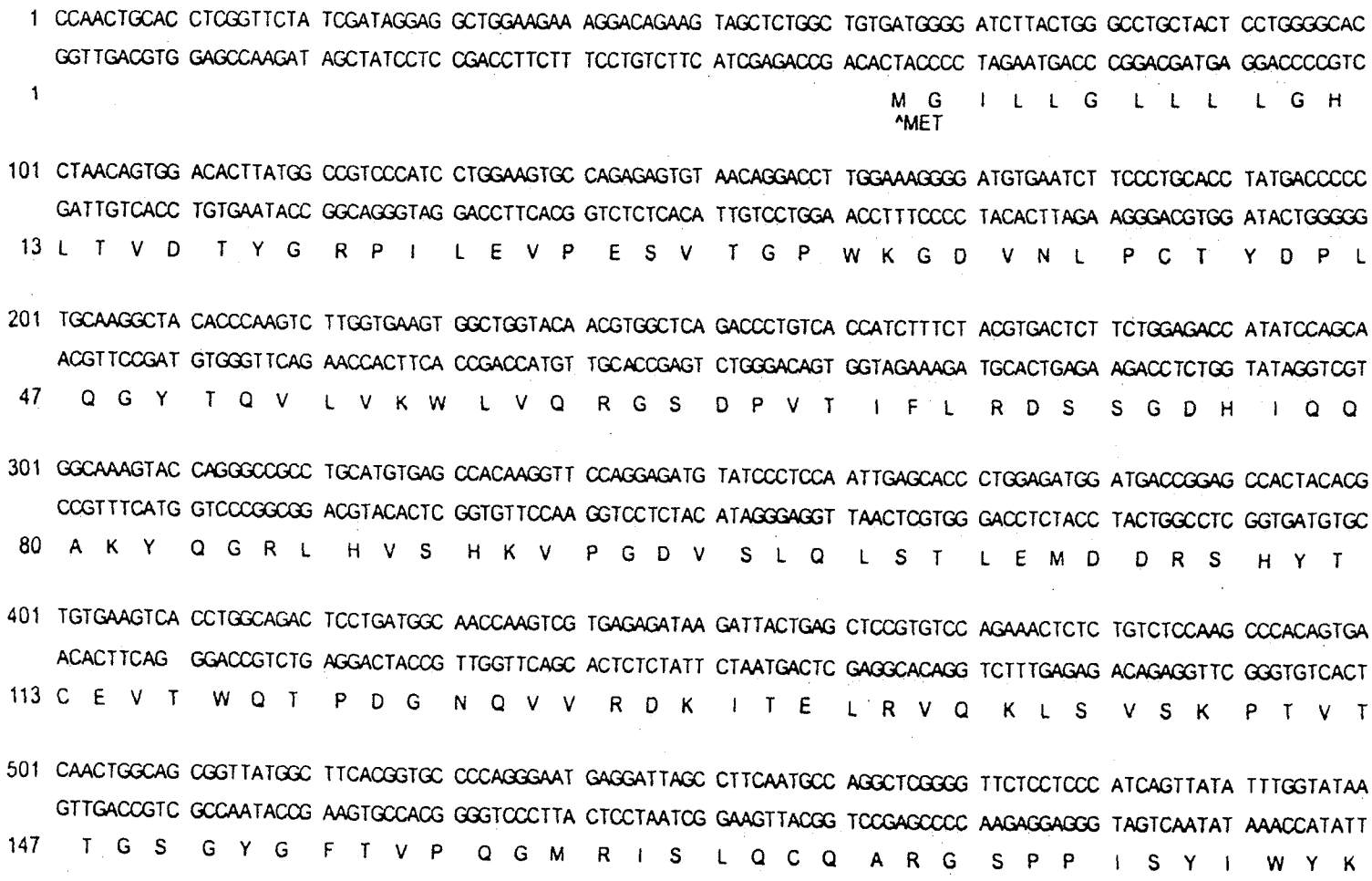

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1 M G I L L G L L L L G H
^MET
101 CTAACAGTGG ACACTTATGG CCGTCCCATC CTGGAAGTGC CAGAGAGTGT AACAGGACCT TGGAAAGGGG ATGTGAATCT TCCCTGCACC TATGACCCCC
GATTGTCACC TGTGAATACC GGCAGGGTAG GACCTTCACG GTCTCTCACA TTGTCCTGGA ACCTTTCCCC TACACTTAGA AGGGACGTGG ATACTGGGGG
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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
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FIG. 1A

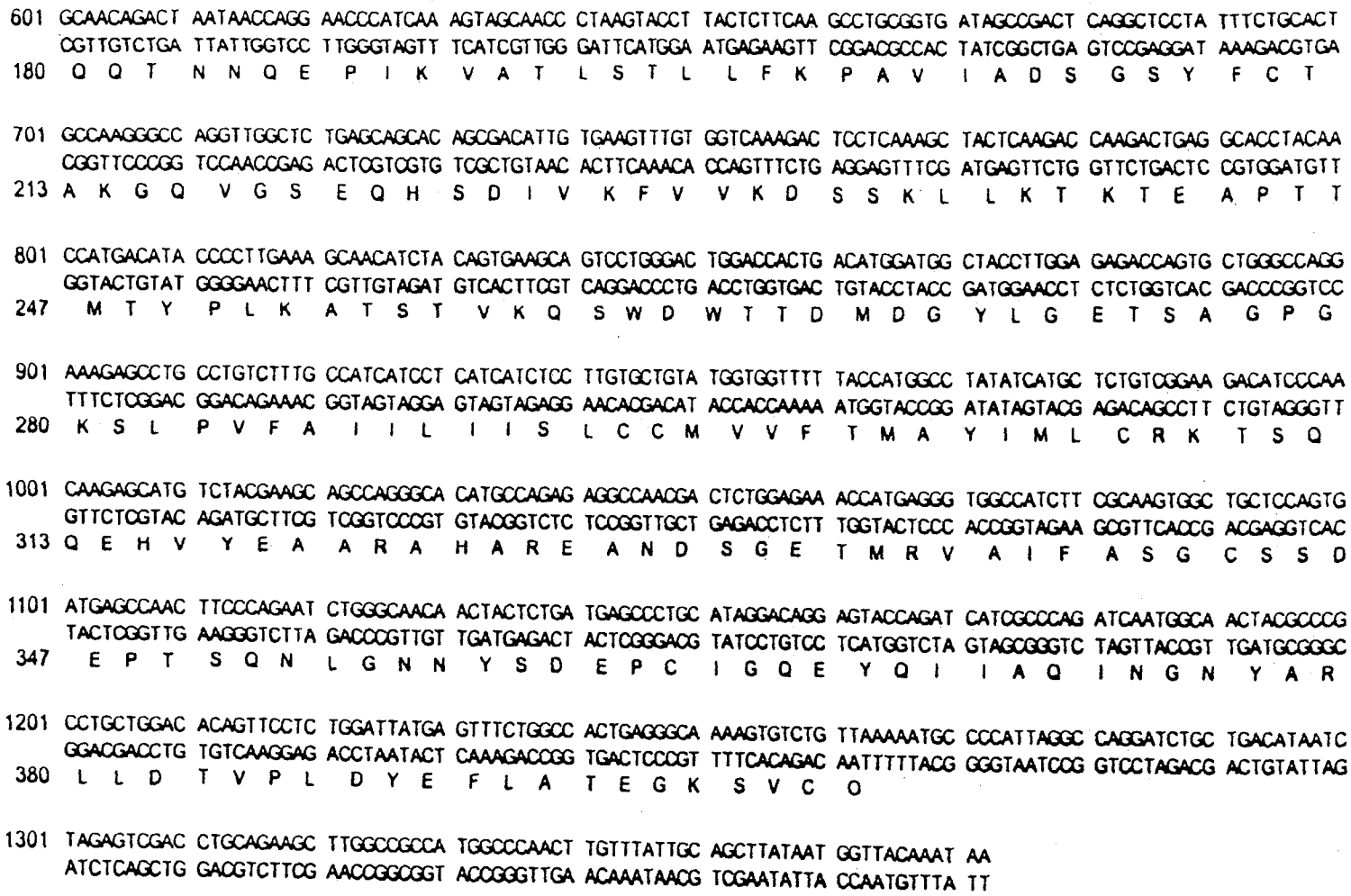

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180 Q Q T N N Q E P I K V A T L S T L L F K P A V I A D S G S Y F C T
701 GCCAAGGGCC AGGTTGGCTC TGAGCAGCAC AGCGACATTG TGAAGTTTGT GGTCAAAGAC TCCTCAAAGC TACTCAAGAC CAAGACTGAG GCACCTACAA
CGGTTCCCGG TCCAACCGAG ACTCGTCGTG TCGCTGTAAC ACTTCAAACA CCAGTTTCTG AGGAGTTTCG ATGAGTTCTG GTTCTGACTC CGTGGATGTT
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GGTACTGTAT GGGGAACTTT CGTTGTAGAT GTCACTTCGT CAGGACCCTG ACCTGGTGAC TGTACCTACC GATGGAACCT CTCTGGTCAC GACCCGGTCC
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GTTCTCGTAC AGATGCTTCG TCGGTCCCGT GTACGGTCTC TCCGGTTGCT GAGACCTCTT TGGTACTCCC 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 O
1301 TAGAGTCGAC CTGCAGAAGC TTGGCCGCCA TGGCCCAACT TGTTTATTGC AGCTTATAAT GGTTACAAAT AA
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FIG. 1B

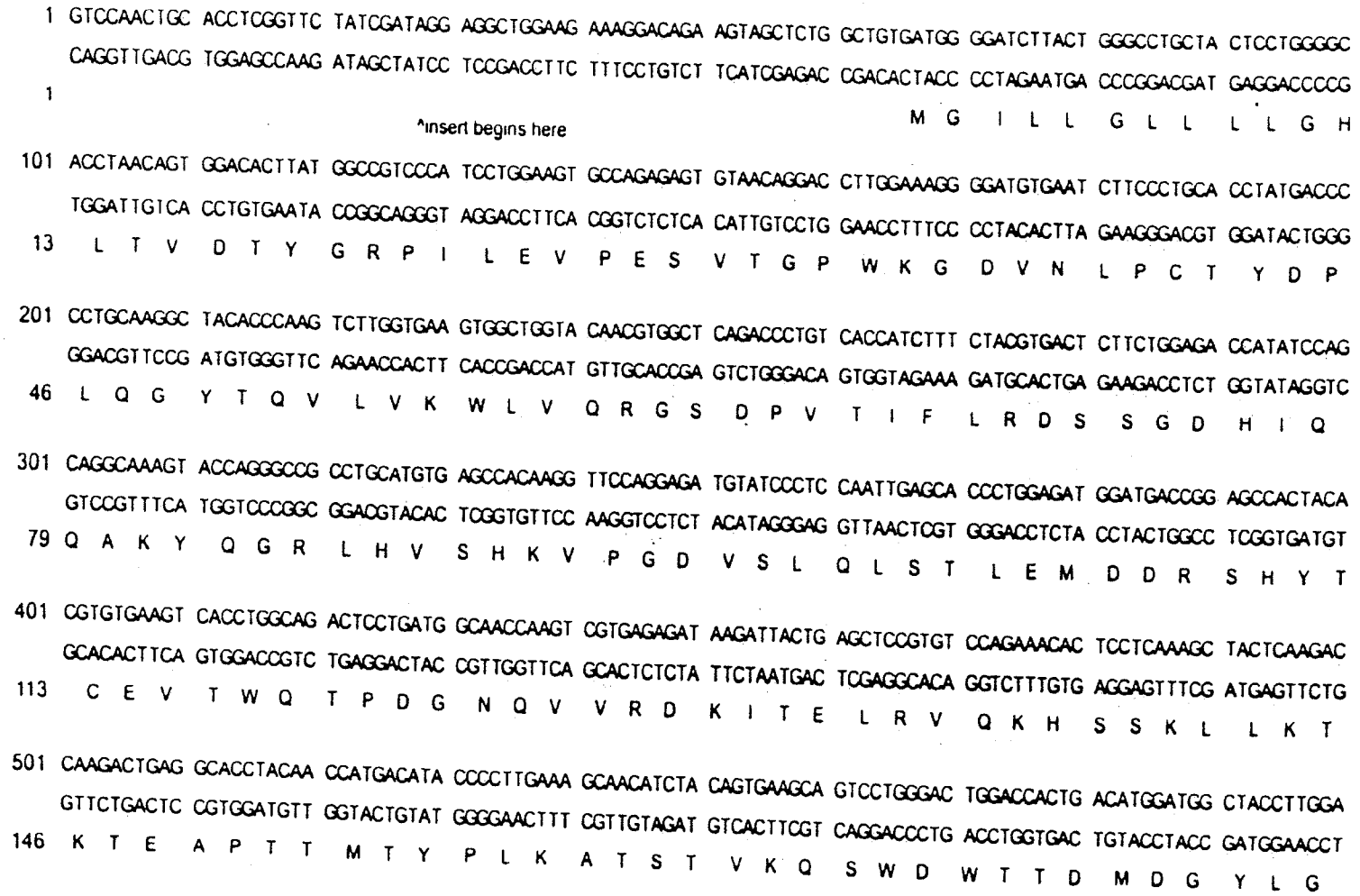

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1
^insert begins here
M G I L L G L L L L G H
101 ACCTAACAGT GGACACTTAT GGCCGTCCCA TCCTGGAAGT GCCAGAGAGT GTAACAGGAC CTTGGAAAGG GGATGTGAAT CTTCCCTGCA CCTATGACCC
TGGATTGTCA CCTGTGAATA CCGGCAGGGT AGGACCTTCA CGGTCTCTCA CATTGTCCTG GAACCTTTCC CCTACACTTA 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
46 L 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
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GTCCGTTTCA TGGTCCCGGC GGACGTACAC TCGGTGTTCC AAGGTCCTCT ACATAGGGAG GTTAACTCGT GGGACCTCTA CCTACTGGCC TCGGTGATGT
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 GGTACTGTAT GGGGAACTTT CGTTGTAGAT GTCACTTCGT CAGGACCCTG ACCTGGTGAC TGTACCTACC GATGGAACCT
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FIG. 2A

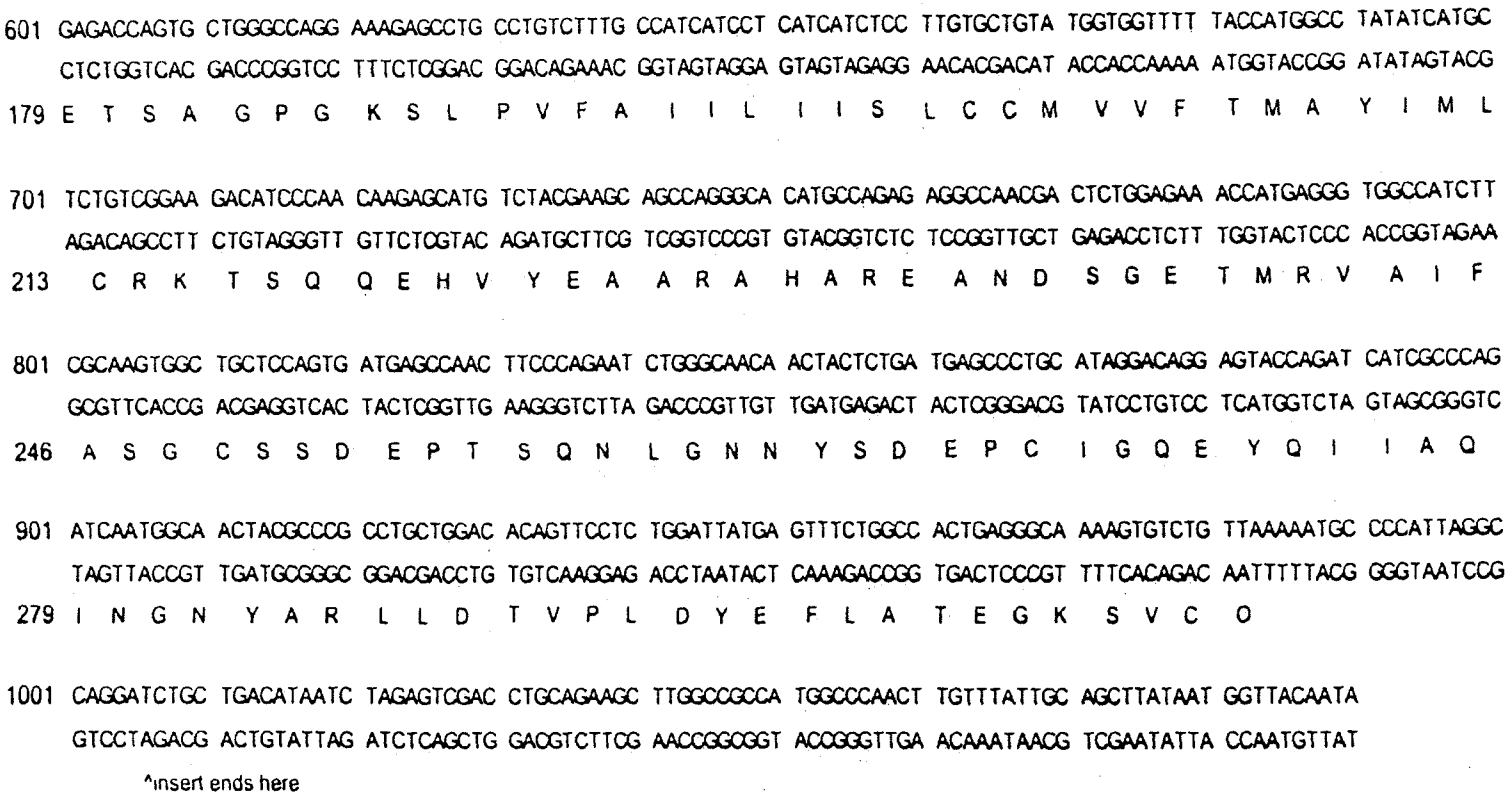

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701 TCTGTCGGAA GACATCCCAA CAAGAGCATG TCTACGAAGC AGCCAGGGCA CATGCCAGAG AGGCCAACGA CTCTGGAGAA ACCATGAGGG TGGCCATCTT
AGACAGCCTT CTGTAGGGTT GTTCTCGTAC AGATGCTTCG TCGGTCCCGT GTACGGTCTC TCCGGTTGCT GAGACCTCTT TGGTACTCCC ACCGGTAGAA
213 C R K T S Q Q E H V Y E A A R A H A R E A N D S G E T M R V A I F
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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
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FIG. 2B

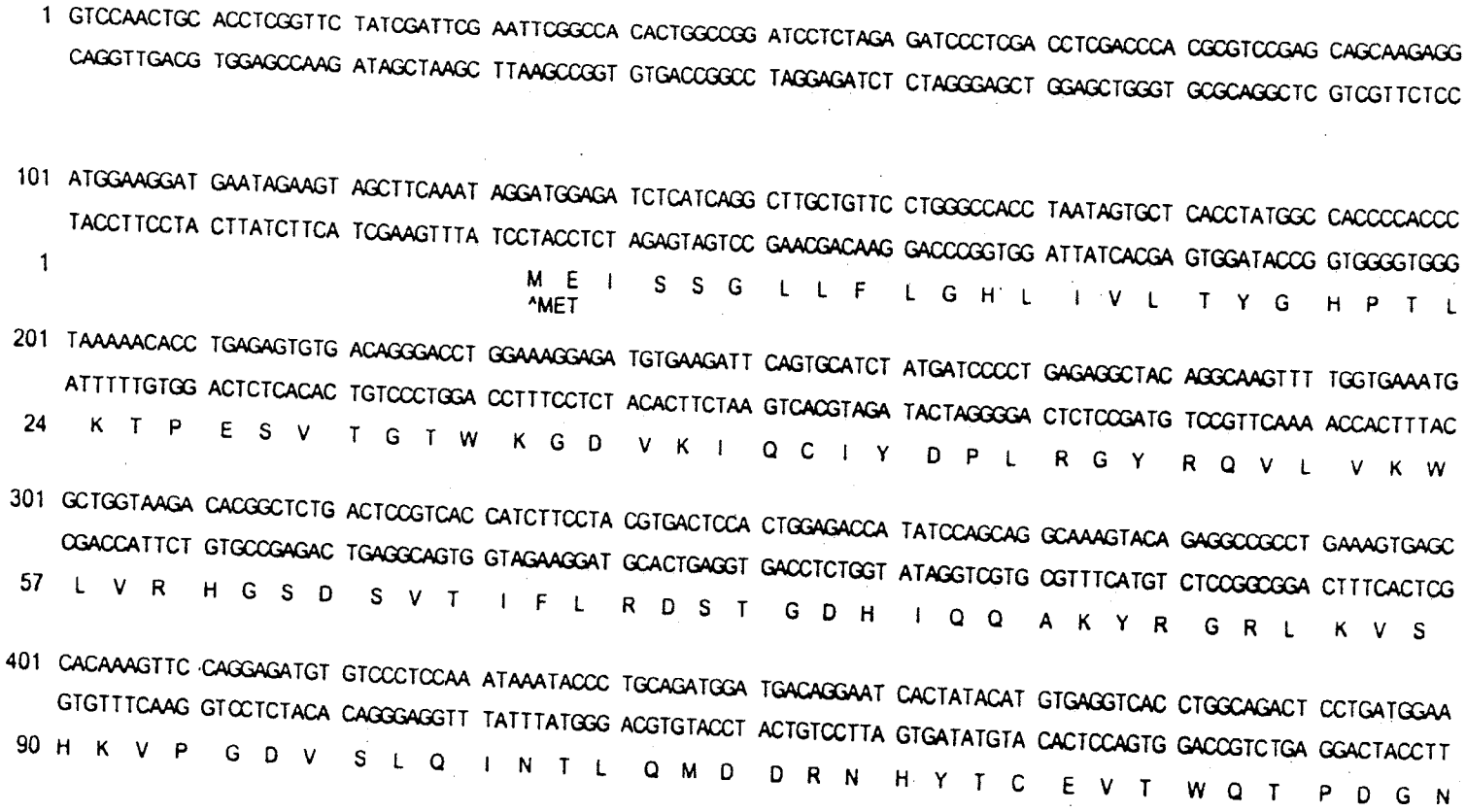

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TACCTTCCTA CTTATCTTCA TCGAAGTTTA TCCTACCTCT AGAGTAGTCC GAACGACAAG GACCCGGTGG ATTATCACGA GTGGATACCG GTGGGGTGGG
1
M E I S S G L L F L G H L I V L T Y G H P T L
^MET
201 TAAAAACACC TGAGAGTGTG ACAGGGACCT GGAAAGGAGA TGTGAAGATT CAGTGCATCT ATGATCCCCT GAGAGGCTAC AGGCAAGTTT TGGTGAAATG
ATTTTTGTGG ACTCTCACAC TGTCCCTGGA CCTTTCCTCT ACACTTCTAA GTCACGTAGA TACTAGGGGA CTCTCCGATG TCCGTTCAAA ACCACTTTAC
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57 L V R H G S D S V T I F L R D S T G D H I Q Q A K Y R G R L K V S
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GTGTTTCAAG GTCCTCTACA CAGGGAGGTT TATTTATGGG ACGTGTACCT ACTGTCCTTA GTGATATGTA CACTCCAGTG GACCGTCTGA GGACTACCTT
90 H K V P G D V S L Q I N T L Q M D D R N H Y T C E V T W Q T P D G N

FIG. 3A


501 ACCAAGTAAT AAGAGATAAG ATCATTGAGC TCCGTGTTCG GAAATATAAT CCACCTAGAA TCAATACTGA AGCACCTACA ACCCTGCACT CCTCTTTGGA
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
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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
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CGGTATTAGA AGTAGTAGAG GGAAACGACG TATCATCGAC AGTGGTATGG AATATAGAAC AAGGCGACGT CCTGTAAGGT TGTTCTCATA CAGATACCTC
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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
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FIG. 3B

1101 TAGCTTTCTC CCTGGCTTCC TTTCTTCTTA GACAACCTAA AGTATCTATC TAGTCTGCCA ATTCTGGGGC CATTGAGAAA TCCTGGGTTT GGCTAAGAAT
ATCGAAAGAG GGACCGAAGG AAAGAAGAAT CTGTTGGATT TCATAGATAG ATCAGACGGT TAAGACCCCG GTAACTCTTT AGGACCCAAA CCGATTCTTA

1201 ATACTACATG CACCTCAAGA AATCTAGCTT CTGGGCTTCA CCCAGAACAA TTTTCTTCCT AGGGCCTTCA CAACTCTTCT CCAAACAGCA GAGAAATTCC
TATGATGTAC GTGGAGTTCT TTAGATCGAA GACCCGAAGT GGGTCTTGTT AAAAGAAGGA TCCCGGAAGT GTTGAGAAGA GGTTTGTCGT CTCTTTAAGG

1301 ATAGCAGTAG AGGTTCTTTA TCATGCCTCC AGACAGCGTG AGTCTCAGTC CTACAAACTC AGACAAGCAC ATGGGTCTAG GATTACTCCT CTTTCTCTAG
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

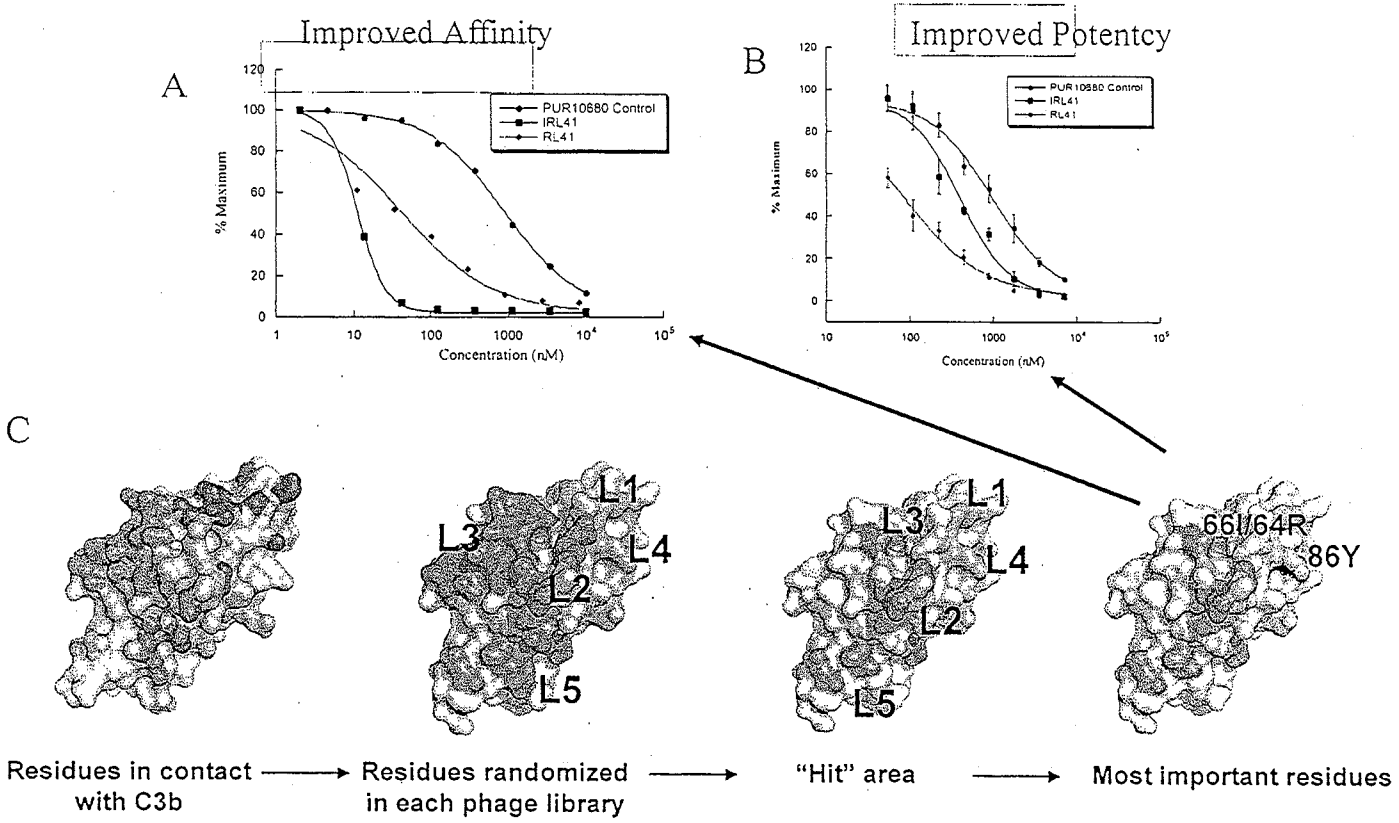
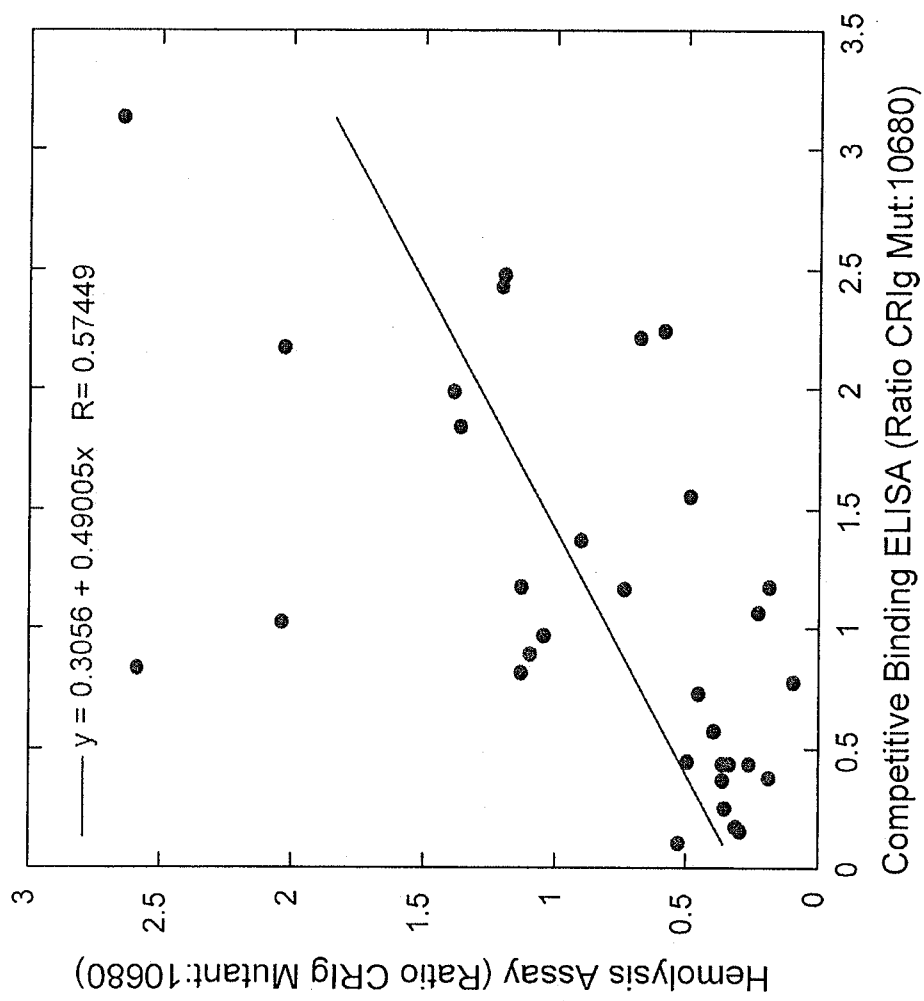


Fig. 4



The correlated between competitive ELISA and Hemolytic Assay

Fig. 5

Improved affinity of the Q64R / M86Y CRIg mutant

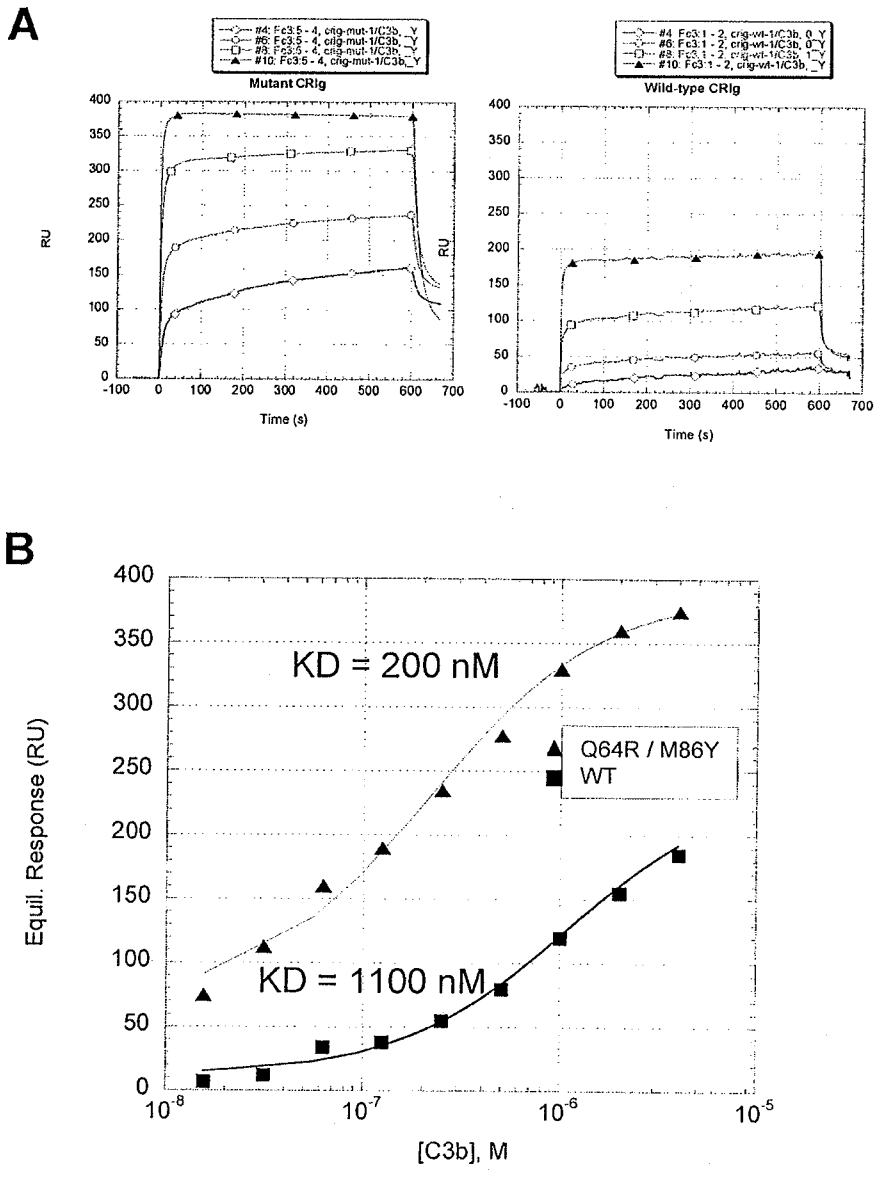


Fig. 6

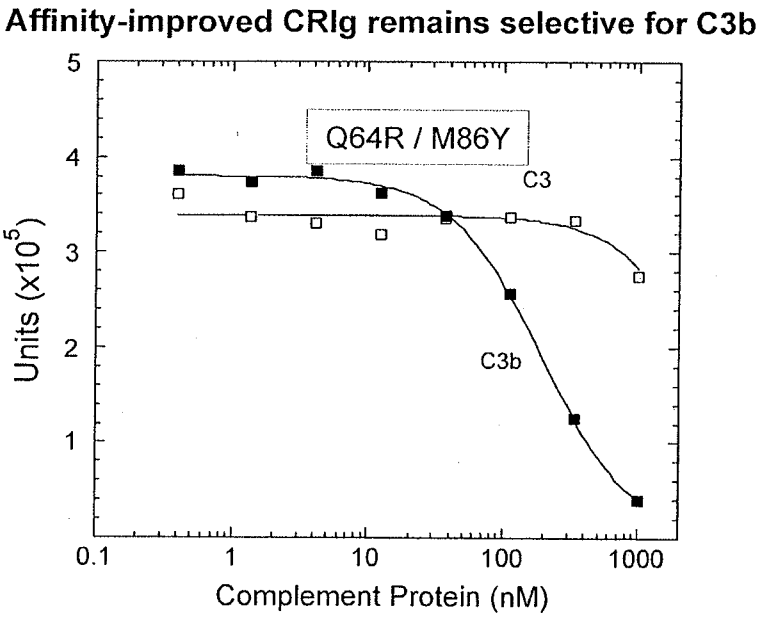

Affinity-improved CRIg remains selective for C3b
Q64R / M86Y
C3
C3b
Units (X10^5)
Complement Protein (nM)
0.1
1
10
100
1000
1
2
3
4
5

Fig. 7

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

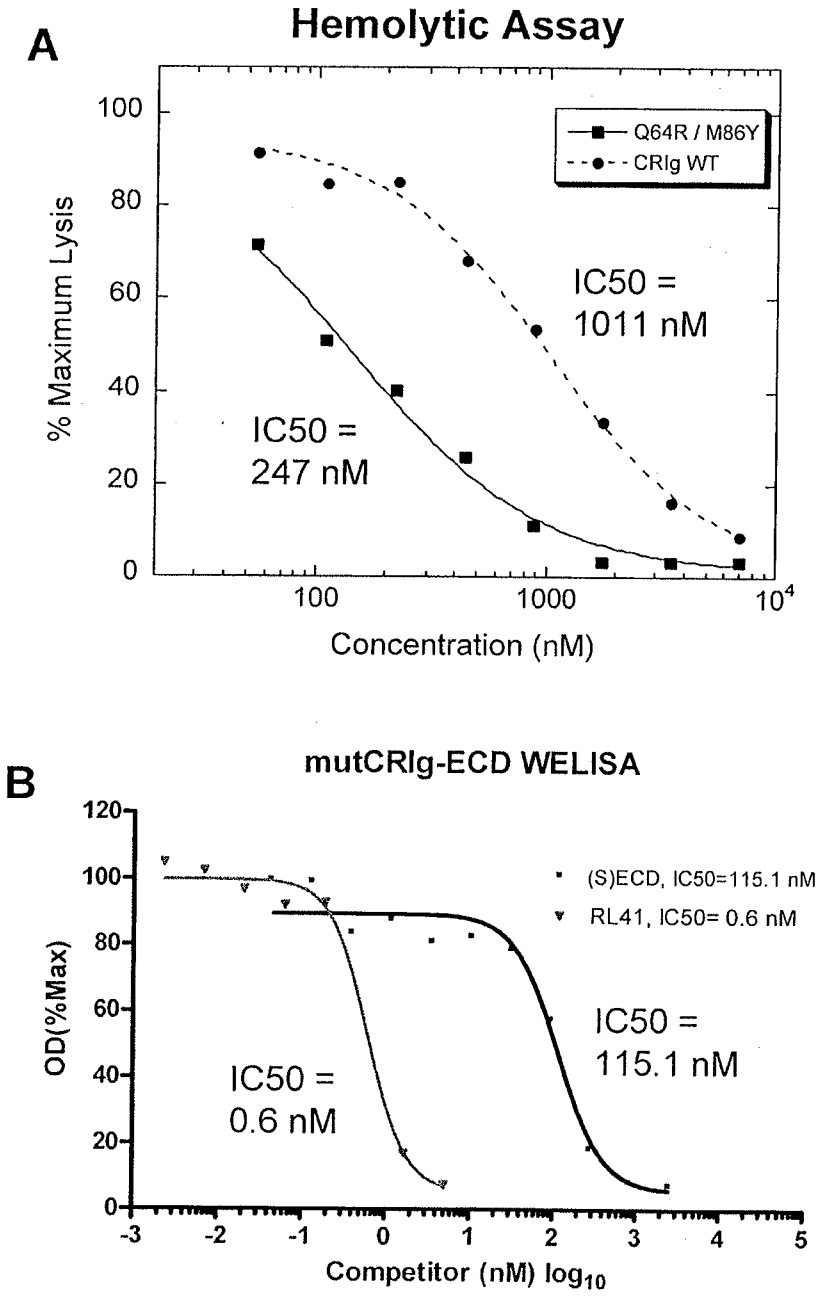


Fig. 8

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

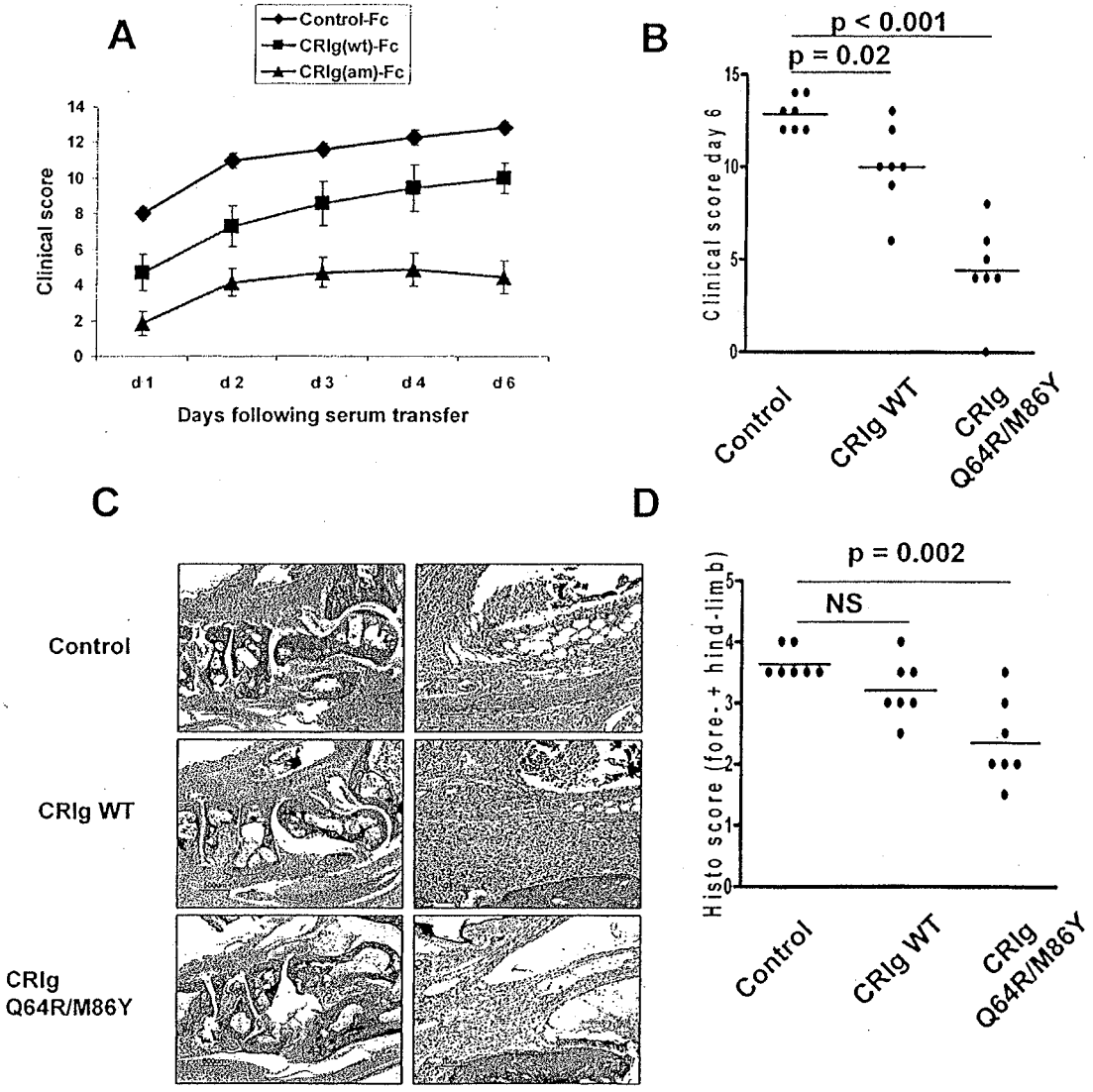


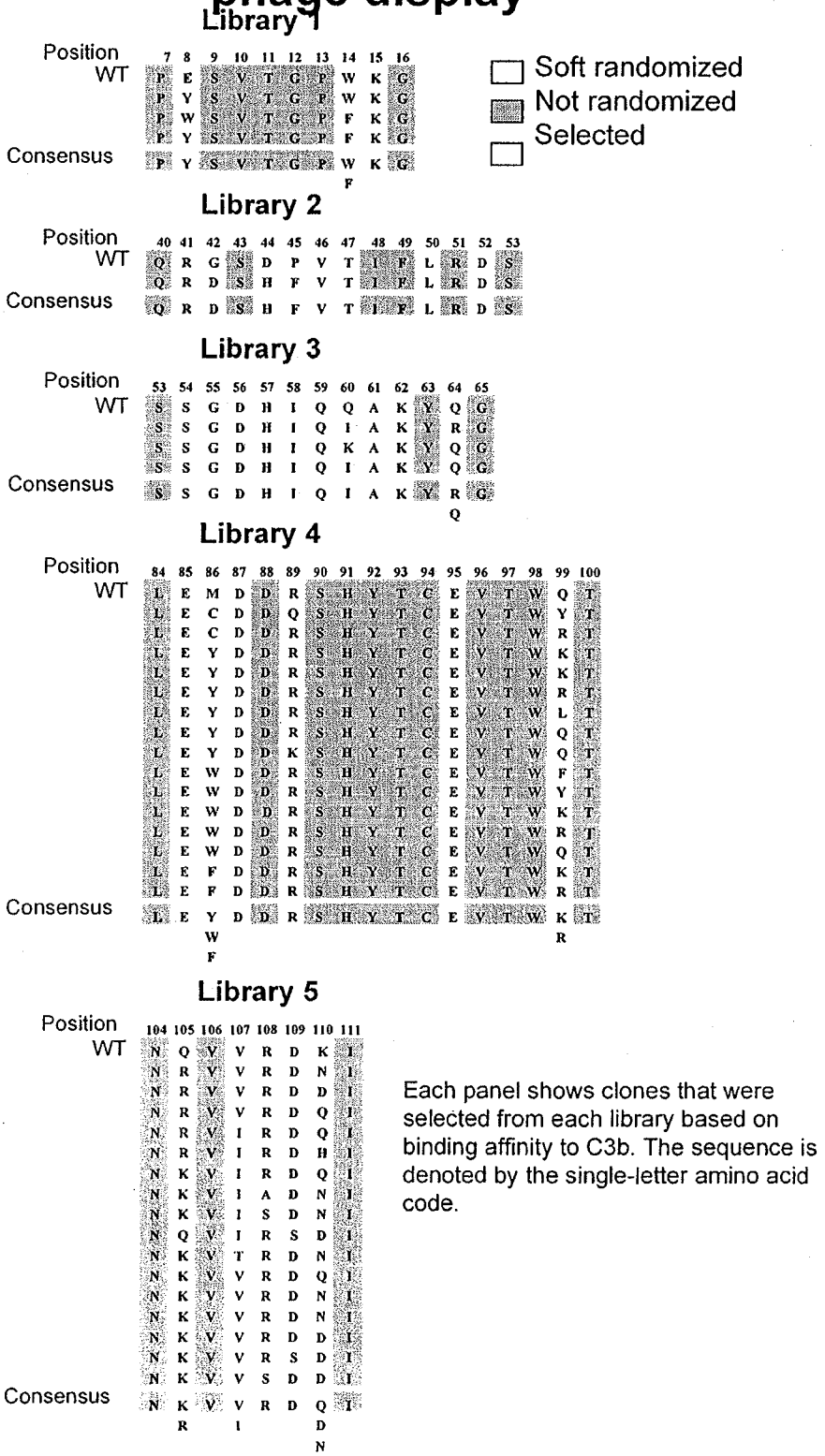
Fig. 9

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}

Table 1

Step-wise generation of higher affinity CRIg by phage display



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.

Table 2

Table 3

			Competitive ELISA			Hemolysis Inhibition		
	Name	Mutants	Mu Ic50 (nM)	WT-P10680 (nM)	WT/Mu	Mu Ic50	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

		Competitive ELISA			Hemolysis Inhibition		
Name	Mutants	Mu Ic50 (nM)	WT-P10680 (nM)	WT/Mu	Mu Ic50	WT-P10680	WT/Mu
L12	E8W	142	332	2X	176	657	4X
WL21	E8W/G42D	400	457	1X	1601	506	0.3X
WL23	E8W/P45F	376	457	1X	2063	506	0.2X
WL42	E8W/M86W	71	457	6X	416	506	1X
WL51	E8W/Q105R/K110N	312	457	1X	475	506	1X
WL55	E8W/K110N	374	457	1X	492	506	1X
WL59	E8W/Q105R/K110N	276	457	12X	2340	506	0.2X
L32	Q64R	34	332	10X	293	549	2X
RL11	Q64R/E8Y	303	712	2X	390	506	1X
RL21	Q64R/G42D	490	712	1X	217	506	2X
RL23	Q64R/P45F	293	712	2X	375	506	1X
RL41	Q64R/M86Y	40	712	18X	85	506	6X
RL42	Q64R/M86W	56	712	13X	170	506	3X
RL46	Q64R/Q99Y	682	712	1X	473	506	1X
RL47	Q64R/Q99F	725	712	1X	669	506	0.8X
RL49	Q64R/Q99R	64	712	11X	177	506	3X
RL51	Q64R/Q105R/K110N	286	712	2X	227	506	2X
RL52	Q64R/Q105K	290	712	3X	222	506	2X
RL59	Q64R/Q105R/K110N	65	712	11X	208	506	2X
L33	Q60I/Q64R	255	332	1X	51	492	10X
IRL11	Q60I-Q64R/E8Y	34	903	27X	327	1052	3X
IRL21	Q60I-Q64R/G42D	29	903	31X	386	1052	3X
IRL23	Q60I-Q64R/P45F	31	903	29X	338	1052	3X
IRL24	Q60I-Q64R/G42D/D44H/P45	4	903	226X	235	1052	4X
IRL41	Q60I-Q64R/M86Y	10	903	90X	358	1052	3X
IRL51	Q60I-Q64R/Q105R	24	903	38X	313	1052	3X
IRL52	Q60I-Q64R/Q105K	22	903	41X	143	1052	7X
IRL55	Q60I-Q64R/K110N	33	903	27X	292	1052	4X
IRL59	Q60I/Q105R/K110N	15	903	60X	277	506	4X
L41	M86Y	123	332	3X	124	657	5X
YL11	M86Y/E8Y	75	457	6X	562	962	2X
YL21	M86Y/G42D/D44H/P45F	71	457	6X	220	962	4X
YL23	M86Y/P45F	7	457	65X	202	962	5X
YL24	M86Y/G42D/D44H/P45F	31	457	15X	248	962	4X
YL44	M86Y/Q99K	91	457	5X	250	962	4X
YL49	M86Y/Q99R	175	457	3X	392	962	2X
YL51	M86Y/Q105R	39	457	12X	417	962	2X
YL52	M86Y/Q105K)	49	457	9X	224	962	4X
YL59	M86Y/Q105R/K110N	18	457	25X	515	962	2X

Table 4