Electronic Supplementary Material (ESI) for Molecular Omics.


**Supplementary file 2: Bioinformatics analysis**

## Single-cell RNA-sequencing data processing

We processed single-cell sequencing data following 10x Genomics analysis workflow for 3' Gene Expression v3 Libraries using Cell Ranger software version 6.1.2. The cellranger-count command was used with default parameters unless otherwise specified. The option --include-introns was set to *false* to exclude intronic reads in count. Sequencing reads were aligned on the human reference genome version GRCh38 using the STAR aligner.
Detailed information about the different processes performed by the Cell Ranger pipeline for 3' Single Cell Gene Expression analysis are available on 10x Genomics website at the following URL: https://support.10xgenomics.com/single-cell-gene-expression/software/pipelines/latest/algorithms/overview.
Filtered gene count expression data produced by the pipeline (filtered_feature_bc_matrix) were further visualized and explored using Loupe Browser software version 6. Cell clusters identified on the UMAP dimensional reduction of the data were further screened for the expression of relevant marker genes. Gene expression plots (violin plots, heatmaps) and gene expression tables for significant clusters were then exported from Loupe Browser for additional analysis.

## Hierarchical clustering

Expression tables of selected genes in the different clusters of interest previously exported from Loupe Browser were uploaded on the iDEP96 web interface (Ge et al., 2018; http://bioinformatics.sdstate.edu/idep96/) to perform hierarchical clustering analysis. The heatmaps of gene expression presented on figure 5B and 7A were generated using the following parameters: the "distance" option was set to *correlation*, the "linkage" option was set to *average* and the "cut-off Z score" was set to 4. The "center genes (subtract of the mean)" and "normalized genes (divide by the SD)" options were also applied.

## Pathway analysis

We used ShinyGO (http://bioinformatics.sdstate.edu/go/) for subsequent pathway analysis. The lists of top representative genes of each cluster of interest (obtained previously from the files exported from Loupe Browser) were uploaded in the web ShinyGO interface for gene set enrichment analysis. The enrichment analysis is

calculated based on hypergeometric distribution followed by false discovery rate (FDR) correction (Ge et al., 2020). The FDR cutoff was set at 0.05 in our study.

**References**

Ge SX, Son EW, Yao R: iDEP: an integrated web application for differential expression and pathway analysis of RNA-Seq data. BMC Bioinformatics 2018, 19(1):534

Ge SX et al, ShinyGO: a graphical gene-set enrichment tool for animals and plants, *Bioinformatics*, Volume 36, Issue 8, April 2020, Pages 2628–2629