

Cross-protective immunity following coronavirus vaccination and coronavirus infection

Tanushree Dangi^{#1}, Nicole Palacio^{#1}, Sarah Sanchez¹, Mincheol Park¹, Jacob Class², Lavanya Visvabharathy³, Thomas Ciucci^{4,5}, Igor J. Koralnik³, Justin Richner^{*2}, Pablo Penaloza-MacMaster^{*1}

¹Department of Microbiology-Immunology, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611, USA. ²Department of Microbiology & Immunology, University of Illinois at Chicago, Chicago, IL 60612, USA. ³Ken and Ruth Davee Department of Neurology, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611, USA. ⁴David H. Smith Center for Vaccine Biology and Immunology, University of Rochester, Rochester, NY 14642, USA. ⁵Department of Microbiology and Immunology, Center for Vaccine Biology and Immunology, University of Rochester, Rochester, NY 14642, USA.

[#]These authors contributed equally

***Correspondence:**

Justin Richner (richner@uic.edu)

835 S Wolcott

Room E703 MC790

Chicago IL 60612-7342

Office: 312-996-4834

Pablo Penaloza-MacMaster (ppm@northwestern.edu)

303 E. Chicago Ave.

Tarry 6-733, Chicago, IL 60611

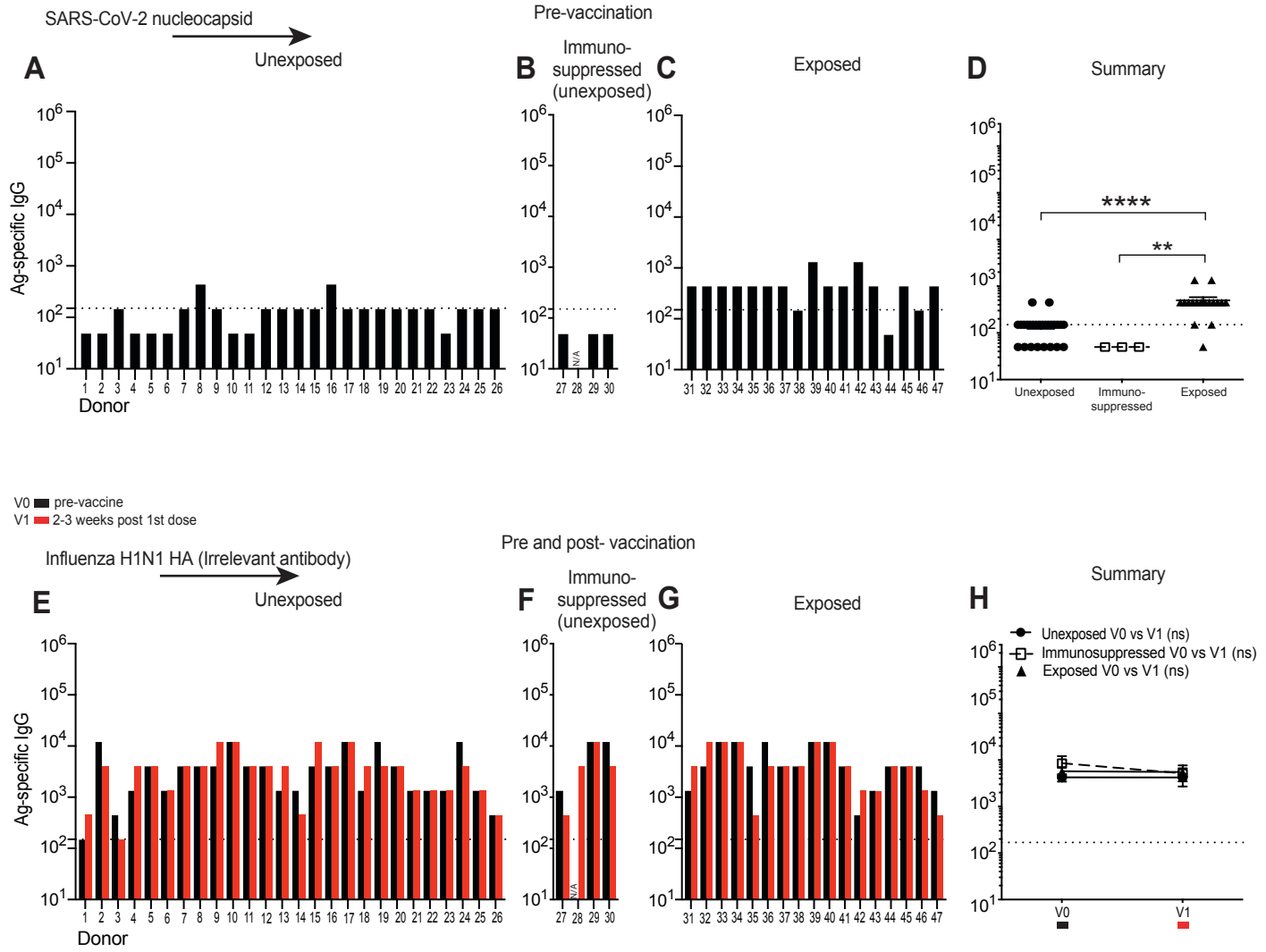
Lab: 312.503.0357

Office: 312.503.5240

Supplemental Figure Legends:

Supplemental Figure 1. Serology screening in humans prior to vaccination and unrelated cross-reactive antibodies post vaccination. SARS-CoV-2 nucleocapsid specific antibody titers prior to vaccination in **(A)** unexposed, **(B)** unexposed immunosuppressed, and **(C)** exposed participants. **(D)** Summary of nucleocapsid specific antibody responses. Influenza virus H1N1 HA specific antibodies pre (V0) and post (V1) vaccination in **(E)** unexposed, **(F)** unexposed immunosuppressed, and **(G)** exposed participants. **(H)** Summary of Influenza virus H1N1 HA specific antibodies for V0 and V1. **, $P < 0.01$, **** $P < 0.0001$, and ns=not significant by one way ANOVA with multiple comparisons in panel D and by paired student t test V0 vs V1 within each cohort (unexposed, immunosuppressed, and exposed) in panel H. Dashed lines represent LOD. D-H, error bars represent SEM.

Fig. S1

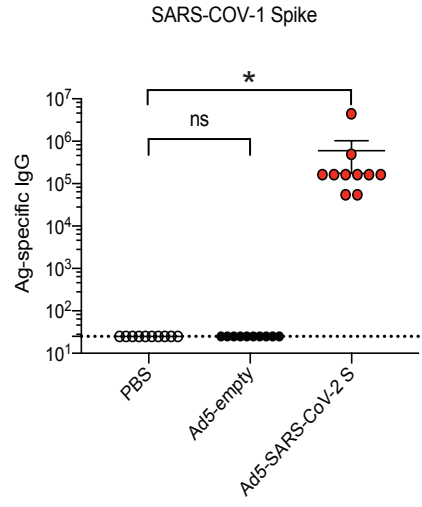
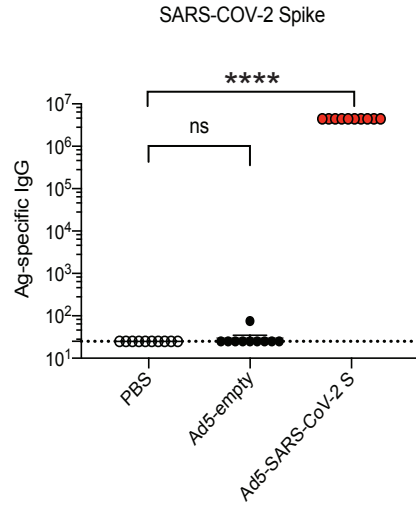
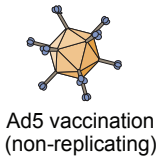


Supplemental Figure 2. SARS-CoV-1 and SARS-CoV-2 spike specific antibody responses in mice immunized with irrelevant vaccine vectors. (A)

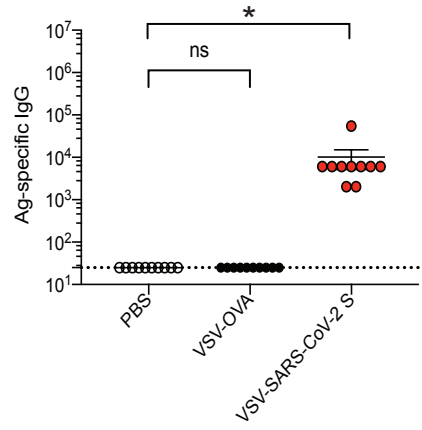
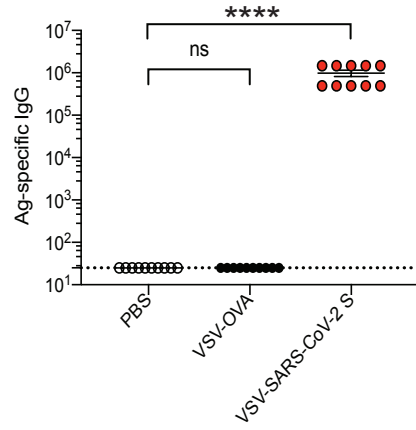
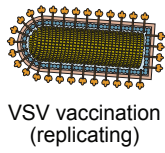
Antibody responses after immunization with PBS, Ad5-empty or Ad5-SARS-CoV-2 spike. **(B)** Antibody responses after immunization with PBS, VSV-Ova or VSV-SARS-CoV-2 spike. **(C)** Antibody responses after immunization with PBS, MVA-SIV or MVA-SARS-CoV-1 spike. Data are from two independent experiments with n=5/group. Data from all experiments are shown. *, P <0.05, ***, P <0.001, and ****, P <0.0001, and ns=not significant by Mann Whitney U Test. Dashed lines represent LOD. Error bars represent SEM.

Fig. S2

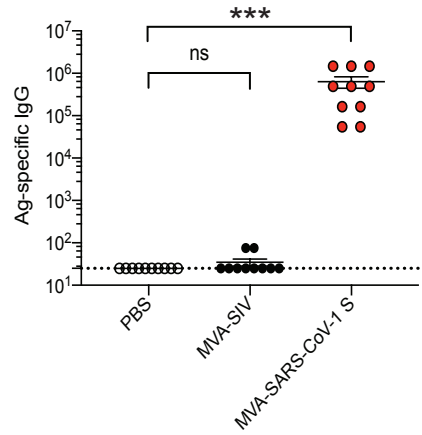
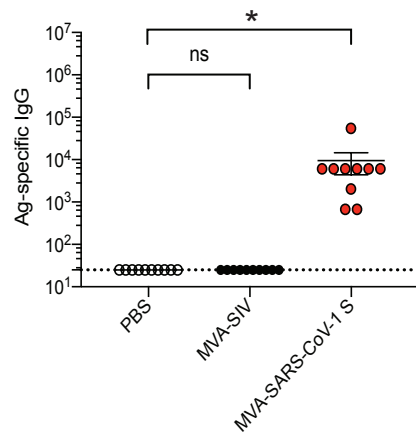
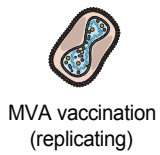
A



B



C



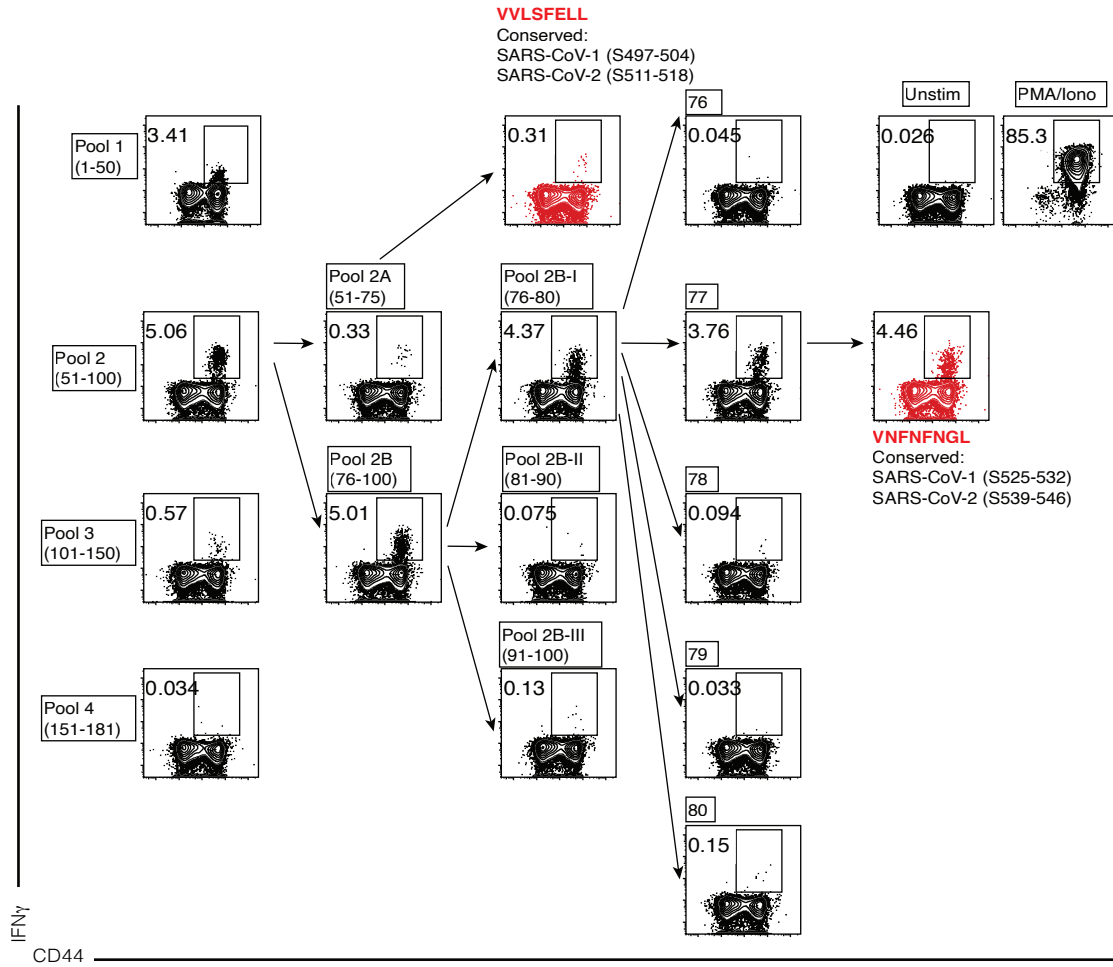
Supplemental Figure 3. Gene alignment of conserved epitopes across various bat coronaviruses. (A) Clustal Omega Multiple Sequence Alignment of SARS-CoV-1, SARS-CoV-2, and other betacoronaviruses, showing conservation of the VVLSFELL and VNFNFNGL epitopes. (B) Phylogenetic tree showing the genetic distance between 2 different coronavirus subgenera: sarbecoviruses and embecoviruses.

Supplemental Figure 4. Mapping of conserved CD8 T cell epitopes

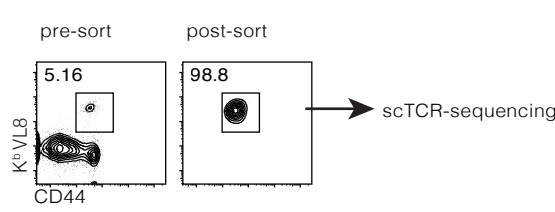
following SARS-CoV-2 vaccination. (A) Epitope mapping using Ad5-SARS-CoV-2 spike immune splenocytes (week 2 post-boost), stimulated with overlapping SARS-CoV-2 spike peptide pools for 5 hr at 37°C in a CO₂ incubator. This study identified 2 potential K^b binding epitopes that are highly conserved among multiple sarbecoviruses listed in Figure S3A: a subdominant VLVSFELL epitope and a dominant VNFNFNGL epitope. Cells are gated on live CD8⁺ lymphocytes. (B) K^b VNFNFNGL (K^b VL8) tetramer⁺ CD8 T cells were FACS-sorted for TCR-sequencing (week 4 post-prime). (C) Top 5 TCR usages (percent of total VL8-specific). (D) TCR sequences in VL8-specific CD8 T cells. (E) TCR Vβ11 frequency in K^b VL8⁺ or K^b VL8⁻ CD8 T cells by confirmatory flow cytometry. Data from panel A represent a pooled sample from 5 spleens. Single-cell TCR-seq data from panels B-D are from 2,032 individual cells from 1 mouse spleen. Experiment was repeated with 2431 individual cells from 1 mouse spleen and similar results were observed (see accession data in Methods). FACS data from panel E are representative from one experiment with n=5 mice (experiment was repeated once with n=5 mice, with similar results).

Fig. S4

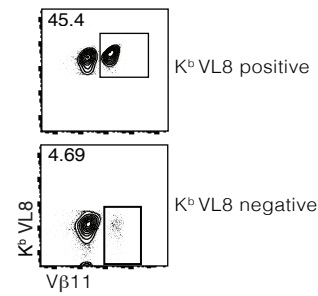
A



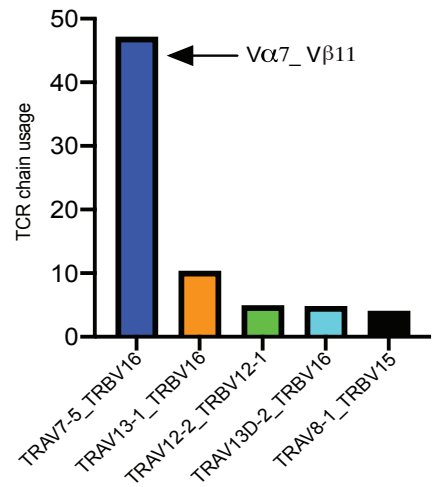
B



E



C



D

TCR α (V7-5, J50)
 CTAGAAGGTTGTGCGCGGGATTTTGTATTGGGGAGAATCCCCAGTGGAGAGAGATAAAGAG
 CAAATGATGAAATCCTTGAGTGTTCACTAGTGGTCTGTGGCTCCAGTTAATGGGTGAGCA
 GCCAGCAGAAGGTGCGCAGAGCCAGAATCCCTCACTGTCTCAGAGGGAGCCATGGCCTCTCT
 CAACTGCAGTTCAGTGATGGTACTTCTAACAACCTCAGGTGGTACAGACAGCATTCTGCGAAA
 GGCCTTGAGGTGCTAGTGTCCATCTCTGATGGTAAAAGGAAGGCAGATTACAGTCTC
 ACCTCAATAGAGCCAACTTGCATGTTCCCTACACATCAGAGAACCACAACCCAGTGACTTGC
 TGCTACCTCTGTGCAGTTATAGCATCTCTCTCCTCAGCAAGTGGTGTGGGCGAGGGGACA
 TCCTTATCAGTCTCCAAACATCCAGAACCCAGAACCTGTGTACCAGTTAAAGATCCTC
 GGTCTCAGGACAGCACCTCTGCCTGTTCCAGACTTTGACT
 CDR3 sequence CAVIASSSFSKLVF

TCR β (V16, J2-5, C2)
 TCTCGTGCTTACGTGGAGTTTCTATGAGTGAAGCCACTGCCTCATCTTGCCATGGCCCCAGGC
 TCCTTTTCTGTCTGGTCTTTGCTCTTGAGAGCAGAACCAACAAATGCTGGTGCATCCAAAC
 ACCTAGGCACAAGGTGACAGGGAAGGACAAGAAGCACTCTGTGGTGTGAGCCAAATTCAGGA
 CATAGTGTGTTTTCTGTACAGACAGACCATTTGTCAGGGCTGGAGTTCCTGACTTACTTTC
 GAAATCAAGCTCTATAGATGATTCAGGGATGCCAAGGAACGATTCTCAGCTCAGATGCCAA
 TCAGTGCACACTCACTGAAATCCAGAGCAGCAACCCAGGACTCAGCGGTATCTTTGT
 GCAAGCAGCTTACTGGGGGGCGCACACCCAGTACTTTGGGCGAGGCACTCGGCTCCTGTGT
 TAGAGGACTGTGAGAAATGTGACTCCACCAAGTCTCCTTGTGAGCCATCAAAGCAGAGAT
 TGCAAACAACAAAAGGCTACCTCTGTGCTTGCC
 CDR3 sequence CASLLGGRDQYF

5'UTR
 C region

Supplemental Figure 5. Cross-reactive antibody responses following coronavirus infections in mice. (A) Antibody responses after infection with OC43 common cold coronavirus. (B) Antibody responses after infection with MHV-1 coronavirus. Mice were intranasally primed and boosted after 3 weeks (see Methods for virus dose information). Antibody responses were evaluated by ELISA at week 2 post-boost. Experiments were done using wild type C57BL/6 mice. Dashed lines represent LOD. Data are from two independent experiments with n=5/group. Data from all experiments are shown. *, P <0.05, ***, P <0.001, and ****, P <0.0001 by Mann Whitney U Test. Error bars represent SEM.

Supplemental Table Legends:

Supplemental Table 1. SARS-CoV-2 spike overlapping peptide pools spanning 1273 amino acids. Each individual peptide consisted of 13-17 amino acids, with 10 amino acid overlaps.

SARS-CoV-2 overlapping peptide pools		
Peptide pools	Peptide	Sequence
Pool 1 (1-50)	1	1-MFVFLVLLPLVSSQCVN-17
	2	8-LPLVSSQCVNLTRRTQL-24
	3	15-CVNLTRRTQLPPAYTNS-31
	4	22-TQLPPAYTNSFTRGVYY-38
	5	29-TNSFTRGVYYPDKVFRS-45
	6	36-VYYPDKVFRSSVLHSTQ-52
	7	43-FRSSLHSTQDLFLPFF-59
	8	50-STQDLFLPFFSNVTWFH-66
	9	57-PFFSNVTWFHAIHVSGT-73
	10	64-WFHAIHVSGTNGTKRFD-80
	11	71-SGTNGTKRFDNPVLPFN-87
	12	78-RFDNPVLPFNDGVYFAS-94
	13	85-PFNDGVYFASTEKSNII-101
	14	92-FASTEKSNIIRGWIFGT-108
	15	99-NIIRGWIFGTTLDSKTQ-115
	16	106-FGTTLDSKTQSLIVNN-122
	17	113-KTQSLIVNNATNVVIK-129
	18	120-VNNATNVVIKVCEFQFC-136
	19	127-VIKVCEFQFCNDPFLGV-143
	20	134-QFCNDPFLGVYYHKNNK-150
	21	141-LGVYYHKNNKSWMESEF-157
	22	148-NNKSWMESEFRVYSSAN-164
	23	155-SEFRVYSSANNCTFEYV-171
	24	162-SANNCTFEYVSQPFLMD-178
	25	169-EYVSQPFLMDLEGKQGN-185
	26	176-LMDLEGKQGNFKNLREF-192
	27	183-QGNFKNLREFVFKNIDG-199
	28	190-REFVFKNIDGYFKIYSK-206
	29	197-IDGYFKIYSKHTPINLV-213
	30	204-YSKHTPINLVRDLPQGF-220
	31	211-NLVRDLPQGFSALEPLV-227
	32	218-QGFSALEPLVDLPIGIN-234
	33	225-PLVDLPIGINITRFQTL-241
	34	232-GINITRFQTLALHRSY-248
	35	239-QTLLALHRSYTPGDSS-255
	36	246-RSYLTPGDSSSGWTAGA-262
	37	253-DSSSGWTAGAAAYVGY-269
	38	260-AGAAAYVGYLQPRTFL-276
	39	267-VGYLQPRTFLLKYNENG-283
	40	274-TFLLKYNENGTITDAVD-290

	41	281-ENGTITDAVDCALDPLS-297
	42	288-AVDCALDPLSETKCTLK-304
	43	295-PLSETKCTLKSFTVEKG-311
	44	302-TLKSFTVEKGIYQTSNF-318
	45	309-EKGIYQTSNFRVQPTES-325
	46	316-SNFRVQPTESIVRFPNI-332
	47	323-TESIVRFPNITNLCDFG-339
	48	330-PNITNLCDFGEVFNATR-346
	49	337-PFGEVFNATRFASVYAW-353
	50	344-ATRFASVYAWNRKRISN-360
Pool 2 (51-100)	51	351-YAWNRKRISNCVADYSV-367
	52	358-ISNCVADYSVLYNSASF-374
	53	365-YSVLYNSASFSTFKCYG-381
	54	372-ASFSTFKCYGVSPTKLN-388
	55	379-CYGVSPKLNLDLFTNV-395
	56	386-KLNDLFTNVYADSFVI-402
	57	393-TNVYADSFVIRGDEVRQ-409
	58	400-FVIRGDEVRQIAPGQTG-416
	59	407-VRQIAPGQTGKIADYNY-423
	60	414-QTGKIADYNYKLPDFT-430
	61	421-YNYKLPDFTGCVIAWN-437
	62	428-DFTGCVIAWNSNNLDSK-444
	63	435-AWNSNNLDSKVGGNYY-451
	64	442-DSKVGGNYYLYRLFRK-458
	65	449-YNYLYRLFRKSNLKPFE-465
	66	456-FRKSNLKPFERDISTEI-472
	67	463-PFERDISTEIQAGSTP-479
	68	470-TEIQAGSTPCNGVEGF-486
	69	477-STPCNGVEGFNCYFPLQ-493
	70	484-EGFNCYFPLQSYGFQPT-500
	71	491-PLQSYGFQPTNGVGYQP-507
	72	498-QPTNGVGYQPYRVVLS-514
	73	505-YQPYRVVLSFELLHAP-521
	74	512-VLSFELLHAPATVCGPK-528
	75	519-HAPATVCGPKKSTNLVK-535
	76	526-GPKKSTNLVKNKCVNFN-542
	77	533-LVKNKCVNFNFNGLTGT-549
	78	540-NFNFNGLTGTGVLTESN-556
	79	547-TGTGVLTESNKKFLPFQ-563
	80	554-ESNKKFLPFQQFGRDIA-570
	81	561-PFQQFGRDIADTTDAVR-577
	82	568-DIADTTDAVRDPQTLEI-584
	83	575-AVRDPQTLEILDITPCS-591

	84	582-LEILDITPCSFGGVSVI-598
	85	589-PCSFGGVSVITPGTNTS-605
	86	596-SVITPGTNTSNQVAVLY-612
	87	603-NTSNQVAVLYQDVNCTE-619
	88	610-VLYQDVNCTEVPVAIHA-626
	89	617-CTEVPVAIHADQLTPTW-633
	90	624-IHADQLTPTWRVYSTGS-640
	91	631-PTWRVYSTGSNVFQTRA-647
	92	638-TGSNVFQTRAGCLIGAE-654
	93	645-TRAGCLIGAEHVNNSYE-661
	94	652-GAEHVNNSYECDIPIGA-668
	95	659-SYECDIPIGAGICASYQ-675
	96	666-IGAGICASYQTQTNSPR-682
	97	673-SYQTQTNSPRRARSVAS-689
	98	680-SPRRARSVASQSIIAYT-696
	99	687-VASQSIIAYTMSLGAEN-703
	100	694-AYTMSLGAENSVAYSNN-710
Pool 3 (101-150)	101	701-AENSVAYSNNIAIPTN-717
	102	708-SNNSIAIPTNFTISVTT-724
	103	715-PTNFTISVTTEILPVSM-731
	104	722-VTTEILPVSMTKTSVDC-738
	105	729-VSMTKTSVDCTMYICGD-745
	106	736-VDCTMYICGDSTECNL-752
	107	743-CGDSTECNLLLQYGSF-759
	108	750-SNLLLQYGSFCTQLNRA-766
	109	757-GSFCTQLNRALTGIAVE-773
	110	764-NRALTGIAVEQDKNTQE-780
	111	771-AVEQDKNTQEVFAQVKQ-787
	112	778-TQEVFAQVKQIYKTPPI-794
	113	785-VKQIYKTPPIKDFGGFN-801
	114	792-PPIKDFGGFNFSQILPD-808
	115	799-GFNFSQILPDPSKPSKR-815
	116	806-LPDPSKPSKRSFIEDLL-822
	117	813-SKRSFIEDLLFNKVTLA-829
	118	820-DLLFNKVTLADAGFIKQ-836
	119	827-TLADAGFIKQYGDCLGD-843
	120	834-IKQYGDCLGDIAARDLI-850
	121	841-LGDIAARDLICAQKFNG-857
	122	848-DLICAQKFNGLTVLPPL-864
	123	855-FNGLTVLPPLLTDEMIA-871
	124	862-PPLLTDEMIAQYTSALL-878
	125	869-MIAQYTSALLAGTITSG-885
	126	876-ALLAGTITSGWTFGAGA-892

	127	883-TSGWTFGAGAALQIPFA-899
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	129	897-PFAMQMAYRFNGIGVTQ-913
	130	904-YRFNGIGVTQNVLYENQ-920
	131	911-VTQNVLYENQKLIANQF-927
	132	918-ENQKLIANQFNSAIGKI-934
	133	925-NQFNSAIGKIQDLSLST-941
	134	932-GKIQDLSLSTASALGKL-948
	135	939-SSTASALGKLQDVVNQN-955
	136	946-GKLQDVVNQNAQALNTL-962
	137	953-NQNAQALNTLVKQLSSN-969
	138	960-NTLVKQLSSNFGAISSV-976
	139	967-SSNFGAISSVLNDILSR-983
	140	974-SSVLNDILSRDKVEAE-990
	141	981-LSRLDKVEAEVQIDRLI-997
	142	988-EAEVQIDRLITGRLQSL-1004
	143	995-RLITGRLQSLQTYVTQQ-1011
	144	1002-QSLQTYVTQQLIRAAEI-1018
	145	1009-TQQLIRAAEIRASANLA-1025
	146	1016-AEIRASANLAATKMSEC-1032
	147	1023-NLAATKMSECVLGQSKR-1039
	148	1030-SECVLGQSKRVDFCGKG-1046
	149	1037-SKRVDFCGKGYHLMSFP-1053
	150	1044-GKGYHLMSFPQSAPHGV-1060
Pool 4 (151-181)	151	1051-SFPQSAPHGVVFLHVTY-1067
	152	1058-HGVVFLHVTYVPAQEKN-1074
	153	1065-VTYVPAQEKNFTTAPAI-1081
	154	1072-EKNFTTAPAICHDGKAH-1088
	155	1079-PAICHDGKAHFPREGVF-1095
	156	1086-KAHFPREGVFSNGTHW-1102
	157	1093-GVFSNGTHWFVTQRNF-1109
	158	1100-THWFVTQRNFYEPQIIT-1116
	159	1107-RNFYEPQIITDNTFVS-1123
	160	1114-IITDNTFVSGNCDVVI-1130
	161	1121-FVSGNCDVVIGIVNNTV-1137
	162	1128-VVIGIVNNTVYDPLQPE-1144
	163	1135-NTVYDPLQPELDSFKEE-1151
	164	1142-QPELDSFKEELDKYFKN-1158
	165	1149-KEELDKYFKNHTSPDVD-1165
	166	1156-FKNHTSPDVDLGDISGI-1172
	167	1163-DVDLGDISGINASVVNI-1179
	168	1170-SGINASVVNIQKEIDRL-1186
	169	1177-VNIQKEIDRLNEVAKNL-1193

170	1184-DRLNEVAKNLNESLIDL-1200
171	1191-KNLNESLIDLQELGKYE-1207
172	1198-IDLQELGKYEQYIKWPW-1214
173	1205-KYEQYIKWPWYIWLGF-1221
174	1212-WPWYIWLGFIAGLIAIV-1228
175	1219-GFIAGLIAIVMTIMLC-1235
176	1226-AIVMTIMLCCMTSCCS-1242
177	1233-MLCCMTSCCSCLKGCCS-1249
178	1240-CCSCLKGCCSCGSCCKF-1256
179	1247-CCSCGSCCKFDEDDSEP-1263
180	1254-CKFDEDDSEPVKGVKL-1270
181	1261-SEPVKGVKLHYT-1273