Article

Correlates of protection against SARS-CoV-2 in rhesus macaques

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Recent studies have reported the protective efficacy of both natural[1] and vaccine-induced[2–7] immunity against challenge with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in rhesus macaques. However, the importance of humoral and cellular immunity for protection against infection with SARS-CoV-2 remains to be determined. Here we show that the adoptive transfer of purified IgG from convalescent rhesus macaques (*Macaca mulatta*) protects naive recipient macaques against challenge with SARS-CoV-2 in a dose-dependent fashion. Depletion of $CD8^+$ T cells in convalescent macaques partially abrogated the protective efficacy of natural immunity against rechallenge with SARS-CoV-2, which suggests a role for cellular immunity in the context of waning or subprotective antibody titres. These data demonstrate that relatively low antibody titres are sufficient for protection against SARS-CoV-2 in rhesus macaques, and that cellular immune responses may contribute to protection if antibody responses are suboptimal. We also show that higher antibody titres are required for treatment of SARS-CoV-2 infection in macaques. These findings have implications for the development of SARS-CoV-2 vaccines and immune-based therapeutic agents.

The protective efficacy of several candidate vaccines for SARS-CoV-2[2–7] and the protective efficacy of natural immunity against re-exposure to SARS-CoV-2[1] have recently been reported in rhesus macaques. Both neutralizing antibody (NAb) titres and Fc functional antibody responses correlated with protection[4,5], but direct data demonstrating the immunological determinants of protection have been lacking. In particular, the relative importance of humoral immunity, cellular immunity and innate immunity in protecting against SARS-CoV-2 has not yet been determined. Such knowledge is important to the development of vaccines, antibody-based therapeutic agents and public health strategies.

IgG adoptive transfer protection study

We previously reported that nine rhesus macaques infected with SARS-CoV-2 by the intranasal and intratracheal routes were protected against rechallenge with SARS-CoV-2[1]. Following rechallenge, these macaques developed increased $\log_{10}$-transformed NAb titres of 2.56–3.38 (median 3.07) using a luciferase-based pseudovirus neutralization assay[1,4,8] (Extended Data Fig. 1). To develop a high-titre IgG stock for adoptive transfer studies, we purified IgG from plasma from each of these 9 macaques and prepared individual IgG stocks at concentrations of 10 mg ml^{-1}. These stocks had NAb titres similar to those of the original plasma samples (Extended Data Fig. 1). We then pooled these individual IgG stocks to create a combined IgG stock with a $\log_{10}$-transformed NAb titre of 3.20 (NAb titre of 1,581).

We assessed the protective efficacy of this purified IgG stock in an adoptive transfer study in rhesus macaques. Twelve male and female rhesus macaques of Indian origin (Extended Data Table 1), divided into four groups of three animals each, received an intravenous infusion of 250 mg per kg bodyweight (mg kg^{-1}) (group I), 25 mg kg^{-1} (group II) or 2.5 mg kg^{-1} (group III) of the SARS-CoV-2 IgG stock, or sham IgG (group IV) on day −3. On day 0, all macaques were challenged with 1.0×10^5 tissue culture infectious dose ($TCID_{50}$) SARS-CoV-2 by the intranasal and intratracheal routes, as has previously been described[5]. Following infusion, serum antibody titres were observed in the recipient macaques as expected. On day 0, the $\log_{10}$-transformed NAb titres were 2.71–2.76 in group I (NAb titres of 511–571), 1.62–1.87 in group II (NAb titres of 42–49), and <1.30–1.36 in group III (NAb titres of <20–23) (Extended Data Fig. 2a). Binding enzyme-linked immunosorbent assay (ELISA) titres to spike (S) and the receptor-binding domain (RBD) similarly showed a titration of antibody titres in the recipient macaques on day 0 (Extended Data Fig. 2b, c). After challenge

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with SARS-CoV-2, NAb and ELISA titres gradually decreased in group I and in a subset of macaques in group II over the 14 days of follow-up. By contrast, NAb and ELISA titres increased sharply in groups III and IV by days 10–14 after challenge, presumably reflecting the autologous antibody response to productive infection[1].

We profiled S-, RBD- and nucleocapsid (N)-specific antibody responses in the recipient macaques by systems serology[9,10], as diverse Fc functional antibody responses have previously been observed in SARS-CoV-2-infected animals[1]. We assessed IgG subclasses, Fc-receptors (FcR2A-1, FcR2A-2 and FcR3A), antibody-dependent neutrophil phagocytosis (ADNP), antibody-dependent complement deposition (ADCD), antibody-dependent cellular phagocytosis (ADCP) and antibody-dependent natural-killer cell activation (ADNKA) responses. Consistent with the NAb and ELISA data, the antibody Fc effector profiles showed higher responses in group I and marginal responses in group III (Extended Data Fig. 3). Moreover, a principal component analysis of the antibody features showed that macaques in group I separated from the other groups.

To assess the protective efficacy of the adoptively transferred IgG, we assessed viral loads in bronchoalveolar lavage and nasal swabs by real-time PCR (RT–PCR) for subgenomic RNA (sgRNA), which is believed to measure replicating virus[1,11]. All sham controls in group IV were infected and had a median peak of 5.18 (range of 3.82–5.45) $\log_{10}$-transformed sgRNA copies per ml in bronchoalveolar lavage (Fig. 1a) and a median peak of 5.72 (range of 5.12–6.62) $\log_{10}$-transformed sgRNA copies per swab in nasal swabs (Fig. 1b), consistent with previous experience with this challenge stock[1,4,5]. As expected, viral replication was observed for 7–10 days in bronchoalveolar lavage and for 10–14 days in nasal swabs. All macaques that received 2.5 mg kg^{-1} IgG in group III were also infected, but generally showed a shorter duration of viral replication of 3–10 days in bronchoalveolar lavage and 7–10 days in nasal swabs. By contrast, 1 of 3 macaques that received 25 mg kg^{-1} IgG in group II, and all macaques that received 250 mg kg^{-1} IgG in group I, were protected with no detectable virus in bronchoalveolar lavage or nasal swabs at any time point following challenge (limit of quantification was 1.69 $\log_{10}$-transformed sgRNA copies per ml or sgRNA copies per swab). These data demonstrate that purified IgG from convalescent macaques—in the absence of cellular and innate immunity—can effectively protect naive recipient macaques against challenge with SARS-CoV-2 in both the upper and lower respiratory tracts, in a dose-dependent fashion.

We used logistic regression models to determine the antibody titre thresholds for protection against SARS-CoV-2 in this experiment. These regression models indicated that pseudovirus NAb titres of approximately 50 (Fig. 2a, d), RBD ELISA titres of approximately 100 (Fig. 2b, e) and S ELISA titres of approximately 400 (Fig. 2c, f) were required for protection. More precise estimates of NAb titres required for protection will require evaluation of titres between those achieved in group II (NAb titres of approximately 50) and those achieved in group I (NAb titres of approximately 500). Correlation analysis showed that NAb titres correlated with protective efficacy ($P = 0.013$ for bronchoalveolar lavage, $P = 0.023$ for nasal swabs, two-sided Spearman rank-correlation tests) (Fig. 2) and that NAb titres effectively differentiated fully protected from nonprotected macaques ($P = 0.0082$, two-sided Mann–Whitney U-test) (Fig. 2). Other antibody features also correlated with protective efficacy, including IgG1, ADCD, ADNKA and ADNP ($P = 0.00032$, $P = 0.00097$, $P = 0.0048$ and $P = 0.0074$, respectively; two-sided Spearman rank-correlation tests) (Extended Data Fig. 4). Future studies could assess whether there are different correlates of protection in the lower and upper respiratory tracts.

IgG adoptive transfer therapy study

Given the clinical interest in the therapeutic use of convalescent plasma for SARS-CoV-2 infection in humans[12,13], we next assessed the therapeutic efficacy of purified IgG in infected rhesus macaques. Six rhesus macaques of Indian origin, divided into three groups of two animals each, were infected with 1.0×10^5 $TCID_{50}$ SARS-CoV-2 by the intranasal and intratracheal routes on day 0 and then received an intravenous infusion of 250 mg kg^{-1} (group 1), 25 mg kg^{-1} (group 2) or 0 mg kg^{-1} (sham) (group 3) of the SARS-CoV-2 IgG stock on day 1. After infusion, serum antibody titres were similar to those observed in the previous experiment (NAb titres of approximately 500 in group 1 and 50 in group 2) (data not shown).

The sham controls in group 3 had a median peak of 5.22 $\log_{10}$-transformed sgRNA copies per ml in bronchoalveolar lavage and 5.62 $\log_{10}$-transformed sgRNA copies per swab in nasal swabs with 7–10 days of virus replication (Fig. 3), similar to the previous experiment. Macaques in group 2 that received 25 mg kg^{-1} IgG had a median peak of 4.58 $\log_{10}$-transformed sgRNA copies per ml in bronchoalveolar lavage and 5.74 $\log_{10}$-transformed sgRNA copies per swab in nasal swabs. By contrast, macaques in group 1 that received 250 mg kg^{-1} IgG had a median peak of 3.38 $\log_{10}$-transformed sgRNA copies per ml in bronchoalveolar lavage and 4.36 $\log_{10}$-transformed sgRNA copies per swab

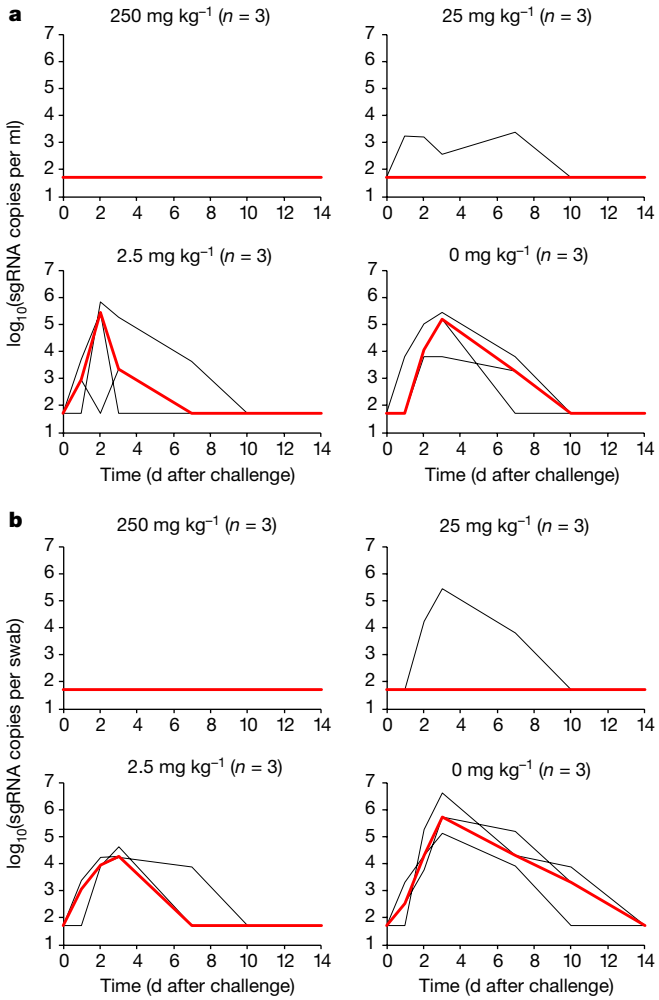

Fig. 1 | Viral loads after prophylactic adoptive transfer of IgG before challenge with SARS-CoV-2. Rhesus macaques were challenged by intranasal and intratracheal routes with 10^5 $TCID_{50}$ SARS-CoV-2. **a**, $\log_{10}$-transformed sgRNA copies per ml (limit of quantification was 50 copies per ml) were assessed in bronchoalveolar lavage after challenge. **b**, $\log_{10}$-transformed sgRNA copies per swab (limit of quantification was 50 copies per swab) were assessed in nasal swabs after challenge. Red lines reflect median values. The number of macaques is denoted in each panel and the median line overlaps with data lines. IgG dosage is indicated above each panel.

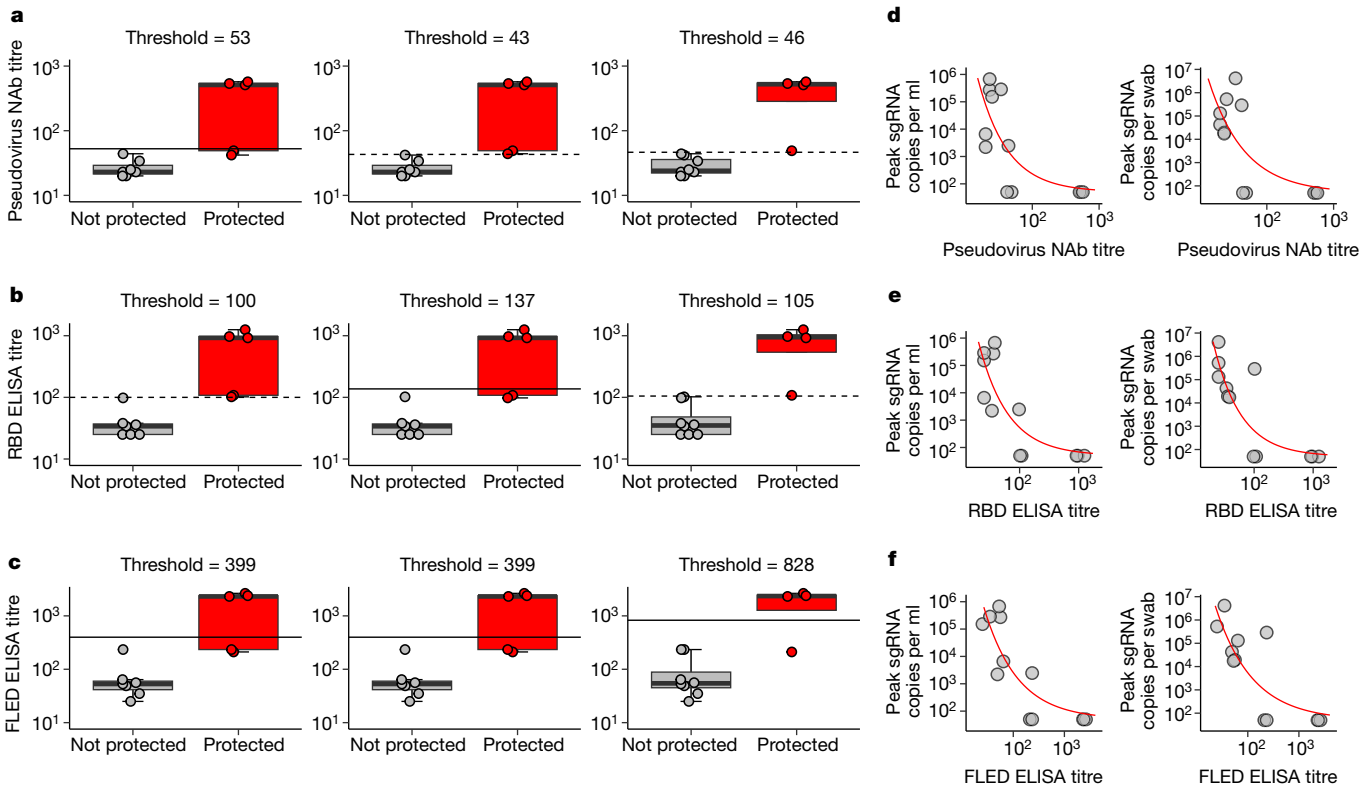

Fig. 2 | Regression analysis of antibody threshold for protection. **a**–**c**, Differences in pseudovirus NAb titres (**a**), RBD ELISA titre (**b**) and S (full-length ectodomain (FLED)) ELISA titre (**c**) between protected and nonprotected macaques. Macaques were considered to be protected if they had no detectable virus in bronchoalveolar lavage ($n = 5$ protected macaques) (left), nasal swab ($n = 5$ protected macaques) (middle) or in both ($n = 4$ protected macaques), right). The threshold titres are indicated as horizontal lines and were obtained by logistic regression for scenarios in which the groups were not completely separable using the corresponding titre (95% predicted probability of protection) (solid lines), or represent the mean between the highest and lowest titres of nonprotected and protected macaques, respectively (dotted lines). In the box plots, whiskers indicate range, and boxes indicates 25th to 75th percentile. **d**–**f**, Relationship between pseudovirus NAb titres (**d**), RBD ELISA titre (**e**) and S (FLED) ELISA titre (**f**) and peak viral loads in bronchoalveolar lavage (left) and nasal swab (right). Red lines indicate fitted exponential curves.

in nasal swabs with 3–7 days of virus replication. These data suggest a 1.84-log reduction of peak sgRNA copies per ml in bronchoalveolar lavage and a 1.26-log reduction of peak sgRNA copies per swab in nasal swabs with the 250 mg kg^{-1} IgG dose, but minimal or no efficacy with the 25 mg kg^{-1} IgG dose.

CD8 depletion study

To evaluate the potential role of cellular immune responses in protection against SARS-CoV-2, we CD8-depleted[14] convalescent rhesus macaques before rechallenge[1]. Thirteen rhesus macaques were infected with 1.0×10^5 $TCID_{50}$ SARS-CoV-2 by the intranasal and intratracheal routes, and all animals demonstrated robust viral replication in bronchoalveolar lavage and nasal swabs as expected (data not shown). At week 4 after infection, the median log_{10}-transformed NAb titre of these convalescent macaques was 2.30, which was lower than the median log_{10}-transformed NAb titre of 3.07 in the rechallenged macaques that were used in the adoptive transfer study ($P = 0.0001$, two-sided Mann–Whitney U-test) (Extended Data Fig. 5). NAb titres in these convalescent macaques declined to a median log_{10}-transformed NAb titre of 1.91 by week 7 ($P = 0.0003$, two-sided Mann–Whitney U-test, comparing week-4 and week-7 titres) (Extended Data Fig. 5).

To evaluate the role of CD8$^+$ T cells in contributing to protective efficacy against rechallenge, macaques received a single intravenous infusion of 50 mg kg^{-1} of MT807R1 (anti-CD8α CDR-grafted rhesus IgG1; MassBiologics) ($n = 5$ macaques), CD8b255R1 (anti-CD8β rhesus IgG1; MassBiologics) ($n = 3$ macaques) or a sham control antibody ($n = 5$ macaques) at week 7 after primary infection (on day −3 relative to rechallenge). The monoclonal anti-CD8α antibody depletes both CD8$^+$ T cells and natural killer cells in rhesus macaques, whereas the monoclonal anti-CD8β antibody depletes only CD8$^+$ T cells[14]. After infusion, all macaques that received anti-CD8α or anti-CD8β antibodies showed complete or near-complete CD8 depletion in peripheral blood, including total CD8$^+$ cells, CD8$^+$CD3$^+$ cells and CD8$^+$CD3$^-$ cells (Extended Data Fig. 6). On day 0, all macaques were rechallenged with 1.0×10^5 $TCID_{50}$ SARS-CoV-2 by the intranasal and intratracheal routes. After rechallenge, S-specific IFNγ$^+$CD8$^+$ T cell responses were undetectable in the CD8-depleted macaques (as expected), but increased in the nondepleted sham controls ($P = 0.0157$, two-sided Mann–Whitney U-test) (Extended Data Fig. 7). By contrast, S-specific IFNγ$^+$CD4$^+$ T cell responses were similar in both groups (Extended Data Fig. 8). NAb responses also increased in both groups after rechallenge (Extended Data Fig. 9), consistent with previous findings[1].

After rechallenge, macaques that received the sham control antibody demonstrated no virus in bronchoalveolar lavage (Fig. 4a) and only transient virus in 1 of 5 macaques in nasal swabs (Fig. 4b) indicative of natural immunity, similar to previous observations[1]. By contrast, in the CD8α-depleted group, 1 of 5 macaques showed breakthrough virus in bronchoalveolar lavage (Fig. 4a), and 5 of 5 macaques exhibited breakthrough virus in nasal swabs (Fig. 4b) after rechallenge. Similarly, in the CD8β-depleted group, 1 of 3 macaques showed breakthrough virus in bronchoalveolar lavage (Fig. 4a), and 3 of 3 macaques exhibited breakthrough virus in nasal swabs (Fig. 4b) after rechallenge. Peak viral loads in nasal swabs were higher in the CD8-depleted macaques than

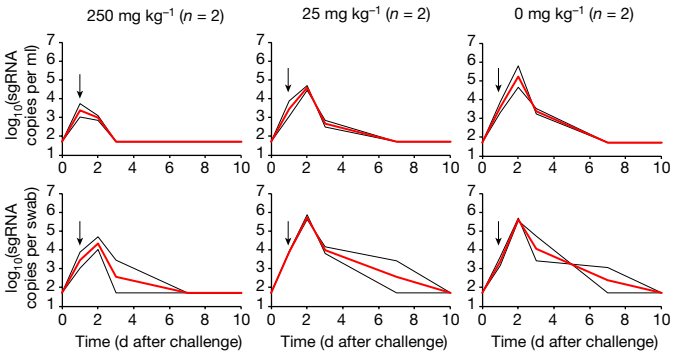

Fig. 3 | Viral loads following therapeutic adoptive transfer of IgG after challenge with SARS-CoV-2. Rhesus macaques were challenged by intranasal and intratracheal routes with 10^5 $TCID_{50}$ SARS-CoV-2 on day 0 and then received intravenous infusion of 250 mg kg^{-1}, 25 mg kg^{-1} or 0 mg kg^{-1} purified SARS-CoV-2 IgG on day 1. log_{10}-transformed sgRNA copies per ml (limit of quantification was 50 copies per ml) were assessed in bronchoalveolar lavage (top panels) and log_{10}-transformed sgRNA copies per swab (limit of quantification was 50 copies per swab) were assessed in nasal swabs (bottom panels) after challenge. Red lines reflect median values. Arrows represent the day of IgG infusion. The number of macaques is denoted in each panel and the median line overlaps with data lines.

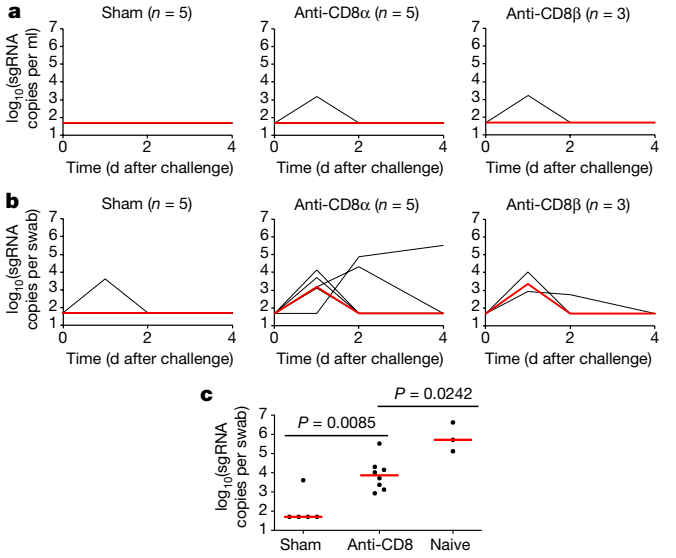

Fig. 4 | Viral loads after CD8 depletion and rechallenge with SARS-CoV-2. Rhesus macaques were infected with SARS-CoV-2 and received 50 mg kg^{-1} anti-CD8α monoclonal antibody, anti-CD8β monoclonal antibody or sham monoclonal antibody at week 7, reflecting day −3 relative to rechallenge. On day 0, all macaques were rechallenged with 10^5 $TCID_{50}$ SARS-CoV-2. **a**, log_{10}-transformed sgRNA copies per ml (limit of quantification was 50 copies per ml) were assessed in bronchoalveolar lavage after rechallenge. **b**, log_{10}-transformed sgRNA copies per swab (limit of quantification was 50 copies per swab) were assessed in nasal swabs after rechallenge. **c**, Comparison of peak log_{10}-transformed sgRNA copies per swab in nasal swabs after rechallenge in sham and anti-CD8 groups. Peak log_{10}-transformed sgRNA copies per swab in nasal swabs from naive macaques following primary challenge from the adoptive transfer study are shown for comparison. Red lines reflect median values. In **a**, **b**, the number of macaques is denoted in each panel; in **c**, sham $n = 5$, anti-CD8 $n = 8$, naive $n = 3$ independent macaques. *P* values reflect two-sided Mann–Whitney *U*-tests.

in the sham controls ($P = 0.0085$, two-sided Mann–Whitney *U*-test) (Fig. 4c) but lower than in naive macaques after primary challenge ($P = 0.0242$, two-sided Mann–Whitney *U*-test) (Fig. 4c). These data suggest that cellular immunity, including $CD8^+$ T cells, can contribute to the protection of convalescent macaques against rechallenge with SARS-CoV-2 in the setting of waning and subprotective antibody titres.

Discussion

Our data demonstrate that adoptive transfer of purified polyclonal IgG from convalescent macaques robustly protected naive recipient rhesus macaques against challenge with SARS-CoV-2 in a dose-dependent fashion. These data indicate that relatively low titres of antibodies are sufficient for protection in both the upper and lower respiratory tracts. CD8 depletion studies also showed that cellular immunity can contribute to protection against rechallenge with SARS-CoV-2 in convalescent macaques with waning antibody titres. Taken together, these data demonstrate proof-of-concept findings that define key immunological determinants for protection against SARS-CoV-2 in rhesus macaques.

These findings extend DNA and Ad26 vaccine studies in rhesus macaques in which we observed that NAb titres and other Fc functional antibody responses correlated with protective efficacy[4,5]. Our data also extend recent studies that have shown that potent RBD-specific monoclonal antibodies can protect against challenge with SARS-CoV-2 in macaques[15,16]. In the present study, we show that polyclonal antibodies from convalescent macaques—in the absence of cellular and innate immune responses—protect when administered at an appropriate titre. NAb titres of approximately 500 fully protected macaques, and titres of approximately 50 partially protected macaques. These titres should be readily achievable by vaccination in humans. These data demonstrate that relatively low NAb titres are sufficient to protect against SARS-CoV-2 in rhesus macaques.

We also observed that protection of convalescent macaques against rechallenge with SARS-CoV-2 was partially abrogated by CD8 depletion before rechallenge. NAb titres declined in convalescent macaques from week 4 to week 7, with over half of the macaques exhibiting NAb titres of <100 by week 7. CD8 depletion in these macaques resulted in a loss of protection in the upper respiratory tract against rechallenge with SARS-CoV-2, which suggests that $CD8^+$ T cells contribute to virological control if NAb titres are suboptimal or subprotective. Future studies could be designed to evaluate the potential protective efficacy of cellular immune responses in the absence of antibody responses.

Our findings have implications for vaccines and immune-based therapeutic agents. Our data suggest the importance of SARS-CoV-2 vaccines to induce potent and durable humoral as well as cellular immune responses. We speculate that NAb titres above a particular threshold are sufficient for protection, but that $CD8^+$ T cells may also contribute to protection when NAb titres decline. However, it is possible that the specific NAb threshold for protection identified in the present study (about 50) may be model-specific and may be dependent on technical details, such as donor plasma characteristics, challenge virus-stock infectivity and inoculum dose, as well as recipient macaque specifics. Further adoptive transfer studies could be performed with IgG purified from plasma from vaccinated macaques or humans.

The present data also demonstrate the therapeutic efficacy of convalescent plasma for treatment of infection with SARS-CoV-2, which is currently being explored in clinical trials[12,13]. However, our data should be interpreted cautiously, because only high serum NAb titres (of approximately 500) in recipient macaques showed therapeutic efficacy in this model, whereas lower serum-NAb titres (of approximately 50) in recipient macaques did not show efficacy. Such high serum NAb titres in recipient macaques probably exceed typical serum NAb titres achieved in human recipients of convalescent human plasma[4,17], and thus implications for the clinical use of convalescent plasma for treatment of SARS-CoV-2 infection remain limited. Monoclonal antibodies achieve substantially higher neutralization titres than convalescent plasma.

Our data provide a proof-of-concept that antibodies can protect against challenge with SARS-CoV-2 in a dose-dependent fashion. If antibodies are below the threshold titre required for protection, then cellular immune responses may be important for rapid virological control. Collectively, these data provide insights into the immunological determinants and correlates of protection against SARS-CoV-2 and have important implications for the development of vaccines, immune-based therapeutic agents and public health strategies.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-020-03041-6.

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Methods

No statistical methods were used to predetermine sample size. The experiments were not randomized, and investigators were not blinded to allocation during experiments and outcome assessment.

Animals and study design

Thirty-one outbred Indian-origin male and female rhesus macaques (*M. mulatta*), 2–5 years old, were randomly allocated to groups. All macaques were housed at Bioqual. In the prophylactic adoptive transfer study ($n = 12$), macaques received an intravenous infusion of 250 mg kg^{-1}, 25 mg kg^{-1} or 2.5 mg kg^{-1} purified SARS-CoV-2 IgG or sham IgG ($n = 3$ macaques per group) on day −3. On day 0, all macaques were challenged with 1.0×10^5 $TCID_{50}$ (1.2×10^8 RNA copies, 1.1×10^4 plaque-forming units) SARS-CoV-2, which was derived from USA-WA1/2020 (NR-52281; BEI Resources) and fully sequenced, revealing no mutations[1]. In the therapeutic adoptive transfer study ($n = 6$), macaques were challenged with 1.0×10^5 $TCID_{50}$ SARS-CoV-2 on day 0 and then received an intravenous infusion of 250 mg kg^{-1}, 25 mg kg^{-1} or 0 mg kg^{-1} purified SARS-CoV-2 IgG ($n = 2$ macaques per group) on day 1. Virus was administered as 1 ml by the intranasal route (0.5 ml in each nare) and 1 ml by the intratracheal route. In the CD8 depletion study ($n = 13$), macaques were infected with 1.0×10^5 $TCID_{50}$ SARS-CoV-2 and then received an intravenous infusion of 50 mg kg^{-1} of the anti-CD8α CDR-grafted rhesus IgG1 antibody MT807R1 (MassBiologics) ($n = 5$), the anti-CD8β rhesus IgG1 antibody CD8b255R1 (MassBiologics) ($n = 3$) or a sham antibody ($n = 5$) at week 7, reflecting day −3 relative to rechallenge. On day 0, all macaques were rechallenged intranasally and intratracheally with SARS-CoV-2 in the adoptive transfer study. All immunological and virological assays were performed blinded. All animal studies were conducted in compliance with all relevant local, state, and federal regulations and were approved by the Bioqual Institutional Animal Care and Use Committee.

IgG purification

Polyclonal IgG was purified from rhesus macaque plasma using NAb protein G spin columns (Thermo Scientific). Plasm was incubated for 1 h with protein G columns preconditioned with protein G IgG binding buffer (Thermo Scientific) to capture maximum IgG. The columns were then washed six times, and IgG was eluted with IgG elution buffer (Thermo Scientific). The eluted IgG was buffer-exchanged with 1× DPBS and analysed by SDS–PAGE and spectrophotometry.

Subgenomic mRNA assay

SARS-CoV-2 *E* gene sgRNA was assessed by RT–PCR using primers and probes as previously described[1,4,11]. In brief, to generate a standard curve, the SARS-CoV-2 *E* gene sgRNA was cloned into a pcDNA3.1 expression plasmid; this insert was transcribed using an AmpliCap-Max T7 High Yield Message Maker Kit (Cellscript) to obtain RNA for standards. Before RT–PCR, samples collected from challenged macaques or standards were reverse-transcribed using Superscript III VILO (Invitrogen) according to the manufacturer's instructions. A Taqman custom gene expression assay (ThermoFisher Scientific) was designed using the sequences targeting the *E* gene sgRNA[11]. Reactions were carried out on a QuantStudio 6 and 7 Flex Real-Time PCR System (Applied Biosystems) according to the manufacturer's specifications. Standard curves were used to calculate sgRNA in copies per ml or per swab; the quantitative assay sensitivity was 50 copies per ml or per swab.

ELISA

Binding antibodies were assessed by ELISA essentially as previously described[1,4]. In brief, 96-well plates were coated with 1 μg/ml SARS-CoV-2 S or RBD protein in 1× DPBS and incubated at 4 °C overnight. After incubation, plates were washed once with wash buffer (0.05% Tween 20 in 1× DPBS) and blocked with 350 μl casein block per well for 2–3 h at room temperature. After incubation, block solution was discarded and plates were blotted dry. Serial dilutions of heat-inactivated serum diluted in casein block were added to wells and plates were incubated for 1 h at room temperature, before three further washes and a 1-h incubation with a 1:1,000 dilution of anti-macaque IgG HRP (NIH NHP Reagent Program) at room temperature in the dark. Plates were then washed 3 times, and 100 μl of SeraCare KPL TMB SureBlue Start solution was added to each well; plate development was halted by the addition of 100 μl SeraCare KPL TMB Stop solution per well. The absorbance at 450 nm was recorded using a VersaMax or Omega microplate reader. ELISA endpoint titres were defined as the highest reciprocal serum dilution that yielded an absorbance >0.2. $\log_{10}$-transformed end point titres are reported.

Pseudovirus neutralization assay

The SARS-CoV-2 pseudoviruses expressing a luciferase reporter gene were generated in an approach similar to that previously described[1,4,8]. In brief, the packaging construct psPAX2 (AIDS Resource and Reagent Program), luciferase reporter plasmid pLenti-CMV Puro-Luc (Addgene),and S protein expressing pcDNA3.1-SARS-CoV-2 SΔCT were cotransfected into HEK293T cells with calcium phosphate. The supernatants containing the pseudotype viruses were collected 48 h after transfection; pseudotype viruses were purified by filtration with a 0.45-μm filter. To determine the neutralization activity of the antisera from vaccinated macaques, HEK293T-hACE2 cells were seeded in 96-well tissue culture plates at a density of 1.75×10^4 cells per well overnight. Twofold serial dilutions of heat-inactivated serum samples were prepared and mixed with 50 μl of pseudovirus. The mixture was incubated at 37 °C for 1 h before adding to HEK293T-hACE2 cells. After 48 h, cells were lysed in Steady-Glo Luciferase Assay (Promega) according to the manufacturer's instructions. SARS-CoV-2 neutralization titres were defined as the sample dilution at which a 50% reduction in relative light units was observed relative to the average of the virus control wells.

Intracellular cytokine staining assay

One million peripheral blood mononuclear cells per well were resuspended in 100 μl of R10 medium supplemented with CD49d monoclonal antibody (1 μg ml^{-1}). Each sample was assessed with mock (100 μl of R10 plus 0.5% DMSO; background control), peptide pools (2 μg ml^{-1}), or 10 pg ml^{-1} phorbol myristate acetate (PMA) and 1 μg ml^{-1} ionomycin (Sigma-Aldrich) (100 μl; positive control) and incubated at 37 °C for 1 h. After incubation, 0.25 μl of GolgiStop and 0.25 μl of GolgiPlug in 50 μl of R10 was added to each well and incubated at 37 °C for 8 h and then held at 4 °C overnight. The next day, the cells were washed twice with DPBS, stained with near IR live/dead dye for 10 min and then stained with predetermined titres of monoclonal antibodies against CD279 (clone EH12.1, BB700), CD38 (clone OKT10, PE), CD28 (clone 28.2, PE CY5), CD4 (clone L200, BV510), CD45 (clone D058-1283, BUV615), CD95 (clone DX2, BUV737) and CD8 (clone SK1, BUV805), for 30 min. Cells were then washed twice with 2% FBS in DPBS buffer and incubated for 15 min with 200 μl of BD CytoFix/CytoPerm Fixation/Permeabilization solution. Cells were washed twice with 1× Perm Wash buffer (BD Perm/Wash Buffer 10× in the CytoFix/CytoPerm Fixation/Permeabilization kit diluted with MilliQ water and passed through a 0.22-μm filter) and stained with intracellularly with monoclonal antibodies against Ki67 (clone B56, FITC), CD69 (clone TP1.55.3, ECD), IL10 (clone JES3-9D7, PE CY7), IL13 (clone JES10-5A2, BV421), TNF (clone Mab11, BV650), IL4 (clone MP4-25D2, BV711), IFNγ (clone B27; BUV395), IL2 (clone MQ1-17H12, APC) and CD3 (clone SP34.2, Alexa 700), for 30 min. Cells were washed twice with 1× Perm Wash buffer and fixed with 250 μl of freshly prepared 1.5% formaldehyde. Fixed cells were transferred to 96-well round-bottom plate and analysed by BD FACSymphony system.

Luminex isotype assay

To determine relative concentrations of antigen-specific antibody isotypes and Fc receptor binding activity, a Luminex isotype assay was

performed[10]. Protein antigens included prefusion stabilized S ectodomain, RBD, nucleocapsid (N) and S1+S2 (Aalto Bio Reagents; Sino Biological). Antigens were covalently coupled to different Luminex microplex carboxylated bead regions (Luminex) using NHS-ester linkages by using EDC and NHS (Thermo Scientific) according to the manufacturer recommendations. Antigen-coupled beads were added to 384-well plates and incubated with diluted samples (1:100 for isotypes, 1:250 for FcR binding) for 2 h to form immune complexes. The plates were then washed. Mouse-anti-NHP secondary detectors were added (40 µl at 0.65 µg ml^{-1}) for each antibody isotype (IgG1, IgG2, IgG3 and IgG4 (Nonhuman Primate Reagent Resource)) and allowed to bind for 1 h. Following a wash, tertiary anti-mouse-IgG–PE detector antibodies were added (45 µl at 0.5 µg ml^{-1}). FcR binding was measured in a similar procedure by using recombinant NHP FcRs (FcR2A-1, FcR2A-2 and FcR3A (Duke Protein Production Facility)) conjugated to PE as secondary detectors. Flow cytometry was performed using an iQue (Intellicyt).

Functional antibody assays

To quantify and compare antibody functionality of plasma samples, bead-based assays were used to measure ADCP, ADNP and ADCD[9]. Biotinylated antigens S, RBD and N were coupled to fluorescent streptavidin beads (Thermo Fisher) and incubated with diluted plasma (ADCP at 1:100, ADNP at 1:50 and ADCD at 1:10) to allow antibody binding to occur. For ADCP, cultured THP-1 cells were incubated with immune complexes for 16 h at 37 °C, during which phagocytosis occurred. For ADNP, primary neutrophils were isolated from whole blood using ammonium–chloride–potassium (ACK) lysis buffer. After phagocytosis of immune complexes for 1 h at 37 °C, neutrophils were stained with an anti-CD66b Pacific Blue detection antibody (Biolegend) before flow cytometry. For ADCD, lyophilized guinea pig complement (Cedarlane) was reconstituted according to manufacturer's instructions and diluted in gelatin veronal buffer with calcium and magnesium (Boston BioProducts). After allowing for complement deposition, C3 bound to immune complexes was detected with FITC–conjugated goat IgG fraction to guinea pig complement C3 (MP Biomedicals). For quantification of antibody-dependent natural killer cell activation, plasma samples diluted at 1:25 were incubated for 2 h at 37 °C in ELISA plates coated with 0.3 µg of antigen per well. Human natural killer cells were isolated the evening before using RosetteSep (STEMCELL Technologies) from healthy buffy coat donors and incubated overnight in 1 ng ml^{-1} human recombinant interleukin 15 (STEMCELL Technologies). Natural killer cells were incubated with immune complexes for 5 h at 37 °C, after which they were stained with CD107a PE–Cy5 (BD), Golgi stop (BD) and brefeldin A (BFA) (Sigma-Aldrich). After incubation, cells were fixed (Perm A, Life Tech) and stained using anti-CD16 APC–Cy7 (BD), anti-CD56 PE–Cy7 (BD) and anti-CD3 Pacific Blue (BD). Intracellular staining using anti-MIP-1β PE (BD) was performed after permeabilizing the natural killer cells using Perm B (Thermo Fisher). Flow cytometry acquisition of all assays was performed using an iQue (Intellicyt) and an S-LAB robot (PAA). For ADCP, phagocytosis events were gated on bead-positive cells. For ADNP, neutrophils were identified by gating on CD3$^-$CD14$^-$CD66b$^+$ cells. Neutrophil phagocytosis was identified by gating on bead-positive cells. A phagocytosis score for ADCP and ADNP was calculated as (percentage of bead-positive cells) × (mean fluorescence intensity of bead-positive cells) divided by 10,000. ADCD quantification was reported as mean fluorescence intensity of FITC–anti-C3. Natural killer cells were identified by gating on CD3$^-$CD16$^+$CD56$^+$ cells. Data are reported as the percentage of cells positive for CD107a and MIP-1β.

Statistical analyses

Comparisons of virological and immunological data were performed using GraphPad Prism 8.4.2 (GraphPad Software). Comparison of data between groups was performed using two-sided Mann–Whitney *U*-tests. *P* values of less than 0.05 were considered significant. For regression analyses, pseudovirus NAb titres, ELISA titres, IgG subclass titres, FcR binding levels, ADCD and the maximum or peak viral loads were $\log_{10}$-transformed. To determine a threshold in antibody titres predicting protection, we classified protected and nonprotected macaques with logistic regression models fitted using the R function glm. The thresholds required for protection were then determined as the titres for which the models predict a 95% probability of being protected. When the titres were higher for all protected macaques than for the nonprotected macaques, the two groups were completely separable, and more data would be required to obtain reliable estimates for the titre threshold corresponding to a 95% probability of protection. In these cases, the mean value of the highest titre of the nonprotected macaques and the lowest titre of the protected macaques is reported. To relate the antibody features to the peak viral loads, we fitted exponential curves, $y = a \times \exp(b \times x) + \log_{10}(50)$, in which x is the $\log_{10}$-transformed antibody titre and y is the $\log_{10}$-transformed peak viral load, using the R function nls. This curve has the asymptote $\log_{10}(50) = 1.7$, which corresponds to the limit of quantification of the assay at 50 copies per ml (bronchoalveolar lavage) or copies per swab (nasal swabs). A principal component analysis was constructed using the R package ropls to compare multivariate profiles. Correlations were assessed using Spearman rank correlations, and *P* values were corrected for multiple testing using the Benjamini–Hochberg procedure. The analyses were performed in R version 3.6.1.

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

All data are available in the Article and its Supplementary Information. Source data are provided with this paper.

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Author contributions D.H.B. designed the study. K.M., J.Y., N.B.M., L.H.T., A. Chandrashekar, J.L., L. Peter, E.A.B., G.D., M.S.G., C.-J.D., Z.L., F.N. and S.P. performed the immunological and virological assays. C.L., C.A., A.Z., D.A.L. and G.A. performed the systems serology and regression modelling. L. Pessaint, A.V.R., K.B., J.Y.-O., M.C., R.B., A. Cook, E.T., H.A. and M.G.L. led the clinical care of the macaques. D.H.B. wrote the paper with all co-authors.

Competing interests D.H.B. is a co-inventor on provisional SARS-CoV-2 vaccine patents (62/969,008; 62/994,630). The remaining authors declare no financial conflicts of interest.

Additional information
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Correspondence and requests for materials should be addressed to D.H.B.
Peer review information *Nature* thanks Ali Ellebedy and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.
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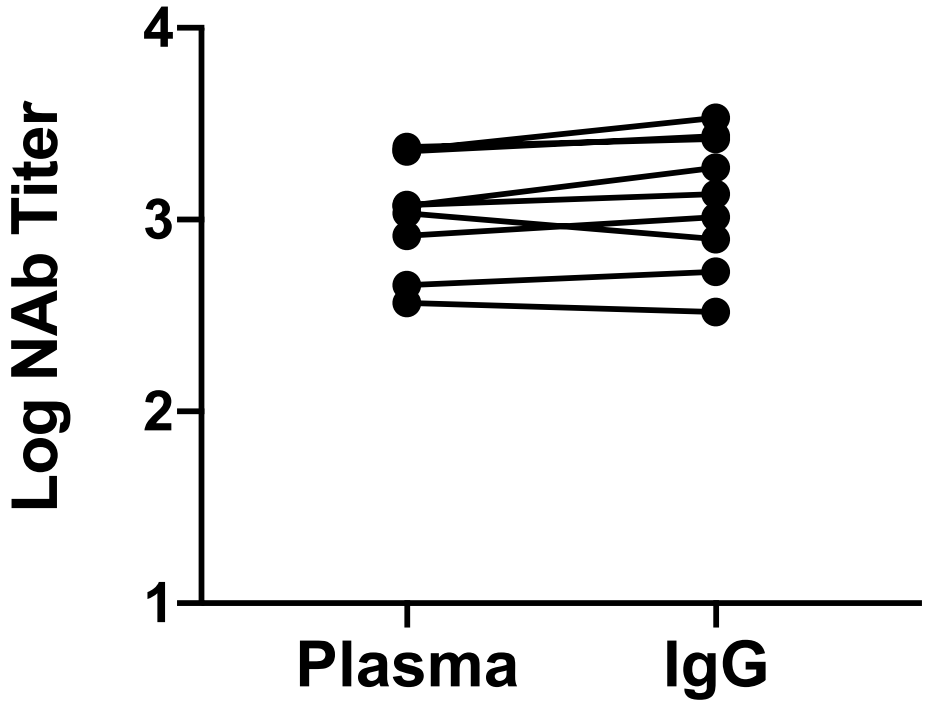
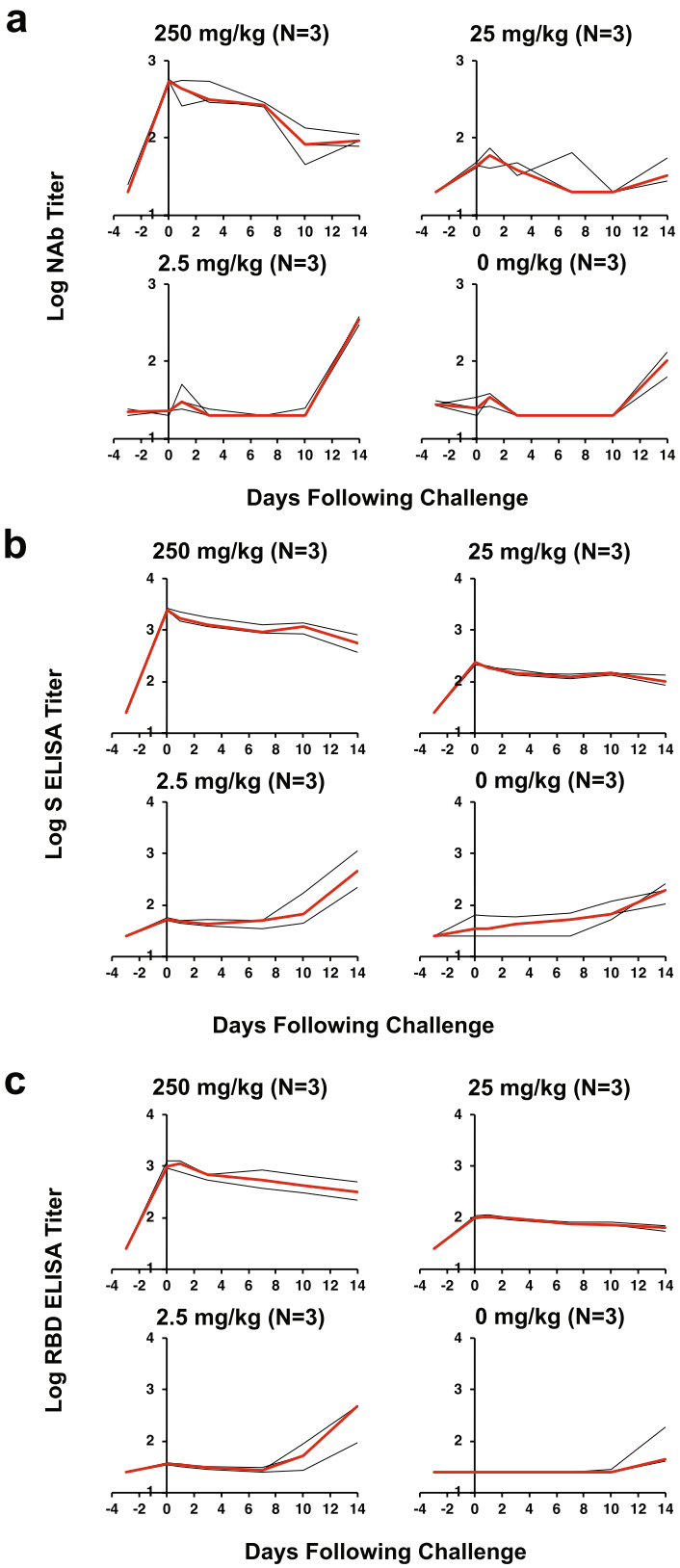
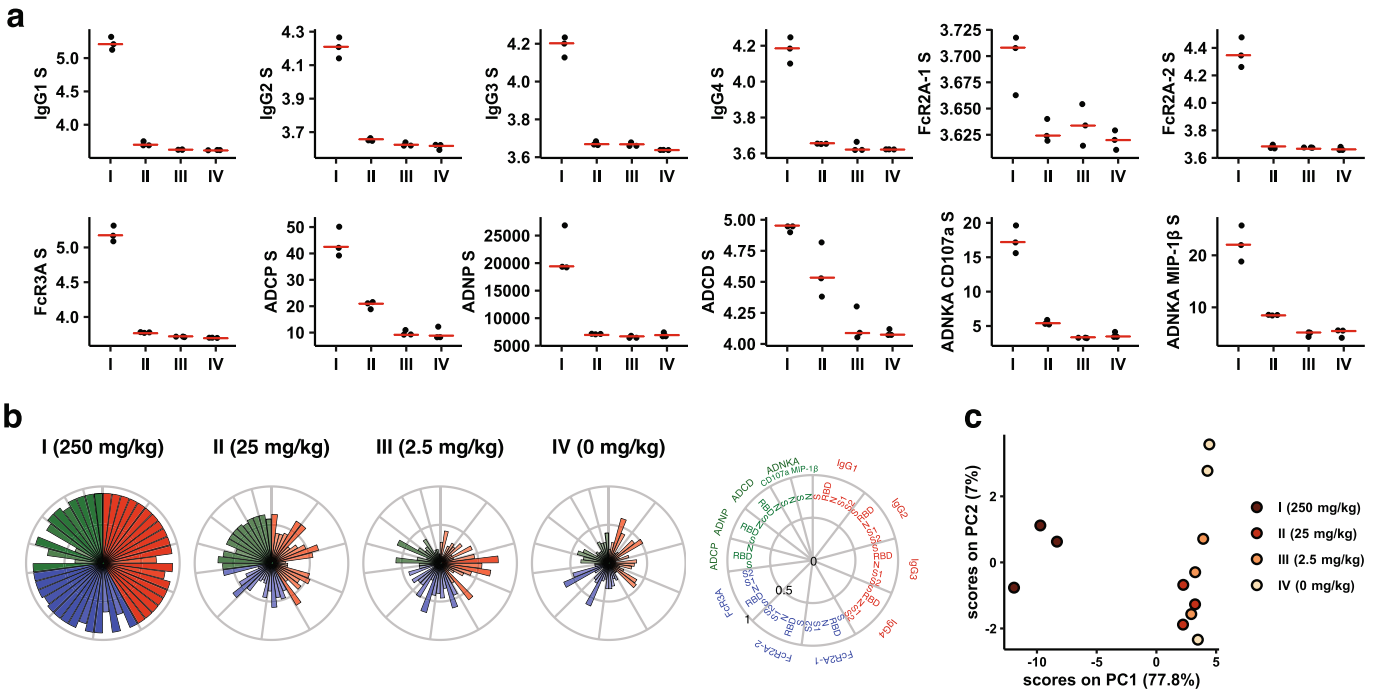


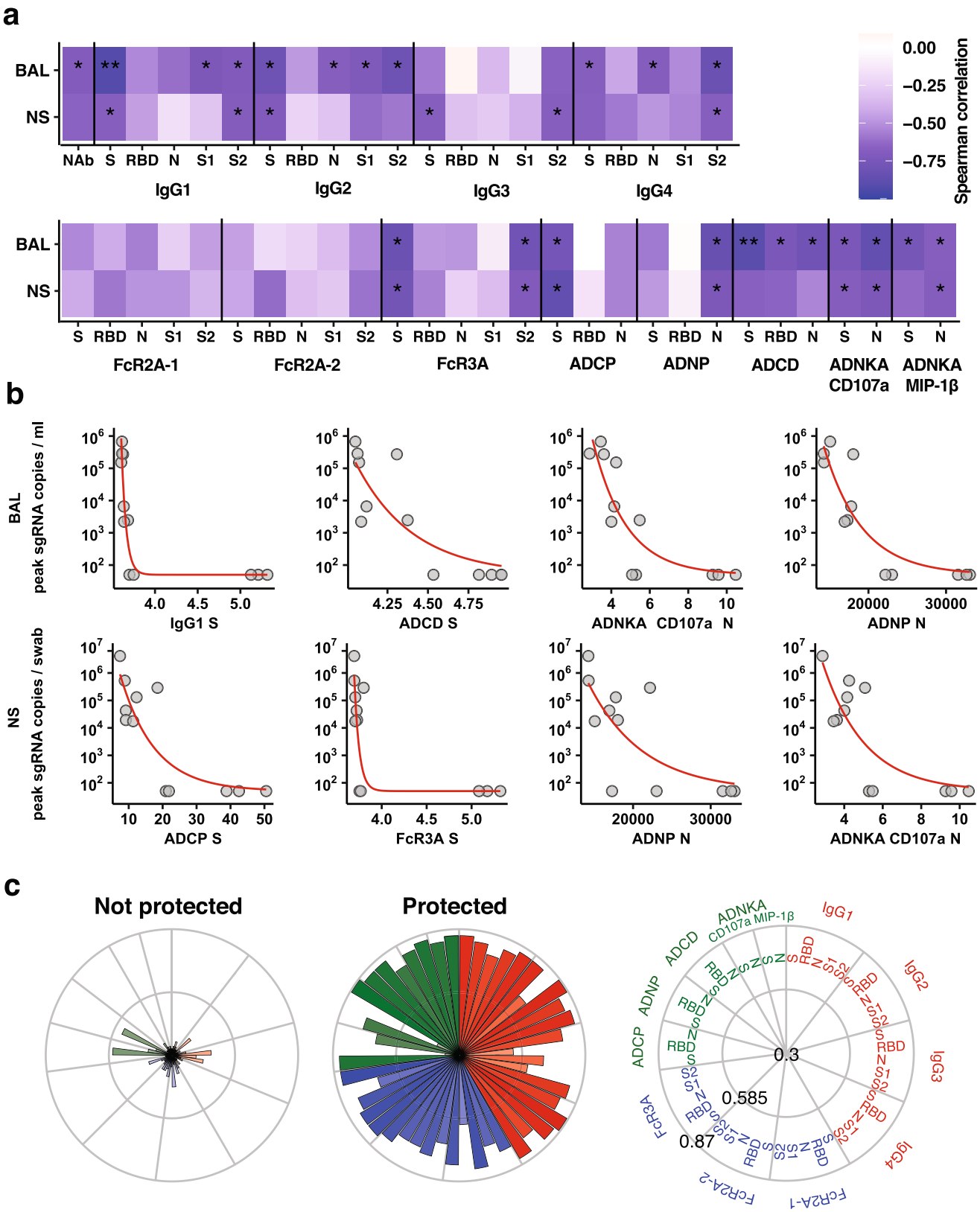
Extended Data Fig. 1 | Pseudovirus NAb titres in plasma and purified IgG of donor macaques. Nine rhesus macaques were infected and rechallenged with SARS-CoV-2. IgG was purified from plasma and formulated at 10 mg ml^{-1}.



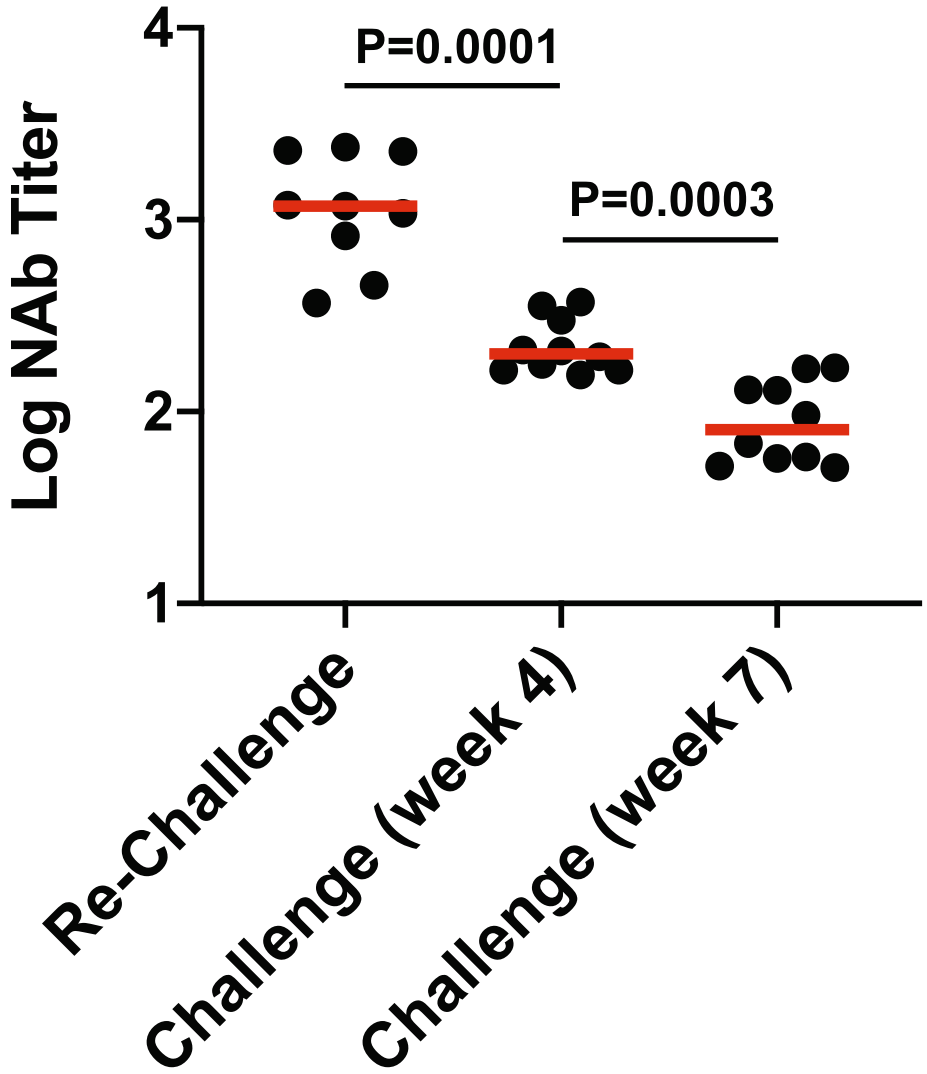
Extended Data Fig. 2 | Antibody titres after prophylactic adoptive transfer of IgG before challenge with SARS-CoV-2. a–**c**, log_{10}-transformed pseudovirus NAb titers (**a**), log_{10}-transformed S-specific ELISA titres (**b**) and log_{10}-transformed RBD-specific ELISA titres (**c**) in rhesus macaques after intravenous infusion of 250 mg kg^{-1}, 25 mg kg^{-1} or 2.5 mg kg^{-1} of purified SARS-CoV-2 IgG, or sham IgG, on day −3. On day 0, all macaques were challenged with 10^5 $TCID_{50}$ SARS-CoV-2. Days following challenge are shown on the *x* axis. Red lines reflect median responses. The number of macaques is denoted in each panel and the median line overlaps with data lines.



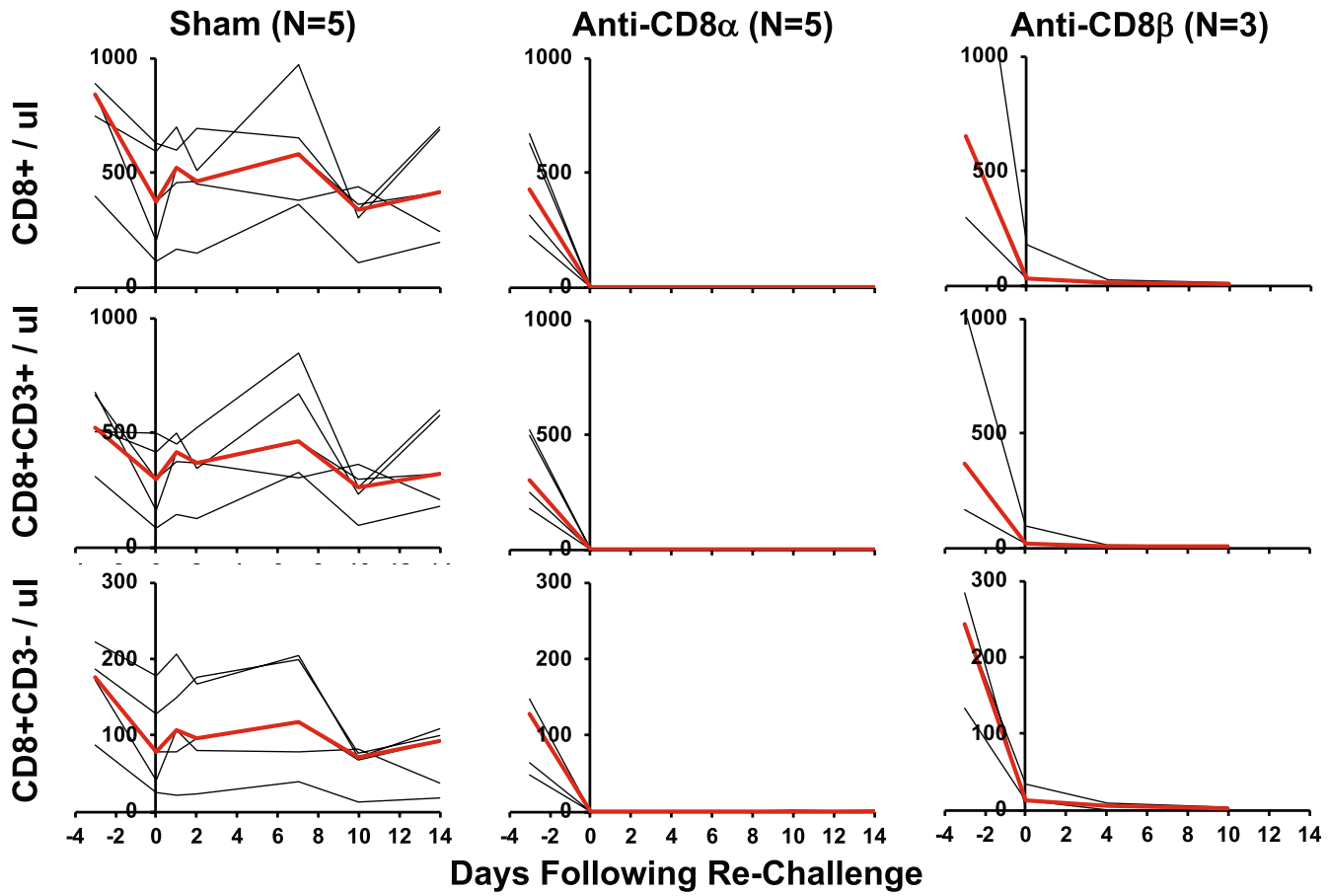
Extended Data Fig. 3 | Systems serology after adoptive transfer of IgG in rhesus macaques. a, S-specific IgG subclass, FcR and ADCP, ADNP, ADCD and ADNKA responses. **b**, Radar plot showing the mean percentile of the antibody levels across passive transfer groups. The size and colour intensity of the wedges indicate the mean percentile of the feature for the corresponding group: antibody subclass, red; FcR binding, blue; effector function, green. **c**, Principal component (PC) analysis plot showing the multivariate antibody profiles across groups. Each dot represents one macaque ($n = 12$ independent macaque) and the colour of the dot indicates the group. Red lines reflect median responses.



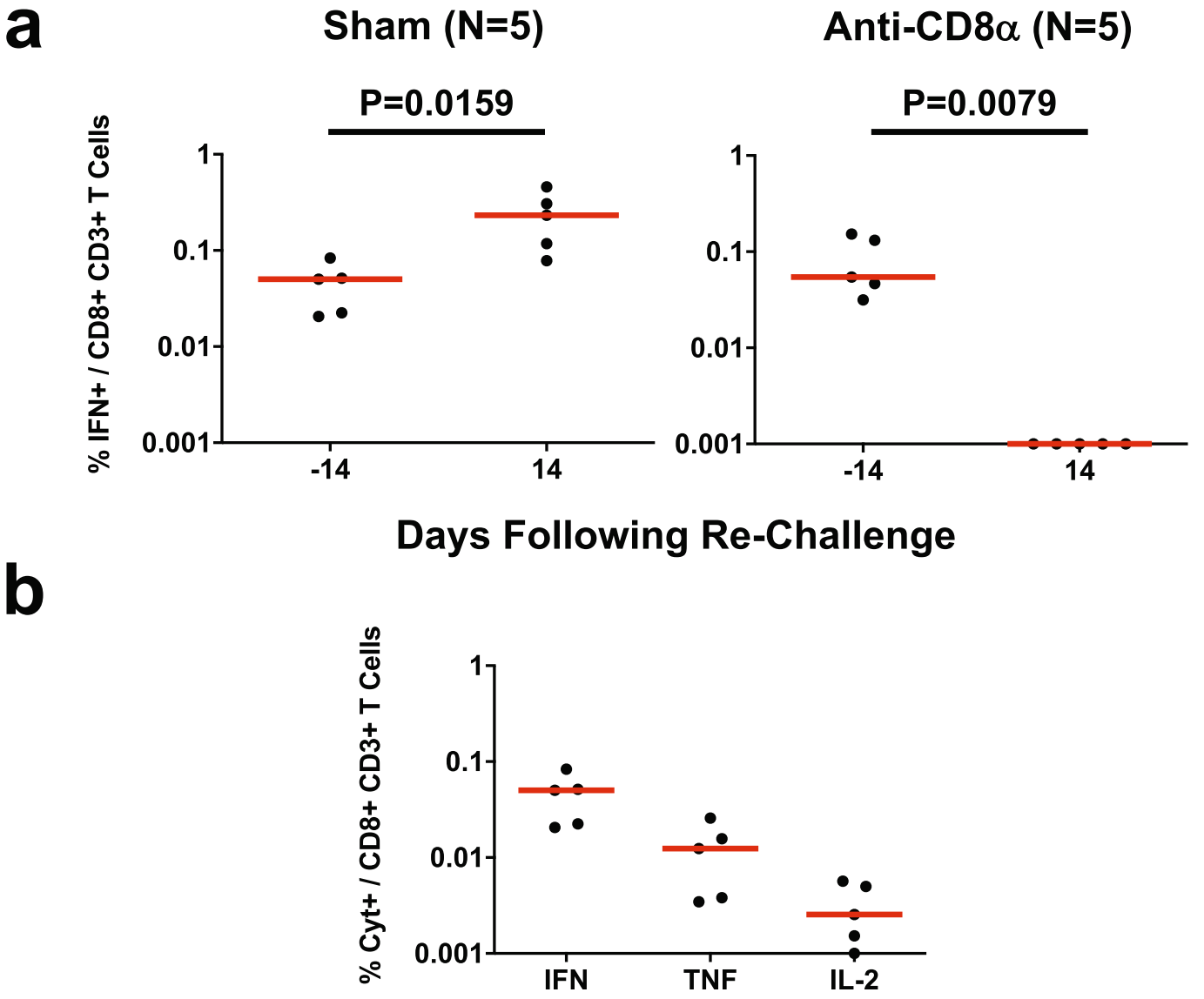
Extended Data Fig. 4 | Systems serology immune correlates. a, The heat map shows the Spearman correlations between antibody features and peak sgRNA in bronchoalveolar lavage (BAL) and nasal swabs (NS). *$q < 0.05$, **$q < 0.01$; q values were obtained by Benjamini–Hochberg correction for multiple testing. **b**, Relations between the top four antibody features ($n = 12$ independent macaques) that are highest correlated to peak viral loads in bronchoalveolar lavage (top) and nasal swabs (bottom). Red lines indicate fitted exponential curves. **c**, The radar plots depict the mean percentile of each antibody feature for protected and nonprotected macaques. The size and colour intensity of the wedges indicate the mean percentile of the feature for the corresponding group: antibody subclass, red; FcR binding, blue; effector function, green.



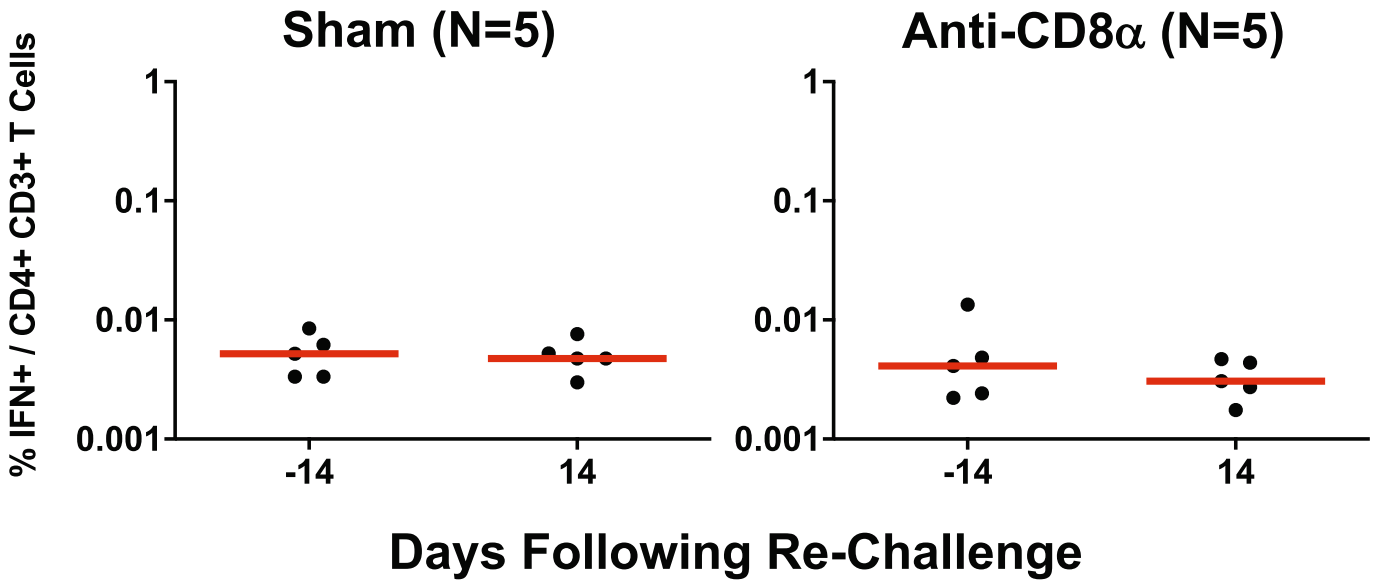
Extended Data Fig. 5 | Decline of NAb titres in convalescent rhesus macaques after infection with SARS-CoV-2. log_{10}-transformed pseudovirus NAb titres in the 9 rechallenged macaques used as donors in the adoptive transfer study and in the 10 macaques used in the CD8 depletion study at week 4 and week 7 after infection with SARS-CoV-2. Red lines reflect median values. *P* values reflect two-sided Mann–Whitney *U*-tests.



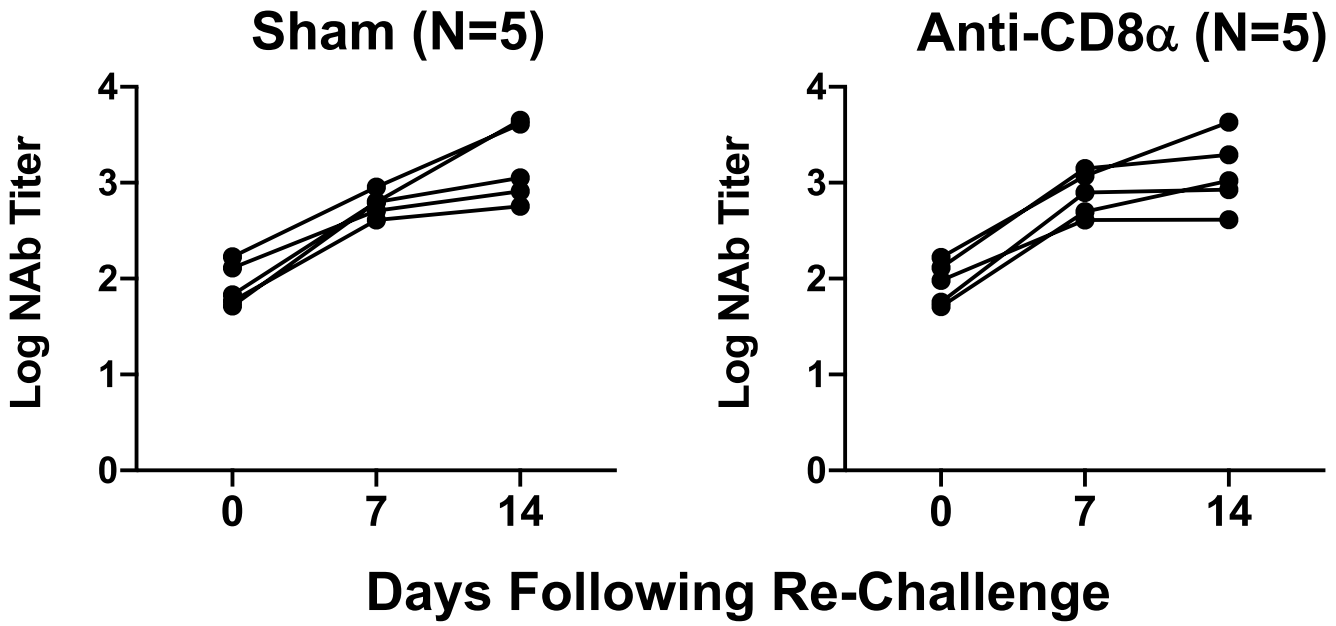
Extended Data Fig. 6 | CD8$^+$ cells after CD8 depletion and rechallenge with SARS-CoV-2. Rhesus macaques were infected with SARS-CoV-2 and received 50 mg kg^{-1} anti-CD8α monoclonal antibody, anti-CD8β monoclonal antibody or sham monoclonal antibody at week 7 (reflecting day −3 relative to rechallenge). On day 0, all macaques were rechallenged with 10^5 $TCID_{50}$ SARS-CoV-2. Total CD8$^+$ cells, CD8$^+$CD3$^+$ cells and CD8$^+$CD3$^-$ cells per µl peripheral blood are shown. Days following challenge are shown on the *x* axis. Red lines reflect median values.



Extended Data Fig. 7 | CD8⁺ T cell responses after CD8 depletion and rechallenge with SARS-CoV-2. a, IFNγ S-specific CD8⁺ T cell responses assessed by intracellular cytokine staining in nondepleted (sham) and CD8-depleted (anti-CD8α) rhesus macaques before and after rechallenge with SARS-CoV-2. Days following rechallenge are shown on the *x* axis. Red lines reflect median values. *P* values reflect two-sided Mann–Whitney *U*-tests. **b**, IFNγ, TNF and IL-2 S-specific CD8⁺ T cell responses in nondepleted (sham) macaques before rechallenge ($n = 5$ independent macaques). Red lines reflect median responses.



Extended Data Fig. 8 | CD4⁺ T cell responses after CD8 depletion and rechallenge with SARS-CoV-2. IFN-γ S-specific CD4⁺ T cell responses assessed by intracellular cytokine staining in nondepleted (Sham) and CD8-depleted (anti-CD8α) rhesus macaques before and after rechallenge with SARS-CoV-2. Days following rechallenge are shown on the *x* axis. Red lines reflect median values.



Extended Data Fig. 9 | NAb responses after CD8 depletion and rechallenge with SARS-CoV-2. log_{10}-transformed pseudovirus NAb titres in nondepleted (sham) and CD8-depleted (anti-CD8α) rhesus macaques before and after rechallenge with SARS-CoV-2. Days following rechallenge are shown on the *x* axis.

Extended Data Table 1 | Characteristics of rhesus macaques

Animal ID	Sex	Age (years)	Weight (kg)
Group I: 250 mg/kg			
18C279	Female	2	3.29
18C300	Female	2	2.5
18C289	Male	2	2.31
Group II: 25 mg/kg			
18C221	Female	2	2.96
18C200	Male	2	2.83
18C277	Male	2	2.92
Group III: 2.5 mg/kg			
18C297	Female	2	2.29
18C278	Male	2	2.76
18C284	Male	2	2.83
Group IV: Sham			
18C290	Female	2	3.28
18C139	Male	2	2.85
18C287	Male	2	2.91

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Last updated by author(s): Nov 7, 2020

Reporting Summary

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

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	QuantStudio 6 was used to collect sgRNA data.
Data analysis	Analysis of virologic and immunologic data was performed using R 3.6.1 and GraphPad Prism 8.4.2 (GraphPad Software).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

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Policy information about availability of data

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

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

All data are available in the manuscript or the supplementary material.

Field-specific reporting

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

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

Life sciences study design

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

Sample size	Sample size includes N=31 vaccinated animals (N=2-3 animals for each adoptive transfer group; N=3-5 animals for each CD8 depletion group; Chandrashekar et al Science 2020). Based on our experience with SARS-CoV-2 in rhesus macaques, this sample size can differentiate large differences in protective efficacy compared with the sham controls.
Data exclusions	No data were excluded.
Replication	Virologic and immunologic measures were performed in duplicate. Technical replicates were minimally different. All attempts at replication were successful.
Randomization	Animals were balanced for age and gender and otherwise randomly allocated to groups for the experiments in Figs. 1, 3, 4.
Blinding	All immunologic and virologic assays were performed blinded.

Reporting for specific materials, systems and methods

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

Materials & experimental systems

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

Methods

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

Antibodies

Antibodies used	For ELISA and ELISPOT assays anti-macaque IgG HRP (NIH NHP Reagent Program), rabbit polyclonal anti-human IFN-γ (U-Cytech); for ICS assays mAbs against CD279 (clone EH12.1, BB700), CD38 (clone OKT10, PE), CD28 (clone 28.2, PE CY5), CD4 (clone L200, BV510), CD45 (clone D058-1283, BUV615), CD95 (clone DX2, BUV737), CD8 (clone SK1, BUV805), Ki67 (clone B56, FITC), CD69 (clone TP1.55.3, ECD), IL10 (clone JES3-9D7, PE CY7), IL13 (clone JES10-5A2, BV421), TNF-α (clone Mab11, BV650), IL4 (clone MP4-25D2, BV711), IFN-γ (clone B27; BUV395), IL2 (clone MQ1-17H12, APC), CD3 (clone SP34.2, Alexa 700) (BD); for 800CW-conjugated goat-anti-human secondary antibody (Li-COR); anti-rhesus IgG1, IgG2, IgG3, IgA, IgM (NIH NHP Reagent Program); tertiary goat anti-mouse IgG-PE antibody (Southern Biotech), anti-CD107a (PE-Cy7, BD), anti-CD56 (PE-Cy7, BD), anti-MIP-1β (PE, BD), mouse anti-human IFN-γ monoclonal antibody (BD), Streptavidin-alkaline phosphatase antibody (Southern Biotech).
Validation	all mAbs used according to manufacturer's instructions and previously published methods; mAbs were validated and titrated for specificity prior to use

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HEK293T, THP-1 cells
Authentication	Commerically purchased (ATCC) and evaluated in control experiments prior to use
Mycoplasma contamination	Negative for mycoplasma

Commonly misidentified lines (See ICLAC register)	None were utilized

Animals and other organisms

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

Laboratory animals	31 outbred Indian-origin adult male and female rhesus macaques (Macaca mulatta), 2-5 years old
Wild animals	None
Field-collected samples	None
Ethics oversight	Bioqual IACUC

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