C3b Panning Results

Project	Library	Enrichment	Clones Screened	Unique Clones
YW136	VH	x 10,000	300 (P4&P3)	~37
YW144	VH/VL	x 37,000	300 (P4&P3)	~120

** Blocking with C3 after 1st round

FIG. 1

C3b Phage Competition Results

Clone	Relative Phage IC_{50}
YW136.1.55	~50nM *
YW136.1.6	~50nM *
YW136.1.54	~100nM
YW144.1.15	~100nM
YW144.1.60	~50nM *
YW144.2.43	~50nM *
YW144.2.45	~50nM *
YW144.2.91	~100nM
YW144.P3.10	~100nM
YW144.P3.24	~10nM **
YW144.P3.39	~100nM
YW144.P3.49	~50nM *
YW144.P3.65	~100nM

FIG. 2

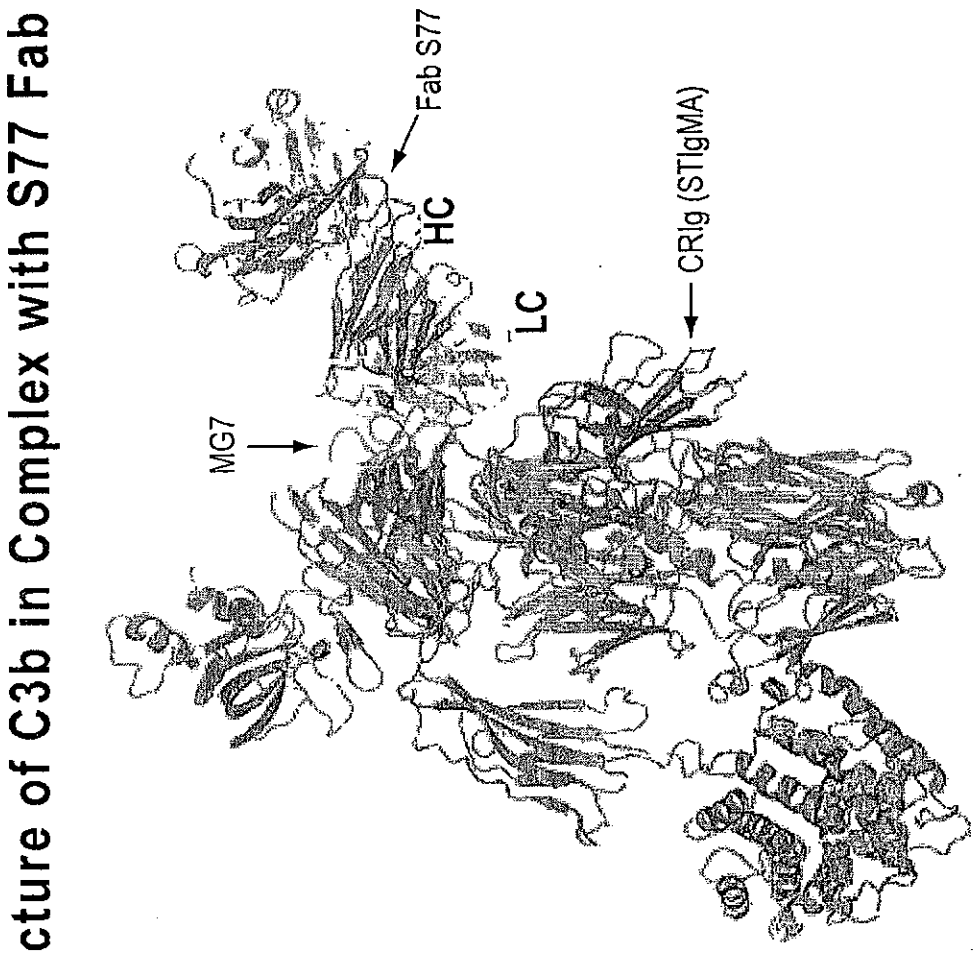

Structure of C3b in Complex with S77 Fab
Fab S77
HC
LC
CRIg (STIgMA)
MG7

FIG. 3

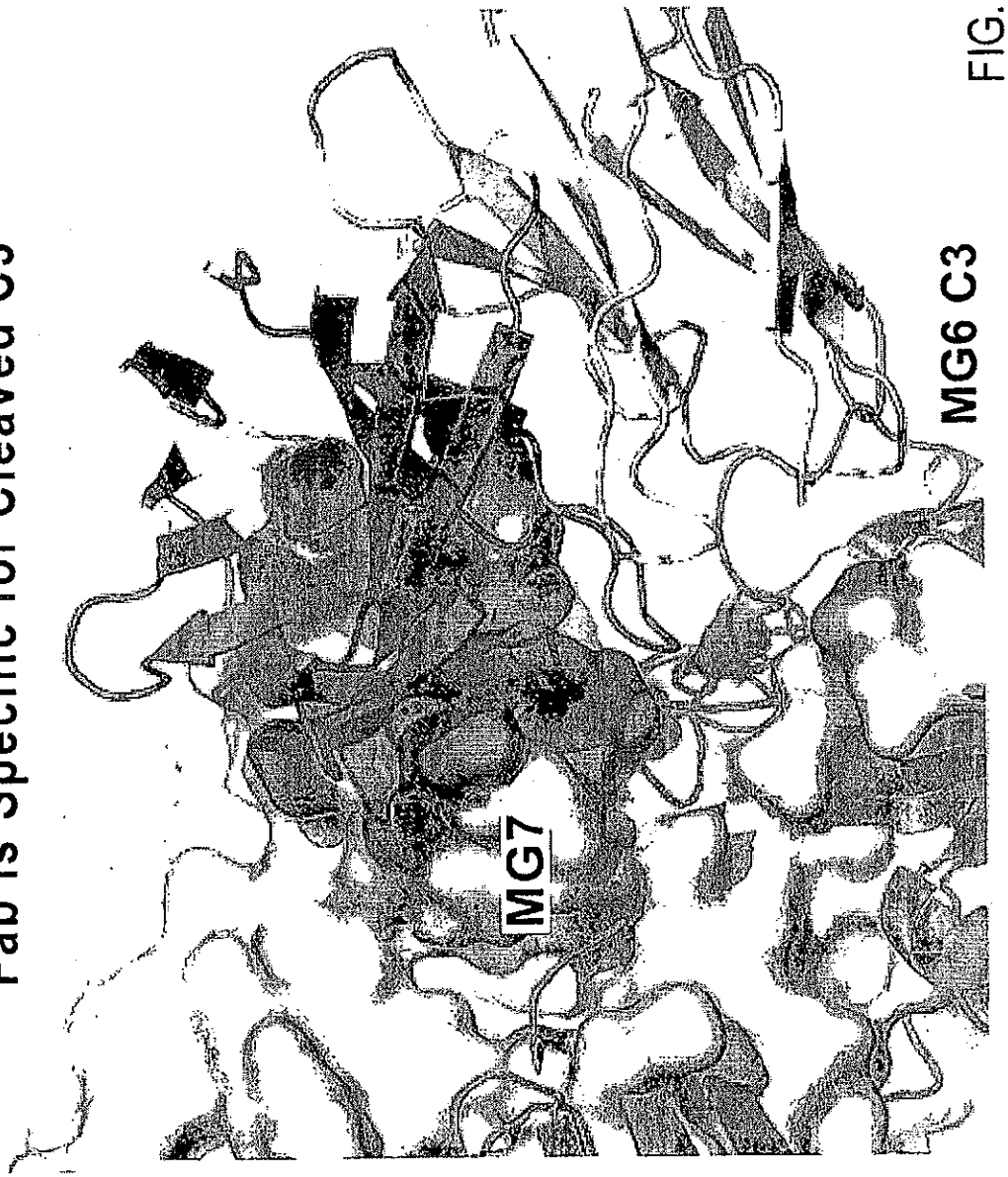


FIG. 4

Sequence of Heavy-and Light Chain of Fab S77 and Residues on S77 in Contact with C3b

S77 HC

MGWSCIILFLVATATGAYAE**V**QLVESGGGLVQPGGSLRLSCAAS
<CDR H1>**GFS**F**TSSS**VSWVRQAPGKGLEWVGL<CDR
H2>I**Y**P**YN**G**F**NYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYC
A**R**<CDR H3>**NALYGSGGY**Y**A**M**DY**WGQGTLVTVSSA
<End reformatted sequence>

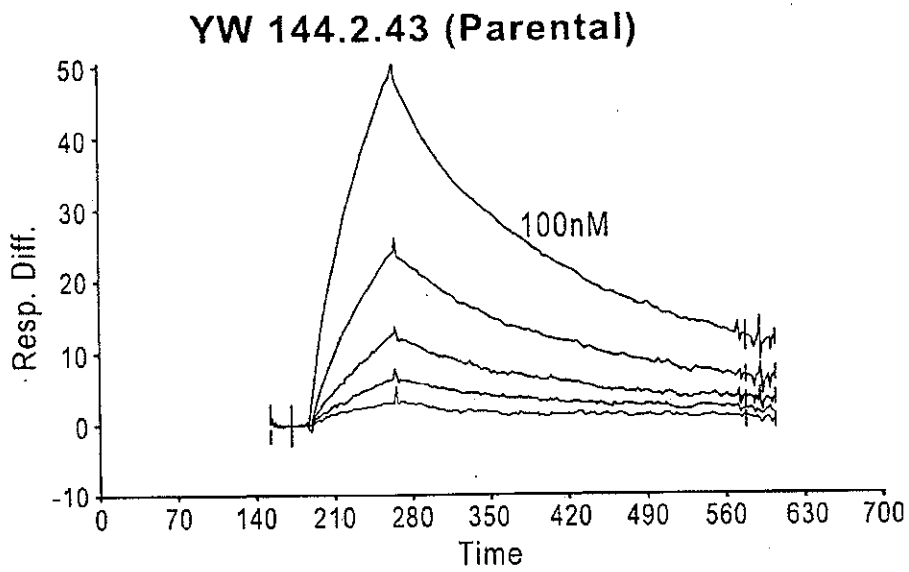
S77 LC

MGWSCIILFLVATATGVHSDIQMTQSPSSLSASVGDRVTITC<CDR
L1>RASQ**D**VSTAVAWYQQKPGKAPK**L**LI**Y**<CDR
L2>SASFL**Y**SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC<CDR
L3>QQS**Y**ATLPTFEQGTKVEIK
<End reformatted sequence>

FIG. 5

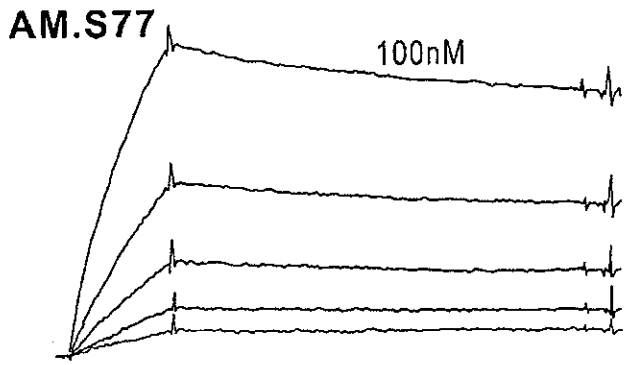
Affinity of YW144.2.43 Fab vs. C3b

FIG. 6A



Clone	ka (1/Ms)	kd (1/s)	KD (M)
YW 144.2.43	2.50E+04	4.49E-03	1.80E-07
YM 144.2.43.S77	7.61E+04	4.92E-04	6.47E-09

FIG. 6B



YW144.2.43.S77 Fab vs. C3b & C3

FIG. 7A

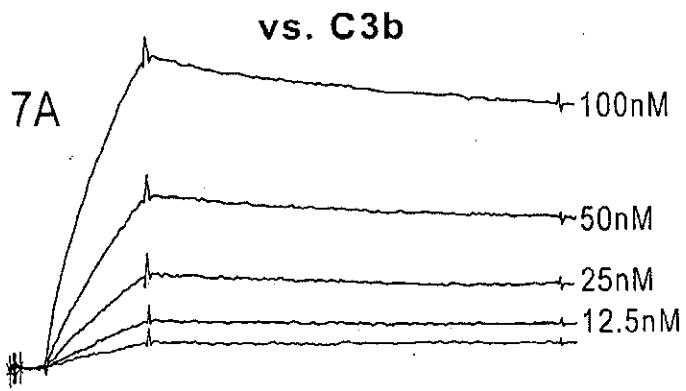


FIG. 7B

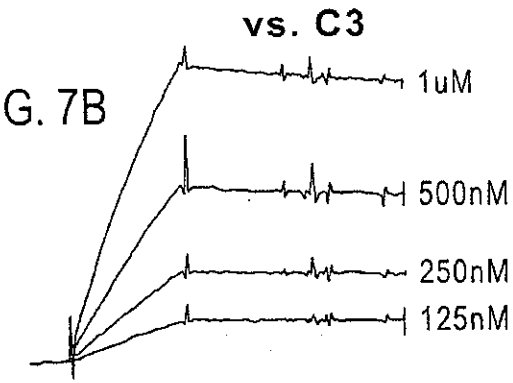
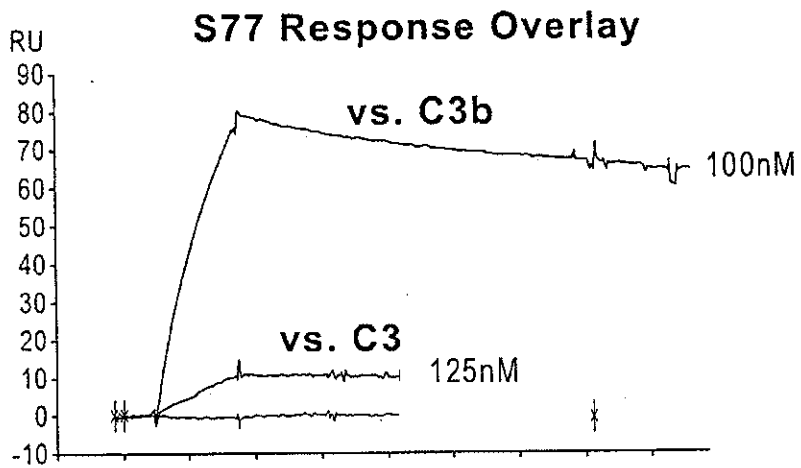


FIG. 7C



S77 Recognizes C3b, but not the Pro-molecule C3

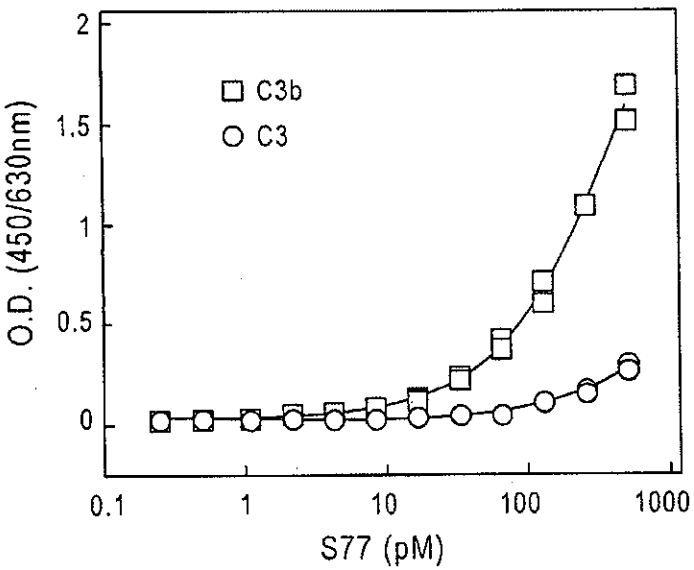


FIG. 8A

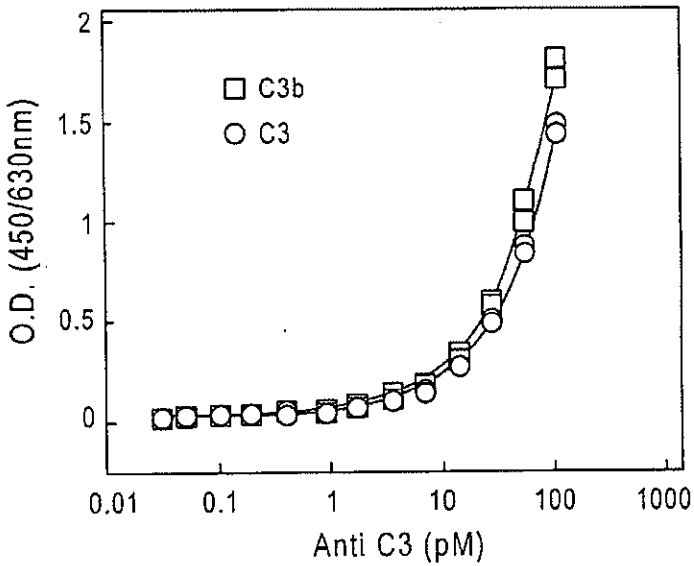


FIG. 8B

C3b abs Specifically Inhibit the Alternative Pathway of Complement

Full length IgG

Clone#	IC50 (nM)
2.43 S77	112

FIG. 9A

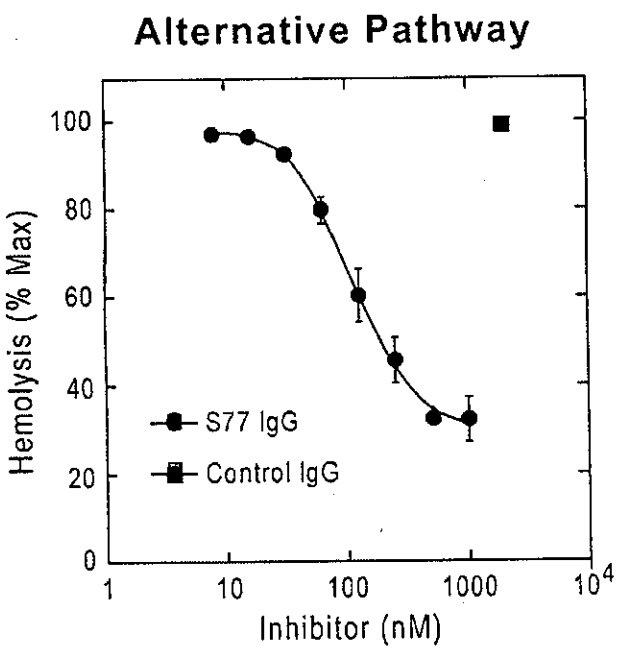


FIG. 9B

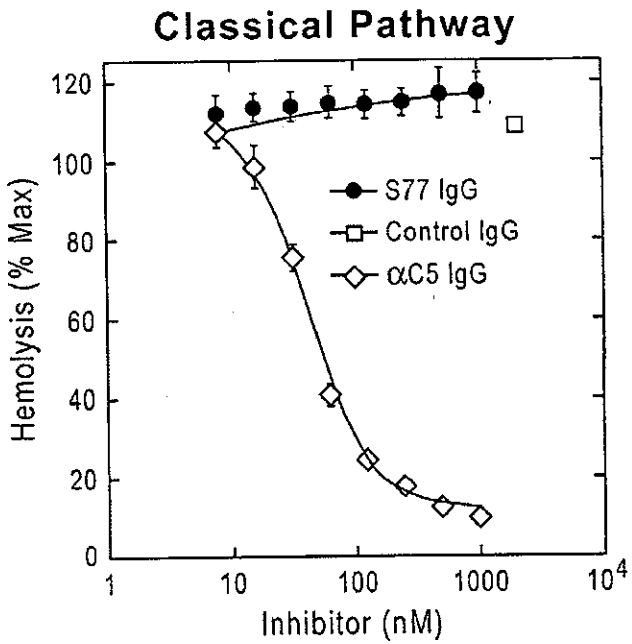


FIG. 9C

Affinity-matured S77 Fab Inhibits Alternative Pathway of Complement

Fab

Clone#	IC50 (nM)	IC90 (nM)
2.43 S77	100	632

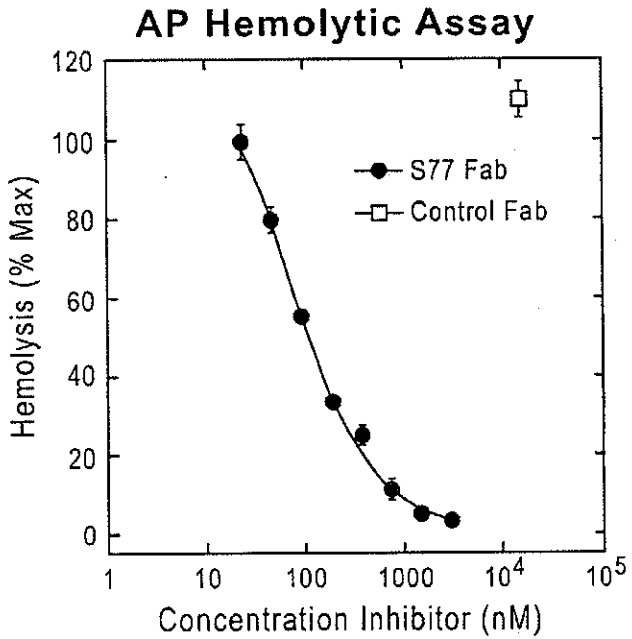


FIG. 10

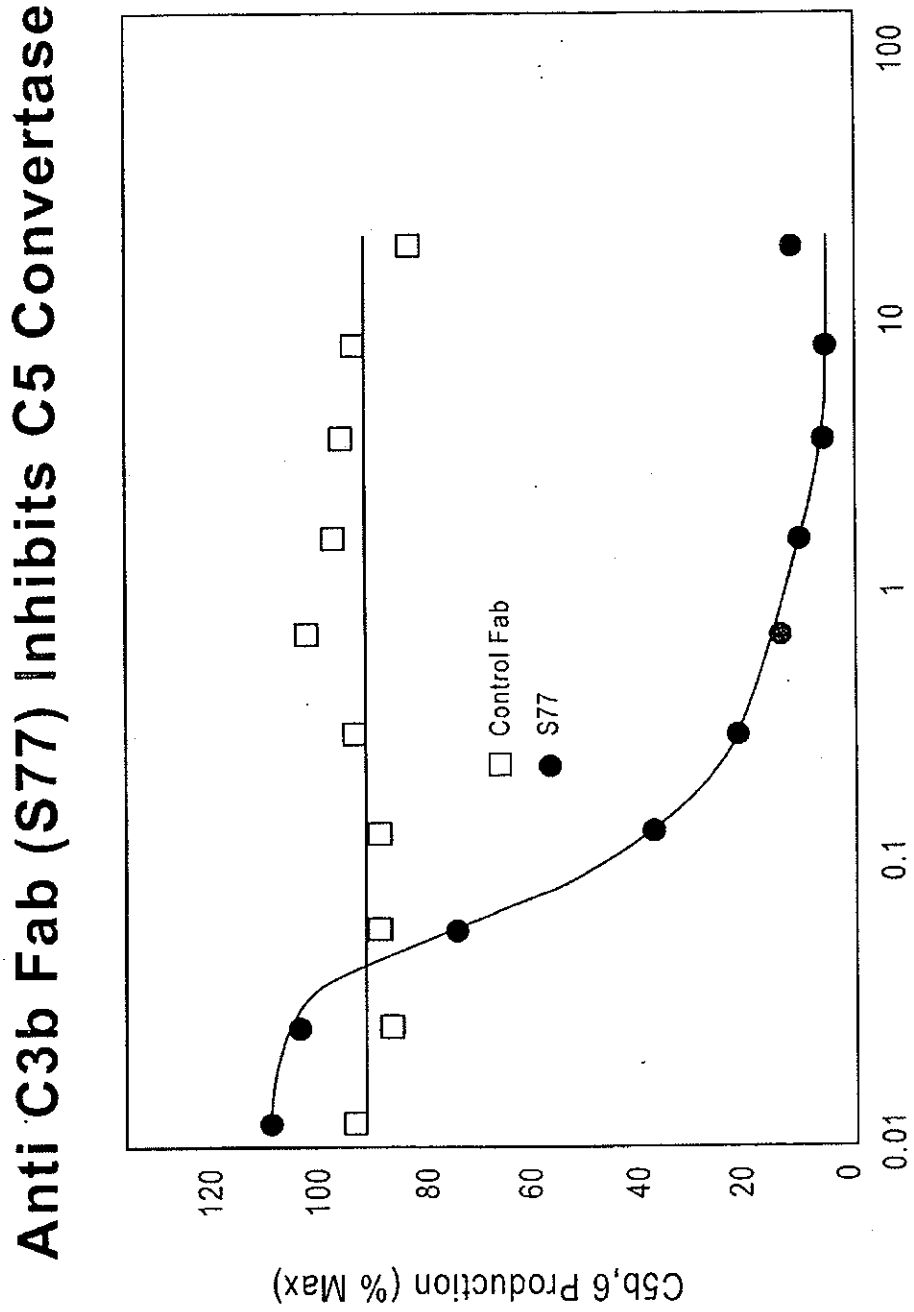

Anti C3b Fab (S77) Inhibits C5 Convertase
C5b,6 Production (% Max)
120
100
80
60
40
20
0
0.01
0.1
1
10
100
µM Fab
Control Fab
S77

FIG. 11

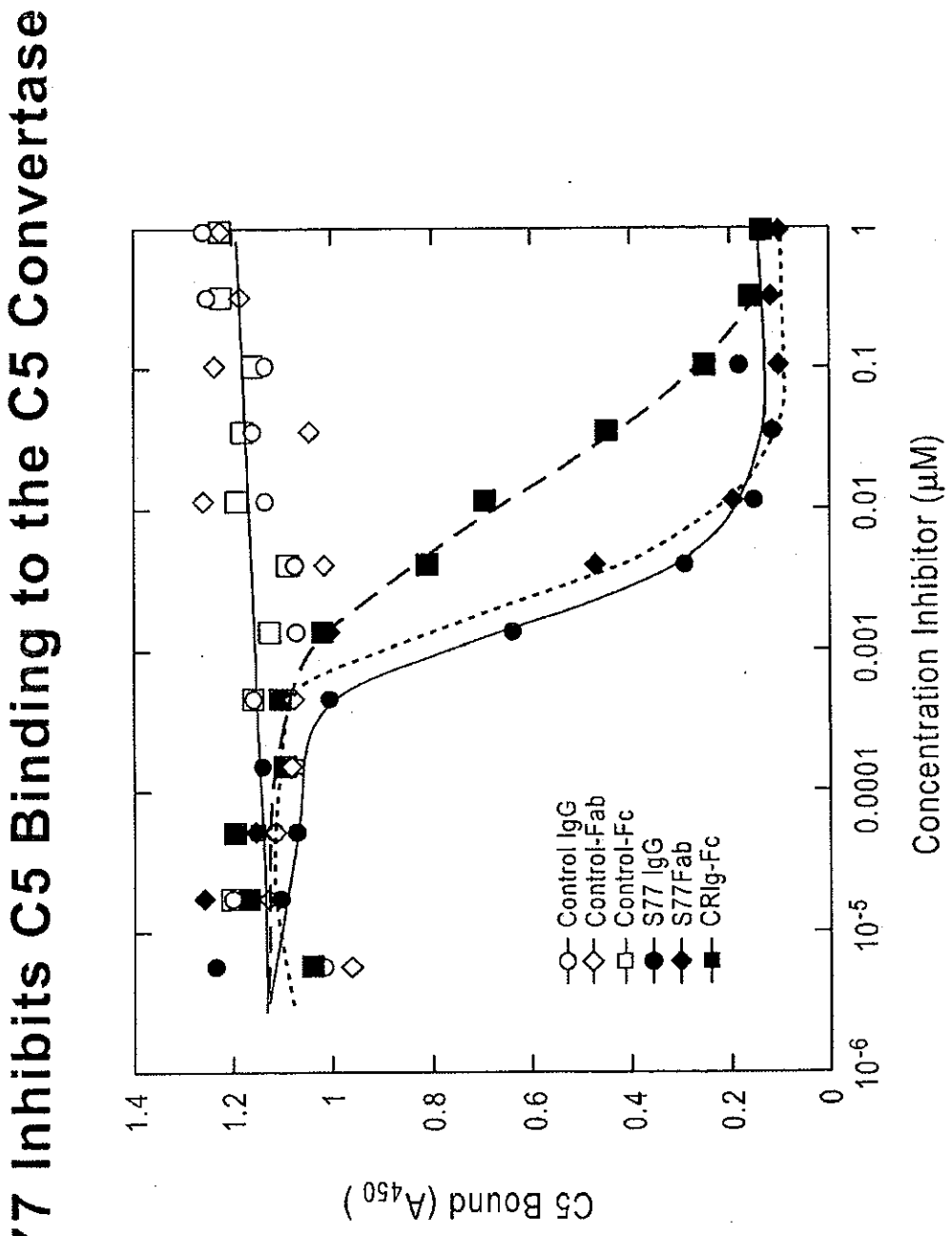

S77 Inhibits C5 Binding to the C5 Convertase
C5 Bound (A_{450})
1.4
1.2
1
0.8
0.6
0.4
0.2
0
10^{-6}
10^{-5}
0.0001
0.001
0.01
0.1
1
Concentration Inhibitor (μM)
Control IgG
Control-Fab
Control-Fc
S77 IgG
S77Fab
CRIg-Fc

FIG. 12

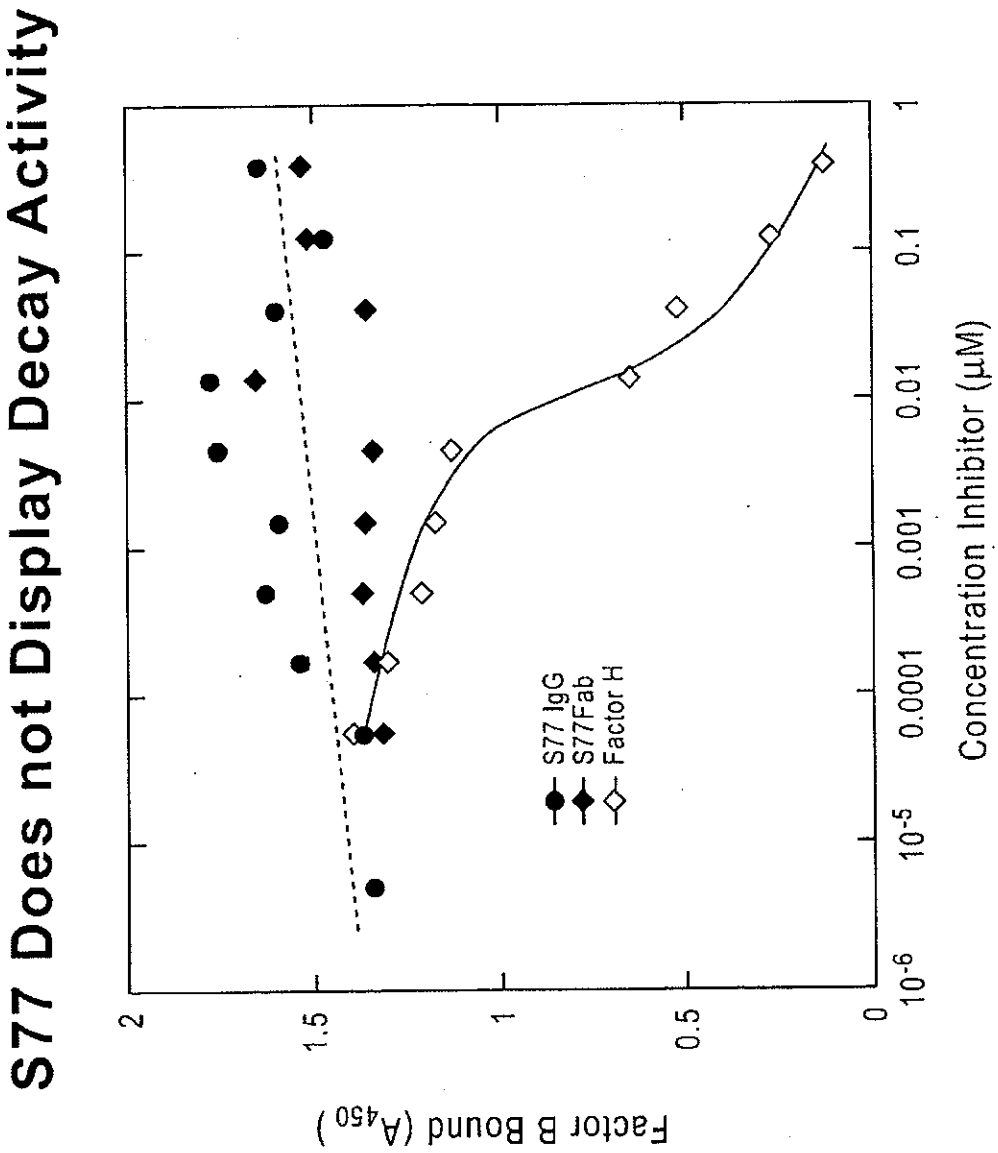

S77 Does not Display Decay Activity
Factor B Bound (A_{450})
2
1.5
1
0.5
0
10^{-6}
10^{-5}
0.0001
0.001
0.01
0.1
1
Concentration Inhibitor (μM)
S77 IgG
S77Fab
Factor H

FIG. 13

S77 Inhibits Binding of Pro-factor B to C3b, and Inhibits Formation of the C3bBb Convertase

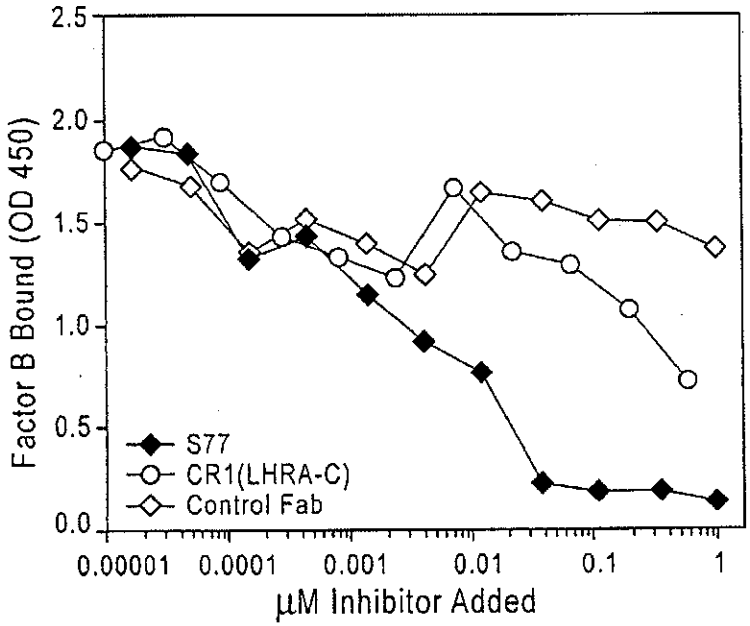


FIG. 14A

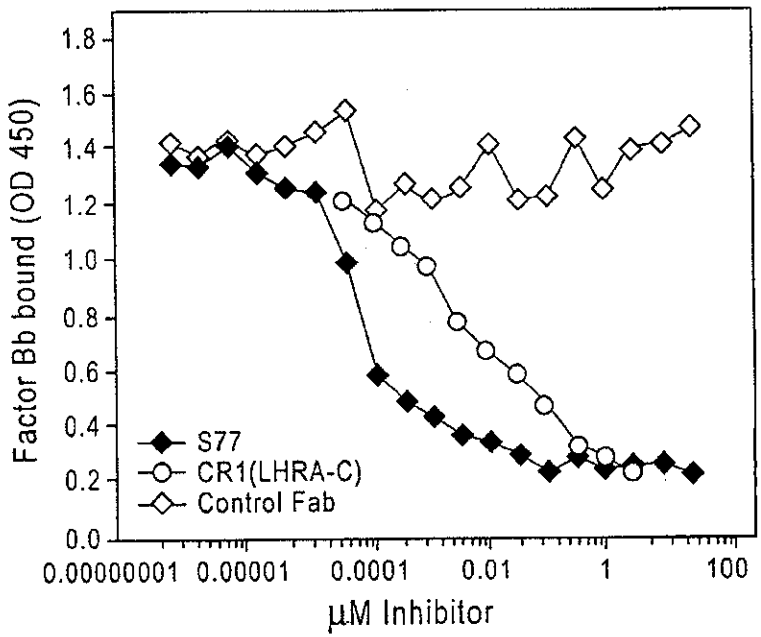


FIG. 14B

S77 Can Bind C3b in the Presence of Bound fBb and Does not Decay the C3 Convertase

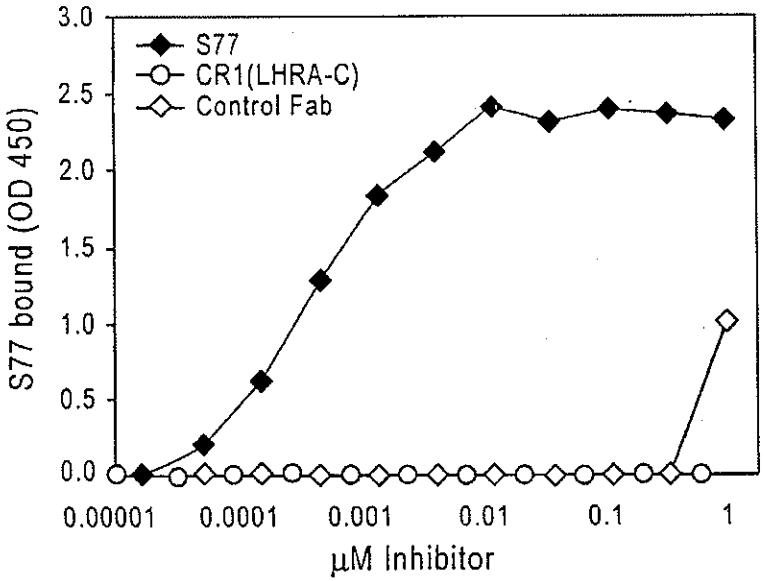


FIG. 15A

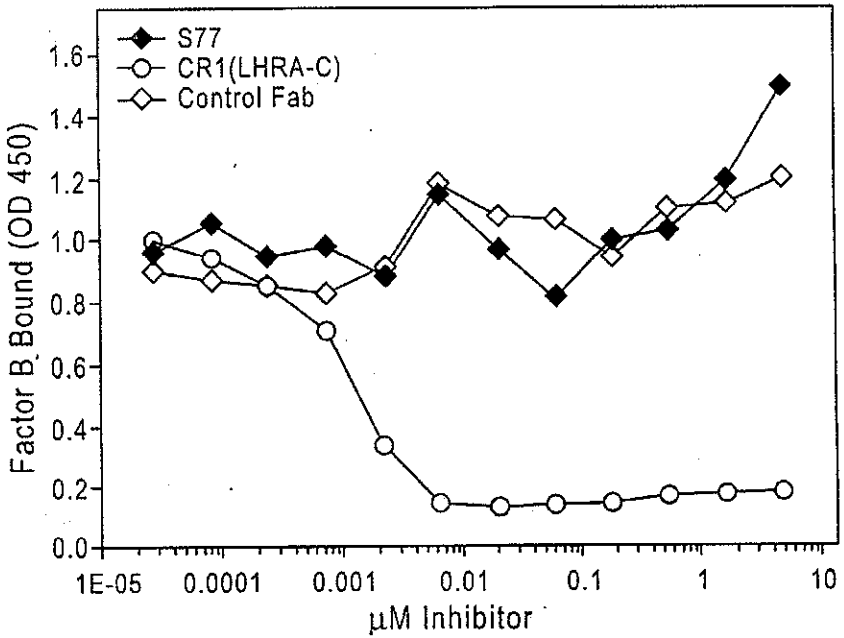


FIG. 15B

S77 Inhibits Factor H Binding to C3b and Inhibits Factor H Co-factor Activity

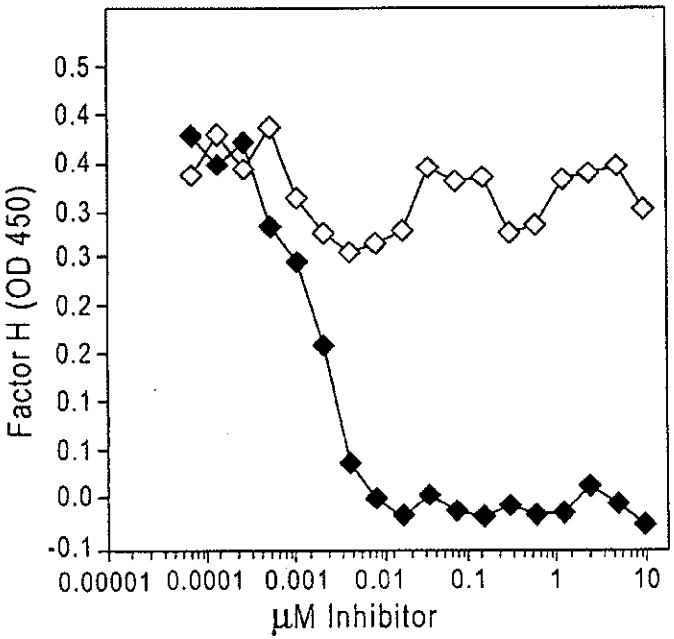


FIG. 16A

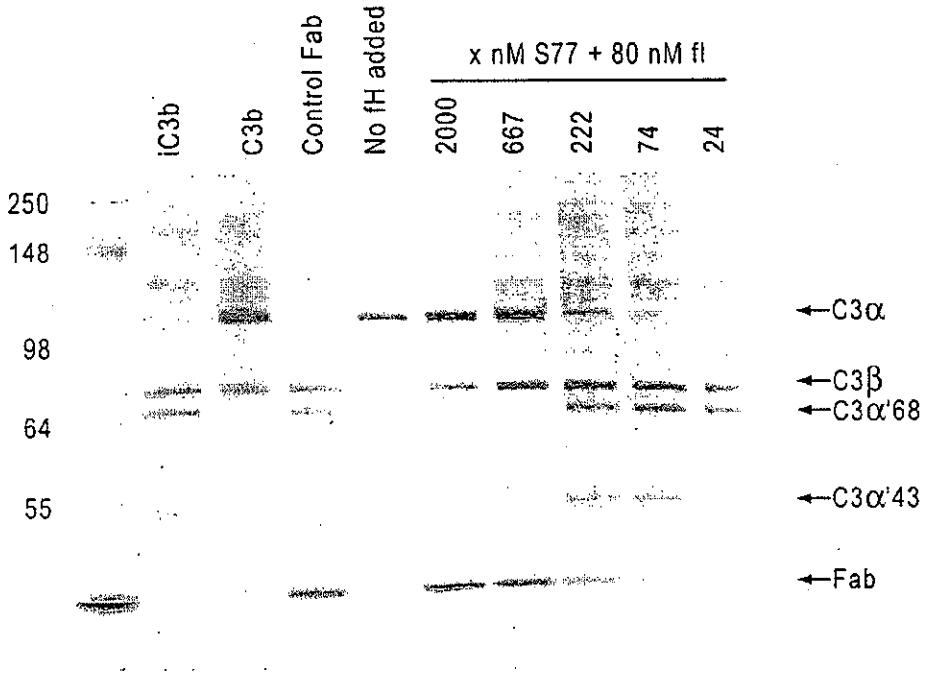


FIG. 16B

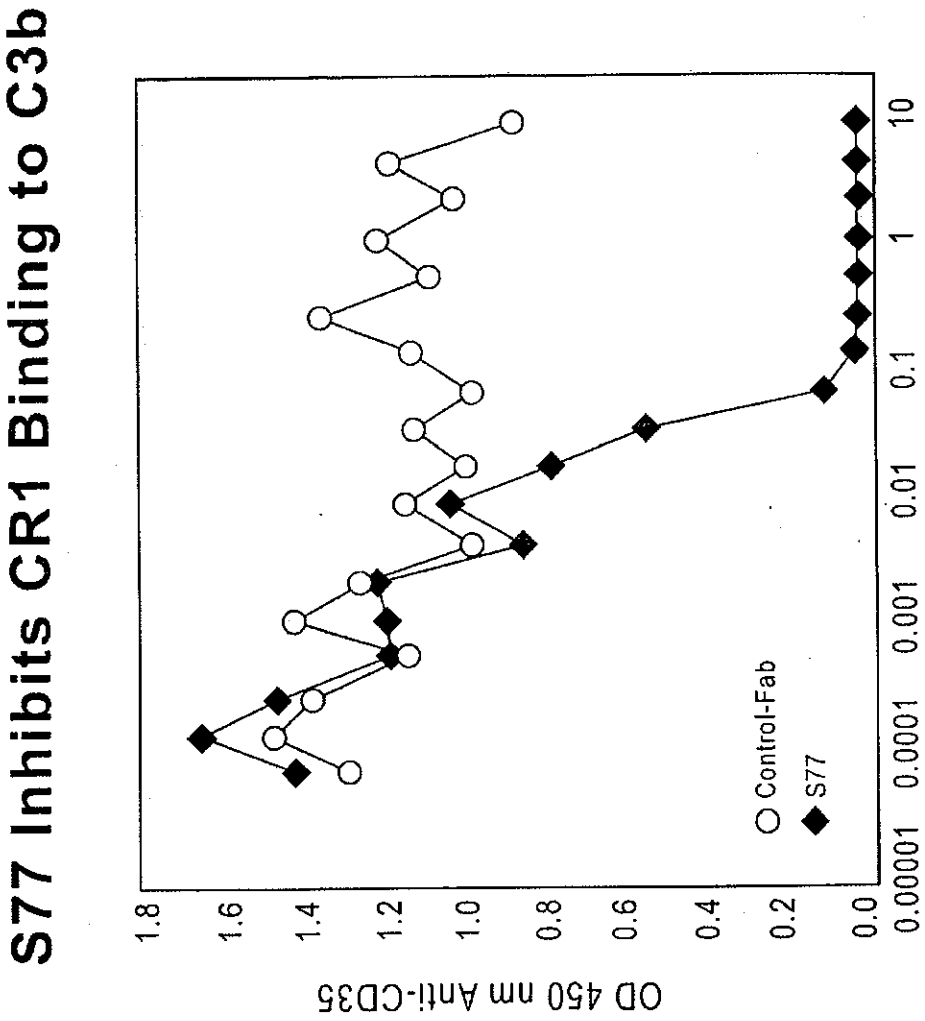

S77 Inhibits CR1 Binding to C3b
OD 450 nm Anti-CD35
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.00001
0.0001
0.001
0.01
0.1
1
10
µM Inhibitor
Control-Fab
S77

FIG. 17

LIGHT CHAIN

```
1                 15                  30                  45
D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q D V N T A V A W Y Q Q K P G K A P K

46                60                  75                  90
L L I Y S A S F L Y S G V P S R F S G S R S G T D F T L T I S S L Q P E D F A T Y Y C Q Q

91                105                 120                 135
H Y T T P P T F G Q G T K V E I K R T V A A P S V F I F P P S D E Q L K S G T A S V V C L

136               150                 165                 180
L N N F Y P R E A K V Q W K V D N A L Q S G N S Q E S V T E Q D S K D S T Y S L S S T L T

181               195                 210  214
L S K A D Y E K H K V Y A C E V T H Q G L S S P V T K S F N R G E C
```

FIG. 18A

HEAVY CHAIN

```
1                 15                  30                  45
E V Q L V E S G G G L V Q P G G S L R L S C A A S G F N I K D T Y I H W V R Q A P G K G L

46                60                  75                  90
E W V A R I Y P T N G Y T R Y A D S V K G R F T I S A D T S K N T A Y L Q M N S L R A E D

91                105                 120                 135
T A V Y Y C S R W G G D G F Y A M D Y W G Q G T L V T V S S A S T K G P S V F P L A P S S

136               150                 165                 180
K S T S G G T A A L G C L V K D Y F P E P V T V S W N S G A L T S G V H T F P A V L Q S S

181               195                 210                 225
G L Y S L S S V V T V P S S S L G T Q T Y I C N V N H K P S N T K V D K K V E P K S C D K

226               240                 255                 270
T H T C P P C P A P E L L G G P S V F L F P P K P K D T L M I S R T P E V T C V V V D V S

271               285                 300                 315
H E D P E V K F N W Y V D G V E V H N A K T K P R E E Q Y N S T Y R V V S V L T V L H Q D

316               330                 345                 360
W L N G K E Y K C K V S N K A L P A P I E K T I S K A K G Q P R E P Q V Y T L P P S R E E

361               375                 390                 405
M T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P V L D S D G

406               420                 435                 449
S F F L Y S K L T V D K S R W Q Q G N V F S C S V M H E A L H N H Y T Q K S L S L S P G
```

FIG. 18B

Residues on C3b in contact within S77

>From C3b:
SER A 548 28.00 34.57% of 81.00
ASP A 550 10.00 7.69% of 130.00
ARG A 551 81.00 60.90% of 133.00
VAL A 554 20.00 34.48% of 58.00
ASP A 732 54.00 54.00% of 100.00
MET A 804 1.00 5.88% of 17.00
GLN A 805 11.00 30.56% of 36.00
ASP A 806 48.00 62.34% of 77.00
ARG A 833 66.00 72.53% of 91.00
GLN A 834 3.00 2.88% of 104.00
ASN A 835 121.00 86.43% of 140.00
GLN A 836 56.00 98.25% of 57.00
GLU A 837 80.00 70.18% of 114.00
LEU A 838 4.00 80.00% of 5.00
LYS A 839 25.00 28.41% of 88.00
PRO A 867 8.00 22.22% of 36.00
PRO A 868 26.00 60.47% of 43.00
TYR A 895 1.00 4.35% of 23.00
HIS A 896 101.00 87.83% of 115.00
HIS A 897 67.00 91.78% of 73.00
PHE A 898 166.00 80.98% of 205.00
ILE A 899 49.00 70.00% of 70.00 CHAIN A DIFF-AREA: 1026.0
(1.47% of 69629.0 total AREA for this chain)

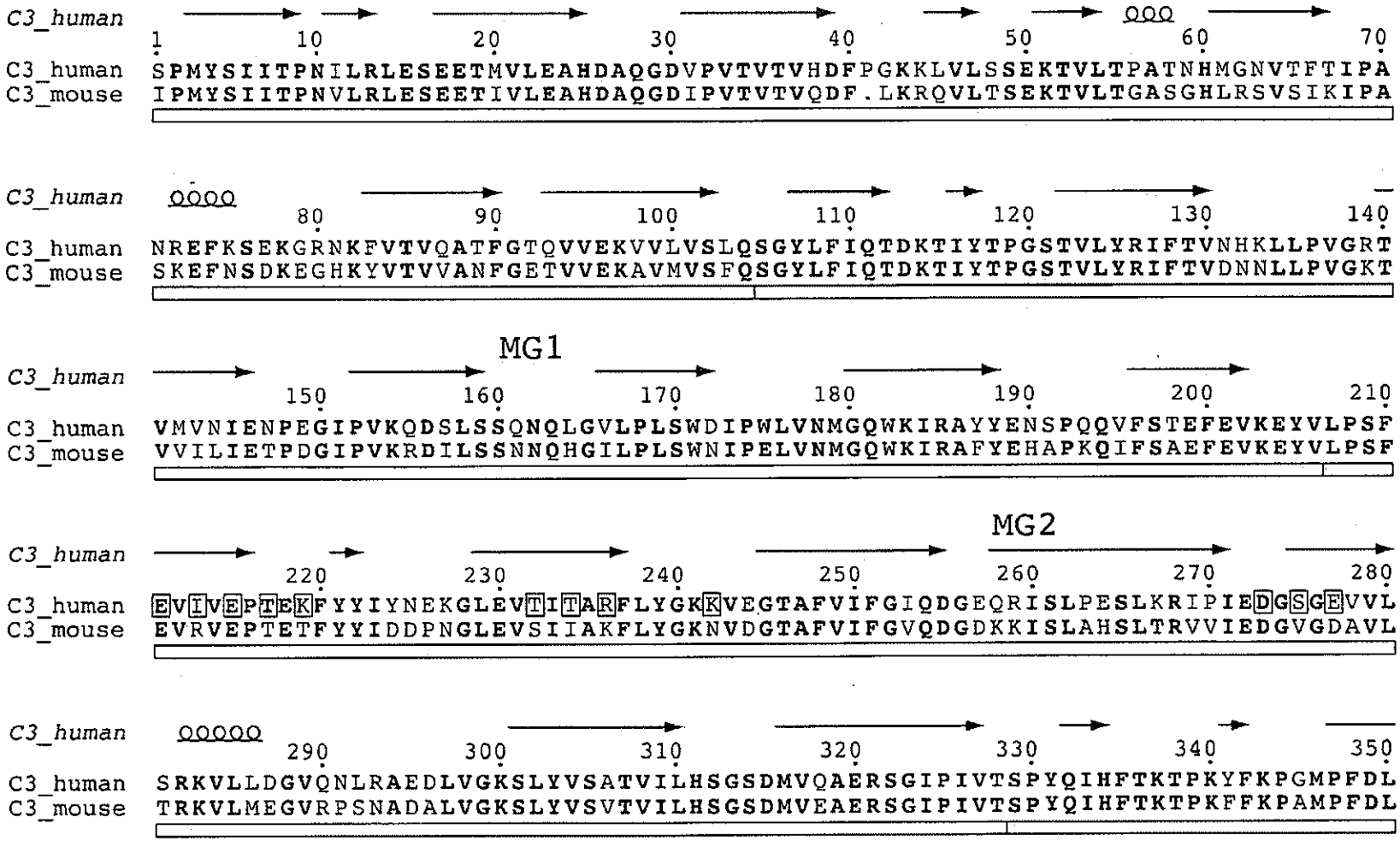
SUPPLEMENTAL FIG. 1

Fab S77 Residues in contact with C3b

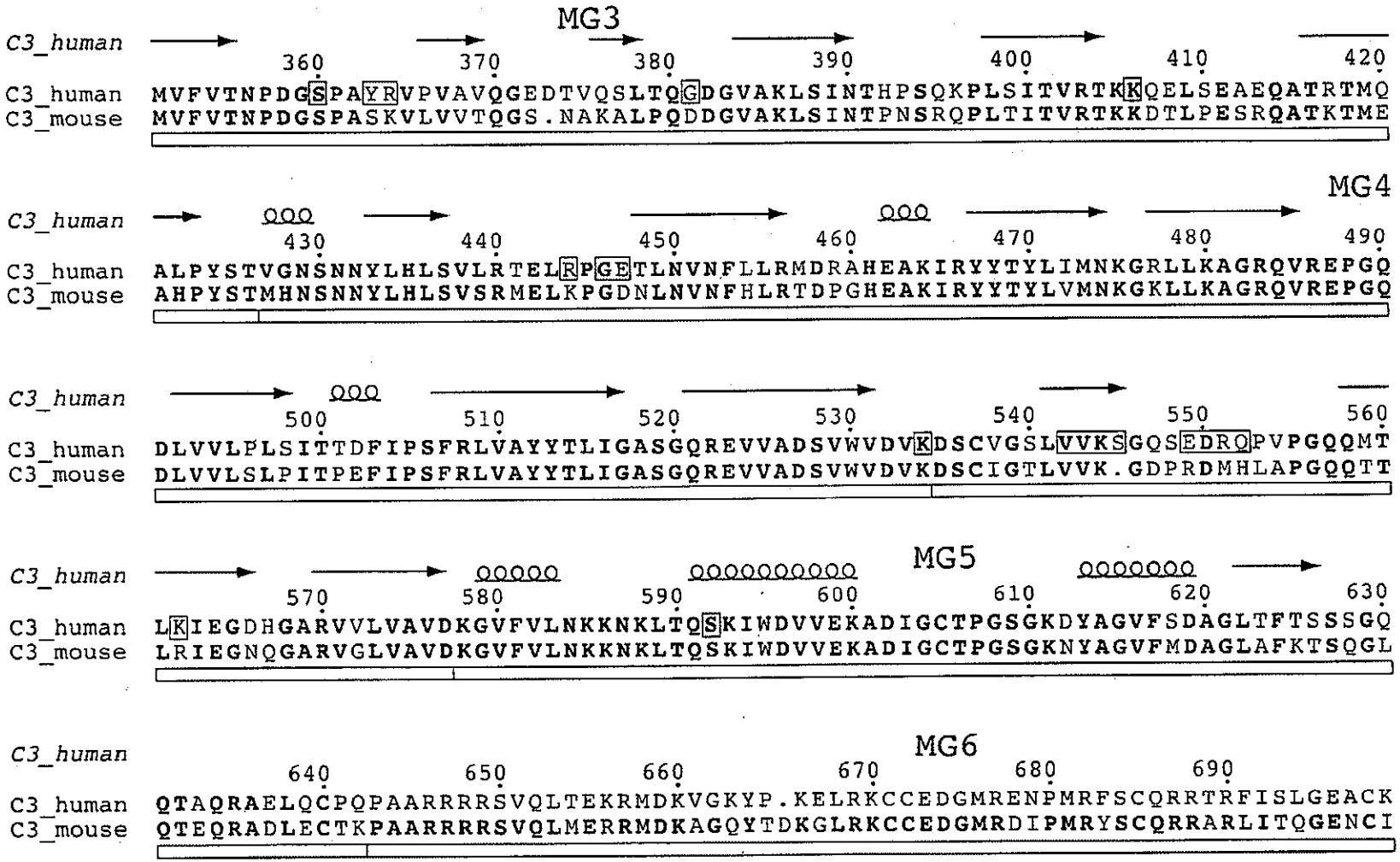
S77LC in contact with C3b:
ASP B 28 41.00 44.09% of 93.00
SER B 30 15.00 37.50% of 40.00
LEU B 46 3.00 33.33% of 9.00
TYR B 49 36.00 55.38% of 65.00
TYR B 55 15.00 30.00% of 50.00
TYR B 92 38.00 40.43% of 94.00

Residues on S77 HC in contact with C3b:
VAL B1002 13.00 56.52% of 23.00
GLY B1026 8.00 13.33% of 60.00
PHE B1027 17.00 58.62% of 29.00
SER B1028 34.00 49.28% of 69.00
THR B1030 31.00 48.44% of 64.00
SER B1031 96.00 100.00% of 96.00
SER B1032 18.00 100.00% of 18.00
SER B1033 3.00 60.00% of 5.00
TYR B1052 65.00 86.67% of 75.00
TYR B1054 76.00 43.93% of 173.00
ASN B1055 22.00 20.75% of 106.00
PHE B1057 16.00 16.67% of 96.00
ARG B1098 41.00 100.00% of 41.00
ASN B1099 8.00 72.73% of 11.00
ALA B1100 30.00 100.00% of 30.00
LEU B1101 98.00 90.74% of 108.00
TYR B1102 37.00 75.51% of 49.00
GLY B1103 10.00 24.39% of 41.00
SER B1104 65.00 67.71% of 96.00
GLY B1105 27.00 29.03% of 93.00
GLY B1106 17.00 68.00% of 25.00
TYR B1107 2.00 5.88% of 34.00
ALA B1109 4.00 100.00% of 4.00
ASP B1111 12.00 46.15% of 26.00
TYR B1112 11.00 14.29% of 77.00

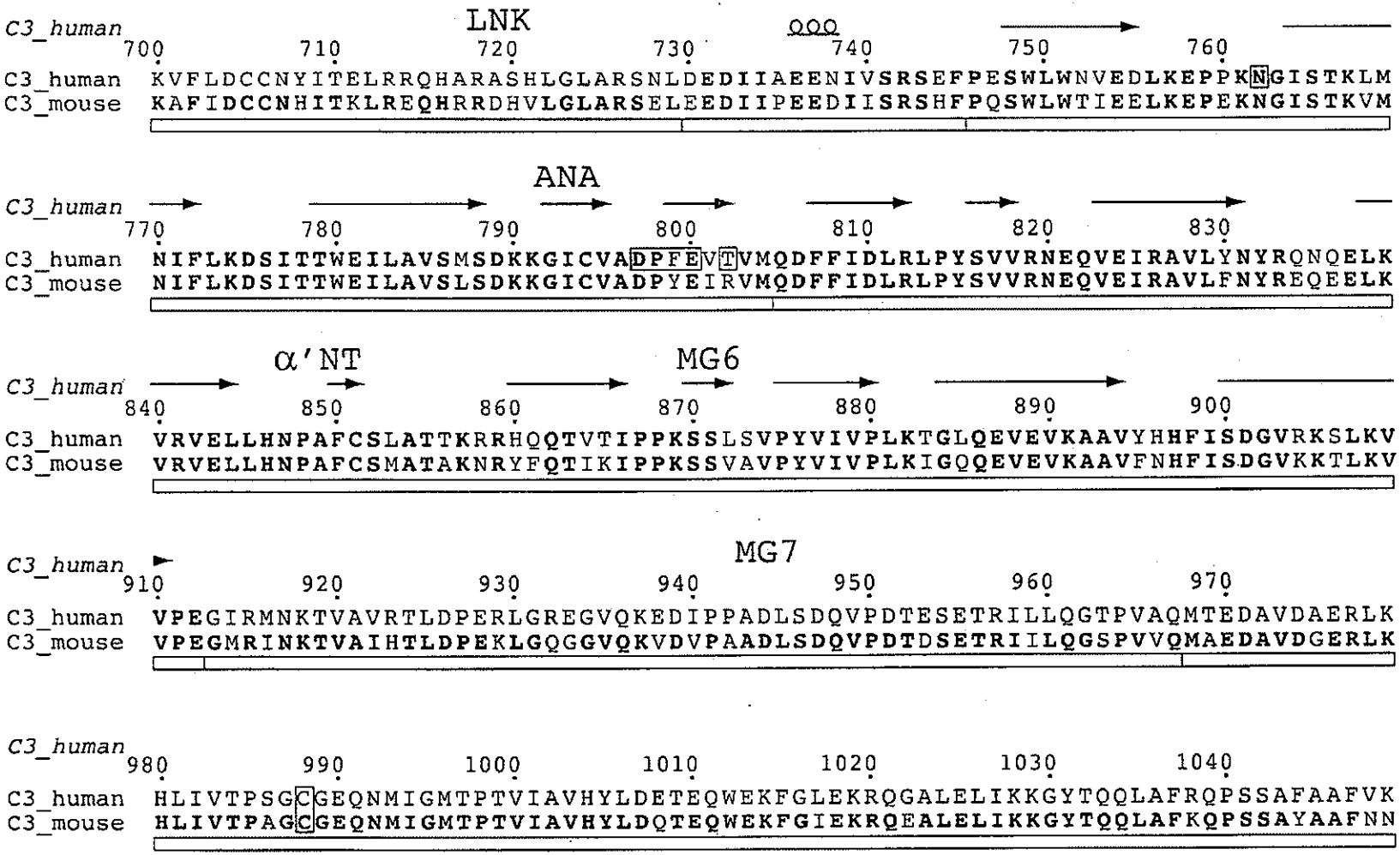
SUPPLEMENTAL FIG. 2



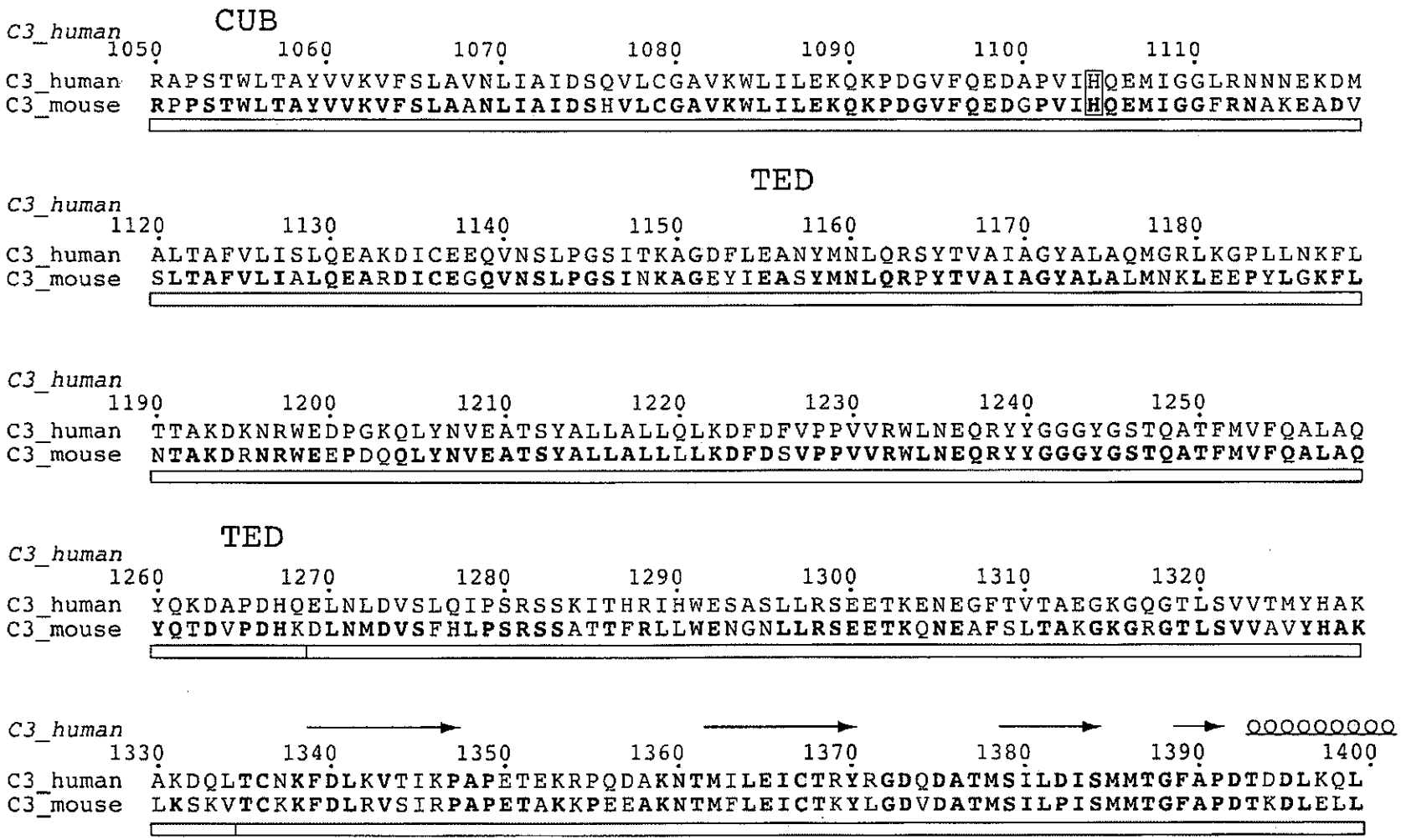
SUPPLEMENTAL FIG. 3 (Part 1)



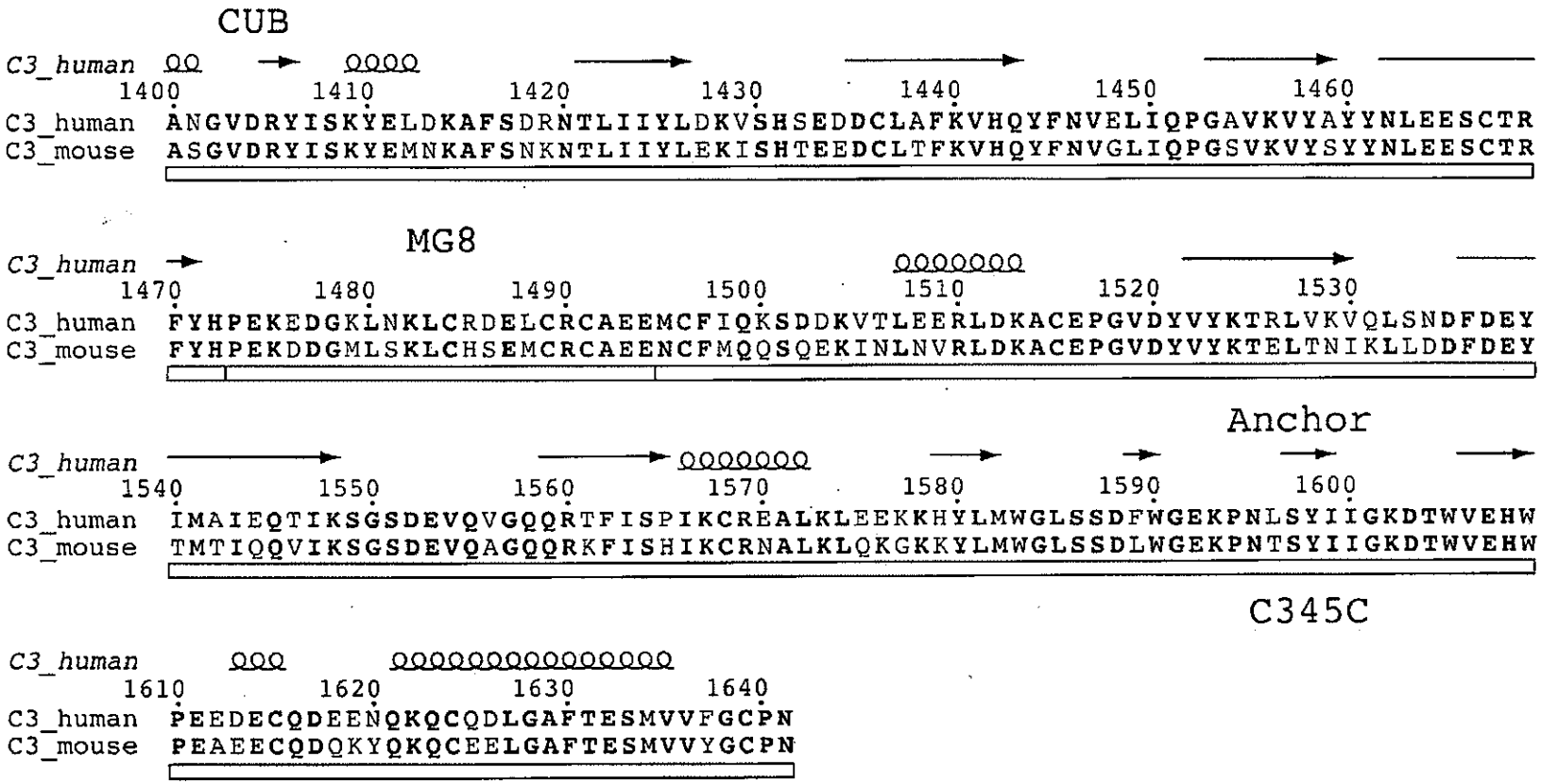
SUPPLEMENTAL FIG. 3 (Part 2)



SUPPLEMENTAL FIG. 3 (Part 3)



SUPPLEMENTAL FIG. 3 (Part 4)



SUPPLEMENTAL FIG. 3 (Part 5)