

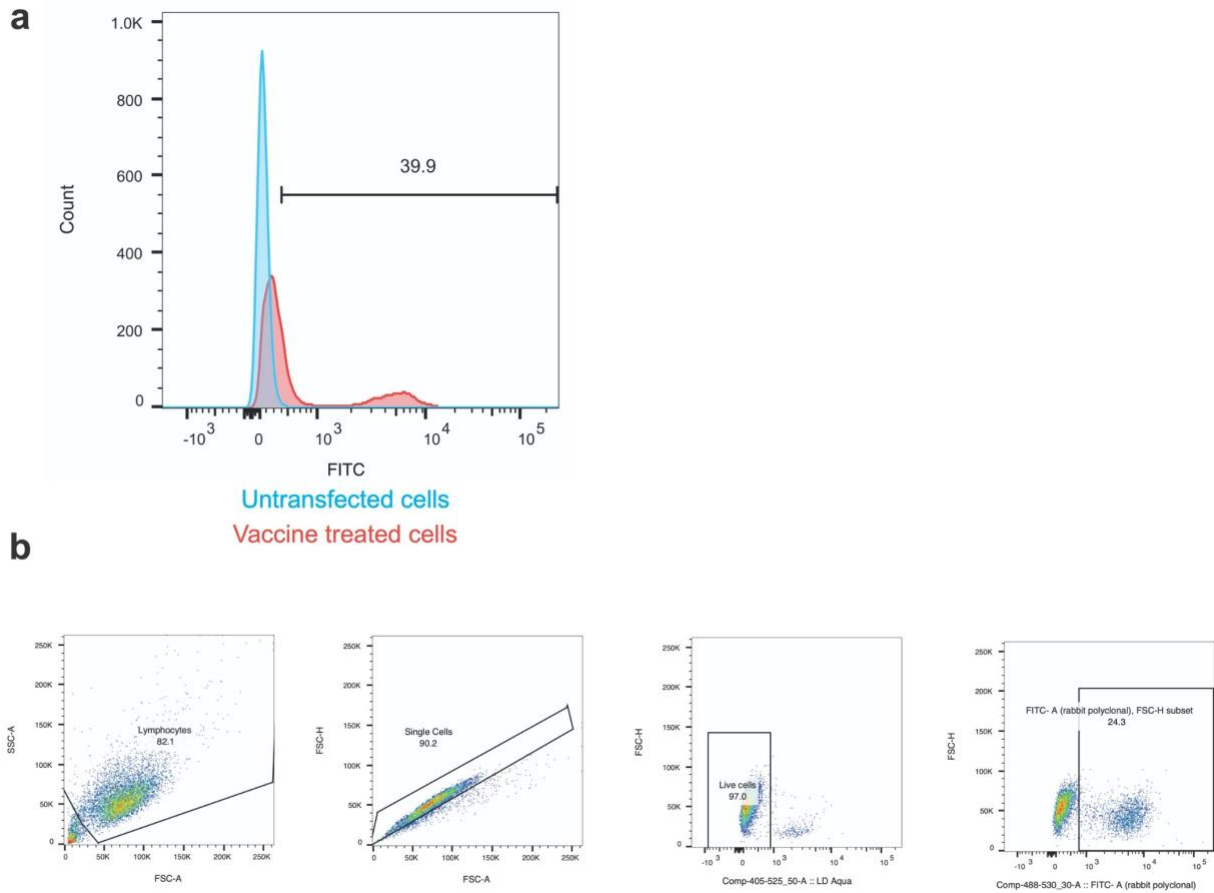
**Supplementary Information for:**  
**Self-amplifying RNA SARS-CoV-2 lipid nanoparticle vaccine candidate induces high  
neutralizing antibody titers in mice**

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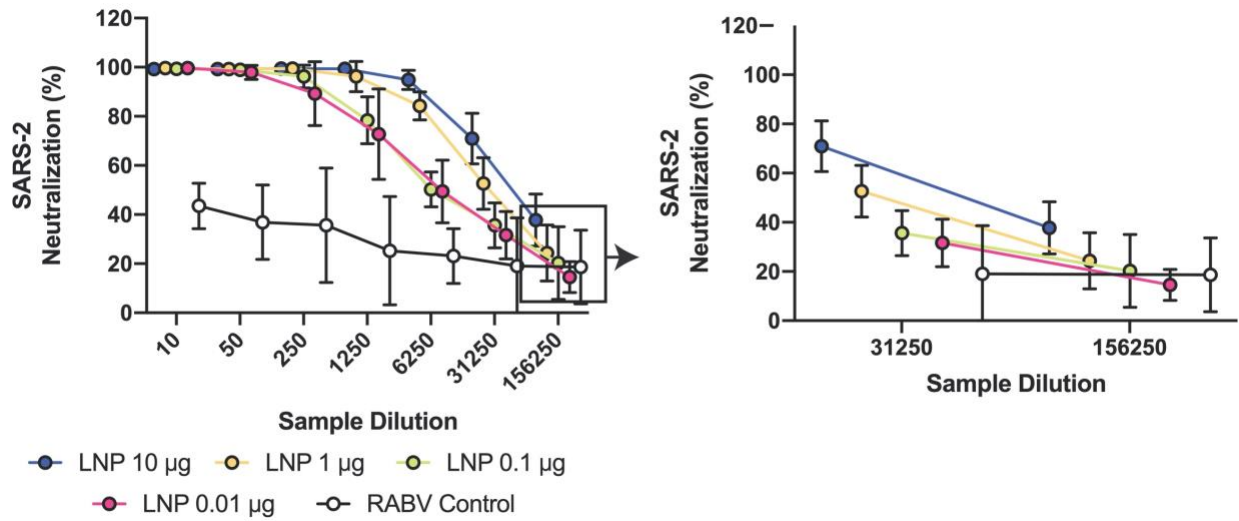
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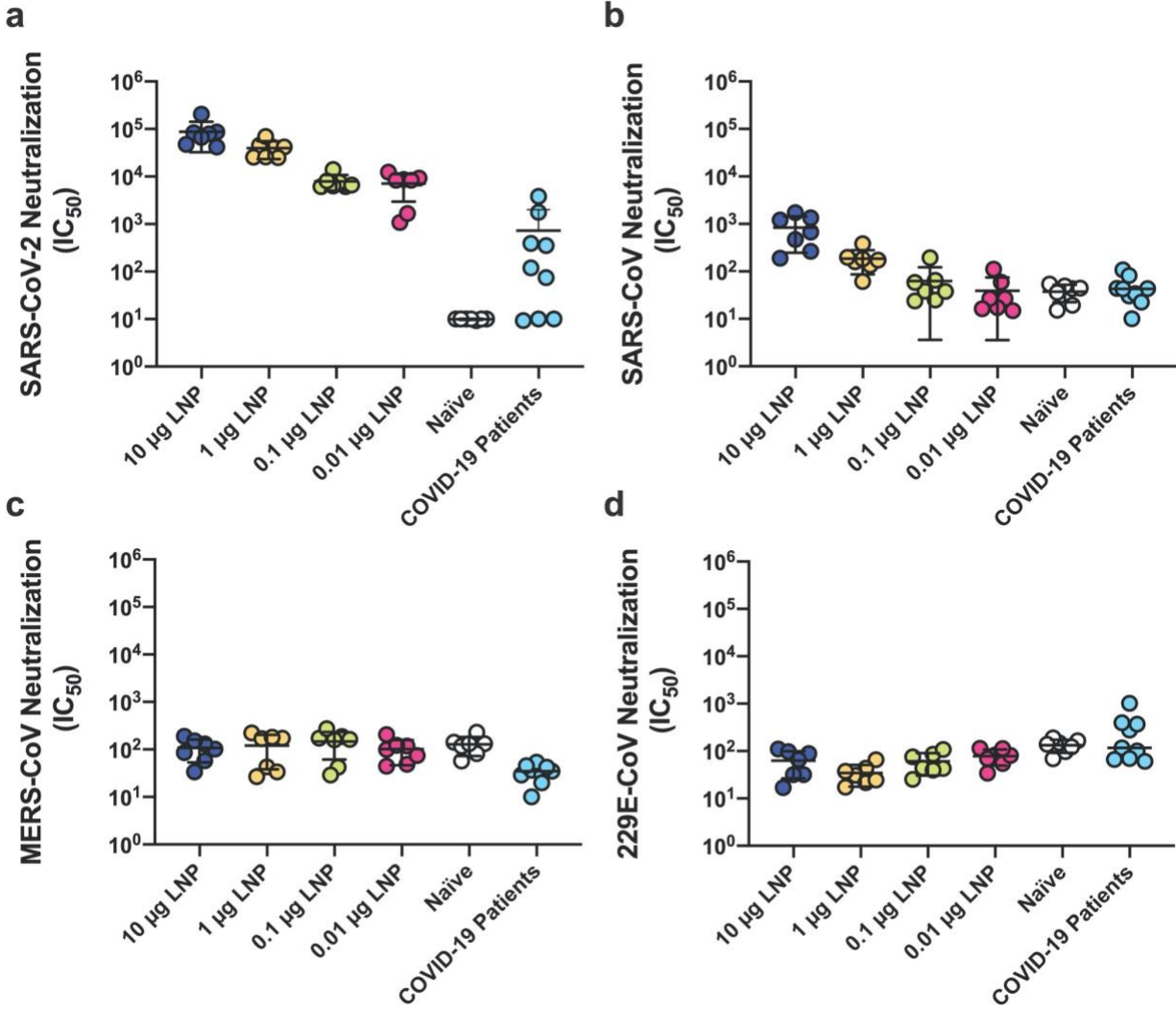
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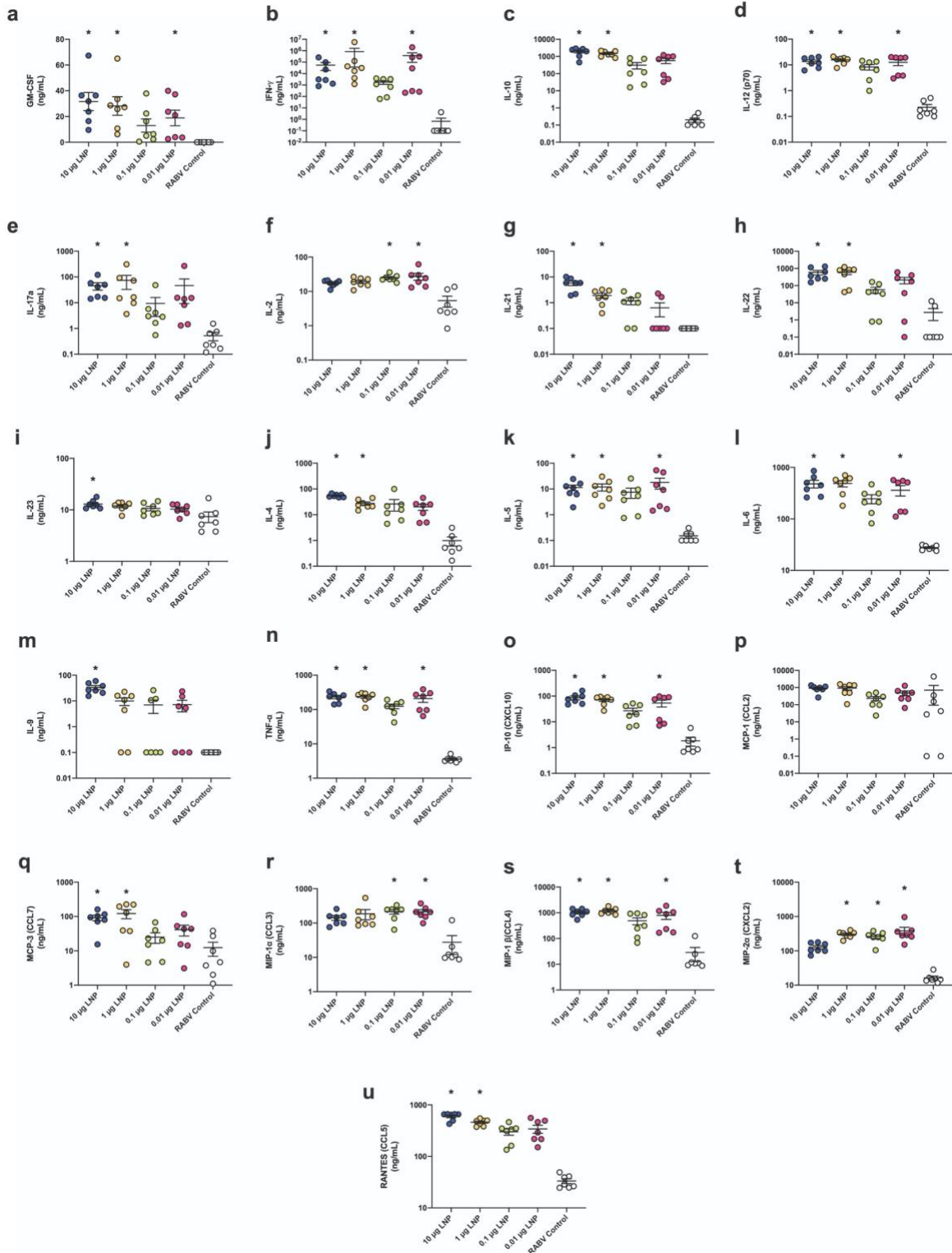
**Supplementary Figure 1.** Flow cytometry of HEK293T.17 cells transfected with saRNA expressing membrane bound SARS-CoV-2 spike protein. HEK293T.17 cells were transfected with 1  $\mu$ g of saRNA encoding a membrane bound SARS-CoV-2 spike protein complexed with Lipofectamine MessengerMAX™ and stained with a fixable aqua live/dead stain, as well as SARS-CoV spike protein polyclonal antibody as a FITC-labelled secondary antibody.



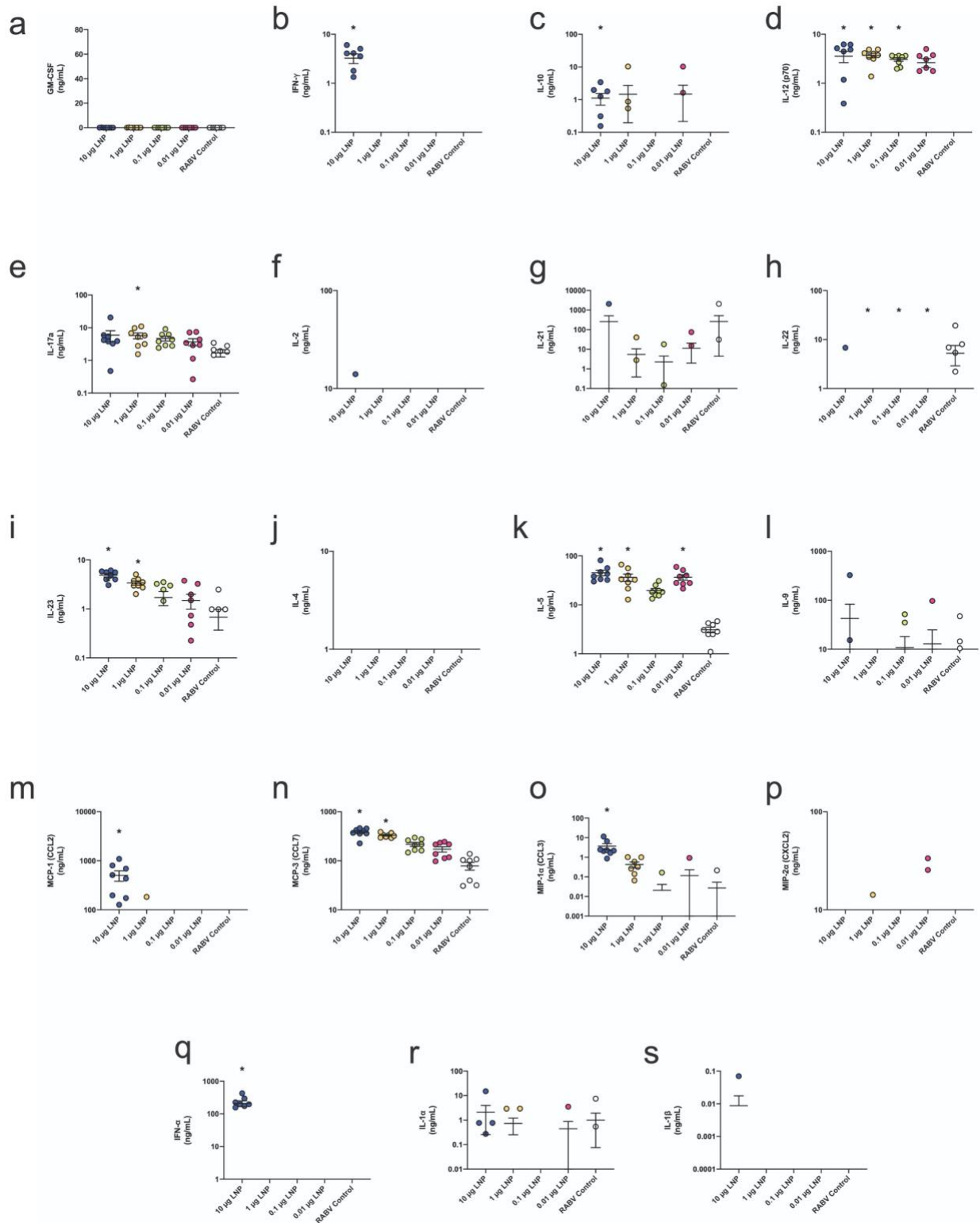
**Supplementary Figure 2.** Quantification of antibody-dependent enhancement (ADE) via wild type virus neutralization of sera from BALB/c mice vaccinated with doses of LNP-formulated saRNA ranging from 0.01-10 µg of saRNA with n=7 biologically independent animals. Line and error bars indicated mean ± SD.



**Supplementary Figure 3.** Pseudotyped virus neutralization of sera from BALB/c mice vaccinated with LNP formulations (n=7 biologically independent animals) and human patients (n=9 biologically independent samples) after recovery from COVID-19. Line and error bars indicated mean  $\pm$  SD.



**Supplementary Figure 4.** Cytokine response of re-stimulated splenocytes of vaccinated mice (n=7 biologically independent animals) with recombinant SARS-CoV-2 protein. \* indicates significance of  $p < 0.05$  as determined by a Kruskal-Wallis test. Line and error bars indicated mean  $\pm$  SD.



**Supplementary Figure 5.** Cytokine response in the sera of mice (n=7 biologically independent animals) 4 hours after vaccination with SARS-CoV-2 LNP vaccine. \* indicates significance of  $p < 0.05$  as determined by a Kruskal-Wallis test. Line and error bars indicated mean  $\pm$  SD.

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**Supplementary Figure 6.** Sequence of saRNA encoding NSP1-4 of VEEV and the spike glycoprotein of SARS-CoV-2.

**Supplementary Table 1.** Primers used for sequencing of SARS-CoV-2 saRNA vector.

<b>Sequencing Primer Name</b>	<b>Sequence</b>
nCoV FOR_1	AGAGCCTGCTGATCGTGAAC
nCoV FOR_2	TACCTGACACCTGGCGATAG
nCoV FOR_3	TGTCCCCTACCAAGCTGAAC
nCoV FOR_4	GAAGTCTGTCATGCCCCCT
nCoV FOR_5	CGCCCAAGTGAAGCAGAT
nCoV FOR_6	CGAGAACCAGAAGCTGATCG
nCoV FOR_7	ATGAGCTTCCCTCAGTCTGC
nCoV FOR_8	AAGAGATCGACCGGCTGAA
nCoV REV_1	GTGGTAGTAGACGCCCAGGA
nCoV REV_2	TCAGCAGGAAGGTTCTAGGC
nCoV REV_3	CAATCTGCCGCACTTCATCT
nCoV REV_4	CAGGCCGTTGAAGTTGAAGT
nCoV REV_5	GGCTGTTTGTCTGTGTCTGG
nCoV REV_6	TGCTAGGATCGGGCAGAAT
nCoV REV_7	GCGCTTGCTGTGCTGCTC
nCoV REV_8	GCGGTGGTCAAATTCTTCTC
nCoV REV_9	GGGCACTTGATGTACTGCT
VEEV sgp FW	ATCATTCAGCTACCTGAGAGG
VEEVrep-Not1-Rev	CTATGTAAGCAGCTTGCCAATTC