**Materials Design Analysis Reporting (MDAR)**

**Checklist for Authors**

The [MDAR framework](https://osf.io/xfpn4/) establishes a minimum set of requirements in transparent reporting mainly applicable to studies in the life sciences.

*eLife* asks authors to **provide detailed information within their article** to facilitate the interpretation and replication of their work. Authors can also upload supporting materials to comply with relevant reporting guidelines for health-related research (see [EQUATOR Network](http://www.equator-network.org/%20)), life science research (see the [BioSharing Information Resource](http://biosharing.org/)), or animal research (see the [ARRIVE Guidelines](http://www.plosbiology.org/article/info%3Adoi/10.1371/journal.pbio.1000412) and the [STRANGE Framework](https://doi.org/10.1038/d41586-020-01751-5); for details, see *eLife*’s [Journal Policies](https://reviewer.elifesciences.org/author-guide/journal-policies)). Where applicable, authors should refer to any relevant reporting standards materials in this form.

For all that apply, please note **where in the article** the information is provided. Please note that we also collect information about data availability and ethics in the submission form.

**Materials:**

|  |  |  |
| --- | --- | --- |
| **Newly created materials** | **Indicate where provided: section/figure legend** | **N/A** |
| The manuscript includes a dedicated "materials availability statement" providing transparent disclosure about availability of newly created materials including details on how materials can be accessed and describing any restrictions on access. | The materials generated in this manuscript are multiple sclerosis PBMC humanized mice, which are single-use animals and therefore not freely available for distribution. However, we make the protocols for their generation freely available:Specifically:Materials & Methods (PBMC isolation: lines 487-497; Mice: lines 508-524).Figure 1- figure supplement 2. |  |
|  |  |  |
| **Antibodies** | **Indicate where provided: section/figure legend** | **N/A** |
| For commercial reagents, provide supplier name, catalogue number and [RRID](https://scicrunch.org/resources), if available. | **PBMC isolation**Ficoll-Histopaque®-1077 (Sigma-Aldrich).Quicklysis™ erythrocyte lysis buffer (Cytognos).**EBV antibody responses**ELISA-VIDITESTS (all Vidia):anti-VCA EBV IgG (ODZ502 265), anti-VCA IgM (ODZ-005), and anti-VCA IgA (ODZ-096), anti-EA (D) EBV IgG (ODZ-006), anti-EBNA-1 EBV IgM (ODZ-002), anti-EBNA-1 EBV IgG (ODZ-001). **EAE induction**Freund's complete adjuvant (FCA) (Sigma-Aldrich). H37Ra Mycobacterium tuberculosis (Difco, BD Biosciences).Bordetella pertussis toxin (Sigma-Aldrich).Myelin-oligodendrocyte glycoprotein (MOG) IIFT assay (Euroimmun, Lubeck, Germany) (Cat#: FA 1156-1010-50)**FACS antibodies**BV 510-hCD45 (clone 2D1) (Biolegend). APC-Cyanine 7-hCD3 (clone HIT3a) (Biolegend). PE-Cyanine 7-hCD4 (clone A161A1) (Biolegend).PerCP-hCD8 (clone SK1) (Biolegend).PE-hCD19 (clone 4G7) (Biolegend).FITC-hCD56 (clone 5.1H11) (Biolegend).APC579 hCD66b (clone G1OF5) (Biolegend). BV-421-hCD14 (clone HCD14) (Biolegend). APC-mCD45 (clone 30-F11) (Biolegend).PerCP-Cyanine 5.5-CD3 (clone HIT3a) (Biolegend). APC-CD4 594 (clone RPA-T4) (BD Biosciences). PerCP-CD8 (clone SK1) (Biolegend). PE-IFN-γ (clone B27) (BD Biosciences).PE-IL-17A (clone eBio64DEC17) (eBioscience).**FACS intracellular staining**Phorbol 12-myristate 13 acetate (PMA) (Sigma-Aldrich).Ionomycin (Sigma-Aldrich).Brefeldin-A (Sigma-Aldrich).carboxylfluorescein succinimidyl ester (CFSE, V12883) (Thermofisher).2- 15 Mercaptoethanol (Sigma-Aldrich).Anti-CD3 (clone HIT3a) (BD Biosciences).PHA (Sigma-Aldrich).**Immunohistochemistry**-**Primary antibodies**Rat anti-hCD3 (SP7) (Epredia #RM-9107-R7).Rabbit anti-hCD4 (4B12) (Agilent-Dako #M7310).Mouse anti-hCD8 (C8/144B) (Agilent-Dako #BSB 5169).Anti-hCD45 (HI30) (Biolegend).Rat anti-mCD45 (30-F11) (Biolegend). Rabbit anti-mIba1 (019-19741) (Wako chemicals).Rabbit anti-mGFAP (Z0334) (Agilent-Dako).Rat anti-MBP (ab7349) (Abcam).**-Secondary antibodies & detection systems**EnVision FLEX High pH kit (Agilent-Dako)Biotinylated secondary anti-IgG antibodies (Vector Laboratories).Vectastain ABC kit (HRP) (Vector Laboratories).Goat anti-rat IgG CF-647 (Biotium). Goat anti-rabbit IgG AlexaFluor 568 (A11011) (Invitrogen).DAPI (D1306) (Invitrogen).3’3’-Diaminobenzidine (DAB) (Vector Laboratories). |  |
|  |  |  |
| **DNA and RNA sequences** | **Indicate where provided: section/figure legend** | **N/A** |
| Short novel DNA or RNA including primers, probes: Sequences should be included or deposited in a public repository. |  | N/A |
|  |  |  |
| **Cell materials** | **Indicate where provided: section/figure legend** | **N/A** |
| Cell lines: Provide species information, strain. Provide accession number in repository OR supplier name, catalog number, clone number, OR RRID. |  | N/A |
| Primary cultures: Provide species, strain, sex of origin, genetic modification status.  | Fresh human peripheral blood monocytes isolated from human MS patients and healthy individual. Cells were used fresh and not maintained in culture. |  |
|  |  |  |
| **Experimental animals** | **Indicate where provided: section/figure legend** | **N/A** |
| Laboratory animals or Model organisms: Provide species, strain, sex, age, genetic modification status. Provide accession number in repository OR supplier name, catalog number, clone number, OR RRID. | B2m-NOD/Shi - scid IL2rgnull (B2m-NOG)stock number 14957-F: NOD.Cg-B2m PrkdcIl2rgJicTac; Taconic Biosciences.NOG mice were engrafted with human PBMC and used for experiments at different time points post-engraftment. |  |
| Animal observed in or captured from the field: Provide species, sex, and age where possible. |  | N/A |
|  |  |  |
| **Plants and microbes** | **Indicate where provided: section/figure legend** | **N/A** |
| Plants: provide species and strain, ecotype and cultivar where relevant, unique accession number if available, and source (including location for collected wild specimens). |  | N/A |
| Microbes: provide species and strain, unique accession number if available, and source. |  | N/A |
|  |  |  |
| **Human research participants** | **Indicate where provided: section/figure legend) or state if these demographics were not collected** | **N/A** |
| If collected and within the bounds of privacy constraints report on age, sex, gender and ethnicity for all study participants. |  | N/A |

**Design:**

|  |  |  |
| --- | --- | --- |
| **Study protocol** | **Indicate where provided: section/figure legend** | **N/A** |
| If the study protocol has been pre-registered, provide DOI. For clinical trials, provide the trial registration number OR cite DOI. |  | N/A |
|  |  |  |
| **Laboratory protocol** | **Indicate where provided: section/figure legend** | **N/A** |
| Provide DOI OR other citation details if detailed step-by-step protocols are available. | Myelin peptide synthesis doi: 10.1016/j.ab.2015.06.010Induction and scoring of EAE in female C57BL/6 mice doi: 10.3389/fimmu.2020.575451 |  |
|  |  |  |
| **Experimental study design (statistics details) \*** |
| **For in vivo studies: State whether and how the following have been done** | **Indicate where provided: section/figure legend. If it could have been done, but was not, write “not done”** | **N/A** |
| Sample size determination | Sample size determination of humanized mice generated from each human donor was limited by the numbers of human PBMC available from 50 ml of freshly drawn blood for engraftment into mice @10x106 cells/mouse | N/A |
| Randomisation |  | N/A |
| Blinding | For all histochemical analysis measurements were performed blinded by a minimum of two independent investigators |  |
| Inclusion/exclusion criteria |  | N/A |
|  |  |  |
| **Sample definition and in-laboratory replication** | **Indicate where provided: section/figure legend** | **N/A** |
| State number of times the experiment was replicated in the laboratory. | Two full experiments were performed using different MS donors |  |
| Define whether data describe technical or biological replicates. | Biological replicates were used for all in vivo and in vitro analyses |  |
|  |  |  |
| **Ethics** | **Indicate where provided: section/submission form** | **N/A** |
| Studies involving human participants: State details of authority granting ethics approval (IRB or equivalent committee(s), provide reference number for approval. | Human peripheral blood sampling was performed under signed informed consent in accordance with the Declaration of Helsinki and approved from the Institutional Ethics Committee of Aeginition Hospital, NKUA with Protocol No: 7BSH46Y8N2-B66, 13/05/2015 |  |
| Studies involving experimental animals: State details of authority granting ethics approval (IRB or equivalent committee(s), provide reference number for approval. | Mouse experiments were reviewed and approved by Committee for Evaluation of Experimental Procedures, Department of Experimental Animal Models, Hellenic Pasteur Institute (Presided by Dr P Andriopoulos pandriopoulos@patt.gov.gr for the Hellenic Republic, General Secretariat for Agricultural Economy, Veterinary and Licenses), and performed under licence numbers 770851/27-11-2019 and 1343917/02-11-2023. |  |
| Studies involving specimen and field samples: State if relevant permits obtained, provide details of authority approving study; if none were required, explain why. |  | N/A |
|  |  |  |
| **Dual Use Research of Concern (DURC)** | **Indicate where provided: section/submission form** | **N/A** |
| If study is subject to dual use research of concern regulations, state the authority granting approval and reference number for the regulatory approval. |  | N/A |

**Analysis:**

|  |  |  |
| --- | --- | --- |
| **Attrition** | **Indicate where provided: section/figure legend** | **N/A** |
| Describe whether exclusion criteria were pre-established. Report if sample or data points were omitted from analysis. If yes, report if this was due to attrition or intentional exclusion and provide justification. |  | N/A |
|  |  |  |
| **Statistics** | **Indicate where provided: section/figure legend** | **N/A** |
| Describe statistical tests used and justify choice of tests. | **Figure 1A, E**: Statistical significance is shown after pairwise comparisons between, (A) peripheral blood and splenocytes from immunized and non-immunized groups of humanized mice, (E) splenocytes isolated at dpt 42 from immunized and non-immunized DR13 MS mice, using Student’s t test (\*p ≤ 0.05, \*\*p ≤ 0.01, \*\*\*p ≤ 0.001).**Figure 2 supplement 1A, B:** Statistical significance after multiple comparisons between lung and liver inflammation in three different immunized mouse groups at the same time point using one-way ANOVA (\*p ≤ 0.05, \*\*p ≤ 0.01, \*\*\*p ≤ 0.001).**Figure 3C**: Statistical significance after pairwise comparisons between white matter (WM) and grey matter (GM) spinal cord lesions in two different non-immunized mouse groups, at the same time point using Student’s t test (\*\*p ≤ 0.01). |  |
|  |  |  |
| **Data availability** | **Indicate where provided: section/submission form** | **N/A** |
| For newly created and reused datasets, the manuscript includes a data availability statement that provides details for access (or notes restrictions on access). |  | N/A |
| When newly created datasets are publicly available, provide accession number in repository OR DOI and licensing details where available. |  | N/A |
| If reused data is publicly available provide accession number in repository OR DOI, OR URL, OR citation. |  | N/A |
|  |  |  |
| **Code availability** | **Indicate where provided: section/figure legend** | **N/A** |
| For any computer code/software/mathematical algorithms essential for replicating the main findings of the study, whether newly generated or re-used, the manuscript includes a data availability statement that provides details for access or notes restrictions. |  | N/A |
| Where newly generated code is publicly available, provide accession number in repository, OR DOI OR URL and licensing details where available. State any restrictions on code availability or accessibility. |  | N/A |
| If reused code is publicly available provide accession number in repository OR DOI OR URL, OR citation. |  | N/A |

**Reporting:**

The MDAR framework recommends adoption of discipline-specific guidelines, established and endorsed through community initiatives.

|  |  |  |
| --- | --- | --- |
| **Adherence to community standards** | **Indicate where provided: section/figure legend** | **N/A** |
| State if relevant guidelines (e.g., ICMJE, MIBBI, ARRIVE, STRANGE) have been followed, and whether a checklist (e.g., CONSORT, PRISMA, ARRIVE) is provided with the manuscript. | The mouse experiments complied with ARRIVE guidelines and were in accordance with the local Ethical Committee guidelines on the use of experimental animals at the Hellenic Pasteur Institute, were approved by the national authorities and complied to EU Directive 2010/63/EU for animal experiments. |  |

\* We provide the following guidance regarding transparent reporting and statistics; we also refer authors to [Ten common statistical mistakes to watch out for when writing or reviewing a manuscript](https://doi.org/10.7554/eLife.48175).

**Sample-size estimation**

* You should state whether an appropriate sample size was computed when the study was being designed
* You should state the statistical method of sample size computation and any required assumptions
* If no explicit power analysis was used, you should describe how you decided what sample (replicate) size (number) to use

**Replicates**

* You should report how often each experiment was performed
* You should include a definition of biological versus technical replication
* The data obtained should be provided and sufficient information should be provided to indicate the number of independent biological and/or technical replicates
* If you encountered any outliers, you should describe how these were handled
* Criteria for exclusion/inclusion of data should be clearly stated
* High-throughput sequence data should be uploaded before submission, with a private link for reviewers provided (these are available from both GEO and ArrayExpress)

**Statistical reporting**

* Statistical analysis methods should be described and justified
* Raw data should be presented in figures whenever informative to do so (typically when N per group is less than 10)
* For each experiment, you should identify the statistical tests used, exact values of N, definitions of center, methods of multiple test correction, and dispersion and precision measures (e.g., mean, median, SD, SEM, confidence intervals; and, for the major substantive results, a measure of effect size (e.g., Pearson's r, Cohen's d)
* Report exact p-values wherever possible alongside the summary statistics and 95% confidence intervals. These should be reported for all key questions and not only when the p-value is less than 0.05.

**Group allocation**

* Indicate how samples were allocated into experimental groups (in the case of clinical studies, please specify allocation to treatment method); if randomization was used, please also state if restricted randomization was applied
* Indicate if masking was used during group allocation, data collection and/or data analysis