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CGTTGACGTG GAGCCAAGAT AGCTATCCTC CGACCTTCTT TCCTGTCTTC ATCGAGACCG ACACTACCCC TAGAATGACC CGGACGATGA GGACCCCGTC
1 M G I L L G L L L L G H
*MET
101 CTAACAGTGG ACACTTATGG CCGTCCCATC CTGGAAGTGC CAGAGAGTGT AACAGGACCT TGGAAAGGGC ATGTGAATCT TCCCTGCACC TATGACCCCC
GATTGTCACC TGTGAATACC GGCAGGGTAG GACCTTCACG GTCTCTCACA TTGTCCTGGA ACCTTTCCCC TACACTTAGA AGGGACGTGG ATACTGGGGG
13 L T V D T Y G R P I L E V P E S V T G P W K G D V N L P C T Y D P L
201 TGCAAGGCTA CACCCAAGTC TTGGTGAAGT GGCTGGTACA ACGTGGCTCA GACCCTGTCA CCATCTTTCT ACGTGACTCT TCTGGAGACC ATATCCAGCA
ACGTTCCGAT GTGGGTTCAG AACCACTTCA CCGACCATGT TGCACCGAGT CTGGGACAGT GGTAGAAAGA TGCACTGAGA AGACCTCTGG TATAGGTCGT
47 Q G Y T Q V L V K W L V Q R G S D P V T I F L R D S S G D H I Q Q
301 GGCAAAGTAC CAGGGCCGCC TGCATGTGAG CCACAAGGTT CCAGGAGATG TATCCGTCCA ATTGAGCACC CTGGAGATGG ATGACCGGAG CCACTACACG
CCGTTTCATG GTCCCGGCGG ACGTACACTC GGTGTTCCAA GGTCCTCTAC ATAGGGAGGT TAACTCGTGG GACCTCTACC TACTGGCCTC GGTGATGTGC
80 A K Y Q G R L H V S H K V P G D V S L Q L S T L E M D D R S H Y T
401 TGTGAAGTCA CCTGGCAGAC TCCTGATGGC AACCAAGTCG TGAGAGATAA GATTACTGAG CTCCGTGTCC AGAAACTCTC TGTCTCCAAG CCCACAGTGA
ACACTTCAG GGACCGTCTG AGGACTACCG TTGGTTCAGC ACTCTCTATT CTAATGACTC GAGGCACAGG TCTTTGAGAG ACAGAGGTTC GGGTGTCACT
113 C E V T W Q T P D G N Q V V R D K I T E L R V Q K L S V S K P T V T
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GTTGACCGTC GCCAATACCG AAGTGCCACG GGGTCCCTTA CTCCTAATCG GAAGTTACGG TCCGAGCCCC AAGAGGAGGG TAGTCAATAT AAACCATATT
147 T G S G Y G F T V P Q G M R I S L Q C Q A R G S P P I S Y I W Y K

FIG. 1A

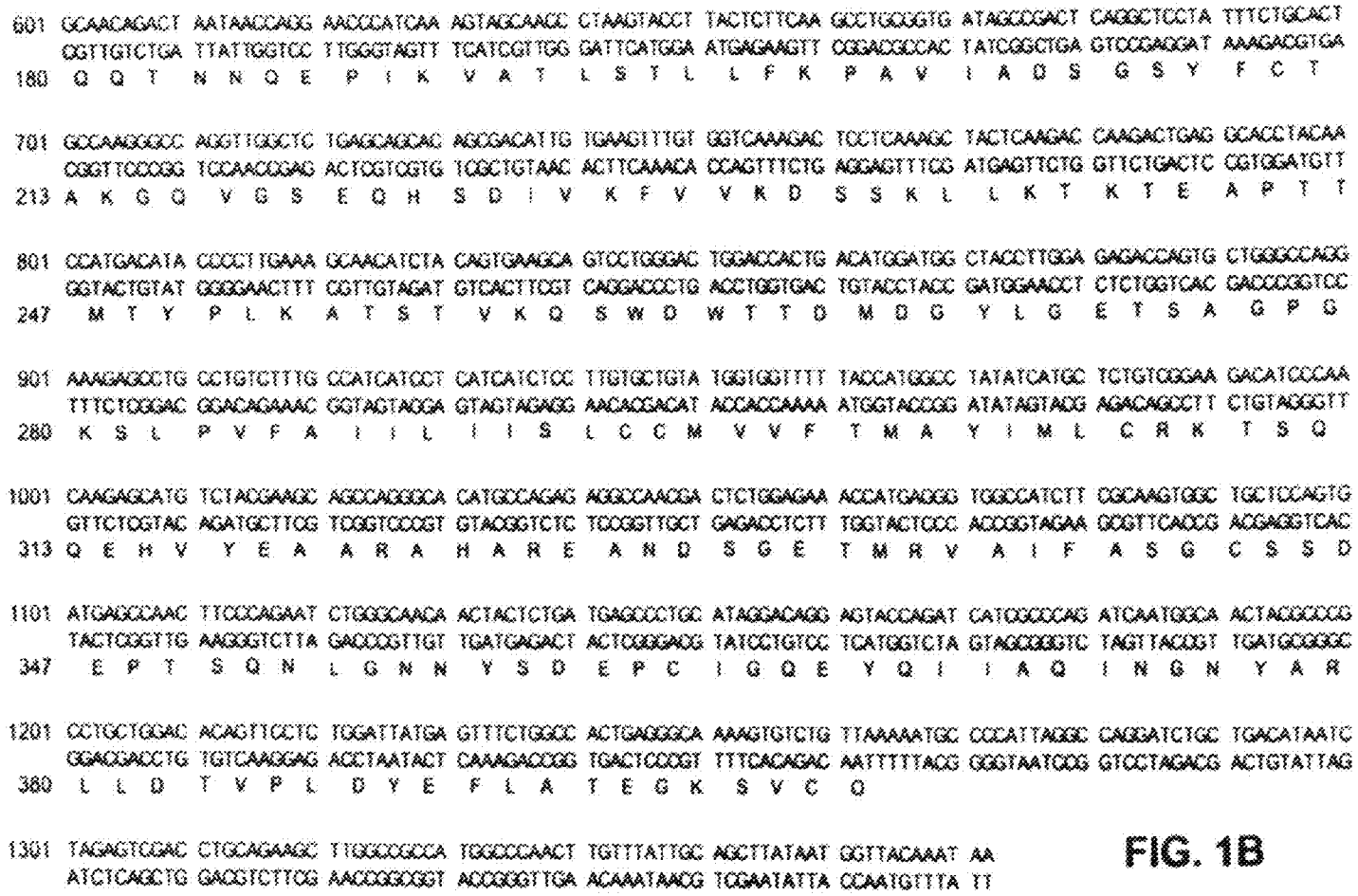

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CGGTTCCCGG TCCAACCGAG ACTCGTCGTG TCGCTGTAAC ACTTCAAACA CCAGTTTCTG AGGAGTTTCG ATGAGTTCTG GTTCTGACTC CGTGGATGTT
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GTTCTCGTAC AGATGCTTCG TCGGTCCCGT GTACGGTCTC TCCGGTTGCT GAGACCTCTT TGGTACTGCC ACCGGTAGAA GCGTTCACCG ACGAGGTCAC
313 Q E H V Y E A A R A H A R E A N D S G E T M R V A I F A S G C S S D
1101 ATGAGCCAAC TTCCCAGAAT CTGGGCAACA ACTACTCTGA TGAGCCCTGC ATAGGACAGG AGTACCAGAT CATCGCCCAG ATCAATGGCA ACTACGCCCG
TACTCGGTTG AAGGGTCTTA GACCCGTTGT TGATGAGACT ACTCGGGACG TATCCTGTCC TCATGGTCTA GTAGCGGGTC TAGTTACCGT TGATGCGGGC
347 E P T S Q N L G N N Y S D E P C I G Q E Y Q I I A Q I N G N Y A R
1201 CCTGCTGGAC ACAGTTCCTC TGGATTATGA GTTTCTGGCC ACTGAGGGCA AAAGTGTCTG TTAAAAATGC CCCATTAGGC CAGGATCTGC TGACATAATC
GGACGACCTG TGTCAAGGAG ACCTAATACT CAAAGACCGG TGACTCCCGT TTTCACAGAC AATTTTTACG GGGTAATCCG GTCCTAGACG ACTGTATTAG
380 L L D T V P L D Y E F L A T E G K S V C Q
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FIG. 1B

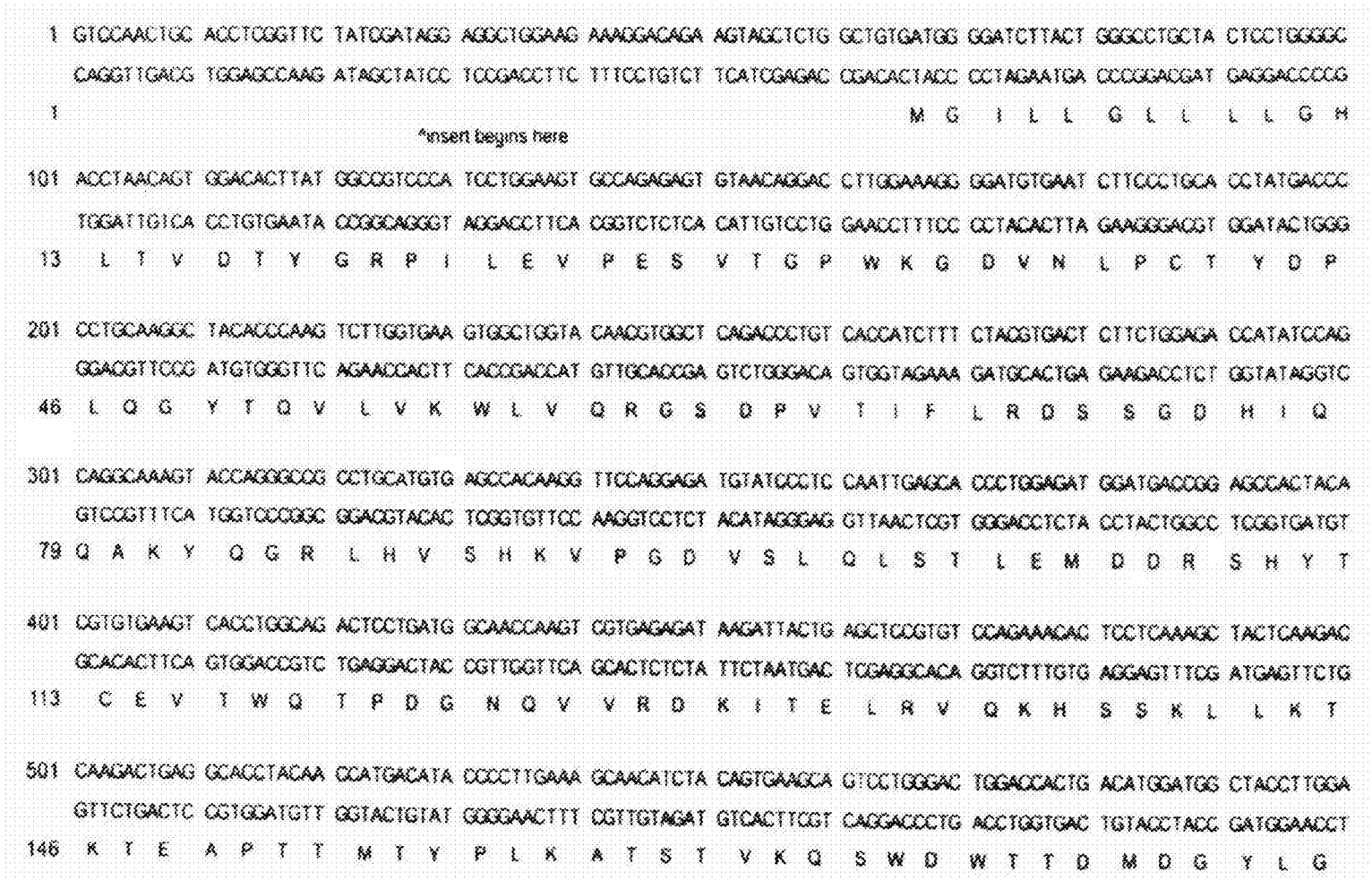

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1 M G I L L G L L L L G H
*insert begins here
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TGGATTGTCA CCTGTGAATA CCGGCAGGGT AGGACCTTCA CGGTCTCTCA CATTGTCCTG GAACCTTTCC CCTACACTTA GAAGGGACGT GGATACTGGG
13 L T V D T Y G R P I L E V P E S V T G P W K G D V N L P C T Y D P
201 CCTGCAAGGC TACACCCAAG TCTTGGTGAA GTGGCTGGTA CAACGTGGCT CAGACCCTGT CACCATCTTT CTACGTGACT CTTCTGGAGA CCATATCCAG
GGACGTTCCG ATGTGGGTTC AGAACCACTT CACCGACCAT GTTGCACCGA GTCTGGGACA GTGGTAGAAA GATGCACTGA GAAGACCTCT GGTATAGGTC
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79 Q A K Y Q G R L H V S H K V P G D V S L Q L S T L E M D D R S H Y T
401 CGTGTGAAGT CACCTGGCAG ACTCCTGATG GCAACCAAGT CGTGAGAGAT AAGATTACTG AGCTCCGTGT CCAGAAACAC TCCTCAAAGC TACTCAAGAC
GCACACTTCA GTGGACCGTC TGAGGACTAC CGTTGGTTCA GCACTCTCTA TTCTAATGAC TCGAGGCACA GGTCTTTGTG AGGAGTTTCG ATGAGTTCTG
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GTTCTGACTC CGTGGATGTT CGTACTGTAT GGGGAACTTT CGTTGTAGAT GTCACTTCGT CAGGACCCTG ACCTGGTGAC TGTACCTACC GATGGAACCT
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FIG. 2A

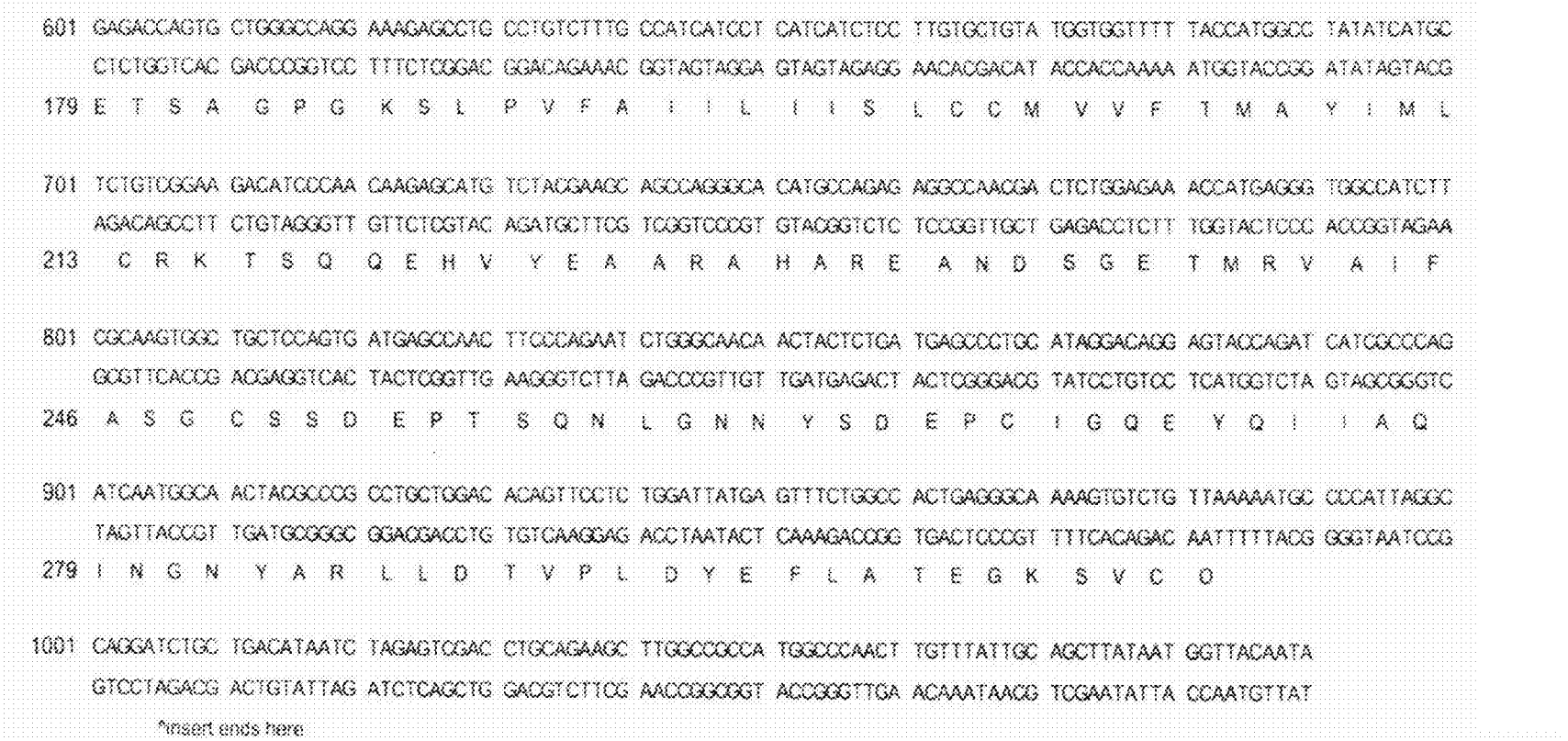

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179 E T S A G P G K S L P V F A I I L I I S L C C M V V F T M A Y I M L
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AGACAGCCTT CTGTAGGGTT GTTCTCGTAC AGATGCTTCG TCGGTCCCGT GTACGGTCTC TCCGGTTGCT GAGACCTCTT TGGTACTCCC ACCGGTAGAA
213 C R K T S Q Q E H V Y E A A R A H A R E A N D S G E T M R V A I F
801 CGCAAGTGGC TGCTCCAGTG ATGAGCCAAC TTCCCAGAAT CTGGGCAACA ACTACTCTGA TGAGCCCTGC ATAGGACAGG AGTACCAGAT CATGGCCCAG
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246 A S G C S S D E P T S Q N L G N N Y S D E P C I G Q E Y Q I I A Q
901 ATCAATGGCA ACTACGCCCG CCTGCTGGAC ACAGTTCCTC TGGATTATGA GTTTCTGGCC ACTGAGGGCA AAAGTGTCTG TTAAAAATGC CCCATTAGGC
TAGTTACCGT TGATGCGGGC GGACGACCTG TGTCAAGGAG ACCTAATACT CAAAGACCGG TGACTCCCGT TTTCACAGAC AATTTTTACG GGGTAATCCG
279 I N G N Y A R L L D T V P L D Y E F L A T E G K S V C O
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^Insert ends here

FIG. 2B

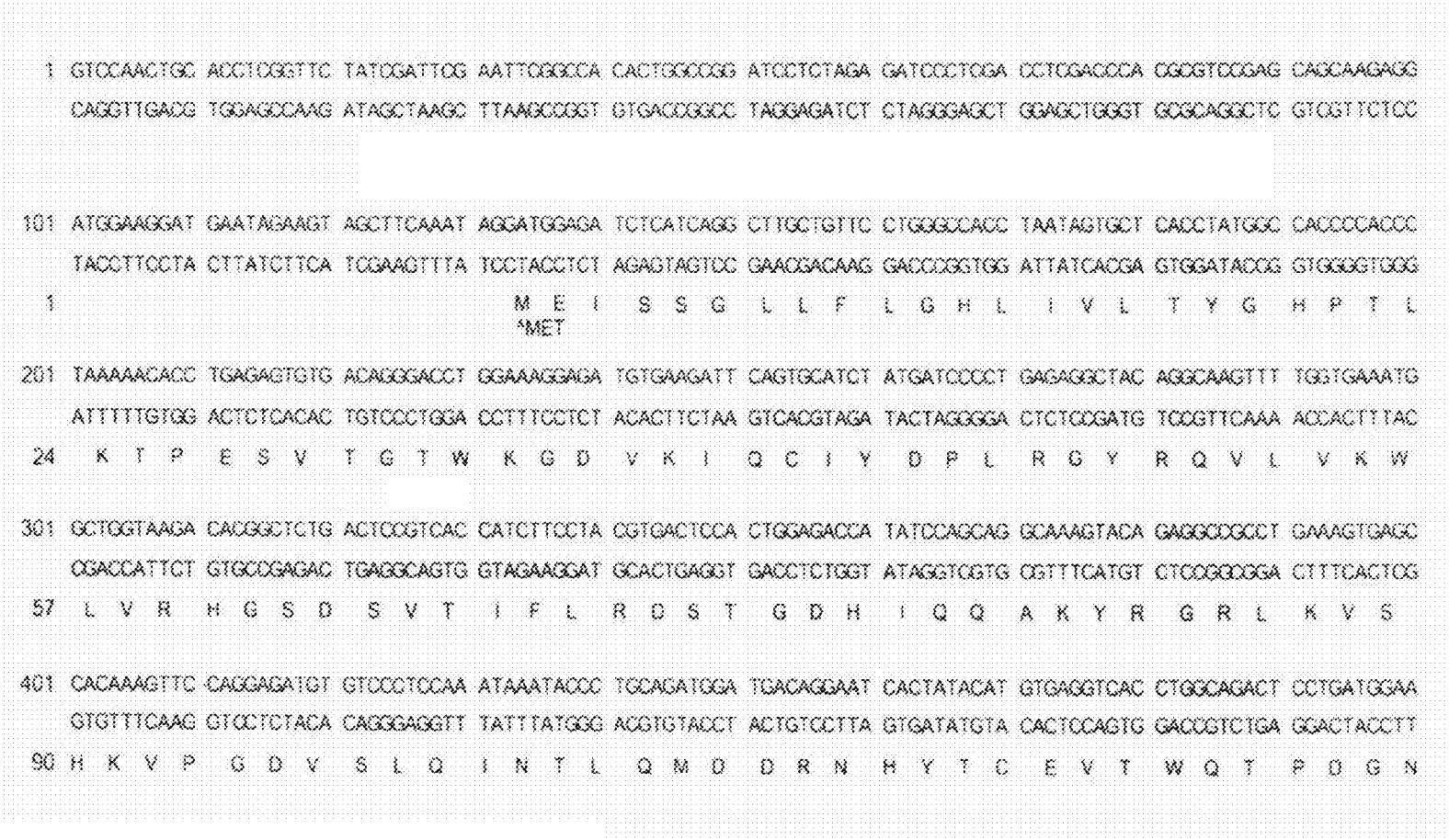

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1 M E I S S G L L F L G H L I V L T Y G H P T L
*MET
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57 L V R H G S D S V T I F L R C S T G D H I Q Q A K Y R G R L K V S
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90 H K V P G D V S L Q I N T L Q M C D R N H Y T C E V T W Q T P D G N

FIG. 3A


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TGGTTCATTA TTCTCTATTC TAGTAACTCG AGGCACAAGC CTTTATATTA GGTGGATCTT AGTTATGACT TCGTGGATGT TGGGACGTGA GGAGAAACCT
124 Q V I R D K I I E L R V R K Y N P P R I N T E A P T T L H S S L E
601 AGCAACAACT ATAATGAGTT CAACCTCTGA CTTGACCACT AATGGGACTG GAAAACTTGA GGAGACCATT GCTGGTTCAG GGAGGAACCT GCCAATCTTT
TCGTTGTTGA TATTACTCAA GTTGGAGACT GAACTGGTGA TTACCCTGAC CTTTTGAACT CCTCTGGTAA CGACCAAGTC CCTCCTTGGA CGGTTAGAAA
157 A T T I M S S T S D L T T N G T G K L E E T I A G S G R N L P I F
701 GCCATAATCT TCATCATCTC CCTTTGCTGC ATAGTAGCTG TCACCATACC TTATATCTTG TTCCGCTGCA GGACATTCCA ACAAGAGTAT CTCTATGGAG
CGGTATTAGA AGTAGTAGAG GGAAACGACG TATCATCGAC AGTGGTATGG AATATAGAAC AAGGCGACGT CCTGTAAGGT TGTTCTCATA GAGATACCTC
190 A I I F I I S L C C I V A V T I P Y I L F R C R T F Q Q E Y V Y G V
801 TGAGCAGGGT GTTTGCCAGG AAGACAAGCA ACTCTGAAGA AACCACAAGG GTGACTACCA TCGCAACTGA TGAACCAGAT TCCCAGGCTC TGATTAGTGA
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GATGAGACTA CTAGGAACGG AGTCGGTCCT CATGGTTTAT TGGTAGTCTA GTTGTTACAG ATAAGGACGG ACGACTTGTG TCAAAGGTCT TTGATTCTTC
257 Y S D D P C L S Q E Y Q I T I R S T M S I P A C O
1001 TTCTTGCTAC TGAAGAAAAT AACATCTGCT AAAATGCCCC TACTAAGTCA AGGTCTACTG GCGTAATTAC CTGTTACTTA TTTACTACTT GCCTTCAACA
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FIG. 3B

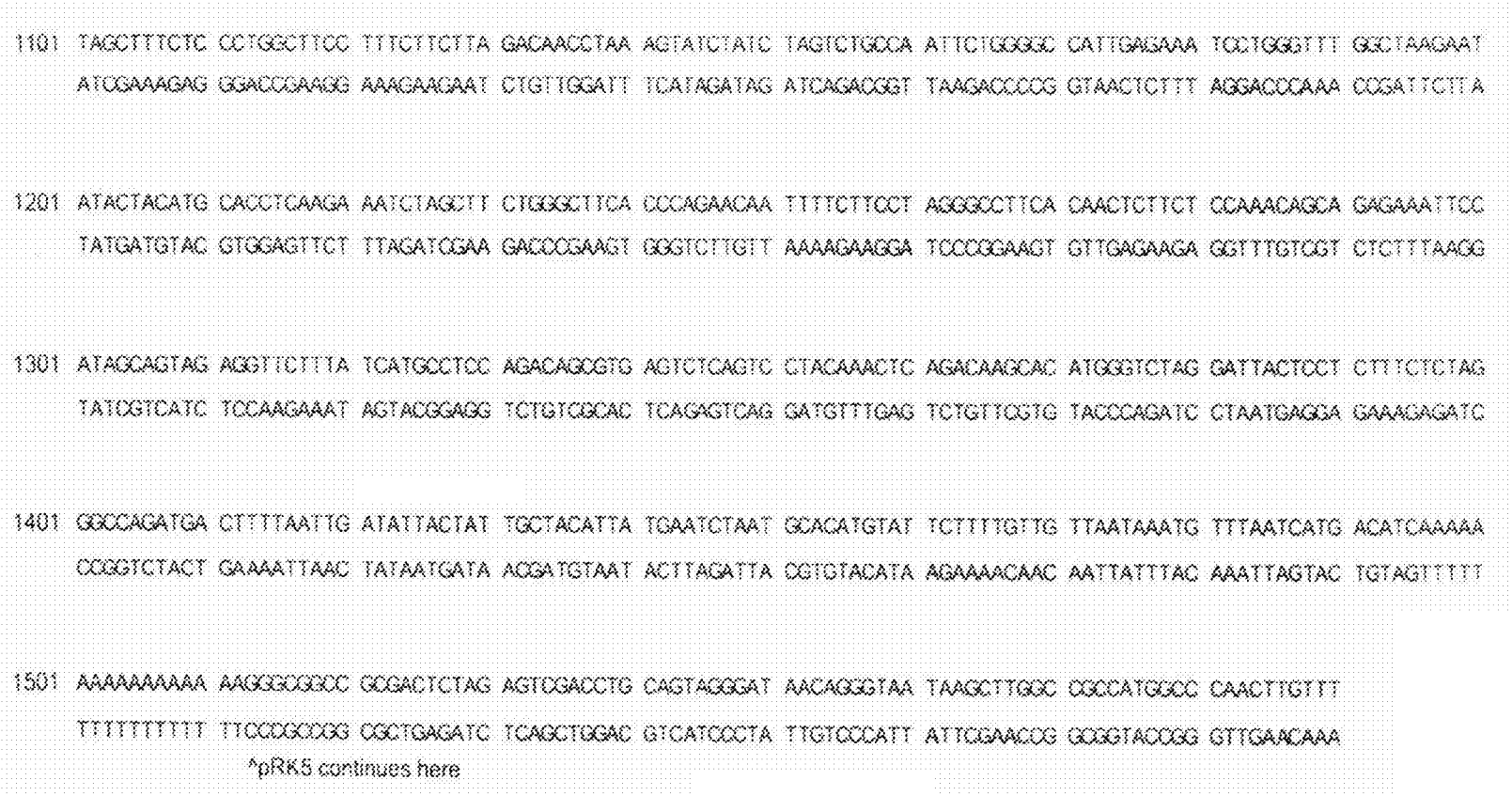

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TATCGTCATC TCCAAGAAAT AGTACGGAGG TCTGTCGCAC TCAGAGTCAG GATGTTTGAG TCTGTTCGTG TACCCAGATC CTAATGAGGA GAAAGAGATC
1401 GGCCAGATGA CTTTTAATTG ATATTACTAT TGCTACATTA TGAATCTAAT GCACATGTAT TCTTTTGTTG TTAATAAATG TTTAATCATG ACATCAAAAA
CCGGTCTACT GAAAATTAAC TATAATGATA ACGATGTAAT ACTTAGATTA CGTGTACATA AGAAAACAAC AATTATTTAC AAATTAGTAC TGTAGTTTTT
1501 AAAAAAAAAA AAGGGCGGCC GCGACTCTAG AGTCGACCTG CAGTAGGGAT AACAGGGTAA TAAGCTTGGC CGCCATGGCC CAACTTGTTT
TTTTTTTTTT TTCCCGCCGG CGCTGAGATC TCAGCTGGAC GTCATCCCTA TTGTCCCATT ATTCGAACCG GCGGTACCGG GTTGAACAAA
^pRK5 continues here

FIG. 3C

Structure-based affinity maturation of CRIg to generate a more potent inhibitor

Fig. 4A

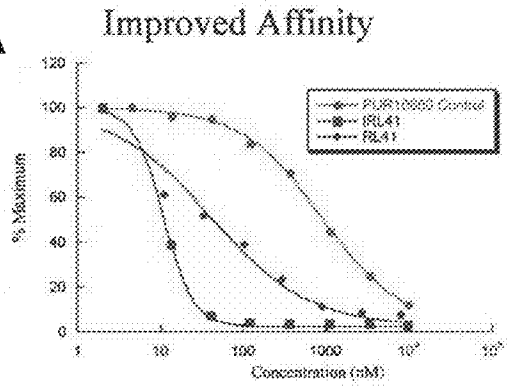
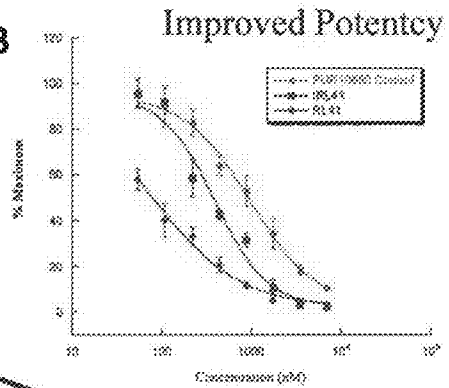


Fig. 4B



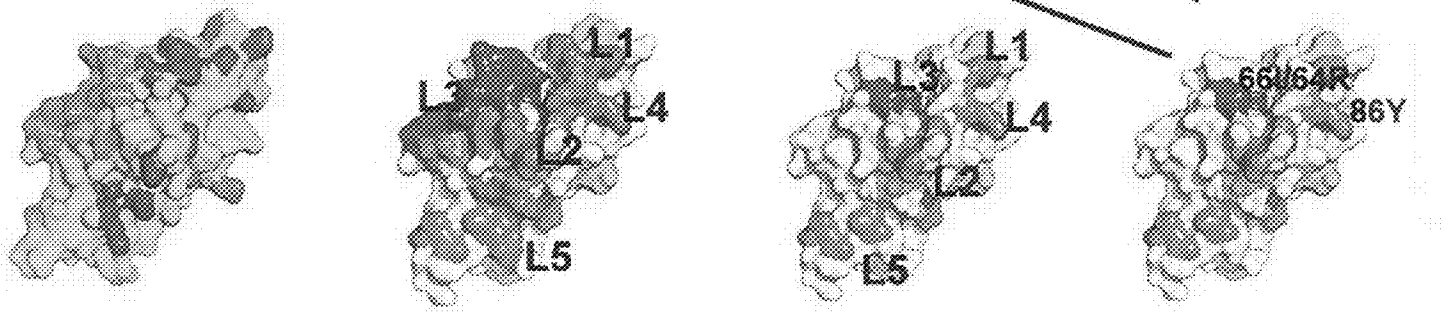
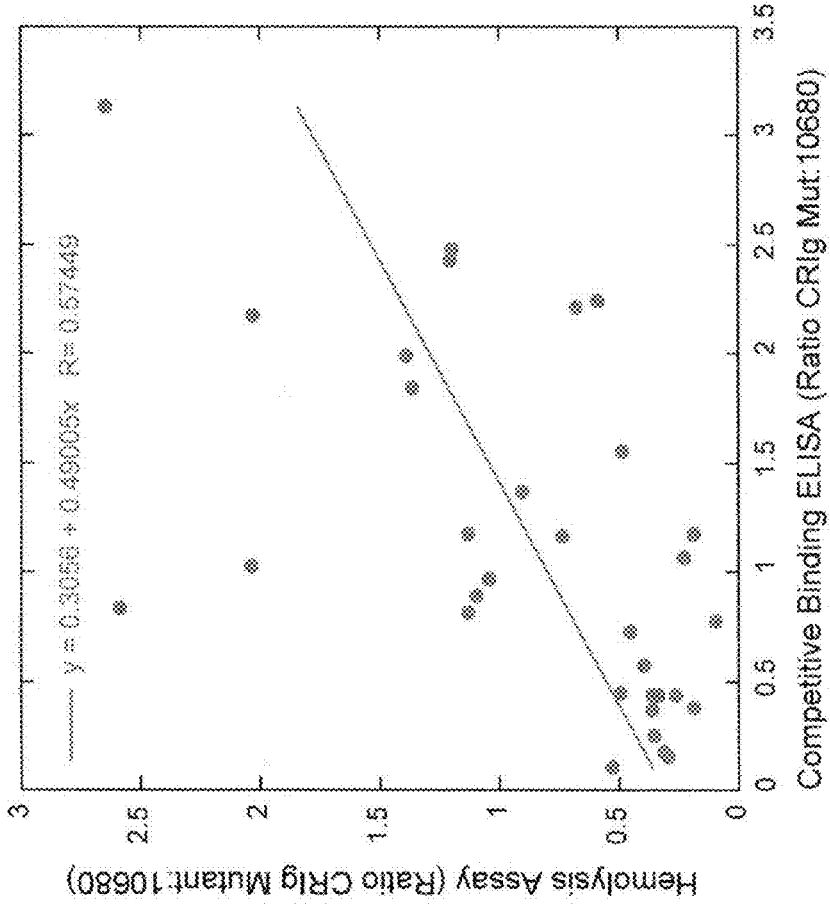


Fig. 4C



The correlated between competitive ELISA and Hemolytic Assay

Fig. 5

Improved affinity of the Q64R / M86Y CRIg mutant

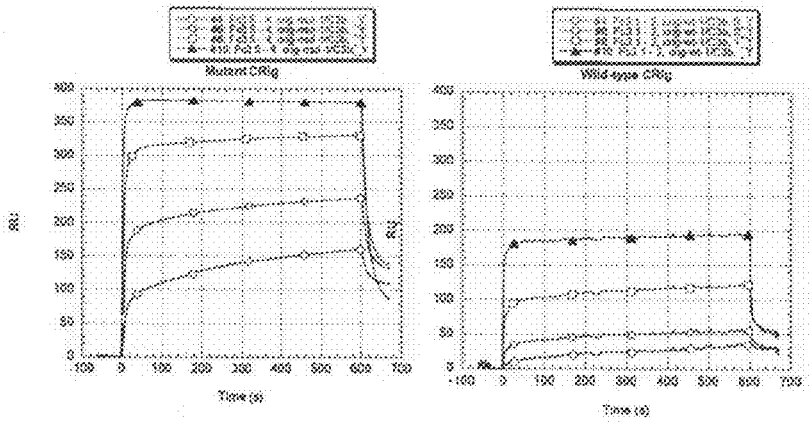


Fig. 6A

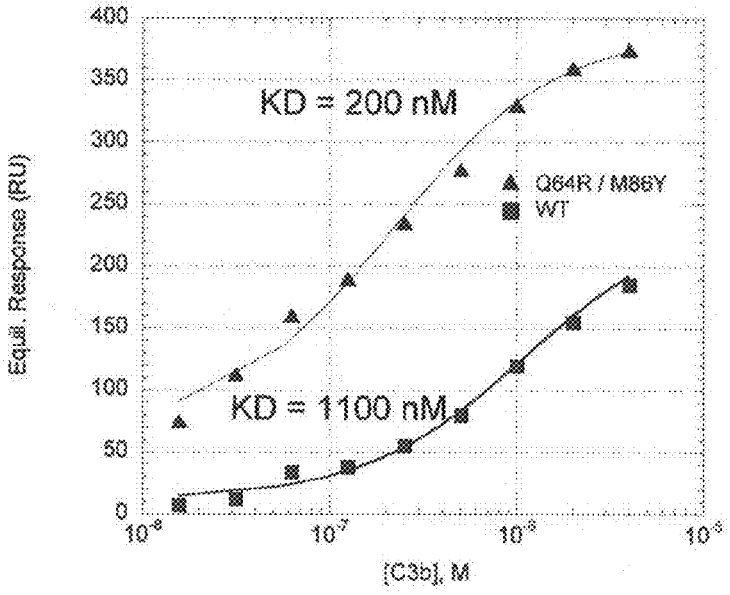


Fig. 6B

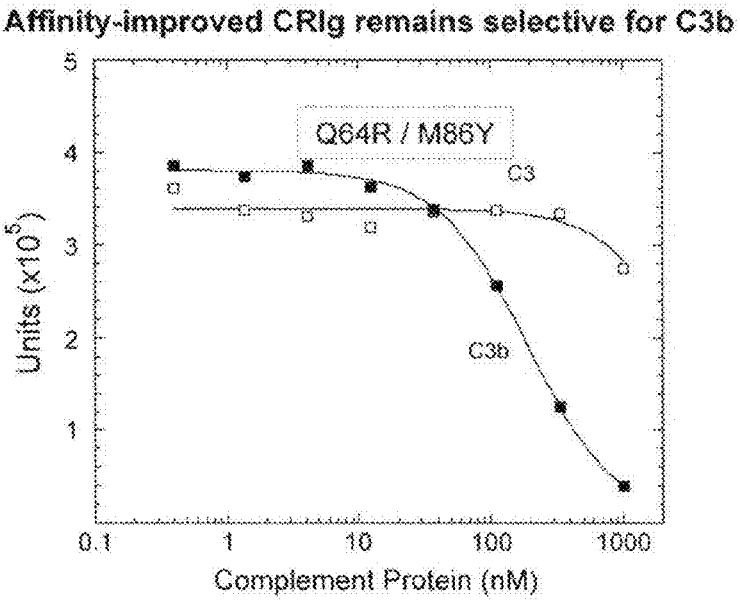
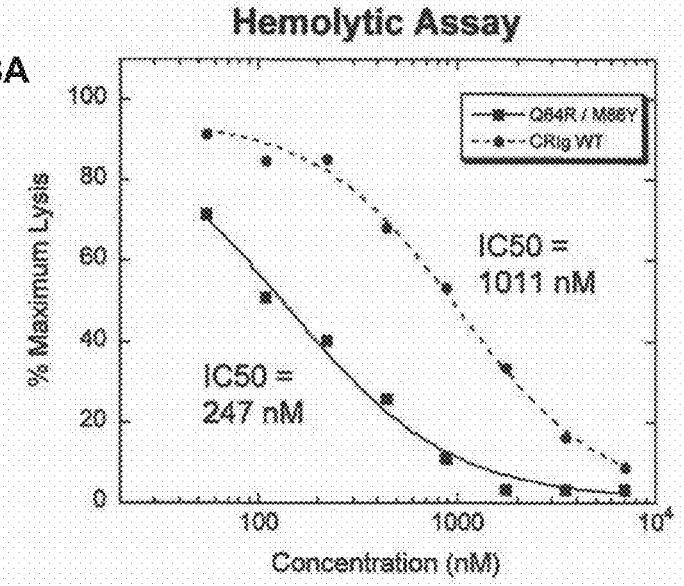

Affinity-improved CRIg remains selective for C3b
Q64R / M86Y
C3
C3b
Units (x10^5)
Complement Protein (nM)
0.1
1
10
100
1000
1
2
3
4
5

Fig. 7

Improved complement inhibitory potency of CRIg Q64R / M86Y *in vitro* compared to wildtype CRIg

Fig. 8A



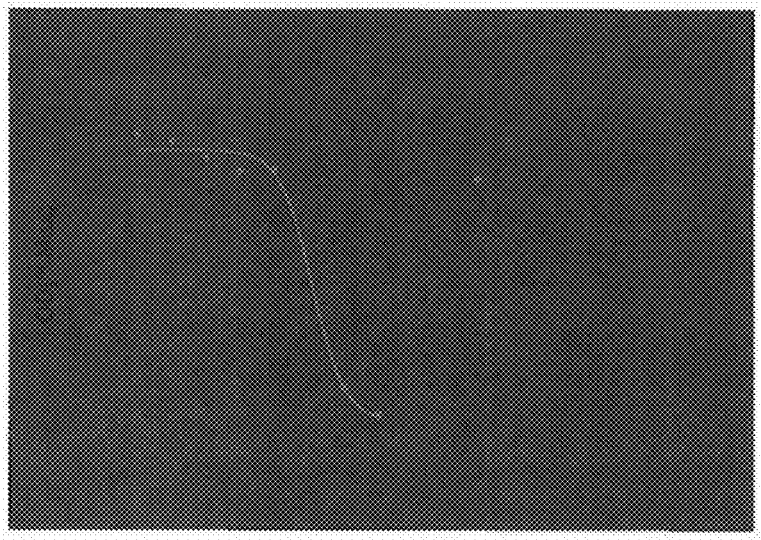


Fig. 8B

CRIg Q64R/M86Y shows improved efficacy *in vivo* over CRIg WT *in vivo*

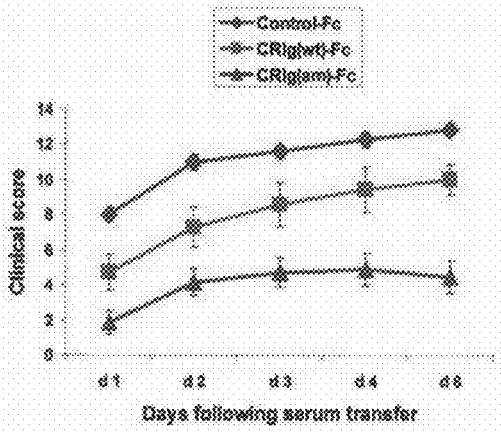


Fig. 9A

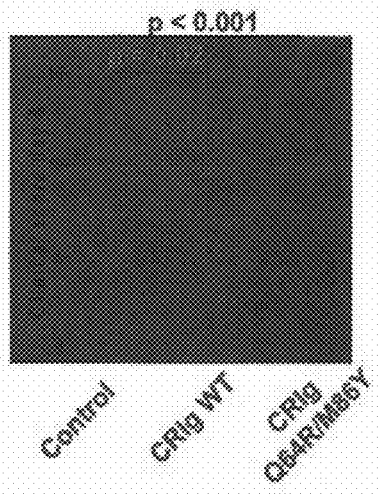


Fig. 9B

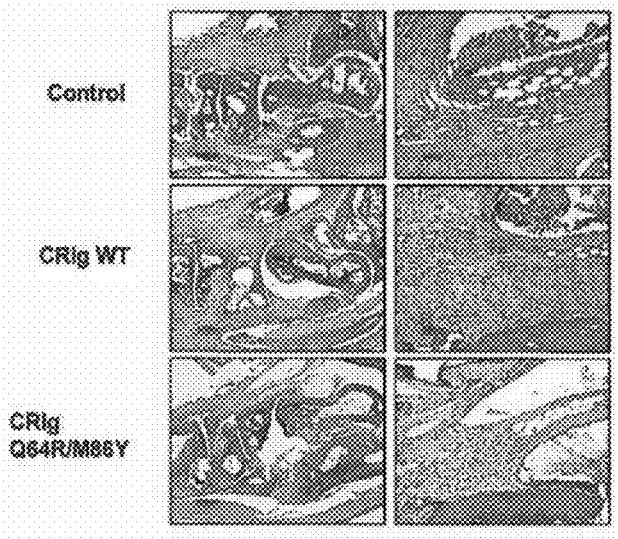


Fig. 9C

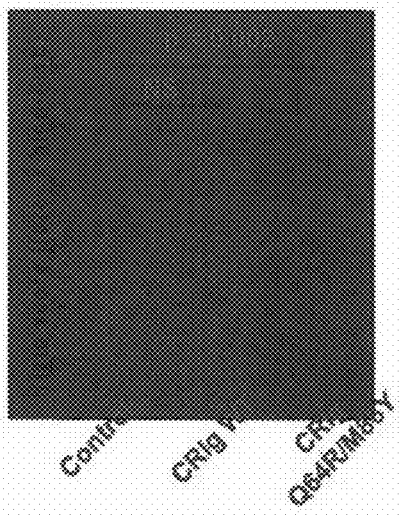


Fig. 9D

Table 1. Phage Libraries

Library	Position	Diversity
Library 1	E8-K15	1.2×10^{10}
Library 2	R41-T47	1.4×10^{10}
Library 3	S54-Q64	1.6×10^{10}
Library 4	E85-Q99	1.2×10^{10}
Library 5	Q105-K111	1.9×10^{10}

Fig. 10

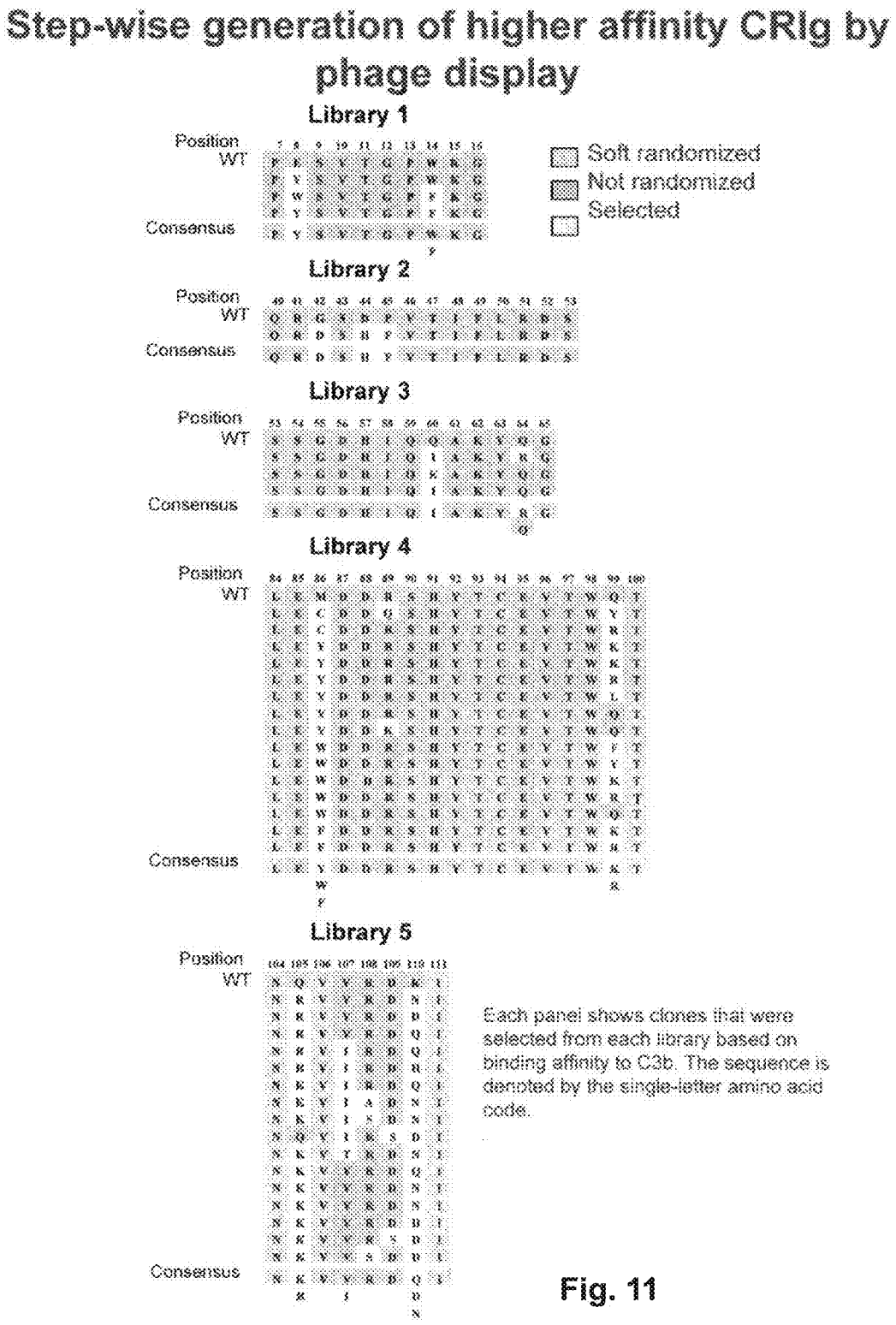
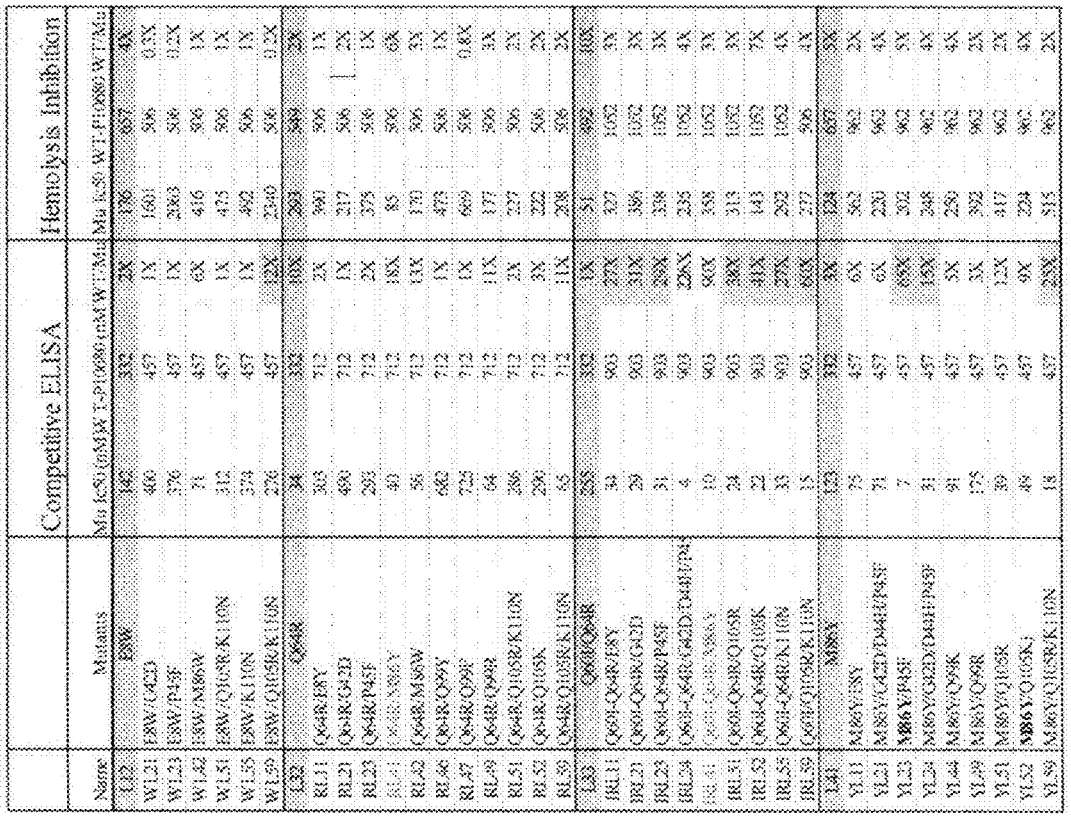

Step-wise generation of higher affinity CRIg by phage display
Library 1
Position
WT
Consensus
Soft randomized
Not randomized
Selected
Library 2
Position
WT
Consensus
Library 3
Position
WT
Consensus
Library 4
Position
WT
Consensus
Library 5
Position
WT
Consensus
Each panel shows clones that were selected from each library based on binding affinity to C3b. The sequence is denoted by the single-letter amino acid code.

Fig. 11

			Competitive ELISA			Hemolysis Inhibition		
	Name	Mutants	Mu IcS0 (nM)	WT-P10680 (nM)	WT/Mu	Mu IcS0	WT-P10680	WT/Mu
Library 1	L11	E8Y	384	332	0.9X	439	591	1X
	L12	E8W	142	332	2X	176	657	4X
	L13	W14F	882	332	0.4X	293	596	2X
	L15	E8Y/W14F	1274	332	0.3X	310	528	2X
Library 2	L21	G42D	453	332	0.7X	378	416	1X
	L22	D44H	338	332	1X	1207	591	0.5X
	L23	P45F	1266	332	0.3X	358	528	1.5X
	L24	G42D/D44/H/P45F	238	332	1X	227	498	2X
Library 3	L31	Q60I	388	332	0.9X	78	416	5X
	L32	Q64R	34	332	10X	293	549	2X
	L33	Q60I/Q64R	255	332	1X	51	492	10X
Library 4	L41	M86Y	123	332	3X	124	657	5X
	L42	M86W	80	332	4X	226	635	3X
	L43	M86F	604	332	0.5X	185	794	4X
	L44	Q99K	268	332	1X	588	520	1X
	L45	Q99R	1397	331	0.2X	818	682	0.8X
	L46	Q99Y	468	332	0.7X	1715	663	0.4X
	L47	Q99F	296	332	1X	570	520	0.9X
	L48	Q99L	1376	332	0.2X	828	682	0.8X
	L49	M86W/Q99R	55	332	6X	201	635	3X
	L410	M86F/Q99R	121	332	3X	273	746	3X
	L411	M86W/Q99K	1594	298	0.2X	1980	747	0.4X
Library 5	L51	Q105R	599	298	0.5X	901	794	0.9X
	L52	Q105K	491	298	0.6X	696	663	1X
	L55	K110D	128	298	2X	196	525	3X
	L56	K110N	132	298	2X	376	746	1.2X
	L58	K110H	1105	298	0.3X	1393	686	0.5X
	L59	Q105R/K110N	127	298	2X	180	525	3X
	L510	Q105R/K110D	1011	298	0.3X	954	686	0.7X
	L511	Q105R/K110Q	166	298	2X	234	592	3X
	L512	Q105K/K110Q	937	298	0.3X	814	596	0.7X
	L514	Q105K/K110D	46	298	6X	167	549	3X

Fig. 12



		Competitive ELISA		Hemolysis Inhibition	
Name	Mutants	Mu Ic50 MAb T-P10686 mAb WT	Mu	Mu Ic50 WT-P10686 WT	Mu
L12	E6W	162	2X	170	497	4X
W1.21	E6W/Q4CD	400	1X	1601	506	0.3X
W1.23	E6W/P45F	376	1X	2083	506	0.2X
W1.42	E6W/N86W	71	6X	416	506	1X
W1.51	E6W/Q16R/K110N	312	1X	425	506	1X
W1.55	E6W/K110N	378	1X	492	506	1X
W1.59	E6W/Q16R/K110N	276	2X	2340	506	0.2X
L32	Q4R	24	10X	200	506	2X
RL11	Q4R/E6Y	303	2X	960	506	1X
RL21	Q4R/Q43D	490	1X	217	506	2X
RL23	Q4R/P45F	295	2X	375	506	1X
RL41	Q4R/N86Y	49	10X	85	506	6X
RL42	Q4R/M86W	86	6X	170	506	3X
RL46	Q4R/Q43Y	682	1X	478	506	1X
RL47	Q4R/Q43F	725	1X	669	506	0.8X
RL48	Q4R/Q43R	64	11X	177	506	3X
RL51	Q4R/Q16R/K110N	286	2X	237	506	2X
RL52	Q4R/Q16N	296	2X	222	506	2X
RL55	Q4R/Q16R/K110N	55	11X	208	506	2X
L33	Q4R/Q43R	255	8X	91	992	10X
IRL11	Q4R/Q43R/E6Y	34	27X	527	1052	2X
IRL21	Q4R/Q43R/Q43D	29	31X	386	1052	2X
IRL23	Q4R/Q43R/P45F	31	29X	338	1052	3X
IRL24	Q4R/Q43R/Q43D/P45F	4	226X	235	1052	4X
IRL41	Q4R/Q43R/N86Y	10	90X	268	1052	4X
IRL51	Q4R/Q43R/Q16R	24	40X	313	1052	3X
IRL52	Q4R/Q43R/Q16N	22	41X	143	1052	7X
IRL55	Q4R/Q43R/K110N	33	27X	262	1052	4X
IRL59	Q4R/Q16R/K110N	15	60X	277	506	4X
L41	N86Y	153	3X	158	497	3X
YL11	N86Y/E6Y	79	6X	362	962	2X
YL21	N86Y/Q43D/P45F	71	6X	220	962	4X
YL23	**N86Y/P45F**	7	69X	302	962	3X
YL24	N86Y/Q43D/P45F	81	59X	248	962	4X
YL44	N86Y/N86K	91	5X	250	962	4X
YL49	N86Y/Q43R	125	3X	392	962	2X
YL51	N86Y/Q16R	89	12X	417	962	2X
YL52	**N86Y/Q16N**	48	9X	234	962	4X
YL59	N86Y/Q16R/K110N	18	25X	515	962	2X

Fig. 13