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Corresponding author(s): David J. Mooney

☐ 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.

The required samples size varied depending on the experiment and for in vivo experiments was determined in G*Power using an a priori analysis, set at significance level 0.05 and power 0.9.

2. Data exclusions

Describe any data exclusions.

All exclusion criteria were pre-established. In the human antigen-specific T cell expansion studies, data obtained from donors in which the baseline frequency of CLG- or GLC-specific CD8+ T cells was below 0.01% in 10000 live single CD8+ events were excluded from analysis for the respective peptide (two incidences occurred). In the in vivo CD19 CAR T cell study, one animal was excluded from analysis as it were given a different dose of therapeutic CAR T cells.

3. Replication

Describe whether the experimental findings were reliably reproduced.

All experiments with the exception of the in vivo study were conducted at least two times and could be reliably reproduced.

4. Randomization

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

Animals were randomized and allocated to different experimental groups within each cage.

5. Blinding

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

During the experiments, the investigator administrating the treatment to the animals was blinded.

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.

Sample size was calculated using G*Power v3 (for in vivo experiments) and statistical testing was performed using GraphPad Prism v6.

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 in this study.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

Antibodies were purchased Biolegend, with the exception of Foxp3, which was purchased from eBioscience. Antibodies used in this study included:

anti-mouse CD3 (100243)
anti-mouse CD28 (102103)
anti-human CD3 (317319)
anti-human CD28 (302903)
anti-mouse CD3 (100227)
anti-mouse CD4 (100405)
anti-mouse CD8 (100711)
anti-human CD3 (344803)
anti-human CD4 (344611)
anti-human CD8 (344705)
anti-human CD62L (304827)
anti-human CCR7 (353213)
nti-mouse granzymeB (515405)
anti-mouse FoxP3 (12-5773)
anti-mouse PD-1 (135205)
anti-human LAG-3 (369303)
anti-human PD-1 (329919)
anti-human HLA-A2 (343307)
anti-human IFNg (506510)
anti-human TNFa (502906)

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

T2 cell line: ATCC
B3Z T cell reporter cell line: ATCC
B16-OVA: ATCC

b. Describe the method of cell line authentication used.

Cells lines were not authenticated.

c. Report whether the cell lines were tested for mycoplasma contamination.

The B16-OVA cell line tested negative for mycoplasma. The B3Z and T2 cell lines were not tested 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.

No such 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.

All animals were female and between 6 and 9 weeks old at the start of the experiment. Specific strains included: C57BL/6J, C57BL/6-Tg(TcraTcrb)1100Mjb/J (OT-I), BALB/c, and NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ mice (NSG)

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 participants were involved in this study.

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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 T cells were obtained from the spleen following mechanical disruption and T cell isolation (Miltenyi). Human PBMCs were obtained following a Ficoll-gradient separation. Human T cells or CD14-depleted PBMCs were obtained following an additional T cell isolation or CD14-depletion step (Miltenyi). Cells were washed and then stained directly with fluorophore-conjugated tetramers, antibodies, or live/dead exclusion stain.
6. Identify the instrument used for data collection.	BD LSR fortessa X-20 (5 laser)
7. Describe the software used to collect and analyze the flow cytometry data.	Treestar FlowJo v10
8. Describe the abundance of the relevant cell populations within post-sort fractions.	At least 10,000 relevant events were acquired for all FACS analysis. For the human antigen-specific T cell expansion experiments, donors in which the baseline frequency of CLG- or GLC-specific CD8+ T cells was below 0.01% in 10000 live single CD8+ events were excluded from further analyses for the respective peptide (two incidences occurred).
9. Describe the gating strategy used.	In general, cells were first gated by FSC-A/SSC-A to exclude debris, then FSC-A/FSC-H to exclude doublets, followed by live/dead staining (Thermo Fisher), and then followed by the specific antibody or tetramer stain (e.g., CD3, CD8, GLC tetramer, etc.). Boundaries between positively- and negatively- stained cells were defined based on fluorescence-minus-one (FMO) controls.

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