

Supporting Information

Effect of clinical isolate or cleavage site mutations in the SARS-CoV-2 spike protein on protein stability, cleavage, and cell–cell fusion

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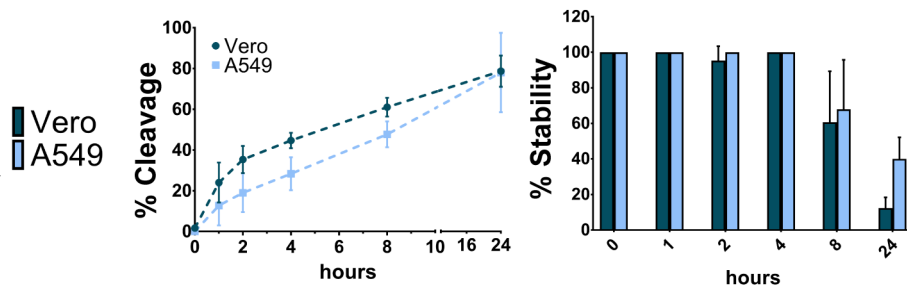
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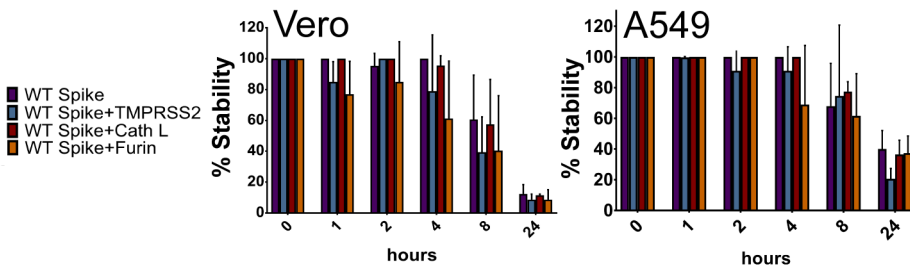
Materials included:

- Three supporting figures

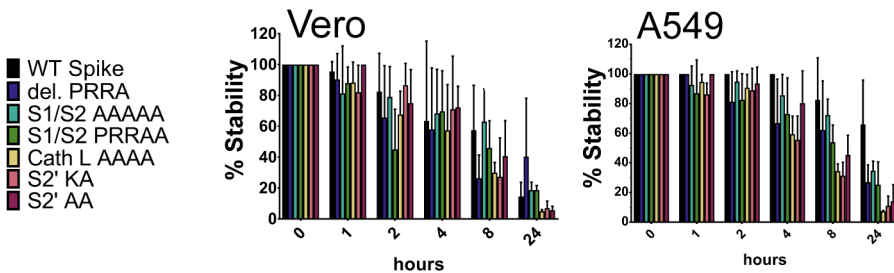
a.
WT Spike
Vero vs. A549



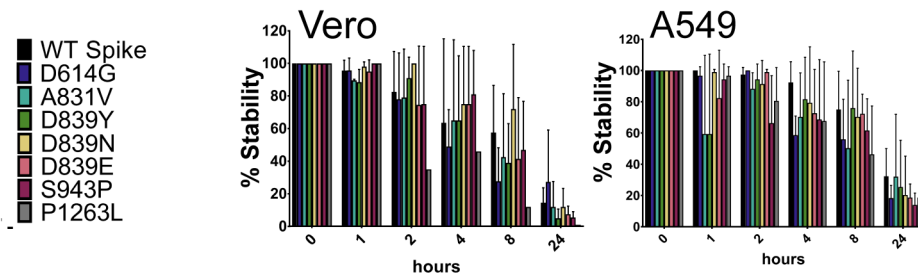
b.
Additional
Proteases



c.
Cleavage
Mutants



d.
Circulating
Mutants



e.

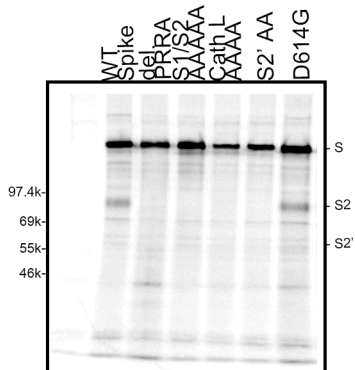


Figure S1: SARS-CoV-2 protein stability comparisons. A) Wt S was transfected into Vero or A549 cells, metabolically labeled for one hour, and chased for times indicated. Blots (shown in Fig 1a) were quantified using band densitometry. Both percent cleavage and percent stability are shown. B) Overall protein stability at each time point from blots in Fig. 1D were quantified. C) Overall protein stability at each time point from blots in Fig. 3B were quantified. D) Overall protein stability at each time point from blots in Fig. 4B were quantified. E) Vero cells expressing wt S or the indicated mutants were metabolically labeled for 5 hours. The predicted position of S2 and S2' are indicated, based on the molecular weight.

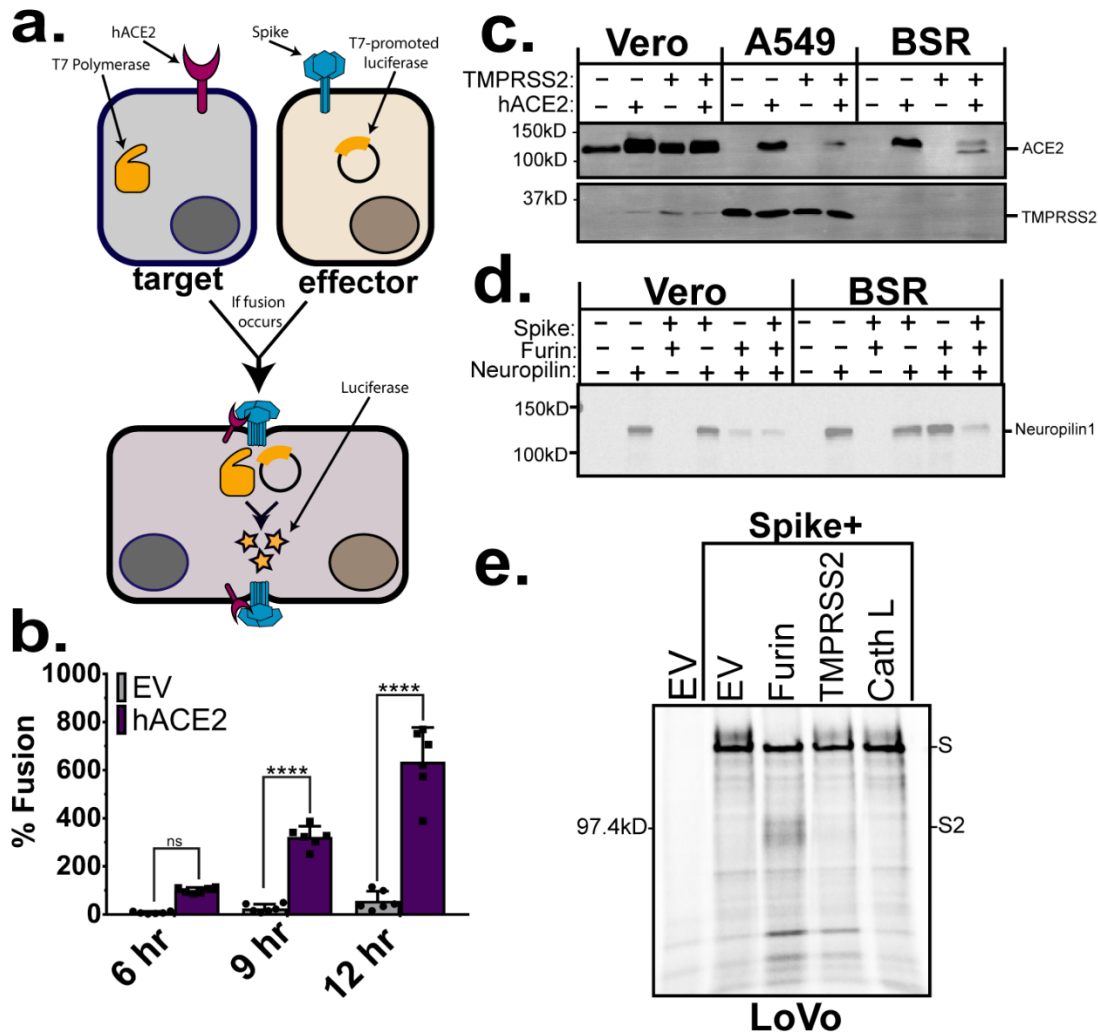


Figure S2: SARS-CoV-2 Spike Luciferase Reporter Gene Assay Controls. a) The experimental schematic for the luciferase reporter gene fusion assay used throughout the manuscript is shown here. In all experiments, the target cells used are BSR/T7 cells that constitutively express a T7 polymerase. Effector cells used are Vero cells in most experiments and A549 cells in Figure 2c. b) Various overlay times were tested in a luciferase reporter gene assay, effector cells (Vero) were transfected with wt S, and target cells were transfected with empty vector (EV) or hACE2. Results represent three independent experiments, each performed in duplicate. Significance was determined by two-way ANOVA, ****; $p < 0.001$. c) Endogenous or transfection levels of TMPRSS2 and hACE2 were assessed in Vero, A549, and BSR/T7 cells by western blot. d) Transfected expression levels of Neuropilin in the experimental conditions from Fig 2d. e) LoVo cells transiently expressing S alone, or S with the indicated protease were metabolically labeled for 6 hours. Results are representative of four independent experiments.

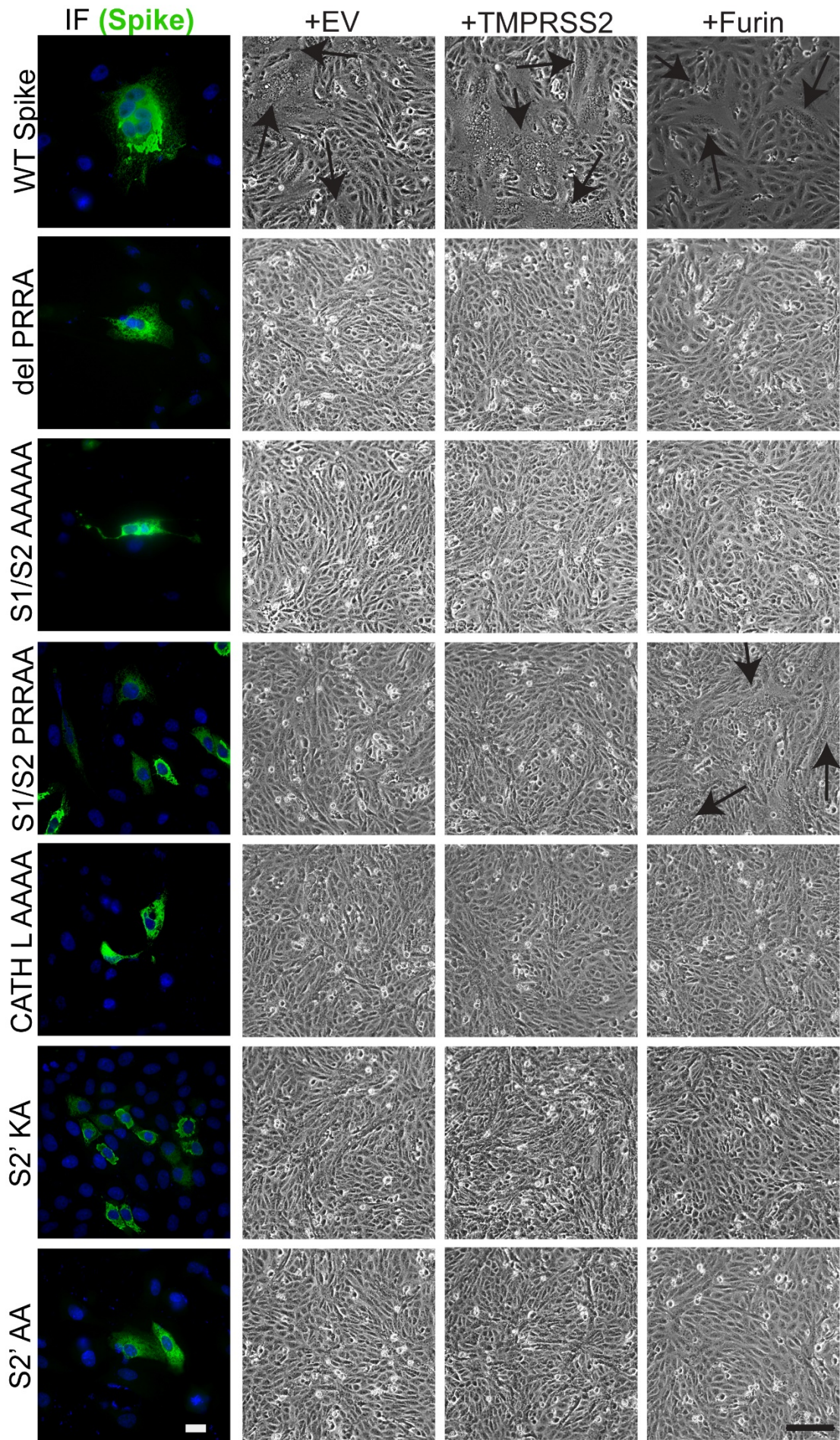


Figure S3: Mutations made at any of the three potential cleavage sites abolish syncytia formation. Vero cells were transfected with wt S or each of the cleavage mutants. The first panel shows immunofluorescence of wt S or S cleavage mutants (green, magnification bar is 20 μ m). The remaining panels show syncytia formation at 24 hours post transfection with S or mutants co-expressed with EV, TMPRSS2, or furin. Syncytia are indicated by the black arrows, magnification bar is 100 μ M.