

Supplementary Information

Mice

Wild-type (WT) C57BL/6 and BALB/c mice were purchased from Walter and Eliza Hall Institute for Medical Research or bred in house. C57BL/6 *Ptprc*^a (CD45.1⁺) mice, C57BL/6 CD39-deficient (*Cd39*^{-/-}) mice (1), C57BL/6 CD73-deficient (*Cd73*^{-/-}) mice, C57BL/6 Adenosine 2A receptor-deficient mice (*Adora2a*^{-/-}) (2); C57BL/6 Adenosine 2B receptor-deficient mice (*Adora2b*^{-/-}), C57BL/6 NALP3-deficient (*Nalp3*^{-/-}) mice, C57BL/6 P2X7-deficient (*P2X7*^{-/-}) mice (3), C57BL/6 ASC-deficient (*Pycard*^{-/-}) mice, C57BL/6 IL-18-deficient mice (*Il18*^{-/-}) (4), C57BL/6 IL-1 receptor-deficient mice (*Il1R*^{-/-}), C57BL/6 IFN γ -deficient mice (*Ifng*^{-/-}), C57BL/6 perforin-deficient (*Pfp*^{-/-}), C57BL/6 *gld* mutant (*gld*) and Nod-rag1-gamma c (NRG) mice were bred in-house and maintained at the QIMR Berghofer Medical Research Institute. Pmel-1 TCR Tg mice have been previously described (5). Mice greater than 6 weeks of age were sex-matched to the appropriate models. The number of mice in each group treatment or strain of mice for each experiment is indicated in the figure legends. In all studies, no mice were excluded based on pre-established criteria and randomization was only applied immediately pre-treatment in therapy experiments to ensure similar mean tumor size was the starting point. Experiments were conducted as approved by the QIMR Berghofer Medical Research Institute Animal Ethics Committee.

Cell Culture

Mouse CT26 colon adenocarcinoma and TUBO mammary carcinoma were cultured in RPMI 1640, supplemented with 10% Fetal Calf Serum (Bovogen), 1% Glutamine

(Gibco), and 1% Penicillin-Streptomycin (Gibco)(complete RPMI). The human SK-MEL-28 melanoma was routinely maintained in complete RPMI.

In vivo treatments

For mouse tumor models, clodronate-containing liposomes for the depletion of phagocytic cells and empty-control liposome preparations was used as described [clodrolip; sodium clodronate tetrahydrate, Farchemia SRL, Treviglio, Italy (6)]. Clodrolip and control liposomes were diluted in PBS and injected i.p. at 2 mg per 20 g body weight, 2 d prior to therapy. Subsequent doses of depleting and control liposome preparations (1 mg/20 g body weight) were administered every 2 d. Some groups of mice were neutralized for TRAIL (N2B2), CD11b (5C6), or IFN γ (H22) using the scheduling and dosing as indicated. Some mice were treated with clg (I-536, Leinco), anti-mouse CD39 (B66, mIgG1, Tizona), anti-mouse CD39 (B66, mIgG1 D265A, Tizona), anti-mouse CD39 (Tz-617, mouse IgG1, Tizona), anti-mouse CD39 (Tz-619, mouse IgG1, Tizona), anti-CD73 (2C5; mIgG1, Tizona), anti-PD1 (RMP1-14), A2AR inhibitor (SCH58261)(Sigma-Aldrich), A2BR inhibitor (PSB1115)(Sigma Aldrich), and CD39 inhibitors (POM1 and ARL-67156)(Santa Cruz Biotechnology and Sigma Aldrich, respectively) with schedules and doses as indicated in the figure legends. For human tumor models, some groups of mice treated either: clg (Palivizumab hG4, MedImmune), anti-CD39 (TTX-030; Tizona), anti-PD1 (Pembrolizumab, Merck) with schedules and doses as indicated in the figure legends.

Enzyme Histochemistry

Enzyme histochemistry (EHC) was performed on mouse MC38 tumors harvested on day 14 in OCT. Slides containing 5 μ M OCT-embedded fresh frozen tissue were brought to RT before fixing in 4% PFA, then transferred to a chamber containing Trizma-maleate sucrose buffer (TMSB 9.5 g/L Trizma maleate, 80 g/L sucrose, pH 7.4) for 60 minutes. The slides were then incubated in substrate buffer containing TMSB with 0.5 mM CaCl_2 , 0.25 mg/mL lead nitrate and ATP before washing with dH₂O. The lead orthophosphate precipitated in the course of nucleotidase activity was visualized as a brown deposit by incubating sections in 0.4% $(\text{NH}_4)_2\text{S}$ followed by three washes in TMSB. Slides were mounted with mounting medium (DAKO) and images were acquired using a digital Aperio slide scanner. Using Aperio ImageScope software, the representative images were captured and the mean of total pixel intensities across sample per unit area of the tumor tissues were calculated for each sample.

IL-18 ELISA

WT mice and *Nalp3*^{-/-} mice were subcutaneously challenged with MC38 tumor cells (1×10^6). Mice were treated i.p. with anti-CD39 or clg (200 μ g) on day 8 after tumor inoculation. Tumor samples were collected 24 h post-treatment, followed by snap-freezing in liquid nitrogen. Frozen tumor samples were mechanically digested in the presence of T-PER Tissue Protein Extraction Reagent (Thermo Fisher Scientific) supplemented with Complete™ Protease Inhibitor Cocktail (Sigma Aldrich). IL-18 levels in tumor lysates were measured by sandwich ELISA using anti-mouse IL-18 mAb (clone 74, MBL international), and biotinylated anti-IL-18 (Clone 93-10C, MBL international), as capture and detection antibodies, respectively (7). Colorimetric

quantification was performed by HRP-streptavidin (Sigma Aldrich) and TMB substrate solution (eBioscience).

Flow cytometry

Tumors, tumor draining lymph nodes, and spleens were harvested from mice untreated or treated with control or therapeutic antibodies as indicated in the figure legends. Tumors and lymph nodes were minced and digested with 1 mg/mL collagenase IV (Worthington Biochemical) and 0.02 mg/mL DNase I (Roche) and homogenized to prepare single cell suspensions. Single cell suspensions of spleens were depleted of erythrocytes. For surface staining, cells were stained in phosphate buffered saline (PBS) containing 2% (v/v) FBS with anti-CD45.2 (104; BD biosciences) or anti-CD45.2 (30-F11; Thermofisher), anti-CD4 (RM4-5; Biolegend) or anti-CD4 (GK1.5; eBioscience), anti-CD8a (53-6.7; Biolegend), anti-TCR β (H57-597; Biolegend), anti-NK1.1 (PK136; BD biosciences), anti-CD39 (Duha59; BioLegend), anti-CD73 (TY/23; BD Bioscience), OVA-tetramer (assembled with biotinylated KbOVA monomer from Prof Andrew Brooks Lab, the University of Melbourne and streptavidin from BD biosciences), anti-CD279 (29F.1A12; BioLegend), anti-Ly6G (1A8; BioLegend), anti-CD11b (M1/70; BioLegend), anti-CD11c (N418; eBioscience), anti-CD64 (X54-5/7.1; BioLegend), anti-MHC II (M5/114.15.2; eBioscience), anti-CD274 (10F.9G2; BioLegend), anti-P2X7R (1F11; BioLegend). For intracellular staining, surface-stained cells were fixed and permeabilized using the Foxp3/Transcription Factor Staining Buffer Set (eBioscience) or BD Cytofix/Cytoperm (BD Biosciences) according to the manufacturer's protocol and stained with anti-Foxp3 (FJK-16s, eBioscience), anti-IFN γ (XMG1.2; BioLegend), anti-Ki67 (16A8; BioLegend) or anti-Ki67 (B56; BD biosciences), and respective

isotype antibodies. For intracellular staining of IFN γ , cells were stimulated ex vivo with eBioscience™ Cell Stimulation Cocktail (plus protein transport inhibitors) for 4 h before surface staining. Populations defined in CD45⁺ live cells: CD8⁺ T cells, TCR β ⁺CD8⁺; CD4⁺Foxp3⁻ T cells, TCR β ⁺CD4⁺Foxp3⁻; regulatory T cells (Treg), TCR β ⁺CD4⁺Foxp3⁺; natural killer (NK) cells, NK1.1⁺TCR β ⁻; neutrophils, Ly6G⁺; eosinophils, Ly6G⁻CD64⁻MHCII⁻CD11b⁺SSC^{hi}; macrophages, Ly6G⁻CD64⁺MHCII⁺; dendritic cells (DC), Ly6G⁻CD64⁻MHCII⁺CD11c⁺; monocytes, Ly6G⁻CD64^{-/lo}MHCII^{-/lo}CD11b^{hi}SSC^{lo}. Cells were acquired on the BD LSR Fortessa V (BD Biosciences) and analysis was carried out using FlowJo (Tree Star). Dead cells stained by 7-AAD (BioLegend) or Zombie Aqua (BioLegend) were excluded from analysis. Lymphoid and myeloid components were gated by markers indicated in Figure S7.

Adoptive transfer of gp100-specific mouse T cells

Cohorts of C57BL/6 WT or *Cd39*^{-/-} mice were s.c. injected with HC.PmelKO.TYRP1-SFhg100 melanoma cells. The melanoma cell line HCmel12 was established from a primary melanoma in the *Hgf-CDK4*^{R24C} mouse model by serial transplantation in our laboratory as previously described (8). The Pmel gene was knocked out using CRISPR/Cas9 technology to generate the HC.PmelKO melanoma monoclonal cell line. Using CRISPiTope we generate HC.PmelKO cells expressing mScarlet fluorescent protein (S), a FLAG-Tag (F) and the human gp100 epitope [KVPRNQDWL (hgp100₂₅₋₃₃)] fused to TYRP1 to generate HC.PmelKO.TYRP1-SFhgp100 cells as described previously (Effern et al. submitted). Once melanomas were palpable, mice received a single dose of cyclophosphamide (2 mg i.p., Baxter) followed the next day by the adoptive transfer of 1 x 10⁶ purified gp100-specific Pmel-1 T cells isolated from

the spleen of Pmel-1 TCRTg mice. On days 3,6 and 9 after cyclophosphamide all mice received intratumor injections of polyI:C/CpG (50 µg/mouse of each, Invivogen) as described previously (9). Mice were randomly allocated for antibody treatment on the day of cyclophosphamide injection. Therapeutic antibody treatment commenced as indicated and mlgG1 or anti-CD39 (B66) (200 µg each i.p.) was given every 3 or 4 days up to a maximum of 5 doses.

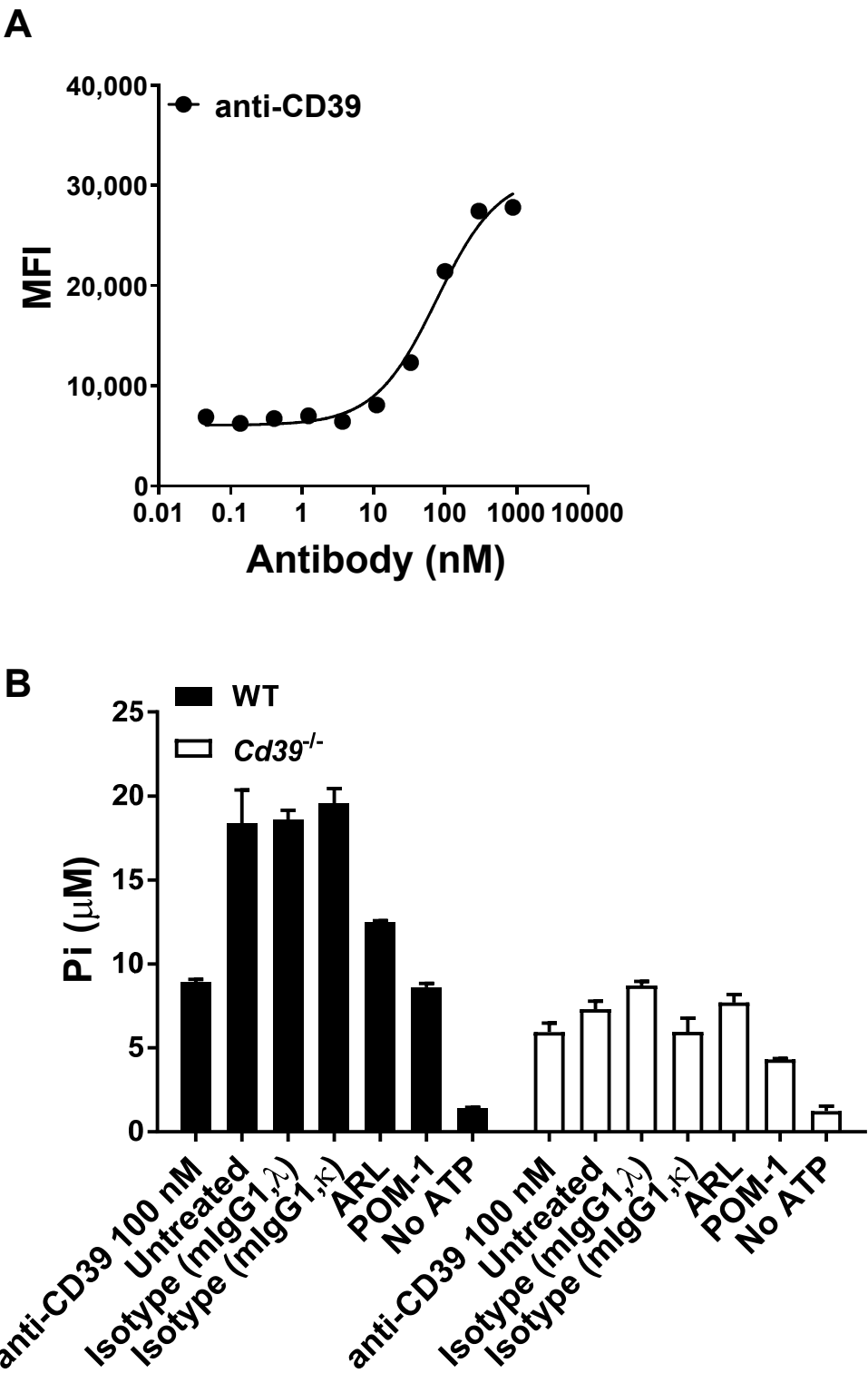
Statistical analysis

Statistical analysis was determined with Graphpad Prism 7 (GraphPad Software). A 1-tailed Mann-Whitney U test was used for comparisons of 2 groups. Significance of differences was also calculated by log-rank t test or Gehan-Breslow-Wilcoxon for Kaplan-Meier survival analysis or 2-way ANOVA as necessary. Tukey's multiple comparisons tests were utilized unless otherwise indicated. A Fisher's exact test was also used to determine significance of proportion of tumor free mice. Differences between groups are shown as the mean $\pm$ SEM. P values of less than 0.05 were considered statistically significant.

References

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Figure S1

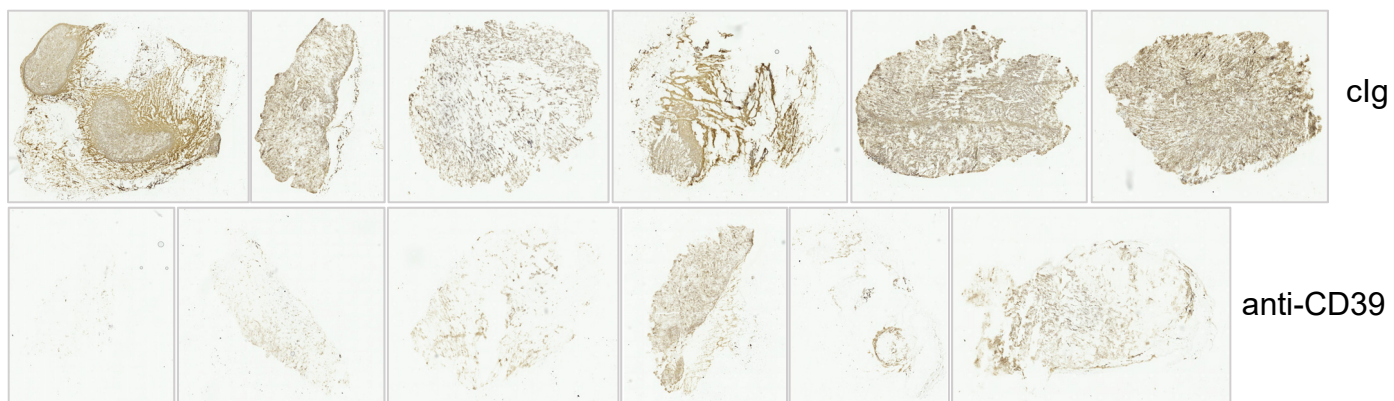


Supplementary Figure Legends

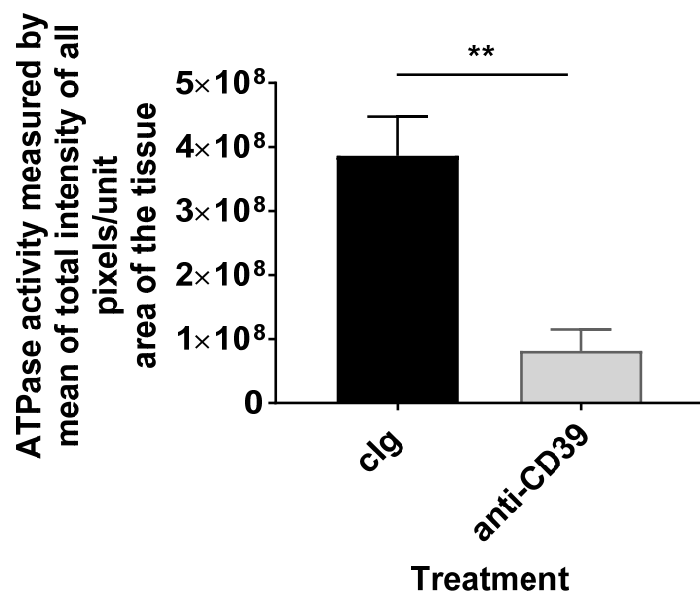
Figure S1. Anti-CD39 mAb binds to BCL1 cells with high affinity and blocks CD39 enzymatic activity in splenocytes. The graphs shown represent the binding affinity of anti-CD39 antibody (B66) to BCL1 cells by titration **(A)** and blockade of CD39 enzymatic activity by isotype antibody controls, B66, ARL and POM-1 in the presence of ATP or absence (no ATP) in splenocytes from WT (closed squares) and CD39^{-/-} (open squares) mice measured by phosphate release **(B)**. In addition, anti-CD39 was determined by Biolayer Interferometry to bind to recombinant mCD39-ECD with a monovalent K_D of 2.65 nM.

Figure S2

A



B



C

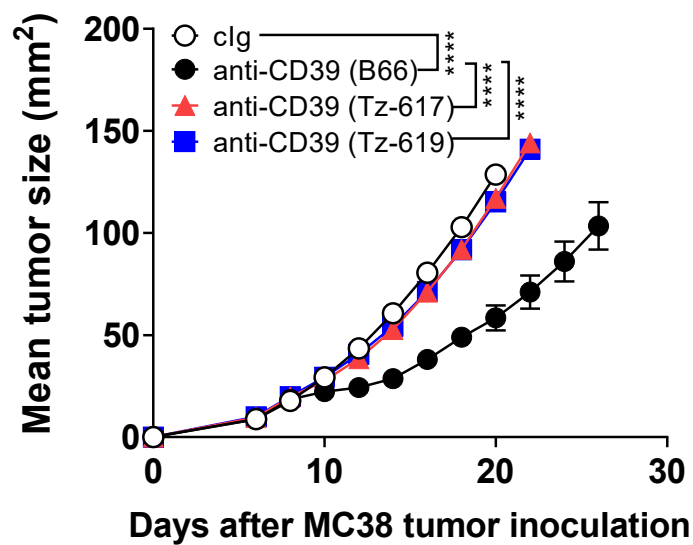
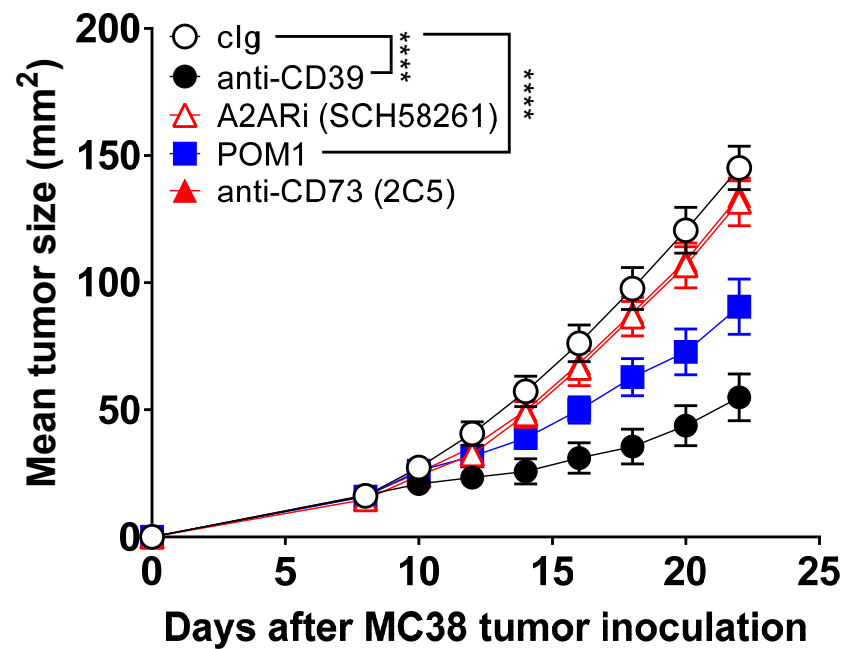


Figure S2. Groups (n = 6/group) of WT mice were injected s.c. with MC38 colon adenocarcinoma cells (1×10^5) on day 0 and treated i.p. with clg (200 μ g) or anti-CD39 (200 μ g) on day 12. The tumor samples were harvested 48 h post-treatment in OCT and EHC was performed as described in Methods. **(A)** Representative EHC images and **(B)** the quantification of staining intensity represented as the means of total pixel intensities across samples per unit area of the tumor tissues, showing reduced ATPase activity in the anti-CD39-treated group compared to clg-treated group. Significant differences were determined by Mann-Whitney test (** $P < 0.01$). Representative of two experiments performed **(C)** Groups (n = 10/group) of WT mice were injected s.c. with MC38 (1×10^5) colon adenocarcinoma cells on day 0 and treated i.p with clg (200 μ g) or various anti-CD39 (B66, Tz-617, Tz-619, 200 μ g) on days 8, 11, 14, and 17. Representative of two experiments performed. Tumor sizes (mm^2) were measured at the indicated time points and presented as mean $\pm$ SEM. All experiments were performed once unless indicated. Significant differences among treatment groups were determined by a two-way ANOVA, followed by Tukey's multiple comparison test (**** $p < 0.0001$).

Figure S3

A



B

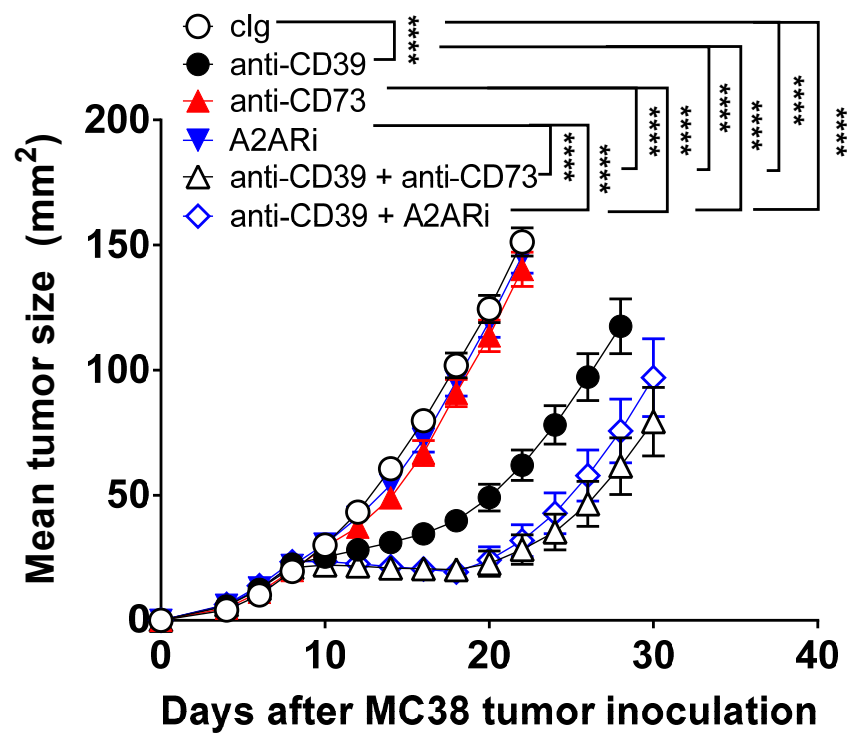


Figure S3. Anti-CD39 mAb is more effective than POM-1 in suppressing MC38 tumor growth. (A) Groups (n = 5/group) of C57BL/6 WT mice were injected s.c. with MC38 (1×10^5) colon adenocarcinoma cells and treated i.p. with clg (200 μ g) anti-CD39 (200 μ g), A2ARi (SCH58261, 10 mg/kg) on days 8, 11, 14 and 17, or 250 μ g POM-1 on days 8, 10, 12, 14, 16 and 18. **(B)** Adenosine blockade further improves anti-CD39 control of MC38 tumor growth. Groups (n = 10/group) of WT mice were injected s.c. with MC38 (1×10^5) colon adenocarcinoma cells on day 0 and treated i.p with clg (200 μ g), anti-CD39 (200 μ g), anti-CD73 (200 μ g), A2ARi (SCH58261)(10 mg/kg) or combination of these as indicated on days 8, 11, 14, and 17. This experiment is representative of two performed. Tumor sizes (mm^2) were measured at the indicated time points and presented as mean $\pm$ SEM. Significant differences among treatment groups were determined by a two-way ANOVA, followed by Tukey's multiple comparison test (**** $P < 0.0001$).

Figure S4

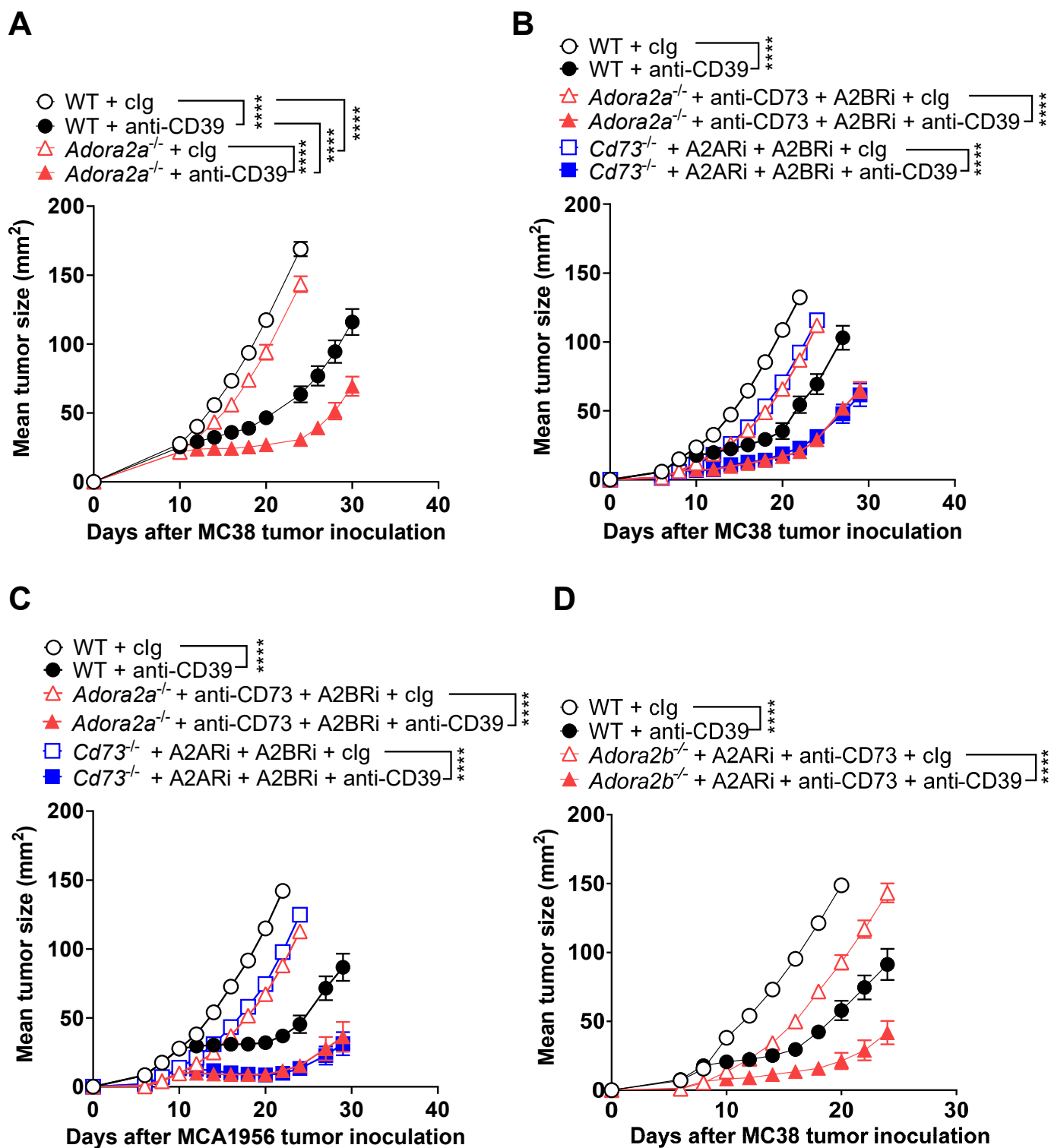
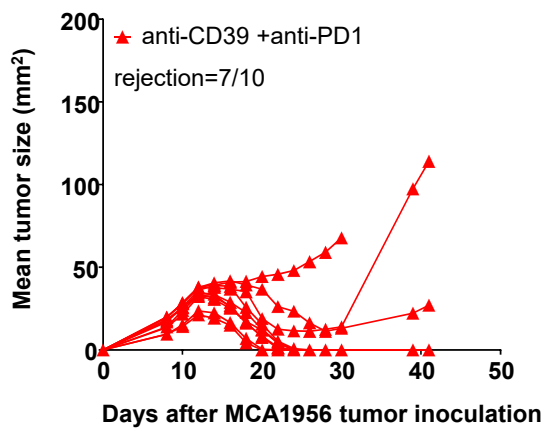
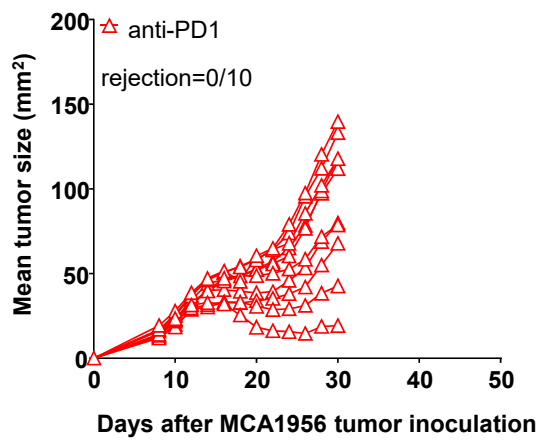
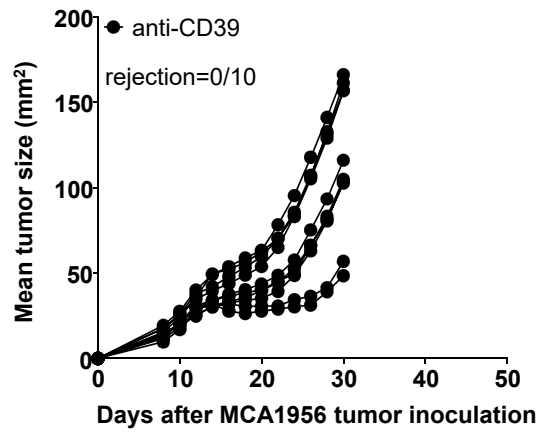
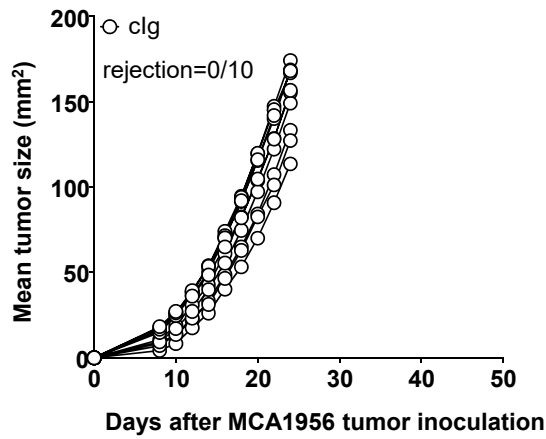


Figure S4. Combination of anti-CD39 with adenosine blockade. (A) Groups (n = 9-10/group) of C57BL/6 WT or *Adora2a*^{-/-} mice were injected subcutaneously (s.c.) with MC38 (1 x 10⁵) colon adenocarcinoma cells and treated i.p. with clg (200 µg) anti-CD39 (200 µg) on days 10, 13, 16 and 19. **(B)** Groups (n = 10/group) of WT, *Adora2a*^{-/-}, and *Cd73*^{-/-} mice were injected s.c. with MC38 (1 x 10⁵) colon adenocarcinoma cells on day 0. *Adora2a*^{-/-} mice were treated i.p with a combination of anti-CD73 (200 µg) and (A2BR inhibitor) PSB1115 (1 mg/kg) and *Cd73*^{-/-} mice treated with a combination of A2AR inhibitor (SCH58261) (10 mg/kg) and (A2BR inhibitor) (PSB1115) (1 mg/kg) on days 0, 3, 6, 9, 12, 15 and 18. All groups of mice were also treated i.p with clg (200 µg) or anti-CD39 (200 µg) on days 8, 11, 14, and 17. **(C)** Groups (n = 7-8/group) of WT, *Adora2a*^{-/-}, and *Cd73*^{-/-} mice were injected s.c. with MCA1956 (1 x 10⁶) colon adenocarcinoma cells on day 0. *Adora2a*^{-/-} mice were treated i.p with a combination of anti-CD73 (200 µg) and (A2BR inhibitor) (PSB1115) (1 mg/kg) and *Cd73*^{-/-} mice treated with a combination of A2AR inhibitor (SCH58261) (10 mg/kg) and PSB1115 (A2BR inhibitor) (1 mg/kg) on days 0, 3, 6, 9, 12, 15 and 18. All groups of mice were also treated i.p with clg (200 µg) or anti-CD39 (200 µg) on days 10, 13, 16, and 19. **(D)** Groups (n = 5/group) of WT and *Adora2b*^{-/-} mice were injected s.c. with MC38 (1 x 10⁵) colon adenocarcinoma cells on day 0. *Adora2b*^{-/-} mice were treated i.p with a combination of anti-CD73 (200 µg) and SCH58261 (10 mg/kg) on days 0, 3, 6, 9, 12, 15 and 18. All groups of mice were also treated i.p with clg (200 µg) or anti-CD39 (200 µg) on days 8, 11, 14, and 17. Tumor sizes (mm²) were measured at the indicated time points and presented as mean ± SEM. Significant differences among treatment groups were determined by a two-way ANOVA, followed by Tukey's multiple comparison test (**** P < 0.0001).

Figure S5

A



B

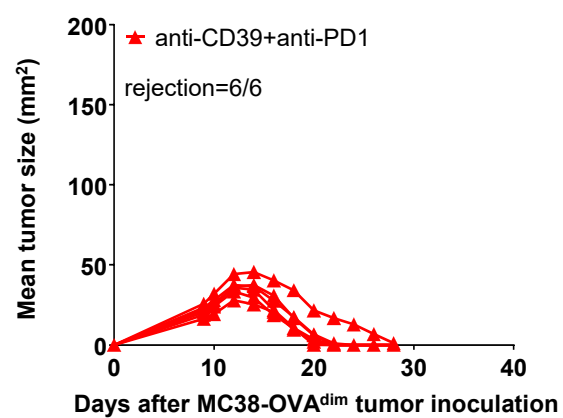
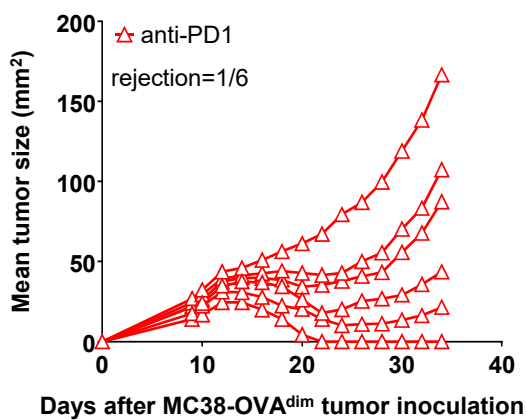
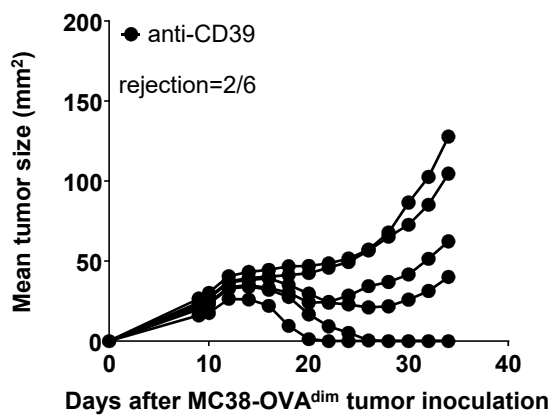
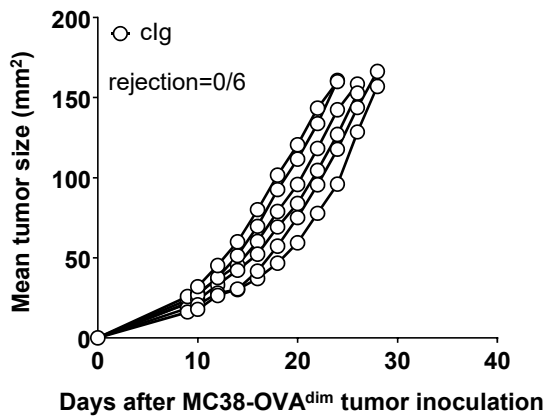
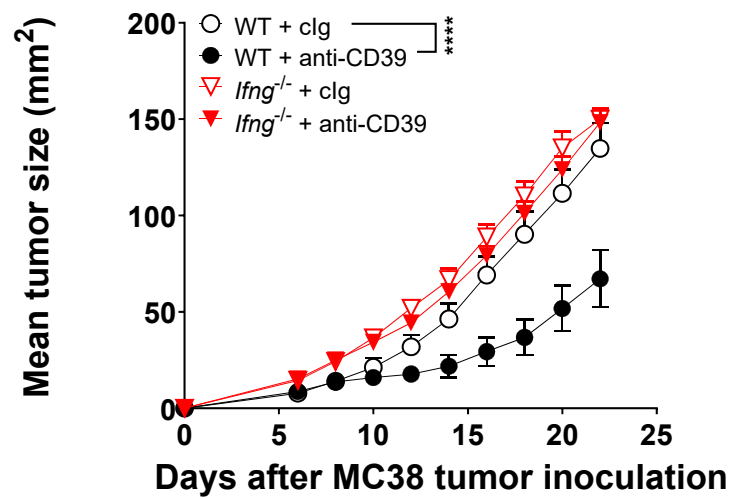


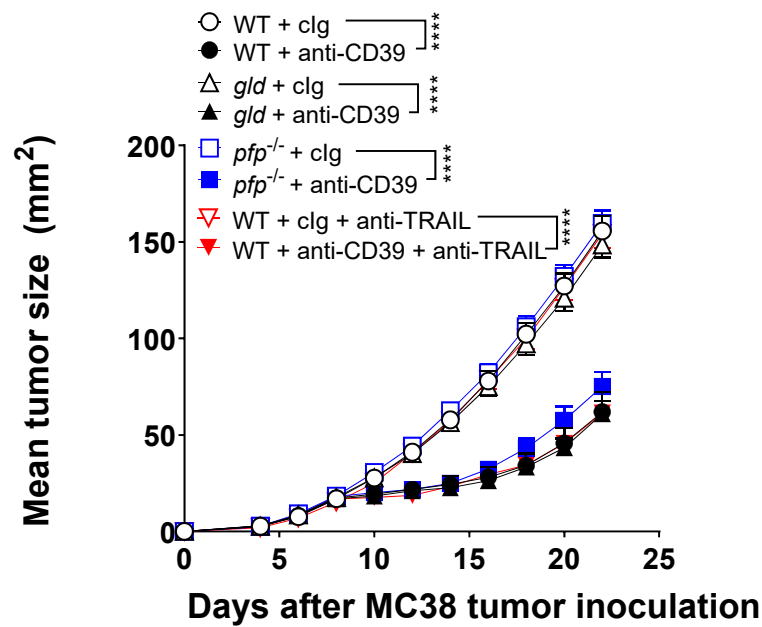
Figure S5. Individual tumor growth curves from anti-CD39- and anti-PD1-treated mice. Individual growth curves plotted from mice in **(A)** Figure 1G (MCA1956) and **(B)** Figure 1H (MC38-OVA^{dim}). Tumor rejection rates are as indicated for each panel/treatment group.

Figure S6

A



B



C

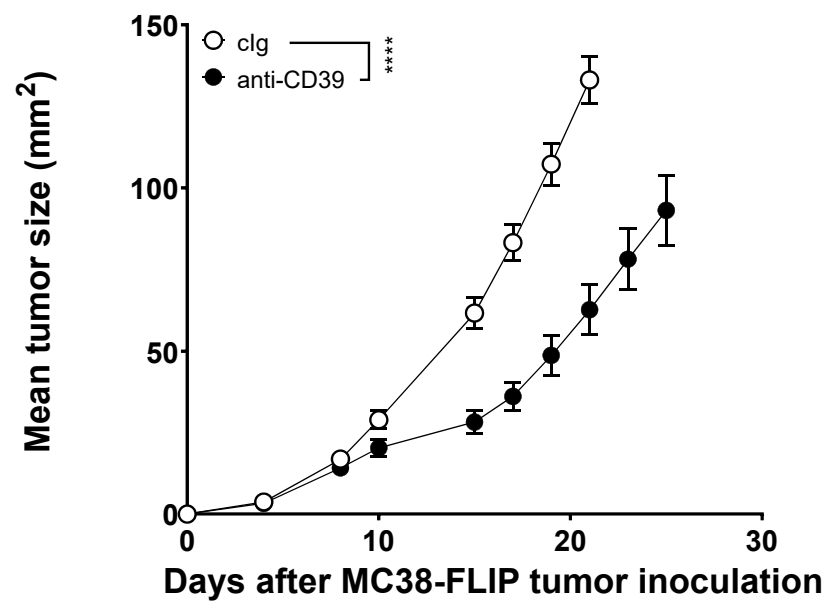
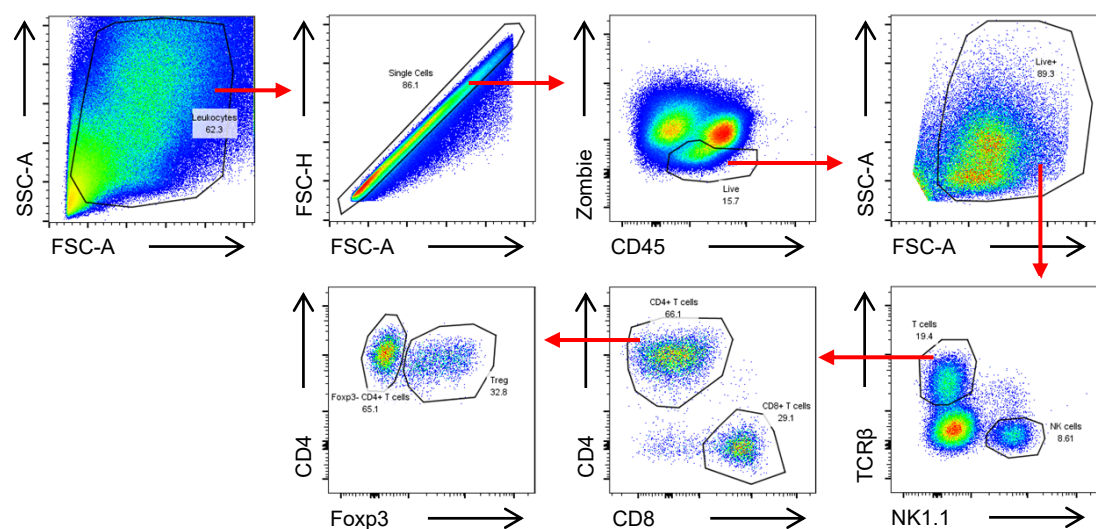


Figure S6. (A) IFN- γ -dependent MC38 tumor growth control by anti-CD39 mAb.

Groups (n= 8-10/group) of C57BL/6 WT or *Ifng*^{-/-} mice were injected s.c. with MC38 (1 x 10⁵) colon adenocarcinoma cells and treated i.p. with clg (200 μ g) or anti-CD39 mAb (200 μ g) on days 8, 11, 14 and 18. **(B-C) Lymphocyte cytotoxic mechanisms were not required for the efficacy of anti-CD39 mAb. (B)** Groups (n = 5/group) of WT, *pfpr*^{-/-}, or *gld* mice were injected s.c. with MC38 (1 x 10⁵) colon adenocarcinoma cells on day 0 and treated i.p with clg (200 μ g) or anti-CD39 (200 μ g) on days 8, 11, 14, and 17. WT mice additionally received i.p. either clg (250 μ g) or anti-TRAIL (N2B2, 250 μ g) on days 7, 8, 12, 14, 17, and 21. **(C)** Groups (n = 10/group) of WT mice were injected s.c. with MC38-FLIP (1 x 10⁵) colon adenocarcinoma cells on day 0 and treated i.p with clg (200 μ g) or anti-CD39 (200 μ g) on days 8, 11, 15, and 17. Tumor sizes (mm²) were measured at the indicated time points and presented as mean $\pm$ SEM. Experiments in A-C performed once. Significant differences among treatment groups were determined by a two-way ANOVA, followed by Tukey's multiple comparison test (**** P < 0.0001).

Figure S7
Lymphocyte Panel



Myeloid Panel

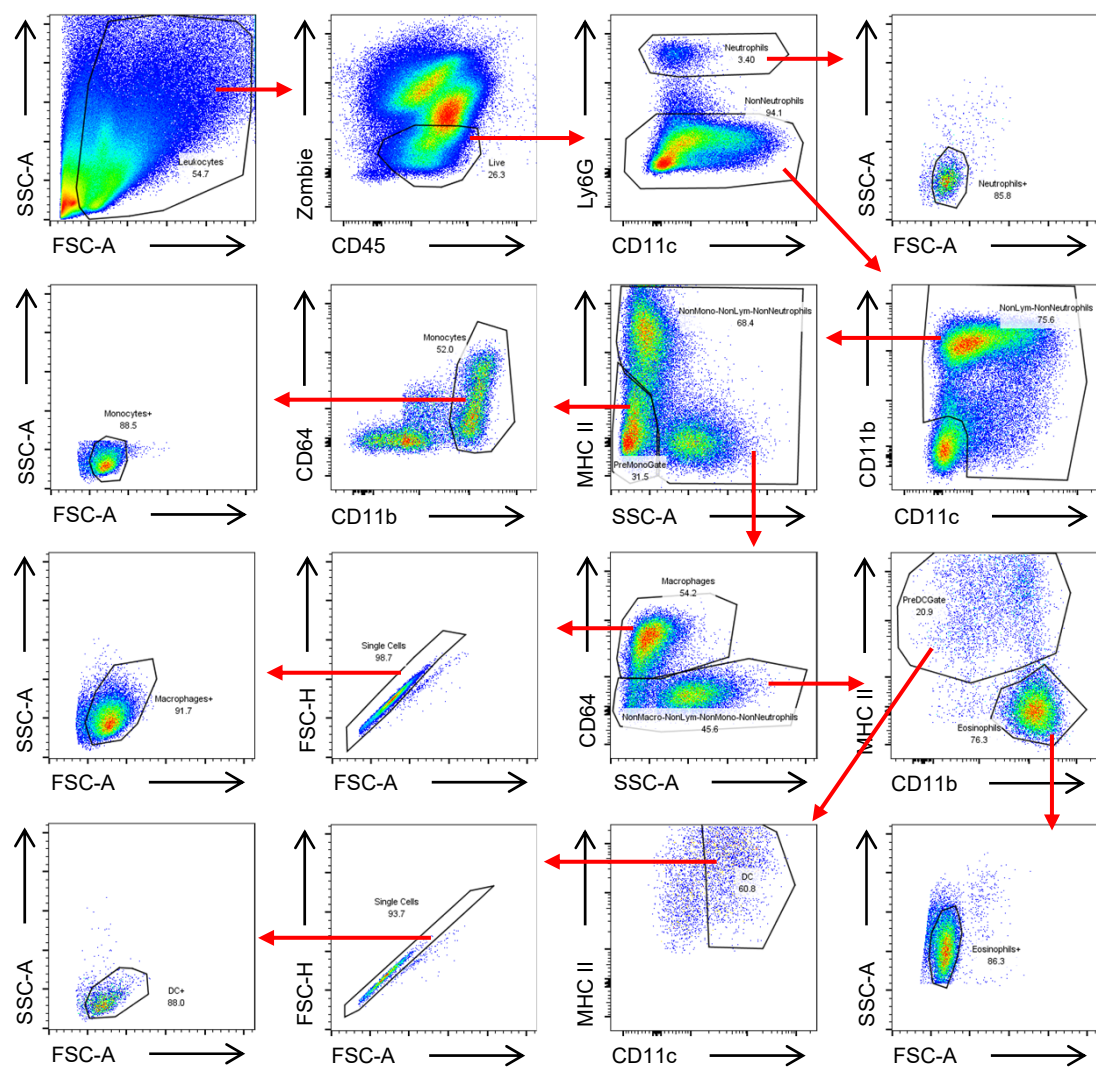


Figure S7. Gating strategies for different populations in FACS analyses. MC38 tumor samples are shown here as examples. For lymphocyte panel, CD45⁺ live cells were gated out from single cells of general leukocyte size. Another gate in forward/side scatter was added to further exclude debris. After that, NK cells were gated out as NK1.1⁺TCRb⁻, CD8⁺ T cells were gated as NK1.1⁻TCRb⁺CD8⁺, Treg as NK1.1⁻TCRb⁺CD4⁺Foxp3⁺, and Foxp3⁻ CD4⁺ T cells as NK1.1⁻TCRb⁺CD4⁺Foxp3⁻. For myeloid panel, CD45⁺ live cells were gated out from leukocytes. Neutrophils were defined as Ly6G⁺. CD11b⁻CD11c⁻ lymphocytes were excluded from Ly6G⁻ populations. From there, monocytes were defined as MHCII^{-/low}SSC^{low}CD11b^{high}CD64^{low/+}, macrophages were defined as MHCII⁺CD64⁺, eosinophils as MHCII⁻CD64⁻CD11b^{high}, dendritic cells as MHCII⁺CD64⁻CD11c⁺. Additional gates in forward and side scatter were added for each of the populations to further exclude doublets and debris.

Figure S8

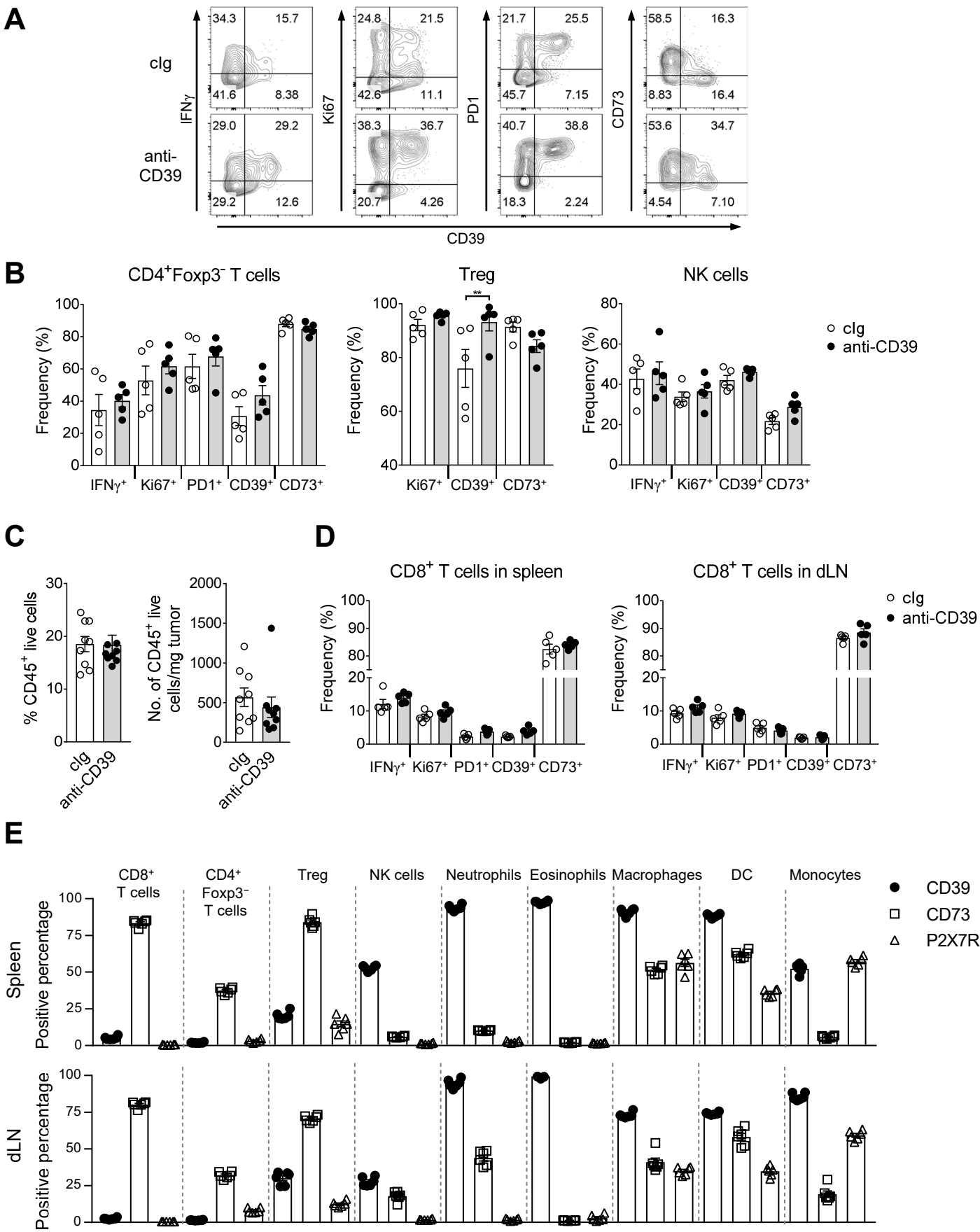
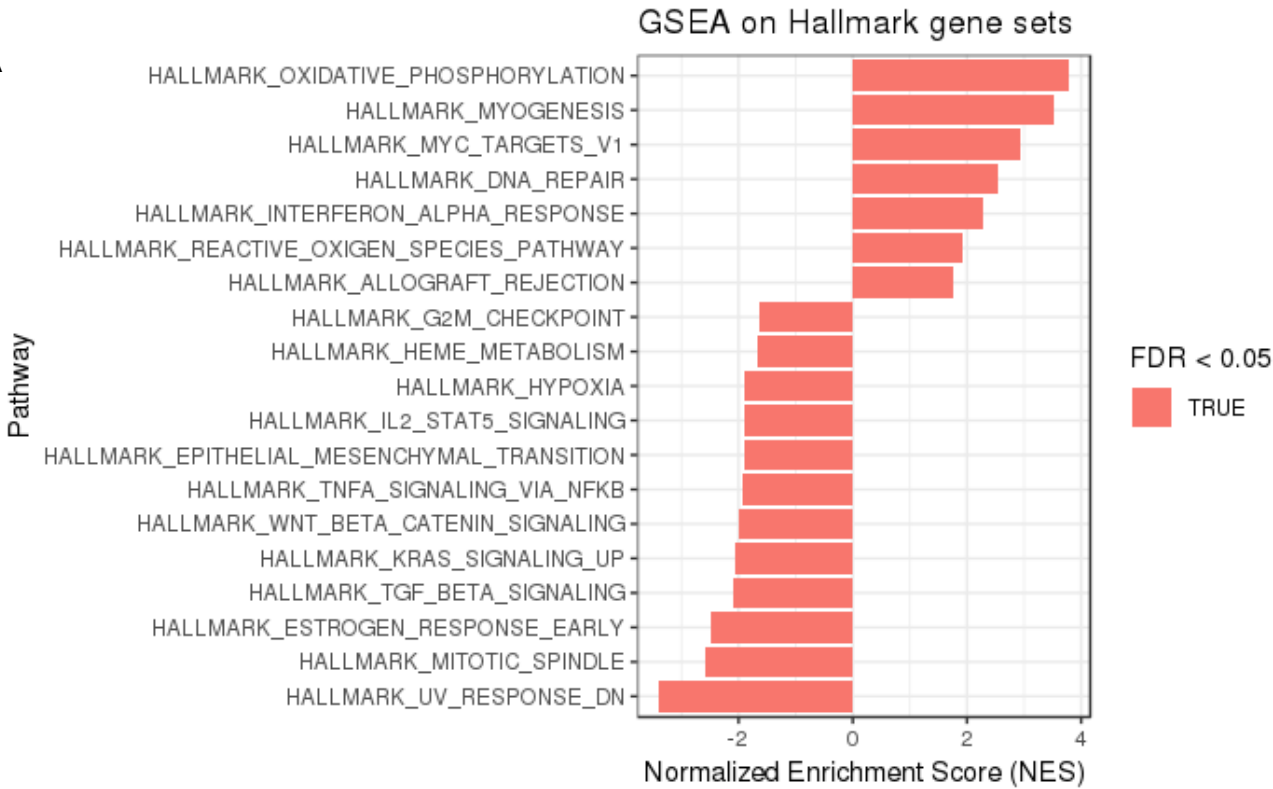


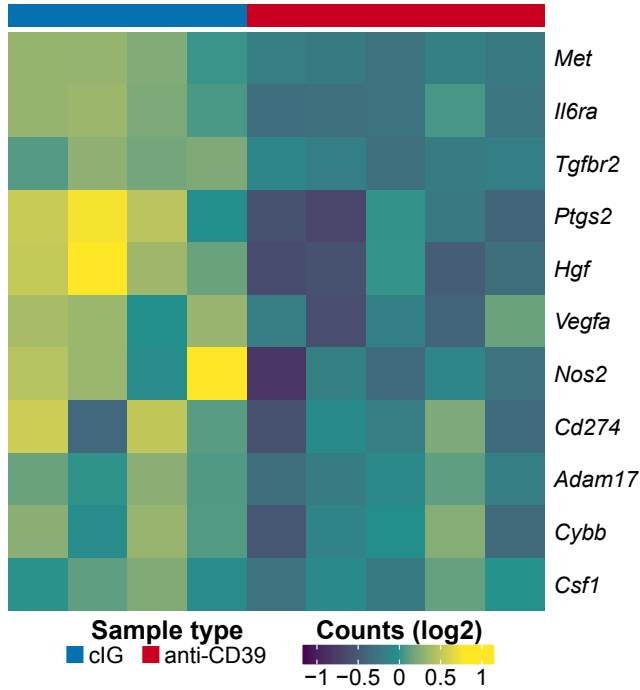
Figure S8. Anti-CD39 treatment modulates the phenotypic features and function of immune cells. From the same experiments as Fig. 2F, 2G and Fig. 4B, groups (n = 4–8/group) of WT mice were injected s.c. with (**A–E**) MC38 (1×10^5) colon adenocarcinoma cells on day 0. Mice were untreated (**E**) or treated i.p. with clg (200 μ g) or anti-CD39 (200 μ g) on day 7 (**A–D**). Samples of tumor, spleen, and tumor draining lymph node (dLN) were collected on day 8 (**E**) or 48 h after mAb injection (**A–D**) and processed for single cell suspensions then subjected to flow cytometry with (**A–D**) or without (**E**) ex-vivo stimulation for 4 h. **A**, Representative FACS plots showing expression of CD39 and indicated surface and intracellular molecules on tumor-infiltrating CD8⁺ T cells. **B**, Frequencies of MC38 tumor-infiltrating CD4⁺Foxp3[−] T cells, Treg cells, and NK cells expressing the indicated surface and intracellular molecules and presented as mean $\pm$ SEM with individual values shown. **C**, Frequencies and numbers of MC38 tumor infiltrating leukocyte. **D**, Frequencies of CD8⁺ T cells from the spleen or dLN of MC38-tumor bearing mice expressing the indicated surface and intracellular molecules and presented as the mean $\pm$ SEM with individual values shown. **E**, Summary bar graphs of frequencies of immune cell subsets expressing CD39, CD73 and P2X7R in the spleen and tumor draining lymph node of MC38-bearing mice, (mean $\pm$ SEM). Significant differences between the indicated groups were determined by a two-way ANOVA, followed by Sidak's multiple comparison test (**B** and **D**) or Mann-Whitney test (**C**). Data from (**C**) are pooled from two experiments while the remaining parts represent one experiment. (**B**) ** P < 0.01.

Figure S9

A



B



C

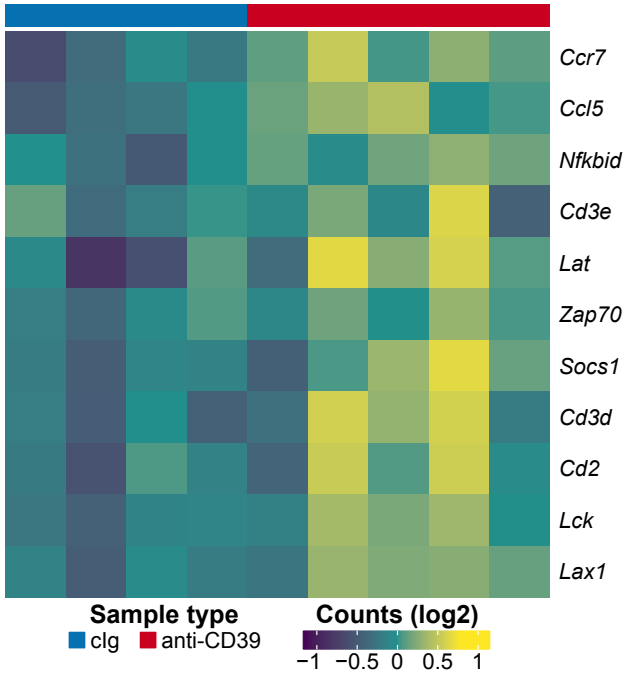


Figure S9. (A) Gene-set enrichment analysis of MC38 tumors treated with clg compared to anti-CD39. From Fig. 3C, significantly regulated Hallmark gene sets between anti-CD39- and clg-treated MC38 tumors (FDR < 0.05). **(B)** Heatmap of expression of key genes within immune suppression signature. **(C)** Heatmap of expression of key genes within T-cell activation signature.

Figure S10

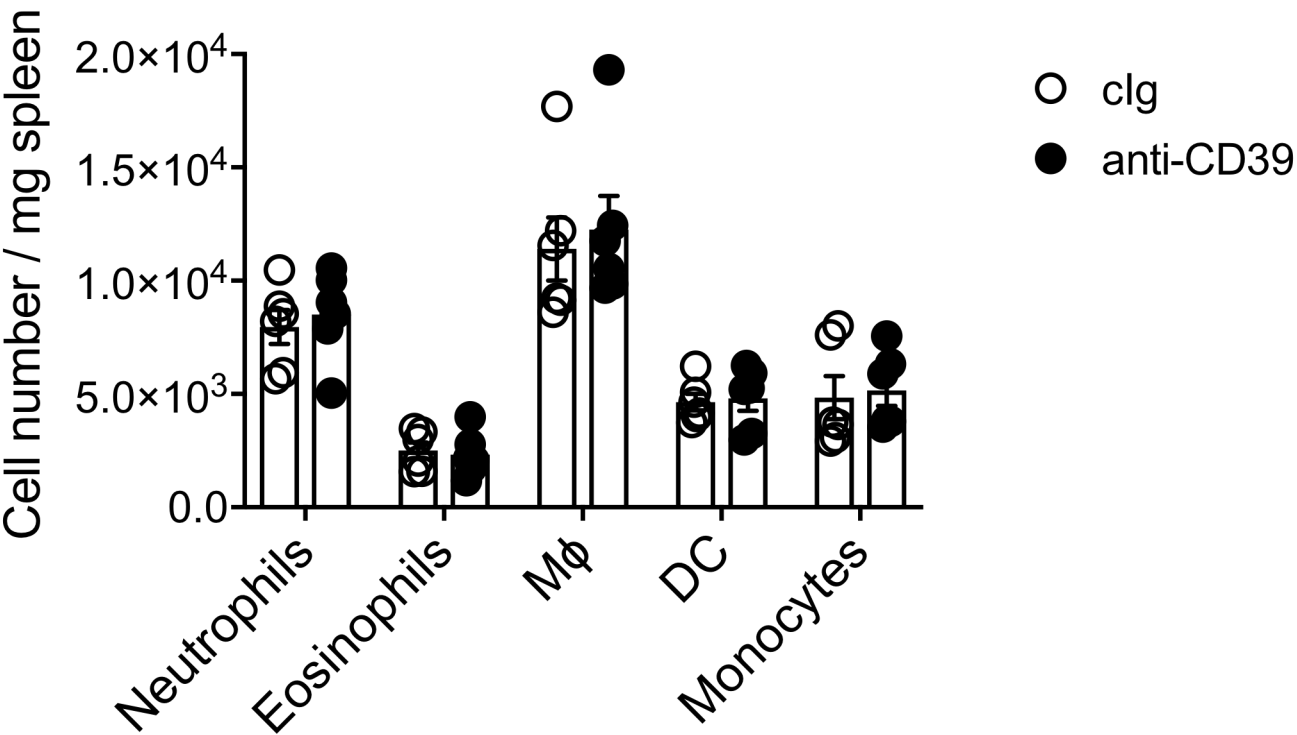


Figure S10. Flow cytometry shows no macrophage reduction in spleen after anti-CD39 treatment. From the same experiments as Figure 3D, groups (n = 6/group) of WT mice were injected s.c. with MC38 (1×10^5) colon adenocarcinoma cells on day 0 and treated i.p. with clg (200 μ g) or anti-CD39 (200 μ g) on day 7. Spleen samples were collected on day 9 and processed for single cell suspensions then subjected to flow cytometry. Summary bar graphs of numbers of myeloid populations in spleen, as mean $\pm$ SEM with individual values shown. Data represents one experiment.

Figure S11

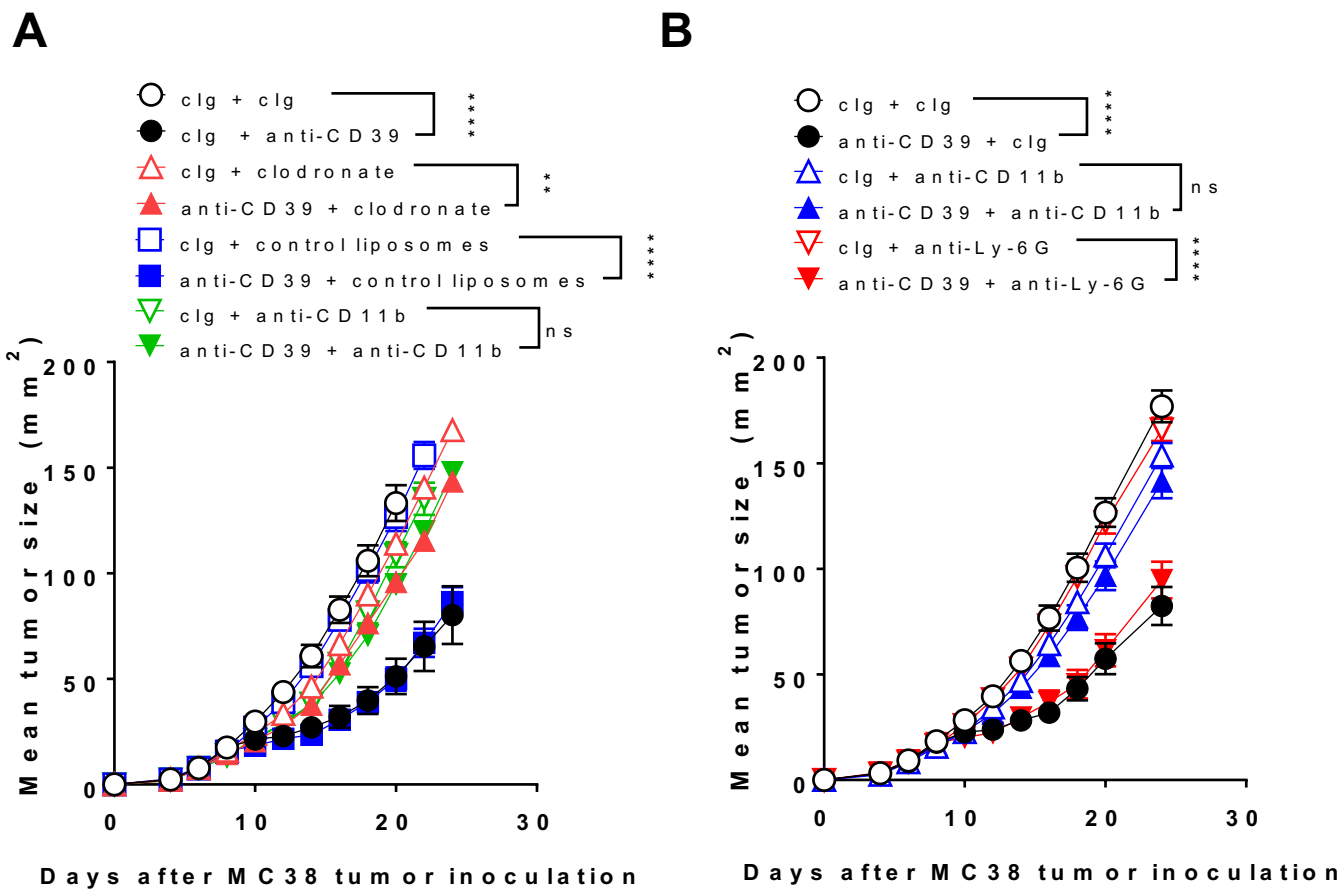
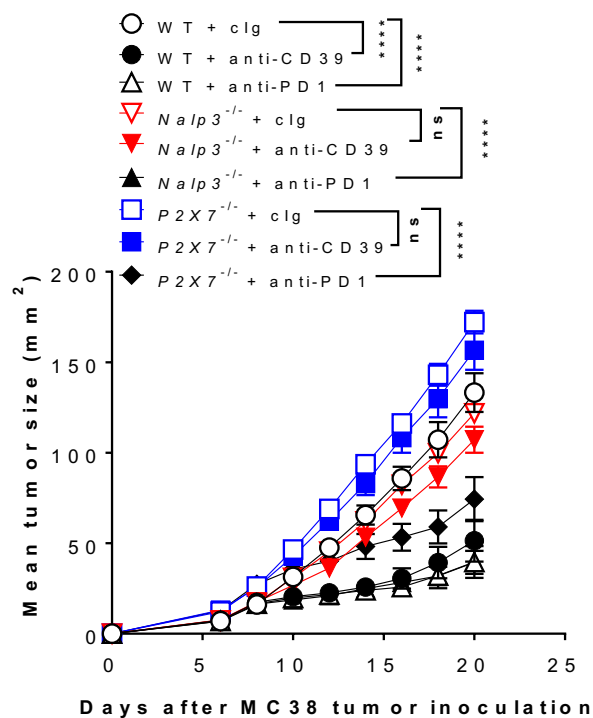


Figure S11. Anti-CD39 mAb efficacy requires CD11b and myeloid cells. **(A)** Groups (n = 5/group) of WT mice were injected s.c. with MC38 (1×10^5) colon adenocarcinoma cells on day 0 and treated i.p with anti-CD39 (200 μ g) or clg (200 μ g) on days 8, 11, 14, and 17. Some groups of mice additionally received clg or anti-CD11b (5C6) (300 μ g i.p.) on days 7,8,15, and 21, or clodronate or control liposomes on days 6 (2 mg/20 g mouse), 8 (1 mg/20 g mouse thereafter), 10, 12, and 14. **(B)** Groups (n = 6/group) of WT mice were injected s.c. with MC38 (1×10^5) colon adenocarcinoma cells on day 0 and treated i.p with anti-CD39 (200 μ g) or clg (200 μ g) on days 8, 11, 14, and 17. Some groups of mice additionally received clg, anti-CD11b (5C6) or anti-Ly-6G (1A8) (300 μ g i.p.) on days 7, 8, 15, and 24. Tumor sizes (mm²) were measured at the indicated time points and presented as mean $\pm$ SEM. Experiment was performed once. Significant differences among treatment groups were determined by a two-way ANOVA, followed by Tukey's multiple comparison test (** P < 0.01; **** P < 0.0001).

Figure S12

A



B

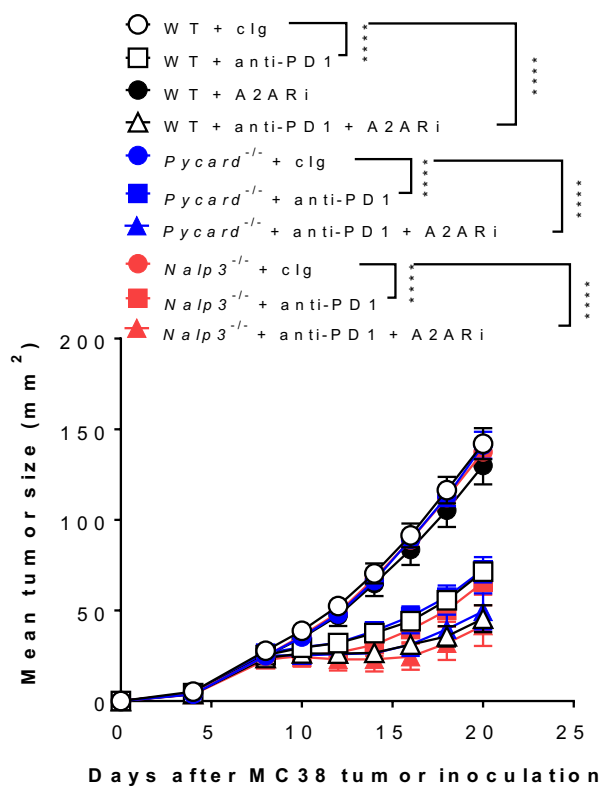
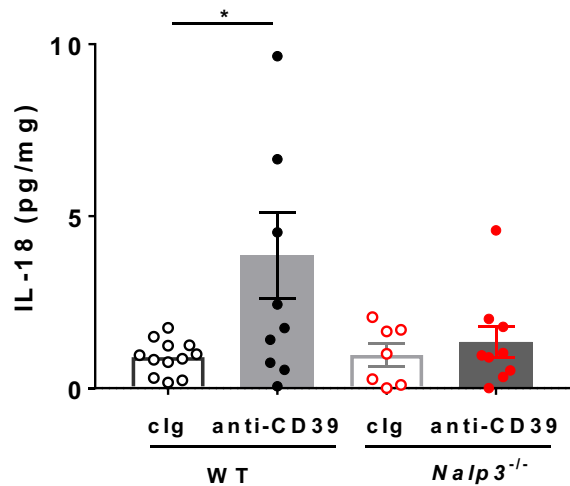


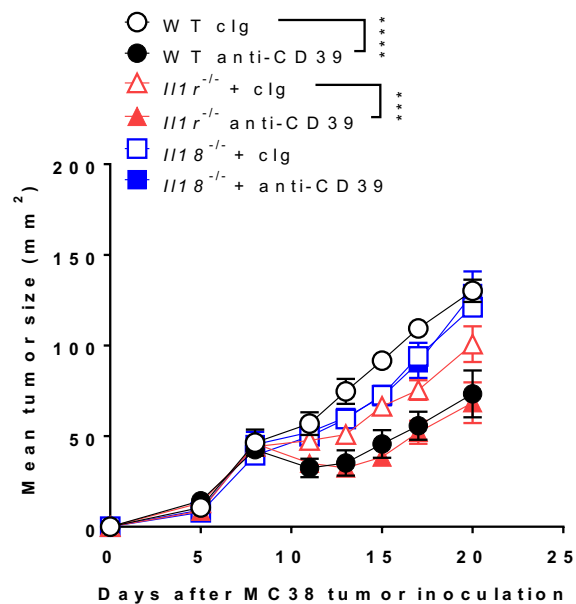
Figure S12. Anti-PD1 and SCH58261 efficacy is NALP3-independent. (A) Groups (n = 4-6/group) of WT, *P2X7*^{-/-}, and *Nalp3*^{-/-} mice were injected s.c. with MC38 (1 x 10⁵) colon adenocarcinoma cells on day 0 and treated i.p with clg (200 µg), anti-CD39 (200 µg), or anti-PD1 (250 µg) on days 8, 11, 14, and 17. **(B)** Groups (n = 5-6/group) of WT, *Pycard*^{-/-}, and *Nalp3*^{-/-} mice were injected s.c. with MC38 (1 x 10⁵) colon adenocarcinoma cells on day 0 and treated i.p with clg (200 µg), anti-PD1 (250 µg) or anti-PD1 (250 µg) and A2AR inhibitor (SCH58261) (10 mg/kg) on days 8, 11, 14, and 17. Tumor sizes (mm²) were measured at the indicated time points and presented as mean ± SEM. Experiments performed once. Significant differences among treatment groups were determined by a two-way ANOVA, followed by Tukey's multiple comparison test (**** P < 0.0001, ns = not significant).

Figure S13

A



B



C

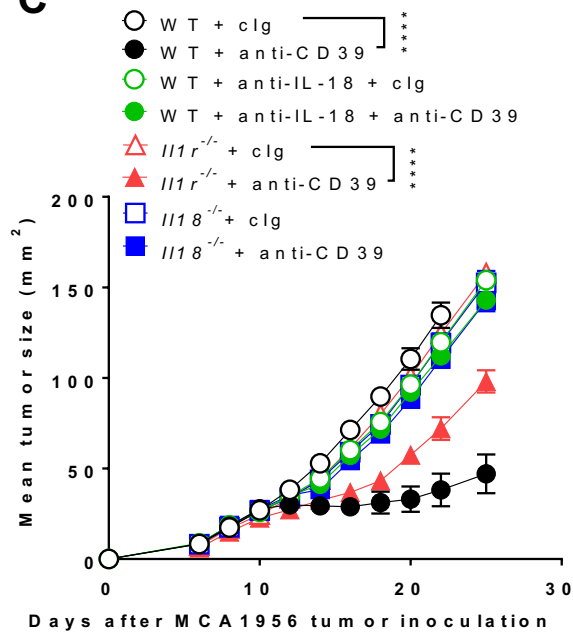
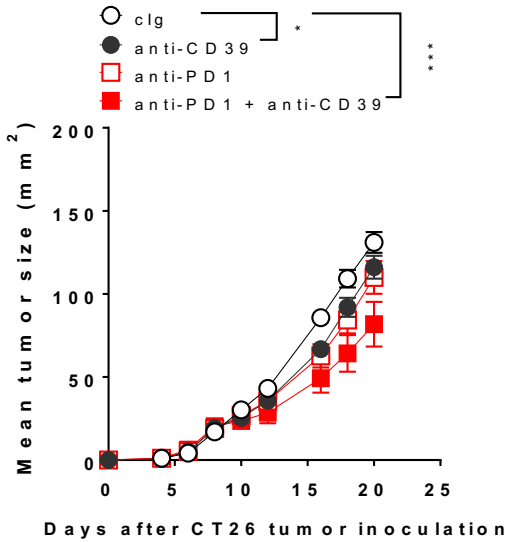


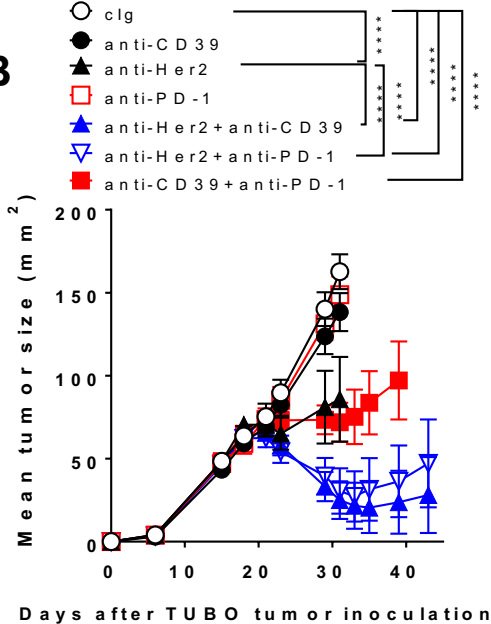
Figure S13. Role of IL-18 in mechanism of action of anti-CD39. (A) Inflammasome IL-18 production in MC38 tumors after anti-CD39 mAb. Groups (n = 7-9/group) of WT and *Nalp3*^{-/-} mice were injected s.c. with MC38 (1 x 10⁵) colon adenocarcinoma cells on day 0 and treated i.p with clg (200 µg) or anti-CD39 (200 µg) on day 8. Tumor lysates were prepared 24 h after antibody treatment and IL-18 levels were determined by ELISA. This experiment is pooled from two performed. **(B)** Groups (n = 4/group) of WT, *Il1r*^{-/-}, or *Il18*^{-/-} mice were injected s.c. with MC38 (5 x 10⁵) colon adenocarcinoma cells on day 0 and treated i.p with clg (200 µg) or anti-CD39 (200 µg) on days 8, 11, and 14. **(C)** Groups (n = 5/group) of WT, *Il1r*^{-/-}, or *Il18*^{-/-} mice were injected s.c. with MCA1956 (1 x 10⁶) sarcoma cells on day 0 and treated i.p with clg (200 µg) or anti-CD39 (200 µg) on days 10, 13, 16, and 19. WT mice additionally received i.p either clg (250 µg) or anti-IL-18 (250 µg) on days 9, 10, 17, and 24 after tumor inoculation. **(B-C)** Experiments performed twice each. Significant differences among treatment groups were determined by a two-way ANOVA, followed by Tukey's multiple comparison test (* P < 0.05, *** P < 0.001, **** P < 0.0001).

Figure S14

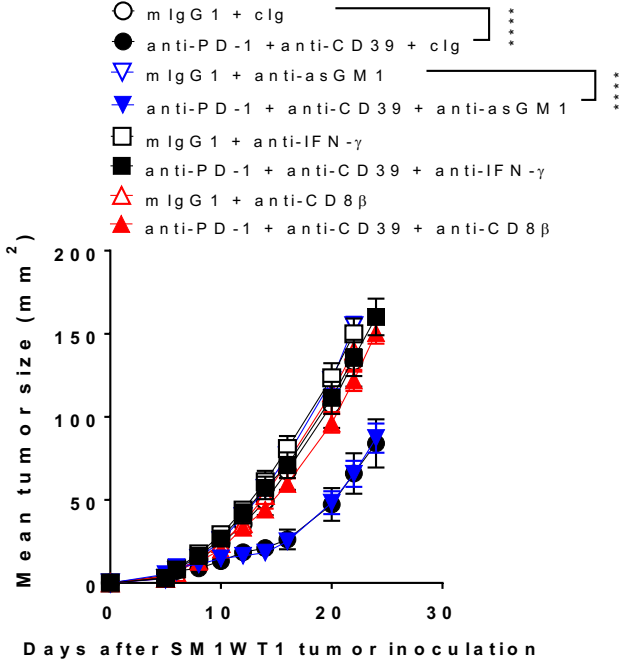
A



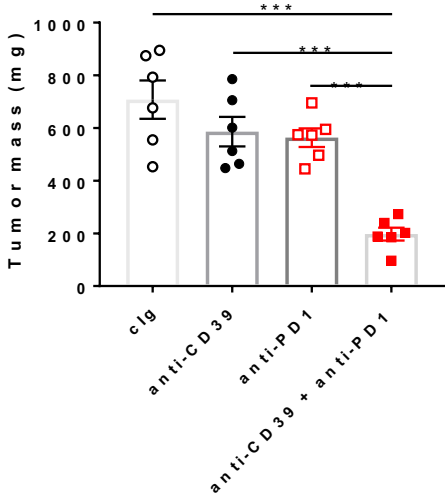
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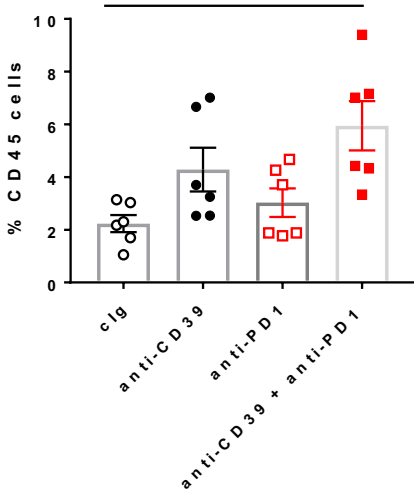
C



D



E



F

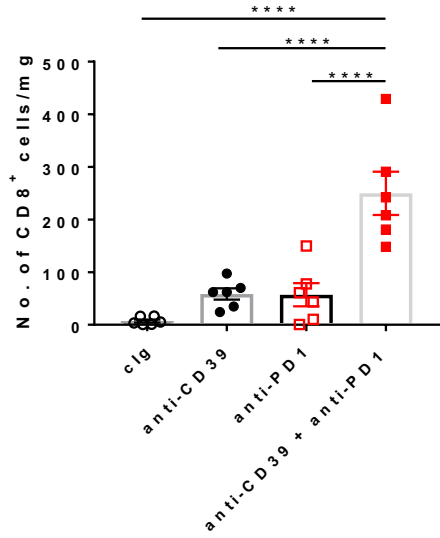
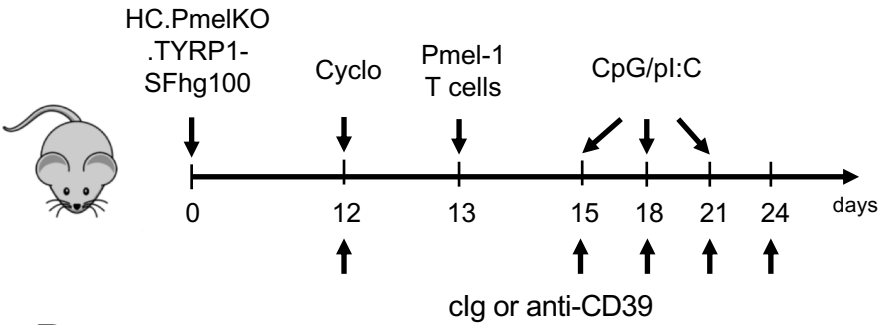


Figure S14. CD39 blockade sensitizes anti-PD1-resistant subcutaneous

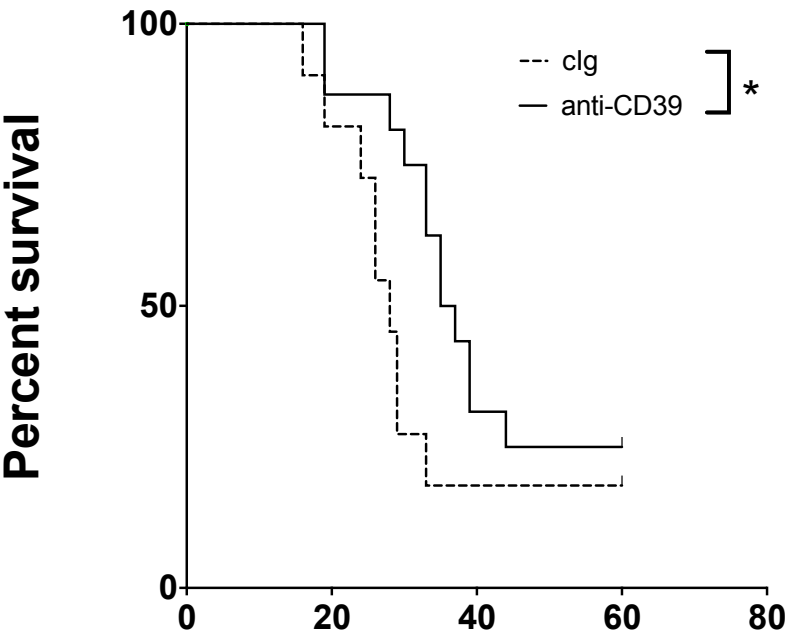
tumors. (A) Anti-CD39 sensitizes anti-PD1-resistant CT26 colon adenocarcinomas. Groups (n = 10/group) of BALB/c WT mice were injected s.c. with **(A)** CT26 (1×10^5) on day 0 and treated i.p. with clg (200 μ g), anti-CD39 (200 μ g), anti-PD1 (250 μ g) and combination of anti-CD39 and anti-PD1 (200 μ g; 250 μ g) as indicated on days 8, 11, 14, 17. **(B)** Anti-CD39 sensitizes anti-PD1-resistant TUBO mammary carcinomas. Groups (n = 7-8/group) of BALB/c WT mice were injected s.c. with TUBO (5×10^5) on day 0 and treated i.p. with clg (200 μ g), anti-CD39 (200 μ g), anti-PD1 (250 μ g), anti-Her2 (7.16.4, 100 μ g) alone and in various combinations as indicated on days 18,21,23, and 29. **(C)** Anti-CD39- and anti-PD1-mediated control of SM1WT1 is IFN- γ and CD8 $^+$ T cell-dependent. Groups (n = 5/group) of WT mice were injected s.c. with SM1WT1 (1×10^6) on day 0 and treated i.p. with clg (200 μ g), anti-CD39 (200 μ g), anti-PD1 (250 μ g) on days 6, 9, 12, and 15 and some groups of mice were also treated i.p. with either clg (100 μ g), anti-asGM1 (50 μ g,) anti-CD8 β (100 μ g) or anti-mIFN- γ (250 μ g) on days 5, 6 13, and 20. Tumor sizes (mm ^{2}) were measured at the indicated time points and presented as mean $\pm$ SEM. All experiments were performed once. Significant differences between the indicated groups were determined by a two-way ANOVA, followed by Tukey's multiple comparison test (* P < 0.05, *** P < 0.001, **** P < 0.0001). **(D-F)** From same experiment as Figure 5F, G. The tumors were collected on day 14, 48 h after the third dose. Graphs showing tumor weight **(D)**, summary bar graph of percentage of CD45 $^+$ cells **(E)**, and numbers of CD8 $^+$ T cell count per mg tumor mass **(F)**. Significant differences in percentage between the selected cell populations were determined by one-way ANOVA, followed by Tukey's multiple comparison test (** P < 0.01, *** P < 0.001, **** P < 0.0001).

Figure S15

A



B

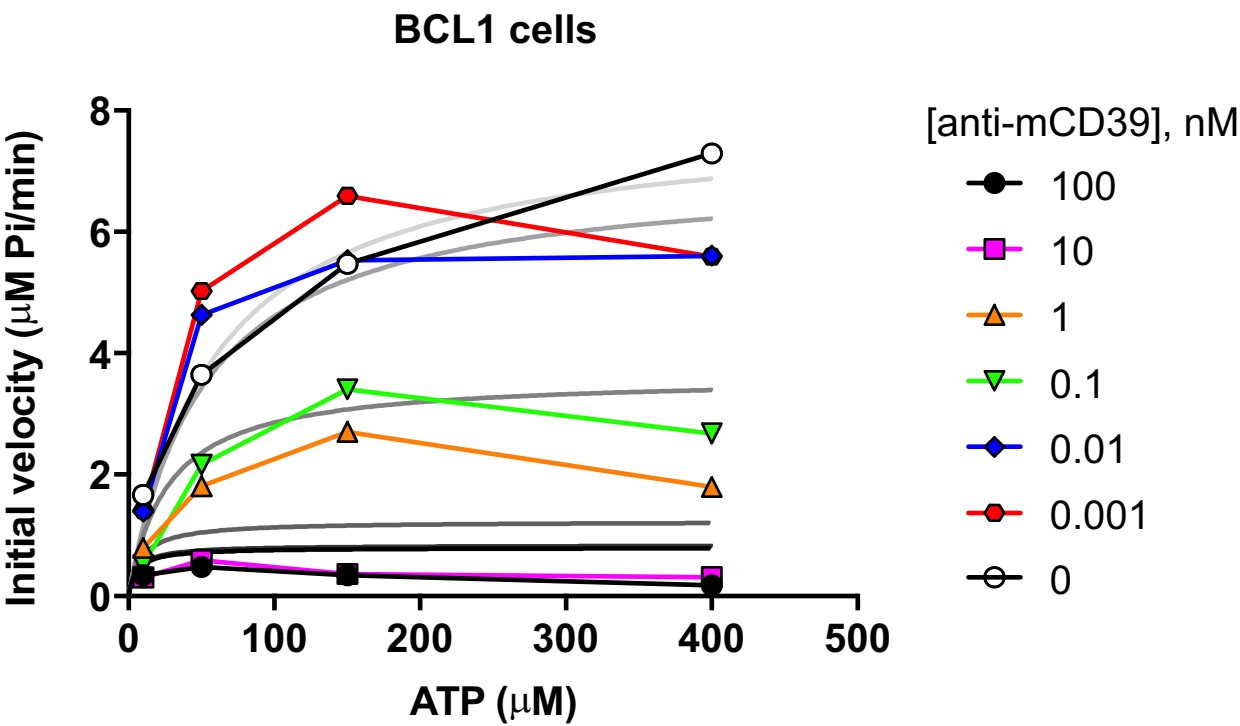


Days after HC.pMEL tumor inoculation

Figure S15. Anti-CD39 enhances the efficacy of adoptive cellular therapy against immune cell poor melanoma. (A) Experimental setup: WT mice were injected with HCmel12 melanoma expressing the human gp100 epitope under the control of the melanocytic lineage gene *Typr1* [HC.pMELKO.TYRP1-SFhg100, HC.pMEL)]. Once tumors were palpable, mice received a single dose of cyclophosphamide (Cyclo)(2 mg) followed by adoptive transfer of 10^6 pMEL-1 T cells and three intratumor injections of CpG/polyI:C (50 μ g each). Cohorts of mice were randomly assigned to either receive control IgG (clg) or anti-CD39 (B66) antibody. **(B)** Corresponding Kaplan-Meier-Plot showing overall survival of mice treated with ACT+clg or ACT+anti-CD39 (n = 11 for clg; n = 16 for anti-CD39). Significant differences between the indicated groups were determined Gehan-Breslow-Wilcoxon test (* P < 0.05).

Figure S16

A



B

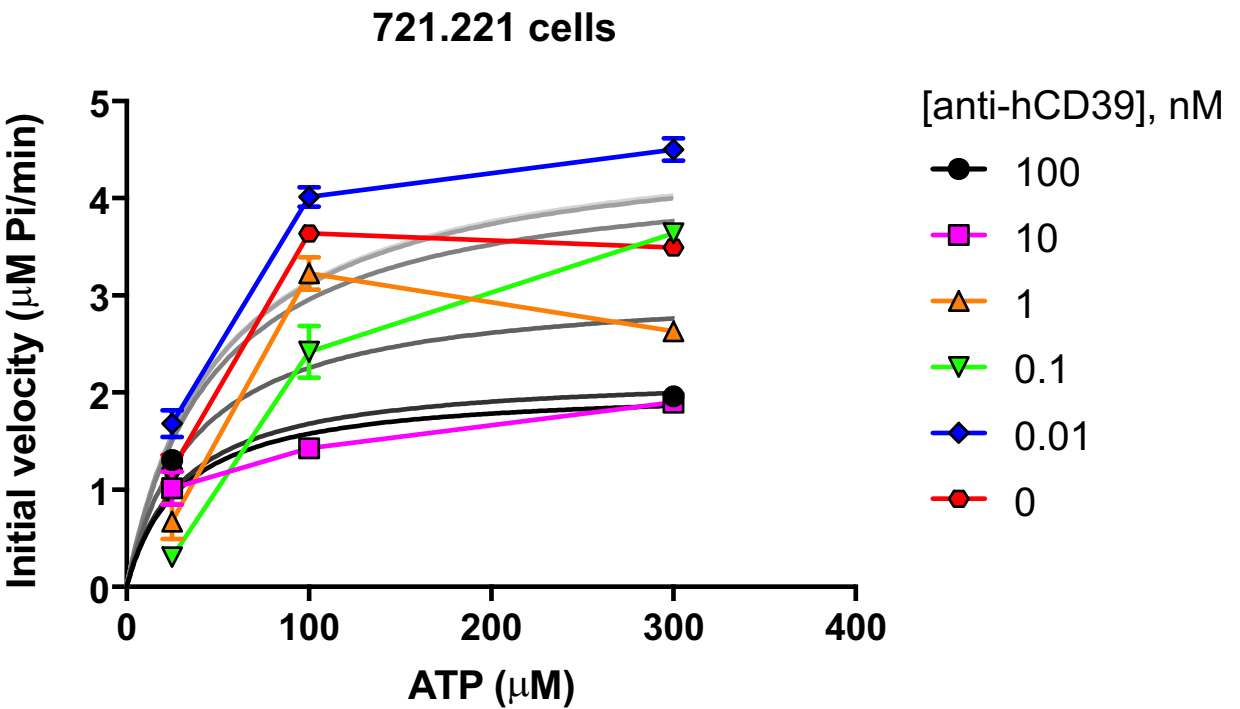


Figure S16. Anti-mCD39 B66 and Anti-hCD39 TTX-030 allosterically inhibit CD39 ATPase activity. (A) Mouse cells endogenously expressing CD39 (BCL1 clone 5B1b) or (B) human cells endogenously expressing CD39 (721.221) were treated with anti-CD39 antibody and ATP and subsequent phosphate production monitored kinetically using the EnzChek Phosphate Assay Kit (Invitrogen, CAT# E-6646). Michaelis-Menten kinetic modeling was used to determine various kinetic parameters including $V_{\max}$ and K_{app} . These were in turn used in a set of mixed model inhibition equations to determine mechanism of inhibition of B66 and TTX-030. In both cases, it was determined that the mAbs inhibit CD39 ATP hydrolysis with an allosteric mechanism ($\alpha < 1$).

Figure S17

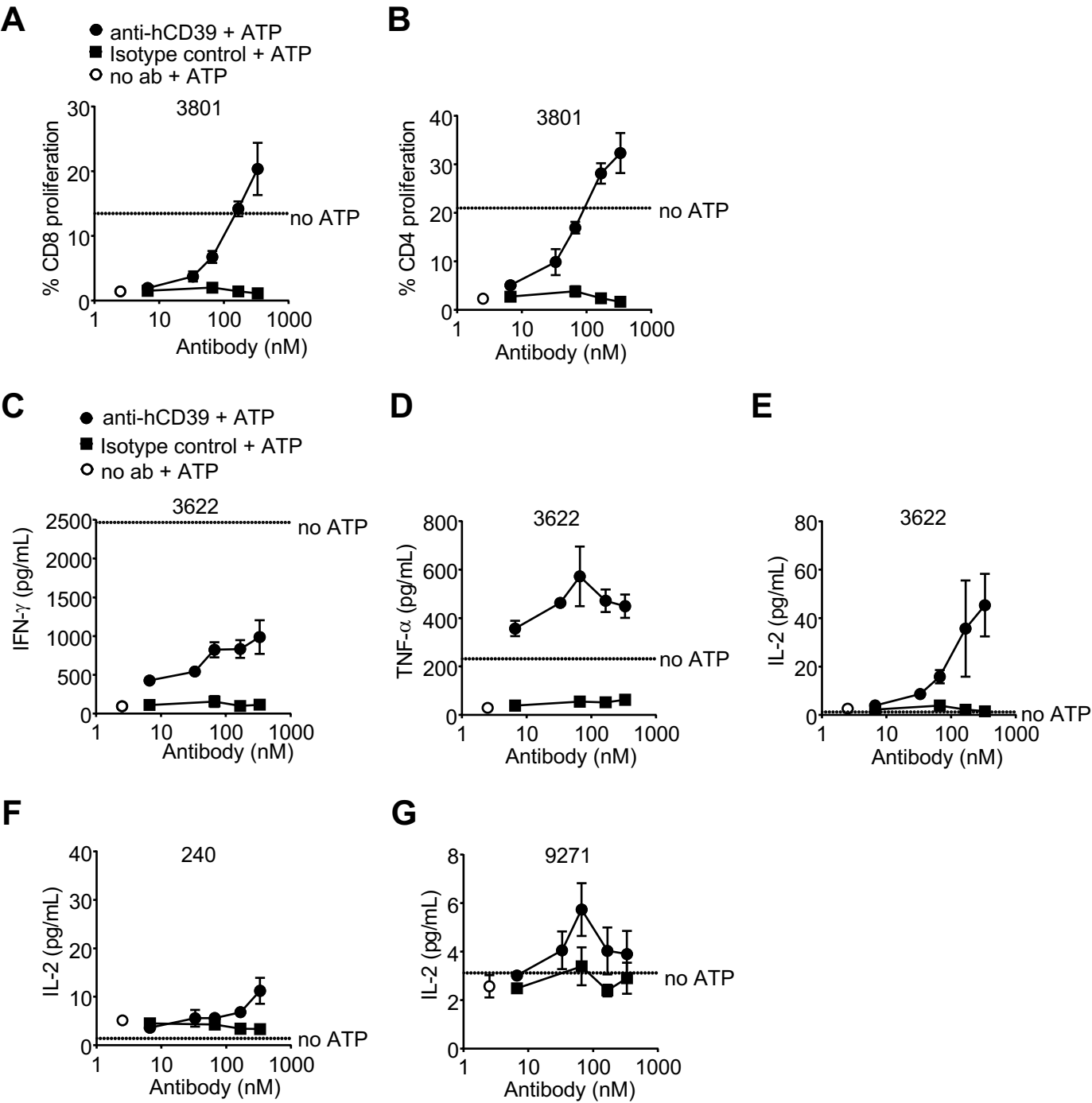


Figure S17. Anti-human CD39 enhances T cell proliferation and Th1 cytokine production. PBMCs from four different donors were harvested, stimulated with anti-CD3/anti-CD28, and assessed in triplicate for T cell proliferation and cytokine release in the presence and absence of ATP by flow cytometry. The graphs represent anti-hCD39-induced increased CD8 (**A**) and CD4 (**B**) T cell proliferation in the presence of ATP (donor 3801), and increased IFN- γ (**C**), TNF- α (**D**) and IL-2 (**E-G**) production in PBMCs (donors 3622, 240 and 9271), treated with anti-hCD39 in the presence of ATP.

Figure S18

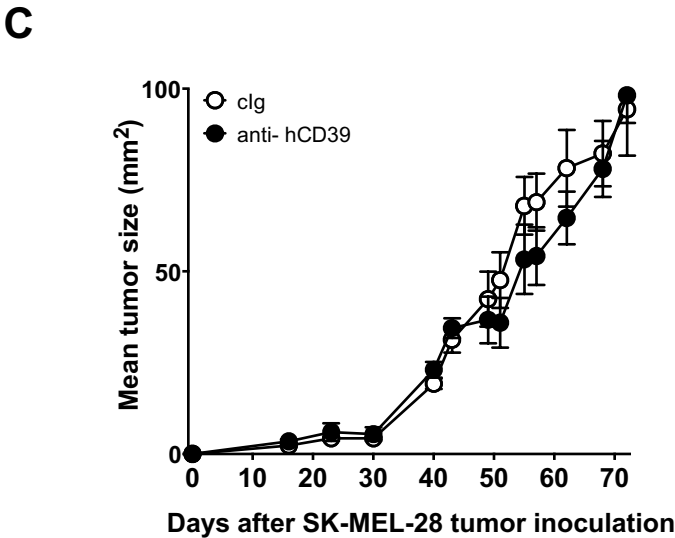
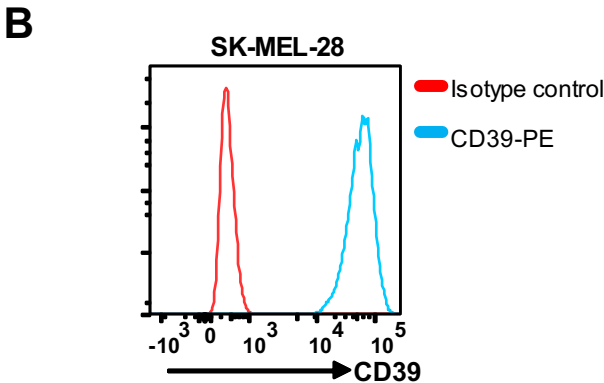
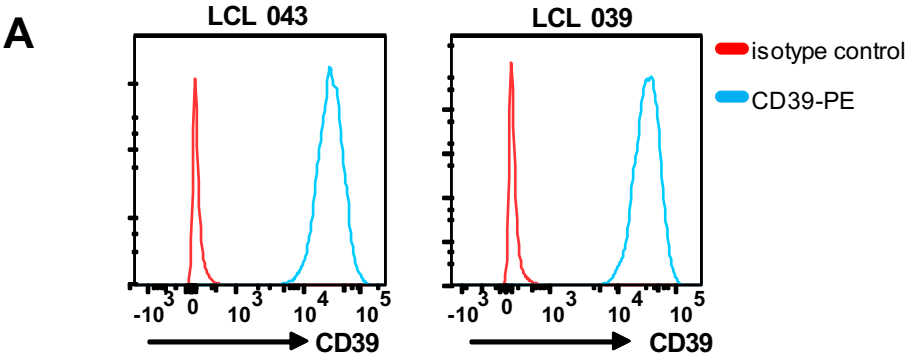


Figure S18. Human CD39. (A-B) Flow cytometry histograms showing CD39 expression on (A) lymphoblastoid cells LCL043, LCL039 or (B) SK-MEL-28 melanoma cells. (C) Groups (n = 7-8/group) of NRG mice were injected s.c. with SK-MEL-28 melanoma cells (2.5×10^6) and treated i.p. with clg (250 μ g) or anti-CD39 (250 μ g) on days 44, 47, 49, 51, 54 and 56. Tumor sizes (mm^2) were measured at the indicated time points and presented as mean $\pm$ SEM. Representative data from two independent experiments.

Figure S19

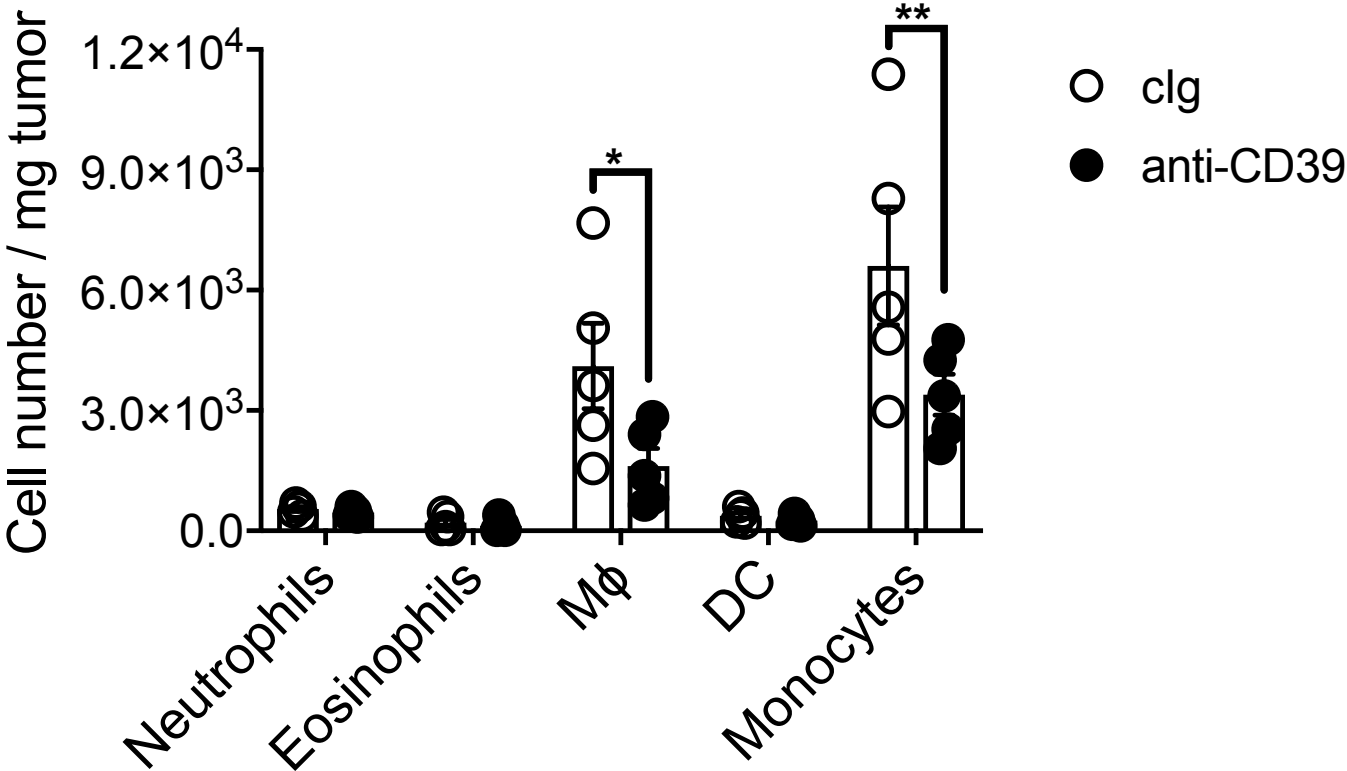


Figure S19. Flow cytometry shows macrophage reduction in RM-1 tumors after anti-CD39 treatment. Groups (n = 5/group) of WT mice were injected s.c. with RM-1 prostate carcinoma cells (5×10^4) on day 0 and treated i.p with clg (200 μ g) or anti-CD39 (200 μ g) on days 6. Tumor samples were collected on day 8 and processed for single cell suspensions then subjected to flow cytometry. Summary bar graphs of numbers of myeloid populations in tumor, as mean $\pm$ SEM with individual values shown. (* P < 0.05, ** P < 0.01, determined by a two-way ANOVA, followed by Sidak's multiple comparison test). Data represents one experiment.

Table S1 Top 150 differentially regulated genes in MC38 tumors treated with clg or anti-CD39 antibody as shown in Figure 3C.

Gene.Name	Associated.Gene.Name	logFC	PValue	Padj
ENSMUSG00000007877	Tcap	1.84315765	4.52E-15	1.08E-12
ENSMUSG00000018893	Mb	1.78940325	2.79E-12	3.18E-10
ENSMUSG000000069622	Gm10273	0.57156842	2.88E-12	3.26E-10
ENSMUSG00000024371	C2	1.61698752	5.00E-19	2.95E-16
ENSMUSG00000001056	Nhp2	0.50561928	4.24E-11	3.47E-09
ENSMUSG000000006360	Crip1	0.63564797	5.96E-14	1.08E-11
ENSMUSG000000057322	Rpl38	0.53745189	4.11E-11	3.41E-09
ENSMUSG000000025283	Sat1	0.45430193	6.95E-11	5.48E-09
ENSMUSG000000037646	Vps13b	-0.6455412	1.55E-17	6.68E-15
ENSMUSG000000022812	Gsk3b	-0.5464004	3.56E-14	7.12E-12
ENSMUSG000000036273	Lrrk2	-0.5978489	1.69E-12	2.06E-10
ENSMUSG000000036792	Mbd5	-0.6413971	1.20E-13	1.91E-11
ENSMUSG000000047414	Flrt2	-0.518313	5.32E-13	7.95E-11
ENSMUSG000000019726	Lyst	-1.048263	8.89E-24	1.11E-20
ENSMUSG000000035284	Vps13c	-0.8443381	9.15E-13	1.23E-10
ENSMUSG000000022641	Bbx	-0.6020853	7.63E-14	1.31E-11
ENSMUSG000000041702	Btbd7	-0.922413	6.32E-17	2.28E-14
ENSMUSG000000067336	Bmpr2	-1.5092389	2.80E-29	1.57E-25
ENSMUSG000000031529	Tnks	-1.0950279	3.75E-25	7.01E-22
ENSMUSG000000034342	Cbl	-1.4523348	3.94E-27	1.10E-23
ENSMUSG000000028246	6230409E13Rik	-0.7027988	2.59E-12	2.99E-10
ENSMUSG000000052812	Atad2b	-1.0771254	2.02E-17	8.37E-15
ENSMUSG000000033855	Ston1	-0.7578346	5.67E-13	8.35E-11
ENSMUSG000000036109	Mbnl3	-1.0663549	1.03E-13	1.71E-11
ENSMUSG000000033960	9430020K01Rik	-0.6637831	8.40E-13	1.15E-10
ENSMUSG000000038024	Dennd4c	-0.5895309	3.71E-12	3.99E-10
ENSMUSG000000094410	Zbed6	-1.4501981	1.44E-25	3.23E-22
ENSMUSG000000005886	Ncoa2	-0.6074981	6.76E-13	9.70E-11
ENSMUSG000000025949	Pikfyve	-0.8992978	8.83E-15	2.02E-12
ENSMUSG000000047036	Zfp445	-0.8754088	1.54E-12	1.92E-10
ENSMUSG000000096401	AC241392.2	-0.8434265	3.65E-12	3.99E-10
ENSMUSG000000069892	9930111J21Rik2	-0.845818	7.74E-19	4.33E-16
ENSMUSG000000022306	Zfpm2	-0.5482655	1.25E-10	9.34E-09
ENSMUSG000000033799	BC016423	-0.5544077	7.25E-11	5.63E-09
ENSMUSG000000030180	Kdm5a	-0.5260105	1.00E-11	9.77E-10

ENSMUSG00000037416	Dmxl1	-0.7724239	4.04E-15	9.85E-13
ENSMUSG00000020181	Nav3	-0.9337723	1.60E-19	1.20E-16
ENSMUSG00000038151	Prdm1	-0.7348829	3.82E-12	4.08E-10
ENSMUSG00000000628	Hk2	-0.488244	6.16E-11	4.93E-09
ENSMUSG00000025612	Bach1	-0.5262225	1.94E-11	1.78E-09
ENSMUSG00000033792	Atp7a	-1.1270831	4.98E-19	2.95E-16
ENSMUSG00000030660	Pik3c2a	-0.5942181	2.36E-14	4.99E-12
ENSMUSG00000040021	Lats1	-0.5127307	1.19E-10	8.99E-09
ENSMUSG00000039087	Rreb1	-0.7009343	4.17E-14	8.06E-12
ENSMUSG00000049470	Aff4	-0.6468274	7.08E-14	1.25E-11
ENSMUSG00000042473	Tbc1d8b	-0.7144496	3.18E-11	2.83E-09
ENSMUSG00000039286	Fndc3b	-0.5161241	3.20E-12	3.58E-10
ENSMUSG00000022883	Robo1	-0.6808323	2.58E-13	4.01E-11
ENSMUSG00000054387	Mdm4	-0.8795125	1.57E-12	1.94E-10
ENSMUSG00000046230	Vps13a	-0.7583028	1.53E-14	3.36E-12
ENSMUSG00000025326	Ube3a	-0.5874177	4.46E-12	4.64E-10
ENSMUSG00000021756	Il6st	-0.5558173	2.20E-12	2.59E-10
ENSMUSG00000055320	Tead1	-1.301947	2.15E-30	2.41E-26
ENSMUSG00000034636	Zyg11b	-0.4959564	1.17E-10	8.93E-09
ENSMUSG00000053877	Srcap	-1.3204004	2.04E-15	5.43E-13
ENSMUSG00000034218	Atm	-0.8619404	9.30E-18	4.16E-15
ENSMUSG00000047497	Adamts12	-1.0162726	1.41E-11	1.35E-09
ENSMUSG00000048279	Sacs	-0.7255095	4.22E-11	3.47E-09
ENSMUSG00000024542	Cep192	-0.6130059	3.34E-13	5.05E-11
ENSMUSG00000069793	Slfn9	-0.6637468	2.57E-12	2.99E-10
ENSMUSG00000026872	Zeb2	-0.6466166	1.55E-11	1.45E-09
ENSMUSG00000038371	Sbf2	-0.5533413	4.91E-12	4.96E-10
ENSMUSG00000039477	Tnrc18	-0.8138057	1.23E-15	3.36E-13
ENSMUSG00000026313	Hdac4	-0.9203918	3.83E-11	3.25E-09
ENSMUSG00000046138	9930021J03Rik	-0.7133291	1.54E-12	1.92E-10
ENSMUSG00000061436	Hipk2	-0.9124621	2.65E-18	1.41E-15
ENSMUSG00000034610	Zcchc11	-0.502338	6.91E-13	9.80E-11
ENSMUSG00000033396	Spg11	-0.4988122	1.96E-11	1.78E-09
ENSMUSG00000058690	Gcap14	-0.5432047	1.13E-12	1.51E-10
ENSMUSG00000057335	Cep170	-0.6342088	1.60E-16	5.14E-14
ENSMUSG00000037876	Jmjd1c	-0.6932789	5.98E-14	1.08E-11
ENSMUSG00000031540	Myst3	-0.7159131	3.12E-17	1.25E-14
ENSMUSG00000022141	Nipbl	-0.7041469	3.05E-16	9.00E-14
ENSMUSG00000039967	Zfp292	-0.7056634	4.32E-14	8.20E-12

ENSMUSG00000003119	Cdk12	-0.652897	2.18E-13	3.44E-11
ENSMUSG000000041852	Tcf20	-0.5490693	1.72E-12	2.08E-10
ENSMUSG000000034751	Mast4	-0.675687	4.75E-12	4.83E-10
ENSMUSG000000051149	Adnp	-1.2889419	6.18E-17	2.28E-14
ENSMUSG000000036202	Rif1	-0.6079938	7.95E-11	6.14E-09
ENSMUSG000000032740	Ccdc88a	-0.6263009	3.83E-11	3.25E-09
ENSMUSG000000022521	Crebbp	-0.8241118	5.66E-14	1.06E-11
ENSMUSG000000061755	Bod1l	-0.632616	1.20E-10	8.99E-09
ENSMUSG000000040225	Prrc2c	-1.0339487	7.83E-18	3.65E-15
ENSMUSG000000019907	Ppp1r12a	-0.6298676	4.47E-12	4.64E-10
ENSMUSG000000036550	Cnot1	-0.4963522	4.04E-11	3.40E-09
ENSMUSG000000019820	Utrn	-0.6246233	8.55E-12	8.39E-10
ENSMUSG000000005871	Apc	-0.5909858	1.11E-10	8.49E-09
ENSMUSG000000037270	4932438A13Rik	-0.7040642	3.32E-15	8.26E-13
ENSMUSG000000034377	Tulp4	-0.5465416	8.12E-12	8.05E-10
ENSMUSG000000031441	Atp11a	-0.528958	5.88E-11	4.74E-09
ENSMUSG000000037112	Sik2	-0.6191487	1.23E-11	1.19E-09
ENSMUSG000000022263	Trio	-0.6239415	3.81E-11	3.25E-09
ENSMUSG000000040524	Zfp609	-0.6794061	7.88E-15	1.84E-12
ENSMUSG000000020640	Itsn2	-0.6132294	6.65E-12	6.65E-10
ENSMUSG000000043940	Wdfy3	-0.6824764	1.04E-13	1.71E-11
ENSMUSG000000037487	Ubr5	-0.5264442	3.73E-11	3.24E-09
ENSMUSG000000015501	Hivep2	-0.585124	6.59E-13	9.59E-11
ENSMUSG000000057230	Aak1	-1.2529859	9.91E-16	2.77E-13
ENSMUSG000000028487	Bnc2	-0.940397	1.11E-14	2.50E-12
ENSMUSG000000038679	Trps1	-0.6059365	1.12E-13	1.82E-11
ENSMUSG000000021782	Dlg5	-0.5703048	3.30E-11	2.88E-09
ENSMUSG000000021767	Myst4	-0.70999	6.88E-11	5.47E-09
ENSMUSG000000068284	Gm608	-0.7013207	2.61E-11	2.34E-09
ENSMUSG000000019841	Rev3l	-0.6890561	1.46E-11	1.39E-09
ENSMUSG000000028053	Ash1l	-0.7291701	3.75E-14	7.36E-12
ENSMUSG000000074305	C230081A13Rik	-0.6721642	1.90E-14	4.08E-12
ENSMUSG000000070565	Rasal2	-0.7054422	7.51E-13	1.05E-10
ENSMUSG000000041444	Arhgap32	-0.7818615	4.25E-12	4.49E-10
ENSMUSG000000030516	Tjp1	-0.6360077	3.71E-12	3.99E-10
ENSMUSG000000034297	Med13	-0.6432913	7.13E-14	1.25E-11
ENSMUSG000000029313	Aff1	-0.6592621	3.36E-14	6.84E-12
ENSMUSG000000022016	Akap11	-0.5441697	3.28E-11	2.88E-09
ENSMUSG000000018076	Med13l	-0.7493125	7.39E-17	2.59E-14

ENSMUSG00000027799	Nbea	-0.7459495	9.51E-17	3.23E-14
ENSMUSG00000036698	Eif2c2	-1.0030473	6.08E-25	9.73E-22
ENSMUSG00000021831	Ero1l	-0.8357147	1.40E-16	4.61E-14
ENSMUSG00000027820	Mme	-0.779831	5.16E-11	4.19E-09
ENSMUSG00000036155	Mgat5	-1.0285818	2.89E-16	8.76E-14
ENSMUSG00000054008	Ndst1	-0.6382115	3.56E-12	3.95E-10
ENSMUSG00000028030	Tbck	-0.8834419	1.55E-11	1.45E-09
ENSMUSG00000039952	Dag1	-0.7110282	3.00E-15	7.64E-13
ENSMUSG00000060012	Kif13b	-0.9032526	1.47E-12	1.87E-10
ENSMUSG00000042390	Gatad2b	-1.0849678	4.64E-22	5.20E-19
ENSMUSG00000053007	Creb5	-1.053161	1.93E-12	2.30E-10
ENSMUSG00000032487	Ptgs2	-0.7902567	2.10E-11	1.90E-09
ENSMUSG00000041324	Inhba	-1.1335198	6.83E-21	5.89E-18
ENSMUSG00000079575	Rbpsuh-rs3	-1.151103	1.34E-24	1.87E-21
ENSMUSG00000019947	Arid5b	-1.0683836	2.65E-16	8.25E-14
ENSMUSG00000073016	Uprt	-0.9924767	1.15E-12	1.51E-10
ENSMUSG00000025017	Pik3ap1	-0.619213	1.73E-11	1.60E-09
ENSMUSG00000069607	Cd300lh	-0.7785191	7.16E-11	5.61E-09
ENSMUSG00000079685	Ulbp1	-0.9958741	3.14E-13	4.82E-11
ENSMUSG00000052414	Atf7	-1.219725	1.31E-12	1.68E-10
ENSMUSG00000063550	Nup98	-0.7725928	3.54E-17	1.37E-14
ENSMUSG00000040274	Cdk6	-0.9396621	3.46E-16	9.95E-14
ENSMUSG00000069893	9930111J21Rik1	-1.4933908	2.31E-28	8.64E-25
ENSMUSG00000029202	Pds5a	-0.8007329	2.78E-18	1.41E-15
ENSMUSG00000037533	Rapgef6	-0.6396602	4.68E-12	4.81E-10
ENSMUSG00000023845	Lnpep	-1.1175084	7.86E-20	6.29E-17
ENSMUSG00000039704	Lmbrd2	-1.3802661	8.79E-22	8.21E-19
ENSMUSG00000021952	Xpo4	-1.0035331	2.20E-15	5.73E-13
ENSMUSG00000035133	Arhgap5	-0.9957967	5.14E-22	5.23E-19
ENSMUSG00000035898	Uba6	-0.7107274	9.47E-14	1.61E-11
ENSMUSG00000024298	Zfp871	-1.524904	7.75E-13	1.07E-10
ENSMUSG00000075470	Alg10b	-0.9623805	4.72E-18	2.30E-15
ENSMUSG00000070639	Lrrc8b	-1.1570397	2.43E-19	1.70E-16
ENSMUSG00000021466	Ptch1	-1.1699887	2.91E-14	6.04E-12
ENSMUSG00000063895	Nupl1	-0.564427	4.10E-11	3.41E-09
ENSMUSG00000020961	Ston2	-0.9794443	1.20E-12	1.57E-10
ENSMUSG00000033624	Pdpr	-1.1331289	2.72E-19	1.79E-16

Table S2

Supplementary Table 2 - Summary of antibody specific IHC conditions				
SI No	Target	Ag Retrieval & pH	Primary Ab clone	Primary Ab dilution
1	CD8	Dako Target Retrieval Solution, pH 9	C8/144B	1:200
2	CD39	Dako Target Retrieval Solution, pH 9	OTI2B10	1:200
3	PD1	Dako Target Retrieval Solution, pH 6	NAT105	1:100
4	CD19	Dako Target Retrieval Solution, pH 9	EPR5906	1:200