



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. b. The experimental unit (e.g. a single animal, litter, or cage of animals).	1a. Detailed descriptions of the <i>in vivo</i> experimental design and the definition of the experimental unit were provided in the Materials and Methods section, Orthotopic xenograft tumor growth assay (line 184-191) and <i>In vivo</i> tumor-initiating cell (TIC) limiting dilution assay (line 203-210), as well as in the legend for Fig. 4 (line 622-636). 1b. The experimental unit was an individual mouse, as described in the Materials and Methods section, Orthotopic xenograft tumor growth assay (line 190-191).
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. b. Explain how the sample size was decided. Provide details of any <i>a priori</i> sample size calculation, if done.	2a. The total sample size for each study group, as well as group allocation and samples used, were described in the Materials and Methods section, Orthotopic xenograft tumor growth assay (line 184-191) and <i>In vivo</i> tumor-initiating cell (TIC) limiting dilution assay (line 203-210), as well as in the legend for Fig. 4 (line 622-636). 2b. Sample sizes were determined based on prior experience and pilot studies, as described in the Materials and Methods section, Sample size determination (line 220-222).
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 <i>a priori</i>. If no criteria were set, state this explicitly. 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. c. For each analysis, report the exact value of <i>n</i> in each experimental group.	3a. No inclusion or exclusion criteria were pre-established. All injected mice were included in the analyses, as described in the Materials and Methods, Inclusion and exclusion criteria (line 224-227). 3b. No animals, experimental units, or data points were excluded from any analysis, as described in the Materials and Methods, Inclusion and exclusion criteria (line 224-227). 3c. The number of experimental animals in each group and their allocation was described in the legend for Fig. 4 (line 622-636).
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. 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.	4a. Mice were randomly assigned to experimental groups after cell preparation and prior to injection, as described in the Materials and Methods section, Randomization and blinding (line 229-231). 4b. Mice were randomly assigned to experimental groups prior to injection. No additional strategies were implemented to minimise potential confounders such as treatment order, measurement order, or animal/cage location. Tumor monitoring, measurements, and data analysis were conducted without blinding, as described in the Materials and Methods section, Randomization and blinding (line 229-231).

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).	5. Tumor monitoring, measurements, and data analysis were conducted without blinding, as described in the Materials and Methods section, Randomization and blinding (line 229-231).
Outcome measures	6	a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	6a. For the tumor growth assays, the primary outcome measure was tumor growth over time, assessed by weekly monitoring of tumor formation at the injection site. Tumor presence and progression were evaluated by palpation, as described in the Materials and Methods section, Orthotopic xenograft tumor growth assay (line 189-196). For the TIC limiting dilution assay, the primary outcome measure was tumor take, defined as the presence or absence of a palpable and progressively growing tumor within a three-month observation period. Tumor take was recorded as a binary outcome (yes/no) and used to estimate tumor-initiating cell frequency, as described in the Materials and Methods section, <i>In vivo</i> tumor-initiating cell (TIC) limiting dilution assay (line 211-218). 6b. No a priori sample size calculation was performed for the <i>in vivo</i> experiments. Sample sizes were determined based on prior experience and pilot studies, as described in the Materials and Methods section, Sample size determination (line 220-222).
Statistical methods	7	a. Provide details of the statistical methods used for each analysis, including software used. 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.	7a. Tumor growth data were analyzed using two-way ANOVA with treatment group and time as independent variables, as described in the legend for Fig. 4 (line 618-632). Tumor-initiating cell (TIC) frequency was estimated using extreme limiting dilution analysis (ELDA) based on a single-hit Poisson model. Maximum likelihood estimation was used to calculate TIC frequency and 95% confidence intervals, and differences between groups were assessed using a likelihood ratio test, as described in the Materials and Methods section, <i>In vivo</i> tumor-initiating cell (TIC) limiting dilution assay (line 211-218). Tumor growth data were analyzed using GraphPad Prism 5.0, as described in the Materials and Methods section, Statistical analysis (line 277-281). 7b. No formal tests were conducted to assess whether the data met the assumptions of the statistical methods, as described in the Materials and Methods section, Statistical analysis (line 280-281).
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. b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	8a. Detailed informations on experimental animals were described in the Materials and Methods section, Orthotopic xenograft tumor growth assay (line 184-189). 8b. Detailed information on experimental animals was described in the Materials and Methods section, Orthotopic xenograft tumor growth assay (line 184-189).
Experimental procedures	9	For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: a. What was done, how it was done and what was used. b. When and how often. c. Where (including detail of any acclimatisation periods). d. Why (provide rationale for procedures).	9a. Detailed informations were described in the Materials and Methods section, Orthotopic xenograft tumor growth assay (line 184-191) and <i>In vivo</i> tumor-initiating cell (TIC) limiting dilution assay (line 203-218). 9b. The details were described in the Materials and Methods section, Orthotopic xenograft tumor growth assay (lines 184-191) and <i>In vivo</i> tumor-initiating cell (TIC) limiting dilution assay (line 203-218). 9c. The details were described in the Materials and Methods section, Orthotopic xenograft tumor growth assay (lines 184-201) and <i>In vivo</i> tumor-initiating cell (TIC) limiting dilution assay (line 203-218).

	9d. The details were described in the Materials and Methods section, Orthotopic xenograft tumor growth assay (lines 184-201) and <i>In vivo</i> tumor-initiating cell (TIC) limiting dilution assay (line 203-218).
Results 10 For each experiment conducted, including independent replications, report: a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). b. If applicable, the effect size with a confidence interval.	10a. Tumor growth data were presented as growth curves with summary statistics for each experimental group, and tumor incidence in the tumor-initiating cell (TIC) limiting dilution assay was reported as the number of tumors formed at each cell dose level, as described in the legend for Fig. 4 (line 622-636). 10b. Tumor-initiating cell (TIC) frequency was reported as an effect size with 95% confidence intervals, estimated using extreme limiting dilution analysis (ELDA). Differences between groups were assessed using a likelihood ratio test, as described in the Materials and Methods section, <i>In vivo</i> tumor-initiating cell (TIC) limiting dilution assay (line 211-218), as well as in the legend for Fig. 4 (line 632-636).