Supplementary Information for

SARS-CoV-2 N protein antagonizes type I interferon signaling by suppressing phosphorylation and nuclear translocation of STAT1 and STAT2

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Other Supplementary Materials for this manuscript include the following:

None

26 **Supplementary Materials and Methods**

27 **Cell culture and transfection**

28 Human embryonic kidney 293T (293T) cells and HepG2 cells were obtained
29 commercially from the American Type Culture Collection (Manassas, VA, USA) and
30 maintained in modified Eagle's medium supplemented with 10% fetal bovine serum
31 (Gibco, South America, USA), 100 U/ml penicillin, and 100 μg/ml streptomycin at
32 37°C in an incubator with 5% CO2. Transfection of cells was conducted using
33 Lipofectamine 2000 or Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA)
34 according to the manufacturer's protocol.

35

36 **Plasmids construction**

37 Construction of pRK-Flag-N (SARS-CoV-2) was described previously[1]. The plasmids
38 of ISRE promoter luciferase reporter and plasmids of STAT1/2 with HA tagged at the
39 N-terminus were kindly provided by Dr. Xinwen Chen (Wuhan, China).

40

41 **Construction of stable expression of Flag-tagged SARS-CoV-2 N protein in 293T**
42 **cells**

43 Full-length cDNA encoding SARS-CoV-2 N was amplified from pRK-Flag-N plasmid
44 and then was inserted into pCDH-CMV-MCS-EF1-CoGFP-T2A-puro vector to obtain
45 Flag-tagged SARS-CoV-2 N overexpressing plasmid. pCDH-Flag-N lentivirus
46 particles were obtained by co-transfecting pCDH-Flag-N with packaging plasmids
47 (pMD2.G and pCMV-dR8.91) into 293T cells, we obtained Flag-N lentivirus particles.

293T cells were infected with concentrated Flag-N lentivirus particles and then were screened by puromycin to obtain stable Flag-N-overexpressed cell line. The protein expression of Flag-N was detected by western blot analysis and immunofluorescence.

Virus infection

SeV was kindly provided by Dr. Xinwen Chen. All virus was amplified and titrated by standard plaque or focus-forming assays[2]. Cells were infected with SeV (50 hemagglutination units per ml). SARS-CoV-2 was kindly provided by Dr. Zhengli Shi. HepG2 cells were infected with SARS-CoV-2 at a multiplicities of infection (MOI) of 0.1 and harvested at 24 hours post infection.

Luciferase assay

Luciferase assay was performed as previously described[3]. The dual-Luciferase Reporter Assay System (Promega) was used for luciferase assays. Briefly, cells were seeded in 24-well plates and transfected luciferase reporter and pRL-TK plasmids combined with a total of 500 ng of target plasmid or empty control plasmid for 24 hrs. Subsequently, cells were stimulated with IFNα 2α (50 ng/mL), IFN-β (50 ng/mL), or left untreated for the indicated time periods and luciferase activity was measured.

Quantitative real-time reverse transcription PCR (qRT-PCR)

The qRT-PCR were performed as described previously[4]. Total cellular RNAs were isolated using TRIzol reagent (Invitrogen) according to the manufacturer's protocols.

70 The quantification of transcripts was analyzed by real-time RT-PCR with the RT reagent
71 Kit (Takara) and TB Green premix Ex Taq II (Takara). The data were normalized to
72 levels of cellular β-actin mRNA in each individual sample. $2^{-\Delta\Delta Ct}$ method was used to
73 calculate relative expression changes. The ratios of gene expression relative to the
74 control were calculated by the $\Delta\Delta C_T$ method as previously described[4]. Data represented
75 the means and standard deviations of three independent experiments. The primers for
76 quantitative RT-PCR are in Supplementary Table S1.
77
78 **Western blotting**
79 The Western blotting were performed as described previously[5]. Cells were washed
80 twice in cold phosphate-buffered saline (PBS) and lysed in lysis buffer (50 mM Tris-
81 HCl [pH 7.4], 150 mM NaCl, 1% NP40, 0.25% deoxycholate, and a protease inhibitor
82 cocktail [6]), and the lysates were subjected to SDS-PAGE on 12% gels by western
83 blotting according to standard procedures with the relevant antibodies. Rabbit anti-
84 GAPDH, HA, STAT1, phospho-STAT1 (Tyr701), STAT2, phospho-STAT2 (Tyr690),
85 antibodies were all purchased from Cell Signaling Technology. Mouse anti-Flag were
86 from Sigma-Aldrich.
87
88 **Co-Immunoprecipitation (Co-IP)**
89 Co-IP was performed as previously described[7]. Cells were rinsed with ice-cold PBS
90 and lysed with RIPA buffer (50 mM Tris, pH7.5, 1 mM EDTA, 1% Triton X-100, 150
91 mM NaCl, 2 mM DTT) and a protease inhibitor cocktail (Roche). The cell lysates were
92 centrifuged at 12000×g at 4 ℃ for 10 min and the supernatants were incubated with
93 anti-HA together with protein-A/G agarose beads (Roche) at 4 ℃ overnight according

94 to the manufacturer's protocol. The immunoprecipitated samples were centrifuged and
95 washed three times and subjected to Western blots using anti-HA and anti-Flag.

96 **Cell fractionation**

97 Cells were rinsed with ice-cold PBS and lysed using Cell fractionation kit (Beyotime,
98 China) according to the manufacturer's instructions. The purified cytoplasmic fraction
99 and the nuclear fraction were subjected to Western blot assays according to standard
100 procedures with the relevant antibodies. Histone H3 (abcam, ab1791), Tubulin (Cell
101 Signaling Technology), phospho-STAT1 (Tyr701), and phospho-STAT2 (Tyr690)
102 antibodies were used.

103

104 **Immunofluorescence assay**

105 The immunofluorescence assays were performed as described previously[8]. Briefly, the
106 cells were fixed with 3.7% paraformaldehyde in PBS for 30 min, permeabilized with
107 0.5% Triton X-100 and blocked in 1% normal goat serum (Boster) in PBS for 30 min
108 on ice. The cells were incubated with anti-HA (1:500 dilution, Invitrogen) or anti-Flag
109 (1:500 dilution, Sigma) primary antibodies. The secondary antibodies were Alexa Fluor
110 568- or 488-conjugated anti-rabbit and anti-mouse antibodies (1:800 dilution,
111 Invitrogen). The nuclear DNA was stained with DAPI (Beyotime).

112 **Reference**

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129 8 Mu, J. *et al.* Autographa californica Multiple Nucleopolyhedrovirus Ac34 Protein Retains
130 Cellular Actin-Related Protein 2/3 Complex in the Nucleus by Subversion of CRM1-
131 Dependent Nuclear Export. *Plos Pathog* **12**, e1005994-e1005994,(2016).

132

133

134

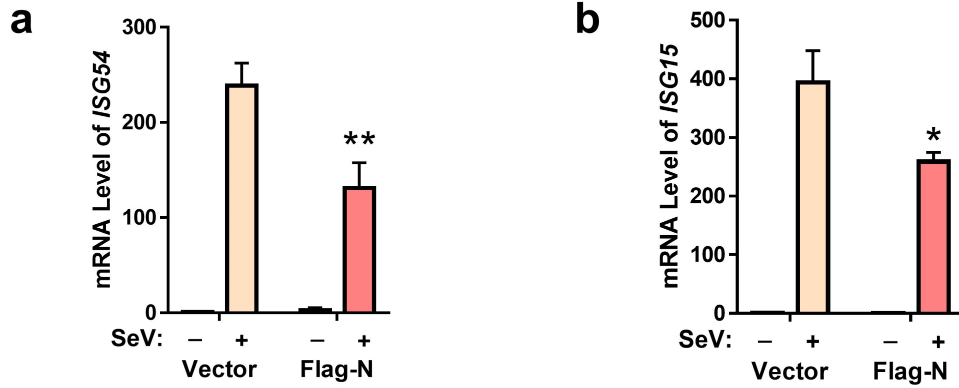
Table S1. The primers for qRT-PCR

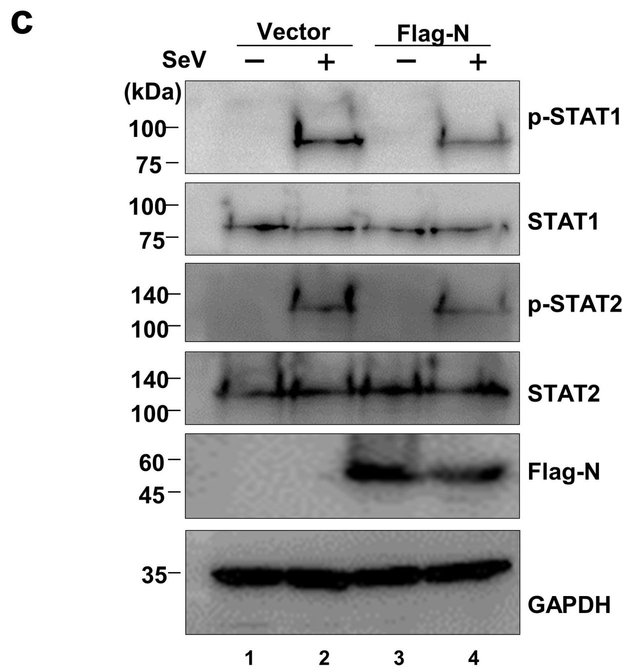
Name	Sequences(5′-3′)
RBD-FOR	CAATGGTTTAACAGGCACAGG
RBD-REV	CTCAAGTGTCTGTGGATCACG
ISG56-FOR	TCCCCTAAGGCAGGCTGTC
ISG56-REV	GACATGTTGGCTAGAGCTTCTTC
ISG15-FOR	GAGAGGCAGCGAACTCATCTT
ISG15-REV	CCAGCATCTTCACCGTCAGG
ISG54-FOR	GGTCTCTTCAGCATTTATTGGTG
ISG54-REV	TGCCGTAGGCTGCTCTCCA
β-actin-FOR	AGAGCTACGAGCTGCCTGAC
β-actin-REV	AGCACTGTGTTGGCGTACAG

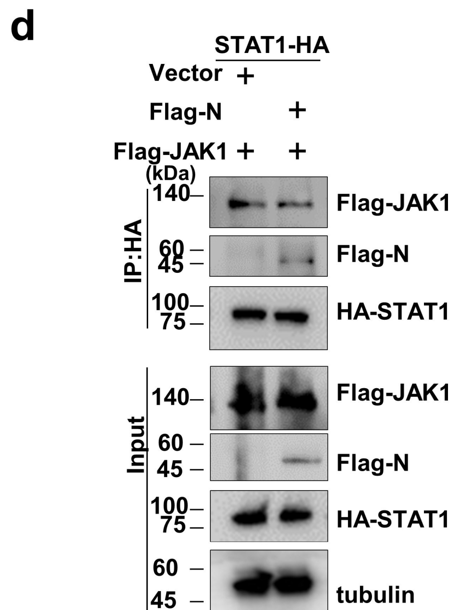
135

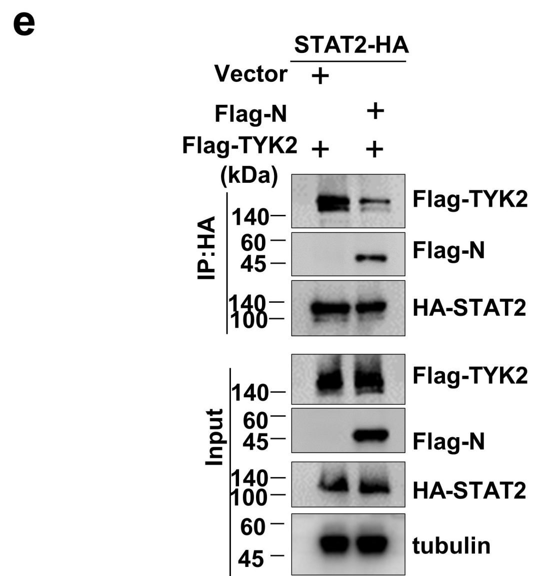
136

Supplementary Fig. S1



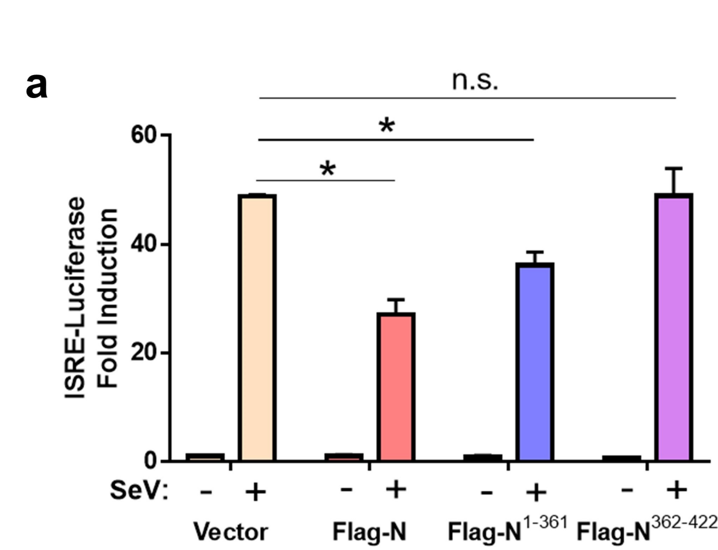


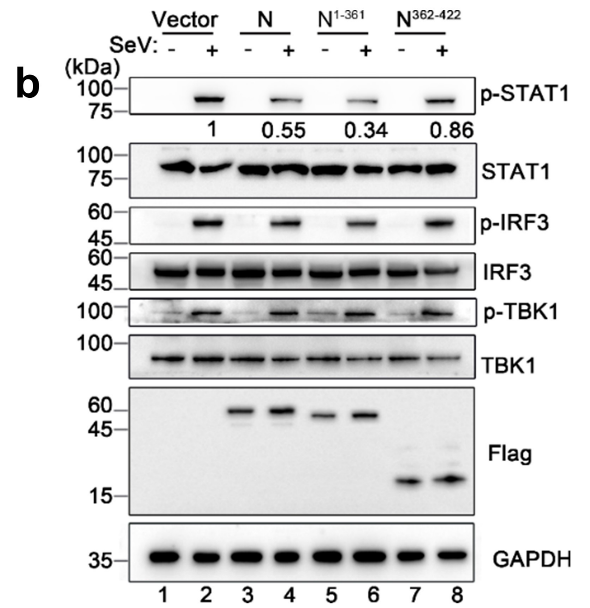


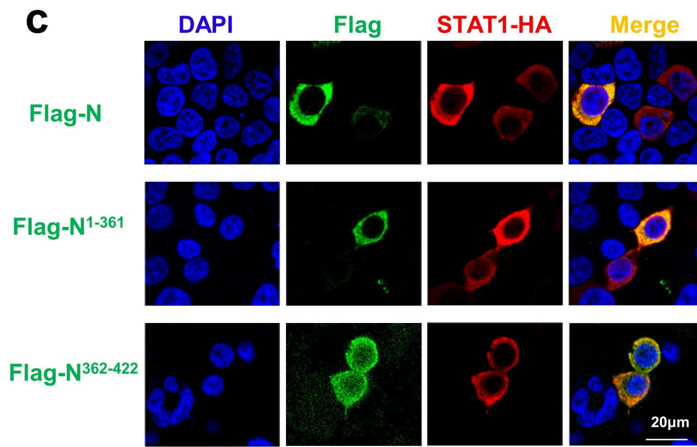


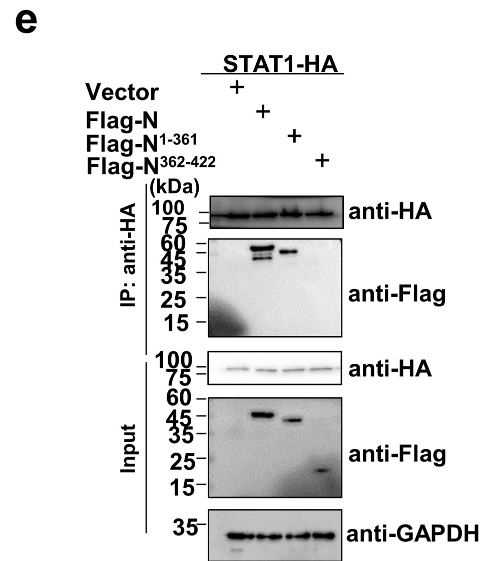
Supplementary Fig. S1 a-b 293T cells were co-transfected with plasmids with either empty vector or the plasmid encoding Flag-N (0.5μg each), as indicated. At 24 hpt, cells were then infected with or without SeV, Total RNAs were extracted at 12 hpi. The mRNA levels of ISG54 and ISG15 were measured by real-time RT-PCR analysis, and normalized to the cellular β-actin mRNA. Student's t-test was used for estimation of statistical significance. *, $P < 0.05$ and **, $P < 0.01$. Data were from three independent experiments. Mean values and standard deviations from three independent experiments per group. **c** we constructed 293T cell lines stably expressing Flag tagged SARS-CoV-2 N protein. Cells were seeded on 6-well plate and then infected with or without SeV for 12 h, The STAT1 and STAT2 protein levels and their phosphorylation levels were detected by Western blot. The concentration of total protein was quantified using the BCA method. GAPDH serves as the loading control. **d-e** 293T cells were transfected with plasmids encoding STAT1-HA and Flag-JAK1 (**d**), or plasmid encoding STAT2-HA and FLAG-TYK2 (**e**), together with either empty vector or the plasmid encoding Flag-N. At 48 hpt, the cells were harvested and subjected to a Co-IP assay using anti-HA. The immunoprecipitated proteins and the inputs were separated by SDS-PAGE and probed with anti-HA and anti-Flag.

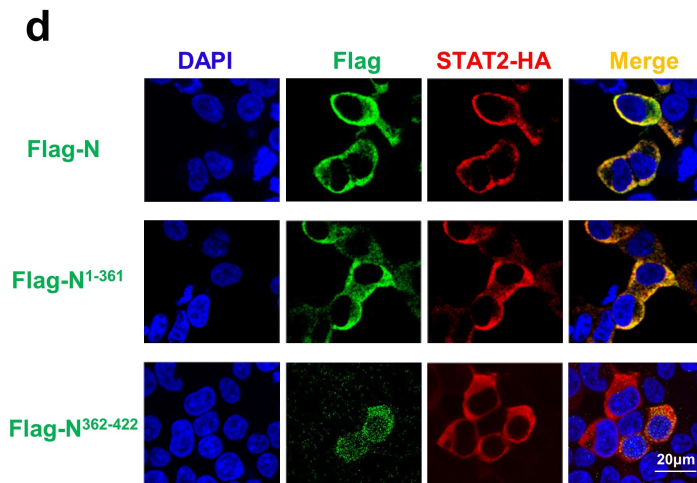
Supplementary Fig. S2

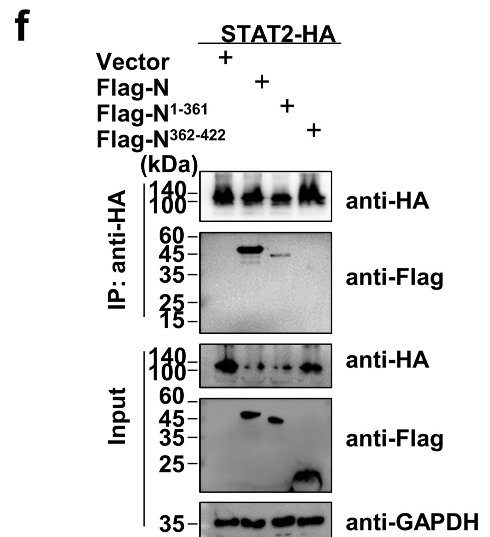












Supplementary Fig. S2 The N-terminal domain of SARS-CoV-2 N protein is sufficient for antagonizing IFN signaling. **a** 293T cells were co-transfected with plasmids with ISRE-Luc and pRL-TK, together with either empty vector or the plasmid encoding Flag-N, Flag-N^{1-361}, Flag-$N^{362-422}$ (0.5μg each), as indicated. At 24 hpt, cells were then infected with or without SeV, at 12 hpi, the reporter activity was detected by dual luciferase reporter system. Student's *t*-test was used for estimation of statistical significance. n.s., there was no significant difference, *, $P < 0.05$. Data are from three independent experiments. Mean values and standard deviations from three independent experiments per group. **b** 293T cells were transfected with either empty vector or the plasmid encoding Flag-N, Flag-N^{1-361}, Flag-$N^{362-422}$ (0.5μg each), as indicated. At 24 hpt, cells were then infected with or without SeV. At 12 hpi, Cell lysates were harvested and analyzed by western blotting with antibodies as indicated. The densities of blots were analyzed by using ImageJ software. **c-d** 293T cells were transfected with plasmids Flag-N, Flag-N^{1-361}, or Flag-$N^{362-422}$, together with the plasmid encoding STAT1-HA (**c**) or the plasmid encoding STAT2-HA (**d**). At 24 hpt, the cells were fixed and subjected to immunofluorescence staining assays. Scale bar, 20μm. **e-f** 293T cells were transfected with plasmids encoding STAT1-HA (**e**), or plasmid encoding STAT2-HA (**f**), together with either empty vector or the plasmid encoding Flag-N, Flag-N^{1-361}, or Flag-$N^{362-422}$, At 48 hpt, the cells were harvested and subjected to a Co-IP assay using anti-HA. The immunoprecipitated proteins and the inputs were separated by SDS-PAGE and probed with anti-HA and anti-Flag.

Supplementary Fig. S3

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                                 20                   40                   60
SARS-CoV-2 YP_009724397  MSDNGPQ-NQ RNAPR-ITFG GPSDSTGSNQ NGERSGARSK QRRPQGLPNN TASWFTALTQ 58
SARS-CoV-2 QJI53879      MSDNGPQ-NQ RNAPR-ITFG GPSDSTGSNQ NGERSGARSK QRRPQGLPNN TASWFTALTQ 58
SARS-CoV-2 QIK50455      MSDNGPQ-NQ RNAPR-ITFG GPSDSTGSNQ NGERSGARSK QRRPQGLPNN TASWFTALTQ 58
SARS-CoV NC_004718       MSDNGPQSNQ RSAPR-ITFG GPTDSTDNNQ NGGRNGARPK QRRPQGLPNN TASWFTALTQ 59
SARS-CoV AY502924        MSDNGPQSNQ RSAPR-ITFG GPTDSTDNNQ NGGRNGARPK QRRPQGLPNN TASWFTALTQ 59
SARS-CoV AY536760        MSDNGPQSNQ RSAPR-ITFG GPTDSTDNNQ NGGRNGARPK QRRPQGLPNN TASWFTALTQ 59
MERS-CoV AXP07352        MASPA----- --APRAVSFA DNNDITNTNL SRGR-GRNPK ---PRAAPNN TVSWYTGLTQ 49
MERS-CoV AVN89309        MASPA----- --APRAVSFA DNNDITNTNL SRGR-GRNPK ---PRAAPNN TVSWYTGLTQ 49
MERS-CoV ATG84873        MASPA----- --APCAVSFA DNNDITNTNL SRGR-GRNPK ---PRAAPNN TVSWYTGLTQ 49
Consensus                MSDNGPQ-NQ RXAPR-ITFG GPXDSTXXNQ NGGRXGARPK QRRPQGLPNN TASWFTALTQ
Conservation (100% / 0%)

                                 80                  100                  120
SARS-CoV-2 YP_009724397  HGKEDLKFPR GQGVPINTNS SPDDQIGYYR RATRRIRGGD GKMKDLSPRW YFYYLGTGPE 118
SARS-CoV-2 QJI53879      HGKEDLKFPR GQGVPINTNS SPDDQIGYYR RATRRIRGGD GKMKDLSPRW YFYYLGTGPE 118
SARS-CoV-2 QIK50455      HGKEDLKFPR GQGVPINTNS SPDDQIGYYR RATRRIRGGD GKMKDLSPRW YFYYLGTGPE 118
SARS-CoV NC_004718       HGKEELRFPR GQGVPINTNS GPDDQIGYYR RATRRVRGGD GKMKELSPRW YFYYLGTGPE 119
SARS-CoV AY502924        HGKEELRFPR GQGVPINTNS GPDDQIGYYR RATRRVRGGD GKMKELSPRW YFYYLGTGPE 119
SARS-CoV AY536760        HGKEELRFPR GQGVPINTNS GPDDQIGYYR RATRRVRGGD GKMKELSPRW YFYYLGTGPE 119
MERS-CoV AXP07352        HGKVPLTFPP GQGVPLNANS TPAQNAGYWR RQDRKINTGN G-IKQLAPRW YFYYTGTGPE 108
MERS-CoV AVN89309        HGKVPLTFPP GQGVPLNANS TPAQNAGYWR RQDRKINTGN G-IKQLAPRW YFYYTGTGPE 108
MERS-CoV ATG84873        HGKVPLTFPP GQGVPLNANS TPAQNAGYWR RQDRKINTGN G-IKQLAPRW YFYYTGTGPE 108
Consensus                HGKEXLXFPR GQGVPINTNS XPDDQIGYYR RATRRIRGGD GKMKXLSPRW YFYYLGTGPE
Conservation (100% / 0%)

                                140                  160                  180
SARS-CoV-2 YP_009724397  AGLPYGANKD GIIWVATEGA LNTPKDHIGT RNPANNAAIV LQLPQGTTLP KGFYAEGSRG 178
SARS-CoV-2 QJI53879      AGLPYGANKD GIIWVATEGA LNTPKDHIGT RNPANNAAIV LQLPQGTTLP KGFYAEGSRG 178
SARS-CoV-2 QIK50455      AGLPYGANKD GIIWVATEGA LNTPKDHIGT RNPANNAAIV LQLPQGTTLP KGFYAEGSRG 178
SARS-CoV NC_004718       ASLPYGANKE GIVWVATEGA LNTPKDHIGT RNPNNNAATV LQLPQGTTLP KGFYAEGSRG 179
SARS-CoV AY502924        ASLPYGANKE GIIWVATEGA LNTPKDHIGT RNPNNNAATV LQLPQGTTLP KGFYAEGSRG 179
SARS-CoV AY536760        ASLPYGANKE GIVWVATEGA LNTPKDHIGT RNPNNNAATV LQLPQGTTLP KGFYAEGSRG 179
MERS-CoV AXP07352        AALPFRAVKD GIVWVHEDGA TDAPST-FGT RNPNNDSAIV TQFAPGTKLP KNFHIEGTGG 167
MERS-CoV AVN89309        AALPFRAVKD GIVWVHEDGA TDAPST-FGT RNPNNDSAIV TQFAPGTKLP KNFHIEGTGG 167
MERS-CoV ATG84873        AALPFRAVKD GIVWVHEDGA TDAPST-FGT RNPNNDSAIV TQFAPGTKLP KNFHIEGTGG 167
Consensus                AXLPYGANKD GIVWVATEGA LNTPKDHIGT RNPNNNAAIV LQLPQGTTLP KGFYAEGSRG
Conservation (100% / 0%)

                                200                  220                  240
SARS-CoV-2 YP_009724397  GSQASSRSSS RSRNSSRNST PGSSRGTS-- -----PARMA GNGGDAALAL LLLDRLNQLE 231
SARS-CoV-2 QJI53879      GSQASSRSSS RSRNSSRNST PGSSRGTS-- -----PARMA GNGGDAALAL LLLDRLNQLE 231
SARS-CoV-2 QIK50455      GSQASSRSSS RSRNSSRNST PGSSRGTS-- -----PARMA GNGGDAALAL LLLDRLNQLE 231
SARS-CoV NC_004718       GSQASSRSSS RSRGNSRNST PGSSRGNS-- -----PARMA SGGGETALAL LLLDRLNQLE 232
SARS-CoV AY502924        GSQASSRSSS RSRGNSRNST PGSSRGNS-- -----PARMA SGGGETALAL LLLDRLNQLE 232
SARS-CoV AY536760        GSQASSRSSS RSRGNSRNST PGSSRGNS-- -----PARMA SGGGETALAL LLLDRLNQLE 232
MERS-CoV AXP07352        NSQSSSRASS VSRNSSRSSS QGSRSGNSTR GTSPGPSGIG AVGGD----L LYLDLLNRLQ 223
MERS-CoV AVN89309        NSQSSSRASS VSRNSSRSSS QGSRSGNSTR GTSPGPSGIG AVGGD----L LYLDLLNRLQ 223
MERS-CoV ATG84873        NSQSSSRASS VSRNSSRSSS QGSRSGNSTR GTSPGPSGIG AVGGD----L LYLDLLNRLQ 223
Consensus                GSQASSRSSS RSRNSSRNST PGSSRGNS-- -----PARMA XXGGDXALAL LLLDRLNQLE
Conservation (100% / 0%)

                                260                  280                  300
SARS-CoV-2 YP_009724397  SKMSGKGQQQ QGQTVTKKSA AEASKKPRQK RTATKAYNVT QAFGRRGPEQ TQGNFGDQEL 291
SARS-CoV-2 QJI53879      SKMSGKGQQQ QGQTVTKKSA AEASKKPRQK RTATKAYNVT QAFGRRGPEQ TQGNFGDQEL 291
SARS-CoV-2 QIK50455      SKMSGKGQQQ QGQTVTKKSA AEASKKPRQK RTATKAYNVT QAFGRRGPEQ TQGNFGDQEL 291
SARS-CoV NC_004718       SKVSGKGQQQ QGQTVTKKSA AEASKKPRQK RTATKQYNVT QAFGRRGPEQ TQGNFGDQDL 292
SARS-CoV AY502924        SKVSGKGQQQ QGQTVTKKSA AEASKKPRQK RTATKQYNVT QAFGRRGPEQ TQGNFGDQDL 292
SARS-CoV AY536760        SKVSGKGQQQ QGQTVTKKSA AEASKKPRQK RTATKQYNVT QAFGRRGPEQ TQGNFGDQDL 292
MERS-CoV AXP07352        ALESGKVKQS QPKVITKKDA AAAKNKMRHK RTSTKSFNMV QAFGLRGPGD LQGNFGDLQL 283
MERS-CoV AVN89309        ALESGKVKQS QPKVITKKDA AAAKNKMRHK RTSTKSFNMV QAFGLRGPGD LQGNFGDLQL 283
MERS-CoV ATG84873        ALESGKVKQS QPKVITKKDA AAAKNKMRHK RTSTKSFNMV QAFGLRGPGD LQGNFGDLQL 283
Consensus                SKXSGKGQQQ QGQTVTKKSA AEASKKPRQK RTATKXYNVT QAFGRRGPEQ TQGNFGDQXL
Conservation (100% / 0%)

                                320                  340                  360
SARS-CoV-2 YP_009724397  IRQGTDYKHW PQIAQFAPSA SAFFGMSRIG MEVTPSGT-- ----WLTYTA AIKLDDKDPN 345
SARS-CoV-2 QJI53879      IRQGTDYKHW PQIAQFAPSA SAFFGMSRIG MEVTPSGT-- ----WLTYTG AIKLDDKDPN 345
SARS-CoV-2 QIK50455      IRQGTDYKHW PQIAQFAPSA SAFFGMSRIG MEVTPSGT-- ----WLTYTG AIKLDDKDPN 345
SARS-CoV NC_004718       IRQGTDYKHW PQIAQFAPSA SAFFGMSRIG MEVTPSGT-- ----WLTYHG AIKLDDKDPQ 346
SARS-CoV AY502924        IRQGTDYKHW PQIAQFAPSA SAFFGMSRIG MEVTPSGT-- ----WLTYHG AIKLDDKDPQ 346
SARS-CoV AY536760        IRQGTDYKHW PQIAQFAPSA SAFFGMSRIG MEVTPSGT-- ----WLTYHG AIKLDDKDPQ 346
MERS-CoV AXP07352        TKLGTEDPRW PQIAELAPTA SAFMGMSQFK LTHQNNDDHG NPVYFLRYSG AIKLDPKNPN 343
MERS-CoV AVN89309        TKLGTEDPRW PQIAELAPTA SAFMGMSQFK LTHQNNDDHG NPVYFLRYSG AIKLDPKNPN 343
MERS-CoV ATG84873        NKLGTEDPRW PQIAELAPTA SAFMGMSQFK LTHQNNDDHG NPVYFLRYSG AIKLDPKNPN 343
Consensus                IRQGTDYKHW PQIAQFAPSA SAFFGMSRIG MEVTPSGT-- ----WLTYXG AIKLDDKDPN
Conservation (100% / 0%)

                                380                  400                  420
SARS-CoV-2 YP_009724397  FKDQVILLNK HIDAYKTFPP TEPKKD--KK KKADETQALP QRQKKQQTVT LLPAADLDDF 403
SARS-CoV-2 QJI53879      FKDQVILLNK HIDAYKTFPP TEPKKD--KK KKADETQALP QRQKKQQTVT LLPAADLDDF 403
SARS-CoV-2 QIK50455      FKDQVILLNK HIDAYKTFPP TEPKKD--KK KKADETQALP QRQKKQQTVT LLPAADLDDF 403
SARS-CoV NC_004718       FKDNVILLNK HIDAYKTFPP TEPKKD--KK KKTDEAQPLP QRQKKQPTVT LLPAADMDDF 404
SARS-CoV AY502924        FKDNVILLNK HIDAYKTFPP TEPKKD--KK KKTDEAQPLP QRQKKQPTVT LLPAADMDDF 404
SARS-CoV AY536760        FKDNVILLNK HIDAYKTFPP TEPKKD--KK KKTDEAQPLP QRQKKQPTVT PLPAADMDDF 404
MERS-CoV AXP07352        YNKWLELLEQ NIDAYKTFPK KEKKQKAPKE ESTDQMSEPP MEQRVQGSIT --------QR 395
MERS-CoV AVN89309        YNKWLELLEQ NIDAYKTFPK KEKKQKAPKE ESTDQMSEPP KEQRVQGSIT --------QR 395
MERS-CoV ATG84873        YNKWLELLEQ NIDAYKTFPK KEKKQKAPKE ESTDQMSEPP KEQRVQGSIT --------QR 395
Consensus                FKDXVILLNK HIDAYKTFPP TEPKKD--KK KKTDEXQXLP QRQKKQXTVT LLPAADXDDF
Conservation (100% / 0%)

SARS-CoV-2 YP_009724397  SKQLQQSMS- -SADSTQA 419
SARS-CoV-2 QJI53879      SKQLQQSMS- -SADSTQA 419
SARS-CoV-2 QIK50455      SKQLQQSMS- -SADSTQA 419
SARS-CoV NC_004718       SRQLQNSMSG ASADSTQA 422
SARS-CoV AY502924        SRQLQNSMSG ASADSTQA 422
SARS-CoV AY536760        SRQLQNSMSG ASADSTQA 422
MERS-CoV AXP07352        TRTRPSVQPG PMIDVNTD 413
MERS-CoV AVN89309        TRTRPSVQPG PMIDVNTD 413
MERS-CoV ATG84873        TRTRPSVQPG PMIDVNTD 413
Consensus                SRQLQXSMSG XSADSTQA
Conservation (100% / 0%)
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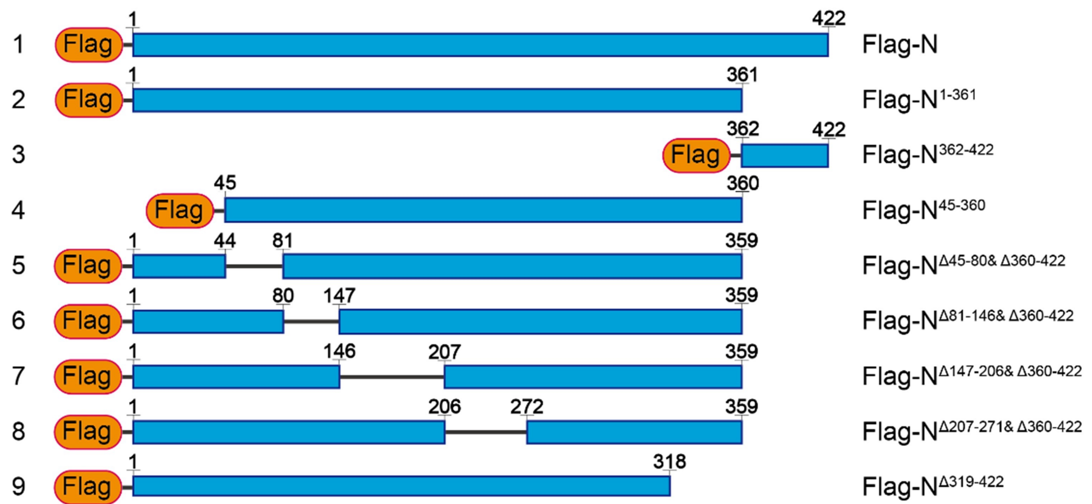
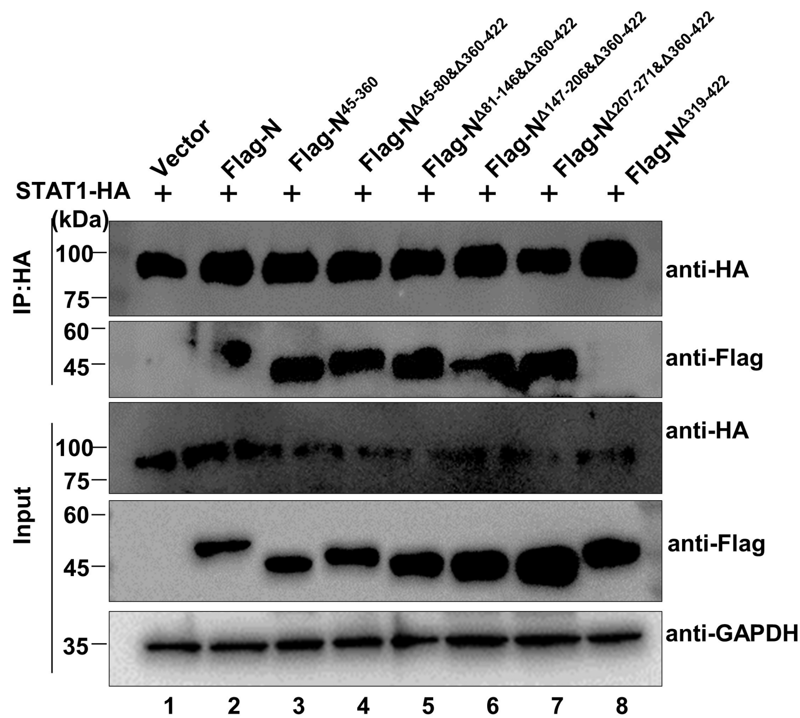
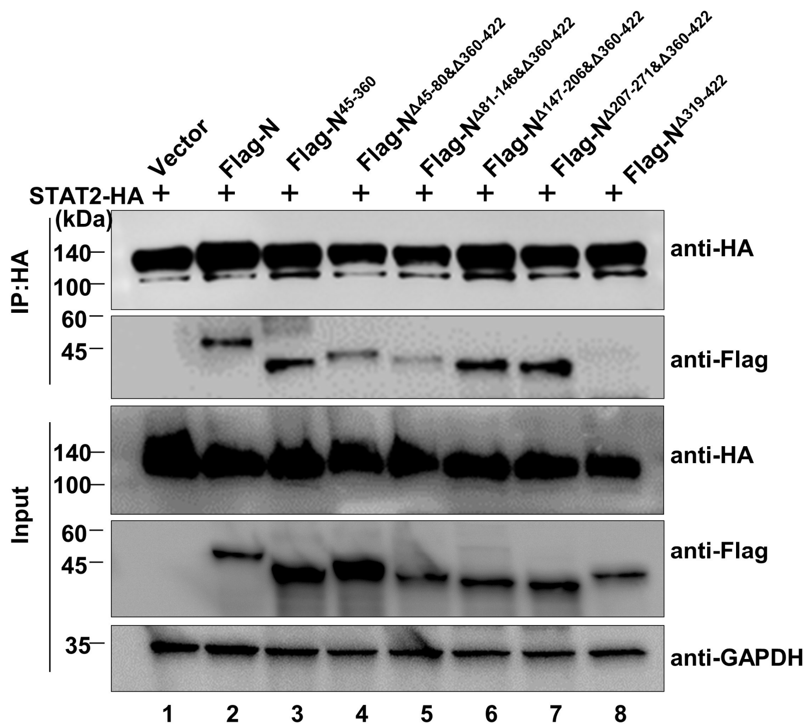
Supplementary Fig. S3 The amino acid sequence alignment of coronavirus N proteins was performed using Clustal Omega.

Supplementary Fig. S4

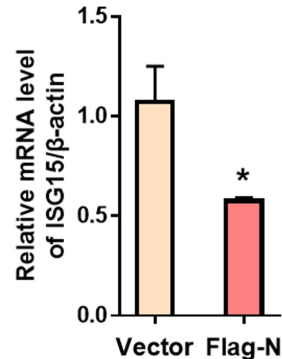






Supplementary Fig. S4 C-terminal 319-422 amino acids of SARS-CoV-2 N protein is indispensable for its binding to STAT1 and STAT2 **a** Schematic diagram of N protein and N truncations. **b-c** 293T cells were transfected with plasmids empty vector, Flag-N, Flag-N^{45-360}, Flag-$N^{\Delta 45-80\& \Delta 360-422}$, Flag-$N^{\Delta 81-146\& \Delta 360-422}$, Flag-$N^{\Delta 147-206\& \Delta 360-422}$, Flag-$N^{\Delta 207-271\& \Delta 360-422}$, or Flag-$N^{319-422}$, together with the plasmid encoding STAT1-HA (b) or the plasmid encoding STAT2-HA (**c**). At 48 hpt, the cells were harvested and subjected to a Co-IP assay using anti-HA. The immunoprecipitated proteins and the inputs were separated by SDS-PAGE and probed with anti-HA and anti-Flag.

Supplementary Fig. S5



Supplementary Fig. S5 Huh7 cells were transfected with either empty vector or the plasmid encoding Flag-N. At 24 hpt, cells were infected with SARS-CoV-2 at a MOI of 0.1. At 24 hpi, total RNAs were extracted and the levels of ISG15 were measured by real-time RT-PCR analysis, normalized to the cellular β-actin mRNA. Mean values and standard deviations from three independent experiments are shown. Student's t-test was used for estimation of statistical significance. *, $P < 0.05$.