Expanded View Figures

Figure EV1. Qualitative and quantitative assessment of syncytia formation.

A U2OS-ACE2 GFP-split cells were infected at MOI 0.01 with the Wuhan, D614G, Alpha, and Beta strains for 20 h. Cells were stained for S protein with the human pan-SARS-CoV-2 102 mAb and Alex647 fluorescent secondary antibody. Representative confocal images of the variant induced syncytia formation: GFP-Split (green), Spike (red), and Hoechst (blue). Scale bars: 50 μm.

B Quantification method for syncytia formation using the Opera Phenix high content imager and harmony software: Total syncytia area (GFP area) is normalized for cell number upon quantifying the number of nuclei (Hoechst).

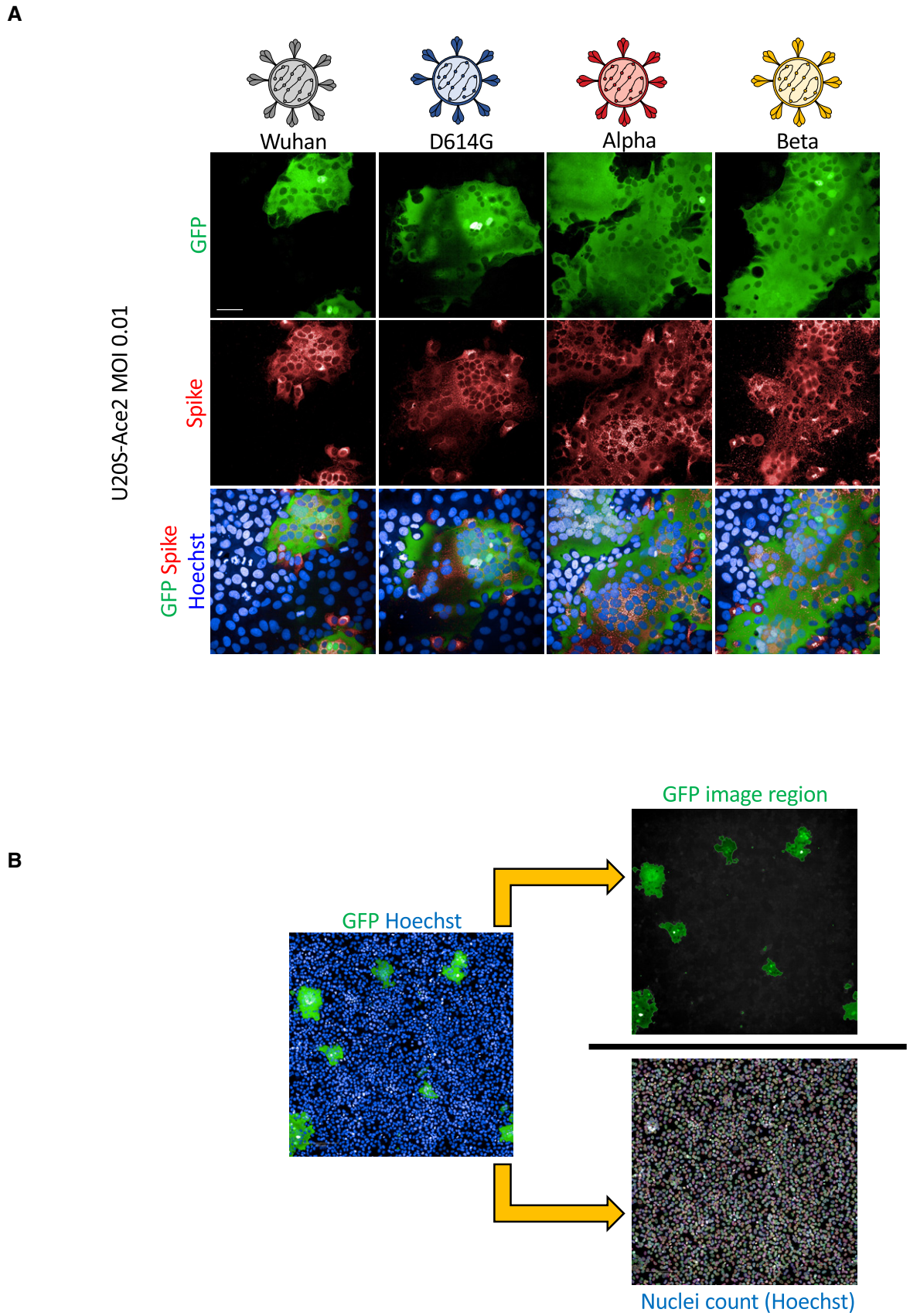
A
Wuhan
D614G
Alpha
Beta
U20S-Ace2 MOI 0.01
GFP
Spike
GFP Spike Hoechst
B
GFP image region
GFP Hoechst
Nuclei count (Hoechst)

Figure EV1.

Figure EV2. SARS-CoV-2 variant S proteins are expressed equally at the cell surface.

293T cells were transfected with variant S proteins for 20 h and stained with human pan-coronavirus mAb10 without permeabilization. 293T cells were chosen because they lack ACE2 and do not fuse upon S transfection; this makes them suitable for single-cell flow cytometry.

A Left Panel: Quantification of percent of cells expressing each S protein at the surface. Right Panel: Representative FACs plots.
B Quantification of median florescent intensity (MFI) of variant S protein at the cell surface and representative histograms of MFI of the Wuhan, D614G, Alpha, Beta, and Alpha + E484K variants S protein using mAb10.
C Quantification of median florescent intensity (MFI) of variant S protein at the cell surface and representative histograms of MFI of the Wuhan, D614G, Alpha, Beta, and Alpha + E484K variants S protein using mAb129.
D Quantification of median florescent intensity (MFI) of variant S protein at the cell surface and representative histograms of MFI of the Delta variant compared with the Alpha and D614G using mAb129.
E Controlled acceptor/donor experiment 293T GFP1-10 donor cells transfected with S protein and verified to have equal S protein expression on the surface (top), were then added to Vero GFP11 acceptor cells (bottom) to assess fusion.

Data information: Flow cytometry data are mean ± SD of at least three independent experiments. Statistical analysis: one-way ANOVA compared with D614G reference, ns: non-significant, *$P < 0.05$, ****$P < 0.0001$.

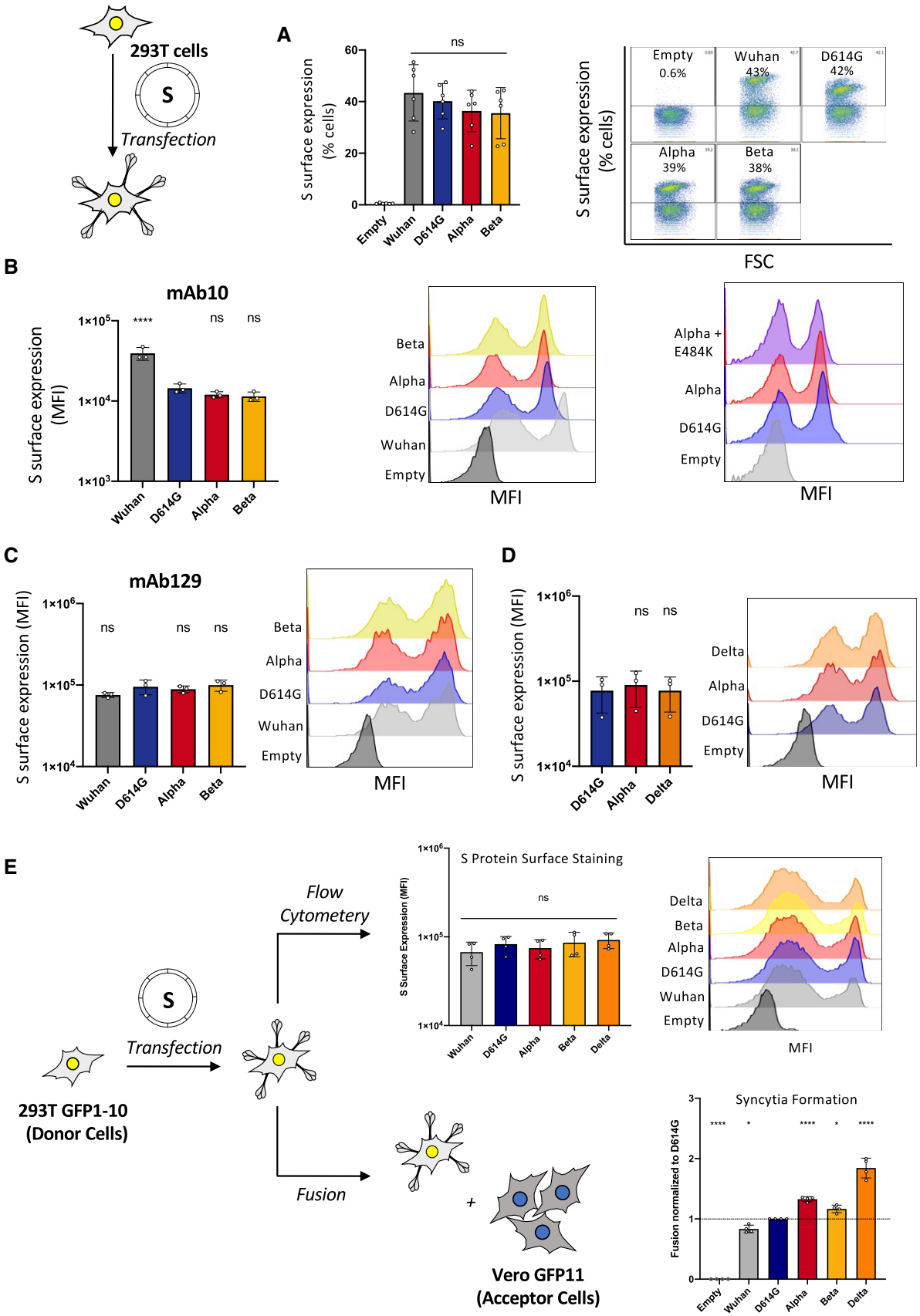
293T cells
S
Transfection
A
S surface expression (% cells)
ns
Empty
Wuhan
D614G
Alpha
Beta
Empty 0.6%
Wuhan 43%
D614G 42%
Alpha 39%
Beta 38%
FSC
B
mAb10
S surface expression (MFI)
****
ns
Alpha + E484K
MFI
C
mAb129
D
Delta
E
Flow Cytometery
S Protein Surface Staining
S Surface Expression (MFI)
293T GFP1-10 (Donor Cells)
Fusion
Vero GFP11 (Acceptor Cells)
Syncytia Formation
Fusion normalized to D614G

Figure EV2.

Figure EV3. Impact of IFN-β1 and IFITMs on SARS-CoV-2 variant replication and S protein-mediated cell–cell fusion.

A Vero cells were pre-treated for 2 h with a serial dilution of IFN-β1 prior to infection with the SARS-CoV-2 variants. Infected cells were maintained in media containing IFN-β1 and analyzed by flow cytometry 48 h post-infection to determine relative infection change.

B U2OS-ACE2 GFP-split cells were pre-treated for 2 h with a serial dilution of IFN-β1 prior to infection with the SARS-CoV-2 variants. Infected cells were maintained in media containing IFN-β1 and relative inhibition of syncytia formation 20 h post-infection was determined via GFP signal.

C–G A co-culture of 293T GFP-Split cells were transfected with combination of S, control, ACE2, TMPRSS2, and IFITM plasmids and then imaged 18 h post-transfection. Effect of IFITMs and TMPRSS2 on the cell–cell fusion induced by different S proteins, (D) Wuhan, (E) D614G, (F) Alpha, and (G) Beta.

Data information: Data are mean ± SD of at least three independent experiments. Statistical analysis: one-way ANOVA compared with D614G reference or control plasmid transfection, ns: non-significant, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

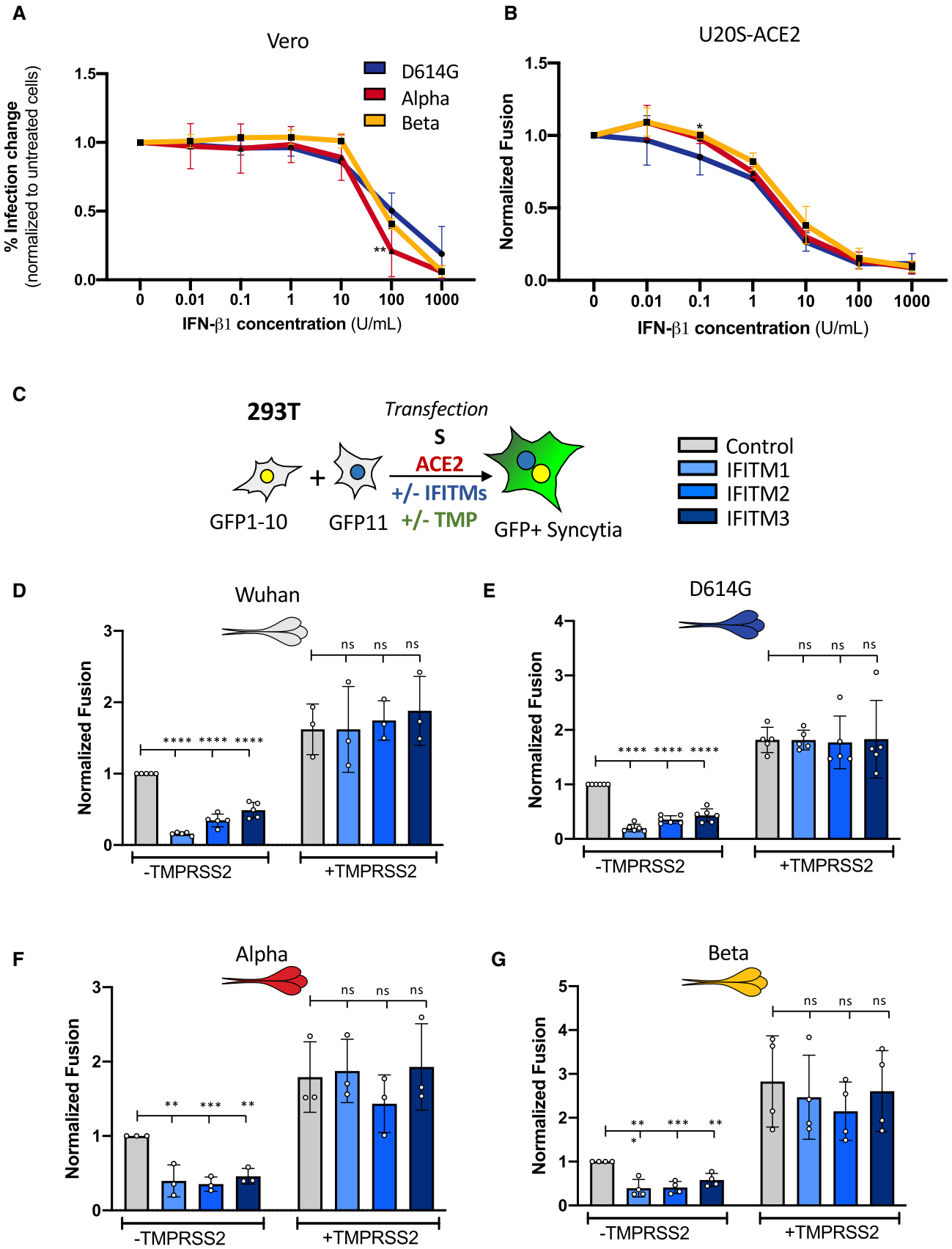
A
Vero
D614G
Alpha
Beta
% Infection change
(normalized to untreated cells)
IFN-β1 concentration (U/mL)
B
U20S-ACE2
Normalized Fusion
IFN-β1 concentration (U/mL)
C
293T
Transfection
S
ACE2
+/- IFITMs
+/- TMP
GFP1-10
GFP11
GFP+ Syncytia
Control
IFITM1
IFITM2
IFITM3
D
Wuhan
E
D614G
F
Alpha
G
Beta
Normalized Fusion
-TMPRSS2
+TMPRSS2
ns

Figure EV3.

Figure EV4. SARS-CoV-2 variant S protein-associated mutations are expressed equally at the cell surface.

293T cells were transfected with S proteins with each of the variant-associated mutations for 18 h and stained with human pan-coronavirus mAb10 without permeabilization.

A Representative FAC plots of percent of cells expressing each mutant S protein at the surface.
B Quantification of percent of cells expressing each S protein at the surface.
C Quantification of median florescent intensity (MFI) of the mutant S protein at the cell surface.
D Representative histograms of MFI of each mutant S protein.
E Representative images of Vero GFP-split cells 20 h after transfection with each Alpha variant-associated mutant S protein, GFP-Split (Green). Scale bars: 200 μm.
F Representative images of Vero GFP-split cells 20 h after transfection with each Beta variant-associated mutant S protein. Scale bars: 200 μm.

Data information: Data are mean ± SD of at least three independent experiments. Statistical analysis: one-way ANOVA compared with D614G reference, ns: non-significant.

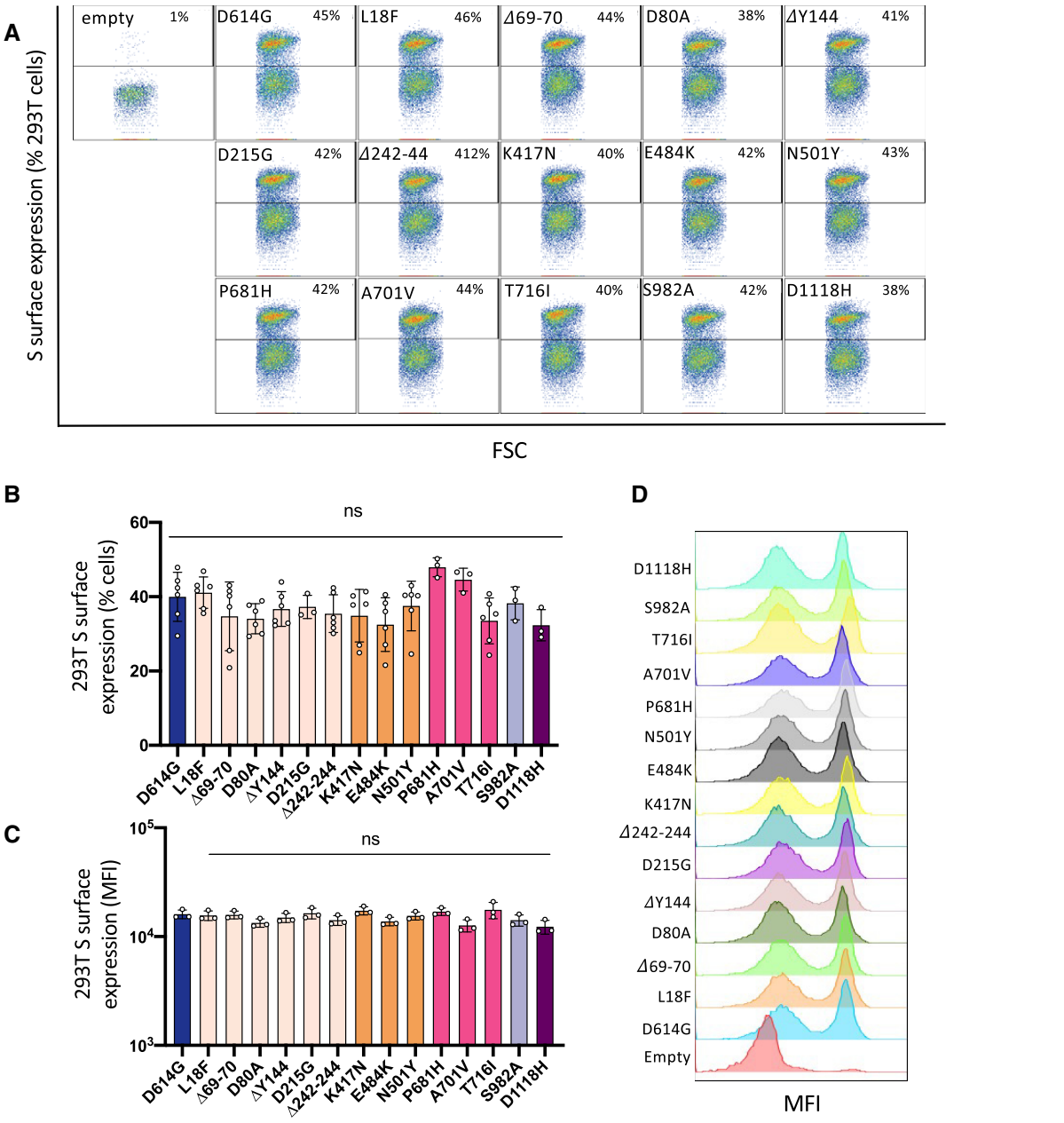
A
S surface expression (% 293T cells)
FSC
empty 1%
D614G 45%
L18F 46%
Δ69-70 44%
D80A 38%
ΔY144 41%
D215G 42%
Δ242-44 412%
K417N 40%
E484K 42%
N501Y 43%
P681H 42%
A701V 44%
T716I 40%
S982A 42%
D1118H 38%
B
293T S surface expression (% cells)
ns
C
293T S surface expression (MFI)
ns
D614G
L18F
Δ69-70
D80A
ΔY144
D215G
Δ242-244
K417N
E484K
N501Y
P681H
A701V
T716I
S982A
D1118H
D
MFI
Empty
E
Alpha associated mutations
D614G
Δ69-70
ΔY144
N501Y
A570D
P681H
T716I
S982A
D1118H
F
Beta associated mutations
D614G
L18F
D80
D215
Δ242-244
K417N
E484K
N501Y
A701

Figure EV4.

Figure EV5. ACE2 binding curves to SARS-CoV-2 variant S proteins and associated mutations.

293T cells were transfected with variant or mutant S proteins for 24 h and stained with a serial dilution of soluble biotinylated ACE2 and revealed by fluorescent streptavidin before analysis by flow cytometry.

A ACE2 binding dilution curves of each variant S protein.

B–E ACE2 binding dilution curves of each variant-associated mutation located in (B) S protein n-terminal domain (NTD), (C) receptor-binding domain (RBD), (D) S1/S2 cleavage site, and (E) heptad repeat 1–2 site (HR1-HR2).

Data information: Data are mean ± SD of three independent experiments.

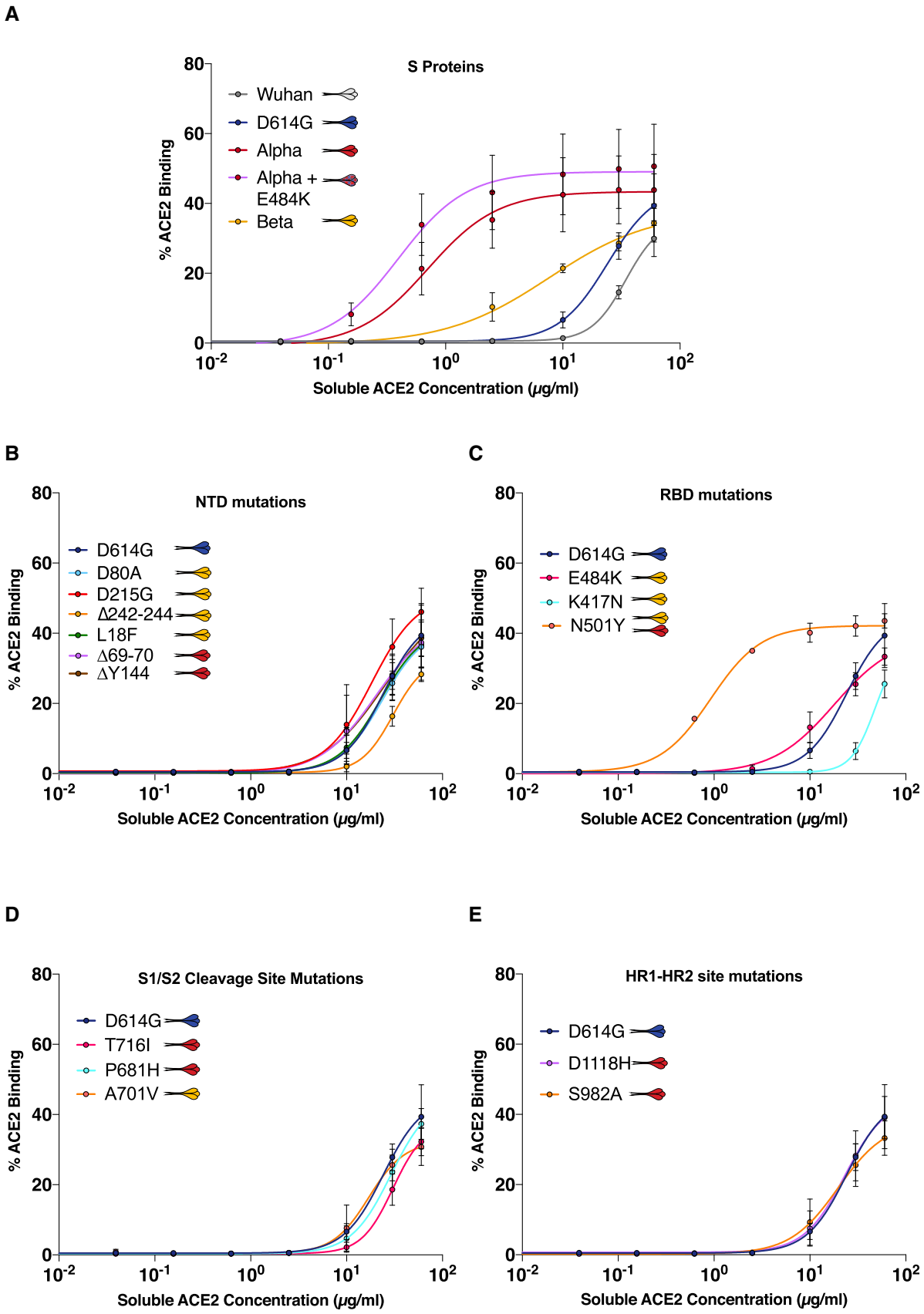

Figure EV5.