

Reporting Summary

Nature Portfolio 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 Portfolio 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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	Data were collected using the following technologies: Flow cytometry: Cytoflex LX Cell sorting: FACS Aria II Bulk and single cell RNA-sequencing: Illumina NovaSeq 6000 System
Data analysis	Data were analysed using the following softwares: Flow cytometry data: FlowJo 10.6.1 and GraphPad Prism 8.4.2 Bulk RNA-sequencing: RStudio (version 4.3.1), Salmon software (version 1.10), DESeq2, Enrich R, Bioplanet database Single cell RNA-sequencing: RStudio (version 4.3.1), Seurat library Bulk TCR analysis: MiXCR software (v3.0.3), RStudio (version 4.3.1), vegan (v2.6-2), ggplot2 (v3.3.6)

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 Portfolio [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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

We included a Data availability section in the manuscript:

All data are included in the Supplementary Information or available from the authors, as are unique reagents used in this Article. The raw numbers for charts and graphs are available in the Source Data file whenever possible. The raw sequencing data generated in this study have been deposited in the SRA database under the accession codes: PRJNA1248774 for the human transcriptomic data and PRJNA1250406 for the murine single-cell transcriptomic data. The processed TCR sequencing data have been deposited in Zenodo database under accession code 10.5281/zenodo.15183541.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender

Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used. Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.

Reporting on race, ethnicity, or other socially relevant groupings

In this study we used healthy blood donor samples obtained from the Etablissement Français du Sang (EFS in Paris) without any race or ethnicity grouping.

Population characteristics

Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."

Recruitment

Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.

Ethics oversight

For the use of human cells, leukapheresis' products were obtained from the "Etablissement Français du Sang", EFS, according to the agreement n°21/EFS/024 between the EFS and our institution. All written consents from healthy volunteers were obtained by the EFS.

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

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](https://www.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

The sample sizes were not determined using a specific statistical method. We followed the 3R rule (Replace, Reduce, and Refine) for our in vivo experiments, which resulted in a reduced number of mice for some figures. However, to assess the statistical significance of our data, we ensured that each group had at least three mice in the majority of experiments, and we repeated the experiments to pool the data.

Data exclusions

No data were excluded from the analyses.

Replication

Most of the experiments were performed at least twice. Some experiments were conducted only once, as we had at least three mice per group, or we assumed that a single experiment would provide the necessary information. This includes the transcriptomic analysis in bulk or single-cell analysis.

Randomization

Human cells were injected equally into male and female NSG mice, with groups being allocated based on the animal stocks. For murine Tregs, when the donor mice were female, the cells were injected into female recipient mice, and the same was done for male mice. However, randomization was performed based on the availability of mice from different animal stocks, ensuring balanced group allocations.

Blinding

The histological analysis was scored blindly by a pathologist.

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

Methods

n/a

Involved in the study

☐

☒

Antibodies

☐

☒

Eukaryotic cell lines

☒

☐

Palaeontology and archaeology

☐

☒

Animals and other organisms

☒

☐

Clinical data

☒

☐

Dual use research of concern

☒

☐

Plants

n/a

Involved in the study

☒

☐

ChIP-seq

☐

☒

Flow cytometry

☒

☐

MRI-based neuroimaging

Antibodies

Antibodies used

The list of antibodies is provided in Supplementary Table 1, which includes the target, fluorochrome, clone, dilution, brand, and catalog number for each antibody.

Validation

All antibodies were validated in PBMCs or splenocytes for the staining of murine cells. Most of the antibodies were titrated before use.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)

293T cells from ATCC (#CRL-11268)
Platinum-E (Plat-E) retroviral packaging cell line from Cell Biolabs (#RV-101)

Authentication

Cell line authentication was performed by the provider.

Mycoplasma contamination

The absence of mycoplasma contamination has been validated by the provider.

Commonly misidentified lines
(See [ICLAC](#) register)

No commonly misidentified cell lines have been used in the study.

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals

B6 Foxp3EGFP Thy1.1 (obtained from a crossing of B6.Cg-Foxp3tm1Kuch/J (#035864) and B6.PL-Thy1a/CyJ (#000406) both lines from Jackson Laboratory) were used as donor mice to collect murine Tregs.
B6.129(Cg)-Foxp3tm3(Hbegf/GFP)Ayr/J, or B6 DTR Thy1.2 (from Jackson Laboratory, #016958) were used as recipient mice for mouse Tregs, when using immunocompetent mice. Murine Tregs were also injected in the immunodeficient mouse line B6.Cg-Rag2tm1.1Cgn/J or RAG mice (Jackson Laboratory, #008448).
Human cells were injected in NOD.Cg-Prkdcscid IL2rgtm1Wjl/SzJ or NSG mice.

Wild animals

No wild animals have been used in this study.

Reporting on sex

Human cells were injected equally into male and female NSG mice, with groups being allocated based on the animal stocks. For murine cells, when the donor mice were female, the cells were injected into female recipient mice, and the same was done for male mice.

Field-collected samples

For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.

Ethics oversight

Ethical approval was given by the "Comité d'éthique pour l'expérimentation animale" Charles Darwin N°5, registered at the "Comité National de Réflexion Ethique sur l'Expérimentation animale", CEEAACD 005, under the agreement number 29481.

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

Plants

Seed stocks	Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.
Novel plant genotypes	Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.
Authentication	Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.

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	The sample preparation have been described in the Methods section
Instrument	Flow cytometry: Cytoflex LX Cell sorting: FACS Aria II
Software	FlowJo 10.6.1 and GraphPad Prism 8.4.2
Cell population abundance	Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.
Gating strategy	The key gating strategies have been indicated in the figures and gating strategies for cell sorting are provided in Supplementary Information.

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