**Methods**

**according to the ARRIVE Guidelines (**<https://doi.org/10.1371/journal.pbio.1000412>**)**

|  |  |
| --- | --- |
| Ethical statement | DELETE THE PREFILLED TEXT IN THIS COLUMN, AND DESCRIBE ALL THE STUDY METHODS ACCORDING TO THE ARRIVE GUIDELINES (ITEMS IN THE TEXT BELOW)  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. |
| Study design | For each experiment, give brief details of the study design, including:   1. The number of experimental and control groups. 2. 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). 3. 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. |
| Experimental procedures | For each experiment and each experimental group, including controls, provide precise details of all  procedures carried out. For example:   1. 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). 2. When (e.g., time of day). 3. Where (e.g., home cage, laboratory, water maze). 4. Why (e.g., rationale for choice of specific anaesthetic, route of administration, drug dose used). |
| Experimental animals | 1. 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). 2. 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 testnaïve, previous procedures, etc. |
| Housing and husbandry | Provide details of:   1. Housing (e.g., 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). 2. 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). 3. Welfare-related assessments and interventions that were carried out before, during, or after the experiment. |
| Sample size | 1. Specify the total number of animals used in each experiment and the number of animals in each experimental group. 2. Explain how the number of animals was decided. Provide details of any sample size calculation used. 3. Indicate the number of independent replications of each experiment, if relevant. |
| Allocating animals to experimental groups | 1. Give full details of how animals were allocated to experimental groups, including randomisation or matching if done. 2. Describe the order in which the animals in the different experimental groups were treated and assessed. |
| Experimental outcomes | Clearly define the primary and secondary experimental outcomes assessed (e.g., cell death, molecular markers, behavioural changes). |
| Statistical methods | 1. Provide details of the statistical methods used for each analysis. 2. Specify the unit of analysis for each dataset (e.g. single animal, group of animals, single neuron). 3. Describe any methods used to assess whether the data met the assumptions of the statistical approach. |