

690 **Fig. S1 Enhanced innate immune responses during Nsp15_{H234A} SARS-CoV-2**
691 **infection.** A549-ACE2 cells were uninfected or infected by Nsp15_{WT}, Nsp15_{H234A},
692 and Nsp15_{N277A} rSARS-CoV-2 at MOI of 5 for 8 and 24 h. (A) Total RNA extracted
693 for rRNA integrity analysis by use of the Agilent 2100 bioanalyzer. (B) Cell lysates
694 harvested for western blot analysis.

695

696 **Fig. S2 Amino acids associated with EndoU activity and uridine specificity within**
697 **SARS-CoV-2 Nsp15.** Cryo-EM structure of SARS-CoV-2 Nsp15 hexamer (PDB ID:
698 7K0R) is shown with the catalytic triad residues (H234, H249, K289) labeled in red,
699 and residues involved in uridine discrimination (N277, S293, Y342) colored yellow.
700 Images were rendered in ChimeraX v1.8.

701

702 **Fig. S3 Sequence identity of Nsp15 across human coronaviruses.** Nsp15 sequences
703 from SARS-CoV-2 (Wuhan-Hu-1 strain, GenBank: NC045512), SARS-CoV (Urbani
704 strain, GenBank: AY278741), MERS-CoV (EMC/2012 strain, GenBank: NC019843),
705 HCoV-OC43 (ATCC VR-759, GenBank: AY585228), HCoV-HKU1 (GenBank:
706 NC006577), HCoV-229E (ATCC VR-740, GenBank: AF304460), HCoV-NL63
707 (Amsterdam 1 strain, GenBank: NC005831) were acquired from the Nucleotide
708 database, NCBI. Asterisk (*) = fully conserved; colon (:) = conserved positions
709 containing residues with strongly similar properties; period (.) = conserved positions
710 containing residues with weakly similar properties. (A) The amino acid sequences
711 from HCoV Nsp15 aligned by Clustal 2.1. (B) The percentage of sequence identity
712 among HCoV Nsp15.

Fig. S2

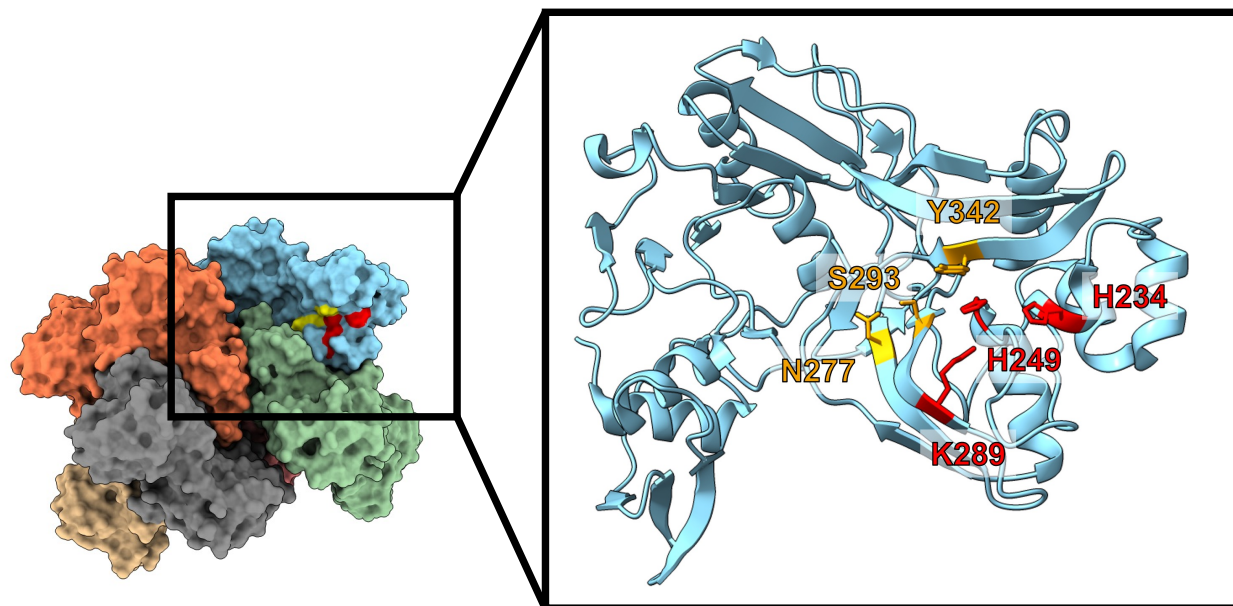


Fig. S3

A.

```

HCoV-229E      GLENIAFNVVNKGSGFVADGELPVAISGDVVFVRDGNLDFVFNKTSLPTNIAFELFAK      60
HCoV-NL63      SLENIAFNVVKKGCFTGVDGELPVAVVDKVFVRYGDVDNLVFTNKTTLPNVAFELFAK      60
HCoV-OC43      SLENNVYLVKTGHYTGQAGEMPCAIIINDKVVAKIDKEDVVFIFINNTTYPTNVAVELFAK      60
HCoV-HKU1      SLENVIYNLVNMGHYDGRTEGELPCAIMNDKVVVKINNVDTVIFKNNTSFPNTIAVELFTK      60
MERS-CoV      GLENIAFNVVKQGHFVIGVEGELPVAVVDKIFTKSGVNDICMFENKTTLPNTIAFELYAK      60
SARS-CoV-2    SLENVAFNVVKNKGHFDDGQGEVPSVIINNTVYTKVDGVDVELFENKTTLPNVAFELWAK      60
SARS-CoV      SLENVAYNVVKNKGHFDDGAGEAPVSIINNAVYTKVDGIDVEIFENKTTLPNVAFELWAK      60
                .***: !*:*: * : * ** * : : . : . * : * !*:*: *.**.*:**:

HCoV-229E      RKVGLTPPLSILKNLGVVATYKFLVLDYEAERPLTSFTKSVCGYDFAED-----VC      112
HCoV-NL63      RKMGLTPPLSILKNLGVVATYKFLVLDYEAERPFTSYTKSVCKYDFNED-----VC      112
HCoV-OC43      RSVRHHPELKLFRNLNIDVCKWHVIWDYARESIFCSNTYGVCMYDCLKFID-----KLN      114
HCoV-HKU1      RSIIRHPELKLFRNLNIDVCKWHVLWDYVKDSLFCSSYGVCKYDCLKFIE-----NLN      114
MERS-CoV      RAVRSHDPDFKLLHNLQADICYKFLVLDYERSNIYGTATIGVCKYDIDVNSALNIC-----      116
SARS-CoV-2    RNIKPVPEIKILNMLGVDIAANTVIWDYKRDAPAHISTIGVCSMTDIAKKPTETICAPLT      120
SARS-CoV      RNIKPVPEIKILNMLGVDIAANTVIWDYKREAPAHVSTIGVCTMTDIAKKPTESACSSLT      120
                * : * ..!.*. : !*:* . * .** **:

HCoV-229E      TCYDNSIQGSYERFTLTNAVLFSATAVKTGGKSLPAIKLNFGLMNGNAIATVKS EDGNI      172
HCoV-NL63      VCFDNSIQGSYERFTLTNAVLFSSTVVIK----NLTPIKLNFGLMNGMPVSSIKGDKGVE      168
HCoV-OC43      VLFDRDNGALEAFKRSNNGVYISTTKVKSLSMIRGPP---RAELNGVVVDK---VGDTD      168
HCoV-HKU1      ILFDGRDNGALEAFKRNKNGVFIISTEKL SRLSMIKGPQ---RADLNGVIVDK---VVELK      168
MERS-CoV      --FDIRDNCSELEKFMSTPNAIFISDRKIKKYPVMVGPD---YAYFNGAIIRD---SDVVK      168
SARS-CoV-2    VFFDGRVDGQVDFRNARNGLVITEGSVKGLQPSVGPQ---QASLNGVTLIG---EAVK-      173
SARS-CoV      VLFDRGVEGQVDFRNARNGLVITEGSVKGLTPSKGPA---QASVNGVTLIG---ESVK-      173
                :* : * : * .: : : . . .** :

HCoV-229E      KNINWVYVYR-----KDGKVPDHYDGFYTG      198
HCoV-NL63      KLVNWIYVYR-----KNGQFQDHYDGFYTG      194
HCoV-OC43      --CVFYFAVRKEGQDVFISQFDSLGVSSNQSPQGNLGSNGKPGNVGGNDALSISTIFTQS      226
HCoV-HKU1      --VEFWAMRKGDDVIFSRDLSLCSHYWSPQGNLGGNC--AGNVIGNDALTRFTIFTQS      225
MERS-CoV      QPVKFYLYKK-----VNEFIDPTECIYTS      194
SARS-CoV-2    --TQFNYYKK-----VDGVVQLPEYFTQS      197
SARS-CoV      --TQFNYYFK-----VDGIIQLPEYFTQS      197
                : : . . .** :

HCoV-229E      RNLQDFLPRSTMEEDFLNMDIGVFIQKYGLEDNFHEHVYGVDSKTTLGLLHLLISQVRL      258
HCoV-NL63      RNLSDFTPRSDMEYDFLNMDMGVFINKYGLEDNFHEHVYGVDSKTTLGLLHLLISQFRL      254
HCoV-OC43      RVISSFTCRTDMEKDFIALDQDVFQKYGLEDYAFEHIVYGNFQKIIGLHLLIGLYRR      286
HCoV-HKU1      RVLSSFEPRSDLERDFIDMDNLFIQKYGLEDYAFDHIVYGSFNHVKVIGLHLLIGLFRR      285
MERS-CoV      RSCSDFLPLSDMEKDFLSDVDFIKKYGLENYAFEHVYGVDFSHHTLGLLHLLIGLYKK      254
SARS-CoV-2    RNLQEFKPRSQMEIDFLELAMDEFIERYKLEGYAFEHIVYGVDFSHSGLGLHLLIGLAKR      257
SARS-CoV      RDLEDFKPRSQMETDFLELAMDEFIQRKLEGYAFEHIVYGVDFSHGQLGLLHLLIGLAKR      257
                * ..* : !* ** : . . .** : * * * * . * : * * * * . . . : * * * * . * :

HCoV-229E      SKMGILKAEFVAASDITLKCCTVTYLNDPSSKTVCTYMDLLDDFVSVLKSLLDVTVSK      318
HCoV-NL63      SKMGVLKADDFVTASDTLRCCTVTYLNELSSKVCTYMDLLDDFVTILKSLDLGVISK      314
HCoV-OC43      QQTSNLVVQEFVSYD--SSIHSYFITDEKSGGSKSVCTVIDILLDDFVALVKSLNLCVSK      345
HCoV-HKU1      KKSNNLLIQEFLQYD--SSIHSYFITDQECGSSKSVCTVIDLLDDFVSVIVKSLNLCVSK      344
MERS-CoV      QQEGHIIMEEMLKGS--STIHNYFITENTAAFKAVCSVIDLKLDDFVMILKSQDLGVVSK      313
SARS-CoV-2    FKESPFLEDFIPMD--STVKNYFITDAQTGSSKVCVSVIDLDDDFVEIISQDLSVSK      316
SARS-CoV      SQDSPLKLEDFIPMD--STVKNYFITDAQTGSSKVCVSVIDLDDDFVEIISQDLSVSK      316
                : . : : : : . : : . * : . * ** : !* : * * * * : * * : * * :

HCoV-229E      VHEVIDNKPWRLWMLWCKDNVATFYPLQ      348
HCoV-NL63      VHEVIDNKPYRWMLWCKDNHLSFYPLQ      344
HCoV-OC43      VVNVNDFKDFQFMLWCNDEKVMTFYPLQ      375
HCoV-HKU1      VVINVDFKDFQFMLWCNDNKIMTFYPKMQ      374
MERS-CoV      VVKVPIDLTMIEFMLWCKDQVQTFYPLQ      343
SARS-CoV-2    VVKVTIDYTEISFMLWCKDGHVTFYPKLQ      346
SARS-CoV      VVKVTIDYAEISFMLWCKDGHVTFYPKLQ      346
                * : : * : * * * * : * * * * :

                234 249
                277 289 293
                342

```

B.

Virus strain	SARS-CoV-2	SARS-CoV	MERS-CoV	HCoV-OC43	HCoV-HKU1	HCoV-229E	HCoV-NL63
SARS-CoV-2	100	88.76	51.47	48.09	48.82	43.95	44.61
SARS-CoV		100	49.71	49.27	48.53	43.36	44.31
MERS-CoV			100	48.51	46.73	47.18	50.76
HCoV-OC43				100	71.66	40.88	43.28
HCoV-HKU1					100	41.3	43.58
HCoV-229E						100	79.07
HCoV-NL63							100