

## **Materials Design Analysis Reporting (MDAR) 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:**

<b>Newly created materials</b>	<b>Indicate where provided: section/figure legend</b>	<b>N/A</b>
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.	No newly created materials were deposited in a public repository. Materials described in this study are available from the authors upon reasonable request.	N/A

<b>Antibodies</b>	<b>Indicate where provided: section/figure legend</b>	<b>N/A</b>
For commercial reagents, provide supplier name, catalogue number and <a href="#">RRID</a> , if available.	Key Resources Table	

<b>DNA and RNA sequences</b>	<b>Indicate where provided: section/figure legend</b>	<b>N/A</b>
Short novel DNA or RNA including primers, probes: Sequences should be included or deposited in a public repository.	Methods Section "Measuring Hedgehog signaling with quantitative PCR"	

<b>Cell materials</b>	<b>Indicate where provided: section/figure legend</b>	<b>N/A</b>
Cell lines: Provide species information, strain. Provide accession number in repository OR supplier name, catalog number, clone number, OR RRID.	Key Resources Table, Methods Section "Cell culture and cell line generation", Methods Section "Measuring Hedgehog signaling with quantitative PCR"	

Primary cultures: Provide species, strain, sex of origin, genetic modification status.	Key Resources Table, Methods Section "Cell culture and cell line generation", Methods Section "Measuring Hedgehog signaling with quantitative PCR"	
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<b>Experimental animals</b>	<b>Indicate where provided: section/figure legend</b>	<b>N/A</b>
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.	No Lab animals	N/A
Animal observed in or captured from the field: Provide species, sex, and age where possible.	No experimental animals	N/A

<b>Plants and microbes</b>	<b>Indicate where provided: section/figure legend</b>	<b>N/A</b>
Plants: provide species and strain, ecotype and cultivar where relevant, unique accession number if available, and source (including location for collected wild specimens).	No plants	N/A
Microbes: provide species and strain, unique accession number if available, and source.	No microbes	N/A

<b>Human research participants</b>	<b>Indicate where provided: section/figure legend) or state if these demographics were not collected</b>	<b>N/A</b>
If collected and within the bounds of privacy constraints report on age, sex, gender and ethnicity for all study participants.	No human participants	N/A

**Design:**

<b>Study protocol</b>	<b>Indicate where provided: section/figure legend</b>	<b>N/A</b>
If the study protocol has been pre-registered, provide DOI. For clinical trials, provide the trial registration number OR cite DOI.	Not pre-registered	N/A

<b>Laboratory protocol</b>	<b>Indicate where provided: section/figure legend</b>	<b>N/A</b>

Provide DOI OR other citation details if detailed step-by-step protocols are available.	Not available	N/A
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<b>Experimental study design (statistics details) *</b>		
<b>For in vivo studies: State whether and how the following have been done</b>	<b>Indicate where provided: section/figure legend. If it could have been done, but was not, write "not done"</b>	<b>N/A</b>
Sample size determination	No in-vivo work in this study	N/A
Randomisation	No in-vivo work in this study	N/A
Blinding	No in-vivo work in this study	N/A
Inclusion/exclusion criteria	No in-vivo work in this study	N/A

<b>Sample definition and in-laboratory replication</b>	<b>Indicate where provided: section/figure legend</b>	<b>N/A</b>
State number of times the experiment was replicated in the laboratory.	Each experiment was replicated a minimum of three separate times on different days in the laboratory. All experimental data represent biological replicates, N=4. This is mentioned in the captions of Figure 3 and 6.	
Define whether data describe technical or biological replicates.	The data depicted shows 4 biological replicates. Each data point was a separate biological sample, plated and treated separately throughout the entirety of the experiment.	

<b>Ethics</b>	<b>Indicate where provided: section/submission form</b>	<b>N/A</b>
Studies involving human participants: State details of authority granting ethics approval (IRB or equivalent committee(s), provide reference number for approval.	No human participants	N/A
Studies involving experimental animals: State details of authority granting ethics approval (IRB or equivalent committee(s), provide reference number for approval.	No experimental animals	N/A

Studies involving specimen and field samples: State if relevant permits obtained, provide details of authority approving study; if none were required, explain why.	No specimen and field samples	N/A
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<b>Dual Use Research of Concern (DURC)</b>	<b>Indicate where provided: section/submission form</b>	<b>N/A</b>
If study is subject to dual use research of concern regulations, state the authority granting approval and reference number for the regulatory approval.	Not subject to dual use research of concern regulations	N/A

### Analysis:

<b>Attrition</b>	<b>Indicate where provided: section/figure legend</b>	<b>N/A</b>
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.	No exclusions	N/A

<b>Statistics</b>	<b>Indicate where provided: section/figure legend</b>	<b>N/A</b>
Describe statistical tests used and justify choice of tests.	<p>A Student's t-test with a Welch's correction was used to compare untreated to treated values in Figure 3 and 6. Mentioned in Captions of figure 3, 6.</p> <p>This test is the gold standard for comparing between two groups because it does not assume equal sample size or variance.</p>	

<b>Data availability</b>	<b>Indicate where provided: section/submission form</b>	<b>N/A</b>
For newly created and reused datasets, the manuscript includes a data availability statement that provides details for access (or notes restrictions on access).	<p>Relevant trajectory files have been shared via Box (<a href="https://uofi.box.com/s/zldrqparb39ax72c637amcxi43hv2yaqj">\url{https://uofi.box.com/s/zldrqparb39ax72c637amcxi43hv2yaqj}</a>) and are available publicly.</p> <p>Relevant .npy files used for the generation of plots has been submitted to a Dryad Repository (<a href="https://doi.org/10.5061/dryad.76hdr7t4w">\url{https://doi.org/10.5061/dryad.76hdr7t4w}</a>).</p>	

	Section "Data Availability"	
When newly created datasets are publicly available, provide accession number in repository OR DOI and licensing details where available.	<p>Relevant trajectory files have been shared via Box (<a href="https://uofi.box.com/s/zldrqp arb39ax72c637amcxi43hv2yaq">\url{https://uofi.box.com/s/zldrqp arb39ax72c637amcxi43hv2yaq}</a>) and are available publicly.</p> <p>Relevant .npy files used for the generation of plots has been submitted to a Dryad Repository (<a href="https://doi.org/10.5061/dryad.76hdr7t4w">\url{https://doi.org/10.5061/dryad.76hdr7t4w}</a>).</p>	
If reused data is publicly available provide accession number in repository OR DOI, OR URL, OR citation.	No reused data	N/A

<b>Code availability</b>	<b>Indicate where provided: section/figure legend</b>	<b>N/A</b>
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.	<p>Python scripts used for analysis are available on GitHub (<a href="https://github.com/ShuklaGroup/Bansal_et_al_Cholesterol_Sm othened_2024">\url{https://github.com/ShuklaGroup/Bansal_et_al_Cholesterol_Sm othened_2024}</a>).</p>	
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.	<p>Python scripts used for analysis are available on GitHub (<a href="https://github.com/ShuklaGroup/Bansal_et_al_Cholesterol_Sm othened_2024">\url{https://github.com/ShuklaGroup/Bansal_et_al_Cholesterol_Sm othened_2024}</a>).</p>	
If reused code is publicly available provide accession number in repository OR DOI OR URL, OR citation.	No reused code	

## Reporting:

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

<b>Adherence to community standards</b>	<b>Indicate where provided: section/figure legend</b>	<b>N/A</b>

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.	Not a clinical trial (CONSORT, ICMJE) Not a systematic review or meta-analysis (PRISMA) Not an animal study (ARRIVE) Not a minimum information standard experiment like microarrays or proteomics (MIBBI) Not a human behavioral or survey study (STRANGE)	N/A
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\* 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](#).

### 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