

Potent neutralization of clinical isolates of SARS-CoV-2 D614 and G614 variants by a monomeric, sub-nanomolar affinity Nanobody.

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Supplemental Figure Legends

Supplemental Figure-1. Evaluation of the alpaca's immune response by ELISA. Sera before and after the second immunization (3 weeks) have been diluted as indicated in the figure and total IgG1, IgG2 & IgG3 have been evaluated to recognize full length Spike before and after immunization, n=4, error bars indicate standard deviation.

Supplemental Figure-2. Controls and expression. A) Immunofluorescence assays of Spike-GFP transfected HeLa cells using purified recombinant W25UACH nanobody as primary antibody. **B)** Immunofluorescence assays of Nucleoprotein- GFP transfected HeLa cells using purified recombinant W25UACH nanobody as primary antibody.

Supplemental Figure-3. Original scan and supplemental neutralization table. A) Original figure 2a. **B)** original Figure 3a **C)** Zoom image of W25 inflection of Figure 2b, **D)** Summary table of neutralizing activity of W25 compared to previously reported Nbs

Nanobodies Sequences

Nucleotide sequences W23 Nanobody

ATGGCTCAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGAGTCTCTGAGACTCTC
CTGTGCAGCCTCTGGAAACATCTTCGGAATCGCTGCCGTGCACTGGTTCCGCAAGGCTCCAGGGAAGGA
GCGCGAGTTTACTGCAGGTTTTGGTAGTGATGGTAGCACAACTATGCAAACCTCCGTGAAGGGCCGATT
CACCATCTCCAGAGACAATGCCAAGAACACGACATATCTGCAAATGAACAGCCTGAAACCTGAGGACAC
GGCCGTCTATTATTGTCATGCGCTAATCAAGAATGAACTTGGATTCTTGATTACTGGGGCCCGGGGACC
CAGGTCACCGTCTCCTCA

Amino acid sequences W23 Nanobody

MAQVQLVESGGGLVQPGESLRLSCAASGNIFGIAAVHWFRKAPGKEREF TAGFGSDGSTNYANSVKGRFTIS
RDNAKNTTYLQMNSLKPEDTAVYYCHALIKNELGFLDYWGPGTQVTVSS

Nucleotide sequences W25 Nanobody

ATGGCTCAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGAGTCTCTGAGACTCTC
CTGTGCAGCCTCTGGAAAGTATCTTCGGAATCTATGCCGTGCACTGGTTCCGCATGGCTCCAGGGAAGGA
GCGCGAGTTTACTGCAGGTTTTGGAAGTCATGGTAGCACAAATTATGCAGCTTCCGTGAAGGGACGATT
CACCATGTCCAGAGACAATGCCAAGAACACGACGTATCTGCAAATGAACAGCCTGAAACCTGCGGACAC
GGCCGTCTATTACTGTCATGCGCTAATAAAGAATGAACTTGGGTTCTTGACTACTGGGGCCCGGGGAC
CCAGGTCACCGTCTCCTCA

Amino acid sequences W25 Nanobody

MAQVQLVESGGGLVQPGESLRLSCAASGSIFGIYAVHWFRMAPGKEREF TAGFGSHGSTNYAASVKGRFT
MSRDNAKNTTYLQMNSLKPADTAVYYCHALIKNELGFLDYWGPGTQVTVSS