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Supplementary appendix

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Appendix for

Neutralising antibody activity against SARS-CoV-2 VOCs B.1.617.2 and B.1.351 by BNT162b2 vaccination

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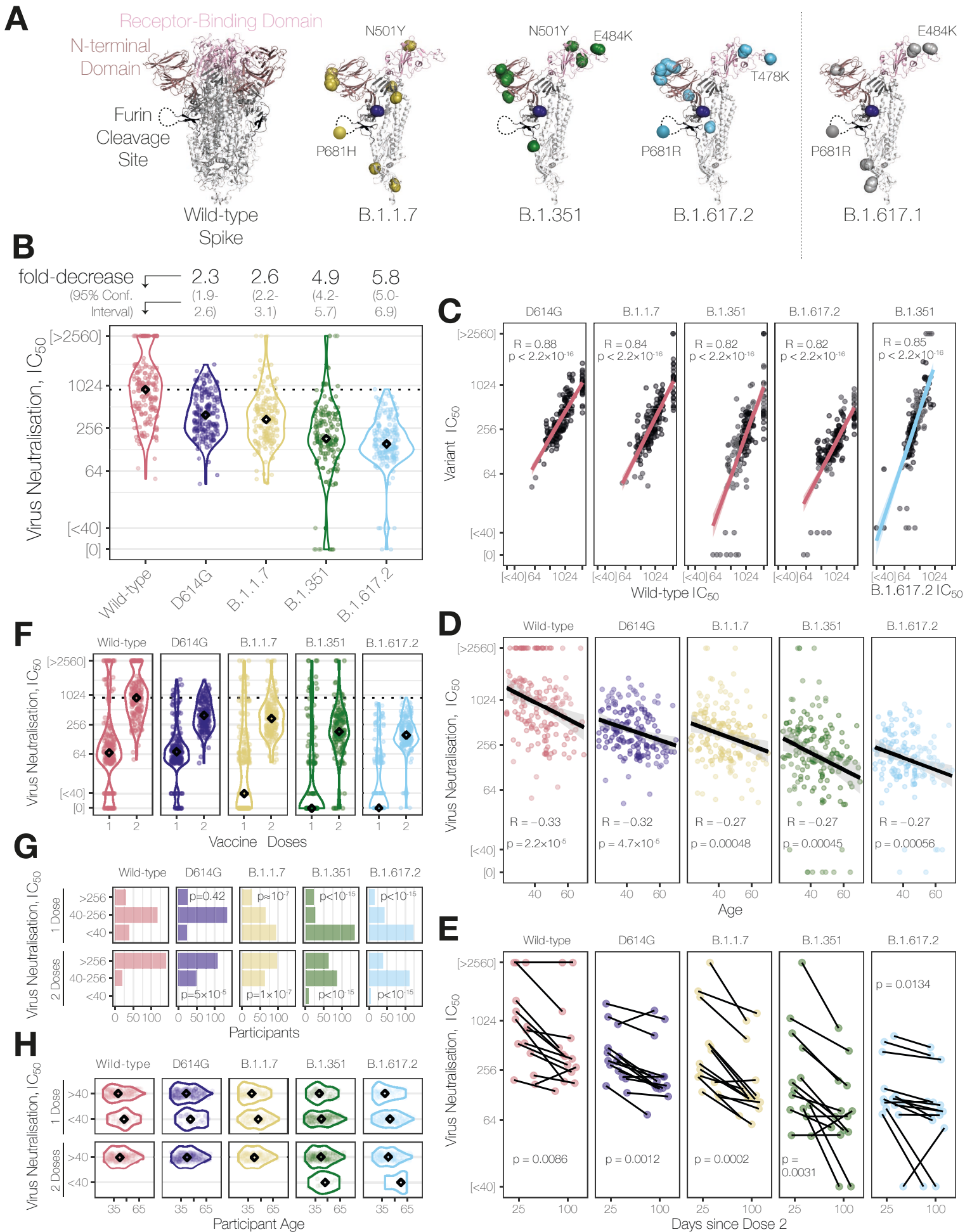
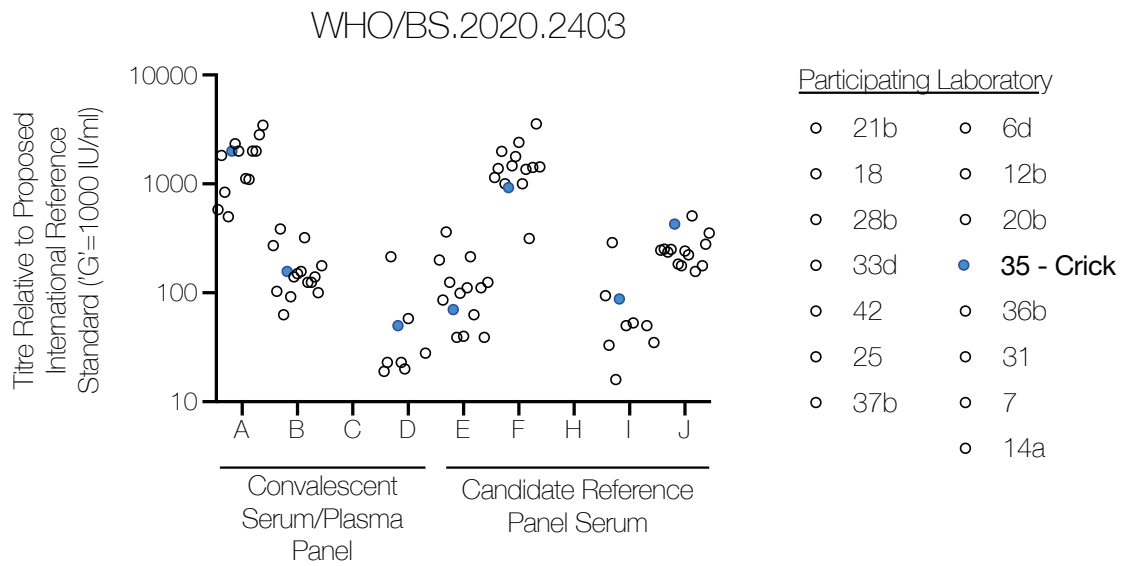
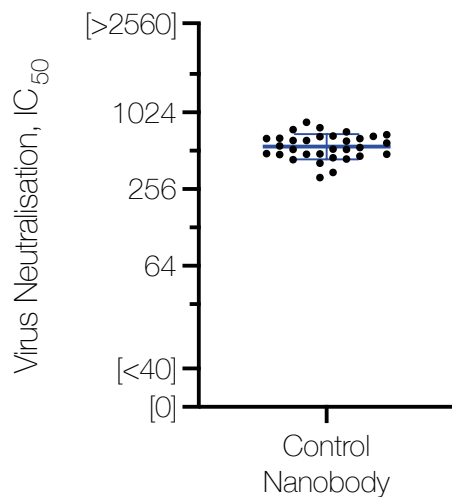
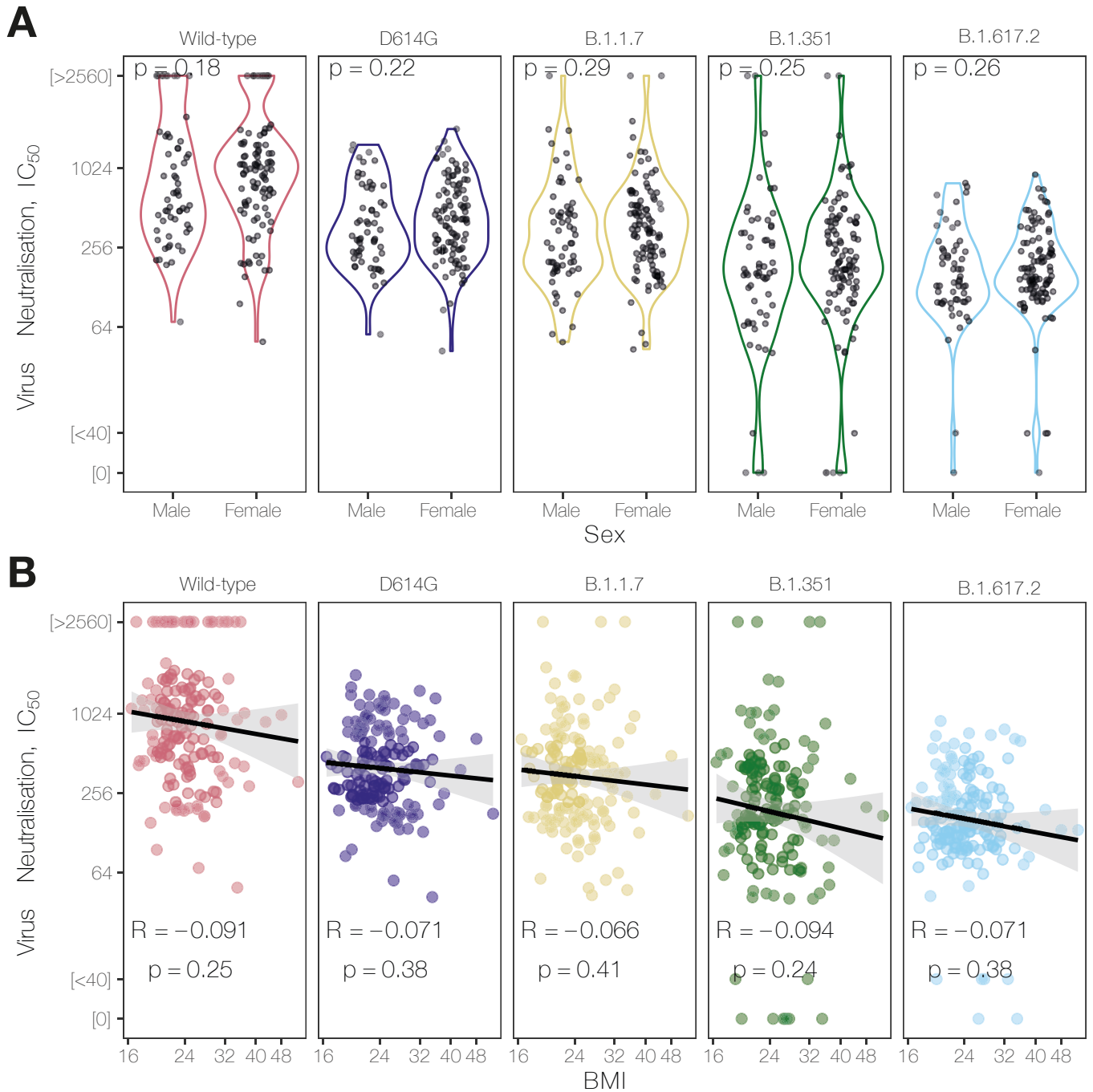


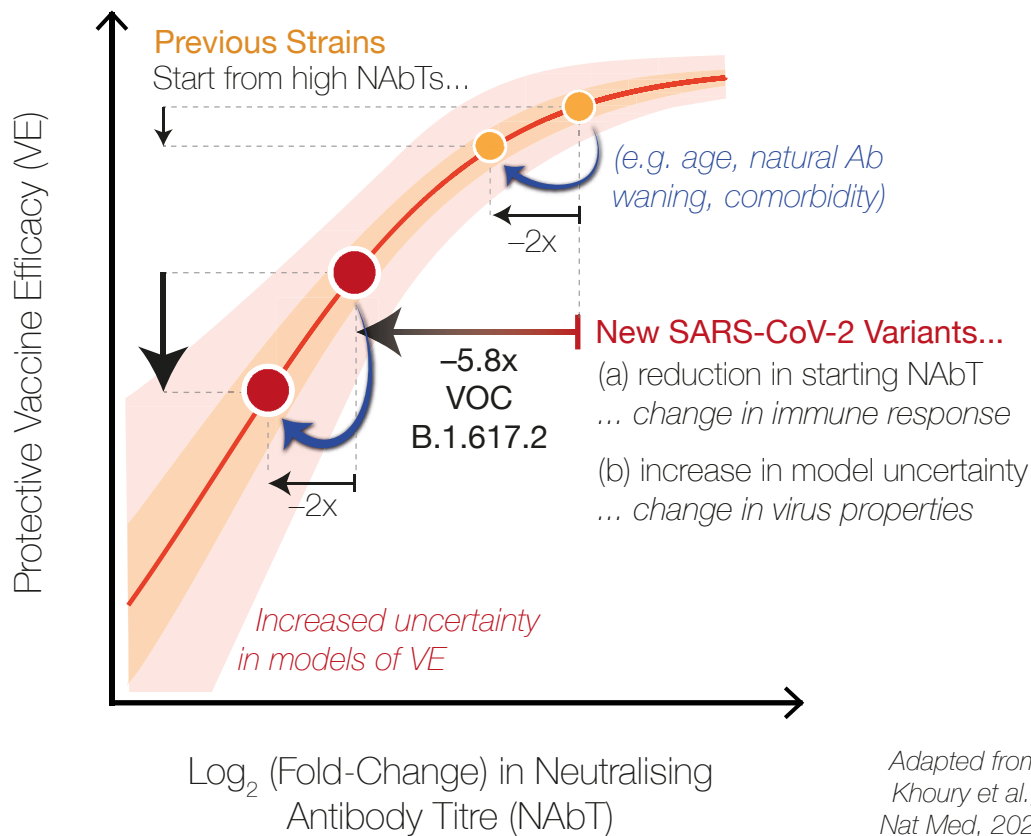
Figure 1. Limits of neutralising antibody activity against SARS-CoV-2 variants of concern B.1.617.2 and B.1.351 elicited by partial or full vaccination with BNT162b2 (Pfizer-BioNTech). (A) Structure of the SARS-CoV-2 spike protein, with selected amino acid mutations in Variants of Concern indicated on monomer. (B) Neutralising antibody titres (NAbTs) against five SARS-CoV-2 strains from 159 study participants who had received 2 doses of BNT162b2. NAbTs are expressed as serum fold-dilution required to achieve 50% virus neutralisation (IC_{50}). (C) Correlation of NAbTs between variants. (D) Correlation between participant age and IC_{50} . (E) NAbTs of participants sampled ~4 and ~10 weeks following the second dose of BNT162b2. (F) Comparison of NAbTs following 1 and 2 doses of BNT162b2, shown (G) grouped into 3 response levels. (H) Participant age distribution following stratification by NAbT following a single dose of BNT162b2.

A**B**

Supplementary Figure 1. High-throughput live-virus neutralisation assay performance during validation with wild-type SARS-CoV-2. (A) Neutralisation assay performance across laboratories participating in the establishment and validation of the WHO International Standard for SARS-CoV-2 antibody neutralisation. The high-throughput neutralisation assay used in this work is “Participating Laboratory 35” (annotated ‘Crick’). Data are from publication WHO/BS.2020.2403. (B) Inter-assay variation of measured neutralisation titre of a monoclonal control nanobody. Blue line and error bars indicate geometric mean and standard deviation.



Supplementary Figure 2. Stratification neutralising antibody titres against SARS-CoV-2 variants by sex (A) and BMI (B) following two doses of BNT162b2. Neither factors significantly affect neutralising antibody titres.



Supplementary Figure 3. Schematic based on the model of Khoury et al. illustrating the relationship between measured neutralising antibody titres (NAbT) against SARS-CoV-2 and observed real-world vaccine efficacy (VE). When NAbTs begin at a high level (e.g. against variants with spike proteins similar to the Wild-type spike in first-generation vaccines), small changes in NAbTs have a small effect on VE. However, when titres begin from a lower level (for example due to reduced activity against VOCs such as B.1.617.2 ‘B.1.617.2’), small subsequent changes in NAbTs that would not have greatly affected VE previously now have a larger effect on VE.

	Unique Participants n = 250		P-Value
	First Dose	Second Dose	
	<i>Mean/Count (SD/%)</i>	<i>Mean/Count (SD/%)</i>	
	n = 149	n = 159	
<i>Site</i>			0.052
Crick	95 (63.8%)	84 (52.8%)	
UCLH	54 (36.2%)	75 (47.2%)	
<i>Age</i>			0.812
	42.7 (11.9)	43.1 (11.6)	
<i>Sex</i>			0.041
Female	109 (73.2%)	99 (62.3%)	
Male	40 (26.8%)	60 (37.7%)	
<i>BMI</i>			0.870
	24.9 (5.4)	24.9 (5.6)	
<i>Ethnicity (Grouped)</i>			0.362
All White Backgrounds	123 (82.6%)	125 (78.6%)	
All South Asian Backgrounds	5 (3.4%)	11 (6.9%)	
All Other Backgrounds	21 (14.1%)	23 (14.5%)	

Supplementary Table 1. Initial analysis of the Legacy study (University College London Hospital and the Francis Crick Institute)

1 Dose

Wald Statistics Response: IC50 (Binned)

Factor	Chi-Square	d.f.	P
Strain (Factor+Higher Order Factors)	155.02	8	<.0001
All Interactions	3.04	4	0.5518
Age (Factor+Higher Order Factors)	16.18	5	0.0064
All Interactions	3.04	4	0.5518
Strain * Age (Factor+Higher Order Factors)	3.04	4	0.5518
TOTAL	163.85	9	<.0001

Supplementary Table 2. ANOVA following fitting of ordered logistical regression model of effect of strain and participant age on neutralising antibody response following a single dose of BNT162b2 ($IC_{50} \sim \text{Strain} * \text{Age}$). Both SARS-CoV-2 strain and participant age independently decrease neutralising antibody titres – but strain-dependent age effects (Strain*Age) are not observed in our sample.

Supplementary Methods

Clinical cohort

Two prospective cohorts of Legacy participants were established in January 2021 (NCT04750356). Participants were included if they were an employee of either UCLH or the Francis Crick Institute and had submitted at least one sample for RT-qPCR occupational health testing for COVID-19 using the Crick testing pipeline. Participants consisted of patient-facing healthcare workers at UCLH, who had received at least one dose of a currently licensed COVID-19 vaccine and Crick staff. Participants were sampled at approximately 3 weeks post-vaccination and invited for follow-up visits at approximately 6 and 12 weeks. All participants were sampled at each visit with additional nasopharyngeal RT-qPCR for SARS CoV-2 (in addition to their occupational health testing) to exclude concurrent active infection, blood was collected for serological assays including anti-spike IgG, IgM and live-virus neutralisation. Participants were analysed by vaccine dose number, date since vaccine dose, age, sex, and BMI.

ELISA

Anti-Spike IgG was detected using an in-house ELISA as previously described¹. Briefly, 96 well plates were coated with purified protein overnight at 4°C, and blocked for 1 hour with blocking buffer. Sera were diluted 1:50 in blocking buffer, added to the plate and incubated for 1 hour at RT. After four washes with PBS-Tween 0.1%, plates were incubated with goat anti-human IgG for 1 hour. Plates were then developed after six washes and read at 405nm on a plate reader (Tecan).

Virus variants & culture

The SARS-CoV-2 reference isolate (referred to as 'Wild-type') was hCoV-19/England/02/2020, obtained from the Respiratory Virus Unit, Public Health England, UK, (GISAID EpiCov accession EPI_ISL_407073). The B.1.1 strain ("D614G") was isolated from a swab from an infected healthcare worker at UCLH, obtained through the SAFER study,² and carries only the D614G mutation in its spike. The B.1.1.7 isolate ("B.1.1.7") was the hCoV-19/England/204690005/2020, which carries the D614G, Δ69-70, Δ144, N501Y, A570D, P681H, T716I, S982A and D1118H mutations,³ obtained from Public Health England (PHE), UK, through Prof. Wendy Barclay, Imperial College London, London, UK through the Genotype-to-Phenotype National Virology Consortium (G2P-UK). The B.1.351 virus isolate was the 501Y.V2.HV001, which carries the D614G, L18F, D80A, D215G, Δ242-244, K417N, E484K, N501Y, A701V mutations, and was kindly provided by Prof. Alex Sigal and Prof. Tulio de Oliveira;⁴ sequencing of viral isolates received identified the Q677H and R682W mutations

at the furin cleavage site in approximately 50% of the genomes, which was maintained upon passage in cell culture. The B.1.617.2 isolate was MS066352H (GISAID accession number EPI_ISL_1731019), which carries the T19R, K77R, G142D, Δ 156-157/R158G, A222V, L452R, T478K, D614G, P681R, D950N, and was kindly provided by Prof. Wendy Barclay, Imperial College London, London, UK through the Genotype-to-Phenotype National Virology Consortium (G2P-UK).

All viral isolates were propagated in Vero V1 cells. Briefly, 50% confluent monolayers of Vero E6 cells were infected with the given SARS CoV-2 strains at an MOI of approx. 0.001. Cells were washed once with DMEM (Sigma; D6429), then 5 ml virus inoculum made up in DMEM was added to each T175 flask and incubated at room temperature for 30 minutes. DMEM + 1% FCS (Biosera; FB-1001/500) was added to each flask. Cells were incubated at 37° C, 5% CO₂ for 4 days until extensive cytopathogenic effect was observed. Supernatant was harvested and clarified by centrifugation at 2000 rpm for 10 minutes in a benchtop centrifuge. Supernatant was aliquoted and frozen at -80°C.

Virus PCR and sequencing

All virus stocks generated for use in neutralisation assays were sequence-validated prior to use. To confirm the identity of cultured VoC samples, 8ul of viral RNA was prepared for sequencing by the ARTIC method (<https://www.protocols.io/view/ncov-2019-sequencing-protocol-v3-locost-bh42j8ye>) and sequenced on the ONT GridION platform to >30k reads / sample. The data was demultiplexed and processed using the viralrecon pipeline (<https://github.com/nf-core/viralrecon>).

High-throughput live virus microneutralisation assay

High-throughput live virus microneutralisation assays were performed as described previously⁵. Briefly, Vero E6 cells (Institute Pasteur) or Vero E6 cells expressing ACE2 and TMPRSS2 (VAT-1) (Centre for Virus Research)⁶ at 90-100% confluency in 384-well format were first titrated with varying MOIs of each SARS-CoV-2 variant and varying concentrations of a control monoclonal nanobody in order to normalise for possible replicative differences between variants and select conditions equivalent to wild-type virus. Following this calibration, cells were infected in the presence of serial dilutions of patient serum samples. After infection (24 hrs Vero E6 Pasteur, 16hrs VAT-1), cells were fixed with 4% final Formaldehyde, permeabilised with 0.2% TritonX-100, 3% BSA in PBS (v/v), and stained for SARS-CoV-2 N protein using Alexa488-labelled-CR3009 antibody produced in-house and cellular DNA using DAPI⁷. Whole-well imaging at 5x was carried out using an Opera Phenix (Perkin Elmer) and fluorescent areas and intensity calculated using the Phenix-associated software Harmony

(Perkin Elmer). Inhibition was estimated from the measured area of infected cells/total area occupied by all cells. The inhibitory profile of each serum sample was estimated by fitting a 4-parameter dose response curve executed in SciPy. Neutralising antibody titres are reported as the fold-dilution of serum required to inhibit 50% of viral replication (IC_{50}), and are further annotated if they lie above the quantitative (complete inhibition) range, below the quantitative range but still within the qualitative range (i.e. partial inhibition is observed but a dose-response curve cannot be fit because it does not sufficiently span the IC_{50}), or if they show no inhibition at all.

Data analysis, statistics, and availability

Study data were collected and managed using REDCap electronic data capture tools hosted at University College London^{8,9}. Data were exported from REDCap into R for visualisation and analysis. IC_{50} values above the quantitative limit of detection of the assay (>25600) were recoded as 5120; IC_{50} values below the quantitative limit of the assay (< 40) but within the qualitative range were recoded as 10 and data below the qualitative range (i.e. no response observed) were recoded as 5. For 2-dose data, these changes do not affect any statistical parameters considered in the analysis (i.e. they lie outside the median and IQR) and we do not perform analyses that consider the absolute value of the points – i.e. rank-based analyses are used instead: statistical significance of the difference in median viral neutralisation IC_{50} values between different strains was performed using a paired Wilcoxon Ranked sum test, and the 95% confidence interval for the difference in median viral neutralisation IC_{50} between different strains was determined using bootstrap statistics, implemented in R using the *bootstrap* and *boot.ci* functions, using the *type="basic"* argument to avoid any assumptions about the normality of the data. Correlation analysis of NAbTs between different virus strains and between NAbT and age and BMI was carried out using Spearman's test using the *cor.test* function in R, and comparisons of NAb response by sex using a paired Wilcoxon Ranked sum test. *p*-values reported have not been corrected for multiple testing. All graphs were generated using the ggplot2 package. Analysis of stratified NAb responses by strain for each dose of vaccine was carried out using ordered logistical regression using the *lrm* function of the Regression Modeling Strategies (*'rms'*) package in R, using the formula $IC_{50} \sim \text{Strain}$ or $IC_{50} \sim \text{Strain} * \text{Age}$, and *p*-values were calculated using the Wald Chi-Square test. Analysis of variance was carried out using the *anova* function in R. All data (anonymised) and full R code to produce all figures and statistical analysis presented in this manuscript are freely-available online on Github: <https://github.com/davidlvb/Crick-UCLH-Legacy-VOCs-2021-05>

Ethics

The Legacy study was approved by London Camden and Kings Cross Health Research Authority (HRA) Research and Ethics committee (REC) IRAS number 286469 and sponsored by University College London.

Role of the funding source

This work was undertaken at UCLH/UCL who received a proportion of funding from the National Institute for Health Research (NIHR) University College London Hospitals Department of Health's NIHR Biomedical Research Centre (BRC). EW, VL and BW are supported by the Centre's funding scheme. This work was supported jointly by the BRC and core funding from the Francis Crick Institute, which receives its funding from Cancer Research UK, the UK Medical Research Council, and the Wellcome Trust. DLVB is additionally supported by the Genotype-to-Phenotype National Virology Consortium (G2P-UK) via UK Research and Innovation and the UK Medical Research Council. The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding authors had full access to all the data and the final responsibility to submit for publication.

Declaration of interests

All authors declare no conflicts of interest

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