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Supplemental information

ZBTB7A promotes virus-host homeostasis during human coronavirus 229E infection

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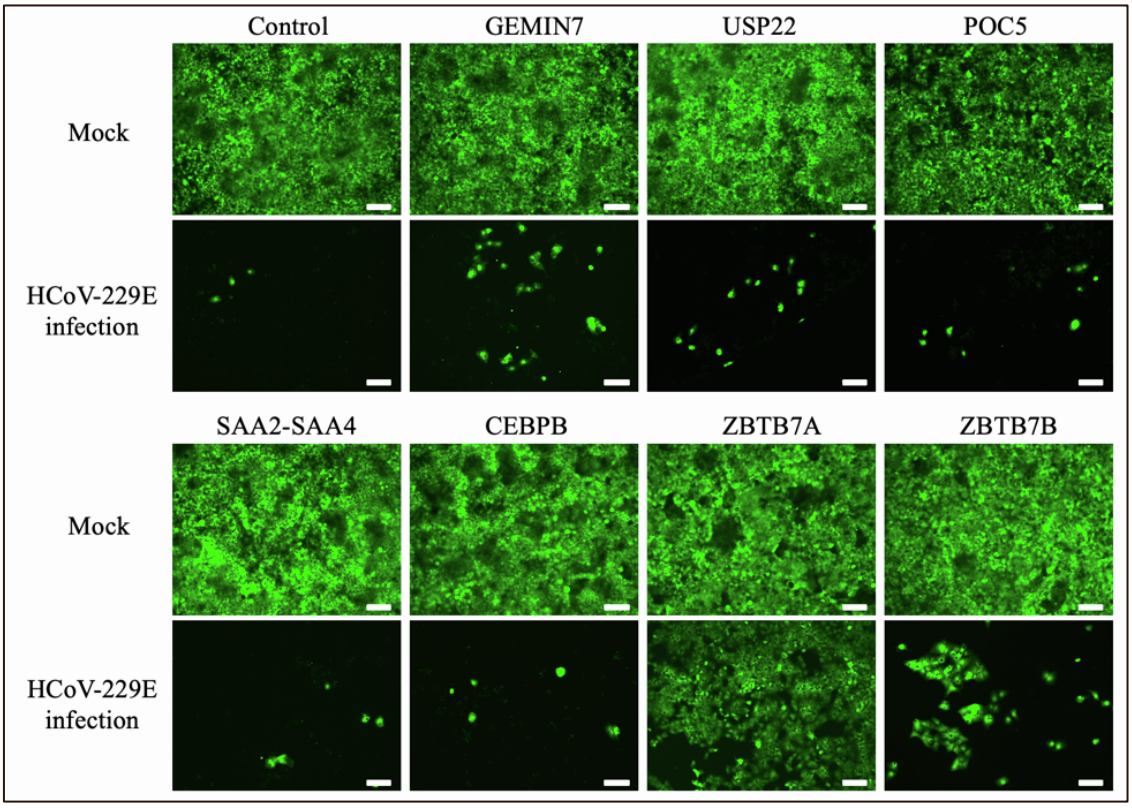

Fig. S1. Survival of Huh7 cells expressing seven candidate genes after HCoV-229E infection, related to Figure 1. Transduced cells were infected with HCoV-229E at 0.5 MOI. Live cells (green) were stained 5 DPI. Images are representative of two independent experiments. Scale bar=100 μm.

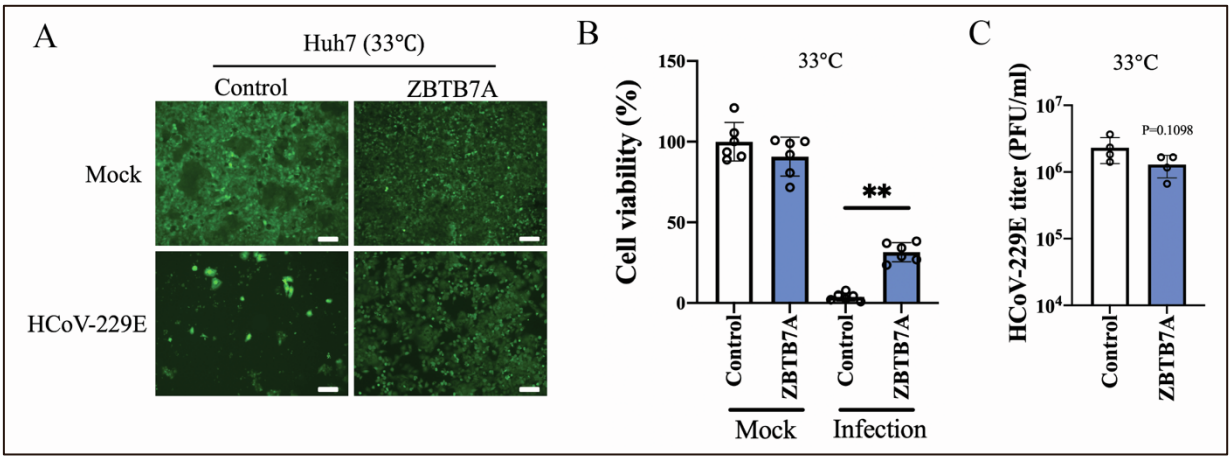

Fig. S2. ZBTB7A promotes cell survival during HCoV-229E infection at 33°C, related to Figure 2. (A) Transduced cells were infected with HCoV-229E at 0.5 MOI. Live cells (green) were stained 3 DPI. Scale bar=100 μm. (B) Cell viability of transduced Huh7 cells after infection. Transduced cells were infected as described in panel A. MTT assay was conducted 3 DPI. N=6 biological replicates. (C) Viral titer tested by plaque assay. The infection was conducted as described in panel A. Samples were collected at 2 DPI. N=4 biological replicates. *P* values were calculated by unpaired two-tailed Student's *t*-tests. For panel B, $P<0.001$, **. All panels are representative of two independent experiments.

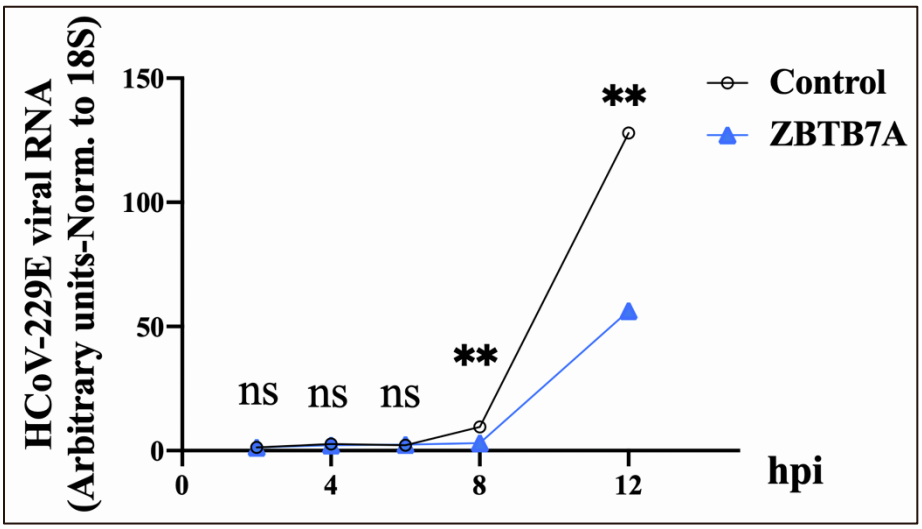

Fig. S3. ZBTB7A slightly affects HCoV-229E early infection, related to Figure 2. Huh7 cells were infected with HCoV-229E at 0.5 MOI. Samples were collected at indicated timepoint. N=4 biological replicates. *P* values were calculated by two-way analysis of variance (ANOVA). $P<0.05$, *; $P<0.001$, **; not significant, ns. Representative of two independent experiments.

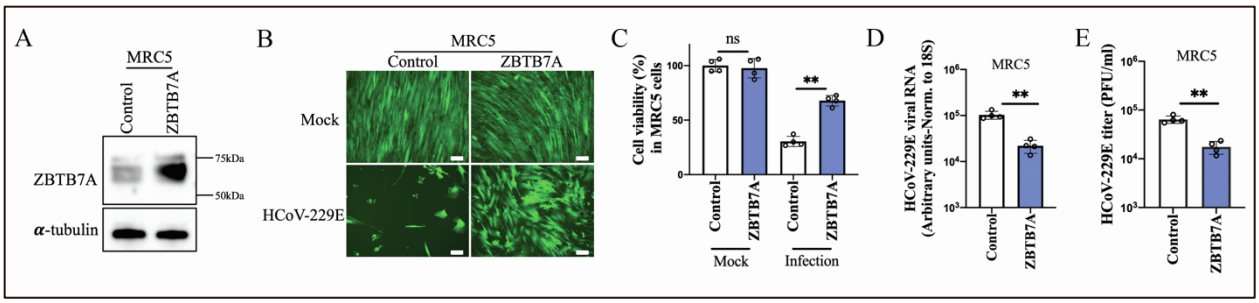

Fig. S4. ZBTB7A promotes survival of MRC5 cells after HCoV-229E infection, related to Figure 2. (A) ZBTB7A expression in transduced MRC5 cells. (B) MRC5 cells expressing ZBTB7A were infected with HCoV-229E at 1 MOI. Live cells were stained 5 DPI. Scale bar=100 μm. (C) Control-MRC5 cells and ZBTB7A-MRC5 cells were infected as described in panel B. MTT assays were conducted 5 DPI. N=4 biological replicates. (D) Viral RNA detected through qRT-PCR. The infection was conducted as described in panel B. Viral RNA was analyzed at 2 DPI. N=4 biological replicates. (E) Viral titer tested by plaque assay. The infection was conducted as described in panel B. N=4 biological replicates. All panels are representative of two independent experiments. *P* values were calculated by unpaired two-tailed Student's *t*-tests. For all panels, $P<0.05$, *; $P<0.001$, **; not significant, ns.

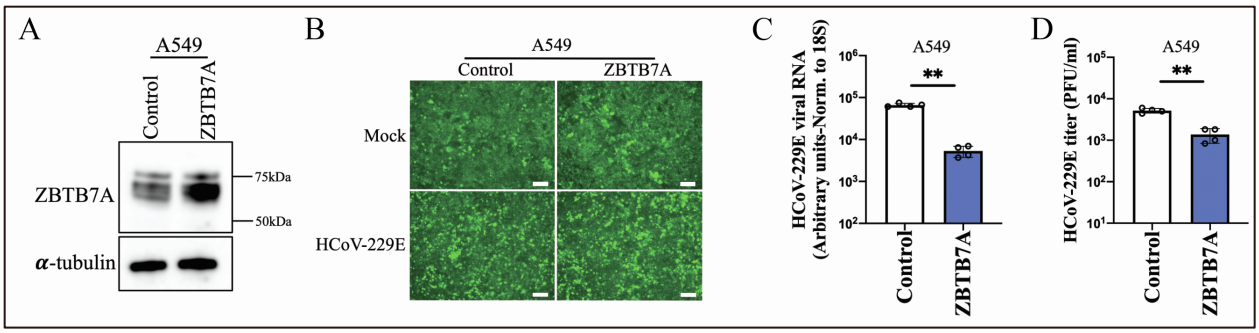

Fig. S5. ZBTB7A expression slightly inhibits HCoV-229E infection in A549 cells, related to Figure 2. (A) ZBTB7A expression in transduced A549 cells. (B) Control-A549 cells and ZBTB7A-A549 cells were infected with HCoV-229E at 0.5 MOI. Live cells were stained 5 DPI. Scale bar=100 μm. (C) Viral RNA detected through qRT-PCR at 2 DPI. Infection was conducted as described in panel B. N=4 biological replicates. $P<0.001$, **. (D) Viral titer was tested by plaque assay. Infection was conducted as described in panel B. N=4 biological replicates. *P* values were calculated by unpaired two-tailed Student's *t*-tests. $P<0.001$, **. All panels are representative of two independent experiments.

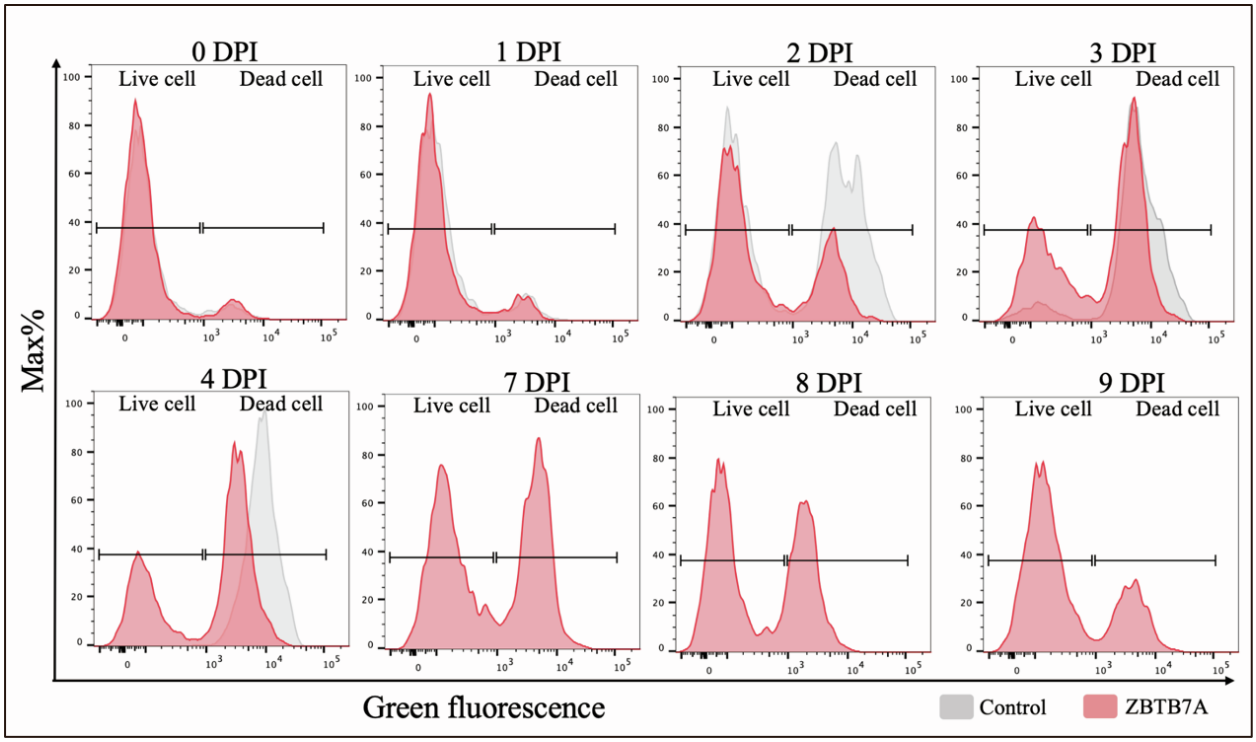

Fig. S6. Live and dead cells assay during HCoV-229E infection, related to Figure 2. Control-Huh7 cells and ZBTB7A-Huh7 cells were infected at 1 MOI. Live and dead cells were stained and analyzed by flow cytometry at the indicated time points. Representative of two independent experiments.

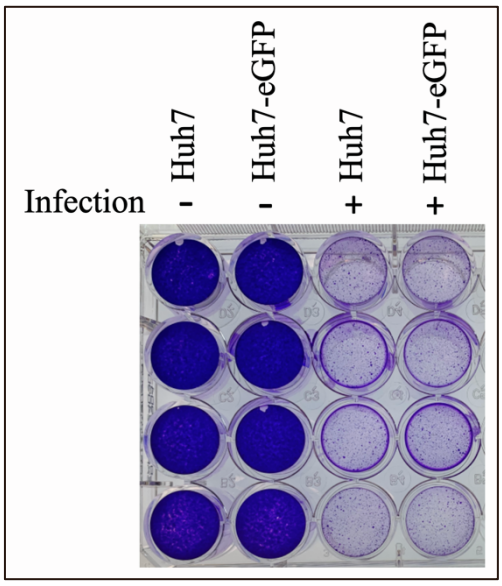

Fig. S7. Crystal violet staining of eGFP transduced Huh7 cells after infection, related to Figure 2. Huh7 cells expressing eGFP were infected with HCoV-229E at 0.5 MOI. The cells were stained 3 DPI. Representative of two independent experiments.

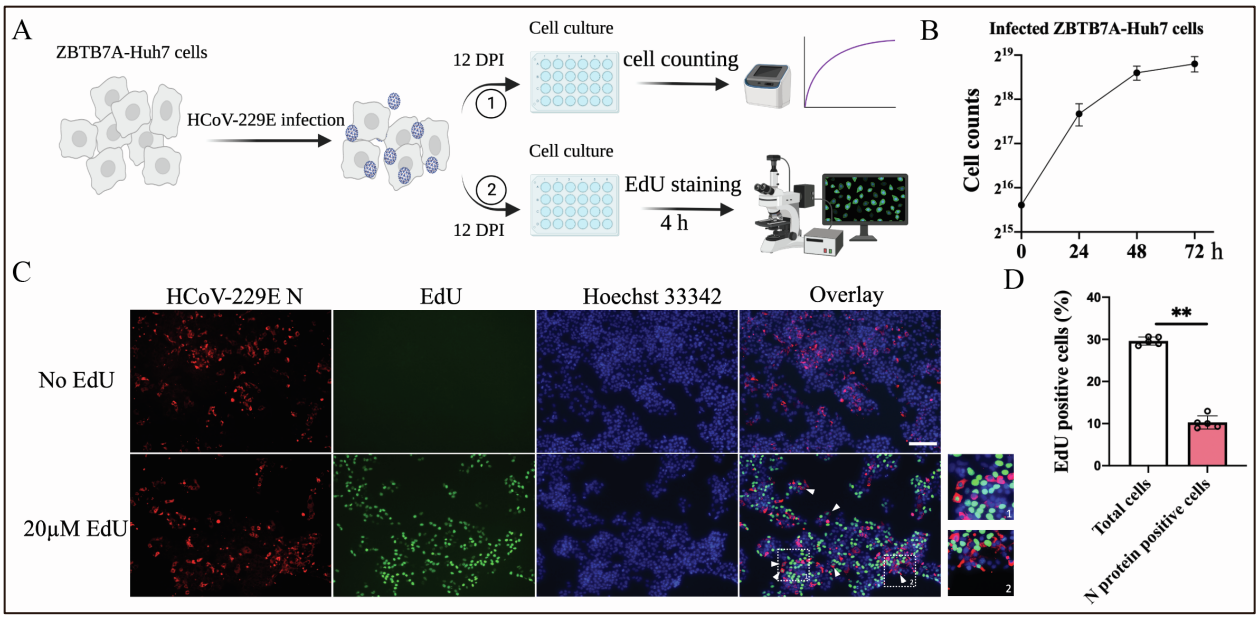

Fig. S8. The proliferation of infected ZBTB7A-Huh7 cells, related to Figure 2. (A) Overview of proliferation analysis for infected ZBTB7A-Huh7 cells. ZBTB7A-Huh7 cells were infected with HCoV-229E at 0.5 MOI. The persistently infected ZBTB7A-Huh7 cells at 12 DPI were seeded in 24-well plate. Cell counts were calculated by automated cell counter to present cell proliferation curve in the time course. In addition, the infected ZBTB7A-Huh7 cells were incubated with EdU for 4 h and further analyzed by fluorescence microscopy. (B) The infected ZBTB7A-Huh7 cells (12 DPI) were cultured and stained with trypan blue solution at indicated timepoint. Cell counts were calculated by automated cell counter. (C) The infected ZBTB7A-Huh7 cells (12 DPI) were treated with 20μM EdU for 4 h. HCoV-229E N and EdU were stained. Images representative of N=5 biological replicates. Scale bars=100 μm. (D) EdU positive cells were calculated from the experiment shown in panel C via Image J. N=5 biological replicates. *P* values were calculated by unpaired two-tailed Student's *t*-tests. $P<0.001$, **. All panels are representative of two independent experiments.

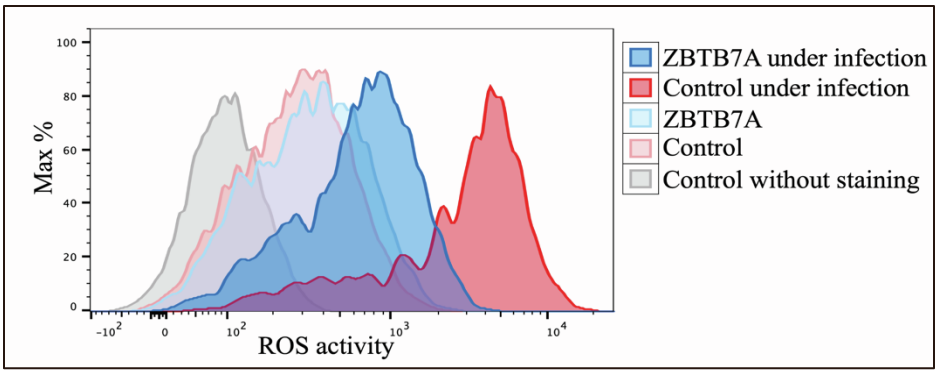

Fig. S9. ROS activity in Huh7 cells during infection, related to Figure 4. ZBTB7A-Huh7 cells and control-Huh7 cells were infected with HCoV-229E at 0.5 MOI. Samples were stained at 2 DPI and analyzed by flow cytometry. Representative of two independent experiments.

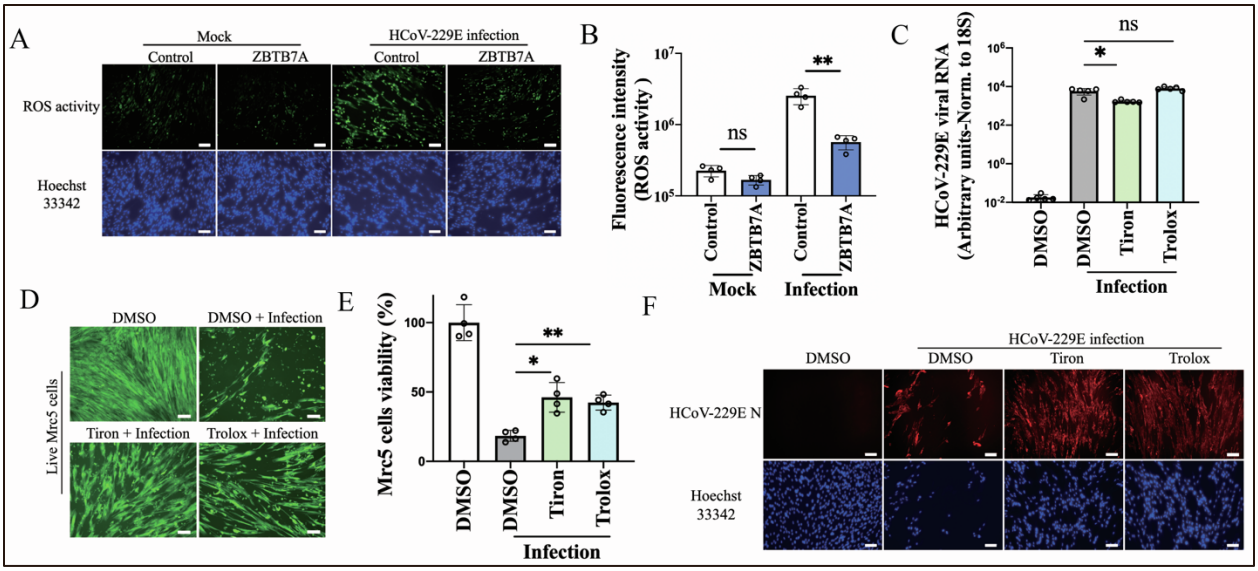

Fig. S10. ZBTB7A controls HCoV-229E-induced ROS activation in MRC5 cells, related to Figure 4. (A) ZBTB7A-MRC5 cells and control-MRC5 cells were infected with HCoV-229E at 0.5 MOI. ROS activity (green) was stained and imaged 2 DPI. (B) Fluorescence intensity of ROS activity in MRC5 cells calculated from panel A by Image J. N=4 biological replicates. (C) Viral RNA detection after superoxide scavenger treatment. MRC5 cells were infected with HCoV-229E at 0.5 MOI and treated with 10 mM tiron or 200 μM trolox were added at 2 hpi. Cells were collected at 2 DPI for qRT-PCR. N=5 biological replicates. (D) Surviving MRC5 cells imaging under tiron or trolox treatment. MRC5 cells were infected and treated as described in panel C. Surviving cells were stained 5 DPI. (E) MRC5 cells were treated as described in panel C and cell viability was determined by MTT assay and normalized to un-infected group. Value was N=4 biological replicates. (F) MRC5 cells were treated as described in panel C and HCoV-229E N was stained 5 DPI. All panels are representative of two independent experiments. For all panels, $P<0.05$, *; $P<0.001$, **; not significant, ns. Scale bar=100 μm.