Supplementary Materials

Table 1. Antibodies for flow cytometry

Target	Fluorophore	Company	Catalog number	Clone
FACSAria III panel for sorting of cord blood DCs				
CD141	BV711	BD	563155	1A4
CD1c	PE-Cy7	Biolegend	331516	L161
CD14	PerCP-Cy5.5	Biolegend	325622	HCD14
CLEC9A	APC	Biolegend	353806	8F9
CD45	AF700	Biolegend	304024	HI30
Dead Cell Stain	Near-IR	Life Technologies	L34976	
FACSAria III panel for enrichment of DCs from tumor digests				
CD45	BV510	Biolegend	304036	HI30
CD3	AF488	Biolegend	317310	OKT3
Dead Cell Stain	Near-IR	Life Technologies	L34976	
LSRFortessa panel for cytokine expression in blood or tumor-derived DCs				
CD11c	BV421	Biolegend	301628	3.9
CD45	BV510	Biolegend	304036	HI30
TNFα	BV605	Biolegend	502936	MAb11
CD56	BV650	Biolegend	318344	HCD56
CD16	BV711	Biolegend	302044	3G8
IFNλ	AF488*	R&D Systems	MAB15981-500	247801
CD14	PerCP-Cy5.5	Biolegend	325622	HCD14
CXCL9	PE	Biolegend	519504	J034D6
CXCL10	PE	Biolegend	357904	J1015E10
CD3	PE-Cy5	BD	555341	HIT3a
CD1c	PE-Cy7	Biolegend	331516	L161
CLEC9A	APC	Biolegend	353806	8F9
CD19	AF700	Biolegend	302226	HIB19
HLA-DR	BUV395	BD	564040	G46-6
Dead Cell Stain	Near-IR	Life Technologies	L34976	

FACSymphony A5 panel for cytokine expression in blood or tumor-derived DCs				
CD3	BUV395	BD	740283	HIT3a
CD19	BUV395	BD	563551	SJ25C1
HLA-DR	BUV496	BD	741157	Tu39
CD16	BUV615	BD	750572	3G8
CD56	BUV661	BD	750478	NCAM16.2
CD45	BUV805	BD	612891	HI30
TNFα	BV605	Biolegend	502936	MAb11
CD14	BV650	BD	740633	MφP9
IFNλ	AF488*	R&D Systems	MAB15981-500	247801
CXCL10	PE	Biolegend	519504	J034D6
CD68	PE-eFluor 610	invitrogen	61-0689-42	Y1/82A
CD1c	PE-Cy7	Biolegend	331516	L161
CLEC9A	APC	Biolegend	353806	8F9
CD11c	AF700	Biolegend	337220	Bu15
Dead Cell Stain	Near-IR	Life Technologies	L34976	

* anti-IFNλ antibodies in AF488: in-house labeling (Invitrogen, #A30005)

Table 2. Overview of scRNA-seq studies

Study_name	Tissue_type	Status	Study
Ovarian_Qian	Ovarian	Solid tumor - Cancer	Qian *et al.*
OV-FTC_Cheng	Ovarian	Solid tumor - Cancer	Cheng *et al.*
Lung_Qian	Lung	Solid tumor - Cancer	Qian *et al.*
Breat_Qian	Breast	Solid tumor - Cancer	Qian *et al.*
THCA_Cheng	Thyroid Carcinoma (THCA)	Solid tumor - Cancer	Cheng *et al.*
ESCA_Cheng	Esophageal Carcinoma (ESCA)	Solid tumor - Cancer	Cheng *et al.*
Kidney_Cheng	Kidney cancer	Solid tumor - Cancer	Cheng *et al.*
PAAD_Cheng	Pancreatic adenocarcinoma (PAAD)	Solid tumor - Cancer	Cheng *et al.*
UCEC_Cheng	Uterine Corpus Endometrial Carcinoma (UCEC)	Solid tumor - Cancer	Cheng *et al.*
HN_Cillo	Head and Neck cancer	Solid tumor - Cancer	Cillo *et al.*
Intestine_Qian	Intestine/Colorectal	Solid tumor - Cancer	Qian *et al.*
Intestine_Lee	Intestine/Colorectal	Solid tumor - Cancer	Lee *et al.*
Liver_Ma	Liver	Solid tumor - Cancer	Ma *et al.*
Lung_Maier	Lung	Solid tumor - Cancer	Maier *et al.*
Pancreatic_Peng	Pancreatic cancer	Solid tumor - Cancer	Peng *et al.*
PBMC_Cillo	Head and Neck PBMC - cancer	Blood sample from cancer patients	Cillo *et al.*
PBMC_Cillo_nc	Head and Neck PBMC - healthy	Blood sample from healthy donors	Cillo *et al.*
Intestine_Smillie	Intestine	Non-cancer	Smillie *et al.*
Pancreatic_Peng_nc	Pancreas	Non-cancer	Peng *et al.*
Lung_Raredon	Lung	Non-cancer	Raredon *et al.*
Lung_Madissoon	Lung	Non-cancer	Madissoon *et al.*
Tonsils_Cillo	Tonsils	Non-cancer	Cillo *et al.*
PBMC_Kotliarov	PBMC	Non-cancer	Kotliarov *et al.*
Liver_Ramachandran	Liver	Non-cancer	Ramachandran *et al.*
Intestine_Martin	Intestine	Non-cancer	Martin *et al.*
CBDCs_He	CBDCs	Cord blood sample	He *et al.*
PBMC_He	PBMC	Blood sample	He *et al.*
Lung_He	Lung	Solid tumor - Cancer, IFNγ+TL8-506 and Poly(I:C)+TL8-506 treated	He *et al.*
Colon_He	Colon	Solid tumor - Cancer, IFNγ+TL8-506 and Poly(I:C)+TL8-506 treated	He *et al.*
Melanoma_He	Melanoma	Solid tumor - Cancer, IFNγ+TL8-506 treated	He *et al.*

Table 3. Parameters for the filtering of high-quality cells in scRNA-seq studies

Study_name	standard_min_genes	standard_min_cells	standard_min_counts	standard_n_genes	standard_percent_mito	standard_max_counts
Ovarian_Qian	800	10	600	6000	0.1	55000
OV-FTC_Cheng	500	10	1000	6000	0.15	50000
Lung_Qian	600	10	1000	6000	0.15	70000
Breat_Qian	400	10	800	5900	0.03	70000
THCA_Cheng	500	10	1000	6000	0.15	50000
ESCA_Cheng	500	10	1000	6000	0.15	50000
Kidney_Cheng	500	10	1000	6000	0.15	50000
PAAD_Cheng	500	10	1000	6000	0.15	50000
UCEC_Cheng	500	10	1000	6000	0.15	50000
HN_Cillo	400	10	1200	7000	0.3	70000
Intestine_Qian	800	10	1200	6000	0.1	55000
Intestine_Lee	500	20	1000	6000	0.1	70000
Liver_Ma	500	30	1500	7000	0.15	60000
Lung_Maier	800	20	1500	5000	0.1	30000
Pancreatic_Peng	500	20	1000	8000	0.1	80000
PBMC_Cillo	600	10	1200	5000	0.15	40000
PBMC_Cillo_nc	600	10	1200	5000	0.15	40000
Intestine_Smillie	800	30	2000	8000	0.2	100000
Pancreatic_Peng_nc	800	30	2000	8000	0.2	100000
Lung_Raredon	700	20	1000	8000	0.15	60000
Lung_Madissoon	700	20	1000	8000	0.1	80000
Tonsils_Cillo	400	10	1700	7000	0.1	70000
PBMC_Kotliarov	500	30	1000	3000	0.05	20000
Liver_Ramachandran	600	20	1000	7000	0.15	60000
Intestine_Martin	500	30	1000	6000	0.2	50000
CBDCs_He	800	10	1000	6500	0.15	50000
PBMC_He	1500	20	1000	7000	0.18	60000
Lung_He	600	10	800	7000	0.15	75000
Colon_He	600	10	800	7000	0.15	75000
Melanoma_He	600	10	800	6500	0.15	50000
Parameters for integrated DC dataset	500	10	1000	6000	0.15	50000

Table 4. List of top 50 DE genes in treated tumor cDCs

	cDC1_medium_vs_ IFNγ + TL8-506		cDC1_medium_vs_ Poly(I:C) + TL8-506		cDC1_IFNγ + TL8-506_vs_Poly(I:C) + TL8-506		cDC2_medium_vs_ IFNγ + TL8-506		cDC2_medium_vs_ Poly(I:C) + TL8-506		cDC2_IFNγ + TL8-506_vs_Poly(I:C) + TL8-506	
	gene	log2FC	gene	log2FC	gene	log2FC	gene	log2FC	gene	log2FC	gene	log2FC
1	IL12B	8.8	ITGB8	30.9	ISG15	3.3	IL12B	6.5	IL6	8.1	SIGLEC6	25.9
2	UBD	6.9	NCF2	30.2	OASL	3.0	IL6	6.3	TCF7L2	5.2	EVA1C	25.4
3	CCL3L1	6.2	WNT4	29.2	PELI1	2.8	CXCL9	5.5	STEAP1B	5.1	DKK1	25.2
4	CCL5	5.6	CCL3L1	8.8	CLCF1	2.2	CXCL10	5.5	CCL4	5.0	FAM9C	25.1
5	LTA	5.3	CCL4L2	7.8	PMAIP1	1.7	UBD	5.4	TNF	5.0	FSTL1	25.1
6	CXCL9	4.9	CCL4	6.7	PSMB8	-1.5	IL36G	5.2	CCL4L2	4.8	IFNL1	25.1
7	GBP5	4.9	CCL3	6.6	CDC42SE2	-1.5	TNF	5.2	LTA	4.7	DAGLA	25.0
8	ITGB8	4.8	G0S2	6.0	WARS1	-1.6	ACOD1	5.1	CCL3L1	4.4	BIK	25.0
9	CCL3	4.8	NIPA1	5.9	CD48	-1.7	LTA	5.0	HERC5	4.4	CCDC68	24.8
10	TCF7L2	4.6	TCF7L2	5.8	STAT1	-1.9	IL27	4.7	CD200	4.4	EFNA4	24.8
11	TNF	4.5	TNF	5.0	PCYT1A	-1.9	CXCL11	4.7	IGSF3	4.2	RANBP3L	24.7
12	PRR5L	4.5	EXT1	5.0	TAP1	-1.9	XIRP1	4.6	FXYD6	4.0	ITGB5	24.7
13	IL1B	4.5	KMO	4.4	RIOK3	-1.9	STEAP1B	4.6	CD40	4.0	MRPL38	24.7
14	SOCS2	4.5	OASL	4.1	CD274	-1.9	IL12A	4.3	TNFSF9	3.8	GRIN1	24.7
15	TBX21	4.4	TFRC	4.0	APOL2	-2.0	KREMEN1	4.2	DHX58	3.8	TPX2	24.6
16	CXCL10	4.4	PMAIP1	3.9	HM13	-2.1	KCNJ2	4.1	IL23A	3.8	MEX3B	24.6
17	IGSF3	4.4	B3GNT7	3.6	STAT3	-2.1	IL1B	4.0	HLA-DOB	3.7	PIGR	24.6
18	IL27	4.2	FSD1L	3.5	LMNB1	-2.2	GBP5	4.0	CCL3	3.7	CCN2	24.6
19	CXCL11	4.1	GNS	3.5	RIPK2	-2.2	CCL4	3.8	SOCS1	3.6	MYOM1	24.5
20	G0S2	4.0	CLCF1	3.5	ITPRIPL2	-2.3	CCL3	3.7	CKB	3.6	PORCN	24.5
21	SLC1A2	4.0	SOD2	3.5	CLIP1	-2.3	PDGFRA	3.7	CSRP2	3.5	FOXC2	24.5
22	ANKRD22	3.8	CXCL8	3.3	CD226	-2.4	CCL5	3.6	CCL5	3.5	ENOX1	24.5
23	FSD1L	3.8	SOCS3	3.0	PSMB9	-2.5	APOL4	3.6	G0S2	3.5	BICD1	24.5
24	CCL4L2	3.8	CDH1	2.9	LAP3	-2.6	GBP1	3.5	FSD1L	3.3	WDR27	24.5
25	BATF	3.7	MACROH2A2	2.9	IRF1	-2.8	ADIRF	3.5	IFNLR1	3.1	NRG2	24.4
26	IL23A	3.5	PLAUR	2.9	UBE2L6	-2.9	TBX21	3.3	IL1B	3.1	FABP3	24.4
27	GBP1	3.4	STAT5A	2.8	CXCL11	-2.9	GPR171	3.3	RAB3IP	3.0	GATD3B	24.3
28	ELOVL7	3.4	SLC7A1	2.6	TRAFD1	-3.1	CCL3L1	3.2	CLCF1	2.9	FANCD2	24.3
29	TNFAIP6	3.3	HLA-DOB	2.6	CSF2RB	-3.1	JAG1	3.2	RIPOR2	2.9	ARHGEF19	24.3
30	ETV7	3.3	CCR7	2.6	GBP2	-3.1	CSF3	3.2	ATP6V0A2	2.9	ANKS6	24.3
31	MYC	3.2	NOP58	2.6	MSRB1	-3.7	G0S2	3.2	EXT1	2.9	TMSB15A	24.2
32	EXT1	3.2	NHP2	2.5	GBP5	-3.8	IGSF3	3.2	ITGA1	2.9	FANCB	24.2
33	RAB33A	3.2	CD44	2.4	CXCL10	-4.4	SOCS1	3.2	PIM1	2.8	ESPL1	24.2
34	GBP4	3.1	B4GALT5	2.4	GBP4	-4.4	IL2RA	3.1	NFKBIZ	2.8	CMTM4	24.2
35	CCND2	3.1	INSIG1	2.4	CXCL9	-5.4	CD274	3.1	TMEM268	2.7	PLOD2	24.2
36	CCL4	3.1	NFKBIZ	2.4	HAPLN3	-5.8	FJX1	3.1	EBI3	2.7	EFCAB11	24.1
37	CD274	3.1	UTP6	2.4	GBP1	-6.8	NFKBIZ	3.0	GALNT3	2.6	FRAT1	24.1
38	SOCS1	3.0	FOXP1	2.4			HECW2	3.0	PMAIP1	2.6	LAMC2	24.1
39	SOCS3	3.0	THAP2	2.3			LIMK2	3.0	FAM126A	2.6	ST6GALNAC2	24.1
40	CREB5	2.9	CD40	2.3			FXYD6	2.9	TUBB2A	2.6	CCDC24	24.1
41	NFKBIZ	2.9	DDX21	2.3			HLA-DOB	2.9	IFI44	2.6	TONSL	24.1
42	CSF2RB	2.8	MAP3K8	2.2			ETV7	2.9	RAB29	2.6	EYA2	24.1
43	SOD2	2.8	PELI1	2.1			SOCS3	2.9	NEDD4L	2.5	LRRC26	24.1
44	TCFL5	2.8	NANS	2.1			IL1A	2.8	SOCS3	2.5	IFT81	24.0
45	IL15	2.8	GTPBP4	2.0			IDO1	2.7	HERC6	2.5	ZSCAN20	24.0
46	PRDM1	2.6	ISG20	1.9			CCL4L2	2.7	OASL	2.5	NLRP2	24.0
47	IL1A	2.6	CDK6	1.9			FSD1L	2.7	FLT1	2.5	TBC1D3L	24.0
48	HAPLN3	2.6	WTAP	1.8			GBP4	2.7	TAGAP	2.5	IL22RA2	24.0
49	CDH1	2.6	MGLL	1.8			CD40	2.6	KIF3B	2.5	DNAH10	24.0
50	KMO	2.6	IL2RA	1.8			PDCD1LG2	2.6	STK17B	2.5	H3C4	24.0

	aDC_tumor in situ_vs_ IFNγ + TL8-506 ex vivo		aDC_tumor in situ_vs_ Poly(I:C) + TL8-506 ex vivo		aDC+intcDC2_tumor in situ_vs_IFNγ + TL8-506 ex vivo		aDC+intcDC2_tumor in situ_vs_Poly(I:C) + TL8-506 ex vivo	
	gene	log2FC	gene	log2FC	gene	log2FC	gene	log2FC
1	LTA	29.4	WNT4	6.7	LTA	29.5	LTA	29.2
2	TBKBP1	6.1	ZBTB32	6.3	CCL3	6.9	IFNB1	8.8
3	CXCL10	5.6	TNF	6.0	TNF	6.0	CSF2	7.8
4	TNF	5.4	CCL3	5.2	IL1B	5.9	IL12A	7.2
5	WNT4	5.4	IL1B	4.7	IL12A	5.8	CCL3	6.9
6	GBP5	5.3	IL6	4.7	CCL4	5.7	WNT4	6.7
7	CXCL11	5.2	CCL4	4.7	CXCL10	5.7	CCL4	6.6
8	TCF7L2	5.0	LAMC1	4.7	CSF2	5.6	TNF	6.4
9	IL27	4.8	TCF7L2	4.6	GBP5	5.3	IL1B	6.2
10	CXCL9	4.8	RASSF8	4.5	CXCL11	5.3	IL6	6.0
11	IL1B	4.7	EXT1	4.3	KCNJ2	5.2	STEAP1B	5.9
12	CCL3	4.5	NIPA1	4.3	IL27	5.1	IL1A	5.8
13	AIM2	4.5	ABAT	4.3	WNT4	5.1	EPB41L5	5.6
14	SNTB1	4.4	GALNT12	4.3	SERPINB2	5.0	ZBTB32	5.4
15	IL6	4.3	TCFL5	4.3	IL6	4.9	CCL5	4.9
16	P2RY6	4.2	IL1R1	4.3	TBKBP1	4.9	RASSF8	4.9
17	SLAMF8	4.2	B3GNT7	4.2	IL1A	4.7	TLCD1	4.8
18	TCFL5	4.2	APOBR	4.1	STEAP1B	4.7	B3GNT7	4.8
19	TLR4	4.1	TLCD1	4.0	CXCL9	4.7	OASL	4.7
20	GBP7	4.1	MGST1	4.0	MEFV	4.6	EXT1	4.7
21	IL12B	4.0	HERC5	3.9	PLEKHN1	4.5	IL1R1	4.6
22	PRR5L	3.9	IGSF3	3.8	CSF3	4.4	BAALC	4.6
23	CDH1	3.9	TACSTD2	3.7	CCL5	4.4	NIPA1	4.6
24	GBP4	3.8	CCL5	3.6	GBP1	4.4	ITGA1	4.5
25	GBP1	3.8	IL2RA	3.6	TCF7L2	4.3	IGSF3	4.5
26	TBX21	3.8	SGMS2	3.6	CACNA1A	4.3	LAMC1	4.4
27	APOBR	3.8	NFKBIZ	3.5	BAALC	4.2	HERC6	4.4
28	IGSF3	3.8	PDGFB	3.4	RAB33A	4.2	TJP1	4.4
29	CMPK2	3.7	IL12B	3.4	AQP9	4.2	IL23A	4.3
30	RAB33A	3.7	PLAUR	3.4	DNAAF1	4.2	AMIGO2	4.2
31	POU3F1	3.6	MBP	3.4	P2RX7	4.2	GALNT12	4.2
32	TLCD1	3.6	GALNT3	3.3	EPB41L5	4.1	HERC5	4.2
33	DLL4	3.6	GRWD1	3.3	CMPK2	4.1	TCF7L2	4.2
34	CCL5	3.6	NEDD4L	3.3	CD300E	4.1	SHROOM1	4.2
35	SMN1	3.6	TCF7	3.2	TLR4	4.1	IL2RA	4.2
36	ZBTB6	3.5	G0S2	3.2	GBP7	4.1	NEDD4L	4.1
37	EXT1	3.4	ZMYND11	3.1	ATP10A	4.1	CLCF1	4.0
38	PAWR	3.4	MAP4K5	3.1	INHBA	4.0	CDH1	3.9
39	CASP10	3.4	CLCF1	3.1	PRR5L	3.9	KIAA1522	3.9
40	NFKBIZ	3.4	CD40	3.1	KCNN4	3.9	IL1RN	3.8
41	SCARF1	3.3	PMAIP1	3.1	EXT1	3.8	IFIT2	3.8
42	IRF8	3.3	IGF2R	3.1	DYRK3	3.8	TRAF3IP2	3.8
43	ZFYVE28	3.3	HAUS6	3.1	TBX21	3.8	TCFL5	3.8
44	ETV7	3.3	RFFL	3.1	NEU4	3.7	IL12B	3.8
45	CCL4	3.2	FOSL1	3.1	OASL	3.7	LIMS2	3.8
46	EPC2	3.2	MFHAS1	3.0	ITGA1	3.7	MACC1	3.7
47	IL2RA	3.2	PLAGL2	3.0	AIM2	3.6	SNTB1	3.7
48	GPATCH4	3.2	FAM126A	3.0	ZFYVE28	3.6	GALNT3	3.7
49	APOL1	3.2	PRKCI	3.0	IGSF3	3.6	DDX60	3.7
50	DYRK3	3.1	CDC42EP3	3.0	GBP4	3.6	G0S2	3.7

Table 5. Overview of patient tumor samples

sample_ID	Tumor Indication	Subtype	Patient Treatment	Assays	% $CD45^+$ cells	Panel for flow cytometry
colon_1	Colon cancer	liver metastasis	untreated	scRNA-seq, flow cytometry	30	FACSAria III , FACSymphony A5
lung_1	Lung cancer	Adeno carcinoma	untreated	scRNA-seq	75	FACSAria III
lung_2	Lung cancer	NSCLC	untreated	flow cytometry, ELISA	44	LSRFortessa
lung_3	Lung cancer	NSCLC	untreated	flow cytometry	72	LSRFortessa
lung_4	Lung cancer	NSCLC	untreated	flow cytometry	74	LSRFortessa
melanoma_1	Melanoma	lymph node metastasis	untreated	scRNA-seq	96	FACSAria III
melanoma_2	Melanoma	lymph node metastasis	untreated	scRNA-seq, flow cytometry	15	FACSAria III, FACSymphony A5
melanoma_3	Melanoma	lymph node metastasis	untreated	flow cytometry	98	FACSymphony A5
melanoma_4	Melanoma	lymph node metastasis	untreated	flow cytometry	15	LSRFortessa
melanoma_5	Melanoma	brain metastasis	LGX818+MEK162, Ipi+Nivo, Nivo	flow cytometry	21	LSRFortessa
melanoma_6	Melanoma	lymph node metastasis	untreated	flow cytometry	18	LSRFortessa
ovarian_1	Ovarian cancer	serous carcinoma	untreated	ELISA	61	-
ovarian_2	Ovarian cancer	serous carcinoma	untreated	ELISA	59	-

Materials and Methods

ScRNA-seq data quality control and pre-processing

Publicly available scRNA-seq datasets of different tissues (online supplemental table 2) were collected either as raw count matrices or fastq files. Internal and external fastq files were aligned and quantified using the Cell Ranger Single-Cell Software [1] with default parameters against the GRCh38 human reference genome. The data was further pre-processed by following the standard workflow in Besca [2]. For individual datasets, the quality of cells was assured by filtering all low-quality cells and removing uninformative genes by using the parameters in online supplemental table 3. The filtering was applied to cells based on the metrics, including minimum and maximum number of genes expressed, minimum and maximum total UMI count, and maximum proportion of mitochondrial gene count. Also, genes that were expressed in less than 10 cells were filtered out. After performing quality control, the raw count data were normalized, logarithmized and used for several downstream studies.

Dimension reduction and unsupervised clustering

For each dataset, the genes showing highest variability using besca.st.highly_variable_genes function were selected (minimal mean = 0.0125, maximal mean = 3 and minimal normalized dispersion = 0.5 cutoffs). Next, the effects of total count per cell and mitochondrial gene percentage effects were regressed out and the data were standardized. Subsequently, a principal component analysis with 50 components was performed and the first 50 components retained to build a nearest neighbor graph (local neighborhood size 15) and to derive clusters using the Leiden community detection

algorithm [3]. To integrate the cells from public datasets into a shared space, the raw datasets were concatenated and a second round of preprocessing, quality control was performed. The calculated PCA matrix was subjected to the Harmony algorithm [4] as an input, and individual studies were kept as a technical covariate for the correction. The batch-corrected PCA coordinates were then used to build the integrated nearest neighbor graph and to find clusters within. For the visualization of identified clusters, the Harmony corrected PCA matrix as an input was used for the UMAP. Further, Gaussian kernel density estimation function from scanpy was used to calculate the density of tumor-derived treated and non-treated immune cells, as well as tumor-derived cells from public datasets in the UMAP space [5].

Cell type annotation

To identify the cell type of the clusters returned by the Leiden algorithm, curated signatures and the sig-annot workflow available in Besca were used [2] based on the expression of signature markers. After identifying major cell types in the pooled dataset, myeloid dendritic cells were separated and the third round of pre-processing and cell-type annotation was performed to explore subpopulations with higher resolution. The third round is similar to the second round of pre-processing, where the raw and unfiltered gene expression concatenated matrix of only the identified myeloid dendritic cells was used and filtering (parameters in online supplemental table 3), HVG identification, PCA computation, and batch correction by Harmony algorithm was performed.

Differential expression analysis

Differential expression (DE) analysis between different cell groups was performed using a Wilcoxon Rank Sum test and multiple hypothesis testing correction using the Benjamini-

Hochberg procedure (function scanpy.tl.rank_genes_groups). To avoid the possible bias resulting from the comparison of imbalanced cell groups, when needed, each cell group was downsampled to the minimum number of all cell groups. The adjusted p-value threshold was kept at 0.05 and genes with less than this value were considered as significantly differentially expressed. Top DE genes were selected based on the highest log2 fold change (log2FC). Top 50 DE genes are listed in online supplemental table 4.

Velocity analysis

The Velocyto 0.17.17 package [6] was used to obtain spliced and unspliced read counts from the previously aligned scRNA-seq files from melanoma, lung and colon cancer samples and RNA velocity was calculated using the scvelo 0.2.3 package [7]. To keep the embedding consistent with the integrated dataset, the batch corrected PCA space was used to calculate the nearest neighbor graph. For each cell, the RNA velocity of genes was used to generate the RNA velocity vector embedding. For this, the first and second-order moments among nearest neighbor cells in reduced PCA space were computed. Further, the data and calculated moments were subjected to the RNA velocity estimation by modeling the full transcriptional dynamics of splicing kinetics (dynamical model).

$CD8^+$ T cell migration assay

Observation windows were filled with 50 µL cold PBS. Collagen mixture (4 mg/mL rat collagen, R&D, #3440-100-01, in 0.1 M HEPES, Life Technologies, #15630-122, 3.7 g/L $NaHCO_3$, Lonza, #BE17-613E) was prepared on ice and 2 µL was pipetted into the middle channel of a cold 3-lane OrganoPlate (MIMETAS, #4004-400-B) to build the extracellular matrix barrier. After 30 min of polymerization at 37°C, 30 µL PBS were added to the inlet

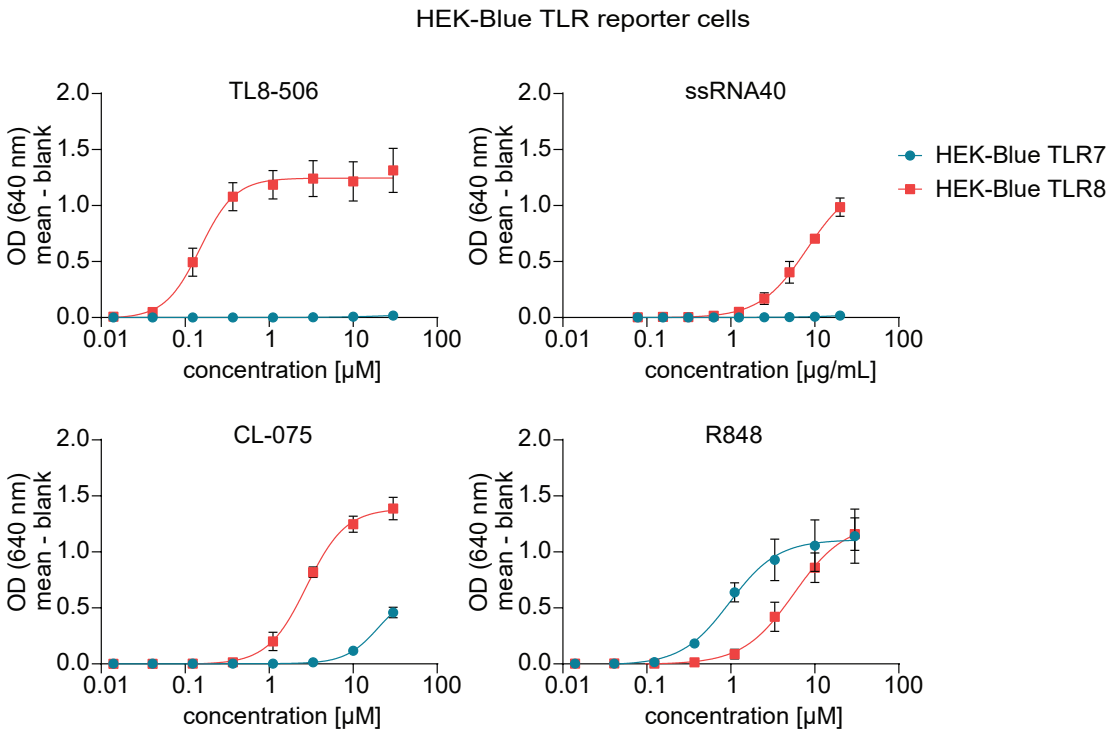
of the collagen channel to prevent collagen drying. OrganoPlate was left overnight in the cell culture incubator. The next day, PBS was removed from the collagen inlets, 2 x 10^4 human umbilical vein endothelial cells (HUVECs, Lonza, #C2517AS) were seeded in 2 µL HUVEC medium (EGM-2, Lonza, #CC-3162/6) into the inlet of the top channel. 50 µL HUVEC medium was added to the inlet of the top channel. HUVECs were allowed to attach to the collagen interface for 2 hours in the cell culture incubator. 50 µL HUVEC medium was then added to the outlet of the top channel. After 6 days of HUVEC vessel formation at 37°C in a CO_2 incubator, HUVEC medium was removed from the inlets and outlets of the top channel. 2 x 10^5 activated $CD8^+$ T cells in 100 µL T cell medium (RPMI, Gibco, #42401-018, 10% FBS, 1% Pen/Strep, 1% Sodium Pyruvate, 1% Non-Essential Amino Acids Solution, Gibco, #11140-050, 1% GlutaMAX, Gibco, #35050-061, 50 µM 2-Mercaptoethanol, Gibco, #31350-010) were plated into the top channel. $CD8^+$ T cells were isolated from PBMCs using the Miltenyi $CD8^+$ T Cell Isolation Kit (#130-096-495) and activated for 4 days using CD3/CD28 activator (STEMCELL, #10971) according to the manufacturer's protocol. $CD8^+$ T cells were labeled with 1 mM CMFDA (Life Technologies, #C7025) for 15 min at 37°C right before plating into the OrganoPlate. Supernatants of stimulated cord blood cDCs, 1:1 diluted in T cell medium were added to the bottom channel. T cell migration was measured in the collagen layer after 48 hours at 37°C in a CO_2 incubator using the PerkinElmer Operetta High Content Imaging System. After 72 hours of migration, cells in the collagen layer were fixed using 0.4% formaldehyde (Sigma, #47608) in PBS for 15 min at room temperature. Cells were washed 2x with PBS and permeabilized using 0.3% Triton X-100 (Sigma, #T8787) for 10 min at room temperature. The plate was washed 1x with 4% FBS in PBS and blocked with 2% FBS, 2% BSA, 0.1% Tween20 (Sigma, #P2287) in PBS for 30 min at room temperature. Cells were stained

with anti-CD8 antibodies (BD, #555635) in 2% FBS, 2% BSA, 0.1% Tween20 in PBS for 4 hours at room temperature. The plate was washed 2x with 4% FBS in PBS, 1x with PBS. Stained cells were detected using the PerkinElmer Operetta High Content Imaging System. Quantification of the migrated T cells was done using ImageJ.

References

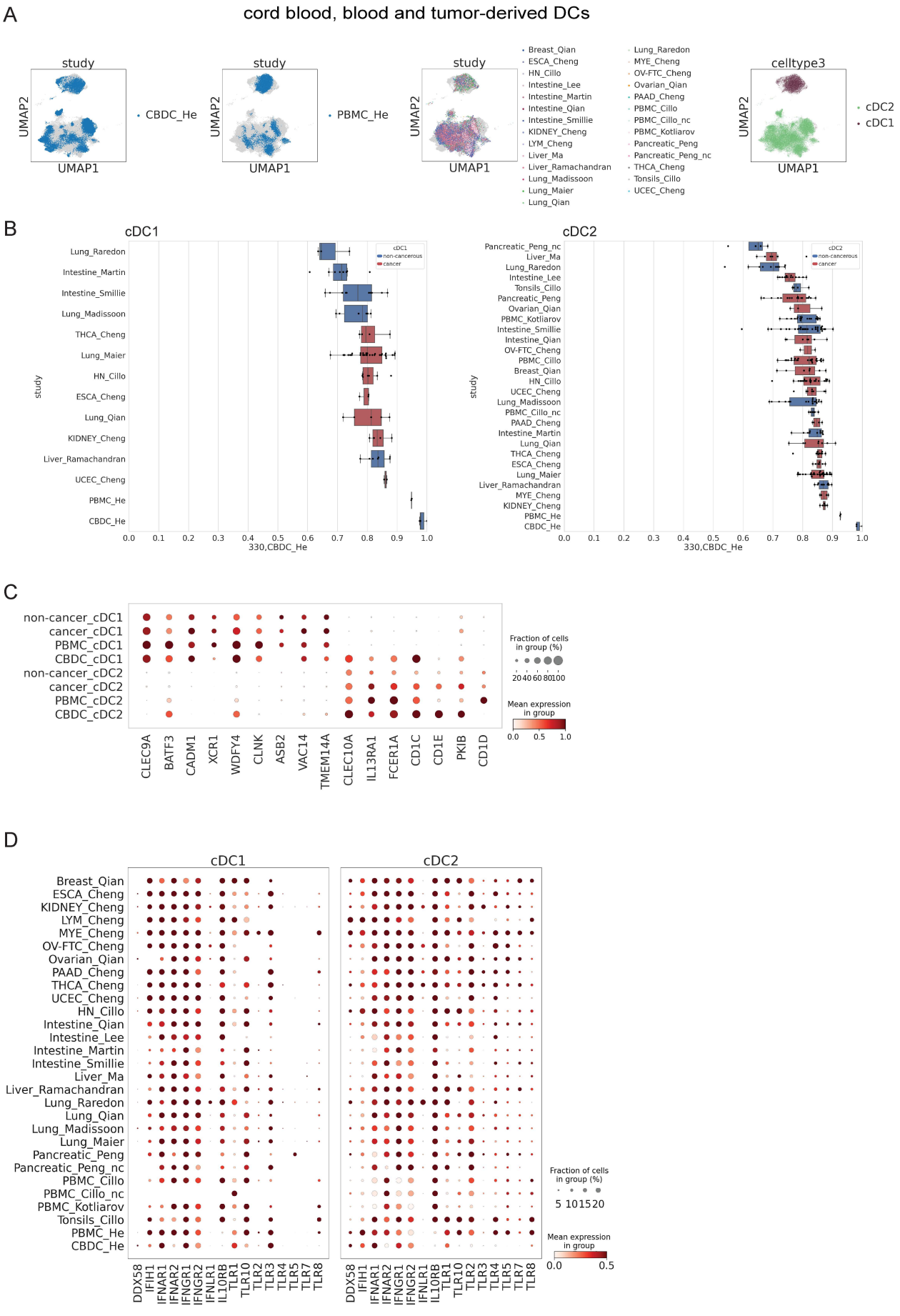
1. Zheng, G.X.Y., et al., *Massively parallel digital transcriptional profiling of single cells.* Nature Communications, 2017. **8**.
2. Madler, S.C., et al., *Besca, a single-cell transcriptomics analysis toolkit to accelerate translational research.* NAR Genom Bioinform, 2021. **3**(4): p. lqab102.
3. Traag, V.A., L. Waltman, and N.J. van Eck, *From Louvain to Leiden: guaranteeing well-connected communities.* Scientific Reports, 2019. **9**.
4. Korsunsky, I., et al., *Fast, sensitive and accurate integration of single-cell data with Harmony.* Nat Methods, 2019. **16**(12): p. 1289-1296.
5. Wolf, F.A., P. Angerer, and F.J. Theis, *SCANPY: large-scale single-cell gene expression data analysis.* Genome Biology, 2018. **19**.
6. La Manno, G., et al., *RNA velocity of single cells.* Nature, 2018. **560**(7719): p. 494-498.
7. Bergen, V., et al., *Generalizing RNA velocity to transient cell states through dynamical modeling.* Nature Biotechnology, 2020. **38**(12).

Supplementary Figures Figure S1



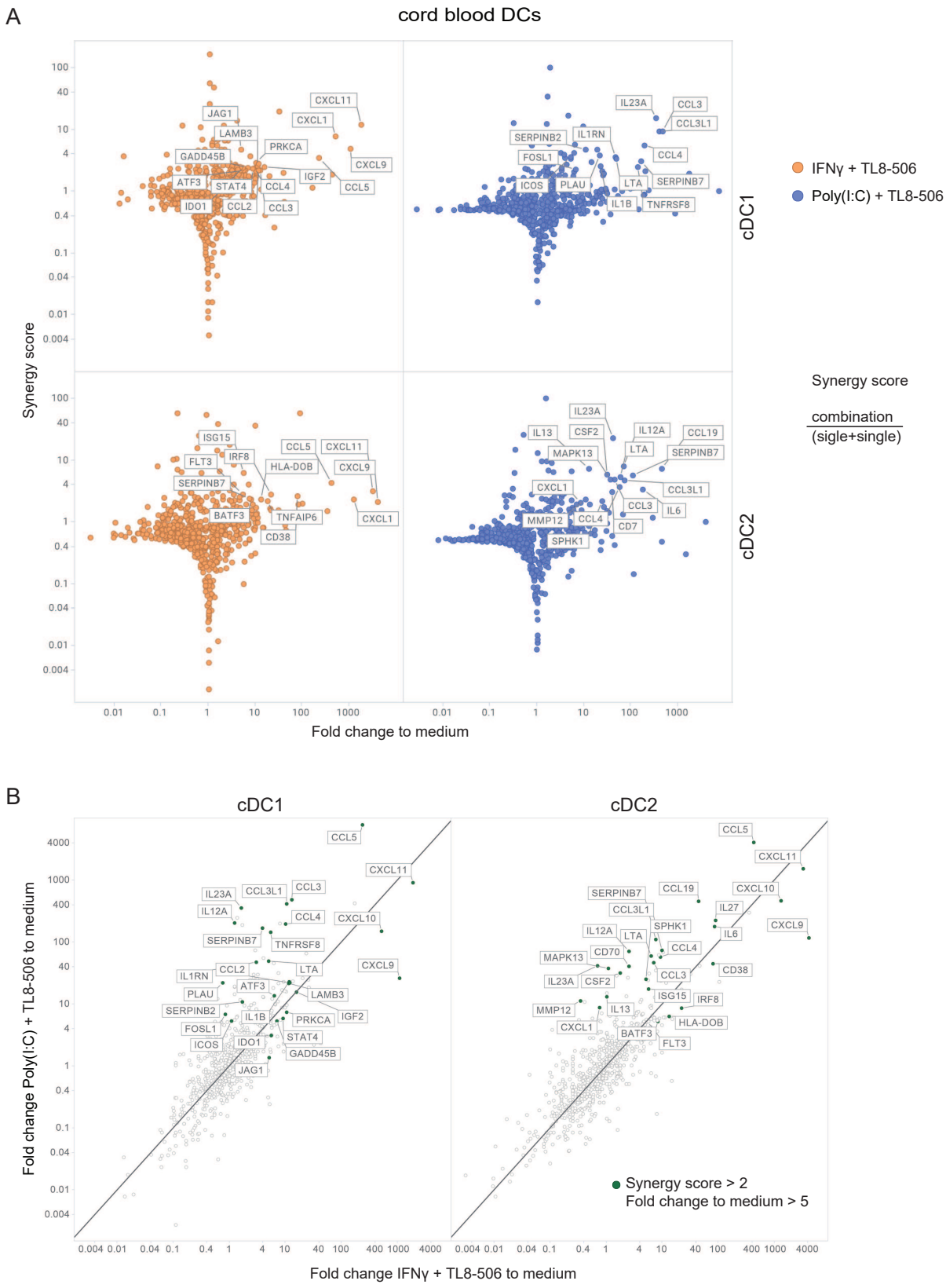
Supp. Figure 1 TL8-506 is selective for human TLR8 and does not activate human TLR7. HEK-Blue cells that were engineered to express the human TLR7 or TLR8 and the NF-κB-inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene were treated with the indicated agonists and concentrations for 18 hours. Real-time detection of SEAP activity by performing the colorimetric enzyme assay in HEK-Blue Detection medium, from three independent experiments, mean+SEM is shown.

Figure S2


A
cord blood, blood and tumor-derived DCs
study
UMAP2
UMAP1
CBDC_He
PBMC_He
Breast_Qian
ESCA_Cheng
HN_Cillo
Intestine_Lee
Intestine_Martin
Intestine_Qian
Intestine_Smillie
KIDNEY_Cheng
LYM_Cheng
Liver_Ma
Liver_Ramachandran
Lung_Madissoon
Lung_Maier
Lung_Qian
Lung_Raredon
MYE_Cheng
OV-FTC_Cheng
Ovarian_Qian
PAAD_Cheng
PBMC_Cillo
PBMC_Cillo_nc
PBMC_Kotliarov
Pancreatic_Peng
Pancreatic_Peng_nc
THCA_Cheng
Tonsils_Cillo
UCEC_Cheng
celltype3
cDC2
cDC1
B
cDC1
cDC2
non-cancerous
cancer
330,CBDC_He
C
non-cancer_cDC1
cancer_cDC1
PBMC_cDC1
CBDC_cDC1
non-cancer_cDC2
cancer_cDC2
PBMC_cDC2
CBDC_cDC2
CLEC9A
BATF3
CADM1
XCR1
WDFY4
CLNK
ASB2
VAC14
TMEM14A
CLEC10A
IL13RA1
FCER1A
CD1C
CD1E
PKIB
CD1D
Fraction of cells in group (%)
Mean expression in group
D
cDC1
cDC2
DDX58
IFIH1
IFNAR1
IFNAR2
IFNGR1
IFNGR2
IFNLR1
IL10RB
TLR1
TLR10
TLR2
TLR3
TLR4
TLR5
TLR7
TLR8
Fraction of cells in group (%)
Mean expression in group

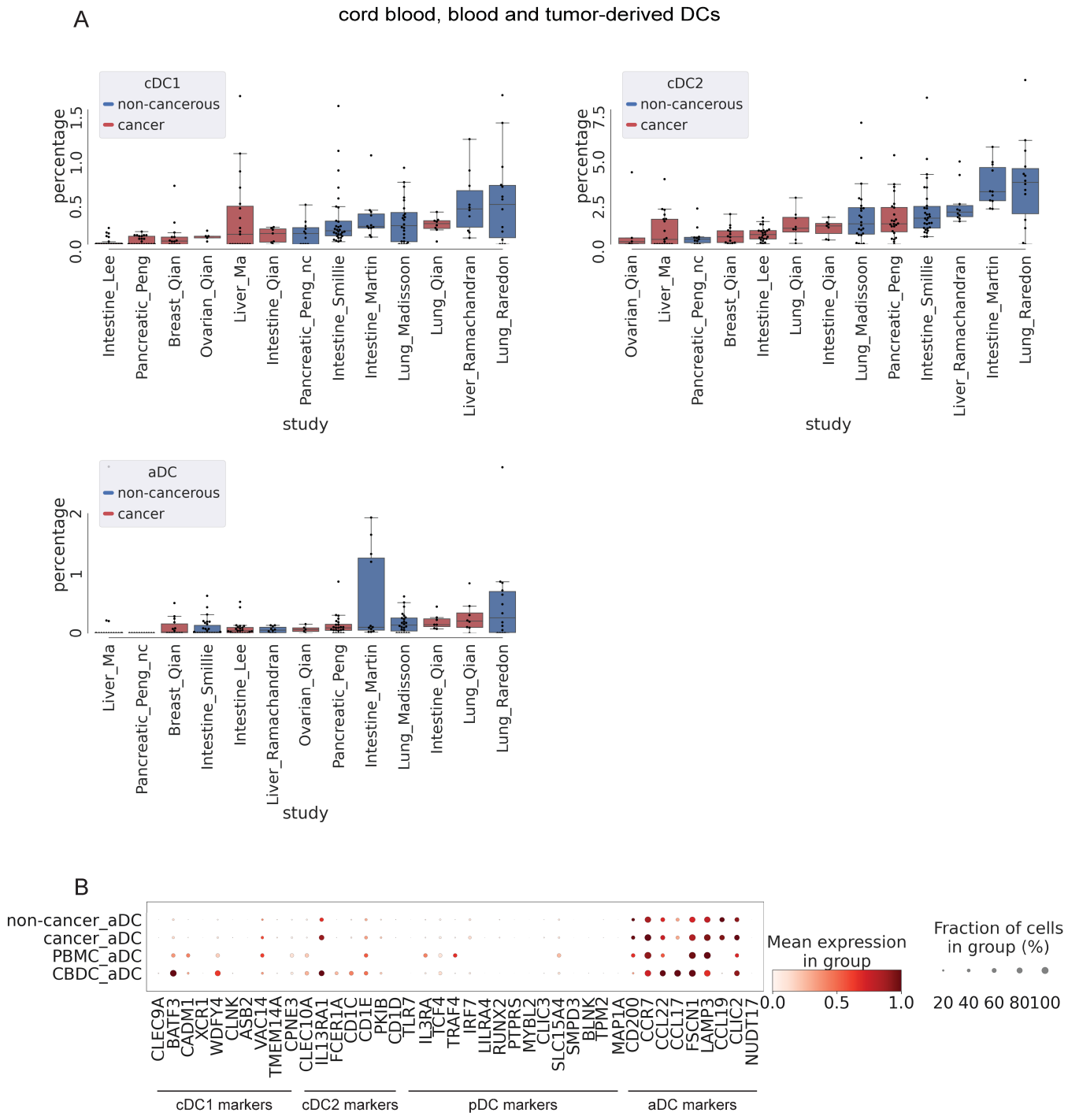
Supp. Figure 2 Human cord blood, blood and tumor cDCs show comparable gene expression. 10x Genomics scRNA-seq was performed on FACS sorted blood cDCs (PBMC), n=2 donors, from one experiment, and in vitro differentiated cord blood cDCs (CBDC) harvested on day 12, n=3 batches of cord blood from mixed donors, 6 donors in total, from two independent experiments. scRNA-seq data of tissue cDCs (non-cancer and cancer) were integrated from different public studies listed in online supplemental table 2. (A) UMAP of 59'025 cDCs profiled across different scRNA-seq studies with each cell color-coded for study or cDC subset. (B) Spearman correlation values are shown comparing cord blood cDCs with blood and tissue cDCs. Correlation values were calculated based on the mean expression of genes per cell subset and donor. (C) Fraction positive and mean expression of different cDC subset-specific markers in cord blood, blood and tissue cDC1s or cDC2s. Mean expression was calculated across all the cells in the group and then scaled to a 0-1 range. (D) Fraction positive and mean expression of different IFNRs and PRRs in cord blood, blood and tissue cDC1s or cDC2s. Mean expression was calculated across all the cells in the group and then scaled to a 0-1 range.

Figure S3


A
cord blood DCs
cDC1
cDC2
Synergy score
Fold change to medium
IFNγ + TL8-506
Poly(I:C) + TL8-506
Synergy score
combination
(sigle+single)
B
cDC1
cDC2
Fold change Poly(I:C) + TL8-506 to medium
Fold change IFNγ + TL8-506 to medium
Synergy score > 2
Fold change to medium > 5

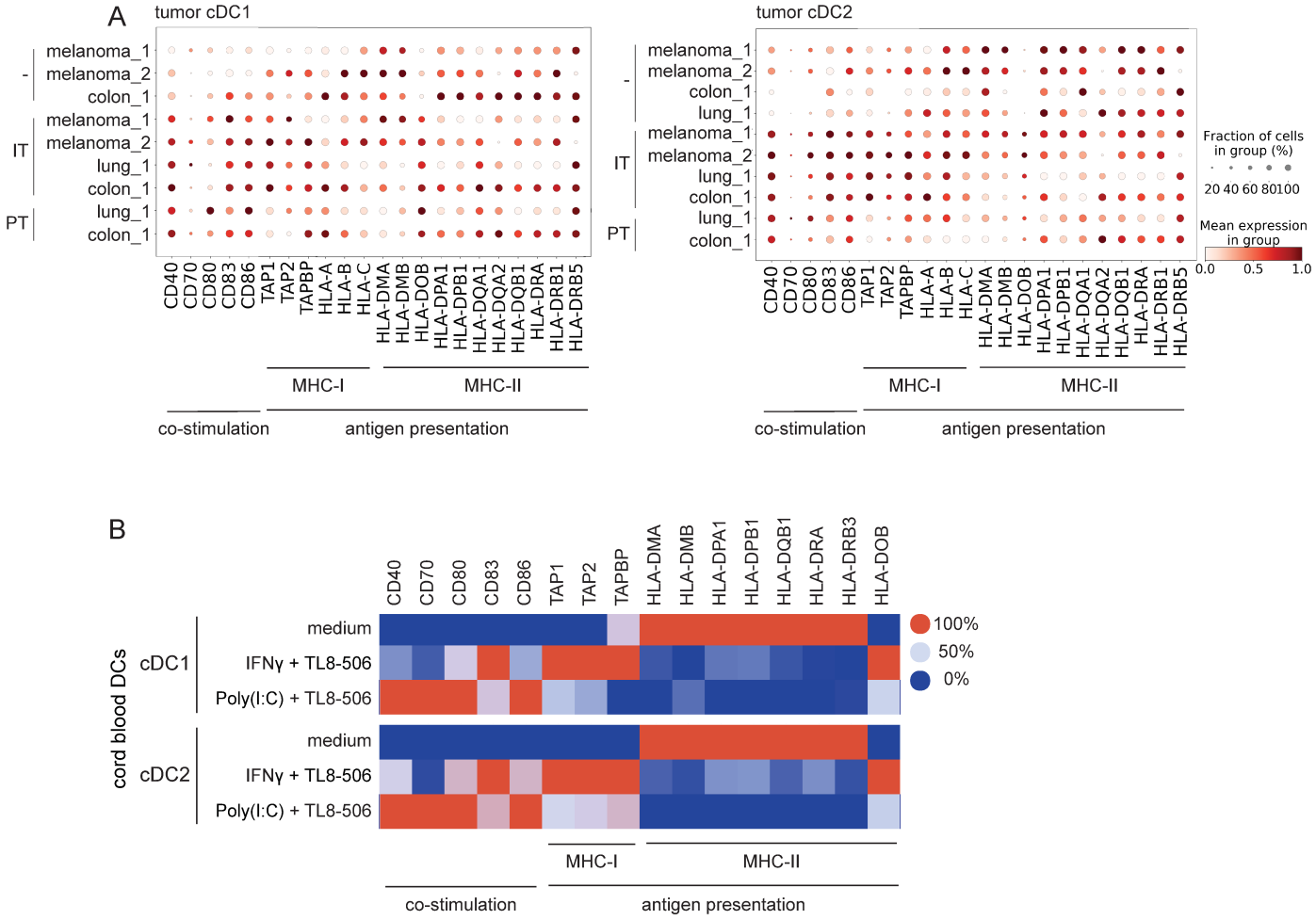
Supp. Figure 3 IFNγ + TL8-506 or Poly(I:C) + TL8-506 synergize to upregulate different genes in cord blood cDCs. Cord blood cDCs were sorted by FACS and stimulated with the indicated stimuli for 15 hours. Gene expression was analyzed in cell lysates using the NanoString human Myeloid Innate Immunity panel, n=2 batches of cord blood from mixed donors, in total 4 donors, from two independent experiments, representative data are shown for 1 batch of cord blood from mixed donors (2 donors). (A) For each gene, synergy scores for combinatorial stimulation is plotted against fold change in expression in stimulated cDCs, calculation of synergy score for each gene is depicted on the right, labeled genes have a synergy score >2 and fold change to medium >5. (B) For each gene, fold change in expression in IFNγ + TL8-506 treated cDCs is plotted against fold change in expression in Poly(I:C) + TL8-506 treated cDCs, comparing the genes highly induced by IFNγ + TL8-506 vs Poly(I:C) + TL8-506 treatment. Labeled genes have a synergy score >2 and fold change to medium >5. The following concentrations were used for DC stimulation: 50'000 U/mL huIFNγ, 10 µg/mL Poly(I:C), 1 µM TL8-506.

Figure S4



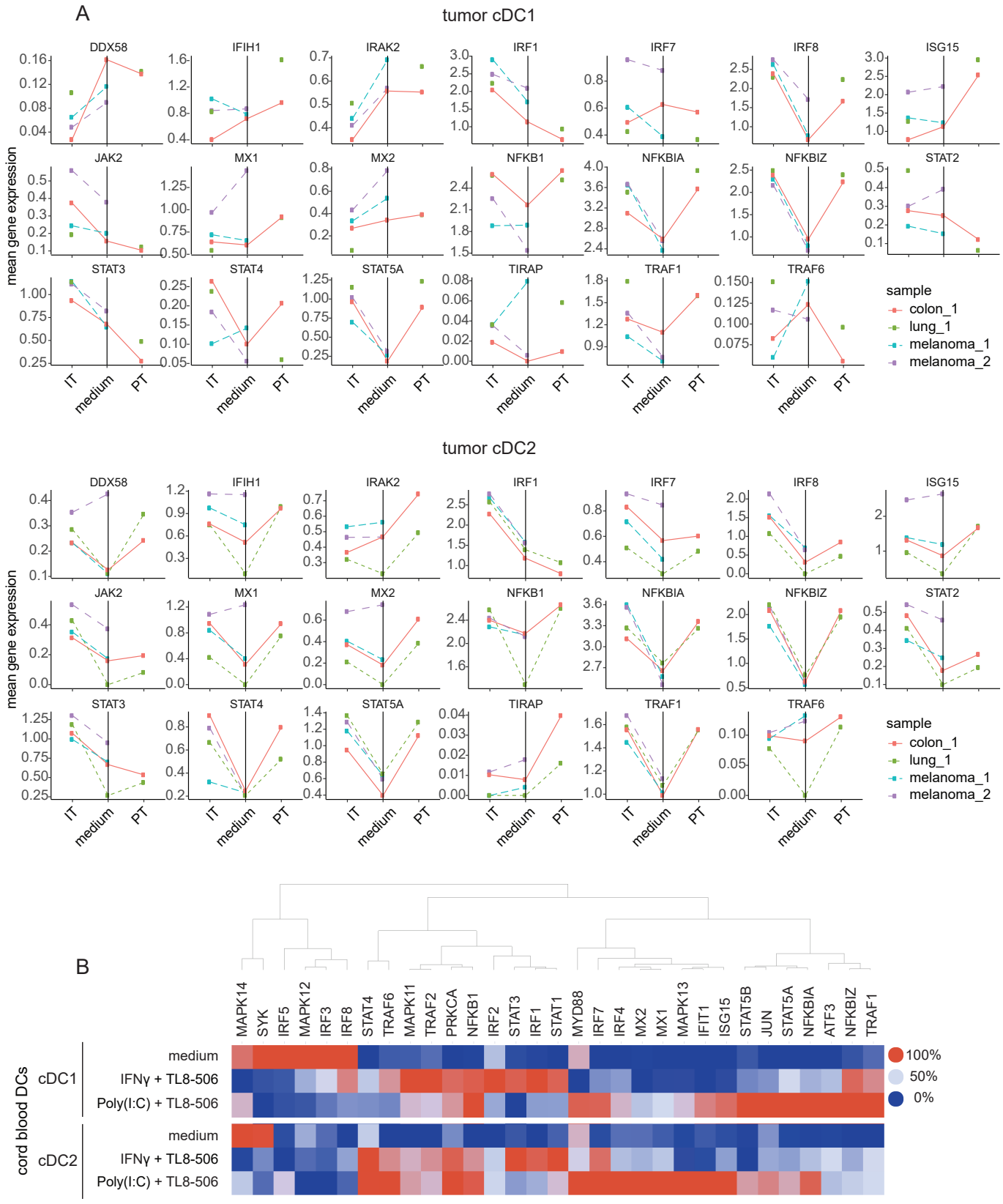
Supp. Figure 4 cDCs with an activated phenotype are rare across different human tumor indications. (A) Percentage of cDC1s, cDC2s and activated DCs (aDCs) of all cells (CD45⁺ and CD45⁻ cells) from the processed tissue samples was calculated and depicted. Only studies in which the entire tissue was proved by scRNA-seq without prior cell type enrichment were included. (B) DC subset-specific markers were analyzed in the activated DC (aDC) population from all scRNA-seq studies. Fraction positive and mean expression of DC subset-specific markers in aDCs from tissue (non-cancer, cancer), blood (PBMC) and cord blood (CBDC) is shown. Mean expression was calculated across all the cells in the group and then scaled to a 0-1 range.

Figure S5



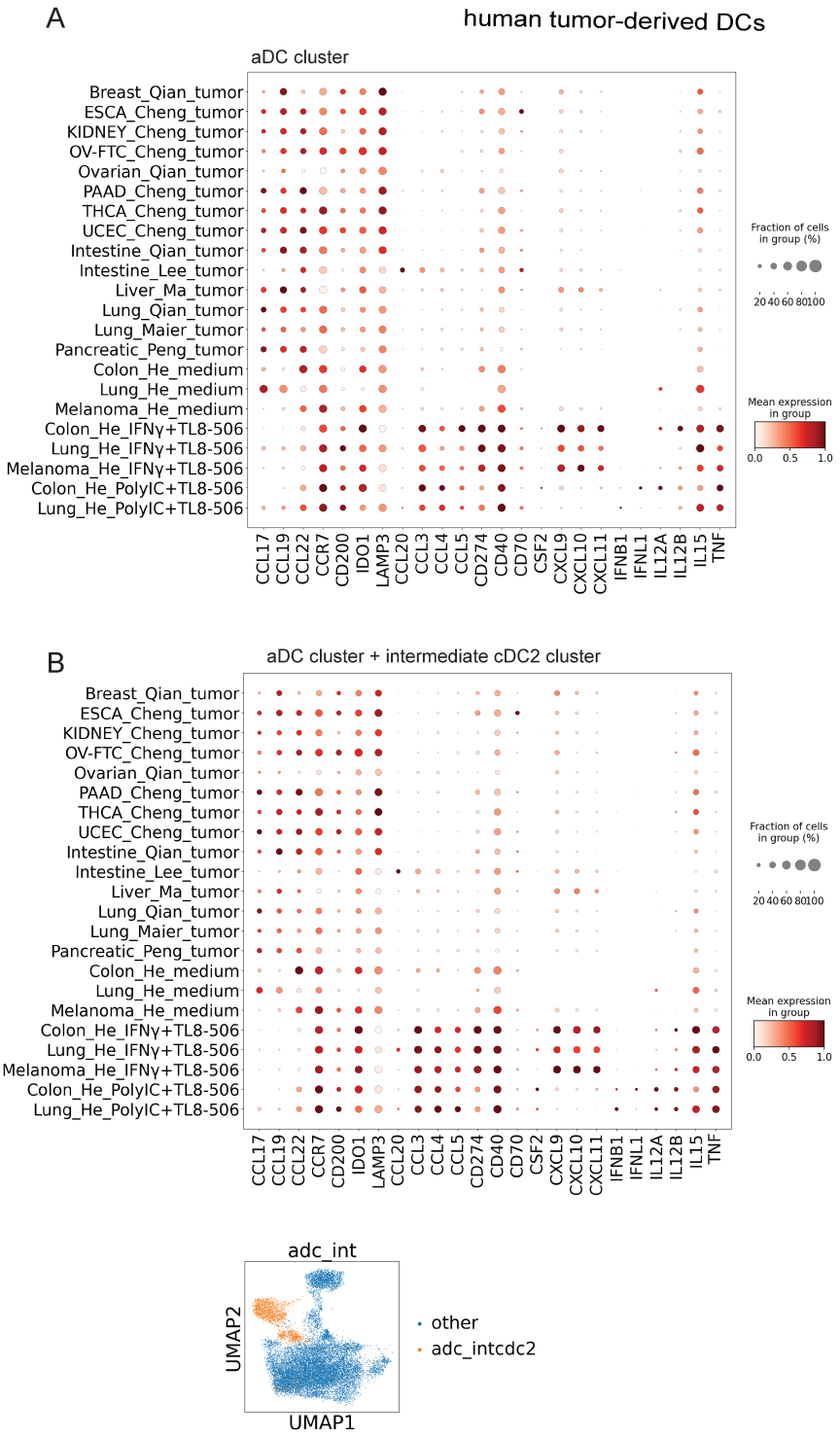
Supp. Figure 5 Tumor cDCs activated by TL8-506 combinations up-regulate genes involved in co-stimulation and antigen presentation. (A) Digested tumor samples from melanoma, CRC and lung cancer patients were treated with 1 µM TL8-506 + 50'000 U/mL IFNγ or 1 µM TL8-506 + 10 µg/mL Poly(I:C) for 4 hours. 10x Genomics scRNA-seq was performed on FACS sorted $CD45^{+}$, $CD3^{-}$ cells, n=4 donors, from three independent experiments. Fraction positive and mean expression of co-stimulatory molecules and genes involved in antigen presentation in tumor-derived cDC1s and cDC2s upon treatment with TL8-506 combinations is shown. Mean expression was calculated across all the cells in the group and then scaled to a 0-1 range, - = medium, IT = IFNγ + TL8-506, PT = Poly(I:C) + TL8-506. (B) Cord blood cDCs were sorted by FACS and stimulated with 1 µM TL8-506 + 50'000 U/mL IFNγ or 1 µM TL8-506 + 10 µg/mL Poly(I:C) for 15 hours. Gene expression was analyzed in cell lysates using the NanoString human Myeloid Innate Immunity panel, n=2 batches of cord blood from mixed donors, 4 donors in total, from two independent experiments, colors displaying the maximum (100%) to minimum (0%) mean gene expression per column.

Figure S6


A
tumor cDC1
tumor cDC2
mean gene expression
DDX58
IFIH1
IRAK2
IRF1
IRF7
IRF8
ISG15
JAK2
MX1
MX2
NFKB1
NFKBIA
NFKBIZ
STAT2
STAT3
STAT4
STAT5A
TIRAP
TRAF1
TRAF6
IT
medium
PT
sample
colon_1
lung_1
melanoma_1
melanoma_2
B
MAPK14
SYK
IRF5
MAPK12
IRF3
IRF8
STAT4
TRAF6
MAPK11
TRAF2
PRKCA
NFKB1
IRF2
STAT3
IRF1
STAT1
MYD88
IRF7
IRF4
MX2
MX1
MAPK13
IFIT1
ISG15
STAT5B
JUN
STAT5A
NFKBIA
ATF3
NFKBIZ
TRAF1
cord blood DCs
cDC1
cDC2
medium
IFNγ + TL8-506
Poly(I:C) + TL8-506
100%
50%
0%

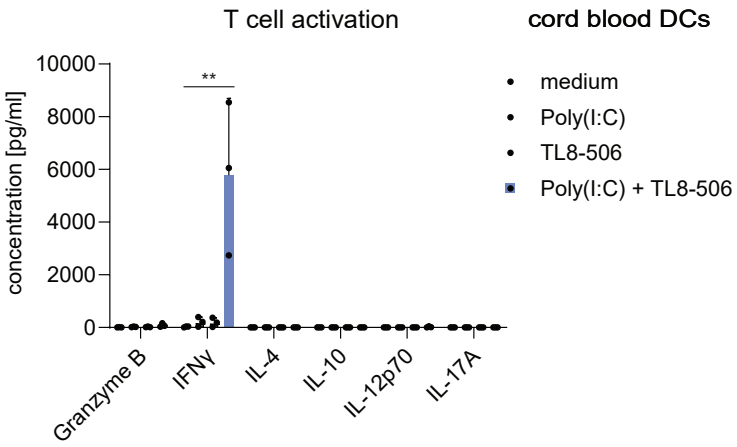
Supp. Figure 6 TL8-506 combinations induce TLR and IFN signaling in treated tumor cDCs. (A) Digested tumor samples from melanoma, CRC and lung cancer patients were treated with 1 µM TL8-506 + 50'000 U/mL IFNγ or 1 µM TL8-506 + 10 µg/mL Poly(I:C) for 4 hours. 10x Genomics scRNA-seq was performed on FACS sorted $CD45^+$, $CD3^-$ cells, n=4 donors, from three independent experiments. Mean gene expression of components of the TLR and IFN pathways in tumor cDC1s and cDC2s is displayed, IT = IFNγ + TL8-506, PT = Poly(I:C) + TL8-506. (B) Cord blood cDCs were sorted by FACS and stimulated with 1 µM TL8-506 + 50'000 U/mL IFNγ or 1 µM TL8-506 + 10 µg/mL Poly(I:C) for 15 hours. Gene expression was analyzed in cell lysates using the NanoString human Myeloid Innate Immunity panel, n=2 batches of cord blood from mixed donors, 4 donors in total, from two independent experiments, colors displaying the maximum (100%) to minimum (0%) mean gene expression per column.

Figure S7



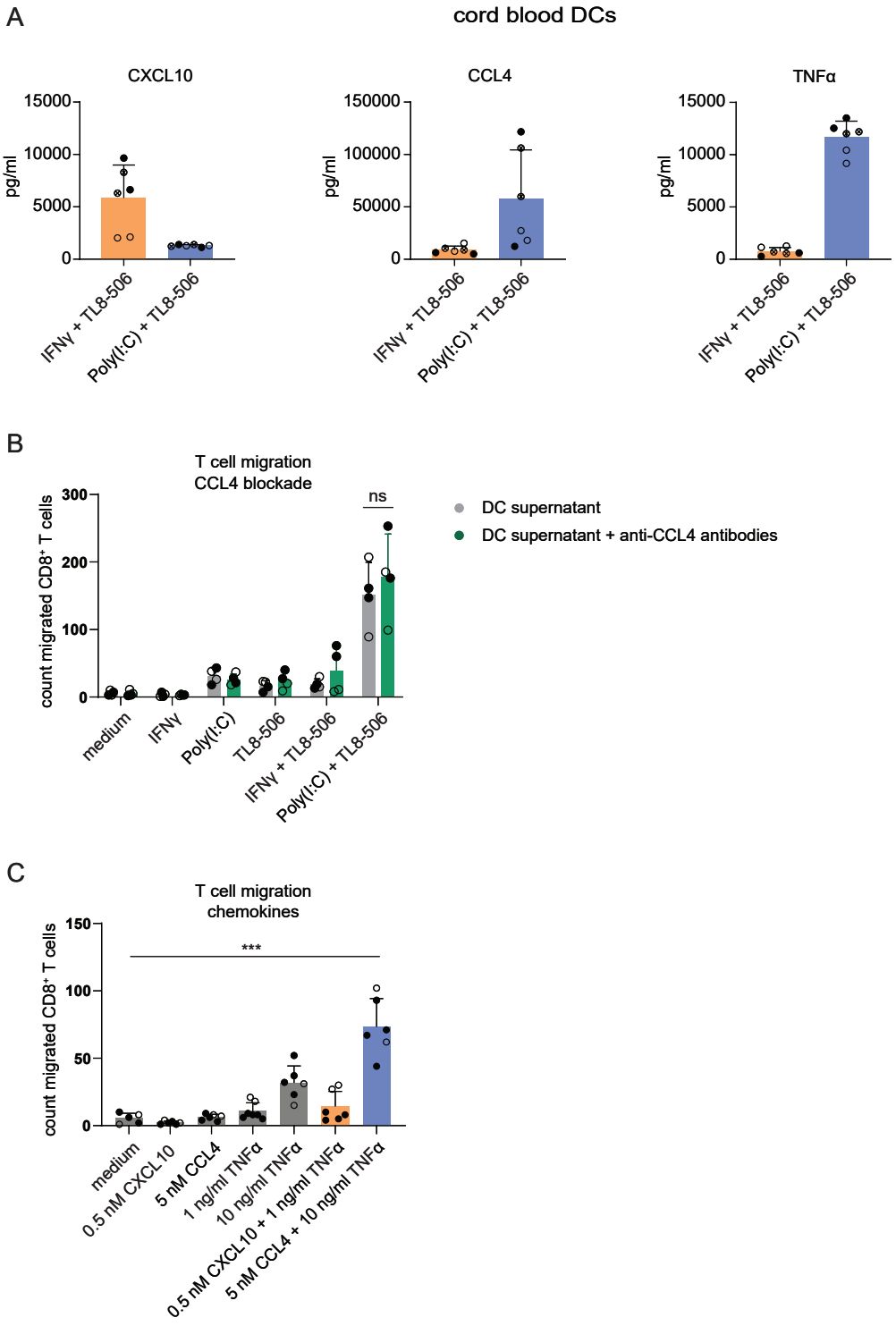
Supp. Figure 7 Tumor-derived cDCs activated by TL8-506 combinations show increased expression of activation markers compared to in situ activated cDCs from different human tumor indications. Digested patient tumor samples were treated with 1 µM TL8-506 + 50'000 U/mL IFNγ or 1 µM TL8-506 + 10 µg/mL Poly(I:C) for 4 hours. scRNA-seq was performed on FACS sorted CD45^{+}, CD3^{-} cells, n=4 donors, from three independent experiments. scRNA-seq data of in situ activated cDCs were integrated from different public studies listed in online supplemental table 2. (A) Fraction positive and mean expression of activation markers in activated DCs (aDCs), stratified per treatment, study and tissue of origin. Only cDCs falling into the aDC cluster were analyzed. Mean expression was calculated across all the cells in the group and then scaled to a 0-1 range. (B) Fraction positive and mean expression of activation markers in activated DCs (extended population), stratified per treatment, study and tissue of origin. Cells color-coded in orange from the aDC cluster and intermediate cluster between aDCs and cDC2s were analyzed, adc_intcdc2 = aDC cluster + intermediate cDC2 cluster. Mean expression was calculated across all the cells in group and then scaled to a 0-1 range.

Figure S8



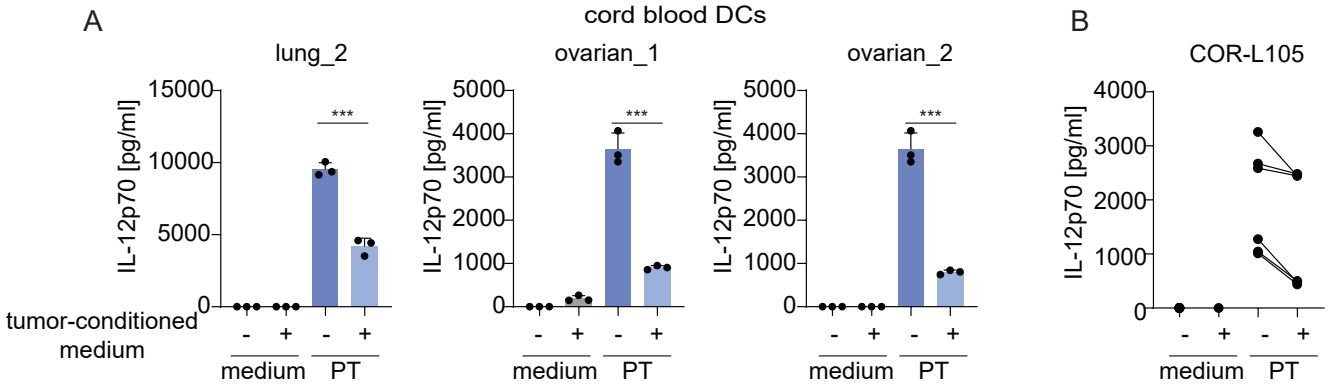
Supp. Figure 8 Poly(I:C) + TL8-506 activate cord blood cDCs to induce the release of IFNγ but not IL-4 or IL-10 in DC/T cell co-cultures. Sorted cord blood cDC2s were stimulated with the indicated stimuli for 18 hours. Treated cDCs were washed and co-cultured with allogeneic naive T cells for 4 days. Cytokine concentrations in the supernatant of co-cultures were determined by ELISA, n=2 batches of cord blood from mixed donors, 3 T cell donors, from two independent experiments, one-way ANOVA was used for statistical analysis, **p≤0.002. The following concentrations were used for DC stimulation: 10 µg/mL Poly(I:C), 1 µM TL8-506.

Figure S9


A
cord blood DCs
CXCL10
CCL4
TNFα
pg/ml
15000
10000
5000
0
150000
100000
50000
IFNγ + TL8-506
Poly(I:C) + TL8-506
B
T cell migration
CCL4 blockade
count migrated CD8+ T cells
300
200
100
ns
DC supernatant
DC supernatant + anti-CCL4 antibodies
medium
IFNγ
Poly(I:C)
TL8-506
C
T cell migration
chemokines
150
50
***
0.5 nM CXCL10
5 nM CCL4
1 ng/ml TNFα
10 ng/ml TNFα
0.5 nM CXCL10 + 1 ng/ml TNFα
5 nM CCL4 + 10 ng/ml TNFα

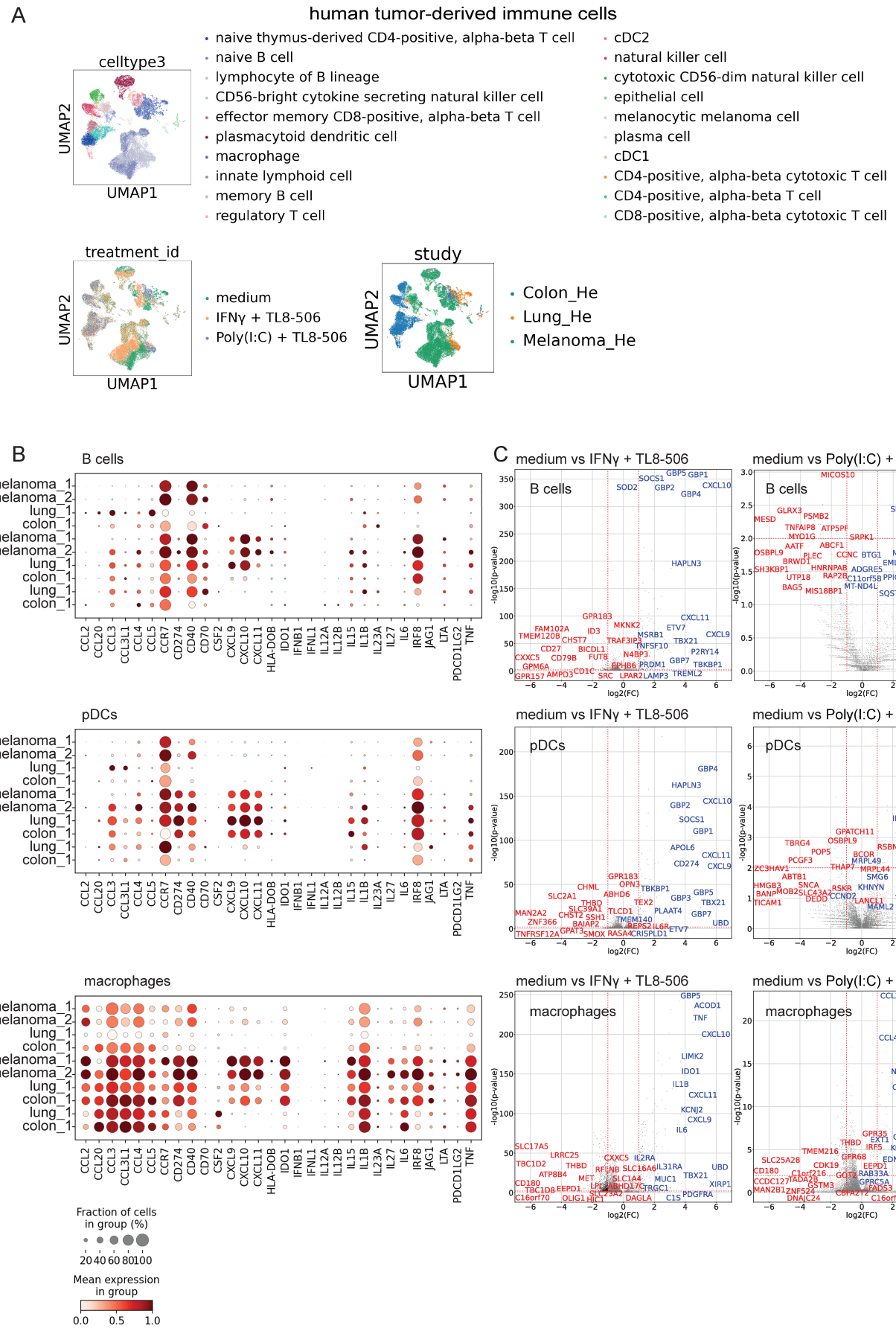
Supp. Figure 9 CCL4 and TNFα induce $CD8^+$ T cell migration in 3D in vitro system. (A) Sorted cord blood cDC2s were stimulated for 18 hours with the indicated stimuli. Cytokine concentrations were determined in the cell culture supernatants by ELISA. CXCL10, CCL4 and TNFα concentrations in the supernatant of cDCs treated with IFNγ + TL8-506 or Poly(I:C) + TL8-506 are shown, n=3 batches of cord blood from mixed donors, 6 donors in total, from three independent experiments as described in Figure 1D. (B) Sorted cord blood cDC2s were stimulated with the indicated compounds for 18 hours. The supernatant was collected and placed into the bottom channel of a 3D tissue culture device. Activated $CD8^+$ T cells were labeled with CMFDA and added to the top channel which was coated with an artificial endothelial vessel. T cell migration was measured after 48 hours by imaging of the collagen layer that separated the two channels. Cell counts of migrated $CD8^+$ T cells in the absence (grey) and presence of 5 µg/mL anti-CCL4 antibodies (green) in supernatants of stimulated cDCs are shown, n=2 donors, from two independent experiments, mean+SD, Student's t-test, ns, not significant. (C) Experimental set-up as described in B, CXCL10 + TNFα or CCL4 + TNFα dilutions in concentrations present in supernatants of IFNγ + TL8-506 or Poly(I:C) + TL8-506 treated cDC2s were placed in the bottom channel. Cell counts of migrated $CD8^+$ T cells towards chemokine dilutions are depicted, n=2 donors, from two independent experiments, mean+SD, one-way ANOVA, $^{**}p \leq 0.002$. The following concentrations were used for DC stimulation: 50'000 U/mL huIFNγ, 10 µg/mL Poly(I:C), 1 µM TL8-506.

Figure S10



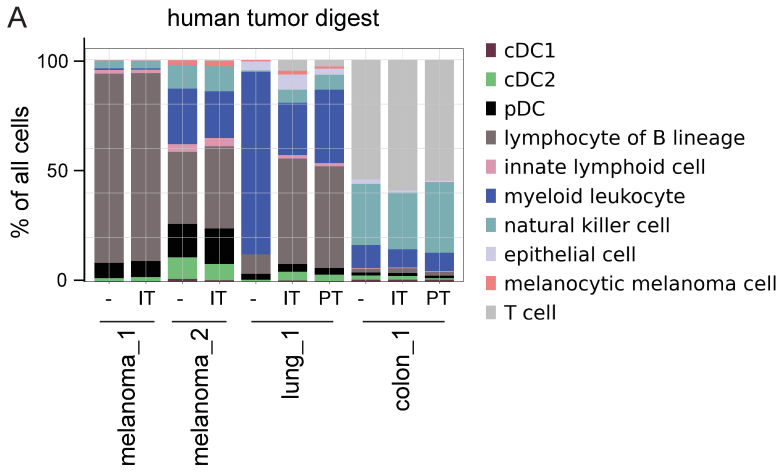
Supp. Figure 10 Poly(I:C) + TL8-506 activate cord blood cDCs in the presence of tumor-conditioned medium to produce IL-12p70. (A) Patient tumor-derived digest was cultured for 6 hours and tumor-conditioned medium was harvested. Sorted cord blood cDCs were stimulated with 1 µM TL8-506 + 10 µg/mL Poly(I:C) in the absence or presence of tumor-conditioned medium (1:1 diluted) for 16 hours. IL-12p70 concentrations were measured in cell culture supernatant by ELISA, 3 patient tumor-derived digests, 2 batches of cord blood from mixed donors (4 donors in total), from two independent experiments, mean+SD of technical replicates is shown, unpaired Student's t-test, ***p≤0.0002, PT = Poly(I:C) + TL8-506. (B) Cell culture supernatant of the human lung cancer cell line COR-L105 was collected. Sorted cord blood cDC2s were activated in the absence or presence of conditioned medium from COR-L105 cells for 16 hours. IL-12p70 concentrations were measured in cell culture supernatant by ELISA, 2 batches of cord blood from mixed donors (4 donors in total), from two independent experiments.

Figure S11

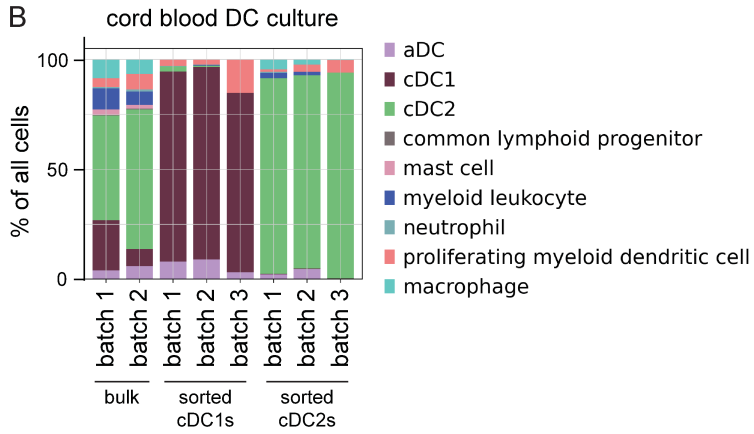

A
human tumor-derived immune cells
celltype3
UMAP2
UMAP1
naive thymus-derived CD4-positive, alpha-beta T cell
naive B cell
lymphocyte of B lineage
CD56-bright cytokine secreting natural killer cell
effector memory CD8-positive, alpha-beta T cell
plasmacytoid dendritic cell
macrophage
innate lymphoid cell
memory B cell
regulatory T cell
cDC2
natural killer cell
cytotoxic CD56-dim natural killer cell
epithelial cell
melanocytic melanoma cell
plasma cell
cDC1
CD4-positive, alpha-beta cytotoxic T cell
CD4-positive, alpha-beta T cell
CD8-positive, alpha-beta cytotoxic T cell
treatment_id
medium
IFNγ + TL8-506
Poly(I:C) + TL8-506
study
Colon_He
Lung_He
Melanoma_He
B
B cells
pDCs
macrophages
medium
IFNγ + TL8-506
Poly(I:C) + TL8-506
melanoma_1
melanoma_2
lung_1
colon_1
CCL2 CCL20 CCL3 CCL3L1 CCL4 CCL5 CCR7 CD274 CD40 CD70 CSF2 CXCL9 CXCL10 CXCL11 HLA-DOB IDO1 IFNB1 IFNL1 IL12A IL12B IL15 IL1B IL23A IL27 IL6 IRF8 JAG1 LTA PDCD1LG2 TNF
Fraction of cells in group (%)
20 40 60 80 100
Mean expression in group
0.0 0.5 1.0
C
medium vs IFNγ + TL8-506
medium vs Poly(I:C) + TL8-506
B cells
pDCs
macrophages
-log10(p-value)
log2(FC)

Supp. Figure 11 TL8-506 combinations up-regulate cytokines in human tumor-derived B cells, plasmacytoid DCs and macrophages in a cell type-specific manner. (A) UMAP of 35’169 cells profiled from IFNγ + TL8-506 or Poly(I:C) + TL8-506 treated and control treated tumor digests, with each cell color-coded for cell type, treatment and tissue. (B) Fraction positive and mean expression of activation markers in tumor-derived B cells, plasmacytoid DCs (pDCs) and macrophages upon treatment with TL8-506 combinations. Mean expression is calculated across all the cells in the group and then scaled to a 0-1 range. (C) Volcano plots showing differentially expressed (DE) genes (blue: up-regulated, red: down-regulated) between stimulated and control treated B cells, pDCs and macrophages from human tumor digests. Top 20 DE genes scored by p-values and fold changes are labeled, Wilcoxon Rank Sum test. The following concentrations were used for stimulation: 50’000 U/mL huIFNγ, 10 µg/mL Poly(I:C), 1 µM TL8-506.

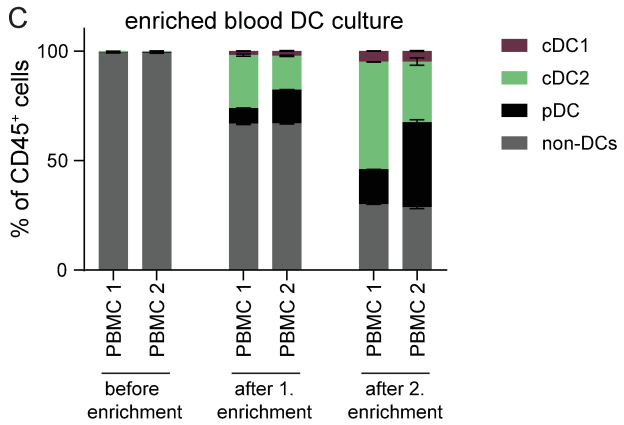
Figure S12



Sample	Treatment	cDC1	cDC2	pDC
melanoma_1	medium	0.2	1.4	6.8
melanoma_1	IFNγ + TL8-506	0.3	1.8	7.2
melanoma_2	medium	1.3	9.6	15.1
melanoma_2	IFNγ + TL8-506	0.6	7.5	16.0
lung_1	medium	0.0	0.8	2.7
lung_1	IFNγ + TL8-506	0.4	4.0	3.6
lung_1	Poly(I:C) + TL8-506	0.3	2.9	3.0
colon_1	medium	0.9	2.0	1.2
colon_1	IFNγ + TL8-506	0.8	1.9	1.2
colon_1	Poly(I:C) + TL8-506	0.9	0.7	1.1



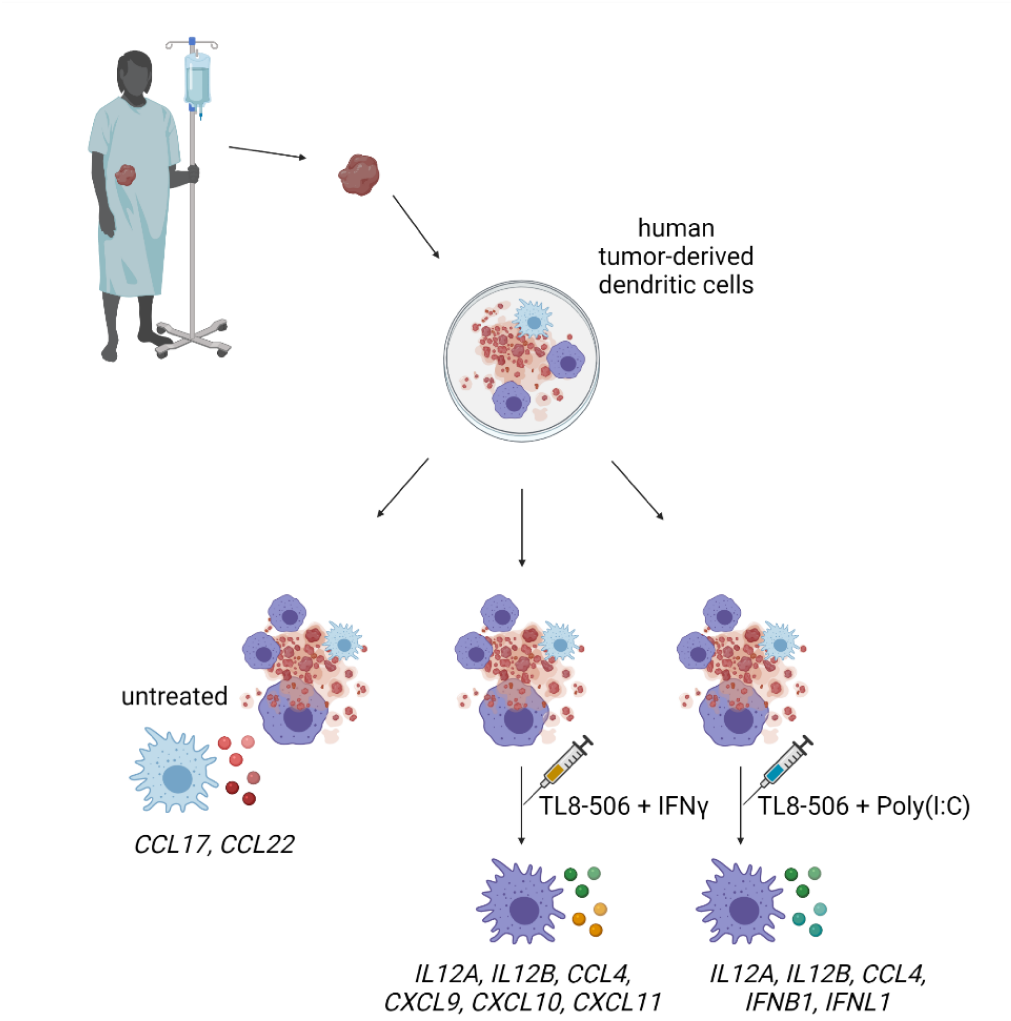
Sample	Enrichment	cDC1	cDC2
batch 1	bulk	22.9	47.5
batch 2	bulk	7.8	63.5
batch 1	sorted for cDC1s	86.4	2.3
batch 2	sorted for cDC1s	87.6	0.5
batch 3	sorted for cDC1s	81.6	0.0
batch 1	sorted for cDC2s	0.1	89.1
batch 2	sorted for cDC2s	0.3	87.8
batch 3	sorted for cDC2s	0.2	93.6



Sample	Enrichment	cDC1	cDC2	pDC
PBMC1	bulk	0.0	0.7	0.2
PBMC2	bulk	0.1	0.5	0.4
PBMC1	1x	1.9	24.2	7.1
PBMC2	1x	2.1	15.5	15.4
PBMC1	2x	4.9	49.1	16.0
PBMC2	2x	4.8	27.7	38.7

Supp. Figure 12 Human cDCs are low in percentage in tumor tissue and blood. (A) Patient tumor-derived tissues were treated with the indicated compounds for 4 hours. 10x Genomics scRNA-seq was performed on FACS sorted CD45⁺, CD3⁻ cells with the exception of colon_1 that was sorted for CD45⁺ cells, n=4 donors, from three independent experiments. Percentages of DCs from all sequenced cells are plotted in a bar graph (left) or are listed in a table (right), - = medium, IT = IFNγ + TL8-506, PT = Poly(I:C) + TL8-506. (B) DCs were differentiated in vitro from cord blood stem cells, cells were harvested on day 12 and sorted for cDC1s, cDC2s or live cells (bulk) by FACS, 10x Genomics scRNA-seq was performed on sorted cells. n=3 batches of cord blood from mixed donors, 6 donors in total, from two independent experiments. Percentages of DCs from all sequenced cells are plotted in a bar graph (left) or are listed in a table (right). (C) PBMCs were isolated from buffy coats of healthy donors (bulk). DCs were enriched from PBMCs 1x or 2x using the Miltenyi Pan-DC Enrichment Kit, n=2 donors, from one experiment. Percentages of DCs from all CD45⁺ cells were quantified by flow cytometry and are plotted in a bar graph (left) or are listed in a table (right), mean+SD of technical replicates is shown.

Graphical Abstract



The graphical abstract was created with BioRender.com.

Combinations of Toll-like receptor 8 agonist TL8-506 activate human tumor-derived dendritic cells

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In Brief

- Human tumor-derived conventional dendritic cells (cDCs) from cancer patients are activated by Toll-like receptor 8 agonist combinations
- Human tumor-derived cDC1s and cDC2s show an immunostimulatory phenotype associated with Th1 responses upon treatment
- Combination-specific induction of activation markers are consistent in human cord blood, blood and tumor-derived cDCs