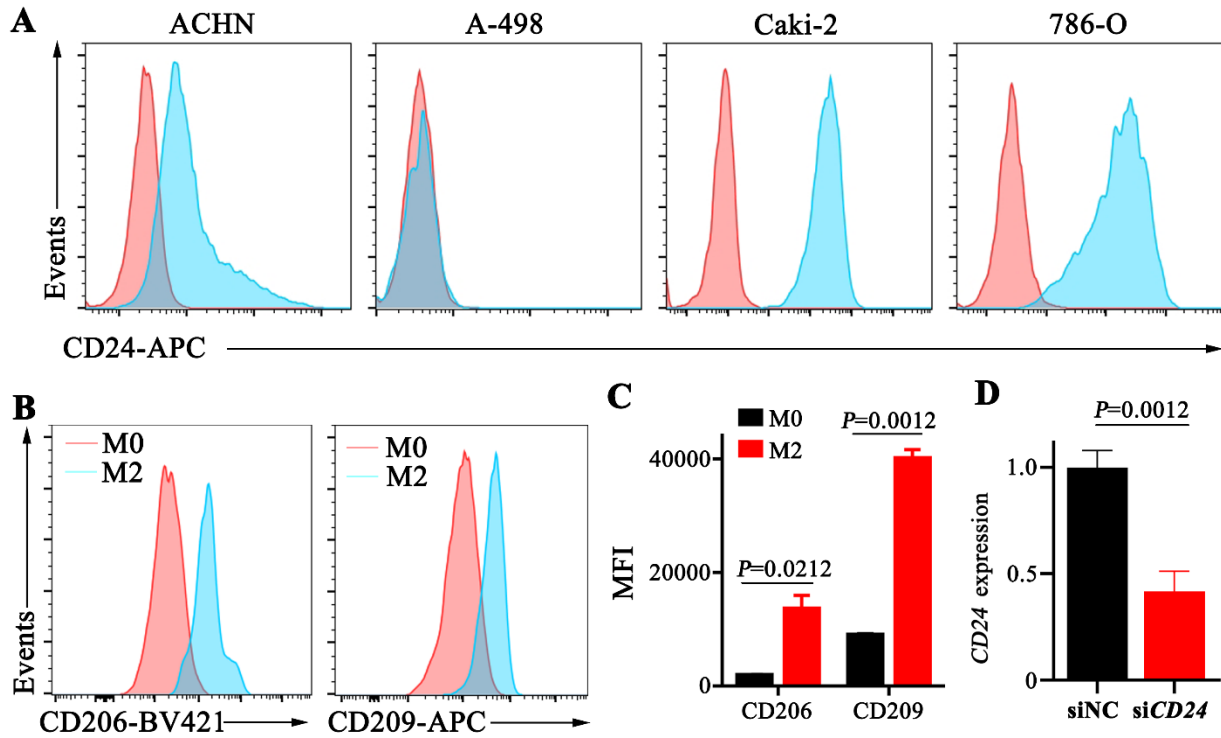


Supplementary information

Innate immune checkpoint Siglec10 in cancers: mining of comprehensive omics data and validation in patient samples



Supplementary Figure 1. Detection of surface markers on the cells. (A) The expression of CD24 on cancer line. (B) Flow cytometry assays of CD206 and CD209 on macrophages and the corresponding mean fluorescence intensity (MFI) on the cells (C). (D) The effects of siCD24 treatment on CD24 expression on Jurkat cells.

Supplementary Table 1. Baseline demographic characteristics of the LGG patients.

No.	Age, y	Gender	Pathology	Stage	Primary location	Laterality	Seizure	headache	Asthenia	Sensation
1	50	F	Glioma	II	Deep Structure	Left	Yes	Yes	No	Discontinuous numb
2	51	F	Glioma	II	Deep Structure	Right	Yes	No	No	Dizziness and nausea
3	52	M	Glioma	II	Cortical	Right	No	No	No	Dizziness
4	31	F	Glioma	III	Deep Structure	Left	Yes	No	No	No
5	53	F	Glioma	III	Deep Structure	Left	No	No	No	Dizziness, nausea and vomit
6	55	F	Glioma	III	Cortical	Right	No	No	No	No
7	34	M	Glioma	IV	Cerebellum	Right	No	No	Yes	Speech impaired

Supplementary Table 2. Baseline demographic characteristics of the KIRC patients obtained from Beijing Chaoyang Hospital and Shanghai Outdo Biotech.

Variable	No. of cases (%)	Siglec-10 expression		P value*
		High, n (%)	Low, n (%)	
<i>KIRC patients' samples from Beijing Chaoyang Hospital, n=41</i>				
Age at diagnosis				0.067
≤ 55	17 (41.5)	8 (47.1)	9 (52.9)	
> 55	24 (58.5)	18 (75)	6 (25)	
Gender				0.181
Male	32 (78)	22 (68.7)	10 (31.3)	
Female	9 (22)	4 (44.4)	5 (55.6)	
Stage				0.016
I	11 (26.8)	4 (36.4)	7 (63.6)	
II	17 (41.5)	10 (58.8)	7 (41.2)	
III-IV	13 (31.7)	12 (92.3)	1 (7.7)	
Unknown	0	0	0	
<i>KIRC patients' samples from Shanghai Outdo Biotech, n=90</i>				
Age at diagnosis				0.49
≤ 55	37 (41.1)	25 (67.6)	12 (32.4)	
> 55	53 (58.9)	32 (60.4)	21 (39.6)	
Gender				0.45
Male	51 (56.7)	34 (66.7)	17 (33.3)	
Female	39 (43.3)	23 (59)	16 (41)	
Stage				0.59
I	60 (66.7)	38 (63.3)	22 (36.7)	
II	18 (20)	11 (61.1)	7 (38.9)	
III-IV	6 (6.7)	5 (83.3)	1 (16.7)	
Unknown	6 (6.7)			

*P values were calculated using a two-sided Fisher's exact test.

Supplementary Table 3. Sequences of primers and siRNAs

Genes	Forward primer (5'→3')	Reverse primer (5'→3')
RT-PCR		
<i>GAPDH</i>	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG
<i>SIGLEC10</i>	AAGGGACTCATCTCAACGGC	TCATTGGAACCTGACTTCTGCATAATC
qRT-PCR		
<i>GAPDH</i>	CTGGGCTACACTGAGCACC	AAGTGGTCGTTGAGGGCAATG
<i>SIGLEC10</i>	AGATTCTACCGAAGAGACGGAC	CGTCGGGACCACATTGATGTA
<i>CD24</i>	CTCCTACCCACGCAGATTTATTC	AGAGTGAGACCACGAAGAGAC
siRNA		
	Sequence 1 (5'→3')	
si <i>CD24</i>	ACAACAACCTGGAACCTCAA	
si <i>Fos</i>	GCAAGGTGGAACAGTTATC	
si <i>GATA1</i>	GGACAGGCCACTACCTATG	

Supplementary Table 4. Cancer types, samples, and databases used in this study.

Databases	Cancer types	Normal tissues	Tumor samples	Normal samples
TCGA	ACC		79	
	BLCA		408	19
	BRCA		1093	112
	CESC		306	3
	CHOL		36	9
	COAD		457	41
	DLBC		48	
	ESCA		184	13
	GBM		163	5
	HNSC		520	44
	KICH		66	25
	KIRC		533	72
	KIRP		290	32
	LAML		173	
	LGG		518	
	LIHC		371	50
	LUAD		515	59
	LUSC		501	51
	MESO		87	
	OV		426	
	PAAD		179	4
	PCPG		182	3
	PRAD		497	52
	READ		166	10
	SARC		262	2
	SKCM		471	1
	STAD		415	36
TGCT		150		
THCA		512	59	
THYM		120	2	
UCEC		545	35	
UCS		57		
UVM		80		
GTEx		Adrenal Gland		128
		Bladder		9
		Breast		179
		Cervix Uteri		10

GTEX		Colon		308
		Blood		337
		Esophagus		273
		Brain		207
		Kidney		28
		Bone Marrow		70
		Brain		207
		Liver		110
		Lung		288
		Ovary		88
		Pancreas		167
		Prostate		100
		Colon		308
		Skin		557
		Stomach		175
		Testis		165
		Thyroid		278
		Blood		337
		Uterus		78
	Oncomine	Bladder Cancer		340
Brain and CNS Cancer			1327	789
Breast Cancer			3629	1084
Cervical Cancer			297	132
Colorectal Cancer			1171	721
Esophageal Cancer			120	130
Gastric Cancer			701	573
Head and Neck Cancer			1106	934
Kidney Cancer			594	544
Leukemia			2248	298
Liver Cancer			287	237
Lung Cancer			1334	1012
Lymphoma			239	124
Melanoma			424	339
Myeloma			140	106
Other Cancer			587	417
Ovarian Cancer			654	567
Pancreatic Cancer			157	111
Prostate Cancer			456	271
Sarcoma			125	72
	Adrenal gland cancer		627	

COSMIC	Autonomic ganglia cancer	1137	
	Biliary tract cancer	767	
	Bone cancer	557	
	Breast cancer	2580	
	Central nervous system cancer	2608	
	Cervix cancer	326	
	Endometrium cancer	712	
	Eye cancer	121	
	Fallopian tube cancer	2	
	Gastrointestinal tract (site indeterminate) cancer	1	
	Genital tract cancer	70	
	Haematopoietic and lymphoid cancer	4860	
	Kidney cancer	2192	
	Large intestine cancer	2547	
	Liver cancer	2215	
	Lung cancer	2609	
	Meninges cancer	130	
	NS cancer	130	
	Oesophagus cancer	1530	
	Ovary cancer	903	
	Pancreas cancer	1840	
	Parathyroid cancer	35	
	Penis cancer	10	
	Peritoneum cancer	20	
	Pituitary cancer	57	
	Placenta cancer	2	
	Pleura cancer	269	
	Prostate cancer	1984	
	Salivary gland cancer	98	
	Skin cancer	1631	
	Small intestine cancer	193	
	Soft tissue cancer	589	
	Stomach cancer	1418	
	Testis cancer	229	
Thymus cancer	152		
Thyroid cancer	1040		
Upper aerodigestive tract cancer	1282		
Urinary tract cancer	695		
Vulva cancer	3		

Total	33	23	64517	13811
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The Oncomine database includes 830 samples that are also included in TCGA database.

Supplementary Table 5. *SIGLEC10* expression in tumor tissues compared to normal tissues in OncoPrint database.

Cancer	Data set	Cancer type	Samples	Fold change	P value
Brain and CNS Cancer	French Brain	Anaplastic Oligoastrocytoma	10	1.807	0.002
	Lee Brain	Glioblastoma	25	7.209	0.002
	Sun Brain	Diffuse Astrocytoma	30	2.007	0.006
Breast Cancer	Curtis Breast	Medullary Breast Carcinoma	176	3.492	2.43E-13
	Curtis Breast	Invasive Ductal and Invasive Lobular Breast Carcinoma	234	1.688	4.49E-26
	Curtis Breast	Ductal Breast Carcinoma in Situ	154	1.806	2.64E-04
	Curtis Breast	Invasive Ductal Breast Carcinoma	1700	1.737	1.30E-64
	Curtis Breast	Benign Breast Neoplasm	147	2.701	0.011
	Curtis Breast	Invasive Lobular Breast Carcinoma	292	1.577	4.99E-29
	Curtis Breast	Breast Carcinoma	158	1.833	2.32E-04
	Ma Breast 4	Invasive Ductal Breast Carcinoma Stroma	23	1.814	0.001
	Ma Breast 4	Ductal Breast Carcinoma in Situ Stroma	25	1.752	0.004
	TCGA Breast	Invasive Lobular Breast Carcinoma	97	2.484	2.10E-11
	Karnoub Breast	Invasive Ductal Breast Carcinoma Stroma	22	1.967	0.012
	Ma Breast 4	Invasive Ductal Breast Carcinoma Epithelia	23	-1.628	5.53E-05
	Ma Breast 4	Ductal Breast Carcinoma in Situ Epithelia	23	-1.524	0.005
Colorectal cancer	TCGA Colorectal	Rectosigmoid Adenocarcinoma	22	-2.86	6.27E-10
Gastric cancer	Cho Gastric	Gastric Mixed Adenocarcinoma	29	-2.048	4.20E-04
	Cho Gastric	Diffuse Gastric Adenocarcinoma	50	-1.576	0.001
Kidney cancer	Yusenko Renal	Chromophobe Renal Cell Carcinoma	9	7.504	0.001
	Yusenko Renal	Papillary renal Cell Carcinoma	24	7.736	5.80E-04
	Yusenko Renal	Clear Cell renal Cell Carcinoma	31	17.018	1.53E-04
Leukemia	Haferlach	B-cell Childhood Acute Leukemia	433	-1.755	8.63E-22
Myeloma	Zhan Myeloma	Monoclonal Gammopathy of Undetermined Significance	66	-1.793	1.17E-04
Other	Skotheim Testis	Testicular Embryonal Carcinoma	8	-1.535	0.009

Supplementary Table 6. Correlations between *Siglec10* and the marker genes of monocytes and macrophages in GEPIA database.

Description	Gene markers	KIRC		LGG	
		R	P	R	P
Monocyte	CD86	0.65	***	0.69	***
	CSF1R	0.78	***	0.74	***
TAM	CCL5	0.49	***	0.54	***
	CD68	0.38	***	0.7	***
	IL10	0.29	***	0.43	***
M1 Macrophage	NOS2	-0.0084	0.85	0.052	0.23
	IRF5	0.29	***	0.65	***
	PTGS2	-0.031	0.48	0.014	0.76
M2 Macrophage	CD163	0.61	***	0.74	***
	VSIG4	0.75	***	0.8	***
	MS4A4A	0.61	***	0.7	***

*P < 0.05, **P < 0.01, ***P < 0.001.

Supplementary Table 7. Correlation analysis between *SIGLEC10* and the marker genes of immune cells in TIMER.

description	Gene markers	KIRC				LGG			
		None		Purity		None		Purity	
		Cor	P	Cor	P	Cor	P	Cor	P
CD8+T cell	CD8A	0.557	***	0.512	***	0.089	*	-0.003	0.952
	CD8B	0.522	***	0.482	***	0.073	0.096	-0.012	0.796
T cell	CD3D	0.593	***	0.549	***	0.308	***	0.265	***
	CD3E	0.621	***	0.578	***	0.356	***	0.332	***
	CD2	0.643	***	0.607	***	0.354	***	0.331	***
B cell	CD19	0.404	***	0.375	***	0.392	***	0.339	***
	CD79A	0.424	***	0.390	***	0.386	***	0.408	***
Monocyte	CD86	0.827	***	0.809	***	0.854	***	0.835	***
	CSF1R	0.846	***	0.833	***	0.811	***	0.783	***
TAM	CCL5	0.464	*	0.464	0.492	0.331	***	0.309	***
	CD68	0.562	***	0.566	***	0.823	***	0.808	***
	IL10	0.525	***	0.466	***	0.512	***	0.465	***
M1 Macrophage	NOS2	0.134	**	0.082	0.079	-0.117	**	-0.132	**
	IRF5	0.436	***	0.437	***	0.787	***	0.757	***
	PTGS2	0.112	**	0.076	0.102	0.133	**	0.078	0.090
M2 Macrophage	CD163	0.684	***	0.664	***	0.545	***	0.548	***
	VSIG4	0.701	***	0.672	***	0.816	***	0.800	***
	MS4A4A	0.699	***	0.668	***	0.719	***	0.715	***
Neutrophils	CEACAM8	0.084	0.053	0.117	*	0.079	0.074	0.078	0.090
	ITGAM	0.724	***	0.713	***	0.747	***	0.709	***
	CCR7	0.533	***	0.493	***	0.254	***	0.240	***
Natural killer cell	KIR2DL1	0.128	**	0.098	*	-0.035	0.430	0.004	0.926
	KIR2DL3	0.171	***	0.163	***	0.131	**	0.147	***
	KIR2DL4	0.304	***	0.278	***	0.410	***	0.422	***
	KIR3DL1	0.149	***	0.159	**	-0.019	0.669	-0.027	0.563
	KIR3DL2	0.217	***	0.219	***	0.151	***	0.158	***
	KIR3DL3	0.104	*	0.087	0.063	0.071	0.105	0.063	0.168
	KIR2DS4	0.104	*	0.080	0.085	0.148	***	0.145	**
Dendritic cell	HLA-DPB1	0.764	***	0.749	***	0.597	***	0.571	***
	HLA-DQB1	0.475	***	0.436	***	0.464	***	0.445	***
	HLA-DRA	0.770	***	0.761	***	0.643	***	0.617	***
	HLA-DPA1	0.750	***	0.731	***	0.604	***	0.581	***
	CD1C	0.342	***	0.278	***	0.310	***	0.310	***
	NRP1	0.197	***	0.136	**	0.094	*	0.131	**
	ITGAX	0.620	***	0.611	***	0.703	***	0.660	***
Th1	TBX21	0.450	***	0.417	***	0.195	***	0.210	***

description	Gene markers	KIRC				LGG			
		None		Purity		None		Purity	
		Cor	P	Cor	P	Cor	P	Cor	P
Th2	STAT4	0.544	***	0.503	***	-0.220	***	-0.276	***
	STAT1	0.621	***	0.586	***	0.311	***	0.305	***
	IFNG	0.510	***	0.461	***	0.156	***	0.123	0.007
	TNF	0.366	***	0.354	***	0.247	***	0.199	***
	GATA3	0.218	***	0.202	***	0.320	***	0.285	***
	STAT6	0.196	***	0.230	***	0.437	***	0.363	***
	STAT5A	0.677	***	0.657	***	0.662	***	0.614	***
Tfh	IL13	0.045	0.297	0.058	0.215	0.078	0.076	0.080	0.080
	BCL6	0.193	***	0.194	***	0.387	***	0.428	***
	IL21	0.251	***	0.240	***	0.134	**	0.114	*
Th17	STAT3	0.353	***	0.321	***	0.479	***	0.498	***
	IL17A	0.062	0.151	0.067	0.150	-0.023	0.600	-0.039	0.395
Treg	FOXP3	0.593	***	0.563	***	-0.189	***	-0.162	***
	CCR8	0.615	***	0.591	***	0.126	**	0.126	**
	STAT5B	0.210	***	0.216	***	0.116	**	0.195	***
	TGFBI	0.376	***	0.318	***	0.292	***	0.297	***
T cell exhaustion	PDCD1	0.572	***	0.545	***	0.453	***	0.441	***
	CTLA4	0.602	***	0.577	***	0.336	***	0.282	***
	LAG3	0.537	***	0.508	***	0.308	***	0.351	***
	HAVCR2	0.303	***	0.258	***	0.842	***	0.824	***

Th, T helper cell; Tfh, Follicular helper T cell; Treg, regulatory T cell; Cor, R value of Spearman's correlation; None, correlation without adjustment. Purity, correlation adjusted by purity. *P < 0.05, **P < 0.01, ***P < 0.001.