Mast cell-T cell axis alters development of colitis-dependent and colitis-independent colorectal tumours: potential for therapeutically targeting via mast cell inhibition

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ABSTRACT

Background Colorectal cancer (CRC) has a high mortality rate and can develop in either colitis-dependent (colitis-associated (CA)-CRC) or colitis-independent (sporadic sCRC) manner. There has been a significant debate about whether mast cells (MCs) promote or inhibit the development of CRC. Herein we investigated MC activity throughout the multistep development of CRC in both human patients and animal models.

Methods We analyzed human patient matched samples of healthy colon vs CRC tissue alongside conducting a The Cancer Genome Atlas-based immunogenomic analysis and multiple experiments employing genetically engineered mouse (GEM) models.

Results Analyzing human CRC samples revealed that MCs can be active or inactive in this disease. An activated MC population decreased the number of tumor-residing CD8 T cells. In mice, MC deficiency decreased the development of CA-CRC lesions, while it increased the density of tumor-based CD8 infiltration. Furthermore, co-culture experiments revealed that tumor-prime MCs promote apoptosis in CRC cells. In MC-deficient mice, we found that MCs inhibited the development of sCRC lesions. Further exploration of this with several GEM models confirmed that different immune responses alter and are altered by MC activity, which directly alters colon tumorigenesis. Since rescuing MC activity with bone marrow transplantation in MC-deficient mice or pharmacologically inhibiting MC effects impacts the development of sCRC lesions, we explored its therapeutic potential against CRC. MC activity promoted CRC cell engraftment by inhibiting CD8+ cell infiltration in tumors, pharmacologically blocking it inhibits the ability of allograft tumors to develop. This therapeutic strategy potentiated the cytotoxic activity of fluorouracil chemotherapy.

Conclusion Therefore, we suggest that MCs have a dual role throughout CRC development and are potential druggable targets against this disease.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The role of mast cells (MCs) in colorectal cancer (CRC) is controversial as they can either promote or inhibit it. Studying this issue may reveal druggable targets for oncostatic therapies.

WHAT THIS STUDY ADDS

⇒ Our study shows that an MC-lymphocyte axis alters the development of both CA-CRC and sporadic CRC. However, several immune mechanisms can also change MCs’ function and CRC development. Moreover, pharmacologically inhibiting MC activity blocks the growth of CRC and melanoma tumors. This therapeutic strategy can be combined with a chemotherapeutic compound.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Our findings define specific conditions in which MCs can promote or block CRC development. They also highlight MCs as a versatile druggable target against melanoma and CRC.

INTRODUCTION

Mast cells (MCs) have been suggested to play a pivotal yet complex role in several immune-mediated reactions in the body.1-3 Bone marrow releases immature MCs that migrate towards their patrolling sites that are then activated according to immediate region-specific needs. Also, MCs synthesize inflammatory factors towards antigens binding to the Fc region of IgE coating their membrane.3,4

In human colorectal cancer (CRC), a reduced MC population has been associated
with a good prognosis, while elevated numbers appear to be associated with poorer disease outcomes. Conversely, there have also been reports suggesting that increased MC numbers in tumors promote patient survival rates. In carcinogen-exposed animal models, which resemble the development of sporadic CRC (sCRC), MC deficiency reduced tumor burden.9 Pharmacologically inhibiting MCs’ activity decreased CRC risk also.10

Other research groups have suggested that MCs promote the development of colitis-associated CRC (CA-CRC) in mice. Haribabu and colleagues reported that MC deficiency promotes the development of colon tumors in mice carrying a heterozygous mutation in the adenomatous polyposis coli (ApcMin/+) gene; thus, MCs might orchestrate an anticycnic immunological reaction during the development of intestinal tumorigenesis.13 Sinnamon et al demonstrated similar observations that MC deficiency increases tumor numbers in ApcMin/+ mice.14 Whether the MC activity can differentially alter the development of CA-CRC and sCRC lesions remains unknown. We have thus challenged the hypothesis that MC activity alters and can be altered by different immune reactions. Our findings not only confirm that a MC-T cell axis alters the development of CA-CRC and sCRC tumors but that its potential can be pharmacologically harnessed to treat CRC in combination with a standard chemotherapeutic compound.

MATERIALS AND METHODS

Human samples and immunogenomic analysis

According to the Declaration of Helsinki for the research use of previously collected human biospecimens in minimal risk studies, this Research Ethics Committee waived the patient informed consent. None of the patients underwent chemotherapy or radiotherapy prior to sample collection. Coded tissue samples ensured patient confidentiality.

Also, we performed an immunogenomic analysis based on 604 CRC cases from The Cancer Genome Atlas (TCGA). It investigated the immune cell population estimated by CIBERSORT,15 and the immune features that our previous report described. Additionally, we selected the TCGA CRC samples classified as ‘genome stable subtype’ and divided them in two groups based on the frequency of activated MC by setting up a dichotomy comparing the top quarter (high MC activated frequency, HMCA) against the bottom quarter (low MC activated frequency, LMCA). The samples with intermediary frequency were excluded from the analysis.

Mouse experiments

The Research Ethics Committee for Animal Use of the School of Pharmaceutical Sciences of Ribeirao Preto of the University of Sao Paulo approved the current study (22.1.253.60.2). The following mouse strains were purchased from the Jackson Laboratories (USA): KitW/ (012861), KitW/W (100410), B2m knockout (KO; #002087), Ciita KO (#003239), Il6 KO (#002650), NOD (001976), NOD-scid (005557), C57BL/6J (#000664; named herein KitB6, B2mWT, or CiitaWT), BALB/c (000651; named herein St2WT), C57BL/6-Tg(CAG-EGFP)131Osb/LeySopJ (#006567; named herein KitB6-Tag(CAG-EGFP)), and Rag1 KO (#002216). The St2KO mouse strain was developed and donated by Dr Andrew McKenzie.17

Cell culture, co-culture, and allograft CRC model

A murine CRC cell line (MC38) was cultured (±95% humidity; 37°C; 5% CO2) in Dulbecco’s Modified Eagle Medium (D5030; 10% foetal bovine serum, 12103C; 100 U/mL penicillin-streptomycin, P0781; 10 mM sodium pyruvate, P5280; Sigma-Aldrich, USA). To harvest tumor-primed MCs, KitB6 mice were intraperitoneally (i.p.) injected with MC38 cells (1×106). Following 7 days from this procedure, MCs were isolated from the peritoneal cavity according to our previous description.18 Tumor-primed MCs were co-cultured with MC38 cells at a ratio of 1:10. MC38 cells (2×106) were subcutaneously injected into both KitW/WT and KitB6 mice. Tumors grew for 14 days and were monitored with a caliper. Treatments started from the seventh day onwards. Treatments were saline, cromolyn (CA; Sigma-Aldrich; C0399; 25 mg/kg/day,19 and 5-fluorouracil (5-FU; Libbs Farmaceutica Ltda, Sao Paulo, Brazil; 70 mg/kg once every 3 days.20

Colon tumorigenic mouse models

The CA-CRC protocol consisted of a single i.p. injection of azoxymethane (AOM; 10 mg/kg; Sigma-Aldrich; A5486) followed by three cycles of 2% dextran sulfate sodium (Sigma-Aldrich; 42867.21 Experiments were ended in a CO2 chamber (10th week).

The sCRC protocol consisted of 6 i.p. injections of AOM throughout 6 weeks. Tumors were detected after other 18 weeks.20 Early tumorigenic lesions (APL) were detected after 6 weeks from the sixth AOM exposure. DNA damage can be studied after 3 days and single APLs (PL) after 1 week from the sixth AOM injection. Our protocol of 3 i.p. AOM injections followed by 3 days or 3 weeks was also applied.22

Bone marrow transplantation

To rescue MC deficiency, KitW/ mice and their counterparts underwent a single lethal dose of whole-body irradiation (IR; RS-2000 Biological Research Irradiator [Rad Source, Kansas City, MO, US]; 7 Gy). After 12 hours, IR-exposed mice received 1×107 freshly isolated mouse bone marrow cells expressing enhanced green fluorescent protein (EGFP) from KitB6-Tag(CAG-EGFP) mice.23 After other 6 weeks, bone marrow transplantation (BMT) was confirmed by counting EGFP cells in flow cytometry (Guava easyCyte 8HT [MerkMillipore, Burlington, MA, USA]; InCyte 2.7 software). Then, mice underwent sCRC protocol.
qPCR
The RNase Mini Kit (74104; Qiagen, DE) was used to prepare RNA samples. Then, cDNA synthesis followed the manufacturer’s guideline for the SuperScript III Reverse Transcriptase kit (Thermo Fisher Scientific, USA; 18080093). Online supplemental table 1 and 2 show probes and primers, respectively. Data were collected and analyzed using an Eppendorf Mastercycler RealPlex2 system (Eppendorf, DE). The fold-change (FC) between reaction control, experimental control, and target groups was calculated using the 2−ΔΔCt method (Gapdh as a housekeeping gene).

Flow cytometry
According to the manufacturer’s guideline (Thermo Fisher Scientific, USA), the Annexin V/ 7AAD kit (#88-8006-74) was used to analyze viability and apoptosis. Also, colonic lymphocytes, tumor immune cellular populations, and tumor cells were isolated as we have described previously. Then, samples were incubated with antibodies against CD45-FITC (1:250), CD45-APC (1:250), CD45-BV421 (1:300), CD117-PE-Cy7 (1:250), FceRI-PE (1:250), CD8α-PE (1:300), CD5-FITC (1:250), CD4+FITC (1:250), and FOXP3-APC (1:300; online supplemental table 3). Cellular viability was checked by 7AAD (#A1310; Thermo Fisher Scientific) or FVS-APC Cy7 (#565388; BD Biosciences). The data acquisition was performed on FACSVerse (BD Biosciences) and analysis carried out by the FlowJo software (FlowJo LLC, USA). Lymphocytes were determined as CD4+, CD4+FOXP3+, CD8*, and CD8*CD3* gated from singlet viable CD45* cells. MCs were determined as CD45+FceRI-CD117+ cells. Values are given as percentage.

Clonogenic assay
For decades the clonogenic assay has been used to test the number of cancer cell colonies in different experimental settings. It can also be applied in co-culturing systems. After tumor-primed MCs were co-cultured with MC38 cells for 5 days, colonies were fixed and stained with 0.2% crystal violet solution. The well area normalized the number of colonies.

Histopathological analysis and multiplex immunohistochemistry
As previously reported, 4μm paraffin-embedded tissue sections were stained with toluidine blue (Sigma-Aldrich; 89640) before determining the density of MCs per sample area (tTB; Axio Imager M2 [Carl-Zeiss, DE]).
To validate this analysis (tTRY), immunohistochemistry (IHC; Picture-Max Dab Kit; Thermo Fisher Scientific, USA; 878983) stained samples following manufacturer’s instructions (anti-MC trypstat [TRY; 1:100; ab2378], ABCAM, USA).
Following our previous description, tumors and preneoplastic tissue samples underwent H&E staining and analysis (lesions per mm²). IHC stained samples with primary antibodies (online supplemental table 1) for analysis (number of positive per crypt).
A multiplex IHC was performed following our protocol. Reactivity to primary antibodies (online supplemental table 3; online supplemental figure 1; online supplemental table 3; online supplemental figure 1) was determined by a Vector AEC Substrate kit (Vector Laboratories, USA; SK-4200). Stained tissue slides were scanned using an Aperio ImageScope AT system (Leica Biosystems, DE). Image processing and visualization were carried out using ImageScope software (Leica Biosystems).

RNAseq panel analysis
According to our previous report, immunoblotting analyses were performed. Following cold protein extraction (RIPA Buffer, R0278; phosphatase inhibitors, P0044; protease inhibitor, P2714; Sigma-Aldrich), protein concentration was determined (Pierce BCA Protein Assay Kit, 23225; Thermo Fisher Scientific). Any kD MiniPROTEAN TGX Stain-Free Protein Gels (#4568123; Bio-Rad Laboratories, USA) were used for electrophoresis. Then, we used the Bio-Rad Trans-Blot Turbo Mini Nitrocellulose Transfer Packs (#1704158) together with the Bio-Rad Trans-Blot Turbo Transfer System. Next, membranes were incubated at 4°C overnight with primary antibodies (online supplemental table 3).

Statistical analysis
The R software was applied for the TCGA-based immunogenomic analysis. Also, we computed the Pearson correlation between immune features and MC populations. A two-tailed T-test was used to analyze the frequency of immune cell populations/signatures between CRC samples with low (LMCA) and HMCA frequencies.

The R software was also applied for analyzing the RNAseq datasets. After the data normalization by DESeq2, the differential expression was calculated (p value adjust<0.05). Test t was used to analyze only two groups, while the one-way analysis of variance (ANOVA) (post hoc test Benjamini–Hochberg) was used to analyze more than two groups. The libraries used were: DESeq2, ComplexHeatmap, gplots and parallel.
Other datasets were analyzed in the GraphPad Prism 9.3 software (Graph Pad Software Inc., USA). The two-tailed Mann-Whitney’s U test analyzed probabilities between two different groups. The one-way ANOVA test (Kruskal-Wallis’ post hoc test) was applied to analyze experiments with more than two groups. The two-way ANOVA test (with Bonferroni’s post hoc test) was applied to analyze different categorical independent endpoints with one dependent variable. A probability of p<0.05 was considered to be statistically significant.

RESULTS
The MC activity alters the lymphocyte population in human CRC cases
Analyzing matching samples of normal colon and CRC tissues reveals that MC numbers might increase or remain unchanged in this pathological condition when compared with normal colon tissue from the same patient (figure 1A,B). Furthermore, TCGA-based immunogenomic analysis of 604 CRC cases shows that MCs reside in tumors as either active or inactive units (figure 1C). We also observed that activated MCs negatively correlated with lymphocyte infiltration in CRC cases, whereas dormant MCs positively correlated with these cells (figure 1C). We further verified these observations by studying CRC cases in which activated MCs were present in either low (LMCA) or high numbers (HMCA). An HMCA appeared to decrease the number of tumor-residing CD8 T cells (figure 1D). Our observations confirm previous reports that MCs may alter the CRC development and CD8 T cell density in those lesions.

The MC activity impacts the development of colitis-dependent or colitis-independent tumors in mice
To test the idea that MCs can directly affect CRC cells, we first harvested high-MC numbers from KitB6 mice bearing MC38 cell-induced ascites (figure 2A). Peritoneal MCs from MC38-bearing mice reduced proliferation of CRC cells in a co-culture model (figure 2B). MCs also promoted apoptosis in MC38 cells (figure 2C,D). Moreover, MCs decreased the number of MC38 colonies in a long-term co-culturing system (figure 2E,F).

To mechanistically explore MC activity throughout the multi-stage development of CRC, tumorigenesis was induced in mice in either a colitis-dependent (CA-CRC) or a colitis-independent fashion (sCRC).21 Induction of CA-CRC in MC-deficient mice (KitW/sh) reduced tumor burden compared with counterparts, even with high-Cd8 expression levels in those tumors (figure 3A,B). However, this experimental condition did not alter Cd3 expression levels (KitB6 – 1.0±0.1; KitW/sh – 1.3±0.2; p=0.38). Histopathological analysis confirmed that MC deficiency promoted a higher number of CD8+ cells in CA-CRC lesions (figure 3B). MC deficiency did not alter the Cd11c (KitB6 – 0.6±0.08; KitW/sh – 0.4±0.17; p=0.48) and Cd4 levels (KitB6 – 1.8±0.2; KitW/sh – 2.5±0.6; p=0.48).

After mice underwent the sCRC induction protocol, we observed that MC deficiency promoted the development of sporadic colorectal tumors (figure 3C). In sCRC lesions, unlike the CA-CRC ones, MC deficiency did not alter the Cd8 expression levels, nor the tumor infiltration of CD8 lymphocytes (figure 3D,E; Suppl online supplemental figure 2A). However, it decreased tumor Cd3 expression levels (KitB6 – 1.0±0.1; KitW/sh – 0.6±0.1; p=0.04). By analyzing a proliferation biomarker, we observed proliferative rates did not change, significantly (figure 3E; online supplemental figure 2A–C).

To explore these findings, we performed an RNAseq analysis to investigate that MC deficiency alters several inflammatory responses (figure 3F; Suppl online supplemental table 4). MC deficiency upregulated the cytokine–cytokine receptor interaction pathway (p=9.8−11; online supplemental table 5). Our findings suggest that MC activity varies according to the various factors inducing the development of colorectal tumors, as opposing results between CA-CRC and sCRC models demonstrate.

The MC-lymphocyte axis impacts the development of early tumorigenic events in the colon
Considering that MC deficiency may promote significant changes in the tumor microenvironment, we explored the hypothesis that MCs play a pivotal role in building this tumor microenvironment from the early tumorigenic steps onwards. First, we observed that the sCRC protocol increased the colonic CD8 T cell population during the development of the early tumorigenic steps in the colon (figure 4A; online supplemental figure 3). This finding led us to rationalize that major histocompatibility complex class I (MHC1) activity directly impacts the lymphocyte population.31 When we induced early tumorigenic lesions in a MHC1 deficiency model (B2m KO), we observed reduced Cd8 expression levels but increased levels of MC biomarkers (MC protease 1, Mcpt1 (epithelial); Mcpt4 (stromal)32; figure 4B–E; online supplemental figure 1). Conversely, MHCII deficiency decreased the expression of Mcpt1 and Mcpt4 in the colon of AOM-exposed mice (figure 4F–I; online supplemental figure 4). To confirm that these early tumorigenic steps required a specific immune response to develop, they were induced in NOD-scidγ immunosuppressed mice. This experiment revealed that immunosuppression decreases the development of early tumorigenic lesions, epithelial cellular proliferation, and the number of MCs (online supplemental figure 5A–C).

As MC-deficient sCRC tumors had low Il6 expression levels (figure 3F), a mouse Il6 deficiency model underwent induction of early tumorigenic lesions. This colonic Il6 deficient microenvironment decreased the development of early tumorigenic lesions and MC numbers (online supplemental figure 5D–E). Considering that we found that high-Ih33 levels occurs in MC deficient sCRC lesions (figure 3F) and a previous study has shown that the interleukin 33 receptor (IH33r) modulated MC-related tumorigenic effects [11], we induced early colon tumorigenic
Figure 1  MC activity (MCA) effects in human CRC cases. (A) Representative images of MCs stained by TB (CRC or normal colon (CT) samples from the same patient; 40× (left-side) and 100× (right-side) magnification; sectioned lines indicate 100×magnified areas; arrows lead to positive cells). (B) Representative images of MCs stained by anti-tryptase antibody (TRY; 20× magnification; increased [upper-left] or unchanged (lower-left) MC numbers) in both CT and CRC samples. (A, B) MC density was found to be increased (A, n=16, *p=0.023; B, n=14, *p=0.026), or remain unchanged (A, n=22; B, n=12; p>0.05). (C) A Immune features (y axes; total MCs (upper panels), or leukocyte infiltration (lower panels)) vs MC activated (left panels) or resting fractions (right panels; x axes) for CRC cases from TCGA. (D) The CRC cohort positive for a MCA profile was divided in two groups of cases with low (LMCA) or high MCA values (HMCA). Lymphocytes and CD8 T cell levels are shown to be increased in LMCA group (****p=0.000105; *p=0.0284). Data are shown as the median, highest and lowest values, and upper and lower quartiles. Dots represent individual tumor samples. The Pearson correlation was applied. P values were calculated using two-tailed Mann-Whitney’s U test. CRC, colorectal cancer; LMCA, low MC activated frequency; MC, mast cell; TB, toluidine blue; TCGA, The Cancer Genome Atlas.
Figure 2  MCs induce apoptosis in murine CRC cells. (A) Tumor-primed MCs were isolated 7 days after KitB6 mice received a single i.p. injection of murine CRC cells (MC38). Once purified, tumor-primed MCs were co cultured with MC38 cells for 2 (B–D) or 5 days (E, F). (B) Gene expression analysis by qPCR for Mki67 (*, p<0.05). (C, D) Representative dot plots of flow cytometric analysis for gating CD45(-) cells or MC38. It was followed by viability and apoptosis analysis. Graph shows percentages of negative or positive MC38 cells for Anexin V (*, p<0.05). (E, F) Representative images of clonogenic assays. Graph shows the relative number of colonies for each group (****, p<0.001). Dots represent individual values together with mean±SEM. P values were calculated using two-tailed Mann-Whitney’s and two-way ANOVA tests (Bonferroni’s post hoc) tests. ANOVA, analysis of variance; CRC, colorectal cancer; MCs, mast cells; ns, not significant.
Figure 3  The dual MC role in colorectal tumorigenesis. (A) Representative images of tumors induced by the CA-CRC protocol (Scale bars=1 cm). Graph shows tumor numbers per mice (n=8; *p=0.0286). (B) Gene expression analysis for Cd8 (p=0.0286). Representative images of tumors stained by an anti-CD8 antibody (40× (left-side) and 100× (right-side) magnification; lines indicate 100× magnified areas; arrows lead to positive cells). (C) Representative images of tumors induced by sCRC protocol (Scale bars=1 cm). Graph shows tumor numbers per mice (n=17; p=0.0164). (D) Gene expression analysis for Cd8 (p>0.05). (E) Representative images of multiplex IHC staining for CD8 lymphocytes (CD8) and proliferating tumor cells (Mki67; magnification at 200 µm). Data are shown as the median, highest and lowest values, and upper and lower quartiles (A, B) or individual data points with mean±SEM. (C, D) P values were calculated using two-tailed Mann-Whitney’s U test. (F) Heatmap of hierarchical clustering indicates differentially expressed genes (rows) between tumor samples from KitB6 and KitW/sh mice (n=10). Red indicates upregulation (Max) and blue indicates downregulation (Min). CA-CRC, colitis-associated colorectal cancer; MC, mast cell; sCRC, sporadic CRC.
Figure 4  The early colorectal tumorigenic steps require complex immune reactions to develop. (A) Representative dot plot graphs of CD4+ and CD8+ lymphocytes gated for CD45+, live cells, and singlet. (B–D, F, G, J–O) Gene expression analysis for Cd8 (B, n=8, *p=0.02; H, n=13, p>0.05; O, n=9, *p=0.03), Mcpt1 (C, n=8, *p=0.02; G, n=13, *p=0.02; M, n=9, *p=0.01), Mcpt4 (D, n=8, *p=0.02; F, n=13, *p=0.02; N, n=9, *p=0.01), Ctnnb1 (J, n=9, *p=0.03), and Mki67 (L, *p=0.03) in B2M KO, Cita KO, and St2 KO mice, and their counterparts. (E, I, P) Representative images of multiplex IHC staining with anti-CD8, anti-CD3, and anti-TRY antibodies (magnification at 50 µm). Data are shown as the median, highest and lowest values, and upper and lower quartiles. P values were calculated using a two-tailed Mann-Whitney's U test. IHC, immunohistochemistry.
lesions in an Il33r deficiency model. The Il33r deficiency reduced CRC-related biomarkers in carcinogen-exposed mice (figure 4J,L). This condition upregulated expression levels of MC biomarkers but downregulated the gene encoding Cd8 (figure 4M–P; online supplemental figure 4).

We further confirmed that an MC-lymphocyte axis impacts the development of early tumorigenic lesions in the colon. Thus, these lesions were induced in MC deficient mice and analyzed after 6 weeks from the sixth carcinogenic exposure. This experiment revealed that MC activity reduces the development of early tumorigenic lesions and the levels of Cd4 and Cd11c (figure 5A–C). We further confirmed that increased Cd4 and Cd11c levels were occurring just a week from the sixth carcinogenic exposure (figure 5D–E). Moreover, MC deficiency decreased the expression of a gene associated to an essential DNA damage repair mechanism against AOM-related DNA adducts known as O6-methylguanine-DNA-methyltransferase (Mgmt) (figure 5F).

To further investigate the MC role in these early colon tumorigenic events, we applied our previous reported strategy to study DNA damage and single early tumorigenic events. DNA damage was analyzed following 72 hours from the third carcinogenic exposure to reveal that MC deficiency did not alter the γH2AX levels in the colon while increasing the Cd11c expression (figure 5G–H). To study the earliest immunological reactions activated by MC deficiency, we performed transcriptomic analyses in the colon of mice at the third week from the third carcinogenic exposure. Out of several immunological reactions (figure 5I; online supplemental table 6), MC deficiency significantly impacted the inflammatory bowel disease pathway (online supplemental table 7).

We next validated these findings by performing BMT in Kit\( ^{W/sh}\) mice before inducing the development of single early colon tumorigenic lesions in them (figure 5J). This experiment revealed that restoring MCs into previously MC deficient mice increased proliferation in the carcinogen-exposed colon (Mki67 (FC), Kit\( ^{B6}\)BMT – 1.3±0.1; Kit\( ^{W/sh}\)BMT – 2.9±0.5; \(p=0.006\)). Interestingly, rescuing MC activity in MC deficient mice before the development of single early colon tumorigenic lesions increased the Mcpt4 levels while blocking the expression of Cd11c (figure 5K,L). These results illustrate that MCs impact and are impacted by other immune reactions activated throughout the development of early colon tumorigenic lesions.

Targeting MCs against CRC lesions

Since our observations suggested that the MC population could promote specific effects in different stages of CRC development, we treated Kit\( ^{W6}\) mice with CA to inhibit MC activity throughout the 3 weeks of carcinogenic exposure or following this period (figure 6A). Although CA treatment did not alter DNA damage levels (figure 6B), inhibiting MC activity throughout the carcinogenic exposure increased sCRC risk, whereas mice given CA post-carcinogen induction did not develop early tumorigenic lesions (figure 6C). This time-related MC inhibition that promoted the development of early tumorigenic lesions also increased proliferation and upregulated the expression levels of Cd4, Foxp3, Cd8, and Cd3 (Cd3 (FC), AOM – 1.3±0.1; AOM+CA – 3.0±0.3; AOM-CA – 2.3±0.7; AOM vs AOM+CA, \(p=0.03\); figure 6D,F–H).

We then performed transcriptomic studies to clarify significant changes in inflammatory reactions promoted by pharmacologically inhibiting the MC activity (figure 6I; online supplemental table 8,9). Out of several immunological changes that the CA treatment induces, it inhibited key elements of the cell adhesion pathway (figure 6I; online supplemental table 10). Conversely, when CA treatment and carcinogenic exposure are given together, it down-regulates the antigen processing and presentation pathway (online supplemental table 11).

These findings led us to verify how much MCs could impact the engraftment potential of CRC cells. This experiment revealed that MC deficiency blocks the development of CRC allograft tumors without altering their proliferative activity (figure 7A; online supplemental figure 6A). Interestingly, this MC deficiency significantly up-regulates the tumor Cd4 and Cd8 expression levels (online supplemental figure 6B). Moreover, it increased the tumor infiltration of CD8 lymphocytes (figure 7B). When we performed an RNAseq analysis to explore potential immune reactions associated with MC deficient tumors, it showed a down-regulation of the proteasome pathway and upregulation of key elements of the antigen processing and presentation mechanism (figure 7C; Suppl. online supplemental table 12,13).

Once we tested the therapeutic potential of MC inhibition against CRC development, it revealed that inhibiting MC activity significantly blocks the development of allograft colorectal tumors (figure 7D; online supplemental figure 7A). To further explore the anticancer therapeutic potential of MC activity blockade, we observed that CA treatment prevented the growth of melanoma tumor allografts (figure 7E; online supplemental figure 8A,B). Surprisingly, blocking MC activity in melanoma reduced the CD8\(^+\)CD3\(^+\) T cell population without altering the MC one (figure 7F; online supplemental figure 8C,D). Encouraged by these findings, we blocked MC activity in colorectal tumors 14 days after cancer implantation (online supplemental figure 9A,B). Again, CA treatment inhibited colorectal tumor development and increased the CD8\(^+\)CD3\(^+\) T cell population without altering the MC numbers (figure 7G–I; online supplemental figure 9C). To challenge the idea that inhibiting MC activity would enhance anticancer response by cytotoxic lymphocytes, we treated CD3-deficient mice with CA for 3 days before implanting MC38 cancer cells. We then monitored the tumor growth for other 11 days. It revealed that without an effective T cell-based response CA treatment did not reduce tumor growth (online supplemental figure 11).

This observation led to testing whether this drug could be combined with a gold-standard chemotherapy
Figure 5  The MC activity protects against early colorectal tumorigenic steps. (A) Representative images (10× and 40× (inset); scale bars=20 µm) of early tumorigenic events preceding the detection of fully grown tumors in the colon. MC deficiency increases the number of them (n=11; *p=0.004). (B, C) Gene expression analysis in colon samples of mice at the sixth week after the sixth carcinogenic exposure for Cd4 (n=11; *p=0.03) and Cd11c (*p=0.03). (D-F) Gene expression analysis in colon samples of mice at the first week after the sixth carcinogenic exposure for Cd11c (n=10; *p=0.01), Cd4 (*p=0.02), and Mgmt (n=11; *p=0.04). (G) DNA damage levels by immunoblotting against γH2AX and GADPH in colon samples of mice at the third day after the third carcinogenic exposure. (H) Gene expression analysis for Cd11c in colon samples of mice at the third day after the third carcinogenic exposure (n=9; p=0.003). (I) Heatmap of hierarchical clustering obtained from RNA-seq data indicates differentially expressed genes between colon samples of KitB6 and KitW/sh mice at the third week after the third carcinogenic exposure (n=6). (J) Representative histograms of the BMT protocol performed in both KitB6 and KitW/sh mice. Spectral color represents EGFP+ cells by fluorescence intensity (GF; x axis) and cell counting (y axis) in different experimental conditions. (K-M) Gene expression analysis for Mcpt4 (*p=0.004), Cd11c (*p=0.01), and Cd8 (p>0.05) in colon samples of mice at the third week after the third carcinogenic exposure following the BMT procedure. Data are shown as individual data points with means±SEM p value was calculated using a two-tailed Mann-Whitney’s U test. BMT, bone marrow transplantation.
Figure 6  The MC activity impacts the development of early tumorigenic lesions in the colon. (A) Timeline for different groups treated with CA (red arrows) and AOM (black arrows) throughout 6 weeks. (B) DNA damage levels by immunoblotting against γH2AX and GADPH in colon samples. (C) Inhibiting MC activity in specific timepoints of carcinogenic exposure can either promote or inhibit the development early tumorigenic events in the colon (n=24; *p=0.042, ****p<0.0001). (D–H) Gene expression analysis for Mki67 (n=24; *p<0.01; AOM vs AOM+CA), Cd11c (p>0.05), Cd4 (*p<0.05; AOM vs AOM+CA), Foxp3 (*p<0.01; AOM vs AOM+CA), and Cd8 (*p<0.01; AOM vs AOM+CA). Data are shown as the median, highest and lowest values, and upper and lower quartiles (C–H). P values were calculated using ANOVA with a Kruskal-Wallis’ post hoc test. (I) Heatmap of hierarchical clustering obtained from RNA-seq data indicates differentially expressed genes (n=12). ANOVA, analysis of variance; AOM, azoxymethane; CA, colitis-associated; MC, mast cell.
in CRC treatment. We found that CA potentialized the anticancer effects of 5-FU (figure 7J; online supplemental figure 7B). Nevertheless, the results of this combination therapy seem to be comparable to the association of chemotherapy with a standard immunotherapeutic antibody (figure 7K). Our data demonstrate that MCs impact the multistage development of CRC and are a druggable target against this disease.

**DISCUSSION**

In this study, we have shown that MCs play an intricate role in CRC development in human patients. Tan *et al* reported that a low MC counting correlates with more invasive tumors, increased number of metastatic lesions, and reduced 5-year survival rates. Another study with 72 cases of CRC showed that a high MC density could promote overall survival and reduce the risk of malignancy-related...
to have some anticancer activity in a context closely interacts with tumor-inflammation.

Whereas MCs would inhibit CD8 cell density but promote that MCs could either promote or inhibit the development our in vivo experiments suggest that the MC effects can stages II and III not receiving adjuvant chemotherapy. They further showed that MC density alters the CD8 T cell population.6 Our findings suggest that MC populations closely interacts with tumor-infiltrating lymphocytes. Our mouse-based dataset provides significant evidence that MCs could either promote or inhibit the development of colon tumors. Although tumor-primed MCs seemed to have some anticancer activity in a co-culture system, our in vivo experiments suggest that the MC effects can vary according to the type of stimuli promoting CRC. Whereas MCs would inhibit CD8 cell density but promote inflammation-related CRC (CA-CRC), they may be protective against the DNA damage-related form of this disease (sCRC). In a Kit mutant mouse strain other than the KitW/sh one (differences described by Grimbaldeston et al10, Wedemeyer and Galli reported that MC deficiency reduced carcinogen-induced colorectal tumor burden.9 We have also observed that KitW/sh and KitW/W- respond differently to the same carcinogen exposure (figures 3A and 5A and online supplemental figure 11).

Sinnamon et al reported that deleting adenomatous polyposis coli (Apc) in mice promotes TRY expression in tumors. By ablating the MC population in ApcMin/+ mice, they found that MC activity blocked tumor development.11 Bodduluri et al have also shown that chemokine-mediated MC recruitment initiates MC-dered leukotriene-regulated CD8+T cell homing and antitumor immunity reducing intestinal tumor burden in ApcMin/+ mice.13 In the CA-CRC model, Tanaka et al reported that MC deficient mice are less susceptible to inflammation-associated colorectal tumors.12 Rigoni et al also observed that MC deficiency increased the number of malignant lesions in the colon.11

We further demonstrated that MC activity alters pivotal immune mechanisms and vice-versa. Whether MC density seemed to determine the growth potential of carcinogen-induced lesions (figures 3, 5 and 6), an effect completely reversed in cancer (figure 7), essential immune factors also changed the MC activity. The balance between MHC-I and MHC-II levels altered MC-related chemokines release in our study. Le Bouteiller et al found that anti-MHC allo-antibodies modulate MC activity.36 When MCs were stimulated by antibody bipolar bridging, Daeron and Voisin showed that MHC-I reverse signaling modulates MC activity.37 38 Moreover, Malbec et al revealed that antigen recognition by FcγR-bound IgG on MCs could mediate transinhibition without co-clustering with FcεRI binding to IgE antibodies, a mechanism inhibiting oncospecific-induced proliferation in mastocytoma.39 Furthermore, MCs seem able to express MHCII in cell culture but not in vivo.1 40 Then, Dudek et al showed that innate-to-innate synapse-like contacts enable DC-to-MC molecule transfers including MHCII proteins and subsequent T cell priming.41

The Il6-related and Il13r-related signaling promoted opposing effects on MC activity during the early colon tumorigenic steps. Desai et al have shown that Il6 stimulation expands MC population and promotes its reactivity.42 It has been demonstrated that that differentiating CD34+ human cord blood cells into MCs requires stem cell factor stimulation but adding IL6 increases cell size and intracellular levels of chymase and histamine.43 In both human CRC and mouse polyposis, MCs can modulate and be modulated by Il10, Il6, Il17, and II2 levels, by which they recruit Treg and promote Treg-induced suppression of host-vs-graft rejection responses or render the Treg proinflammatory role furthering CRC development.43 Interestingly, Il13r seems required for CRC cellular expansion,45 while Il33 deficiency inhibited intestinal tumor burden and reduced intratumor MC density and MC-derived proteases and cytokines release.45 46 Moreover, Il11-induced tumor-derived Il33 signaling activates MCs and promotes gastric cancer development in mice. Genetically ablating Il33r blocks MC-dependent recruitment of macrophages limiting tumor growth.46

We further explored the idea that MCs are druggable targets against CRC. Pharmacologically inhibiting MC activity blocks tumor growth and can be combined with standard chemotherapy strategies. Pharmacologically inhibiting MCs activity has been shown an effective anti-cancer strategy against different types of tumors.48–50 Moreover, drug-based MC inhibition has been combined with gemcitabine, decreasing pancreatic cancer’s tumor growth.49 Also, it has also been associated with antiangiogenic therapy to treat murine lymphoma and pancreatic cancer.51 These facts illustrate that MCs are a druggable target that can potentialize the anticancer effects of current therapies. However, a potential limitation of our study is that in addition to inhibiting MC activity CA can impact cancer cell signaling and survival as well as other immune responses.52–55 The fact that CA treatment requires T lymphocytes to block tumor growth and can be combined with 5-FU strengthens the idea that CRC patients may be benefit from MC-based therapies.

Therefore, we suggest that MC activity can significantly impact CRC development in either a beneficial or harmful fashion; this effect seems to be substantially determined during the early stages of this disease. Moreover, this MC activity appears to be a druggable target with significant potential for anti-CRC therapeutic strategies.
REFERENCES

1. Chatterjee V, Gashev AA. Mast cell-directed recruitment of MHC class II positive cells and eosinophils towards mesenteric lymphatic vessels in adulthood and elderly. *Lymphat Res Biol* 2014;12:37–47.

2. Villa I, Skokos D, Tkaczyk C, et al. Capacity of mouse mast cells to prime T cells and to induce specific antibody responses in vivo. *Immunology* 2001;102:165–72.

3. Frandji P, Tkaczyk C, Oskeritzian C, et al. Exogenous and endogenous antigens are differently presented by mast cells to CD4+T lymphocytes. *Eur J Immunol* 1996;26:2517–20.

4. Dimitriadou V, Mecht S, Koutoulidis M, et al. Expression of functional major histocompatibility complex class II molecules on HMC-1 human mast cells. *J Leukoc Biol* 1998;64:791–9.

5. Mafette A, Selvestrin N, Saponaro C. High density of tryptase-positive mast cells in human colorectal cancer: a poor prognostic factor related to protease-activated receptor 2 expression. *J Cell Mol Med* 2013;17:1025–37.

6. Mao Y, Feng Q, Zhang P, et al. Low tumor infiltrating mast cell density confers prognostic benefit and reflects immunomodulation in colorectal cancer. *Int J Cancer* 2018;143:2271–80.

7. Mehdiawi L, Osman J, Topi G, et al. High tumor mast cell density is associated with longer survival of colon cancer patients. *Acta Oncol* 2016;55:1434–42.

8. Nielsen HJ, Hansen U, Christensen IJ, et al. Independent prognostic value of eosinophil and mast cell infiltration in colorectal cancer tissue. *J Pathol* 1999;189:487–95.

9. Wedemeyer J, Galli SJ. Decreased susceptibility of mast cell-deficient Kit(W/Kit(W-v)) mice to the development of 1,2-diarylhydrazine-induced intestinal tumors. *Lab Invest* 2005;85:588–98.

10. Matowi TK, El-Maraghy SA, ElMashad AN, et al. Cromolyn chitosan nanoparticles as a novel protective approach for colorectal cancer. *Chem Biol Interact* 2017;275:1–12.

11. Rigoni A, Bongiovanni L, Burocchi A, et al. Mast cells infiltrating inflamed or transformed gut alternatively sustain mucosal healing or tumor growth. *Cancer Res* 2015;75:3760–70.

12. Tanaka T, Ishikawa H. Mast cells and inflammation-associated colorectal carcinogenesis. *Semin Immunopathol* 2013;35:245–54.

13. Bodduluri SR, Mathis S, Maturu P, et al. Mast Cell-Dependent CD8+ T-cell Recruitment Mediates Immune Surveillance of Intestinal Tumors in Apo+-/- Mice. *Cancer Immunol Res* 2018;6:332–47.

14. Sinnamon MJ, Carter KJ, Sims LP, et al. A protective role of mast cells in intestinal tumorigenesis. *Carcinogenesis* 2008;29:880–6.

15. Newman AM, Liu CL, Green MR, et al. Robust enumeration of cell subsets from tissue expression profiles. *Nat Methods* 2015;12:453–7.

16. Thorsson V, Gibbs DL, Brown SD, et al. The immune landscape of cancer. *Immunity* 2019;51:411–2.

17. Townsend MJ, Fallon PG, Matthews DJ, et al. T1/ST2-deficient mice demonstrate the importance of T1/ST2 in developing primary T helper cell type 2 responses. *J Exp Med* 2000;191:1069–76.

18. Carlos D, Sá-Nunes A, da Paula L, et al. Histamine modulates mast cell degranulation through an indirect mechanism in a model IgE-mediated reaction. *Eur J Immunol* 2006;36:1494–503.

19. Wargovich MJ, Chen CD, Jimenez A, et al. Aberrant crypts as a biomarker for colon cancer: evaluation of potential chemopreventive agents in the rat. *Cancer Epidemiol Biomarkers Prev* 1996;5:355–60.

20. Kubota T, Fujita S, Kodaiz A, Antitumor activity of fluoropyrimidines and thymidylate synthetase inhibition. *Jpn J Cancer Res* 1991;82:476–82.

21. Neufert C, Becker C, Neurath MF. An inducible mouse model of colon carcinogenesis for the analysis of sporadic and inflammation-driven tumor progression. *Nat Protoc* 2007;2:1999–2004.

22. Sakita JY, Bader M, Santos ES, et al. Serotonin synthesis protects the mouse colonic crypt from DNA damage and colorectal tumorigenesis. *J Pathol* 2019;249:102–13.

23. Finneberg NK, Hart LS, Dolf D, et al. High-resolution imaging and antitumor effects of GFP(+) bone marrow-derived cells homing to syngeneic mouse colon tumors. *Am J Pathol* 2011;179:2169–76.

24. Leite CA, Mota JM, de Lima KA, et al. Paradoxical interaction between cancer and long-term postsepsis disorder: impairment of de novo carcinogenesis versus favoring the growth of established tumors. *J Immunother Cancer* 2020;8:8000129.

25. Ravindran A, Rönnberger D, Dahlin JS, et al. An optimized protocol for the isolation and functional analysis of human lung mast cells. *Front Immunol* 2019;8:2193.

26. Puck TT, Marcus PI. A rapid method for viable cell titration and clone production with HeLa cells in tissue culture: the use of x-irradiated cells to supply conditioning factors. *Proc Natl Acad Sci U S A* 1955;41:432–7.
27 Samoszuk M, Tan J, Chorn G. Clonogenic growth of human breast cancer cells co-cultured in direct contact with serum-activated fibroblasts. *Breast Cancer Res* 2005;7:R274–83.

28 Puebla-Osorio N, Sarchio SNE, Ullrich SE, et al. Detection of infiltrating mast cells using a modified toluidine blue staining. *Methods Mol Biol* 2017;1627:213–22.

29 Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-Seq data with DESeq2. *Genome Biol* 2014;15:550.

30 Computing RFIS. *A language and environment for statistical computing*, 2021.

31 Koller BH, Marrack P, Kappler JW, et al. Normal development of mice deficient in beta 2M, MHC class I proteins, and CD8+ T cells. *Science* 1990;248:1227–30.

32 Xing W, Austen KF, Gurish MF, et al. Protease phenotype of constitutive connective tissue and of induced mucosal mast cells in mice is regulated by the tissue. *Proc Natl Acad Sci U S A* 2011;108:14210–5.

33 Tan S-Y, Fan Y, Luo H-S, et al. Prognostic significance of cell infiltrations of immunosurveillance in colorectal cancer. *World J Gastroenterol* 2005;11:1210–4.

34 Tanis E, Jülie C, Emile J-F, et al. Prognostic impact of immune response in resectable colorectal liver metastases treated by surgery alone or surgery with perioperative FOLFOX in the randomised EORTC study 40983. *J Am Pathol* 2005;167:835–48.

35 Le Bouteiller P, Daëron M, Duc HT, et al. An ultrastructural study of two different responses of mouse mast cells to transplantation antibodies directed against the same transplantation antigens. *Eur J Immunol* 1976;6:326–32.

36 Daëron M, Voisin GA. H-2 antigens, on mast cell membrane, as target antigens for anaphylactic degranulation. *Cell Immunol* 1978;37:467–72.

37 Daëron M, Voisin GA. Mast cell membrane antigens and Fc receptors in anaphylaxis. I. products of the major histocompatibility complex involved in alloantibody-induced mast cell activation. *Immunology* 1979;38:447–58.

38 Malbec O, Cassard L, Albanesi M, et al. Trans-inhibition of activation and proliferation signals by Fc receptors in mast cells and basophils. *Sci Signal* 2016;9:ra126.

39 Kambayashi T, Allenspach EJ, Chang JT, et al. Inducible MHC class II expression by mast cells supports effector and regulatory T cell activation. *J Immunol* 2009;182:4686–95.

40 Dudeck J, Medyukhina A, Fröbel J, et al. Mast cells acquire MHCII from dendritic cells during skin inflammation. *J Exp Med* 2017;214:3791–811.

41 Desai A, Jung M-Y, Olivera A, et al. IL-6 promotes an increase in human mast cell numbers and reactivity through suppression of suppressor of cytokine signaling 3. *J Allergy Clin Immunol* 2016;137:1863–71.

42 Conti P, Kempuraj D, Di Gioacchino M, et al. Interleukin-6 and mast cells. *Allergy Asthma Proc* 2002;23:331–5.

43 Blatner NR, Bonertz A, Beckhove P, et al. In colorectal cancer mast cells contribute to systemic regulatory T-cell dysfunction. *Proc Natl Acad Sci U S A* 2010;107:9430–5.

44 O’Donnell C, Mahmoud A, Keane J, et al. An antitumorigenic role for the IL-33 receptor, ST2L, in colon cancer. *Br J Cancer* 2016;114:37–43.

45 Maywald RL, Doener SK, Pastorelli L, et al. IL-33 activates tumor stroma to promote intestinal polyposis. *Proc Natl Acad Sci U S A* 2015;112:E2487–96.

46 He Z, Chen L, Souto FO, et al. Epithelial-derived IL-33 promotes intestinal tumorgenesis in ApcMin/− mice. *Sci Rep* 2017;7:5520.

47 Eissmann MF, Dijkstra C, Jarnicki A, et al. IL-33-mediated mast cell activation promotes gastric cancer through macrophage mobilization. *Nat Commun* 2019;10:2735.

48 Johnson C, Huyhn V, Hargrove L, et al. Inhibition of mast cell-derived histamine decreases human cholangiocarcinoma growth and differentiation via c-KIT/sh cytokine-dependent signaling. *Am J Pathol* 2016;186:123–33.

49 Arumugam T, Ramachandran V, Logsdon CD. Effect of Cromolyn on S100P interactions with RAGE and pancreatic cancer growth and invasion in mouse models. *J Natl Cancer Inst* 2006;98:1806–18.

50 Wrobleswski M, Stauer R, Cubas Córdova M, et al. Mast cells decrease efficacy of anti-angiogenic therapy by secreting matrix-degrading granzyme B. *Nat Commun* 2017;8:269.

51 Matowii TMK, Bustanjy Y, El-Maraghy S, et al. Evaluation of naproxen and cromolyn activities against cancer cells viability, proliferation, apoptosis, p53, and gene expression of survivin and caspase-3. *J Enzyme Inhib Med Chem* 2014;29:153–61.

52 Puzzovio PG, Brüggemann TR, Pahima H, et al. Cromolyn sodium differentially regulates human mast cell and mouse leukocyte responses to control allergic inflammation. *Pharmacol Res* 2022;178:106172.
Supplementary Figures

| Mki67 | TRY | CD8 | CD3 |
|-------|-----|-----|-----|
| 1st Atb | Tumor | | |
| 2nd Atb | Tumor | | |
| 1st Atb | Brain | | |
| 2nd Atb | Brain | | |
| 1st Atb | Spleen | | |
| 2nd Atb | Spleen | | |

Suppl. Fig. 1. *Testing primary and secondary antibodies against positive and negative tissue samples.* To verify the working condition of our primary and secondary antibodies, we tested different antibodies (Suppl. Tab. 3) against samples from tumour (target tissue), spleen (positive control tissue), and brain (negative control tissue). Antibodies worked according to the manufacturer’s guideline.
Suppl. Fig. 2. Representative single images of multiplex IHC staining, and histopathological and gene expression analyses.

(A) Representative single images of multiplex IHC staining with anti-CD8 and anti-Mki67 antibodies (magnification at 200 µm).

(B, C) Histopathological (n = 8) and gene expression (n = 12) analyses for Mki67 (p > 0.05). Data are shown as the median, highest and lowest values, along with upper and lower quartiles. *P*-values were calculated using a two-tailed Mann-Whitney test.
**Suppl. Fig. 3.** Carcinogenic exposure alters CD8 T cell population in the colon.

(A – C) Graphs show percentages of CD8+, CD4+, and CD4+FOXP3+ T cells isolated from colon samples (n = 10; *p = 0.03). Data are shown as the median, highest and lowest values, along with upper and lower quartiles. *P*-values were calculated using a two-tailed Mann-Whitney test.
Suppl. Fig. 4. Multiplex IHC staining. Representative single images of multiplex IHC staining with anti-CD8, anti-CD3, and anti-TRY antibodies (magnification at 50 µm).
Suppl. Fig. 5. Inflammatory response alters MC population and colon tumorigenesis since its early steps.

(A – E) Histopathological analyses for single early tumorigenic lesions (A, n = 14, *p =0.0006; D, n = 8, *p =0.02), proliferation (B, n = 11, *p =0.006), and MC numbers (C, n = 8, *p =0.02; E, n = 8, *p =0.02) in colonic samples from NOD scidγ and Il6KO mice and their counterparts. Data are shown as individual data points with mean ± SEM. p-values were calculated using a two-tailed Mann-Whitney test.
Suppl. Fig. 6. The MC activity alter the immune response against CRC in mice.

(A – C) Gene expression analysis for Mki67 (n = 9; *p >0.05), Cd4 (*p =0.01), and Cd8 (*p =0.009). Data are shown as the median, highest and lowest values, along with upper and lower quartiles. P-values were calculated using a two-tailed Mann-Whitney test.
Suppl. Fig. 7. The MC activity can be therapeutically targeted against CRC in mice.

(A) Relative tumour growth between CA-treated (CA) and untreated (CT) mice for 15 days (n = 8).

(B) Relative tumour growth among untreated (CT), 5-FU-treated, and combined treatment of 5-FU and CA mice for 17 days (n = 15). Data are shown as mean ± SEM.
Suppl. Fig. 8. The MC activity can be therapeutically targeted against melanoma in mice. (A) Relative tumour growth between CA-treated (CA) and untreated (CT) mice for 15 days (n = 11). Data are shown as mean ± SEM. (B) Tumour weight between untreated and treated mice for 15 days (n = 11; *p = 0.03). (C, D) Graphs show percentages of MCs (CD45⁺CD117⁺FcεRI⁺) and CD45⁺CD8⁺CD3⁺ T cells isolated from tumour samples (n = 8; *p = 0.002). Data are shown as the median, highest and lowest values, along with upper and lower quartiles. P-values were calculated using a two-tailed Mann-Whitney test.
Suppl. Fig. 9. The MC activity can be therapeutically targeted against colorectal tumours in mice.

(A, B) Relative tumour growth and body weight between CA-treated (CA) and untreated (CT) mice for 15 days (n = 17). Data are shown as mean ± SEM.

(C) Graphs show percentages of MCs (CD45<sup>+</sup>CD117<sup>+</sup>FcεRI<sup>+</sup>) isolated from tumour samples (n = 8). Data are shown as the median, highest and lowest values, along with upper and lower quartiles. P-values were calculated using a two-tailed Mann-Whitney test.
Suppl. Fig. 10. The anticancer CA effects against colorectal tumours require T lymphocytes activity in mice.

(A – C) Relative tumour growth in CA treated and untreated Rag1KO mice for 14 days (n = 10; p > 0.05). Data are shown as individual data points with mean ± SEM. p-values were calculated using two-tailed Mann Whitney’s test.
Suppl. Fig. 11. *The MC activity alter colorectal tumorigenesis in mice.*

(A, B) Histopathological analyses for single early tumorigenic lesions (A, n = 11, *p* = 0.004), and proliferation (B, n = 11, *p* =0.002) in colonic samples from Kit^W/W^- mice and their counterparts. Data are shown as individual data points with mean ± SEM. *p*-values were calculated using a two-tailed Mann-Whitney test.
### Suppl. Tab. 1. List of TaqMan Probes used in the study.

| Targets | Probes ID |
|---------|-----------|
| Ctnnb1  | Mm00483039|
| Gapdh   | Mm99999915|
| Mki67   | Mm01278617|
| Mgmt    | Mm00485014|

### Suppl. Tab. 2. List of qPCR SYBR rimer sequences used in the study.

| Primers | Sequences                     |
|---------|-------------------------------|
| Cd11c F | TCTTCTGCTGGTGTTGGGTTTGG       |
| Cd11c R | CAGTTGCGCCTGTGTGATAGCC         |
| Cd4 F   | TCTGGCAACCTGTGACTCTGAC         |
| Cd4 R   | TCATCACCACCATGTCCTGCA          |
| Cd8 F   | CAGAGACCAGAAGATTGTCTCG         |
| Cd8 R   | TGATCAAGGACAGCAGAAAGG          |
| Cd3 F   | ATGCAGGTGGAACACTTTCCTTG        |
| Cd3 R   | GCACGTCAAACCTCACTAAGGTTG       |
| Foxp3 F | GCTGATCAATGCGTGGGTGGT         |
| Foxp3 R | GCCCTTCTCAGAGGACAGA           |
| Mcpt1 R | TCCAGGCGACATATGCAGAG          |
| Mcpt4 F | TCCAGGTGAGAGGTTGCTCA          |
| Mcpt4 R | CATGAAGCTCAAGGTTGAAC          |
| Mcpt6 F | ACGTGGCCCTGCTGAGGTCTG         |
| Mcpt6 R | CAGCAGATGTCCTCCCTGAGGG        |
| Gapdh F | TGGTGAAAGTCGGTGTGAAC          |
| Gapdh R | GACAGGCTTTCCATTTCTCG          |

### Suppl. Tab. 3. List of primary antibodies used in the study.

| Antibodies | ID          | Concentration |
|------------|-------------|---------------|
| Cd8        | Ab203035    | 1:150         |
| Cd3        | Ab5690      | 1:500         |
| GAPDH      | Ab181602    | 1:10000       |
| Mki67      | Ab16667     | 1:150         |
| Triptase   | Ab2378      | 1:150         |
| γH2AX      | Ab81299     | 1:5000        |
| CD45-FITC  | 11-0451-82  | 1:250         |
| CD45-APC   | 561873      | 1:250         |
| CD45-BV421 | 103133      | 1:300         |
| CD117-PE-Cy7 | 55863   | 1:250         |
| FcerRI-PE  | 134307      | 1:250         |
| CD8a-PE    | 553032      | 1:300         |
| CD3-FITC   | 557354      | 1:250         |
| CD4-FITC   | 100405      | 1:300         |
| Foxp3-APC  | 77-5775-40  | 1:300         |
Suppl. Tab. 4. Significantly altered genes by MC deficiency in sCRC lesions. Data were obtained by RNA-seq.

| Name                                           | Acronym | log2FC  | p-Adj     | p-Value       |
|------------------------------------------------|---------|---------|-----------|---------------|
| Chemokine (C-X-C motif) ligand 2               | Cxcl2   | 2.91762 | 5.50E-05  | 1.20E-07      |
| Interleukin 6                                  | Il6     | 2.76768 | 6.93E-03  | 2.88E-04      |
| Chemokine (C-X-C motif) ligand 1               | Cxcl1   | 2.41013 | 2.15E-03  | 3.29E-05      |
| Colony stimulating factor 2                    | Csf2    | 2.39663 | 2.15E-03  | 3.16E-05      |
| Interleukin 1 α                                | Il1a    | 2.32562 | 1.70E-03  | 1.67E-05      |
| Chemokine (C-X-C motif) ligand 3               | Cxcl3   | 2.17899 | 3.52E-03  | 1.04E-04      |
| Formyl peptide receptor 1                      | Fpr1    | 2.13251 | 3.99E-04  | 2.18E-06      |
| Chemokine (C-C motif) ligand 4                 | Ccl4    | 2.1307  | 4.82E-03  | 1.57E-04      |
| Interleukin 17A                                | Il17a   | 2.11911 | 1.90E-02  | 1.12E-03      |
| Interleukin 1 β                                | Il1b    | 2.107   | 4.82E-03  | 1.58E-04      |
| Chemokine (C-C motif) ligand 3                 | Ccl3    | 1.89559 | 1.13E-02  | 5.05E-04      |
| Nitric oxide synthase 2, inducible             | Nos2    | 1.73595 | 1.70E-03  | 1.51E-05      |
| Chemokine (C-C motif) receptor 10              | Ccr10   | 1.68575 | 2.81E-02  | 2.25E-03      |
| Chemokine (C-X-C motif) receptor 2             | Cxcr2   | 1.6386  | 1.13E-03  | 7.43E-06      |
| Histidine decarboxylase                        | Hdc     | 1.59195 | 3.94E-04  | 1.72E-06      |
| Regulator of G-protein signaling 16            | Rgs16   | 1.54682 | 2.90E-03  | 6.99E-05      |
| Chitinase-like 1                               | Chil1   | 1.50777 | 3.08E-03  | 8.43E-05      |
| G0/G1 switch gene 2                            | G0s2    | 1.49325 | 2.94E-02  | 2.41E-03      |
| Tumor necrosis factor receptor superfamily, member 9 | Tnfrsf9 | 1.47377 | 3.63E-02  | 3.01E-03      |
| Arginase 2                                     | Arg2    | 1.38296 | 2.15E-03  | 2.75E-05      |
| Formyl peptide receptor 2                      | Fpr2    | 1.38215 | 1.22E-02  | 6.10E-04      |
| Tumor necrosis factor (ligand) superfamily, member 9 | Tnfsf9 | 1.33416 | 6.27E-03  | 2.54E-04      |
| Interleukin 33                                 | Il33    | 1.29891 | 5.47E-03  | 2.05E-04      |
| CD80 antigen                                   | Cd80    | 1.25429 | 1.97E-02  | 1.19E-03      |
| Selectin, platelet                            | Selp    | 1.2436  | 2.66E-03  | 5.53E-05      |
| Granzyme B                                     | Gzmb    | 1.10501 | 5.47E-03  | 2.15E-04      |
| Inositol polyphosphate-4-phosphatase, type II  | Inpp4b  | 1.04564 | 2.68E-02  | 2.09E-03      |
| Serine hydroxymethyltransferase 2 (mitochondrial) | Shmt2 | 0.97879 | 3.63E-04  | 1.19E-06      |
| Nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor, α | Nfkbia | 0.96189 | 1.70E-03  | 1.39E-05      |
| CD14 antigen                                   | Cd14    | 0.87987 | 2.19E-03  | 3.82E-05      |
| Helicase, lymphoid specific                    | Hells   | 0.87468 | 2.19E-03  | 4.01E-05      |
| Cell division cycle 7                          | Cdc7    | 0.83777 | 2.05E-02  | 1.31E-03      |
### Tumor necrosis factor receptor superfamily, member 1b

**Tnfrsf1b**

| Gene         | Fold Change | p-value | q-value |
|--------------|-------------|---------|---------|
|              | 0.71521     | 2.02E-03| 2.20E-05|

### Serine/arginine-rich splicing factor 7

**Srsf7**

| Gene         | Fold Change | p-value | q-value |
|--------------|-------------|---------|---------|
|              | 0.71185     | 5.47E-03| 2.09E-04|

### BORA Aurora Kinase A Activator

**Bora**

| Gene         | Fold Change | p-value | q-value |
|--------------|-------------|---------|---------|
|              | 0.69255     | 2.40E-02| 1.73E-03|

### Activating transcription factor 4

**Atf4**

| Gene         | Fold Change | p-value | q-value |
|--------------|-------------|---------|---------|
|              | 0.68931     | 2.12E-02| 1.41E-03|

### Dystrobrevin, beta

**Dtnb**

| Gene         | Fold Change | p-value | q-value |
|--------------|-------------|---------|---------|
|              | 0.65154     | 3.81E-02| 3.25E-03|

### Regulatory factor X-associated protein

**Rfxap**

| Gene         | Fold Change | p-value | q-value |
|--------------|-------------|---------|---------|
|              | 0.56539     | 4.28E-02| 3.74E-03|

### Sphingomyelin synthase 1

**Sgms1**

| Gene         | Fold Change | p-value | q-value |
|--------------|-------------|---------|---------|
|              | 0.46723     | 2.99E-03| 7.83E-05|

### DEAD (Asp-Glu-Ala-Asp) box polypeptide 17

**Ddx17**

| Gene         | Fold Change | p-value | q-value |
|--------------|-------------|---------|---------|
|              | 0.43006     | 2.29E-02| 1.58E-03|

### Downregulated genes

| Gene                                                      | Fold Change | p-value  | q-value  |
|-----------------------------------------------------------|-------------|----------|----------|
| Serine (or cysteine) peptidase inhibitor, clade A, member 1D | -5.0011     | 1.22E-02 | 6.16E-04|
| Somatostatin receptor 3                                   | -4.0509     | 7.35E-03 | 3.14E-04|
| Carboxypeptidase A3, mast cell                           | -3.6243     | 2.15E-03 | 3.08E-05|
| Chromogranin B                                           | -2.3208     | 4.90E-03 | 1.66E-04|
| Endothelin 2                                              | -2.1053     | 1.14E-02 | 5.22E-04|
| Monoamine oxidase B                                      | -2.0844     | 1.97E-02 | 1.21E-03|
| Collagen, type VIII, alpha 2                             | -1.9191     | 4.87E-02 | 4.38E-03|
| Carcinoembryonic antigen-related cell adhesion molecule 13 | -1.9147     | 1.14E-02 | 5.49E-04|
| RAR-related orphan receptor gamma                         | -1.9034     | 1.14E-02 | 5.49E-04|
| Vitamin D receptor                                       | -1.8635     | 2.40E-02 | 1.72E-03|
| Chemokine (C motif) ligand 1                             | -1.8057     | 5.47E-03 | 2.08E-04|
| Solute carrier family 24 (sodium/potassium/calcium exchanger), member 3 | -1.7958     | 1.37E-02 | 7.17E-04|
| Hydroxyprostaglandin dehydrogenase 15 (NAD)              | -1.7713     | 2.05E-02 | 1.35E-03|
| Kit oncogene                                              | -1.6707     | 5.47E-03 | 2.11E-04|
| Fibroblast growth factor 18                              | -1.6239     | 3.35E-03 | 9.53E-05|
| RAB38, member RAS oncogene family                        | -1.4745     | 2.05E-02 | 1.34E-03|
| Myosin XVB                                               | -1.4343     | 2.68E-02 | 2.07E-03|
| MHC I like leukocyte 2                                    | -1.4073     | 2.58E-02 | 1.92E-03|
| SH3 domain and tetra tri peptide repeats 1               | -1.3104     | 1.38E-02 | 7.41E-04|
| Glucosaminyl (N-acetyl) transferase 1, core 2            | -1.1739     | 4.10E-02 | 3.54E-03|
| Myocyte enhancer factor 2C                               | -1.1546     | 3.68E-03 | 1.13E-04|
| Melanophilin                                              | -1.1311     | 2.58E-02 | 1.89E-03|
| KN motif and ankyrin repeat domains 2                    | -1.0783     | 1.38E-02 | 7.55E-04|
| Gene Name                                                                 | Symbol | log2FC | padj  | fold change |
|--------------------------------------------------------------------------|--------|--------|-------|-------------|
| Prostaglandin-endoperoxide synthase 1                                   | Ptgs1  | -1.0425| 1.71E-02| 9.74E-04    |
| Inositol polyphosphate-4-phosphatase, type II                           | Inpp4b | -1.0258| 2.84E-03| 6.53E-05    |
| Epithelial membrane protein 1                                            | Emp1   | -0.9828| 1.26E-02| 6.50E-04    |
| Erb-b2 receptor tyrosine kinase 2                                        | Erbb2  | -0.9277| 2.99E-03| 7.84E-05    |
| Interleukin 15                                                           | Il15   | -0.8982| 3.80E-02| 3.21E-03    |
| Avian musculoaponeurotic fibrosarcoma oncogene homolog                   | Kank2  | -0.888 | 2.81E-02| 2.27E-03    |
| RNA binding motif protein 3                                              | Rbm3   | -0.8148| 4.50E-02| 3.99E-03    |
| Zinc finger E-box binding homeobox 1                                     | Zeb1   | -0.8086| 2.19E-03| 4.21E-05    |
| ATPase Phospholipid Transporting 9A (Putative)                          | Atp9a  | -0.7628| 1.88E-02| 1.09E-03    |
| Serine (or cysteine) peptidase inhibitor, clade B, member 6a             | Serpinb6a | -0.7483| 2.19E-02| 1.48E-03    |
| Cathepsin S                                                              | Ctss   | -0.7402| 4.87E-02| 4.42E-03    |
| Glypican 4                                                               | Gpc4   | -0.7059| 2.03E-05| 2.22E-08    |
| Forkhead box N3                                                          | Foxn3  | -0.6665| 2.19E-03| 4.31E-05    |
| Sphingomyelin phosphodiesterase 3, neutral                               | Smpd3  | -0.6579| 2.68E-02| 2.10E-03    |
| Proteasome (prosome, macropain) 26S subunit, non-ATPase, 11              | Psmd11 | -0.6125| 2.75E-03| 6.02E-05    |
| Nuclear mitotic apparatus protein 1                                      | Numa1  | -0.5496| 1.71E-02| 9.65E-04    |
| Catalase                                                                 | Cat    | -0.5231| 1.13E-02| 5.07E-04    |
| Protein phosphatase 1, regulatory (inhibitor) subunit 2                  | Ppp1r2 | -0.5019| 2.68E-02| 2.11E-03    |
| von Willebrand factor A domain containing 5A                             | Vwa5a  | -0.4409| 1.97E-02| 1.23E-03    |
| Inhibitor of kappaB kinase γ                                             | Ikbkγ  | -0.4047| 2.29E-02| 1.60E-03    |

According to their log2FC values, genes are shown in descending order.
## Suppl. Tab. 5: Pathways significantly altered by MC deficiency in sCRC lesions. Data were obtained by RNA-seq and analysed on DAVID (Bioinformatic Database).

| Pathway                              | Status     | p-Value    | Genes                  |
|--------------------------------------|------------|------------|------------------------|
| Cytokine-cytokine receptor interaction | Upregulated| 9.80E-11   | Ccl3, Ccl4, Ccr10, Cxcl1, Cxcl2, Cxcr2, Csf2, Il1a, Il1b, Il17a, Il6, Tnfsf9, Tnfrsf1b, Tnfrsf9 |
|                                      | Downregulated| 9.80E-11  | Xcl1, Il15             |
| TNF signaling                        | Upregulated| 4.40E-09   | Atf4; Cxcl1, Cxcl2, Cxcl3, Csf2, Il1b, Il6, Nfkbia, Tnfrsf1b |
|                                      | Downregulated| 4.40E-09  | Ikbkg, Il15            |
| Toll-like receptor signaling         | Upregulated| 7.50E-06   | Cd14, Cd80, Cxcl3, Cxcl4, Il1b, Il6, Nfkbia |
|                                      | Downregulated| 7.50E-06  | Ikbkg                  |
| Chemokine signaling                  | Upregulated| 1.00E-05   | Ccl3, Ccl4, Ccr10, Cxcl1, Cxcl2, Cxcl3, Cxcr2, Nfkbia |
|                                      | Downregulated| 1.00E-05  | Ikbkg, Cxcl1           |
| NOD-like receptor signaling          | Upregulated| 5.40E-05   | Cxcl1, Cxcl2, Il1b, Il6, Nfkbia |
|                                      | Downregulated| 5.40E-05  | Ikbkg                  |
| Inflammatory bowel disease (IBD)     | Upregulated| 6.30E-05   | Il1a, Il1b, Il17a, Il6 |
| Cytosolic DNA-sensing                | Upregulated| 9.40E-05   | Ccl4, Il1b, Il33, Il6, Nfkbia |
|                                      | Downregulated| 9.40E-05  | Ikbkg                  |
| Transcriptional dysregulation in cancer | Upregulated| 1.20E-03   | Cd14, Csfr2, Gzmb, Il6 |
|                                      | Downregulated| 1.20E-03  | Maf, Hpgd, Mef2c       |
| Intestinal immune network for IgA production | Upregulated| 3.40E-03  | Cd80, Ccr10, Il15, Il6 |
| NF-κβ signaling                      | Upregulated| 5.30E-03   | Cd14, Ccl4, Il1b, Nfkbia |

Acronyms meaning is given in Suppl. Tab. 4.
**Suppl. Tab. 6.** Significantly altered genes by MC deficiency in early colon tumorigenic lesions. Data were obtained by RNA-seq.

| Name                                           | Acronym | log2FC | p-Adj    | p-Value  |
|------------------------------------------------|---------|--------|----------|----------|
| Chemokine (C-X-C motif) ligand 5               | Cxcl5   | 3.22413| 1.82E-02 | 1.62E-03 |
| Chemokine (C-X-C motif) ligand 13              | Cxcl13  | 2.58874| 2.24E-06 | 4.79E-08 |
| Chemokine (C-X-C motif) receptor 5             | Cxcr5   | 2.51089| 2.29E-06 | 5.14E-08 |
| Z-DNA binding protein 1                        | Zbp1    | 2.1316 | 3.00E-04 | 1.11E-05 |
| Interferon-induced protein with tetratricopeptide repeats 3 | Ifit3  | 1.76255| 6.40E-03 | 4.42E-04 |
| Interleukin 18                                 | Il18    | 1.52986| 4.68E-03 | 3.00E-04 |
| Chemokine (C-C motif) receptor 6               | Ccr6    | 1.37639| 1.35E-03 | 6.35E-05 |
| Epithelial membrane protein 1                  | Emp1    | 1.28044| 7.14E-06 | 1.77E-07 |
| Histocompatibility 2, class II antigen A, β 1  | H2-Ab1  | 1.23486| 3.00E-04 | 1.11E-05 |
| Interleukin 21 receptor                        | Il21r   | 1.21134| 5.61E-04 | 2.27E-05 |
| Tumor necrosis factor receptor superfamily, member 10b | Tnfrsf10b | 1.19788| 1.50E-02 | 1.28E-03 |
| Nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor, α | Nfkbia | 1.1736 | 8.73E-04 | 3.81E-05 |
| Guanylate binding protein 2b                   | Gbp2b   | 1.15584| 1.28E-02 | 1.03E-03 |
| Anti-Mullerian hormone type 2 receptor         | Amhr2   | 1.08817| 8.73E-04 | 3.82E-05 |
| Selectin, platelet (p-selectin) ligand         | Selplg  | 1.02693| 3.21E-02 | 3.35E-03 |
| Mucosal vascular addressin cell adhesion molecule 1 | Madcam1 | 1.01299| 4.93E-03 | 3.21E-04 |
| TRAF3 interacting protein 3                    | Traf3ip3| 0.93662| 1.10E-02 | 8.53E-04 |
| Cytidine deaminase                            | Cda     | 0.9289 | 6.94E-03 | 4.99E-04 |
| 2'-5' oligoadenylate synthetase 1A             | Oas1a   | 0.87346| 2.94E-05 | 8.59E-07 |
| Histocompatibility 2, class II antigen E β2    | H2-Eb2  | 0.83882| 1.43E-02 | 1.21E-03 |
| Chemokine (C-C motif) ligand 19                | Ccl19   | 0.80013| 1.84E-03 | 9.30E-05 |
### Histocompatibility 2, class II antigen A, α

| Gene      | Log2 Fold Change | p-Value     |
|-----------|-----------------|-------------|
| H2-Aa     | 0.70998         | 8.82E-03    |

### Lymphotoxin B

| Gene | Log2 Fold Change | p-Value     |
|------|-----------------|-------------|
| Ltb  | 0.70568         | 2.76E-02    |

### RAB27A, member RAS oncogene family

| Gene   | Log2 Fold Change | p-Value     |
|--------|-----------------|-------------|
| Rab27a | 0.69102         | 2.03E-02    |

### Guanylate binding protein 2

| Gene | Log2 Fold Change | p-Value     |
|------|-----------------|-------------|
| Gbp2 | 0.65227         | 2.63E-02    |

### Endothelin 1

| Gene | Log2 Fold Change | p-Value     |
|------|-----------------|-------------|
| Edn1 | 0.6305          | 2.63E-03    |

### Transmembrane and immunoglobulin domain containing 1

| Gene   | Log2 Fold Change | p-Value     |
|--------|-----------------|-------------|
| Tmigd1 | 0.62949         | 2.04E-03    |

### Interferon regulatory factor 7

| Gene | Log2 Fold Change | p-Value     |
|------|-----------------|-------------|
| Irf7  | 0.61944         | 1.94E-03    |

### Heparan sulfate (glucosamine) 3-O-sulfotransferase 3B1

| Gene   | Log2 Fold Change | p-Value     |
|--------|-----------------|-------------|
| Hs3st3b1 | 0.56872     | 9.59E-03    |

### SMAD family member 3

| Gene | Log2 Fold Change | p-Value     |
|------|-----------------|-------------|
| Smad3 | 0.53498        | 2.51E-02    |

### Nuclear antigen Sp100

| Gene   | Log2 Fold Change | p-Value     |
|--------|-----------------|-------------|
| Sp100  | 0.48685         | 4.98E-02    |

### Downregulated genes

| Gene   | Log2 Fold Change | p-Value     |
|--------|-----------------|-------------|
| Cr2    | -5.2193         | 4.19E-02    |
| Mt2    | -4.0879         | 9.89E-06    |
| Ccl2   | -3.6923         | 2.63E-03    |
| Cd2    | -3.4363         | 6.10E-08    |
| Pla2g7 | -3.2754         | 8.39E-09    |
| Cybb   | -3.2693         | 3.25E-06    |
| Oas3   | -3.2321         | 2.98E-43    |
| H2-M3  | -3.1173         | 1.28E-09    |
| Tnf    | -3.0967         | 2.07E-38    |
| Ceacam13 | -3.0565      | 7.45E-13    |
| Bstl   | -3.0226         | 3.00E-04    |
| Cd48   | -2.9478         | 2.17E-07    |
| Oasl1  | -2.9434         | 2.68E-37    |

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| Gene Name                                      | Symbol | Log2 Fold Change | P-Value  | Adjusted P-Value |
|-----------------------------------------------|--------|-----------------|----------|-----------------|
| Chemokine (C-X-C motif) ligand 1              | Cxcl1  | -2.9432         | 8.20E-05 | 2.58E-06        |
| SMAD family member 2                         | Smad2  | -2.5089         | 4.98E-02 | 5.73E-03        |
| Histone cluster 1, H1c                       | Hist1h1c| -2.4994         | 1.96E-12 | 1.54E-14        |
| Chemokine (C-X-C motif) ligand 2             | Cxcl2  | -2.4367         | 1.95E-09 | 2.85E-11        |
| Fucosyltransferase 4                         | Fut4   | -2.4197         | 8.04E-11 | 7.23E-13        |
| CD177 antigen                                 | Cd177  | -2.3846         | 4.40E-03 | 2.72E-04        |
| Carbonic anhydrase 4                         | Car4   | -2.3158         | 1.39E-03 | 6.86E-05        |
| B lymphoid kinase                            | Blk    | -2.1328         | 1.23E-05 | 3.32E-07        |
| Lymphocyte antigen 75                        | Ly75   | -2.0604         | 8.95E-04 | 4.12E-05        |
| Histocompatibility 2, T region locus 24      | H2-T24 | -2.0561         | 1.29E-02 | 1.07E-03        |
| CD74 antigen                                  | Cd74   | -1.9534         | 5.91E-04 | 2.46E-05        |
| T cell receptor associated transmembrane adaptor 1 | Trat1 | -1.9484         | 3.03E-02 | 3.07E-03        |
| Carboxypeptidase A3, mast cell               | Cpa3   | -1.8726         | 1.90E-02 | 1.73E-03        |
| Kit oncogene                                  | Kit    | -1.8279         | 2.24E-13 | 1.26E-15        |
| Lymphocyte transmembrane adaptor 1           | Lax1   | -1.8135         | 3.12E-02 | 3.19E-03        |
| Kynureninase (L-kynurenine hydrolase)        | Knu    | -1.7685         | 1.34E-09 | 1.81E-11        |
| Interferon induced transmembrane protein 3   | Ifitm3 | -1.7126         | 6.18E-05 | 1.87E-06        |
| Interferon regulatory factor 1               | Irfl   | -1.7119         | 2.69E-02 | 2.66E-03        |
| CD22 antigen                                  | Cd22   | -1.6939         | 1.26E-02 | 1.00E-03        |
| B and T lymphocyte associated                 | Btla   | -1.6385         | 9.68E-10 | 9.78E-12        |
| CD69 antigen                                  | Cd69   | -1.6102         | 1.48E-05 | 4.15E-07        |
| Malic enzyme 1, NADP(+)‐dependent, cytosolic | Me1    | -1.5944         | 1.90E-02 | 1.71E-03        |
| DEAD (Asp-Glu-Ala-Asp) box polypeptide 58    | Ddx58  | -1.5393         | 2.30E-03 | 1.27E-04        |
| Beta-2 microglobulin                         | B2m    | -1.5158         | 4.51E-03 | 2.84E-04        |
| Spi-B transcription factor (Spi-1/PU.1 related) | Spib  | -1.4302         | 1.34E-09 | 1.69E-11        |
| Gene Name                      | Symbol | log2 Fold Change | p-value     | FDR           |
|-------------------------------|--------|-----------------|-------------|--------------|
| Glypican 4                    | Gpc4   | -1.4035         | 4.95E-02    | 5.56E-03     |
| CD52 antigen                  | Cd52   | -1.3942         | 5.39E-03    | 3.58E-04     |
| 2'-5' oligoadenylate synthetase 1G | Oas1g  | -1.3506         | 4.18E-04    | 1.64E-05     |
| Three prime repair exonuclease 1 | Trex1  | -1.3436         | 2.29E-03    | 1.23E-04     |
| Optineurin                    | Optn   | -1.3247         | 1.25E-08    | 2.11E-10     |
| Apolipoprotein D              | Apod   | -1.2874         | 6.43E-03    | 5.00E-04     |
| Myocyte enhancer factor 2C   | Mef2c  | -1.2523         | 1.53E-04    | 4.55E-04     |
| Lectin, galactose binding, soluble 9 | Lgals9 | -1.2305         | 1.06E-02    | 8.10E-04     |
| 2'-5' oligoadenylate synthetase 1B | Oas1b  | -1.2252         | 3.00E-03    | 1.79E-04     |
| Signal transducer and activator of transcription 3 | Stat3  | -1.2118         | 3.33E-02    | 3.52E-03     |
| DnaJ heat shock protein family (Hsp40) member B1 | Dnajb1 | -1.1764         | 3.00E-04    | 1.08E-05     |
| LPS-responsive beige-like anchor | Lrba   | -1.1744         | 8.80E-04    | 3.95E-05     |
| Myelin and lymphocyte protein, T cell differentiation protein | Mal    | -1.1117         | 4.98E-02    | 5.76E-03     |
| Immunoglobulin kappa constant | Igkc   | -1.1016         | 2.44E-03    | 1.37E-04     |
| Interferon Induced Transmembrane Protein 3 | Iftm3  | -1.1015         | 2.44E-03    | 1.37E-04     |
| Serine (or cysteine) preptidase inhibitor, clade A, member 1B | Serpina1b | -0.9767        | 1.92E-02    | 1.77E-03     |
| Acid phosphatase, prostate     | Acpp   | -0.9537         | 4.04E-04    | 1.54E-05     |
| Membrane-spanning 4-domains, subfamily A, member 1 | Ms4a1  | -0.9392         | 3.14E-02    | 3.24E-03     |
| B cell scaffold protein with ankyrin repeats 1 | Bank1  | -0.8961         | 5.07E-07    | 1.03E-08     |
| Indoleamine 2,3-dioxygenase 1 | Idol   | -0.8774         | 1.63E-02    | 1.43E-03     |
| 2'-5' oligoadenylate synthetase 2 | Oas2   | -0.8606         | 2.10E-02    | 1.99E-03     |
| Protein tyrosine phosphatase, receptor type, C | Ptprc  | -0.8081         | 1.63E-02    | 1.43E-03     |
| Transient receptor potential cation channel, subfamily V, member 6 | Trpv6  | -0.7858         | 6.26E-03    | 4.22E-04     |
| IKAROS family zinc finger 2   | Ikarz2 | -0.7351         | 6.40E-03    | 4.46E-04     |
| Histocompatibility 2, O region alpha locus | H2-Oa  | -0.6812         | 1.28E-02    | 1.05E-03     |
| Gene Name | Symbol | log2FC Value | P-value | Fold Change |
|-----------|--------|--------------|---------|-------------|
| Carcinoembryonic antigen-related cell adhesion molecule 12 | Ceacam12 | -0.6733 | 4.92E-02 | 5.48E-03 |
| Zinc and ring finger 1 | Znrf1 | -0.6444 | 3.93E-02 | 4.24E-03 |
| Regulator of G-protein signaling 16 | Rgs16 | -0.6152 | 7.96E-03 | 5.82E-04 |
| Paired box 5 | Pax5 | -0.5946 | 2.49E-02 | 2.38E-03 |
| CD19 antigen | Cd19 | -0.5853 | 3.25E-03 | 1.97E-04 |
| Bone marrow stromal cell antigen 2 | Bst2 | -1.582 | 4.19E-02 | 4.61E-03 |
| CD72 antigen | Cd72 | -1.565 | 1.11E-02 | 8.72E-04 |
| Lysophosphatidylcholine acyltransferase 4 | Lpcat4 | -1.355 | 2.42E-15 | 1.09E-17 |
| Protein tyrosine phosphatase, receptor type, J | Ptpn1 | -1.196 | 1.37E-03 | 6.61E-05 |

Genes are shown in descending order (log2FC values).
Suppl. Tab. 7: Pathways significantly altered by MC deficiency in sCRC preneoplastic lesions. Data were obtained by RNA-seq and analysed on DAVID (Bioinformatic Database).

| Pathway                                      | Status       | p-Value      | Genes                                                                 |
|----------------------------------------------|--------------|--------------|----------------------------------------------------------------------|
| Inflammatory bowel disease (IBD)             | Upregulated  | 3.40E-08     | Smad3, H2-Aa, H2-Ab1, Il18, Il21r                                   |
|                                              | Downregulated| 3.40E-08     | Smad2, H2-Oa, Stat3, Tnf                                            |
| Cytokine-cytokine receptor interaction       | Upregulated  | 1.20E-07     | Amhr2, Ccl19, Ccr6, Cxcl13, Cxcl5, Cxcr5, Il18, Il21r, Ltb, Tnfsf10b|
|                                              | Downregulated| 1.20E-07     | Ccl2, Cxcl1, Cxcl2, Tnf                                            |
| Antigen processing and presentation          | Upregulated  | 6.30E-06     | H2-Aa, H2-Ab1                                                       |
|                                              | Downregulated| 6.30E-06     | Cd74, B2m, H2-M3, H2-Oa, H2-T24, Tnf                                |
| Cell adhesion molecules (CAMs)               | Upregulated  | 9.80E-06     | H2-Aa, H2-Ab1, Madcam1, Selplg                                      |
|                                              | Downregulated| 9.80E-06     | Cd2, Cd22, H2-M3, H2-Oa, H2-T24, Pipec                                |
| Chemokine signaling pathway                 | Upregulated  | 4.50E-05     | Ccl19, Ccr6, Cxcl13, Cxcl5, Cxcr5, Nfipia                           |
|                                              | Downregulated| 4.50E-05     | Ccl2, Cxcl1, Cxcl2, Stat3                                           |
| NOD-like receptor signaling pathway          | Downregulated| 1.30E-04     | Ccl2, Cxcl1, Cxcl2, Tnf                                            |
| Cytosolic DNA-sensing pathway                | Upregulated  | 2.20E-04     | Zbp1, Irf7, Il18, Nfkbia                                           |
|                                              | Downregulated| 2.20E-04     | Ddx58, Trex1                                                      |
| TNF signaling                                | Downregulated| 2.50E-03     | Ccl2, Cxcl1, Cxcl2, Tnf                                            |
| B cell receptor signaling                    | Downregulated| 3.10E-03     | Cd19, Cd22, Cd72, Cr2                                               |
| TGF-beta signaling                           | Upregulated  | 3.80E-02     | Smad3, Amhr2                                                        |
|                                              | Downregulated| 3.80E-02     | Smad2, Tnf                                                         |

Acronyms meaning is given in Suppl. Tab. 6.
Suppl. Tab. 8. Mast cells are drugable targets altering the development of early colon tumorigenic events (AOM vs AOM+CA). Data were obtained by RNA-seq.

| Name                                           | Acronym | log2FC | p-Adj  | p-Value  |
|------------------------------------------------|---------|--------|--------|----------|
| **Upregulated genes**                          |         |        |        |          |
| ATP-binding cassette, sub-family G (WHITE), member 2 | Abcg2   | 0.9441 | 1.38E-03 | 1.15E-05 |
| CDC14 cell division cycle 14A                   | Cdc14a  | 0.6245 | 7.49E-03 | 1.34E-04 |
| Frizzled class receptor 2                       | Fzd2    | 0.5464 | 4.26E-02 | 1.87E-03 |
| Caspase 1                                       | Casp1   | 0.4698 | 4.26E-02 | 1.77E-03 |
| Abhydrolase domain containing 2                 | Abhd2   | 0.4607 | 4.64E-02 | 2.10E-03 |
| Nedd4 family interacting protein 1              | Ndfip1  | 0.4589 | 1.95E-02 | 5.34E-04 |
| Chemokine (C-X-C motif) ligand 12                | Cxcl12  | 0.4473 | 2.23E-02 | 7.68E-04 |
| **Downregulated genes**                         |         |        |        |          |
| Chemokine (C-C motif) ligand 19                  | Ccl19   | -3.5472| 6.04E-04 | 3.59E-06 |
| Interleukin 13                                   | Il13    | -3.4608| 4.91E-02 | 2.40E-03 |
| T cell receptor associated transmembrane adaptor 1 | Trat1   | -2.8763| 2.23E-02 | 7.54E-04 |
| Membrane-spanning 4-domains, subfamily A, member 1 | Ms4a1  | -2.3658| 4.91E-02 | 2.34E-03 |
| Complement receptor 2                           | Cr2     | -2.3086| 4.90E-03 | 7.57E-05 |
| CD19 antigen                                    | Cd19    | -1.9972| 1.29E-03 | 9.18E-06 |
| Carcinoembryonic antigen-related cell adhesion molecule 12 | Ceacam1 | -1.9074| 1.53E-02 | 3.81E-04 |
| Chemokine (C-X-C motif) ligand 5                | Cxcl5   | -1.794 | 8.22E-03 | 1.63E-04 |
| Nitric oxide synthase 2, inducible              | Nos2    | -1.7376| 4.26E-02 | 1.83E-03 |
| Ubiquitin D                                     | Ubd     | -1.5843| 3.95E-04 | 1.88E-06 |
| CD22 antigen                                    | Cd22    | -1.5688| 2.08E-02 | 5.93E-04 |
| Dual specificity phosphatase 2                  | Dusp2   | -1.5562| 4.90E-03 | 7.42E-05 |
| Granzyme A                                      | Gzma    | -1.5448| 1.12E-02 | 2.40E-04 |
| Suppressor of cytokine signaling 1              | Socs1   | -1.4443| 1.38E-02 | 3.21E-04 |
| Gene Name                                           | Log2FC | Log2FoldChange | Adjusted p-Value |
|-----------------------------------------------------|--------|----------------|-----------------|
| Spi-B transcription factor                          | -1.4357| 2.23E-02       | 7.69E-04        |
| Chemokine (C-C motif) receptor 6                    | -1.3625| 4.71E-02       | 2.19E-03        |
| Zeta-chain associated protein kinase                | -1.1181| 2.25E-02       | 8.01E-04        |
| CD72 antigen                                        | -1.0918| 3.63E-02       | 1.38E-03        |
| Serine peptidase inhibitor, clade A, member 3N      | -1.0806| 1.38E-02       | 3.27E-04        |
| Proteasome subunit, beta type 9                     | -1.0404| 1.91E-06       | 2.27E-09        |
| Chemokine (C-C motif) ligand 5                      | -1.0292| 1.58E-04       | 5.63E-07        |
| Z-DNA binding protein 1                             | -0.964 | 3.60E-03       | 4.70E-05        |
| Natural killer cell group 7 sequence                | -0.8674| 2.32E-03       | 2.21E-05        |
| Transporter 1, ATP-binding cassette, sub-family B   | -0.8594| 8.22E-03       | 1.66E-04        |
| CD74 antigen                                        | -0.7109| 4.47E-05       | 1.06E-07        |
| Centromere protein F                                | -0.705 | 1.64E-02       | 4.28E-04        |
| Histocompatibility 2, class II antigen A, \(\beta \)1 | -0.7036| 6.03E-03       | 1.00E-04        |
| Antigen identified by monoclonal antibody Ki 67     | -0.6661| 2.39E-02       | 8.81E-04        |
| Baculoviral IAP repeat-containing 5                 | -0.6435| 2.23E-02       | 7.02E-04        |
| Histocompatibility 2, Q region locus 4              | -0.624 | 2.22E-02       | 6.60E-04        |
| Histocompatibility 2, class II antigen A, \(\alpha \) | -0.5918| 2.86E-03       | 3.40E-05        |
| Histocompatibility 2, class II antigen E, \(\beta \) | -0.5903| 2.86E-03       | 3.20E-05        |
| Histocompatibility 2, M region locus 3              | -0.5693| 3.97E-02       | 1.56E-03        |
| Proteasome subunit, beta type 10                    | -0.4267| 3.99E-02       | 1.61E-03        |

According to their log2FC values, genes are shown in descending order.
### Suppl. Tab. 9. Mast cells are drugable targets altering the development of early tumorigenic events in the colon (AOM vs AOM → CA). Data were obtained by RNA-seq.

| Name                                                                 | Acronym | log2FC  | p-Adj     | p-Value   |
|----------------------------------------------------------------------|---------|---------|-----------|-----------|
| **Upregulated genes**                                                |         |         |           |           |
| γ-glutamyltransferase 1                                             | Ggt1    | 2.69378 | 4.80E-02  | 2.10E-03  |
| Chemokine (C-C motif) ligand 3                                      | Ccl3    | 1.53774 | 4.76E-02  | 2.00E-03  |
| Leucine rich repeat containing 32                                   | Lrrc32  | 1.29369 | 2.59E-02  | 6.35E-04  |
| CD36 antigen                                                        | Cd36    | 1.083   | 2.87E-02  | 7.37E-04  |
| Interleukin 15                                                      | Il15    | 1.04341 | 4.90E-02  | 2.23E-03  |
| Hairy/enhancer-of-split related with YRPW motif 1                   | Hey1    | 1.00961 | 4.76E-02  | 1.96E-03  |
| Alanyl (membrane) aminopeptidase                                     | Anpnp   | 0.83758 | 3.15E-02  | 1.06E-03  |
| Spondin 2. extracellular matrix protein                              | Spon2   | 0.80936 | 1.41E-02  | 1.97E-04  |
| Cysteine-rich secretory protein LCCL domain containing 2            | Crispld2| 0.7985  | 2.38E-02  | 4.99E-04  |
| CD34 antigen                                                        | Cd34    | 0.79222 | 1.23E-02  | 1.20E-04  |
| Collagen. type III. alpha 1                                         | Col3a1  | 0.61973 | 2.10E-02  | 3.91E-04  |
| Frizzled class receptor 2                                           | Fzd2    | 0.6149  | 2.16E-02  | 4.28E-04  |
| Vascular endothelial growth factor C                                 | Vegfc   | 0.59837 | 4.80E-02  | 2.13E-03  |
| Acid phosphatase. prostate                                          | Acpp    | 0.59654 | 4.44E-02  | 1.76E-03  |
| Insulin-like growth factor binding protein 5                        | Igbp5   | 0.56027 | 3.11E-02  | 9.31E-04  |
| Chemokine (C-X-C motif) ligand 12                                    | Cxcl12  | 0.54604 | 6.64E-03  | 3.87E-05  |
| CDC14 cell division cycle 14A                                        | Cdc14a  | 0.536   | 3.11E-02  | 9.68E-04  |
| **Downregulated genes**                                             |         |         |           |           |
| Chemokine (C-C motif) ligand 19                                      | Ccl19   | -4.111  | 4.56E-05  | 5.32E-08  |
| T cell receptor associated transmembrane adaptor 1                  | Trat1   | -3.2211 | 1.23E-02  | 1.58E-04  |
| Cochlin                                                             | Coch    | -3.1581 | 4.44E-02  | 1.74E-03  |
| Carcinoembryonic antigen-related cell adhesion molecule 12          | Ceacam12| -2.6061 | 2.15E-04  | 7.51E-07  |
| Gene Name                                                                 | Symbol | Log2 Fold Change | p-Value       | q-Value       |
|--------------------------------------------------------------------------|--------|-----------------|---------------|---------------|
| Membrane-spanning 4-domains. subfamily A. member 1                        | Ms4a1  | -2.5536         | 3.11E-02      | 1.01E-03      |
| Chemokine (C-C motif) receptor 7                                          | Ccr7   | -2.3446         | 1.23E-02      | 1.56E-04      |
| Complement receptor 2                                                     | Ctr2   | -2.3063         | 1.06E-02      | 7.38E-05      |
| CD19 antigen                                                              | Cd19   | -2.2856         | 1.40E-04      | 3.26E-07      |
| Cystatin F (leukocystatin)                                               | Cst7   | -2.2784         | 4.95E-02      | 2.31E-03      |
| Histocompatibility 2. class II antigen E β2                              | H2-Eb2 | -1.8299         | 2.55E-02      | 5.95E-04      |
| CD22 antigen                                                             | Cd22   | -1.7512         | 1.23E-02      | 1.18E-04      |
| Histocompatibility 2. O region α locus                                    | H2-Oa  | -1.7194         | 3.56E-02      | 1.25E-03      |
| B cell scaffold protein with ankyrin repeats 1                           | Bank1  | -1.5358         | 2.55E-02      | 5.92E-04      |
| Chemokine (C-X-C motif) ligand 5                                         | Cxcl5  | -1.5001         | 4.42E-02      | 1.65E-03      |
| Chemokine (C-C motif) receptor 6                                         | Ccr6   | -1.4843         | 2.91E-02      | 7.79E-04      |
| Dual specificity phosphatase 2                                           | Dusp2  | -1.4269         | 1.70E-02      | 2.77E-04      |
| Spi-B transcription factor                                               | Spib   | -1.4116         | 3.11E-02      | 9.09E-04      |
| Nuclear receptor subfamily 4. group A. member 2                          | Nr4a2  | -1.3889         | 1.55E-02      | 2.35E-04      |
| Tumor necrosis factor receptor superfamily. member 8                     | Tnfrsf8| -1.2221         | 4.99E-02      | 2.40E-03      |
| Histocompatibility 2. T region locus 24                                  | H2-T24 | -1.1875         | 4.42E-02      | 1.64E-03      |
| Histocompatibility 2. M region locus 2                                    | H2-M2  | -1.1742         | 4.99E-02      | 2.44E-03      |
| Z-DNA binding protein 1                                                   | Zbp1   | -0.9897         | 6.06E-03      | 2.82E-05      |
| Epithelial membrane protein 1                                            | Emp1   | -0.9596         | 1.92E-02      | 3.36E-04      |
| Melanophilin                                                             | Mlph   | -0.936          | 1.23E-02      | 1.56E-04      |
| Protein tyrosine phosphatase. receptor type. C                           | Ptprc  | -0.8487         | 3.11E-02      | 9.96E-04      |
| Centromere protein F                                                     | Cenpf  | -0.705          | 1.64E-02      | 4.28E-04      |
| Histocompatibility 2. class II antigen A, β 1                            | H2-Ab1 | -0.7036         | 6.03E-03      | 1.00E-04      |
| Antigen identified by monoclonal antibody Ki 67                          | Mki67  | -0.6661         | 2.39E-02      | 8.81E-04      |
| Baculoviral IAP repeat-containing 5                                       | Birc5  | -0.6435         | 2.23E-02      | 7.02E-04      |
| Gene Description                                      | Gene Symbol | log2FC Value | p-value 1 | p-value 2 |
|-------------------------------------------------------|-------------|--------------|-----------|-----------|
| Histocompatibility 2, Q region locus 4                | H2-Q4       | -0.624       | 2.22E-02  | 6.60E-04  |
| Histocompatibility 2, class II antigen A, α           | H2-Aa       | -0.5918      | 2.86E-03  | 3.40E-05  |
| Histocompatibility 2, class II antigen E, β           | H2-Eb1      | -0.5903      | 2.86E-03  | 3.20E-05  |
| Histocompatibility 2, M region locus 3                | H2-M3       | -0.5693      | 3.97E-02  | 1.56E-03  |
| Proteasome subunit, beta type 10                      | Psmb10      | -0.4267      | 3.99E-02  | 1.61E-03  |

Genes are shown in descending order (log2FC values).
**Suppl. Tab. 10:** Pathways significantly altered by MC deficiency in early tumorigenic events in the colon (AOM vs AOM $\rightarrow$ CA). Data were obtained by RNA-seq and analysed on DAVID (Bioinformatic Database).

| Pathway                                           | Status   | $p$-Value | Genes                                      |
|---------------------------------------------------|----------|-----------|--------------------------------------------|
| Cell adhesion molecules                           | Downregulated | 1.2E-8    | $Cd34$, $H2-M2$, $H2-M3$, $H2-Oa$, $H2-T24$, $H2-Ab1$, $H2-Aa$, $H2-Eb1$, $Ptprc$ |
| Intestinal immune network for IgA production      | Downregulated | 6.9E-7    | $H2-Oa$, $H2-Aa$, $H2-Ab1$, $H2-Eb1$       |
| Intestinal immune network for IgA production      | Upregulated | 6.9E-7    | $Cxcl12$, $Il15$                          |
| Antigen processing and presentation               | Downregulated | 8.9E-7    | $H2-M2$, $H2-M3$, $H2-Oa$, $H2-T24$, $H2-Ab1$, $H2-Aa$, $H2-Eb1$ |
| Cytokine-cytokine receptor interaction            | Upregulated | 4.8E-5    | $Ccl3$, $Il15$, $Cxcl12$                  |
|                                                  | Downregulated | 4.8E-5    | $Ccl19$, $Ccr6$, $Ccr7$, $Cxcl5$, $Tnfrsf8$ |
| Chemokine signaling pathway                       | Upregulated | 1.2E-3    | $Ccl9$, $Ccl3$, $Cxcl12$                  |
|                                                  | Downregulated | 1.2E-3    | $Ccr6$, $Ccr7$, $Cxcl5$                   |
| B cell receptor signaling pathway                 | Downregulated | 3.4E-2    | $Cd19$, $Cd22$, $Cr2$                     |

Acronyms meaning is given in Suppl. Tab. 9.
Suppl. Tab 11: Pathways significantly altered by MC deficiency in early tumorigenic events in the colon (AOM vs AOM+CA). Data were obtained by RNA-seq and analysed on DAVID (Bioinformatic Database).

| Pathway                                | Status          | p-Value  | Genes                                      |
|----------------------------------------|-----------------|----------|---------------------------------------------|
| Antigen processing and presentation    | Downregulated   | 1.2E-5   | Cld74, H2-M3, H2-Aa, H2-Ab1, H2-Eb1, Tap1  |
| Intestinal immune network for IgA production | Downregulated | 5.0E-4   | H2-Aa, H2-Ab1, H2-Eb1                       |
| Inflammatory bowel disease (IBD)       | Downregulated   | 1.4E-3   | H2-Aa, H2-Ab1, H2-Eb1, Il13                |
| Cytokine-cytokine receptor interaction | Downregulated   | 1.9E-3   | Ccl19, Ccl5, Ccr6, Cxcl5, Il13             |
| B cell receptor signaling pathway      | Downregulated   | 2.2E-3   | Cld9, Cld22, Cld72, Cr2                    |
| Cell adhesion molecules (CAMs)         | Downregulated   | 3.0E-3   | Cld22, H2-M3, H2-Aa, H2-Ab1, H2-Eb1        |
| Chemokine signaling pathway            | Downregulated   | 5.9E-3   | Ccl19, Ccl5, Ccr6, Cxcl5                   |
| NF-kappa B signaling pathway           | Downregulated   | 5.1E-2   | Ccl19, Zap70                               |

Acronyms meaning is given in Suppl. Tab. 9.
### Suppl. Tab. 12. Significantly altered genes by MC deficiency in allograft tumors (Kit<sup>B6</sup> vs Kit<sup>W/sh</sup>). Data were obtained by RNA-seq.

| Name                                           | Acronym | log2FC  | p-Adj  | p-Value  |
|------------------------------------------------|---------|---------|--------|----------|
| Upregulated genes                              |         |         |        |          |
| RNA binding motif protein 3                    | Rbm3    | 8.39E+14| 1.37E+14| 1.77E+09 |
| Cell adhesion molecule 1                       | Cadm1   | 8.37E+14| 1.18E+14| 3.38E+14 |
| Proteasome (prosome, macropain) subunit, alpha type 2 | Psma2   | 8.01E+14| 7.53E+14| 1.79E+14 |
| Proteasome (prosome, macropain) subunit, beta type 7 | Psmb7   | 7.85E+14| 1.44E+14| 4.74E+14 |
| Proteasome (prosome, macropain) inhibitor subunit 1 | Psnf1   | 7.58E+14| 4.88E+14| 3.53E+14 |
| Caspase 1                                      | Casp1   | 7.57E+14| 1.63E+14| 2.56E+09 |
| Tryptase alpha/beta 1                          | Tpsab1  | 7.50E+14| 1.07E+02| 3.07E+01 |
| Proteasome (prosome, macropain) subunit, beta type 5 | Psmb5   | 7.13E+14| 4.49E+14| 2.70E+14 |
| Carboxypeptidase A3, mast cell                  | Cpa3    | 6.78E+14| 6.11E-26| 8.74E-29 |
| Interferon induced transmembrane protein 3      | Ifitm3  | 6.53E+14| 1.18E+14| 3.31E+14 |
| Tryptase beta 2                                 | Tpsb2   | 6.49E+14| 1.57E+03| 6.75E+00 |
| Proteasome (prosome, macropain) activator subunit 1 (PA28 alpha) | Psme1   | 6.39E+13| 2.00E+14| 8.02E+14 |
| Proteasome (prosome, macropain) 26S subunit, non-atpase, 6 | Psmd6   | 6.13E+14| 3.25E+14| 1.48E+14 |
| Proteasome (prosome, macropain) subunit, alpha type 4 | Psma4   | 5.97E+14| 3.47E+14| 1.74E+14 |
| Lectin, galactose binding, soluble 9            | Lgals9  | 5.50E+14| 4.88E+14| 3.10E+14 |
| Chemokine (C-C motif) receptor 5                | Ccr5    | 5.50E+14| 1.58E+14| 5.87E+14 |
| Vascular cell adhesion molecule 1               | Vcam1   | 5.10E+14| 3.47E+14| 1.71E+14 |
| Macrophage scavenger receptor 1                 | Msr1    | 4.89E+14| 4.88E+14| 3.30E+14 |
| Proteasome (prosome, macropain) activator subunit 2 (PA28 beta) | Psme2   | 4.84E+14| 1.84E+14| 7.09E+14 |
| Catalase                                       | Cat     | 4.55E+14| 4.46E+14| 2.62E+14 |
| Regulator of G-protein signaling 2              | Rgs2    | 4.49E+14| 4.36E+14| 2.43E+14 |
| Gene Name                                      | Log2 Fold Change | Fold Change | p Value  |
|-----------------------------------------------|------------------|-------------|----------|
| Hepatitis A virus cellular receptor 2         | Haver2           | 4.38E+14    | 4.88E+14 | 3.37E+13 |
| Beta-2 microglobulin                          | B2m              | 3.92E+14    | 4.18E+14 | 2.16E+14 |
| Chemokine (C motif) ligand 1                  | Xcl1             | 1.00E+14    | 2.56E+13 | 1.09E+14 |

| Gene Name                                      | Log2 Fold Change | Fold Change | p Value  |
|-----------------------------------------------|------------------|-------------|----------|
| Downregulated genes                           |                  |             |          |
| Basic leucine zipper transcription factor, ATF-like | Batf             | -5.87E+14   | 4.88E+14 | 3.56E+14 |
| Chemokine (C-C motif) receptor 3              | Ccr3             | -2.03E+14   | 2.56E+13 | 1.10E+14 |
| CD163 antigen                                 | Cd163            | -3.19E+12   | 5.71E+14 | 5.72E+08 |
| CD5 antigen                                   | Cd5              | -1.27E+13   | 1.18E+14 | 3.34E+14 |
| Class II transactivator                       | Ciita            | -8.39E+14   | 1.91E+14 | 3.27E+09 |
| C-type lectin domain family 10, member A      | Clec10a          | -1.99E+14   | 7.53E+14 | 1.83E+14 |
| Colony stimulating factor 1 receptor          | Csf1r            | -6.71E+14   | 4.27E+14 | 2.32E+14 |
| Dual specificity phosphatase 5                | Dusp5            | -8.73E+14   | 4.27E+14 | 2.32E+14 |
| Coagulation factor XIII, A1 subunit           | F13a1            | -1.34E+14   | 1.46E+14 | 5.09E+14 |
| Histocompatibility 2, class II antigen A, beta 1 | H2-Ab1           | -6.65E+14   | 9.83E+14 | 1.12E+09 |
| Histocompatibility 2, class II antigen E beta | H2-Eb1           | -8.66E+14   | 3.16E+14 | 2.71E+08 |
| Histone cluster 1, h1c                         | Hist1h1c         | -8.62E+14   | 3.25E+14 | 1.49E+14 |
| Interleukin 13                                 | Il13             | -1.89E+14   | 4.88E+14 | 3.56E+14 |
| K(lysine) acetyltransferase 6a                 | Kat6a            | -5.78E+13   | 1.23E+14 | 3.78E+14 |
| Leptin receptor overlapping transcript         | Leprot           | -8.37E+14   | 1.63E+14 | 2.50E+09 |
| MAP-kinase activating death domain             | Madd             | -7.40E+14   | 4.44E+14 | 2.54E+14 |
| Avian musculoaponeurotic fibrosarcoma oncogene homolog | Maf               | -1.22E+14   | 1.46E+14 | 5.23E+13 |
| Nuclear factor of kappa light polypeptide gene enhancer in B cells 1, p105 | Nfkb1           | -4.57E+14   | 6.19E+14 | 1.24E+13 |
| Nudix (nucleoside diphosphate linked moiety X)-type motif 9 | Nudt9           | -6.03E+14   | 1.23E+14 | 3.87E+14 |
| Programmed cell death 1 ligand 2              | Pdcd11g2         | -1.56E+14   | 6.66E+14 | 1.43E+14 |
| Proteoglycan 2, bone marrow                   | Prg2             | -4.22E+14   | 2.19E+14 | 4.07E+09 |
| Solute carrier family 15 (H+/peptide transporter), member 2 | Slc15a2        | -8.60E+13   | 1.67E+14 | 1.20E+08 |
| Pathway                                      | Status                  | p-Value  | Genes                                                                 |
|---------------------------------------------|-------------------------|----------|------------------------------------------------------------------------|
| Proteasome                                  | Downregulated           | 8.6E-10  | Psmd6, Psme1, Psme2, Psmf1, Psma2, Psma4, Psmb5, Psmb7                |
| Antigen processing and presentation         | Upregulated             | 3.1E-5   | Ciita, H2-Ab1, H2-Eb1                                                 |
|                                             | Downregulated           | 3.1E-5   | B2m, Psme1, Psme2                                                     |
| Inflammatory bowel disease (IBD)            | Upregulated             | 1.4E-4   | Maf, H2-Ab1, H2-Eb1, Il13, Nfkbl                                      |
| Cell adhesion molecules (CAMs)              | Upregulated             | 6.0E-3   | H2-Ab1, H2-Eb1, Pdccllg2                                              |
|                                             | Downregulated           | 6.0E-3   | Cadm1, Vcam1                                                          |
| Cytokine-cytokine receptor interaction      | Downregulated           | 2.3E-2   | Ccr5, Xcl1                                                            |
|                                             | Upregulated             | 2.3E-2   | Ccr3, Csf1r, Il13                                                     |
| Chemokine signaling pathway                 | Downregulated           | 5.9E-2   | Xcl1, Ccr5                                                            |
|                                             | Upregulated             | 5.9E-2   | Ccr3, Nfkbl                                                           |

According to their log2FC values, genes are shown in descending order.

**Suppl. Tab. 13: Pathways significantly altered by MC deficiency in in allograftic tumors.**

Data were obtained by RNA-seq and analysed on DAVID (Bioinformatic Database).

Acronyms meaning is given in Suppl. Tab. 12.
