Supplementary Fig. S1 Gating strategy to identify MAIT cells. (a) Single cell suspensions were isolated from unaffected colon, colon tumors and peripheral blood, and frequencies of MAIT cells among CD3+ cells determined by flow cytometry. (b) Dot plots show one example of MAIT cell gating using a single cell suspension from a colon tumor. Live, singlet cells from a lymphocyte gate were further gated as CD45+CD3+ T cells, and then Vα7.2 and CD161 or MR1 tetramers were used to identify MAIT cells. (c) Distribution of CD8+ and DN MAIT cells in cell suspensions from unaffected colon, colon tumors and peripheral blood. Symbols represent individual values. * p<0.05, *** p<0.001, using the Friedman test followed by Dunn’s post test for multiple comparisons. n=41
Supplementary Fig. S2 PD-1 expression in tissue MAIT cells. Single cell suspensions were isolated from unaffected colon and colon tumors, and MAIT cells were analyzed for their expression of PD-1 by flow cytometry. (a) Mean fluorescence intensity (MFI) of PD-1 staining on MAIT cells from unaffected colon and colon tumors. (b) Representative histogram showing PD-1 expression on MAIT cells isolated from a colon tumor and the corresponding unaffected tissue. Symbols represent individual values and the line the median. ** p<0.01, n=15
Supplementary Fig. S3. Correlation between exhaustion markers in intratumoral MAIT cells. Single cell suspensions were isolated from colon tumors, and expression of Tim-3, PD-1, CD39, and Ki67 by CD8+ MAIT cells evaluated by flow cytometry. Graphs show correlation between percentage of Tim-3+ cells and percentage of (a) PD-1+, (b) CD39+, and (c) Ki67+ cells using linear regression. n=11-31
Supplementary Fig. S4 Frequencies of exhausted MAIT cells in relation to patient and tumor characteristics. Single cell suspensions were prepared from colon tumors, and frequencies of CD8⁺ MAIT cells co-expressing PD-1 and Tim-3 determined by flow cytometry in freshly isolated cells. n=26-31
Supplementary Fig. S5 Polyfunctionality in tumor-infiltrating MAIT cells. Single cell suspensions were isolated from colon tumors, and production of IFN-γ, IL-2, and TNF by MAIT cells evaluated in vitro by flow cytometry after polyclonal stimulation with PMA and Ionomycin. (a) Percentage of PD-1highTim-3+ and PD-1 Tim-3− MAIT cells from individual patients expressing 3 (red), 2 (yellow), 1 (green), or 0 (turquoise) of the analyzed cytokines illustrated as pie charts. (b) Frequencies of PD-1highTim-3+ (white bars) and PD-1 Tim-3− (grey bars) MAIT cells expressing 3, 2, 1 or 0 of the analyzed effector molecules, shown as the percentage of total MAIT cells. (c) Frequencies of PD-1highTim-3+ (white bars) and PD-1 Tim-3− (grey bars) MAIT cell subsets expressing a combination of IFN-γ, IL-2, and TNF shown as the percentage of total MAIT cells. Symbols represent individual values and bars the mean. *p<0.05 using Wilcoxon matched-pairs signed rank test, n=7p
Supplementary Fig. S6 Representative flow cytometry analysis showing the expression of PD-L1 and PD-L2 among putative monocytes/immature macrophages (CD14+CD11c+) and dendritic cells (CD14 CD11c+) gated from live CD45^+CD3^-CD19^- single cells isolated from a colon tumor. Gates are set according to FMO controls gated from the respective cell suspensions.
**Supplementary Fig. 7** MAIT cell activation after PD-1 blocking. Single cell suspensions were isolated from unaffected colon, colon tumors and peripheral blood, and stimulated with THP-1 cells pre-incubated with *E. coli* in the presence or absence of blocking antibodies to PD-1. MAIT cell expression of HLA-DR (a) and CD38 (b) was evaluated by flow cytometry. Symbols represent individual values, and are connected to show corresponding values in the same individuals.