

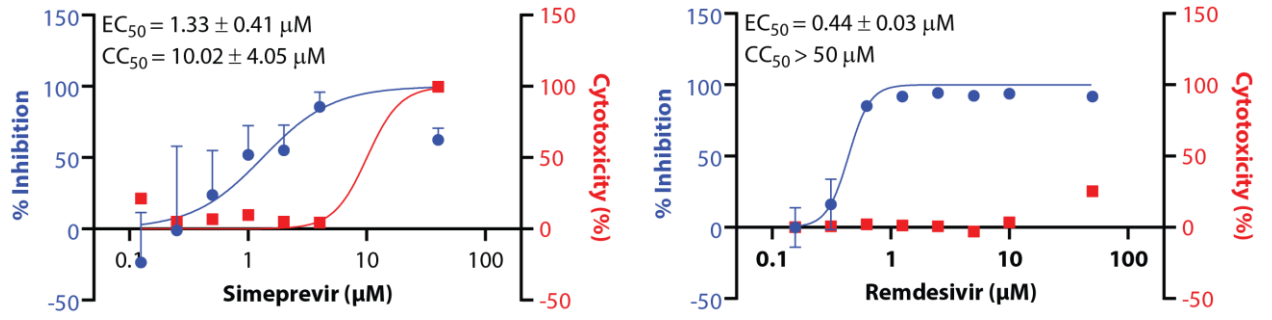
## Supporting Information

### Figures

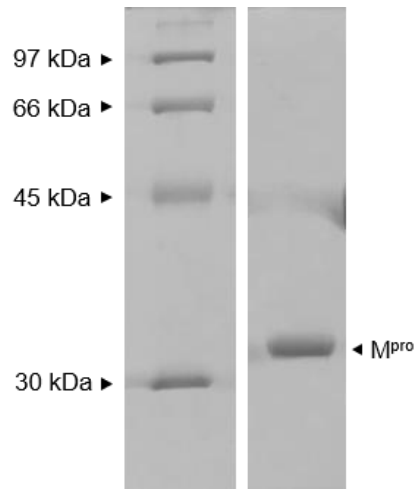
1. Dose-Response Curve of Simeprevir and Remdesivir in A549-ACE2 Cells
2. SDS-PAGE analysis of recombinant SARS-CoV-2 M<sup>pro</sup>
3. Supplementary Data of M<sup>pro</sup> FRET Assay
4. Docking of Simeprevir on M<sup>pro</sup>
5. Supplementary Data of PL<sup>pro</sup> Fluorescence Assay with Ubiquitin Substrate
6. Supplementary Data of PL<sup>pro</sup> Gel-Based Assay
7. Docking of Simeprevir and Other Inhibitors against SARS-CoV-2 nsp12
8. Supplementary Data of RdRp Gel-Based Assay
9. Gene Set Enrichment Analysis (GSEA) of SARS-CoV-2 infection
10. Focused Analyses of Interferon-Related Genes in RNA-seq Data
11. Viral Titration Assay and qPCR of A549-ACE2 cells under Simeprevir treatment
12. qPCR of A549-ACE2 cells under JAK inhibitor and Simeprevir treatment

### Tables

1. A List of FDA-approved repurposable drug candidates
2. Gene Set Enrichment Analysis (GSEA) of Simeprevir in infected samples

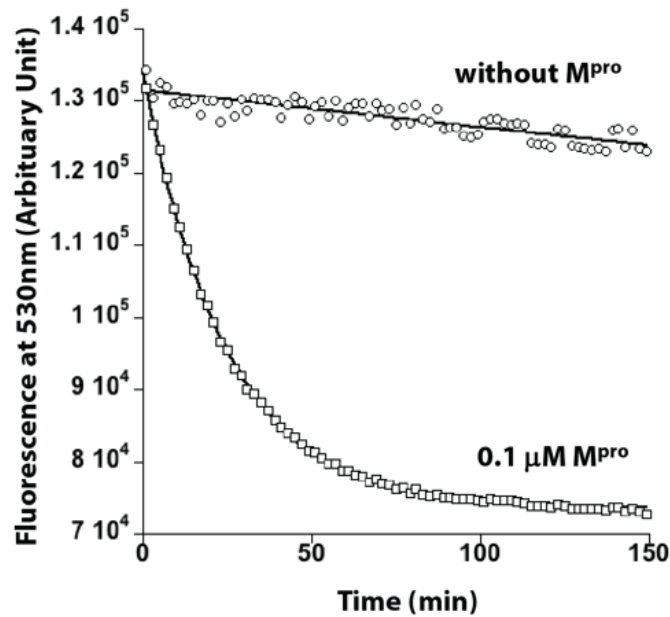


**Supplementary Fig. 1** Dose-response curves in the suppression of SARS-CoV-2 replication in A549-ACE2 cells and cytotoxicity for simeprevir (**Left**) and remdesivir (**Right**) are shown. Data points in all plots represent mean  $\pm$  S.E.M.. For all data points,  $n = 3$  replicates.

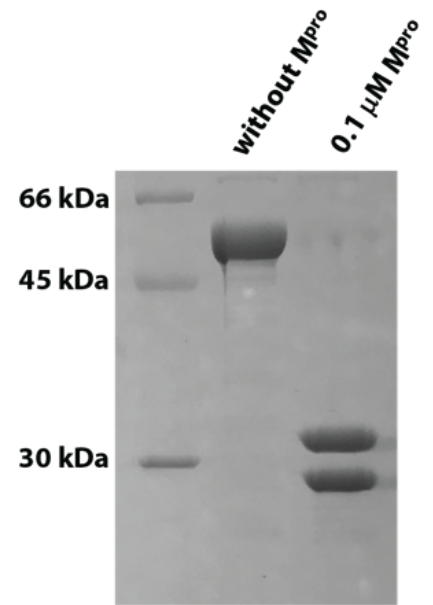


**Supplementary Fig. 2** SDS-PAGE analysis of recombinant SARS-CoV-2 M<sup>pro</sup>.

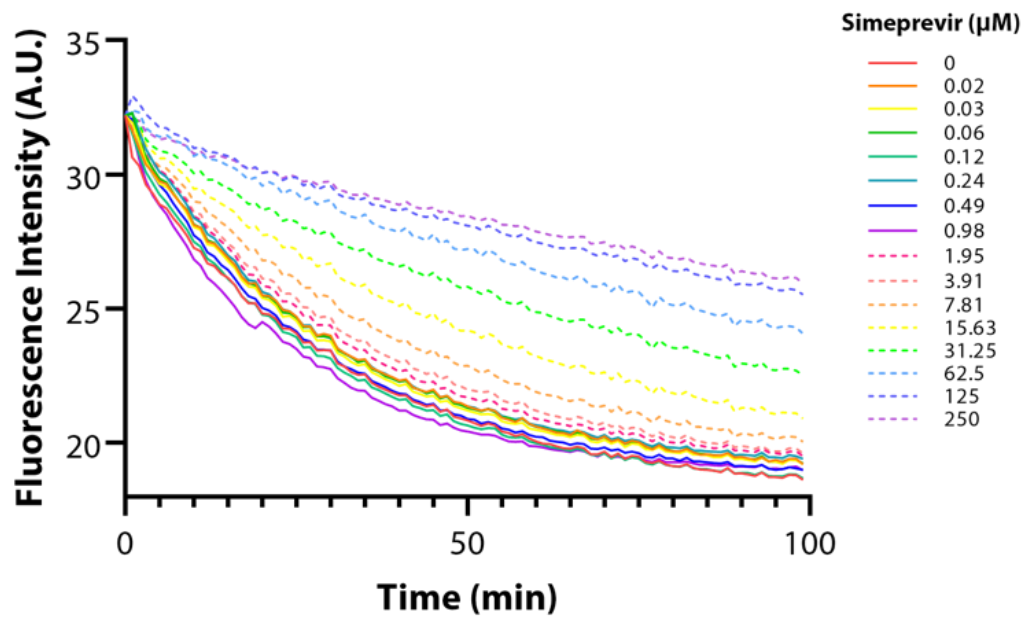
A



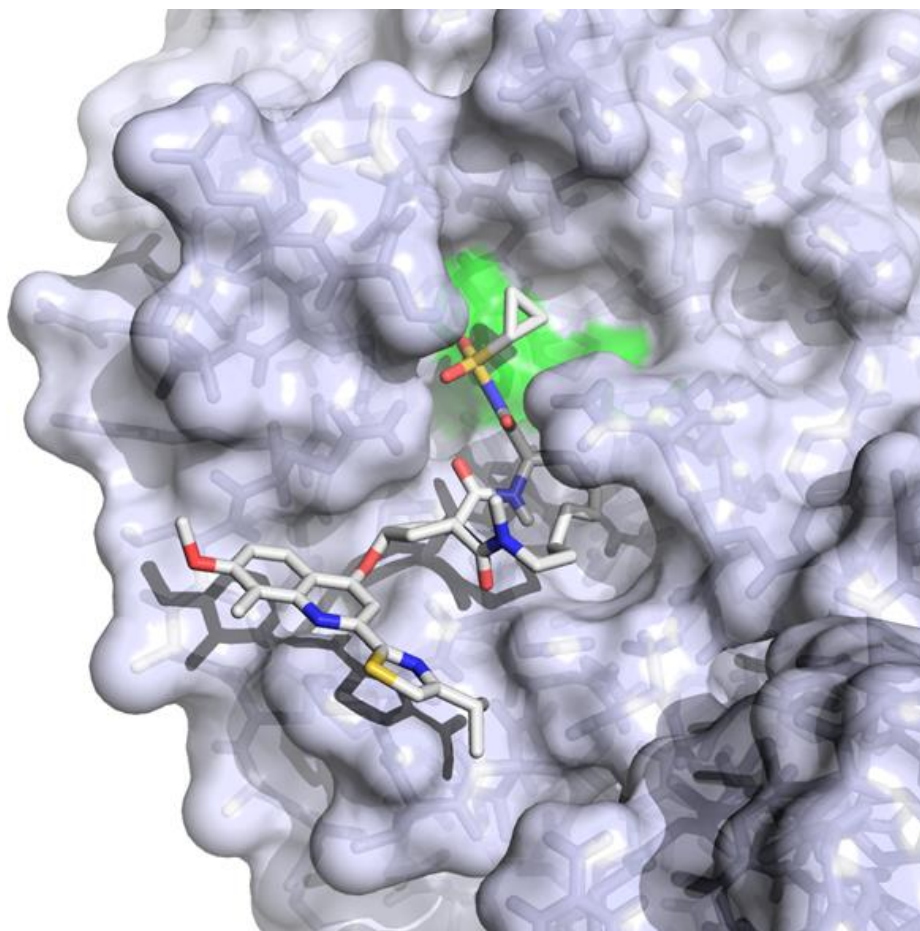
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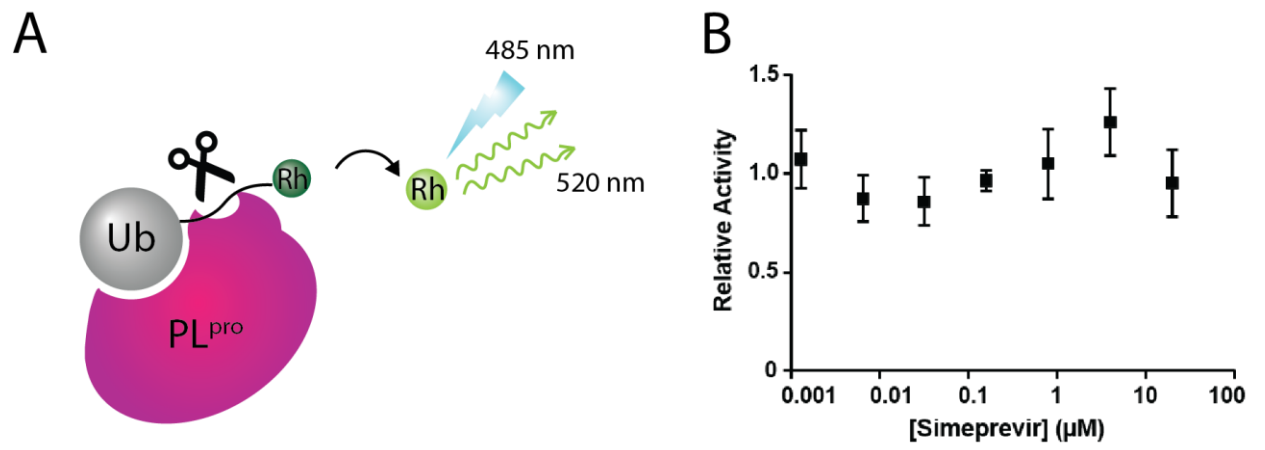
C



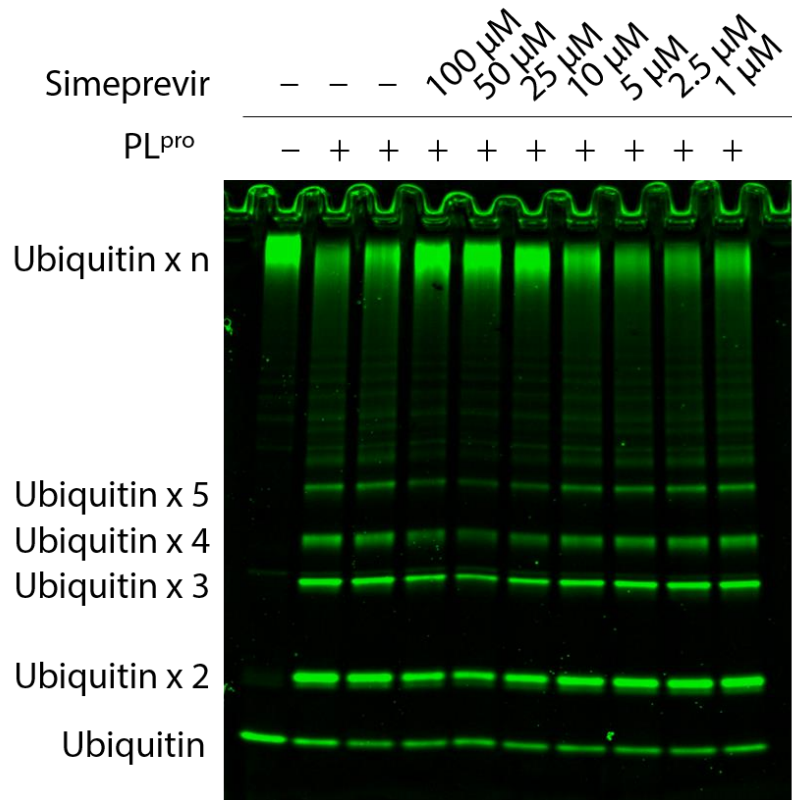
**Supplementary Fig. 3 (A)** The addition of SARS-CoV-2 M<sup>pro</sup> led to the cleavage of the substrate, causing detectable decline in FRET signal with 430-nm excitation and 530-nm emission. **(B)** Confirmation of substrate cleavage by M<sup>pro</sup> using SDS-PAGE. **(C)** The addition of simeprevir at varying concentrations attenuated the rate of FRET substrate cleavage by M<sup>pro</sup>.



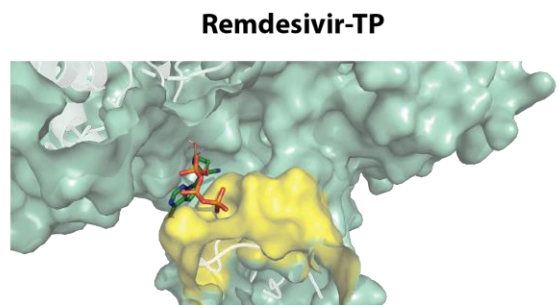
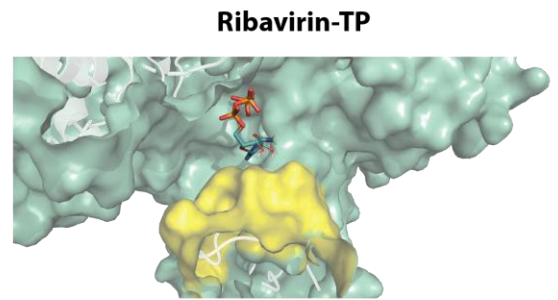
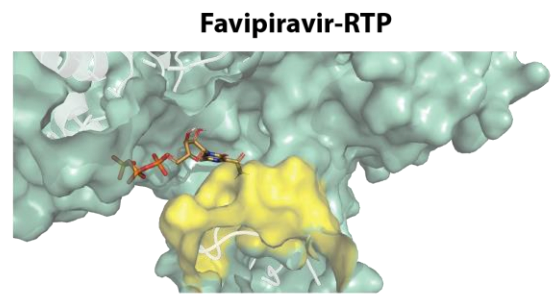
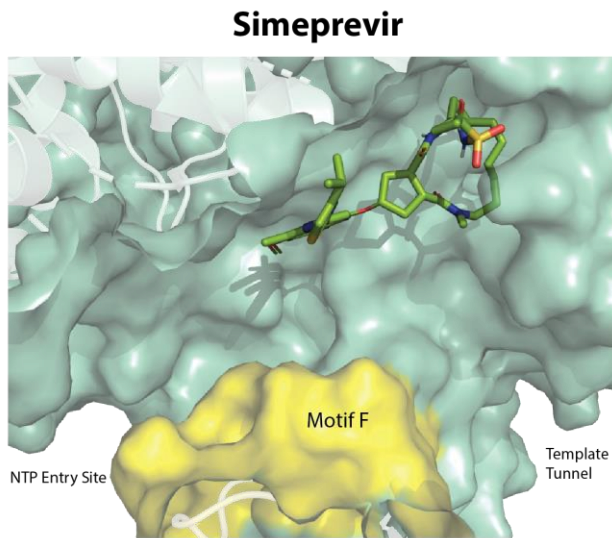
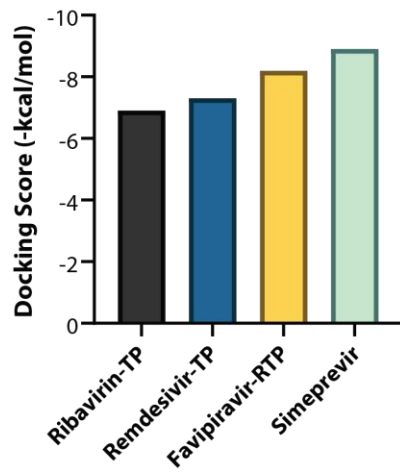
**Supplementary Fig. 4** Docking of simeprevir on SARS-CoV-2 M<sup>pro</sup> (performed with AutoDock Vina version 1.1.2). The M<sup>pro</sup> structure was based on an apo protein crystal structure (PDB ID: 6YB7); the A:B dimer was generated by crystallographic symmetry. Docking was run with the substrate-binding residues set to be flexible; and a 30 x 30 x 30 Å<sup>3</sup> search box centered near the side-chain Nε2 atom of His163. The top docking mode shown here scored -9.9 kcal mol<sup>-1</sup>. The protein is shown as a semi-transparent molecular surface encasing its stick model with the catalytic residues His41 and Cys145 in green. The ligand is shown as a stick model with oxygen atoms in red; nitrogen atoms in blue, sulfur atoms in yellow and carbon atoms in white.



**Supplementary Fig. 5** Simeprevir did not inhibit PL<sup>pro</sup> activity in a repeated PL<sup>pro</sup> cleavage assay using a different substrate ubiquitin-rhodamine.

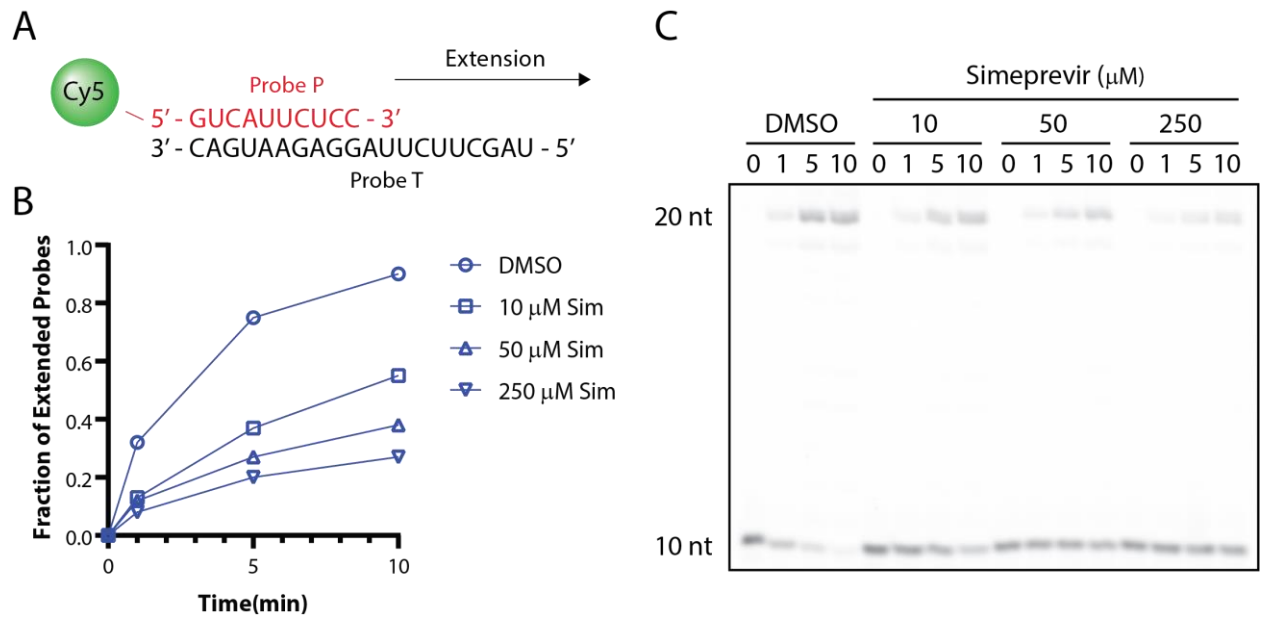


**Supplementary Fig. 6** Inhibitor deubiquitination assay of PL<sup>pro</sup> shows PL<sup>pro</sup> (SARS-CoV-2) actively cleaves poly-ubiquitinated substrate into di-ubiquitin or longer ubiquitin chain. While simeprevir was added in reaction solution, the activity of PL<sup>pro</sup> was not profoundly inhibited compared to control experiments (no inhibitor and no inhibitor with 2% DMSO).

**A****B**

**Supplementary Fig. 7 (A)** Binding mode of simeprevir and other inhibitors against SARS-CoV-2 nsp12 (PDB ID: 6M71). Motif F is highlighted in yellow. **(B)** Docking scores of drug candidates against nsp12.

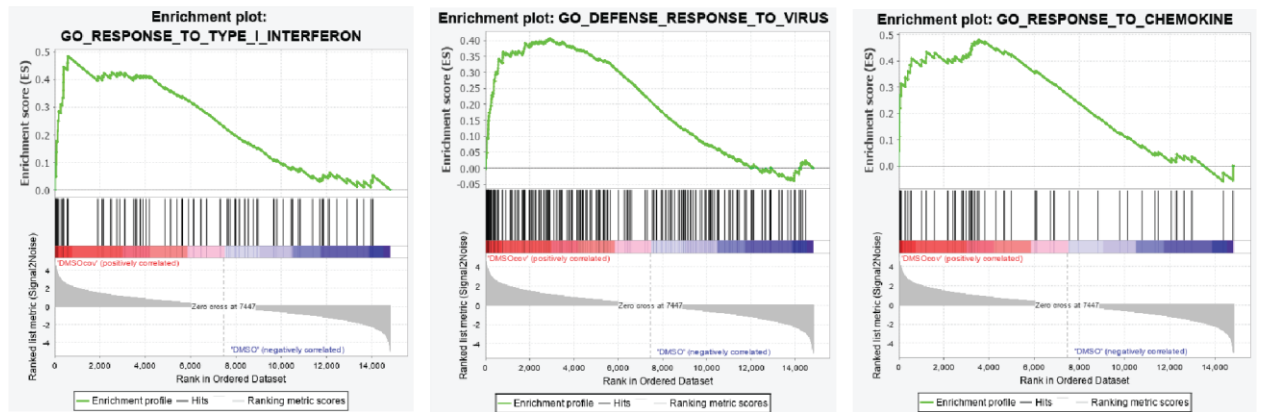




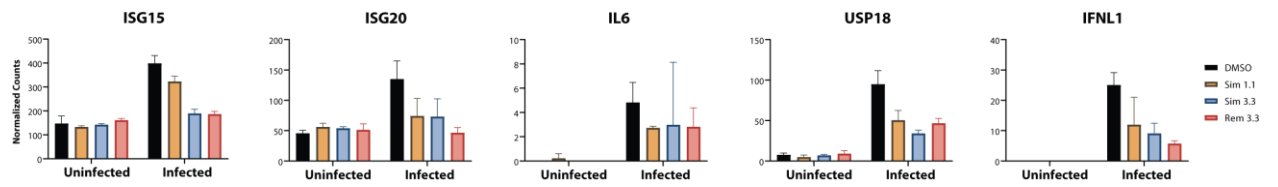
**Supplementary Fig. 8 (A)** Gel-based assay scheme for SARS-CoV-RdRp. Two partially complementary RNA probes are extended by the enzyme, and the extent of extension is visualized by detecting the Cy5 labeling of the shorter probe P. **(B)** Time-dependent elongation of probe P. Fraction of extension is determined by densitometry of extended product versus total RNA. **(C)** RNA-PAGE imaged with Cy5 mode.

## DMSO infected vs. DMSO uninfected

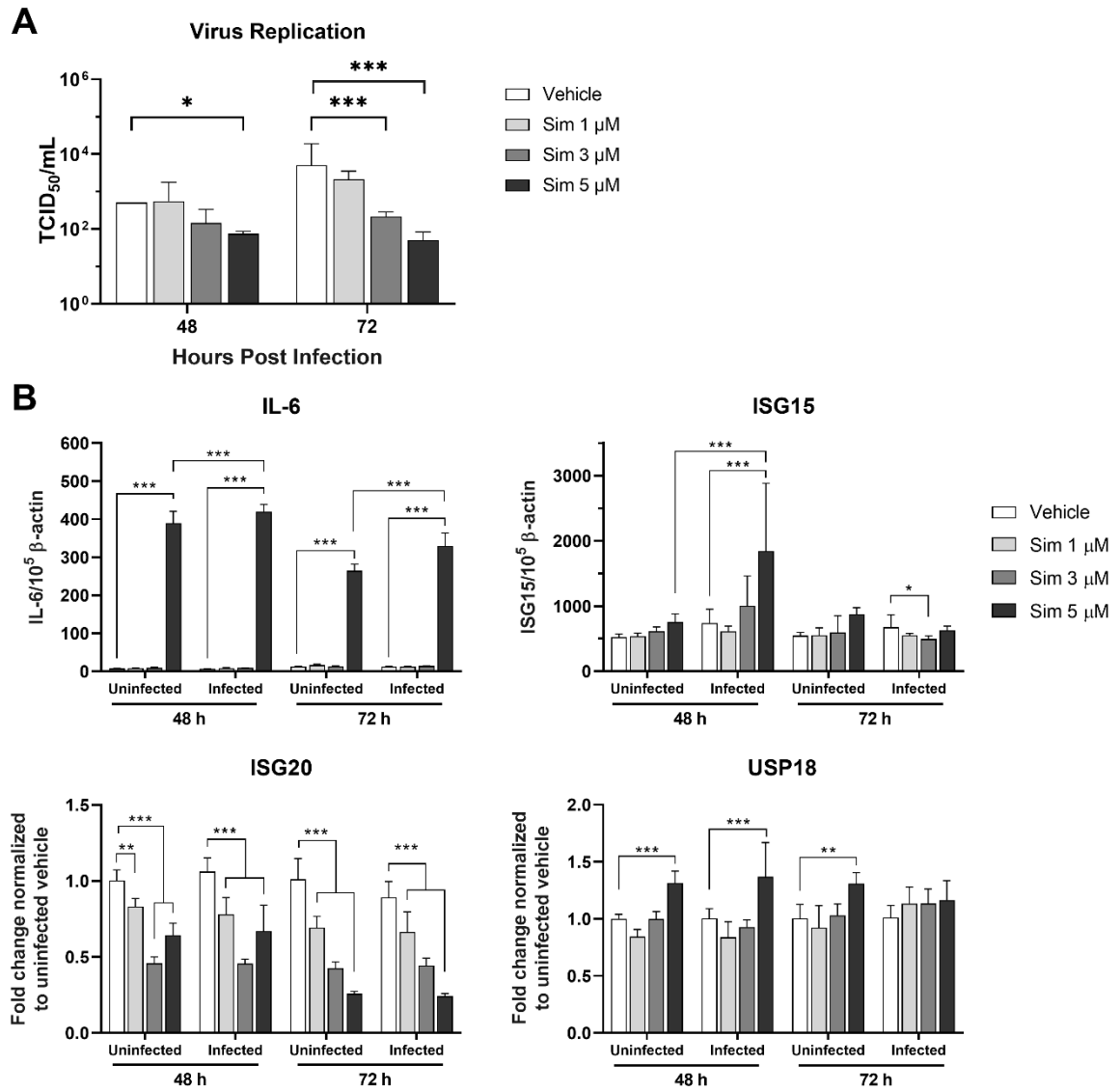
Gene Set	NES	FDR q-val
RESPONSE_TO_TYPE_I_INTERFERON	1.88	0.101
DEFENSE_RESPONSE_TO_VIRUS	1.51	0.101
RESPONSE_TO_CHEMOKINE	1.36	0.133



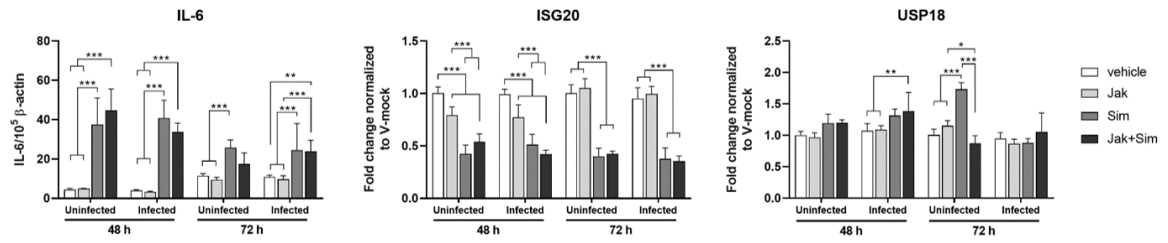
**Supplementary Fig. 9** Gene set enrichment analysis of SARS-CoV-2 infection. Relevant statistics and enrichment plots for three gene sets in response to virus infection are shown.



**Supplementary Fig. 10** Bar charts showing normalized counts from RNA-seq data of five genes involved in antiviral response in Vero E6 cells. Data points in this plot represent mean  $\pm$  S.E.M. For all data points,  $n = 3$  replicates.



**Supplementary Fig. 11 (A)** Viral titration assay using A549-ACE2 cells treated with various concentrations of simeprevir. **(B)** Relative RNA level of various genes in uninfected or infected A549-ACE2 cells, treated with various concentrations of simeprevir. Data points in all plots represent mean  $\pm$  S.E.M.. For all data points,  $n = 3$  replicates. \*: p-value < 0.05, \*\*\*: p-value < 0.005.



**Supplementary Fig. 12** Relative RNA level of various genes in uninfected or infected A549-ACE2 cells, treated with 4  $\mu$ M Simeprevir and/or 1  $\mu$ M JAK inhibitor I. Data points in all plots represent mean  $\pm$  S.E.M.. For all data points,  $n = 3$  replicates. \*: p-value < 0.05, \*\*: p-value < 0.01; \*\*\*: p-value < 0.005.

<b>Drug candidate</b>	<b>Approved indication(s)</b>	<b>Common/notable adverse effects</b>	<b>Drug formulation(s) available</b>	<b>Contraindications (except known hypersensitivity to the drug)</b>
Simeprevir	Chronic HCV infection	Photosensitivity, rash, fatigue, myalgia, dyspnea	Oral	None
Daclatasvir	Chronic HCV infection	Fatigue, nausea, anemia, diarrhea	Oral	None
Saquinavir	HIV infection	Nausea, diarrhea, QT prolongation	Oral	Prolonged QT interval, Patients at risk/having complete AV block
Bromocriptine	Hyperprolactinemia, pituitary prolactinoma	Constipation, dizziness, nausea, fatigue, orthostatic hypotension, vasospasm, abdominal pain	Oral	Uncontrolled hypertension, psychosis, syncopal migraine
Asunaprevir	HIV infection	Fatigue, rash, nausea, neutropenia, anemia, deranged liver function tests	Oral	Moderate/severe hepatic impairment (Child B or C)
Bictegravir	HIV infection	Increased serum creatine kinase, deranged liver function tests, neutropenia	Oral	None
Entecavir	HBV infection	Deranged liver function tests	Oral	Moderate/severe hepatic impairment (Child B or C)
Zidovudine	HIV infection	Headache, malaise, rash, nausea, neutropenia, anemia	Oral	None
Sofosbuvir	Chronic HCV infection	Fatigue, headache, insomnia, nausea, diarrhea, anemia, myalgia, rash	Oral	None
Atovaquone	Protozoal infection or prophylaxis (Pneumocystis jirovecii, Plasmodium spp. etc)	Headache, insomnia, rash, diarrhea, myalgia, drug fever, hyponatremia, neutropenia	Oral	None
Remdesivir	COVID-19	Deranged liver function tests	Intravenous	Serum alanine transaminase $\geq 5$ x the upper-limit of normal

**Supplementary Table 1** FDA-approved repurposable drug candidates tested in this study.

NAME	SIZE	NES	FDR q-val
REACTOME_PKMTS_METHYLATE_HISTONE_LYSINES	46	2.035874	0.044
REACTOME_MITOTIC_PROPHASE	109	1.795425	0.213909
REACTOME_RMTS_METHYLATE_HISTONE_ARGININES	49	1.748761	0.228738
REACTOME_NRIF_SIGNALS_CELL_DEATH_FROM_THE_NUCLEUS	15	1.739992	0.193812
REACTOME_SCF_SKP2_MEDIATED_DEGRADATION_OF_P27_P21	56	1.705256	0.24902
REACTOME_RHO_GTPASES_ACTIVATE_PKNS	58	1.70523	0.21485
REACTOME_CELLULAR_SENESCENCE	154	1.700467	0.203287
REACTOME_MEIOTIC_RECOMBINATION	51	1.70042	0.183376
REACTOME_MEIOSIS	77	1.698298	0.17955
REACTOME_AUF1_HNRNP_D0_BINDS_AND_DESTABILIZES_MRNA	51	1.689022	0.170498
REACTOME_GENE_SILENCING_BY_RNA	98	1.680767	0.185941
REACTOME_ASYMMETRIC_LOCALIZATION_OF_PCP_PROTEINS	58	1.662479	0.215862
REACTOME_CONDENSATION_OF_PROPHASE_CHROMOSOMES	43	1.661445	0.209014
REACTOME_MICRORNA_MIRNA_BIOGENESIS	24	1.645813	0.21082
REACTOME_INTERLEUKIN_7_SIGNALING	24	1.643195	0.199699
REACTOME_TRANSCRIPTIONAL_REGULATION_BY_SMALL_RNAS	75	1.642892	0.189968
REACTOME_RUNX1_REGULATES_TRANSCRIPTION_OF_GENES_INVOLVED_IN_DIFFERENTIATION_OF_HSCS	93	1.639989	0.183625
REACTOME_HDMS_DEMETHYLATE_HISTONES	30	1.624097	0.189556
REACTOME_ESTROGEN_DEPENDENT_GENE_EXPRESSION	114	1.623586	0.181895
REACTOME_G1_S_DNA_DAMAGE_CHECKPOINTS	63	1.622633	0.177329
REACTOME_REGULATION_OF_MRNA_STABILITY_BY_PROTEINS_THAT_BIND_AU_RICH_ELEMENTS	81	1.619633	0.179618
REACTOME_DEFECTIVE_CFTR_CAUSES_CYSTIC_FIBROSIS	56	1.616455	0.179718
REACTOME_APC_C_CDH1_MEDIATED_DEGRADATION_OF_CDC20_AND_OTHER_APC_C_CDH1_TARGETED_PROTEINS_IN_LATE_MITOSIS_EARLY_G1	67	1.613433	0.177683

REACTOME_PRC2_METHYLATES_HISTONES_AND_DNA	41	1.606214	0.17865
REACTOME_EUKARYOTIC_TRANSLATION_INITIATION	81	1.603774	0.174798
REACTOME_HDACS_DEACETYLATE_HISTONES	55	1.59932	0.175764
REACTOME_CELLULAR_RESPONSE_TO_HYPOXIA	67	1.596512	0.170884
REACTOME_RNA_POLYMERASE_I_TRANSCRIPTION	78	1.595998	0.166353
REACTOME_HATS_ACETYLATE_HISTONES	103	1.594675	0.169724
REACTOME_RNA_POLYMERASE_I_PROMOTER_ESCAPE	59	1.593383	0.167005
REACTOME_FORMATION_OF_THE_EARLY_ELONGATION_COMPLEX	32	1.593123	0.163037
REACTOME_SWITCHING_OF_ORIGINS_TO_A_POST_REPLICATIVE_STATE	84	1.590331	0.165325
REACTOME_FANCONI_ANEMIA_PATHWAY	35	1.587149	0.16675
REACTOME_APOPTOTIC_FACTOR_MEDIATED_RESPONSE	16	1.585396	0.16314
REACTOME_ACTIVATION_OF_THE_MRNA_UPON_BINDING_OF_THE_CAP_BINDING_COMPLEX_AND_EIF5_AND_SUBSEQUENT_BINDING_TO_43S	42	1.585282	0.159736
REACTOME_ERCC6_CSB_AND_EHMT2_G9A_POSITIVELY_REGULATE_RRNA_EXPRESSION	44	1.577036	0.163907
REACTOME_ACTIVATION_OF_ANTERIOR_HOX_GENES_IN_HINDBRAIN_DEVELOPMENT_DURING_EARLY_EMBRYOGENESIS	87	1.5695	0.168932
REACTOME_HCMV_LATE_EVENTS	77	1.569343	0.165644
REACTOME_STABILIZATION_OF_P53	52	1.5621	0.176659
REACTOME_HIV_TRANSCRIPTION_ELONGATION	40	1.56044	0.175868
REACTOME_NEGATIVE_EPIGENETIC_REGULATION_OF_RRNA_EXPRESSION	76	1.560153	0.172651
REACTOME_FORMATION_OF_TC_NER_PRE_INCISION_COMPLEX	52	1.556269	0.171755
REACTOME_P75NTR_SIGNALS_VIA_NF_KB	15	1.55442	0.170508
REACTOME_ACTIVATED_PKN1_STIMULATES_TRANSCRIPTION_OF_AR_ANDROGEN_RECEPTOR_REGULATED_GENES_KLK2_AND_KLK3	33	1.550302	0.172005
REACTOME_TRANSCRIPTIONAL_REGULATION_OF GRANULOPOIESIS	56	1.549487	0.169161
REACTOME_FORMATION_OF_THE_BETA_CATENIN_TCF_TRANSACTIVATING_COMPLEX	61	1.548597	0.167521



REACTOME_EPIGENETIC_REGULATION_OF_GENE_EXPRESSION	113	1.545807	0.169608
REACTOME_MRNA_CAPPING	28	1.539692	0.171467
REACTOME_B_WICH_COMPLEX_POSITIVELY_REGULATES_RRNA_EXPRESSION	58	1.535715	0.172508
REACTOME_CYCLIN_A_CDK2_ASSOCIATED_EVENTS_AT_S_PHASE_ENTRY	80	1.533588	0.171664
REACTOME_OXIDATIVE_STRESS_INDUCED_SENESCENCE	88	1.532286	0.170114
REACTOME_AMYLOID_FIBER_FORMATION	64	1.531269	0.168856
REACTOME_INFLUENZA_INFECTION	117	1.524853	0.177163
REACTOME_RRNA_PROCESSING	161	1.523799	0.175456
REACTOME_ABORTIVE_ELONGATION_OF_HIV_1_TRANSCRIPT_IN_THE_ABSENCE_OF_TAT	22	1.523257	0.175333
REACTOME_POSITIVE_EPIGENETIC_REGULATION_OF_RRNA_EXPRESSION	72	1.519612	0.179256
REACTOME_METABOLISM_OF_POLYAMINES	54	1.513546	0.18058
REACTOME_NONSENSE_MEDIATED_DECAY_NMD	79	1.510758	0.182073
REACTOME_DEGRADATION_OF_DVL	53	1.50713	0.1833
REACTOME_SIRT1_NEGATIVELY_REGULATES_RRNA_EXPRESSION	37	1.502177	0.186786
REACTOME_PRE_NOTCH_EXPRESSION_AND_PROCESSING	80	1.502052	0.184445
REACTOME_DNA_METHYLATION	34	1.49901	0.186451
REACTOME_REPRODUCTION	91	1.497232	0.188127
REACTOME_TRANSCRIPTION_COUPLED_NUCLEOTIDE_EXCISION_REPAIR_TC_NER	76	1.493491	0.188147
REACTOME_REGULATION_OF_EXPRESSION_OF_SLITS_AND_ROBOS	129	1.488774	0.191595
REACTOME_SRP_DEPENDENT_COTRANSLATIONAL_PROTEIN_TARGETING_TO_MEMBRANE	75	1.481835	0.201191
REACTOME_CELLULAR_RESPONSES_TO_EXTERNAL_STIMULI	486	1.480829	0.198844
REACTOME_EUKARYOTIC_TRANSLATION_ELONGATION	58	1.474574	0.207194
REACTOME_ORC1_REMOVAL_FROM_CHROMATIN	66	1.471954	0.208534
REACTOME_ASSEMBLY_OF_THE_PRE_REPLICATIVE_COMPLEX	63	1.470746	0.208734

REACTOME_KERATAN_SULFATE_BIOSYNTHESIS	21	1.464303	0.215357
REACTOME_SENESCENCE_ASSOCIATED_SECRETORY_PHENOTYPE_SASP	75	1.464137	0.213509
REACTOME_TRANSLATION	240	1.45829	0.223907
REACTOME_VOLTAGE_GATED_POTASSIUM_CHANNELS	20	1.454349	0.226557
REACTOME_DNA_DAMAGE_RECOGNITION_IN_GG_NER	37	1.447582	0.233274
REACTOME_INTERCONVERSION_OF_NUCLEOTIDE_DI_AND_TRIPHOSPHATES	25	1.447349	0.231582
REACTOME_APC_C_MEDIATED_DEGRADATION_OF_CELL_CYCLE_PROTEINS	81	1.443777	0.23438
REACTOME_POLYMERASE_SWITCHING_ON_THE_C_STRAND_OF_THE_TELOMERE	16	1.439511	0.23541
REACTOME_TNFR1_INDUCED_NFKAPPAB_SIGNALING_PATHWAY	27	1.437981	0.233472
REACTOME_DUAL_INCISION_IN_TC_NER	63	1.437643	0.231104
REACTOME_REGULATION_OF_RUNX3_EXPRESSION_AND_ACTIVITY	51	1.432937	0.234545
REACTOME_KERATAN_SULFATE_KERATIN_METABOLISM	27	1.430326	0.236033
REACTOME_PCP_CE_PATHWAY	85	1.427146	0.237307
REACTOME_NEGATIVE_REGULATION_OF_NOTCH4_SIGNALING	51	1.42502	0.237688
REACTOME_SIGNALING_BY_NOTCH4	78	1.420591	0.246818
REACTOME_KSRP_KHSRP_BINDS_AND_DESTABILIZES_MRNA	16	1.420209	0.244459
REACTOME_TRANSCRIPTIONAL_REGULATION_BY_RUNX2	108	1.419713	0.243666
REACTOME_DECTIN_1_MEDIATED_NONCANONICAL_NFKB_SIGNALING	58	1.419439	0.241926
REACTOME_RESPONSE_OF_EIF2AK4_GCN2_TO_AMINO_ACID_DEFICIENCY	66	1.418276	0.240912
REACTOME_RUNX1_REGULATES_GENES_INVOLVED_IN_MEGAKARYOCYTE_DIFFERENTIATION_AND_PLATELET_FUNCTION	64	1.417405	0.239215
REACTOME_ESR_MEDIATED_SIGNALING	178	1.414636	0.242566
REACTOME_DNA_REPLICATION	119	1.407263	0.249869
REACTOME_NUCLEOTIDE_SALVAGE	20	1.406257	0.249112

**Supplementary Table 2.** Gene sets enriched in Sim 1.1 vs. DMSO in infected samples according to Gene set enrichment analysis (GSEA).