nature research

Corresponding author(s): Sofie Van Gassen

Last updated by author(s): Feb 26, 2021

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 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	BD Diva software was used to collect the cytometry data.
Data analysis	Data was analysed using R 4.0.3. All code is available at https://github.com/saeyslab/FlowSOM_protocol

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 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

The data is available at FlowRepository ("FR-FCM-ZZQY" and "FR-FCM-Z2TQ")

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 sample size calculation was performed upfront, as this protocol only illustrates the application of the algorithm on a small example dataset. No biological interpretations are made out of the comparisons presented.
Data exclusions	No data was excluded from this experiment
Replication	No replications were included in the small example dataset.
Randomization	All samples were measured in one batch, no randomization was required.
Blinding	No blinding was applied, as this data served as an illustratory example only.

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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used	Conjugated monoclonal antibodies used for the generation of the example data: CD16, CD32, MHCII, CD3, CD19, CD11c, CD49b, CD11b, CD64, FcER1, NK1.1, Ly6G
Validation	This study did not involve any additional validation.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	A20fl/fl; NKp46-iCre Tg/+ (natural killer cell deficient) knock-out mice (n = 4) and non-treated C57BtwL/6J wild type mice (n = 3)
Wild animals	The study did not involve wild animals
Field-collected samples	The study did not involve samples collected in the field
Ethics oversight	All animal experiments were performed in accordance with institutional guidelines for animal care of the VIB site Ghent/Ghent University Faculty of Sciences and in accordance with ethical committee EC2014_043.

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	Spleens were first minced, digested for 30 min in RPMI 1640 (Gibco) containing Liberase TM (0.02 mg/ml; Roche) and DNase I (10 U/ml; Roche) in a shaking water bath at 37°C, filtered through a 70-µM filter, and subjected to osmotic lysis. Single-cell suspensions were stained with monoclonal antibodies labeled with fluorochromes or biotin.
Instrument	BD LSR Fortessa
Software	BD Diva software was used to collect the cytometry data. Data was analysed using R 4.0.3. All code is available at https://github.com/saeyslab/FlowSOM_protocol
Cell population abundance	Cell population percentages are specified in Supplementary figure 1.
Gating strategy	B cells (live, CD19+), T cells (live, CD19−NK1.1−CD3+), macrophages (live, CD19−NK1.1−CD3−CD64+ autofluorescent), dendritic cells (live, CD19−NK1.1−CD3− [not macrophages] CD11c+MHCII+), NK cells (live, CD19−CD3−NK1.1+), NK T cells (live, CD19−CD3+NK1.1+), neutrophils (live, CD19−NK1.1−CD3− [not DC] CD11b+Ly6G+).

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