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☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.

Group sizes (N>5) were chosen to ensure proper statistical ANOVA analysis for efficacy studies.

2. Data exclusions

Describe any data exclusions.

Mice requiring veterinary care for any reason were excluded from the experiments.

3. Replication

Describe whether the experimental findings were reliably reproduced.

All experimental replications were successful, and are noted in the manuscript as 1 of x independent experiments.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

Mice were inoculated at the same time and then randomly assigned to a group for similar average tumor sizes at the beginning of the study.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

There was no blinding in most efficacy experiments. The antibody-blocking and immunological studies were blinded; in these, the measurements and experiments were taken on the basis of mouse ear-tag number, and the treatment group for each mouse was revealed after the analysis.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	FlowJo, OriginPro, and GraphPad Prism were used for data analysis.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	No unique materials were used.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	Anti-Cox2 (clone 33, BD), anti-H2AX provided by HCS DNA Damage Kit (Life Technology), anti-CD16/32 (clone 93), CD45 (30-F11), CD3e (145-2C11), CD4 (GK1.5), CD8 (53-6.7), Foxp3 (FJK-16s), CD11b (M1/70), Ly6C (HK1.4), Ly6G (RB6-8C5), F4/80 (BM8), B220 (RA3-6B2), CTLA4 (UC10-4B9), PD-1 (RMP1-30), Tim-3 (8B.2C12), eomes (Dan11mag), CD20 (AISB12) and PI staining solution (all from eBioscience and Biolegend unless otherwise noted).

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	Human head and neck cancer cells SQ20B, JSQ3, SCC61, and HNSCC135, murine breast cancer cell TUBO, and murine prostate cancer cell TRAMP-C2 were donated by Dr. Stephen J. Kron at University of Chicago. Human GBM cells U251 and U87MG and murine GBM cell GL261 were kindly provided by Dr. Maciej S. Lesniak at Northwestern University. Murine colon adenocarcinoma cell CT26 was purchased from the American Type Culture Collection (Rockville, MD, USA). Human colorectal cancer cell HT29, human ovarian cancer cell A2780cisR, human breast cancer cell MCF-7, and human pancreatic cancer cell BxPC-3 were purchased from Developmental Therapeutics Core at Northwestern University. SQ20B, JSQ3, SCC61, and HNSCC135 were cultured in DME/F12 medium (GE Healthcare, USA) supplemented with 20% FBS (Hyclone, USA). TUBO, CT26, and A2780cis were cultured in RPMI 1640 medium (GE Healthcare, USA) supplemented with 10% FBS. TRAMP-C2, U251, U87MG, GL261, MCF-7, and BxPC-3 were cultured in DMEM medium (GE Healthcare, USA), supplemented with 10% FBS. HT29 was cultured in McCoy 5A (Gibco, USA) supplemented with 10% FBS. Mycoplasma was tested before use by MycoAlert detection kit (Lonza Nottingham, Ltd.).
b. Describe the method of cell line authentication used.	None of the cell lines used have been authenticated.
c. Report whether the cell lines were tested for mycoplasma contamination.	The cells were tested by using the MycoAlert detection kit (Lonza Nottingham, Ltd).
d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.	No commonly misidentified cell lines were used.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.	Athymic male or female nude mice (6–8 weeks, 24–26 g) and BALB/c male or female mice (6–8 weeks, 20–22 g) were obtained from Harlan-Envigo Laboratories, Inc (USA). Rag2-/- BALB/c mice were obtained from Taconic Biosciences (USA).

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.	The study did not involve human research participants.

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Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

Data presentation

For all flow cytometry data, confirm that:

☒ 1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ 2. The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ 3. All plots are contour plots with outliers or pseudocolor plots.

☒ 4. A numerical value for number of cells or percentage (with statistics) is provided.

Methodological details

5. Describe the sample preparation.	Tumour-draining lymph nodes were harvested and ground by the rubber end of a syringe. Tumours were harvested, treated with 1 mg/mL collagenase I (Gibco™, USA) for 1 h, and ground by the rubber end of a syringe. Cells were filtered through nylon mesh filters and washed with PBS. The single-cell suspension was incubated with anti-CD16/32 (clone 93; 23 eBiosciences) to reduce nonspecific binding to FcRs. Cells were further stained with the following fluorochrome-conjugated antibodies: CD45 (30-F11), CD3e (145-2C11), CD4 (GK1.5), CD8 (53-6.7), Foxp3 (FJK-16s), CD11b (M1/70), Ly6C (HK1.4), Ly6G (RB6-8C5), F4/80 (BM8), B220 (RA3-6B2), CTLA4 (UC10-4B9), PD-1 (RMP1-30), Tim-3 (8B.2C12), eomes (Dan11mag), CD20 (AISB12) and PI staining solution (all from eBioscience and Biolegend). LSR FORTESSA cell analyzer (BD Biosciences) was used for flow cytometry, and data analysis was carried out using FlowJo software (TreeStar, Ashland, OR).
6. Identify the instrument used for data collection.	FACS LSR Fortessa with 4 lasers and 15 fluorescence detectors.
7. Describe the software used to collect and analyze the flow cytometry data.	BD FACSDiva and Flowjo were used to collect and analyze data.
8. Describe the abundance of the relevant cell populations within post-sort fractions.	Flow cytometry was used for quantification only. No post-sort fractions were collected.
9. Describe the gating strategy used.	Provided in Supplementary Fig. 43.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information. ☒