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Cross-protective immunity following coronavirus vaccination and coronavirus infection

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Abstract

Although Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) vaccines have shown efficacy against SARS-CoV-2, it is unknown if coronavirus vaccines can also protect against other coronaviruses that may infect humans in the future. Here, we show that coronavirus vaccines elicit cross-protective immune responses against heterologous coronaviruses. In particular, we show that a Severe Acute Respiratory Syndrome Coronavirus 1 (SARS-CoV-1) vaccine developed in 2004 and known to protect against SARS-CoV-1, confers robust heterologous protection against SARS-CoV-2 in mice. Similarly, prior coronavirus infections conferred heterologous protection against distinct coronaviruses. Cross-reactive immunity was also reported in Coronavirus Disease 2019 (COVID-19) patients and humans who received SARS-CoV-2 vaccines, and transfer of plasma from these individuals into mice improved protection against coronavirus challenges. These findings provide the first demonstration that coronavirus vaccines (and prior coronavirus infections) can confer broad protection against heterologous coronaviruses, providing a rationale for universal coronavirus vaccines.

Introduction

Coronaviruses have garnered attention for their potential to cause pandemics. In less than 20 years, there have been outbreaks from three coronaviruses: Severe Acute Respiratory Syndrome 1 Coronavirus (SARS-CoV-1), Middle Eastern Respiratory Syndrome Coronavirus (MERS), and recently, Severe Acute Respiratory Syndrome 2 Coronavirus (SARS-CoV-2). Various vaccines have shown efficacy at preventing Coronavirus Disease 2019 (COVID-19), but whether these vaccines protect against other coronaviruses remains unknown. It is also unclear whether prior coronavirus infections confer protection against other coronaviruses. Knowing whether coronavirus vaccines confer broad protection against different coronaviruses is crucial for vaccine development, because it would suggest that coronavirus vaccines can protect even if they are not completely matched to a specific coronavirus antigen. Moreover, knowing whether prior coronavirus infections confer cross-protection against other coronaviruses could help explain differences in COVID-19 susceptibility among humans.

In this study, we evaluated cross-reactive and cross-protective immunity elicited by coronavirus vaccines and coronavirus infections. Our studies show that coronavirus vaccines and coronavirus infections confer protection against heterologous coronaviruses.

Results

SARS-CoV-2 vaccines induce cross-reactive antibody responses against other coronaviruses in humans

We first measured antibody responses following vaccination of humans with SARS-CoV-2 vaccines (Pfizer/BioNTek, Moderna and J&J). Plasma samples from human volunteers were obtained before vaccination, and at several time points after vaccination. These vaccinated individuals were identified as being: unexposed to SARS-CoV-2 (asymptomatic and serology negative); unexposed to SARS-CoV-2 on immunosuppressive drugs (asymptomatic and serology negative); or previously exposed to SARS-CoV-2 (symptomatic and being PCR+, prior to vaccination). Consistent with the PCR results, we observed nucleocapsid-specific antibodies in most exposed individuals, but not in unexposed individuals (Supplemental Figure 1A-1D). As expected, vaccination of humans with SARS-CoV-2 vaccines resulted in an increase in SARS-CoV-2 spike-specific antibodies (Figure 1A-1D). Consistent with prior reports(1, 2), the vaccine prime induced a more substantial increase in SARS-CoV-2-specific antibodies in people who were previously exposed to SARS-CoV-2 (Figure 1C-1D). Importantly, the SARS-CoV-2 vaccines also induced an increase in SARS-CoV-1 spike-specific antibodies, and previously exposed individuals showed more pronounced antibody responses, relative to unexposed individuals (Figure 1E-1H).

We then quantified antibody responses against the spike protein of OC43, which is an endemic coronavirus that causes common colds in humans. All patients had high levels of pre-existing antibody titers against OC43, but SARS-CoV-2 vaccination increased antibody titers against this endemic coronavirus in most unexposed (including immunosuppressed) participants, 22/29 (76%) (Figure 1I-1L), consistent with prior studies(3). Prior to vaccination, antibody responses to OC43 tended to be higher in people who were previously exposed to SARS-CoV-2 (Figure 1L).

We also evaluated bystander antibody levels before and after vaccination to examine whether SARS-CoV-2 vaccination increased non-coronavirus-specific immune responses. Antibodies against the Influenza virus HA protein were not increased following SARS-CoV-2 vaccination, demonstrating that the increase of antibodies post-vaccination was specific to coronaviruses (Supplemental Figure 1E-1H). Taken together, these data show that SARS-CoV-2 vaccination elicits cross-reactive antibody against other coronaviruses, besides SARS-CoV-2.

COVID-19 patients show cross-reactive antibody responses against other coronaviruses

We then interrogated if cross-reactive antibodies could also be observed during a natural SARS-CoV-2 infection. We compared antibody responses in plasma from PCR+, symptomatic COVID-19 individuals ranging from mild to severe COVID-19,

as well as healthy control plasma harvested before 2019. As expected(4), COVID-19 individuals showed higher levels of SARS-CoV-2 spike-specific antibodies (Figure 2A), as well as SARS-CoV-1 spike-specific (Figure 2B) and OC43-specific (Figure 2C) antibodies, relative to control individuals. Antibody levels against the SARS-CoV-2 nucleocapsid protein were also measured for these two groups, and shown to be significantly higher in COVID-19 patients (Figure 2D). We did not observe any increase in Influenza-specific antibodies in the COVID-19 cohort (Figure 2E). These data demonstrate that COVID-19 patients develop cross-reactive antibody responses that recognize other coronaviruses.

Characterization of cross-reactive antibody responses with multiple SARS-CoV-2 vaccine modalities

Our experiments above showed that SARS-CoV-2 vaccines induce antibody responses against heterologous coronaviruses in humans. Most of the vaccinated volunteers received mRNA vaccines, and we then interrogated whether this effect was generalizable to other vaccine platforms. We primed C57BL/6 mice intramuscularly with various SARS-CoV-2 vaccines similar to the approved vaccines or experimental vaccines that have been used around the world during the COVID-19 pandemic, including adenovirus-based, vesicular stomatitis virus (VSV) based, mRNA-based, RBD protein-based, spike protein-based, and inactivated virus-based vaccines. We boosted mice homologously at

approximately 3 weeks to recapitulate the regimen in most human trials, and we evaluated antibody responses at 2 weeks post-boost.

Consistent with our data in humans, vaccination of mice with an adenovirus vector expressing SARS-CoV-2 spike (Ad5-SARS-CoV-2 spike) resulted in potent antibody responses against SARS-CoV-2 and SARS-CoV-1, and a more modest but statistically significant increase in antibody responses against more distant coronaviruses, including OC43 and mouse hepatitis virus (MHV-1) (Figure 3A). Cross-reactive antibody responses were also elicited by VSV-based, mRNA-based, RBD protein-based, spike protein-based, and inactivated virus-based vaccines (Figure 3B-3F). We also performed control experiments to measure cross-reactive antibody levels in mice that received “sham vaccines” lacking coronavirus spike transgenes. Vaccination with sham vectors did not elicit SARS-CoV-1 and CoV-2 specific antibodies (Supplemental Figure 2). Altogether, these data showed that multiple SARS-CoV-2 vaccine platforms are able to elicit cross-reactive antibody responses that recognize other coronaviruses.

We then interrogated whether a vaccine against a different SARS coronavirus spike protein could also induce cross-reactive antibodies. Similarly, cross-reactive antibodies were observed with an experimental SARS-CoV-1 spike vaccine developed in 2004, based on modified vaccinia Ankara (MVA-SARS-1 spike), which was previously shown to protect mice and macaques against a SARS-CoV-1 challenge(5, 6) (Figure 4A). Interestingly, sera from MVA-SARS-CoV-1-

vaccinated mice partially neutralized SARS-CoV-2 pseudovirus in vitro (Figure 4B-4D). These data show that immunization with a SARS-CoV-1 vaccine also elicits cross-reactive neutralizing antibodies against SARS-CoV-2 and other coronaviruses.

Following a viral infection, viral control is facilitated by CD8 T cells. To measure cross-reactive CD8 T cell responses, we harvested splenocytes from mice that received the SARS-CoV-1 vaccine, and stimulated these cells with SARS-CoV-2 spike peptides (Table S1) for 5 hr, followed by intracellular cytokine stain (ICS) to detect cross-reactive (SARS-CoV-2 spike-specific) CD8 T cells. Interestingly, the SARS-CoV-1 vaccine elicited SARS-CoV-2 specific CD8 T cell responses (Figure 4E), suggesting the presence of conserved CD8 T cell epitopes in SARS-CoV-1 and SARS-CoV-2. To identify cross-reactive CD8 T cell epitopes, we performed spike sequence alignment (Supplemental Figure 3) followed by epitope mapping. We identified two highly conserved epitopes in the spike protein, in particular the VVLSFELL and VNFNFNGL epitopes, which are highly conserved among other SARS-like coronaviruses (Supplemental Figure 4A). These two epitopes were identified in a prior study in SARS-CoV-2 infected mice(7). The VNFNFNGL CD8 T cell response has also been reported to be elicited after SARS-CoV-1 infection in C57BL/6 mice(8), and we show that it is also immunodominant after SARS-CoV-2 vaccination (Supplemental Figure 4A). Both VVLSFELL and VNFNFNGL were predicted to bind the mouse MHC-I K^b by utilizing MHC-I epitope prediction algorithms (see Methods).

We reasoned that K^b VNFNFNGL tetramers could be used to track cross-reactive CD8 T cells following SARS-CoV-1 or SARS-CoV-2 vaccination across multiple vaccine platforms. The spike protein vaccine and the inactivated virus vaccine did not generate robust K^b VNFNFNGL (K^b VL8) CD8 T cell responses (Figure 4F), likely because CD8 T cell priming is dependent on intracellular protein expression. However, robust K^b VL8 CD8 T cell responses above the limit of detection were observed after vaccination with viral vectors or mRNA (Figure 4F). Among the different vaccines, adenovirus-based, MVA-based, and mRNA-based vaccines generated the greatest K^b VL8 CD8 T cell response (Figure 4F-4G).

We then performed single cell TCR-seq analyses to interrogate whether the cross-reactive K^b VL8 response exhibited a biased TCR usage. We show at the RNA and protein level that that most of the K^b VL8 response contained a TCR composed of Vα7/Vβ11 (Supplemental Figure 4B-4E). We are currently using this single cell TCR sequencing information to develop a TCR transgenic mouse that could be used to study cross-reactive CD8 T cells among different sarbecovirus infections. Altogether, our data showed that a SARS-CoV-1 vaccine also generates antibody and T cell responses that recognize other coronaviruses. In particular, these data suggested that an old SARS-CoV-1 vaccine could protect against SARS-CoV-2.

A SARS-CoV-1 vaccine protects against a SARS-CoV-2 challenge

There are concerns about emerging SARS-CoV-2 variants and the possibility that they could escape vaccine-elicited protection(9). Furthermore, it is possible that SARS-CoV-1 may spill over again into the human population. Thus, a critical question is whether SARS-CoV-2 vaccines could also protect against SARS-CoV-1, as well as other bat coronaviruses. To answer this simple question, we performed challenge experiments to evaluate whether coronavirus vaccines could protect against heterologous coronaviruses. SARS-CoV-1 is a select agent, so we were not able to challenge SARS-CoV-2 vaccinated animals with SARS-CoV-1 in our BL3 facilities. Instead, we evaluated whether an old SARS-CoV-1 vaccine developed in 2004 could protect against a SARS-CoV-2 challenge. We immunized mice with a SARS-CoV-1 spike vaccine developed by Dr. B. Moss (MVA-SARS-1 spike)(5), and then challenged mice intranasally with SARS-CoV-2. At day 5 post-challenge, we harvested lungs and measured viral loads by PCR. Strikingly, this SARS-CoV-1 vaccine conferred a 282-fold decrease in viral loads following a SARS-CoV-2 challenge (Figure 5A). Improved control of SARS-CoV-2 was also observed at an earlier timepoint (day 3) (Figure 5A). These data demonstrate that a sarbecovirus vaccine with a large antigenic mismatch (only 76% identity) can still confer robust protection following a heterologous sarbecovirus challenge.

Humans are constantly exposed to endemic coronaviruses, including the embecovirus OC43, and our next question was whether SARS-CoV-2 vaccines protected against this endemic coronavirus. To answer this question, we immunized mice with an Ad5 vector expressing either SARS-CoV-2 spike or

nucleocapsid, and then challenged these mice intranasally with OC43. At day 5 post-challenge, we harvested lungs and measured viral loads by PCR. The SARS-CoV-2 nucleocapsid vaccine conferred a 3.7-fold viral load decrease relative to control, following this common cold coronavirus challenge (Figure 5B). No significant heterologous protection was observed with the spike-based vaccine (Figure 5B). These data suggest that the degree of cross-protection is affected by the genetic similarity between the vaccine antigen and the challenge antigen. In other words, a sarbecovirus vaccine conferred robust protection against a related sarbecovirus challenge (Figure 5A); but only slight (or negligible) protection against an embecovirus challenge (Figure 5B).

Prior coronavirus infections confer protection against future coronavirus infections

Similar to our data with COVID-19 patients, coronavirus infections in mice also induced cross-reactive antibody responses. In particular, a common cold coronavirus (OC43) infection elicited cross-reactive antibodies against SARS-CoV-2, SARS-CoV-1 and MHV-1 (Supplemental Figure 5A). Cross-reactive antibody was also generated after an MHV-1 infection (Supplemental Figure 5B). We thus interrogated whether mice that had prior coronavirus infections were better protected following heterologous coronavirus challenges. In our first model, we challenged OC43-immune mice with MHV-1. Note that OC43 and MHV-1 are two embecoviruses that are more genetically distant, relative to SARS-CoV-1 and

SARS-CoV-2. Interestingly, OC43-immune mice exhibited a 408-fold viral load reduction following a heterologous MHV-1 challenge (Figure 5C).

In our second model, we challenged MHV-1-immune mice with MHV-A59. Although these 2 coronaviruses have similar names, they are genetically distinct. Note that MHV-1 and MHV-A59 are more genetically distant than SARS-CoV-2 and RaTG13. Interestingly, MHV-1-immune mice exhibited sterilizing immune protection against a heterologous MHV-A59 challenge (Figure 5D). These data demonstrate that prior coronavirus infections can confer protection against subsequent infections with related coronaviruses. Moreover, the degree of heterologous protection appeared to be influenced by the genetic similarity between the initial coronavirus infection and the subsequent coronavirus infection (5C-5D).

Mechanism: Antibodies are sufficient for cross-protection

Measuring cross-protection in humans is difficult because most people are already seropositive for endemic coronaviruses. In addition, susceptibility to coronavirus infection can be influenced by many variables, including the immune histories of the host, comorbidities, age, and socio-economic status, rendering it difficult to determine whether SARS-CoV-2 vaccination or SARS-CoV-2 infection protect against other coronaviruses. Therefore, we developed a reductionist animal model that allowed us to better discern heterologous immune protection by vaccine-

elicited antibodies, using plasma from humans who received SARS-CoV-2 vaccines. In our first experiment, we obtained longitudinal plasma from human volunteers, before and after receiving SARS-CoV-2 vaccines. We then transferred these donor-matched human plasma into naïve C57BL/6 mice via intraperitoneal injection, and on the following day, we challenged these mice with common cold coronavirus OC43. Mice that received post-vaccine human plasma exhibited a 12-fold lower OC43 viral loads, relative to mice that received pre-vaccine human plasma (Figure 5E). To explore the mechanism of immune protection, we performed plaque reduction neutralization titer assays (PRNT) using these donor-matched plasma (pre and post vaccination). Human plasma harvested 2-3 weeks after SARS-CoV-2 vaccination showed more robust in vitro OC43 neutralization, relative to matched pre-vaccination plasma (Figure 5F). These data show that SARS-CoV-2 vaccination in humans elicits humoral responses that confer protection against a different coronavirus.

In our second experiment, we obtained plasma from COVID-19 patients versus individuals before the 2019 pandemic. We transferred these human plasma into naïve C57BL/6 mice, and on the following day, we challenged these mice with OC43. Plasma from COVID-19 patients induced sterilizing immunity to OC43 in 80% of mice, whereas all of the mice that received pre-2019 human plasma showed detectable viral loads (Figure 5G). Human plasma from COVID-19 patients also showed more robust in vitro OC43 neutralization by PRNT, relative to pre-2019 plasma (Figure 5H). These data showed that antibody responses

elicited by SARS-CoV-2 infection in humans confer protection against an endemic coronavirus. Altogether, these data demonstrate that immunity elicited by SARS-CoV-2 vaccination or SARS-CoV-2 infection can cross-protect against common cold coronavirus infections.

Discussion

Several SARS-CoV-2 vaccines have been deployed for human use, but it is unknown if these vaccines could also protect against other viruses, including pandemic or endemic coronaviruses. In this study, we show that SARS-CoV-2 vaccination in humans elicits cross-reactive antibodies against SARS-CoV-1 and common cold coronavirus OC43. Our subsequent studies in mice demonstrate that a SARS-CoV-1 vaccine protects against a SARS-CoV-2 challenge, and that prior coronavirus infections can protect against subsequent infections with other coronaviruses.

Coronavirus vaccines have been previously shown to elicit cross-reactive antibodies(10-12), but until now, it has been unclear if these antibodies cross-protect in vivo. Our data bring clarity to the question of cross-protection, and suggest that cross-protection is proportional to the level of genetic conservation. For example, vaccination with a SARS-CoV-1 spike vaccine confers robust protection against a SARS-CoV-2 challenge (which is 76% antigen-matched), but cross-protection is more limited when the challenge virus is more distant (Figure 5A-5B). Similarly, a positive correlation between genetic similarity and cross-protection was observed in the context of coronavirus infections. For example, OC43-immune mice showed partial protection against an MHV-1 challenge (73% antigenically matched). However, MHV-1-immune mice showed sterilizing protection against an MHV-A59 challenge (94% antigenically matched).

Furthermore, our challenge data in Figure 5A-5D suggest that it is more likely for a vaccine to confer cross-protection within (but not across) subgenera.

Immune cross-reactivity in the context of coronavirus vaccination or coronavirus infection is based on genetic conservation. Interestingly, vaccination with “whole” spike protein induced higher levels of cross-reactive antibody, relative to vaccination with RBD protein (Figure 3D-3E). This is likely due to the higher number of conserved epitopes in the “whole” spike protein, relative to RBD only. Our data also suggest that the level of OC43/MHV cross-reactivity may vary between viral vector platforms, as replicating viral vectors (Figures 3B and 4A) tended to generate higher levels of these cross-reactive responses, compared to non-replicating Ad5 vectors (Figure 3A).

Additionally, our human plasma transfer experiments suggest that SARS-CoV-2 spike-based vaccination confers partial protection against OC43 (Figure 5E-5F). However, SARS-CoV-2 spike-based vaccination in mice did not confer significant protection against OC43 (Figure 5B). This difference may be explained by the high levels of OC43-specific antibody in human plasma. It is possible that pre-existing humoral immunity to OC43 improves the maturation of OC43-specific antibody in humans, upon SARS-CoV-2 vaccination.

Prior studies have suggested that recent endemic coronavirus infections in humans are associated with less severe COVID-19(13). However, other studies

have shown contradicting results(10). Such discrepancy can be explained by the heterogeneous “immune histories” of humans, including the fact that humans are frequently re-exposed with endemic coronaviruses. In addition, age, gender, and pre-existing conditions in humans can significantly influence COVID-19 susceptibility, making it difficult to quantify the antiviral effect of cross-reactive immune responses elicited by prior coronavirus infections. Our plasma transfer studies bring clarity to this issue of heterologous protection, as we transferred donor-matched plasma from humans (before and after vaccination) into naïve, sex-matched, genetically-identical recipient mice without any pre-existing immunity to any coronavirus (Figure 5E-5F).

A limitation of our study is that we only evaluated heterologous immune protection at an early time post-vaccination or post-infection, and it is possible that cross-protection declines over time. Future studies will determine the durability of cross-protection, and whether cross-reactive antibodies are produced by plasma cells or short-lived plasmablasts. There is a critical point that is worth discussing further, as it could facilitate vaccine preparedness for future pandemics. We show that a single coronavirus vaccine based on the original SARS-CoV-1 can confer robust heterologous protection against SARS-CoV-2, demonstrating that the vaccine antigen does not need to fully match the viral challenge. There are ongoing discussions about how to prepare better for future coronavirus pandemics, and our data suggest that it would be reasonable to archive a stockpile of vaccine candidates, based on known sequenced coronaviruses. Upon the start of an

outbreak, the most “antigenically-matched” vaccine could be immediately tested from the pre-existing catalog, saving time in vaccine manufacturing time. Even if vaccine protection is only partial with an antigenically-mismatched vaccine, this approach may slow down viral transmission and mitigate clinical outcomes, until more antigenically-matched vaccines are developed. Overall, these findings provide a framework for the rational design of pancoronavirus vaccines and may help vaccine preparedness for future pandemics.

Methods

Human Subjects

Inclusion criteria consisted of being 18 years of age or greater, a SARS-CoV-2 infection and/or a scheduled vaccination for COVID-19, and ability and willingness to provide informed consent. Exclusion criteria consisted of less than 18 years of age or unwillingness or inability to provide informed consent. Enrollment started on August 2020 and is expected to be completed by August 2022. Subject population consisted of residents across the Chicago area. Adults of different ages, races, and ethnicities were included in the study. Subjects were de-identified by assigning a 4-letter study code, which will be used for the duration of the study. Participants considered as exposed before vaccination had a positive PCR test for SARS-CoV-2 any time prior to vaccination. Blood was collected by phlebotomy using BD Vacutainer 10 mL tubes containing sodium heparin. Anticoagulated blood was added to LeucoSep tubes (Greiner Bio) and plasma was separated by density gradient centrifugation. To protect subject's identity, all samples were labeled with their assigned 4-letter study code and stored in the principal investigator's laboratory freezers.

Mice, vaccinations, infections, and challenges

6-8-week-old C57BL/6, BALB/c, A/J mice were used. For VSV-SARS-2 spike vaccinations, k18-hACE2 (on C57BL/6 background) mice were used. All mice were purchased from Jackson laboratories (approximately half males and half females)

and housed at the Northwestern University Center for Comparative Medicine (CCM) or the University of Illinois at Chicago (UIC). Mice were immunized intramuscularly (50 µl per quadriceps) with: adenovirus serotype 5 expressing SARS-CoV-2-spike protein (Ad5-SARS-CoV-2 spike; 10^9 PFU), vesicular stomatitis virus expressing SARS-CoV-2-spike protein (VSV-SARS-CoV-2-spike; 10^7 PFU), mRNA-based vaccine encoding SARS-CoV-2-spike protein (mRNA-SARS-CoV-2 spike; 5 µg), SARS-CoV-2 "whole spike" protein (SARS-CoV-2 spike; 100 µg with 1:5 Adju-Phos), SARS-CoV-2 RBD protein (SARS-CoV-2 RBD protein; 100 µg with 1:5 Adju-Phos), gamma-irradiated SARS-CoV-2 (inactivated SARS-CoV-2; $2.5x10^5$ PFU), and modified vaccinia Ankara expressing SARS-CoV-1 spike protein (MVA-SARS-CoV-1 spike; 10^7 PFU). The vaccine doses were chosen empirically based on prior studies by us and others(5, 14-22).

We obtained Ad5-SARS-CoV-2 spike from the University of Iowa viral vector core (VVC-U-7643); VSV-SARS-CoV-2 spike from Dr. Sean Whelan (Washington University in St. Louis, MO); and MVA-SARS-CoV-1 spike from the NIH Biodefense and Emerging Infections Research Resources Repository, NIAID, (NR-623, originally developed by Dr. Bernard Moss at the NIH in Bethesda, MD (5). We obtained Ad5-SARS-CoV-2 nucleocapsid from Dr. David Masopust (University of Minnesota, Minneapolis, MN); this vector has been used in prior publications(15, 23).

We synthesized mRNA vaccines encoding for the codon-optimized SARS-CoV-2 Spike protein from strain USA-WA1/2020. Constructs were purchased from Integrated DNA Technologies (IDT) and contained a T7 promoter site for in vitro transcription of mRNA, 5' UTR and 3' UTRs. The sequence of the 5' and 3' UTRs were identical to previous publications with a Dengue virus mRNA vaccine(19). mRNA was synthesized from linearized DNA with T7 in vitro transcription kits from CellScript and following manufacturer's protocol. RNA was generated with pseudouridine in place of uridine with the Incognito mRNA synthesis kit (Cat# C-ICTY110510). 5' cap-1 structure and 3' poly-A tail were enzymatically added. mRNA was encapsulated into lipid nanoparticles using the PNI Nanosystems NanoAssemblr Benchtop system. mRNA was dissolved in PNI Formulation Buffer (Cat# NWW0043) and was run through a laminar flow cartridge with GenVoy ILM (Cat# NWW0041) encapsulation lipids at a flow ratio of 3:1 (RNA in PNI Buffer : Genvoy ILM) at total flow rate of 12 mL/min to produce mRNA-LNPs. These mRNA-LNPs were characterized for encapsulation efficiency and mRNA concentration via RiboGreen Assay using Invitrogen's Quant-iT Ribogreen RNA Assay Kit (Cat# R11490).

SARS-CoV-2 spike and RBD proteins used for vaccinations were produced at the Northwestern University Recombinant Protein Production Core by Dr. Sergii Pshenychnyi using plasmids that were produced under HHSN272201400008C and obtained through BEI Resources, NIAID, NIH: Vector pCAGGS containing the SARS-related coronavirus 2, Wuhan-Hu-1 spike glycoprotein gene (soluble,

stabilized), NR-52394 and receptor binding domain (RBD), NR-52309. Protein vaccines were administered with 1:10 AdjuPhos (Invivogen).

Inactivated SARS-CoV-2 was obtained from BEI resources, NIAID (SARS-related Coronavirus 2, isolate USA-WA1/2020, gamma-irradiated, NR-52287). MHV-1 was purchased from ATCC (VR-261) and OC43 was received from BEI (NR-52725). MHV-A59 was a kind gift from Dr. Susan Weiss (University of Pennsylvania, Philadelphia, PA).

OC43 and MHV challenges: Mice were infected IN (25 µl per nostril) with OC43 ($2x10^6$ PFU) or mouse hepatitis virus (MHV-1/MHV-A59; 10^6 PFU). All mouse experiments with BL2 agents were performed with approval from the Northwestern University Institutional Animal Care and Use Committee (IACUC).

SARS-CoV-2 challenges: Mouse adapted SARS-CoV-2 (MA10) was kindly provided by Dr. Ralph Baric (University of North Carolina, Chapel Hill, NC)(24). SARS-CoV-2 (MA10) was propagated and tittered on Vero-E6 cells (ATCC, CRL1586). BALB/c mice were anesthetized with isoflurane and challenged via intranasal inoculation with $8x10^3$ FFU of SARS-CoV-2 (MA10). Lungs were isolated from mice at 5 days post infection and homogenized in PBS. RNA was extracted from lung homogenate using a Zymo Research Quick-RNA 96 Kit (R1052). Viral genomes were quantified via qPCR with N1 primer/probe kit from IDT (Cat. # 10006713). SARS-CoV-2 infections were performed at the University

of Illinois at Chicago (UIC) following BL3 guidelines with approval by the UIC Institutional Animal Care and Use Committee.

Protein-specific ELISA (SARS-CoV-2 spike, RBD, nucleocapsid; SARS-CoV-1 spike; OC43 spike)

Antigen-specific total antibody titers were measured by ELISA as described previously(16, 25). Briefly, 96-well flat-bottom MaxiSorp plates (Thermo Scientific) were coated with 1 μg/ml of respective protein, for 48 hr at 4°C. Plates were washed three times with wash buffer (PBS + 0.05% Tween 20). Blocking was performed with blocking solution (200 μl of PBS + 0.05% Tween 20 + 2% bovine serum albumin), for 4 hr at room temperature. 6 μl of sera (plasma for human ELISAs) were added to 144 μl of blocking solution in the first column of the plate, 1:3 serial dilutions were performed until row 12 for each sample, and plates were incubated for 60 min at room temperature. Plates were washed three times with wash buffer followed by addition of secondary antibody conjugated to horseradish peroxidase, goat anti-mouse IgG (Southern Biotech) diluted in blocking solution (1:5000) at 100 μl/well were added and incubated for 60 min at room temperature. For the ELISAs with human plasma samples, goat anti-human IgG (H + L) conjugated with horseradish peroxidase (1:1000) (Jackson ImmunoResearch) was used. After washing plates three times with wash buffer, 100 μl/well of Sure Blue substrate (SeraCare) was added for 1 min. Reaction was stopped using 100 μl/well of KPL TMB Stop Solution (SeraCare). Absorbance was measured at 450 nm using a Spectramax Plus 384 (Molecular Devices). In all ELISA plots, the Y axis

indicate endpoint titer (the sera or plasma dilution at which absorbance was >2x average for negative controls (human pre-2019 plasma, or mouse naive sera). SARS-CoV-2 spike and RBD proteins used for ELISA were produced at the Northwestern Recombinant Protein Production Core by Dr. Sergii Pshenychnyi using plasmids that were produced under HHSN272201400008C and obtained from BEI Resources, NIAID, NIH: Vector pCAGGS containing the SARS-related coronavirus 2, Wuhan-Hu-1 spike glycoprotein gene (soluble, stabilized), NR-52394 and receptor binding domain (RBD), NR-52309. SARS-CoV-2 nucleocapsid protein was obtained through BEI Resources, NIAID, NIH (NR-53797). SARS-CoV-1 spike protein was obtained through BEI Resources, NIAID, NIH (NR-722). OC43-spike protein was purchased from Sino Biologicals (40607-V08B).

Virus-specific ELISA (OC43; MHV-1; MHV-A59)

Virus-specific ELISAs were performed as described earlier(16, 25). In brief, 96-well flat-bottom MaxiSorp plates (Thermo Scientific) were coated with 100 µl/well of the respective viral lysate (OC43, MHV-1 or MHV-A59 infected cell lysates) diluted 1:10 in PBS, for 48 hr at room temperature. Plates were washed three times with wash buffer (PBS + 0.5% Tween 20) followed by blocking with blocking solution (200 µl/well of PBS + 0.2% Tween 20 + 10% FCS) for 2 hr at room temperature. 5 µl of sera (plasma for human ELISAs) were added to 145 µl of blocking solution in the first column of the plate, 1:3 serial dilutions were performed until row 12 for each sample, followed by incubation at room temperature for 90 min. Plates were washed three times with wash buffer, followed by addition of 100

µl/well of a secondary antibody conjugated to horseradish peroxidase, goat anti-mouse IgG (Southern Biotech) diluted in blocking solution (1:5000). Plates were incubated for 90 min at room temperature. Goat anti-human IgG (H + L) conjugated with horseradish peroxidase (1:1000) (Jackson ImmunoResearch) was used when ELISA was performed with human samples. After washing plates three times with wash buffer, 100 µl/well of Sure Blue substrate (SeraCare) was added for 8 min. Reaction was stopped using 100 µl/well of KPL TMB Stop Solution (SeraCare). Absorbance was measured at 450 nm using a Spectramax Plus 384 (Molecular Devices).

Virus propagation

OC43 was propagated in an 80-90% confluent monolayer of HCT-8 cells (ATCC, CCL-244) in T175 flasks at a multiplicity of infection (MOI) of 0.01 diluted in 5 mL of RPMI supplemented with 2% FBS, 1% penicillin/streptomycin, and 1% L-glutamine. Infected cells were incubated at 33°C for 2 hr in a humidified 5% CO2 incubator. After incubation, flasks were supplemented with 20 ml of 2% RPMI and incubated for 5 days at 33°C in a CO_2 incubator. MHV-A59 and MHV-1 were expanded in 17CL-1 cells (kind gift from Dr. Susan Weiss, University of Pennsylvania, Philadelphia, PA) following a prior protocol(26).

OC43 and MHV quantification by plaque assay

For MHV quantification, 10^6 cells per well of L2 cells (kind gift from Dr. Susan Weiss, University of Pennsylvania, Philadelphia, PA) were seeded into 6-well

plates in 10% DMEM (10% FBS, 1% penicillin/streptomycin, and L-glutamine). After 2 days, when cells reached ~100% confluency, media were removed. 10-fold serial dilutions of viral stock or homogenized lung were prepared in 1% DMEM (1% FBS, 1% penicillin/streptomycin, and L-glutamine), added to wells, and incubated at 37°C for 1 hr, gently rocking plates every 10 minutes. After incubation, 3.5 mL of 1% agarose diluted 1:1 with 20% 2X-199 media (2X-199 media supplemented with 20% FBS, 1% penicillin/streptomycin, and L-glutamine) was overlaid onto the monolayer and the plates were incubated at 37°C 5% CO_2 for 2 days. On day 2, the agar overlay was removed gently and the monolayer was stained with 1% crystal violet for 15 minutes. After staining, the crystal violet was aspirated, plates were washed once with 2 ml water per well, and then dried to visualize plaques. Quantification of OC43 stocks for challenge studies was similar to the quantification of MHV-A59(26) except that 5 ml of agar overlay was added on an infected monolayer of L2 cells and incubated at 33°C in CO_2 incubator for 5-6 days. Monolayer was stained with 1% crystal violet and plaques quantified by manual counting. For viral load quantification in lung, tissue was collected in round-bottom 14-ml tubes (Falcon) containing 2 ml of 1% FBS DMEM. Tissues were ruptured using a Tissue Ruptor homogenizer (Qiagen). Homogenized tissues were clarified using a 100-µm strainer (Scientific Inc.) to remove debris, and clarified tissue lysates were used for plaque assay.

Quantification of OC43 by RT-PCR

Lungs were isolated from mice and homogenized in 1% FBS DMEM. RNA was extracted from lung homogenate using PureLink Viral RNA/DNA Mini kit (Invitrogen), according to the manufacturer's instructions. OC43 viral loads in lungs were determined using one-step quantitative real-time reverse transcriptase polymerase chain reaction (qRT-PCR). qRT-PCR was performed using OC43-nucleocapsid specific TaqMan primers and a probe 18 fluorescent-labelled with a 5'-FAM reporter dye and 3'-BHQ quencher (IDT) and AgPath-ID™ One-Step RT-PCR kit (AgPath AM1005, Applied Biosystems) on an ABI QuantStudio 3 platform (Thermo Fisher). Each sample was tested in duplicates in 25 µl reactions containing 12.5 µl of a 2X RT-PCR buffer, 1 µl of 25X RT-PCR enzyme mix provided with the AgPath kit, 0.5 µl (450 nM) forward primer, 0.5 µl (450 nM) reverse primer, 0.5 µl (100 nM) of probe, and 10 µl RNA. In parallel, each sample was also tested for beta-actin gene as an internal control to verify RNA extraction quality using mouse beta-actin-specific Taqman primers/probe labelled with 5'-FAM and 3'-BHQ (IDT). Thermal cycling involved reverse transcription at 45°C for 10 min, denaturation at 95°C for 15 min, followed by 45 cycles of amplification (15 sec at 95°C and 1 min at 60°C.) To avoid cross-contamination, single use aliquots were prepared for all reagents including primers, probes, buffers, and enzymes.

Quantification of SARS-CoV-2 by RT-PCR

Lungs were isolated from mice and homogenized in PBS. RNA was extracted from lung homogenate using a Zymo Research Quick-RNA 96 Kit (R1052). Viral genomes were quantified via RT-qPCR with the TaqMan RNA-to-Ct 1-step kit

(ThermoFisher, Cat # 4392653) and primer/probe sets with the following sequences: Forward 5' GAC CCC AAA ATC AGC GAA AT 3', Reverse 5' TCT GGT TAC TGC CAG TTG AAT CTG 3', Probe 5' ACC CCG CAT TAC GTT TGG TGG ACC 3' (Integrated DNA Technologies, Cat # 10006713). A SARS CoV-2 copy number control was obtained from BEI (NR-52358) and used to quantify SARS-CoV-2 genomes.

Reagents, flow cytometry, and equipment

Dead cells were gated out using Live/Dead fixable dead cell stain (Invitrogen). The SARS-CoV-2 spike overlapping peptide pools obtained from BEI resources, NIH (NR-52402) were used for intracellular cytokine staining. Biotinylated MHC class I monomers (K^b VNFNFNGL, abbreviated as K^b VL8) were obtained from the NIH tetramer facility at Emory University. Cells were stained with fluorescently-labelled antibodies against CD44 (IM7 on Pacific Blue, Biolegend cat. 103020), CD8α (53-6.7 on PerCP-Cy5.5, BD Pharmingen cat. 551162), IFNγ (XMG1.2 on APC, BD Pharmingen cat. 554413), and Vβ11 (RR3-15 on FITC, Biolegend cat. 125905). Fluorescently-labelled antibodies were purchased from BD Pharmingen, except for anti-CD44 (which was from Biolegend). Flow cytometry samples were acquired with a Becton Dickinson Canto II or an LSRII and analyzed using FlowJo (Treestar).

SARS-CoV-2 pseudovirus neutralization assays

A SARS-CoV-2 pseudovirus was generated by transfection of HEK-293T cells (ATCC, CRL-1573) with a pCAGGS vector expressing the SARS-CoV-2 spike glycoprotein (BEI resources, NIAID, NIH: NR-52310). 24 hr later, transfected cells were infected with VSVΔG*G-GFP at a multiplicity of infection (MOI) of 0.5. After 24 hr, GFP foci were visualized, and the supernatant was harvested and passed through a 0.45 μM filter. This SARS-CoV-2 pseudovirus was concentrated using an Amicon Ultra-15 filter (UFC910024, Sigma-Aldrich), and then stored at −80°C. Titers were measured by infecting HEK-293T-hACE2 cells (BEI NR-52511) and counting GFP foci under a fluorescence microscope after 24 hr.

The SARS-CoV-2 pseudovirus neutralization assay was performed by mixing serial dilutions of MVA-SARS-CoV-1 immune mice sera (or naïve sera) with 200 FFU of SARS-CoV-2 pseudovirus in a 96-well plate and incubated for 2 hr. After incubation, 100 μl of the sera-virus mixture was transferred to a half area 96-well plate containing HEK-293T-hACE2 cells. The next day, GFP foci were counted in each well under a fluorescent microscope.

MHC-I binding predictions

The MHC-I binding predictions were made on 5/17/2021 using the IEDB analysis resource NetMHCpan (ver. 4.1) tool(27), at http://tools.iedb.org/mhci/.

Single cell TCR-Seq (scTCR-Seq) Data Acquisition and Analysis

C57BL/6 mice were immunized intramuscularly with 10^9 PFU of Ad5-SARS-2 spike, and at day 28, splenic CD8 T cells were MACS-sorted using negative selection (STEMCELL). Purified CD8 T cells were stained with K^b VL8, Live/Dead stain, and flow cytometry antibodies for CD8 and CD44. Live, CD8+, CD44+, K^b VL8+ cells were FACS-sorted to ~99% purity on a FACS Aria cytometer (BD Biosciences) and delivered to the Northwestern University NU-Seq core for scTCR-seq using Chromium NextGem 5' v2 kit (10X Genomics). Once the library was sequenced, the output file in BCL format was converted to fastq files and aligned to mouse genome in order to generate a matrix file using the Cell Ranger pipeline. These upstream QC steps were performed by Drs. Ching Man Wai and Matthew Schipma at the Northwestern University NUSeq core. TCR analyses were performed using the scRepertoire package(28). Only cells expressing both TCRα and TCRβ chains were selected. For cells with more than 2 TCR chains, only the top 2 expressed chains were used. scTCR-Seq accession data uploaded at: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE173567

Adoptive plasma transfers

C57BL/6 mice received 50 µL of heat inactivated human plasma from different human donors (pre-vaccination, post-vaccination, pre-2019, or SARS-CoV-2 convalescent). Each mouse received plasma from 1 different human subject. On the next day, mice were infected intranasally with $5x10^7$ PFU of OC43. Lungs were harvested at day 5 post infection and ruptured using a Tissue Ruptor homogenizer (Qiagen). Viral loads were quantified by qRT-PCR as described above.

Statistics

Most statistical analyses used the Mann-Whitney test, unless specified otherwise in the figure legend. Dashed lines in ELISA/plaque assay figures represent the limit of detection (LOD). Data were analyzed using Prism (Graphpad).

Study Approval

Human specimens

All protocols used for subject recruitment, enrollment, blood collection, sample processing, and immunological assays with human samples were approved by the Northwestern University Institutional review board (STU00212583) (Chicago, IL). All participants voluntarily enrolled in the study by signing an informed consent form after receiving detailed information of the clinical study.

Mouse studies

All mouse experiments were performed with approval from the Northwestern University / University of Illinois in Chicago Institutional Animal Care and Use Committee (IACUC): Study approval numbers are IS00003258 and 20-107.

Author contributions

TD, NP, PPM designed and conducted all mouse BSL-2 experiments. SS helped with the MHV-1 challenge studies. MP expressed the nucleocapsid protein and helped to analyze viral sequences. TC performed the scTCR-seq analyses. LV

performed the human blood draws; LV and IK provided feedback on the human antibody studies. JR and JC made the mRNA vaccine, and performed the SARS-CoV-2 challenge studies. PPM, TD, and NP wrote the paper, with feedback from all other authors.

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Conflict of interest: PPM reports being Task Force Advisor to the Illinois Department of Public Health (IDPH) on SARS-CoV-2 vaccines in the state of Illinois.

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Figure 1

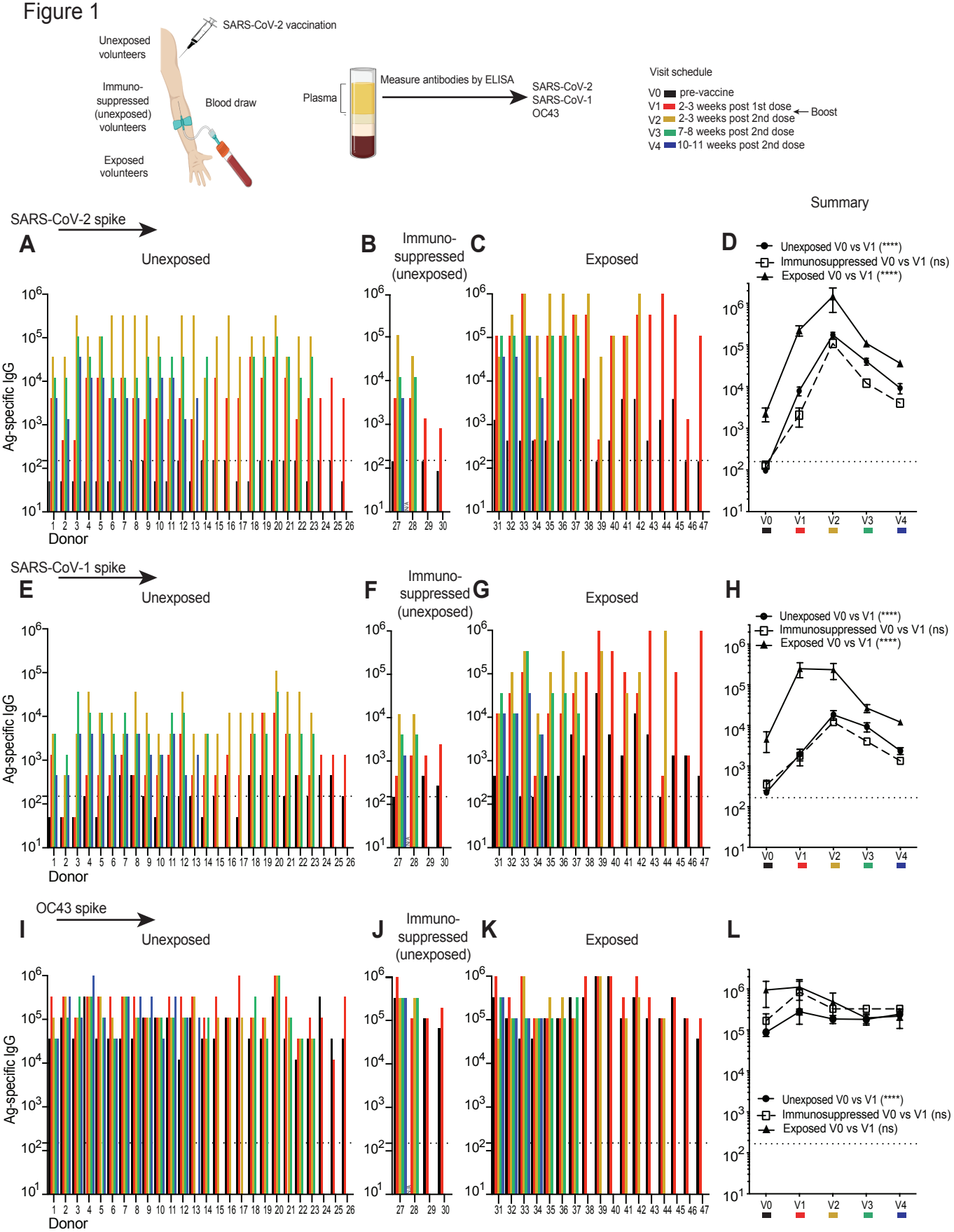
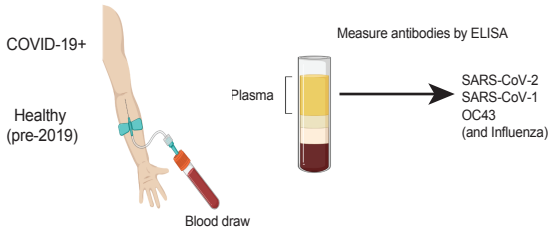

SARS-CoV-2 vaccination
Unexposed volunteers
Immuno-suppressed (unexposed) volunteers
Exposed volunteers
Blood draw
Plasma
Measure antibodies by ELISA
SARS-CoV-2
SARS-CoV-1
OC43
Visit schedule
V0 pre-vaccine
V1 2-3 weeks post 1st dose
V2 2-3 weeks post 2nd dose
V3 7-8 weeks post 2nd dose
V4 10-11 weeks post 2nd dose
Boost
Summary
SARS-CoV-2 spike
A
Unexposed
B
Immuno-suppressed (unexposed)
C
Exposed
D
Unexposed V0 vs V1 (****)
Immunosuppressed V0 vs V1 (ns)
Exposed V0 vs V1 (****)
Ag-specific IgG
Donor
SARS-CoV-1 spike
E
Unexposed
F
Immuno-suppressed (unexposed)
G
Exposed
H
Unexposed V0 vs V1 (****)
Immunosuppressed V0 vs V1 (ns)
Exposed V0 vs V1 (****)
OC43 spike
I
Unexposed
J
Immuno-suppressed (unexposed)
K
Exposed
L
Unexposed V0 vs V1 (****)
Immunosuppressed V0 vs V1 (ns)
Exposed V0 vs V1 (ns)

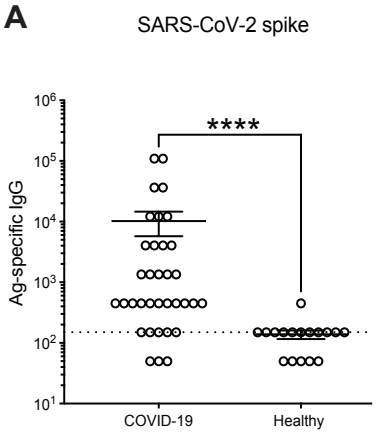
Figure Legends:

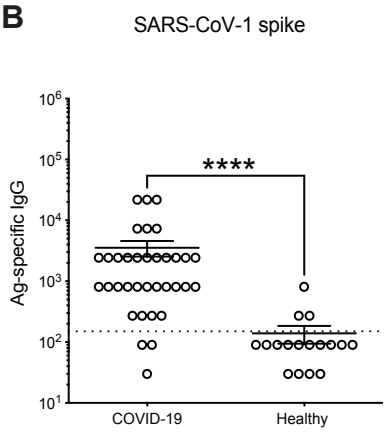
Figure 1. Cross-reactive antibody responses following SARS-CoV-2 vaccination. Antibody responses after SARS-CoV-2 vaccination. Participants received the Pfizer/BioNTek vaccine, except for 18-23 which received Moderna and 30 received the J & J vaccine. Participants were determined unexposed (1-26) prior to vaccination based on negative serology for SARS-CoV-2 spike and nucleocapsid protein before vaccination (0-7 days prior to vaccination). Participants 27-30 were unexposed, under immunosuppressive regimens, and did not interrupt treatments at the time of vaccination. Treatments: 27: Azathioprine and prednisone, 28: anti-IL-6 monoclonal antibody, 29: prednisone, and 30: methotrexate. Exposed participants, 31-47, tested positive for SARS-CoV-2 by PCR prior to vaccination. SARS-CoV-2 spike-specific antibody responses after vaccination in (**A**) unexposed, (**B**) unexposed immunosuppressed, and (**C**) exposed participants. (**D**) Summary of SARS-CoV-2 spike antibody responses. SARS-CoV-1 spike-specific antibody responses after vaccination in (**E**) unexposed, (**F**) unexposed immunosuppressed, and (**G**) exposed participants. (**H**) Summary of SARS-CoV-1 spike antibody responses. OC43 spike-specific antibody responses after vaccination in (**I**) unexposed, (**J**) unexposed immunosuppressed, and (**K**) exposed participants. (**L**) Summary of OC43 spike antibody responses. Y axis indicates endpoint titer (the highest plasma dilution at which absorbance was >2x than negative controls: human pre-2019 plasma, see Methods). Data shown are from an ongoing longitudinal study where participants were vaccinated on different dates, hence the heterogeneity in available timepoints

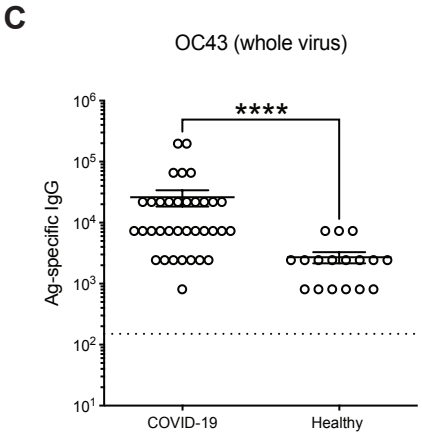
post vaccination. Antibody responses were evaluated by ELISA. Dashed lines represent limit of detection (LOD). In panels D, H and L, indicated P values compare V0 and V1 from each group by paired Wilcoxon test. ****, P <0.0001, ns=not significant (P > 0.05). All Participants but 28 (lack of V0 data) were included in the analysis. Error bars represent SEM.

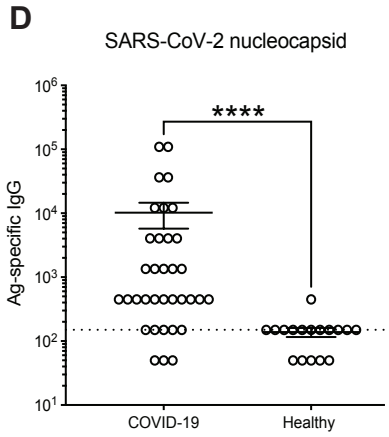
Figure 2


COVID-19+
Healthy
(pre-2019)
Blood draw
Measure antibodies by ELISA
Plasma
SARS-CoV-2
SARS-CoV-1
OC43
(and Influenza)


A
SARS-CoV-2 spike
Ag-specific IgG
****
COVID-19
Healthy


B
SARS-CoV-1 spike
Ag-specific IgG
****
COVID-19
Healthy


C
OC43 (whole virus)
Ag-specific IgG
****
COVID-19
Healthy


D
SARS-CoV-2 nucleocapsid
Ag-specific IgG
****
COVID-19
Healthy

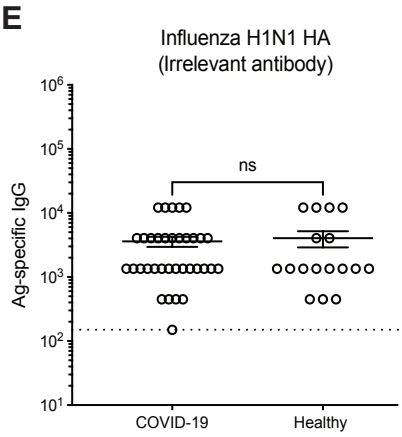

E
Influenza H1N1 HA
(Irrelevant antibody)
Ag-specific IgG
ns
COVID-19
Healthy

Figure 2. Cross-reactive antibody responses following SARS-CoV-2 infection in humans. Antibody responses after SARS-CoV-2 infection. Participants in the COVID-19 group had a positive PCR test accompanied by mild to severe symptoms. Serum samples (35 COVID-19 and 17 healthy controls) were collected once from week 3 to week 45 following symptom onset for the COVID-19 cohort. Healthy control cohort refers to human plasma collected prior to 2019. (**A**) SARS-CoV-2 spike-specific antibody responses. (**B**) SARS-CoV-1 spike-specific antibody responses. (**C**) OC43-specific antibody responses. OC43 infected cell lysate was used as coating antigen **(D)** SARS-CoV-2 nucleocapsid-specific antibody responses. **(E)** Influenza virus H1N1 HA-specific antibodies. Antibody responses were evaluated by ELISA. Dashed lines represent LOD. ****, P <0.0001, ns=not significant by non-parametric Mann Whitney U Test. Error bars represent SEM.

Figure 3

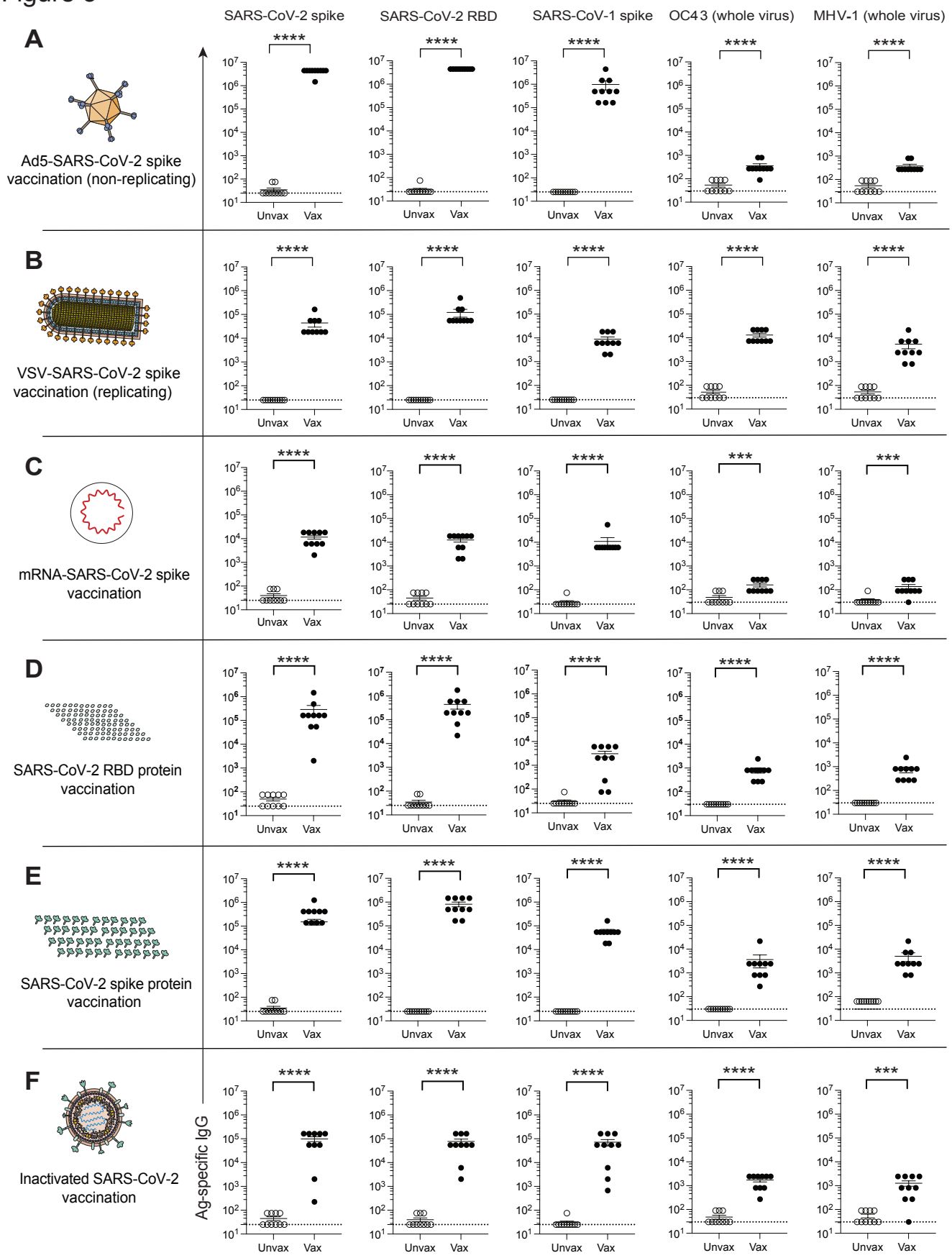

SARS-CoV-2 spike
SARS-CoV-2 RBD
SARS-CoV-1 spike
OC43 (whole virus)
MHV-1 (whole virus)
A
Ad5-SARS-CoV-2 spike vaccination (non-replicating)
B
VSV-SARS-CoV-2 spike vaccination (replicating)
C
mRNA-SARS-CoV-2 spike vaccination
D
SARS-CoV-2 RBD protein vaccination
E
SARS-CoV-2 spike protein vaccination
F
Inactivated SARS-CoV-2 vaccination
Ag-specific IgG
Unvax
Vax
****
***

Figure 3. Cross-reactive antibody responses following SARS-CoV-2 vaccination in mice. (**A**) Antibody responses after Ad5-SARS-CoV-2 spike vaccination. (**B**) Antibody responses after VSV-SARS-CoV-2 spike vaccination. (**C**) Antibody responses after mRNA-SARS-CoV-2 spike vaccination. (**D**) Antibody responses after SARS-CoV-2 RBD vaccination. (**E**) Antibody responses after SARS-CoV-2 "whole" spike vaccination. (**F**) Antibody responses after inactivated SARS-CoV-2 vaccination. Mice were primed intramuscularly and boosted after 3 weeks (see Methods for vaccine dosing information). Antibody responses were evaluated by ELISA at week 2 post-boost. Experiments were done using wild type C57BL/6 mice, except for VSV-SARS-CoV-2 spike vaccination, which used k18-hACE2 (C57BL/6) mice. Dashed lines represent LOD. Data are from two independent experiments with n=5/group. Data from all experiments are shown. ***, P <0.001,**** P <0.0001 by Mann Whitney U Test. Error bars represent SEM.

Figure 4

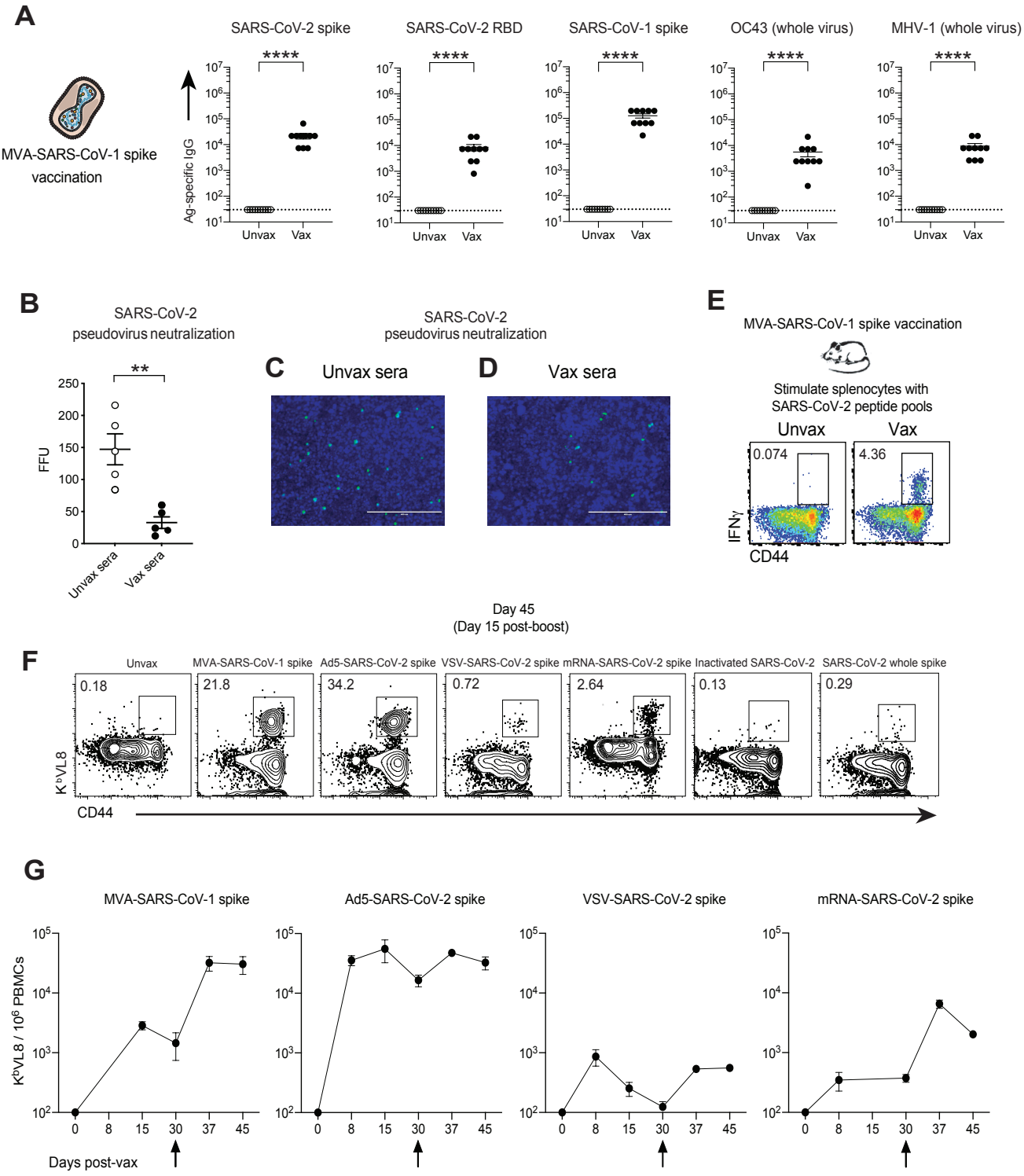

A
MVA-SARS-CoV-1 spike vaccination
SARS-CoV-2 spike
SARS-CoV-2 RBD
SARS-CoV-1 spike
OC43 (whole virus)
MHV-1 (whole virus)
Ag-specific IgG
****
Unvax
Vax
B
SARS-CoV-2 pseudovirus neutralization
FFU
**
Unvax sera
Vax sera
C
Unvax sera
D
Vax sera
E
MVA-SARS-CoV-1 spike vaccination
Stimulate splenocytes with SARS-CoV-2 peptide pools
Unvax
Vax
0.074
4.36
IFNγ
CD44
Day 45
(Day 15 post-boost)
F
Unvax
MVA-SARS-CoV-1 spike
Ad5-SARS-CoV-2 spike
VSV-SARS-CoV-2 spike
mRNA-SARS-CoV-2 spike
Inactivated SARS-CoV-2
SARS-CoV-2 whole spike
0.18
21.8
34.2
0.72
2.64
0.13
0.29
KbVL8
CD44
G
MVA-SARS-CoV-1 spike
Ad5-SARS-CoV-2 spike
VSV-SARS-CoV-2 spike
mRNA-SARS-CoV-2 spike
KbVL8 / 10^6 PBMCs
Days post-vax

Figure 4. SARS-CoV-1 vaccination induces cross-reactive antibodies and T cells. (**A**) Antibody responses after MVA-SARS-CoV-1 spike vaccination. (**B**) SARS-CoV-2 pseudovirus neutralization assay. 200 foci forming units (FFU) of SARS-CoV-2 pseudovirus were incubated with mouse sera diluted 1:4 prior to addition onto a HEK293-hACE2 cell monolayer. (**C**) Representative microscopy image of SARS-CoV-2 pseudovirus neutralization using sera from unvaccinated mice (**D**) or SARS-CoV-1 vaccinated (**D**) mice. Scale bar is 400μm. (**E**) Representative FACS plots showing cross-reactive SARS-CoV-2 specific CD8 T cells in SARS-CoV-1 vaccinated mice. Cross-reactive CD8 T cells were detected by intracellular cytokine staining after 5 hr stimulation with SARS-CoV-2 spike overlapping peptide pools, in a 37°C 5% CO_2 incubator. Cells are gated on live CD8+ lymphocytes. Data are from spleen at day 15 post-boost. (**F**) Representative FACS plots showing cross-reactive (VNFNFNGL-specific) CD8 T cells in mice vaccinated with a SARS-CoV-1 vaccine, and various SARS-CoV-2 vaccines. Cells are gated on live CD8+ lymphocytes. Data are from PBMCs at day 15 post-boost. (**G**) Summary of CD8 T cell responses among vaccine platforms. All mice were primed and boosted intramuscularly (see Methods for vaccine dosing information). Vertical arrows in panel G indicate time of boosting. Experiments were done using wild type C57BL/6 mice, except for VSV-SARS-CoV-2 spike vaccination, which used k18-hACE2 (C57BL/6) mice. In panel A, data are from two independent experiments with n=5/group, data from all experiments are shown and dashed lines represent LOD. In panel B, data are from one experiment with n=5/group. E-F, representative panels of experiment

performed twice with n=5/group are shown. Panel G shows summary of the two experiments combined. **, P <0.01, ****, P <0.0001 by Mann Whitney U Test. Error bars represent SEM.

Figure 5

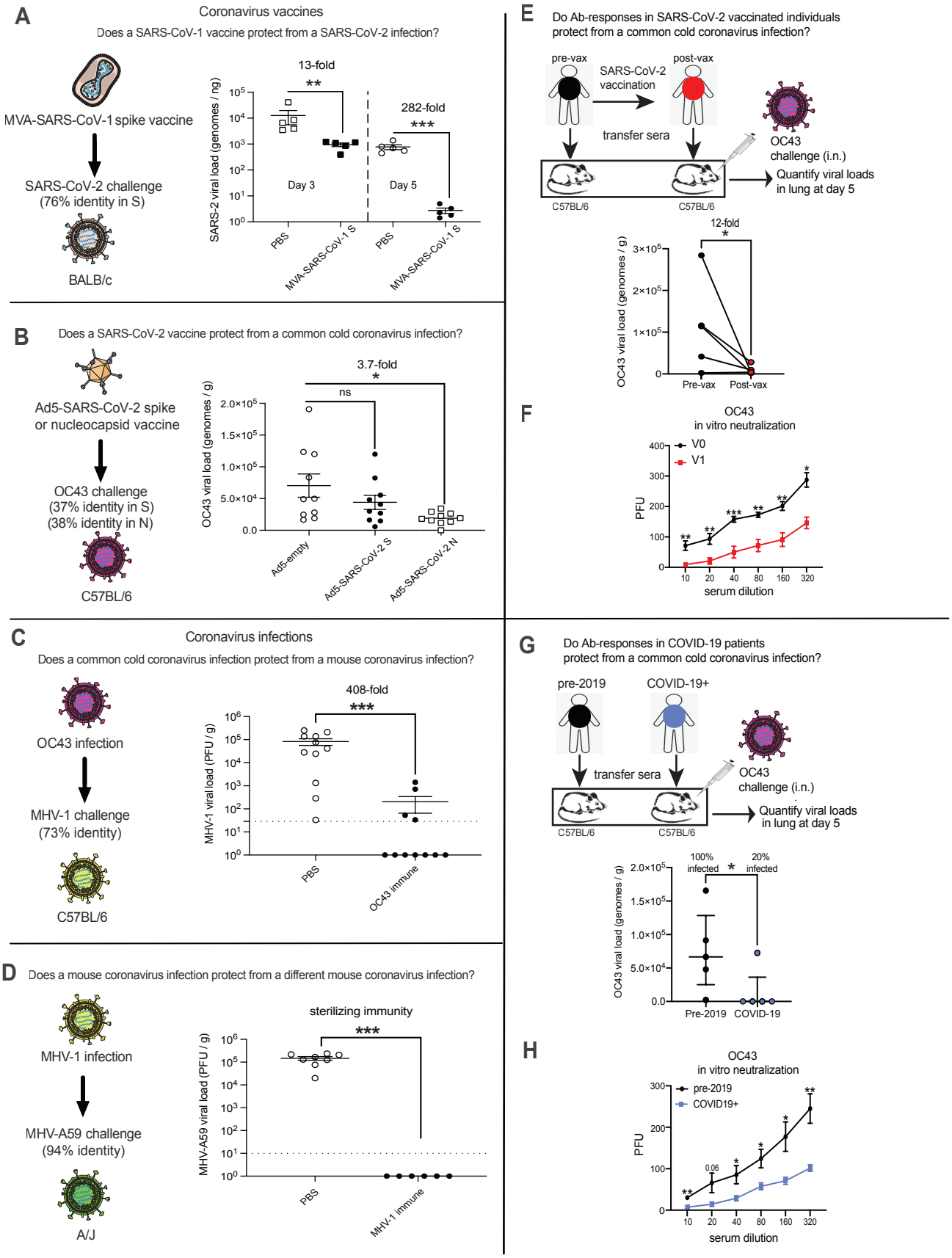

A
Coronavirus vaccines
Does a SARS-CoV-1 vaccine protect from a SARS-CoV-2 infection?
MVA-SARS-CoV-1 spike vaccine
SARS-CoV-2 challenge
(76% identity in S)
BALB/c
13-fold
**
282-fold
***
SARS-2 viral load (genomes / ng)
Day 3
Day 5
PBS
MVA-SARS-CoV-1 S
B
Does a SARS-CoV-2 vaccine protect from a common cold coronavirus infection?
Ad5-SARS-CoV-2 spike
or nucleocapsid vaccine
OC43 challenge
(37% identity in S)
(38% identity in N)
C57BL/6
3.7-fold
*
ns
OC43 viral load (genomes / g)
Ad5-empty
Ad5-SARS-CoV-2 S
Ad5-SARS-CoV-2 N
C
Coronavirus infections
Does a common cold coronavirus infection protect from a mouse coronavirus infection?
OC43 infection
MHV-1 challenge
(73% identity)
C57BL/6
408-fold
***
MHV-1 viral load (PFU / g)
PBS
OC43 immune
D
Does a mouse coronavirus infection protect from a different mouse coronavirus infection?
MHV-1 infection
MHV-A59 challenge
(94% identity)
A/J
sterilizing immunity
***
MHV-A59 viral load (PFU / g)
PBS
MHV-1 immune
E
Do Ab-responses in SARS-CoV-2 vaccinated individuals
protect from a common cold coronavirus infection?
pre-vax
post-vax
SARS-CoV-2
vaccination
transfer sera
OC43
challenge (i.n.)
Quantify viral loads
in lung at day 5
C57BL/6
C57BL/6
12-fold
*
OC43 viral load (genomes / g)
Pre-vax
Post-vax
F
OC43
in vitro neutralization
V0
V1
PFU
serum dilution
G
Do Ab-responses in COVID-19 patients
protect from a common cold coronavirus infection?
pre-2019
COVID-19+
transfer sera
OC43
challenge (i.n.)
Quantify viral loads
in lung at day 5
C57BL/6
C57BL/6
100%
infected
*
20%
infected
OC43 viral load (genomes / g)
Pre-2019
COVID-19
H
OC43
in vitro neutralization
pre-2019
COVID19+
PFU
serum dilution

Figure 5. Cross-protective immunity following coronavirus vaccination or coronavirus infection. (**A**) Viral loads after SARS-CoV-2 (MA10) challenge in SARS-CoV-1 vaccinated mice. LOD is 0.007 genomes / ng. (**B**) Viral loads after OC43 challenge in SARS-CoV-2 vaccinated mice. LOD is 27 genomes / g. (**C**) Viral loads after MHV-1 challenge in OC43-immune mice. (**D**) Viral loads after MHV-A59 challenge in MHV-1-immune mice. In panels A-D, mice were intramuscularly primed and boosted after 3 weeks (see Methods). Mice were challenged intranasally 2 weeks post-boost. (**E**) Viral loads after OC43 challenge in mice that received 50 μL of human plasma (pre or post-vaccination). Plasma was adoptively transferred into naïve mice, and at day 1 post-transfer, mice were challenged intranasally with OC43. OC43 IgG titers pre-vaccination (V0) ranged from 12150-109350, and post-vaccination (V1) ranged from 328050-984150. (**F**) OC43 PRNT in plasma pre and post-vaccination. (**G**) Viral loads after OC43 challenge in mice that received 50 μL of human plasma (pre-2019 vs COVID-19). Plasma was adoptively transferred into naïve mice, and at day 1 post-transfer, mice were challenged intranasally with OC43. OC43 IgG titer was 4050 for all pre-2019 and 63450 for all COVID-19 samples. (**H**) OC43 PRNT in plasma from pre-2019 and COVID-19 patients. Lung viral loads were quantified by PCR (panels A, B, E, G) or plaque assays (panels C, D, F and H). Data are from day 5 post-challenge unless indicated otherwise. Dashed lines represent LOD. A-D data are from two independent experiments with n=3-5/group. Panels E-H are from 1 experiment with n=5/group. Data from all experiments are shown. In panels A-D, F and H error bars represent SEM. Panels E and G show paired

values. Below LOD values in log scales represent zero values.*, P <0.05, **, P <0.01, ***, P <0.001, ns=not significant by non-parametric Mann Whitney U Test, except E and G (paired t test).