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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 (*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	Leica AF6000LX and DMi8 Fluorescence microscopes were used for scanning immunostained slides. VisualSonic Vevo 2100 was used for echocardiography data collection and analysis. BD FACSAria™ Fusion flow cytometer (BD Biosciences), BD Influx™ cell sorter for FACS sorting of cells. BD Fortessa for flow cytometery analysis.
Data analysis	Statistics: GraphPad Prism software (v9) Histology analysis: ImageJ and Aperio ImageScope Viewer (Germany) Echocardiography analysis: VevoLab software (V3.1.0) Flow cytometery analysis: FlowJo v9 RNA sequencing analysis: Degust web tool (David R. Powell. Degust: interactive RNA-seq analysis, DOI: 10.5281/zenodo.3258932) Heatmap generation: pheatmap package (version 1.0.12) and RColorBrewer package (version 1.1-3)

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 data associated with this study are present in the main text or the supplementary materials. Source data are provided with this paper. All RNA sequencing data presented were generated for this study and have been deposited in NCBI's Gene Expression Omnibus (GEO) database under accession code GSE228871 for bulk RNA-seq of endogenous Tregs, GSE230173 for mini-bulk RNA-seq of macrophages from Treg-treated mice, GSE230177 for mini-bulk RNA-seq of recovered exogenous Tregs and GSE268828 for bulk RNA-seq of macrophages from Treg-depleted mice.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

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 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.*
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	*Identify the organization(s) that approved the study protocol.*

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

Life sciences study design

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

Sample size	No statistical methods were used to predetermine sample size. Estimates were made based on our previous experience, availability and feasibility required to obtain statistically significant results. This methodology was followed based on previously published research articles (e.g. Nature Communications DOI: s41467-018-04908-z), while complying with the 3Rs rule on reducing, replacing and refining the use of animals for scientific purpose. The simple size (n) for each experiment is provided in the figure legends.
Data exclusions	Data were not excluded from study reporting. Animals who suffered perioperative (same day) death and heart rupture were not included in study reporting.
Replication	Results shown are representative of several independently performed experiments. Each dot in the bar plots indicates a different animal.
Randomization	All studies were performed with randomization when possible. For all in vivo experiments, mice were preselected based on age and then randomly assigned to treatment groups.
Blinding	The surgeon was blinded to administration of the treatment group and electrocardiographic analysis of cardiac function. Blinding was not performed for all other experiments due to limited availability of researchers.

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
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used

Anti-mouse antibodies to detect lymphocytes:
1. PE/Cyanine7 anti-CD3 (BioLegend, Clone 17A2, 2.7 µg/ml)
2. APC anti-CD4 (BioLegend, Clone GK1.5, 2 µg/ml)
3. BV605 anti-CD4 (BioLegend, Clone GK1.5, 2 µg/ml)
4. BV421 anti-CD8 (BioLegend, Clone 53-6.7, 2 µg/ml)
5. PE anti-CD25 (BioLegend, Clone PC61, 2 µg/ml)
6. FITC anti-mouse Foxp3 (Tonbo Biosciences, Clone 3G3, 10 µg/ml)

Anti-mouse antibodies to detect myeloid cells:
1. BV711, PE and APC-Fire750 anti-CD11b (BioLegend, Clone M1/70, 2 µg/ml)
2. PE and BV711 anti-F4/80 (BioLegend, Clone BM8, 8 µg/ml)
3. PE/Cyanine7 anti-CD206 (BioLegend, Clone C068C2, 2 µg/ml)
4. BV421, BV711 and PerCP/Cyanine5.5 anti-Ly6G (BioLegend, Clone 1A8, 2 µg/ml)
5. FITC anti-Ly6C (BioLegend, Clone HK1.4, 6.7 µg/ml)
6. PerCP/Cyanine5.5 anti—Siglec-F (BioLegend, Clone S17007L, 2 µg/ml)
7. PE/Cyanine7 anti-CD115 (BioLegend, Clone AFS98, 2.7 µg/ml)
8. BV421 anti-CCR2 (BioLegend, Clone SA203G11, 2 µg/ml)

Immunostaining primary antibodies:
1. rabbit anti- mouse CD31 (Abcam, #ab124432, 2 µg/ml)
2. rabbit anti- mouse phospho-histone 3 (pH3) (Abcam, #ab32107, 1 µg/ml)
3. mouse anti- mouse cardiac troponin T (cTnT) (Thermo Fisher Scientific, #13-11, 5 µg/ml)
4. rat anti-mouse CD163 (Abcam, #ab289979, 2 µg/ml)
5. rabbit anti-mouse F4/80 (Abcam, #ab111101, 1 µg/ml)
6. rabbit anti-mouse CD206 (Abcam, #ab64693, 1 µg/ml)
7. rabbit anti-mouse LYVE1 (Abcam, #ab14917, 2 µg/ml)
8. rabbit anti-mouse SPARC (Abcam, #ab290636, 3 µg/ml)
9. rat anti-mouse F4/80 (Thermo, #14-4801-82, 5 µg/ml)

Immunostaining secondary antibodies:
1. Goat anti-rabbit Alexa Fluor-488 (Thermo Fisher Scientific, # A-11008, 2 µg/ml)
2. Goat anti-rabbit Alexa Fluor-594 (Thermo Fisher Scientific, # A-11012, 2 µg/ml)
3. Goat anti-mouse Alexa Fluor-647 (Thermo Fisher Scientific, # A-21235, 2 µg/ml)
4. anti-wheat germ agglutinin (WGA) antibody conjugated with FITC (Sigma-Aldrich, # L4895-2MG, 20 µg/ml)
5. Goat anti-rat Alexa Fluor-594 (Thermo Fisher Scientific, #A-11012, 2 µg/ml)
6. Goat anti-rat Alexa Fluor-488 (Thermo Fisher Scientific, #A-11006 2 µg/ml)

Antibody used for CCR2+ cell systemic depletion in vivo: anti-CCR2 monoclonal antibody, Mouse IgG2a, Fc Silent, clone MC21 (Absolute Antibody, Ab02444-2.3)

Validation

All flow cytometry conjugated primary antibodies from biolegend were validated by the manufacturer on C57BL/6 mouse bone marrow cells, C57BL/6 mouse splenocytes or BALB/c mouse peritoneal macrophages.
1. Rabbit anti-mouse CD31 (Abcam, #ab124432, 2 µg/ml) was validated independently by the manufacturer using formalin-fixed, paraffin-embedded ischemic and normal mouse hindlimb tissue stained for CD31 using ab124432 at 1/1000 dilution.
2. Rabbit anti-mouse phospho-histone 3 (pH3) (Abcam, #ab32107, 1 µg/ml) was validated independently by the manufacturer using HeLa (Human epithelial cell line from cervix adenocarcinoma) labeling Histone H3 (phospho S10 + T11) with ab32107 at a dilution of 1/500.
3. Mouse anti-mouse cardiac troponin T (cTnT) (Thermo Fisher Scientific, #13-11, 5 µg/ml) was validated independently by the manufacturer using frozen sections of mouse heart tissue.
4. Rat anti-mouse CD163 (Abcam, #ab289979, 2 µg/ml) was validated independently by the manufacturer using paraffin-embedded Rat spleen tissue labeling CD163 with ab289979 at 1/1000 (1.066 µg/ml).

5. Rabbit anti-mouse F4/80 (Abcam, #ab111101, 1 µg/ml) was validated independently by the manufacturer using formalin/PFA-fixed paraffin-embedded sections) analysis of Mouse liver tissue sections labeling F4/80 with ab111101 at 1/250 dilution (0.48 µg/ml).
6. Rabbit anti-mouse CD206 (Abcam, #ab64693, 1 µg/ml) was validated independently by the manufacturer using formalin fixed paraffin embedded human lung labelling mannose receptor with ab64693 at 0.1 µg/ml.
7. Rabbit anti-mouse LYVE1 (Abcam, #ab14917, 2 µg/ml) was validated independently by the manufacturer using formalin fixed paraffin embedded mouse tuberculosis infected lung samples at 1/100 dilution.
8. Rabbit anti-mouse SPARC (Abcam, #ab290636, 3 µg/ml) was validated independently by the manufacturer by immunoprecipitating from rat brain tissue lysate with ab290636 at 1/30 dilution (2 µg in 0.35 mg lysates), and western blot was performed on the immunoprecipitate using ab290636 at 1/1000 dilution.
9. Rat anti-mouse F4/80 (Thermo Fisher Scientific, #14-4801-82, 5 µg/ml) was validated independently by the manufacturer using formalin-fixed paraffin embedded mouse spleen at 10 µg/ml.

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	C57BL6/J were purchased from the Monash Animal Research Platform. Foxp3(DTR/GFP) were bred in house using the Jackson laboratory (strain 016958). Foxp3(IRES-mRFP) mice were bred in house from the Jackson laboratory (strain 008374). B6.129P2-Il10tm1Cgn/J (l10 knockout) mice were purchased from Jackson laboratory (strain 002251). All mice were housed in 12:12 light:dark light cycles at room temperatures ranging between 20°C and 24°C and humidities between 40 and 60%. All mice were housed in 12:12 light:dark light cycles at room temperatures ranging between 20°C and 24°C and humidities between 40 and 60%. Food and water were available ad libitum to the mice. Regular diet (BARASTOC) was provided. All animals displayed normal behaviour directly after recovery from anaesthesia. At any significant sign of distress or at the end of the experiments, animals were euthanized by cervical dislocation or CO2 asphyxiation.
Wild animals	No wild animals were used.
Reporting on sex	Male mice were used for surgeries in this study. Female mice would have provided further insight on the gender differences in response to the treatment. However, due to consistency and to ensure that the treatment variabilities are not due to gender differences we chose to utilize only male mice for surgery. For sorting of Tregs from spleen, both female and male mice were used.
Field-collected samples	No field-collected samples used.
Ethics oversight	Animal experiments were approved by the Monash Animal Research Platform ethics committee (15216, 38561).

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	Hearts were perfused with 5 L/min of HBSS for 5 min immediately after cervical dislocation of the animal. After perfusion, hearts were removed from the chest and chopped in 0.5 mm pieces and transferred in 5 ml of digestion solution (2 mg/ml collagenase II and 100 µg/ml DNase I in 5% HI FBS/HBSS). Digestion was performed at 37°C for 30 min for two serial digestions. Digestion was terminated by adding RPMI (for T cells) or DMEM/F10 (for Mo/MΦ) containing 10% heat-inactivated (HI) FBS and 10 mM EDTA and passed through a 70 µm filter. Samples were centrifuged for 10 min at 400 x g, and the pellets were resuspended in 5 ml 5% HI FBS and 5 mM EDTA in PBS (FACS buffer). T cells and myeloid cells in the heart were stained with anti-mouse antibodies for 30 min at 4°C in FACS buffer. For intracellular staining, cells are fixed with the eBioscience Fixation/Permeabilization diluent and concentrate (ThermoFisher, #00-5223-56, #00-5123-43), then stained with anti-mouse antibodies in 0.5% saponin for 30 min at 4°C. If intracellular staining was required, viability was measured by staining the cells with LIVE/DEAD™ Fixable Aqua Dead Cell Stain Kit, for 405 nm excitation (ThermoFisher, #L34957, 1:500) prior to surface staining. Otherwise, viability was detected with 4′, 6-diamidino-2-phenylindole dihydrochloride (DAPI) (BV421).
Instrument	Flow cytometery: BD Fortessa flow cytometery FACS sorting: BD FACSAria™ Fusion flow cytometer (BD Biosciences) and BD Influx™ cell sorter

Software	FlowJo Software v9 for analysis
Cell population abundance	For sorting of Tregs from spleen, the population is almost 100% pure and makes up 20% of the CD3+ CD4+ population in the spleen. For sorting of Tregs from the heart, the population is almost 100% pure as we exclude myeloid cells that are likely sticking to the T cells. This ensures we get a pure Treg population and it makes up 20% of the CD3+ CD4+ population. For sorting of macrophages (CD11b+ F4/80+) the population is almost 100% pure as we exclude the cells that will likely stick to macrophages like neutrophils and eosinophils.
Gating strategy	All the gating strategies are based on FMOs (Fluorescence Minus One): Spleen Tregs CD4+GFP+(or RFP+) (or CD25+) cells were gated from CD3+ CD4+, live cells, singlets and cells gate. Heart Tregs CD4+ GFP+ (or RFP+) cells were gated fron CD3+ CD4+ cells after exclusion of myeloid cells (CD11b+, F4/80+). Heart Macrophages (F4/80+ CD11b+) were gated by excluding Ly6G and Siglec-F populations from the live cells. Heart Neutrophils (CD11b+ Ly6G+) were gated from total live cells. Heart Monocytes (Ly6CHi+ CCR2+) were gated from CD11b+ cells. Heart pro-repair macrophages CD206+ were gated from CD11b+ F4/80+ cells. Heart CD8 T cells were gated from CD3+ T cells. Heart CD4 T cells were gated from CD3+ T cells.

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