

Table S1: Catalogue numbers and clones of used antibodies.

Antibodies	Fluorochrome	Brand	Catalog number	Clone
7AAD		Sigma	A9400-1MG	
BDCA-3	BV711	BD Biosciences	563155	1A4
CD103	BV711	Biologend	334334	5C3
CD11c	APC-Cy7	Biologend	337218	Bu15
CD14	FITC	BD Biosciences	345784	MΦP9
CD16	BV786	BD Biosciences	563690	3G8
CD1c	PE-Cy7	Sony	2257580	L161
CD25	APC	BD Biosciences	340907	2A3
CD25	FITC	BD Biosciences	345796	2A3
CD3	BV711	BD Biosciences	563725	UCHT1
CD3	PerCP-Cy5.5	BD Biosciences	332771	SK7
CD4	AF700	BD Biosciences	557922	RPA-T4
CD45	AF700	Biologend	304024	HI30
CD45	PE-Cy7	BD Biosciences	557748	HI30
CD45RA	APC-H7	BD Biosciences	560674	HI100
CD56	BV510	BD Biosciences	563041	NCAM16.2
CD69	FITC	BD Biosciences	347823	L78
CD8	V500	BD Biosciences	561618	SK1
CD80	BV650	BD Biosciences	564158	L307.4
CD83	PE-CF594	BD Biosciences	562631	HB15e
CD86	PE	BD Biosciences	555658	2331(FUN-1)
CD88	BV711	BD Biosciences	742319	D53-1473
CD89	BV421	BD Biosciences	744374	A59
CTLA-4	PE-CF594	BD Biosciences	562742	BNI3
DNAM1	AF700	RnD Systems	FAB666N	102511
Epcam	BV421	BD Biosciences	563180	EBA-1

Epcam	FITC	Biologend	324204	9C4
FoxP3	PE	eBioscience	12-4776-42	PCH101
HLA-ABC	PE	ThermoFisher	MA1-19662	W6/32
HLA-DR	APC	BD Biosciences	340907	2A3
HLA-E	PE	eBioscience	12-9953-42	3D12HLA-E
HLA-G	PE	Biologend	335906	87G
KIR2D	FITC	Miltenyi Biotech	130-098-689	NKVFS1
Lag3	PE-Cy7	eBioscience	25-2239-42	3DS223H
MICA/B	PE	Biologend	320906	6D4
Nectin-2	PE	Biologend	337409	TX31
NKG2A	PE-Vio770	Miltenyi Biotech	130-114-093	REA110
NKG2C	PE	Miltenyi Biotech	130-119-776	REA205
NKG2D	APC	BD Biosciences	558071	1D11
NKp30	PE	Biologend	325208	P30-15
NKp44	PE-Vio770	Miltenyi Biotech	130-120-356	REA1163
NKp46	APC	Miltenyi Biotech	130-092-609	9E2
PD-1	BV786	BD Biosciences	563789	EH12.1
PD-L1	BV786	BD Biosciences	563739	MIH1
PVR	PE	Biologend	337610	SK11.4
Tim3	BV421	Biologend	345008	F38-2E2
ULBP 1	PE	R&D systems	FAB1380P	170818
ULBP 2/5/6	PE	R&D systems	FAB1298P	165903
ULBP 3	PE	R&D systems	FAB1517P	166510

Table S2: Changes in NK cell receptor expression after 5 days upon co-culture of dissociated primary colorectal cancer samples in the presence and absence of sample and/or R848. NK cell:SCS ratio: 1:1. NK baseline is receptor expression of NK cells before co-culture. Abbreviations: T = tumor. R = R848. NK = NK cells

Marker	N	Conditions - Mean (SEM)				Analysis type	p-value	Multiple Comparison Analysis					
		NK baseline	NK	T + NK	T + NK + R848			NKbaseline-NK	NKbaseline -T+NK	NKbaseline - T+NK+R	NK-T+NK	NK-T+NK+R	T+NK - T+NK+R
%KIR2D	5	9.6 (2.0)	8.8 (1.6)	10.3 (1.6)	11.5 (1.5)	Friedman	ns	ns	ns	ns	ns	ns	ns
%NKG2A	5	56.6 (4.3)	64.3 (5.5)	66.2 (5.6)	68.5 (6.2)	ANOVA	**	ns	*	**	ns	ns	ns
%NKG2C	5	7.1 (1.0)	20.7 (4.1)	24.7 (4.7)	25.6 (3.6)	ANOVA	**	*	**	**	ns	ns	ns
%NKG2D	5	74.2 (5.0)	75.6 (3.8)	67.7 (6.2)	76.6 (6.7)	ANOVA	ns	ns	ns	ns	ns	ns	ns
%DNAM-1	5	68.4 (5.2)	78.9 (1.5)	78.3 (3.1)	79.6 (1.9)	ANOVA	ns	ns	ns	ns	ns	ns	ns
%CD25	5	6.0 (1.2)	34.0 (8.1)	33.9 (8.4)	41.5 (11.7)	ANOVA	**	*	*	**	ns	ns	ns
%NKp30	5	38.9 (10.7)	26.8 (6.9)	10.5 (5.4)	38.9 (10.7)	Friedman	ns	ns	ns	ns	ns	ns	ns
%NKp44	5	44.4 (6.4)	48.3 (5.9)	28.3 (12.3)	30.8 (13.6)	ANOVA	ns	ns	ns	ns	ns	ns	ns
%NKp46	5	76.1 (1.63)	86.2 (0.867)	85.7 (1.06)	86.4 (1.21)	Friedman	**	*	ns	ns	ns	ns	ns
%CD16	5	2.1 (0.3)	3.32 (0.4)	0.8 (0.6)	1.5 (0.6)	Friedman	ns	ns	ns	ns	ns	ns	ns

Table S3: Changes in NK cell receptor expression after 5 days upon co-culture of dissociated metastatic colorectal cancer samples in the presence and absence of sample and/or R848. NK cell:SCS ratio: 1:1. NK baseline is receptor expression of NK cells before co-culture Abbreviations: T = tumor. R = R848. NK = NK cells

Marker	N	Conditions - Mean (SEM)				Analysis type	p-value	Multiple Comparison Analysis					
		NK baseline	NK	T + NK	T + NK + R848			NKbaseline-NK	NKbaseline -T+NK	NKbaseline - T+NK+R	NK-T+NK	NK-T+NK+R	T+NK - T+NK+R
%KIR2D	6	6.6 (0.7)	4.2 (0.6)	6.3 (1.3)	6.3 (1.1)	Friedman	*	*	ns	ns	ns	ns	ns
%NKG2A	6	68.5 (5.2)	62.3 (2.2)	69.9 (3.6)	74.8 (2.6)	Friedman	*	ns	ns	ns	ns	*	ns
%NKG2C	6	6.4 (0.9)	15.8 (2.7)	13.3 (3.2)	12.2 (1.8)	Friedman	*	*	ns	ns	ns	ns	ns
%NKG2D	6	84.3 (3.3)	87.7 (2.0)	84.9 (1.7)	87.4 (1.2)	Friedman	ns	ns	ns	ns	ns	ns	ns
%DNAM-1	6	84.2 (4.4)	87.9 (2.4)	84.9 (2.2)	88 (2.1)	Friedman	ns	ns	ns	ns	ns	ns	ns
%CD25	6	2.7 (0.7)	19.9 (3.4)	19.8 (5.1)	25.5 (5.2)	Friedman	****	ns	ns	***	ns	ns	ns
%NKp30	6	13.2 (6.8)	6.0 (1.0)	5.6 (0.3)	5.3 (1.5)	Friedman	ns	ns	ns	ns	ns	ns	ns
%NKp44	6	78.9 (4.0)	17.2 (1.2)	28.4 (8.9)	30.7 (10.2)	ANOVA	****	****	***	***	ns	ns	ns
%NKp46	6	83.7 (1.99)	86.3 (1.76)	87.7 (1.01)	89.9 (0.920)	Friedman	*	ns	ns	*	ns	ns	ns
%CD16	6	12.0 (1.4)	6.0 (1.1)	10.5 (1.46)	9.7 (1.2)	Friedman	*	*	ns	ns	ns	ns	ns

Table S4: Changes in the myeloid and lymphocyte compartments and in the cytokine and chemokine concentrations upon culture of dissociated primary colorectal cancer samples in the presence and absence of NK cells and/or R848. NK cell:SCS ratio: 1:1. Abbreviations: T = tumor. R = R848. NK = NK cells.

Marker	N	Conditions - Mean (SEM)				Analysis type	p-value	Multiple Comparison Analysis					
		T	T+NK	T+R848	T+NK+R848			T – T+NK	T – T+R	T – T+R+NK	T+NK – T+R	T+NK – T+R+NK	T+R – T+R+NK
Myeloid Analysis Day 5													
% CD14 neg	9	16.9 (3.6)	28.3 (5.1)	30.8 (4.2)	48.2 (5.1)	ANOVA	****	**	****	****	ns	****	****
% CD14 dim	9	51.1 (7.4)	50.8 (5.3)	50.3 (5.0)	36.3 (4.9)	ANOVA	***	ns	ns	**	ns	**	**
% CD14 high	9	24.0 (6.1)	7.6 (2.8)	12.0 (3.4)	3.9 (1.7)	ANOVA	****	***	*	****	ns	ns	ns
CD14 neg CD80 MFI	9	19990 (5172)	20881 (5143)	22298 (5672)	32833 (6519)	ANOVA	****	ns	ns	***	ns	***	**
CD14 neg CD83 MFI	9	3691 (434.8)	4059 (457.3)	4715 (502.2)	6176 (692.8)	ANOVA	****	ns	ns	****	ns	***	*
CD14 neg CD86 MFI	4	10214 (3052)	10265 (2743)	15865 (2113)	29587 (4401)	ANOVA	**	ns	ns	**	ns	**	*
CD14 neg PD-L1 MFI	9	2609 (581)	2108 (355.5)	2186 (388.1)	2444 (491.7)	ANOVA	ns	ns	ns	ns	ns	ns	ns
CD14 neg Tim3 MFI	9	9802 (1813)	9108 (1687)	11231 (2369)	11961 (2152)	ANOVA	***	ns	ns	*	*	**	ns
CD14 neg BDCA3 MFI	9	6897 (1433)	5442 (970.9)	5944 (1087)	6211 (1068)	ANOVA	ns	ns	ns	ns	ns	ns	ns
Lymphocyte Analysis Day 5													
Ab. N. CD8+ T cells	4	238.7 (61.6)	232.2 (45.9)	213.5 (41.3)	196.4 (22.5)	ANOVA	ns	ns	ns	ns	ns	ns	ns
Ab. N. CD4+ T helper	4	679.6 (202.8)	601.7 (105.4)	640.3 (131.1)	533.4 (26.5)	ANOVA	ns	ns	ns	ns	ns	ns	ns
Absolut N aTreg	4	111.8 (34.0)	50.6 (12.3)	67.8 (15.8)	34.3 (12.2)	ANOVA	**	*	ns	**	ns	ns	ns
% aTreg	9	13.7 (1.8)	8.0 (1.3)	10.4 (1.7)	4.8 (1.1)	ANOVA	****	**	ns	****	ns	ns	**
CD8+ T cells %CD25	9	20.3 (3.5)	35.2 (5.2)	25.0 (3.6)	44.1 (4.9)	ANOVA	****	**	ns	****	ns	ns	***
CD8+ T cells %CD69	9	26.2 (6.1)	31.5 (6.0)	34.6 (7.1)	43.3 (5.9)	ANOVA	****	ns	*	****	ns	***	*
CD8+ T cells %CTLA4	9	15.9 (2.5)	12.2 (2.8)	14.9 (2.6)	16.0 (2.9)	ANOVA	ns	ns	ns	ns	ns	ns	ns

CD8⁺ T cells %PD-1	9	45.3 (7.6)	39.7 (7.6)	46.5 (7.9)	40.1 (8.0)	ANOVA	ns	ns	ns	ns	ns	ns	ns
CD8⁺ T cells %Tim3	9	13.6 (4.4)	26.0 (7.8)	16.7 (4.8)	31.9 (8.2)	Friedman	***	ns	ns	***	ns	ns	**
CD4⁺ T helper %CD25	9	18.6 (2.4)	28.7 (4.9)	22.3 (2.2)	30.9 (4.7)	ANOVA	**	*	ns	**	ns	ns	ns
CD4⁺ T helper %CD69	9	15.3 (2.6)	14.0 (3.5)	19.6 (3.4)	17.5 (3.8)	Friedman	***	ns	*	ns	***	*	ns
CD4⁺ T helper %CTLA4	9	43.6 (3.7)	35.2 (4.2)	41.4 (3.7)	36.9 (3.6)	ANOVA	ns	ns	ns	ns	ns	ns	ns
CD4⁺ T helper %Lag3	9	4.2 (1.4)	5.4 (1.5)	4.2 (1.1)	3.8 (0.6)	Friedman	ns	ns	ns	ns	ns	ns	ns
CD4⁺ T helper %PD-1	9	31.9 (6.1)	31.6 (5.3)	37.4 (6.2)	30.9 (5.5)	ANOVA	*	ns	ns	ns	ns	ns	ns
CD4⁺ T helper %Tim3	9	8.0 (2.6)	10.0 (3.6)	9.4 (2.5)	13.5 (4.7)	Friedman	ns	ns	ns	ns	ns	ns	ns
Cytokine Analysis Day 5 (pg/mL)													
IL-12p70	9	1.9 (0.6)	1.7 (0.5)	6.2 (2.4)	3.7 (1.7)	Friedman	***	ns	***	*	**	ns	ns
IFNα	9	2.6 (0.8)	4.6 (2.5)	51.7 (25.0)	79.7 (34.5)	Friedman	***	ns	ns	**	ns	**	ns
IL-10	9	47.3 (10.9)	21.2 (5.9)	109.1 (16.9)	58.1 (9.3)	Friedman	****	ns	ns	ns	****	*	ns
IL-6	9	38545 (6326)	21121 (5081)	42940 (6708)	14976 (3200)	Friedman	**	ns	ns	ns	ns	ns	*
IL-2	9	19.1 (4.0)	32.4 (7.6)	22.0 (6.8)	55.9 (10.5)	ANOVA	**	ns	ns	**	ns	ns	*
IFNγ	9	51 (21.12)	3495 (2005)	358.8 (122.1)	10342 (1775)	Friedman	****	*	ns	****	ns	ns	*
TNF	9	16.3 (5.5)	119.1 (62.6)	54.2 (14.8)	120.5 (26.9)	Friedman	***	ns	ns	***	ns	ns	ns
IL-15	9	3.2 (1.0)	8.1 (4.9)	3.8 (1.7)	8.2 (4.0)	Friedman	ns	ns	ns	ns	ns	ns	ns
IL-18	9	40.0 (11.4)	35.8 (6.09)	41.3 (9.0)	42.5 (8.5)	Friedman	ns	ns	ns	ns	ns	ns	ns
Chemokine Analysis Day 5 (pg/mL)													
CCL4	9	19.1 (4.5)	41.6 (12.2)	28.2 (5.8)	63.3 (16.4)	ANOVA	***	ns	ns	***	ns	**	ns
CCL5	9	84.0 (13.5)	254.7 (66.1)	164.9 (38.2)	530.1 (137.1)	Friedman	****	*	ns	****	ns	ns	*

CXCL9	9	124.7 (45.3)	308.9 (147.9)	189.4 (52.7)	302.9 (138.4)	Friedman	ns	ns	ns	ns	ns	ns	ns
CXCL10	9	650.4 (236.9)	332.0 (56.6)	1763.4 (669.9)	755.1 (156.6)	Friedman	*	ns	ns	ns	*	ns	ns
CXCL11	9	5.6 (2.3)	2.6 (0.7)	8.5 (3.9)	3.3 (0.6)	Friedman	*	ns	ns	ns	ns	ns	ns
CCL20	9	40.7 (11.5)	17.6 (6.0)	23.4 (5.6)	13.0 (6.3)	Friedman	*	ns	ns	*	ns	ns	ns

Table S5: Changes in the myeloid and lymphocyte compartments and in the cytokine and chemokine concentrations upon culture of dissociated metastatic colorectal cancer samples in the presence and absence of NK cells and/or R848. NK cell:SCS ratio: 1:1. Abbreviations: T = tumor. R = R848. NK = NK cells.

Marker	N	Conditions				Analysis type	p-value	Multiple Comparison Analysis					
		T	T+NK	T+R848	T+NK+R848			T – T+NK	T – T+R	T – T+R+NK	T+NK – T+R	T+NK – T+R+NK	T+R – T+R+NK
Myeloid Analysis Day 5													
% CD14 neg	6	7.7 (0.9)	21.8 (5.2)	17.6 (3.3)	27.9 (5.2)	ANOVA	***	**	ns	***	ns	ns	ns
% CD14 dim	6	17.7 (1.3)	47.3 (5.6)	35.1 (5.7)	37.1 (4.1)	ANOVA	**	***	*	*	ns	ns	ns
% CD14 high	6	74.1 (1.3)	29.3 (10.2)	46.4 (7.5)	33.4 (8.4)	ANOVA	***	***	*	**	ns	ns	ns
CD14 neg CD80 MFI	6	57217 (11402)	49960 (11464)	21954 (4030)	40163 (16987)	Friedman	ns	ns	ns	ns	ns	ns	ns
CD14 neg CD83 MFI	6	7945 (898.6)	7896 (1258)	6538 (1117)	6239 (1338)	Friedman	ns	ns	ns	ns	ns	ns	ns
CD14 neg CD86 MFI	6	35604 (21419)	33465 (21824)	9900 (1338)	16316 (8341)	Friedman	ns	ns	ns	ns	ns	ns	ns
CD14 neg PD-L1 MFI	6	7119 (668.2)	8797 (1570)	7429 (1129)	5127 (1319)	Friedman	ns	ns	ns	ns	ns	ns	ns
CD14 neg Tim3 MFI	6	17786 (825.6)	19517 (1856)	16339 (811.7)	16990 (1911)	ANOVA	ns	ns	ns	ns	ns	ns	ns
CD14 neg BDCA3 MFI	6	7575 (1666)	5809 (1598)	4230 (827.8)	3398 (674.9)	ANOVA	**	ns	ns	*	ns	ns	ns
Lymphocyte Analysis Day 5													
Ab. N. CD8 ⁺ T cells	6	1028 (174.9)	1225 (314.1)	1323 (373.5)	1211 (387.3)	ANOVA	ns	ns	ns	ns	ns	ns	ns
Ab. N. CD4 ⁺ T helper	6	1944 (438)	2246 (577.4)	2467 (894.9)	2526 (1206)	Friedman	ns	ns	ns	ns	ns	ns	ns
Absolut N aTreg	6	121 (38.3)	78.7 (22.9)	58.6 (18.3)	29.0 (6.3)	ANOVA	ns	ns	ns	*	ns	ns	ns
% aTreg	7	5.9 (1.2)	2.9 (0.5)	3.5 (1.1)	1.5 (0.3)	ANOVA	**	*	ns	**	ns	ns	ns
CD8 ⁺ T cells %CD25	7	8.8 (2.8)	18.8 (3.3)	10.0 (2.5)	27.4 (5.3)	ANOVA	***	ns	ns	***	ns	ns	***
CD8 ⁺ T cells %CD69	7	14.4 (3.9)	15.2 (3.1)	19.9 (5.5)	18.7 (3.1)	ANOVA	ns	ns	ns	ns	ns	ns	ns

CD8⁺ T cells %CTLA4	7	16.1 (2.4)	12.3 (3.0)	18.7 (3.5)	16.5 (3.8)	ANOVA	*	ns	ns	ns	*	ns	ns
CD8⁺ T cells %PD-1	7	27.0 (8.2)	27.3 (8.4)	28.0 (8.4)	27.9 (7.2)	ANOVA	ns	ns	ns	ns	ns	ns	ns
CD8⁺ T cells %Tim3	7	5.6 (1.3)	10.8 (3.6)	9.9 (3.6)	11.5 (5.1)	Friedman	ns	ns	ns	ns	ns	ns	ns
CD4⁺ T helper %CD25	7	7.7 (1.1)	24.5 (7.1)	11.2 (1.9)	30.8 (9.0)	Friedman	***	ns	ns	***	ns	ns	*
CD4⁺ T helper %CD69	7	16.3 (2.9)	16.0 (4.0)	17.7 (4.6)	16.2 (2.9)	ANOVA	ns	ns	ns	ns	ns	ns	ns
CD4⁺ T helper %CTLA4	7	40.8 (5.3)	30.7 (5.4)	42.8 (5.6)	35.4 (6.6)	ANOVA	***	**	ns	ns	***	ns	*
CD4⁺ T helper %PD-1	7	31.4 (7.6)	30.1 (8.3)	28.5 (7.6)	26.3 (6.8)	ANOVA	ns	ns	ns	ns	ns	ns	ns
CD4⁺ T helper %Tim3	7	7.7 (3.2)	16.6 (7.5)	10.5 (4.6)	19.7 (9.3)	Friedman	ns	ns	ns	ns	ns	ns	ns
Cytokine Analysis Day 5 (pg/mL)													
IL-12p70	6	0.5 (0.005)	0.6 (0.04)	1.6 (0.1)	0.9 (0.1)	Friedman	****	ns	***	ns	ns	ns	ns
IFNα	6	0.5 (0.04)	0.8 (0.2)	9.1 (4.3)	5.05 (2.9)	Friedman	***	ns	*	*	ns	ns	ns
IL-10	6	205 (78.6)	36.3 (14.4)	1573.2 (413.0)	609.8 (234.7)	Friedman	****	ns	ns	ns	***	*	ns
IL-6	6	87230 (12801)	10278 (6135)	98956 (13835)	7887 (2534)	Friedman	***	ns	ns	*	ns	ns	*
IL-2	6	11.23 (2.52)	33.38 (4.162)	29.52 (3.117)	59 (3.716)	ANOVA	****	**	**	****	ns	***	***
IFNγ	6	7.992 (3.748)	1834 (636.8)	433.9 (145.6)	13067 (841.4)	Friedman	****	*	ns	***	ns	ns	*
TNF	6	17.29 (7.94)	111.1 (18.87)	2488 (987.5)	1342 (513.4)	Friedman	****	ns	***	*	ns	ns	ns
IL-15	6	4.358 (0.04833)	43 (13.46)	5.322 (0.4977)	43.62 (12.02)	Friedman	**	*	ns	ns	ns	ns	ns
IL-18	6	142.9 (40.95)	108.3 (44.84)	296 (105.7)	252.4 (96.35)	Friedman	**	ns	ns	ns	*	*	ns
Chemokine Analysis Day 5 (pg/mL)													
CCL4	6	12.0 (3.0)	31.8 (7.6)	93.7 (30.1)	82.9 (17.0)	ANOVA	**	ns	*	*	ns	ns	ns

CCL5	6	47.9 (18.7)	133.6 (31.2)	424.4 (253.6)	504.6 (163.1)	Friedman	***	ns	ns	**	ns	ns	ns
CXCL9	6	70.1 (35.8)	569.5 (90.9)	384.8 (110.8)	465 (138.4)	Friedman	**	*	ns	ns	ns	ns	ns
CXCL10	6	217.8 (129)	1453 (661)	2002 (1237)	1939 (1171)	Friedman	*	ns	*	ns	ns	ns	ns
CXCL11	6	1.2 (0.4)	3.2 (0.9)	8.4 (5.1)	6.3 (3.6)	Friedman	ns	ns	ns	ns	ns	ns	ns
CCL20	6	640.5 (347.7)	89.8 (56.1)	1011 (470.5)	125 (83.1)	Friedman	****	ns	ns	ns	**	ns	*