

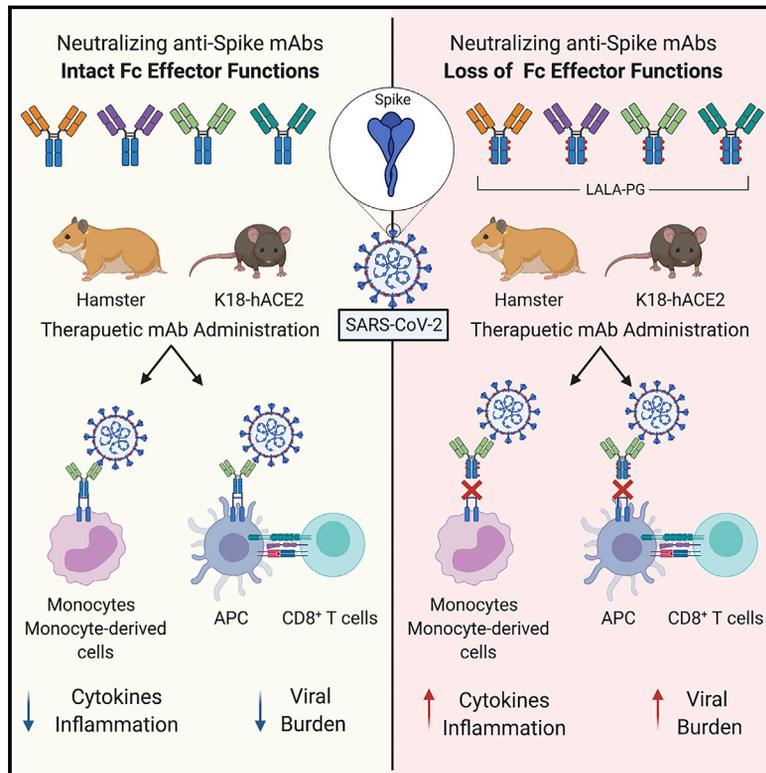


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Human neutralizing antibodies against SARS-CoV-2 require intact Fc effector functions for optimal therapeutic protection

Graphical abstract



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In brief

Neutralizing human monoclonal antibodies (mAbs) against SARS-CoV-2 require Fc effector functions for optimal protection during post-exposure therapy, with intact mAbs reducing SARS-CoV-2 burden and lung disease in rodent models better than LALA-PG loss-of-function Fc variant mAbs and requiring monocytes and CD8⁺ T cells for optimal clinical and virological benefit.

Highlights

- Neutralizing mAbs do not require Fc effector functions when given as prophylaxis
- MAbs against SARS-CoV-2 require Fc effector functions for therapeutic protection
- Fc engagement of mAbs decreases viral burden and mitigates lung inflammation
- CD8⁺ T cells and monocytes are necessary for optimal Fc-dependent mAb protection



Article

Human neutralizing antibodies against SARS-CoV-2 require intact Fc effector functions for optimal therapeutic protection

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SUMMARY

SARS-CoV-2 has caused the global COVID-19 pandemic. Although passively delivered neutralizing antibodies against SARS-CoV-2 show promise in clinical trials, their mechanism of action *in vivo* is incompletely understood. Here, we define correlates of protection of neutralizing human monoclonal antibodies (mAbs) in SARS-CoV-2-infected animals. Whereas Fc effector functions are dispensable when representative neutralizing mAbs are administered as prophylaxis, they are required for optimal protection as therapy. When given after infection, intact mAbs reduce SARS-CoV-2 burden and lung disease in mice and hamsters better than loss-of-function Fc variant mAbs. Fc engagement of neutralizing antibodies mitigates inflammation and improves respiratory mechanics, and transcriptional profiling suggests these phenotypes are associated with diminished innate immune signaling and preserved tissue repair. Immune cell depletions establish that neutralizing mAbs require monocytes and CD8⁺ T cells for optimal clinical and virological benefit. Thus, potently neutralizing mAbs utilize Fc effector functions during therapy to mitigate lung infection and disease.

INTRODUCTION

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is the recently emerged RNA virus responsible for the coronavirus disease 2019 (COVID-19) pandemic that has led to over 116 million infections and over 2.5 million deaths (Dong et al., 2020). The need for effective countermeasures of COVID-19 is urgent, because only dexamethasone and remdesivir have demonstrated clinical efficacy in specific indications. One approach for both prevention and treatment of COVID-19 has been the development of SARS-CoV-2-neutralizing monoclonal antibodies (mAbs) isolated from the B cells of individuals with recent SARS-CoV-2 infection (Abraham, 2020; Marovich et al., 2020). Multiple neutralizing mAbs directed against the spike (S) glycoprotein of SARS-CoV-2 have been identified that target non-

overlapping epitopes and show differences in neutralization potency (Alsoussi et al., 2020; Cao et al., 2020; Hansen et al., 2020; Ju et al., 2020; Liu et al., 2020; Noy-Porat et al., 2020; Pinto et al., 2020; Robbiani et al., 2020; Zost et al., 2020a). The majority of these mAbs bind to the S1 subunit, specifically the receptor binding domain (RBD), and inhibit virus engagement with its cell surface receptor, angiotensin converting enzyme (ACE)2.

Prophylactic and therapeutic efficacy of anti-S mAbs has been demonstrated *in vivo* in murine, hamster, and non-human primate models of SARS-CoV-2 pathogenesis (Alsoussi et al., 2020; Baum et al., 2020; Fagre et al., 2020; Hansen et al., 2020; Hassan et al., 2020; Kreye et al., 2020; Rogers et al., 2020; Shi et al., 2020; Zost et al., 2020a), with varying degrees of reduction in viral burden and dampened inflammation of the lung. However, the mechanisms of protection *in vivo* can be



due to multiple factors including direct virus neutralization and engagement of complement or Fc gamma receptors (Fc γ R) on leukocytes. Fc effector functions of antibodies can promote immune-mediated cellular clearance, enhance antigen presentation and CD8⁺ T cell responses, and reshape inflammation through engagement of Fc γ R on specific cells (Lu et al., 2018). In contrast, under certain circumstances, Fc-Fc γ R interactions can promote antibody-dependent enhancement of virus infection (ADE) (Halstead, 1994) or pathological immune skewing (Bolles et al., 2011; Ruckwardt et al., 2019), which is at least a theoretical concern of antibody-based therapies and vaccines against SARS-CoV-2 (Diamond and Pierson, 2020). Thus, a more thorough understanding of the contribution of Fc effector functions in the context of antibody-based therapies is needed.

Here, we use the K18-hACE2 transgenic mouse model of SARS-CoV-2 pathogenesis (Golden et al., 2020; Winkler et al., 2020) and a Fc region genetic variant of immunoglobulin G (IgG) (LALA-PG) of potent RBD-binding neutralizing mAbs that cannot engage Fc γ R or complement to define the role of Fc effector functions in antibody protection. We find that Fc effector functions are dispensable when neutralizing mAbs are administered as prophylaxis but are required for optimal protection when given as post-exposure therapy. When administered after SARS-CoV-2 infection, intact, but not LALA-PG, mAbs reduce viral burden and lung disease. Fc engagement by antibodies decreases immune cell activation and levels of inflammatory cytokines, and this activity is linked to improved respiratory mechanics and outcome. RNA sequencing analysis of lung homogenates reveals distinct gene signatures associated with Fc engagement including decreased innate immune signaling and extracellular matrix remodeling but maintained expression of genes that mediate tissue repair. Immune cell depletions showed that neutralizing mAbs require monocytes and CD8⁺ T cells for maximal clinical and virological benefit. We confirmed these findings in hamsters, a second mammalian species, that also required Fc effector functions of a neutralizing mAb to prevent weight loss, control viral infection, and limit inflammation. Overall, these studies establish that Fc effector functions of neutralizing antibodies are necessary for optimal therapeutic outcome after SARS-CoV-2 infection.

RESULTS

Pre-exposure protection against SARS-CoV-2 infection in mice by a neutralizing mAb does not require Fc effector functions

Passive transfer of neutralizing mAbs targeting the S protein confers protection in multiple pre-clinical models of SARS CoV-2 infection (Alsoussi et al., 2020; Baum et al., 2020; Hansen et al., 2020; Hassan et al., 2020; Kreye et al., 2020; Rogers et al., 2020; Zost et al., 2020a). However, *in vivo* antiviral efficacy can be due to multiple mechanisms including Fab-dependent direct virus neutralization and Fc-dependent engagement that promotes opsonization of virus, clearance of virally infected cells, and modulation of innate and adaptive immune responses (Bournazos et al., 2014b, 2019; DiLillo et al., 2014, 2016; Fox et al., 2019). To evaluate the contribution of Fc effector functions, we introduced loss-of-function LALA-PG (L234A, L235A, and

P329G) mutations into the Fc region of the human IgG1 heavy chain of COV2-2050, a neutralizing human mAb that binds the RBD of S and blocks ACE2 binding (Zost et al., 2020a), to abolish antibody Fc interactions with Fc γ R and complement proteins (Lo et al., 2017). Intact or LALA-PG variants of COV2-2050 neutralized SARS-CoV-2 equivalently in cell culture (Figure 1A), and introduction of the LALA-PG mutation prevented binding to mouse Fc γ RI, Fc γ RIV, or Fc γ RIIb (Figure 1B). To test their relative efficacy *in vivo*, we used the K18-hACE2 transgenic mouse model of SARS-CoV-2 pathogenesis (McCray et al., 2007; Winkler et al., 2020). In prophylaxis studies, K18-hACE2 transgenic mice received decreasing doses of COV2-2050 or COV2-2050 LALA-PG (200 μ g, 40 μ g, 8 μ g, or 1.6 μ g) by intraperitoneal injection 16 h prior to intranasal inoculation with SARS-CoV-2 (10^3 plaque-forming unit [PFU], strain 2019 n-CoV/USA_WA1/2020). An isotype control human mAb (DENV-2D22) was delivered at a single 200 μ g dose for comparison. At the time of SARS-CoV-2 challenge, the serum levels of COV2-2050 or COV2-2050 LALA-PG were equivalent (Figure 1C). As expected, lower doses of COV2-2050 resulted in decreasing neutralizing antibody titers in serum at the time of virus inoculation (Figure 1D).

Effects on SARS-CoV-2-induced weight loss were comparable between COV2-2050 or COV2-2050 LALA-PG at all doses with a loss of protection observed only at the 1.6 μ g dose (Figures 1E and 1F). Levels of COV2-2050 correlated with clinical protection (Figures 1G and 1H), with a minimum serum neutralizing titer (NT₅₀) of 104 and concentration of 212 ng/mL required to prevent weight loss in this stringent challenge model. Although weight loss was prevented at a dose of 86 μ g per animal, a higher 40 μ g dose was required to reduce viral burden in the lung at 7 days post-infection (dpi) (Figure 1I). Serum levels of COV2-2050 also correlated inversely with SARS-CoV-2 RNA in the lung (Figures 1J and 1K) with a minimum neutralizing titer of 381 and mAb concentration of 851 ng/mL required to reduce viral infection at 7 dpi. Differences in viral burden were not observed at several different doses of COV2-2050 and COV2-2050 LALA-PG treatment. Importantly, no evidence of antibody-dependent enhancement of clinical disease or viral burden (Lee et al., 2020; Taylor et al., 2015) was detected even when levels were below the protective dose. Thus, the Fc-dependent effector functions of COV2-2050 are dispensable for clinical and virological protection when a potentially neutralizing antibody is administered as prophylaxis.

Fc effector functions enhance therapeutic activity of neutralizing mAbs

Although we did not detect a requirement for Fc effector functions when COV2-2050 was administered as prophylaxis, we re-evaluated this in the setting of post-exposure administration. We inoculated K18-hACE2 mice with SARS-CoV-2 by the intranasal route and then delivered a single 200 μ g dose of COV2-2050 or COV2-2050 LALA-PG mAbs at 1 (D+1), 2 (D+2), or 3 (D+3) dpi by intraperitoneal injection. Whereas passive transfer of the intact COV2-2050 prevented weight loss compared to isotype control mAb-treated animals, this protection was lost in animals treated with the COV2-2050 LALA-PG variant (Figures 2A and 2B). Although treatment beginning at 3 dpi with either

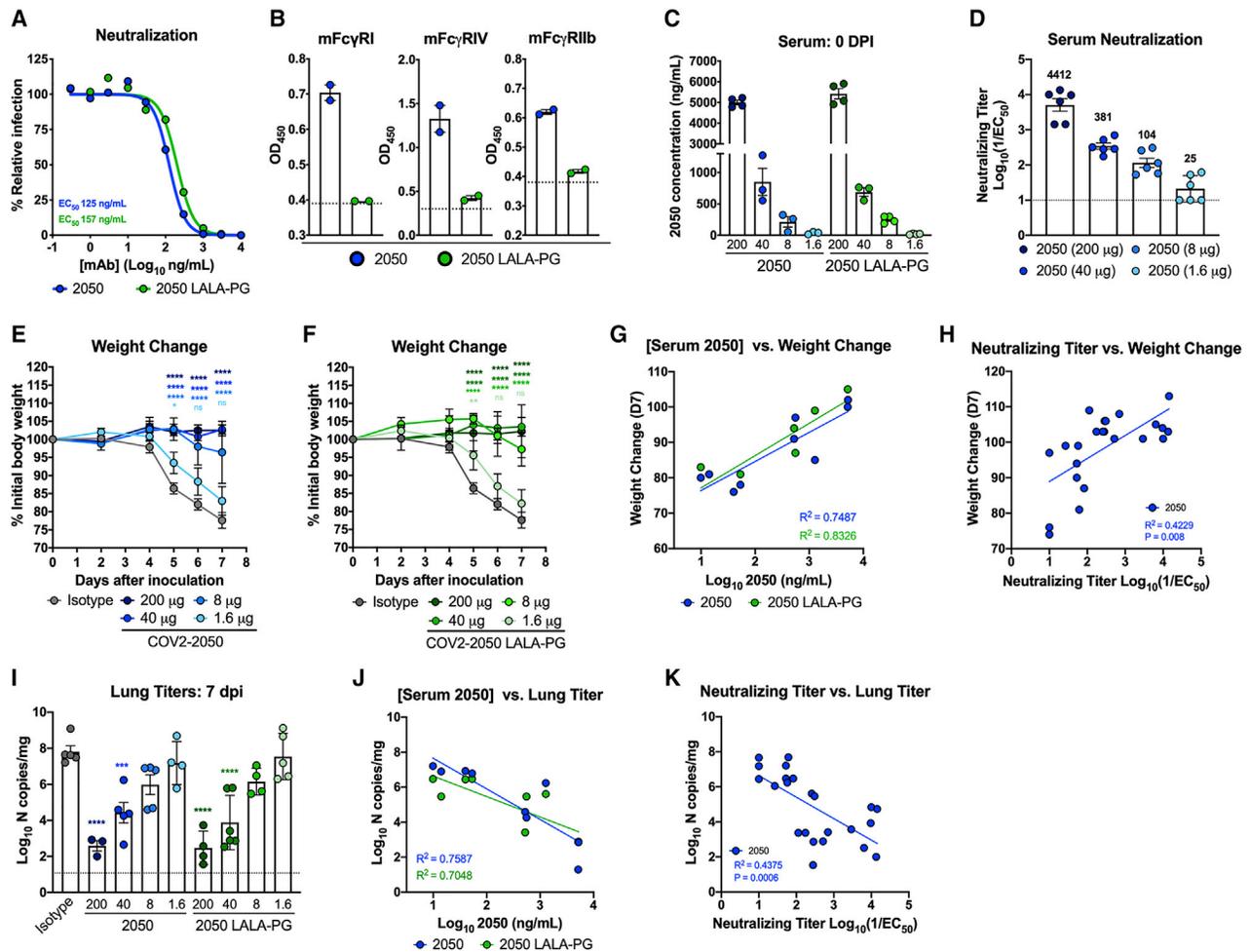
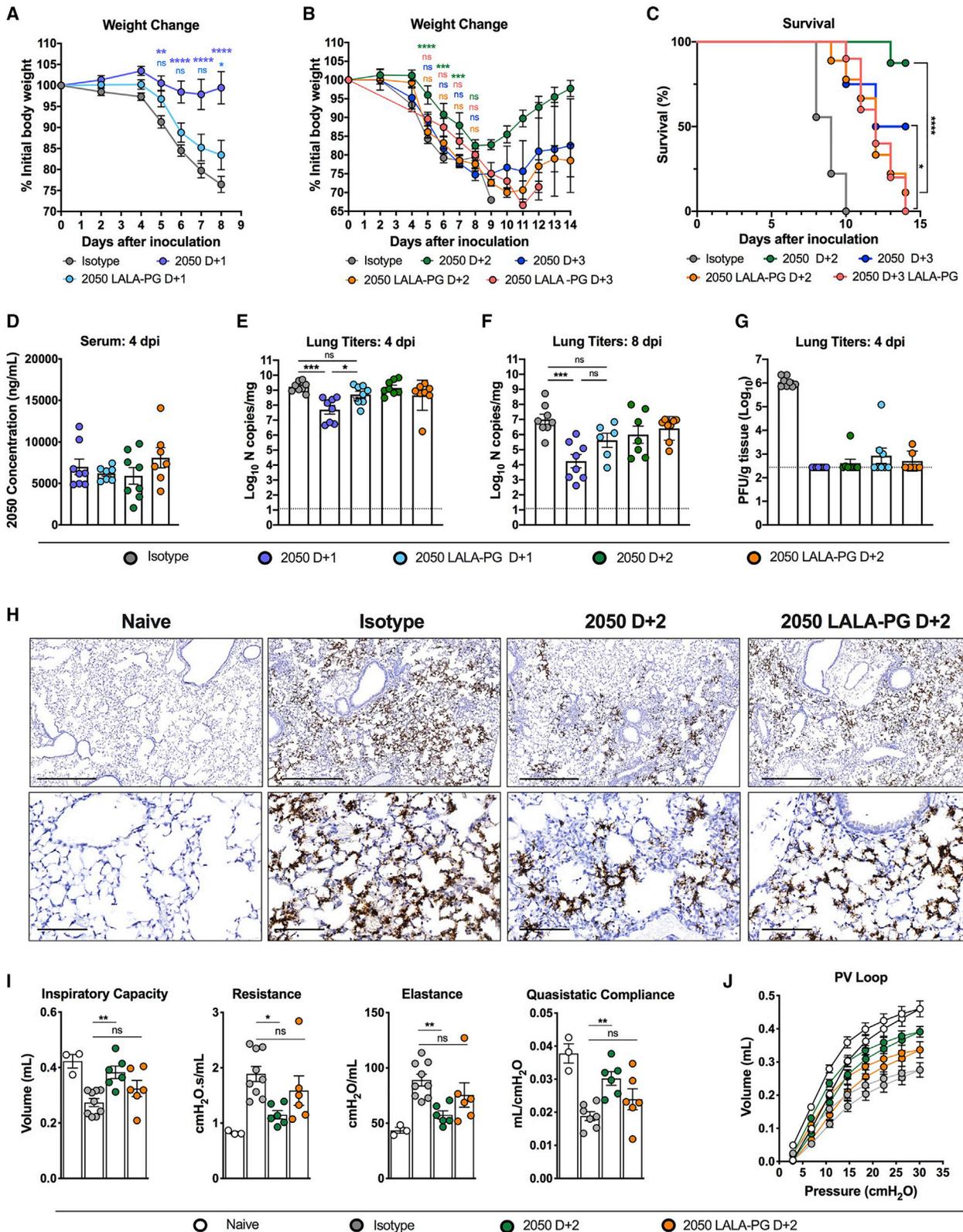


Figure 1. Neutralizing activity is sufficient for prophylactic efficacy of mAbs against SARS-CoV-2 infection in K18-hACE2 mice

(A) mAbs (COV2-2050 and COV2-2050 LALA-PG) were incubated with 10^2 focus-forming units (FFU) of SARS-CoV-2 for 1 h followed by addition to Vero E6 cells. Wells containing mAb were compared to wells without mAb to determine relative infection. One experiment with the mean of 2 technical replicates is shown. (B) Binding of COV2-2050 or COV2-2050 LALA-PG to murine Fc γ RI, Fc γ RIV, or Fc γ RIIb by ELISA (two experiments). The dotted line indicates the limit of detection (LOD), as determined by background binding to a negative control. (C–K) Eight-week-old female and male K18-hACE2 mice received 200, 40, 8, or 1.6 μ g of COV2-2050 or COV2-2050 LALA-PG by intraperitoneal injection 1 day prior to intranasal inoculation with 10^3 PFU of SARS-CoV-2. (D) Serum concentrations (ng/mL) of COV2-2050 or COV2-2050 LALA-PG at the time of challenge (0 dpi) (mean \pm SEM; n = 3–4, 2 experiments). (E) Neutralizing titers in serum of indicated groups at 0 dpi as measured by FRNT (mean \pm SEM; n = 6, 2 experiments). (E and F) Weight change following COV2-2050 (E) or COV2-2050 LALA-PG (F) administration (mean \pm SEM; n = 4–6, 2 experiments). (G) Correlation analyses comparing COV2-2050 or COV2-2050 LALA-PG serum concentrations (day 0 [D0]) against weight change (D+7) (n = 4–6, 2 experiments). (H) Correlation analyses comparing COV2-2050 neutralizing titers in serum (D0) against weight change (D+7) (n = 6–8, 3 experiments). (I) Viral RNA levels at 7 dpi in the lung (n = 4–6, 2 experiments). (J) Correlation analyses comparing COV2-2050 or COV2-2050 LALA-PG serum concentrations (D0) against lung viral titer (D+7) (n = 4–6, 2 experiments). (K) Correlation analyses comparing serum neutralizing titers (D0) against lung viral titer (D7) (n = 6–8, 2 experiments). (E and F) Two-way ANOVA with Sidak's post-test: ns not significant, *p < 0.05, **p < 0.01, ****p < 0.0001; comparison to the isotype control mAb-treated group. (G, H, J, and K) Pearson's correlations: (G) COV2-2050, p = 0.0026; COV2-2050 LALA-PG, p = 0.0006; (H) COV2-2050, p = 0.0008; (J) COV2-2050, p = 0.0022; COV2-2050 LALA-PG, p = 0.0095; and (K) COV2-2050, p = 0.0006. (I) One-way ANOVA with Turkey's post-test: ***p < 0.001, ****p < 0.0001, comparison to the isotype control mAb-treated group.

COV2-2050 or COV2-2050 LALA-PG did not prevent weight loss, therapy with COV2-2050, but not COV2-2050 LALA-PG, reduced lethality (Figures 2B and 2C). The differences in protection were not due to disparate levels of COV2-2050 and COV2-2050 LALA-PG, as equivalent amounts were detected in serum at 0 and 4 dpi (Figure 2D). Treatment at 1 dpi with COV2-2050,

but not COV2-2050 LALA-PG, reduced SARS-CoV-2 viral RNA levels in the lung at 4 and 8 dpi substantially (Figures 2E and 2F). In contrast, D+2 treatment with either intact COV2-2050 or COV2-2050 LALA-PG did not reduce viral RNA levels in the lung when compared to isotype-treated controls. However, COV2-2050 and COV2-2050 LALA-PG mAbs both reduced



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levels of infectious virus in the lung at 4 dpi (Figure 2G). Given the disparity in viral RNA levels, this effect might be due in part to *ex vivo* neutralization after lung tissue homogenization, as reported for other respiratory viruses (Subbarao et al., 2004; Wells et al., 1981). Visualization of viral RNA by *in situ* hybridization showed that although expression of SARS-CoV-2 RNA was spread diffusely throughout the lung in groups treated at D+2 with isotype control mAb or 2050 LALA-PG, viral RNA was more focal in the lungs of mice receiving intact COV2-2050 at D+2 with evidence of cleared regions and a smaller affected proportion (Figure 2H). Additionally, K18-hACE2 mice receiving intact COV2-2050 at D+2, but not COV2-2050 LALA-PG, showed functional improvement in pulmonary mechanics (e.g., inspiratory capacity, resistance, and elastance), and lung compliance and distensibility (Figures 2I and 2J).

To expand on these results, we tested the therapeutic activity of three additional neutralizing human mAbs that bind the RBD, COV2-3025, COV2-2072, and COV2-2381 (Zost et al., 2020b), and compared them to LALA-PG variants of each mAb. COV2-3025, COV2-2072, and COV2-2381 neutralized SARS-CoV-2 equivalently compared to their respective LALA-PG variants (Figures 3A, 3E, and 3I), and introduction of the LALA-PG mutation abolished binding to murine Fc γ RI or Fc γ RIV (Figures 3B, 3F, and 3J). Administration of intact COV2-3025 at D+1 recapitulated the same pattern of protection observed with COV2-2050 treatment: COV2-3025 LALA-PG failed to protect mice from weight loss or reduce viral titers compared to the intact COV2-3025 (Figures 3C and 3D). In comparison, COV2-2381 LALA-PG still showed a modest level of protection against weight loss, and viral burden was reduced in mice receiving either COV2-2381 or COV2-2381 LALA-PG compared to isotype-treated controls (Figures 3G and 3H). Administration of COV2-2072 LALA-PG at D+1 or D+2 partially protected against SARS-CoV-2-induced weight loss (D+1) or lethality (D+2) compared to the isotype-treated controls, but failed to reduce viral titers, unlike the intact COV2-2072 (Figures 3K–3M). The differences in therapeutic activity of COV2-2050 LALA-PG and COV2-3025 LALA-PG compared to COV2-2381 LALA-PG and COV2-2072 LALA-PG could be due to differential neutralizing activity, pharmacokinetics, or bioavailability *in vivo*. Regardless, these results indicate that intact Fc effector functions of neutral-

izing antibodies are needed for optimal therapeutic activity to mitigate clinical disease caused by SARS-CoV-2 infection.

To confirm the requirement of Fc-mediated effector functions in protection in a species-homologous experimental system, we generated chimeric versions of COV2-2072 in which the human IgG1 Fc region was replaced with ones from murine (m)IgG2a, murine (m)IgG1, or mIgG1 containing a loss-of-function D265A mutation that abolishes binding to murine Fc γ Rs (Baudino et al., 2008; Lo et al., 2017). Chimeras of COV2-2072 with murine Fc regions neutralized SARS-CoV-2 equivalently compared to parental COV2-2072 human IgG1 mAb (Figure S1A). As expected, mIgG1 and mIgG1 D265A bound mFc γ RI or mFc γ RIV poorly compared to the mIgG2a (Figure S1B). Notably, D+1 administration of COV2-2072 mIgG1 or COV2-2072 mIgG1 D265A failed to protect mice from weight loss. In contrast, COV2-2072 mIgG2a prevented weight loss and reduced viral titers similarly to the human IgG1 form of COV2-2072 (Figures S1C and S1D). These data establish the importance of Fc effector functions to antibody protection when administered in a corresponding system with murine Fc regions and murine Fc γ Rs.

Fc effector functions of a neutralizing mAb modulate the immune responses to SARS-CoV-2 infection

An excessive pro-inflammatory host response to SARS-CoV-2 infection is hypothesized to contribute to pulmonary pathology and severe COVID-19 (Giamarellos-Bourboulis et al., 2020; Tay et al., 2020). Given that COV2-2050-mediated improvement in pulmonary function was not associated with reduced viral RNA levels in the lung when given at D+2, we speculated that the intact mAb might modulate immune cell and inflammatory responses. Analysis of hematoxylin and eosin-stained lung sections from isotype mAb-treated mice at 8 dpi showed perivascular and parenchymal immune cell accumulation with accompanying edema and lung consolidation (Figure 4A). Animals receiving COV2-2050 at D+1 showed markedly reduced lung inflammation, but this protection was lost in mice receiving COV2-2050 LALA-PG at D+1 or D+2. Although COV2-2050 treatment at D+2 did not completely reduce pathology, immune cell infiltration was more focal with patches of clear airspaces throughout the lung. Measurements of pro-inflammatory cytokine and chemokines in the lung at 8 dpi showed decreased

Figure 2. Fc effector functions enhance the therapeutic activity of a neutralizing antibody against SARS-CoV-2 in K18-hACE2 mice

(A–J) Eight-week-old K18-hACE2 mice were inoculated by the intranasal route with 10^3 PFU of SARS-CoV-2. At 1 (D+1), 2 (D+2), or 3 (D+3) dpi, mice were given 200 μ g of COV2-2050 or COV2-2050 LALA-PG by intraperitoneal injection. Naive animals were mock-infected with sterile PBS.

(A and B) Weight change. For (B), statistical analysis was performed only at time points when all mice were alive to avoid survivor bias (mean \pm SEM; n = 8–10, 3 experiments).

(C) Survival analysis (n = 8–19, 3 experiments).

(D) Serum concentrations (ng/mL) of COV2-2050 or COV2-2050 LALA-PG at 4 dpi (mean \pm SEM; n = 8, 2 experiments).

(E and F) Viral RNA levels at 4 and 8 dpi in the lung (n = 6–10, 3 experiments).

(G) Infectious virus at 4 dpi in the lung (n = 6–8, 2 experiments).

(H) SARS-CoV-2 RNA *in situ* hybridization of lung sections. Images show low- (top; scale bars, 500 μ m) and high-power magnification (bottom; scale bars, 100 μ m). Images are representative of n = 4 per group.

(I) Parameters of respiratory mechanics: inspiratory capacity, resistance, elastance, tissue damping, and quasistatic compliance measured at 8 dpi (n = 3–6, 2 experiments).

(J) Pressure volume loops (n = 3–6, 2 experiments).

(A and B) Two-way ANOVA with Sidak's post-test: *p < 0.05, ***p < 0.001, ****p < 0.0001; comparison to the isotype control mAb-treated group. (C) Mantel-Cox log-rank test for survival: *p < 0.05, ****p < 0.0001. (E, F, and I) One-way (E and F) or two-way (I and J) ANOVA with Turkey's post-test: ns not significant, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, comparison to the isotype control mAb-treated group.

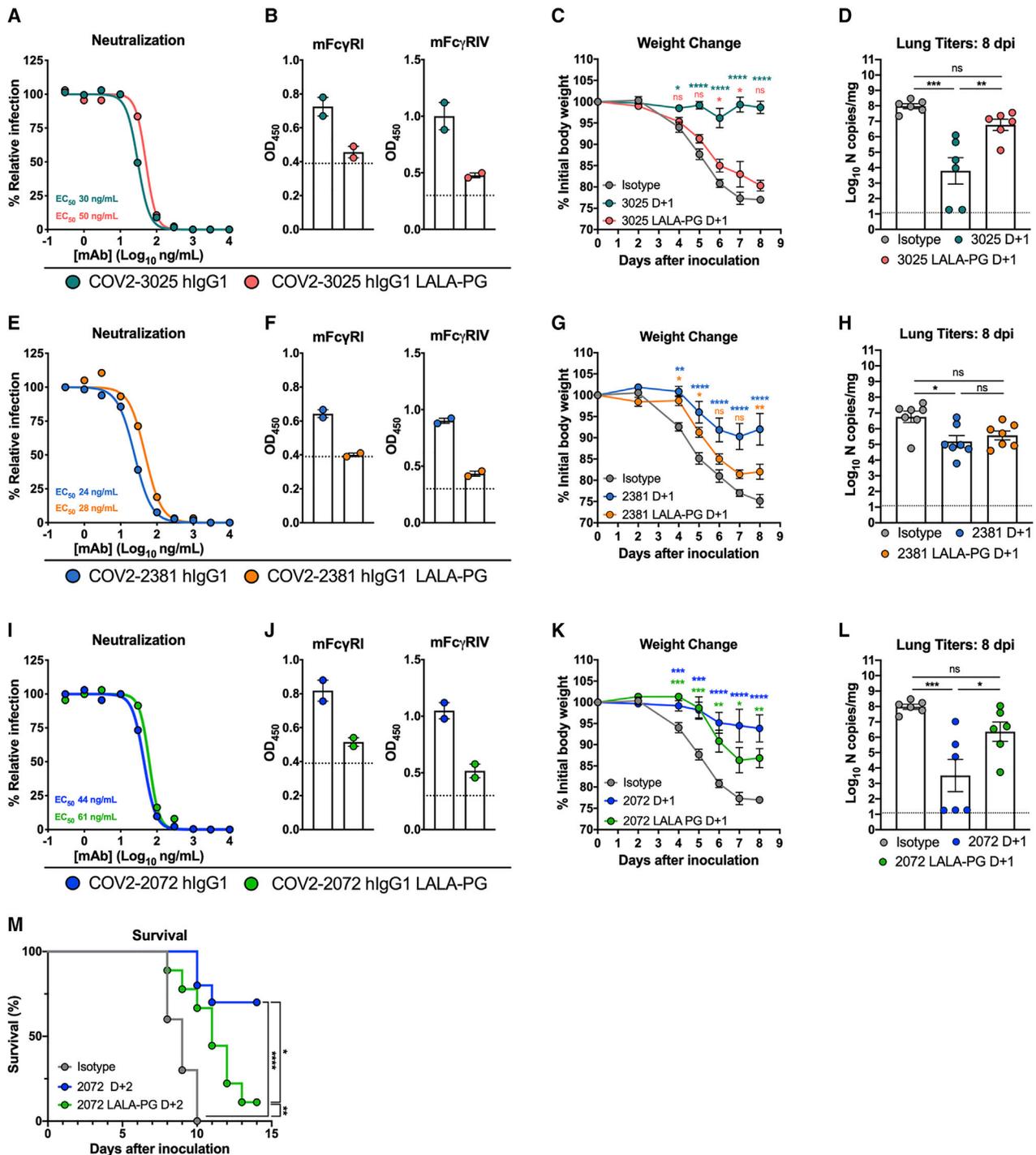


Figure 3. Requirement of Fc effector functions for different anti-RBD mAb *in vivo*

(A, E, and I) Anti-SARS-CoV-2 mAbs: COV2-3025 and COV2-3025 LALA-PG (A); COV2-2381, COV2-2381 LALA-PG (E); and COV2-2072, and COV2-2072 LALA-PG (I); MABs were incubated with 10² focus-forming units (FFU) of SARS-CoV-2 before adding to Vero E6 cells. Wells containing mAb were compared to those without mAb to determine relative infection. One experiment with the mean of 2 technical replicates is shown.

(B, F, and J) Binding to recombinant murine FcγRI or FcγRIV of COV2-3025 or COV2-3025 LALA-PG (B); COV2-2381 or COV2-2381 LALA-PG (F); and COV2-2072 or COV2-2072 LALA-PG (J). Data are from 2 experiments. The dotted line indicates the LOD.

(C, D, G, H, K, and L) Eight-week-old K18-hACE2 mice were inoculated by the intranasal route with 10³ PFU of SARS-CoV-2. At 1 dpi (D+1), mice were given 200 μg of each respective mAb by intraperitoneal injection. (C, G, and K) Weight change (mean ± SEM; n = 6–8, 2 experiments: two-way ANOVA with Sidak's

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levels of CXCL10, G-CSF, interleukin (IL)-6, interferon (IFN)- γ , CCL2, CCL3, CCL4, CCL19, and CXCL1 following both D+1 and D+2 COV2-2050 treatment, which did not occur in animals receiving COV2-2050 LALA-PG (Figures 4B and S2).

Cellular analysis of bronchoalveolar lavage (BAL) fluid at 4 dpi (Figure S3) showed significantly reduced numbers of CD8⁺ T cells with a trend toward fewer monocytes after administration of intact COV2-2050 at D+1. Differences in BAL fluid cell numbers were not observed at 4 dpi when treatment with COV2-2050 or COV2-2050 LALA-PG was started at D+2 (Figure 4C). At 8 dpi, we observed a significant reduction in the numbers of CD45⁺ cells, neutrophils, CD11b⁺DCs, Ly6C^{hi} monocytes, and CD8⁺ T cells in the BAL fluid of mice treated at D+1 with COV2-2050 compared to COV2-2050 LALA-PG (Figure 4D) but no difference in BAL cell number when comparing COV2-2050 compared to COV2-2050 LALA-PG following D+2 treatment (Figure 4D). In comparison, flow cytometric analysis of the lung tissues at 8 dpi showed increased numbers of CD8⁺ T cells (Figure 4E) and a higher percentage of activated CD44⁺CD62L⁻ CD8⁺ T cells (Figure 4F) associated with intact COV2-2050 D+1 treatment compared to animals treated with isotype control mAb or COV2-2050 LALA-PG at D+1. Additionally, monocytes and monocyte-derived CD11b⁺ DCs showed decreased expression of tumor necrosis factor alpha (TNF- α) and inducible nitric oxide synthase (iNOS) as well as the activation marker CD80⁺ following COV2-2050 D+1 treatment (Figures 4G and 4H). These results showing that greater antibody-dependent CD8⁺ T cell responses and skewing of myeloid cell inflammatory responses in the lung correlate with protection are consistent with studies showing that Fc engagement can enhance antiviral CD8⁺ T cell responses (Bournazos et al., 2020), and myeloid cell dysfunction may drive severe COVID-19 (Merad and Martin, 2020; Sánchez-Cerrillo et al., 2020).

Distinct transcriptional signatures in the lung after treatment with COV2-2050 or COV2-2050 LALA-PG

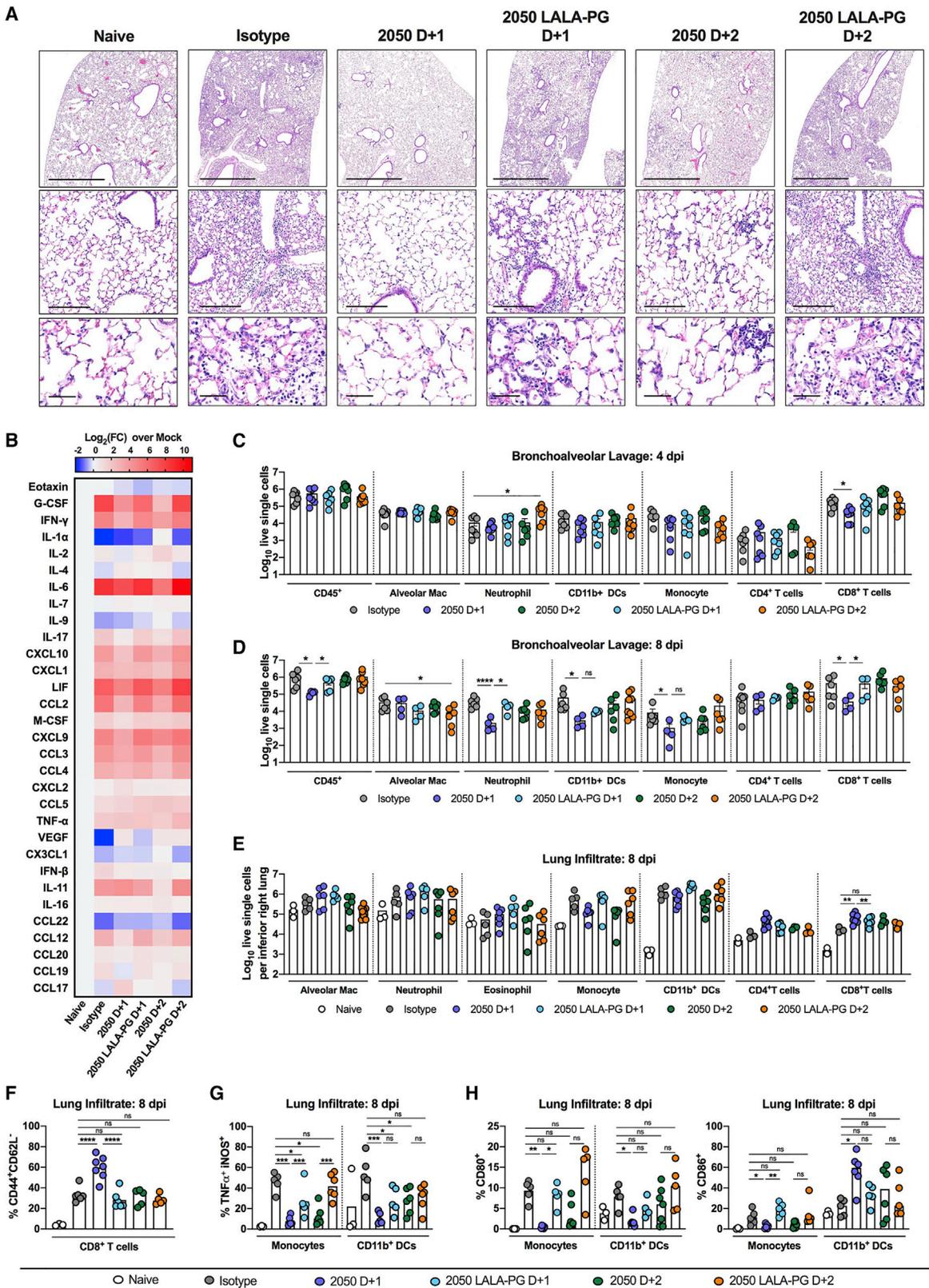
To interrogate further the impact of Fc effector functions on protection, we performed RNA sequencing of lung homogenates at 8 dpi in mice receiving an isotype control mAb, COV2-2050, or COV2-2050 LALA-PG mAbs at 1 (D+1) or 2 (D+2) and compared the results to those from samples from naive mice. Principal component analysis (PCA) revealed distinct transcriptional signatures associated with SARS-CoV-2 infection when compared to mock-infected naive animals. Treatment with intact COV2-2050 demonstrated a clear transcriptional shift toward the naive group, whereas the profiles from COV2-2050 LALA-PG-treated mice were more similar to isotype control mAb-treated animals (Figure 5A). Indeed, only 91 differentially expressed genes (DEGs) were identified when comparing the isotype control mAb to the COV2-2050 LALA-PG D+1 group, whereas 2,056 and 1,975 DEGs were detected when comparing COV2-2050 D+1 to isotype control and COV-2050 LALA-PG D+1, respec-

tively (Figure 5B). A similar large number of DEGs was observed in the lungs at 8 dpi from mice treated at D+2 with COV2-2050 or COV2-2050 LALA-PG (Figure 5B). Gene ontology analysis of the top downregulated genes comparing COV2-2050 to COV2-2050 LALA-PG showed immune gene clusters including cytokine-mediated signaling (e.g., *Il10ra*, *Il15*, *Il17ra*, *Socs1*, and *Jak3*), type I IFN signaling (e.g., *Ifnar2*, *Stat2*, *Ifit1*, *Ifit2*, *Ifit3*, and *Irf7*), and leukocyte chemotaxis (e.g., *Ccl2*, *Cxcl10*, and *Ccl17*) as well as genes involved in cell proliferation (e.g., *Egfr*, *Fgfr1*, *Fos1*, *Myc*, and *Cdkn2b*) and metalloproteinase-mediated extracellular matrix organization (e.g., *Adam15*, *Adam19*, *Col1a*, *Mmp14*, and *Itgam*) (Figure S4; Table S1).

We next performed CompBio analysis (v2.0, PercayAI), as we did previously (Adamo et al., 2020; Gehrig et al., 2019), to identify biological themes uniquely enriched in the COV2-2050-treated groups compared to the isotype control and COV2-2050 LALA-PG-treated groups (Figure 5C; Table S2). Pathways unique to the COV2-2050 D+1 treatment group compared to the isotype-treated group included genes involved in actinomyosin-associated cell adhesion (e.g., *Kif1c*, *Ctnnd1*, *Nectin3*, *Unc45b*, and *Prkca*) and Rho GTPase signaling (e.g., *Rhoq*, *Mapk3*, *Prkca*, and *Cdc42bpa*), processes that are typically associated with wound repair programs (Verboon and Parkhurst, 2015) (Figure 5D). Pathways downregulated in the COV2-2050 D+1 group included genes involved in type I IFN and nuclear factor κ B (NF- κ B)-dependent signaling (e.g., *Irf7*, *Stat2*, *Nfkb2*, *Bst2*, *Isg15*, and *Ikbk3*) (Figure 5D), which may in part be due to the lower levels of viral RNA detected (Figures 2E and 2F). The expression pattern of down- or upregulated gene sets in the COV2-2050 D+1 treated animals was similar to that in naive animals, suggesting that the intact antibody limited the transcriptional changes usually seen during SARS-CoV-2 infection.

Pathways that were downregulated in the D+2 COV2-2050-treated group compared to the isotype control or D+2 COV2-2050 LALA-PG-treated animals included S100A8-associated innate immune signaling (e.g., *Reg3g*, *Saa3*, *Itgma*, *Mmp8*, and *S100a8*), oncostatin M receptor associated signaling (e.g., *Il6*, *Osmr*, *Csf1*, and *Socs3*), and extracellular matrix remodeling (e.g., *Adamts15*, *Col5a1*, *Vcam1*, and *Lama4*) (Figure S5; Table S2). Thus, COV2-2050 treatment at D+2 and the resultant Fc-dependent effector responses may differentially modulate neutrophil activation, gp130 signaling, and tissue damage due to matrix metalloproteinase activation. Unlike what was observed in the analysis of gene sets unique to D+1, these downregulated programs at D+2 were not lower in naive animals. Therefore, intact COV2-2050 administration at D+2 may induce a separate protective transcriptional program that is not simply preventing virus-induced transcriptional changes. Of note, we also identified diminished expression of genes involved in immune complex clearance following COV2-2050 versus COV2-2050 LALA-PG administration at D+2, which may reflect a negative feedback loop (Daëron and Lesourne, 2006). Collectively,

post-test: *p < 0.05, **p < 0.01, ****p < 0.0001; comparison to the isotype control mAb-treated group). (D, H, and L) Viral RNA levels at 8 dpi in the lung (n = 6–8, 2 experiments, one-way ANOVA with Dunnett's test; ****p < 0.0001). (K) Eight-week-old male K18-hACE2 mice were inoculated by the intranasal route with 10³ PFU of SARS-CoV-2. At 2 (D+2) dpi, mice were given 200 μ g of COV2-2072 or COV2-2072 LALA-PG by intraperitoneal injection and survival was monitored (n = 8–10, 2 experiments; Mantel-Cox log-rank test for survival: *p < 0.05, **p < 0.01, ****p < 0.0001). See also Figure S1.



(legend on next page)

our results with intact COV2-2050 suggest that Fc engagement by Fc γ R and/or complement can induce distinct and protective transcriptional programs in the SARS-CoV-2-infected lung depending on the timing of therapy.

Monocytes and CD8⁺ T cells are required for optimal therapeutic activity of neutralizing mAb

Fc engagement of Fc γ Rs on innate immune cells including macrophages, monocytes, natural killer (NK) cells, and neutrophils can lead to antibody-dependent cellular cytotoxicity (ADCC) or phagocytosis (ADCP), modulation of inflammatory mediators produced by these cells, and enhanced adaptive immunity (Bournazos et al., 2020; Lu et al., 2018). To determine which immune cells contributed to the antibody-mediated protection observed *in vivo*, we depleted Ly6C^{hi} monocytes (anti-CCR2) (Figures 6A and S6), natural killer (NK) cells (anti-NK1.1) (Figures 6B and S6), mature neutrophils (anti-Ly6G) (Figures 6C and S6) or CD8⁺ T cells (anti-CD8) (Figures 6D and S6) in combination with COV2-2050 treatment at D+1. Depletion of mature neutrophils, NK cells, or CD8⁺ T cells had no impact on weight loss in the presence of COV2-2050 or the isotype control mAb (Figures 6B–6D). When monocytes were depleted, COV2-2050 failed to prevent the weight loss phenotype seen in non-depleted, COV2-2050-treated mice (Figure 6A). Monocyte depletion during COV2-2050 therapy also was associated with a loss of improvement in lung pathology (Figure 6E) and higher levels of some cytokines and chemokines (CXCL10, G-CSF, IL-6, IFN- γ , and CCL2) compared to treatment with COV2-2050 without cell depletion (Figures 6F and S7). As expected, the numbers of monocytes and CD11b⁺ DCs in the lung were lower after anti-CCR2 treatment, but other cell subsets were unaffected. Because these myeloid cells show a less inflammatory phenotype after COV2-2050, but not COV2-2050 LALA-PG, treatment (Figures 4G and 4H), Fc γ R engagement may shape cellular responses to limit immunopathology independently of viral clearance.

Although monocyte depletion in combination with COV2-2050 treatment resulted in increased weight loss, it was not associated with changes in viral RNA levels at 8 dpi (Figure 6H), suggesting that Fc interactions with monocytes do not contribute directly to viral clearance. In contrast, and despite no difference in weight loss, when CD8⁺ T cells were depleted, COV2-2050 treatment less efficiently reduced viral RNA levels (Figure 6H). These data in conjunction with data showing increased CD8⁺ T cell activation following COV2-2050 D+1

treatment (Figure 4F) suggest that Fc effector functions can enhance CD8⁺ T cell responses, possibly through increased antigen presentation (Bournazos et al., 2020), to mediate viral clearance.

Fc effector functions enhance the therapeutic activity of neutralizing mAbs against SARS-CoV-2 in Syrian hamsters

To validate the requirement for Fc effector functions in therapeutic antibody protection, we used a golden Syrian hamster model. This animal model supports viral replication with corresponding weight loss and interstitial pneumonia (Rosenke et al., 2020; Sia et al., 2020) and has been used to evaluate mAb-based therapies and vaccines (Liu et al., 2020; Rogers et al., 2020; Tostanoski et al., 2020) against SARS-CoV-2. We inoculated 7-month-old hamsters with SARS-CoV-2 (5 \times 10⁵ PFU, 2019 n-CoV/USA_WA1/2020 strain) by the intranasal route and then delivered a single 1-mg dose of COV2-2050 or COV2-2050 LALA-PG mAbs at 1 dpi (D+1) by intraperitoneal injection. Consistent with observations in mice, passive transfer of intact COV2-2050 prevented weight loss compared to isotype mAb-treated animals at 5 and 6 dpi, and this protection was lost in animals treated with the COV2-2050 LALA-PG variant (Figure 7A). Furthermore, hamsters treated with intact COV2-2050, but not COV2-2050 LALA-PG, showed reductions in viral RNA levels at 6 dpi (Figure 7B). The improved viral burden with COV2-2050 was associated with lower levels of the inflammatory mediators *Cxcl10*, *Ccl2*, *Ccl3*, *Ccl5*, and *Ifit3* (Figure 7C). Thus, therapeutic efficacy following neutralizing mAb administration also depends on Fc interactions in a second, relevant small animal model.

DISCUSSION

Neutralizing mAbs against SARS-CoV-2 are a promising option for the treatment of COVID-19 and have demonstrated efficacy in a variety of pre-clinical models (Abraham, 2020; Marovich et al., 2020). Antibody programs have reported encouraging results in clinical trials (Chen et al., 2021), and the Food and Drug Administration (FDA) granted Emergency Use Authorization to the anti-SARS-CoV-2 S mAbs bamlanivimab and casirivimab/imdevimab for mild-to-moderately ill, high-risk patients. However, the mechanisms of protection *in vivo* have not been fully investigated and are assumed to be related to the neutralizing capacity of the mAb. In this study, we examined the contributions of Fc effector functions for clinical and virological

Figure 4. Fc effector functions of a neutralizing antibody modulate the immune responses to SARS-CoV-2 infection

Eight-week-old K18-hACE2 mice were inoculated by the intranasal route with 10⁹ PFU of SARS-CoV-2. At 1 (D+1) or 2 (D+2) dpi, mice were given 200 μ g of COV2-2050 or COV2-2050 LALA-PG by intraperitoneal injection. Naive animals were mock-infected with sterile PBS.

(A) Hematoxylin and eosin staining of lung sections at 8 dpi or from a mock-infected animal. Images show low- (top; scale bars, 1 mm), medium-power (middle; scale bars, 200 μ m), and high-power (bottom; scale bars, 50 μ m). Representative images from n = 5 per group.

(B) Heat-maps of cytokine levels in lung tissue of SARS-CoV-2-infected mice at 8 dpi. Fold-change was calculated compared to mock-infected animals, and log₂(fold-change) was plotted in the corresponding heat-map (3 experiments, n = 5 per group except naive (n = 2), statistics reported in Figure S2).

(C and D) Flow cytometric analysis of cells harvested from BAL fluid at (C) 4 and (D) 8 dpi (2 experiments, n = 4–8 per group; Bars, mean number of cells). Gating scheme in Figure S3.

(E–H) Flow cytometric analysis of lung tissues at 8 dpi. Gating scheme in Figure S3. (E) Number of immune cells harvested from the right inferior lobe. Bars, mean number of cells. (F) Proportion of CD44⁺CD62L[−] CD8⁺ T cells. (G and H) Proportion of CD80⁺, CD86⁺, or TNF α ⁺iNOS⁺ monocytes or CD11b⁺ DCs. Bars, mean percentage of positive cells. (E–H) Two experiments, n = 3–8 per group. (C–H) One-way ANOVA with Dunnett's test; *p < 0.05, **p < 0.01, ****p < 0.0001).

See also Figures S2 and S3.

protection in murine and hamster models of SARS-CoV-2 infection. Whereas intact Fc effector functions were not required when neutralizing mAbs were given as prophylaxis, for several anti-RBD human mAbs tested in mice, a functional Fc region was required for optimal protection as post-exposure therapy. This enhanced therapeutic efficacy of intact compared to their LALA-PG IgG loss-of-function Fc region variants correlated with decreased viral burden, improved pulmonary function, diminished inflammatory responses, and preserved tissue repair processes. In mice, cell depletion studies identified both monocytes and CD8⁺ T cells as key immune cell type for antibody-dependent clinical and virological protection.

Fc effector function interactions were not required when neutralizing mAbs were administered as prophylaxis, suggesting their mechanism of protection in a pre-exposure setting depends largely on the neutralizing capacity of the antibody to prevent initial viral infection and limit dissemination. In the stringent K18-hACE2 model of SARS-CoV-2 pathogenesis (Golden et al., 2020; Winkler et al., 2020), we defined minimum serum neutralizing titers (NT₅₀) and concentrations of 104 and 212 ng/mL for the prevention of weight loss and 381 and 851 ng/mL for reduction of viral burden in the lung. Establishing serum correlates of protection for mAb- and vaccine-based therapies in pre-clinical models is an important step for translation and analysis of human studies. Levels of neutralizing antibody required for protection against disease in humans might be lower due to the slower kinetics of disease pathogenesis and/or a longer half-life of the antibody. Last, even at sub-protective doses of intact COV2-2050, we did not observe evidence of antibody-dependent enhancement (ADE) of infection or immune enhancement, consistent with other studies (Baum et al., 2020; Cao et al., 2020; Halstead and Katzelnick, 2020; Laczkó et al., 2020; Luo et al., 2018; Qin et al., 2006; Rogers et al., 2020).

In contrast to prophylactic administration, Fc effector functions were required for optimal efficacy when neutralizing mAbs were administered as post-exposure therapy. The treatment window to reduce viral RNA levels in the lung in the stringent K18-hACE2 model was essentially 1 day, suggesting that antibody therapy optimally should be given prior to the peak of viral replication, which is 2 dpi in this model (Winkler et al.,

2020). The narrow therapeutic window for reducing viral load we observed could be related to the stoichiometry of antibody binding required for virus neutralization (Pierson and Diamond, 2015), such that when too much viral antigen is present in the lung, neutralization is limited. Against this hypothesis, infectious virus levels were neutralized and equivalently low at 4 dpi in mice treated with intact or LALA-PG versions of antibodies at 1 or 2 dpi, although it is possible that this effect is due to *ex vivo* neutralization of virus after tissue homogenization (Subbarao et al., 2004; Wells et al., 1981). More likely, the Fc effector functions of neutralizing antibodies serve to clear SARS-CoV-2 infected cells through enhanced antigen presentation and induction of antigen-specific CD8⁺ T cell responses (Bournazou et al., 2020). Although the precise mechanism awaits delineation, our data suggest that in the post-exposure therapeutic setting, the neutralizing activity of most RBD-specific antibodies may no longer be sufficient for optimal protection, and additional mechanisms mediated by Fc effector functions are required. We acknowledge that some anti-RBD mAbs with superior neutralizing activity *in vivo* may confer protection in the absence of Fc effector functions, at least at early time points post-infection. However, at later time points, once infection is established and high amounts of viral RNA are produced in the lung, Fc effector functions of even “elite” neutralizing mAbs may be needed for immune modulation and the most beneficial clinical outcome.

With the lower levels of viral RNA in lungs of mice treated at 1 dpi with COV2-2050 but not COV2-2050 LALA-PG, we also found reduced levels of pro-inflammatory cytokines, immune cell infiltration, and expression of genes downstream of type I IFN and NF- κ B-dependent signaling pathways. Transcriptional signatures associated with COV2-2050 therapy also included enrichment of gene sets involved in tissue repair processes. This same pattern of expression was observed in naive mice, suggesting that Fc effector functions of antibodies may limit virus-induced perturbations and maintain certain reparative homeostatic transcriptional programs. Remarkably, administration of neutralizing mAb at 2 or 3 dpi still improved survival in an Fc-dependent manner without substantive reduction in viral RNA levels. The gene signatures uniquely associated with intact COV2-2050 mAb administration at 2 dpi differed from those at 1 dpi, as they showed reduced

Figure 5. Distinct transcriptional signatures in the lung are associated with COV2-2050 with intact Fc effector functions

RNA sequencing analysis from the lung homogenates of naive K18-hACE2 mice or mice infected with SARS-CoV-2 infection at 8 dpi. At 1 (D+1) or 2 (D+2) dpi, mice were given 200 μ g of COV2-2050 or COV2-2050 LALA-PG by intraperitoneal injection (n = 5 per group except naive where n = 6).

(A) Three-dimensional map from principal component analysis (PCA) of the RNA sequencing data in the study. The PCA has been performed using 12,157 unique genes with count per million reads ≥ 1 in at least 5 of the study samples (n = 31). Each group is represented by an ellipse and the color-matched solid circle, which is the centroid of each group. The size of the ellipse is the centroid with 1 SD. The dashed red lines with numbers indicate the spatial distance between centroids of the 6 groups, which is calculated by using the three-dimensional coordinates for the centroids.

(B) Venn diagrams of overlapping genes identified in differential expression analysis when comparing isotype control, COV2-2050 D+1, and COV2-2050 LALA-PG D+1 or isotype control, COV2-2050 D+2, and COV2-2050 LALA-PG D+2. Numbers in the parenthesis under each comparison indicate the number of differentially expressed genes (fold-change ≥ 2 at p < 0.05) followed by the proportion that are up- or downregulated.

(C) The significantly enriched biological themes defined by a “CompBio” pathway analysis tool comparing treatments with isotype control mAb, COV2-2050 (D+1 and D+2), and COV2-2050 LALA-PG (D+1 and D+2). Only those themes enriched in at least two comparisons are displayed. These themes either are upregulated (brown) or downregulated (blue) in the COV2-2050-treated group (at D+1 or D+2) when compared to the isotype control or COV2-2050 LALA-PG-treated groups. A comparison between the naive and isotype mAb-treated animals for each identified theme also was made. The scaled color blocks represent the mean fold-change of enriched genes with an enrichment score of 10 or greater in the comparison.

(D) Heatmaps of selected relevant biological themes (RIG-I/MDA-5 mediated signaling, TNF receptor-associated signaling, actinomyosin cell adhesion, Rho GTPases related signaling) enriched in COV2-2050 D+1 versus isotype control or COV2-2050 LALA-PG D+1. Genes shown are common in the pair of comparisons having an enrichment score of 100 or greater.

See also Figures S4 and S5 and Tables S1 and S2.

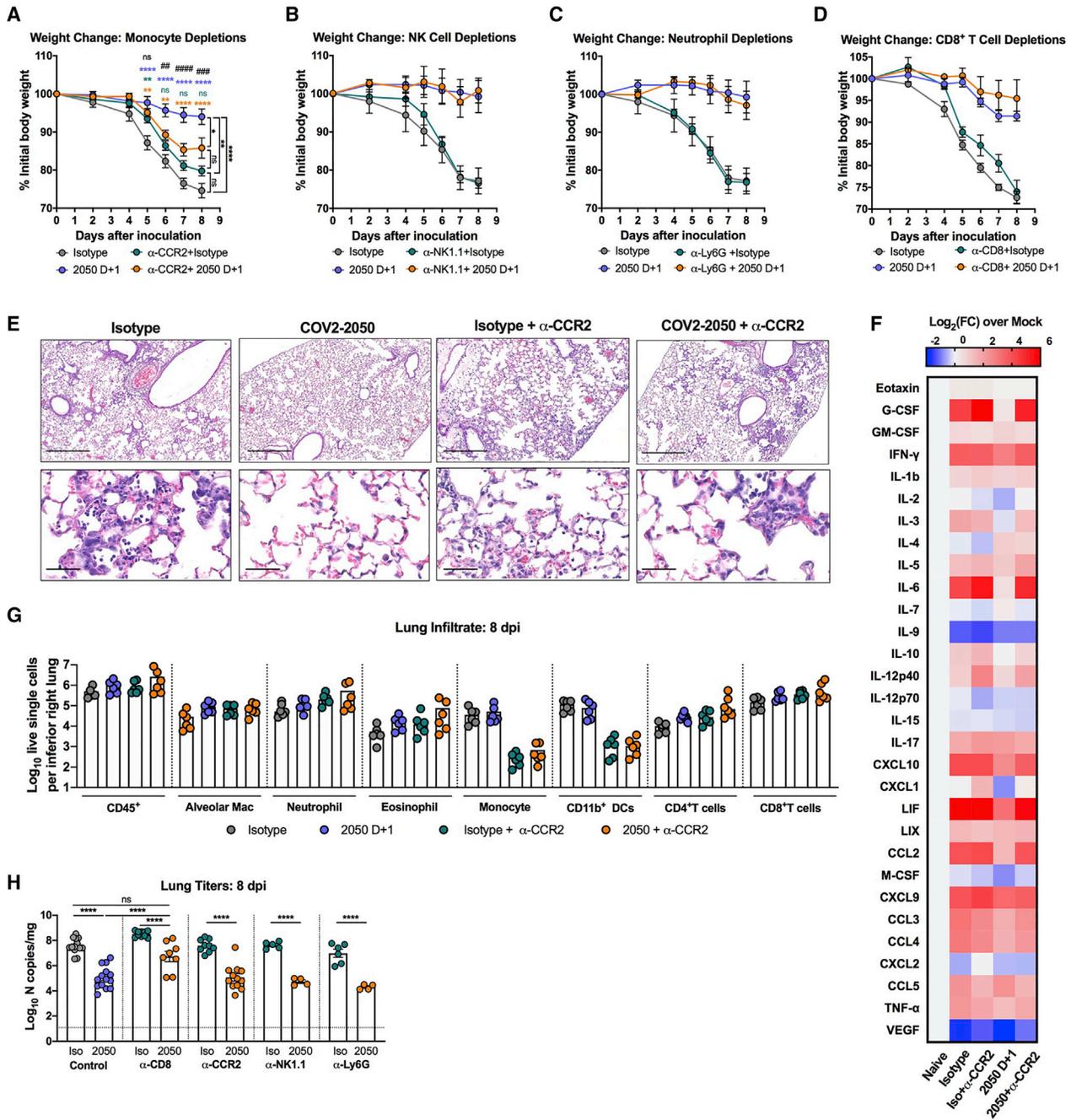


Figure 6. Monocytes and CD8⁺ T cells are necessary for protection following mAb therapy

(A–H) Eight-week-old K18-hACE2 mice received anti-CCR2 (50 μ g/dose) (A), anti-NK1.1 (200 μ g/dose) (B), anti-Ly6G (250 μ g/dose) (C), or anti-CD8 (500 μ g/dose) (D) or corresponding isotype controls at D–1, D+1, D+3, D+5, and D+7 relative to SARS-CoV-2 infection. At D0, animals were inoculated by the intranasal route with 10^3 PFU of SARS-CoV-2. At 1 (D+1) dpi, mice were administered 200 μ g of COV2-2050 by intraperitoneal injection. (A–D) Weight change (mean \pm SEM; n = 8–12, 2–3 experiments: two-way ANOVA with Sidak’s post-test: *p < 0.001, ***p < 0.001, ****p < 0.0001; comparison to the isotype control mAb-treated group).

(E) Hematoxylin and eosin staining of lung sections at 8 dpi. Images show low-power (top; scale bars, 250 μ m), or high-power (bottom; scale bars, 50 μ m). Representative images from n = 3 per group.

(F) Heat-maps of cytokine levels in lung tissues of SARS-CoV-2-infected mice at 8 dpi. For each cytokine, fold-change was calculated compared to mock-infected animals, and log₂ (fold-change) was plotted in the corresponding heat-map (3 experiments, n = 5–6 per group except naive (n = 4)).

(G) Flow cytometric analysis of lung tissues at 8 dpi (2 experiments, n = 8 per group; Bars, mean number of cells).

(H) Viral RNA levels at 8 dpi in the lung (n = 4–12, 2–3 experiments: one-way ANOVA with Turkey’s post-test: ns not significant, ****p < 0.0001, comparison to the isotype control mAb-treated group).

See Figures S6 and S7.

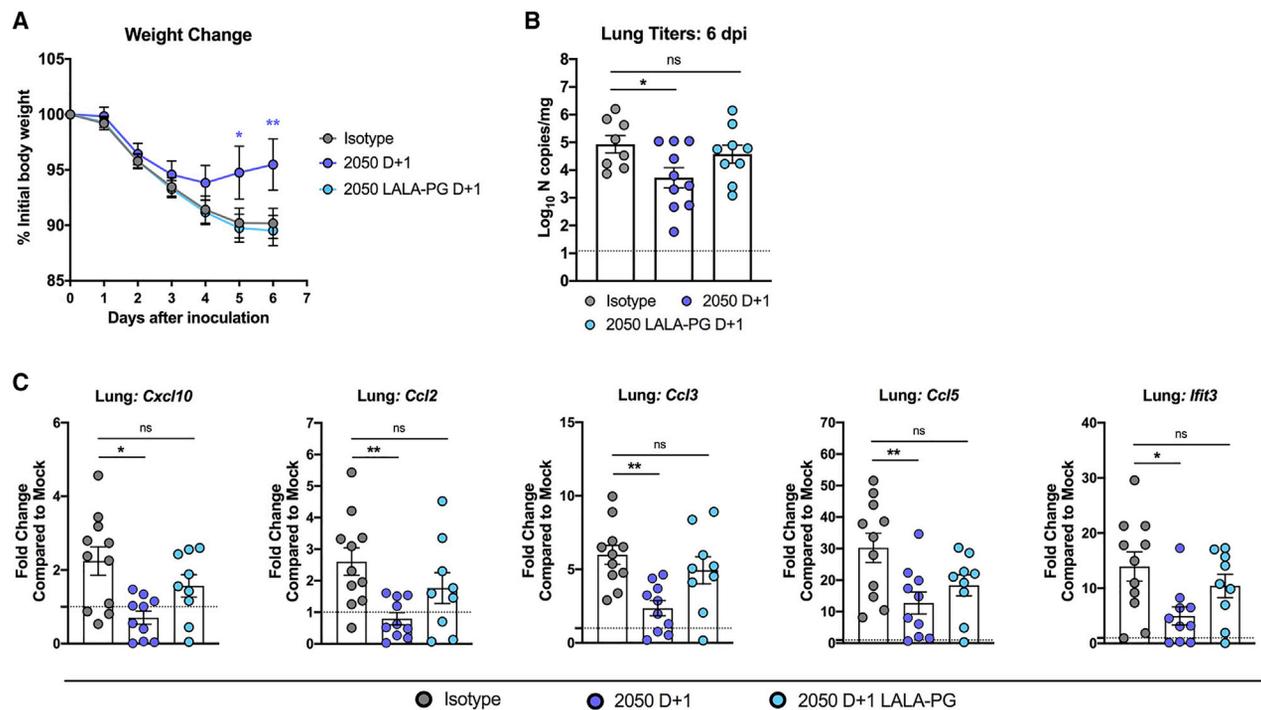


Figure 7. Fc effector functions enhance the therapeutic activity of neutralizing antibodies against SARS-CoV-2 in Syrian hamsters

(A–C) Seven-month-old female Syrian hamsters were inoculated by the intranasal route with 5×10^5 PFU of SARS-CoV-2. At 1 dpi (D+1), hamsters were given 1 mg of COV2-2050 or COV2-2050 LALA-PG by intraperitoneal injection.

(A) Weight change (mean \pm SEM; $n = 8$ –10, 2 experiments: two-way ANOVA with Sidak's post-test: * $p < 0.05$, ** $p < 0.01$; comparison to the isotype control mAb-treated group).

(B) Viral RNA levels at 6 dpi in the lung ($n = 8$ –10, 2 experiments: one-way ANOVA with Turkey's post-test: ns not significant, * $p < 0.05$, comparison to the isotype control mAb-treated group).

(C) Fold change in gene expression of indicated cytokines and chemokines in lung homogenates. Data are normalized to *Rpl18* and compared to naive controls (2 experiments, $n = 8$ –10 per group, one-way ANOVA with Dunnett's test; * $p < 0.05$; ** $p < 0.01$). Dotted lines indicate the mean cytokine or chemokine transcript levels in naive hamsters.

expression of genes involved in neutrophil activation, IL-6 signaling, and metalloproteinase-mediated extracellular matrix remodeling pathways. Altogether, these results suggest that at least some neutralizing mAbs protect *in vivo* through multiple Fc-dependent mechanisms including both viral clearance and modulation of the immune response to enhance resolution of inflammation.

The importance of Fc effector functions for antibody efficacy *in vivo* has been illustrated in other viral models (Bournazos et al., 2020; DiLillo et al., 2014, 2016; Fox et al., 2019; Hessel et al., 2007; Li et al., 2017; Liu et al., 2017; Vogt et al., 2011), although the specific cells mediating protection have been characterized in relatively few cases (Fox et al., 2019; He et al., 2017; Laidlaw et al., 2013). Our immune cell depletion studies showed that monocytes, but not neutrophils or NK cells were necessary for mAb-dependent clinical protection and diminished pro-inflammatory cytokine responses following SARS-CoV-2 infection in mice, although monocyte depletion did not affect viral burden. Circulating CCR2⁺ monocytes can differentiate into myeloid cell subsets including interstitial macrophages and monocyte-derived CD11b⁺ dendritic cells following migration to the lung (Jakubzick et al.,

2017). The phenotype of these myeloid cells in the lung can vary from TNF/iNOS producing DCs (Tip-DCs) (Serbina et al., 2003) to interstitial macrophages involved in tissue repair. Fc γ R-mediated signaling can impact myeloid cell function, differentiation, and polarization (Clynes et al., 1999; Dhodapkar et al., 2007). Moreover, myeloid cell dysregulation has been linked to severe COVID-19 in humans (Schulte-Schrepping et al., 2020), and blocking of TNF- α and IFN γ reduces lethality following SARS-CoV-2 infection in K18-hACE2 mice (Karki et al., 2021). Administration of intact COV2-2050 but not COV2-2050 LALA-PG, even at D+2, when a difference in viral titers was not detected between these two groups, reduced the proportion of monocytes and monocyte-derived CD11b⁺DCs producing TNF- α and iNOS without impacting the total number of myeloid cells in the lung. Thus, Fc effector engagement on monocyte-derived cells in the lung may not directly impact viral clearance, but rather improve clinical outcome through reprogramming of the immune response.

In comparison, CD8⁺ T cells were required for Fc-effector function-dependent reductions in viral burden. Phagocytosis of IgG immune complexes by activating Fc γ Rs on antigen-presenting cells, such as dendritic cells, can enhance antigen

processing (Amigorena et al., 1992; Bergtold et al., 2005; Hoffmann et al., 2012) and accelerate CD8⁺ T cell priming and cytolysis of virally infected cells (Lu et al., 2016; Niessl et al., 2020). Our data suggest a compartmentalization of cellular interactions mediated by antibody Fc regions in the context of SARS-CoV-2 infection: myeloid cell interactions regulate inflammation, and enhanced CD8⁺ T cell activity promotes clearance of virus-infected cells. Engineering of the Fc domain sequences to optimize selected effector functions (Bournazos et al., 2020; Lazar et al., 2006; Saunders, 2019) on specific cell types could augment the therapeutic activity of neutralizing mAbs against SARS-CoV-2.

Limitations of study

Consistent with previous findings (Schäfer et al., 2021), our results highlight the importance of Fc effector functions of antibody in two animal models of SARS-CoV-2 infection. Although LALA-PG mutations abrogate binding to both Fc γ Rs and C1q, it remains unknown which of these effector molecules or particular receptors (e.g., Fc γ RI, Fc γ RIII, or Fc γ RIV) dominantly associate with loss of anti-SARS-CoV-2 mAb therapeutic activity. Confirmatory studies also are needed in non-human primates, a model that more closely recapitulates human mAb dosing kinetics *in vivo*. Although we used human IgG1 anti-SARS-CoV-2 antibodies in mice and hamsters, we validated our findings in a species-homologous system with chimeric versions of COV2-2072 with murine Fc moieties in mice. Fc γ R expression patterns on immune cells and human IgG-mouse/hamster Fc γ R interactions or even mouse IgG-mouse Fc γ R interactions may not fully recapitulate patterns observed with human IgG, human Fc γ Rs, and human cells (Bournazos et al., 2014a). Although animals expressing human Fc γ Rs exist (Smith et al., 2012), they have not been crossed to the K18-hACE2 transgenic mice. Future studies with human Fc γ R mice, mouse-adapted strains of SARS-CoV-2 (Leist et al., 2020), and additional human anti-SARS-CoV-2 mAbs may be useful for confirmation of the phenotypes described.

The requirement of Fc effector functions for protection by other anti-SARS-CoV-2 mAbs warrants more study. Indeed, *in vitro* neutralization potency does not uniformly correlate with *in vivo* protection (Schäfer et al., 2021), and compromised Fc effector functions correlates with increased mortality in humans (Zohar et al., 2020). Our studies used neutralizing antibodies that bind the RBD on S and block ACE2 receptor engagement (Zost et al., 2020b). Other neutralizing mAbs against the N-terminal domain or some classes of non-neutralizing mAbs also may require Fc effector functions for therapeutic activity. Even among anti-RBD mAbs, we observed variation, as clinical protection with COV2-2381 and COV2-2072 was only partially Fc-dependent when administered as post-exposure therapy at D+1, yet virological protection was lost with COV2-2072 LALA-PG, but not COV2-2381 LALA-PG administration. In conclusion, our study highlights the contributions of Fc effector functions for therapeutic activity of neutralizing mAbs through reductions in viral burden and/or immunopathology. Accordingly, the design of antibody-based combinations against SARS-CoV-2 likely should optimize both neutralization and multiple Fc effector function pathways to enhance the win-

dow of treatment and provide the greatest antiviral and clinical protection.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.cell.2021.02.026>.

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AUTHOR CONTRIBUTIONS

P.G., S.J.Z., R.E.S., and R.H.C. designed and generated human antibodies. E.S.W., A.L.B., and R.E.C. performed mouse experiments. E.S.W. and Z.C. analyzed viral burden and performed immune cell processing. E.S.W. and A.L.B. performed pulmonary mechanics analysis. E.S.W. and J.B.C. performed neutralization analyses. J.Y. and R.H. performed RNA sequencing

and analysis. H.J., Y.H., J.D.A., and T.M.R. performed hamster experiments. T.L.D. and A.C.M.B. validated reagents for hamster gene expression. M.M. provided anti-CCR2 antibody. J.E.C., T.M.R., A.C.M.B., and M.S.D. obtained funding and supervised research. E.S.W. and M.S.D. wrote the initial draft, with the other authors providing editorial comments.

DECLARATION OF INTERESTS

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
COV2-2050	Zost et al., 2020b	N/A
COV2-2050 LALA-PG	This paper	N/A
COV2-2072	Zost et al., 2020b	N/A
COV2-2072 LALA-PG	This paper	N/A
COV2-3025	Zost et al., 2020b	N/A
COV2-3025 LALA-PG	This paper	N/A
COV2-2381	Zost et al., 2020b	N/A
COV2-2381 LALA-PG	This paper	N/A
COV2-2072 mlgG1	This paper	N/A
COV2-2072 mlgG1 D265A	This paper	N/A
COV2-2072 mlgG2a	This paper	N/A
DENV-2D22	Fibriansah et al., 2015	N/A
HRP conjugated goat anti-mouse IgG (H + L)	Jackson ImmunoResearch	115-035-062; RRID: AB_2338504
CR3022, anti-SARS-CoV-2 mAb	Yuan et al., 2020	N/A
Goat anti-human lambda, mouse ads-UNLB	Southern Biotech	RRID:AB_2795760
Goat anti-human kappa, mouse ads-UNLB	Southern Biotech	RRID:AB_2795728
Goat anti-human IgG Fc, Multispecies ads-HRP	Southern Biotech	RRID:AB_2795580
BUV395 anti-CD45	BD BioSciences	RRID:AB_2651134
Brilliant Violet 650 anti-mouse Ly-6G	BioLegend	RRID:AB_2565881
Pacific Blue anti-mouse Ly-6C	BioLegend	RRID:AB_1732090
PE/Dazzle 594 anti-mouse/human CD11b	BioLegend	RRID:AB_2563648
Brilliant Violet 711 anti-mouse CD3 Antibody	BioLegend	RRID:AB_2563945
Brilliant Violet 421 anti-mouse CD3 Antibody	BioLegend	RRID:AB_10900227
APC anti-mouse CD8b Antibody	BioLegend	RRID:AB_2562774
PE anti-mouse/rat XCR1 Antibody	BioLegend	RRID:AB_2563843
Brilliant Violet 785 anti-mouse CD4 Antibody	BioLegend	RRID:AB_2565843
BV421 Rat Anti-Mouse CD335	BD BioSciences	RRID:AB_2737837
FITC anti-mouse NK-1.1 Antibody	BioLegend	RRID:AB_313393
PE-Cy7 Hamster Anti-Mouse CD11c	BD BioSciences	RRID:AB_2033997
Alexa Fluor® 700 anti-mouse I-A/I-E	BioLegend	RRID:AB_493726
eBioscience Fixable Viability Dye eFluor 506	Thermo Fisher	65-0866-14
FITC Anti-Neutrophil (Anti-Ly6B) Antibody	Abcam	RRID:AB_881408
TruStain FcX (anti-mouse CD16/32) Antibody	BioLegend	RRID:AB_1574973
Brilliant Violet 711 anti-mouse/human CD45R/B220 Antibody	BioLegend	RRID:AB_2563491
Brilliant Violet 711 anti-mouse/human CD11b Antibody	BioLegend	RRID:AB_2563310
FITC anti-mouse CD24 Antibody	BioLegend	RRID:AB_312839
PerCP/Cyanine5.5 anti-mouse Ly-6G Antibody	BioLegend	RRID:AB_1877271
PE/Cyanine7 anti-mouse CD64 (FcγRI) Antibody	BioLegend	RRID:AB_2563904
PE Rat Anti-Mouse Siglec-F	BD BioSciences	RRID:AB_394341
Brilliant Violet 605 anti-mouse CD80 Antibody	BioLegend	RRID:AB_11126141
PE/Cyanine5 anti-mouse CD86 Antibody	BioLegend	RRID:AB_493602

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Brilliant Violet 605 anti-mouse CD62L Antibody	BioLegend	RRID:AB_2563058
APC/Cyanine7 anti-mouse/human CD44 Antibod6	BioLegend	RRID:AB_830785
PE/Cyanine7 anti-mouse TNF- α Antibody	BioLegend	RRID:AB_2256076
Brilliant Violet 421 anti-mouse CD64 (Fc γ RI) Antibody	BioLegend	RRID:AB_2562694
APC-Cy7 Hamster Anti-Mouse CD11c	BD BioSciences	RRID:AB_10611727
NOS Monoclonal Antibody (CXNFT), APC, eBioscience	Thermo Fisher	RRID:AB_2573244
InVivoMAb rat IgG2b isotype control, anti-keyhole limpet hemocyanin	BioXCell	RRID:AB_1107780
InVivoMAb anti-mouse Ly6G	BioXCell	RRID:AB_1107721
InVivoMAb rat IgG2a isotype control, anti-trinitrophenol	BioXCell	RRID:AB_1107769
InVivoPlus anti-mouse CD8 α	BioXCell	RRID:AB_10950145
InVivoMAb anti-mouse NK1.1	BioXCell	RRID:AB_1107737
InVivoMAb mouse IgG2a isotype control, unknown specificity	BioXCell	RRID:AB_1107771
anti-CCR2 (clone MC-21)	Matthias Mack	N/A
Chemicals, peptides, and recombinant proteins		
Recombinant Mouse Fc gamma RIIB/CD32b Protein, CF	R and D Systems	1460-CD-050
Recombinant Mouse Fc gamma RI/CD64 Protein, CF	R and D Systems	2074-FC-050
Recombinant Mouse Fc γ R4/CD16-2 Protein, CF	R and D Systems	1974-CD-050
Critical commercial assays		
GIBCO ExpiCHO Expression System	ThermoFisher Scientific	A29133
MagMAX-96 Viral RNA Isolation Kit	Thermo Fisher	AM1836
MagMAX mirVana Total RNA Isolation Kit	Thermo Fisher	A27828
TaqMan RNA-to-Ct 1-Step Kit	Thermo Fisher	4392939
Mouse Cytokine Array / Chemokine Array 44-Plex (MD44)	Eve Technologies	MD44
Mouse Cytokine Array / Chemokine Array 31-Plex (MD31)	Eve Technologies	MD31
Ambion DNase I (RNase-free)	Thermo Fisher	AM2222
High-Capacity cDNA Reverse Transcription Kit with RNase Inhibitor	Thermo Fisher	4374966
Foxp3/Transcription Factor Staining Buffer Set	Thermo Fisher	00-5523-00
Endotoxin PTS201F cartridge	Charles River	PTS201F
SeqPlex RNA Amplification Kit	Millipore Sigma	EQR-500RXN
Virus and bacterial strains		
SARS-CoV-2 (strain 2019 n-CoV/USA_WA1/2020)	CDC/BEI Resources	NR52281
Experimental models: Cell lines		
Vero CCL-81	ATCC	CCL-81; RRID: CVCL_0059
Vero E6	ATCC	CRL-1586; RRID:CVCL_0574
Hamster: ExpiCHO-S	ThermoFisher Scientific	Cat#A29127; RRID: CVCL_5J31
Vero Furin	Mukherjee et al., 2016	N/A
Experimental models: Organisms/strains		
Mouse: B6.Cg-Tg(K18-ACE2)2PrImn/J	Jackson Laboratory	Cat# 034860; RRID:IMSR_JAX:03486
Golden Syrian Hamster	Charles River Laboratory	Strain Code: 049
Oligonucleotides		
SARS-CoV-2 N F: 5'-ATGCTGCAATCGTGCTACAA-3'	32838945	N/A
SARS-CoV-2 N R: 5'-GACTGCCGCCTCTGCTC-3'	32838945	N/A
SARS-CoV-2 N Probe: 5'-/56-FAM/TCAAGGAAC/ZEN/AACATTGCCAA/3IABkFQ/-3'	32838945	N/A
SARS-CoV-2 RNA ISH probe (S gene)	Advanced Cell Diagnostics	Cat# 4848561
Hamster <i>Rp18</i> TaqMan F: GTTTATGAGTCGCACTAACCG	This paper	N/A
Hamster <i>Rp18</i> TaqMan R: TGTCTCTCGGCCAGGAA	This paper	N/A

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Hamster <i>Rpl18</i> TaqMan Probe : YAK-TCTGTCCCTGTCCCGGATGATC-BBQ	This paper	N/A
Hamster <i>Cxcl10</i> TaqMan F : GCCATTCATCCACAGTTGACA	This paper	N/A
Hamster <i>Cxcl10</i> TaqMan R : CATGGTGCTGACAGTGGAGTCT	This paper	N/A
Hamster <i>Cxcl10</i> TaqMan Probe : 6FAM-CGTCCCGAGCCAGCCAACGA-BBQ	This paper	N/A
Hamster <i>Ccl2</i> TaqMan F : CTCACCTGCTGCTACTCATT	This paper	N/A
Hamster <i>Ccl2</i> TaqMan R : CTCTCTCTTGAGCTTGGTGATG	This paper	N/A
Hamster <i>Ccl2</i> TaqMan Probe : 6FAM-CAGCAGCAAGTGTCCTCCAAAGAAGC-BBQ	This paper	N/A
Hamster <i>Ccl3</i> TaqMan F : CTCACCTGCTGCTACTCATT	This paper	N/A
Hamster <i>Ccl3</i> TaqMan R : CTCTCTCTTGAGCTTGGTGATG	This paper	N/A
Hamster <i>Ccl3</i> TaqMan Probe : 6FAM-CAGCAGCAAGTGTCCTCCAAAGAAGC-BBQ	This paper	N/A
Hamster <i>Ccl5</i> TaqMan F : TGCTTTGACTACCTCTCCTTTAC	This paper	N/A
Hamster <i>Ccl5</i> TaqMan R : GGTTCTTCGGGTGACAAA	This paper	N/A
Hamster <i>Ccl5</i> TaqMan Probe : 6FAM-TGCCTCGTGTTCACATCAAGGAGT-BBQ	This paper	N/A
Hamster <i>Ifit3</i> TaqMan F : CTGATACCAACTGAGACTCCTG	This paper	N/A
Hamster <i>Ifit3</i> TaqMan R : CTTCTGTCTTCTCGGATTAG	This paper	N/A
Hamster <i>Ifit3</i> TaqMan Probe : 6FAM-ACCGTACAGTCCACACCCAACCTT-BBQ	This paper	N/A

Software and algorithms

FlowJo	FlowJo, LLC	v10
GraphPad Prism	GraphPad	v 9.0.0
Biorender	biorender.com	N/A
flexiWare	SCIREQ Inc.	v8.1.3
STAR program	Dobin et al., 2013	v 2.5.1a
EdgeR	Robinson et al., 2010	N/A
limma	Ritchie et al., 2015	N/A
RSeQC	Liao et al., 2014	v2.6.2
Nanozoomer Digital Pathology	Hamamatsu	v2

Recombinant DNA

Plasmid: rCOV2-2050 in pTwist-mCis_hG1	Zost et al., 2020b	N/A
Plasmid: rCOV2-2050 in pTwist-mCis_hG1 LALA-PG	This study	N/A
Plasmid: rCOV2-2381 in pTwist-mCis_hG1	Zost et al., 2020b	N/A
Plasmid: rCOV2-2381 in pTwist-mCis_hG1 LALA-PG	This study	N/A
Plasmid: rCOV2-2072 in pTwist-mCis_hG1	Zost et al., 2020b	N/A
Plasmid: rCOV2-2072 in pTwist-mCis_hG1 LALA-PG	This study	N/A
Plasmid: rCOV2-3025 in pTwist-mCis_hG1	Zost et al., 2020b	N/A
Plasmid: rCOV2-3025 in pTwist-mCis_hG1 LALA-PG	This study	N/A
Plasmid: rCOV2-2072 in pTwist-mCis_mG1	This study	N/A
Plasmid: rCOV2-2072 in pTwist-mCis_mG1 D265A	This study	N/A
Plasmid: rCOV2-2072 in pTwist-mCis_mG2a	This study	N/A
Plasmid: rCOV2-2072 in pTwist-mCis_mK	This study	N/A

Deposited data

Lung RNA-Seq Data	This paper	GEO: GSE161615
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Other

HiTrap MabSelect SuRe	Cytvia	11003493
CountBright Absolute Counting Beads	Thermo Fisher	C36950

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to the Lead Contact, Michael S. Diamond (diamond@wusm.wustl.edu).

Materials availability

All requests for resources and reagents should be directed to the Lead Contact author. This includes mice, antibodies, viruses, and proteins. All reagents will be made available on request after completion of a Materials Transfer Agreement.

Data and code availability

All data supporting the findings of this study are available within the paper and are available from the corresponding author upon request. RNA sequencing datasets have been uploaded and are available at GSE161615.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Cells and viruses

Vero E6 (CRL-1586, American Type Culture Collection (ATCC)), Vero CCL81 (ATCC), and Vero-furin cells ([Mukherjee et al., 2016](#)) were cultured at 37°C in Dulbecco's Modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 10 mM HEPES pH 7.3, 1 mM sodium pyruvate, 1 × non-essential amino acids, and 100 U/ml of penicillin–streptomycin. The 2019n-CoV/USA_WA1/2019 isolate of SARS-CoV-2 was obtained from the US Centers for Disease Control (CDC). Infectious stocks were propagated by inoculating Vero CCL81 cells and collecting supernatant upon observation of cytopathic effect; debris was removed by centrifugation and passage through a 0.22 μm filter. Supernatant was aliquoted and stored at –80°C. All work with infectious SARS-CoV-2 was performed in Institutional Biosafety Committee approved BSL3 and A-BSL3 facilities at Washington University School of Medicine using appropriate positive pressure air respirators and protective equipment.

Antibodies

The human antibodies studied in this paper were isolated from blood samples from two individuals in North America with previous laboratory-confirmed symptomatic SARS-CoV-2 infection that was acquired in China. The original clinical studies to obtain specimens after written informed consent were previously described ([Zost et al., 2020b](#)) and had been approved by the Institutional Review Board of Vanderbilt University Medical Center, the Institutional Review Board of the University of Washington and the Research Ethics Board of the University of Toronto.

Mouse experiments

Animal studies were carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocols were approved by the Institutional Animal Care and Use Committee at the Washington University School of Medicine (assurance number A3381–01). Virus inoculations were performed under anesthesia that was induced and maintained with ketamine hydrochloride and xylazine, and all efforts were made to minimize animal suffering.

Heterozygous K18-hACE c57BL/6J mice (strain: 2B6.Cg-Tg(K18-ACE2)2Prlmn/J) were obtained from The Jackson Laboratory. Animals were housed in groups and fed standard chow diets. Eight- to nine-week-old mice of both sexes were administered 10³ PFU of SARS-CoV-2 by intranasal administration.

Hamster experiments

Seven-month-old female Syrian hamsters were purchased from Charles River Laboratories and housed in microisolator units. All hamsters were allowed free access to food and water and cared for under United States Department of Agriculture (USDA) guidelines for laboratory animals. Hamsters were administered with 5 × 10⁵ PFU of SARS-CoV-2 (2019-nCoV/USA-WA1/2020) by the intranasal route in a final volume of 100 μL. One day later, hamsters were administered by intraperitoneal injection COV2-2050, COV2-2050 LALA-PG, or isotype control (10 mg/kg). All hamsters were monitored for body weight loss until humanely euthanized at 6 dpi. All procedures were approved by the University of Georgia Institutional Animal Care and Use Committee (IACUC number A2020 04-024-Y1-A4). Virus inoculations and antibody transfers were performed under anesthesia that was induced and maintained with 5% isoflurane. All efforts were made to minimize animal suffering.

METHOD DETAILS

mAb production and purification

COV2-2050, COV2-2381, COV2-2072, and COV2-3025 were isolated from B cells from a SARS-CoV-2 convalescent patient and described previously ([Zost et al., 2020a, 2020b](#)). mAb 2D22 was described previously ([Fibriansah et al., 2015](#)). Sequences of mAbs that had been synthesized (Twist Bioscience) and cloned into an IgG1 monocistronic expression vector (designated as

pTwist-mCis_G1) or IgG1 monocistronic expression vector containing L234A, L235A, and P329G mutations in the Fc region of the heavy chain (designated as pTwist-mCis_LALA-PG) were used for mAb secretion in mammalian cell culture. This vector contains an enhanced 2A sequence and GSG linker that allows the simultaneous expression of mAb heavy and light chain genes from a single construct upon transfection (Chng et al., 2015). For the design of chimeric mAbs, the human kappa light chain constant region was replaced with mouse kappa light chain constant region, and the human IgG1 heavy chain constant region was exchanged for the murine IgG1, mouse IgG1 D265A, or mouse IgG2a constant region. Sequences of the light and heavy chains for these chimeric mAbs were synthesized (Twist Bioscience), cloned into separate expression vectors (designated as pTwist-mG1 and pTwist-mK), and were used for expression in mammalian cell culture. mAb proteins were produced after transient transfection using the GIBCO ExpiCHO Expression System (ThermoFisher Scientific) following the manufacturer's protocol. Culture supernatants were purified using HiTrap MabSelect SuRe columns (Cytiva, formerly GE Healthcare Life Sciences) on an AKTA Pure chromatographer (GE Healthcare Life Sciences). Purified mAbs were buffer-exchanged into PBS, concentrated using Amicon Ultra-4 50-kDa centrifugal filter units (Millipore Sigma) and stored at -80°C until use. Purified mAbs were tested routinely for endotoxin levels (found to be less than 30 EU per mg IgG). Endotoxin testing was performed using the PTS201F cartridge (Charles River), with a sensitivity range from 10 to 0.1 EU per ml, and an Endosafe Nexgen-MCS instrument (Charles River).

Recombinant Fc γ R protein ELISA

Recombinant murine Fc γ RI, Fc γ RIIb, and Fc γ RIV protein (R&D Systems) was immobilized on Maxisorp ELISA plates (Thermo Fisher) overnight in sodium bicarbonate buffer, pH 9.3. Plates were rinsed with PBS and 0.05% Tween-20 and blocked with 4% BSA for 1 h at 25°C . Intact or LALA-PG mAbs were diluted in 2% BSA and added to plates for 1 h at 25°C . After rinsing, primary mAbs were detected with horseradish peroxidase conjugated goat-anti human IgG F(ab')₂ (1:5,000 dilution, Jackson ImmunoResearch) for 1 h at 25°C . Plates were washed and developed with 3,3'-5,5' tetramethylbenzidine substrate (Thermo Fisher), stopped with 2N H₂SO₄, and read at 450 nm using a TriStar Microplate Reader (Berthold).

Human mAb serum ELISA

Goat anti-human kappa (cross-absorbed against mouse IgG) (Southern Biotech) and goat anti-human lambda (cross-absorbed against mouse IgG) (Southern Biotech) were coated onto 96-well Nunc Maxisorp flat-bottomed plates at 2 $\mu\text{g}/\text{mL}$ in coating buffer (0.1 M sodium carbonate, 0.1 M sodium bicarbonate, 0.02% sodium azide, pH 9.6) overnight at 4°C . Coating buffers were removed, and wells were blocked with 2% BSA (blocking buffer) (Thermo Fisher), for 1 h at 37°C . Heat-inactivated serum samples were diluted in blocking buffer in a separate polypropylene plate. The plates then were washed four times with PBS + 0.05% Tween-20 (PBST), followed by addition of 50 μL of respective serum dilutions, and then incubated for 1 h at 4°C . The plates were again washed four times in PBST, followed by addition of 50 μL of 1:2,000 Goat Anti-Human IgG Fc, Multi-Species (Southern Biotech). Plates were incubated at room temperature for 1 h. Plates were washed with four times in PBST, followed by 100 μL of TMB-ELISA substrate (Thermo Fisher) and incubated at room temperature for 3 to 5 min. Color development was monitored, and reactions were stopped with 50 μL of 2N H₂SO₄. Optical density (450 nm) measurements were determined using a microplate reader (Bio-Rad).

Plaque forming assay

Vero-furin cells (Mukherjee et al., 2016) were seeded at a density of 2.5×10^5 cells per well in flat-bottom 12-well tissue culture plates. The following day, medium was removed and replaced with 200 μL of 10-fold serial dilutions of the material to be titered, diluted in DMEM+2% FBS. One hour later, 1 mL of methylcellulose overlay was added. Plates were incubated for 72 h, then fixed with 4% paraformaldehyde (final concentration) in phosphate-buffered saline for 20 min. Plates were stained with 0.05% (w/v) crystal violet in 20% methanol and washed twice with distilled, deionized water.

Measurement of viral burden

Tissues were weighed and homogenized with zirconia beads in a MagNA Lyser instrument (Roche Life Science) in 1000 μL of DMEM media supplemented with 2% heat-inactivated FBS. Tissue homogenates were clarified by centrifugation at 10,000 rpm for 5 min and stored at -80°C . RNA was extracted using the MagMax mirVana Total RNA isolation kit (Thermo Scientific) on the Kingfisher Flex extraction robot (Thermo Scientific). RNA was reverse transcribed and amplified using the TaqMan RNA-to-CT 1-Step Kit (ThermoFisher). Reverse transcription was carried out at 48°C for 15 min followed by 2 min at 95°C . Amplification was accomplished over 50 cycles as follows: 95°C for 15 s and 60°C for 1 min. Copies of SARS-CoV-2 N gene RNA in samples were determined using a previously published assay (Case et al., 2020). Briefly, a TaqMan assay was designed to target a highly conserved region of the N gene (Forward primer: ATGCTGCAATCGTGCTACAA; Reverse primer: GACTGCCGCTCTGCTC; Probe: /56-FAM/TCAAGGAAC/ZEN/AACATTGCCAA/3IABkFQ/). This region was included in an RNA standard to allow for copy number determination down to 10 copies per reaction. The reaction mixture contained final concentrations of primers and probe of 500 and 100 nM, respectively.

Cytokine and chemokine mRNA measurements

RNA was isolated from lung homogenates as described above. cDNA was synthesized from DNase-treated RNA using the High-Capacity cDNA Reverse Transcription kit (Thermo Scientific) with the addition of RNase inhibitor following the manufacturer's protocol. Mouse cytokine and chemokine expression was determined using TaqMan Fast Universal PCR master mix (Thermo Scientific)

with commercial primers/probe sets specific for *IFN-g* (IDT: Mm.PT.58.41769240), *IL-6* (Mm.PT.58.10005566), *CXCL10* (Mm.PT.58.43575827), *CCL2* (Mm.PT.58.42151692), and results were normalized to *GAPDH* (Mm.PT.39a.1) levels. Fold change was determined using the $2^{-\Delta\Delta C_t}$ method comparing treated mice to naive controls. Hamster cytokine and chemokine expression was determined using TaqMan Fast Universal PCR master mix (Thermo Scientific) with primers/probe sets specific for *Rpl18* (For: GTTTATGAGTCGCACTAACCG, Rev: TGTTCTCTCGGCCAGGAA, Probe: YAK-TCTGTCCCTGTCCCGGATGATC-BBQ), *Cxcl10* (For: GCCATTCATCCACAGTTGACA, Rev: CATGGTGCTGACAGTGGAGTCT, Probe: 6FAM-CGTCCCGAGCCAGCCAACGA-BBQ), *Ccl2* (For: CTCACCTGCTGCTACTCATT, Rev: CTCTCTCTTGTAGCTTGGTGATG, Probe: 6FAM-CAGCAGCAAGTGTC CAAAGAAGC-BBQ), *Ccl3* (For: CTCACCTGCTGCTACTCATT, Rev: CTCTCTCTTGTAGCTTGGTGATG, Probe: 6FAM-CAGCAG CAAGTGTCCCAAAGAAGC-BBQ), *Ccl5* (For: TGCTTTGACTACCTCTCCTTTAC, Rev: GGTTCCCTCGGGTGACAAA, Probe: 6FAM-TGCCTCGTGTTCACATCAAGGAGT-BBQ), *Iffit3* (For: CTGATACCAACTGAGACTCCTG, Rev: CTTCTGTCCTTCTCGGATTAG, Probe: 6FAM-ACCGTACAGTCCACACCAACTTT-BBQ). Fold change was determined using the $2^{-\Delta\Delta C_t}$ method comparing treated hamster to naive controls.

Cytokine and chemokine protein measurements

Lung homogenates were incubated with Triton X-100 (1% final concentration) for 1 h at room temperature to inactivate SARS-CoV-2. Homogenates then were analyzed for cytokines and chemokines by Eve Technologies Corporation (Calgary, AB, Canada) using their Mouse Cytokine Array/Chemokine Array 44-Plex (MD44) platform or Mouse Cytokine Array/Chemokine Array 31-Plex (MD31) platform.

Lung histology

Animals were euthanized before harvest and fixation of tissues. The left lung was first tied off at the left main bronchus and collected for viral RNA analysis. The right lung then was inflated with ~1.2 mL of 10% neutral buffered formalin using a 3-mL syringe and catheter inserted into the trachea. Tissues were embedded in paraffin, and sections were stained with hematoxylin and eosin. RNA *in situ* hybridization was performed using the RNAscope 2.5 HD Assay (Brown Kit) according to the manufacturer's instructions (Advanced Cell Diagnostics). Briefly, sections were deparaffinized and treated with H₂O₂ and Protease Plus before probe hybridization. Probes specifically targeting hACE2 (catalog number 848151) or SARS-CoV-2 spike sequence (catalog number 848561) were hybridized followed by proprietary signal amplification and detection with 3,3'-diaminobenzidine. Tissues were counterstained with Gill's hematoxylin. An uninfected mouse was used as a negative control and stained in parallel. Images were captured using the Nanozoomer (Hamamatsu) at the Alafi Neuroimaging Core at Washington University.

Flow cytometry analysis of immune cell infiltrates

For analysis of BAL fluid, mice were sacrificed by ketamine overdose, followed by cannulation of the trachea with a 19-G canula. BAL was performed with three washes of 0.8 mL of sterile PBS. BAL fluid was centrifuged, and single cell suspensions were generated for staining. For analysis of lung tissues, mice were perfused with sterile PBS, and the right inferior lung lobes were digested at 37°C with 630 µg/mL collagenase D (Roche) and 75 U µL of DNase I (Sigma-Aldrich) for 2 h. Single cell suspensions of BAL fluid and lung digest were preincubated with Fc Block antibody (BD PharMingen) in PBS + 2% heat-inactivated FBS for 10 min at room temperature before staining. Cells were incubated with antibodies against the following markers: efluor506 Viability Dye, BUV395 anti-CD45, BV711 anti-CD11b, APC-Cy7 anti-CD11c, Percp-Cy5.5 anti-Ly6G, Pacific Blue anti-Ly6C, FITC anti-CD24, PE anti-Siglec F, PE-Cy7 anti-CD64 or BV421 anti-CD64, AF700 anti-MHCII, BV605 anti-CD80, PeCy5 anti-CD86, BV421 anti-CD3, BV785 anti-CD4, APC anti-CD8α, BV711 anti-B220, APC-Cy7 anti-CD44, BV605 anti-CD62L. All antibodies were used at a dilution of 1:200. Cells were stained for 20 min at 4°C, washed, fixed and permeabilized for intracellular staining with Foxp3/Transcription Factor Staining Buffer Set (eBioscience) according to manufacturer's instructions. Myeloid cells were incubated overnight at 4°C with APC anti-NOS and PeCy7 anti-TNFα, washed, re-fixed with 4% PFA (EMS) for 20 min and resuspended in permeabilization buffer. Absolute cell counts were determined using TruCount beads (BD). Flow cytometric data were acquired on a cytometer (BD-X20; BD Biosciences) and analyzed using FlowJo software (Tree Star).

Antibody depletion of immune cell subsets

For neutrophil depletion, anti-Ly6G (BioXCell; clone 1A8; 250 µg) or an isotype control (BioXCell; clone 2A3; 250 µg) was administered to mice by intraperitoneal injection one day before infection and at D+1, D+3, and D+6 relative to SARS-CoV-2 inoculation. For monocyte depletion, anti-CCR2 (clone MC-21; 50 µg) (Mack et al., 2001) or an isotype control mAb (BioXCell; clone LTF-2; 50 µg) was administered to mice by intraperitoneal injection one day before infection and at D+1, D+3, D+5, and D+7 relative to SARS-CoV-2 inoculation. For NK cell depletion, anti-NK1.1 (clone PK136; 200 µg) or an isotype control mAb (BioXCell; clone C1.18.4; 200 µg) was administered to mice by intraperitoneal injection one day before infection and at D+1, D+3, D+5, and D+7 relative to SARS-CoV-2 inoculation. For CD8⁺ T cell depletions, anti-CD8a (clone 2.43; 500 µg) or an isotype control mAb (BioXCell; clone LTF-2; 500 µg) was administered to mice by intraperitoneal injection one day before infection and at D+1, D+3, D+5, and D+7 relative to SARS-CoV-2 inoculation.

For analysis of immune cell depletion, peripheral blood or the spleen was collected on the day of harvest. Erythrocytes were lysed with ACK lysis buffer (GIBCO) and resuspended in RPMI supplemented with 10% FBS. For anti-Ly6G or anti-CCR2 depletion,

single-cell suspensions were preincubated with Fc Block antibody (BD PharMingen) in PBS + 2% heat-inactivated FBS for 10 min at room temperature and then stained with antibodies against CD45 BUV395, CD11b PE/Dazzle 594, Ly6C Pacific Blue, Ly6B FITC, Ly6G BV650, and fixable viability dye (eFluor 506). For NK cell depletions, single cell suspensions were blocked for Fc γ R binding and stained with antibodies against CD45 BUV395, CD19 BV711, CD3 BV711, NK1.1 FITC, and Nkp56 Pacific Blue. For CD8⁺ T cell depletions, single-cell suspensions were blocked for Fc γ R binding and stained with antibodies against CD45 BUV395, CD3 421, CD4 BV785, CD8b APC, CXCR1-PE, B220 BV711, MHCII-AF700, and CD11c PE-Cy7.

Respiratory mechanics

Mice were anesthetized with ketamine/xylazine (100 mg/kg and 10 mg/kg, i.p., respectively). The trachea was isolated by dissection of the neck area and cannulated using an 18-gauge blunt metal cannula (typical resistance of 0.18 cmH₂O.s/mL), which was secured in place with a nylon suture. The mouse then was connected to the flexiVent computer-controlled piston ventilator (SCIREQ Inc.) via the cannula, which was attached to the FX adaptor Y-tubing. Mechanical ventilation was initiated, and mice were given an additional 100 mg/kg of ketamine and 0.1 mg/mouse of the paralytic pancuronium bromide by intraperitoneal route to prevent breathing efforts against the ventilator and during measurements. Mice were ventilated using default settings for mice, which consisted in a positive end expiratory pressure at 3 cm H₂O, a 10 mL/kg tidal volume (V_t), a respiratory rate at 150 breaths per minute (bpm), and a fraction of inspired oxygen (F_IO₂) of 0.21 (i.e., room air). Respiratory mechanics were assessed using the forced oscillation technique, as previously described (McGovern et al., 2013), using the latest version of the flexiVent operating software (flexiWare v8.1.3). Pressure-volume loops and measurements of inspiratory capacity also were done.

Neutralization assay

Serial dilutions of mAbs were incubated with 10² focus-forming units (FFU) of SARS-CoV-2 for 1 h at 37°C. mAb-virus complexes were added to Vero E6 cell monolayers in 96-well plates and incubated at 37°C for 1 h. Subsequently, cells were overlaid with 1% (w/v) methylcellulose in MEM supplemented with 2% FBS. Plates were harvested 30 h later by removing overlays and fixed with 4% PFA in PBS for 20 min at room temperature. Plates were washed and sequentially incubated with 1 μg/mL of a recombinant IgG based on the sequence of CR3022 (Yuan et al., 2020) anti-S antibody and HRP-conjugated goat anti-human IgG in PBS supplemented with 0.1% saponin and 0.1% bovine serum albumin. SARS-CoV-2-infected cell foci were visualized using TrueBlue peroxidase substrate (KPL) and quantitated on an ImmunoSpot microanalyzer (Cellular Technologies).

RNA sequencing

cDNA libraries were constructed starting with 10 ng of total RNA from lung tissues of each sample that was extracted using the Mag-Max Mirvana kit per manufacturer's instructions. cDNA was generated using the Seqplex kit (Sigma-Aldrich, St. Louis, MO) with amplification of 20 cycles. Library construction was performed using 100 ng of cDNA undergoing end repair, A-tailing, ligation of universal TruSeq adapters, and 8 cycles of amplification to incorporate unique dual index sequences. Libraries were sequenced on the NovaSeq 6000 (Illumina, San Diego, CA) targeting 40 million read pairs and extending 150 cycles with paired end reads. RNA-seq reads were aligned to the mouse Ensembl GRCh38.76 primary assembly and SARS-CoV-2 NCBI NC_045512 Wuhan-Hu-1 genome with STAR program (version 2.5.1a) (Dobin et al., 2013). Gene counts were derived from the number of uniquely aligned unambiguous reads by Subread:featureCount (version 1.4.6-p5) (Liao et al., 2014). The ribosomal fraction, known junction saturation, and read distribution over known gene models were quantified with RSeQC (version 2.6.2) (Liao et al., 2014). All gene counts were preprocessed with the R package EdgeR (Robinson et al., 2010) to adjust samples for differences in library size using the trimmed mean of M values (TMM) normalization procedure. Viral and ribosomal genes and genes not expressed in at least five samples (the smallest group size) at a level greater than or equal to 1 count per million reads were excluded, resulting 12,157 unique genes in further analysis. The R package limma (Ritchie et al., 2015) with voomWithQualityWeights function (Liu et al., 2015) was utilized to calculate the weighted likelihoods for all samples, based on the observed mean-variance relationship of every gene and sample. Differentially expressed genes were defined as those with at least 2-fold difference between two individual groups at the Benjamini-Hochberg false-discovery rate (FDR) (<https://www.jstor.org/stable/2346101?seq=1>) adjusted *P* value (i.e., *q*-value < 0.05).

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical significance was assigned when *P* values were < 0.05 using Prism version 8 (GraphPad). Tests, number of animals (*n*), median values, and statistical comparison groups are indicated in the Figure legends. Analysis of weight change was determined by two-way ANOVA. Changes in functional parameters or immune parameters were compared to isotype-treated animals and were analyzed by one-way ANOVA or one-way ANOVA with Dunnett's test.

Supplemental figures

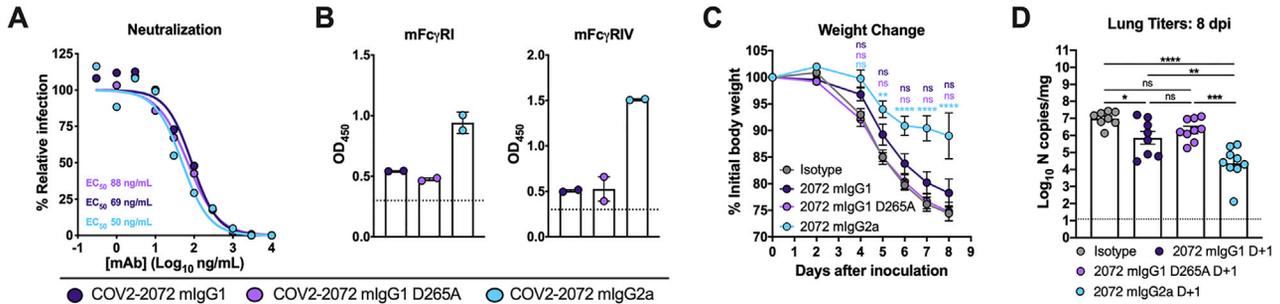


Figure S1. Protective effects of chimeric COV2-2072 with murine Fc regions, related to Figure 3

(A) Chimeric COV2-2072 (mlgG1, mlgG1 D265A, or mlgG2a) were incubated with 10^2 FFU of SARS-CoV-2 for 1 h at 37°C before adding to Vero E6 cells for a FRNT. Wells containing mAb were compared to wells without mAb to determine relative infection. One experiment of two, with similar results, is shown. The mean of two technical replicates is shown. (B) Binding of COV2-2072 mlgG1, mlgG1 D265A, and mlgG2a to recombinant mouse Fc γ RI or Fc γ RIV as measured by ELISA (two independent experiments). The dotted line indicates the limit of detection, as determined by background binding to a negative control. For C-D, eight-week-old female or male K18-hACE2 transgenic mice were inoculated by the intranasal route with 10^3 PFU of SARS-CoV-2. At D+1, mice were given a single 200 μg dose of COV2-2072 mlgG1, mlgG1 D265A, and mlgG2a by intraperitoneal injection. (C) Weight change (mean \pm SEM; $n = 7-8$, two experiments: two-way ANOVA with Sidak's post-test: ns not significant, ** $p < 0.01$, **** $p < 0.0001$; comparison to the isotype control mAb-treated group). (D) Viral RNA levels at 8 dpi in the lung as determined by qRT-PCR ($n = 8$, two experiments, one-way ANOVA with Dunnett's test; ns not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

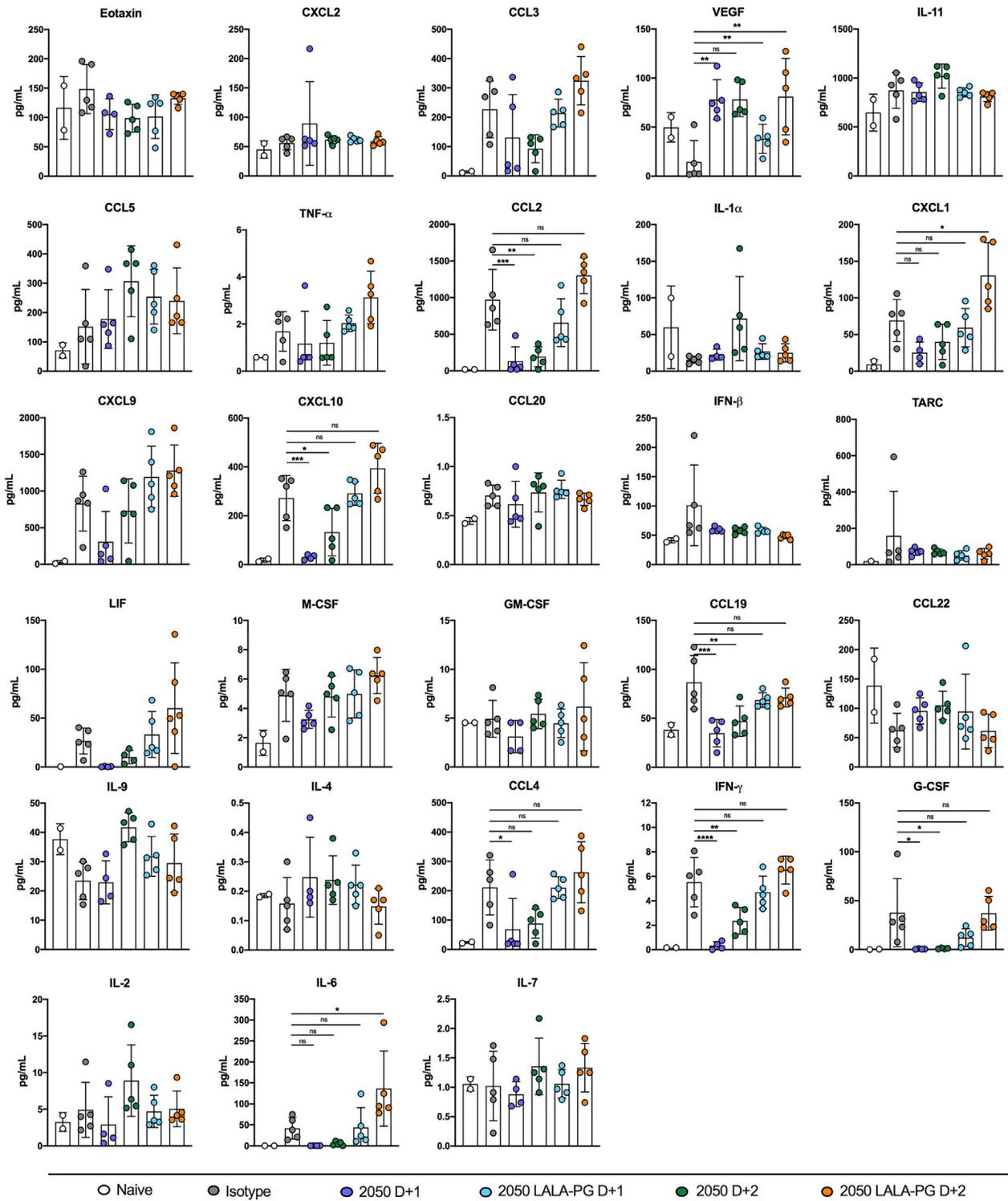


Figure S2. Cytokine induction following SARS-CoV-2 infection, related to Figure 4

Cytokine levels as measured by multiplex platform in the lungs of SARS-CoV-2 infected mice at 8 dpi following isotype, COV2-2050, or COV2-2050 LALA-PG treatment at 1 dpi (D+1) or 2 dpi (D+2) (three experiments, n = 5 per group except naive in which n = 2. One-way ANOVA with Dunnett's test; *p < 0.05; **p < 0.01; ***p < 0.001.) Asterisks indicate statistical significance compared to the isotype-control mAb-treated group.

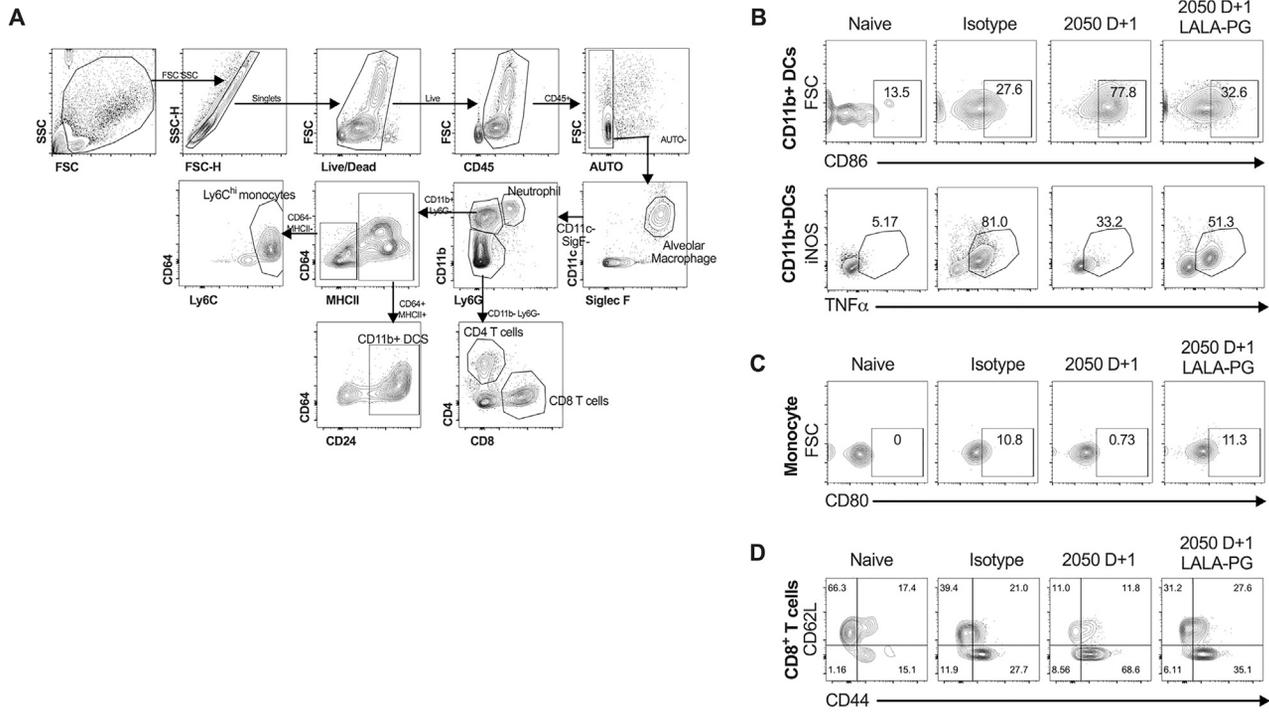
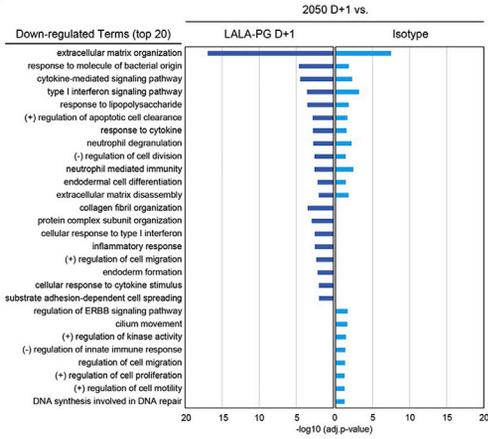


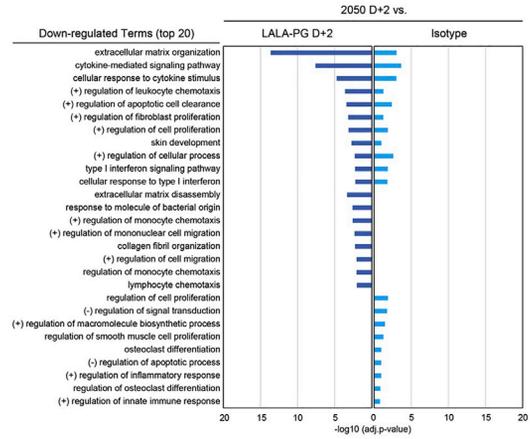
Figure S3. Flow cytometric gating strategy for BAL fluid analysis, related to Figure 4

(A) For BAL fluid and lung tissue flow cytometric analysis, cells were gated on live, single, autofluorescent-negative CD45⁺ cells to identify hematopoietic cells. Alveolar macrophages were identified as SiglecF^{hi} CD11c^{hi} cells. Neutrophils were identified as Ly6G^{hi} CD11b^{hi} cells. CD11b⁻ cells were gated further into CD4⁺ and CD8⁺ T cells. CD11b^{hi}Ly6G⁻ cells were gated subsequently using CD64, CD24, and MHC-II. MHCII^{hi} CD24^{hi} were defined as CD11b⁺ DCs. MHCII^{lo} Ly6^{hi} cells were defined as monocytes. (B) Representative flow cytometry plots of CD86 expression and TNF α /iNOS expression on CD11b⁺ DCs isolated from the lung tissues of indicated treatment groups. (C) Representative flow cytometry plots of CD80 expression on monocytes isolated from the lung tissues of indicated treatment groups. (D) Representative flow cytometry plots of CD44 and CD62L expression on CD8⁺ T cells isolated from the lung tissues of indicated treatment groups.

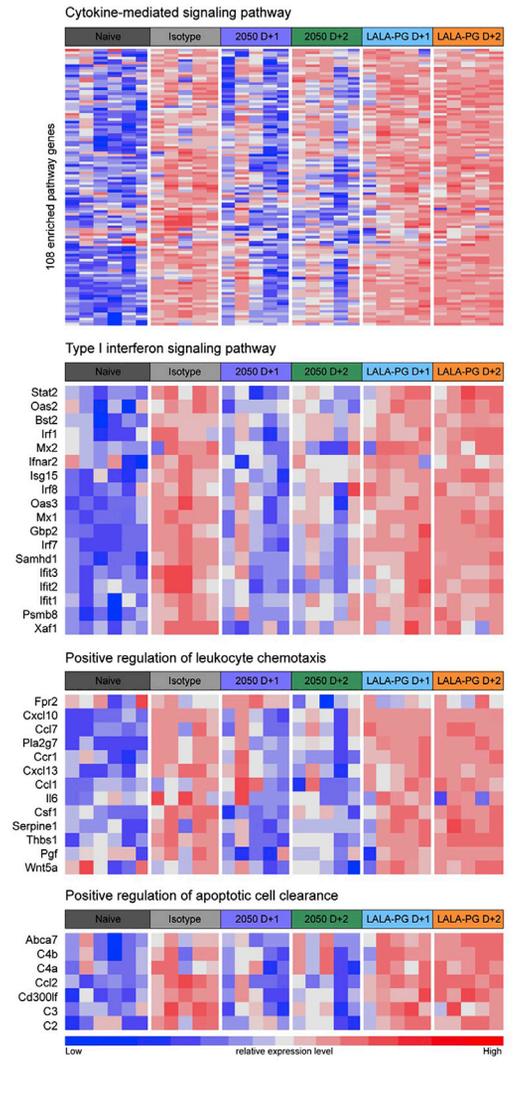
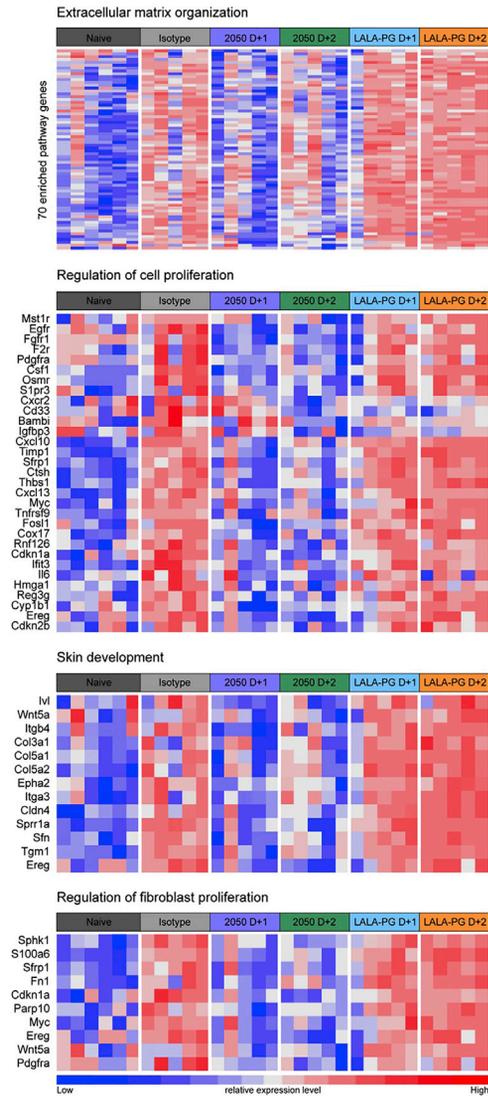
A



B



C



(legend on next page)

Figure S4. Gene ontology analysis of RNA-seq data, related to Figure 5

(A-C) RNA sequencing analysis from the lung homogenates of naive K18-hACE2 mice or mice inoculated with SARS-CoV-2 at 8 dpi. At 1 (D+1) or 2 (D+2) dpi, mice were given a single 200 μ g dose of COV2-2050 or COV2-2050 LALA-PG by intraperitoneal injection. (A-B) Gene Ontology (GO) Enrichment Analysis of biological process terms enriched in downregulated genes from comparisons of isotype control, COV2-2050, and COV2-2050 LALA-PG when given at D+1 (A) or isotype control, COV2-2050, and COV2-2050 LALA-PG when given at D+2 (B). Terms were ranked by the false discovery rate (q-value), and the top 20 are listed after eliminating redundant terms. (C) Heatmaps of significantly downregulated gene sets corresponding with intact COV2-2050 treatment identified through GO analysis. Genes shown in each pathway are the union of the differentially expressed genes (DEGs) from the five comparisons (isotype control, COV2-2050 D+1, COV2-2050 D+2, COV2-2050 LALA-PG D+1, or COV2-2050 LALA-PG D+2 versus mock-infected). Columns represent samples and rows represent genes. Gene expression levels in the heatmaps are z score-normalized values determined from log₂cpm values.

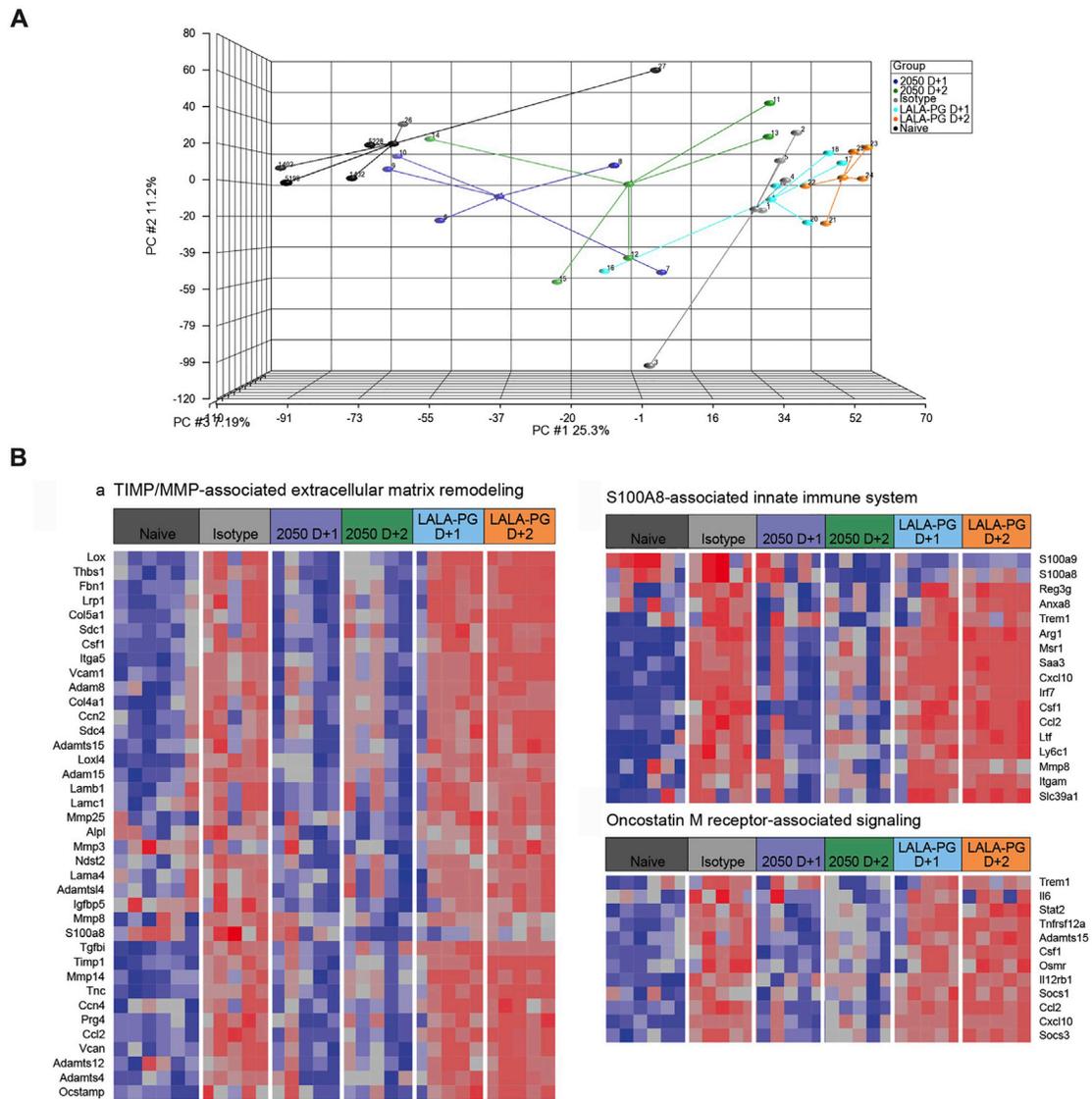


Figure S5. CompBio analysis comparing COV2-2050 D+2, isotype control, and COV2-2050 LALA-PG D+2, related to Figure 5

(A) Three-dimensional map from principal component analysis (PCA) of the RNA-seq data in the study. The PCA was performed using 12,157 unique genes with counts per million reads ≥ 1 in at least 5 of the study samples ($n = 31$). Each mouse is represented by an individual point that is color-matched to its corresponding experimental group and connected to the centroid point (with a cross bar and ID number) of the corresponding group. (B) Heatmaps of selected relevant biological themes enriched in COV2-2050 D+2 versus isotype control and COV2- LALA-PG D+2. Genes shown are common in the pair of comparisons with an enrichment score of 100 or greater in either of the paired comparisons.

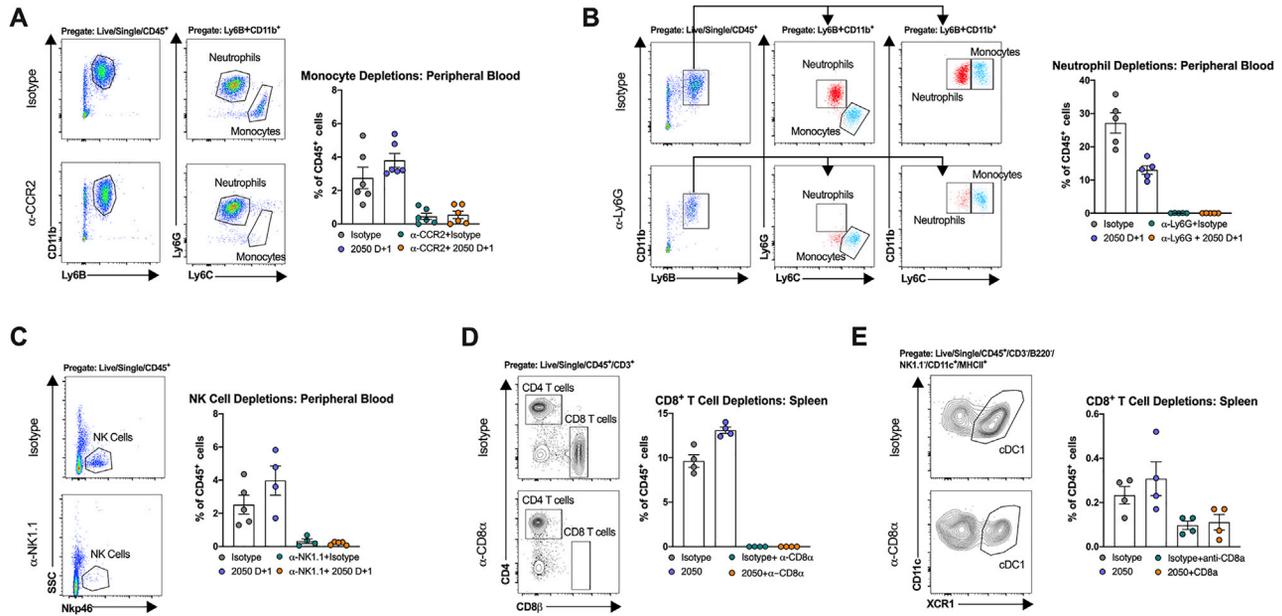
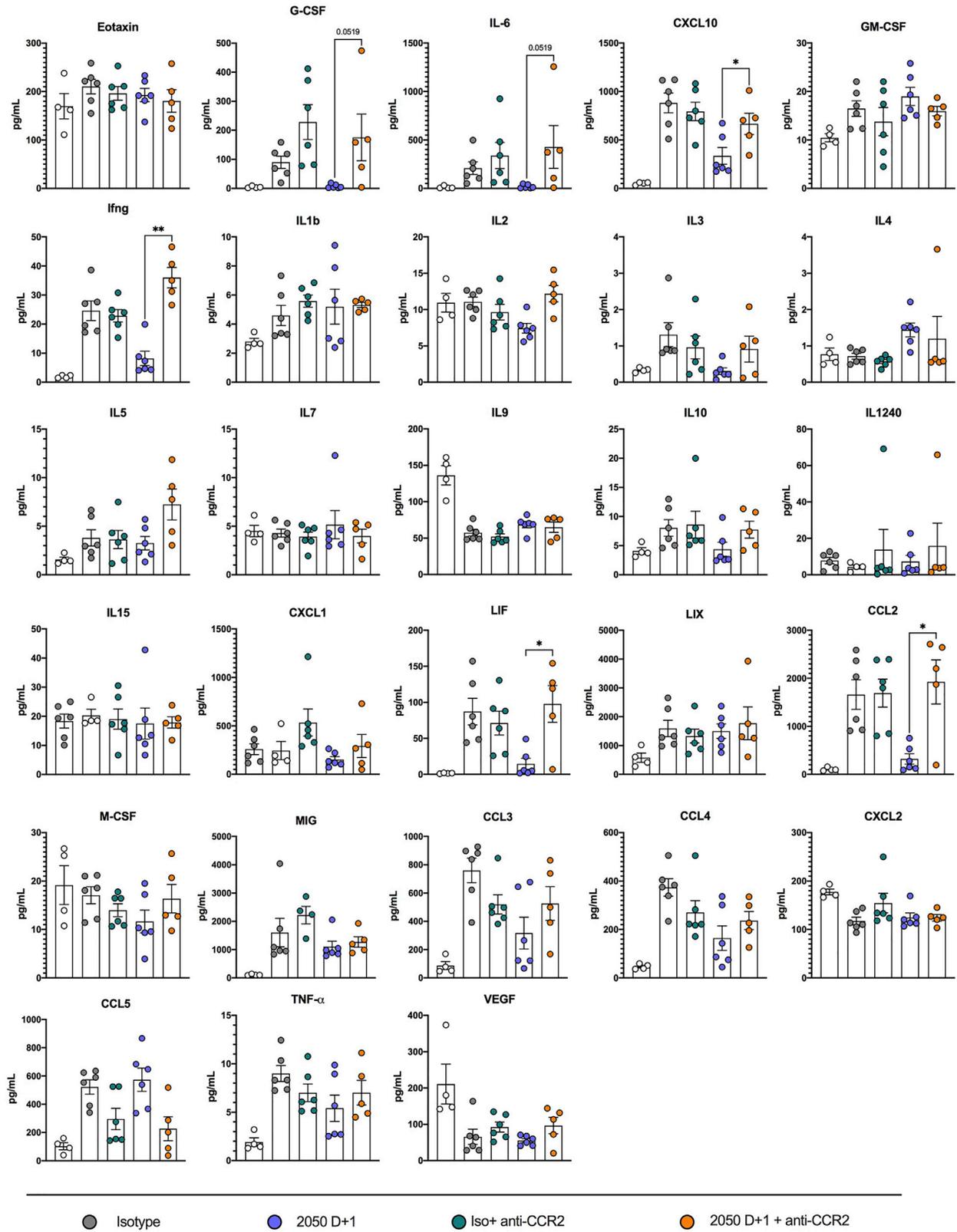


Figure S6. Confirmation of cellular depletions, related to Figure 6

(A) Representative flow cytometry plots of monocytes and neutrophils from peripheral blood at 8 dpi following intraperitoneal injection of a depleting anti-CCR2 mAb or isotype control mAb and frequency of Ly6C^{hi} monocytes and neutrophils in blood at 8 dpi following anti-CCR2 or isotype control mAb administration in isotype control or COV2-2050-treated mice (two experiments, n = 6 per group). (B) Representative flow cytometry plots of peripheral blood at 8 dpi following intraperitoneal injection of a depleting anti-Ly6G mAb or isotype control mAb and frequency of Ly6C^{hi} monocytes and mature Ly6G⁺ neutrophils in blood at 8 dpi following anti-Ly6G or isotype control mAb administration in isotype control or COV2-2050-treated mice (two experiments, n = 5-6 per group). In the groups treated with anti-Ly6G, neutrophils were identified as CD11b⁺ Ly6B⁺ Ly6C^{int} cells. Red dots (neutrophils) and monocytes (blue dots) correspond to the same population identified in both plots (neutrophils: CD11b⁺ Ly6C⁺ Ly6G⁺ Ly6C^{int} and monocytes: CD11b⁺ Ly6C⁺ Ly6G⁻ Ly6C^{high}). (C) Representative flow cytometry plots of peripheral blood at 8 dpi following intraperitoneal injection of a depleting anti-NK1.1 mAb or isotype control mAb. Also shown is the frequency of NK cells in blood at 8 dpi following anti-NK1.1 or isotype control mAb administration in isotype control or COV2-2050-treated mice (two experiments, n = 4-5 per group). (D) Representative flow cytometry plots of splenocytes gated on CD4⁺ and CD8⁺ T cells at 8 dpi following intraperitoneal injection of a depleting anti-CD8 α mAb or isotype control mAb (left). Frequency of CD8⁺ T cells in the spleen at 8 dpi following anti-CD8 α or isotype control mAb administration in isotype mAb control or COV2-2050-treated mice (right) (two experiments, n = 4-5 per group). (E) Representative flow cytometry plots of splenocytes gated on cDC1s at 8 dpi following intraperitoneal injection of a depleting anti-CD8 α mAb or isotype control mAb (left). Frequency of cDC1 cells in the spleen at 8 dpi following anti-CD8 α or isotype control mAb administration in isotype control mAb or COV2-2050-treated mice (right) (two experiments, n = 4-5 per group).



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Figure S7. Cytokine induction following SARS-CoV-2 infection and monocyte depletion, related to Figure 6

Cytokine levels as measured by multiplex platform in the lungs of mock-infected mice or SARS-CoV-2-infected mice at 8 dpi following treatment with isotype mAb, COV2-2050 D+1, isotype mAb + anti-CCR2, or COV2-2050 D+1 + anti-CCR2 (two experiments, n = 4-6 per group. One-way ANOVA with Dunnett's test; *p < 0.05; **p < 0.01; ***p < 0.001.) One animal was excluded from cytokine analysis in the 2050 D+1 + anti-CCR2 group due to incomplete monocyte depletion in peripheral blood (monocytes still made up ~3% of CD45⁺ cells).