

Supplementary Figure S1

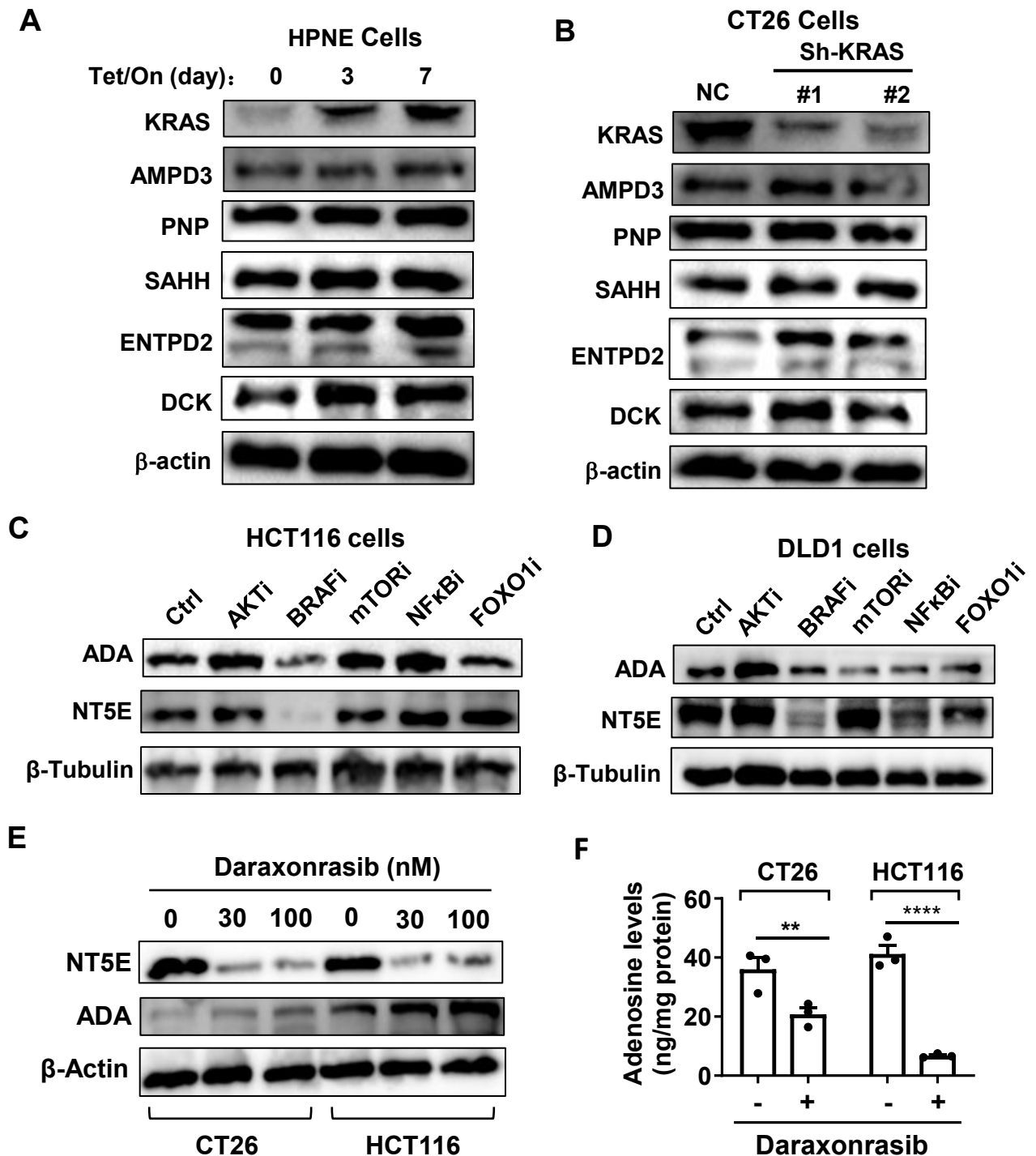
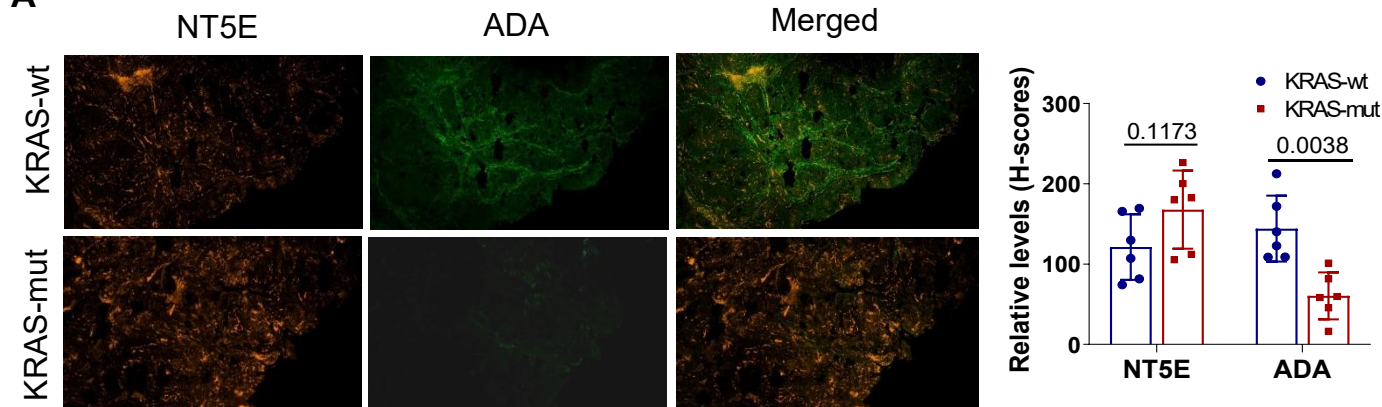


Figure S1. Protein and metabolite analyses in HPNE, CT26, HCT116, and DLD1 cell models. (A) Western blot analysis of KRAS and the key enzymes involved in adenosine metabolism (AMPD3, PNP, SAHH, ENTPD2, DCK) in HPNE cells cultured under Tet-On conditions for 0, 3, and 7 days. Protein extracts were prepared at the indicated time points for Western blot analysis. (B) Western blot analysis of KRAS and the key enzymes involved in

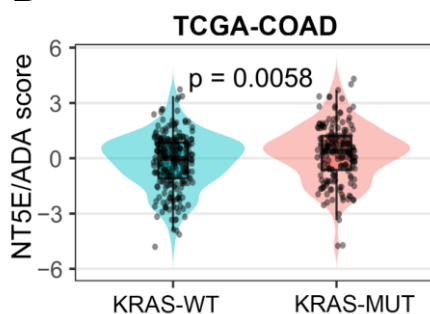
adenosine metabolism (AMPD3, PNP, SAHH, ENTPD2, DCK) in CT26 cells transfected with control (NC) or KRAS-specific shRNA (#1, #2). Protein expression was assessed 48 h after transfection; β -actin was blotted as a loading control. (C-D) Western blot analysis of NT5E and ADA protein expression in HCT116 and DLD1 cells treated with the indicated pharmacological inhibitors. AKTi: MK2206 (50 nM); BRAFi: SB590885 (20 nM); mTORi: Rapamycin (20 nM); NF κ Bi: BAY 11-7082 (5 μ M); FOXO1i: AS1842856 (50 nM). Protein extracts were prepared 24 hours after drug treatment. Western blot analysis was performed using the procedures described under Methods. (E) Western blot analysis of NT5E and ADA in CT26 and HCT116 cells treated in vitro with daraxonrasib at 0, 30, or 100 nM for 24h. (F) Extracellular adenosine levels in conditioned culture medium from HCT116 and CT26 cells treated in vitro with vehicle or daraxonrasib at 30 nM for 24h. Adenosine was quantified by mass spectrometry and normalized to total cellular protein. Each dot represents an independent biological replicate; bars show mean $\pm$ SEM. Statistical significance was determined by unpaired two-tailed Student's t-test. **P < 0.01; ****P < 0.0001.

Supplementary Figure S2

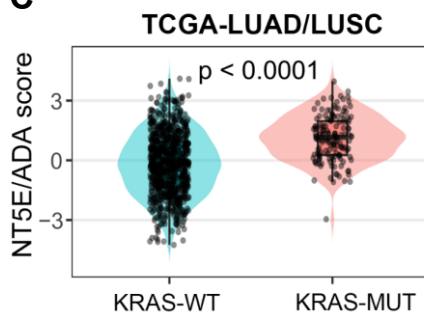
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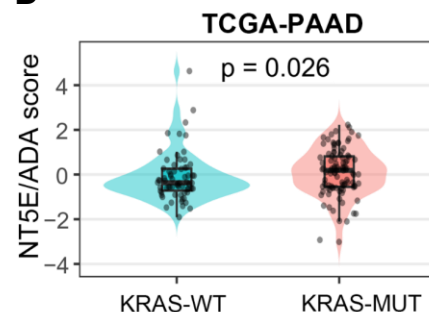
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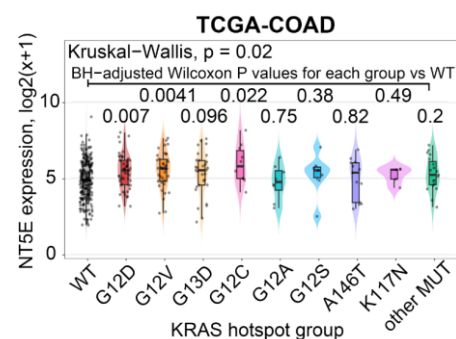
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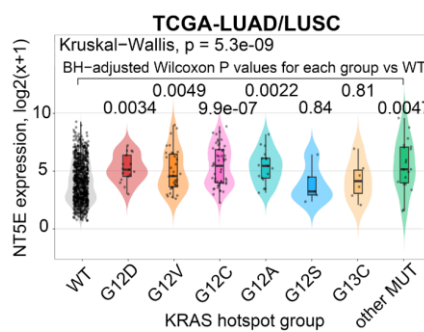
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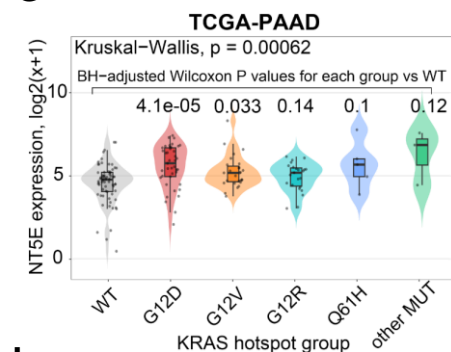
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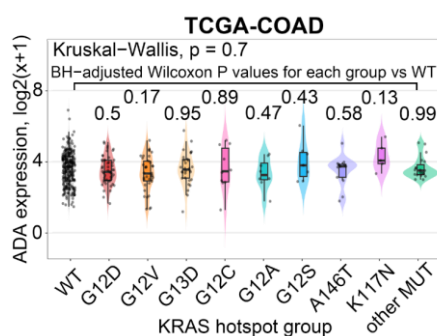
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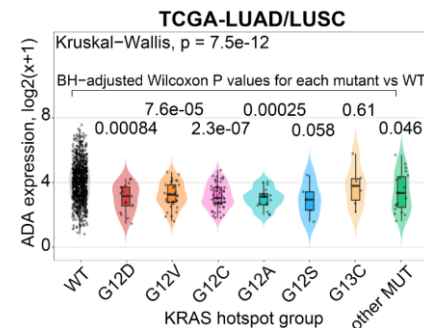
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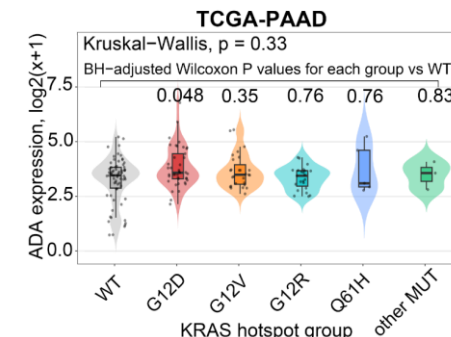
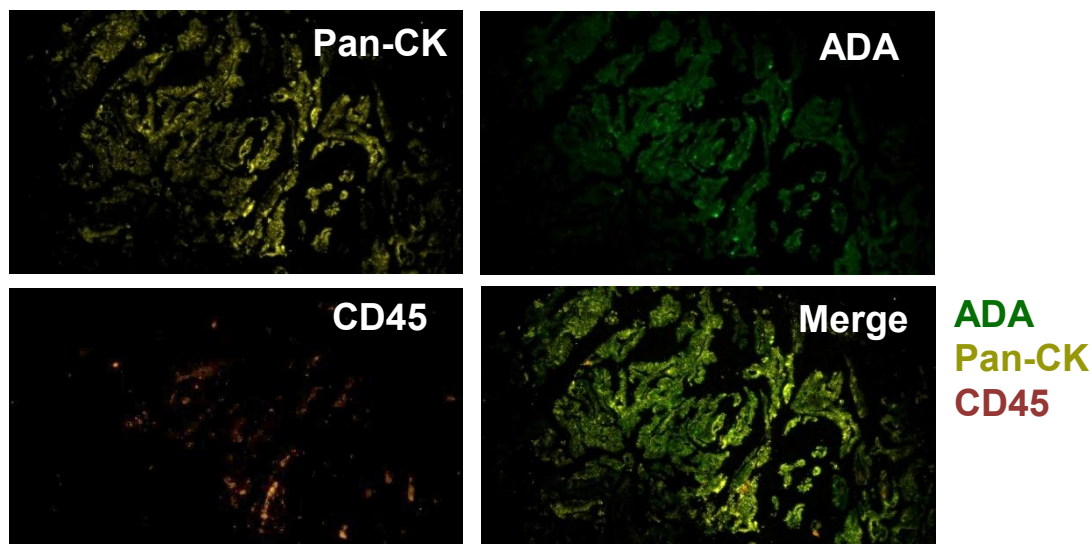


Figure S2. Multiplex immunohistochemistry and TCGA transcriptomic analyses of NT5E, CD73, and ADA expression in KRAS-wild-type and KRAS-mutant tumors. (A) Representative multiplex immunohistochemistry images of human surgical tumor specimens with wt-KRAS or mut-

KRAS (G12D, G12S). NT5E and ADA were detected on the same tissue sections, and are shown as single-channel and merged images. NT5E is shown in orange and ADA in green. Quantification of NT5E and ADA staining is presented as a relative H-score for KRAS-wild-type and KRAS-mutant tumors. **(B–D)** Violin plots showing the NT5E/ADA score in wt-KRAS and mutant-KRAS tumors from TCGA colorectal adenocarcinoma (TCGA-COAD), lung adenocarcinoma/lung squamous cell carcinoma (TCGA-LUAD/LUSC), and pancreatic adenocarcinoma (TCGA-PAAD) cohorts. **(E–G)** NT5E mRNA expression, shown as $\log_2(x+1)$, across KRAS hotspot mutation subgroups in TCGA-COAD, TCGA-LUAD/LUSC, and TCGA-PAAD cohorts. **(H–J)** ADA expression across KRAS hotspot mutation subgroups in the corresponding TCGA cohorts. Individual KRAS hotspot mutation groups were compared with KRAS-wild-type tumors.

Supplementary Figure S3

A



B

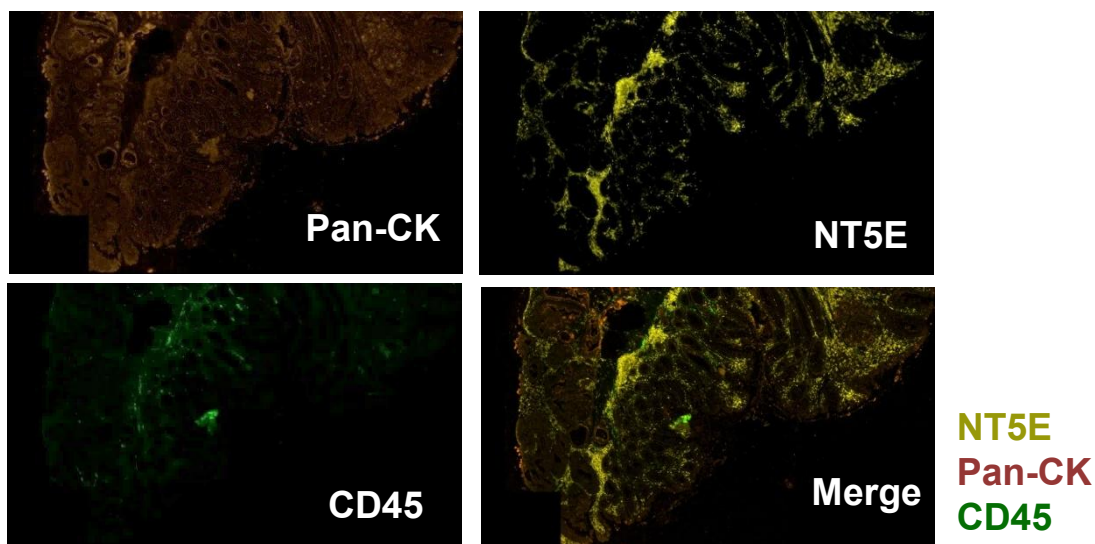
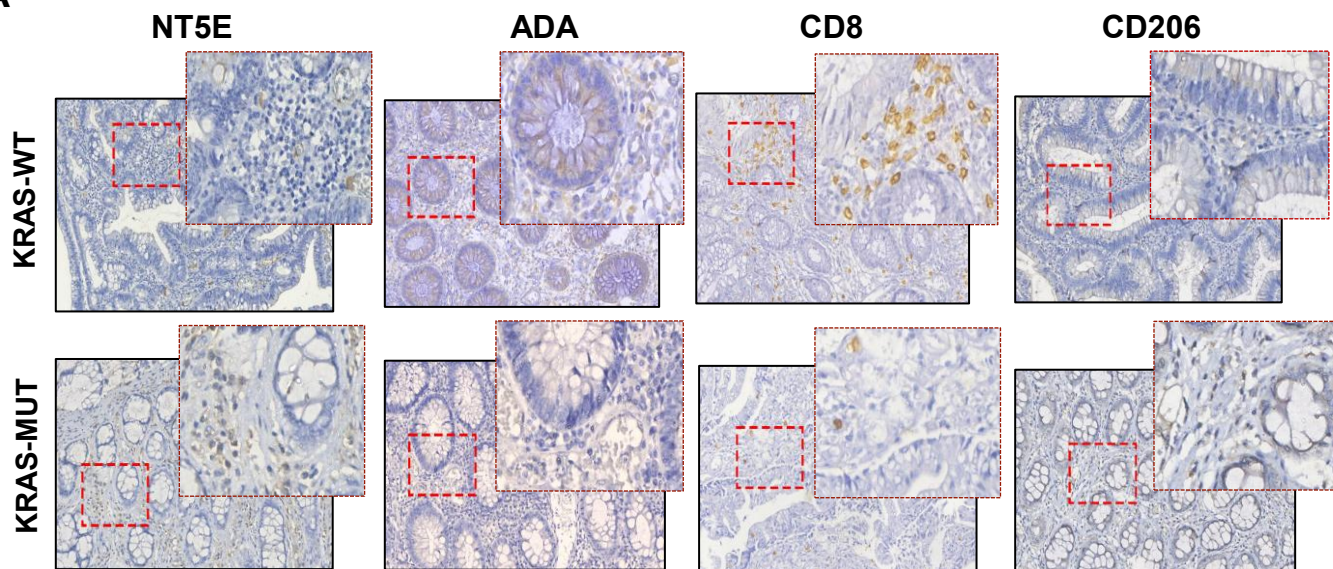


Figure S3. Multiplex immunohistochemistry analysis of ADA and NT5E in human colorectal cancer specimens. (A) Representative multiplex immunohistochemistry images of human colorectal cancer specimens with KRAS G12D mutation stained for ADA, pan-cytokeratin (Pan-CK), and CD45. Single-channel images and the corresponding merged images are shown. ADA is shown in green, Pan-CK in yellow, and CD45 in orange. **(B)** Representative multiplex immunohistochemistry images of human colorectal cancer specimens with KRAS mutation (G12D) stained for NT5E, Pan-CK, and CD45. Single-channel images and the corresponding merged image are shown. NT5E is shown in yellow-green, Pan-CK in red/orange, and CD45 in green..

Supplementary Figure S4

A



B

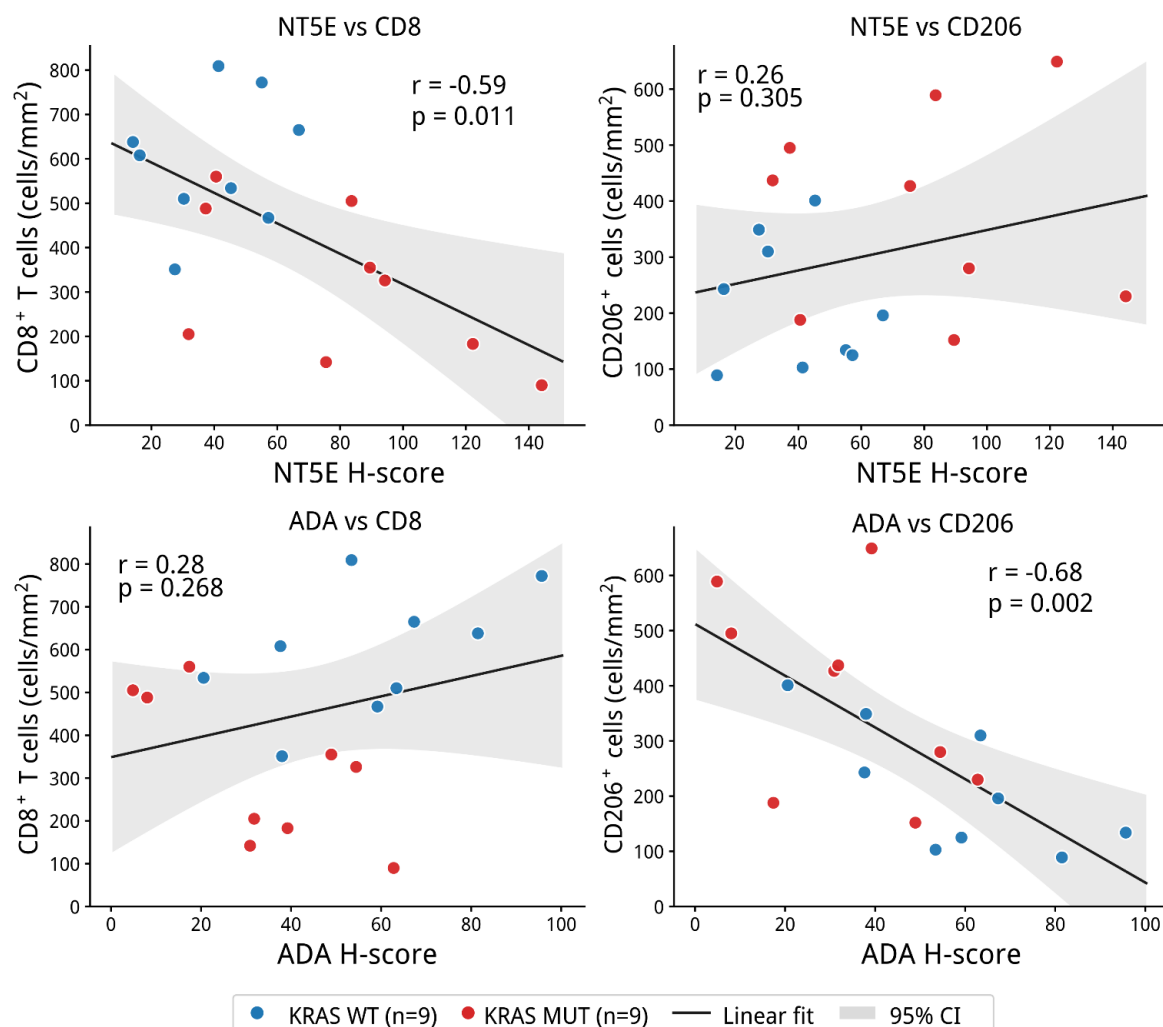


Figure S4. Correlation between the expression of adenosine metabolism-related enzymes and immune cell infiltration in human CRC tissues. (A) Representative immunohistochemistry

images of NT5E, ADA, CD8 and CD206 staining in human colorectal cancer tissues with KRAS mutation (MUT) or KRAS wild-type (WT) status as indicated. NT5E and ADA were analyzed in tumor epithelial compartments, while CD8 and CD206 were used as markers to show the infiltrating immune cells in the tumor microenvironment. **(B)** Correlation analyses between tumoral NT5E and ADA expression (H-score) and immune-cell infiltration density in 18 tumor samples (KRAS WT, n = 9; KRAS MUT, n = 9). The four panels show NT5E versus CD8, ADA versus CD8, NT5E versus CD206, and ADA versus CD206, respectively. Blue and red circles indicate KRAS wild-type and KRAS-mutant samples. Solid lines represent linear regression fits; shaded areas denote 95% confidence intervals. Pearson correlation coefficients and two-tailed p-values are indicated in each panel.

Supplementary Figure S5

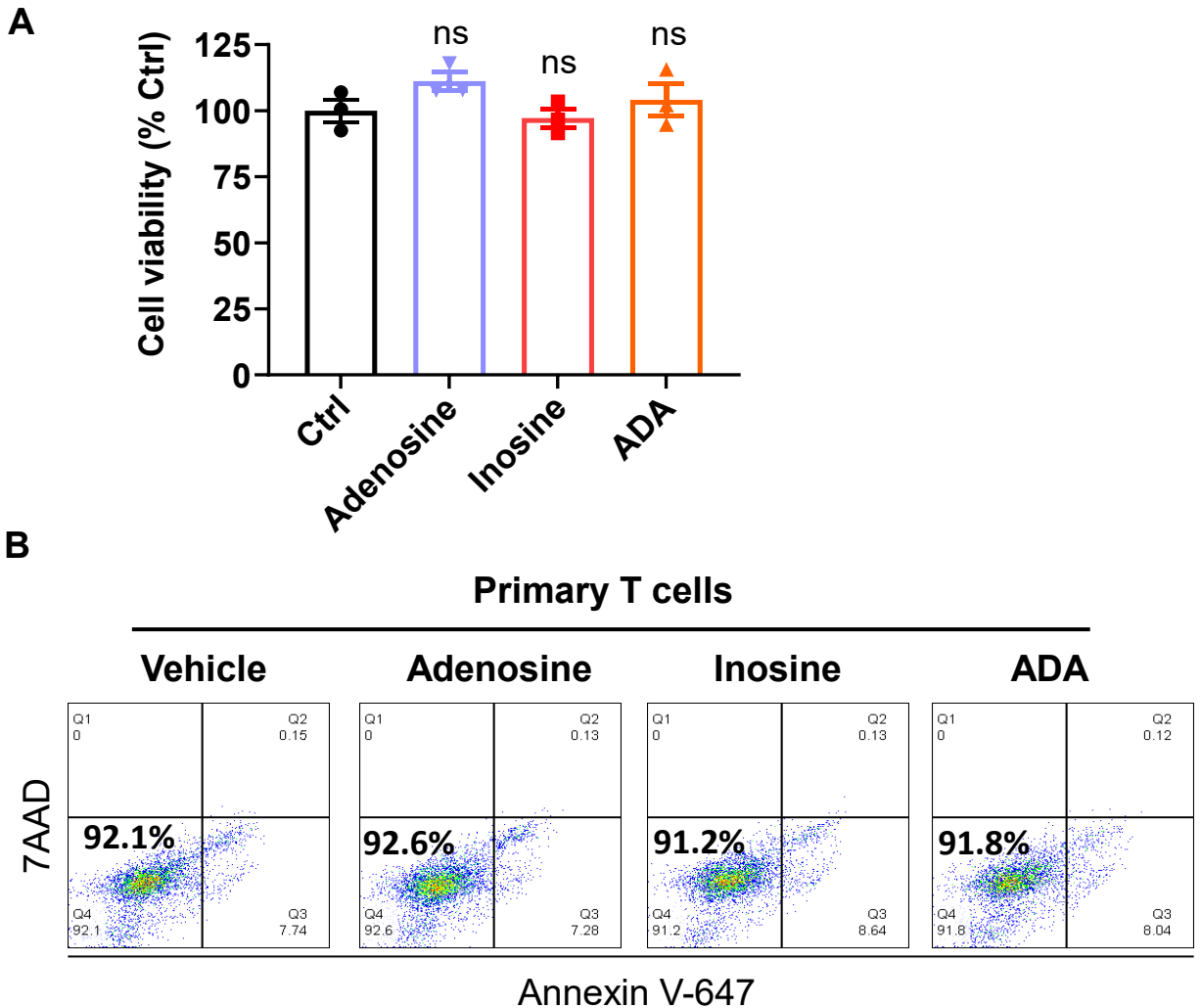
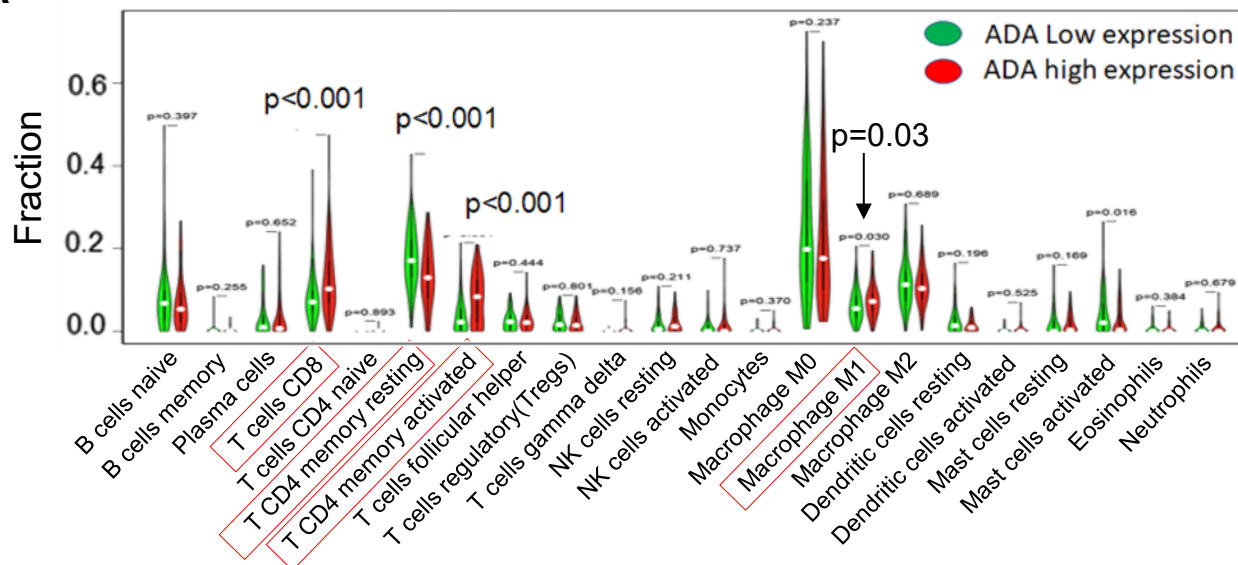


Figure S5. Effect of adenosine, inosine and ADA on cell viability. **(A)** Effect of adenosine (5 μ M), inosine (5 μ M), and ADA (10 nM) on cell viability of B3Z-CT26 in co-culture for 24 h. Cell viability was measured by MTS assay. Data are presented as mean $\pm$ SD. Statistical significance was determined using one-way ANOVA analysis, ns, not significant. **(B)** Representative flow cytometry plots of Annexin V/7AAD staining of mouse primary T cells treated with adenosine, inosine, or ADA as indicated. T cells were then gated for apoptosis analysis. The percentage within each chart indicates % of viable cells.

Supplementary Figure S6

A



B

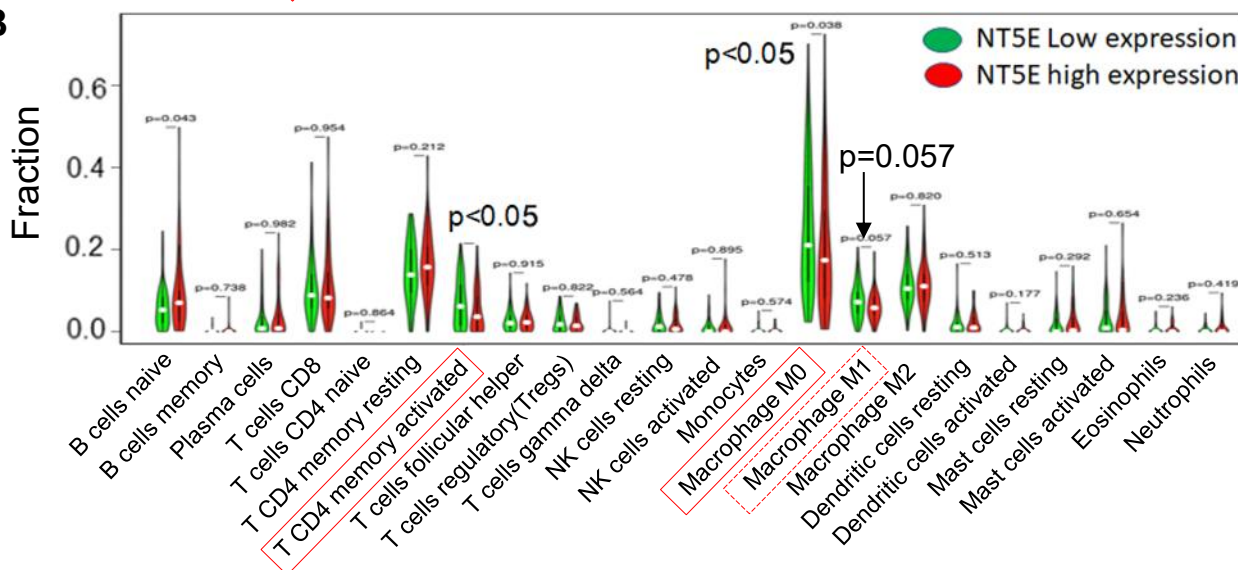


Figure S6. Infiltration of immune cells in CRC patient samples with different levels of ADA or NT5E expression. Transcriptomic immune cell deconvolution was performed using the CIBERSORT algorithm (LM22 leukocyte gene signature) and the normalized RNA-seq data from the Cancer Genome Atlas (TCGA) colorectal cancer cohort (TCGA-COAD dataset). The patient samples were stratified into ADA-high and ADA-low groups (**A**) or NT5E-high and NT5E-low groups (**B**) based on the median transcript per million (TPM, $\log_2$ -transformed) values. The estimated fractions of the indicated 22 subsets of immune cells are shown as violin plots, with green color indicating samples with low expression of ADA or NT5E and red color indicating samples with high expression of ADA or NT5E. The statistical difference between the high- and low-expression groups was analyzed using two-sided Wilcoxon rank-sum test, and p values are indicated above each pair of comparison. Immune cell types with significant difference ($p < 0.05$) related to ADA or NT5E expression are highlighted with red boxes. Note that the p value for M1 macrophages in panel (B) was 0.057, indicated with a dashed red box.

Supplementary Figure S7

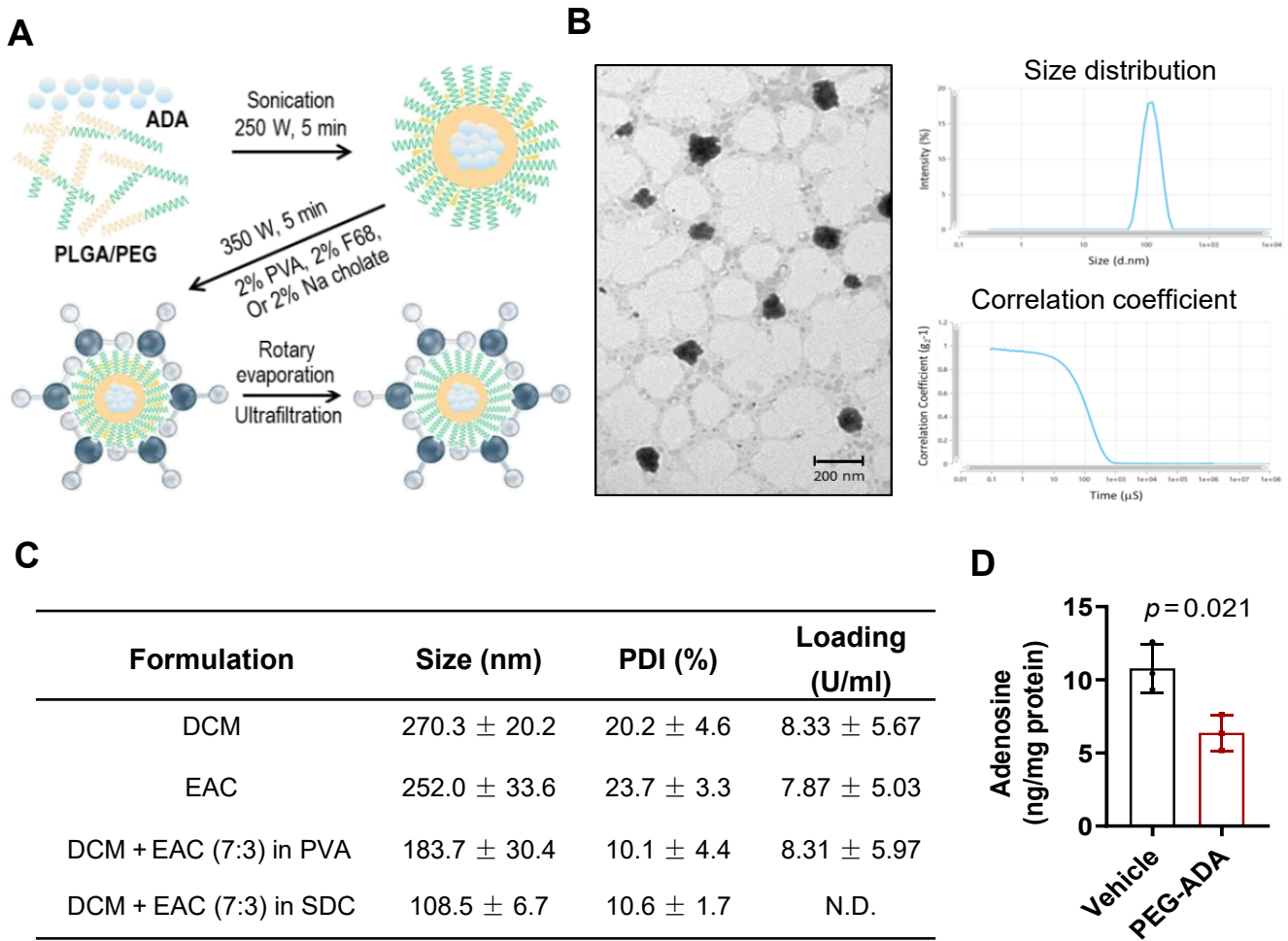


Figure S7. Preparation and characterization of PEG-ADA nanoparticles. **(A)** Schematic illustration of PEG-ADA nanoparticle preparation. The detail steps are described under Methods. **(B)** Transmission electron microscopy (TEM) image showing the morphology of the PEG-ADA nanoparticles. Scale bar = 200 nm. The right panel shows the size distribution and the correlation coefficient of PEG-ADA nanoparticles, measured by dynamic light scattering (DLS). **(C)** Particle sizes, polydispersity index (PDI), and loading efficiency of PEG-ADA nanoparticles prepared under the indicated conditions. Particle size and loading efficiency were determined by DLS using UV-VIS spectrophotometry. **(D)** Quantification of adenosine levels in cells treated with or without PEG-ADA nanoparticles. Adenosine levels were measured using LC-MS/MS.

Supplementary Figure S8

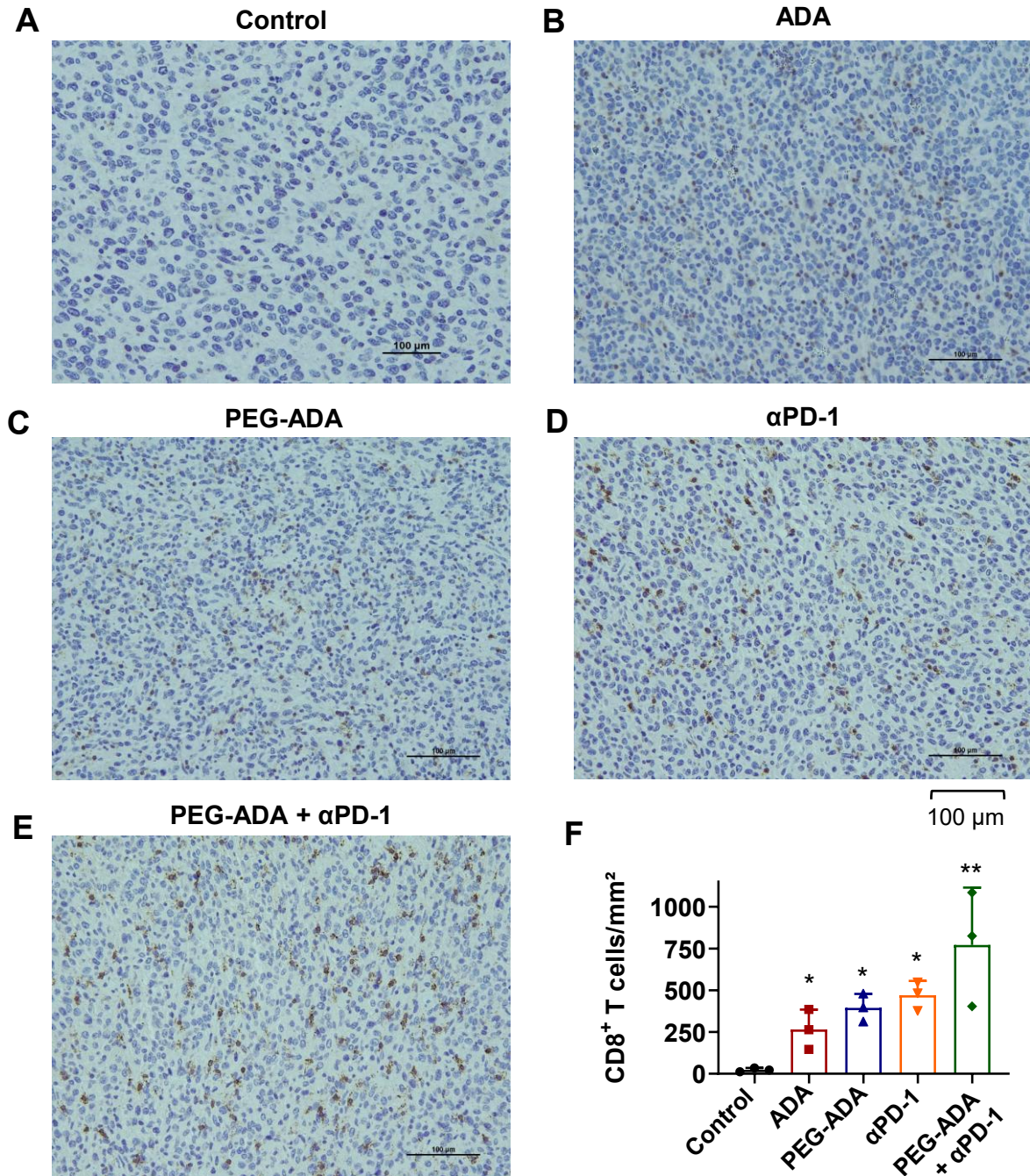


Figure S8. Effect of ADA, PEG-ADA, and PD-1 antibody treatment on CD8⁺ T cell infiltration in tumor tissues. (A-E) BALB/c mice were inoculated with mouse CRC cells (CT26), and treated with saline (Control), ADA, PEG-ADA, PD-1 antibody (α PD-1), or PEG-ADA + α PD-1 as indicated. Tumor tissues were isolated at the end of the experiment, sliced, and stained for CD8⁺ T cells by IHC. Representative images are shown. Scale bar, 100 μ m. (F) Quantitation of CD8⁺ T cells in tumor tissues from the indicated mouse groups. Data are mean $\pm$ SD, n=3, *, p < 0.05; **, p < 0.01 (One-way ANOVA).

Supplementary Figure S9

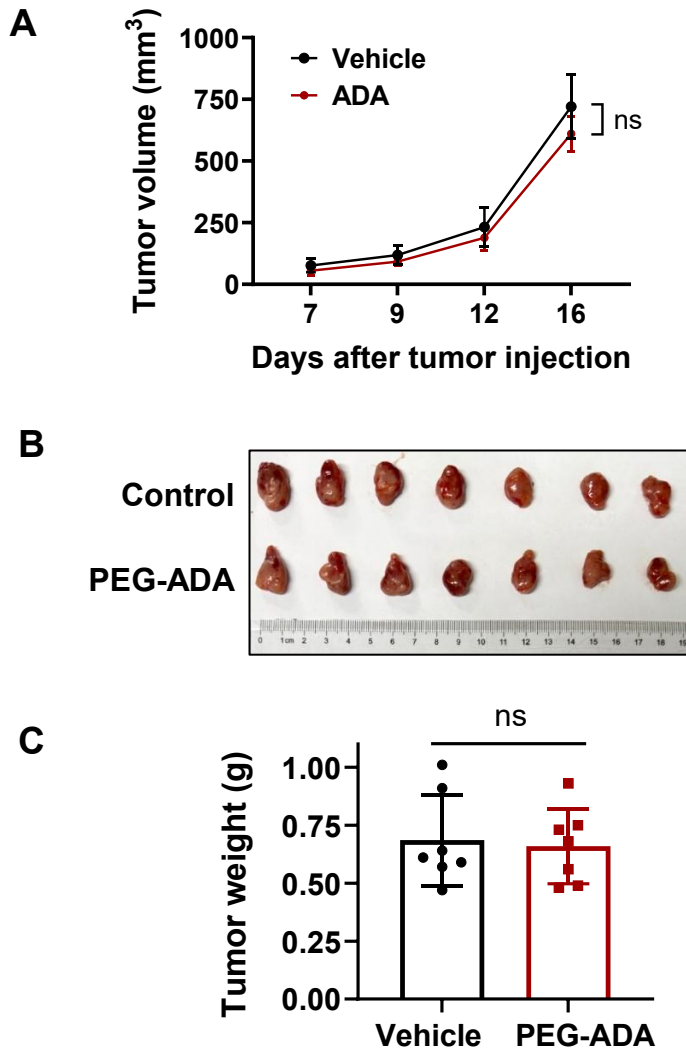
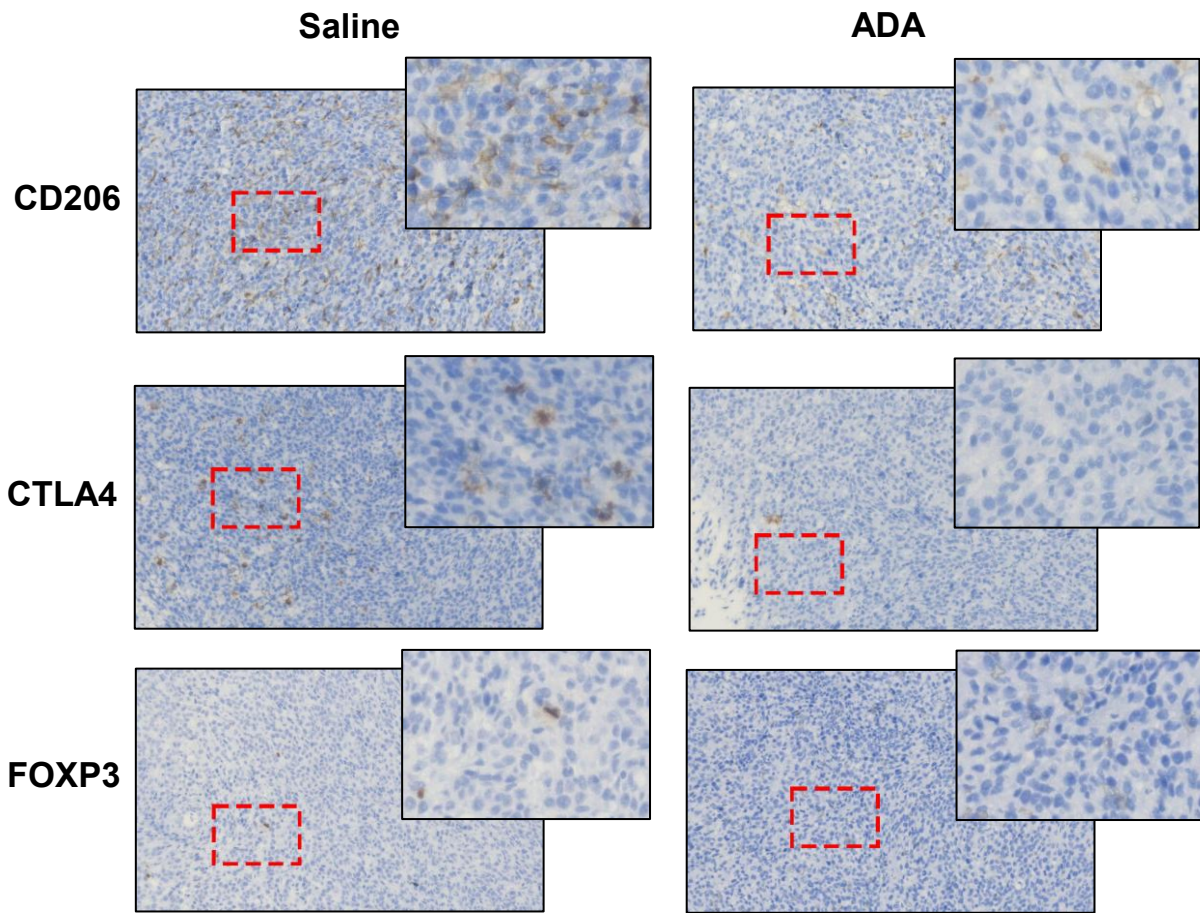


Figure S9. Effect of PEG-ADA treatment on CT26 tumor growth in nude mice. (A) Tumor growth curves of subcutaneous CT26 xenografts in nude mice treated with vehicle or with PEG-ADA (100 µg/mouse, i.t., every other day). **(B)** Photos of CT26 tumors from vehicle- or PEG-ADA-treated mice at the experimental endpoint. **(C)** Tumor weights of the control and PEG-ADA-treated groups. Data are presented as mean $\pm$ SEM; n = 7 mice per group.

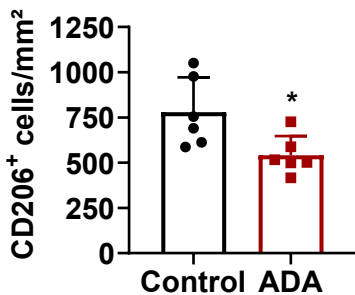
Supplementary Figure S10

A

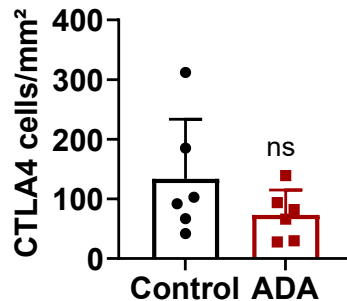
In vivo treatment



B



C



D

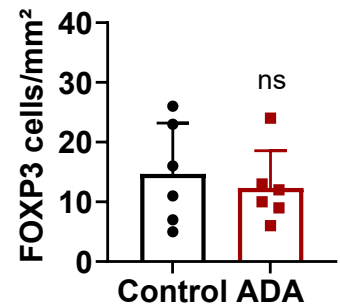
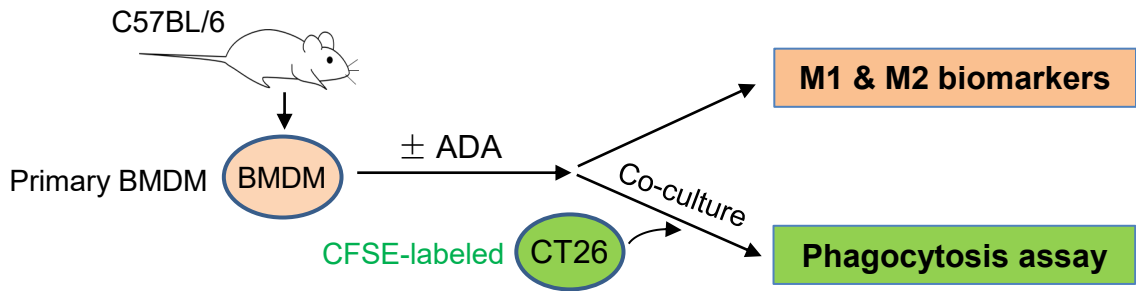


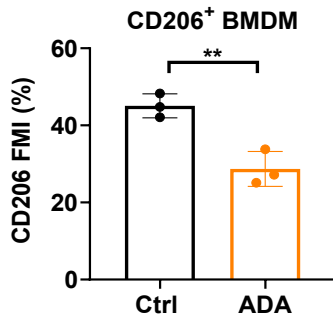
Figure S10. IHC staining of CD206, CTLA4, and FOXP3 in tumor tissues from mice treated with saline or ADA. (A) Representative IHC staining of tumor sections from mice bearing CT26 xenografts treated with saline or ADA (100 µg/mouse, i.t., every other day). Tumor tissues were stained for CD206, CTLA4, and FOXP3. **(B-D)** Quantification of CD206⁺ cells (B), CTLA4⁺ cells (C), and FOXP3⁺ cells (D) in tumor sections. Each dot represents an individual tumor sample. Bars indicate the mean and standard errors. ns, not significant; *, $P < 0.05$.

Supplementary Figure S11

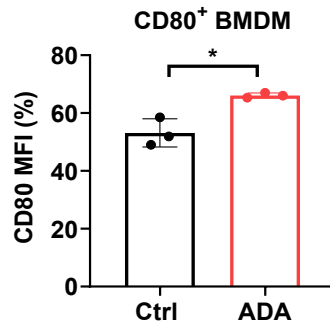
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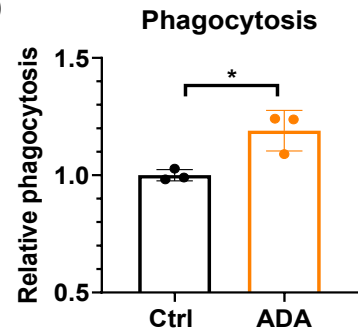
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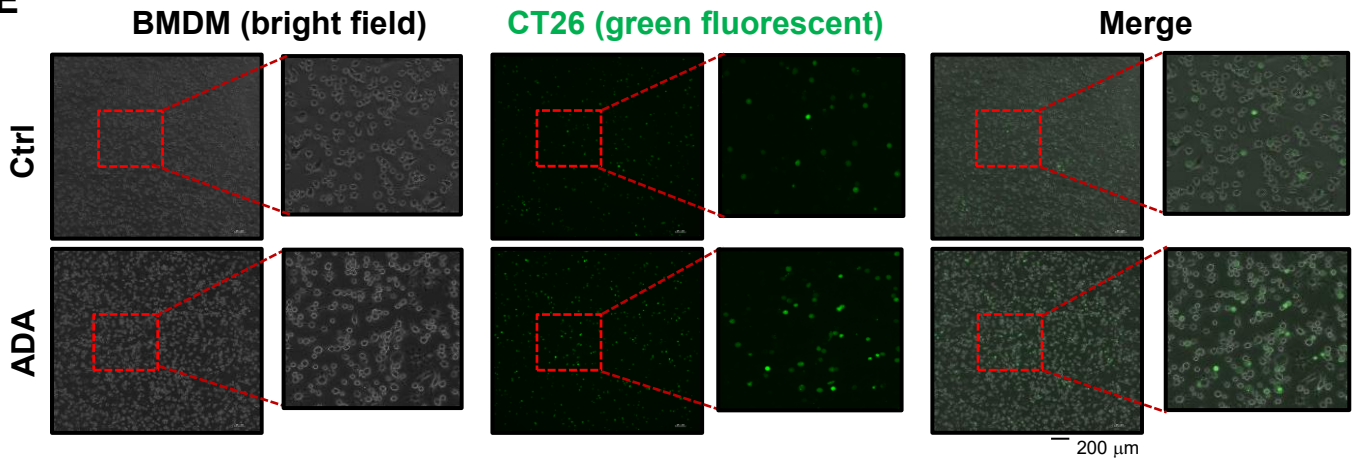
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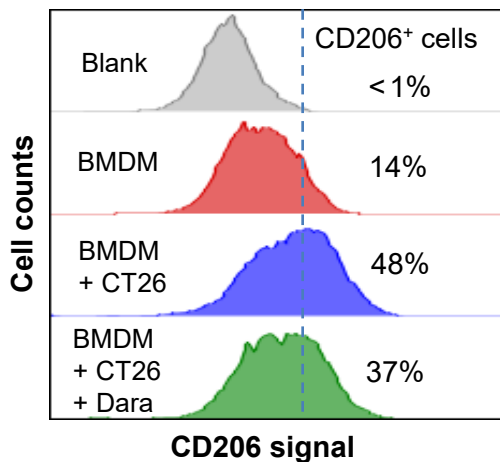
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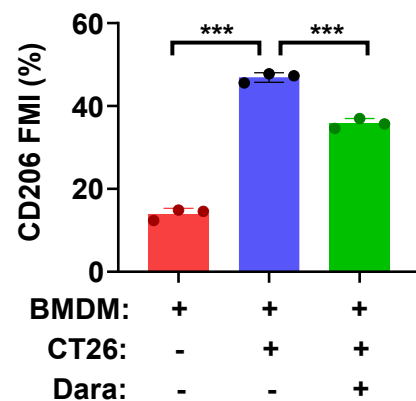
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F



G



FigS11. Effect of ADA and daraxonrasib on polarization and phagocytic function of bone marrow-derived macrophage (BMDM). (A) Schematic illustration of the experimental workflow.

(B, C) Quantitation of CD206 and CD80 expression in primary BMDM cells incubated with or without ADA (100 nM, 24 h). The expression of CD206 or CD80 is shown as mean fluorescence intensity (MFI). Bars represent mean $\pm$ SD from three independent experiments.

(D) Effect of ADA on phagocytosis function of bone marrow-derived macrophages. CT26 tumor cells were labeled with CFSE green dye, treated with 2 mM H₂O₂ to induce apoptosis as the substrates for phagocytosis, and then co-cultured with mouse primary BMDMs pre-treated with or without ADA (100 nM) for 16 h. After removal of the free CT26 cells, the mean fluorescence intensity of the CFSE-labeled CT26 debris internalized by BMDMs was measured by flow cytometry analysis. Bars represent mean $\pm$ SD from three independent experiments.

(E) Representative fluorescence images showing the CFSE-labeled CT26 cells (green) engulfed by BMDMs. Scale bar, 200 μ m.

(F) Effect of daraxonrasib (Dara) on CD206 expression in BMDMs. Representative flow cytometry histograms of CD206 expression in BMDMs co-cultured with or without CT26 tumor cells and daraxonrasib (10 nM, 24 h) as indicated.

(G) Quantitation of CD206 expression under the indicated conditions. Bars represent mean $\pm$ SD from three independent experiments.

Supplementary Figure S12

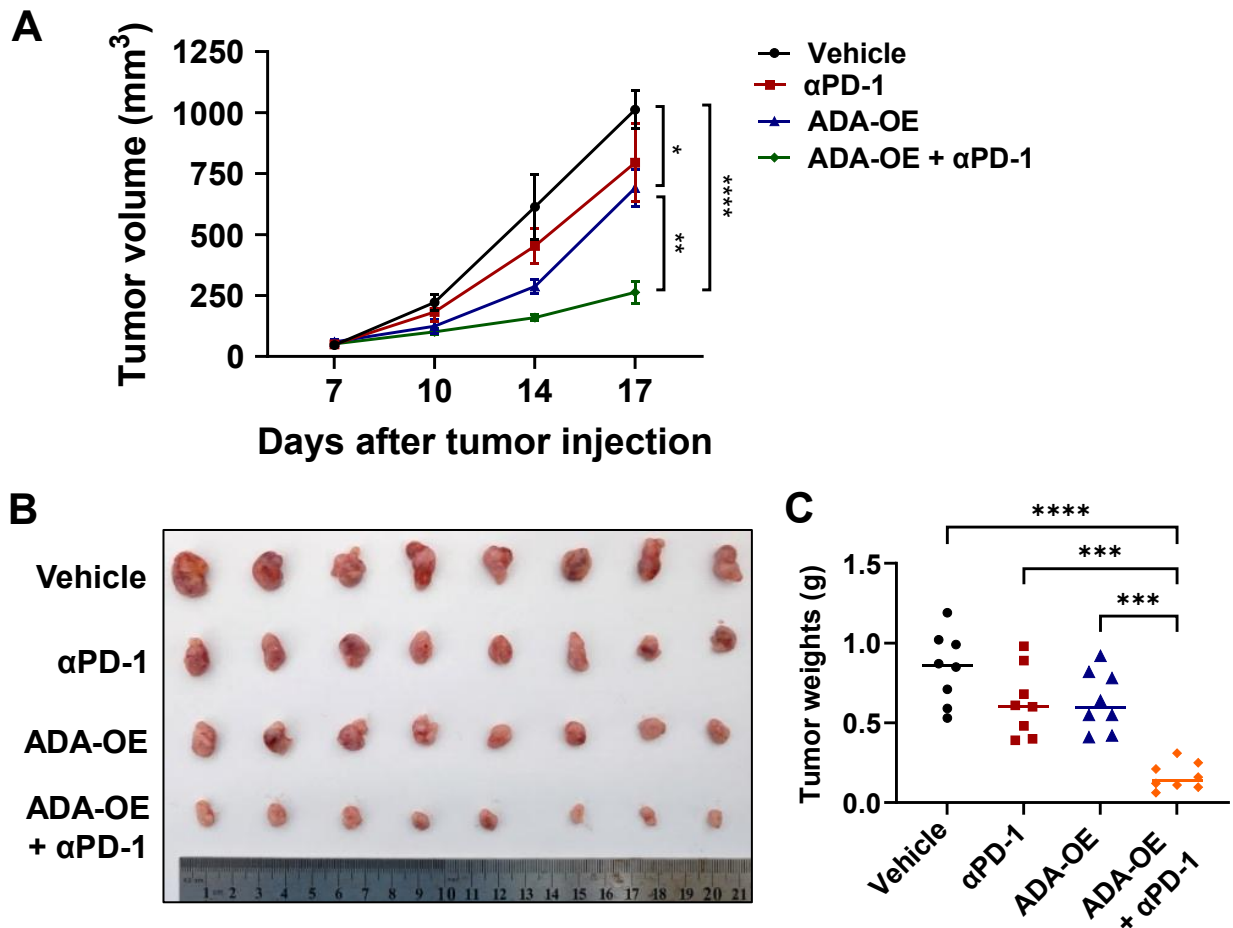


Figure S12. Effect of ADA expression on antitumor activity of anti-PD-1 antibody in mice bearing CT26 tumor xenografts. (A) Tumor growth curves of subcutaneous CT26 tumors generated by inoculation of CT26 cells harboring the control vector or ADA-overexpressing (ADA-OE) into BALB/c mice treated with vehicle or anti-PD-1 antibody. Anti-PD-1 antibody was administered by intraperitoneal injection at 150 μ g per mouse twice weekly. Tumor volumes were measured at the indicated time points. **(B)** Photos of tumors excised from BALB/c mice at the experimental endpoint. **(C)** Tumor weights of CT26 tumors from the indicated groups. Data are presented as mean $\pm$ SEM. Tumor growth curves were analyzed by two-way ANOVA with multiple-comparison correction. Endpoint tumor weights were analyzed by one-way ANOVA with multiple-comparison correction unless otherwise indicated. ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

Supplementary Figure S13

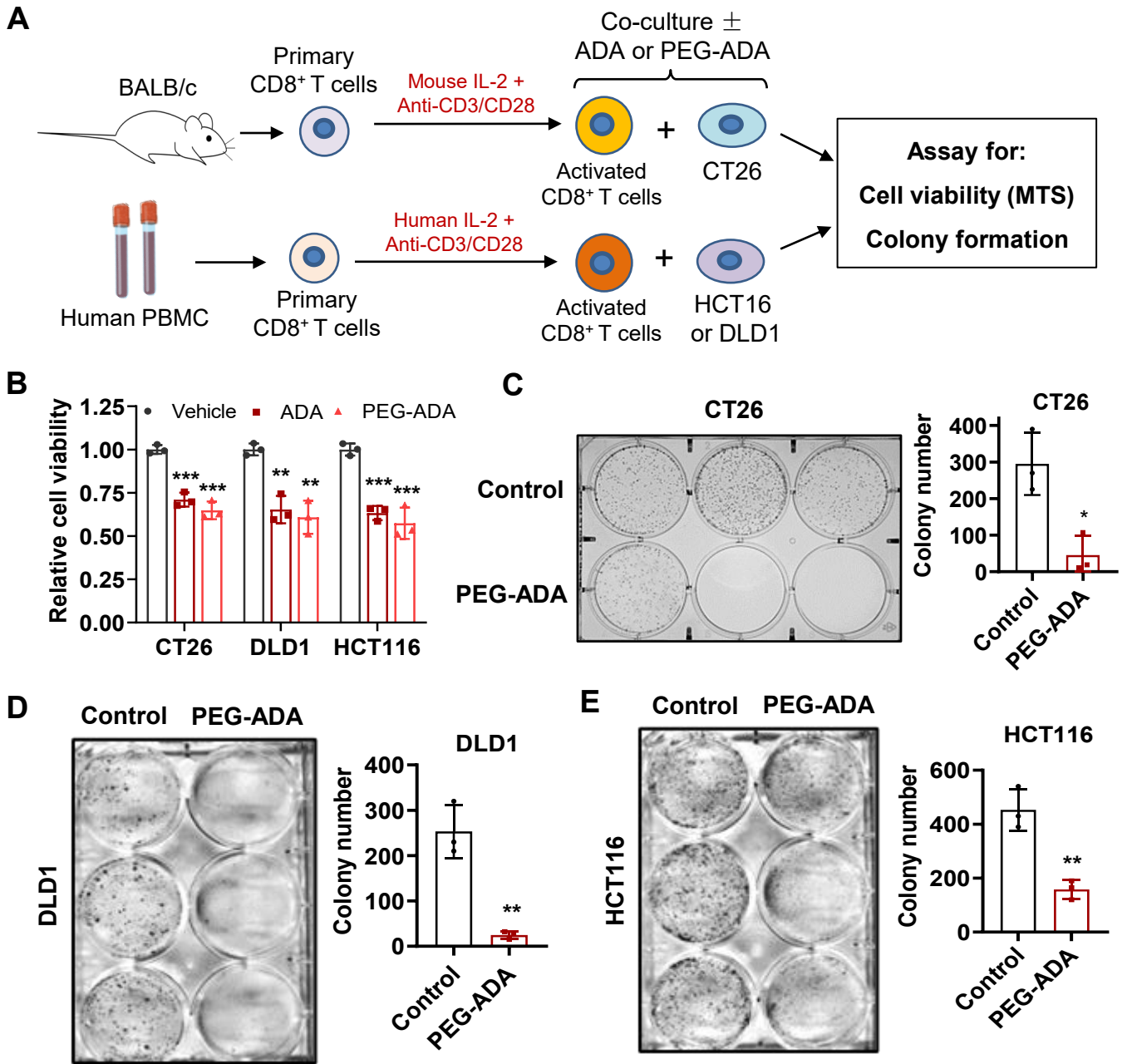


Figure S13. Effect of ADA and PEG-ADA on T cell-mediated cytotoxicity against tumor cells. (A) Schematic illustration of the experimental design. (B) Effect of ADA or PEG-ADA on T cell cytotoxicity against tumor cells in co-culture of pre-activated CD8⁺ T cells and CRC cells (48 h). Cell viability was measured using MTS assay. (C-E) Colony formation in DLD1, HCT116, and CT26 cells co-cultured with activated CD8⁺ T cells in the presence or absence of PEG-ADA. Colony formation assays were performed 48 h after co-culture. The plates were incubated for additional 10-14 days. Data are presented as mean $\pm$ SD. Statistical significance was determined using one-way ANOVA analysis. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Supplementary Figure S14

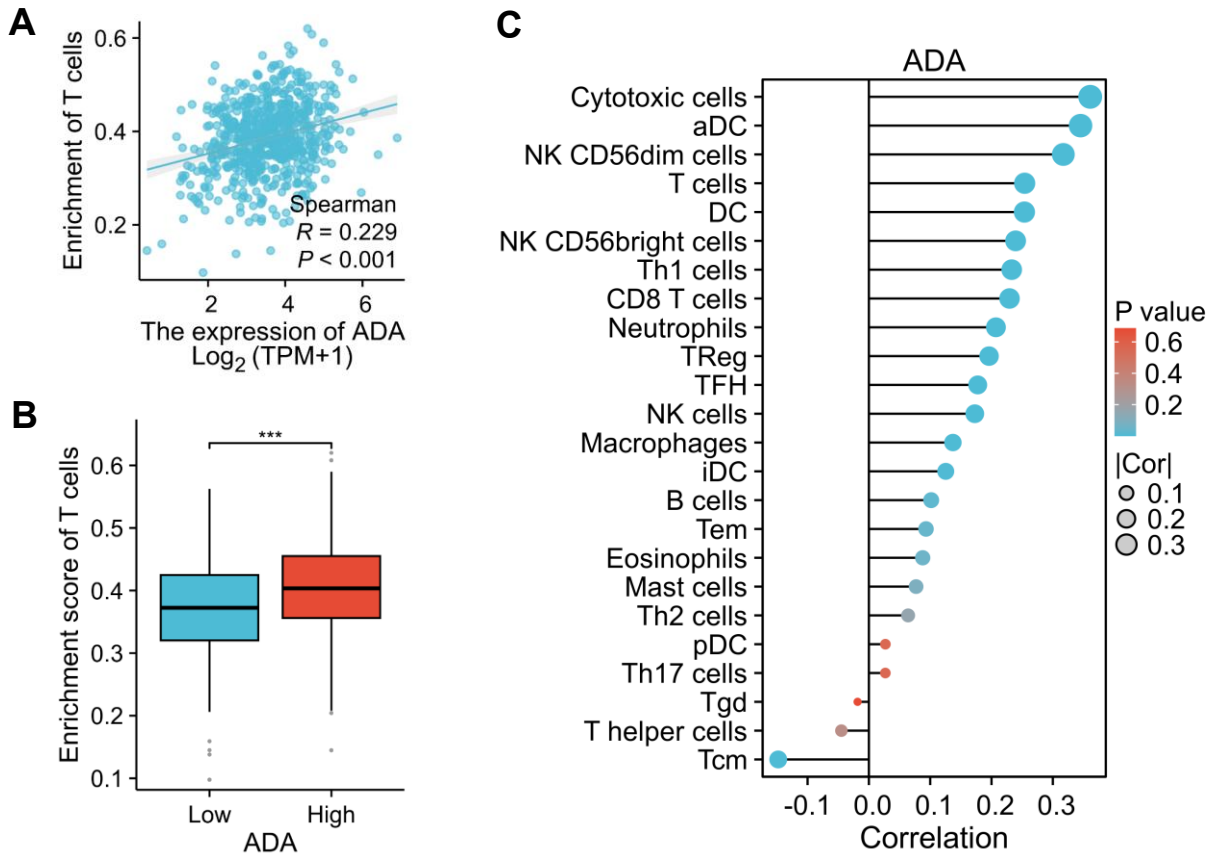
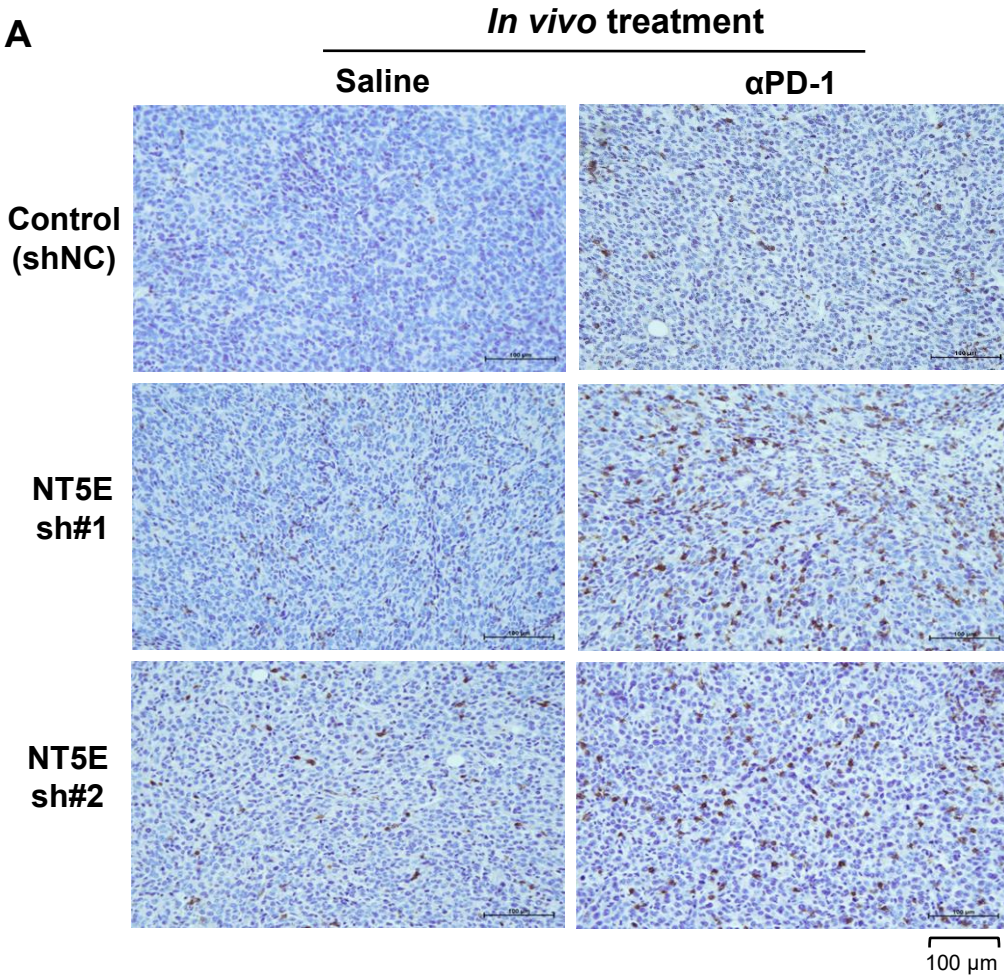


Figure S14. Correlation between ADA expression and immune cells enrichment in colorectal cancer tissues. **(A)** Bioinformatic analysis of The Cancer Genome Atlas (TCGA) colorectal cancer cohorts (TCGA-COAD and TCGA-READ, dbGaP study accession: phs000178) showing the correlation between ADA expression and immune cell infiltration in colorectal cancer tumor tissues, estimated by Spearman's correlation analysis. **(B)** Comparison of T cell enrichment in CRC tumors with high or low ADA expression with high statistical significance (***, $p < 0.001$). **(C)** Bubble plot illustrating the correlation between ADA expression levels and immune cell subpopulations in CRC tissues. The plot shows the p values of the correlation coefficients for the indicated immune cell subtypes.

Supplementary Figure S15

A



B

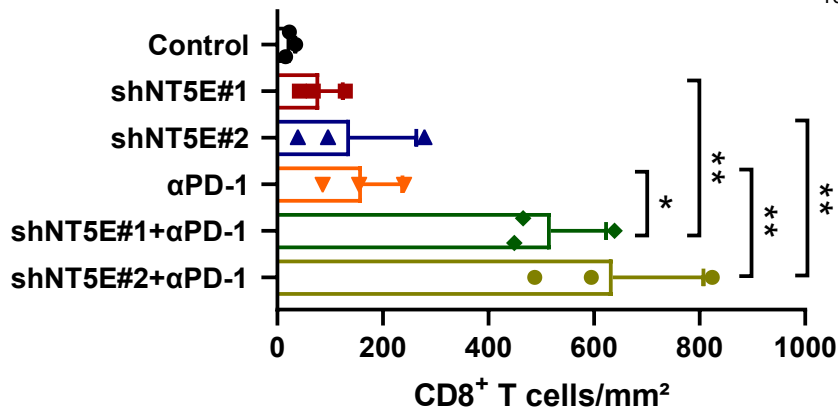


Figure S15. Effect of NT5E expression and PD-1 antibody on CD8⁺ T cell infiltration in tumor tissues. (A) BALB/c mice were inoculated with CT26 cells transfected with control shRNA (CN) or shRNA against NT5E (#1 and #2). Each group of the tumor-bearing mice were divided into two subgroups for treatment with saline or PD-1 antibody (α PD-1). Tumor tissues were isolated at the end of the experiment, sliced, and stained for CD8⁺ T cells using IHC. (B) Quantitation of CD8⁺ T cells in tumor tissues from the indicated mouse groups. Data represent mean $\pm$ SD (n=3); *, p < 0.05; **, p < 0.01 (One-way ANOVA).

Supplementary Figure S16

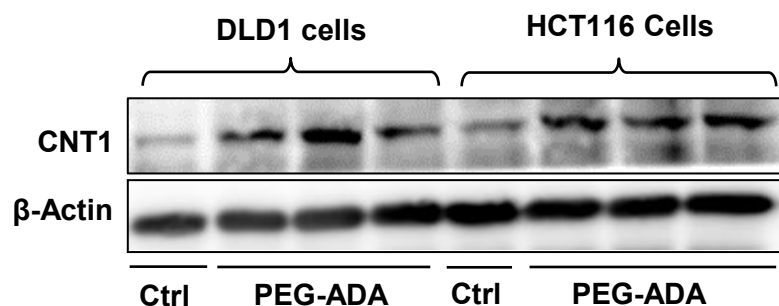


Figure S16. Effect of PEG-ADA on CNT1 expression in colon cancer cells. DLD1 and HCT116 cells were treated with PEG-ADA or solvent control (PBS) for 48 h. Cell extracts were prepared for Western blot analysis of CNT1 (concentrative nucleoside transporter 1) protein expression. β -actin was blotted as a loading control.

Supplementary Figure S17

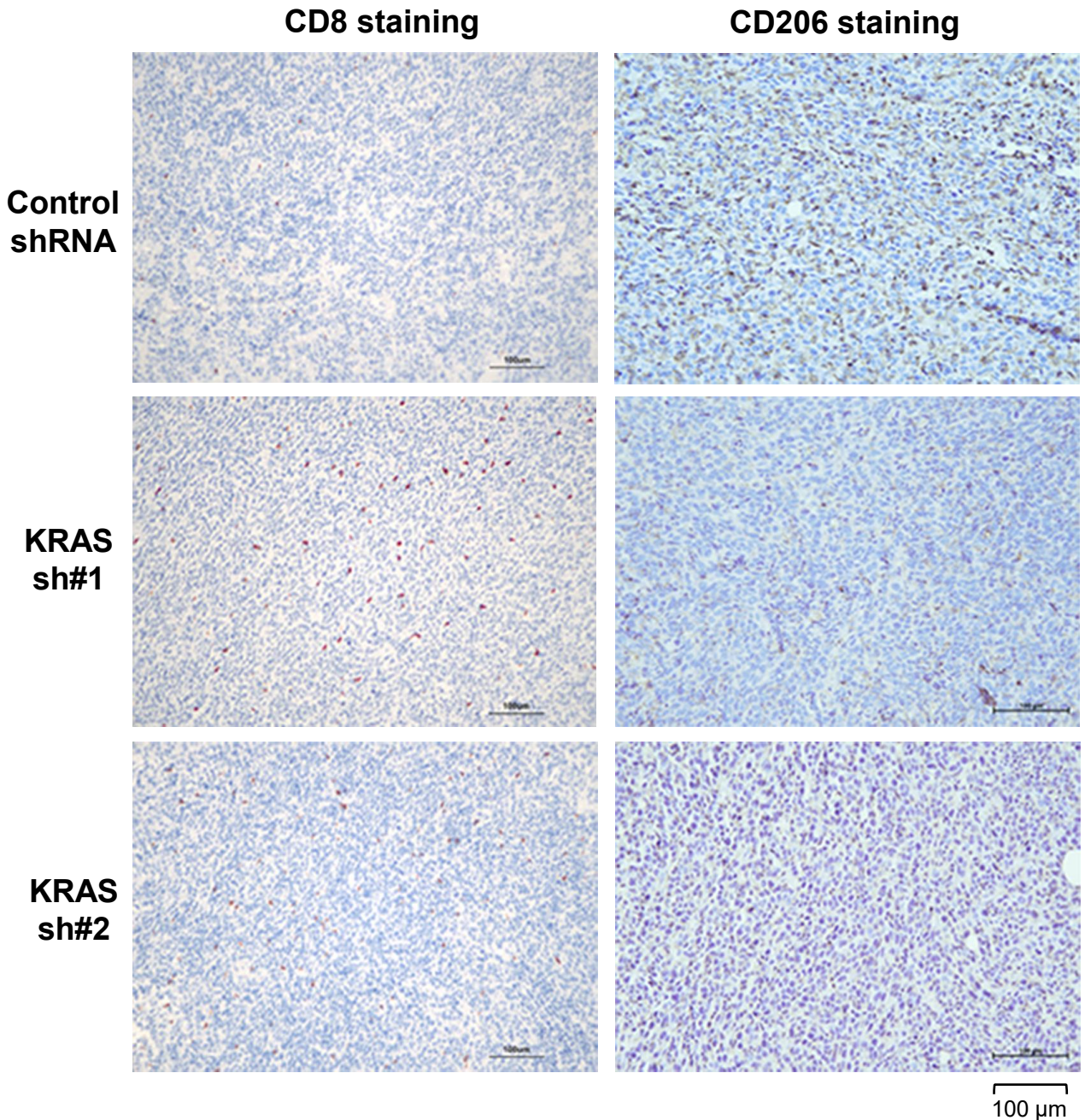
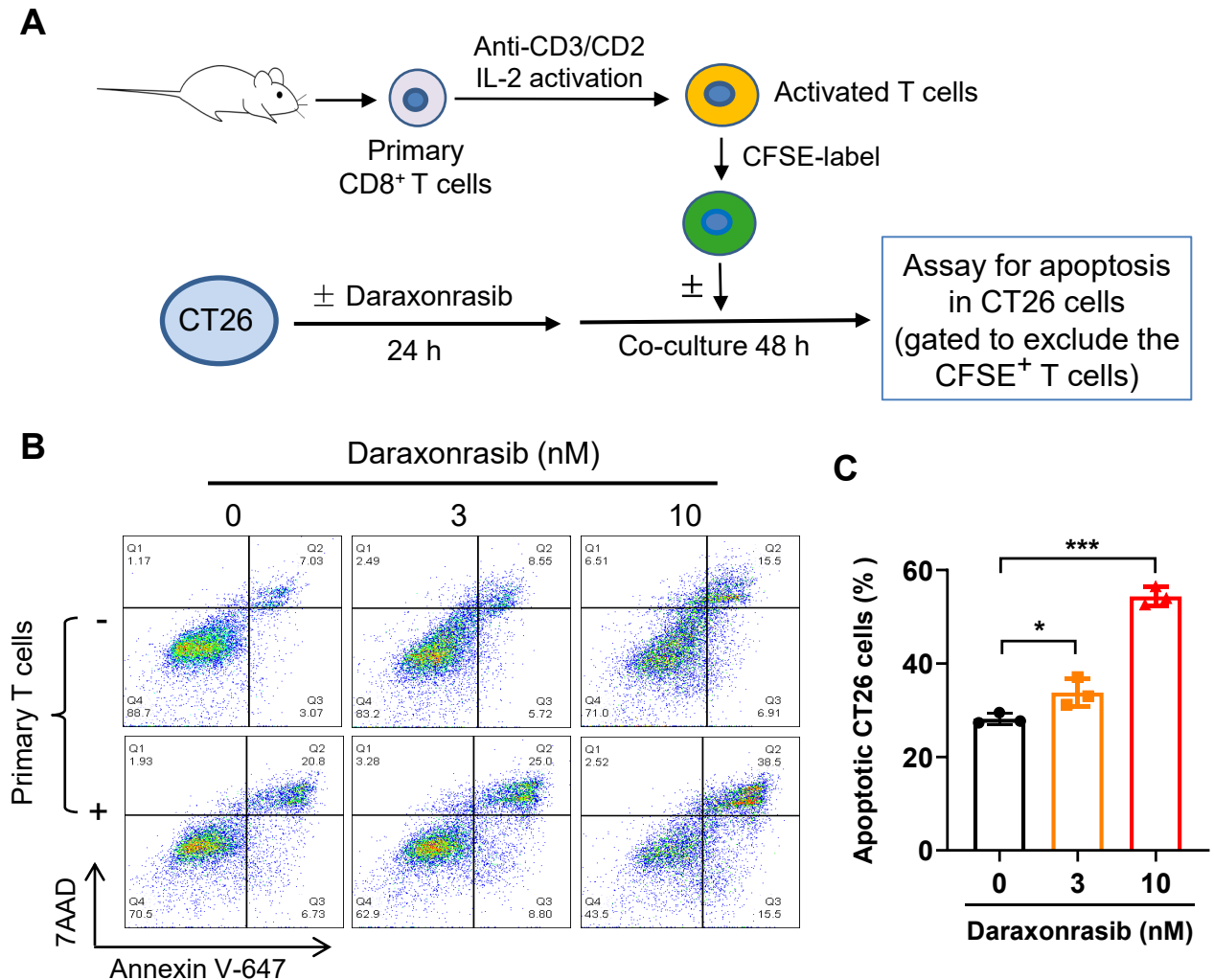


Figure S17. Effect of KRAS on the infiltration of immune cells in tumor tissues. BALB/c mice were inoculated with CT26 cells transfected with control shRNA or specific shRNA against KRAS as indicated. Tumor tissues were isolated at the end of the experiment, sliced, and stained for CD8⁺ T cells or CD206⁺ macrophages using immunohistochemistry staining. Representative images showing CD8⁺ T cells or CD206⁺ macrophages in tumor tissues are shown. Scale bar = 100 μm.

Supplementary Figure S18



FigS18. Effect of KRAS inhibitor daraxonrasib on cell viability of CT26 cells co-cultured with primary T cells. (A) Schematic illustration of the experimental workflow. **(B)** Representative plots of flow cytometry analysis of CT26 cell viability under the indicated conditions. Annexin V/7AAD double-staining was used to reveal apoptotic cells; CT26 cells were gated based on lack of CFSE green fluorescence. The upper panels depict CT26 cells treated with daraxonrasib in the absence of T cells; the lower panels show CT26 cells co-cultured with primary T cells and treated with daraxonrasib. **(C)** Quantitation CT26 cell death induced by daraxonrasib in co-culture with activated primary T cells.

Supplementary Figure S19

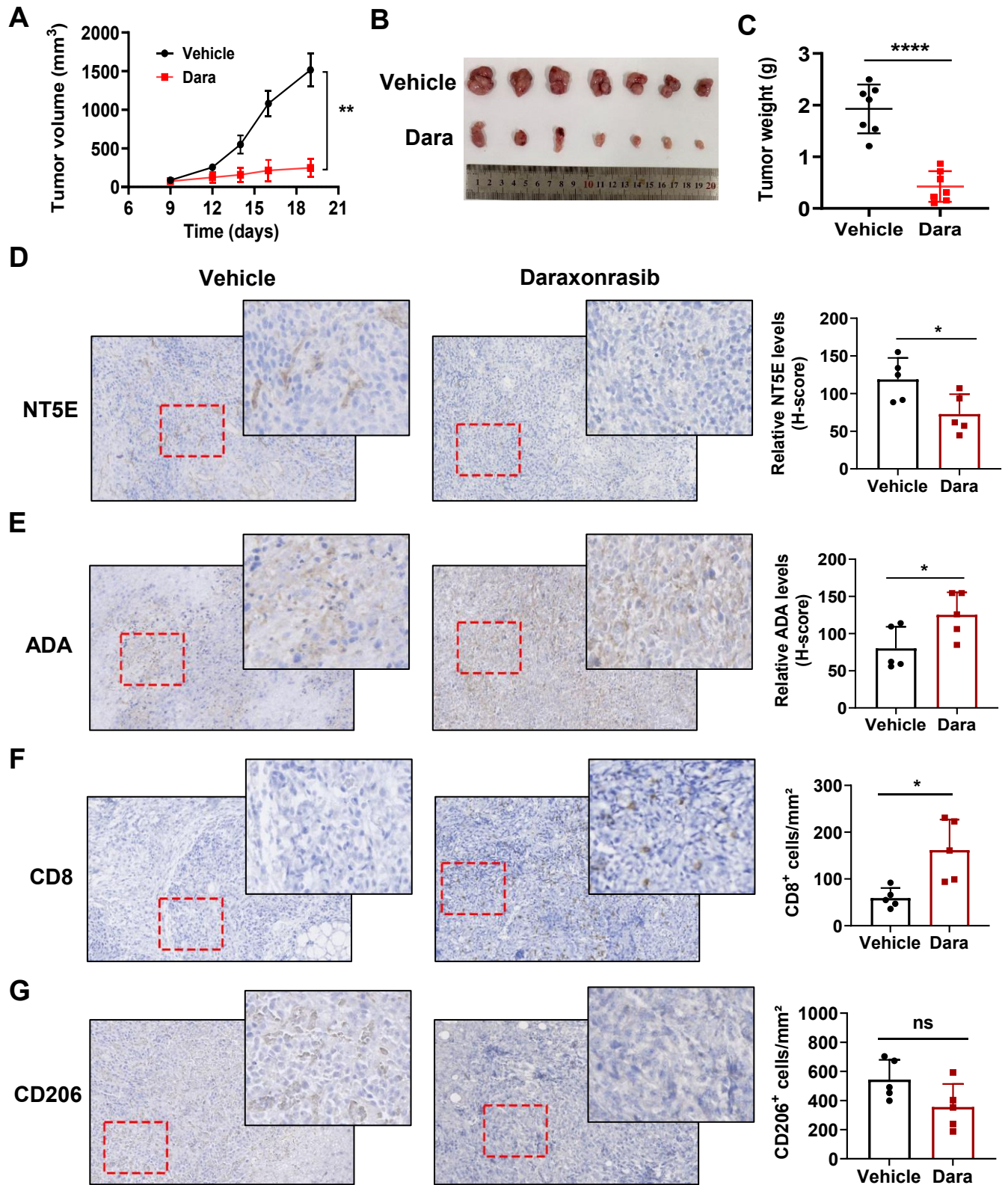


Figure S19. Impact of KRAS inhibitor daraxonrasib on tumor growth, ADA and NT5E expression, and infiltration of immune cells *in vivo*. (A) BALB/c mice bearing subcutaneous

CT26 xenografts were randomized into two groups (n=7 per group) and treated with daraxonrasib (Dara, 20 mg/kg) or vehicle by oral gavage every other day. Tumor volumes were measured at the indicated time points; **(B, C)** Tumor photographs and tumor weights at the experiment endpoint; **(D, E)** IHC staining of NT5E-expressing and ADA-expressing cells in tumor tissues isolated from the control (vehicle) and daraxonrasib-treated mice at experiment endpoint. Quantitative data are shown on the right panels; **(F)** IHC staining of CD8⁺ cells in tumor tissues isolated from the control (vehicle) and daraxonrasib-treated mice at experiment endpoint. Quantitative data are shown on the right panel; **(G)** IHC staining of CD206⁺ cells in tumor tissues isolated from the control (vehicle) and daraxonrasib-treated mice at experiment endpoint. Quantitative data are shown on the right panel. scale bar, 100 μ m; Data are presented as mean $\pm$ SD. *, p<0.05; **, p<0.01; ****, p<0.0001; ns, not significant; Statistical analysis: unpaired two-tailed Student's t-test.

Supplementary Figure S20

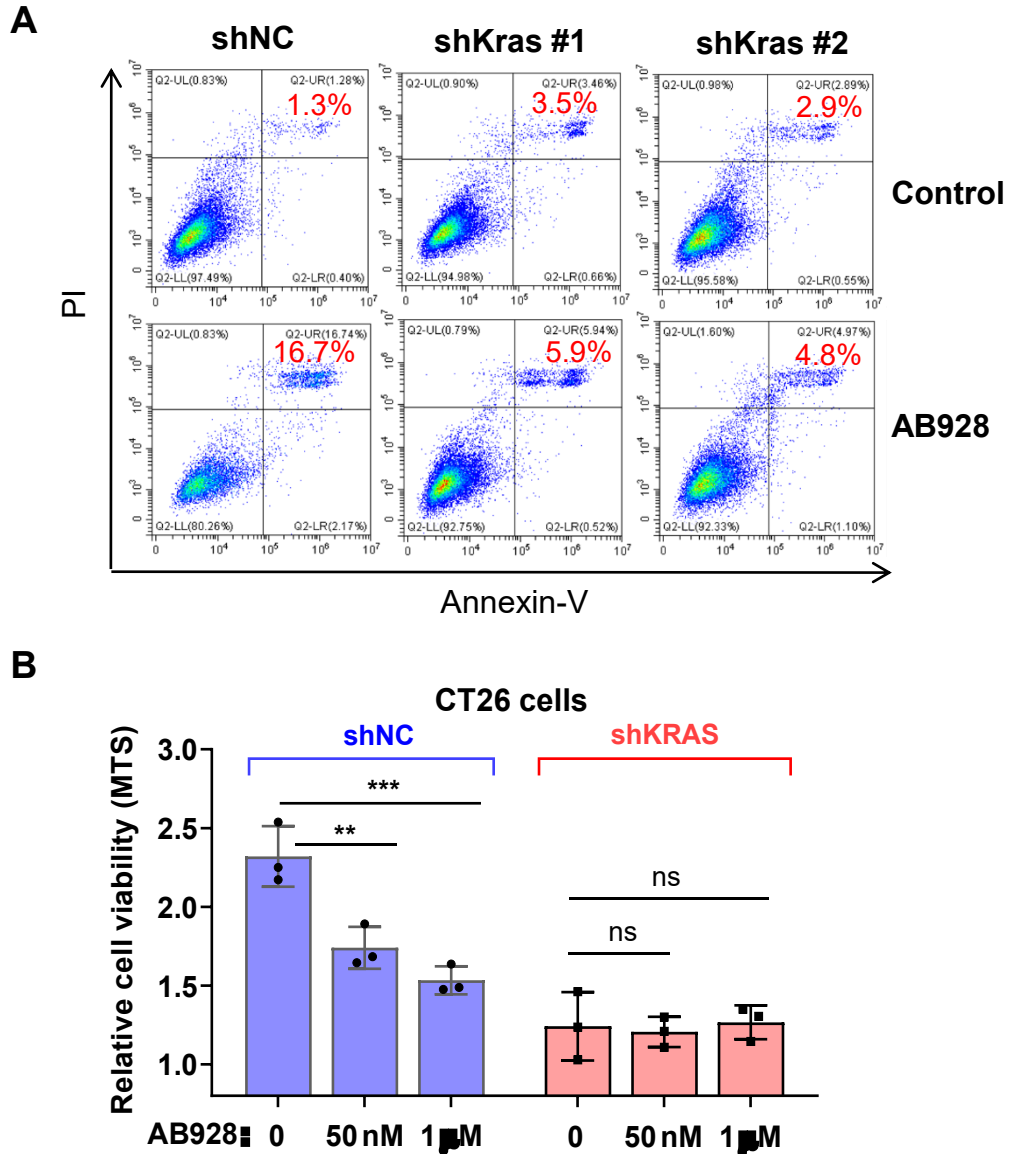


Figure S20. Effect of KRAS knockdown on cellular sensitivity to A2AR inhibition.

(A) CT26 cells with knockdown of Kras by shRNA (shKras#1 and shKras#2) or with control shRNA (NC) were treated with the A2AR inhibitor AB928 or with solvent control for 48 h. Apoptotic cells were stained using annexin-V/PI, followed by quantitation by flow cytometry analysis. **(B)** CT26 cells with or without KRAS knockdown were treated with the indicated concentrations of AB928, and cell viability was assessed by MTS assay. Data are presented as mean $\pm$ SD. Statistical significance was determined using one-way ANOVA test. **, $p < 0.01$; ***, $p < 0.001$.

Supplementary Figure S21

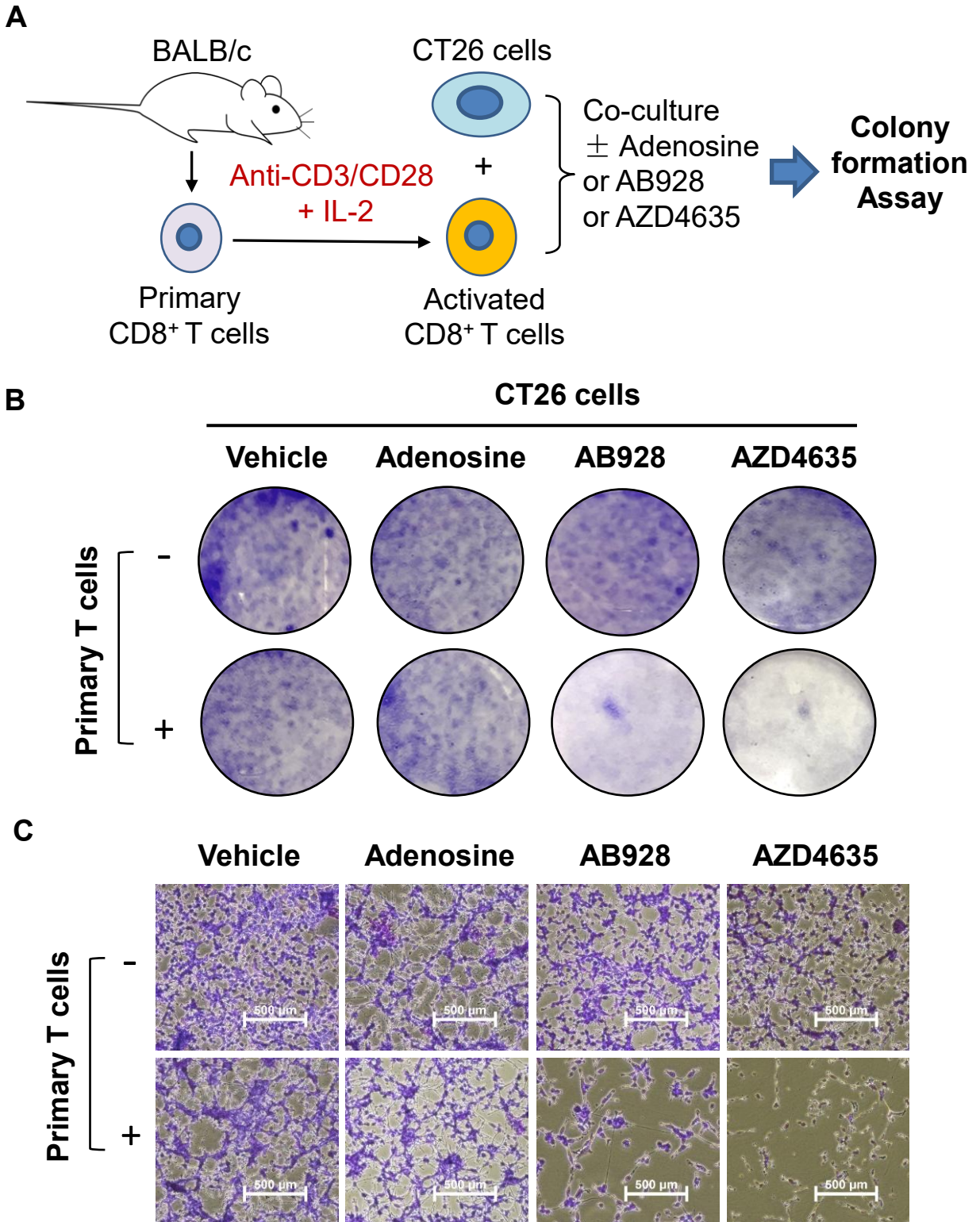


Figure S21. Effect of adenosine receptor antagonists on CT26 tumor cells in the presence or absence of T cells. (A) Schematic illustration of the co-culture system to test the effect of adenosine receptor antagonists on T cell-mediated cytotoxicity against tumor cell.

Primary T cells were isolated from the spleens of BALB/c mice, using the MojoSort™ mouse CD8 T cell isolation Kit. The purified CD8⁺ T cells were then activated with anti-CD3/CD28 + IL-2, followed by co-culture with CT26 tumor cells in the presence or absence of AB928 and AZD4635 as indicated. The adhered CT26 cells were allowed to form colonies for two weeks. **(B)** Representative images of CT26 colonies formed under the indicated conditions. Note that CT26 cells had a tendency to spread in culture and grew as cell clusters without typical colony shapes. **(C)** Representative microscopic images (10×) of CT26 cell clusters in colony formation assay from (B). Scale bar: 500 μm.