

## Appendix 1

### *Cell lines and cell culture*

The HEK293T cell line was obtained from the American Type Culture Collection (ATCC; USA). Cell cultivation was carried out in the prescribed medium (DMEM) with the addition of 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (p/s) at 37 °C in a 5% CO<sub>2</sub> atmosphere.

### *Over expression plasmid construction*

The STAT1 coding sequence (CDS) fragment was excised from the aforementioned plasmid and subsequently introduced into the pcDNA3.1 vector (Invitrogen). The complete human STAT1 cDNA templates were amplified from HEK293T cells utilizing the One-Step RT-PCR System kit (Invitrogen) and subsequently subcloned into the pcDNA3.1 vector.

The primers for Predicted ACP2 promoter are as follows: forward sequencing (5'-3')—ATAGGTACCCTTCCTCTCTTTAGG, reverse sequencing (5'-3')—ATAACGCGTCAAGCTGGAACCCGC.

### *Transient transfections*

Transfections were performed using either Lipofectamine 2000 or Lipofectamine RNAiMAX (for siRNA) transfection reagent (Invitrogen) in accordance with the manufacturer's guidelines.

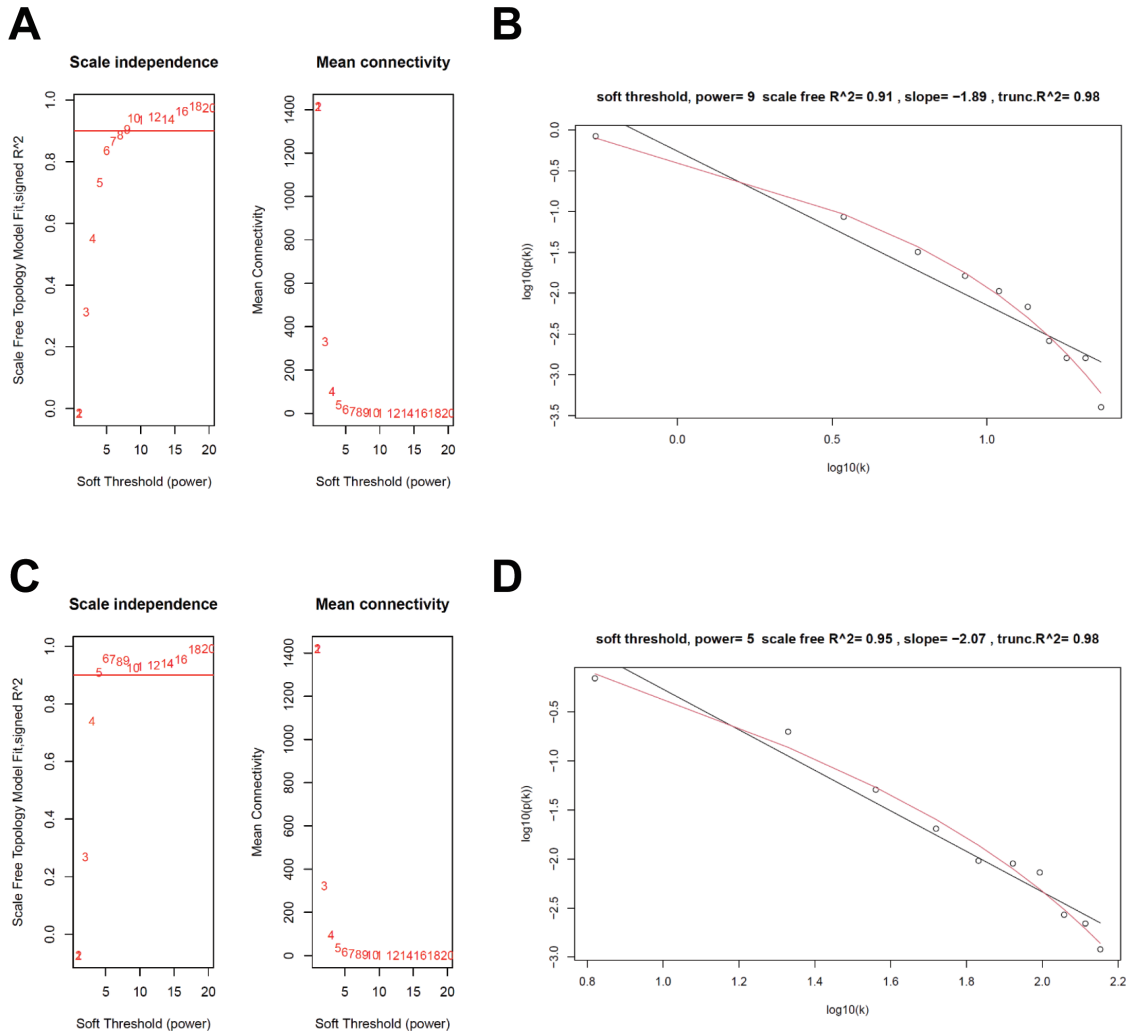
### *Luciferase assay*

The human STAT1 promoter region (spanning from -2,000 bp to +1 bp) was amplified from genomic DNA extracted from HEK293T cells and subsequently integrated into the pGL3 basic vector (Promega), positioned upstream of the luciferase gene. Cells were co-transfected with 50–100 ng of the designated reporter construct and 2.0 ng of pRenilla. Twenty-four hours post-DNA transfection, cells were harvested and subjected to analysis using a dual-luciferase reporter assay kit (Promega). Firefly luminescence signals were normalized based on the corresponding Renilla signals, enabling the calculation of relative luciferase activity. Mean luciferase activity levels were determined from three independent experiments.

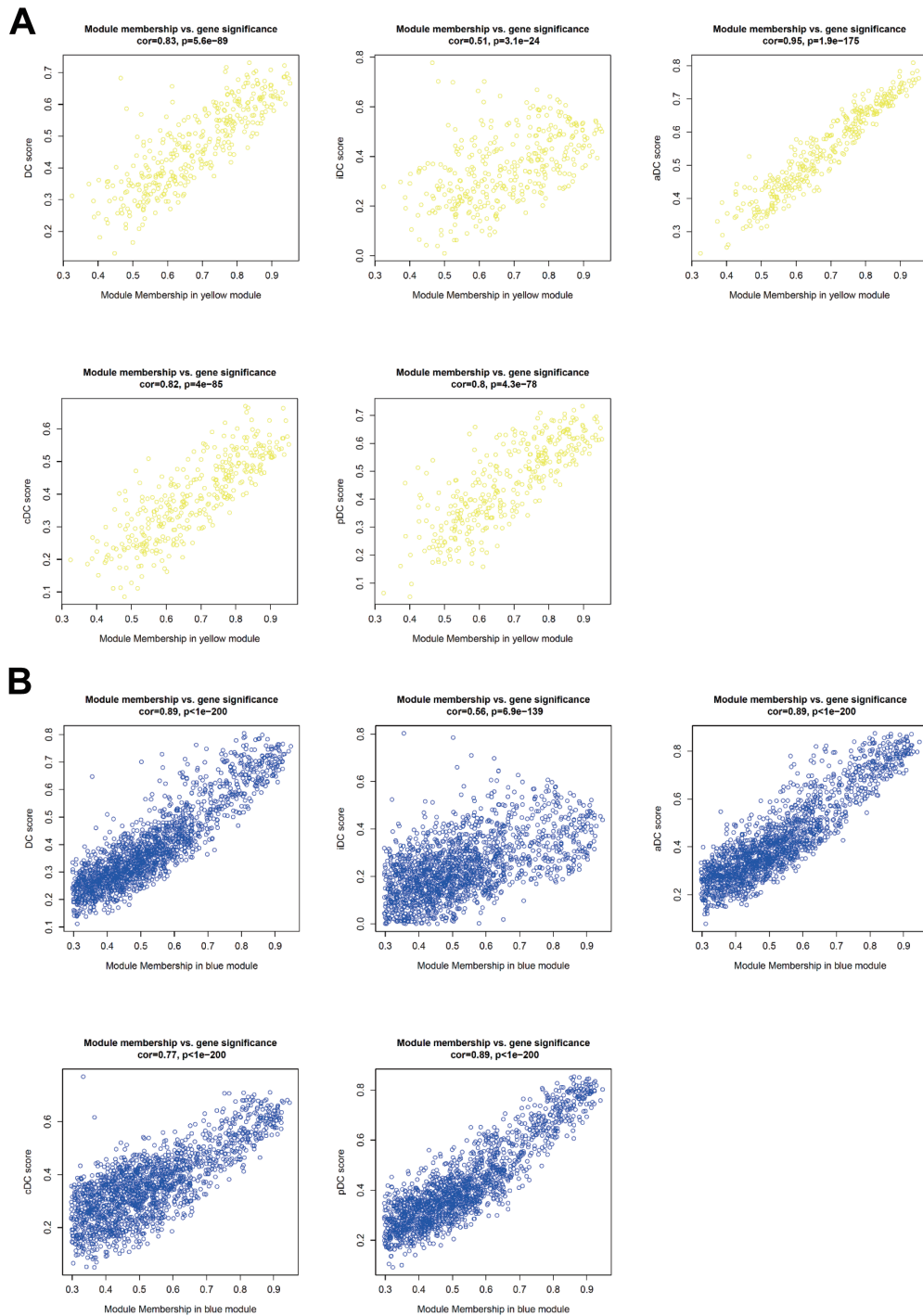
### *The quantitative real-time PCR (qRT-PCR)*

Total RNA was isolated from either fresh tissue samples or cells using TRIzol Reagent (Invitrogen), followed by reverse transcription utilizing an MLV-reverse transcriptase kit (Invitrogen). Quantitative real-time polymerase chain reaction (qRT-PCR) was conducted with SYBR Green (Tiangen, China) in triplicate assays on an ABI 7500 Real-Time Detection system (Applied Biosystems), following the manufacturer's protocol. GAPDH served as the internal standard. Experimental procedures were repeated in triplicate, and data analysis was performed using the  $2^{-\Delta\Delta C_t}$  method.

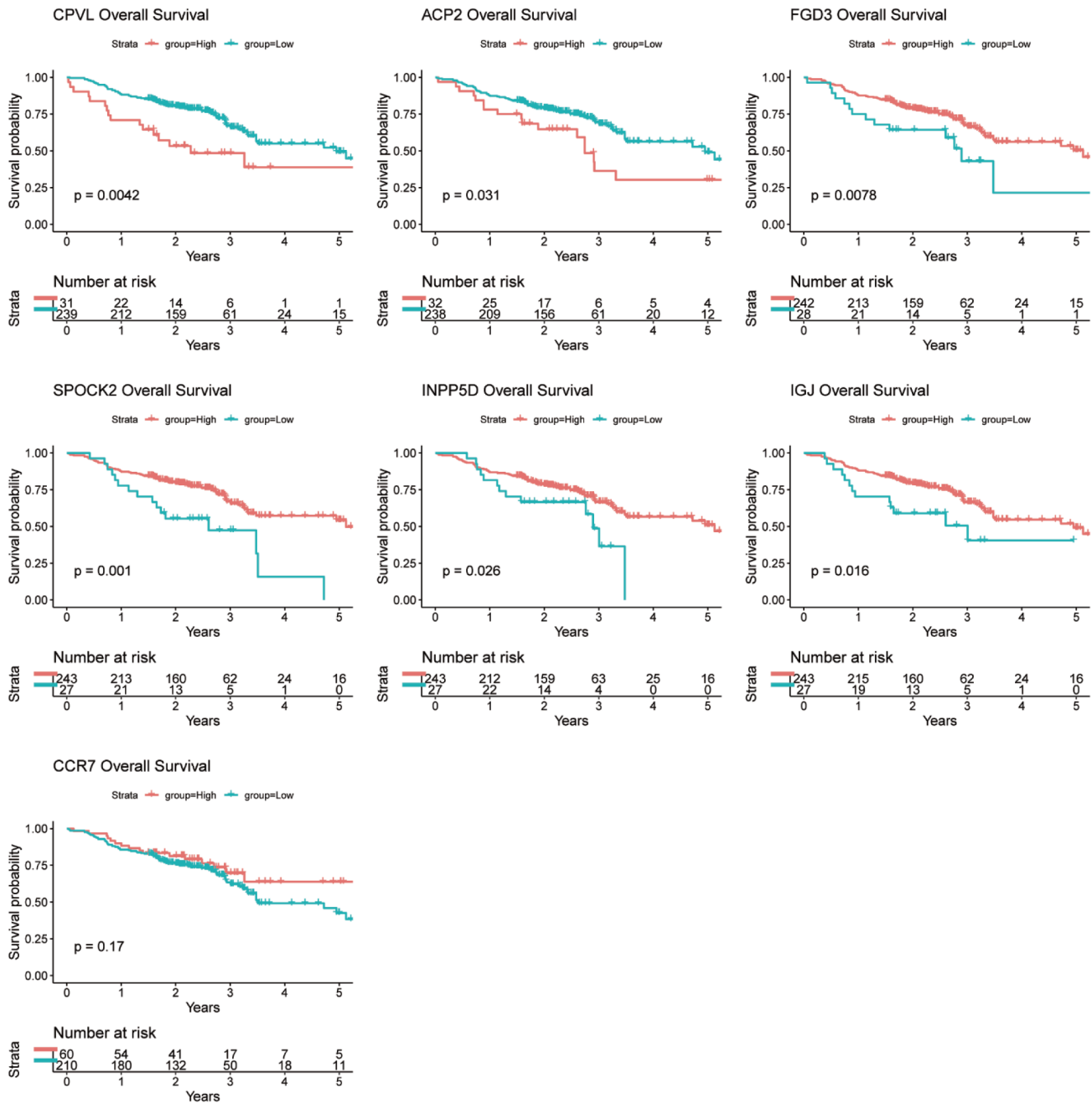
The primers for STAT1 are as follows: forward sequencing (5'-3')—TGTATGCCATCCTCGAGAGC, reverse sequencing (5'-3')—AGACATCCTGCCACCTTGTG. The primers for ACP2 are as follows: forward sequencing (5'-3')—GAAACCAATGAGCACCCAGC, reverse sequencing (5'-3')—GATGAATACCAGTCCACCCGCAAC. The primers for GAPDH are as follows: forward sequencing (5'-3')—GAAGGTGAAGGTCCGAGTC, reverse sequencing (5'-3')—GAAGATGGTGATGGGATTTC.



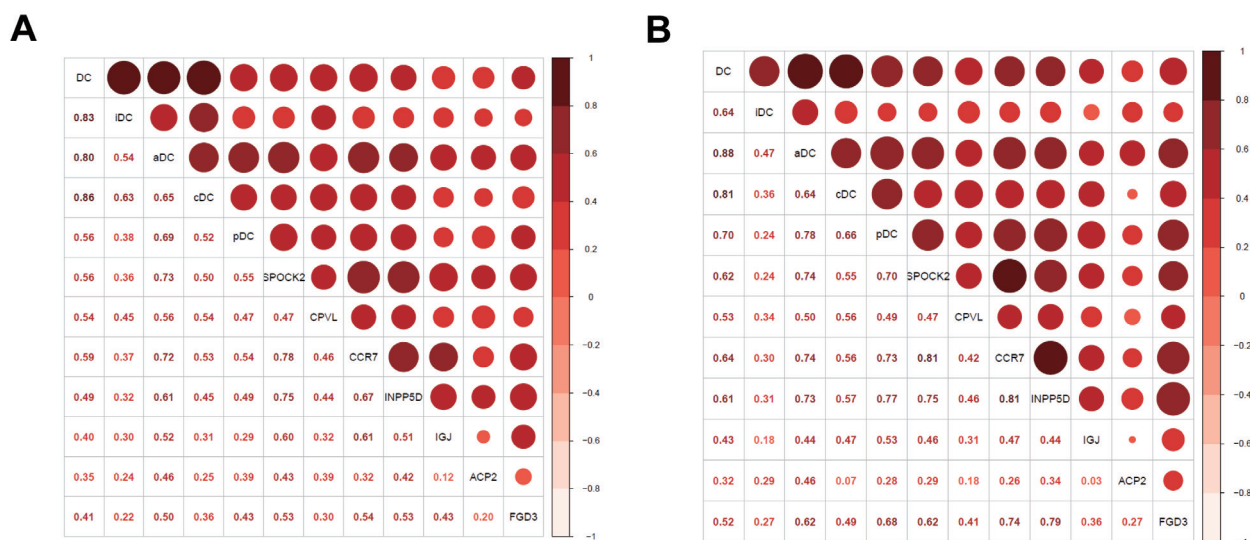
**Figure S1** The soft-thresholding power selection of WGCNA in TCGA-HNSCC and GSE65858. The soft-thresholding power of 9 was selected based on the scale-free topology criterion in TCGA-HNSCC (A,B). The soft-thresholding power of 5 was selected based on the scale-free topology criterion in GSE65858 (C,D). WGCNA, weighted gene co-expression network analysis; TCGA, The Cancer Genome Atlas; HNSCC, head and neck squamous cell carcinoma.



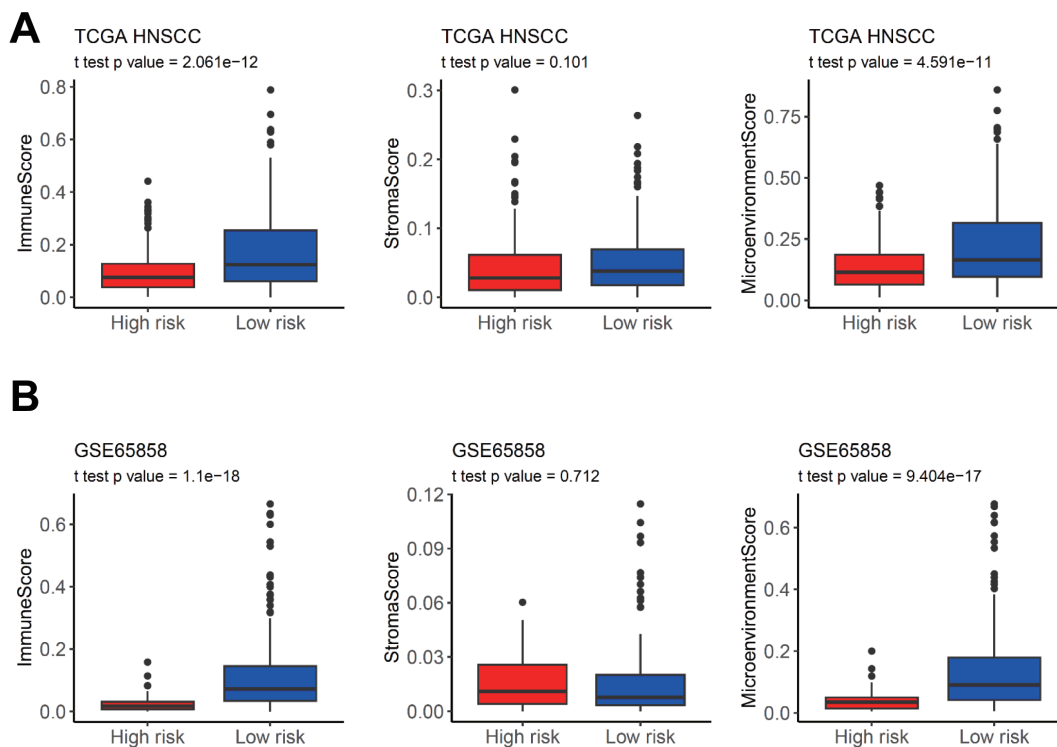
**Figure S2** The selection of module genes by WGCNA in TCGA-HNSCC and GSE65858. Scatter plots of the module membership and multi-estimated DC signatures as well as stromal score in the yellow module of TCGA-HNSCC (A). Scatter plots of the module membership and multi-estimated DC signatures as well as stromal score in the blue module of GSE65858 (B). WGCNA, weighted gene co-expression network analysis; TCGA, The Cancer Genome Atlas; HNSCC, head and neck squamous cell carcinoma; DC, dendritic cell.



**Figure S3** Prognostic role of prioritized candidates in GSE65858. Kaplan-Meier plots were generated for the 5-year overall survival in both low and high-level of 7 prioritized candidates for GSE65858.



**Figure S4** The association between multi-estimated DC signatures and 7 prioritized candidates. Graphs illustrating the seven prioritized candidate genes demonstrated a notably positive correlation with multiple DC signatures in both TCGA-HNSCC cohort (A) and the GSE65858 cohort (B), as evidenced by Spearman correlation analysis. DC, dendritic cell; TCGA, The Cancer Genome Atlas; HNSCC, head and neck squamous cell carcinoma.



**Figure S5** ESTIMATE data immune features in low and high-risk groups in TCGA-HNSCC and GSE65858. The box plots illustrate the distribution of immune, stromal, and microenvironment scores between low and high-risk clusters in TCGA-HNSCC (A) and GSE65858 (B). ESTIMATE, Estimation of STromal and Immune cells in MAlignant Tumours using Expression; TCGA, The Cancer Genome Atlas; HNSCC, head and neck squamous cell carcinoma.

**Table S1** xCells subtypes difference between high and low risk groups of TCGA-HNSCC

	High risk (n=248)	Low risk (n=251)	P
aDC, mean (SD)	0.24 (0.13)	0.33 (0.17)	<0.001
Adipocytes, mean (SD)	0.00 (0.01)	0.00 (0.01)	0.911
Astrocytes, mean (SD)	0.09 (0.11)	0.08 (0.09)	0.128
B cells, mean (SD)	0.01 (0.02)	0.08 (0.12)	<0.001
Basophils, mean (SD)	0.04 (0.04)	0.06 (0.05)	<0.001
CD4 memory T cells, mean (SD)	0.01 (0.01)	0.02 (0.02)	<0.001
CD4 naive T cells, mean (SD)	0.00 (0.01)	0.02 (0.03)	<0.001
CD4 T cells, mean (SD)	0.00 (0.01)	0.01 (0.02)	<0.001
CD4 Tcm, mean (SD)	0.04 (0.02)	0.05 (0.03)	<0.001
CD4 Tem, mean (SD)	0.00 (0.01)	0.01 (0.01)	0.003
CD8 naive T cells, mean (SD)	0.01 (0.01)	0.01 (0.01)	0.351
CD8 T cells, mean (SD)	0.03 (0.04)	0.05 (0.06)	<0.001
CD8 Tcm, mean (SD)	0.02 (0.04)	0.06 (0.07)	<0.001
CD8 Tem, mean (SD)	0.01 (0.03)	0.03 (0.04)	<0.001
cDC, mean (SD)	0.08 (0.09)	0.11 (0.11)	<0.001
Chondrocytes, mean (SD)	0.02 (0.03)	0.02 (0.03)	0.248
Class switched memory B cells, mean (SD)	0.01 (0.01)	0.03 (0.03)	<0.001
CLP, mean (SD)	0.03 (0.02)	0.02 (0.01)	<0.001
CMP, mean (SD)	0.00 (0.00)	0.00 (0.00)	<0.001
DC, mean (SD)	0.02 (0.02)	0.03 (0.03)	<0.001
Endothelial cells, mean (SD)	0.04 (0.04)	0.04 (0.04)	0.459
Eosinophils, mean (SD)	0.00 (0.00)	0.00 (0.00)	0.212
Epithelial cells, mean (SD)	0.36 (0.08)	0.37 (0.06)	0.112
Erythrocytes, mean (SD)	0.00 (0.00)	0.00 (0.00)	0.318
Fibroblasts, mean (SD)	0.04 (0.06)	0.05 (0.06)	0.054
GMP, mean (SD)	0.00 (0.01)	0.01 (0.01)	0.035
Hepatocytes, mean (SD)	0.00 (0.00)	0.00 (0.00)	<0.001
HSC, mean (SD)	0.05 (0.05)	0.06 (0.06)	0.017
iDC, mean (SD)	0.19 (0.21)	0.22 (0.24)	0.089
Keratinocytes, mean (SD)	0.21 (0.05)	0.20 (0.04)	0.015
ly Endothelial cells, mean (SD)	0.02 (0.02)	0.02 (0.02)	0.121
Macrophages, mean (SD)	0.03 (0.04)	0.03 (0.04)	0.246
Macrophages M1, mean (SD)	0.03 (0.03)	0.03 (0.03)	0.951
Macrophages M2, mean (SD)	0.01 (0.01)	0.01 (0.01)	0.045
Mast cells, mean (SD)	0.02 (0.01)	0.02 (0.01)	<0.001
Megakaryocytes, mean (SD)	0.00 (0.00)	0.00 (0.00)	<0.001
Melanocytes, mean (SD)	0.00 (0.00)	0.00 (0.00)	0.001
Memory B cells, mean (SD)	0.00 (0.01)	0.02 (0.04)	<0.001

Table S1 (continued)

**Table S1** (continued)

	High risk (n=248)	Low risk (n=251)	P
MEP, mean (SD)	0.05 (0.03)	0.05 (0.03)	0.608
Mesangial cells, mean (SD)	0.02 (0.02)	0.02 (0.02)	0.079
Monocytes, mean (SD)	0.02 (0.02)	0.02 (0.03)	0.275
MPP, mean (SD)	0.00 (0.00)	0.00 (0.00)	0.008
MSC, mean (SD)	0.15 (0.12)	0.10 (0.12)	<0.001
mv Endothelial cells, mean (SD)	0.02 (0.02)	0.02 (0.02)	0.133
Myocytes, mean (SD)	0.09 (0.10)	0.06 (0.09)	<0.001
naive B cells, mean (SD)	0.00 (0.00)	0.01 (0.02)	<0.001
Neurons, mean (SD)	0.00 (0.00)	0.00 (0.00)	0.672
Neutrophils, mean (SD)	0.00 (0.01)	0.00 (0.01)	0.007
NK cells, mean (SD)	0.00 (0.00)	0.00 (0.00)	0.96
NKT, mean (SD)	0.05 (0.04)	0.04 (0.04)	0.1
Osteoblast, mean (SD)	0.00 (0.01)	0.00 (0.01)	0.526
pDC, mean (SD)	0.04 (0.04)	0.06 (0.06)	<0.001
Pericytes, mean (SD)	0.04 (0.06)	0.04 (0.05)	0.997
Plasma cells, mean (SD)	0.01 (0.01)	0.02 (0.02)	<0.001
Platelets, mean (SD)	0.00 (0.00)	0.01 (0.01)	<0.001
Preadipocytes, mean (SD)	0.01 (0.02)	0.01 (0.03)	0.002
pro B cells, mean (SD)	0.01 (0.01)	0.01 (0.02)	<0.001
Sebocytes, mean (SD)	0.17 (0.06)	0.17 (0.06)	0.708
Skeletal muscle, mean (SD)	0.11 (0.17)	0.07 (0.15)	0.013
Smooth muscle, mean (SD)	0.18 (0.08)	0.14 (0.08)	<0.001
Tgd cells, mean (SD)	0.00 (0.01)	0.01 (0.01)	<0.001
Th1 cells, mean (SD)	0.05 (0.05)	0.04 (0.05)	0.212
Th2 cells, mean (SD)	0.14 (0.08)	0.14 (0.07)	0.447
Tregs, mean (SD)	0.02 (0.03)	0.03 (0.04)	<0.001
ImmuneScore, mean (SD)	0.09 (0.08)	0.17 (0.15)	<0.001
StromaScore, mean (SD)	0.04 (0.05)	0.05 (0.04)	0.101
MicroenvironmentScore, mean (SD)	0.14 (0.10)	0.22 (0.17)	<0.001

**Table S2** xCells subtypes difference between high and low risk groups of GSE65858

	High risk (n=60)	Low risk (n=210)	P
aDC, mean (SD)	0.08 (0.08)	0.18 (0.12)	<0.001
Adipocytes, mean (SD)	0.00 (0.00)	0.00 (0.00)	0.454
Astrocytes, mean (SD)	0.02 (0.03)	0.02 (0.03)	0.41
B cells, mean (SD)	0.01 (0.01)	0.09 (0.14)	<0.001
Basophils, mean (SD)	0.06 (0.04)	0.08 (0.05)	0.002
CD4 memory T cells, mean (SD)	0.00 (0.01)	0.01 (0.02)	<0.001
CD4 naive T cells, mean (SD)	0.00 (0.00)	0.01 (0.03)	<0.001
CD4 T cells, mean (SD)	0.00 (0.00)	0.00 (0.01)	0.03
CD4 Tcm, mean (SD)	0.00 (0.00)	0.00 (0.01)	0.043
CD4 Tem, mean (SD)	0.00 (0.01)	0.01 (0.01)	0.005
CD8 naive T cells, mean (SD)	0.01 (0.01)	0.01 (0.01)	0.301
CD8 T cells, mean (SD)	0.01 (0.02)	0.05 (0.04)	<0.001
CD8 Tcm, mean (SD)	0.02 (0.03)	0.08 (0.06)	<0.001
CD8 Tem, mean (SD)	0.00 (0.01)	0.02 (0.03)	<0.001
cDC, mean (SD)	0.06 (0.05)	0.11 (0.08)	<0.001
Chondrocytes, mean (SD)	0.00 (0.01)	0.00 (0.01)	0.341
Class switched memory B cells, mean (SD)	0.02 (0.02)	0.07 (0.07)	<0.001
CLP, mean (SD)	0.01 (0.01)	0.01 (0.01)	0.361
CMP, mean (SD)	0.00 (0.01)	0.00 (0.01)	0.093
DC, mean (SD)	0.01 (0.01)	0.02 (0.02)	<0.001
Endothelial cells, mean (SD)	0.01 (0.01)	0.01 (0.01)	0.66
Eosinophils, mean (SD)	0.00 (0.00)	0.00 (0.00)	0.511
Epithelial cells, mean (SD)	0.08 (0.03)	0.08 (0.03)	0.599
Erythrocytes, mean (SD)	0.00 (0.00)	0.00 (0.00)	0.094
Fibroblasts, mean (SD)	0.02 (0.02)	0.02 (0.03)	0.553
GMP, mean (SD)	0.01 (0.01)	0.01 (0.01)	0.539
Hepatocytes, mean (SD)	0.00 (0.00)	0.00 (0.00)	0.114
HSC, mean (SD)	0.02 (0.03)	0.02 (0.03)	0.334
iDC, mean (SD)	0.06 (0.09)	0.06 (0.09)	0.611
Keratinocytes, mean (SD)	0.04 (0.02)	0.04 (0.02)	0.168
ly Endothelial cells, mean (SD)	0.00 (0.01)	0.01 (0.01)	0.207
Macrophages, mean (SD)	0.00 (0.01)	0.01 (0.01)	0.021
Macrophages M1, mean (SD)	0.01 (0.01)	0.01 (0.02)	0.003
Macrophages M2, mean (SD)	0.00 (0.00)	0.00 (0.00)	<0.001
Mast cells, mean (SD)	0.00 (0.00)	0.01 (0.01)	0.002
Megakaryocytes, mean (SD)	0.00 (0.00)	0.00 (0.00)	0.042
Melanocytes, mean (SD)	0.00 (0.00)	0.00 (0.00)	0.024
Memory B cells, mean (SD)	0.00 (0.00)	0.03 (0.06)	<0.001

**Table S2** (continued)



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	High risk (n=60)	Low risk (n=210)	P
MEP, mean (SD)	0.03 (0.01)	0.03 (0.01)	0.043
Mesangial cells, mean (SD)	0.02 (0.02)	0.02 (0.02)	0.424
Monocytes, mean (SD)	0.01 (0.01)	0.01 (0.02)	0.084
MPP, mean (SD)	0.00 (0.00)	0.00 (0.00)	0.272
MSC, mean (SD)	0.08 (0.07)	0.07 (0.07)	0.611
mv Endothelial cells, mean (SD)	0.01 (0.01)	0.01 (0.01)	0.154
Myocytes, mean (SD)	0.02 (0.03)	0.02 (0.04)	0.76
naive B cells, mean (SD)	0.00 (0.00)	0.00 (0.01)	0.075
Neurons, mean (SD)	0.00 (0.00)	0.00 (0.00)	0.603
Neutrophils, mean (SD)	0.00 (0.00)	0.00 (0.01)	0.512
NK cells, mean (SD)	0.00 (0.00)	0.00 (0.00)	0.541
NKT, mean (SD)	0.02 (0.02)	0.03 (0.03)	0.047
Osteoblast, mean (SD)	0.02 (0.01)	0.01 (0.02)	0.037
pDC, mean (SD)	0.03 (0.03)	0.07 (0.05)	<0.001
Pericytes, mean (SD)	0.01 (0.02)	0.01 (0.02)	0.789
Plasma cells, mean (SD)	0.01 (0.01)	0.02 (0.02)	0.001
Platelets, mean (SD)	0.00 (0.01)	0.00 (0.01)	0.837
Preadipocytes, mean (SD)	0.00 (0.00)	0.01 (0.02)	0.003
pro B cells, mean (SD)	0.00 (0.00)	0.01 (0.02)	<0.001
Sebocytes, mean (SD)	0.02 (0.01)	0.02 (0.01)	0.756
Skeletal muscle, mean (SD)	0.01 (0.04)	0.02 (0.05)	0.379
Smooth muscle, mean (SD)	0.15 (0.05)	0.11 (0.05)	<0.001
Tgd cells, mean (SD)	0.00 (0.00)	0.00 (0.00)	<0.001
Th1 cells, mean (SD)	0.02 (0.02)	0.02 (0.03)	0.684
Th2 cells, mean (SD)	0.05 (0.04)	0.05 (0.03)	0.41
Tregs, mean (SD)	0.01 (0.02)	0.02 (0.02)	<0.001
ImmuneScore, mean (SD)	0.03 (0.03)	0.12 (0.13)	<0.001
StromaScore, mean (SD)	0.02 (0.02)	0.02 (0.02)	0.75
MicroenvironmentScore, mean (SD)	0.04 (0.04)	0.13 (0.14)	<0.001