



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: <ol style="list-style-type: none"> The groups being compared, including control groups. If no control group has been used, the rationale should be stated. The experimental unit (e.g. a single animal, litter, or cage of animals). 	a. Abstract/ line 8-17. b.Methods/ line 129-195. a single rat.
Sample size	2 <ol style="list-style-type: none"> 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. Explain how the sample size was decided. Provide details of any <i>a priori</i> sample size calculation, if done. 	a. Animals and model establishment/ line 108, 113 and 123. b. N/A. A small sample size was selected for the pilot study.
Inclusion and exclusion criteria	3 <ol style="list-style-type: none"> 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 <i>a priori</i>. If no criteria were set, state this explicitly. 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. For each analysis, report the exact value of <i>n</i> in each experimental group. 	a. Animals and model establishment/ line 121. b.Animals and model establishment/ line 123, 207. The animal model was successfully constructed, so there were no exclusions. c. Animals and model establishment/line 113 and 123.
Randomisation	4 <ol style="list-style-type: none"> 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. 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. 	a. Animals and model establishment/ Line 111. b.Animals and model establishment/ Line 119, 125 and 126.
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).	CEUS image analysis/ line 166. The investigator administering the treatment is the only person who aware of group allocation.
Outcome measures	6 <ol style="list-style-type: none"> Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size. 	a.Results/ line 206-246. b.Results/ line 216-240. The result of CEUS and SWE of the three groups are the primary measure.
Statistical methods	7 <ol style="list-style-type: none"> Provide details of the statistical methods used for each analysis, including software used. 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. 	a.Statistical Analysis/ line 196-205. b.Statistical Analysis/ line 196-202.
Experimental animals	8 <ol style="list-style-type: none"> Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures. 	a.Animals and model establishment/ Line 108-109. b. Animals and model establishment/ Line 108-111.
Experimental procedures	9 For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: <ol style="list-style-type: none"> What was done, how it was done and what was used. When and how often. Where (including detail of any acclimatisation periods). Why (provide rationale for procedures). 	a.Method/ Line 109-195. b.Method/ Line 109-195. c.Animals and model establishment/ Line 112. d.Method/ Line 109-195.
Results	10 For each experiment conducted, including independent replications, report: <ol style="list-style-type: none"> Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). If applicable, the effect size with a confidence interval. 	a. Results/ Line 207-232. Present data clearly as text, in tables, or in graphs. b. Results /Line 236-240.

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 1-31.
Background	12 a. Include sufficient scientific background to understand the rationale and context for the study, and explain the experimental approach. b. Explain how the animal species and model used address the scientific objectives and, where appropriate, the relevance to human biology.	a. Introduction/ line 46-98. b. Introduction/ line 99-103.
Objectives	13 Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested.	Introduction/ line 99-103.
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.	Animals and model establishment/ line 106-107. Ethical Statement/ line 365-367.
Housing and husbandry	15 Provide details of housing and husbandry conditions, including any environmental enrichment.	Animals and model establishment/ line 109-111.
Animal care and monitoring	16 a. Describe any interventions or steps taken in the experimental protocols to reduce pain, suffering and distress. b. Report any expected or unexpected adverse events. 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.	a. Animals and model establishment/ Line 127-128. b. Results/ line 207. c. Animals and model establishment/ Line 121 and 127.
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. b. Comment on the study limitations including potential sources of bias, limitations of the animal model, and imprecision associated with the results.	a. Discussion/line 268-333. b. Discussion/line 334-343.
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).	Conclusions/ line 345-349.
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.	A protocol was prepared before the study without registration.
Data access	20 Provide a statement describing if and where study data are available.	Open Access Statement/ line 369-375.
Declaration of interests	21 a. Declare any potential conflicts of interest, including financial and non-financial. If none exist, this should be stated. b. List all funding sources (including grant identifier) and the role of the funder(s) in the design, analysis and reporting of the study.	a. Conflicts of Interest/ line 360-361. b. Acknowledgments/ line 351-355.

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