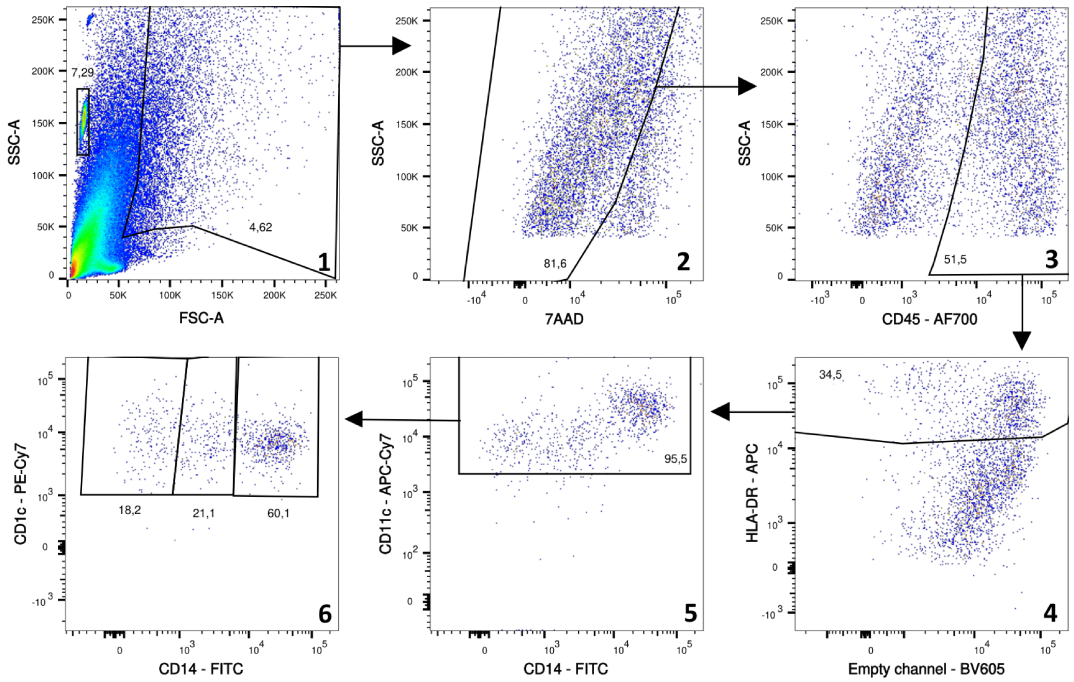

Figure S1: *Example of myeloid gating strategy. Percentages of the gated populations are listed in the plots.*

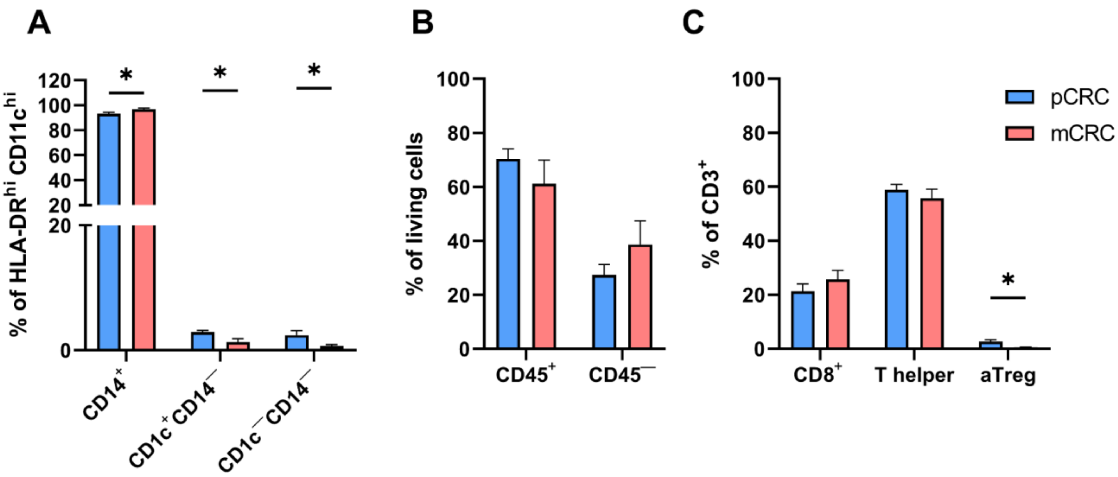

Figure S2: *Baseline characterization of the tumor microenvironment.* Differences between pCRC and mCRC in the myeloid subsets defined by the expression of (**A**) CD14 and CD1c (**B)** in the percentages of CD45^{+} and CD45^{-} cells and in **C)** the distribution of CD8^{+}, T helper and aTreg. pCRC: *n* = 9. mCRC: *n* = 5. The data are presented as mean ± SEM. Significance is presented as p <0.05 *. p-values were determined by Mann-Whitney test (**A, C**) or two-tailed T-test (**B**). Abbreviations: pCRC = primary colorectal cancer, mCRC = metastatic colorectal cancer, aTreg = activated regulatory T cells.

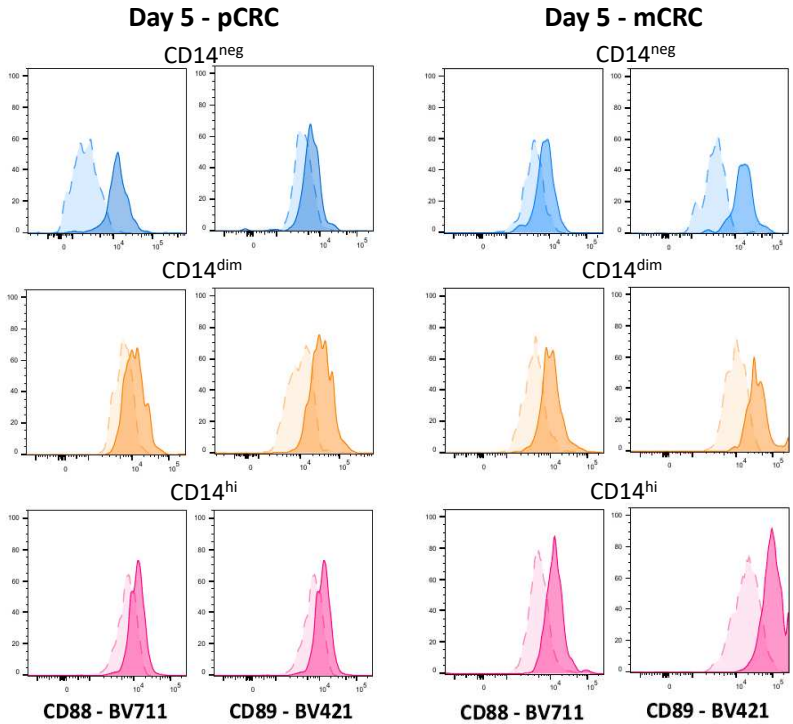

Figure S3: *Expression of CD88 and CD89 on the different myeloid subsets defined by the expression of CD14 (CD14^{neg}, CD14^{dim}, CD14^{hi}) after 5-day culture.* Concatenated data; pCRC: *n* = 4, mCRC: *n* = 6. Darker color: stained; lighter color: FMO. Abbreviations: pCRC = primary colorectal cancer, mCRC = metastatic colorectal cancer.

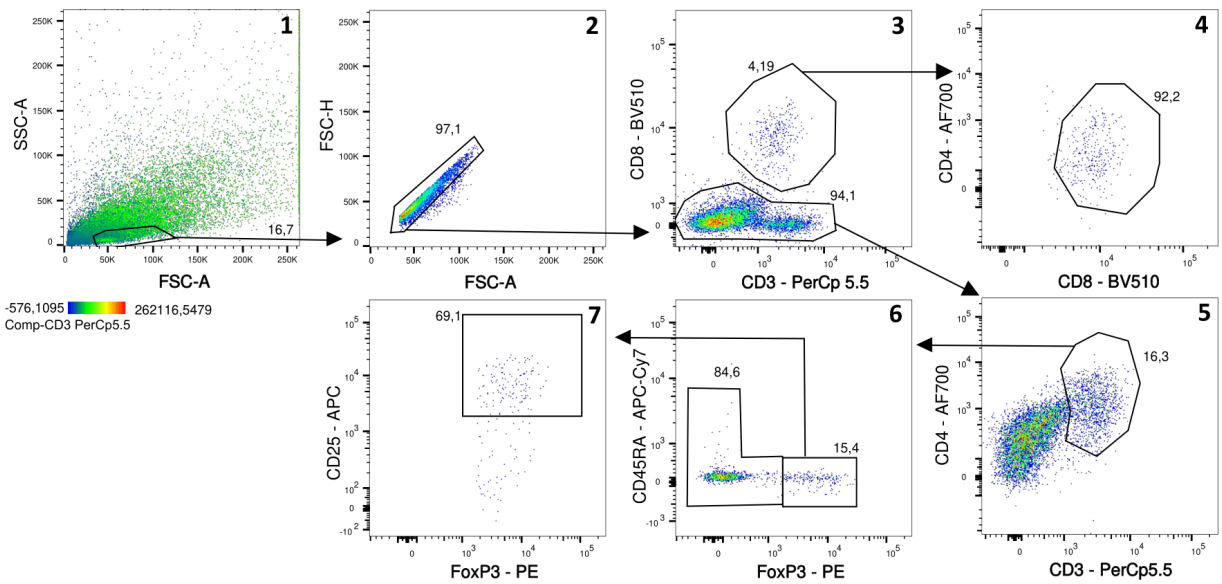

Figure S4: *Example of T cell gating strategy*. Perventages of gated cells are shown in the plots.

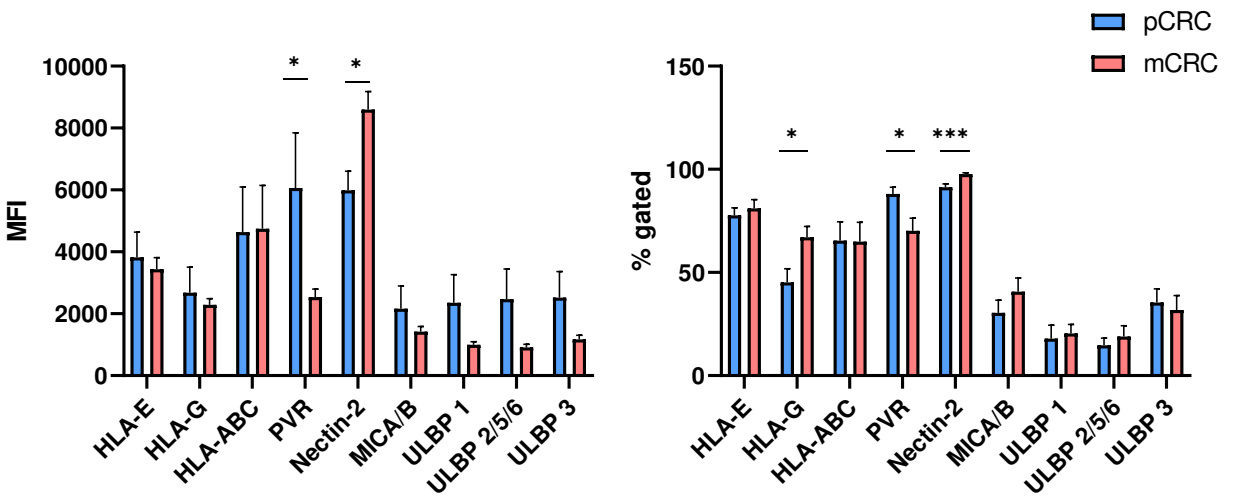

Figure S5: *Expression of NK cell ligands on the surface of CRC tumor cells, defined as Epcam⁺CD45⁻.* The data are presented as median fluorescence intensity (MFI) and percentage of positive cells. pCRC *n* = 12 (HLA-ABC *n* = 10), mCRC *n* = 12 (Nectin-2 *n* = 7). The data are presented as mean ± SEM. Significance is presented as *p* <0.05 *, <0.01 **, 0.001 ***. *p*-values are determined by Mann-Whitney test.

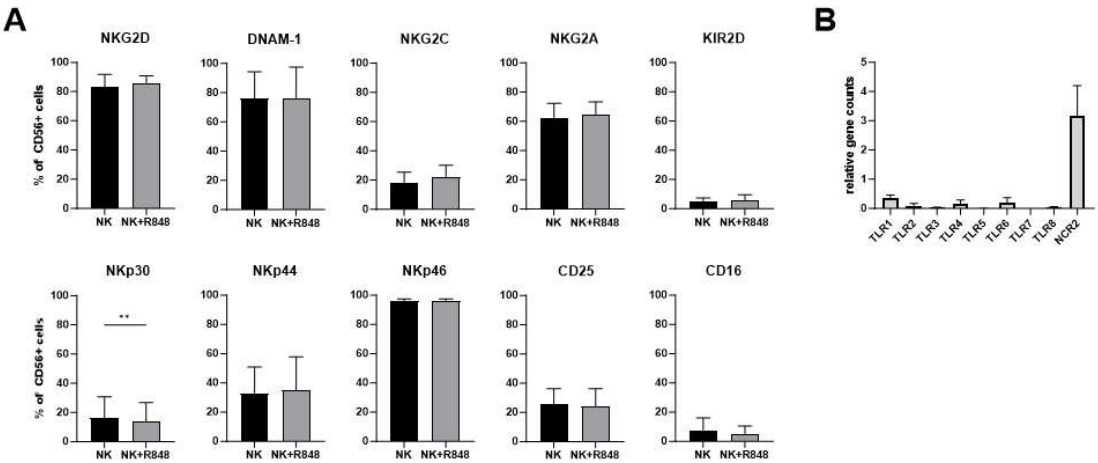

Figure S6: *Effect of R848 on NK phenotype and TLR expression on NK cells.* (**A**) Receptor expression (in percentages) at day 5 of NK cells alone or in co-culture with R848 (*n*=6). Significance is presented as <0.01 **. *p*-values were determined by two-tailed paired T-test analysis. (**B**) TLR expression obtained via whole transcriptome RNA sequencing, represented as relative gene counts. Relative gene count is calculated as ratio of the average count per gene based on 18647 analyzed genes. NCR2 (NKp44) is added as control of a gene that is highly expressed and with a similar gene size (*n*=9).

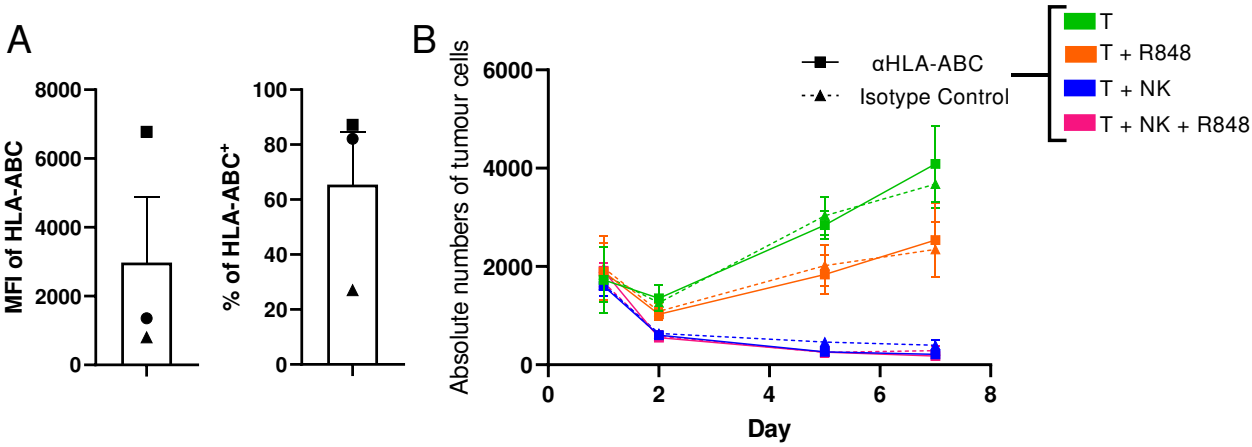

Figure S7: *Effect of NK cells and CD8⁺ T cells on tumor growth control*. (**A**) Expression of HLA-ABC on Epcam⁺CD45⁻ cells. The data are presented as median fluorescence intensity or percentage of positive cells. The symbols correspond to the different dissociated tumor samples used in the cytotoxicity assay (**B**). *n* = 3. (**B**) Cytotoxicity assay performed by co-culturing dissociated pCRC tissue in the presence and absence of NK cells, R848 and either HLA-ABC blocking antibodies (10 ug/mL, Thermofisher, cat: MA1-19027) or an IgG2a isotype control (10 ug/mL, Biolegend, cat: 401504) for 1,2,5 and 7 days. The readout is based on the absolute numbers of alive tumor cells (Epcam⁺CD45⁻7AAD⁻) quantified with QUANTI BEADS (Invitrogen, Thermo Fisher Scientific). NK cell:SCS ratio: 1:1. *n* = 3; 2 NK donors. The data are presented as mean ± SEM. *p*-values are determined by two-way ANOVA with Tukey's multiple comparisons analysis (**B**). Abbreviations: T = tumor, NK = NK cells.

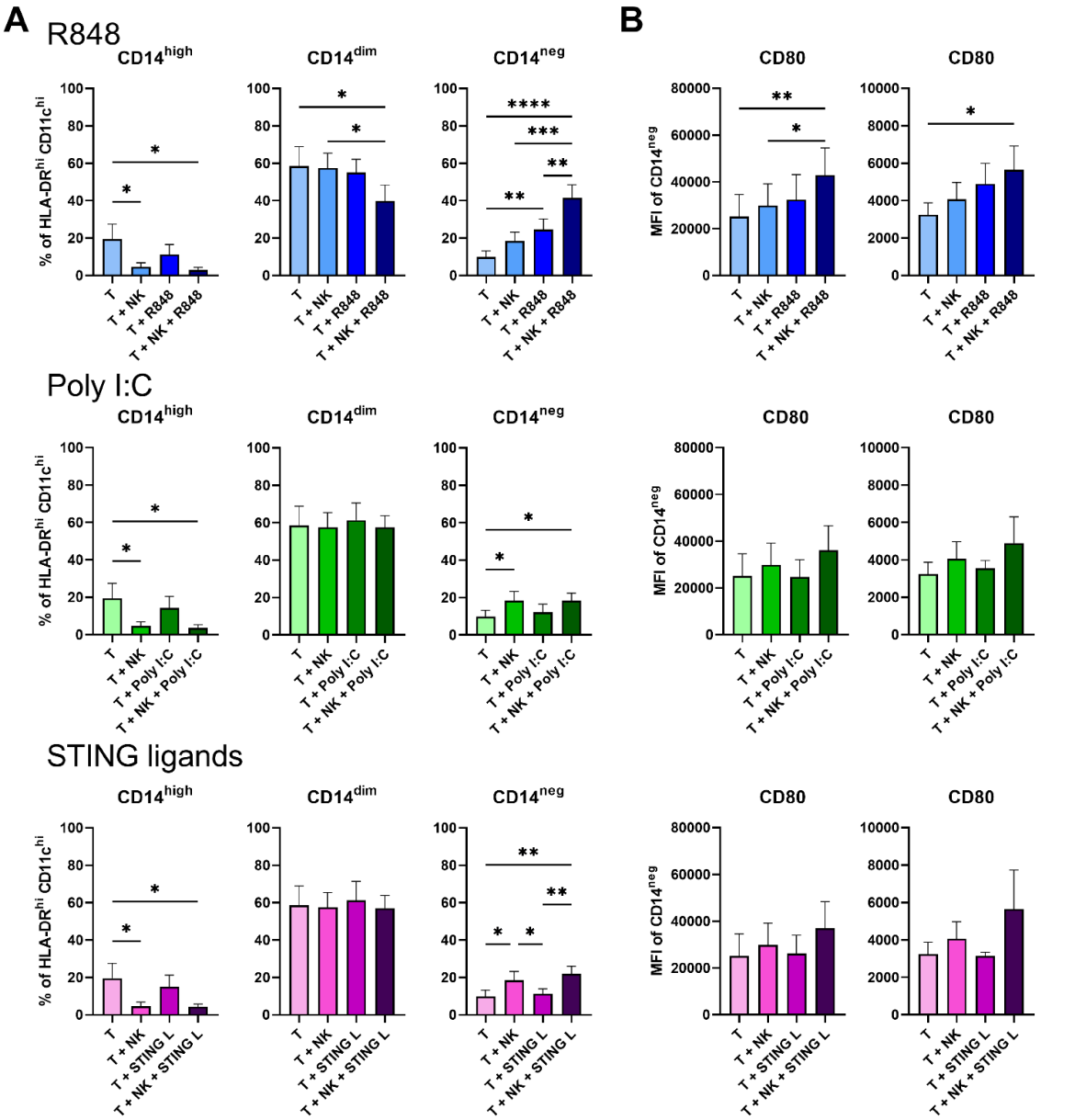

Figure S8: *The combination of NK cells with R848 induces a stronger monocyte-to-dendritic cell conversion and CD80 and CD80 upregulation on CD14^{neg} MoMC compared the combination of the NK cells with Poly IC or STING ligands after a 5-day co-culture with pCRC-SCS.*

(**A**) Changes in the percentages of CD14^{hi}, CD14^{dim}, CD14^{neg} (n=5) and (**B**) in the expression level (in Mean Fluorescence Intensity [MFI]) of CD80 and CD83 on CD14^{neg} cells (pCRC: *n*=4; 2 NK donors). Poly I:C (20 µL/mL, Invivogen, SanDiego CA). STING ligand (cyclic-di-AMP and rr-cyclic-di-AMP, 10 µL/mL Invivogen, San Diego CA). The data are presented as mean ± SEM. Significance is presented as *p* <0.05 *, <0.01 **, 0.001 ***, 0.0001 ****. *p*-values are determined by one-way ANOVA with Tukey multiple comparison analysis. Abbreviations: pCRC = primary colorectal cancer, T = tumor, NK = NK cells, STING L: STING ligands.

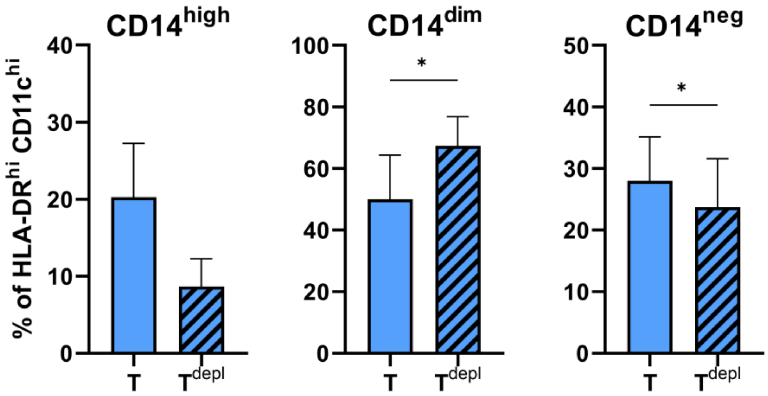

Figure S9: *Effects of tumor cell depletion on the myeloid compartment of dissociated pCRC samples.* Changes in the percentages of $CD14^{high}$, $CD14^{dim}$, $CD14^{neg}$ monocyte-derived myeloid cells after 5-day culture of dissociated pCRC or tumor-depleted dissociated pCRC samples. The myeloid cells were defined as $CD45^{+}HLA\text{-}DR^{high}CD11c^{high}$. *n* = 3; 2 NK donors. The data are presented as mean ± SEM. Significance is presented as $p < 0.05$ *. *p*-values are determined by two-tailed paired T-test. Abbreviations: T = tumor, T^{depl}: tumor depleted.

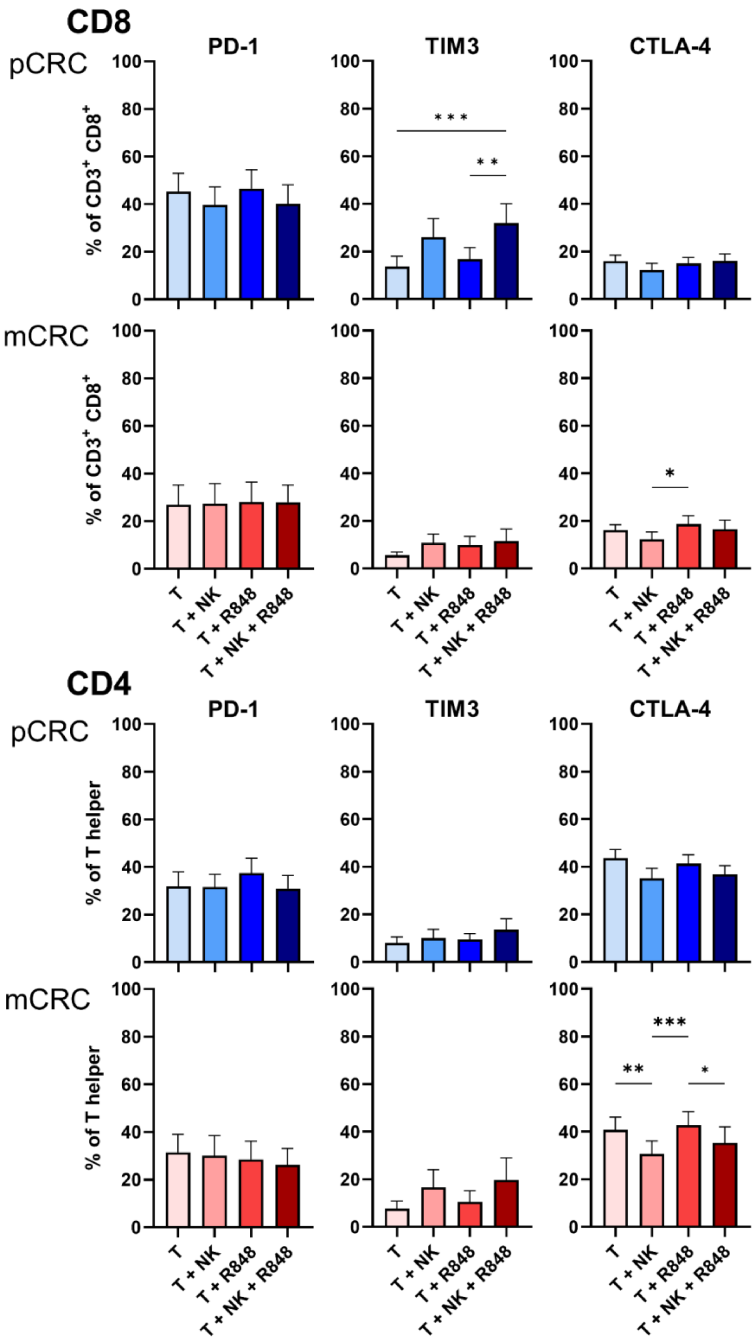

Figure S10: *Changes in the expression of immune checkpoints on CD8⁺ T cells and CD4⁺ T helper cells after 5-day culture of dissociated pCRC or mCRC samples in the presence and absence of NK cells and/or R848*. NK cell:SCS ratio: 1:1. pCRC: n = 9; 3 NK donors. mCRC: n = 7; 3 NK donors. The data are presented as mean ± SEM. *p*-values are determined by one-way ANOVA with Tukey multiple comparison analysis or Friedman test with Dunn multiple comparison analysis (pCRC and mCRC: TIM3). Abbreviations: pCRC = primary colorectal cancer, mCRC = metastatic colorectal cancer, T = tumor, NK = NK cells.

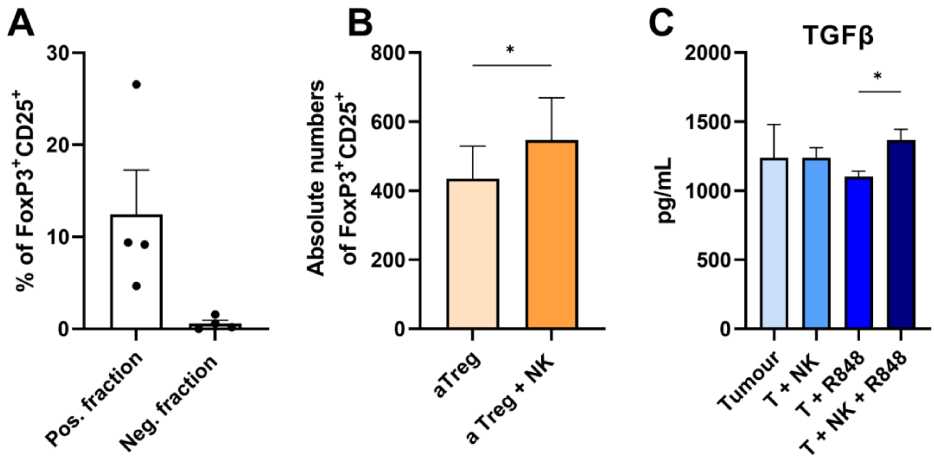

Figure S11: *Effect of NK cells on aTreg.* (**A**) Enrichement of aTreg (defined as FoxP3$^+$CD25$^+$CD45RA$^-$) of dissociated pCRC samples executed by performing multiple magnetic bead-activated cell sorting (MACS). First the tumor cells were depleted using CD326 (EpCAM) MicroBeads (Miltenyi Biotech, cat: 130-061-101). Next, CD4$^+$ T cells were isolated with CD4$^+$ T cell isolation kit (Miltenyi Biotech, cat: 130-096-533). Finally, CD25$^+$ CD4$^+$ T cells were isolated using CD25 Microbeads II (Miltenyi Biotech, cat: 130-092-983). All the MACS kit were used according to manufacturer's instructions. *n* = 4. (**B**) Absolute number of FoxP3$^+$CD25$^+$CD45RA$^-$ cells after 24h culture of aTreg enriched dissociated pCRC and NK cells. NK cell:SCS ratio 1:1. *n* = 3 pCRC and 2 NK donors. (**C**) TGFβ levels in the supernatant of dissociated pCRC samples cultured for 5 days in the presence and absence of NK cells and/or R848, determined with the TGF-β1 ELISA kit (R&D, cat: DY240). NK cell:SCS ratio: 1:1. *n* = 4. Significance is presented as p <0.05 *. p-values are determined by two-tailed paired T test (B) or one-way ANOVA with Tukey multiple comparison analysis (C). Abbreviations: T = tumor, aTreg = activated regulatory T cells, NK = NK cells, aTreg = activated regulatory T cells.

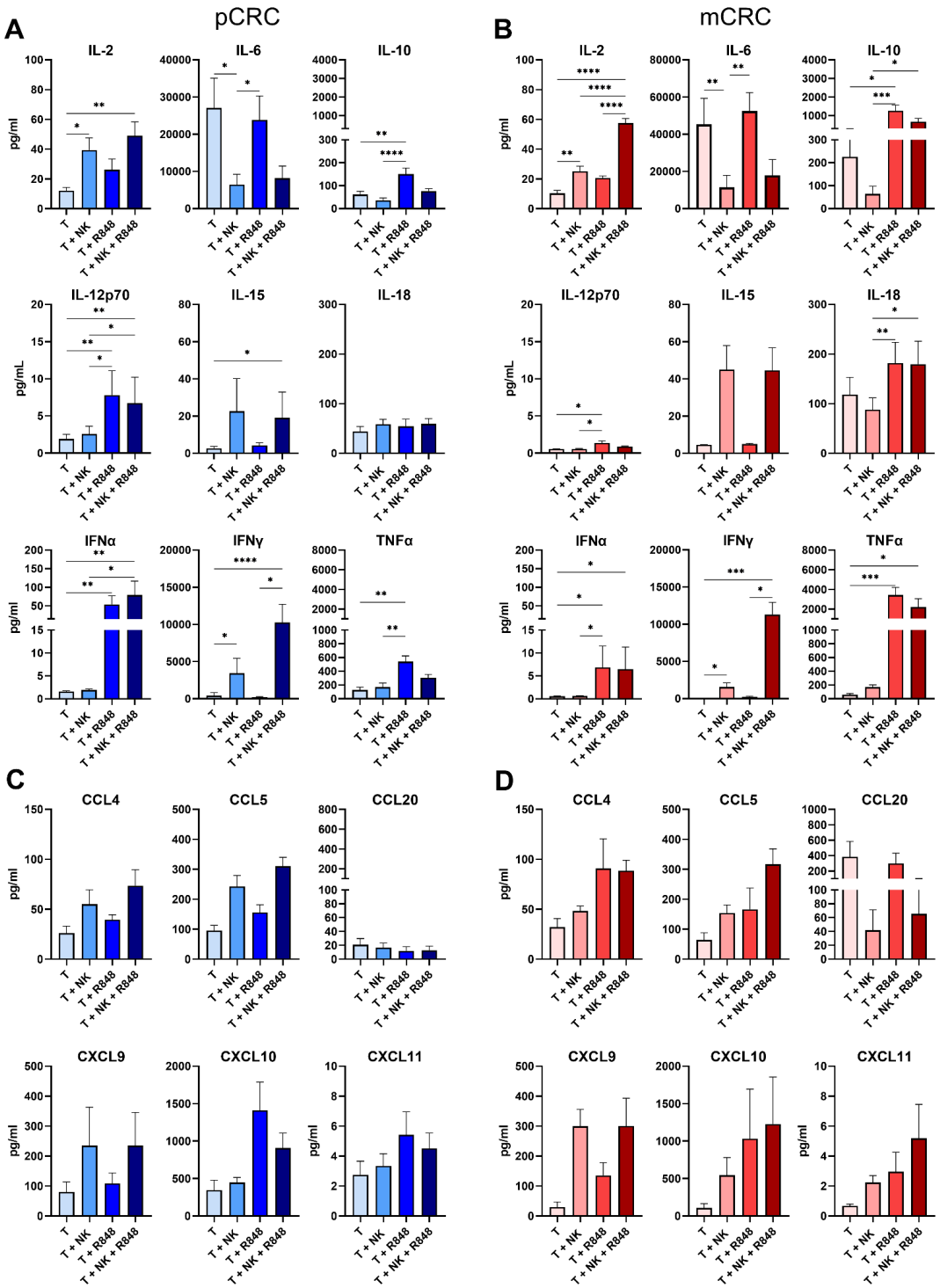

Figure S12 *Changes in the cytokine and chemokine profile upon a 2 day co-culture of pCRC- or mCRC-SCS with or without NK cells and/or R848*. Cytometry Bead Array performed on the supernatant of pCRC- or mCRC-SCS cultured for 2 days in the presence and absence of NK cells and/or R848. NK cell:SCS ratio 1:1. Cytokine levels: (**A**) pCRC- and (**B**) mCRC-SCS. Chemokine levels: (**C**) pCRC- and (**D**) mCRC-SCS. PCRC: *n* = 9; 3 NK donors (IL-6 *n* = 5), mCRC: *n* = 6; 3 NK donors. The data are presented as mean ± SEM. Significance is presented as *p* <0.05 *, <0.01 **, 0.001 ***, 0.0001 ****. *p*-values are determined by Friedman ANOVA with Dunn multiple comparison analysis or one-way ANOVA with Tukey multiple comparison analysis (pCRC: IL-2, CCL4, CCL5; mCRC: IL-2, CCL4). Abbreviations: pCRC = primary colorectal cancer, mCRC = metastatic colorectal cancer, T = tumor, NK = NK cells.