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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.

Sample sizes were determined to allow the statistical significance of differences of 50% or greater, and according to similar studies conducted in the field. The specific sample size required depended on the experiment.

2. Data exclusions

Describe any data exclusions.

No samples were excluded

3. Replication

Describe whether the experimental findings were reliably reproduced.

Results from each experiment were reproduced in the lab at least twice

4. Randomization

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

Animals were randomly sorted to various experimental or control groups.

5. Blinding

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

No formal blinding was used, but the various steps in key studies were performed by different investigators, who were each independent of the steps performed by the other investigator.

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.	Statistical analysis was performed using GraphPad Prism.

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 materials used are readily available from the authors or common commercial sources (provided in the manuscript).

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	List of antibodies used, including manufacturing source and clone, were provided in the manuscript.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	B16F10 - ATCC; TC-1 - Johns Hopkins University, CT26 - ATCC
b. Describe the method of cell line authentication used.	ATCC Cell lines were authenticated using STR profiling by ATCC, no further authentication was performed
c. Report whether the cell lines were tested for mycoplasma contamination.	TC-1 cells were tested free 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.	N/A

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.	C57BL/6J or Balb/c mice were obtained from Jackson Laboratory. Female, 6-10 weeks old at the start of experiment were used. All animal studies were performed in accordance with NIH guidelines, under approval of Harvard University's Institutional Animal Care and Use Committee.

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.	N/A

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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.	For scaffold samples, the scaffolds were processed through mechanical disruption and digested for 30 min. at 37 C in 250 U/ml Collagenase IV in RPMI. The resulting cell suspension was then filtered through a 40µm cell strainer to isolate the cells from the larger sized MSRs. The cells and small remaining MSR particles were pelleted and washed with cold PBS. For LN samples, they were processed through mechanical disruption and digested for 30 min. at 37 °C in RPMI containing 0.5mg/ml Collagenase 4 and 0.1 mg/ml DNAse. Cells were then filtered through a 40µm cell and washed in cold PBS. Tumor sample processing is detailed in the methods section. For all samples, cells were first stained with a dead exclusion dye, followed by antibodies against surface antigens. In some experiments, cells were subsequently fixed, permeablized and stained for intracellular antigens.
6. Identify the instrument used for data collection.	LSRFortessa, BD
7. Describe the software used to collect and analyze the flow cytometry data.	Flowjo 7.6 and Flowjo 10.4
8. Describe the abundance of the relevant cell populations within post-sort fractions.	No sorting was performed
9. Describe the gating strategy used.	In general, cells were first gated on FSC/SSC. Singlet cells were gated using FSC-H and FSC-A. Dead cells were then excluded and further surface and intracellular antigen gating was performed on the live cell population.

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