Supplementary Materials and Methods

In silico analysis of IL33 transcript levels

IL33 transcript levels from Affymetrix probeset 209821_at were extracted from Affymetrix GeneChip Human Genome U133 Plus 2.0 datasets and normalized using the GCRMA procedure. Data from CRC and normal large intestine tissue samples were obtained from the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/). Data from cancer cell lines were obtained from the cancer cell line encyclopedia resource. [1] Tumor and normal tissue expression values were compared using the Wilcoxon rank sum test implemented in R (http://www.r-project.org/).

Grading of intestinal tumors in mice

The following criteria were used for grading of mouse tumors; Grade 0: no tumor, healthy intestinal epithelium; Grade 1: adenoma with mild dysplasia (low grade); Grade 2: adenoma with moderate dysplasia (low grade); Grade 3: adenoma with severe dysplasia (high grade); Grade 4: adenoma with high grade dysplasia and infiltration of the *lamina propria* (intramucosal neoplasia); Grade 5: invasive carcinoma.

Immunohistochemistry of mouse tissue

Mouse tissue was fixed in 4% formaldehyde for 24 hours prior to dehydration and paraffin embedding. Antigen retrieval for IL-33 for IHC was performed with treatment in a 1 mM Tris / 1 mM EDTA pH 9.0 solution for 18 minutes. After an endogenous peroxidase block (5 minutes at room temperature with 3% H_2O_2 and 15 mM sodium azide), primary goat anti-mouse IL-33 (R&D systems, AF3626) was used at a dilution of 1:100. A secondary rabbit anti-goat antibody (Dako, E0466) was used at a dilution of 1:100. A tertiary horseradish

peroxidase (HRP)-linked anti-rabbit antibody (Dako, K400311) was used undiluted to detect positive cells.

Tumor induction protocol for gene expression studies over time

Gene expression studies over time (Supplementary Figure 4A and B) were performed in a different facility. Because of changes in commensal communities between mouse facilities, a different protocol of AOM/DSS was applied to yield robust tumor formation in C57BL/6J mice, which was comparable to the one observed in the other mouse facility. In brief, animals were injected with AOM. After 5 days, mice were given one cycle of 2% DSS for 5 days, followed by 16 days of water. In the next two feeding cycles, DSS was increased to 2.7%. Animals were analyzed on days 20, 60 and 90.

Generation of bone marrow chimeras

Mice were irradiated with two doses of 650 cGy at an interval of 4 hours. Mice were then adoptively transfused with 1×10^7 bone marrow (BM) cells from donor animals and treated with the antibiotics Bactrim (Roche) and Baytril (Bayer) in the drinking water for two weeks. Following antibiotic treatment, BM recipient mice were co-housed with untreated WT mice for 4 weeks prior to DSS or AOM/DSS treatment to accelerate the re-colonization of the intestinal microflora. Also, soiled bedding was exchanged between cages at least weekly for matching of commensal communities between experimental groups.

ELISA

A sandwich ELISA for IL-6 was performed on 96-flat bottom Nunc MaxiSorp® plates (eBisoscience, 44-2404) using a purified anti-mouse IL-6 capture antibody (eBisoscience, 14-7061-81). A second biotinylated anti-mouse IL-6 antibody (eBioscience, 13-7062) followed by addition of Avidin HRP (eBioscience, 13-7062-81) were used for detection. Following

addition of Substrate Reagent Pack (R&D Systems, DY999) and addition of 1 M sulfuric acid used to stop color development, signals were analyzed on a SpectraMax M2e device (Bucher Biotec).

Quantitative PCR analysis

RNA form mouse tissue or cells was purified using TRI-reagent (Sigma, T9424) or TRIzol (Life Technologies, 15596-026). Transcription of cDNA from RNA was performed with M-MLV Reverse Transcriptase (Promega, M1701). FastStart SYBR Green Master (Roche, 04673492001) and commercial primers (Qiagen) were used to detect *Arg1* (QT00134288), *Ccl2* (QT00167832), *Il6 (*QT00098875), *Tnf* (QT00104006), *Il11* (QT00122122), *Il1b* (QT01048355), *Il17a* (QT00103278) and *Gapdh* (QT01658692) transcript levels. Quantification of 16S rRNA was performed as previously described. [2] Alternatively, PCR reactions were run using the QuantiTect Probe RT-PCR Kit (Qiagen, 204443) and TaqMan Inventoried Gene Expression Arrays (Life Technologies, 4331182) to detect *Il33* (Mm00505403_m1), *Cxcl1* (Mm00433859_m1), *Cxcl2* (Mm00436450_m1), *Il6* (Mm99999064_m1), *Mpo (*Mm00447886_m1*), Nos2* (Mm00440502_m1), or human or mouse *St2* (Hs00545033_m1 or Mm00516117_m1) transcript levels and 18S rRNA (4319413E). Reactions were run on a StepOnePlus Real-Time PCR System (Life Technologies) or on an ABI 7500 Real-Time PCR System (Life Technologies). Expression levels of genes were normalized to *Gapdh* mRNA or 18S rRNA and tumor-free / unstimulated versus tumor / stimulated tissues were compared.

Human intestinal epithelial cell lines

The human intestinal epithelial cell lines Caco-2, COLO 205, DLD-1, HT-29, LS 174T, LS 180, SW480, SW620 and T84 were obtained from ATCC.

STAT3 phosphorylation in human intestinal tumor cell lines

HT-29 and Caco-2 cells were resuspended in MEM containing 0.5% FCS, Glutamax, and Penicillin/Streptomycin at 4×10^6 cells/ml, and equilibrated at 37°C in a water bath for 40 minutes. IL-6 was added at the indicated concentrations for 15 minutes, paraformaldehyde was added to a final concentration of 2% and plates were returned to 37°C for another 15 minutes. Cells were pelleted, resuspended in ice-cold 90% methanol, and incubated at -20°C for at least 30 minutes. Cells were washed three times in FACS buffer (FB, PBS containing 2% FCS, 10 mM EDTA). Mouse-anti-human-pSTAT3 (pY705)-Alexa647, (BD Biosciences, 557815) was added at room temperature for 45 minutes. Cells were washed and resuspended in FB and analyzed on FACS Canto II (BD Biosciences) using the Diva software. Data were analyzed using FlowJo (Tree Star Inc.).

Proliferation of human intestinal tumor cell lines

10^4 Caco-2, HT-29 and LS 174T cells were seeded in their respective culture media in 96-well flat-bottom plates (75µl/well), adhered for 5 hours and incubated for 24 hours with the indicated concentrations of IL-33. Cell proliferation was measured using a colorimetric, BrdU-based cell proliferation ELISA (Roche, 11647229001).

Determination of disease activity score

The following criteria were used for determining the disease activity score, by adding for each mouse the value of these three distinct parameters; Weight loss compared with initial weight (Score 0, <1% weight loss; Score 1, 1-5% weight loss; Score 2, 5-15% weight loss; Score 4, 15-20% weight loss); Stool consistency (Score 0, normal stool; Score 2, loose stool; Score 4, diarrhea); Blood loss (Score 0, negative; Score 2, positive).

Mouse endoscopy

Colonoscopy was performed on isoflurane-anesthetized mice using a straight-type rigid miniature endoscope with a high resolution Karl Storz IMAGE1^{TM} System (Storz).

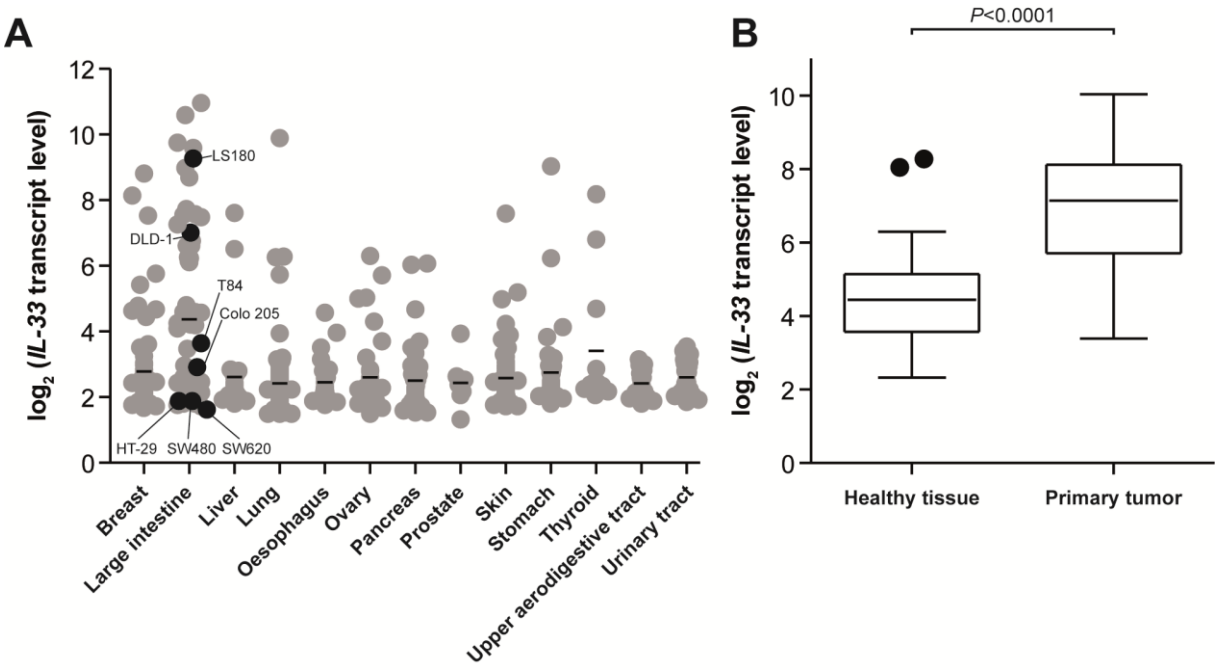
References

1. Barretina J, Caponigro G, Stransky N, Venkatesan K, Margolin AA, Kim S, Wilson CJ, Lehar J, Kryukov GV, Sonkin D, et al. The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. Nature 2012; 483:603-7.

2. Croswell A, Amir E, Teggatz P, Barman M, Salzman NH. Prolonged impact of antibiotics on intestinal microbial ecology and susceptibility to enteric Salmonella infection. Infect Immun 2009; 77:2741-53.

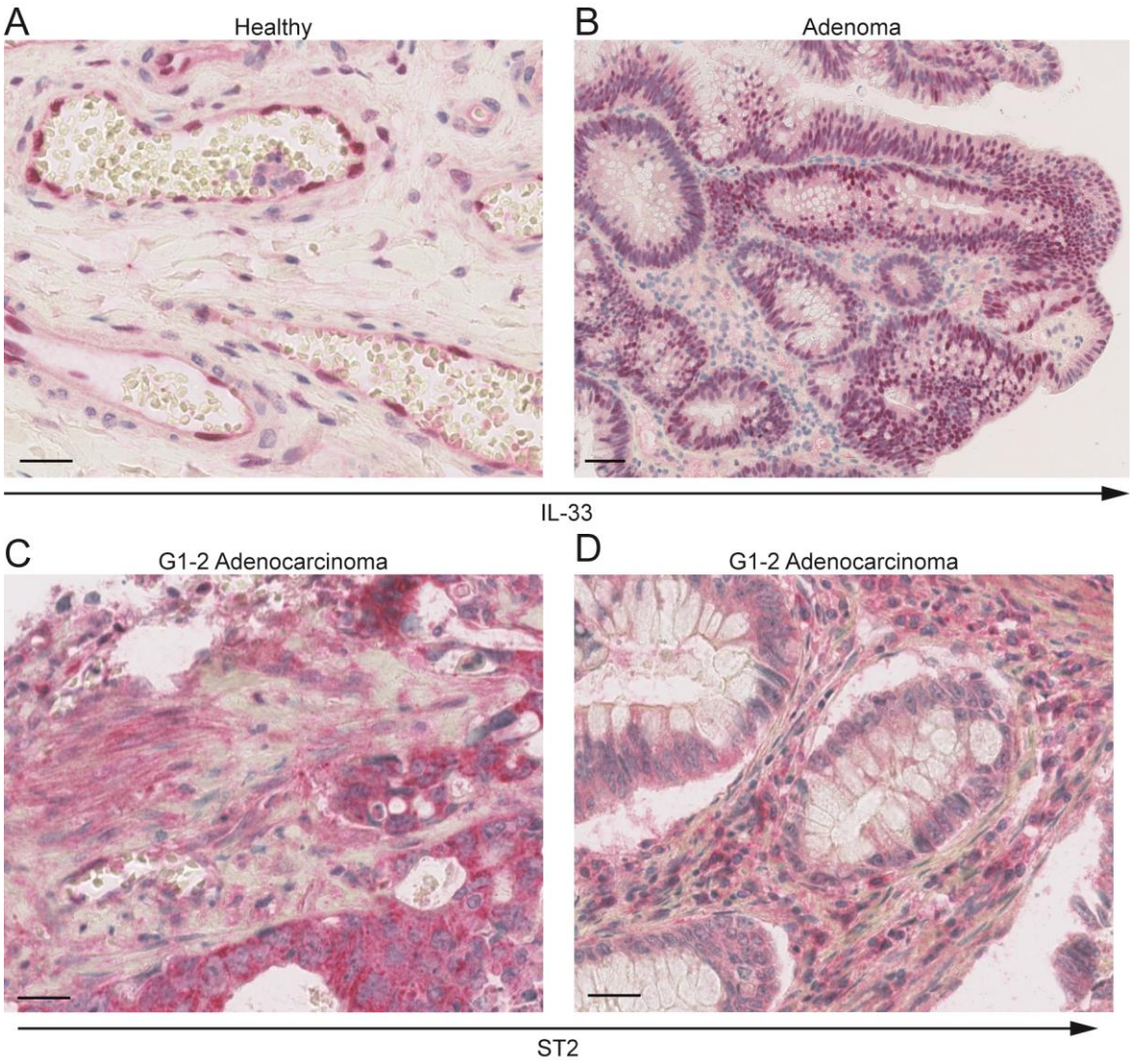
Supplementary Figures

Supplementary Figure 1



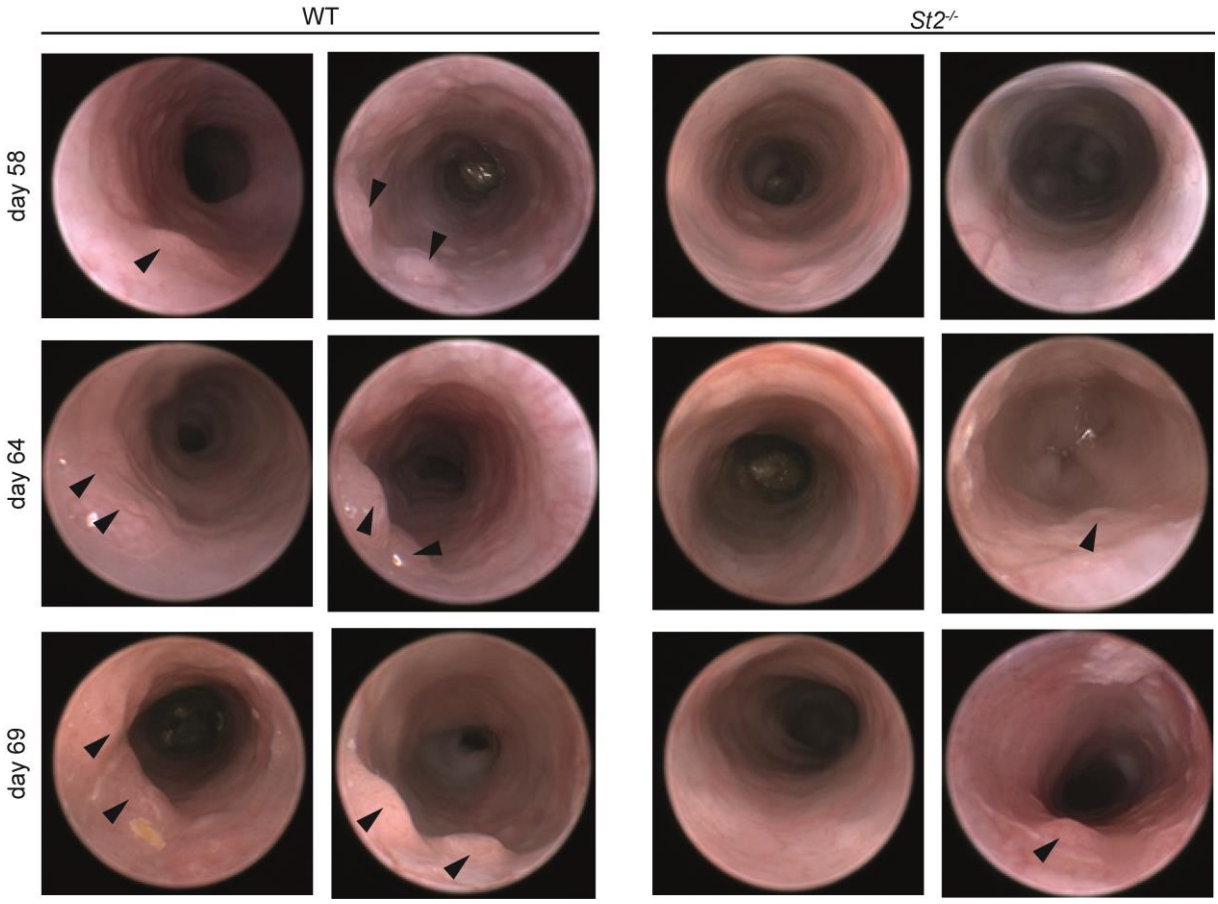
Cancer cell lines and primary tumors from the large intestine show increased *IL33* transcription. (A) *IL33* transcript levels in established cancer cell lines from indicated tissues. *IL33* transcripts in the large intestine are significantly upregulated compared to lung, pancreas ($P<0.001$), breast, liver, esophagus, ovary and skin ($P<0.01$). Means and identities of selected CRC cell lines are indicated. (B) Relative expression levels of *IL33* are increased in primary tumor samples ($n=81$) compared with normal large intestine tissue samples ($n=24$). Data were obtained from the GEO dataset GSE20916 (http://www.ncbi.nlm.nih.gov/pubmed/20957034). A similar observation was made upon analysis of two other independent GEO datasets (data not shown). Statistical analyses were performed using (A) Kruskal-Wallis analysis with Dunn's post-test comparing cells from the large intestine against all other cell lines and (B) Student *t* test.

Supplementary Figure 2



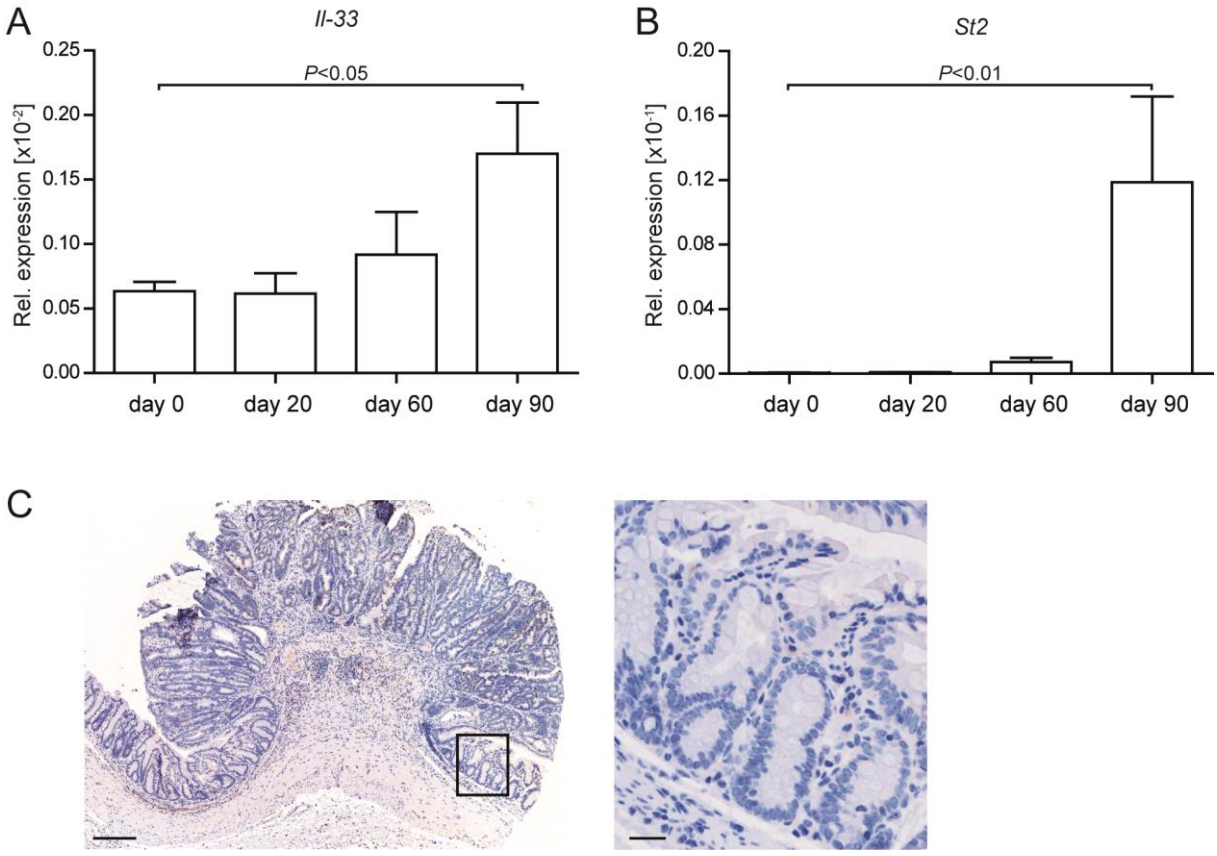
IL-33 and ST2 expression in human colon. (A) Endothelial cells in the healthy mucosa express IL-33. (B) IECs in adenomas express IL-33. (C) ST2 was also expressed by myofibrolasts, endothelial cells and infiltrating immune cells. (D) ST2 expression on infiltrating immune cells. Scale bars: (A, C, D) 25 µm or (B) 50 µm.

Supplementary Figure 3



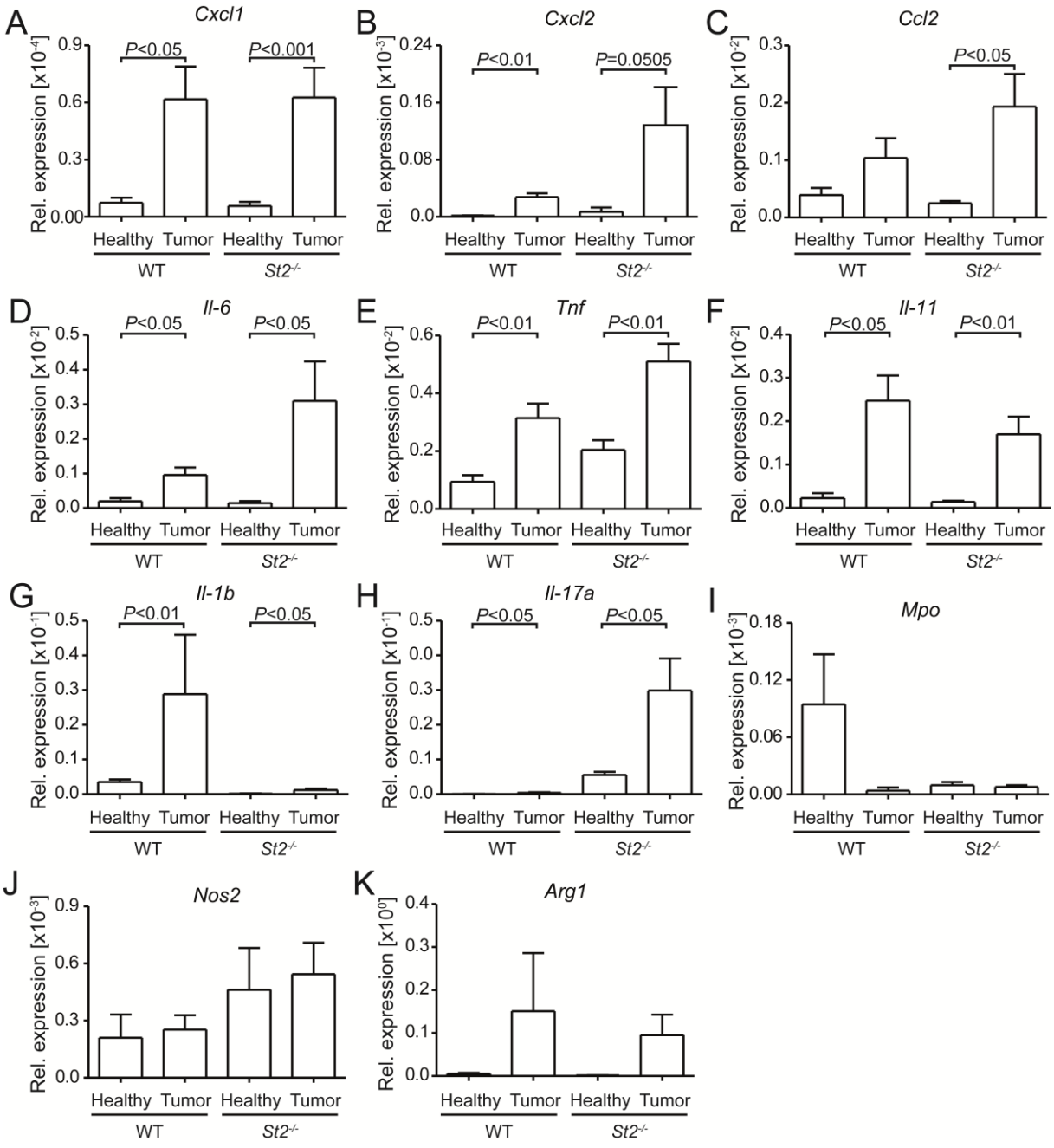
Disruption of IL-33/ST2 signaling delays the development of CRC in the AOM/DSS model. Tumor development was assessed by miniature endoscopy. Upper and corresponding lower panels show the same locations in the colons of the same animals on the indicated days after the start of AOM/DSS treatment. Representative endoscopic pictures are shown for two out of five mice per group. Tumors are indicated with arrowheads.

Supplementary Figure 4



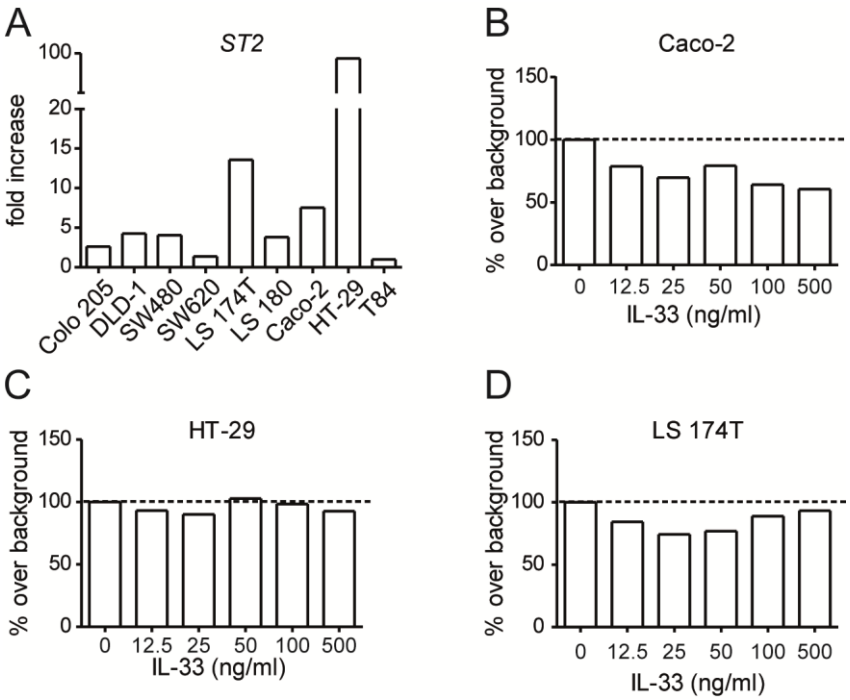
Expression levels of the IL-33/ST2 pathway during AOM/DSS treatment. Colonic (A) *Il33* and (B) *St2* transcript levels become progressively upregulated in AOM/DSS-treated WT mice. Data represent means ± SEM; *n*=5 samples per time point. (C) IHC for IL-33 of WT colorectal tumor showing the same tissue sample as in Figure 3. Inlay shows tumor-free tissue. Scale bars: overview: 200 µm; inlay 25 µm. Statistical analyses were performed using (A) Student *t* test or (B) Mann-Whitney test.

Supplementary Figure 5



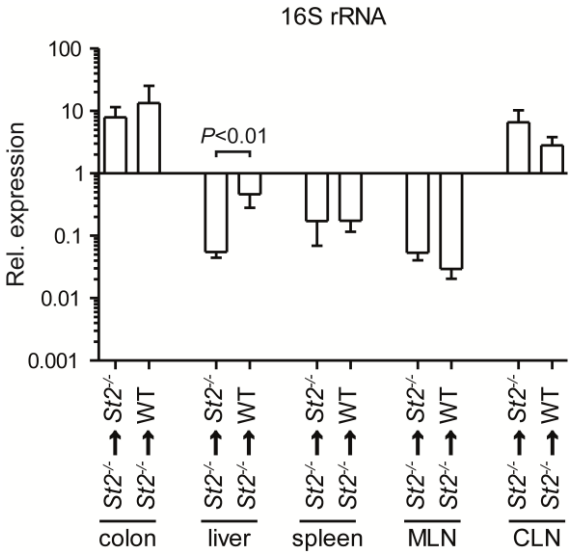
Differential gene expression in tumor versus adjacent tumor-free tissue. Transcript levels were quantified in WT versus *St2*$^{-/-}$ mice for (A) *Cxcl1*, (B) *Cxcl2*, (C) *Ccl2*, (D) *Il6*, (E) *Tnf*, (F) *Il11*, (G) *Il1b* and (H) *Il17a*, (I) *Mpo*, (J) *Nos2*, (K) *Arg1*. Data represent means ± SEM; *n*=9 samples per group. Statistical analyses were performed using paired *t* test.

Supplementary Figure 6



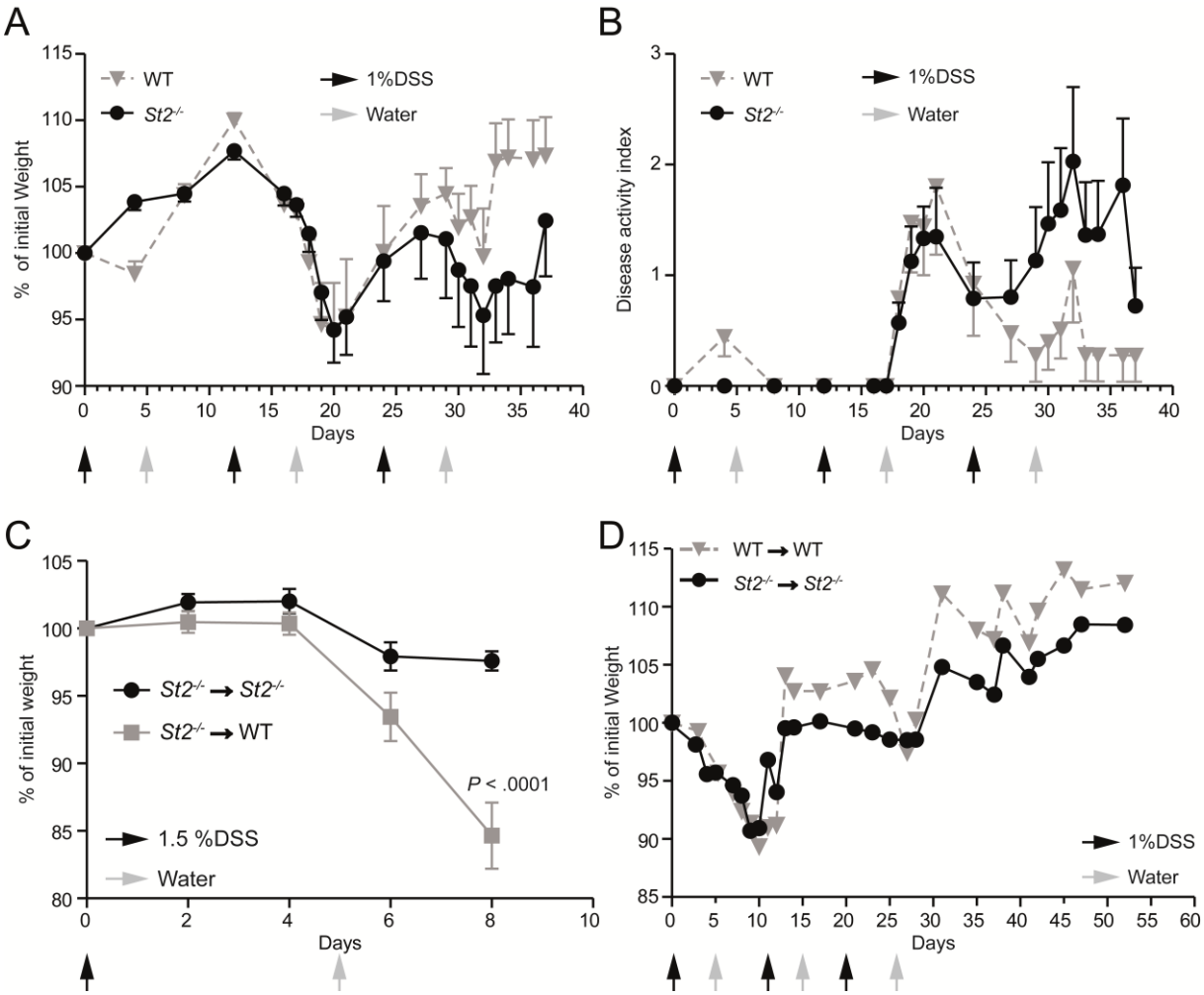
Activation of the IL-33/ST2 pathway does not promote IEC proliferation. (A) *ST2* transcript levels in the indicated human CRC cell lines are represented relatively to *ST2* expression in T84 cells. IL-33 did not stimulate proliferation of (B) HT-29, (C) Caco-2 cells and (D) LS 174T cells. Data represent means of experimental duplicates.

Supplementary Figure 7



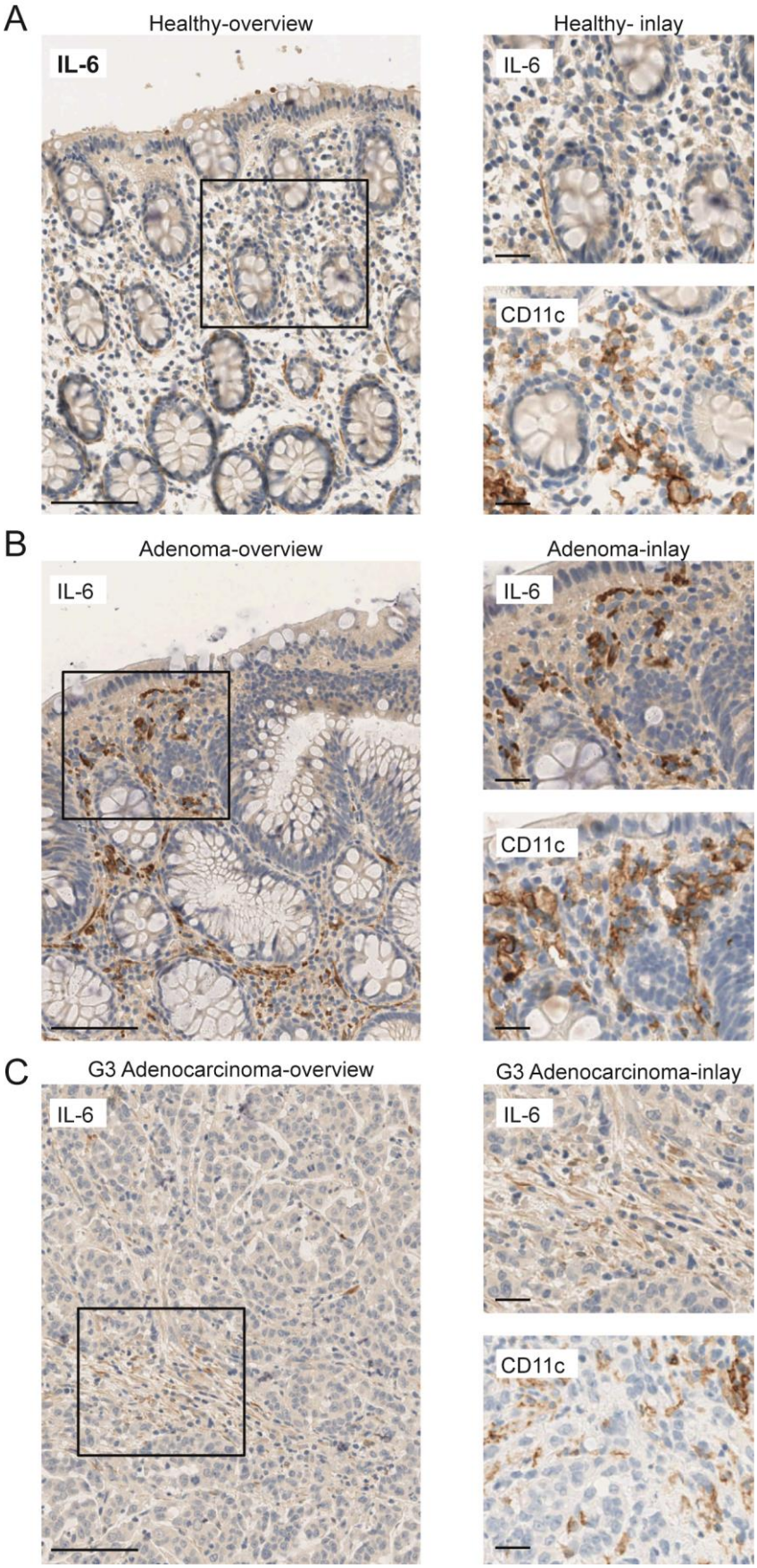
Function of the IL-33/ST2 pathway in radio-resistant cells. Indicated groups of BM chimeras were treated with DSS. Presence of bacteria in indicated organs was assessed by quantifying 16S rRNA in the indicated organs. Data represent means ± SEM and show one representative from two independent experiments; *n*=5-9 mice per group. MLN, mesenteric lymph nodes. Statistical analysis was performed using Mann-Whitney test.

Supplementary Figure 8



Disruption of IL-33/ST2 signaling delays the kinetics of the clinical symptoms associated with DSS/water treatment. (A) *St2*$^{-/-}$ and WT mice previously injected with AOM were given three cycles of DSS/water at the indicated frequency and variation in body weight and (B) disease activity index were recorded; *n*=9 samples per group. (C) and (D) Indicated sets of BM chimeric mice given DSS/water at the indicated frequency and variation in body weight was recorded. In (C), *n*=6-10 samples per group. In (D), mice were previously injected with AOM and *n*=8-9 samples per group. Data represent means ± SEM. Statistical analyses were performed using a two-way ANOVA with a Bonferroni post-test.

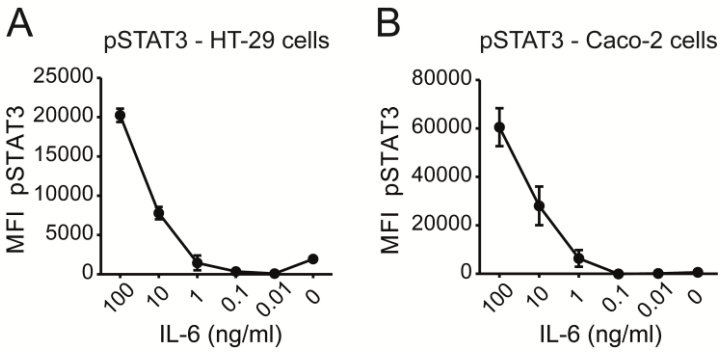
Supplementary Figure 9


A
Healthy-overview
IL-6
Healthy- inlay
IL-6
CD11c
B
Adenoma-overview
IL-6
Adenoma-inlay
IL-6
CD11c
C
G3 Adenocarcinoma-overview
IL-6
G3 Adenocarcinoma-inlay
IL-6
CD11c

188

Serial sections of human colon were stained for IL-6 or CD11c. (A) Healthy mucosa. (B) Adenoma. (C) High-grade adenocarcinoma. Scale bars: representative overview 100 µm; inlay 25 µm.

Supplementary Figure 10



IL-6 induced STAT3 phosphorylation in (A) HT-29 and (B) Caco-2 cells in a dose-dependent manner. Data represent means of experimental triplicates ± SEM.

Supplementary Table 1: Association of clinicopathological features and ST2/IL-33 combinations

Feature		ST2/IL-33 combinations				P-value
		Low/Low *n*=231; 50.6%	Low/High *n*=22; 4.8%	High/Low *n*=168; 36.8%	High/High *n*=36; 7.9%	
Patient age (*n*=457) Median (range)		76 (29-100)	70 (46-88)	76 (15-98)	75 (40-90)	0.1636
Gender (*n*=457)						
	Male	134 (58.0)	12 (54.6)	98 (58.3)	15 (41.7)	0.2959
	Female	97 (42.0)	10 (45.5)	70 (41.7)	21 (58.3)	
Tumor location (*n*=447)						
	Left	89 (39.0)	6 (28.6)	81 (49.7)	19 (54.3)	0.0604
	Rectum	14 (6.1)	1 (4.8)	15 (9.2)	1 (2.9)	
	Right	125 (54.8)	14 (66.7)	67 (41.1)	15 (42.9)	
pT (*n*=457)						
	pT1-2	32 (13.9)	3 (13.6)	20 (11.9)	12 (33.3)	0.0107
	pT3-4	199 (86.2)	19 (86.4)	148 (88.1)	24 (66.7)	
pN (*n*=454)						
	pN0	119 (51.7)	9 (40.9)	102 (61.5)	27 (750.)	0.0119
	pN1-2	111 (48.3)	13 (59.1)	64 (38.6)	9 (25.0)	
pM (*n*=456)						
	pM0	200 (86.6)	17 (77.3)	143 (85.1)	30 (85.7)	0.6959
	pM1	31 (13.4)	5 (22.7)	25 (14.9)	5 (14.3)	
UICC Stage (*n*=454)						
	I	21 (9.1)	2 (9.1)	18 (10.8)	11 (30.6)	0.0023
	II	88 (38.3)	6 (27.3)	78 (47.0)	15 (41.7)	
	III	90 (39.1)	9 (40.9)	45 (27.1)	5 (13.9)	
	IV	31 (13.5)	5 (22.7)	25 (15.1)	5 (13.9)	
Tumor grade (*n*=452)						
	G1-2	158 (69.3)	17 (77.3)	135 (81.3)	31 (86.1)	0.0186
	G3	70 (30.7)	5 (22.7)	31 (18.7)	5 (13.9)	
Lymphatic invasion (*n*=406)						
	L0	161 (84.7)	20 (90.9)	141 (89.2)	33 (91.7)	0.4651
	L1	29 (15.3)	2 (9.1)	17 (10.8)	3 (8.3)	
Venous invasion (*n*=417)						
	V0	149 (75.6)	20 (90.9)	134 (82.7)	34 (94.4)	0.0228
	V1-2	48 (24.4)	2 (9.1)	28 (17.3)	2 (5.6)	
Adjuvant therapy (*n*=291)						
	None	88 (68.8)	11 (57.9)	85 (71.4)	20 (80.0)	0.435
	Treated	40 (31.3)	8 (42.1)	34 (28.6)	5 (20.0)	
Overall survival (%) (*n*=272)						
	Median (95%CI)	66.0 (34-n.e.)	61 (3-n.e.)	50 (38-n.e.)	n.e.	0.6634

n.e., not evaluable

205 **Supplementary Table 2: Patient characteristics**

206

Patient cohort		Zurich	Liestal
Characteristic		Total	Total
Patient age at surgical resection[1] (year, range)		70/73 (21-95)	73/76 (15-100)
Observation period[1] (months, range)		28/21 (2-95)	38/28 (4-151)
Gender (%)			
	Male	124 (49)	266 (56)
	Female	129 (51)	205 (44)
Tumor location (%)			
	Left	106 (51)	219 (49)
	Rectum	80 (38)	196 (44)
	Right	23 (11)	32 (7)
pT (%)			
	pT1-2	47 (19)	71 (15)
	pT3-4	204 (81)	400 (85)
pN (%)			
	pN0	126 (50)	264 (57)
	pN1-2	125 (50)	202 (43)
pM (%)			
	pM0	6 (12)	402 (85)
	pM1	43 (88)	69 (15)
UICC stage (%)			
	0	2 (1)	0 (0)
	I	34 (13)	54 (12)
	II	88 (35)	190 (40)
	III	86 (34)	154 (33)
	IV	43 (17)	69 (15)
Grade (%)			
	G1-G2	189 (75)	349 (77)
	G3	62 (25)	101 (22)
Lymphatic invasion (%)			
	L0	n.a.	365 (87)
	L1	n.a.	54 (13)
Venous invasion (%)			
	V0	17 (34)	347 (81)
	V1	33 (66)	83 (19)
	V2	1 (2)	4 (1)
Adjuvant therapy (%)			
	None	n.a.	207 (69)
	Treated	n.a.	93 (31)
Overall survival (%)			
	Median (95%CI)	62 (50-73)	87 (77-97)

[1] Data are presented as mean / median values, n.a., information not available

207