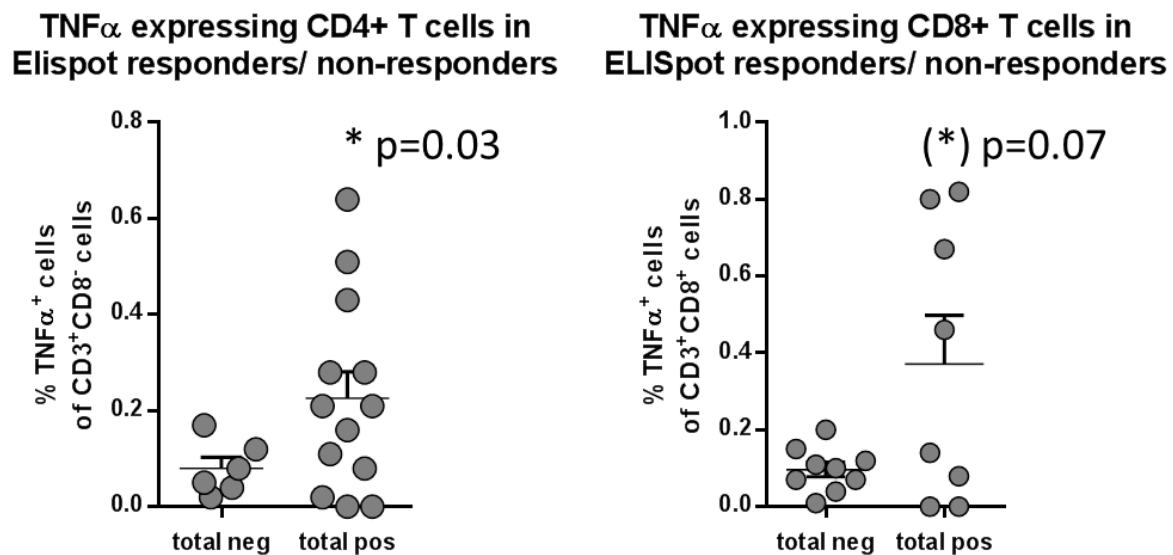
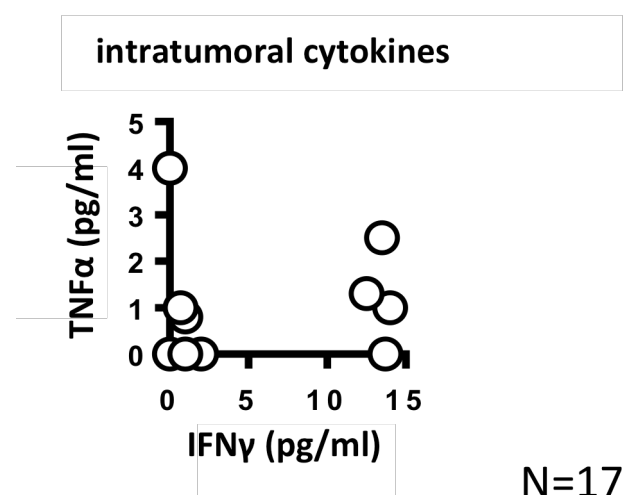


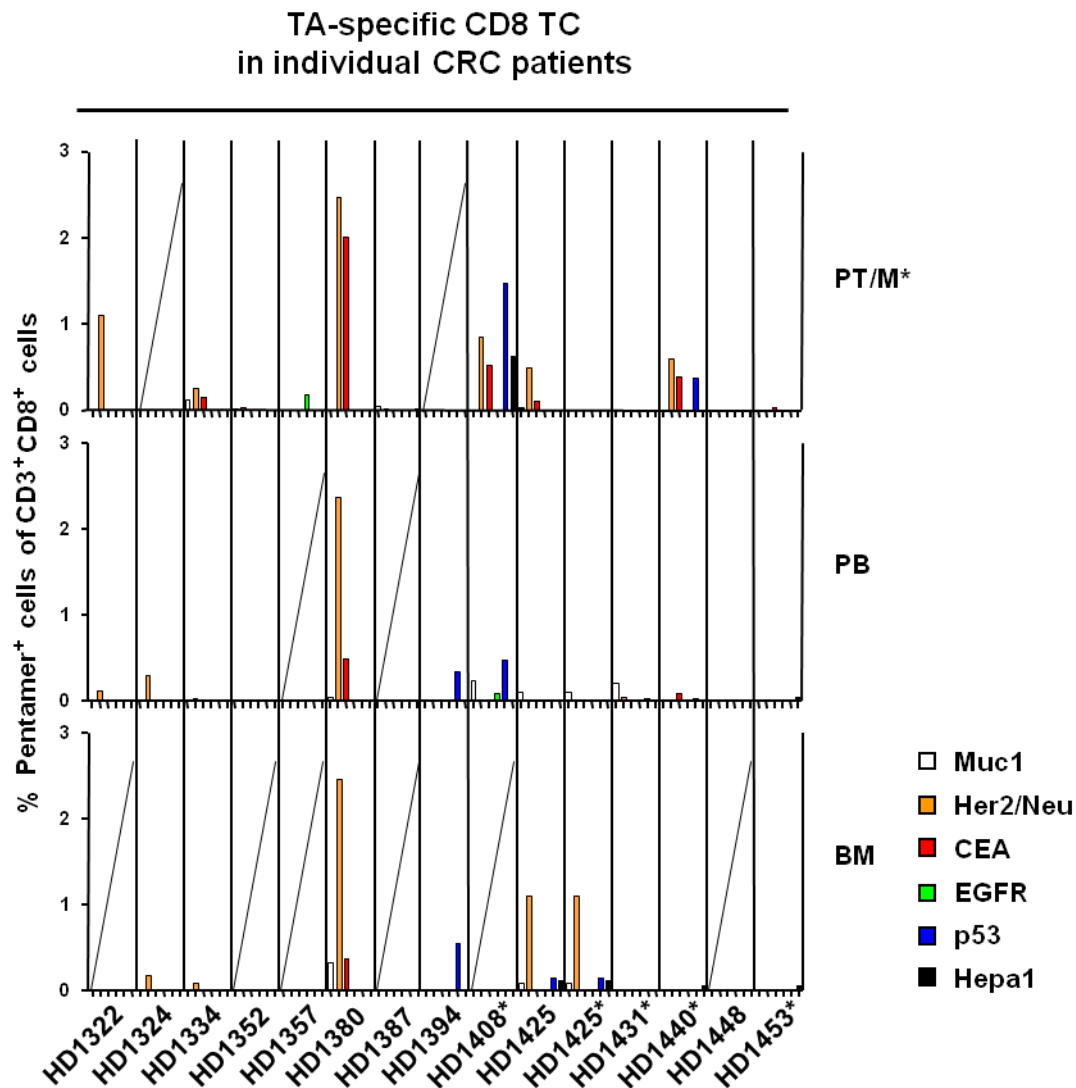
Supplemental Material



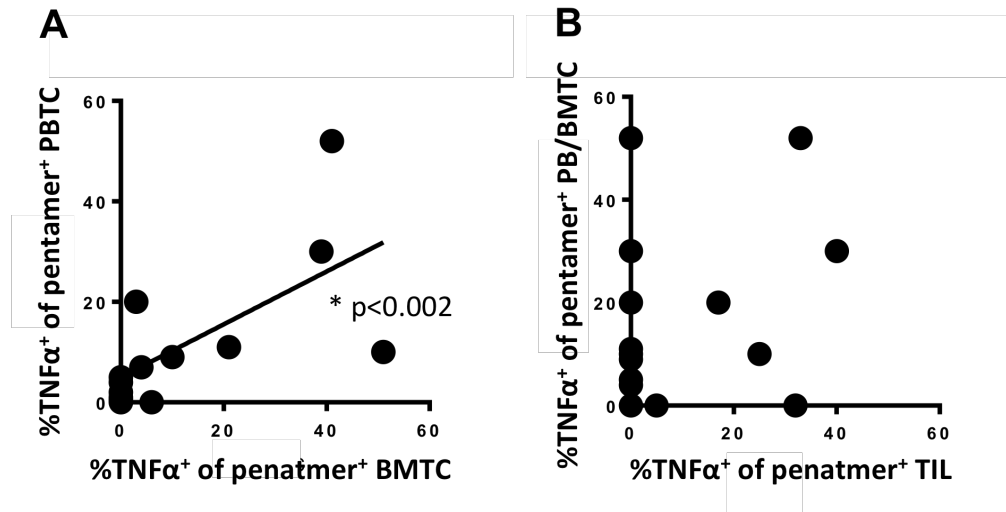
Supplemental Figure S1. Frequency (%) of TNF α expressing CD4+ (left graph) or CD8+ (right graph) TIL from patients having (total pos; n=13 (CD4⁺) and n=8 (CD8⁺)) or lacking (total neg; n=6 (CD4⁺) and n=9 (CD8⁺)) tumor reactive T cells in their blood or bone marrow as determined by IFN-gamma ELISpot analysis. Mean + SEM are indicated by black lines and error bars, respectively. Statistical difference is indicated by *; (p<0.05) as determined by paired student's T test.



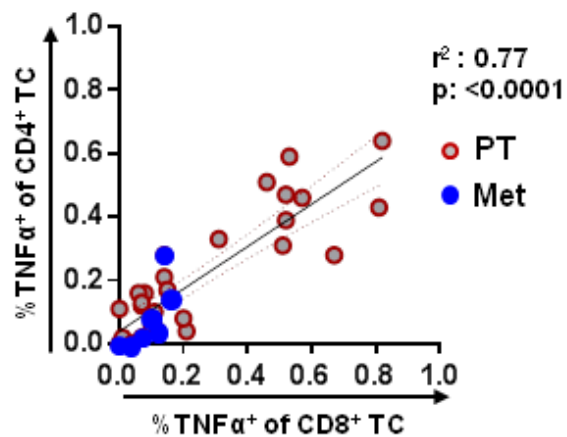
Supplemental Figure S2. Relation among intratumoral TNF α and IFN γ expression in individual CRCs as estimated by ELISA of tumor lysates of CRC tissues from 17 different patients (white circles). No direct correlation was observed.



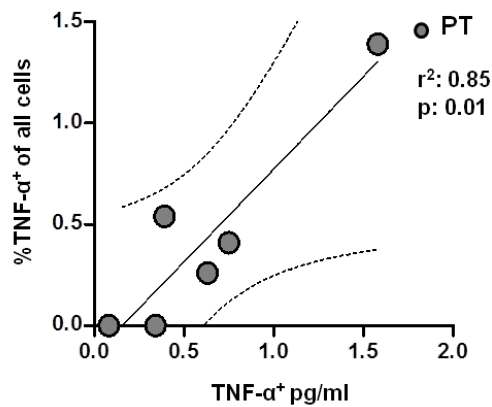
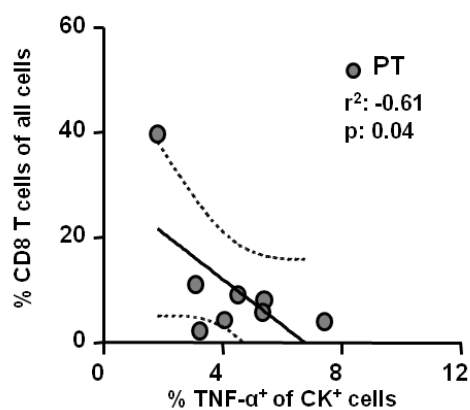
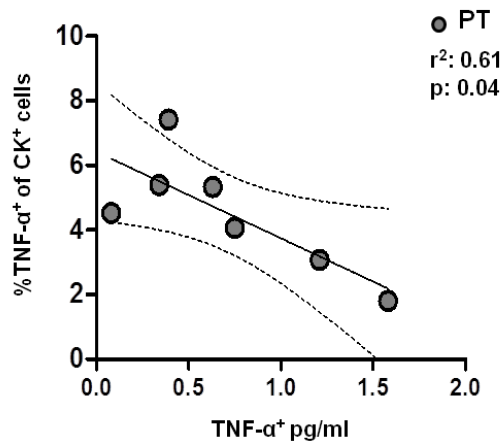
Supplemental Figure S3. Proportions of TA-specific CTL among CD8⁺ T cells in primary (PT) or metastatic (M) tumor tissue, peripheral blood (PB) or bone marrow (BM) of 15 individual CRC patients. CTL frequencies are individually shown for different TAs by different colouring as indicated in the figure legend. *, represent patients with metastatic CRC.



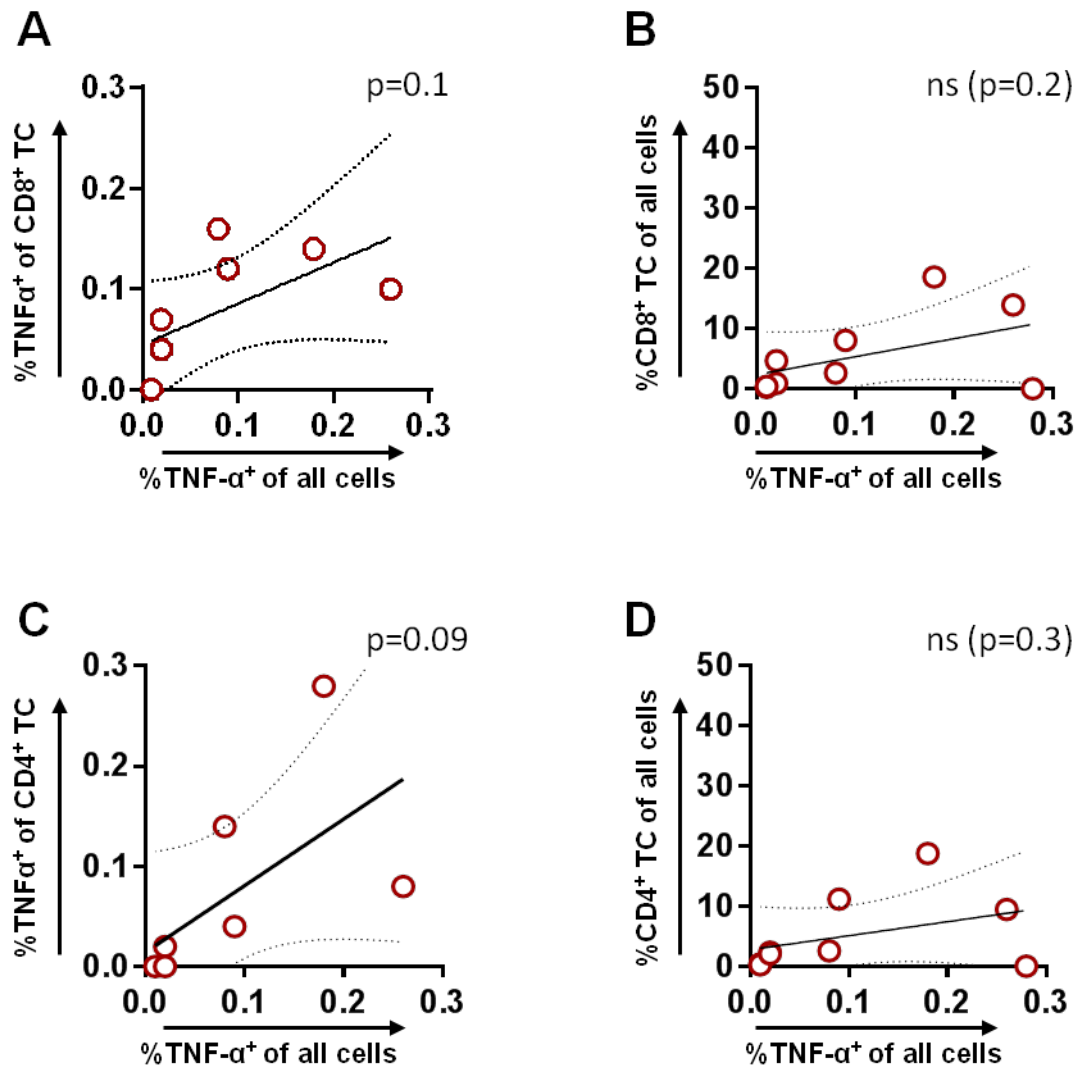
Supplemental Figure S4. **A.** Correlation of the frequency of TNF α ⁺ antigen specific TCs in the blood and bone marrow of 22 CRC patients as determined by pentamer staining. Best fit line of the linear regression and p-value are shown. **B.** Correlation between the frequency of TNF α ⁺ antigen specific TIL and T cells in the periphery of CRC patients (n=22). No direct correlation was observed.



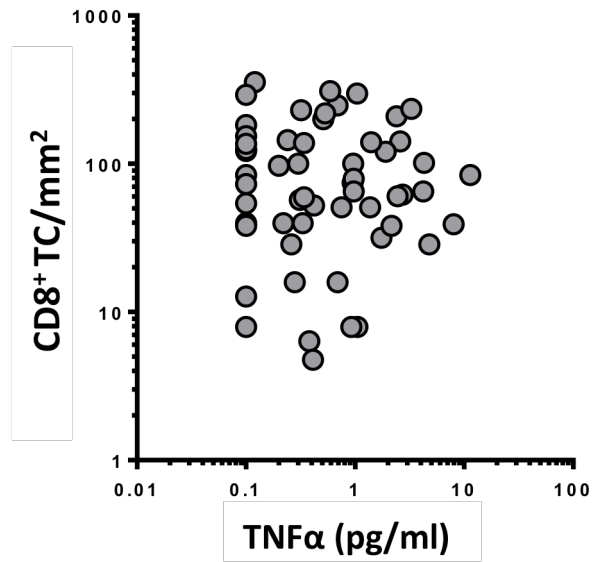
Supplemental Figure S5. Linear regression curve showing proportions of TNF α expressing T cells among CD4⁺ or CD8⁺ T cells in primary CRC (PT; red bordered grey circles) or CRC metastases (met; blue circles). Dots represent data from individual tumors. Best fit line and confidence bands indicating the 95% confidence interval are shown by full and dotted lines, respectively. r^2 ; correlation coefficient.

A**B**

Supplemental Figure S6. A, B. Linear regression curves demonstrating that TNFα protein levels in primary CRCs positively correlate with frequencies of total TNFα expressing tumor infiltrating cells (A) but show an inverse correlation with frequencies of TNFα cytokeatin (CK) expressing tumor cells (B). C. Linear regression curve demonstrating an inverse correlation between proportions of CTL among tumor infiltrating cells and proportions of TNFα expressing tumor cells. Dots represent data from individual tumors. Best fit lines and confidence bands indicating the 95% confidence interval are shown by full and dotted lines, respectively. r^2 : correlation coefficient.



Supplemental Figure S7. Proportions of $\text{TNF}\alpha$ positive among CD8^+ (A) or CD4^+ (C) TIL and of total CD8^+ (B) or CD4^+ (D) among all tumor infiltrating cells compared to the proportions of $\text{TNF}\alpha$ positive among all tumor infiltrating cells (A-D). Circles represent individual data from 9 different CRC metastases. Strength and significance of respective correlations are indicated by r^2 and p values.



Supplemental Figure S8. Correlation between the number of CD8⁺ TIL in CRC tumors and the level of TNFα expression in tumor tissue as evaluated by ELISA (n=56). No direct correlation was observed.

Supplemental Table 1

Infiltration of Treg (regulatory T cell) and CD4⁺ in colorectal cancer tissue (CRC) and healthy colon tissue (HT) in the different Tumor invasion (T), lymph node infiltration (N), metastasis (M) and UICC stages of 101 patients. Numbers are demonstrated as median and IQR per high power field. *: primary CRC, only

	Treg				CD4 ⁺			
	CRC	p-value	HT	p-value	CRC	p-value	HT	p-value
T								
2	3.5 (1.5 - 6.5)		0 (0 - 0.1)		6.4 (3.7 - 8.8)		5 (2.1 - 47)	
3	1.5 (0.4 - 2.9)	0.07	0.1 (0.1 - 0.1)	n.s.	5.2 (3 - 7.5)	n.s.	5.7 (4.3 - 7.3)	n.s.
4	1.5 (0.6 - 3.5)		0.1 (0 - 0.1)		5.4 (3.8 - 8.8)		3.7 (3.2 - 6.4)	
N								
0	2 (0.8 - 3.3)		0.1 (0 - 0.1)		6.5 (3.9 - 8.8)		5.7 (4.2 - 8.1)	
1	0.8 (0.3 - 2.8)	0.03	0.1 (0.1 - 0.1)	n.s.	4.7 (2.7 - 6.6)	n.s.	5.1 (3.5 - 6.9)	n.s.
2	0.7 (0.2 - 2.7)		0.1 (0 - 0.1)		4.4 (2.3 - 7.5)		4.9 (3.5 - 6.9)	
M								
0	2 (0.8 - 3.3)		0.1 (0.1 - 0.1)		6.5 (4.3 - 8.8)		5.6 (4.3 - 7.5)	
1	0.2 (0.1 - 0.9)	0.001	0	0.001	2 (1.8 - 3.7)	0.001	3.8 (2.6 - 5.9)	0.04
UICC								
2	2.3 (1.5 - 3.5)		0.1 (0.1 - 0.1)		6.9 (4.5 - 9.3)		6.2 (4.5 - 8.8)	
3	1.4 (0.4 - 3)	0.001	0.1 (0.1 - 0.1)	0.001	6 (4.2 - 7.8)	0.001	5.2 (4.3 - 6.9)	0.06
4	0.2 (0.1 - 0.9)		0		2 (1.8 - 3.7)		3.8 (2.6 - 5.9)	

Supplemental Table 2

TNF α and TGF β values in colorectal cancer tissue (CRC) and healthy colon tissue (HT) in the different Tumor invasion (T), lymph node infiltration (N), metastasis (M) and UICC stages of 101 patients. Numbers are demonstrated as median + IQR. * primary CRC only

	TNF α				TGF β			
	CRC	p-value	HT	p-value	CRC	p-value	HT	p-value
T								
2	0.34 (0.02 - 4.3)		0.04 (0.03 - 0.05)		638 (331 - 765)		144 (38 - 238)	
3	0.32 (0.07 - 1)	n.s.	0 (0 - 0.08)	n.s.	445 (228 - 660)	0.04	76 (53 - 133)	n.s.
4	0.64 (0.3 - 1.6)		0.02 (0 - 0.08)		740 (442 - 1107)		76 (55 - 164)	
N								
0	0.27 (0.05 - 1)		0 (0 - 0.02)		371 (202 - 772)		82 (52 - 137)	
1	0.4 (0.1 - 0.9)	n.s.	0.06 (0.02 - 0.19)	0.001	599 (265 - 767)	n.s.	79 (56 - 136)	n.s.
2	0.6 (0.2 - 1.6)		0.04 (0 - 0.7)		583 (382 - 743)		70 (53 - 139)	
M								
0	0.3 (0.05 - 1)		0 (0 - 0.04)		463 (259 - 743)		67 (52 - 106)	
1	0.7 (0.3 - 2.8)	0.009	0.08 (0.08 - 0.4)	0.001	587 (252 - 796)	n.s.	144 (119 - 164)	0.001
UICC								
2	0.2 (0.01 - 0.8)		0 (0 - 0.2)		441 (235 - 764)		78 (51 - 116)	
3	0.4 (0.07 - 1)	0.02	0.03 (0.03 - 0.16)	0.001	524 (333 - 733)	n.s.	59 (53 - 81)	0.001
4	0.7 (0.3 - 2.8)		0.08 (0.08 - 0.4)		587 (252 - 796)		144 (119 - 164)	

Supplemental Table 3

Infiltration of mast cells and CD8⁺ in colorectal cancer tissue (CRC) and healthy colon tissue (HT) in the different Tumor invasion (T), lymph node infiltration (N), metastasis (M) and UICC stages of 101 patients. Numbers are demonstrated as median and IQR per high power field. * primary CRC only

	Mastcell				CD8			
	CRC	p-value	HT	p-value	CRC	p-value	HT	p-value
T								
2	27.4 (11.1 – 34.7)				130 (59.8 – 301.1)			
3	33.3 (19 – 49.2)	0.06.	72.6 (54.1 - 101)	0.8.	79.3 (39.6 – 153.9)	0.6	19.1 (9.5 – 42.8)	0.3
4	17.4 (7.9 – 40.4)		64.2 (45.2 - 114)		74.6 (15.8 – 211.9)		14.3 (0 – 52.3)	
N								
0	33.3 (15.5 – 52.4)		76.2 (55.9 - 113)		99.2 (42.1 – 195.6)		14.3 (8.3 – 45.2)	
1	23 (11.5 – 45.6)	0.4	60.7 (44.6 – 101)	0.5	90.5 (51.1 – 132.9)	0.2	19.1 (11.9 – 40.5)	0.7
2	29.5 (15.8 - 46)		70.2 (47.6 – 101)		57.1 (31.7 – 144.4)		17.8 (13.1 – 45.2)	
M								
0	33.3 (15.8 – 52.4)		73.8 (55.9 – 105)		83.3 (36.5 – 162.3)		19.1 (9.5 – 45.2)	
1	25 (12.7 – 36.5)	0.06	57.1 (26.2 - 105)	0.3	65 (39.7 – 146)	0.9	7.1 (4.7 – 16.1)	0.04
UICC								
2	33.3 (14.3 – 52.3)		85.7 (58.3 – 113)		82.5 (26.9 - 192)		16.6 (9.5 – 47.6)	
3	36.5 (19 – 49.2)	0.2	63.1 (47.6 – 99.4)	0.2	84.1 (38.1 – 144.4)	0.9	20.2 (14.3 – 41.7)	0.1
4	25 (12.7 - 36.5)		57.1 (26.1 – 105)		65.1 (39.7 - 146)		7.1 (4.7 – 16.1)	

Supplemental Table 4A

Correlation of general patient characteristics with CD4.

	CD4 CRC Median (range)	p-value	CD 4 HT Median (range)	p-value
Gender				
Male	5.1 (3.4 – 8.6)	0.92	5.5 (4.2 – 8)	0.84
Female	5.3 (3 – 7.8)		5.2 (3.7 – 7)	
Age				
≤ 60 years	4.9 (2.7 – 8.6)	0.73	5.4 (3.5 – 6.9)	0.70
> 60 years	5.3 (3.5 – 7.6)		5.3 (4.1 – 7.4)	
tumor localisation				
colon ascendens	5.1 (3.1 – 6.3)	0.77	4.9 (2.9 – 5.8)	0.07
c. transversum	5.8 (3.5 – 9.9)		10.1 (6.7 – 14.4)	
c. descendens	5.4 (4.5 – 7.1)		4.7 (3.7 – 5.7)	
c. sigmoideum	6.5 (3.1 – 9.2)		4.6 (3.3 – 9.4)	
rectum	5.1 (2.8 – 8.7)		6 (4.4 – 7.1)	

Supplemental Table 4B

Correlation of general patient characteristics with CD8.

	CD8 CRC Median (range)	p-value	CD 8 HT Median (range)	p-value
Gender				
Male	84.1 (39.6 – 172.6)	0.47	20.2 (9.5 – 44)	0.30
Female	65 (34.9 – 142)		16.6 (4.7 – 33.3)	
Age				
≤ 60 years	91.2 (48 – 194)	0.22	19 (13 – 34.5)	0.94
> 60 years	66.6 (28.5 – 146)		19 (9.5 – 47.6)	
tumor localisation				
colon ascendens	60.3 (22.2 – 137.3)	0.49	7.1 (4.7 – 19.6)	0.23
c. transversum	55.5 (47.6 – 151.1)		29.7 (14.8 – 42.8)	
c. descendens	59.5 (26.9 – 230.9)		21.4 (1.7 – 40.4)	
c. sigmoideum	79.3 (33.3 – 141.2)		23.8 (10.7 – 57.1)	
rectum	98.4 (50.7 – 199.2)		19 (10.7 – 47.6)	

Supplemental Table 4C

Correlation of general patient characteristics with Treg.

	Treg CRC Median (range)	p-value	Treg HT Median (range)	p-value
Gender				
Male	1.5 (0.6 – 3)	0.59	0.1 (0 – 0.1)	0.47
Female	1.6 (0.4 – 3.2)		0.1 (0.1 – 0.1)	
Age				
≤ 60 years	1.1 (0.5 – 2.9)	0.58	0.1 (0.07 – 0.1)	0.54
> 60 years	1.8 (0.5 – 3.1)		0.1 (0 – 0.1)	
tumor localisation				
colon ascendens	1.6 (0.4 – 2.2)	0.98	0.1 (0 – 0.1)	0.68
c. transversum	1.2 (0.4 – 3.6)		0.1 (0 – 0.1)	
c. descendens	2 (0.5 – 3.3)		0.1 (0.07 – 0.1)	
c. sigmoideum	2 (0.6 – 3)		0.1 (0.1 – 0.1)	
rectum	1.3 (0.4 – 3.3)		0.1 (0 – 0.1)	

Supplemental Table 4D

Correlation of general patient characteristics with Mast cells.

	Mast cells CRC Median (range)	p-value	Mast cells HT Median (range)	p-value
Gender				
Male	36.5 (19 – 52.4)	0.08	77.3 (57.1 – 106.5)	0.17
Female	23.8 (11.1 – 42)		57.1 (47.6 – 104.7)	
Age				
≤ 60 years	36.5 (19 – 47.6)	0.24	77.3 (57.1 – 113)	0.23
> 60 years	25 (14.3 – 47.6)		64.2 (47.6 – 100)	
tumor localisation				
colon ascendens	25.4 (11.9 – 43.6)	0.45	57.1 (46.4 – 81.5)	0.13
c. transversum	29.1 (10.3 – 58.3)		54.7 (41.6 – 78.5)	
c. descendens	44.4 (26.7 – 49.2)		94 (64.2 – 120)	
c. sigmoideum	30.1 (17.4 – 37.3)		67.8 (55.3 – 94)	
rectum	36.5 (17.4 – 51.5)		98.8 (57.1 – 114)	

Supplemental Table 4E

Correlation of general patient characteristics with TNFα.

	TNFα CRC Median (range)	p-value	TNFα HT Median (range)	p-value
Gender				
Male	0.42 (0.05 – 1.04)	0.87	0 (0 – 0.08)	0.78
Female	0.33 (0.08 – 1.07)		0.03 (0 – 0.08)	
Age				
≤ 60 years	0.52 (0.08 – 1.65)	0.41	0.02 (0 – 0.05)	0.67
> 60 years	0.32 (0.08 – 0.98)		0 (0 – 0.08)	
tumor localisation				
colon ascendens	0.62 (0.33 – 1.1)	0.21	0.07 (0.04 – 0.18)	0.09
c. transversum	0.98 (0.31 – 2.41)		0 (0 – 0)	
c. descendens	0.32 (0 – 1.06)		0 (0 – 1.8)	
c. sigmoideum	0.2 (0.04 – 0.95)		0 (0 – 0.3)	
rectum	0.3 (0.06 – 1.48)		0.02 (0 – 0.08)	

Supplemental Table 4F

Correlation of general patient characteristics with TGFβ

	TGFβ CRC Median (range)	p-value	TGFβ HT Median (range)	p-value
Gender				
Male	476 (245 – 767)	0.75	82 (52 – 144)	0.41
Female	504 (298 – 754)		72 (5 – 125)	
Age				
≤ 60 years	587 (315 – 755)	0.49	77 (53 – 157)	1
> 60 years	460 (243 – 764)		77 (53 – 141)	
tumor localisation				
colon ascendens	551 (344 – 974)	0.11	142 (56 – 152)	0.17
c. transversum	764 (686 – 971)		66 (17 – 125)	
c. descendens	344 (133 – 1070)		52 (44 – 79)	
c. sigmoideum	356 (229 – 594)		82 (58 – 182)	
rectum	538 (269 – 738)		76 (50 – 121)	

Supplemental Table 5A

Patients at risk dependent on the concentration of TNF α (median 0.51).

	Months										
	0	12	24	36	48	60	72	84	96	108	120
High (>median)	17	17	16	13	12	12	12	11	11	11	11
Low (<median)	18	17	16	13	12	9	6	5	5	5	5

Supplemental Table 5B

Patients at risk dependent on the tumor infiltration by Treg (median 1.45/HPF).

	Months										
	0	12	24	36	48	60	72	84	96	108	120
High (>median)	18	17	17	17	16	14	12	10	10	10	10
Low (<median)	18	18	15	9	8	7	6	6	6	6	6

Supplemental Table 5C

Patients at risk dependent on the tumor infiltration by CD4⁺ conv.T cells (median 5.9/HPF).

	Months										
	0	12	24	36	48	60	72	84	96	108	120
High (>median)	18	18	18	17	16	14	11	10	10	10	10
Low (<median)	18	17	14	9	8	7	7	6	6	6	6

Supplemental Table 5D

Patients at risk dependent on the tumor infiltration by CD8⁺ T cells (median 81,7/HPF).

	Months										
	0	12	24	36	48	60	72	84	96	108	120
High (>median)	17	17	14	13	11	10	8	7	7	7	7
Low (<median)	17	16	16	11	11	9	8	7	7	7	7