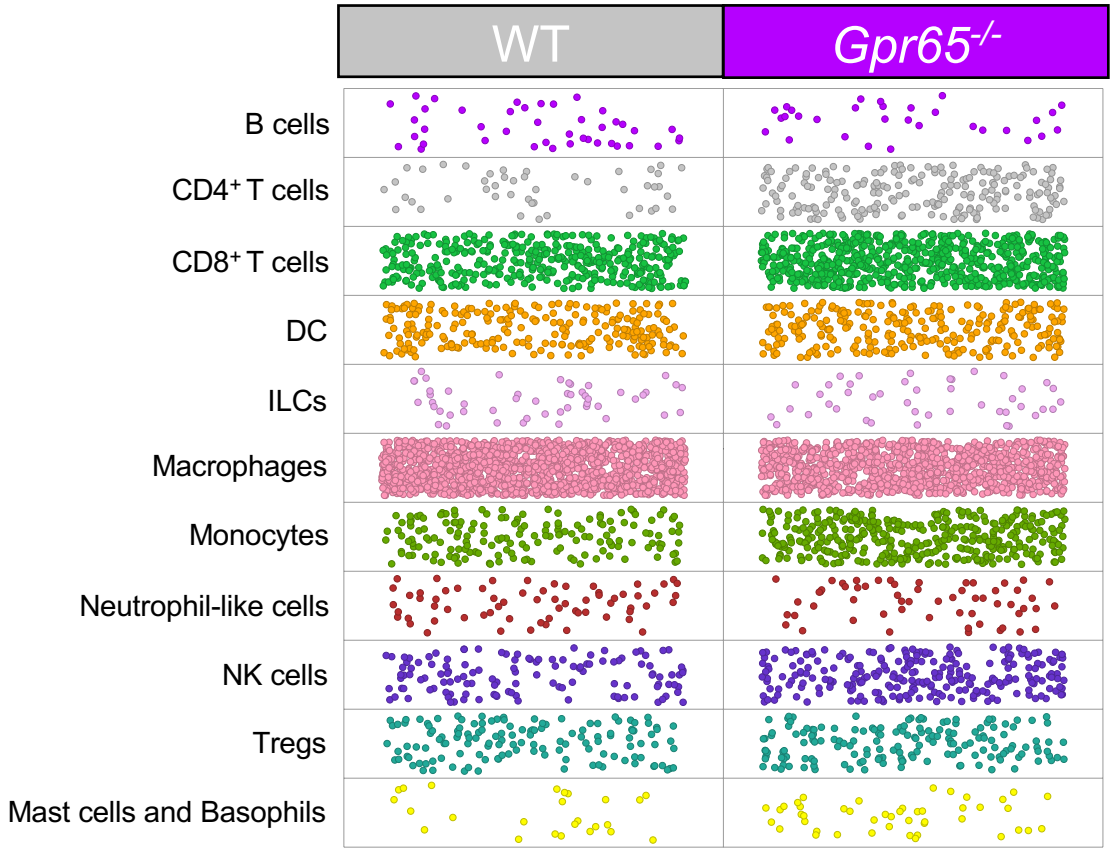
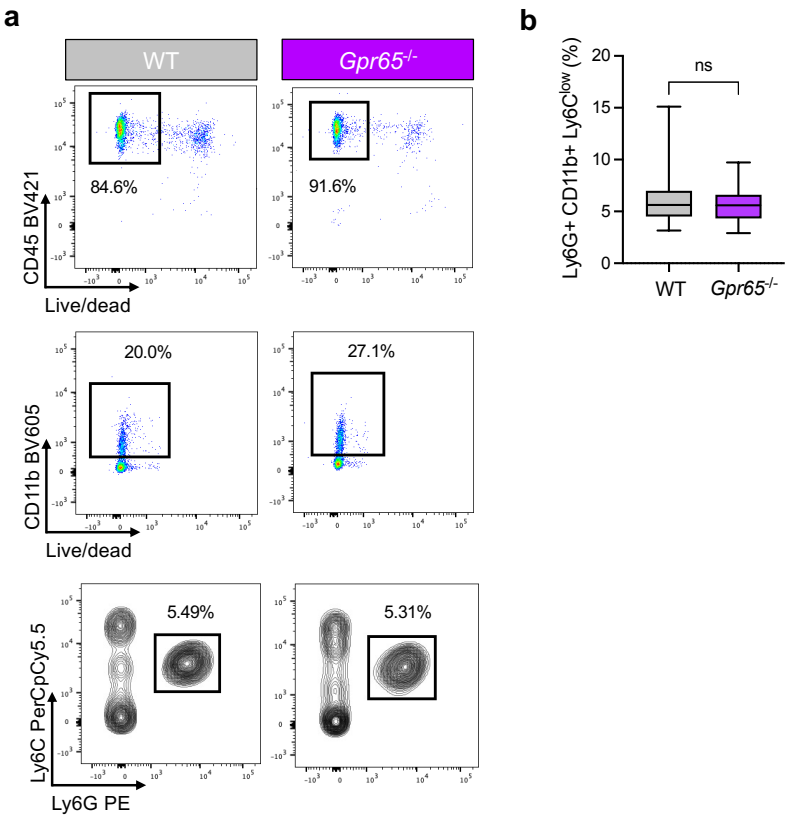
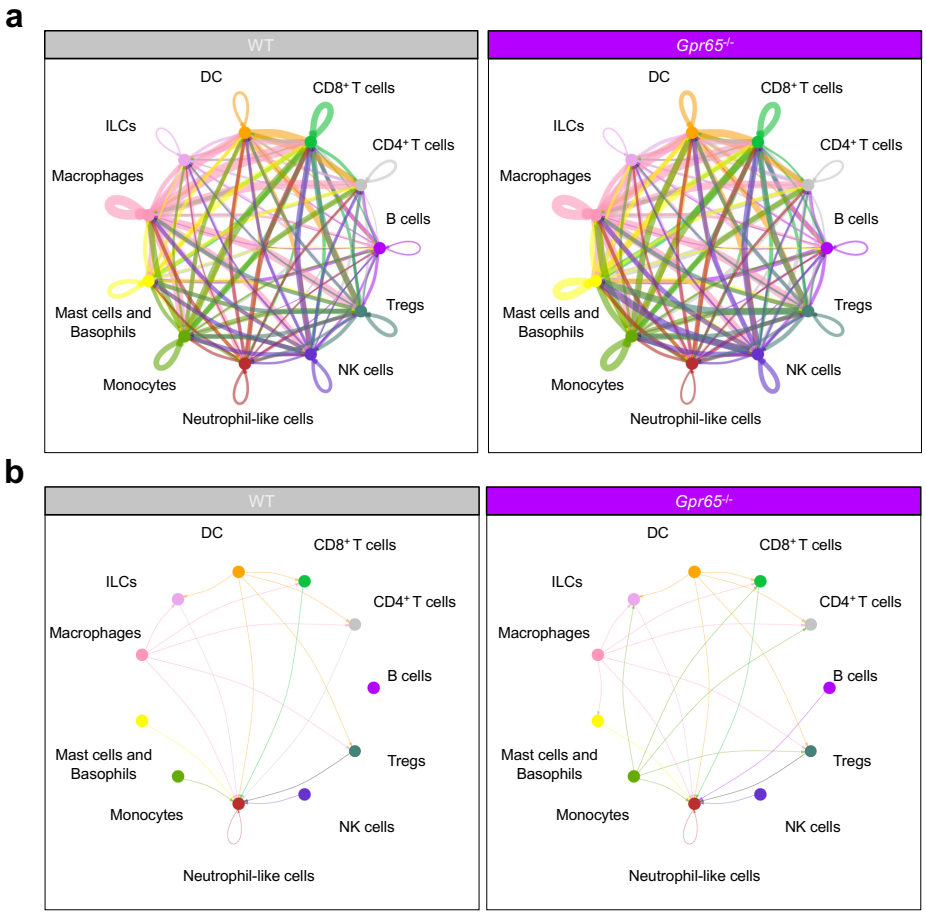


Supp. Fig. 1 TME composition of weakly acidic tumors.
$1x10^6$ MC38 colon adenocarcinoma cells were inoculated s.c. into the right flank of *Gpr65*-competent (WT, n=7) and *Gpr65*-deficient mice (*Gpr65*$^{-/-}$, n= 9). Tumor measurement and volume calculation were performed as described in Methods. On day 16 after tumor cell inoculation, $CD45^+$ TILs were isolated and scRNA-seq was performed (WT: n=2, *Gpr65*$^{-/-}$: n=3). Distribution of the individual cells of each predicted cell population is shown as a scatter plot.

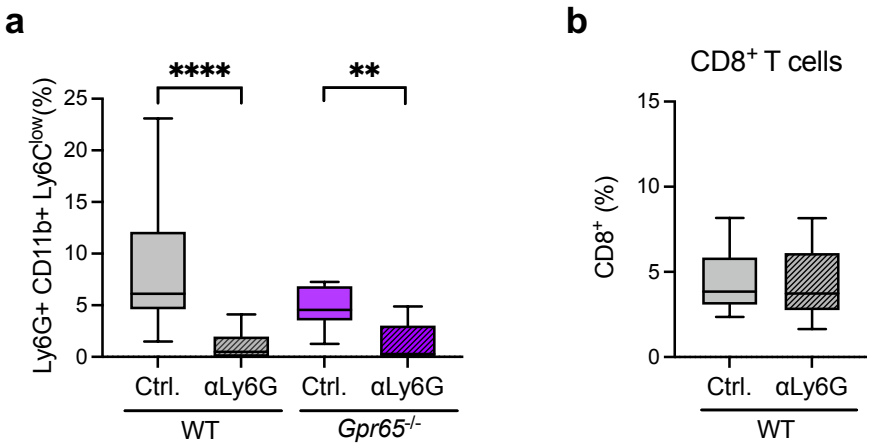


Supp. Fig. 2 Neutrophil development in *Gpr65*-deficient and WT control mice.
Blood samples of *Gpr65*-deficient (*Gpr65*$^{-/-}$, n=19) and WT control mice (n=20) were collected and number of neutrophils was analyzed by flow cytometry. In **a**, representative gating strategy from one sample is shown and in **b**, results of all analyses are represented as box plots. Center lines represent the median, whiskers indicate minimum and maximum values, boxes indicate interquartile range.

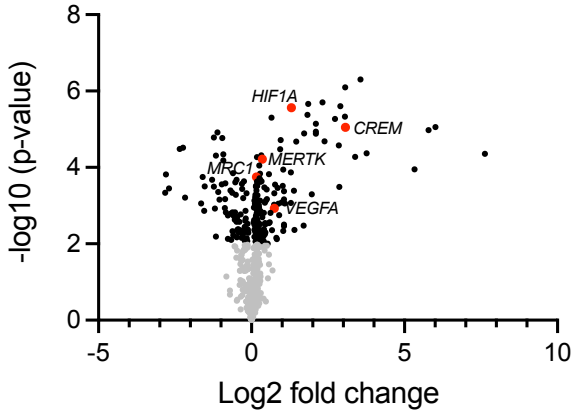


Supp. Fig. 3 Network analyses in weakly acidic tumors grown in *Gpr65*-deficient and wildtype control mice.

$1x10^6$ MC38 colon adenocarcinoma cells were inoculated s.c. into the right flank of *Gpr65*-competent (WT, n=7) and *Gpr65*-deficient mice ($Gpr65^{-/-}$, n= 9). Tumor measurement and volume calculation were performed as described in Methods. On day 16 after tumor cell inoculation, $CD45^+$ TILs were isolated and scRNA-seq was performed (WT: n=2, $Gpr65^{-/-}$: n=3). Cell-cell communication of individual cell clusters was analyzed by CellChat. Communication plots of *Gpr65*-deficient mice and respective littermate controls are shown. General communication is presented in **a** and CXCL-dependent communication in **b**.

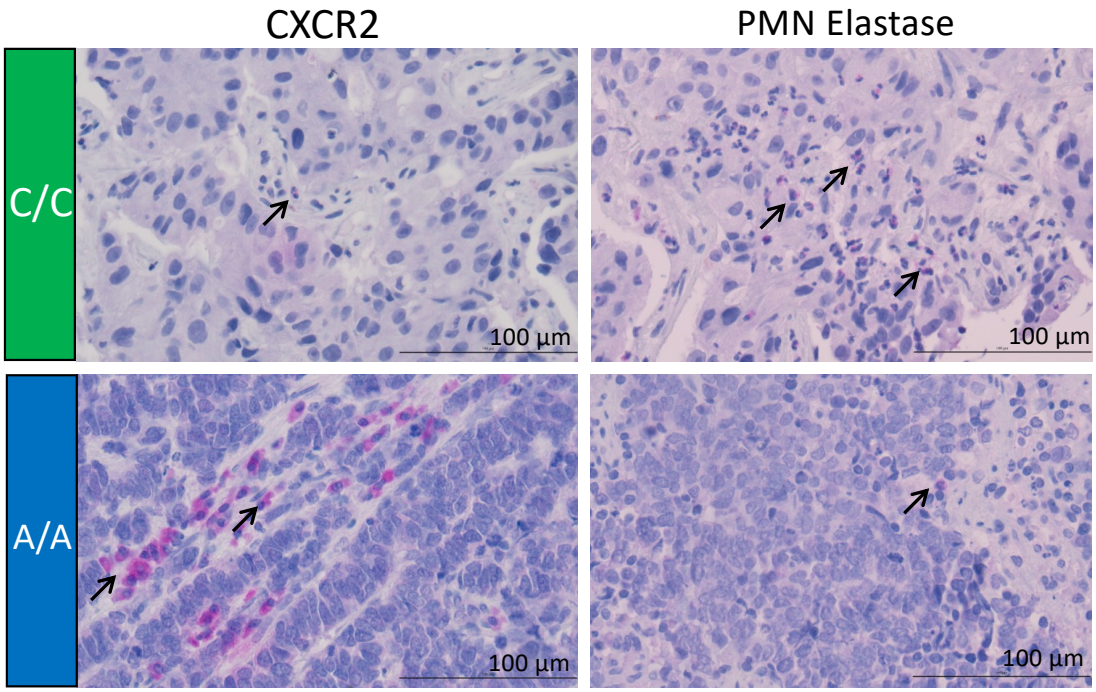


Supp. Fig. 4 Depletion of neutrophil-like cells.
B16F10-bearing *Gpr65*-competent (WT) and Gpr65-deficient (*Gpr65*$^{-/-}$) mice were treated with Ly6G-antibody (αLy6G) or respective isotype control (Ctrl.), n=10 per group. Inoculation of tumor cells and antibodies was performed as described in Methods. On day 20, tumors were harvested and percentages of tumor infiltrated neutrophil-like cells (**a**) and CD8^{+} T cells (**b**) were analyzed by flow cytometry. Results are shown as box plots. Center lines represent the median, whiskers indicate minimum and maximum values, boxes indicate interquartile range. In **a**, statistical analyses were done using Mann-Whitney test, p-value WT Ctrl. vs. WT αLy6G < 0.0001, p-value *Gpr65*$^{-/-}$ Ctrl. vs. *Gpr65*$^{-/-}$ αLy6G = 0.0062.

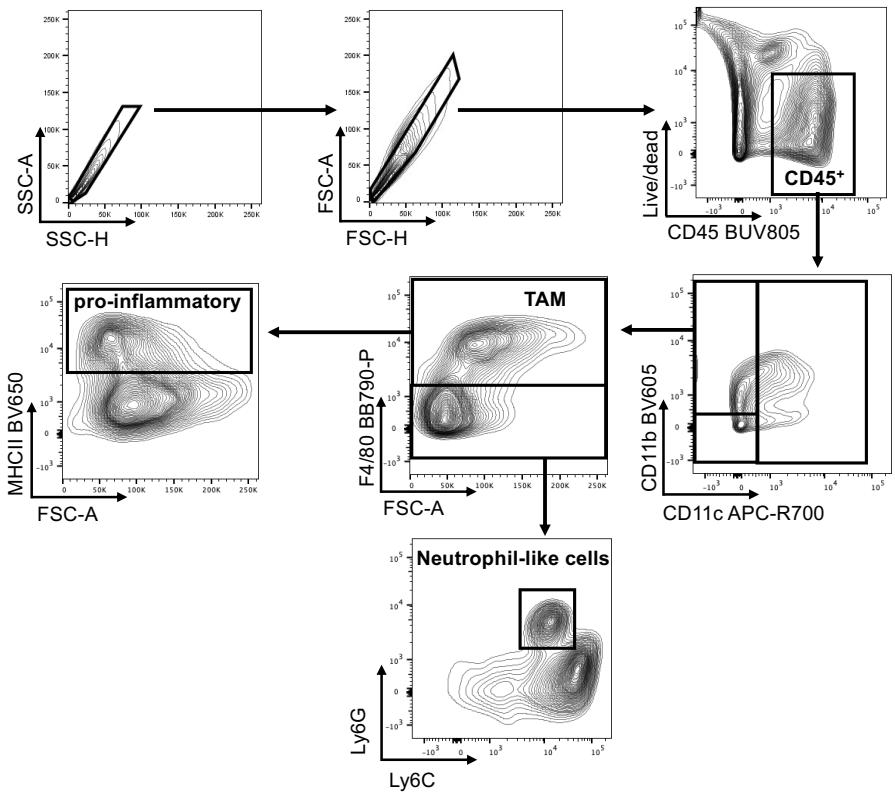


Supp. Fig. 5 Expression of anti-inflammatory marker genes in human macrophages under acidic conditions.

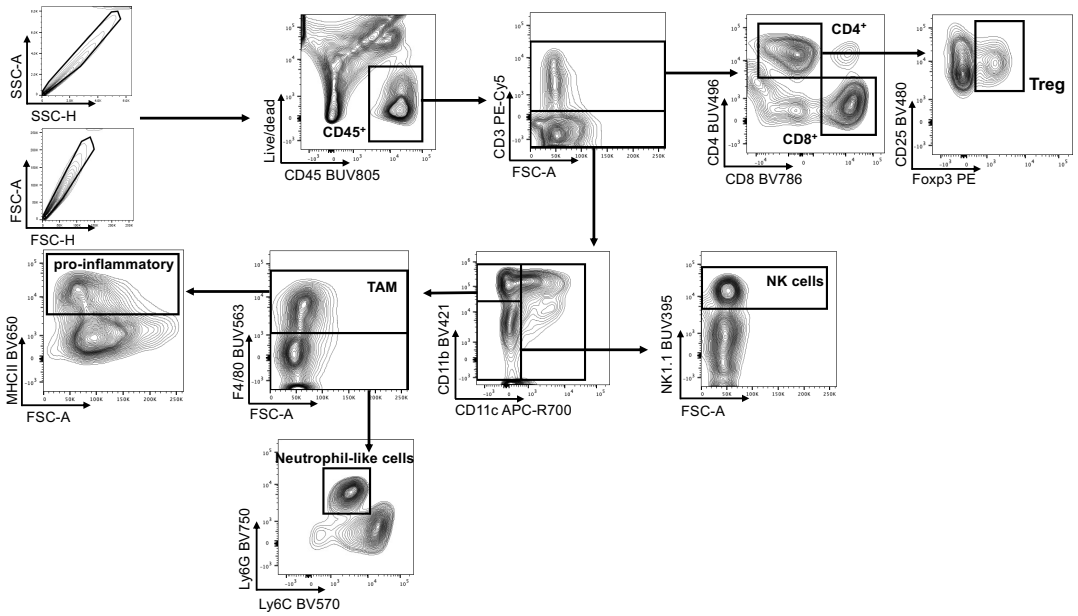
Primary human macrophages were cultured under acidic and neutral conditions as described in Methods. Significantly regulated genes under acidic conditions are plotted as volcano plot. Each dot represents one gene. Genes associated with an anti-inflammatory macrophage phenotype are highlighted in red. Shown are representative results from one of two independent experiments.



Supp. Fig. 6 Immunohistochemistry of CXCR2 and PMN Elastase in lung cancer of homozygous SNP I231L carrier.
Non-small-cell lung cancer tissue samples from patients carrying the I231L SNP in the *GPR65* locus (C/C) and WT allele controls (A/A) were stained with antibodies against CXCR2 (left panels) and PMN Elastase (right panes). Staining was performed as described in methods. Representative images are shown.



Supp. Fig. 7 Gating strategy of flow cytometric analyses of the "neutrophil-like cell depletion" experiment (Fig. 4). Starting from singlets, dead cells were excluded and $CD45^+$ tumor infiltrated TILs were gated. Following, pro-inflammatory TAM and neutrophil-like cells were identified.



Supp. Fig. 8 Gating strategy of flow cytometric analyses of the "CXCR2 antagonism and ICB treatment" experiment (Fig. 5). Starting from singlets, dead cells were excluded and $CD45^+$ tumor infiltrated TILs were gated. Following, pro-inflammatory TAM, Treg, NK cells, and neutrophil-like cells were identified.

Supplemental Methods

Single cell mRNA sequencing

$1x10^6$ MC38 colon adenocarcinoma cells were inoculated in *Gpr65*$^{-/-}$ mice and respective littermate control mice as described in the section "Subcutaneous tumor mouse models". 16 days after inoculation, tumors were harvested and dissociated to a single cell suspension using the murine Tumor Dissociation Kit (Miltenyi Biotec). $CD45^+$ tumor-infiltrating leucocytes (TIL) were isolated from single cell suspension by CD45 (TIL) MircoBeads (Miltenyi Biotec) using magnetic separation (MACS, Magnetic Activated Cell Sorting). To enhance purity, viable $CD45^+$ $CD11b^+$ $F4/80^+$ were enriched by flow cytometry-based cell sorting using FACS Aria II machine (BD Biosciences). Cell surface staining was performed as described in the flow cytometry section. Single cell capturing, isolation of RNA and synthesis of cDNA was performed according to the BD Rhapsody Single-Cell Analysis System (BD Rhapsody) following the manufacturer's guidelines. Each sample was tagged with a unique sample tag in order to allow multiplexing of samples on the same cartridge. Whole transcriptome libraries and sample tag libraries were created using BD WTA Amplification Kit (BD Rhapsody) with random primers followed by amplification and insertion of sequencing adaptors following the BD Rhapsody System mRNA WTA and Sample Tag Library Preparation Protocol (BD Biosciences). Quality and quantity of cDNA libraries was checked on a Bioanalyzer2100 (Agilent) and sequencing (150 Paired-End) was performed on a NovaSeq 6000 (Illumina) at Novogene (Cambridge, UK).

Resulting raw data was preprocessed according to the Illumina standard protocol and transcript alignment, counting and demultiplexing was performed using the BD Rhapsody WTA analysis pipeline yielding to 7925 called putative cells overall with a mean of 140,000 aligned reads per cell. Pipeline output RSEC Mols per Cell files were imported in Partek Flow software (Version 10.0) and cells that contained less than 500 detected genes and a percentage of mitochondrial counts higher than 10% were removed from subsequent analysis resulting in 5941 cells for

subsequent analyses. Single cell counts were normalized by Partek Flows recommended normalization order (CPM (counts_per_million), Add:1 and log2). For dimensionality reduction PCA was used, followed by Graph-based clustering (using the SmartLocalMoving (SLM) clustering algorithm) and t-SNE algorithm for visualization. Populations shown as scatter plot were annotated according to ImmGen‘s (https://www.immgen.org/) databrowser.