NOTE: Please save this file locally before filling in the table, DO NOT work on the file within your internet browser as changes will not be saved. Adobe Acrobat Reader (available free here) is recommended for completion.

The ARRIVE guidelines 2.0: author checklist

The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

Item		Recommendation	Section/line number, or reason for not reporting
Study design	1	For each experiment, provide brief details of study design including:	
		a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated.	Methods/Line 94-98
		b. The experimental unit (e.g. a single animal, litter, or cage of animals).	Litter
Sample size	2	a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used.	Methods/Line 93-104
		b. Explain how the sample size was decided. Provide details of any *a priori* sample size calculation, if done.	Use the Power and sample size software
Inclusion and exclusion criteria	3	a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established *a priori.* If no criteria were set, state this explicitly.	Methods/Line 111-113
		b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so.	Methods/Line 111-113
		c. For each analysis, report the exact value of *n* in each experimental group.	In each Figure Legend
Randomisation	4	a. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence.	The mouse numbers were labeled and random grouping was achieved by generating random numbers using SPSS software.
		b. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.	Methods/Line 116-117
Blinding	5	Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	The different stages of the experiment were carried out according to the group numbers. Results the evaluation and data analysis results were entered according to the corresponding numbers of each group. According to the allocation of different stages of the experiment, there are corresponding records on the label outside the cage. Correspondingly, the result evaluation and data analysis are analyzed according to the random cage unit.
Outcome measures	6	a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes).	Methods/Line 111-113
		b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	Methods/Line 111-113
Statistical methods	7	a. Provide details of the statistical methods used for each analysis, including software used.	Methods/Line 171-175
		b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	Methods/Line 171-175
Experimental animals	8	a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight.	Methods/Line 93-94
		b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	Methods/Line 109-111
Experimental procedures	9	For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including:	Methods
		a. What was done, how it was done and what was used.	Methods/Line 93-117
		b. When and how often.	Methods/Line 93-117
		c. Where (including detail of any acclimatisation periods).	Methods/Line 93-117
		d. Why (provide rationale for procedures).	Methods/Line 106-108
Results	10	For each experiment conducted, including independent replications, report:	The sample size of each round included at least three animals in each case. Data are presented asthe mean ± standard deviation (s.d.). Groups were compared by the one-way analysis of variance (ANOVA) using GraphPad Prism Software.Statistical significance of $P < 0.05$ was accepted.
		a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range).	According to the effect of 50% increase and 25% standard deviation compared with the control group. The acceptable error rate is 5%.
		b. If applicable, the effect size with a confidence interval.	

The Recommended Set

These items complement the Essential 10 and add important context to the study. Reporting the items in both sets represents best practice.

Item		Recommendation	Section/line number, or reason for not reporting
Abstract	11	Provide an accurate summary of the research objectives, animal species, strain and sex, key methods, principal findings, and study conclusions.	Abstract/Line 18-40
Background	12	a. Include sufficient scientific background to understand the rationale and context for the study, and explain the experimental approach.	Background/Line 19-23
		b. Explain how the animal species and model used address the scientific objectives and, where appropriate, the relevance to human biology.	Methods/Line 109-113
Objectives	13	Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested.	Introduction/Line 88-89
Ethical statement	14	Provide the name of the ethical review committee or equivalent that has approved the use of animals in this study, and any relevant licence or protocol numbers (if applicable). If ethical approval was not sought or granted, provide a justification.	Methods/Line 106-108
Housing and husbandry	15	Provide details of housing and husbandry conditions, including any environmental enrichment.	Methods/Line 104-106
Animal care and monitoring	16	a. Describe any interventions or steps taken in the experimental protocols to reduce pain, suffering and distress.	The mice were anesthetized with 2% isoflurane prior to sacrifice.
		b. Report any expected or unexpected adverse events.	If there are unexpected adverse events, the samples will be excluded. Then the experiment would be repeated.
		c. Describe the humane endpoints established for the study, the signs that were monitored and the frequency of monitoring. If the study did not have humane endpoints, state this.	If the experiment may cause adverse consequences such as movement difficulty, severe infection, severe pain and other adverse consequences, we administered anesthesia and analgesia and other methods for nursing upon observation twice per day . If the symptoms are not relieved after nursing, a humane end point of animal euthanasia is carried out. The way to euthanasia is to inhale excess carbon dioxide.
Interpretation/ scientific implications	17	a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature.	Discussion/Line 250-335
		b. Comment on the study limitations including potential sources of bias, limitations of the animal model, and imprecision associated with the results.	Discussion/Line 329-331
Generalisability/ translation	18	Comment on whether, and how, the findings of this study are likely to generalise to other species or experimental conditions, including any relevance to human biology (where appropriate).	Discussion/Line 333-335
Protocol registration	19	Provide a statement indicating whether a protocol (including the research question, key design features, and analysis plan) was prepared before the study, and if and where this protocol was registered.	Methods/Line 117
Data access	20	Provide a statement describing if and where study data are available.	Study data is available in the online version of the paper.
Declaration of interests	21	a. Declare any potential conflicts of interest, including financial and non-financial. If none exist, this should be stated.	Footnote/Line 347-348
		b. List all funding sources (including grant identifier) and the role of the funder(s) in the design, analysis and reporting of the study.	Acknowledgements/Line 341-344

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