注：Method 描述模板仅供参考，请根据实际情况修改使用。

10x Visium spatial RNA-seq data preprocessing

The FASTQ files were processed and aligned to GRCh38 human reference genome using Space Ranger software (version 2.0.1) from 10x Genomics, with unique molecular identifier (UMI) counts summarized for each barcode. To distinguish tissue overlaying spots from the background, tissue overlaying spots were detected according to the images. The filtered UMI count matrix was then analyzed using Seurat[1] (version 4.3.0) R package.

Sctransform[2] was used to normalize data and identify top 3000 highly variable genes (HVGs). Principal component analysis (PCA) was conducted to reduce dimensionality on the log transformed gene-barcode matrices of top variable genes. 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 = bimod) was used to identify marker genes of each cluster.

To infer the cell-type composition of each spot, RCTD[3] (version 1.1.0) was applied. Specifically, the default parameters was used in the creat.RCTD function, except for minimum number of cell > 1 for each cell types and minimum of unique molecular identifier(UMI) counts > 1 per pixel, and doublet_mode parameter was set to FALSE in the run.RCTD function .

Differentially expressed genes(DEGs) were selected using the Seurat[2] function FindMarkers (test.use = presto). P value < 0.05 and |log2foldchange| > 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.

The sequencing and bioinformatics analysis were provided by OE Biotech Co., Ltd. (Shanghai, China).

批注 [A1]: 人：GRCh38 human
小鼠：mm10 mouse

批注 [A2]: 如果样本间存在批次，可将这句话替换为：
The RunHarmony function in harmony (version 0.1.0) R package was performed to remove the batch effects.

Korsunsky, I., Millard, N., Fan, J. et al. Fast, sensitive and accurate integration of single-cell data with Harmony. Nat Methods 16, 1289–1296 (2019)

批注 [A3]: 如最后文章没有用到 RCTD，则本段舍弃。

Reference

[1] Stuart, Tim, et al. "Comprehensive integration of single-cell data." Cell 177.7 (2019): 1888-1902.

[2] Hafemeister, Christoph, and Rahul Satija. "Normalization and variance stabilization of single-cell RNA-seq data using regularized negative binomial regression." Genome biology 20.1 (2019): 296.

[3] Cable, Dylan M., et al. "Robust decomposition of cell type mixtures in spatial transcriptomics." Nature biotechnology 40.4 (2022): 517-526.