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Supplemental Information

A Replication-Competent Vesicular Stomatitis Virus for Studies of SARS-CoV-2 Spike-Mediated Cell Entry and Its Inhibition

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Fig. S1

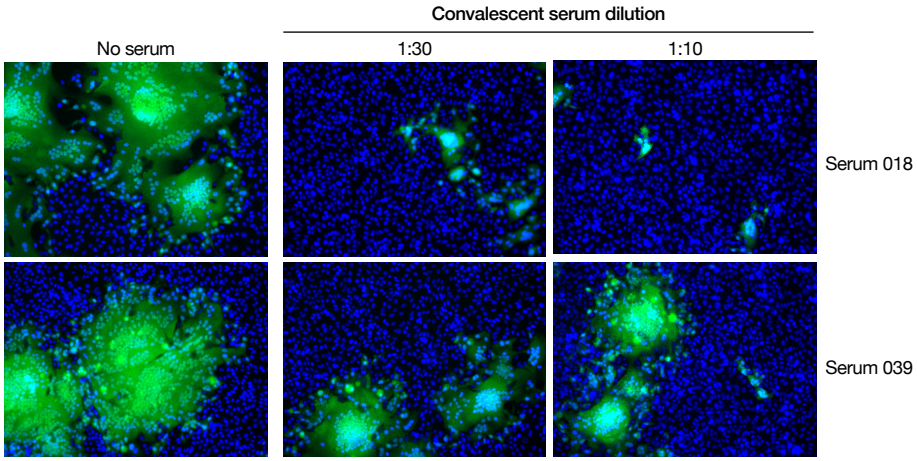

Fig S1. Inhibition of syncytium formation by sera from convalescent donors. Related to Figure 1. Vero cells were infected with pre-titrated amounts of rVSV-SARS-CoV-2. At 2 h post-infection, cells were washed with PBS to remove residual virus and then exposed to the indicated dilutions of each convalescent serum. Cells were fixed, their nuclei counterstained, and they were imaged for syncytium formation by eGFP expression at 16 h post-infection. Representative images from one of two independent experiments are shown.

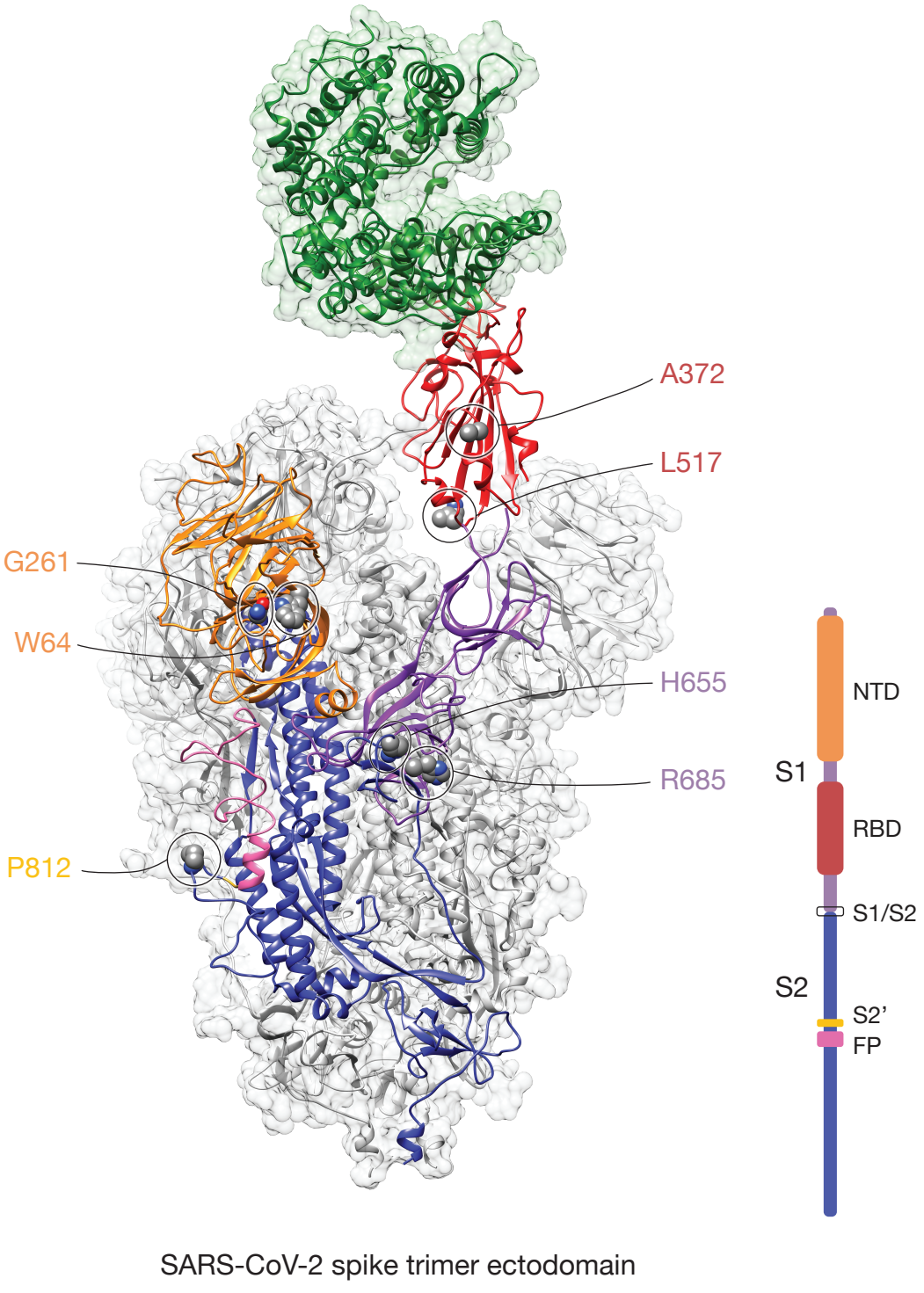

Fig S2. Locations of selected rVSV-SARS-CoV-2 S mutations in the viral spike glycoprotein. Related to Figure 1. Selected mutations (see Table S1) are indicated on a structural model of the pre-fusion confor-mation of the SARS-CoV-2 spike trimer ectodomain in complex with its receptor hACE2 (green). In order to model the SARS-CoV-2 spike trimer bound to hACE, we combined X-ray structures for the SARS-CoV-2 spike trimer (PDB IDs: 6VXX, 6VSB) with the SARS-CoV-2 S1-RBD domain bound to hACE2 (PDB IDs: 6JM0, 6LGZ) by structural superposition using Chimera. Missing loops within the spike protein were modeled using the modeller plugin in Chimera. Domains within one monomer of the spike trimer are depicted in different colors (S1: purple; S1-NTD [N–terminal domain]: orangve; S1-RBD: Red; S2: dark blue; S2-fusion peptide [FP]: yellow; S2-S2' cleavage site: pink). A linear diagram of the spike protein sequence is shown at right. NTD, N–terminal domain, RBD, receptor-binding domain. FP, fusion peptide. See text for details.

Fig. S3

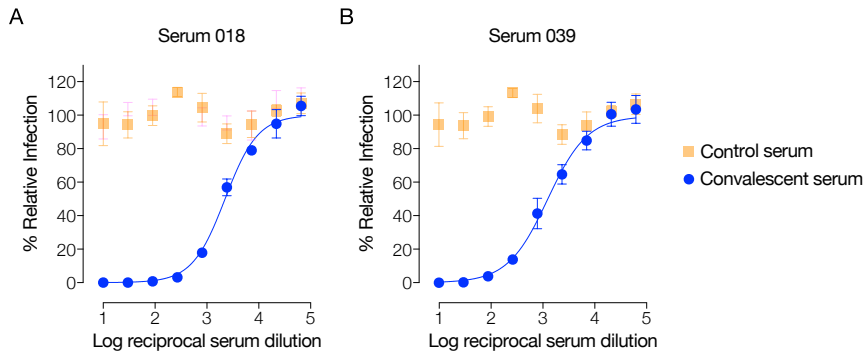

Fig S3. rVSV-SARS-CoV-2 S neutralization dose-curves with antisera from convalescent donors. Related to Figures 5 and 6. Pre-titrated amounts of rVSV-SARS-CoV-2 S were incubated with serial 3-fold dilutions of antisera from two COVID-19 convalescent donors or negative control at 37°C for 1 h. Virus:serum mixtures were then applied to monolayers of Vero cells. At 16-18 h post-infection, cells were fixed, nuclei were counterstained, and infected cells were scored for eGFP expression (Average ± SD, n = 4 from 2 independent experiments).

Fig. S4

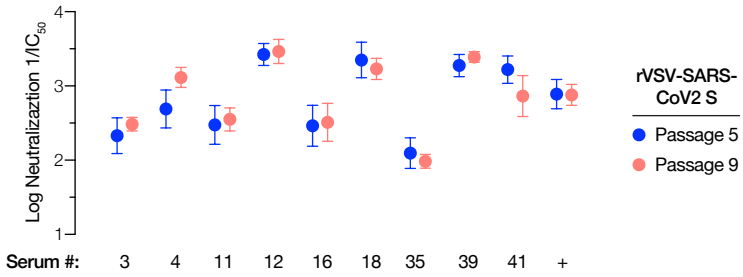

Fig S4. Capacities of selected convalescent antisera to neutralize two rVSV-SARS-CoV-2 S passage stocks. Related to Figure 6. Pre-titrated amounts of rVSV-SARS-CoV-2 S from passage 5 and passage 9 were incubated with serial 3-fold dilutions of antisera from nine COVID-19 convalescent donors or positive and negative controls at 37°C for 1 h. Virus:serum mixtures were then applied to monolayers of Vero cells. At 16-18 h post-infection, cells were fixed, nuclei were counterstained, and infected cells were scored for eGFP expression. IC_{50} values were extracted from logistic curve fits of the neutralization dose curves and are shown above (±95% confidence intervals).

Table S1: Spike missense and nonsense mutations acquired by rVSV-SARS-CoV-2 S during serial passage. Related to Figure 1.

Passage #	Mutations									
	W64R	D253N	G261R	A372T	L517S	H655Y	R685G	P812R	C1250*[a]	C1253*[a]
1										
5				X	X			X	X	X
7	X		X	X	X			X	X	X
8	X		X	X	X			X	X	X
9[b]	X		X	X	X			X	X	X
Plaque #1 #2	X		X	X		X	X			X
Plaque #3	X	X	X	X		X				X
Plaque #4 #5	X	X	X	X		X	X			X
Plaque #6	X		X	X			X			X

[a]Mutation introduces stop codon.
[b]Plaque isolates are derived from passage 9.