

Supplemental material for

Thick ascending limb injury critically impacts kidney allograft survival after T-cell-mediated rejection

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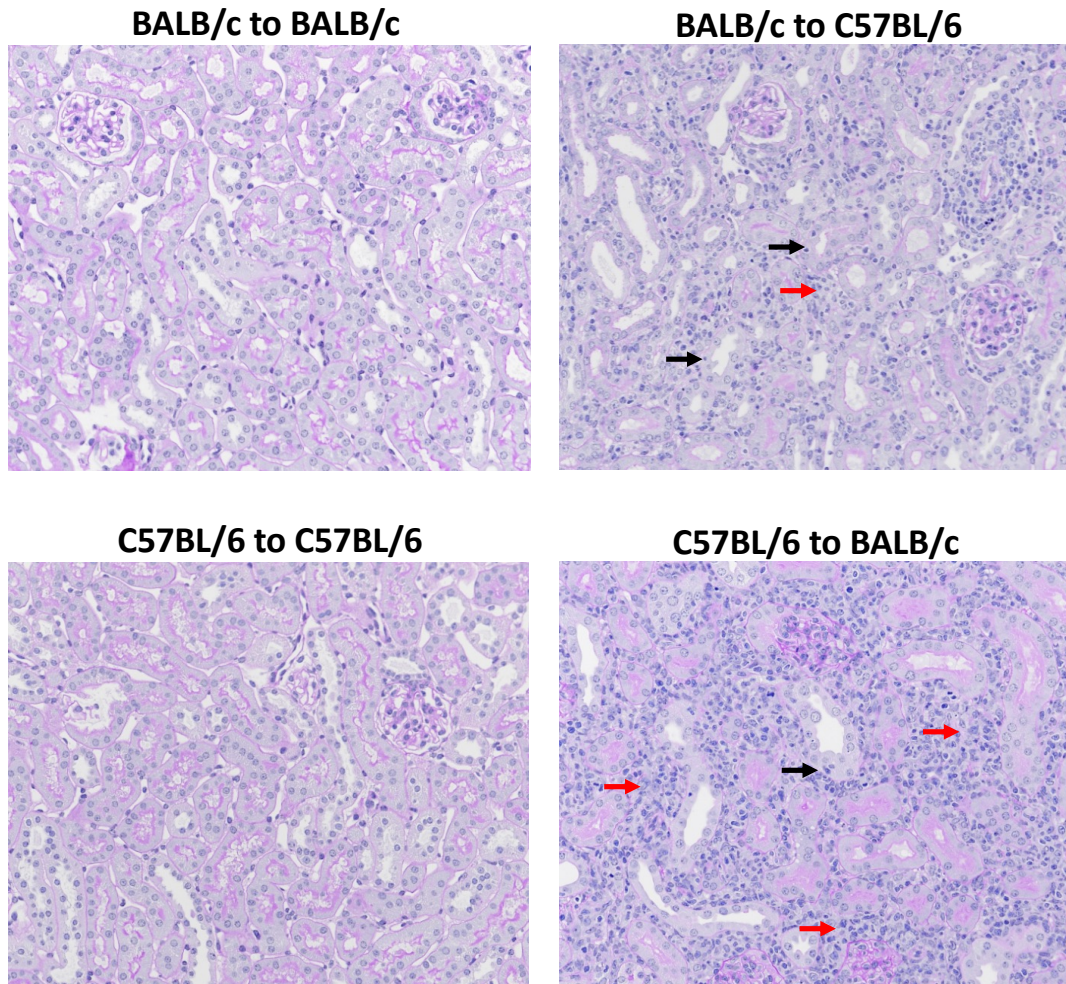
* shared first authors

shared senior and corresponding authors

A

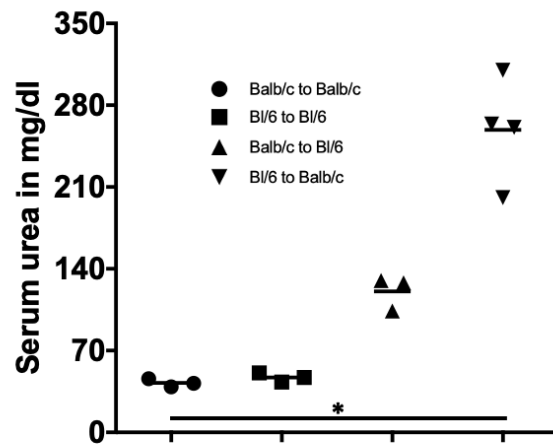
Group	Tx combination	Mouse IDs	Sample name in manuscript (if applicable)	Used for clinical analyses (creatinine etc.)	snRNA-seq	ST	Banff scoring		
							i	t	v
SYN	C57BL/6 - C57BL/6	M1	C57BL/6 to C57BL/6 1	x	x	x	0	0	0
SYN		M2		x			0	0	0
SYN		M3		x			0	0	0
SYN	Balb/c - Balb/c	M1	BALB/c to BALB/c 1	x	x	x	0	0	0
SYN		M2	BALB/c to BALB/c 2	x	x		0	0	0
SYN		M3		x			0	0	0
ALLO	Balb/c - C57BL/6	M1	BALB/c to C57BL/6 1	x	x	x	2	1	1
ALLO		M2		x			2	2	1
ALLO		M3	BALB/c to C57BL/6 3	x	x	x	3	1	1
ALLO	C57BL/6 - Balb/c	M1	C57BL/6 to BALB/c 1	x	x	x	3	1	1
ALLO		M2	C57BL/6 to BALB/c 2	x	x	x	3	3	1
ALLO		M3	C57BL/6 to BALB/c 3	x	x	x	3	2	1
ALLO		M4		x			3	2	1

B



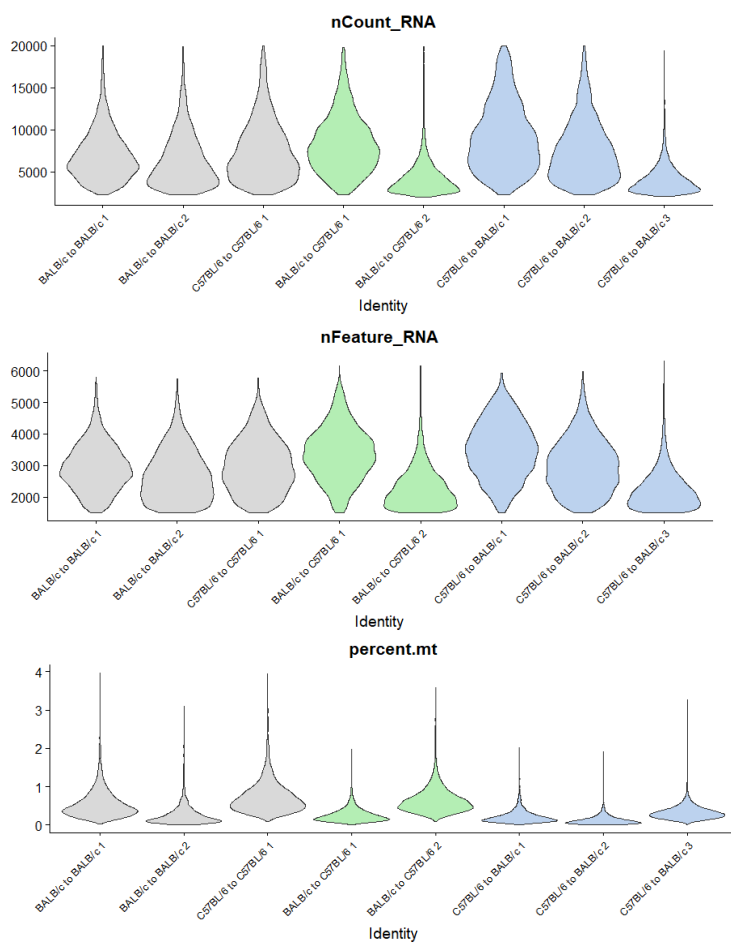
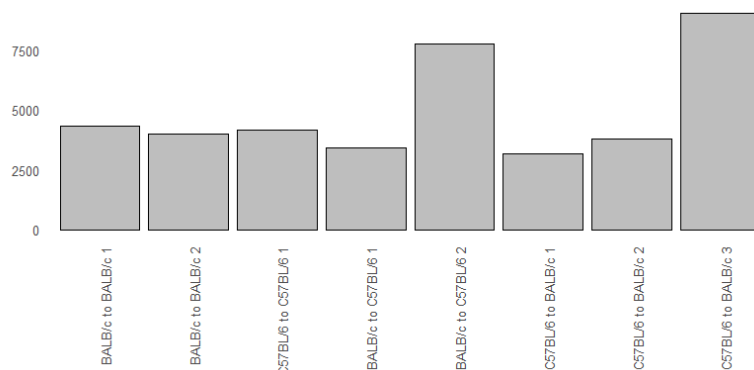
Supplemental Figure S1: Sample information and histology of mouse kidneys from syngeneic and allogeneic groups. A. Sample information for mouse kidney samples. **B.** Representative periodic acid-Schiff (PAS) stained images of the BALB/c and C57BL/6 kidney

isografts and the kidney allografts BALB/c to C57BL/6, C57BL/6 to BALB/c at post-operative day 7, showing interstitial inflammation (red arrows) and tubulitis (black arrow). Note that part of the C57BL/6 to BALB/c allograft is depicted in Fig. 1 in the main body.



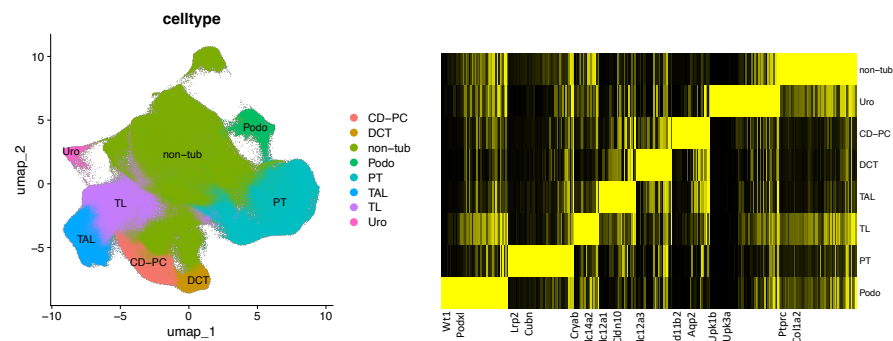
Supplemental Figure S2: Serum urea measurements from syngeneic and allogeneic mice.

P-value: * < 0.05.

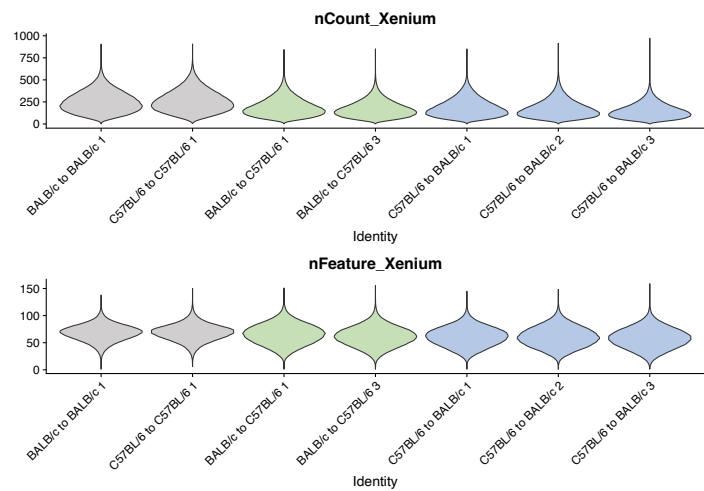
A**B**

Supplemental Figure S3: Quality metrics for mouse snRNA-seq data. A. Number of detected genes (nFeature_RNA), transcripts (nCount_RNA) and percentage of mitochondrial reads (percent.mt) per sample. **B.** Number of detected cells per sample.

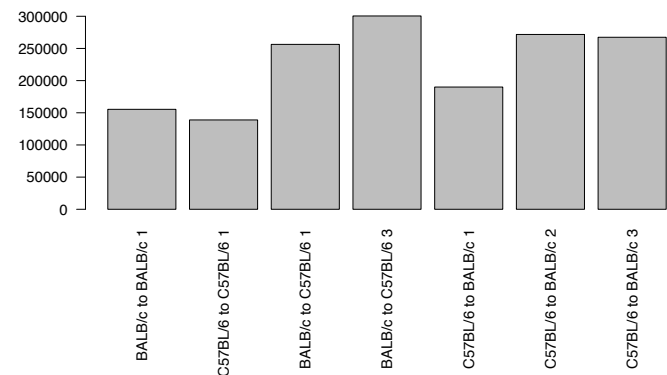
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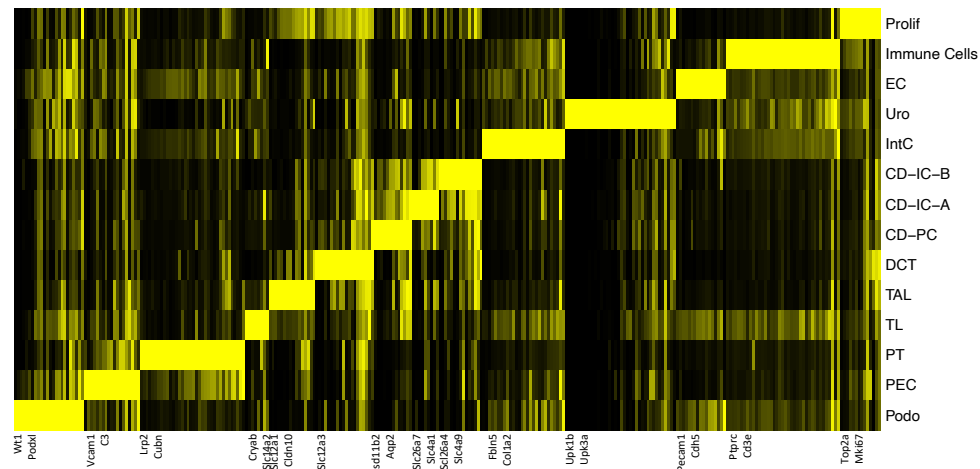
B



C

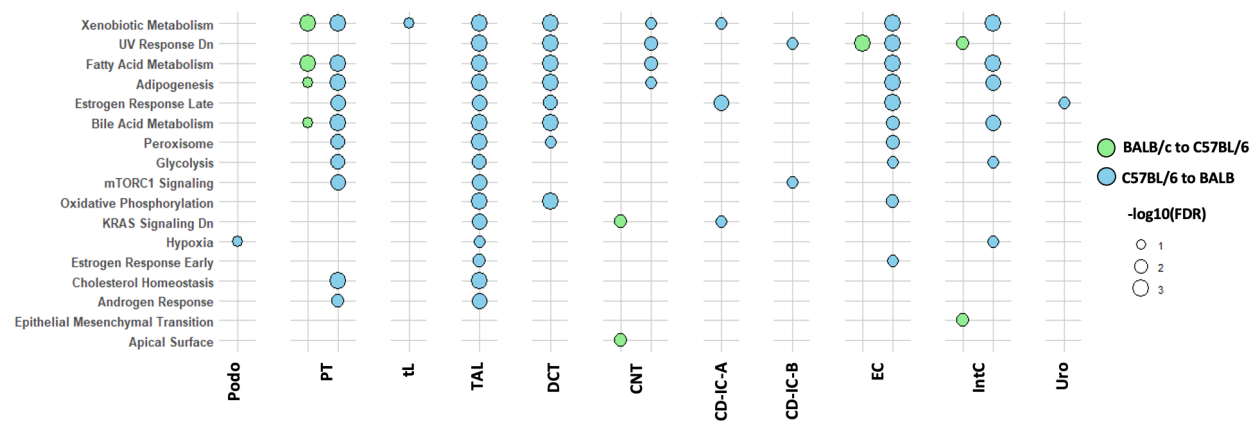


D



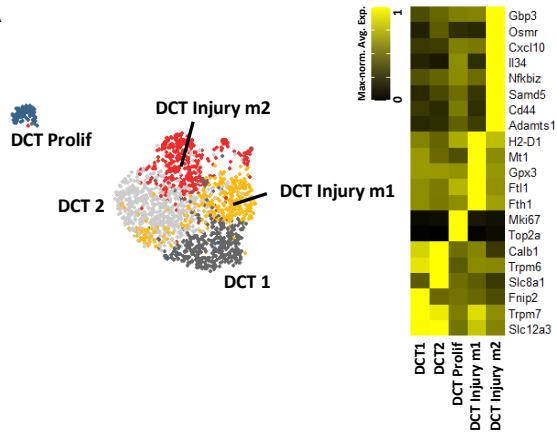
Supplemental Figure S4: Quality metrics and marker gene analysis for mouse ST data. A.

Broad cell type assignment was performed as detailed in the methods, utilizing marker gene expression and histological localization. The left panel displays a UMAP highlighting broad cell types, while the right panel presents a heatmap showing marker gene expression, with values normalized to the maximum average expression per gene. **B.** Number of detected genes (nFeature_Xenium) and transcripts (nCount_Xenium) per sample. **C.** Number of detected and segmented cells per sample. **D.** Heatmap showing marker gene expression for refined cell types representing all major cell types from snRNA-seq data (values normalized to the maximum average expression per gene).

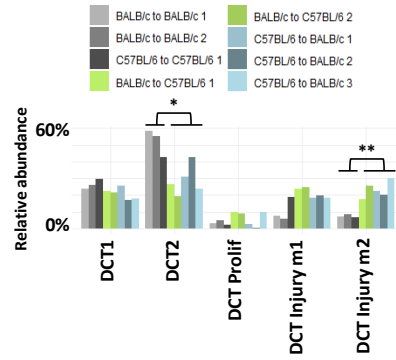


Supplemental Figure S5: Pathway enrichment analysis of differentially downregulated genes.

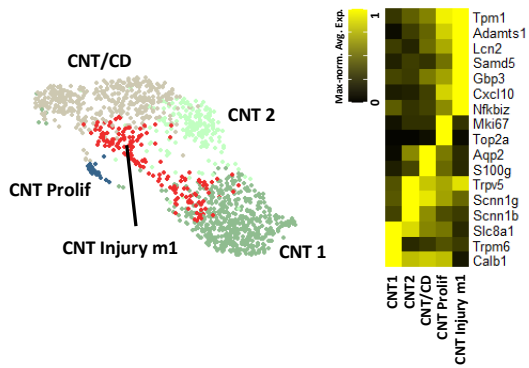
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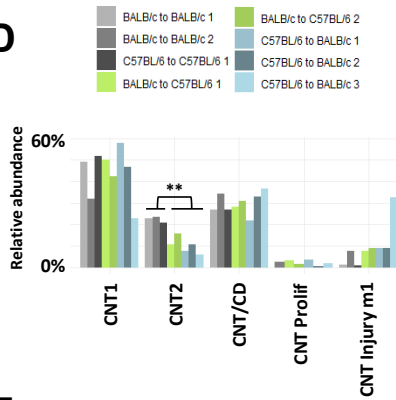
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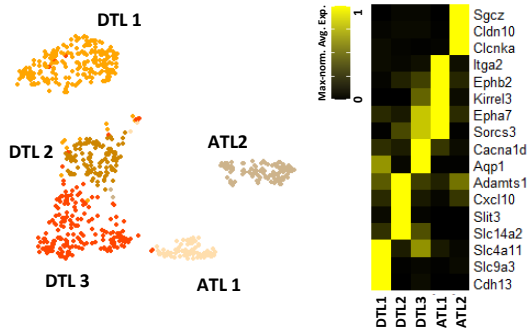
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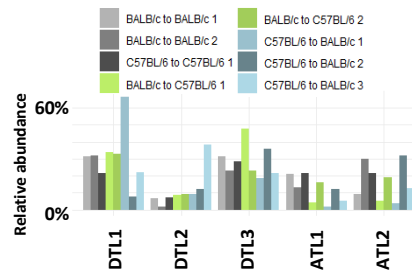
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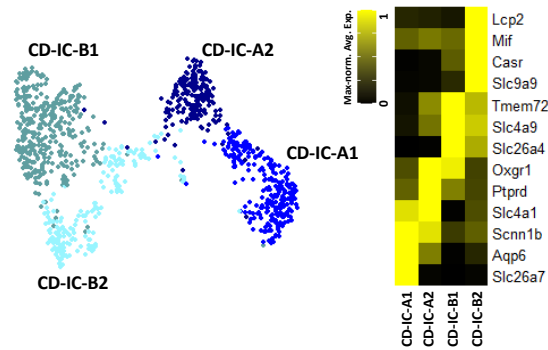
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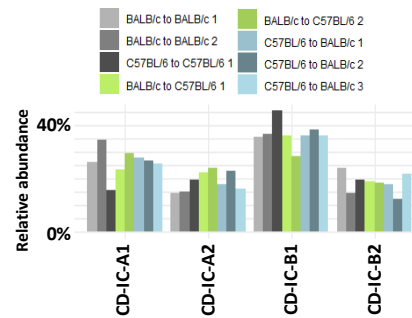
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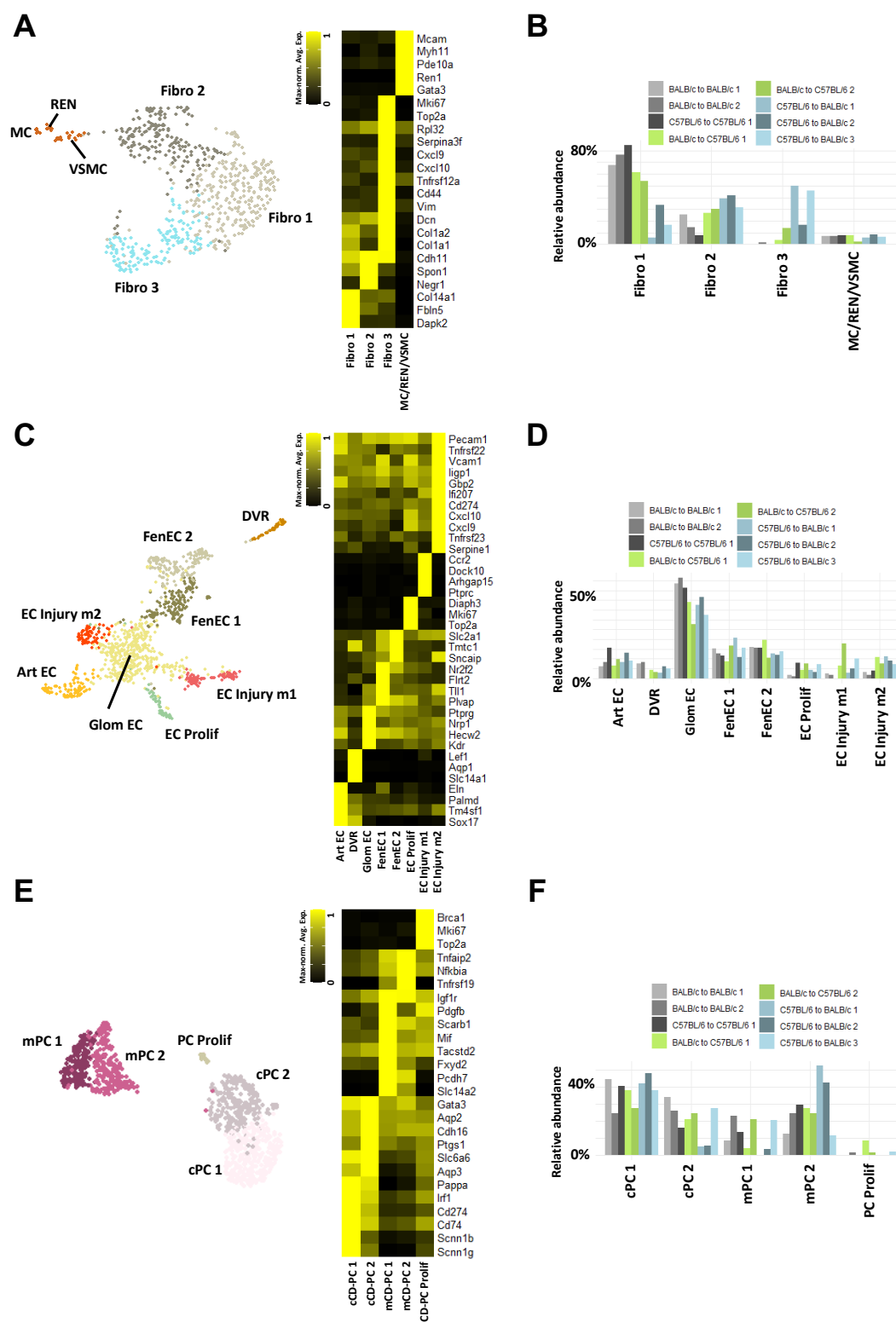
G



H

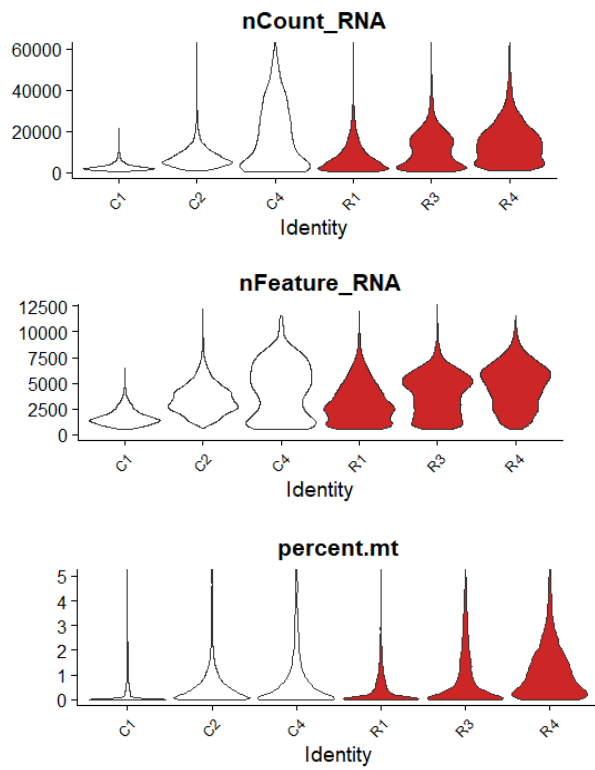
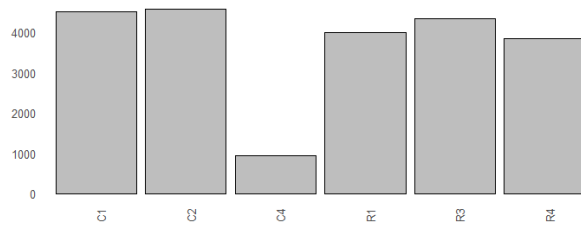


Supplemental Figure S6: Results from subclustering analysis of remaining major cell types. Note that subclustering leukocytes is included in the main figures. Analyses as indicated for DCT (**A.** and **B.**), CNT (**C.** and **D.**), TL (**E.** and **F.**, ATL – ascending thin limb, DTL – descending thin limb) and CD-IC (**A.** and **B.**). Heatmaps show marker gene expression (counts per million, maximum-normalized per gene).

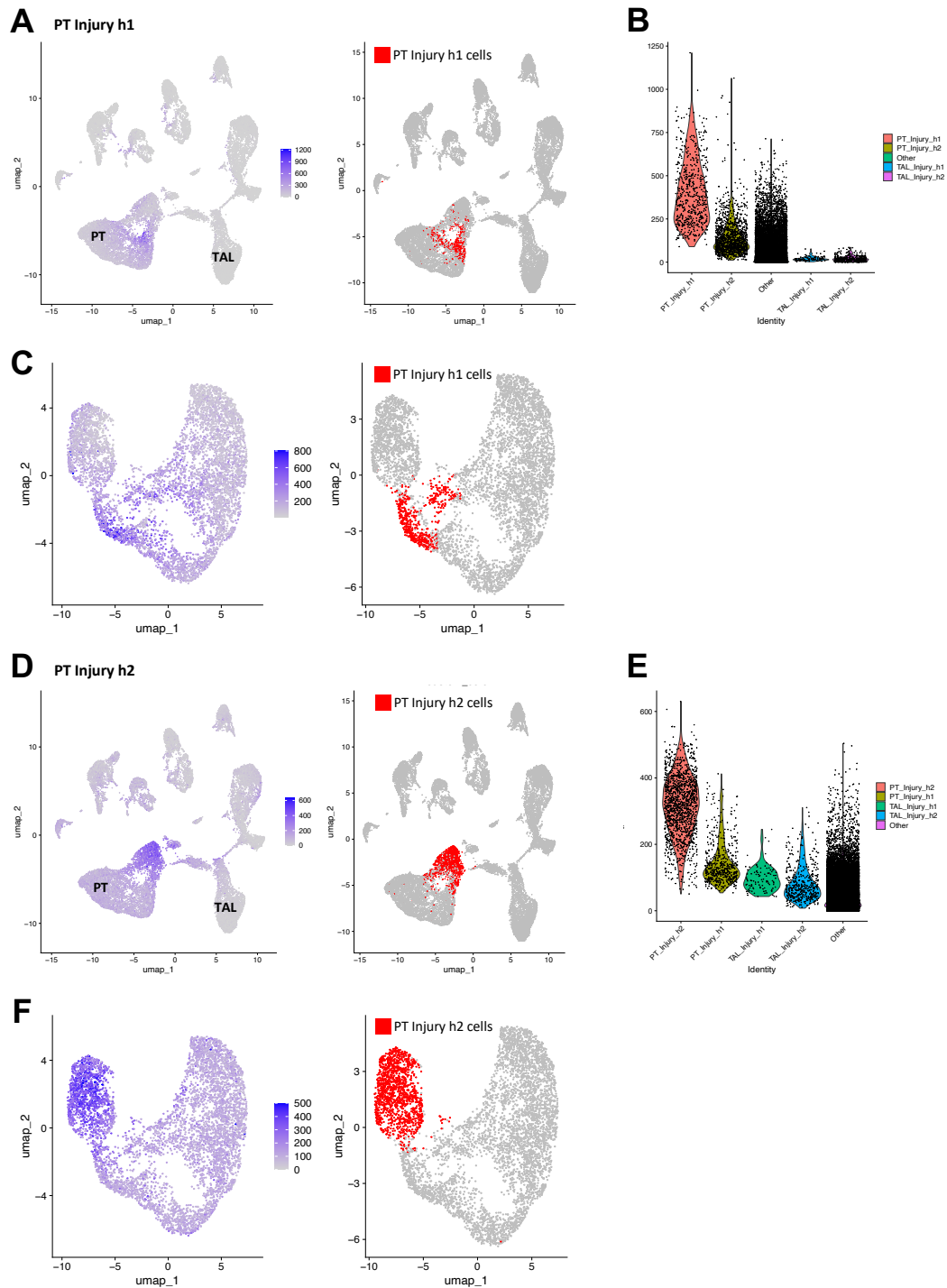


Supplemental Figure S7: Results from subclustering analysis of remaining major cell types. Note that subclustering leukocytes is included in the main figures. Analyses as indicated

for interstitial cells (**A.** and **B.**, MC – mesangial cells, REN – renin-producing cells, VSMC – vascular smooth muscle cells, Fibro - fibroblasts), EC (**C.** and **D.**, FenEC – fenestrated EC, Glom EC – glomerular EC, Art EC – arterial EC, DVR – descending vasa recta) and CD-PC (**E.** and **F.**, mPC – medullary principal cells, cPC – cortical principal cells). Heatmaps show marker gene expression (counts per million, maximum-normalized per gene).

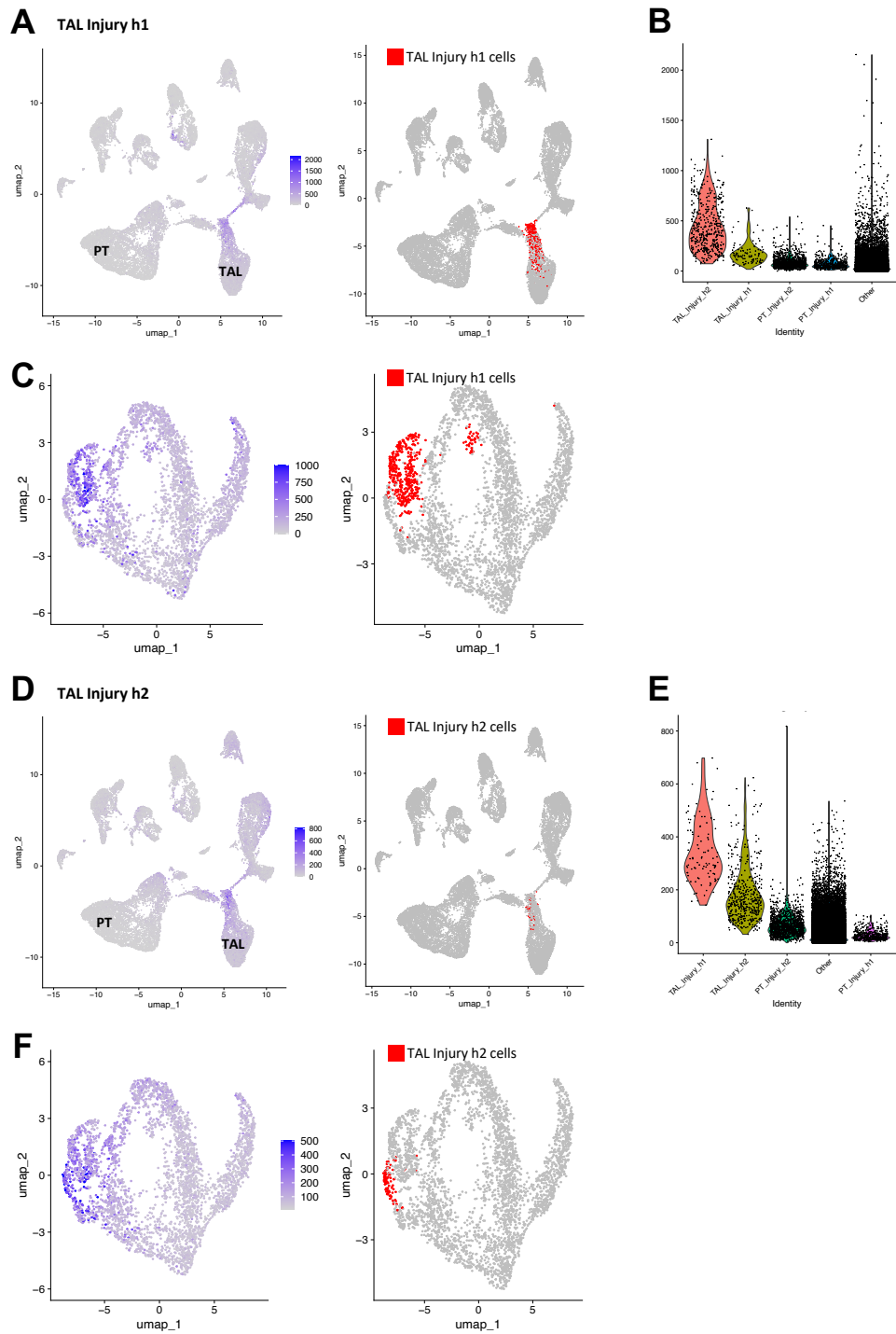
A**B**

Supplemental Figure S7: Quality metric for human snRNA-seq data. A. Number of detected genes (nFeature_RNA), transcripts (nCount_RNA) and percentage of mitochondrial reads (percent.mt) per sample. **B.** Number of detected cells per sample.



Supplemental Figure S8: Expression patterns for PT gene set scores on human snRNA-seq data. **A.** Feature plot for gene set score for PT Injury h1 on all human cells (average counts per million) (left plot), UMAP highlighting PT Injury h1 cells (right plot) and **B.** violin plot showing average counts per million per cell in all PT and TAL injury clusters and all other cells ("Other").

C. Feature plot for gene set score for PT Injury h1 on all human PT cells (left plot) and UMAP highlighting PT Injury h1 cells (right plot). **D.-F.** Analogous plots for PT Injury h2.



Supplemental Figure S9: Expression patterns for TAL gene set scores on human snRNA-seq data. Plots are analogous to Fig. S8 for TAL Injury h1 and h2 cells.

This subclustering includes AKI-induced injured PT cell states PT-New 1-4. Feature plots and violin plots are showing the average counts per million score per gene set on the indicated cell populations. **B.** Analogous plots for AKI-induced TAL cell states TAL-New 1-4.

References:

- 1 Hinze, C. *et al.* Single-cell transcriptomics reveals common epithelial response patterns in human acute kidney injury. *Genome Med* **14**, 103, doi:10.1186/s13073-022-01108-9 (2022).