

SUPPLEMENTAL INFORMATION

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Fig. S3. TonEBP NT does not affect expression of proteins in the MDA5–IFN- β signaling pathway and phosphorylation of TBK1 and IRF3.

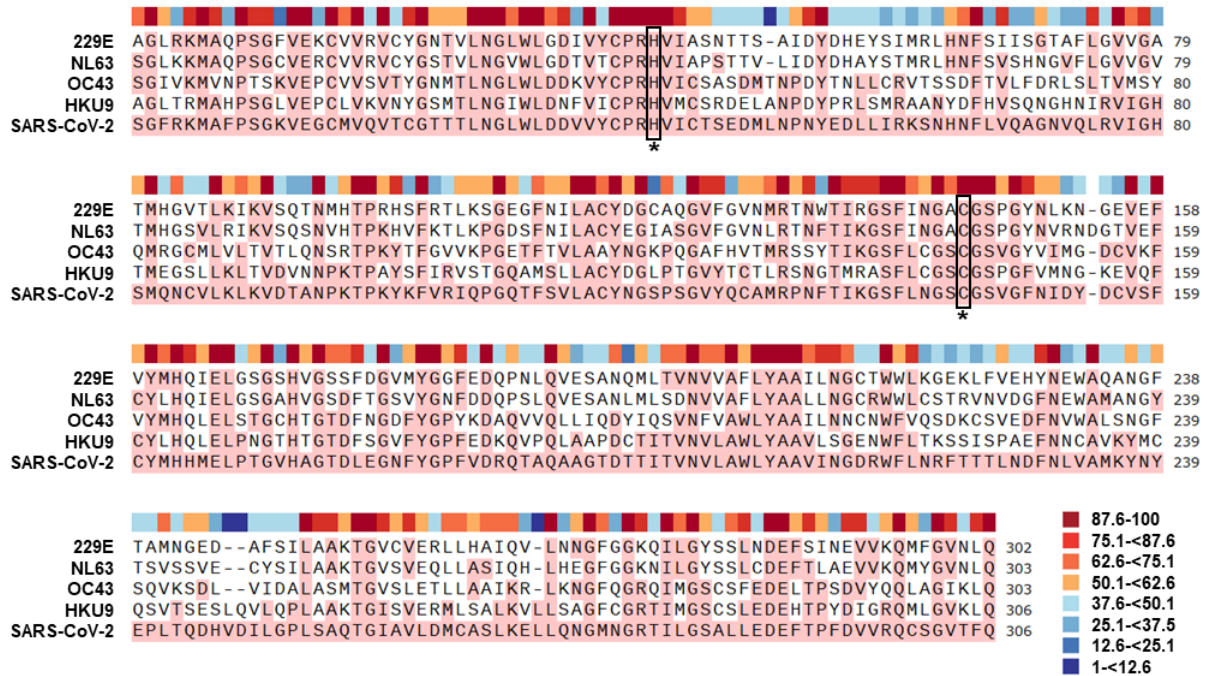


Figure. S1 Sequence alignment of coronavirus NSP5. Sequences of human coronavirus 229E, NL63, OC43, bat coronavirus HKU9, and SARS-CoV-2 NSP5 were aligned. The catalytic dyad residues (His/Cys) are boxed. The amino acids identified to SARS-CoV-2 is marked by pink and conserved amino acids are shown with color score range.

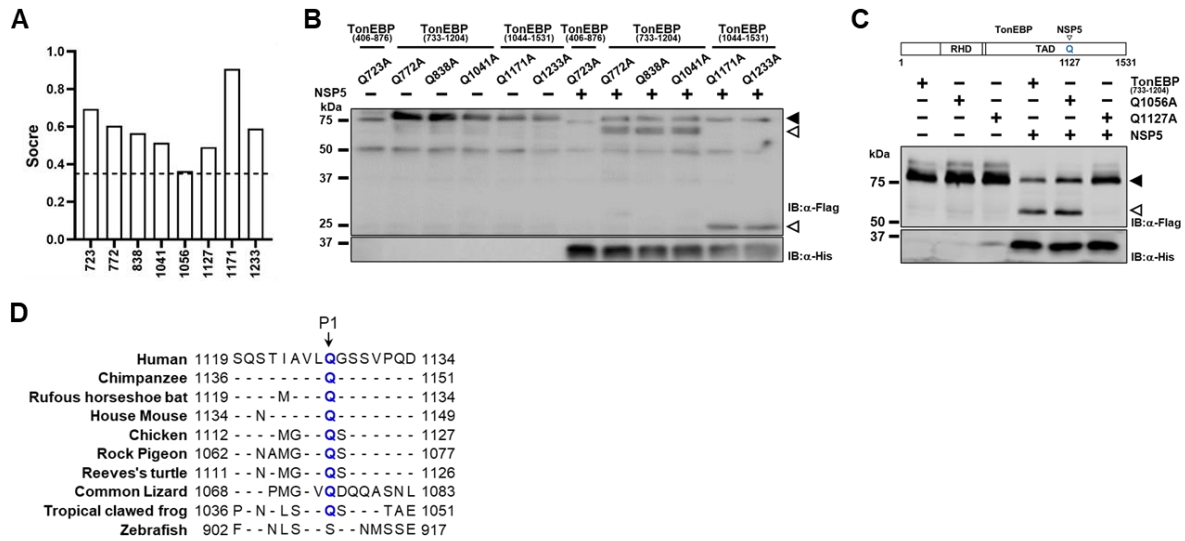


Figure. S2 Site-specific cleavage of TonEBP at Q1127 by SARS-CoV-2 NSP5. **(A)** NetCorona 1.0 prediction of the potential TonEBP cleavage sites used by SARS-CoV-2 NSP5. The dotted line indicates the threshold (0.35). The predicted sites of TonEBP have been arranged in the order of the amino acid sequence number of TonEBP. **(B)** HEK293T cells were transfected with plasmids expressing Flag-tagged truncated TonEBP at positions indicated which were mutated at the indicated putative cleavage site, and His-tagged SARS-CoV-2 NSP5. Solid arrowhead denotes truncated TonEBP, whereas open arrowhead denotes cleavage product. **(C)** Schematic of TonEBP showing the location of the NSP5 cleavage site (top). Cells were transfected with various combinations of expression plasmids for Flag-TonEBP (733–1204) wild type, Q1026A mutant, and Q1127A, and His-tagged SARS-CoV-2 NSP5. **(D)** Amino acid sequence alignment of the region of TonEBP targeted by NSP5 from various species. The putative cleavage sites (Q) are in blue and labeled as P1.

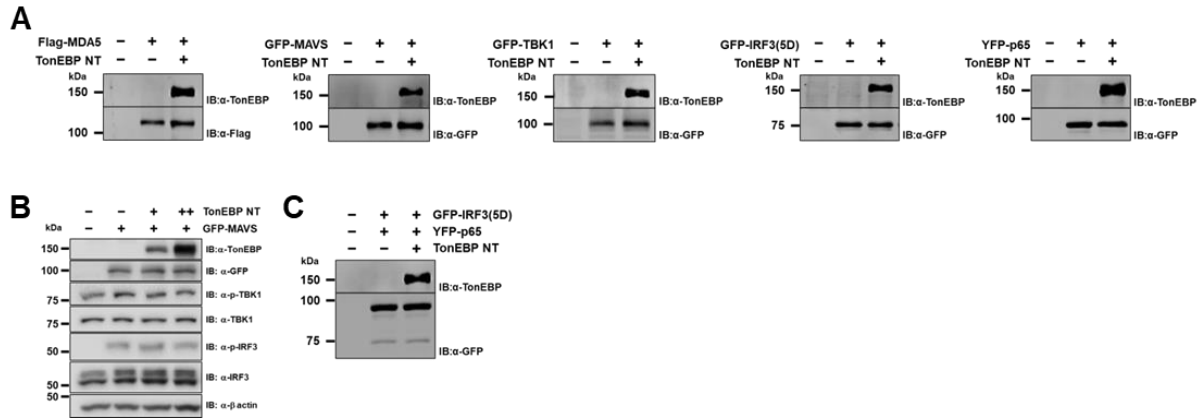


Figure. S3 TonEBP NT does not affect expression of proteins in the MDA5–IFN- β signaling pathway and phosphorylation of TBK1 and IRF3. (A) HEK293T cells were transfected with either an empty vector or a plasmid expressing Flag-TonEBP NT, along with plasmids expressing Flag-MDA5, GFP-MAVS, GFP-TBK1, GFP-IRF3-5D, or YFP-p65. (B) Cells were transfected for 24 h with a plasmid expressing GFP-MAVS along with various amounts of plasmids expressing Flag-TonEBP NT. Phosphorylated TBK1 and IRF3 were immunoblotted. (C) Cells were transfected with various combinations of plasmids expressing TonEBP NT, GFP-IRF3-5D and YFP-p65, as indicated.