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

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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 10^8iu AZD1222 (ChAdOx1 nCoV-19), AZD2816 (ChAdOx1 nCoV-19 B.1.351) or with 10^8iu 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 IFNγ 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 IFNγ 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 IFNγ, 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 IFNγ and TNFα 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 virus [5]. 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 surface [9]. The L452R change is present in B.1.429, B.1.617.1 and B.1.617.2 shown to reduce sensitivity of neutralising antibodies [7]. 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 antibodies [12]. 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 spike [15-17], 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 described [20]. In brief, the B.1.351 glycoprotein (S) gene [4] 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 vector [21] 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^{o}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 10^8 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 template[22]. Lentiviral-based SARS-CoV-2 pseudotyped viruses were generated in HEK293T cells incubated at 37 °C, 5% CO_2 as previously described [23]. Briefly, cells were seeded at a density of 7.5 x 10^5 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 × 10^4 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 10^5 to 10^6 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 10^4 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 IFNγ 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 IFNγ (AN18). After 18-20 hours of stimulation at 37°C, IFNγ spot forming cells (SFC) were detected by staining membranes with anti-mouse IFNγ 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 "$IFN\gamma^+$ or $TNF\alpha^+$" 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 $\log_{10}$ 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.

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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.

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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.

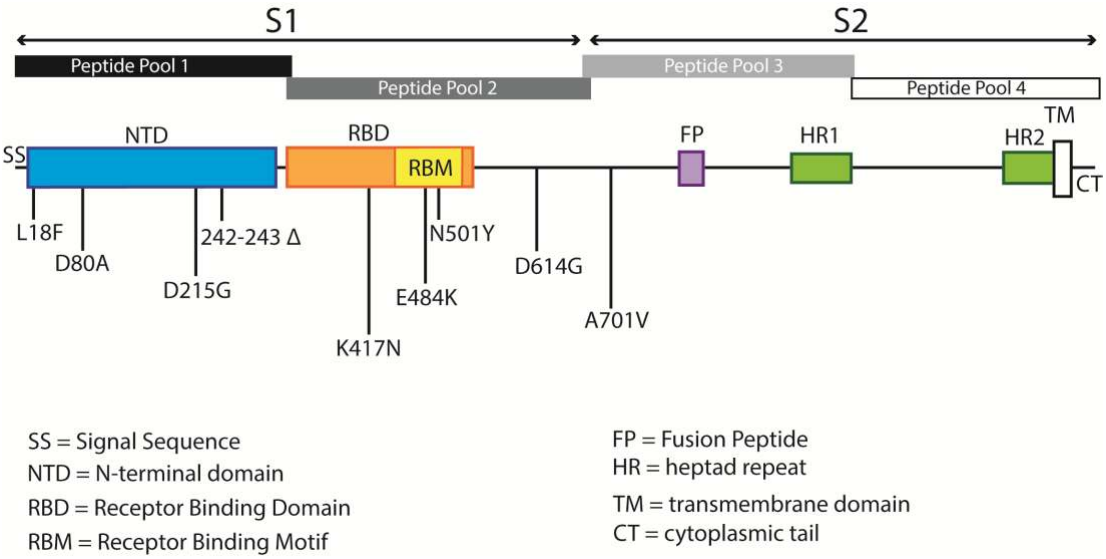

Figure 1: Schematic of SARS-CoV-2 spike protein and peptide pools used in studies

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.

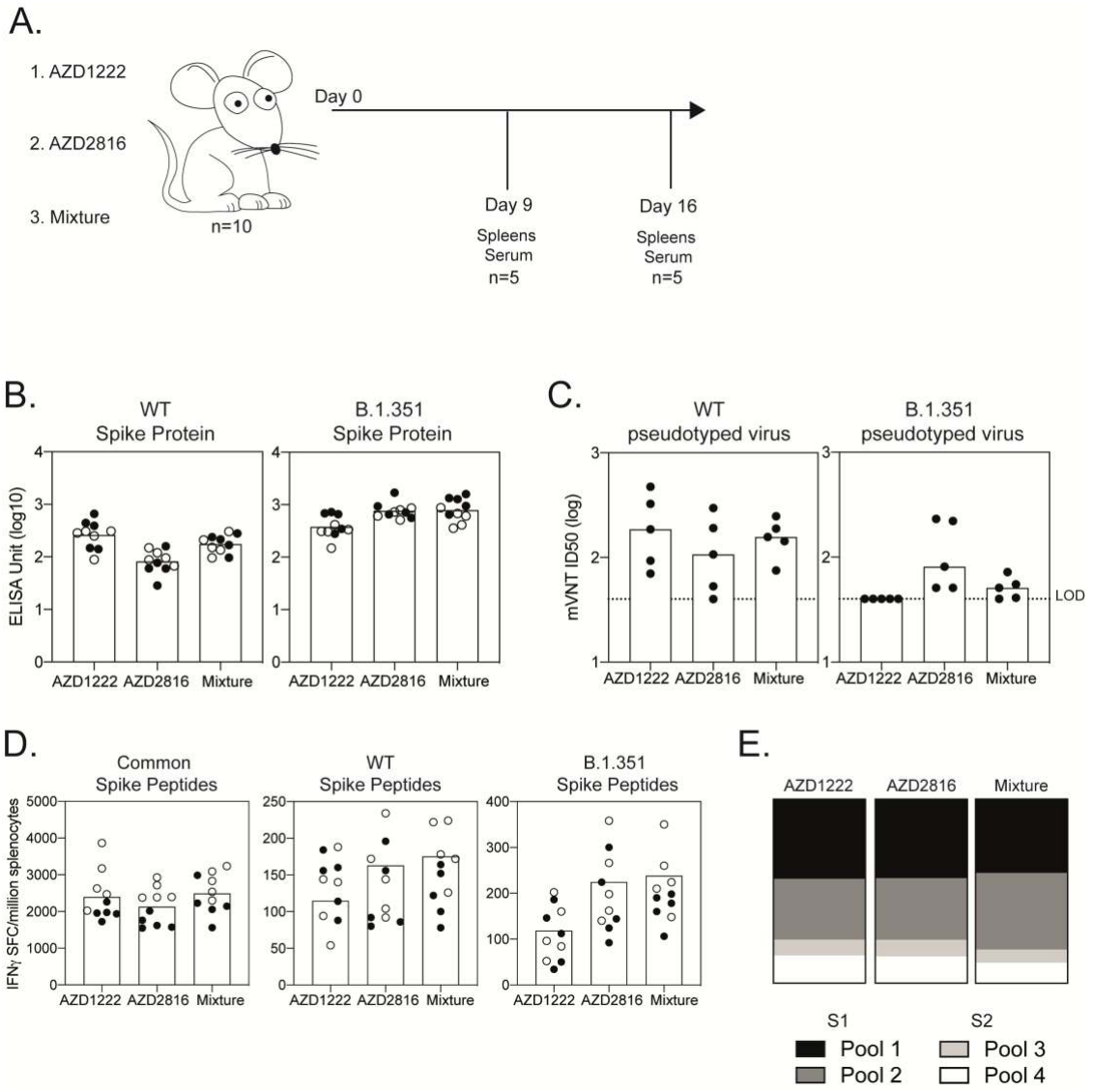

Figure 2: Immune response following a single dose of ChAdOx1 vaccines

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.) IFNγ 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 IFNγ 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.

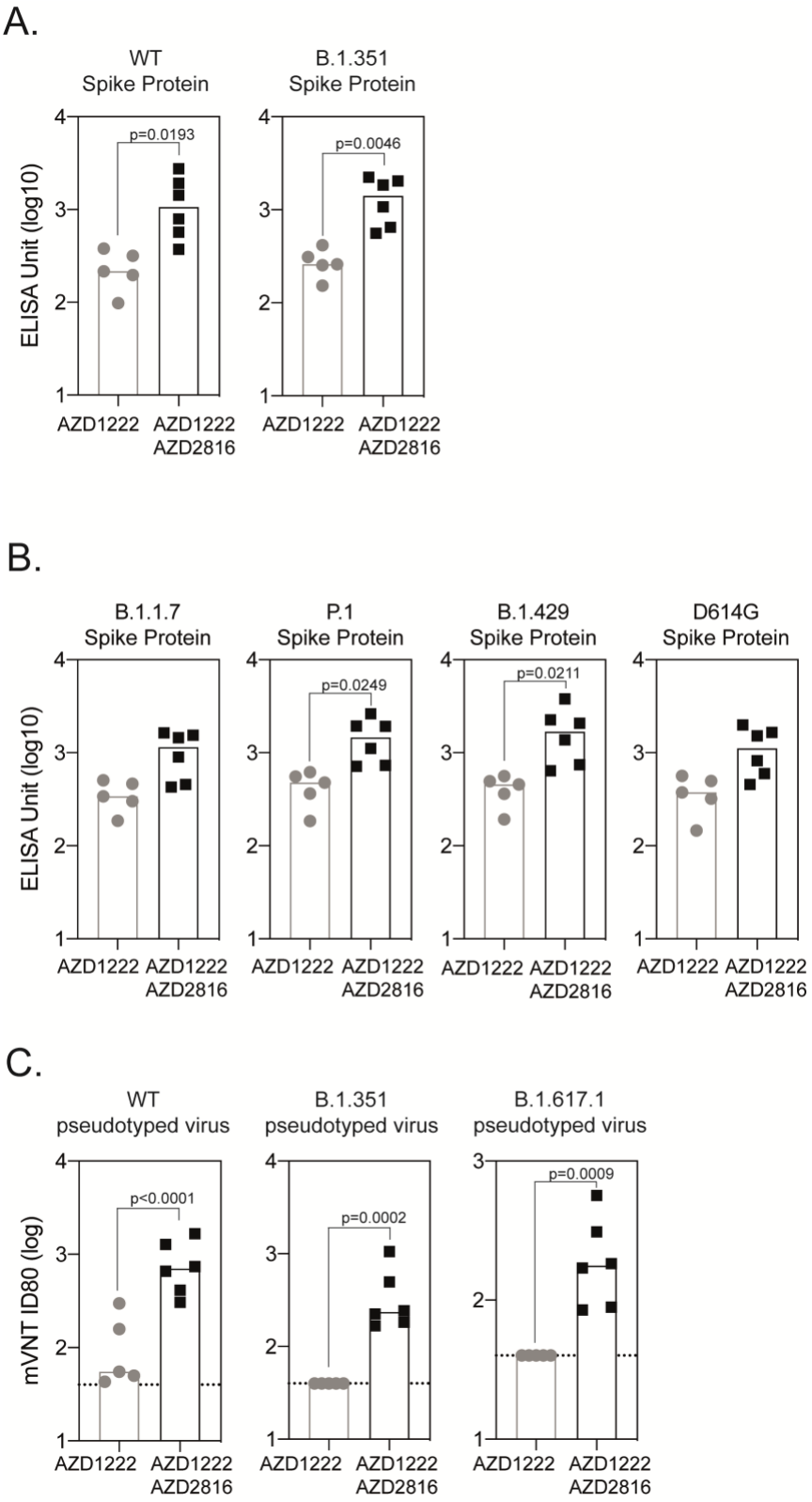

Figure 3: Antibody titres and breadth are increased following a booster dose with AZD2816 vaccine

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.

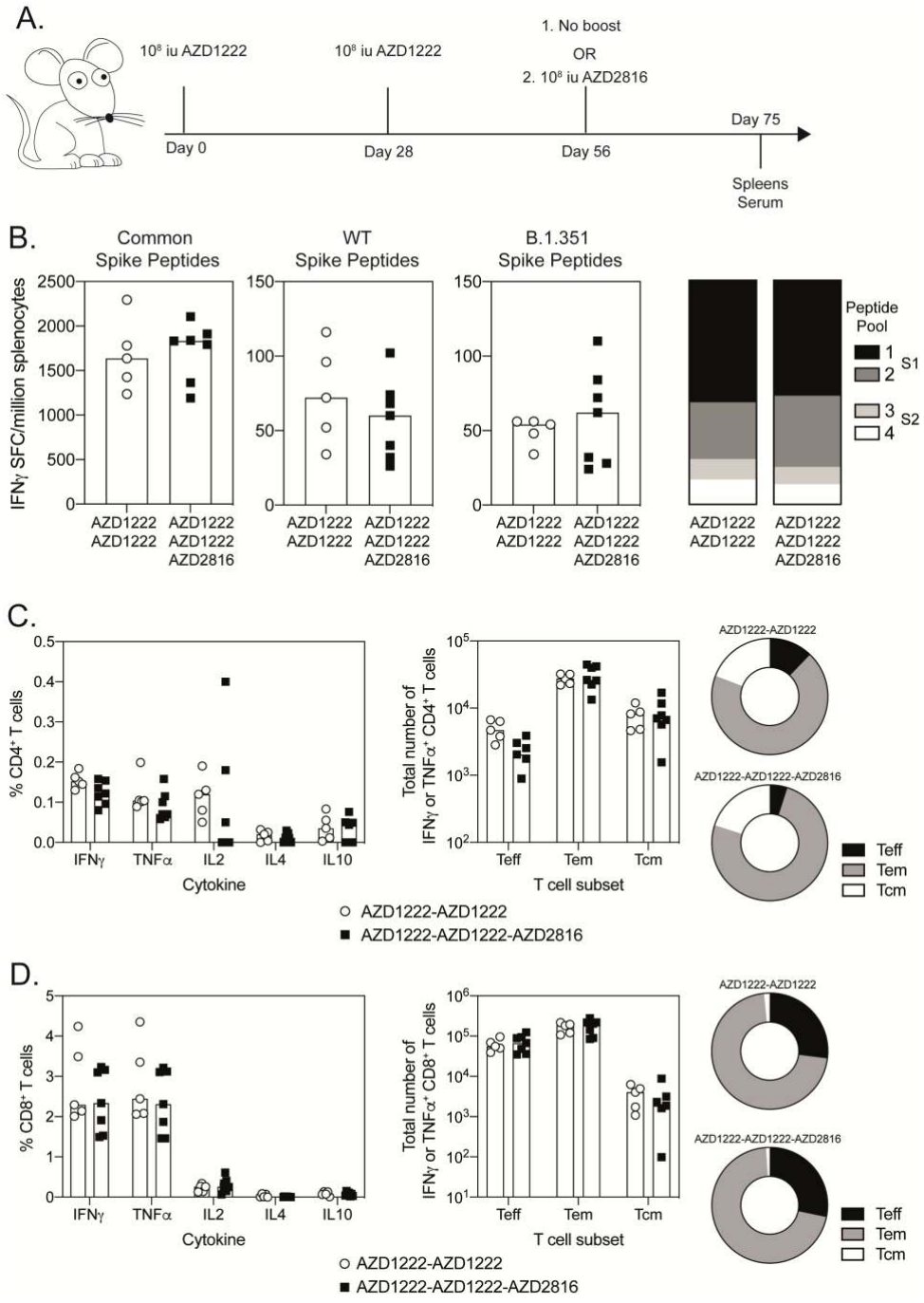

Figure 4: T cells responses are boosted by vaccination with variant vaccine AZD2816

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 IFNγ 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 IFNγ 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) $IFN\gamma^+$ or $TNF\alpha^+$ $CD4^+$ cells (middle), or the proportion of total $IFN\gamma^+$ or $TNF\alpha^+$ $CD4^+$ 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) $IFN\gamma^+$ or $TNF\alpha^+$ CD8+ cells (middle), or the proportion of total $IFN\gamma^+$ or $TNF\alpha^+$ CD8+ T cells of each T cell subset (right).

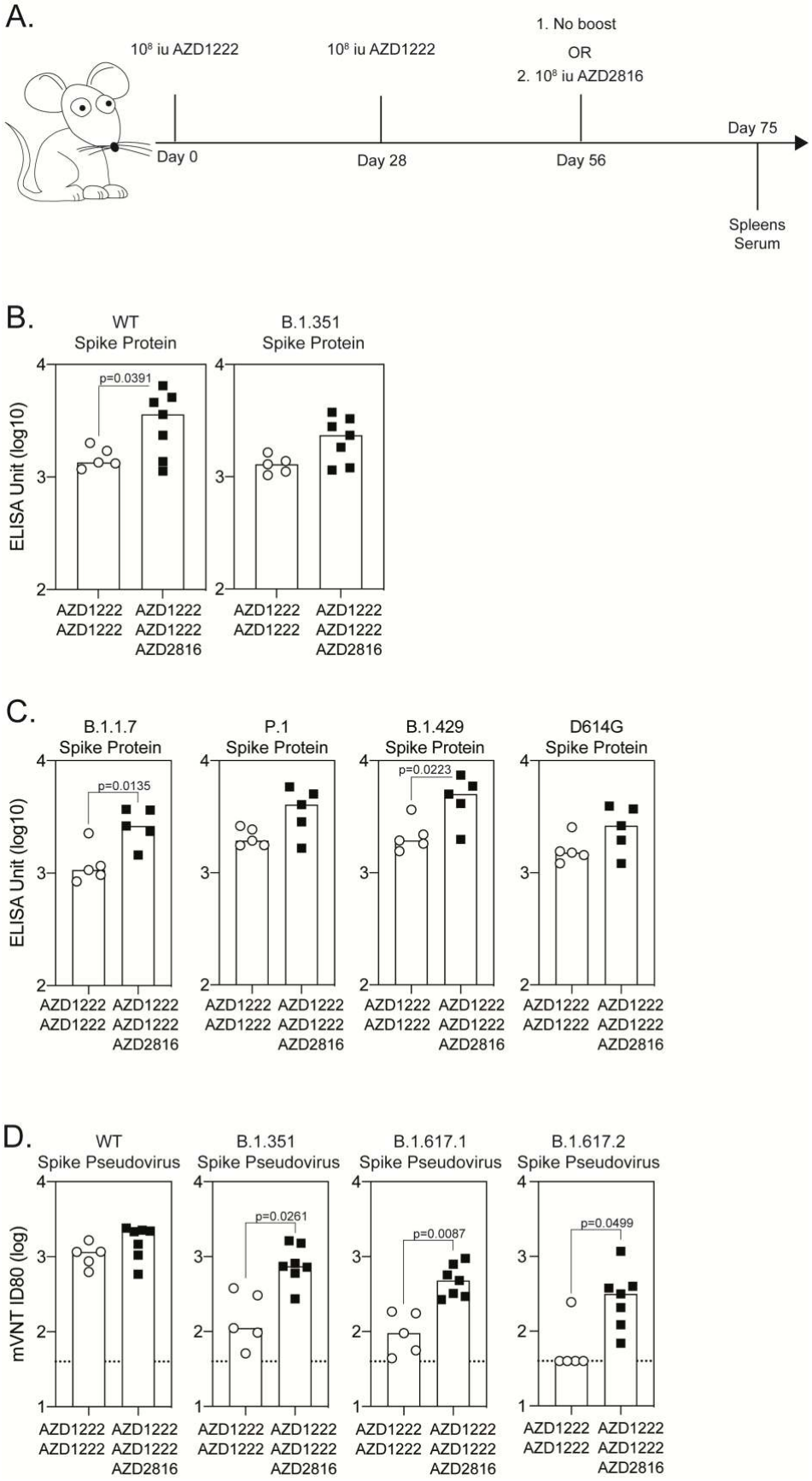

Figure 5: Immune response are boosted by immunisation with AZD2816

A.) In the same experiment as described in Figure 4, BALB/c mice received two doses of 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 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	EKGIYQTSNFRVQPT		
2		LVLLPLVSSQCVNLT	LVLLPLVSSQCVNFT	79	YQTSNFRVQPTESIV		
3		PLVSSQCVNLTTRTQ	PLVSSQCVNFTTRTQ	80	NFRVQPTESIVRFPN		
4		SQCVNLTTRTQLPPA	SQCVNFTTRTQLPPA	81	QPTESIVRFPNITNL		
5		NLTTRTQLPPAYTNS	NFTTRTQLPPAYTNS	82	SIVRFPNITNLCPFG		
6	RTQLPPAYTNSFTRG			83	FPNITNLCPFGEVFN		
7	PPAYTNSFTRGVYYP			84	TNLCPFGEVFNATRF		
8	TNSFTRGVYYPDKVF			85	PFGEVFNATRFASVY		
9	TRGVYYPDKVFRSSV			86	VFNATRFASVYAWNR		
10	YYPDKVFRSSVLHST			87	TRFASVYAWNRKRIS		
11	KVFRSSVLHSTQDLF			88	SVYAWNRKRISNCVA		
12	SSVLHSTQDLFLPFF			89	WNRKRISNCVADYSV		
13	HSTQDLFLPFFSNVT			90	RISNCVADYSVLYNS		
14	DLFLPFFSNVTWFHA			91	CVADYSVLYNSASFS		
15	PFFSNVTWFHAIHVS			92	YSVLYNSASFSTFKC		
16	NVTWFHAIHVSGTNG			93	YNSASFSTFKCYGVS		
17	FHAIHVSGTNGTKRF			94	SFSTFKCYGVSPTKL		
18		HVSGTNGTKRFDNPV	HVSGTNGTKRFANPV	95	FKCYGVSPTKLNDLC		
19		TNGTKRFDNPVLPFN	TNGTKRFANPVLPFN	96	GVSPTKLNDLCFTNV		
20		KRFDNPVLPFNDGVY	KRFANPVLPFNDGVY	97	TKLNDLCFTNVYADS		
21	NPVLPFNDGVYFAST			98	DLCFTNVYADSFVIR		
22	PFNDGVYFASTEKSN			99	TNVYADSFVIRGDEV		
23	GVYFASTEKSNIIRG			100	ADSFVIRGDEVRQIA		
24	ASTEKSNIIRGWIFG			101	VIRGDEVRQIAPGQT		
25	KSNIIRGWIFGTTLD			102		DEVRQIAPGQTGKIA	DEVRQIAPGQTGNIA
26	IRGWIFGTTLDSKTQ			103		QIAPGQTGKIADYNY	QIAPGQTGNIADYNY
27	IFGTTLDSKTQSLLI			104		GQTGKIADYNYKLPD	GQTGNIADYNYKLPD
28	TLDSKTQSLLIVNNA			105		KIADYNYKLPDDFTG	NIADYNYKLPDDFTG
29	KTQSLLIVNNATNVV			106	YNYKLPDDFTGCVIA		
30	LLIVNNATNVVIKVC			107	LPDDFTGCVIAWNSN		
31	NNATNVVIKVCEFQF			108	FTGCVIAWNSNNLDS		
32	NVVIKVCEFQFCNDP			109	VIAWNSNNLDSKVGG		
33	KVCEFQFCNDPFLGV			110	NSNNLDSKVGGNYNY		
34	FQFCNDPFLGVYYHK			111	LDSKVGGNYNYLYRL		
35	NDPFLGVYYHKNNKS			112	VGGNYNYLYRLFRKS		
36	LGVYYHKNNKSWMES			113	YNYLYRLFRKSNLKP		
37	YHKNNKSWMESEFRV			114	YRLFRKSNLKPFERD		
38	NKSWMESEFRVYSSA			115	RKSNLKPFERDISTE		
39	MESEFRVYSSANNCT			116	LKPFERDISTEIYQA		
40	FRVYSSANNCTFEYV			117	ERDISTEIYQAGSTP		
41	SSANNCTFEYVSQPF			118	STEIYQAGSTPCNGV		
42	NCTFEYVSQPFLMDL			119		YQAGSTPCNGVEGFN	YQAGSTPCNGVKGFN
43	EYVSQPFLMDLEGKQ			120		STPCNGVEGFNCYFP	STPCNGVKGFNCYFP
44	QPFLMDLEGKQGNFK			121		NGVEGFNCYFPLQSY	NGVKGFNCYFPLQSY
45	MDLEGKQGNFKNLRE			122	GFNCYFPLQSYGFQP		
46	GKQGNFKNLREFVFK			123		YFPLQSYGFQPTNGV	YFPLQSYGFQPTYGV
47	NFKNLREFVFKNIDG			124		QSYGFQPTNGVGYQP	QSYGFQPTYGVGYQP
48	LREFVFKNIDGYFKI			125		FQPTNGVGYQPYRVV	FQPTYGVGYQPYRVV
49	VFKNIDGYFKIYSKH			126		NGVGYQPYRVVVLSF	YGVGYQPYRVVVLSF
50	IDGYFKIYSKHTPIN			127	YQPYRVVVLSFELLH		
51		FKIYSKHTPINLVRD	FKIYSKHTPINLVRG	128	RVVVLSFELLHAPAT		
52		SKHTPINLVRDLPQG	SKHTPINLVRGLPQG	129	LSFELLHAPATVCGP		
53		PINLVRDLPQGFSAL	PINLVRGLPQGFSAL	130	LLHAPATVCGPKKST		
54		VRDLPQGFSALEPLV	VRGLPQGFSALEPLV	131	PATVCGPKKSTNLVK		
55	PQGFSALEPLVDLPI			132	CGPKKSTNLVKNKCV		
56	SALEPLVDLPIGINI			133	KSTNLVKNKCVNFNF		
57	PLVDLPIGINITRFQ			134	LVKNKCVNFNFNGLT		
58		LPIGINITRFQTLLA	LPIGINITRFQTLHR	135	KCVNFNFNGLTGTGV		
59		INITRFQTLLALHRS	INITRFQTLHRSYLT	136	FNFNGLTGTGVLTES		
60		RFQTLLALHRSYLTP	RFQTLHRSYLTPGDS	137	GLTGTGVLTESNKKF		
61		LLALHRSYLTPGDSS	LHRSYLTPGDSSSGW	138	TGVLTESNKKFLPFQ		
62	HRSYLTPGDSSSGWT			139	TESNKKFLPFQQFGR		
63	LTPGDSSSGWTAGAA			140	KKFLPFQQFGRDIAD		
64	DSSSGWTAGAAAYYV			141	PFQQFGRDIADTTDA		
65	GWTAGAAAYYVGYLQ			142	FGRDIADTTDAVRDP		
66	GAAAYYVGYLQPRTF			143	IADTTDAVRDPQTLE		
67	YYVGYLQPRTFLLKY			144	TDAVRDPQTLEILDI		
68	YLQPRTFLLKYNENG			145	RDPQTLEILDITPCS		
69	RTFLLKYNENGTITD			146	TLEILDITPCSFGGV		
70	LKYNENGTITDAVDC			147	LDITPCSFGGVSVIT		
71	ENGTITDAVDCALDP			148	PCSFGGVSVITPGTN		
72	ITDAVDCALDPLSET			149	GGVSVITPGTNTSNQ		
73	VDCALDPLSETKCTL			150	VITPGTNTSNQVAVL		
74	LDPLSETKCTLKSFT			151		GTNTSNQVAVLYQDV	GTNTSNQVAVLYQGV
75	SETKCTLKSFTVEKG			152		SNQVAVLYQDVNCTE	SNQVAVLYQGVNCTE
76	CTLKSFTVEKGIYQT			153		AVLYQDVNCTEVPVA	AVLYQGVNCTEVPVA
77	SFTVEKGIYQTSNFR			154		QDVNCTEVPVAIHAD	QGVNCTEVPVAIHAD
				155	CTEVPVAIHADQLTP		
tpa	MDAMKRGLCCVLLLC			156	PVAIHADQLTPTWRV		

tpa	RGLCCVLLLCGAVFV			157	HADQLTPTWRVYSTG		
tpa	VLLLCGAVFVSASQE			158	LTPTWRVYSTGSNVF		
tpa	GAVFVSASQEIHARF			159	WRVYSTGSNVFQTRA		
tpa	SASQEIHARFRRIHS			160	STGSNVFQTRAGCLI		
				161	NVFQTRAGCLIGAEH		
				162	TRAGCLIGAEHVNNS		
				163	CLIGAEHVNNSYECD		
				164	AEHVNNSYECDIPIG		
				165	NNSYECDIPIGAGIC		
				166	ECDIPIGAGICASYQ		
				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	GICASYQTQTNSPRR			242	QLSSNFGAISSVLND		
169	SYQTQTNSPRRARSV			243	NFGAISSVLNDILSR		
170	QTNSPRRARSVASQS			244	ISSVLNDILSRLDKV		
171	PRRARSVASQSIIAY			245	LNDILSRLDKVEAEV		
172	RSVASQSIIAYTMSL			246	LSRLDKVEAEVQIDR		
173		SQSIIAYTMSLGAEN	SQSIIAYTMSLGVEN	247	DKVEAEVQIDRLITG		
174		IAYTMSLGAENSVAY	IAYTMSLGVENSVAY	248	AEVQIDRLITGRLQS		
175		MSLGAENSVAYSNNS	MSLGVENSVAYSNNS	249	IDRLITGRLQSLQTY		
176		AENSVAYSNNSIAIP	VENSVAYSNNSIAIP	250	ITGRLQSLQTYVTQQ		
177	VAYSNNSIAIPTNFT			251	LQSLQTYVTQQLIRA		
178	NNSIAIPTNFTISVT			252	QTYVTQQLIRAAEIR		
179	AIPTNFTISVTTEIL			253	TQQLIRAAEIRASAN		
180	NFTISVTTEILPVSM			254	IRAAEIRASANLAAT		
181	SVTTEILPVSMTKTS			255	EIRASANLAATKMSE		
182	EILPVSMTKTSVDCT			256	SANLAATKMSECVLG		
183	VSMTKTSVDCTMYIC			257	AATKMSECVLGQSKR		
184	KTSVDCTMYICGDST			258	MSECVLGQSKRVDFC		
185	DCTMYICGDSTECSN			259	VLGQSKRVDFCGKGY		
186	YICGDSTECSNLLLQ			260	SKRVDFCGKGYHLMS		
187	DSTECSNLLLQYGSF			261	DFCGKGYHLMSFPQS		
188	CSNLLLQYGSFCTQL			262	KGYHLMSFPQSAPHG		
189	LLQYGSFCTQLNRAL			263	LMSFPQSAPHGVVFL		
190	GSFCTQLNRALTGIA			264	PQSAPHGVVFLHVTY		
191	TQLNRALTGIAVEQD			265	PHGVVFLHVTYVPAQ		
192	RALTGIAVEQDKNTQ			266	VFLHVTYVPAQEKNF		
193	GIAVEQDKNTQEVFA			267	VTYVPAQEKNFTTAP		
194	EQDKNTQEVFAQVKQ			268	PAQEKNFTTAPAICH		
195	NTQEVFAQVKQIYKT			269	KNFTTAPAICHDGKA		
196	VFAQVKQIYKTPPIK			270	TAPAICHDGKAHFPR		
197	VKQIYKTPPIKDFGG			271	ICHDGKAHFPREGVF		
198	YKTPPIKDFGGFNFS			272	GKAHFPREGVFVSNG		
199	PIKDFGGFNFSQILP			273	FPREGVFVSNGTHWF		
200	FGGFNFSQILPDPSK			274	GVFVSNGTHWFVTQR		
201	NFSQILPDPSKPSKR			275	SNGTHWFVTQRNFYE		
202	ILPDPSKPSKRSFIE			276	HWFVTQRNFYEPQII		
203	PSKPSKRSFIEDLLF			277	TQRNFYEPQIITTDN		
204	SKRSFIEDLLFNKVT			278	FYEPQIITTDNTFVS		
205	FIEDLLFNKVTLADA			279	QIITTDNTFVSGNCD		
206	LLFNKVTLADAGFIK			280	TDNTFVSGNCDVVIG		
207	KVTLADAGFIKQYGD			281	FVSGNCDVVIGIVNN		
208	ADAGFIKQYGDCLGD			282	NCDVVIGIVNNTVYD		
209	FIKQYGDCLGDIAAR			283	VIGIVNNTVYDPLQP		
210	YGDCLGDIAARDLIC			284	VNNTVYDPLQPELDS		
211	LGDIAARDLICAQKF			285	VYDPLQPELDSFKEE		
212	AARDLICAQKFNGLT			286	LQPELDSFKEELDKY		
213	LICAQKFNGLTVLPP			287	LDSFKEELDKYFKNH		
214	QKFNGLTVLPPLLTD			288	KEELDKYFKNHTSPD		
215	GLTVLPPLLTDEMIA			289	DKYFKNHTSPDVDLG		
216	LPPLLTDEMIAQYTS			290	KNHTSPDVDLGDISG		
217	LTDEMIAQYTSALLA			291	SPDVDLGDISGINAS		
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	QMAYRFNGIGVTQNV			300	LIDLQELGKYEQYIK		
227	RFNGIGVTQNVLYEN			301	QELGKYEQYIKWPWY		
228	IGVTQNVLYENQKLI			302	KYEQYIKWPWYIWLG		
229	QNVLYENQKLIANQF			303	YIKWPWYIWLGFIAG		
230	YENQKLIANQFNSAI			304	PWYIWLGFIAGLIAI		
231	KLIANQFNSAIGKIQ			305	WLGFIAGLIAIVMVT		
232	NQFNSAIGKIQDSLS			306	IAGLIAIVMVTIMLC		
233	SAIGKIQDSLSSTAS			307	IAIVMVTIMLCCMTS		
234	KIQDSLSSTASALGK			308	MVTIMLCCMTSCCSC		
235	SLSSTASALGKLQDV			309	MLCCMTSCCSCLKGC		
236	TASALGKLQDVVNQN			310	MTSCCSCLKGCCSCG		
237	LGKLQDVVNQNAQAL			311	CSCLKGCCSCGSCCK		

238	QDVVNQNAQALNTLV			312	KGCCSCGSCCKFDED		
239	NQNAQALNTLVKQLS			313	SCGSCCKFDEDDSEP		
240	QALNTLVKQLSSNFG			314	CCKFDEDDSEPVLKG		
241	TLVKQLSSNFGAISS			315	DEDDSEPVLKGVKLH		
				316	DDSEPVLKGVKLHYT		

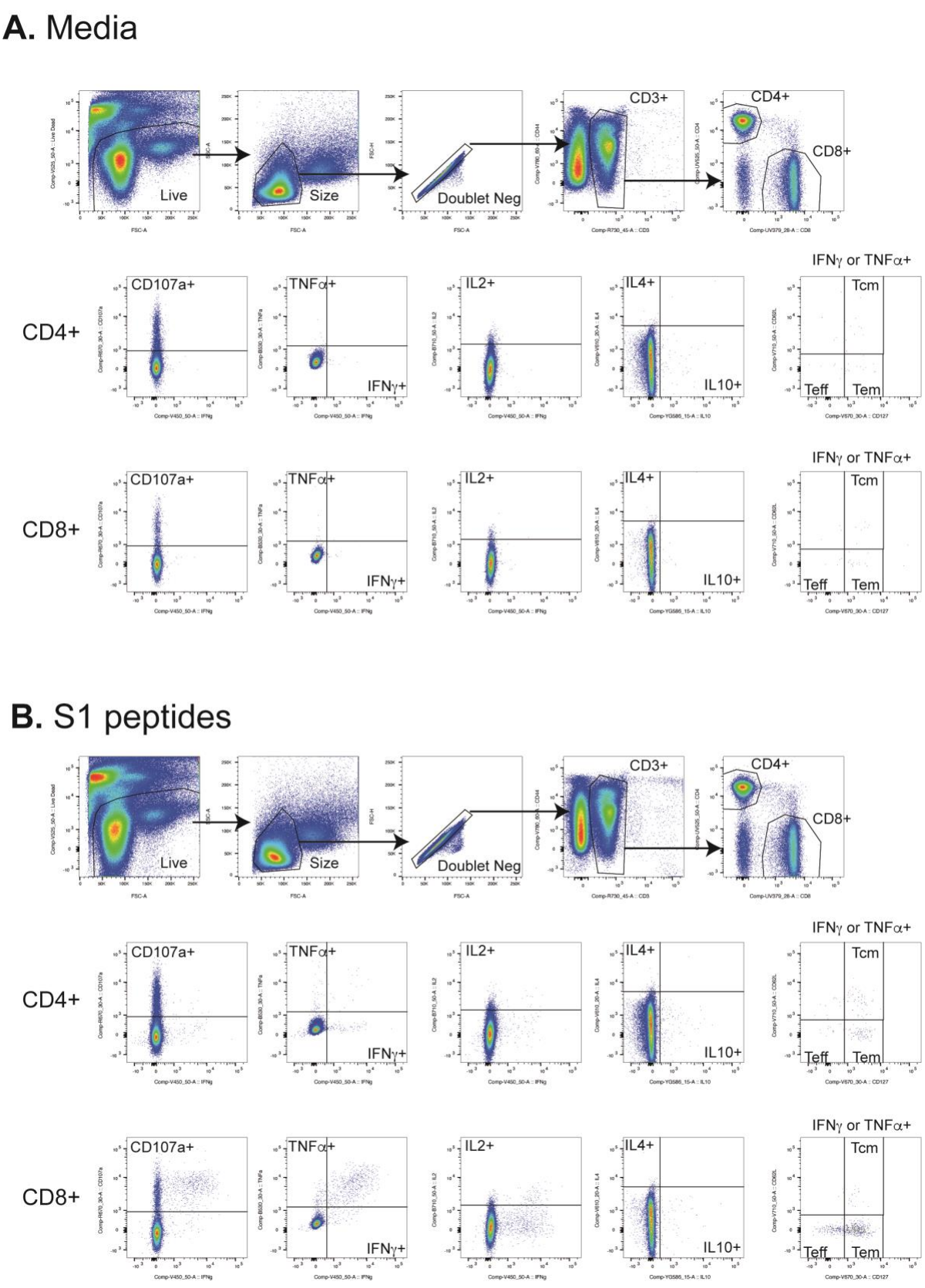

Figure S1: Flow cytometry gating strategy

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 "$IFN\gamma^+$ or $TNF\alpha^+$" 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.