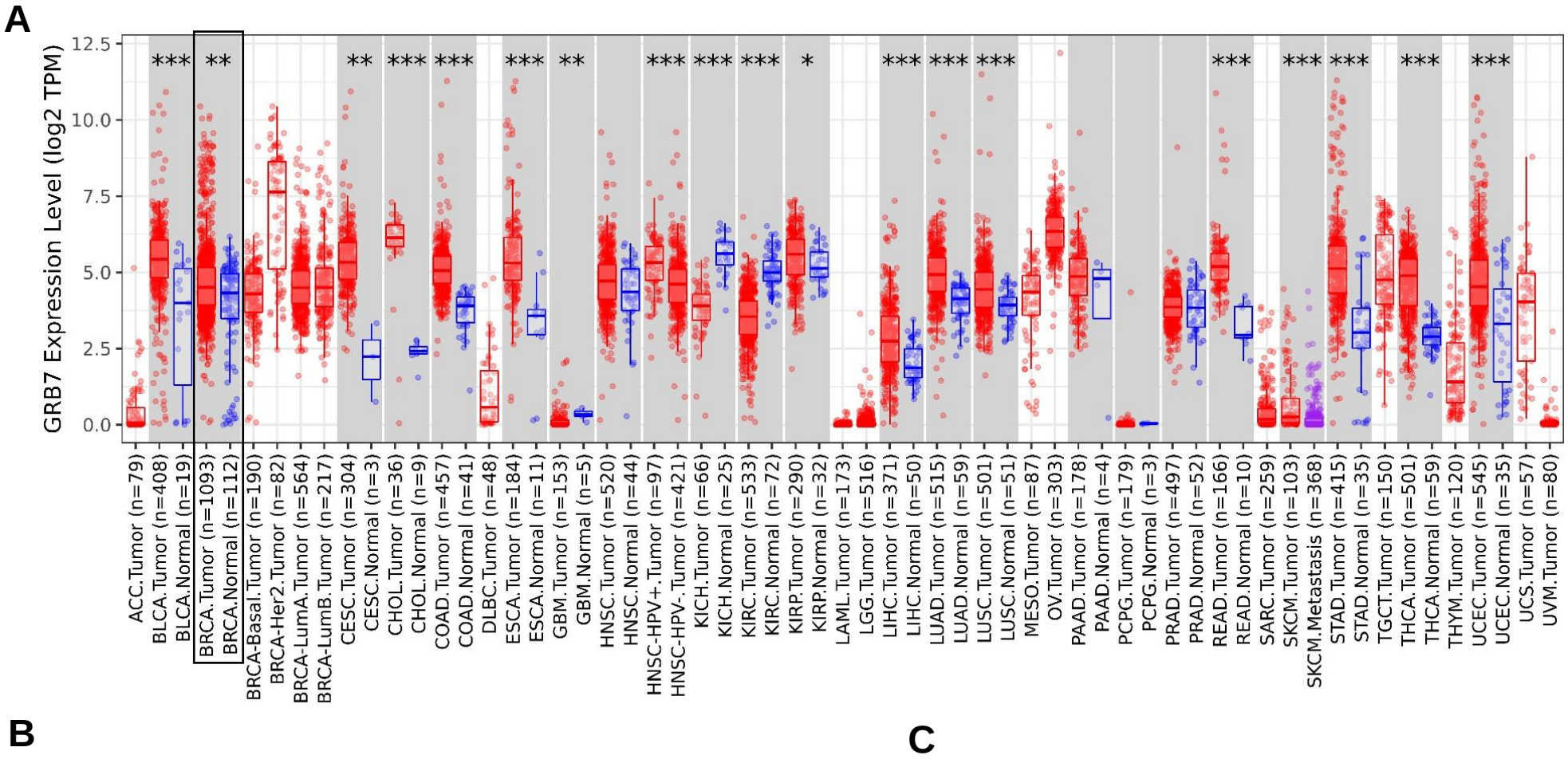
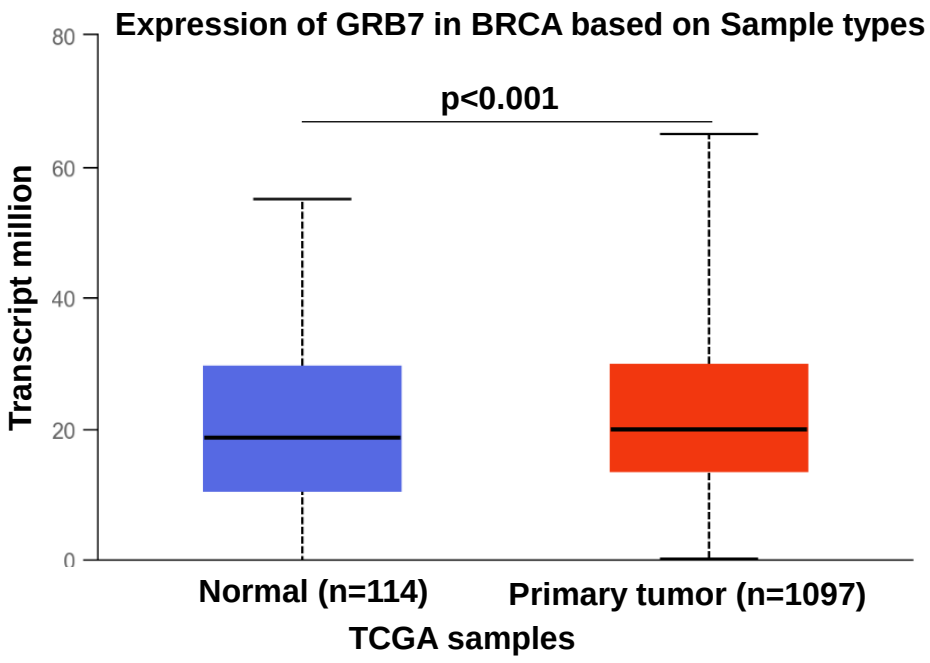
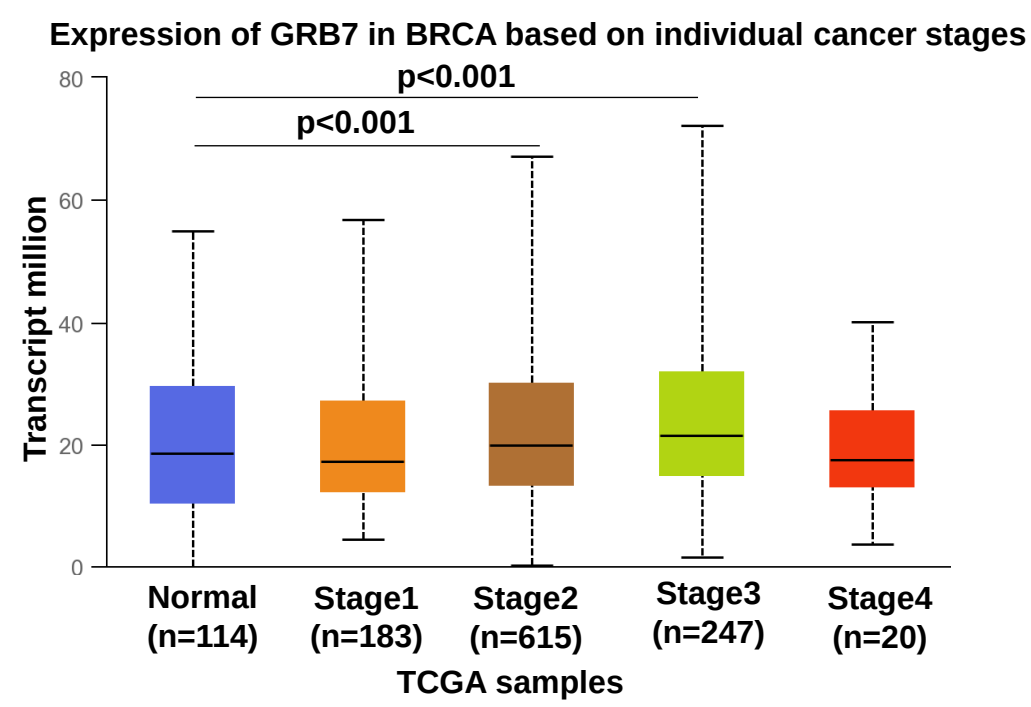
A


GRB7 Expression Level (log2 TPM)
12.5
10.0
7.5
5.0
2.5
0.0
***
**
ACC.Tumor (n=79)
BLCA.Tumor (n=408)
BLCA.Normal (n=19)
BRCA.Tumor (n=1093)
BRCA.Normal (n=112)
BRCA-Basal.Tumor (n=190)
BRCA-Her2.Tumor (n=82)
BRCA-LumA.Tumor (n=564)
BRCA-LumB.Tumor (n=217)
CESC.Tumor (n=304)
CESC.Normal (n=3)
CHOL.Tumor (n=36)
CHOL.Normal (n=9)
COAD.Tumor (n=457)
COAD.Normal (n=41)
DLBC.Tumor (n=48)
ESCA.Tumor (n=184)
ESCA.Normal (n=11)
GBM.Tumor (n=153)
GBM.Normal (n=5)
HNSC.Tumor (n=520)
HNSC.Normal (n=44)
HNSC-HPV+.Tumor (n=97)
HNSC-HPV-.Tumor (n=421)
KICH.Tumor (n=66)
KICH.Normal (n=25)
KIRC.Tumor (n=533)
KIRC.Normal (n=72)
KIRP.Tumor (n=290)
KIRP.Normal (n=32)
LAML.Tumor (n=173)
LGG.Tumor (n=516)
LIHC.Tumor (n=371)
LIHC.Normal (n=50)
LUAD.Tumor (n=515)
LUAD.Normal (n=59)
LUSC.Tumor (n=501)
LUSC.Normal (n=51)
MESO.Tumor (n=87)
OV.Tumor (n=303)
PAAD.Tumor (n=178)
PAAD.Normal (n=4)
PCPG.Tumor (n=179)
PCPG.Normal (n=3)
PRAD.Tumor (n=497)
PRAD.Normal (n=52)
READ.Tumor (n=166)
READ.Normal (n=10)
SARC.Tumor (n=259)
SKCM.Tumor (n=103)
SKCM.Metastasis (n=368)
STAD.Tumor (n=415)
STAD.Normal (n=35)
TGCT.Tumor (n=150)
THCA.Tumor (n=501)
THCA.Normal (n=59)
THYM.Tumor (n=120)
UCEC.Tumor (n=545)
UCEC.Normal (n=35)
UCS.Tumor (n=57)
UVM.Tumor (n=80)

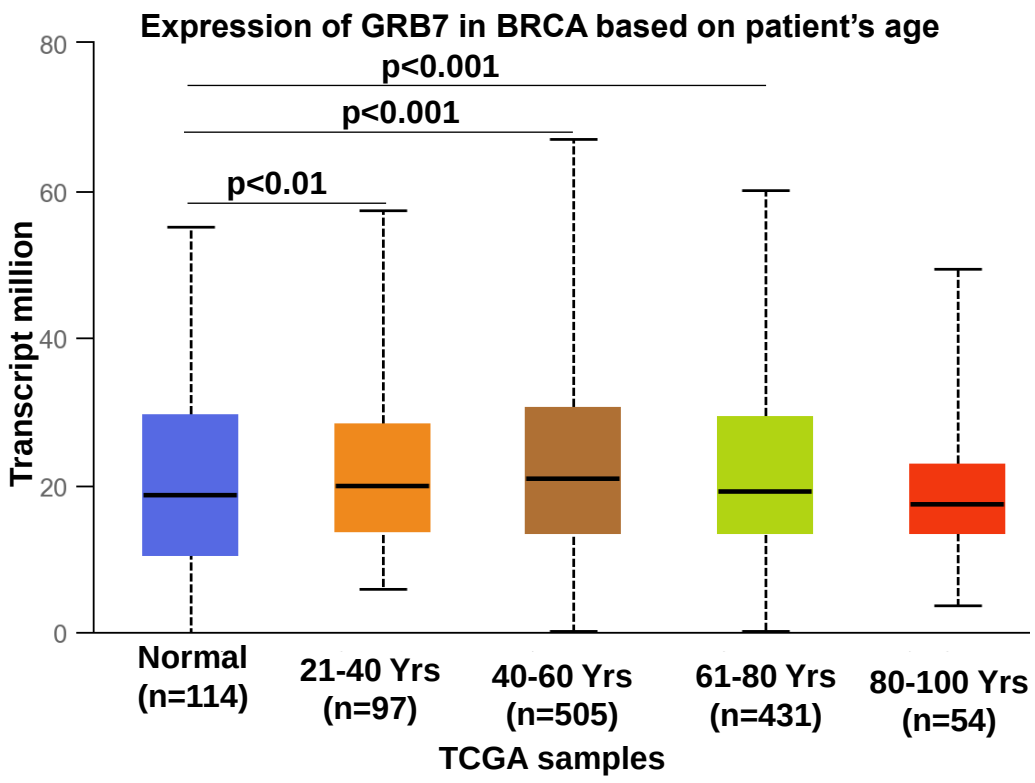
B


Expression of GRB7 in BRCA based on Sample types
p<0.001
Transcript million
80
60
40
20
0
Normal (n=114)
Primary tumor (n=1097)
TCGA samples

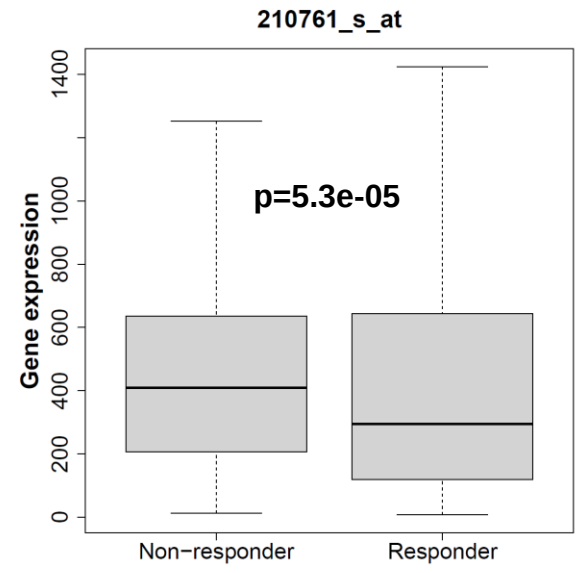
C


Expression of GRB7 in BRCA based on individual cancer stages
p<0.001
p<0.001
Transcript million
80
60
40
20
0
Normal (n=114)
Stage1 (n=183)
Stage2 (n=615)
Stage3 (n=247)
Stage4 (n=20)
TCGA samples

D


Expression of GRB7 in BRCA based on patient's age
p<0.001
p<0.001
p<0.01
Transcript million
80
60
40
20
0
Normal (n=114)
21-40 Yrs (n=97)
40-60 Yrs (n=505)
61-80 Yrs (n=431)
80-100 Yrs (n=54)
TCGA samples

E


210761_s_at
Gene expression
1400
1000
800
600
400
200
0
p=5.3e-05
Non−responder
Responder

F

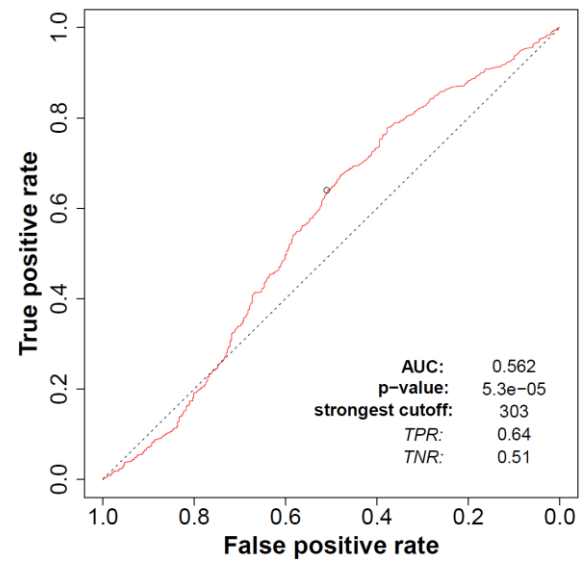

True positive rate
1.0
0.8
0.6
0.4
0.2
0.0
AUC: 0.562
p−value: 5.3e−05
strongest cutoff: 303
TPR: 0.64
TNR: 0.51
False positive rate

Figure S1. GRB7 is highly expressed in BC and affects prognosis. (A) Pan-cancer expression profile of GRB7 from the TIMER database. **(B)** Expression of GRB7 in BC and normal breast tissue. **(C-D)** Expression level of GRB7 in different stages and ages of BC patients from the TCGA database. **(E)** Box plot showing increased GRB7 levels in non-responders (any chemotherapy) compared to responders in BC. **(F)** AUC curve of GRB7 based on the 5-year RFS of BC after any chemotherapy. All data were from http://www. rocplot. org. Data are shown as the mean ± SD. **p*<0.05, ***p*<0.01, ****p*<0.001.

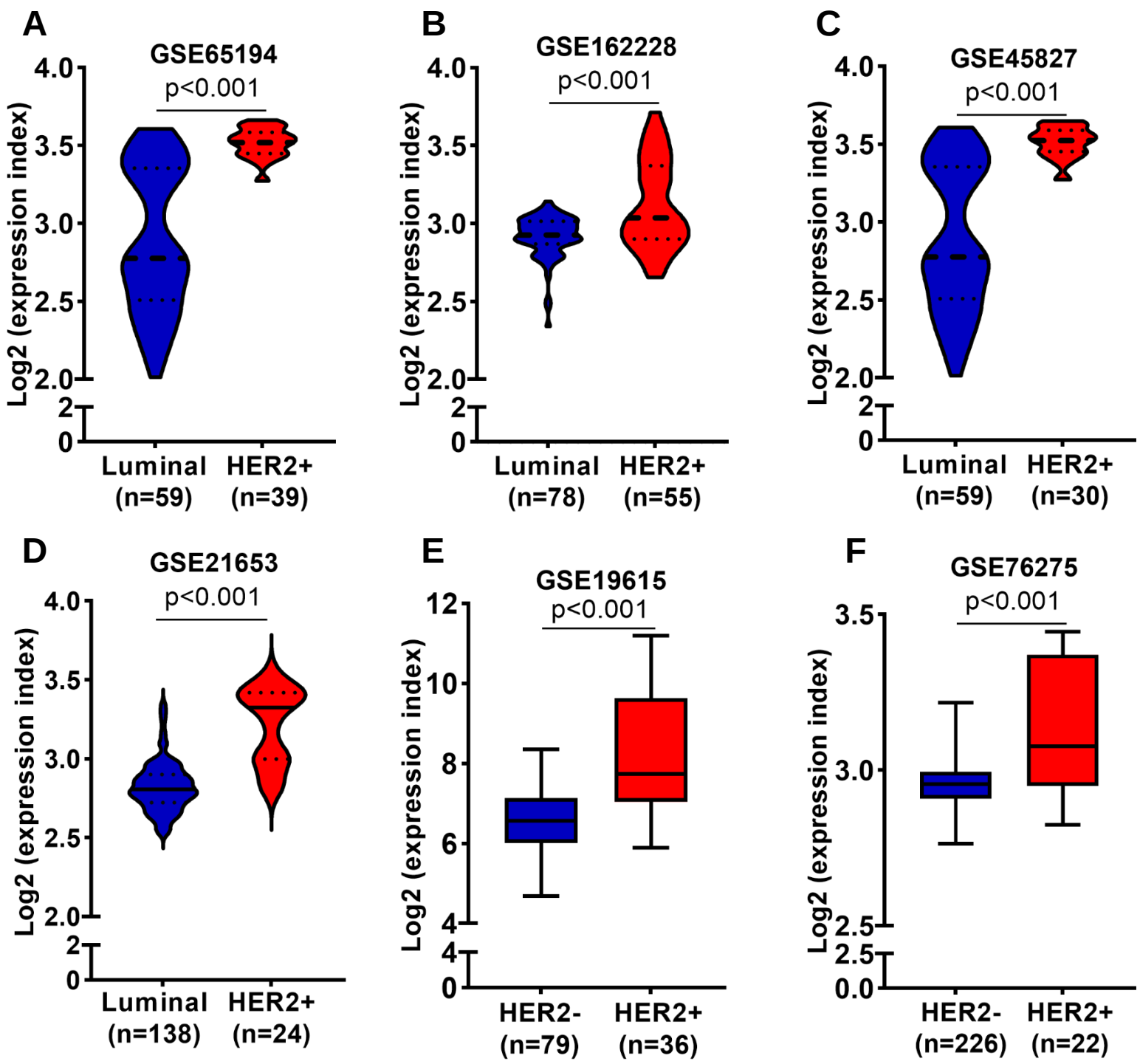
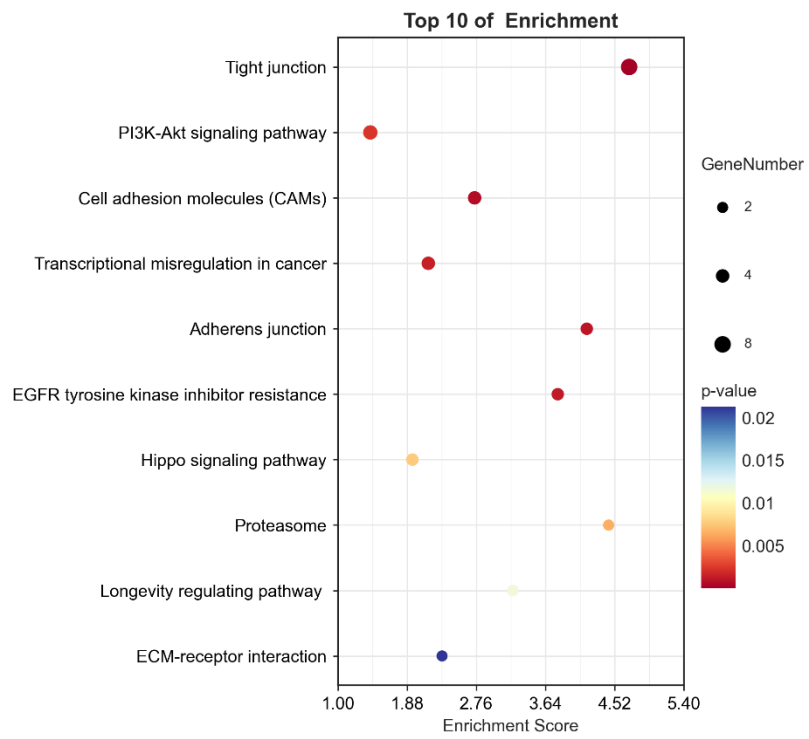
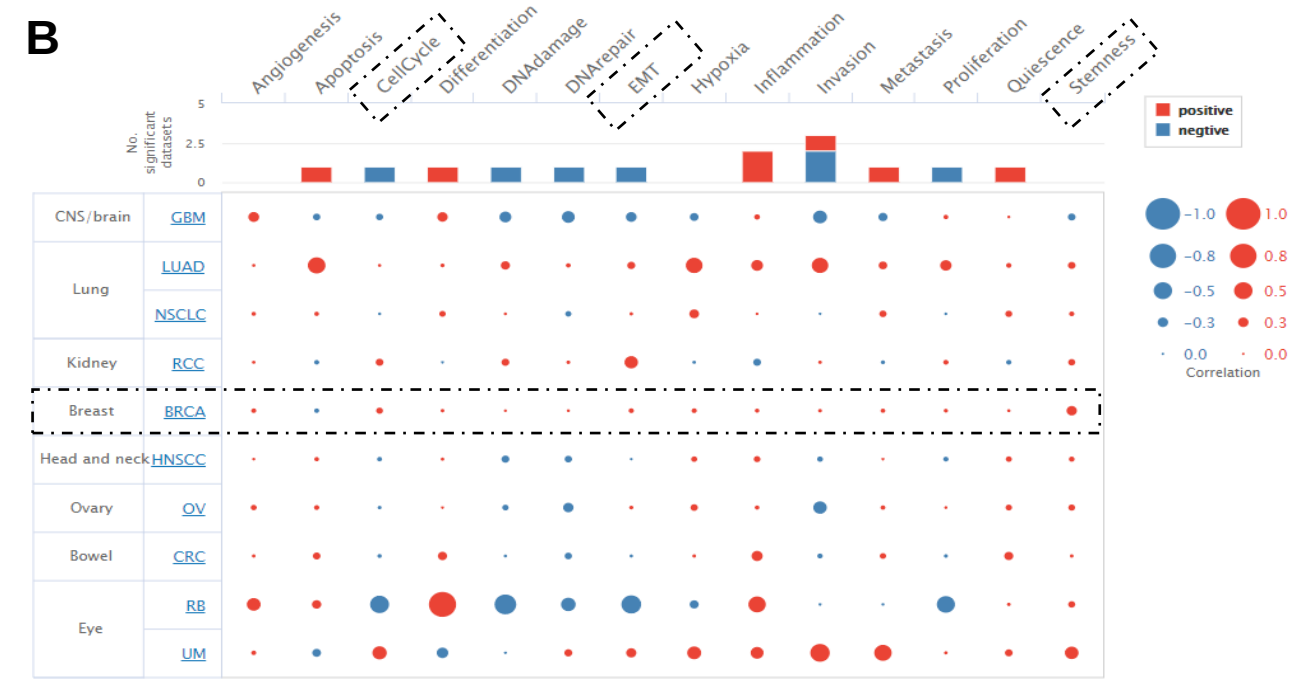

A
GSE65194
p<0.001
Log2 (expression index)
4.0
3.5
3.0
2.5
2.0
2
0
Luminal (n=59)
HER2+ (n=39)
B
GSE162228
p<0.001
Log2 (expression index)
4.0
3.5
3.0
2.5
2.0
2
0
Luminal (n=78)
HER2+ (n=55)
C
GSE45827
p<0.001
Log2 (expression index)
4.0
3.5
3.0
2.5
2.0
2
0
Luminal (n=59)
HER2+ (n=30)
D
GSE21653
p<0.001
Log2 (expression index)
4.0
3.5
3.0
2.5
2.0
2
0
Luminal (n=138)
HER2+ (n=24)
E
GSE19615
p<0.001
Log2 (expression index)
12
10
8
6
4
4
0
HER2- (n=79)
HER2+ (n=36)
F
GSE76275
p<0.001
Log2 (expression index)
3.5
3.0
2.5
2.5
0.0
HER2- (n=226)
HER2+ (n=22)

Figure S2. Expression of GRB7 in GEO datasets. (A-C) Expression levels of GRB7 in luminal and HER2+ BC were compared in the GSE65194, GSE162228, GSE45827 and GSE21653 datasets. **(D-F)** Expression levels of GRB7 in HER2- and HER2+ BC were compared in the GSE19615 and GSE76275 datasets.

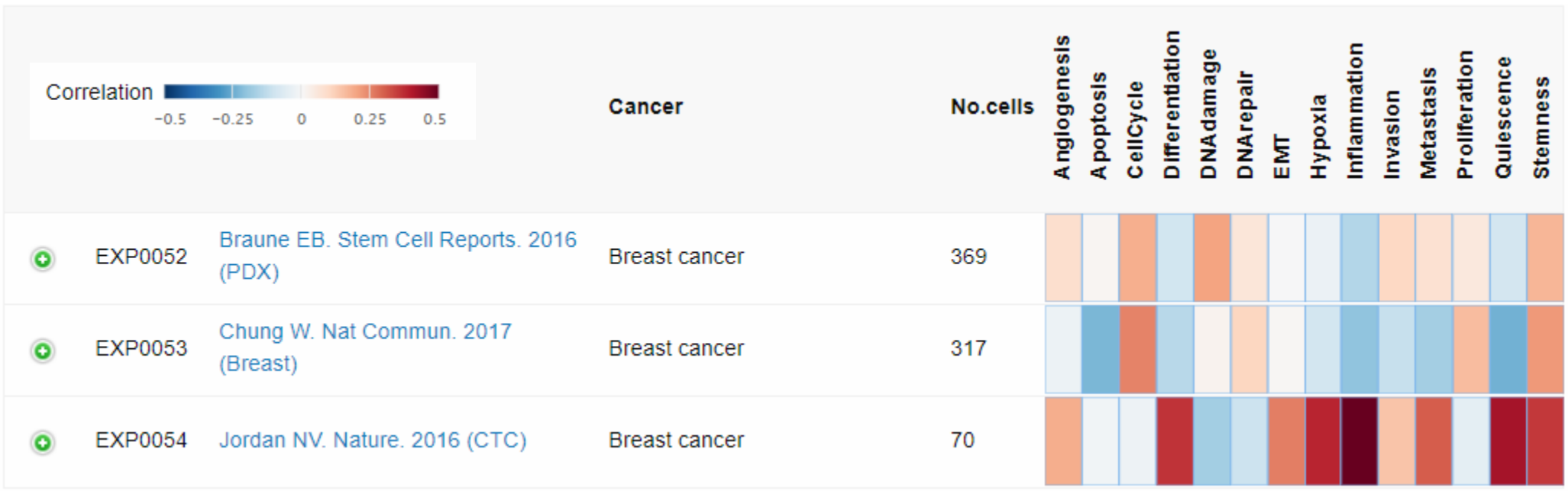
A


Top 10 of Enrichment
Tight junction
PI3K-Akt signaling pathway
Cell adhesion molecules (CAMs)
Transcriptional misregulation in cancer
Adherens junction
EGFR tyrosine kinase inhibitor resistance
Hippo signaling pathway
Proteasome
Longevity regulating pathway
ECM-receptor interaction
1.00
1.88
2.76
3.64
4.52
5.40
Enrichment Score
GeneNumber
2
4
8
p-value
0.02
0.015
0.01
0.005

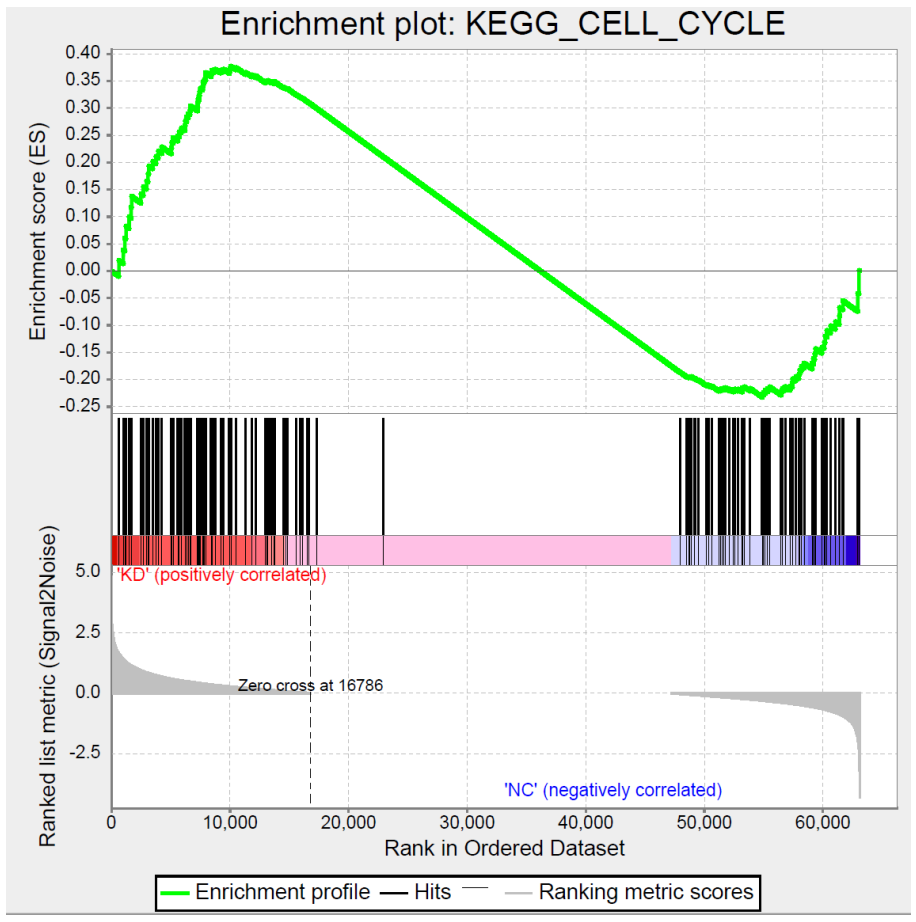
B


Angiogenesis
Apoptosis
CellCycle
Differentiation
DNAdamage
DNArepair
EMT
Hypoxia
Inflammation
Invasion
Metastasis
Proliferation
Quiescence
Stemness
No. significant datasets
positive
negtive
CNS/brain
GBM
Lung
LUAD
NSCLC
Kidney
RCC
Breast
BRCA
Head and neck
HNSCC
Ovary
OV
Bowel
CRC
Eye
RB
UM
Correlation

C


Correlation
−0.5
−0.25
0
0.25
0.5
Cancer
No.cells
Anglogenesis
Apoptosis
CellCycle
Differentiation
DNAdamage
DNArepair
EMT
Hypoxia
Inflammation
Invasion
Metastasis
Proliferation
Quiescence
Stemness
EXP0052
Braune EB. Stem Cell Reports. 2016 (PDX)
Breast cancer
369
EXP0053
Chung W. Nat Commun. 2017 (Breast)
Breast cancer
317
EXP0054
Jordan NV. Nature. 2016 (CTC)
Breast cancer
70

D


Enrichment plot: KEGG_CELL_CYCLE
Enrichment score (ES)
'KD' (positively correlated)
Zero cross at 16786
'NC' (negatively correlated)
Ranked list metric (Signal2Noise)
Rank in Ordered Dataset
Enrichment profile
Hits
Ranking metric scores

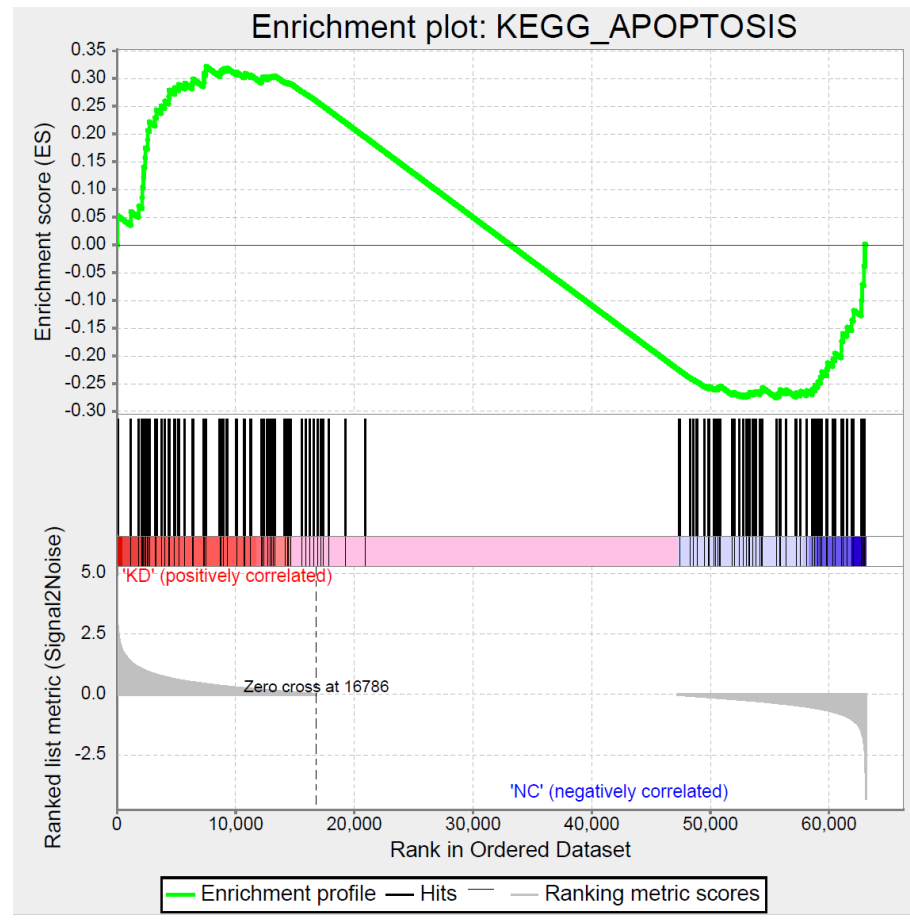
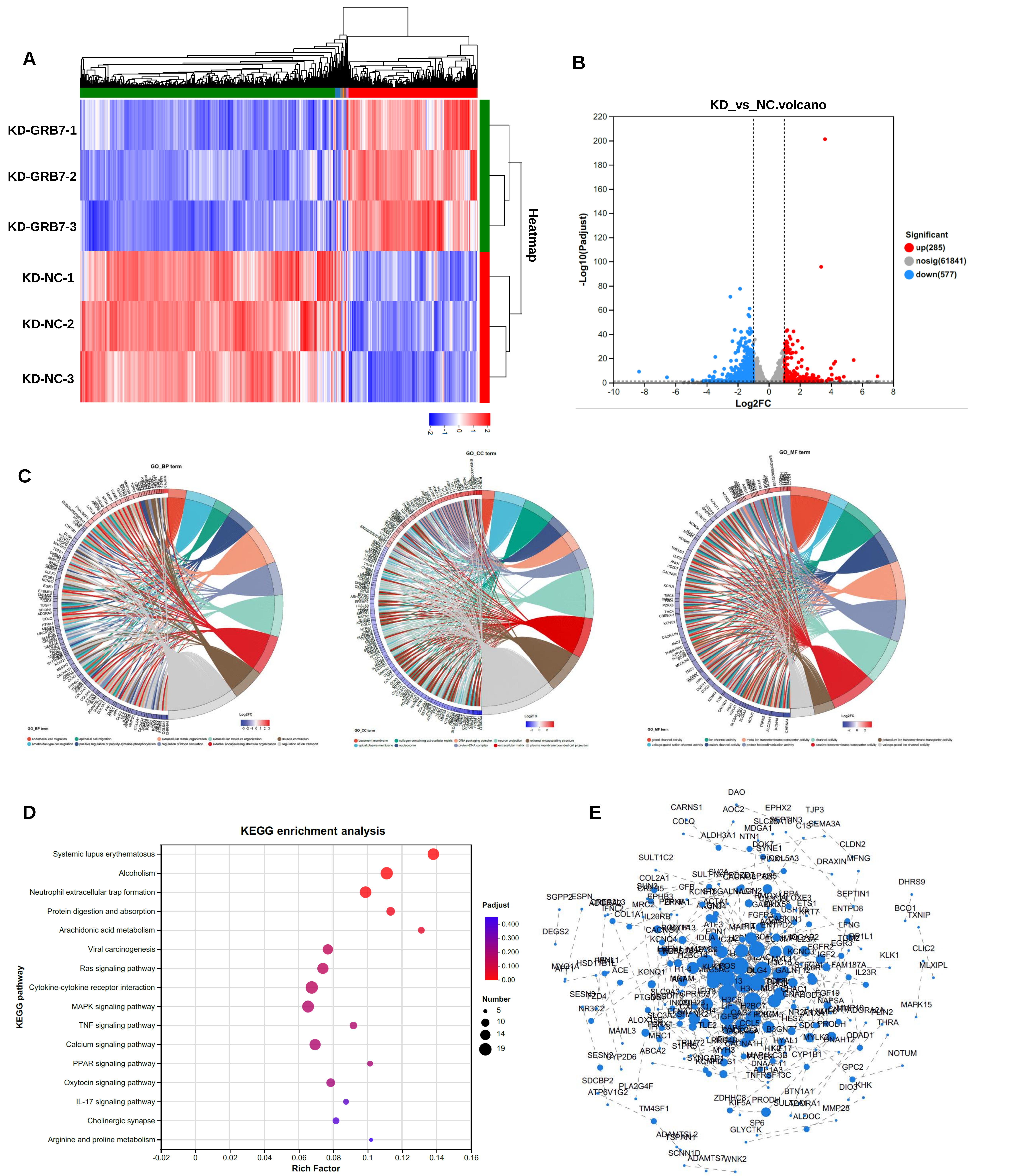

Enrichment plot: KEGG_APOPTOSIS
Enrichment score (ES)
'KD' (positively correlated)
Zero cross at 16786
'NC' (negatively correlated)
Ranked list metric (Signal2Noise)
Rank in Ordered Dataset
Enrichment profile
Hits
Ranking metric scores

Figure S3. Predictive analysis of GRB7 in cancer functional status. (A) KEGG enrichment analysis of GRB7 co-expressed genes. **(B)** The function of GRB7 in various tumors was analyzed using the CancerSEA database. **(C-D)** The biological role of GRB7 in BC was analyzed from the CancerSEA database and GSEA database.



A
KD-GRB7-1
KD-GRB7-2
KD-GRB7-3
KD-NC-1
KD-NC-2
KD-NC-3
Heatmap
-2 -1 0 1 2
B
KD_vs_NC.volcano
-Log10(Padjust)
Log2FC
Significant
up(285)
nosig(61841)
down(577)
C
GO_BP term
GO_CC term
GO_MF term
Log2FC
endothelial cell migration
epithelial cell migration
extracellular matrix organization
extracellular structure organization
muscle contraction
ameboidal-type cell migration
positive regulation of peptidyl-tyrosine phosphorylation
regulation of blood circulation
external encapsulating structure organization
regulation of ion transport
basement membrane
collagen-containing extracellular matrix
DNA packaging complex
neuron projection
external encapsulating structure
apical plasma membrane
nucleosome
protein-DNA complex
extracellular matrix
plasma membrane bounded cell projection
gated channel activity
ion channel activity
metal ion transmembrane transporter activity
channel activity
potassium ion transmembrane transporter activity
voltage-gated cation channel activity
cation channel activity
protein heterodimerization activity
passive transmembrane transporter activity
voltage-gated ion channel activity
D
KEGG enrichment analysis
Systemic lupus erythematosus
Alcoholism
Neutrophil extracellular trap formation
Protein digestion and absorption
Arachidonic acid metabolism
Viral carcinogenesis
Ras signaling pathway
Cytokine-cytokine receptor interaction
MAPK signaling pathway
TNF signaling pathway
Calcium signaling pathway
PPAR signaling pathway
Oxytocin signaling pathway
IL-17 signaling pathway
Cholinergic synapse
Arginine and proline metabolism
KEGG pathway
Rich Factor
Padjust
Number
E

Figure S4. Enrichment analysis of differential genes after knockdown of GRB7. **(A)** Heatmap of the transcriptional changes. **(B)** Volcano plot showing up- and downregulated genes in GRB7-depleted SK-BR-3 cells. **(C)** Based on the different genes of GRB7 knockdown, the GO chord plots were visualized. **(D)** KEGG enrichment analysis of transcriptionally altered genes identified by RNA-seq in GRB7-deficient SK-BR-3 cells. **(E)** Protein interaction network analysis of differentially expressed genes after knockdown of GRB7.

A

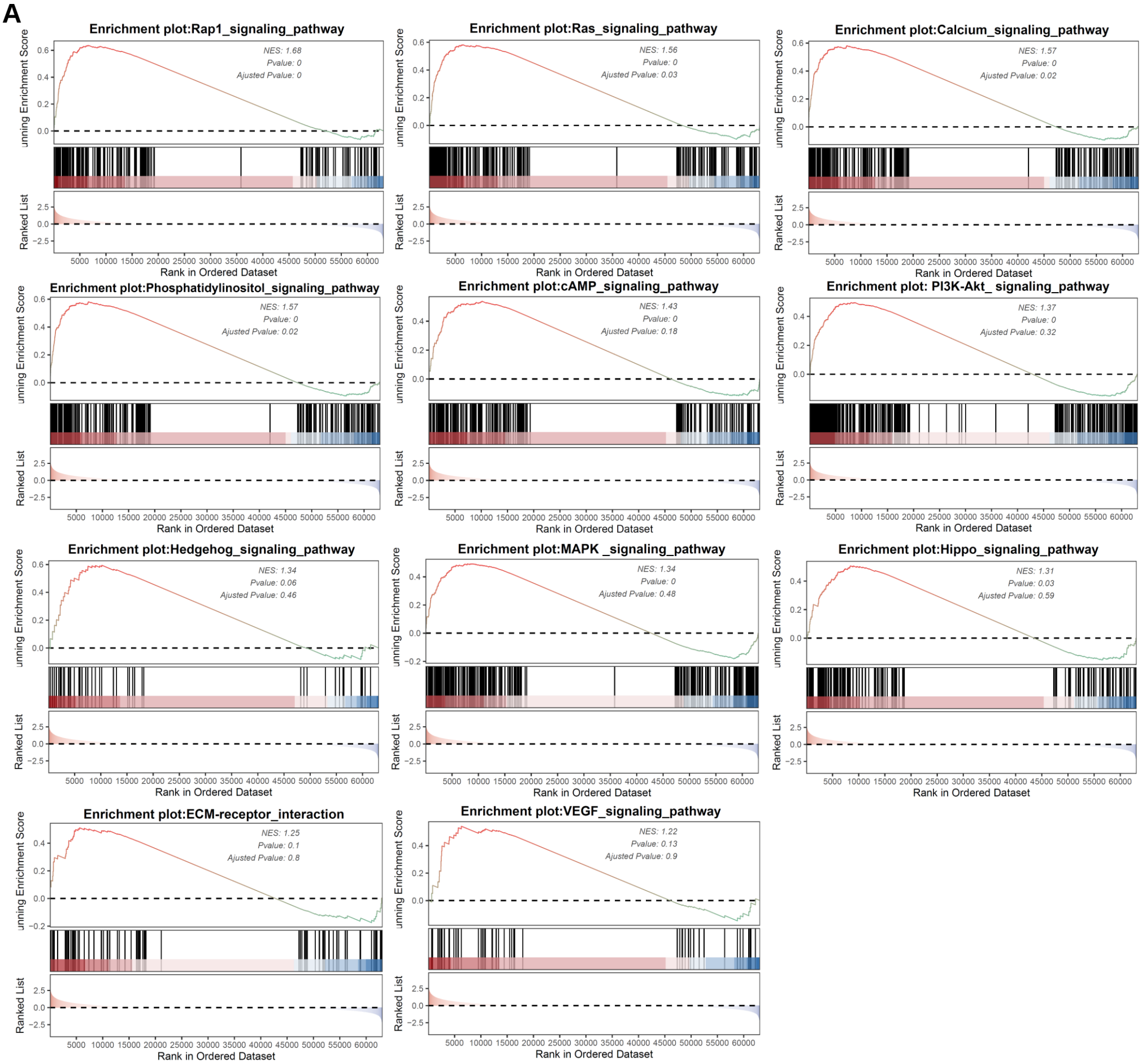
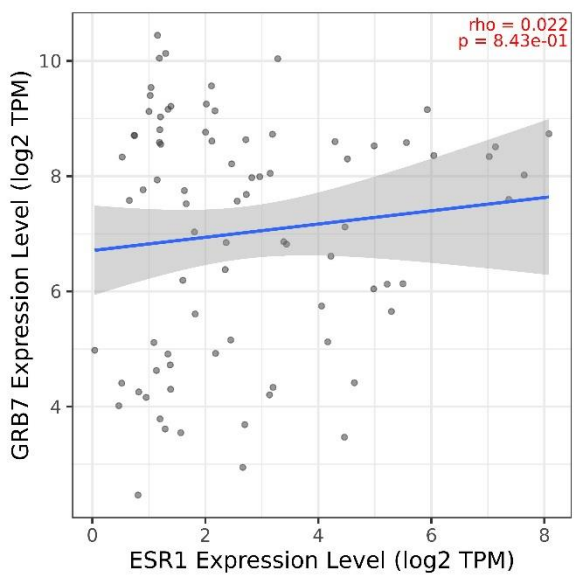
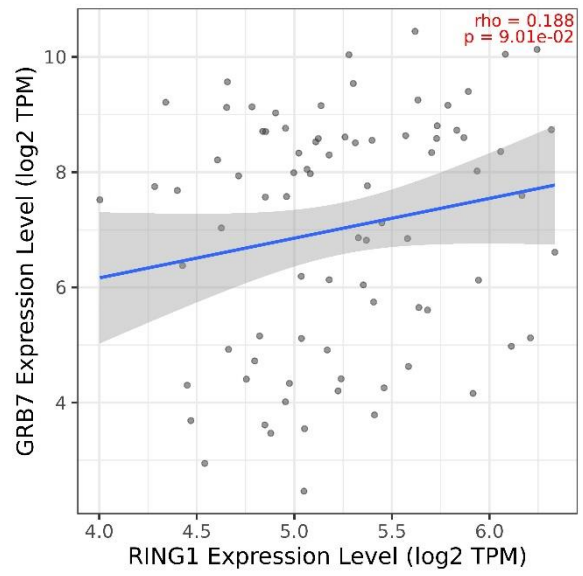
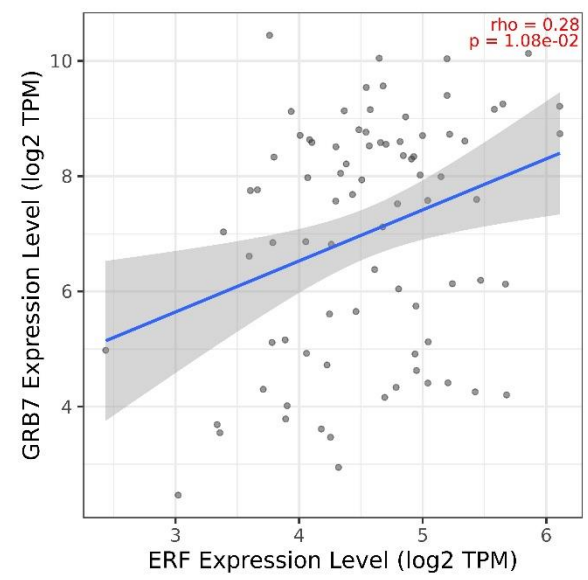

Enrichment plot:Rap1_signaling_pathway
NES: 1.68
Pvalue: 0
Ajusted Pvalue: 0
Enrichment plot:Ras_signaling_pathway
NES: 1.56
Pvalue: 0
Ajusted Pvalue: 0.03
Enrichment plot:Calcium_signaling_pathway
NES: 1.57
Pvalue: 0
Ajusted Pvalue: 0.02
Enrichment plot:Phosphatidylinositol_signaling_pathway
NES: 1.57
Pvalue: 0
Ajusted Pvalue: 0.02
Enrichment plot:cAMP_signaling_pathway
NES: 1.43
Pvalue: 0
Ajusted Pvalue: 0.18
Enrichment plot: PI3K-Akt_ signaling_pathway
NES: 1.37
Pvalue: 0
Ajusted Pvalue: 0.32
Enrichment plot:Hedgehog_signaling_pathway
NES: 1.34
Pvalue: 0.06
Ajusted Pvalue: 0.46
Enrichment plot:MAPK _signaling_pathway
NES: 1.34
Pvalue: 0
Ajusted Pvalue: 0.48
Enrichment plot:Hippo_signaling_pathway
NES: 1.31
Pvalue: 0.03
Ajusted Pvalue: 0.59
Enrichment plot:ECM-receptor_interaction
NES: 1.25
Pvalue: 0.1
Ajusted Pvalue: 0.8
Enrichment plot:VEGF_signaling_pathway
NES: 1.22
Pvalue: 0.13
Ajusted Pvalue: 0.9
unning Enrichment Score
Ranked List
Rank in Ordered Dataset

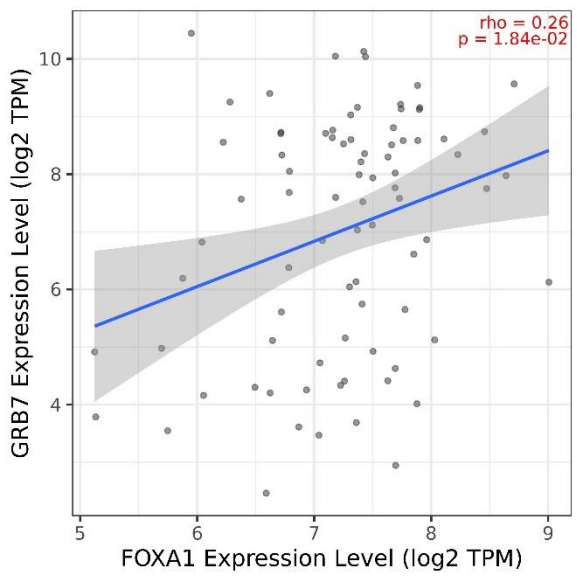
Figure S5. GSEA of GRB7 with several signaling pathways.

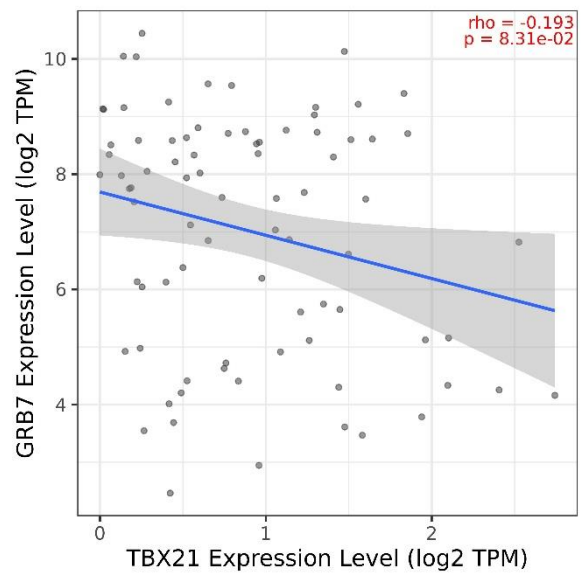
A

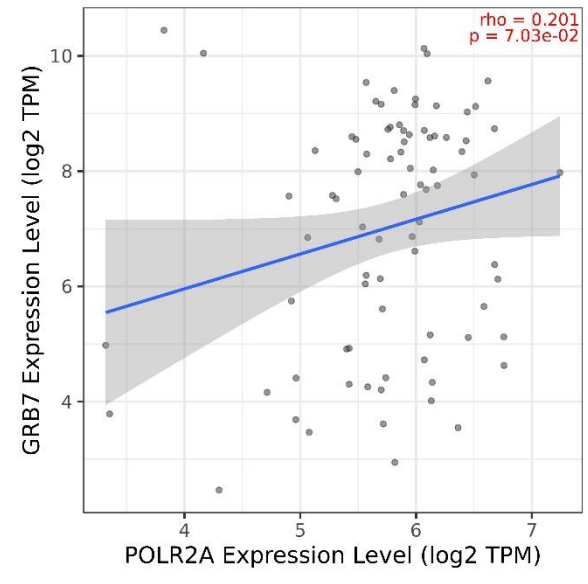

rho = 0.022
p = 8.43e-01
GRB7 Expression Level (log2 TPM)
ESR1 Expression Level (log2 TPM)

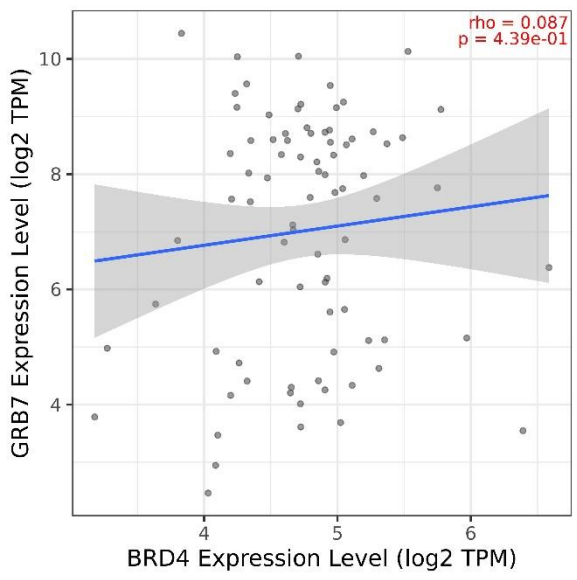

rho = 0.188
p = 9.01e-02
GRB7 Expression Level (log2 TPM)
RING1 Expression Level (log2 TPM)

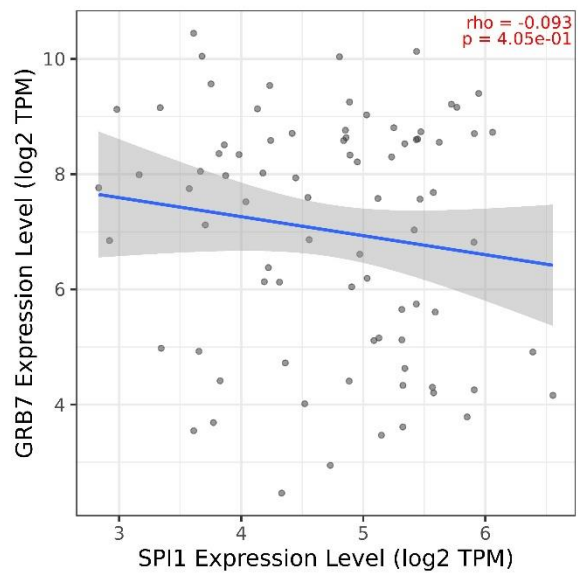

rho = 0.28
p = 1.08e-02
GRB7 Expression Level (log2 TPM)
ERF Expression Level (log2 TPM)


rho = 0.26
p = 1.84e-02
GRB7 Expression Level (log2 TPM)
FOXA1 Expression Level (log2 TPM)


rho = -0.193
p = 8.31e-02
GRB7 Expression Level (log2 TPM)
TBX21 Expression Level (log2 TPM)


rho = 0.201
p = 7.03e-02
GRB7 Expression Level (log2 TPM)
POLR2A Expression Level (log2 TPM)


rho = 0.087
p = 4.39e-01
GRB7 Expression Level (log2 TPM)
BRD4 Expression Level (log2 TPM)


rho = -0.093
p = 4.05e-01
GRB7 Expression Level (log2 TPM)
SPI1 Expression Level (log2 TPM)

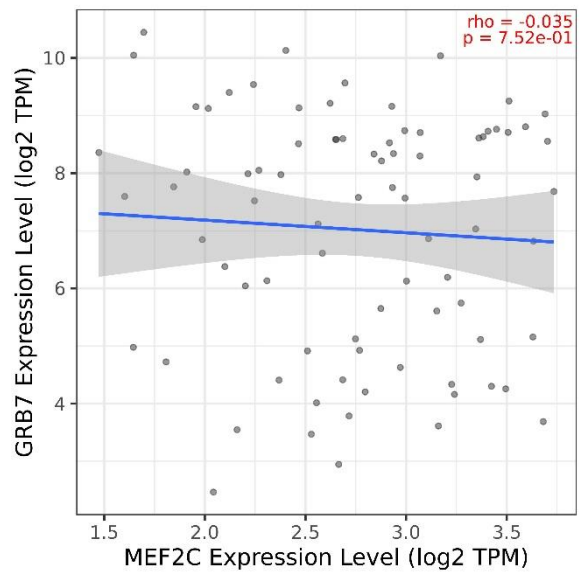
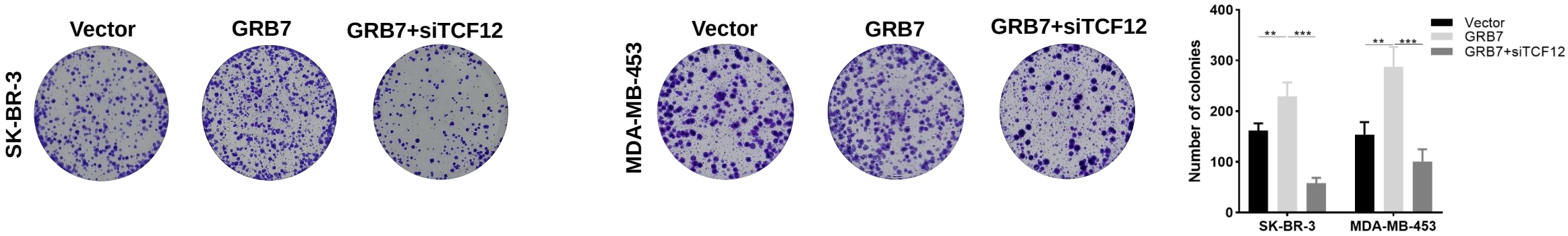
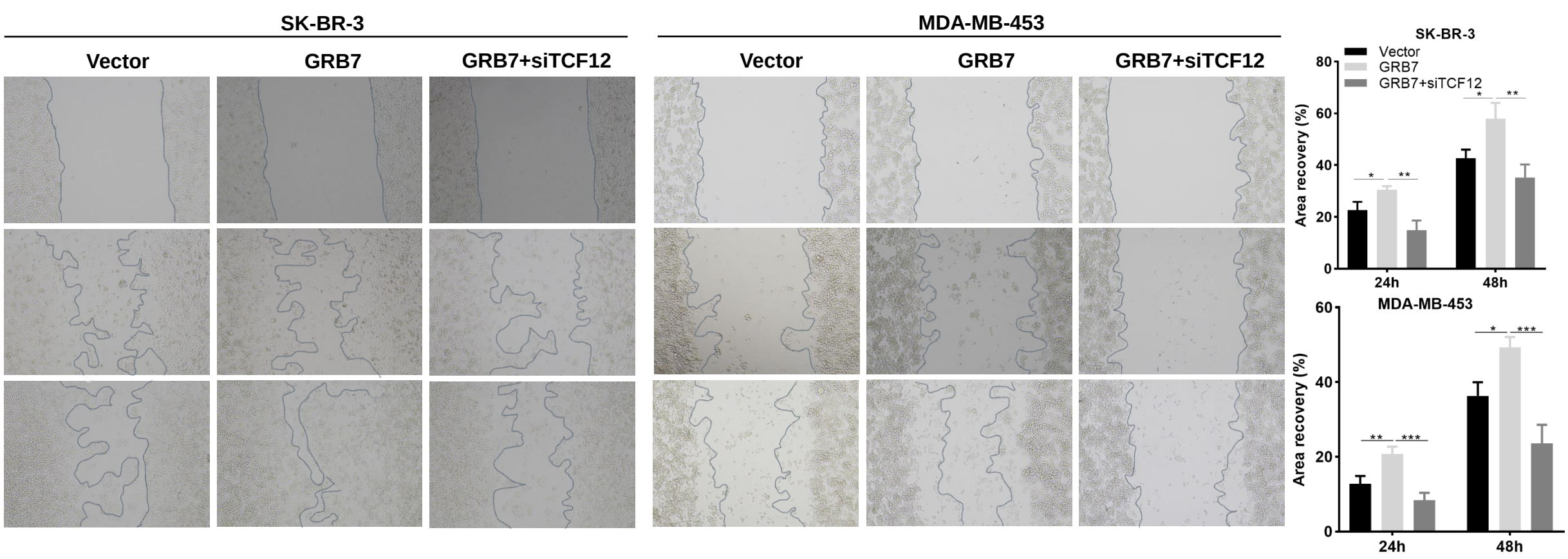

rho = -0.035
p = 7.52e-01
GRB7 Expression Level (log2 TPM)
MEF2C Expression Level (log2 TPM)

Figure S6. Correlation analysis of several TFs with GRB7.

A


Vector
GRB7
GRB7+siTCF12
SK-BR-3
MDA-MB-453
Number of colonies
SK-BR-3
MDA-MB-453

B


SK-BR-3
MDA-MB-453
Vector
GRB7
GRB7+siTCF12
0h
24h
48h
Area recovery (%)
24h
48h

C

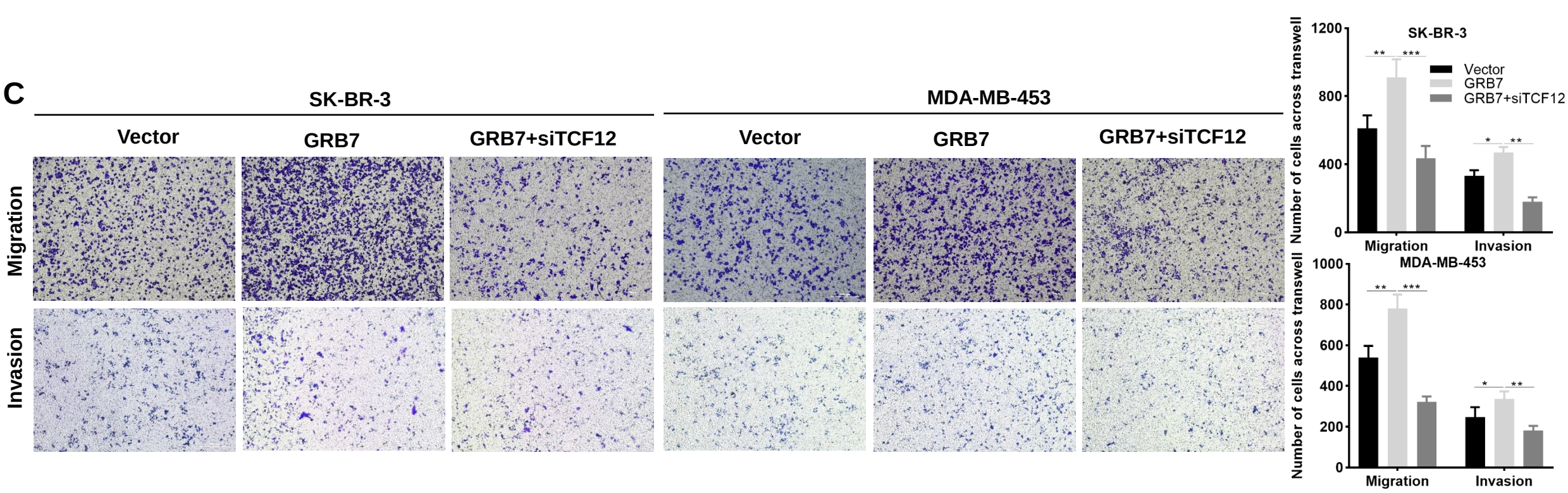

SK-BR-3
MDA-MB-453
Vector
GRB7
GRB7+siTCF12
Migration
Invasion
Number of cells across transwell
Migration
Invasion

Figure S7. TCF12 deletion significantly reversed the proliferation, migration and invasion of HER2+ cells induced by GRB7 overexpression. (A) Colony formation assay showed that GRB7 could partially reverse the inhibitory effect of TCF12 knockdown on cell proliferation. **(B)** Wound healing assays and **(C)** transwell assays indicated that overexpression of GRB7 partially restored the inhibitory effect of TCF12 downregulation on cell migration and invasion. Data are shown as the mean ± SD, N ≥3. *p<0.05, **p<0.01, ***p<0.001.