

# <u>Materials Design Analysis Reporting (MDAR)</u> **Checklist for Authors**

The MDAR framework 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), life science research (see the **BioSharing Information Resource**), or animal research (see the **ARRIVE Guidelines** and the STRANGE Framework; for details, see *eLife*'s 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.		N/A

Antibodies	Indicate where provided: section/figure legend	N/A
For commercial reagents, provide supplier name, catalogue number and <u>RRID</u> , if available.		
CD19 MicroBeads. (Miltenyi Biotec Cat# 130-121- 301, RRID: AB_2827612)	Figure legends for Figure 1-figure supplement 1. Figures 2 to 6 and their supplements. MATERIALS AND METHODS	
Mouse B Cell Isolation Kit. Miltenyi Biotec. Cat no: 130-090-862.	Figures 2 to 6 and their supplements. MATERIALS AND METHODS	
Peritoneum Macrophage Isolation Kit. Miltenyi Biotec. Cat no: 130-110-434.	Figure legends for Figure 7. Figure 7-figure supplement 1.	
InVivoPlus rat IgG1 (HRPN, BioXcell), cat no: BE0088, RRID: AB_1107775.	Figure legends for Figure 4. Figure 4-figure supplement 2, Figure 7.	

	Figure 7-figure supplement 1. MATERIALS AND METHODS
InVivoMAb rat IgG2b (LTF-2, BioXcell), cat no: BE0090, RRID: AB_1107780.	Figure legends for Figure 4. Figure 4-figure supplement 2. MATERIALS AND METHODS
InVivo polyclonal Armenian hamster IgG (BioXcell), cat no: BE0091, RRID: AB_1107773.	Figure legends for Figure 4-figure supplement 3. MATERIALS AND METHODS
InVivoMAb anti-mouse IL-6R (15A7, BioXcell), cat no: BE0047, RRID: AB_1107588.	Figure legends for Figure 4. Figure 4-figure supplement 2. MATERIALS AND METHODS
InVivoPlus anti-mouse IL-10R (1B1.3A, BioXcell), cat no: BE0050, RRID: AB_1107611.	Figure legends for Figure 4. Figure 4-figure supplement 2. MATERIALS AND METHODS
InVivoMAb anti-mouse/rat IL-1β (B122, BioXcell), cat no: BE0246, RRID: AB_2687727.	Figure legends for Figure 4-figure supplement 3. MATERIALS AND METHODS
f(ab') <sub>2</sub> fragments of goat anti-mouse IgM antibody (eBioscience, San Diego, CA), cat no: 16-5092-85, RRID: AB_2573088.	Figure legends for Figures 1, 2, 3, 4, 6 and 7. Figure 2-figure supplement 1. Figure 2-figure supplement 2. Figure 3-figure supplement 1. Figure 4-figure supplement 2. Figure 4-figure supplement 3. Figure 4-figure supplement 4. Figure 5-figure supplement 2. Figure 6-figure supplement 1. Figure 6-figure supplement 3. Figure 7, figure supplement 1, MATERIALS AND METHODS
anti-phospho STAT1 Tyr <sup>701</sup> (58D6), Cell Signaling Technology. Cat no: 9167, RRID: AB_561284.	Figure legends for Figure 2. MATERIALS AND

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anti-STAT1 (42H3), Cell Signaling Technology. Cat no: 9175, RRID: AB_2197984.	Figure legends for Figure 2. MATERIALS AND METHODS
anti-phospho STAT3 Tyr <sup>706</sup> (D3A7). Cell Signaling Technology. Cat no: 9145, RRID: AB_2491009.	Figure legends for Figure 2. Figure 3. Figure 3-figure supplement 1. Figure 4. Figure 4-figure supplement 1. Figure 4-figure supplement 2. Figure 5. MATERIALS AND METHODS
anti-STAT3 (79D7). Cell Signaling Technology. Cat no: 4904, RRID: AB_331269.	Figure legends for Figure 2. Figure 3. Figure 3-figure supplement 1. Figure 4. Figure 4-figure supplement 1. Figure 4-figure supplement 2. Figure 5. MATERIALS AND METHODS
anti-phospho STAT5 Tyr <sup>694</sup> (D47E7). Cell Signaling Technology. Cat no: 4322, RRID: AB_10544692.	Figure legends for Figure 2. Figure 3. Figure 3-figure supplement 1. Figure 4. Figure 5. Figure 6. Figure 6-figure supplement 2. MATERIALS AND METHODS
anti-STAT5 (D2O6Y), Cell Signaling Technology. Cat no: 94205, RRID: AB_2737403.	Figure legends for Figure 2. Figure 3. Figure 3-figure supplement 1. Figure 4. Figure 5. Figure 6. Figure 6-figure supplement 2. MATERIALS AND METHODS

anti-beta-actin (D6A8), Cell Signaling Technology. Cat no: 8457, RRID: AB_10950489.	Figure legends for Figure 2. Figure 3. Figure 6. MATERIALS AND METHODS
anti-phospho PKC (pan) betaII Ser <sup>660</sup> . Cell Signaling Technology. Cat no: 9371, RRID: AB_2168219.	Figure legends for Figure 6. Figure 6-figure supplement 2. MATERIALS AND METHODS
anti-phospho FAK Tyr <sup>397</sup> . Cell Signaling Technology. Cat no: 3283, RRID: AB_2173659.	Figure legends for Figure 6. Figure 6-figure supplement 2. MATERIALS AND METHODS
anti-FAK, Cell Signaling Technology. Cat no: 3285, RRID: AB_2269034.	Figure legends for Figure 6. Figure 6-figure supplement 2. MATERIALS AND METHODS
anti-phospho Akt Ser <sup>473</sup> (D9E). Cell Signaling Technology. Cat no: 4060, RRID: AB_2315049.	Figure legends for Figure 2-figure supplement 2. MATERIALS AND METHODS
anti-Akt (pan) (C67E7). Cell Signaling Technology. Cat no: 4691, RRID: AB_915783.	Figure legends for Figure 2-figure supplement 2. MATERIALS AND METHODS
anti-phospho p38 MAPK Thr <sup>180</sup> /Tyr <sup>181</sup> (D3F9). Cell Signaling Technology. Cat no: 4511, RRID: AB_2139682.	Figure legends for Figure 2. MATERIALS AND METHODS
anti-p38 MAPK (D13E1). Cell Signaling Technology. Cat no: 8690, RRID: AB_10999090.	Figure legends for Figure 2. MATERIALS AND METHODS
anti-phospho SAPK/JNK Thr <sup>183</sup> /Tyr <sup>185</sup> (81E11). Cell Signaling Technology. Cat no: 4668, RRID: AB_823588.	Figure legends for Figure 2. MATERIALS AND METHODS
anti-SAPK/JNK. Cell Signaling Technology. Cat no: 9252, RRID: AB_2250373.	Figure legends for Figure 2. MATERIALS AND

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anti-phospho p44/42 MAPK (Erk1/2) Thr <sup>202</sup> /Tyr <sup>204</sup> (D13.14.4E). Cell Signaling Technology. Cat no: 4370, RRID:AB_2315112.	Figure legends for Figure 2-figure supplement 2. MATERIALS AND METHODS
anti-p44/42 MAPK (Erk1/2) (137F5). Cell Signaling Technology. Cat no: 4695, RRID: AB_390779.	Figure legends for Figure 2-figure supplement 2. MATERIALS AND METHODS
anti-phospho NF-κB p65 Ser <sup>536</sup> (93H1). Cell Signaling Technology. Cat no: 3033, RRID: AB_331284.	Figure legends for Figure 6-figure supplement 2. MATERIALS AND METHODS
anti-NF-κB p65 (D14E12). Cell Signaling Technology. Cat no: 8242, RRID: AB_10859369.	Figure legends for Figure 6-figure supplement 2. MATERIALS AND METHODS
anti-phospho IκBα Ser <sup>32</sup> (14D4). Cell Signaling Technology. Cat no: 2859, RRID: AB_561111.	Figure legends for Figure 6-figure supplement 2. MATERIALS AND METHODS
IκBα (44D4). Cell Signaling Technology. Cat no: 4812, RRID: AB_10694416.	Figure legends for Figure 6-figure supplement 2. MATERIALS AND METHODS
anti-rabbit IgG, HRP-linked. Cell Signaling Technology. Cat no: 7074, RRID: AB_2099233.	MATERIALS AND METHODS
anti-PKC (A-3). Santa Cruz. Cat no: sc-17769, RRID: AB_628139.	Figure legends for Figure 6. Figure 6-figure supplement 2. MATERIALS AND METHODS
anti-phospho FAK Tyr <sup>576</sup> (2H74L24). Thermo Fisher Scientific. Cat no: 700013, RRID: AB_2532268.	Figure legends for Figure 6. MATERIALS AND METHODS
phycoerythrin (PE)-anti-mouse CD43 (S11), Biolegend. Cat no: 143205, RRID: AB_11142681.	Figure legends for Figure 1. Figure 1-figure supplement 2. Figure 1-figure supplement 3. Figure 1-figure supplement 4. MATERIALS AND

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Pacific Blue-anti-mouse CD19 (6D5), Biolegend. Cat no: 115526, RRID: AB_493341.	Figure legends for Figure 1. Figure 1-figure supplement 2. Figure 1-figure supplement 3. Figure 1-figure supplement 4. Figure 3-figure supplement 1. Figure 6-figure supplement 1. MATERIALS AND METHODS
fluorescein isothiocyanate (FITC)-anti-mouse CD19 (1D3/CD19), Biolegend, Cat no: 152403, RRID: AB_2629812.	MATERIALS AND METHODS
allophycocyanin (APC)-Cy7-anti-mouse CD19 (6D5), Biolegend. Cat no: 115529, RRID: AB_830706.	MATERIALS AND METHODS
Brilliant Violet (BV) 510-anti-mouse/human CD45R/B220 (RA3-6B2), Biolegend. Cat no: 103247, RRID: AB_2561394.	Figure legends for Figure 5. Figure 5-figure supplement 1. MATERIALS AND METHODS
PE-anti-mouse CD126 (IL-6Rα chain) (D7715A7), Biolegend. Cat no: 115805, RRID: AB_313676.	Figure legends for Figure 5. MATERIALS AND METHODS
FITC-anti-mouse IL-10 (JES5-16E3), Biolegend. Cat no: 505005, RRID: AB_315359.	Figure legends for Figure 1. Figure 1-figure supplement 3. Figure 6-figure supplement 3. MATERIALS AND METHODS
PE-anti-mouse IL-6 (MP5-20F3), Biolegend. Cat no: 504503, RRID: AB_315337.	Figure legends for Figure 3-figure supplement 1. MATERIALS AND METHODS
FAK (Phospho-Tyr <sup>397</sup> ) (CF405M). Biorbyt. Cat no: orb106222.	Figure legends for Figure 6-figure supplement 1. MATERIALS AND METHODS
FAK (FITC). Biorbyt. Cat no: orb15594.	MATERIALS AND METHODS

APC-anti-mouse CD130 (gp130) (KGP130). Thermo Fisher Scientific. Cat no: 17-1302-82, RRID: AB_10670874.	Figure legends for Figure 5-figure supplement 1. MATERIALS AND METHODS	
Alexa Fluor <sup>®</sup> 488-Donkey anti-Rabbit IgG (H+L). Jackson ImmunoResearch Labs. Cat no: 711-545-152, RRID: AB_2313584.	MATERIALS AND METHODS	
FITC-anti-mouse IgM (RMM-1), Biolegend, Cat no: 406506, RRID: AB_315056.	MATERIALS AND METHODS	
BV605-anti-mouse IgD (11-26c.2α), BD Biosciences, Cat no: 563003, RRID: AB_2737944.	MATERIALS AND METHODS	

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.		
<i>Gapdh</i> (Mm999999915_g1) TaqMan probes. Thermo Fisher Scientific. Cat no: 4331182	MATERIALS AND METHODS	
<i>Il10</i> (Mm01288386_m1) TaqMan probes. Thermo Fisher Scientific. Cat no: 4331182	Figure legends for Figure 1. Figure 3. Figure 4. Figure 4-figure supplement 3. MATERIALS AND METHODS	
<i>Il6</i> (Mm00446190_m1) TaqMan probes. Thermo Fisher Scientific. Cat no: 4331182	Figure legends for Figure 3. Figure 6. MATERIALS AND METHODS	
<i>Tnf</i> (Mm00443258_m1) TaqMan probes. Thermo Fisher Scientific. Cat no: 4331182	Figure legends for Figure 4. MATERIALS AND METHODS	
<i>Il1b</i> (Mm00434228_m1) TaqMan probes. Thermo Fisher Scientific. Cat no: 4331182	Figure legends for Figure 4-figure supplement 3. MATERIALS AND METHODS	
<i>ll6ra</i> (Mm01211445_m1) TaqMan probes. Thermo Fisher Scientific. Cat no: 4331182	MATERIALS AND METHODS	

Accell Mouse Stat5a siRNA-SMART pool. Horizon Discovery Dharmacon. Cat no: E-063202-00. GAUUCAUCCUUCUUGCUUU, CGUGGAAGAACUUUUACGC, UGAACUACCUUAUCUACGU, UUGUGGUCUCAGAAAUCGC	Figure legends for Figure 3. MATERIALS AND METHODS	
Accell Non-targeting siRNA Pool. Horizon Discovery Dharmacon. Cat no: D-001910-10. UGGUUUACAUGUCGACUAA, UGGUUUACAUGUUUUCUGA, UGGUUUACAUGUUUUCCUA, UGGUUUACAUGUUGUGUGA	Figure legends for Figure 3. MATERIALS AND METHODS	

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.		
Splenic B cells isolated from 6-8 day old (neonate) or 8-10 weeks old (adult, female) C57BL/6J mice (Jackson Laboratory, Cat no: 000664) or 6-8 day old (neonate) IL-6 <sup>-/-</sup> (IL-6 KO) (B6.129S2- <i>Il6</i> <sup>tm1Kopf</sup> /J) mice (Jackson Laboratory, Cat no: 002650).	Figure legends for Figures 1 to 7 and their supplements.	
Peritoneal macrophages isolated from 8-10 weeks old (adult, female) C57BL/6J mice (Jackson Laboratory, Cat no: 000664).	Figure legends for Figures 7. Figure 7-figure supplement 1.	

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.		
6-8 day old (neonate) or 8-10 weeks old (adult, female) C57BL/6J mouse. Jackson Laboratory. Cat no: 000664.	Figure legends for Figures 1 to 7 and their supplements.	

6-8 day old (neonate) IL-6 <sup>-/-</sup> (IL-6 KO) (B6.129S2- <i>Il6<sup>tm1Kopf/JJ</sup></i> ) mouse. Jackson Laboratory. Cat no: 002650.	Figure legends for Figures 4. Figure 4-figure supplement 2. Figure 6-figure supplement 3.	
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.		N/A

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,	N/A

	write "not done"	
Sample size determination		N/A
Randomisation		N/A
Blinding		N/A
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.		
Three times	Indicated in each figure legend.	
Define whether data describe technical or biological replicates.		
Biological replicates	Indicated in each figure legend.	

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.		N/A
Studies involving experimental animals: State details of authority granting ethics approval (IRB or equivalent committee(s), provide reference number for approval.		
C57BL/6J mouse and IL-6KO mice	US FDA/Center for Biologics Evaluation and Research Institutional Animal Care and Use Committee guidelines (permit 2002-31 and 2017-45).	
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.		
Two-tailed Student's <i>t</i> -test to compare two conditions.	Indicated in each figure legend.	
One-way analysis of variance (ANOVA) with a Dunnett's multiple comparisons test to compare multiple conditions at a timepoint.	Indicated in each figure legend.	
Two-way ANOVA to compare multiple conditions in a time course.	Indicated in each figure legend.	

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).		
Raw data of RNA sequencing	Data Availability	
When newly created datasets are publicly available, provide accession number in repository OR DOI and licensing details where available.		

Raw data of RNA sequencing	Sequencing data have been deposited in GEO under accession code GSE200955 https://www.ncbi.nlm.nih.gov/g eo/query/acc.cgi?acc=GSE2009 55	
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.		N/A

\* We provide the following guidance regarding transparent reporting and statistics; we also refer authors to <u>Ten common statistical mistakes to watch out for when writing or reviewing a manuscript</u>.

#### 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