iScience, Volume *26*

Supplemental information

Endogenous IFITMs boost SARS-coronavirus 1 and 2 replication whereas overexpression inhibits infection by relocalizing ACE2

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SUPPLEMENTAL FIGURES

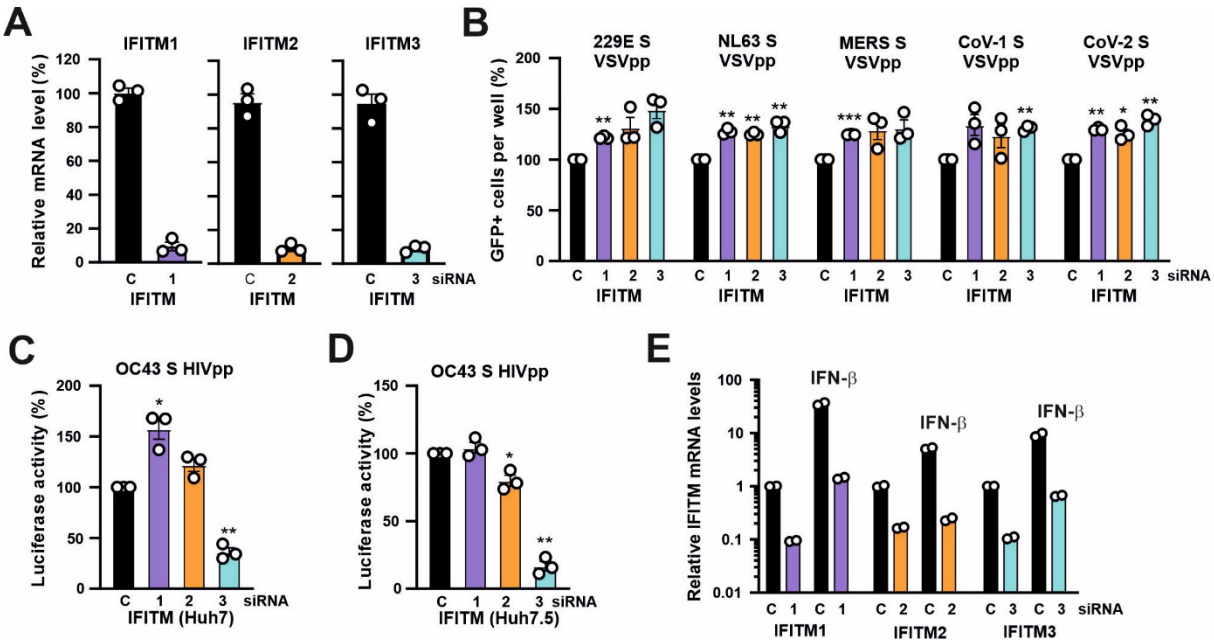

Figure S1 (related to Figure 2). Effect of IFITM siRNA silencing on infection by S-containing viral pseudo-particles.

(**A**) Levels of IFITM mRNAs in Huh7 cells transfected with non-targeting siRNA or siRNAs targeting the indicated IFITMs. Levels of the respective IFITM mRNAs are shown relative to those measured in in cells treated the control siRNA (100%). Bars in panels A-D represent the mean of three independent experiments (±SEM). *, $p<0.05$; **; $p<0.01$; ***, $p<0.001$. (**B**) Huh7 cells were transfected with non-targeting siRNA or siRNAs targeting the indicated IFITMs and infected with 229E, NL63, MERS, CoV-1 or CoV-2 S-containing VSVpp at the next day. Cytation for GFP+ cells was performed 24h post-infection. (**C**) Huh7 or (**D**) Huh7.5 cells were transfected with the indicate siRNAs, infected with OC43 S HIVpp on the following day and luciferase activities were measured 48h post-infection. (**E**) Levels of IFITM mRNAs in Huh7 cells that were left untreated or treated with IFN-β and transfected with non-targeting siRNA or siRNAs targeting the indicated IFITMs. Levels of the respective IFITM mRNAs are shown relative to those measured in the absence of IFN-β in cells treated the control siRNA (100%).

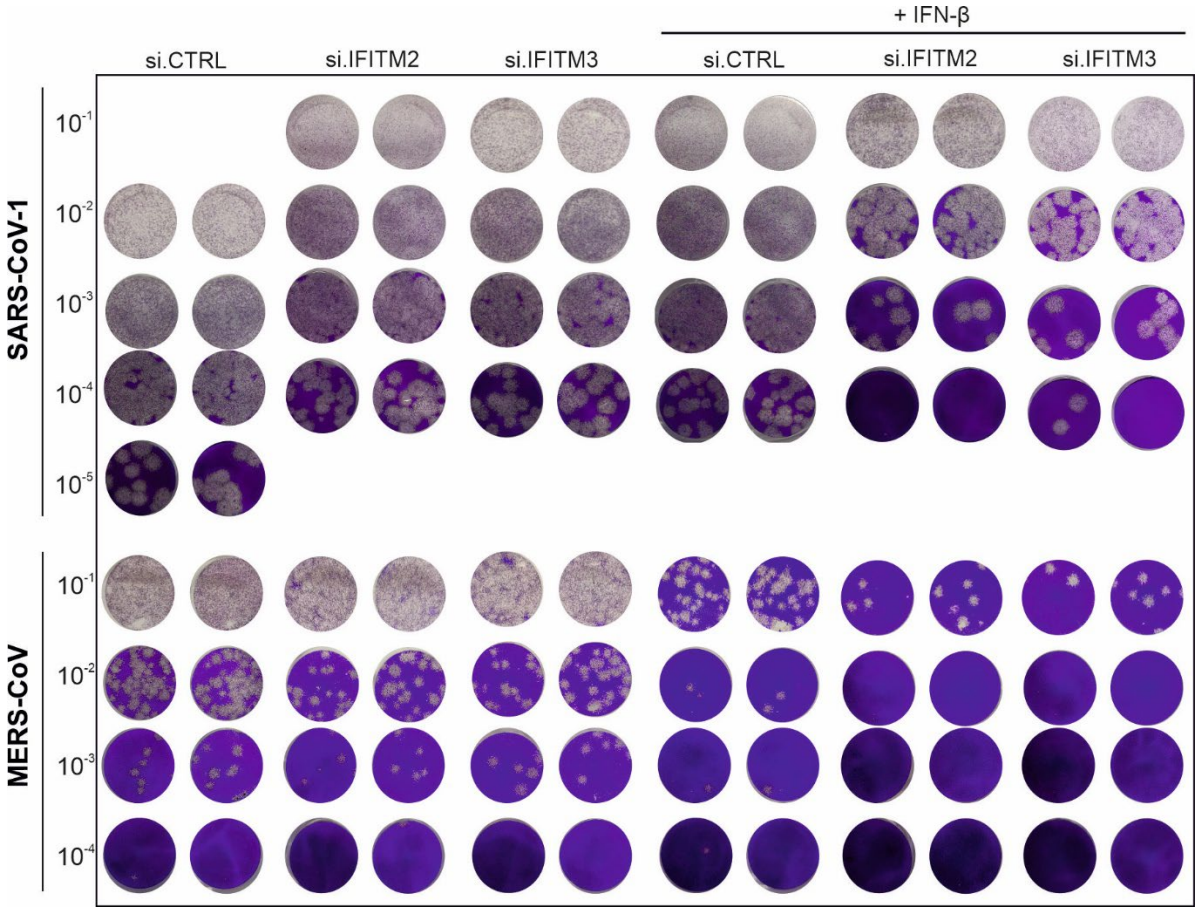

Figure S2 (related to Figure 4). Impact of endogenous IFIM2 or IFITM3 expression on production of infectious SARS-CoV-1 or MERS-CoV particles.

Shown are primary data of plaque-forming unit assays using the supernatants of the experiment shown in Figure 4D and 4E.

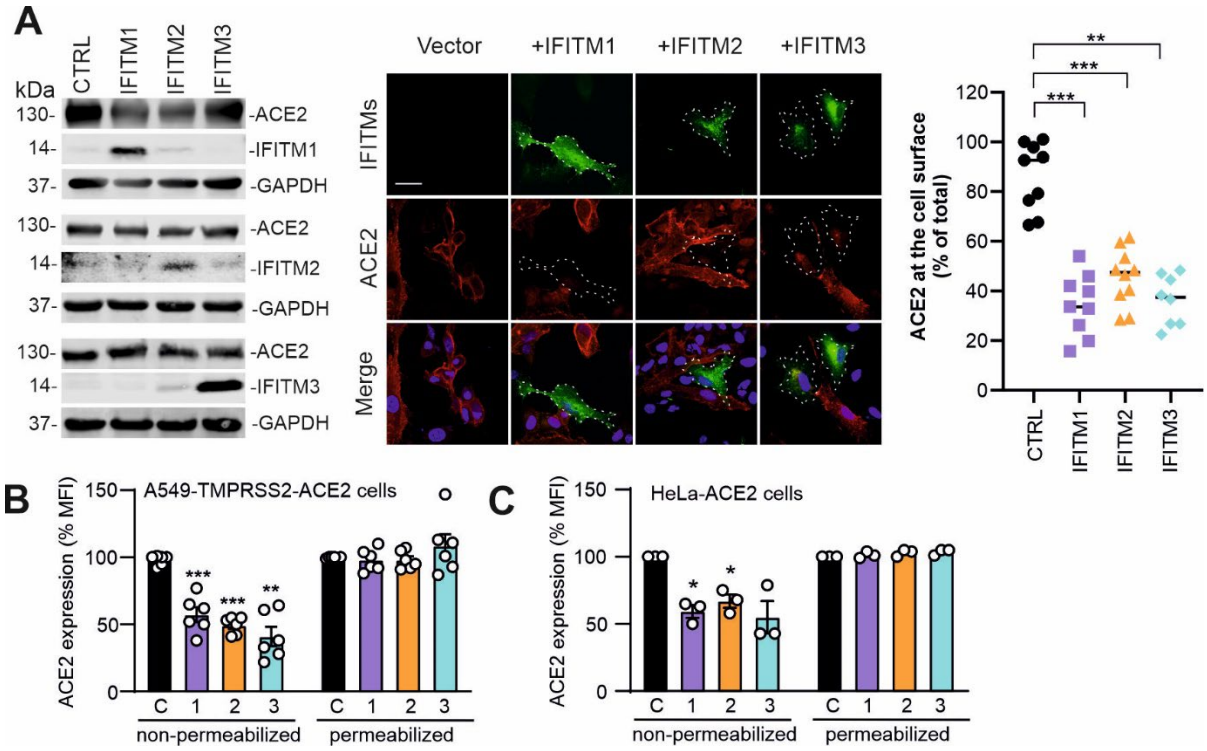

Figure S3 (related to Figure 6). IFITM overexpression impairs cell surface expression of ACE2. (**A**) A549-TMPRSS2-ACE2 were transfected with either IFITM1, IFITM2 or IFITM3 expression constructs or an empty control vector. Forty-eight hours post-transfection cells were harvested for Western blot analysis (left) or stained with anti-ACE2 and examined by confocal microscopy (middle). ACE2 signal intensities at the cell surface and in the cytoplasm were quantified using Image J (right). (**B, C**) A549-TMPRSS2-ACE2 cells (B) of Hela stably expressing ACE2 (C) were transfected with either IFITM1, IFITM2 or IFITM3 expression constructs. 48 h post-transfection, cells were permeabilized (left panels) or not (right panels), stained with anti-ACE2 antibody and analyzed by flow cytometry. Shown are mean fluorescence intensities (MFIs) measured in cells transfected with IFITM expression vectors relative to those that received the control construct (100%). Bars represent the mean of three independent experiments (±SEM), measured in duplicates (B), *, $p<0.05$; **, $p<0.01$; ***$p<0.001$.