



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: - 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).	Sham and SAH groups were compared in the in vivo validation experiment. The individual mouse was the experimental unit.
Sample size	2 - 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.	were allocated to the Sham and SAH groups (Sham, n = 6; SAH, n = 6), and six additional donor mice were used for arterial blood collection for SAH induction. Donor mice were not included in the outcome analyses. The sample size of six recipient mice per group was determined based on previous experimental experience and commonly used sample sizes in similar experimental SAH studies. No formal <i>a priori</i> sample size calculation was performed.
Inclusion and exclusion criteria	3 - 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.	No separate prespecified inclusion or exclusion criteria were established beyond successful completion of the assigned Sham or SAH procedure. No recipient animals or corresponding data points were excluded from the final in vivo analyses. The final sample size was <i>n</i> = 6 mice per group for all reported in vivo analyses.
Randomisation	4 - 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.	Recipient mice were allocated to the Sham and SAH groups using a random number generator. No formal computer-generated or random-number sequence was used for group allocation. All surgical procedures, postoperative handling, tissue collection, and outcome assessments were performed under standardised experimental conditions. Cage position and the order of procedures or measurements were not specifically randomized.
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).	Investigators responsible for outcome assessment and data analysis were blinded to group allocation. Personnel performing the Sham or SAH surgical procedures were aware of group allocation because of the nature of the intervention.
Outcome measures	6 - 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.	The in vivo outcome measures included AI22-based neuronal density and Western blot analysis of p53, p65, and phosphorylated p65, together with correlation analysis between AI22 expression and p53 activation. No single primary outcome was prespecified for formal sample-size determination because no <i>a priori</i> sample-size calculation was performed.
Statistical methods	7 - 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.	by Tukey's <i>post hoc</i> test. The in vivo Pearson correlation analysis shown in Figure 6C was performed using GraphPad Prism 8. Transcriptional Pearson correlation analyses were performed using R version 4.3.2. No obvious major departures from the assumptions underlying the parametric tests were identified. If substantial violations had been observed, appropriate non-parametric alternatives would have been used.
Experimental animals	8 - 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.	Male C57BL/6J mice aged 8–10 weeks and weighing 25 ± 1 g were used for the in vivo SAH experiments. Both recipient and donor mice were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd., Beijing, China. The animals were not genetically modified and had not undergone previous experimental procedures.
Experimental procedures	9 For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: - 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).	Use of physiological saline. Isoflurane anaesthesia was used during SAH surgery, whereas xylazine plus ketamine was used for terminal anaesthesia before euthanasia and tissue collection. Brain tissues were collected at the predefined experimental endpoint immediately after euthanasia. The in vivo analyses reported in this manuscript were based on a single post-SAH endpoint. The experimental SAH model was used to provide in vivo validation of the association between AI22 expression and p53 activation identified in the neuronal and transcriptional analyses.
Results	10 For each experiment conducted, including independent replications, report: - 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.	In vivo data are presented as mean ± SEM, with <i>n</i> = 6 mice per group. Size measures for the correlation analyses. The in vivo group comparisons were designed primarily as exploratory validation rather than as formal treatment-effect estimation; therefore, separate standardized effect sizes and confidence intervals were not prespecified.

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.	ents, as specified in the Methods section.
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.	The Introduction provides the scientific background and rationale for investigating AIH2/NF- κ B signaling in SAH. The experimental SAH mouse model was used to assess the in vivo relevance of the findings obtained from the neuronal and transcriptomic analyses.
Objectives	13	Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested.	al in vivo validation in experimental SAH.
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.	Animal Research Ethics Committee of Wenzhou Medical University; Approval No. wydw2025-0495.
Housing and husbandry	15	Provide details of housing and husbandry conditions, including any environmental enrichment.	ved the institutional animal-facility SOP.
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.	20. At the experimental endpoint, apomorphine plus ketamine was used for terminal anaesthesia before euthanasia and tissue collection. Animals were observed during perioperative recovery, and all invasive procedures were performed in accordance with institutional animal-care requirements. No unexpected adverse events were recorded during the reported in vivo experiments. 21. During humane assessment, no signs of pain or distress. Humane endpoints were applied in accordance with institutional animal-care requirements; animals showing severe or persistent distress or a moribund condition would have been euthanized promptly.
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.	The Discussion interprets the findings in relation to the study objectives, current knowledge of AIH2/NF- κ B signaling, and previous experimental SAH studies. 20. Although the objective of a randomised sequence, blinding during outcome assessment and data analysis was used to reduce observer bias. In addition, the in vivo analyses primarily provide associative validation rather than direct evidence of causality.
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).	The in vivo findings should be interpreted in the context of male C57BL/6J mice and the specific experimental SAH model used. Generalisability to female animals, other mouse strains, other species, alternative SAH models, and human disease remains to be established. Further validation in diverse experimental models and clinical samples is required.
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 prespecified animal experimental protocol was prepared before the study as part of ethics application No. 18906 and was reviewed by the Animal Research Ethics Committee of Wenzhou Medical University. The protocol was not registered in a public protocol registry.
Data access	20	Provide a statement describing if and where study data are available.	er accession numbers GSE36791 and GSE73378.
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.	The authors declare no conflicts of interest. Research funding, participated in the study design, research supervision, data analysis, interpretation of the results, and manuscript preparation and revision. The funding agencies themselves had no role in the decision to publish.