

Figure S1. Titration of antibodies for flow cytometry. In total, 5×10^5 PBMCs were stained with indicated dilutions of fluorochrome-conjugated antibodies. For titration of EpCAM, PBMCs were mixed at a 1:1 ratio with SNU-C5 cells. Detection of CD137 and CD107a was performed after o.n. stimulation with anti-CD3/CD28 beads (1:1, Miltenyi) and addition of GolgiStop. Optimal antibody dilutions were defined by separation index.

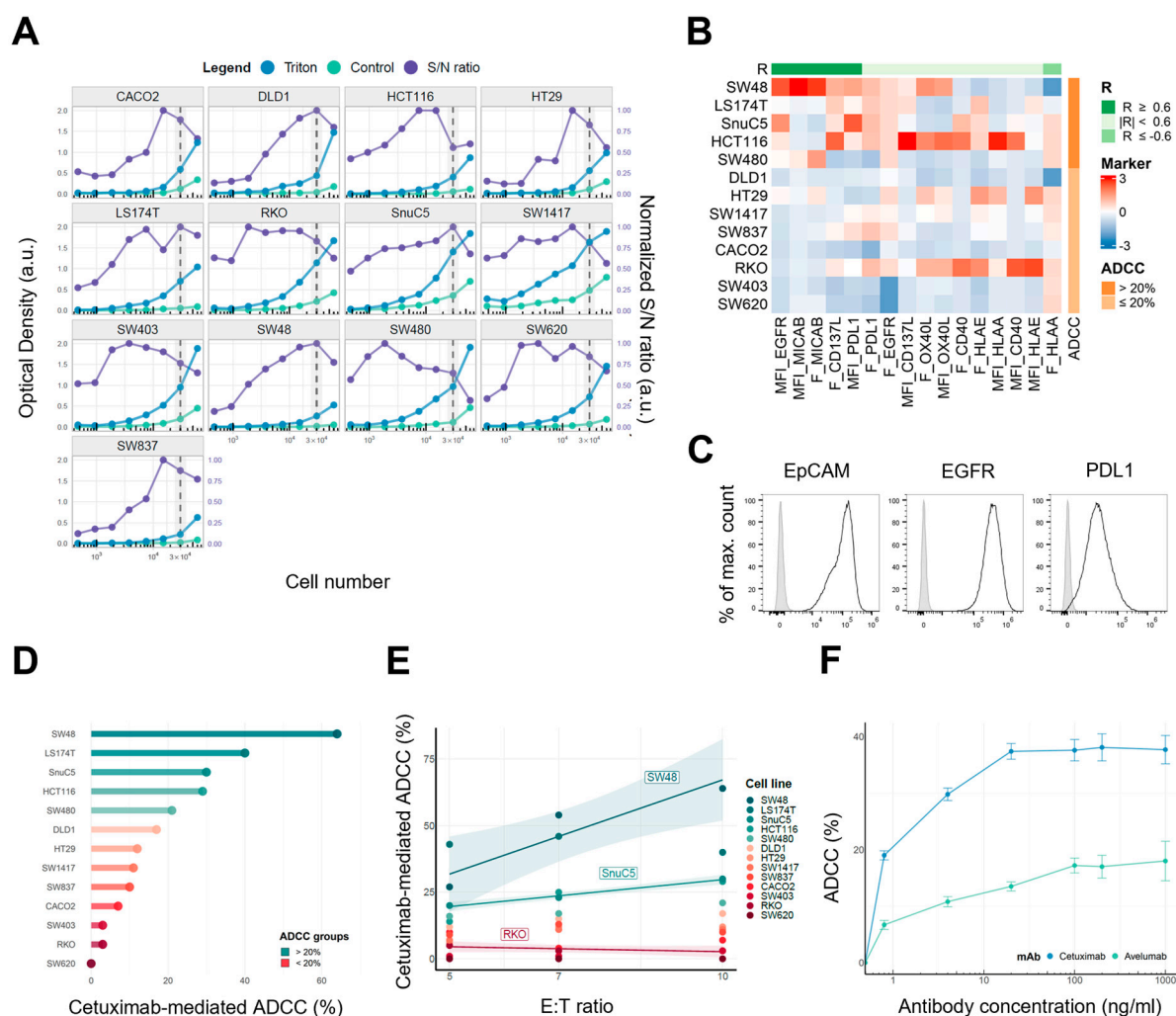


Figure S2. Characterization of 13 colorectal cancer cell lines. (A) Detection of signal:noise (S/N) ratios of 13 different CRC cell lines at different cell concentrations. Calculating the ratios between unstimulated samples (control) to the potential maximal release induced by Triton-X-100 revealed a high and stable S/N ratio even for low cell counts. Each dot represents the mean of triplicates. (B) All cell lines were stained with PE-conjugated antibodies against the depicted cell surface markers and frequencies of positive cells (F) as well as the MFI of each marker was analyzed. In the heat map, cell lines are ordered according to ADCC sensitivity (see (D)) and markers are arranged based on descending Pearson's correlation coefficient (R) between marker expression and ADCC. (C) Histograms of EpCAM, EGFR, and PDL1 expression on SNU-C5 cells. The solid black line shows marker expression while the respective isotype control is shown in gray. (D) Sensitivity of CRC cell lines to cetuximab-mediated ADCC detected after 24h co-cultures (E:T = 10:1). ADCC was assessed after stimulation with 100ng/mL cetuximab by LDH release assay. Each bar represents the mean of technical triplicates from a representative experiment of at least two individual assays. A cut-off of 20% ADCC was introduced to distinguish cetuximab-resistant from sensitive cell lines. (E) ADCC sensitivity was also tested at different E:T ratios. While SW48 shows a slightly higher ADCC compared to SNU-C5, their strong adherence in cell culture resulted in high intra-assay variations illustrated by the shaded areas representing the assay standard deviation. (F) Titration series of cetuximab and avelumab-induced ADCC against SNU-C5 cells indicates a maximum induced lysis at 100ng/mL. ADCC was determined after 24h at a 10:1 (E:T) ratio by LDH release and each point represents the mean and SEM of a representative experiment.

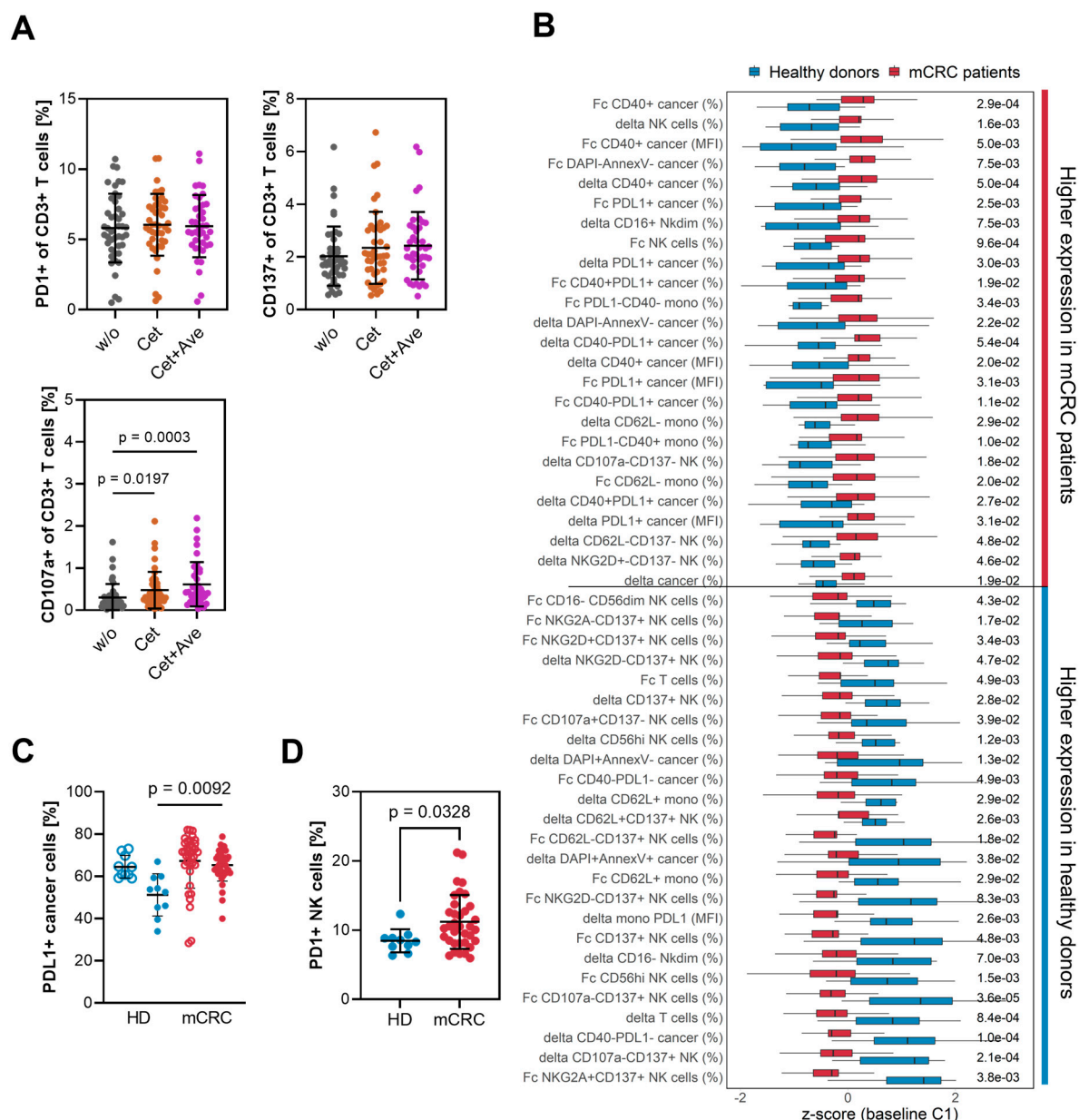


Figure S3. Differentially regulated parameters by cetuximab stimulation. (A) Frequencies of PD1, CD137, or CD107a on CD3+ T cells after co-cultures with SNU-C5 cells and stimulation with cetuximab (Cet) alone or in combination with avelumab (Cet+Ave). Data were generated from experiments with 10 HD and 35 mCRC patients and significance was tested by Kruskal–Wallis test. (B) Boxplots summarize all significantly differentially regulated parameters after cetuximab stimulation between healthy donors and mCRC patients. Bars show the mean and SEM of z-score normalized values together with the p-value determined by Brunner–Munzel test for non-normal data with unequal variance. Parameters are ordered based on the mean difference between healthy donors and mCRC patients. (C) Frequencies of PDL1+ SNU-C5 cancer cells in unstimulated (outlined circles) or cetuximab-stimulated (filled circles) co-cultures with PBMCs from HD or mCRC patients. Statistics were performed using one-way ANOVA followed by Kruskal–Wallis test. D) Frequency of PD1+ NK cells detected in co-cultures without antibody stimulation. Mann–Whitney test was used to test the significance between groups.

Table S1. Study cohort.

Sample ID	Group	Age	Gender	FcyRIIIa
FCY-001	Healthy control	74	male	V/F
FCY-002	Healthy control	70	female	F/F
FCY-003	Healthy control	69	male	V/F
FCY-004	Healthy control	67	female	V/V
FCY-005	Healthy control	68	female	F/F
FCY-006	Healthy control	69	male	V/V
FCY-007	Healthy control	68	male	V/F
FCY-008	Healthy control	68	female	V/F
FCY-009	Healthy control	61	female	V/F
FCY-010	Healthy control	66	male	V/F
FCY-011	Healthy control	38	female	V/V
FCY-012	Healthy control	35	female	V/F
FCY-013	Healthy control	36	male	V/V
FCY-014	Healthy control	35	male	V/F
FCY-015	Healthy control	38	female	V/F
FCY-016	Healthy control	36	male	F/F
FCY-017	Healthy control	34	male	F/F
FCY-018	Healthy control	36	male	V/V
FCY-019	Healthy control	35	female	F/F
FCY-020	Healthy control	33	female	V/F
FCY-021	Healthy control	33	female	V/V
FCY-022	Healthy control	44	female	V/V
FCY-023	Healthy control	42	male	V/V
FCY-024	Healthy control	35	female	V/F
FCY-025	Healthy control	33	male	F/F
FCY-026	Healthy control	26	male	F/F
FCY-027	Healthy control	25	female	F/F
FCY-028	Healthy control	26	female	F/F
FCY-029	Healthy control	48	male	F/F
FCY-030	Healthy control	32	female	F/F
FCY-031	Healthy control	28	female	F/F
FCY-032	Healthy control	26	male	F/F
FCY-033	Healthy control	26	female	V/F
FCY-034	Healthy control	41	female	V/F
FCY-035	Healthy control	49	female	F/F
FCY-036	Healthy control	37	male	V/V
FCY-037	Healthy control	36	male	V/F
FCY-038	Healthy control	31	female	F/F
FCY-039	Healthy control	25	female	V/F
Pat-01	mCRC patient	74	male	F/F
Pat-02	mCRC patient	78	male	F/F
Pat-03	mCRC patient	61	male	F/F
Pat-04	mCRC patient	75	male	F/F
Pat-05	mCRC patient	76	male	F/V
Pat-06	mCRC patient	57	female	F/V
Pat-07	mCRC patient	62	female	F/V
Pat-08	mCRC patient	58	male	F/F
Pat-09	mCRC patient	61	male	F/V
Pat-10	mCRC patient	81	male	F/F
Pat-11	mCRC patient	65	male	F/V
Pat-12	mCRC patient	47	male	F/F
Pat-13	mCRC patient	74	female	F/V
Pat-14	mCRC patient	63	male	F/V
Pat-15	mCRC patient	57	male	V/V
Pat-16	mCRC patient	62	male	F/V
Pat-17	mCRC patient	63	male	F/V
Pat-18	mCRC patient	63	male	V/V
Pat-19	mCRC patient	72	male	F/V
Pat-20	mCRC patient	72	female	F/F
Pat-21	mCRC patient	78	male	F/F
Pat-22	mCRC patient	56	male	V/V
Pat-23	mCRC patient	68	female	V/V
Pat-24	mCRC patient	57	male	F/F

Pat-25	mCRC patient	55	male	F/F
Pat-26	mCRC patient	75	male	V/V
Pat-27	mCRC patient	70	male	F/F
Pat-28	mCRC patient	47	male	F/V
Pat-29	mCRC patient	66	male	F/V
Pat-30	mCRC patient	68	male	F/V
Pat-31	mCRC patient	53	male	F/V
Pat-32	mCRC patient	74	male	F/F
Pat-33	mCRC patient	51	male	V/V
Pat-34	mCRC patient	47	male	F/F
Pat-35	mCRC patient	60	male	F/F
Pat-36	mCRC patient	71	male	F/V
Pat-37	mCRC patient	56	female	V/V
Pat-38	mCRC patient	53	male	F/F
Pat-39	mCRC patient	49	male	F/V
Pat-40	mCRC patient	72	male	F/V
Pat-41	mCRC patient	53	male	F/V
Pat-42	mCRC patient	45	female	F/F
Pat-43	mCRC patient	58	male	F/F
Pat-44	mCRC patient	55	male	V/V
Pat-45	mCRC patient	69	female	F/V
Pat-46	mCRC patient	63	male	F/V
Pat-47	mCRC patient	35	female	V/V
Pat-48	mCRC patient	53	male	F/F
Pat-49	mCRC patient	73	female	F/V
Pat-50	mCRC patient	60	male	F/F
Pat-51	mCRC patient	60	male	F/F
Pat-52	mCRC patient	78	male	F/F

Table S2. Flow cytometry antibodies of the FcγRIIIa panel.

Category	Antibody	Fluorochrome	Clone	Dilution	μl/staining	Company	Catalog No.
Cell lineage	CD3	BV421	UCHT1	1:50	1	Biolegend	300434
	CD14	BV785	M5E2	1:50	1	Biolegend	301840
	CD56	BUV395	NCAM16.2	1:200	0.25	BD Biosciences	563554
FcγRIIIa phenotyping	CD16 MEM	FITC	MEM154	1:50	1	Santa Cruz	sc-51525 FITC
	CD16 LNK	AF647	LNK16	1:50	1	BioRad Antibodies	MCA1193A647T
FcγR block	Beriglobin	-	-	1:50	1	CSL Behring	PZN-046161

Abbreviations: BV, brilliant violet; BUV, Brilliant Ultraviolet; FITC, Fluorescein isothiocyanate; AF, Alexa Fluor.

Table S3. Flow cytometry antibodies for CRC cell line characterization.

Antibody	Fluorochrome	Clone	Dilution	μl/staining	Company	Catalog No.
EpCAM	AF700	9C4	1:400	0.125	Biolegend	324243
EGFR	PE	AY13	1:100	0.5	Biolegend	352903
PDL1	PE	MIH3	1:100	0.5	Biolegend	374512
HLA-A,B,C	PE	W6/32	1:50	1	Biolegend	311406
HLA-E	PE	3D12	1:100	0.5	Biolegend	342603
MICA/B	PE	6D4	1:100	0.5	Biolegend	320906
CD137L	PE	5F4	1:100	0.5	Biolegend	311504
CD40	PE	5C3	1:100	0.5	Biolegend	334308
OX40L	PE	11C3.1	1:100	0.5	Biolegend	326307

Abbreviations: PE, Phycoerythrin; AF, Alexa Fluor.

Table S4. Flow cytometry antibodies of the ADCC panel.

Category	Antibody	Fluorochrome	Clone	Dilution	µl/staining	Company	Catalog No.
Cell lineage	CD3	BV421	UCHT1	1:50	1	Biolegend	300434
	CD14	BV785	M5E2	1:50	1	Biolegend	301840
	CD56	BUV395	NCAM16.2	1:200	0.25	BD Biosciences	563554
	EpCAM	AF700	9C4	1:400	0.125	Biolegend	324243
	CD16	APC-Fire750	3G8	1:100	0.5	Biolegend	302060
Checkpoint receptors and activation markers	CD107a	AF488	H4A3	1:100	0.5	Biolegend	328610
	CD137	APC	4B4-1	1:200	0.25	Biolegend	309809
	CD62L	BV605	DREG-56	1:200	0.25	Biolegend	304834
	NKG2A	Pe-Cy5	S19004C	1:200	0.25	Biolegend	375112
	NKG2D	BV510	1D11	1:50	1	Biolegend	320815
Checkpoint ligands	PD1	BV650	EH12.1	1:100	0.5	BD Biosciences	3564104
	PDL1	Pe-Cy5	MIH3	1:200	0.25	Biolegend	374512
	CD40	Pe-Cy7	5C3	1:200	0.25	Biolegend	374512
	DAPI	-	-	0.4µM	0.8	Sigma-Aldrich	D9542-5MG
Cell death	Annexin V	PeDazzle	-	1:50	1	Biolegend	640956
FcyR block	Beriglobin	-	-	1:50	1	CSL Behring	PZN-046161

Abbreviations: BV, brilliant violet; BUV, Brilliant Ultraviolet; APC, Allophycocyanin; AF, Alexa Fluor; Pe, Phycoerythrin; Cy, Cyanine.

Table S5. Selected parameters to establish a FcγRIIIa prediction model.

[illegible]

Abbreviations: MFI, Median fluorescent intensity.

Table S6. Characteristics of colorectal cancer cell lines.

Cell Line	Disease	Medium	Additives	RRID
CACO2	Colorectal adenocarcinoma	DMEM	10% FBS, 1% P/S, 1% GlutaMax	CVCL_0025
DLD1	Dukes' type C, colorectal adenocarcinoma	RPMI1640	10% FBS, 1% P/S, 1% GlutaMax	CVCL_0248
HT29	Colorectal adenocarcinoma	DMEM	10% FBS, 1% P/S, 1% GlutaMax	CVCL_0320
HCT116	Colorectal adenocarcinoma	DMEM	10% FBS, 1% P/S, 1% GlutaMax	CVCL_0291
LS174T	Dukes' type B, colorectal adenocarcinoma	DMEM	10% FBS, 1% P/S, 1% GlutaMax	CVCL_1384
RKO	Carcinoma, papilloma	DMEM	10% FBS, 1% P/S, 1% GlutaMax	CVCL_0504
SNU-C5	Cecum adenocarcinoma	RPMI1640	10% FBS, 1% P/S, 1% GlutaMax	CVCL_5112
SW48	Dukes' type C, colorectal adenocarcinoma	Leibovitz's L-15	10% FBS, 1% P/S, 1% GlutaMax	CVCL_1724
SW403	Dukes' type C, colorectal adenocarcinoma	Leibovitz's L-15	10% FBS, 1% P/S, 1% GlutaMax	CVCL_0545
SW480	Dukes' type B, colorectal adenocarcinoma	Leibovitz's L-15	10% FBS, 1% P/S, 1% GlutaMax	CVCL_0546
SW620	Dukes' type C, colorectal adenocarcinoma	Leibovitz's L-15	10% FBS, 1% P/S, 1% GlutaMax	CVCL_0547
SW837	Colorectal adenocarcinoma	Leibovitz's L-15	10% FBS, 1% P/S, 1% GlutaMax	CVCL_1729
SW1417	Dukes' type C, colorectal adenocarcinoma	Leibovitz's L-15	10% FBS, 1% P/S, 1% GlutaMax	CVCL_1717

Abbreviations: RRID, Research Resource Identifier; FBS, Fetale bovine serum; P/S, Penicillin-Streptomycin.