



# Dipetidyl peptidase-4 and transferrin receptor serve as prognostic biomarkers for acute myeloid leukemia

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**Background:** Acute myeloid leukemia (AML) is the most common hematological malignancy in adult patients. Ferroptosis-related signatures have been shown to act as regulators of the progression of multiple cancer types, but the role of ferroptosis in AML remains to be elucidated. We performed the present study to preliminarily investigate the roles of ferroptosis-related genes (FRGs) in AML.

**Methods:** The transcriptome data of AML patients was downloaded from The Cancer Genome Atlas (TCGA) and the transcriptome data of normal samples was obtained from the Genotype-Tissue Expression (GTEx) database. FRGs were selected via public articles. Expression levels of FRGs between AML and normal samples were analyzed. The prognostic model based on FRGs was constructed via lasso regression. The expression levels and prognostic role of FRGs were identified from the risk model. We also performed validation experiments to verify the expression levels of the final selected genes via immunohistochemistry, polymerase chain reaction (PCR), and RNA-seq. Finally, we explored the associations between immune infiltration, drug sensitivity, and the selected FRGs.

**Results:** The transcriptome data of 151 AML samples were retrieved from TCGA and 70 bone marrow normal samples were retrieved from the GTEx database. Additionally, 23 FRGs were collected from the published articles. There were 22 differentially expressed FRGs, and among them, dipetidyl peptidase-4 (DPP4) (P= 0.011, HR =1.504), GPX4 (P=0.055, HR =1.569), LPCAT3 (P<0.001, HR =2.243), SLC7A11 (P=0.012, HR =2.243), and transferrin receptor (TFRC) (P=0.029, 0.774) had a significant influence on the prognosis of AML patients via lasso regression. The area under the curve (AUC) values of the 1-, 3-, and 5-year receiver operating characteristic (ROC) curves of the FRG signatures indicated that this model is novel and effective method for predicting the prognosis of AML patients. DPP4 (P<0.001) was overexpressed while LPCAT3 (P<0.001), TFRC (P<0.001), GPX4 (P<0.001), and SLC7A11 (P<0.001) were downregulated, further validation experiment results indicated that DPP4 was significantly downregulated but TFRC was upregulated in AML samples. Dysregulation of DPP4 and TFRC influence numbers of chemotherapy regimens sensitivity.

**Conclusions:** DPP4 and TFRC act as biomarkers for predicting and diagnosing AML, and their expression levels also have significant correlations with drug resistance in AML.

**Keywords:** Acute myeloid leukemia (AML); DPP4; TFRC; prognostics biomarker

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## 1 Introduction

2 Acute myeloid leukemia (AML) is characterized by a  
3 loss of control of myeloid precursor cell proliferation  
4 and undifferentiation (1). If AML patients do not  
5 undergo appropriate treatment, death can rapidly occur.  
6 Anthracycline and cytarabine have remained the standard  
7 therapy regimens for AML patients since the 1970s (2).  
8 Despite advances in diagnostic and therapeutic methods, the  
9 overall survival (OS) of AML patients has not significantly  
10 improved. Over the past decade, with the introduction  
11 of targeted therapy agents combined with traditional  
12 chemotherapy, the rates of complete remission (CR) have  
13 been improved, but the rate of relapse is still unchanged.  
14 Relapse of disease remains an obstacle for lengthening  
15 the OS of AML patients. For high-risk patients, the rate  
16 of disease relapse is more than 60% and results in a short  
17 median disease-free survival (DFS) of less than 1 year (range,  
18 4 to 11 months) (3). To date, several driver mutations  
19 have been observed in AML patients, and these mutations  
20 have deep influence on the prognosis of AML patients.  
21 Kishtagari *et al.* study summarizes the driver mutations  
22 in AML, based on the functions of driver genes, they are  
23 divided into signal transduction (FLT3, NRAS, KRAS,  
24 and KIT), splicing mutations (SF3B1, ZRSR2, U2AF1,  
25 and SRSF2), tumor suppressors (TP53, WT1, and TET2),  
26 AML licensing mutations (NMP1), epigenetic modifiers  
27 (IDH1, IDH2, TET2, SRSF2, BCOR, BCORL, TET2,  
28 ASXL1, and EH22), transcription factors (RUNX1,  
29 CEBPA, and GATA2), and chromatin modifiers (Cohesin,  
30 ASXL1, and EH22) (4). Patients with the mutated NMP1,  
31 RUNX1, and TP53 lead to poor prognosis, but biallelic  
32 mutated CEBPA indicate favorable prognosis (5). Several  
33 studies have also demonstrated the occurrence of targeted  
34 therapy resistance (6,7). The target regimens enasidenib  
35 and ivosidenib have been used to treat IDH mutated AML  
36 patients (8). Sorafenib was used to therapy the with FLT3-  
37 ITD mutated AML patients (9). However, the resistance of  
38 these target therapy has been found (6,10). These indicated  
39 that some unique mutation can be sever as the diagnostic  
40 and prognostic biomarkers for AML patients, as well as  
41 assessing the drug resistance, relapse risk, and therapy  
42 targets markers. Drug resistance and disease relapse may  
43 be the main reasons leading to the poor outcomes of AML  
44 patients, but the underlying mechanisms are still unclear.  
45 It is therefore important to find novel biomarkers for  
46 diagnosis, assessing prognosis, monitoring drug resistance,  
47 and even supplementary therapy methods for AML patients.

49 Iron is a fundamental inorganic nutrient which has a  
50 critical role in multiple biological processes such as DNA and  
51 RNA synthesis, cellular respiration, immune responses, and  
52 detoxification processes, among others (11). Ferroptosis was  
53 introduced in 2012 and is defined as a unique iron-dependent  
54 form of cell death. The features of ferroptosis include  
55 smaller mitochondria with increased membrane density,  
56 and decreased mitochondrial cristae (12). Ferroptosis strike  
57 the death balance in common cells and tissues (13). Several  
58 studies have demonstrated that ferroptosis is a significant  
59 regulator of tumor progression (14-16). Ferroptosis is  
60 regulated via several factors, and ferroptosis-related genes  
61 (FRGs) may be the most significant regulators among them.  
62 FRGs have been observed to be differentially expressed and  
63 play key roles in the prognosis of various cancer types such  
64 as pancreatic cancer, glioma, and hepatocellular carcinoma  
65 (17-20). From these findings, it is clear that FRGs have  
66 been well investigated in solid tumors. In regards to AML,  
67 several studies have explored the mechanism of drug-  
68 induced ferroptosis (21-23). Du *et al.*'s study indicated that  
69 DHA can inhibit leukemia cell proliferation via inducing  
70 ferroptosis (21). Furthermore, Du *et al.* revealed that  
71 inhibition of ferroptosis can promote ATPR-induced AML  
72 cell differentiation by regulating the ROS-autophagy-  
73 lysosomal pathway (22). Zhu *et al.* showed that typhaneoside  
74 inhibited leukemia cell proliferation via inducing ferroptosis-  
75 related autophagy (23). These findings indicate that inducing  
76 ferroptosis may be a novel potential anticancer method  
77 for AML. However, there have been no studies that have  
78 investigated FRG expression levels, their prognostic role, and  
79 their association with the tumor microenvironment (TME)  
80 and drug resistance in AML patients. In the present study,  
81 we used bioinformatics to analyze FRG expression levels,  
82 their prognostic role, and their association with immune  
83 infiltration and drug sensitivity. Furthermore, we collected  
84 normal samples and AML patient samples to validate the  
85 gene expression levels via immunohistochemistry, polymerase  
86 chain reaction (PCR), and next-generation sequencing  
87 (NGS). We present the following article in accordance  
88 with the REMARK reporting checklist (<https://dx.doi.org/10.21037/atm-21-3368>).

## Methods

### Raw data

94 The transcriptome data and clinical data of 151 AML  
95 samples from The Cancer Genome Atlas (TCGA) database  
96

97 and 70 bone marrow normal samples from the Genotype-  
 98 Tissue Expression (GTEx) database were collected from  
 99 the University of California Santa Cruz database (UCSC  
 100 Xena, <https://xenabrowser.net/datapages/>). Subsequently,  
 101 log<sub>2</sub> (FPKM+1) normalization was performed on the  
 102 transcriptome data. We searched and extracted 23 FRGs  
 103 from PubMed (24-26).

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### Screening differentially expressed FRGs

107 We screened differentially expressed FRGs between the  
 108 TCGA-LAML cohort (tumor) and the GTEx cohort  
 109 (normal) for further analysis. Differential analysis was  
 110 carried out with the Wilcoxon test in R software. A heatmap  
 111 plot of differentially expressed genes was generated via  
 112 the ggplot2 package. P<0.05 was considered statistically  
 113 significant.

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### Construction of the ferroptosis-related prognostic signature

117 We obtained prognostic FRGs via univariate cox regression  
 118 based on differential expression of FRGs, then used  
 119 lasso regression to obtain a more refined signature by  
 120 constructing a penalty function. Multivariate cox regression  
 121 (stepwise) was used to construct the final prognostic  
 122 signature. KM survival analysis was used to generate  
 123 the survival curves based on median values, and log-  
 124 rank P<0.05 was considered statistically significant. The  
 125 receiver operating characteristic (ROC) curves, nomogram,  
 126 and calibration curve of the prognostic signature were  
 127 generated via the R packages survivalROC, survminer, and  
 128 rms, respectively. P<0.05 was considered as statistically  
 129 significant.

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### Tumor immune infiltration analysis

133 We used the CIBERSORT algorithm of tumor immune cell  
 134 infiltration to calculate the abundance of 22 immune cells  
 135 in the TCGA-LAML cohort. The correlation analysis of  
 136 immune cells was carried out via the Spearman method.

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### Immunohistochemistry

140 Bone marrow smears of AML and normal cases were  
 141 collected, fixed with 10% neutral formalin, dehydrated  
 142 with gradient alcohol, and stained with hematoxylin  
 143 and eosin (HE). The following antibodies were used for  
 144 immunostaining: dipetidyl peptidase-4 (DPP4) (Abcam,

ab187048), GPX4 (Proteintech, 14432-1-AP), LPCAT3  
 (Abcam, ab239585), SLC7A11 (Proteintech, 26864-1-  
 AP), and transferrin receptor (TFRC) (Proteintech,  
 10084-2-AP).

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### PCR

EDTA anticoagulant tubes were used to collect the  
 peripheral blood of healthy adults and AML patients,  
 and Trizol (Invitrogen, China) was used to extract total  
 RNA. Then, the concentration of total RNA was detected  
 by a nucleic acid analyzer. GeneRuler DNA Ladder Mix  
 and Maxima Reverse Transcriptase were used to reverse  
 transcribe RNA into cDNA, and gene expression levels  
 were detected according to the 2X SG Fast qPCR Master  
 Mix (High Rox, B639273, BBI, ABI) kit instructions.  
 GAPDH was used as an internal reference, and the results  
 were calculated using the 2<sup>-ΔΔCt</sup> method.

### RNA-sequence (RNA-seq)

EDTA anticoagulant tubes were used to collect the  
 peripheral blood of healthy adults and AML patients, and  
 Trizol (Invitrogen, China) was used to extract total RNA.  
 RNA samples were used to perform NGS. The library  
 construction and transcriptome sequencing were completed  
 by Sheng Gong Bioengineering (Shanghai) Co., Ltd.

### Drug sensitivity analysis based on risk score

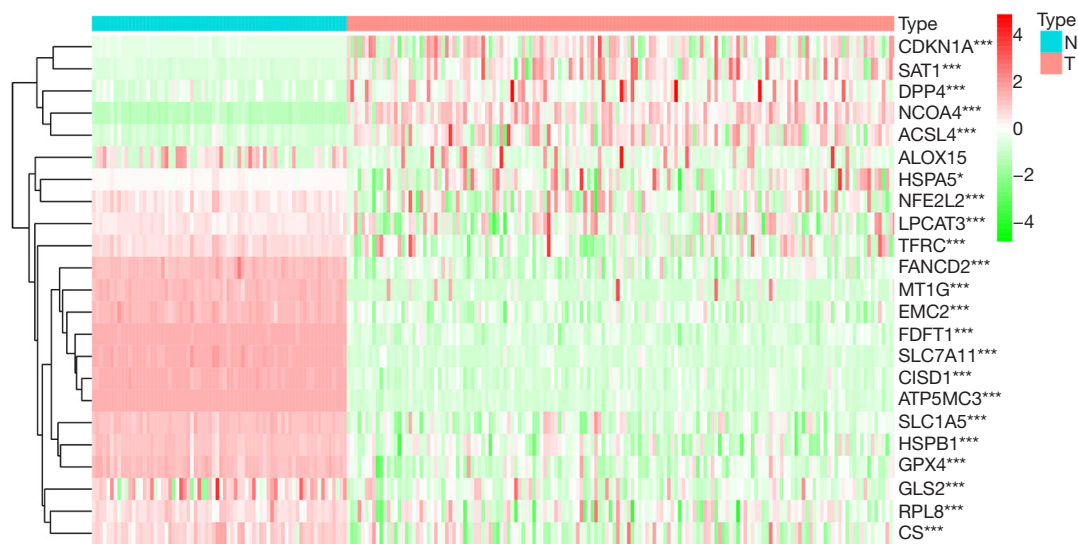
The R package pRRophetic was used to perform the drug  
 sensitivity analysis.

### Statistical analysis

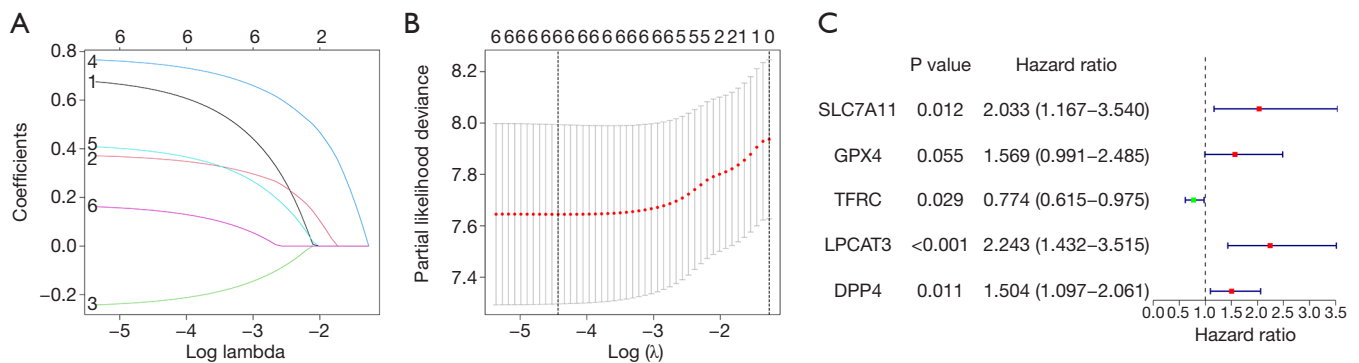
The differential FRGs were screened through the Wilcoxon  
 method. Kaplan-Meier (KM) plots were used to analyze the  
 differential survival between groups, and log-rank P<0.05  
 was considered statistically significant. Univariate cox  
 regression, lasso regression, and multivariate (stepwise) cox  
 regression were used to construct the prognostic signature.  
 Wilcoxon and Spearman tests were used for difference  
 analysis and correlation analysis, respectively. P<0.05 was  
 considered statistically significant.

### Ethical statement

The study was conducted in accordance with the



**Figure 1** Differential expression of ferroptosis-related genes in acute myeloid leukemia patients. Red represents genes with high expression, and green represents genes with low expression. \*, \*\*\* represent  $P < 0.05$ , and  $P < 0.001$ , respectively.



**Figure 2** Construction of the FRG prognostic signature for acute myeloid leukemia. (A,B) Selection of the optimal  $\lambda$  threshold for lasso regression. (C) The forest graph of the FRG prognostic signature. FRG, ferroptosis-related gene.

193 Declaration of Helsinki (as revised in 2013).

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195 **Results**

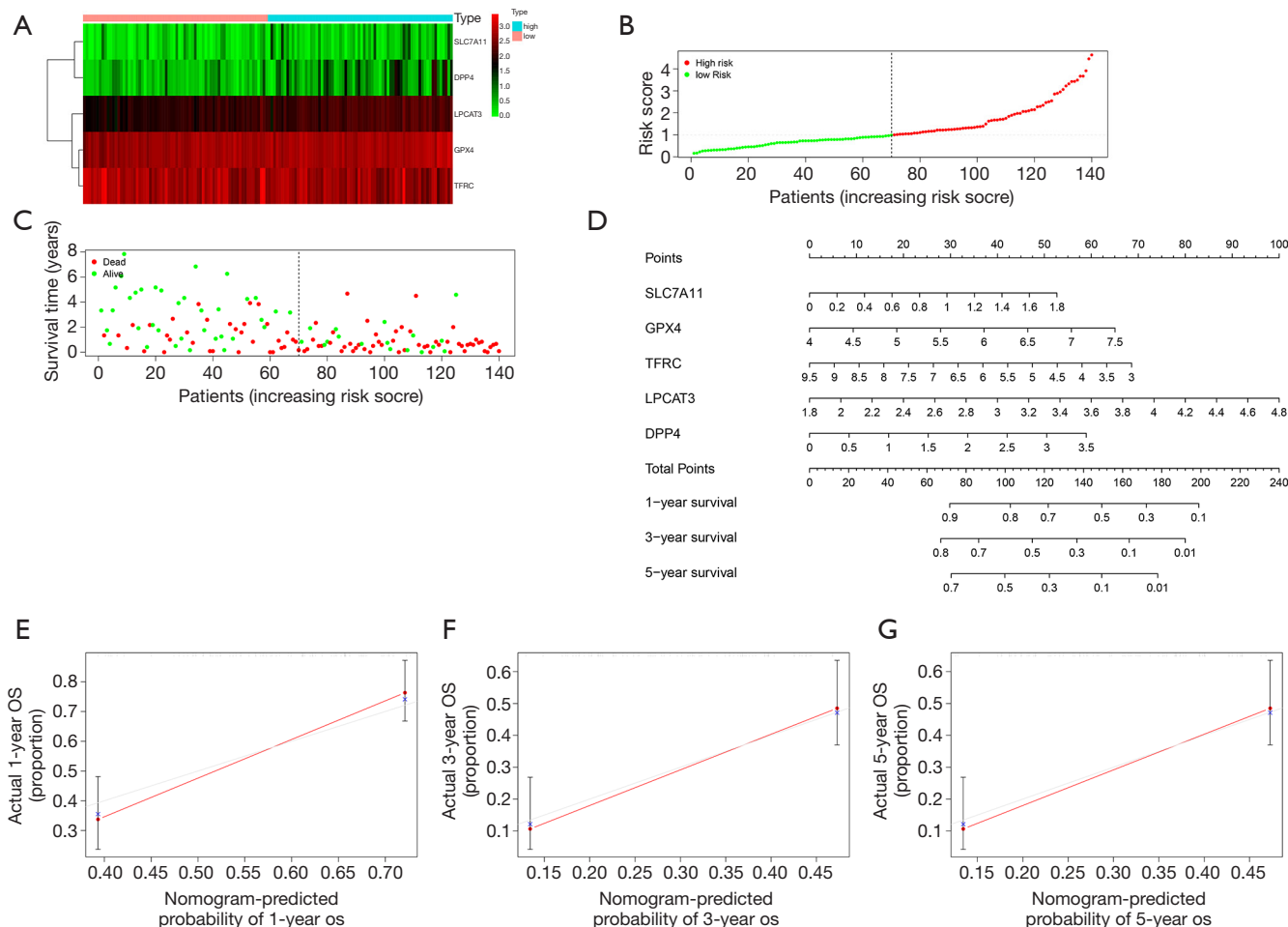
196 *Differential expression of FRGs in AML patients*

197 We retrieved 23 FRGs from PubMed and analyzed the  
 198 differential expression of FRGs between AML (n=151)  
 199 and normal bone marrow (n=70). The heatmap plot  
 200 showed that there were 22 differentially expressed FRGs  
 201 (Figure 1).  
 202  
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*Establishment of the FRG prognostic signature for AML*

204 We obtained 7 FRGs that affected the OS of AML patients  
 205 via univariate cox regression of differentially expressed  
 206 FRGs. The results of lasso regression indicated that  $\lambda = -4.4$   
 207 was the optimal value, then 6 FRGs were obtained for  
 208 further analysis (Figure 2A,2B). Finally, a 5-FRG prognostic  
 209 signature was established for AML (Figure 2C).  
 210

211 A heatmap was generated showing the FRG signature's  
 212 gene expression in low-risk and high-risk samples  
 213 (Figure 3A). The risk score curve and survival status plot  
 214



**Figure 3** The expression of the signature genes, risk score curve, survival status, nomogram, and calibration curve of the FRG prognostic signature. (A) Heatmap of the expression of FRG signature genes in low- and high-risk samples. Red represents high expression and green represents low expression. (B) Risk score curve of the FRG prognostic signature. Dotted lines represent the boundaries between high- and low-risk groupings. (C) Survival status plot of the FRG prognostic signature. (D) Nomogram of the FRG prognostic signature. The 1-year (E), 3-year (F), and 5-year (G) calibration curves of the nomogram. X-axis and Y-axis represent the predicted survival and actual survival probability of patients' overall survival, respectively. FRG, ferroptosis-related gene. FRG, ferroptosis-related gene.

215 indicated that low and high-risk could well distinguish  
 216 between surviving and dead patients (*Figure 3B,3C*). The  
 217 nomogram and calibration curves demonstrated that the  
 218 FRG prognostic signature had perfect predictive ability  
 219 (*Figure 3D-3G*).

#### 220 *KM survival analysis and ROC curve of the FRG signature*

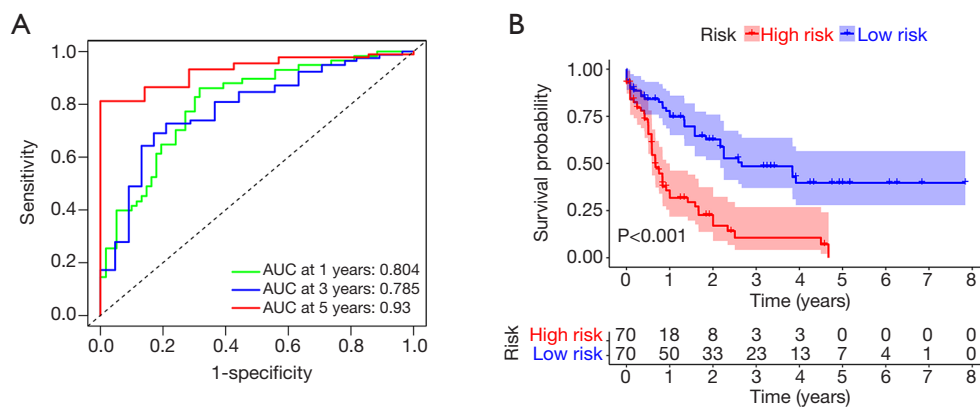
221  
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 223 The area under curve (AUC) values of the 1-, 3-, and 5-year  
 224 ROC curves of the FRG signature were 0.804, 0.785,  
 225 and 0.930, respectively (*Figure 4A*). KM survival analysis  
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227 indicated that patients with low risk had a better OS for  
 228 AML (log-rank  $P < 0.001$ ) (*Figure 4B*).

#### 229 *FRG expression levels and their association with prognosis*

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 232 There were 5 genes in the FRG signature, namely *DPP4*,  
 233 *LPCAT3*, *TFRC*, *GPX4*, and *SLC7A11*. *DPP4* was highly  
 234 expressed in tumors compared with normal samples  
 235 (*Figure 5A*), while *LPCAT3*, *TFRC*, *GPX4*, and *SLC7A11*  
 236 were lowly expressed in tumor samples (*Figure 5B-5E*). In  
 237 terms of prognosis, high expression of *DPP4*, *LPCAT3*,  
 238





**Figure 4** KM survival analysis and ROC curve of the FRG signature. (A) The ROC curves of the FRG signature. Green, blue, and red represent 1-year, 3-year, and 5-year ROC curves, respectively. (B) KM survival analysis of high and low risk of FRG signature. KM, Kaplan-Meier; ROC, receiver operating characteristic; AUC, area under curve; FRG, ferroptosis-related gene.

239 *GPX4*, and *SLC7A11* resulted in a shorter OS, while high  
240 expression of TFRC resulted in a better OS in AML  
241 patients (Figure 5F-5J).

#### 242 243 *The relative abundance and correlation of 22 immune cells* 244 *in the TCGA-LAML cohort*

246 The histogram shows the relative abundance of 22 immune  
247 cells in the TCGA-LAML cohort (Figure 6A). The heatmap  
248 of correlations between the 22 immune cells indicated  
249 that M2 macrophages were negatively correlated with  
250 other immune cells, and resting mast cells were positively  
251 correlated with other immune cells (Figure 6B).

#### 252 253 *Prognostic immune cells in AML patients*

255 KM survival analysis indicated that high infiltration  
256 of resting mast cells resulted in a better OS in AML  
257 patients (Figure 7A). Nevertheless, high infiltration of M2  
258 macrophages resulted in a poor prognosis (Figure 7B).

#### 259 260 *Correlation between the FRG signature biomarker and the* 261 *abundance of resting mast cells and M2 macrophages*

263 Spearman correlation analysis demonstrated that DPP4  
264 was negatively correlated with resting mast cells and M2  
265 macrophages (Figure 8A). GPX4 was positively correlated  
266 with resting mast cells but negatively correlated with  
267 M2 macrophages (Figure 8B). LPCAT3 was positively  
268 correlated with resting mast cells but negatively correlated  
269

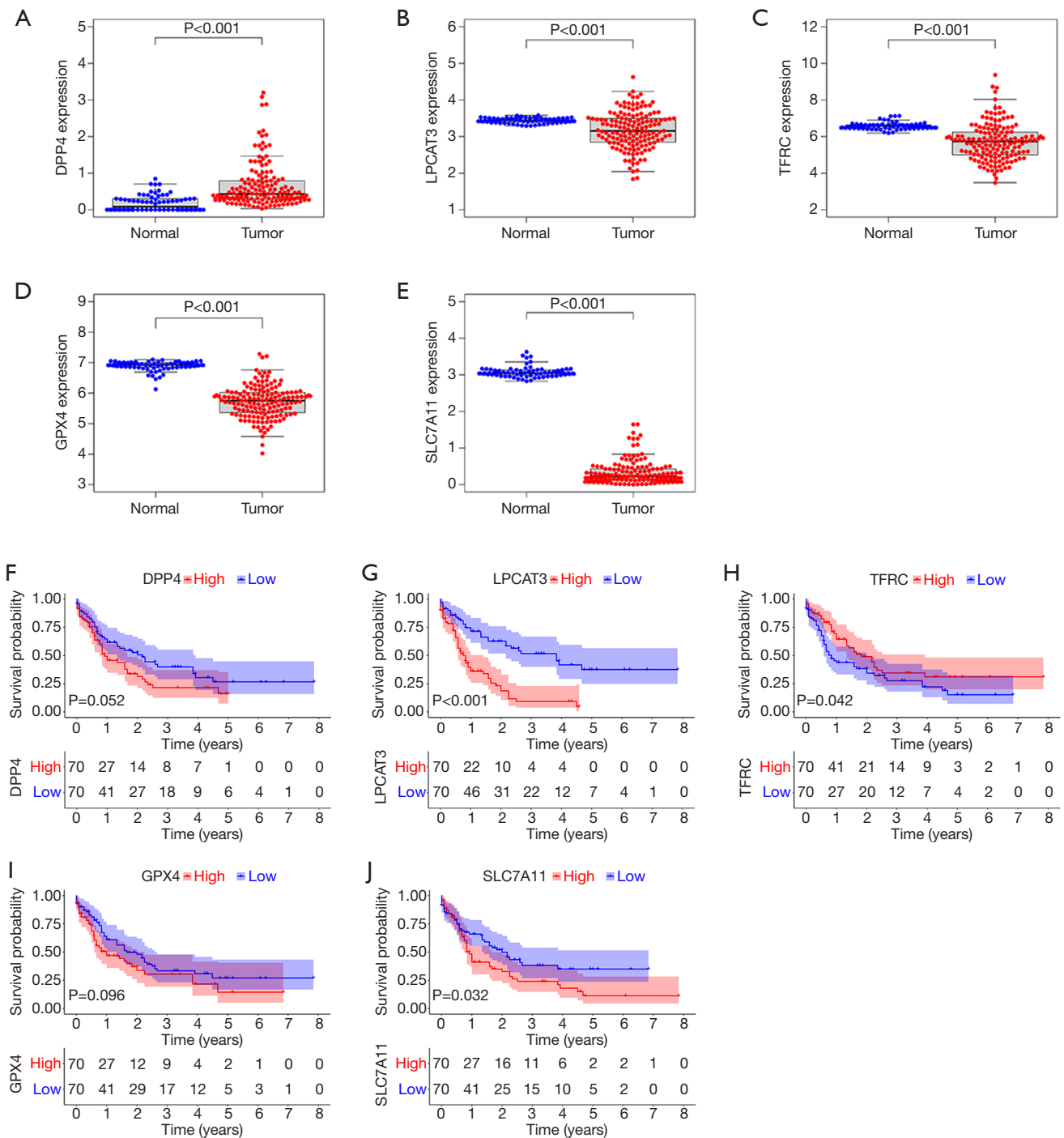
270 with M2 macrophages (Figure 8C). *SLC7A11* was positively  
271 correlated with resting mast cells but negatively correlated  
272 with M2 macrophages (Figure 8D). TFRC was negatively  
273 correlated with resting mast cells but positively correlated  
274 with M2 macrophages (Figure 8E).

#### 275 276 *The results of validation experiments*

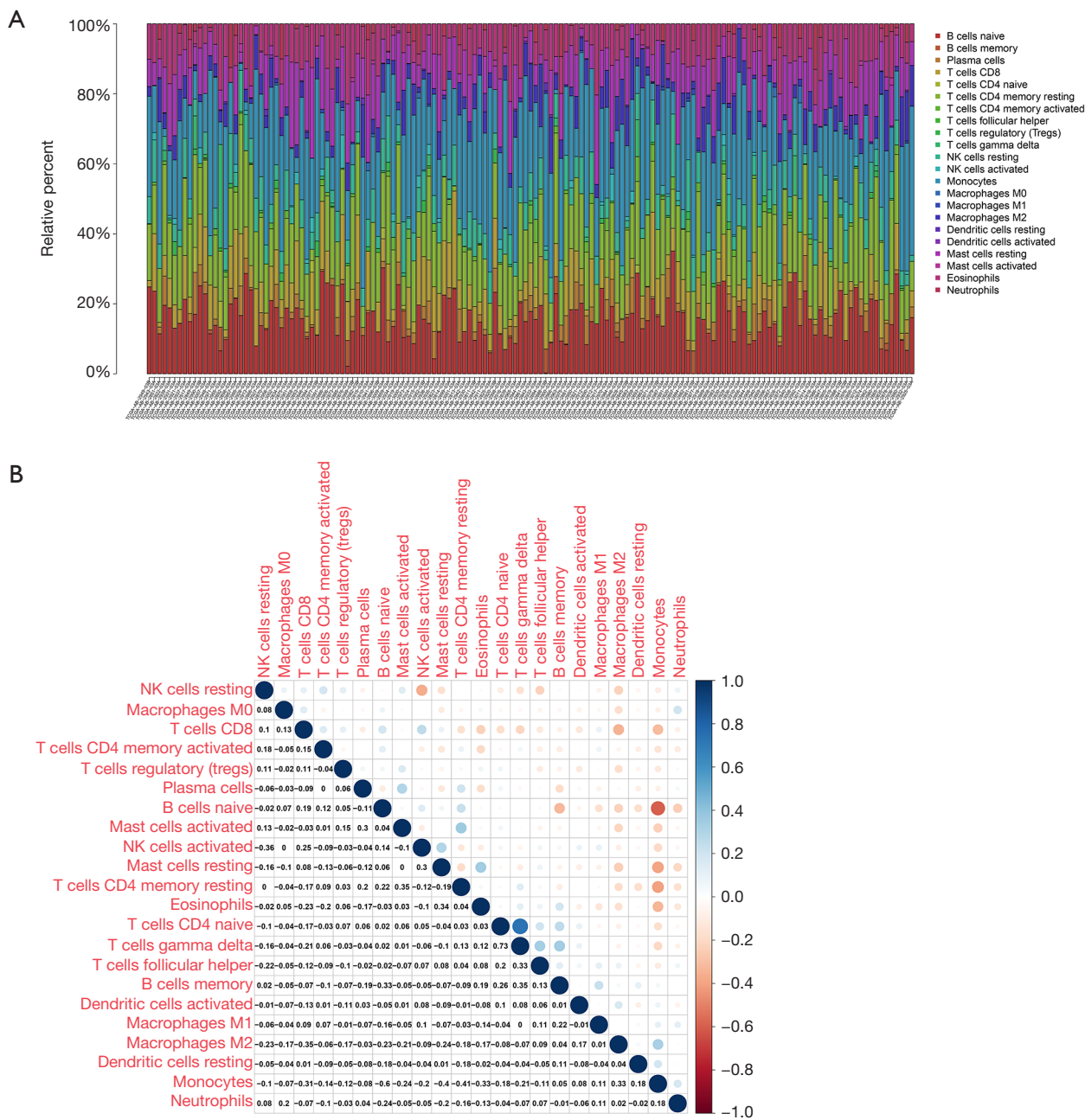
278 The results of immunohistochemistry indicated that  
279 DPP4, GPX4, LPCAT3, *SLC7A11*, and TFRC had higher  
280 expression in AML bone marrow samples (Figure 9).  
281 Furthermore, PCR results showed that TFRC ( $P < 0.01$ )  
282 was significantly overexpressed, but DPP4 ( $P < 0.01$ ), GPX4  
283 ( $P < 0.01$ ), LPCAT3 ( $P < 0.01$ ), and *SLC7A11* ( $P < 0.01$ )  
284 were significantly downregulated in AML samples (Figure 10). To  
285 further validate these selected gene expression levels between  
286 normal and AML samples, RNA-seq was performed, and the  
287 results showed that TFRC was significantly overexpressed  
288 in AML samples ( $P = 2.13 \times 10^{-6}$ ), while DPP4 ( $P = 0.016$ ) was  
289 significantly downregulated in AML samples (<https://cdn.amegroups.cn/static/public/atm-21-3368-1.xls>).  
290

#### 291 292 *Drug sensitivity*

294 The ultimate goal of cancer research is finding novel or  
295 complementary therapy regimens for cancer patients. We  
296 used TFRC and DPP4 to divide AML patients into high-  
297 and low-risk score groups, and explored the association  
298 between risk score and drug sensitivity. The results showed  
299 that patients with downregulation of TFRC were resistant  
300



**Figure 5** FRG expression levels and their association with prognosis. Expression levels of (A) DPP4, (B) LPCAT3, (C) TFRC, (D) GPX4, and (E) SLC7A11 in tumor and normal samples. Kaplan-Meier survival analysis of expression levels and overall survival based on (F) DPP4, (G) LPCAT3, (H) TFRC, (I) GPX4, and (J) SLC7A11. FRG, ferroptosis-related gene.



**Figure 6** The relative abundance and correlation of 22 immune cells in the TCGA-LAML cohort. (A) Histogram of the relative abundance of 22 immune cells. (B) Heatmap of correlations between the 22 immune cells. Blue and red represent positive and negative correlation, respectively.

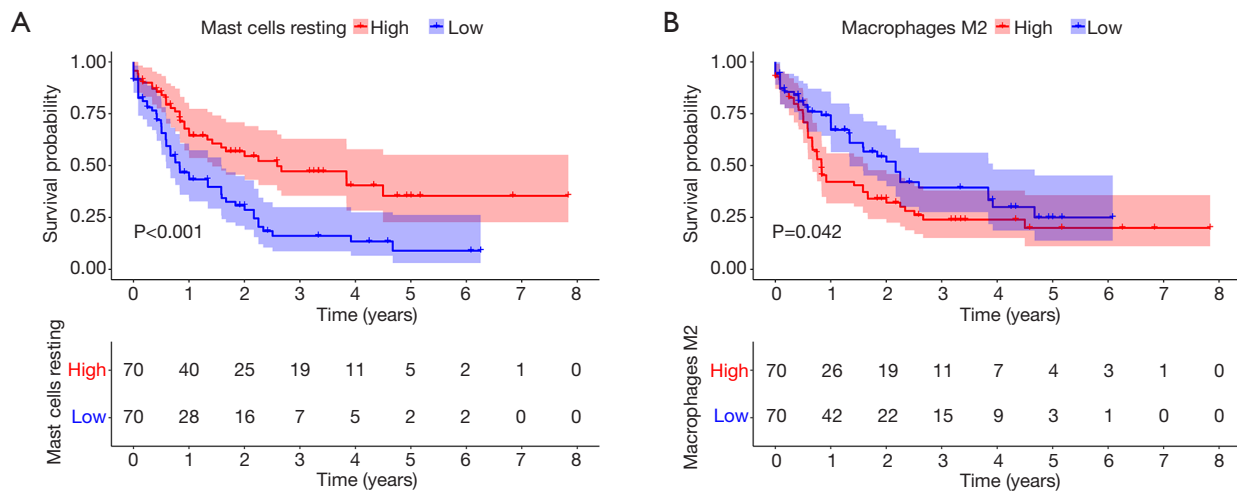
to many drugs such as ATRA, axitinib, and vinorelbine, among others, but sensitive to dasatinib, bryostatins, and so on (Figure 11). According to DPP4, the sensitivity analysis revealed that patients with scores based on the DPP4 group were resistant to CMK and cytarabine, and among others,

but sensitive to dasatinib (Figure 12).

### Discussion

With the better learning the critical role of ferroptosis in





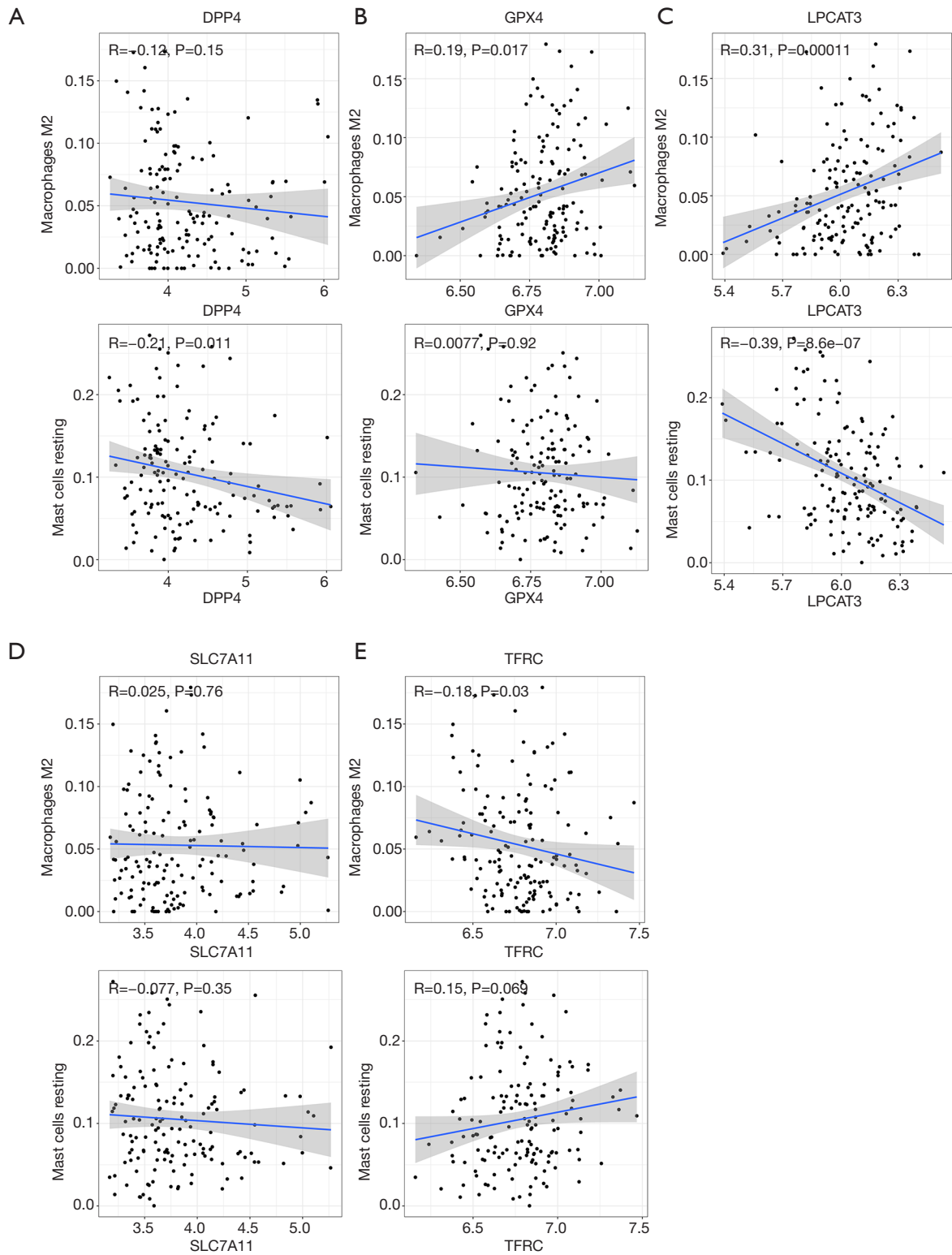
**Figure 7** Immune cells that affected the overall survival in AML patients. Kaplan-Meier survival analysis of (A) resting mast cells and (B) M2 macrophages. AML, acute myeloid leukemia.

tumorigenesis, therapy response, drug resistance in various cancer types. FRGs have been shown to be important factors that significantly influence tumor progression in multiple cancer types such as hepatocellular carcinoma, clear cell renal cell carcinoma, and breast cancer (27-29). AML, as the most common hematological malignancy in adult patients, is still an incurable disease and poses a big challenge for public health. A number of studies have shown that ferroptosis-related signatures take part in several important processes in solid cancer, but no study has revealed the underlying mechanism and role of FRGs in AML. We therefore attempted to investigate their expression levels, prognostic role, influence on the TME, and the effect of drug resistance in AML.

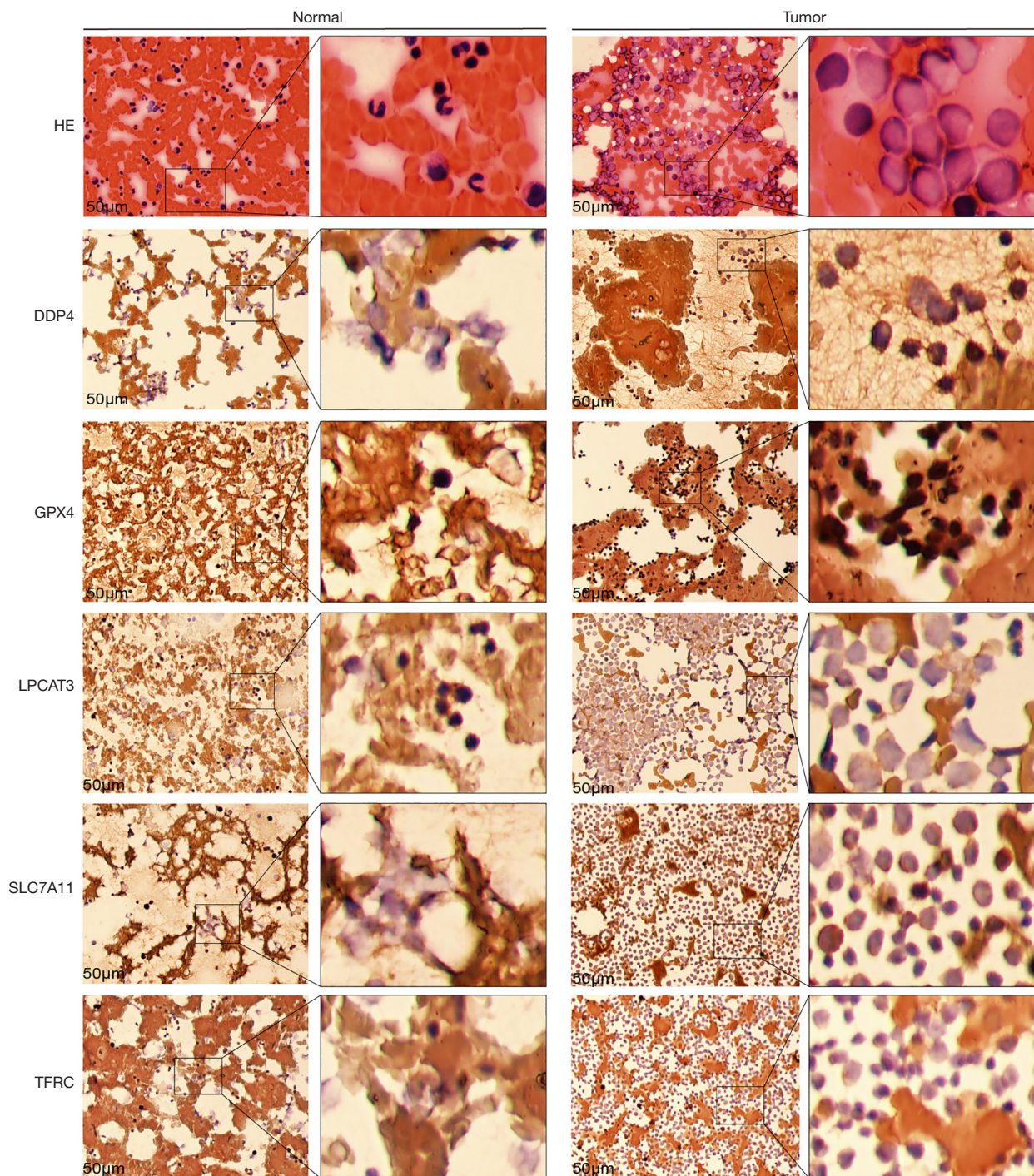
The transcriptome data of AML patients was downloaded from TCGA and the transcriptome data of normal samples was obtained from the GTEx database, and FRGs were selected via public articles. We analyzed the expression levels of FRGs between AML and normal samples. A prognostic model based on FRGs was constructed via lasso regression. Among the genes, SLC7A11, GPX4, TFRC, LPCAT3, and DPP4 were further investigated in terms of their expression levels and prognostic role in AML. We performed validation experiments to verify the final selected gene expression levels via immunohistochemistry, PCR, and RNA-seq. Finally, we explored whether there was an association between immune infiltration and drug sensitivity, and finally selected FRGs.

Recently, more and more studies have revealed the

significant role of ferroptosis in cancer. Apart from being a unique form of cell death, ferroptosis has been shown to play important roles in cancer stem cells and the TME (30-32). As the most important regulators in the ferroptosis process, FRGs have been confirmed to play critical roles in the prognosis and resistance of glioma (33,34). In our study, DPP4 was overexpressed, while LPCAT3, TFRC, GPX4, and SLC7A11 were downregulated in AML samples compared to normal samples. Interestingly, several gene expression levels were inconsistent in the public dataset analysis. DPP4, GPX4, LPCAT3, SLC7A11, and TFRC all had higher expression in AML bone marrow samples. TFRC was significantly overexpressed, but DPP4, GPX4, LPCAT3, and SLC7A11 were significantly downregulated in AML samples via PCR analysis. RNA-seq results showed that TFRC was significantly overexpressed while DPP4 was significantly downregulated in AML samples. The prognostic model showed that SLC7A11, GPX4, TFRC, LPCAT3, and DPP4 significantly influenced the prognosis of AML patients. DPP4, LPCAT3, GPX4, and SLC7A11 may act as adverse biomarkers, while controversially, TFRC may act as a protective factor for AML patients. DPP4 acts as an adverse signature for breast, prostate, and pancreatic cancer, and inhibition of DPP4 can improve the prognosis of these patients (35). Zhang *et al.*'s study indicated that overexpression of glutathione peroxidase 4 (GPX4) could enhance cisplatin resistance *in vitro* (36). Guerriero *et al.* revealed that GPX4 was significantly overexpressed in human hepatocellular carcinoma, further indicating that



**Figure 8** Correlation between the FRG signature biomarker and the abundance of resting mast cells and M2 macrophages. (A) DPP4, (B) GPX4, (C) LPCAT3, (D) SLC7A11, and (E) TFRC. FRG, ferroptosis-related gene.

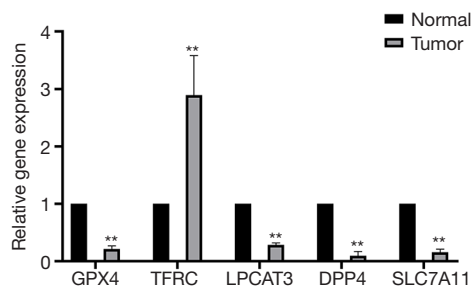


**Figure 9** Immunohistochemistry findings of DPP4, GPX4, LPCAT3, SLC7A11, and TFRC expression.

expression levels may be impacted by cancer status (37). Ma *et al.* revealed that SLC7A11 was overexpressed in laryngeal squamous cell carcinoma, and the upregulation of

SLC7A11 promoted tumor progression (38). From these findings, we can conclude that DPP4, LPCAT3, GPX4, and SLC7A11 have essential biological functions in multiple





**Figure 10** The PCR results of DPP4, GPX4, LPCAT3, SLC7A11, and TFRC expression. \*\*,  $P < 0.01$ . PCR, polymerase chain reaction.

cancer types, and most of them act as tumor promoters. In regards to AML, only GPX4 has been investigated in terms of its expression and prognostic role. Wei *et al.* showed that GPX4 was significantly downregulated in AML patient samples, and overexpression of GPX4 indicated a better outcome (39). In regards to TFRC, Huang *et al.* revealed that TFRC accelerated the progression of epithelial ovarian cancer via upregulating AXIN2 expression (40). In another study, TFRC also acted as a promoter of liver cancer cells, and inhibition of TFRC could suppress cancer cell growth and survival (41). From these findings, TFRC may be an oncogene for liver cancer and epithelial ovarian cancer, which is inconsistent with its prognostic role in AML patients. There has been no study that has explored the role of TFRC in AML.

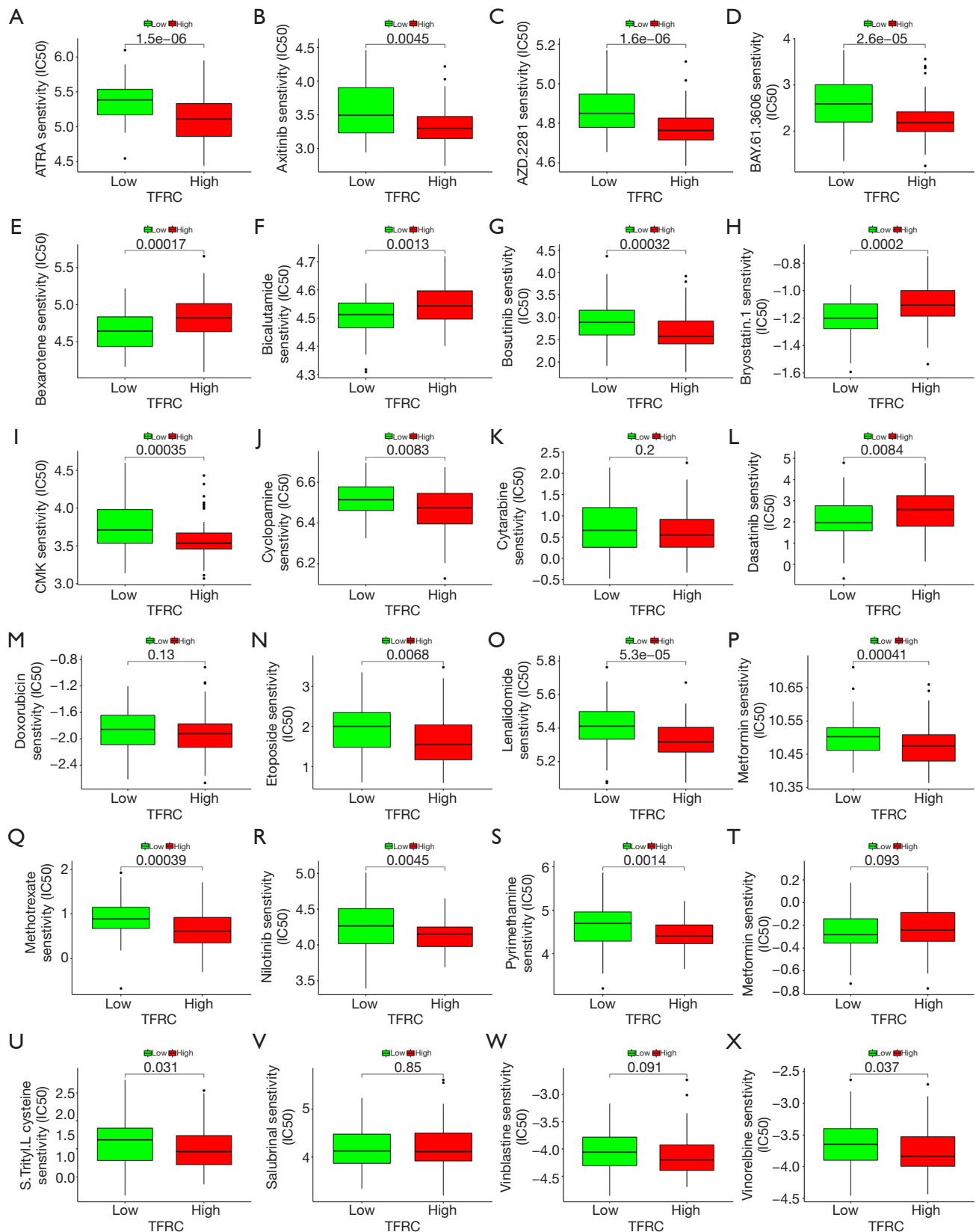
Based on the fundamental function of ferroptosis in immune responses, we also performed an analysis of the relationship between final selected FRGs and immune cell infiltration. Based on the validation experiment results, we finally selected DPP4 and TFRC for this analysis. The results showed that TFRC and DPP4 were negatively correlated with resting mast cells but positively correlated with M2 macrophages. The TME is one of the critical regulators of immunotherapy, chemotherapy response, and tumor progression (42-44). Research on the TME in solid tumors has been prosperous, but the underlying mechanisms of the TME in therapy response, prognosis, and tumor progression are still unclear. Based on the complexity of the microenvironment of AML, only a few studies have preliminarily investigated the TME of AML (45-48). Carter

*et al.* revealed that the TME can significantly influence the drug sensitivity of AML (45). Furthermore, our results showed that resting mast cell infiltration resulted in a better OS, but high infiltration of M2 macrophages resulted in a poor prognosis for AML patients. Lan *et al.* revealed that M2 macrophage-derived exosomes promoted the invasion and migration ability of colon cancer cells (49). M2 macrophages also served as promoters of multiple cancer types such as breast, gastric, and bladder cancer (50,51). The fundamental biological function of resting mast cells in cancer still remains to be elucidated, but several studies have shown that they may have a strong influence on cancer (52-54). Xu *et al.* indicated that M2 macrophages were enriched in AML, and led to poor outcomes (55). The other type of macrophages, M1 macrophages, may serve as protective factors in AML (56). These results also highlight the important role of the TME in AML, but there is still a long way to go.

The ultimate goal of the present study was to find a reasonable novel or complimentary therapy regimen for AML patients. We analyzed the association between DPP4, TFRC, and drug sensitivity in AML patients. The results showed that patients with downregulation of TFRC lead to resistant to ATRA, AZD.2281, CMK, and metformin, and upregulated TFRC induce resistant to bexarotene, bicalutamide, and dasatinib. According to DPP4, patients with high-risk scores were resistant to CMK and cytarabine, and among others, but sensitive to dasatinib. The dysregulated expression of DPP4 can influence the sensitivity to cytarabine, and cytarabine is one of the first-line therapy regimens in AML. Therefore, more reasonable chemotherapy regimens can be selected via this analysis.

## Conclusions

In our study, we found that FRGs can serve as diagnostic and prognostic biomarkers for AML patients. FRGs not only have a strong influence on the TME of AML, but also drug resistance. The findings of this study provide useful information for clinicians to select therapy regimens based on FRG expression levels, and pave the way for future fundamental research to understand the underlying mechanisms of ferroptosis in AML.



**Figure 11** Relationship between risk score and drug sensitivity via the R package pRRophetic (TFRC).



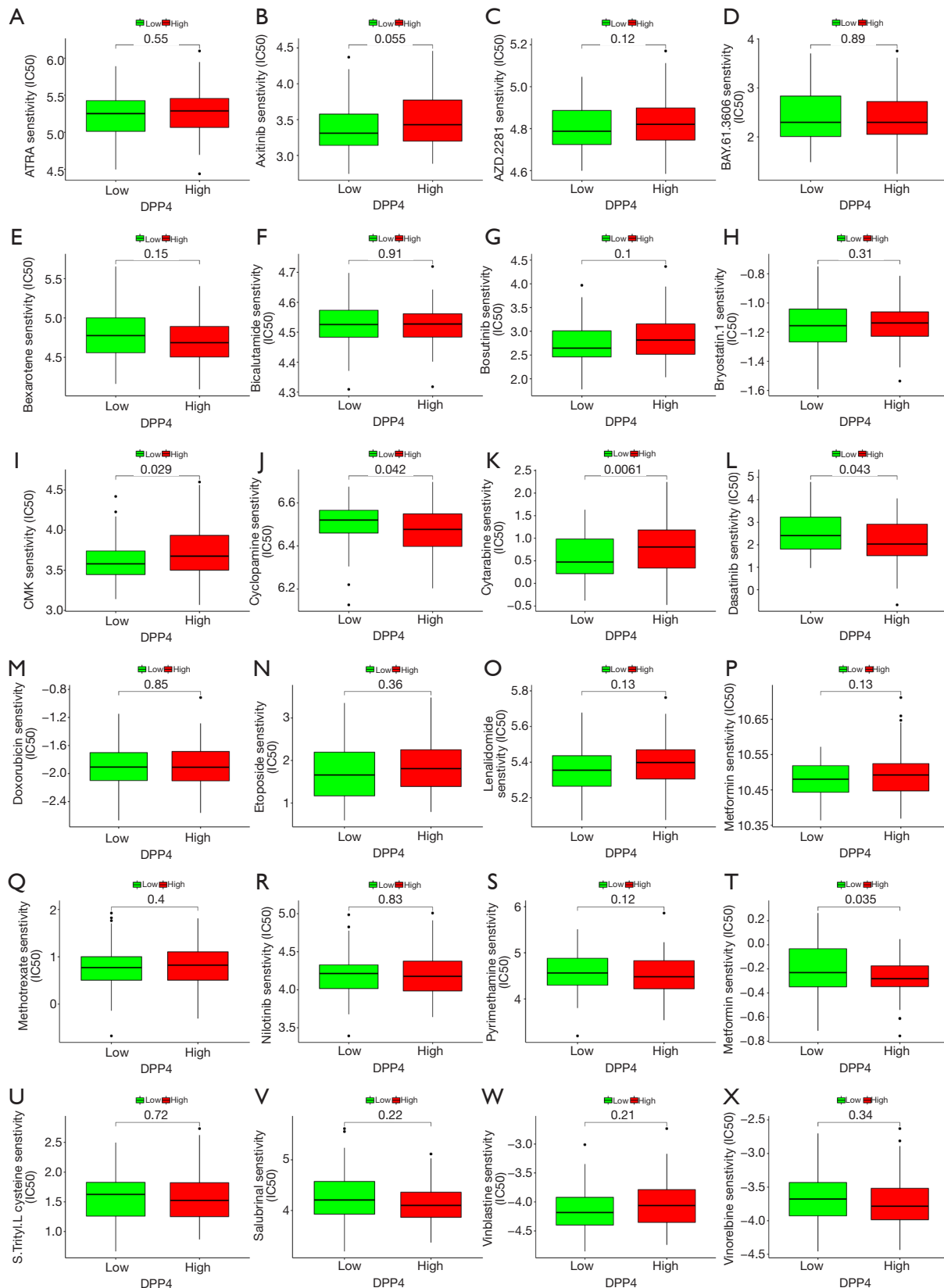


Figure 12 Relationship between risk score and drug sensitivity via the R package pRRophetic (DPP4).

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## Footnote

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