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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 tagmentation-based whole-genome bisulfite sequencing, the sample size was estimated by the required coverage for each CpG for analysis. We determined an average of 10-15-fold coverage for each CpG per population. Three independent replicates per group were analyzed and an average of 20-fold coverage for each CpG per population was finally achieved.

2. Data exclusions

Describe any data exclusions.

We did not exclude data from the analysis.

3. Replication

Describe whether the experimental findings were reliably reproduced.

We reproduced RNA-sequencing data with real-time PCR and flow cytometry experiments and TWGBS data with amplicon sequencing experiments.

4. Randomization

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

We did not randomize groups. To prove that no samples were mixed up, we confirmed RNA and TWGBS data with real-time PCR, flow cytometry and amplicon sequencing.

5. Blinding

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

No blinding was performed in this study.

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.	We used Prism GraphPad to perform ANOVA and t test statistics. RNA-seq and TWGBS statistics were based on R scripts as described in the material and methods section.

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.	All materials and mice used in this study are commercially available.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	All antibodies are listed in the materials section. Antibodies were tested for specificity (e.g. specific staining on Treg cells) and staining patterns were validated, where applicable, with real-time PCR or other methods.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	We did not use cell lines in this study.
b. Describe the method of cell line authentication used.	NA
c. Report whether the cell lines were tested for mycoplasma contamination.	NA
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.	NA

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.	We used animal-derived skin tissue, fat tissue, liver tissue, and lymph node as well as spleen tissue. Detailed information about tissue extraction, tissue digestion and genetic background of animals is listed in the materials and methods section.

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.	This study does not involve human material.

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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.	Samples from tissues were digested as described in the materials and methods section. After red blood cell lysis, samples were stained for 30 minutes at 4°C in FCS-containing PBS. Intracellular staining or cytokine restimulation is described in detail in the materials and methods section. Samples were washed at least two times and filtered immediately before acquisition.
6. Identify the instrument used for data collection.	Samples were measured either on a BD LSR II, BD LSR Fortessa II, or BD FACS ARIA II flow cytometer with four or five laser configuration.
7. Describe the software used to collect and analyze the flow cytometry data.	Flow cytometry data were recorded using BD FACS DIVA software. FCS files were analyzed using FlowJo and, if applicable, samples were concatenated using FCSConcat software.
8. Describe the abundance of the relevant cell populations within post-sort fractions.	Post-sort purity control for samples subjected to RNA sequencing or TBWGS or amplicon-based sequencing are shown in Supplementary Figure 1. For all other experiments where no post-sort control is shown in main or supplementary figures, a stringent quality control was performed, but not shown.
9. Describe the gating strategy used.	The gating strategy to isolate Treg and Tconv cells from tissues is shown in Supplementary Figure 1. For samples subjected to amplicon-based sequencing, post-sort QC as exemplary gating strategy is shown in Supplementary Figures.

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