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Supplemental Information

Critical Role of Type III Interferon in Controlling SARS-CoV-2 Infection in Human Intestinal Epithelial Cells

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Supplementary Figure legends

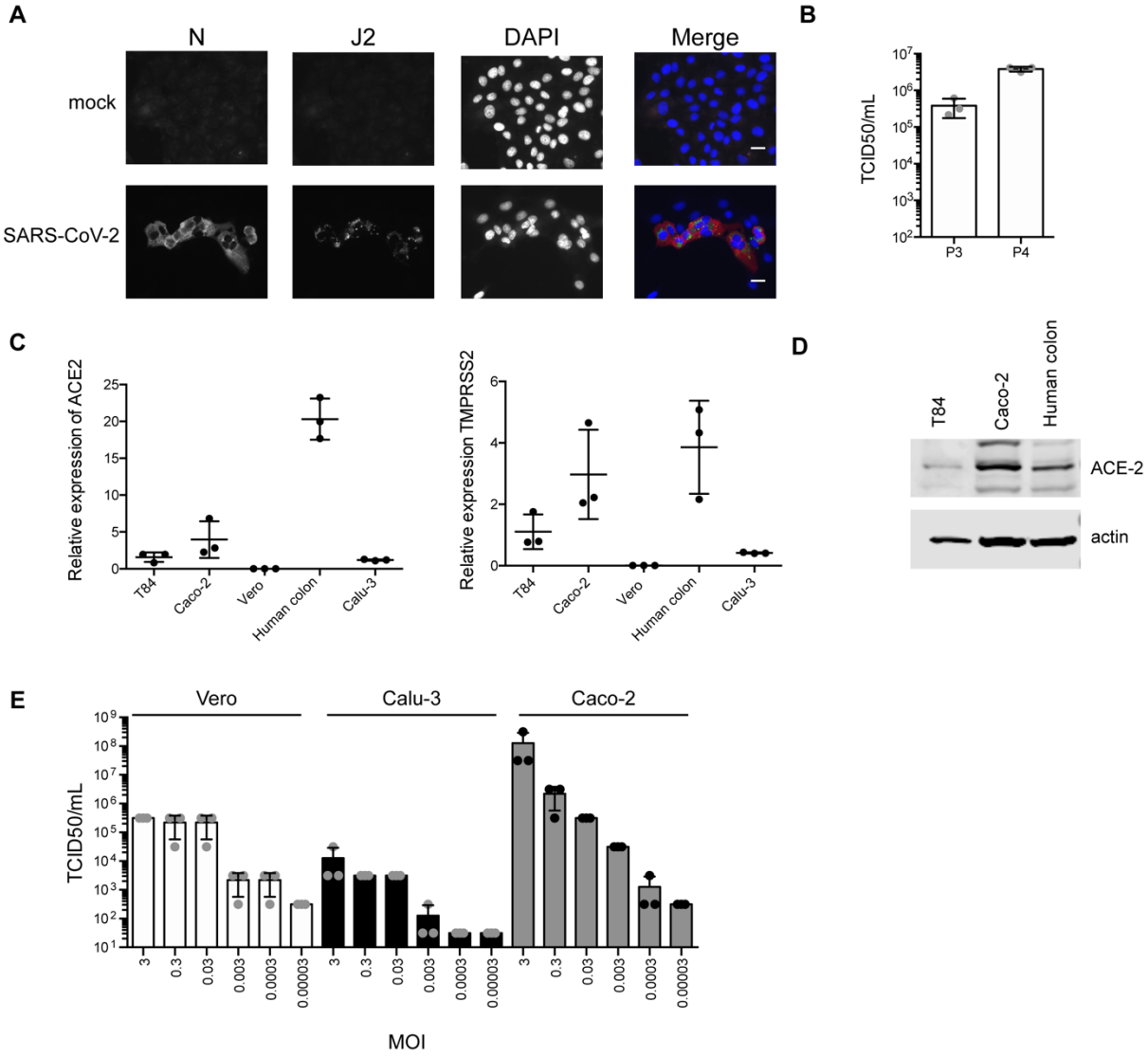

Figure S1. Related to Figure 1: Vero cells support SARS-CoV-2 infection. (A) Vero cells were infected with SARS-CoV-2 and 24 hpi viral replication was evaluated by indirect immunofluorescence for the viral N protein (red) and dsRNA (green). Nuclei were stained with DAPI (blue). Three biological replicates were performed, representative images are shown. Scale bars=10um. Related to Fig.1. (B) Viral titers from the passage P3 and P4 stocks was determined by TCID50. N=3 biological replicates. Error bars indicate standard deviation. Related to Fig.1. (C) q-RT-PCR analysis of the levels of ACE-2 and TMPRSS2 in human colon carcinoma cells T84, and Caco-2 cells, Vero E6 cells, human lung adenocarcinoma Calu-3 cells and human colon organoids. Expression is normalized to housekeeping gene TBP. N=3 biological replicates. Related to Fig.2 and Fig.4. (D) Western blot of ACE-2 from T84, Caco-2 and human colon organoids. Actin is used as a loading control. Three biological replicates were performed; representative image is shown. Related to Fig.2 and Fig.4. (E) Vero E6, Calu-3 and Caco-2 cells were infected with SARS-CoV-2 virus at a MOI 3, 0.3, 0.03, 0.003, 0.0003, and 0.00003. 24 hours post-infection supernatants were collected, and the amount of *de-novo* infectious viruses produced was quantitated on naïve Vero E6 cells. Related to Fig.1. N=3 biological replicates. Error bars indicate standard deviation.

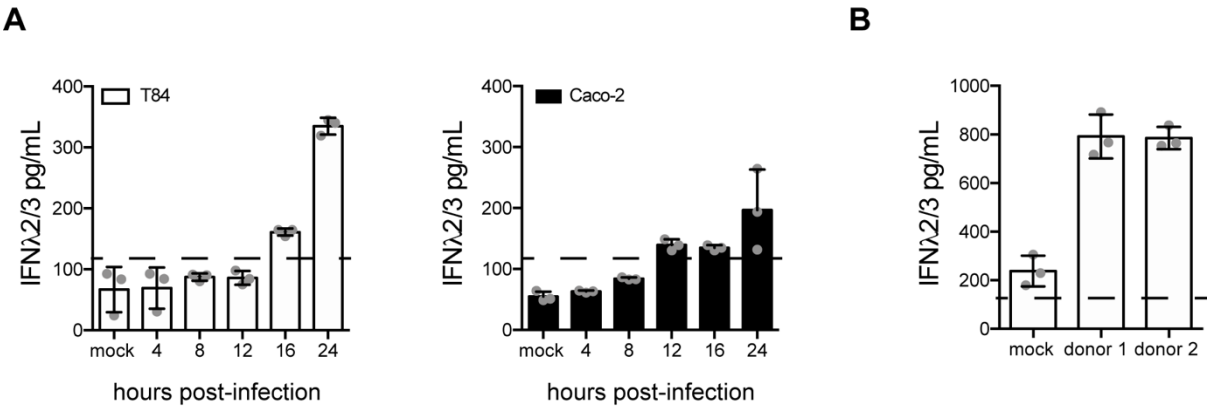

Figure S2. Related to Figure 2 and 4. Human intestinal epithelial cells secrete type III IFN. (A) At indicated time points supernatants were collected from infected T84 and Caco-2 cells (Related to Fig.2). The amount of IFNλ2/3 present in the supernatants was determined by ELISA. N=3 biological replicates. (B) Same as A except two colon organoid donors were tested 24 hour post-infection. (Related to Fig. 4) (A-B) N=3 biological replicates. Error bars indicate standard deviation. Dotted line indicates limit of detection in assay.

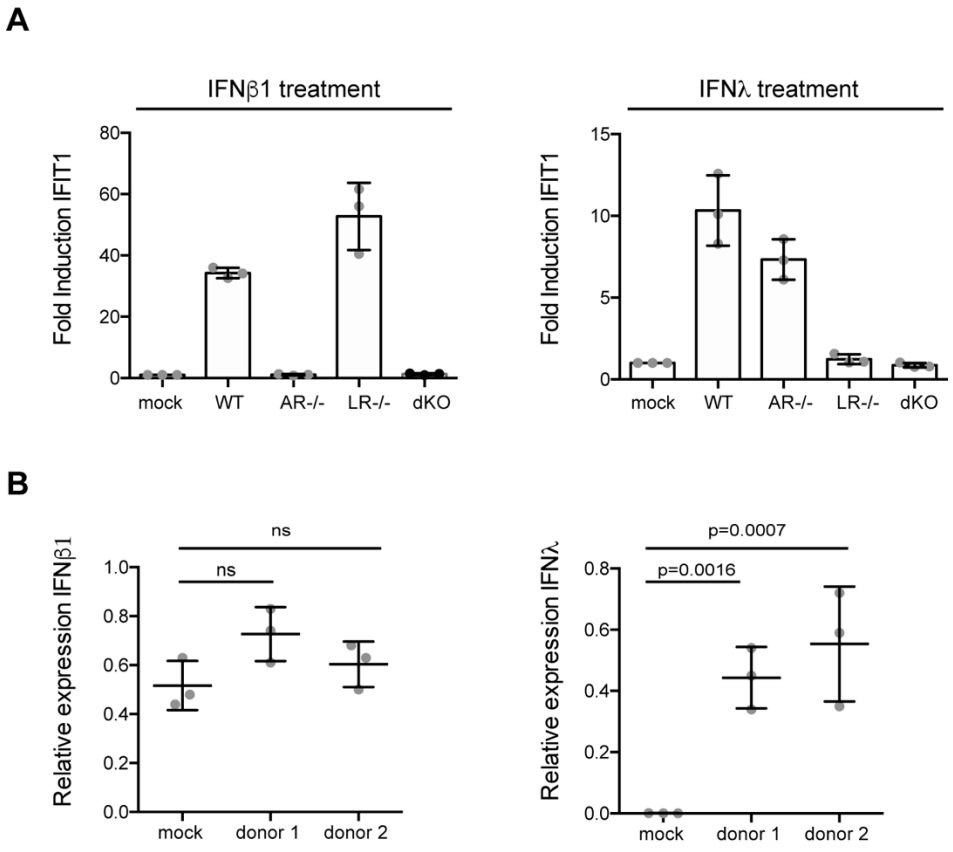

Figure S3. Related to Figure 3 and 4. Upregulation of IFIT1 in T84 wild and IFN receptor knock-out cells and Normalized fold of IFNβ1 and IFNλ 2/3induction in colon organoids. (A) Wild type T84 cells and T84 cells depleted of the type I IFN receptor (AR-/-), the type III IFN receptor (LR-/-) or both (dKO) from Fig. 3 were treated with IFNβ1 and IFNλ 2/3 24hrs post-treatment, RNA was harvested and the upregulation of IFIT1 was evaluated by q-RT-PCR. N=3 biological replicates. Error bars indicate standard deviation. **(B)** Colon organoids were infected with SARS-CoV-2. 24 hpi the upregulation of IFNβ1 and IFNλ 2/3 was evaluated by q-RT-PCR. N=3 biological replicates. Same data as Fig. 4G but values are expressed as normalized to the TBP housekeeping gene. Error bars indicate standard deviation. P values were determined by unpaired t-test.

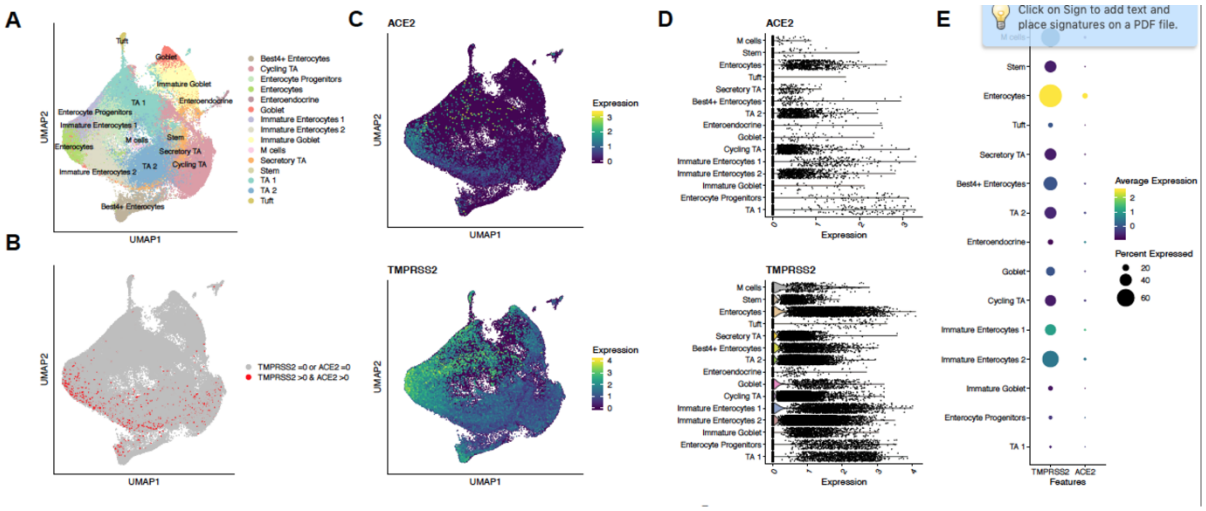

Figure S4. Related to Figure 4. Expression of ACE2 and TMPRSS2 in Human Colon Epithelial cells. (A) Uniform manifold approximation and projection (UMAP) of 99,107 cells from (Smillie et al., 2019) colored by their cell type. (B) UMAP visualization, cells co-expressing ACE2 and TMPRSS2 are highlighted. (C) UMAP visualization, colored by the normalized expression of ACE2 and TMPRSS2. (D) Expression values of ACE2 and TMPRSS2 in the epithelial cell types of the colon. (E) Dot plot of ACE2 and TMPRSS2 for each cell type. Dot size represents percentage of cells expressing the gene, and colour intensity average expression across the cell type.

Compound	*Final concentration*
Basal media	
Ad DMEM/F12 +GlutaMAX +HEPES +P/S	
L-WRN	50% by volume
B27	1:50
N-acetyl-cysteine	1mM
EGF	50ng/mL
A83-01	500nM
IGF-1	100ng/mL
FGF basic	50ng/mL
Gastrin	10mM
Differentiation Media	
Ad DMEM/F12 +GlutaMAX +HEPES +P/S	
B27	1:50
N-acetyl-cysteine	1mM
R-spondin	5% by volume
Noggin	50ng/mL
EGF	50ng/mL
Gastrin	10mM
A83-01	500nM

Supplementary Table 1. Related to STAR Methods. Human organoid media composition.