

以 GTEx edQTL 分析 IgA 肾病中的 RNA 编辑

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1 摘要

2 前言

最近的研究表明，RNA 编辑是常见炎症性疾病遗传风险的基础，相当的 cis-RNA editing QTLs (edQTLs) 在自身免疫和免疫介导疾病的全基因组关联研究信号中显着富集¹。RNA 编辑蛋白 ADAR 介导的腺昔-肌昔 (A-to-I) RNA 编辑是防止细胞自身双链 RNA (dsRNA) 引发先天免疫干扰素应答的重要转录后事件，因遗传因素引起的双链 RNA 编辑水平的降低，是导致炎症性疾病遗传风险升高的重要因素¹。RNA 编辑拓宽了自身免疫性疾病治疗和机制探究的领域。IgA 肾病 (IgA nephropathy) 是 CKD 和肾衰竭的主要原因²，发病机制尚不明确，被推測为一种遗传 (genetic) 相关疾病³。然而，还没有研究从 RNA 编辑的角度阐述 IgA 肾病的发生或发展机制。在本研究中，选用了一组 IgA 肾病的单细胞数据集 (scRNA-seq)，并借助 GTEx 的 edQTL 数据集，探究 IgA 肾病细胞中可能的 RNA 编辑事件。

3 材料和方法

3.1 材料

All used GEO expression data and their design:

- **GSE171314:** Single-cell RNA sequencing (scRNA-seq) was applied to kidney biopsies from 4 IgAN and 1 control subjects to define the transcriptomic landscape at the single-cell resolution.

3.2 方法

Mainly used method:

- Package biomaRt used for gene annotation.⁴
- CellChat used for cell communication analysis.⁵
- The edQTL data were obtained from GTEx database.⁶
- ClusterProfiler used for gene enrichment analysis.⁷
- GEO <https://www.ncbi.nlm.nih.gov/geo/> used for expression dataset acquisition .
- ClusterProfiler used for GSEA enrichment.⁷
- .
- RISC used for scRNA-seq data integration.⁸
- Seurat used for scRNA-seq processing.^{9,10}
- Other R packages used for statistic analysis or data visualization.

4 分析结果

4.1 IgA 肾病组织和 Normal 组织

Figure 1 (下方图) 为图 MAIN IgA and Normal dataset 概览。

(对应文件为 ./Figure+Table/fig1.pdf)

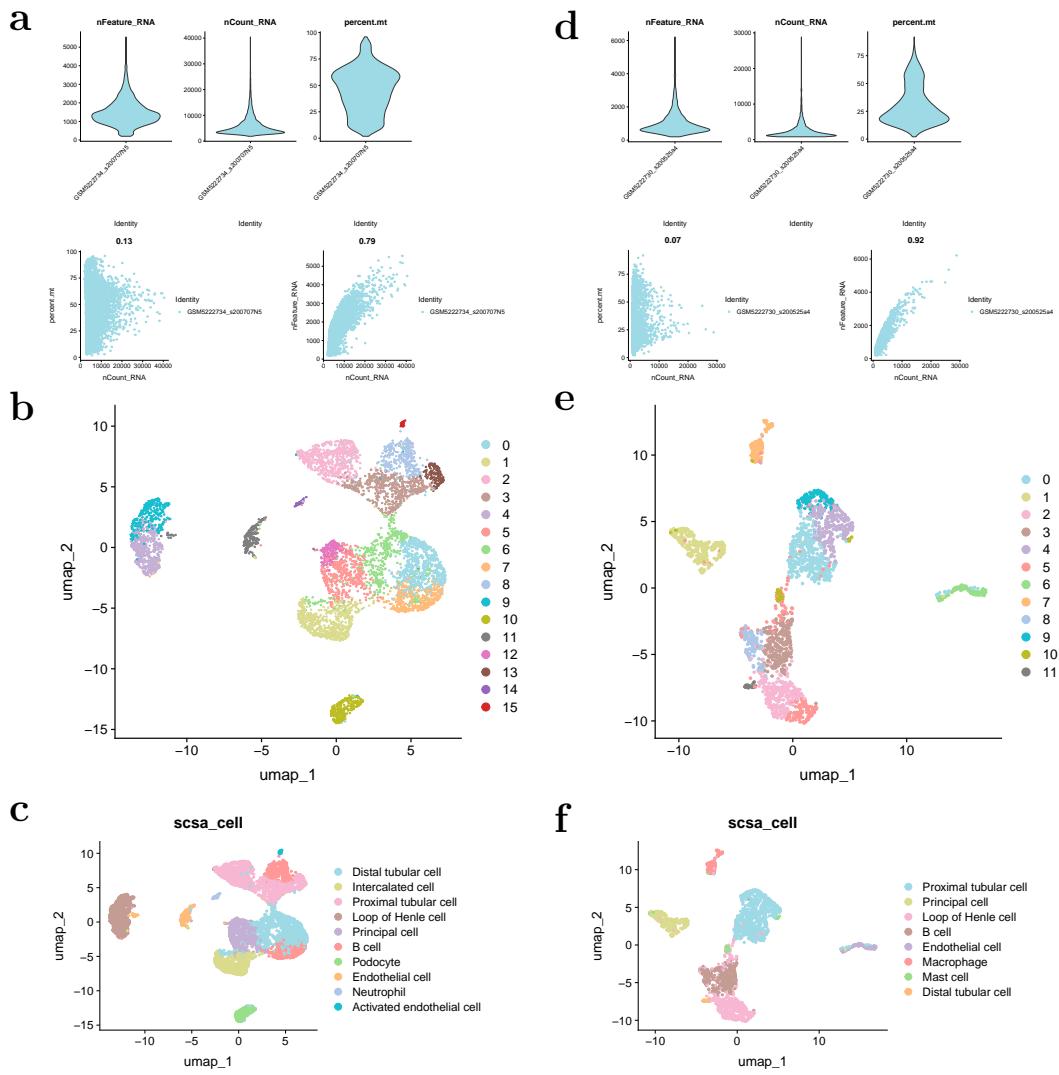


Figure 1: MAIN IgA and Normal dataset

4.2 IgA 肾病组织的细胞通讯

4.3 IgA 和 Normal 的集成分析

Figure 2 (下方图) 为图 MAIN integrated dataset of B cells 概览。

(对应文件为 ./Figure+Table/fig2.pdf)

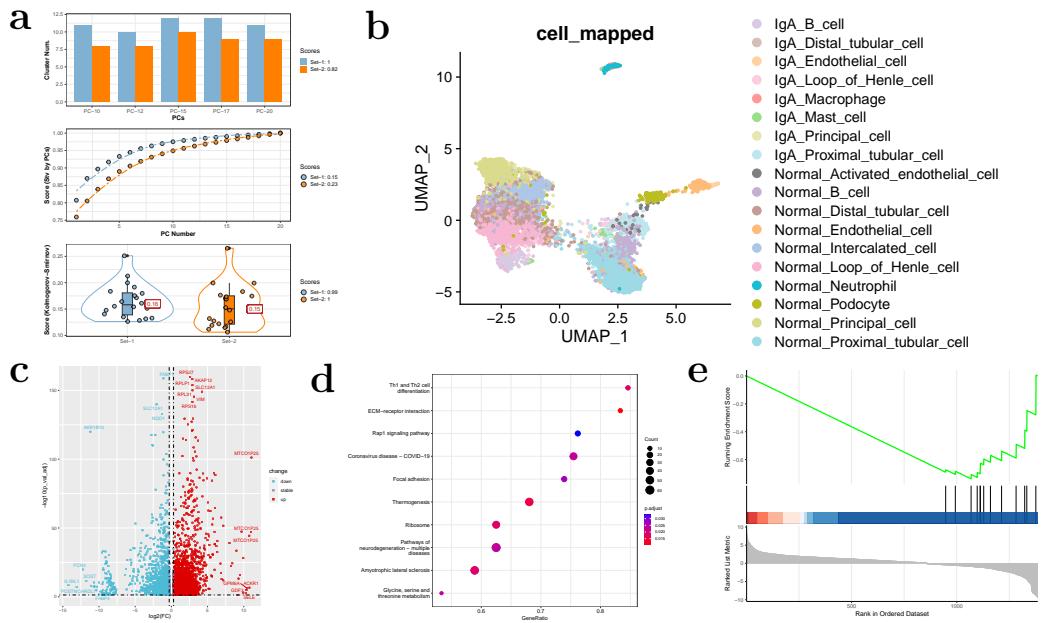


Figure 2: MAIN integrated dataset of B cells

4.4 IgA 肾病 B 细胞可能的 RNA 编辑

Figure 3 (下方图) 为图 MAIN possibly RNA editing site in B cells 概览。

(对应文件为 ./Figure+Table/fig3.pdf)

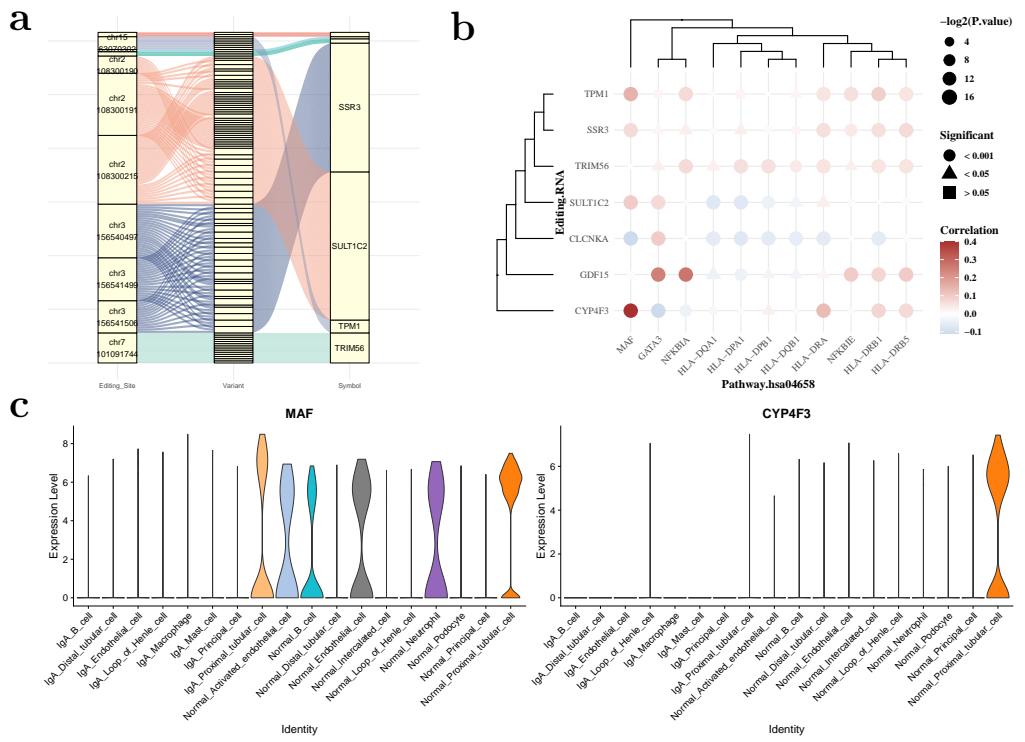


Figure 3: MAIN possibly RNA editing site in B cells

5 结论

6 附：分析流程

6.1 IgA 肾病和正常组织相比

6.1.1 IgA scRNA-seq

Figure 4 (下方图) 为图 IgA Quality Control 概览。

(对应文件为 Figure+Table/IgA-Quality-Control.pdf)

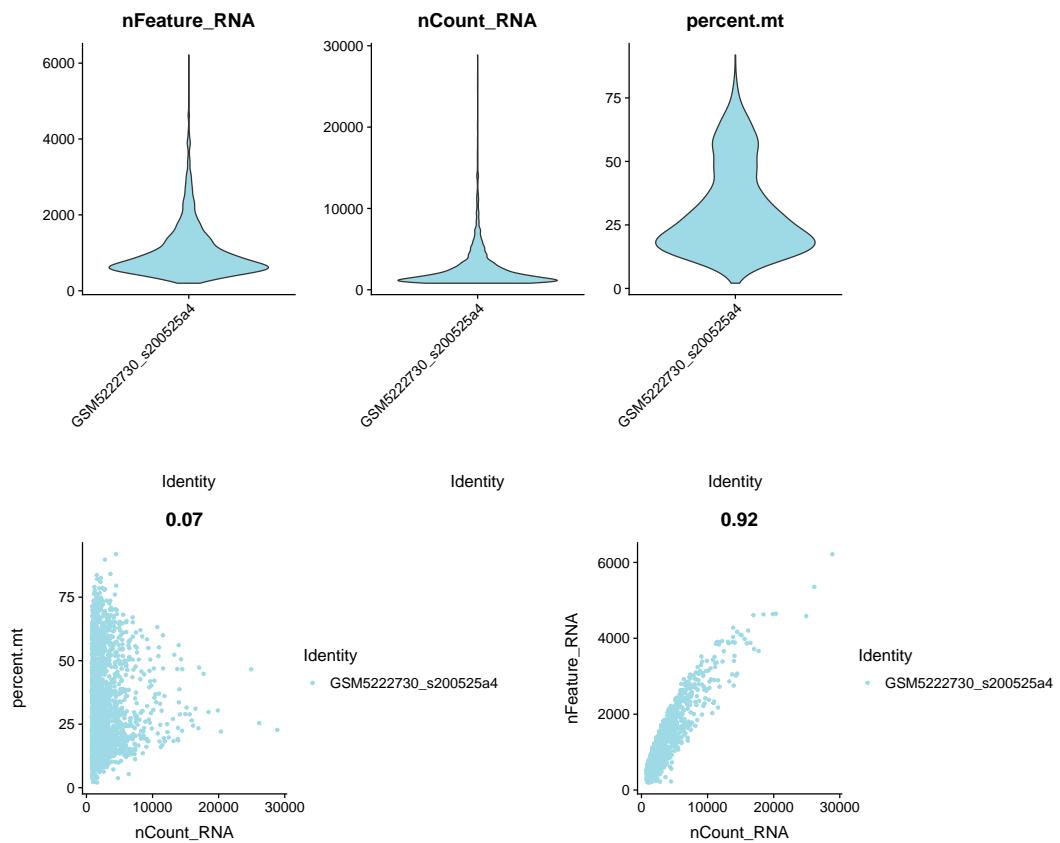


Figure 4: IgA Quality Control

Figure 5 (下方图) 为图 IgA Ranking of principle components 概览。

(对应文件为 Figure+Table/IgA-Ranking-of-principle-components.pdf)

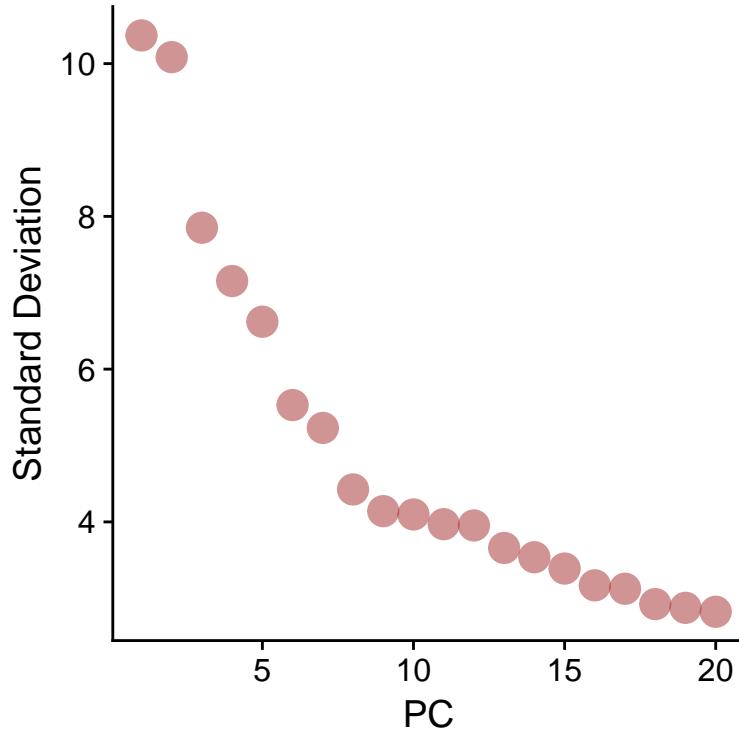


Figure 5: IgA Ranking of principle components

Figure 6 (下方图) 为图 IgA UMAP Clustering 概览。

(对应文件为 Figure+Table/IgA-UMAP-Clustering.pdf)

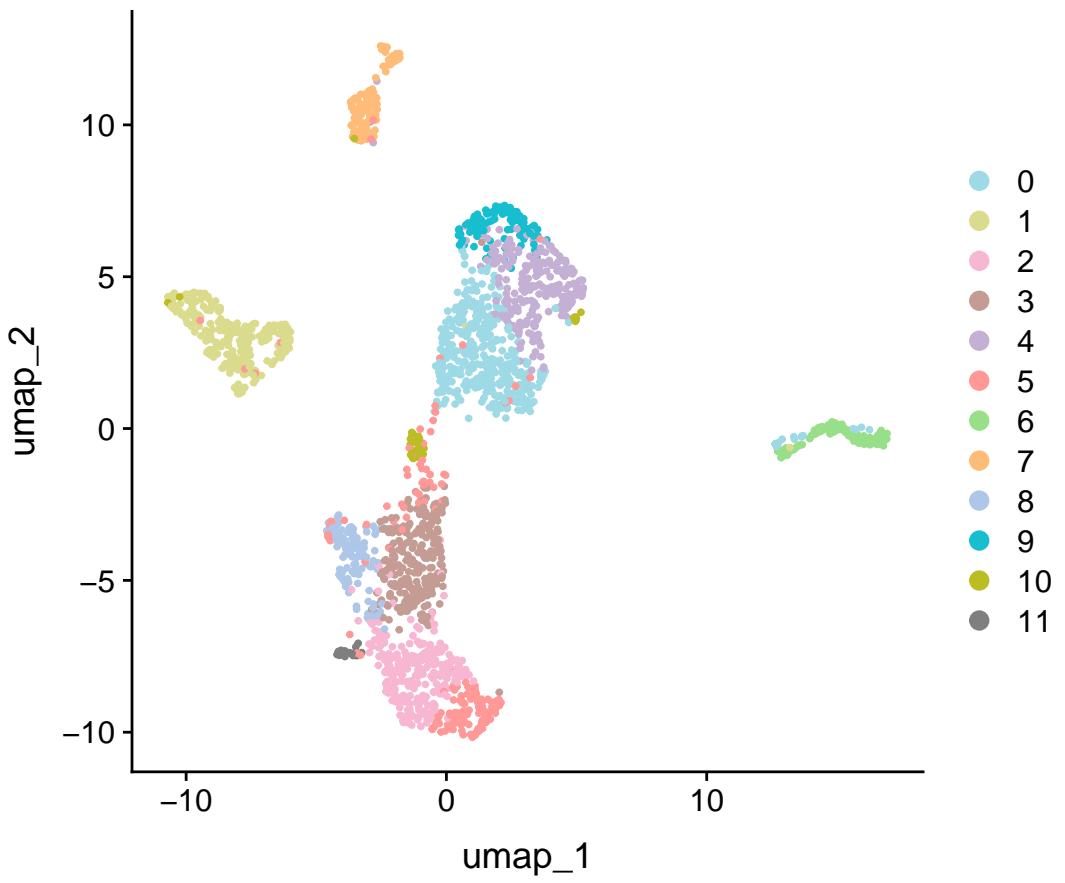


Figure 6: IgA UMAP Clustering

Figure 7 (下方图) 为图 IgA SCSA Cell type annotation 概览。

(对应文件为 Figure+Table/IgA-SCSA-Cell-type-annotation.pdf)

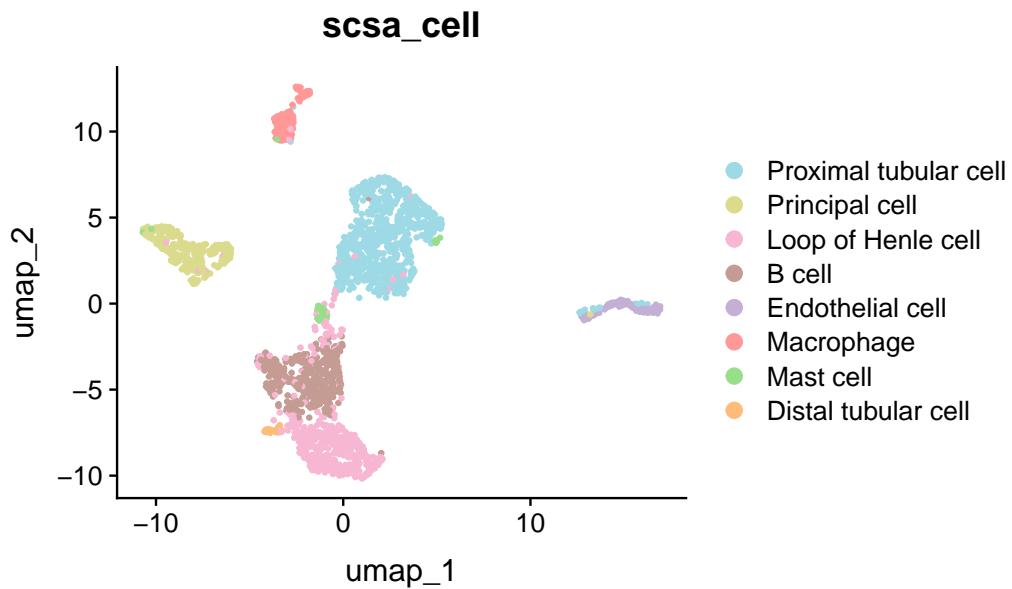


Figure 7: IgA SCSA Cell type annotation

6.1.2 Normal scRNA-seq

Figure 8 (下方图) 为图 Normal Quality Control 概览。

(对应文件为 Figure+Table/Normal-Quality-Control.pdf)

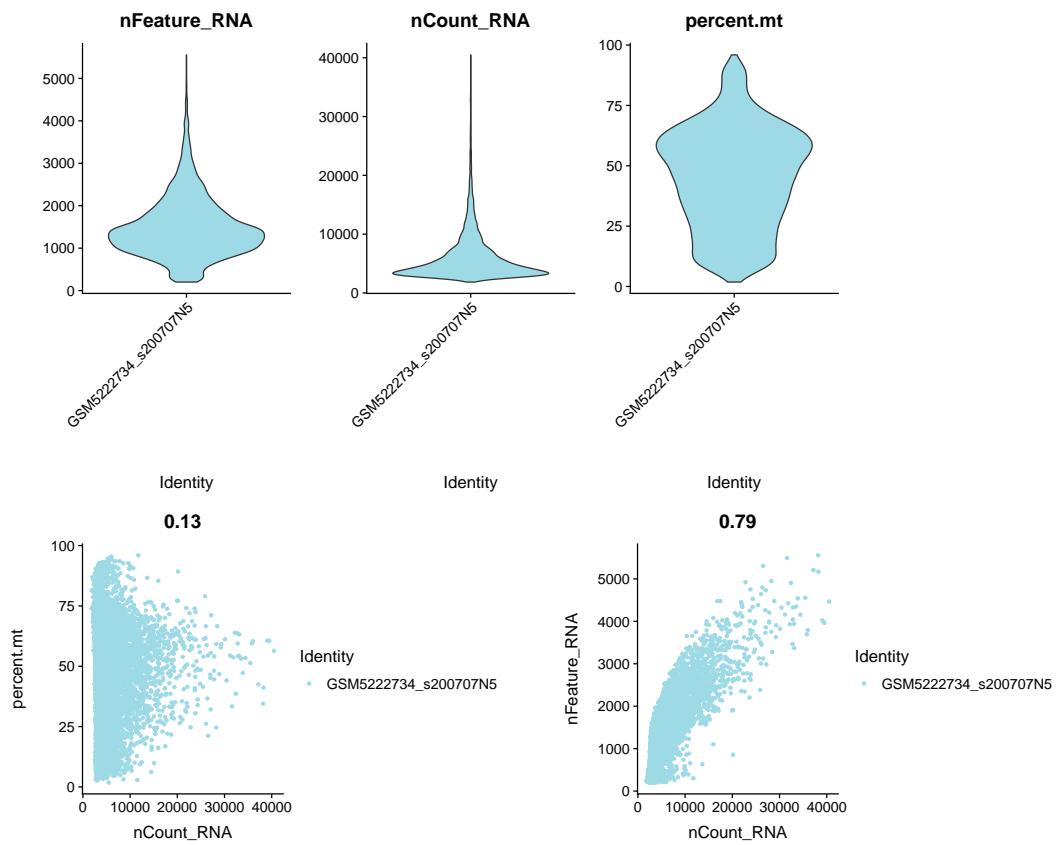


Figure 8: Normal Quality Control

Figure 9 (下方图) 为图 Normal Ranking of principle components 概览。

(对应文件为 Figure+Table/Normal-Ranking-of-principle-components.pdf)

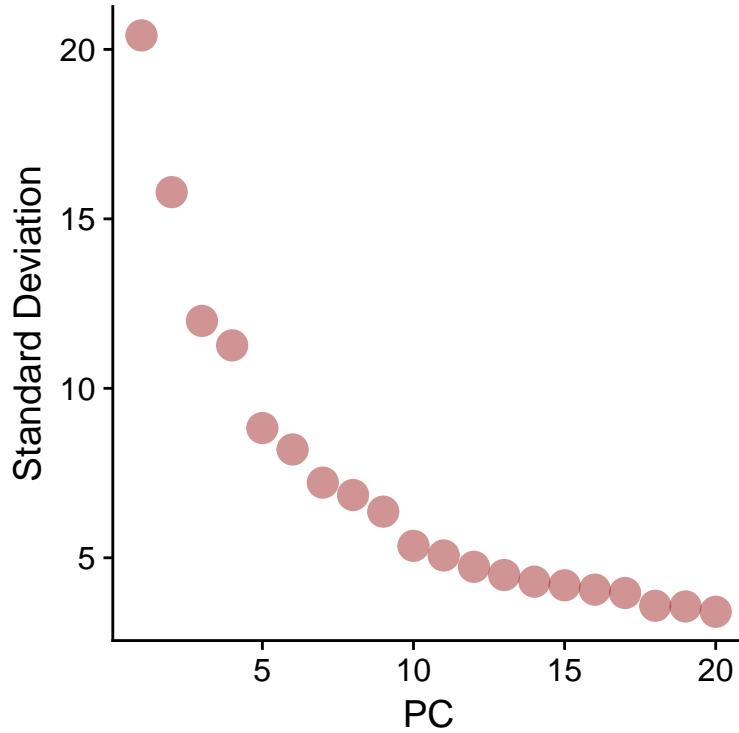


Figure 9: Normal Ranking of principle components

Figure 10 (下方图) 为图 Normal UMAP Clustering 概览。

(对应文件为 Figure+Table/Normal-UMAP-Clustering.pdf)

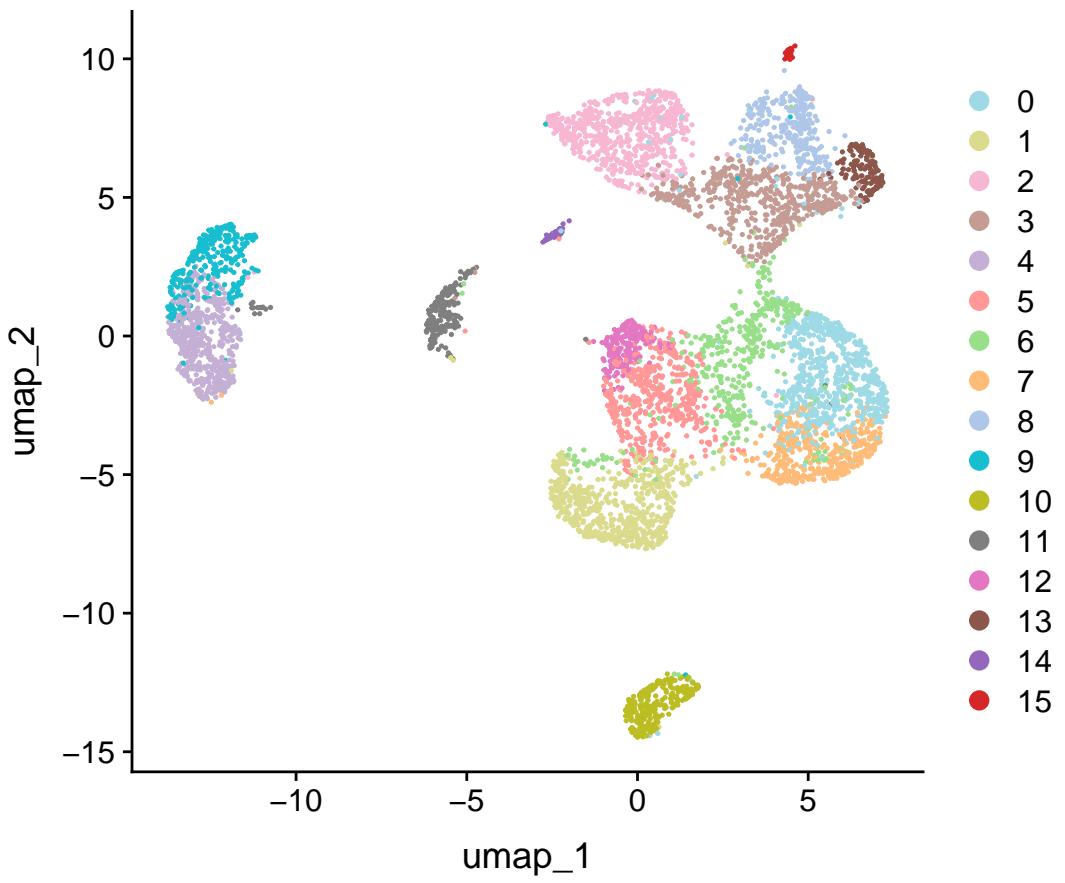


Figure 10: Normal UMAP Clustering

Figure 11 (下方图) 为图 Normal SCSA Cell type annotation 概览。

(对应文件为 Figure+Table/Normal-SCSA-Cell-type-annotation.pdf)

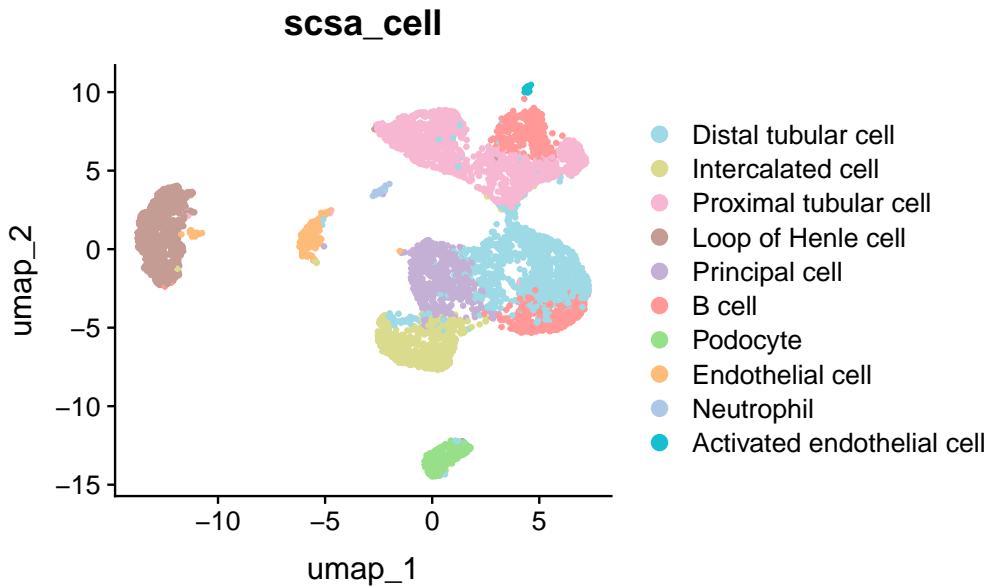


Figure 11: Normal SCSA Cell type annotation

6.2 IgA 肾病细胞的通讯

Figure 12 (下方图) 为图 IgA overall communication count 概览。

(对应文件为 Figure+Table/IgA-overall-communication-count.pdf)

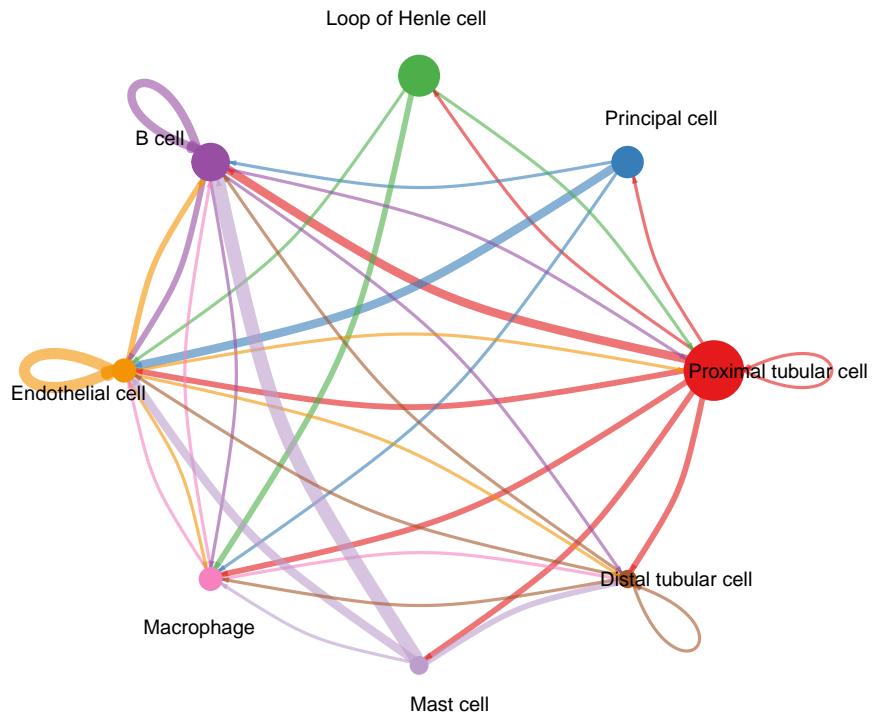


Figure 12: IgA overall communication count

Figure 13 (下方图) 为图 IgA Cell communication heatmap 概览。

(对应文件为 [Figure+Table/IgA-Cell-communication-heatmap.pdf](#))

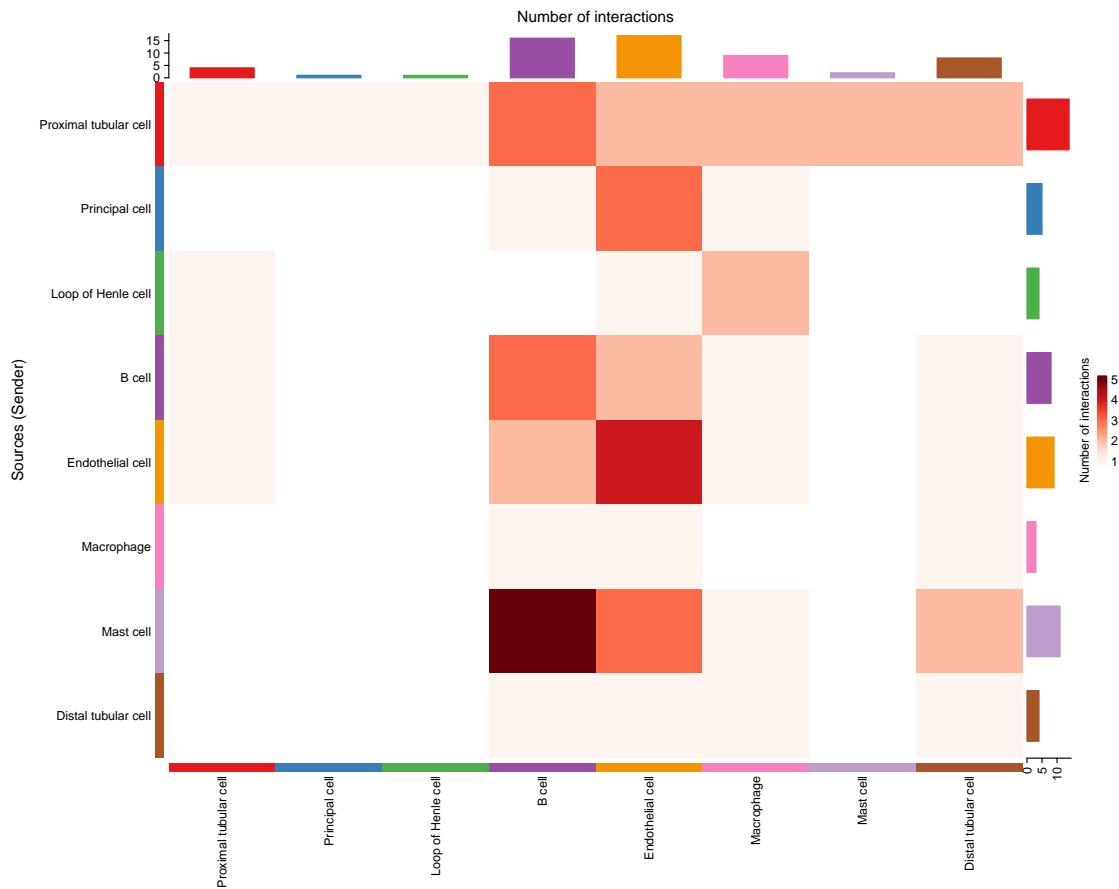


Figure 13: IgA Cell communication heatmap

Figure 14 (下方图) 为图 IgA All ligand receptor role 概览。

(对应文件为 Figure+Table/IgA-All-ligand-receptor-role.pdf)

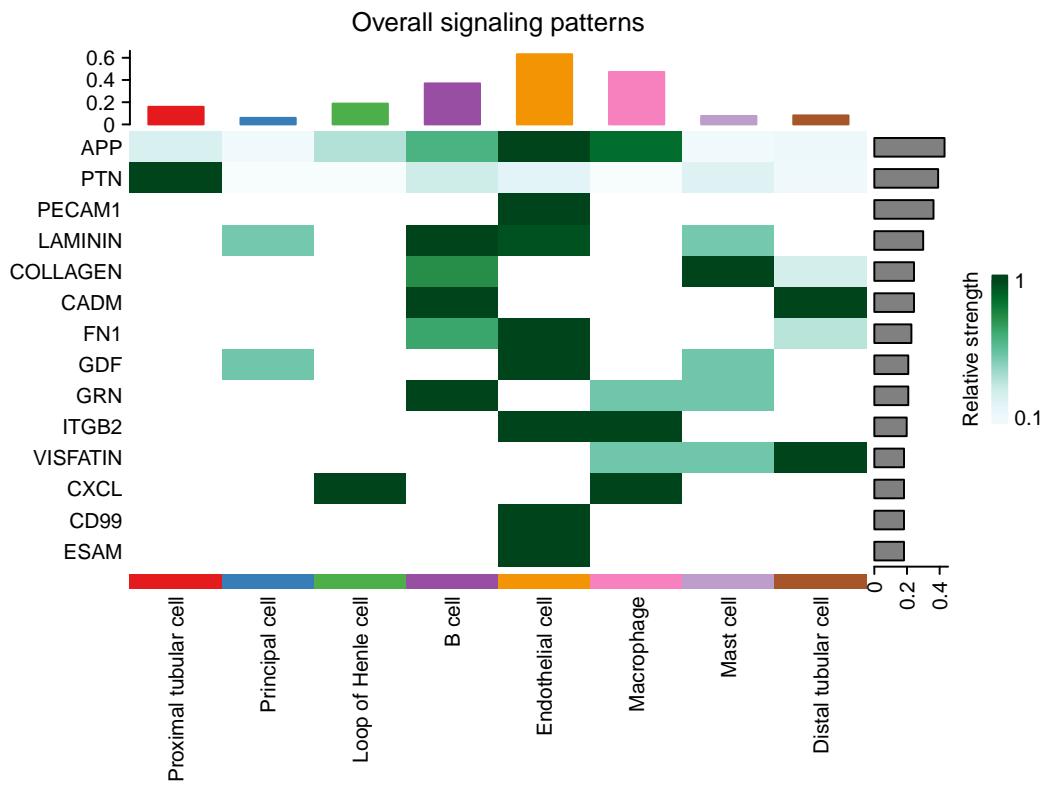


Figure 14: IgA All ligand receptor role

Figure 15 (下方图) 为图 IgA ligand receptor of B cell communicate with Mast cell 概览。

(对应文件为 Figure+Table/IgA-ligand-receptor-of-B-cell-communicate-with-Mast-cell.pdf)

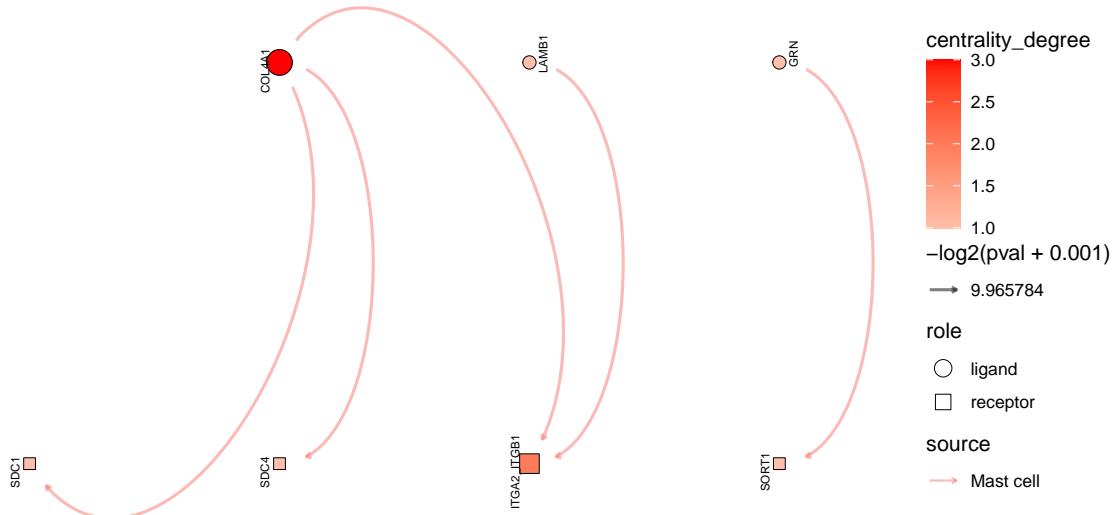


Figure 15: IgA ligand receptor of B cell communicate with Mast cell

6.3 IgA 和 Normal 的集成分析

6.3.1 RISC integration

Figure 16 (下方图) 为图 Integrated dataset select reference dataset for integration 概览。

(对应文件为 Figure+Table/Integrated-dataset-select-reference-dataset-for-integration.pdf)

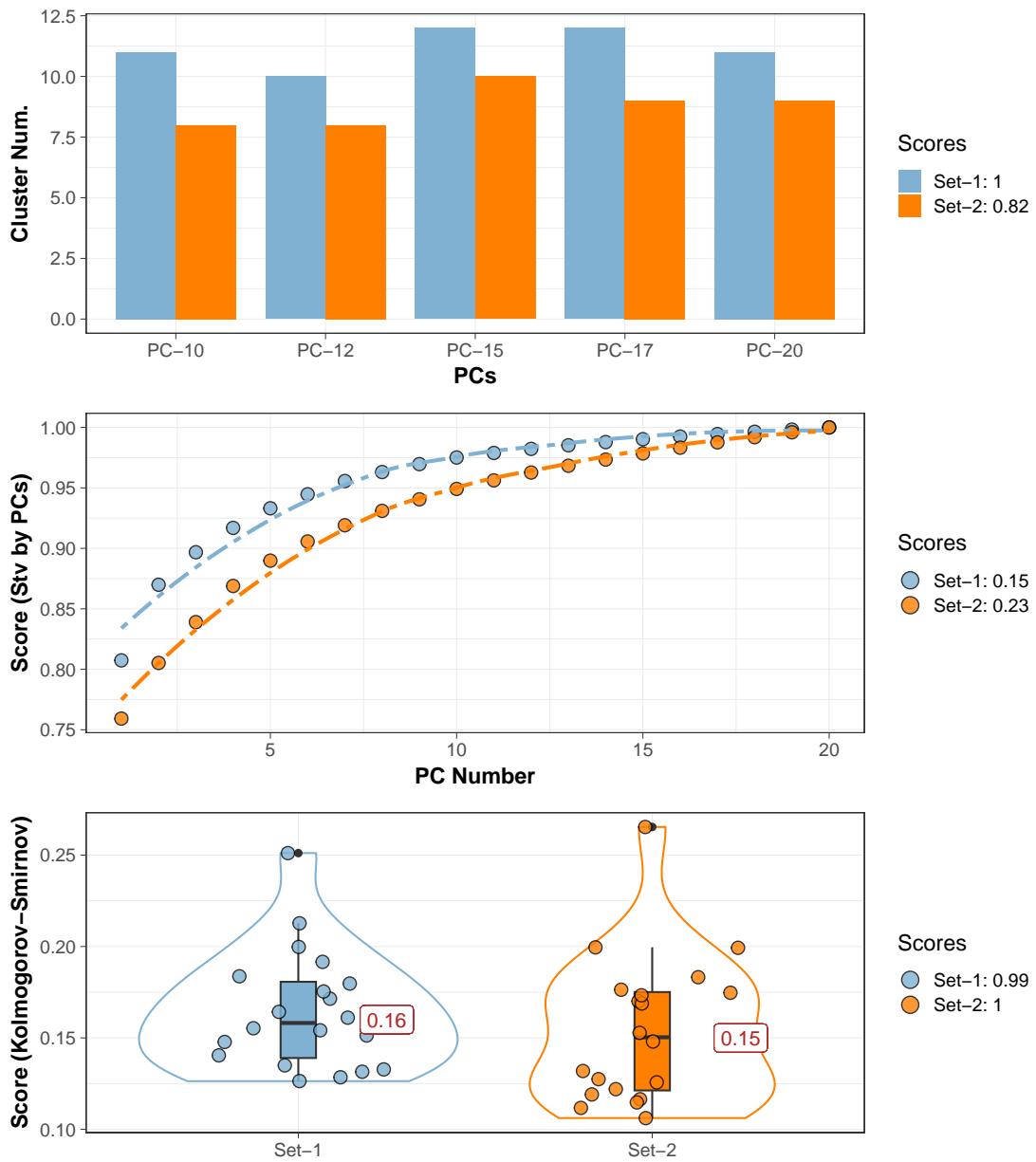


Figure 16: Integrated dataset select reference dataset for integration

Figure 17 (下方图) 为图 Integrated dataset The cell mapped 概览。

(对应文件为 Figure+Table/Integrated-dataset-The-cell-mapped.pdf)

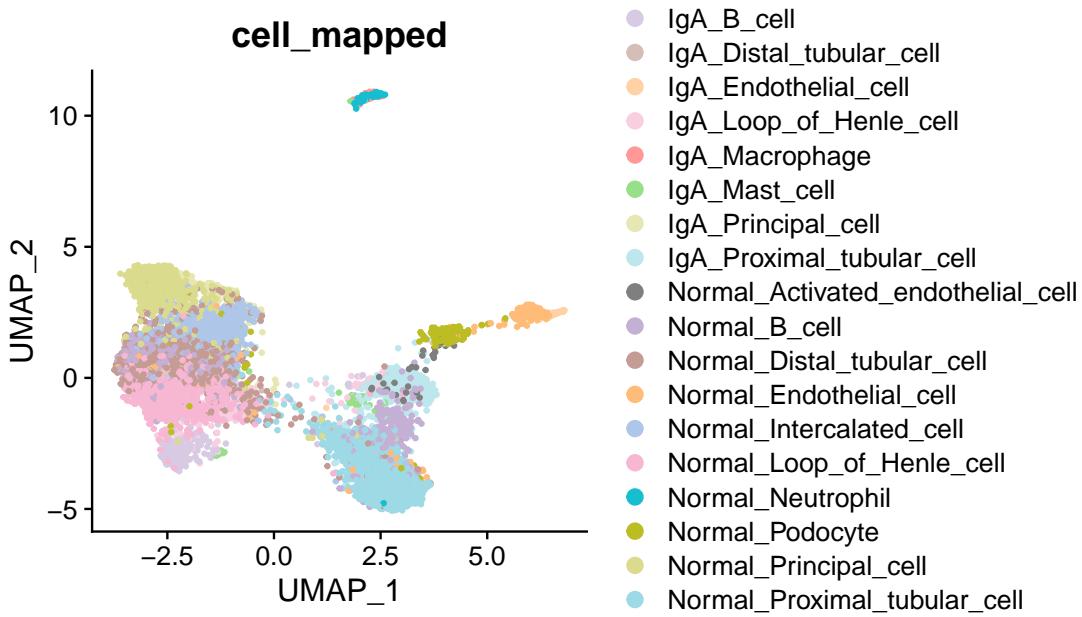


Figure 17: Integrated dataset The cell mapped

6.3.2 AS Seurat object

Figure 18 (下方图) 为图 Integrated IgA B cell vs Normal B cell 概览。

(对应文件为 Figure+Table/Integrated-IgA-B-cell-vs-Normal-B-cell.pdf)

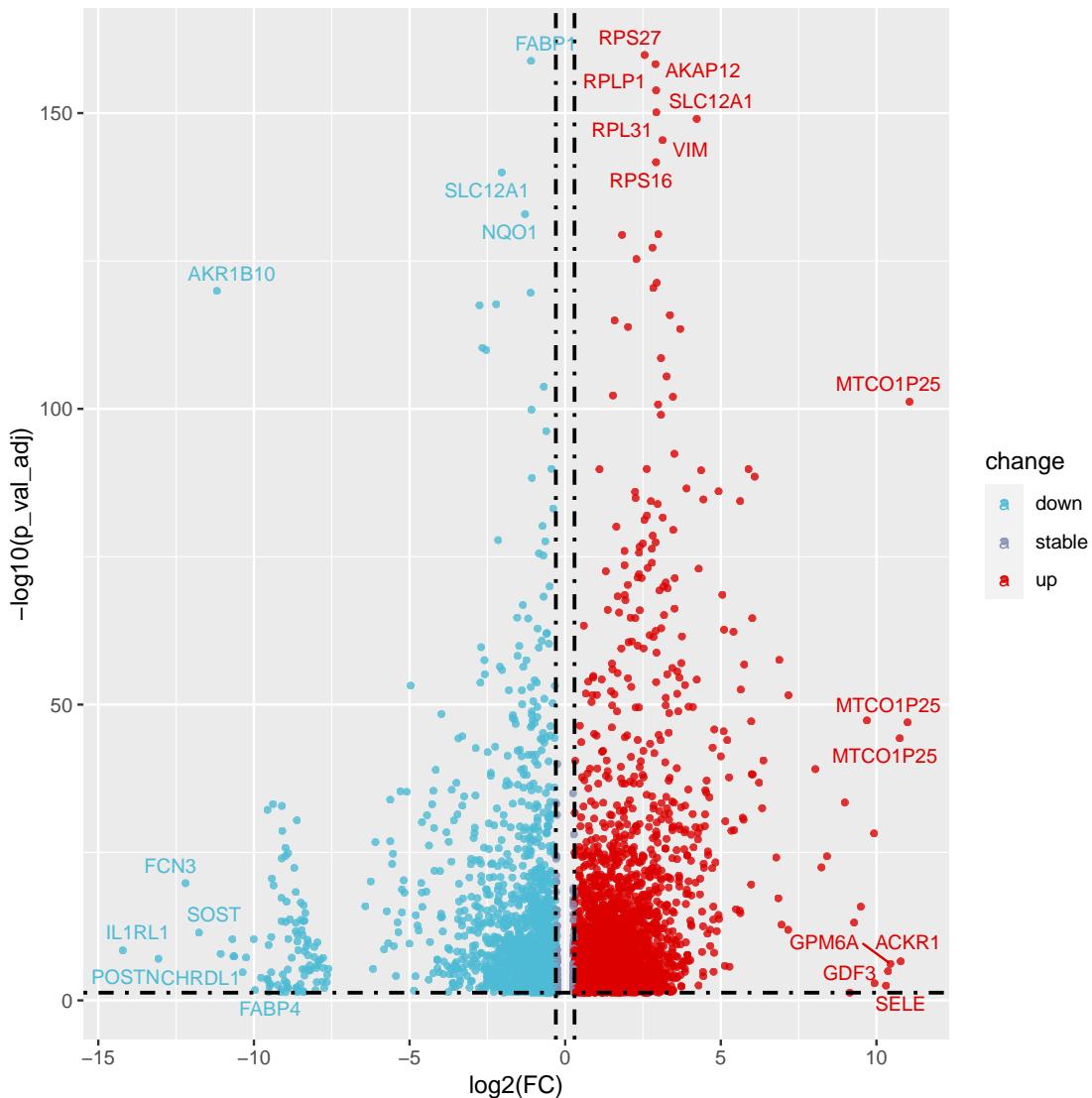


Figure 18: Integrated IgA B cell vs Normal B cell

在 R 中，重新将 RISC 对象转化为 Seurat 对象进行差异分析。

6.3.3 Enrichment

Figure 19 (下方图) 为图 Integrated dataset IgA B cell vs Normal B cell KEGG enrichment 概览。

(对应文件为 Figure+Table/Integrated-dataset-IgA-B-cell-vs-Normal-B-cell-KEGG-enrichment.pdf)

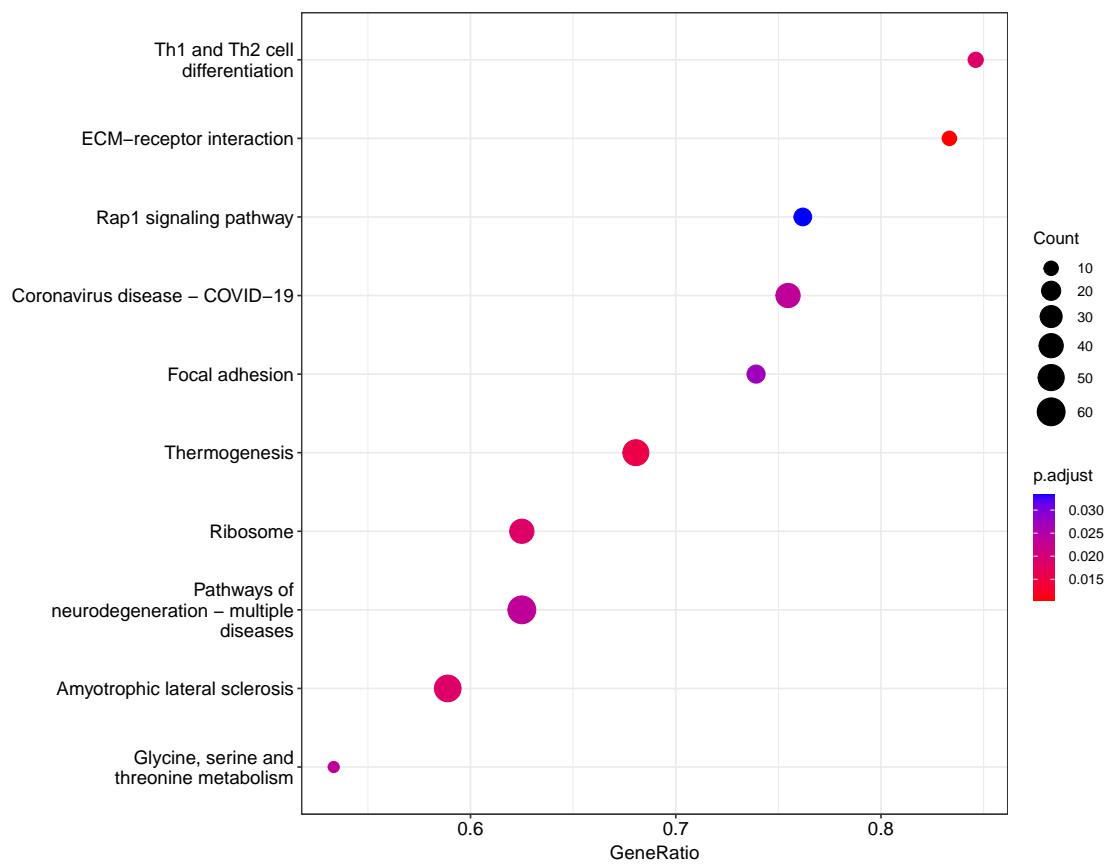


Figure 19: Integrated dataset IgA B cell vs Normal B cell KEGG enrichment

Figure 20 (下方图) 为图 Integrated dataset IgA B cell vs Normal B cell GO enrichment 概览。

(对应文件为 [Figure+Table/Integrated-dataset-IgA-B-cell-vs-Normal-B-cell-GO-enrichment.pdf](#))

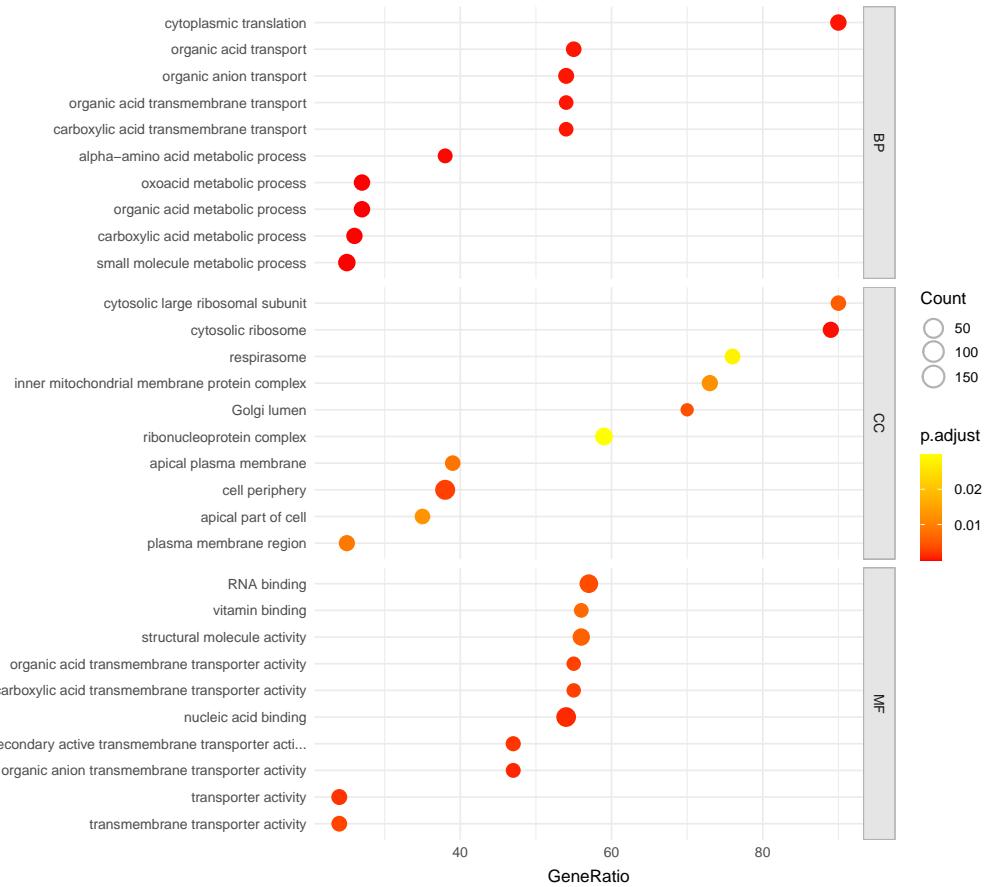


Figure 20: Integrated dataset IgA B cell vs Normal B cell GO enrichment

Figure 21 (下方图) 为图 Integrated dataset IgA B cell vs Normal B cell GSEA plot of pathway 概览。

(对应文件为 Figure+Table/Integrated-dataset-IgA-B-cell-vs-Normal-B-cell-GSEA-plot-of-pathway.pdf)

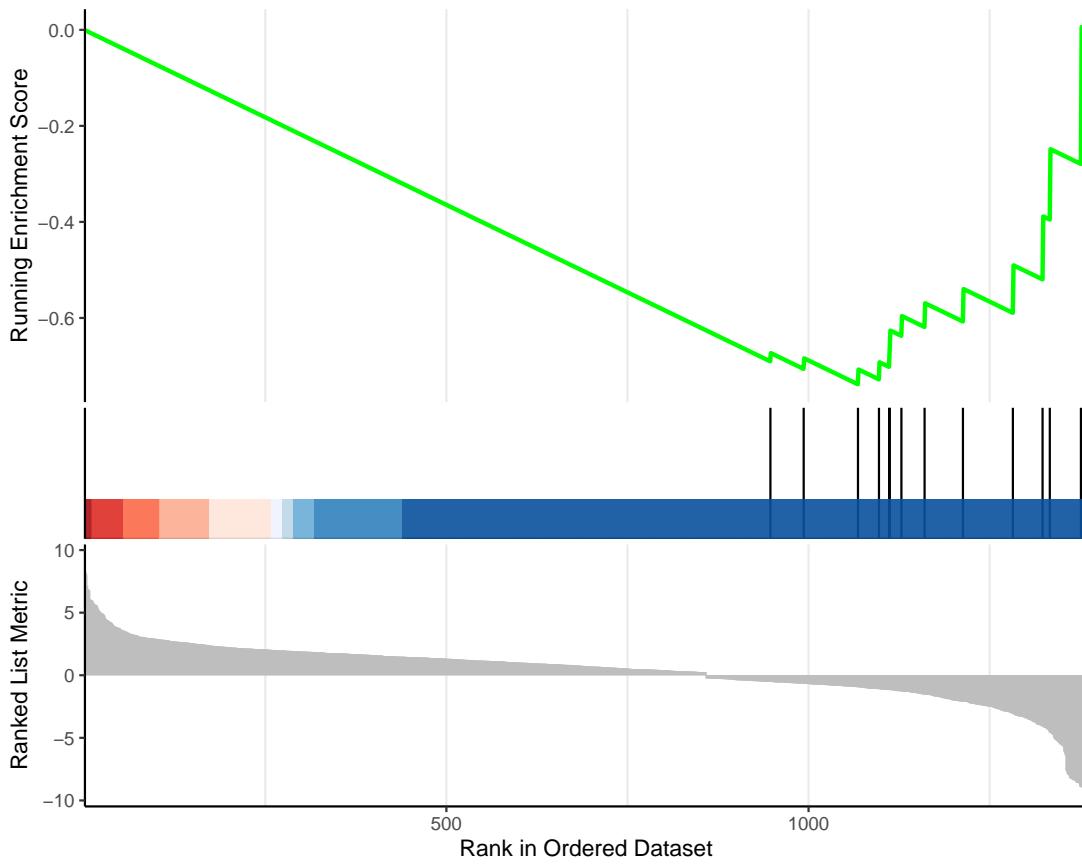


Figure 21: Integrated dataset IgA B cell vs Normal B cell GSEA plot of pathway

6.4 GTEx edQTLs 数据

- <https://gtexportal.org/home/downloads/adult-gtex#variants>

6.4.1 The matched RNA editing site (using top DEGs)

Figure 22 (下方图) 为图 Top DEGs The matched RNA editing site 概览。

(对应文件为 [Figure+Table/Top-DEGs-The-matched-RNA-editing-site.pdf](#))

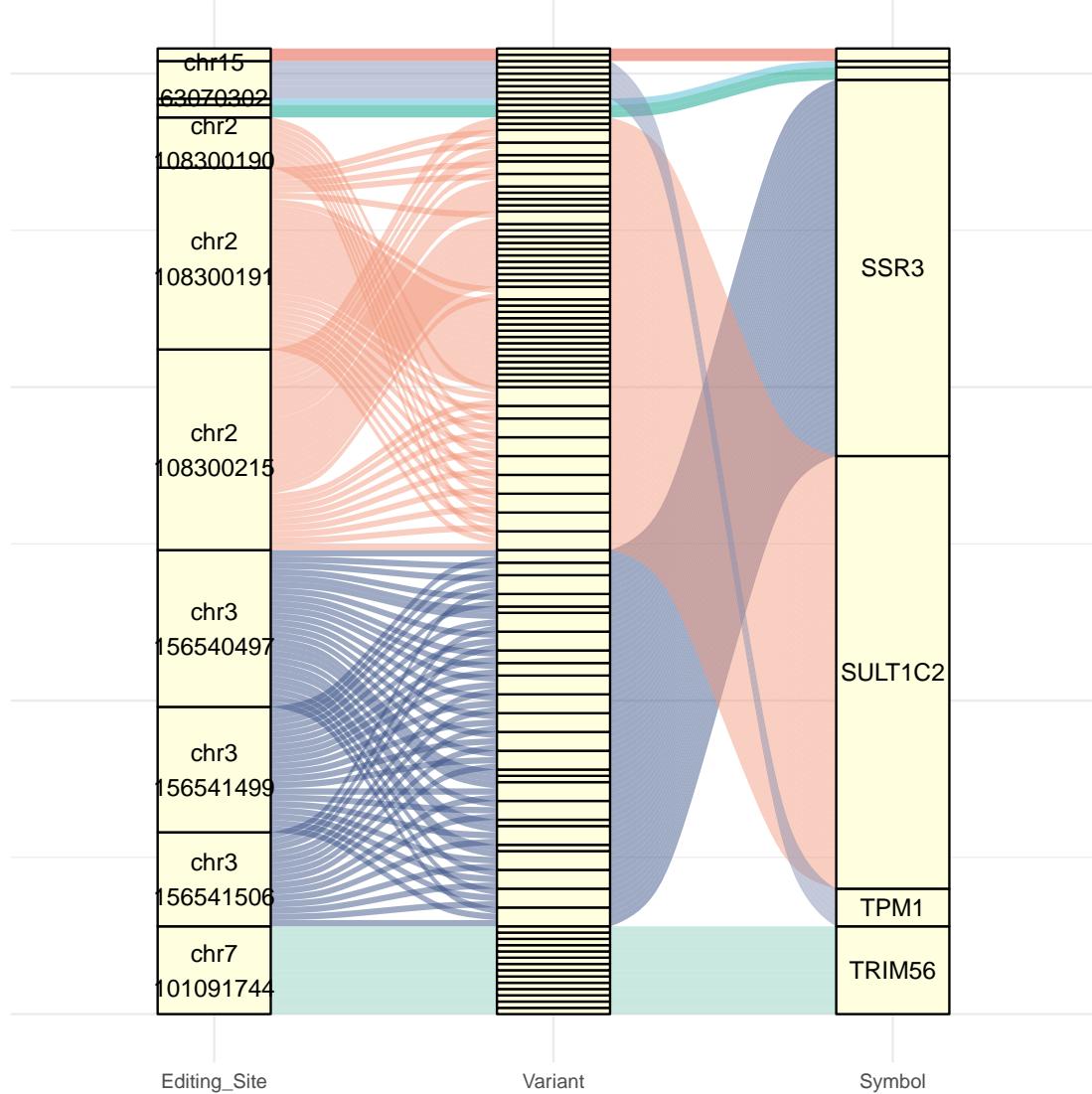


Figure 22: Top DEGs The matched RNA editing site

Table 1 (下方表格) 为表格 Top DEGs The matched RNA editing site DATA 概览。

(对应文件为 [Figure+Table/Top-DEGs-The-matched-RNA-editing-site-DATA.csv](#))

注：表格共有 154 行 13 列，以下预览的表格可能省略部分数据；表格含有 7 个唯一‘ref_gene’。

Table 1: Top DEGs The matched RNA editing site DATA

ref_gene	gene_id	varia...	tss_d...	ma_sa...	ma_coun...	maf	pval_.....8	slope	slope_se	pval_.....11	...
CLCNKA	chr1_...	-12705	28	30	0.205479	2.748...	-	0.151189	6.655...	...	1.08673

ref_gene	gene_id	varia...	tss_d...	ma_sa...	ma_coun...	maf	pval_...	8	slope	slope_se	pval_...	11	...
CLCNK	Ahr1_...	chr1_...	-6	28	30	0.205479	2.748...	-	0.151189	6.655...	...		
									1.08673				
TPM1	chr15...	chr15...	-10628	10	11	0.075...	3.571...	-	0.337299	6.188...	...		
									1.97185				
TPM1	chr15...	chr15...	-7409	38	46	0.315068	5.453...	1.52632	0.184497	6.188...	...		
TPM1	chr15...	chr15...	5614	15	16	0.109589	1.700...	-	0.283645	6.188...	...		
									1.89669				
TPM1	chr15...	chr15...	7177	11	12	0.082...	3.314...	-	0.33122	6.188...	...		
									1.94319				
TPM1	chr15...	chr15...	14803	40	49	0.340278	1.892...	-	0.237289	6.188...	...		
									1.42903				
TPM1	chr15...	chr15...	22273	45	60	0.410959	1.032...	-	0.199004	6.188...	...		
									1.23182				
CYP4F3	chr19...	chr19...	-	32	33	0.226027	1.024...	-0.94...	0.138261	5.444...	...		
			869875										
GDF15	chr19...	chr19...	15	22	23	0.157534	2.261...	-	0.188924	4.188...	...		
									1.24849				
GDF15	chr19...	chr19...	987	21	22	0.150685	1.557...	-1.217	0.200289	4.188...	...		
SULT1C2	chr2_...	chr2_...	-12306	23	28	0.191781	2.907...	-	0.137543	1.135...	...		
									0.89944				
SULT1C2	chr2_...	chr2_...	-3973	22	30	0.205479	3.388...	-0.89...	0.125638	1.135...	...		
SULT1C2	chr2_...	chr2_...	-3634	22	29	0.19863	1.141...	-0.92...	0.124944	1.135...	...		
SULT1C2	chr2_...	chr2_...	-3556	22	29	0.19863	1.141...	-0.92...	0.124944	1.135...	...		
...

6.4.2 Correlation

Figure 23 (下方图) 为图 Possibly RNA editing site correlation with the pathway genes 概览。

(对应文件为 Figure+Table/Possibly-RNA-editing-site-correlation-with-the-pathway-genes.pdf)

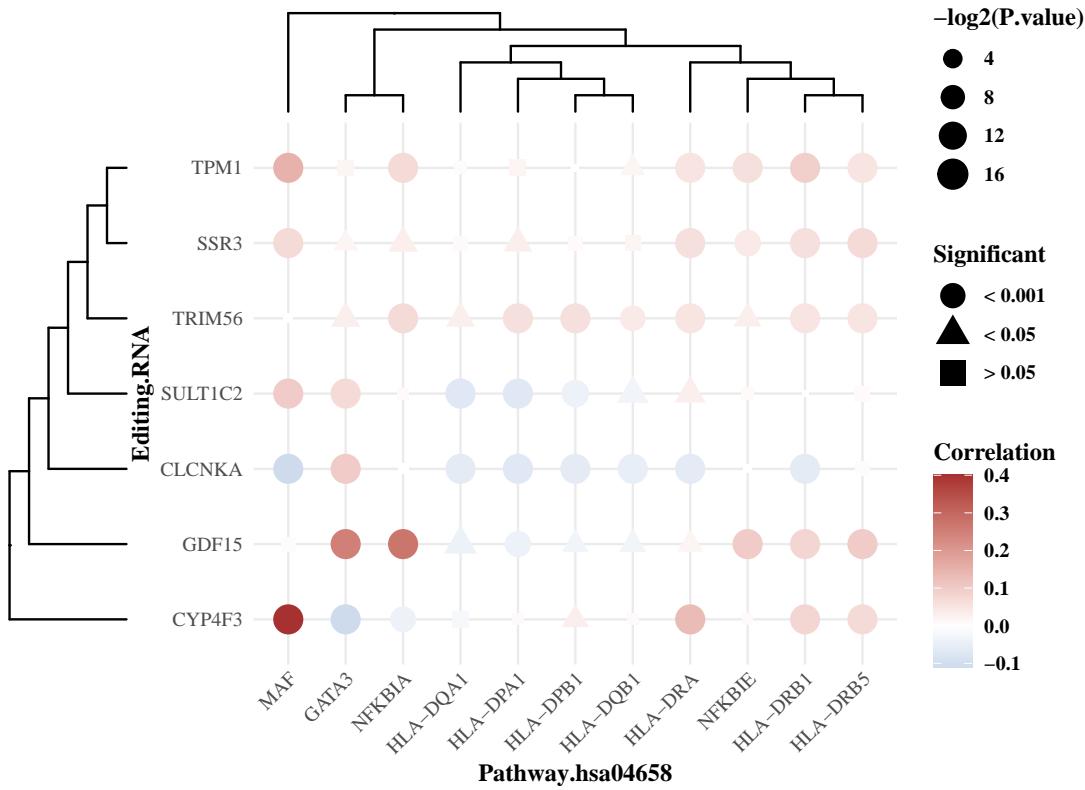


Figure 23: Possibly RNA editing site correlation with the pathway genes

6.4.3 Expression level

Figure 24 (下方图) 为图 Integrated violin plot of expression level of the genes 概览。

(对应文件为 Figure+Table/Integrated-violin-plot-of-expression-level-of-the-genes.pdf)

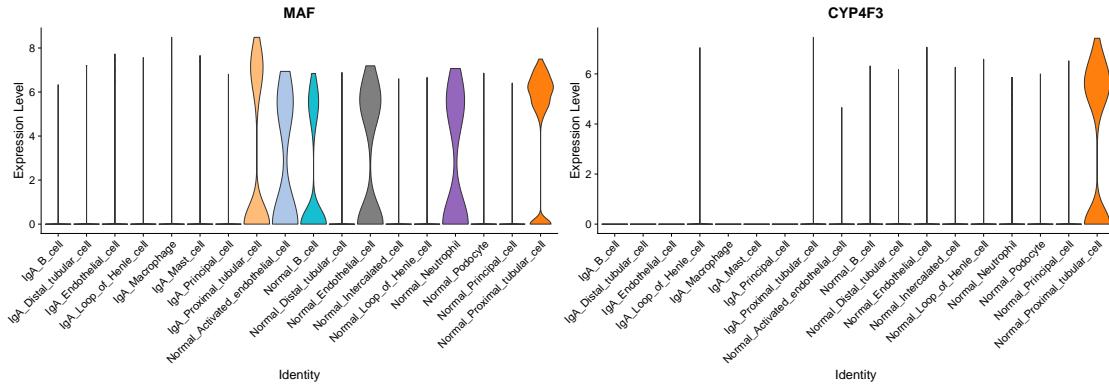


Figure 24: Integrated violin plot of expression level of the genes

Reference

- Li, Q. et al. RNA editing underlies genetic risk of common inflammatory diseases. *Nature* **608**, 569–577 (2022).

2. Rodrigues, J. C., Haas, M. & Reich, H. N. IgA nephropathy. *Clinical journal of the American Society of Nephrology : CJASN* **12**, 677–686 (2017).
3. Gentile, M. *et al.* Immune abnormalities in iga nephropathy. *Clinical Kidney Journal* **16**, 1059–1070 (2023).
4. Durinck, S., Spellman, P. T., Birney, E. & Huber, W. Mapping identifiers for the integration of genomic datasets with the r/bioconductor package biomaRt. *Nature protocols* **4**, 1184–1191 (2009).
5. Jin, S. *et al.* Inference and analysis of cell-cell communication using cellchat. *Nature Communications* **12**, (2021).
6. None, N. *et al.* The gtex consortium atlas of genetic regulatory effects across human tissues. *Science* **369**, 1318–1330 (2020).
7. Wu, T. *et al.* ClusterProfiler 4.0: A universal enrichment tool for interpreting omics data. *The Innovation* **2**, (2021).
8. Liu, Y., Wang, T., Zhou, B. & Zheng, D. Robust integration of multiple single-cell rna sequencing datasets using a single reference space. *Nature biotechnology* **39**, 877–884 (2021).
9. Hao, Y. *et al.* Integrated analysis of multimodal single-cell data. *Cell* **184**, (2021).
10. Stuart, T. *et al.* Comprehensive integration of single-cell data. *Cell* **177**, (2019).