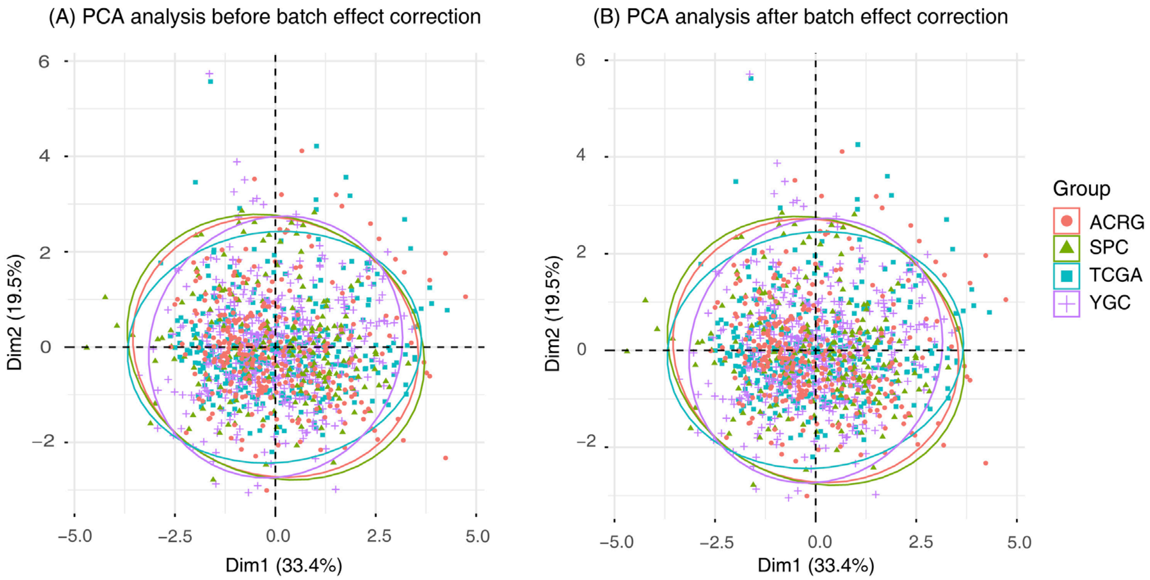


Figure S1 PCA analysis before and after batch effect correction. PCA showing sample distribution before and after batch effect correction. (A) PCA analysis results before batch effect correction. (B) PCA analysis results after batch effect correction. Different colored points represent four different cohorts. Dim1 and Dim2 indicate the first and second principal components, respectively. PCA, Principal component analysis; TCGA, The Cancer Genome Atlas; ACRG, Asian Cancer Research Group; SPC, Singapore Patient Cohort; YGC, Yonsei Gastric Cancer Cohort.

https://dx.doi.org/10.21037/tcr-2025-aw-2368

Table S1 Demographic and clinical characteristics of newly diagnosed gastric cancer patients in different datasets

Characteristics	Discovery	External Validation			Overall
	Training	Testing 1	Testing 2	Testing 3	
	TCGA	ACRG	SPC	YGC	
Area	America	Asia	Singapore	South Korea	-
Number of samples	383	300	191	431	1,305
Age at diagnosis (years)	65.0±10.4	61.9±11.4	64.3±13.2	60.0±11.6	62.6±11.6
Survival status					
Alive	226 (59.0)	148 (49.3)	96(50.3)	224 (52.0)	694 (53.2)
Dead	157 (41.0)	152 (50.7)	95 (49.7)	207 (48.0)	611 (46.8)
Unknown	0	0	0	0	0
Gender					
Male	250 (65.3)	199 (66.3)	124 (64.9)	294 (68.2)	867(66.4)
Female	133 (34.7)	101 (33.7)	667(35.1)	137 (31.8)	438(33.6)
Unknown	0	0	0	0	0
AJCC Stage					
I	53 (13.8)	30 (10.0)	31 (16.2)	19 (4.4)	133 (10.2)
II	125 (32.6)	97 (32.3)	29 (15.2)	108 (25.1)	359 (27.5)
III	168 (43.9)	96 (32.0)	72 (37.7)	288 (66.8)	624 (47.8)
IV	37 (9.7)	77 (25.7)	59 (0.9)	16 (3.7)	189 (14.5)
Unknown	0	0	0	0	0
T Stage					
I	18 (4.7)	0 (0)	-	11 (2.6)	29 (2.2)
II	82 (21.4)	188 (62.7)	-	38 (8.8)	308 (23.6)
III	171 (44.6)	91 (30.3)	-	92 (21.3)	354 (27.1)
IV	108 (28.2)	21 (7)	-	290 (67.3)	419 (32.1)
Unknown	4	0	182	0	186
N Stage					
0	116 (31.1)	38 (12.7)	-	80 (18.6)	234 (17.9)
1	103 (28.0)	131 (43.7)	-	187 (43.4)	421 (32.3)
2	74 (19.6)	80 (26.7)	-	132 (30.6)	286 (21.9)
3	79 (21.3)	51 (17.0)	-	32 (7.4)	162 (12.4)
Unknown	11	0	182	0	193
M Stage					
0	344 (93.8)	273 (91.0)	-	-	617 (47.3)
1	23 (6.2)	27 (9.0)	-	-	50 (3.8)
Unknown	16	0	182	431	629

TCGA, The Cancer Genome Atlas; ACRG, Asian Cancer Research Group; SPC, Singapore Patient Cohort; YGC, Yonsei Gastric Cancer Cohort; AJCC, American Joint Committee on Cancer; T Stage, Tumor Stage; N Stage, Node Stage; M Stage, Metastasis Stage. Continuous variables (e.g., Age) are presented as mean ± standard deviation (SD). Categorical variables are presented as number (percentage, %). Internal validation: bootstrap resampling (n = 1000) on TCGA data.

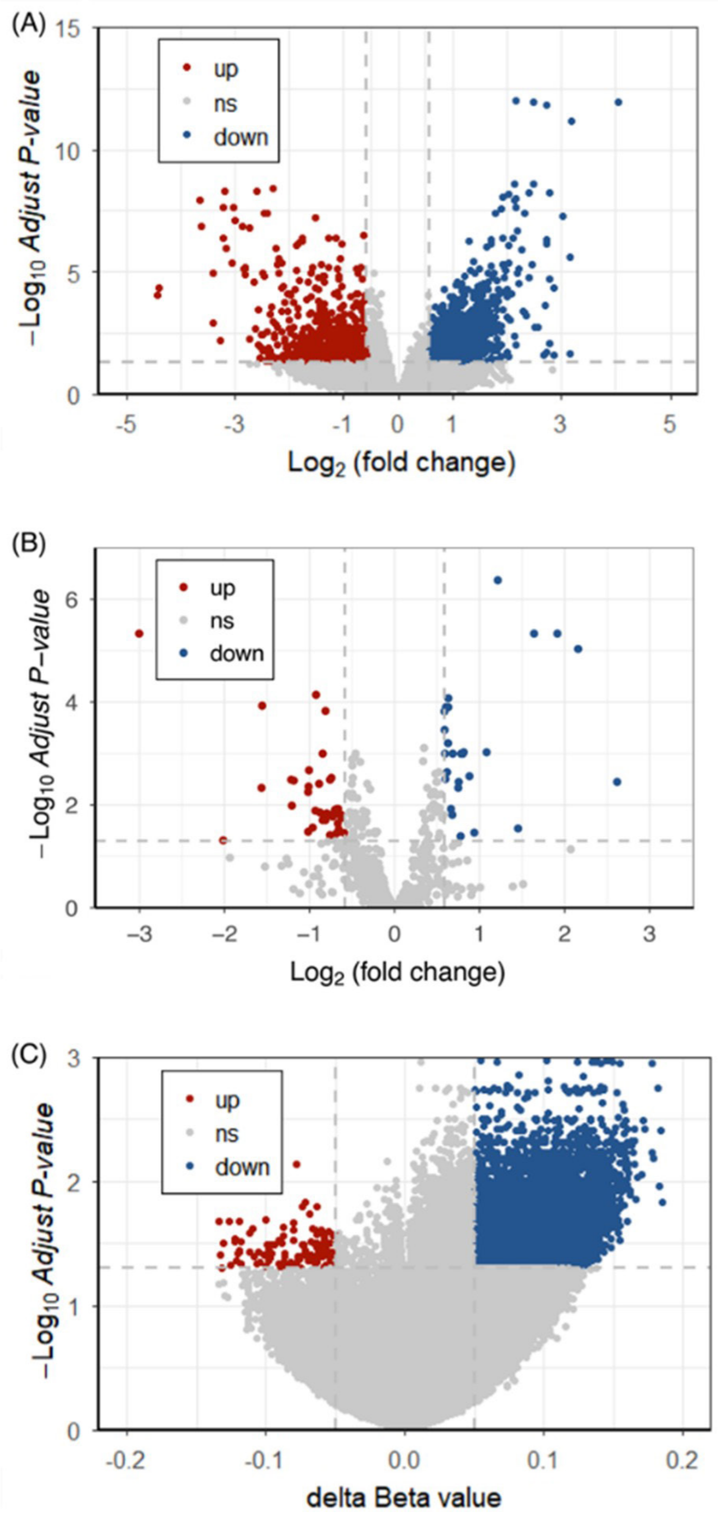


Figure S2 Volcano plots of differential analysis for gene expression, miRNA expression, and DNA methylation in early- vs advanced-stage gastric cancer patients. Volcano plot for results of (A)differential expression analysis, (B)differential miRNA analysis and (C)differential methylation analysis between early-(I, ref) and advanced-stage(III, IV).Red dots represent significantly up-regulated sites and Blue dots represent significantly down-regulated sites.

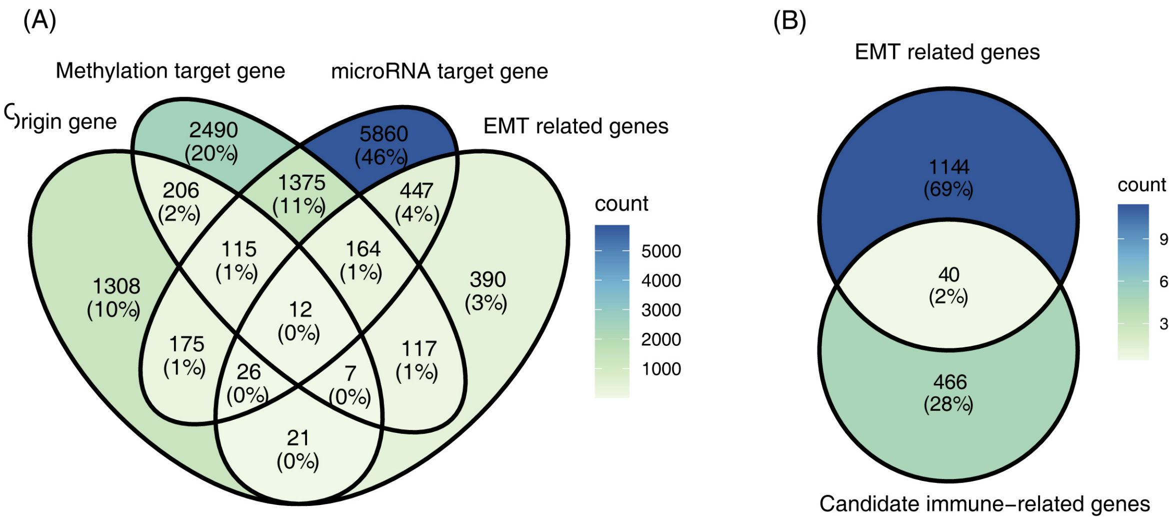


Figure S3 The overlap genes between four differentially expressed omic sets. The differential miRNAs and CpG sites are mapped to corresponding genes using the miRTarBase and ChAMP databases. (A) Venn diagram illustrating the overlap among four gene sets. Each set and the intersecting areas are color-coded using the GnBu palette, with numbers in the intersections indicating the count of unique genes in that region. A total of 127 genes were identified as being associated with the progression of gastric cancer across all three omics layers. (B) Venn diagram showing the overlap between candidate immune-related genes and EMT-related genes. EMT, Epithelial–mesenchymal transition.

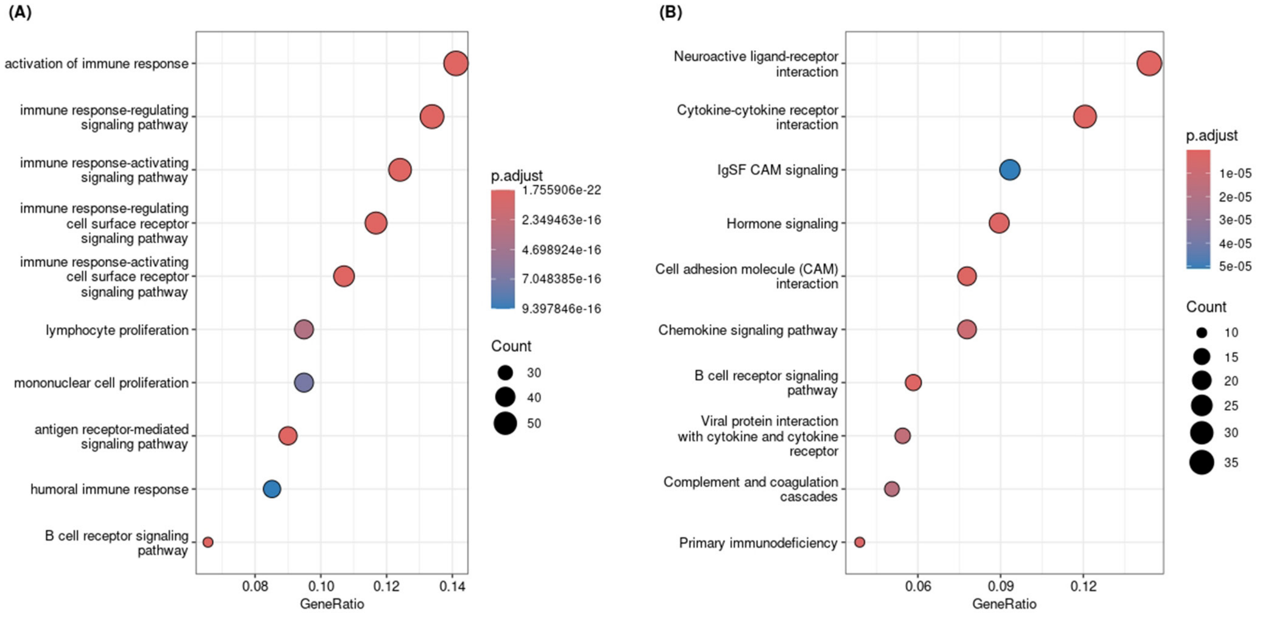


Figure S4 Functional and pathway enrichment analysis of 506 candidate immune-related genes. Dot plots of the enrichment analysis for candidate immune-related genes. (A) Results of GO biological process enrichment analysis. (B) Results of KEGG pathway enrichment analysis. In each plot, the color of the dots represents the adjusted P value (p.adjust), with a color gradient (from red to blue) indicating increasing statistical significance; the size of the dots represents the number of genes (Count) enriched in that term. The X-axis is the gene ratio (GeneRatio). Only the top significant enrichment terms are displayed. GO, Gene oncology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Table S2 Association results of 35 genes with significant main effects derived from Cox regression models adjusted for covariates

Index	Gene	Discovery phase		Validation phase	
		HR (95% CI)	P	HR (95% CI)	P
1	*FLRT2*	1.3776 (1.1865-1.5994)	2.61E-05	1.3361 (1.1289-1.5813)	7.50E-04
2	*KCNQ1*	0.7629 (0.6510-0.8942)	8.36E-04	0.7786 (0.6639-0.9133)	2.10E-03
3	*ANGPTL1*	1.3278 (1.1395-1.5473)	2.80E-04	1.3167 (1.1602-1.4943)	2.02E-05
4	*BOC*	1.3271 (1.1333-1.5540)	4.42E-04	1.3548 (1.1588-1.5839)	1.39E-04
5	*C1QTNF7*	1.2637 (1.0829-1.4747)	2.97E-03	1.2799 (1.1044-1.4833)	1.04E-03
6	*CALD1*	1.3196 (1.1296-1.5415)	4.72E-04	1.2288 (1.0666-1.4157)	4.34E-03
7	*COL14A1*	1.2781 (1.0917-1.4963)	2.28E-03	1.2863 (1.1153-1.4836)	5.43E-04
8	*EBF2*	1.2741 (1.1226-1.4461)	1.77E-04	1.2542 (1.0858-1.4488)	2.08E-03
9	*ECM2*	1.3743 (1.1697-1.6147)	1.11E-04	1.3369 (1.1350-1.5747)	5.10E-04
10	*ELN*	1.2503 (1.0601-1.4747)	7.98E-03	1.2440 (1.0672-1.4501)	5.26E-03
11	*FBLN5*	1.3076 (1.1061-1.5458)	1.68E-03	1.3399 (1.1366-1.5796)	4.92E-04
12	*FERMT2*	1.3803 (1.1754-1.6210)	8.44E-05	1.5160 (1.3058-1.7601)	4.69E-08
13	*FGF7*	1.2598 (1.0781-1.4722)	3.66E-03	1.3301 (1.1302-1.5654)	5.96E-04
14	*FHL1*	1.2747 (1.0859-1.4963)	3.01E-03	1.2925 (1.1194-1.4924)	4.69E-04
15	*FRZB*	1.2527 (1.0626-1.4769)	7.31E-03	1.3547 (1.1467-1.6005)	3.57E-04
16	*GEM*	1.3174 (1.1138-1.5582)	1.29E-03	1.2910 (1.0874-1.5327)	3.54E-03
17	*GHR*	1.3893 (1.2005-1.6078)	1.02E-05	1.4118 (1.2162-1.6389)	5.85E-06
18	*KCNMA1*	1.2921 (1.1094-1.5050)	9.88E-04	1.2503 (1.0969-1.4251)	8.24E-04
19	*LAMA2*	1.2883 (1.0960-1.5142)	2.13E-03	1.4434 (1.2062-1.7272)	6.17E-05
20	*MEOX2*	1.3900 (1.1874-1.6271)	4.17E-05	1.2744 (1.1057-1.4689)	8.18E-04
21	*NEGR1*	1.3405 (1.1453-1.5689)	2.63E-04	1.3812 (1.1786-1.6186)	6.57E-05
22	*NFASC*	1.2588 (1.0788-1.4687)	3.46E-03	1.2179 (1.0527-1.4090)	8.05E-03
23	*NPTX1*	1.3013 (1.1283-1.5009)	2.96E-04	1.2436 (1.0983-1.4080)	5.83E-04
24	*NRXN2*	1.2510 (1.0736-1.4577)	4.10E-03	1.4008 (1.2223-1.6054)	1.26E-06
25	*NRXN3*	1.2588 (1.0735-1.4762)	4.62E-03	1.2474 (1.0907-1.4266)	1.25E-03
26	*OMD*	1.3587 (1.1585-1.5935)	1.64E-04	1.4261 (1.2296-1.6539)	2.69E-06
27	*PGM5*	1.2545 (1.0726-1.4673)	4.56E-03	1.2897 (1.1122-1.4955)	7.59E-04
28	*PTGER3*	1.3202 (1.1241-1.5505)	7.09E-04	1.3280 (1.1357-1.5530)	3.80E-04
29	*SFRP2*	1.2658 (1.0667-1.5019)	6.93E-03	1.2409 (1.0509-1.4651)	1.09E-02
30	*SLIT2*	1.3375 (1.1407-1.5682)	3.41E-04	1.3740 (1.1877-1.5895)	1.92E-05
31	*SPARCL1*	1.3499 (1.1474-1.5881)	2.96E-04	1.4010 (1.1838-1.6581)	8.73E-05
32	*SRPX*	1.2991 (1.1096-1.5210)	1.14E-03	1.3948 (1.1902-1.6346)	3.95E-05
33	*SSPN*	1.3389 (1.1370-1.5766)	4.65E-04	1.3847 (1.1839-1.6196)	4.68E-05
34	*TGFB3*	1.2805 (1.0911-1.5027)	2.46E-03	1.2717 (1.0941-1.4782)	1.74E-03
35	*TPM2*	1.2495 (1.0656-1.4652)	6.10E-03	1.3867 (1.1846-1.6231)	4.73E-05

HR, hazard ratio; 95% CI, 95% confidence interval. HR, 95% CI, and P value were derived from the Cox proportional hazards model adjusted for age, gender and American Joint Committee on Cancer (AJCC) pathological stage.

https://dx.doi.org/10.21037/tcr-2025-aw-2368

Table S3 Association results of 55 pairs of genes with significant gene-gene interactions derived from Cox regression models adjusted for covariates

Index	Gene 1	Gene 2	Discovery phase		Validation phase	
			HR (95% CI)	P	HR (95% CI)	P
1	*NPY*	*ITGBL1*	1.2645 (1.0975-1.4569)	1.16E-03	1.2303 (1.0595-1.4287)	6.57E-03
2	*ATP4B*	*CD84*	0.7988 (0.6729-0.9482)	1.02E-02	0.7871 (0.6682-0.9271)	4.15E-03
3	*A2M*	*ARC*	1.5303 (1.2684-1.8463)	8.90E-06	1.2672 (1.0131-1.5851)	3.81E-02
4	*ATP4B*	*LCK*	0.6716 (0.5513-0.8183)	7.79E-05	0.7766 (0.6475-0.9313)	6.39E-03
5	*ATP4B*	*UMODL1*	1.3852 (1.1583-1.6564)	3.56E-04	1.4094 (1.0896-1.8230)	8.95E-03
6	*ATP4B*	*IL10RA*	0.7411 (0.6247-0.8792)	5.87E-04	0.8190 (0.6893-0.9731)	2.32E-02
7	*ATP4B*	*CD28*	0.7316 (0.6102-0.8771)	7.31E-04	0.8020 (0.6724-0.9566)	1.41E-02
8	*ATP4B*	*FYB1*	0.7691 (0.6578-0.8992)	9.94E-04	0.8218 (0.6900-0.9788)	2.78E-02
9	*ATP4B*	*VAV1*	0.7594 (0.6449-0.8943)	9.67E-04	0.8191 (0.6820-0.9837)	3.26E-02
10	*ATP4B*	*APOBEC3G*	0.7469 (0.6253-0.8921)	1.28E-03	0.7650 (0.6299-0.9291)	6.89E-03
11	*ATP4B*	*APLN*	1.3201 (1.1024-1.5806)	2.52E-03	1.1873 (1.0367-1.3598)	1.31E-02
12	*ATP4B*	*CD72*	0.7545 (0.6266-0.9085)	2.96E-03	0.8174 (0.6840-0.9769)	2.66E-02
13	*ATP4B*	*CCR5*	0.7552 (0.6272-0.9093)	3.04E-03	0.7996 (0.6697-0.9546)	1.34E-02
14	*ATP4B*	*IL21R*	0.7369 (0.6015-0.9027)	3.20E-03	0.7291 (0.5812-0.9147)	6.32E-03
15	*ATP4B*	*CD8A*	0.7569 (0.6285-0.9114)	3.30E-03	0.7357 (0.6045-0.8954)	2.20E-03
16	*ATP4B*	*SIGLEC10*	0.7612 (0.6344-0.9133)	3.33E-03	0.8421 (0.7171-0.9889)	3.61E-02
17	*ATP4B*	*ITGAL*	0.7686 (0.6442-0.9169)	3.46E-03	0.7692 (0.6364-0.9297)	6.65E-03
18	*ATP4B*	*CD96*	0.7426 (0.6082-0.9067)	3.48E-03	0.7542 (0.6141-0.9264)	7.17E-03
19	*ATP4B*	*FCGR2B*	0.7569 (0.6239-0.9182)	4.72E-03	0.7180 (0.5372-0.9598)	2.53E-02
20	*ATP4B*	*CCL5*	0.7638 (0.6340-0.9203)	4.60E-03	0.7009 (0.5688-0.8637)	8.50E-04
21	*ATP4B*	*TIGIT*	0.7616 (0.6304-0.9201)	4.76E-03	0.8186 (0.6775-0.9891)	3.81E-02
22	*ATP4B*	*IGHD*	0.7450 (0.6021-0.9218)	6.73E-03	0.8289 (0.6920-0.9928)	4.16E-02
23	*ATP4B*	*THEMIS*	0.7564 (0.6179-0.9259)	6.80E-03	0.8090 (0.6694-0.9777)	2.83E-02
24	*ATP4B*	*TRAV20*	0.7081 (0.5501-0.9114)	7.35E-03	0.7776 (0.6370-0.9492)	1.34E-02
25	*ATP4B*	*CR2*	0.7457 (0.5988-0.9285)	8.71E-03	0.8228 (0.6778-0.9990)	4.88E-02
26	*ATP4B*	*SIGLEC8*	0.7616 (0.6213-0.9336)	8.75E-03	0.8258 (0.6876-0.9917)	4.04E-02
27	*ATP4B*	*ZAP70*	0.7468 (0.5999-0.9296)	8.97E-03	0.8366 (0.7120-0.9830)	3.01E-02
28	*ATP4B*	*SH2D1A*	0.7731 (0.6371-0.9382)	9.15E-03	0.8004 (0.6719-0.9534)	1.26E-02
29	*ATP4B*	*CRTAM*	0.7607 (0.6192-0.9345)	9.17E-03	0.7601 (0.6322-0.9139)	3.52E-03
30	*ATP4B*	*TDGF1P3*	1.1854 (1.0430-1.3472)	9.18E-03	1.2708 (1.0659-1.5150)	7.54E-03
31	*ATP4B*	*CCR2*	0.7786 (0.6434-0.9423)	1.01E-02	0.8234 (0.7051-0.9617)	1.42E-02
32	*AVPR1A*	*ARC*	1.2995 (1.1377-1.4843)	1.12E-04	1.1818 (1.0339-1.3508)	1.43E-02
33	*AVPR1A*	*NLGN3*	1.2303 (1.1013-1.3744)	2.45E-04	1.2275 (1.0525-1.4316)	9.02E-03

Table S3 (*continued*)

Table S3 (*continued*)

Index	Gene 1	Gene 2	Discovery phase		Validation phase	
			HR (95% CI)	P	HR (95% CI)	P
34	*C1S*	*NLGN3*	1.4463 (1.2345-1.6945)	4.92E-06	1.2050 (1.0392-1.3971)	1.35E-02
35	*C1S*	*CSRP1*	1.4420 (1.2010-1.7314)	8.77E-05	1.2078 (1.0139-1.4388)	3.44E-02
36	*C1S*	*IL17RD*	1.3799 (1.1691-1.6287)	1.41E-04	1.2152 (1.0108-1.4609)	3.81E-02
37	*CD79B*	*PROX1*	1.4377 (1.1892-1.7381)	1.77E-04	1.2254 (1.0214-1.4702)	2.87E-02
38	*COL14A1*	*ARC*	1.4303 (1.2155-1.6830)	1.63E-05	1.2943 (1.0660-1.5715)	9.18E-03
39	*FGB*	*PTPRC*	0.7789 (0.6659-0.9110)	1.77E-03	0.7644 (0.6105-0.9572)	1.92E-02
40	*GAST*	*CD84*	0.7400 (0.6142-0.8916)	1.54E-03	0.7833 (0.6295-0.9746)	2.85E-02
41	*GAST*	*TNFSF13B*	0.7530 (0.6224-0.9110)	3.51E-03	0.7571 (0.6084-0.9423)	1.27E-02
42	*ITGA8*	*ARC*	1.2634 (1.1180-1.4277)	1.79E-04	1.1580 (1.0117-1.3254)	3.33E-02
43	*NLGN3*	*COLEC12*	1.2758 (1.1287-1.4421)	9.79E-05	1.2254 (1.0671-1.4073)	3.98E-03
44	*NPY*	*COL20A1*	1.4967 (1.2627-1.7739)	3.31E-06	1.2086 (1.0354-1.4109)	1.64E-02
45	*NPY*	*SFRP2*	1.5486 (1.2595-1.9039)	3.34E-05	1.1937 (1.0147-1.4042)	3.27E-02
46	*NPY*	*GREM1*	1.4397 (1.1917-1.7392)	1.58E-04	1.2725 (1.0390-1.5584)	1.98E-02
47	*NPY*	*LEFTY2*	1.1700 (1.0738-1.2747)	3.35E-04	1.2363 (1.0780-1.4178)	2.40E-03
48	*NPY*	*COL14A1*	1.3164 (1.1341-1.5280)	3.00E-04	1.1825 (1.0101-1.3844)	3.70E-02
49	*NPY*	*GREM2*	1.2900 (1.1224-1.4825)	3.34E-04	1.1544 (1.0173-1.3100)	2.60E-02
50	*NPY*	*NR2F1*	1.2845 (1.1039-1.4946)	1.20E-03	1.2230 (1.0484-1.4267)	1.04E-02
51	*NPY*	*FBLN5*	1.2950 (1.1073-1.5145)	1.21E-03	1.2363 (1.0456-1.4616)	1.31E-02
52	*NPY*	*PTGIS*	1.2932 (1.0987-1.5222)	1.99E-03	1.3005 (1.0758-1.5720)	6.62E-03
53	*NPY*	*NRXN2*	1.2179 (1.0728-1.3826)	2.32E-03	1.1715 (1.0089-1.3603)	3.78E-02
54	*NTN1*	*COL20A1*	1.5883 (1.3080-1.9286)	3.00E-06	1.1598 (1.0174-1.3222)	2.66E-02
55	*PRKAR2B*	*NLGN3*	1.3056 (1.1419-1.4929)	9.60E-05	1.2489 (1.0683-1.4600)	5.30E-03

HR, hazard ratio; 95% CI, 95% confidence interval. HR, 95% CI, and P value were derived from the Cox proportional hazards model adjusted for age, gender and American Joint Committee on Cancer (AJCC) pathological stage.

Table S4 Association results of genes with main effects or gene-gene interactions derived from a multivariable Cox regression model in the TCGA training

Gene	β (95% CI)	z score	P
Age	0.0390 (0.0216, 0.0563)	4.4032	1.07E-05
Stage			
I	ref		
II	0.3495 (-0.2919, 0.9909)	1.0680	2.86E-01
III	0.8790 (0.2782, 1.4798)	2.8676	4.00E-03
IV	1.6134 (0.9121, 2.3148)	4.5089	6.52E-06
Main effect			
FLRT2	0.3635 (0.1326, 0.5944)	3.0857	2.00E-03
KCNQ1	-0.2264 (-0.3944, -0.0584)	-2.6411	8.00E-03
G×G interaction			
NPY	0.0135 (-0.1607, 0.1876)	0.1514	8.80E-01
ITGBL1	-0.0584 (-0.2833, 0.1666)	-0.5086	6.11E-01
*NPY*ITGBL1*	0.2175 (0.0790, 0.3559)	3.0784	2.00E-03
ATP4B	-0.0445 (-0.2332, 0.1442)	-0.4620	6.44E-01
CD84	-0.0890 (-0.2714, 0.0934)	-0.9563	3.39E-01
*ATP4B*CD84*	-0.2645 (-0.4529, -0.0762)	-2.7525	6.00E-03

Abbreviations: TCGA, The Cancer Genome Atlas; 95% CI, 95% confidence interval; G×G interaction, gene-gene interaction.

Table S5 Internal validation of the clinical model in the TCGA cohort

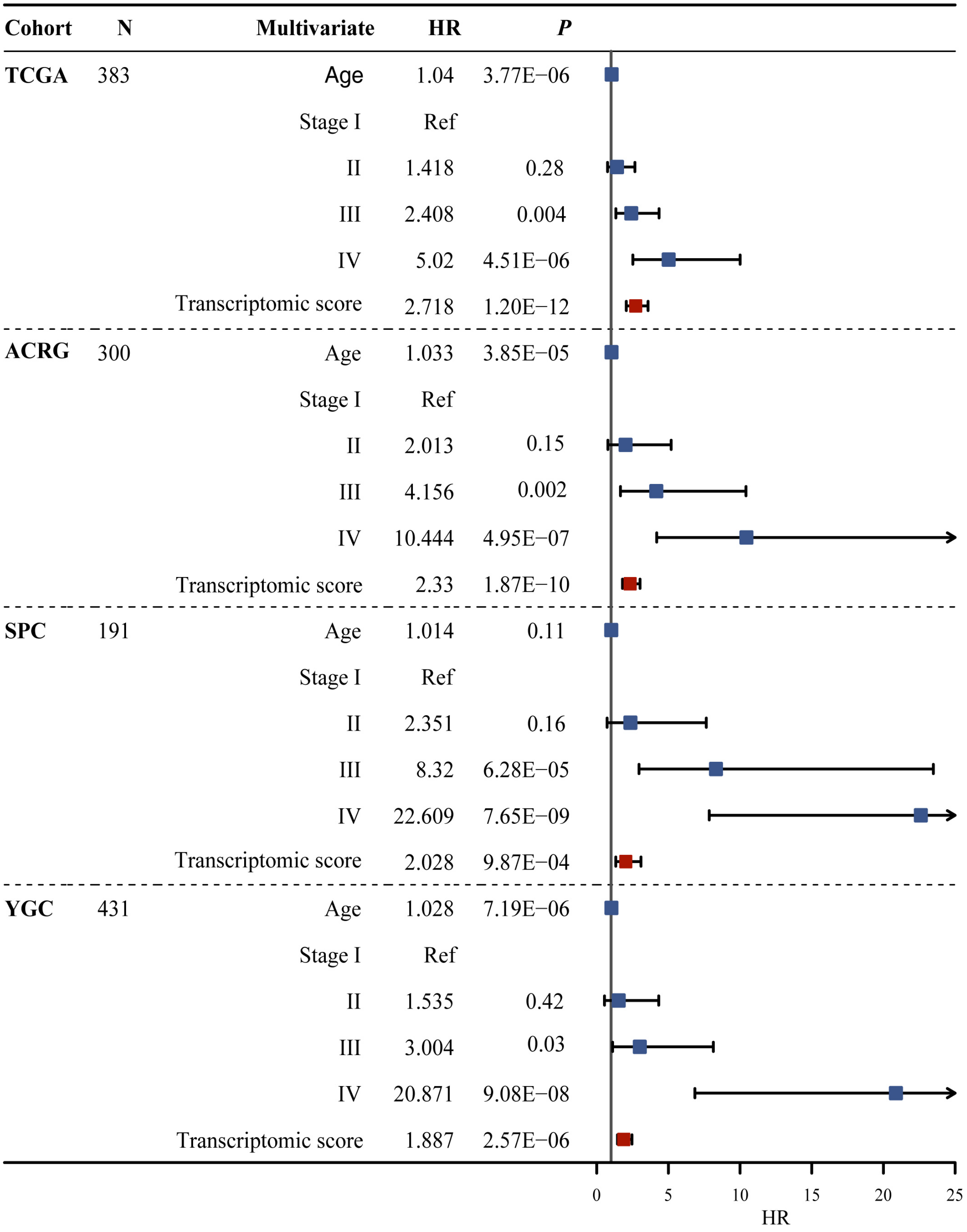
Model	C-index (95% CI)
Clinic Model	0.6394 (0.5910, 0.6878)
Bootstrap (mean)	0.6313 (0.5841, 0.6805)

TCGA, The Cancer Genome Atlas; C-index, concordance index; 95% CI, 95% confidence interval. Clinical Model includes age and stage as predictive factor. The C-index for the Bootstrap (mean) model represents the mean value and its 95% CI calculated from 1000 bootstrap samples, assessing the model's robustness against sampling variability.

Table S6 Internal validation of the ITPG score in the TCGA cohort

Data	C-index (95% CI)
ITPG	0.7034 (0.6603, 0.74652)
Bootstrap (mean)	0.6895 (0.6728, 0.7062)

ITPG, Immune-related Transcriptomic Predictive Model for Gastric Cancer Prognosis; TCGA, The Cancer Genome Atlas; C-index, concordance index; 95% CI, 95% confidence interval. ITPG model includes age, stage, *KCNQ1*, *FLRT2*, and the interaction terms *NPY*×*ITGBL1* and *ATP4B*×*CD84* as predictive factors. The C-index for the Bootstrap (mean) model represents the mean value and its 95% CI for the same ITPG model calculated from 1000 bootstrap samples, assessing the internal stability of the final model.



Cohort	N	Multivariate	HR	*P*
TCGA	383	Age	1.04	3.77E−06
		Stage I	Ref	
		II	1.418	0.28
		III	2.408	0.004
		IV	5.02	4.51E−06
		Transcriptomic score	2.718	1.20E−12
ACRG	300	Age	1.033	3.85E−05
		Stage I	Ref	
		II	2.013	0.15
		III	4.156	0.002
		IV	10.444	4.95E−07
		Transcriptomic score	2.33	1.87E−10
SPC	191	Age	1.014	0.11
		Stage I	Ref	
		II	2.351	0.16
		III	8.32	6.28E−05
		IV	22.609	7.65E−09
		Transcriptomic score	2.028	9.87E−04
YGC	431	Age	1.028	7.19E−06
		Stage I	Ref	
		II	1.535	0.42
		III	3.004	0.03
		IV	20.871	9.08E−08
		Transcriptomic score	1.887	2.57E−06

Figure S5 Multivariate Cox analysis evaluating independently predictive ability of transcriptomic score for overall survival of GC patients. Forest plot showing hazard ratios (HRs) from multivariate Cox proportional hazards models adjusted for age and tumor stage (I-IV). Stage I served as the reference group. The square data markers indicate the point estimates of hazard ratios and the error bars represent 95% CIs. $P < 0.05$ was considered statistically significant. GC, gastric cancer; TCGA, The Cancer Genome Atlas; ACRG, Asian Cancer Research Group; SPC, Singapore Patient Cohort; YGC, Yonsei Gastric Cancer Cohort; HR: hazard ratio; CI, confidence interval.

Cohort	N	Multivariate	HR	*P*
TCGA	383	Age	1.034	2.41E−05
		Stage I	Ref	
		II	1.434	0.26
		III/IV	2.729	7.15E−04
		Transcriptomic score	2.664	2.69E−12
ACRG	300	Age	1.034	5.60E−05
		Stage I	Ref	
		II	2.043	0.14
		III/IV	6.138	7.34E−05
		Transcriptomic score	2.377	1.88E−10
SPC	191	Age	1.011	0.19
		Stage I	Ref	
		II	2.3	0.17
		III/IV	11.375	2.64E−06
		Transcriptomic score	1.978	0.001
YGC	431	Age	1.026	4.32E−05
		Stage I	Ref	
		II	1.539	0.41
		III/IV	3.242	0.02
		Transcriptomic score	1.834	5.99E−06

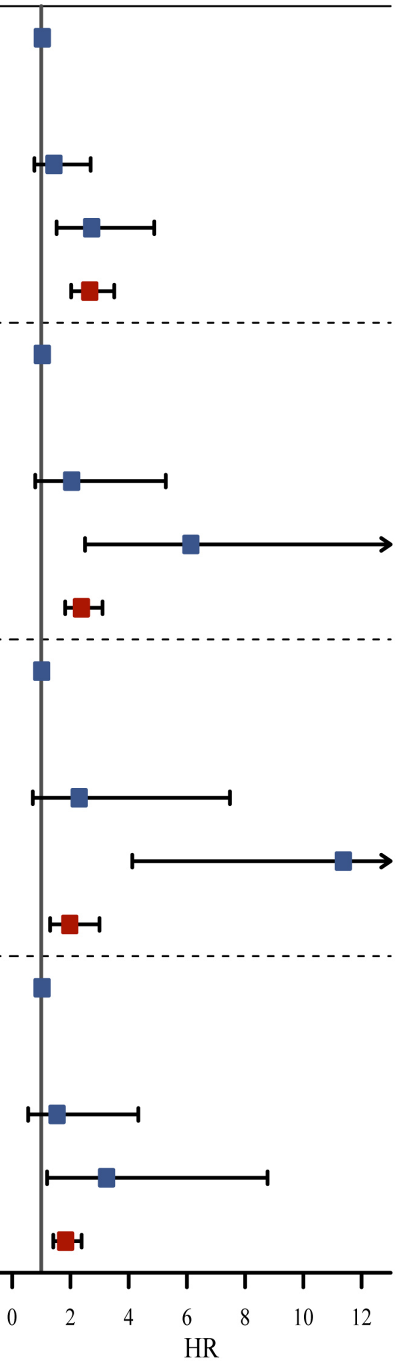


Figure S6 Sensitivity analysis: Multivariate Cox analysis evaluating independently predictive ability of transcriptomic score for overall survival of GC patients. Forest plot displaying hazard ratios (HRs) from multivariate Cox proportional hazards models adjusted for age and tumor stage (I-III/IV). Stage III and IV were combined into a single category. Stage I served as the reference group. The square data markers indicate the point estimates of hazard ratios and the error bars represent 95% CIs. P < 0.05 was considered statistically significant. GC, gastric cancer; TCGA, The Cancer Genome Atlas; ACRG, Asian Cancer Research Group; SPC, Singapore Patient Cohort; YGC, Yonsei Gastric Cancer Cohort; HR: hazard ratio; CI, confidence interval.

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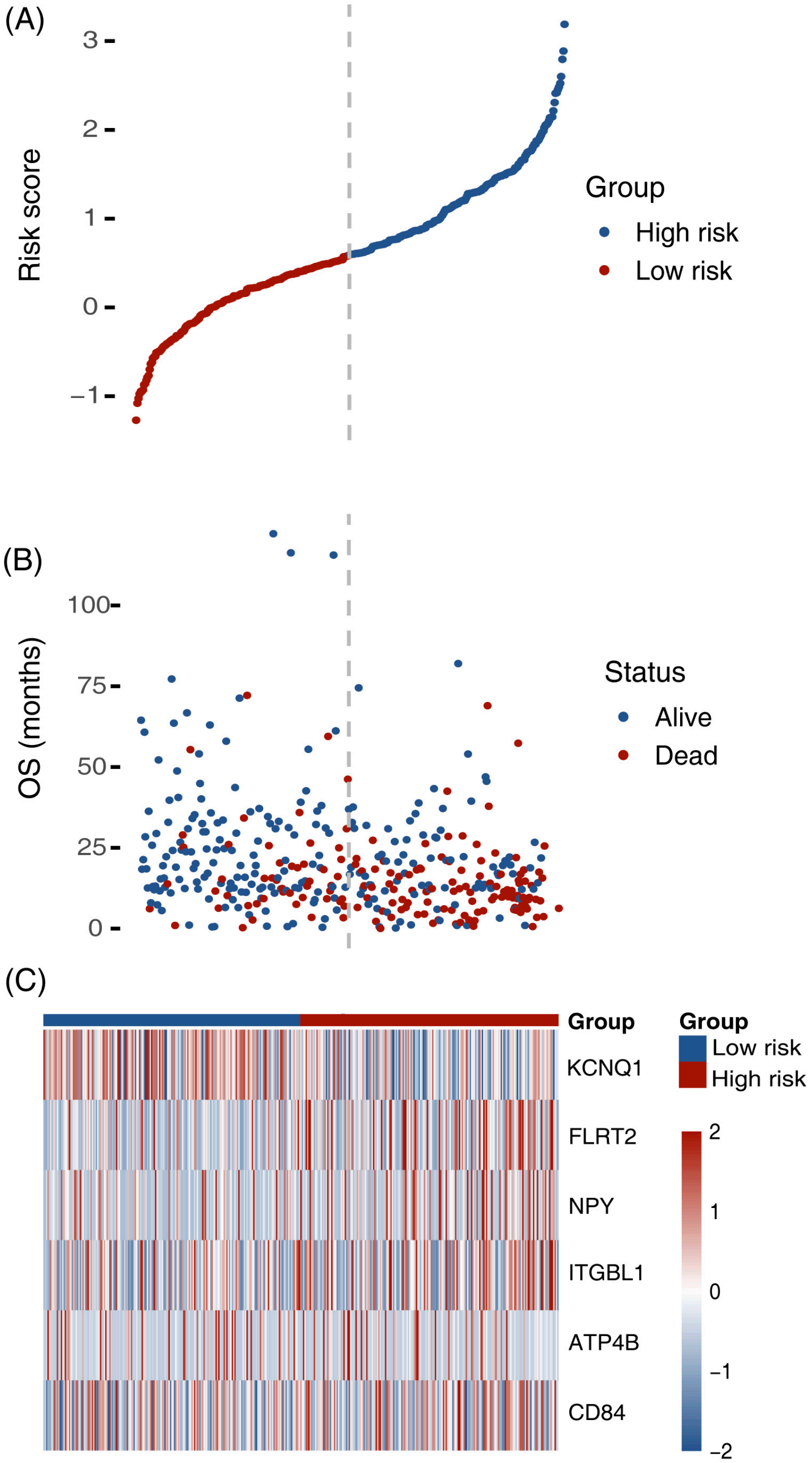

Figure S7 The distribution of risk scores, overall survival and the expression of transcriptomic predictors in the TCGA cohort. The distribution of (A) risk scores and (B) OS status between the high-risk and low-risk groups, as defined by the median ITPG score in the TCGA cohort. (C) Heatmap plot for the expression levels of six genes serving as predictors between the high-risk and low-risk groups. TCGA, The Cancer Genome Atlas; OS, overall survival; ITPG, Immune-related Transcriptomic Predictive Model for Gastric Cancer Prognosis.

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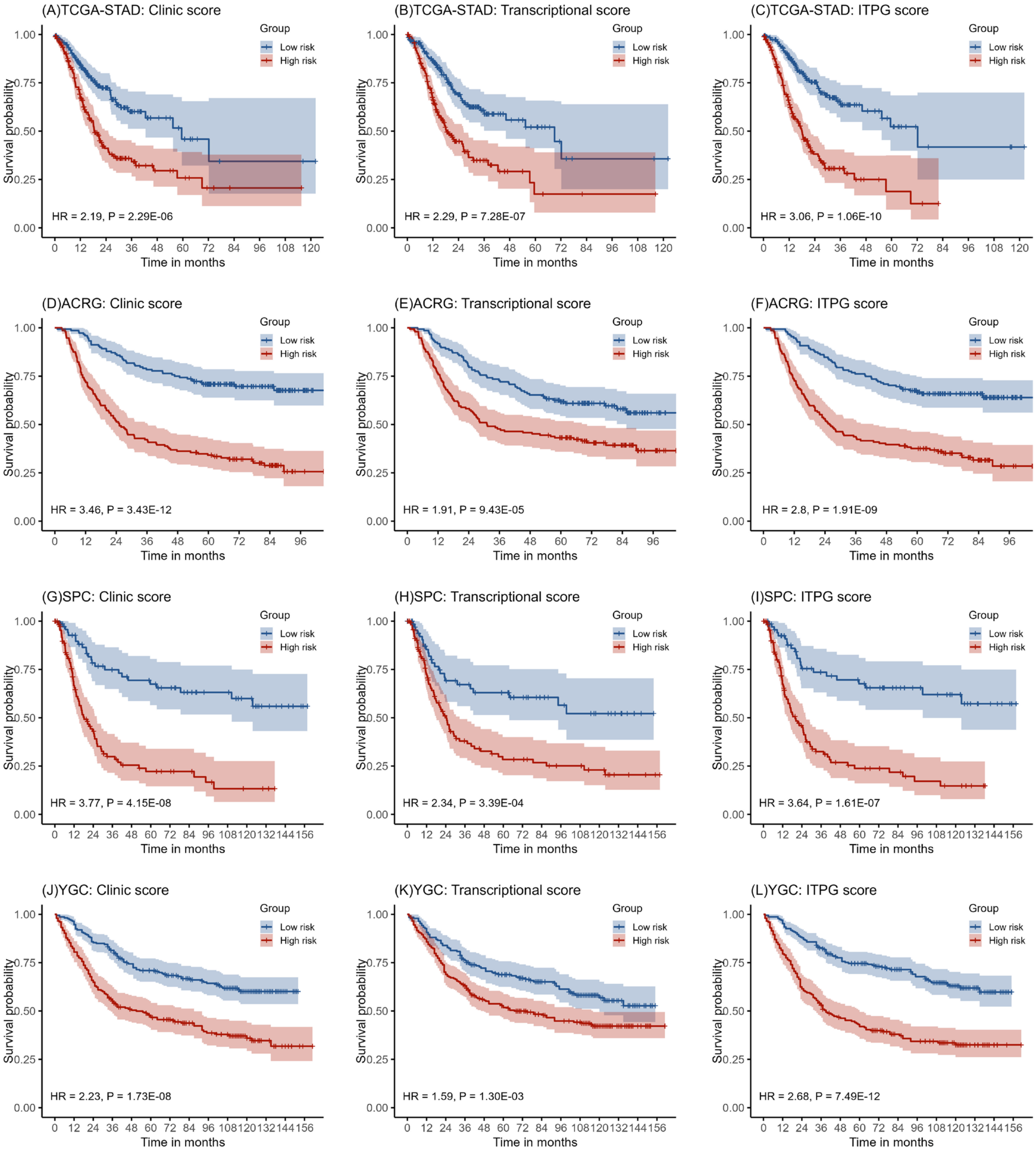

Figure S8 Kaplan-Meier survival curves for high- and low-risk gastric cancer patients stratified by different models in four independent cohorts. Kaplan-Meier survival curves comparing three prognostic scoring models across four independent gastric cancer cohorts. High- and low-risk groups for all cohorts and all scores were stratified using uniform thresholds, defined as the median values of each respective score in the TCGA-STAD discovery cohort. Four cohorts are as follows: TCGA-STAD(A-C), ACRG(D-F), SPC(G-I), YJGC(J-L). Three prognostic scoring models are as follows: Clinical score (A, D, G, J), Transcriptomic score (B, E, H, K), ITPG score (C, F, I, L). P-values show the survival difference between risk groups within each cohort based on the Log-rank test. TCGA, The Cancer Genome Atlas; TCGA-STAD, TCGA stomach adenocarcinoma cohort; ACRG, Asian Cancer Research Group; SPC, Singapore Patient Cohort; YGC, Yonsei Gastric Cancer Cohort; HR: hazard ratio; ITPG, Immune-related Transcriptomic Predictive Model for Gastric Cancer Prognosis.

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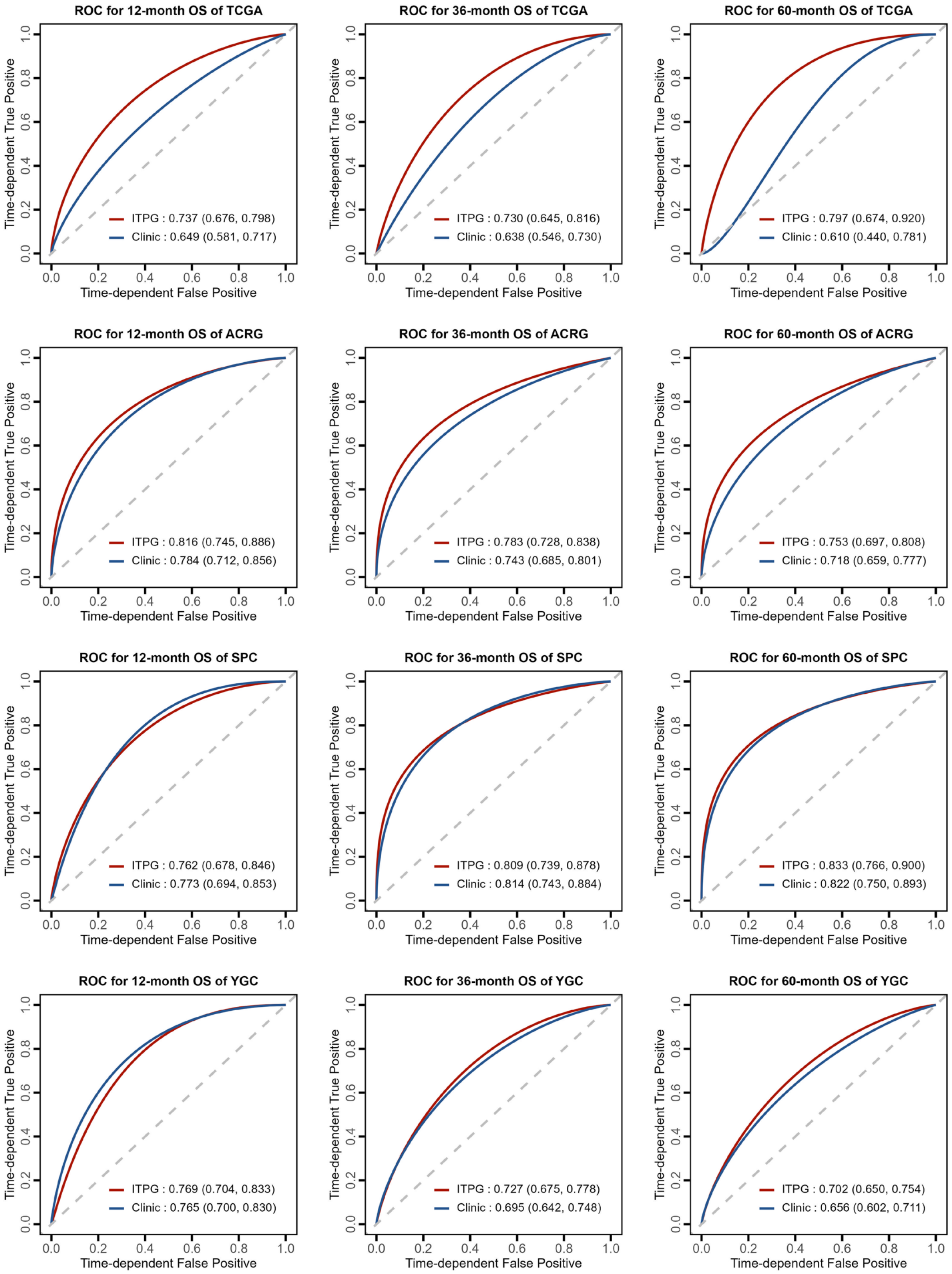

Figure S9 Time-dependent ROC curves of different prognostic prediction models based on clinical information and transcriptomic predictors. ROC curves are presented for 1-year, 3-year and 5-year survival prediction in four independent gastric cancer cohorts (TCGA-STAD, ACRG, SPC, and YGC). The AUC increase (%) is evaluated by comparing ITPG model and the model with clinic model, calculated by R package *timeROC*. TCGA, The Cancer Genome Atlas; ACRG, Asian Cancer Research Group; SPC, Singapore Patient Cohort; YGC, Yonsei Gastric Cancer Cohort; ITPG, Immune-related Transcriptomic Predictive Model for Gastric Cancer Prognosis; ROC, Receiver operating characteristic; OS, overall survival.

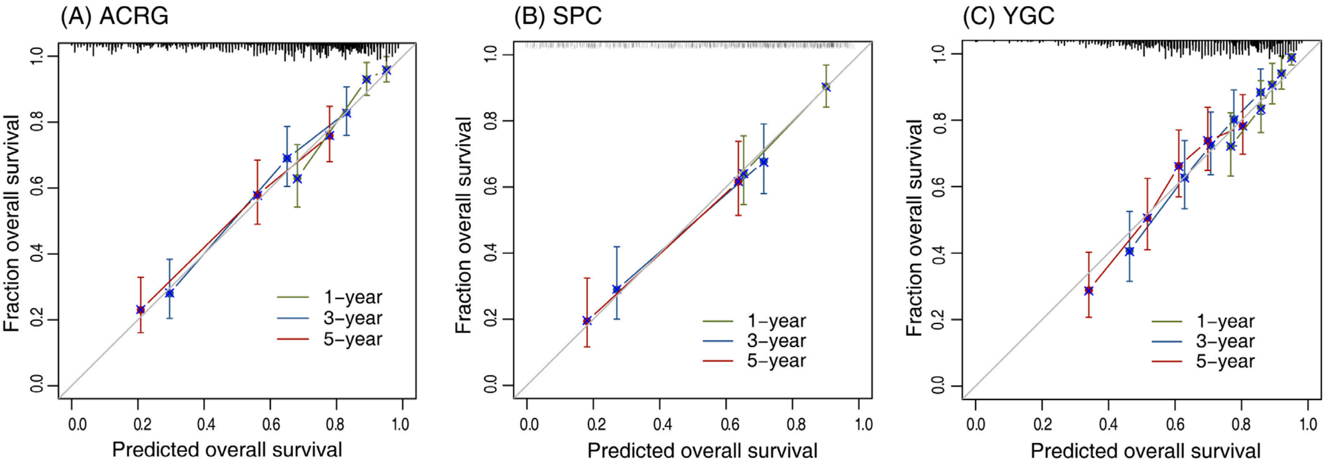

Figure S10 Calibration curves for ITPG in the testing datasets. Nomograph of (A) ACRG, (B) SPC and (C) YGC. The x-axis shows the nomogram-predicted survival probability, and the y-axis shows the actual survival probability estimated by Kaplan-Meier analysis. ITPG, Immune-related Transcriptomic Predictive Model for Gastric Cancer Prognosis; ACRG, Asian Cancer Research Group; SPC, Singapore Patient Cohort; YGC, Yonsei Gastric Cancer Cohort.

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Table S7 The results of gene enrichment pathway analysis of genes as predictors of ITPG based on the KEGG and GO databases

Database	ID	Description	Ratio	P	Adjusted P	Gene Entrez ID
KEGG	hsa04971	Gastric acid secretion	76/8859	2.17E-04	2.81E-03	3784/496
GO (BP)	GO:0055075	potassium ion homeostasis	34/18614	4.84E-05	1.65E-02	3784/496
GO (BP)	GO:1990573	potassium ion import across plasma membrane	47/18614	9.30E-05	1.65E-02	3784/496
GO (BP)	GO:0140115	export across plasma membrane	80/18614	2.71E-04	3.19E-02	3784/496
GO (BP)	GO:1903305	regulation of regulated secretory pathway	130/18614	7.13E-04	3.50E-02	4852/8832
GO (BP)	GO:0048565	digestive tract development	134/18614	7.57E-04	3.50E-02	3784/4852
GO (BP)	GO:0098659	inorganic cation import across plasma membrane	136/18614	7.80E-04	3.50E-02	3784/496
GO (BP)	GO:0099587	inorganic ion import across plasma membrane	136/18614	7.80E-04	3.50E-02	3784/496
GO (BP)	GO:0055123	digestive system development	146/18614	8.98E-04	3.50E-02	3784/4852
GO (BP)	GO:0035725	sodium ion transmembrane transport	181/18614	1.37E-03	3.50E-02	3784/496
GO (BP)	GO:0008217	regulation of blood pressure	187/18614	1.47E-03	3.50E-02	3784/4852
GO (BP)	GO:0050777	negative regulation of immune response	191/18614	1.53E-03	3.50E-02	4852/8832
GO (BP)	GO:0017157	regulation of exocytosis	194/18614	1.58E-03	3.50E-02	4852/8832
GO (BP)	GO:0098739	import across plasma membrane	212/18614	1.88E-03	3.50E-02	3784/496
GO (BP)	GO:0071805	potassium ion transmembrane transport	217/18614	1.97E-03	3.50E-02	3784/496
GO (BP)	GO:0045055	regulated exocytosis	228/18614	2.17E-03	3.50E-02	4852/8832
GO (BP)	GO:0002274	myeloid leukocyte activation	240/18614	2.40E-03	3.50E-02	4852/8832
GO (BP)	GO:0006813	potassium ion transport	241/18614	2.42E-03	3.50E-02	3784/496
GO (BP)	GO:0006814	sodium ion transport	254/18614	2.68E-03	3.50E-02	3784/496
GO (BP)	GO:0098657	import into cell	265/18614	2.92E-03	3.50E-02	3784/496
GO (BP)	GO:0033007	negative regulation of mast cell activation involved in immune response	10/18614	3.22E-03	3.50E-02	8832
GO (CC)	GO:0005890	sodium:potassium-exchanging ATPase complex	12/19518	3.68E-03	2.77E-02	496
GO (CC)	GO:0016324	apical plasma membrane	381/19518	5.41E-03	2.77E-02	3784/496
GO (CC)	GO:0090533	cation-transporting ATPase complex	18/19518	5.52E-03	2.77E-02	496
GO (CC)	GO:1902495	transmembrane transporter complex	392/19518	5.72E-03	2.77E-02	3784/496
GO (CC)	GO:1990351	transporter complex	418/19518	6.48E-03	2.77E-02	3784/496
GO (CC)	GO:0005925	focal adhesion	422/19518	6.60E-03	2.77E-02	23768/9358
GO (CC)	GO:0030055	cell-substrate junction	432/19518	6.91E-03	2.77E-02	23768/9358
GO (CC)	GO:0045177	apical part of cell	446/19518	7.35E-03	2.77E-02	3784/496
GO (CC)	GO:0098533	ATPase dependent transmembrane transport complex	24/19518	7.36E-03	2.77E-02	496
GO (CC)	GO:0098992	neuronal dense core vesicle	28/19518	8.58E-03	2.77E-02	4852

Table S7 (*continued*)

Table S7 (*continued*)

Database	ID	Description	Ratio	P	Adjusted P	Gene Entrez ID
GO (CC)	GO:0043025	neuronal cell body	500/19518	9.17E-03	2.77E-02	3784/4852
GO (CC)	GO:0008305	integrin complex	31/19518	9.49E-03	2.77E-02	9358
GO (CC)	GO:0031045	dense core granule	41/19518	1.25E-02	3.35E-02	4852
GO (CC)	GO:0043195	terminal bouton	47/19518	1.44E-02	3.35E-02	4852
GO (CC)	GO:0097546	ciliary base	47/19518	1.44E-02	3.35E-02	3784
GO (CC)	GO:0098636	protein complex involved in cell adhesion	57/19518	1.74E-02	3.81E-02	9358
GO (CC)	GO:0008076	voltage-gated potassium channel complex	78/19518	2.37E-02	4.89E-02	3784
GO (CC)	GO:0098982	GABA-ergic synapse	84/19518	2.55E-02	4.97E-02	4852
GO (CC)	GO:0034705	potassium channel complex	89/19518	2.71E-02	4.98E-02	3784
GO (MF)	GO:0015079	potassium ion transmembrane transporter activity	157/18369	1.06E-03	3.28E-02	3784/496
GO (MF)	GO:0034236	protein kinase A catalytic subunit binding	13/18369	4.24E-03	3.28E-02	3784
GO (MF)	GO:1902282	voltage-gated potassium channel activity involved in ventricular cardiac muscle cell action potential repolarization	13/18369	4.24E-03	3.28E-02	3784
GO (MF)	GO:0015271	outward rectifier potassium channel activity	14/18369	4.56E-03	3.28E-02	3784
GO (MF)	GO:0086008	voltage-gated potassium channel activity involved in cardiac muscle cell action potential repolarization	16/18369	5.22E-03	3.28E-02	3784
GO (MF)	GO:0015662	P-type ion transporter activity	20/18369	6.52E-03	3.28E-02	496
GO (MF)	GO:0008157	protein phosphatase 1 binding	23/18369	7.49E-03	3.28E-02	3784
GO (MF)	GO:0140358	P-type transmembrane transporter activity	24/18369	7.81E-03	3.28E-02	496
GO (MF)	GO:0046873	metal ion transmembrane transporter activity	435/18369	7.88E-03	3.28E-02	3784/496
GO (MF)	GO:0005104	fibroblast growth factor receptor binding	25/18369	8.14E-03	3.28E-02	23768
GO (MF)	GO:0034237	protein kinase A regulatory subunit binding	25/18369	8.14E-03	3.28E-02	3784
GO (MF)	GO:0001671	ATPase activator activity	27/18369	8.79E-03	3.28E-02	496
GO (MF)	GO:0009678	pyrophosphate hydrolysis-driven proton transmembrane transporter activity	27/18369	8.79E-03	3.28E-02	496
GO (MF)	GO:0045499	chemorepellent activity	28/18369	9.11E-03	3.28E-02	23768
GO (MF)	GO:0005251	delayed rectifier potassium channel activity	29/18369	9.44E-03	3.28E-02	3784
GO (MF)	GO:0005184	neuropeptide hormone activity	30/18369	9.76E-03	3.28E-02	4852
GO (MF)	GO:0048018	receptor ligand activity	497/18369	1.02E-02	3.28E-02	23768/4852
GO (MF)	GO:0071855	neuropeptide receptor binding	33/18369	1.07E-02	3.28E-02	4852
GO (MF)	GO:0060590	ATPase regulator activity	46/18369	1.49E-02	4.32E-02	496
GO (MF)	GO:0005246	calcium channel regulator activity	54/18369	1.75E-02	4.38E-02	4852

ITPG, Immune-related Transcriptomic Predictive Model for Gastric Cancer Prognosis; GO, Gene oncology; KEGG, Kyoto Encyclopedia of Genes and Genomes; BP, biological process; MF, molecular function; CC, cellular component.

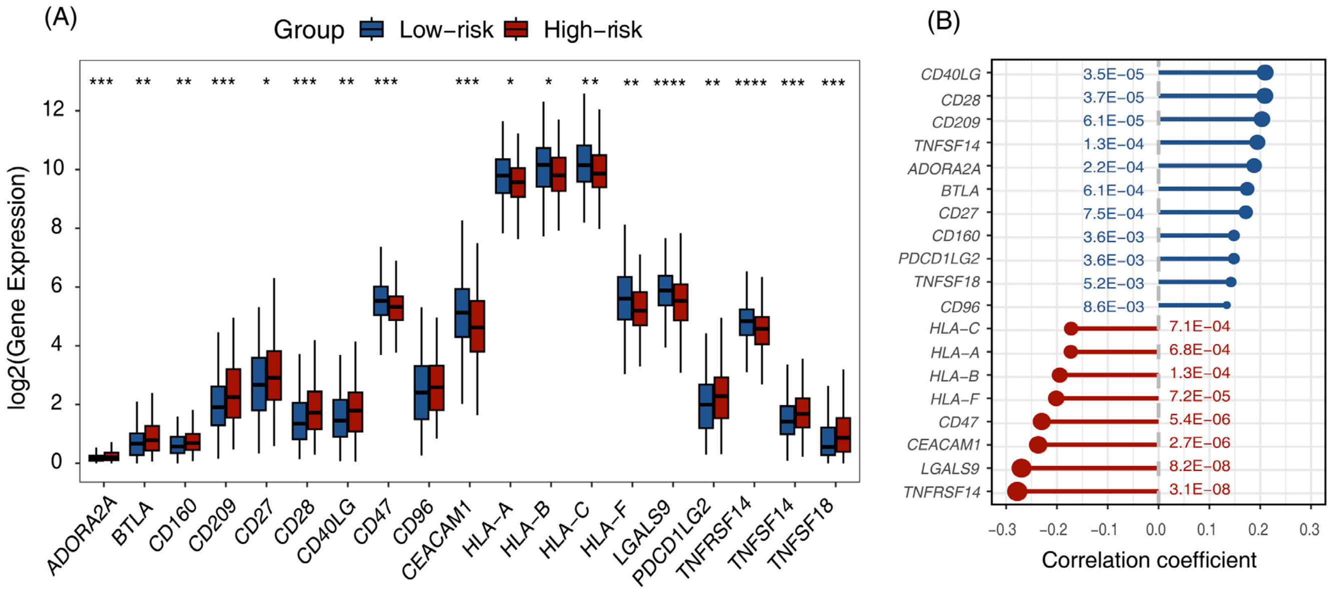

Figure S11 Association results between the transcriptomic score and immune checkpoint gene expressions. (A) Boxplots represent the differential expression of immune checkpoint genes between low- and high-risk groups categorized by median value of the transcriptomic score. Statistical significance is indicated as *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001. (B) Lollipop chart of correlations among checkpoints and transcriptomic score. Correlation coefficients were derived from Pearson correlation analysis.

Table S8 Potential targeted drug for genes as predictors of ITPG derived from DrugBank database

Gene symbol	Drug name	Drug group	Change	Immunity-related drug
FLRT2	Cytarabine	Approved, Investigational	downregulated	✓
	Entinostat	Investigational	downregulated	✓
	Formaldehyde	Approved, Vet Approved	upregulated	✓
	Panobinostat	Approved, Investigational	downregulated	✓
KCNQ1	Daunorubicin	Approved	downregulated	✓
	Doxorubicin	Approved, Investigational	downregulated	✓
	Entinostat	Investigational	upregulated	✓
	Mitoxantrone	Approved, Investigational	downregulated	✓
	Tretinoin	Approved, Investigational Nutraceutical	downregulated	✓
NPY	Arsenic trioxide	Approved, Investigational	upregulated	✓
	Belinostat	Approved, Investigational	upregulated	✓
	Cytarabine	Approved, Investigational	downregulated	✓
	Entinostat	Investigational	upregulated	✓
	Panobinostat	Approved, Investigational	upregulated	✓
	Tretinoin	Approved, Investigational, Nutraceutical	downregulated	✓
	Vorinostat	Approved, Investigational	upregulated	✓
ITGBL1	Arsenic trioxide	Approved, Investigational	downregulated	✓
	Methotrexate	Approved	downregulated	✓
ATP4B	Carmustine	Approved, Investigational	downregulated	✓
CD84	Arsenic trioxide	Approved, Investigational	upregulated	✓
	Cyclophosphamide	Approved, Investigational	upregulated	✓
	Prednisolone	Approved, Vet Approved	upregulated	✓
	Sirolimus	Approved, Investigational	upregulated	✓

ITPG, Immune-related Transcriptomic Predictive Model for Gastric Cancer Prognosis. The drug-gene results were obtained from Pharmaco-transcriptomics, a module in the DrugBank database (https://go.drugbank.com/pharmaco/transcriptomics).

Table S9 EMT-related gene in the predictator selection process

Geneset	EMT-related genes
EMT-related genes with differential expression across three omics levels	*COL8A1*, *FERMT2*, *FBLN5*, *IGSF8*, *AXIN2*, *JAK3*, *CDK14*, *GATA3*, *SCUBE3*, *CCR2*, *ESR1*, *ELF5*
EMT-related genes among the candidate predictive factors.	*AQP3*, *ASCL1*, *CDH11*, *CCR5*, *COL8A1*, *CRMP1*, *EPO*, *ESR1*, *F2*, *FHL1*, *FOXC1*, *FLNA*, *CXCL8*, *JAK3*, *KRAS*, *L1CAM*, *P2RY2*, *PCSK1*, *PRKCQ*, *PROX1*, *S100A2*, *S100A8*, *CCL5*, *CCL19*, *SFRP2*, *TDGF1*, *TGFB3*, *WNT1*, *ADIPOQ*, *HAND1*, *NTN1*, *HDAC9*, *FBLN5*, *FERMT2*, *GREM1*, *IL23A*, *IL17RD*, *IGSF8*, *MUC16*, *CCR2*

EMT, Epithelial-mesenchymal transition.

Table S10 Association results of *ZEB1* and *PD-L1* derived from Cox regression model adjusted for covariates in the TCGA training

Gene	β (95% CI)	z score	P
ZEB1	0.2953 (0.1319, 0.4586)	3.5431	3.95E-04
PD-L1	-0.1325 (-0.3036, 0.0386)	-1.5179	1.29E-01

TCGA, The Cancer Genome Atlas; 95% CI, 95% confidence interval. β (95% CI), z score, and P value were derived from the Cox proportional hazards model adjusted for age and AJCC pathological stage.

Table S11 Association results of genes with main effects or gene-gene interactions derived from a multivariable Cox regression model (ITPG_plus) in the TCGA training

Gene	β (95% CI)	z score	P
Age	0.0402 (0.0227, 0.0577)	4.5016	6.74E-06
Stage			
I	ref		
II	0.3719 (-0.2711, 1.0149)	1.1335	2.57E-01
III	0.8744 (0.2741, 1.4747)	2.8549	4.00E-03
IV	1.6322 (0.9266, 2.3378)	4.5339	5.79E-06
Main effect			
FLRT2	0.3116 (0.0343, 0.5888)	2.2024	2.80E-02
KCNQ1	-0.2363 (-0.4085, -0.0640)	-2.6884	7.00E-03
ZEB1	0.0018 (-0.2683, 0.2720)	0.0131	9.90E-01
PD-L1	-0.1690 (-0.4003, 0.0623)	-1.4322	1.52E-01
G×G interaction			
NPY	-0.0000 (-0.1895, 0.1894)	-0.0004	1.00E+00
ITGBL1	-0.0633 (-0.2922, 0.1656)	-0.5419	5.88E-01
*NPY*ITGBL1*	0.2270 (0.0866, 0.3673)	3.1698	2.00E-03
ATP4B	-0.0598 (-0.2519, 0.1323)	-0.6102	5.42E-01
CD84	0.0331 (-0.2149, 0.2810)	0.2614	7.94E-01
*ATP4B*CD84*	-0.2725 (-0.4625, -0.0825)	-2.8111	5.00E-03

ITPG, Immune-related Transcriptomic Predictive Model for Gastric Cancer Prognosis; TCGA, The Cancer Genome Atlas; 95% CI, 95% confidence interval; G×G interaction, gene-gene interaction.

Table S12 Predictive performance of the ITPG_plus model (incorporating *ZEB1* and *PD-L1* as predictive factors) across different cohorts

Cohort	ITPG_plus				ITPG			
	C-index	1-year AUC	3-year AUC	5-year AUC	C-index	1-year AUC	3-year AUC	5-year AUC
TCGA	0.704	0.7375	0.7194	0.7938	0.703	0.7373	0.7305	0.7971
ACRG	0.726	0.8037	0.7772	0.7518	0.729	0.8160	0.7831	0.7525
SPC	0.712	0.7558	0.8024	0.8297	0.715	0.7623	0.8086	0.8327
YGC	0.665	0.7436	0.7165	0.6923	0.675	0.7686	0.7267	0.7023

ITPG, Immune-related Transcriptomic Predictive Model for Gastric Cancer Prognosis; TCGA, The Cancer Genome Atlas; ACRG, Asian Cancer Research Group; SPC, Singapore Patient Cohort; YGC, Yonsei Gastric Cancer Cohort; C-index, concordance index; AUC, Area under the curve.

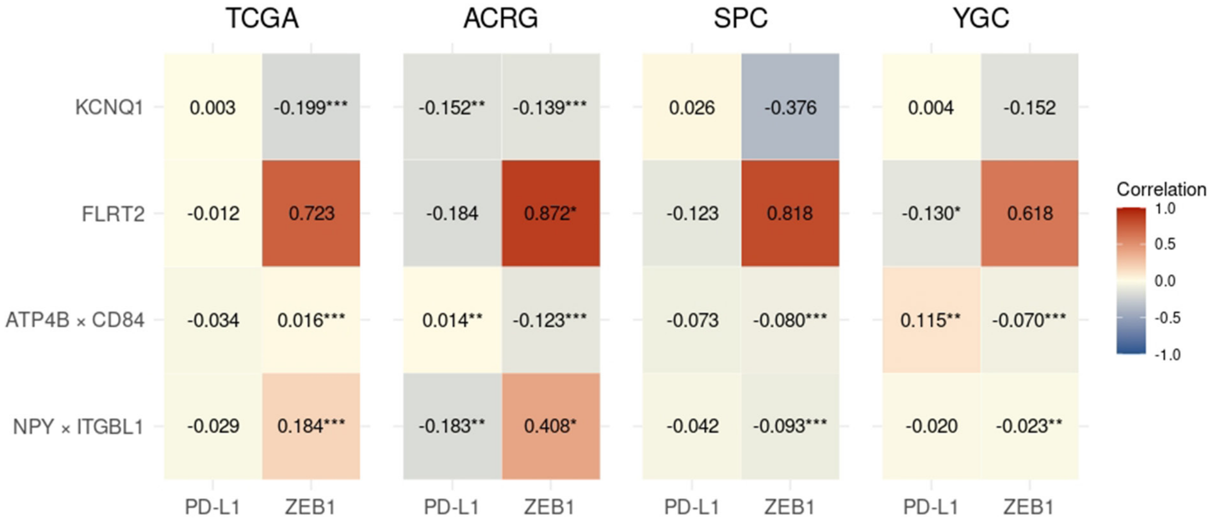

Figure S12 Correlation heatmap between transcriptomic model genes and key regulators across multiple cancer cohorts. Heatmap illustrates the correlation of expression between genes selected for the transcriptomic model and two key regulators (*PD-L1* and *ZEB1*) across four gastric cancer cohorts (TCGA, ACRG, SPC, and YGC). The selected genes include two genes with main effects (*KCNQ1*, *FLRT2*) and two pairs of gene-gene interaction terms (*ATP4B*×*CD84*, *NPY*×*ITGBL1*). Color intensity (from blue to red) represents the strength and direction of correlation (blue for negative, red for positive). Statistical significance is indicated as *P<0.05, **P<0.01, ***P<0.001. TCGA, The Cancer Genome Atlas; ACRG, Asian Cancer Research Group; SPC, Singapore Patient Cohort; YGC, Yonsei Gastric Cancer Cohort.