



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).	2.7 A total of four groups were compared in this study, including two control groups (shCont + DMSO and shCont + DHA) and two experimental groups (shRALB + DMSO and shRALB + DHA). 2.7 Five mice were used in each group.
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.	2.7 Five mice were allocated to each group, totaling 20 mice across all groups. 2.7 The sample size was determined based on preliminary pilot experiments and previous literature.
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.	2.7 Inclusion: male BALB/c nude mice, 6 weeks, 18–22 g, healthy. Exclusion: no tumor formation. All criteria were established <i>a priori</i> . 2.7 No animals, experimental units, or data points were excluded from the analysis, and all data were incorporated in the final analysis. 2.7 <i>n</i> = 5 per group
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.	2.7 Yes 2.7 Fixed dosing time; standardized measurement sequence; cages randomly positioned and rearranged twice weekly; blinded assessment and quantification
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).	2.9 H&E and IHC (P62/LC3B) slides were coded and evaluated in a double-blinded manner by two independent pathologists. Group allocation was revealed after all analyses were completed.
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.	2.9, 2.10 Autophagy markers: protein expression levels of P62 and LC3B measured by Western blot, and their expression/labelization in tongue tissues assessed by IHC. 3.6 This study is a hypothesis-testing study (comparing the shCont group with the shRALB group).
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.	2.11 All data are presented as mean $\pm$ SD. For comparisons between two groups, the t-test was used when data were normally distributed. For comparisons among more than two groups, one-way ANOVA was employed after confirming homogeneity of variance. 2.11 Data were first assessed for normal distribution (regular distribution) and homogeneity of variance. When data met these assumptions, parametric tests (t-test or ANOVA) were applied.
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.	2.7 Male BALB/c nude mice (BALB/c Nude), 6 weeks old, weighing 18–22 g, were used in this study. 2.7 Mice (SPF, 1-cell/100121, 100121) were obtained from GenPharmatech and Sipeifu, housed under 12h light/dark cycles with free access to food/water, and acclimatized for one week before experiments.
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).	2.7 Intratongue injection of 1×10^6 Cal-27 cells (shCont/shRALB); DHA 25 mg/kg 2.7 Daily for 10 days from day 7 post-injection 2.7 SPF barrier facility; 1 week acclimatisation 2.7 Dose/timing based on literature
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.	2.11 All data expressed as mean $\pm$ SD; details in corresponding figures Not applicable. Effect sizes were not calculated for this study; therefore, confidence intervals are not reported.