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Last updated by author(s): Mar 25, 2022

Reporting Summary

Nature Research 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 Research policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	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 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 hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical 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 statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	No software used
Data analysis	GraphPad PRISM 8 version. 8.0.2 (263) for graphs including cytokine levels, antibody titers, neutralization (graphing and statistics). Kaluza Analysis Software v. 2.1 (BD) for flow cytometry analysis. Xcalibur and TraceFinder (ThermoFisher) were used for data acquisition and analysis respectively. KC Junior (Biotek Instruments) for ELISA. MARS software (Clariostar) v. 5.70 R2 For ELISA. Excel (Microsoft Office) for data analysis.

For manuscripts utilizing 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 reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Authors can confirm that all relevant data are included in the paper and/or its supplementary information files
The data that support the findings of this study are available from the corresponding

author upon reasonable request.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample sizes were calculated based on the resource equation approach explained by Arifin and Zahiruddin (2017) - Sample size calculation in animal studies using resource equation approach. Malays J Med Sci. 2017;24(5):101–105. (https://doi.org/10.21315/mjms2017.24.5.11).
Data exclusions	No data was excluded
Replication	Pn the initial experiments, we tested two modes of injection of the EDV vaccine: intravenous and subcutaneous. Then we did a pilot study using intramuscular injection and two different concentrations of the vaccine. Based on our pilot study results, we proceeded to do the complete experiment using two concentrations of the vaccine delivered intramuscularly at three different time points (8hr,1 wk,4 wks -boost post 3 wks)
Randomization	N=4-10 mice were used per group (depending on the experiment). Serum and tissue samples (spleen, bone marrow) from all mice in each group were used to perform corresponding assays
Blinding	Mice were identified based on ear-tag numbering system in each group. Tissues/serum from each mouse were assayed individually

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☐	☒ Human research participants
☐	☒ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

SARS-CoV-2 (COVID-19) spike antibody against the S1 and S2 subunits, Genetex (Cat. #GTX135356), pAb.
SARS-CoV-2 (COVID-19) spike antibody against the S1 and S2 subunits, Genetex (Cat. #GTX632604), clone 1A9
SARS-CoV-2 (2019-nCoV) spike RBD rabbit PAb, antigen affinity purified (Cat. #40592-T62, Sino Biological).
ELISA antibodies used for cytokine analysis.
Mouse TNFα DuoSet R&D Systems ; DY41005
Mouse IFNαall subtype Verikine-HS PBL ; 42115
Mouse IFNγ DuoSet R&D Systems ; DY48505
Mouse IL12/p40 DuoSet R&D Systems; DY239805
Mouse IL4 DuoSet R&D Systems ; DY40405
Mouse IL10 DuoSet R&D Systems ; DY41705
Mouse IL6 DuoSet R&D Systems; DY40605
Mouse IL2 DuoSet R&D Systems; DY402
Mouse IL21 LEGEND MAX Mouse IL-21 ELISA Kit (Biolegend); 446107
T cell staining panel.
CD45 Anti-mouse CD45-AF488 Biolegend 103122; Clone 30F11; Rat IgG2b, κ
CD4 Anti-mouse CD4 [GK1.5] (PE-Texas Red®) Abcam ab51467; Clone GK1.5; Rat IgG2b, κ

OX40 Anti-mouse CD134 (OX-40)-APC Biolegend 119413; Clone OX-86; Rat IgG1, κ
CD3 Anti-mouse CD3-PE-Cy7 Biolegend 100220; Clone 17A2; Rat IgG2b, κ
CD8 Anti-human/mouse CD8-AF647 Biolegend 372906; Clone C8/144B; Mouse IgG1, κ
CD69 Anti-mouse CD69-BV421 Biolegend 104527; Clone H1/2F3
7-AAD Viability Staining Solution Biolegend 420404
Clinical B cell analysis; DuraClone IM B cells tube (Cat # B53318, Beckman Coulter)
Clinical T cell analysis; DuraClone IM T cell subsets tube (Cat # B53328, Beckman Coulter)

Validation

https://www.genetex.com/Product/Detail/SARS-CoV-2-COVID-19-Spike-antibody/GTX135356#references
https://www.genetex.com/Product/Detail/SARS-CoV-SARS-CoV-2-COVID-19-spike-antibody-1A9/GTX632604#references
SARS-CoV-2 (2019-nCoV) spike RBD rabbit PAb, (Cat. #40592-T62, Sino Biological). Validated applications, WB, ELISA https://www.sinobiological.com/antibodies/cov-spike-40592-t62
Mouse DuoSet ELISA kits- "Contains carefully selected and validated antibodies, reducing development time; Includes mass-calibrated recombinant standard, reducing assay variability". https://www.rndsystems.com/products/duoset-elisa-development-systems
CD45 Anti-mouse CD45-AF488 Biolegend 103122; " FC quality tested". https://www.biolegend.com/en-us/search-results/alexa-fluor-488-anti-mouse-cd45-antibody-3100
CD4 Anti-mouse CD4 [GK1.5] (PE-Texas Red®) Abcam ab51467; Clone GK1.5; Rat IgG2b, κ; " FC quality tested". https://www.biolegend.com/en-us/products/pe-anti-mouse-cd4-antibody-250?GroupID=BLG4745
OX40 Anti-mouse CD134 (OX-40)-APC Biolegend 119413; Clone OX-86; Rat IgG1, κ; " FC quality tested". https://www.biolegend.com/en-us/products/apc-anti-mouse-cd134-ox-40-antibody-8305?GroupID=BLG4698
CD3 Anti-mouse CD3-PE-Cy7 Biolegend 100220; Clone 17A2; Rat IgG2b, κ; " FC quality tested". https://www.biolegend.com/en-gb/products/pe-cyanine7-anti-mouse-cd3-antibody-6060?GroupID=BLG242
CD8 Anti-human/mouse CD8-AF647 Biolegend 372906; Clone C8/144B; Mouse IgG1, κ; " FC quality tested". https://www.biolegend.com/fr-ch/products/alexa-fluor-647-anti-human-cd8a-antibody-14127
CD69 Anti-mouse CD69-BV421 Biolegend 104527; Clone H1/2F3; " FC quality tested". https://www.biolegend.com/en-us/search-results/brilliant-violet-421-anti-mouse-cd69-antibody-7358
7-AAD Viability Staining Solution; " FC quality tested".Biolegend 420404; https://www.biolegend.com/en-gb/products/7-aad-viability-staining-solution-1649?GroupID=BLG13283
Duraclone kits-"Proven marker combination; Designed for assay precision and lot-to-lot consistency."

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	JAWSII mouse bone marrow derived dendritic cells (ATCC® CRL-11904™)
Authentication	Not authenticated, authentication by ATCC®
Mycoplasma contamination	Cell line confirmed mycoplasma negative
Commonly misidentified lines (See ICLAC register)	*Name any commonly misidentified cell lines used in the study and provide a rationale for their use.*

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Female BALB/C mice, 6-8 weeks old were obtained from the Animal Resource Centre in Western Australia
Wild animals	Study did not involve wild animals.
Field-collected samples	Study did not involve samples collected from field.
Ethics oversight	EnGeneIC Animal Ethics Committee according to the "Australian code for the use and care of animals for scientific purposes"

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

Human research participants

Policy information about studies involving human research participants

Population characteristics	Individuals provided informed consent and were enrolled in the study (https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=382580&isReview=true) with approval from St. Vincent's Hospital Melbourne Human Research Ethics Committee. All participants were otherwise healthy and did not report any history of chronic health conditions. Subjects were identified as SARS-CoV-2 naive via PCR test and naïve for prior COVID-19 vaccines.
Recruitment	Recruitment via St. Vincent's Hospital Melbourne, Australia.
Ethics oversight	St. Vincent's Hospital Melbourne, Australia, Human Research Ethics Committee.

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

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	(https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=382580&isReview=true)
Study protocol	(https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=382580&isReview=true)
Data collection	Blood sample collection at St. Vincent's Hospital Melbourne, Australia. PBMC isolation and data collection at EnGeneIC laboratories. Samples were collected at 4 time points: pre-vaccine baseline (time point 1), day 21 before the booster vaccination (time point 2), and day 28 one week post-boost (time point 4). Subjects are also scheduled to return for a 3 month and 6 month time point. Each study visit included collection of 20 mL of peripheral blood. The study began in September 2021 and at time of submission there are a number of volunteers that have come forward to be part of the study.
Outcomes	(1) Safety measured by clinical parameters, hematology, biochemistry, inflammatory cytokine analyses. (2) Efficacy measured by SARS-CoV-2 S-protein specific antibody and cellular immune responses in serum and PBMC and comparing with similar corresponding published data from approved mRNA vaccines.

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	The cultured JAWSII cells were stained with PE anti-mouse αGC:CD1d complex antibody and S1 protein polyclonal primary antibody at room temperature stained with Alexa Fluor 647 goat anti-rabbit IgG (H+L) highly cross-adsorbed secondary antibody at 4 degrees celsius for a further 20 min. The mouse spleen were isolated from dissected spleens of treated BALB/C mice using a Dounce homogenizer and resuspended in RPMI-1640 medium. The homogenized tissue was then filtered through sterile 70 µm MACS SmartStrainers and incubated with Red Cell Lysing Buffer Hybri-Max™ as recommended by the manufacturer. Cells were then resuspended in 2.5 mL of autoMACS running buffer and passed through a 70 µm MACS SmartStrainer to obtain a single-cell suspension. Human PBMCs were isolated from whole blood by adding it to SepMate tubes with Ficoll, then collect the portion above the Sepmate inserts following centrifugation. The samples were then washed once with PBS and once with AIMV serum free medium.
Instrument	The instrument used was a Beckman Galios flow cytometer
Software	The software used is Kaluza Analysis Version 2.1
Cell population abundance	The source materials (JAWSII cells, spleens, PBMCs) contained a high abundance of CD45+ immune cells. CD4+ and CD8+ T cells were also of high abundance within the immune cell population. The cells were stained for their specific markers. There was no sorting involved.
Gating strategy	The cells were selected through SSC-A/FSC-A. The singlets were selected through either FSC-A/FSC-W or FSC-H/FSC-A. Live cell population was determined by a negative 7AAD staining and the total immune cell population was determined by a positive CD45 population. Positive/negative boundaries were determined by unstained controls.

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