Analysis

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1 摘要

筛选丹参酮治疗脓毒症（sepsis）的关键差异表达基因及相关信号通路。

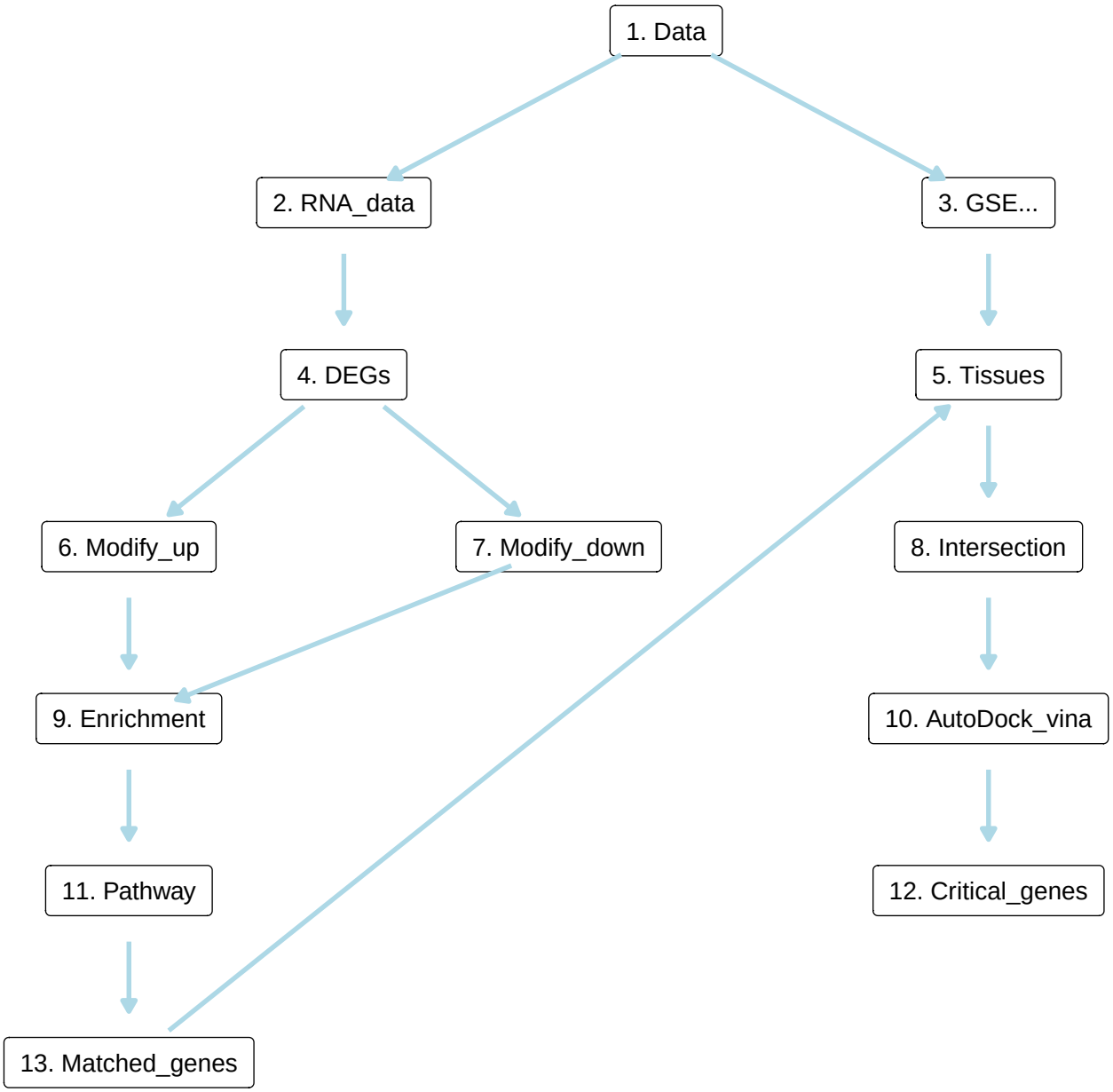
注：补充分析请参考 7（进一步筛选与肠道微生物和免疫微环境相关的差异表达基因）

2 材料和方法

测序数据：Caco-2 细胞系，对照组 con，脂多糖组 LPS，丹参酮组 TNA（LPS+TNA）。

GEO 数据：GSE237861

3 研究设计流程图



4 分析结果

单以测序数据集筛选到 1797 个靶点，富集分析聚焦到 Hippo 通路（Fig. 7）。

以 GEO 数据 GSE237861 分析发现，6 种不同组织的 sepsis 病例存在 51 个共同的差异表达基因（Disease vs control）。进一步分析发现：无同时存在于 6 或 5 种组织的 Hippo 通路基因（同时也是 Tanshinone IIA 的作用靶点）；*BIRC3*、*ID1* 在 4 种组织中差异表达；*DLG4* 在 3 种组织中差异表达（Fig. 10）。分子对接显示，SMAD7, SOX2, TGFBR2, DLG4, DLG2 具有良好亲和度（Fig. 11）。综上，*DLG4* 在 3 种 sepsis 组织中差异表达，且 DLG4 可与 Tanshinone IIA 结合，因此，*DLG4* 可能是 TNA 治疗 sepsis 的关键靶点之一，对应信号通路为 Hippo。

5 结论

DLG4 可能是 TNA 治疗 sepsis 的关键靶点，相关信号通路为 Hippo。

6 附：分析流程

6.1 测序数据

6.1.1 差异分析

Figure 1 (下方图) 为图 Low expression filtering 概览。

(对应文件为 `Figure+Table/Low-expression-filtering.pdf`)

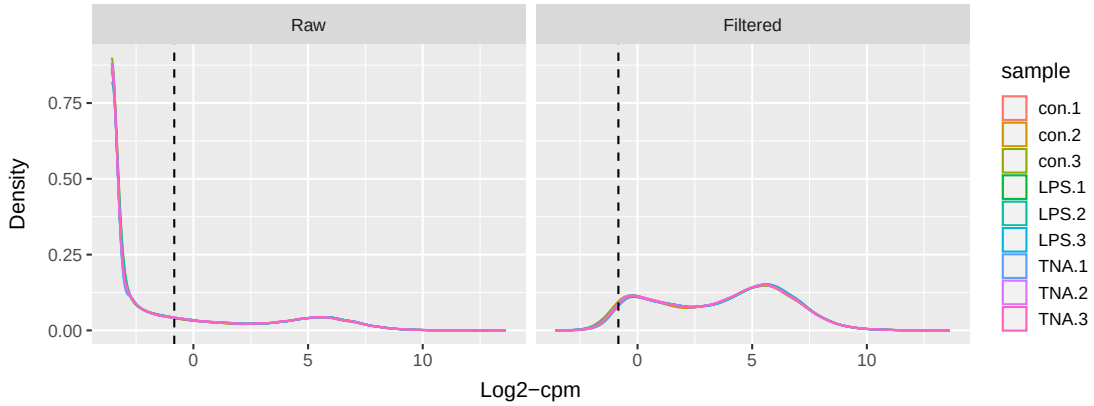


Figure 1: Low expression filtering

Figure 2 (下方图) 为图 expression normalization 概览。

(对应文件为 `Figure+Table/expression-normalization.pdf`)

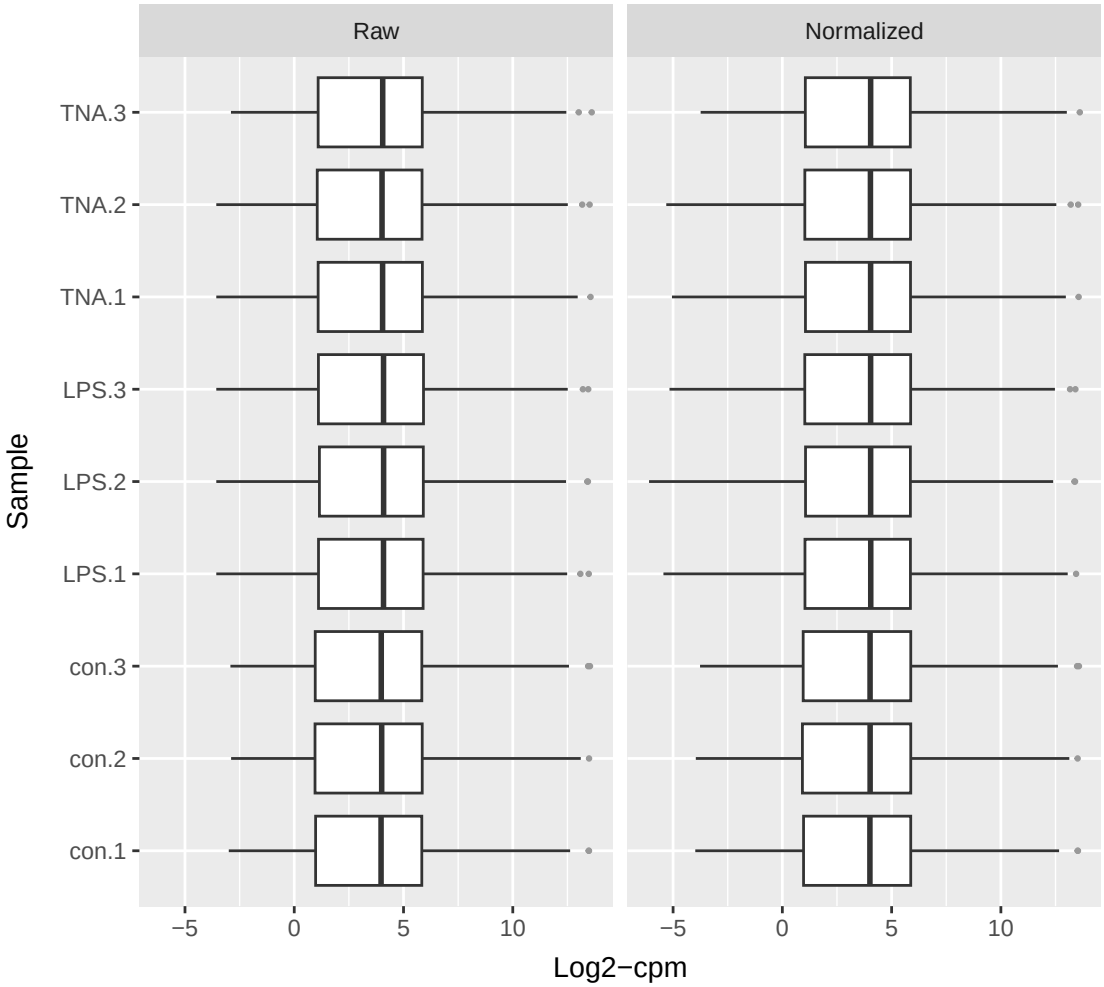


Figure 2: Expression normalization

Figure 3 (下方图) 为图 DEGs of model versus control 概览。

(对应文件为 `Figure+Table/DEGs-of-model-versus-control.pdf`)

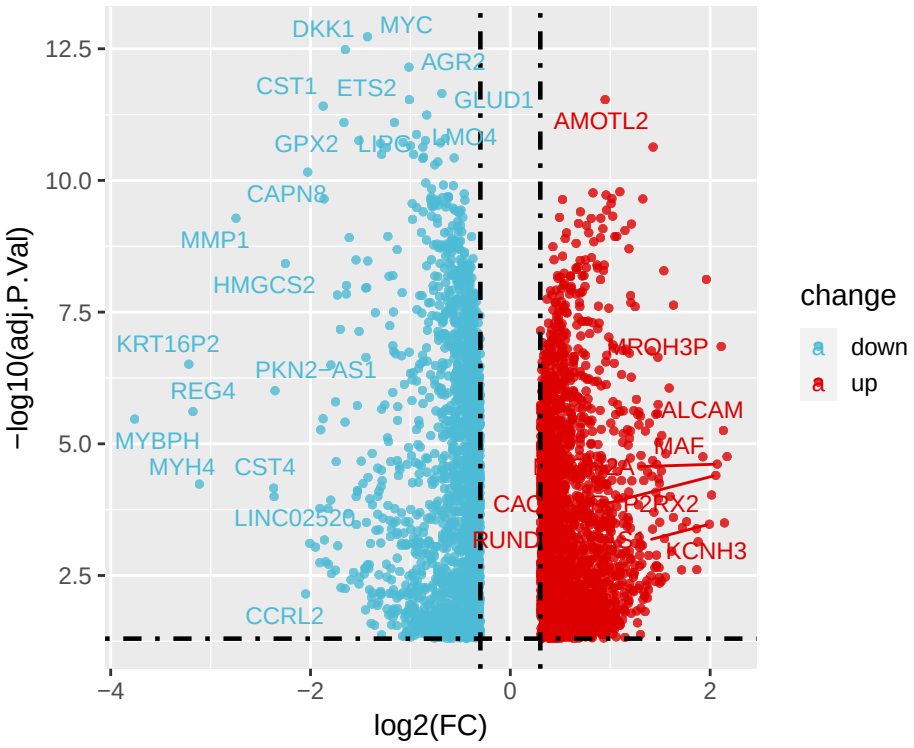


Figure 3: DEGs of model versus control

Table 1 (下方表格) 为表格 table of DEGs of model versus control 概览。

(对应文件为 `Figure+Table/table-of-DEGs-of-model-versus-control.xlsx`)

> 注：表格共有 3865 行 15 列，以下预览的表格可能省略部分数据；表格含有 3865 个唯一 'ensembl_gene_id'。

Table 1: Table of DEGs of model versus control

ensem……1	ensem……2	entre…	hgnc_…	refse…	chrom…	start…	end_p…	descr…	logFC	…
ENSG0…	ENST0…	4609	MYC		8	12773…	12774…	MYC p…	-1.42…	…
ENSG0…	ENST0…	22943	DKK1	NM_01…	10	52314281	52318042	dickk…	-1.65…	…
ENSG0…	ENST0…	10551	AGR2	NM_00…	7	16791811	16833433	anter…	-1.01…	…
ENSG0…	ENST0…	2746	GLUD1	NM_00…	10	87050202	87094843	gluta…	-0.68…	…
ENSG0…	ENST0…	51421	AMOTL2	NM_01…	3	13435…	13437…	angio…	0.948…	…
ENSG0…	ENST0…	2114	ETS2	NM_00…	21	38805183	38824955	ETS p…	-1.01…	…
ENSG0…	ENST0…	8543	LMO4	NM_00…	1	87328880	87348923	LIM d…	-0.83…	…
ENSG0…	ENST0…	1469	CST1	NM_00…	20	23747562	23751268	cysta…	-1.87…	…
ENSG0…	ENST0…	9388	LIPG		18	49560699	49599185	lipas…	-1.16…	…
ENSG0…	ENST0…	2877	GPX2		14	64939152	64942746	gluta…	-1.66…	…
ENSG0…	ENST0…	11163	NUDT4	NM_19…	12	93377883	93408146	nudix…	-0.93…	…
ENSG0…	ENST0…	11260	XPOT	NM_00…	12	64404392	64451125	expor…	-0.65…	…

ensem……1	ensem……2	entre…	hgnc_…	refse…	chrom…	start…	end_p…	descr…	logFC	…
ENSG0…	ENST0…	25902	MTHFD1L	NM_01…	6	15086…	15110…	methy…	-0.84…	…
ENSG0…	ENST0…	91461	PKDCC	NM_13…	2	42048021	42058517	prote…	-0.69…	…
ENSG0…	ENST0…	1080	CFTR		7	11728…	11771…	CF tr…	-1.07…	…
…	…	…	…	…	…	…	…	…	…	…

Figure 4 (下方图) 为图 DEGs of treatment versus model 概览。

(对应文件为 `Figure+Table/DEGs-of-treatment-versus-model.pdf`)

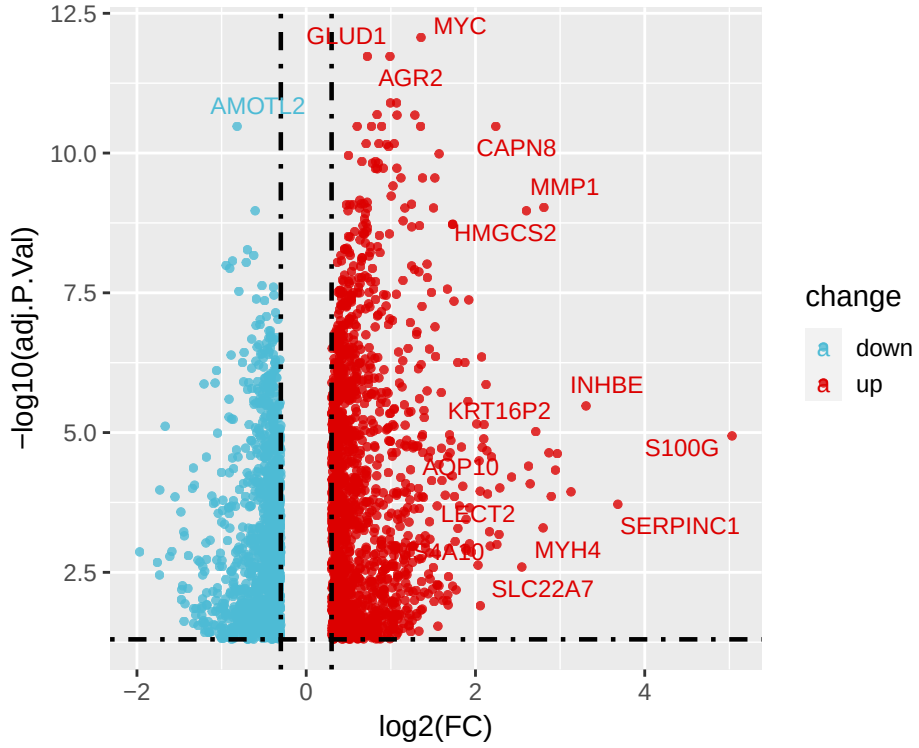


Figure 4: DEGs of treatment versus model

Table 2 (下方表格) 为表格 table of DEGs of treatment versus model 概览。

(对应文件为 `Figure+Table/table-of-DEGs-of-treatment-versus-model.xlsx`)

> 注：表格共有 2762 行 15 列，以下预览的表格可能省略部分数据；表格含有 2762 个唯一 'ensembl_gene_id'。

Table 2: Table of DEGs of treatment versus model

ensem……1	ensem……2	entre…	hgnc_…	refse…	chrom…	start…	end_p…	descr…	logFC	…
ENSG0…	ENST0…	4609	MYC		8	12773…	12774…	MYC p…	1.356…	…
ENSG0…	ENST0…	2746	GLUD1	NM_00…	10	87050202	87094843	gluta…	0.722…	…

ensem……1	ensem……2	entre…	hgnc_…	refse…	chrom…	start…	end_p…	descr…	logFC	…
ENSG0…	ENST0…	10551	AGR2	NM_00…	7	16791811	16833433	anter…	0.989…	…
ENSG0…	ENST0…	8614	STC2	NM_00…	5	17331…	17332…	stann…	1.067…	…
ENSG0…	ENST0…	63910	SLC17A9	NM_02…	20	62952707	62969585	solut…	0.994…	…
ENSG0…	ENST0…	7422	VEGFA		6	43770184	43786487	vascu…	0.837…	…
ENSG0…	ENST0…	22943	DKK1	NM_01…	10	52314281	52318042	dickk…	1.283…	…
ENSG0…	ENST0…	2118	ETV4	NM_00…	17	43527844	43579620	ETS v…	1.073…	…
ENSG0…	ENST0…	8543	LMO4	NM_00…	1	87328880	87348923	LIM d…	0.771…	…
ENSG0…	ENST0…	2114	ETS2	NM_00…	21	38805183	38824955	ETS p…	0.891…	…
ENSG0…	ENST0…	51421	AMOTL2	NM_01…	3	13435…	13437…	angio…	-0.81…	…
ENSG0…	ENST0…	336	APOA2		1	16122…	16122…	apoli…	1.352…	…
ENSG0…	ENST0…	1152	CKB	NM_00…	14	10351…	10352…	creat…	0.602…	…
ENSG0…	ENST0…	56649	TMPRSS4		11	11807…	11812…	trans…	0.859…	…
ENSG0…	ENST0…	10797	MTHFD2		2	74186172	74217565	methy…	0.708…	…
…	…	…	…	…	…	…	…	…	…	…

丹参酮的疗效有两种情况：

- 模型组相比对照组，基因上调；而以丹参酮处理后，基因下调（相比于模型组）。
- 模型组相比对照组，基因下调；而以丹参酮处理后，基因上调（相比于模型组）。

Figure 5 (下方图) 为图 intersection of disease genes expression and treatment effect of TNA 概览。

(对应文件为 `Figure+Table/intersection-of-disease-genes-expression-and-treatment-effect-of-TNA.pdf`)

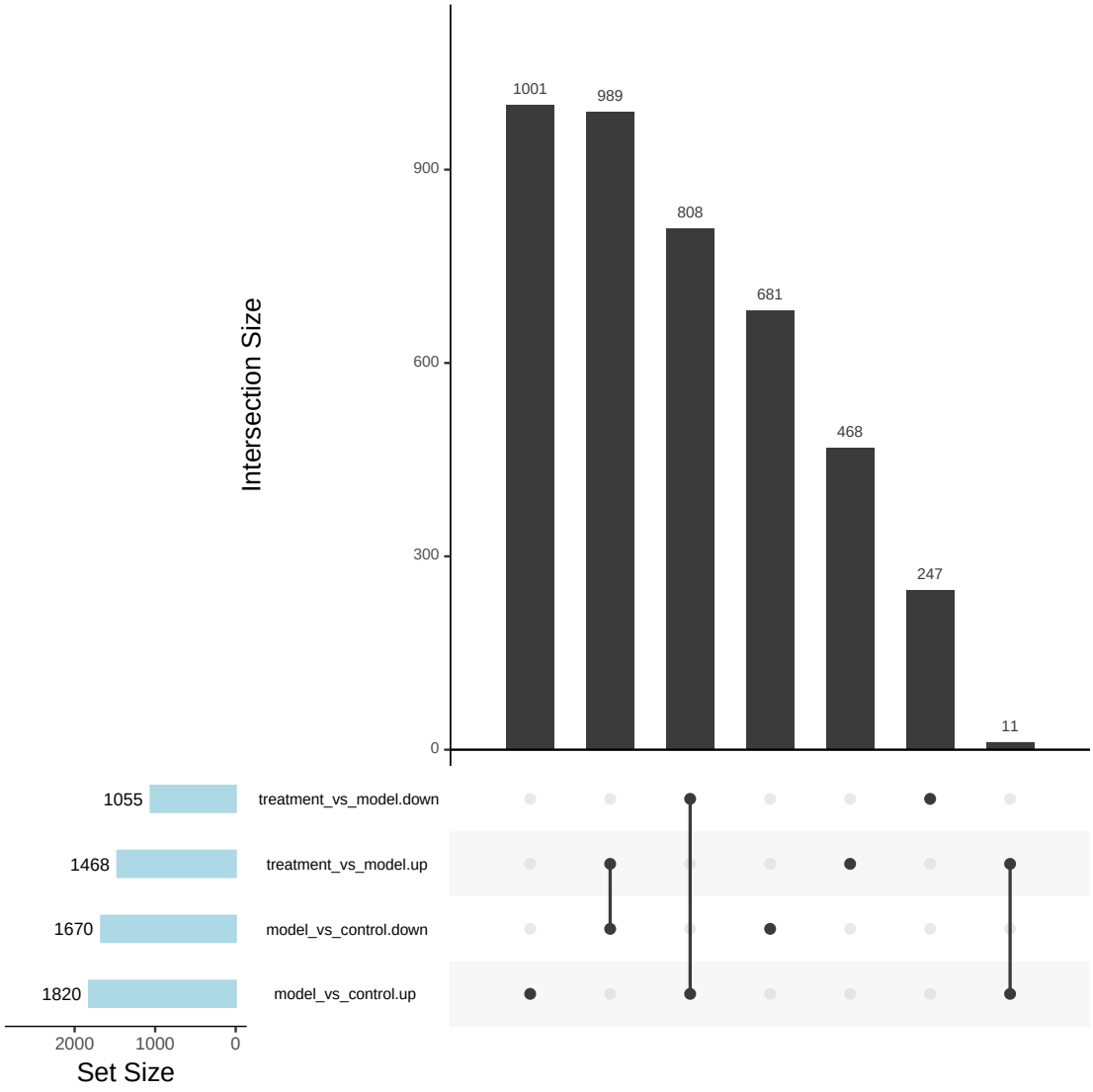


Figure 5: Intersection of disease genes expression and treatment effect of TNA

取 Fig. 5 的两组交集的合集（989 + 808），。

6.1.2 富集分析

以上述合集做富集分析。

Figure 6 (下方图) 为图 KEGG enrichment 概览。

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

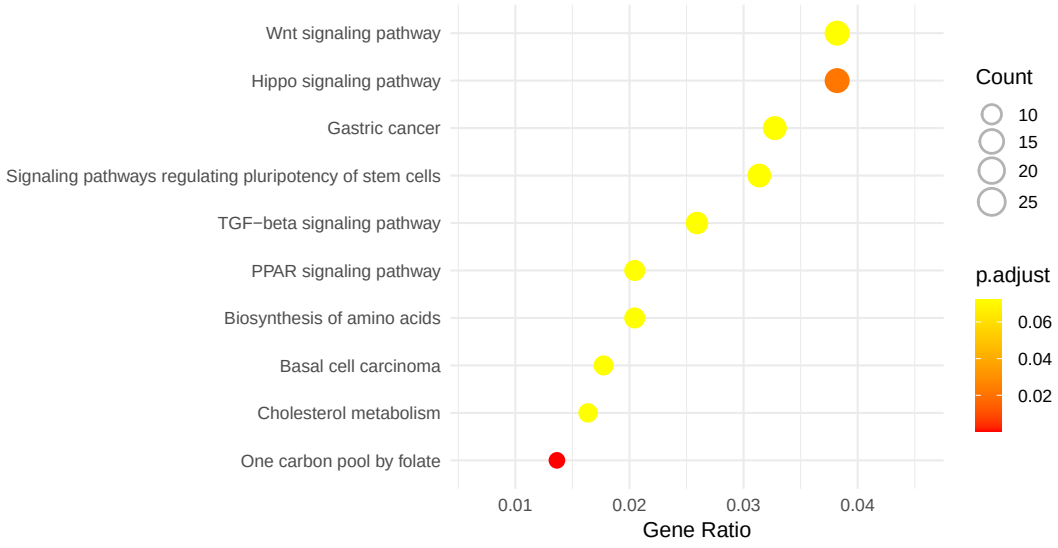


Figure 6: KEGG enrichment

Hippo 通路为显著富集通路。

Figure 7 (下方图) 为图 genes enriched in hippo signiling pathway 概览。

(对应文件为 `Figure+Table/hsa04390.pathview.png`)

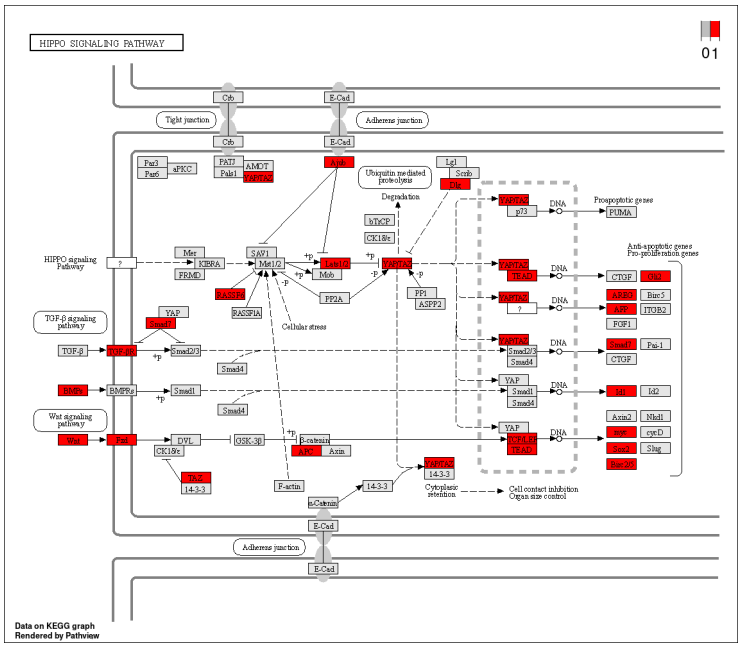


Figure 7: Genes enriched in hippo signiling pathway

6.2 GEO sepsis

6.2.1 GSE237861: Transcriptome analysis of six tissues obtained post mortem from sepsis patients

data_processing :

The libraries were quantified by Qubit dsDNA High Sensitivity Assay Kit (Life Technologies Corporation, Carlsbad, CA, United States) and the median sizes were determined by TapeStation 4200 (Agilent Technologies, USA), using the High Sensitivity D1000 ScreenTape assay, to form an equimolar pool.

data_processing.1 :

Sequencing was performed as a 75-bp single-read, single-index run on a NextSeq 500 next-generation sequencer (Illumina, San Diego, CA, United States) with High Output kit.

data_processing.2 :

Quality control analysis was performed using FastQC software, showing a Phred value superior to 30

data_processing.3 :

Trimmomatic software was used to trim low-quality reads and adapters. Raw reads were aligned to the hg38 reference through HISAT2 software. Quantification of the gene expression data was performed through the function featureCounts of the R package Rsubread and the counts were normalized according to log2CPM.

data_processing.4 :

Differential expression analysis was performed by the R package edgeR (FDR < 0.1 was considered significant), comparing each male patient with sepsis with all male uninfected controls and the female patients with sepsis with all female uninfected controls.

data_processing.5 :

Assembly: hg38

data_processing.6 :

Supplementary files format and content: tab-delimited text file contains results of differential expression analysis in edgeR

data_processing.7 :

Supplementary files format and content: columns indicate gene ID, logFc, p-value and FDR

Table 3 (下方表格) 为表格 metadata of GSE237861 概览。

(对应文件为 `Figure+Table/metadata-of-GSE237861.csv`)

> 注：表格共有 82 行 2 列，以下预览的表格可能省略部分数据；表格含有 82 个唯一 'title'。

Table 3: Metadata of GSE237861

title	tissu…
sepsi…	prefr…
sepsi…	hippo…
sepsi…	heart
sepsi…	lung
sepsi…	kidney
sepsi…	colon
sepsi…	brain…
sepsi…	hippo…
sepsi…	heart
sepsi…	lung
sepsi…	kidney
sepsi…	brain…
sepsi…	hippo…
sepsi…	heart
sepsi…	lung
…	…

Figure 8 (下方图) 为图 DEGs number in sepsis of mutiple tissue of GEO dataset 概览。

(对应文件为 `Figure+Table/DEGs-number-in-sepsis-of-mutiple-tissue-of-GEO-dataset.pdf`)

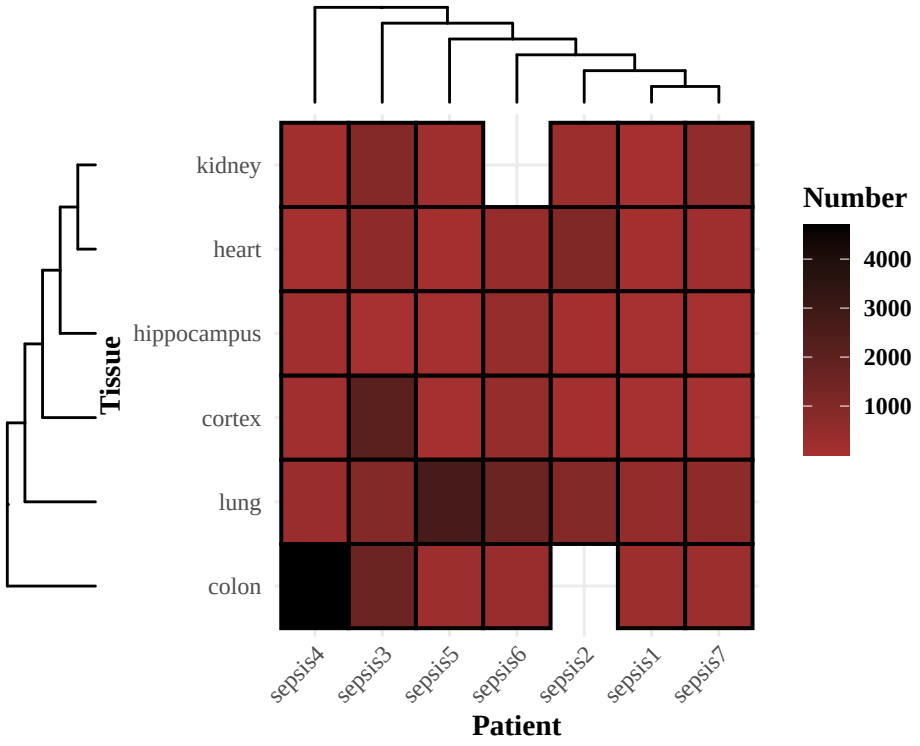


Figure 8: DEGs number in sepsis of mutiple tissue of GEO dataset

在六种不同的 sepsis 组织中，共有 51 个共同的交集基因（Fig. 9）。

Figure 9 (下方图) 为图 intersection of DEGs of mutiple tissue of sepsis 概览。

(对应文件为 `Figure+Table/intersection-of-DEGs-of-mutiple-tissue-of-sepsis.pdf`)

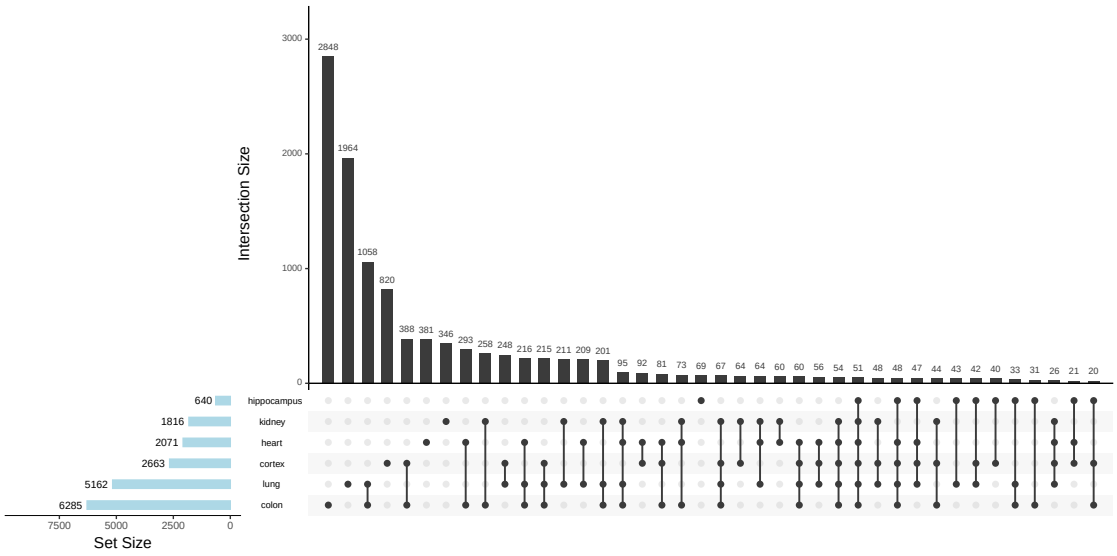


Figure 9: Intersection of DEGs of mutiple tissue of sepsis

6.3 整合：测序数据和 GEO 数据

6.3.1 关联基因

以 GSE237861 验证 TNA 作用的 Hippo 通路基因，属于 sepsis 哪些组织的差异表达基因，以确认 TNA 是否对其具有疗效。

- BIRC3、ID1 在 4 种组织中差异表达
- DLG4 在 3 种组织中差异表达
- ...

Figure 10 (下方图) 为图 Target genes of TNA in mutiple tissue of sepsis of Hippo pathway 概览。

(对应文件为 `Figure+Table/Target-genes-of-TNA-in-mutiple-tissue-of-sepsis-of-Hippo-pathway.pdf`)

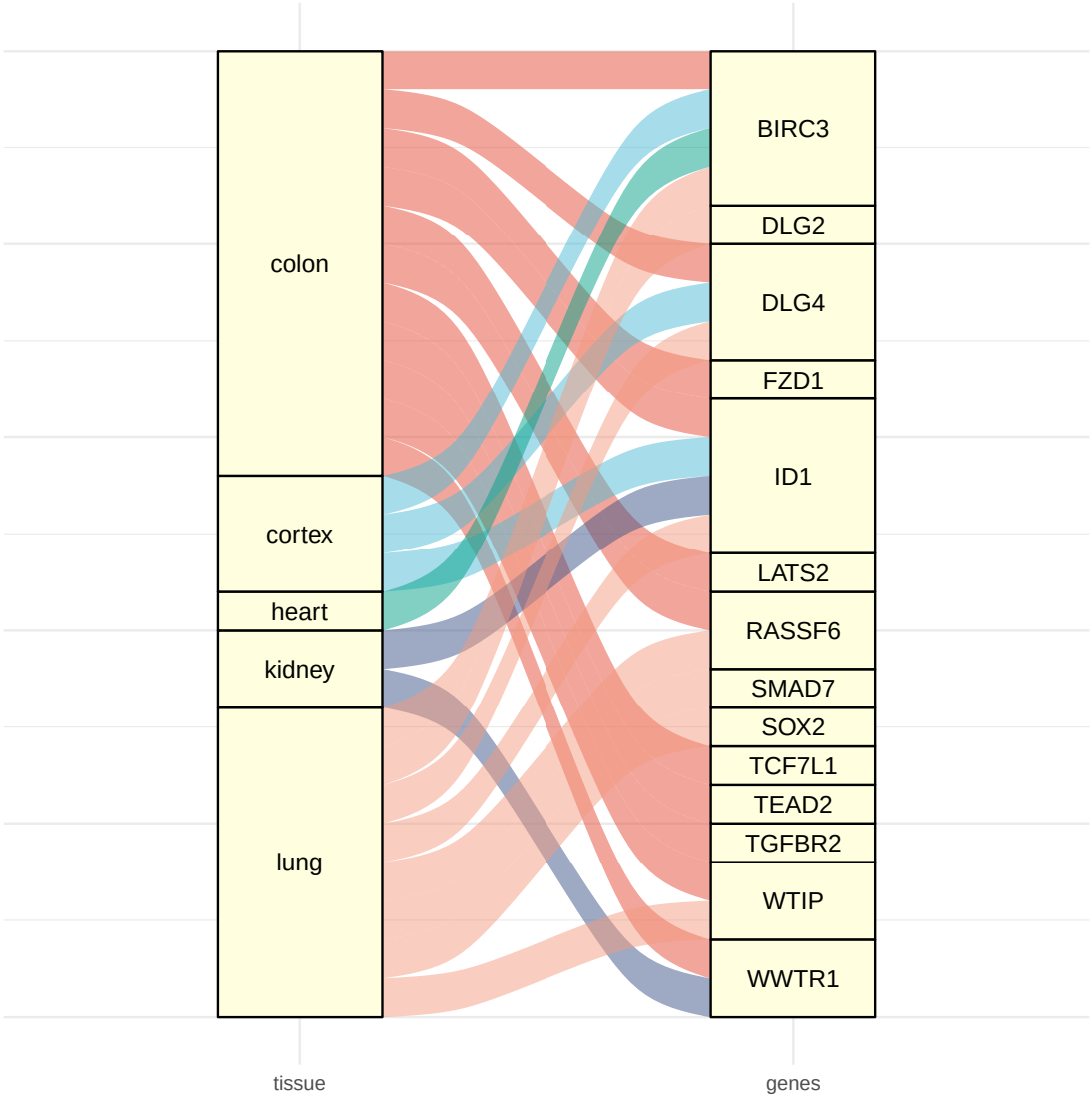


Figure 10: Target genes of TNA in mutiple tissue of sepsis of Hippo pathway

6.3.2 分子对接

丹参酮 I（Tanshinone IIA, CID:164676）

以 AutoDock Vina 对 Fig. 10 所示基因的蛋白以 Tanshinone IIA 分子对接。

结果显示，SMAD7, SOX2, TGFBR2, DLG4, DLG2 具有良好亲和度。

结合 Fig. 10 所示的多组织差异表达，DLG4 同时在 3 种组织 sepsis 差异表达，且为 TNA 作用靶点，表现良好对接亲和度，可能是 TNA 治疗的关键靶点之一。

Figure 11 (下方图) 为图 docking affinity 概览。

(对应文件为 `Figure+Table/docking-affinity.pdf`)

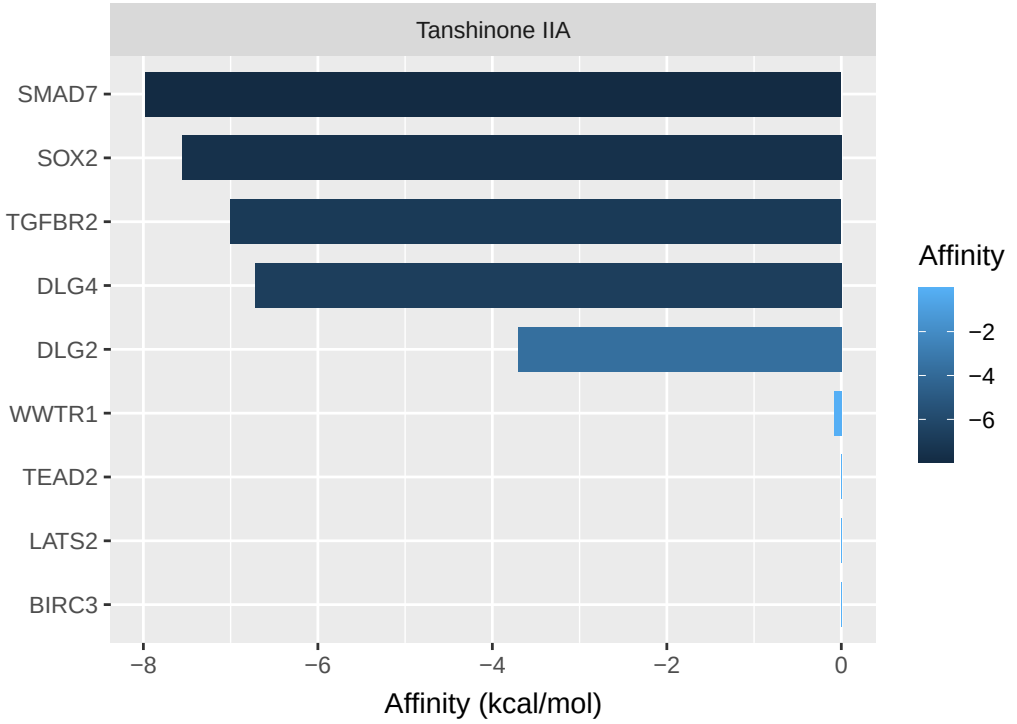


Figure 11: Docking affinity

Figure 12 (下方图) 为图 Tanshinone IIA binding with protein DLG4 概览。

(对应文件为 `Figure+Table/164676_into_1kef.png`)

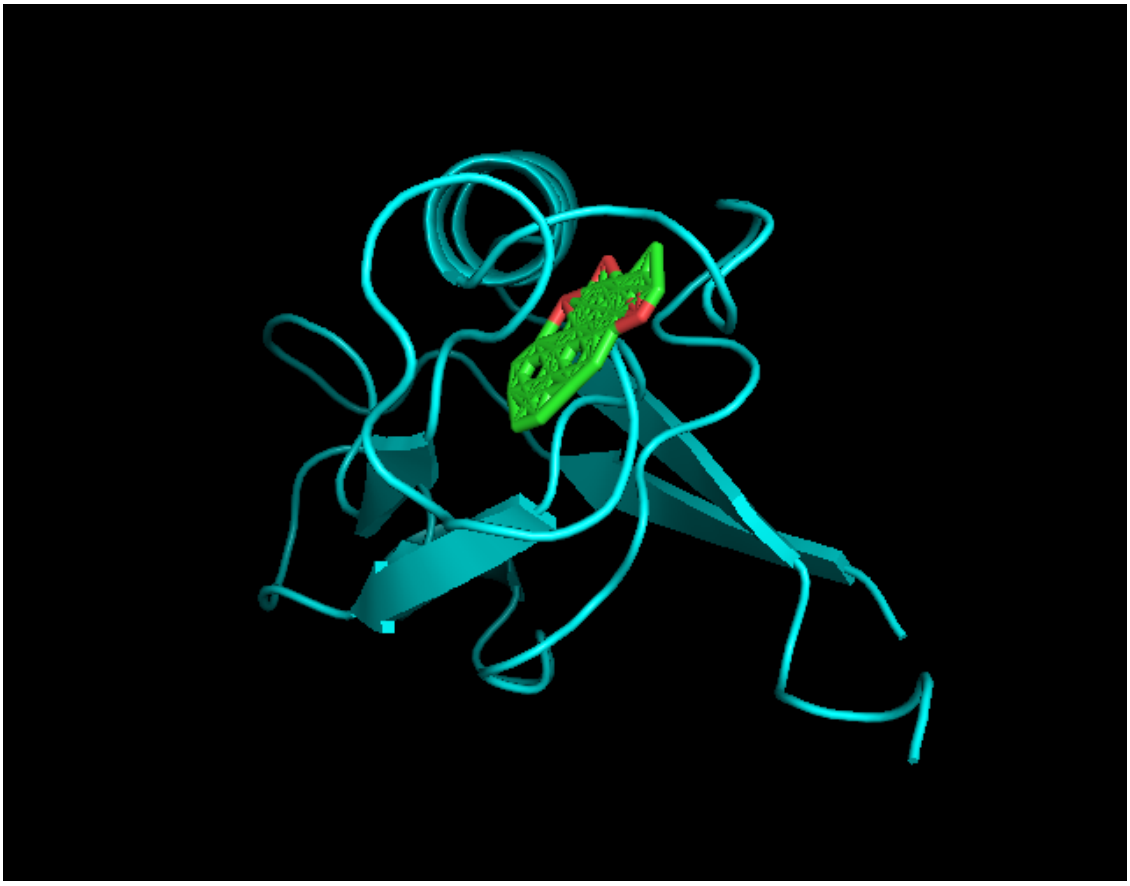


Figure 12: Tanshinone IIA binding with protein DLG4

7 附：补充分析——肠道微生物关联

补充分析：进一步筛选与肠道微生物和免疫微环境相关的差异表达基因

7.1 Sepsis 的代谢物研究

获取文献相关资料：

- Integrative analysis of metabolomics and proteomics reveals amino acid metabolism disorder in sepsis[1]
  - PMID: 35287674
  - Blood samples were collected from patients diagnosed with sepsis at admission to the ICU. Blood samples of patients and NC subjects were drawn using serum separation tubes and allowed to clot at room temperature for 60 min. The samples were centrifuged for 10 min within 30 min (1600×g, 4 °C) to remove insoluble solids. Each aliquot of serum was collected and immediately stored at – 80 °C until ultrahigh-performance liquid chromatography with quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF/MS) analysis.

7.1.1 Sepsis 差异代谢物

获取上述文献差异代谢物数据[1]：

Table 4 (下方表格) 为表格 LITERATURE Positive ionmode Differentially expressed metabolites of the comparison between sepsis patients and NC subjects 概览。

(对应文件为 Figure+Table/LITERATURE-Positive-ionmode-Differentially-expressed-metabolites-of-the-comparis

> 注：表格共有 106 行 16 列，以下预览的表格可能省略部分数据；表格含有 106 个唯一 'Metabolic'。

Table 4: LITERATURE Positive ionmode Differentially expressed metabolites of the comparison between sepsis patients and NC subjects

Metab...	mz	rt	Ref_Name	Precu...	Score...	...6	Score...	...7	HMDB	KEGG	Super...	Class	Subclass...	
M235T129	235.1...	138.765	His-Pro	[M+H-...	0.9955		0.9994		NA	NA	Organ...	Carbo...	Amino...	...
M246T534	246.1...	538.772	Tramadol	[M+H-...	0.9466		0.9997		HMDB0...	C07153	Benze...	Pheno...	Anisoles	...
M158T215	158.1...	214.803	2-(4-...	[M+H]+	0.9424		0.9952		NA	NA	Organ...	Carbo...	Amino...	...
M286T128	286.1...	167.9745	N-ace...	[M+H]+	0.9925		0.9928		NA	NA	NA	NA	NA	...
M261T430	261.1...	430.267	Diave...	[M+H]+	0.9896		1		NA	NA	Benze...	Benze...	Metho...	...
M151T145	151.0616	145.8605	3-met...	[M+H-...	0.9995		0.9998		HMDB0...	C05587	Benze...	Phenols	Metho...	...
M168T42	168.1...	41.67385	Pheny...	[M+H]+	0.9947		0.9988		HMDB0...	C07441	Benze...	Phenols	1-hyd...	...
M430T248	430.3...	288.673	Milte...	[M+Na]+	0.8708		0.9996		NA	NA	Organ...	Organ...	Quate...	...
M207T62	207.1...	65.8776	Pyrantel	[M+H]+	0.9992		0.9997		NA	C07409	Organ...	Diazines	Pyrim...	...
M334T433	334.1...	419.257	Tebuf...	[M+H]+	0.9111		0.9973		NA	C11126	Benze...	Benze...	Pheny...	...
M257T165	257.1...	165.785	Ptero...	[M+H]+	0.9503		0.9999		HMDB0...	C10287	NA	NA	NA	...
M175T275	175.0...	219.69	3,6-d...	[M+H]+	0.8791		0.9514		NA	NA	Organ...	Benzo...	1-ben...	...
M229T329	229.1...	316.932	Tri(p...	[M+Na]+	0.9965		0.9997		NA	NA	Organ...	Organ...	Alcoh...	...
M225T367	225.1...	367.1115	Pogos...	[M+H]+	0.9287		0.9357		HMDB0...	NA	Organ...	Organ...	Carbo...	...
M290T...	290.1...	411.1295	Adipo...	[M+H]+	0.9802		0.9809		NA	NA	Lipid...	Fatty...	Fatty...	...
...	...	...	...	...	...		...		...	...	...	...	...	...

Table 5 (下方表格) 为表格 LITERATURE Negtive ionmode Differentially expressed metabolites of the comparison between sepsis patients and NC subjects 概览。

(对应文件为 Figure+Table/LITERATURE-Negtive-ionmode-Differentially-expressed-metabolites-of-the-comparisc

> 注：表格共有 76 行 16 列，以下预览的表格可能省略部分数据；表格含有 76 个唯一 'Metabolic'。

Table 5: LITERATURE Negtive ionmode Differentially expressed metabolites of the comparison between sepsis patients and NC subjects

Metab...	mz	rt	Ref_Name	Precu...	Score.....6	Score.....7	HMDB	KEGG	Super...	Class	Subclass...	
M287T328	287.0...	324.445	Oroti...	[M-H]-	0.9944	0.9953	HMDB00	NA	NA	NA	NA	...
M246T...	246.0747	40.3787	Asp-Asn	[M-H]-	0.9185	0.9955	NA	NA	Organ...	Carbo...	Amino...	...
M179T...	179.0...	398.696	inositol	[M-H]-	0.9973	0.9977	HMDB00	C00137	Organ...	Organ...	Alcoh...	...
M151T41	151.0...	40.7983	Creso...	[M-H]-	0.9999	0.9999	HMDB00	C14088	Benze...	Benze...	Benzo...	...
M147T92	147.0...	92.21745	3-iso...	[M-H]-	0.8552	0.9127	NA	C07728	Organ...	Benzo...	2-ben...	...
M151T211	151.0...	210.531	4-hyd...	[M-H]-	0.927	0.999	HMDB00	C00642	Benze...	Phenols	1-hyd...	...
M300T...	300.0...	166.016	N-Ace...	[M-H]-	0.9797	0.9817	HMDB00	C04256	NA	NA	NA	...
M165T92	165.0...	91.6485	Pheny...	[M-H]-	0.9949	0.9951	HMDB00	C01479	NA	NA	NA	...
M245T32	245.0...	33.9236	Asn-Asn	[M-H]-	0.9475	0.9987	NA	NA	Organ...	Carbo...	Amino...	...
M415T37	415.2158	37.4836	Ramipril	[M-H]-	0.9887	0.9942	HMDB00	NA	Organ...	Carbo...	Amino...	...
M125T302	125.0...	398.69	1,2,4...	[M-H]-	0.9697	0.9755	HMDB00	C02814	Benze...	Phenols	Benze...	...
M286T28	286.1...	259.84	Norox...	[M-H]-	0.908	0.9959	HMDB00	NA	NA	NA	NA	...
M144T294	144.0...	297.591	4-ace...	[M-H]-	0.9479	0.9977	HMDB00	C02946	Organ...	Carbo...	Amino...	...
M190T219	190.0...	212.844	N-ace...	[M-H]-	0.9816	0.9853	HMDB00	C02712	Organ...	Carbo...	Amino...	...
M125T...	124.9...	165.0135	Ethyl...	[M-H]-	0.9683	0.9909	HMDB00	NA	Organ...	Organ...	Sulfu...	...
...	...	...	...	...	...	...	...	...	...	...	...	...

7.1.2 从代谢物到肠道微生物

利用 gutMDisorder[2] 数据库，检索与差异代谢物相关的肠道微生物。

Table 6 (下方表格) 为表格 databse of gutMDisorder 概览。

(对应文件为 `Figure+Table/databse-of-gutMDisorder.xlsx`)

注：表格共有 724 行 12 列，以下预览的表格可能省略部分数据；表格含有 289 个唯一 'Gut Microbiota'。

Table 6: Databse of gutMDisorder

Gut M……1	Gut M……2	Gut M……3	Class…	Subst……5	Subst……6	Subst……7	Subst……8	…
Chris…	NA	gm0883	strain	D-Glu…	5793	HMDB0…	CHEBI…	…
Chris…	NA	gm0883	strain	Salicin	439503	HMDB0…	CHEBI…	…
Chris…	NA	gm0883	strain	D-Xylose	135191	HMDB0…	CHEBI…	…
Chris…	NA	gm0883	strain	L-Ara…	439195	HMDB0…	CHEBI…	…
Chris…	NA	gm0883	strain	L-Rha…	25310	HMDB0…	CHEBI…	…
Chris…	NA	gm0883	strain	D-Man…	18950	HMDB0…	CHEBI…	…
Chris…	NA	gm0883	strain	D-Glu…	5793	HMDB0…	CHEBI…	…
Chris…	NA	gm0883	strain	Salicin	439503	HMDB0…	CHEBI…	…
Chris…	NA	gm0883	strain	D-Xylose	135191	HMDB0…	CHEBI…	…
Chris…	NA	gm0883	strain	L-Ara…	439195	HMDB0…	CHEBI…	…
Chris…	NA	gm0883	strain	L-Rha…	25310	HMDB0…	CHEBI…	…
Chris…	NA	gm0883	strain	D-Man…	18950	HMDB0…	CHEBI…	…
Enter…	1343173	gm0884	species	Orientin	5281675	HMDB0…	CHEBI…	…
Clost…	29347	gm0885	strain	Bile …	439520		CHEBI…	…
Clost…	29347	gm0885	strain	Choli…	221493	HMDB0…	CHEBI…	…
…	…	…	…	…	…	…	…	…

从 gutMDisorder 中匹配 Tab. 4 与 Tab. 5 对应的化合物:

Figure 13 (下方图) 为图 hited metabolites and microbiota in gutMDisorder 概览。

(对应文件为 `Figure+Table/hited-metabolites-and-microbiota-in-gutMDisorder.pdf`)

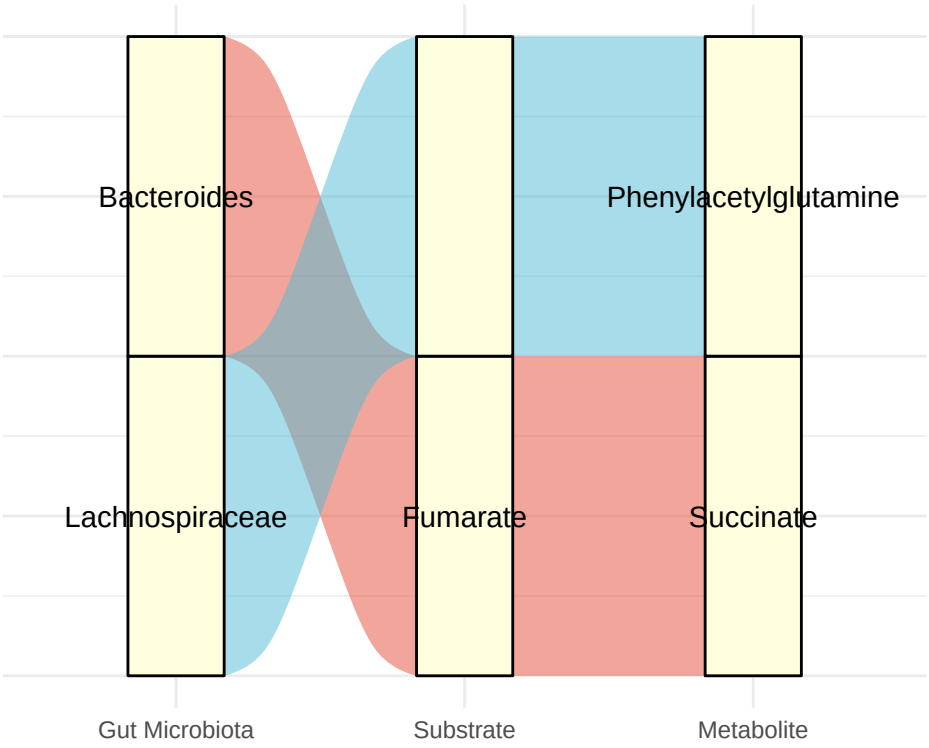


Figure 13: Hited metabolites and microbiota in gutMDisorder

Table 7 (下方表格) 为表格 table of the hitted compounds 概览。

(对应文件为 `Figure+Table/table-of-the-hitted-compounds.csv`)

> 注：表格共有 2 行 7 列，以下预览的表格可能省略部分数据；表格含有 2 个唯一 'Query'。

Table 7: Table of the hitted compounds

Query	Match	HMDB	PubChem	KEGG	SMILES	Comment
Pheny…	Alpha…	HMDB0…	92258	C04148	C1=CC…	1
Fumarate	Fumar…	HMDB0…	444972	C00122	C(=C/…	1

7.1.3 （选定的）代谢物的富集分析

将 Tab. 7 中的两种化合物富集分析。

FELLA （pagerank 算法）用于差异代谢物的富集分析[3]。

Figure 14 (下方图) 为图 FELLA enrichment 概览。

(对应文件为 `Figure+Table/FELLA-enrichment.pdf`)

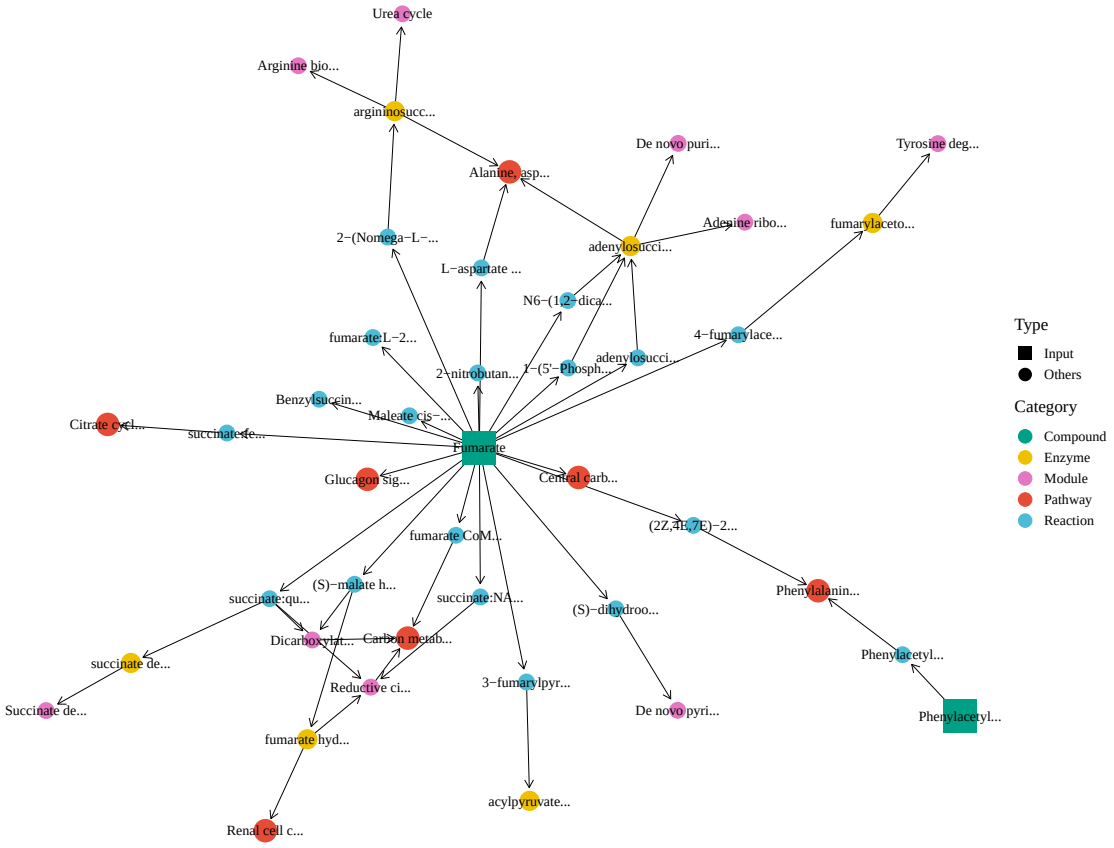


Figure 14: FELLA enrichment

Table 8 (下方表格) 为表格 table of FELLA enrichment result 概览。

(对应文件为 `Figure+Table/table-of-FELLA-enrichment-result.xlsx`)

> 注：表格共有 43 行 7 列，以下预览的表格可能省略部分数据；表格含有 43 个唯一 'name'。

Table 8: Table of FELLA enrichment result

name	com	NAME	label	input	abbre...	type
hsa00020	1	Citra...	Citra...	Others	Citra...	Pathway
hsa00250	1	Alani...	Alani...	Others	Alani...	Pathway
hsa00360	1	Pheny...	Pheny...	Others	Pheny...	Pathway
hsa01200	1	Carbo...	Carbo...	Others	Carbo...	Pathway
hsa04922	1	Gluca...	Gluca...	Others	Gluca...	Pathway
hsa05211	1	Renal...	Renal...	Others	Renal...	Pathway
hsa05230	1	Centr...	Centr...	Others	Centr...	Pathway
M00029	2	Urea ...	Urea ...	Others	Urea ...	Module
M00044	2	Tyros...	Tyros...	Others	Tyros...	Module
M00048	2	De no...	De no...	Others	De no...	Module

name	com	NAME	label	input	abbre…	type
M00049	2	Adeni…	Adeni…	Others	Adeni…	Module
M00051	2	De no…	De no…	Others	De no…	Module
M00148	2	Succi…	Succi…	Others	Succi…	Module
M00173	2	Reduc…	Reduc…	Others	Reduc…	Module
M00374	2	Dicar…	Dicar…	Others	Dicar…	Module
…	…	…	…	…	…	…

首要关注 “Phenylalanine metabolism” 代谢通路。

7.2 Sepsis 的蛋白质研究

7.2.1 Sepsis 差异蛋白

- Serum proteomics reveals disorder of lipoprotein metabolism in sepsis[4]
  - Blood samples of patients and NC subjects were collected and allowed to clot at room temperature for 60 min. Serum was separated by centrifugation at 1,600g for 10 min within 30 min to remove insoluble solids and stored at −80°C until proteomic analysis and ELISA (Tammen, 2008). Removal of high-abundance proteins in serum, such as albumin and IgG, was performed using ProteoPrep Blue Albumin & IgG Depletion Kit (PROTBA; Sigma-Aldrich) according to the manufacturer’s instructions. Removal of impurities from the protein extraction was performed using a 2-D clean kit (GE Healthcare) before the determination of the sample concentration.

从上述文献获取差异蛋白数据[4]：

Table 9 (下方表格) 为表格 LITERATURE Comparison of protein abundance in patients with sepsis and normal control subjects 概览。

(对应文件为 `Figure+Table/LITERATURE-Comparison-of-protein-abundance-in-patients-with-sepsis-and-normal-co`

> 注：表格共有 243 行 5 列，以下预览的表格可能省略部分数据；表格含有 243 个唯一 ‘Protein.IDs’。

Table 9: LITERATURE Comparison of protein abundance in patients with sepsis and normal control subjects

Prote…	Gene….	log2….	P.value	adjus…
ENSBT…	ENSBT…	-0.72…	0.007…	0.013…
P00761	P00761	0.289…	0.000…	0.000…
P02768	ALB	0.144…	0.001…	0.002…
Q28107	Q28107	-1.06…	0.000…	0.000…
Q61782	Q61782	-1.42…	6.878…	3.783…
Q86YZ3	HRNR	-1.04…	0.000…	0.001…
P07996	THBS1	-1.03…	3.808…	1.523…
O14791	APOL1	-0.42…	6.701…	2.175…

Prote...	Gene....	log2....	P.value	adjus...
O75460	ERN1	0.777...	1.357...	4.714...
O75636	FCN3	-0.27...	0.007...	0.014...
O75882	ATRN	-0.36...	5.825...	2.196...
O95445	APOM	-1.43...	3.389...	5.161...
O95497	VNN1	-1.19...	0.000...	0.000...
P00326	ADH1C	2.087...	0.003...	0.007...
P00441	SOD1	2.111...	6.761...	2.176...
...	...	...	...	...

7.3 TNA 治疗靶点和 Sepsis 差异蛋白质的关联（从基因到蛋白质）

7.3.1 交集蛋白（基因）

Figure 15 (下方图) 为图 intersection of TNA targets and Sepsis significant protein 概览。

(对应文件为 `Figure+Table/intersection-of-TNA-targets-and-Sepsis-significant-protein.pdf`)

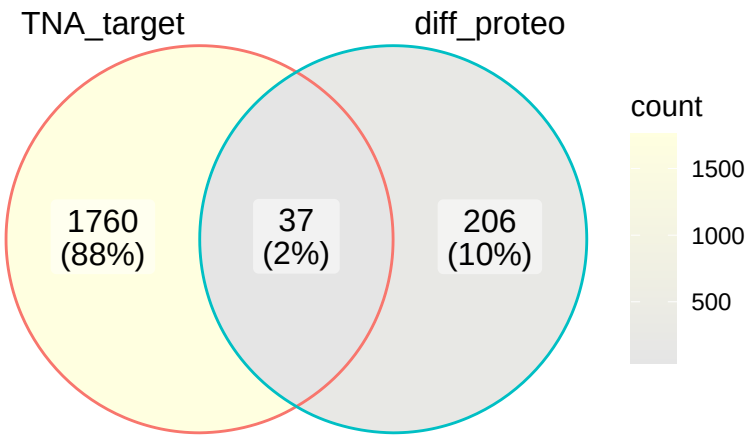


Figure 15: Intersection of TNA targets and Sepsis significant protein

Fig. 15，共有 37 个交集基因（蛋白质）。

intersection :

APOA2, RBP4, AGT, SERPINA1, TTR, PLTP, ENO1, ITIH2, AHSG, F2, OAF, C4BPA, TKT, CST3, F7, APOC3, MDN1, IGFBP4, ALDOB, ASGR2, HABP2, F12, VWF, APOC1, ALB, APOA4, CFI, CA2, THBS1, HRNR, PDE4DIP, LCAT, IL1RAP, SEMA4B, VASN, C8G, CTBS

7.3.2 富集分析

Figure 16 (下方图) 为图 KEGG enrichment of intersection of TNA targets and Sepsis significant protein 概览。

(对应文件为 `Figure+Table/KEGG-enrichment-of-intersection-of-TNA-targets-and-Sepsis-significant-protein.pd`

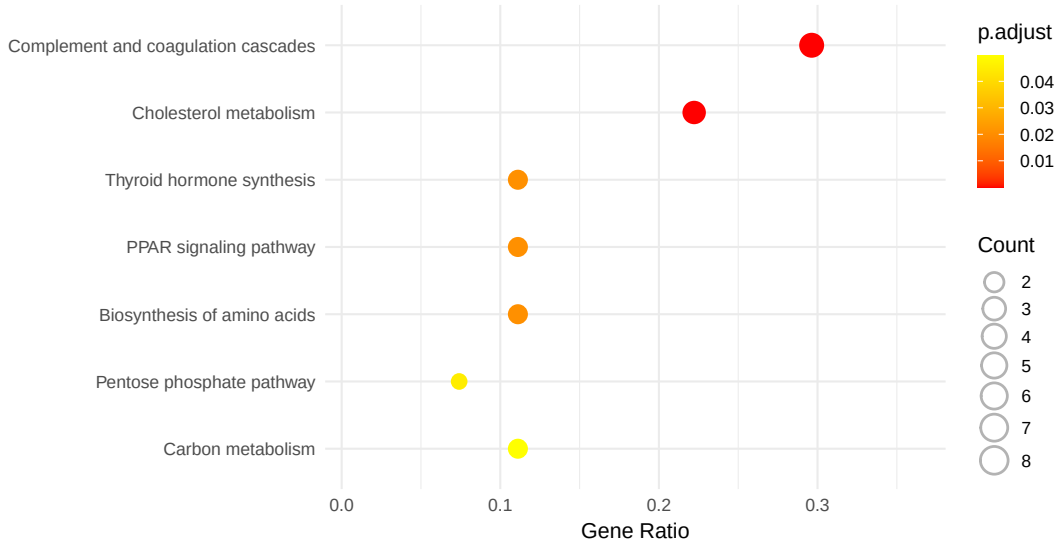


Figure 16: KEGG enrichment of intersection of TNA targets and Sepsis significant protein

Fig. 16，关注到 “Biosynthesis of amino acids” 通路，这一结果与文献[1] 一致。此外，根据 Fig. 17，Phenylalanine 的存在受该通路调控（Phenylalanine metabolism 在 Fig. 14 中富集）。

因此，这里建立了从差异蛋白到 TNA 治疗的差异表达基因的联系，又联系到了差异代谢物的分析（7.1.1），而相应代谢物又联系到了肠道微生物（Fig. 13）。

Figure 17 (下方图) 为图 KEGG pathway of Biosynthesis of amino acids 概览。

(对应文件为 `Figure+Table/hsa01230.png`)

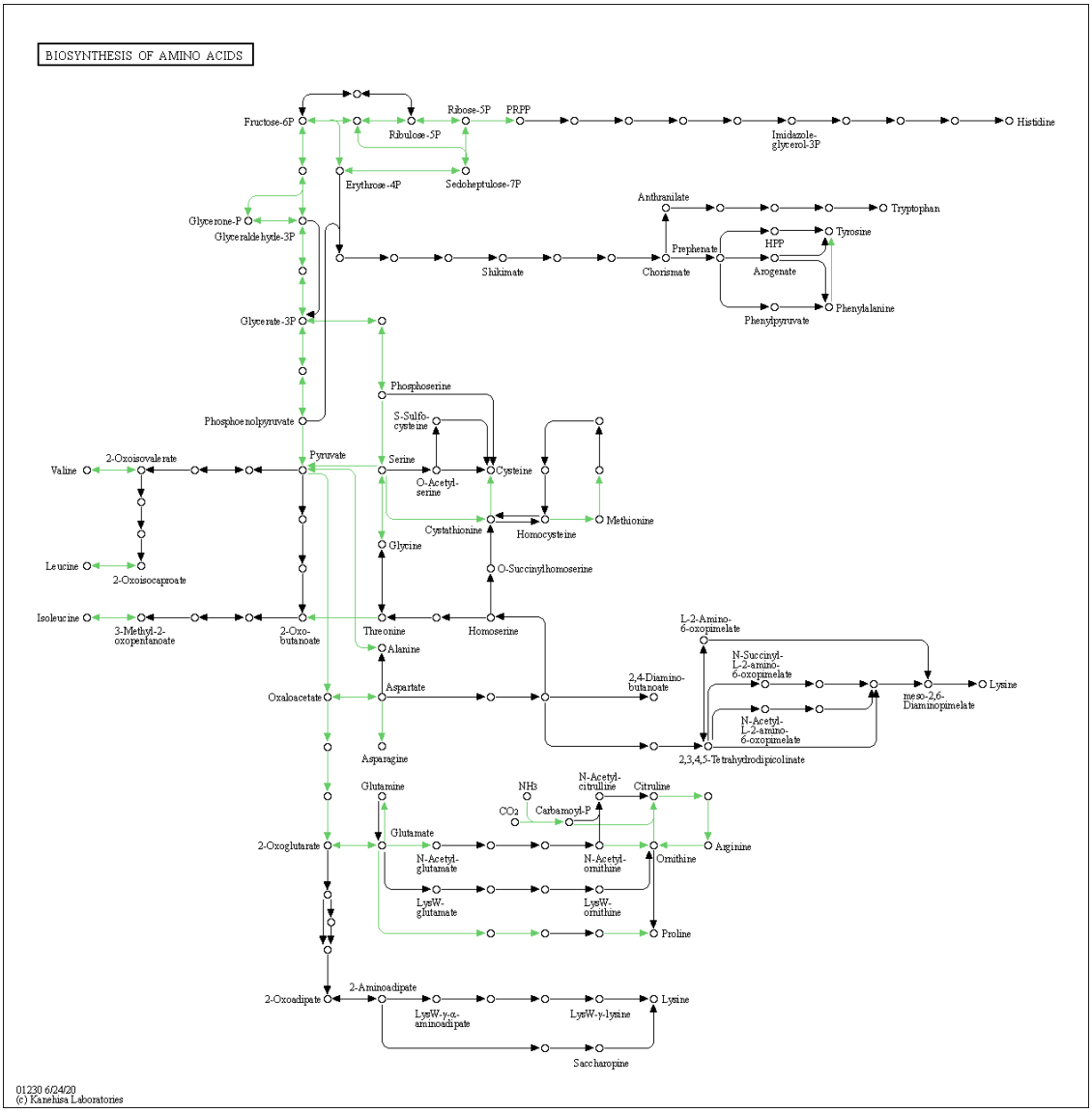


Figure 17: KEGG pathway of Biosynthesis of amino acids

hited_genes :

ENO1, TKT, ALDOB

7.4 蛋白质组学和代谢组学的关联性分析

见文献[4]。这部分，由于原作者[4] 未上传代谢物的检测含量数据，因此无法重现代谢物和蛋白质的关联分析。不过，原作者已得出结论，即：

- The characteristic proteins and metabolites identified formed a complex network to depict the crucial immunometabolism linked to sepsis. Amino acid-related pathways, including phenylalanine metabolism, tyrosine metabolism and tryptophan biosynthesis, were illustrated to be essential

mechanisms of sepsis.

因此，蛋白质组学和代谢组学的联合表明，phenylalanine metabolism 是 Sepsis 关键通路之一。

以下为该文献[4] 提供的 Figure：

Figure 18（下方图）为图 LITERATURE Integrative network analysis of proteomics and untargeted metabolomics data 概览。

(对应文件为 `Figure+Table/literature_omics_fig5.jpeg`)

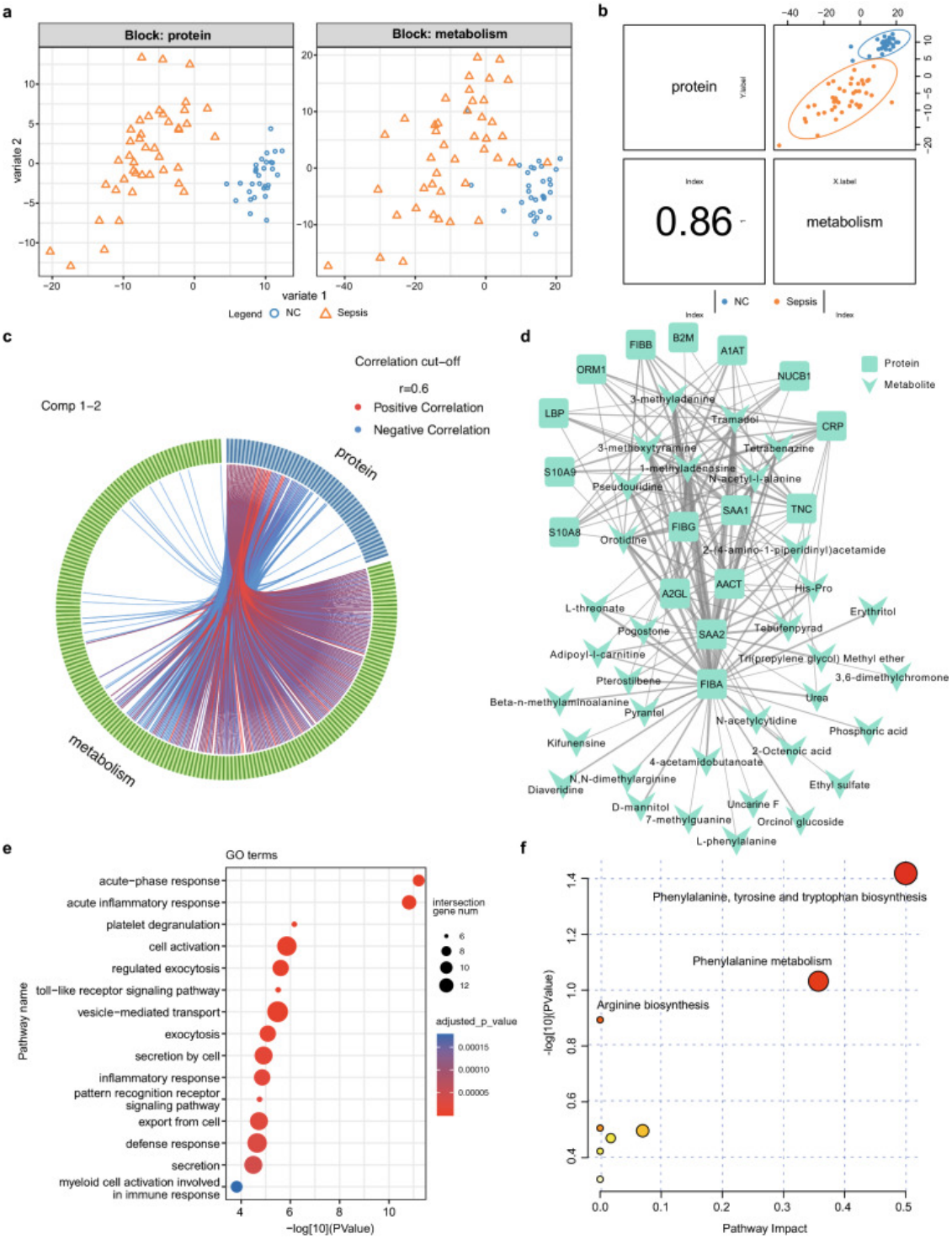


Figure 18: LITERATURE Integrative network analysis of proteomics and untargeted metabolomics data

Figure 19 (下方图) 为图 LITERATURE Schematic diagram of the crucial pathways 概览。

(对应文件为 `Figure+Table/literature_omics_fig6.jpeg`)

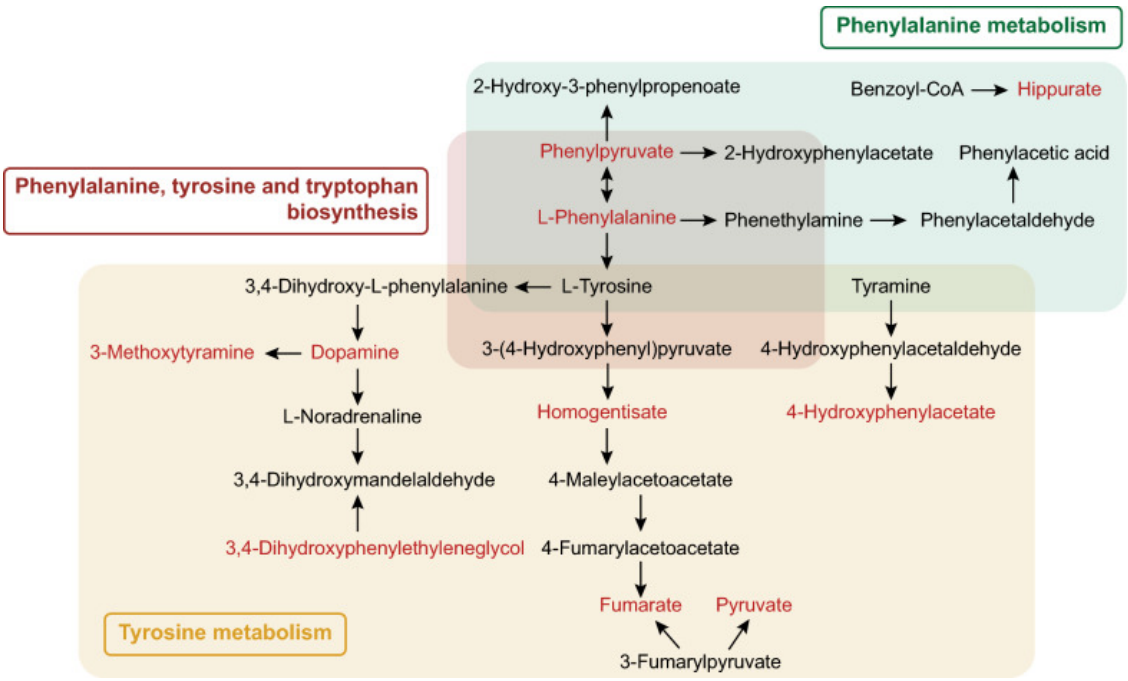


Figure 19: LITERATURE Schematic diagram of the crucial pathways

7.5 总结：TNA 治疗 Sepsis 可能涉及的肠道微生物-代谢物-蛋白质关联轴

- 以 Sepsis 差异代谢物（Tab. 4，Tab. 5）和 gutMDisorder 数据库[2] 发现了 Sepsis 差异代谢物和肠道菌的联系（Fig. 13）。
- 上述代谢物（与肠道菌关联的）富集到了通路 Phenylalanine metabolism（Fig. 14）
- TNA 治疗靶点和 Sepsis 差异蛋白的交集共有 37 个基因（Fig. 15）。
- 上述交集基因富集到通路 Biosynthesis of amino acids（Fig. 16），涉及 Phenylalanine 及其相关化合物的调控。
- 文献中[1]，蛋白组学和代谢组学分析结果聚焦于 “Amino acid metabolism”，包含化合物 Phenylalanine 等（Fig. 18，Fig. 19）。

因此，TNA 治疗 Sepsis 可能涉及的肠道微生物-代谢物-蛋白质（基因）关联轴：

- 肠道微生物：Lachnospiraceae
- 代谢物：Phenylacetylglutamine
- 基因（蛋白）：ENO1, TKT, ALDOB
- 通路：Biosynthesis of amino acids

8 附：补充分析——铁死亡

补充分析：筛选差异表达基因是否涉及与铁死亡相关的信号通路。

Ferroptosis[5,6]

- In general, ferroptosis is a type of oxidative cell death driven by lipid peroxidation. Various oxidation and antioxidant pathways couple autophagy and membrane repair mechanisms to shape the process of

lipid peroxidation and plasma membrane damage during ferroptosis

8.1 铁死亡关联方法

铁死亡涉及的信号通路较为复杂，这里为了检测 TNA 治疗的 DEGs 是否涉及铁死亡，调研了涉及 Ferroptosis 检测的方法学文献。

Table 10 (下方表格) 为表格 Methods for Ferroptosis 概览。

(对应文件为 `Figure+Table/Methods-for-Ferroptosis.csv`)

> 注: 表格共有 12 行 5 列，以下预览的表格可能省略部分数据；表格含有 12 个唯一 'Title'。

Table 10: Methods for Ferroptosis

.id	Title	FullJ…	Name	Id
BMC g…	Explo…	BMC g…	Ran M…	37430218
BMC g…	Const…	BMC g…	Chen …	37259023
BMC g…	Compe…	BMC g…	Chen …	36755220
Nucle…	FerrD…	Nucle…	Zhou …	36305834
BMC g…	Const…	BMC g…	Shi W…	36414988
BMC g…	Ident…	BMC g…	Chen …	35354376
BMC g…	Ferro…	BMC g…	Xu Y|…	35042463
Brief…	Ferro…	Brief…	Tang …	34553745
BMC g…	Ident…	BMC g…	Fan J…	35033021
BMC g…	Ident…	BMC g…	Lin R…	34315405
Nucle…	Lipid…	Nucle…	Lin W…	34048582
BMC g…	Compa…	BMC g…	Zhang…	33952209

其中，FerrDb V2[7] 为 Nucleic Acids Res 2023 年的方法。

8.2 以 FerrDb V2 数据库富集分析

- FerrDb V2: update of the manually curated database of ferroptosis regulators and ferroptosis-disease associations[7]
  - http://www.zhounan.org/ferrdb/current/

Table 11 (下方表格) 为表格 database FerrDb V2 概览。

(对应文件为 `Figure+Table/database-FerrDb-V2.xlsx`)

> 注: 表格共有 844 行 19 列，以下预览的表格可能省略部分数据；表格含有 4 个唯一 'term'。

Table 11: Database FerrDb V2

term	symbol	id	id	rcd	hgncid	ensgs...	evidence	testin	pathway	confi...	exper...	caution	unipr...	...
Ferro...	PTGS2	marker	1	Ferro...	HGNC:...	ENSG0...	Simpl...	Human	*NA*	Valid...	0	*NA*	P35354	...
Ferro...	CHAC1	marker	2	Ferro...	HGNC:...	ENSG0...	Up-re...	Human	*NA*	Valid...	0	*NA*	Q9BUX1	...
Ferro...	SLC40A1	marker	3	Ferro...	HGNC:...	ENSG0...	Erast...	Mice	*NA*	Valid...	0	Used...	Q9NP59	...
Ferro...	TF	marker	4	Ferro...	HGNC:...	ENSG0...	Erast...	Mice	*NA*	Valid...	0	Used...	P02787	...
Ferro...	TFRC	marker	5	Ferro...	HGNC:...	ENSG0...	Erast...	Mice	*NA*	Valid...	0	Used...	P02786	...
Ferro...	FTH1	marker	6	Ferro...	HGNC:...	ENSG0...	Erast...	Mice	*NA*	Valid...	0	Used...	P02794	...
Ferro...	GPX4	marker	7	Ferro...	HGNC:...	ENSG0...	Erast...	Mice	*NA*	Valid...	0	Used...	P36969	...
Ferro...	HSPB1	marker	8	Ferro...	HGNC:...	ENSG0...	Erast...	Mice	*NA*	Valid...	0	Used...	P04792	...
Ferro...	NFE2L2	marker	9	Ferro...	HGNC:...	ENSG0...	Erast...	Mice	*NA*	Valid...	0	Used...	Q16236	...
Ferro...	GPX4	marker	10	Ferro...	HGNC:...	ENSG0...	Upreg...	Rat	*NA*	Valid...	0	Inhib...	P36969	...
Ferro...	FTH1	marker	12	Ferro...	HGNC:...	ENSG0...	The p...	Human	*NA*	Valid...	0	*NA*	P02794	...
Ferro...	RPL8	driver	1	Ferro...	HGNC:...	ENSG0...	Requi...	Human	RPL8...	Valid...	0	*NA*	P62917	...
Ferro...	IREB2	driver	2	Ferro...	HGNC:...	ENSG0...	Requi...	Human	IREB2...	Valid...	0	*NA*	P48200	...
Ferro...	ATP5MC3	driver	3	Ferro...	HGNC:845	ENSG0...	Requi...	Human	ATP5MV...	Valid...	0	*NA*	P48201	...
Ferro...	CS	driver	4	Ferro...	HGNC:...	ENSG0...	Requi...	Human	CS :+...	Valid...	0	*NA*	O75390	...
...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

Tab. 11 为以下分析所包含的所有 FerrDb V2 数据内容，以及相关文献来源证明等信息。

8.2.1 所有的 DEGs 与 FerrDb V2 的交集

Figure 20 (下方图) 为图 Proportion of intersected genes of DEGs with Ferrdb 概览。

(对应文件为 `Figure+Table/Proportion-of-intersected-genes-of-DEGs-with-Ferrdb.pdf`)

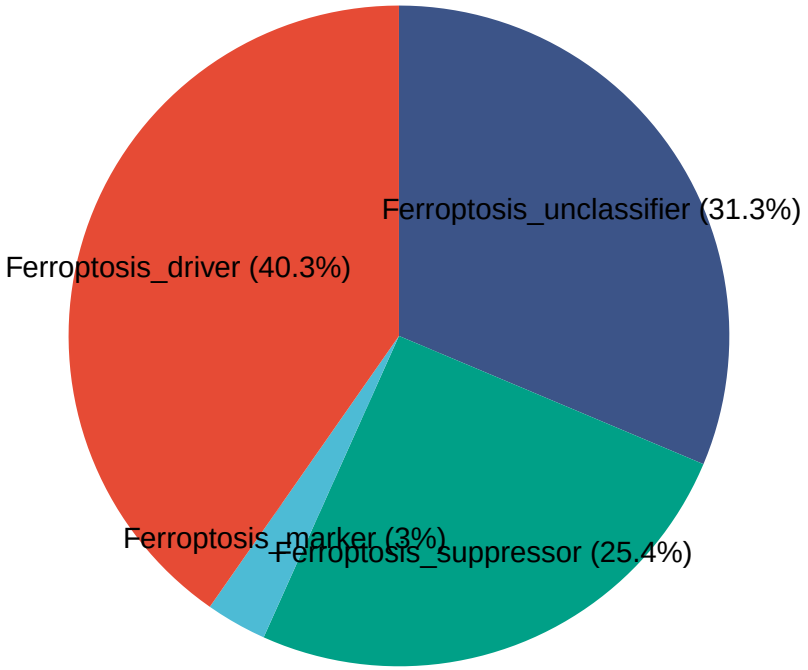


Figure 20: Proportion of intersected genes of DEGs with Ferrdb

Table 12 (下方表格) 为表格 intersected genes of DEGs with Ferrdb 概览。

(对应文件为 `Figure+Table/intersected-genes-of-DEGs-with-Ferrdb.xlsx`)

> 注：表格共有 92 行 19 列，以下预览的表格可能省略部分数据；表格含有 59 个唯一 'symbol'。

Table 12: Intersected genes of DEGs with Ferrdb

term	symbol	.id	id	rcd	hgncid	ensgs...	evidence	testin	pathway	confi...	exper...	caution	unipr...	...
Ferro...	CHAC1	marker	2	Ferro...	HGNC:...	ENSG0...	Up-re...	Human	*NA*	Valid...	0	*NA*	Q9BUX1	...
Ferro...	HSPB1	marker	8	Ferro...	HGNC:...	ENSG0...	Erast...	Mice	*NA*	Valid...	0	Used ...	P04792	...
Ferro...	NOX1	driver	7	Ferro...	HGNC:...	ENSG0...	Suppr...	Human	NOX1 ...	Deduced	0	The p...	Q9Y5S8	...
Ferro...	TFR2	driver	28	Ferro...	HGNC:...	ENSG0...	RNAi ...	Mice	TFR2 ...	Valid...	0	*NA*	Q9UP52	...
Ferro...	SLC38A1	driver	29	Ferro...	HGNC:...	ENSG0...	RNAi ...	Mice	SLC38...	Valid...	0	*NA*	Q9H2H9	...
Ferro...	SLC1A5	driver	30	Ferro...	HGNC:...	ENSG0...	Pharm...	Mice	SLC1A...	Valid...	0	*NA*	Q15758	...
Ferro...	CARS1	driver	33	Ferro...	HGNC:...	ENSG0...	Requi...	Human	CARS1...	Valid...	0	*NA*	P49589	...

term	symbol	.id	id	rcd	hgncid	ensgs...	evidence	testin	pathway	confi...	exper...	caution	unipr...	...
Ferro...	SLC38A1	driver	55	Ferro...	HGNC:...	ENSG0...	The g...	Mice	SLC38...	Screened	0	*NA*	Q9H2H9	...
Ferro...	GABARAP	driver	65	Ferro...	HGNC:...	ENSG0...	Poten...	Mice	GABARAP	Screened	0	*NA*	Q9H0R8	...
Ferro...	WIPI1	driver	67	Ferro...	HGNC:...	ENSG0...	Poten...	Mice	WIPI1	Screened	0	*NA*	Q5MNZ9	...
Ferro...	SLC1A5	driver	103	Ferro...	HGNC:...	ENSG0...	Overe...	Human	SLC1A...	Valid...	0	*NA*	Q15758	...
Ferro...	CHAC1	driver	104	Ferro...	HGNC:...	ENSG0...	CHAC1...	Human	CHAC1	Valid...	0	*NA*	Q9BUX1	...
Ferro...	SIRT1	driver	142	Ferro...	HGNC:...	ENSG0...	Knock...	Mice	SIRT1...	Predi...	0	*NA*	Q96EB6	...
Ferro...	LONP1	driver	150	Ferro...	HGNC:...	ENSG0...	Inhib...	Human	LONP1	Valid...	0	*NA*	P36776	...
Ferro...	PTEN	driver	160	Ferro...	HGNC:...	ENSG0...	Notab...	Human	PTEN ...	Deduced	0	*NA*	*NA*	...
...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

8.2.2 所有的 DEGs 的富集分析 (GSEA)

将 Fig. 5 的 1797 个 DEGs 以 FerrDb V2 的基因集用于 GSEA 富集分析。

注：结果均不显著 (Tab. 13)，仅用于参考 TNA 的治疗与 Ferroptosis 有哪些联系。

Figure 21 (下方图) 为图 All DEGs of GSEA enrichment via FerrDb V2 概览。

(对应文件为 `Figure+Table/All-DEGs-of-GSEA-enrichment-via-FerrDb-V2.pdf`)

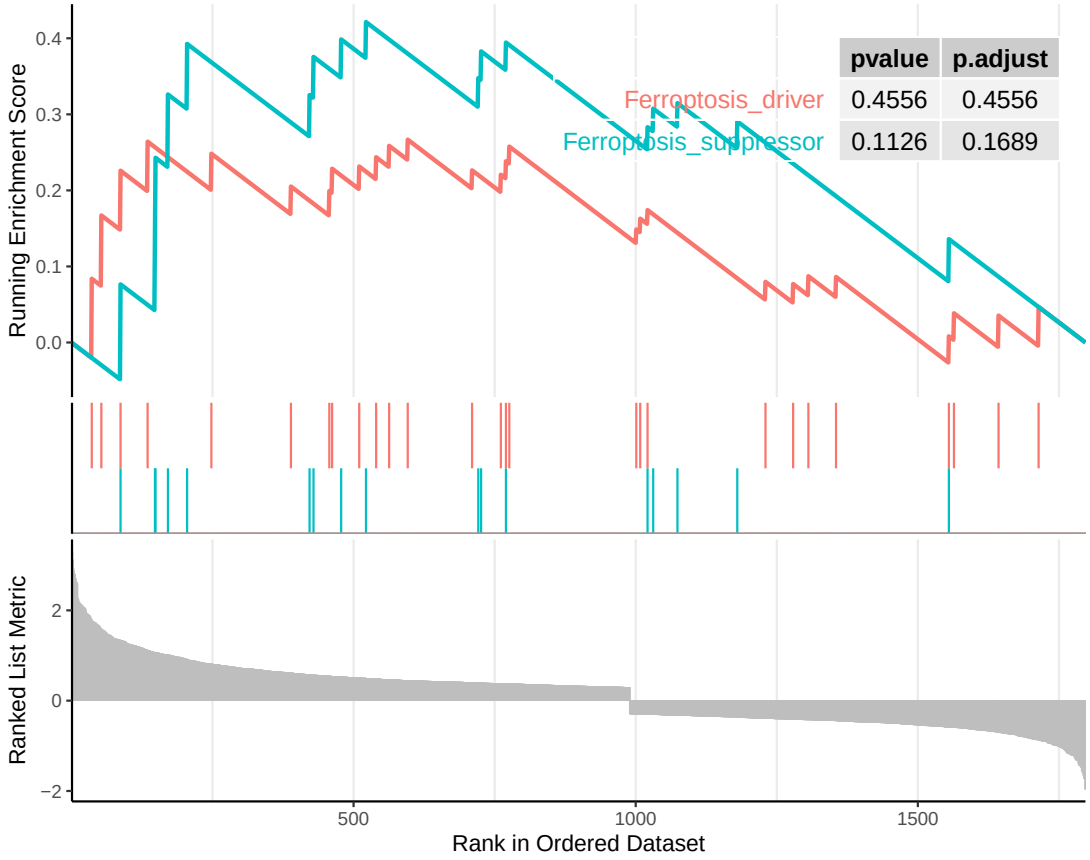


Figure 21: All DEGs of GSEA enrichment via FerrDb V2

Table 13 (下方表格) 为表格 table of All DEGs of GSEA enrichment via FerrDb V2 概览。

(对应文件为 `Figure+Table/table-of-All-DEGs-of-GSEA-enrichment-via-FerrDb-V2.xlsx`)

注：表格共有 3 行 13 列，以下预览的表格可能省略部分数据；表格含有 3 个唯一 'ID'。

Table 13: Table of All DEGs of GSEA enrichment via FerrDb V2

ID	Descr...	setSize	enric...	NES	pvalue	p.adjust	qvalue	rank	leadi...	core_...	GeneR...	Count
Ferro...	Ferro...	21	0.576...	2.006...	0.001...	0.004...	0.003...	379	tags=...	TSC22...	62	13
Ferro...	Ferro...	17	0.421...	1.372...	0.112...	0.168...	0.118...	522	tags=...	SLC7A...	53	9
Ferro...	Ferro...	27	0.266...	1.002...	0.455...	0.455...	0.319...	596	tags=...	CHAC1...	44	12

8.2.3 仅 Ferroptosis 相关基因的富集分析

注：以下富集是过拟合的，因为预先根据 FerrDb 数据筛选了基因用于分析。仅供参考。

Figure 22 (下方图) 为图 KEGG enrichment over Fitting 概览。

(对应文件为 `Figure+Table/KEGG-enrichment-over-Fitting.pdf`)

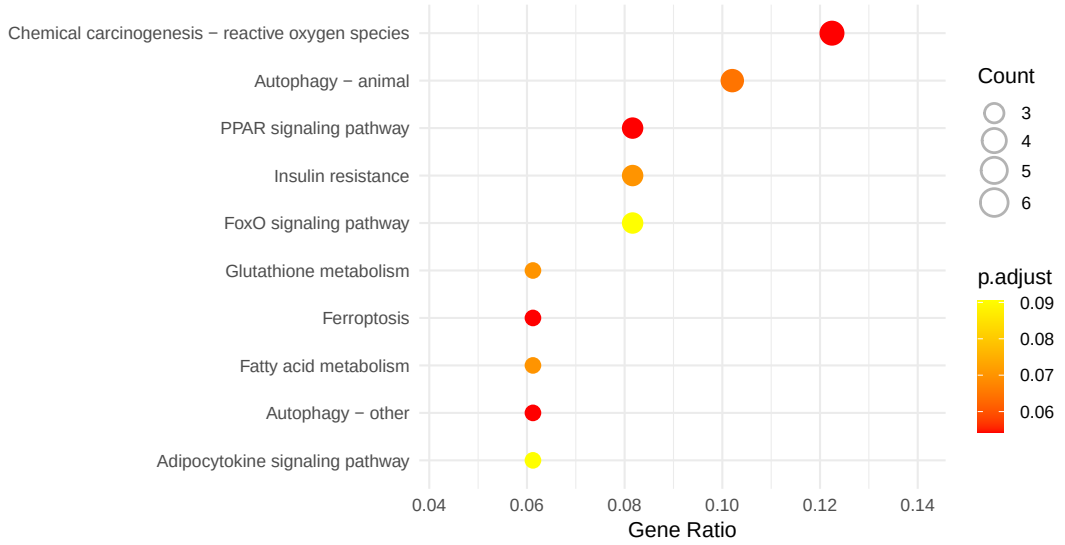


Figure 22: KEGG enrichment over Fitting

Table 14 (下方表格) 为表格 tables of KEGG enrichment over Fitting 概览。

(对应文件为 `Figure+Table/tables-of-KEGG-enrichment-over-Fitting.xlsx`)

注：表格共有 136 行 9 列，以下预览的表格可能省略部分数据；表格含有 136 个唯一 'ID'。

Table 14: Tables of KEGG enrichment over Fitting

ID	Descr...	GeneR...	BgRatio	pvalue	p.adjust	qvalue	geneID	Count
hsa04136	Autop...	3/49	32/8586	0.000...	0.054...	0.047...	23710...	3
hsa03320	PPAR ...	4/49	75/8586	0.000...	0.054...	0.047...	5468/...	4
hsa05208	Chemi...	6/49	223/8586	0.001...	0.054...	0.047...	7422/...	6
hsa04216	Ferro...	3/49	41/8586	0.001...	0.054...	0.047...	55240...	3
hsa04140	Autop...	5/49	165/8586	0.002...	0.064...	0.057...	54541...	5
hsa04931	Insul...	4/49	108/8586	0.003...	0.069...	0.061...	6513/...	4
hsa00480	Gluta...	3/49	57/8586	0.004...	0.069...	0.061...	4257/...	3
hsa01212	Fatty...	3/49	57/8586	0.004...	0.069...	0.061...	2180/...	3
hsa04068	FoxO ...	4/49	131/8586	0.006...	0.090...	0.079...	5728/...	4
hsa04920	Adipo...	3/49	69/8586	0.007...	0.090...	0.079...	6513/...	3
hsa05230	Centr...	3/49	70/8586	0.007...	0.090...	0.079...	6513/...	3
hsa01230	Biosy...	3/49	75/8586	0.008...	0.100...	0.088...	440/2...	3
hsa00250	Alani...	2/49	37/8586	0.018...	0.181...	0.160...	440/8...	2
hsa00760	Nicot...	2/49	37/8586	0.018...	0.181...	0.160...	23411...	2
hsa04922	Gluca...	3/49	107/8586	0.022...	0.207...	0.183...	6513/...	3
...	...	...	...	...	...	...	...	...

Figure 23 (下方图) 为图 GO enrichment over Fitting 概览。

(对应文件为 `Figure+Table/GO-enrichment-over-Fitting.pdf`)

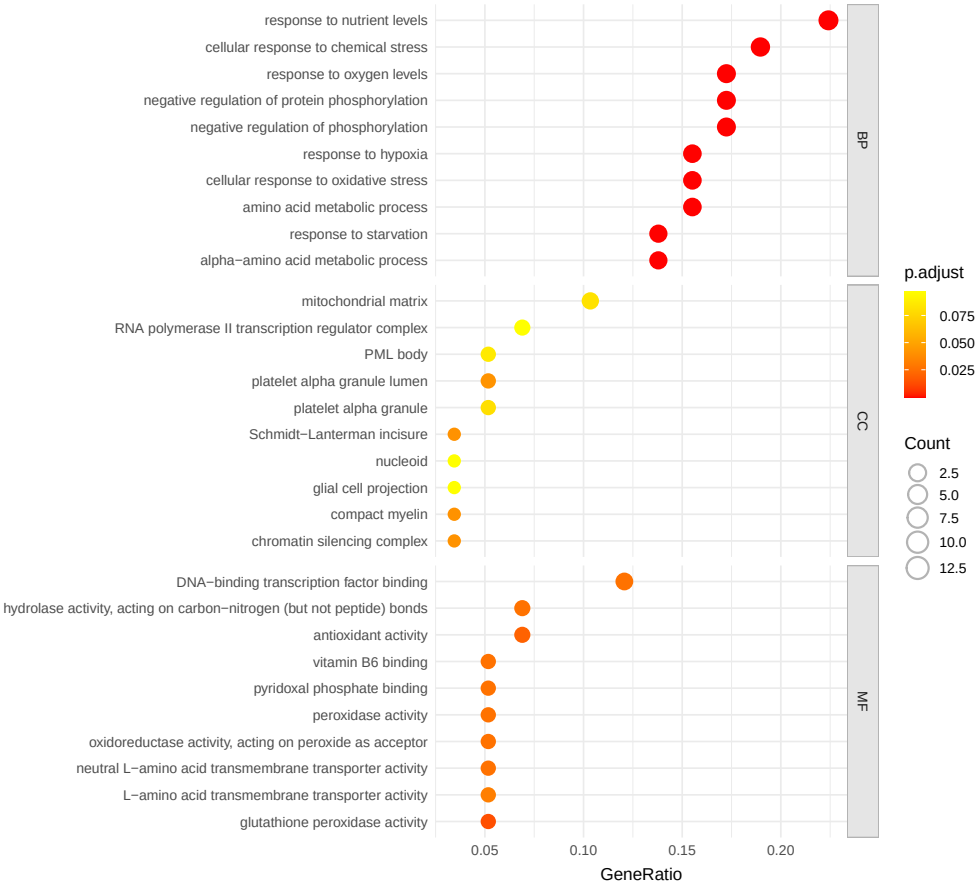


Figure 23: GO enrichment over Fitting

Figure 24 (下方图) 为图 visualization of pathway of Ferroptosis over Fitting 概览。

(对应文件为 `Figure+Table/hsa04216.pathview.png`)

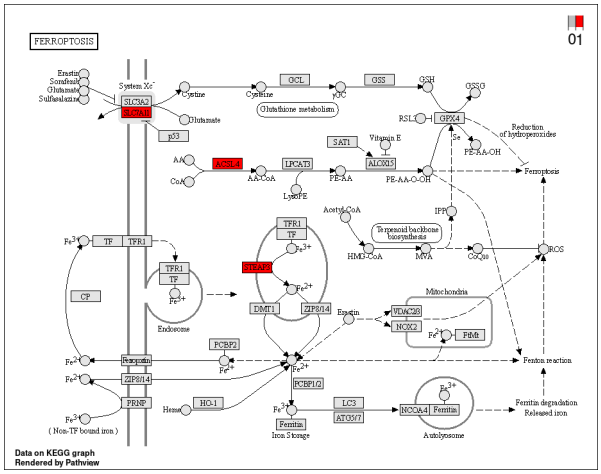


Figure 24: Visualization of pathway of Ferroptosis over Fitting

8.3 总结

- TNA 治疗的 DEGs 包含一部分铁死亡（Ferroptosis）相关的基因（这些基因见 Tab. 12，包含通路注释和文献佐证等内容），但是关联并不密切（不显著）（Fig. 21）。
- 其余分析供参考。

Reference

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