

The ChAdOx1 vectored vaccine, AZD2816, induces strong immunogenicity against SARS-CoV-2 B.1.351 and other variants of concern in preclinical studies

Alexandra J Spencer^{1*}, Susan Morris¹, Marta Ulaszewska¹, Claire Powers¹, Reshma Kailath¹, Cameron Bissett¹, Adam Truby¹, Nazia Thakur^{1,2}, Joseph Newman², Elizabeth R Allen¹, Chang Liu^{3,4}, Wanwisa Dejnirattisai³, Juthathip Mongkolsapaya³, Hannah Davies¹, Francesca R Donnellan¹, David Pulido¹, Thomas P. Peacock⁵, Wendy S. Barclay⁵, Helen Bright⁶, Kuishu Ren⁶, Gavin Screaton³, Patrick McTammy⁶, Dalan Bailey², Sarah C Gilbert¹, Teresa Lambe¹

1 The Jenner Institute, Nuffield Department of Medicine, University of Oxford, United Kingdom

2 The Pirbright Institute, Woking, Surrey, United Kingdom

3 The Wellcome Trust Centre for Human Genetics, Nuffield Department of Medicine, University of Oxford, United Kingdom

4 Chinese Academy of Medical Science (CAMS) Oxford Institute (COI), University of Oxford, United Kingdom

5 Department of Infectious Disease, Imperial College London, United Kingdom

6 AstraZeneca, Gaithersburg 20878, Maryland, United States

**Correspondence to: Alexandra J Spencer, The Jenner Institute, ORCRB, Roosevelt Drive, Oxford*

OX3 7DQ. Email: alex.spencer@ndm.ox.ac.uk.

Abstract:

There is an ongoing global effort, to design, manufacture, and clinically assess vaccines against SARS-CoV-2. Over the course of the ongoing pandemic a number of new SARS-CoV-2 virus isolates or variants of concern (VoC) have been identified containing mutations that negatively impact the role of neutralising antibodies. In this study we describe the generation and preclinical assessment of a ChAdOx1-vectored vaccine against the variant of concern B.1.351 (AZD2816). We demonstrate AZD2816 is immunogenic after a single dose and when used as a booster dose in animals primed with original vaccine AZD1222, we see no evidence of original antigenic sin but high titre antibodies against a number of variant spike proteins. In addition, neutralisation titres against B.1.351 (Beta), B.1.617.1 (Kappa) and B.1.617.2 (Delta), are induced in these boost regimens. These data support the ongoing clinical development and testing of this new variant vaccine.

Introduction:

Since the first reports of infections caused by a novel coronavirus, there has been an unprecedented global effort to design, manufacture and test multiple vaccines against SARS-CoV-2. All authorised vaccines, to date, target the full-length spike protein of SARS-CoV-2 and induce neutralising antibodies to varying levels. COVID-19 vaccines are being deployed world-wide and

effectiveness data is now demonstrating the impact vaccination has on preventing COVID related hospital admissions and death ¹⁻³.

Over the course of the pandemic a number of variants of concern (VoC) have been identified, each containing multiple mutations within the viral genome. Variants with mutations in the spike protein and in particular the receptor binding domain (RBD), which facilitates viral cell entry via the angiotensin-converting enzyme 2 (ACE2) receptor, are of most concern. There is the potential for VoC to escape vaccine-induced host immunity due to the reduced ability of neutralising antibodies to bind and prevent cell entry of the VoC, resulting in infection and disease even in vaccinated individuals.

The B.1.351 variant (Beta)⁴, first identified in October 2020, contains 10 changes across the spike protein with 3 amino acid changes in the RBD region. These changes in RBD are reported to increase binding between spike and ACE2, leading to overall reduced ability of antibodies induced against the original virus to block cell entry ⁵. Efforts are underway to produce second generation SARS CoV-2 vaccines targeting VoC.

In this study we describe the generation and preclinical assessment of ChAdOx1 expressing B.1.351 spike protein; AZD2816. Importantly both binding and neutralising antibodies against B.1.351 are measured after single dose vaccination. When AZD2816 is used as a booster dose in mice already primed with the original ChAdOx1 nCoV-19 (AZD1222) we measure strong antibody binding against both the original wild-type and B.1.351 spike protein, with booster doses increasing the antibody response and neutralising ability against other variants. These data support the clinical testing of AZD2816 either alone or in prime-boost regimens with heterologous spike proteins.

Results:

Single dose of vaccine induces cross-reactive immunity.

Following reports of the new SARS-CoV-2 variant B.1.351 expressing multiple mutations across the spike protein and reduced ability of vaccine induced and convalescent sera to neutralise this variant virus ⁵, we generated a new ChAdOx1 vector expressing spike containing the key B.1.351 mutations (Figure 1). To assess the immunogenicity, BALB/c mice were immunised with 10⁸iu AZD1222 (ChAdOx1 nCoV-19), AZD2816 (ChAdOx1 nCoV-19 B.1.351) or with 10⁸iu of each vaccine mixed together prior to immunisation (Figure 2A). Comparable levels of anti-spike antibodies were observed in all groups of vaccinated mice against both wild-type spike and B.1.351 spike protein (Figure 2B). Mixing both vaccines together did not compromise the antibody response to either spike protein, nor was there a difference between total ELISA Units measured on day 9 or day 16 post-vaccination (Figure 2B). This rapid onset of a measurable antibody response suggests this vaccine is highly immunogenic. Neutralising antibodies, measured in pseudotyped virus neutralisation assay, were detected against original wild-type and B.1.351 (Figure 2C).

T cell responses were measured by IFN γ ELISpot with splenocytes stimulated with peptide pools containing peptides common to both vaccines, wild-type spike peptides or B.1.351 peptides (Table S2). Equivalent numbers of IFN γ producing cells were detected against all pools of spike peptides at both timepoints measured (Figure 2D), with responses to the common peptides dominating the response and minimal responses observed against variant regions. Consistent with earlier studies ⁶, the T cell response was dominant towards the first 2 peptide pools corresponding to the S1 portion of the protein (Figure 2E) across all vaccine groups.

Antibody responses are boosted by vaccination with variant vaccine AZD2816

To determine whether a booster vaccination with a variant vaccine was impacted in the presence of a response to the wild-type spike protein, mice were immunised with one dose of AZD1222 prior to boosting with AZD2816 and antibody responses compared across relevant groups. Total IgG responses, measured by ELISA, showed that a booster dose of AZD2816 increased the antibody titre against wild-type spike and B.1.351 spike (Figure 3A). In addition, boosting AZD1222 primed mice with AZD2816 increased the antibody titre against other variant proteins including P.1 (Gamma) and B.1.429 (Epsilon) when compared with a single dose of AZD1222 (Figure 3B). Neutralising antibody titres were also higher against the wild-type, B.1.351, B.1.617.1 (Kappa) or B.1.617.2 (Delta) (variants of concern) in two dose regimens with AZD1222 and AZD2816 (Figure 3C and Table 1).

AZD2816 as a third dose maintains T cell responses

To maximise the vaccine induced immune response and associated vaccine efficacy against disease, AZD1222 has been authorised for use in a 2-dose vaccination regimen. To determine the impact of immunisation with AZD2816 after the clinically recommended dosing regimen, BALB/c mice received two doses of AZD1222 4 weeks apart and were boosted with 10^8 iu of AZD2816 or remained unboosted (Figure 4A). Although a booster dose with AZD2816 did not further increase the frequency of antigen specific T cells (Figure 4B), the breadth of the cellular immune response remained consistent (Figure 4B). The cellular immune response is dominated by responses to common SARS-CoV-2 spike peptides with minimal reactivity against peptides from either original spike WT or B.1.153 as measured after a single dose of vaccine (Figure 2). Most importantly, a third dose vaccination with AZD2816 did not alter T cell response with CD4⁺ T cells shown to produce primarily IFN γ , and no-significant difference in the proportion or number of T effector (Teff), T effector memory (Tem) or T central memory (Tcm) CD4⁺ T cells observed, (Figure 4C). Consistent with previous data in mice ⁶, the anti-spike cell-mediated response was dominated by CD8⁺ T cells, with a high frequency of CD8⁺ T cells producing IFN γ and TNF α was observed in both groups of mice (Figure 4D left), with a response dominated by Teff and Tem CD8⁺ T cells, that was similar between vaccine regimens.

AZD2816 as a third dose can further enhance antibody responses induced by two doses of AZD1222

Antibody responses were also compared between mice receiving a homologous AZD1222 two dose regimen or with a third dose AZD2816 vaccination. A significant increase in total IgG ELISA units was observed against wild-type spike following a booster dose, while a small (albeit not statistically significant) increase against B.1.351 protein was observed (Figure 5B). Antibody binding was observed against all variant spike proteins (Figure 5C), with significantly higher responses observed in AZD2816 boosted mice against variant proteins B.1.1.7 (Alpha) and B.1.429 (Figure 5C). Neutralising responses were detected in all vaccine groups against wild-type spike, B.1.351, B.1.617.1 and B.1.617.2 (Table 1), with higher neutralisation titres against the two variant proteins observed in AZD2816 boosted animals (Figure 5D).

Overall the data shows that a booster dose with a new ChAdOx1 against the new variant B.1.351 (AZD2816), can further enhance antibody responses against SARS-CoV-2 B.1.351 and provide cross-reactivity against other variant proteins.

Discussion:

In populations where vaccination against SARS-CoV-2 has been widely used the impact on prevention of severe disease, hospitalisation and death has been demonstrated, but variant

viruses with mutations in the spike protein are now in circulation, and the efficacy of the original vaccines against VoCs may be reduced.

The VoC B.1.351 was first identified in South Africa and was thought to have driven the second wave of infection resulting in a larger proportion of young individuals being infected than previously seen, with health officials indicating that B.1.351 spreads faster than other variants. B.1.351 contains several mutations across the S1 portion of spike protein. In particular, three mutations involved in binding of spike to the ACE2 receptor have been shown to increase the strength of spike-ACE2 binding, with some antibodies from convalescent or vaccinated individuals showing reduced ability to neutralise this variant virus⁵. A number of common amino acid changes within the RBD and NTD region of the spike protein have been identified amongst SARS-CoV-2 variants (Table S2). The D614G identified in all VoC, increases virus infectivity^{7,8}, potentially through increased density of spike on the virion surface⁹. The L452R change is present in B.1.429, B.1.617.1 and B.1.617.2 shown to reduce sensitivity of neutralising antibodies⁷. The E484K is present in B.1.351 and P.1 isolates is believed to enhance binding affinity of RBD to ACE2^{10,11} and evasion from antibodies¹². The N501Y is present in B.1.351, B.1.1.7 and P.1 variants alone does not appear to significantly impact neutralisation, but N501Y in combination with E484K and D614G can affect sera neutralisation titres^{13,14}. A high proportion of isolated neutralising anti-spike antibody bind to the RBD domain of spike¹⁵⁻¹⁷, there is concern these cumulative changes are leading to the reduced ability of antibodies induced against WT SARS-CoV-2 to neutralise VoCs^{5,18,19}.

The mRNA and viral vector technologies that allowed rapid production of vaccines against SARS-CoV-2 in early 2020 can be readily employed to express the spike protein from VoCs rather than the original virus. Here we generated AZD2816, a new ChAdOx1 nCoV-19 vaccine expressing B.1.351 spike protein and assessed the immunogenicity in mice. As priming of the immune response to the original wild-type spike protein may impact the ability to switch specificity of the response to B.1.351, we measured antibody and T cell responses after one or two doses of the original ChAdOx1 nCoV-19 vaccine (AZD1222) followed by a single dose of AZD2816.

While a single dose of either AZD1222 or AZD2816 induces rapid T cells and antibodies capable of binding and neutralising wild-type and B.1.351 spike protein, antibody responses can be increased with a booster dose of either AZD1222 or AZD2816. Importantly, we saw no evidence that priming of the immune system response was detrimental when mice received a booster dose of ChAdOx1 expressing B.1.351 expressing protein. Equivalent high levels of T cells were observed, with equivalent cytokines produced and populations of effector and memory T cells (Figure 4). Boosting mice with one dose of AZD2816 after a one or two doses of AZD1222 led to an increase in binding antibody titres in addition to neutralisation against B.1.351 and both B.1.617 variants. In addition, higher antibody titres against P.1, B.1.1.7, B.1.429 and D614G spike was also observed.

Ongoing surveillance has identified B.1.617.2 as a VoC that is spreading rapidly within the UK and elsewhere. Two dose vaccination with AZD1222 induces antibodies capable of neutralising B.1.617.1 and B.1.617.2 (Table 1), and early real-world evidence suggest that the effectiveness of this regimen against hospitalisation and death is maintained (https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/988619/Variants_of_Concern_VOC_Technical_Briefing_12_England.pdf). Encouragingly neutralisation can be further enhanced with a 3rd dose of vaccine (Table 1), supporting the clinical assessment of these regimens.

The data presented herein demonstrates that vaccination with ChAdOx1 nCoV-19 (AZD1222) induces high titre cross-reactive antibodies capable of neutralising a number of SARS-CoV-2 variants of concern, B.1.351, B.1.617.1 and B.1.617.2. Most importantly these responses can be further enhanced by a booster dose of vaccine expressing the spike protein from B.1.351. These

data support clinical assessment of AZD2816 in vaccine naïve individuals as well as those previously vaccinated with AZD1222.

Methods:

Vector Construction;

AZD2816 vaccine was constructed as previously described²⁰. In brief, the B.1.351 glycoprotein (S) gene⁴ was codon-optimized for expression in human cell lines and synthesized with the tissue plasminogen activator (tPA) leader sequence at the 5' end by GeneArt Gene Synthesis (Thermo Fisher Scientific). The S gene was inserted into the Gateway[®] recombination cassette of the shuttle plasmid containing a human cytomegalovirus major immediate early promoter (IE CMV), which includes intron A and two tetracycline operator 2 sites, and the bovine growth hormone polyadenylation signal. BACs containing the ChAdOx1 SARS-CoV-2 B.1.351 Spike protein were prepared by Gateway[®] recombination between the ChAdOx1 destination DNA BAC vector²¹ and the shuttle plasmids containing the SARS CoV-2 S gene expression cassettes using standard protocols resulting in the insertion of the SARS-CoV-2 expression cassette at the E1 locus. The ChAdOx1 SARS CoV-2 S adenovirus genome was excised from the BAC using unique PmeI sites flanking the adenovirus genome sequence. ChAdOx1 SARS CoV-2 S viral vectors were rescued in T-REx[™] cells (Invitrogen, Cat. R71007), a derivative of HEK293 cells which constitutively express the Tet repressor protein and prevent antigen expression during virus production. The resultant virus, ChAdOx1 nCov-19 B.1.351 (AZD2816), was purified by CsCl gradient ultracentrifugation as described previously. The titres were determined on T-REx[™] cells using anti-hexon immunostaining assay based on the QuickTiter[™] Adenovirus Titer Immunoassay kit (Cell Biolabs Inc).

Ethics Statement; Mice were used in accordance with the UK Animals (Scientific Procedures) Act 1986 under project license number P9804B4F1 granted by the UK Home Office with approval from the local Animal Welfare and Ethical Review Board (AWERB) at the University of Oxford. Age matched animals were purchased from commercial suppliers as a batch for each experiment and randomly split into groups on arrival at our facility. Animals were group housed in IVCs under SPF conditions, with constant temperature (20-24°C) and humidity (45-65%) with lighting on a 13:11 light-dark cycle (7am to 8pm). For induction of short-term anaesthesia, animals were anaesthetised using vaporised IsoFlo[®]. All animals were humanely sacrificed at the end of each experiment by an approved Schedule 1 method.

Animals and Immunizations; Inbred BALB/cOlaHsd (BALB/c) (Envigo) (n=5 to 7 mice per group), were immunized intramuscularly (i.m.) in the musculus tibialis with 10⁸ infectious units (iu) of ChAdOx1 vector. Mice were boosted with the relevant vaccine candidate 4 weeks later. All mice were sacrificed 3 weeks (or at a time indicated on figure legend) after the final vaccination with serum and spleens collected for analysis of humoral and cell-mediated immunity.

Antigen specific IgG ELISA; MaxiSorp plates (Nunc) were coated with 250ng/well of full-length SARS-CoV-2 wild-type (WT) spike (NC_045512), B.1.351 spike, B.1.1.7 spike, P.1 spike, B.1.429 spike and original wild-type spike sequence with a D to G amino acid substitution at position 614 (D614G) protein (Table S1) overnight at 4 °C, prior to washing in PBS/Tween (0.05% v/v) and blocking with Blocker Casein in PBS (Thermo Fisher Scientific) for 1 hour at room temperature (RT). Standard positive serum (pool of mouse serum with high endpoint titre against original wild-type spike protein), individual mouse serum samples, negative and an internal control (diluted in

casein) were incubated for 2 hrs at RT. Following washing, bound antibodies were detected by addition of a 1 in 5000 dilution of alkaline phosphatase (AP)-conjugated goat anti-mouse IgG (Sigma-Aldrich) for 1 hour at RT and addition of p-Nitrophenyl Phosphate, Disodium Salt substrate (Sigma-Aldrich). An arbitrary number of ELISA units (EU) were assigned to the reference pool and optical density values of each dilution were fitted to a 4-parameter logistic curve using SOFTmax PRO software. ELISA units were calculated for each sample using the optical density values of the sample and the parameters of the standard curve. All data was log-transformed for presentation and statistical analyses.

Micro neutralisation test (mVNT) using lentiviral-based pseudotypes bearing the SARS-CoV-2 Spike; Spike-expressing plasmid constructs were generated using the QuikChange Lightning Multi Site-Directed Mutagenesis kit (Agilent) on a previously described Wuhan-hu-1 template²². Lentiviral-based SARS-CoV-2 pseudotyped viruses were generated in HEK293T cells incubated at 37 °C, 5% CO₂ as previously described²³. Briefly, cells were seeded at a density of 7.5 x 10⁵ in 6 well dishes, before being transfected with plasmids as follows: 500 ng of SARS-CoV-2 spike (NC_045512, B.1.351, B.1.617.1, B.1.617.2) (Table S1), 600 ng p8.91 (encoding for HIV-1 gag-pol), 600 ng CSFLW (lentivirus backbone expressing a firefly luciferase reporter gene), in Opti-MEM (Gibco) along with 10 µL PEI (1 µg/mL) transfection reagent. A 'no glycoprotein' control was also set up using the pcDNA3.1 vector instead of the SARS-CoV-2 Spike expressing plasmid. The following day, the transfection mix was replaced with 3 mL DMEM with 10% FBS (DMEM-10%) and incubated for 48 and 72 hours, after which supernatants containing pseudotyped SARS-CoV-2 (SARS-CoV-2 pps) were harvested, pooled and centrifuged at 1,300 x g for 10 minutes at 4 °C to remove cellular debris. Target HEK293T cells, previously transfected with 500 ng of a human ACE2 expression plasmid (Addgene, Cambridge, MA, USA) were seeded at a density of 2 x 10⁴ in 100 µL DMEM-10% in a white flat-bottomed 96-well plate one day prior to harvesting SARS-CoV-2 pps. The following day, SARS-CoV-2 pps were titrated 10-fold on target cells, and the remainder stored at -80 °C. For mVNTs, sera was diluted 1 in 20 in serum-free media and 50 µL was added to a 96-well plate in triplicate and titrated 2-fold. A fixed titred volume of SARS-CoV-2 pps was added at a dilution equivalent to 10⁵ to 10⁶ signal luciferase units in 50 µL DMEM-10% and incubated with sera for 1 hour at 37 °C, 5% CO₂ (giving a final sera dilution of 1 in 40). Target cells expressing human ACE2 were then added at a density of 2 x 10⁴ in 100 µL and incubated at 37 °C, 5% CO₂ for 72 hours. Firefly luciferase activity was then measured with BrightGlo luciferase reagent and a Glomax-Multi+ Detection System (Promega, Southampton, UK). Pseudotyped virus neutralisation titres were calculated by interpolating the point at which there was 80% reduction in luciferase activity, relative to untreated controls (80% neutralisation, inhibitory dilution 80, ID80).

ELISpot and ICS staining; Spleen single cell suspension were prepared by passing cells through 70µM cell strainers and treatment with ammonium potassium chloride lysis solution prior to resuspension in complete media. Splenocytes were stimulated 15mer peptides (overlapping by 11) spanning the length of SARS-CoV-2 protein and tpa promoter, with peptide pools subdivided into common and variant peptide regions within the S1 and S2 region of spike (Figure 1A) (Table S2). For analysis of IFN γ production by ELISpot, splenocytes were stimulated with two pools of S1 peptides (pools 1 and 2) and two pools of S2 peptides (pools 3 and 4) (final concentration of 2µg/mL) on hydrophobic-PVDF ELISpot plates (Merck) coated with 5µg/mL anti-mouse IFN γ (AN18). After 18-20 hours of stimulation at 37°C, IFN γ spot forming cells (SFC) were detected by staining membranes with anti-mouse IFN γ biotin (1mg/mL) (R46A2) followed by streptavidin-Alkaline Phosphatase (1mg/mL) and development with AP conjugate substrate kit (BioRad, UK). Spots were enumerated using an AID ELISpot reader and software (AID).

For analysis of intracellular cytokine production, cells were stimulated at 37°C for 6 hours with 2µg/mL a pool of S1 (ELISpot pools 1 and 2) or S2 (ELISpot pools 3 and 4) total original spike peptides (Table S2), media or positive control cell stimulation cocktail (containing PMA-Ionomycin, BioLegend), together with 1µg/mL Golgi-plug (BD) and 2µl/mL CD107a-Alexa647 (Clone 1D4B). Following surface staining with CD3-A700 (Clone 17A2, 1 in 100), CD4-BUV496 (Clone GK1.5, 1 in 200), CD8-BUV395 (Clone 53-6.7, 1 in 200), CD11a-PECy7 (Clone H155-78, 1 in 200), CD44-BV780 (Clone IM7, 1 in 100), CD62L-BV711 (Clone MEL-14, 1 in 100), CD69-PECy7 (Clone H1.2F3, 1 in 100), CD103-APCCy7 (Clone 2E7, 1 in 100) and CD127-BV650 (Clone A7R34, 1 in 100) cells were fixed with 4% paraformaldehyde and stained intracellularly with IL2-PerCPCy5.5 (Clone JES6-5H4, 1 in 100), IL4-BV605 (Clone 11B11, 1 in 100), IL10-PE (Clone JES5-16E3, 1 in 100), IFNγ-e450 (Clone XMG1.2, 1 in 100) and TNFα-A488 (Clone MP6-XT22, 1 in 100) diluted in Perm-Wash buffer (BD). Sample acquisition was performed on a Fortessa (BD) and data analyzed in FlowJo V10 (TreeStar). An acquisition threshold was set at a minimum of 5000 events in the live CD3⁺ gate. Antigen specific T cells were identified by gating on LIVE/DEAD negative, size (FSC-A vs SSC), doublet negative (FSC-H vs FSC-A), CD3⁺, CD4⁺ or CD8⁺ cells and each individual cytokine. T cell subsets were gated within the population of “IFNγ⁺ or TNFα⁺” responses and are presented after subtraction of the background response detected in the corresponding media stimulated control sample for each mouse, and summing together the response detected to each pool of peptides. T effector (Teff) cells were defined as CD62L^{low} CD127^{low}, T effector memory (Tem) cells defined as CD62L^{low} CD127^{hi} and T central memory (Tcm) cells defined as CD62L^{hi} CD127^{hi} (Figure S1). The total number of cells was calculated by multiplying the frequency of the background corrected population (expressed as a percentage of total lymphocytes) by the total number of lymphocytes counted in each individual spleen sample.

Statistical analysis; All graphs and statistical analysis were performed using Prism v9 (Graphpad). For analysis of vaccination regimen against a single variable (eg IgG level), data was analysed with a one-way anova (Kruskal-Wallis) followed by post-hoc Dunn’s multiple comparison test. For analysis of vaccination regimen against multiple variables (eg each individual cytokine or T cell subset) the data was analysed with a two-way analysis of variance, where a significant difference was observed, a post-hoc analysis was performed to compare the overall effect of vaccination regimen. In graphs where a significant difference was observed between multiple vaccine groups, the highest p value is displayed on the graph. All data displayed on a logarithmic scale was log₁₀ transformed prior to statistical analysis (ELISA Units, Neutralisation Titres, Total Cell Numbers).

Data availability; The data that support the findings of this study are available within the article and its Supplementary Information files or are available from the corresponding author upon reasonable request. Source data are provided with this paper.

Acknowledgments; The authors would like to thank the BMS staff for animal husbandry and A. Worth, J.Furze, M. Mykhaylyk and R. Evans for facilities support.

Funding; This research was funded by AstraZeneca. JN, TPP, WSB and DB are funded by the G2P-UK National Virology consortium, MRC/UKRI (grant ref: MR/W005611/1).

Author Contributions; SM, RK, CP cloned and produced virus preparations; AJS, MU, AT, CB & ERA performed animal procedures and/or sample processing; AJS, MU, NT, JN, CB performed

experiments; AJS, NT, DA analyzed data; CL, WD, JM, HD, FRD, DP, TPP, WSB, HB, KR, GS, PM provided reagents; AJS, TL & SG designed the study. AJS & TL wrote the manuscript. All authors reviewed the final version of the manuscript.

Competing interests; SCG is co-founder and board member of Vaccitech and named as an inventor on a patent covering use of ChAdOx1-vectored vaccines and a patent application covering the ChAdOx1 nCoV-19 (AZD1222) vaccine. TL is named as an inventor on a patent application covering the ChAdOx1 nCoV-19 (AZD1222) vaccine and was consultant to Vaccitech. PM was an employee of AstraZeneca, KR is an employee of AstraZeneca. HB is an employee of AstraZeneca and is a named inventor on a patent application covering the AZD2816 vaccine.

References:

- 1 Vasileiou, E. *et al.* Interim findings from first-dose mass COVID-19 vaccination roll-out and COVID-19 hospital admissions in Scotland: a national prospective cohort study. *Lancet* **397**, 1646-1657, doi:10.1016/S0140-6736(21)00677-2 (2021).
- 2 Hall, V. J. *et al.* COVID-19 vaccine coverage in health-care workers in England and effectiveness of BNT162b2 mRNA vaccine against infection (SIREN): a prospective, multicentre, cohort study. *Lancet* **397**, 1725-1735, doi:10.1016/S0140-6736(21)00790-X (2021).
- 3 Mahase, E. Covid-19: One dose of vaccine cuts risk of passing on infection by as much as 50%, research shows. *BMJ* **373**, n1112, doi:10.1136/bmj.n1112 (2021).
- 4 Tegally, H. *et al.* Emergence and rapid spread of a new severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) lineage with multiple spike mutations in South Africa. *medRxiv*, 2020.2012.2021.20248640, doi:10.1101/2020.12.21.20248640 (2020).
- 5 Zhou, D. *et al.* Evidence of escape of SARS-CoV-2 variant B.1.351 from natural and vaccine-induced sera. *Cell* **184**, 2348-2361 e2346, doi:10.1016/j.cell.2021.02.037 (2021).
- 6 Spencer, A. J. *et al.* Heterologous vaccination regimens with self-amplifying RNA and adenoviral COVID vaccines induce robust immune responses in mice. *Nature communications* **12**, 2893, doi:10.1038/s41467-021-23173-1 (2021).
- 7 Li, Q. *et al.* The Impact of Mutations in SARS-CoV-2 Spike on Viral Infectivity and Antigenicity. *Cell* **182**, 1284-1294 e1289, doi:10.1016/j.cell.2020.07.012 (2020).
- 8 Hou, Y. J. *et al.* SARS-CoV-2 D614G variant exhibits efficient replication ex vivo and transmission in vivo. *Science* **370**, 1464-1468, doi:10.1126/science.abe8499 (2020).
- 9 Zhang, L. *et al.* SARS-CoV-2 spike-protein D614G mutation increases virion spike density and infectivity. *Nature communications* **11**, 6013, doi:10.1038/s41467-020-19808-4 (2020).
- 10 Nelson, G. *et al.* Molecular dynamic simulation reveals E484K mutation enhances spike RBD-ACE2 affinity and the combination of E484K, K417N and N501Y mutations (501Y.V2 variant) induces conformational change greater than N501Y mutant alone, potentially resulting in an escape mutant. *bioRxiv*, 2021.2001.2013.426558, doi:10.1101/2021.01.13.426558 (2021).
- 11 Wang, W. B. *et al.* E484K mutation in SARS-CoV-2 RBD enhances binding affinity with hACE2 but reduces interactions with neutralizing antibodies and nanobodies: Binding free energy calculation studies. *bioRxiv*, 2021.2002.2017.431566, doi:10.1101/2021.02.17.431566 (2021).
- 12 Greaney, A. J. *et al.* Complete Mapping of Mutations to the SARS-CoV-2 Spike Receptor-Binding Domain that Escape Antibody Recognition. *Cell Host Microbe* **29**, 44-57 e49, doi:10.1016/j.chom.2020.11.007 (2021).
- 13 Xie, X. *et al.* Neutralization of SARS-CoV-2 spike 69/70 deletion, E484K and N501Y variants by BNT162b2 vaccine-elicited sera. *Nat Med* **27**, 620-621, doi:10.1038/s41591-021-01270-4 (2021).
- 14 Li, Q. *et al.* SARS-CoV-2 501Y.V2 variants lack higher infectivity but do have immune escape. *Cell* **184**, 2362-2371 e2369, doi:10.1016/j.cell.2021.02.042 (2021).
- 15 Zost, S. J. *et al.* Potently neutralizing and protective human antibodies against SARS-CoV-2. *Nature* **584**, 443-449, doi:10.1038/s41586-020-2548-6 (2020).
- 16 Cerutti, G. *et al.* Potent SARS-CoV-2 neutralizing antibodies directed against spike N-terminal domain target a single supersite. *Cell Host Microbe* **29**, 819-833 e817, doi:10.1016/j.chom.2021.03.005 (2021).

- 17 Yang, L. *et al.* COVID-19 antibody therapeutics tracker: a global online database of antibody therapeutics for the prevention and treatment of COVID-19. *Antib Ther* **3**, 205-212, doi:10.1093/abt/tbaa020 (2020).
- 18 Collier, D. A. *et al.* Sensitivity of SARS-CoV-2 B.1.1.7 to mRNA vaccine-elicited antibodies. *Nature* **593**, 136-141, doi:10.1038/s41586-021-03412-7 (2021).
- 19 Wibmer, C. K. *et al.* SARS-CoV-2 501Y.V2 escapes neutralization by South African COVID-19 donor plasma. *Nat Med* **27**, 622-625, doi:10.1038/s41591-021-01285-x (2021).
- 20 van Doremalen, N. *et al.* ChAdOx1 nCoV-19 vaccine prevents SARS-CoV-2 pneumonia in rhesus macaques. *Nature* **586**, 578-582, doi:10.1038/s41586-020-2608-y (2020).
- 21 Dicks, M. D. *et al.* A novel chimpanzee adenovirus vector with low human seroprevalence: improved systems for vector derivation and comparative immunogenicity. *PLoS One* **7**, e40385, doi:10.1371/journal.pone.0040385 (2012).
- 22 McKay, P. F. *et al.* Self-amplifying RNA SARS-CoV-2 lipid nanoparticle vaccine candidate induces high neutralizing antibody titers in mice. *Nature communications* **11**, 3523, doi:10.1038/s41467-020-17409-9 (2020).
- 23 Graham, S. P. *et al.* Evaluation of the immunogenicity of prime-boost vaccination with the replication-deficient viral vectored COVID-19 vaccine candidate ChAdOx1 nCoV-19. *NPJ Vaccines* **5**, 69, doi:10.1038/s41541-020-00221-3 (2020).

Table 1: Microneutralisation Titres

Prime	Boost	Boost	Time post last vaccine	Original wild-type spike		B.1.351 (Beta)		B.1.617.1 (Kappa)		B.1.617.2 (Delta)	
				ID50	ID80	ID50	ID80	ID50	ID80	ID50	ID80
AZD1222			16 days	186 (70 to 474)	55 (43 to 297)	40	40	40	40	40	40 (40 to 41)
AZD2816			16 days	107 (40 to 297)	40 (40 to 118)	81 (51 to 231)	55 (40 to 163)	40 (40 to 42)	40	40	40
AZD1222 & AZD2816			16 days	157 (75 to 248)	65 (40 to 93)	51 (40 to 72)	41 (40 to 51)	40 (40 to 63)	40	40	40
AZD1222	AZD2816		20 days	1285 (541 to 2560)	700 (307 to 1661)	661 (212 to 1719)	235 (167 to 1057)	276 (126 to 964)	177 (85 to 565)	226 (54 to 751)	145 (43 to 467)
AZD1222	AZD1222		48 days	2546 (1789 to 2560)	1158 (627 to 1658)	350 (69 to 630)	111 (51 to 380)	132 (54 to 490)	95 (44 to 185)	40 (40 to 582)	40 (40 to 245)
AZD1222	AZD1222	AZD2816	20 days	2560 (1452 to 2560)	2159 (584 to 2408)	1148 (383 to 2475)	742 (273 to 1628)	724 (397 to 1874)	481 (267 to 947)	637 (87 to 1656)	316 (69 to 1172)

Functional ability of antibodies to neutralise pseudotyped virus expressing original spike, B.1.351 or B.1.617 spike protein was measured in the serum of vaccinated mice. Pseudotyped virus neutralization titres are expressed as the reciprocal of the serum dilution that inhibited luciferase expression by 50% (ID50) or 80% (ID80). Table shows the median (min to max) per group.

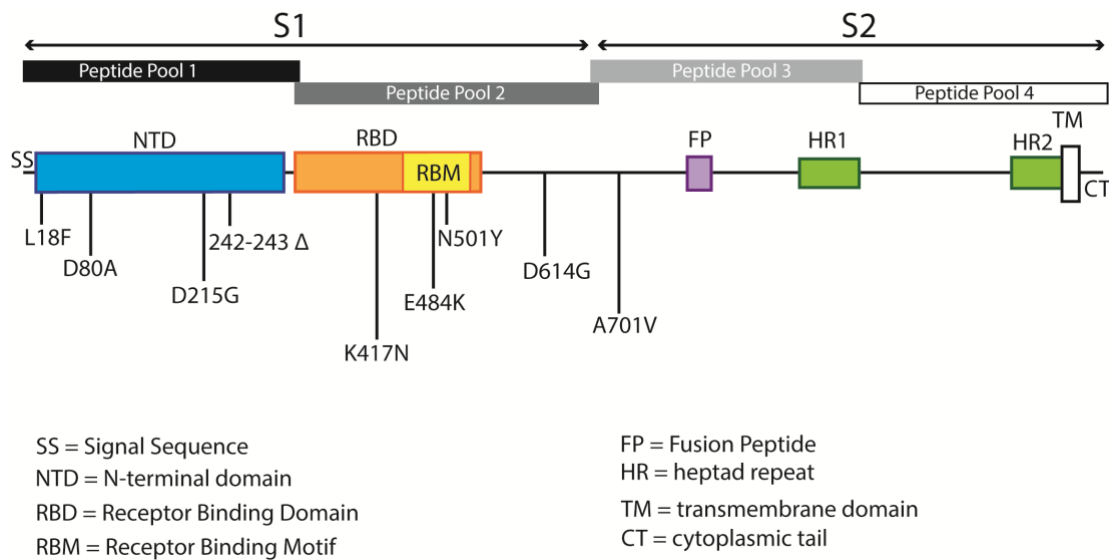
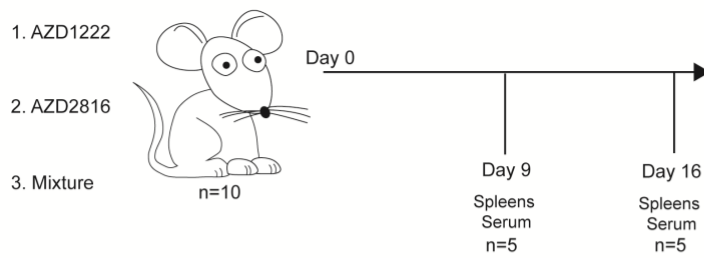


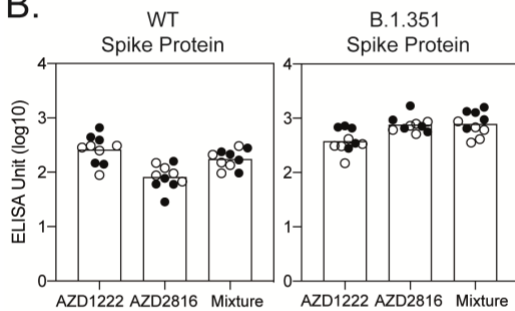
Figure 1: Schematic of SARS-CoV-2 spike protein and peptide pools used in studies

Schematic is a graphical representation of spike protein indicating location of the signal sequence (SS), N-terminal domain (NTD), receptor binding domain (RBD, receptor binding motif (RBM), fusion peptide (FP), heptad repeat (HR) regions, transmembrane domain (TM) and cytoplasmic tail (CT). Peptide pools used to stimulate splenocytes were sub-divided into 4 pools to cover the S1 and S2 regions of spike. Amino acid changes between original and B.1.351 variant virus and encoded in the AZD2816 vaccine construct are indicated.

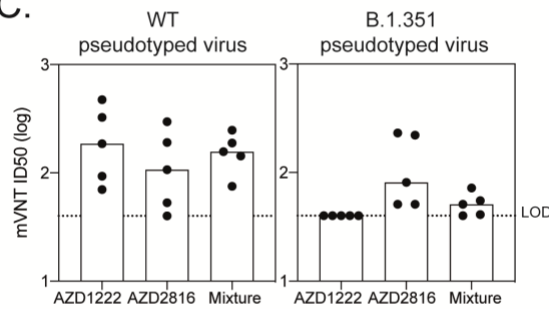
A.



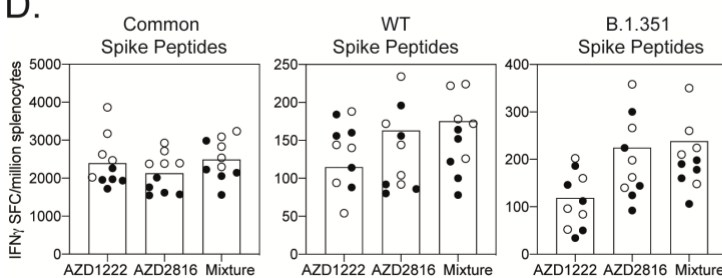
B.



C.



D.



E.

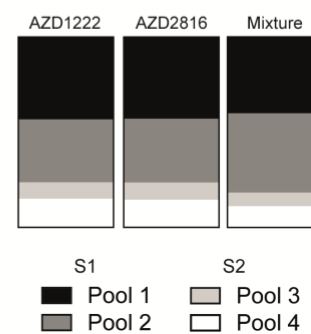


Figure 2: Immune response following a single dose of ChAdOx1 vaccines

A.) BALB/c mice (n=10) were vaccinated with 10^8 iu of AZD1222 (ChAdOx1 nCoV-19), AZD2816 (ChAdOx1 nCoV-19 B.1.351) or 10^8 iu of each vaccine mixed together. Mice were sacrificed 9 or 16 days later to measure antibody and T cell responses.

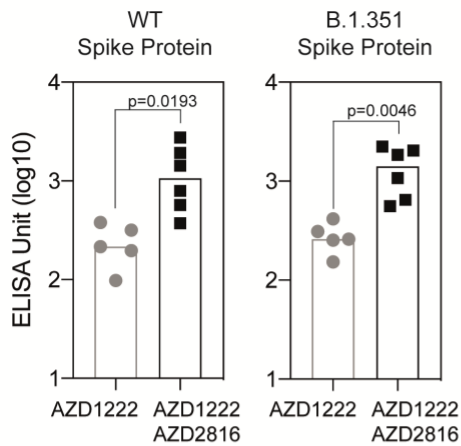
B.) Total IgG levels measured in the serum of mice against original spike protein (WT) or B.1.351 spike protein.

C.) Microneutralisation titres mVNT (ND50) measured in the serum of mice day 16 post vaccination, against pseudotyped virus expressing original spike (WT) or B.1.351 protein. Limit of detection (LOD) in the assay is defined as a titre of 40.

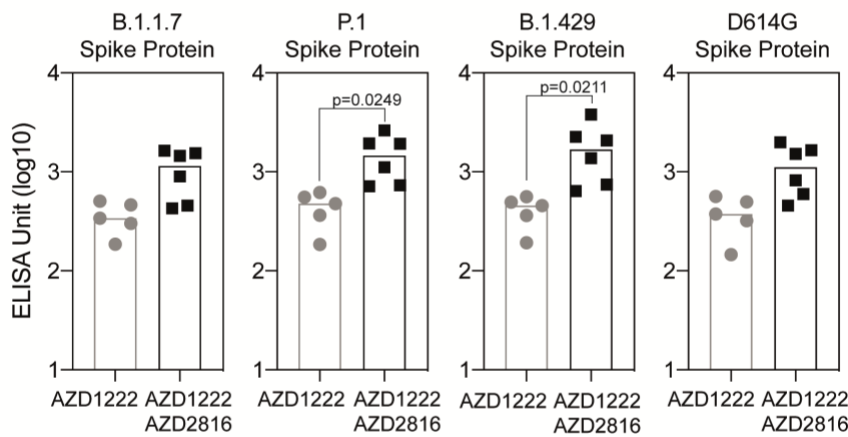
D.) IFN γ secreting cells measured by ELISpot on day 9 or day 16, with splenocytes stimulated with pools of common peptides, original (WT) spike peptides or corresponding B.1.351 peptides covering the regions of difference between SARS-CoV-2 isolates.

E.) Proportion of IFN γ secreting cells measured against spike common peptides, sub-divided into S1 (pool 1 and pool 2) or S2 (pool 3 or pool 4) regions of spike protein.

A.



B.



C.

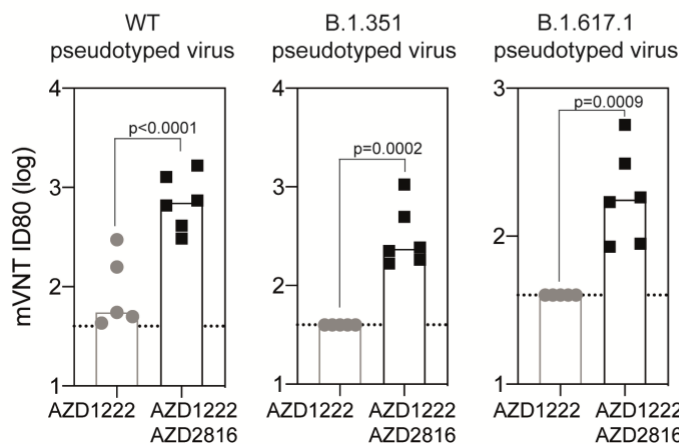


Figure 3: Antibody titres and breadth are increased following a booster dose with AZD2816 vaccine

A.) Graphs show total IgG response against original spike protein (WT) or B.1.351 measured in the serum of mice collected 16 days after vaccination with AZD1222 (n=5) (animals from Figure 2) or a prime-boost regimen of AZD1222 followed 4 weeks later by AZD2816 (n=6).

B.) Graphs show total IgG responses measured against B.1.17, P.1, B.1.429 or D614G spike proteins in serum collected 16 days and 3 weeks after the final vaccination.

All ELISAs were performed simultaneously, data log transformed and analysed with a 2-way anova (repeated measure) with a post-hoc positive test, statistically significant differences between groups ($p < 0.05$) are indicated.

C.) Microneutralisation titre of serum (ND80) collected day 16 post-vaccination (animals Figure 2) and 21 days after prime-boost vaccination against pseudotyped virus expressing original (WT), B.1.351 or B.1.617.1 spike protein. Limit of detection in the assay is defined as a titre of 40 (dotted line). Data was log-transformed and analysed with a 2-way anova (repeated measure) and post-hoc positive test, statistically significant differences ($p < 0.05$) between groups are indicated.

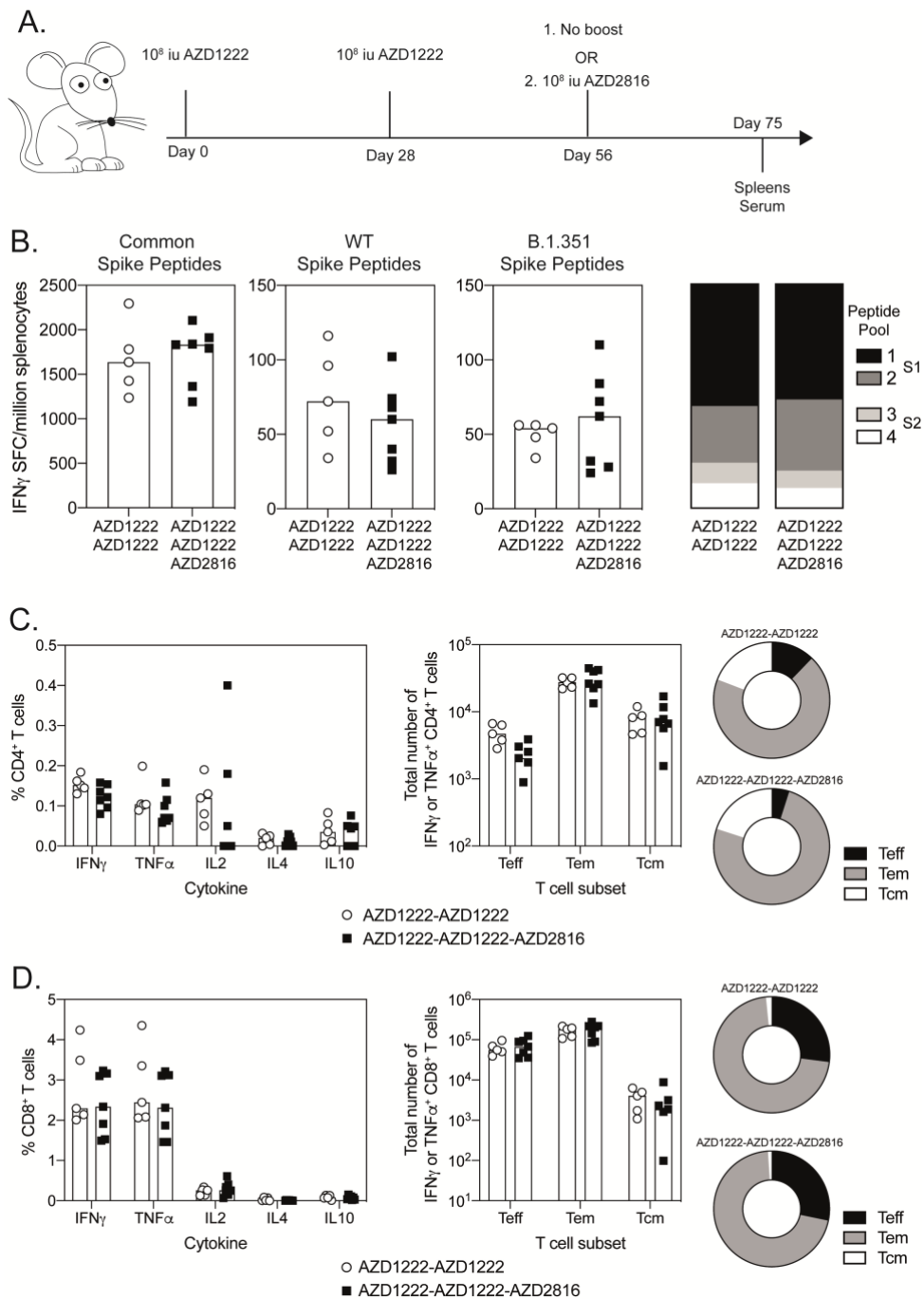


Figure 4: T cells responses are boosted by vaccination with variant vaccine AZD2816

A.) BALB/c mice received two doses of 10^8 iu of AZD1222 (ChAdOx1 nCoV-19) 4 weeks apart and were boosted with 10^8 iu of AZD2816 (ChAdOx1 nCoV-19 B.1.351) ($n=7$) or did not receive a final boost ($n=5$). Mice were sacrificed a further 3 weeks later and splenocytes stimulated with

overlapping SARS-CoV-2 peptides to measure cytokine production by ELISpot or intracellular cytokine staining.

B.) Graphs show IFN γ secreting cells measured by ELISpot with splenocytes stimulated with pools of common peptides, original (WT) spike peptides or corresponding B.1.351 peptides covering the regions of difference between SARS-CoV-2 isolates. Bars graphs represent show the proportion of IFN γ secreting cells measured against spike common peptides, sub-divided into S1 (pool 1 and pool 2) or S2 (pool 3 or pool 4) regions of spike protein.

C.) Graphs show the frequency of cytokine positive CD4⁺ T cells (left), total number of T effector (Tem), T effector memory (Tem) or T central memory cells (Tcm) IFN γ ⁺ or TNF α ⁺ CD4⁺ cells (middle), or the proportion of total IFN γ ⁺ or TNF α ⁺ CD4⁺ T cells of each T cell subset (right).

D.) Graphs show the frequency of cytokine positive CD8⁺ T cells (left), total number of T effector (Tem), T effector memory (Tem) or T central memory cells (Tcm) IFN γ ⁺ or TNF α ⁺ CD8⁺ cells (middle), or the proportion of total IFN γ ⁺ or TNF α ⁺ CD8⁺ T cells of each T cell subset (right).

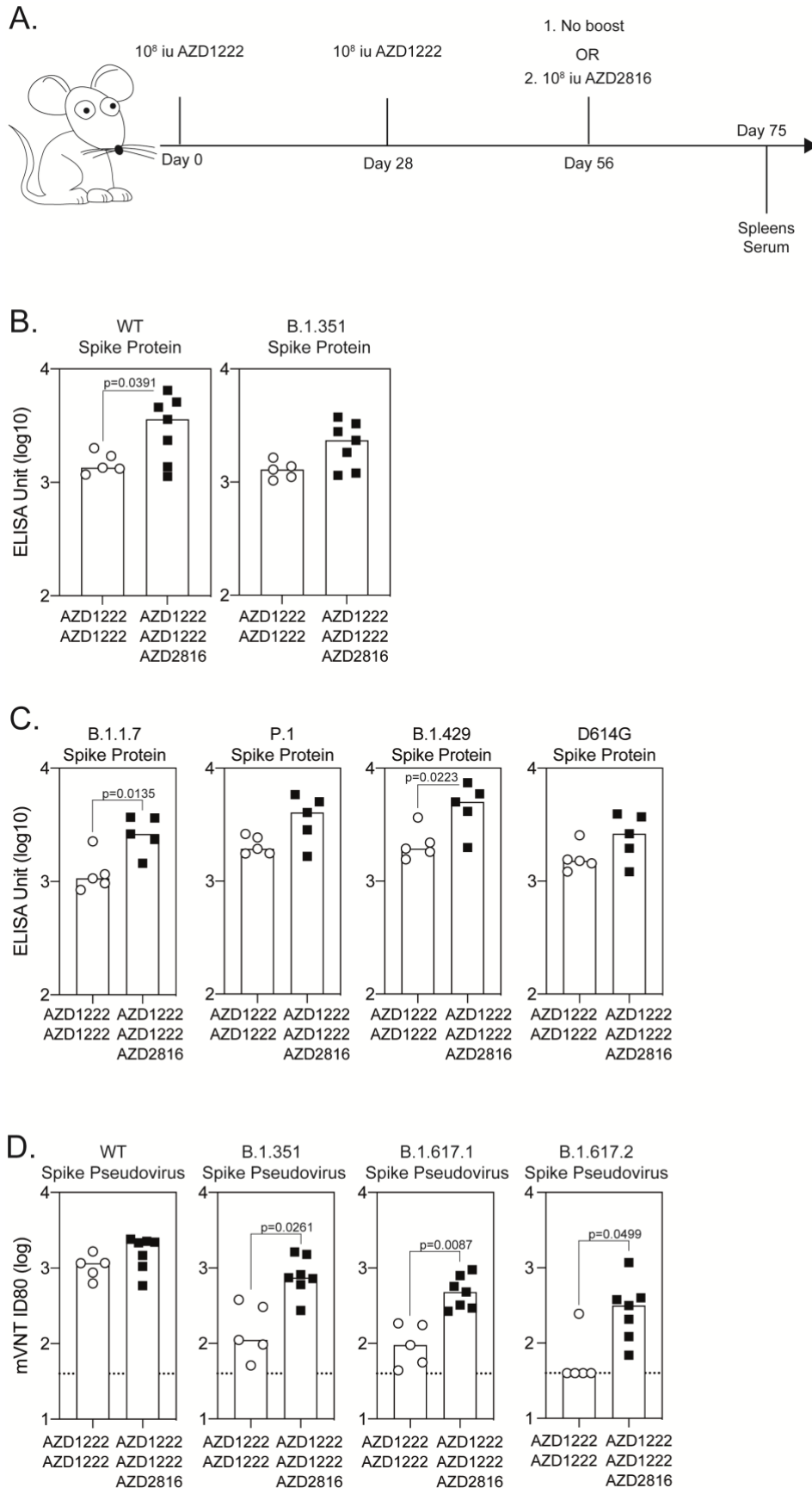


Figure 5: Immune response are boosted by immunisation with AZD2816

A.) In the same experiment as described in Figure 4, BALB/c mice received two doses of 10⁸ iu of AZD1222 (ChAdOx1 nCoV-19) 4 weeks apart and were boosted with 10⁸ iu of AZD2816 (ChAdOx1 nCoV-19 B.1.351) (n=7) or did not receive a final boost (n=5). Mice were sacrificed a further 3 weeks later and antibody responses measured in the serum of mice.

B.) Graphs show the total IgG level measured by ELISA against original spike protein (WT) or B.1.351 spike protein. Data was log transformed and analysed with a two-way analysis of variance (repeated measure) and post-hoc positive test, significance between groups ($p < 0.05$) is indicated.

C.) Graphs show total IgG antibody responses measured by ELISA against B.1.1.7, P.1, B.1.429 or D614G spike protein. Data was log transformed and analysed with a two-way analysis of variance (repeated measure) and post-hoc positive test, significance between groups ($p < 0.05$) is indicated.

D.) Graphs show microneutralisation titres mVNT (ND80) measured against pseudotyped virus expressing original (WT), B.1.351, B.1.617.1 or B.1.617.2 spike protein. Limit of detection in the assay is defined as a titre of 40 (dotted line). Data was log transformed and analysed with a two-way analysis of variance (repeated measure) and post-hoc positive test, significance between groups ($p < 0.05$) is indicated.

Table S1: Sequence changes to SARS-CoV-2 spike protein

	Original Sequence	B.1.351 Beta	B.1.1.7 Alpha	P.1 Gamma	B.1.429 Epsilon	D614G	B.1.617.1 Kappa	B.1.617.2 Delta
NTD	S13				I			
	L18	F		F				
	L19							R
	T20			N				
	P26			S				
	H69-V70		Δ					
	D80	A						
	T95						I	
	D138			Y				
	G142						D	D
	Y144		Δ					
	W152					C		
	E154						K	
	E156-F157							Δ
	R158							G
	R190				S			
	D215	G						
L242-A243	Δ							
RBD	K417	N		T				
	L452				R		R	R
	T478							K
	E484	K		K			Q	
	N501	Y	Y	Y				
Other	A570		D					
	D614	G	G	G	G	G	G	G
	H655			Y				
	P681		H				R	R
	A701	V						
	T716		I					
	D950							N
	S982		A					
	T1027			I				
	Q1071						H	
	D1118		H					
V1176				F				

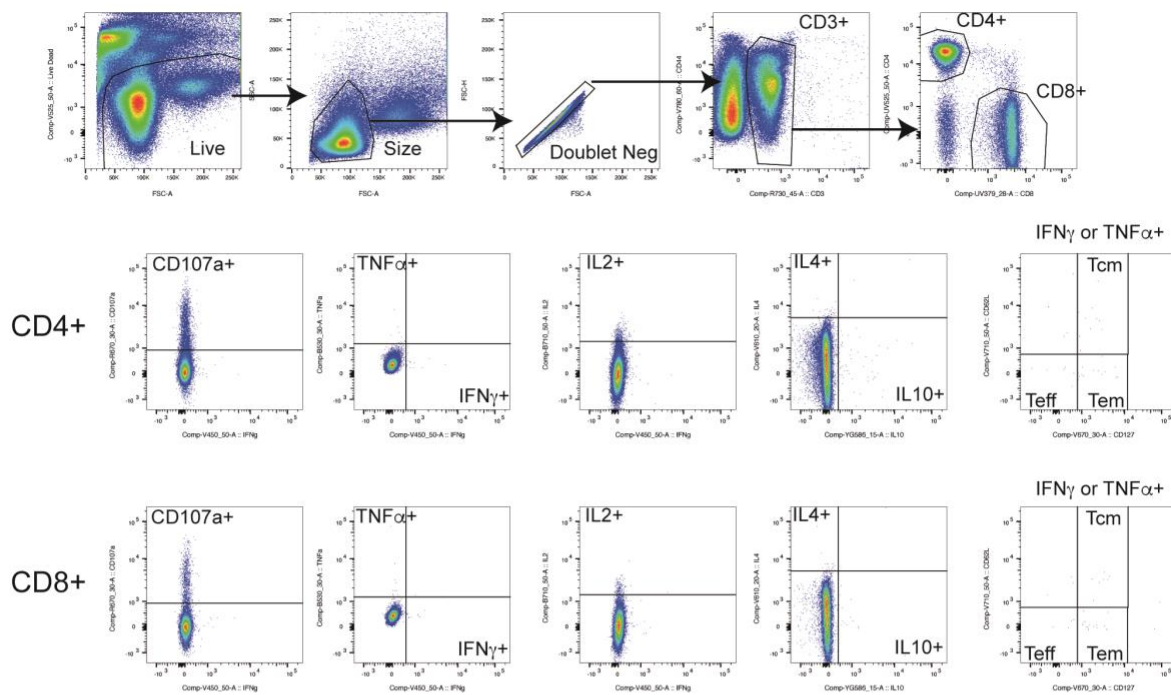
Table S2: Overlapping SARS-CoV-2 spike peptide sequences

S1 region							
Pool 1			Pool 2				
#	Common Peptides	Original NC_045512 Sequence	B.1.351 Sequence	#	Common Peptides	Original NC_045512 Sequence	B.1.351 Sequence
1	MFVFLVLLPLVSSQC			78	EKGIVQTSNFRVQPT		
2		LVLLPLVSSQCVNLT	LVLLPLVSSQCVNFT	79	YQTSNFRVQPTESIV		
3		PLVSSQCVNLTTRTQ	PLVSSQCVNFTTRTQ	80	NFRVQPTESIVRFPN		
4		SQCVNLTTRTQLPPA	SQCVNFTTRTQLPPA	81	QPTESIVRFPNITNL		
5		NLTTRTQLPPAYTNS	NFTTRTQLPPAYTNS	82	SIVRFPNITNLCDFG		
6	RTQLPPAYTNSFTRG			83	FPNITNLCDFGVEFN		
7	PPAYTNSFTRGVYYP			84	TNLCDFGVEFNATRF		
8	TNSFTRGVYYPDKVF			85	PFGEVFNATRFASVY		
9	TRGVYYPDKVFRSSV			86	VFNATRFASVYAWN		
10	YYPDKVFRSSVLHST			87	TRFASVYAWNKRKIS		
11	KVFRSSVLHSTQDLF			88	SVYAWNKRKISNCVA		
12	SSVLHSTQDLFLPFF			89	WNRKRISNCVADYSV		
13	HSTQDLFLPFFSNVT			90	RISNCVADYSVLYNS		
14	DLFLPFFSNVTWFHA			91	CVADYSVLYNSASF		
15	PFFSNVTWFHAIHVS			92	YSVLYNSASFSTFKC		
16	NVTWFHAIHVS			93	YNSASFSTFKCYGVS		
17	FHAIHVS			94	SFSTFKCYGVSPTKL		
18		HVSGTNGTKRFDNPV	HVSGTNGTKRFDNPV	95	FKCYGVSPTKLNLDL		
19		TNGTKRFDNPVLPFN	TNGTKRFDNPVLPFN	96	GVSPKLNLDLCTNV		
20		KRFDNPVLPFNDGVY	KRFDNPVLPFNDGVY	97	TKLNLDLCTNVYADS		
21	NPVLPFNDGVYFAST			98	DLCTNVYADSVIR		
22	PFNDGVYFAST			99	TNVYADSVIRGDEV		
23	GVYFAST			100	ADSVIRGDEV		
24	ASTEKSNIRGWIFG			101	VIRGDEV		
25	KSNIRGWIFG			102		DEVRIAPGQTGKIA	DEVRIAPGQTGNIA
26	IRGWIFG			103		QIAPGQTGKIADYNY	QIAPGQTGNIADYNY
27	IFGTTLD			104		GQTGKIADYNYKLPD	GQTGNIADYNYKLPD
28	TLD			105		KIADYNYKLPDDFTG	NIADYNYKLPDDFTG
29	KTQSL			106	YNYKLPDDFTG		
30	LLIVN			107	LPDDFTG		
31	NNATN			108	FTGCVI		
32	NVVIK			109	VIWNSN		
33	KVCE			110	NSNLD		
34	FQFC			111	LDSKV		
35	NDP			112	VGGNY		
36	LG			113	YNYL		
37	YH			114	YRFR		
38	NK			115	RKSN		
39	ME			116	LKPF		
40	FR			117	ERD		
41	SS			118	STEI		
42	NCT			119		YQAG	YQAG
43	EY			120		STPC	STPC
44	QP			121		NGVE	NGVE
45	MD			122	GF		
46	GK			123		YFPL	YFPL
47	NF			124		QSYG	QSYG
48	L			125		FQPT	FQPT
49	V			126		NGVG	NGVG
50	ID			127	YQ		
51		FKI	FKI	128	R		
52		SK	SK	129	L		
53		P	P	130	L		
54		V	V	131	P		
55		P	P	132	C		
56		S	S	133	K		
57		P	P	134	L		
58		L	L	135	K		
59		I	I	136	F		
60		R	R	137	G		
61		L	L	138	T		
62		H	H	139	T		
63		L	L	140	K		
64		D	D	141	P		
65		G	G	142	F		
66		G	G	143	I		
67		Y	Y	144	T		
68		Y	Y	145	R		
69		R	R	146	L		
70		L	L	147	L		
71		E	E	148	P		
72		I	I	149	G		
73		V	V	150	V		
74		L	L	151		GTNT	GTNT
75		S	S	152		SNQ	SNQ
76		C	C	153		AVL	AVL
77		S	S	154		QDV	QDV
				155	C		
tpa	MDAMK			156	P		

tpa	RGLCCVLLLCGAVFV			157	HADQLTPTWRVYSTG		
tpa	VLLLCGAVFVSASQE			158	LTPTWRVYSTGSNVF		
tpa	GAVFVSASQEIHFARF			159	WRVYSTGSNVFQTRA		
tpa	SASQEIHFARFRRIHS			160	STGSNVFQTRAGCLI		
				161	NVFQTRAGCLIGAHEH		
				162	TRAGCLIGAHEHVNNS		
				163	CLIGAHEHVNNSYECD		
				164	AEHVNNSYECDPIG		
				165	NNSYECDPIGAGIC		
				166	ECDPIGAGICASYQ		
				167	PIGAGICASYQTQTN		
S2 region							
Pool 3				Pool 4			
#	Common Peptides	Original NC_045512 Sequence	B.1.351 Sequence	#	Common Peptides	Original NC_045512 Sequence	B.1.351 Sequence
168	GICASYQTQNSPRR			242	QLSSNFGAISSVLND		
169	SVQTQNSPRRARSV			243	NFGAISSVLNDILSR		
170	QNSPRRARSVASQS			244	ISSVLNDILSRDKV		
171	PRRARSVASQSIIAY			245	LNDILSRDKVEAEV		
172	RSVASQSIIAYTMSL			246	LSRLDKVEAEVQIDR		
173		SQSIIAYTMSLGAEN	SQSIIAYTMSLGVEN	247	DKVEAEVQIDRLITG		
174		IAYTMSLGAENSVAY	IAYTMSLGVENSVAY	248	AEVQIDRLITGRLLQS		
175		MSLGAENSVAYSNNNS	MSLGVENSVAYSNNNS	249	IDRLITGRLLQSLQTY		
176		AENSVAYSNNNSIAIP	VENSVAYSNNNSIAIP	250	ITGRLLQSLQTYVTQQ		
177	VAYSNNNSIAIPTNFT			251	LQSLQTYVTQQIIRA		
178	NNSIAIPTNFTISVT			252	QTYVTQQIIRAAEIR		
179	AIPTNFTISVTTEIL			253	TQQIIRAAEIRASAN		
180	NFTISVTTEILPVSMT			254	IRAAEIRASANLAAT		
181	SVTTEILPVSMTKTS			255	EIRASANLAATKMSE		
182	EILPVSMTKTSVDCT			256	SANLAATKMSECVLG		
183	VSMTKTSVDCTMYIC			257	AATKMSECVLGGQSKR		
184	KTSVDCTMYICGDST			258	MSECVLGGQSKRVDFC		
185	DCTMYICGDSTECNS			259	VLGGQSKRVDFCGKGY		
186	YICGDSTECNSLLQ			260	SKRVDFCGKGYHLM		
187	DSTECNSLLQYGSF			261	DFCGKGYHLMFSPQS		
188	CSNLLQYGSFCTQL			262	KGYHLMFSPQSAPHG		
189	LLQYGSFCTQLNRAL			263	LMSFSPQSAPHGVVFL		
190	GSFCTQLNRALTGIA			264	PQSAPHGVVFLHVITY		
191	TQLNRALTGIAVEQD			265	PHGVVFLHVITYVPAQ		
192	RALTGIAVEQDKNTQ			266	VFLHVITYVPAQEKNF		
193	GIAVEQDKNTQEVFA			267	VTVVPAQEKNFITAP		
194	EQDKNTQEVFAQVKQ			268	PAQEKNFITAPAICH		
195	NTQEVFAQVKQYKT			269	KNFITAPAICHGKA		
196	VFAQVKQYKTPPIK			270	TAPAICHGKAHFPR		
197	VKQYKTPPIKDFGG			271	ICHGKAHFPREGVF		
198	YKTPPIKDFGGFNFS			272	GKAHFPREGVFVSN		
199	PIKDFGGFNFSQILP			273	FPREGVFVSNGTHWF		
200	FGGFNFSQILPDP			274	GVFVSNGTHWVFTQR		
201	NFSQILPDPSPSKR			275	SNGTHWVFTQRNFYE		
202	ILPDPSPSKRSFIE			276	HWVFTQRNFYEPQII		
203	PSKRSFIEDLLF			277	TQRNFYEPQIITD		
204	SKRSFIEDLLFNKVT			278	FYEPQIITDNTFVS		
205	FIEDLLFNKVTLADA			279	QIITDNTFVSGNCD		
206	LLFNKVTLADAGFIK			280	TDNTFVSGNCDVWIG		
207	KVTLADAGFIKQYGD			281	FVSGNCDVWIGVNN		
208	ADAGFIKQYGDCLGD			282	NCDVWIGVNNVYD		
209	FIKQYGDCLGDIAAR			283	VIGVNNVYDPLQP		
210	YGDCLGDIAARDLIC			284	VNNTVYDPLQPELDS		
211	LGDIARDLICAQKF			285	VYDPLQPELDSFKEE		
212	AARDLICAQKFNGLT			286	LQPELDSFKEELDKY		
213	LICAQKFNGLTVLPP			287	LDSFKEELDKYFKNH		
214	QKFNGLTVLPLLT			288	KEELDKYFKNHTSPD		
215	GLTVLPLLTDEMI			289	DKYFKNHTSPDVLG		
216	LPPLLTDEMIQYTS			290	KNHTSPDVLGDISG		
217	LTDEMIQYTSALLA			291	SPDVLGDISGINAS		
218	MIAQYTSALLAGTIT			292	DLGDISGINASVVNI		
219	YTSALLAGTITSGWT			293	ISGINASVVNIQKEI		
220	LLAGTITSGWTFGAG			294	NASVVNIQKEIDRLN		
221	TITSGWTFGAGAALQ			295	VNIQKEIDRLNEVAK		
222	GWTFGAGAALQIPFA			296	KEIDRLNEVAKNLNE		
223	GAGAALQIPFAMQMA			297	RLNEVAKNLNESLID		
224	ALQIPFAMQMAYRFN			298	VAKNLNESLIDLQEL		
225	PFAMQMAYRFNGIGV			299	LNESLIDLQELGKYE		
226	QMAYRFNGIGVTVQNV			300	LIDLQELGKYEYQIK		
227	RFNGIGVTVQNVLYEN			301	QELGKYEYQIKWPWY		
228	IGVTVQNVLYENQKLI			302	KYEYQIKWPWYIWL		
229	QNVLYENQKLIANQF			303	YIKWPWYIWLGFIA		
230	YENQKLIANQFN			304	PWYIWLGFIAGLIAI		
231	KLIANQFN			305	WLGFIAGLIAIVMVT		
232	NQFN			306	IAGLIAIVMVTIMLC		
233	SAIGKIQDLSSTAS			307	IAIVMVTIMLCMCTS		
234	KIQDLSSTASALGK			308	MVTIMLCMTCSCCSC		
235	SLSSTASALGKLQDV			309	MLCMTSCCCLKGCG		
236	TASALGKLQDVVNQN			310	MTSCCCLKGCCSCG		
237	LGKLQDVVNQNAQAL			311	CSCLKGCCSCGSCCK		

238	QDVVNQNAQALNTLV			312	KGCCSCGSCCKFDED		
239	NQNAQALNTLVKQLS			313	SCGSCCKFDEDDSEP		
240	QALNTLVKQLSSNFG			314	CCKFDEDDSEPVKLG		
241	TLVKQLSSNFGAISS			315	DEDDSEPVKGVKLVH		
				316	DDSEPVKGVKLVHT		

A. Media



B. S1 peptides

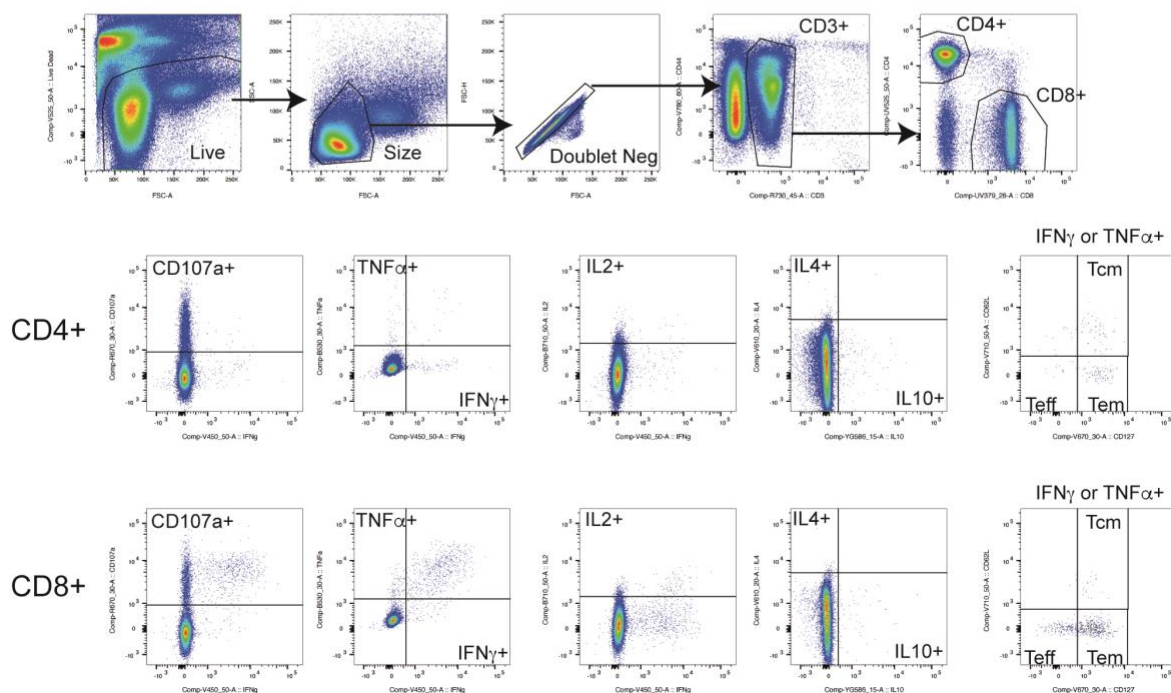


Figure S1: Flow cytometry gating strategy

Antigen specific T cells were identified by gating on LIVE/DEAD negative, size (FSC-A vs SSC), doublet negative (FSC-H vs FSC-A), CD3⁺, CD4⁺ or CD8⁺ cells and each individual cytokine. T effector (Teff) cells were defined as CD62L^{low} CD127^{low}, T effector memory (Tem) cells defined as CD62L^{low} CD127^{hi} and T central memory (Tcm) cells defined as CD62L^{hi} CD127^{hi}. T cell subsets were gated within the population of "IFN γ ⁺ or TNF α ⁺" responses and are presented after subtraction of the background response detected in the corresponding media stimulated control sample (A.) from the S1 (B.) or S2 peptide stimulated sample for each mouse, and summing together the response detected to each pool of peptides.