

Table S1 Properties and culture conditions of cancer cell lines

Cancer	Cell line	Histopathological Feature	Source	Culture media [†]	Cell number*
Breast	MDA-MB-231	Pleural effusion of metastatic triple negative adenocarcinoma	Addexbio Cat# C0006002 RRID: CVCL_0062	RPMI 1640	200'000
	MCF-7	Pleural effusion of metastatic adenocarcinoma	NCI-DTP Cat# MCF7, RRID: CVCL_0031	DMEM	400'000
Cervical	HeLa	Cervical adenocarcinoma, HPV+	ATCC Cat# CCL-2 RRID: CVCL_0030	DMEM	150'000
	SiHa	Cervical squamous cell carcinoma, HPV+	AddexBio Cat# C0008002 RRID: CVCL_0032	DMEM	200'000
Colorectal	HT-29	Colorectal adenocarcinoma	CLS Cat#330215 RRID: CVCL_0320	RPMI 1640	250'000
	SW-620	Lymph node metastasis of colorectal adenocarcinoma	CLS Cat#300466 RRID: CVCL_0547	DMEM	250'000
Lung	A549	Adenocarcinoma	NCI-DTP Cat# A549, RRID: CVCL_0023	RPMI 1640	250'000
	H226	Pleural effusion of metastatic squamous cell carcinoma	AddexBio Cat#C0016011 RRID: CVCL_1544	RPMI 1640	350'000
Ovarian	TOV-112D	Ovarian endometrioid adenocarcinoma	ATCC Cat# CRL-11731 RRID: CVCL_3612	DMEM	300'000
	SKOV-3	Ascites metastasis of ovarian serous cystadenocarcinoma	ATCC Cat# HTB-77, RRID: CVCL_0532	DMEM	250'000
Pancreatic	MIA PaCa-2	Pancreatic ductal carcinoma	ATCC Cat# CRL-1420, RRID: CVCL_0428	DMEM	300'000
	Capan-1	Liver metastasis of pancreatic ductal carcinoma	CLS Cat# 300143 RRID: CVCL_0237	RPMI 1640	450'000
	BxPC-3	Pancreatic ductal adenocarcinoma	ATCC Cat# CRL-1687 RRID: CVCL_0186	RPMI 1640	250'000
	PANC-1	Pancreatic ductal adenocarcinoma	ATCC Cat# CRL-1469 RRID: CVCL_0480	DMEM	400'000

[†]All media has been supplemented with 10% FBS, 1% Sodium pyruvate and 1% Pen-Strep (Gibco, ThermoFisher Scientific, USA)

*Cell number seeded for experiments in normoxia and hypoxia

DMEM: Dulbecco's Modified Eagle Medium; RPMI: Roswell Park Memorial Institute

Table S2 TaqMan Gene Expression Assays

HGNC Symbol	Assay ID	Product size (bp)
<i>VEGFA</i>	Hs00900055_m1	59
<i>NDRG1</i>	Hs00608387_m1	54
<i>SLC2A1</i>	Hs00892681_m1	76
<i>P4HA1</i>	Hs00914594_m1	99
<i>ANGPTL4</i>	Hs01101127_m1	92
<i>LDHA</i>	Hs01378790_g1	66
<i>BNIP3</i>	Hs00969291_m1	125
<i>PGK1</i>	Hs99999906_m1	75
<i>DDIT4</i>	Hs01111686_g1	68
<i>ADM</i>	Hs00181605_m1	76
<i>CA9</i>	Hs00154208_m1	78
<i>LOX</i>	Hs00942483_m1	77
<i>MXI1</i>	Hs00365651_m1	102
<i>CORO1C</i>	Hs00902568_m1	62
<i>CCND1</i>	Hs00765553_m1	57
<i>ACTB</i>	Hs99999903_m1	171
<i>18S</i>	Hs99999901_s1	187

Table S3 Eighteen-gene tumor inflammation signature

Gene	Biological role
<i>PSMB10</i>	Antigen presenting cell abundance
<i>HLA-DQA1</i>	
<i>HLA-DRB1</i>	
<i>CMKLR1</i>	
<i>HLA-E</i>	T Cell/ NK Cell Abundance
<i>NKG7</i>	
<i>CD8A</i>	
<i>CCL5</i>	IFN Activity
<i>CXCL9</i>	
<i>CD27</i>	
<i>CXCR6</i>	
<i>IDO1</i>	
<i>STAT1</i>	
<i>TIGIT</i>	T Cell Exhaustion
<i>LAG3</i>	
<i>CD274</i>	
<i>PDCD1LG2</i>	
<i>CD276</i>	

Table S4 Prognostic and predictive hypoxia gene signatures

s.n.	Signature [#]	Prognostic / predictive capacity	Number of genes	Overlapping genes (%)
1	Chi JT et al., 2006 (1)	Breast cancer, ovarian cancer, lung cancer	123	17.89 [†]
2	Seigneuric R et al., 2007 (2)	Breast cancer; neuroblastoma	15	0.00
3	Winter SC et al., 2007 (3)	Breast cancer; head and neck squamous cell carcinoma; lung cancer	99	31.31
4	Hu Z et al., 2009 (4)	Breast cancer; lung cancer; glioma	13	53.85
5	Buffa FM et al., 2010 (5)	Breast cancer; lung cancer	51	56.86
6	van Malenstein H et al., 2010 (6)	Hepatocellular carcinoma	7	28.57
7	Fardin P et al., 2010 (7)	Neuroblastoma	32	78.13
8	Toustrup K et al., 2011 (8)	Head and neck squamous cell carcinoma*; soft tissue sarcoma	15	100.00
9	Halle C et al., 2012 (9)	Cervical cancer	31	54.84
10	Eustace A et al., 2013 (10)	Laryngeal cancer [§]	26	73.08
11	Ragnum HB et al., 2015 (11)	Prostate cancer	32	21.88
12	Fjeldbo S et al., 2016 (12)	Uterine cervical cancer	6	100.00
13	Buart S et al., 2017 (13)	Melanoma [§]	2	100.00
14	Loftus SK et al., 2017 (14)	Melanoma	10	30.00
15	Suh YE et al., 2017 (15)	Oropharyngeal squamous cell carcinoma	21	9.52
16	Yang L et al., 2017 (16)	Bladder cancer*	24	25.00
17	Yang L et al., 2018 (17)	Soft tissue sarcoma	24	79.17
18	Yang L et al., 2018 (18)	Prostate cancer; bladder cancer [§]	28	14.29
19	Ye IC et al., 2018 (19)	Basal subtype breast cancer	42	73.81
20	Dao Trong P et al., 2018 (20)	IDH1 ^{mut} lower grade glioma	5	40.00
21	Lee JH et al., 2019 (21)	Colorectal cancer	4	0.00
22	Zou YF et al., 2019 (22)	Colorectal cancer	14	7.14
23	Deng F et al., 2020 (23)	Hepatocellular carcinoma	4	100.00
24	Lin W et al., 2020 (24)	Glioma	4	100.00
25	Mo Z et al., 2020 (25)	Lung adenocarcinoma	4	75.00
26	Sun J et al., 2020 (26)	Lung adenocarcinoma	16	12.50
27	Wang J et al., 2020 (27)	Breast cancer	14	35.71
28	Wang Z et al., 2020 (28)	Glioma	5	0.00
29	Karn T et al., 2020 (29)	Breast cancer [§]	3	66.67

[#] Prognostic and predictive hypoxia gene signatures since 2006 that satisfied at least one of the following conditions: showed independent prognostic association / was validated in independent dataset(s) / was associated with validated markers of hypoxia

* Both prognostic and predictive of therapeutic response

[§] Only predictive of therapeutic response

[†] Based on the genes reported in the paper

IDH1^{mut}: Isocitrate Dehydrogenase 1 mutant

Table S5 Mapped unique genes in published prognostic and predictive hypoxia gene signatures

Unique Genes											
ACACA	C4orf47	CSTB	ESRP1	GPI	ISG15	METTL22	PDIK1L	RHOC	SIK1	TFAP2C	TRMT5
ACOT7	CA12	CTSV	FABP5	GPN3	ITGA5	MIR210HG	PDLIM2	RIMKLA	SLC16A3	TFR2	TUBA1B
ADAMTS4	CAD	CXCL1	FAM117B	GRHPR	JAG2	MNAT1	PDSS1	RLF	SLC25A32	TFRC	TUBA1C
ADORA2B	CALD1	CXCL3	FAM120B	GRIN2D	KDM4A	MRPL13	PDZD11	RNF24	SLC2A14	TGFBI	TUBB2A
AGTRAP	CASP14	CXCL8	FAM13A	GRM3	KDM6A	MRPL14	PEX14	RNPS1	SLC2A1-AS1	THBD	TUBB6
AHNAK2	CASP6	CYB5R3	FAM83B	GSS	KIF20A	MRPL15	PFKL	RPL36A	SLC2A5	THBS1	TXLNG
AK2	CAV1	DAAM1	FBP1	GULP1	KIF4A	MS4A6A	PGAM4	RRAGD	SLC5A12	THBS4	TXNIP
AKR7A2P1	CCN1	DCBLD1	FBXO45	GYS1	KLF10	MTFP1	PGF	RRM2	SLC6A10P	TIMM23	UBE2D2
AMH	CCND1	DELEC1	FER1L4	H2AJ	KLF6	MTMR2	PGM1	RRP1B	SLCO1B3	TIMP2	UBR1
ANG	CCNG1	DNAH11	FGF21	HAUS2	LALBA	MTX1	PIN4	RUNX3	SLIRP	TIPARP	UCHL1
ANKRD9	CCNG2	DNAJC28	FLVCR2	HES2	LIF	MVD	PLAU	RUVBL2	SNTA1	TM4SF1	UNG
APOL1	CCT2	DONSON	FOSB	HJURP	LIFR	NAMPT	PLEKHG3	S100A10	SNX24	TMEM189	UTP11
ASF1B	CDC25C	DPM2	FOSL1	HLA-DRB5	LRP1	NCOA7	POU2AF1	S100A3	SOCS3	TMEM30B	VAPB
ASPM	CDCA4	DPYSL2	FOSL2	HMMR	LRP2BP	NDUFA4L2	PPAT	S100A7	SOD2	TMEM45A	VEZT
ATF3	CDCP1	DSC2	FOXM1	HMOX1	LRRC31	NEBL	PPM1J	SAV1	SORL1	TMPRSS11D	VHL
ATP5MPL	CDK18	DSP	FZD7	HOMER1	LRRC42	NIT1	PPP4R1	SCARB1	SPAG7	TMTC3	WDR45B
B3GNT4	CDK20	DTL	G6PD	HSPA1L	LYVE1	NME1	PRELID2	SCD	SPTB	TNIP1	WDSUB1
B4GALT2	CENPE	DTWD1	GAL	HSPB1	MAD2L2	NR0B2	PRSS53	SDAD1P1	SRPK1	TNS4	WNT6
BACH1	CENPU	E2F3	GAS6	ID3	MAFF	NR4A3	PSIP1	SDC1	SRPX	TPBG	XPNPEP1
BCAR1	CHCHD2	E2F6	GEM	IFI6	MAP7D1	NUDT15	PSMB7	SDC3	STBD1	TPD52	XPO5
BCO1	CLK3	EGR1	GEMIN2	IGF1R	MAPT	ORAI2	PSMD2	SEC61G	STC1	TPD52L2	XRCC6
BIRC5	CMTM3	EGR2	GJB6	IGFBP3	MAT1A	ORAI3	PSRC1	SEH1L	SYDE1	TPM4	YKT6
BMS1	CNIH4	EGR3	GLG1	IGFL2	MCL1	OTP	PTGFRN	SELE	SYNGR2	TRAF3	ZBTB44
BNC1	COL4A5	EIF2S1	GMFB	IL20RB	MCM2	PACS1	PUM1	SERPING1	TANC2	TRAM2	ZFP36
BTG2	COL4A6	ELAC1	GNAI1	IMMP2L	MCTS1	PAPPA	RAN	SH3GL3	TCAF2	TRAPPC1	ZNF292
BUB3	COL5A1	ENO2	GNRH1	INPP5J	MDM2	PAWR	RCL1	SHCBP1	TDG	TRIM9	ZWINT
C16orf74	CSRNP1	ERRFI1	GPC6	INSIG2	MEP1A	PCNA	RHOB	SIAH2	TEAD4	TRIP13	

Table S6 Common genes and their frequency in published prognostic and predictive hypoxia gene signatures

Common genes	Count	Common genes	Count	Common genes	Count	Common genes	Count
<i>NDRG1</i>	10	<i>PFKFB4</i>	5	<i>PFKP</i>	3	<i>MIF</i>	2
<i>VEGFA</i>	10	<i>SLC2A3</i>	5	<i>PGAM1</i>	3	<i>PFKFB3</i>	2
<i>SLC2A1</i>	9	<i>BHLHE40</i>	4	<i>PLOD1</i>	3	<i>PKM</i>	2
<i>P4HA1</i>	8	<i>ERO1A</i>	4	<i>AK4</i>	2	<i>PNP</i>	2
<i>ANGPTL4</i>	7	<i>FAM162A</i>	4	<i>BTG1</i>	2	<i>PPARD</i>	2
<i>BNIP3</i>	7	<i>LOX</i>	4	<i>C4ORF3</i>	2	<i>PPFIA4</i>	2
<i>LDHA</i>	7	<i>MRGBP</i>	4	<i>CDKN3</i>	2	<i>PPP1R15A</i>	2
<i>P4HA2</i>	7	<i>PDK1</i>	4	<i>CORO1C</i>	2	<i>PSMA7</i>	2
<i>ALDOA</i>	6	<i>SLC16A1</i>	4	<i>CYP1B1</i>	2	<i>PVR</i>	2
<i>DDIT4</i>	6	<i>TPI1</i>	4	<i>DARS1</i>	2	<i>PYGL</i>	2
<i>GAPDH</i>	6	<i>AK3</i>	3	<i>DDIT3</i>	2	<i>RNASE4</i>	2
<i>PGK1</i>	6	<i>ALDOC</i>	3	<i>ECE2</i>	2	<i>S100A2</i>	2
<i>ADM</i>	5	<i>ANLN</i>	3	<i>EGLN1</i>	2	<i>SLC6A8</i>	2
<i>ANKRD37</i>	5	<i>ENO1</i>	3	<i>FGF11</i>	2	<i>SPAG4</i>	2
<i>BNIP3L</i>	5	<i>FUT11</i>	3	<i>ISG20</i>	2	<i>STC2</i>	2
<i>CA9</i>	5	<i>GBE1</i>	3	<i>JUNB</i>	2	<i>UPK1A</i>	2
<i>EGLN3</i>	5	<i>HK2</i>	3	<i>KDM3A</i>	2	<i>VLDLR</i>	2
<i>HILPDA</i>	5	<i>MRPS17</i>	3	<i>KRT17</i>	2	<i>ZNF395</i>	2
<i>KCTD11</i>	5	<i>MXI1</i>	3	<i>LDLR</i>	2		

Table S7 Univariate PH Cox analysis of potential prognostic parameters in PDA datasets

Cancer dataset	Clinical endpoint	Variable	Hazard ratio
PAAD Data-1	OS	HS (low vs high)	1.9 (1.2-2.9) p = 0.004
		Age (<64 vs ≥64)	1.7 (1.1-2.6) p = 0.025
		Gender (female vs male)	0.87 (0.57-1.3) p = 0.501
		Grade (G1+G2 vs G3+G4)	<i>1.5 (0.97-2.4) p = 0.068</i>
		Stage (I, IA, IB, IIA vs IIB)	2.3 (1.3-4) p = 0.003
	DSS	HS (low vs high)	2 (1.2-3.2) p = 0.005
		Age (<64 vs ≥64)	1.3 (0.81-2.1) p = 0.263
		Gender (female vs male)	0.81 (0.51-1.3) p = 0.394
		Grade (G1+G2 vs G3+G4)	<i>1.6 (0.95-2.6) p = 0.077</i>
		Stage (I, IA, IB, IIA vs IIB)	3.1 (1.6-6) p = 0.001
	PFS	HS (low vs high)	1.7 (1.1-2.5) p = 0.011
		Age (<64 vs ≥64)	1.1 (0.72-1.6) p = 0.708
		Gender (female vs male)	1 (0.96-1.5) p = 0.885
		Grade (G1+G2 vs G3+G4)	1.7 (1.1-2.6) p = 0.014
		Stage (I, IA, IB, IIA vs IIB)	1.8 (1.1-3) p = 0.011
PAAD Data-2	OS	HS (low vs high)	2.1 (1.6-2.8) p < 0.001
		Gender (female vs male)	1.2 (0.9- 1.6) p = 0.206
		Grade (G1+G2 vs G3)	1.3 (0.96-1.8) p = 0.084
		Stage (IA, IB, IIA vs IIB)	1.7 (1.1-2.4) p = 0.007
		Molecular subtype [#]	1.5 (1.3-1.7) p < 0.001
	DFS	HS (low vs high)	1.8 (1.3-2.3) p < 0.001
		Gender (female vs male)	1.3 (0.96- 1.7) p = 0.098
		Grade (G1+G2 vs G3)	1.4 (1-1.8) p = 0.023
		Stage (IA, IB, IIA vs IIB)	1.7 (1.2-2.4) p = 0.001
		Molecular subtype [#]	1.4 (1.2-1.6) p < 0.001

*In bold are significant p-values < 0.05. In italic are p-values of borderline significance. The 95% confidence interval is reported in brackets.

[#] Immune classical used as reference vs pure classical, desmoplastic, stroma activated and pure basal-like

HS: hypoxia score; OS: overall survival; DSS: disease specific survival; PFS: progression free survival; DFS: disease-free survival

Table S8 Grouping PDA cohorts based on Immune score and Hypoxia score

Group	PAAD Data-1	PAAD Data-2
G1: Hypoxia Low / Immune High	56	91
G2: Hypoxia High / Immune Low	43	87
G3: Hypoxia Low / Immune Low	42	82
G4: Hypoxia High / Immune High	23	49