**SUPPLEMENTARY MATERIALS AND METHODS**

**Mice**

Female BALB/c and C57BL/6 mice (6 to 8 weeks of age) were purchased from Envigo, Frederick, MD. Animals were provided with standard laboratory chow and water ad libitum and allowed to acclimate for 7 days after arrival at the Lilly animal facilities (Indianapolis, IN or New York, NY). All experimental procedures were done in accordance with the guidelines of the NIH “Guide for Care and Use of Animals” and approved protocols were reviewed by the Institutional Animal Care and Use Committee at each site.

***In vivo* tumor studies**

BALB/c or C57BL/6 mice were implanted into the flank subcutaneously with 1 x 106 Colon26 or 0.5x106 MC38 cells purchased from DTP and NCI DCTD Tumor Repository, respectively. Mice were randomized post implantation as indicated into individual treatment groups (n=10-15 mice per group). Tumor volume was calculated using the formula: Tumor Volume (mm3) = π/6 \* Length \* Width2. Animals were sacrificed due to progressive disease if tumor burden was greater than 2,500mm3.Tumor volume compared to control (%T/C) was calculated as %T/C = 100 x T/C. Statistical comparisons were done with two-way repeated measures analysis of variance (ANOVA), and The Bliss Independence method is then applied to determine interaction effect, Kaplan-Meier analysis method was performed on the tumor growth data to obtain log-rank p-values for the comparison of groups for median survival. For combination analysis of Figure 5C, Combination analysis using Categorical Response Analysis Bayesian Ordinal Logistic Regression.

**Flow cytometry**

Single cell suspensions were stained with corresponding surface antigen Ab cocktails containing mixtures of indicated markers for both mouse and human (T cell panel: CD3, CD4, CD8, CD11b, CD45, PD-1, Tim3, CD137, GITR, CD25, Granzyme B, DX5; Myeloid Cell Panel: CD3, CD11b, CD19, CD45, CD11c, Ly6C, Ly6G, F480, MHC-I, PD-L1, MHC-II, CD86) and a fixable viability dye (Affymetrix, Thermofisher). Intracellular staining was done with Foxp3 Fix/Perm buffer (intracellular antibody staining cocktail (Ki67, FoxP3). Mitochondrial mass was determined by pre-staining samples using Mitotracker Deep Red (Thermofisher) prior to cell surface staining. 123 count beads were added to each well. Samples were collected on a 4-laser Fortessa X-20 cytometer (BD Biosciences) and analyzed with FlowJo V10 software (Flowjo, LLC, Ashland, OR). Absolute cell counts were calculated: Absolute counts(cells/mg)=(cell count\*50\*tumor volume)/(bead count\*150\*tumor weight)\*beads concentration.

**Isolation, expansion and treatment of primary human T cells *in vitro***

Primary human T cell were initially stimulated as described in Schaer and Beckman et al (*18*) briefly T cells were cultured with DMSO, pemetrexed (0.05 uM), or paclitaxel (0.2 uM), counted before being analyzed as described.

**Metabolic assessments of primary mouse T cells**

Mouse splenic T cells stimulated with CD3/CD28 were cultured in the presence of pemetrexed as indicated. Oxygen consumption rate (OCR) was analyzed using Seahorse XF Cell Mito Stress Test kit and Seahorse XFe96 instrument (Agilent, Santa Clara, CA). Cells were sequentially stimulated with oligomycin (1 M), FCCP (1.5 M), and rotenone/antimycin A (0.5 M each) and the spare respiratory capacity (SRC) was measured as the difference between basal OCR values and maximal OCR values obtained after FCCP uncoupling. To assess T cell ability to metabolize fatty acids, XF Palmitate:BSA FAO substrate (Agilent) was incorporated into XF Cell Mito Stress Test assay. Wave 2.4 software (Agilent) was used for data acquisition and analysis of Seahorse data.

**Metabolomic Analysis**

Liquid chromatography–mass spectrometry (LC-MS) was used for broad metabolomic analyses of both plasma and tumor tissue samples. Tumor tissue homogenates collected and analyzed described previously (*19*) on a SCIEX 5500 triple quadrupole mass spectrometer equipped with a Turbo V source in Electrospray mode, which was coupled to a NEXERA UPLC system (Shimadzu). Metabolite data was processed using MultiQuant software (Sciex). Raw signal intensities for each metabolite were normalized across all samples and then the intensity for each individual sample was calculated relative to the average value.

**Assessment of tumor antigen-specific T cells responses**

CD8+ T cells were isolated from spleens, tumor-draining lymph nodes (TDLNs) and tumors using CD8+ T cell isolation kits (Miltenyi cat# 130104075 and 130116478). Mouse IFN-gamma enzyme-linked immune absorbent spot (ELISpot) assay (Cellular Technology LTD) was performed with 1X105 CD8 cells in the presence or absence or of 2 x104 EMT6 or Colon26 tumor cells. For flow cytometry analysis, CD8+ T cells were cultured with or without cell stimulation cocktail (Ebioscience Cat#00497093) at 1,000X dilution in the presence of brefeldin A for 4 hours. After blocking, the cells were stained with live dead dye (Life Technologies), CD8 Ab (BD Biosciences) and H-2Ld MuLV gp70 Tetramer (MBL International). Intracellular staining were done with TNF-alpha Ab (BD Biosciences) after fixation and permeabilization.

**Tumor cell killing assay**

Splenocytes from ovalbumin-specific T cell receptor transgenic OT-1 mice were incubated in the presence of 0.1nM of SIINFEKL peptide and IL-2 for 5 days. CD8+ T cells were then isolated and cultured with B16 tumor cells that had been previously labeled with cell tracer BV421 and pulsed with 100 nM of SIINFEKL peptide for 2 hours, at a 10:1 effector to target ratio. Tumor cell death was then analyzed by 7AAD incorporation by flow cytometry after 4 hours of coculture.

***In vitro* assessment of immunogenic cell death (ICD)**

Colon26 and MC38 tumor cell lines were treated with chemotherapeutic agents (pemetrexed, carboplatin, paclitaxel, gemcitabine, doxorubicin) for 96 hours. At the end of treatment, culture supernatants were collected and high mobility group B1 (HMGB1) protein and calreticulin (CRT) release was measured using commercially available kits (IBL International, Hamburg, Germany and Cloud Clone Corporation, Katy, TX respectively). The viability of remaining cells was measured by Cell Titer-Glo assay (Promega) according to manufacturer’s protocol.

**QuantiGene® Plex gene expression analysis**

Samples were analyzed as described previously (*18*), Briefly, 500 ng of total RNA was isolated from snap-frozen tumor tissue and a custom oligonucleotide QuantiGene probeset designed by Affymetrix (Affymetrix, Santa Clara, CA) was used to detect immune activation. Magnetic capture beads, the probesets, lysis mixture were processed according to manufactures specifications. Beads were identified and level of Phycoerythrin was detected on the FLEXMAP 3D Luminex instrument (ThermoFisher, Waltham, MA). Relative gene expression was calculated by normalizing adjusted net MFI to the geometric mean of housekeeping genes (HKG) (adjusted net MFI/geometric mean HKG MFI). Fold change for each gene was then determined compared with the control group. Statistical analysis of the mean fold change for each gene of each treatment group analyzed was then compared with control using ANOVA tests to determine the significance of the difference between groups. Data visualizations were done using TIBCO Spotfire software (Spotfire).

**nCounter gene expression analysis and pathway analysis**

For high-content gene expression analysis, total RNA (50-100 ng) was used from collected samples in the nCounter® assay (nanoString® Technologies, Seattle, WA) using mouse-specific nCounter® PanCancer Immune Profiling and Myeloid/Innate Immunity codesets to analyze MC38 and Colon26 tumors and human specific nCounter® PanCancer Immune Profiling codeset to analyze pemetrexed-treated primary human T cells . Samples were measured on nanoString Digital Analyzer. The normalization and analysis of gene expression was performed with an internally-developed, automated workflow named ILAstring prior to ingenuity pathway analysis as describe previously (18). Planned contrasts were performed to identify differentially expressed genes (DEGs) between the treatment groups and untreated controls

**Quantification and statistical analysis**

Group wise statistical comparisons were performed as indicated in each figure using standard paired T tests, one-way ANOVA, or two-way ANOVA models with Tukey’s adjustment per time point, comparing treatment/dose and time point.