nature research

Corresponding author(s): Burkhard Becher

Last updated by author(s): May 31, 2019

# Reporting Summary

Nature Research 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 Research policies, see Authors & Referees 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<br>*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<br>*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 | Data collection Fluidigm CyTOF was used for mass cytometry acquisition, BD Diva software was used for flow cytometry acquisition. |
| Data analysis | R and R studio (Version 0.99.489) was used for data analysis. MatLab R_2013b was used for data preprocessing and analysis. FlowJo 10.1 was used for preprocessing of the data and analysis. Imaris Imaging software for image immunofluorescence. |

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/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

Mass cytometry and flow cytometry data analyzed in the manuscript (Figure 1-6) are deposited in a public repository and accession codes can be found at  are available at repository http://flowrepository.org/experiments/2166/. Patient-related data not included in the manuscript may be subject to patient confidentiality. The R-based workflow and source codes can be accessed found at https://github.com/GalliES/MS_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 | Sample size was determined by a power calculation of 0.80 given a FDR of 5% |
| Data exclusions | During the analysis samples with fewer than 1000 cells (or specific subpopulation with less than 50 cells) were excluded as stated in the manuscript. As requested by reviewers, older controls were excluded from the analysis as stated in the manuscript. |
| Replication | Validation of the findings have been achieved with the acquisition of an independent validation cohort that confirmed our initial findings. |
| Randomization | Samples were equally randomized among different runs |
| Blinding | Mass cyometric experiments and initial analysis was performed blinded with regards to disease. Unblinding was required for the further clinical stratification. However, the experimental approach and the automateted-data analysis workflow employed strongly reduce per sé the potential investigator bias. |

# 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 |
| ☒ | ☐ Animals and other organisms |
| ☐ | ☒ Human research participants |
| ☒ | ☐ Clinical data |

## Methods

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

## Human research participants

Policy information about studies involving human research participants

| | |
|---|---|
| Population characteristics | Patients included in the study were selected based on the MS McDonald criteria. Patients with other non-inflammatory neurological diseases, inflammatory diseases clinically isolated syndrome and healthy donor were also included. For the discovery cohort, the mean age was 36.5 years old (female = 56, male = 43), In the validation cohort, the average age was of 30.4 years old (male = 20, female = 38). In the DMF cohort, treated patients were longitudinally analyzed before and after therapy as (1 year follow up, DMF dose =240 mg twice per day). The average age of patients was of 40.3 years old, with 6 female and 3 male patients investigated. In the CNS cohort, patients' average age was 41.9, with 2 male and 7 female enrolled. |
| Recruitment | For the discovery, validation and CNS cohorts, all samples were obtained from our in-house biobank containing samples collected during routine neurological diagnostic work up or during clinical follow-up. Samples from patients included in this study were selected based on demographic matching with controls, diagnosis and therapy. The ethical review board of the Karolinska Institute and Stockhom approved the study (Diary Numbers: 2003/2-548, 2009/2107-31-2 and 2014/1201-31/1).<br>For the DMF cohort, only treatment-naïve adult patients fulfilling the prescription criteria for DMF (delayed release formulation, 240 mg twice daily, as approved by Swissmedic) were enrolled. All eligible individuals with intention to DMF-treatment visiting the multiple sclerosis (MS) centre of the University Hospital of Basel were assessed for study participation and consecutively recruited. The study was approved by the local ethics committee for northwest and central Switzerland (EKNZ) and performed in accordance with the Declaration of Helsinki.<br>Written informed consent was obtained from all patients and controls included in the whole study. No additional bias was introduced in the recruitment phase. |
| Ethics oversight | Stockholm Regional Ethical Vetting Board; |

| | |
|---|---|
| Ethics oversight | Regional ethical review board Goettingen;<br>Ethics Committee of northwest/central Switzerland. |

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 | Peripheral blood mononuclear cells and cerebrospinal fluid cells were used for cytometry experiment. Detailed protocols are reported in the Method section of the manuscript. |
| Instrument | CyTOF2, BD FACSymphony |
| Software | BD Diva software was used during data acquisition. Customized code used for data analysis will be uploaded in a GitHub repository before publication. |
| 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 | For all the experiment, single live cells gating have been used. Further analysis is based on automated data clustering. Cytokine positivity threshold have been assessed based on the .99 percentile of the corresponding cytokine in an unstimulated sample. Further details are stated in the manuscript. |

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