

空代 + 空转 + 单细胞的联合分析

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1 摘要

在本研究中，我们通过空间代谢组学和空间转录组学方法分析了肺癌患者样本，以探讨癌细胞和癌周组织之间的代谢和基因表达差异。发现了癌细胞与癌周组织的转化途径。进一步的差异分析揭示了多个差异代谢物，通过 scFEA 工具预测细胞水平的代谢通量变化，结合单细胞数据和空间代谢组数据验证了 3 个共同的差异代谢物，Glutamic acid (C00025), Uridine 5'-monophosphate (C00105), Adenosine 5'-monophosphate (C00020)。拟时分析进一步揭示了癌变过程中的关键基因，并通过 PPI 网络构建发现了这些基因与代谢变化相关的直接物理作用，有 4 个基因被筛选为可进一步分析验证：SOX2, A2M, CD74, DSP。

曹卓订单，空代 + 空转 + 单细胞的联合分析

2 前言

3 材料和方法

3.1 材料

3.2 方法

Mainly used method:

- The R package `Cardinal` used for analyzing mass spectrometry imaging datasets¹.
- R package `ClusterProfiler` used for gene enrichment analysis².
- R package `Seurat` used for multiple dataset integration^{3,4}.
- R package `FELLA` used for metabolite enrichment analysis⁵.
- The `scFEA` (python) was used to estimate cell-wise metabolic via single cell RNA-seq data⁶.
- R package `Limma` and `edgeR` used for differential expression analysis^{7,8}.
- R package `Monocle3` used for cell pseudotime analysis^{9,10}.
- R package `STEINGdb` used for PPI network construction^{11,12}.
- R package `Seurat` used for spatial scRNA-seq analysis^{3,4}.
- The R package `Seurat` used for scRNA-seq processing^{3,4}.
- `SCSA` (python) used for cell type annotation¹³.
- The MCC score was calculated referring to algorithm of `CytoHubba`¹².
- R version 4.4.0 (2024-04-24); Other R packages (eg., `dplyr` and `ggplot2`) used for statistic analysis or data visualization.

4 分析结果

4.1 空间代谢组分析

在空间代谢组分析部分，对数据集预处理后，首先对样本进行 PCA 聚类，见 Fig. 1a，Tumor 和 Peritumor 的聚类结果呈现了同一性和异同性，提示两者之间的转化途径。随后，我们对两组数据进行差异分析，差异代谢物见 Tab. 2，Fig. 1b 展示了 Top 10 的代谢物的峰强度。将差异代谢物以 FELLA 包提供的 PageRank

(Fig. 1c) 和 Hypergeom (Fig. 1d) 算法进行 KEGG 富集分析。PageRank 提供的富集网络可以帮助理解差异代谢物彼此之间关联以及与通路、模块、酶反应之间的联系。一共有 12 个差异代谢物被富集。Fig. 1d, “Nucleotide metabolism” 等通路的富集, 揭示了癌变后细胞代谢的变化 (癌细胞快速增殖, 需要大量的核苷酸来支持 DNA 和 RNA 的合成)。值得注意的是, “bile secretion” 等通路, 在单细胞数据集的拟时分析中得到印证 (Fig. 5c)。



Figure 1 (下方图) 为图 MAIN Fig 1 概览。

(对应文件为 Figure+Table/MAIN-Fig-1.pdf)

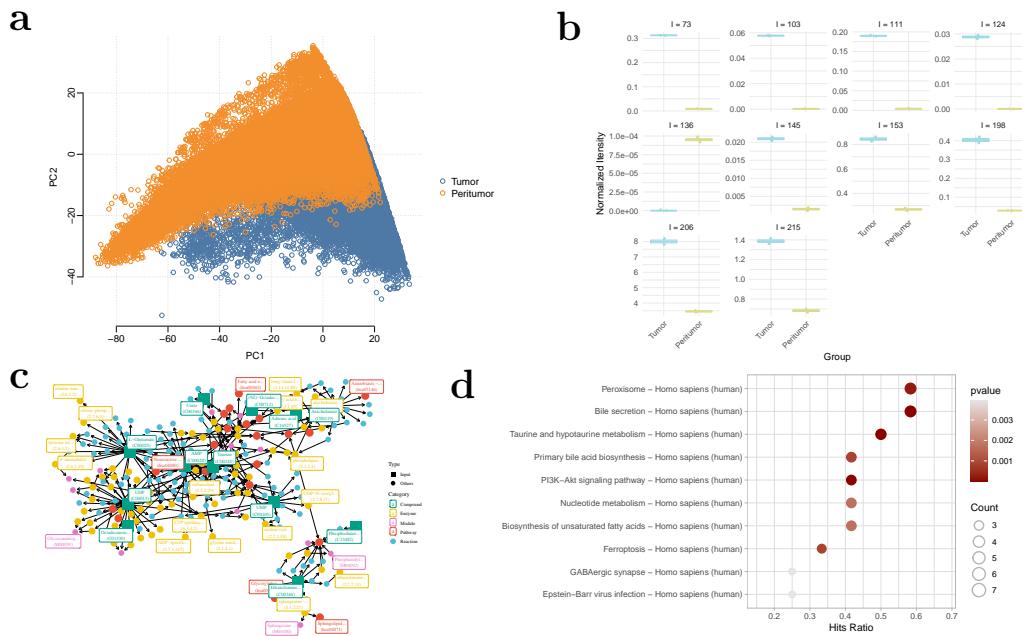


Figure 1: MAIN Fig 1

Composition :

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- ./Figure+Table/INTEGRATE-boxplot-of-top-features.pdf
- ./Figure+Table/TOPS-enrichment-with-algorithm-PageRank.pdf
- ./Figure+Table/Tops-Compounds-hypergeom-KEGG-enrichment.pdf



4.2 空间转录组分析

以癌组织切片和癌周组织切片进行空间转录组数据分析。我们首先分别对两组数据进行细胞聚类、注释，Fig. 2a、b。其中，癌组织以 copyKAT 预测非整倍体 (Fig. 2c)，判断为癌细胞。(注，这部分内容取自前一次该客户的分析结果，没有重新分析。) 随后，将两部分的单细胞数据集成，以备后续分析 (Fig. 2d)。随后，我们将主要关注于 *Epithelial_cell_Peritumoral_cell*, *Cancer_cell*。



Figure 2 (下方图) 为图 MAIN Fig 2 概览。

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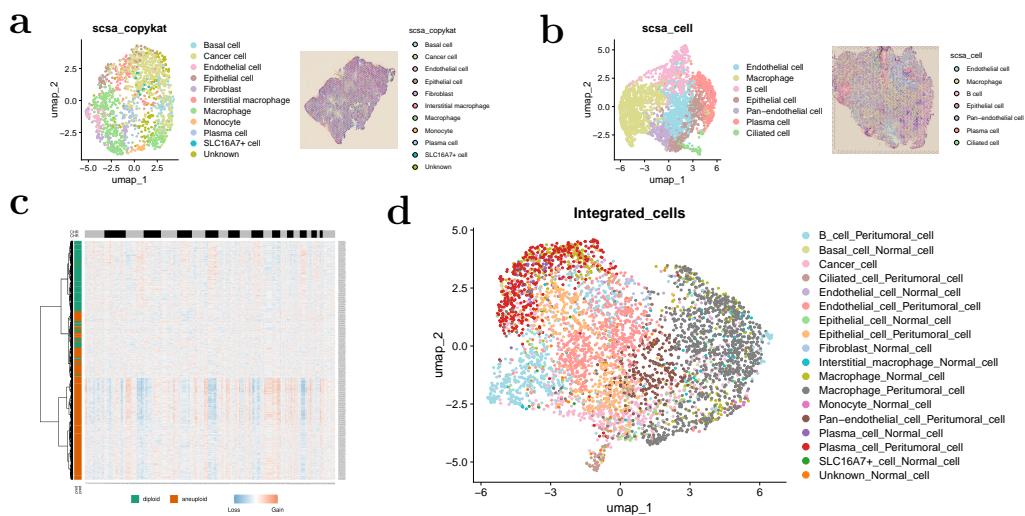


Figure 2: MAIN Fig 2

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- ./Figure+Table/PERI-The-scsa-cell.pdf
- ./Figure+Table/copyKAT-prediction-of-aneuploidy.png
- ./Figure+Table/The-Integrated-cells.pdf



4.3 单细胞数据集预测代谢通量

空间转录组和空间代谢组的样本数据都来自于肺癌患者的样本数据，那么它们的本质将揭示相同的病机。应该认为，代谢变化与单细胞水平的基因表达变化是相互反映的。这里，通过借用 scFEA 工具，以单细胞数据(取 Epithelial_cell_Peritumoral_cell, Cancer_cell) 预测细胞水平的代谢通量变化，随后和我们的空间代谢组分析结果相互印证。Fig. 3a 为 scFEA 训练数据时的收敛曲线。预测细胞水平的代谢通量变化，随后进行差异分析 Fig. 3b。差异分析结果表格见 Tab. 5。之后，将这里代谢通量变化的对应代谢物，与空间转录组的差异代谢物，取交集，Fig. 3c。我们发现了三个同时在空间转录组水平和空间代谢组水平反映的差异代谢物：Glutamic acid (C00025), Uridine 5'-monophosphate (C00105), Adenosine 5'-monophosphate (C00020)。回到空间代谢组数据考察三个代谢物在癌症组织和癌周组织的含量水平。一共有 4 个 Feature 被鉴定为这三个代谢物 (可能是不同的加合离子型)，Fig. 3d、e、f、g。

针对这三个代谢物，我们重新进行了富集分析，Fig. 4a、b。富集结果与此前的富集分析结果一致，体现了癌变后细胞内的特征性代谢变化。此外，Fig. 4b 的网络图体现了三个代谢物在机体内的密切关联。随后，我们通过获取 scFEA 反映代谢通量变化的基因，对其进行 KEGG 富集分析，Fig. 4d。Fig. 4a 和 Fig. 4d 共同揭示了一些通路，我们将其汇总于 Fig. 4c。“Nicotinate and nicotinamide metabolism”的发现，与文献报道的相一致¹⁴。



Figure 3 (下方图) 为图 MAIN Fig 3 概览。

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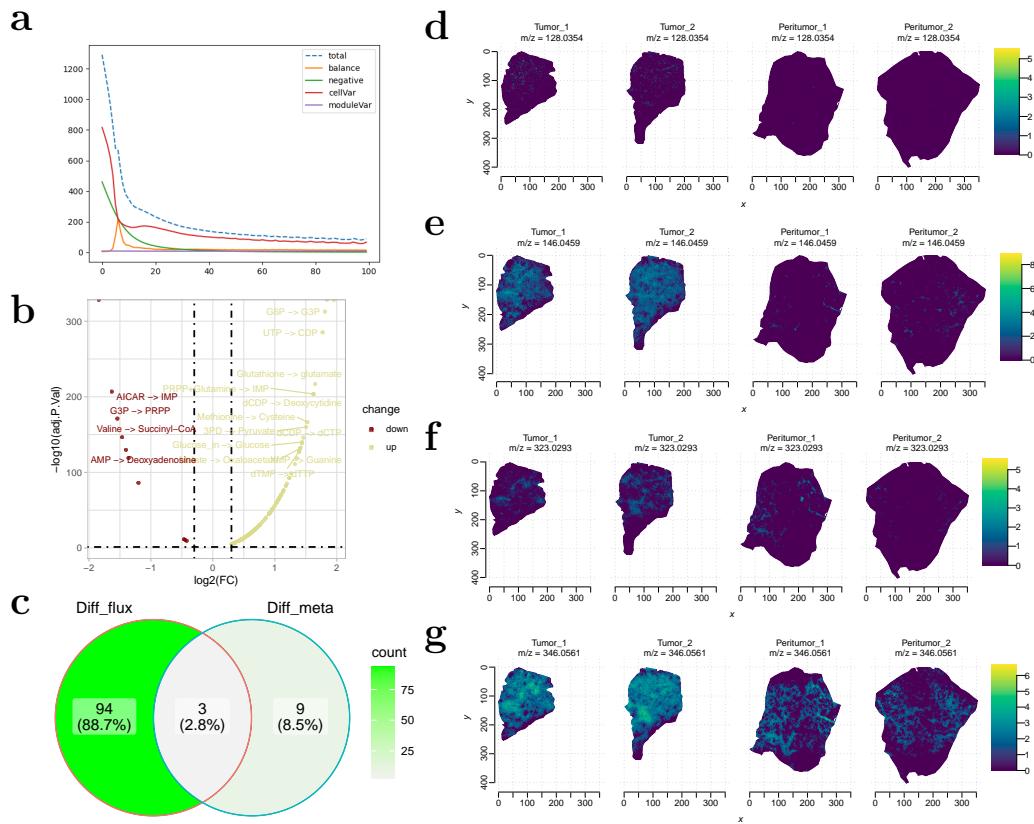


Figure 3: MAIN Fig 3

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- ./Figure+Table/FLUX-Cancer-cell-vs-Epithelial-cell-Peritumoral-cell-DEGs.pdf
- ./Figure+Table/Intersection-of-Diff-flux-with-Diff-meta.pdf
- ./Figure+Table/Feature-image-visualizations/1_Feature_13_C00025.pdf
- ./Figure+Table/Feature-image-visualizations/2_Feature_25_C00025.pdf
- ./Figure+Table/Feature-image-visualizations/3_Feature_123_C00105.pdf
- ./Figure+Table/Feature-image-visualizations/4_Feature_131_C00020.pdf



Figure 4 (下方图) 为图 MAIN Fig 4 概览。

(对应文件为 Figure+Table/MAIN-Fig-4.pdf)

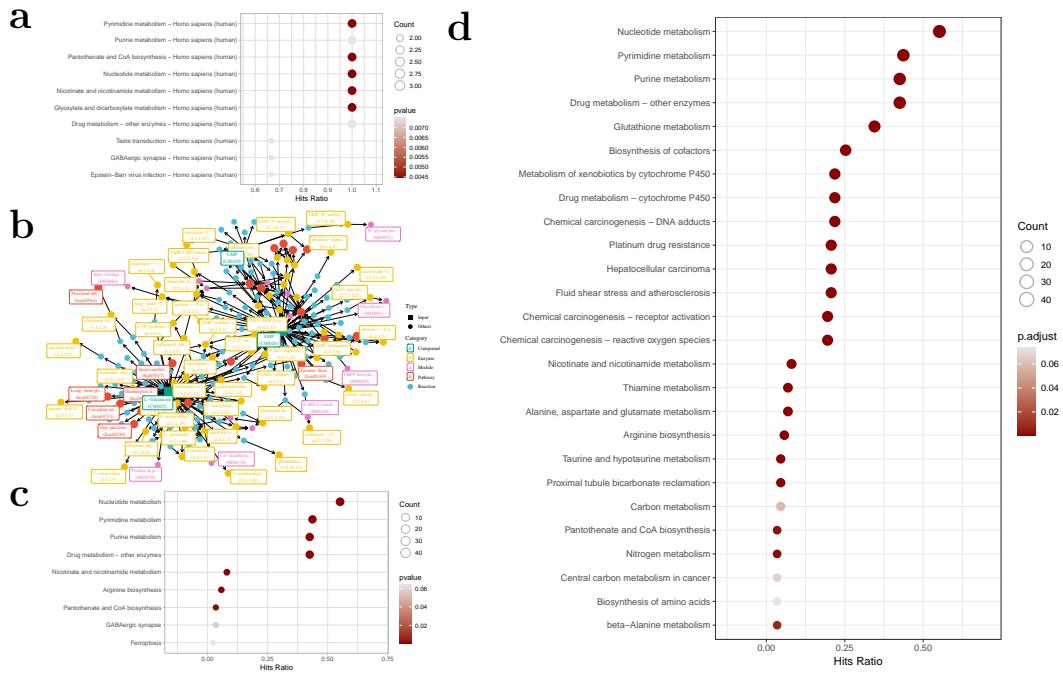


Figure 4: MAIN Fig 4

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- ./Figure+Table/FLUX-Co-enriched-KEGG-pathway.pdf
- ./Figure+Table/FLUX-KEGG-enrichment.pdf



4.4 癌细胞拟时分析

对 Epithelial_cell_Peritumoral_cell, Cancer_cell 进行拟时分析。拟时分析结果揭示了明确的癌变过程, Fig. 5a、b。随后, 根据拟时分析图像, 以“Graph test”筛选拟时变化过程中的关键基因。对 Top 50 的基因进行了富集分析, 以及拟时热图注释。

随后，为了发现拟时变化过程中的关键基因与此前揭示的代谢物之间（控制代谢变化相关的基因，即 scFEA 与代谢通量相关的基因）的关联，我们将这些对应基因以 STRINGdb 构建 PPI 网络 Fig. 6a。通过深入挖掘这些蛋白质（基因）之间的关联，获取 Fig. 6b，可以发现，一共有 4 个拟时变化过程中的关键基因（蛋白质），与代谢变化对应的基因（蛋白质）有直接物理作用（physical）。这些基因为：SOX2, A2M, CD74, DSP。其中，SOX2 是与其它蛋白质关联最为密切的对象（MCC 得分）。它们在单细胞数据集中的表达见 Fig. 6c。其中，SOX2 主要在癌细胞中高表达，而 A2M 在癌细胞中的表达量下降了（结合 Fig. 5a、b）。这些基因在空间转录组的组织切片中的表达见 Fig. 6d。



Figure 5 (下方图) 为图 MAIN Fig 5 概览。

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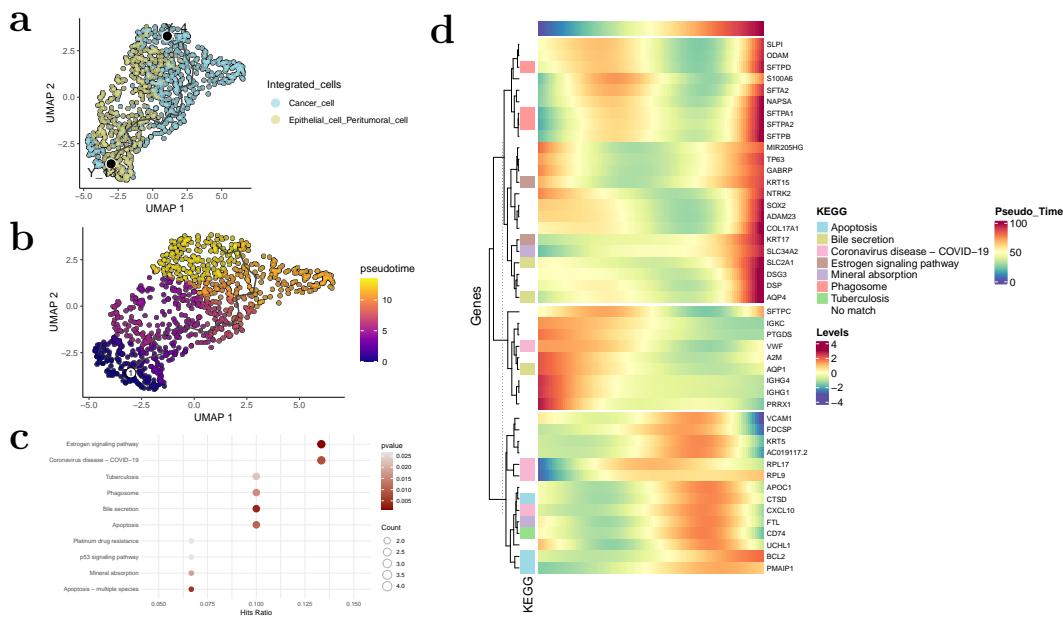


Figure 5: MAIN Fig 5

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- ./Figure+Table/INTEGRATED-pseudotime.pdf
- ./Figure+Table/GRAPHTOPS-KEGG-enrichment.pdf
- ./Figure+Table/INTEGRATED-Pseudotime-heatmap-of-genes.pdf





Figure 6 (下方图) 为图 MAIN Fig 6 概览。

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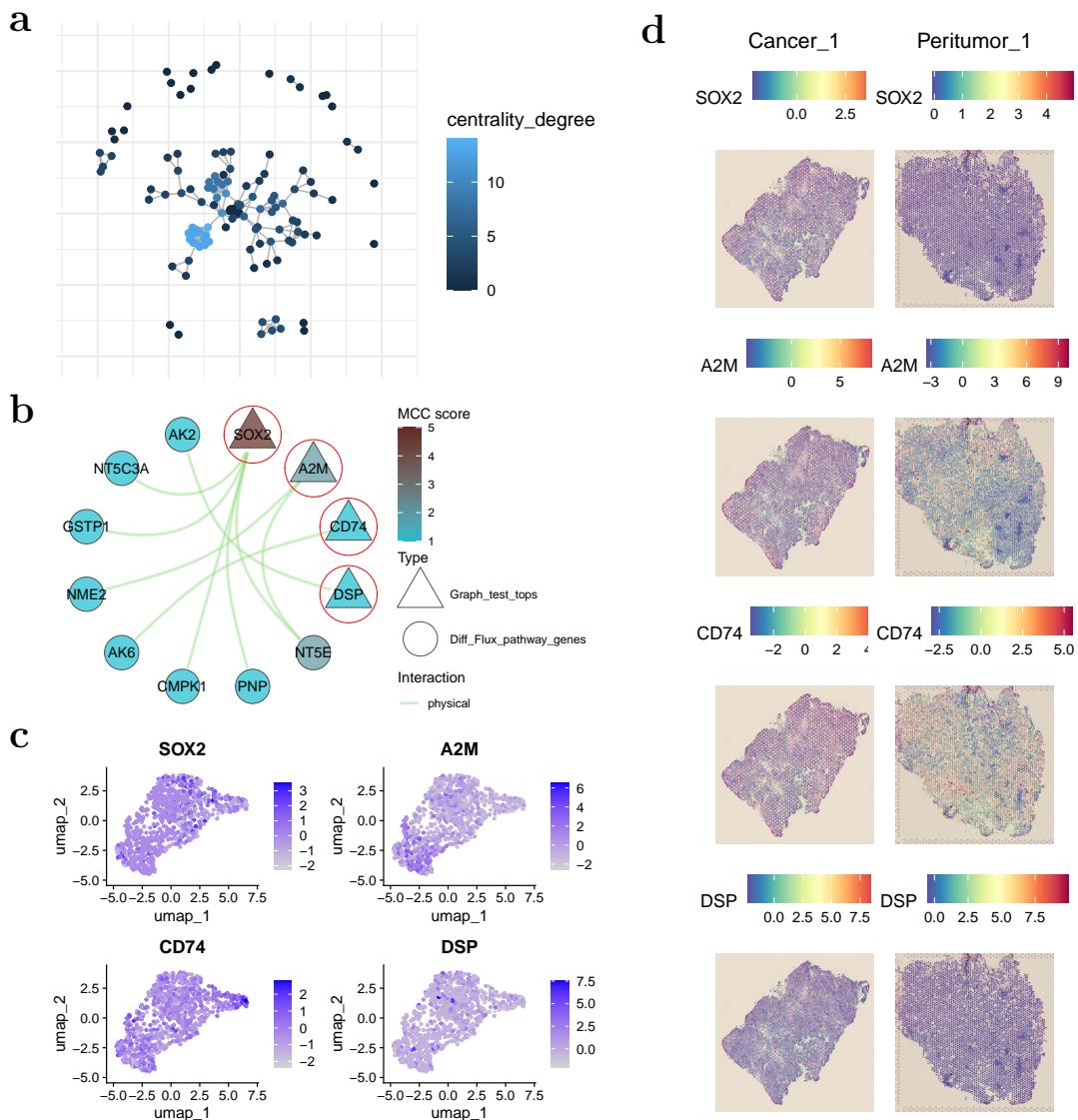


Figure 6: MAIN Fig 6

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./Figure+Table/Spatial-feature-plot.pdf



‘Tiff figures’ 数据已全部提供。

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5. MAIN-Fig-5.tiff
6. ...



5 结论

见 1 和 4

6 附：分析流程

6.1 空间代谢组数据分析

空间代谢组数据分析包括数据收集、预处理和分析。使用不同的分析工具和方法，我们可以揭示样本中代谢物的空间分布和变化情况。这部分的重点是数据的整合和分析流程的描述，以确保结果的准确性和可重复性。

6.1.1 Cardinal 空间代谢组数据分析 (INTEGRATE)

Cardinal 是一种用于空间代谢组数据分析的强大工具，能够处理和分析高维数据。通过对数据进行整合和分析，我们可以揭示样本中代谢物的空间分布和动态变化，从而为进一步的生物学研究提供线索。

Table 1 (下方表格) 为表格 INTEGRATE all features 概览。

(对应文件为 Figure+Table/INTEGRATE-all-features.xlsx)

注：表格共有 218 行 15 列，以下预览的表格可能省略部分数据；含有 218 个唯一 ‘i’。

Table 1: INTEGRATE all features

i	mz	count	freq	mz.sub	m.z	Name	Notation	Formula	KEGG
1	96.000...	118	0.0005...	96.0007	96.000...	NA	[M-H]-	NA	NA
2	96.960...	230943	0.9866...	96.9603	96.960...	sulfate	[M-H]-	H2O4S	NA
3	98.956...	1	4.2722...	98.956	98.956...	NA	[M-H-H]2-	NA	NA
4	99.925...	7	2.9905...	99.926	99.926...	NA	[M-H]-	NA	NA
5	102.05...	2	8.5444...	102.0562	102.05...	Dimeth...	[M-H]-	C4H9NO2	C01026
6	108.00...	379	0.0016...	108.0006	108.00...	NA	[M-H]-	NA	NA
7	111.00...	26	0.0001...	111.0089	111.00...	Glutac...	[M-H-H]...	C5H6O4	C02214
8	115.92...	1	4.2722...	115.9207	115.92...	NA	[M-H]-	NA	NA
9	118.89...	9	3.8449...	118.8996	118.89...	NA	[M-H]-	NA	NA
10	120.00...	135	0.0005...	120.0006	120.00...	NA	[M-H-H]2-	NA	NA
11	121.00...	26	0.0001...	121.0083	121.00...	NA	[M-H]-	NA	NA
12	124.00...	233542	0.9977...	124.0074	124.00...	Taurine	[M-H]-	C2H7NO3S	C00245
13	128.03...	2469	0.0105...	128.0354	128.03...	Glutam...	[M-H-H]...	C5H9NO4	C00025
14	128.89...	170277	0.7274...	128.8922	128.89...	NA	[M-H]-	NA	NA
15	132.00...	321	0.0013...	132.0006	132.00...	NA	[M-H]-	NA	NA
...

Figure 7 (下方图) 为图 INTEGRATE PCA plot 概览。

(对应文件为 Figure+Table/INTEGRATE-PCA-plot.pdf)

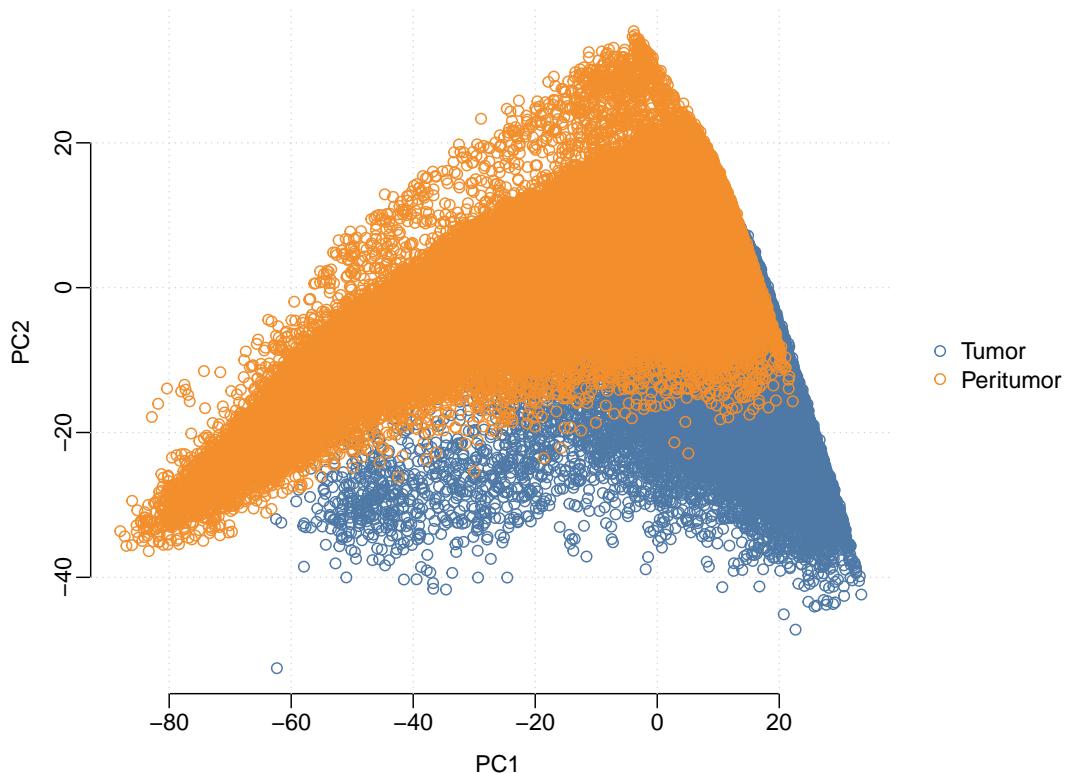


Figure 7: INTEGRATE PCA plot

Table 2 (下方表格) 为表格 INTEGRATE Significant differences features 概览。

(对应文件为 Figure+Table/INTEGRATE-Significant-differences-features.xlsx)

注：表格共有 97 行 16 列，以下预览的表格可能省略部分数据；含有 97 个唯一 ‘i’。

1. pvalue: 显著性 P。

Table 2: INTEGRATE Significant differences features

i	mz	statistic	pvalue	fdr	mz.sub	m.z	Name	Notation	Formula
2	96.960...	15.186...	9.7420...	0.0005...	96.9603	96.960...	sulfate	[M-H]-	H2O4S
11	121.00...	8.3623...	0.0038...	0.0104...	121.0083	121.00...	NA	[M-H]-	NA
12	124.00...	20.761...	5.2004...	8.0979...	124.0074	124.00...	Taurine	[M-H]-	C2H7NO3S
13	128.03...	19.401...	1.0590...	0.0001...	128.0354	128.03...	Glutam...	[M-H-H...]	C5H9NO4
21	140.02...	15.018...	0.0001...	0.0006...	140.0119	140.01...	O-Phos...	[M-H]-	C2H8NO4P
24	144.86...	11.260...	0.0007...	0.0029...	144.8697	144.86...	NA	[M-H]-	NA
25	146.04...	17.324...	3.1515...	0.0002...	146.0459	146.04...	Glutam...	[M-H]-	C5H9NO4
26	146.86...	9.0877...	0.0025...	0.0073...	146.8668	146.86...	NA	[M-H]-	NA
28	151.89...	9.8327...	0.0017...	0.0054...	NA	NA	NA	NA	NA
29	152.99...	6.5953...	0.0102...	0.0254...	152.9958	152.99...	3-phos...	[M-H]-	C3H7O5P
31	154.91...	11.696...	0.0006...	0.0024...	154.9168	154.91...	NA	[M-H]-	NA
36	160.00...	6.5840...	0.0102...	0.0254...	160.0074	160.00...	NA	[M-H]-	NA
37	160.84...	17.149...	3.4541...	0.0002...	160.8421	160.84...	NA	[M-H]-	NA
41	168.04...	9.6952...	0.0018...	0.0057...	168.0432	168.04...	Phosph...	[M-H]-	C4H12NO4P
43	169.90...	8.7556...	0.0030...	0.0087...	NA	NA	NA	NA	NA
...

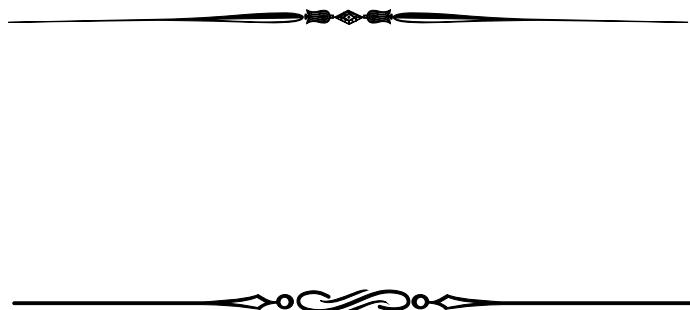


Figure 8 (下方图) 为图 INTEGRATE boxplot of top features 概览。

(对应文件为 [Figure+Table/INTEGRATE-boxplot-of-top-features.pdf](#))

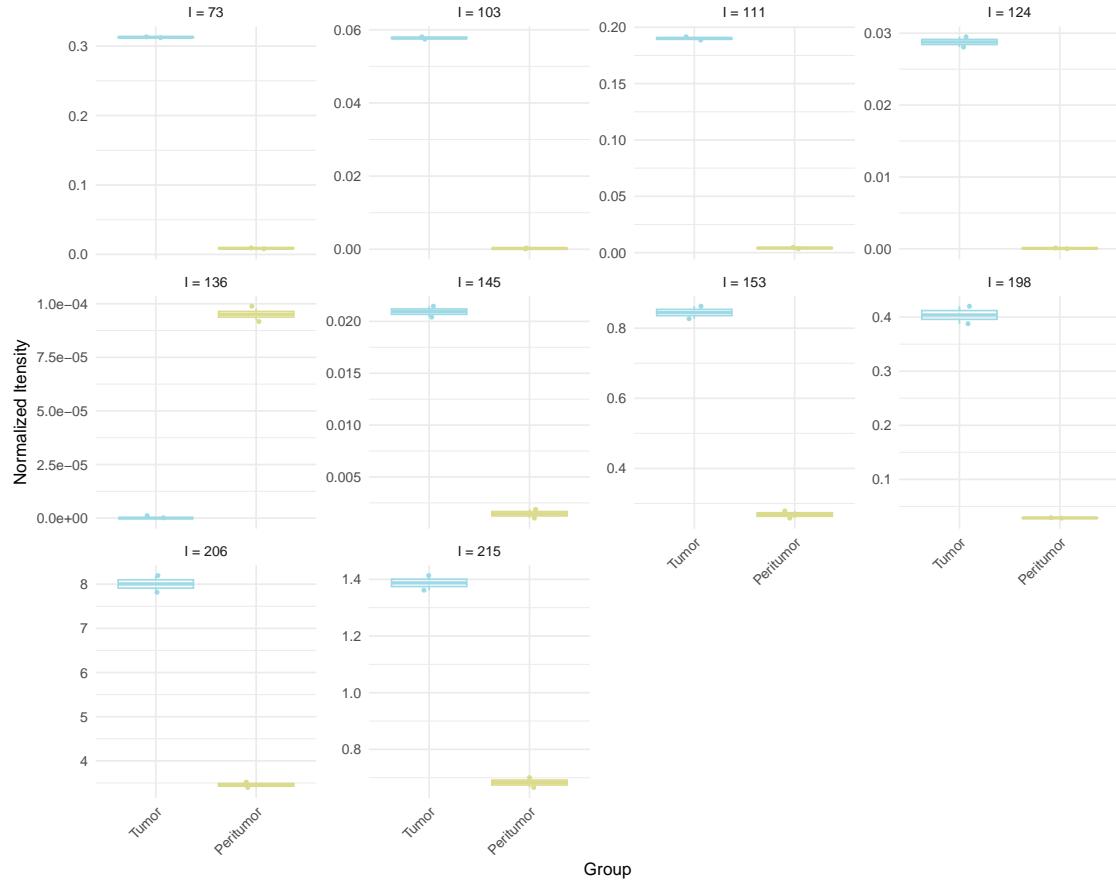


Figure 8: INTEGRATE boxplot of top features



6.1.2 FELLA 代谢物富集分析 (TOPS)

FELLA 工具用于代谢物富集分析，通过将代谢物与已知的代谢通路进行比对，可以识别出富集的代谢通路。这有助于我们理解代谢物的功能和其在生物过程中的角色。



Figure 9 (下方图) 为图 TOPS enrichment with algorithm PageRank 概览。

(对应文件为 Figure+Table/TOPS-enrichment-with-algorithm-PageRank.pdf)

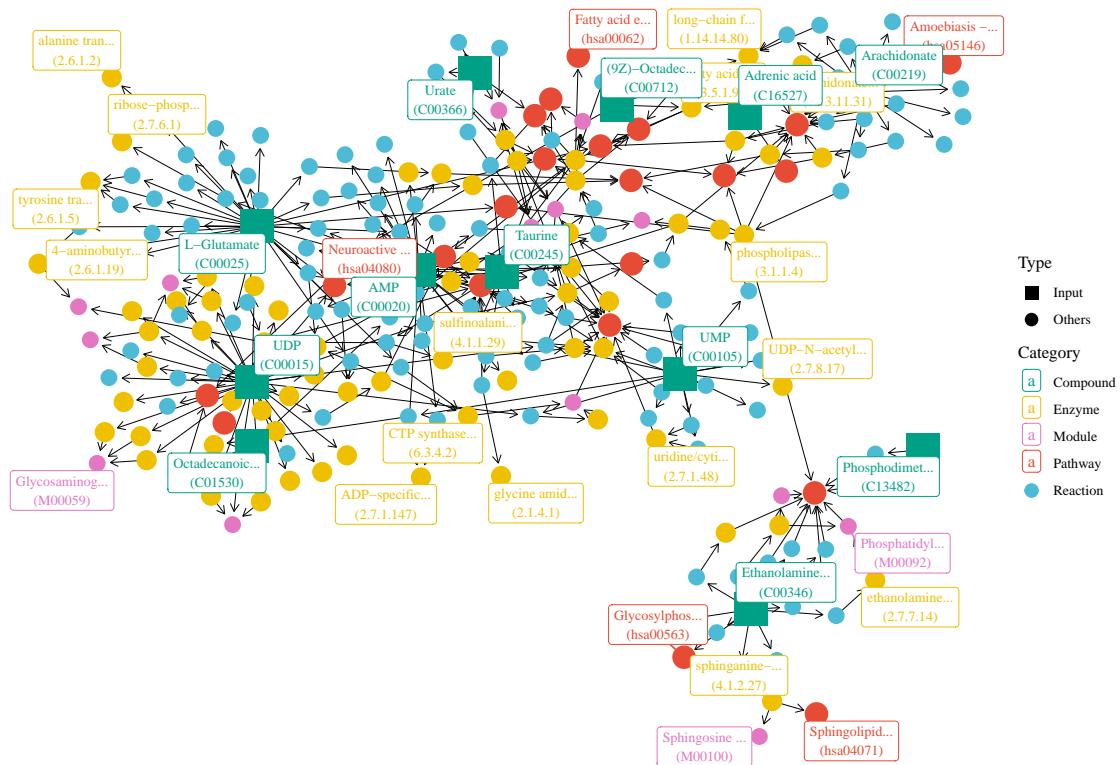


Figure 9: TOPS enrichment with algorithm PageRank

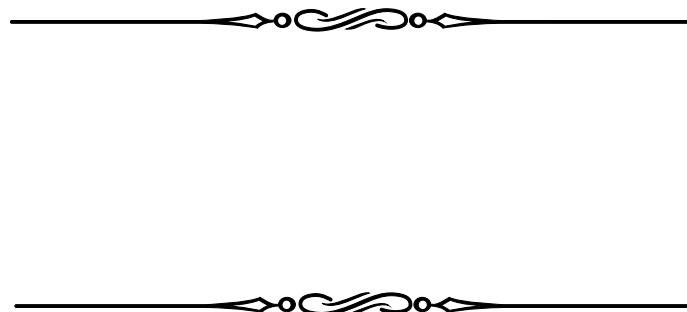


Figure 10 (下方图) 为图 Tops Compounds hypergeom KEGG enrichment 概览。

(对应文件为 Figure+Table/Tops-Compounds-hypergeom-KEGG-enrichment.pdf)

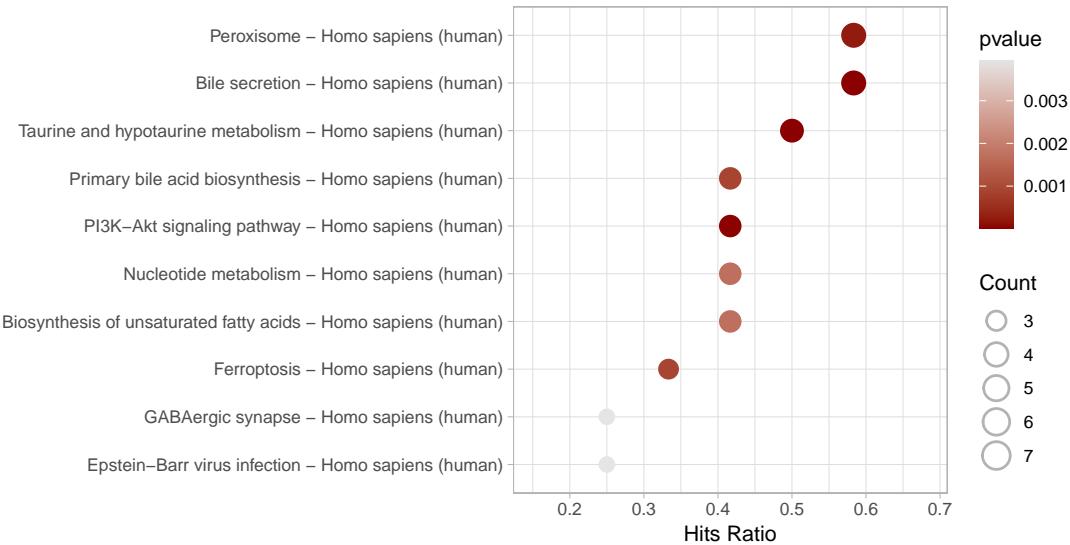


Figure 10: Tops Compounds hypergeom KEGG enrichment

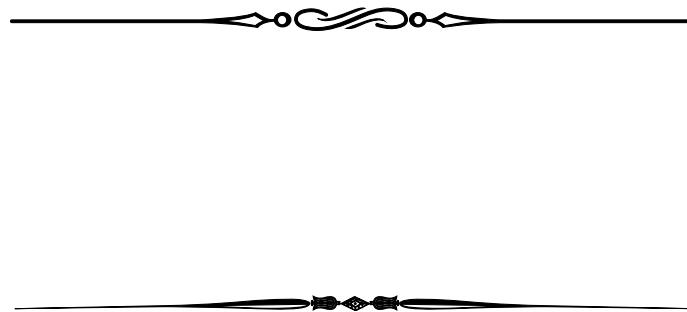


Table 3 (下方表格) 为表格 TOPS data of enrichment with algorithm PageRank 概览。

(对应文件为 Figure+Table/TOPS-data-of-enrichment-with-algorithm-PageRank.xlsx)

注：表格共有 249 行 7 列，以下预览的表格可能省略部分数据；含有 249 个唯一 ‘name’。

Table 3: TOPS data of enrichment with algorithm PageRank

name	com	NAME	label	input	abbrev.name	type
hsa00061	1	Fatty acid...	Fatty acid...	Others	Fatty acid...	Pathway
hsa00062	1	Fatty acid...	Fatty acid...	Others	Fatty acid...	Pathway
hsa00120	1	Primary bi...	Primary bi...	Others	Primary bi...	Pathway
hsa00230	1	Purine met...	Purine met...	Others	Purine met...	Pathway
hsa00430	1	Taurine an...	Taurine an...	Others	Taurine an...	Pathway
hsa00563	1	Glycosylph...	Glycosylph...	Others	Glycosylph...	Pathway
hsa00564	1	Glyceropho...	Glyceropho...	Others	Glyceropho...	Pathway
hsa00590	1	Arachidoni...	Arachidoni...	Others	Arachidoni...	Pathway
hsa01040	1	Biosynthes...	Biosynthes...	Others	Biosynthes...	Pathway
hsa01232	1	Nucleotide...	Nucleotide...	Others	Nucleotide...	Pathway

name	com	NAME	label	input	abbrev.name	type
hsa04071	1	Sphingolip...	Sphingolip...	Others	Sphingolip...	Pathway
hsa04080	1	Neuroactiv...	Neuroactiv...	Others	Neuroactiv...	Pathway
hsa04146	1	Peroxisome...	Peroxisome...	Others	Peroxisome...	Pathway
hsa04151	1	PI3K-Akt s...	PI3K-Akt s...	Others	PI3K-Akt s...	Pathway
hsa04216	1	Ferroptosi...	Ferroptosi...	Others	Ferroptosi...	Pathway
...

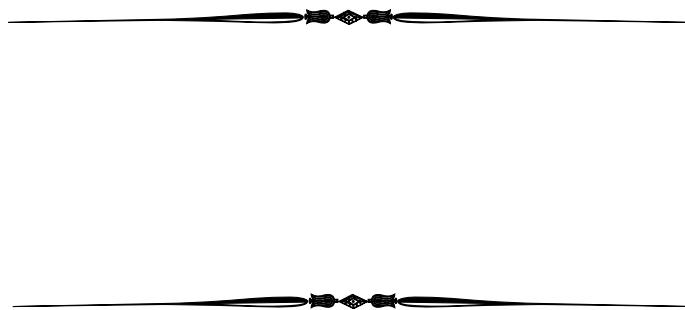


Table 4 (下方表格) 为表格 TOPS data of enrichment with algorithm Hypergeom 概览。

(对应文件为 `Figure+Table/TOPS-data-of-enrichment-with-algorithm-Hypergeom.csv`)

注: 表格共有 15 行 6 列, 以下预览的表格可能省略部分数据; 含有 15 个唯一 ‘KEGG.id’。

1. pvalue: 显著性 P。

Table 4: TOPS data of enrichment with algorithm Hypergeom

KEGG.id	Description	Count	CompoundsL...	pvalue	Compound_R...
hsa00430	Taurine an...	6	93	1.26333822...	0.5
hsa04976	Bile secre...	7	161	1.26333822...	0.58333333...
hsa04151	PI3K-Akt s...	5	57	2.94176160...	0.41666666...
hsa04146	Peroxisome...	7	279	0.00023384...	0.58333333...
hsa00120	Primary bi...	5	130	0.00090942...	0.41666666...
hsa04216	Ferroptosi...	4	59	0.00090942...	0.33333333...
hsa01040	Biosynthes...	5	157	0.00171085...	0.41666666...
hsa01232	Nucleotide...	5	154	0.00171085...	0.41666666...
hsa04727	GABAergic ...	3	35	0.00394520...	0.25
hsa05169	Epstein-Ba...	3	36	0.00394520...	0.25
hsa04723	Retrograde...	3	39	0.00456905...	0.25
hsa04080	Neuroactiv...	3	41	0.00487000...	0.25
hsa00230	Purine met...	5	218	0.00508159...	0.41666666...
hsa01523	Antifolate...	3	45	0.00552169...	0.25

KEGG.id	Description	Count	CompoundsL...	pvalue	Compound_R...
hsa00770	Pantothena...	4	125	0.00655730...	0.33333333...



6.2 空间转录组分析

空间转录组分析通过整合空间信息和基因表达数据，揭示细胞在空间上的异质性和基因表达的空间分布。这部分的重点在于数据的获取、处理及分析方法的选择，以确保结果的准确性和生物学意义。

6.2.1 Seurat 空间转录组分析 (CANCER)

Seurat 是一种广泛使用的单细胞数据分析工具，适用于空间转录组数据。通过 Seurat，我们可以分析癌症样本中的基因表达和细胞异质性，揭示其在空间上的分布和功能。

注：这部分内容取自上一回该客户业务的结果。



Figure 11 (下方图) 为图 copyKAT prediction of aneuploidy 概览。

(对应文件为 [Figure+Table/copyKAT-prediction-of-aneuploidy.png](#))

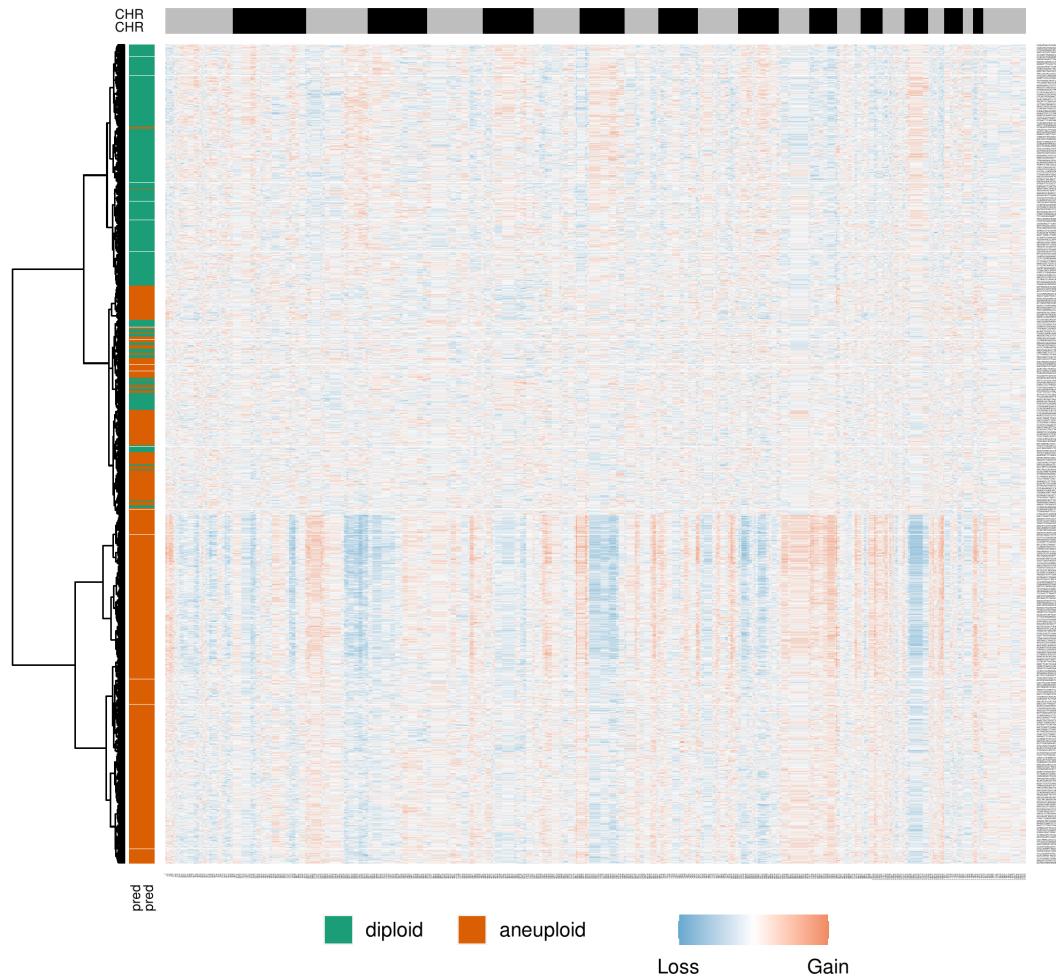


Figure 11: CopyKAT prediction of aneuploidy

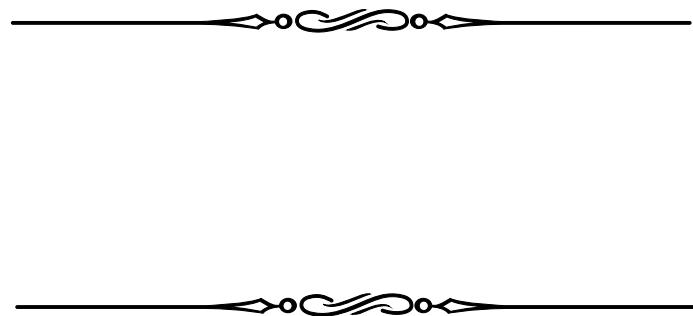


Figure 12 (下方图) 为图 CANCER The scsa cell 概览。

(对应文件为 Figure+Table/CANCER-The-scsa-cell.pdf)

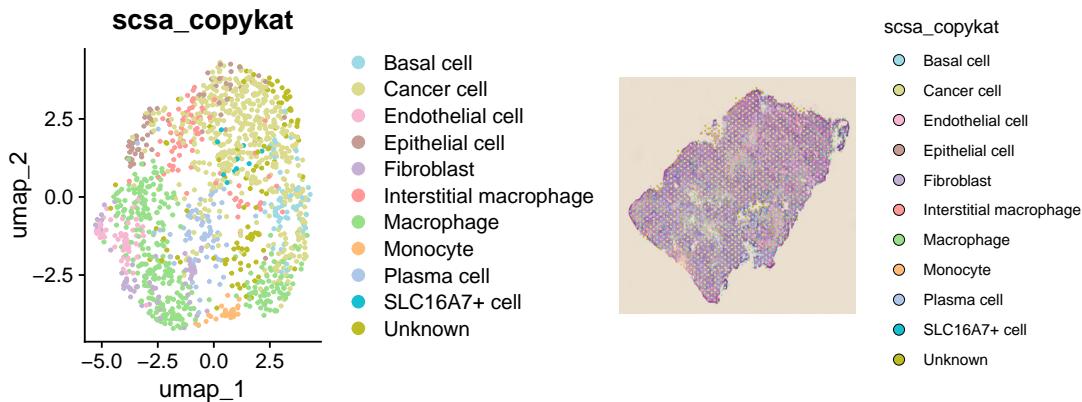


Figure 12: CANER The scsa cell



6.2.2 Seurat 空间转录组分析 (PERI)

使用 Seurat 工具对空间转录组数据进行分析，可以揭示样本中基因表达的空间模式。PERI 分析特别关注特定区域的基因表达情况，帮助我们理解这些区域的功能和其在生物过程中的角色。

注：这部分内容取自上一回该客户业务的结果。



Figure 13 (下方图) 为图 PERI The scsa cell 概览。

(对应文件为 Figure+Table/PERI-The-scsa-cell.pdf)

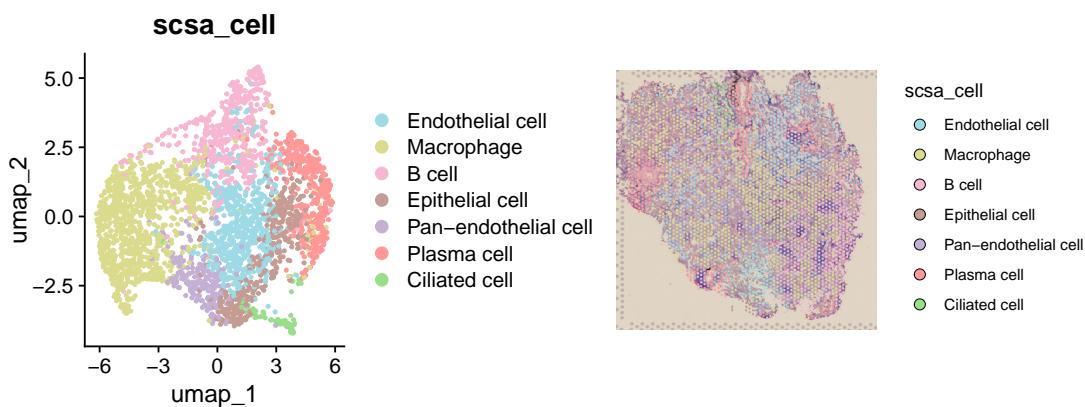


Figure 13: PERI The scsa cell

6.2.3 集成单细胞数据分析

集成单细胞数据分析通过整合来自不同来源的单细胞数据，揭示细胞异质性和基因表达的多样性。这部分的重点在于数据的整合和分析方法的选择，以确保结果的准确性和生物学意义。

6.2.3.1 Seurat 集成单细胞数据分析

Seurat 工具适用于集成不同单细胞数据集，通过整合分析，我们可以揭示不同样本或条件下细胞的异质性和基因表达差异。



Figure 14 (下方图) 为图 The Integrated cells 概览。

(对应文件为 Figure+Table/The-Integrated-cells.pdf)

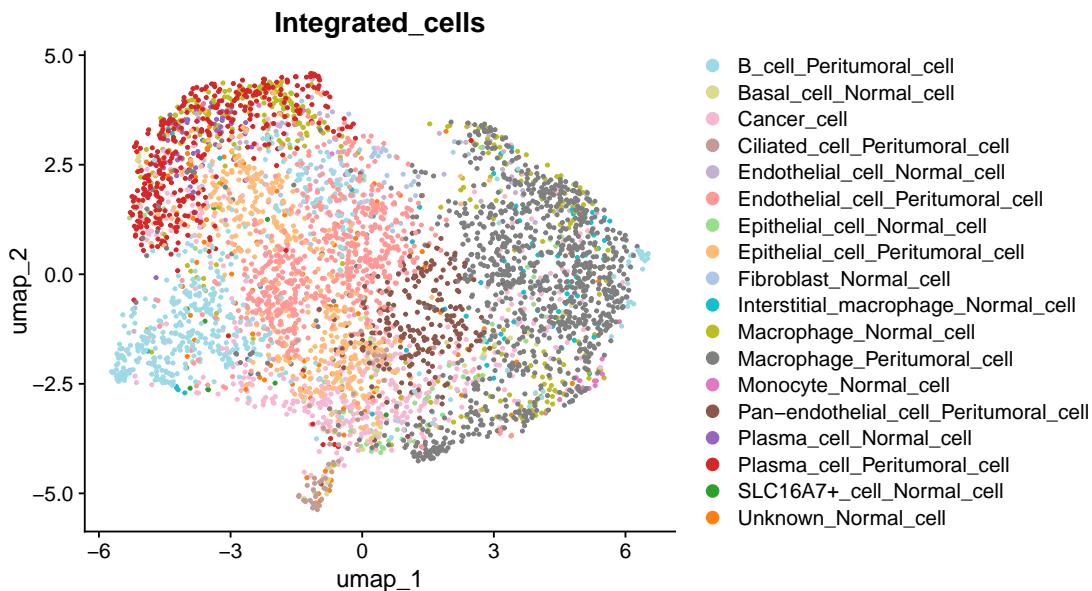


Figure 14: The Integrated cells



6.2.3.2 ClusterProfiler 富集分析 (STNCANCER)

ClusterProfiler 是一种用于基因富集分析的工具，通过识别出富集的基因集，可以帮助我们理解基因在生物过程中的功能及其在癌症研究中的角色。



Figure 15 (下方图) 为图 STNCANCER Cancer cell vs Epithelial cell Peritumoral cell KEGG enrichment 概览。

(对应文件为 Figure+Table/STNCANCER-Cancer-cell-vs-Epithelial-cell-Peritumoral-cell-KEGG-enrichment.pdf)

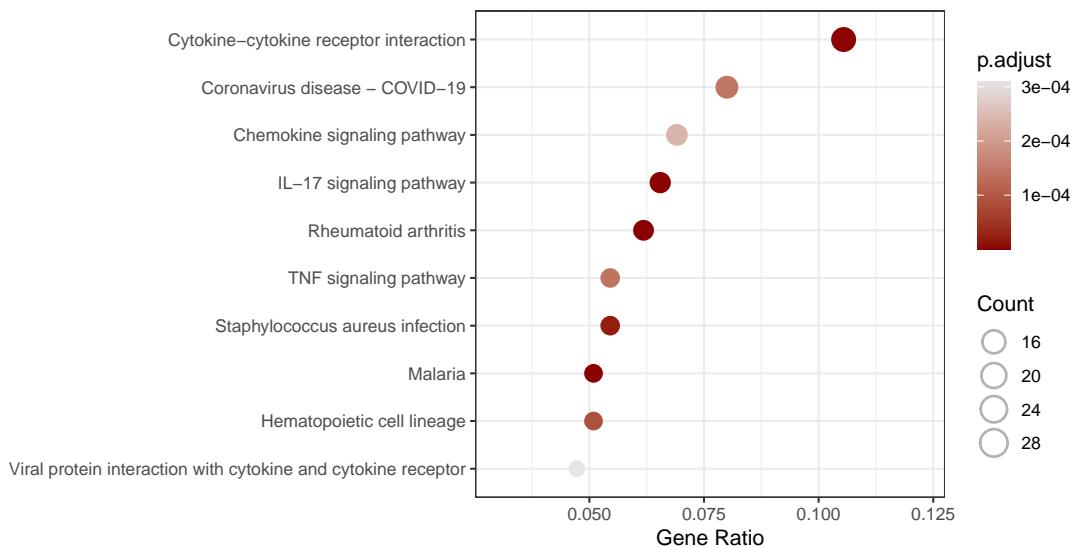


Figure 15: STNCANCER Cancer cell vs Epithelial cell Peritumoral cell KEGG enrichment



6.2.3.3 scFEA 单细胞数据的代谢通量预测 (INTEGRATED)

scFEA 工具用于单细胞数据的代谢通量预测，通过分析单细胞数据，可以预测细胞在不同条件下的代谢活动，揭示其代谢特征和变化。



Figure 16 (下方图) 为图 INTEGRATED Convergency of the loss terms during training 概览。

(对应文件为 Figure+Table/INTEGRATED-Convergenc-y-of-the-loss-terms-during-training.png)

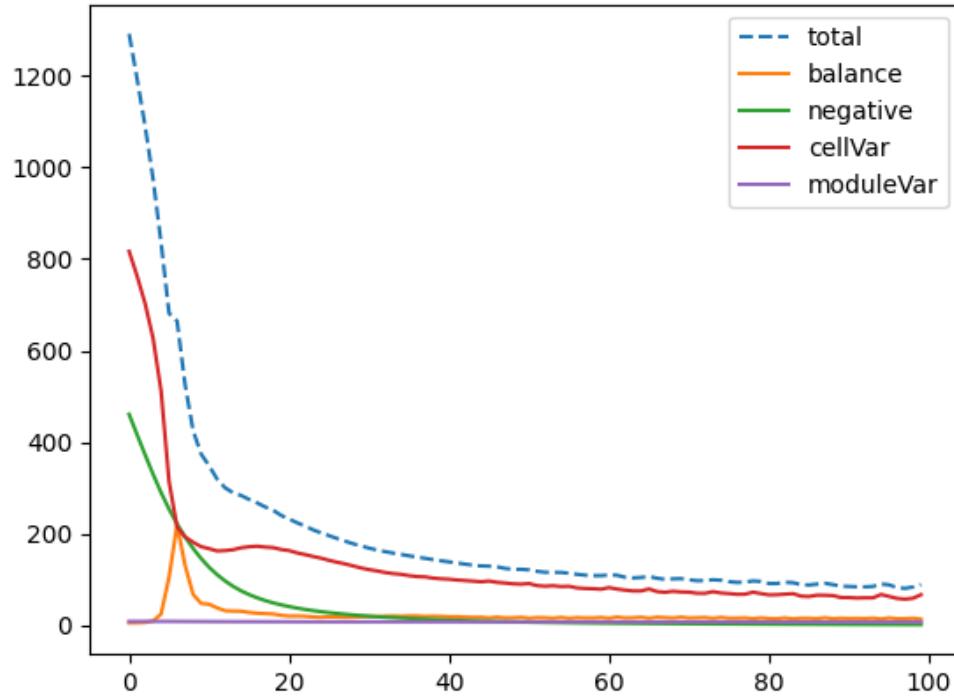


Figure 16: INTEGRATED Convergence of the loss terms during training



6.2.3.4 Limma 代谢通量差异分析 (FLUX)

Limma 工具用于代谢通量的差异分析，通过比较不同条件下的代谢通量，可以揭示其在生物过程中的变化和作用。



Figure 17 (下方图) 为图 FLUX Cancer cell vs Epithelial cell Peritumoral cell DEGs 概览。

(对应文件为 Figure+Table/FLUX-Cancer-cell-vs-Epithelial-cell-Peritumoral-cell-DEGs.pdf)

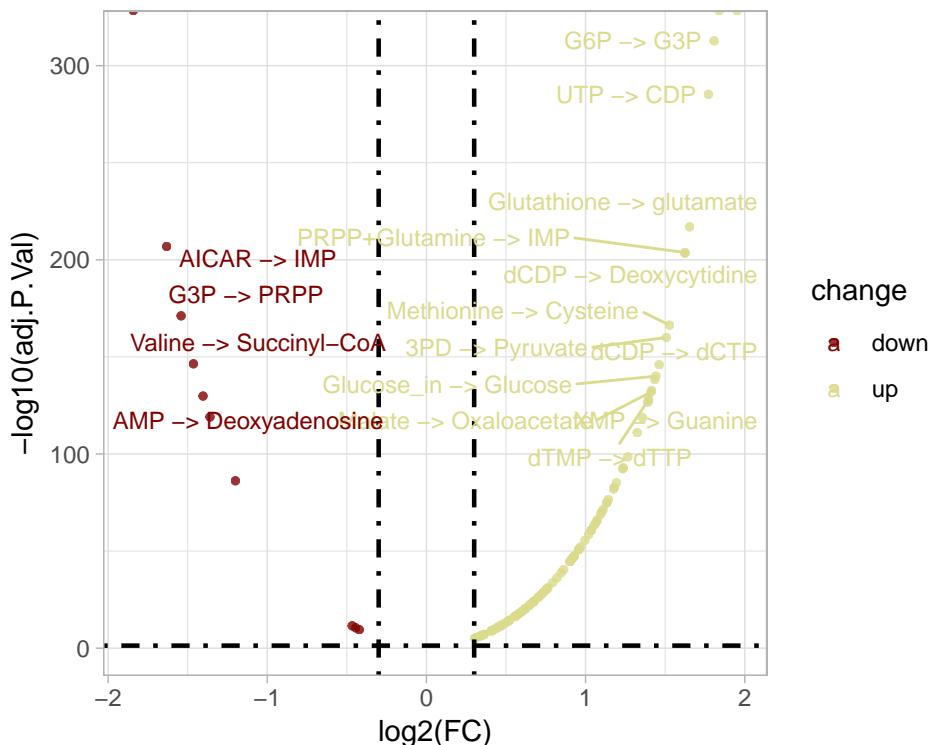


Figure 17: FLUX Cancer cell vs Epithelial cell Peritumoral cell DEGs

adj.P.Val cut-off :

0.05

Log2(FC) cut-off :

0.3

(上述信息框内容已保存至 Figure+Table/FLUX-Cancer-cell-vs-Epithelial-cell-Peritumoral-cell-DEGs-content)

Table 5 (下方表格) 为表格 FLUX data Cancer cell vs Epithelial cell Peritumoral cell DEGs 概览。

(对应文件为 Figure+Table/FLUX-data-Cancer-cell-vs-Epithelial-cell-Peritumoral-cell-DEGs.xlsx)

注：表格共有 122 行 11 列，以下预览的表格可能省略部分数据；含有 122 个唯一 ‘rownames’。

1. logFC: estimate of the log2-fold-change corresponding to the effect or contrast (for ‘topTableF’ there may be several columns of log-fold-changes)
2. AveExpr: average log2-expression for the probe over all arrays and channels, same as ‘Amean’ in the ‘MarrayLM’ object
3. t: moderated t-statistic (omitted for ‘topTableF’)
4. P.Value: raw p-value
5. B: log-odds that the gene is differentially expressed (omitted for ‘topTreat’)

Table 5: FLUX data Cancer cell vs Epithelial cell Peritumoral cell DEGs

rownames	logFC	AveExpr	t	P.Value	adj.P.Val	B	name	gene	compounds
M_3	1.9518...	1.1240...	104.99...	0	0	1169.3...	G3P ->...	BPGM ...	G3P 3PD
M_153	-1.841...	1.0340...	-65.06...	0	0	783.55...	UMP ->...	CANT1 ...	UMP CDP
M_6	1.8399...	-9.576...	64.736...	0	0	779.68...	Pyruva...	LDHA ...	Pyruva...
M_2	1.8075...	-9.279...	59.080...	4.0796...	1.7032...	711.97...	G6P ->...	ALDOA ...	G6P G3P
M_155	1.7719...	-4.982...	54.039...	1.7285...	5.7733...	648.36...	UTP ->...	CTPS1 ...	UTP CDP
M_25	1.6541...	-1.192...	42.502...	4.1359...	1.1511...	490.93...	Glutat...	GGCT ...	Glutat...
M_135	-1.632...	1.4551...	-40.88...	5.5177...	1.3163...	467.61...	AICAR ...	ATIC ...	AICAR ...
M_133	1.6248...	6.1442...	40.382...	8.2789...	1.7282...	460.30...	PRPP+G...	ADSL ...	PRPP+G...
M_158	1.6239...	1.3231...	40.322...	1.9993...	3.7098...	459.42...	dCDP -...	CMPK1 ...	dCDP ...
M_33	-1.541...	-1.347...	-35.28...	4.3329...	7.2359...	384.97...	G3P ->...	ALDOA ...	G3P ...
M_30	1.5272...	-1.458...	34.550...	2.8313...	4.2984...	373.89...	Methio...	AHCY ...	Methio...
M_4	1.5080...	7.6787...	33.574...	6.8791...	9.5734...	359.19...	3PD ->...	BPGM ...	3PD ...
M_54	-1.464...	8.1183...	-31.50...	2.8664...	3.6823...	327.84...	Valine...	ABAT ...	Valine...
M_161	1.4624...	1.9116...	31.429...	8.4922...	1.0130...	326.75...	dCDP -...	NME1 ...	dCDP ...
M_71	1.4416...	2.5366...	30.525...	7.6516...	8.5188...	313.05...	Glucos...	SLC2A1...	Glucos...
...



6.3 联合空间代谢和空间转录组分析

联合空间代谢和空间转录组分析通过整合这两种数据类型，揭示基因表达和代谢活动在空间上的相互关系。这部分的重点在于数据的整合和分析方法的选择，以确保结果的准确性和生物学意义。

6.3.1 交集：差异代谢物 + 单细胞差异代谢通量相关代谢物

通过识别差异代谢物和单细胞差异代谢通量相关的代谢物，我们可以揭示它们在生物过程中的相互作用和关系。



Figure 18 (下方图) 为图 Intersection of Diff flux with Diff meta 概览。

(对应文件为 [Figure+Table/Intersection-of-Diff-flux-with-Diff-meta.pdf](#))

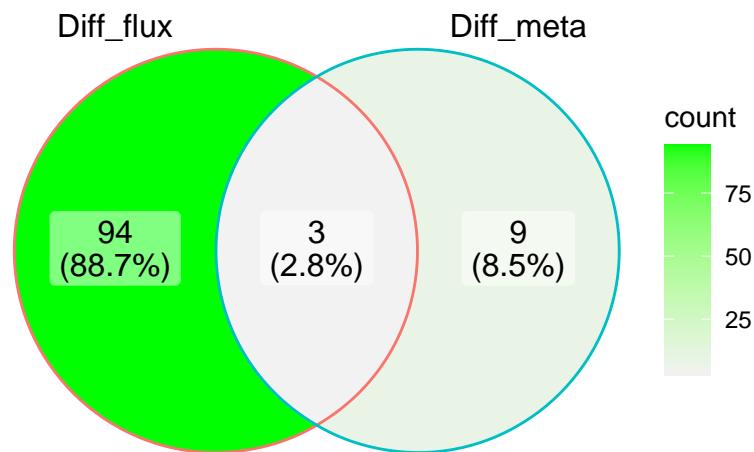


Figure 18: Intersection of Diff flux with Diff meta



All_intersection :

C00105, C00025, C00020

(上述信息框内容已保存至 [Figure+Table/Intersection-of-Diff-flux-with-Diff-meta-content](#))



Table 6 (下方表格) 为表格 FLUX intersection flux data 概览。

(对应文件为 [Figure+Table/FLUX-intersection-flux-data.csv](#))

注：表格共有 11 行 12 列，以下预览的表格可能省略部分数据；含有 11 个唯一 ‘rownames’。

1. logFC: estimate of the log2-fold-change corresponding to the effect or contrast (for ‘topTableF’ there may be several columns of log-fold-changes)
2. AveExpr: average log2-expression for the probe over all arrays and channels, same as ‘Amean’ in the ‘MarrayLM’ object
3. t: moderated t-statistic (omitted for ‘topTableF’)
4. P.Value: raw p-value
5. B: log-odds that the gene is differentially expressed (omitted for ‘topTreat’)

Table 6: FLUX intersection flux data

rownames	logFC	AveExpr	t	P.Value	adj.P.Val	B	name	gene	compounds
M_153	-1.841...	1.0340...	-65.06...	0	0	783.55...	UMP ->...	CANT1 ...	UMP CDP
M_25	1.6541...	-1.192...	42.502...	4.1359...	1.1511...	490.93...	Glutat...	GGCT ...	Glutat...
M_138	-1.403...	3.0980...	-28.95...	1.6973...	1.4918...	289.23...	AMP ->...	AK1 ...	AMP ...
M_48	1.3245...	8.1225...	26.060...	1.5888...	1.0613...	245.58...	Glutam...	GLS ...	Glutam...
M_136	1.0740...	2.0540...	18.875...	2.0959...	8.9750...	141.81...	IMP ->...	ADSL ...	IMP AMP
M_151	1.0350...	-1.063...	17.944...	5.9310...	2.2511...	129.29...	Orotid...	UMPS	Orotid...
M_150	0.9279...	-6.182...	15.560...	1.2410...	3.9857...	98.702...	PRPP -...	UMPS	PRPP ...
M_139	0.9134...	-1.242...	15.256...	5.2227...	1.5858...	94.975...	AMP ->...	ADK ...	AMP ...
M_26	0.8463...	4.3980...	13.888...	5.9781...	1.6921...	78.784...	Glutam...	GCLC ...	Glutam...
M_51	0.6412...	3.5247...	10.081...	9.6264...	2.0349...	39.403...	Glutam...	GLUD1 ...	Glutam...
M_152	0.5810...	2.7901...	9.0453...	8.6957...	1.6885...	30.383...	UMP ->...	DPYD ...	UMP ...

Table 7 (下方表格) 为表格 INTEGRATE metadata of visualized metabolites 概览。

(对应文件为 Figure+Table/INTEGRATE-metadata-of-visualized-metabolites.xlsx)

注：表格共有 4 行 15 列，以下预览的表格可能省略部分数据；含有 4 个唯一 ‘i’。

Table 7: INTEGRATE metadata of visualized metabolites

i	mz	count	freq	mz.sub	m.z	Name	Notation	Formula	KEGG
13	128.03...	2469	0.0105...	128.0354	128.03...	Glutam...	[M-H-H...]	C5H9NO4	C00025
25	146.04...	53307	0.2277...	146.0459	146.04...	Glutam...	[M-H]-	C5H9NO4	C00025
123	323.02...	19079	0.0815...	323.0284	323.02...	Uridin...	[M-H]-	C9H13N...	C00105
131	346.05...	112118	0.4789...	346.0561	346.05...	Adenos...	[M-H]-	C10H14...	C00020

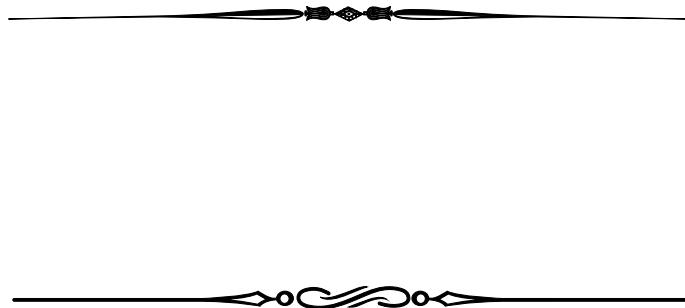


Figure 19 (下方图) 为图 INTEGRATE Feature 25 C00025 image visualization 概览。

(对应文件为 Figure+Table/INTEGRATE-Feature-25-C00025-image-visualization.pdf)

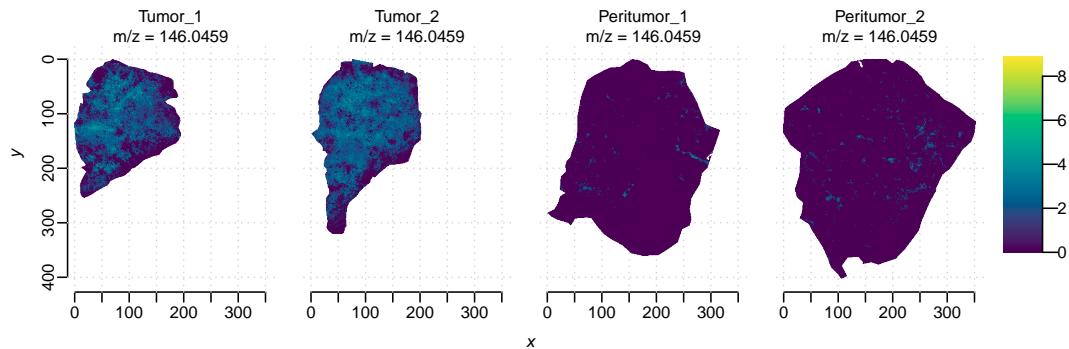
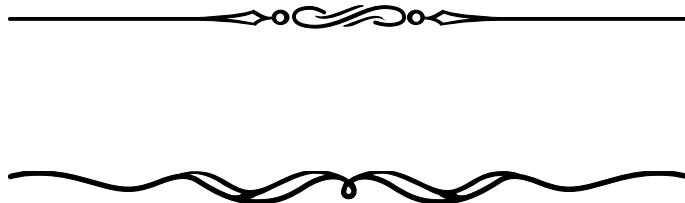


Figure 19: INTEGRATE Feature 25 C00025 image visualization



‘Feature image visualizations’ 数据已全部提供。

(对应文件为 Figure+Table/Feature-image-visualizations)

注：文件夹 Figure+Table/Feature-image-visualizations 共包含 4 个文件。

1. 1_Feature_13_C00025.pdf
2. 2_Feature_25_C00025.pdf
3. 3_Feature_123_C00105.pdf
4. 4_Feature_131_C00020.pdf



6.3.2 FELLA 代谢物富集分析 (INTERSECT)

FELLA 工具用于富集分析，通过识别出富集的代谢通路，可以帮助我们理解代谢物在生物过程中的功能及其相互关系。



Table 8 (下方表格) 为表格 INTERSECT data of enrichment with algorithm Hypergeom 概览。

(对应文件为 Figure+Table/INTERSECT-data-of-enrichment-with-algorithm-Hypergeom.csv)

注：表格共有 15 行 6 列，以下预览的表格可能省略部分数据；含有 15 个唯一 ‘KEGG.id’。

1. pvalue: 显著性 P。

Table 8: INTERSECT data of enrichment with algorithm Hypergeom

KEGG.id	Description	Count	CompoundsI...	pvalue	Compound_R...
hsa00240	Pyrimidine...	3	173	0.00448664...	1
hsa00630	Glyoxylate...	3	147	0.00448664...	1
hsa00760	Nicotinate...	3	169	0.00448664...	1
hsa00770	Pantothena...	3	125	0.00448664...	1
hsa01232	Nucleotide...	3	154	0.00448664...	1
hsa00230	Purine met...	3	218	0.00748364...	1
hsa00983	Drug metab...	3	258	0.00748364...	1
hsa04727	GABAergic ...	2	35	0.00748364...	0.66666666...
hsa04742	Taste tran...	2	36	0.00748364...	0.66666666...
hsa05169	Epstein-Ba...	2	36	0.00748364...	0.66666666...
hsa04068	FoxO signa...	2	42	0.00885843...	0.66666666...
hsa01523	Antifolate...	2	45	0.00933248...	0.66666666...

KEGG.id	Description	Count	CompoundsL...	pvalue	Compound_R...
hsa04216	Ferroptosis	2	59	0.01485597...	0.66666666...
hsa00740	Riboflavin	2	65	0.01675415...	0.66666666...
hsa00220	Arginine b...	2	70	0.01700766...	0.66666666...

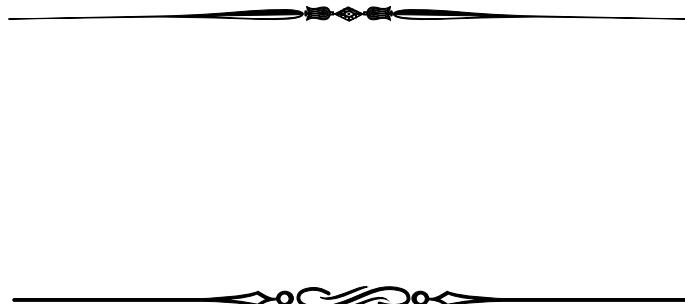


Figure 20 (下方图) 为图 INTERSECT Compounds hypergeom KEGG enrichment 概览。

(对应文件为 Figure+Table/INTERSECT-Compounds-hypergeom-KEGG-enrichment.pdf)

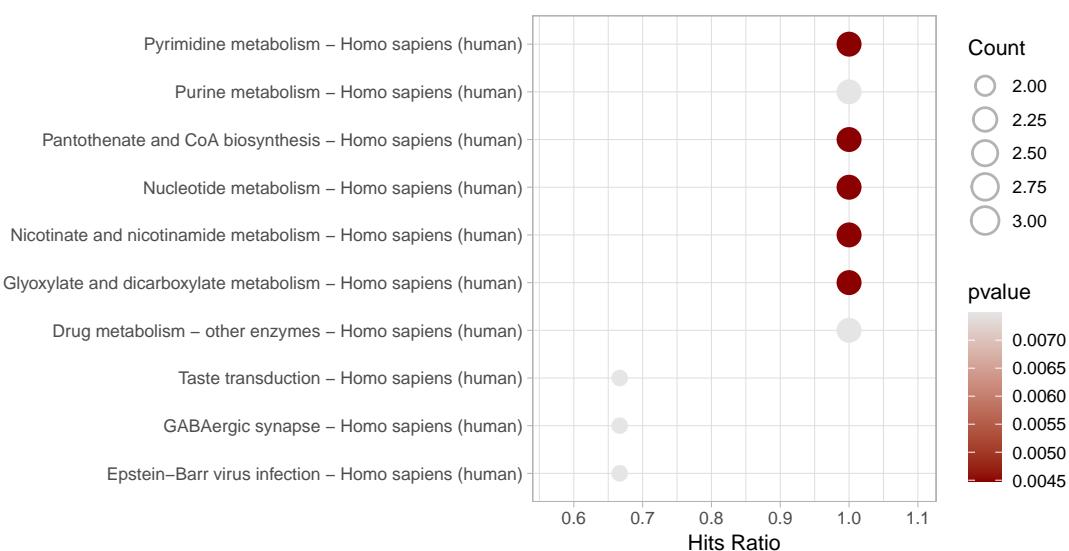


Figure 20: INTERSECT Compounds hypergeom KEGG enrichment

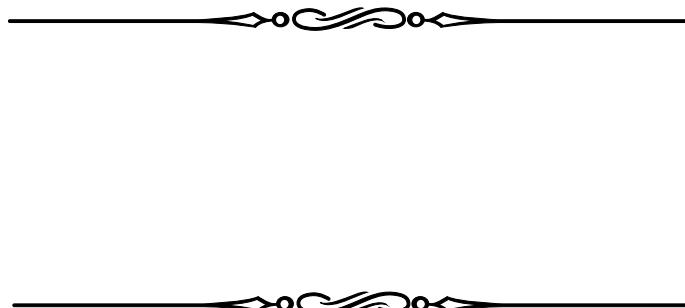


Figure 21 (下方图) 为图 INTERSECT enrichment with algorithm PageRank 概览。

(对应文件为 Figure+Table/INTERSECT-enrichment-with-algorithm-PageRank.pdf)

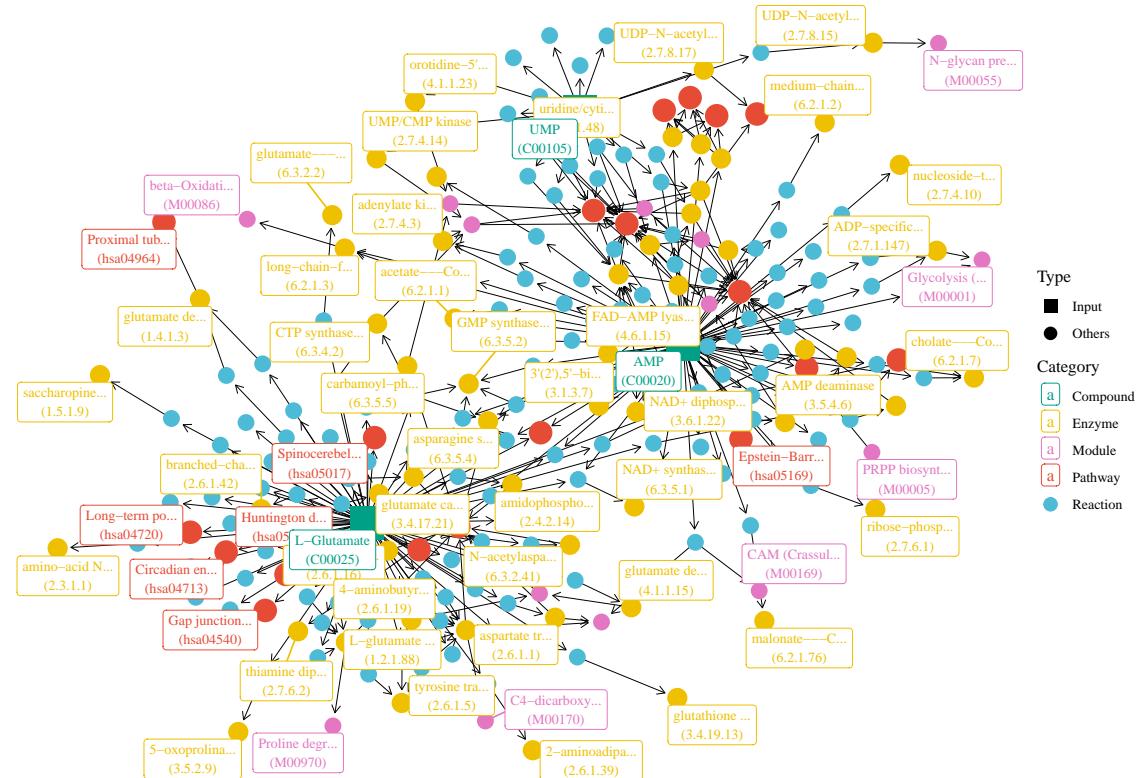


Figure 21: INTERSECT enrichment with algorithm PageRank



6.3.3 ClusterProfiler 富集分析 (FLUX)

ClusterProfiler 工具用于基因富集分析，通过识别出富集的基因集，可以帮助我们理解基因在生物过程中的功能及其在代谢研究中的角色。



Figure 22 (下方图) 为图 FLUX GO enrichment 概览。

(对应文件为 Figure+Table/FLUX-GO-enrichment.pdf)

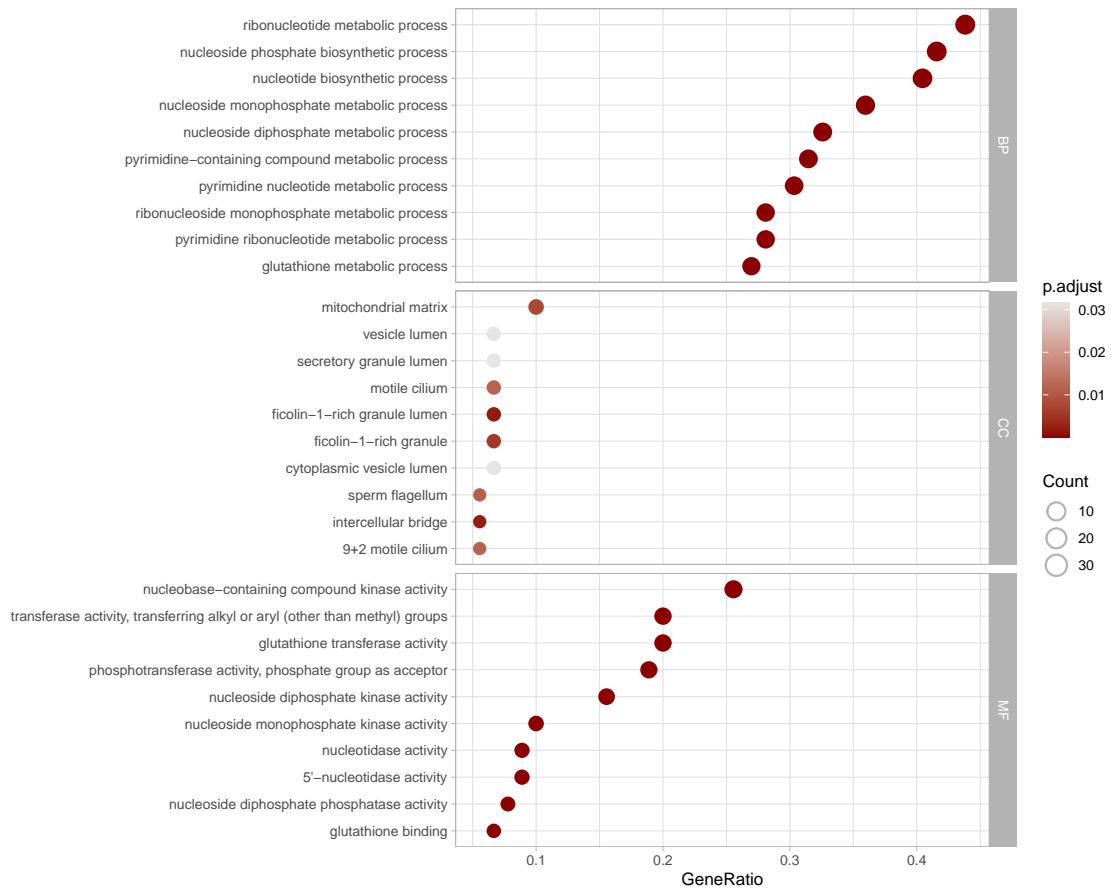


Figure 22: FLUX GO enrichment

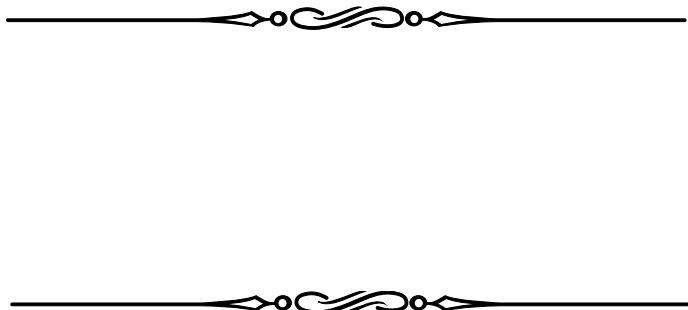


Figure 23 (下方图) 为图 FLUX KEGG enrichment 概览。

(对应文件为 Figure+Table/FLUX-KEGG-enrichment.pdf)

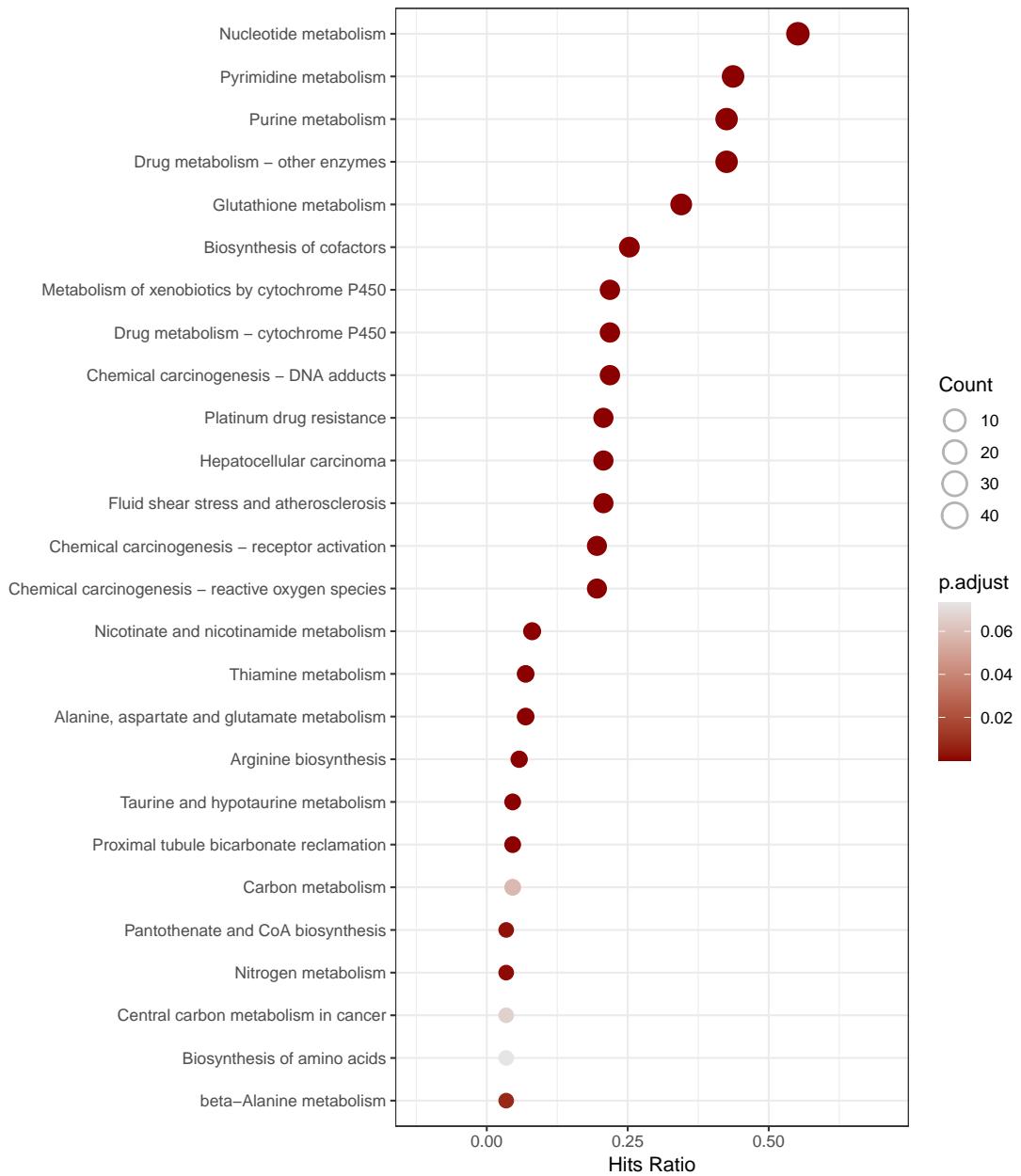


Figure 23: FLUX KEGG enrichment



6.3.4 代谢物与基因共同富集的 KEGG 通路

通过分析代谢物与基因在 KEGG 通路中的共同富集情况，我们可以揭示其在生物过程中的相互作用和功能。



Figure 24 (下方图) 为图 FLUX Co enriched KEGG pathway 概览。

(对应文件为 [Figure+Table/FLUX-Co-enriched-KEGG-pathway.pdf](#))

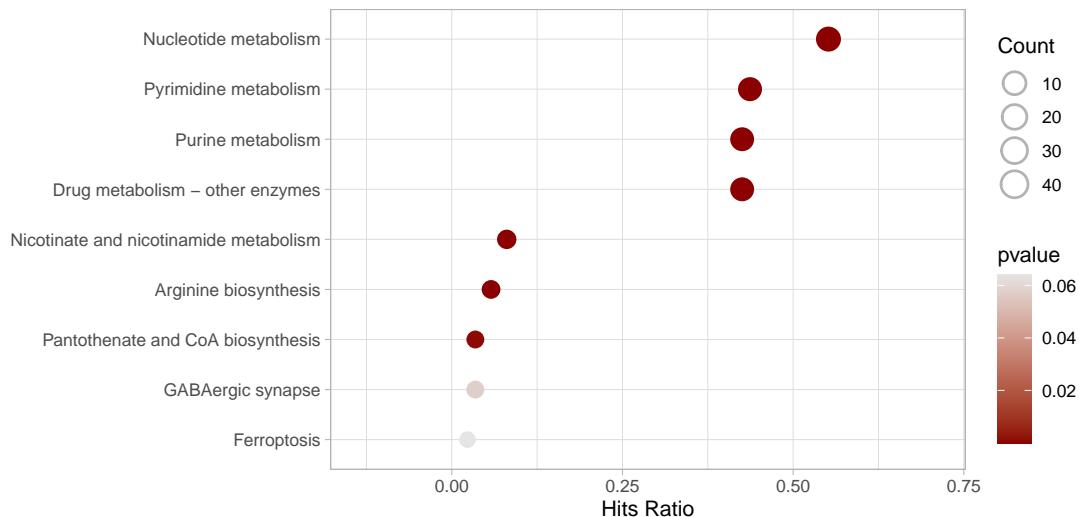


Figure 24: FLUX Co enriched KEGG pathway



Table 9 (下方表格) 为表格 FLUX Co enriched KEGG pathway data 概览。

(对应文件为 [Figure+Table/FLUX-Co-enriched-KEGG-pathway-data.csv](#))

注：表格共有 11 行 11 列，以下预览的表格可能省略部分数据；含有 11 个唯一 ‘ID’。

1. pvalue: 显著性 P。

Table 9: FLUX Co enriched KEGG pathway data

ID	Descri...	GeneRatio	BgRatio	pvalue	p.adjust	qvalue	geneID	Count	geneID...
hsa01232	Nucleo...	48/87	85/8840	6.1247...	3.1235...	1.6117...	132/15...	48	132 ...
hsa00240	Pyrimi...	38/87	58/8840	6.7352...	1.7174...	8.8621...	124583...	38	124583...
hsa00983	Drug m...	37/87	81/8840	8.8804...	1.5096...	7.7898...	51727/...	37	51727 ...
hsa00230	Purine...	37/87	128/8840	8.9327...	9.1114...	4.7014...	132/15...	37	132 ...

ID	Descri...	GeneRatio	BgRatio	pvalue	p.adjust	qvalue	geneID	Count	geneID...
hsa00760	Nicot...	7/87	38/8840	6.9016...	2.1999...	1.1351...	30833/...	7	30833 ...
hsa00220	Argini...	5/87	23/8840	2.4063...	6.8180...	3.5181...	2744/2...	5	2744 ...
hsa00770	Pantot...	3/87	21/8840	0.0010...	0.0024...	0.0012...	1806/1...	3	1806 ...
hsa04727	GABAer...	3/87	89/8840	0.0571...	0.1079...	0.0556...	2744/2...	3	2744 ...
hsa04216	Ferrop...	2/87	42/8840	0.0639...	0.1165...	0.0601...	2729/2937	2	2729 ...
hsa00630	Glyoxy...	1/87	31/8840	0.2644...	0.3549...	0.1831...	2752	1	2752
hsa05169	Epstei...	3/87	203/8840	0.3226...	0.4219...	0.2177...	953/95...	3	953 ...



6.3.5 拟时分析 (发现拟时变化中的差异基因)

拟时分析用于揭示基因表达随时间变化的模式，通过识别差异基因，可以帮助我们理解这些基因在生物过程中的动态变化。

6.3.5.1 Monocle3 拟时分析 (INTEGRATED)

Monocle3 是一种用于拟时分析的工具，通过分析基因表达随时间的变化，揭示其动态变化模式和生物学意义。



Figure 25 (下方图) 为图 INTEGRATED principal points 概览。

(对应文件为 Figure+Table/INTEGRATED-principal-points.pdf)

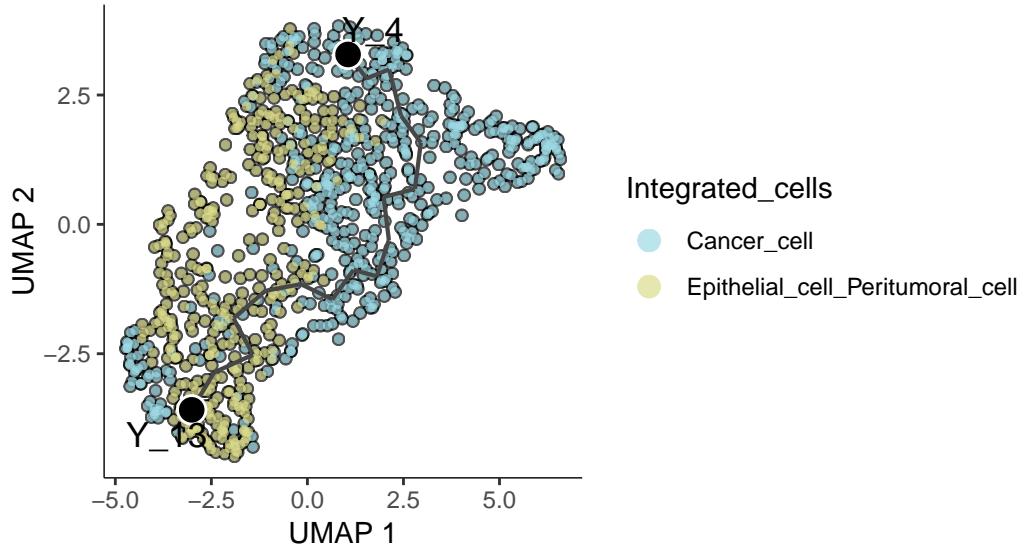


Figure 25: INTEGRATED principal points

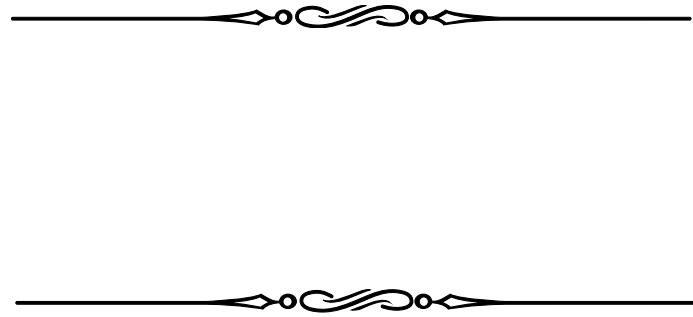


Figure 26 (下方图) 为图 INTEGRATED pseudotime 概览。

(对应文件为 Figure+Table/INTEGRATED-pseudotime.pdf)

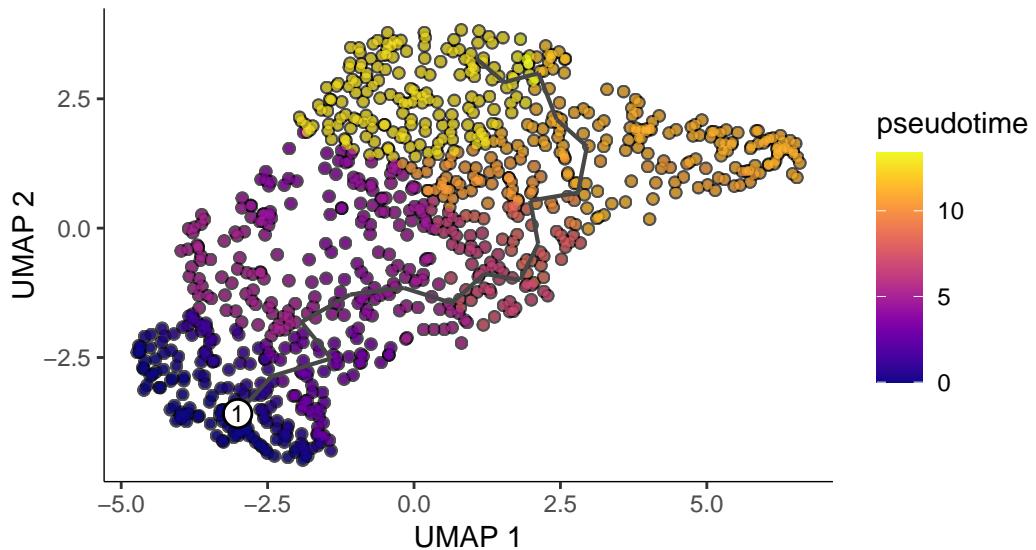


Figure 26: INTEGRATED pseudotime

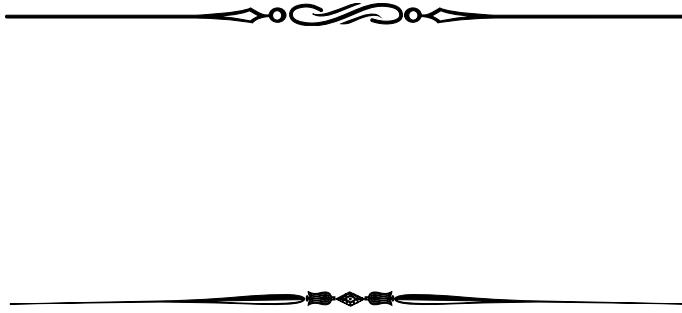


Table 10 (下方表格) 为表格 Graph Test Significant genes Top50 概览。

(对应文件为 Figure+Table/Graph-Test-Significant-genes-Top50.csv)

注：表格共有 50 行 6 列，以下预览的表格可能省略部分数据；含有 50 个唯一 ‘gene_id’。

1. gene_id: GENCODE/Ensembl gene ID

Table 10: Graph Test Significant genes Top50

gene_id	status	p_value	morans_tes...	morans_I	q_value
IGKC	OK	0	75.8062198...	0.67942383...	0
SFTPC	OK	0	69.5950802...	0.62419283...	0
IQCH	OK	0	68.4928396...	0.61428628...	0
MIR205HG	OK	0	68.3310378...	0.61282346...	0
NTRK2	OK	0	68.1731853...	0.61140579...	0
FDCSP	OK	0	68.0735909...	0.61049602...	0
AC019117.2	OK	0	67.3543910...	0.60403901...	0
NAPSA	OK	0	67.3373767...	0.60388367...	0
KRT15	OK	0	67.3218228...	0.60374698...	0
DSG3	OK	0	66.5485380...	0.59678432...	0
ADAM23	OK	0	66.2024864...	0.59369367...	0
PKP1	OK	0	66.1815915...	0.59350259...	0
DSP	OK	0	66.1350653...	0.59304932...	0
SFTPД	OK	0	66.0821697...	0.59261178...	0
SLC34A2	OK	0	65.7432638...	0.58956078...	0
...



6.3.5.2 ClusterProfiler 富集分析 (GRAPHTOPS)

ClusterProfiler 工具用于基因富集分析，通过识别出富集的基因集，可以帮助我们理解基因在拟时分析中的功能及其在生物过程中的角色。



Figure 27 (下方图) 为图 GRAPHTOPS KEGG enrichment 概览。

(对应文件为 [Figure+Table/GRAPHTOPS-KEGG-enrichment.pdf](#))

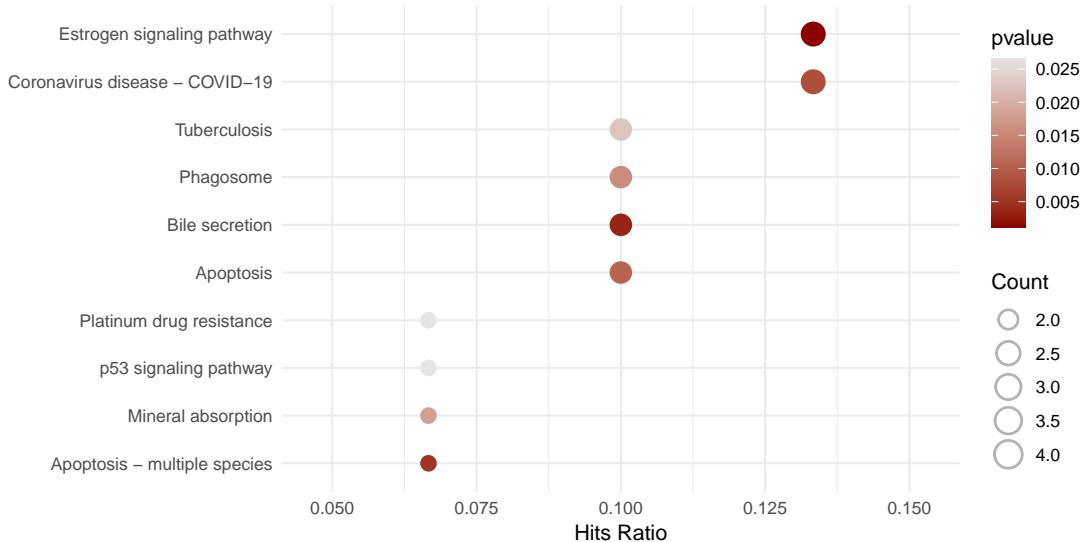


Figure 27: GRAPHTOPS KEGG enrichment



Table 11 (下方表格) 为表格 GRAPHTOPS KEGG enrichment data 概览。

(对应文件为 [Figure+Table/GRAPHTOPS-KEGG-enrichment-data.xlsx](#))

注：表格共有 102 行 11 列，以下预览的表格可能省略部分数据；含有 102 个唯一 ‘ID’。

1. pvalue: 显著性 P。

Table 11: GRAPHTOPS KEGG enrichment data

ID	Descri...	GeneRatio	BgRatio	pvalue	p.adjust	qvalue	geneID	Count	geneID...
hsa04915	Estrog...	4/30	139/8840	0.0011...	0.1191...	0.1057...	596/15...	4	596 ...
hsa04976	Bile s...	3/30	90/8840	0.0033...	0.1732...	0.1537...	358/36...	3	358 ...
hsa04215	Apopto...	2/30	32/8840	0.0051...	0.1762...	0.1564...	596/5366	2	596 ...
hsa05171	Corona...	4/30	238/8840	0.0080...	0.2065...	0.1832...	3627/6...	4	3627 ...
hsa04210	Apoptosis	3/30	136/8840	0.0106...	0.2177...	0.1932...	596/15...	3	596 ...
hsa04145	Phagosome	3/30	157/8840	0.0157...	0.2622...	0.2327...	653509...	3	653509...
hsa04978	Minera...	2/30	61/8840	0.0179...	0.2622...	0.2327...	2512/1...	2	2512 ...
hsa05152	Tuberc...	3/30	180/8840	0.0225...	0.2643...	0.2345...	596/97...	3	596 ...
hsa01524	Platin...	2/30	75/8840	0.0265...	0.2643...	0.2345...	596/5366	2	596 ...
hsa04115	p53 si...	2/30	75/8840	0.0265...	0.2643...	0.2345...	596/5366	2	596 ...
hsa05133	Pertussis	2/30	78/8840	0.0285...	0.2643...	0.2345...	653509...	2	653509...
hsa05210	Colore...	2/30	87/8840	0.0348...	0.2792...	0.2478...	596/5366	2	596 ...
hsa04610	Comple...	2/30	88/8840	0.0355...	0.2792...	0.2478...	2/7450	2	2 7450
hsa05150	Staphy...	2/30	100/8840	0.0448...	0.3106...	0.2756...	3866/3872	2	3866 ...
hsa04933	AGE-RA...	2/30	101/8840	0.0456...	0.3106...	0.2756...	596/7412	2	596 ...
...



6.3.5.3 Pseudotime Heatmap

通过拟时分析的热图，我们可以直观地展示基因表达随时间的变化模式，帮助我们理解这些基因在生物过程中的动态变化。



Figure 28 (下方图) 为图 INTEGRATED Pseudotime heatmap of genes 概览。

(对应文件为 Figure+Table/INTEGRATED-Pseudotime-heatmap-of-genes.pdf)

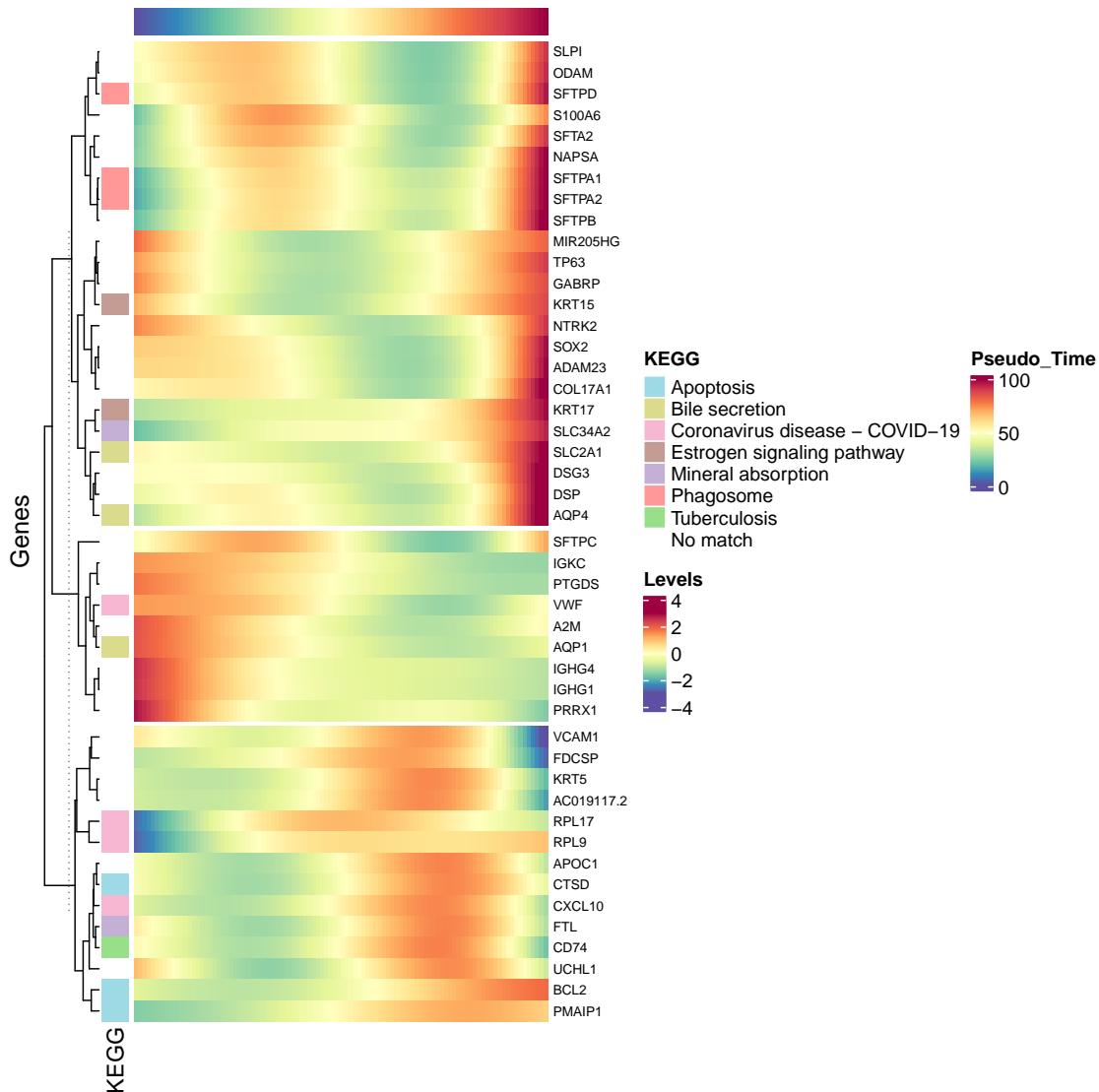


Figure 28: INTEGRATED Pseudotime heatmap of genes



6.3.5.4 STRINGdb PPI 分析 (PPI)

STRINGdb 工具用于蛋白质-蛋白质相互作用 (PPI) 分析，通过识别蛋白质之间的相互作用，可以帮助我们理解这些蛋白质在生物过程中的功能及其相互关系。



Figure 29 (下方图) 为图 PPI raw PPI network 概览。
(对应文件为 Figure+Table/PPI-raw-PPI-network.pdf)

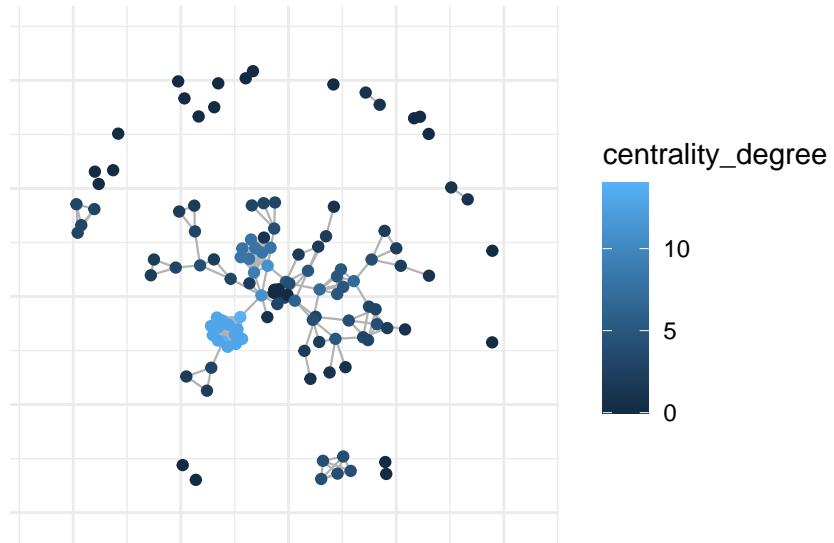


Figure 29: PPI raw PPI network

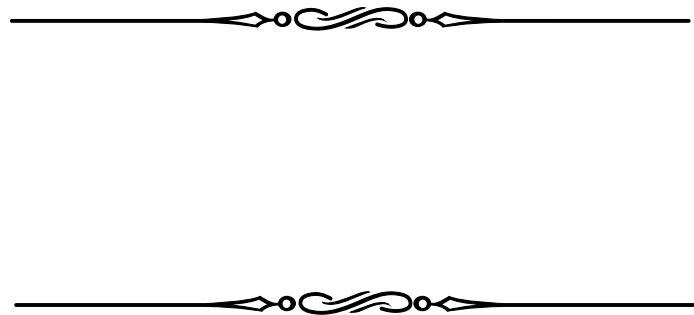


Figure 30 (下方图) 为图 PPI Top MCC score 概览。
(对应文件为 Figure+Table/PPI-Top-MCC-score.pdf)

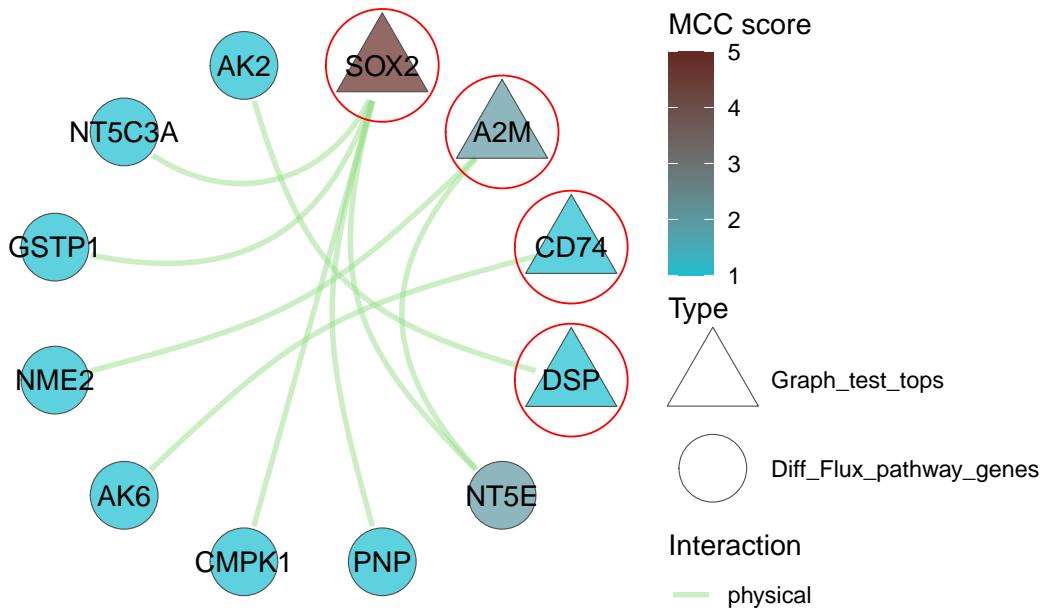


Figure 30: PPI Top MCC score

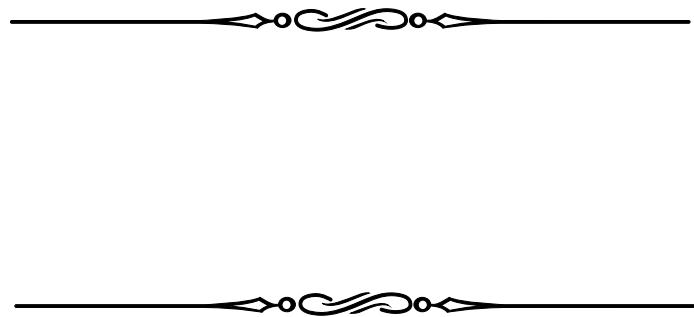


Figure 31 (下方图) 为图 Dimension plot of expression level of the genes 概览。

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

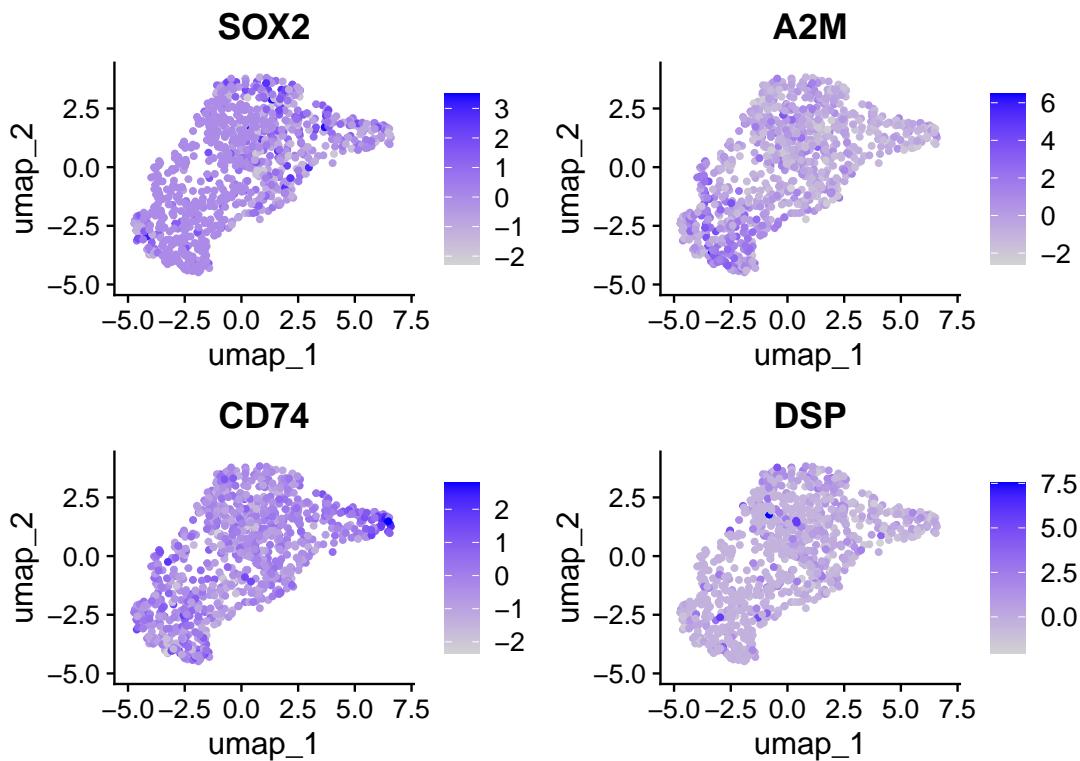


Figure 31: Dimension plot of expression level of the genes

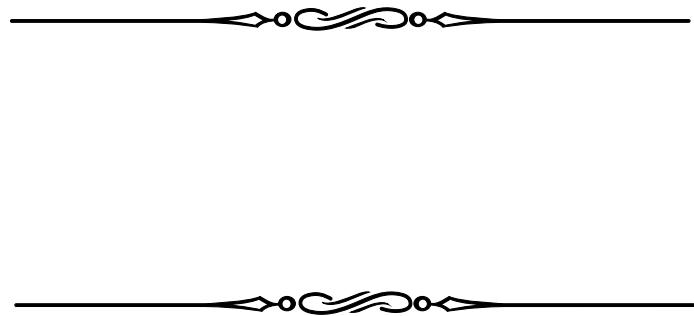


Figure 32 (下方图) 为图 Spatial feature plot 概览。

(对应文件为 Figure+Table/Spatial-feature-plot.pdf)

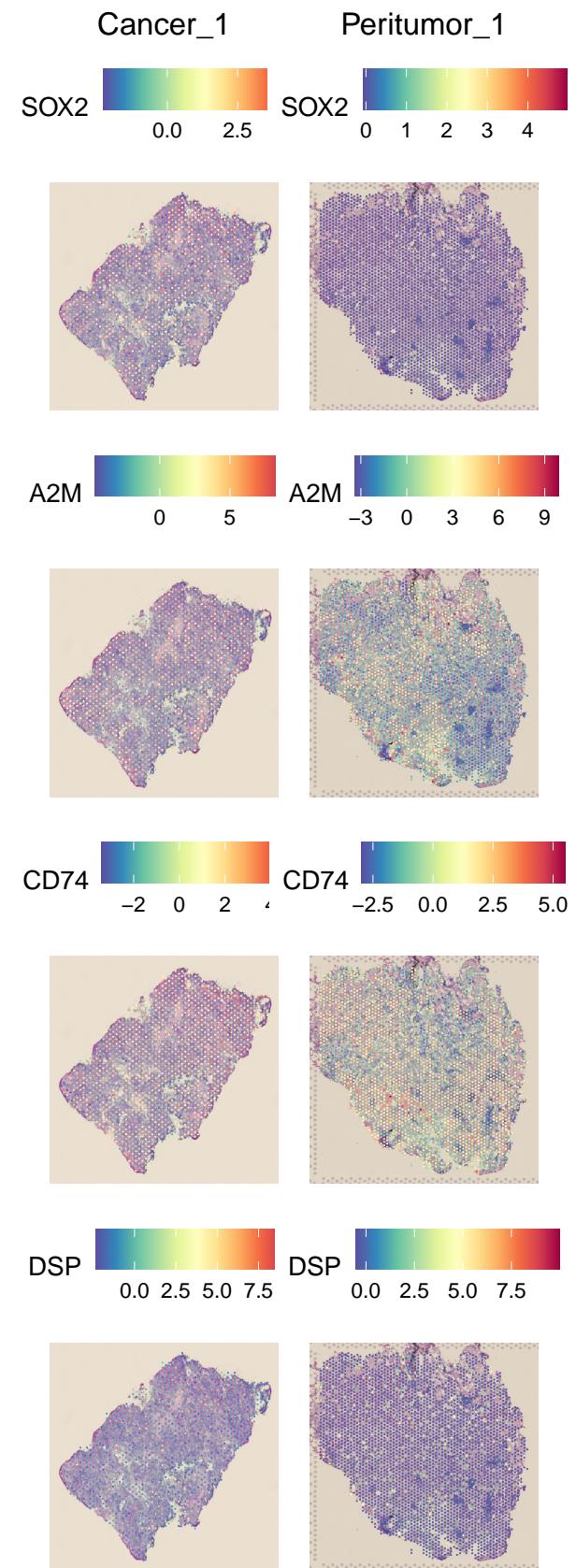


Figure 32: Spatial feature plot



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