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Supplemental Information

Remdesivir Inhibits SARS-CoV-2 in Human Lung Cells and Chimeric SARS-CoV Expressing the SARS-CoV-2 RNA Polymerase in Mice

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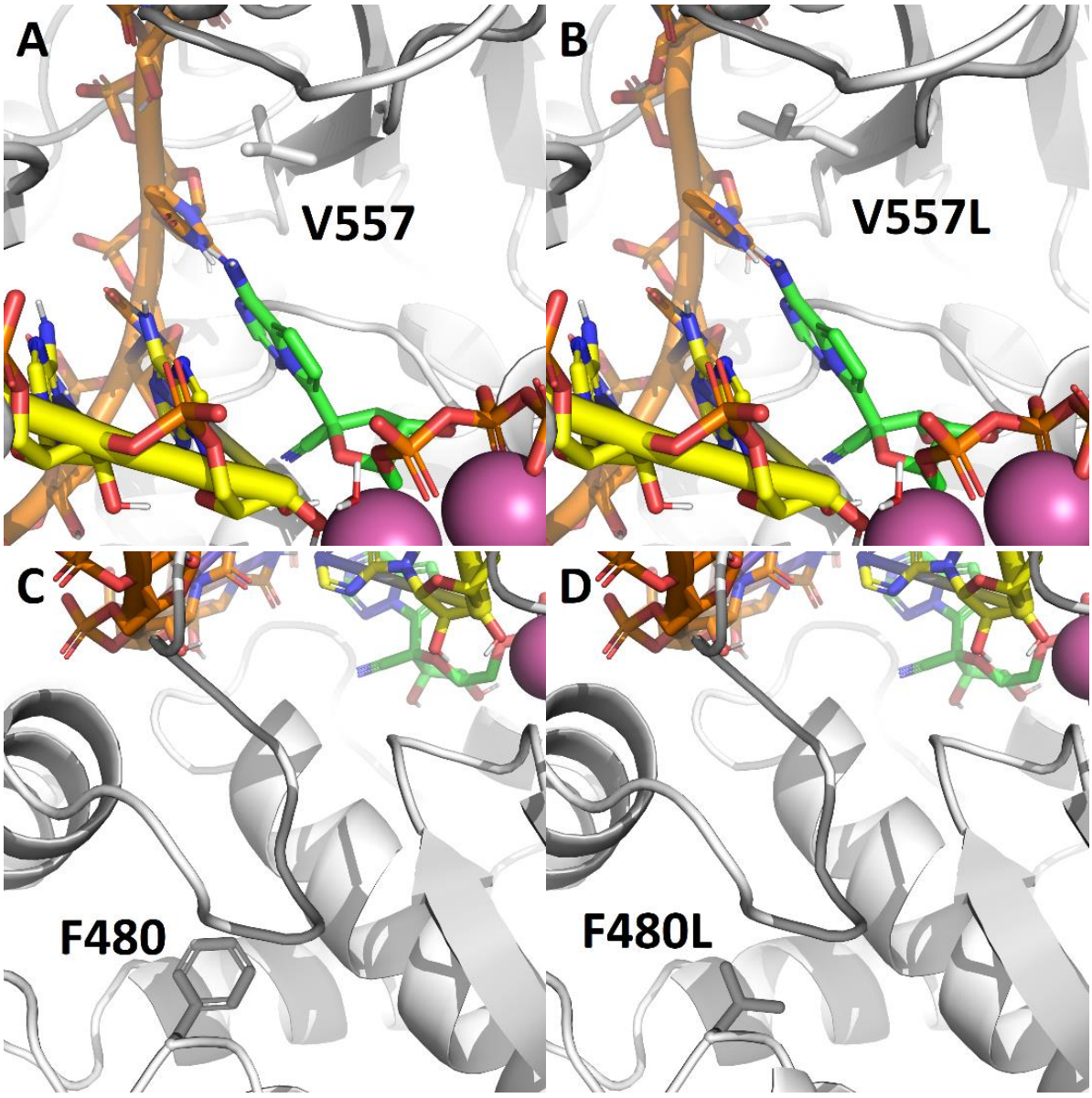

Figure S1. A. Influence of RDV resistance mutations in MHV selected by virus passage in the presence drug. WT V557 is in direct contact with the template base. **B.** V557L leads to a modest repositioning of the template, and by extension, RDV (green). **C.** WT F480 lies outside of the active site. **D.** F480L leads to minor adjustments in structural elements that form both the active site and RNA binding pocket. Related to Figure 1.

Figure S2. Nucleotide sequence conservation of *nsp12* from different SARS-CoV-2 isolates in RDV studies. Alignment of full nsp12 nucleotide sequences from isolates hCoV-19_Wuhan_WIV04_2019 (GISAID EpiFlu™ Database Accession ID: EPI_ISL_402124), hCoV-19_Hong Kong_VM20001061_2020 (GISAID EpiFlu™ Database Accession ID: EPI_ISL_412028), SARS-CoV-2/human/CHN/IQTC01/2020 (GenBank Accession number MT123290.1), and 2019-nCoV/USA-WA1/2020 (GenBank Accession number MN985325.1). The nsp12 sequences are 100% conserved at the nucleotide level. Related to Figure 1.

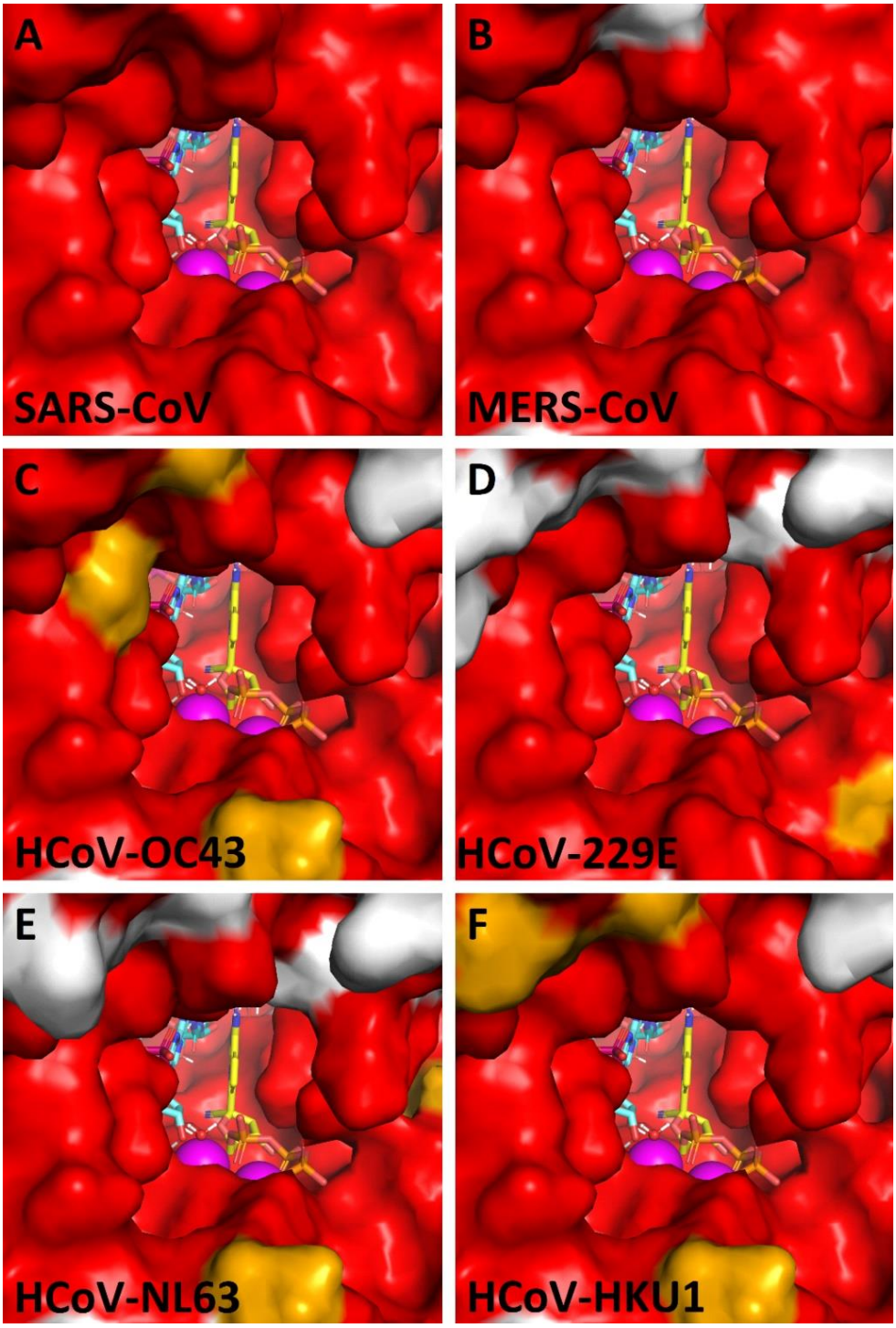

Figure S3. Models of RDV-TP in other human coronaviruses. **A.** SARS-CoV [AAP13442.1] **B.** MERS-CoV [AFS88944.1] **C.** HCoV-OC43 [AAX85675.1] **D.** HCoV-229E [AFR79248.1] **E.** HCoV-NL63 [AFV53147.1] **F.** HCoV-HKU1 [ABD75567.1]. Residues in red are conserved relative to SARS-CoV-2 [QHD43415.1]. Residues in gold are similar. Residues in white are dissimilar. SARS-CoV-2 is identical to SARS-CoV out to a radius of 18 Å from the active site. While differences are visible on the periphery of the active site, residues that interact directly with the RDV-TP are highly conserved for all human CoVs. Related to Figure 1.

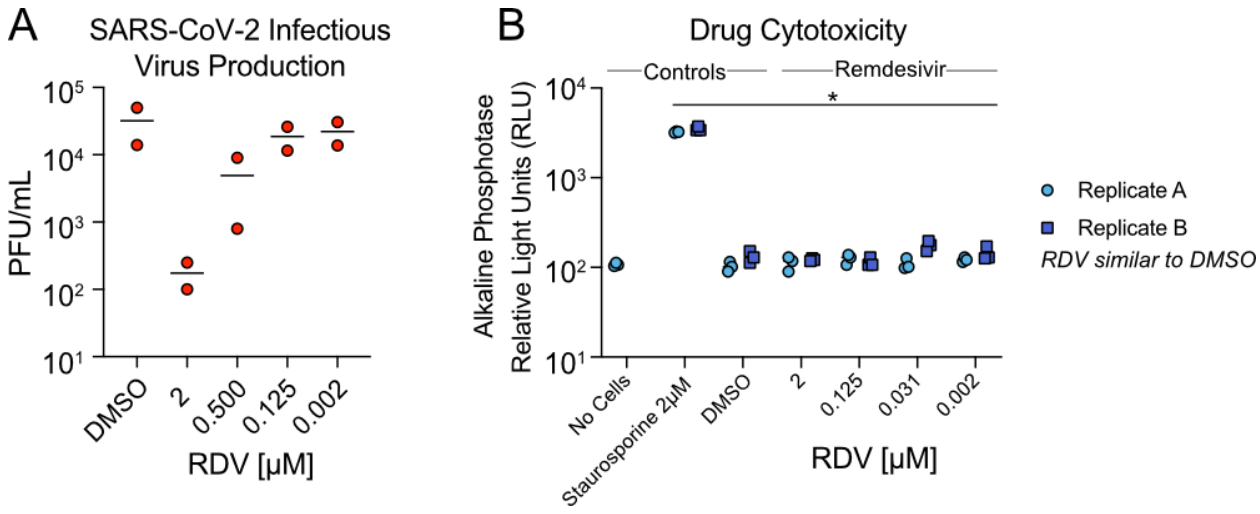

Figure S4. RDV is potently antiviral against SARS-CoV-2 in primary human airway epithelial (HAE) cultures without cellular toxicity. HAE cultures were infected with a SARS-CoV-2 clinical isolate (2019-nCoV/USA-WA1/2020) at MOI = 0.5 PFU/cell for 2 h, after which virus was removed and cultures were washed 3 times, followed by incubation 37˚C for 48 h. **A.** SARS-CoV-2 infectious virus production in one independent study. Virus in apical washes at 48 h post-infection was titered via plaque assay. Each symbol represents the titer from a single culture, and line is drawn at the mean. **B.** Cytotoxicity measured in companion uninfected cultures. HAE from the same donor as in A were exposed a dose response of RDV, DMSO, or positive control 2µM staurosporin in duplicate. After 48hr, cytotoxicity was measured using Toxilight Assay which measures alkaline phosphatase released into in culture medium from dying cells. Staurosporin was significantly different than vehicle and all RDV conditions by Two-Way ANOVA with a Sidak's multiple comparison test. All $P < 0.0001$. Related to Figure 3.

Treatment	Time (h)	Metabolite levels (pmol / million cells)[a, b, c]							
		NTP		NDP		NMP		Nucleoside	
		Vero E6[d]	Calu3[e]	Vero E6[d]	Calu3[e]	Vero E6[d]	Calu3[e]	Vero E6[d]	Calu3[e]
RDV	8	1.21 ± 1.67	2.87 ± 0.84	0.14 ± 0.02	2.90 ± 2.03	0.23 ± 0.02	2.43 ± 2.14	BLQ	0.95 ± 0.20
	24	0.50 ± 0.15	2.17 ± 0.14	0.15 ± 0.05	1.12 ± 0.11	0.20 ± 0.06	0.50 ± 0.03	BLQ	0.64 ± 0.04
	48	0.61 ± 0.14	2.00 ± 0.31	0.15 ± 0.03	1.13 ± 0.03	0.13 ± 0.01	0.31 ± 0.06	BLQ	1.02 ± 0.07
GS-441524	8	2.17 ± 1.11	0.67 ± 0.09	0.37 ± 0.05	0.35 ± 0.04	0.20 ± 0.02	0.06 ± 0.02	2.96 ± 0.80	2.96 ± 0.59
	24	1.78 ± 0.68	0.85 ± 0.16	0.31 ± 0.09	0.73 ± 0.45	0.16 ± 0.04	0.08 ± 0.04	1.93 ± 1.16	3.56 ± 0.46
	48	1.42 ± 0.46	0.72 ± 0.34	0.22 ± 0.04	0.72 ± 0.16	0.13 ± 0.01	0.13 ± 0.07	1.78 ± 1.02	4.31 ± 0.60

Table S1. Metabolite levels following RDV or GS-441524 treatment of Vero E6 and Calu3 cell lines. Related to Table 1 and Figure 4.

[a] Vero E6 cell volume: 0.59-0.74 pL/cell. From: Noorafshan A, et al. 2011. Microbiology Research, 2:18.https://doi.org/10.4081/mr.2011.e18

[b] Calu-3 cell volume: 2.7 pL/cell. From: Min KA, et al. 2013. Pharm Res. 30:2118. doi: 10.1007/s11095-013-1069-5

[c] BLQ (below limit of quantitation): NDP, 0.156 pmol/sample; NMP, 0.039 pmol/sample; Nucleoside, 0.625 pmol/sample

[d] Values represent mean ± SD from four independent replicates

[e] Values represent mean ± SD from two independent replicates

Donor[a]	Time (h)	Remdesivir metabolite levels (pmol / million cells)[b,c]			
		RDV-TP	RDV-DP	RDV-MP	GS-441524
1	8	18.3 ± 3.22	2.10 ± 0.14	0.54 ± 0.10	BLQ
	24	15.3 ± 1.73	3.45 ± 0.46	1.31 ± 0.32	BLQ
	48	2.45 ± 0.36	BLQ	BLQ	BLQ
2	8	6.58 ± 1.18	0.87 ± 0.13	0.57 ± 0.15	BLQ
	24	5.78 ± 0.84	1.72 ± 0.28	1.19 ± 0.20	BLQ
	48	0.73 ± 0.07	BLQ	BLQ	BLQ

Table S2. Metabolite levels following RDV treatment of primary HAE cultures. Related to Table 1 and Figure 4.

[a] Origin of tissues are from healthy, non-smoker donors. Donor 1 = 56-year-old black female; Donor 2 = 62-year-old black female

[b] Values represent mean ± SD from four independent replicates for each donor

[c] BLQ (below limit of quantitation); Limit of quantification for each analyte is as follows: RDV-DP, 0.156 pmol/sample; RDV-MP, 0.156 pmol/sample; GS-441524, 0.625 pmol/sample

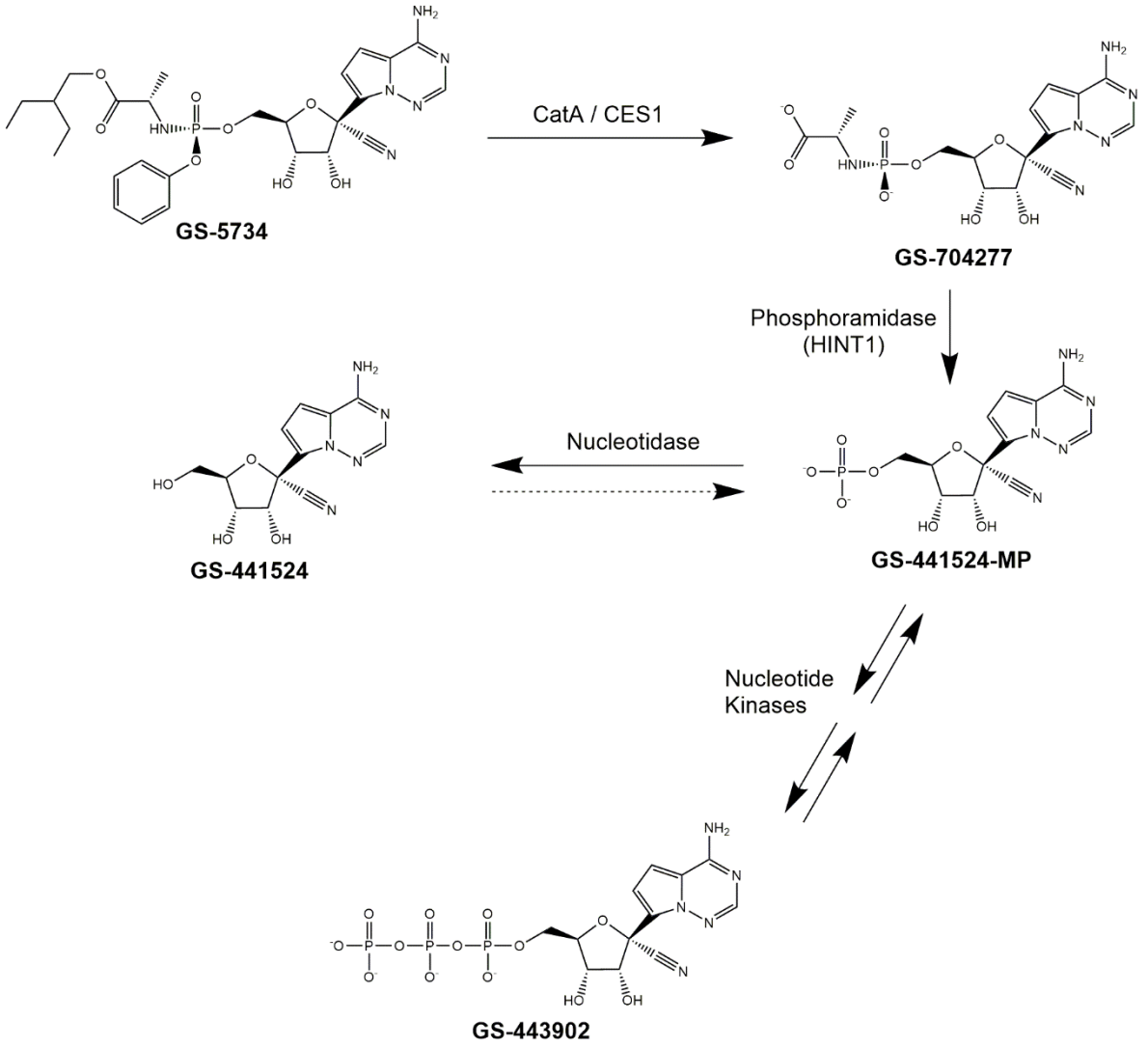

Figure S5. Generalized intracellular metabolic pathway of remdesivir. Combined results from pharmacology and pharmacokinetic studies have led to the proposed intracellular metabolic pathway. Remdesivir (GS-5734) is activated to the pharmacologically active nucleoside analog triphosphate, GS-443902, by a sequential metabolic activation pathway. Cellular hydrolases (CatA and CES1) removes the ester, then a spontaneous chemical step forms the intermediate metabolite GS-704277. HINT1 (a phosphoramidase) subsequently cleaves the phosphoramide bond, liberating the nucleoside analog monophosphate (GS-441524-MP). GS-441524-MP is either catalyzed to the active triphosphate, GS-443902, by nucleotide kinases or dephosphorylated to the nucleoside analog GS-441524. Related to Table 1, Figure 2, and Figure 4.