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Last updated by author(s): Jul 2, 2026

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

All software for data collection are publicly or commercially available. We did not generate custom computer code in this manuscript.

Flow cytometry
- CytExpert Software for the CytoFLEX platform
- BD FACSDiva™ (BD Biosciences)

Spectral Cell Sorter with High-Speed Cell Imaging
BD FACSChorus™ Software (BD Biosciences)

10X RNA sequencing
- NovaSeq Control Software v1.8

Droplet-digital PCR
QuantaSoft (Biorad)

Histological image acquisition
- BZ-X800 software (Keyence https://www.keyence.com/landing/microscope/lp_fluorescence.jsp)

Confocal image acquisition
-The ZEISS LSM 800 utilizes ZEN imaging software

Bulk RNA sequencing:
-- NovaSeq Control Software v1.8

Behavioural testing
- Gemini software
- Ethovision XT 17.5 (Noldus information technology tracking system)
- Activity Monitor Software-811 (Med associates Inc)

Echocardiography
-The FUJIFILM VisualSonics Vevo 2100 ultrasound system uses Vevo 2100 Imaging System Software

Seahorse XF Analyzers
-Seahorse Wave Pro Software

Western Blot
-Image Studio™ Software

Data analysis

- FlowJo 10.8.2 (FlowJo, LLC) was used for analysis of flow cytometry data
- Prism version 11 (GraphPad software) for graphing and statistical analysis
- Microsoft Excel 16.75.2

Analysis of single cell RNA-sequencing data
- CellRanger software from 10x Genomics (https://support.10xgenomics.com/single-cell-gene-expression/software/overview/welcome)
- Seurat package (v5.1)
- Seurat package (v5.1)
- MAST (differential expression, two-sided)
- EnhancedVolcano (volcano plots)
- Metascape (enrichment network analysis)
- msigdbr (v7.5.1) package (v4.0)

Analysis of Bulk RNA0seq
Quality Control and Read Alignment
FastQC (v0.11.9): Used for sequencing data quality control.
fastq-mcf (v1.05) & cutadapt (v4.7): Trimmed low-quality reads and removed adapter sequences.
Bowtie2 (v2.5.3): Filtered out unwanted sequences such as mitochondrial, ribosomal, and transfer RNA sequences.
STAR (v2.7.11b): Aligned paired-end reads to the Ensembl Mus musculus genome (GRCm39).
HTSeq (v2.0.5): Estimated raw read counts from aligned reads.
Cufflinks (v2.2.1): Calculated FPKM (Fragments Per Kilobase per Million) expression values.

Data Analysis and Visualization
R (v4.1.5): Used for statistical analysis and visualization.
prcomp function: Performed Principal Component Analysis (PCA) on FPKM values.
pheatmap (v1.0.12): Generated hierarchical clustering and heatmaps.
Differential Expression and Pathway Analysis
DESeq2 (v1.40.2): Conducted differential gene expression analysis between experimental groups.
FGSEA (v1.26): Performed Gene Set Enrichment Analysis (GSEA) using biological process gene sets from MSigDB (v7.5.1).

Electrocardiography:
-Vevo LAB Software

Seahorse assays:
-Seahorse Wave Pro Software

Spectral Cell Sorter with High-Speed Cell Imaging
-"BD FACSChorus™ Software
(incorporating BD SpectralFX™ Technology and BD CellView™ Image Technology)"

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

The single-cell RNA-sequencing and bulk RNA-sequencing datasets generated and analysed in this study have been deposited in the NCBI Gene Expression Omnibus (GEO) under the SuperSeries accession GSE288476 (single-cell RNA-seq, GSE288475; bulk RNA-seq, GSE288474) and will be made publicly available on publication. Source data are provided with this paper. Any additional data supporting the findings are available from the corresponding author on request.

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	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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 method was used to predetermine sample size. Sample sizes were based on our prior experience with this microglia replacement model and on cohort sizes commonly used in comparable Friedreich's ataxia mouse studies, chosen to detect the large effect sizes observed while minimizing animal use. For single-cell RNA-seq, cells were pooled from three mice per condition to obtain ~100,000 viable sorted cells per population. In vitro assays were performed with at least three independent biological replicates
Data exclusions	No data were excluded from the analyses, other than low-quality sequencing reads and non-viable cells removed during standard RNA-sequencing quality control and flow-cytometry gating, as described in the Methods.
Replication	For in vivo experiments, n denotes individual mice (biological replicates) as specified in each figure legend; each value derives from a distinct animal. Representative images and immunoblots are shown for experiments performed on the number of mice indicated in the legends, all with similar results. Bulk RNA-seq used three mice per group; single-cell RNA-seq was performed once on cells pooled from three mice per condition. In vitro assays (co-culture, Seahorse, ddPCR) were performed in at least three independent experiments. All replication attempts were successful.
Randomization	Animals were assigned to experimental groups on the basis of genotype and donor/treatment condition, which cannot be randomised; within genotypes, age- and sex-matched littermates were allocated across conditions. All animals in each group were measured, without selection.
Blinding	Behavioural testing and analysis were performed by an experimenter blinded to experimental conditions. Full blinding was not possible for all in vivo measurements because affected FA mice are overtly distinguishable from unaffected mice by their evident disease phenotype (e.g., reduced body weight and impaired condition), and group allocation was defined by genotype and fluorescent-reporter status inherent to the experimental design. To minimise bias, imaging and molecular data were quantified using identical, objective, and where possible automated pipelines applied uniformly across all groups.

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
☒	☐ Plants

Methods

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

Antibodies

Antibodies used

Antigen Conjugation Clone RRID Company name Catalog number Dilution Lot
BD Fc BloBlock™ unconjugated 2.4G2 AB_394656 BD Biosciences BDB553142 100 296888
CD45.2 BV650 104 AB_11203374 Biolegend 109836 100 B381041
Ly-6C (Gr-1) PE-Cy7 RB6-8C5 AB_313381 Biolegend 108416 100 B379114
CD11b PE-Cy7 M1/70 AB_312799 BioLegend 101216 100 B360932
TER-119 AF700 TER-119 AB_528963 Biolegend 116220 200 B372502
CD4 APC RM4-5 AB_312719 Biolegend 100516 100 B356935
CD8a APC 53-6.7 AB_312751 Biolegend 100712 100 B365419
CD19 APC/Cy7 6D5 AB_830707 BioLegend 115530 100 B356278

CD45 PE-Cy7 30-F11 AB_312979 BioLegend 103114 100 B362196
CD11b BV650 M1/70 AB_11125575 BioLegend 101239 100 B382474
TER-119 AF700 TER-119 AB_528963 BioLegend 116220 200 B372502
CD90.2 BV421 REA969 AB_2632888 BioLegend 105341 100 B366801
ACSA-2 APC 2B8 AB_2727362 Miltenyi Biotec 130-116-142 100 1323031258
O4 PE O4 AB_2733887 Miltenyi Biotec 130-117-357 100 1323031248

CD11b APC M1/70 AB_312795 BioLegend 101212 100 B352669
CD90 APC 5E 10 AB_893440 BioLegend 328113 100 B364585
CD45R (B220) APC-Cy7 RA3-6B2 AB_313047 BioLegend 103224 100 B357799

Synaptophysin unconjugated D8F6H AB_2799098 Cell Signaling Technology 36406S 1000 3
PSD95 unconjugated K28/43 AB_11211891 EMD Millipore MABN68 1000 4201125
GAPDH unconjugated Polyclonal AB_2107310 Thermo Fisher Scientific PA1-988 3000 XE350046
"OXPHOS
Complexes I–V" unconjugated Cocktail of monoclonal antibodies AB_2629281 Abcam ab110413 1000 2101063088

Synaptophysin unconjugated D8F6H AB_2799098 Cell Signaling Technology 36406S 200 3
"Phospho-DRP1
(Ser616)" unconjugated Polyclonal AB_2304689 Cell Signaling Technology 3455S 200 7
IBA1 unconjugated Polyclonal AB_8395040 "FUJIFILM Wako Pure
Chemical Corporation" 19-19741 200 SEM1769
F4/80 unconjugated BM8.1 AB_2938669 Cell Signaling Technology 71299S 200 1
CD68 unconjugated FA-11 AB_2572857 Thermo Fisher Scientific 14-0681-82 200 2626320

Validation

Antibodies used for flow cytometry: All antibodies used herein were validated using several types of positive and negative controls. Positive controls included mouse peripheral blood mononuclear cells (PBMCs) and brain single-cell suspensions. Negative controls included fluorescence-minus-one (FMO) controls, unstained samples, and cells from a species for which the antibody is not expected to show cross-reactivity. Furthermore, all antibodies used here for flow cytometry have been previously reported and are in routine use. All vendors (BioLegend, BD Biosciences, and eBioscience/Invitrogen/Thermo Fisher Scientific) report quality-control measures to ensure that antibodies sold are valid and reproducible; details of each manufacturer's validation procedures are available at https://www.biolegend.com/de-de/quality-control and https://www.thermofisher.com/us/en/home/life-science/antibodies/invitrogen-antibody-validation.html.

Antibodies used for histology: Negative-control slides from each tissue, stained with secondary antibodies only (no primary antibody), were processed in parallel with fully stained slides (primary plus secondary antibodies) to identify and subtract non-specific fluorescence signal. Each primary antibody was additionally validated by the manufacturer for the relevant species and application, as indicated by the RRIDs and catalogue numbers listed in the antibody table above.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

Human Friedreich's ataxia (FA) dermal fibroblasts were obtained from the Coriell Institute for Medical Research (Camden, NJ, USA; catalogue number [GM04078). Mouse FA fibroblasts were isolated in-house from ear-pinna skin of YG8-800 mice, and

	wild-type mouse fibroblasts from C57BL/6J mice (6–8 weeks old). Primary bone-marrow-derived macrophages were isolated in-house from GFP/mKate2 double-positive (DP) mice (10 weeks old).
Authentication	The human FA fibroblasts were obtained directly from the Coriell Institute, a certified biorepository, and were used at low passage; authentication by checking for the expanded allele using PCR. The mouse fibroblasts and bone-marrow-derived macrophages were primary cells freshly isolated from mice of defined genotype (confirmed by genotyping and by GFP/mKate2 fluorescence, as applicable) and were not authenticated by demonstrating presence of human transgenic BAC using ddPCR.
Mycoplasma contamination	All cultures were short term and not tested for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this 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	Species: Mus musculus. All strains are on a C57BL/6 background and were obtained from The Jackson Laboratory, except the double-positive donor line, which was generated in-house. C57BL/6J (wild-type; JAX #000664) — adult (8–10 weeks old); used as wild-type controls and for Busulfan conditioning (25 mg/kg/day for 4 days, total 100 mg/kg) prior to transplantation. YG8-800 Friedreich's ataxia model, Fxn^em2.1Lutzy Tg(FXN)YG8Pook/800J (JAX #030395) — used in conditioning, bone-marrow transplantation, behavioural, cardiac, histological and RNA-sequencing experiments. Double-positive (DP) transgenic donors (C57BL/6 background) — generated in-house by crossing C57BL/6-Tg(CAG-EGFP) (JAX #006567) with C57BL/6-Tg(CAG-mito:mKate) (JAX #032188); used as GFP/mKate2 bone-marrow donors for transplantation studies and as the source of primary bone-marrow-derived macrophages. Ages by experiment: conditioning/recipients, 8–10 weeks; bone-marrow donors, 8–12 weeks; primary-macrophage donors, 10 weeks; ear-skin fibroblast donors, 6–8 weeks; echocardiography and behavioral testing, 29 weeks; survival and body weight, 8–20 weeks (female) and 8–26 weeks (male); brain histology, single-cell RNA-seq, ulk RNA-seq and heart, 30 weeks after BMT. Sex: both male and female mice were used for microglia-replacement, growth and survival studies (data disaggregated by sex); behavioral, cardiac and RNA-sequencing analyses used female mice.
Wild animals	This study did not involve wild animals.
Reporting on sex	Sex was considered in the study design. Microglia-replacement, body-weight and survival experiments included both male and female mice, with data reported disaggregated by sex (e.g., Fig. 3C,D female and Fig. 3F,G male). Behavioural, cardiac and single-cell/bulk RNA-sequencing analyses were conducted in female mice to reduce variability due to sex differences. Results were consistent between sexes where both were assessed.
Field-collected samples	This study did not involve samples collected from the field
Ethics oversight	All animal procedures were approved by the Stanford University Administrative Panel on Laboratory Animal Care (APLAC; IACUC protocol 33941) and complied with National Institutes of Health institutional guidelines

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

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A

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 isolation of cells from mouse tissues (brain, spleen, bone marrow, peripheral blood, peritoneum, and heart) and their staining for flow cytometry analyses is described in the Methods section.
Instrument	BD FACSAria II cell sorter and BD FACSDiscover™ S8 Cell Sorter (image-enabled spectral flow cytometer)
Software	Data collection: BD FACSDiva software and "BD FACSChorus™ Software (incorporating BD SpectralFX™ Technology and BD CellView™ Image Technology)" Data analysis: FlowJo 10.8.2 (FlowJo, LLC). "BD FACSChorus™ Software (incorporating BD SpectralFX™ Technology and BD CellView™ Image Technology)"
Cell population abundance	None of the populations analyzed by flow cytometry were rare.
Gating strategy	Figures exemplifying the gating strategies are provided in the Supplementary Figures 1 and 2.

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