# nature portfolio

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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.

| Stati   | istics   |
|---------|----------|
| For all | statisti |

| 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. <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>                        |
|     | 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 <u>statistics for biologists</u> contains articles on many of the points above.  |
| So  | oftware and code   |

Policy information about availability of computer code

Data collection

BD FACSDiva Software (flow cytometry), ZEISS ZEN lite (immunofluresence)

Data analysis

graphpad prism (statistical analysis), flowjo (flow cytometry), ImageJ, (immunofluresence MFI), Aperio Imagescope (Immunohistochemistry

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

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| Research inv | volving hu | man participants, their data, or biological material |
|--------------|------------|--|
| ,            |            | vith   |

### **Antibodies**

Antibodies used

The antibodies used in flowcytometry staining for human PBMCs. antigen conjugate channel laser supplier catalogue no. CD3 BV510 Amcyan violet Biolegend 300447

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CD4 Alexa Fluor 488 FITC blue Life technologies 11-0048-41
CD8 PE-Cy7 PE-Cy7 yellow Life technologies 25-0084-82
CD25 BV786 Qdot800 violet BD biosciences 563701
CD127 PE-Cy5 PE-Cy5 Yellow Life technologies 15-1278-42
FOXP3 PerCP-Cy5.5 PerCP-Cy5.5 blue BD biosciences 561493
IFNy APC-eFluor 780 APC-Cy7 red Life technologies 47-7319-42
IL17A PE PE yellow BD biosciences 560487
IL21 APC APC red BD biosciences 560493
IL22 PerCP-eFluor 710 PerCP-Cy5.5 blue Life technologies 46-7229-42
RORyt BV650 Qdot655 violet BD biosciences 563424
Viability fixable dye eFluor 455 (UV) Indo-1 (violet) UV Life technologies 65-0868-14
The antibodies used in the flow cytometry staining for mouse splenocytes.
antigen conjugate channel laser supplier catalogue no.
CD3 BV510 Amcyan violet Biolegend 100233
CD4 Alexa Fluor 488 FITC blue Life technologies 53-0041-82
CD8 PE-Cy7 PE-Cy7 yellow Life technologies 25-0081-81
CD45 APC-Cy7 APC-Cy7 red BD biosciences 561037
CD117 APC APC red BD biosciences 561074
CD127 PerCP-eFluor 710 PerCP-Cy5.5 blue Life technologies 46-1271-80
CD138 PerCP-Cy5.5 PerCP-Cy5.5
CD169 FITC FITC blue Life technologies MA5-28189
IFNy APC-eFluor 780 APC-Cy7 red Life technologies 47-7311-80
IgG3 Alexa Fluor 594 Texas red yellow BD biosciences A-21155
IgM Alexa Fluor 350 Indo-1 (violet) UV Life technologies A-31552
IL17A PE PE yellow Life technologies 12-7177-81
IL21 APC APC red Life technologies 17-7211-80
IL22 PerCP-eFluor 710 PerCP-Cy5.5 blue Life technologies 46-7221-80
Linage (Lin) eFluor 450 Alexa Fluor 405 violet Life technologies 88-7772-72
Ly6G FITC FITC blue Life technologies 11-9668-80
RORyt BV650 Qdot655 violet BD biosciences 564722
Viability fixable dye eFluor 455 (UV) Indo-1 (violet) UV Life technologies 65-0868-14
Viability fixable dye eFluor 506 Amcyan violet Life technologies 65-0866-14
fixable dye eFluor 780 APC-Cy7 red Life technologies 65-0865-14
The antibodies used for immune fluorescence staining of mouse spleen sections.
anitgen excitation emission supplier catalogue no
IgM 343 nm 441 nm Life technologies A-31552
IgG 590 nm 618 nm Life technologies A-21155
Ly6G 498 nm 517 nm Life technologies 11-9668-80
The antibodies used in immunohistochemistry for mouse skin sections.
antigen supplier catalogue no
CD4 Santa Cruz sc-19641
IL17A R&D Systems AF317-NA
Lv6G BD biosciences 551459
Other materials used in human PBMCs polarization experiments.
Item supplier catalogue no.
Human CD3/CD28 dynabeads Life technologies 11161D
Human IL-17A ELISA Biolegend 433914
Human IL21 ELISA Biolegend 433804
Other materials used in mouse experiments.
Item supplier catalogue no.
```

Cell stimulation cocktail Life technologies 00-4975-93

Easysep mouse T Cell isolation kit Stemcell 19851Mouse CD3/CD28 dynabeads Life technologies 11456D

IgG (total) mouse ELISA kit Life technologies 88-50400-22

IgG3 mouse ELISA kit Life technologies 88-50440-22

Validation

For all antibodies validation can be accessed on manufacturer's website using the catalogue number

#### 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 | C57BL/6 and Balb/c mice |
|--------------------|-------------------------|
| Wild animals       | N/A                     |
| Reporting on sex   | N/A                     |

| Field-collected samples | Patients samples were collected from the hospital sites and processed within 1 hour of collection                                     |
|-------------------------|---|
| Ethics oversight        | McGill University and McGill University Health Centre In vitro experiments using blood samples animal study (animal ethics committee) |

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

#### Clinical data

| DOI | icv  | inforr | nation | ahout  | clinical  | studies |
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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 | N/A |
|-----------------------------|-----|
| Study protocol              | N/A |
| Data collection             | N/A |
| Outcomes                    | N/A |

#### **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        | PBMCs, Th1, Treg, Th17 polarized PBMCs  |
|---------------------------|---|
|                           | Mouse splenocytes isolated activated and cytokines stained intracellularly      |
| Instrument                | BD LSR-Fortessa flow cytometer  |
| Software                  | BD FACSDiva™ Software   |
| Cell population abundance | The least cell count 15000 and up to 200000 cell per sample per acquisition     |
| Gating strategy           | Cells>single cells>live cells>CD3+>CD4+>IL17A, IL21, IL22, IFN                  |
|                           | Cells>single cells>live cells>CD3+>CD4+>IL17A, IFN                              |
|                           | Cells>single cells>live cells>CD3+>CD4+>CD25hi>CD127lo>Foxp3+ (gating provided) |
|                           | mouse splenocytes   |
|                           | Cells>single cells>live cells>CD3+>CD4+>IL17A, IL21, IL22 or IFN                |
|                           | Cells>single cells>live cells>CD3->Lin->CD117+>CD127+                           |
|                           | Cells>single cells>live cells>CD3->CD19+CD183+>IgM vs IgG3                      |

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