Expanded View Figures

Figure EV1. ACE2 and IFITM expression in U2OS-ACE2 cell derivatives.

A ACE2 expression assessed by Western blot in U2OS-ACE2 GFP-split cells expressing or not IFITM1. Cell lysates were analyzed by for ACE2 and actin as a loading control.

B–E Analysis of ACE2 and IFITMs levels by flow cytometry. (B) Gating strategy. Cells were gated by size and granularity. Positive and negative gates were then set on control U2OS cells lacking the protein of interest. (C) ACE2 levels on parental U2OS (control) and various IFITM derivatives. (D) IFITM1 levels on parental U2OS (control) and U2OS-ACE2 IFITM1 cells. (E) IFITM2 and IFITM3 levels on parental U2OS (control) and U2OS-ACE2 IFITM2 and U2OS-ACE2 IFITM3 cells.

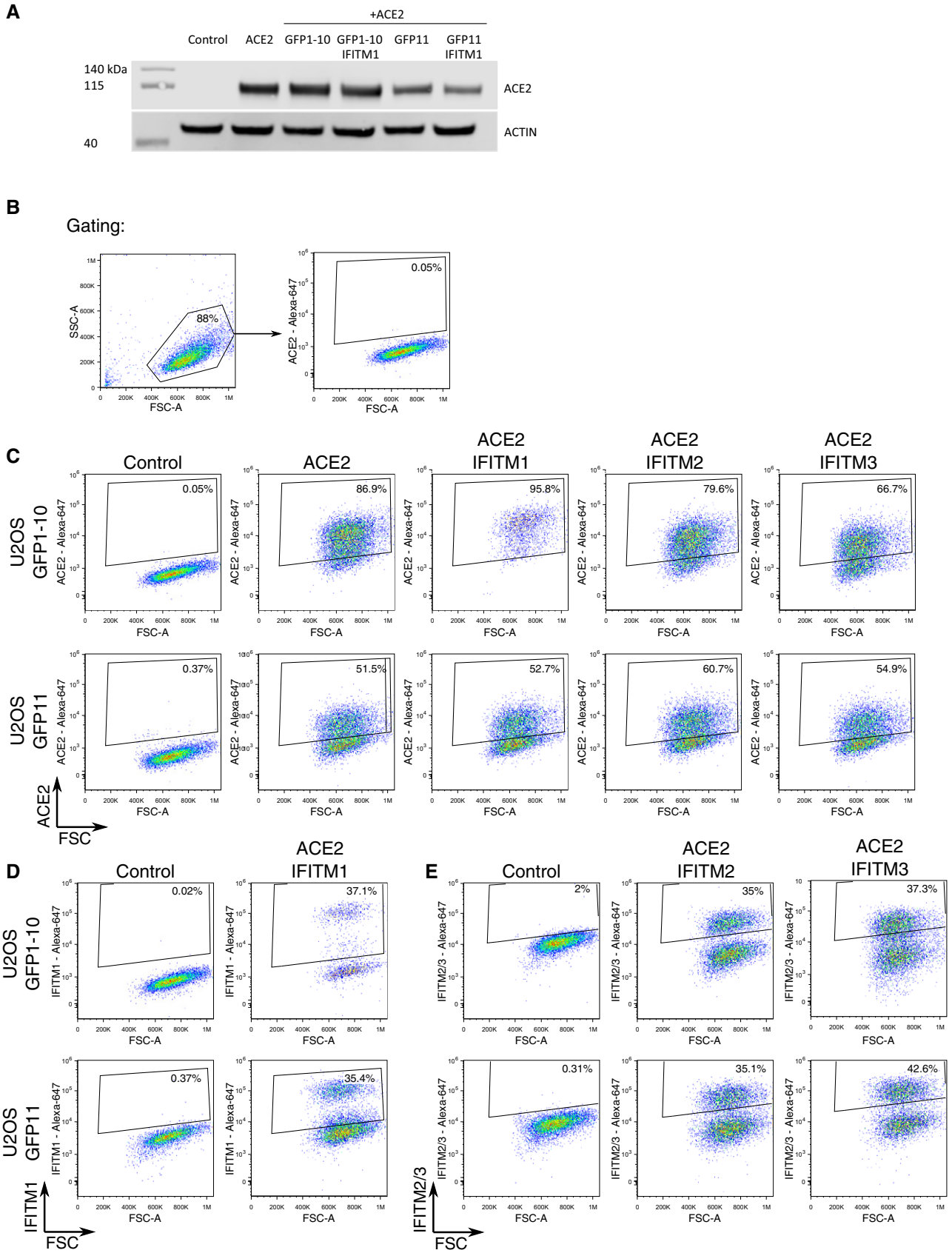

Figure EV1.

Figure EV2. Image quantification methodology.

A Quantification of fusion and viability. To measure the extent of cell–cell fusion, the GFP area was automatically delimited, measured, and then divided by the total cell area. For cell viability, nuclei were automatically counted, and the total number of nuclei per well was normalized to that of non-infected control cells.

B Quantification of syncytia expressing IFITMs. For IFITM-GFP overlap quantification, the $IFITM^+$ area was first selected on the 647 nm channel (image 2) and the GFP-positive area was quantified within the $IFITM^+$ area (image 3). Image 4 shows the overlap area in gray for simpler visualization.

C Quantification of infected cells expressing the S protein. The cells were stained with anti-S antibodies, and the nuclei were detected with Hoechst. The S^+ area was delimited (each selected object is pseudo-colored). Nuclei present within the S^+ area were scored and divided by the total number of nuclei, to calculate the number of infected cells per well.

Data information: Scale bars: 100 μm. The same field was used in this example for A and C, Hoechst images are therefore identical in A and C.

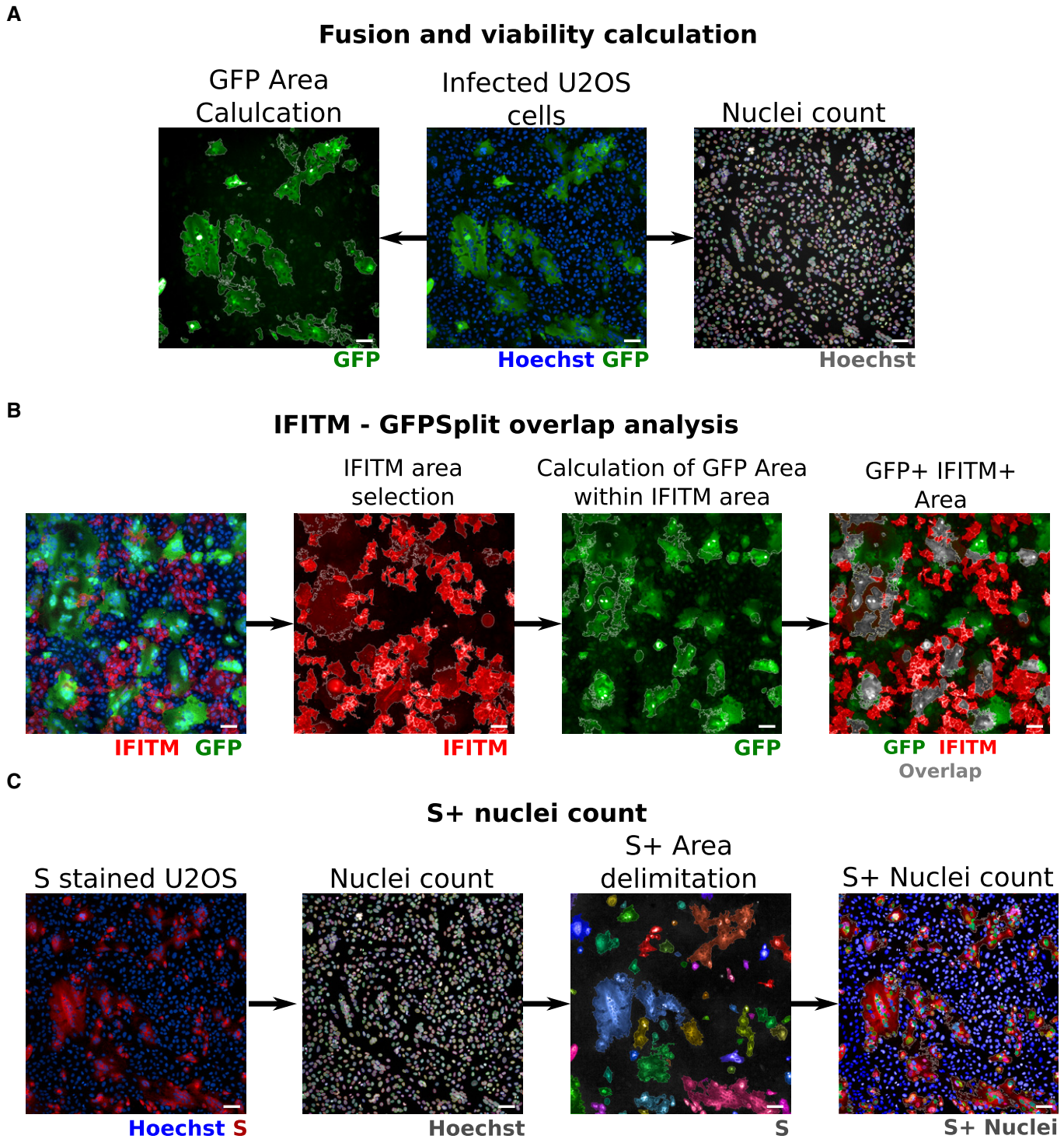

Figure EV2.

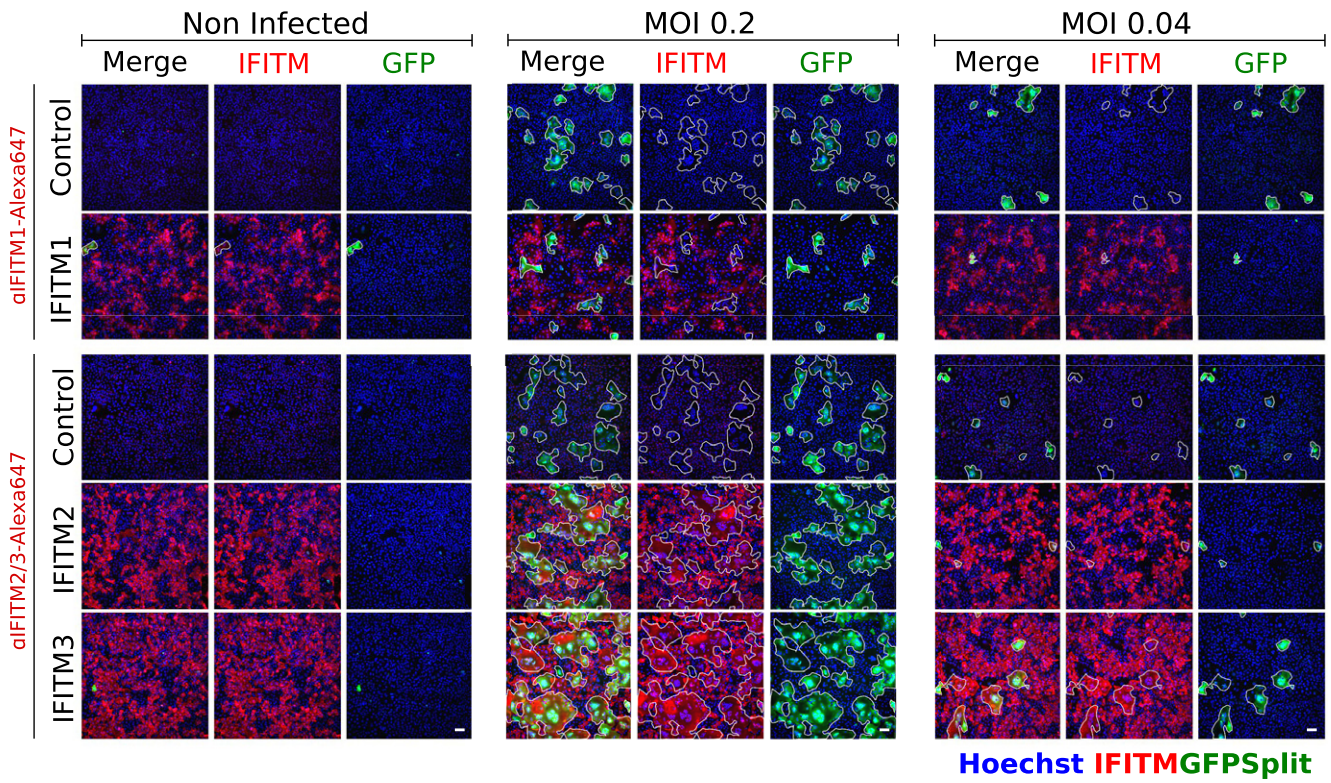

Figure EV3. Expression of IFITM1, IFITM2, and IFITM3 in SARS-CoV-2-infected syncytia.
SARS-CoV-2-induced syncytia in U2OS cells are IFITM1 dim or negative, but IFITM2/3 positive. Left: Non-infected U2OS-ACE2 cells. Middle and right: Cells infected with SARS-CoV-2 at the indicated MOI. The GFP area is delimited in white. With IFITM1, the fused GFP area does not overlap with the red IFITM1 area. For IFITM2 and IFITM3, an overlap with the GFP is visible. Scale bars: 100 μm. Quantifications are presented Fig 2C.

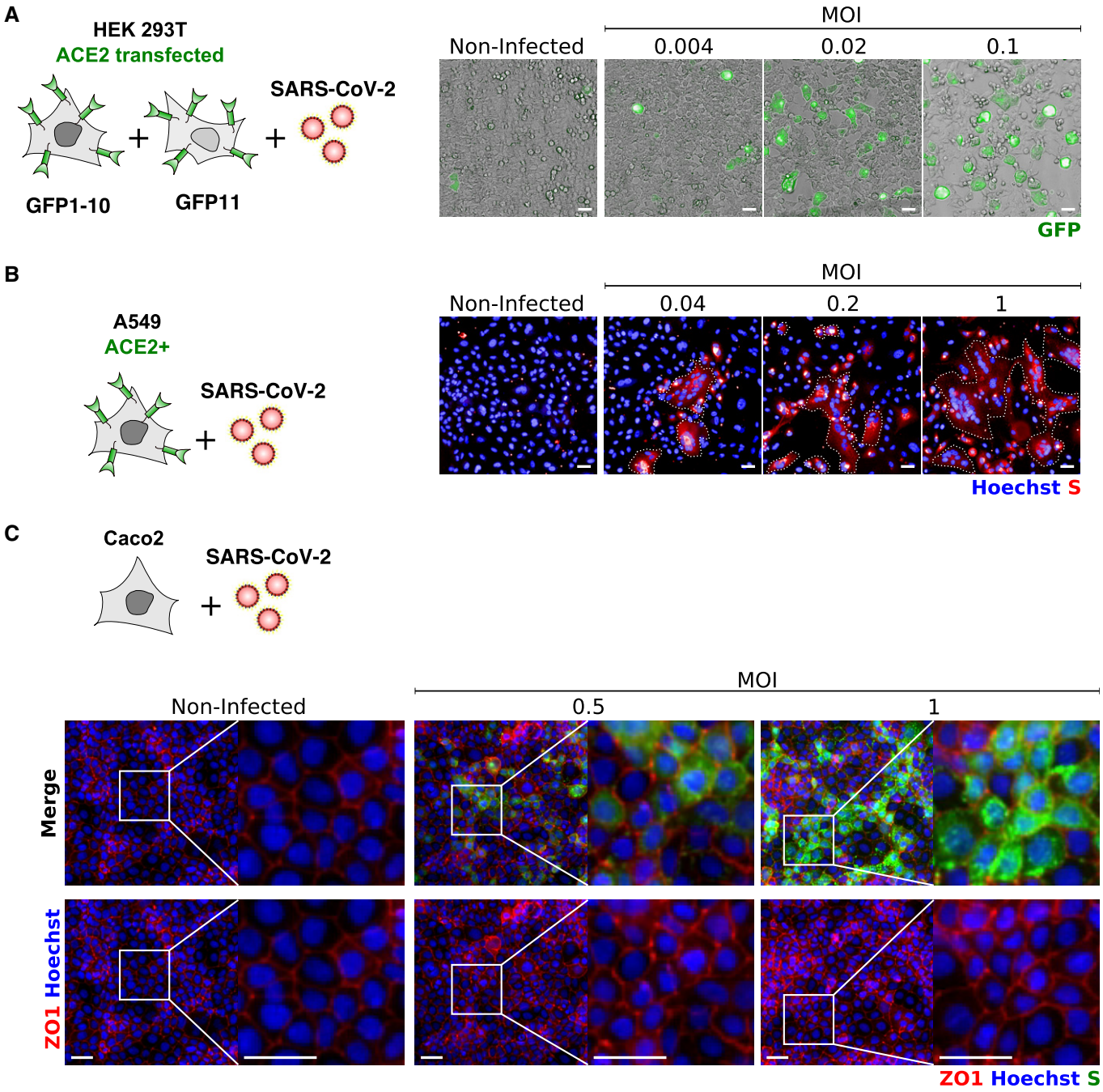

Figure EV4. Characterization of SARS-CoV-2-induced syncytia in different cell lines.

A 293T-GFP1-10 and -GFP11 (1:1 ratio) were transfected with ACE2 and infected with SARS-CoV-2 at the indicated MOIs. Cell fusion was visualized by the GFP^+ area 18 h post-infection. One representative image per condition is shown, and GFP area is delimited in white.

B A549-ACE2 cells were infected with SARS-CoV-2 at the indicated MOIs for 24 h and stained with anti-S antibodies and Hoechst. One representative image per condition is shown, and syncytia are manually delimited in white.

C Caco2 cells were infected with SARS-CoV-2 at the indicated MOIs for 24 h and stained for ZO1 tight junction marker, Hoechst and S. No clear syncytia formation was observed at any of the MOIs tested.

Data information: Scale bar: 50 μm. Data are representative of three independent experiments.

Figure EV5. Characterization of syncytia formation in Vero cells.

A Vero-GFP1-10 and -GFP11 (1:1 ratio) were infected with SARS-CoV-2 for 24 h and stained with anti-S antibodies and Hoechst. Cell fusion was visualized by the GFP^+ area. No syncytia were observed in infected Vero cells.

B Vero-GFP1-10 and -GFP11 (1:1 ratio) were transfected with S expression plasmid and cell fusion was visualized by the GFP^+ area at 18 h post-transfection. S expression induced extensive syncytia formation.

C S-expressing 293T GFP11 (Donor cells) were co-cultured overnight with Vero GFP1-10 (Acceptor cells). Cell fusion was quantified by measuring the GFP^+ area. S-expressing 293Ts readily fuse with Vero cells.

D SARS-CoV-2-infected U2OS $ACE2^+$ GFP1-10 (Donor cells) were co-cultured for 8 h with Vero GFP11 (Acceptor cells). Cell fusion was quantified by measuring the GFP^+ area. Infected U2OS fuse with Vero cells.

Data information: Scale bar: 50 μm. Representative images of three independent experiments.

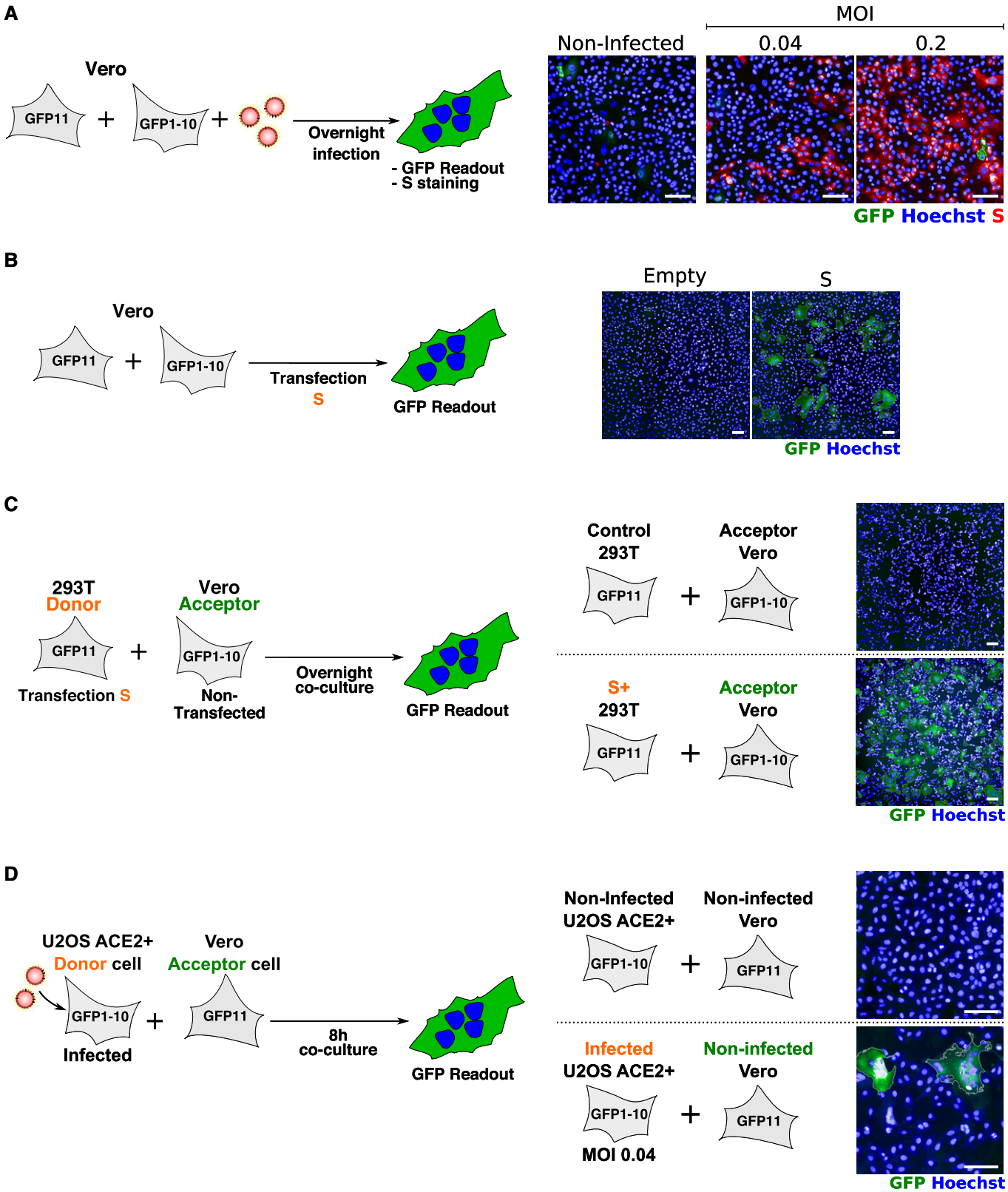

Figure EV5.

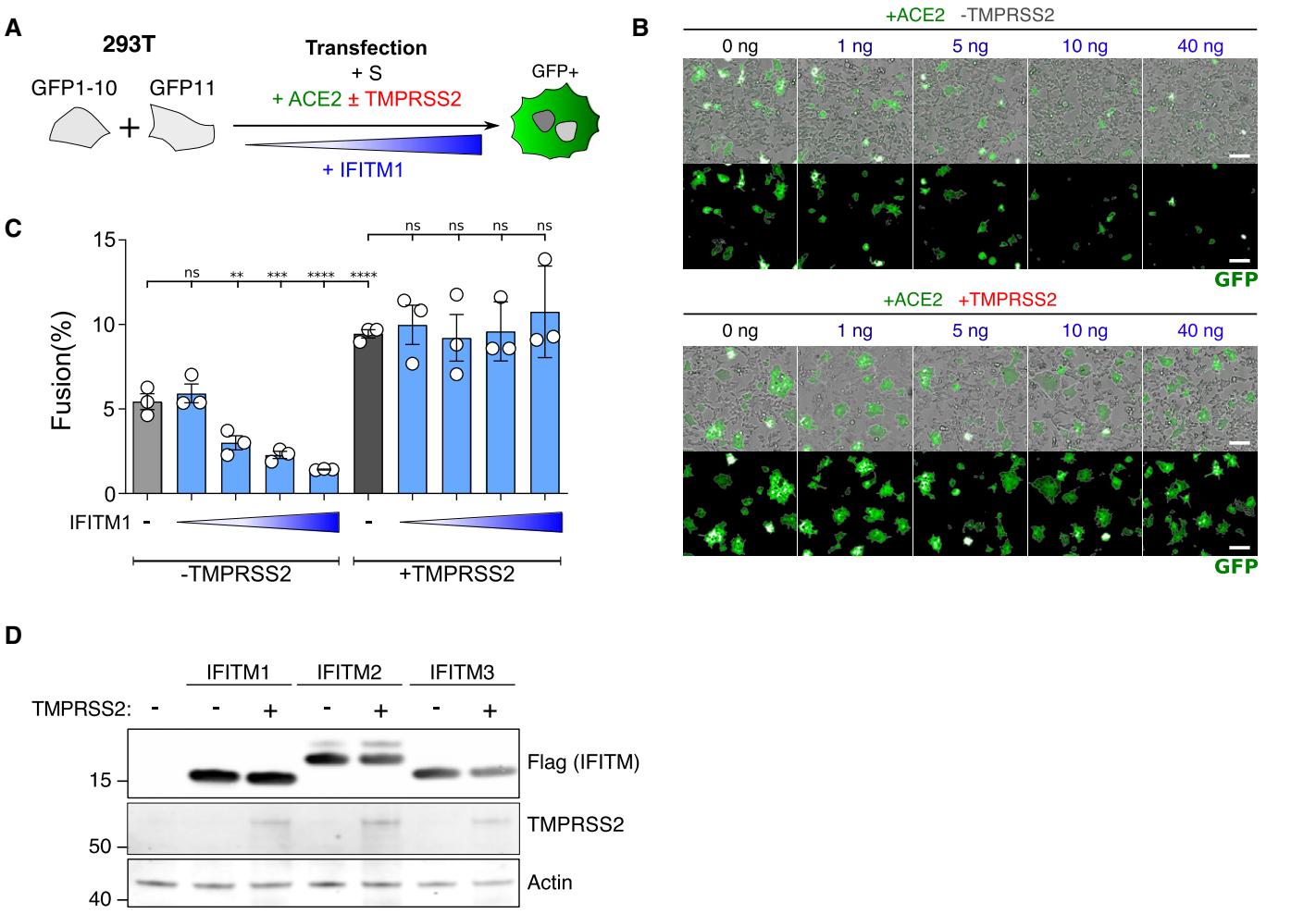

Figure EV6. Dose-response analysis of IFITM1 activity and impact of TMPRSS2 on IFITM1, 2, and 3 levels measured by Western blot.

A 293T-GFP1-10 and -GFP11 (1:1 ratio) were co-transfected with S, ACE2, TMPRSS2, and increasing amounts of IFITM1 plasmids. Cell fusion was quantified by measuring the GFP^+ area by high-content imaging at 18 h post-transfection

B Representative images of cell–cell fusion, with the indicated amounts of transfected plasmids. Scale bar: 100 μm.

C Quantification of GFP^+ area. Results are mean ± SD from three independent experiments. Statistical analysis: One-way ANOVA, ns: non-significant, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

D 293T cells were co-transfected with TMPRSS2 and IFITM1, IFITM2, IFITM3, or control plasmids, and analyzed 18 h post-transfection by Western blot. TMPRSS2 does not cleave or reduce the levels of IFITMs.

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