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Supplementary appendix

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Supplementary Appendix

Appendix 1 Inclusion/exclusion criteria, additional methods, and statistical analyses

Inclusion/exclusion criteria

Inclusion criteria include: healthy men or non-pregnant women, defined as having no clinically significant illness or surgery within 28 days, no clinically significant history of major disease, non-smoker, and willing to use contraception during the study and for 3 months after the last dose; body mass index >18 and <34 kg/m^2; body weight ≥50 kg for men and ≥45 kg for women; and able to provide written informed consent, attend all study visits, and comply with all study procedures. Exclusion criteria include: active COVID-19 infection (positive nasal/oropharyngeal swab and/or serum antibody response) at screening/Day 1; close contact with someone with active or previous COVID-19 infection, or previous SARS-CoV-1 or Middle East respiratory syndrome (MERS) infection; any clinically significant abnormality, including hypertension, high blood sugar (non-fasting), abnormal lab test results, positive test for human immunodeficiency virus (HIV), hepatitis B, or hepatitis C, or any chronic illness that may preclude participation; known or suspected impairment of the immune system or respiratory system disorder; history of allergic reactions or hypersensitivity to vaccines or to any excipient, including the adjuvant; positive pregnancy test, urine drug screen, or alcohol breath test at screening; history of alcohol or drug abuse; prescription medications (except contraceptives or medications exempted by the investigator) within 14 days or over-the-counter medications (except paracetamol <2 g/day) within 7 days of first dose of study vaccine; any medications that affect the immune system within 90 days before enrolment; any vaccine within 30 days of enrolment or plans to receive non-study vaccines within 28 days of second dose of study vaccine; any other investigational SARS-CoV-2 vaccine before or during the study; and plasma donation within 7 days, blood donation or loss of 50-499 mL within 30 days or >499 mL within 56 days, or receipt of blood products within 2 months before first dose of study vaccine.

Nipah virus Fclamp

The Nipah virus (NiV) Fclamp antigen comprises amino acids 1–483 of NiV F protein together with the identical clamp sequence used for SARS-CoV-2 Sclamp. NiV Fclamp was expressed using expiCHO expression kit (Thermo Fisher Scientific). Tissue culture supernatant containing secreted, molecular clamp-stabilised NiV F was harvested at 7 days post-transfection, clarified by centrifugation (3000× *g*), passed through a 22 µM filter, and purified by immunoaffinity chromatography with an HiTrap NHS-activated column (GE Healthcare) conjugated with a clamp-specific monoclonal antibody. Purified protein was buffer-exchanged to phosphate-buffered saline (PBS), purity assessed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis, and protein concentration quantified by Nanodrop™ spectrophotometry (Thermo Fisher Scientific).

Serum antibody responses and quantification of relative clamp-specific response by ELISA

Antigen-specific ELISA plates were coated with 2 µg/mL (10 nM) SARS-CoV-2 Sclamp or 0.72 µg/mL (10 nM) NiV Fclamp in PBS overnight at 4˚C. Plates were then blocked with 150 µL/well of 5% KPL Milk Diluent/Blocking Solution Concentrate (SeraCare Life Sciences) in PBS with 0.05% Tween-20 detergent for 1 h at room temperature. Blocking buffer was removed and 5-fold serial dilutions of sera collected at Day 57 from each participant were prepared in duplicate for each antigen. Plates were incubated for 1 h at 37˚C. Plates were washed three times with water before incubation with 5 µg/mL of a 1:2000 dilution of a horseradish peroxidase (HRP)-conjugated goat anti-human secondary antibody (#A18829, Invitrogen) for 1 h at 37˚C. After a final wash, plates were developed for 5 min using TMB Chromogen Single Solution (Thermo Fisher Scientific) before the reaction was stopped by addition of 2N H_2SO_4. Absorbance at 450 nm was then read on a Spectramax 190 Microplate reader (Molecular Devices).

For data analysis, 12 background readings for each plate were averaged and the average was subtracted from each other reading. Background-subtracted absorbance readings were graphed against the log of the serum dilution factor and a 4-parameter curve was fit using non-linear regression in GraphPad Prism 9.0.0. The bottom value was constrained to 0 (as background was already subtracted). Endpoint was calculated as twice the background by interpolating X at Y = background as previously described[1] (note, 1x background already subtracted). The serum endpoint for NiV Fclamp was divided by the endpoint for SARS-CoV-2 Sclamp and multiplied by 100 to calculate the relative percentage of response to the clamp domain.

Coronavirus multiplex assay

A custom multiplex bead array was designed to investigate the isotypes and subclasses of pathogen-specific antibodies present in collected plasma samples, as previously described.[2] Briefly, antigens coupled in the assay included SARS-CoV-2 spike 1 (Sino Biological), spike 2 (ACRO Biosystems), RBD (BEI Resources), as well as human coronavirus (229E, NL63, HKU1, OC43) spikes (Sino Biological) and both SARS-CoV-2 Sclamp and Nipah Fclamp. Tetanus toxoid (Sigma-Aldrich) and influenza hemagglutinin (H1Cal2009; Sino Biological) were also included in the assay as positive controls. All antigens were covalently coupled to individual magnetic

carboxylated bead regions (Bio Rad) using a two-step carbodiimide reaction and blocked with 0.1% bovine serum albumin, before being resuspended and stored in PBS 0.05% sodium azide until use.

To set up the multiplex bead array, 20 µL of working bead mixture (1000 beads per bead region) and 20 µL of diluted plasma (final dilution 1:200) were added per well and incubated overnight at 4°C on a shaker. Ten different detectors were used to assess pathogen-specific antibodies. Single-step detection was done using phycoerythrin (PE)-conjugated mouse anti-human pan-IgG, IgG1-4, and IgA1-2 (Southern Biotech; 1.3 µg/mL, 25 µL/well). For the detection of IgM, biotinylated mouse anti-human IgM (mab MT22; Mabtech; 1.3 µg/mL, 25 µL/well) was first added to beads, washed, followed by the addition of Streptavidin R-PE (SAPE; Thermo Fisher Scientific). For the detection of FcγR-binding, soluble recombinant FcγR dimers (higher affinity polymorphism FcγRIIa-H131 and FcγRIIIa-V158; 1.3 µg/mL, 25 µL/well) were first added to the beads, washed, and followed by the addition of SAPE. Assays were read on the Flexmap 3D and performed in duplicate.

Microneutralisation method

Neutralising activity against infectious SARS-CoV-2 was assessed as previously described.[5] Briefly, SARS-CoV-2 isolate CoV/Australia/VIC01/2020[4] was passaged in African green monkey kidney cells (Vero cells) and stored at -80°C. Serum samples were heat-inactivated at 56°C for 30 min and serially diluted 1:20 to 1:10,240 before addition of 100 times the median tissue culture infectious dose ($TCID_{50}$) of SARS-CoV-2 in minimum essential medium/0.5% bovine serum albumin and incubation at room temperature for 1 h. Residual virus infectivity in the plasma/virus mixtures was assessed in quadruplicate wells of Vero cells incubated in serum-free media containing 1 µg/mL of TPCK trypsin at 37°C/5% CO_2; viral cytopathic effect was read on Day 5. The neutralising antibody titre was calculated using the Reed/Muench method as previously described.[5] The limit of detection was defined as the reciprocal of the highest concentration of sera tested; any values falling below the limit of detection were reported as half the limit of detection.

Pseudovirus neutralisation assay

HIV reporter virus pseudotyped with SARS-CoV-2 S-glycoprotein was produced by lipofectamine cotransfection of 8 µg of different SARS-CoV-2 S-glycoprotein plasmids together with 16 µg of the viral backbone plasmid (pDR-NL Δenv FLUC) into 8 x 10^6 HEK-293T cells. SARS-CoV-2 S-glycoprotein plasmids included pcDNA 3.1 (Invitrogen) with original SARS-CoV-2 S-glycoprotein corresponding to the virus isolated in Wuhan and mutations S477N/D614G, G485R/D614G, and N501Y/D614G. The last 18 C-terminal amino acids were deleted to remove the endoplasmic reticulum retention signal.

HeLa cells expressing angiotensin converting enzyme-2 (ACE2) were produced by transduction with lentiviral vector expressing the human ACE2 under the EF1a promoter. Transduced cells were sorted for ACE2 expression and single cell clones with the highest ACE2 expression were selected.

Pseudovirus was harvested 48 h after transfection and clarified by filtration through a 45-µm filter, aliquoted, and stored at -80°C. Virus stock titres, reported as relative luciferase units (RLU) infectious dose, were calculated by limiting dilution infections in HeLa-ACE2 cells and measuring luciferase activity. Pseudovirus neutralisation was measured and expressed as the patient sera dilution required for 50% reduction in RLU compared with controls. To test neutralisation activity, 1 in 3 serial dilutions of antibody samples (Dulbecco's Modified Eagle Medium [DMEM] without foetal bovine serum) were mixed with 10,000-50,000 RLU of virus in 120 µL. The virus-antibody mix was incubated for 1 h at 37°C to allow neutralisation to occur. After incubation, the virus-antibody mix was added to 1 x 10^4 HeLa-ACE2 cells seeded on a 96-well plate (total volume cell-virus-antibody 250 µL) and spinoculated for 2 h at 1500 g. Finally, cells were incubated at 37°C, 5% CO_2 for 72 h. At the end of this incubation, 150 µL of media was removed from the cells leaving 100 µL residual media on the plate and an equal volume of Britelite Plus reagent (Luciferase enzyme substrate Cat# 6066769, Perkin Elmer, MA, USA) was added for 2 min to allow cell lysis and enzymatic conversion to a luminescence product. To read virus infectivity, 100 µL of the cell lysate was transferred to a black-walled plate and luminescence signal was measured on a FLUOStar Omega microplate reader (BMG Labtech, Germany). Samples were tested in duplicate and the percentage of neutralisation was determined by calculating the difference in average RLU between virus control (virus incubated with DMEM) and test wells, dividing this result by the difference in average RLU between virus control and uninfected cell control wells (background), and multiplying by 100.

Neutralising antibody titres are expressed as the serum dilution required for 50% reduction of RLUs compared to the level in the virus control wells. We employed Prism (GraphPad Software, Inc., San Diego, CA) to plot the

percent neutralisation versus patient sera dilution and to perform a non-linear regression model (log(inhibitor) vs. response [three parameters] least squares fit, setting constraints Top=100, Bottom=0).

Surrogate virus neutralisation test

The surrogate virus neutralisation test was conducted using a commercial kit (SARS-CoV-2 surrogate virus neutralisation test, GenScript, Piscataway, NJ, US) as previously described.[9] In brief, samples and controls were pre-incubated with HRP-conjugated recombinant SARS-CoV-2 receptor binding domain fragment (HRP-RBD). The mixture was added to the solid phase of the assay, which consisted of human ACE-2-coated microtitre plate wells. Unbound HRP-RBD and any HRP-RBD bound to non-neutralising antibody was captured on the plate. Neutralising antibodies bound to HRP-RBD were then washed away and TMB substrate solution was added to produce a blue colour, followed by the stop solution. The absorbance at 450 nm is inversely proportional to the titre of the anti-SARS-CoV-2 neutralising antibodies.

CD4+ and CD8+ T-cell cytokine/cytotoxic marker analysis by flow cytometry

Expression of cytokines (interferon [IFN]-γ, tumour necrosis factor [TNF]-α, interleukin [IL]-2) and cytotoxic markers (cluster of differentiation [CD]107a, granzyme B, granzyme K, and T-cell intracellular antigen 1 [TIA-1]) by $CD4^+$ and $CD8^+$ T cells from fresh whole blood samples were assessed using flow cytometry as described previously.[3-5] Briefly, to assess cytokine and CD107a expression, 235 µl of whole blood was incubated with 5 µg/mL of SARS-CoV-2 S peptide pool (18-mer with ten amino acids overlap between adjacent peptides) spanning the S ectodomain encoded in the vaccine, together with 1 µg/mL of costimulatory antibodies anti-CD28 (clone L293, BD Biosciences) and anti-CD49d (clone L25, BD Biosciences) at 37□C for 6 h. After 1 h of initial stimulation, brefeldin A (BioLegend) was added to each well. Positive (phorbol myristate acetate at 0.025 µg/mL and ionomycin at 1µg/mL) and negative (dimethyl sulfoxide) controls were also performed with each sample. Cells were stained for surface markers CD3 (Pacific Blue, clone SP34-2, BD Biosciences), CD4 (Brilliant Violet 510, clone L200, BD Biosciences), CD8 (Brilliant Violet 650, clone SK1, BD Biosciences), CD107a (APV-H7, clone H4A3, BD Biosciences) and Fixable Viability Stain 440UV or FV620 (BD Biosciences) in the dark at room temperature for 30 min. Next, erythrocytes were lysed using BD FACS™ lysing solution (BD Biosciences) in the dark at room temperature for 10 min. Cells were permeabilised using BD Permeabilization solution (BD Biosciences), also in the dark, at room temperature for 10 min, and cells were washed using FACS stain buffer (FBS, BD Biosciences) before intracellular staining. Antibodies for detection of IFN-γ (antigen-presenting cells [APC], clone B27, BD Biosciences), TNF (PE-Cy7™, clone MAb11, BD Biosciences), IL-2 (fluorescein isothiocyanate [FITC], clone MQ1-17H12, BD Biosciences), IL-4 (PerCP/Cy™5.5, clone 8D4-8, BD Biosciences) and IL-13 (Pseudomonas exotoxin [PE], clone JES10-5A2, BD Biosciences) were used to stain for cytokine expression, and anti-granzyme B (APC, clone GB11. ThermoFisher), granzyme K (FITC, clone GM6C3, Santa Cruz Biotechnology), and TIA-1 (PE, clone 2G9A10F5, Beckman Coulter) antibodies were used to evaluate cytotoxic marker expression. Note that the cytotoxic marker staining set (granzyme B, granzyme K, and TIA-1) was not stimulated with SARS-CoV-2 S peptide pool as the study assessed the difference of expression pre-vaccination to post-vaccination. The cytokine/CD107a and cytotoxic marker sets were stained separately in the dark at room temperature for 40 min. Finally, cells were washed with PBS and fixed with stabilising fixative (BD Biosciences). Approximately 600,000 events per sample were acquired on a flow cytometer (BD LSRII Fortessa™, BD Scientific) and data were analysed using FlowJo software version 10 (FlowJo LLC, Ashland, OR, USA; https://www.flowjo.com/solutions/flowjo).

Whole blood ASC, Tfh1, and Th1 assays

Fresh whole blood (200 µl per stain) was used to measure $CD3^-CD19^+CD27^{hi}CD38^{hi}$ antibody-secreting cells [ASCs)], activated $CD4^+CXCR5^+CXCR3^+CCR6^-$ $ICOS^+PD1^+$ follicular T cells type-1 (Tfh1), and activated $CD4^+CXCR5^-$ $ICOS^+PD1^+$ Th1 populations as previously described.[6] The flow cytometry panel comprised the following antibodies: CD3 PE-CF594 (#562280), CD4 APC-H7 (#560158), CD8a FITC (#555634), CD19 BV510 (#562947), CD20 BV711 (#563126), CD27 AF700 (#560611), CD38 BV786 (#563964), CD45 PerCP-Cy5.5 (#340953), CXCR3 APC (#550967), CXCR5 BV421 (#562747), CCR6 BV650 (#563922), ICOS PE (#557802), PD-1 PeCy7 (#561272) (BD Biosciences), and human leukocyte antigen – DR isotype BV605 (#307640, Biolegend). After whole blood was stained for 30 mins at room temperature in the dark, samples were lysed with BD FACS™ lysing solution (BD Biosciences), washed, and fixed with 1% paraformaldehyde. AccuCheck Counting Beads, 30 µl, (Thermo Fisher Scientific) were added and all samples were acquired on a flow cytometer (BD LSRII Fortessa™, BD Scientific). Flow cytometry data were analysed using FlowJo software version 10 (FlowJo LLC, Ashland, OR, USA; https://www.flowjo.com/solutions/flowjo).

Statistical analysis
Adjusted geometric means with 95% confidence intervals are calculated for immunogenicity data using a linear mixed model (response variable = base 10-log transformed titre value [or fold increase]), fixed effect = treatment, covariate = base 10-log transformed baseline titre. The model was fit using ordinary least squares and the model-adjusted geometric means at each level of treatment were calculated using the average value of the covariate (baseline titre). Differences in immunogenicity endpoints between treatment groups and between SARS-CoV-2 Sclamp vaccine and placebo were compared using the Dunnett's test for multiple comparisons.

For systems serology multiplex analysis, H1Cal2009 antigens (positive controls) were excluded. Right shifting was performed on each feature (detector-antigen pair) individually if it contained any negative values, by adding the minimum value for that feature back to all samples within that feature. Following this, all data were log-transformed using the following equation, where x is the right-shifted data and y is the right-shifted log-transformed data: $y = \log10(x+1)$. This process transformed the majority of the features to having a normal distribution. In all subsequent analyses, the data were furthered normalised by mean centring and variance scaling each feature using the zscore function in Matlab. Principal Component Analysis (PCA) was used to visualise the variance in the samples based on all of SARS-CoV-2 measured antibody features. Separation of groups on the scores plot indicates unsupervised separation of groups. PCA was performed using the Statistics and Machine Learning toolbox in Matlab.

The percentage of total $CD4^+$ or $CD8^+$ T cells responding to SARS-CoV-2 (summation analysis) for each participant was calculated as (number of $CD4^+$ or $CD8^+$ T cells expressing IFN-γ^+ + TNF^+ + IL-2^+ + IFN-γ^+TNF^+ + IFN-γ^+IL-2^+ + TNF^+IL-2^+ + IFN-γ^+TNF^+IL-2^+) / number of $CD4^+$ or $CD8^+$ T cells × 100%). Note that the single, double, or triple positive were calculated by avoiding any overlap (figure 4.1, appendix 4).

Statistical significance between groups was determined using a fitted mixed model of one- or two-way analysis of variance (ANOVA) and significance reported as either Dunn's multiple comparison test, Dunnett's multiple comparison test, or Sidak's multiple comparison test. Statistical analysis was performed using GraphPad Prism v9 software (https://www.graphpad.com/scientific-software/prism/).

Correlations were assessed using Spearman's correlation coefficient (r_s) and visualised in R 4.0.2 as circos plots using the circlize package[7] or heatmaps using the corrplot package; p-values of correlations were corrected for multiple comparisons by False Discovery Rate (FDR) in R 4.0.2. Volcano plots were created in R using a Wilcoxon rank-sum test (equivalent to the Mann-Whitney test) with the *wilcox.test* function in R and statistics were corrected with FDR adjustment.

Table 1.1: Toxicity Grading for Solicited Local (Administration Site) Adverse Events

Local Reactions to Injection	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Pain (at the vaccination site)	Does not interfere with activity	Repeated use of non-narcotic pain reliever >24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest
Erythema/Redness	2.5 – 5 cm	5.1 – 10 cm	>10 cm
Induration/Swelling	2.5 – 5 cm and does not interfere with activity	5.1 – 10 cm or interferes with activity	>10 cm or prevents daily activity

Table 1.2: Toxicity Grading Scales for Solicited Systemic Adverse Events

Systemic Solicited Adverse Event	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Fever °C (Oral*)	38.0 – 38.4	38.5 – 38.9	39.0 - 40
Chills	Present, but does not interfere with activity	Interferes with activity	Prevents daily activity
Nausea	Nausea present but not interfering with daily activity	Nausea present leading to decreased oral intake	Nausea present leading to minimal to no oral intake
Vomiting	No interference with activity or 1 – 2 episodes in 24 hours	Some interference with activity or >2 episodes in 24 hours	Prevents daily activity, ≥4 episodes in 24 hours, or 2 or more per day prolonged on several days
Muscle pain	Present, but does not interfere with activity	Interferes with activity, or some use of non-narcotic pain reliever	Prevents daily activity; use of narcotic pain reliever
Joint pain	Present, but does not interfere with activity	Interferes with activity, or some use of non-narcotic pain reliever	Prevents daily activity; use of narcotic pain reliever
Headache	Present, but does not interfere with activity	Interferes with activity, or some use of non-narcotic pain reliever	Prevents daily activity; use of narcotic pain reliever
Fatigue/Somnolence	Present, but does not interfere with activity	Interferes with activity,	Prevents daily activity;
Diarrhoea	2 – 3 loose stools or <400 g / 24 hours	4 – 5 stools or 400 – 800 g / 24 hours	6 or more watery stools or >800 g / 24 hours or requires outpatient IV hydration
Malaise (general discomfort)	No interference with activity	Some interference with usual and social activity, no treatment	Significant, prevents usual daily and social, activity or requires treatment

* Oral temperature; no recent hot or cold beverages or smoking

Appendix 2 Efficacy and safety data

Table 2.1: Unsolicited adverse events

System Organ Class Preferred Term	Placebo (N = 24)	SARS-CoV-2 Sclamp 2 x 5 µg (N = 24)	SARS-CoV-2 Sclamp 2 x 15 µg (N = 24)	SARS-CoV-2 Sclamp 2 x 45 µg (N = 24)	SARS-CoV-2 Sclamp 1 x 45 µg (N = 24)
Any unsolicited AE	7 (29.2)	13 (54.2)	7 (29.2)	7 (29.2)	8 (33.3)
Any treatment-related unsolicited AE	1 (4.2)	4 (16.7)	3 (12.5)	1 (4.2)	4 (16.7)
Any severe unsolicited AE	0	0	0	0	0
Infections and infestations					
Pharyngitis	0	0	1 (4.2)[a]	1 (4.2)	0
Tonsillitis	2 (8.3)	1 (4.2)	1 (4.2)	0	0
Upper respiratory tract infection	0	0	1 (4.2)[a]	1 (4.2)	0
Gastroenteritis	0	0	0	0	1 (4.2)
Herpes zoster	0	0	1 (4.2)[a]	0	0
Viral upper respiratory tract infection	1 (4.2)	0	0	0	0
Nervous system disorders					
Headache	1 (4.2)	1 (4.2)	2 (8.3)	0	2 (8.3)
Paraesthesia	1 (4.2)	0	0	0	1 (4.2)[a]
Radicular pain	0	0	0	1 (4.2)[a]	0
Respiratory, thoracic, and mediastinal disorders					
Oropharyngeal pain	0	1 (4.2)[a]	1 (4.2)	0	0
Cough	1 (4.2)	0	0	0	1 (4.2)
Dyspnoea	0	1 (4.2)	0	0	0
Rhinorrhoea	0	1 (4.2)[a]	0	0	0
Musculoskeletal and connective tissue disorders					
Back pain	0	2 (8.3)	0	0	0
Haemarthrosis	0	1 (4.2)[a]	0	0	0
Muscle twitching	0	0	0	0	1 (4.2)[a]
Pain in extremity	0	1 (4.2)	0	0	0
Gastrointestinal disorders					
Constipation	0	0	0	0	1 (4.2)[a]
Diarrhoea	0	0	1 (4.2)	0	0
Mouth ulceration	0	1 (4.2)	0	0	0

Rectal haemorrhage	0	0	0	1 (4.2)	0
Abdominal discomfort	1 (4.2)	0	0	0	0
Skin and subcutaneous tissue disorders					
Alopecia	0	1 (4.2)	0	0	0
Dermatitis contact	0	0	0	1 (4.2)	0
Pruritus	0	1 (4.2)	0	0	0
Rash macular	0	0	0	1 (4.2)	0
Rash pruritic	1 (4.2)[a]	0	0	0	0
General disorders and administration site conditions					
Fatigue	0	1 (4.2)[a]	0	0	0
Injection site pain	0	1 (4.2)[a]	0	0	0
Vessel puncture site bruise	0	0	0	0	1 (4.2)
Injury, poisoning, and procedural complications					
Arthropod bite	0	0	0	1 (4.2)	0
Sunburn	0	0	0	0	1 (4.2)
Thermal burn	0	0	0	1 (4.2)	0
Investigations					
Blood creatine phosphokinase increased	0	1 (4.2)	1 (4.2)	0	0
Lipase increased	0	0	0	1 (4.2)	0
Metabolism and nutrition disorders					
Hypoglycaemia	0	1 (4.2)	0	0	0
Vitamin D deficiency	0	0	0	1 (4.2)	0
Blood and lymphatic system disorders					
Lymphadenopathy	0	0	0	0	1 (4.2)[a]
Ear and labyrinth disorders					
Eustachian tube dysfunction	0	0	0	1 (4.2)	0
Product issues					
Device physical property issue	0	1 (4.2)	0	0	0
Surgical and medical procedures					
Elective procedures	0	0	0	0	1 (4.2)
Vascular disorders					
Hot flush	0	1 (4.2)	0	0	0

Data are n (%).

[a]Considered vaccine-related.

Abbreviations: AE = adverse event, SARS-CoV-2 Sclamp = Severe Acute Respiratory Syndrome Coronavirus 2 S-glycoprotein-clamp vaccine.

Table 2.2: **Solicited local adverse events within *7 days of first and second dose***

	Placebo			SARS-CoV-2 Sclamp					
	(dose 1)	(dose 2)	(dose 2*)	5µg (dose 1)	5µg (dose 2)	15µg (dose 1)	15µg (dose 2)	45µg (dose 1)	45µg (dose 2)
Dose	**(N = 24)**	**2 (N=22)**	**(N = 24)**	**1 (N = 24)**	**2 (N=24)**	**1 (N = 24)**	**2 (N=22)**	**1 (N = 48)**	**2 (N=21)**
Injection Site Pain	**5 (20.8%)**	**3 (12.5%)**	**0**	**15 (62.5%)**	**13 (54.2%)**	**11 (45.8%)**	**12 (50.0%)**	**24 (50%)**	**13 (54.2%)**
Grade 1	4	3	0	14	13	11	12	23	12
Grade 2	0	0	0	0	0	0	0	1	0
Grade 3	1	0	0	1	0	0	0	0	1
Injection Site Tenderness	**3 (12.5%)**	**2 (8.3%)**	**4 (16.7%)**	**12 (50.0%)**	**15 (62.5%)**	**13 (54.2%)**	**14 (58.3%)**	**23 (47.9%)**	**14 (58.3%)**
Grade 1	3	2	4	10	15	9	14	21	13
Grade 2	0	0	0	2	0	4	0	2	1
Grade 3	0	0	0	0	0	0	0	0	0
Erythema/ Redness	**0**	**0**	**1 (4.2%)**	**0**	**0**	**0**	**0**	**1 (2.1%)**	**0**
Grade 1	0	0	0	0	0	0	0	0	0
Grade 2	0	0	1	0	0	0	0	1	0
Grade 3	0	0	0	0	0	0	0	0	0
Induration/ Swelling	**0**	**0**	**0**	**2 (8.3%)**	**0**	**2 (8.3%)**	**0**	**2 (4.2%)**	**0**
Grade 1	0	0	0	2	0	2	0	2	0
Grade 2	0	0	0	0	0	0	0	0	0
Grade 3	0	0	0	0	0	0	0	0	0

Data are n (%)
Data for the first dose of Treatments C (2 x 45-µg SARS-CoV-2 Sclamp) and D (1 x 45-µg SARS-CoV-2 Sclamp, followed by 1 x placebo) were pooled (N=48). *Data from the second dose of Treatment D is shown separately as 'Placebo (dose 2*)'.
Abbreviations: SARS-CoV-2 Sclamp = Severe Acute Respiratory Syndrome Coronavirus 2 S-glycoprotein-clamp vaccine.

Table 2.3: **Solicited systemic adverse events within *7 days of first and second dose***

	Placebo			SARS-CoV-2 Sclamp					
	(dose 1)	(dose 2)	(dose 2*)	5µg (dose 1)	5µg (dose 2)	15µg (dose 1)	15µg (dose 2)	45µg (dose 1)	45µg (dose 2)
Dose	**(N = 24)**	**2 (N=22)**	**(N = 24)**	**1 (N = 24)**	**2 (N=24)**	**1 (N = 24)**	**2 (N=22)**	**1 (N = 48)**	**2 (N=21)**
Chills	**2 (8.3%)**	**4 (16.7%)**	**1 (4.2%)**	**0**	**1 (4.2%)**	**0**	**0**	**6 (12.5%)**	**0**
Grade 1	2	4	1	0	1	0	0	5	0
Grade 2	0	0	0	0	0	0	0	0	0
Grade 3	0	0	0	0	0	0	0	1	0
Nausea	**0**	**1 (4.2%)**	**2 (8.3%)**	**1 (4.2%)**	**1 (4.2%)**	**0**	**2 (8.3%)**	**5 (10.4%)**	**1 (4.2%)**
Grade 1	0	1	2	1	1	0	1	5	1
Grade 2	0	0	0	0	0	0	1	0	0
Grade 3	0	0	0	0	0	0	0	0	0
Vomiting	**1 (4.2%)**	**0**	**0**	**1 (4.2%)**	**1 (4.2%)**	**0**	**2 (8.3%)**	**1 (2.1%)**	**0**
Grade 1	0	0	0	1	1	0	1	1	0
Grade 2	0	0	0	0	0	0	1	0	0
Grade 3	1	0	0	0	0	0	0	0	0
Fever	**0**	**0**	**0**	**0**	**1 (4.2%)**	**0**	**0**	**0**	**0**
Grade 1	0	0	0	0	0	0	0	0	0
Grade 2	0	0	0	0	1	0	0	0	0
Grade 3	0	0	0	0	0	0	0	0	0
Headache	**11 (45.8%)**	**4 (16.7%)**	**6 (25.0%)**	**4 (16.7%)**	**1 (4.2%)**	**10 (41.7%)**	**6 (25.0%)**	**9 (18.8%)**	**4 (16.7%)**
Grade 1	11	2	5	4	1	10	6	7	4
Grade 2	0	1	1	0	0	0	0	1	0
Grade 3	0	1	0	0	0	0	0	1	0
Fatigue/ Somnolence	**4 (16.7%)**	**3 (12.5%)**	**10 (41.7%)**	**5 (20.8%)**	**1 (4.2%)**	**6 (25.0%)**	**3 (12.5%)**	**15 (31.3%)**	**4 (16.7%)**
Grade 1	3	3	9	5	1	5	3	12	4
Grade 2	1	0	1	0	0	1	0	2	0
Grade 3	0	0	0	0	0	0	0	1	0
Diarrhoea	**2 (8.3%)**	**2 (8.3%)**	**1 (4.2%)**	**0**	**0**	**3 (12.5%)**	**1 (4.2%)**	**4 (8.3%)**	**1 (4.2%)**
Grade 1	2	2	1	0	0	3	1	4	1
Grade 2	0	0	0	0	0	0	0	0	0
Grade 3	0	0	0	0	0	0	0	0	0
Malaise	**4 (16.7%)**	**3 (12.5%)**	**7 (29.2%)**	**3 (12.5%)**	**0**	**2 (8.3%)**	**5 (20.8%)**	**11 (22.9%)**	**4 (16.7%)**
Grade 1	4	3	6	3	0	2	4	8	3
Grade 2	0	0	1	0	0	0	1	2	0
Grade 3	0	0	0	0	0	0	0	1	1

Muscle Pain	**2 (8.3%)**	**1 (4.2%)**	**2 (8.3%)**	**1 (4.2%)**	**1 (4.2%)**	**5 (20.8%)**	**3 (12.5%)**	**6 (12.5%)**	**2 (8.3%)**
Grade 1	2	1	1	1	1	3	2	5	2
Grade 2	0	0	1	0	0	2	1	0	0
Grade 3	0	0	0	0	0	0	0	1	0

Joint Pain	**2 (8.3%)**	**1 (4.2%)**	**0**	**2 (8.3%)**	**0**	**3 (12.5%)**	**1 (4.2%)**	**6 (12.5%)**	**1 (4.2%)**
Grade 1	2	1	0	2	0	2	1	6	1
Grade 2	0	0	0	0	0	1	0	0	0
Grade 3	0	0	0	0	0	0	0	0	0

Data are n (%)
Data for the first dose of Treatments C (2 x 45-µg SARS-CoV-2 Sclamp) and D (1 x 45-µg SARS-CoV-2 Sclamp, followed by 1 x placebo) were pooled (N=48). *Data from the second dose of Treatment D is shown separately as 'Placebo (dose 2*)'.
Abbreviations: SARS-CoV-2 Sclamp = Severe Acute Respiratory Syndrome Coronavirus 2 S-glycoprotein-clamp vaccine.

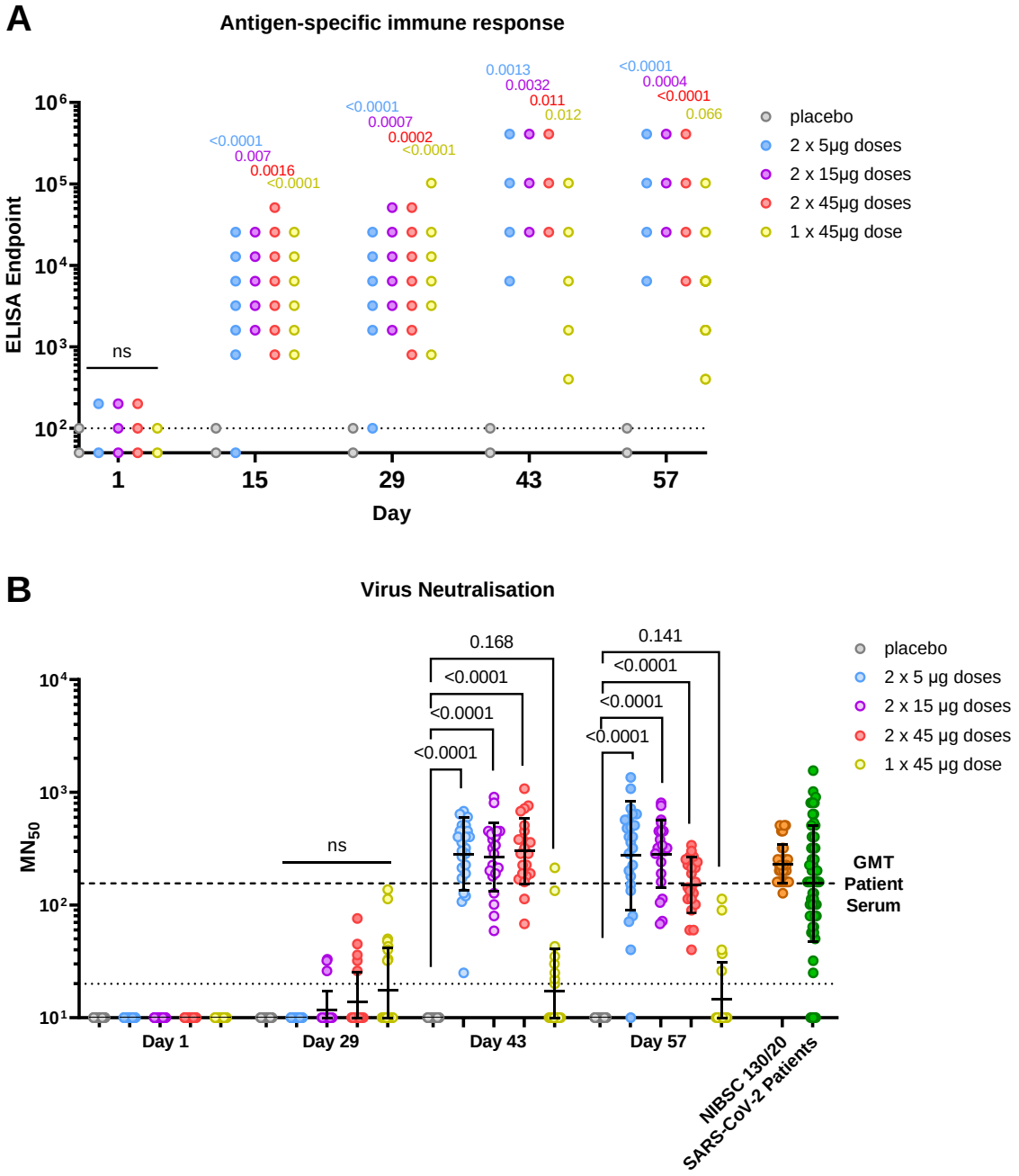

Figure 2.1: **Additional humoral immune response analysis**
(A) Anti-SARS-CoV-2 Sclamp antibody response measured by ELISA. Individual data points for each dose of study vaccine and placebo at each time point. **(B)** Live SARS-CoV-2 microneutralisation in trial participants showing raw un-normalised and spread of internal assay control, convalescent patient serum sample - NIBSC 130/20. Adjusted p-value vs placebo (two-way ANOVA Dunnett's multiple comparison test): Abbreviations: ELISA = enzyme-linked immunosorbent assay, GMT = geometric mean titre, LoD = limit of detection, MN_{50}, 50% microneutralisation; ns = not significant, SARS-CoV-2 Sclamp = Severe Acute Respiratory Syndrome Coronavirus 2 S-glycoprotein-clamp vaccine.

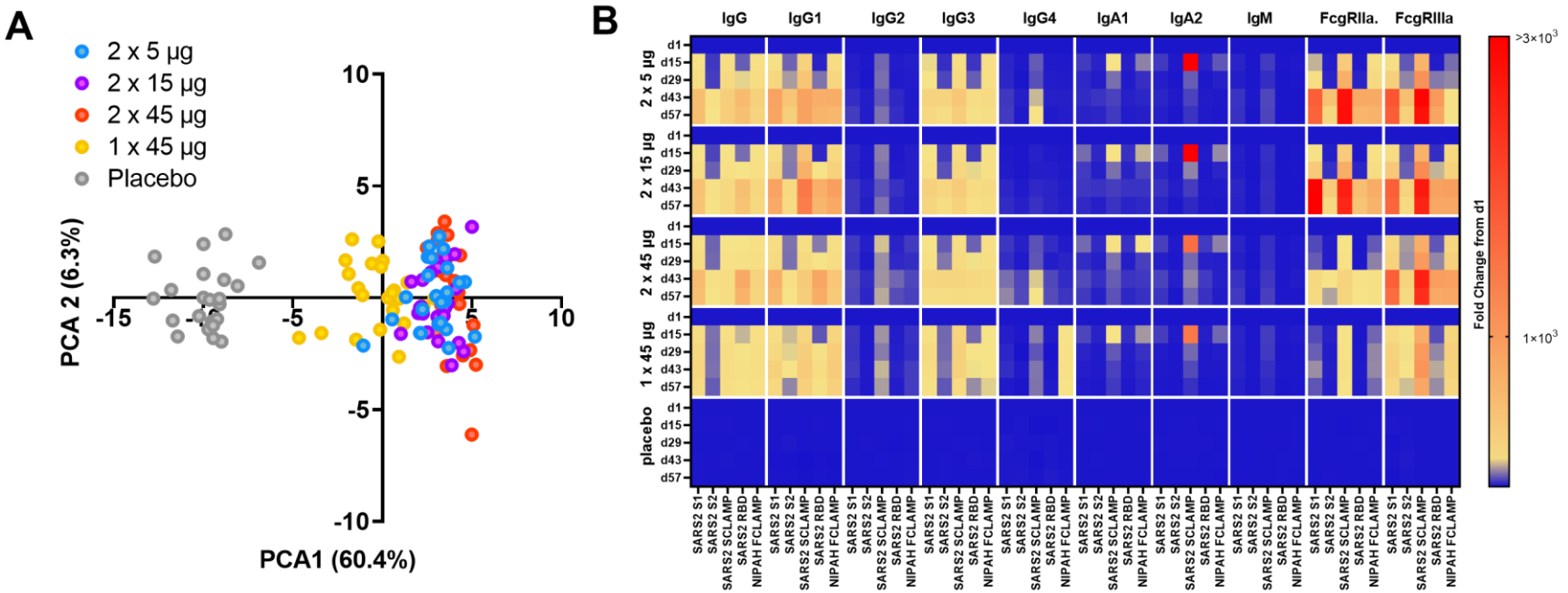

Figure 2.2: **Analysis of serum antibody responses via multiplex assay**
(A) Principal component analysis shows that all vaccine regimens induced vastly different SARS-CoV-2 antibody signatures compared with placebo with main separation across the first dimension (X variance 60.4%). All two-dose regimens induced similar overlapping SARS-CoV-2 antibody signatures, while the single high-dose regimen induced comparatively weak antibody responses. **(B)** SARS-CoV-2 vaccine antigen-specific isotype, subclass, and soluble dimeric FcγR binding responses were assessed for fold change differences from baseline across all vaccine cohorts. Heatmap shows median fold change relative to Day 1. SARS-CoV-2 vaccine antigen-specific IgG responses were boosted by a second dose, which also contributed to enhanced engagement of FcγR dimers; however, intriguingly, IgA responses were elevated at the first vaccination, then rapidly waned, despite a second dose. Abbreviations: d1 = Day 1, FcγR = Fc gamma receptor, Ig = immunoglobulin, PCA = principal component analysis, SARS-CoV-2 = Severe Acute Respiratory Syndrome Coronavirus 2, Sclamp = S-glycoprotein clamp.

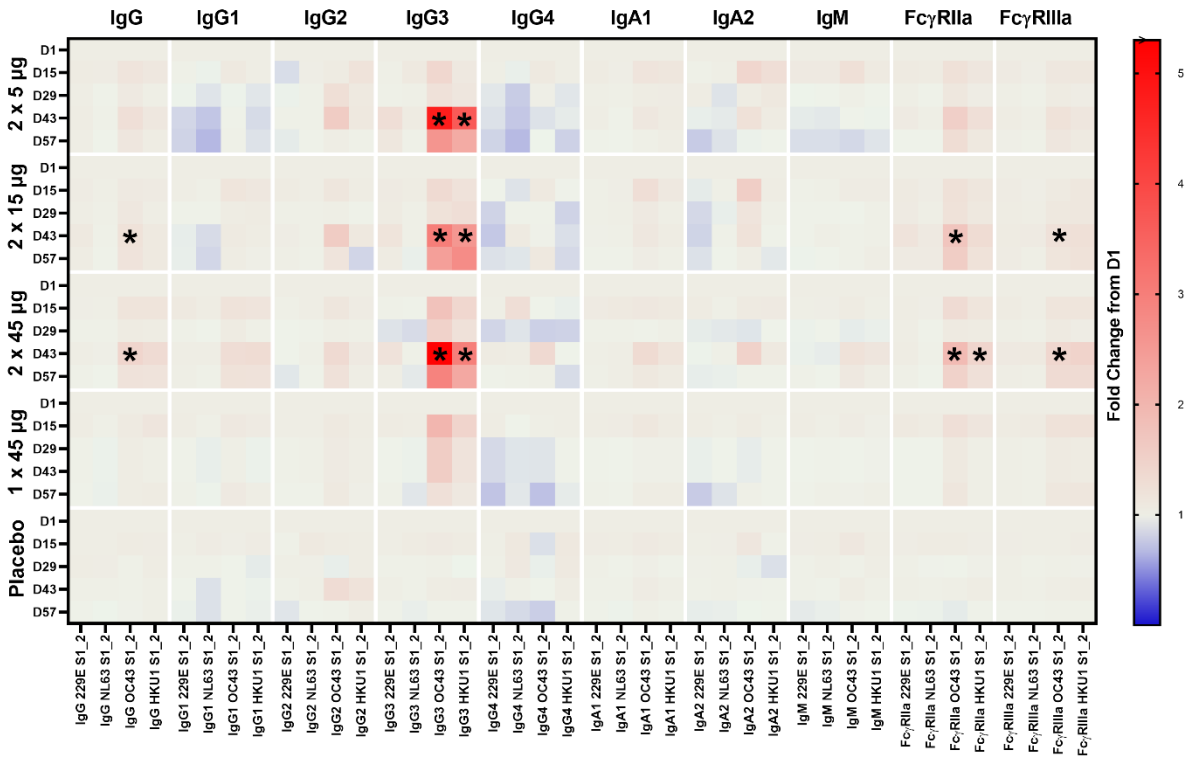

Figure 2.3: **Analysis of vaccine-induced antibody responses to human beta coronaviruses**
Heat map of fold change relative to Day 1. Abbreviations: D = day, FcγR = Fc gamma receptor, hCoV = human coronavirus, Ig = immunoglobulin. *p<0·05.

Appendix 3 HIV diagnostics

Table 3.1: **HIV diagnostic via Western blot**

SARS-CoV-2 Sclamp group	HIV-1 Western blot	p17	p24	p31	p39	gp41	p51	p55	p66	gp120	gp160	Nucleic acid RT-PCR
Two 5-µg doses	Indeterminate	-	-	-	-	-	-	-	-	1+	3+	Neg
Two 5-µg doses	Indeterminate	-	-	-	-	-	-	-	-	1+	2+	Neg
Two 5-µg doses	Indeterminate	-	-	-	-	-	-	-	-	1+	2+	Neg
Two 5-µg doses	Indeterminate	-	-	-	-	-	-	-	-	-	1+	Neg
Two 5-µg doses	Indeterminate	-	-	-	-	-	-	-	-	trace	2+	Neg
Two 5-µg doses	Indeterminate	-	-	-	-	-	-	-	-	1+	3+	Neg
Two 5-µg doses	Indeterminate	-	-	-	-	-	-	-	-	-	1+	Neg
Two 5-µg doses	Indeterminate	-	-	-	-	-	-	-	-	-	1+	Neg
Two 5-µg doses	Indeterminate	-	-	-	-	-	-	-	-	trace	2+	Neg
Two 5-µg doses	Indeterminate	-	-	-	-	-	-	-	-	-	1+	Neg
Two 5-µg doses	Indeterminate	-	-	-	-	-	-	-	-	-	2+	Neg
Two 5-µg doses	Indeterminate	-	-	-	-	-	-	-	-	-	trace	Neg
Two 5-µg doses	Indeterminate	-	-	-	-	-	-	-	-	-	2+	Neg
Two 5-µg doses	Indeterminate	-	-	-	-	-	-	-	-	-	1+	Neg
Two 15-µg doses	Indeterminate	-	-	-	-	-	-	-	-	trace	3+	Neg
Two 15-µg doses	Indeterminate	-	-	-	-	-	-	-	-	-	3+	Neg
Two 15-µg doses	Indeterminate	-	-	-	-	-	-	-	-	trace	3+	Neg
Two 15-µg doses	Indeterminate	-	-	-	-	-	-	-	-	trace	3+	Neg
Two 15-µg doses	Indeterminate	-	-	-	-	-	-	-	-	trace	3+	Neg
Two 15-µg doses	Indeterminate	-	-	-	-	-	-	-	-	trace	3+	Neg
Two 15-µg doses	Indeterminate	-	-	-	-	-	-	-	-	trace	3+	Neg
Two 15-µg doses	Indeterminate	-	1+	-	-	-	-	-	-	trace	3+	Neg
Two 15-µg doses	Indeterminate	-	-	-	-	trace	-	-	-	trace	3+	Neg
Two 15-µg doses	Indeterminate	-	-	-	-	-	-	-	-	trace	3+	Neg
Two 15-µg doses	Indeterminate	-	-	-	-	-	-	-	-	trace	3+	Neg
Two 15-µg doses	Indeterminate	-	-	-	-	-	-	-	-	trace	3+	Neg
Two 15-µg doses	Indeterminate	-	-	-	-	-	-	-	-	-	2+	Neg
Two 15-µg doses	Indeterminate	-	-	-	-	-	-	-	-	trace	3+	Neg
Two 15-µg doses	Reactive	-	trace	-	-	-	-	-	-	trace	3+	Neg
Two 15-µg doses	Indeterminate	-	1+	-	-	-	-	-	-	-	2+	Neg
Two 15-µg doses	Reactive	2+	-	-	-	-	-	-	-	1+	3+	Neg
Two 15-µg doses	Indeterminate	-	-	-	-	-	-	-	-	1+	3+	Neg
Two 15-µg doses	Indeterminate	-	-	-	-	-	-	-	-	-	2+	Neg
Two 15-µg doses	Indeterminate	-	1+	-	-	-	-	-	-	-	3+	Neg
Two 15-µg doses	Indeterminate	-	-	-	-	-	-	-	-	1+	3+	Neg
Two 45-µg doses	Indeterminate	-	-	-	-	-	-	-	-	trace	3+	Neg
Two 45-µg doses	Indeterminate	-	-	-	-	-	-	-	-	-	2+	Neg
Two 45-µg doses	Indeterminate	-	-	-	-	-	-	-	-	-	2+	Neg
Two 45-µg doses	Indeterminate	-	1+	-	-	-	-	-	-	-	3+	Neg

Two 45-µg doses	Indeterminate	-	trace	-	-	-	-	-	-	-	2+	Neg
Two 45-µg doses	Indeterminate	-	-	-	-	-	-	-	-	-	2+	Neg
Two 45-µg doses	See comment[1]	High bg	High bg	High bg	High bg	High bg	High bg	High bg	High bg	High bg	3+	Neg
Two 45-µg doses	Indeterminate	1+	-	-	-	-	-	-	-	-	2+	Neg
Two 45-µg doses	Indeterminate	-	-	-	-	-	-	-	-	trace	2+	Neg
Two 45-µg doses	Indeterminate	-	1+	-	-	-	-	-	-	-	2+	Neg
Two 45-µg doses	Indeterminate	-	-	-	-	-	-	-	-	trace	3+	Neg
Two 45-µg doses	Indeterminate	-	trace	-	-	-	-	-	-	-	2+	Neg
Two 45-µg doses	Indeterminate	-	-	-	-	-	-	-	-	-	2+	Neg
Two 45-µg doses	Indeterminate	-	-	-	-	-	-	-	-	trace	3+	Neg
Two 45-µg doses	Indeterminate	-	-	-	-	-	-	-	-	trace	3+	Neg
Two 45-µg doses	Indeterminate	-	-	-	-	-	-	-	-	trace	2+	Neg
Two 45-µg doses	Indeterminate	-	-	-	-	-	-	-	-	-	2+	Neg
Two 45-µg doses	Indeterminate	-	-	-	-	-	-	-	-	-	1+	Neg
Two 45-µg doses	Indeterminate	-	trace	-	-	-	-	-	-	-	2+	Neg
Two 45-µg doses	Indeterminate	-	trace	-	-	-	-	-	-	-	2+	Neg
One 45-µg dose	Indeterminate	-	-	-	-	-	-	-	-	-	1+	Neg
One 45-µg dose	Indeterminate	-	-	-	-	-	-	-	-	-	3+	Neg
One 45-µg dose	Indeterminate	-	trace	-	-	-	-	-	-	-	1+	Neg
One 45-µg dose	Indeterminate	-	-	-	-	-	-	-	-	-	trace	Neg
One 45-µg dose	Indeterminate	-	2+	-	-	-	-	-	-	-	1+	Neg
One 45-µg dose	Non-reactive	-	-	-	-	-	-	-	-	-	-	Neg
One 45-µg dose	Indeterminate	-	-	-	-	-	-	-	-	-	1+	Neg
One 45-µg dose	Non-reactive	-	-	-	-	-	-	-	-	-	-	Neg
One 45-µg dose	Non-reactive	-	-	-	-	-	-	-	-	-	-	Neg
One 45-µg dose	Indeterminate	-	1+	-	-	-	-	-	-	-	1+	Neg
One 45-µg dose	See comment[1]	High bg	High bg	High bg	High bg	High bg	High bg	High bg	High bg	High bg	High bg	Neg
One 45-µg dose	Indeterminate	-	-	-	-	-	-	-	-	-	trace	Neg
One 45-µg dose	Indeterminate	-	-	-	-	-	-	-	-	-	trace	Neg
One 45-µg dose	Indeterminate	-	-	-	-	-	-	-	-	-	trace	Neg
One 45-µg dose	Indeterminate	-	2+	-	-	-	-	-	-	-	trace	Neg
One 45-µg dose	Indeterminate	-	-	-	-	-	-	-	-	-	trace	Neg

[1]Could not detect weak bands due to high background.

The Western blot detects antibodies to specific denatured HIV-1 proteins including core (p17, p24, p39, and p55), polymerase (p31, p51, p66), and envelope (gp41, gp120, and gp160) proteins.

Abbreviations: bg = background, neg = negligible, RT-PCR = reverse transcription-polymerase chain reaction, HIV-1 = human immunodeficiency virus type 1, QHPS = Queensland Health Pathology Service.

Table 3.2: **Summary of additional HIV diagnostics assessed**

Assay	Format	Antigens	gp41 cross-reactivity
ARCHITECT(®) HIV Ag/Ab Combo Assay (Abbott) **[QHPS Tier 1]*	Laboratory	Antibodies to HIV-1 groups M/O, and HIV-2; five recombinant proteins and two synthetic peptides derived from native TMP sequences of HIV-1 groups M/O, and HIV-2.	Cross-reactive
Western Blot **[QHPS Tier 2]*	Laboratory	gp160/41; p24	Indeterminate
Nucleic Acid Test (NAT) **[QHPS Tier 3]*	Laboratory	RNA Qualitative Assay	Negative
Alere / Abbott - Determine HIV-1/2 - HIV1/HIV2 antibody IVD, kit, immunochromatographic test (ICT), rapid	Point-of-care	Recombinant and synthetic peptides for HIV-1 gp41 and HIV-2 gp36	Cross-reactive
Uni Gold HIV Test (Immuno)	Point-of-care	Recombinant proteins: HIV-1 gp41, HIV-1 gp120, HIV-2 gp36	Cross-reactive
OraQuick Advance HIV-1/2 (Integrated Sciences)	Point-of-care	gp41 peptides	Negative
Bio-Rad Geenius HIV 1/2 Confirmatory Assay	Point-of-care/ laboratory	gp36 peptide, gp140 peptide, p31 peptide, gp160 recombinant protein, p24 recombinant protein, gp41 peptides, protein A control	Negative
Atomo HIV Self-Test	Self-test	Recombinant gp41	Cross-reactive
Siemens Atellica	Laboratory	Recombinant gp41	Cross-reactive
DisSorin Liaison	Laboratory	Recombinant gp41	Cross-reactive
Bio-Rad Genscreen Ultra	Laboratory	gp41 peptides	Negative
Roche Elecsys HIV combi HIV 1/2 Ag-Ab ECLIA	Laboratory	Recombinant gp41	Cross-reactive

*Testing conducted at QHPS as per routine testing procedure.

Abbreviations: Ab = antibody, Ag = antigen, gp = glycoprotein, ECLIA = electrochemiluminescence immunoassay, HIV = human immunodeficiency virus, QHPS = Queensland Health Pathology Service.

Appendix 4 Cellular immunogenicity

SARS-CoV-2 Sclamp elicited robust SARS-CoV-2 S-specific polyfunctional cluster of differentiation (CD)4^+ T-cell responses. Th1 summation analysis showed that the total $CD4^+$ T-cells responding to SARS-CoV-2 Sclamp were significantly elevated in all four treatment groups at Days 15, 36, 43, and 57 compared with placebo and baseline (figure 4.3, p 22-23).; the response decreased at Day 29, just before the second dose. The highest SARS-CoV-2 S-specific $CD4^+$ T-cell responses were observed 2 weeks following the second dose (Day 43) with two doses of vaccine inducing higher responses compared with single 45-µg dose. Importantly, no significant differences in $CD4^+$ T-cell responses were observed with increasing dose levels. Unlike $CD4^+$ T-cell responses, there were no significant differences in S-specific $CD8^+$ T-cell responses compared with placebo at any time point, although some individual samples did exhibit a $CD8^+$ T-cell response (figure 4.4, p 25).

Cytokine expression patterns of the different mono- or polyfunctional subsets were analysed to further characterise the type 1 response. Compared with placebo, two doses of SARS-CoV-2 Sclamp, irrespective of dose level, showed significantly higher S-specific $CD4^+$ T-cell responses for each type 1 cytokine, with the magnitude of expression being the highest for tumour necrosis factor (TNF)-α followed by interleukin (IL)-2 and interferon (IFN)-γ (figure 4.3, p 22-23), and significantly higher TNF-α^+ IL-2^+, TNF-α^+ IL-2^+ IFN-γ^+, and TNF-α^+ IFN-γ^+ polyfunctional $CD4^+$ T-cell responses, although the increase in the latter subset was subtle (figure 4.3, p 22-23). SARS-CoV-2 Sclamp had no effect on regulating polyfunctional $CD8^+$ T-cell subsets (figure 4.4, 4.5, p 25-26).

Th2 summation analysis for IL-4 and IL-13 production showed that the total frequency of $CD4^+$ T-cells responding to SARS-CoV-2 Sclamp vaccine was elevated in all dose groups compared with placebo, mainly at Day 57 (figure 4.3, p 22-23). However, there were no concomitant $CD8^+$ T-cell responses (figure 4.4, p 25). Compared with placebo, two doses of 15-µg and 45-µg vaccine elicited a higher frequency $CD4^+$ T-cells expressing IL-13 at different time intervals (figure 4.3, p 22-23). There was no increase in frequency of $CD4^+$ T-cells expressing IL-4 with SARS-CoV-2 Sclamp, but there was a significant and dose-dependent increase in the double positive subset of IL-4 and IL-13 producing Th2 cells following one or two doses (figure 4.3, p 22-23). There was no increase in the degranulation marker CD107a or in cytotoxic markers (granzyme K, granzyme B, T-cell intracellular antigen 1, cytotoxic granule-associated RNA-binding protein) over time in any dose group compared with placebo or baseline (figure 4.6, appendix 4 p 26).

Two doses of SARS-CoV-2 Sclamp induced elevated antibody-secreting cells (ASCs) and follicular T-cells type-1 (Tfh1) activity in a dose-dependent manner. Following the first dose at Day 8, a very modest and insignificant increase in ASCs and activated circulating Tfh1 (cTfh1) cells was observed in the 15-µg and 45-µg groups. However, whole blood analysis showed that activated inducible co-stimulator $(ICOS)^+CD38^+$ Th1 cells were significantly increased in both dose groups compared with placebo. At Day 36 (8 days after the second dose), the frequency of ASCs increased significantly in the 5-µg and 15-µg groups compared with placebo, indicating that a second booster dose was needed to significantly expand both ASCs (and activated $ICOS^+CD38^+$ Th1 cells. Increased activated $ICOS^+$programmed cell death protein 1 $(PD\text{-}1)^+$ cTfh1 populations were also observed following two doses of 5-µg and 15-µg vaccine compared with a single 45-µg dose. However, a significant increase in cTfh1 cells was only observed with the 5-µg dose compared with placebo.

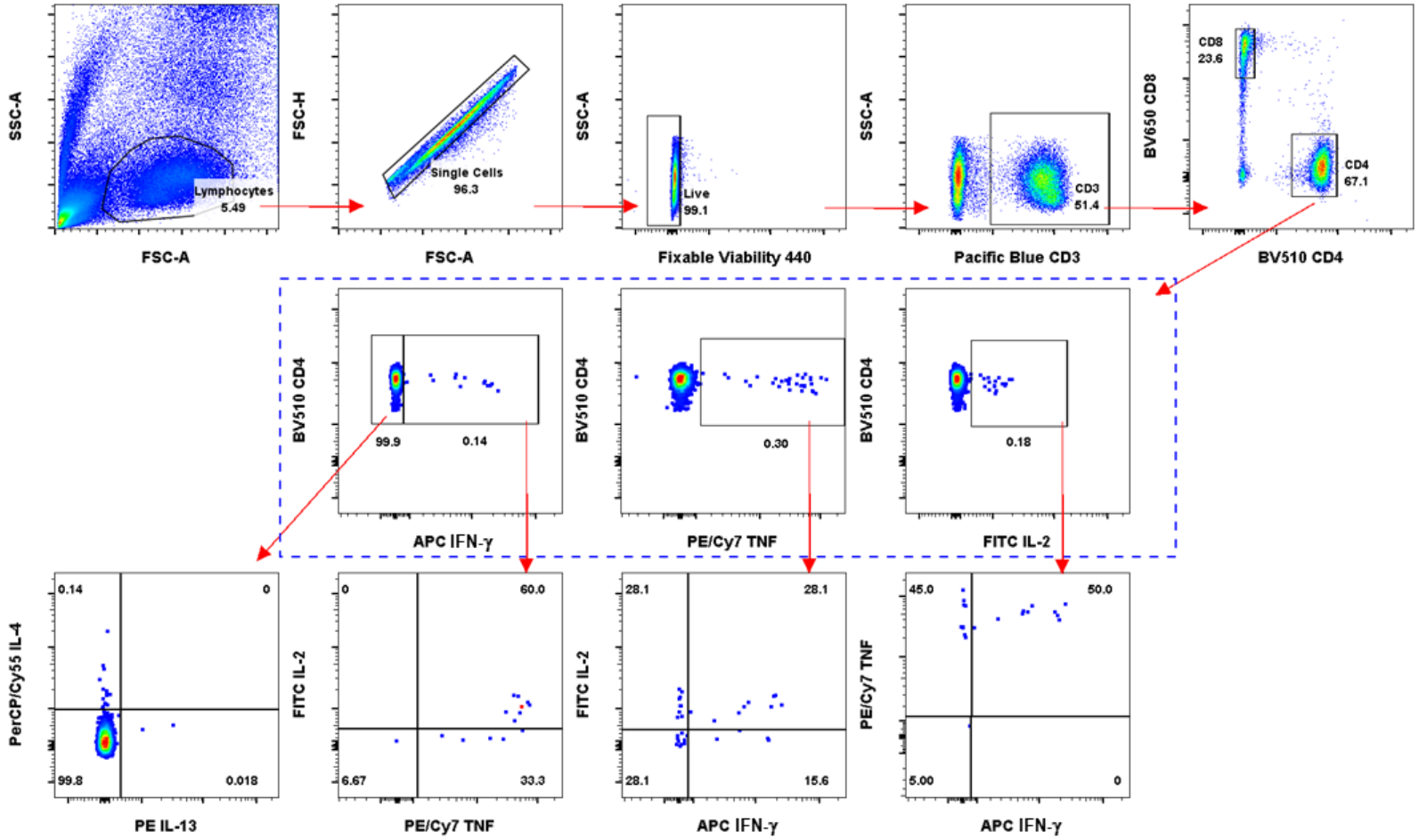

Figure 4.1: **Representative gating strategy – cytokine and polyfunctional analysis**

The FACS analysis to delineate cytokine-producing cells used a modified Boolean gating (logic gating). Firstly, doublet-discriminated live (Fixable Viability Stain negative) T cells were gated as $CD3^+$ $CD4^+$ or $CD3^+$ $CD8^+$ cells from the total lymphocyte population. Next, cytokine-positive cells were gated for $CD4^+$ or $CD8^+$ T-cell subsets. For example, as per FlowJo analysis of the $CD4^+$ IFN-γ^+ population, a quad gate was placed on the plot where IL-2 was on the y-axis and TNF on the x-axis. The resulting four quadrants were defined as single positive (Q4: IFN-γ^+ TNF^- IL-2^-), double positive (Q1: IFN-γ^+ TNF^+ IL-2^-, Q3: IFN-γ^+ TNF^- IL-2^+), and triple positive (Q2: IFN-γ^+ TNF^+ IL-

2^+) populations. Then, a similar gating strategy was placed on the TNF^+ population with IL-2 on the y-axis and IFN-γ on the x-axis, and the same was applied for the $IL\text{-}2^+$ population. IL-4 and IL-13 expression were evaluated from the $IFN\text{-}\gamma^-$ population. All gates were placed based on negative and positive controls for each sample. This enabled us to cross-check the polyfunctional gating from different parent populations to make sure that all the gates were accurate. This strategy enabled us to effectively calculate the single-, double-, or triple-positive populations without overlap, specifically when performing the summation analyses. For example, summation analysis of $CD4^+$ or $CD8^+$ T cells = $IFN\text{-}\gamma^+ + TNF^+ + IL\text{-}2^+ + IFN\text{-}\gamma^+TNF^+ + IFN\text{-}\gamma^+IL\text{-}2^+ + TNF^+IL\text{-}2^+ + IFN\text{-}\gamma^+TNF^+IL\text{-}2^+$ without overlap. Abbreviations: APC = antigen-presenting cells, CD = cluster of differentiation, FACS = fluorescence-activated cell sorting, FITC = fluorescein isothiocyanate, FSC-A = forward scatter, IFN-γ = interferon-γ, IL = interleukin, PE = Pseudomonas exotoxin, SARS-CoV-2 Sclamp = Severe Acute Respiratory Syndrome Coronavirus 2 S-glycoprotein-clamp vaccine, SSC-A = side scatter, TNF = tumour necrosis factor.

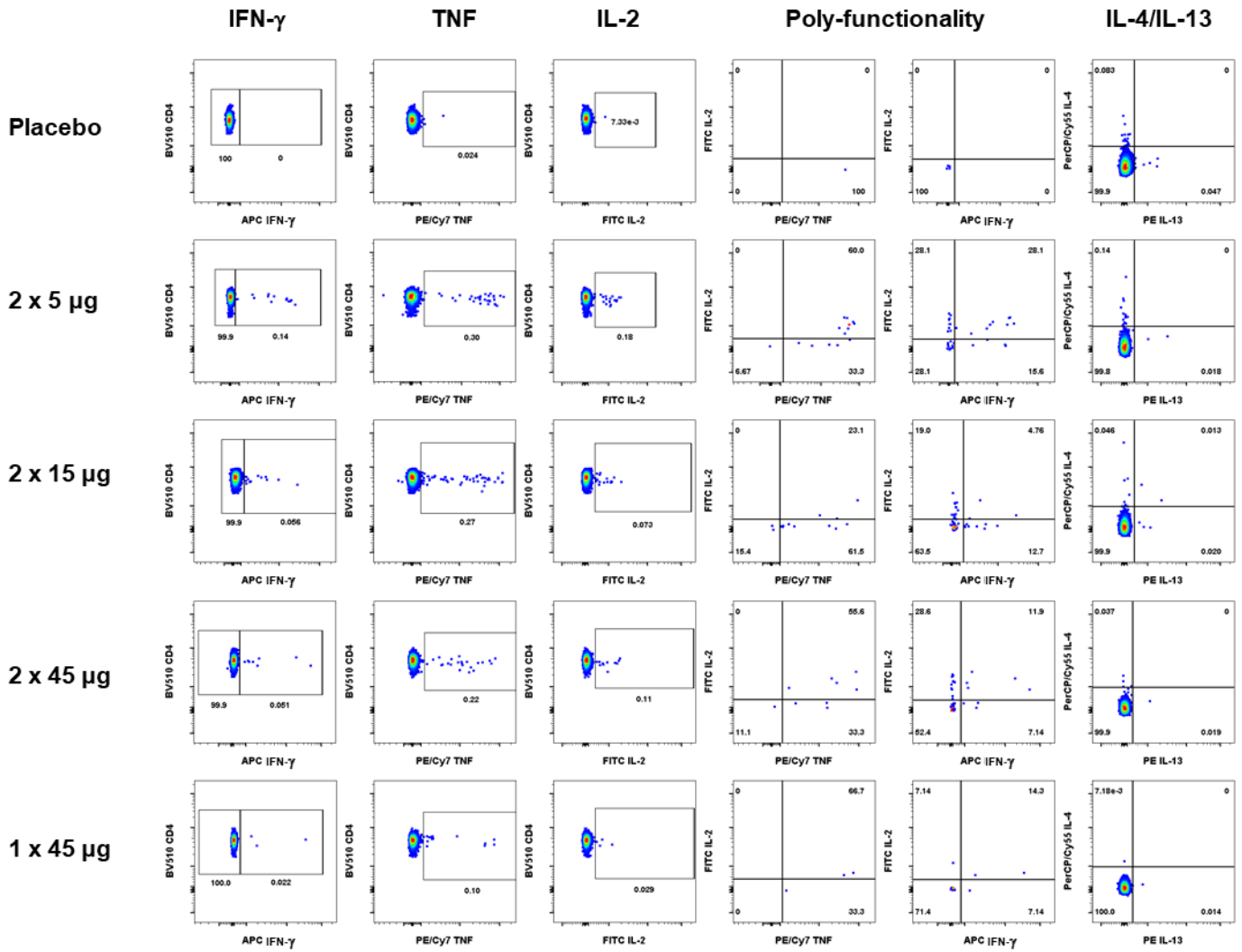

Figure 4.2: **SARS-CoV-2 specific CD4$^+$ T-cell responses – representative FACS plots**

The representative FACS plots show SARS-CoV-2 S-specific mono- or polyfunctional CD4$^+$ T cells with respect to the expression of IFN-γ, TNF, and IL-2, or IL-4 and IL-13 on the IFN-γ negative T-cell subset at Day 43 in the different treatment groups analysed. Abbreviations: APC = antigen-presenting cells, CD = cluster of differentiation,

FACS = fluorescence-activated cell sorting, FITC = fluorescein isothiocyanate, IFN-γ = interferon-γ, IL = interleukin, PE = Pseudomonas exotoxin, SARS-CoV-2 Sclamp = Severe Acute Respiratory Syndrome Coronavirus 2 S-glycoprotein-clamp vaccine, TNF = tumour necrosis factor.

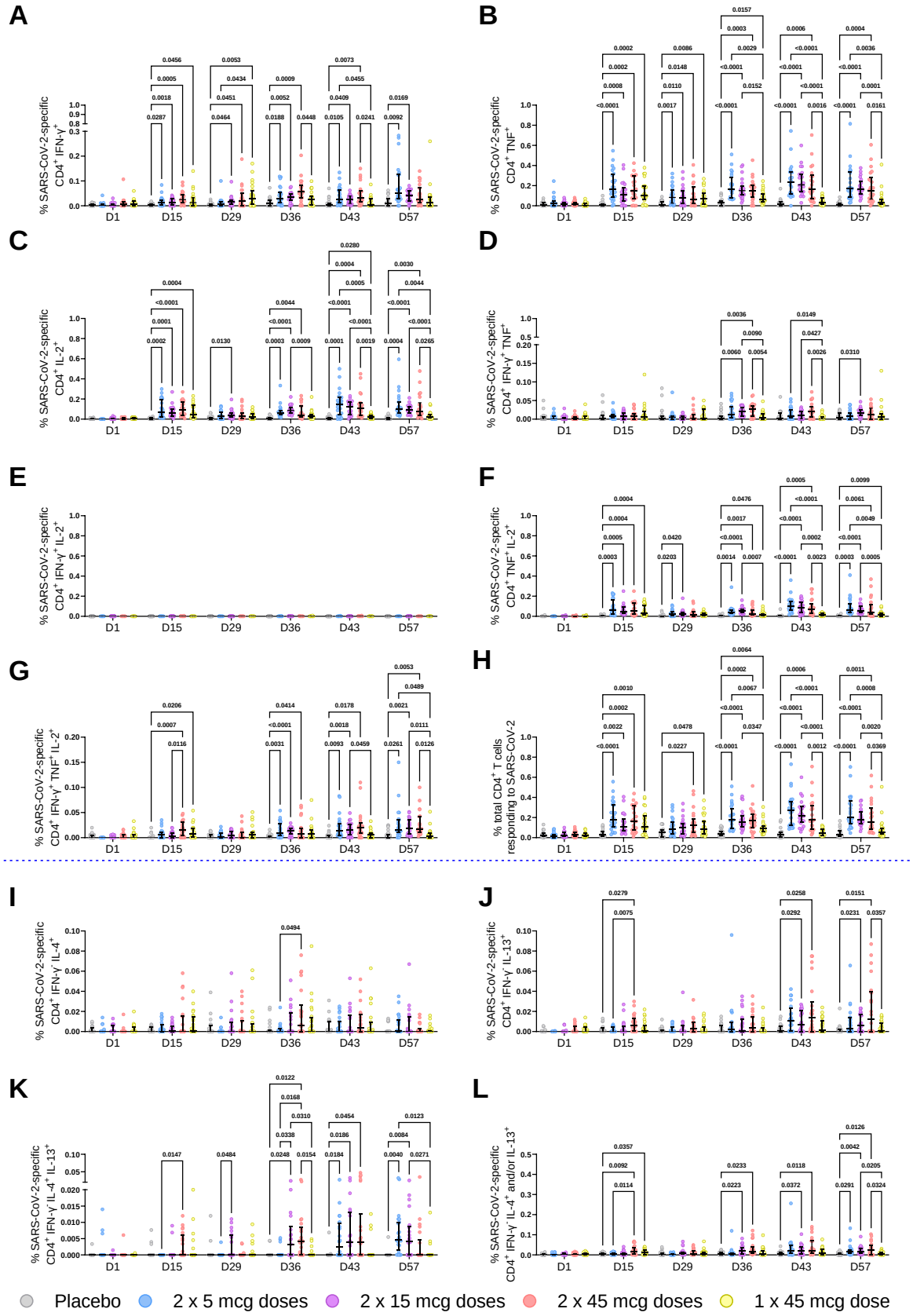

Figure 4.3: ***Kinetics of the SARS-CoV-2 S-specific CD4⁺ T-cell responses***
The graphs indicate percentage of SARS-CoV-2 S-specific CD4⁺ T cells that produced single or multiple (i.e. double positive) Th1 or Th2 cytokines. The monofunctional IFN-γ (A), TNF (B), and IL-2 (C) subsets, polyfunctional Th1 subsets (D-G), and add-up analysis (H), as well as Th2 (IL-4 and/or IL-13) subsets (I-K), and

add-upanalysis are shown. Lines and error bars indicate the median and IQR. Statistical significance between groups was determined using a fitted mixed-model two-way ANOVA. Abbreviations: ANOVA = analysis of variance, CD = cluster of differentiation, D = day, IFN-γ = interferon-γ, IL = interleukin, IQR = interquartile range, SARS-CoV-2 Sclamp = Severe Acute Respiratory Syndrome Coronavirus 2 S-glycoprotein-clamp vaccine, Th1 = T-helper 1, Th2 = T-helper 2, TNF = tumour necrosis factor.

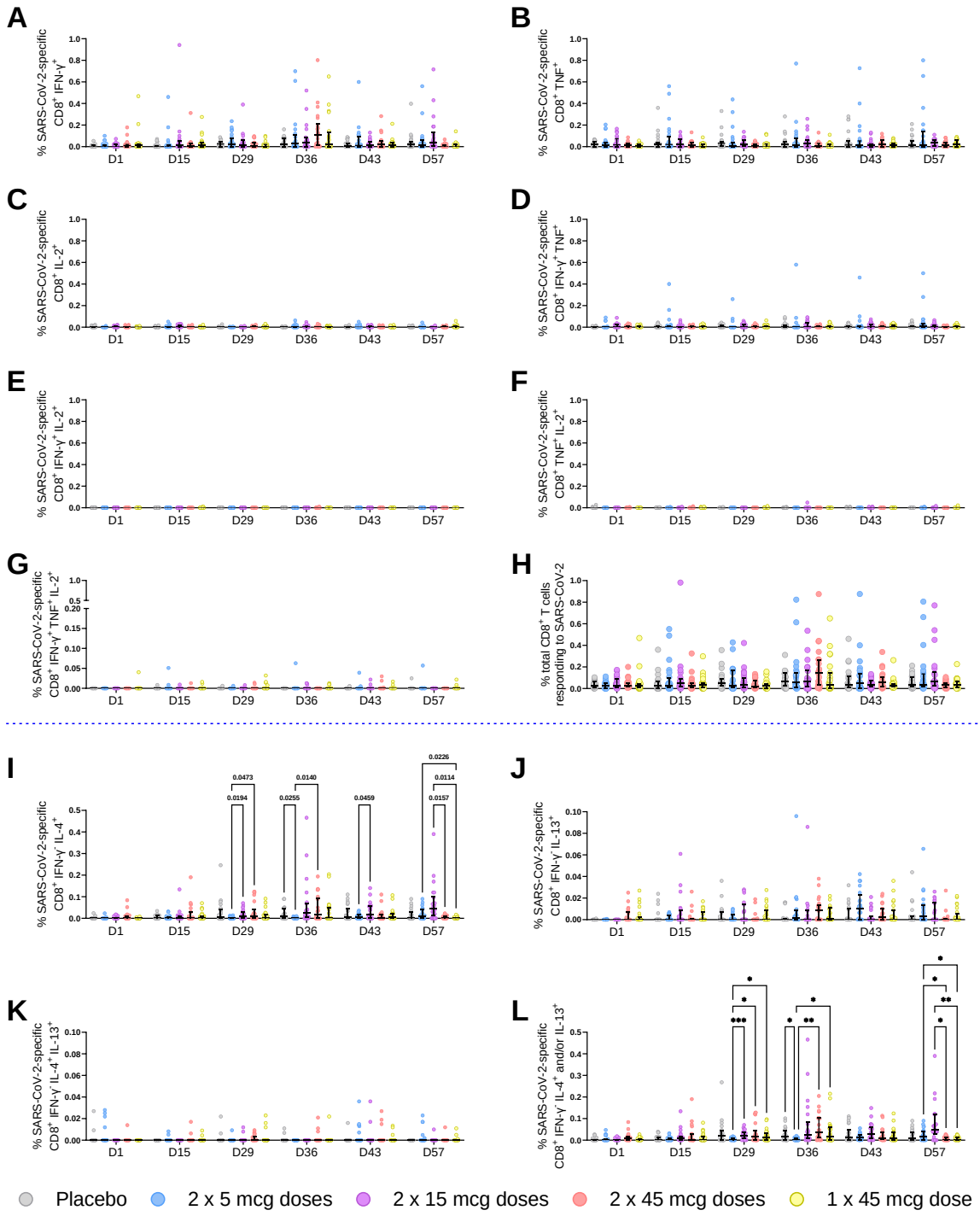

Figure 4.4: **Kinetics of the SARS-CoV-2 S-specific CD8$^+$ T-cell responses**
The graphs indicate percentage of SARS-CoV-2 S-specific CD8$^+$ T cells that produced single or multiple (i.e. double positive) Th1 or Th2 cytokines. The monofunctional IFN-γ (A), TNF (B), and IL-2 (C) subsets, polyfunctional Th1 subsets (D-G, and add-up analysis (H), as well as Th2 (IL-4 and/or IL-13) subsets (I-K) and add-up analysis (L) are shown. Lines and error bars indicate the median and IQR. Statistical significance between groups was determined using a fitted mixed-model two-way ANOVA. Abbreviations: ANOVA = analysis of variance, CD = cluster of differentiation, D = day, IFN-γ = interferon-γ, IL = interleukin, IQR = interquartile range, SARS-CoV-2 Sclamp = Severe Acute Respiratory Syndrome Coronavirus 2 S-glycoprotein-clamp vaccine, Th1 = T-helper 1, Th2 = T-helper 2, TNF = tumour necrosis factor.

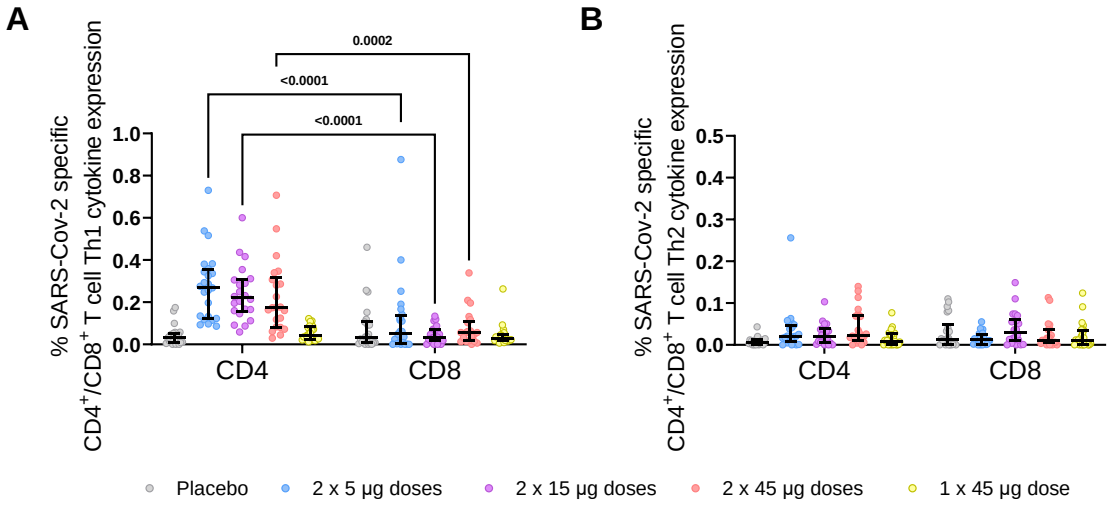

Figure 4.5: **Comparisons of total SARS-CoV-2 S-specific CD4+ and CD8+ T-cell responses**
The two plots show the comparison of total SARS-CoV-2 specific $CD4^+$ or $CD8^+$ T-cell subsets that exhibited a Th1 (A) or Th2 (B) cytokine production profile. Lines and error bars indicate the median and IQR. Statistical significance between groups was determined using a fitted mixed-model two-way ANOVA.. Abbreviations: ANOVA = analysis of variance, CD = cluster of differentiation, IQR = interquartile range, SARS-CoV-2 Sclamp = Severe Acute Respiratory Syndrome Coronavirus 2 S-glycoprotein-clamp vaccine, Th1 = T-helper 1, Th2 = T-helper 2.

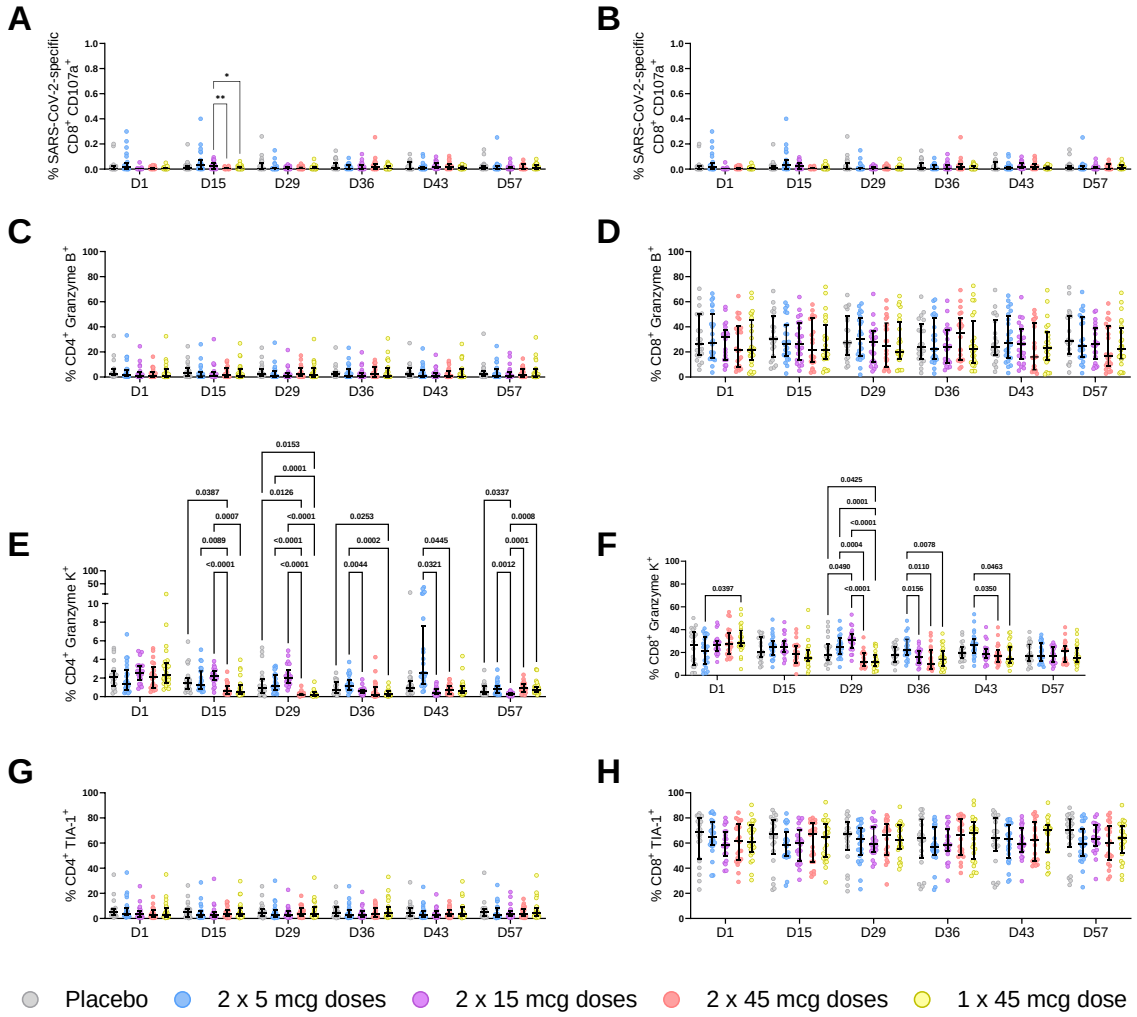

Figure 4.6: **Expression of CD4$^+$ and CD8$^+$ T-cell cytotoxic markers**
Percentage of SARS-CoV-2 S-specific CD107 expression by CD4$^+$ (A) and CD8$^+$ (B) T cells. Percentage of granzyme B, granzyme K, and TIA-1 expression by CD4$^+$ (C, E, G) and CD8$^+$ (D, F, H) T cells, with no SARS-CoV-2 S-peptide pool stimulation. Lines and error bars indicate the median and IQR. Statistical significance between groups was determined using a fitted mixed-model two-way ANOVA. Abbreviations: ANOVA = analysis of variance, CD = cluster of differentiation, D = day, IQR = interquartile range, SARS-CoV-2 Sclamp = Severe Acute Respiratory Syndrome Coronavirus 2 S-glycoprotein-clamp vaccine, TIA-1 = T-cell intracellular antigen 1.

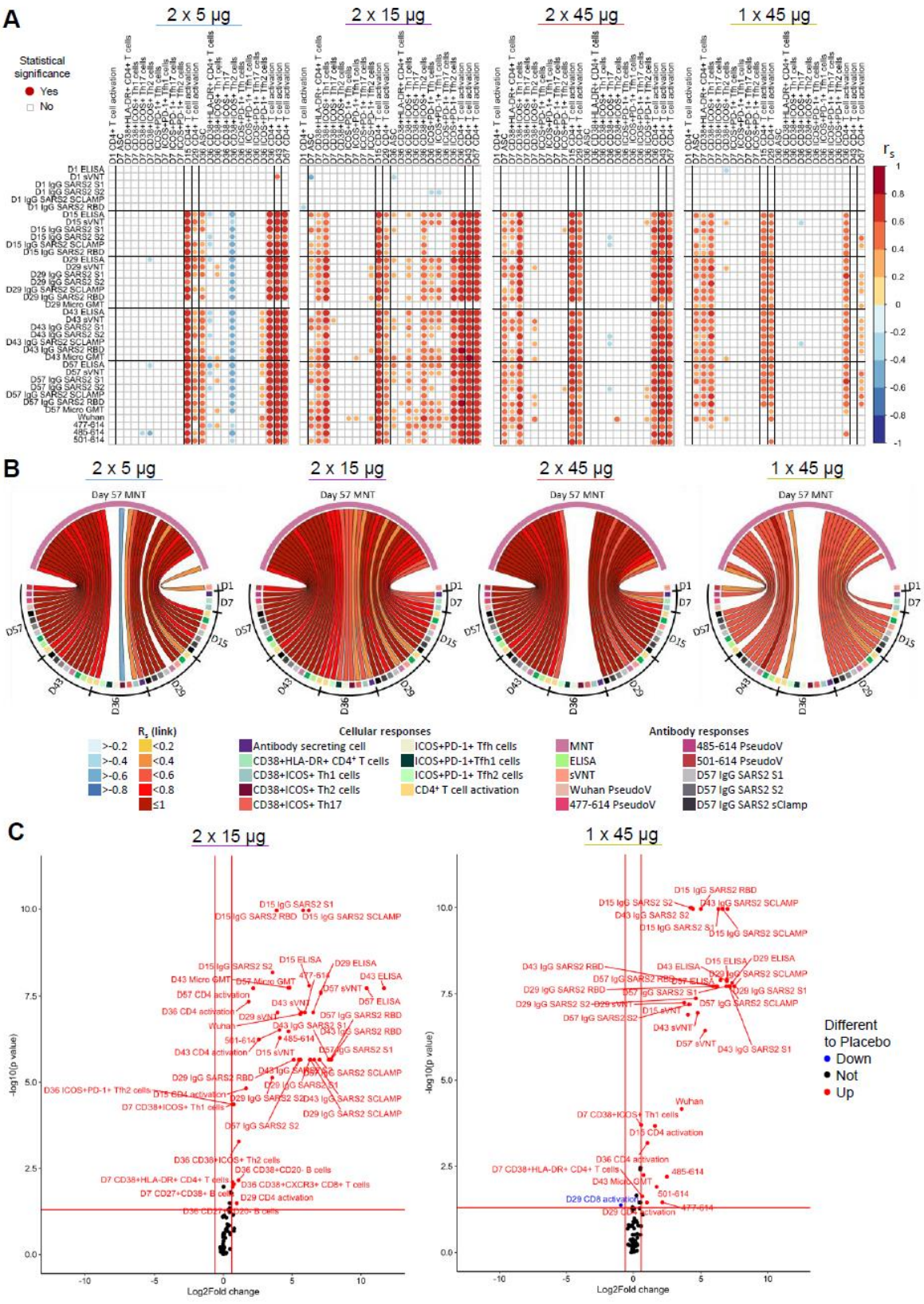

Figure 4.7: **Correlations between humoral and cellular immune responses**
Heat map of antibody and cellular responses **(A)**. Cellular responses of ASCs, Th, and Tfh cells were calculated as the fold change based on frequency data. $CD4^+$ T-cell activation and antibody data were based on actual data values. Each dataset included the placebo group as a negative control. Only significant correlations ($p<0{\cdot}05$) are shown. Circos plots correlating to Day 57 microneutralisation responses with the other cellular and antibody

responses per treatment group (**B**). Volcano plot of 100 total immune features for two doses of 15-µg vaccine and the single high-dose (45-µg) vaccine, with key representative features labelled (**C**).
Abbreviations: ASC = antibody-secreting cells, CD = cluster of differentiation, D = day, ELISA = enzyme-linked immunosorbent assay, GMT = geometric mean titre, HLA-DR = human leukocyte antigen – DR isotype, ICOS = inducible T-cell co-stimulator, MNT = microneutralisation, PD-1 = programmed cell death-protein 1, PseudoV = pseudovirus, RBD = receptor-binding domain, SARS2 = Severe Acute Respiratory Syndrome Coronavirus 2, SCLAMP = spike glycoprotein-clamp, sVNT = surrogate virus neutralisation test, Th = T-helper, Tfh = follicular T-helper.

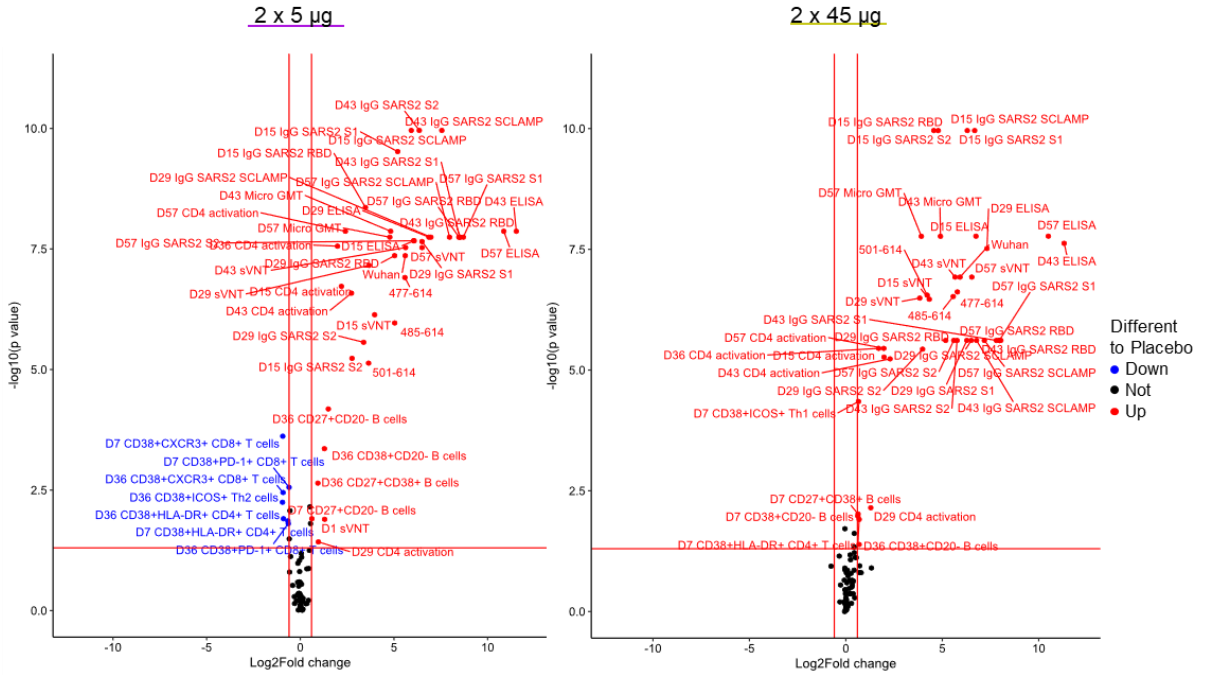

Figure 4.8: **Volcano plots of correlations between humoral and cellular immune responses**
Volcano plot of 100 total immune features for two doses of 5-µg vaccine and two doses of 15-µg vaccine, with key representative features labelled.
Abbreviations: CD = cluster of differentiation, D = day, ELISA = enzyme-linked immunosorbent assay, GMT = geometric mean titre, HLA-DR = human leukocyte antigen – DR isotype, ICOS = inducible co-stimulator, Ig = immunoglobulin, PD-1 = programmed cell death-protein 1, RBD = receptor-binding domain, sVNT = surrogate virus neutralisation, SARS-CoV-2 Sclamp = Severe Acute Respiratory Syndrome Coronavirus 2 S-glycoprotein-clamp vaccine, Th = T-helper.

Appendix 5 References

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