Supplementary materials

Supplementary results

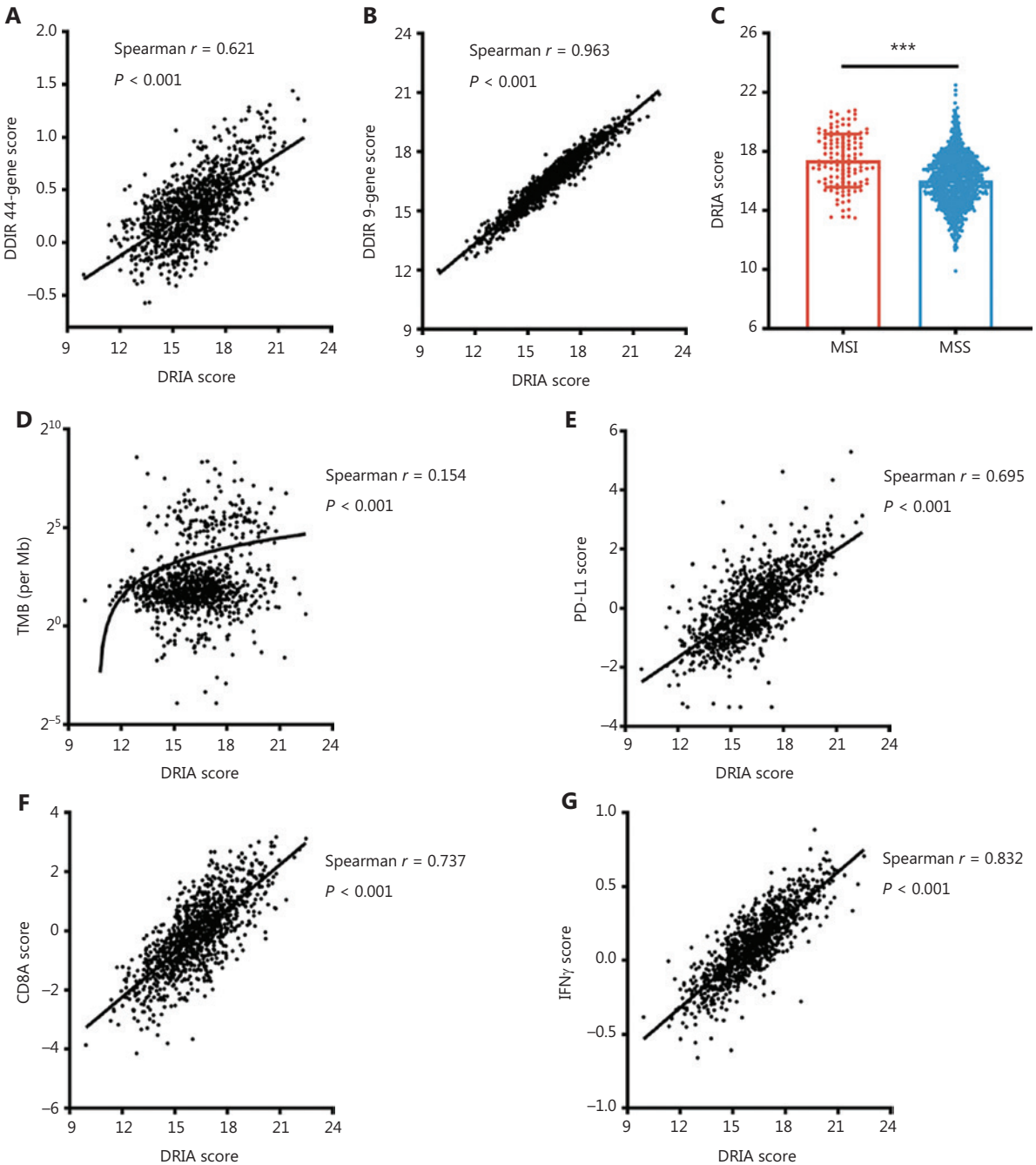

Figure S1 Immune characteristics of DRIA in GI cancer. (A-B) Correlation of the DRIA score with DDIR 44- (A) and DDIR 9-gene scores (B) among GI cancer tissues from the TCGA cohort. (C) Comparison of the DRIA score according to MSI status. (D-G) Correlation of the DRIA score with the TMB level (D), PD-L1 score (E), CD8A score (F), and IFN-γ score (G). DRIA, DNA damage response-related immune activation; GI, gastrointestinal; DDIR, DNA damage immune activation; MSI, microsatellite instability; MSS, microsatellite-stable; TMB, tumor mutational burden. ***$P < 0.001$.

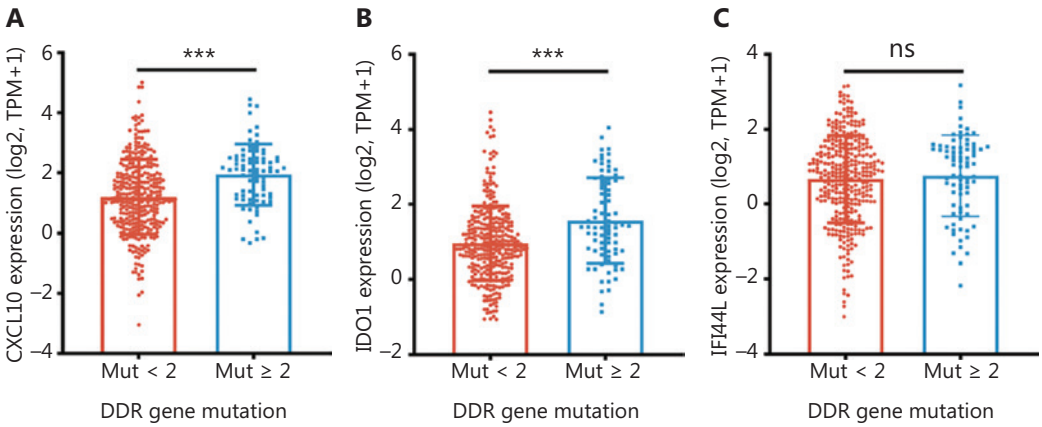

Figure S2 Correlation of the expression of three genes with DDR gene mutations. (A-C) Comparison of the level of CXCL10 (A), IDO1 (B), and IFI44L expression (C) between patients with ≥ 2 and < 2 DDR gene mutations in the TCGA-STAD cohort. DDR, DNA damage response. ***P < 0.001; ns, P > 0.05.

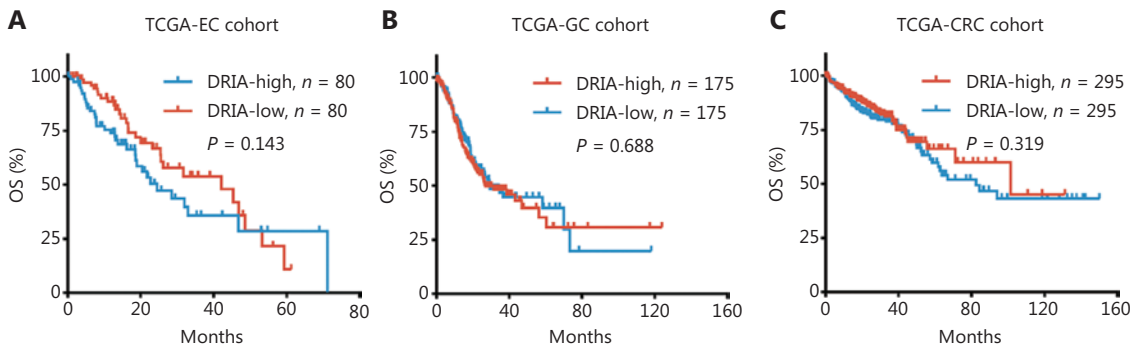

Figure S3 Prognostic role of DRIA in GI cancer without ICI therapy. (A-C) Kaplan–Meier plots of OS segregated by DRIA score in patients with EC (A), GC (B), and CRC (C) from the TCGA cohort. DRIA, DNA damage response-related immune activation; GI, gastrointestinal; ICI, immune checkpoint inhibitors; OS, overall survival; TCGA, The Cancer Genome Atlas; EC, esophageal cancer; GC, gastric cancer; CRC, colorectal cancer.

A

Pairwise combination	AUC (95% CI)
DRIA & PD-L1	0.947 (0.876–1.000)
DRIA & MSI	0.897 (0.766–1.000)
DRIA & EBV	0.897 (0.766–1.000)
DRIA & TMB	0.890 (0.757–1.000)
PD-L1 & MSI	0.900 (0.805–0.995)
PD-L1 & EBV	0.900 (0.805–0.995)
PD-L1 & TMB	0.900 (0.805–0.995)
MSI & EBV	0.917 (0.790–1.000)
MSI & TMB	0.741 (0.549–0.933)
EBV & TMB	0.903 (0.773–1.000)

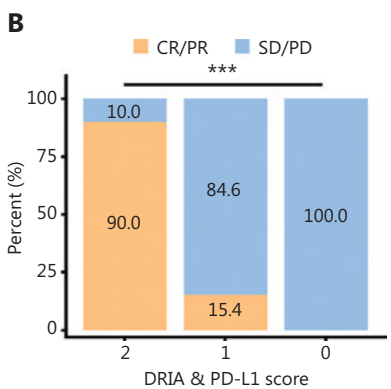

Figure S4 Pairwise combination of DRIA and known clinical biomarkers for prediction of therapeutic response to ICI therapy in gastric cancer. (A) The predictive AUCs of pairwise combination of DRIA and known clinical biomarkers for response to ICI therapy in the Kim18-gastric cancer cohort. (B) Clinical response to ICI therapy by the DRIA and PD-L1 scores in the Kim18-gastric cancer cohort. DRIA, DNA damage response-related immune activation; ICIs, immune checkpoint inhibitors; AUC, area under the ROC curve; MSI, microsatellite instability; EBV, Epstein–Barr virus; TMB, tumor mutational burden; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease. ***P < 0.001.

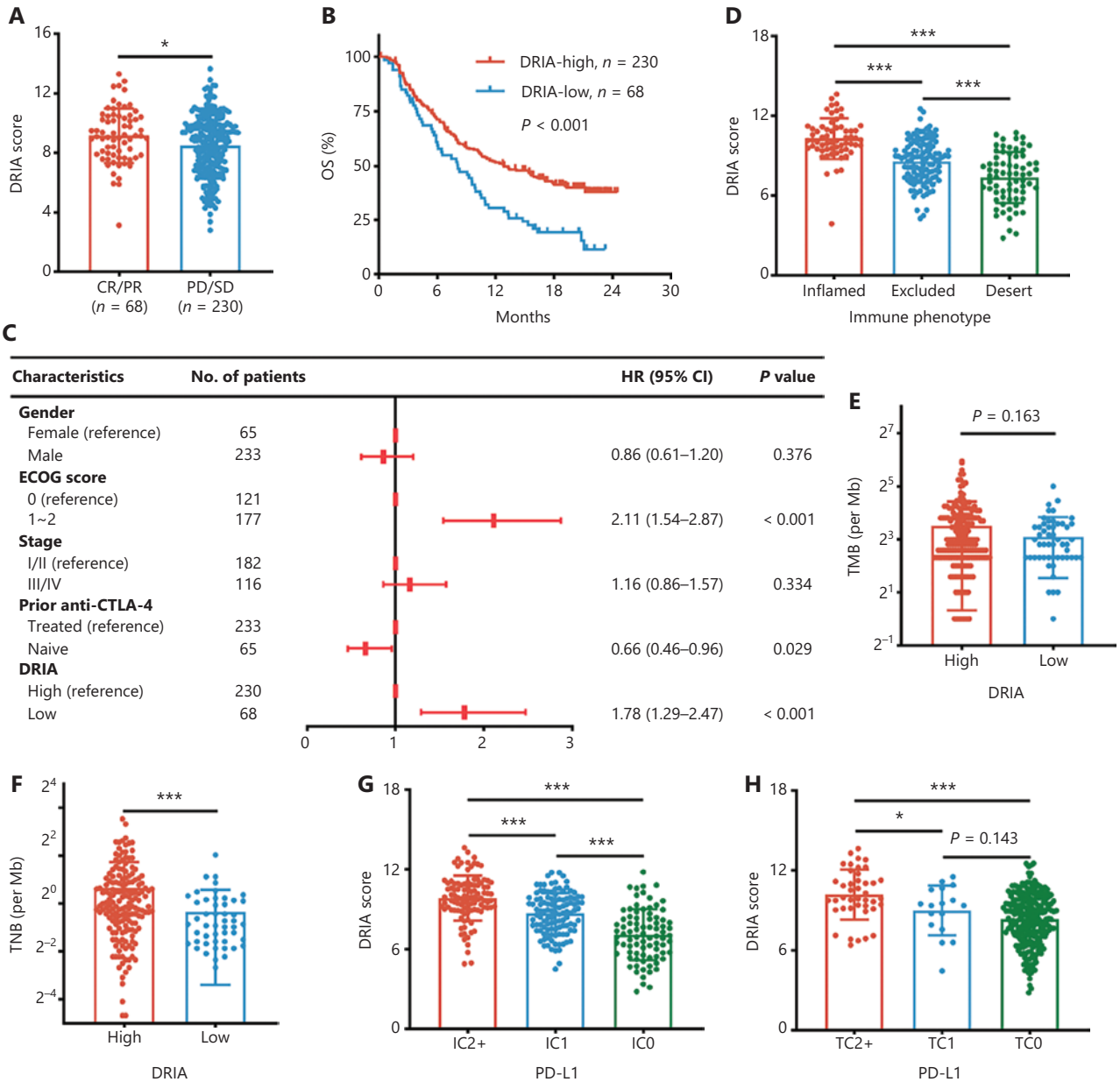

Figure S5 Predictive performance of DRIA for clinical benefit from ICI therapy in the IMvigor210 urothelial cancer cohort. (A) Comparison of the DRIA score between responders and non-responders. (B) Kaplan–Meier survival curve comparing OS between DRIA-high and -low groups. (C) Multivariate Cox regression analyses of DRIA and clinicopathologic factors associated with OS. (D) Comparison of DRIA score between three classic immune phenotypes according to CD8+ T cell infiltration. (E-F) Comparison of TMB (E) and TNB (F) according to the DRIA status. (G-H) Comparison of the DRIA score between subgroups with different PD-L1 expression on tumor-infiltrating immune cells (G) and tumor cells (H). DRIA, DNA damage response-related immune activation; ICIs, immune checkpoint inhibitors; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; OS, overall survival; TMB, tumor mutational burden; TNB, tumor neoantigen burden; IC, immune cell; TC, tumor cell. *P < 0.05; ***P < 0.001.

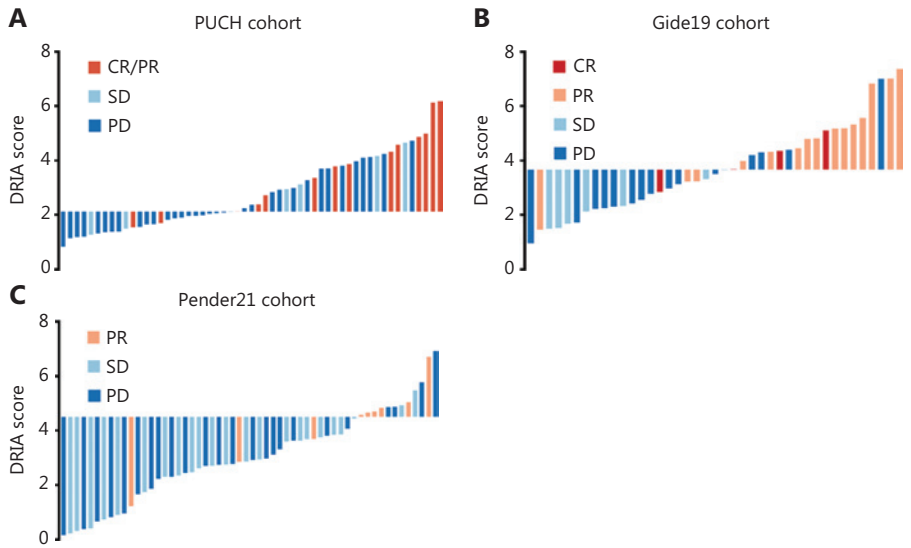

Figure S6 Clinical responses to ICI therapy in the pan-cancer cohorts. (A-C) The DRIA scores of individual patients and the clinical responses to ICI therapy in PUCH melanoma (A), Gide19 melanoma (B), and Pender21 pan-cancer cohorts (C). DRIA, DNA damage response-related immune activation; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ICIs, immune checkpoint inhibitors.

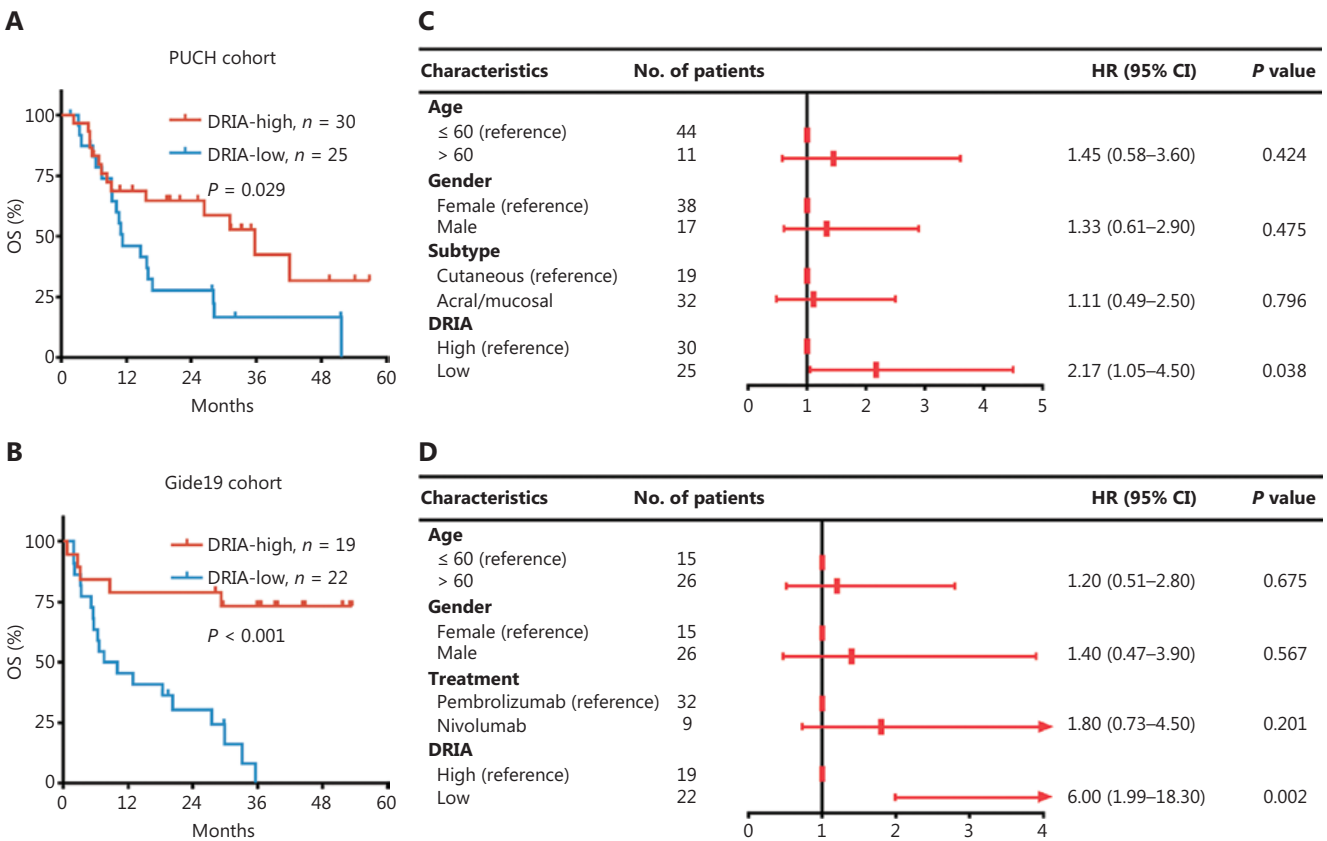

Figure S7 Association between DRIA and OS in two melanoma immunotherapy cohorts. (A-B) Kaplan–Meier plots of OS segregated by DRIA score in PUCH melanoma (A) and Gide19 melanoma cohorts (B). (C-D) Multivariate Cox regression analyses of DRIA and clinicopathologic factors associated with OS in PUCH melanoma (C) and Gide19 melanoma cohorts (D). DRIA, DNA damage response-related immune activation; OS, overall survival; HR, hazard ratio; CI, confidence interval.

Table S1 Data sources

Cohort	Tumor type	Treatment	*N*	RNA-seq platform	Clinical outcome	Reference
Kim18	Gastric cancer	Anti-PD-1 therapy	45	Illumina NextSeq 550	ORR	Nat Med. 2018; 24: 1449-58
Parikh22	MSS colorectal/ pancreatic adenocarcinoma	Anti-PD-1+anti-CTLA-4+radiation therapy	22	Illumina NextSeq 500	ORR, PFS, OS	Nat Cancer. 2021; 2: 1124-35
Gide19	Melanoma	Anti-PD-1 therapy	41	Illumina Hiseq 2500	ORR, PFS, OS	Cancer Cell. 2019; 35: 238-55.e6
IMvigor210	Urothelial cancer	Anti-PD-L1 therapy	298	Illumina HiSeq 2500	ORR, OS	Nature. 2018; 554: 544-8
Pender21	Pan-cancer	Anti-PD-(L)1 therapy	56	Illumina HiSeq 2500/ NextSeq 500	ORR, PFS	Clin Cancer Res. 2021; 27: 202-12

MSS, microsatellite-stable; ORR, objective response rate; PFS, progression-free survival; OS, overall survival.

Table S2 Genes included in two reported DDIR signatures

DDIR panel	Gene symbol	Weight	Gene function
44-gene	CXCL10	0.023	Immune response
	MX1	0.0226	Immune response
	IDO1	0.0221	Immune response
	IFI44L	0.0191	Immune response
	CD2	0.019	Immune response
	GBP5	0.0181	Immune response
	PRAME	0.0177	Cell proliferation differentiation and mitosis
	ITGAL	0.0176	Immune response
	LRP4	−0.0159	Cell adhesion and cell signaling
	APOL3	0.0151	Lipid transport and localization
	CDR1	−0.0149	Immune response
	FYB	0.0149	Cell proliferation and adhesion
	TSPAN7	−0.0148	Signal transduction, growth and motility
	RAC2	0.0148	Signal transduction, growth and motility
	KLHDC7B	0.014	Protein binding
	GRB14	0.0137	Signal transduction, growth and metabolism
	AC138128.1	−0.0136	Unreported
	KIF26A	−0.0136	Transport and signaling
	CD274	0.0133	Immune response
	CD109	−0.0129	Immune response
	ETV7	0.0124	Transcriptional regulation
	MFAP5	−0.0121	ECM remodeling
	OLFM4	−0.0117	Mediates cell-adhesion
	PI15	−0.0115	Proteolysis
	FOSB	−0.0111	Immune response

Table S2 Continued

DDIR panel	Gene symbol	Weight	Gene function
	FAM19A5	−0.011	Immune response
	NLRC5	0.0101	Immune response
	PRICKLE1	−0.0089	Nuclear trafficking
	EGR1	−0.0086	Transcriptional regulation
	CLDN10	−0.0086	Cell adhesion
	ADAMTS4	−0.0085	Proteolysis
	SP140L	0.0084	DNA, protein and metal ion binding
	ANXA1	−0.0082	Immune response
	RSAD2	0.0081	Immune response
	ESR1	0.0079	Transcriptional regulation
	IKZF3	0.0073	Immune response
	OR2I1P	0.007	Olfactory receptor activity
	EGFR	−0.0066	Proliferation and apoptosis
	NAT1	0.0065	Metabolism
	LATS2	−0.0063	Proliferation and mitosis
	CYP2B6	0.0061	Metabolism
	PTPRC	0.0051	Proliferation, differentiation and mitosis
	PPP1R1A	−0.0041	Potentiation and meiosis
	AL137218.1	−0.0017	Unreported
9-gene	CCL5	-	Immune response
	CXCL10	-	Immune response
	CXCL9	-	Immune response
	GABBR1	-	Signal transduction, growth and metabolism
	IDO1	-	Immune response
	IFI44L	-	Immune response
	IFIT1	-	Immune response
	OAS2	-	Immune response
	SLAMF7	-	Immune response

Table S3 The DNA damage response and repair gene panel

MMR	NER	HR	FA	Checkpoint	Others
MLH1	ERCC2	BRCA1	BRCA2	ATM	POLE
MSH2	ERCC3	MRE11A	BRIP1	ATR	MUTYH
MSH6	ERCC4	NBN	FANCA	CHEK1	PARP1
PMS1	ERCC5	RAD50	FANCC	CHEK2	RECQL4
PMS2		RAD51	PALB2	MDC1	
		RAD51B	RAD51C		
		RAD51D	BLM		
		RAD52			
		RAD54L			

MMR, mismatch repair; NER, nucleotide excision repair; HR, homologous recombination; FA, Fanconi anemia.

Table S4 Published immune-related gene signatures used in the current study

Signature name	Genes	Reference
Cytolytic activity	GZMA, PRF1	Cell. 2015; 160: 48-61
13 T-cell signature	CD8A, CCL2, CCL3, CCL4, CXCL9, CXCL10, ICOS, GZMK, IRF1, HLA-DMA, HLA-DMB, HLA-DOA, HLA-DOB	Proc Natl Acad Sci U S A. 2016; 113: E7759-68
TLS	CCL2, CCL3, CCL4, CCL5, CCL8, CCL18, CCL19, CCL21, CXCL9, CXCL10, CXCL11, CXCL13	Sci Rep. 2012; 2: 765
18-gene IFN signature	CD3D, IDO1, CIITA, CD3E, CCL5, GZMK, CD2, HLA-DRA, CXCL13, IL2RG, NKG7, HLA-E, CXCR6, LAG3, TAGAP, CXCL10, STAT1, GZMB	J Clin Invest. 2017; 127: 2930-40
T-cell inflamed GEP	CCL5, CD27, CD274, CD276, CD8A, CMKLR1, CXCL9, CXCR6, HLA-DQA1, HLA-DRB1, HLA-E, IDO1, LAG3, NKG7, PDCD1LG2, PSMB10, STAT1, TIGIT	Science. 2018; 362: eaar3593
Teff gene signature	CD8A, EOMES, PRF1, IFNG, CD274	Nat Med. 2018; 24: 749-57
Immune checkpoint	CD274, CTLA4, HAVCR2, LAG3, PDCD1, PDCD1LG2, TIGIT	Lancet Oncol. 2020; 21: 283-93

TLS, tertiary lymphoid structures; IFN, interferon; GEP, gene expression profiling.

Table S5 Clinical baseline characteristics of six immunotherapy cohorts included in the current study

Patient characteristic	Kim18	Parikh22	PUCH	Gide19	IMvigor210	Pender21
Tumor type	Gastric cancer	Colorectal/pancreatic cancer	Melanoma	Melanoma	Urothelial cancer	Pan-cancer
No. of patients	45	22	55	41	298	56
Median age in yrs (range)	-	-	51 (27–72)	66 (37–90)	-	56.5 (25–86)
Gender, *n* (%)						
Male	-	-	17 (30.9)	26 (63.4)	233 (78.2)	25 (44.6)
Female	-	-	38 (69.1)	15 (36.6)	65 (21.8)	31 (55.4)
Race, *n* (%)						
Yellow	45 (100)	-	55 (100)	-	7 (2.3)	-
White	-	-	-	-	270 (90.6)	-
Black	-	-	-	-	9 (3.0)	-
Other	-	-	-	-	12 (4.0)	-
Previous ICI, *n* (%)						
Naïve	45 (100)	-	-	-	298 (100)	46 (82.1)
Treated	-	-	-	-	-	10 (17.9)
Treatment, *n* (%)						
Anti-PD-1	45 (100)	-	55 (100)	41 (100)	-	44 (78.6)
Anti-PD-L1	-	-	-	-	298 (100)	12 (21.4)
Anti-PD-1 & anti-CTLA-4	-	22 (100)	-	-	-	-
Best overall response, *n* (%)						
CR	3 (6.7)	1 (4.5)	1 (1.8)	4 (9.8)	25 (8.4)	0 (0)
PR	9 (20.0)	2 (9.1)	13 (23.6)	15 (36.6)	43 (14.4)	9 (16.1)
SD	15 (33.3)	1 (4.5)	6 (10.9)	6 (14.6)	63 (21.1)	23 (41.1)
PD	18 (40.0)	11 (50.0)	35 (63.6)	16 (39.0)	167 (56.0)	24 (42.9)
NE	-	7 (31.8)	-	-	-	-
Median PFS (months)	-	2.5	3.8	9.0	-	4.7
Median OS (months)	-	6.4	28.1	29.3	10.6	13.4

ICI, immune checkpoint inhibitors; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluated; PFS, progression-free survival; OS, overall survival.

Table S6 Predictive performance of DRIA and two previously reported DDIR signatures for response to ICI therapy in five immunotherapy cohorts

Cohort	AUC (95% CI)		
	DRIA	9-gene DDIR	44-gene DDIR
Kim18	0.838 (0.680–0.997)	0.818 (0.649–0.988)	0.881 (0.749–1.000)
Parikh22	0.806 (0.580–1.000)	0.833 (0.612–1.000)	0.694 (0.419–0.970)
PUCH	0.760 (0.612–0.907)	0.782 (0.630–0.935)	0.775 (0.645–0.905)
Gide19	0.845 (0.715–0.974)	0.854 (0.727–0.981)	0.813 (0.671–0.956)
Pender21	0.766 (0.596–0.936)	0.738 (0.553–0.922)	0.780 (0.632–0.929)

DRIA, DNA damage response (DDR)-related immune activation; DDIR, DNA damage immune activation; ICIs, immune checkpoint inhibitors; AUC, area under the ROC curve; CI, confidence interval.