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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.

At least 5 animals of target gene knock out and control mice were used to adequately power biological validation experiments throughout the article. Statistical differences provide the rationale for sufficiency of the sample sizes.

2. Data exclusions

Describe any data exclusions.

None

3. Replication

Describe whether the experimental findings were reliably reproduced.

Experiments were repeated multiple times to ensure reproducibility of results and confirmed findings were reliably reproduced.

4. Randomization

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

No randomization was used as animals were genotyped prior to use.

5. Blinding

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

Since mice were genotyped before the experiments , no randomization was used. However, investigators injected tumor randomly and tumor-size was assessed randomly to avoid any bias as much as possible. We will indicate this in the method section in the revised version.

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 software (Tree Star) was used for flow cytometry analysis. Prism software (GraphPad) was used for statistic analysis of biological experiments. R software was used for RNA-seq, ATAC-seq, ChIP-seq, gene expression 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.	All unique materials used are readily available from the authors or from standard commercial sources.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	CD4 (RM4-5), CD8 (53-6.7), PD-1 (RMP1-30), Lag-3 (C9B7W), TIGIT (GIGD7), and Tim-3 (5D12), and Pdpn (8.1.1.) were obtained from BioLegend (San Diego, CA). Reproducibility is indicated in https://www.biolegend.com/reproducibility. Procr (eBio1560) and Fixable viability dye eF506 (eBioscience). Reproducibility is indicated in https://www.thermofisher.com/jp/ja/home/life-science/antibodies/invitrogen-antibody-validation.html. Antibodies of CyTOF is validated by comparing each molecule expression with Flow Cytometry.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	We obtained cell line; B16F10 from ATCC (CRL-6475), B16F10-Ova from Kai Wucherpfennig (Dana-Farber Cancer Institute, Boston, MA) and MC38-Ova from Mark Smyth (QIMR Berghofer, Queensland Institute of Medical Research, Brisbane Australia). This is indicated in method section.
b. Describe the method of cell line authentication used.	Morphology check by microscope and growth curve analysis were performed periodically.
c. Report whether the cell lines were tested for mycoplasma contamination.	All cell lines tested negative for mycoplasma contamination.
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.	None of the cell lines used are listed in the ICLAC database

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.	All mice used are C57BL/6 background, both male and female, 6-12 weeks of age, 15-25g. Each experiment was performed using age, sex matched controls.

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.	Yes, it is indicated in method section (page #43)
6. Identify the instrument used for data collection.	We used BD LSR2™ for FACS analysis and BD FACSAria™ for cell sorting
7. Describe the software used to collect and analyze the flow cytometry data.	We used FlowJo software (Tree Star)
8. Describe the abundance of the relevant cell populations within post-sort fractions.	The cell purity was confirmed as >95% by occationally re-running the obtained samples using BD FACSAria™
9. Describe the gating strategy used.	Obtained data were analyzed by FSC/SSC gates of starting cell population (a figure exemplifying the gating strategy is provided as a supplementary information). Positive gates were set based on fluorescence minus one (FMO) controls in each setting for cell surface molecules and based on unstimulated sample for ICC staining. This is standard for analysis of flow-cytometric data.

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