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Supplemental Information

Kidney single-cell transcriptome profile reveals distinct response of proximal tubule cells to SGLT2i and ARB treatment in diabetic mice

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Figure S1 to S10
Legends for tables S1 to S8
Table S5 and Table S8

Other Supplemental Material for this manuscript include the following:

Table S1 to S4
Table S6 and S7

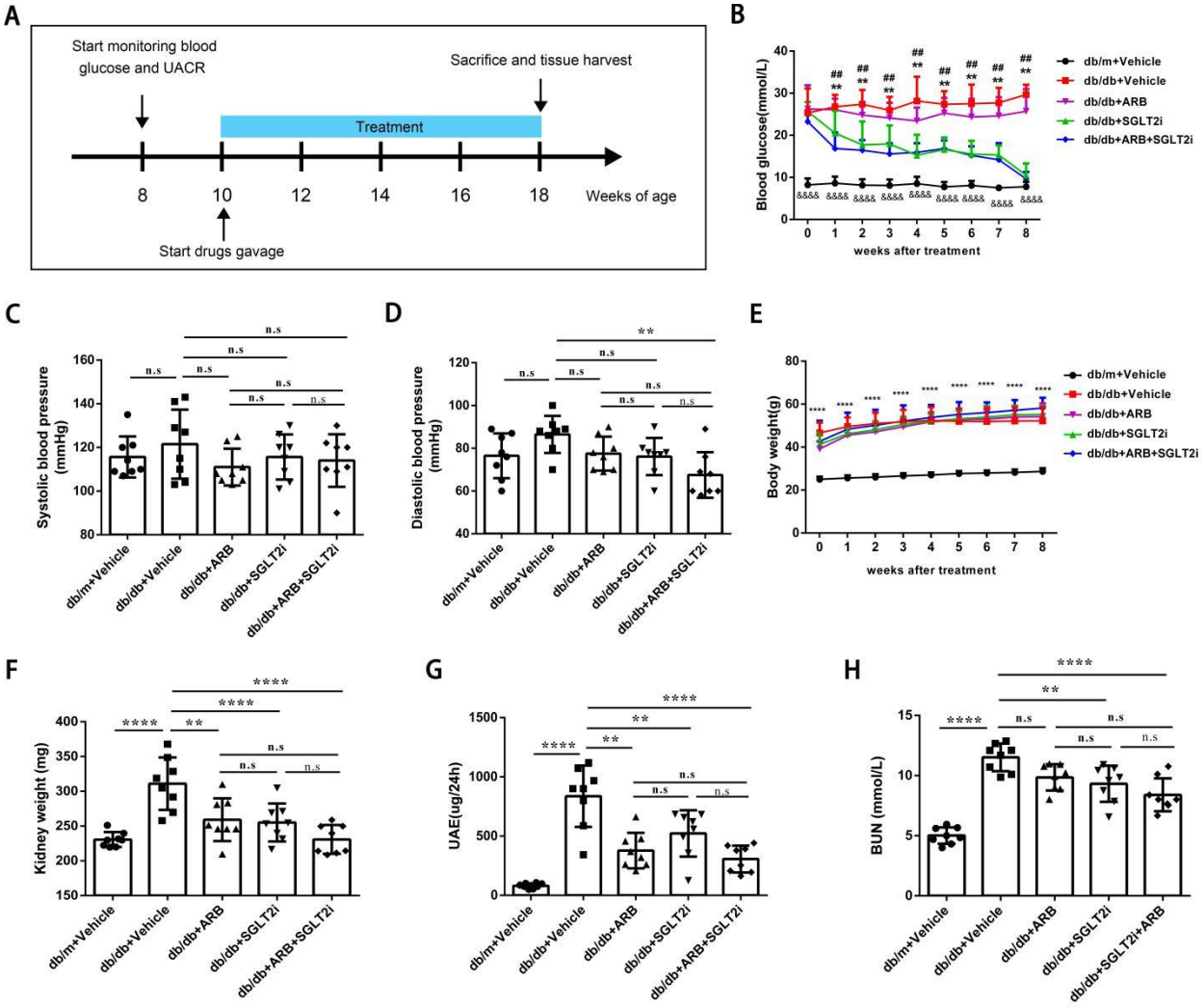

Figure S1. Characterization of mice after 8 weeks of treatments. (A) Schematics of the experimental design. Blood glucose and UACR were monitored in 8-week old db/db mice. Treatment (PBS, SGLT2i, ARB and SGLT2i+ARB) was started after db/db mice developed DKD approximately 10 weeks of age. All mice were sacrificed 8 weeks after treatment, n = 8 mice per group. **(B)** Blood glucose levels after different drug treatments for 8 weeks. Week 0 (10 weeks of age) indicates the baseline characteristics before the treatments. $^{**}P < 0.01$, db/db+Vehicle vs db/db+SGLT2i; $^{\#\#}P < 0.01$, db/db+Vehicle vs db/db+ARB+SGLT2i; $^{\&\&\&\&}P < 0.0001$, db/db+Vehicle vs db/m+Vehicle. **(C)** Systolic blood pressure (mmHg) after 8 weeks of treatment. n.s: not significant. **(D)** Diastolic blood pressure (mmHg) after 8 weeks of treatment. n.s: not significant, $^{**}P < 0.01$, db/db+Vehicle vs db/db+ARB+SGLT2i. **(E)** The body weight of mice. $^{****}P < 0.0001$, db/db+Vehicle vs. db/m+Vehicle. **(F)** The kidney weight of mice. $^{**}P < 0.01$, $^{****}P < 0.0001$. **(G)** Albumin excretion over 24 hours. **(H)** The blood urea (BUN) after 8 weeks of treatment. $^{**}P < 0.01$, $^{****}P < 0.0001$. *P* values between groups by 1-way ANOVA with Tukey's multiple comparisons test; n=8 mice were included in each group.

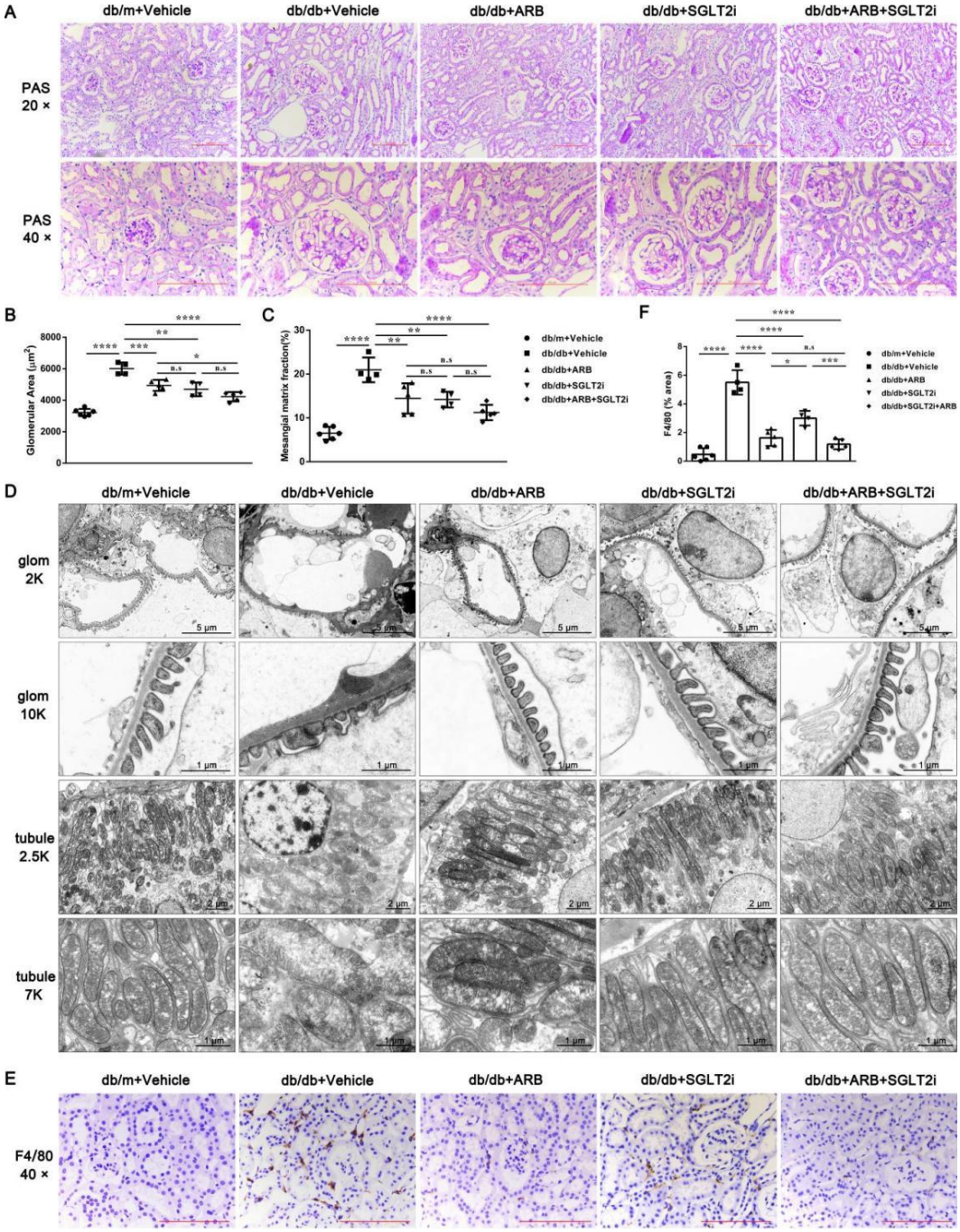

Figure S2. Pathology changes after 8 weeks of treatments. (A) Representative images of PAS-stained kidney sections of db/db or db/m mice at 8 weeks post-treatment. Original magnification, ×200 (upper panels); ×400 (lower panels). Scale bars:100 μm. **(B** and **C)** Quantification of glomerular area (B) and percentage of mesangial matrix (C) are shown. Data are represented as the mean±SD. **P* < 0.05, ***P* < 0.01, *****P* < 0.0001, n.s: not significant. *P* value from 1-way ANOVA with Tukey's multiple comparisons test. **(D)** Representative transmission electron microscopy images of glomerular and tubules at low and high magnifications (n = 4 mice per group). **(E** and **F)** Representative immunohistochemistry images (E) and quantification (F) from each group for F4/80. Scale bars:100 μm. N=4-6 were included in each group.

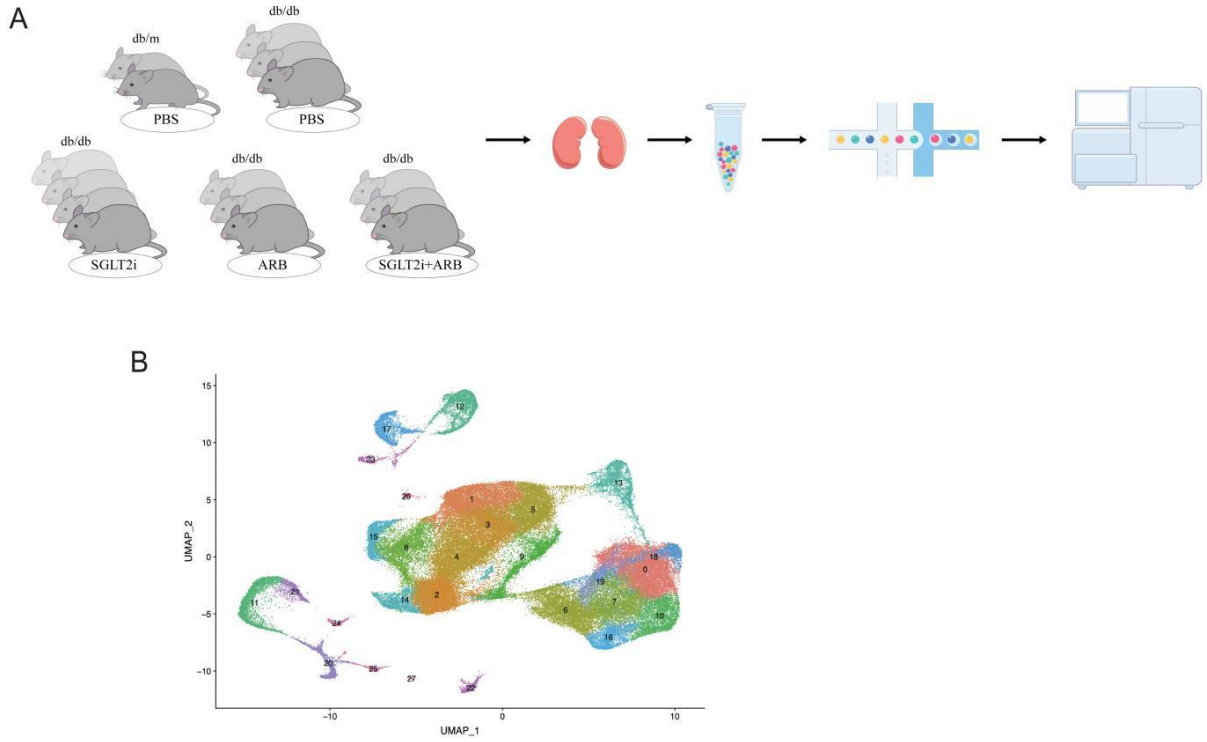

Figure S3. Schematic diagram of experiments, cell clusters identified by UMAP and novel cell markers. (A) Schematic diagram of experiment. **(B)** Total cell cluster identified by UMAP.

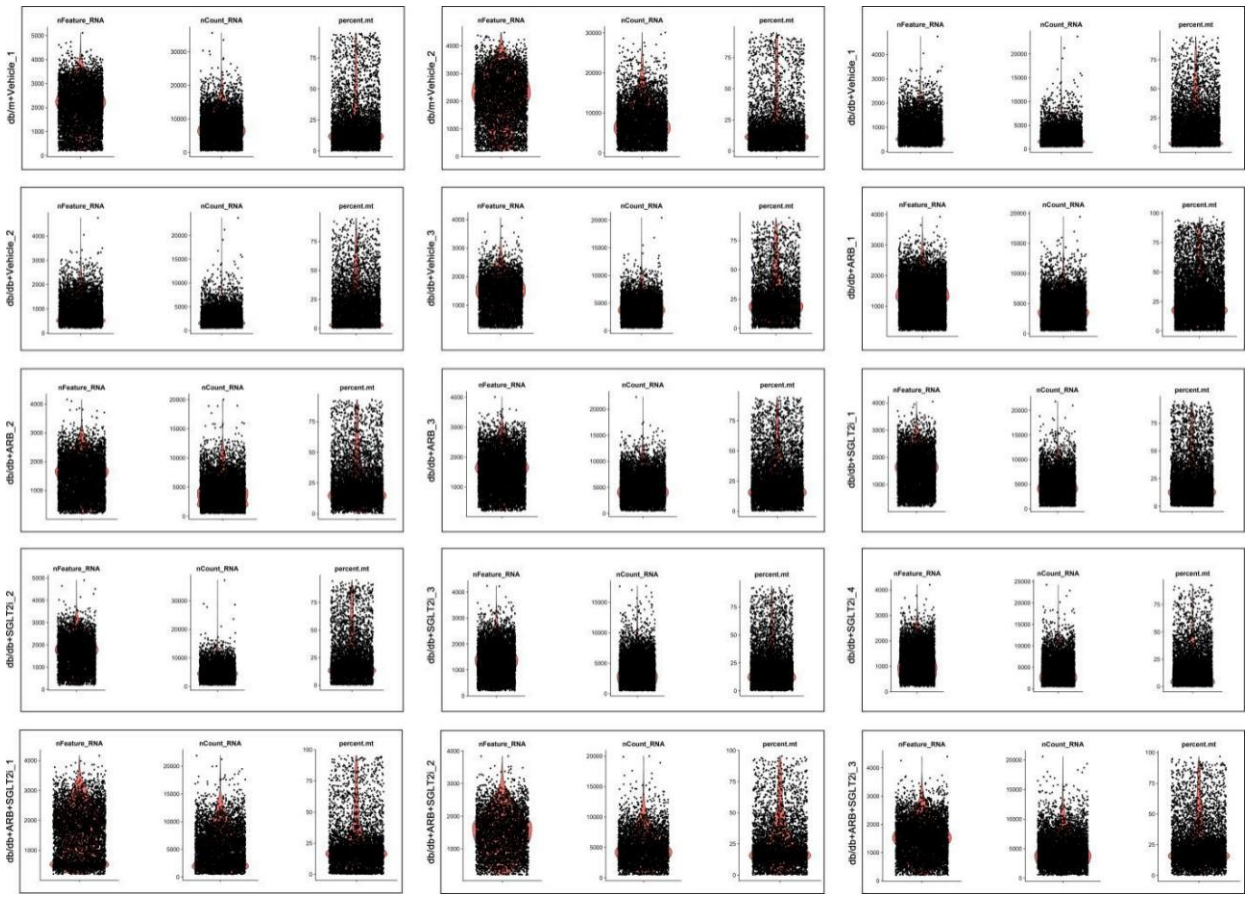

Figure S4. Quality control of all samples. Violin plots shows the number of genes (nFeature_RNA), and cells (nCount_RNA) and the percentage of mitochondrial (percent.mt) genes for all cells in each sample.

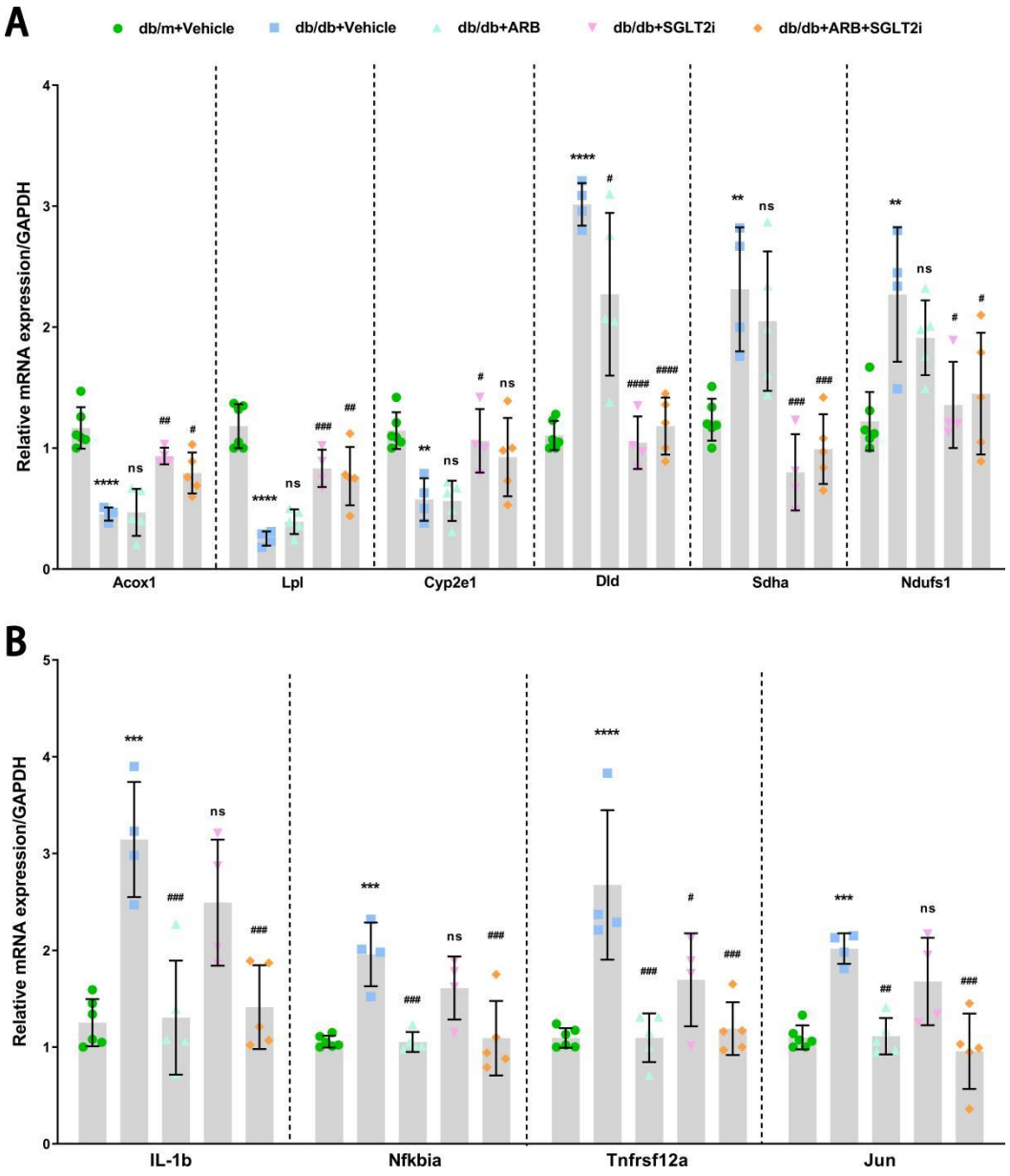

Figure S5. Validation of key genes relate to fatty acid metabolism, ATP synthesis, inflammation and EMT using quantitative PCR. **P < 0.01, ***P < 0.001 and ****P < 0.0001 between db/db vs db/m, $^{\#}$P < 0.05, $^{\#\#}$P < 0.01, $^{\#\#\#}$P< 0.001 and $^{\#\#\#\#}$P< 0.0001 between db/db+vehicle vs db/db+ARB, db/db+SGLT2i or db/db+ARB+SGLT2i. *P* values between groups by 1-way ANOVA with Tukey's multiple comparisons test.

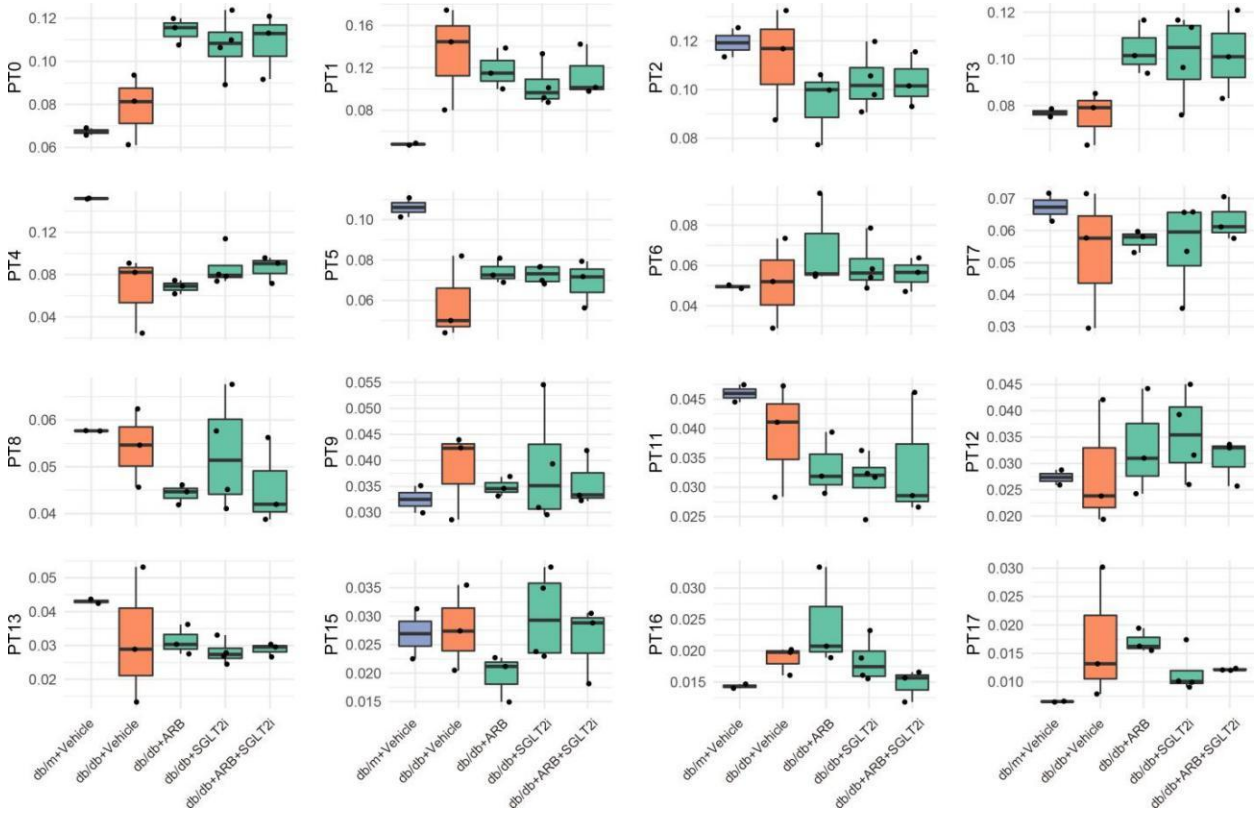

Figure S6. The relative proportion of all PT subclusters by using UMAP. Box-plot shows the quantification of all subclusters of PT among the 5 groups.

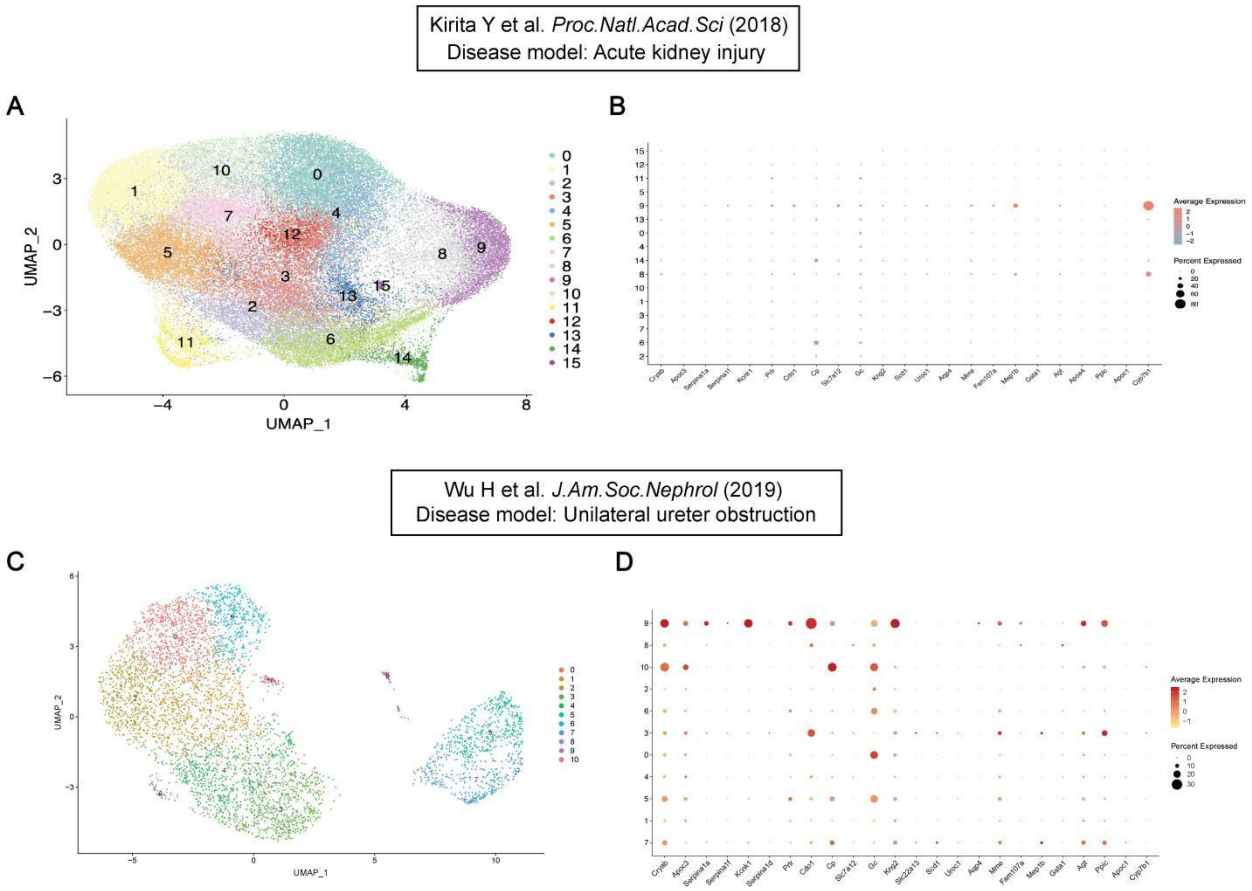

Figure S7. Search of public datasets to identify PT 10 cluster. (A) UMAP plot for PT cells from the dataset of Kirita Y et al. (PMID: 32571916). **(B)** Dot-plot shows the marker genes of PT10 subcluster in the PT cells of AKI animal model from the Kirita Y et al. dataset. **(C)** UMAP plot for PT cells from the dataset of Wu H et al. (PMID: 30510133). **(D)** Dot-plot shows the marker genes of PT10 subcluster in the PT cells from the Wu H et al. dataset.

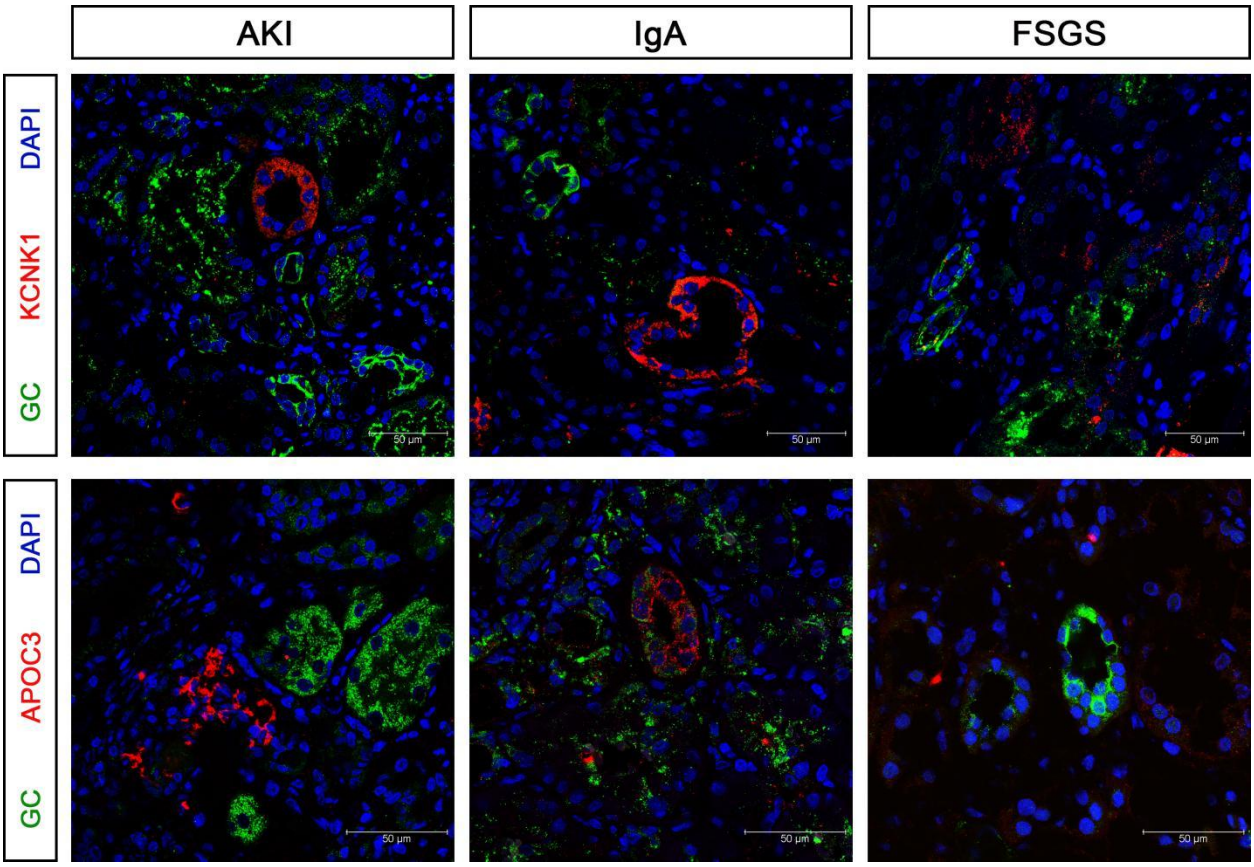

Figure S8. Validation of new PT subcluster-PT10 in non-DKD patients. Representative immunofluorescence images from AKI, IgA and FSGS patients for GC (green) and KCNK1 (red) (upper panel). Representative immunofluorescence images from AKI, IgA and FSGS patients for GC (green) and APOC3 (red) (lower panel). Original magnification, ×63.

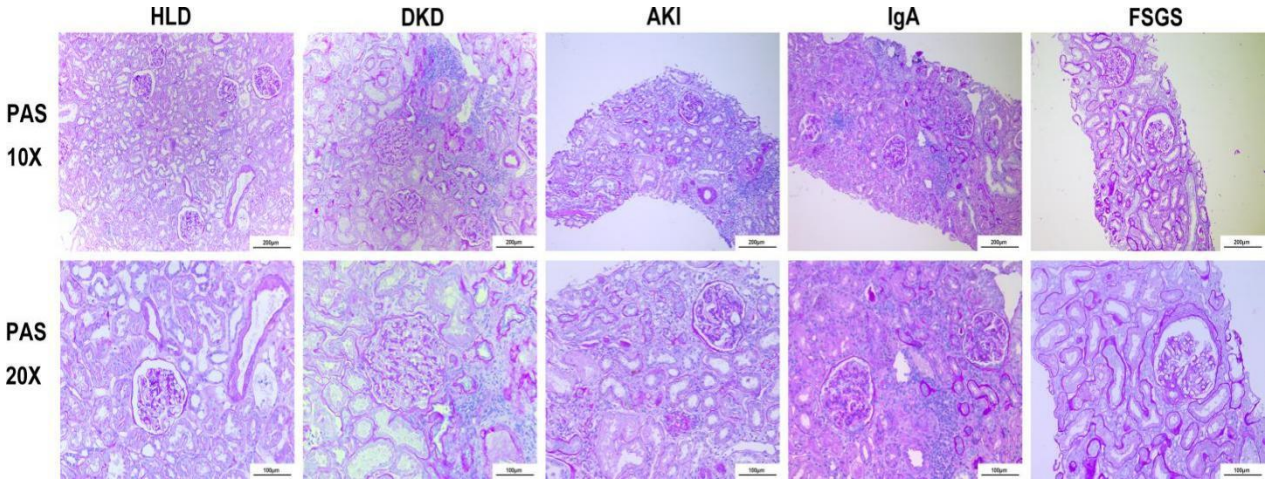

Figure S9. Pathology changes of DKD and non-DKD patients. Representative images of PAS-stained kidney sections of healthy living donor (HLD), DKD, AKI, IgA nephropathy and FSGS patients. Original magnification, ×100 (upper panels); ×200 (lower panels). Scale bars: 100 μm.

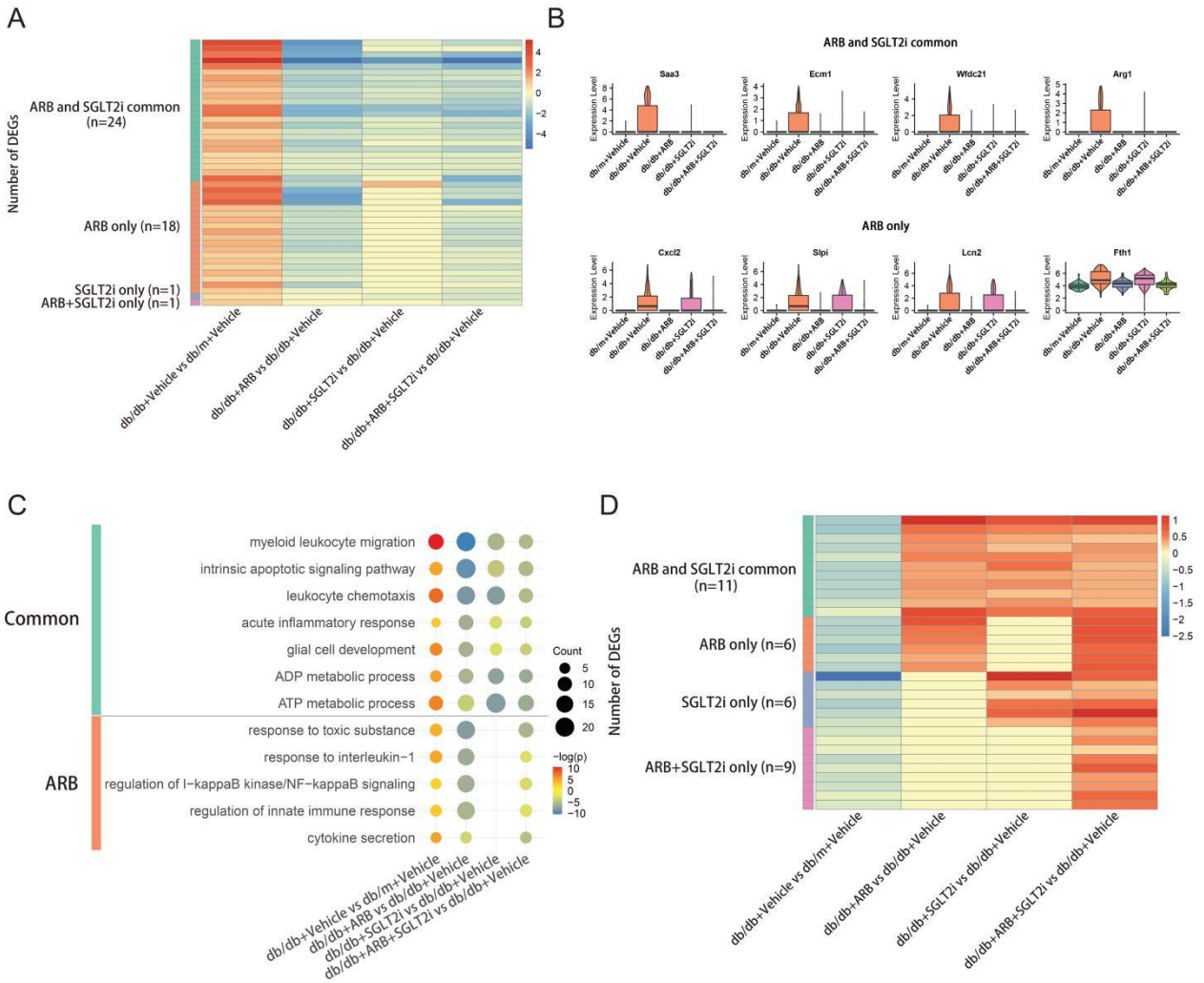

Figure S10. Differential gene expression analysis reveals macrophage-specific responses to DKD injury and different treatments. (**A**) Heat-map shows the number of DEGs that were upregulated in db/db mice compared to db/m mice but downregulated by treatment (updown pattern). ARB and SGLT2i Common: DEGs were down-regulated by both ARB and SGLT2i treatment; ARB only: DEGs downregulated specifically by ARB treatment; SGLT2i only: DEGs downregulated specifically by SGLT2i treatment; ARB+SGLT2i only: DEGs downregulated only by combination treatment with ARB and SGLT2i. The color scale represents the log fold change in the expression levels of genes. (**B**) violin-plot shows the representative DEGs that follow the updown pattern in common and ARB treated groups. (**C**) Dot-plot of Gene Ontology (GO) terms in DEGs that followed the updown pattern. (**D**) Heat-map shows the DEGs downregulated in db/db mice compared to db/m mice but upregulated by treatment (downup pattern). The color scale represents the log fold change in the expression levels of genes. For the Heat-map, red and blue color indicates up- and down-regulated genes between diabetic and control mice in the first column and genes reversed by treatments in the other three columns. The yellow color indicates no changes.

Table S1- Full list of marker genes in each cluster to support annotation.

Marker genes of all kidney cell types we identified by pooling all samples together.

Table S2- Differentially expressed genes between db/db mice vs db/m mice and db/db mice with or without treatment (PT).

List of differentially expressed genes (DEGs) that are significantly different between db/m vs db/db mice, between db/db+vehicle vs db/db+ARB or SGLT2i or combined treatment. The number indicates the log Fold change value.

Table S3- GO term enrichment analysis (PT).

List of GO terms that are enriched for DEGs in different treatment groups. We consider $P<10^{-6}$ as significant GO terms.The number indicates the -log (P) value.

Table S4- Gene lists used for gene scoring analysis.

Gene lists used for gene scoring analysis for fatty acid metabolic, ATP synthesis coupled electron transport, response to inflammation, and epithelial to mesenchymal transition.

Table S5- Clinical parameters of the patients used to validate new PT in Figure 5.

Group (N)	Gender	Age	HbA1c (%)	Serum Creatinine	Proteinuria	eGFR	Glomerulosclerosis
Control (n=4)	F	55	NA	70	No	87.7	None (<10%)
	F	78	NA	54	No	90.5	None (<10%)
	M	72	NA	73	NA	100.9	None (<10%)
	M	57	NA	89	NA	108.9	None (<10%)
Diabetic kidney disease (n=4)	F	62	7	70	2+	83.5	Moderate (26-50%)
	F	66	6.5	48	No	87.245	Mild (11-25%)
	M	49	NA	74	+	102	Mild (11-25%)
	F	71	7.4	45	+	45	Moderate (26-50%)
Acute kidney injury (n=5)	M	36	3.9	203.2	+	34.99	None (<10%)
	M	18	5.6	180.9	2+	45.7	None (<10%)
	F	50	5.8	259	2+	17.96	None (<10%)
	M	51	6	725	No	6.77	None (<10%)
	F	39	6	131	No	43.9	None (<10%)
IgA nephropathy (n=3)	M	23	5.3	188.9	2+	41.88	Severe (>50%)
	F	60	6.2	81.8	+	67.6	None (<10%)
	M	39	5.7	108.6	+	73.56	Mild (11-25%)

Focal segmental glomerular sclerosis (n=3)	M	37	5.4	251	2+	27.1	Mild (11-25%)
	F	66	6	81	2+	65.41	Mild (11-25%)
	M	50	5.4	106.8	3+	69.53	Mild (11-25%)

Table S6- Differentially expressed genes between db/db mice vs db/m mice and db/db mice with or without treatment (macrophage).

List of differentially expressed genes (DEGs) that are significantly different between db/m vs db/db mice, between db/db+vehicle vs db/db+ARB or SGLT2i or combined treatment. The number indicates the log Fold change value.

Table S7- GO term enrichment analysis (macrophage).

List of GO terms that are enriched for DEGs in different treatment groups followed updown pattern. We consider $P<10^{-6}$ as significant GO terms.The number indicates the -log (P) value.

Table S8- Sequence of the qPCR primers used in Figure S5.

Gene	Sequences (5'-3')		NCBI GeneID
	Forward	Reverse	
Acox1	TAACTTCCTCACTCGAAGCCA	AGTTCCATGACCCATCTCTGTC	11430
Lpl	GGGAGTTTGGCTCCAGAGTTT	TGTGTCTTCAGGGGTCCTTAG	16956
Cyp2e1	CGTTGCCTTGCTTGTCTGGA	AAGAAAGGAATTGGGAAAGGTCC	13106
Dld	GAGCTGGAGTCGTGTGTACC	CCTATCACTGTCACGTCAGCC	13382
Sdha	GGAACACTCCAAAAACAGACCT	CCACCACTGGGTATTGAGTAGAA	66945
Ndufs1	AGGATATGTTCGCACAACTGG	TCATGGTAACAGAATCGAGGGA	227197
IL-1b	GCAACTGTTCCTGAACTCAACT	ATCTTTTGGGGTCCGTCAACT	16176
Nfkbia	TGAAGGACGAGGAGTACGAGC	TTCGTGGATGATTGCCAAGTG	18035
Tnfrsf12a	GTGTTGGGATTCGGCTTGGT	GTCCATGCACTTGTCGAGGTC	27279
Jun	CCTTCTACGACGATGCCCTC	GGTTCAAGGTCATGCTCTGTTT	16476