| Item No | RECOMMENDATION                                                                                                                                                                                                 | Reported on Page Number/Line Number | Reported on Section/Paragraph |
|---------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|------------------------------|
| 1       | Provide as accurate and concise a description of the content of the article as possible.                                                                                                                        | Page 1, Line 3-4                    | Title                        |
| 2       | Provide an accurate summary of the background, research objectives, including details of the species or strain of animal used, key methods, principal findings and conclusions of the study.                             | Page 2, Line 1-28                  | Abstract                     |
|         | **INTRODUCTION**                                                                                                                                                                                                                                                         |
| 3       | a. Include sufficient scientific background (including relevant references to previous work) to understand the motivation and context for the study, and explain the experimental approach and rationale.    | Page 2/Line 32-Page 3/Line 27       | Introduction/Paragraph 1-2   |
|         | b. Explain how and why the animal species and model being used can address the scientific objectives and, where appropriate, the study’s relevance to human biology.                                             |                                      |                              |
| 4       | Clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested.                                                                                              | Page 3, Line 28-34                  | Introduction/Paragraph 3    |
|         | **METHODS**                                                                                                                                                                                                     |
| 5       | Indicate the nature of the ethical review permissions, relevant licences (e.g. Animal [Scientific Procedures] Act 1986), and national or institutional guidelines for the care and use of animals, that cover the research. | Page 4/Line 13-17, and Page 7/Line 29-32 | Methods/Paragraph 1 and 11 |
| 6       | For each experiment, give brief details of the study design including:                                                                                                                                         | Page 4/Line 5-Page 8/Line 4         | Methods/Method               |
|         | a. The number of experimental and control groups.                                                                                                                                                               |                                      |                              |
|         | b. Any steps taken to minimise the effects of subjective bias when allocating animals to treatment (e.g. randomisation procedure) and when assessing results (e.g. if done, describe who was blinded and when). |                                      |                              |
|         | c. The experimental unit (e.g. a single animal, group or cage of animals). A time-line diagram or flow chart can be useful to illustrate how complex study designs were carried out.                             |                                      |                              |
| 7       | For each experiment and each experimental group, including controls, provide precise details of all procedures carried out. For example:                                                                        | Page 4/Line 19-Page 7/Line 32       | Methods/Paragraph 2-11       |
|         | a. How (e.g. drug formulation and dose, site and route of administration, anaesthesia and analgesia used [including monitoring], surgical procedure, method of euthanasia). Provide details of any specialist equipment used, including supplier(s). |                                      |                              |
|         | b. When (e.g. time of day).                                                                                                                                                                                    |                                      |                              |
|         | c. Where (e.g. home cage, laboratory, water maze).                                                                                                                                                            |                                      |                              |
|         | d. Why (e.g. rationale for choice of specific anaesthetic, route of administration, drug dose used).                                                                                                         |                                      |                              |
| Section | Row | Details |
|---------|-----|---------|
| Experimental animals | 8 | a. Provide details of the animals used, including species, strain, sex, developmental stage (e.g. mean or median age plus age range) and weight (e.g. mean or median weight plus weight range).<br>b. Provide further relevant information such as the source of animals, international strain nomenclature, genetic modification status (e.g. knock-out or transgenic), genotype, health/immune status, drug or test naïve, previous procedures, etc. |
| Housing and husbandry | 9 | Provide details of:<br>a. Housing (type of facility e.g. specific pathogen free [SPF]; type of cage or housing; bedding material; number of cage companions; tank shape and material etc. for fish).<br>b. Husbandry conditions (e.g. breeding programme, light/dark cycle, temperature, quality of water etc for fish, type of food, access to food and water, environmental enrichment).<br>c. Welfare-related assessments and interventions that were carried out prior to, during, or after the experiment. |
| Sample size | 10 | a. Specify the total number of animals used in each experiment, and the number of animals in each experimental group. <br>b. Explain how the number of animals was arrived at. Provide details of any sample size calculation used. <br>c. Indicate the number of independent replications of each experiment, if relevant. |
| Allocating animals to experimental groups | 11 | a. Give full details of how animals were allocated to experimental groups, including randomisation or matching if done. <br>b. Describe the order in which the animals in the different experimental groups were treated and assessed. |
| Experimental outcomes | 12 | Clearly define the primary and secondary experimental outcomes assessed (e.g. cell death, molecular markers, behavioural changes). |
| Statistical methods | 13 | a. Provide details of the statistical methods used for each analysis. <br>b. Specify the unit of analysis for each dataset (e.g. single animal, group of animals, single neuron). <br>c. Describe any methods used to assess whether the data met the assumptions of the statistical approach. |
| RESULTS | Baseline data | 14 | For each experimental group, report relevant characteristics and health status of animals (e.g. weight, microbiological status, and drug or test naïve) prior to treatment or testing. (This information can often be tabulated). |
| Numbers analysed | 15 | a. Report the number of animals in each group included in each analysis. Report absolute numbers (e.g. 10/20, not 50%).<br>b. If any animals or data were not included in the analysis, explain why. |
| Outcomes and estimation | 16 | Report the results for each analysis carried out, with a measure of precision (e.g. standard error or confidence interval). |
| Adverse events | 17 | a. Give details of all important adverse events in each experimental group. <br>b. Describe any modifications to the experimental protocols made to reduce adverse events. |
| DISCUSSION |
|---|---|---|
| Interpretation/ scientific implications | 18 | a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature.
   b. Comment on the study limitations including any potential sources of bias, any limitations of the animal model, and the imprecision associated with the results.
   c. Describe any implications of your experimental methods or findings for the replacement, refinement or reduction (the 3Rs) of the use of animals in research. | N/A | The animal experiments in this article refer to the experimental methods of predecessors and do not explain the limitations of animal experiments |
| Generalisability/ translation | 19 | Comment on whether, and how, the findings of this study are likely to translate to other species or systems, including any relevance to human biology. | N/A | No indication of possible applicability to other species or systems |
| Funding | 20 | List all funding sources (including grant number) and the role of the funder(s) in the study. | Page 12 Line 15-17 | Acknowledgments 1 |

From:
Animal Research: Reporting In Vivo Experiments
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References:
1. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG (2010) Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. PLoS Biol 8(6): e1000412. doi:10.1371/journal.pbio.1000412
2. Schulz KF, Altman DG, Moher D, the CONSORT Group (2010) CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. BMJ 340:c332.

Article information: http://dx.doi.org/10.21037/tau-21-244
*As the checklist was provided upon initial submission, the page number/line number reported may be changed due to copyediting and may not be referable in the published version. In this case, the section/paragraph may be used as an alternative reference.
**Materials Design Analysis Reporting (MDAR)**

Checklist for Authors

The MDAR framework establishes a minimum set of requirements in transparent reporting applicable to studies in the life sciences (see Statement of Task: doi:10.31222/osf.io/9sm4x). The MDAR checklist is a tool for authors, editors and others seeking to adopt the MDAR framework for transparent reporting in manuscripts and other outputs. Please refer to the MDAR Elaboration Document for additional context for the MDAR framework.
### Materials

| Section                  | Provided? | (Indicate where provided: section/paragraph) | RRID  |
|--------------------------|-----------|---------------------------------------------|-------|
| **Antibodies**           | Yes       | (Indicate where provided: section/paragraph) | n/a   |
| For commercial reagents, provide supplier name, catalogue number and RRID, if available. | Yes       |                                             |       |
| **Cell materials**       | Yes       | (Indicate where provided: section/paragraph) | n/a   |
| Cell lines: Provide species information, strain. Provide accession number in repository OR supplier name, catalog number, clone number, OR RRID | Yes       |                                             |       |
| Primary cultures: Provide species, strain, sex of origin, genetic modification status. | Yes       |                                             |       |
| **Experimental animals** | Yes       | (Indicate where provided: section/paragraph) | n/a   |
| Laboratory animals: Provide species, strain, sex, age, genetic modification status. Provide accession number in repository OR supplier name, catalog number, clone number, OR RRID | Yes       |                                             |       |
| Animal observed in or captured from the field: Provide species, sex and age where possible | n/a       |                                             |       |
| Model organisms: Provide Accession number in repository (where relevant) OR RRID | n/a       |                                             |       |
| **Plants and microbes**  | Yes       | (Indicate where provided: section/paragraph) | n/a   |
| Plants: provide species and strain, 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** | Yes       | (Indicate where provided: section/paragraph) | n/a   |
| Identify authority granting ethics approval (IRB or equivalent committee(s), provide reference number for approval. | Yes       |                                             |       |
| Provide statement confirming informed consent obtained from study participants. | Yes       |                                             |       |
| Report on age and sex for all study participants. | Yes       |                                             |       |
## Design

| Study protocol |
|----------------|
| Yes (indicate where provided: section/paragraph) |
| n/a |
| For clinical trials, provide the trial registration number OR cite DOI in manuscript. |

| Laboratory protocol |
|---------------------|
| Yes (indicate where provided: section/paragraph) |
| n/a |
| Provide DOI or other citation details if detailed step-by-step protocols are available. |

| Experimental study design (statistics details) |
|-----------------------------------------------|
| Yes (indicate where provided: section/paragraph) |
| n/a |
| State whether and how the following have been done, or if they were not carried out. |
| Sample size determination |
| Randomisation |
| Blinding |
| Inclusion/exclusion criteria |

| Sample definition and in-laboratory replication |
|-----------------------------------------------|
| Yes (indicate where provided: section/paragraph) |
| n/a |
| State number of times the experiment was replicated in laboratory |
| Define whether data describe technical or biological replicates |

| Ethics |
|--------|
| Yes (indicate where provided: section/paragraph) |
| n/a |
| Studies involving human participants: State details of authority granting ethics approval (IRB or equivalent committee(s), provide reference number for approval. |
| Studies involving experimental animals: State details of authority granting ethics approval (IRB or equivalent committee(s), provide reference number for approval. |
| Studies involving specimen and field samples: State if relevant permits obtained, provide details of authority approving study; if none were required, explain why. |

| Dual Use Research of Concern (DURC) |
|------------------------------------|
| Yes (indicate where provided: section/paragraph) |
| n/a |
| If study is subject to dual use research of concern, state the authority granting approval and reference number for the regulatory approval |
### Analysis

| Attrition | Yes (indicate where provided: section/paragraph) | n/a |
|-----------|-------------------------------------------------|-----|
| State if sample or data point from the analysis is excluded, and whether the criteria for exclusion were determined and specified in advance. | Yes | |

| Statistics | Yes (indicate where provided: section/paragraph) | n/a |
|------------|-------------------------------------------------|-----|
| Describe statistical tests used and justify choice of tests. | Yes | |

| Data Availability | Yes (indicate where provided: section/paragraph) | n/a |
|-------------------|-------------------------------------------------|-----|
| State whether newly created datasets are available, including protocols for access or restriction on access. | Yes | |
| If data are publicly available, provide accession number in repository or DOI or URL. | No | |
| If publicly available data are reused, provide accession number in repository or DOI or URL, where possible. | No | |

| Code Availability | Yes (indicate where provided: section/paragraph) | n/a |
|-------------------|-------------------------------------------------|-----|
| For all newly generated code and software essential for replicating the main findings of the study: | | |
| State whether the code or software is available. | n/a | |
| If code is publicly available, provide accession number in repository, or DOI or URL. | n/a | |

### Reporting

| Adherence to community standards | Yes (indicate where provided: section/paragraph) | n/a |
|---------------------------------|-------------------------------------------------|-----|
| MDAR framework recommends adoption of discipline-specific guidelines, established and endorsed through community initiatives. Journals have their own policy about requiring specific guidelines and recommendations to complement MDAR. | | |
| State if relevant guidelines (e.g., ICMJE, MIBBI, ARRIVE) have been followed, and whether a checklist (e.g., CONSORT, PRISMA, ARRIVE) is provided with the manuscript. | ICMJE guidelines were followed, as the journal follows ICMJE recommendations for publication. | |

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