SUPPLEMENTARY METHODS

Cell clustering, visualization, and annotation

The gene-barcode matrix was generated using Seurat version 2.3.4 software (Seurat Technologies, Wilmington, MA, USA) in the R environment version 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria). Cells with unique feature counts >2,500 or <200, and those with >5% mitochondrial counts were filtered as low-quality cells. The R package Doublet Finder was used to remove doublets. The remaining data were normalized and 2,000 features per dataset were selected by default for subsequent bioinformatic analysis. Principal components analysis (PCA) was performed on the scaled data for reducing the data complexity. Based on the 10 principal components derived from the PCA reduction, a nearest-neighbor graph was constructed using the FindNeighbors algorithm. The Louvain algorithm was applied to cluster cells with a resolution parameter set at 0.6. For data visualization and exploration, the t-distributed stochastic neighbor embedding (t-SNE) algorithm was utilized. A marker gene was defined as having a $P<0.05$ and $P>0.25$ log-fold higher than the mean expression value in other clusters. Canonical markers were used to match the unbiased clustering to known cell types in the dataset.

Analysis of intercellular crosstalk

The R package CellChat version 1.1.3 (http://www.cellchat.org) was employed to analyze intercellular crosstalk for revealing major signaling inputs and outputs in all cell populations under normal rats and type 2 diabetes mellitus conditions. Cell clustering, conducted using Seurat, served as a prerequisite for CellChat analysis. A comprehensive database, encompassing known ligand-receptor interactions and their structural compositions, was used for this analysis. The Trimean calculation method was applied to calculate the average gene expression per cell group with a truncated mean of 10%.

Pseudotime trajectory analysis

Trajectory analysis was conducted using Monocle 2 version 2.10.1 with the following parameters: mean expression >0.1 and num cells expressed ≥10. The data pre-processing for trajectory analysis was identical to that of the clustering analysis. Differential gene expression between branches was assessed using the BEAM function in Monocle 2, and co-expressed gene modules were identified using the graph test function. Genes expressed in at least 5% of all cells were selected for ordering, and the trajectory root was programmatically determined.