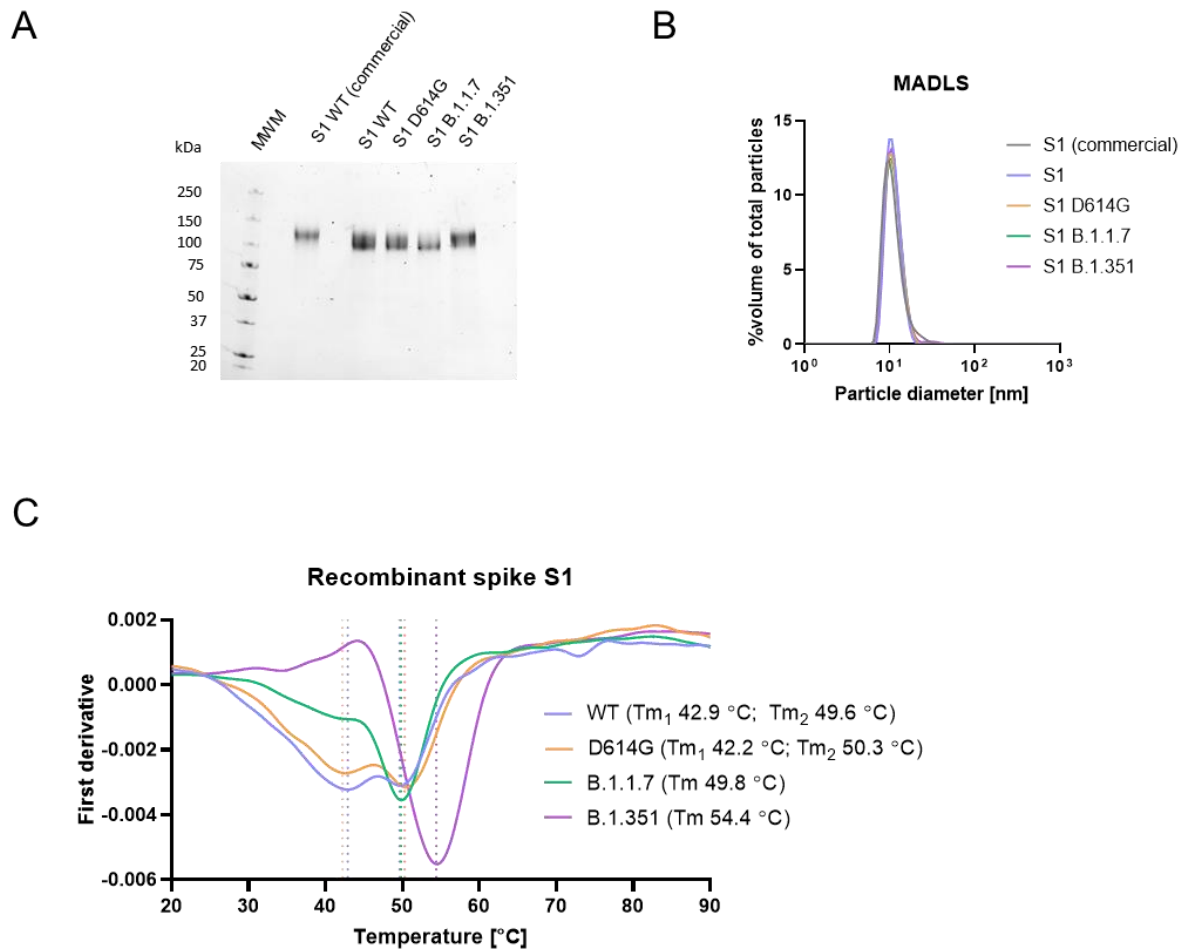


SUPPLEMENTARY MATERIAL

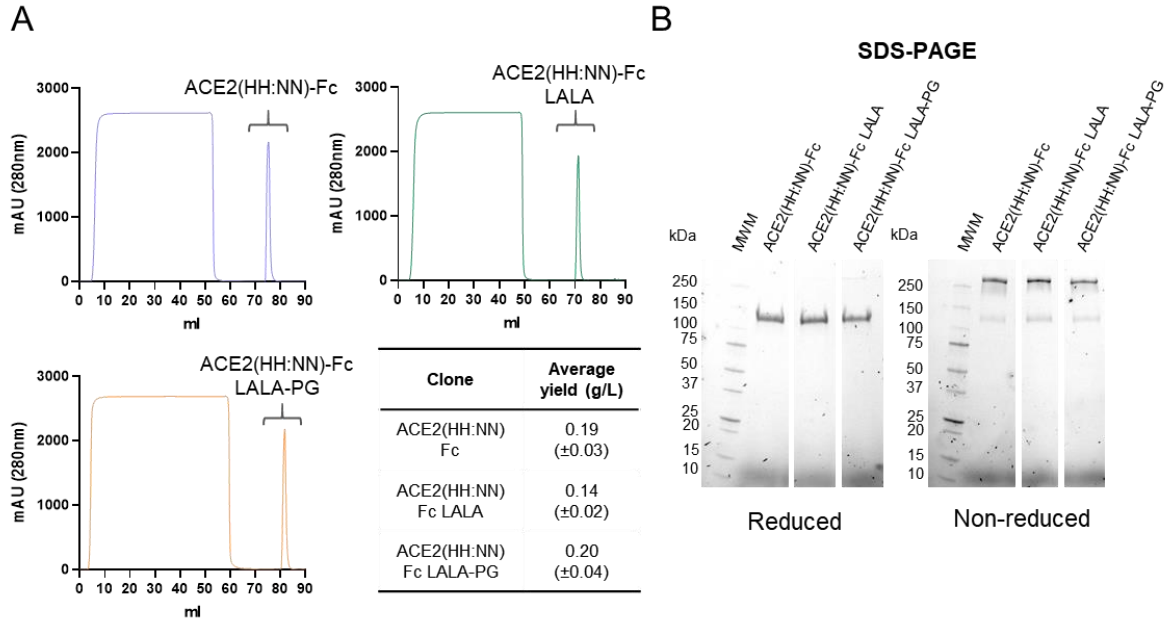


Supplementary Figure 1. Characterisation of SARS-CoV-2 S1 variants

A) SDS-PAGE of SARS-CoV-2 S1 purified proteins from WT, D614G, B.1.1.7 and B.1.351 variants, in comparison to a commercially sourced recombinant S1 protein. All proteins showed >95% purity.

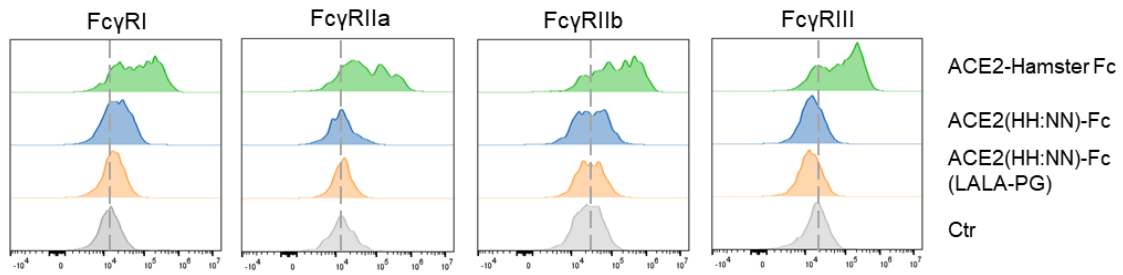
B) Particle size distribution of recombinant purified SARS-CoV-2 S1 variants analysed via MADLS.

C) Thermal stability analysis of purified SARS-CoV-2 variants showing increased T_m for B.1.1.7 and B.1.351 variants compared to WT S1.



Supplementary figure 2. Purification profile of ACE2(HH:NN)-Fc variants

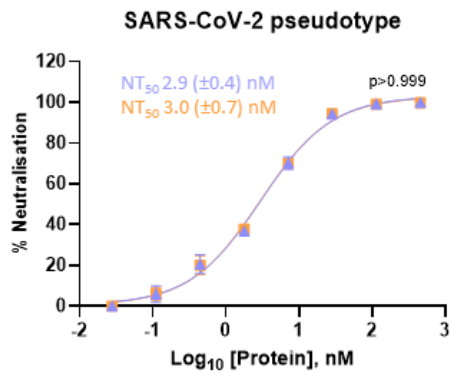
A) Representative protein A purification profile of ExpiCHO expressed ACE2(HH:NN)-Fc (blue), ACE2(HH:NN)-Fc LALA (green) and ACE2(HH:NN)-Fc LALA-PG (orange) fusion proteins using AKTA pure and Mab select Prisma columns with in-line HiTrap desalting column. n=4, n=3, n=10, respectively; Mean (±SD). B) Representative SDS-PAGE of purified ACE2(HH:NN)-Fc, ACE2(HH:NN)-Fc LALA and ACE2(HH:NN)-Fc LALA-PG in reducing and non-reducing conditions showing > 95% purity.



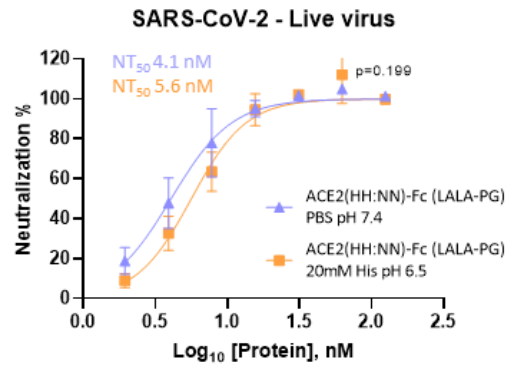
Supplementary figure 3. Hamster Fc γ R binding

Fc-mediated binding capacity of ACE2(HH:NN) WT Fc (blue), LALA-PG Fc (orange) or ACE2(HH:NN) hamster Fc (green) to HEK293T cells expressing hamster Fc γ RI, Fc γ RIIa, Fc γ RIIb and Fc γ RIII receptors, detected with biotinylated SARS-CoV-2 S1 and streptavidin conjugated secondary agent. No binding was detected with ACE2(HH:NN)-Fc constructs carrying the LALA-PG mutations on the hamster Fc γ Rs, while limited binding was detected with the ACE2(HH:NN) WT Fc.

A



B



Supplementary figure 4. Comparison of neutralisation efficiency

Neutralisation assay of SARS-CoV-2 pseudotype (A) or live virus (B) with ACE2(HH:NN)-Fc LALA-PG in PBS pH 7.4 (blue) and 20 mM His pH 6.5 (orange). Both formulations show comparable neutralisation efficiencies (Mean ± SD). Unpaired t test of AUC (A $t=0.000$, $df=32$; B $t=1.317$, $df=28$).

Supplementary Table 1 – Cell microarray antigens

Xlsx table in attachment file