

## **Bioinformatics protocol**

### **1. Explore immune conditions of AML patients**

- a. Download the complete gene expression profiles and clinical information of 173 AML patients from TCGA.
- b. Calculate immune scores and stromal scores by employing the ESTIMATE algorithm.
- c. Analyze the relationship of immune/stromal scores with subtype classification and cytogenetic risk by one-way analysis of variance.
- d. Classify the 173 AML patients into high- and low-score groups (the top 87 scores are the high score group and the rest are the low score group). Explore the association of overall survival with immune/stromal scores by Kaplan-Meier survival analysis.

### **2. Identify DEGs based on immune scores and stromal scores**

- a. Identify DEGs between high immune/stromal score group and low immune/stromal score group by the package edgeR (Set p value  $< 0.05$  and  $|\text{fold change}| > 1.5$  as cut-off criteria).
- b. Identify common DEGs from the immune score/stromal score group through integrated bioinformatics analysis.

### **3. GO term and KEGG pathway enrichment analyses of DEGs**

- a. Perform GO analysis of the DEGs by the DAVID gene annotation tool (thresholds: count 2, ease 0.1; display: Benjamini).
- b. Conduct interrelation analysis by assessing BP, CC, and MF for the DEGs in the ClueGO plug-in Cytoscape software (Set p value  $< 0.05$  as cut-off criteria).
- c. Perform Pathway enrichment by the KEGG pathway enrichment (thresholds: count 2, ease 0.1; display: Benjamini) and the REACTOME online database.

### **4. Survival analysis of DEGs**

Construct Kaplan-Meier survival curves of the DEGs (log-rank test,  $p < 0.05$ ).

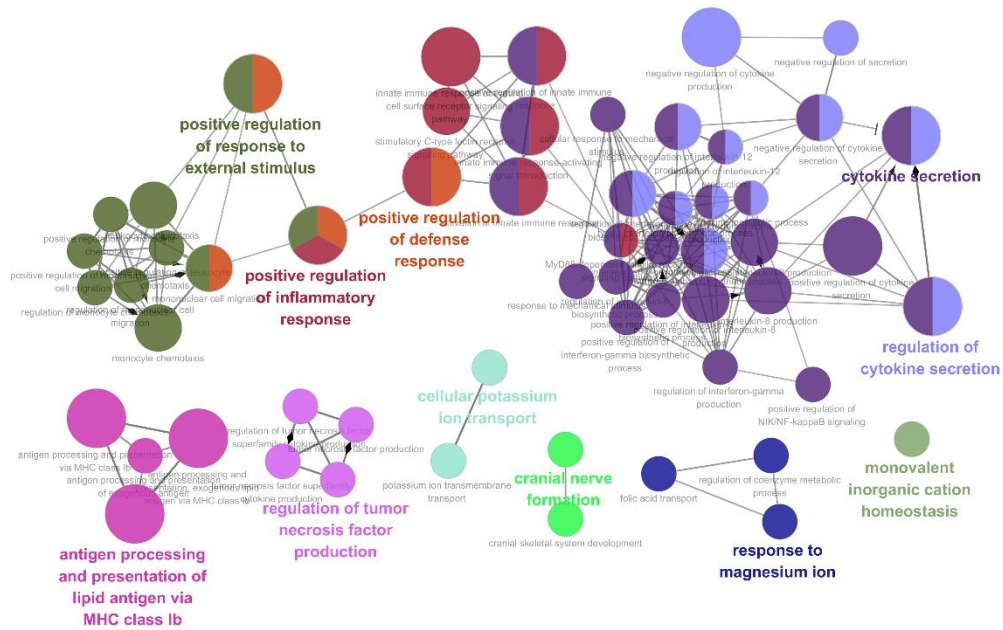
### **5. PPI network construction and functional enrichment of genes of prognostic value**

- a. Construct a PPI network by the STRING online database (minimum required interaction score: medium confidence 0.400) and Cytoscape software.
- b. Explore clustering analysis of the PPI network by the Cytotype MCODE.
- c. Perform GO term and pathway enrichment analysis by DAVID and REACTOME database.
- d. Conduct interrelation analysis by assessing the immune system process in the ClueGO (Set p value  $< 0.05$  as cut-off criteria).

### **6. Validation in the GEO database**

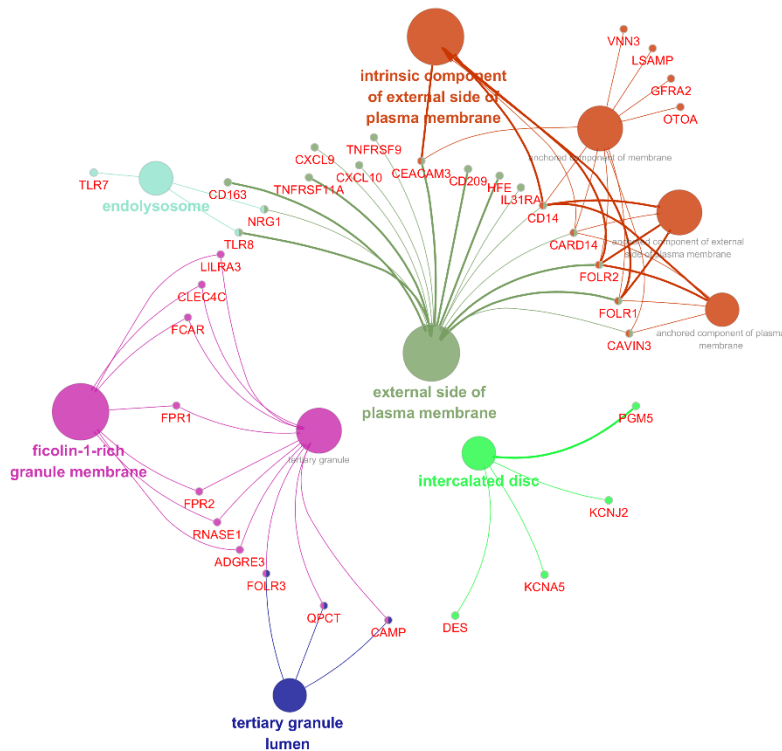
Identify the prognostic values of genes by the PrognosScan online tool (GSE12417 and GSE5122 datasets, Kaplan-Meier plot,  $p < 0.05$ ).

**Supplementary Fig.1**



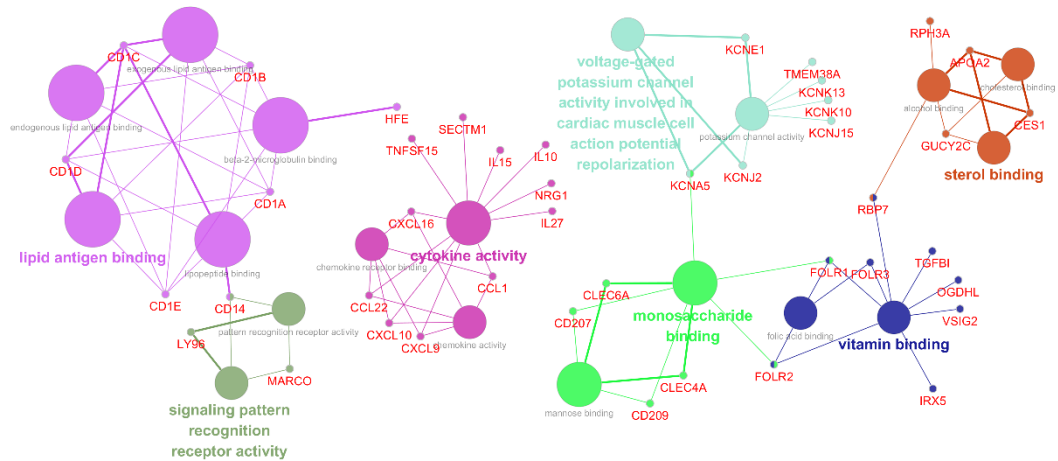
**Supplementary Fig.1** Interrelation analysis was performed by assessing the biological processes of the common DEGs

**Supplementary Fig.2**



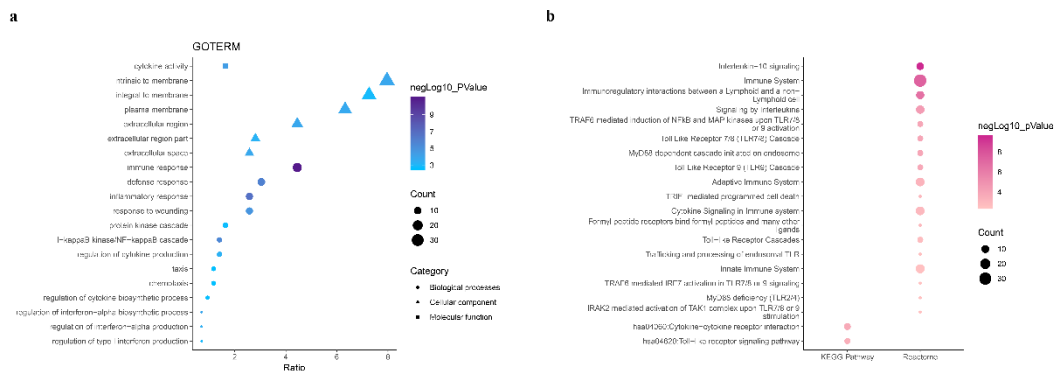
**Supplementary Fig.2** Interrelation analysis was performed by assessing the cellular components of the common DEGs, showing genes shared between terms

### Supplementary Fig.3



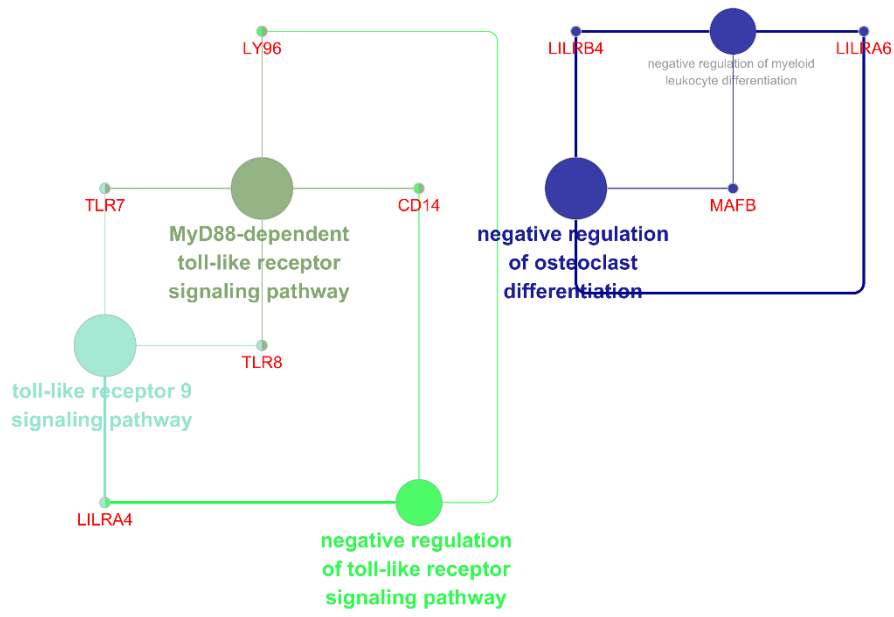
**Supplementary Fig.3** Interrelation analysis was performed by assessing the molecular functions of the common DEGs, showing genes shared between terms

### Supplementary Fig.4



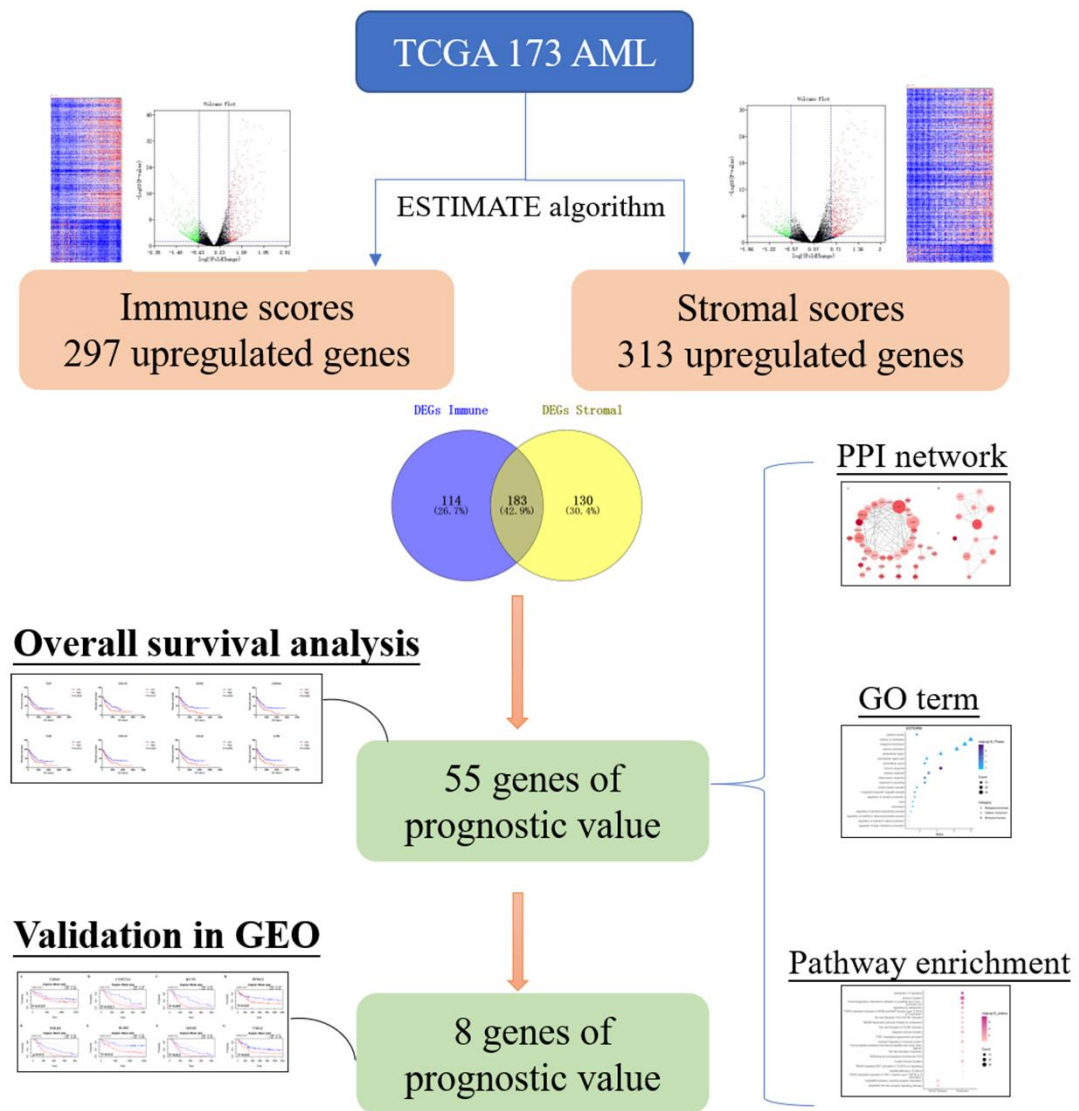
**Supplementary Fig.4** Functional enrichment analysis of DEGs with prognostic value. **a** The top 20 significantly enriched GO terms, including 3 subontologies, biological processes, molecular function and cellular component, are shown. **b** KEGG and REACTOME pathway enrichment

**Supplementary Fig.5**



**Supplementary Fig.5** Interrelation analysis of immune system processes of DEGs with prognostic value, showing genes shared between terms

Supplementary Fig.6



Supplementary Fig.6 Work flow of the current study

**Supplementary Table 1** TCGA IDs of 173 AML patients

	<b>Sample ID</b>
1	TCGA-AB-2803-03
2	TCGA-AB-2805-03
3	TCGA-AB-2806-03
4	TCGA-AB-2807-03
5	TCGA-AB-2808-03
6	TCGA-AB-2810-03
7	TCGA-AB-2811-03
8	TCGA-AB-2812-03
9	TCGA-AB-2813-03
10	TCGA-AB-2814-03
11	TCGA-AB-2815-03
12	TCGA-AB-2816-03
13	TCGA-AB-2817-03
14	TCGA-AB-2818-03
15	TCGA-AB-2819-03
16	TCGA-AB-2820-03
17	TCGA-AB-2821-03
18	TCGA-AB-2822-03
19	TCGA-AB-2823-03
20	TCGA-AB-2824-03
21	TCGA-AB-2825-03
22	TCGA-AB-2826-03
23	TCGA-AB-2828-03
24	TCGA-AB-2830-03
25	TCGA-AB-2832-03
26	TCGA-AB-2833-03
27	TCGA-AB-2834-03
28	TCGA-AB-2835-03
29	TCGA-AB-2836-03
30	TCGA-AB-2837-03
31	TCGA-AB-2838-03
32	TCGA-AB-2839-03
33	TCGA-AB-2840-03
34	TCGA-AB-2841-03
35	TCGA-AB-2842-03
36	TCGA-AB-2843-03
37	TCGA-AB-2844-03
38	TCGA-AB-2845-03
39	TCGA-AB-2846-03
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157	TCGA-AB-2992-03
158	TCGA-AB-2993-03
159	TCGA-AB-2994-03
160	TCGA-AB-2995-03
161	TCGA-AB-2996-03
162	TCGA-AB-2998-03
163	TCGA-AB-2999-03
164	TCGA-AB-3000-03
165	TCGA-AB-3001-03
166	TCGA-AB-3002-03
167	TCGA-AB-3005-03
168	TCGA-AB-3006-03
169	TCGA-AB-3007-03
170	TCGA-AB-3008-03

171	TCGA-AB-3009-03
172	TCGA-AB-3011-03
173	TCGA-AB-3012-03

**Supplementary Table 2** DEGs whose expression is significant in overall survival of AML

	<b>Gene symbol</b>	<b>P value</b>
1	IL1R2	2.08×10 <sup>-4</sup>
2	TNNI2	3.04×10 <sup>-4</sup>
3	HTR7	6.00×10 <sup>-4</sup>
4	FOLR1	6.40×10 <sup>-4</sup>
5	MYO7A	8.85×10 <sup>-4</sup>
6	KCNE1	0.001952
7	PGA4	0.001953
8	IL10	0.001964
9	MS4A4A	0.002529
10	LILRA5	0.003267
11	PADI6	0.004276
12	LILRA4	0.004406
13	LILRA6	0.004797
14	SECTM1	0.004891
15	CRIP3	0.005001
16	SLC15A3	0.005808
17	MYOF	0.006201
18	LILRB4	0.006783
19	FXVD6	0.006852
20	CXCL10	0.006991
21	CD163	0.007108
22	PGA3	0.007246
23	FFAR2	0.007293
24	ADAMDEC1	0.008078
25	LY96	0.010615
26	ACOX2	0.010731
27	TLR8	0.012352
28	PPM1J	0.012384
29	CCL22	0.013998
30	CXCL16	0.016146
31	OTOA	0.017145
32	VSIG2	0.01801
33	VNN3	0.020111
34	MSR1	0.023489
35	IL15	0.023621
36	LILRA3	0.024431
37	MAFB	0.025357

38	NLRP12	0.025726
39	CASP5	0.026741
40	KCNA5	0.027809
41	FPR1	0.028491
42	CD14	0.029046
43	GFRA2	0.030473
44	CYP27A1	0.030825
45	TLR7	0.031755
46	LGALS2	0.033073
47	SIGLEC11	0.033596
48	S100A12	0.03477
49	CD300E	0.035733
50	LOC152225	0.042654
51	ABCC3	0.044616
52	MEFV	0.047176
53	TWIST2	0.047975
54	TNFSF15	0.048976
55	CD1C	0.049767

**Supplementary Table 3** The description of the 8 genes

<b>Gene</b>	<b>Description</b>	<b>Relation to AML (negatively associated with AML prognosis)</b>
CD163	CD163 molecule	GSE5122 (HR=1.44, P=0.0297) GSE12417 (HR=2.11, P=0.0215)
CYP27A1	Cytochrome P450 family 27 subfamily A member 1	GSE5122 (HR=1.60, P=0.0232) GSE12417(HR=1.71, P=0.0459)
KCNA5	Potassium voltage-gated channel subfamily A member 5	GSE5122(HR=1.45, P=0.009)
PPM1J	Protein phosphatase, Mg <sup>2+</sup> /Mn <sup>2+</sup> -dependent 1J	GSE12417 (HR=1.70, P=0.042)
FOLR1	Folate receptor beta	GSE5122 (HR=1.41, P=0.011)
IL1R2	Interleukin 1 receptor type 2	GSE12417 (HR=1.63, P=0.012)
MYOF	Myoferlin	GSE5122 (HR=1.38, P=0.023)
VSIG2	V-set and immunoglobulin domain containing 2	GSE12417 (HR=7.81, P=0.042)