Supplementary Information

A yeast-based system to study SARS-CoV-2 M^{pro} structure and to identify nirmatrelvir resistant mutations

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Table S1. Plasmids used in this study

Number	[1]Plasmid	Gene
JCB462	pGBW-m4046203	SARS-CoV-2 M^{pro} ($3CL^{pro}$)
JCB464	pGBW-m4046418	SARS-CoV-2 N (Nucleocapsid)
JCB465	pGBW-m4046249	SARS-CoV-2 NSP12 (RNA-dependent RNAP)
JCB467	pGBW-m4046574	SARS-CoV-2 S (Spike protein)
JCB504	pGBW-m4046277	SARS-CoV-2 M (Membrane protein)
JCB505	pGBW-m4046231	SARS-CoV-2 NSP8
JCB506	pGBW-m4046455	SARS-CoV-2 E (Envelope protein)
JCB507	pGBW-m4046246	SARS-CoV-2 NSP7
JCB508	pGBW-m4046415	SARS-CoV-2 NSP13 (Helicase)
JCB509	pGBW-m4046483	SARS-CoV-2 NSP3 (PL^{pro})

[1] Plasmids were gifts from Ginkgo Bioworks & Benjie Chen and obtained from Addgene.

Table S2. Primer Sequences

Mutant	F or R	Sequence
C145A	Forward Primer	5'-GGTTCTGCTGGCTCCGTTGGTTTTA-3'
	Reverse Primer	5'-GGAGCCAGCAGAACCATTCAAAAAA-3'
E166R	Forward Primer	5'-TCACATGAGATTGCCTACAGGTGTTCACGC-3'
	Reverse Primer	5'-GGCAATCTCATGTGATGCATGTAACAGAAACTG-3'
E166N	Forward Primer	5'-TCACATGAACTTGCCTACAGGTGTTCACGC-3'
	Reverse Primer	5'-GGCAAGTTCATGTGATGCATGTAACAGAAACTG-3'
E166D	Forward Primer	5'-TCACATGGACTTGCCTACAGGTGTTCACGC-3'
	Reverse Primer	5'-GGCAAGTCCATGTGATGCATGTAACAGAAACTG-3'
P132H	Forward Primer	5'-TATGAGGCACAACTTCACTATTAAGGGTTCTTTTT-3'
	Reverse Primer	5'-AAGTTGTGCCTCATAGCGCACTGGTATACACC-3'
N142A	Forward Primer	5'-TTTTTTGGCTGGTTCTTGTGGCTCCGTT-3'
	Reverse Primer	5'- GAACCAGCCAAAAAAGAACCCTTAATAGTGAAG-3'

Table S3. X-ray Data Collection and Refinement Statistics

Data Collection

Structure (PDB ID)	8DDI	8DDM
Mutation	E166N	E166R
Ligand	Apo	GC376
Space Group	C2	C2
Cell Dimensions		
a, *b*, *c* (Å)	116.138	113.931
	53.928	53.401
	44.636	46.482
α, β, γ (°)	90	90
	101.27	102.12
	90	90
Resolution (Å)	50-2.80	50-2.78
No. Reflections	6641	6825
R_{merge} (%)	11.6	9.8
I / *óI*	12.18 (2.33)	13.22 (2.11)
Completeness (%)	98.2	97.7
Redundancy	3.8 (1.9)	4.3 (2.4)
Refinement		
Resolution (Å)	43.81-2.80	48.20-2.78
R_{work}/R_{free} (%)	17.8/24.7	19.1/24.6
No. Heavy Atoms		
Protein	2376	2385
Ligand/Ion	0	29
Water	22	20
***B*-Factors (Å^2)**		
Protein	49.03	49.38
Ligand/Ion	0	43.41
Water	33.4	34.49
Ramanchandran Plot		
Favored Region (%)	97.4	97.0
Allowed Region (%)	2.3	2.6
Outlier Region (%)	0.3	0.3

* Values in parentheses represent highest resolution shells

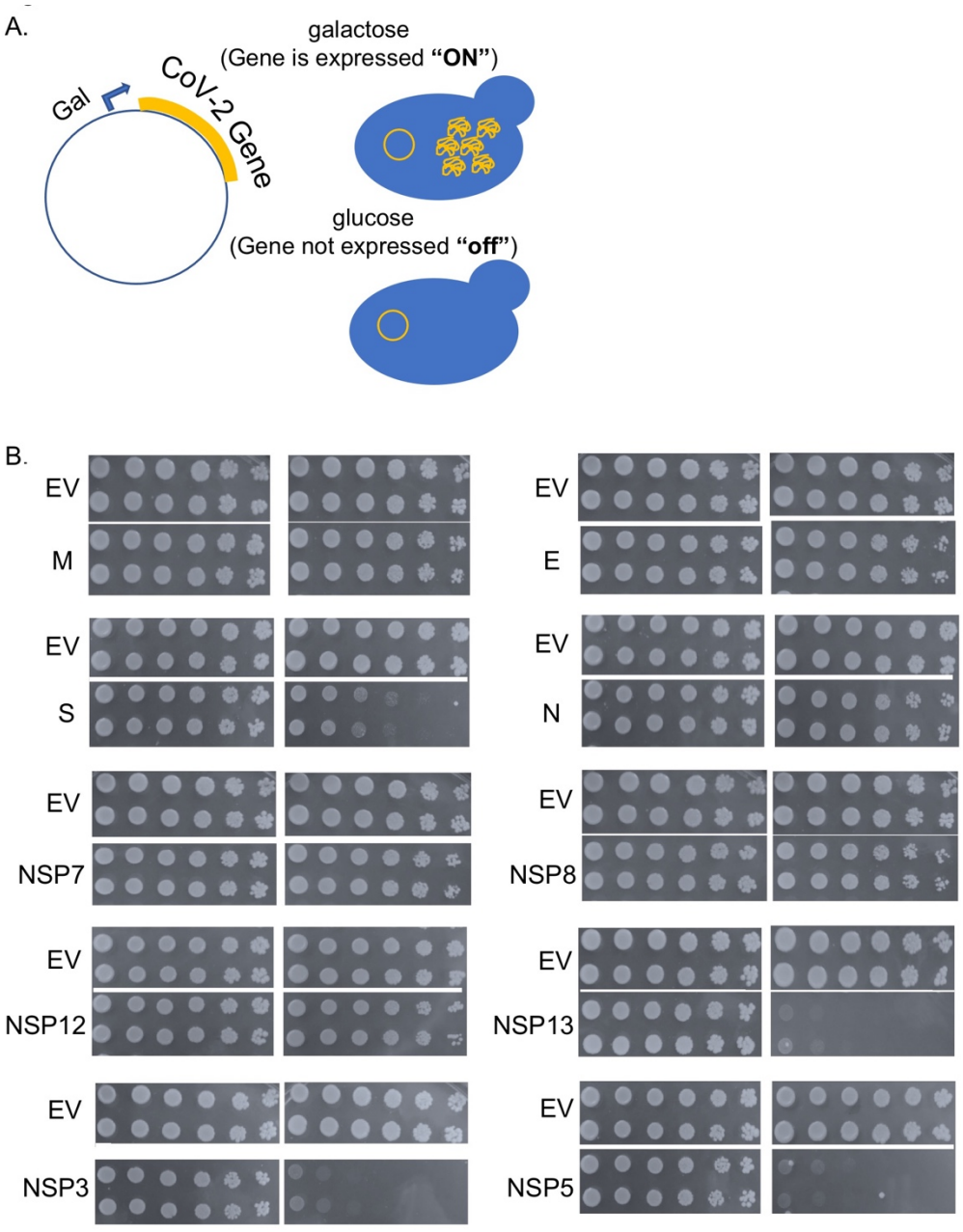

Fig. S1. Expression of SARS-CoV-2 Genes in yeast. A) SARS-CoV-2 genes were cloned into a high copy yeast plasmid and regulated by a galactose inducible promoter (Gal1). B) Spot assays were performed on glucose (left) or galactose (right) containing plates to determine the growth effects conferred by expression of the indicated genes. Two biological replicates were performed for each (two rows) EV and indicated gene. Samples (3ul) of a 5-fold serial dilution are spotted in each row. EV refers to yeast carrying an empty vector. M, E, S, N are structural proteins and all others are non-structural proteins (NSP).

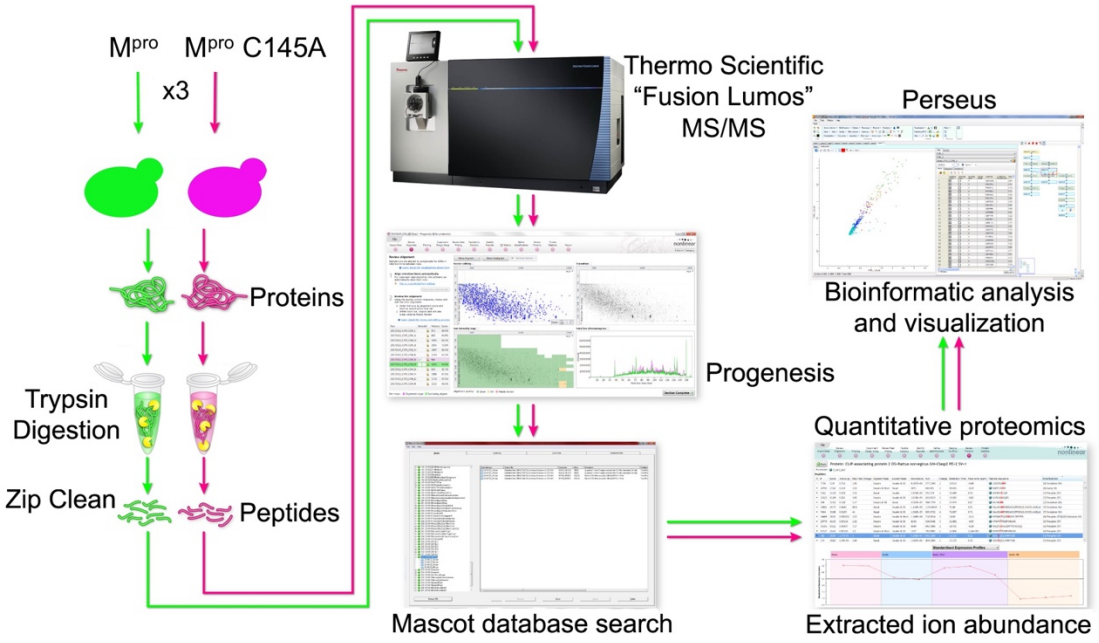

Fig. S2. Determining potential yeast substrates of M^{pro}. Schematic of the work flow to perform proteomics from yeast strains expressing wild-type M^{pro} or catalytically inactive M^{pro} C145A.

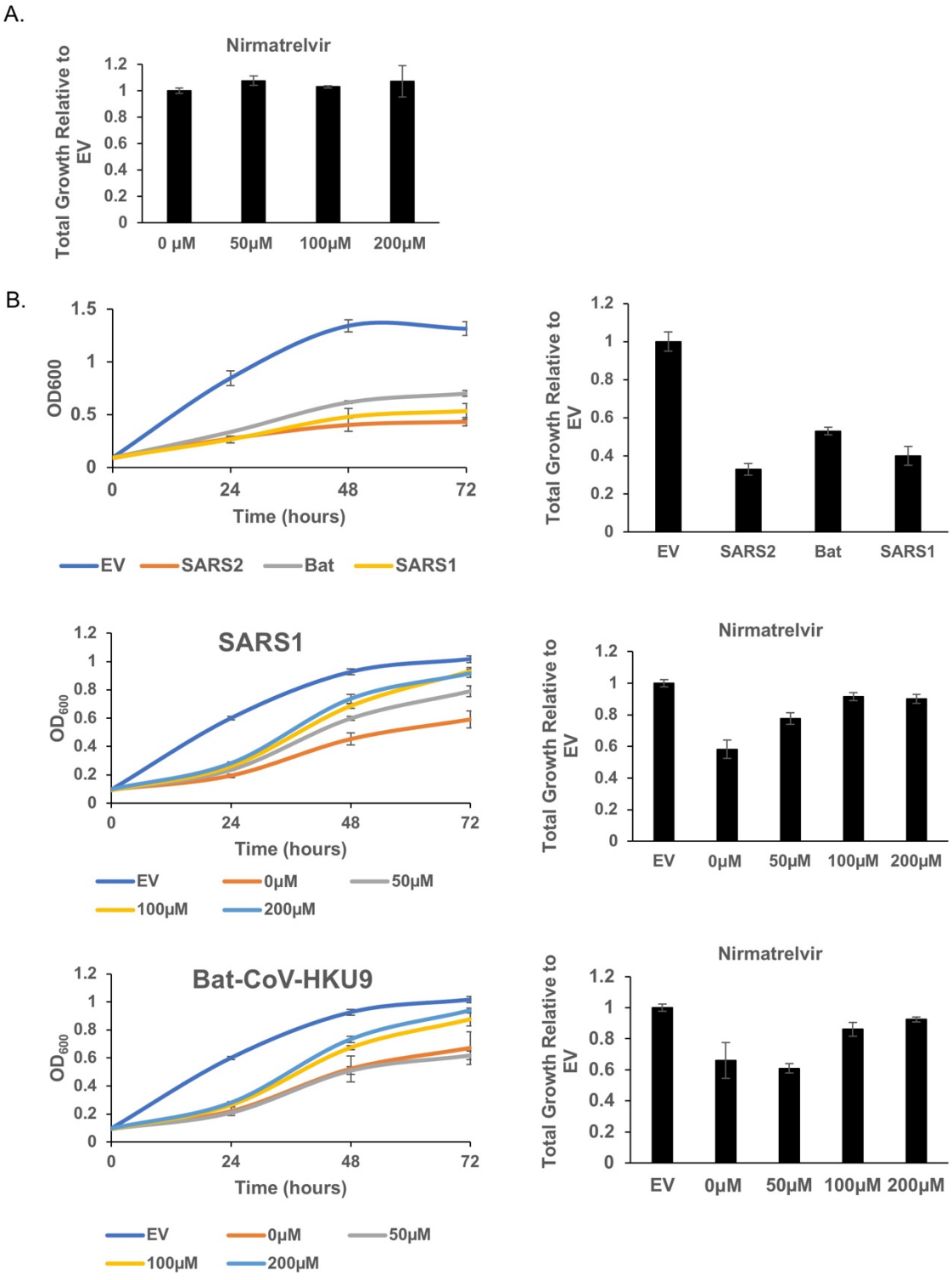

Fig. S3. Effects of nirmatrelvir on yeast expressing Mpro from SARS-CoV-1 and Bat-CoV-HKU9. A) No changes in growth were observed in yeast treated with nirmatrelvir up to 200µM. B) Growth of yeast expressing M^{pro} from the indicated coronavirus is plotted in the presence or

absence of nirmatrelvir. M^{pro} from both viruses remain responsive to nirmatrelvir as indicated improved growth (nearly matching the empty vector (EV) control) in the presence of nirmatrelvir.

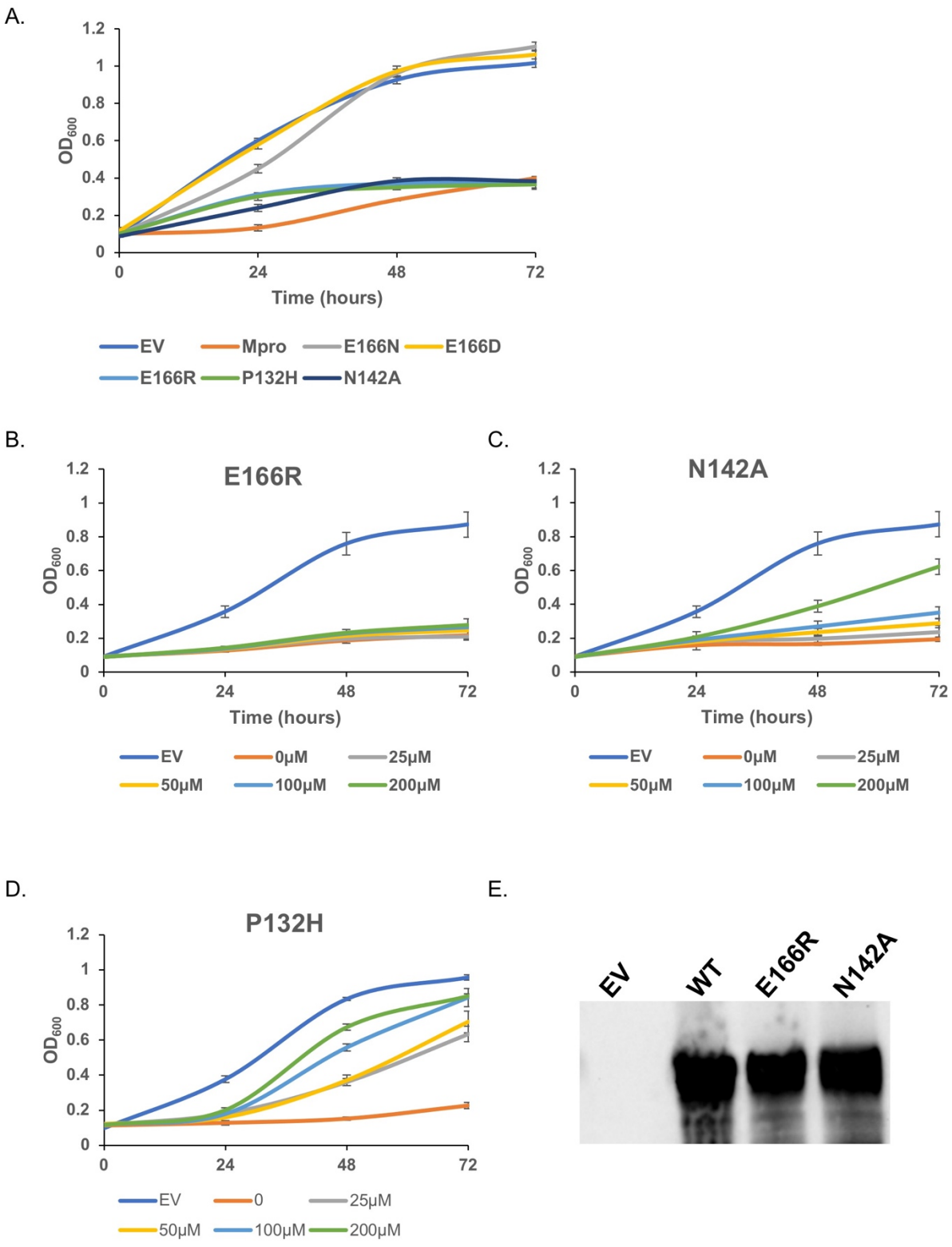

Fig. S4. Growth curves for yeast expressing SARS-CoV-2 M^{pro}. A) Indicated M^{pro} mutants were expressed in yeast. Wild-type M^{pro}, M^{pro} E166R, M^{pro} P132H, and M^{pro} N142A all confer a

strong growth defect compared to empty vector (EV) control. On the other hand M^{pro} E166N and M^{pro} E166D do not confer a growth defect and grow as well as EV control. B-D) Growth curves for M^{pro} E166R, M^{pro} P132H, and M^{pro} N142A treated with 0, 25, 50, 100, 200μM of nirmatrelvir. E) Western blot showing levels of wild-type Mpro, M^{pro} E166R, and M^{pro} N142A.