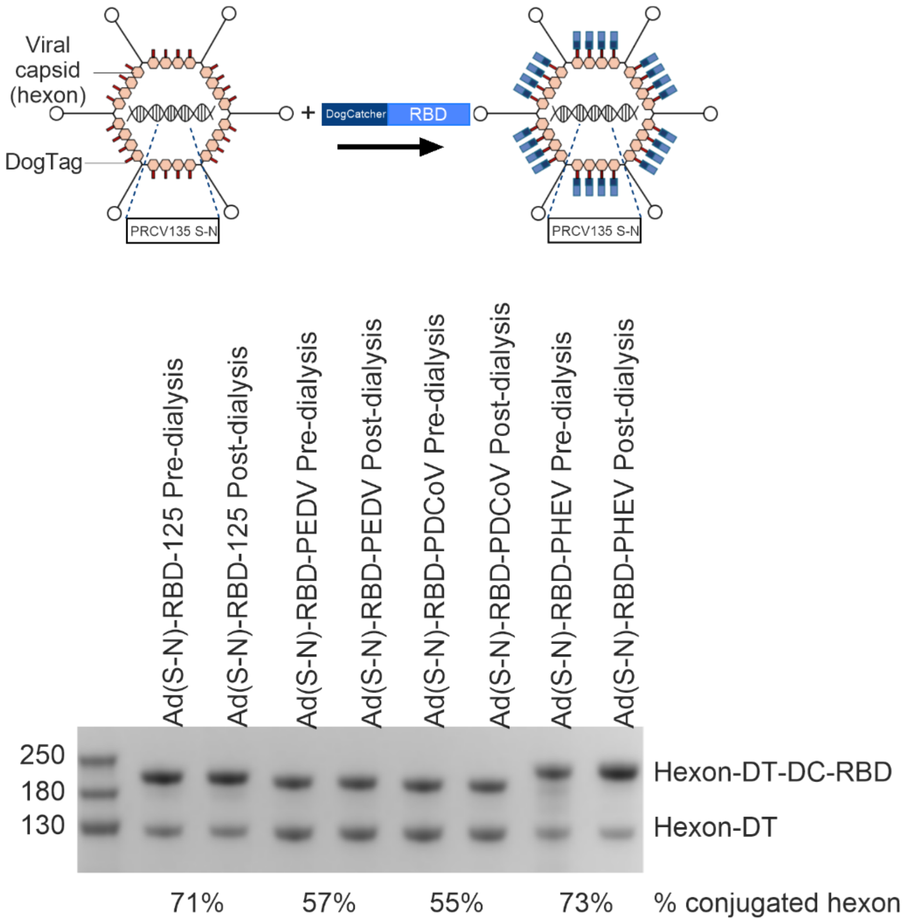
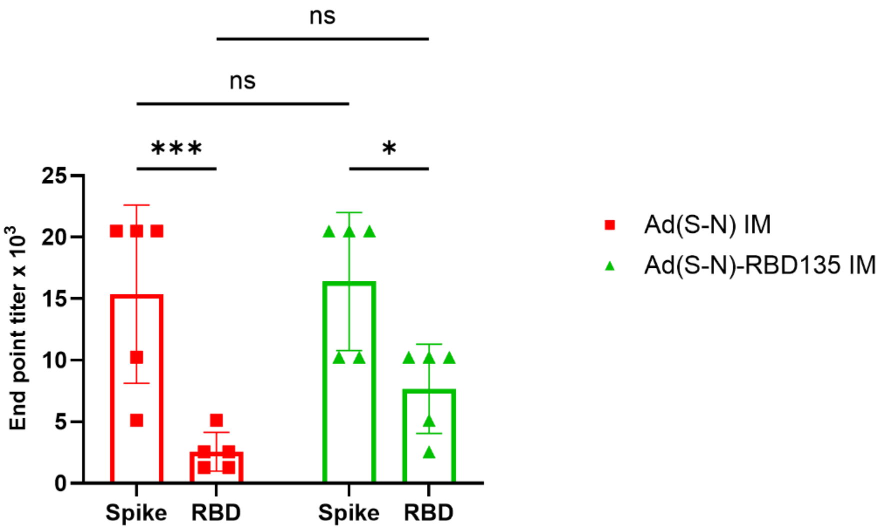
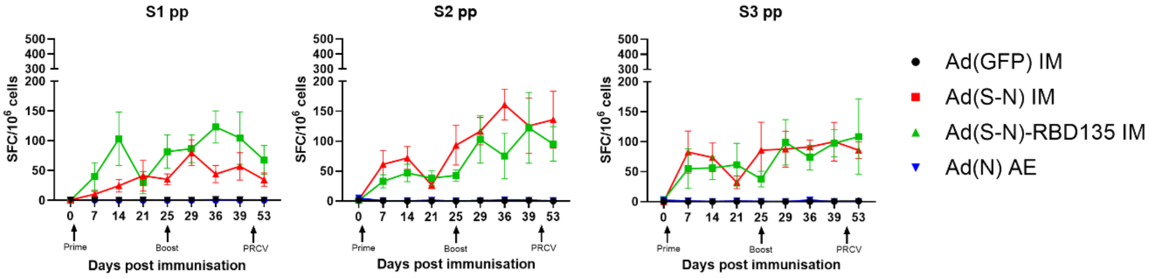


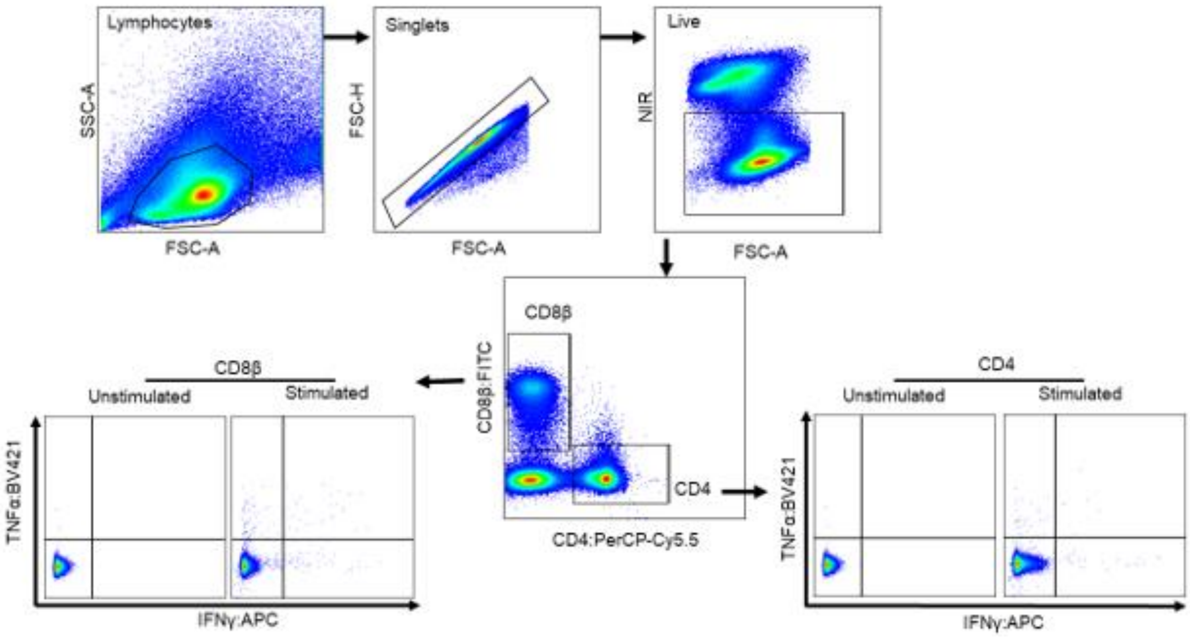
Supplementary Figure S1: Vaccine production. A) Schematic representation of modular display of DogCatcher-fused RBD on the surface of the adenovirus capsid via covalent conjugation with DogTag inserted into hexon surface loop 5 (HVR5). B) Representative SDS-PAGE and Coomassie blue staining analysis of Ad virions displaying DogTag at HVR5 (1E+10 viral particles), incubated with 3.5 μM of the 4 different DogCatcher-RBD proteins at 4 °C for 16 h (Pre-dialysis), followed by dialysis into sucrose storage buffer (Post-dialysis) to remove excess unbound DogCatcher-RBD, prior to immunisation.



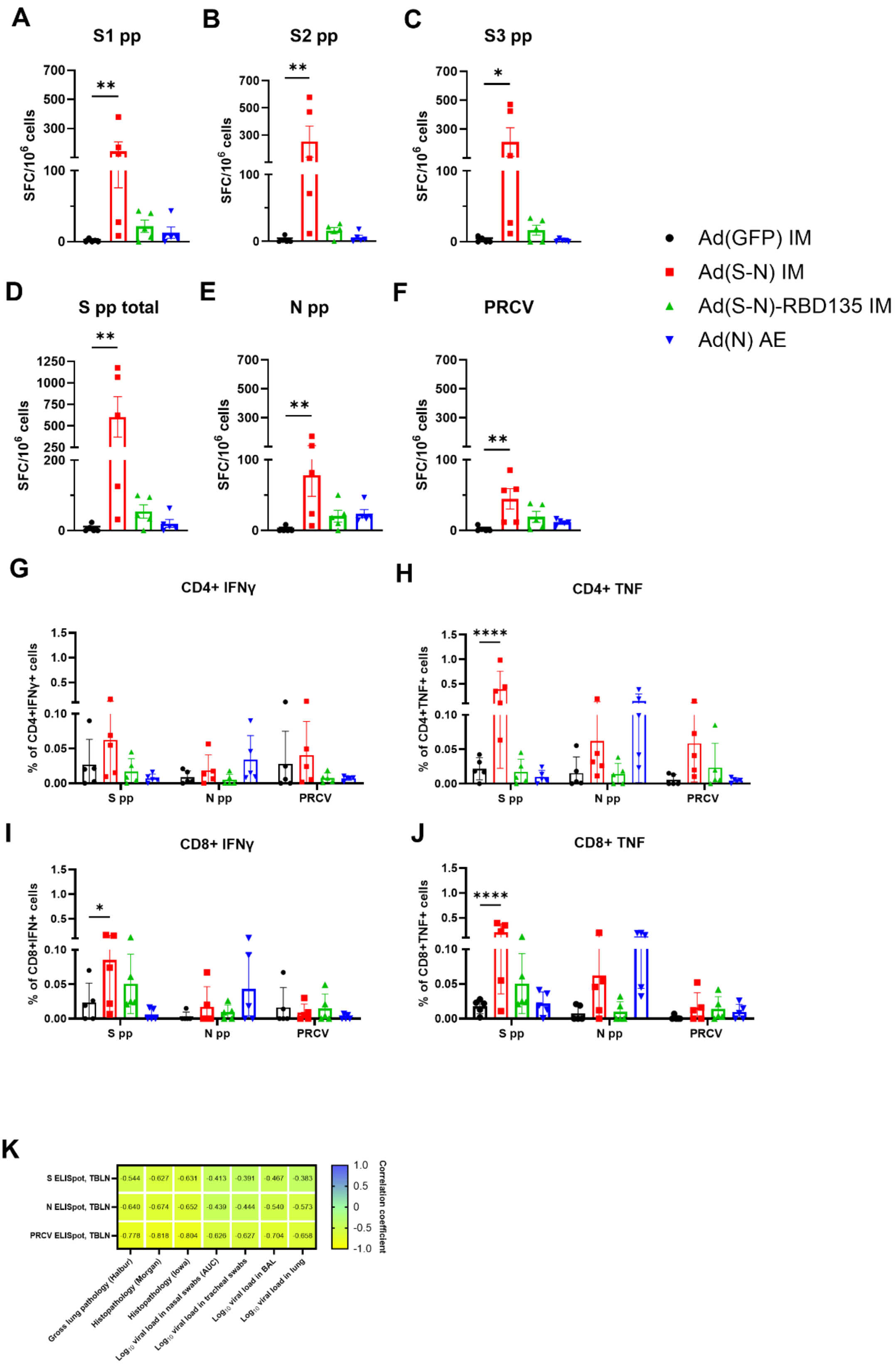
Supplementary Figure S2: RBD and full-length spike ELISA. Comparison between Spike-specific and RBD specific IgG response between S-N and S-N RBD group were analyzed by two-way ANOVA followed by Fisher LSD test.



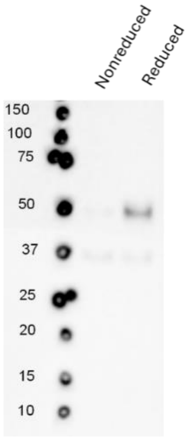
Supplementary Figure S3: IFNγ ELISPOT PBMC. Cells producing IFNγ in response to peptides spanning S were measured by ELISpot.



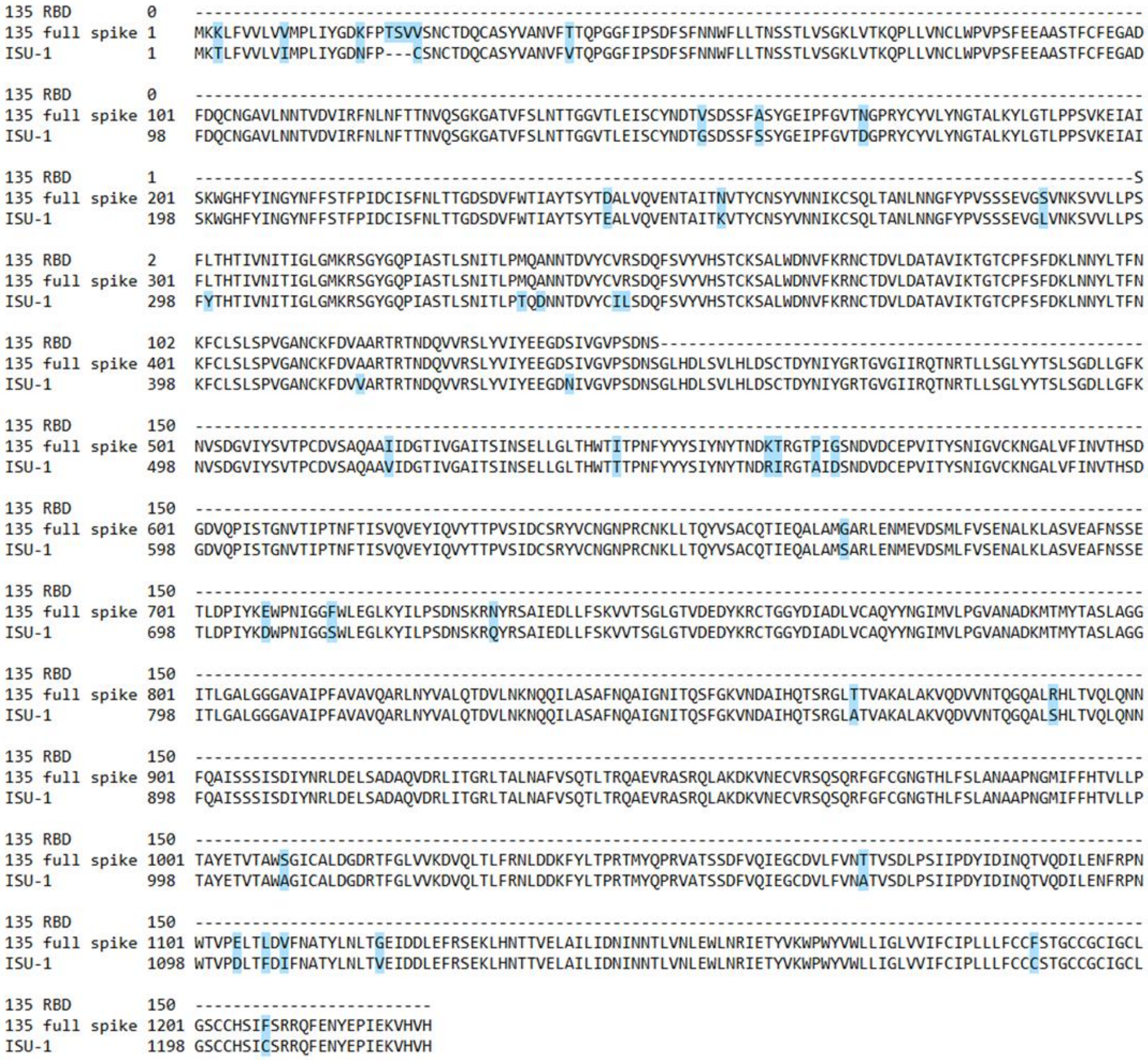
Supplementary figure S4: Gating strategy for intracellular staining of T cells. Cryopreserved BAL samples were thawed and stimulated with PRCV135, Spike or N peptide pools or medium. Flow cytometry staining was performed. Sequential gating strategy was applied on lymphocytes, singlets, live cells and CD4 or CD8-beta. The frequencies of IFNγ and TNF secreting CD4+ and CD8+ T cells were also analyzed.



Supplementary Figure S5: Cytokine responses in TBLN. Cells producing IFNγ in response to peptides spanning S (A-D) or N (E) proteins, or live PRCV 135 virus (F) were measured by ELISpot. Symbols in A-F represent mean spot-forming units per group, with error bars representing SEM. IFNγ- and TNFα-producing CD4+ and CD8+ T cells were measured following stimulation with whole virus or S or N peptides by intracellular cytokine staining (G-J). Data is presented as mean ± SEM, with symbols indicating individual animals (A-J). Normally distributed data (D, F) was analysed by one-way ANOVA followed by Dunnet's post-hoc test, while data not normally distributed was analysed by Kruskal Wallis and Dunn's multiple comparison test (A-C, E). ICS data were analyzed and compared using Two-way ANOVA and Dunnett's test (G-J). Significant difference to Ad-(GFP) control is indicated by * ($p < 0.05$), ** ($p < 0.01$), **** ($p < 0.0001$).



Supplementary Figure S6: Western blot analysis of PRCV135 N protein from unpurified supernatant. Expressed PRCV135 N protein was detected in supernatant by western blot following SDS-PAGE. N protein was detected using anti-C-tag, conjugated to biotin and probed with streptavidin-HRP. Presence of a band at approximately 50kDa indicated expression of N protein.



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135 RBD         0    ----------------------------------------------------------------------------------------------------
135 full spike 1     MKKLFVVLVVMPLIYGDKFPTSVVSNCTDQCASYVANVFTTQPGGFIPSDFSFNNWFLLTNSSTLVSGKLVTKQPLLVNCLWPVPSFEEAASTFCFEGAD
ISU-1           1    MKTLFVVLVIMPLIYGDNFP---CSNCTDQCASYVANVFVTQPGGFIPSDFSFNNWFLLTNSSTLVSGKLVTKQPLLVNCLWPVPSFEEAASTFCFEGAD

135 RBD         0    ----------------------------------------------------------------------------------------------------
135 full spike 101   FDQCNGAVLNNTVDVIRFNLNFTTNVQSGKGATVFSLNTTGGVTLEISCYNDTVSDSSFASYGEIPFGVTNGPRYCYVLYNGTALKYLGTLPPSVKEIAI
ISU-1           98   FDQCNGAVLNNTVDVIRFNLNFTTNVQSGKGATVFSLNTTGGVTLEISCYNDTGSDSSFSSYGEIPFGVTDGPRYCYVLYNGTALKYLGTLPPSVKEIAI

135 RBD         1    ---------------------------------------------------------------------------------------------------S
135 full spike 201   SKWGHFYINGYNFFSTFPIDCISFNLTTGDSDVFWTIAYTSYTDALVQVENTAITNVTYCNSYVNNIKCSQLTANLNNGFYPVSSSEVGSVNKSVVLLPS
ISU-1           198  SKWGHFYINGYNFFSTFPIDCISFNLTTGDSDVFWTIAYTSYTEALVQVENTAITKVTYCNSYVNNIKCSQLTANLNNGFYPVSSSEVGLVNKSVVLLPS

135 RBD         2    FLTHTIVNITIGLGMKRSGYGQPIASTLSNITLPMQANNTDVYCVRSDQFSVYVHSTCKSALWDNVFKRNCTDVLDATAVIKTGTCPFSFDKLNNYLTFN
135 full spike 301   FLTHTIVNITIGLGMKRSGYGQPIASTLSNITLPMQANNTDVYCVRSDQFSVYVHSTCKSALWDNVFKRNCTDVLDATAVIKTGTCPFSFDKLNNYLTFN
ISU-1           298  FYTHTIVNITIGLGMKRSGYGQPIASTLSNITLPTQDNNTDVYCILSDQFSVYVHSTCKSALWDNVFKRNCTDVLDATAVIKTGTCPFSFDKLNNYLTFN

135 RBD         102  KFCLSLSPVGANCKFDVAARTRTNDQVVRSLYVIYEEGDSIVGVPSDNS---------------------------------------------------
135 full spike 401   KFCLSLSPVGANCKFDVAARTRTNDQVVRSLYVIYEEGDSIVGVPSDNSGLHDLSVLHLDSCTDYNIYGRTGVGIIRQTNRTLLSGLYYTSLSGDLLGFK
ISU-1           398  KFCLSLSPVGANCKFDVVARTRTNDQVVRSLYVIYEEGDNIVGVPSDNSGLHDLSVLHLDSCTDYNIYGRTGVGIIRQTNRTLLSGLYYTSLSGDLLGFK

135 RBD         150  ----------------------------------------------------------------------------------------------------
135 full spike 501   NVSDGVIYSVTPCDVSAQAAIIDGTIVGAITSINSELLGLTHWTITPNFYYYSIYNYTNDKTRGTPIGSNDVDCEPVITYSNIGVCKNGALVFINVTHSD
ISU-1           498  NVSDGVIYSVTPCDVSAQAAVIDGTIVGAITSINSELLGLTHWTTTPNFYYYSIYNYTNDRIRGTAIDSNDVDCEPVITYSNIGVCKNGALVFINVTHSD

135 RBD         150  ----------------------------------------------------------------------------------------------------
135 full spike 601   GDVQPISTGNVTIPTNFTISVQVEYIQVYTTPVSIDCSRYVCNGNPRCNKLLTQYVSACQTIEQALAMGARLENMEVDSMLFVSENALKLASVEAFNSSE
ISU-1           598  GDVQPISTGNVTIPTNFTISVQVEYIQVYTTPVSIDCSRYVCNGNPRCNKLLTQYVSACQTIEQALAMSARLENMEVDSMLFVSENALKLASVEAFNSSE

135 RBD         150  ----------------------------------------------------------------------------------------------------
135 full spike 701   TLDPIYKEWPNIGGFWLEGLKYILPSDNSKRNYRSAIEDLLFSKVVTSGLGTVDEDYKRCTGGYDIADLVCAQYYNGIMVLPGVANADKMTMYTASLAGG
ISU-1           698  TLDPIYKDWPNIGGSWLEGLKYILPSDNSKRQYRSAIEDLLFSKVVTSGLGTVDEDYKRCTGGYDIADLVCAQYYNGIMVLPGVANADKMTMYTASLAGG

135 RBD         150  ----------------------------------------------------------------------------------------------------
135 full spike 801   ITLGALGGGAVAIPFAVAVQARLNYVALQTDVLNKNQQILASAFNQAIGNITQSFGKVNDAIHQTSRGLTTVAKALAKVQDVVNTQGQALRHLTVQLQNN
ISU-1           798  ITLGALGGGAVAIPFAVAVQARLNYVALQTDVLNKNQQILASAFNQAIGNITQSFGKVNDAIHQTSRGLATVAKALAKVQDVVNTQGQALSHLTVQLQNN

135 RBD         150  ----------------------------------------------------------------------------------------------------
135 full spike 901   FQAISSSISDIYNRLDELSADAQVDRLITGRLTALNAFVSQTLTRQAEVRASRQLAKDKVNECVRSQSQRFGFCGNGTHLFSLANAAPNGMIFFHTVLLP
ISU-1           898  FQAISSSISDIYNRLDELSADAQVDRLITGRLTALNAFVSQTLTRQAEVRASRQLAKDKVNECVRSQSQRFGFCGNGTHLFSLANAAPNGMIFFHTVLLP

135 RBD         150  ----------------------------------------------------------------------------------------------------
135 full spike 1001 TAYETVTAWSGICALDGDRTFGLVVKDVQLTLFRNLDDKFYLTPRTMYQPRVATSSDFVQIEGCDVLFVNTTVSDLPSIIPDYIDINQTVQDILENFRPN
ISU-1           998  TAYETVTAWAGICALDGDRTFGLVVKDVQLTLFRNLDDKFYLTPRTMYQPRVATSSDFVQIEGCDVLFVNATVSDLPSIIPDYIDINQTVQDILENFRPN

135 RBD         150  ----------------------------------------------------------------------------------------------------
135 full spike 1101 WTVPELTLDVFNATYLNLTGEIDDLEFRSEKLHNTTVELAILIDNINNTLVNLEWLNRIETYVKWPWYVWLLIGLVVIFCIPLLLFCCFSTGCCGCIGCL
ISU-1           1098 WTVPDLTFDIFNATYLNLTVEIDDLEFRSEKLHNTTVELAILIDNINNTLVNLEWLNRIETYVKWPWYVWLLIGLVVIFCIPLLLFCCCSTGCCGCIGCL

135 RBD         150  -------------------------
135 full spike 1201 GSCCHSIFSRRQFENYEPIEKVHVH
ISU-1           1198 GSCCHSICSRRQFENYEPIEKVHVH
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Supplementary Figure S7: Sequence alignment of ISU-1 and 135 spike proteins. Amino acid sequences for the 135 and ISU-1 spike proteins were aligned using MUSCLE. Highlighted amino acids differ from the consensus sequence.