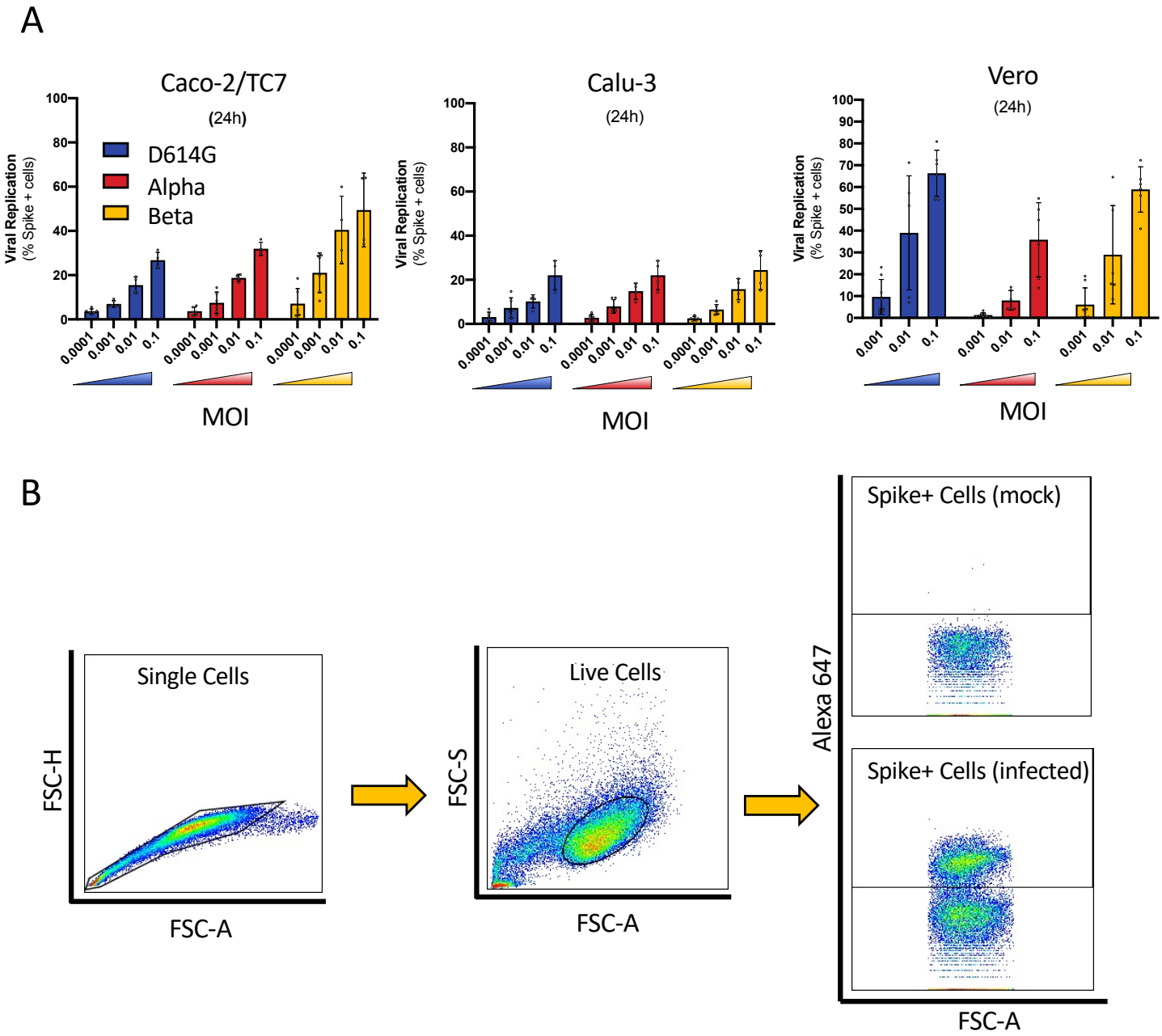
Appendix Table of Contents:

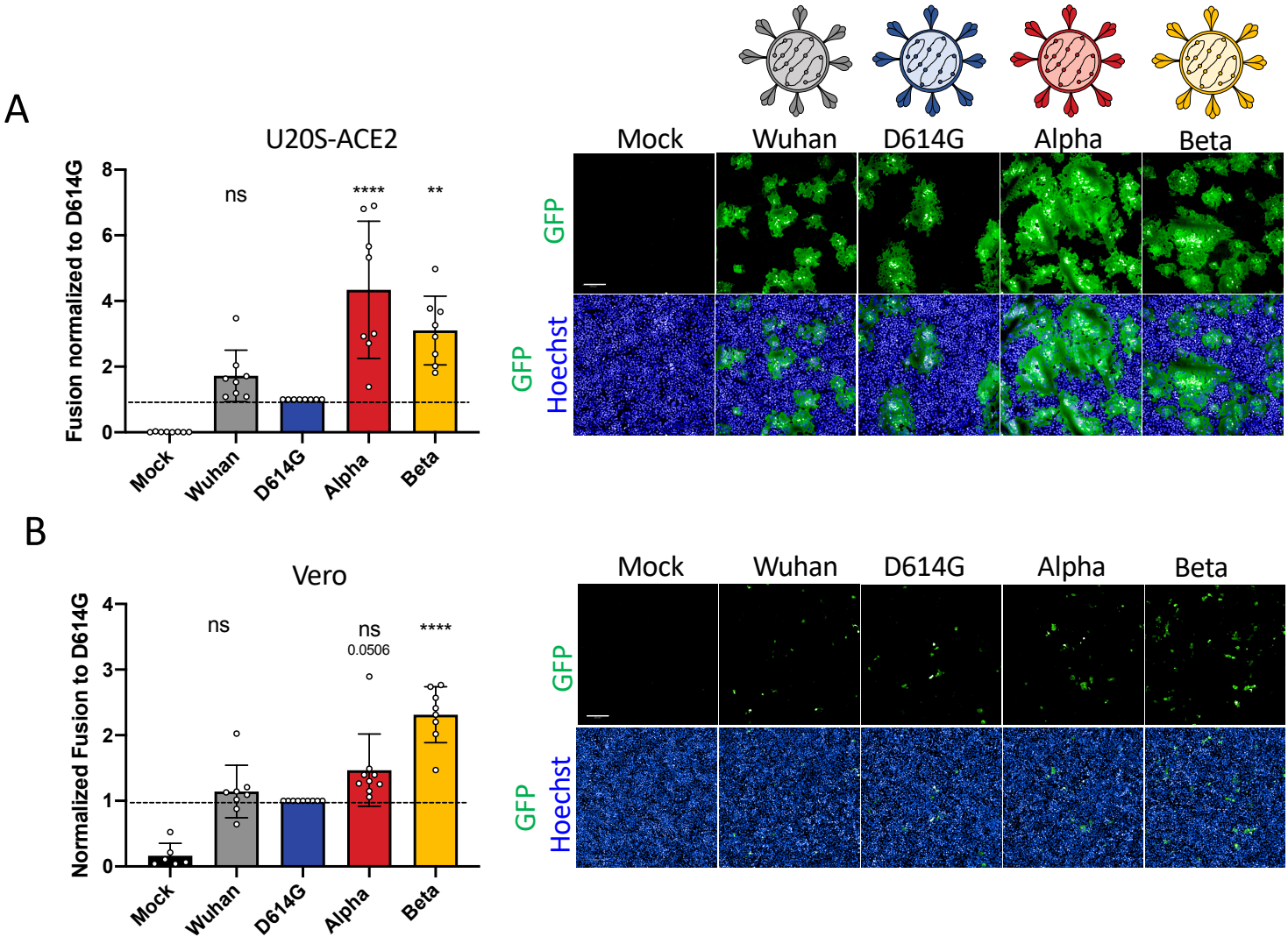


Appendix Figure S1 . Assessment of viral replication by flow cytometry in cell lines

(A) Caco-2/TC7 (left), Calu-3 (middle), and Vero cells (right) were infected at the indicated MOIs with SARS-CoV-2 variants for 24h. The number of S protein positive cells was determined by flow cytometry upon staining with human pan-SARS-CoV-2 mAb102. Only MOIs that were not saturating were used to generate replication curves for each cell line studied.

(B) Representative image of gating strategy used for flow cytometry to determine S protein positive cells.

Data information: Data are mean ± SD of at least 3 biological replicates

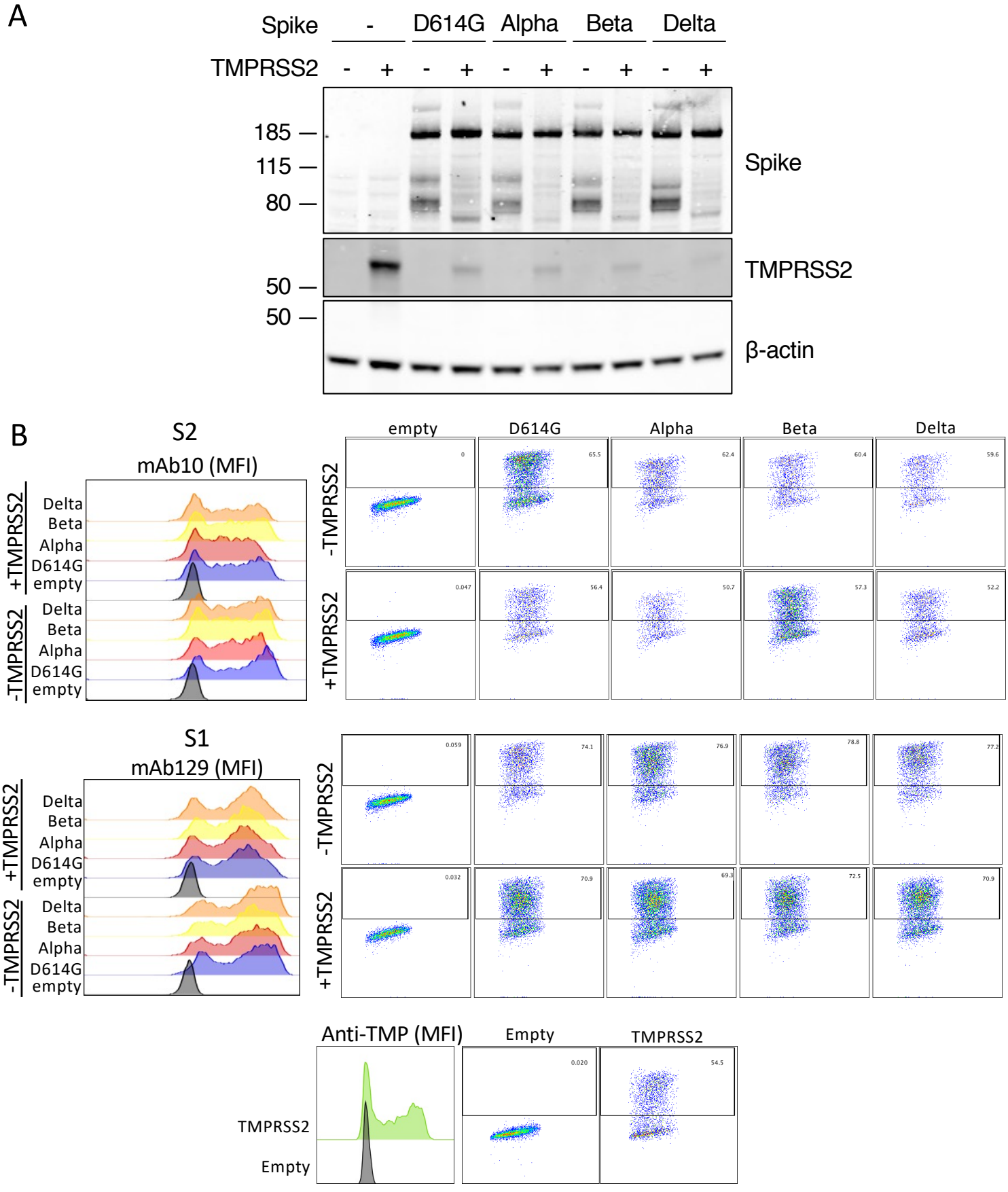


Appendix Figure S2. Syncytia formation by SARS-CoV-2 variants

(A) Left Panel: Fusion normalized to D614G for U2OS-ACE2 20h post infection at MOI 0.01. **Right Panel:** Representative images of U2OS-ACE2 20h post infection, GFP (Green) and Hoechst (Blue). Top and bottom are the same images with and without Hoechst channel.

(B) Left Panel: Quantified fusion of Vero cells infected at MOI 0.1. **Right Panel:** Representative images of Vero cells 48h post infection.

Data information: Scale bars: 200 µm. Data are mean ± SD of at least 3 independent experiments. Statistical analysis: One-way ANOVA compared to D614G reference, ns: non-significant, **P < 0.01, ****P < 0.0001.



Appendix Figure S3. Variant S protein processing by TMPRSS2

(A) Comparison of the impact of TMPRSS2 on variant S protein processing measured by western blot. Plasmids encoding for S protein were co-transfected with or without plasmids expressing TMPRSS2 in 293T cells for 24h.

(B) Representative histograms of median florescent intensity (MFI) and FACs plots of 293T cells co-transfected with S protein with and without TMPRSS2 and stained with anti-S1 and anti-S2 .

Data information: Representative image of 2 experiments.