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Supplemental information

Systematic functional analysis of SARS-CoV-2 proteins uncovers viral innate immune antagonists and remaining vulnerabilities

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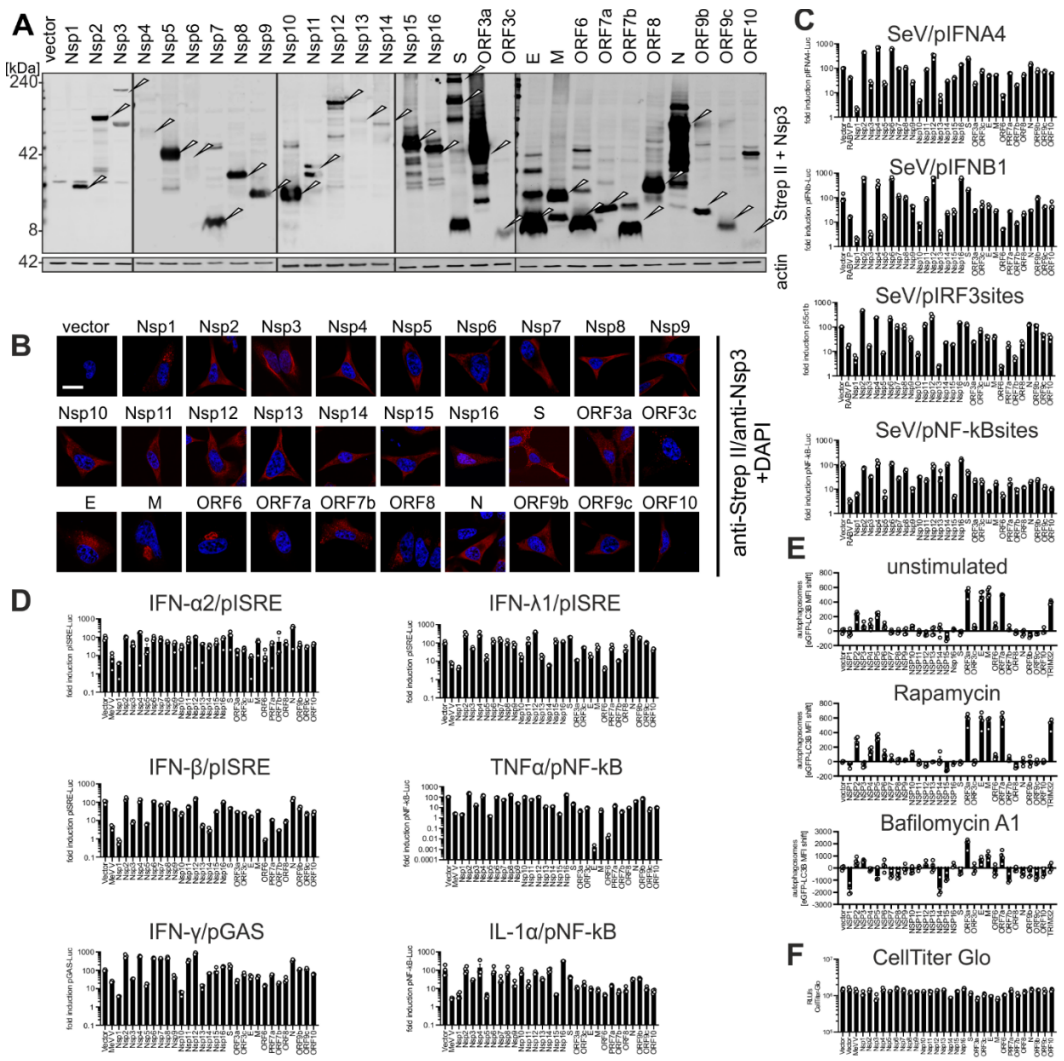

Figure S1. Expression and impact of 29 SARS-CoV-2 proteins on innate immune activation. **A,** Exemplary immunoblots of whole cell lysates (WCLs) of HEK293T cells transiently transfected with the indicated SARS-CoV-2 expression plasmids and stained with anti-Strep II and anti-actin antibodies. **B,** Exemplary confocal microscopy images of Hela cells expressing the indicated Strep II-tagged SARS-CoV-2 proteins (red) or untagged Nsp3. Nuclei, DAPI (blue). Scale bar, 10 µm. **C,** Transiently transfected HEK293T cells expressing the indicated SARS-CoV-2 proteins as well as different Firefly luciferase promotor constructs were infected with Sendai virus or left uninfected. 24 h p.i., Firefly luciferase activities were quantified as relative light units (RLU/s). Shown are mean values of n=3±SEM (biological replicates), stimulated vector control set to 100%. **D,** Luciferase reporter gene assay in HEK293T cells transiently transfected with the indicated SARS-CoV-2 expression plasmids and the indicated Firefly luciferase promotor plasmids. 24 h post transfection cells were stimulated with the respective interferons and cytokines. Firefly luciferase activities were quantified as relative light units (RLU/s) 8-24 h post stimulation and normalized to cell metabolic activity (CellTiter Glo). Shown are mean values of n=3±SEM (biological replicates), stimulated vector control set to 100%. **E,** HEK293T cells stably expressing GFP-LC3B were transiently transfected with the indicated SARS-CoV-2 plasmids. Cells were stimulated with the indicated compounds or left untreated. Autophagosomes were quantified by flow cytometry as mean fluorescence intensity of GFP-LC3B-positive vesicles in saponin-permeabilised cells. Bars represent the mean of n=4±SEM (biological replicates). **F,** Exemplary cell metabolic activity of HEK293T cells transfected and stimulated as described in (D) measured using the CellTiter Glo Assay Kit (Promega) according to the manufacturer's recommendations. Bars represent mean values of n=3±SEM (biological replicates). Related to Figure 1.

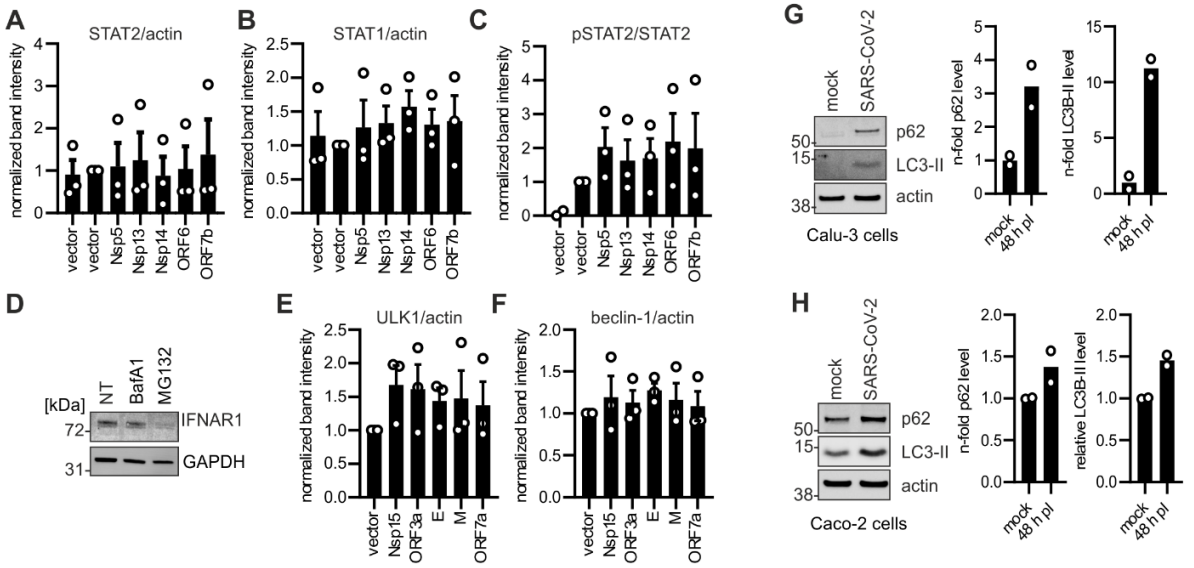

Figure S2. Quantification of the impact of SARS-CoV-2 proteins on IFN and autophagy response. A-C, Quantification of the band intensities of immunoblots in Figure 2. Ratios were calculated as indicated and normalized to the stimulated vector control. Shown are mean values of n=3±SEM (biological replicates). **D**, Immunoblot of endogenous IFNAR1 levels in vector transfected HEK293T cells after 6 h of treatment with BafilomycinA1 (BafA1, 2 µM) or MG132 (50 µM). NT, not treated. **E-F,** Quantification of the band intensities of immunoblots in Figure 3. Ratios were calculated as indicated and normalized to the vector control. Shown are mean values of n=3±SEM (biological replicates). **G-H**, WCLs of Calu-3 (G) or Caco-2 (H) cells infected with SARS-CoV-2 (MOI 0.05, Calu-3; MOI 1, Caco-2; both 48 h p.i.). Blots were stained with anti-p62, anti-LC3 and anti-actin. Ratios were calculated as indicated on the y-axis and normalized to the mock control. Bars represent mean values of n=2 (biological replicates). Related to Figure 2 and 3.

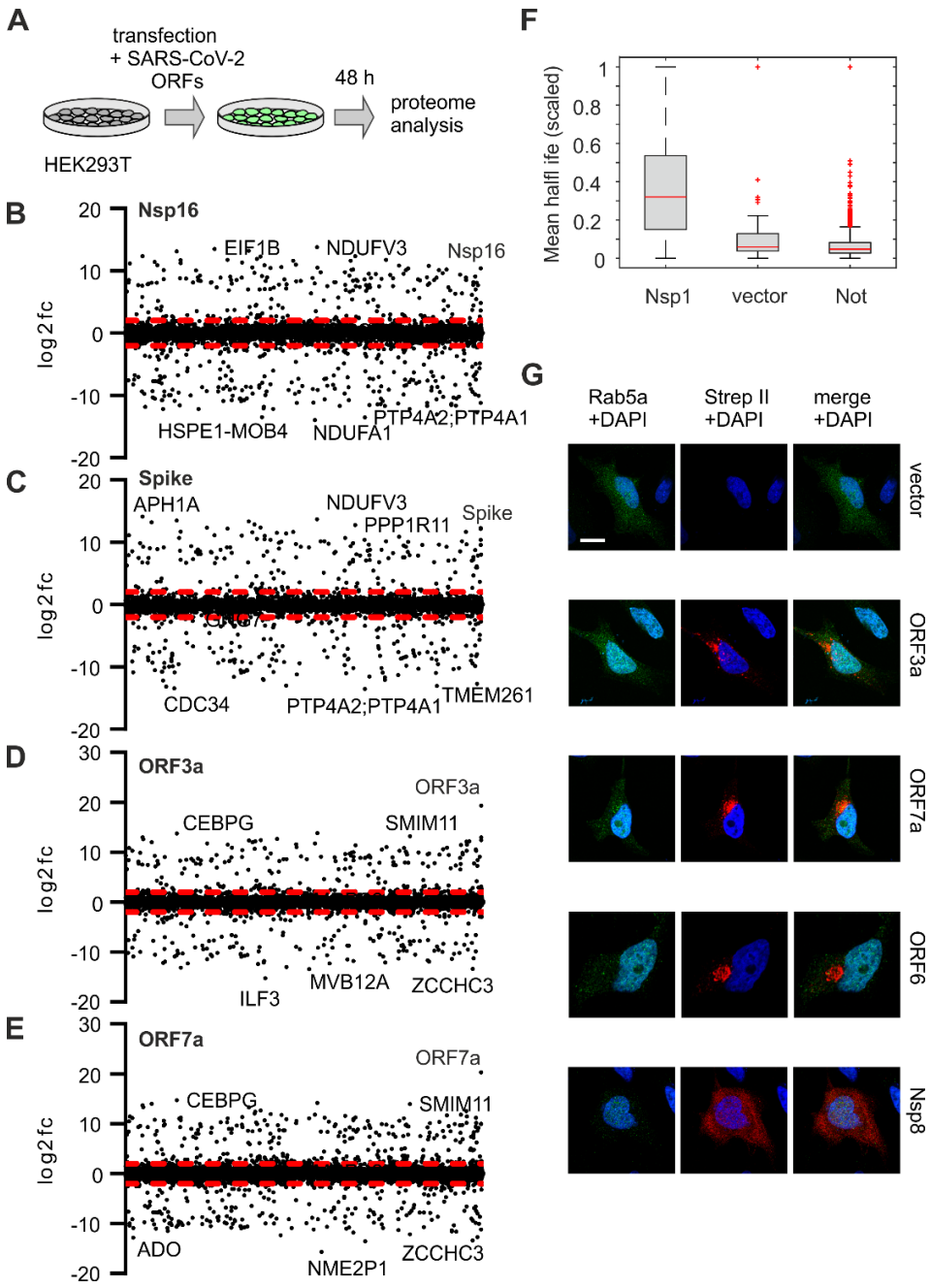

Figure S3. Proteome analysis and localisation of ORF3a and ORF7a. A, Schematic depiction illustrating transient transfection of HEK293T cells with SARS-CoV-2 expression constructs for proteome analysis. **B – E,** Scatter plots of log2 fold changes in expression of proteins in HEK293T cells expressing the indicated SARS-CoV-2 ORFs. Red lines, 4-fold changes. **F,** Box and whisker plot showing the half-life of the proteins regulated by overexpression of Nsp1 or the respective controls. **G,** Exemplary confocal laser scanning microscopy images of HeLa cells transiently transfected with FLAG-Rab5α and the indicate Strep II-tagged viral proteins. Cells were stained with anti-FLAG (green), anti-Strep II (red). DAPI, nuclei (blue). Scale bar, 10 µm. Related to Figure 4.

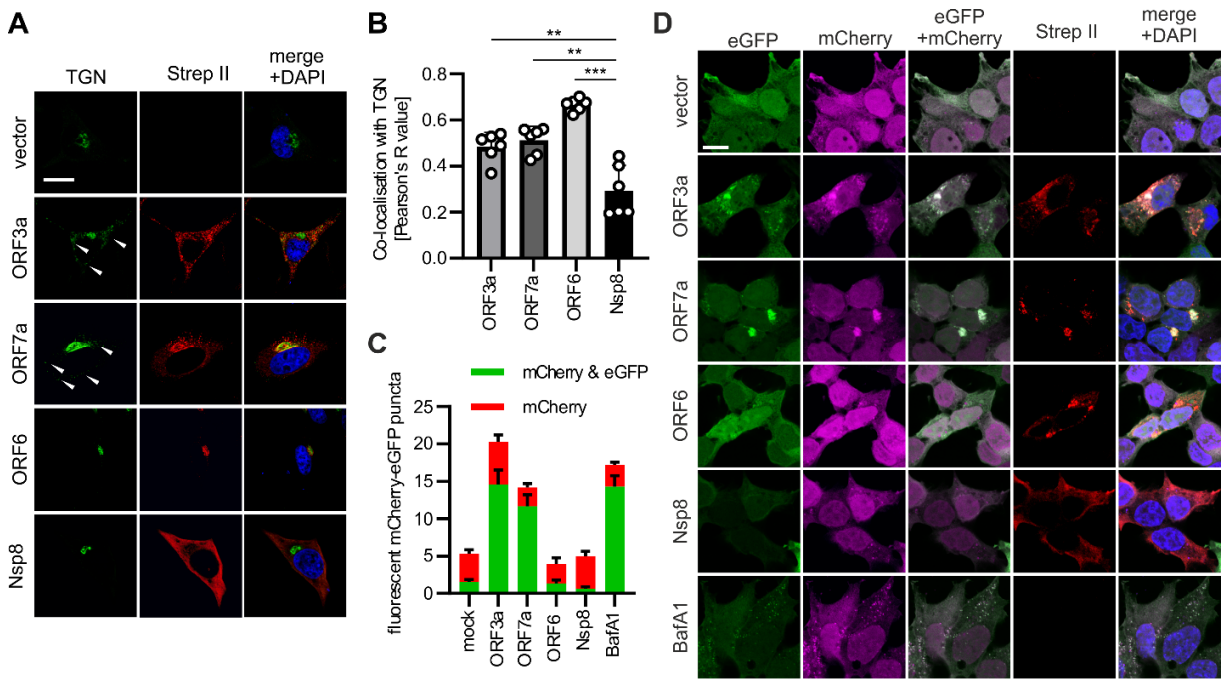

Figure S4. Co-localisation of SARS-CoV-2 proteins with the TGN and impact on mCherry-eGFP-LC3B processing. A, Exemplary confocal microscopy images of HeLa cells transfected with indicated expression vectors and stained with anti-TGN46 (green) and anti-Strep II (red). Nuclei, DAPI (blue). Scale bar, 10 µm. **B**, Pearson's correlation indicating co-localization between TGN46 and the indicated viral proteins from the image in (A). Bars represent the mean of n=6±SEM (individual cells). **C**, Distribution of eGFP and mCherry or mCherry puncta in mCherry-eGFP- LC3B expressing HEK293T cells transiently transfected with the indicated expression constructs. Bars represent the mean of n=12-100±SEM (individual cells). **D**, Exemplary confocal microscopy images of mCherry (magenta)-eGFP (green)- LC3B expressing cells, stained with anti-Strep II (red). Nuclei, DAPI (blue). Scale bar, 10 µm. Related to Figure 4.

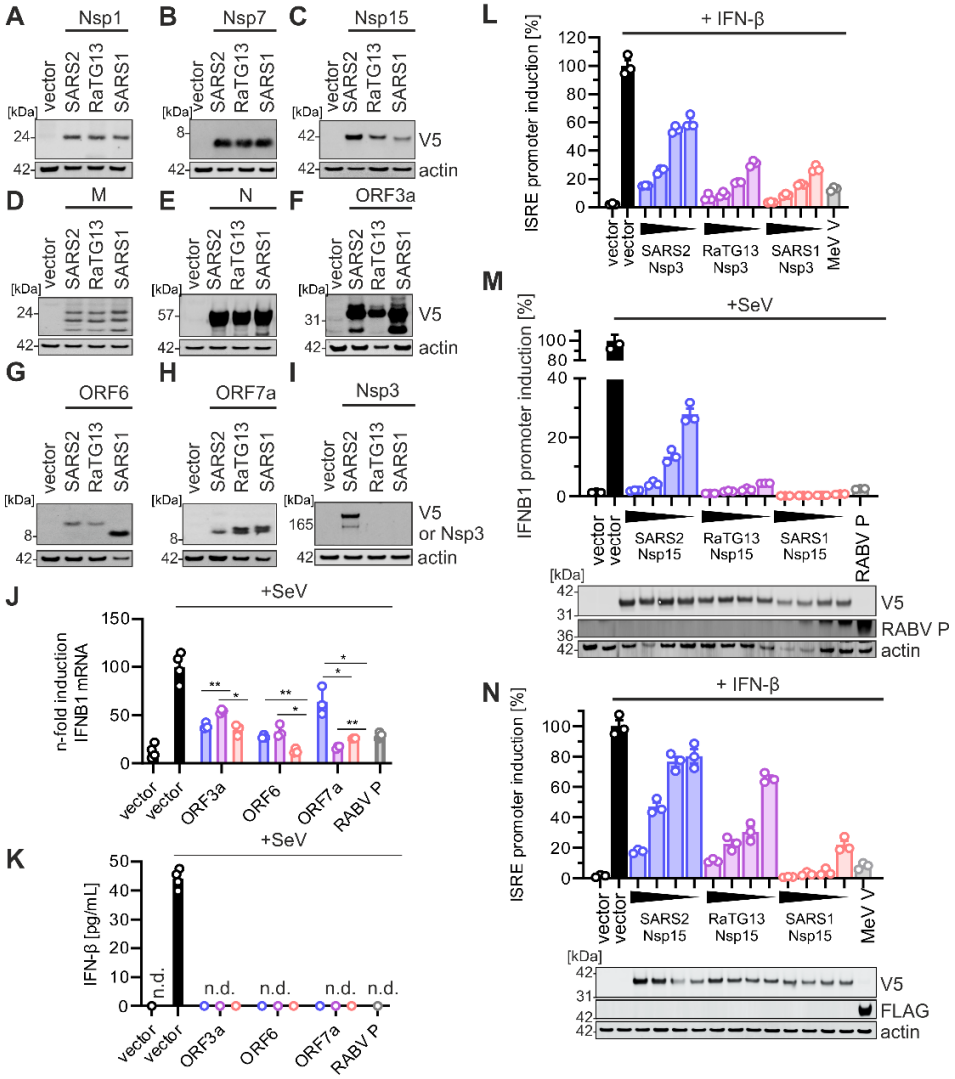

Figure S5. Expression of SARS-CoV-2, RaTG-13-CoV and SARS-CoV-1 constructs and functional comparison. A – I, Immunoblots of WCLs of HEK293T cells transiently transfected with indicated SARS-CoV-2, RaTG13-CoV or SARS-CoV-1 expression plasmids and stained with anti-V5 (A-H) and anti-actin antibodies or anti SARS-CoV-2 Nsp3 (I). **J**, Quantitative real-time PCR analysis of *IFNB1* mRNA levels in HEK293T cells expressing indicated proteins, normalized to GAPDH mRNA. Samples stimulated with Sendai Virus are indicated. Vector transfected samples were set to 100%. Bars represent the mean of n=3-4±SEM (biological replicates). **K**, Legendplex ELISA analysis of IFN-β levels (1:5 dilution) in the supernatant of HEK293T cells expressing the indicated proteins 24 h post infection. Bars represent the mean of n=3-4±SEM (biological replicates). n.d, not detectable. **L,** pISRE Firefly luciferase promotor induction in IFN-β stimulated HEK293T cells previously transfected with the indicated amounts of NSP3 of SARS-CoV-2, RaTG13-CoV or SARS-CoV-1. Firefly luciferase activities were quantified as RLU/s and normalized to cell metabolic activity (CellTiter Glo); stimulated vector control was set to 100%. FLAG-tagged MeV V was used as a control. Bars represent the mean values of n=3±SEM (biological replicates). **M,** Comparison of IFN-β antagonism of the V5-tagged NSP15 protein of SARS-CoV-2, RaTG13-CoV and SARS-CoV-1 as described in (J). RABV P was used as a control. Immunoblots of WCLs stained with anti-V5, anti-RABV P, anti-actin. **N,** Comparison of IFN-β antagonism of the V5-tagged NSP15 protein of SARS-CoV-2, RaTG13-CoV and SARS-CoV-1 as described in (J). FLAG-tagged MeV V was used as a control. Immunoblots of WCLs stained with anti-V5, anti-FLAG, anti-actin. Related to Figure 5.

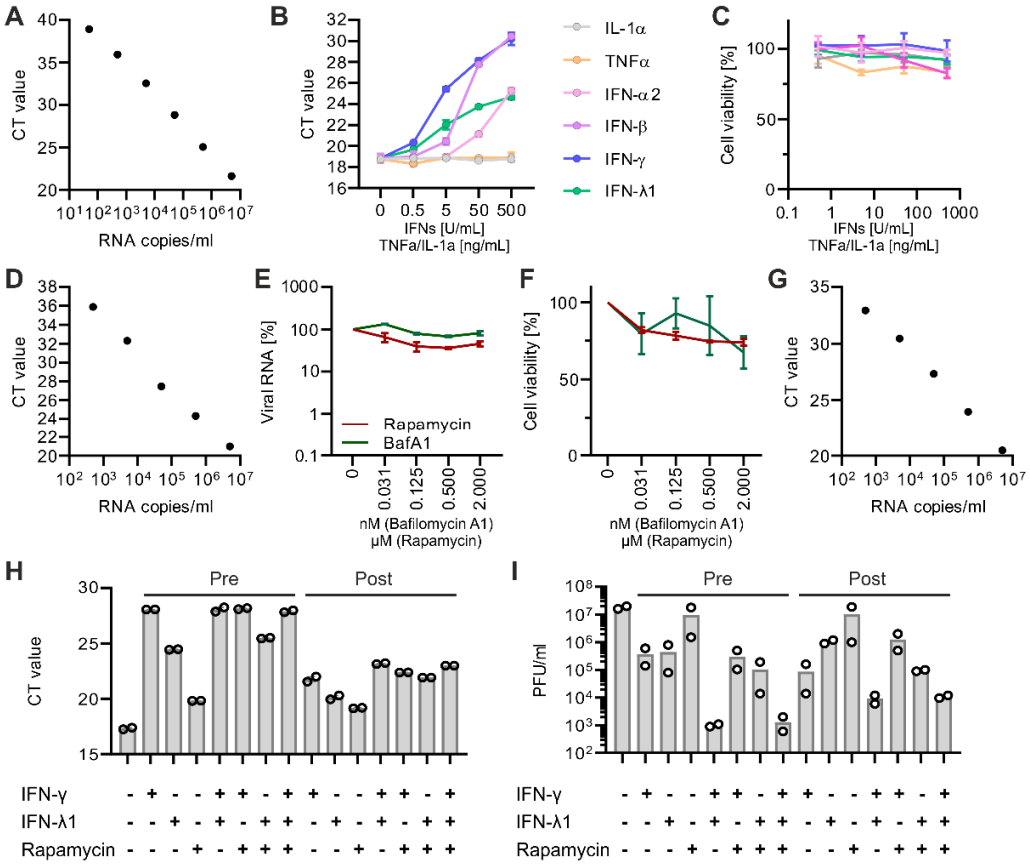

Figure S6. Impact of IFN and autophagy activation on SARS-CoV-2 replication. A, Exemplary standard curve of raw qRT-PCR CT values corresponding to the SARS-CoV-2 N RNA copy numbers per ml supernatant for one replicate. **B,** Effect of indicated IFNs or pro-inflammatory cytokines in different concentrations on SARS-CoV-2 replication in Calu-3 cells. Shown are raw CT values of one replicate, n=3±SD (technical replicates). **C,** Effect of cytokine treatment on cell viability of Calu-3 cells treated as in A, as assessed by intracellular ATP levels. Lines represent the mean of n=3±SEM (biological replicates). **D,** Exemplary standard curve of raw qRT-PCR CT values corresponding to the SARS-CoV-2 RNA copy numbers per ml for one replicate. **E,** SARS-CoV-2 N RNA in the supernatant of SARS-CoV-2 (MOI 0.05, 48h p.i.) infected Calu-3 cells that were left untreated and/or were treated with the indicated amounts of Rapamycin or Bafilomycin A1 (BafA1). Lines represent the mean of n=3±SD. **F,** Effect of autophagy modulating drugs on cell viability of Calu-3 cells treated as indicated, as assessed by intracellular ATP levels. Lines represent the mean of n=3±SEM (biological replicates). **G,** Exemplary standard curve of raw qRT-PCR CT values corresponding to the SARS-CoV-2 RNA copy numbers per ml for one replicate. **H,** Exemplary CT values of N RNA in the supernatant of SARS-CoV-2 infected Calu-3 cells treated as indicated n=2 (technical replicates) for one replicate. Treatment 24 h before infection (Pre), treatments 6 h post infection (Post). **I**, Quantification of plaques on Vero cells infected with serial dilutions of the supernatant from Figure S6H/6D. Bars represent the mean of n=2 (biological replicates). Related to Figure 6.