nature research

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Last updated by author(s): Jan 5, 2021

Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☒	☐	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☒	☐	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☒	☐	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☒	☐	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	FACS Diva Version 8.0.3; Nanosight NTA 3.3; LightCycler® 480 SW 1.5.1; MACSQuantify 2.6; ELISA Reader
Data analysis	FCS Express Version 6; FlowJo Version 10.0.8; GraphPad Prism 8; LightCycler® 480 SW 1.5.1

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

We have included a full data availability statement in our manuscript, including a doi to the data repository (figshare)
Figures 4 and 5 have associated raw data, which are added as "Source data" and have been deposited in figshare

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	For animal experiments we used sufficient numbers (usually n>5) to achieve statistical relevance.
Data exclusions	Mice were excluded from further experiments, if humanization levels are <40%
Replication	Representative data sets based on many biological replicas are shown.
Randomization	Not relevant, since all available mice were used for humanization.
Blinding	Blinding not relevant here, since the read-out for CAR T cell presence is quantified by FACS and qPCR which are independent from experimentor's influence.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	see Table 1 + 2
Validation	Antibodies as listed in Table 1 and 2 have been validated as described on the providers' websites. Additionally, absence of signals in antigen-negative control samples is routinously confirmed.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	see Materials in the manuscript
Authentication	Authentication certificates were obtained for the most important cell lines from the DSMZ (Germany).
Mycoplasma contamination	Cell lines are regularly checked for mycoplasma contaminations.
Commonly misidentified lines (See ICLAC register)	Contaminations were excluded by authentication.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	See manuscript.
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	NOD/SCIDgC-/- (NSG) mice were housed in the animal facility "Plateau de Biologie Expérimentale de la Souris (PBES)" (ENS de Lyon, Lyon, France). Experiments shown in Fig. 5 were performed in accordance with the European Union guidelines upon approval of the animal experimentation protocols by the local ethical and the French government (Authorization agreement number C2EA -15: CECCAPP, Lyon, France). Experiments shown in Fig. 6 were performed in accordance with the European Union guidelines upon approval of the animal experimentation protocols by the local ethical and the French government (Authorization agreement number C2EA-15: CECCAPP, Lyon, France).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ 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).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	see manuscript
Instrument	FACS Diva Version 8.0.3; Nanosight NTA 3.3; LightCycler® 480 SW 1.5.1; MACSQuantify 2.6; ELISA Reader
Software	FCS Express Version 6; FlowJo Verion 10.0.8; GraphPad Prism 8; LightCycler® 480 SW 1.5.1; Prism
Cell population abundance	see manuscript
Gating strategy	see manuscript

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