注：Method 描述模板仅供参考，请根据实际情况修改使用。

Single-cell RNA-seq data preprocessing:

The FASTQ files were processed and aligned to GRCh38 human reference genome using Cell Ranger software (version 7.0.1) from 10x Genomics, with unique molecular identifier (UMI) counts summarized for each barcode. The UMI count matrix was then analyzed using Seurat[1] (version 4.0.0) R package. To remove low-quality cells and likely multiplet captures, a set of criteria were conducted: Cells were filtered by (1) gene numbers (gene numbers < 200), (2) UMI (UMI <1000), (3) log10GenesPerUMI (log10GenesPerUMI < 0.7), (4) percentage of mitochondrial RNA UMIs (proportion of UMIs mapped to mitochondrial genes > 10%) and (5) percentage of hemoglobin RNA UMIs (proportion of UMIs mapped to hemoglobin genes > 5%). Subsequently, the DoubletFinder[2] package (version 2.0.3) was used to identify potential doublets. To obtain the normalized gene expression data, library size normalization was processed using the NormalizeData function. Specifically, the global-scaling normalization method "LogNormalize" normalized the gene expression measurements for each cell by the total expression, multiplied by a scaling factor (10,000 by default), and log-transformed the results.

Top 2000 highly variable genes (HVGs) were calculated using the Seurat function FindVariableGenes (mean.function=FastExpMean, dispersion.function= FastLogVMR). Principal-component analysis (PCA) was performed to reduce the dimensionality with RunPCA function. Graph-based clustering was performed to cluster cells according to their gene expression profile with the FindClusters function. Cells were visualized using a 2-dimensional Uniform Manifold Approximation and Projection (UMAP) algorithm with the RunUMAP function. The FindAllMarkers function (test.use = presto) was used to identify marker genes of each cluster. Differentially expressed genes(DEGs) were selected using the function FindMarkers (test.use = presto). P value < 0.05 and $|\log_2 \text{foldchange}| > 0.58$ was set as the threshold for significantly differential expression. GO enrichment and KEGG pathway enrichment analysis of DEGs were respectively performed using R(version 4.0.3) based on the hypergeometric distribution.

批注 [A1]: 人：GRCh38 human
小鼠：mm10 mouse

批注 [A2]: 默认 UMI/gene/线粒体 质控过滤标准，如有调整可自行修改

批注 [A3]: 如果进行了样本间去批次处理，可将这句话替换为：
To remove the batch effects in single-cell RNA-sequencing data, the mutual nearest neighbors(MNN) presented by Haghverdi et al was performed with the R package batchelor(version 1.6.3).

Haghverdi L, Lun A T L, Morgan M D, et al. Batch effects in single-cell RNA-sequencing data are corrected by matching mutual nearest neighbors[J]. Nature biotechnology, 2018, 36(5): 421-427.

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[1] Hao, Yuhan, et al. "Integrated analysis of multimodal single-cell data." Cell 184.13 (2021): 3573-3587.

[2] McGinnis, Christopher S., Lyndsay M. Murrow, and Zev J. Gartner. "DoubletFinder: doublet detection in single-cell RNA sequencing data using artificial nearest neighbors." Cell systems 8.4 (2019): 329-337.