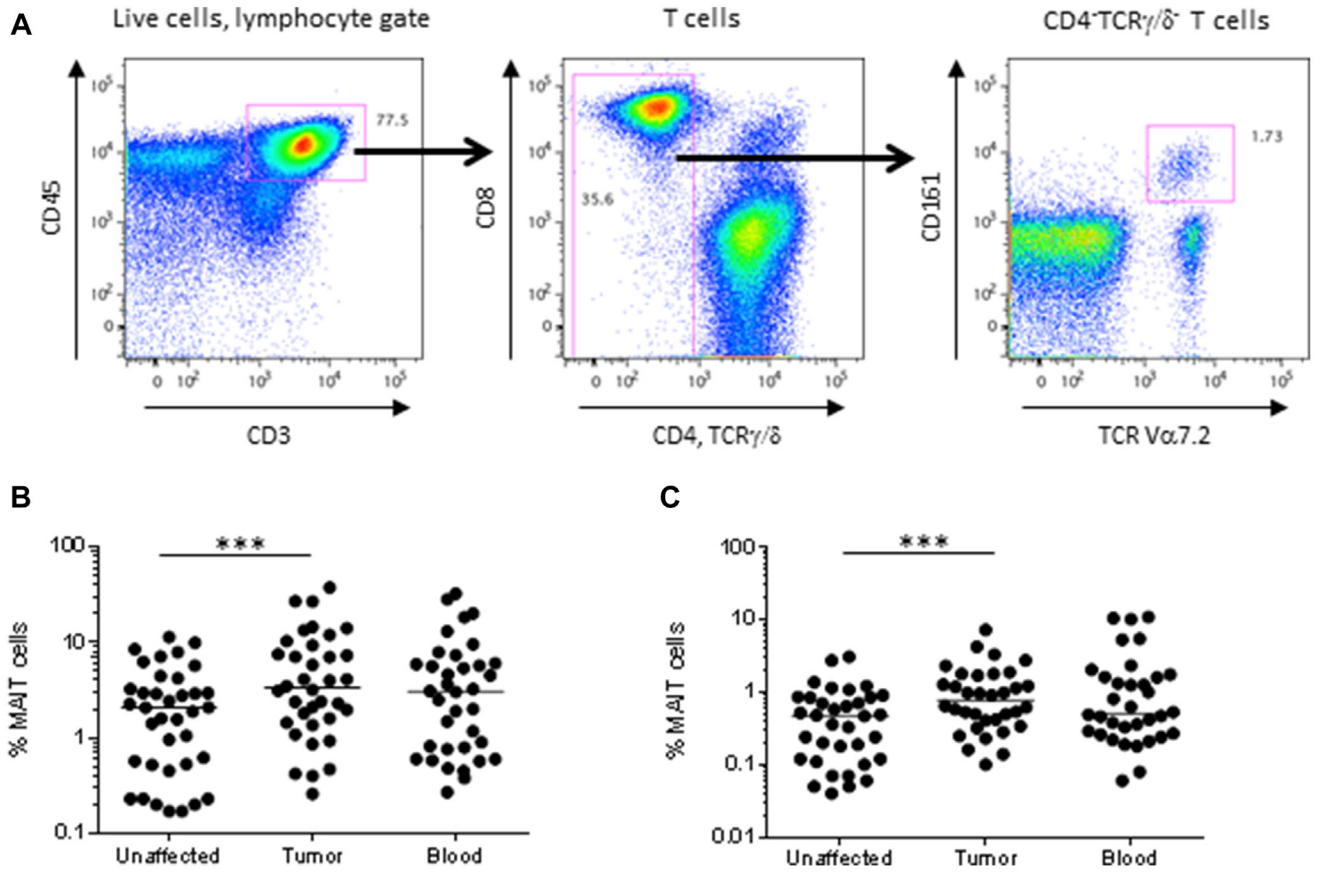
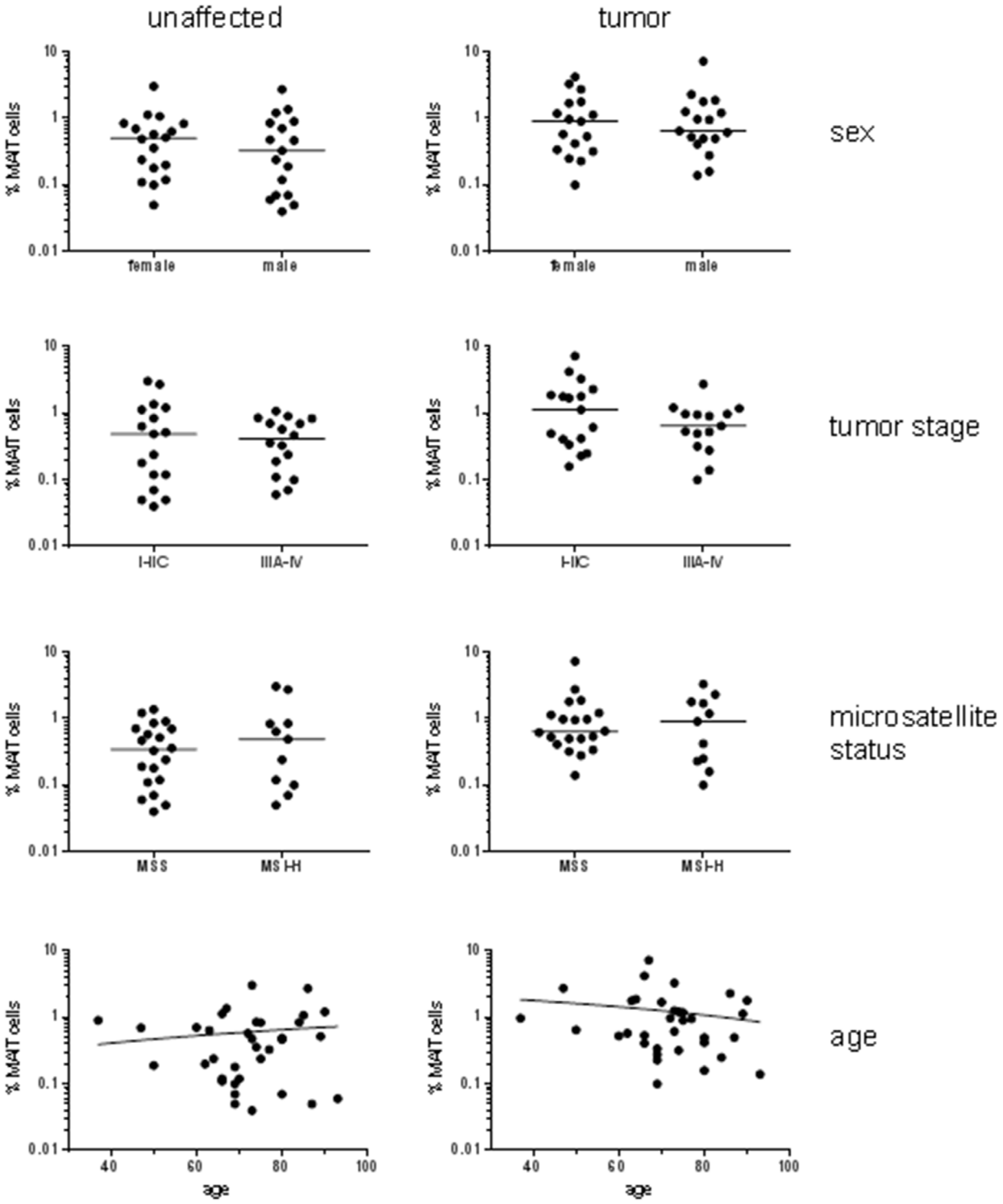
Tumor-infiltrating mucosal-associated invariant T (MAIT) cells retain expression of cytotoxic effector molecules

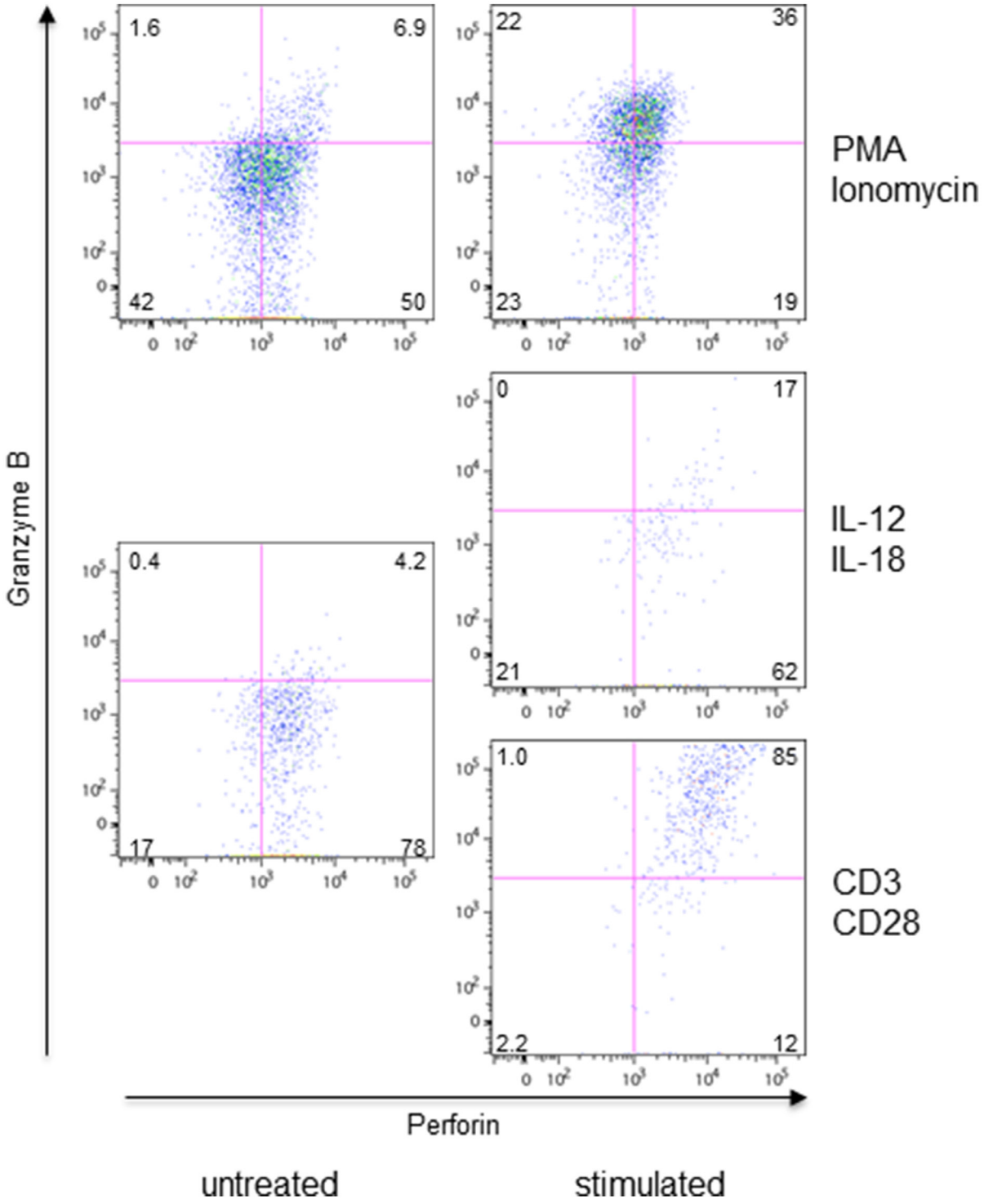
SUPPLEMENTARY MATERIALS



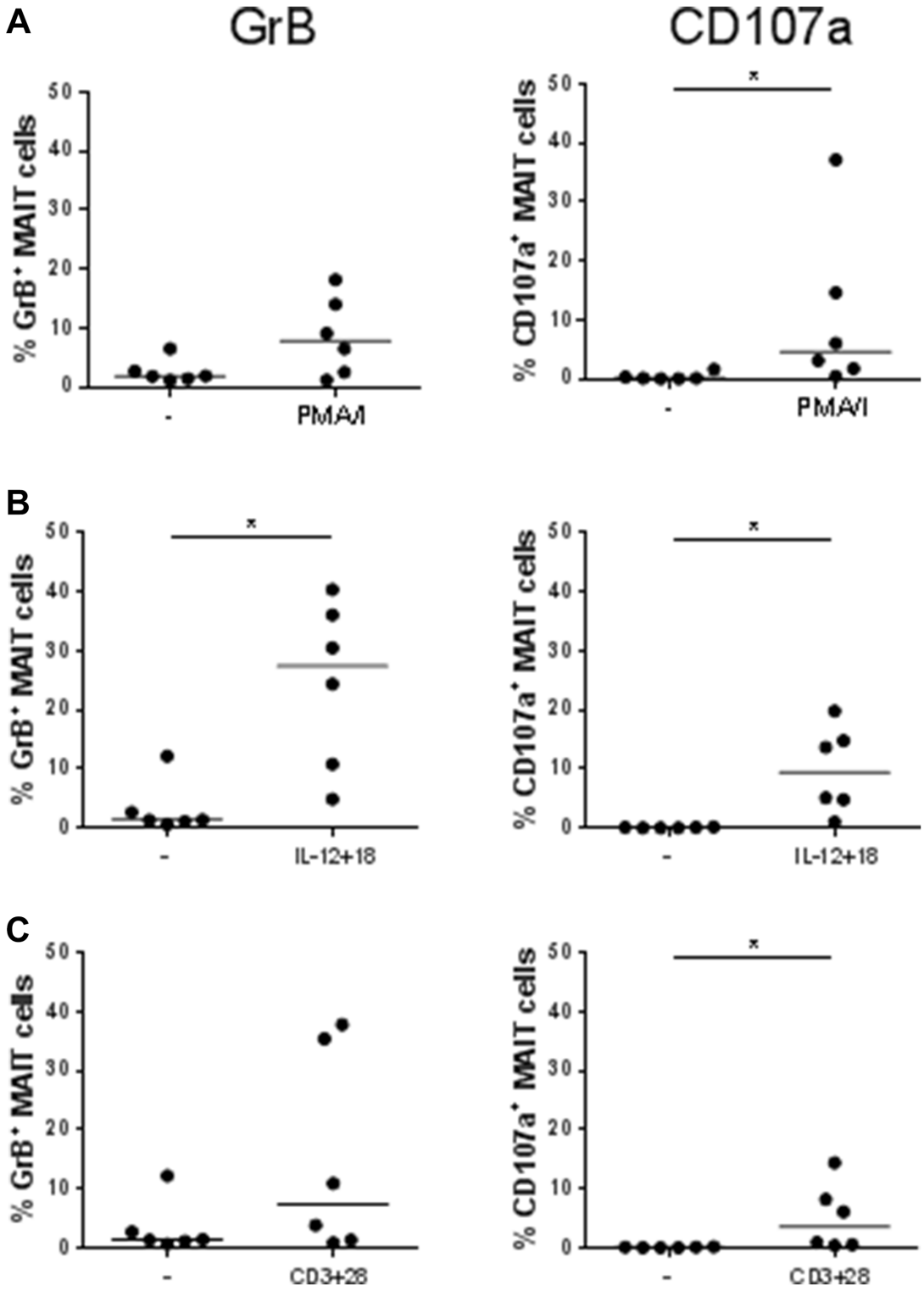
Supplementary Figure 1: Gating strategy to identify MAIT cells. (**A**) Dot plot shows one example of MAIT cell gating using a single cell suspension from a tumor. Live cells from a lymphocyte gate were further gated as $CD45^{+}CD3^{+}$T cells, and then $CD8^{+}$ and double negative T cells were used to determine the frequency of $Va7.2^{+}CD161^{high}$ MAIT cells. (**B**) Frequencies of MAIT cells among $CD8^{+}$ and double negative T cells and (**C**) among all $CD3^{+}$T cells isolated from unaffected colon, tumor tissue and peripheral blood. Symbols represent individual values and the line the median $^{***}P < 0.001$, $n = 35$.



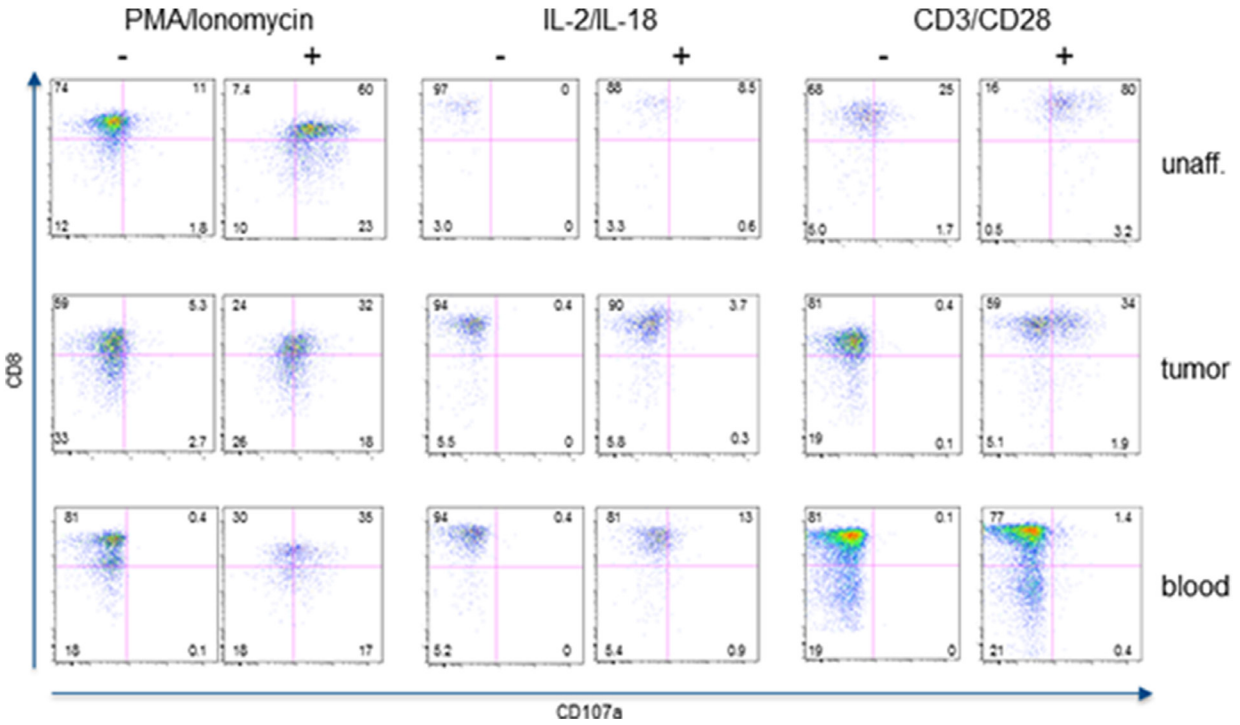
Supplementary Figure 2: MAIT cell frequencies in relation to sex, tumor stage, microsatellite instability and age. Single cell suspensions were prepared from unaffected colon tissue and tumors from the same patients, and the frequencies of MAIT cells in relation to all $CD3^+$ cells determined by flow cytometry. The other variables were retrieved from the pathology report $n = 35$.



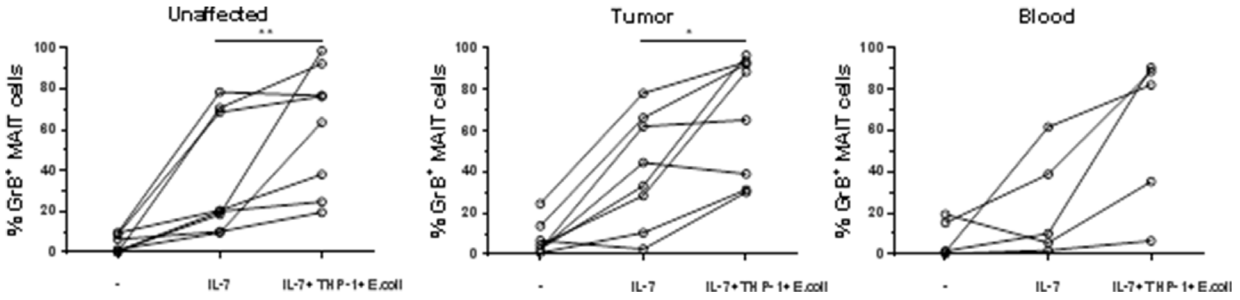
Supplementary Figure 3: Representative FACS plots showing Granzyme B and Perforin staining in MAIT cells from colon tumors. Single cell suspensions were isolated from colon tumors, and stimulated with PMA and Ionomycin, IL-12 and IL-18, or anti-CD3 and anti-CD28. MAIT cells were gated as in supplementary Figure 1, and expression of GrB and Perforin was examined by flow cytometry in untreated and stimulated cells.



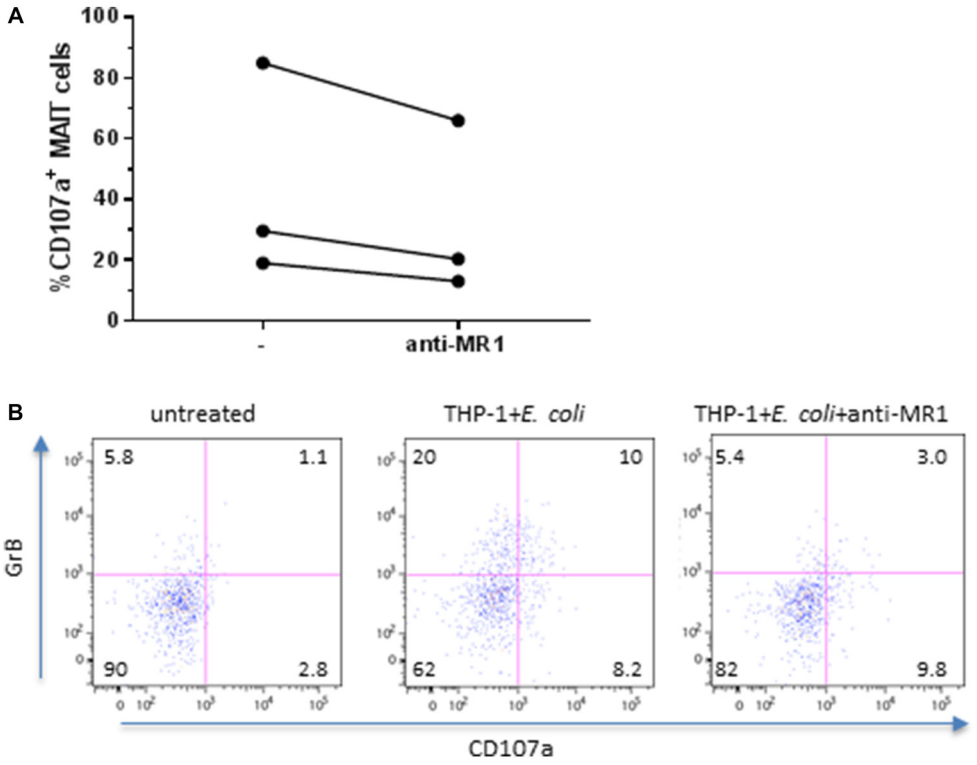
Supplementary Figure 4: Frequencies of GrB$^+$ and CD107a$^+$ circulating MAIT cells from healthy volunteers. Single cell suspensions were isolated from peripheral blood from healthy volunteers and stimulated with (**A**) PMA and lonomycin, (**B**) IL-12 and IL-18, or (**C**) anti-CD3 and anti-CD28. Expression of GrB and CD107a was examined by flow cytometry. Symbols represent individual values and the line the median. $n = 6$ $^*p < 0.05$.



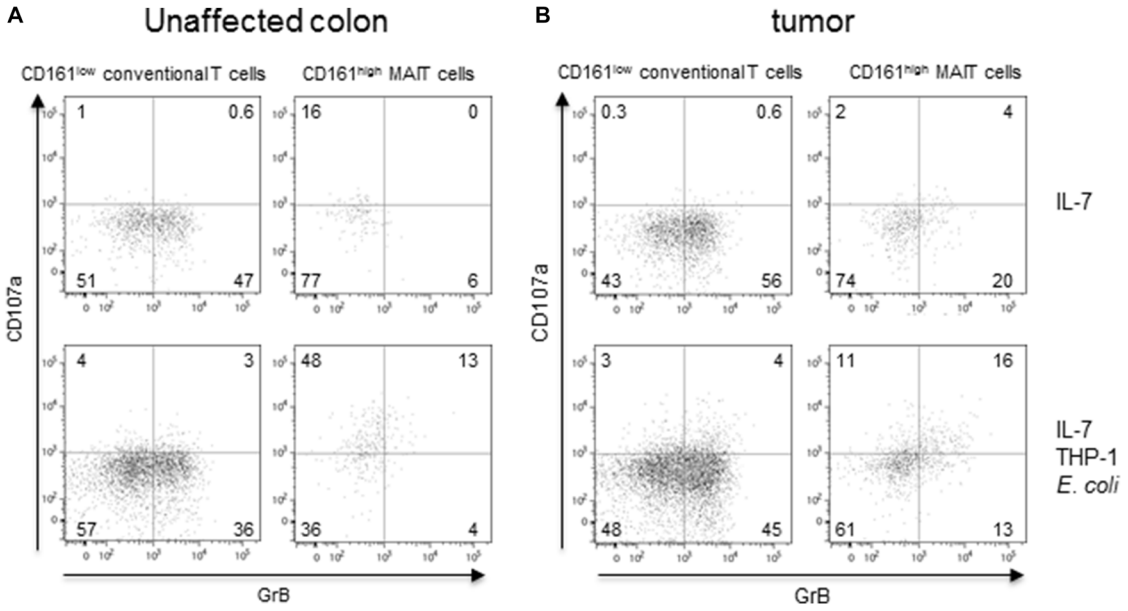
Supplementary Figure 5: Degranulation of MAIT cells after stimulation. Single cell suspensions were isolated from unaffected colon, colon tumors and peripheral blood, and stimulated with PMA and Ionomycin, IL-12 and IL-18, or anti-CD3 and anti-CD28. Expression if CD107a was examined by flow cytometry.



Supplementary Figure 6: Frequencies of GrB$^+$ MAIT cells after antigen stimulation. Single cell suspensions were isolated from unaffected colon, colon tumors and peripheral blood, and pre-incubated with IL-7 for 48 hours. They were then either left in IL-7 alone or co-cultured with THP-1 cells pre-incubated with fixed E. coli for 4 hours. Expression of GrB was examined by flow cytometry. Symbols represent individual values. $n = 5–8$ $^{*}p < 0.05$, $^{**}p < 0.01$.



Supplementary Figure 7: Degranulation of MAIT cells in the presence of antibodies to MR1. Single cell suspensions were isolated from colon tumors and pre-incubated with IL-7 for 48 hours. They were then stimulated for 18 hours with THP-1 cells pre-incubated with fixed *E. coli* in the presence or absence of 10 mg/ml of monoclonal antibody to MR1. Expression of CD107a on MAIT cells was examined by flow cytometry. Symbols represent individual values from 3 different donors (**A**), and dot plots (**B**) show representative staining of MAIT cells isolated from a colon tumor and left unstimulated, or stimulated in the absence of presence of anti-MR1.



Supplementary Figure 8: Degranulation by isolated colonic MAIT cells. $CD8^+$ T cells were isolated by negative magnetic bead separation of single cell suspensions from unaffected colon and colon tumors, and incubated with IL-7 for 40 hours. $V\alpha7.2^+$ cells were then isolated by positive magnetic bead separation and co-cultured with THP-1 cells, which had previously been pre-incubated with fixed *E. coli* for four hours. Expression of GrB and CD107a on $Va7.2^+CD161^{high}$ and $Va7.2^+CD161^{low}$ T cells isolated from (**A**) unaffected colon and (**B**) tumor tissue was determined by flow cytometry in cells only pre-incubated with IL-7 or co-cultured with *E. coli*-loaded THP-1 cells. One representative experiment out of three is shown.

Supplementary Table 1: Characteristics of the colon cancer patients included in the study

		females	males
	n	19	16
	age	47–92	37–93
Tumor location	ascending	10	11
	transverse	5	2
	descending	4	3
TNM stage	I	4	4
	IIA	5	4
	IIB	1	-
	IIC	-	-
	IIIA	-	-
	IIIB	5	5
	IIIC	4	3
	IV	-	-
Microsatellite stability	MSS	8	14
	MSI-H	9	2
	unknown	2	-