Single-cell RNA sequencing reveals the effects of Hederasaponin C in

the treatment of diabetic nephropathy

Supplementary Information Containing:

Supplementary Results
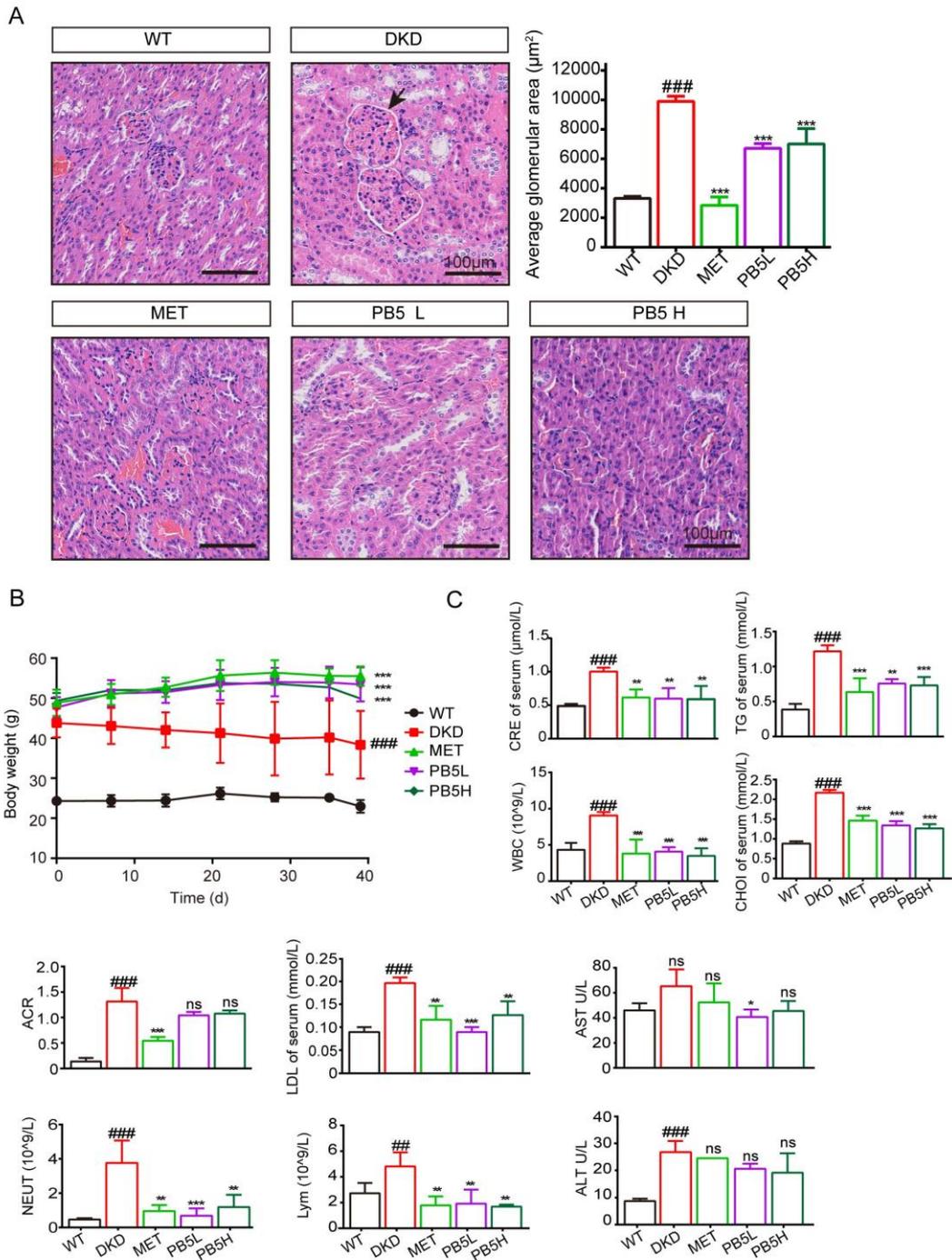
Figure S1.

(A) H&E staining of renal tissues from normal mice, T2DM model or PB5 treatment.
(B) Body weight test of mouse across 3 groups.
(C) Detect results of CRE, TG, WBC, CHOI, ACR, LDL, Lym, NEUT and AST.

$^{*}P <0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus DKD group and ### $P < 0.001$ versus WT group.
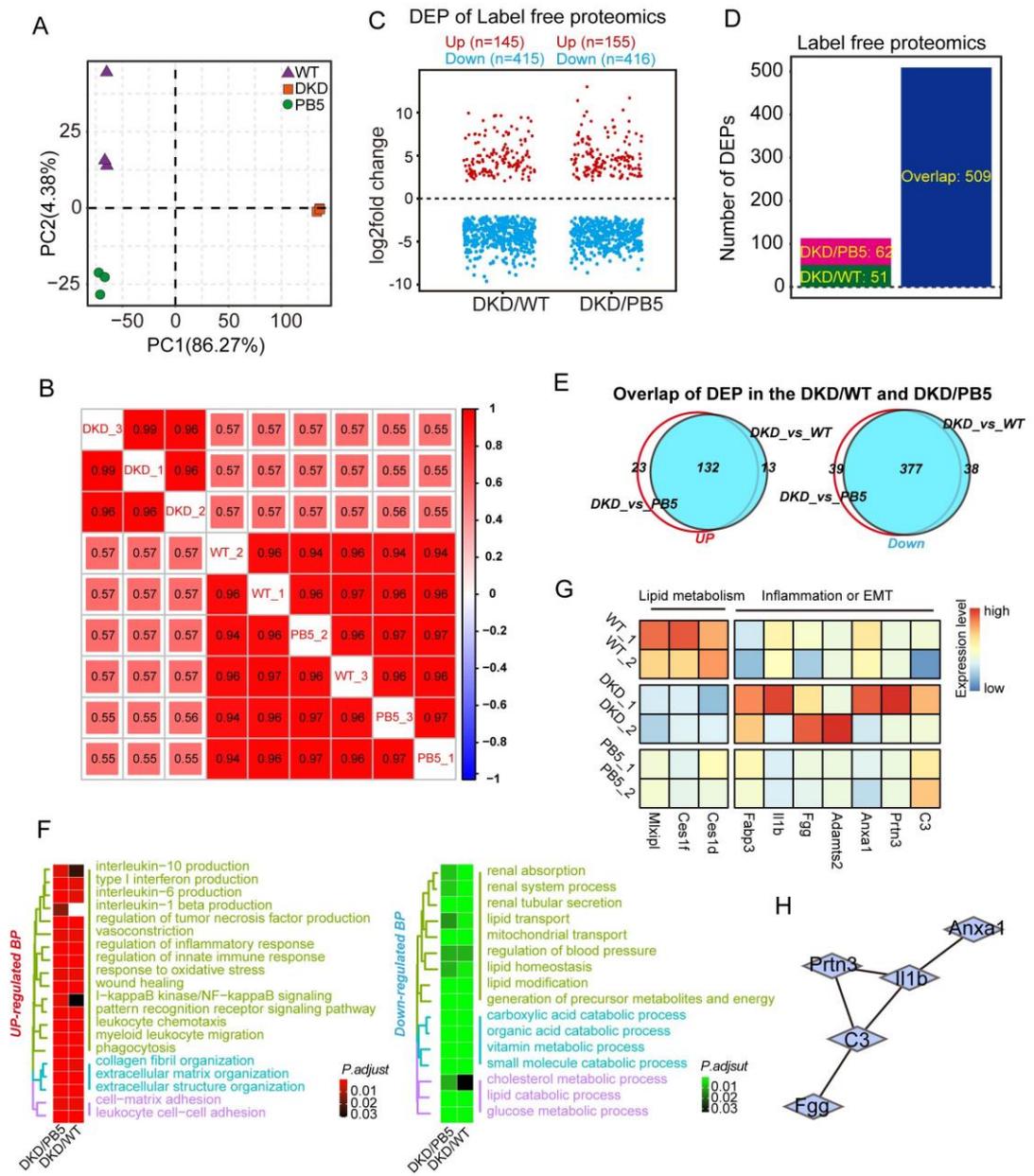
Figure S2

(A) The dot plot presenting the distribution of two principal components (PC1 and PC2) of gene expression level across 3 groups.

(B) Heatmap showing correlation coefficient of the protein expression level across 3 group.

(C) Volcano graph showing the distribution of differential expressed proteins (DEPs) between two comparisons (DKD/WT and DKD/PB5).

(D) Bar plot displaying the number of DEPs between 2 comparisons.

(E) Venn diagram displaying up-regulated and down-regulated DEPs between two comparisons.

(F) Heatmap displaying the up/down-regulated enriched pathways between two comparisons.
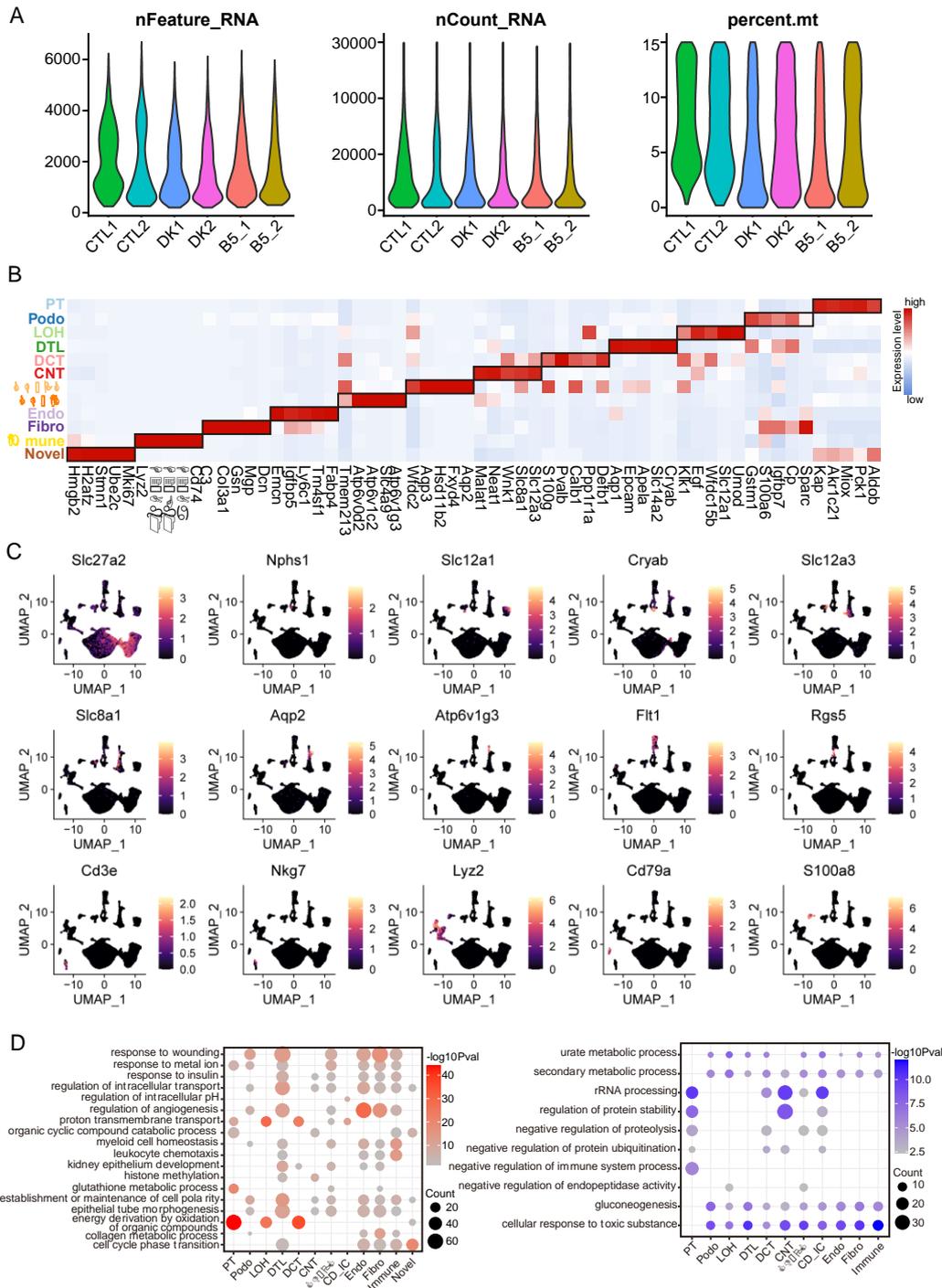
Figure. S3.

(A) Violin plot displaying the distribution of scRNA-seq data after quality control.

(B) Heatmap showing the expression level of cell-specific marker genes in each cell types.

(C) UMAP visualization displaying the expression level of canonical marker genes of each cell type.

(D) Dot plot showing the enriched pathways of DEGs between each cell types (red

represents up-regulated pathways and blue represents down-regulated pathways).
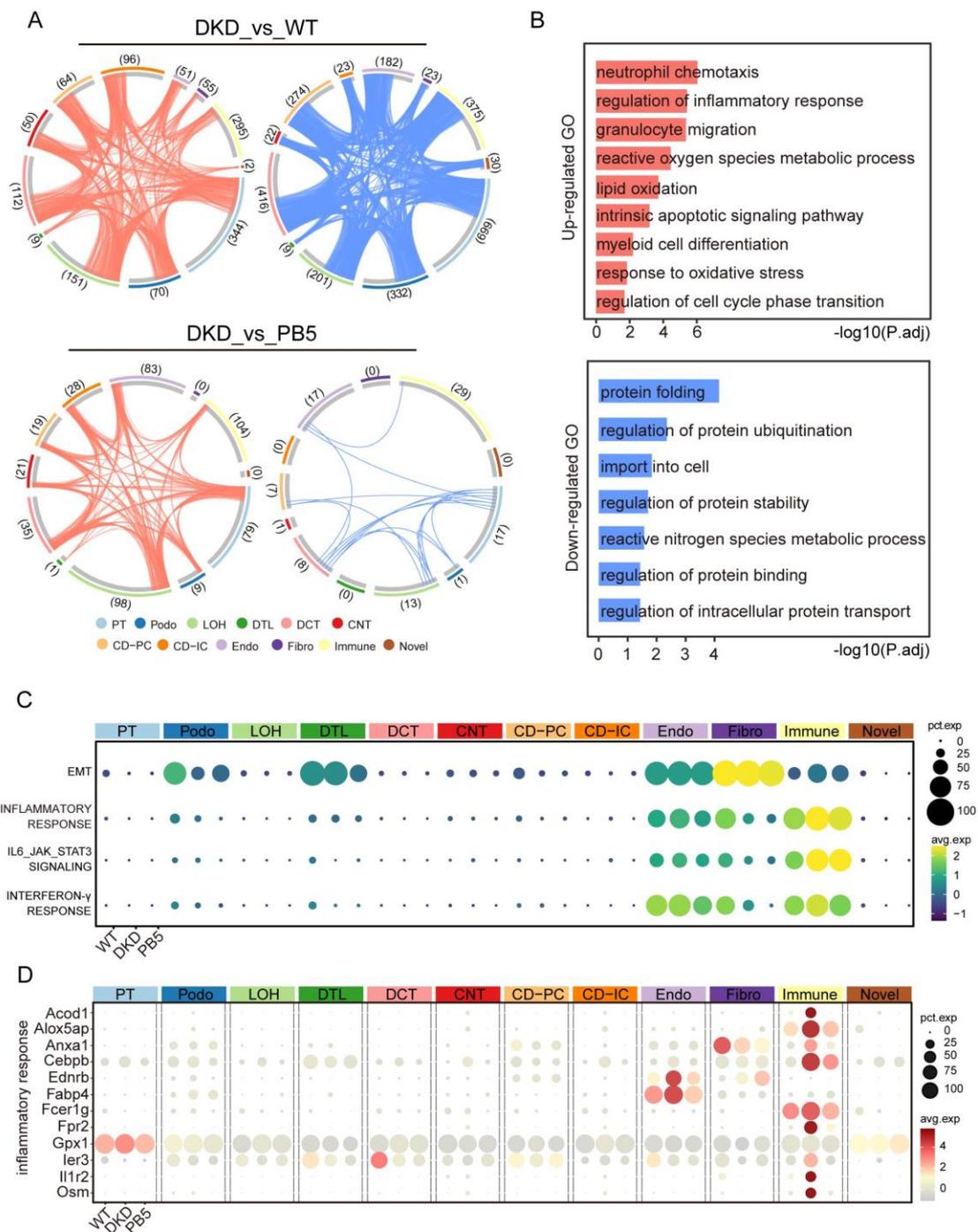
Figure S4.

(A) Circos plot showing up-regualted (red) and down-regulated (blue) DEGs in the 12 cell types in two comparison (DKD_vs_WT and DKD_vs_PB5). Each link represents a overlapped DEG.

(B) Bar plot showing the enriched pathways of overlapped DEGs between DKD_vs_WT and DKD_vs_PB5.

(C) Dot plot showing the expression level of enrichment score of gene sets in each cell types across three groups.

Figure S5.

(A) UMAP plot showing the unsupervised clustering in each sample.

(B) Bar graph displaying proportion of proximal tubule cells from each sample across three groups.

(C) Heatmap showing the expression levels of cell-specific marker genes.

(D) Dot plot showing up-regulated or down-regulated enriched pathways of overlapped DEGs in PT cells (left). Venn diagram presenting the number of DEGs between two comparison (DKD_vs_WT and DKD_vs_PB5) in right.

(E) Bar graph showing enriched pathways based on GSVA analysis both DKD/WT (up) and DKD/PB5 comparison (down).

(F) Bubble plot displaying the expression pattern of APOPTOSIS, EMT and ROS pathways based on GSVA score in each subtype across three groups.
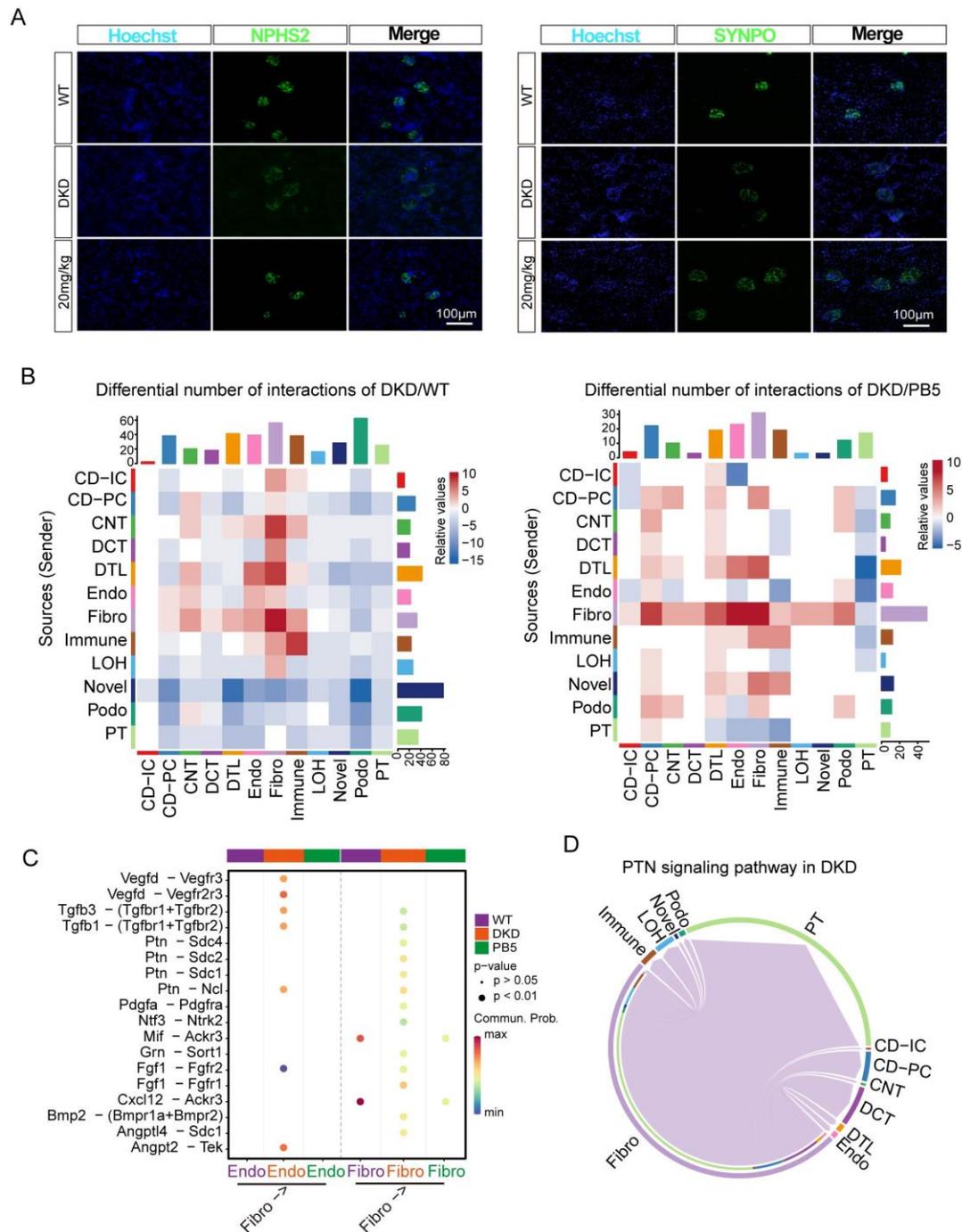
Figure S6.

(A) Immunofluorescence staining of Hoechst (right, blue), NPHS2 and SYNPO (right,

green) in renal tissues. Scale bar: 100 μm.

(B) Distribution of intercellular interactions in all of kidney cell types across three groups.

(C) The bubble plot displaying up-regulated LR pairs between Fibro and Endos across three groups.

(D) Chordal plot showing interaction network in all of renal cell types for specific signaling pathway.
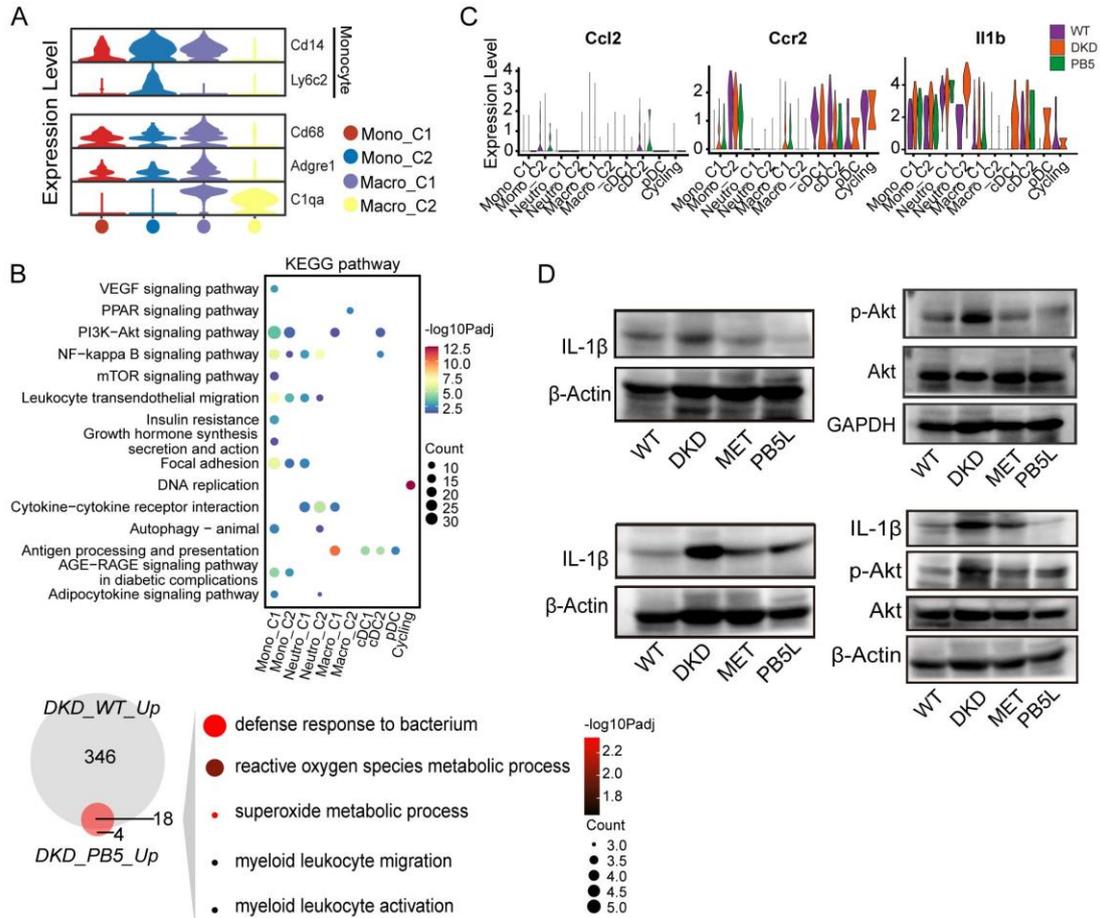
Figure S7.

(A) Violin plot displaying the expression level of markers in monocytes and macrophages.

(B) Dot plot showing up-regulated KEGG pathways between subtypes of myeloid. Venn diagram presenting the number of DEGs between two comparisons (DKD/WT and DKD/PB5), and the dot plot displaying enriched pathways of overlapping DEGs.

(C) Violin plot displaying the relative expression level of *Ccl2*, *Ccr2* and *Il1b* in each subtype across three groups.

(D) Western blotting detected the protein level of IL-1β, p-Akt and Akt.
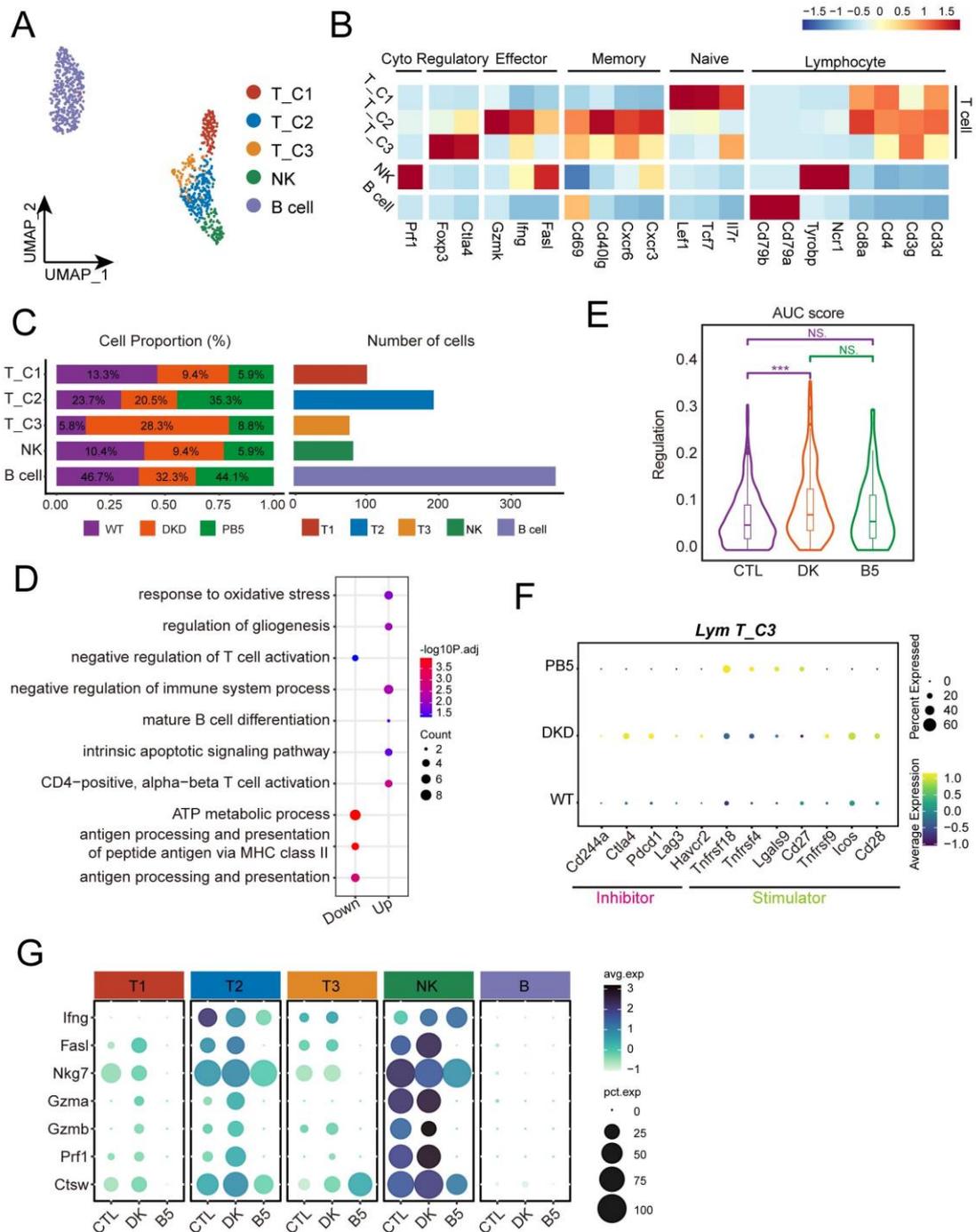
Figure S8.

(A) UMAP visualization

(B) Heatmap showing the expression levels of cell-specific marker genes in five immune clusters.

(C) Bar plot showing the composition of cell subtypes (left) and number of cells for each subtype (right).

(D) Overlapped GO terms between DKD_vs_WT and DKD_CTL for DEGs in T_C3.

(E) Violin plot showing the distribution of enrichment scores for immune regulation in the three groups.

(F) Dot plot showing the relative expression levels of regulators (including stimulators and inhibitors).

(G) Bubble plot showing the expression levels of cytotoxic genes in each subtype across the three groups.