本文中设计到的参数，仅供写文章参考

Sample Preparation for scRNA-seq

The tissues were digested for XX h at XX temperature in the enzyme solution(XX,XX,XX). After collection of cells, centrifuge at a speed not exceeding 400rcf. Carefully discard the supernatant and retain the cell precipitation.The cell pellet was resuspended by 1mL 1X PBS containing 0.04% BSA and the washing procedure was repeated twice. After washing, appropriate volume PBS was added to the cell precipitation to obtain single-cell dispersion suspension with a concentration close to the goal number. A type of wide-bore pipette tip was used in cell resuspension for a lower cell damage. Cell filter were applied to remove cell debris and clusters by 30μm MACS SmartStrainers and 40μm FlowmiTM Cell Strainer.Automatic cytometry was used to determine the cell concentration((Thermo fisher scientific; AMAQAF1000). And the sample volume was calculated based on the optimal cell sampling concentration supplied by 10X official website and the target capture number.Once the desired cell suspension was obtained, immediately place the samples on ice for subsequent GEMs preparation and reverse transcription by Annoroad Gene Tech. (Beijing) Co., Ltd.

scRNA-seq Library Construction and Sequencing

scRNA-seq libraries were prepared according to manufacturer protocol of Chromium Next GEM Automated Chip G Single Cell Kit (10x Genomics, PN-1000146). Briefly, the cells、the Beads and partitioning Oil was loaded onto a Chromium Chip G. Resulting single-cell GEMs were collected and linear amplification was conducted in a C1000 Touch Thermal cycler as 72℃ for 5 min, 98℃ for 30 s, cycled 12X: 98℃ for 10 s, 63℃ for 30 s and 72 ℃ for 1 min. Emulsions were then coalesced using the Recovery Agent and cleaned up using Dynabeads. Indexed sequencing libraries were then constructed, purified and sequenced on an Illumina NovaSeq instrument at Annoroad Gene Tech. (Beijing) Co., Ltd.

scRNA-seq Data Analysis

CellRanger software was applied to demultiplex the Illumina BCL output into FASTQ files. The Cell Ranger count was then applied to each FASTQ file to align reads to the reference genome and generate barcode and unique molecular identifier counts. We followed the Seurat integrated analysis and comparative analysis workflows to do all scRNA-seq analyses (Stuart et al., 2019). For quality control and filtering out low quality cells, only cells expressing more than 200 genes (defined as genes detected in at least 3 cells) and fewer than 20% mitochondrial genes were selected.All single cells passed quality control for further batch correction and unbiased clustering.

The datasets were integrated based on ‘anchors’ identified between datasets (nfeatures=2000, normalization.method=‘SCT’) before performing linear dimensional reduction by principal-component (PC) analysis. The top 30 PCs were included in a UMAP dimensionality reduction. After obtaining the top 30 PCs, we computed the shared nearest-neighbor graph and we identified clusters in the network using the Louvain algorithm with the resolution . The UMAP (Uniform Manifold Approximation and Projection) method was used for visualization of

unsupervised clustering. Differential gene expression or marker gene was determined by the ‘findMarkers’ function with the default Wilcoxon’s rank-sum test either as one versus the rest or as a direct comparison with parameters min.pct=0.1 and logfc.threshold=0.25. Cell cluster identities were determined using scibet software. We used the clusterProfiler package for differential expression gene GO and KEGG pathway annotations and enrichment analysis.