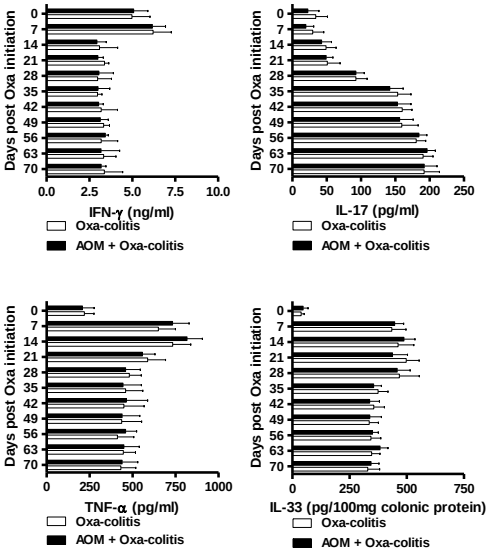
Figure S1

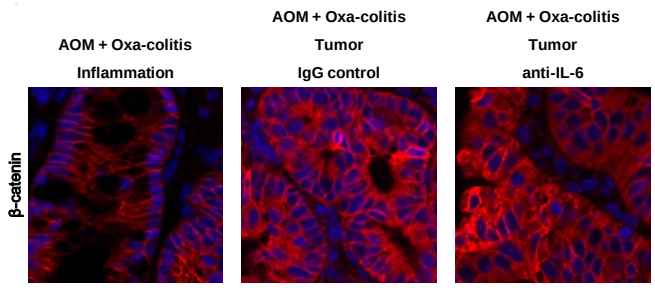


Cytokine secretion profile during the course of chronic oxa-colitis and chronic oxa-colitis with AOM-induced tumors. Cytokine expression during chronic oxa-colitis and chronic oxa-colitis with AOM-induced tumors. Cells were extracted from the lamina propria and stimulated for 48h. Cytokine concentrations were determined in culture supernatants by ELISA. Data shown are mean values ± SEM from individual cultures of cells derived from mice in two separate experiments (n=6 mice per group). *, $p \leq 0.05$.

The capacity of cultured LPMC to secrete IFN-γ and IL-17 determined by stimulation with plate-bound anti-CD3 antibody (10µg/ml) and soluble anti-CD28 antibody (1µg/ml) (BD Biosciences, San Jose, California, USA) or Staphylococcus aureus Cowan I (1:10000 dilution of Pansorbin) (EMD Biosciences) and IFN-γ (1000U/ml) (R&D Systems) for the detection of TNF-α. IL-33 concentration was measured from total colonic protein. Snap-frozen colonic samples were mechanically homogenized in liquid nitrogen, and total protein extracts were collected in buffer containing 10mM HEPES, 10 mM KCl, 0.1 mM EDTA, and 0.2 mM EGTA, supplemented with 1 mM DTT, 10 mg/ml leupeptin, and 1 mM phenylmethanesulphone. Extracts containing

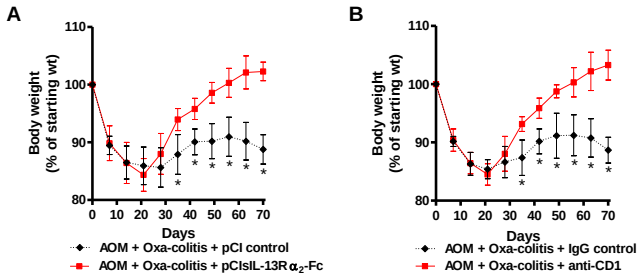
100 mg of proteins were then analyzed for IL-33 expression by cytokine-specific ELISA kits according to the manufacturer’s instructions.

Figure S2



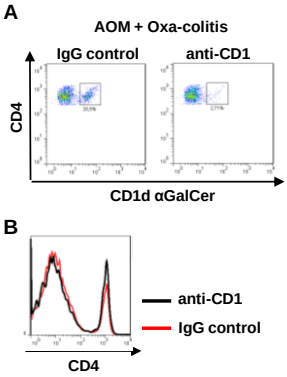
Epithelial cell E-cadherin expression pattern is not affected by anti-IL-6 antibody treatment. Immunofluorescence staining for β-catenin of representative inflamed area and tumor areas of colon sections on day 49 during chronic oxa-colitis with AOM-induced tumors (40x magnification).

Figure S3



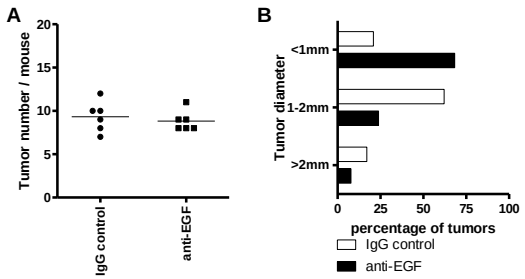
Inhibition of IL-13 and depletion of NKT-cells prevents progression of chronic oxa-colitis with AOM-induced tumors (AOM+oxa-colitis). (**A**) Body weight after treatment with pCI-sIL-13Rα_2-Fc. Data shown are mean values ± SEM (n=6 mice per group). *, p ≤0.05 tested for pCI-control-group and pCI-sIL-13Rα_2-Fc-group. (**B**) Body weight after treatment with anti-CD1 antibody. Data shown are mean values ± SEM (n=6 mice per group). *, p ≤0.05 tested for IgG control-group and anti-CD1-group.

Figure S4



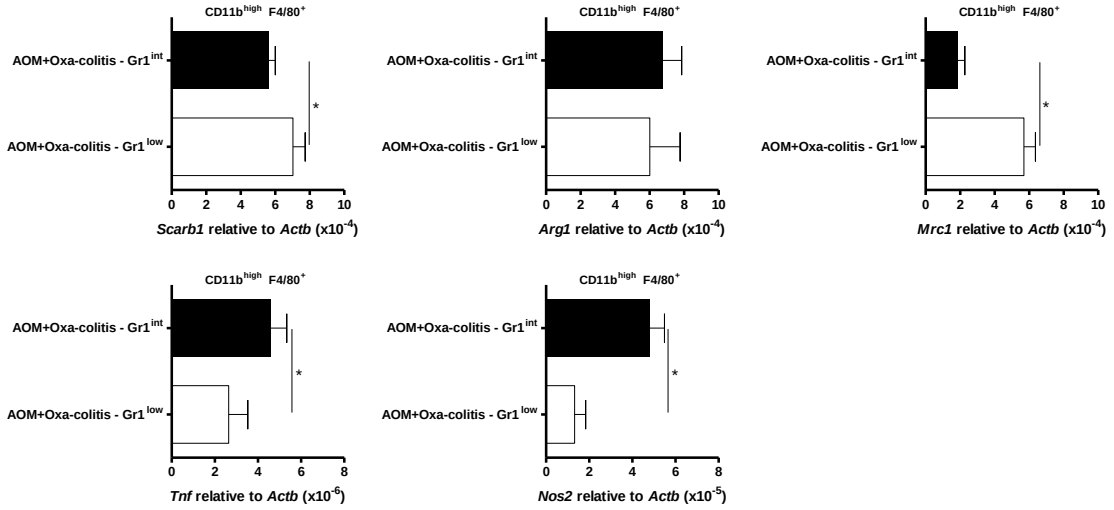
Efficacy and specificity of anti-CD1 antibody treatment. (**A**) Distribution of NKT cells on day 49 of chronic oxa-colitis with AOM-induced tumor growth shows a 90% reduction of $CD4^{+}CD1d\alpha GalCer^{+}$ NKT cells in the lamina propria following anti-CD1 antibody treatment. Cells were extracted from the lamina propria on day 49 and stained with anti-CD4 antibody and αGalCer-loaded CD1d tetramer. Blot shown is gated on $CD4^{+}$ cells. Similar results could be verified for spleen cells (data not shown). (**B**) The number of $CD4^{+}$ T cells in the lamina propria on day 49 of oxa-colitis with tumor growth was unchanged by anti-CD1 antibody treatment. Cells were extracted from the lamina propria on day 49 and stained with anti-CD45 antibody, anti-CD4 antibody and αGalCer tetramer. Blot shown is gated on $CD45^{+}CD1d\alpha GalCer^{-}$ cells. Similar results could be verified for spleen cells (data not shown). Further evaluation revealed that $B220^{+}CD19^{+}$ B cell numbers remained unchanged by anti-CD1 antibody treatment (data not shown).

Figure S5



Inhibition of EGF results in decreased tumor size, but does not influence tumor number. (**A**) Number of tumor nodules on day 49 of chronic oxa-colitis with AOM-induced tumor growth after treatment with anti-EGF antibody. Anti-EGF antibody was administered (500µg IP weekly) starting on day 20. Horizontal bars are mean values (n=6 mice per group). Individual points represent one mouse. *, p ≤0.05. (**B**) Diameter of tumor nodules on day 49 of chronic oxa-colitis with AOM-induced tumor growth after treatment with anti-EGF antibody. Measurements of tumor nodules refer to diameter and were made using a digitally obtained image of the whole tissue section. Evaluation was performed using the Mirax Viewer software (Carl Zeiss AG, Germany).

Figure S6



F4/80^{+}CD11b^{high}Gr1^{low} macrophages display an M2 phenotype. mRNA expression of F4/80^{+}CD11b^{high}Gr1^{low} and F4/80^{+}CD11b^{high}Gr1^{intermediate} cells on day 49 of chronic oxa-colitis with AOM-induced tumors. Cells were extracted from the lamina propria and F4/80^{+}CD11b^{high}Gr1^{low} and F4/80^{+}CD11b^{high}Gr1^{intermediate} cells were isolated by flow cytometric sorting. *Scarb1, Arg1, Mrc1, Tnf, Nos2* mRNA was determined by quantitative PCR. Data shown are mean values ± SEM (n=3-5 mice per group). *, p ≤0.05.

Aliquots of isolated cells were stored in RNAlater solution (Ambion, Austin, Texas, USA) and then subjected to RNA extraction using RNeasy tissue kit (Qiagen, Hilden, Germany). Occasionally, MessageAmp aRNA Amplification Kit (Ambion, Austin, Texas, USA) was used to obtain sufficient RNA template. A total of 100ng template RNA was reverse transcribed with Superscript III RT-PCR Kit (Invitrogen, Carlsbad, California, USA).

Primer sequences:

Scarb1: GGGCTCGATATTGATGGAGA and GGAAGCATGTCTGGGAGGTA

Arg1: GTGAAGAACCCACGGTCTGT and CTGGTTGTCAGGGGAGTGTT

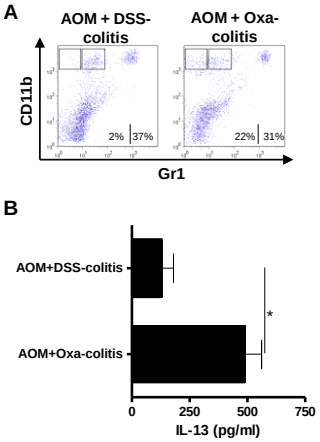
Mrc1: ATGCCAAGTGGGAAAATCTG and TGTAGCAGTGGCCTGCATAG

Tnf: GAACTGGCAGAAGAGGCACT and AGGGTCTGGGCCATAGAACT

Nos2: CACCTTGGAGTTCACCCAGT and ACCACTCGTACTTGGGATGC

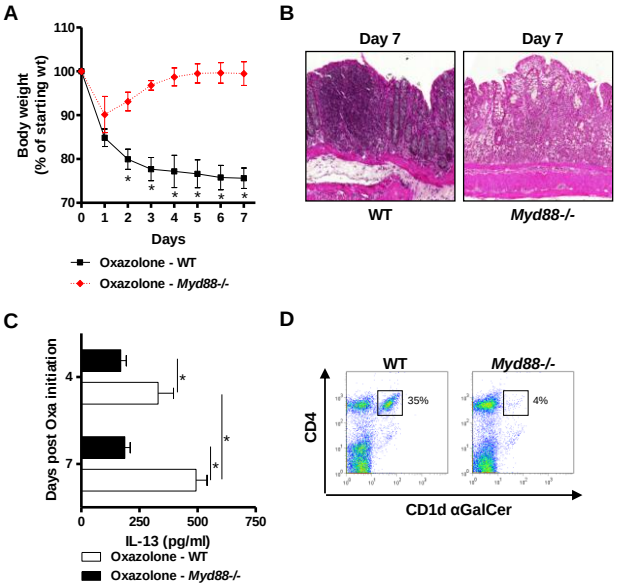
Actb: AGCCATGTACGTAGCCATCC and CTCTCAGCTGTGGTGGTGAA.

Figure S7



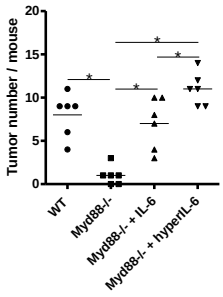
Comparison of DSS-colitis and chronic oxa-colitis with AOM-induced tumors. (**A**) Macrophage populations in DSS-colitis and chronic oxa-colitis with AOM-induced tumor growth. Cells were extracted from the lamina propria on day 49 and stained with anti-F4/80 antibody, anti-CD11b antibody, and anti-Gr1 antibody. Flow cytometric study shown is gated on $F4/80^{+}$ cells. Percentages denote $F4/80^{+}CD11b^{high}Gr1^{low}$ and $F4/80^{+}CD11b^{high}Gr1^{intermediate}$ cells amongst total $F4/80^{+}CD11b^{high}$ cells. (**B**) IL-13 production on day 49 of DSS-colitis with AOM-induced tumor growth and chronic oxa-colitis with tumor growth. Cells were extracted from the lamina propria on day 49 and stimulated for 48h. Cytokine concentrations were determined in culture supernatants by ELISA. Data shown are mean values ± SEM from individual cultures of cells derived from mice in two separate experiments (n=6 mice per group). *, $p \leq 0.05$.

Figure S8



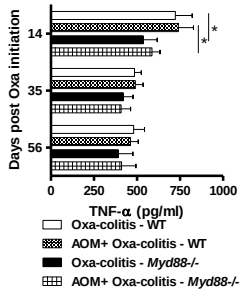
The acute phase of oxa-colitis is partly dependent on MyD88 signaling. (**A**) Body weight as a percent of starting weight in wild type and *Myd88*$^{-/-}$. Data shown are mean values ± SEM (n=6 mice per group). (**B**) H&E staining of representative inflamed areas of colon sections (10x magnification) on day 7 of acute oxa-colitis in wild type and *Myd88*$^{-/-}$ mice. (**C**) IL-13 production on indicated time points of oxa-colitis in wild type and *Myd88*$^{-/-}$ mice. Cells were extracted from the lamina propria and stimulated for 48h. Cytokine concentrations were determined in culture supernatants by ELISA. Data shown are mean values ± SEM from individual cultures of cells derived from mice in two separate experiments (n=6 mice per group). *, p ≤0.05. (**D**) NKT-cell expression on indicated time points of oxa-colitis in wild type and *Myd88*$^{-/-}$ mice. Cells were extracted from the lamina propria and stained with anti-CD4 antibody and CD1dαGalCer tetramers. Percentages denote CD4^{+}CD1dαGalCer^{+} cells amongst total CD4^{+} cells.

Figure S9



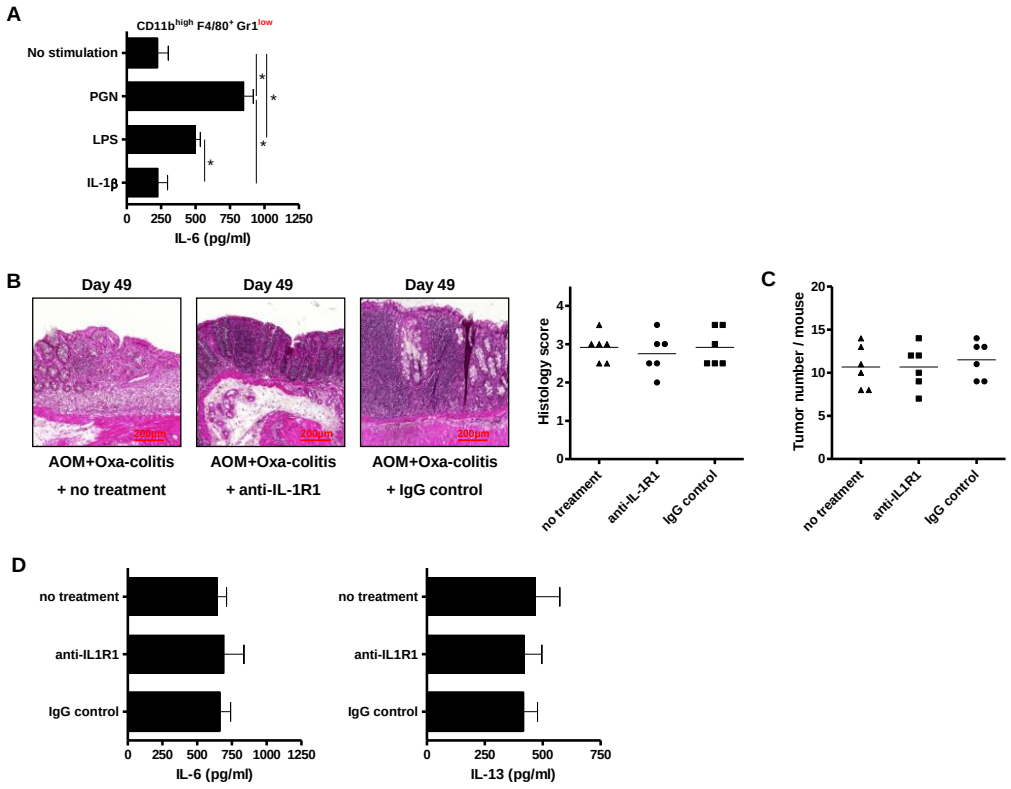
Tumor growth during oxa-colitis with AOM-induced tumors in *Myd88*-deficient animals can be restored by the administration of recombinant IL-6. Number of tumor nodules on day 49 of oxa-colitis with AOM-induced tumors after reconstitution of *Myd88*$^{-/-}$ mice with recombinant IL-6 or hyperIL-6. Horizontal bars are mean values (n=6 mice per group). Individual points represent one mouse. *, $p \leq 0.05$.

Figure S10



TNF-α production on indicated time points of chronic oxa-colitis and chronic oxa-colitis with AOM-induced tumors in wild type and *Myd88*$^{-/-}$ mice. Cells were extracted from the lamina propria and stimulated for 48h. Cytokine concentrations were determined in culture supernatants by ELISA. Data shown are mean values ± SEM from individual cultures of cells derived from mice in two separate experiments (n=6 mice per group). *, $p \leq 0.05$.

Figure S11

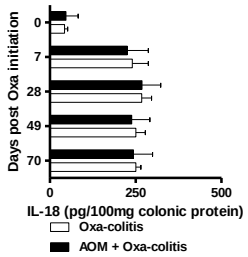


Inhibition of IL-1R signaling does not interfere with the development of chronic oxa-colitis or tumor growth. 100µg anti-IL-1R1 antibody was administered IP every other day starting on day 20 as previously described (1). (**A**) IL-6 expression of $F4/80^{+}CD11b^{high}Gr1^{low}$ of chronic oxa-colitis with AOM-induced tumor growth following stimulation with PGN, LPS, or IL-1β. Cells were extracted from the lamina propria and stimulated for 48h. Cytokine concentrations were determined in culture supernatants by ELISA. Data shown are mean values ± SEM from individual cultures of cells derived from mice in two separate experiments (n=6 mice per group). *, p ≤0.05. (**B**) H&E staining of representative inflamed areas of colon sections (10x magnification) and histology score on day 49 of chronic oxa-colitis with AOM-induced tumor growth following treatment with anti-IL-1R antibody. Horizontal bars are mean values (n=6 mice per group). Individual points represent one mouse. *, p ≤0.05. (**C**) Number of tumor nodules on day 49 of chronic oxa-colitis with tumor growth after treatment with anti-IL-1R antibody. Horizontal bars are mean values (n=6 mice per

group). Individual points represent one mouse. *, p ≤0.05. (**D**) IL-13 and IL-6 production on day 49 of chronic oxa-colitis with AOM-induced tumor growth after treatment with anti-IL-1R antibody. Cells were extracted from the lamina propria on day 49 and stimulated for 48h. Cytokine concentrations were determined in culture supernatants by ELISA. Data shown are mean values ± SEM from individual cultures of cells derived from mice in two separate experiments (n=6 mice per group). *, p ≤0.05.

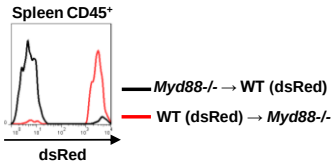
(1) Meng G, Zhang F, Fuss I, Kitani A, Strober W. 2009. A mutation in the Nlrp3 gene causing inflammasome hyperactivation potentiates Th17 cell-dominant immune responses. *Immunity* 30:860-74.

Figure S12



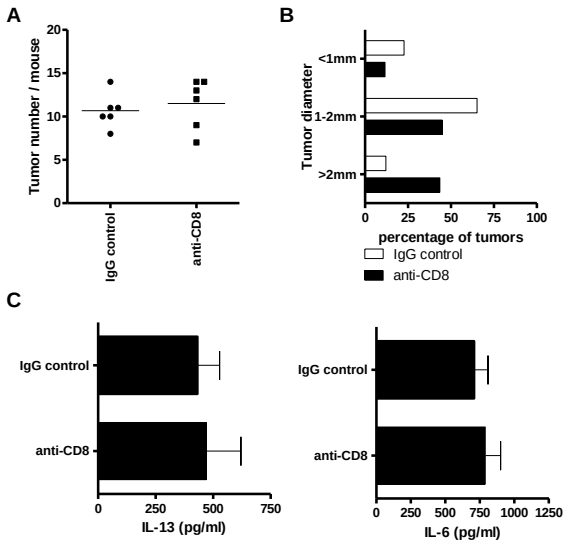
The profiles of IL-18 secretion during the course of chronic oxa-colitis and chronic oxa-colitis with AOM-induced tumor growth were equivalent. IL-18 concentration was measured in total colonic protein extracted from 5 mice each group at indicated time points. Data shown are mean values ± SEM from individual cultures of cells derived from mice in two separate experiments (n=5 mice per group). *, $p \leq 0.05$. Snap-frozen colonic samples were mechanically homogenized in liquid nitrogen, and total protein extracts were collected in buffer containing 10mM HEPES, 10 mM KCl, 0.1 mM EDTA, and 0.2 mM EGTA, supplemented with 1 mM DTT, 10 mg/ml leupeptin, and 1 mM phenylmethanesulphone. Extracts containing 100 mg of proteins were then analyzed for IL-18 expression by cytokine-specific ELISA kits according to the manufacturer's instructions.

Figure S13



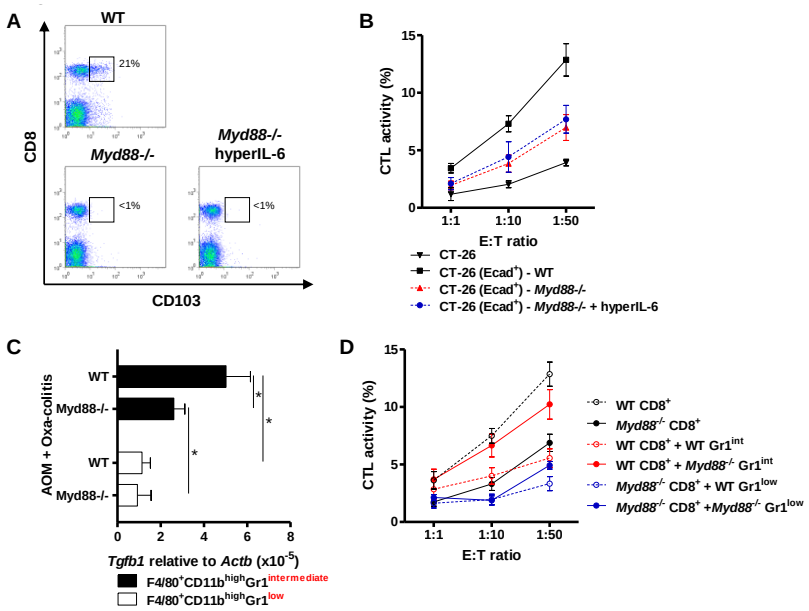
Analysis of BM chimeric mice to determine the role of MyD88 in hematopoietic cells and non-hematopoietic cells during chronic oxa-colitis with AOM-induced tumor growth. Using dsRed-Tg mice, chimerism was confirmed by examining $dsRed^{+}CD45^{+}$ cells in splenocytes 8 weeks after transplantation by flow cytometry. Using this protocol no irradiation-induced histological damage to the intestinal mucosa was noticed at 8 weeks after BM transplantation. To generate BM chimera, six-week-old WT or *Myd88*$^{-/-}$ recipient mice were irradiated with 10 Gy from a 137Cs source delivered in 2 doses of 5 Gy each, 3 hours apart. BM cells were isolated from 6–8-week-old WT or *Myd88*$^{-/-}$ donor mice by flushing the bone shafts of the femurs and tibias with RPMI and $2.5x10^{6}$ BM cells thus obtained were intravenously injected into the recipient mice 3 hours after the last irradiation. WT mice were transplanted with *Myd88*$^{-/-}$ BM (*Myd88*$^{-/-}$ → WT) and *Myd88*$^{-/-}$ mice were transplanted with WT BM (WT → *Myd88*$^{-/-}$). As controls, WT mice were transplanted with WT BM and *Myd88*$^{-/-}$ mice transplanted with *Myd88*$^{-/-}$ BM.

Figure S14



Tumor nodules in mice depletion of $CD8^+$ T cells display increased size. (**A**) Number of tumor nodules on day 49 of chronic oxa-colitis with AOM-induced tumors after treatment with anti-CD8 antibody. 500µg anti-CD8 antibody was administered IP weekly starting on day 20. Horizontal bars are mean values (n=6 mice per group). Individual points represent one mouse. *, $p \leq 0.05$. (**B**) Diameter of tumor nodules on day 49 of chronic oxa-colitis with AOM-induced tumor growth after treatment with anti-CD8 antibody. Measurements of tumor nodules refer to diameter and were made using a digitally obtained image of the whole tissue section. Evaluation was performed using the Mirax Viewer software (Carl Zeiss AG, Germany). (**C**) IL-13 and IL-6 production on day 49 of chronic oxa-colitis with AOM-induced tumor growth after treatment with anti-CD8 antibody. Cells were extracted from the lamina propria on day 49 and stimulated for 48h. Cytokine concentrations were determined in culture supernatants by ELISA. Data shown are mean values ± SEM from individual cultures of cells derived from mice in two separate experiments (n=6 mice per group). *, $p \leq 0.05$.

Figure S15



Myd88-deficiency reduces $CD8^+$ T cell cytotoxicity and immunosurveillance. (**A**) $CD8^+CD103^+$ T-cell expression on day 49 of chronic oxa-colitis with AOM-induced tumors in wild type and *Myd88*$^{-/-}$ mice in the presence or absence of hyper-IL-6. Cells were extracted from the lamina propria and stained with anti-CD8 antibody and anti-CD103 antibody. Percentages denote $CD8^+CD103^+$ cells amongst total $CD8^+$ cells. (**B**) Cytotoxicity of wild type or *Myd88*$^{-/-}$ $CD8^+$ T cells against CT-26 cells or E-cadherin$^+$ CT-26 cells in the presence or absence of hyper-IL-6. $CD8^+$ T cells were isolated from the colon on day 49 of chronic oxa-colitis with AOM-induced tumors and co-cultured with CT-26 cells or E-cadherin$^+$ CT-26 cells target cells. Cytotoxicity was measured after 24h cytolytic activity. (**C**) *Tgfb1* mRNA expression of wild type or *Myd88*$^{-/-}$ $F4/80^+CD11b^{high}Gr1^{low}$ and $F4/80^+CD11b^{high}Gr1^{intermediate}$ cells on day 49 of chronic oxa-colitis with AOM-induced tumors. Cells were extracted from the lamina propria and $F4/80^+CD11b^{high}Gr1^{low}$ and $F4/80^+CD11b^{high}Gr1^{intermediate}$ cells were isolated by flow cytometric sorting. *Tgfb1* mRNA expression was determined by quantitative PCR. Data shown are mean values ± SEM (n=3-5 mice per group). *, $p \leq 0.05$. (**D**) Suppression of cytotoxicity of wild type or *Myd88*$^{-/-}$ $CD8^+$ T cells against E-

cadherin^{+} CT-26 cells mediated by wild type or *Myd88*$^{-/-}$ macrophage populations. Wild type or *Myd88*$^{-/-}$ CD8^{+} T cells and F4/80^{+}CD11b^{high}Gr1^{low} and F4/80^{+}CD11b^{high}Gr1^{intermediate} cells were isolated from the colon on day 49 of chronic oxa-colitis with AOM-induced tumors. Cytotoxicity was measured after 24h cytolytic activity.