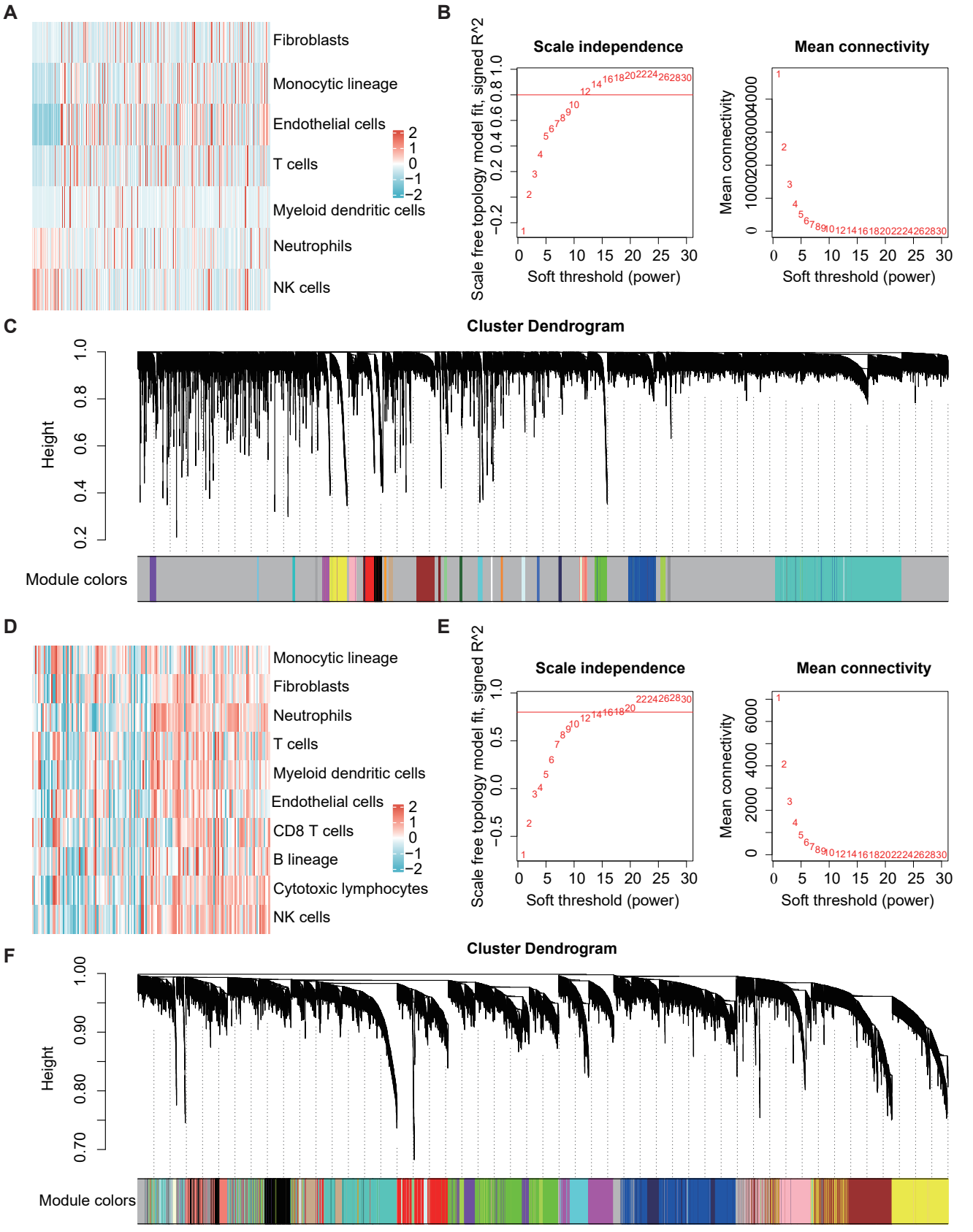
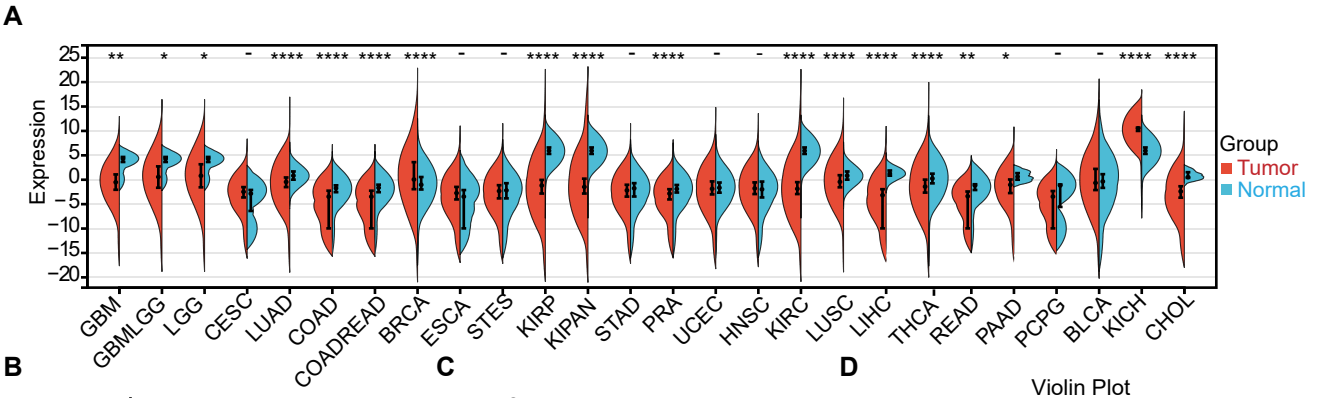
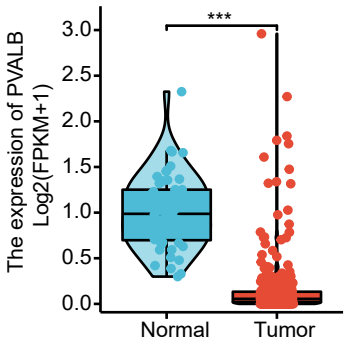
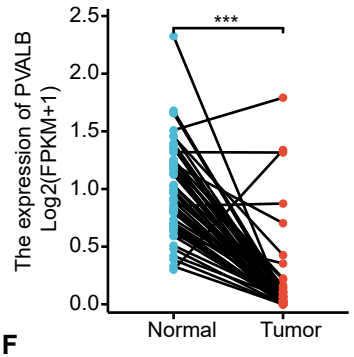
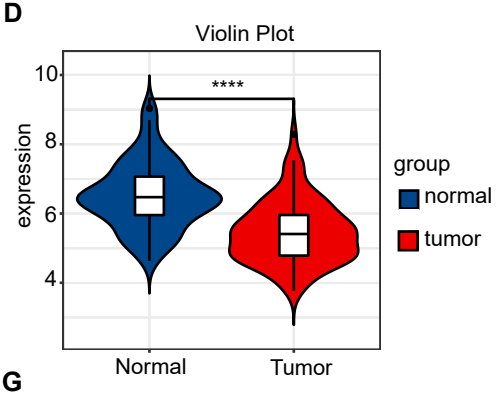

A
Fibroblasts
Monocytic lineage
Endothelial cells
T cells
Myeloid dendritic cells
Neutrophils
NK cells
2
1
0
−1
−2
B
Scale independence
Scale free topology model fit, signed R^2
Soft threshold (power)
Mean connectivity
Mean connectivity
Soft threshold (power)
C
Cluster Dendrogram
Height
Module colors
D
Monocytic lineage
Fibroblasts
Neutrophils
T cells
Myeloid dendritic cells
Endothelial cells
CD8 T cells
B lineage
Cytotoxic lymphocytes
NK cells
2
1
0
−1
−2
E
Scale independence
Scale free topology model fit, signed R^2
Soft threshold (power)
Mean connectivity
Mean connectivity
Soft threshold (power)
F
Cluster Dendrogram
Height
Module colors

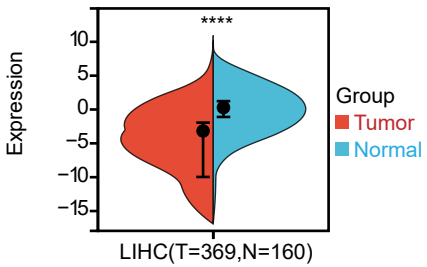
Supplementary Figure 1. Calculation of immune cell abundance and construction of weighted co-expression network. Heatmap illustrated the abundance of immune cell infiltration in **(A)** TCGA cohort. Determination of scale independence and average connectivity of **(B)** TCGA cohort in WGCNA. Dendrograms of all differentially expressed genes clustered in the **(C)** TCGA cohort. Heatmap illustrated the abundance of immune cell infiltration in **(D)** GSE54236 cohort. Determination of scale independence and average connectivity of **(E)** GSE54236 cohort in WGCNA. Dendrograms of all differentially expressed genes clustered in the **(F)** GSE54236 cohort.

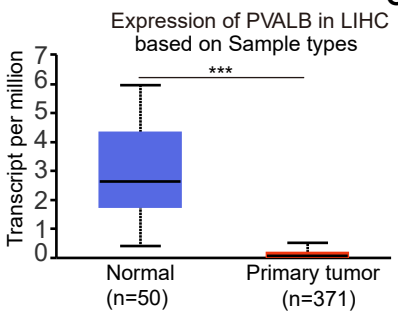


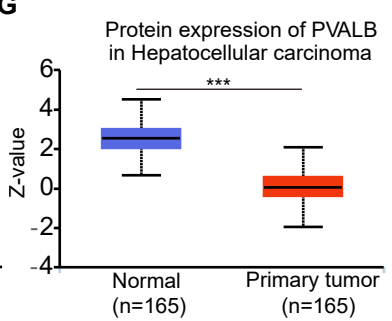






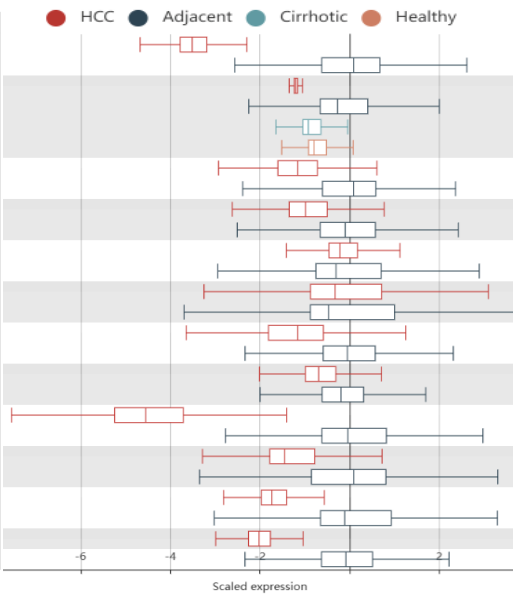




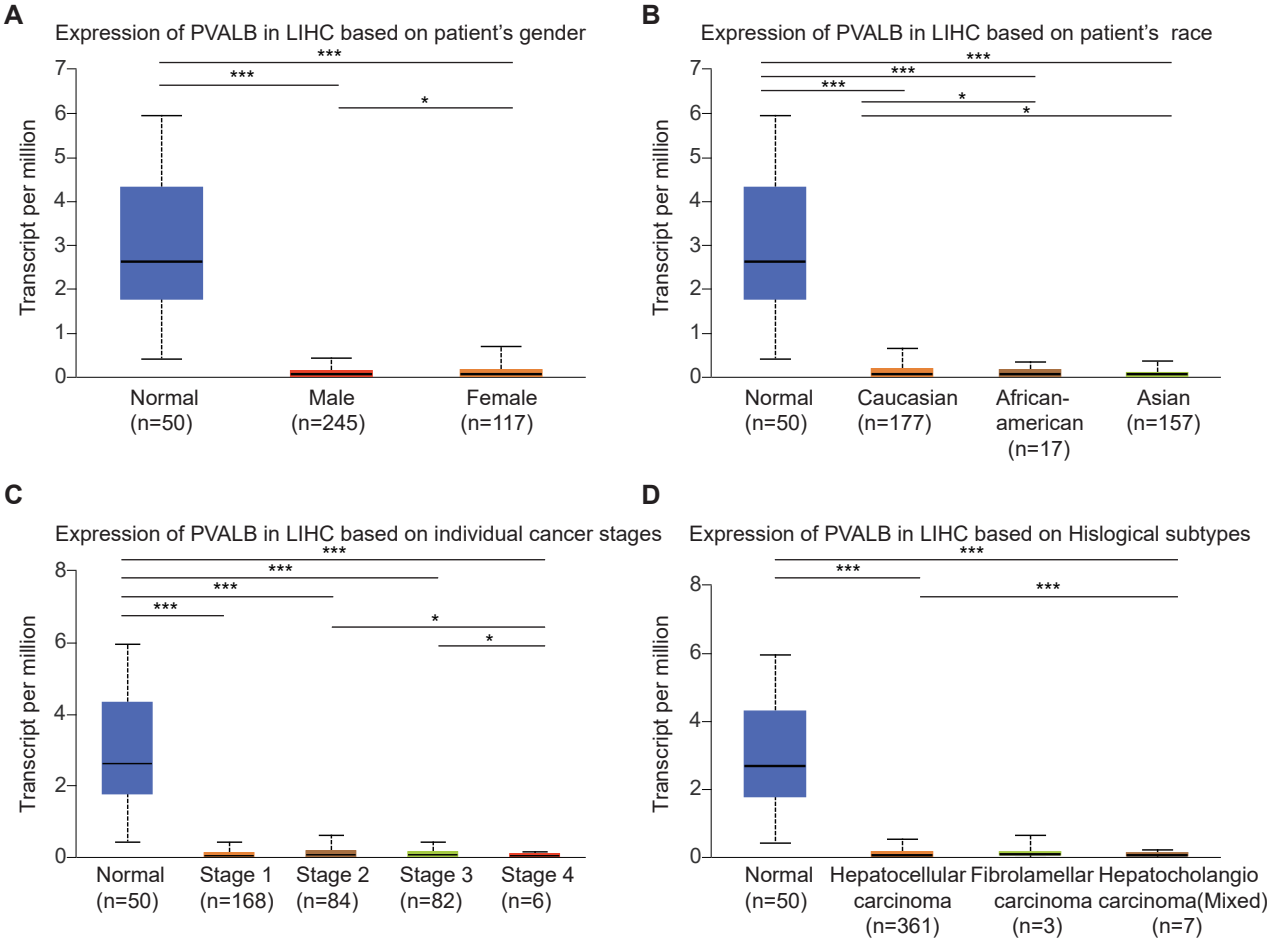


H

Dataset	P-value	Type	Nums	Mean	STD	IQR
HCCDB1	3.430e-65	HCC	100	4.254	0.5435	0.4771
		Adjacent	97	6.994	0.8021	1.038
HCCDB3	1.400e-46	HCC	268	0.04286	0.03886	0.01325
		Adjacent	243	0.2501	0.1790	0.1900
		Cirrhotic	40	0.1035	0.06422	0.07175
		Healthy	6	0.1212	0.06684	0.07125
HCCDB4	3.030e-30	HCC	240	6.441	0.2118	0.2230
		Adjacent	193	6.725	0.2526	0.2999
HCCDB6	2.700e-21	HCC	225	3.493	0.1747	0.1880
		Adjacent	220	3.683	0.2222	0.2738
HCCDB7	0.7106	HCC	80	9.370	0.4142	0.3262
		Adjacent	82	9.397	0.5145	0.7492
HCCDB11	0.7941	HCC	88	6.884	1.480	2.716
		Adjacent	48	6.960	1.714	3.214
HCCDB12	1.710e-11	HCC	81	5.458	0.8981	1.172
		Adjacent	80	6.520	0.9578	1.112
HCCDB13	0.00009030	HCC	228	3.875	0.4639	0.2202
		Adjacent	168	4.032	0.3241	0.2995
HCCDB15	4.320e-39	HCC	351	1.016	1.270	1.485
		Adjacent	49	5.076	0.9687	1.390
HCCDB16	5.670e-12	HCC	60	5.392	0.3119	0.4190
		Adjacent	60	5.913	0.4187	0.6961
HCCDB17	2.130e-15	HCC	115	6.706	0.3013	0.1950
		Adjacent	52	7.243	0.3468	0.5475
HCCDB18	1.140e-46	HCC	212	0.2604	0.6254	0.2700
		Adjacent	177	1.247	0.5534	0.6300

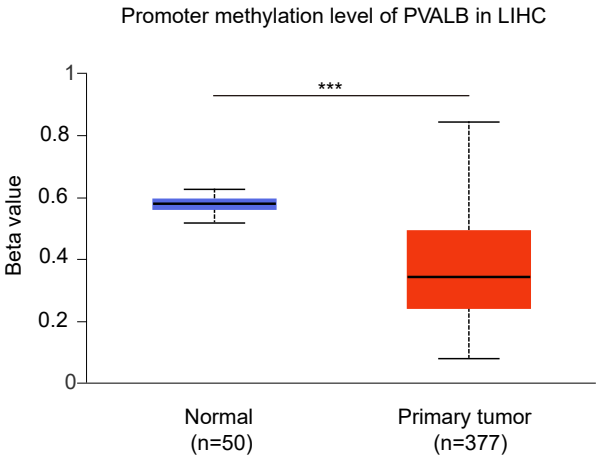


Supplementary Figure 2. The expression of PVALB in HCC. (A) Differential expression of PVALB in tumor and normal tissue across distinct cancers. **(B-C)** Expression level of PVALB in HCC tissue and normal tissue based on TCGA database. Expression level of PVALB in HCC were lower than normal tissue by **(D)** GSE54236 dataset and **(E)** TCGA +GTEx databases. The **(F)** mRNA and **(G)** protein expression level of PVALB in HCC tissue and normal tissue by UALCAN online website. **(H)** Comparison of PVALB expression level in HCC tissue and adiacent tissue in 12 cohorts of HCCDB, of which 10 cohorts were significant.(****$p < 0.0001$, ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$)

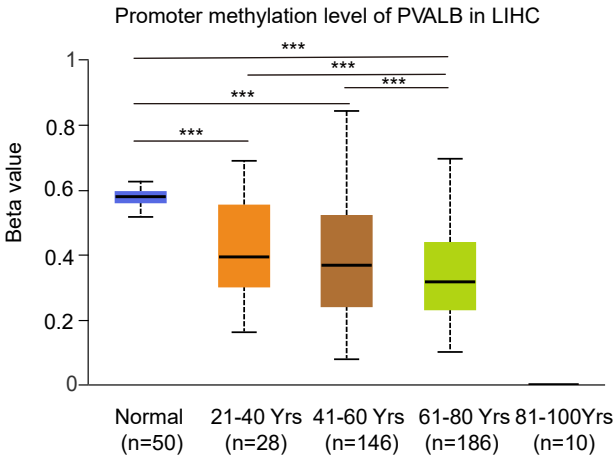


Supplementary Figure 3. Correlation of PVALB expression with clinicopathologic features in HCC patients based on the UALCAN online site. **(A)** gender; **(B)** race; **(C)** cancer stages. **(D)** histologic subtypes.(***p < 0.001, *p < 0.05)

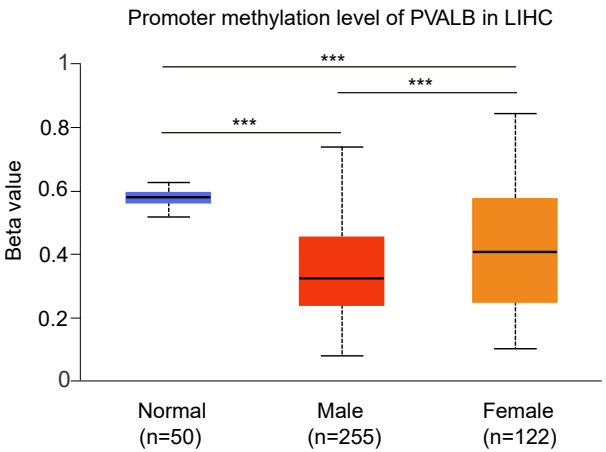
A


Promoter methylation level of PVALB in LIHC
Beta value
1
0.8
0.6
0.4
0.2
0
***
Normal
(n=50)
Primary tumor
(n=377)

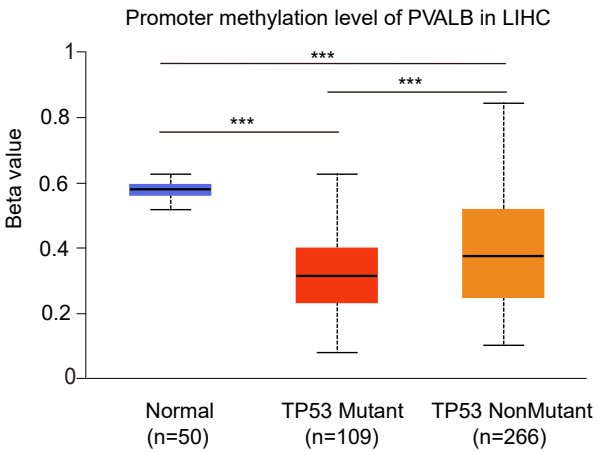
B


Promoter methylation level of PVALB in LIHC
Beta value
1
0.8
0.6
0.4
0.2
0
***
***
***
***
***
Normal
(n=50)
21-40 Yrs
(n=28)
41-60 Yrs
(n=146)
61-80 Yrs
(n=186)
81-100Yrs
(n=10)

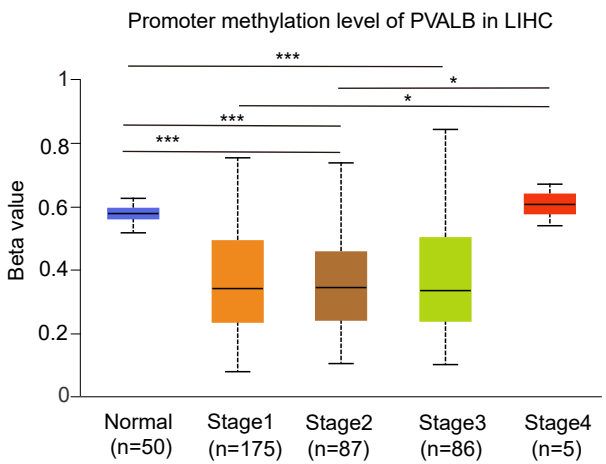
C


Promoter methylation level of PVALB in LIHC
Beta value
1
0.8
0.6
0.4
0.2
0
***
***
***
Normal
(n=50)
Male
(n=255)
Female
(n=122)

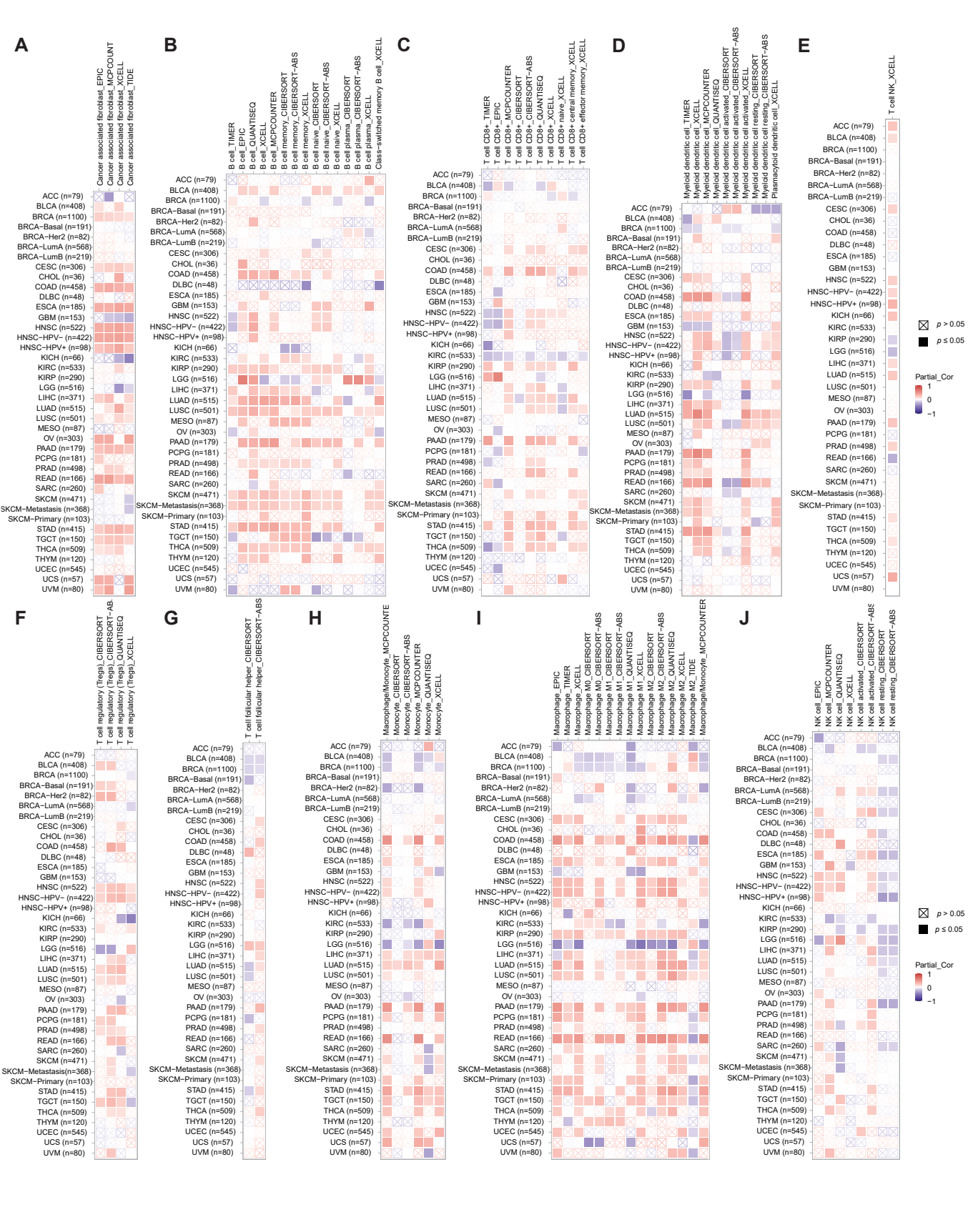
D


Promoter methylation level of PVALB in LIHC
Beta value
1
0.8
0.6
0.4
0.2
0
***
***
***
Normal
(n=50)
TP53 Mutant
(n=109)
TP53 NonMutant
(n=266)

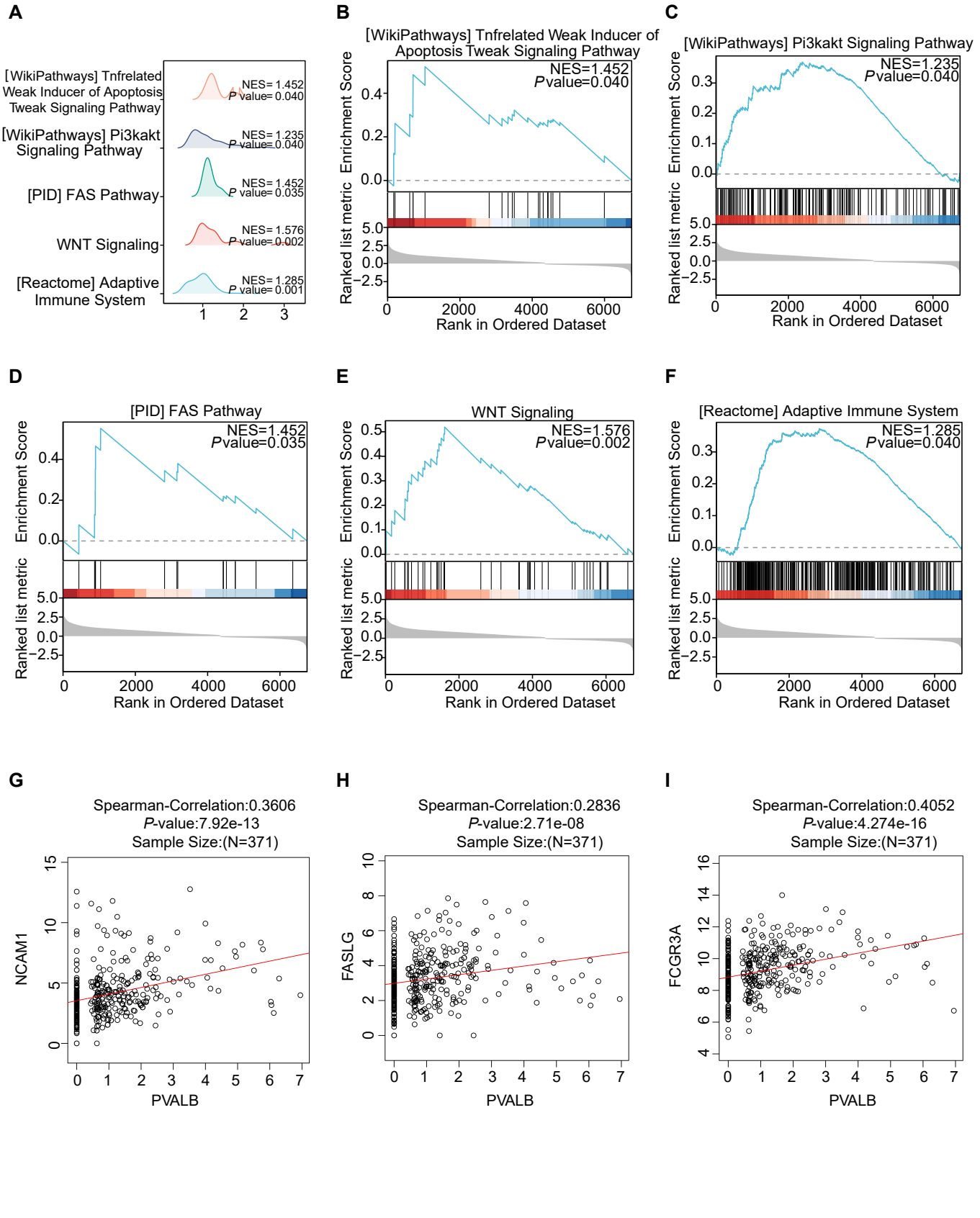
E


Promoter methylation level of PVALB in LIHC
Beta value
1
0.8
0.6
0.4
0.2
0
***
*
*
***
***
Normal
(n=50)
Stage1
(n=175)
Stage2
(n=87)
Stage3
(n=86)
Stage4
(n=5)

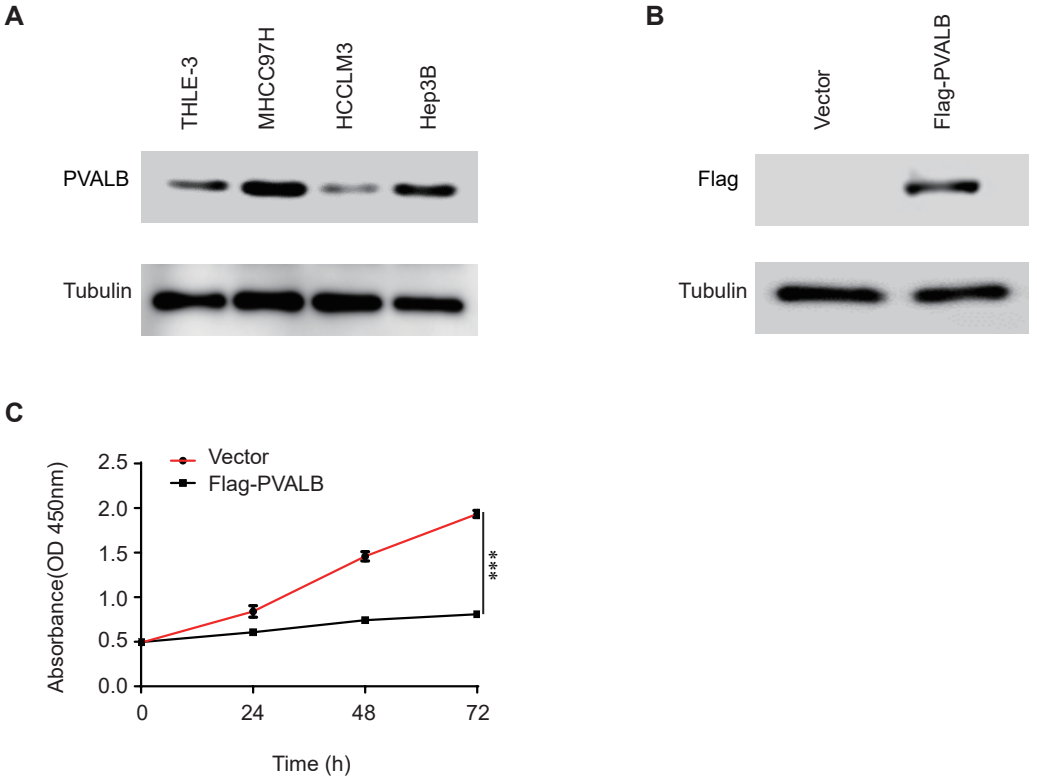
Supplementary Figure 4. PVALB promoter methylation level were associated with clinicopathological features. **(A)** Sample type **(B)** Patient age **(C)** Patient gender **(D)** TP53 mutation status **(E)** Cancer stage.(***p < 0.001, *p < 0.05)


A
Cancer associated fibroblast_EPIC
Cancer associated fibroblast_MCPCOUNT
Cancer associated fibroblast_XCELL
Cancer associated fibroblast_TIDE
B
B cell_TIMER
B cell_EPIC
B cell_QUANTISEQ
B cell_XCELL
B cell_MCPCOUNTER
B cell memory_CIBERSORT
B cell memory_CIBERSORT−ABS
B cell memory_XCELL
B cell naive_CIBERSORT
B cell naive_CIBERSORT−ABS
B cell naive_XCELL
B cell plasma_CIBERSORT
B cell plasma_CIBERSORT−ABS
B cell plasma_XCELL
Class−switched memory B cell_XCELL
C
T cell CD8+_TIMER
T cell CD8+_EPIC
T cell CD8+_MCPCOUNTER
T cell CD8+_CIBERSORT
T cell CD8+_CIBERSORT−ABS
T cell CD8+_QUANTISEQ
T cell CD8+_XCELL
T cell CD8+ naive_XCELL
T cell CD8+ central memory_XCELL
T cell CD8+ effector memory_XCELL
D
Myeloid dendritic cell_TIMER
Myeloid dendritic cell_XCELL
Myeloid dendritic cell_MCPCOUNTER
Myeloid dendritic cell_QUANTISEQ
Myeloid dendritic cell activated_CIBERSORT
Myeloid dendritic cell activated_CIBERSORT−ABS
Myeloid dendritic cell activated_XCELL
Myeloid dendritic cell resting_CIBERSORT
Myeloid dendritic cell resting_CIBERSORT−ABS
Plasmacytoid dendritic cell_XCELL
E
T cell NK_XCELL
ACC (n=79)
BLCA (n=408)
BRCA (n=1100)
BRCA−Basal (n=191)
BRCA−Her2 (n=82)
BRCA−LumA (n=568)
BRCA−LumB (n=219)
CESC (n=306)
CHOL (n=36)
COAD (n=458)
DLBC (n=48)
ESCA (n=185)
GBM (n=153)
HNSC (n=522)
HNSC−HPV− (n=422)
HNSC−HPV+ (n=98)
KICH (n=66)
KIRC (n=533)
KIRP (n=290)
LGG (n=516)
LIHC (n=371)
LUAD (n=515)
LUSC (n=501)
MESO (n=87)
OV (n=303)
PAAD (n=179)
PCPG (n=181)
PRAD (n=498)
READ (n=166)
SARC (n=260)
SKCM (n=471)
SKCM−Metastasis (n=368)
SKCM−Primary (n=103)
STAD (n=415)
TGCT (n=150)
THCA (n=509)
THYM (n=120)
UCEC (n=545)
UCS (n=57)
UVM (n=80)
p > 0.05
p ≤ 0.05
Partial_Cor
1
0
−1
F
T cell regulatory (Tregs)_CIBERSORT
T cell regulatory (Tregs)_CIBERSORT−ABS
T cell regulatory (Tregs)_QUANTISEQ
T cell regulatory (Tregs)_XCELL
G
T cell follicular helper_CIBERSORT
T cell follicular helper_CIBERSORT−ABS
H
Macrophage/Monocyte_MCPCOUNTER
Monocyte_CIBERSORT
Monocyte_CIBERSORT−ABS
Monocyte_MCPCOUNTER
Monocyte_QUANTISEQ
Monocyte_XCELL
I
Macrophage_EPIC
Macrophage_TIMER
Macrophage_XCELL
Macrophage M0_CIBERSORT
Macrophage M0_CIBERSORT−ABS
Macrophage M1_CIBERSORT
Macrophage M1_CIBERSORT−ABS
Macrophage M1_QUANTISEQ
Macrophage M1_XCELL
Macrophage M2_CIBERSORT
Macrophage M2_CIBERSORT−ABS
Macrophage M2_QUANTISEQ
Macrophage M2_XCELL
Macrophage M2_TIDE
Macrophage/Monocyte_MCPCOUNTER
J
NK cell_EPIC
NK cell_MCPCOUNTER
NK cell_QUANTISEQ
NK cell_XCELL
NK cell activated_CIBERSORT
NK cell activated_CIBERSORT−ABS
NK cell resting_CIBERSORT
NK cell resting_CIBERSORT−ABS

Supplementary Figure 5. Correlation between PVALB and immune infiltration in pan- cancer obtained based on different algorithms (A) Cancer associated fibroblast **(B)** B cell **(C)** T cell CD8+ **(D)** Myeloid dendritic cell **(E)** T cell NK **(F)** T cell regulatory **(G)** T cell follicular helper **(H)** Monocyte **(I)** Macrophage **(J)** NK cell.


A
[WikiPathways] Tnfrelated Weak Inducer of Apoptosis Tweak Signaling Pathway
NES= 1.452
P value= 0.040
[WikiPathways] Pi3kakt Signaling Pathway
NES= 1.235
P value= 0.040
[PID] FAS Pathway
NES= 1.452
P value= 0.035
WNT Signaling
NES= 1.576
P value= 0.002
[Reactome] Adaptive Immune System
NES= 1.285
P value= 0.001
B
[WikiPathways] Tnfrelated Weak Inducer of Apoptosis Tweak Signaling Pathway
NES=1.452
Pvalue=0.040
Enrichment Score
Ranked list metric
Rank in Ordered Dataset
C
[WikiPathways] Pi3kakt Signaling Pathway
NES=1.235
Pvalue=0.040
D
[PID] FAS Pathway
NES=1.452
Pvalue=0.035
E
WNT Signaling
NES=1.576
Pvalue=0.002
F
[Reactome] Adaptive Immune System
NES=1.285
Pvalue=0.040
G
Spearman-Correlation:0.3606
P-value:7.92e-13
Sample Size:(N=371)
NCAM1
PVALB
H
Spearman-Correlation:0.2836
P-value:2.71e-08
Sample Size:(N=371)
FASLG
PVALB
I
Spearman-Correlation:0.4052
P-value:4.274e-16
Sample Size:(N=371)
FCGR3A
PVALB

Supplementary Figure 6. PVALB and Fas/FasL signaling pathway. (A) Mountain range plot showed the five pathways enriched for PVALB. GSEA results showed differential enrichment of genes with high PVALB expression **(B)**Tnfrelated Weak Inducer of Apoptosis Tweak Signaling Pathway; **(C)** Pi3kalkt Signaling Pathway; **(D)** Fas Pathway; **(E)** WNT Pathway; **(F)** Adaptive Immune System. NES, normalized enrichment score; P <0.05. **(G-I)** Scatter plots showed correlation between FCGR3A (CD16), NCAM1 (CD56), FASLG (FasL) and PVALB in HCC.



Supplementary Figure 7. Selection and validation of cell lines and CCK8 assay. (A) Expression of PVALB in different cell lines **(B)** The validation of the transfection efficiency of the PVALB gene before doing the phenotypic function experiment**(C)** CCK8. (***p < 0.001)