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SUPPLEMENTAL DATA

Immune-evasion-associated prognostic gene expression score stratifies survival and immune microenvironment features in breast cancer

Bozhi Zhao[1#], Hongyu Wang[2#], Qunyu Hu[1], Shu Fang[3], Qihui Zhang[4], Yixin Cai[1],Yuansong Qu[1], Kexin Zhang[1], Dongwei Zhang[1*]

[1]Department of General Surgery, The Second Affiliated Hospital of Harbin Medical University, Harbin, Heilongjiang, China;

[2]Department of General Surgery, The Affiliated Hospital of Xuzhou Medical College, Xuzhou, Jiangsu, China;

[3]Department of Neurosurgery, The First People's Hospital Of Jiande, Jiande, Hangzhou, Zhejiang, China;

[4]Department of Biochemistry and Molecular Biology, Harbin Medical University, Harbin, Heilongjiang, China.

*Correspondence to **Dongwei Zhang**: mugong0@163.com

[#]Equally contributed to this work: Bozhi Zhao and Hongyu Wang.

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Table S1. List of 182 immune-evasion-associated genes

B2M
IFNGR1
JAK2
SOCS1
TAP1
TAP2
TAPBP
ADAR
ATG10
ATG101
CFLAR
FITM2
GPAA1
GPI1
IFNGR2
IKBKG
IRF1
JAK1
OTUD5
PTPN2
RBCK1
STAT1
STAT2
TNFAIP3
TNFRSF1A
TRAF2
ATG12
ATG3
ATG5
BECN1
CHIC2

DNTTIP1
EMC8
ERAP1
ERP44
FADD
HDAC1
IST1
MAP3K7
MEN1
MOGS
NDUFA13
NXT1
OTULIN
PDIA3
PIGS
PIGU
PPP2R2A
PSMB8
RB1CC1
RBM15
RIC8
RNF31
SCAF4
SMG7
TAB1
TAB2
TMEM127
TNFRSF1B
UBE2G2
UBE2N
VDAC2
VPS29

VPS35
VPS4B
WIPI2
ZCCHC14
ACAD9
ACTB
AGO2
AHSA1
ANAPC15
ARF6
ATG14
ATG7
ATG9A
ATP13A1
ATXN7L3
BC003331
BCL2L1
BOLA3
BRAT1
BRPF1
CAD
CALR
CEP55
CHMP5
COX6C
CREBBP
CUL3
DCP1A
DET1
DICER1
DNAJC13
DOT1L

DPH5
EIF3H
EMC2
EMC3
EMC4
EMC6
F8A
FAM58B
FAS
FNTB
GALE
GLS
HLA-A
HCFC2
HDGFRP2
HEXIM1
HIRA
HSPA13
IFNAR1
IFNAR2
IKBKB
INO80
IPPK
IRF9
JAGN1
JMJD6
KAT6A
KLF16
KMT2A
LIPT2
MAPK1
MED16

MED23
MED24
MGAT1
MTA2
N6AMT1
NCBP1
NDUFAF6
NPLOC4
NUP188
PCBP2
PCED1B
PDCD6IP
PDSS2
PI4KB
PIGK
PIGT
PKN2
PPP1CA
PPP1R8
PPP2R3C
PRKCSH
PSMB9
PSMG1
PTAR1
RCE1
RGP1
RIC1
S100PBP
SARNP
SETD1A
SETDB1
SLC25A32

SPCS1
SRRT
SRSF7
STOML2
SUSD6
TBK1
TFRC
TGFBR2
TMEM208
TRADD
TRPM7
UBR5
UFC1
UFL1
USP7
UXS1
VPS13A
VPS16
WDR7
WDR83
WWP2
YAP1
ZC3H3

Table S2. Univariate Cox regression results for 44 prognostic candidate immediate early genes

ID	HR	HR.95L	HR.95H	*p* value
ADAR	0.734031232	0.562785615	0.957383834	0.022531958
AHSA1	0.648837623	0.42114571	0.999630889	0.049804628
ATG101	0.729620359	0.532956122	0.998854963	0.049170142
ATP13A1	0.527135614	0.369541057	0.751937977	0.000410643
ATXN7L3	0.479613831	0.301845129	0.762077652	0.001870988
CALR	0.54820483	0.409468449	0.733947967	5.40E-05
CEP55	1.330630719	1.167643538	1.516368698	1.83E-05
CFLAR	1.672754552	1.217192435	2.298821213	0.001515758
CHIC2	1.78591665	1.260764151	2.529813588	0.00109767
COX6C	0.863559683	0.767527024	0.971607907	0.014735056
DCP1A	1.596341262	1.089354094	2.339281083	0.016442868
DNTTIP1	0.627162676	0.439856239	0.894230858	0.009949265
FADD	0.784584336	0.616876722	0.997885896	0.04801764
FITM2	1.359841468	1.056243164	1.750703702	0.017104694
GALE	0.807748158	0.660291929	0.988134275	0.037891064
GLS	1.271998497	1.000091317	1.617832441	0.049913078
HCFC2	1.801699803	1.199978767	2.705149682	0.004523935
IFNAR1	0.532465442	0.376950893	0.752138945	0.000348579
IFNGR1	1.497659931	1.177639022	1.904645845	0.000990873
IRF1	0.743756213	0.594455453	0.930554682	0.009610455
IRF9	0.626463995	0.453339964	0.865701611	0.004599152
IST1	1.751716235	1.120328063	2.738938594	0.01396394
KMT2A	1.493896385	1.095866733	2.036494349	0.011115311
MEN1	0.558940213	0.394333774	0.792258189	0.001081884
MOGS	0.52122676	0.359983373	0.754694121	0.000559944
PDIA3	2.150824991	1.403211999	3.296756404	0.00044042
PPP1CA	0.599596315	0.436790975	0.823084178	0.001553506
PSMB8	0.727102959	0.601260716	0.879283642	0.001013446
PSMB9	0.829847065	0.702300014	0.980558363	0.028484448

PTAR1	1.419302742	1.044838766	1.927972372	0.025050009
SETDB1	0.649291692	0.435164595	0.968782171	0.034403895
SMG7	0.665456884	0.454562153	0.974196513	0.036224594
SOCS1	0.810477796	0.657899526	0.998441603	0.048311312
SPCS1	0.632162154	0.433582722	0.921690299	0.017133155
SRRT	0.507346649	0.353775649	0.727581514	0.000225251
STAT1	0.786384651	0.664333006	0.930859696	0.005229541
TAB2	1.437786132	1.090953084	1.894883466	0.009936606
TAP1	0.750014304	0.631766692	0.890394291	0.001015723
TAPBP	0.488679709	0.361184636	0.661179448	3.45E-06
TGFBR2	0.757079789	0.659076546	0.869655901	8.34E-05
TRAF2	0.591587744	0.454898575	0.769349649	9.00E-05
WDR83	0.602515324	0.433354151	0.837709098	0.002585683
YAP1	1.42505871	1.152528362	1.762032409	0.001072412
ZC3H3	0.723996802	0.549127406	0.954553284	0.022037912

Abbreviation: HR, hazard ratio.

Table S3. The 12-gene IEPS model and LASSO Cox coefficients

Gene	**Coefficient**
STAT1	−0.012668072
SRRT	−0.072984414
IFNAR1	−0.038095815
CEP55	0.040678133
ATXN7L3	−0.076770468
PDIA3	0.047437547
PSMB8	−0.004615234
SPCS1	−0.079478778
TAPBP	−0.294418523
CHIC2	0.198791894
TGFBR2	−0.003230231
CALR	−0.131945694

Table S4. Primer sequences utilized in this study

Primers	Sequence (5'-3')
GAPDH F	CTGGGCTACACTGAGCACC
GAPDH R	AAGTGGTCGTTGAGGGCAATG
PDIA3 F	AGCAAAGGTTGATTGCACTGC
PDIA3 R	TCAAGTGGCTGACAATTCCATC

Abbreviations: F, forward; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PDIA3, protein disulfide isomerase family A member 3; R, reverse.

Table S5. Representative published prognostic signatures related to the immune or tumor microenvironment in breast cancer

Published study	**Signature type**	**Main data source**	**Modeling strategy**	**Quantitative benchmarking in this study**	**Reason direct benchmarking was not performed**
Ding et al., 2021 [16]	Immune-related TME signature	TCGA/external cohort	Cox/LASSO-based model	No; summarized qualitatively only	Original coefficients/cutoff not fully applicable; differences in preprocessing and platform annotation
Chen et al., 2022[17]	ssGSEA-based immune-related signature	TCGA-BRCA	ssGSEA + Cox/LASSO model	No; summarized qualitatively only	ssGSEA-based score requires harmonized immune gene-set scoring and cutoff

					recalibration
Liu et al., 2022 [18]	Immune checkpoint-related TNBC signature	TCGA/METABRIC	LASSO Cox model	No; summarized qualitatively only	Developed for TNBC; GSE20685 lacks complete harmonized subtype
					annotation
Du et al., 2022 [19]	Immune-related signature in HR+/HER2− breast cancer	METABRIC/TCGA/GEO	Cox/LASSO-based model	No; summarized qualitatively only	Developed for HR+/HER2− patients; not applicable to the full GSE20685 cohort
Xu et al., 2022 [20]	TME/immune-infiltration-related score	TCGA/METABRIC/GEO	Immune infiltration + Cox/LASSO model	No; summarized qualitatively only	TME/immune-infiltration score depends on immune estimation method and preprocessing differences

This table offers a qualitative summary of published prognostic signatures related to the immune or tumor microenvironment in breast cancer. This study did not perform formal quantitative benchmarking. **Abbreviations**: GEO, Gene Expression Omnibus; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; LASSO, least absolute shrinkage and selection operator; METABRIC, Molecular Taxonomy of Breast Cancer International Consortium; ssGSEA, single-sample gene set enrichment analysis; TCGA, The Cancer Genome Atlas; TCGA-BRCA, The Cancer Genome Atlas Breast Invasive Carcinoma; TME,

tumor microenvironment; TNBC, triple-negative breast cancer.

Table S6. Incremental prognostic value of the IEPS beyond available clinicopathological variables

Cohort	Model	Variables included	C-index	3-year AUC	5-year AUC	10-year AUC
TCGA-BRCA	Clinical model	Age, T stage, N stage, M stage	0.651	0.667	0.681	0.704
TCGA-BRCA	Clinical model + IEPS	Age, T stage, N stage, M stage, IEPS	0.674	0.689	0.703	0.729
GSE20685	Clinical model	Age, overall clinical stage	0.579	0.568	0.584	0.596
GSE20685	Clinical model + IEPS	Age, overall clinical stage, IEPS	0.598	0.589	0.605	0.615

Abbreviations: AUC, area under the curve; C-index, concordance index; GSE20685, Gene Expression Omnibus Series 20685; IEPS, immune-evasion-associated prognostic score; M, distant metastasis; N, regional lymph node; T, primary tumor; TCGA-BRCA, The Cancer Genome Atlas Breast Invasive Carcinoma.

Table S7. Apparent, bootstrap optimism-corrected, and external validation performance of the IEPS

Cohort	Validation method	3-year AUC	5-year AUC	10-year AUC
TCGA-BRCA	Apparent estimate	0.617	0.624	0.713
TCGA-BRCA	Bootstrap optimism-corrected estimate	0.602	0.609	0.689
GSE20685	External validation	0.56	0.572	0.581

Abbreviations: AUC, area under the curve; GSE20685, Gene Expression Omnibus Series 20685; IEPS, immune-evasion-associated prognostic score; TCGA-BRCA, The Cancer Genome Atlas Breast Invasive Carcinoma.

Table S8. RT-qPCR validation of PDIA3 knockdown in SUM-159-PT and MDA-MB-231 cells

Figure 8A.SUM-159-PT RT-qPCR	si-Ctrl	si-PDIA3#1	si-PDIA3#2	si-PDIA3#3
	0.975613009	0.550773896	0.778012407	0.771305932
	0.995657631	0.556368933	0.703510254	0.683681507
	1.029466908	0.578798083	0.68080091	0.773801143
Figure 8A.MDA-MB-231 RT-qPCR	si-Ctrl	si-PDIA3#1	si-PDIA3#2	si-PDIA3#3
	1.080773616	0.119183936	0.160688902	0.141157795
	1.047724083	0.140528486	0.161244761	0.149891408
	0.883117191	0.173016029	0.158898711	0.139794437

The complete quantitative source data for Figure 8 are available in the accompanying Excel file. **Abbreviations**: Ctrl, control; RT-qPCR, reverse transcription quantitative polymerase chain reaction; si-Ctrl, control small interfering RNA; siRNA, small interfering RNA.

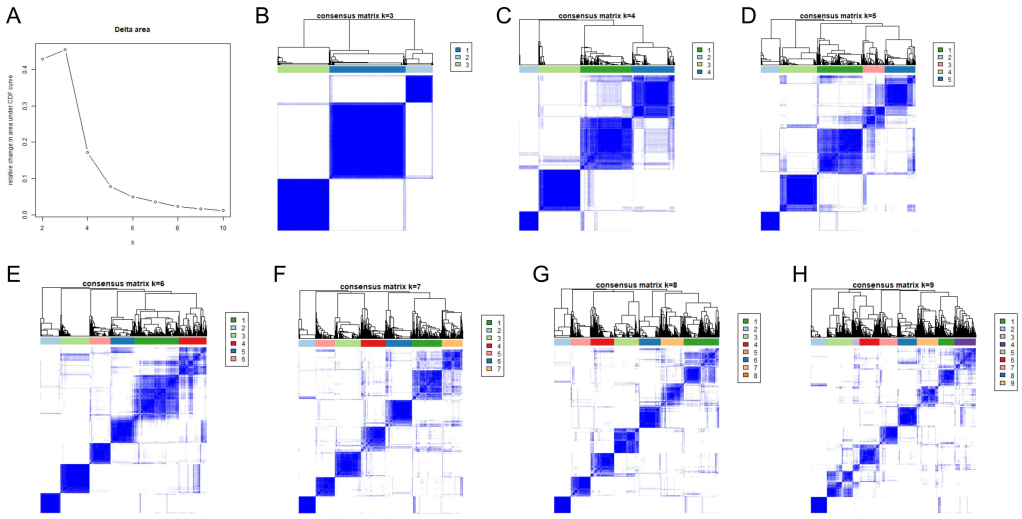


Figure S1. Consensus clustering assessment for selecting the optimal number of immune-evasion-associated breast cancer clusters. (A) Delta-area plot of the consensus cumulative distribution function across different k values, used to evaluate clustering stability. **(B–H)** Consensus matrices with hierarchical dendrograms for k = 3–9, showing increasing cluster fragmentation at higher k values. Together with the consensus results shown in Figure 2, these analyses supported the selection of a two-cluster solution. **Abbreviations**: CDF, cumulative distribution function; k, number of clusters.

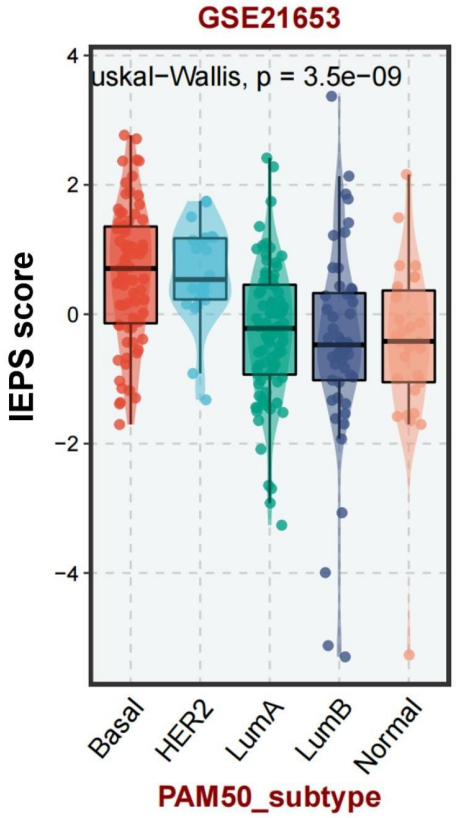


Figure S2. IEPS distribution across PAM50 molecular subtypes in the GSE21653 cohort. Violin and box plots show IEPS values across Basal-like, HER2-enriched, Luminal A, Luminal B, and Normal-like breast cancer subtypes. IEPS values were calculated using the fixed 12-gene formula and TCGA-derived coefficients. Differences across PAM50 subtypes were assessed using the Kruskal–Wallis test, showing a significant subtype-related distribution of IEPS values ($P = 3.5 \times 10^{-9}$). Basal-like and HER2-enriched tumors tended to have higher IEPS values, whereas Luminal B and Normal-like tumors showed relatively lower values, suggesting that IEPS may partly reflect established molecular subtype-related transcriptomic heterogeneity. **Abbreviations**: GSE21653, Gene Expression Omnibus Series 21653; HER2, human epidermal growth factor receptor 2; IEPS, immune-evasion-associated prognostic score; PAM50, prediction analysis of microarray 50-gene subtype classifier; TCGA, The Cancer Genome Atlas.