

**OMTM, Volume 24**

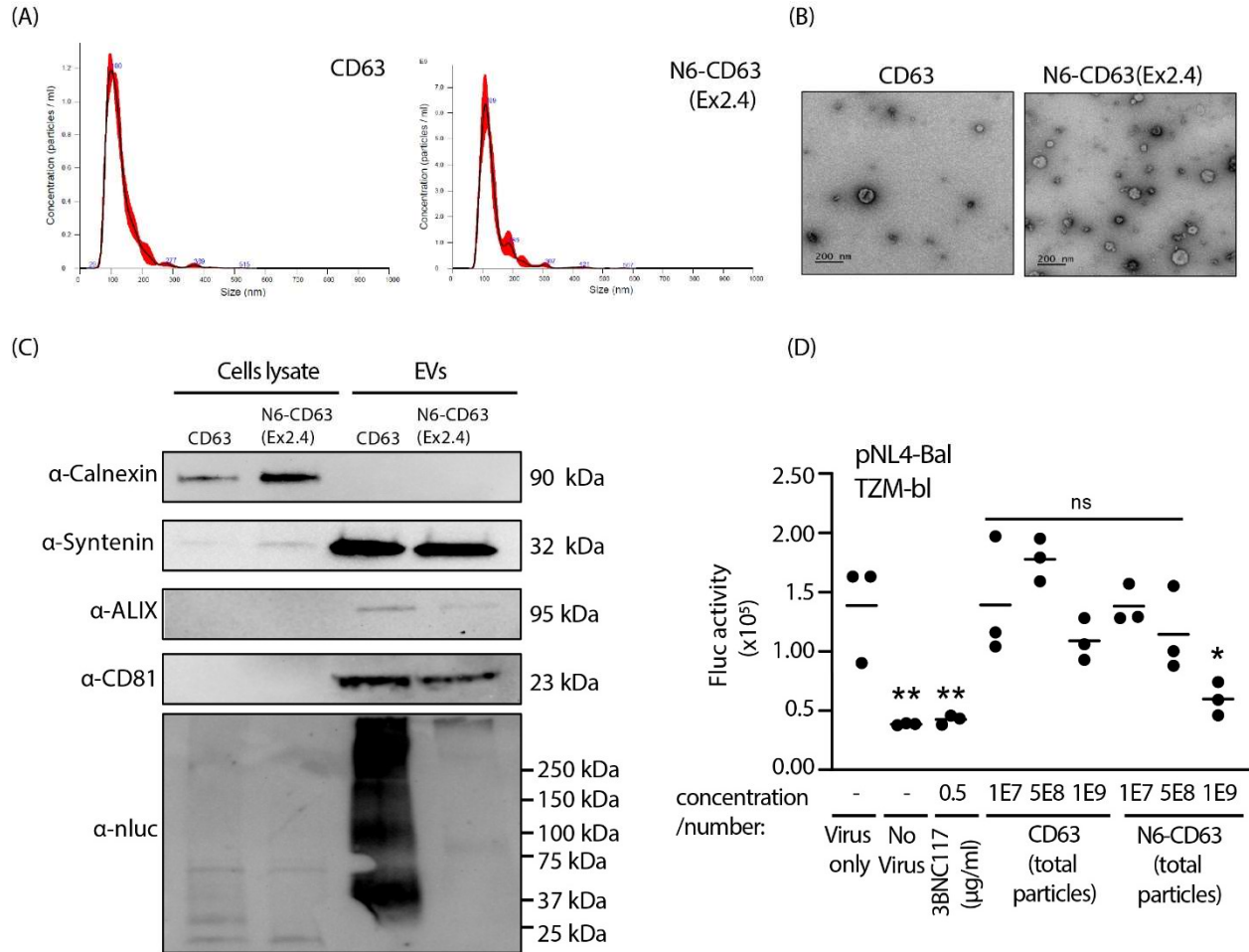
**Supplemental information**

**Engineered extracellular vesicles directed  
to the spike protein inhibit SARS-CoV-2**

**Tristan A. Scott, Aroon Supramaniam, Adi Idris, Angelo A. Cardoso, Surya Shrivastava, Gabrielle Kelly, Nicole A. Grepo, Citradewi Soemardy, Roslyn M. Ray, Nigel A.J. McMillan, and Kevin V. Morris**

## Supplemental information

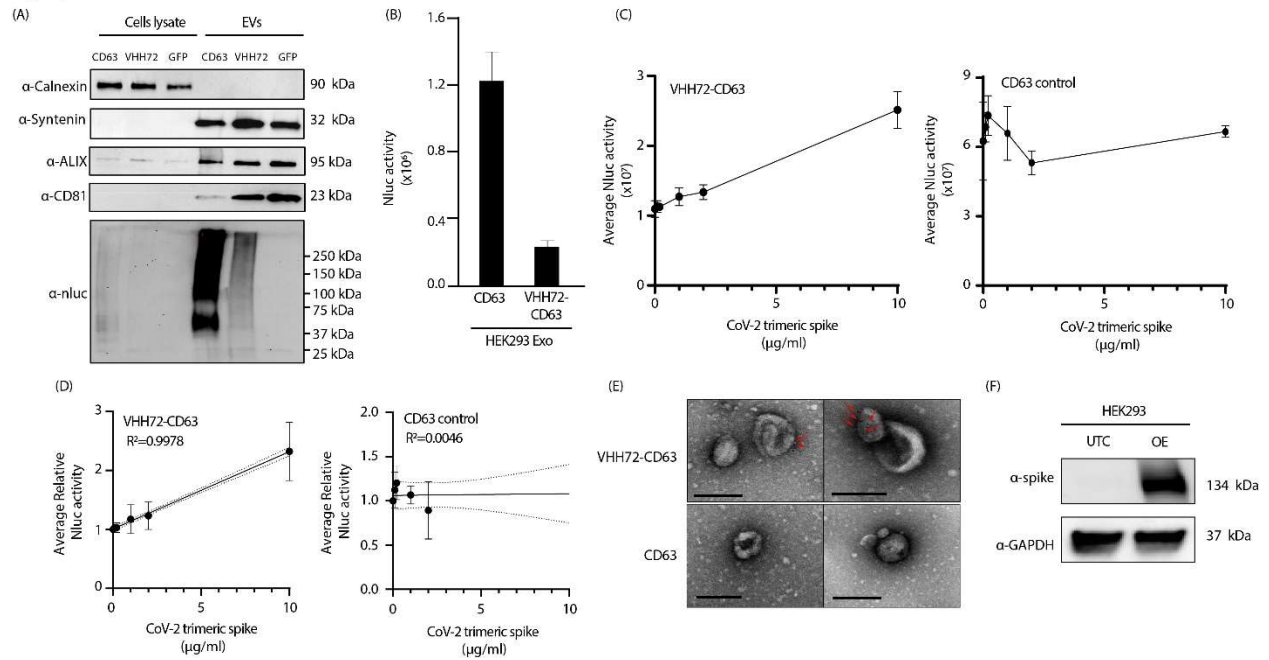
Supp. Figure 1



**Figure S1: Characterization of the N6-CD63 EVs.** (A) NTA and (B) TEM analysis for the CD63 control and N6-CD63 EVs. (C) EVs and cell lysates from CD63 and N6-CD63 samples were assessed by western blot for known EV markers (Syntenin, ALIX, CD81) and an exclusion marker, Calnexin, as well as Nluc, a component of the CD63 fusion protein. (D) Increasing amounts of the CD63 or N6-CD63 EVs were incubated with infectious pNL4-Bal HIV-1 (MOI 0.2) and then TZM-bl cells were infected with the EV:virus mixture. The levels of Fluc luciferase activity were assessed at 48 hrs post-infection. The line represents the mean from an experiment performed in

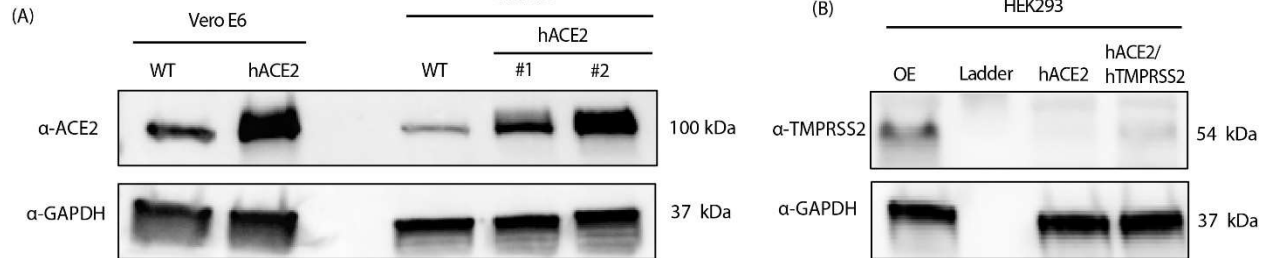
triplicate. The p-values were generated using a one-way ANOVA compared to the virus only control (D) (\*p<0.05,\*\*p<0.01).

Supp. Figure 2



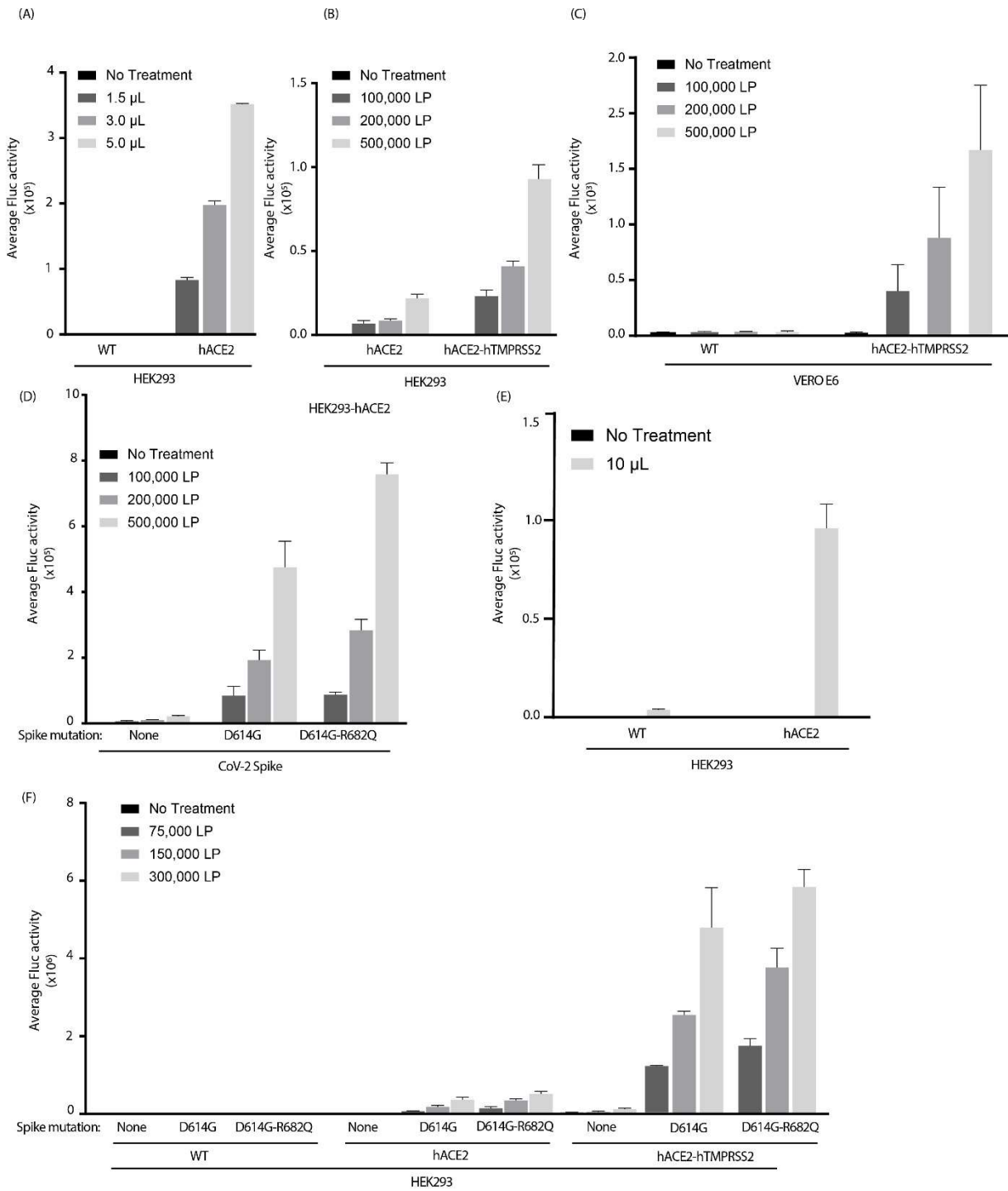
**Figure S2: Characterization of the VHH72-CD63 EVs.** (A) EVs and cell lysates from CD63 and VHH72-CD63 samples were assessed by western blot for inclusion EV markers (Syntenin, ALIX, CD81) and an exclusion marker, Calnexin, and a component of the CD63 fusion protein (Nluc). EVs generated from HEK293 cells transfected with a GFP vector were included as a Nluc detection control. (B) The levels of Nluc activity from equal amounts of CD63 and VHH72-CD63 EVs. Error bars were generated by samples detected in triplicate. (C) Average Nluc activity of EVs bound to spike beads and (D) non-linear line regression of Nluc activity versus the amount of CoV-2 trimeric spike. (E) Additional TEM images of the VHH72-CD63 and CD63 EVs bound to gold-nanoparticle labelled SARS-CoV-2 RBD. Red arrows highlight bound gold particles. Scale bar represents 200 nm. (F) HEK293 cells were transfected with a SARS-CoV-2 spike expression vector and western blot analysis was performed to detect protein levels compared to a untransfected (UTC) control. GAPDH was detected as a loading control.

Supp. Figure 3



**Figure S3: Detection of receptors in stable cell lines.** Protein expression analysis on Vero E6 or HEK293 cells that stably expressing (A) hACE2 and (B) hTMPRSS2. HEK293-hACE2 #1 and #2 represent two different clones that stably expressing hACE. OE = cells transfected with an overexpression hTMPRSS2 vector. GAPDH was detected as a loading control. Expected molecular weights are indicated.

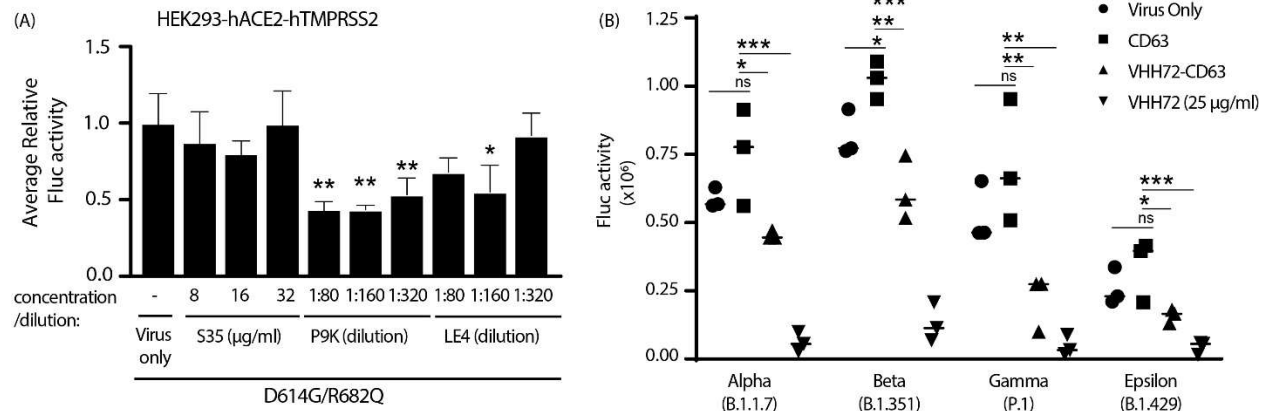
Supp. Figure 4



**Figure S4: Optimization of transduction using COV-2 S pseudotyped vectors. (A)** A lentiviral vector containing a GFP-Fluc pseudotyped with WT spike was transduced in increasing volumes

on WT HEK293 or HEK293-hACE2 cells. **(B)** Increasing amounts of WT spike lentiviral particles were used to transduced HEK293-hACE2 and HEK293-hACE2-hTMPRSS2. **(C)** Increasing amounts of WT spike lentiviral particles were used to transduced Vero E6-ACE2-TMPRSS2 cells. **(D)** A lentiviral vector was pseudotyped with WT spike, spike-D614G, or spike-D614G-R682Q and transduced in increasing lentiviral particles amounts on HEK293-hACE2 cells. **(E)** Lentiviral vectors pseudotyped with a C-terminal truncated spike protein were transduced on HEK293 WT or HEK293-hACE2 cells. **(F)** An experiment combining all the above relevant optimized conditions. In the above experiments, the lentiviral particles were packaged with a GFP-Fluc transgene and the levels of luciferase were assessed at 48 hrs after transduction. Error bars represent standard deviation generated by samples treated in quadruplicate.

Supp. Figure 5



**Figure S5: Neutralization assays using VHH72-CD63 EVs.** **(A)** The lentiviral particles pseudotyped with the D614G-R682Q spike were incubated with the S35 mAb or CCP (P9K and LE4) at the described concentration/dilutions and then HEK293-hACE2-hTMPRSS2 cells were transduced. Error bars represent standard deviation from an experiment performed in triplicate. The p-values were generated using a one-way ANOVA compared to the virus only control (\* $p < 0.05$ , \*\* $p < 0.01$ ). **(B)** The pseudotyped lentiviral particles with the spike proteins from the Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), and Epsilon (B.1.429) variants were incubated with 2E8 EVs and then HEK293-hACE2-hTMPRSS2 cells were transduced. The line represents the mean from samples treated in triplicate. The p-values were generated using a one-way ANOVA compared to CD63 control (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ). For **(A)** and **(B)**, the levels of Fluc were assessed at 48 hrs post-transduction.



**Supplemental Table 1:** Primer sequences used for cloning procedures.

<b>oligomer</b>	<b>Sequence (5'-3')</b>	<b>nt</b>
Spike-F	GCAGGGCAACTTCAAGAACCTGA	23
D614G-R	CAGGCACCTCAGTACAGTTCAC <sub>tc</sub> CCTGGTAGAGCACAGCCACC	44
D614G-F	GTGAACTGTACTGAGGTGCCTG	22
Spike-R	TGTGCTGTCTCCACAGATATACATTGTAC	29
R682Q-F	CAGACCCAGACCAACAGCCCA <sub>ca</sub> GAGGGCAAGGTCTGTGGCAA	43
R682Q-R	GGGCTGTTGGTCTGGGTCTG	20
Spike-F2	CTGTCTCATCATTTTGGCAAAGAATTCGCCACCATG <sub>ttc</sub> GTGTTTTT GGTCCTCTTGCC	59
Spike-R2	GTGTCCACTCCCAGGTCCAAGTTTAAACTTTAATACGACTCACTA TAGGGGCCGCCACCAAGCTTGGTACCATGTTTGTGTTCCCTGGTGC TGC	93
tCD4-D1D2- CD63 F	AGCTGGCTAGCGTTTAAACTTAAGCTTGCCACCATGGTCCGAGGG GTACCGTTTCGAC	58
tCD4-D1D2- CD63-R	TTTCATTCCTCCTTCCACCGCCATGGACCCGCCTCCGCCGAAGAA GATGCCCAGGCCGA	59

**Supplemental Table 2:** gBlock sequences used for cloning procedures.

gBlock	Sequence (5'-3')
hACE2-T2A-puromycin	CGAAAAAGAGGAAGCGGAGAGGGCAGGGGGAGTCTGTTGACCTGTGGGG ACGTAGAAGAGAACCCGGGTCCAATGACTGAGTACAAACCTACTGTTTCGC TTGGCTACTCGAGATGATGTACCTAGAGCGGTTTCGCACCCTGGCCGCGGC TTTCGCGGATTACCCAGCCACCAGGCACACGGTGGACCCAGACAGACACA TTGAGCGAGTGACTGAACTTCAGGAACTTTTCTGACGCGGGTTGGACTC GACATCGGTAAAGTTTGGGTGGCAGATGACGGCGCAGCCGTCGCAGTTTG GACTACCCCTGAATCAGTCGAAGCAGGCAGTGTTTGTGAAATCGGTC CGCGGATGGCCGAACCTTAGTGGGTACGACTCGCGGCACAACAACAGATG GAGGGTCTCCTTGCGCCACATCGACCTAAGGAACCCGCGTGGTTCCTTGC AACCGTAGGTGTCTACCCGACCATCAAGGGAAAGGTCTTGGAAGTGCAG TGGTGCTGCCGGCGTGGAAGCGGCTGAGCGGGCTGGGGTACCAGCCTTC CTGGAAACTTCTGCCCAAGAAACCTGCCGTTTTACGAGAGGCTGGGGTT TACTGTGACAGCCGACGTGGAGGTACCCGAGGGACCGAGGACGTGGTGTA TGACGCGAAAGCCCGGTGCTTAGTAAGCGGCCGCCCGGGCTGCAGGAAT TCGA
hTMPRSS2-T2A-Hygomycin	AGCGTTTAAACGGGCCCTCTAGAGCCACCATGGCGTTGAACTCAGGTAGC CCGCCCGCGATTGGTCCGTAATGAGAACCACGGATACCAACCTGAGAA CCCTTACCCGGCACAGCCACGGTCGTCGACCGTCTATGAAGTCCATCC CGCCCAATACTACCCTTACCCGTCGTCGAGTATGCTCCGAGAGTTCTCAC ACAAGCTAGCAACCCAGTGGTATGCACTCAACCAAGTCACCTAGTGGGA CCGTTTGTACCTCCAAAATAAAAAGGCTCTTTGTATAACACTTACACTTG GAACGTTCTTGGTTGGGGCAGCTCTTGCGGCGGGCCTGCTCTGGAAATTTA TGGGAAGCAAGTGTAGTAATTCCGGAATTGAGTGCGACTCCAGCGGTACA TGCATAAATCCGTCCAACCTGGTGTGACGGCGTTTCACATTGTCCCGGTGGC GAAGACGAGAATCGGTGTGTGACGGCTCTACGGACCTAATTTTATACTGCA AGTGTACAGCAGTCAGAGGAAGTCATGGCATCCCGTTTGCCAGGACGACT GGAATGAAAATATGGTTCGCGCTGCATGTGAGATATGGGGTACAAAAAT AATTTTTATTATCCAGGGCATCGTAGATGACTCCGGTAGCACGAGCTTC ATGAAACTGAATACAAGCGCTGGGAACGTTGACATTTACAAGAAGCTGTA CCACTCTGACGCTTGTTTCTAAGGCGGTGGTTAGCTTGCCTGCATTGC CTGTGGAGTAAATTTGAATTCTAGCCGACAAAGTCGCATTGTTGGGGGAG AAAGTGCTTTGCCGGCGCTTGGCCATGGCAGGTATCTTTGCATGTGCAG AACGTCCACGTGTGCGGGGGGAGTATCATAACCCAGAAATGGATTGTGAC GCGGCGCATTGTGTGAAAAGCCTCTGAACAATCCATGGCACTGGACTG CTTTCGCCGGGATCCTCAGGCAATCATTTATGTTCTATGGTGCCGGGTATC AAGTTGAGAAGGTCATTAGTCATCCGAACTACGATTCCAAAATAAGAAC AACGACATCGCATTGATGAAGCTGCAGAAGCCTCTGACTTTCAACGACCT TGTAAGCCAGTCTGCCTTCCCTAATCCTGGAATGATGCTCCAGCCTGAACA ACTTTGCTGGATCAGCGGATGGGGCGCCACCGAGGAAAAGGGTAAGACTT CCGAAGTCCTTAATGCGGCTAAGGTACTCCTGATTGAGACTCAGAGATGC AATTCCAGATATGTATATGACAACCTGATCACTCCGGCGATGATTTGTGCC GGCTTCTGCAAGGCAATGTAGACTCCTGTCAGGGCGATTCCGGTGGACC TTTGGTTACCTCAAAGAACAACATATGGTGGTTGATCGGCGATACTTCTTG GGGGAGCGGTTGTGCAAAGGCTTATCGGCCCGGTGTGTACGGGAATGTCA TGGTCTTTACGGACTGGATATATAGACAAATGAGGGCCGACGGCACTCGC CGAAAACGGGGTAGCGGCGAAGGTCGAGGCTCCTTGCTGACCTGTGGGGA TGTGGAAGAAAATCCGGGCCCGATGAAAAGCCCGAACTCACCGCTACTT

CAGTAGAAAAATTCTGATAGAGAAGTTTGATTTCAGTTTCTGATCTCATGC  
 AGCTTTCTGAAGGTGAAGAATCTCGGGCTTTTTTCATTTCGATGTAGGTGGCC  
 GGGGATATGTTCTCCGAGTGAATTCTTGGCGCTGACGGTTTCTACAAAGACC  
 GATACGTATATCGACATTTTCGCTAGTGCGGCCCTCCCCATCCCGGAAGTCC  
 TGGATATTGGGAATTTTCCGAGTCCCTTACATACTGTATCTCCCGACGAG  
 CACAAGGTGTCACACTCCAAGACCTCCCGGAGACCGAGCTGCCAGCTGTT  
 CTGCAACCCGTAGCCGAGGCAATGGATGCGATTGCCGCTGCGGATTTGAG  
 CCAGACGAGCGGCTTTGGACCATTCCGACCGCAGGGTATCGGTCAATATA  
 CGACCTGGCGCGACTTTATTTGCGCAATAGCTGACCCCCACGTGTACCATT  
 GGCAAACGTGTGATGGACGACACAGTCTCTGCTTCCGTAGCACAGGCACTG  
 GACGAACTCATGCTTTGGGCTGAAGACTGTCCGGAGGTGCGGCATCTGGT  
 GCATGCCGACTTTGGATCAAACAACGTTCTGACAGACAATGGAAGGATTA  
 CGGCAGTGATAGATTGGAGTGAGGCTATGTTTGGGGATTCCCAGTACGAG  
 GTTGCAAACATTTTCTTCTGGAGGCCATGGTTGGCCTGTATGGAGCAACAG  
 ACACGATATTTTCGAGCGCAGGCACCCTGAGCTTGCTGGATCCCCCGATT  
 GAGAGCATAACATGTTGCGGATAGGGCTCGATCAGCTGTATCAATCCCTGG  
 TAGACGGCAATTTTCGACGACGCGGCCTGGGCGCAGGGTAGGTGTGATGCC  
 ATAGTCCGCTCCGGTGCAGGAACTGTTGGTTCGCACGCAGATTGCTCGCAG  
 GTCCGCTGCGGTGTGGACTGATGGATGCGTTCGAAGTTTTGGCCGACTCAG  
 GTAATAGGCGGCCAAGCACACGCCCCCGCGCTAAGTAGTAAGCGGCCGCC  
 CCGGGCTGCAGGAATTCGATATCAAGC

N6-Ex1.1

CCAAGCTGGCTAGCGTTTAACTTAAGCTTGCCACCATGGCGGTGGAAGGAGGAATGAAAT  
 GTGTGAAGTTCTTGCTCTACGTCCTCTGCTGGCCTTTTTCGCCTGTGCAGTGGGACTGATT  
 GCCGTGGGTGTGGGGCACAGCTTGTCTGGGAGGCGGAAGTGGAGGCGGTTCTGGTGG  
 AGGATCAGGGGGTGGTAGTAGGGCTCACCTTGTACAATCCGGAAGTCCGATGAAAAGCC  
 GGGGGCATCTGTGCGGGTATCTTGTCAAACCTCAGGATACACATTTACCGCACATATTTTGT  
 TCTGGTTCCGGCAGGCCCGGGTAGGGGATTGGAATGGGTAGGTTGGATCAAACCTCAATA  
 CGGAGCTGTCAACTTTGGAGGTGGTTTCAGGGATAGGGTCACATTGACTAGAGATGTCTAC  
 CGCGAAATTGCGTATATGGACATTCGCGGCCTTAAGCCAGACGACACTGCAGTCTATTATTG  
 TGCGCGAGACAGATCATATGGGGACAGCAGCTGGGCGCTTATGCGTGGGGGCAGGGGA  
 CCACAGTGGTCGTCTCCGAGGCGGAGGTAGTGGGGGTGGGTCCGGGGGTGGAAGTGGC  
 GGTGGGAGCTATATACAGTTACTCAATCCCCAAGCAGCCTGTCTGTCAGTATTGGGGATA  
 GAGTTACAATTAAGTCCAAACCTCACAGGGCGTTGGGTCCGATCTCCACTGGTACCAGCAT  
 AAACCAGGCAGGGCGCCAAACTGCTTATACACCATACCAGCTCCGTGGAAGACGGAGTCC  
 CTAGTCGGTTCAGTGGATCAGGGTTCCACACAAGCTTTAACCTGACCATCAGTGACCTTCAG  
 GCGGATGACATAGCGACATACTACTGCCAGGTGCTGCAATTCTTCGGTTCGAGGATCCCGAC  
 TGCACATAAAAGGCGGCGGCAGTGGCGGCGGTAGTGGGGGAGGAAGCGGAGGAGGCTC  
 GATAATCCAGGGGGCTACCCCTGGCTCTCTGTTGCCAGTGGTCATCATCGCAGTGGGTGCT  
 TCCTCTTCTGGTGGCTTTTGTGGGCTGCTGCGGGGCTGCAAGGAGAAGTATTGTCTTATG  
 ATCACGTTTGCCATCTTTCTGTCTTATCATGTTGGTGGAGGTGGCCGAGCCATTGCTGGC  
 TATGTGTTTAGAGATAAGGTGATGTCAGAGTTAATAACAACCTCCGGCAGCAGATGGAGA  
 ATTACCCGAAAACAACCACTGCTTCGATCCTGGACAGGATGCAGGCAGATTTAAGTG  
 CTGTGGGGCTGCTAACTACACAGATTGGGAGAAAATCCCTTCCATGTCGAAGAACCGAGTC  
 CCCGACTCCTGCTGCATTAATGTTACTGTGGGCTGTGGGATTAATTTCAACGAGAAGGCGAT  
 CCATAAGGAGGGCTGTGTGGAGAAGATTGGGGGCTGGCTGAGGAAAAATGTGCTGGTGG  
 TAGCTGCA

<p>N6-Ex2.2</p>	<p>CCAAGCTGGCTAGCGTTTAAACTTAAGCTTGCCACCATGGCGGTGGAAGGAGGAATGAAAT  GTGTGAAGTTCTTGCTCTACGTCCTCCTGCTGGCCTTTTGCCTGTGCAGTGGGACTGATT  GCCGTGGGTGTCGGGGCACAGCTTGCCTGAGTCAGACCATAATCCAGGGGGCTACCCCTG  GCTCTCTGTTGCCAGTGGTCATCATCGCAGTGGGTGCTTCTCCTCCTGGTGGCTTTTGTGG  GCTGCTGCGGGGCTGCAAGGAGAACTATTGTCTTATGATCACGTTTGCCATCTTTCTGTCT  CTTATCATGTTGGTGGAGGTGGCCGAGCCATTGCTGGCTATGTGTTTAGAGATAAGGTGA  TGTCAGAGTTTAATAACAACCTCCGGCAGCAGATGGAGAATTACCCGAAAAACAACCCACAC  TGCTTCGATCCTGGACAGGATGCAGGCAGATTTTAAGTGCTGTGGGGGAGGCGGAAGTGG  AGGCGGTTCTGGTGGAGGATCAGGGGGTGGTAGTAGGGCTCACCTTGTACAATCCGGAAC  TGCGATGAAAAAGCCGGGGGCATCTGTGCGGGTATCTTGTCAAACCTCAGGATACACATTT  ACCGCACATATTTTGTCTGGTTCCGGCAGGCCCGGGTAGGGGATTGGAATGGGTAGGTT  GGATCAAACCTCAATACGGAGCTGTCAACTTTGGAGGTGGTTTCAGGGATAGGGTCACATT  GACTAGAGATGTCTACCGCGAAATTGCGTATATGGACATTCGCGGCCTTAAGCCAGACGAC  ACTGCAGTCTATTATTGTGCGCGAGACAGATCATATGGGGACAGCAGCTGGGCGCTTGATG  CGTGGGGGCAGGGGACCACAGTGGTCGCTCCGCAGGCGGAGGTAGTGGGGGTGGGTCC  GGGGGTGGAAGTGGCGGTGGGAGCTATATACACGTTACTCAATCCCAAGCAGCCTGTCTG  TCAGTATTGGGGATAGAGTTACAATTAAGTCCAAACCTCACAGGGCGTTGGGTCCGATCTC  CACTGGTACCAGCATAAACCAGGCAGGGCGCCAAAACCTGCTTATACACCATAACCAGCTCCGT  CGAAGACGGAGTCCCTAGTCGGTTCAGTGGATCAGGGTCCACACAAGCTTTAACCTGACC  ATCAGTGACCTTCAGGCGGATGACATAGCGACATACTACTGCCAGGTGCTGCAATTCTTCGG  TCGAGGATCCCGACTGCACATAAAAGGCGGCGGCAGTGGCGGCGGTAGTGGGGGAGGAA  GCGGAGGAGGCTCGGGCTGTGTGGAGAAGATTGGGGGCTGGCTGAGGAAAAATGTGCTG  GTGGTAGCTGCA</p>
<p>N6-Ex2.3</p>	<p>CCAAGCTGGCTAGCGTTTAAACTTAAGCTTGCCACCATGGCGGTGGAAGGAGGAATGAAAT  GTGTGAAGTTCTTGCTCTACGTCCTCCTGCTGGCCTTTTGCCTGTGCAGTGGGACTGATT  GCCGTGGGTGTCGGGGCACAGCTTGCCTGAGTCAGACCATAATCCAGGGGGCTACCCCTG  GCTCTCTGTTGCCAGTGGTCATCATCGCAGTGGGTGCTTCTCCTCCTGGTGGCTTTTGTGG  GCTGCTGCGGGGCTGCAAGGAGAACTATTGTCTTATGATCACGTTTGCCATCTTTCTGTCT  CTTATCATGTTGGTGGAGGTGGCCGAGCCATTGCTGGCTATGTGTTTAGAGATAAGGTGA  TGTCAGAGTTTAATAACAACCTCCGGCAGCAGATGGAGAATTACCCGAAAAACAACCCACAC  TGCTTCGATCCTGGACAGGATGCAGGCAGATTTTAAGTGCTGTGGGGGAGGCGGAAGTGG  AGGCGGTTCTGGTGGAGGATCAGGGGGTGGTAGTAGGGCTCACCTTGTACAATCCGGAAC  TGCGATGAAAAAGCCGGGGGCATCTGTGCGGGTATCTTGTCAAACCTCAGGATACACATTT  ACCGCACATATTTTGTCTGGTTCCGGCAGGCCCGGGTAGGGGATTGGAATGGGTAGGTT  GGATCAAACCTCAATACGGAGCTGTCAACTTTGGAGGTGGTTTCAGGGATAGGGTCACATT  GACTAGAGATGTCTACCGCGAAATTGCGTATATGGACATTCGCGGCCTTAAGCCAGACGAC  ACTGCAGTCTATTATTGTGCGCGAGACAGATCATATGGGGACAGCAGCTGGGCGCTTGATG  CGTGGGGGCAGGGGACCACAGTGGTCGCTCCGCAGGCGGAGGTAGTGGGGGTGGGTCC  GGGGGTGGAAGTGGCGGTGGGAGCTATATACACGTTACTCAATCCCAAGCAGCCTGTCTG  TCAGTATTGGGGATAGAGTTACAATTAAGTCCAAACCTCACAGGGCGTTGGGTCCGATCTC  CACTGGTACCAGCATAAACCAGGCAGGGCGCCAAAACCTGCTTATACACCATAACCAGCTCCGT  CGAAGACGGAGTCCCTAGTCGGTTCAGTGGATCAGGGTCCACACAAGCTTTAACCTGACC  ATCAGTGACCTTCAGGCGGATGACATAGCGACATACTACTGCCAGGTGCTGCAATTCTTCGG  TCGAGGATCCCGACTGCACATAAAAGGCGGCGGCAGTGGCGGCGGTAGTGGGGGAGGAA  GCGGAGGAGGCTCGTGTGCTGCTAATGTTACTGTGGGCTGTGGGATTAATTTCAACGAGAA  GGCGATCCATAAGGAGGGCTGTGTGGAGAAGATTGGGGGCTGGCTGAGGAAAAATGTGC  TGTTGGTAGCTGCA</p>

<p>N6-Ex2.4</p>	<p>CCAAGCTGGCTAGCGTTTAAACTTAAGCTTGCCACCATGGCGGTGGAAGGAGGAATGAAAT  GTGTGAAGTTCTTGCTCTACGTCCTCTGCTGGCCTTTTGCCTGTGCAGTGGGACTGATT  GCCGTGGGTGTCGGGGCACAGCTTGTCTGAGTCAGACCATAATCCAGGGGGCTACCCCTG  GCTCTCTGTTGCCAGTGGTCATCATCGCAGTGGGTGTCTTCTCTTCTGCTGGTGGCTTTTGTGG  GCTGCTGCGGGGCTGCAAGGAGAACTATTGTCTTATGATCACGTTTGCCATCTTTCTGTCT  CTTATCATGTTGGTGGAGGTGGCCGAGCCATTGCTGGCTATGTGTTTAGAGATAAGGTGA  TGTCAGAGTTTAATAACAACCTCCGGCAGCAGATGGAGAATTACCCGAAAAACAACCCACAC  TGCTTCGATCCTGGACAGGATGCAGGCAGATTTTAAGTGCTGTGGGGCTGCTAACTACACA  GATTGGGAGAAAATCCCTTCCATGTGGAAGAACCGAGTCCCCGACTCCTGCTGCGGAGGCG  GAAGTGGAGGCGGTTCTGGTGGAGGATCAGGGGGTGGTAGTAGGGCTCACCTTGTACAAT  CCGGAACCTGCGATGAAAAAGCCGGGGGCATCTGTGCGGGTATCTTGTCAAACCTCAGGATA  CACATTTACCGCACATAATTTGTTCTGGTCCGGCAGGCCCGGGTAGGGGATTGGAATGG  GTAGGTTGGATCAAACCTCAATACGGAGCTGTCAACTTTGGAGGTGGTTTCAGGGATAGGG  TCACATTGACTAGAGATGTCTACCGCGAAATTGCGTATATGGACATTGCGGGCCTTAAGCCA  GACGACACTGCAGTCTATTATTGTGCGCGAGACAGATCATATGGGGACAGCAGCTGGGGCG  TTGATGCGTGGGGGCAGGGGACCACAGTGGTCTCTCCGACGGCGGAGGTAGTGGGGGT  GGGTCCGGGGGTGGAAGTGGCGTGGGAGCTATATACACGTTACTCAATCCCCAAGCAGC  CTGTCTGTCAGTATTGGGGATAGAGTTACAATTAAGTCCAAACCTCACAGGGCGTTGGGTC  CGATCTCCACTGGTACCAGCATAAACCAGGCAGGGCGCCAAAACCTGTTATACACCATACCA  GCTCCGTCGAAGACGGAGTCCCTAGTCGGTTCAGTGGATCAGGGTTCCACACAAGCTTTAA  CCTGACCATCAGTGACCTTCAGGCGGATGACATAGCGACATACTACTGCCAGGTGCTGCAA  TTCTTCGGTTCGAGGATCCCCGACTGCACATAAAGGCGGCGGCAGTGGCGGCGGTAGTGGG  GGAGGAAGCGGAGGAGGCTCGGGCTGTGTGGAGAAGATTGGGGGCTGGCTGAGGAAAA  ATGTGCTGGTGGTAGCTGCA</p>
<p>VHH72-CD63- Ex2.4 gBLOCK1</p>	<p>CCAAGCTGGCTAGCGTTTAAACTTAAGCTTGCCACCATGGCGGTGGAAGGAGGAATGAAAT  GTGTGAAGTTCTTGCTCTACGTCCTCTGCTGGCCTTTTGCCTGTGCAGTGGGACTGATT  GCCGTGGGTGTCGGGGCACAGCTTGTCTGAGTCAGACCATAATCCAGGGGGCTACCCCTG  GCTCTCTGTTGCCAGTGGTCATCATCGCAGTGGGTGTCTTCTCTTCTGCTGGTGGCTTTTGTGG  GCTGCTGCGGGGCTGCAAGGAGAACTATTGTCTTATGATCACGTTTGCCATCTTTCTGTCT  CTTATCATGTTGGTGGAGGTGGCCGAGCCATTGCTGGCTATGTGTTTAGAGATAAGGTGA  TGTCAGAGTTTAATAACAACCTCCGGCAGCAGATGGAGAATTACCCGAAAAACAACCCACAC  TGCTTCGATCCTGGACAGGATGCAGGCAGATTTTAAGTGCTGTGGGGCTGCTAACTACACA  GATTGGGAGAAAATCCCTTCCATGTGGAAGAACCGAGTCCCCGACTCCTGCTGCGGAGGCG  GAAGTGGAGGCGGTTCTGGTGGAGGATCAGGGGGTGGTAGTCAAGTGCAGCTGCAGGAG  AGCGGCGGAGGCCTGGTGCAGGCCGGCGGATCTCTGAGACTGAGCTGCGCCGCTTCTGGC  AGAACCTTTAGCGAGTACGCCATGGGCTGGTCCGGCAGGCCCTGGCAAGGAACGGGAA  TTCGTGGCCACCATCAGCTGGTCCGGAGGCAGCACCTACTACACCGACAGCGTGAAGGGCA  GATTCACAATCAGCAGAGATAATGCCAAAAACACCGTGTACCTGCAGATGAACAGCCTGAA  GCCCCGACGACACCGCGTGTATTACTGTGCCGCTGCTGGCCTGGGCACCGTCGTTTCTGAGT  GGGACTACGACTACGATTACTGGGGCCAGGGCACACAGGTGACAGTGTCCAGCGGCGGCG  GTAGTGGTGGTGGTCTGGGGGTGGTAGTGGAGGAGGGAGT</p>
<p>VHH72-CD63- Ex2.4 gBLOCK2</p>	<p>GGGGTGGTAGTGGAGGAGGGAGTCAAGTGCAGCTGCAGGAGAGCGGCGGAGGCCTGGT  GCAGGCCGGCGGATCTCTGAGACTGAGCTGCGCCGCTTCTGGCAGAACCTTTAGCGAGTAC  GCCATGGGCTGGTCCGGCAGGCCCTGGCAAGGAACGGGAATTCGTGGCCACCATCAGCT  GGTCCGGAGGCAGCACCTACTACACCGACAGCGTGAAGGGCAGATTACAATCAGCAGAG  ATAATGCCAAAAACACCGTGTACCTGCAGATGAACAGCCTGAAGCCCCGACGACACCGCCGT  GTATTACTGTGCTGCCGCTGGCCTGGGCACCGTCGTTTCTGAGTGGGACTACGACTACGATT  ACTGGGGCCAGGGCACACAGGTGACAGTGTCCAGCGGCGGCGCAGTGGCGGCGGTAGT</p>

GGGGGAGGAAGCGGAGGAGGCTCGGGCTGTGTGGAGAAGATTGGGGGCTGGCTGAGGA  
AAAATGTGCTGGTGGTAGCTGCA

**Supplemental Table 3:** Amino Acid sequence of the CD63 fusion proteins expressed from the fusion vectors. Bold sequence denotes GS linkers, Underlined sequences denotes the N6 Hv sequence, underlined and italicized sequences denote the VHH723 sequence, and all other sequences reflect the CD63 sequence.

Vector	Amino acid sequence
N6-Ex1.1	MAVEGGMKCVKFLLYVLLAFCAVGLIAGVGAQLVL <b>GGGSGGGSGGGSGGG</b> RAHLVQSGTAMKKPGASVRVSCQTSGYTFTAHLFWFRQAPGRGLEWVGWIKPQYGAVNFGGGFRDRVTLTRDVYREIAYMDIRGLKPDDTAVYYCARDRSYGDSSWALDAWGQTTVVVSAGGGSGGGSGGGSGGG <b><u>SYIHVTQSPSSLSV</u></b> <b><u>SIGDRVTINCQTSQGVGSDLHWYQH</u></b> <b><u>KPGRAPKLLIHTSSVEDGVPSRFSGSGFHT</u></b> <b><u>SFNLTISDLQADDIATYYCQVLQFFGRG</u></b> <b><u>SRLHIKGGGSGGGSGGGSGGG</u></b> SIIQGATPGSLLPVVIAVGVFLFLVAFVCCGACKENYCLMITFAIFLSLIMLVEVAAAIAGYVFRDKVMSEFNFRQMMENYPKNNHTASILDRMQADFKCCGAANYTDWEKIPSMKRNRPDSCCINVTGCGINFNEKAIHKEGCVEKIGWLRKNVLVAAAAALGIAFVEVLGIVFACCLVKSIRSGYEVME <b>FGGGGSMVFTLEDFVGDWRQTAGYNLDQVLEQGGVSSLFQNLGVS</b> <b><u>VTPIQRIVLSGENGLKIDIHVIIPYEGLSGDQMGQIEKIFKVVYPVDDH</u></b> <b><u>HFVKVILHYGTLVIDGVT</u></b> <b><u>PNMIDYFGRPYEGIAVFDGKKITVTGTLWNGNKI</u></b> <b><u>IDERLINPDGSLLFRVTI</u></b> <b><u>NGVTGWRLCERILA</u></b>
N6-Ex2.2	MAVEGGMKCVKFLLYVLLAFCAVGLIAGVGAQLVLSQTIIQGATPGSLLPVVIAVGVFLFLVAFVCCGACKENYCLMITFAIFLSLIMLVEVAAAIAGYVFRDKVMSEFNFRQMMENYPKNNHTASILDRMQADFKCC <b>GGGSGGGSGGGSGGG</b> RAHLVQSGTAMKKPGASVRVSCQTSGYTFTAHLFWFRQAPGRGLEWVGWIKPQYGAVNFGGGFRDRVTLTRDVYREIAYMDIRGLKPDDTAVYYCARDRSYGDSSWALDAWGQTTVVVSAGGGSGGGSGGGSGGG <b><u>SYIHVTQSPSSLSV</u></b> <b><u>SIGDRVTINCQTSQGVGSDLHWYQH</u></b> <b><u>KPGRAPKLLIHTSSVEDGVPSRFSGSGFHTS</u></b> <b><u>FNLTISDLQADDIATYYCQVLQFFGRG</u></b> <b><u>SRHLHIKGGGSGGGSGGGSGGG</u></b> GCVEKIGWLRKNVLVAAAAALGIAFVEVLGIVFACCLVKSIRSGYEVME <b>FGGGGSMVFTLEDFVGDWRQTAGYNLDQVLEQGGVSSLFQNLGVS</b> <b><u>VTPIQRIVLSGENGLKIDIHVIIPYEGLSGDQMGQIEKIFKVVYPVDDH</u></b> <b><u>HFVKVILHYGTLVIDGVT</u></b> <b><u>PNMIDYFGRPYEGIAVFDGKKITVTGTLWNGNKI</u></b> <b><u>IDERLINPDGSLLFRVTINGVTGWRLCERILA</u></b>
N6-Ex2.3	MAVEGGMKCVKFLLYVLLAFCAVGLIAGVGAQLVLSQTIIQGATPGSLLPVVIAVGVFLFLVAFVCCGACKENYCLMITFAIFLSLIMLVEVAAAIAGYVFRDKVMSEFNFRQMMENYPKNNHTASILDRMQADFKCC <b>GGGSGGGSGGGSGGG</b> RAHLVQSGTAMKKPGASVRVSCQTSGYTFTAHLFWFRQAPGRGLEWVGWIKPQYGAVNFGGGFRDRVTLTRDVYREIAYMDIRGLKPDDTAVYYCARDRSYGDSSWALDAWGQTTVVVSAGGGSGGGSGGGSGGG <b><u>SYIHVTQSPSSLSV</u></b> <b><u>SIGDRVTINCQTSQGVGSDLHWYQH</u></b> <b><u>KPGRAPKLLIHTSSVEDGVPSRFSGSGFHTS</u></b> <b><u>FNLTISDLQADDIATYYCQVLQFFGRG</u></b> <b><u>SRHLHIKGGGSGGGSGGGSGGG</u></b> CCINVTGCGINFNEKAIHKEGCVEKIGWLRKNVLVAAAAALGIAFVEVLGIVFACCLVKSIRSGYEVME <b>FGGGGSMVFTLEDFVGDWRQTAGYNLDQVLEQGGVSSLFQNLGVS</b> <b><u>VTPIQRIVLSGENGLKIDIHVIIPYEGLSGDQMGQIEKIFKVVYPVDDH</u></b> <b><u>HFVKVILHYGTLVIDGVT</u></b> <b><u>PNMIDYFGRPYEGIAVFDGKKITVTGTLWNGNKI</u></b> <b><u>IDERLINPDGSLLFRVTINGVTGWRLCERILA</u></b>

N6-Ex2.4	<p>MAVEGGMKCVKFLLYVLLAFCAVGLIAGVGAQLVLSQTIIQGATPGSLLPVVIIAVGVFLFLVAFV  GCCGACKENYCLMITFAIFLSLIMLVEVAAAIAGYVFRDKVMSEFNNNFRQQMENYPKNNHTASILDR  MQADFKCCGAANYTDWEKIPSMKSNRVPDSCCGGGSGGGSGGGSGGGSGGGRAHLVQSGTAMKKPG  ASVRVSCQTSGYTFTAHLFWFRQAPGRGLEWVGWIKPQYGAVNFGGGFRDRVTLTRDVYREIAYM  DIRGLKPDDTAVYYCARDRSYGDSSWALDAWGQGTTVVVSAGGGSGGGSGGGSGGGSGGGSYIHVTQSP  SSLSVSI<del>GD</del>RV<del>TINC</del>QTSQGVGSDLHWYQHHPGRAPKLLIHTSSVEDGVPSRFSGSGFHTSFNLTIS  DLQADDIATYYCQVLQFFGRGSR<del>LHIK</del>GGGSGGGSGGGSGGGSGGGSGCVEKIGGWLRKNVLVAAAAAL  GIAFVEVLGIVFACCLVKSIRSGYEVMEF<del>GGGGS</del>MVFTLEDVFGDWRQTAGYNLDQVLEQGGVSSLF  QNLGVSVTPIQRIVLSGENGLKIDIHVIIPEGLSGDQMGQIEKIFKVVPVDDHHFKVILHYGTLVIDGV  TPNMIDYFGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLINPDGSLFRVTINGVTGWRLCERILA</p>
VHH-72- ex2.4	<p>MAVEGGMKCVKFLLYVLLAFCAVGLIAGVGAQLVLSQTIIQGATPGSLLPVVIIAVGVFLFLVAFV  GCCGACKENYCLMITFAIFLSLIMLVEVAAAIAGYVFRDKVMSEFNNNFRQQMENYPKNNHTASILDR  MQADFKCCGAANYTDWEKIPSMKSNRVPDSCCGGGSGGGSGGGSGGGSGGGQVQLQESGGGLVQAG  GSLRLS<del>CAAS</del>GRTFSEYAMGWFRQAPGKEREFVATISWSSGGSTYYTDSVKGRFTISRDNANTVYLQ  MNSLKPDDTAVYYCAAAGLGTVVSEWDYDYDYWGQGTQVTVSSGGGSGGGSGGGSGGGSGGGQVQL  QESGGGLVQAGGSLRLS<del>CAAS</del>GRTFSEYAMGWFRQAPGKEREFVATISWSSGGSTYYTDSVKGRFTIS  RDNANTVY<del>LQ</del>MNSLKPDDTAVYYCAAAGLGTVVSEWDYDYDYWGQGTQVTVSSGGGSGGGSG  GGSGGGSGCVEKIGGWLRKNVLVAAAAALGIAFVEVLGIVFACCLVKSIRSGYEVMEF<del>GGGGS</del>MVFT  LEDVFGDWRQTAGYNLDQVLEQGGVSSLFQNLGVSVTPIQRIVLSGENGLKIDIHVIIPEGLSGDQM  GQIEKIFKVVPVDDHHFKVILHYGTLVIDGVTPNMIDYFGRPYEGIAVFDGKKITVTGTLWNGNKIIDE  RLINPDGSLFRVTINGVTGWRLCERILA</p>