

Supplementary Figures

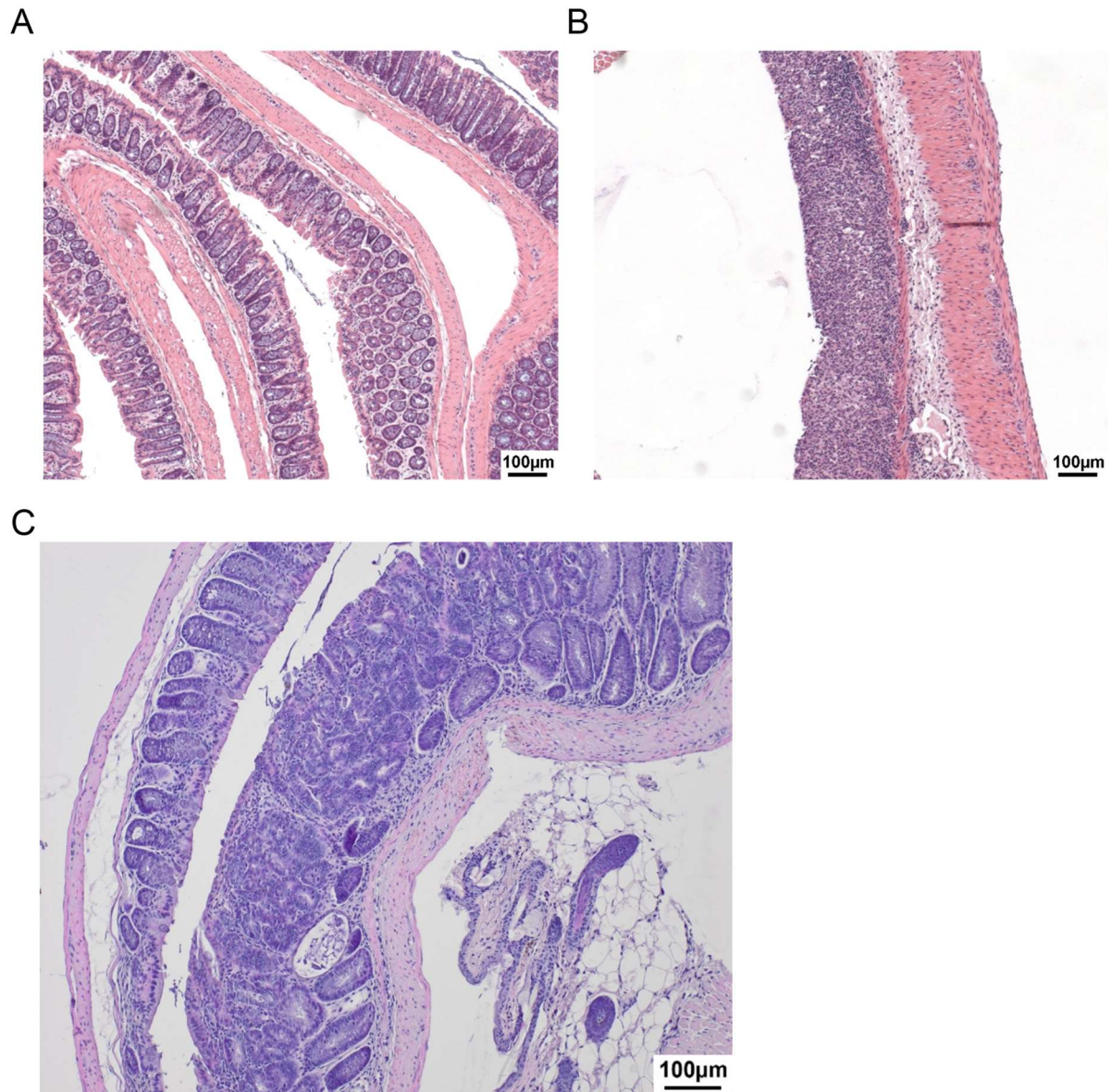


Figure S1. Representative H&E examples of the mouse models.

(A) H&E staining of a healthy colon from a wild type mouse. (B) Colon of a wild-type mouse treated with 5% DSS for five days and then with normal water for 3 days. (C) Colon of a wild-type mouse in the AOM/DSS model of day 61. The left side of the colon represents normal tissue, whereas on the right side a representative tumor tissue is shown.

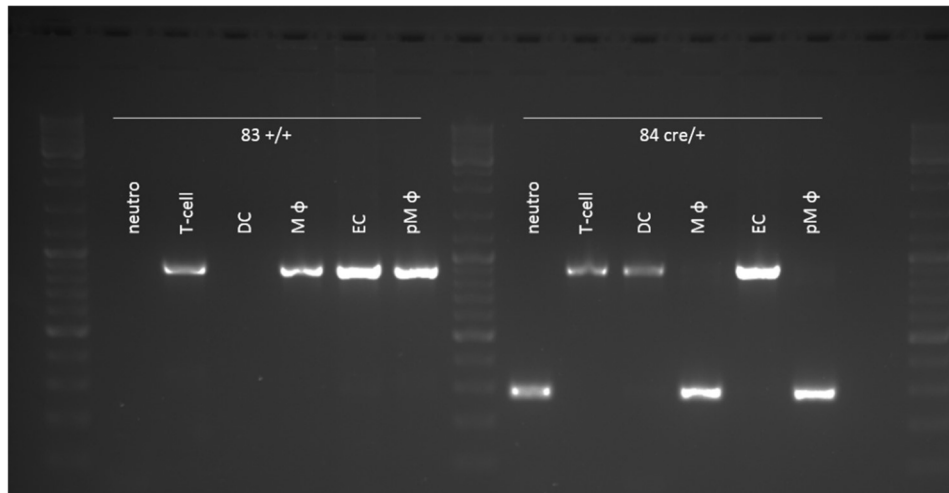


Figure S2. Rictor is efficiently deleted in colonic macrophages.

T-cells, dendritic cells, macrophages, epithelial cells were sorted from the colon of healthy mice. Peritoneal macrophages were isolated from the peritoneum. Recombination efficiency was detected by PCR as described in (63).

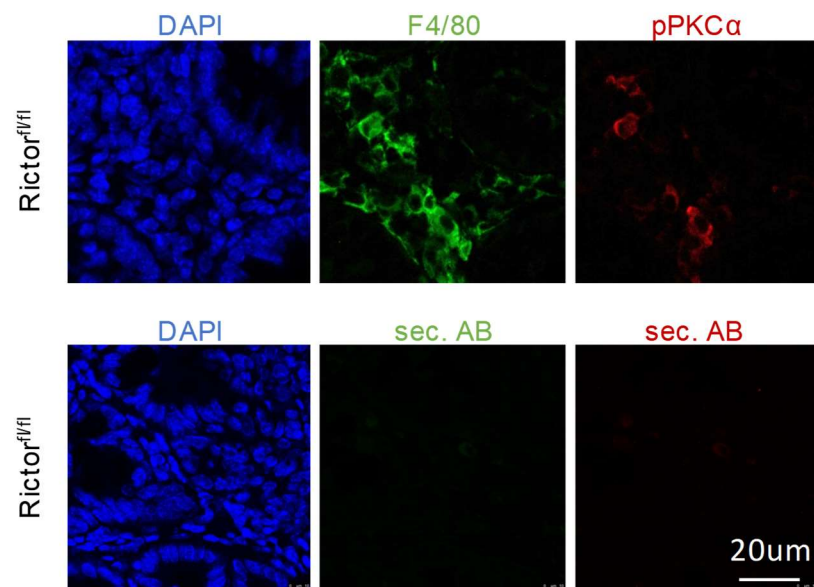


Figure S3. Specificity of F4/80 and pPKC α antibodies.

Immunofluorescence staining showing the *Rictor^{fl/fl}* sample from Figure 3H (upper panel) together with a control staining with secondary antibodies only (lower panel).

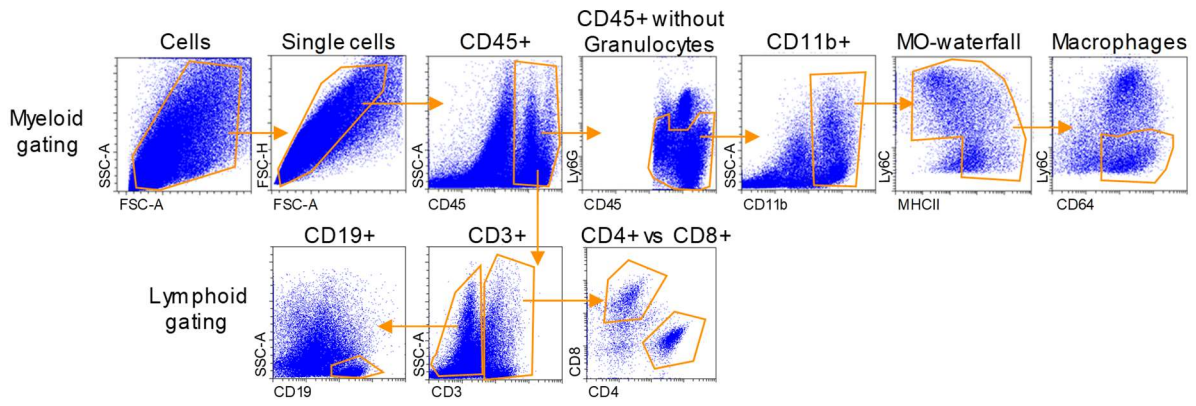


Figure S4. Gating scheme of myeloid and lymphoid staining.

Gating strategies for the myeloid and lymphoid staining are shown. The populations are defined as follows: leukocytes (CD45+), macrophages (CD45+ CD11b+ CD64+ MHCII+ Ly6C-), granulocytes (CD45+ CD11b+ Ly6G+), B-cells (CD45+ CD19+ MHCII+), CD4+ T-cells (CD45+ CD3+ CD4+) and CD8+ T-cells (CD45+ CD3+ CD8+).

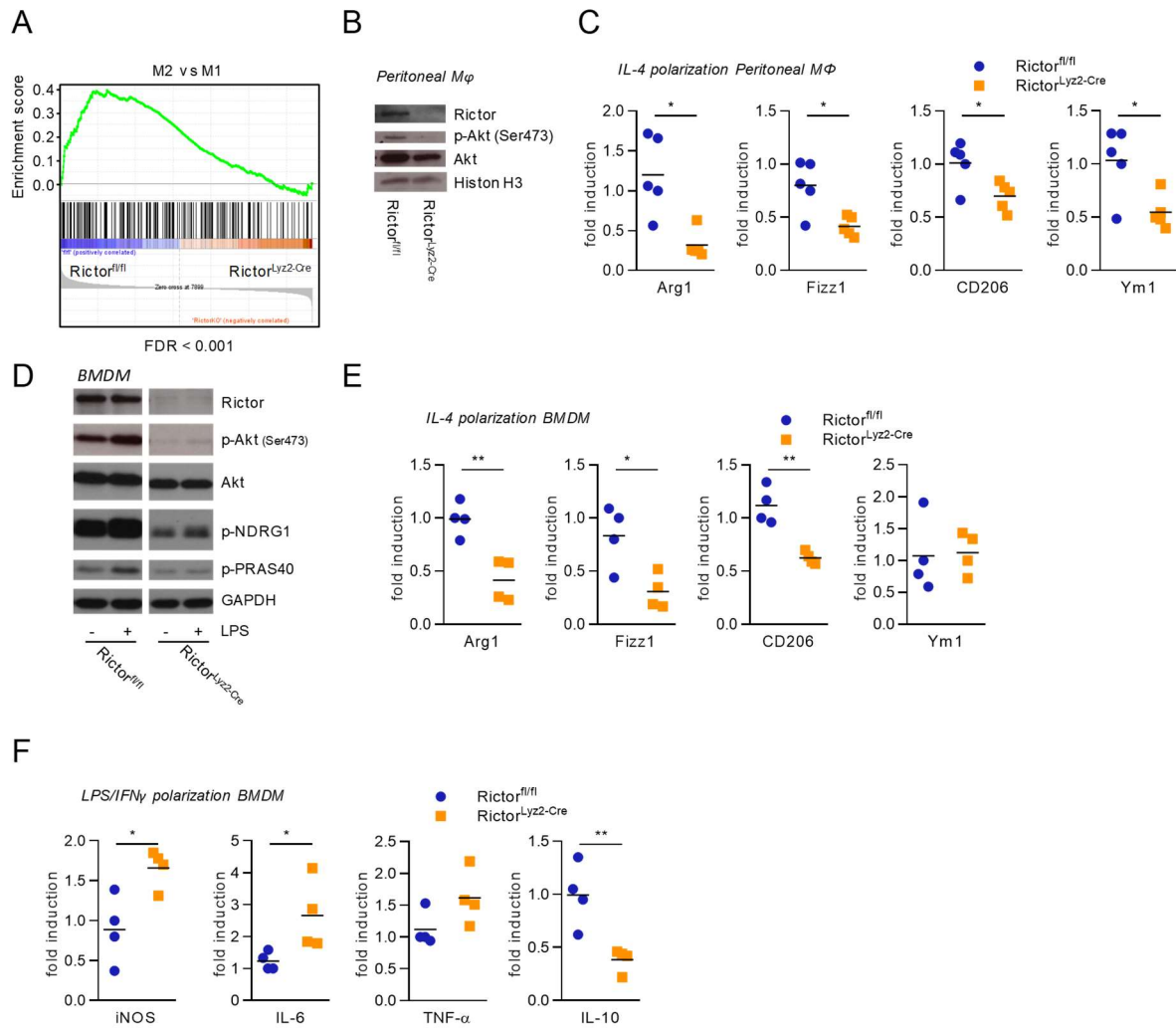


Figure S5. Macrophage polarization of *Rictor^{fl/fl}* and *Rictor^{Lyz2-Cre}* mice.

(A) Microarray data of sorted *Rictor^{fl/fl}* and *Rictor^{Lyz2-Cre}* colonic macrophages on day 8 during DSS-induced colitis was subjected to gene-set enrichment analysis (GSEA). GSEA plot of the M2 vs M1 signature in *Rictor^{fl/fl}* colonic macrophages relative to *Rictor^{Lyz2-Cre}* colonic macrophages is shown. (B) Immunoblot with the indicated antibodies of peritoneal macrophages. (C) Peritoneal macrophages were stimulated for 48 hours with 10 ng/ml IL-4. Levels of the indicated mRNAs were determined by RT-PCR, normalized to β -actin. (D) Immunoblot with the indicated antibodies of unstimulated BMDM and BMDM stimulated for 1 h with 100 ng/ml LPS. (E,F) BMDM were stimulated for 48 hours with 10 ng/ml IL-4 (E) or 100 ng/ml LPS and 20 ng/ml IFN γ (F). Arg-1, Fizz1, CD206, YM1 and IL-10 were used as M2 markers while iNOS, IL-6 and TNF α were used as M1 markers. Lines represent the means (n \geq 4). P values were determined by unpaired two-tailed Student's t-test. *p < 0.05, **p < 0.01.

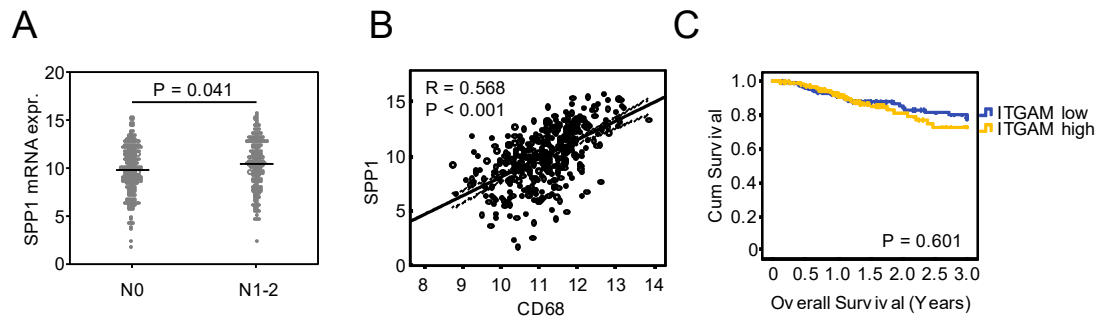
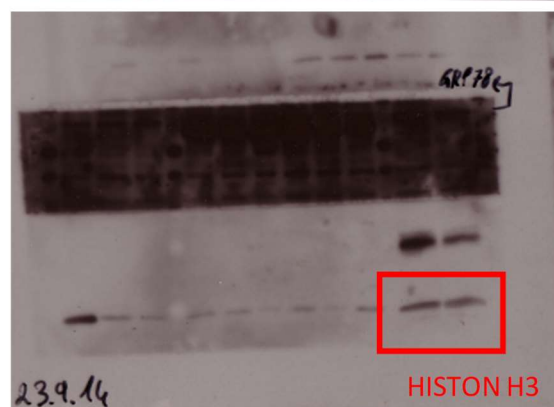
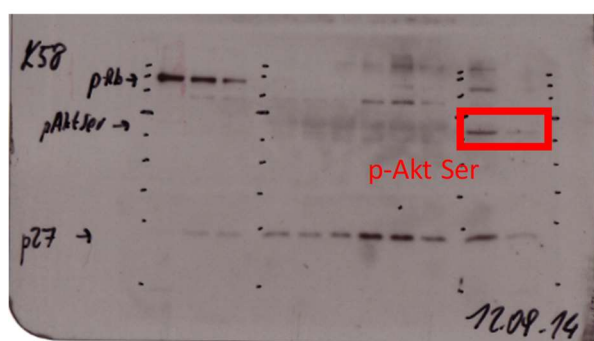
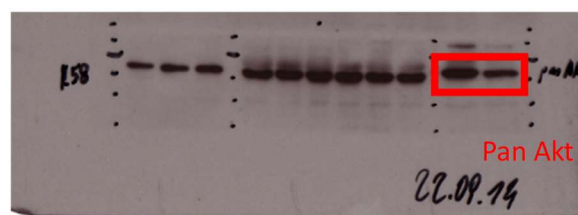
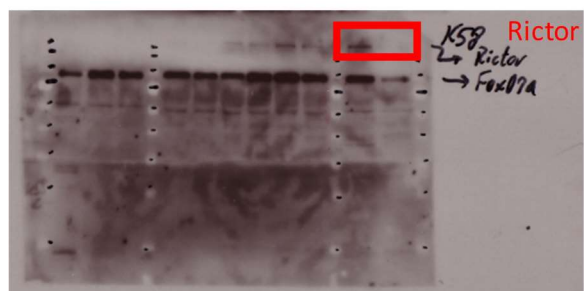


Figure S6. SPP1 correlates with tumor size and poor survival in human colorectal cancer patients.

(A) Comparison of SPP1 mRNA expression in colorectal primary tumor samples of negative (N0) and positive (N1-2) N stage in the TCGA dataset. (B) Scatter plot showing correlations between SPP1 and CD68. Regression lines with corresponding 95% confidence intervals are presented within the graph. (C) Kaplan Meier analysis of colorectal primary tumor samples distributed into low ($n = 181$) and high ($n = 180$) ITGAM expression (A-C) Data are displayed as log2 mRNA expression intensity. Unpaired two-tailed t-test was used to calculate p-values. To determine p-values for the Kaplan Meier analysis, we performed Log-rank test.

Uncropped blots for Fig. S5B



Western blot analysis showing Akt phosphorylation (pAkt Ser473) and total Akt levels. The blot is probed with anti-pAkt Ser473 (top) and anti-Akt (middle). Histone H9 (bottom) serves as a loading control. Two lanes are highlighted with red boxes, indicating increased pAkt Ser473 levels. Labels include 'K123', 'Rictor', 'pAkt Ser473', and 'Histone H9'.

