

Supplementary Figures and Tables

Figure S1. Comparison of the incidence of the CCT-CGG-CGG-GCA stretch of codons and other sequences synonymously encoding -PRRA- in sense and antisense strands of protein-coding human sequences (see Supplementary Table S2 for tabulation of sequence incidence).

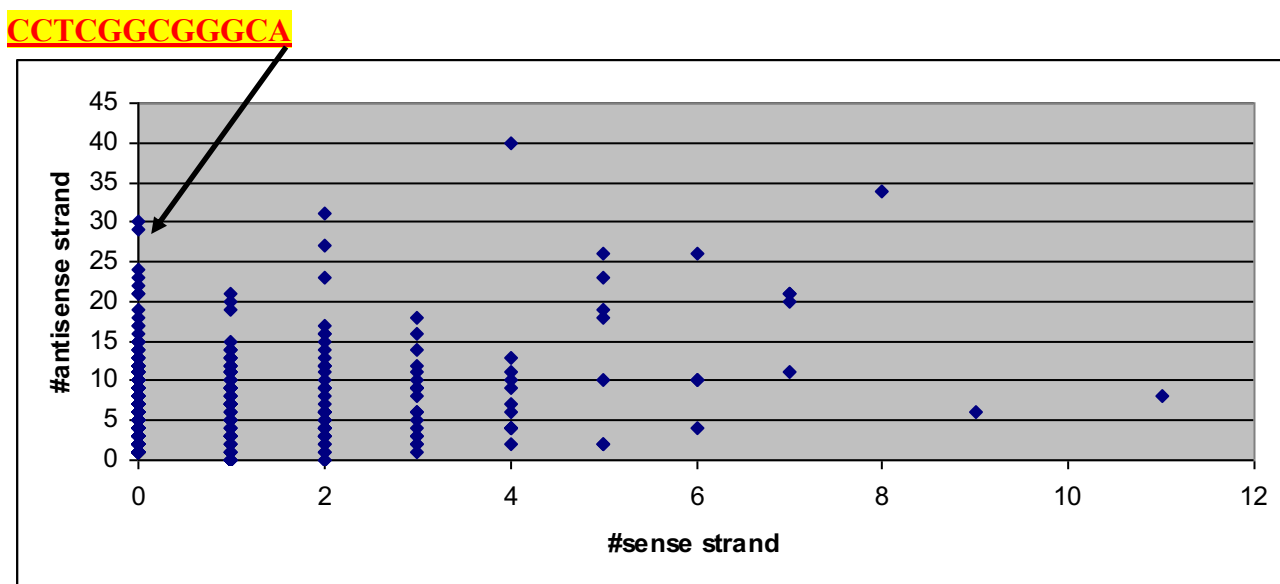


Figure S2. Expression of original and codon-optimized S proteins in LV cell lysates (A) and in Expi293 cell lysates (B). In (A), left panel was imaged at normal gain, while right panel was imaged at higher gain to visualize original S protein expression (S original). Experiments were repeated several times.

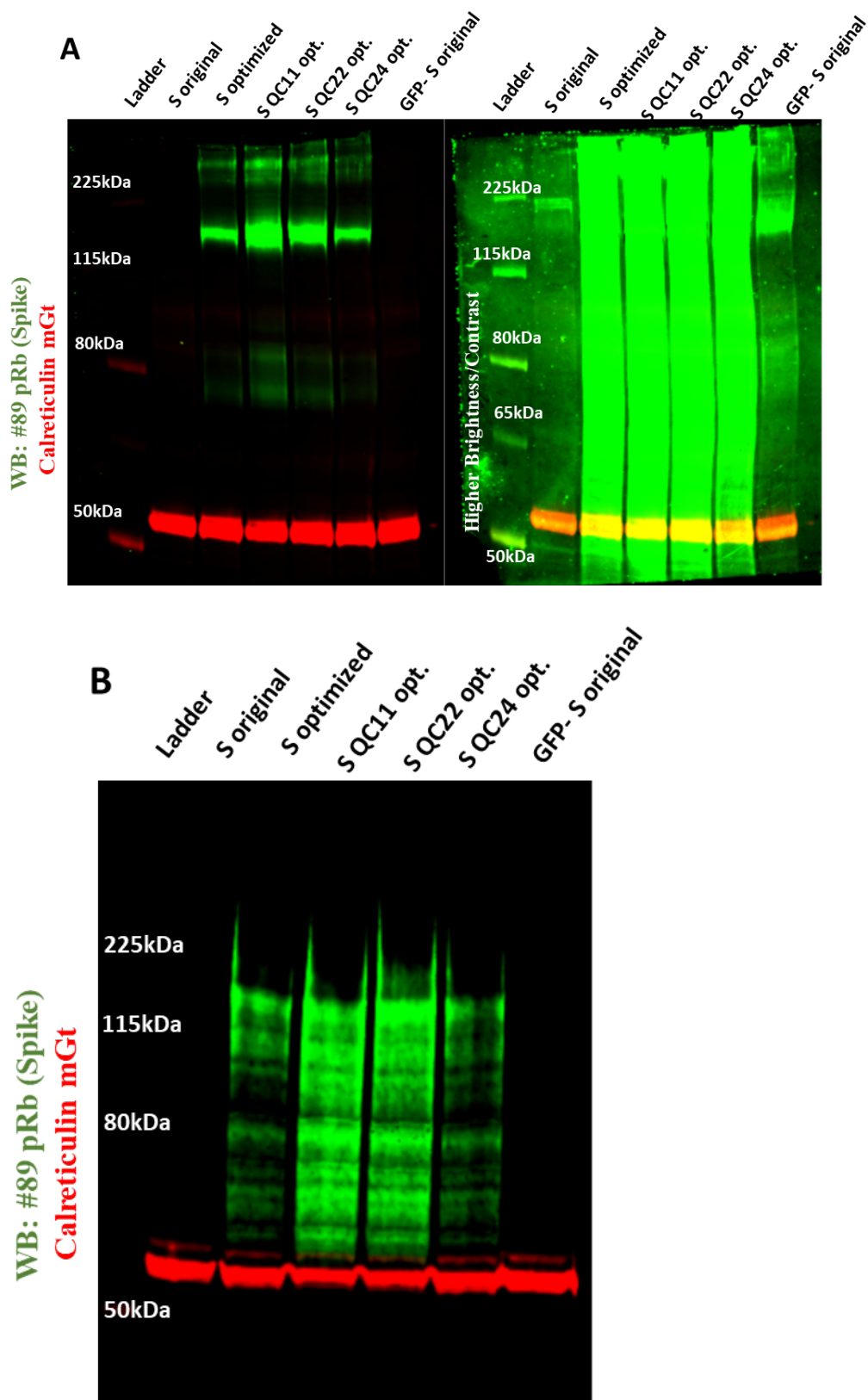


Figure S3. Lentiviral pseudovirion titers for WPRE (upper) and LTR (lower) quantitated by qPCR. Error bars indicate the standard deviation.

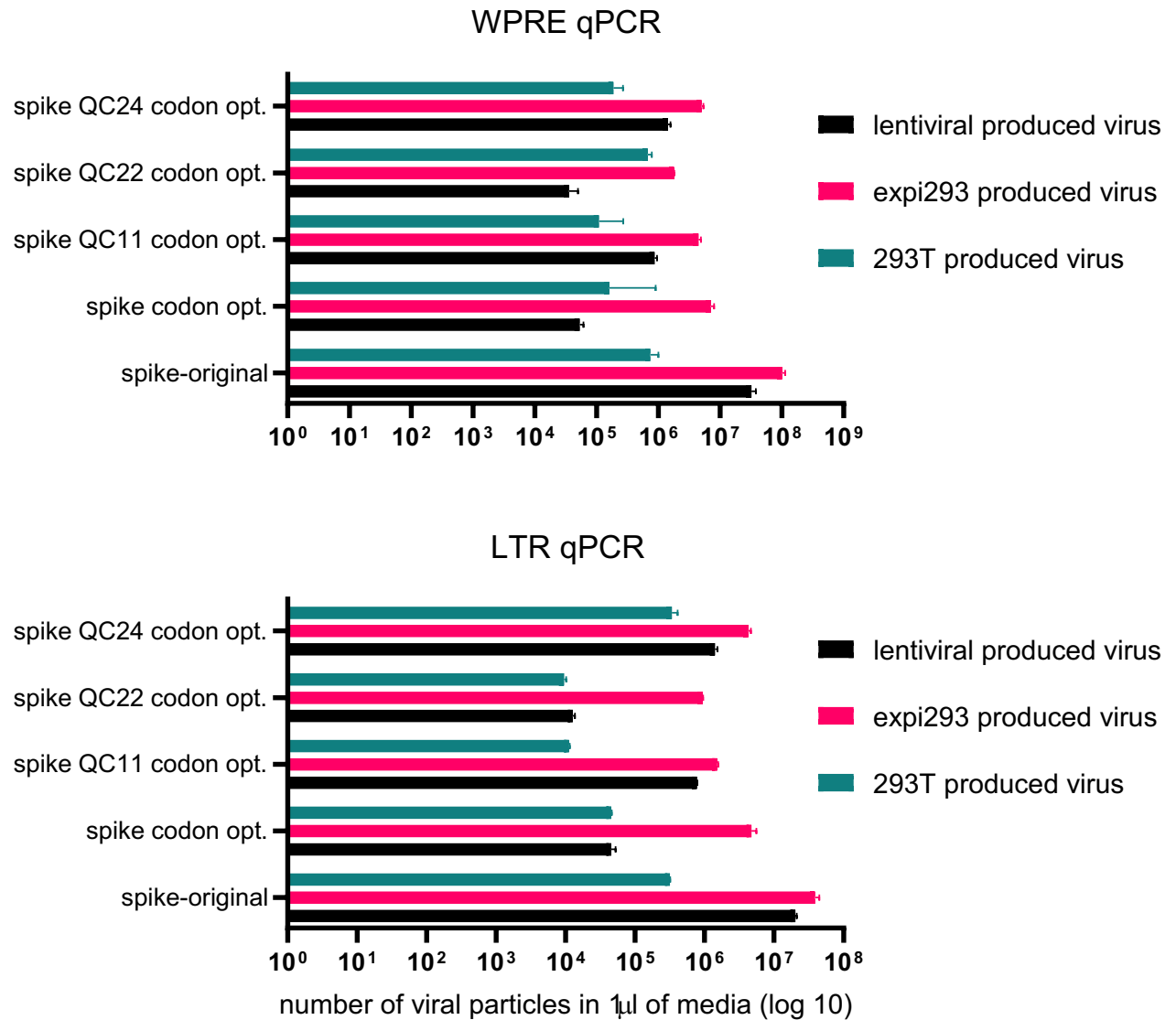


Figure S4. ARPE-19 cell infection with Expi293 produced lentiviral pseudovirions.
Experiments were repeated several times. Scale bar=180 μ m.

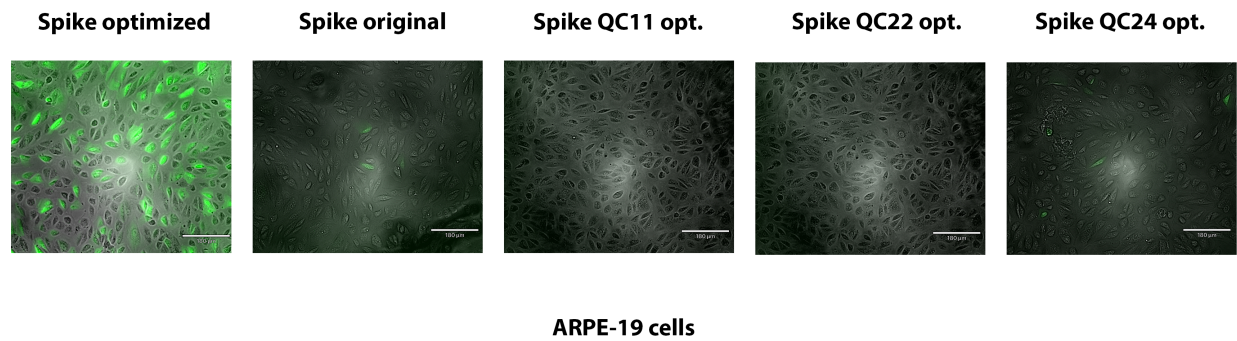


Figure S5. Vero E6 infection with Expi293-produced pseudovirions.
Experiments were repeated several times. Scale bar=180 μ m.

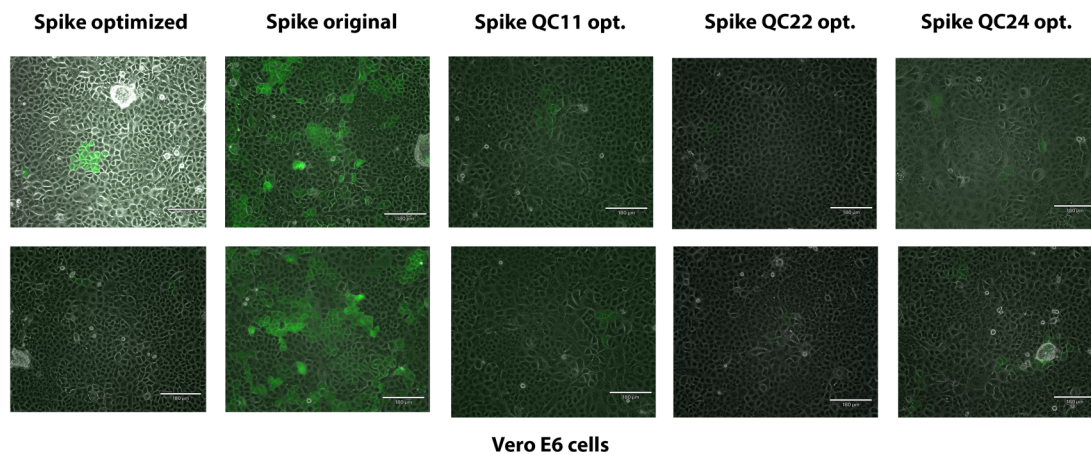


Table S1. Codon usage in protein-coding genes of viral and human genomes. Codons (CCT, CGG, and GCA) featuring in the -PRRA- insert are highlighted in yellow. CAT and CTT codons are highlighted in blue. CG-containing codons are highlighted in green. CDS = protein-coding sequences.

Codon	SARS-CoV2 CDS	SARS CoV-2 CDS, %	Human CDS	Human CDS, %
AAA	2448	6.63	993621	2.45
AAC	280	0.76	776603	1.92
AAG	1097	2.97	1295568	3.20
AAT	2873	7.78	689701	1.70
ACA	1458	3.95	614523	1.52
ACC	54	0.15	768147	1.90
ACG	63	0.17	246105	0.61
ACT	632	1.71	533609	1.32
AGA	602	1.63	494682	1.22
AGC	47	0.13	791383	1.95
AGG	47	0.13	486463	1.20
AGT	478	1.3	493429	1.22
ATA	1106	3.	304565	0.75
ATC	191	0.52	846466	2.09
ATG	762	2.06	896005	2.21
ATT	1955	5.3	650473	1.61
CAA	625	1.69	501911	1.24
CAC	29	0.08	613713	1.51
CAG	298	0.81	1391973	3.43
CAT	576	1.56	441711	1.09
CCA	476	1.29	688038	1.70
CCC	11	0.03	804620	1.99
CCG	48	0.13	281570	0.69
CCT	446	1.21	713233	1.76
CGA	120	0.33	250760	0.62
CGC	29	0.08	423516	1.05
CGG	9	0.02	464485	1.15
CGT	316	0.86	184609	0.46
CTA	75	0.2	290751	0.72
CTC	49	0.13	796638	1.97
CTG	29	0.08	1611801	3.98
CTT	780	2.11	536515	1.32
GAA	2049	5.55	1177632	2.91
GAC	233	0.63	1020595	2.52
GAG	380	1.03	1609975	3.97
GAT	2750	7.45	885429	2.18
GCA	1026	2.78	643471	1.59
GCC	28	0.08	1127679	2.78
GCG	76	0.21	299495	0.74
GCT	452	1.22	750096	1.85
GGA	537	1.46	669873	1.65
GGC	68	0.18	903565	2.23
GGG	31	0.08	669768	1.65
GGT	986	2.67	437126	1.08
GTA	844	2.29	287712	0.71
GTC	53	0.14	588138	1.45
GTG	79	0.21	1143534	2.82
GTT	923	2.5	448607	1.11
TAC	134	0.36	622407	1.54
TAT	1998	5.41	495699	1.22
TCA	1010	2.74	496448	1.22
TCC	51	0.14	718892	1.77
TCG	37	0.1	179419	0.44
TCT	853	2.31	618711	1.53
TGC	69	0.19	513028	1.27
TGG	342	0.93	535595	1.32
TGT	485	1.31	430311	1.06
TTA	1468	3.98	311881	0.77
TTC	332	0.9	824692	2.03
TTG	374	1.01	525688	1.30
TTT	1229	3.33	714298	1.76

Table S2. Statistics of the CCT-CGG-CGG-GCA stretch of codons and other sequences encoding -PRRA- in sense and antisense strands of human protein-coding sequences. All three reading frames of the antisense strand were analyzed. Human protein-coding sequences were obtained from the CCDS database (release 22, <ftp.ncbi.nlm.nih.gov/pub/CCDS/>). The SARS-CoV-2 S protein RNA -CCTCGGCGGGCA- insert is indicated in red.

Stretch of codons	Sense	Antisense
CCCAGGAGGGCC	4	40
CCCCGCCGGGCC	8	34
CCCCGCAGGGCC	2	31
CCCCGCAGGGCT	0	30
CCTCGGCGGGCA	0	29
CCCAGGAGGGCA	2	27
CCCAGAAGAGCA	5	26
CCCCGCCGCGCC	6	26
CCTCGAAGAGCT	0	24
CCACGAAGAGCT	0	23
CCCCGGAGAGCC	2	23
CCCCGGCGGGCA	5	23
CCCAGAAGAGCT	0	22
CCTCGGAGAGCA	0	21
CCCCGCAGGGCA	0	21
CCCAGAAGGGCT	1	21
CCGCGCCGCGCG	7	21
CCGCGCCGGGCC	7	21
CCCAGGAGAGCA	1	20
CCGCGCCGCGCC	7	20
CCCAGAAGGGCA	0	19
CCACGCCGTGCT	1	19
CCAAGAAGAGCC	5	19
CCTCGAAGAGCA	0	18
CCCAGAAGAGCC	3	18
CCTCGGAGGGCA	5	18
CCACGTAGGGCT	0	17
CCTCGTAGAGCT	0	17
CCCAGGAGGGCG	2	17
CCACGGCGGGCA	0	16
CCCCGCCGGGCA	2	16
CCCAGGAGAGCC	2	16
CCCAGACGGGCC	3	16
CCGAGGAGGGCT	0	15
CCACGAAGAGCA	0	15
CCTCGAAGGGCC	0	15
CCTCGCAGAGCC	0	15
CCTCGGCGGGCG	1	15
CCCAGGAGGGCT	2	15
CCTCGGCGCGCA	0	14
CCACGAAGAGCC	0	14
CCTCGCAGGGCT	0	14
CCACGGCGGGCT	0	14
CCACGCCGGGCG	1	14
CCCCGCCGGGCT	1	14

CCTCGGAGGGCC	1	14
CCGCGGCGCGCT	2	14
CCCAGGCGGGCC	3	14
CCTCGTAGGGCG	0	13
CCGAGAAGAGCA	0	13
CCTCGGCGTGCT	0	13
CCACGGAGGGCG	0	13
CCACGCCGGGCA	0	13
CCGCGCCGGGCG	1	13
CCACGAAGGGCA	1	13
CCTCGGAGAGCT	1	13
CCACGCAGGGCC	1	13
CCGCGGCGCGCA	2	13
CCCAGGCGGGCA	4	13
CCGCGGCGTGCA	0	12
CCGAGGCGGGCG	0	12
CCTCGTAGAGCG	0	12
CCTCGAAGAGCG	0	12
CCACGTCGGGCA	0	12
CCTCGGCGCGCG	0	12
CCTCGGCGCGCT	0	12
CCCCGCAGAGCA	0	12
CCACGGAGGGCT	0	12
CCCCGGCGGGCT	0	12
CCGAGGAGGGCA	1	12
CCAAGAAGGGCA	1	12
CCTCGGAGGGCT	1	12
CCAAGAAGAGCT	1	12
CCGCGGAGGGCC	2	12
CCGAGGAGGGCC	2	12
CCTCGCCGGGCA	2	12
CCGAGGAGAGCA	3	12
CCACGTAGAGCA	0	11
CCGAGGCGGGCA	0	11
CCTCGTCGCGCT	0	11
CCTCGGAGGGCG	0	11
CCTCGAAGGGCT	0	11
CCACGGCGCGCA	0	11
CCACGAAGGGCC	0	11
CCTCGCAGAGCT	0	11
CCCAGGAGAGCT	0	11
CCGAGGCGAGCC	1	11
CCCCGAAGAGCT	1	11
CCCCGGCGCGCA	1	11
CCCCGGAGGGCA	1	11
CCCCGGCGCGCC	1	11
CCACGCAGGGCA	2	11
CCGAGGCGGGCT	3	11
CCCCGGAGAGCA	4	11
CCACGCAGAGCG	7	11
CCACGTCGTGCA	0	10
CCGCGTCGCGCT	0	10
CCGCGCCGAGCA	0	10

CCACGAAGAGCG	0	10
CCGCGTCGGGCC	0	10
CCGCGCCGCGCA	0	10
CCACGGCGTGCA	0	10
CCTCGTAGAGCA	0	10
CCACGTCGGGCT	0	10
CCACGTAGAGCT	0	10
CCGAGGAGAGCT	0	10
CCCCGACGGGCA	0	10
CCTCGGCGAGCT	0	10
CCCCGACGCGCT	0	10
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CCGCGGCGGGCC	0	10
CCCCGACGGGCT	0	10
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CCCCGGCGGGCC	0	10
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CCACGCAGGGCT	1	10
CCACGCCGGGCC	1	10
CCCAGGCGGGCG	2	10
CCAAGAAGAGCA	3	10
CCCCGGAGGGCC	4	10
CCGCGGCGGGCG	5	10
CCGCGGCGCGCG	6	10
CCCCGCCGGGCG	6	10
CCGCGCCGTGCA	0	9
CCACGCCGTGCG	0	9
CCTCGTCGGGCA	0	9
CCTCGCAGGGCG	0	9
CCTCGTAGGGCT	0	9
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CCAAGGAGGGCA	3	9
CCCCGCAGAGCT	3	9

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CCCCGCCGCGCG	3	8
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CCTCGACGGGCA	0	1
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CCTAGAAGGGCC	0	1

CCACGACGGGCG	1	1
CCTAGAAGAGCG	1	1
CCTCGGCGAGCA	1	1
CCTAGGCGAGCC	1	1
CCGCGCCGAGCG	2	1
CCACGACGTGCT	2	1
CCTCGCCGTGCT	3	1
CCGCGACGTGCC	1	0
CCGCGACGGGCA	1	0
CCCAGACGAGCG	1	0
CCCCGTCGTGCC	1	0
CCACGCCGCGCG	1	0
CCAAGACGTGCC	1	0
CCTAGGAGGGCT	1	0
CCCAGACGTGCC	2	0
CCAAGACGAGCC	2	0

Table S3. Peptide identification highlighted on sequence coverage maps for different S protein products. Analysis by liquid chromatography-tandem mass spectrometry of peptides from in-gel trypsinization. A, Codon-optimized S protein (full size); B, QC24 (S2 fragment); C, QC11 (S2 fragment); D, QC24 full S protein; E, QC11 full S protein; and F, QC22 full S protein.

A. Codon-optimized S protein (full size)

Accession	Description	False Discovery Rate
A0A087WVQ6	Clathrin heavy chain OS=Homo sapiens OX=9606 GN=CLTC PE=1 SV=1	0
Q86UP2	Kinectin OS=Homo sapiens OX=9606 GN=KTN1 PE=1 SV=1	0
Q9NYU2	UDP-glucose:glycoprotein glucosyltransferase 1 OS=Homo sapiens OX=9606 GN=UGGT1 PE=1 SV=3	0
Q15075	Early endosome antigen 1 OS=Homo sapiens OX=9606 GN=EEA1 PE=1 SV=2	0
P0DTC2	Spike glycoprotein (Full Length) OS=Severe acute respiratory syndrome coronavirus 2 OX=2697049 GN=S PE=1 SV=1	0
P07814	Bifunctional glutamate/proline--tRNA ligase OS=Homo sapiens OX=9606 GN=EPRS PE=1 SV=5	0
Q14152	Eukaryotic translation initiation factor 3 subunit A OS=Homo sapiens OX=9606 GN=EIF3A PE=1 SV=1	0
Q13576	Ras GTPase-activating-like protein IQGAP2 OS=Homo sapiens OX=9606 GN=IQGAP2 PE=1 SV=4	0
Q9NTJ3	Structural maintenance of chromosomes protein 4 OS=Homo sapiens OX=9606 GN=SMC4 PE=1 SV=2	0

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SSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPGGFSALEPLVDLPIGINITRFQT
LLALHRSYLTPGDSSSGWTAGAAAYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETKCTLSFTVEKGIYQTSNFRV
OPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLVNSASFSTFKCYGVSPTKLNDLCFTNVYADSF
VIRGDEVROIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYYNYLYRLFRKSNLKPFERDISTEIYQAGSTPC
NGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFL
PFQQFGRDIADTTDAVRDPQTLILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGS
NVFQTRAGCLIGAHEVNNSECDIPIGAGICASYQTQTNSPRRARSVASQSIAYTMSLGAENSVAYSNNNSIAIPTNFTI
SVTTEILPVSMTKTSVDCTMYICGDSTECNLLLQYGSFCTQLNRLALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGF
NFSQILPDPSKPSKRSFIEDLLFNKVTADAGFIKQYGDCLGDIARLDLCAQKFNGLTVLPPLTDEMIAQYTSALLAG
TITSGWTFGAGAALQIPFAMQMAYRFENGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSTASALGKLQDVVNQNAQALN
TLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGRLOSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRV
DFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHGDKAHFPREGVFVSNGTHWFVTQRNFYEPQIITDNT
FVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDL
QELGKYEYIKWPWYIWLGFIAGLIAIVMVTIMLCMTSCCSCLKGCCSCGSCCKFDEDDSEPVLKGVKLHYT

413/1273*100 =32% coverage

B. QC24 (S2 fragment)

Prohibitin OS=Homo sapiens OX=9606 GN=PHB PE=1 SV=1	0
Eukaryotic translation initiation factor 3 subunit B OS=Homo sapiens OX=9606 GN=EIF3B PE=1 SV=3	0
Bifunctional glutamate/proline--tRNA ligase OS=Homo sapiens OX=9606 GN=EPRS PE=1 SV=5	0
RNA-binding protein 26 OS=Homo sapiens OX=9606 GN=RBM26 PE=1 SV=3	0
Drebrin OS=Homo sapiens OX=9606 GN=DBN1 PE=1 SV=4	0
Interleukin enhancer-binding factor 2 OS=Homo sapiens OX=9606 GN=ILF2 PE=1 SV=1	0
Spike glycoprotein (C-terminal Fragment) OS=Severe acute respiratory syndrome coronavirus 2 OX=2697049 GN=S PE=1 SV=1	0

MFVFLVLLPLVSSQCVNLTRTQLPPAYTNSFTRGVVYPDKVFRSSVLHSTQDLFLPFFSNVTWFFHAIHVSGTNGTKRFD
 NPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSTQSLIVNNATNVVIKVCEFCNDPFLGVVYHKNNKSWMESEFRVY
 SSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVKFNIDGYFKIYKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQT
 LLALHRSYLTPGDSSSGWTAGAAAYVGYLQPRTFLLKYNENGTITDAVDCALDPLSEKCTLKSTVEKGIYQTSNFRV
 QPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPKLNLDLCFTNVYADSF
 VIRGDEVQRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNVNYLYRLFRKSNLKPFERDISTEIQAGSTPC
 NGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFL
 PFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGS
 NVFQTRAGCLIGAEHVNNSYECDIPIGAGICASYQTQTNSPRRARSVASQSIIAYTMSLGAENSVAYSNNNSIAIPTNFTI
 SVTTEILPVSMTKTSVDCTMYICGDSTECNLLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGF
 NFSQILPDPSKPSKRSFIEDLLFNK**VTLADAGFIK**QYGDCLGDIAARDLICAQKFNGLTVLPPLTDEMIAQYTSALLAG
 TITSGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGK**QDSLSSTASALGK****LQDVVNQNAQALN**
TLVKQLSSNFGAISSVLNDILSRDKVEAEVQIDRLITGR**LQSLQTYVTQQLIR**AAEIRASANLAATK**MSECVLGQSKRV**
 DFCGKGYHLMSFPQSPHGVVFLHVTYVPAQEKNFTTAPAICHGKAHFPREGVFSNGTHWVFTQRNFYEPQIITDNT
 FVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDL
 QELGKYEQYIKWPWYIWLGFIAGLIAIVMTIMLCMTSCCCLKGCCSCGSCCKFDEDDSEPVKGVKLHYT

C. QC11 (S2 fragment)

Q16643	Drebrin OS=Homo sapiens OX=9606 GN=DBN1 PE=1 SV=4	0
P25205	DNA replication licensing factor MCM3 OS=Homo sapiens OX=9606 GN=MCM3 PE=1 SV=3	0
E9PLK3	Aminopeptidase OS=Homo sapiens OX=9606 GN=NPEPPS PE=1 SV=1	0
P35221	Catenin alpha-1 OS=Homo sapiens OX=9606 GN=CTNNA1 PE=1 SV=1	0
A0A0A0MT26	Sodium/potassium-transporting ATPase subunit alpha-3 OS=Homo sapiens OX=9606 GN=ATP1A3 PE=1 SV=1	0
P0DTC2	Spike glycoprotein (Predominantly C-terminal Fragment) OS=Severe acute respiratory syndrome coronavirus 2 OX=2697049 GN=S PE=1 SV=1	0

MFVFLVLLPLVSSQCVNLTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVS GTNGTKRFD
 NPVLPFNDGVYFASTEKSNIIIRGWIFGTTLDSTQSLIIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNKSWMESEFRVY
 SSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFNKIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQT
 LLALHRSYLTPGDSSSGWTAGAAAYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETKCTLKSFTVEKGIYQTSNFRV
 QPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLNSASFSTFKCYGVSPTKLNDLCFTNVYADSF
 VIRGDEVQRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIIYQAGSTPC
 NGVEGFNCYFPLQSYGFQPTNGVGYPYRVVLSFELHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFL
 PFQQFGRDIADTTDAVRDPQTEILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGS
 NVFQTRAGCLIGAHEVNNSYECDIPIGAGICASYQTQTNSPRRARSVASQSI IAYTMSLGAENSVAYSNNNSIAIPTNFTI
 SVTTEILPVSMTKTSVDCTMYICGDSTECNLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGF
 NFSQILPDPSKPSKR SFIEDLLFNKVTLADAGFIKQYGDCLGDIAARDLICAQKFNGLTVLPPLLTDemiaQYTSALLAG
 TITSGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSTASALGKLQDVVNQNAQALN
 TLVKQLSSNFGAIISSVLNDILSRLDKVEAEVQIDRLITGRLLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRV
 DFCGKGyHLMsFPQSAPHGVVFLHVTYVPAQEKNTTAPAI CHDGKAHFREGVFVSNGTHWFVTQRNFYEPQIIITDNT
 FVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDL
 QELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCCLKGCCSCGSCCKFDEDDSEPVLKGVKLHYT

D. QC24 full S protein

P07814	Bifunctional glutamate/proline--tRNA ligase OS=Homo sapiens OX=9606 GN=EPRS PE=1 SV=5	0
P39060	Collagen alpha-1(XVIII) chain OS=Homo sapiens OX=9606 GN=COL18A1 PE=1 SV=5	0
P13645	Keratin, type I cytoskeletal 10 OS=Homo sapiens OX=9606 GN=KRT10 PE=1 SV=6	0
O60841	Eukaryotic translation initiation factor 5B OS=Homo sapiens OX=9606 GN=EIF5B PE=1 SV=4	0
Q9NTJ3	Structural maintenance of chromosomes protein 4 OS=Homo sapiens OX=9606 GN=SMC4 PE=1 SV=2	0
P0DTC2	Spike glycoprotein (Full Length) OS=Severe acute respiratory syndrome coronavirus 2 OX=2697049 GN=S PE=1 SV=1	0

MFVFLVLLPLVSSQCVNLTR**TQLPPAYTNSFTR**GVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFWHAIHVSGTNGTKR**FD**
NPVLPFNDGVYFASTEKSNIIR**GWIFGTTLDSK**TQSLIVNNATNVVIKVEFCNDPFLGVYYHKNNKSWMESEFRVY
 SSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYS**HTPINLVR**DLPQGFSALEPLVDLPIGINITR**FQT**
LLALHRSYLTPGDSSSGWTAGAAAYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETKCTLSFTVEK**GIYQTSNFRV**
QPTESIVRFPNITNLCPFGEVFNATRFASVYAWNKRISNCVADYSVLVNSASFSTFK**CYGVSPTK**LNDLCFTNVYADSF
 VIRGDEV**RQIAPGQTGKIADYNYK**LPDDFTGCVIAWNSNNLDSK**VGGNYNYLYR**LFRKSNLKPFERDISTEIYQAGSTPC
 NGVEGFNCYFPLQSYGFQPTNGVGYPYR**VVLSFELLHAPATVCGPK**KSTNLVKNKCVNFNFNGLTGTGVLTESNKK**FL**
PFQQFGRDIADTTDAVRDPQTEILDITPCSGGVSIVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWR**VYSTGS**
NVFQTRAGCLIGAEHVNSYECDIPIGAGICASYQTQTNSPRRARSVASQSIIAYTMSLGAENSVAYSNNISAIPTNFTI
 SVTTEILPVSMTKTSVDCTMYICGDSTECSNLLQYGSFCTQLNR**ALTGIAVEQDK****NTQEVFAQVK**QIYKTPPIKDFGGF
 NFSQILPDPSKPSKR**SFIEDLLFNK****VTLADAGFIKQYGDCLGDIAAR**DLICAQKFNGLTVLPPLTDEMIAQYTSALLAG
 TITSGWTFGAGAALQIPFAMQMAYR**FNGIGVTQNVLYENQK****LIANQFNSAIGKIQDSLSSTASALGKLQDVVNQNAQALN**
TLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGR**LQSLQTYVTQQLIR**AAEIR**ASANLAATKMSECVLGQSKRV**
 DFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHGKAHFPREGVFSNGTHWVFTQRNFYEPQIITDNT
 FVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVLGDISGINASVVNIQKEIDRLNEVAK**NLNESLIDL**
QELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCMTSCCCLKGCCSCGSCCK**FDEDDSEPVLK**GVKLHYT

28% coverage

E. QC11 full S protein

A0A087WVQ6	Clathrin heavy chain OS=Homo sapiens OX=9606 GN=CLTC PE=1 SV=1
Q86UP2	Kinectin OS=Homo sapiens OX=9606 GN=KTN1 PE=1 SV=1
Q15075	Early endosome antigen 1 OS=Homo sapiens OX=9606 GN=EEA1 PE=1 SV=2
Q9NYU2	UDP-glucose:glycoprotein glucosyltransferase 1 OS=Homo sapiens OX=9606 GN=UGGT1 PE=1 SV=3
PODTC2	Spike glycoprotein OS=Severe acute respiratory syndrome coronavirus 2 OX=2697049 GN=S PE=1 SV=1

MFVFLVLLPLVSSQCVNLTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVS GTNGTKRFD
 NPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLIVNNATNVVIKVCEFCFNDPFLGVVYHKNNKSWMESEFRVY
 SSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFNIDGYFKIYSKHTPINLVRDL PQGFSALEPLVDLPIGINITRFQT
 LLALHRSYLT PGDSSSGWTAGAAAYYVGYLQPRTFLLKY NENG TITDAVDCALDPLSETKCTLSFTVEKGIYQTSNFRV
 QPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRRKRSNCVADYSVLYNSASFSTFKCYGVSPTKLN DL CFTNVYADSF
 VIRGDEVROIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVG GNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPC
 NGVEGFNCYFP LQSYGFQPTNGVGYQPYRWWLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFL
 PFQQFGRDIADTTDAVRDPQ TLEILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGS
 NVFQTRAGCLIGA EHVNNSEYCDIPIGAGICASYQTQTNSPRRARSVASQSIIAYTMSLGAENSVAYSNN SIAIPTNFTI
 SVTTEILPVSMTKTSVDCTMYICGDSTEC SNLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGF
 NFSQILPDPSKPSKRSFIEDLLFNKVT LADAGFIKQYGDCLGDIAARDLICAQKFNGLTVLPPLL TDEMIAQYTSALLAG
 TITSGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSTASALGKLQDVVNQNAQALN
 TLVKQLSSNFGAISSVLNDILSR LDKVEAEVQIDRLITGR LQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRV
 DFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICH DGKAHFPREGV FVSNGTHW FVTQRNFYEPQIITDNT
 FVSGNCDVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPD VDLGDISGINASV VNIQKEIDRLNEVAKNLNESLIDL
 QELGKYEQYIKWPWYIWLGFIAGLIAIVMTIMLCCMTSCC SCLGCCSCGSCCKFDEDDSEPV LKGVKLHYT

463/1273*100=36% coverage

F. QC22 full S protein

Q14152	Eukaryotic translation initiation factor 3 subunit A OS=Homo sapiens OX=9606 GN=EIF3A PE=1 SV=1
Q9NTJ3	Structural maintenance of chromosomes protein 4 OS=Homo sapiens OX=9606 GN=SMC4 PE=1 SV=2
O60841	Eukaryotic translation initiation factor 5B OS=Homo sapiens OX=9606 GN=EIF5B PE=1 SV=4
P39060	Collagen alpha-1(XVIII) chain OS=Homo sapiens OX=9606 GN=COL18A1 PE=1 SV=5
Q6P2E9	Enhancer of mRNA-decapping protein 4 OS=Homo sapiens OX=9606 GN=EDC4 PE=1 SV=1
P0DTC2	Spike glycoprotein OS=Severe acute respiratory syndrome coronavirus 2 OX=2697049 GN=S PE=1 SV=1

MFVFLVLLPLVSSQCVNLTR**TQLPPAYTNSFTR**GVVYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVS GTNGTKR**FD**
NPVLPFNDGVYFASTEKSNIIIR**GWIFGTTLDSK**TQSLIVNNATNVVIKVCE FQFCNDPFLGVVYHKNNK**SWMESEFR**VY
 SSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSK**HTPINLVR**DLPQGFSALEPLVDLPIGINITR**FQT**
LLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGITITDAVDCALDPLSETKCTLKSFTVEK**GIYQTSNFRV**
QPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKR**SNCVADYSVLVNSASFSTFKCYGVSP**TKLNDLCFTNVYADSF
 VIRGDEV RQIAPGQTGK**IADYNYKL**PDDFTGCVIAWNSNNLDSK**VGGNYNYLYR**LFRKSNLKPFERDISTEIYQAGSTPC
 NGVEGFNCYFPLQSYGFQPTNGVGYPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKK**FL**
PFQQFGRDIADTTDAVRDPQTL EILDITPCSGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWR**VYSTGS**
NVFQTRAGCLIGAEHVNNSECDIPIGAGICASYQTQTNSPRRARSVASQSIAYTMSLGAENSVAYSNNISIAIPTNFTI
 SVTTEILPVSMTKTSVDCTMYICGDSTEC SNLLLQYGSFCTQLNR**ALTGIAVEQDK****NTQEVFAQVK**QIYKTPPIKDFGGF
 NFSQILPDPSKPSKRSFIEDLLFNK**VTLADAGFIK****QYGDCLGDIAAR**DLICAQKFNGLTVLPPLL TDEMIAQYTSALLAG
 TITSGWTFGAGAAALQIPFAMQMAYR**FNGIGVTQNVLYENQK****LIANQFN**SAIGK**IQDSL**SSTASALGK**LQDVVNQNAQA**LN
TLVKQLSSNFGAISSVLNDILSR**LDKVEAEVQIDR**LITGR**LQSLQTYVTQQLIR**AAEIR**ASANLAATK****MSECVLGQSK**RV
 DFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICH DGKAHFPREGVFVSNGTHW FVTQRNFYEPQIITTDNT
 FVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVLGDISGINASV VNIQKEIDRLNEVAKNLNESLIDL
 QELGKYEQYIKWPWYIWLGFIAGLIAVMVTIMLCCMTSCC SCLGCCSCGSCCK**FDEDDSEPV**LKGVKLHYT

331/1273*100=26% coverage

Table S4. Expression and infectivity of pseudovirions produced in different cells. WPRE qPCR, LTR qPCR, p24 and %GFP positive Vero E6 infected cells with lentiviral pseudovirions containing S proteins produced in LV (lenti), Expi293 (expi) and HEK293T cells. The data are presented as means \pm S.D. of 3 measurements.

qPCR WPRE particle/ μ l	LV		Expi293		HEK293T	
Spike original	3.24E+07	\pm 5.52E+06	1.02E+08	\pm 1.15E+07	7.43E+05	\pm 2.62E+05
Spike codon opt.	5.31E+04	\pm 8.44E+03	7.13E+06	\pm 8.79E+05	1.61E+05	\pm 7.43E+05
Spike QC11 codon opt.	8.67E+05	\pm 8.51E+04	4.48E+06	\pm 4.11E+05	1.09E+05	\pm 1.61E+05
Spike QC22 codon opt.	3.56E+04	\pm 1.46E+04	1.81E+06	\pm 1.31E+04	6.76E+05	\pm 1.09E+05
Spike QC24 codon opt.	1.44E+06	\pm 1.32E+05	5.02E+06	\pm 3.90E+05	1.88E+05	\pm 8.18E+04

qPCR LTR particle/ μ l	LV		Expi293		HEK293T	
Spike original	2.02E+07	\pm 9.57E+05	3.85E+07	\pm 6.29E+06	3.165E+05	\pm 2.84E+03
Spike codon opt.	4.51E+04	\pm 7.35E+03	4.74E+06	\pm 8.41E+05	4.538E+04	\pm 5.81E+02
Spike QC11 codon opt.	7.74E+05	\pm 1.24E+04	1.51E+06	\pm 5.70E+04	1.118E+04	\pm 2.94E+02
Spike QC22 codon opt.	1.27E+04	\pm 9.76E+02	9.41E+05	\pm 1.56E+04	9.441E+03	\pm 7.52E+02
Spike QC24 codon opt.	1.41E+06	\pm 1.18E+05	4.29E+06	\pm 3.66E+05	3.39E+05	\pm 6.87E+04

%GFP at 48h post-infection	LV		Expi293		HEK293T	
Spike original	95.21	\pm 0.28	58.69	\pm 1.2	34.36	\pm 2.8
Spike codon opt.	0.01	\pm 0.0014	1.97	\pm 0.5	10.34	\pm 0.44
Spike QC11 codon opt.	0.61	\pm 0.24	1.19	\pm 0.26	15.78	\pm 0.53
Spike QC22 codon opt.	0.01	\pm 0.006	1.06	\pm 0.09	24.78	\pm 3
Spike QC24 codon opt.	1.45	\pm 0.01	1.47	\pm 0.04	20.3	\pm 0.14

p24 ng/ml	LV	Expi293	HEK293T
Spike original	16631	11262	4137
Spike codon opt.	172	2202	2005
Spike QC11 codon opt.	257	1594	668
Spike QC22 codon opt.	50	700	1820
Spike QC24 codon opt.	237	2832	164