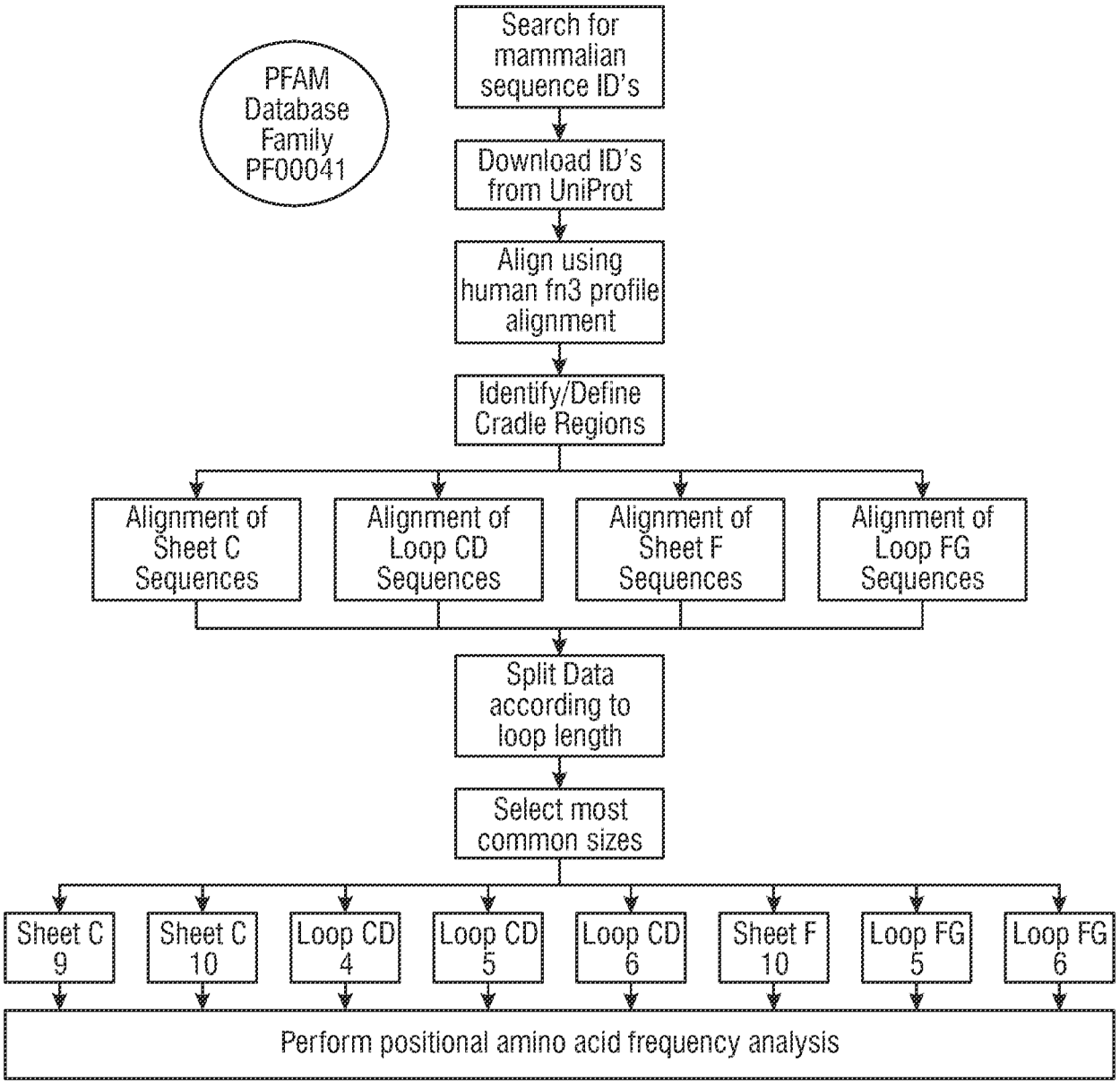
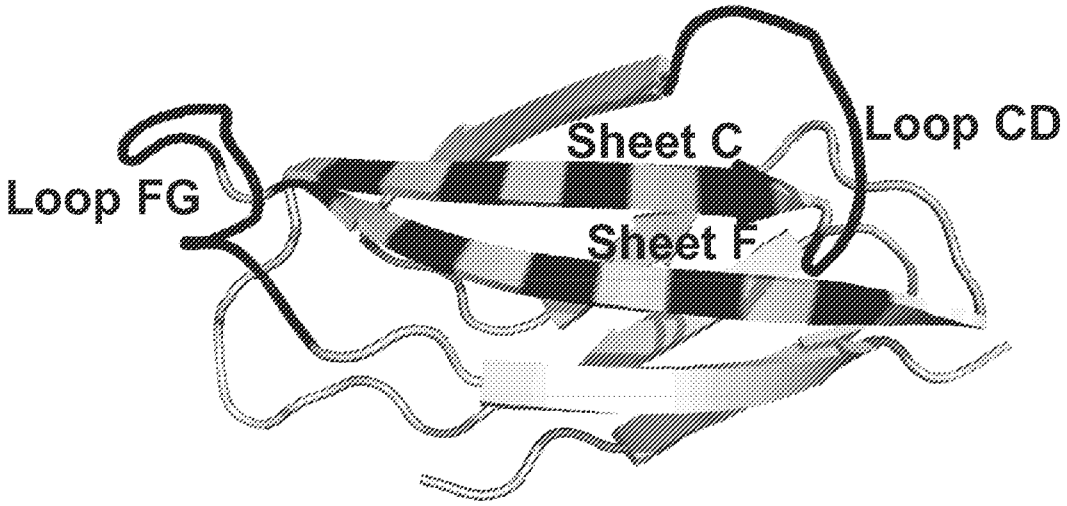

PFAM Database Family PF00041
Search for mammalian sequence ID's
Download ID's from UniProt
Align using human fn3 profile alignment
Identify/Define Cradle Regions
Alignment of Sheet C Sequences
Alignment of Loop CD Sequences
Alignment of Sheet F Sequences
Alignment of Loop FG Sequences
Split Data according to loop length
Select most common sizes
Sheet C 9
Sheet C 10
Loop CD 4
Loop CD 5
Loop CD 6
Sheet F 10
Loop FG 5
Loop FG 6
Perform positional amino acid frequency analysis

Figure 1



Sheet C Loop CD
VSDVPRDLEVVAATPTSLLISWDAPAVTVRYYRITYGETGGNSPVQ
EFTVPGSKSTATISGLKPGVDYTITVYAVTGRGDSPASSKPISINYRT
Sheet F Loop FG

Figure 2A

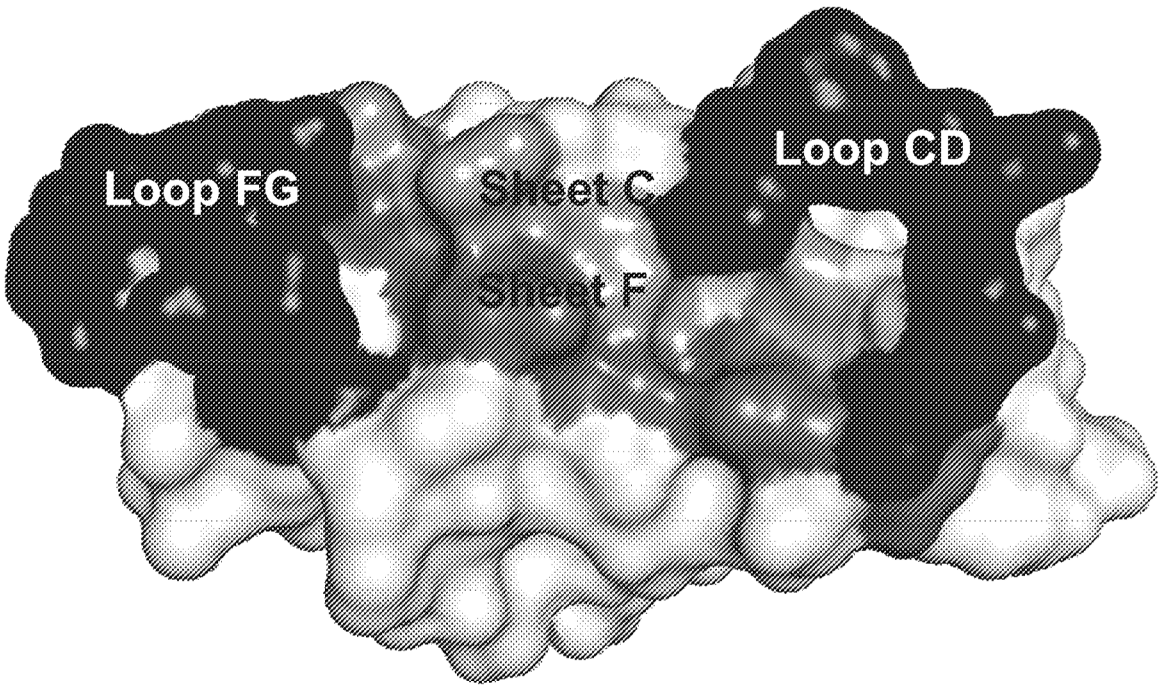


Figure 2B

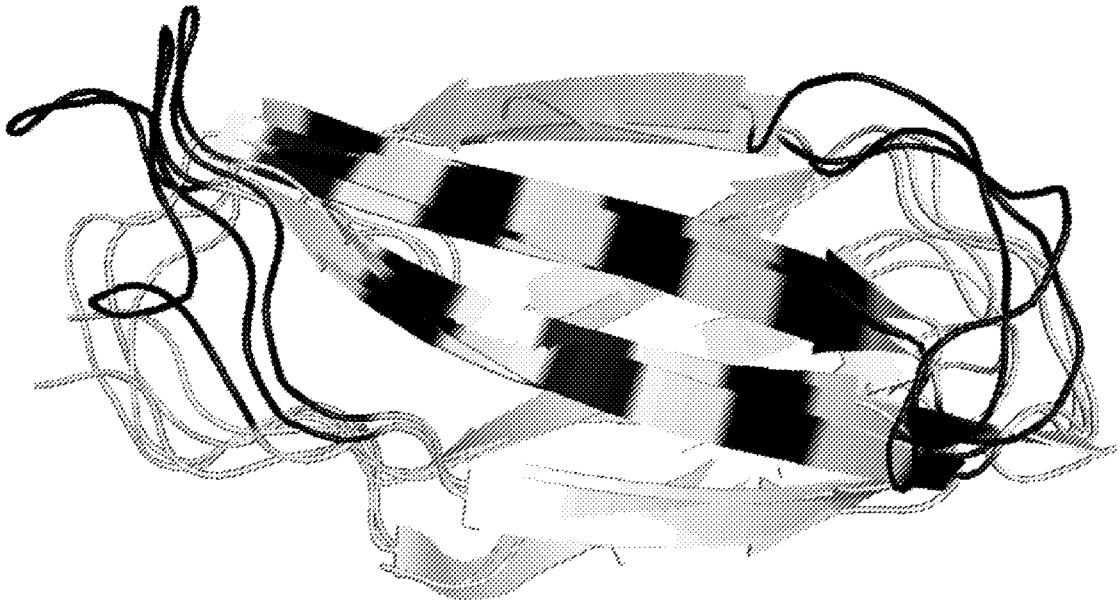


Figure 3A

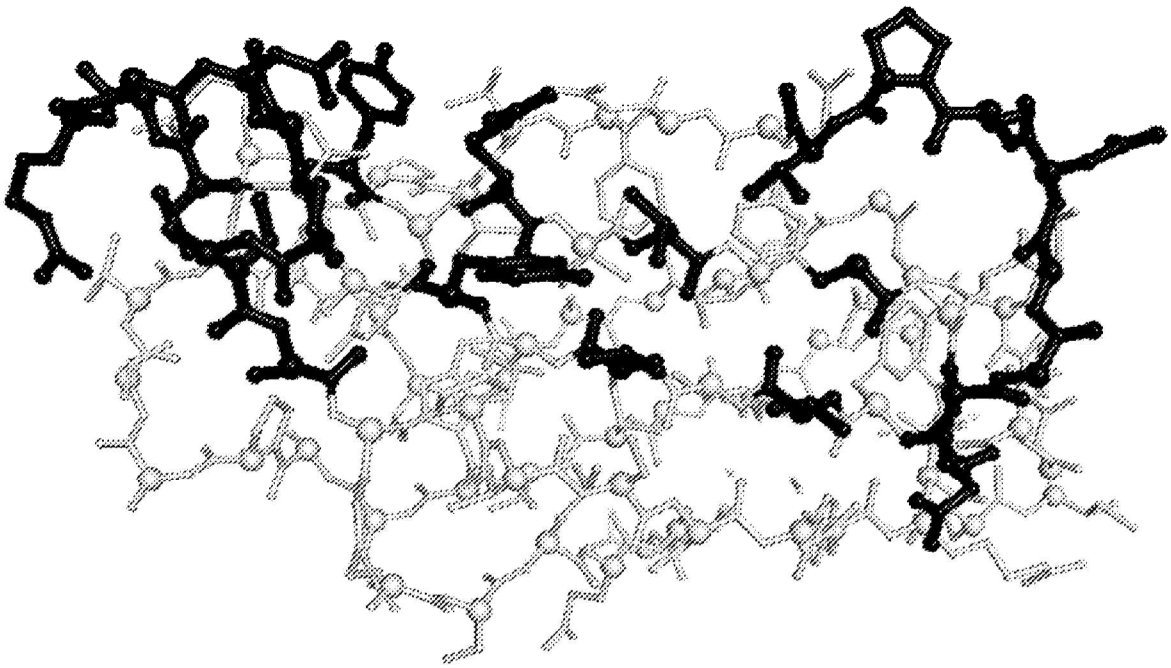


Figure 3B

Length	%
6	0.6
7	0.4
8	2.1
9	70.7
10	15.2
11	6.2
12	2.2
13	0.5
14	2.1

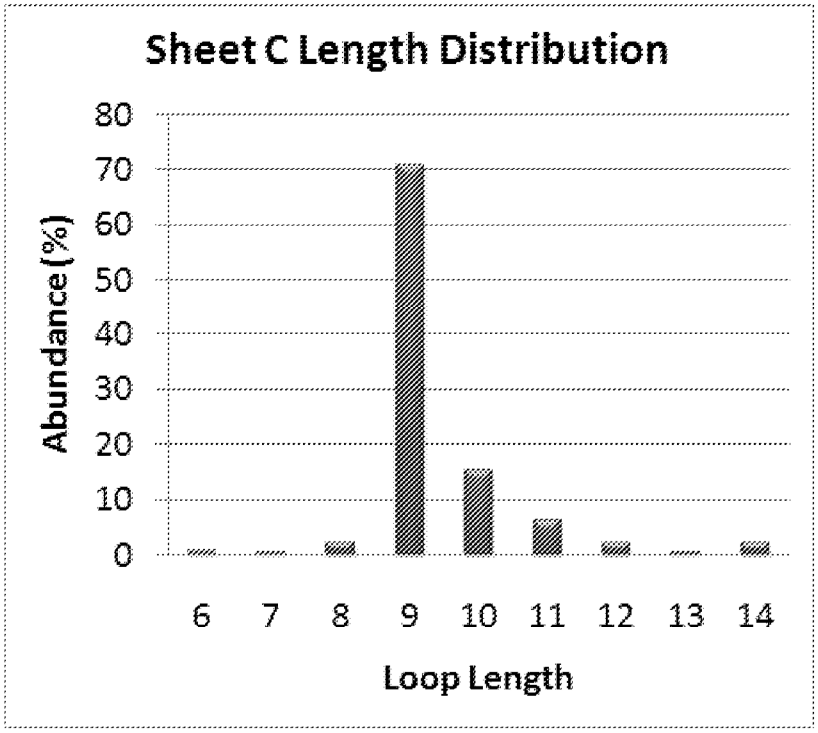


Figure 4A

Length	%
1	0.2
2	0.1
3	7.4
4	24.8
5	31.6
6	24.8
7	4.5
8	3.1
9	1.8
10	0.4
11	1.5

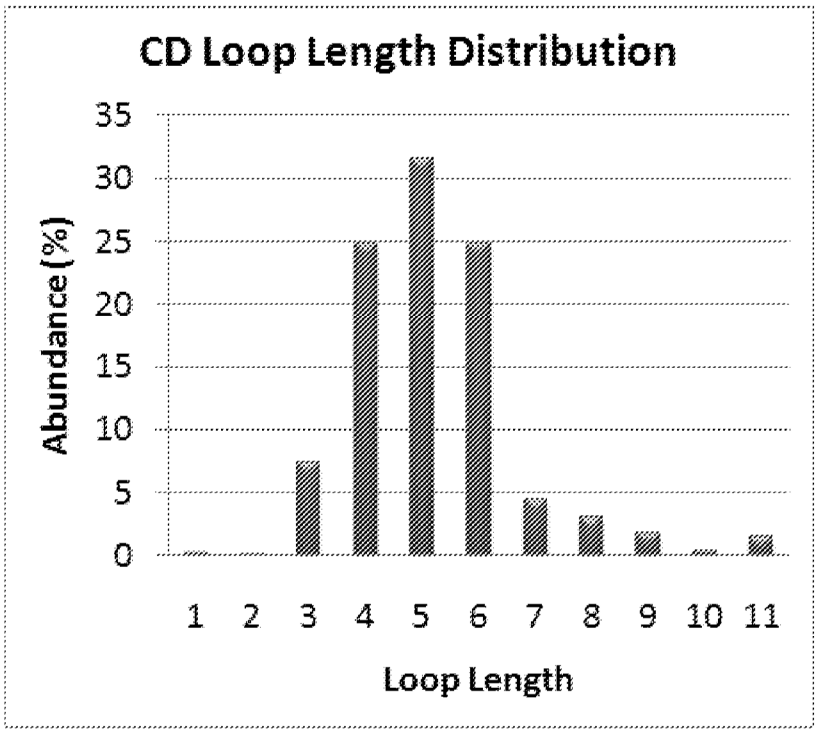


Figure 4B

Length	%
8	0.4
9	1.8
10	94.9
11	2.1
12	0.6
13	0.3

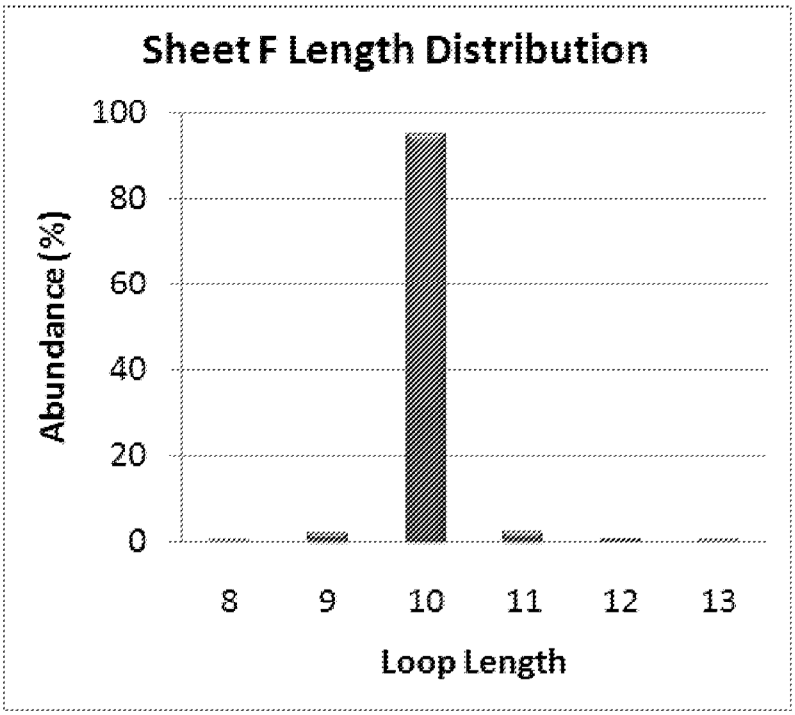


Figure 4C

Length	%
2	0.1
3	0.1
4	0.3
5	32.6
6	62.1
7	3.4
8	1.0
10	0.4

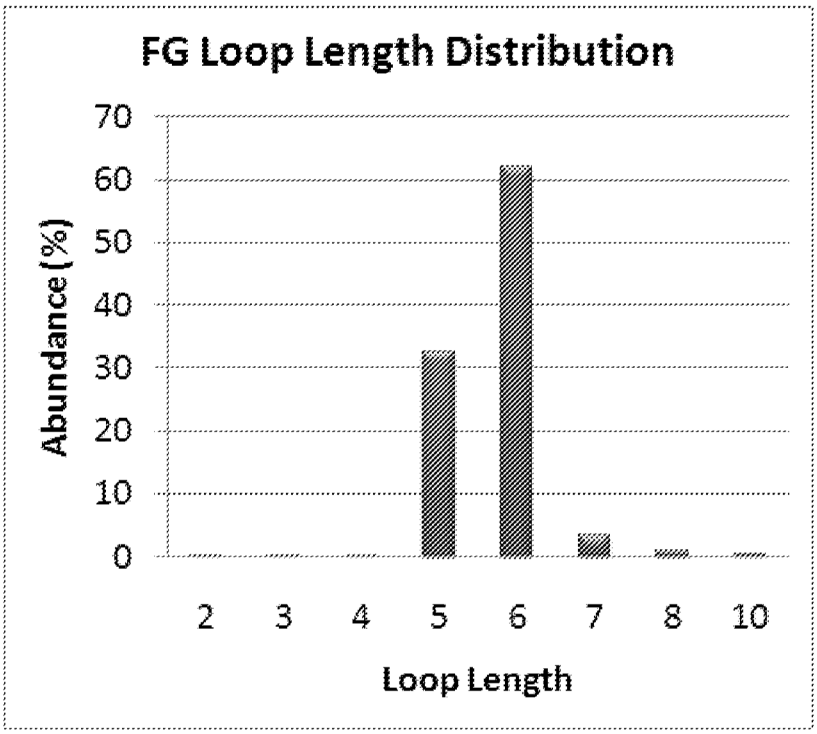


Figure 4D

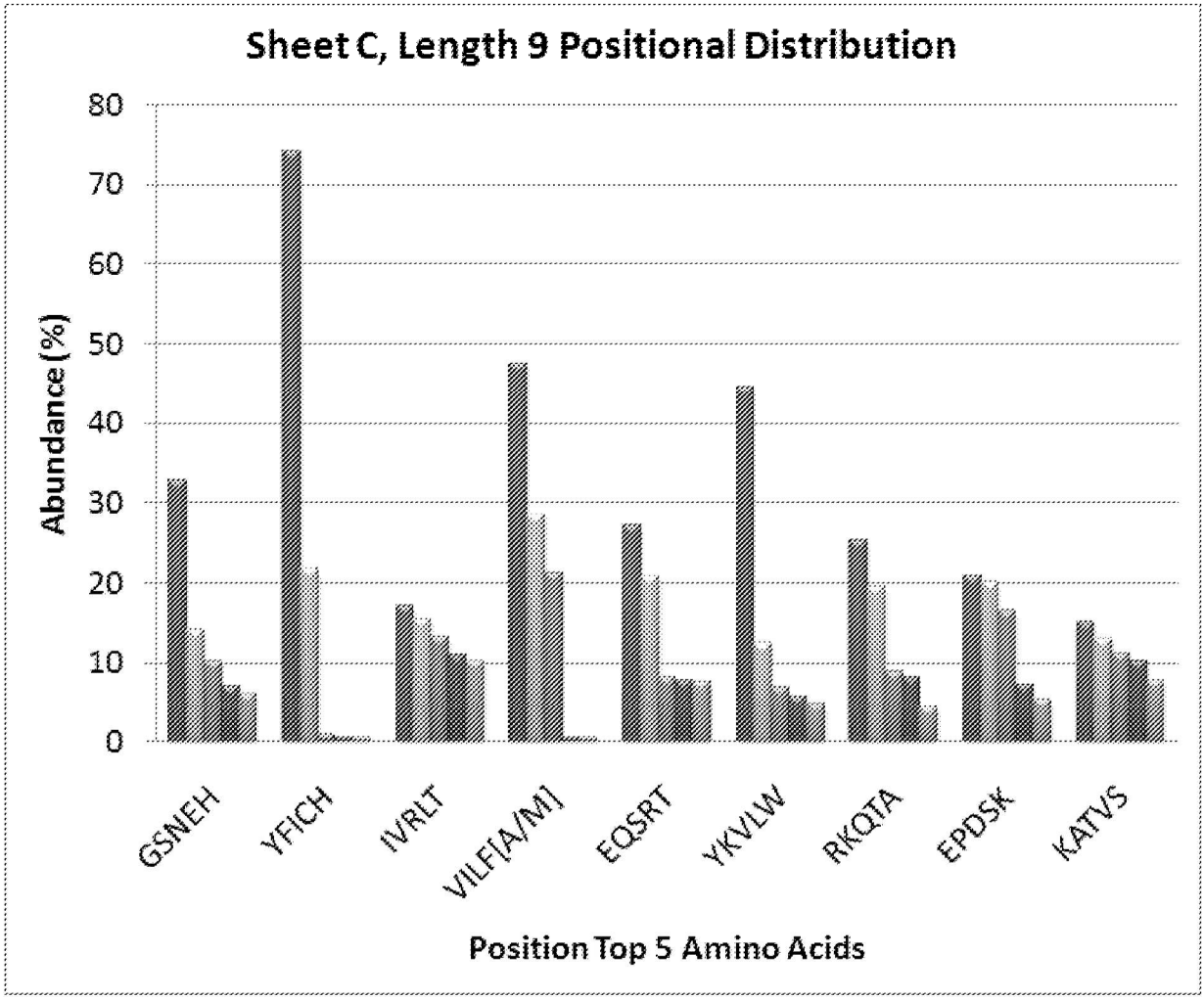

Sheet C, Length 9 Positional Distribution
Abundance (%)
80
70
60
50
40
30
20
10
0
GSNEH
YFICH
IVRLT
VILF[A/M]
EQSRT
YKVLW
RKQTA
EPDSK
KATVS
Position Top 5 Amino Acids

Figure 5A

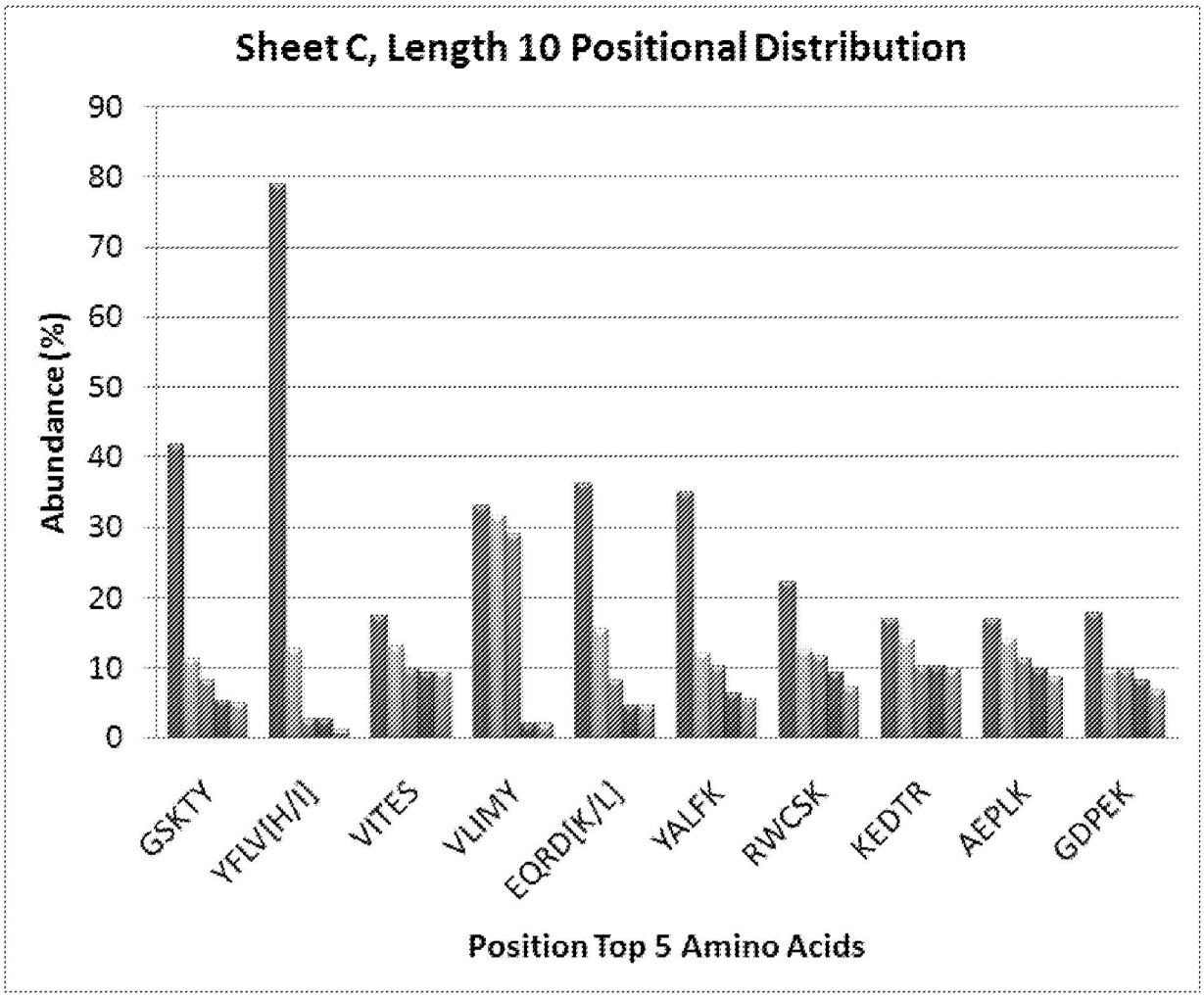

Sheet C, Length 10 Positional Distribution
Abundance (%)
90
80
70
60
50
40
30
20
10
0
GSKTY
YFLV[H/I]
VITES
VLIMY
EQRD[K/L]
YALFK
RWCSK
KEDTR
AEPLK
GDPEK
Position Top 5 Amino Acids

Figure 5B

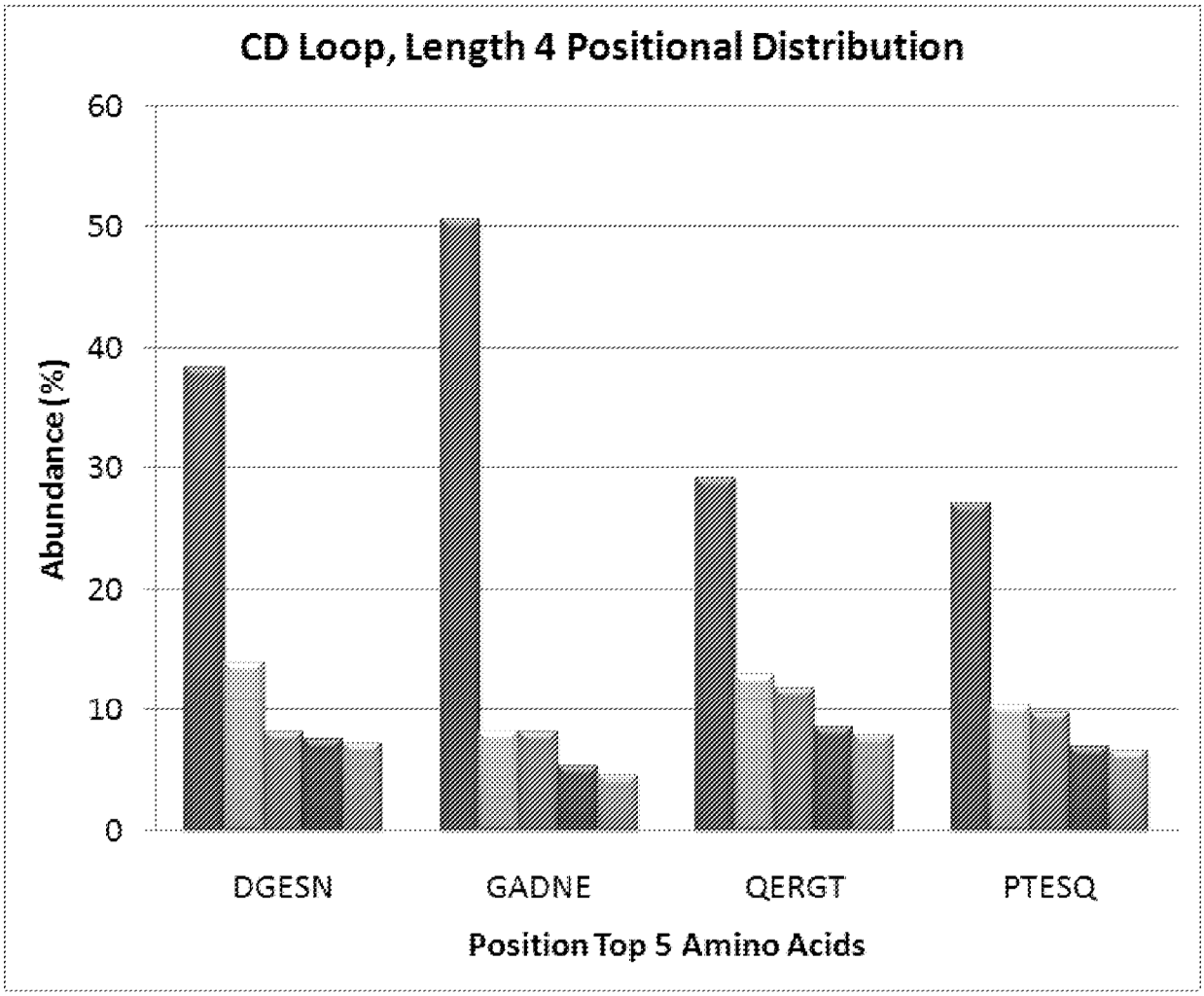

CD Loop, Length 4 Positional Distribution
60
50
40
30
20
10
0
Abundance (%)
DGESN
GADNE
QERGT
PTESQ
Position Top 5 Amino Acids

Figure 5C

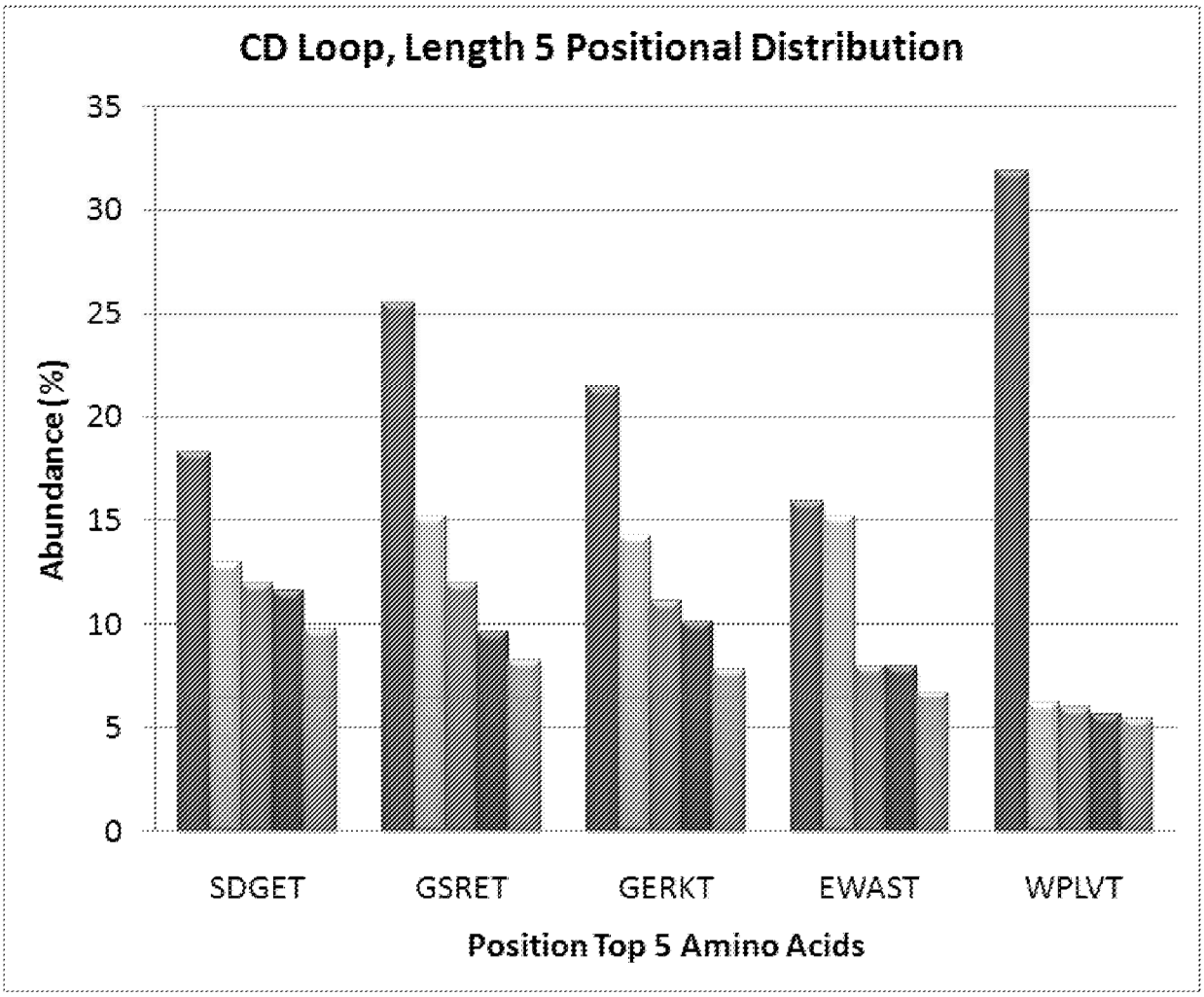

CD Loop, Length 5 Positional Distribution
Abundance (%)
35
30
25
20
15
10
5
0
SDGET
GSRET
GERKT
EWAST
WPLVT
Position Top 5 Amino Acids

Figure 5D

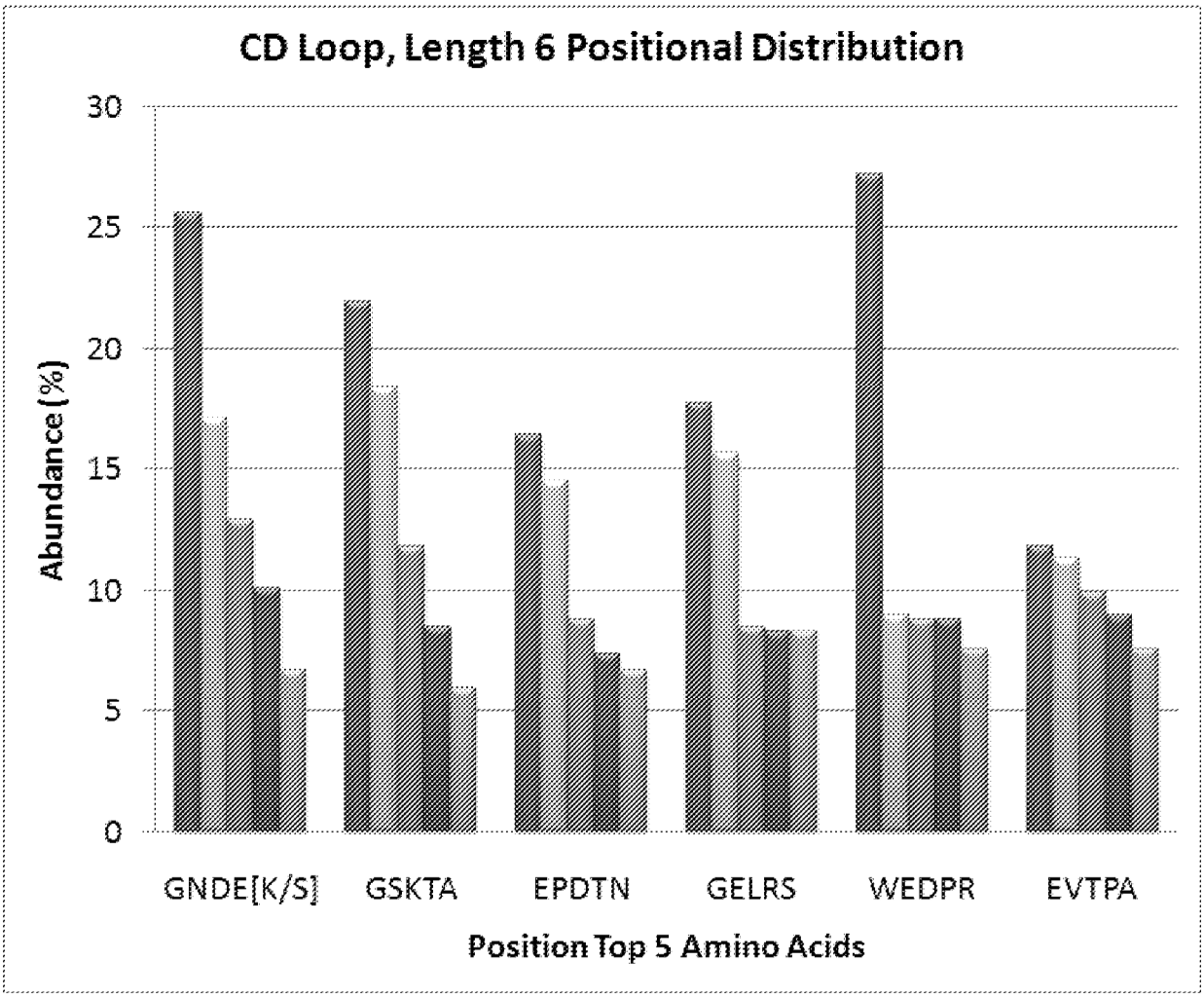

CD Loop, Length 6 Positional Distribution
30
25
20
15
10
5
0
Abundance (%)
GNDE[K/S]
GSKTA
EPDTN
GELRS
WEDPR
EVTPA
Position Top 5 Amino Acids

Figure 5E

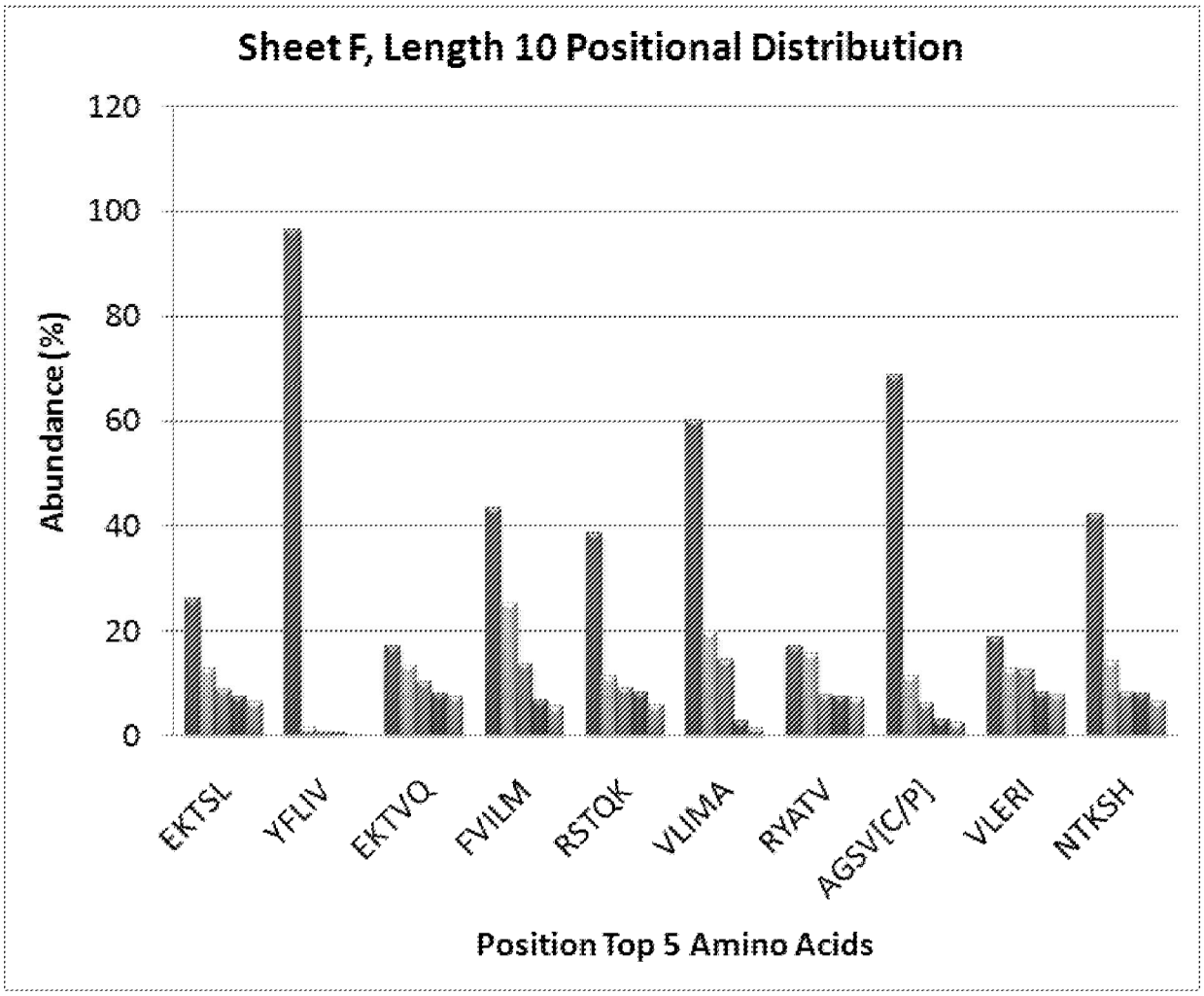

Sheet F, Length 10 Positional Distribution
Abundance (%)
120
100
80
60
40
20
0
EKTSL
YFLIV
EKTVQ
FVILM
RSTQK
VLIMA
RYATV
AGSV[C/P]
VLERI
NTKSH
Position Top 5 Amino Acids

Figure 5F

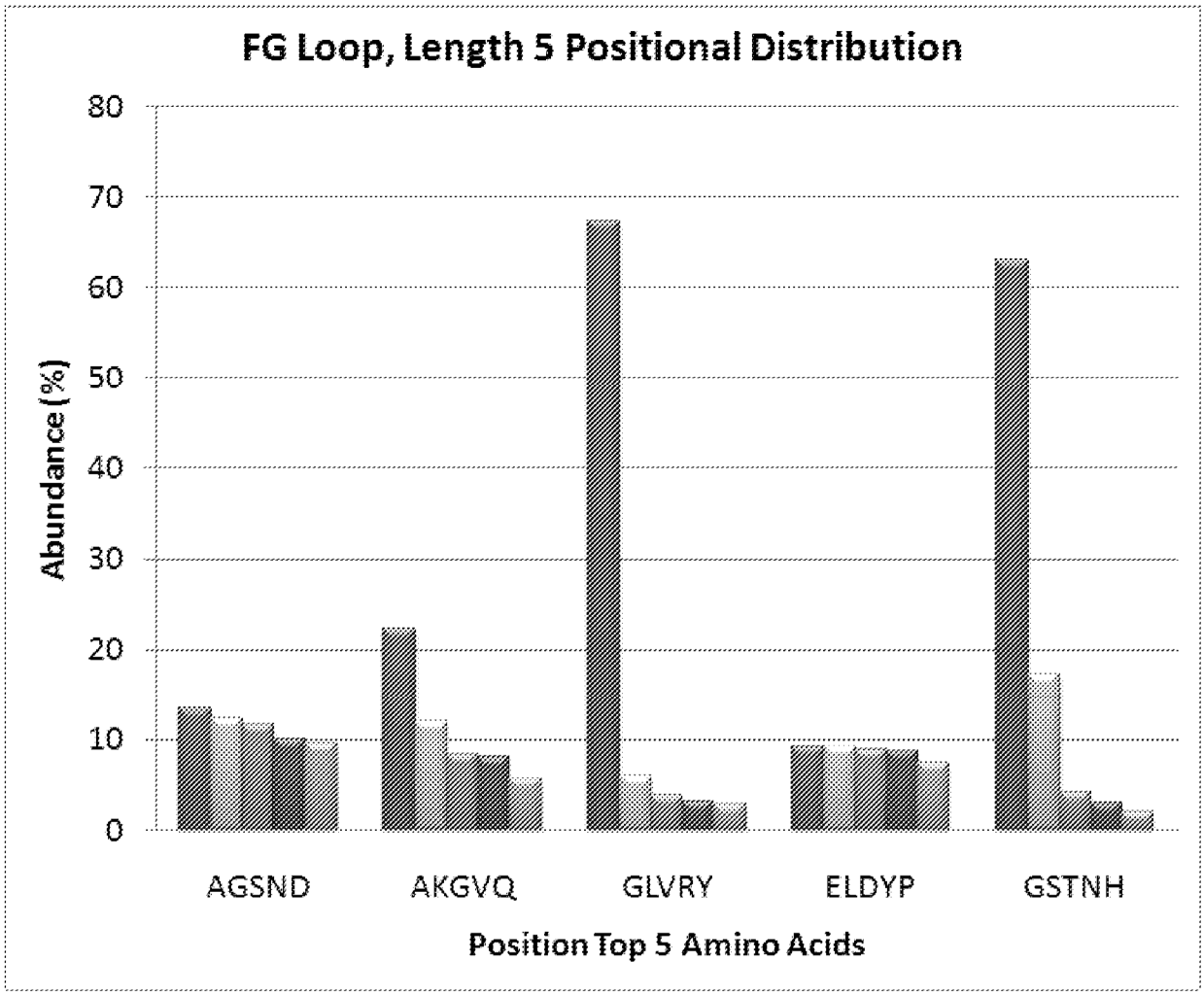

FG Loop, Length 5 Positional Distribution
Abundance (%)
80
70
60
50
40
30
20
10
0
AGSND
AKGVQ
GLVRY
ELDYP
GSTNH
Position Top 5 Amino Acids

Figure 5G

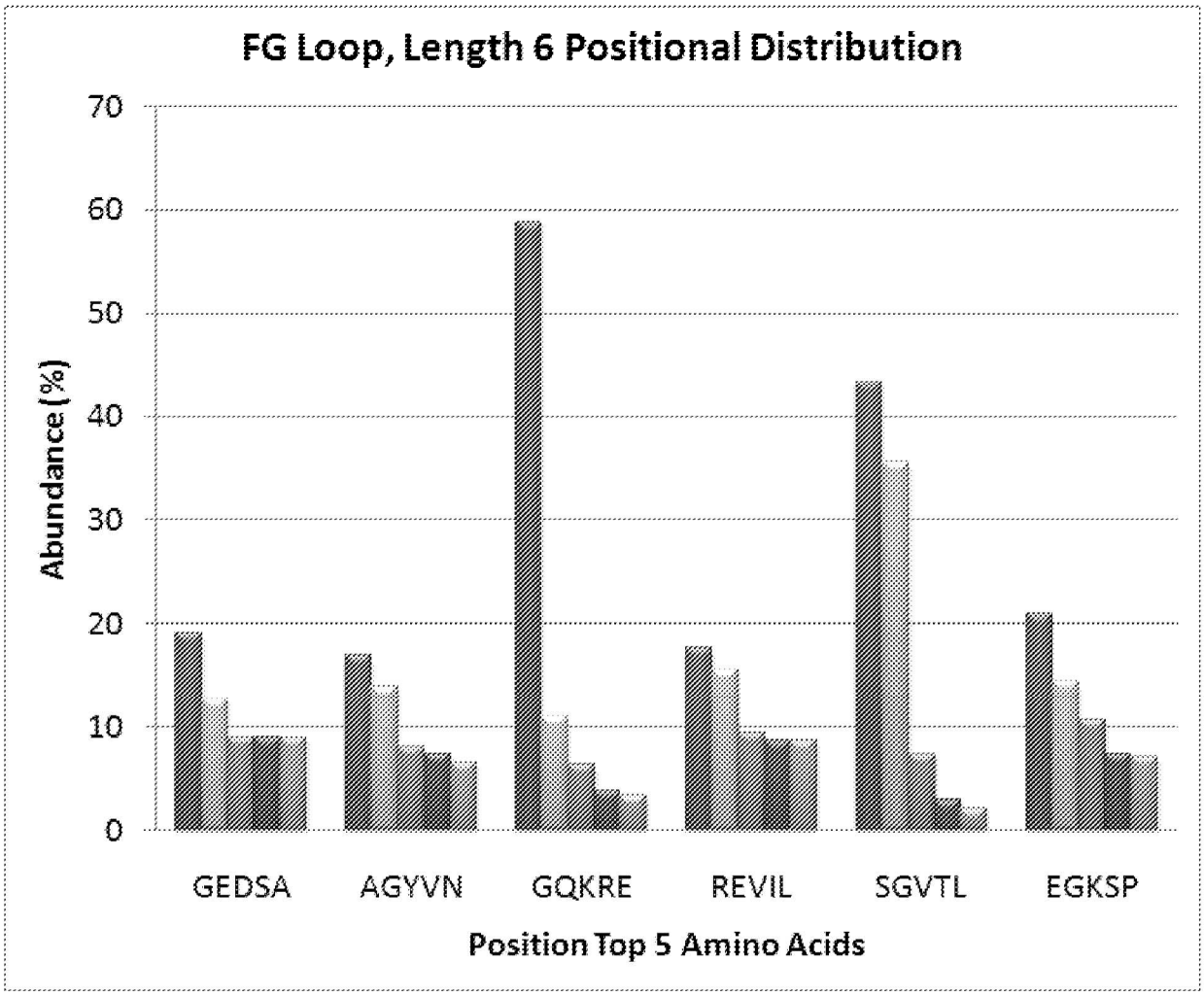
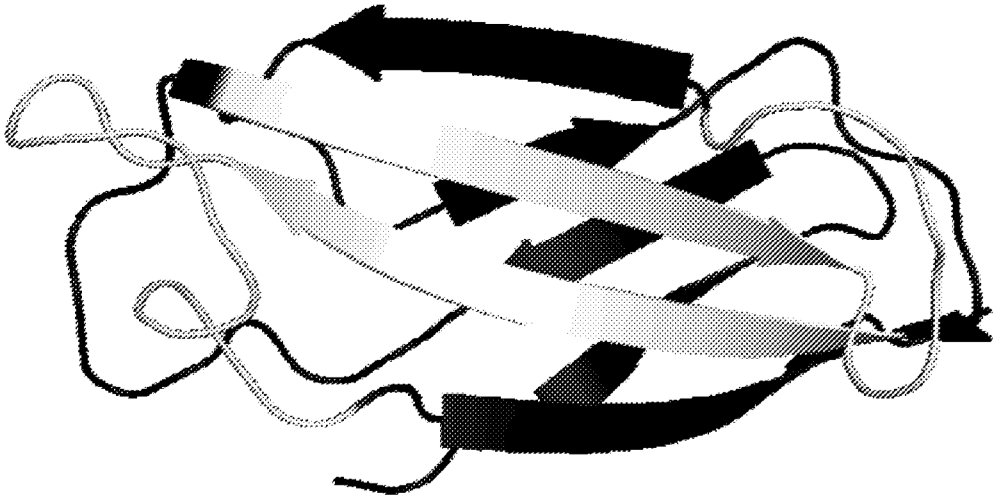
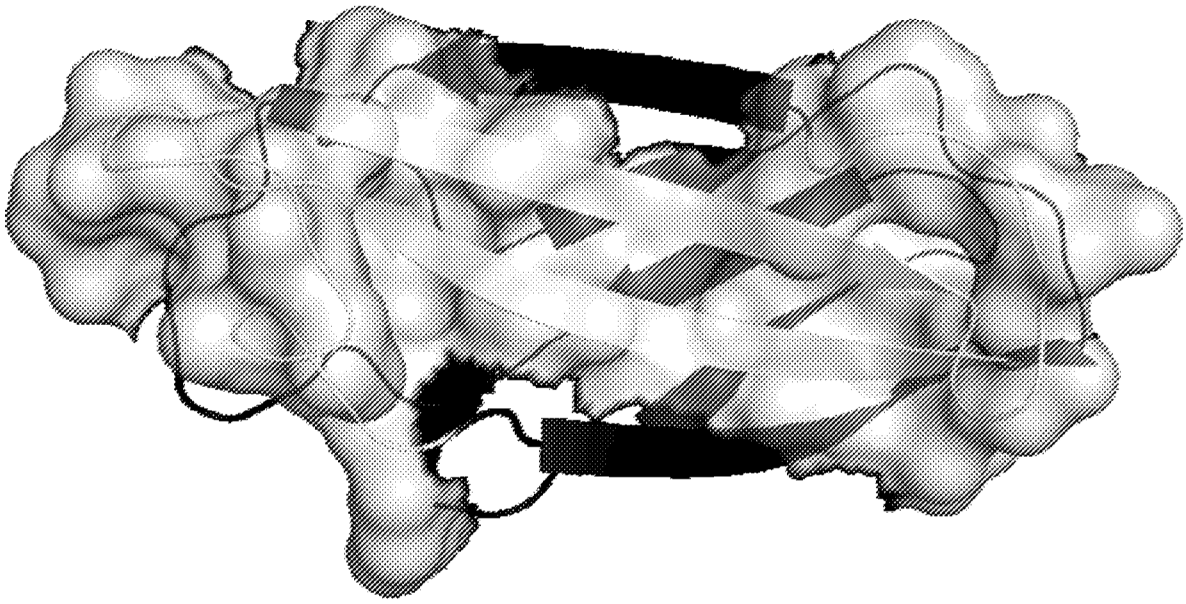

FG Loop, Length 6 Positional Distribution
Abundance (%)
70
60
50
40
30
20
10
0
GEDSA
AGYVN
GQKRE
REVIL
SGVTL
EGKSP
Position Top 5 Amino Acids

Figure 5H



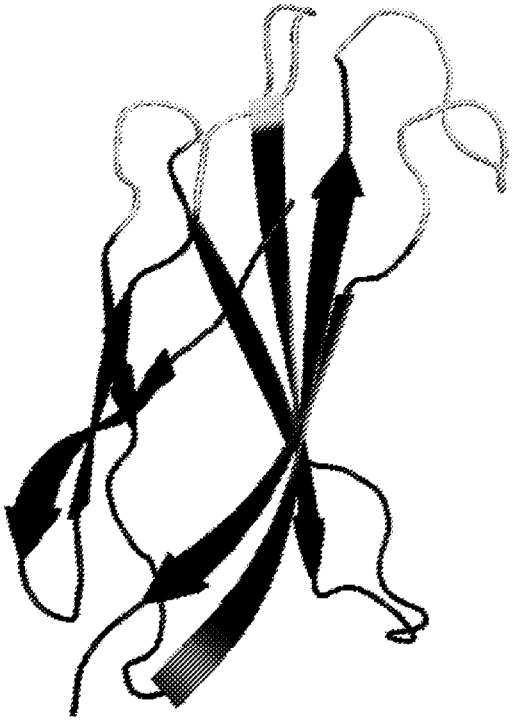
FnIII^{10} Cradle

Figure 6A



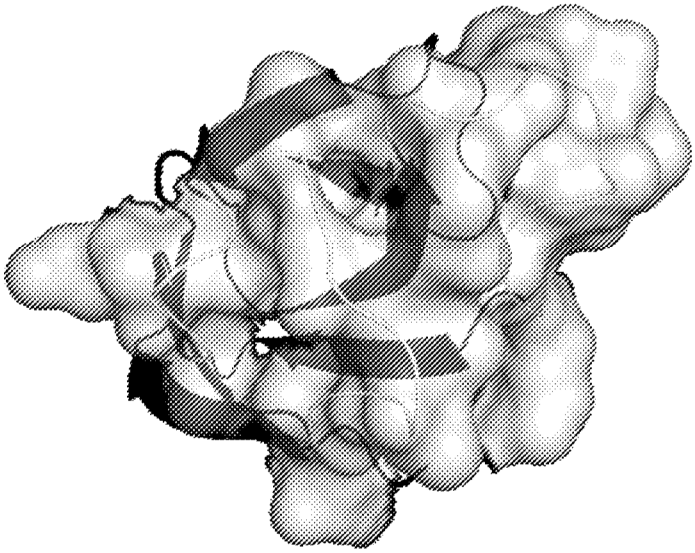
FnIII^{10} Cradle Surface = 2457 Å^2

Figure 6B



FnIII[10] Top Side

Figure 6C



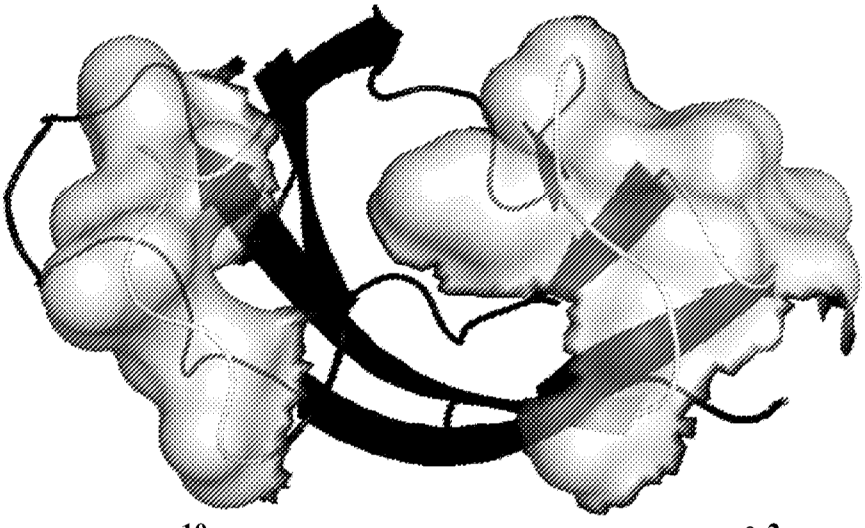
FnIII[10] Top Side Surface = 1834 Å[2]

Figure 6D



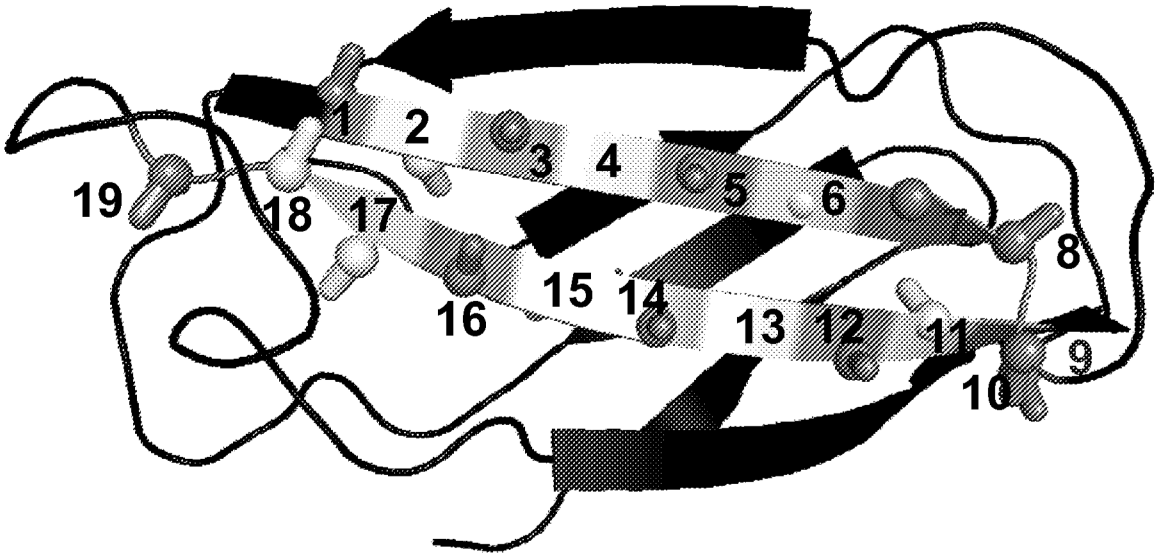
FnIII^{10} Bottom Side

Figure 6E



FnIII^{10} Bottom Side Surface = 1140 Å^2

Figure 6F



FnIII^{10} Cradle β-Sheets
Sheet C: Positions 1 - 9
Sheet F: Positions 10 - 19
Gray = Varied in Cradle

Figure 7A

Position	SF	CP	H	C	Varied
1	50.9	41.5	7.1	0.6	Yes
2	0.2	1.2	98.0	0.6	No
3	15.1	35.4	49.4	0.2	Yes
4	0.7	0.5	98.8	0.1	No
5	17.3	69.0	13.7	0.2	Yes
6	4.8	18.9	73.4	3.7	No
7	19.5	63.6	14.7	2.3	Yes
8	38.4	54.3	6.9	0.3	Yes
9	43.4	34.1	22.5	0.0	Yes
10	24.1	60.1	15.4	0.8	Yes
11	0.0	0.1	99.7	0.1	No
12	19.2	54.6	25.9	0.4	Yes
13	3.6	0.1	94.4	1.0	No
14	23.5	64.4	11.0	1.3	Yes
15	2.1	0.0	98.0	0.0	No
16	24.2	29.1	44.6	2.3	Yes
17	91.5	0.8	5.2	2.7	No
18	10.4	31.1	56.2	2.4	No
19	25.4	63.6	10.6	0.4	Yes

Sheet C: Positions 1 - 9
Sheet F: Positions 10 - 19
SF: Small/Flexible amino acids - A, G, P, S, T
CP: Charged/Polar amino acids - D, E, H, K, N, Q, R
H: Hydrophobic amino acids - F, I, L, M, V, W, Y
C: Cysteine

Figure 7B

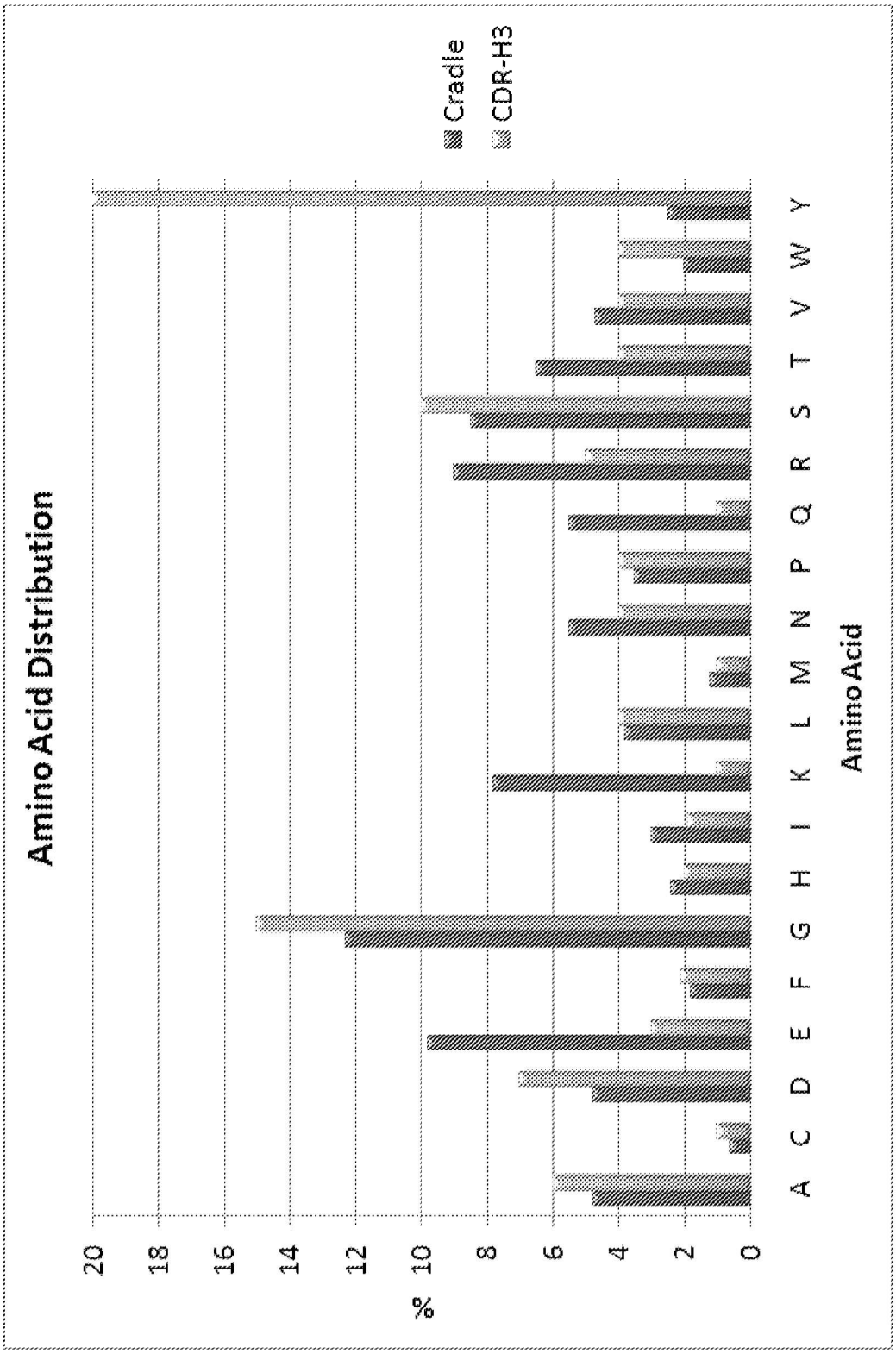

Amino Acid Distribution
Cradle
CDR-H3
20
18
16
14
12
10
8
6
4
2
0
%
A C D E F G H I K L M N P Q R S T V W Y
Amino Acid

Figure 8

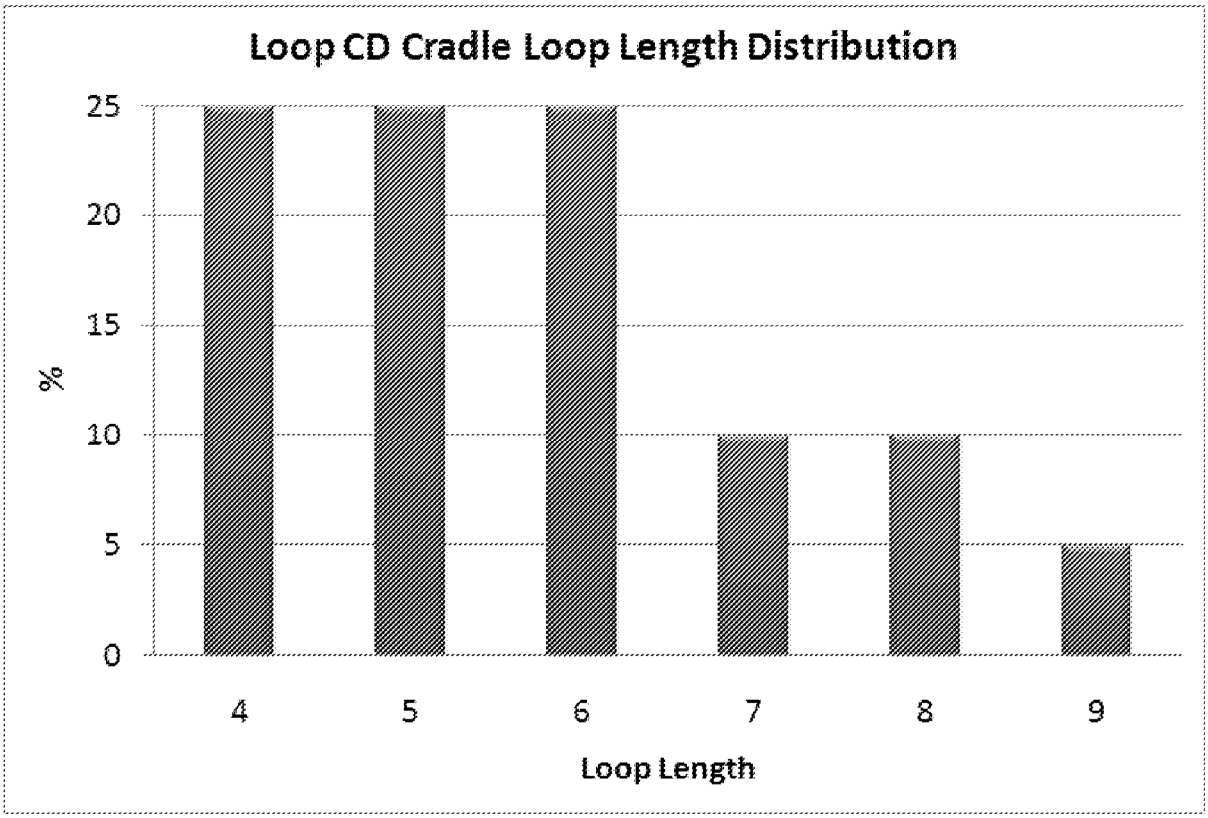

Loop CD Cradle Loop Length Distribution
25
20
15
10
5
0
%
4
5
6
7
8
9
Loop Length

Figure 9A

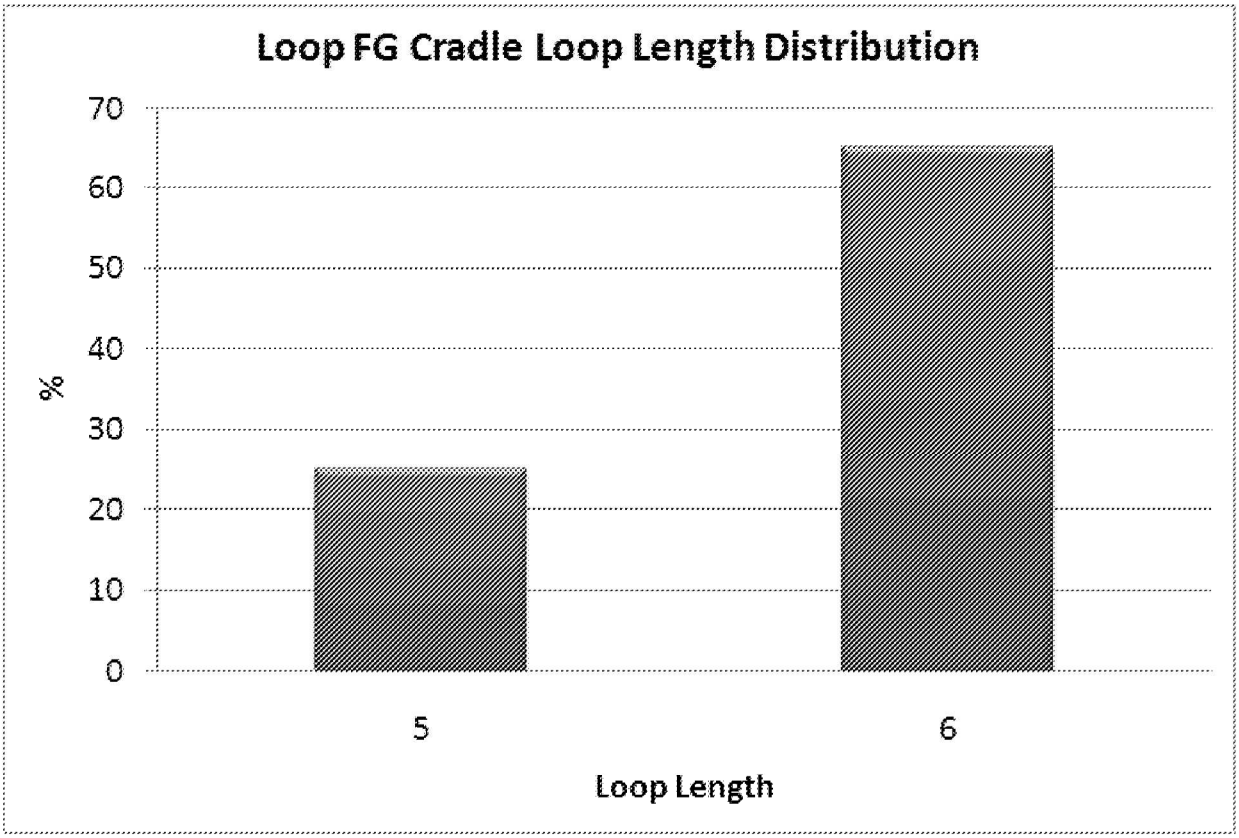

Loop FG Cradle Loop Length Distribution
70
60
50
40
30
20
10
0
%
5
6
Loop Length

Figure 9B

Amino Acid	Sheet Distribution X (%)	Loop Distribution Y (%)
Alanine/Ala/A	6	6
Cysteine/Cys/C	0	0
Aspartic Acid/Asp/D	7	7
Glutamic Acid/Glu/E	4	4
Phenylalanine/Phe/F	2	2
Glycine/Gly/G	17	17
Histidine/His/H	2	2
Isoleucine/Ile/I	2	2
Lysine/Lys/K	2	2
Leucine/Leu/L	4	4
Methionine/Met/M	1	1
Asparagine/Asn/N	4	4
Proline/Pro/P	0	2
Glutamine/Gln/Q	2	2
Arginine/Arg/R	5	5
Serine/Ser/S	12	10
Threonine/Thr/T	4	4
Valine/Val/V	4	4
Tryptophan/Trp/W	3	3
Tyrosine/Tyr/Y	19	19

Figure 9C

Figure 9D


A AB B BC C CD
FnIII07 PLSPPTNLHLEANPDTGVLTVSWERSTTPDITXYXIXTXXX[Y4-9]
FnIII10 VSDVPRDLEVVAATPT-SLLISWD-APAVTVRXYXIXYXXX[Y4-9]
FnIII14 NVSPPRRARVTDATET-TITISWR-TKTETITXFXVXAXPX[Y4-9]
D DE E EF F FG G
FnIII07 LEEVVHADQSSCTFDNLSPGLXYXVXVXTVX[Y5-6]PISDTIIP
FnIII10 QEFTVPGSKSTATISGLKPGVXYXIXVXAVX[Y5-6]PISINYRT
FnIII14 IQRTIKPDVRSYTITGLQPGTXYXIXLXTLX[Y5-6]PVVIDAST

Figure 9E

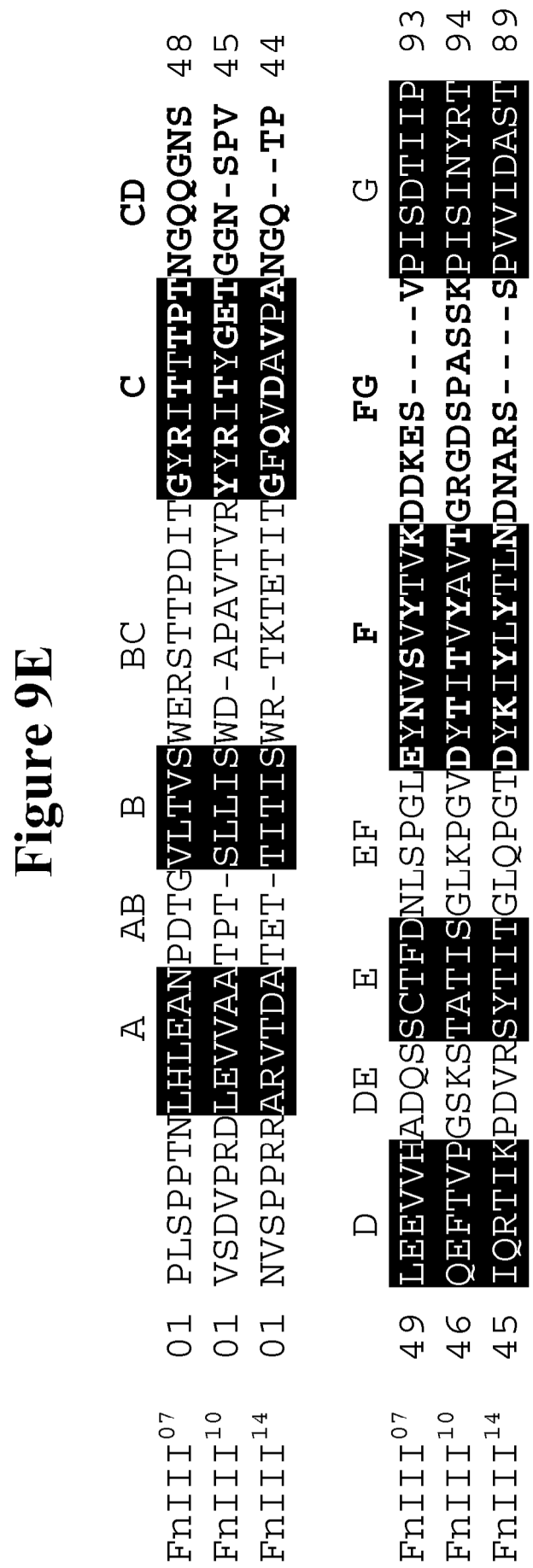

A AB B BC C CD
FnIII07 01 PLSPPTNLHLEANPDTGVLTVSWERSTTPDITGYRITTTPTNGQQGNS 48
FnIII10 01 VSDVPRDLEVVAATPT-SLLISWD-APAVTVRYYRITYGETGGN-SPV 45
FnIII14 01 NVSPPRRARVTDATET-TITISWR-TKTETITGFQVDAVPANGQ--TP 44
D DE E EF F FG G
FnIII07 49 LEEVVHADQSSCTFDNLSPGLEYNVSVYTVKDDKES----VPISDTIIP 93
FnIII10 46 QEFTVPGSKSTATISGLKPGVDYTITVYAVTGRGDSPASSKPISINYRT 94
FnIII14 45 IQRTIKPDVRSYTITGLQPGTDYKIYLYTLNDNARS----SPVVIDAST 89

Figure 9F

FnIII structural element residue ranges

FnIII	A	AB	B	BC	C	CD	D	DE	E	EF	F	FG	G
07	8-13	14-17	18-22	23-32	33-41	42-48	49-54	55-58	59-63	64-69	70-79	80-85	86-93
10	8-13	14-16	17-21	22-30	31-39	40-45	46-51	52-55	56-60	61-66	67-76	77-86	87-94
14	8-13	14-16	17-12	22-30	31-39	40-44	45-50	51-54	55-59	60-65	66-75	76-81	82-89

FnIII Cradle residues ranges

FnIII	Sheet C	Loop CD	Sheet F	Loop FG
07	33,35,37,39-41	42-48	70,72,74,76,79	80-85
10	31,33,35,37-39	40-45	67,69,71,73,76	77-86
14	31,33,35,37,39	40-44	66,68,70,72,75	76-81

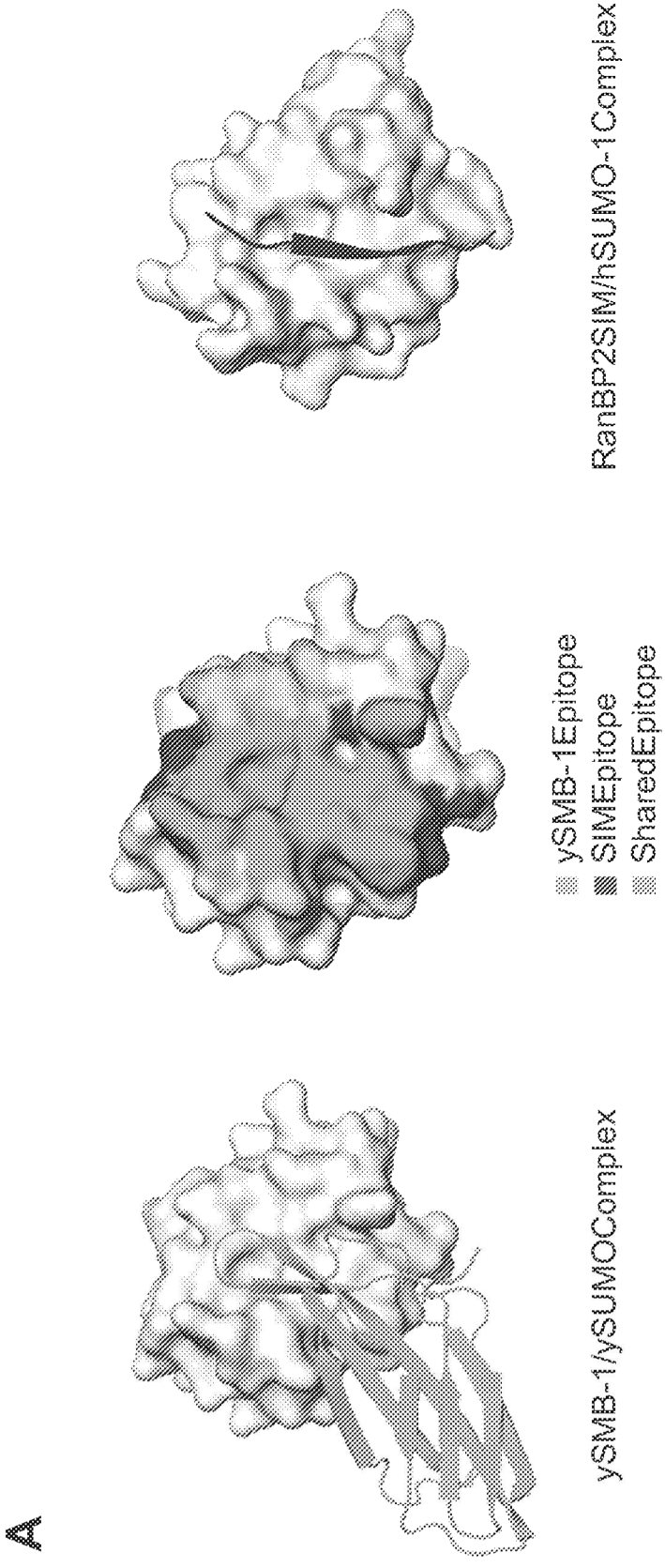

A
ySMB-1/ySUMOComplex
ySMB-1Epitope
SIMEpitope
SharedEpitope
RanBP2SIM/hSUMO-1Complex

Figure 10A

B
ResidueNumber(ySUMO)
ySUMO
hSUMO-1
hSUMO-2
ConservationScore
SIMBindingSite/ySMB-1Epitope
180°
SIMBindingSite
C
Residue Number (ySUMO)
ySUMO
hSUMO-1
hSUMO-2

Figure 10B-C

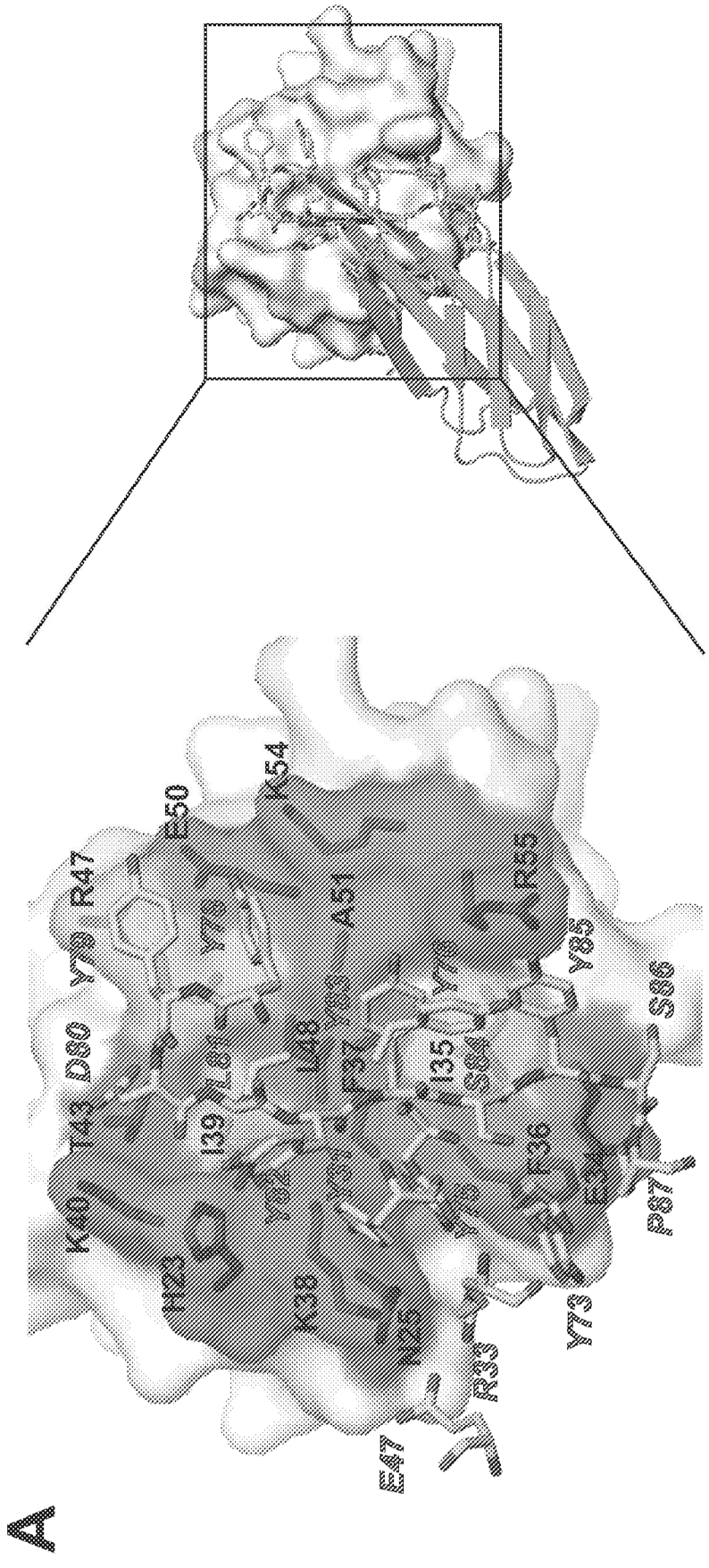

A
E47
R33
K25
K38
N23
K40
T43
D80
Y79
R47
E50
K54
Y78
L44
L46
A51
R55
Y85
S86
I39
F37
I35
S84
F36
E34
P67
Y73

Figure 11A

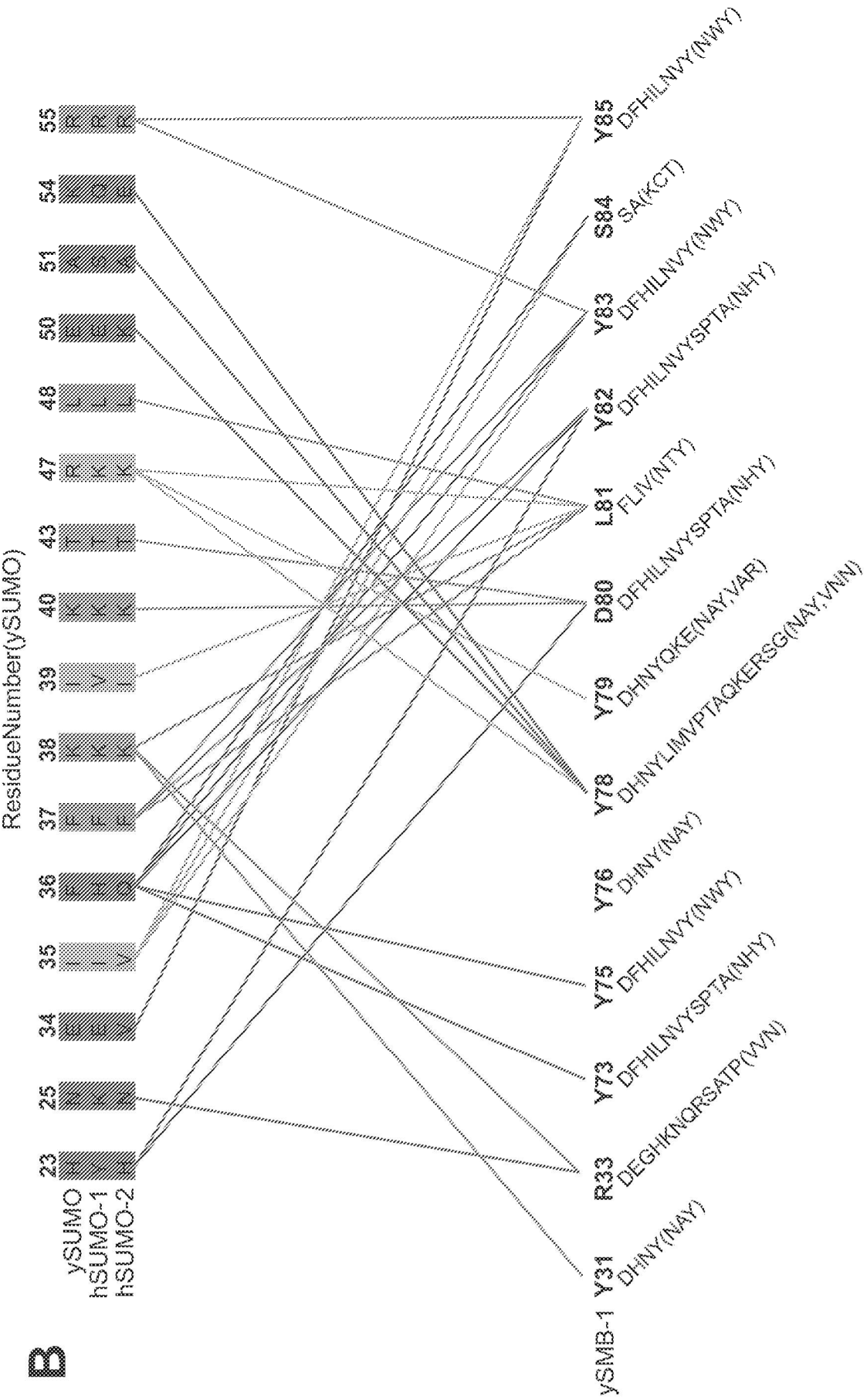

B
ResidueNumber(ySUMO)
ySUMO
hSUMO-1
hSUMO-2
23 25 34 35 36 37 38 39 40 43 47 48 50 51 54 55
ySMB-1
Y31 DHNY(NAY)
R33 DEGHKNQRSATP(V/N)
Y73 DFHILNVYSPTA(NHY)
Y75 DFHILNVY(NWY)
Y76 DHNY(NAY)
Y78 DHNYLIMVPTAQKERSG(NAY/V/NN)
Y79 DHNYQKE(NAY/VAR)
D80 DFHILNVYSPTA(NHY)
L81 FLIV(NTY)
Y82 DFHILNVYSPTA(NHY)
Y83 DFHILNVY(NWY)
S84 SA(KCT)
Y85 DFHILNVY(NWY)

Figure 11B

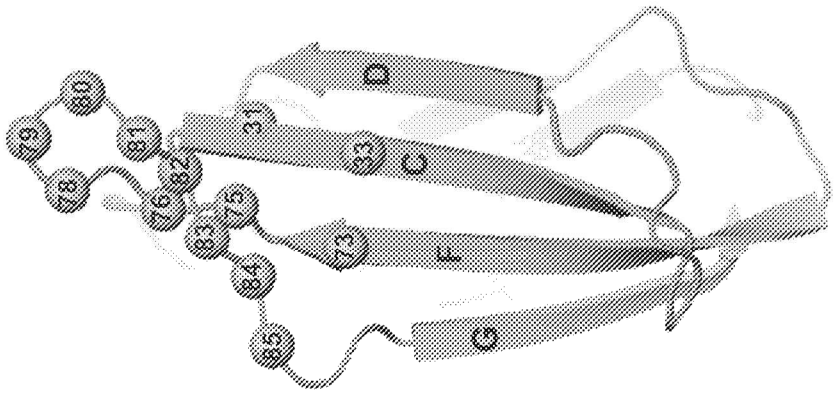


Figure 11C

A
ySUMO
hSUMO-1
Position
Diversity
31 [Y]DHNY
33 [R]DEGHKNQRSATP
73 [Y]DFHILNVYSPTA
75 [Y]DFHILNVY
76 [Y]DHNY
78 [Y]DHNYLIMVPTAQKERSG
79 [Y]DHNYQKE
80 [D]DFHILNVYSPTA
81 [L]FLIV
82 [Y]DFHILNVYSPTA
83 [Y]DFHILNVY
84 [S]SA
85 [Y]DFHILNVY

Figure 12A

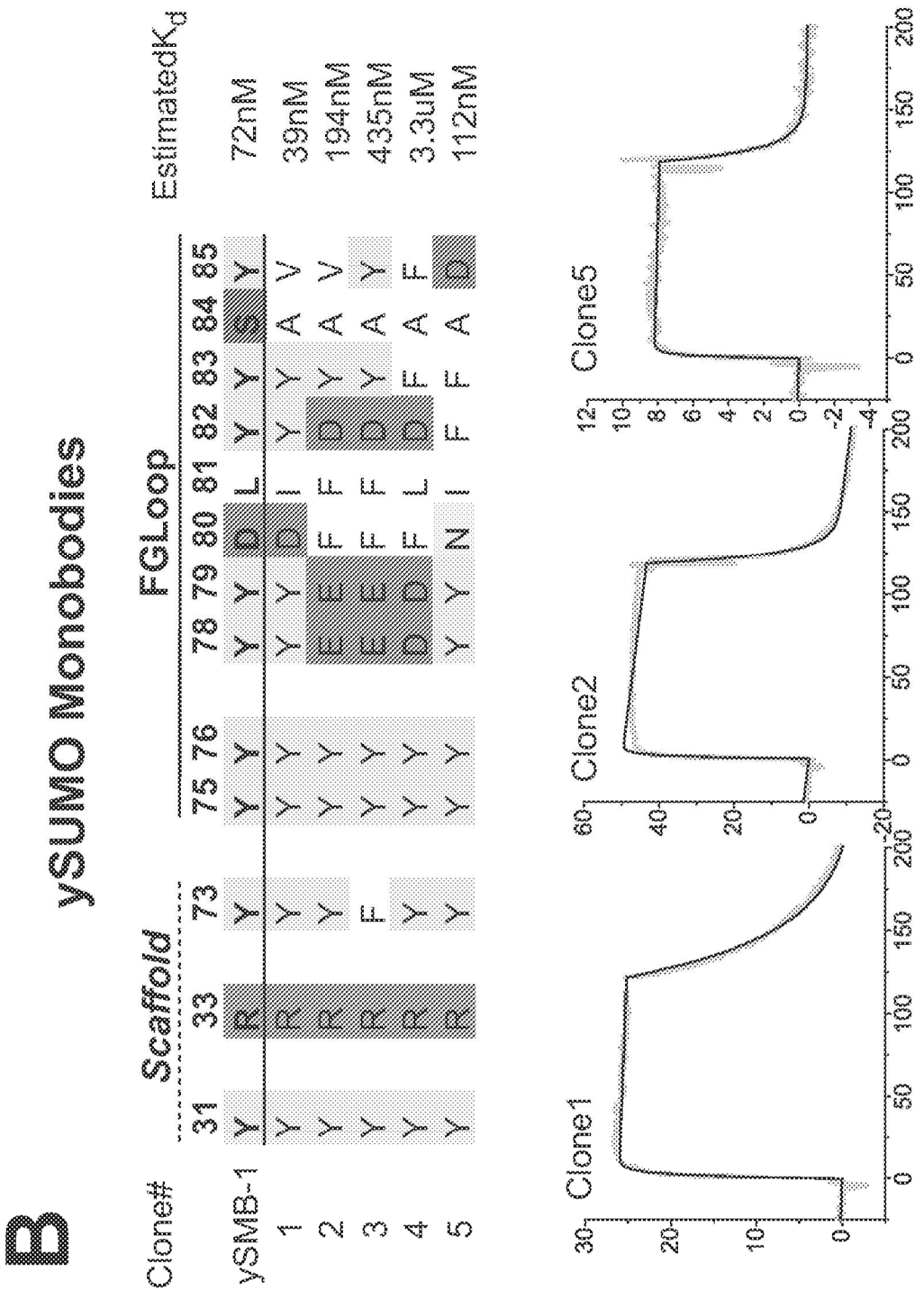

B
ySUMO Monobodies
Clone#
Scaffold
FGLoop
EstimatedKd
31 33 73 75 76 78 79 80 81 82 83 84 85
ySMB-1 Y R Y Y Y Y Y D L Y Y S Y
1 Y R Y Y Y Y Y D I Y Y A V 72nM
2 Y R Y Y Y E E F F D Y A V 39nM
3 Y R F Y Y E E F F D Y A Y 194nM
4 Y R Y Y Y D D F L D F A F 435nM
5 Y R Y Y Y Y Y N I F F A D 3.3uM 112nM
Clone1
Clone2
Clone5

Figure 12B

Figure 12B
(Continued)

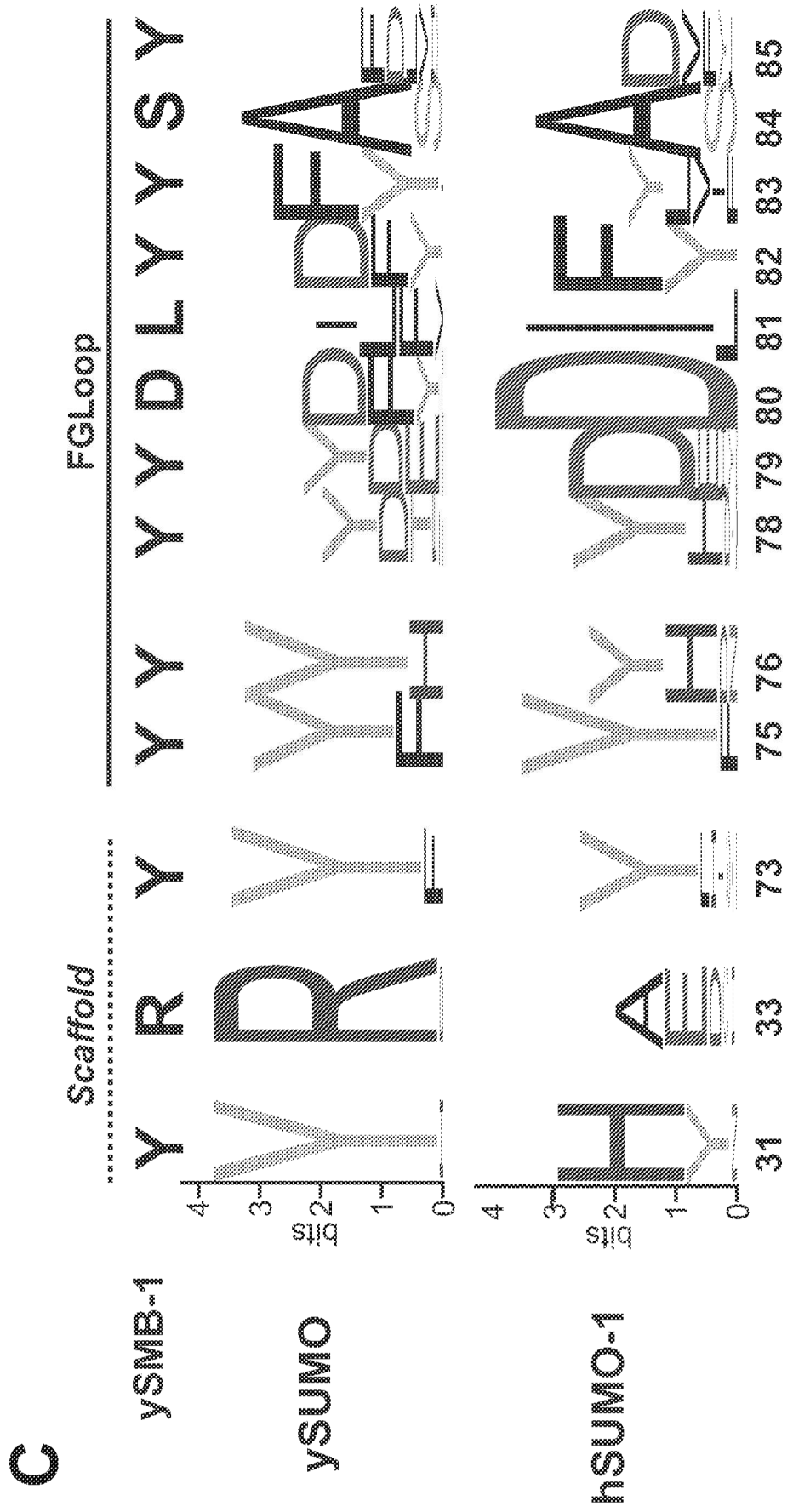
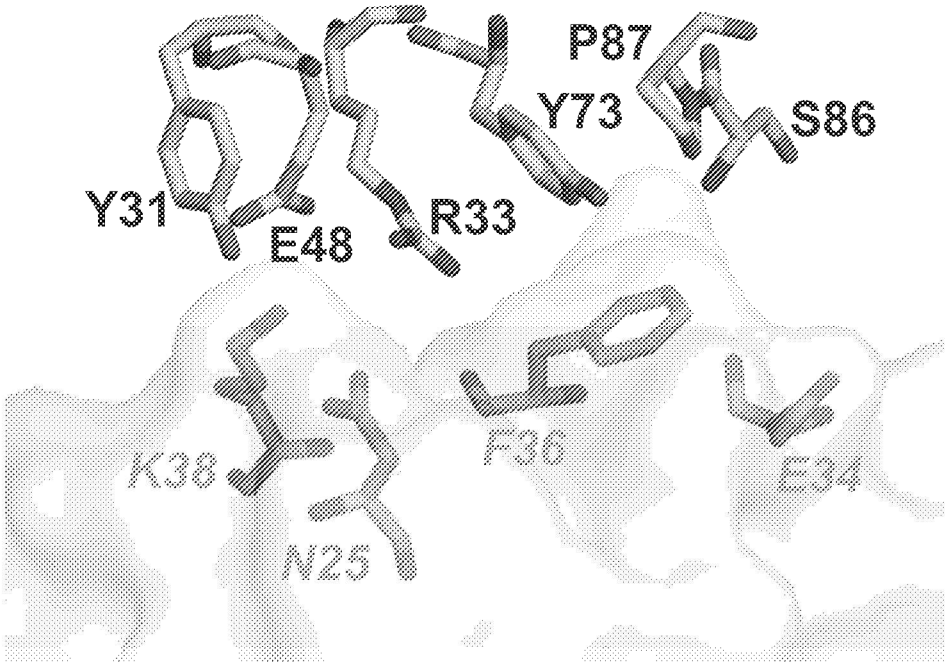
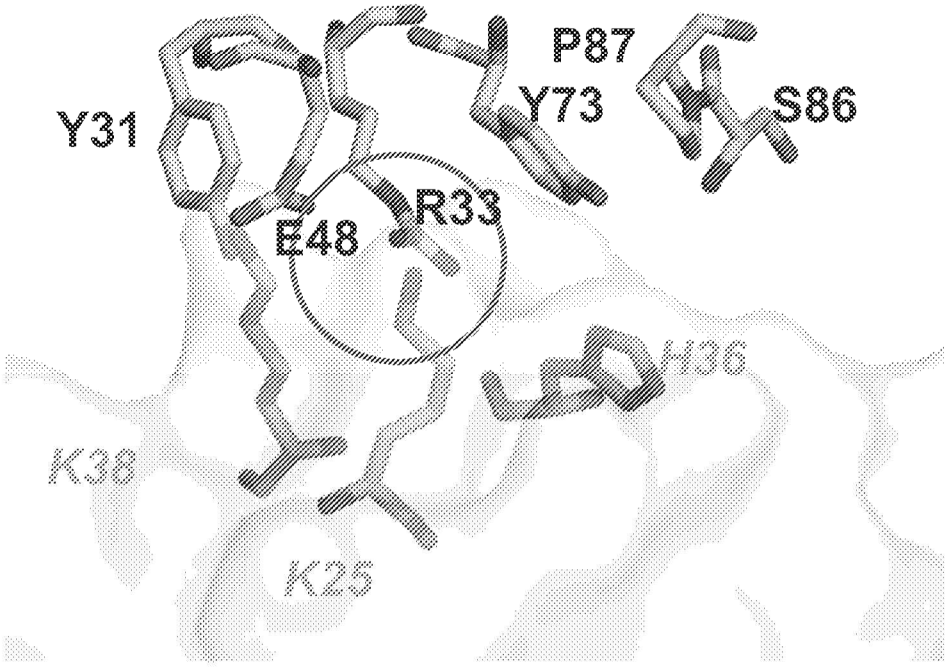

C
ySMB-1
ySUMO
hSUMO-1
bits
Scaffold
FGLoop
Y R Y Y Y Y Y D L Y Y S Y
31 33 73 75 76 78 79 80 81 82 83 84 85
ResidueNumber

Figure 12C



ySMB-1/ySUMO



ySMB-1/hSUMO1Model

Figure 13

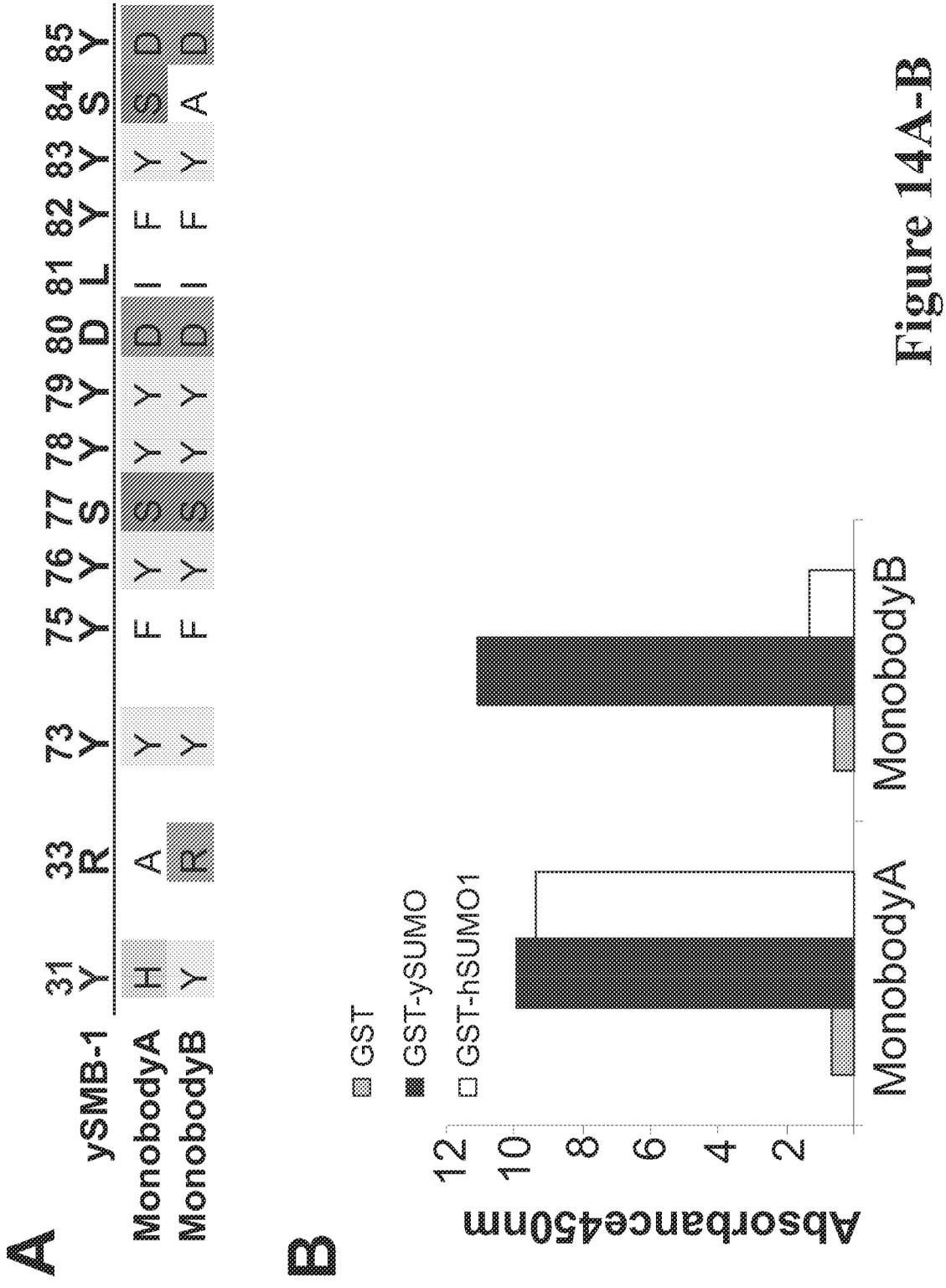

A
ySMB-1
31 33 73 75 76 77 78 79 80 81 82 83 84 85
Y R Y Y Y S Y Y D L Y Y S Y
MonobodyA H A Y F Y S Y Y D I F Y S D
MonobodyB Y R Y F Y S Y Y D I F Y A D
B
GST
GST-ySUMO
GST-hSUMO1
Absorbance450nm
12
10
8
6
4
2
MonobodyA
MonobodyB

Figure 14A-B

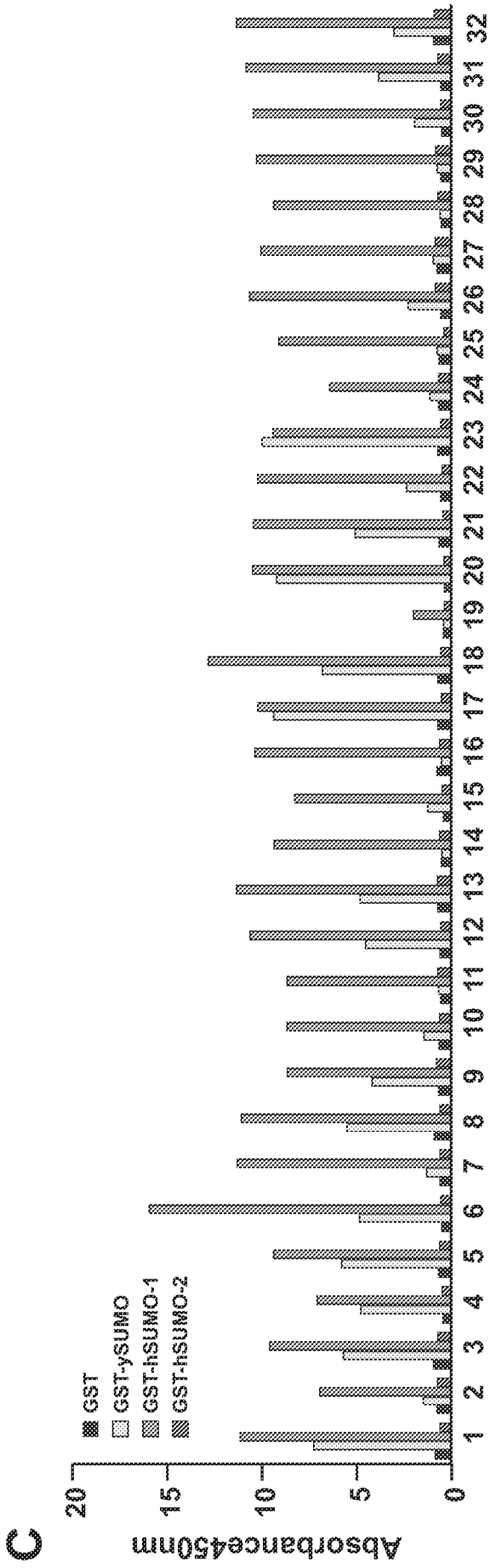

C
Absorbance450nm
0
5
10
15
20
GST
GST-ySUMO
GST-hSUMO-1
GST-hSUMO-2
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32

Figure 14C

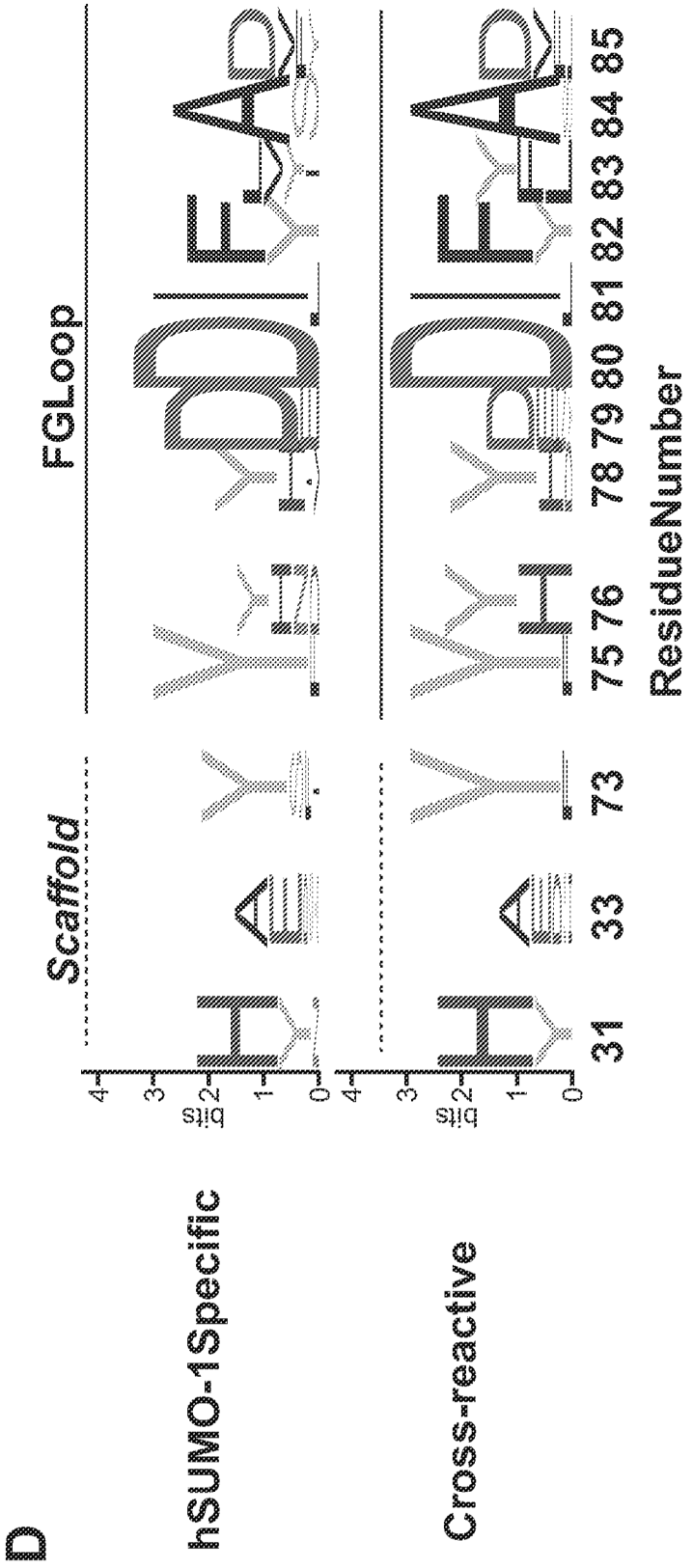

D
hSUMO-1Specific
Cross-reactive
bits
Scaffold
FGLoop
31 33 73 75 76 78 79 80 81 82 83 84 85
ResidueNumber

Figure 14D

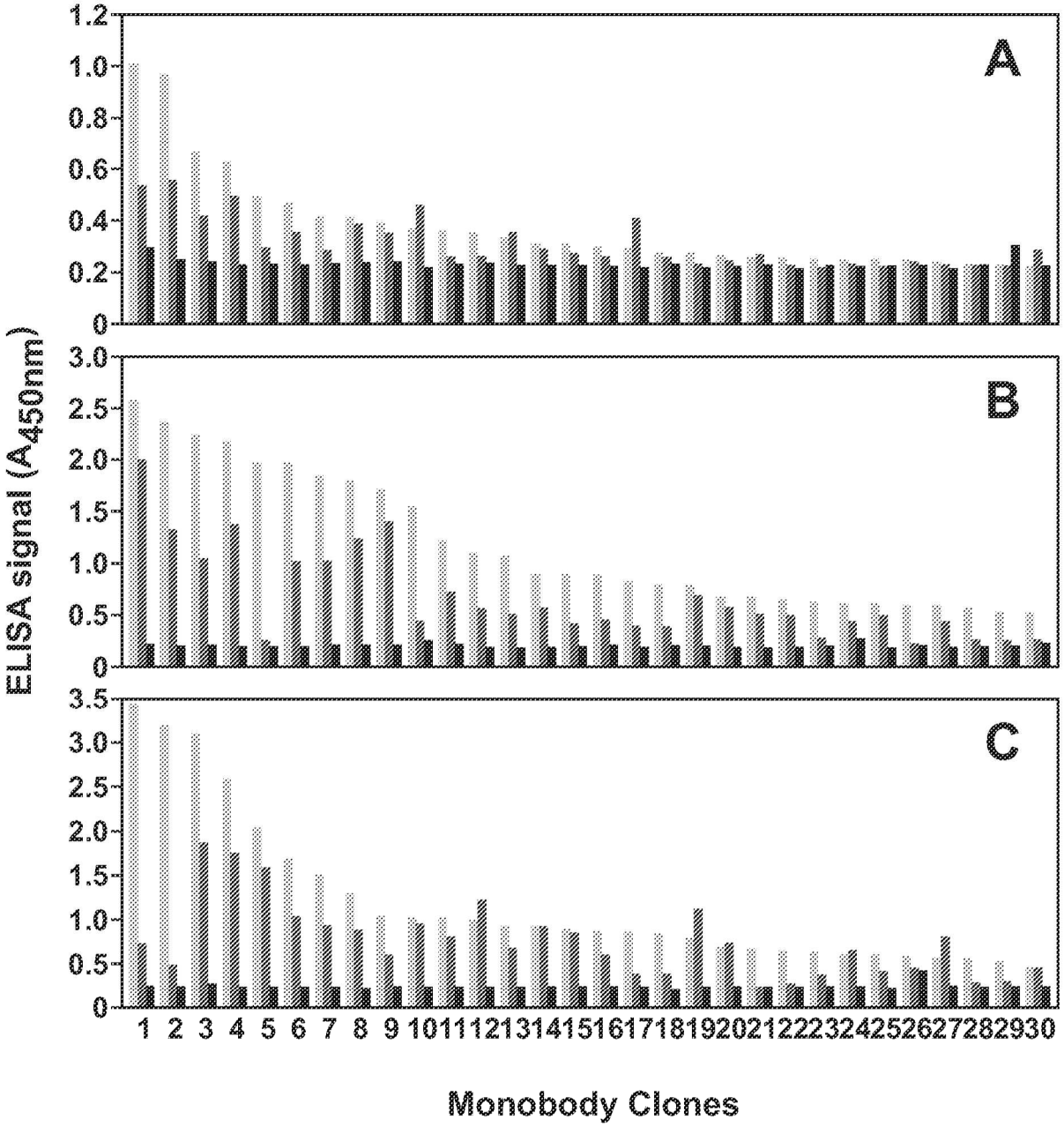
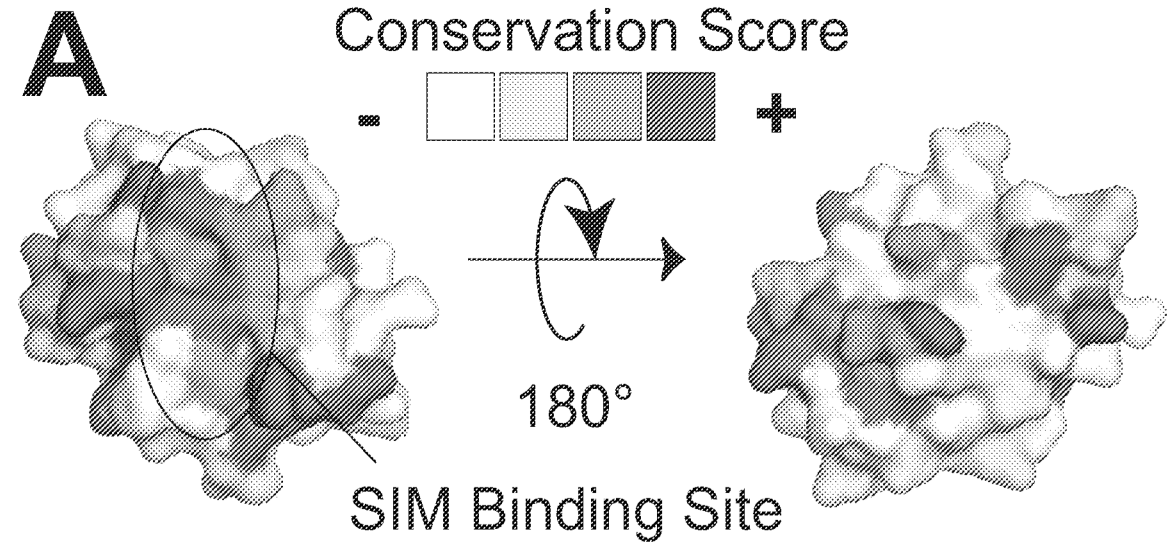


Figure 15A-C


A
Conservation Score
-
+
180°
SIM Binding Site

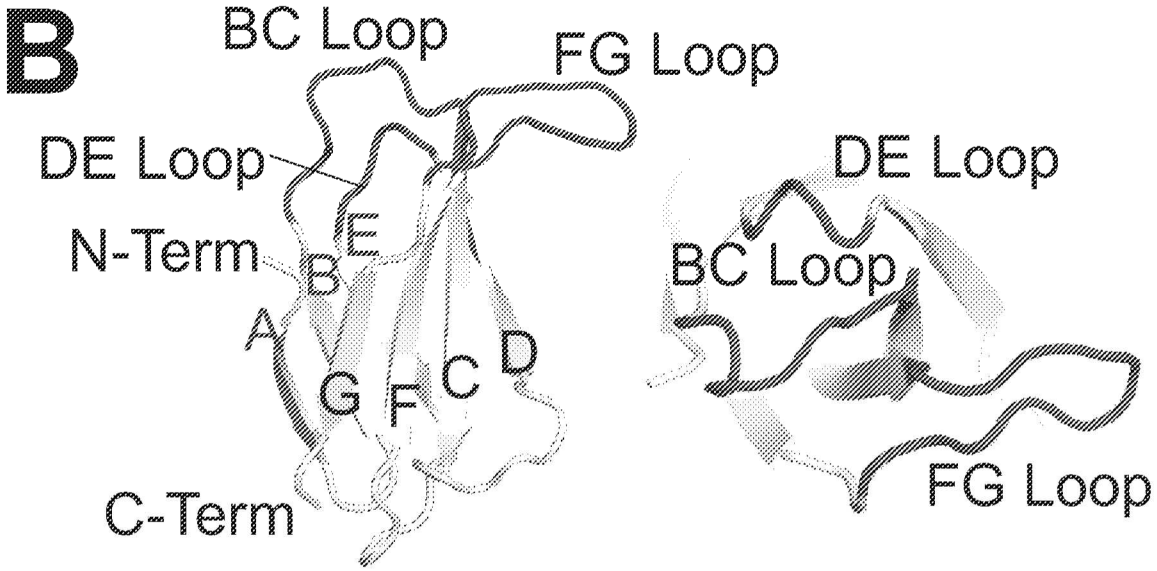

B
BC Loop
FG Loop
DE Loop
N-Term
E
B
A
G
F
C
D
C-Term
DE Loop
BC Loop
FG Loop

Figure 16A-B

C

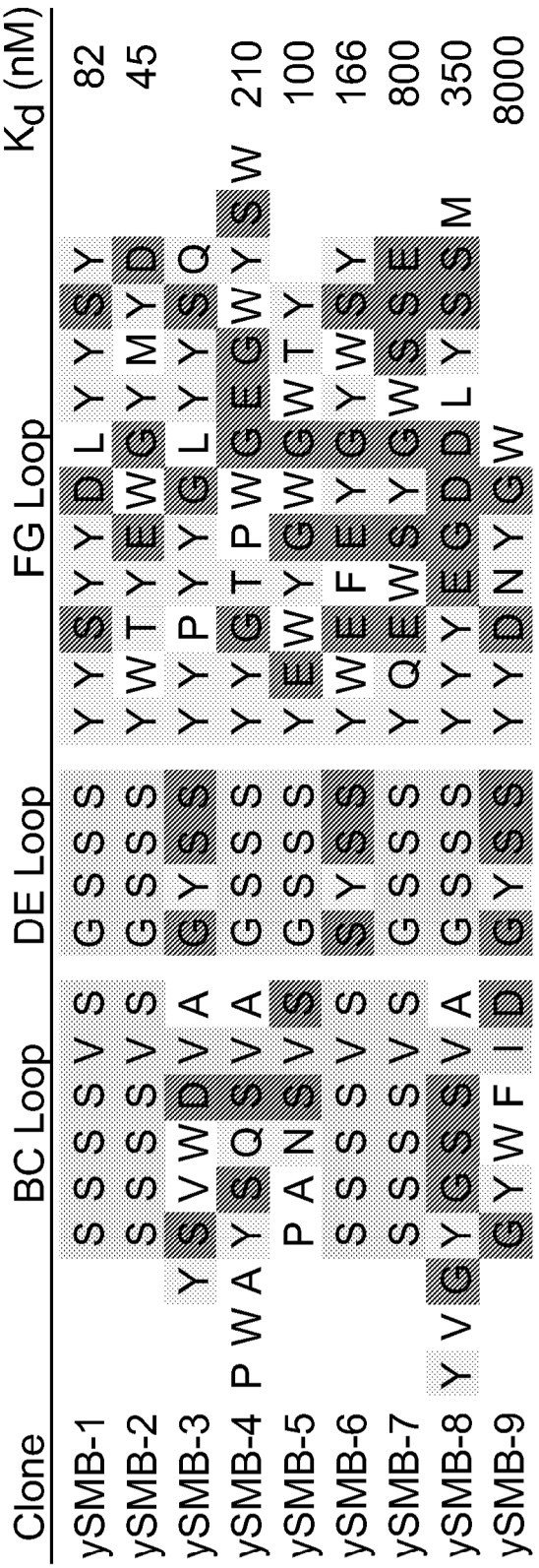

Clone
BC Loop
DE Loop
FG Loop
K_d (nM)
ySMB-1 S S S S V S G S S S S Y Y S Y Y D L Y Y S Y 82
ySMB-2 S S S S V S G S S S S Y W T Y E W G Y M Y D 45
ySMB-3 Y S V W D V A G Y S S S Y Y P Y Y G L Y Y S Q
ySMB-4 P W A Y S Q S V A G S S S Y Y G T P W G E G W Y S W 210
ySMB-5 P A N S V S G S S S Y E W Y G W G W T Y 100
ySMB-6 S S S S V S S Y S S S Y W E F E Y G Y W S Y 166
ySMB-7 S S S S V S G S S S S Y Q E W S Y G W S S E 800
ySMB-8 Y V G Y G S S V A G S S S S Y Y E G D D L Y S S M 350
ySMB-9 G Y W F I D G Y S S Y Y D N Y G W 8000

Figure 16C

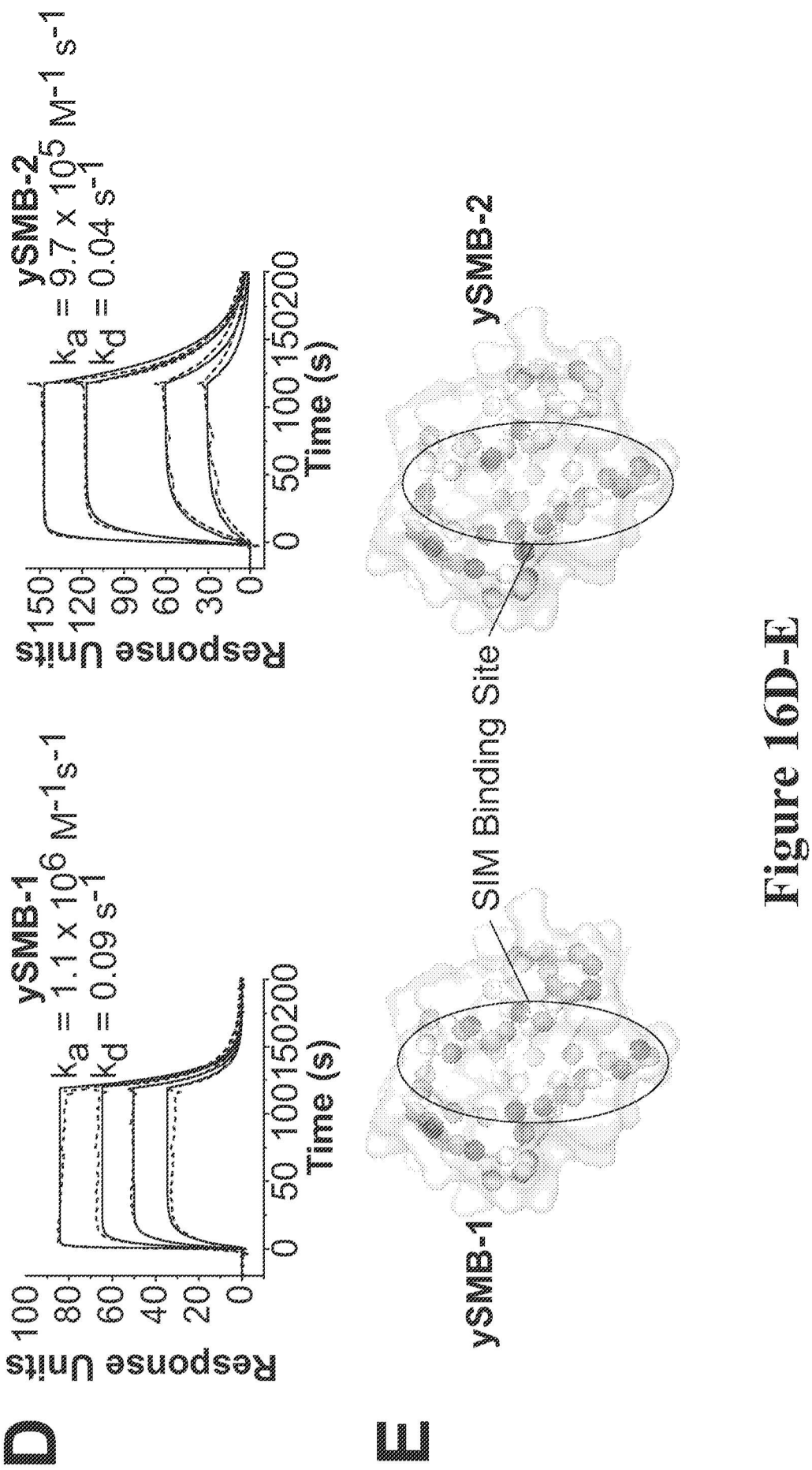

D
ySMB-1
ka = 1.1 x 10^6 M^-1s^-1
kd = 0.09 s^-1
Response Units
Time (s)
ySMB-2
ka = 9.7 x 10^5 M^-1 s^-1
kd = 0.04 s^-1
Response Units
Time (s)
E
ySMB-1
SIM Binding Site
ySMB-2

Figure 16D-E

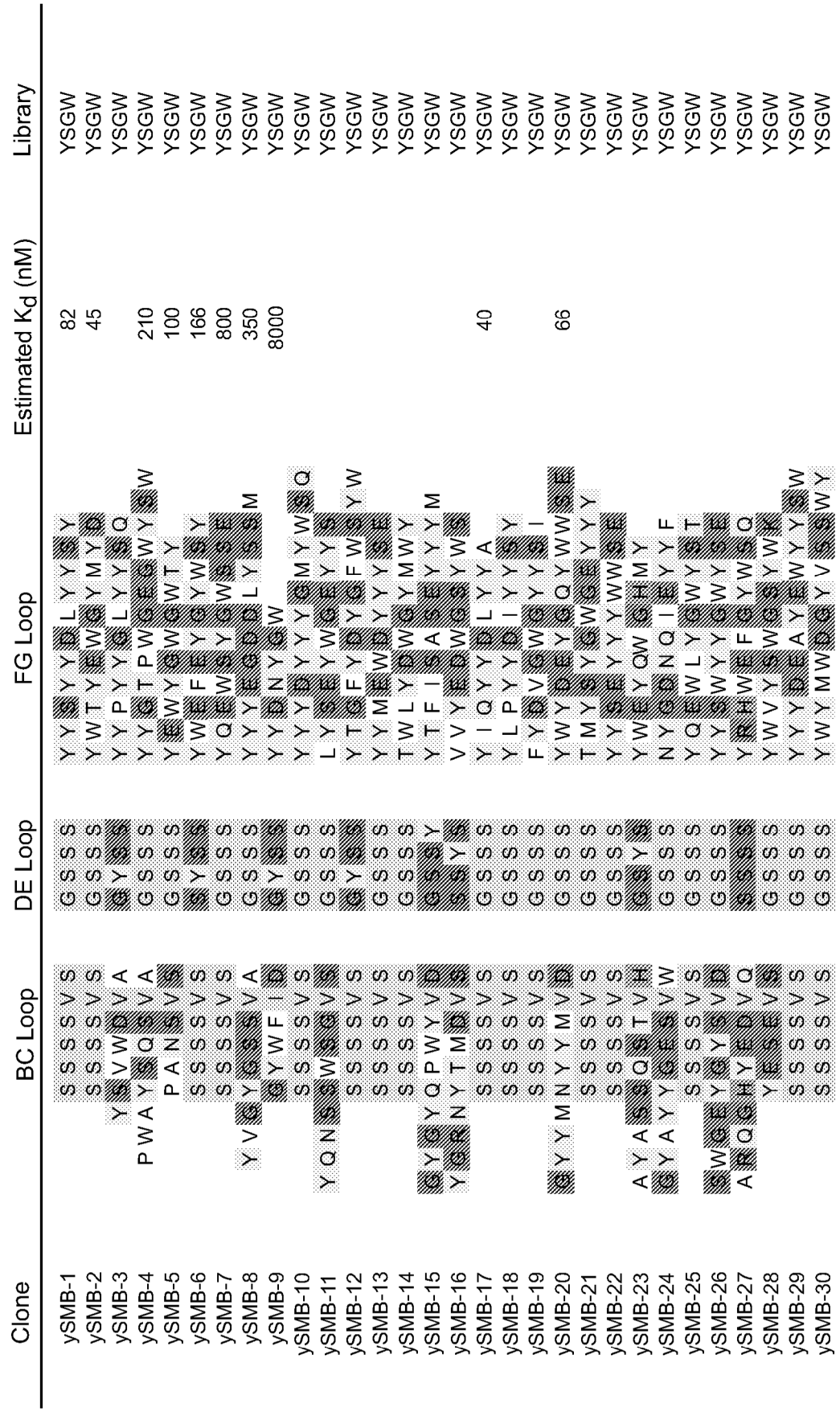

Clone
BC Loop
DE Loop
FG Loop
Estimated Kd (nM)
Library
ySMB-1 SSSSVS GSSS YYSYYDLYYSY 82 YSGW
ySMB-2 SSSSVS GSSS YWTYEWGYMYD 45 YSGW
ySMB-3 YSVWDVA GYES YYPYYGLYYSQ YSGW
ySMB-4 PWAYSQSVA GSSS YYQTPWDEGWYSW 210 YSGW
ySMB-5 PANSVS GSSS YEWYGWGWTY 100 YSGW
ySMB-6 SSSSVS GYES YYWEFEYGYWSY 166 YSGW
ySMB-7 SSSSVS GSSS YQEWYSWGGEE 800 YSGW
ySMB-8 YVGYGSSVA GSSS YYYEGDDLYSSM 350 YSGW
ySMB-9 GYWFID GYES YYDNYGW 8000 YSGW
ySMB-10 SSSSVS GSSS YYYDYYGMYWSQ YSGW
ySMB-11 YQNSWSGVS GSSS LYSEYWGEYYS YSGW
ySMB-12 SSSSVS GYES YTGFYDYGFWSYW YSGW
ySMB-13 SSSSVS GSSS YYMEWDYYYSE YSGW
ySMB-14 SSSSVS GSSS TWLYDWGYMWY YSGW
ySMB-15 GYGYQPWYVD GGSY YTFI99AEYYYM YSGW
ySMB-16 YGRNYTMDVE GGSY VVYEDWGSYWG YSGW
ySMB-17 SSSSVS GSSS YIQYYDLYYA 40 YSGW
ySMB-18 SSSSVS GSSS YLPYYDIYYSY YSGW
ySMB-19 SSSSVS GSSS FYDVGWGYYSI YSGW
ySMB-20 GYYMNYYMVD GSSS YWYDEYSQYWWSE 66 YSGW
ySMB-21 SSSSVS GSSS TMYSYGWGEYYY YSGW
ySMB-22 SSSSVS GSSS YYYSEYYWWSE YSGW
ySMB-23 AYASQSTVH GGYS YWEYQWGHMY YSGW
ySMB-24 GYAYYGESVW GSSS NYCDNQIEYYF YSGW
ySMB-25 SSSSVS GSSS YQEWLYGWYSTT YSGW
ySMB-26 SWGEYGYSVD GSSS YYSWYYGWYSE YSGW
ySMB-27 ARQGHYFDVQ SSES YRHWFEGYWSQ YSGW
ySMB-28 YEGEVS GSSS YWVYSWSSYWK YSGW
ySMB-29 SSSSVS GSSS YYYDEAYEWYYSW YSGW
ySMB-30 SSSSVS GSSS YWYMWDGYVSSWY YSGW

Figure 17

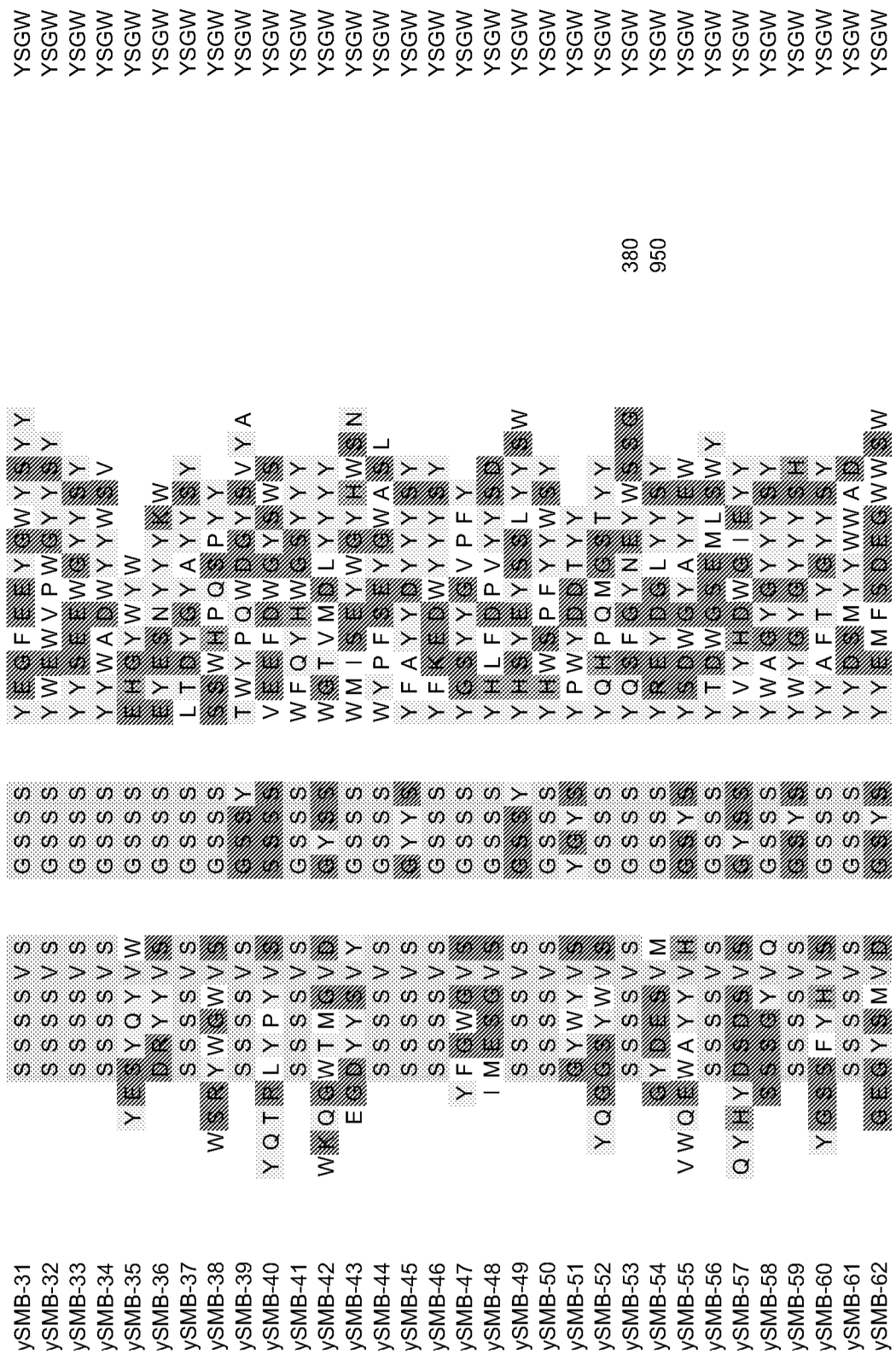


Figure 17
(Continued)

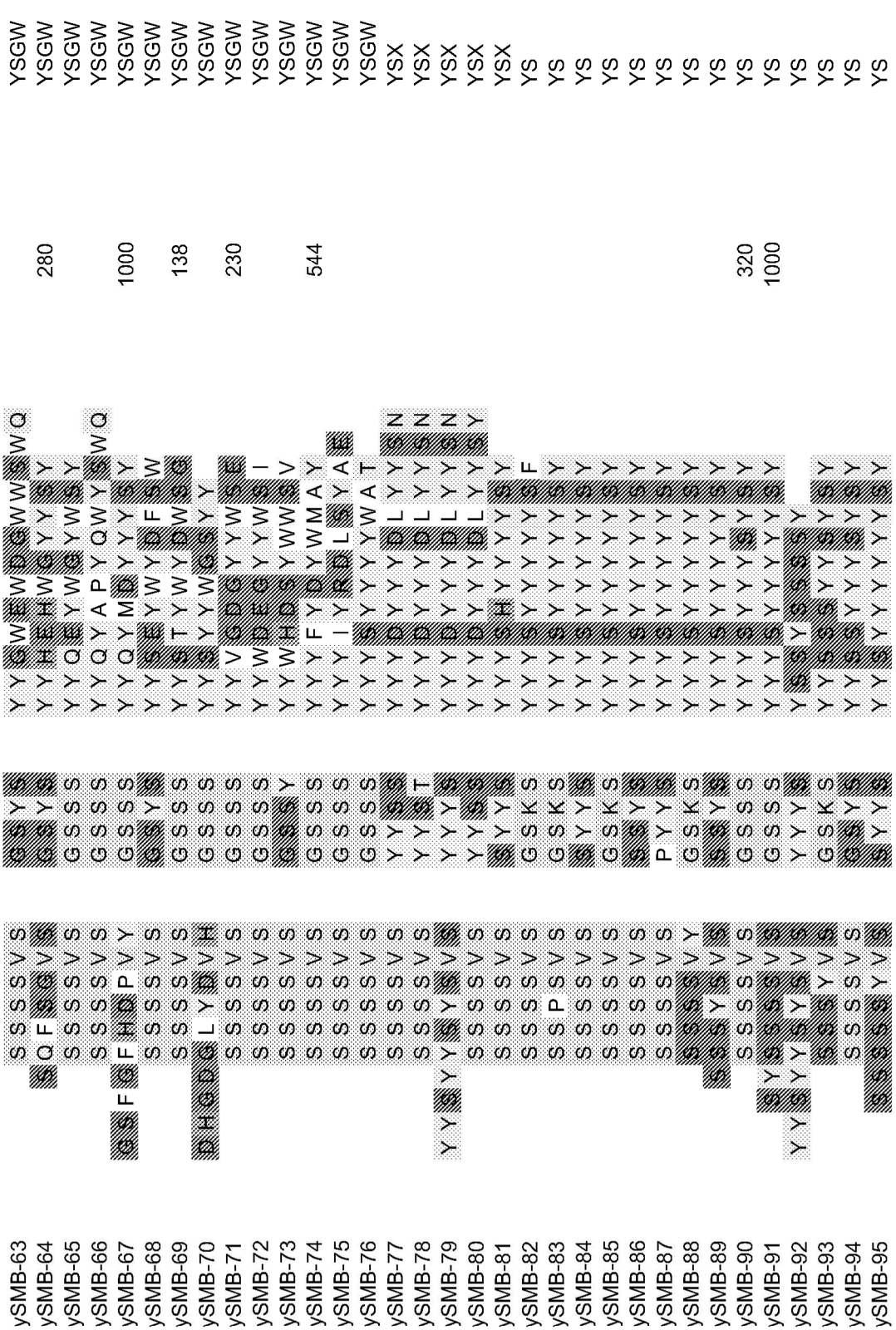


Figure 17
(Continued)

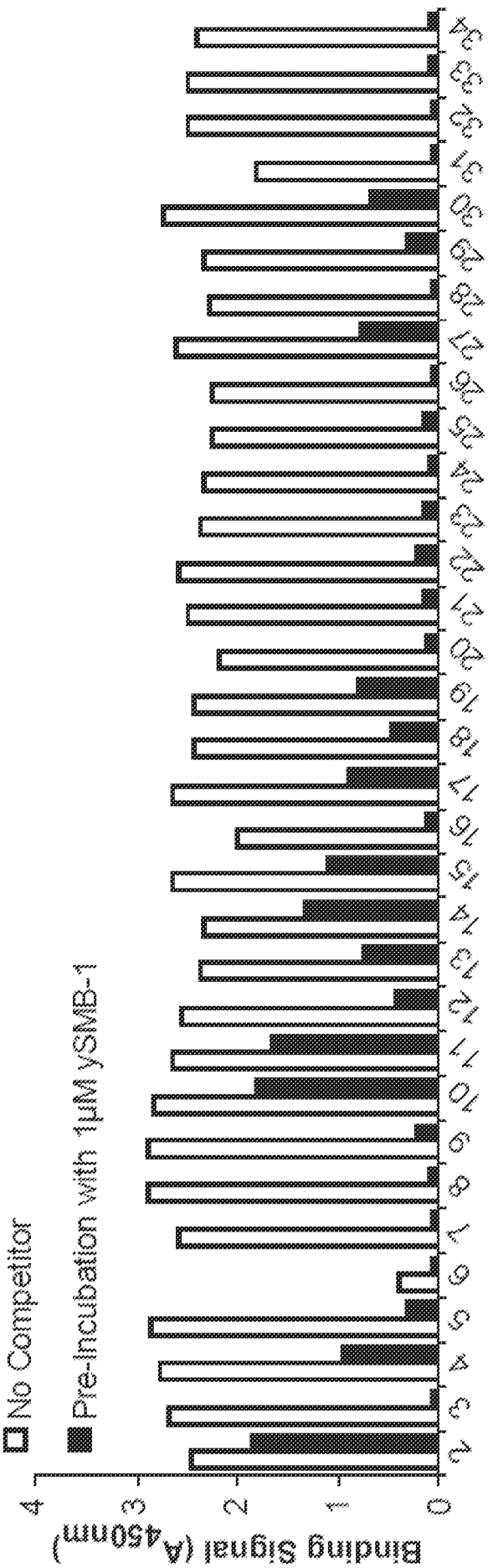

Binding Signal (A_{450nm})
0
1
2
3
4
No Competitor
Pre-Incubation with 1μM ySMB-1
ySMB Clone #

Figure 18

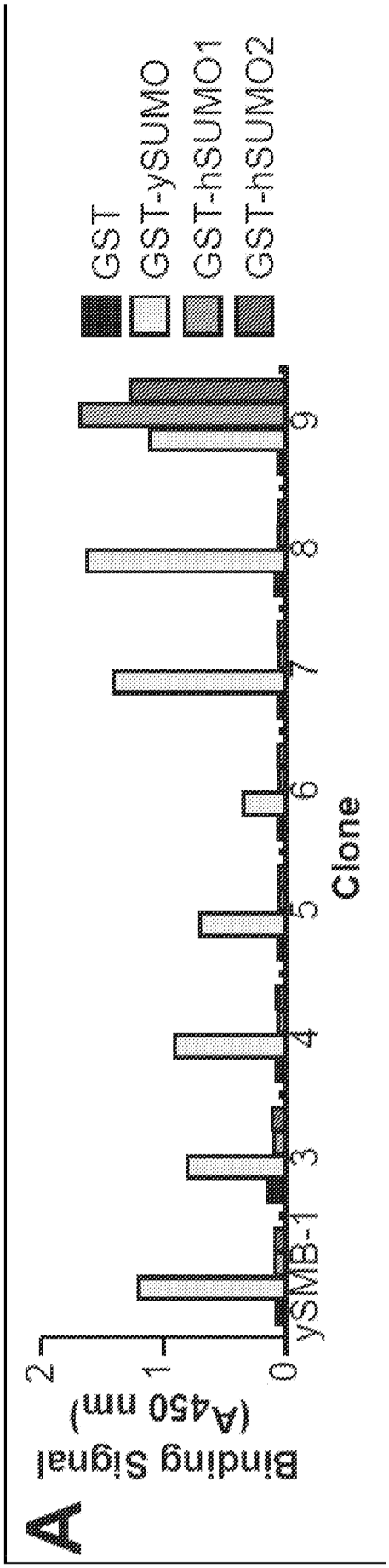

A
Binding Signal
(A450 nm)
2
1
0
ySMB-1
3
4
5
6
7
8
9
Clone
GST
GST-ySUMO
GST-hSUMO1
GST-hSUMO2

Figure 19A

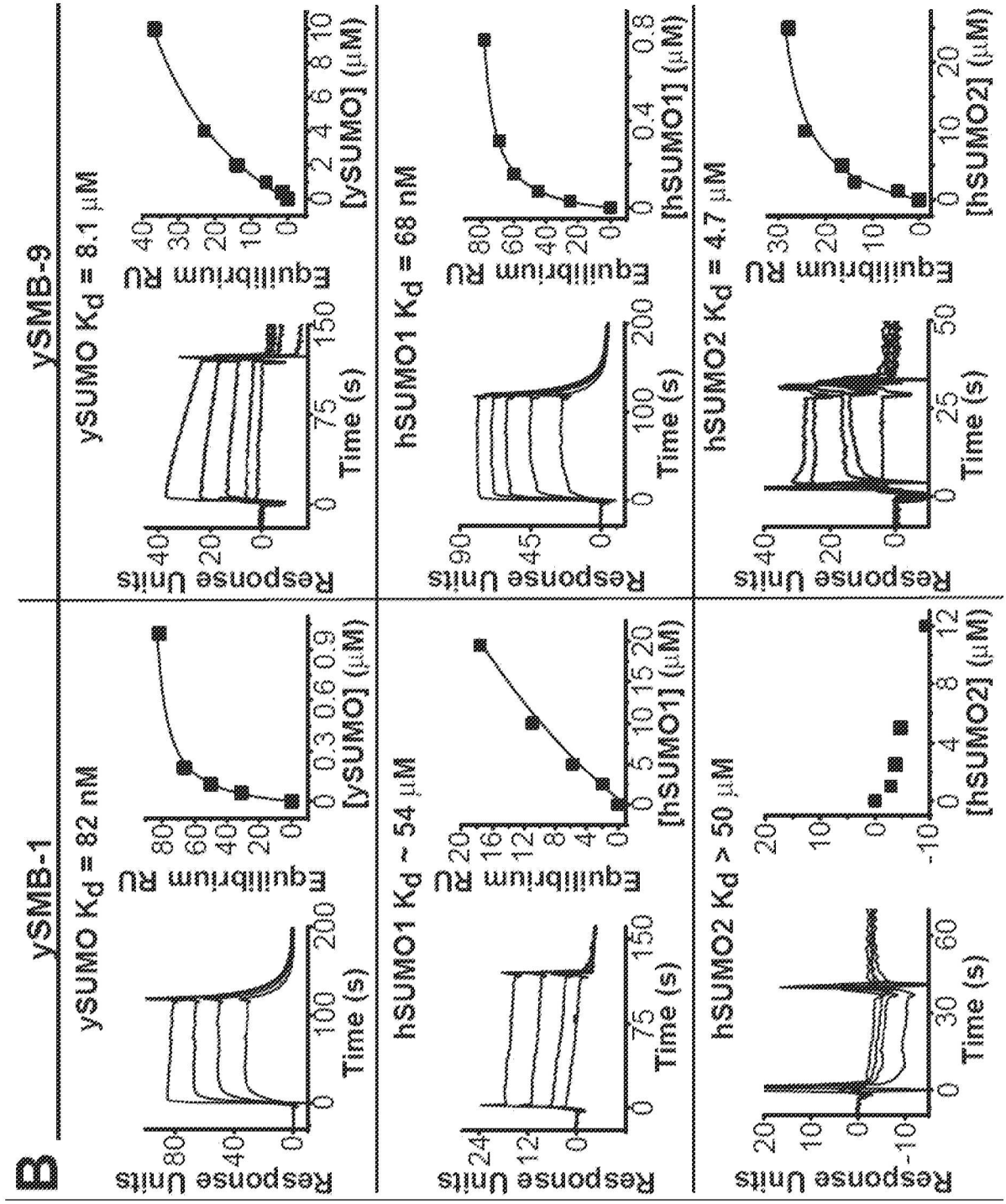

B
ySMB-1
ySMB-9
ySUMO KD = 82 nM
ySUMO KD = 8.1 μM
hSUMO1 KD ~ 54 μM
hSUMO1 KD = 68 nM
hSUMO2 KD > 50 μM
hSUMO2 KD = 4.7 μM
Response Units
Time (s)
Equilibrium RU
[ySUMO] (μM)
[hSUMO1] (μM)
[hSUMO2] (μM)

Figure 19B

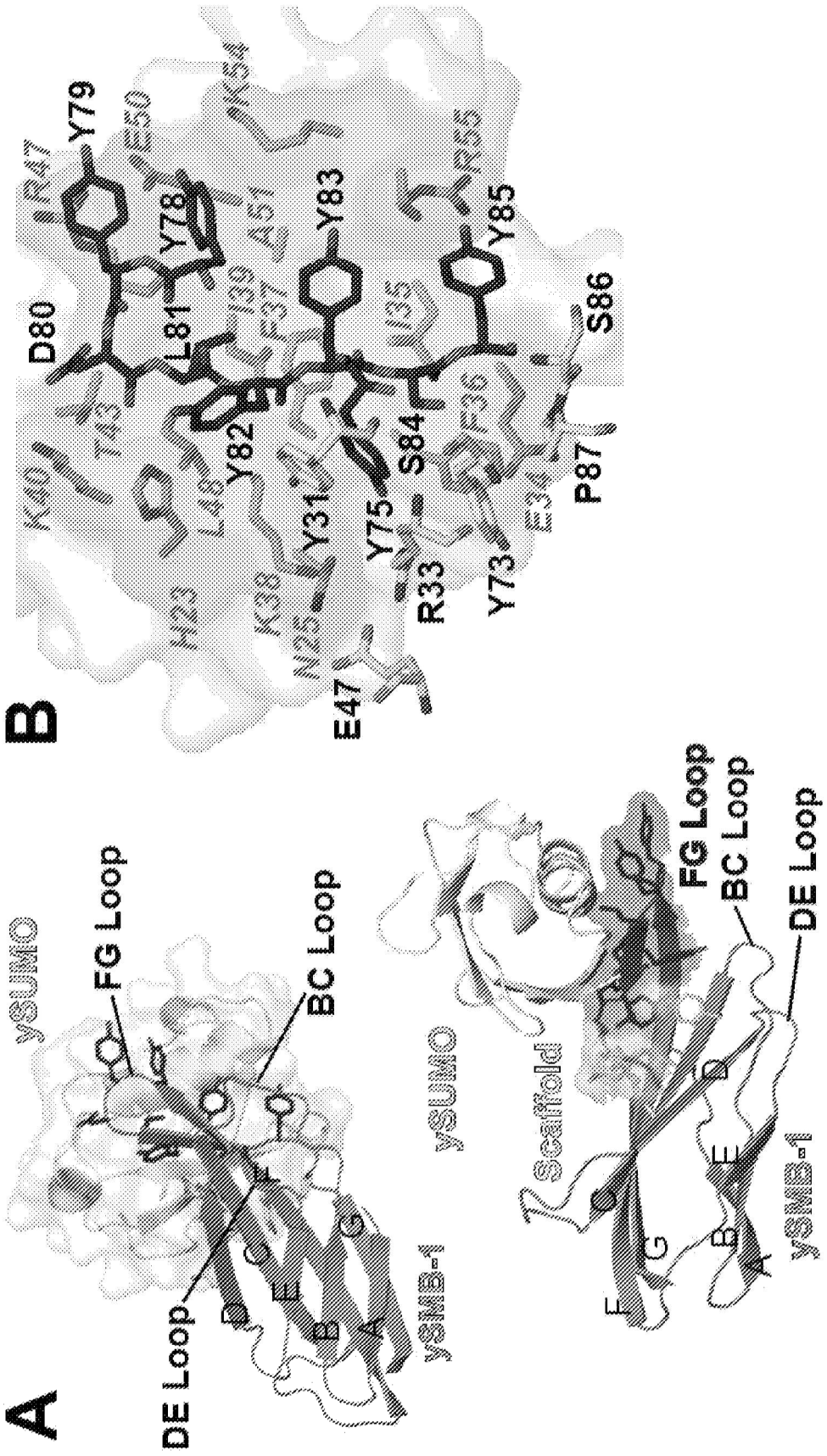

A
ySUMO
FG Loop
BC Loop
DE Loop
ySMB-1
ySUMO
Scaffold
FG Loop
BC Loop
DE Loop
ySMB-1
B
K40
T43
H23
L48
Y82
K38
N25
Y31
E47
Y75
S84
R33
Y73
E34
P87
S86
D80
L81
Y78
Y79
R47
E50
K54
A51
F37
Y83
I85
A55
Y85
F36

Figure 20A-B

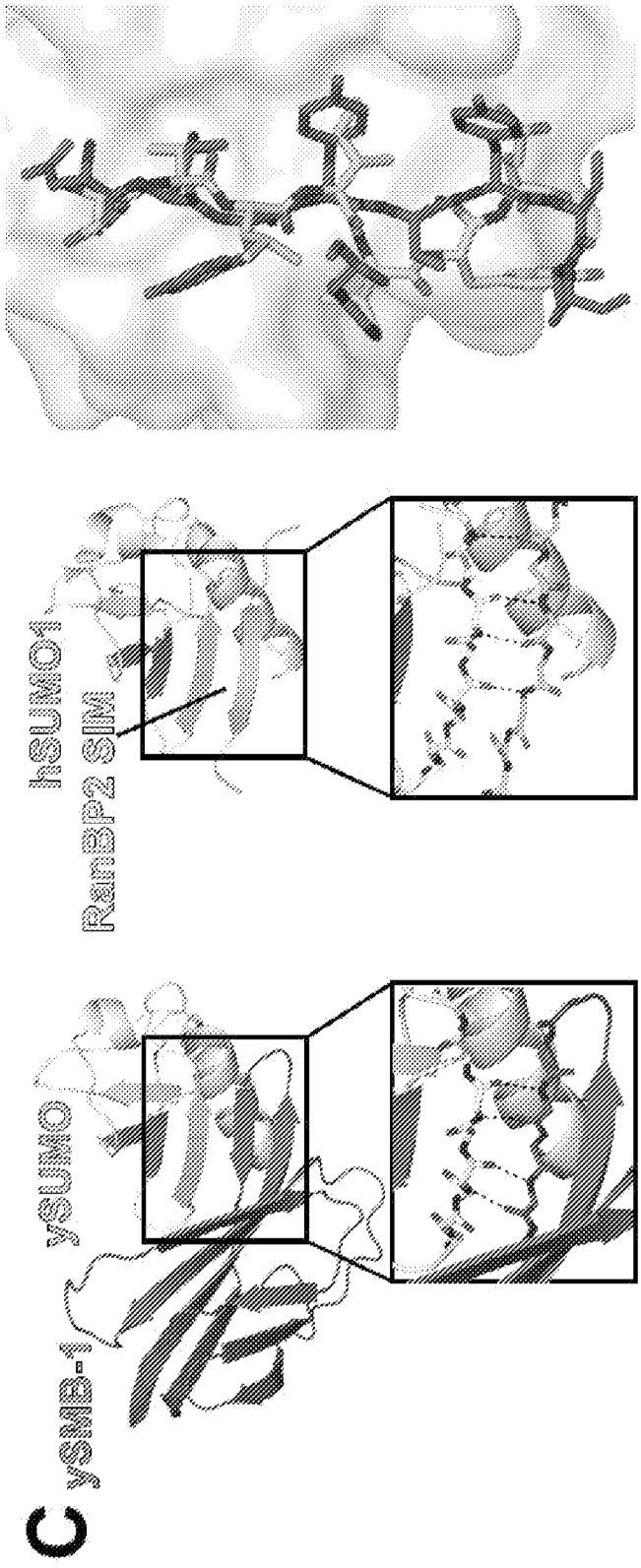


Figure 20C

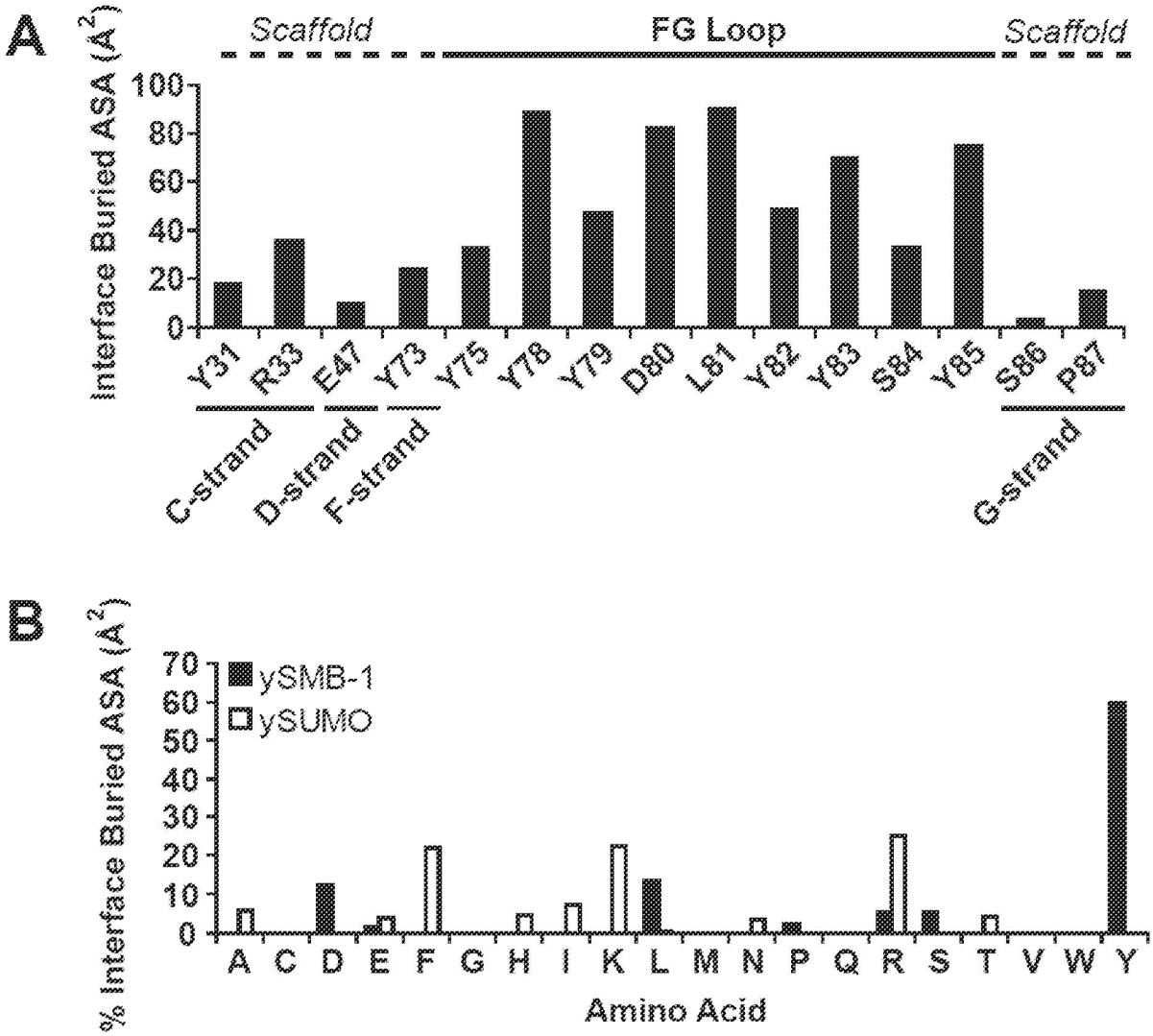

A
Scaffold
FG Loop
Scaffold
Interface Buried ASA (Å^2)
100
80
60
40
20
0
Y31
R33
E47
Y73
Y75
Y78
Y79
D80
L81
Y82
Y83
S84
Y85
S86
P87
C-strand
D-strand
F-strand
G-strand
B
% Interface Buried ASA (Å^2)
70
60
50
40
30
20
10
0
ySMB-1
ySUMO
A C D E F G H I K L M N P Q R S T V W Y
Amino Acid

Figure 21A-B

A
K40 (K,N)
T43 (T,T)
R47 (K,K)
E50 (E,K)
K54 (Q,E)
H29 (H,H)
L48 (L,L)
K38 (K,K)
I30 (V,I)
F37 (F,F)
A31 (S,A)
N25 (N,N)
I35 (I,I)
33
R55 (R,R)
F36 (H,Q) 73
E34 (E,V)
Position Diversity
31 [Y] DHNY
33 [R] DEGHKNQRSATP
73 [Y] DFHILNVYSPTA
75 [Y] DFHILNVY
76 [Y] DHNY
78 [Y] DHNYLIMVPTAQKERSG
79 [Y] DHNYQKE
80 [D] DFHILNVYSPTA
81 [L] FLIV
82 [Y] DFHILNVYSPTA
83 [Y] DFHILNVY
84 [S] SA
85 [Y] DFHILNVY
B
Clone # Scaffold FG Loop Kd
31 33 73 75 76 78 79 80 81 82 83 84 85
vSMB-1 Y R Y Y Y Y Y D L Y Y S Y ~54 μM
hS1MB-1 H H Y F Y D D L Y F A F 652 nM
hS1MB-2 H Q Y Y H Y D D I Y Y A L 290 nM
hS1MB-3 H A Y Y H Y D D I F L A D 519 nM
hS1MB-4 H E Y Y Y H E D I F Y A V 118 nM
hS1MB-5 H E A Y H Y H D I F Y A V 284 nM
hS1MB-6 H E T Y D Y Y D I Y I A Y 2.8 μM
hS1MB-7 Y E I F Y H D D I Y I S D 2.5 μM
hS1MB-8 H A Y Y Y Y D D L Y V S G 3.6 μM
hS1MB-9 H A F Y Y Y D D I Y Y A Y 738 nM
hS1MB-10 H D H Y Y Y D D I Y V A I 2.2 μM
hS1MB-22 H A Y F Y Y Y D I F Y S D
vSMB-ST6 Y R Y F Y Y Y D I F Y A D
hS1MB-2
hS1MB-4
hS1MB-5
RU
Time (s)

Figure 22A-B

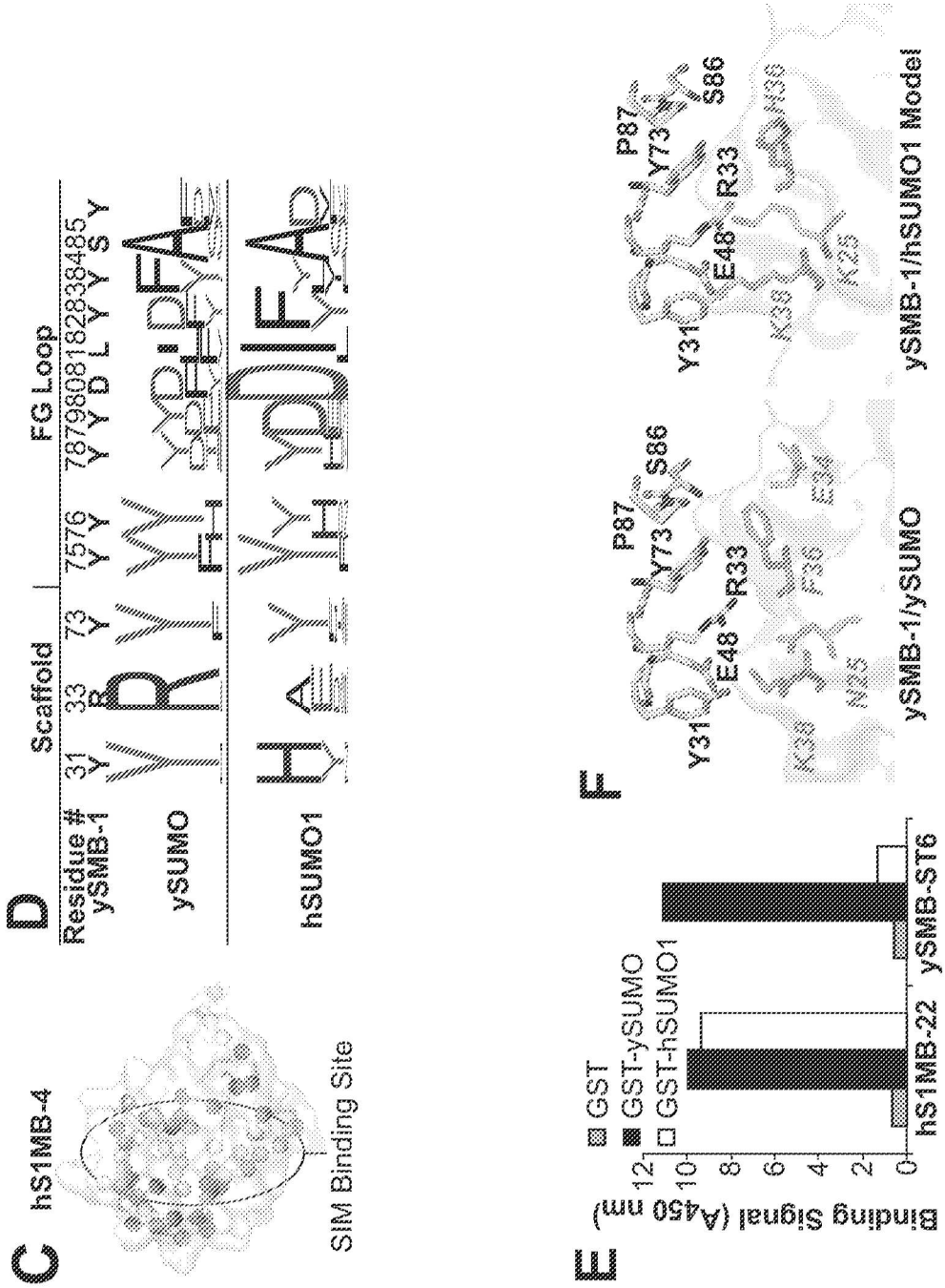

C
hS1MB-4
SIM Binding Site
D
Scaffold
FG Loop
Residue #
ySMB-1
ySUMO
hSUMO1
E
GST
GST-ySUMO
GST-hSUMO1
Binding Signal (A450 nm)
hS1MB-22
ySMB-ST6
F
ySMB-1/ySUMO
ySMB-1/hSUMO1 Model
Y31
E48
R33
P87
Y73
S86
K39
K25
F36
E34
K30

Figure 22C-F

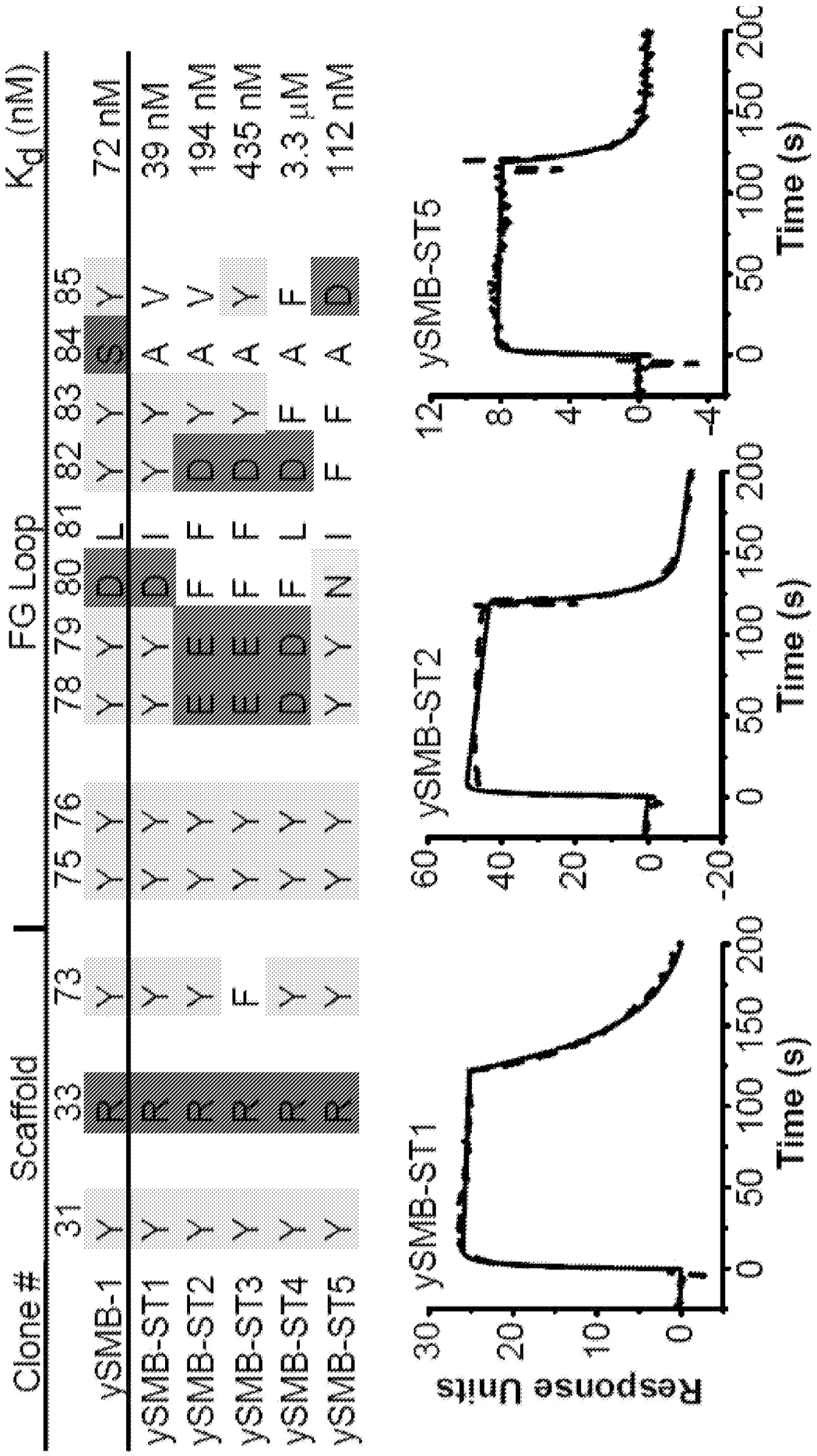

Clone #
Scaffold
FG Loop
K_d (nM)
31 33 73 75 76 78 79 80 81 82 83 84 85
ySMB-1 Y αC Y Y Y Y Y D L Y Y S Y
ySMB-ST1 Y αC Y Y Y Y Y D I Y Y A V 72 nM
ySMB-ST2 Y αC Y Y Y E E F F D Y A V 39 nM
ySMB-ST3 Y αC F Y Y E E F F D Y A Y 194 nM
ySMB-ST4 Y αC Y Y Y D Q F L D F A F 435 nM
ySMB-ST5 Y αC Y Y Y Q Y N I F F A D 3.3 μM 112 nM
Response Units
ySMB-ST1
ySMB-ST2
ySMB-ST5
Time (s)

Figure 23

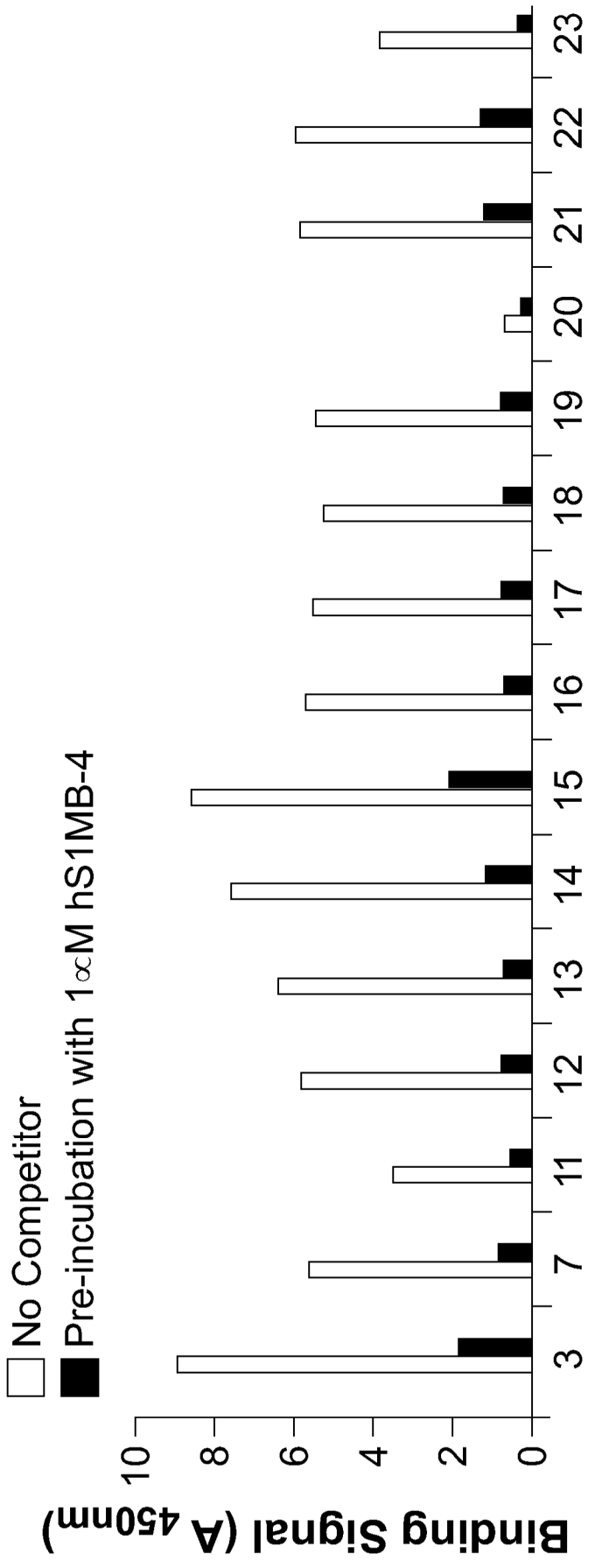

No Competitor
Pre-incubation with 1∝M hS1MB-4
Binding Signal (A 450nm)
0
2
4
6
8
10
3
7
11
12
13
14
15
16
17
18
19
20
21
22
23
hS1MB Clone #

Figure 24

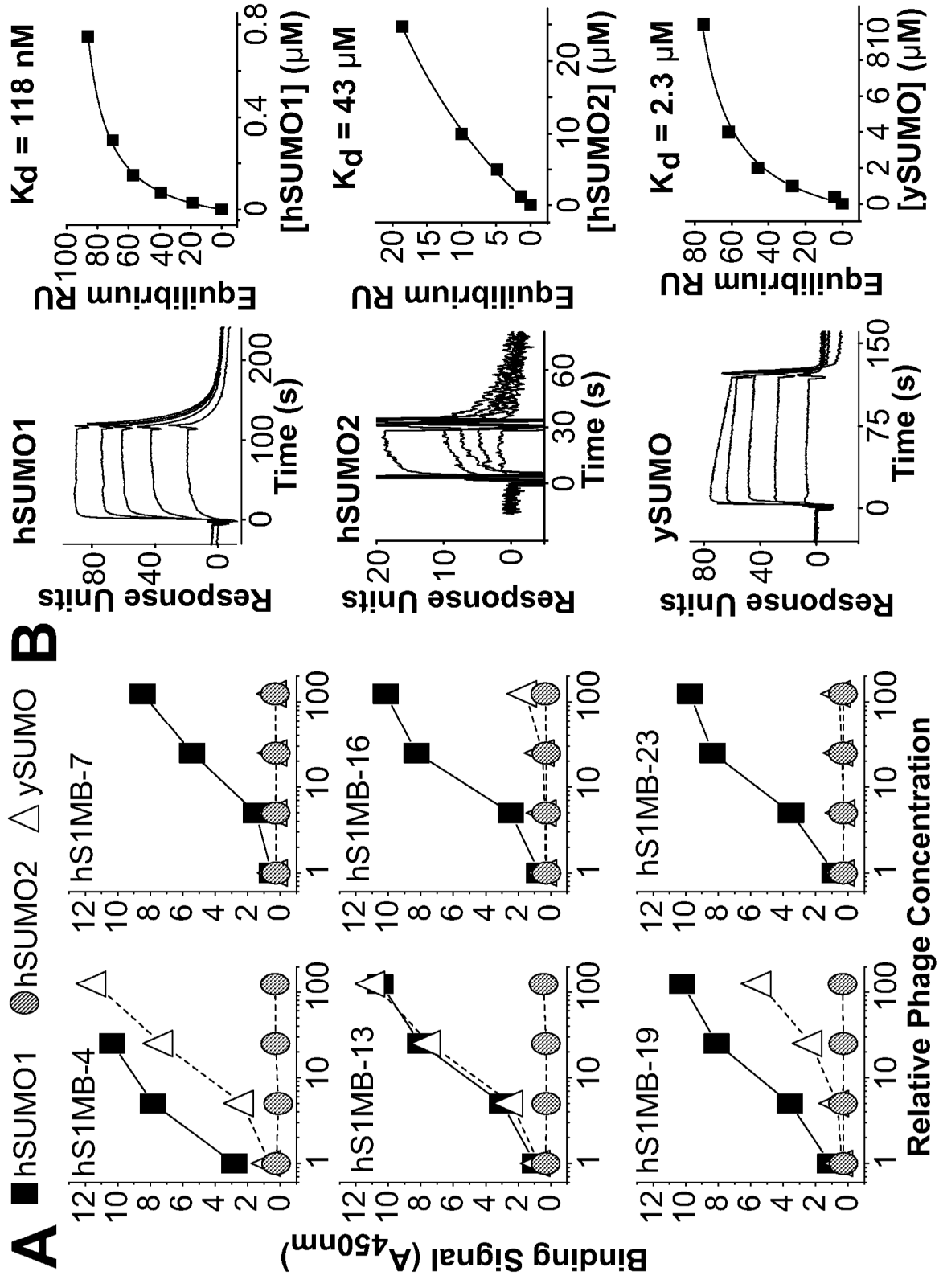

A
hSUMO1
hSUMO2
ySUMO
B
hS1MB-4
hS1MB-7
hS1MB-13
hS1MB-16
hS1MB-19
hS1MB-23
Binding Signal (A450nm)
Relative Phage Concentration
hSUMO1
hSUMO2
ySUMO
Response Units
Time (s)
Equilibrium RU
[hSUMO1] (μM)
[hSUMO2] (μM)
[ySUMO] (μM)
Kd = 118 nM
Kd = 43 μM
Kd = 2.3 μM

Figure 25A-B

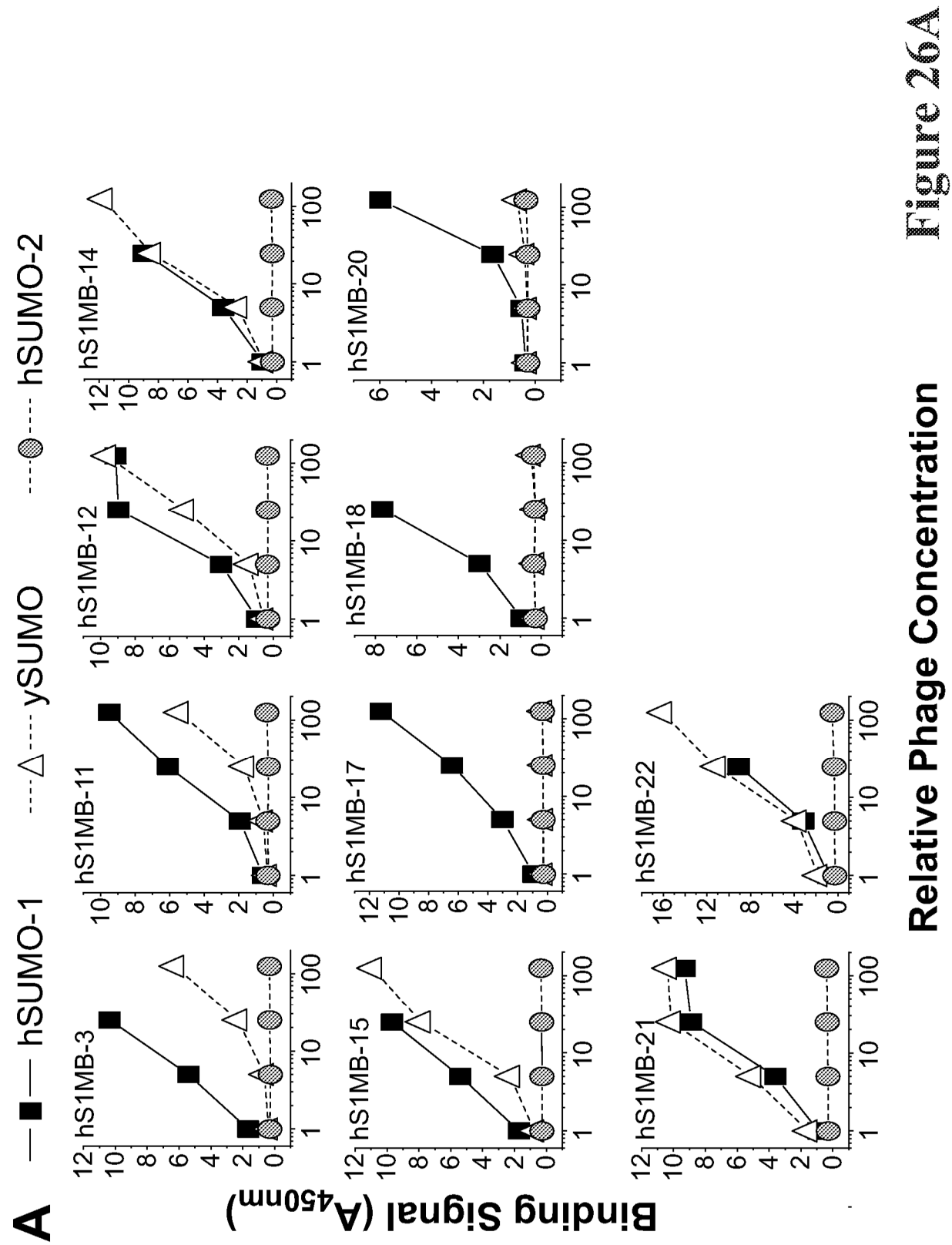

A
hSUMO-1
ySUMO
hSUMO-2
hS1MB-3
hS1MB-11
hS1MB-12
hS1MB-14
hS1MB-15
hS1MB-17
hS1MB-18
hS1MB-20
hS1MB-21
hS1MB-22
Binding Signal (A450nm)
Relative Phage Concentration

Figure 26A

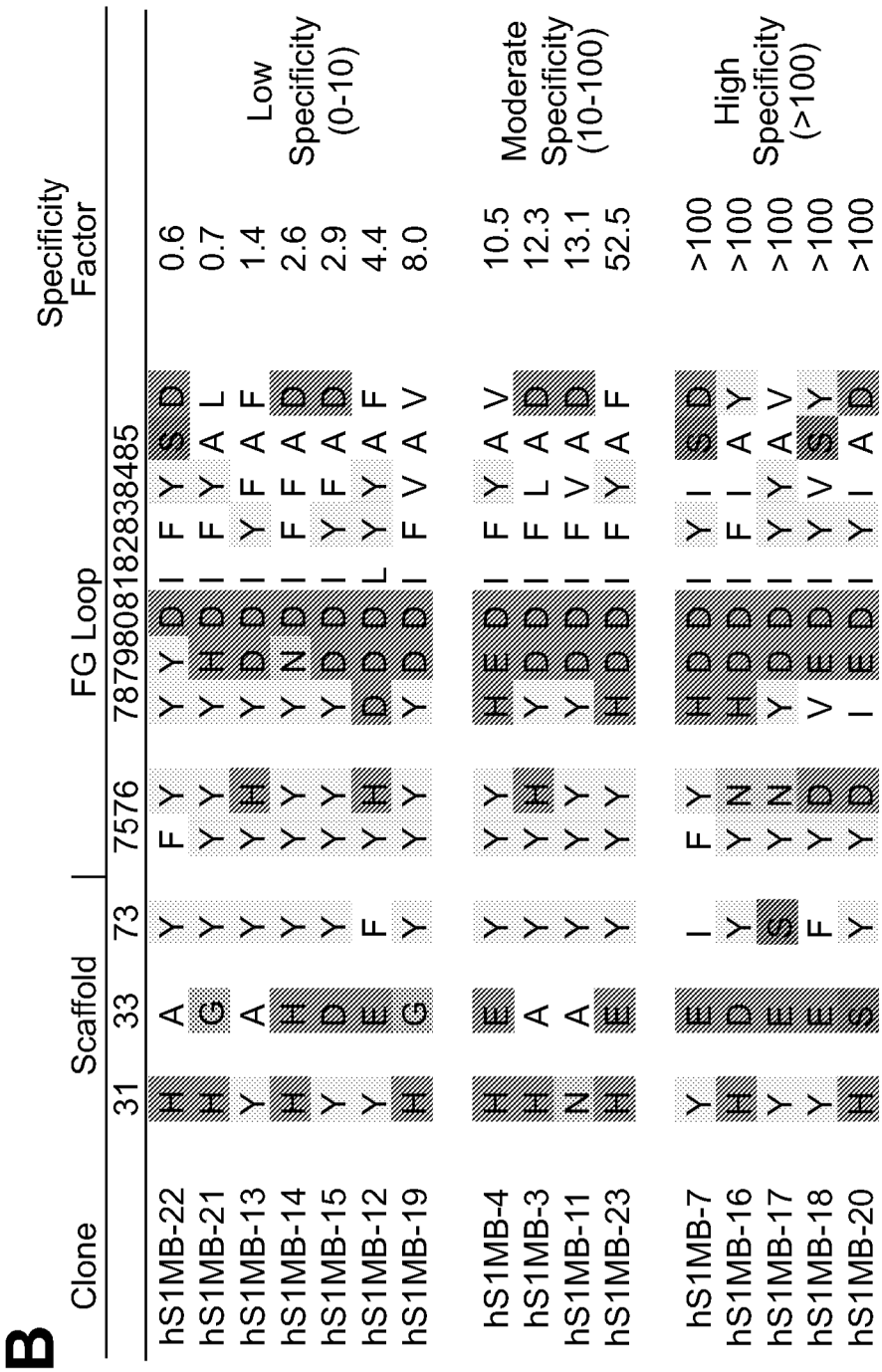

B
Clone
Scaffold
FG Loop
Specificity Factor
31 33 73 7576 787980818283848 5
hS1MB-22 H A Y F Y Y Y D I F Y S D 0.6
hS1MB-21 H G Y Y Y Y H D I F Y A L 0.7
hS1MB-13 Y A Y Y H Y D D I Y F A F 1.4
hS1MB-14 H H Y Y Y Y N D I F F A D 2.6
hS1MB-15 Y D Y Y Y Y D D I Y F A D 2.9
hS1MB-12 Y E F Y H D D D L Y Y A F 4.4
hS1MB-19 H G Y Y Y Y D D I F V A V 8.0
hS1MB-4 H E Y Y Y H E D I F Y A V 10.5
hS1MB-3 H A Y Y H Y D D I F L A D 12.3
hS1MB-11 N A Y Y Y Y D D I F V A D 13.1
hS1MB-23 H E Y Y Y H D D I F Y A F 52.5
hS1MB-7 Y E I F Y H D D I Y I S D >100
hS1MB-16 H D Y Y N H D D I F I A Y >100
hS1MB-17 Y D S Y N Y D D I Y Y A V >100
hS1MB-18 Y E F Y D V E D I Y V S Y >100
hS1MB-20 H S Y Y D I E D I Y I A D >100
Low Specificity (0-10)
Moderate Specificity (10-100)
High Specificity (>100)

Figure 26B

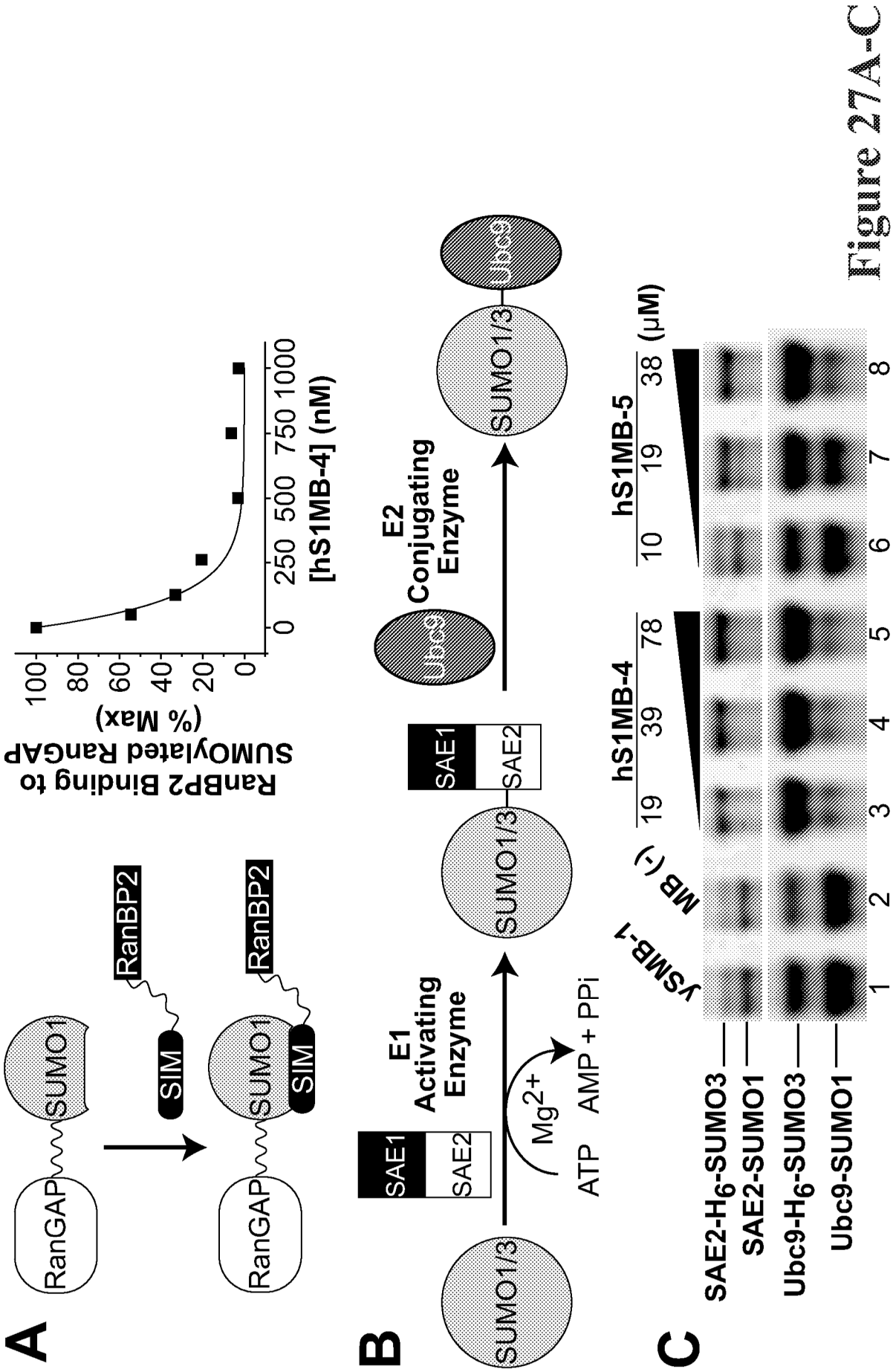

A
RanGAP
SUMO1
RanBP2
SIM
RanGAP
SUMO1
RanBP2
SIM
RanBP2 Binding to SUMOylated RanGAP
(% Max)
100
80
60
40
20
0
0
250
500
750
1000
[hS1MB-4] (nM)
B
SUMO1/3
SAE1
SAE2
E1 Activating Enzyme
Mg2+
ATP
AMP + PPi
SUMO1/3
SAE1
SAE2
E2 Conjugating Enzyme
Ubc9
SUMO1/3
Ubc9
C
ySMB-1
MB (-)
hS1MB-4
19
39
78
hS1MB-5
10
19
38 (μM)
1
2
3
4
5
6
7
8
SAE2-H6-SUMO3
SAE2-SUMO1
Ubc9-H6-SUMO3
Ubc9-SUMO1

Figure 27A-C

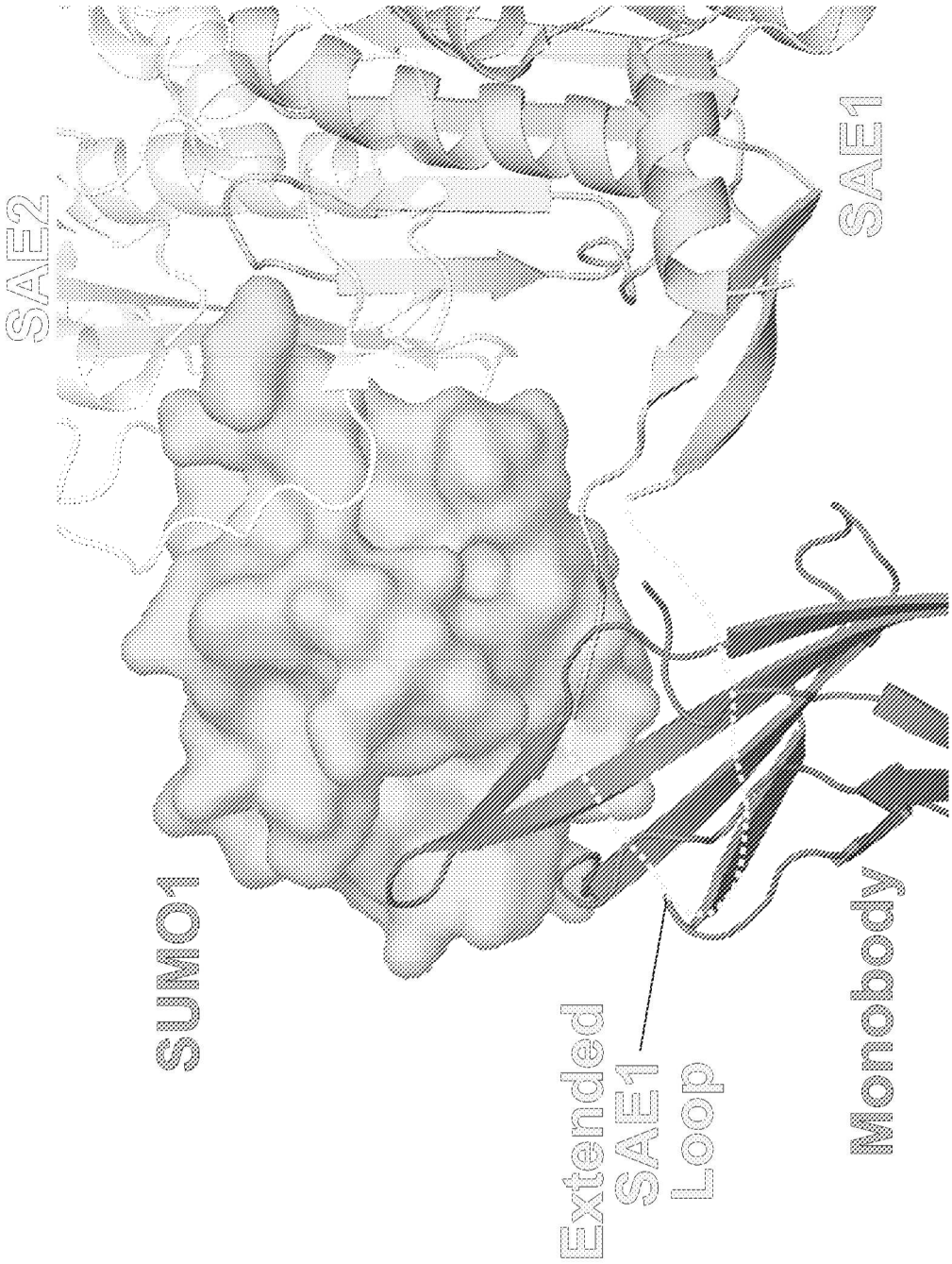


Figure 28

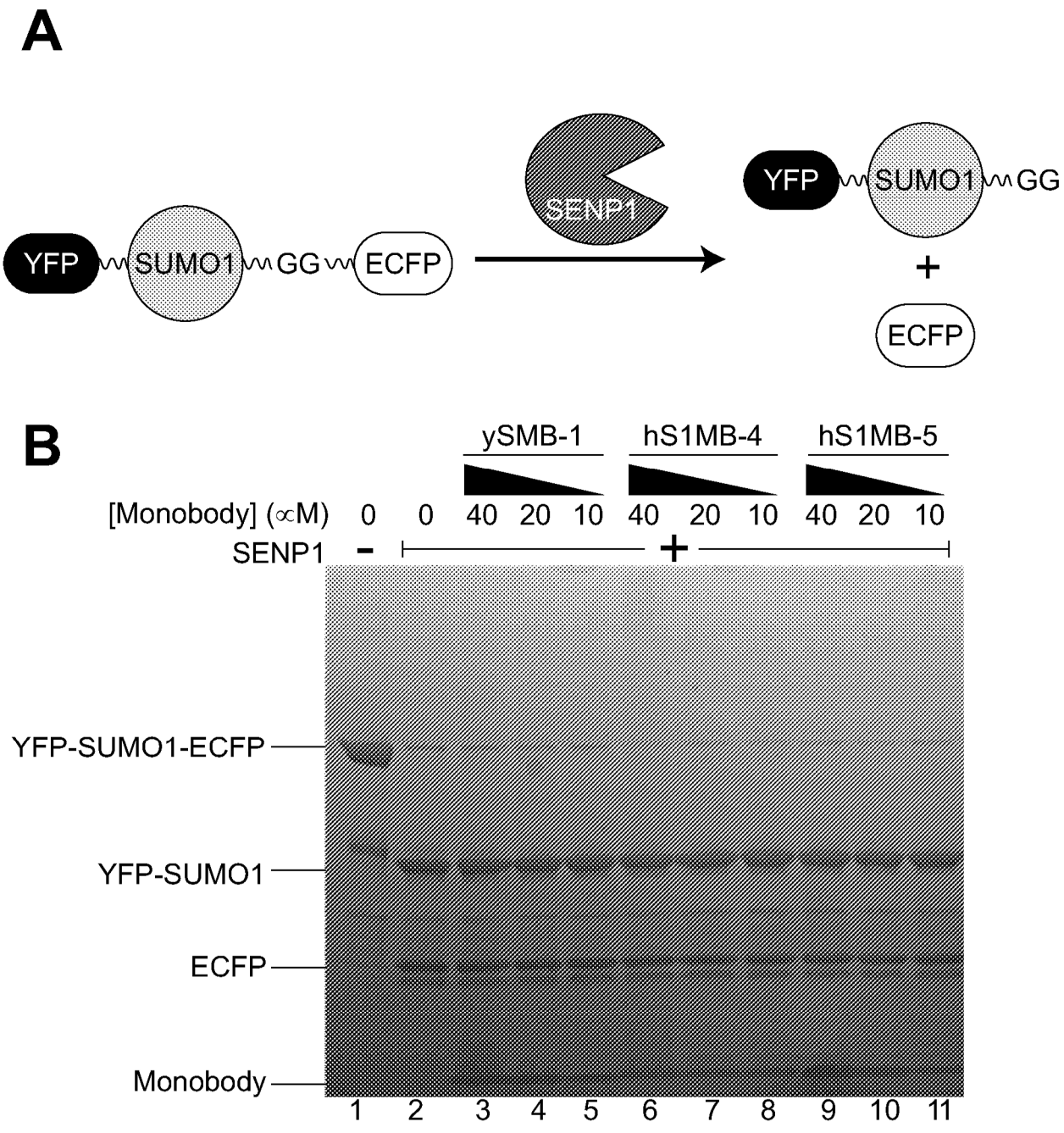

A
YFP
SUMO1
GG
ECFP
SENP1
YFP
SUMO1
GG
+
ECFP
B
ySMB-1
hS1MB-4
hS1MB-5
[Monobody] (∝M)
0 0 40 20 10 40 20 10 40 20 10
SENP1
-
+
YFP-SUMO1-ECFP
YFP-SUMO1
ECFP
Monobody
1 2 3 4 5 6 7 8 9 10 11

Figure 29A-B

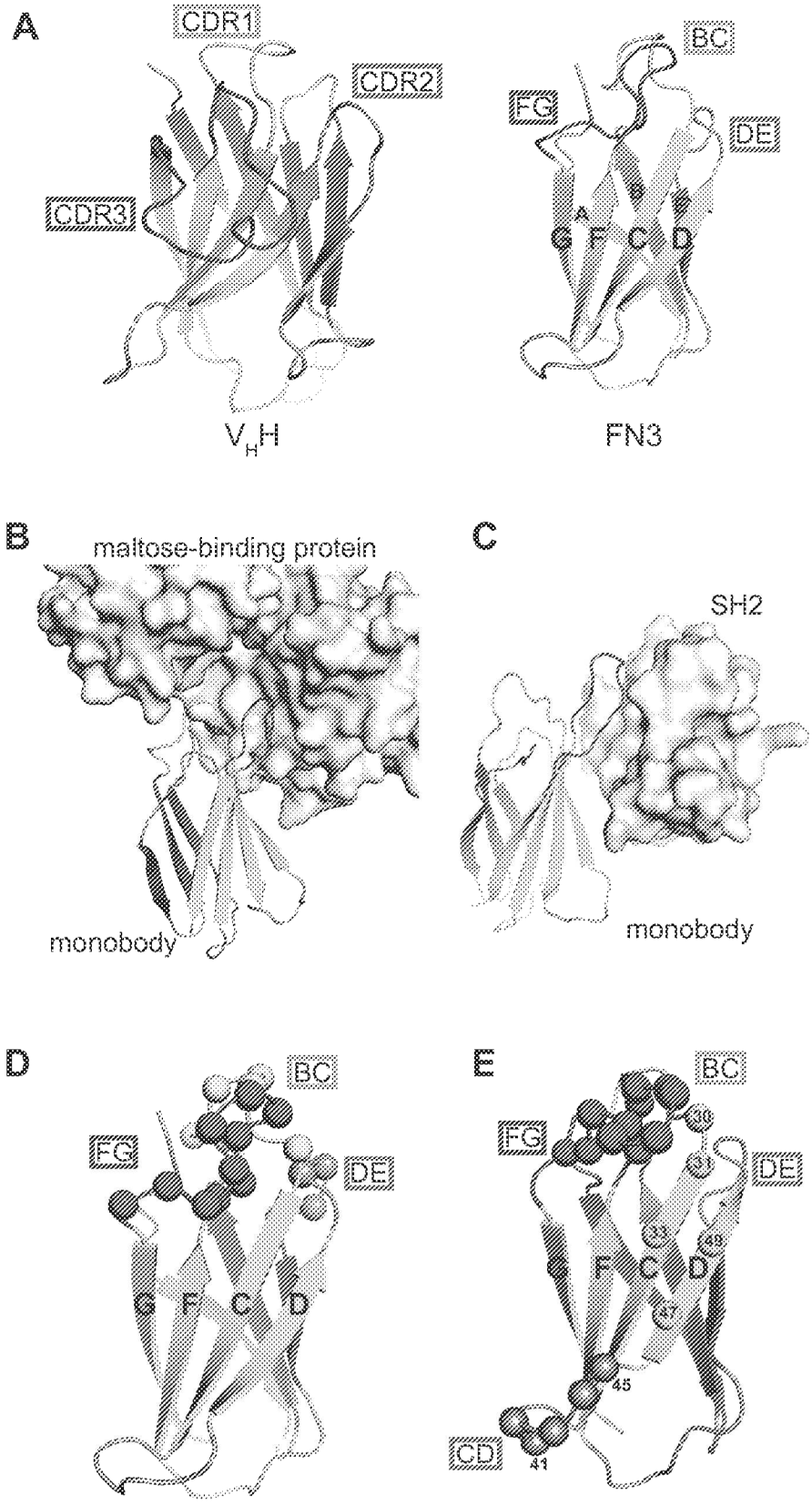

A
CDR1
CDR2
CDR3
V_HH
BC
FG
DE
A
G F C D
FN3
B
maltose-binding protein
monobody
C
SH2
monobody
D
BC
FG
DE
G F C D
E
BC
FG
DE
40
41
43
48
F C D
47
45
CD
41

Figure 30A-E

A

Target	Clone	Kd (nM)	Amino acid sequence
			10 20 βC 40 CD βD 50 60 70 FG 90
	library		VSSVPTKLEVVAATPTSLLISWDAPAV T----VOUYOITYGETG(X_{5-6}) QZF ZVPGSKSTATISGLSPGVDYTITVY A(X_{7-13}) --------SPISINYR T
hSUMO1	SS18	114±30	VSSVPTKLEVVAATPTSLLISWDAPAV T----VDYYVITYGETGGNSPVQEFTVPGSKSTATISGLSPGVDYTITVY AWSDEYTHGYSYSPISINYR T
hSUMO1	SS20	748±156	VSSVPTKLEVVAATPTSLLISWDAPAV T----VDYYVITYGETGGYAGVQEFTVPGSKSTATISGLSPGVDYTITVY AYTQYENIFVGYQ-SPISINYR T
AblSH2	AS15	9±2	VSSVPTKLEVVAATPTSLLISWDAPAV T----VDYYHITYGETGGGSSGQEFTVPGSKSTATISGLSPGVDYTITVY AWMDGYEYTYQGTSPISINYR T
AblSH2	AS25	32±4	VSSVPTKLEVVAATPTSLLISW DAPAVT----VDYYVITYGETGGWSGYQEFEVPGSKSTATISGLSPGVDYTITVY AYGYPYVKYNK ---SPISINYR T
AblSH2	AS27	28±0.4	VSSVPTKLEVVAATPTSLLISWDAPAV T----VDYYHITYGETGAAEGYQEFTVPGSKSTATISGLSPGVDYTITVY AYGYPYVKYNK ---SPISINYR T
AblSH2	SH13	6800±200	VSSVPTKLEVVAATPTSLLISWDAPAV T----VDEYVITYGETGGNSPVQEFTVPGSKSTATISGLSPGVDYTITVY AGYSDSYNWPY ---SPISINYR T
GFP	GS2	25±16	VSSVPTKLEVVAATPTSLLISWDAPAV T----VDYYHITYGETGHYWYQAFAVPGSKSTATISGLSPGVDYTITVY APESVPVM -------SPISINYR T
GFP	GS5	62±7	VSSVPTKLEVVAATPTSLLISWDAPAV T----VDYYHITYGETGHYWYQAFAVPGSKSTATISGLSPGVDYTITVY ASVLYGSRYYY -----SPISINYR T

B

Target	Clone	Kd (nM)	Amino acid sequence
			10 20 BC 30 40 50 DE 60 70 FG 90
	library		VSSVPTKLEVVAATPTSLLISWD A(X_{4-8}) ---VXYYRITYGETGGNSPVQEFTV PBJJJ TATISGLSPGVDYTITVY A(X_{7-13}) --------SPISINYR T
hSUMO1	SL8	10±1	VSSVPTKLEVVAATPTSLLISWD AGRWF ---- VEYYRITYGETGGNSPVQEFTV PYSSSTATISGLSPGVDYTITVY AHYWSTWY ------SPISINYR T
hSUMO1	SL13	270±60	VSSVPTKLEVVAATPTSLLISWD ANSWY ----- VTYYRITYGETGGNSPVQEFTV PYSSSTATISGLSPGVDYTITVY AHYWSTWY --------SPISINYR T
hSUMO1	SL14	179±42	VSSVPTKLEVVAATPTSLLISWD AGYWY ---- VQYYRITYGETGGNSPVQEFTV PGSSSTATISGLSPGVDYTITVY AYYDDWEW ------SPISINYR T
hSUMO1	ySMB9	68	VSSVPTKLEVVAATPTSLLISWD AGYWF---- I DYYRITYGETGGNSPVQEFTV PGYSSTATISGLSPGVDYTITVY AYYDNYGW ------SPISINYR T
AblSH2	AL38	11±1	VSSVPTKLEVVAATPTSLLISWD ALHYSGRGKVIYYRITYGETGGNSPVQEFTV PGSSSTATISGLSPGVDYTITVY AYYQSYGYWYYM-SPISINYR T
AblSH2	AL2	70±16	VSSVXTKLEVV AATPTSLLISWDAGSSWYYY-I SYYRITYGETGGNSPVQEFTV PGSSYTATISGLSPGVDYTITVY AWYDQYYYLVES-SPISINYR T
GFP	GL4	478±72	VSSVPTKLEVVAATPTSLLISWD ASSSS ----- VSYYRITYGETGGNSPVQEFTV PGSSSTATISGLSPGVDYTITVY AYWWRYYYSY--SPISINYR T
GFP	GL6	208±22	VSSVPTKLEVVAATPTSLLISWD AEYEY ----- VSYYRITYGETGGNSPVQEFTV PGSYSTATISGLSPGVDYTITVY AYDQYSSYG -----SPISINYR T
GFP	GL8	257±82	VSSVPTKLEVVAATPTSLLISWD ASSSS ----- VSYYRITYGETGGNSPVQEFTV PGSSSTATISGLSPGVDYTITVY AYWYQYSYYQY--SPISINYR T

Figure 31A-B

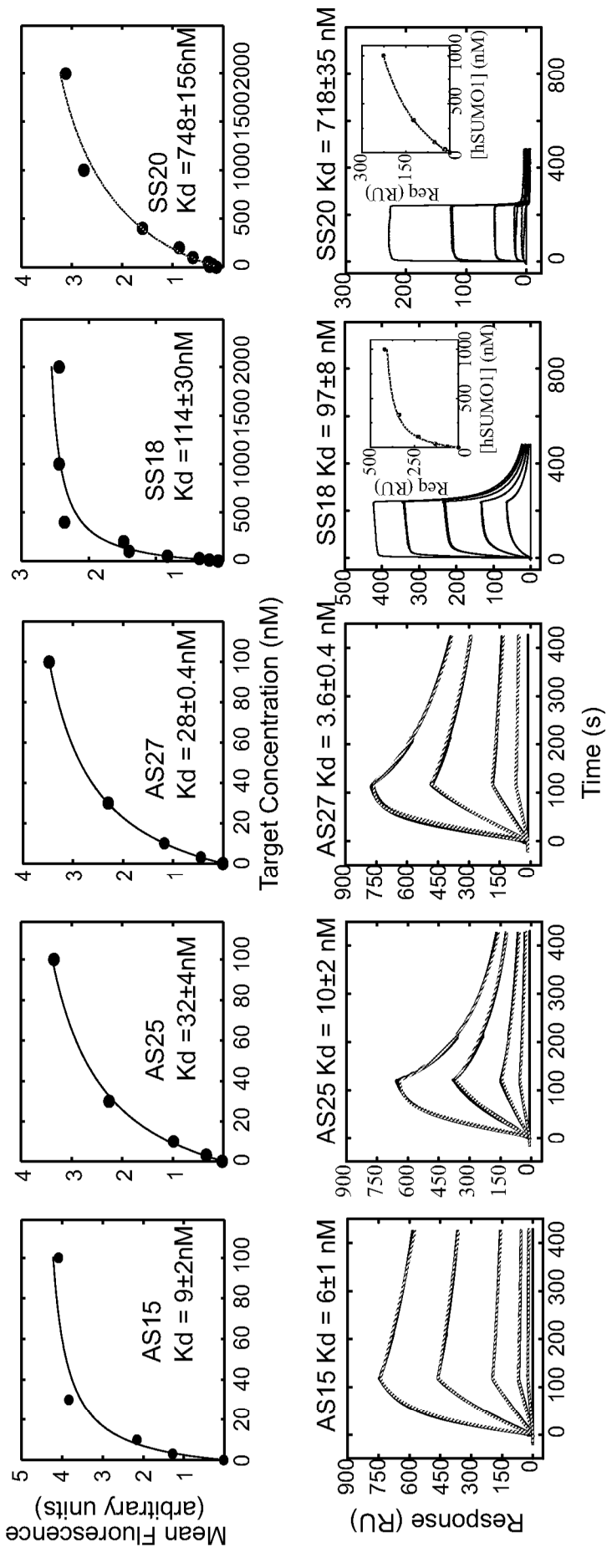

C
D
Mean Fluorescence (arbitrary units)
Target Concentration (nM)
AS15 Kd = 9±2nM
AS25 Kd =32±4nM
AS27 Kd = 28±0.4nM
SS18 Kd =114±30nM
SS20 Kd =748±156nM
Response (RU)
Time (s)
AS15 Kd = 6±1 nM
AS25 Kd = 10±2 nM
AS27 Kd = 3.6±0.4 nM
SS18 Kd = 97±8 nM
SS20 Kd = 718±35 nM
Req (RU)
[hSUMO1] (nM)

Figure 31C-D

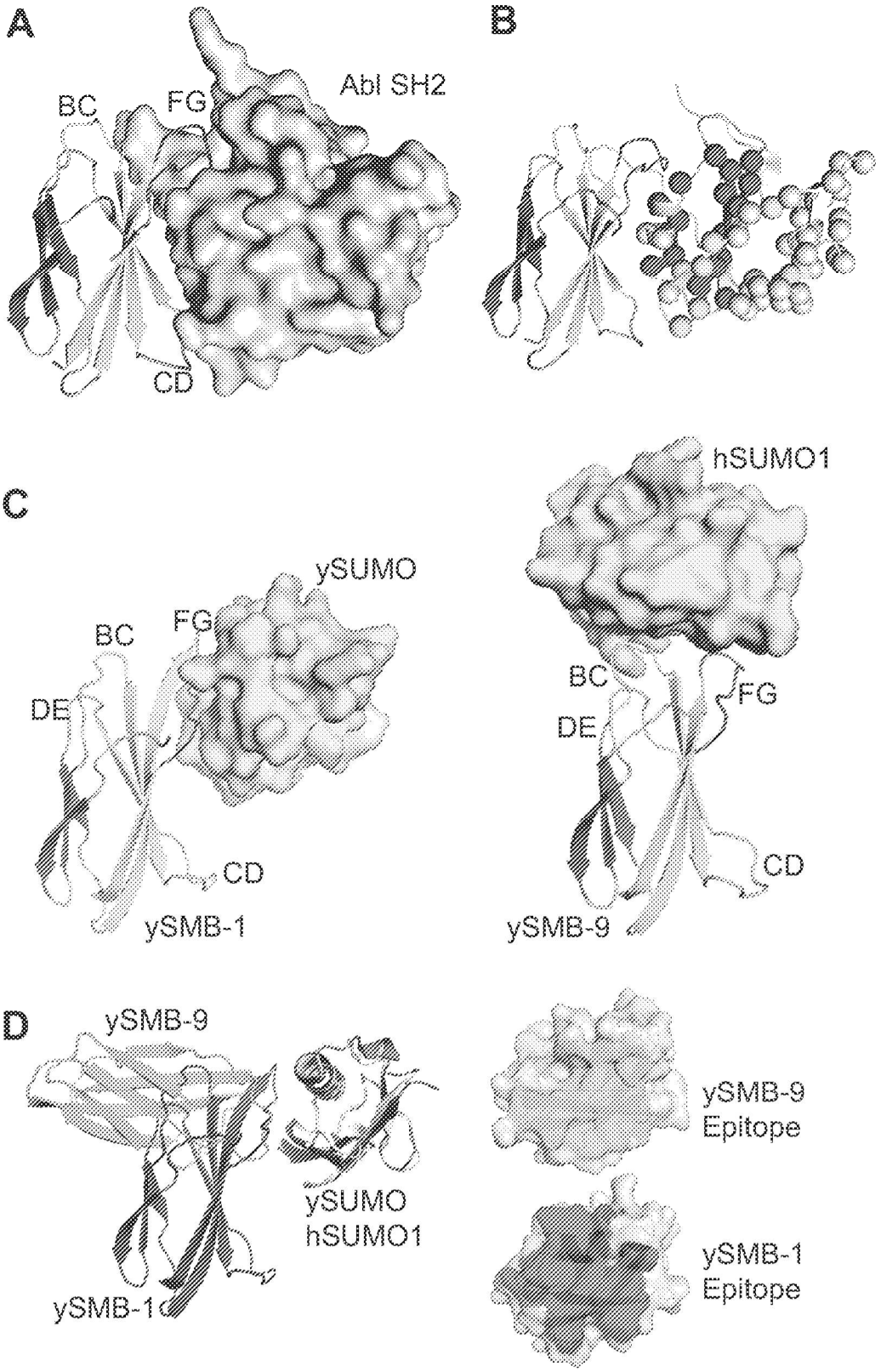

A
BC
FG
Abl SH2
CD
B
C
ySUMO
BC
FG
DE
CD
ySMB-1
hSUMO1
BC
FG
DE
CD
ySMB-9
D
ySMB-9
ySUMO
hSUMO1
ySMB-1
ySMB-9
Epitope
ySMB-1
Epitope

Figure 32A-D

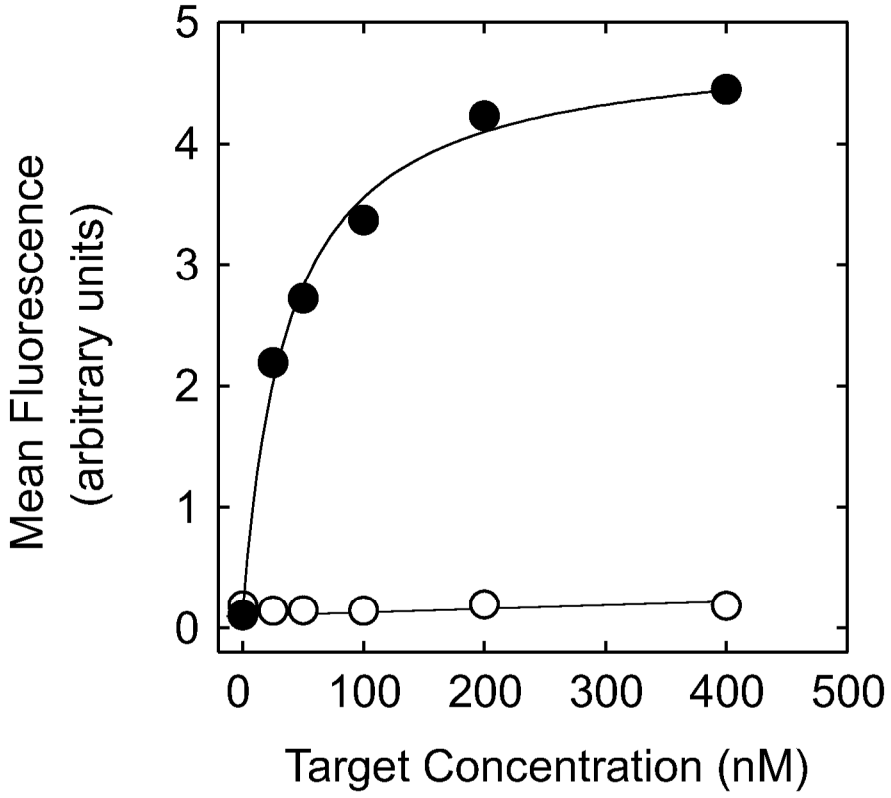

Mean Fluorescence
(arbitrary units)
0
1
2
3
4
5
0
100
200
300
400
500
Target Concentration (nM)

Figure 33