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Supplemental information

Impact of mutations defining SARS-CoV-2 Omicron subvariants BA.2.12.1 and BA.4/5 on Spike function and neutralization

Chiara Pastorio, Sabrina Noettger, Rayhane Nchioua, Fabian Zech, Konstantin M.J. Sparrer, and Frank Kirchhoff

SUPPLEMENTARY FIGURE LEGENDS

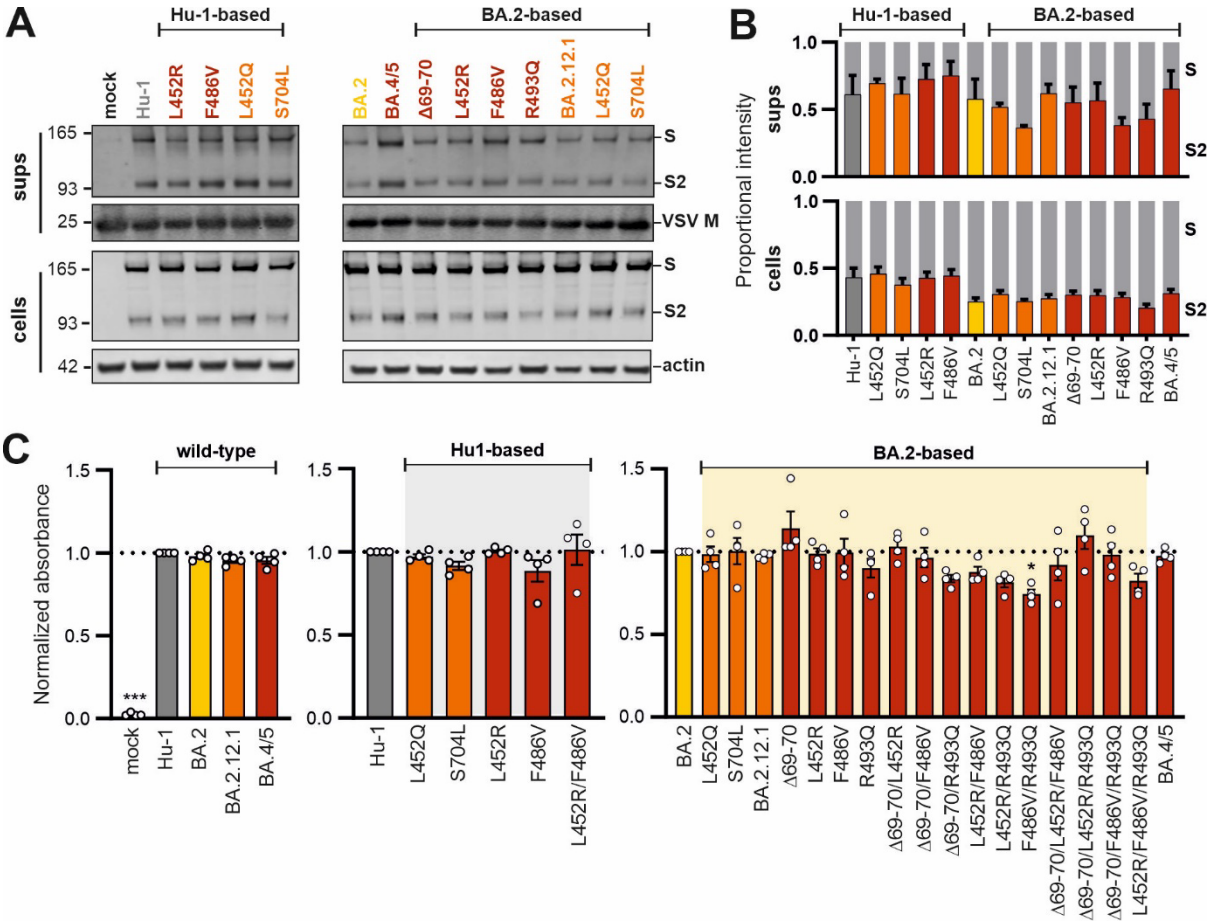

Figure S1 (related to Figure 1). Impact of mutations present in BA.2.12.1 and BA.4/5 S proteins on expression, processing and ACE2 interaction. (**A**) Exemplary immunoblots of whole cells lysates (WCLs) and VSVpp containing supernatants of HEK293T cells transfected with vectors expressing Hu-1, BA.2, BA.2.12.1, BA.4/5 or mutant SARS-CoV-2 S proteins and infected with VSVΔG-GFP. Blots were stained with anti-V5 (Spike), anti-ß-actin and anti-VSV-M. (**B**) Ratio of the expression levels of uncleaved, full-length S protein (S, grey bars) or the S2 subunit (coloured bars) and total S expression levels (S+S2), set to 1. Expression levels of S and S2 were determined by quantification of the band intensities obtained in western blot analyses using LI-COR Image Studio version 5 as described in the methods section. Results show mean values (+SEM) obtained in three independent experiments. (**C**) Binding of the indicated Hu-1 and mutant S proteins to ACE2 using whole cell lysates of transfected HEK293T. Extracts of S-expressing HEK293T cells were added to ACE2-coated wells. After extensive washing, Spike bound to ACE2 was detected using a mouse Ab against the V5 tag of S proteins and quantified using an anti-MS-HRP Ab. Absorption was measured at 450 nm with a baseline correction at 650 nm as outlined in the methods section. The middle panel shows Hu-based S mutants, absorbance is normalized on the Hu-1 S (set at 1) and p-values indicate difference to the Hu-1 S. The panel on the right shows BA.2-based S mutants and infection events are normalized on the BA.2 S (set at 1) and p-values indicate difference to the BA.2 S. Bars represent the mean of four independent experiments (±SEM). Statistical significance was tested by one-way ANOVA. *P < 0.05; **P < 0.001.

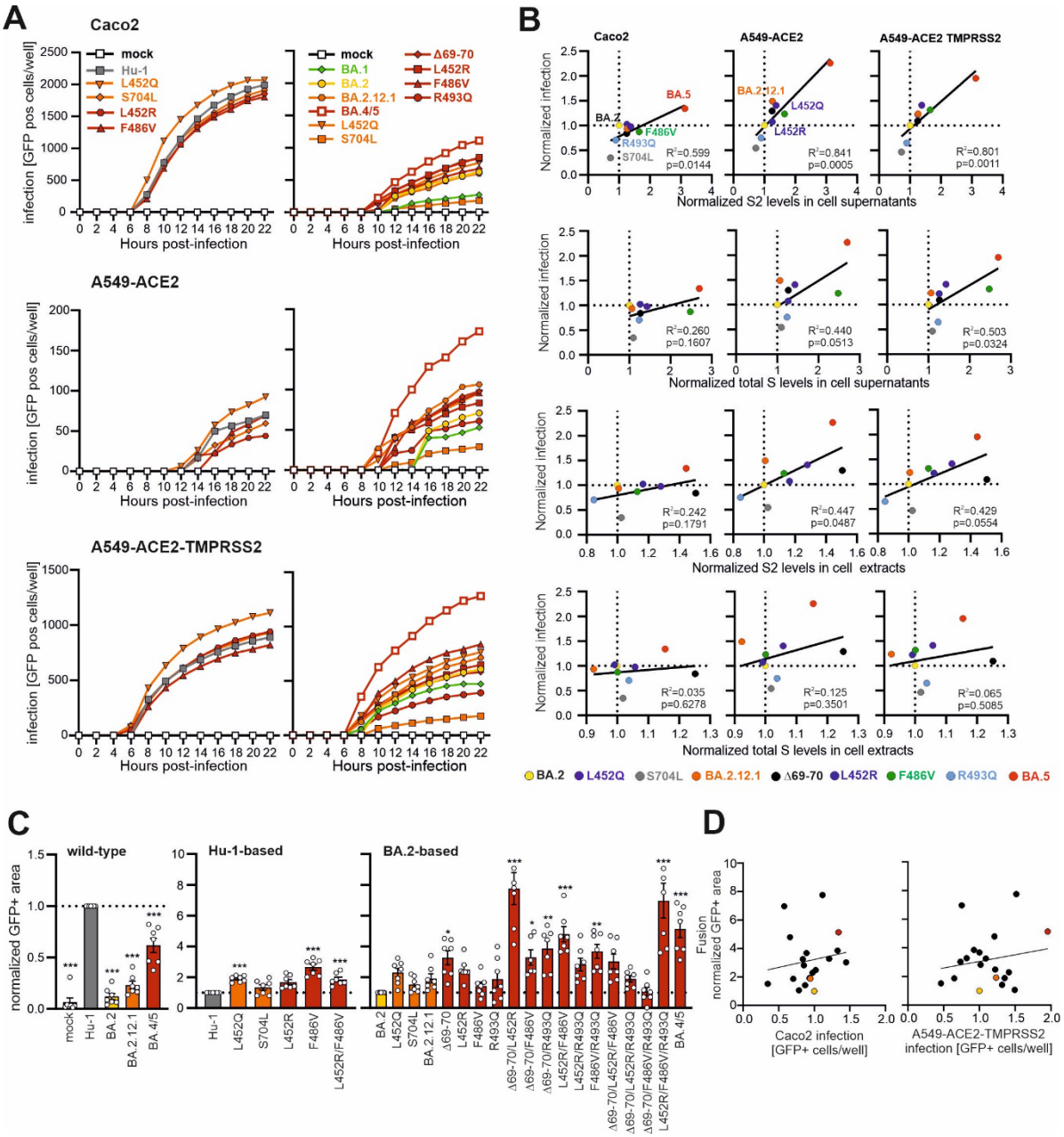

Figure S2 (related to Figure 2). Impact of BA.2.12.1 and BA.4/5 mutations on S-mediated VSVpp infection and cell-to-cell fusion. (**A**) Infection kinetics of Caco2 (upper part), A549-ACE2 (middle part), A549-ACE2-TMPRSS2 (lower part) cells by VSVpp containing the indicated Hu-based (left panel) and BA.2-based (right panel) mutant S proteins. Infected GFP+ cells were automatically quantified over a period of 22 h. (**B**) Correlation of S-mediated VSVpp infection events and the levels of total Spike and processed S2 protein in HEK293T cell extracts and VSVpp containing supernatants. Each dot represents the average value derived from three infection experiments or western blots (example shown in Figure S1A). (**C**) Automatic quantification of syncytia formation of HEK293T cells, expressing the indicated S proteins and ACE2 in the GFP-split complementation system. The upper-right panel show Hu-based S mutants and the GFP+ area is normalized on the Hu-1 (set at 1). The lower panel show BA.2-based S mutants and the GFP+ area is normalized on the BA.2 (set at 1). Bars represent the mean of seven independent experiments (±SEM). Statistical significance was tested by one-way ANOVA. *P < 0.05; **P < 0.01; ***P < 0.001. (**D**) Correlation of syncytia formation (indicated as normalized GFP+ area) with VSVpp infection events of wild type or mutant S proteins in Caco2 (left panel) and A549-ACE2-TMPRSS2 (right panel) cells.

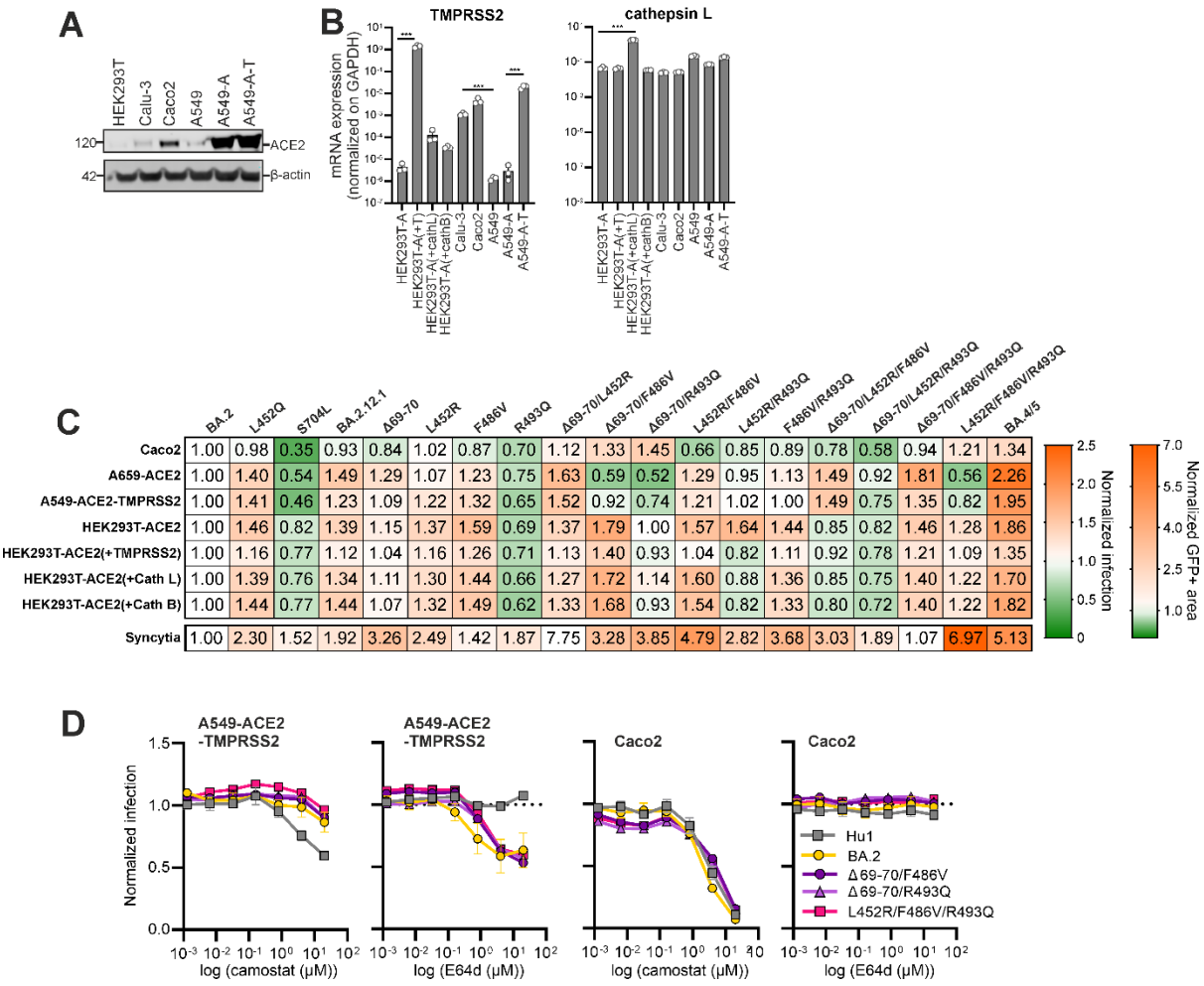

Figure S3 (related to Figure 3). Expression levels of ACE2, TMPRSS2 and cathepsin L and overview on the impact of amino acid changes on S function. (**A**) Exemplary immunoblots of whole cells lysates (WCLs) of HEK293T, Calu-3, Caco2, A549, A549-ACE2, A549-ACE2-TMPRSS2 cells stained with anti-ACE2 and anti-ß-actin protein. (**B**) Quantification of mRNA expression levels of TMPRSS2 (left panel) or cathepsin L (right panel) normalized on GAPDH in HEK293T-ACE2 cells alone or transfected with TMPRSS2, cathepsin L or cathepsin B, Calu-3, Caco2, A549, A549-ACE2, A549-ACE2-TMPRSS2 cells. Bars represent the mean of three independent experiments (±SEM). Statistical significance was tested by one-way ANOVA. *P < 0.05; **P < 0.01; ***P < 0.001. (**C**) For each wild type or indicated mutations in Spike, the table lists: normalized VSVpp infection of Caco2, A549-ACE2, A549-ACE2-TMPRSS2, HEK293T-ACE2 alone or transfected with TMPRSS2, cathepsin L or cathepsin B and the automated quantification of syncytia formation in HEK293T cells carrying the GFP-split complementation system expressing the indicated mutant S proteins and human ACE2. (**D**) Automated quantification of GFP fluorescence of A549-ACE2-TMPRSS2 (left panel) or Caco2 (right panel) cells infected with VSVΔG-GFP pseudotyped with the indicated S variants. Cells were pre-treated (1 hour, 37 °C) with the with 20 µM of camostat or E64d in the highest concentration and diluted in a 1:5 titration row. Lines represent the mean of three independent experiments (±SEM).

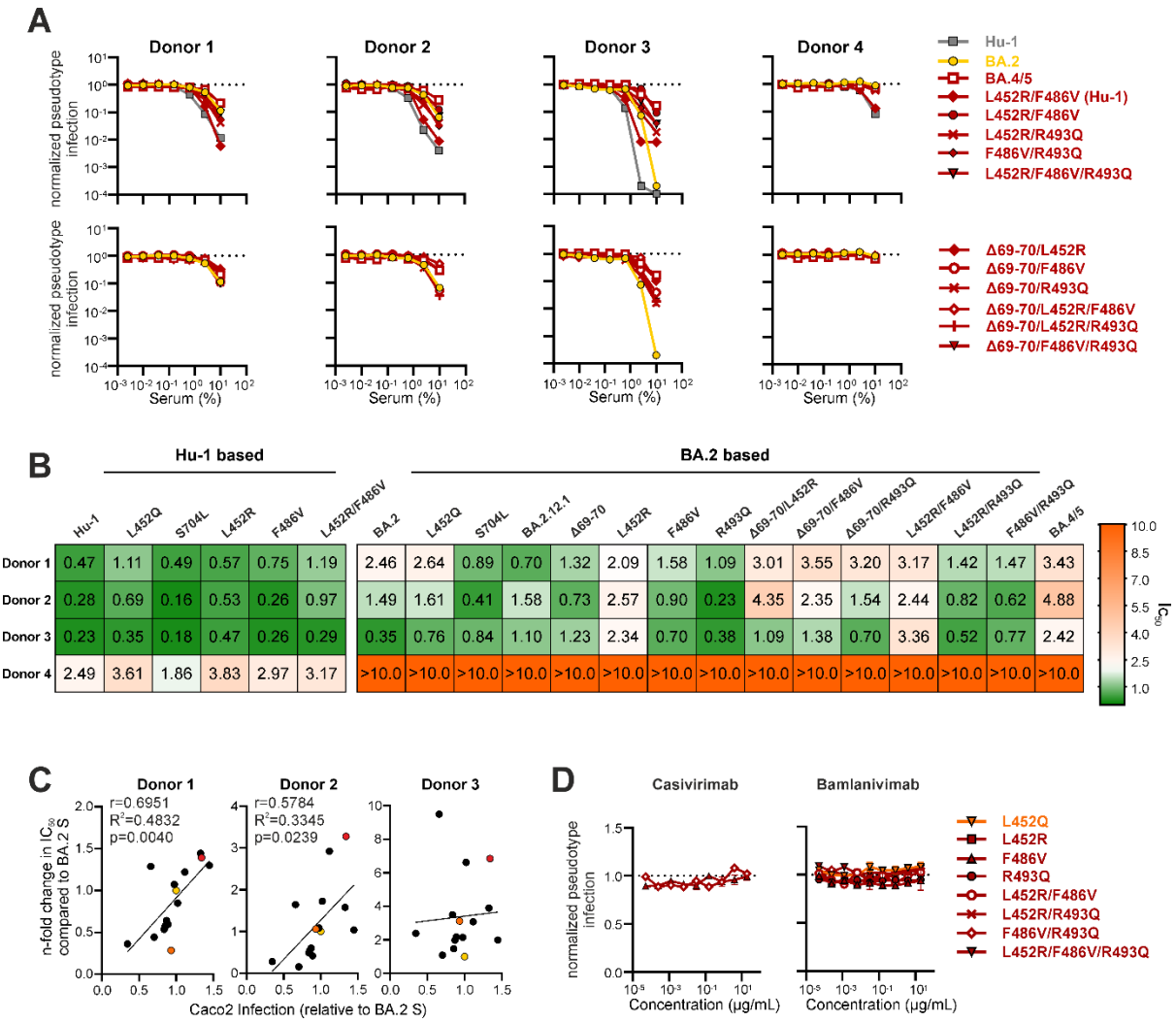

Figure S4 (related to Figure 4). Impact of combined mutations of BA.4/5 S on neutralization by sera and therapeutic Nabs. (**A**) Neutralization of VSVpp carrying the indicated wildtype and mutant S proteins by sera obtained from two AZN/BNT (left panel) and two BNT/BNT (right panel) vaccinated individuals compared to the untreated control (set to one). Infection was measured in Caco2 cells. (**B**) The table indicate TCID50 values obtained for neutralization of the indicated mutant Hu-based (left panel) or BA.2-based (right panel) S proteins by sera from four vaccinated individuals. (**C**) Correlation of n-fold changes in TCID50 values obtained for neutralization of the wild type S or indicated mutant S proteins by sera from 3 vaccinated individuals with VSVpp infection events in Caco2 cells relative to BA.2 S. Coefficient of correlation (r), coefficient of determination (R^2-values) and two tailed P values are provided. (**D**) Automated quantification of GFP fluorescence of Caco2 cells infected with VSVΔG-GFP pseudotyped with the indicated S variants. VSVpp were pre-treated (30 min, RT) with the indicated amounts of Casivirimab or Bamlanivimab. Lines represent the mean of three independent experiments (±SEM).