nature portfolio

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|----------------------------|--------------|
| Last updated by author(s): | Jan 5, 2024 |

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 <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

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| 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 |
| | \square 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. <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> |
| \boxtimes | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| X | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| X | 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 <u>statistics for biologists</u> contains articles on many of the points above. |
| _ | |

Software and code

Policy information about availability of computer code

Data collection

no software was used

Data analysis

FlowJo X

GraphPad Prism 9.4.0

All the a diversity indices in our samples were calculated with QIIME(Version 1.7.0) and displayed with R software(Version 2. 15.3) Sequences analysis were performed by Uparse software (Uparse v7.0. 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 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

All the 16S sequencing data generated in our study are publicly available on the Sequence Read Archive (SRA) of the National Center for Information (NCBI) with the

accession number PRJNA1033804. The metabolism data in this study was deposited in Metabolights with the accession number MTBLS8813, which is an open-access database repository for metabolomics data. All other data are available from the corresponding author.

| Research | involving | human | narticinant | s their data | or higher | gical material |
|-----------|------------|---------|-------------|----------------|-------------|----------------|
| NC3Carcii | IIIVOIVIII | Hullian | participant | o, tiicii aata | , OI DIOIOE | ,icai matemai |

n/a

Data exclusions

| Policy information about stomer and sexual orientation and | udies with <u>human participants or human data</u> . See also policy information about <u>sex, gender (identity/presentation),</u> race, ethnicity and racism. |
|---|---|
| Reporting on sex and gen | der Both male and female participants are involved in this study. |
| Reporting on race, ethnic other socially relevant groupings | The participants were selected from the First Affiliated Hospital of China Medical University, who are Han ethnicity. |
| Population characteristics | The inclusion criteria for patients with HT were as follows: age 18–65 years and the presence of euthyroidism (normal FT3, FT4, and TSH plasma levels, without hormone therapy). The exclusion criteria were as follows: age <18 years; use of iodine drugs within the previous 3 months; pregnancy; use of antibiotics; probiotics; or prebiotics within the previous 3 months; use of hormonal medication or Chinese herbal medicine; chronic diarrhea; and history of inflammatory diseaseno |
| Recruitment | We recruited 23 initially untreated patients with HT and 25 healthy individuals from the First Affiliated Hospital of China Medical University. The two groups were matched for age and sex. The participants were given time to think about the study, read over the informed content and askquestions. When the patients agreed to participate, the informed consent was signed. No biases were present during the recruiment process. |
| Ethics oversight | The study was approved by the Medical Ethics Committee of China Medical University (No. 2022-247). All the procedures were performed according to the relevant laws and guidelines. |
| Note that full information on th | ne 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. |
| X Life sciences | Behavioural & social sciences |
| | ent with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u> |
| | |
| _ife sciences | study design |
| All studies must disclose on | these points even when the disclosure is negative. |
| Sample size Samples | s sizes were determined based on preliminary experiments. |
| Data exclusions no data | were excluded |
| Replication All atter | mpts to replicate the results were successful |
| Randomization Animals | were randomly assigned for indicated treatments. |
| Blinding | ents were not blinded to the assignments. |
| | |
| Behavioural | & social sciences study design |
| All studies must disclose on | these points even when the disclosure is negative. |
| Study description | n/a |
| Research sample | n/a |
| Sampling strategy | n/a |
| Data collection | n/a |
| Timing | n/a |

| Non-participation | n/a |
|--|--|
| Randomization | n/a |
| Randomization | liva . |
| Ecological o | valutionary 2. anvironmental sciences study design |
| | volutionary & environmental sciences study design |
| | these points even when the disclosure is negative. |
| Study description | n/a |
| Research sample | n/a |
| Sampling strategy | n/a |
| Data collection | n/a |
| Timing and spatial scale | (n/a |
| Data exclusions | n/a |
| Reproducibility | n/a |
| Randomization | n/a |
| Blinding | n/a |
| Did the study involve field | d work? |
| Did the study involve her | d work? Yes No |
| ield work, collec | tion and transport |
| | |
| Field conditions | Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall). |
| Location | State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth). |
| Access & import/export | Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information). |
| Disturbance | Describe any disturbance caused by the study and how it was minimized. |
| Ve require information from a | er specific materials, systems and methods authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, evant 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 & experime | ental systems Methods |
| n/a Involved in the study Antibodies | n/a Involved in the study ChIP-seq |
| Eukaryotic cell lines | |
| Palaeontology and archaeology MRI-based neuroimaging | |
| Animals and other o | organisms . |
| Clinical data | |
| Dual use research o | t concern |
| | |
| Antibodies | |
| Antibodies used | Antobody used:CD4-FITC |

Supplier name: Invitrogen

Catalog number:11-0041082
Antibody used: anti-mouse CD25-APC
Supplier name: Invitrogen
Catalog number:17-0251-81
Antobody used:anti-mouse Foxp3-PE
Supplier name:Invitrogen
Catalog number:12-5773-82

Supplier name:Invitrogen
Catalog number:12-5773-82
Antibody used: IFN-y-APC
Supplier name: Invitrogen
Catalog number: 17-0251-81
Antibody used: IL-4-PE
Supplier name:Invitrogen
Catalog number:12-7041-82
Antibody used:IL-17- PerCP
Supplier name: Invitrogen
Catalog number:45-7177-82

Validation

anti-mouse CD4-FITC: This Antibody was verified by Relative expression to ensure that the antibody binds to the antigen stated. Sun et al. Respiratory mucosal vaccination of peptide-poloxamine-DNA nanoparticles provides complete protection against lethal SARS-CoV-2 challenge. Biomaterials. 2023 Jan

-292-121907

anti-mouse CD25-APC:This Antibody was verified by Cell treatment to ensure that the antibody binds to the antigen stated. Snehanshu et al. Intracellular Acetyl CoA Potentiates the Therapeutic Efficacy of Antitumor CD8+ T Cells. Cancers Res 2022 Jul 18;82(14)2640-2655

anti-mouse Foxp3-PE:This Antibody was verified by Relative expression to ensure that the antibody binds to the antigen stated. Kuniyuki et al. Itaconate ameliorates autoimmunity by modulating T cell imbalance via metabolic and epigenetic reprogramming. Nature Communications. 2023 Feb 27;14(1):984

IFN-γ-APC:This Antibody was verified by Cell treatment to ensure that the antibody binds to the antigen stated. Snehanshu et al. Intracellular Acetyl CoA Potentiates the Therapeutic Efficacy of Antitumor CD8+ T Cells. Cancer Res 2022.Jul 18;82(14) 2640-2655

IL-4-PE:Wang et al.Dendritic cell Piezo1 directs the differentiation of TH1 and Treg cells in cancer. Elife 2022 Aug 22:11:e79957

IL-17- PerCP: Jason et al. Transcription factor ROR α enforces stability of the Th17 cell effector program by binding to a Rorc cisregulatory element. Immunity 2022 Nov 8;55(11):2027-2043.e9

Eukaryotic cell lines

Cell line source(s)

Authentication

Policy information about cell lines and Sex and Gender in Research

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State the source of each cell line used and the sex of all primary cell lines and cells derived from human participants or vertebrate models.

Describe the authentication procedures for each cell line used OR declare that none of the cell lines used were authenticated.

Mycoplasma contamination

Confirm that all cell lines tested negative for mycoplasma contamination OR describe the results of the testing for mycoplasma contamination OR declare that the cell lines were not tested for mycoplasma contamination.

Commonly misidentified lines (See ICLAC register)

. Name any commonly misidentified cell lines used in the study and provide a rationale for their use.

Palaeontology and Archaeology

Specimen provenance

Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information). Permits should encompass collection and, where applicable, export.

Specimen deposition

Indicate where the specimens have been deposited to permit free access by other researchers.

Dating methods

If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.

Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight

Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.

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

Animals and other research organisms

Policy information about <u>studies involving animals</u>; <u>ARRIVE guidelines</u> recommended for reporting animal research, and <u>Sex and Gender in Research</u>

| Laboratory animals | Species: Mus musculus Strain: NOD.H-2h4, the spontaneously develop thyroiditis mouse model was purchased from Jackson Laboratory Inc.(Strain #:004447) sex: Male and Female Age: 5-6 weeks |
|-------------------------|--|
| Wild animals | This study not involved wild animals |
| Reporting on sex | both male and female |
| Field-collected samples | This study not involved samples collected from the field. |
| Ethics oversight | The animal experiments were approved by the Laboratory Animal Welfare and Ethical Review of China medical University (No. CMU2020186). |

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

Clinical data

Policy information about <u>clinical studies</u>

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration

Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.

Study protocol

Note where the full trial protocol can be accessed OR if not available, explain why.

Data collection

Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.

Outcomes

Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.

Dual use research of concern

Policy information about <u>dual use research of concern</u>

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

| No | Yes |
|----|----------------------------|
| | Public health |
| | National security |
| | Crops and/or livestock |
| | Ecosystems |
| | Any other significant area |

| Experiments of conce | | | | | and the second second | _ |
|----------------------|--------|-----|-----|----------|-----------------------|----|
| | \cap | CEI | con | \cap t | (neriments | ۲- |

| Doe | Does the work involve any of these experiments of concern: | | | |
|-----|---|--|--|--|
| No | Yes | | | |
| | Demonstrate how to render a vaccine ineffective | | | |
| | Confer resistance to therapeutically useful antibiotics or antiviral agents | | | |
| | Enhance the virulence of a pathogen or render a nonpathogen virulent | | | |
| | Increase transmissibility of a pathogen | | | |
| | Alter the host range of a pathogen | | | |
| | Enable evasion of diagnostic/detection modalities | | | |
| | Enable the weaponization of a biological agent or toxin | | | |
| | Any other potentially harmful combination of experiments and agents | | | |

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, mosiacism, off-target gene editing) were examined.

ChIP-seq

Data deposition

Confirm that both raw and final processed data have been deposited in a public database such as GEO. Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks. Data access links For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data. May remain private before publication. Provide a list of all files available in the database submission. Files in database submission Genome browser session Provide a link to an anonymized aenome browser session for "Initial submission" and "Revised version" documents only, to (e.g. UCSC)

enable peer review. Write "no longer applicable" for "Final submission" documents.

Methodology

Replicates Describe the experimental replicates, specifying number, type and replicate agreement. Sequencing depth Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end. **Antibodies** Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number Peak calling parameters Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment. Data quality Software Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.

Flow Cytometry

Plots

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|----|-------|------|------|
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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 For Th1/Th2/Th17/Treg cell staining, spleen mononuclear cells were obtained and incubated with cell activation cocktail

(00-4975-03, Invitrogen)at 37° C for 5 hours. Then the cells were incubated with Fc-block at room temperature for 10

minutes to avoid non-specific binding.

Instrument FACScan Flow Cytometry

Software FlowJo X software and GraphPad Prism were used to analyze flow cytometry data.

Cell population abundance 50000

Gating strategy The flow cytometer gating strategy was provided in supplementary fig. S8

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

Magnetic resonance imaging

Experimental design

Design type

Indicate task or resting state; event-related or block design.

Design specifications

Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.

Behavioral performance measures

State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).

Acquisition

Imaging type(s)

Specify: functional, structural, diffusion, perfusion.

Field strength

Specify in Tesla

Sequence & imaging parameters

Used

Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.

Area of acquisition

State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.

Diffusion MRI

─ Not used

Preprocessing

Preprocessing software

Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).

Normalization

If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.

Normalization template

Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.

Noise and artifact removal

Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).

| Volume censoring Defini | Define your software ana/or method and criteria for volume censoring, and state the extent of such censoring. | | | |
|--|---|--|--|--|
| Statistical modeling & inference | | | | |
| | Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation). | | | |
| | Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used. | | | |
| Specify type of analysis: Whole b | prain ROI-based Both | | | |
| Statistic type for inference Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods. | | | | |
| (See Eklund et al. 2016) | | | | |
| Correction | ibe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo). | | | |
| Models & analysis | | | | |
| n/a Involved in the study Functional and/or effective connuction Graph analysis Multivariate modeling or prediction | | | | |
| Functional and/or effective connectivi | Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information). | | | |
| Graph analysis | Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.). | | | |

Multivariate modeling and predictive analysis | Specify independent variables, features extraction and dimension reduction, model, training and evaluation

metrics.