

1 In vitro analysis and dynamic modeling of SARS-CoV-2  
2 infection inhibition by sigma-1 receptor antagonist PB28

3 **Supplementary Information**

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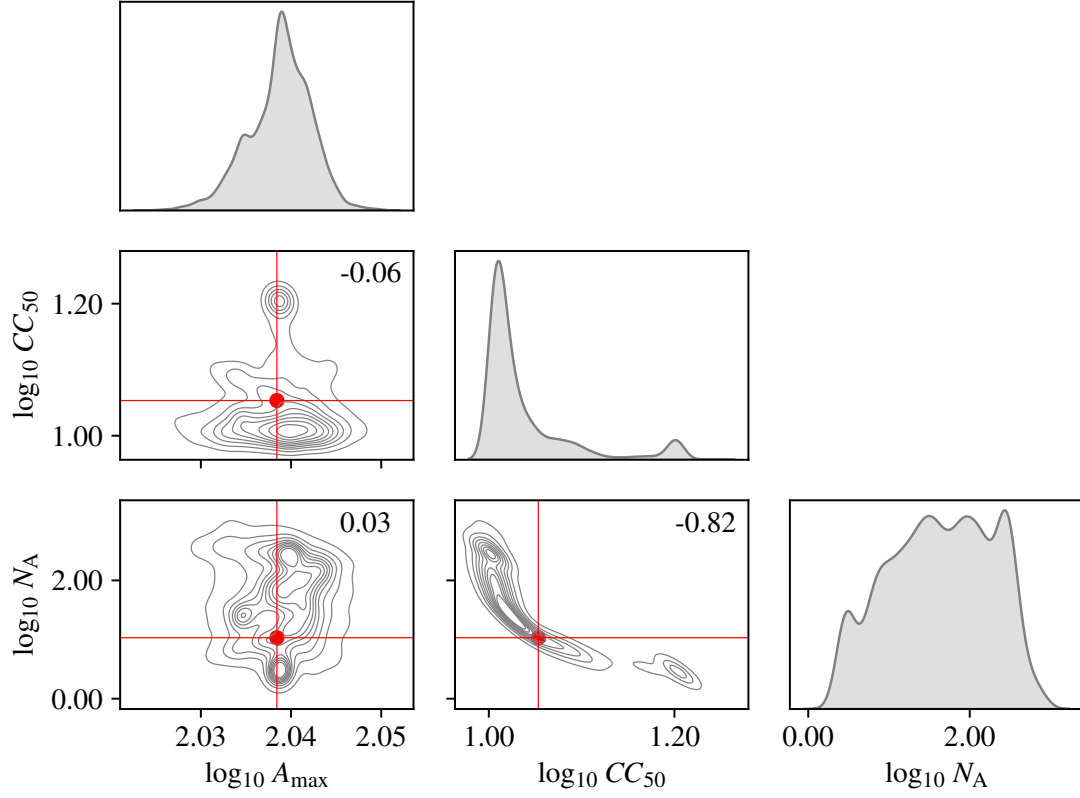
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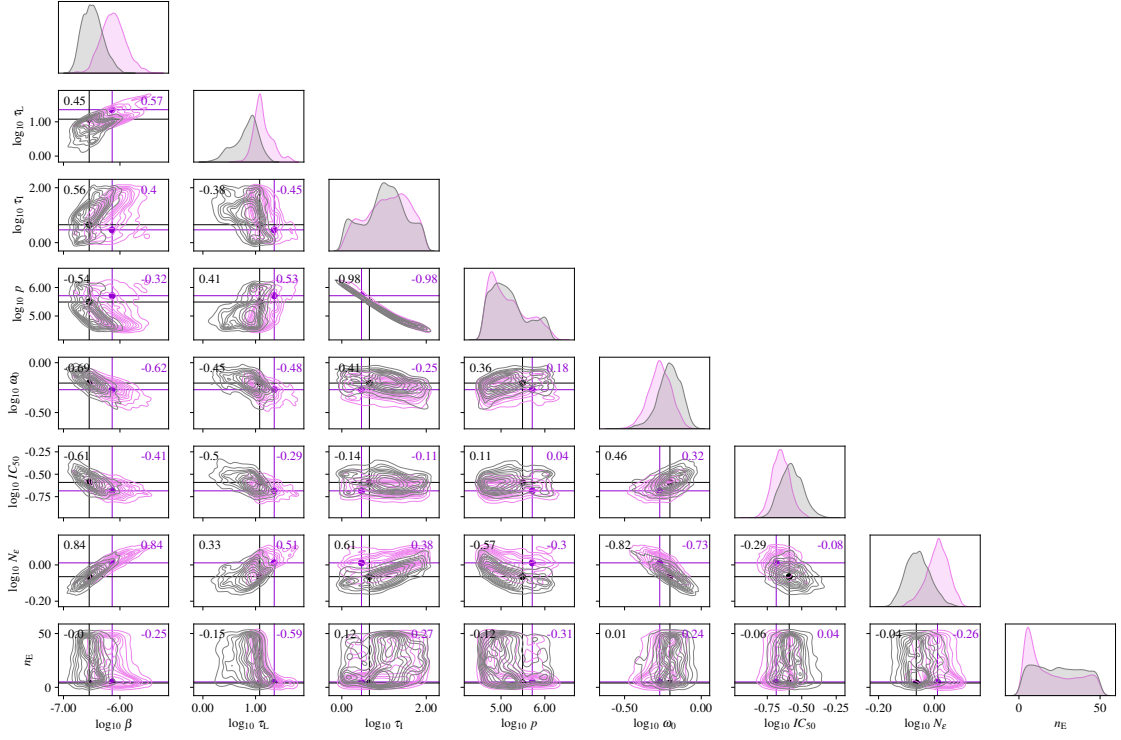
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**Figure S1. PB28 parameters influencing A549-ACE2 cell viability.** Equation (13) in the main text was fitted to the relative percentages of viable cells (relative to the control) using MCMC (details are in Materials and Methods in the main text). A total of 6 independent chains were run for 20,000 steps, with a burn-in of 10,000 steps. Thinning was applied by accepting every 10th step. A total of 6,000 accepted parameter sets were considered. The value in the upper-right corner represents the Pearson correlation coefficient for the corresponding parameter pair. The best-fit parameter value (Table 2 in the main text) is denoted by a red point.



**Figure S2. SARS-CoV-2 infection parameters for time-resolved experimental infection scheme.** Posterior histograms and pair-wise parameter plots were obtained from simultaneously fitting of viral dynamics model (Equations (1)–(5) in the main text) to viral load data obtained from either only time-resolved (pink, Figure S12G–I) or both, end-point and time-resolved (grey, Figures 3A–F and 3G–I in the main text, respectively) experimental infection schemes using MCMC (details are in Materials and Methods in the main text). A total of 16 independent chains were run for 20,000 steps, with a burn-in of 10,000 steps. Thinning was applied by accepting every 10th step. A total of 16,000 accepted parameter sets were considered. The values in the upper-left and upper-right corners represent the Pearson correlation coefficients for the corresponding parameter pair. The best-fit parameter values (Table 1 in the main text) are denoted by filled points.

## 20 Local sensitivity analysis

21 To quantify the impact of parameter variability on the behavior of the viral dynamics  
 22 model (Equations (1)–(5) in the main text), we conducted a one-at-a-time sensitivity  
 23 analysis. The parameters examined were the infection rate constant,  $\beta$ , the latent-phase  
 24 duration,  $\tau_L$ , the infectious-phase duration,  $\tau_I$ , the number of latent compartments,  $n_L$ ,  
 25 the viral production rate constant,  $p$ , the parameter governing the washing process,  $\omega_0$ ,  
 26 the half-maximal inhibitory concentration,  $IC_{50,\epsilon}$ , and the Hill coefficient describing coop-  
 27 erativity in the PB28 effect,  $N_\epsilon$ . Each parameter was perturbed independently by  $\pm 50\%$   
 28 relative to its maximum-likelihood (best-fit) estimate, while all remaining parameters were  
 29 held fixed.

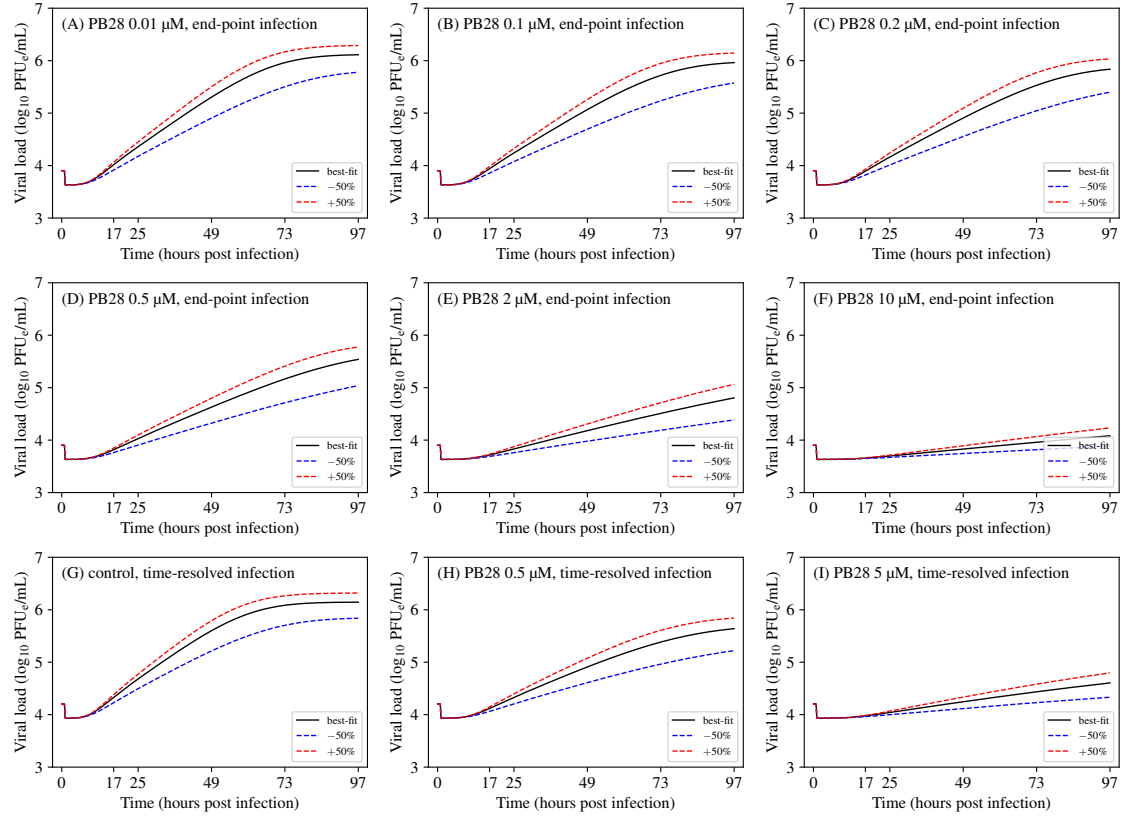
30 The parameters most sensitive to perturbations in their best-fit values were  $\tau_I$  (Figure S3),  
 31  $p$  (Figure S4),  $\beta$  (Figure S5), and  $\tau_L$  (Figure S6). Increases (decreases) in  $\tau_I$ ,  $p$ , and  $\beta$  led  
 32 to corresponding increases (decreases) in viral load. Specifically, increasing  $\beta$  raised the  
 33 proportion of infected cells and, consequently, virus production. Similarly, increases (de-  
 34 creases) in  $\tau_I$  allowed infected cells to produce virus for longer (shorter) periods, resulting  
 35 in greater (lower) viral accumulation. In contrast, increases (decreases) in  $\tau_L$  produced  
 36 decreases (increases) in viral load, reflecting the effect of a prolonged (shortened) delay in  
 37 virus production.

38 Variations in  $\omega_0$  did not significantly affect viral load (Figure S7), and the parameter  $n_L$   
 39 was not sensitive to perturbations in its best-fit value (Figure S8).

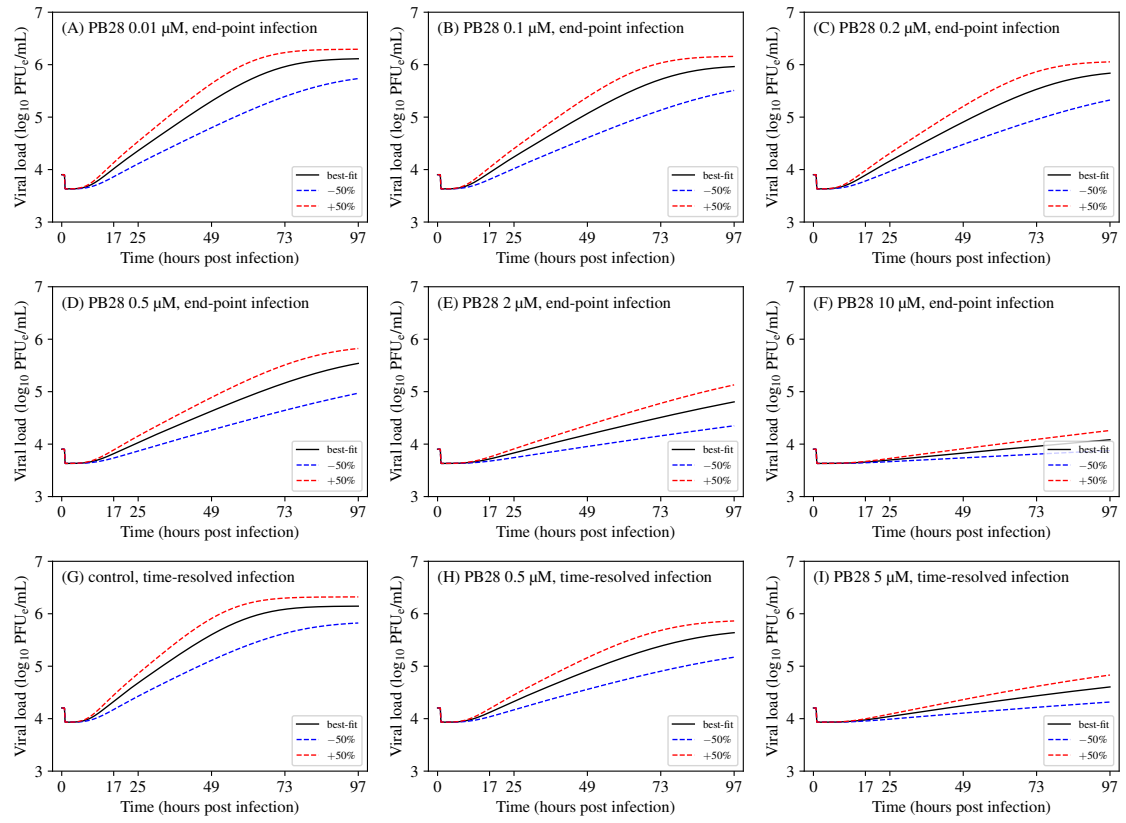
40 Sensitivity of parameters characterizing PB28 inhibition efficacy,  $IC_{50,\epsilon}$  and  $N_\epsilon$ , to per-  
 41 turbations in their best-fit values varied with PB28 concentration (Figures S9 and S10).  
 42 Increases (decreases) in  $IC_{50,\epsilon}$ , and thus decreases (increases) in the PB28 inhibition effi-  
 43 cacy,  $\epsilon$ , resulted in increased (decreased) viral load. Increases (decreases) in  $N_\epsilon$  resulted  
 44 in increases (decreases) in viral load when the PB28 concentration was lower than  $IC_{50,\epsilon}$ .  
 45 Indeed, if  $IC_{50,\epsilon} > C$  (i.e.,  $\frac{IC_{50,\epsilon}}{C} > 1$ ) and assuming  $\epsilon_{\max} = 1$ , then for any  $N_\epsilon^* > N_\epsilon$ , it  
 46 follows that, it follows that

$$\epsilon^* = \frac{1}{\left(\frac{IC_{50,\epsilon}}{C}\right)^{N_\epsilon^*} + 1} < \epsilon = \frac{1}{\left(\frac{IC_{50,\epsilon}}{C}\right)^{N_\epsilon} + 1},$$

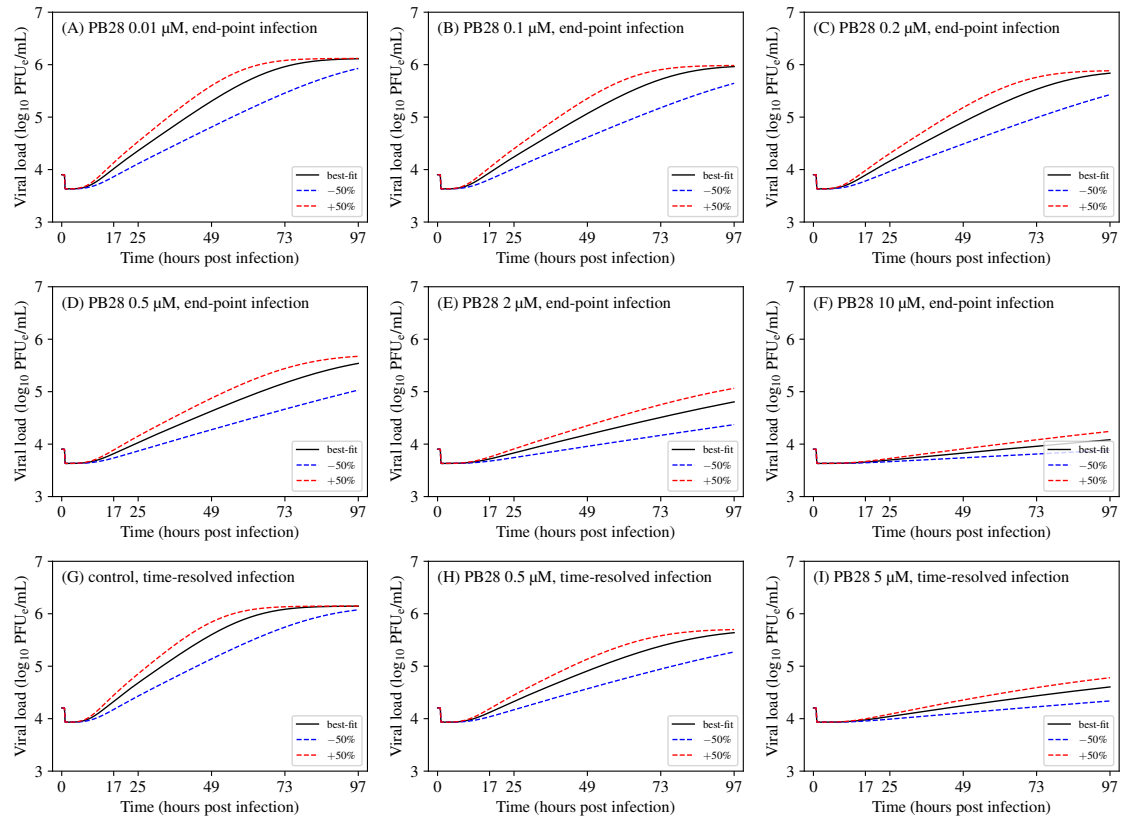
47 resulting in lower inhibition efficacy and higher viral load. Similarly, increases (decreases)  
 48 in  $N_\epsilon$  resulted in corresponding decreases (increases) in viral load when the PB28 concen-  
 49 tration,  $C$ , exceeded  $IC_{50,\epsilon}$ .



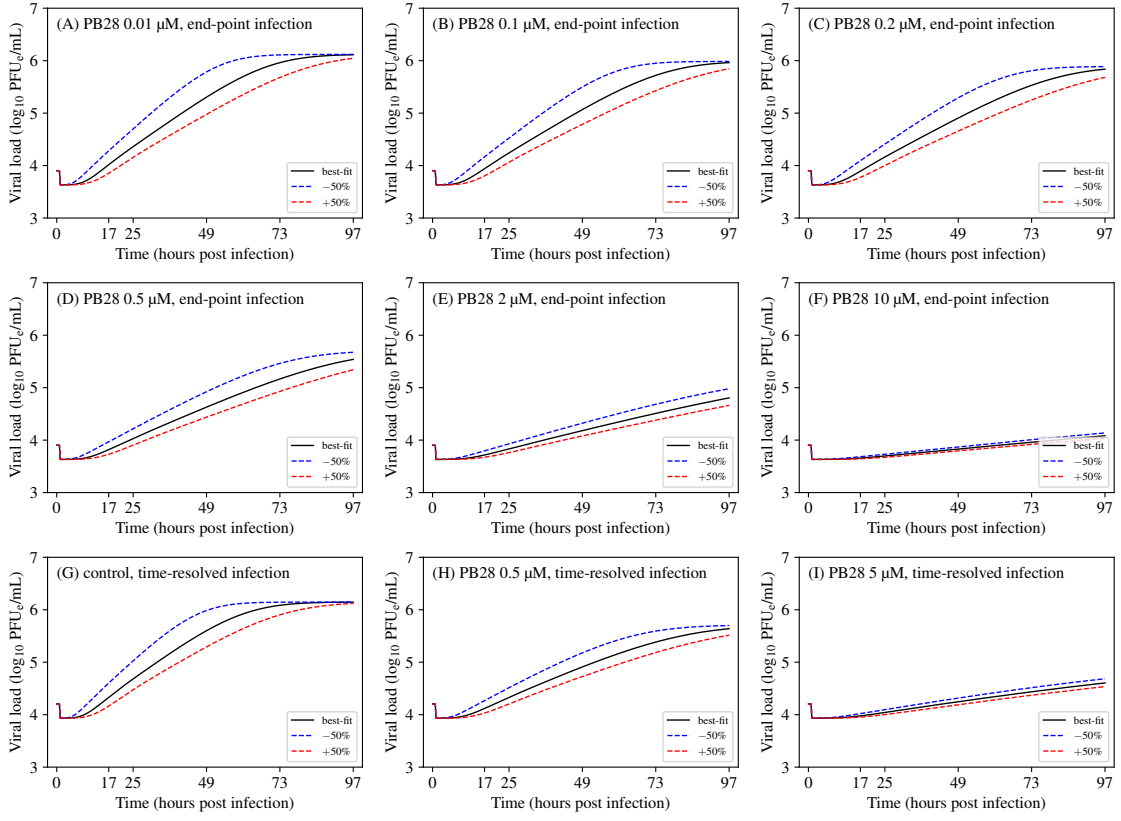
**Figure S3. Local sensitivity analysis of infectious phase duration,  $\tau_I$ .** The parameter  $\tau_I$  was varied by 50% from the best-fit value (Table 1 in the main text), and the corresponding solutions of Equations (1)–(5) in the main text were displayed as follows: a black solid line was used for the solutions associated with the best-fit parameter values; a red dashed line was used for the solutions associated with perturbations of +50%; and a blue dashed line was used for the solutions associated with perturbations in the best-fit parameter values by  $-50\%$ .



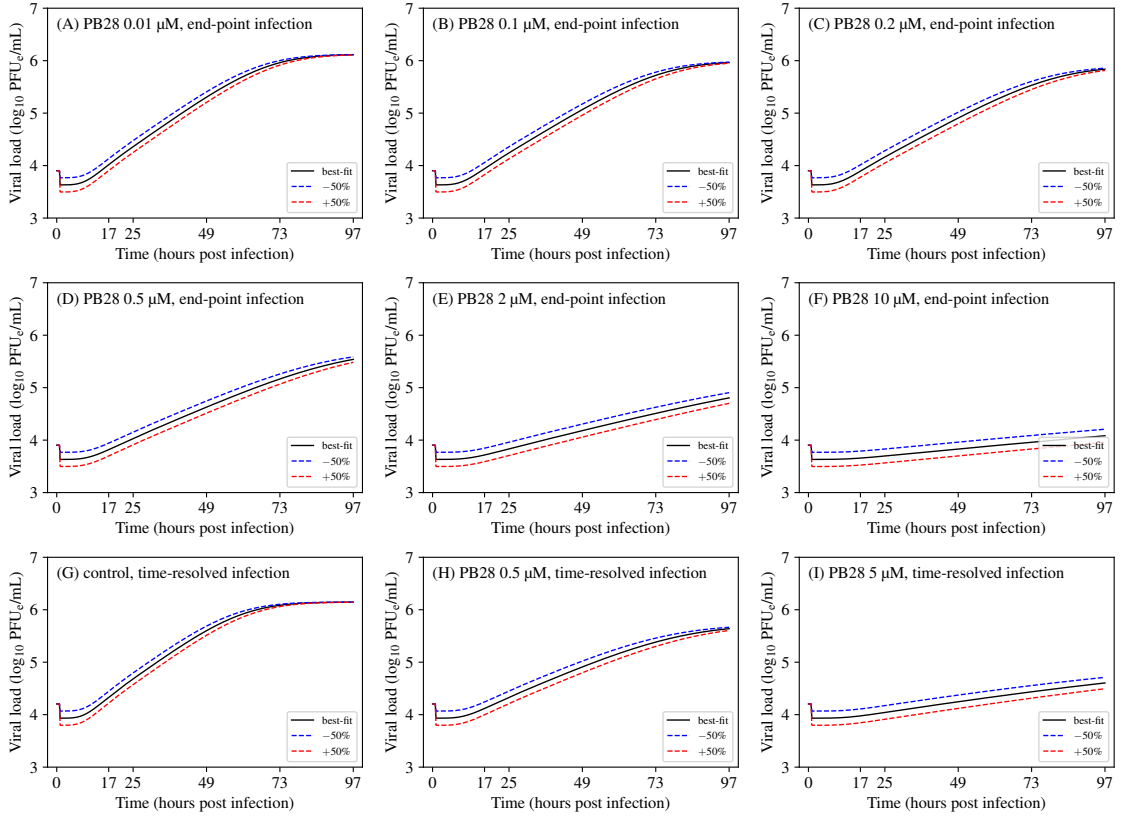
**Figure S4. Local sensitivity analysis of virus production rate constant,  $p$ .** The parameter  $p$  was varied by 50% from the best-fit value (Table 1 in the main text), and the corresponding solutions of Equations (1)–(5) in the main text were displayed as follows: a black solid line was used for the solutions associated with the best-fit parameter values; a red dashed line was used for the solutions associated with perturbations of +50%; and a blue dashed line was used for the solutions associated with perturbations in the best-fit parameter values by −50%.



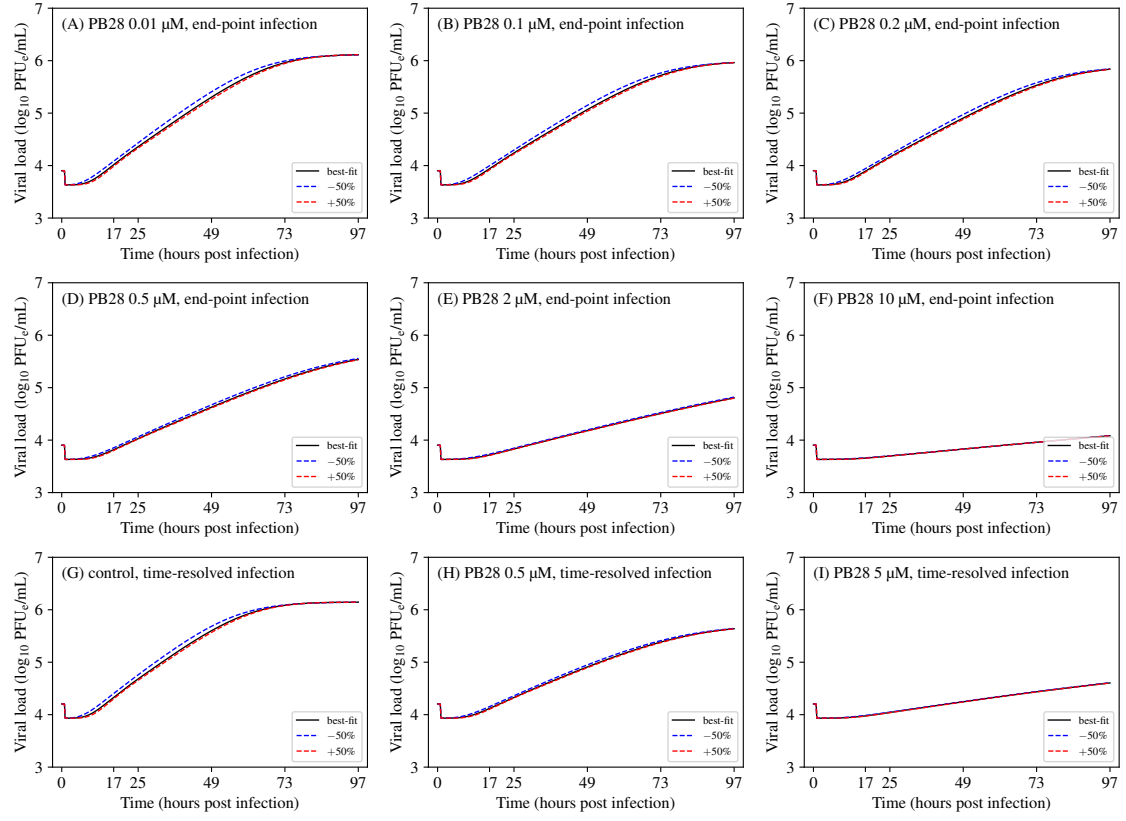
**Figure S5. Local sensitivity analysis of the infection rate constant,  $\beta$ .** The parameter  $\beta$  was varied by 50% from the best-fit value (Table 1 in the main text), and the corresponding solutions of Equations (1)–(5) in the main text were displayed as follows: a black solid line was used for the solutions associated with the best-fit parameter values; a red dashed line was used for the solutions associated with perturbations of +50%; and a blue dashed line was used for the solutions associated with perturbations in the best-fit parameter values by –50%.



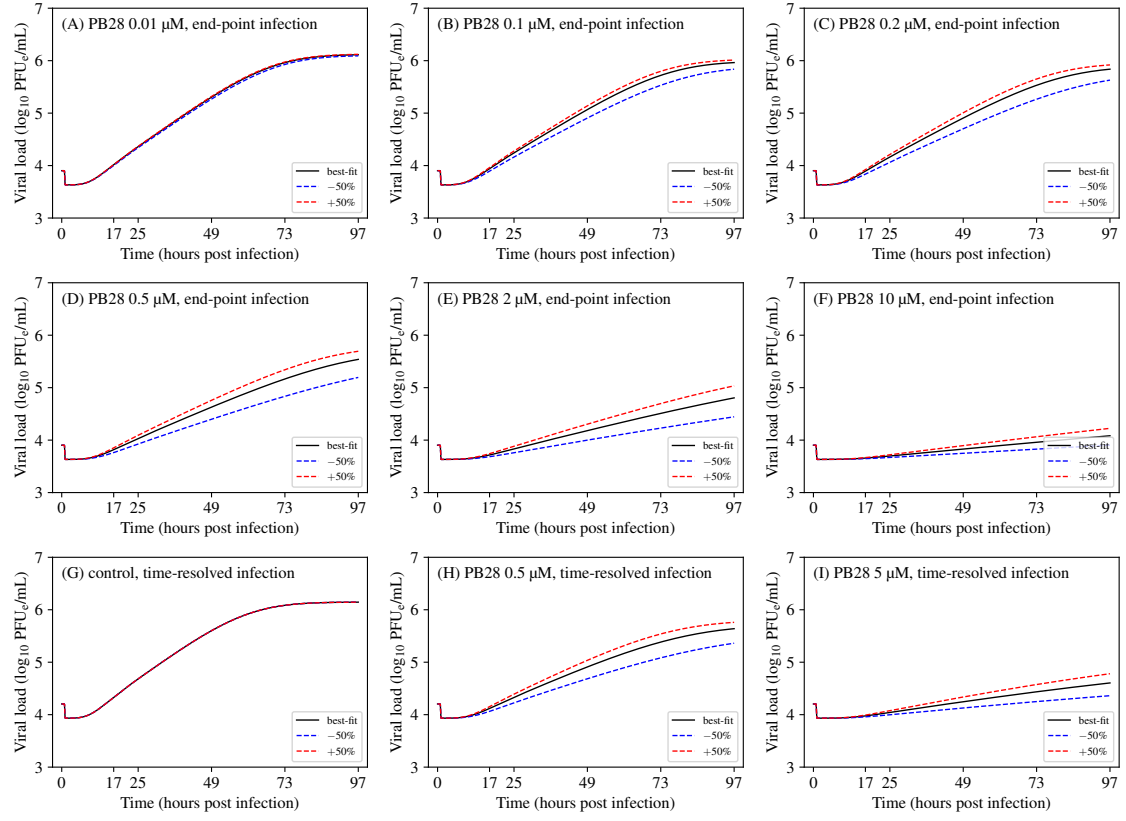
**Figure S6. Local sensitivity analysis of latent phase duration,  $\tau_L$ .** The parameter  $\tau_L$  was varied by 50% from the best-fit value (Table 1 in the main text), and the corresponding solutions of Equations (1)–(5) in the main text were displayed as follows: a black solid line was used for the solutions associated with the best-fit parameter values; a red dashed line was used for the solutions associated with perturbations of +50%; and a blue dashed line was used for the solutions associated with perturbations in the best-fit parameter values by  $-50\%$ .



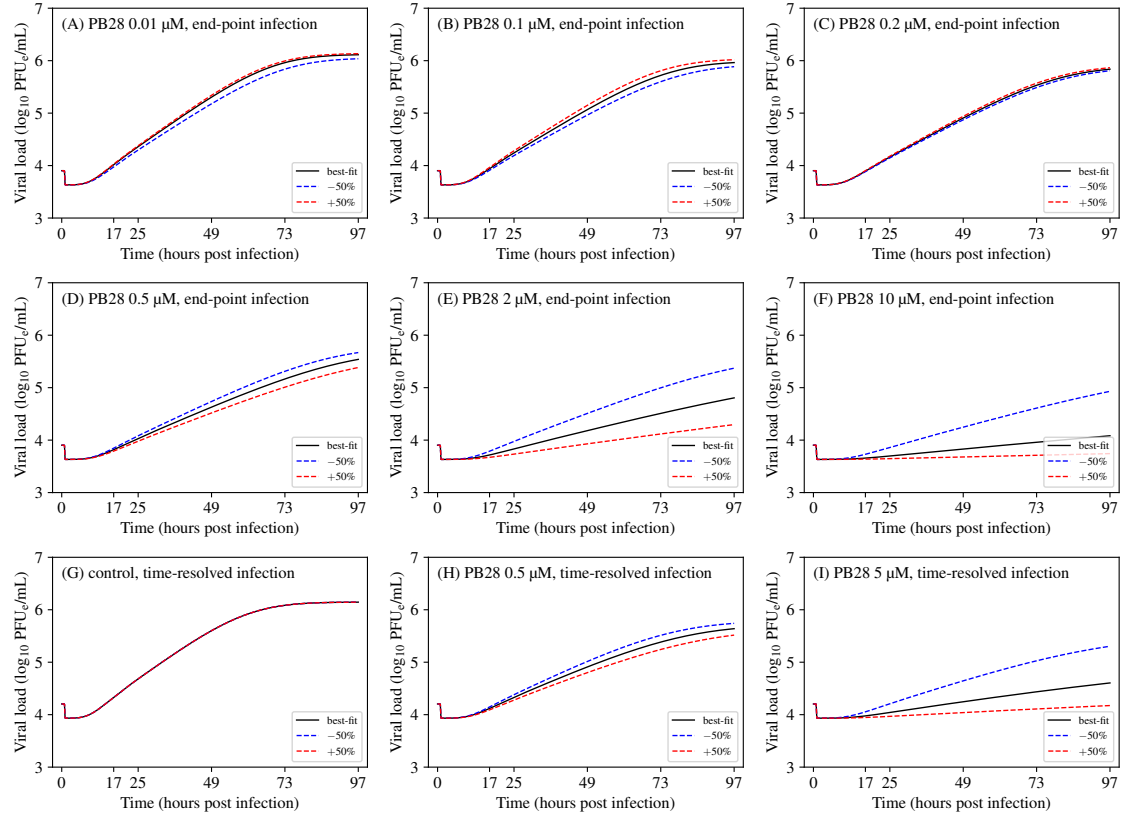
**Figure S7. Local sensitivity analysis of washing rate constant,  $\omega_0$ .** The parameter  $\omega_0$  was varied by 50% from the best-fit value (Table 1 in the main text), and the corresponding solutions of Equations (1)–(5) in the main text were displayed as follows: a black solid line was used for the solutions associated with the best-fit parameter values; a red dashed line was used for the solutions associated with perturbations of +50%; and a blue dashed line was used for the solutions associated with perturbations in the best-fit parameter values by –50%.



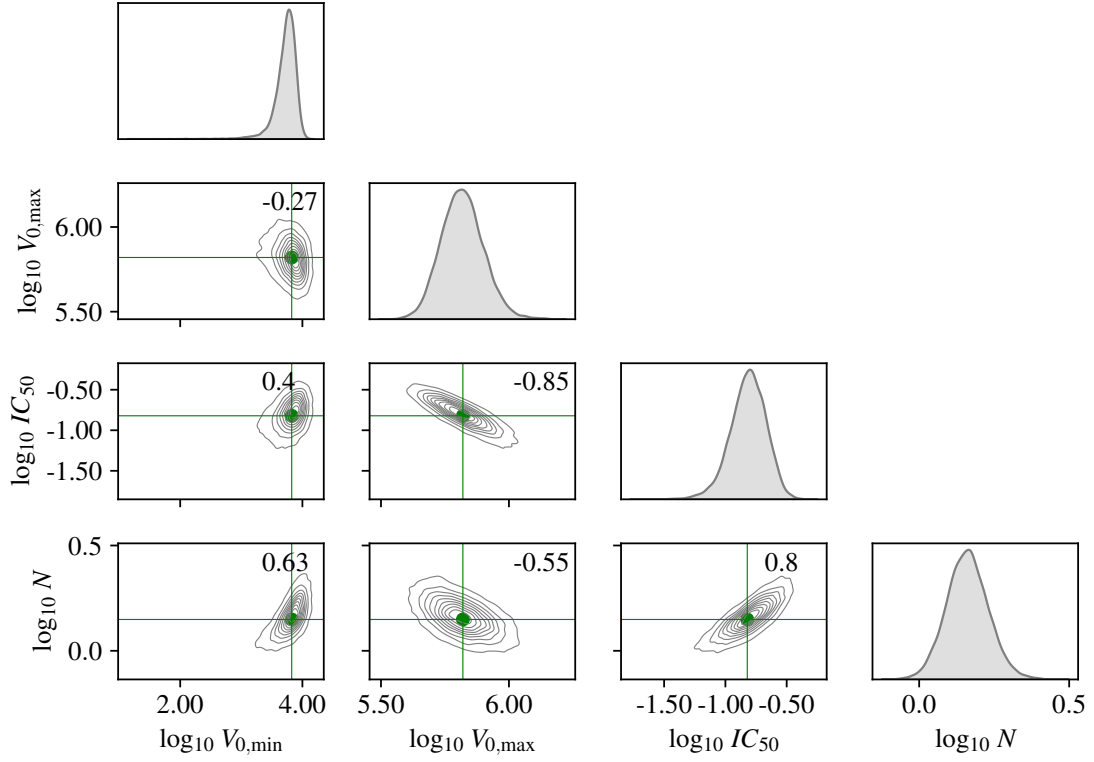
**Figure S8. Local sensitivity analysis of number of latent phase compartments,  $n_L$ .** The parameter  $n_L$  was varied by 50% from the best-fit value (Table 1 in the main text), and the corresponding solutions of Equations (1)–(5) in the main text were displayed as follows: a black solid line was used for the solutions associated with the best-fit parameter values; a red dashed line was used for the solutions associated with perturbations of +50%; and a blue dashed line was used for the solutions associated with perturbations in the best-fit parameter values by –50%.



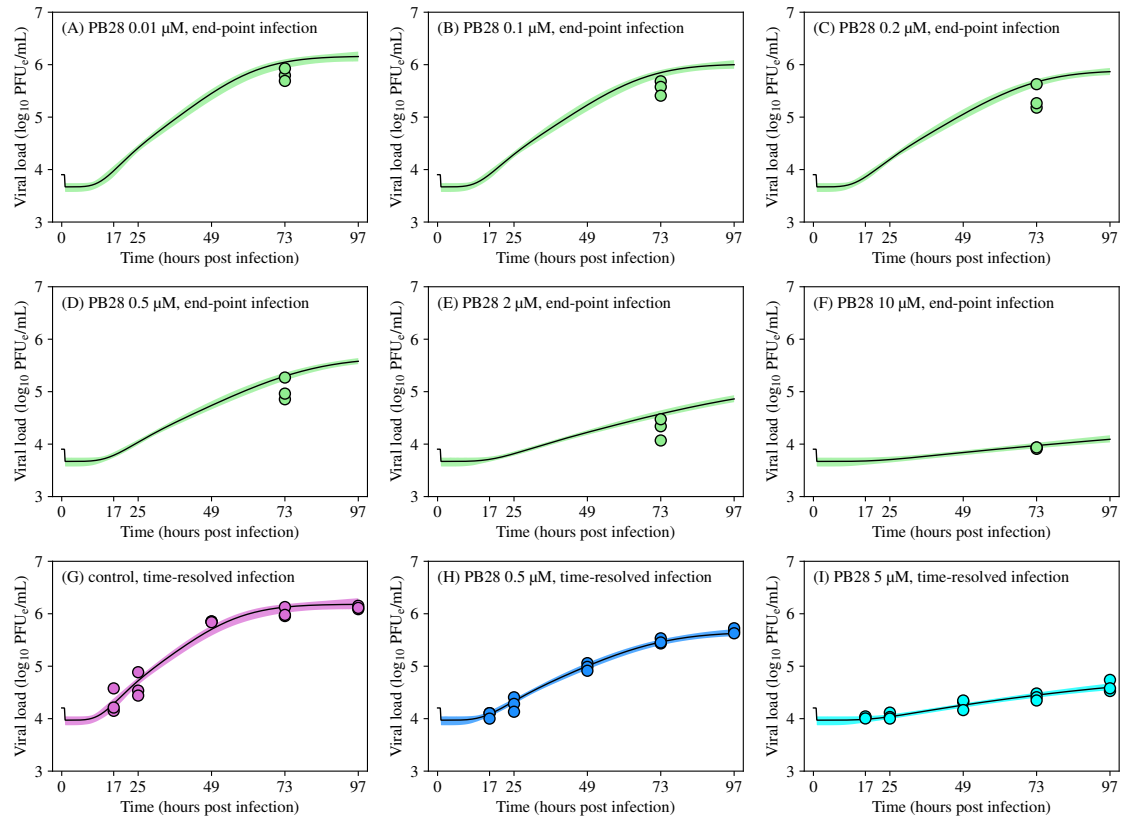
**Figure S9. Local sensitivity analysis of PB28 half-maximum inhibition constant,  $IC_{50}$ .** The parameter  $IC_{50}$  was varied by 50% from the best-fit value (Table 1 in the main text), and the corresponding solutions of Equations (1)–(5) in the main text were displayed as follows: a black solid line was used for the solutions associated with the best-fit parameter values; a red dashed line was used for the solutions associated with perturbations of +50%; and a blue dashed line was used for the solutions associated with perturbations in the best-fit parameter values by –50%.



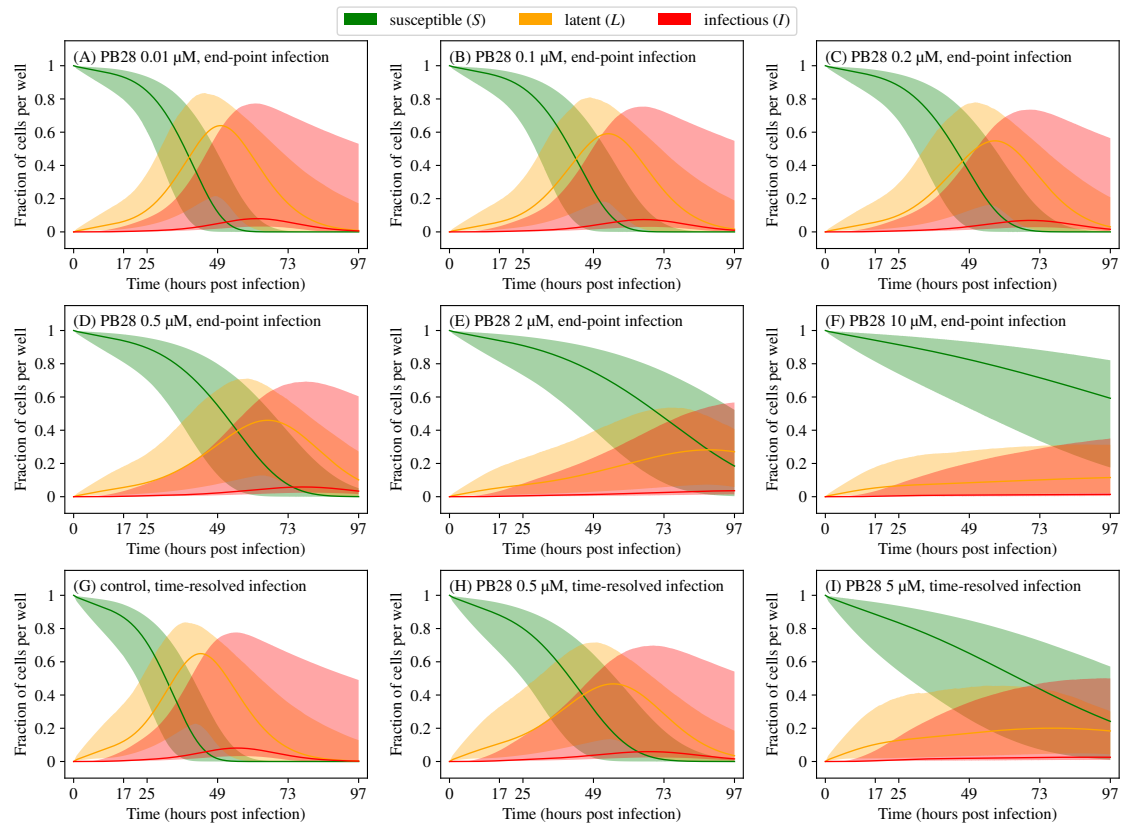
**Figure S10. Local sensitivity analysis of Hill coefficient,  $N_{\epsilon}$ .** The parameter  $N_{\epsilon}$  was varied by 50% from the best-fit value (Table 1 in the main text), and the corresponding solutions of Equations (1)–(5) in the main text were displayed as follows: a black solid line was used for the solutions associated with the best-fit parameter values; a red dashed line was used for the solutions associated with perturbations of +50%; and a blue dashed line was used for the solutions associated with perturbations in the best-fit parameter values by  $-50\%$ .



**Figure S11. PB28 parameters influencing SARS-CoV-2 infection dynamics in A549-ACE2 cells.** Equation (11) in the main text was fitted to SARS-CoV-2 viral loads using MCMC (details are in Materials and Methods in the main text). A total of 8 independent chains were run for 20,000 steps, with a burn-in of 10,000 steps. Thinning was applied by accepting every 5th step. A total of 16,000 accepted parameter sets were considered. The value in the upper-right corner represents the Pearson correlation coefficient for the corresponding parameter pair. The best-fit parameter value (Table 3 in the main text) is denoted by a green point.



**Figure S12. Fits of the viral dynamics model to SARS-CoV-2 viral load data.** Equations (1)–(5) in the main text were fitted exclusively to SARS-CoV-2 viral loads obtained from time-resolved experimental infections (panels (G)–(I)) using MCMC (details are in Materials and Methods in the main text). A total of 16 independent chains were run for 20,000 steps, with a burn-in of 10,000 steps. Thinning was applied by accepting every 10th step. A total of 16,000 accepted parameter sets were considered. The maximum likelihood (best-fit) solutions are displayed as black solid lines. The 95% credible bands are displayed as filled areas. Legend as in Figure 1 in the main text.



**Figure S13. A549-ACE2 cell dynamics.** Proportions of cells associated with fits of Equations (1)–(5) in the main text to SARS-CoV-2 viral loads obtained from time-resolved experimental infections (Figure S12G–I). Proportions of cells were calculated using the MCMC-accepted parameters, as described in Figure S12. The maximum likelihood (best-fit) solutions are displayed as solid lines. The 95% credible bands are displayed as filled areas.

## 50 PB28-induced inhibition of SARS-CoV-2 entry into A549- 51 ACE2 cells

52 To explore how PB28-induced inhibition of SARS-CoV-2 entry into A549-ACE2 cells af-  
53 fects viral dynamics, we modified the viral dynamics model (Equations (1)–(5) in the main  
54 text) such that the antiviral effect of PB28 acts on the infection rate rather than on the  
55 virus production rate:

$$\frac{dS}{dt} = -(1 - \epsilon) \beta V(t) S(t), \quad (\text{S.1})$$

$$\frac{dL_1}{dt} = (1 - \epsilon) \beta V(t) S(t) - \frac{n_L}{\tau_L} L_1(t) \quad (\text{S.2})$$

$$\frac{dL_i}{dt} = \frac{n_L}{\tau_L} (L_{i-1}(t) - L_i(t)) \text{ for } i = 2, \dots, n_L, \quad (\text{S.3})$$

$$\frac{dI}{dt} = \frac{n_L}{\tau_L} L_{n_L}(t) - \frac{1}{\tau_I} I(t), \quad (\text{S.4})$$

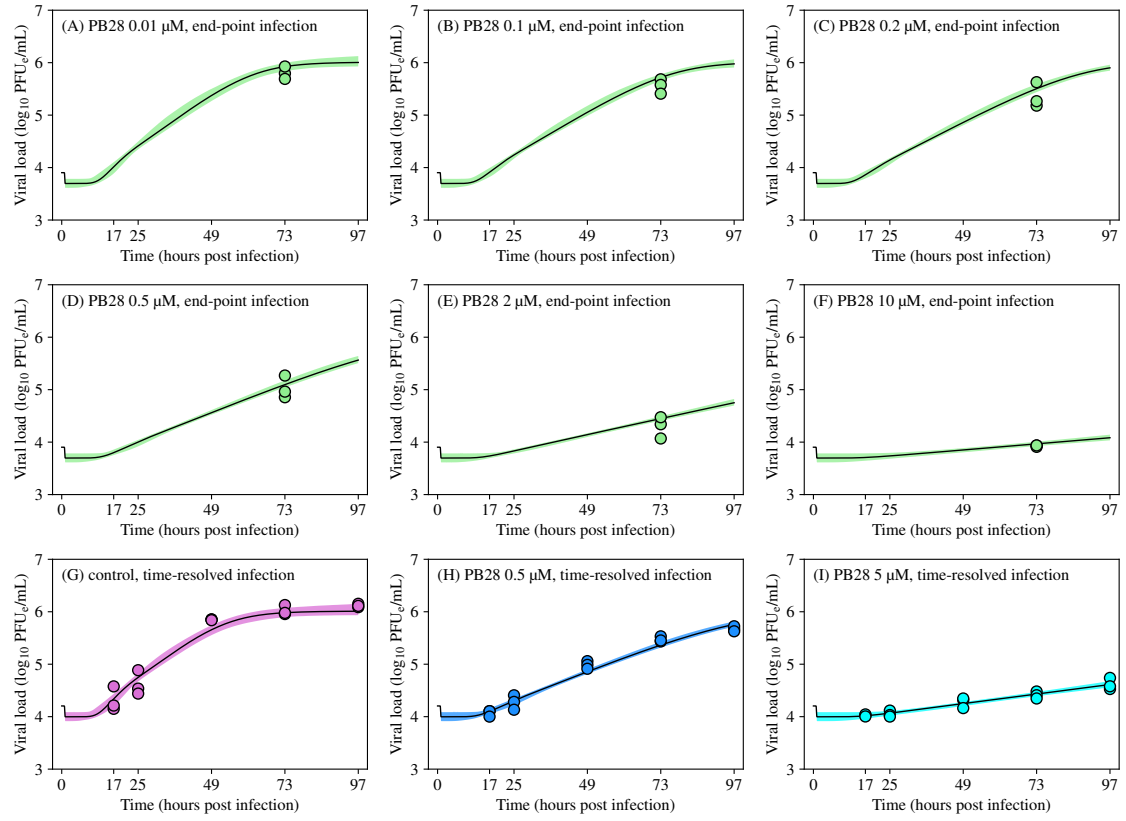
$$\frac{dV}{dt} = p I(t) - \omega(t) V(t). \quad (\text{S.5})$$

56 We assume that PB28 reduces the rate at which the virus infects cells by a factor  $(1 - \epsilon)$ ,  
57 where  $\epsilon$  can vary between 0 (no reduction of viral entry into the cells) and 1 (complete inhi-  
58 bition of virus entry into the cells). The efficacy,  $\epsilon$ , of PB28 in inhibiting the viral infection  
59 in Equations (S.1)–(S.2) is again modeled using Equation (6) in the main text.

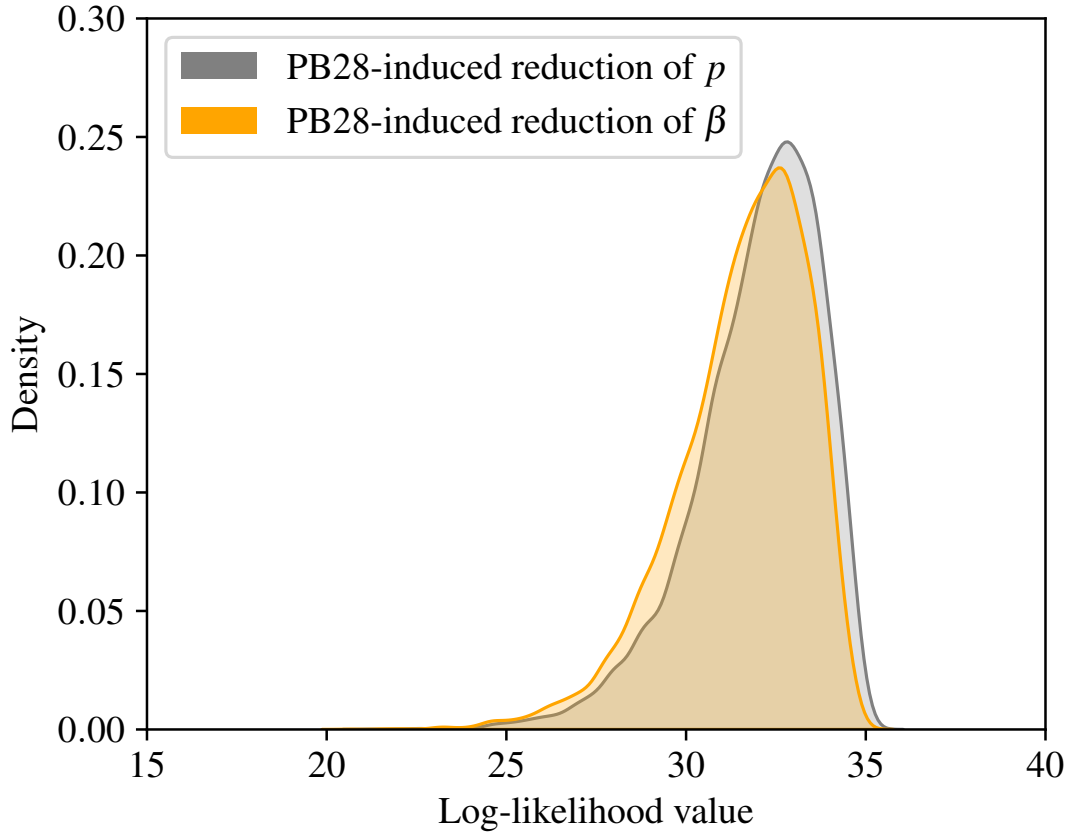
60 We simultaneously fitted Equations (S.1)–(S.5) to viral load data obtained from end-point  
61 and time-resolved infections using the Markov chain Monte Carlo (MCMC) approach  
62 (details are in Materials and Methods in the main text). The parameters estimated were  
63 the infection rate constant,  $\beta$ ; latent phase duration,  $\tau_L$ ; infectious phase duration,  $\tau_I$ ;  
64 the number of compartments for the latent phase,  $n_L$ ; the virus production rate constant,  
65  $p$ ; the parameter characterizing the washing process,  $\omega_0$ ; PB28 half-maximum inhibition  
66 constant,  $IC_{50,\epsilon}$ ; and Hill coefficient,  $N_\epsilon$ . Parameter values and their 95% CIs are given in  
67 Table S1. Fits of Equations (S.1)–(S.5) to viral load data are shown in Figure S14.

**Table S1.** SARS-CoV-2 and PB28 parameter estimates obtained from infection of A549-ACE2 cells (Equations (S.1)–(S.5)).

| Parameter          | Description                           | Units                    | ML value              | 95% CI                         |
|--------------------|---------------------------------------|--------------------------|-----------------------|--------------------------------|
| $\beta$            | infection rate constant               | mL/(PFU <sub>e</sub> ×h) | $6.66 \times 10^{-7}$ | $[2.38, 38.69] \times 10^{-7}$ |
| $\tau_L$           | duration of latent phase              | h                        | 11.49                 | [4.80, 30.71]                  |
| $\tau_I$           | duration of infectious phase          | h                        | 9.28                  | [1.14, 82.16]                  |
| $n_L$              | number of latent phase compartments   | -                        | 20                    | [3, 48]                        |
| $p$                | virus production rate constant        | PFU <sub>e</sub> /(h×mL) | $1.1 \times 10^5$     | $[0.271, 9.34] \times 10^5$    |
| $\omega_0$         | washing rate constant                 | -                        | 0.48                  | [0.27, 0.67]                   |
| $\epsilon_{\max}$  | maximum inhibition efficacy           | -                        | 1                     | fixed                          |
| $IC_{50,\epsilon}$ | PB28 half-maximum inhibition constant | μM                       | 0.11                  | [0.06, 0.23]                   |
| $N_\epsilon$       | Hill coefficient                      | -                        | 0.86                  | [0.74, 1.11]                   |



**Figure S14. Fits of the viral dynamics model to SARS-CoV-2 viral load data assuming PB28-induced reduction of infection rate.** Equations (S.1)–(S.5) were simultaneously fitted to SARS-CoV-2 viral loads obtained from end-point (panels A–F) and time-resolved (panels G–I) experimental infections using MCMC (details are in Materials and Methods). A total of 16 independent chains were run for 20,000 steps, with a burn-in of 10,000 steps. Thinning was applied by accepting every 10th step. A total of 16,000 accepted parameter sets were considered. The maximum likelihood (best-fit) solutions are displayed as black solid lines. The 95% credible bands are displayed as filled areas. Legend as in Figure 1 in the main text.



**Figure S15. Goodness of fit of the viral dynamics model assuming PB28-induced reduction in virus production or the infection rate.** Distributions of log-likelihood values (Equation (8) in the main text) resulting from fitting the viral dynamics model with PB28-induced reduction in virus production rate  $p$  (depicted in grey, Equations (1)–(5) in the main text) and with PB28-induced reduction of viral infection rate  $\beta$  (depicted in orange, Equations (S.1)–(S.5)) to viral load data obtained from endpoint and time-resolved experimental infection schemes (Figures 3 in the main text and S14, respectively) using MCMC (details are in Materials and Methods in the main text and in the captions of Figures 3 in the main text and S14, respectively). Higher values indicate a better fit.