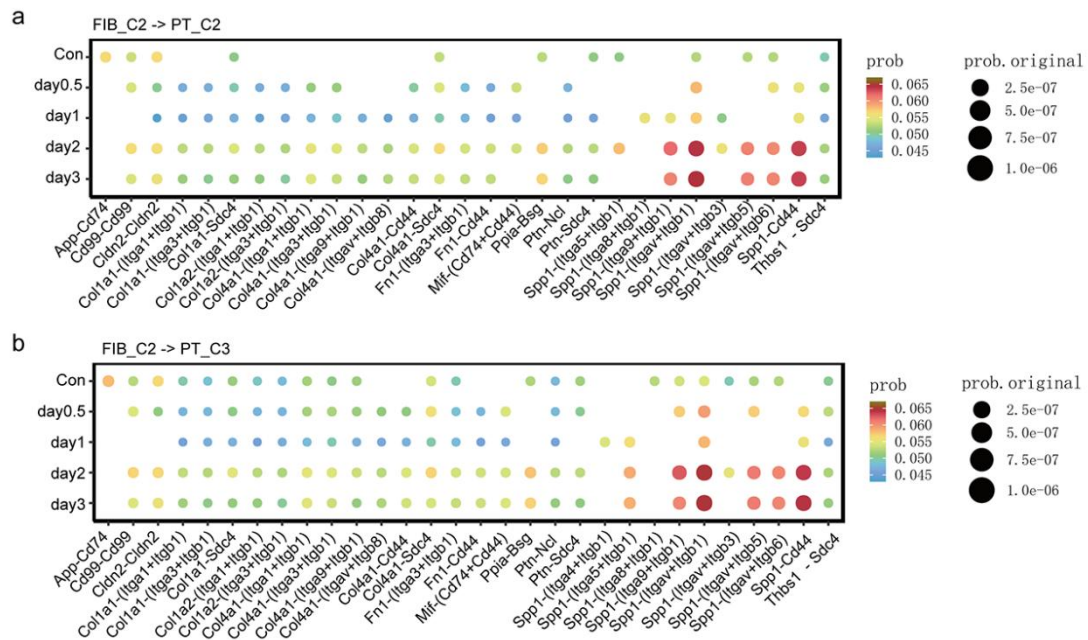
d UMAP visualization of snRNA-seq, annotated by FIB_C1, FIB_C2 and FIB_C3.

e Dot plots of conserved marker genes for identified lymphocyte (LYM), dendritic cell (DC) and Macrophage (MAC) across snRNA-seq datasets. Dot size: gene fraction; color: mean expression.

f Stacked bar plots showing temporal distribution of LYM, DC and Mac across control and IRI groups (day0.5 to day21).

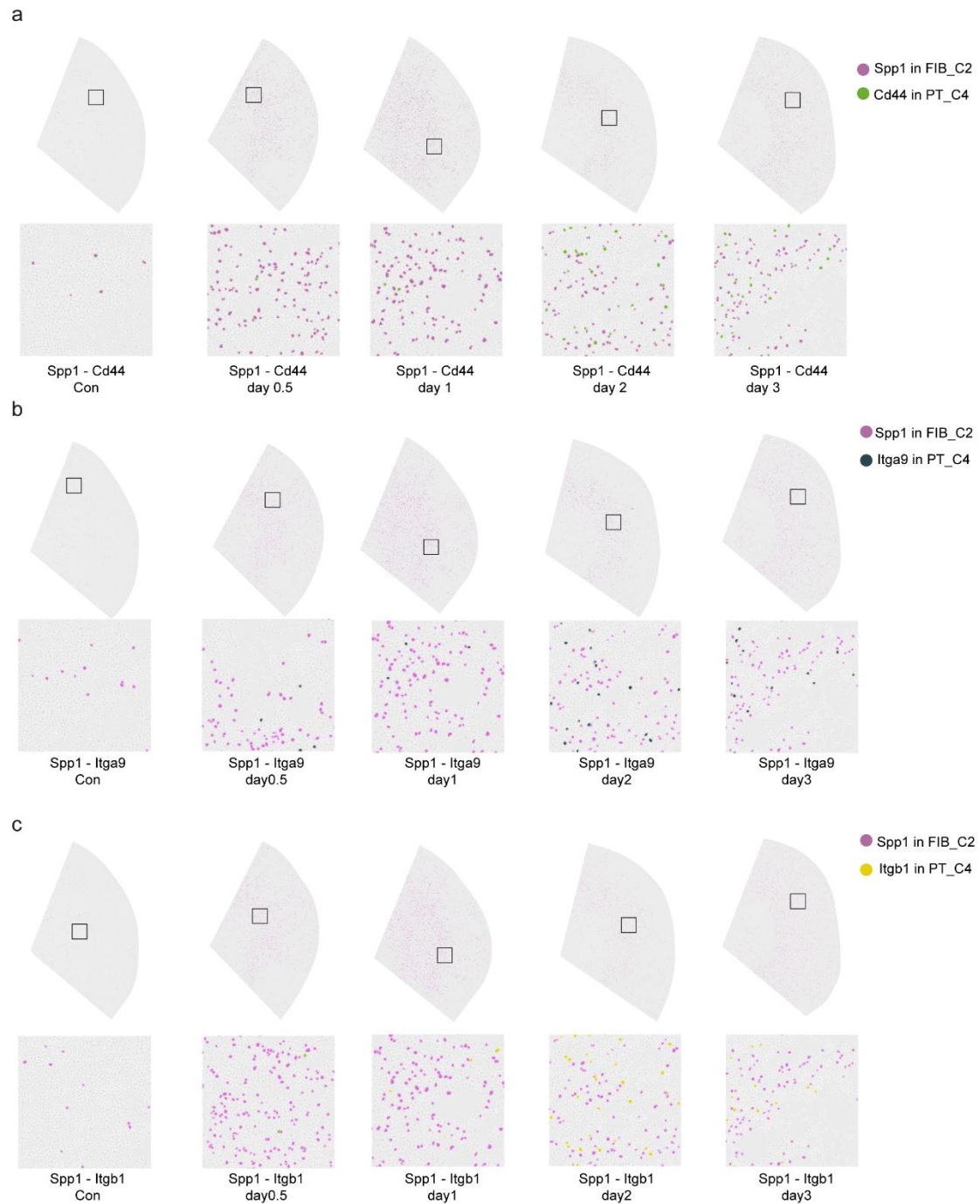
g Heatmap showing relative levels of Ro/e in LYM, DC and Mac clusters at different timepoints (Con and day0.5 to day21) post IRI.

h UMAP visualization of snRNA-seq, annotated by LYM, DC and Mac.



Supplementary Figure 29 | cell-cell communication related pathway between FIB_C2 and PT cells.

a-b Bubble chart to explore the interaction situations between FIB_C2 and PT C2 or PT_C3 by showing the approximately 30 ligand-receptor pairs with the highest prob values at different time points (con, day0.5, day1, day2, day3) post-IRI.



Supplementary Figure 30 | Spatial distribution pattern of Spp1 in FIB_C2 and its ligand in PT_C4 (Cd44, Itga9 and Itgb1).

a-c Spatial context of Spp1 in FIB_C2 and Cd44/Itga9/ Itgb1 in PT_C4 at different time points (con, day0.5, day1, day2, day3) post-IRI.

Supplementary Table 1

Subclass (Full Name)	Annotate	Subclass Level 1	Positive Markers
Proximal Tubule Epithelial Cell	PT	PT	Slc34a1, Slc22a2, Aqp1, Lrp2
Proximal Tubule Epithelial Cell Segment 1	PT-S1	PT	Lrp2, Slc5a12, Slc5a2
Proximal Tubule Epithelial Cell Segment 2	PT-S2	PT	Lrp2, Cyp4b1, Cyp2e1,
Proximal Tubule Epithelial Cell Segment 3	PT-S3	PT	Napsa, Ghr, Cyp7b1
Loop of Henle	LOH	LOH	Umod, Slc12a1, Egf
Distal Convoluted Tubule	DCT	DCT	Slc8a1, Slc12a3, Wnk1, Calb1, Pgam2
Connecting tubule	CNT	CNT	Aqp3, Krt7
Intercalated Cell	IC	CD	Atp6v1g3, Atp6v0d2, Slc4a1, Slc26a4
Principal Cell	PC	CD	Aqp2, Scnn1b, Fxyd4, Hsd11b2
Immune cell	IMM	IMM	Ptpcr, C1qb, Ctss, Lyz2, Cst3
Podocyte	POD	POD	Ptpqr, Nphs1, Nphs2, Clic5, Podxl, Magi2
Endothelium cell	EC	EC	Egfl7, Flt1, Emcn, Ednrb
Fibroblast	FIB	FIB	Plac8, Colla1, Colla2, Cxcl12, Fst, Col3a1
Pericyte	Pericyte	Pericyte	Pdgfrb, Myh11, Rgs5, Tm4sf1