Supplementary Information for

CellTICS: an explainable neural network for cell-type identification and interpretation based on single-cell RNA-seq data

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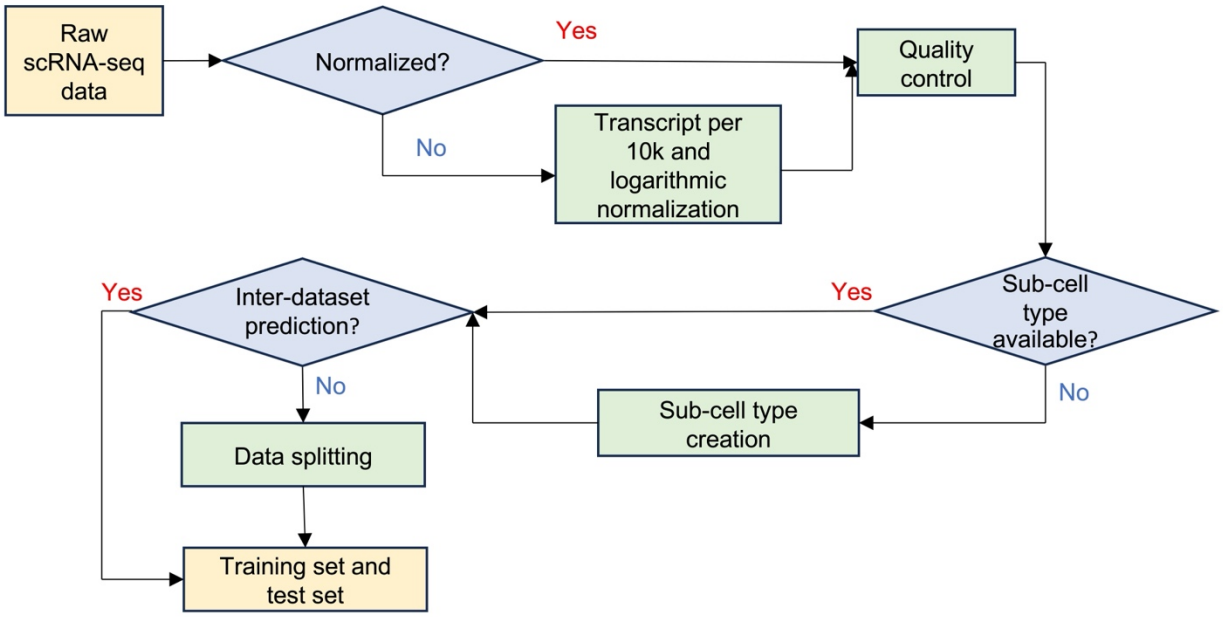
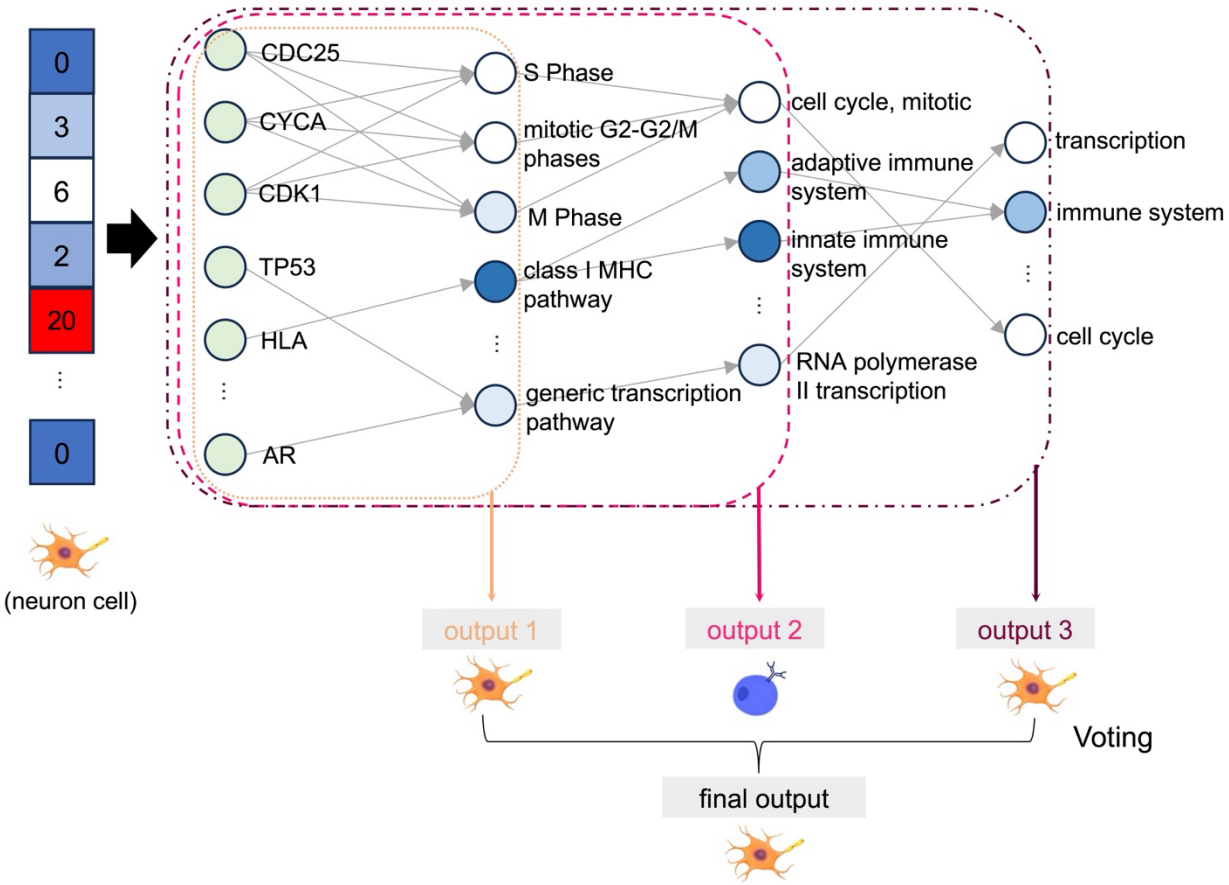
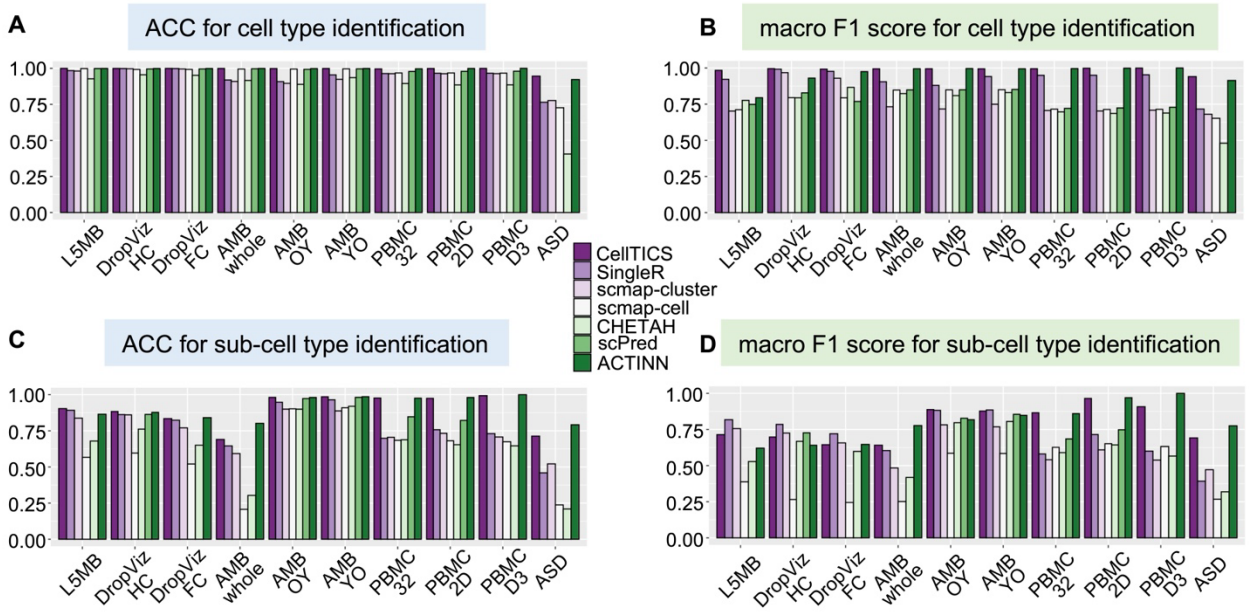


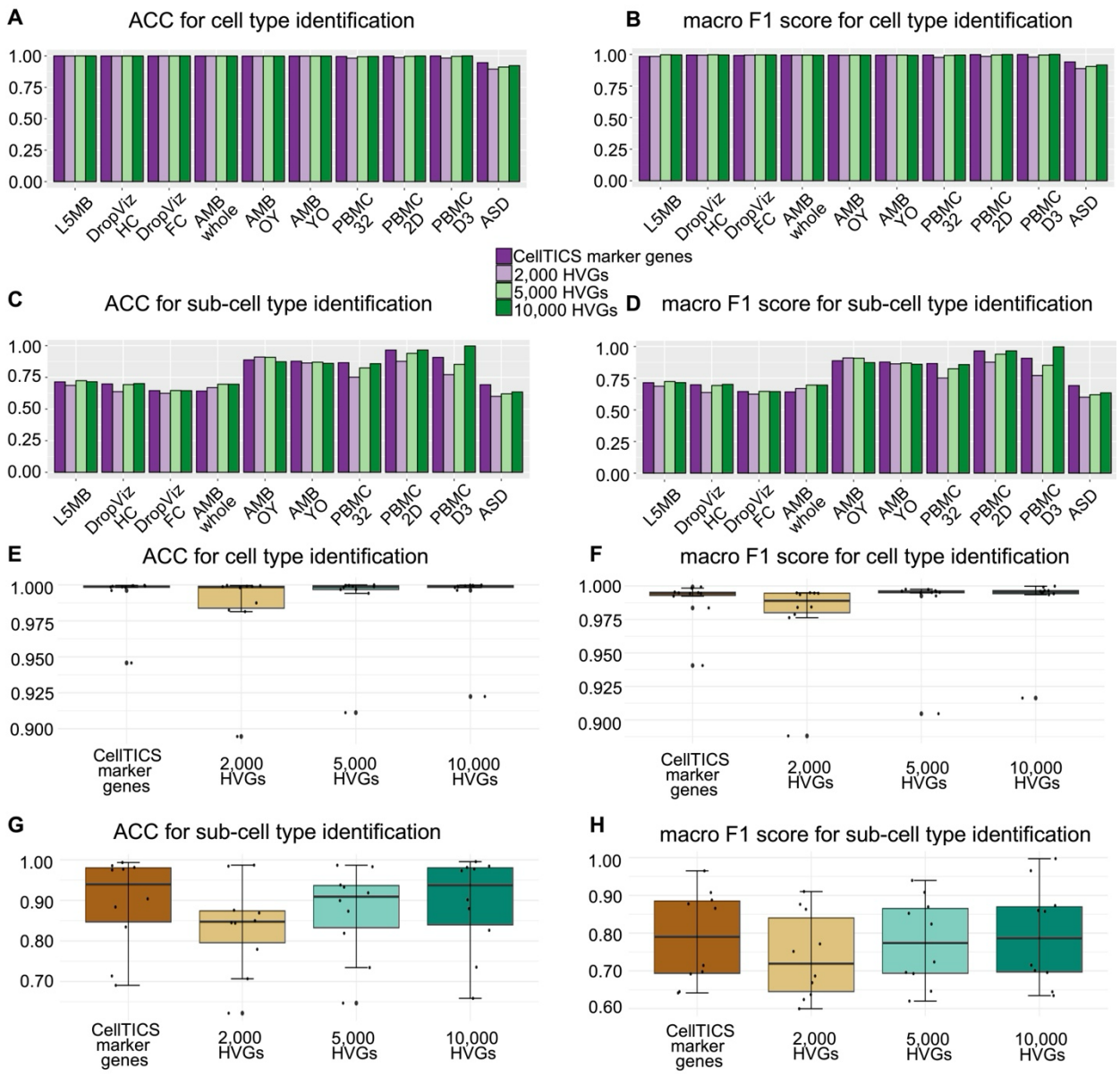
Supplementary Figure S1. Data processing flowchart. Following the acquisition of raw scRNA-seq data, normalization was carried out, contingent upon whether the data had already undergone normalization. Subsequently, after conducting quality control assessments, sub-cell types were generated in cases where they were not initially provided. If the data were not intended for inter-dataset prediction, they were divided into both training and test sets.



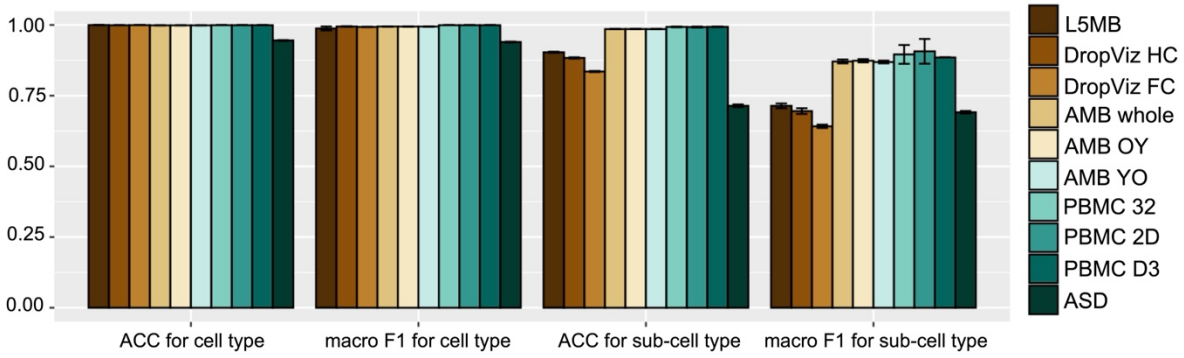
Supplementary Figure S2. The architecture of the CellTICS neural network. In the network, each cell's input comprises the expression levels of all genes within that cell. The input layer corresponds to genes, while the hidden layers model the hierarchical relationships among pathways. After each hidden layer, a predictive layer is introduced to represent cell types. Throughout the training process, significant pathways are identified by harnessing activation values. The predictive results from all predictive layers are then consolidated through a voting mechanism.



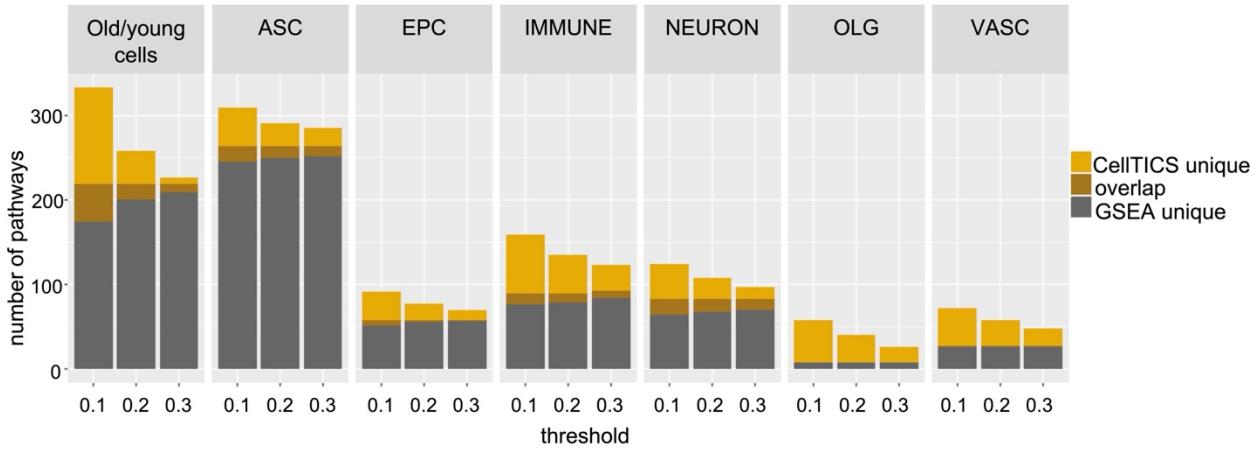
Supplementary Figure S3. The prediction performance of CellTICS and other cell-type identification methods for each analysis task. (A) The ACC for cell-type identification, **(B)** The macro F1 score for cell-type identification, **(C)** The ACC for sub-cell-type identification, **(D)** The macro F1 score for sub-cell-type identification. The ACC and macro F1 scores were average values computed based on five analysis repeats.



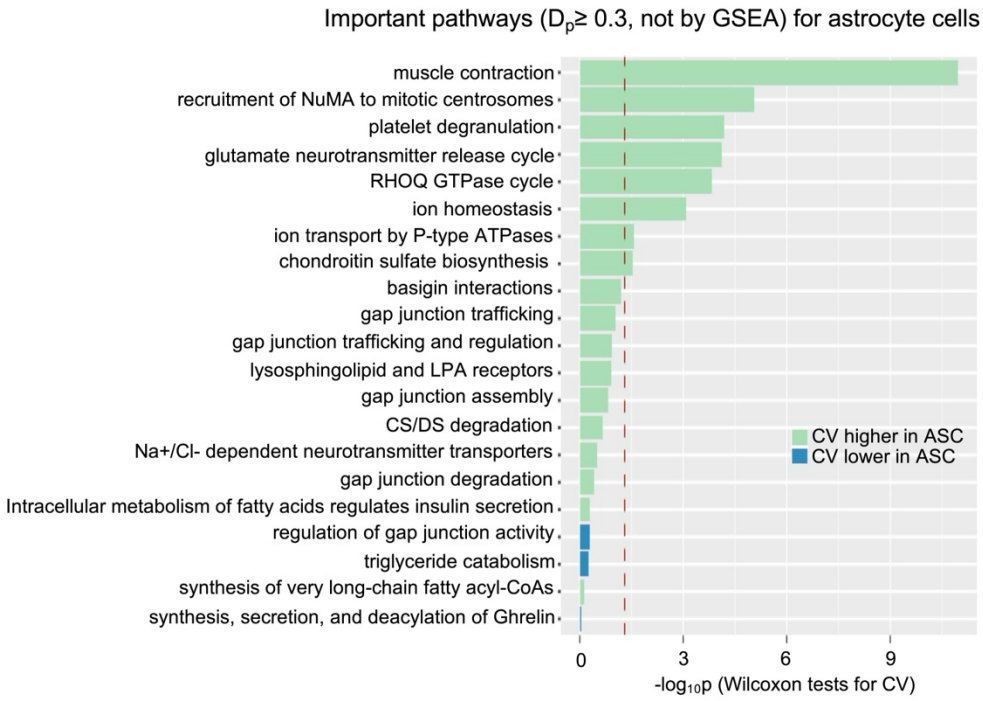
Supplementary Figure S4. Comparison between original CellTICS and CellTICS but using highly variable genes (HVGs). A total of 2000, 5000, or 10000 HVGs were used. A-D are the average values for each dataset. (**A**) The ACC score for cell-type identification. (**B**) The macro F1 score for cell-type identification. (**C**) The ACC score for sub-cell-type identification. (**D**) The macro F1 score for sub-cell-type identification. E-H are boxplots with 10 values for each method. (**E**) The ACC for cell-type identification, (**F**) The macro F1 score for cell-type identification, (**G**) The ACC for sub-cell-type identification, (**H**) The macro F1 score for sub-cell-type identification.



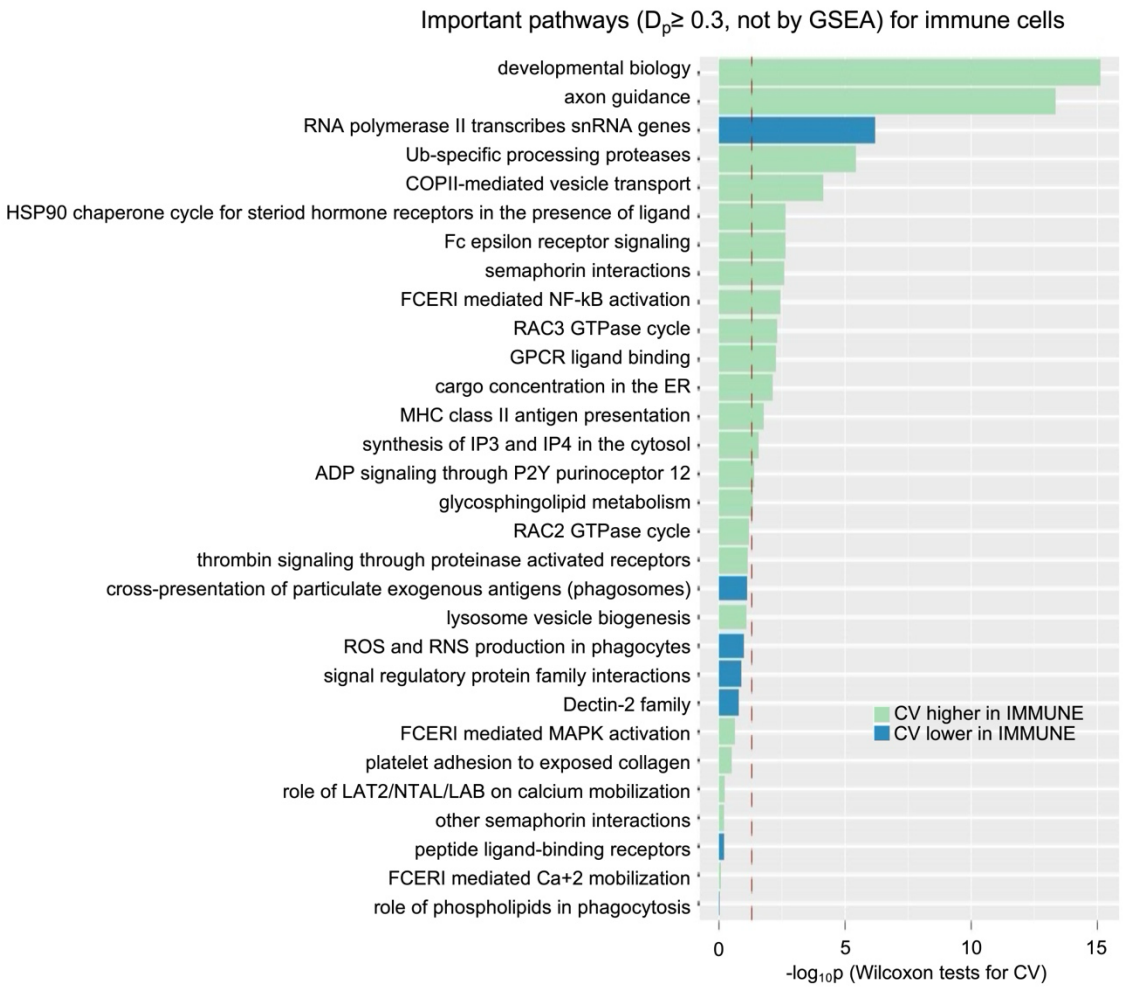
Supplementary Figure S5. Robustness of CellTICS. The average ACC and macro F1 scores of CellTICS for cell-type and sub-cell-type identification across 20 repeats in each dataset are shown. The error bars represent the standard deviation.



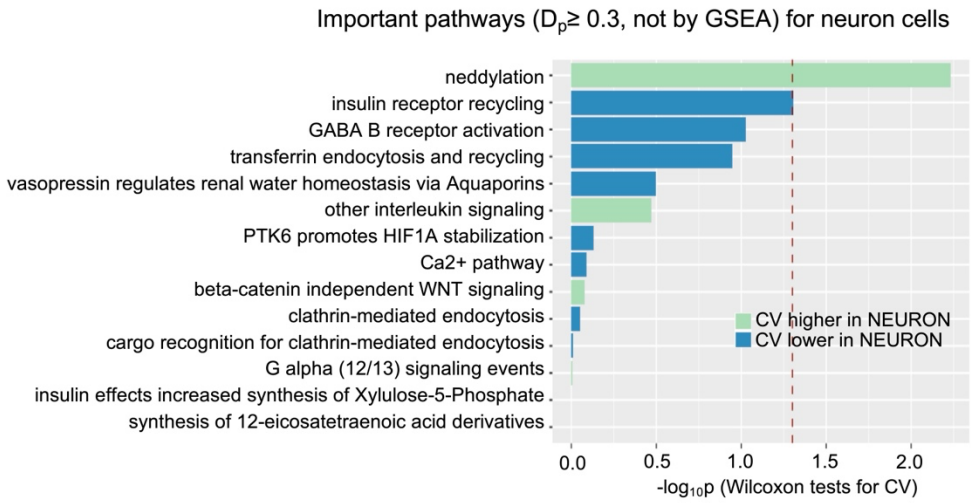
Supplementary Figure S6. Pathway identification of CellTICS with different D_p thresholds. Their overlaps with pathways identified by GSEA are also shown.



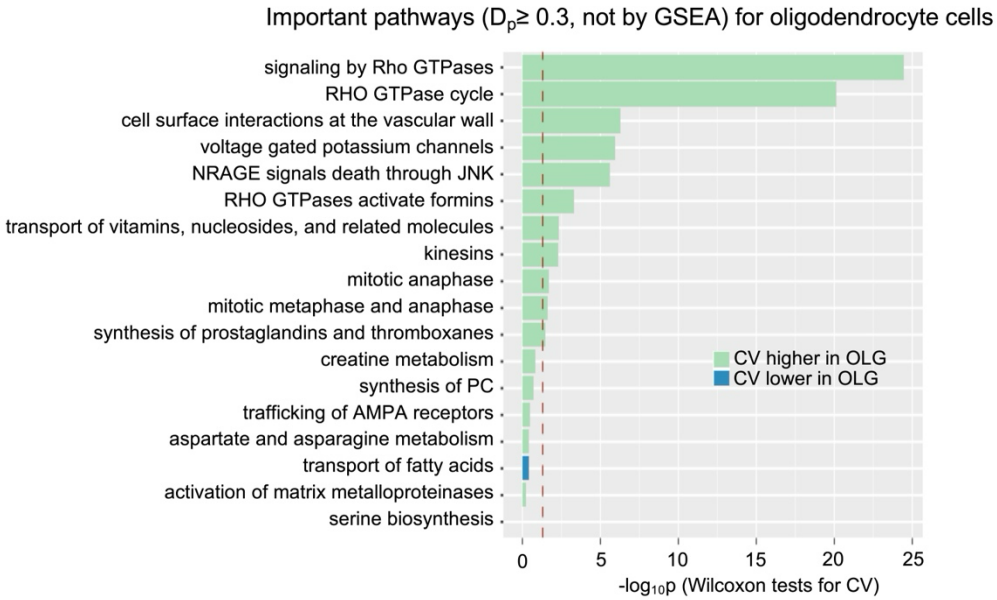
Supplementary Figure S7. Differential expression stochasticity of important pathways identified for astrocytes. Wilcoxon tests are performed to measure the differential expression stochasticity. The red dashed line marks the p-value of 0.05. The color of each pathway indicates whether the group exhibits a higher or lower coefficient of variation.



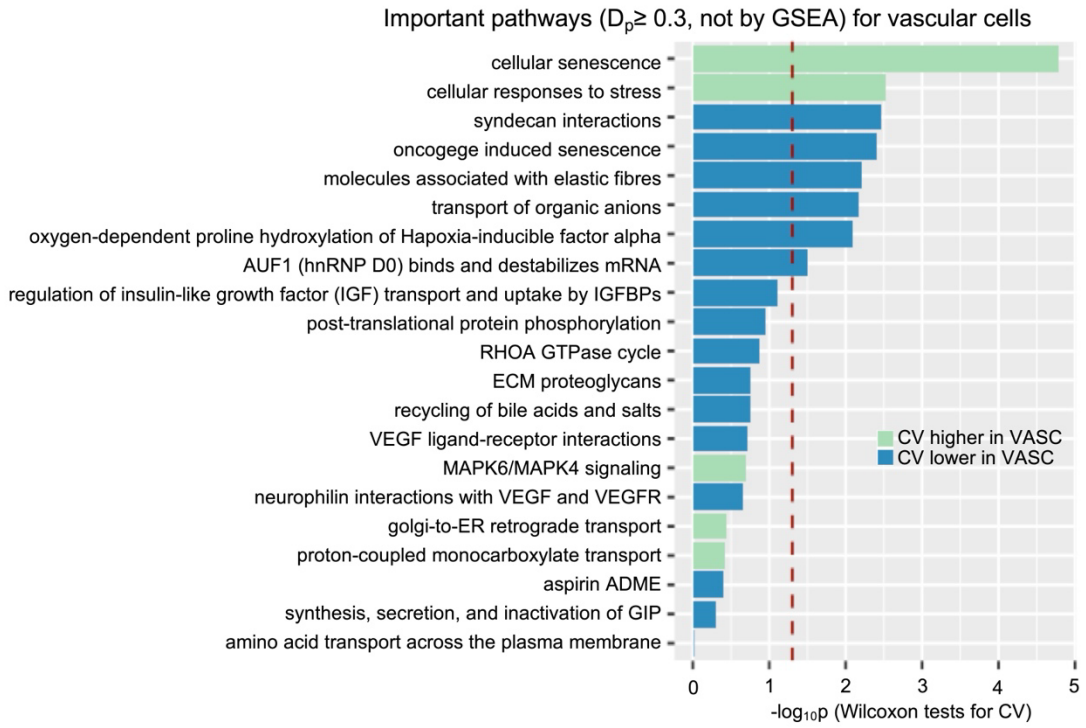
Supplementary Figure S8. Differential expression stochasticity of important pathways identified for immune cells. Wilcoxon tests are performed to measure the differential expression stochasticity. The red dashed line marks the p-value of 0.05. The color of each pathway indicates whether the group exhibits a higher or lower coefficient of variation.



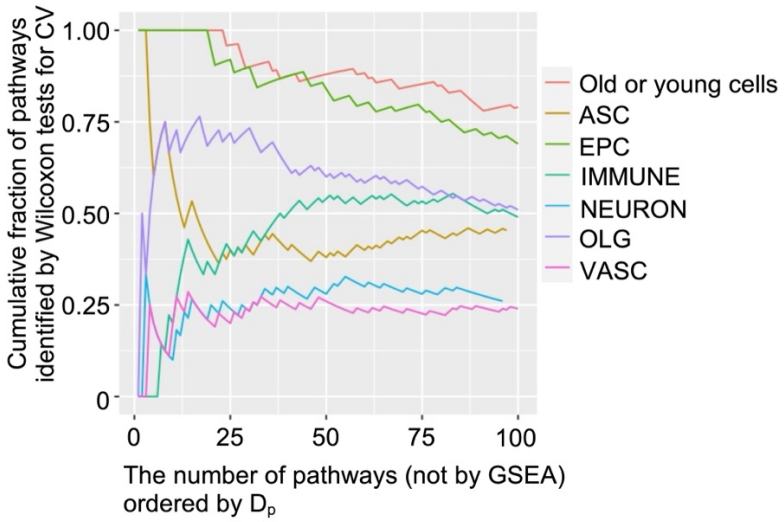
Supplementary Figure S9. Differential expression stochasticity of important pathways identified for neuron cells. Wilcoxon tests are performed to measure the differential expression stochasticity. The red dashed line marks the p-value of 0.05. The color of each pathway indicates whether the group exhibits a higher or lower coefficient of variation.



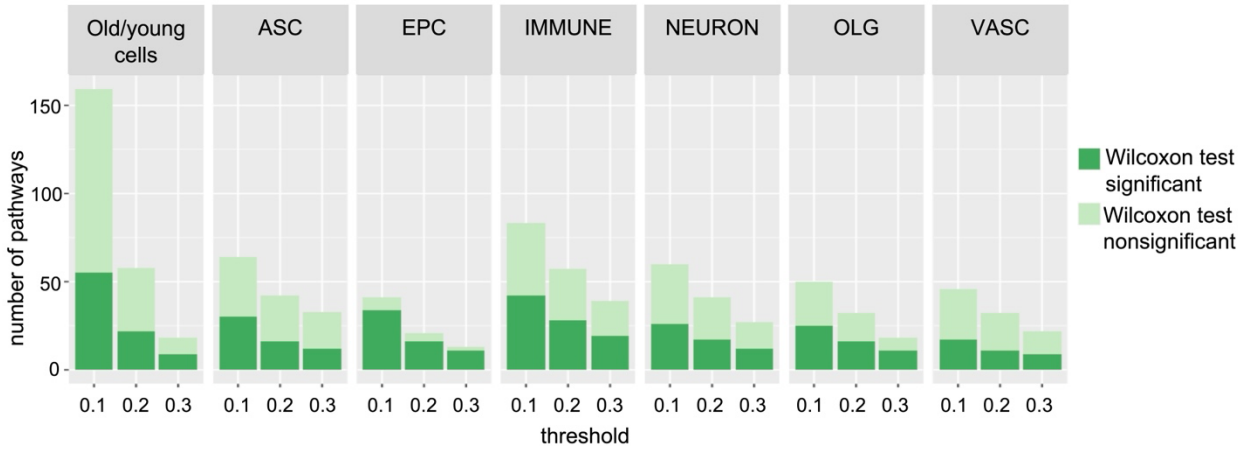
Supplementary Figure S10. Differential expression stochasticity of important pathways identified for oligodendrocytes. Wilcoxon tests are performed to measure the differential expression stochasticity. The red dashed line marks the p-value of 0.05. The color of each pathway indicates whether the group exhibits a higher or lower coefficient of variation.



Supplementary Figure S11. Differential expression stochasticity of important pathways identified for vascular cells. Wilcoxon tests are performed to measure the differential expression stochasticity. The red dashed line marks the p-value of 0.05. The color of each pathway indicates whether the group exhibits a higher or lower coefficient of variation.



Supplementary Figure S12. Shared pathways between CellTICS and Wilcoxon tests on CVs. The cumulative fraction of pathways identified by Wilcoxon tests on CVs is plotted along pathways with decreasing D_p values in CellTICS. These pathways are not identified by GSEA.



Supplementary Figure S13. Differential expression stochasticity of CellTICS-unique pathways. These pathways are identified by CellTICS with different D_p thresholds, and they are not identified by GSEA. Differential expression stochasticity is measured by the Wilcoxon test (p-value ≤ 0.05).

Supplementary Table S1. Information about the datasets used in CellTICS for predicting cell types or sub-cell types.

Dataset	Description	Number of cell types	Number of sub-cell types	Number of genes	Number of cells	Data file size	Link
L5MB	Level 5 adolescent mouse brain cells	7	Training set: 237	27998	Training set: 20731	1080 MB	http://mousebrain.org/
			Test set: 220		Test set: 6811	365MB	
DropViz HC	DropViz mouse brain hippocampus cells	11	Training set: 101	17874	Training set: 19347	661MB	http://dropviz.org/
			Test set: 100		Test set: 6443	220MB	
DropViz FC	DropViz mouse brain frontal cortex cells	9	Training set: 80	18533	Training set: 25157	891MB	
			Test set: 78		Test set: 8380	297MB	
AMB whole	Aging mouse brain cells of all mice	6	12 (old and young for each cell type)	13669	Training set: 27453	987 MB	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE129788
					Test set: 9148	419 MB	
AMB OY	Aging mouse brain cells, using old to predict young	6	25	13669	Old: 20746	950 MB	
					Young: 15855	632 MB	
AMB YO	Aging mouse brain cells, using old to predict young	6	25	13669	Young: 15855	632 MB	
					Old: 20746	950 MB	
PBMC 32	Peripheral blood mononuclear cells, using 10Xv3 to predict 10Xv2	3	10Xv3: 8	21905	10Xv3: 19690	650 MB	https://doi.org/10.5281/zenodo.3357167
			10Xv2: 9	22280	10Xv2: 23154	772 MB	
PBMC 2D	Peripheral blood mononuclear cells, using 10Xv2 to predict	3	9	22280	23154	772 MB	
				16480		730 MB	

	Drop-seq						
PBMC D3	Peripheral blood mononuclear cells, using Drop-seq to predict 10Xv3	3	Drop-seq: 9	16480	Drop-seq: 23154	730 MB	
			10Xv3: 8	21905	10Xv3: 19690	650 MB	
ASD	Autism spectrum disorder human cells	11	22 (ASD and control for each cell type)	36501	Training set: 45641	4760 MB	https://cells.ucsc.edu/?ds=autism
					Test set: 15208	1580 MB	

Supplementary Table S2. Run time of CellTICS across all datasets.

Dataset	L5MB	DropViz HC	DropViz FC	AMB whole	AMB OY	AMB YO	PBMC 32	PBMC 2D	PBMC D3	ASD
Run time(s)	1413	1382	1559	1600	1291	1100	1223	1320	1324	4643