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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.

For flow cytometry-based phagocytosis assays, sample size was chosen to ensure a greater than 95% probability of identifying, by two-tailed t-test, an effect of >20% between treatment conditions, assuming a technical variation of 15%. Unless otherwise indicated, phagocytosis experiments were performed using eight biological replicates (i.e. independent macrophage donors). For in vivo experiments, sample size was chosen to ensure a greater than 95% probability of identifying, by two-tailed t-test, an effect between cell lines of >50%, assuming a technical variation of 50%.

2. Data exclusions

Describe any data exclusions.

In some cases, additional replicates of specific experimental conditions (e.g. DLD1 phagocytosis assay with human macrophages, PBS treated versus Hu5F9-G4-treated) were repeated many times as control, or as part of pilot experiments, or as confirmatory replicates, but only a discrete set of data (e.g. DLD1 phagocytosis assays with eight independent macrophage donors, performed under identical conditions across two distinct experimental instances) was specifically reported. For in vivo experiments, in some cases across additional experiments, including pilot experiments, additional mice were engrafted subcutaneously with relevant cell lines and followed for non-standard periods of time, or assessed for tumor growth at non-standard intervals, or injected with antibody adjuvant treatments, but only a discrete set of mice assessed under identical conditions was reported. Upon reviewer request, both subcutaneous and intravenous injection of B16-F10 cells followed by treatment with antibody TA99 were performed, but were ultimately not reported in the final manuscript.

3. Replication

Describe whether the experimental findings were reliably reproduced.

Unless otherwise indicated, all in vitro experiments were performed in multiple biological replicates, across multiple experimental instances. In the case of in vivo experiments, wherever practical multiple cohorts across multiple experimental instances were performed.

4. Randomization

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

Following engraftment and initial tumor measurement (when relevant), mice were randomized into treatment cohorts using the list randomization tools at random.org.

5. Blinding

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

All experiments, including in vivo experiments in mice, were performed in a non-blinded fashion.

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.	Flow cytometry data was analyzed using FACSDiva (BD) and FlowJo (Tree Star). Graphs and statistical analysis were generated using Prism (GraphPad). CRISPR deletion analysis was performed using the published TIDE software, as described in the references and methods.

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.	APL1 cells are not available from public sources, but are available from Irving Weissman upon request.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	Hu5F9-G4 is a clinical stage humanized anti-human CD47 antibody, and was validated as described in Liu et al. PLoS One (2015). Surface CD47 levels were assessed by antibody staining with clone B6H12 (BioLegend) at a dilution of 1:100; this antibody was validated by comparing flow cytometry staining of unmodified versus CD47 knock out cells. HLA-A/B/C was assessed by antibody staining with clone W6/32 (BioLegend) at a dilution of 1:50; this antibody was validated by comparing flow cytometry staining of unmodified versus B2M knockout cells. LILRB1 was assessed by antibody staining with clone GHI/75 (BioLegend) at a dilution of 1:25; this antibody was validated by comparing flow cytometry staining of non-myeloid versus myeloid cells, and normal macrophages versus macrophages electroporated with LILRB1-targeting CRISPR complexes. LILRB2 was assessed by antibody staining with clone 27D2 (BioLegend) at a dilution of 1:25; this antibody was validated by comparing flow cytometry staining of non-myeloid versus myeloid cells. All other antibody clones (e.g. immune subset markers, IgG controls) were used based on literature reports or data from manufacturer, and were not independently validated for the purposes of this study.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	All cell lines were obtained from ATCC, with the exception of APL1, which was derived from a primary patient tumor as described in Krampitz et al. PNAS (2016).
b. Describe the method of cell line authentication used.	Cell lines were not independently authenticated, beyond the identity provided from the supplier (e.g. ATCC).
c. Report whether the cell lines were tested for mycoplasma contamination.	Stocks of all cell lines were tested for mycoplasma contamination prior to submission. All were negative.
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 cell lines used in this study are in the database of commonly misidentified cell lines.

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.	Relevant information regarding animals and animal experiments are described throughout manuscript and methods.

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.	No human research subjects were utilized in this work.

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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.	Mouse tumor samples were excised and incubated with 10 mL RPMI supplemented with 10 µg/mL DNase I (Sigma Aldrich) and 25 µg/mL Liberase (Roche) for 10-15 minutes at 37°C. Tumors were homogenized by pipetting and filtering through a 100 µm nylon filter. Following the dissociation, 5 x 106 cells were pelleted and resuspended in FACS buffer and blocked with anti CD16/32 monoclonal antibody (Trustain fcX, Biolegend) for 10 minutes prior to staining. All tumors were stained with CD45 (Biolegend, Clone 30-F11), TCRβ (BD, Clone H57-97), NK1.1 (eBioscience, Clone PK136), CD11b (Biolegend, Clone M1/70), F4/80 (Biolegend, Clone BM8), Ly6C (BD, Clone AL-21), CD11c (Biolegened, Blone N418), and Gr-1 (Biolegend, Clone RB6-8C5). Dead cells were eliminated from analysis by excluding Hoechst+ cells. All samples were assayed using a FACSAria cell sorter (BD Biosciences), and channel compensations were performed using single-stained UltraComp eBeads (Affymetrix) or cells. Data was analyzed using FlowJo and outliers in each group were removed using Prism Outlier Calculator (http://graphpad.com/quickcalcs/Grubbs1.cfm).
6. Identify the instrument used for data collection.	Samples were analyzed on an LSR Fortessa (BD), or Aria II SORP unit (BD)
7. Describe the software used to collect and analyze the flow cytometry data.	Data was collected using FACSDiva (BD) and analyzed using FACSDiva (BD) or FlowJo (Tree Star). Graphing and statistical analysis were performed using Prism (GraphPad).
8. Describe the abundance of the relevant cell populations within post-sort fractions.	Not applicable.
9. Describe the gating strategy used.	Unless otherwise indicated, positive and negative gates were set using fluorescence minus one (FMO) controls, or isotype- and fluorophore-matched IgG controls. The gating strategy for analysis of tumor-infiltrating immune cells is detailed in Supplementary Figure 6e.

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