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Supplemental information

Modeling explains prolonged SARS-CoV-2 nasal shedding relative to lung shedding in remdesivir-treated rhesus macaques

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Supplemental figures

Figure S1: Individual fitting to nasal viral loads in remdesivir treated animals using Ke et al.'s model [17], Related to Figure 5 and Table S5. **A)** Fits to 6 treated animals who received 10 mg/kg at day 0.5 and 5 mg/kg at days 1, 2, 3, 4, 5 and 6 (solid lines). Dashed lines represent fits to vehicle group. Pink and blue dots are nasal swabs and BAL data points, respectively. Dots overlying the dotted line are below the limit of detection. Time is in days from infection. **B)** Simulated percentage of dead lung target cells as a proxy for lung damage.

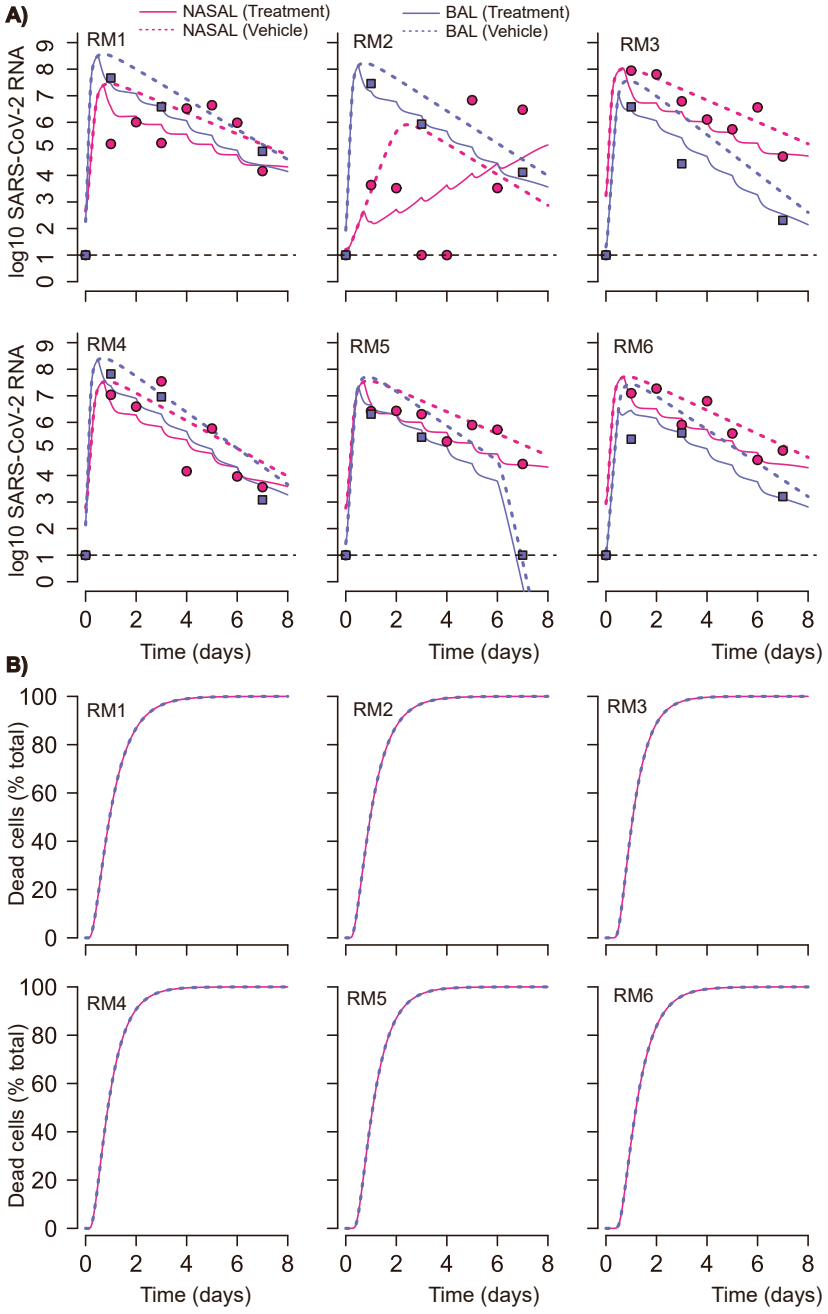


Figure S2: Individual fitting to nasal viral loads in remdesivir treated animals, Related to Figures 5 and 7. Fits to 5 treated animals who received 10 mg/kg at day 0.5 and 5 mg/kg at days 1, 2, 3, 4, 5 and 6. We excluded RM2 from the fitting procedure as our model cannot reproduce viral rebound on treatment, as seen in RM2. Pink dots are nasal swabs datapoints and lines are model projections. Dots overlying the dotted line are below the limit of detection. Time is in days from infection.

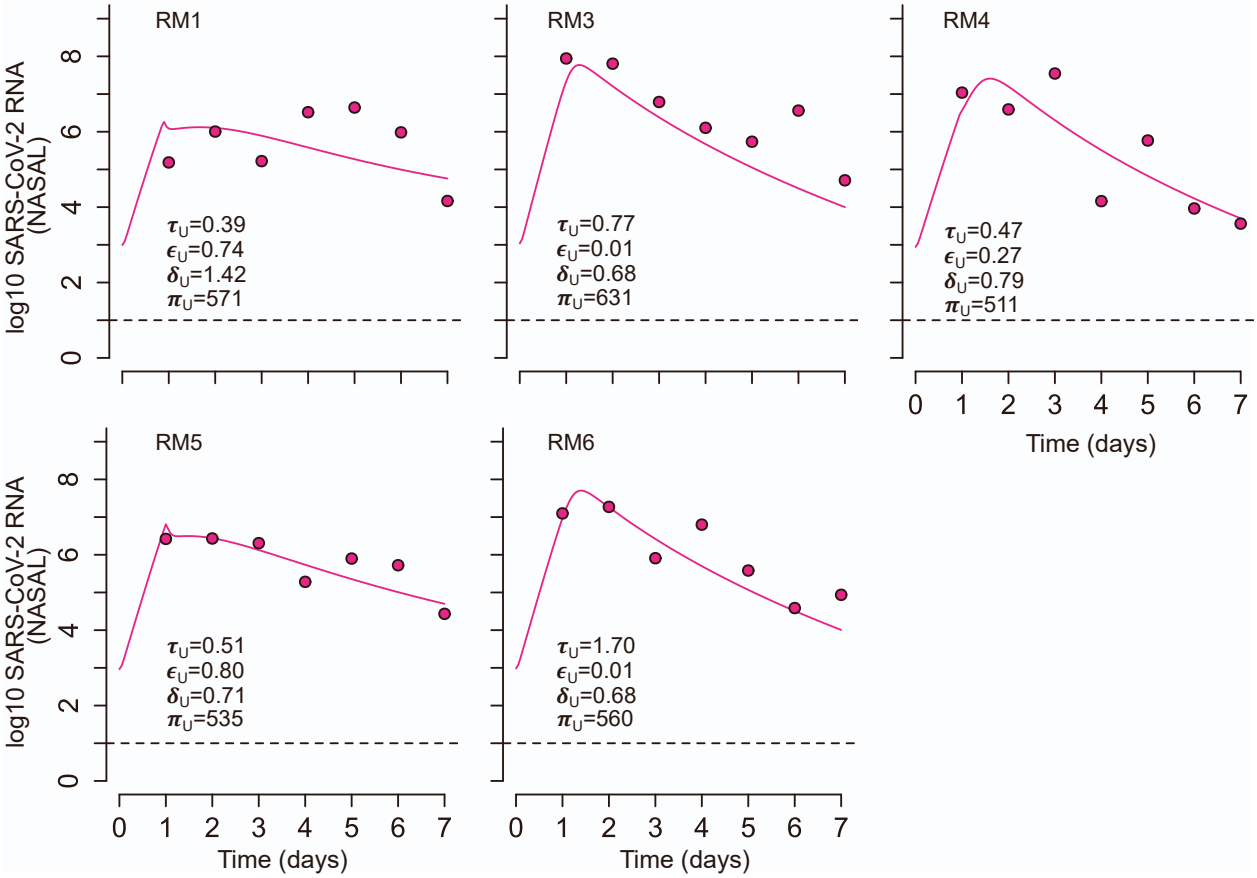


Figure S3: Mechanisms of lung protection in remdesivir treated animals, Related to Figure 5. The number of susceptible cells is projected for simulations fit to treatment data (solid lines) and counterfactual simulations without therapy (dashed lines). In nasal passages, therapy limits initial depletion of susceptible cells which allows for persistent viral replication rather than elimination. In the lung (BAL specimens), depletion of susceptible cells occurs in part due to the cells entering a refractory state: treatment efficacy lowers the number of susceptible cells that become refractory to infection. The depletion of susceptible cells prevents persistent shedding. Simulations are based on data from RM 1-6. Time is in days from infection.

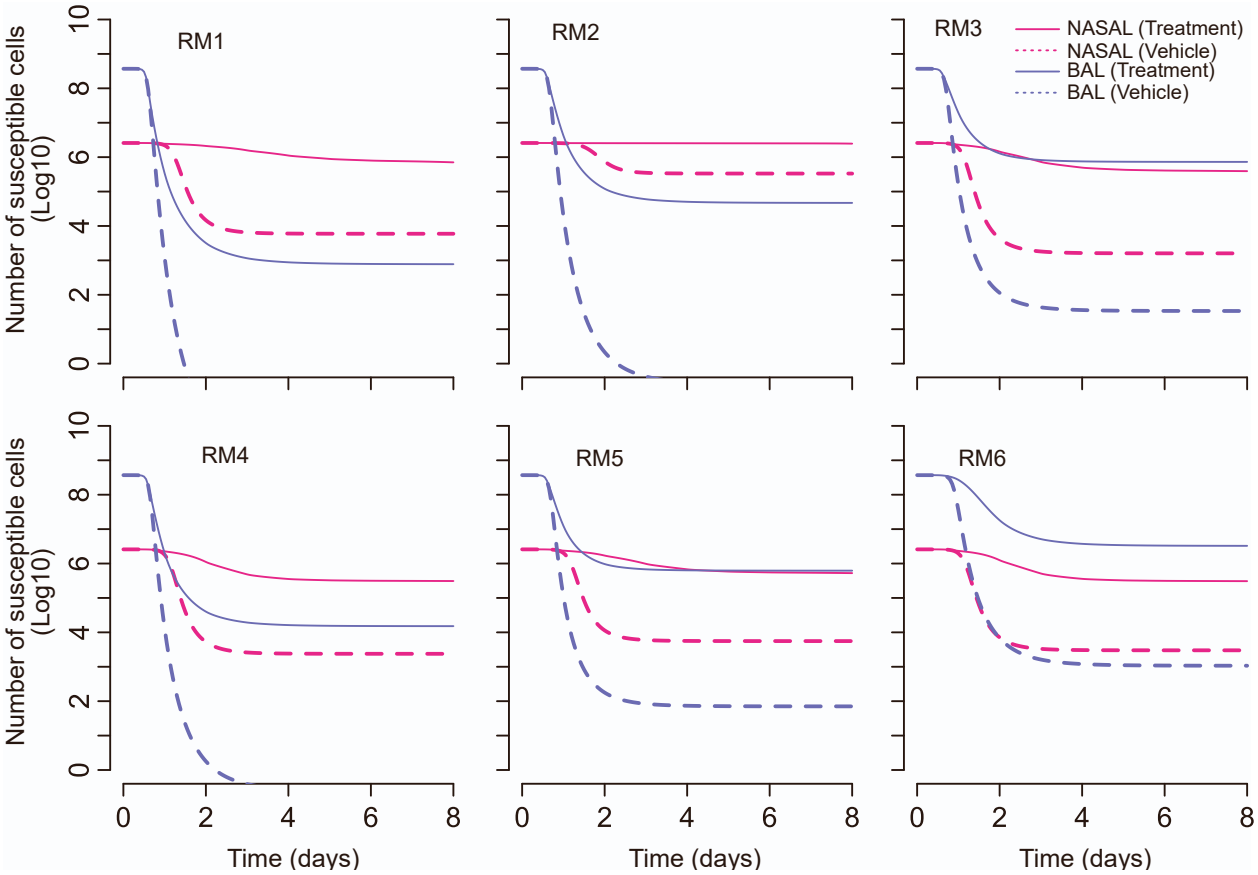


Figure S4: Simulated percentage of dead lung target cells as a proxy for lung damage, Related to Figure 5. We define dead cells as the initial total of susceptible cells minus susceptible cells, infected cells and refractory cells in lung at each time point. Treatment lowers the percent of dead cells relative to the counterfactual simulations without therapy. Simulations are based on data from RM 1-6. Time is in days from infection.

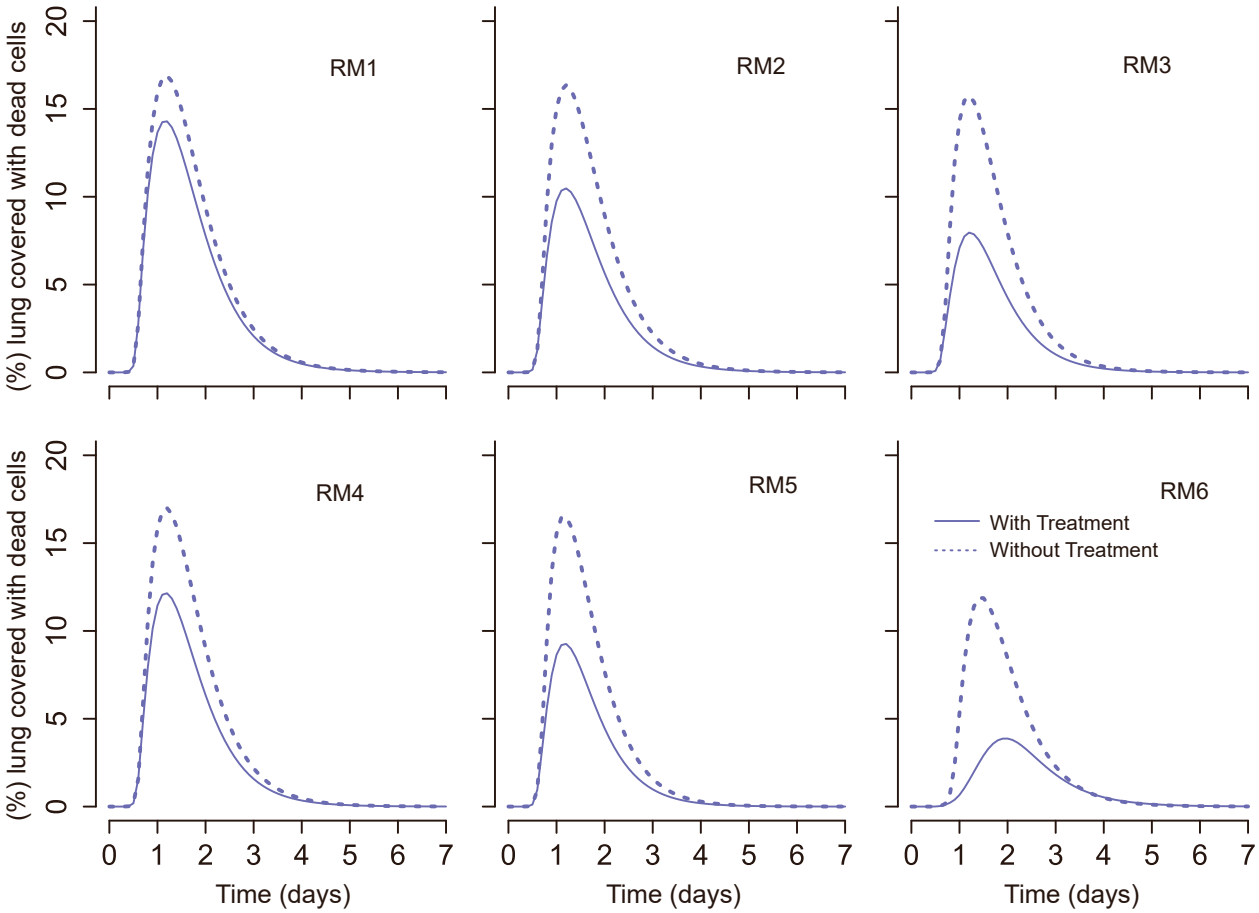


Figure S5: Predicted outcome of more potent remdesivir therapy, Related to Figure 5. Therapy is simulated after lowering the *in vivo* EC_{50} 10-fold and 100-fold relative to data fitting in **Fig 5b.** Treatment is started 0.5 days after infection. **A.** Simulations of nasal viral load. **B.** Simulations of lung viral loads. Simulations are based on data from RM 1-6.

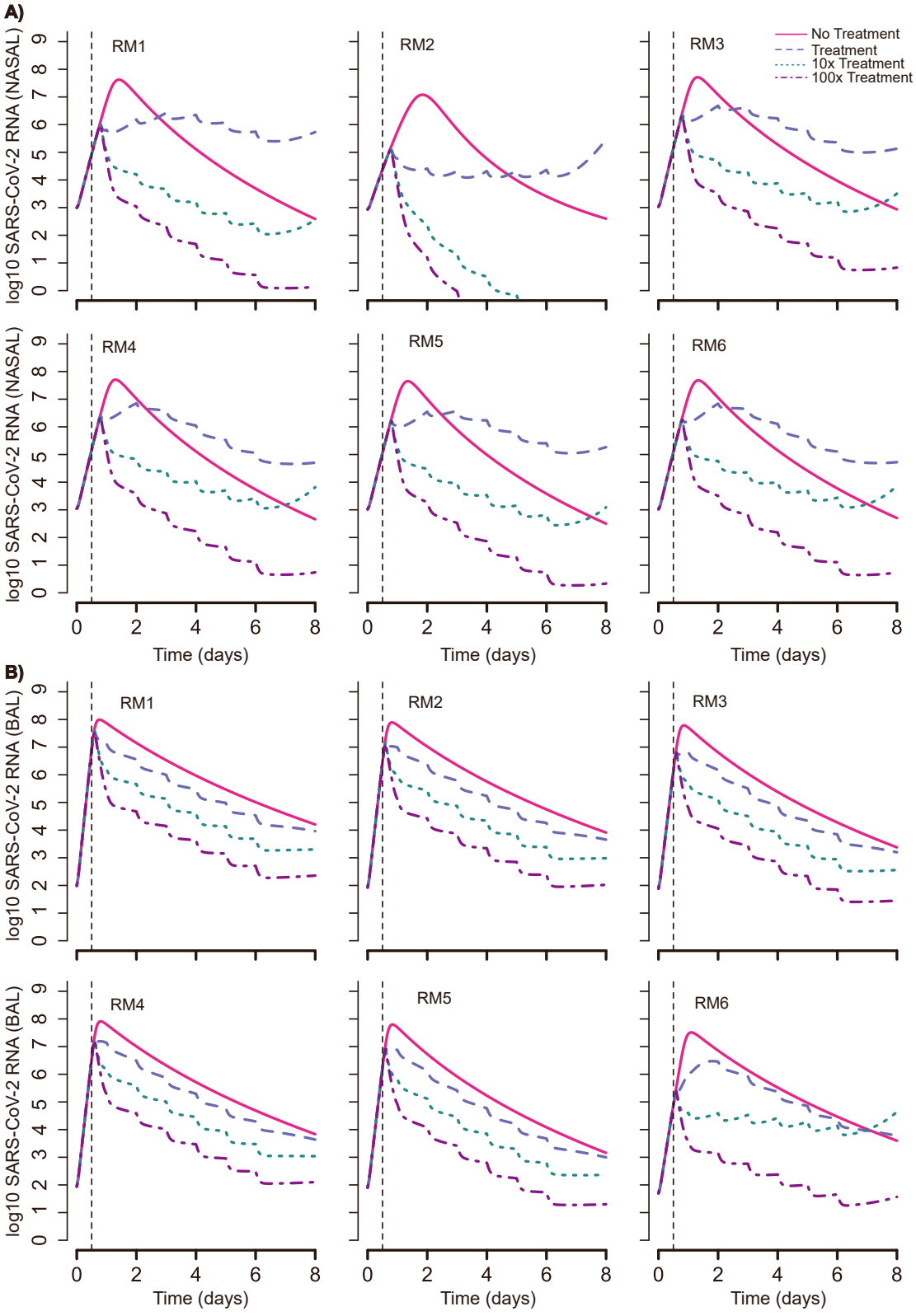
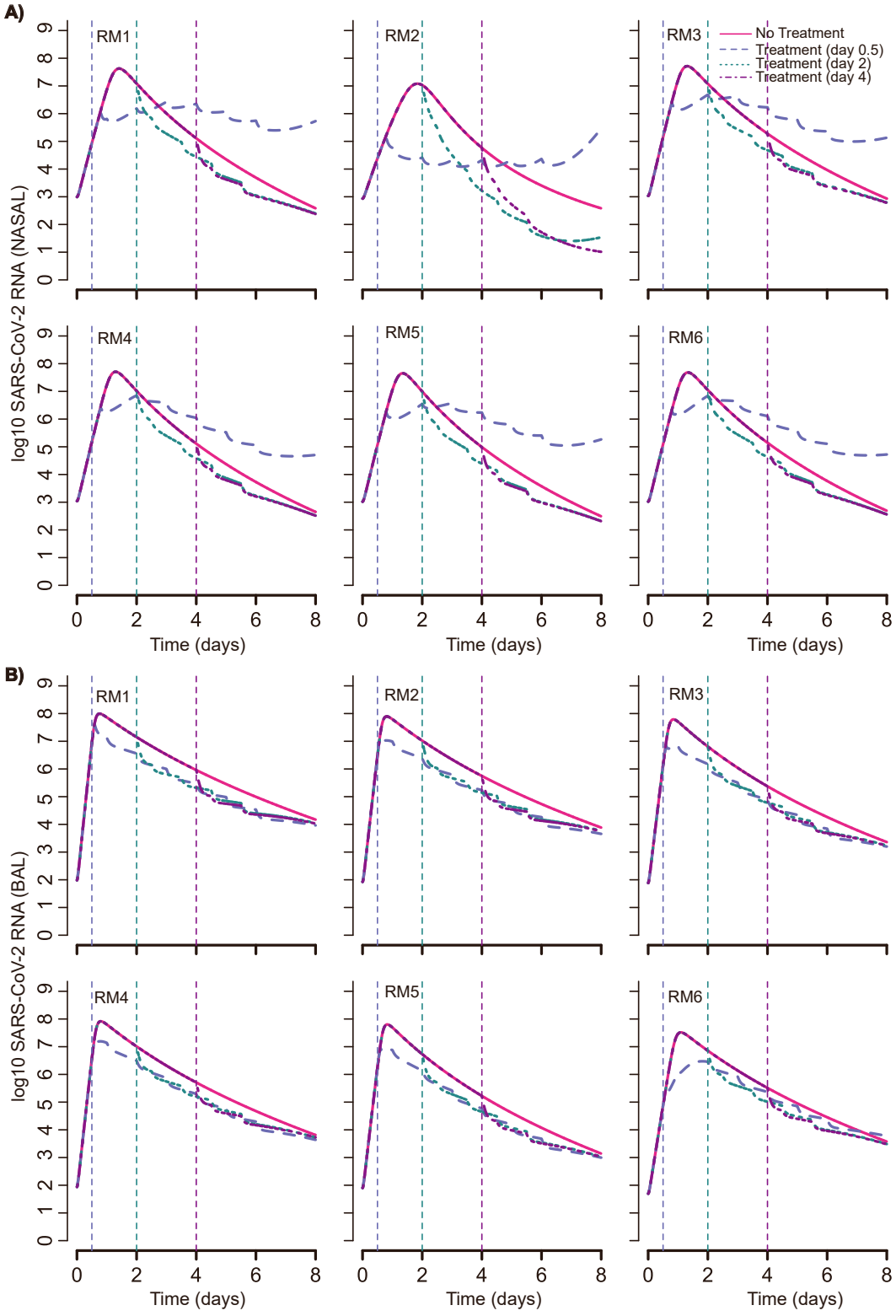


Figure S6: Predicted outcome of later remdesivir therapy, Related to Figures 5 and 7. Therapy is simulated with the same antiviral potencies estimated from RM 1-RM6 treated at day 0.5 but with initiation at later time points (days 2 and 4). **A.** Simulations of nasal viral load. **B.** Simulations of lung viral loads.



Supplementary tables

Table S1. Pharmacokinetic model parameters for six individual animals, RM1-RM6, Related to Figure 3**.** Parameter units are: k_{1T}, /day; k_{2T}, /day; k_{1e}, /day; k_{2e}, /day; k_{12}, /day; k_{23}, /day; k_{34}, /day; k_3, /day; V_1, mL; V_2, mL; V_3, mL; V_{3T}, gm; V_{4T}, gm.

ID	k_{1T}	k_{2T}	k_{1e}	k_{2e}	k_{12}	k_{23}	k_{34}	k_3	V_1	V_2	V_3	V_{3T}	V_{4T}
RM1	33.0	5.86×10^3	0.02	12.3	1.0	989.6	158.4	7.9	2.8×10^{-3}	1.1×10^{-7}	1.7×10^{-5}	3.5×10^{-3}	5.8×10^{-5}
RM2	33.0	5.86×10^3	0.02	12.3	1.0	989.9	168.9	7.9	2.8×10^{-3}	1.1×10^{-7}	1.8×10^{-5}	4.0×10^{-3}	5.8×10^{-5}
RM3	33.0	5.86×10^3	0.02	12.3	1.0	989.7	162.4	7.9	2.8×10^{-3}	1.1×10^{-7}	1.7×10^{-5}	3.7×10^{-3}	5.8×10^{-5}
RM4	33.0	5.86×10^3	0.02	12.3	1.0	990.1	173.4	7.9	2.8×10^{-3}	1.1×10^{-7}	1.8×10^{-5}	4.2×10^{-3}	5.8×10^{-5}
RM5	33.0	5.86×10^3	0.02	12.3	1.0	990.1	166.9	7.9	2.8×10^{-3}	1.1×10^{-7}	1.8×10^{-5}	3.9×10^{-3}	5.8×10^{-5}
RM6	33.0	5.86×10^3	0.02	12.3	1.0	989.8	175.8	7.9	2.8×10^{-3}	1.1×10^{-7}	1.8×10^{-5}	4.3×10^{-3}	5.8×10^{-5}

Table S2: Pharmacokinetic model parameters at the population level, Related to Figure 3**.** Here, NA represents not available. Parameter units are: k_{1T}, /day; k_{2T}, /day; k_{1e}, /day; k_{2e}, /day; k_{12}, /day; k_{23}, /day; k_{34}, /day; k_3, /day; V_1, mL; V_2, mL; V_3, mL; V_{3T}, gm; V_{4T}, gm.

Parameter	**Fixed Effects**	**Standard deviation of the random effects**	**(%) RSE**
k_{1T}	33.0	NA	5.8
Log10(k_{2T})	3.77	NA	36.8
k_{1e}	0.02	NA	17
k_{2e}	12.3	NA	737
k_{12}	1.0	NA	10.4
Log10(k_{23})	3.0	0.008	105
k_{34}	167.9	0.25	5.2×10^3
k_3	7.9	NA	7.8
Log10(V_1)	-2.55	0.008	1.4
Log10(V_2)	-6.96	0.03	14.8
Log10(V_3)	-4.77	0.009	68.3
Log10(V_{3T})	-2.42	0.06	749
Log10(V_{4T})	-4.24	0.10	6.0

Table S3: Different model structures explored while fitting nasal and BAL viral loads in only untreated animals, Related to Figures 4 and 5**.** The model with the lowest Akaike information criteria (AIC) is best supported by the data (denoted in bold). 'Same' implies that the parameter takes the value from the same distribution in two spatial compartments whereas 'different' implies that the parameter has different distributions in two compartments. Here, -2LL represent -2 times the log-likelihood and MOI denotes multiplicity of infection. * and ** represents the case where the viral peak (either in Nasal or lung compartment) is achieved instantly and thus, these cases do not represent biological reality and are excluded from the model selection despite some of them having lower AIC.

δ_i	π_i	k_i	MOI	AIC	-2LL
Diff	Diff	Diff	10^{-3}	308.1	278.1
Diff	Diff	Diff	10^{-4}	308.4	278.4 **
Diff	Diff	Diff	10^{-5}	307.1	277.1 **
Diff	Diff	Diff	10^{-6}	335.4	305.4 *
Same	Diff	Diff	10^{-3}	307.8	281.8 *
Same	Diff	Diff	10^{-4}	307.5	281.5 **
Same	Diff	Diff	10^{-5}	306.5	280.5**
Same	Diff	Diff	10^{-6}	329.8	303.8 *
Diff	Same	Diff	10^{-3}	302.5	276.5 **
Diff	Same	Diff	10^{-4}	301.6	275.6 **
Diff	Same	Diff	10^{-5}	305.3	279.3 **
Diff	Same	Diff	10^{-6}	306.6	286.6 **
Diff	Diff	Same	10^{-3}	303.4	277.4 **
Diff	Diff	Same	10^{-4}	303.3	277.3 **
Diff	**Diff**	**Same**	10^{-5}	**307.9**	**281.9**
Diff	Diff	Same	10^{-6}	313.7	287.7 *
Same	Same	Diff	10^{-3}	302.8	280.8 *
Same	Same	Diff	10^{-4}	302.3	280.3 *
Same	Same	Diff	10^{-5}	306.3	284.3 *
Same	Same	Diff	10^{-6}	310.6	288.6 *
Same	Diff	Same	10^{-3}	304.5	282.5 *
Same	Diff	Same	10^{-4}	303.4	281.4 *
Same	Diff	Same	10^{-5}	304.9	282.9 *
Same	Diff	Same	10^{-6}	306.4	284.4 *
Diff	Same	Same	10^{-3}	NA	NA
Diff	Same	Same	10^{-4}	297.7	275.7 **
Diff	Same	Same	10^{-5}	297.1	275.1 **
Diff	Same	Same	10^{-6}	299.4	277.4 *
Same	Same	Same	10^{-3}	302.1	284.1
Same	Same	Same	10^{-4}	300.0	282.0 *
Same	Same	Same	10^{-5}	301.4	283.4 *
Same	Same	Same	10^{-6}	303.2	285.2 **
Diff	Diff	$k_i = 0$	10^{-5}	318.3	296.3
Diff	Diff	$k_L = 0, k_U \neq 0$	10^{-5}	313.2	287.2
Diff	Diff	$k_U = 0, k_L \neq 0$	10^{-5}	318.3	292.3

Table S4: Estimated population parameters from the fitting of 14 untreated animals using the best model in Table S2, Related to Figure 5**.** Here, we have MOI(m)=10^{-5} and RSE represent the random deviation of the standard effects. Here, NA represents not available. Parameter units are: β's, virions^{-1} day^{-1}; δ's, day^{-1} cells^{-k}; k's, unitless; π's day^{-1}.

Parameter	**Fixed Effects (standard deviation of the random effects)**	**(%) RSE**
Log10(β_U) (or, Log10(β_L))	-6.73 (NA)	2.44
δ_U	0.95 (0.36)	18.6
k_U (or, k_L)	0.09 (0.06)	25.6
π_U	2.77 (0.02)	5.8
δ_L	0.4 (0.13)	33.4
π_L	1.0 (0.16)	16.1

Table S5: Different model structures explored while fitting nasal and BAL viral loads in untreated and remdesivir treated animals, Related to Figures 4 and 5**.** The model with the lowest Akaike information criteria (AIC) is best supported by the data (denoted in bold). Models with the inclusion of refractory cells (in the form of $\rho_i S_i$ and $\phi_i I_i$ unless otherwise mentioned with *) and the delayed proliferation of susceptible cells in lung but not in the nasal passage are better equipped to explain the reduced lung damage in treated animals. All version of models assume $\theta_{LU} = 0$ and $\theta_{UL} = 0$ as their counterpart models with $\theta_{LU} \neq 0$ and $\theta_{UL} \neq 0$ have higher AIC. Moreover, [1] denote parameters for which both fixed and random effects are estimated, [2] denote parameters for which only fixed effects are estimated and [3] denote parameters for which fixed effects with fixed random effects (=0.1) are estimated, [4] denote parameters for which fixed effects with fixed random effects (=0.5) are estimated and [5] denote parameters for which fixed effects with fixed random effects (=1.0) are estimated. Here, [6] represents the situation where we assume the same distribution for EC_{50L} and EC_{50U}. Here, to avoid non-identifiability issues, population parameters such as β_i, δ_i, k_i and π_i were kept fixed from Table S4. We also tried Ke et al.'s approach [1] using the refractory model and the effector cell model to describe data from NASAL and BAL samples. In the refractory model, target cells are assumed can become refractory to infection through the activity of soluble immune mediators released by infected cells, such as interferon. In the immune effector cell model, innate and adaptive immune cells are assumed to be activated and recruited to eliminate infected cells, leading to increased viral clearance. Ke et al.'s model fit the data with AIC=565.4. For more details on Ke et al.'s model structure, see [1].

Refractory cells	**Proliferation terms**	**Delay in the antiviral activity**	**Estimated parameters details**	**AIC**
No (ρ_i's=0, ϕ_i's=0 and ζ_i's=0)	No (r_i's=0 and τ_i's=0)	No (ν_i's =0)	EC_{50i}[1]	736.3
Yes (ρ_i's=0, ϕ_i's≠0 and ζ_i's=0)	No (r_i's=0 and τ_i's=0)	No (ν_i's =0)	EC_{50i}[1] and ϕ_i[1]	732.1
Yes (ρ_i's=0, ϕ_i's≠0 and ζ_i's=0)	No (r_i's=0 and τ_i's=0)	No (ν_i's =0)	EC_{50i}[6,2] and ϕ_i[1]	717.1
Yes (ρ_i's≠0, ϕ_i's=0 and ζ_i's=0)	No (r_i's=0 and τ_i's=0)	No (ν_i's =0)	EC_{50i}[1] and ρ_i[1]	726.5
Yes (ρ_i's≠0, ϕ_i's=0 and ζ_i's=0)	No (r_i's=0 and τ_i's=0)	No (ν_i's =0)	EC_{50i}[6,2] and ρ_i[1]	714.1
Yes (*$\rho_i S_i I_i$) (ρ_i's≠0, ϕ_i's=0 and ζ_i's=0)	No (r_i's=0 and τ_i's=0)	No (ν_i's =0)	EC_{50i}[1] and ρ_i[1]	717.9
Yes (*$\rho_i S_i I_i$) (ρ_i's≠0, ϕ_i's=0 and ζ_i's=0)	No (r_i's=0 and τ_i's=0)	No (ν_i's =0)	EC_{50}[6,1] and ρ_i[1]	707.7
Yes (*$\rho_i S_i I_i$) (ρ_i's≠0, ϕ_i's=0 and ζ_i's=0)	No (r_i's=0 and τ_i's=0)	No (ν_i's =0)	EC_{50}[6,2] and ρ_i[1]	701.6
Yes (*$\rho_i S_i I_i$) (ρ_i's≠0, ϕ_i's=0 and ζ_i's=0)	No (r_i's=0 and τ_i's=0)	No (ν_i's =0)	EC_{50}[6,3] and ρ_i[1]	714.1
No	Yes	No	EC_{50i}[1], r_i[2] and τ_i[2]	732.8

(ρ_i's=0, ϕ_i's=0 and ζ_i's=0)	(r_U=0, r_L≠0, τ_U=0 and τ_L≠0)	(ν_i's =0)		
No (ρ_i's=0, ϕ_i's=0 and ζ_i's=0)	Yes (r_U=0, r_L≠0, τ_U=0 and τ_L≠0)	No (ν_i's =0)	$EC_{50i}^{6,2}$, r_i^2 and τ_i^2	722.7
Yes (ρ_i's=0, ϕ_i's≠0 and ζ_i's=0)	Yes ($r_U = 0, r_L \neq 0, \tau_U = 0$ and $\tau_L \neq 0$)	No (ν_i's =0)	$EC_{50i}^{6,2}$, r_i^2, τ_i^2 and ϕ_i^1	701.8
Yes (ρ_i's≠0, ϕ_i's=0 and ζ_i's=0)	Yes ($r_U = 0, r_L \neq 0, \tau_U = 0$ and $\tau_L \neq 0$)	No (ν_i's =0)	$EC_{50i}^{6,2}$, r_i^2, τ_i^2 and ρ_i^1	700.4
Yes (*$\rho_i S_i I_i$) (ρ_i's ≠ 0, ϕ_i's =0 and ζ_i's=0)	Yes ($r_U = 0, r_L \neq 0, \tau_U = 0$ and $\tau_L \neq 0$)	No (ν_i's=0)	$EC_{50i}^{6,2}$, r_i^2, τ_i^2 and ρ_i^1	698.0
Yes (*$\rho_i S_i I_i$) (ρ_i's ≠ 0, ϕ_i's =0 and ζ_i's=0)	Yes ($r_U = 0, r_L \neq 0, \tau_U = 0$ and $\tau_L \neq 0$)	Yes (ν_i's ≠0)	$EC_{50i}^{6,2}$, r_i^2, τ_i^2, ρ_i^2 and ν_i^2	677.6
Yes (*$\rho_i S_i I_i$) (ρ_i's ≠ 0, ϕ_i's =0 and ζ_i's=0)	Yes ($r_U = 0, r_L \neq 0, \tau_U = 0$ and $\tau_L \neq 0$)	Yes (ν_i's ≠0)	$EC_{50i}^{6,2}$, r_i^2, τ_i^2, ρ_i^3 and ν_i^3	668.7
Yes (*$\rho_i S_i I_i$) (ρ_i's ≠ 0, ϕ_i's =0 and ζ_i's=0)	**Yes ($r_U = 0, r_L \neq 0, \tau_U = 0$ and $\tau_L \neq 0$)**	**Yes (ν_i's ≠0)**	**$EC_{50i}^{6,2}$, r_i^2, τ_i=0, ρ_i^3 and ν_i^3**	**666.8**
Yes (*$\rho_i S_i I_i$) (ρ_i's ≠ 0, ϕ_i's =0 and ζ_i's=0)	Yes ($r_U = 0, r_L \neq 0, \tau_U = 0$ and $\tau_L \neq 0$)	Yes (ν_i's ≠0)	$EC_{50i}^{6,2}$, r_i^2, τ_i^2, ρ_i^3 and ν_U^3, $\nu_L = 0$	670.7
Yes (*$\rho_i S_i I_i$) (ρ_i's ≠ 0, ϕ_i's =0 and ζ_i's=0)	Yes ($r_U = 0, r_L \neq 0, \tau_U = 0$ and $\tau_L \neq 0$)	Yes (ν_i's ≠0)	$EC_{50i}^{6,2}$, r_i^2, τ_i^2, ρ_i^4 and ν_i^4	683.8
Yes (*$\rho_i S_i I_i$) (ρ_i's ≠ 0, ϕ_i's =0 and ζ_i's=0)	Yes ($r_U = 0, r_L \neq 0, \tau_U = 0$ and $\tau_L \neq 0$)	Yes (ν_i's ≠0)	$EC_{50i}^{6,2}$, r_i^2, τ_i^2, ρ_i^3 and $\nu_i^{6,3}$	680.0

Table S6: Estimated parameters under the model depicted in Figure 4b, Related to Figures 4 and 5**.** We also estimated only the fixed effects of four parameters: $EC_{50U} = EC_{50L} = 2.2$ nM/gm, $\tau_L = 0$ days and r_L=2.0/day while fixing β=1.9×10^{-7} virions^{-1}.day^{-1}. In absence of the data on active metabolite concentration, the concentration of nucleoside GS-441524 in tissue (Nuc) was employed as a proxy to calculate EC_{50} [2]. Parameter units are: δ's, day^{-1} cells^{-k}; k's, unitless; π's, day^{-1}; ρ_L; day^{-1} cells^{-1}.

	$k_U = k_L$	δ_U	Log10 (π_U)	δ_L	Log10 (π_L)	Log10 (ρ_L)	ν_U (days)	ν_L (days)	Category
RM1	0.09	0.97	2.76	0.37	1.15	-6.89	0.29	0.09	RDV
RM2	0.09	2.34	2.70	0.40	1.09	-6.88	0.28	0.09	RDV
RM3	0.09	0.83	2.80	0.46	1.05	-6.84	0.29	0.09	RDV
RM4	0.09	0.92	2.80	0.41	1.10	-6.89	0.29	0.09	RDV
RM5	0.09	0.96	2.78	0.49	1.06	-6.84	0.29	0.09	RDV
RM6	0.09	0.91	2.79	0.42	0.86	-6.86	0.29	0.09	RDV
RM7	0.09	1.33	2.78	0.37	1.07	-6.90	NA	NA	VEHICLE
RM8	0.09	1.44	2.77	0.41	0.94	-6.86	NA	NA	VEHICLE
RM9	0.09	1.10	2.78	0.41	1.0	-6.88	NA	NA	VEHICLE
RM10	0.08	0.77	2.78	0.38	1.06	-6.89	NA	NA	VEHICLE
RM11	0.08	1.11	2.76	0.37	1.03	-6.88	NA	NA	VEHICLE
RM12	0.09	0.82	2.77	0.41	1.01	-6.88	NA	NA	VEHICLE
RM13	0.09	0.85	2.78	0.40	1.00	-6.88	NA	NA	VEHICLE
RM14	0.09	0.83	2.77	0.40	1.00	-6.88	NA	NA	VEHICLE
RM15	0.09	0.95	2.77	0.40	1.00	-6.88	NA	NA	VEHICLE
RM16	0.09	0.94	2.76	0.40	1.00	-6.88	NA	NA	VEHICLE
RM17	0.09	0.81	2.77	0.38	1.06	-6.89	NA	NA	VEHICLE
RM18	0.09	0.69	2.77	0.42	0.96	-6.86	NA	NA	VEHICLE
RM19	0.09	0.68	2.77	0.38	1.06	-6.89	NA	NA	VEHICLE
RM20	0.09	1.23	2.77	0.38	1.06	-6.89	NA	NA	VEHICLE
Median	0.09	0.94	2.79	0.42	1.08	-6.87	0.29	0.09	**RDV**
Median	0.09	0.89	2.77	0.40	1.01	-6.88	NA	NA	**VEHICLE**

Table S7: Pharmacokinetic model parameters at the population level from the fitting of 14 untreated animals and 6 treated animals using the best model in Table S5, Related to Figures 4 and 5**.** Here, NA represents not available. Parameter units are: EC_{50}, nM/gm; r_L, day^{-1}; ν's, day; ρ_L; day^{-1} cells^{-1}.

Parameter	**Fixed Effects**	**Standard deviation of the random effects**	**(%) RSE**
Log10(EC_{50})	0.34	NA	32
r_L	2	NA	0.08
ν_U	0.29	0.1 (fixed)	42
ν_L	0.09	0.1 (fixed)	176
Log10(ρ_L)	-6.88	0.1 (fixed)	3.17

References

1. Ke, R., et al., Daily sampling of early SARS-CoV-2 infection reveals substantial heterogeneity in infectiousness. medRxiv, 2021: p. 2021.07.12.21260208.
2. Williamson, B.N., et al., Clinical benefit of remdesivir in rhesus macaques infected with SARS-CoV-2. Nature, 2020.