ature portfolio | reporting summary

March 202

nature portfolio

Corresponding author(s): DBPR-npjvaccines-00137R

Last updated by author(s): Jun 14, 2021

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

Statistics				
For all statistical ar	nalyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.			
n/a Confirmed				
☐ ☐ The exact	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement			
A stateme	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly			
The statistical test(s) used AND whether they are one- or two-sided Only common tests should be described solely by name; describe more complex techniques in the Methods section.				
A description of all covariates tested				
A descript	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons			
A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)				
For null h	ypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted less as exact values whenever suitable.			
For Bayes	ian analysis, information on the choice of priors and Markov chain Monte Carlo settings			
For hierar	chical and complex designs, identification of the appropriate level for tests and full reporting of outcomes			
Estimates	of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated			
	Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.			
Software an	d code			
Policy information	about availability of computer code			
Data collection	Data was collected and set up in Microsoft Excel 2010 and saved in .xls and afterwards imported into R for analysis.			
Data analysis	R graph and statistical analysis packages: ggplot2, ggpubr, nortest, Rmisc, PMCMR, ez, corrplot, graphics. Other R packages: PMCMRplus, plyr, dplyr, PMCMR, tidyverse, DescTools, scales.			
	g custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.			
Data				
All manuscripts m	about <u>availability of data</u> nust include a <u>data availability statement</u> . This statement should provide the following information, where applicable: s, unique identifiers, or web links for publicly available datasets			

- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The datasets generated for this study are available on request. The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Field-spe	ecific reportin	g · · · · · · · · · · · · · · · · · · ·		
		or your research. If you are not sure, read the appropriate sections before making your selection.		
Life sciences				
For a reference copy of	the document with all sections, see <u>na</u>	ature.com/documents/nr-reporting-summary-flat.pdf		
Lifo scior	nces study de	cian		
		then the disclosure is negative.		
Sample size		imple-size calculation was performed. Experimental groups based on 4-5 animals for the PTB infection model have been used before ning statistically significant results (Arrazuria et al Res Vet Sci 2015, Arrazuria et al. Vet Res 2016, Arrazuria et al Front Microbiol 2016 an uria et al Vet Sci 2020)		
Data exclusions	No data were excluded from th	ta were excluded from the analyses.		
Replication	Front Microbiol 2016 and Arraz management system (). Anima Standard UNE 166002:2014 for sectors) and in the natural envi transfer since 2005 – ER-1201-2	ne experimental infection model has shown to be reproducible Arrazuria et al Res Vet Sci 2015, Arrazuria et al. Vet Res 2016, Arrazuria et al ront Microbiol 2016 and Arrazuria et al Vet Sci 2020). All protocols are standarized and documented in the Research Institute's Quality and lanagement system (). Animals, samples and reagents are also tracked. NEIKER has an R&D&I management system in compliance with randard UNE 166002:2014 for research, development and innovation activities in the field of agri-food (agricultural, livestock and forestry ectors) and in the natural environment. Number: IDI-0009/2019, ISO 9001 Certification for the management of R&D projects and technology ansfer since 2005 – ER-1201-2005 and Accreditation no. 615/LE 1321 from ENAC for the carrying out of physical and chemical, iicrobiological, immunological and molecular tests on agri-food products at the Department of Animal Health.		
Randomization	Allocation of organisms to expe	ation of organisms to experimental groups was random. All animals belonged to the same lot, with similar characteristics.		
Blinding	blinded. Fluidigm service only h	organisms were identified with a number that was linked to a experimental group. Map tissue culture, IgA levels and PPA3 ELISA were nded. Fluidigm service only had knowledege about organisms assigned to the non-challenged group. The rest of the studied parameters are performed by researchers that knew which experimental group they were analyzing at each time.		
system or method lis	sted is relevant to your study. If yo	es of materials, experimental systems and methods used in many studies. Here, indicate whether each materi ou are not sure if a list item applies to your research, read the appropriate section before selecting a response		
	perimental systems	Methods		
n/a Involved in the study		n/a Involved in the study		
Antibodies		∑ ChIP-seq		
Eukaryotic cell lines		Flow cytometry		
K	logy and archaeology and other organisms	MRI-based neuroimaging		
	search participants	Aberingal Dauberts and he report for any street and are a second and the second and the second and the second		
Clinical da		the believes on the contract of the contract o		
	esearch of concern			
Z Z Z Z Z Z Z Z Z Z Z Z Z Z Z Z Z Z Z				
Animals and	other organisms			
12		als; ARRIVE guidelines recommended for reporting animal research		
		bbits females of 7 weeks of age at beginning of experiment		
caught and transported		nals observed in or captured in the field; report species, sex and age where possible. Describe how animals wer In and what happened to captive animals after the study (if killed, explain why and describe method; if release		
	say where and when) (OR state that the study did not involve wild animals.		
Field-collected s		ith field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, of-experiment protocol OR state that the study did not involve samples collected from the field.		

All animal procedures were carried out following European, National and Regional regulations on animals used in experimentation and other scientific purposes. The protocols were evaluated and approved by the Ethics Committee at NEIKER (NEIKER-OEBA-2018-0001) and authorized by the Regional Council (BFA-38012).

Ethics oversight

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

Confirm that:

- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

PMNs were isolated fresh from Acid Citrate Dextrose peripherla blood by dextran sedimentation and density gradient on histopaque. Suspensions were prepared in RPMI without phenol red supplemented with 2% FCS at 107 cells/ml.

Instrument

Beckman Coulter Cytoflex

Software

Beckman Coulter CytExpert v2.3 software

Cell population abundance

The isolation protocol yielded PMNs with 94.4 ± 5.9 % purity and 98.68 ± 1.1 % viability

Gating strategy

Gating strategy used for the phagocytosis analysis. (a) PMNs without live-dead staining and without Map-GFP, (b) PMNs with live-dead staining and without Map-GFP, (c) PMNs of the NC group with live-dead staining and with Map-GFP, (d) PMNs of the NC group with live-dead staining and with Map-GFP. In the first column details about leukocyte gating based on FSC and SSC; in the second step PMN-gating based on FSC and SSC (purity information); in the third column doublet discrimination strategy; in the fourth column dead cell discrimination strategy by fluorescence in the PB450 channel; in the fifth column GFP associated PMN selection by fluorescence in the FITC channel and in the sixth column a scatter plot based on FITC-FSC of single-alive-PMNs.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.