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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.

   Methods section, "Statistical analysis" and "ACT tumor immunotherapy". Sample sizes were based on previous experience of variability. In general, 5-10 mice per group is sufficient for in vivo experiments involving adoptive transfer.

2. Data exclusions

   Describe any data exclusions.

   Methods section, "Statistical analysis" and "ACT tumor immunotherapy". Criteria for tumor measurements were pre-established. Mice having >400 mm sq tumor size were euthanized for humane reasons. If a mouse dies due to other condition than tumor burden, it was excluded from the analysis.

3. Replication

   Describe whether the experimental findings were reliably reproduced.

   As reported in the figure legends, main text and Methods section, the findings were reliably reproduced.

4. Randomization

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

   Methods section, "Statistical analysis" and "ACT tumor immunotherapy". No randomization was deemed necessary since all animals received same treatment across comparison groups.

5. Blinding

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

   Methods section, "Statistical analysis" and "ACT tumor immunotherapy". Investigators transplanting tumors, performing treatments, and measuring and recording tumor measurements were blinded to the information regarding group allocations. (In-vivo relevance section)

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.	Prism (GraphPad), FlowJo version 7.5 (FlowJo), BowTie versions 1 and 2, TCGA2STAT package for R, R Studio, Ingenuity Pathway Analysis (QIAGEN)

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.	There are no restrictions on availability of unique materials.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	Methods section "ELISA, confocal imaging, western blots and flow cytometry" contain information regarding clones and catalog number for each antibody.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	Mel624.38, Mel1300, Mel938, Mel526, SK23 and 2245R are published (patient derived) cell-lines from Surgery Branch, NCI. A375 and B16 cells were obtained from the American Type Culture Collection. Murine melanoma B2905 cells were derived in Glenn Merlino lab (NCI).
b. Describe the method of cell line authentication used.	We have confirmed with the commercial and collaborative sources from where the cells were obtained that cell lines were authentic and free of cross-contamination.
c. Report whether the cell lines were tested for mycoplasma contamination.	All cell lines tested were 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 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.	Methods section "Mice". C57BL/6J were purchased from The Jackson Laboratory. Female mice aged 6-8 weeks were used for tumour implantation experiments.

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.	One melanoma patient (SB-4044) reported in this paper was enrolled under NCI-14-C-0022 clinical protocol in Surgery Branch, NCI. Specific details of this patient will be released to other investigators upon reasonable request.

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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.	Methods sections 'Two Cell-type (2CT) T cell and Tumour Cell Co-culture Assay' and 'Flow Cytometry and Immunoassays' contain experimental details of sample preparation.
6. Identify the instrument used for data collection.	BD FACS Canto II (#338960) and BD LSR-Fortessa Cell Analyzer (#647177)
7. Describe the software used to collect and analyze the flow cytometry data.	BD FACS Diva software was used to collect the data and FlowJo was used to analyze the data.
8. Describe the abundance of the relevant cell populations within post-sort fractions.	No cell sorting was performed.
9. Describe the gating strategy used.	Tumors and lymphocytes were first gated by FSC and SSC for size and granularity, followed by Live-dead gate. Tumors were gated by CD3e- population. T cells were gated by CD8+ population.

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