FIG. 1A

Light chain variable region

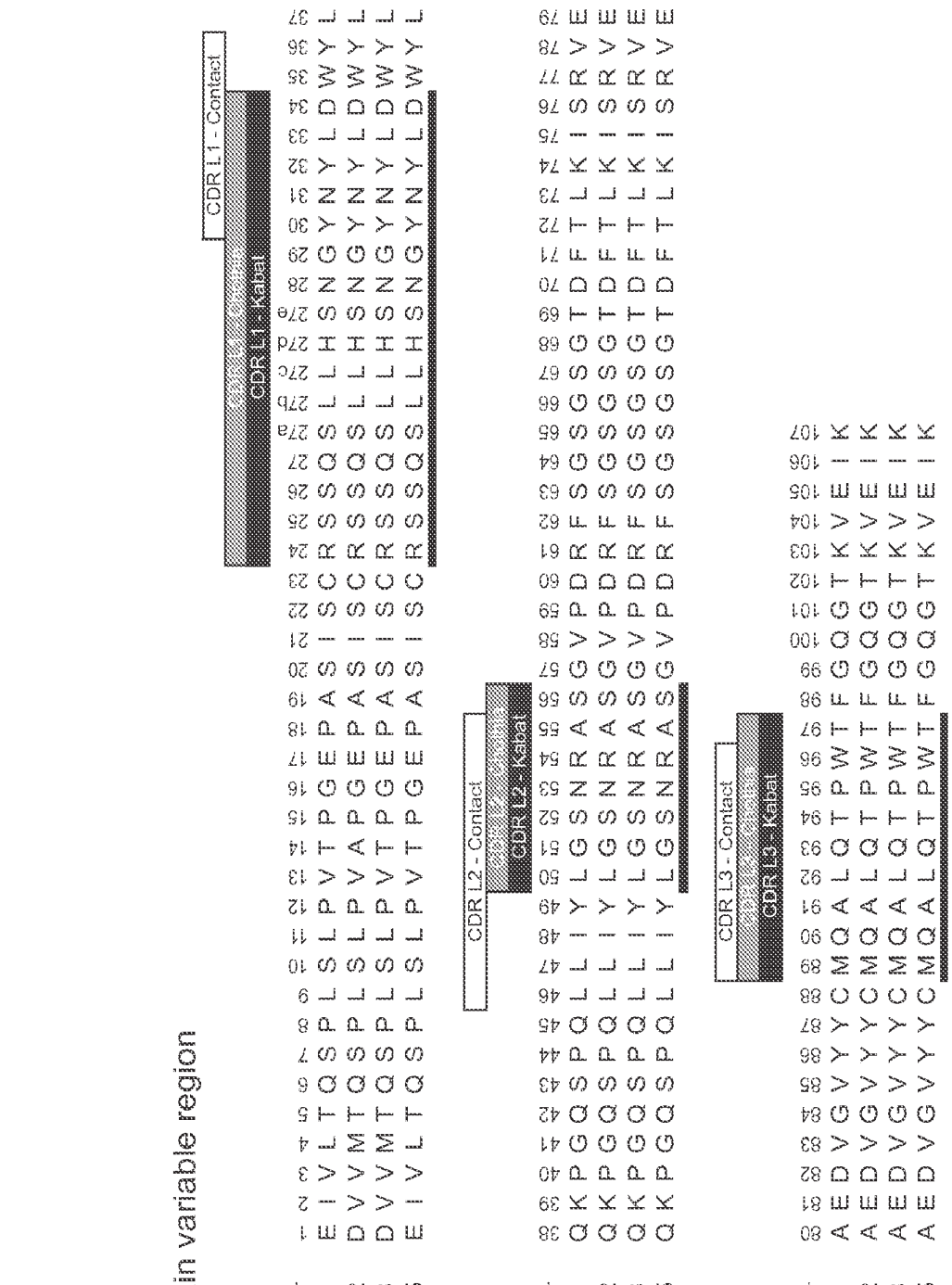


FIG. 1B

Heavy chain variable region

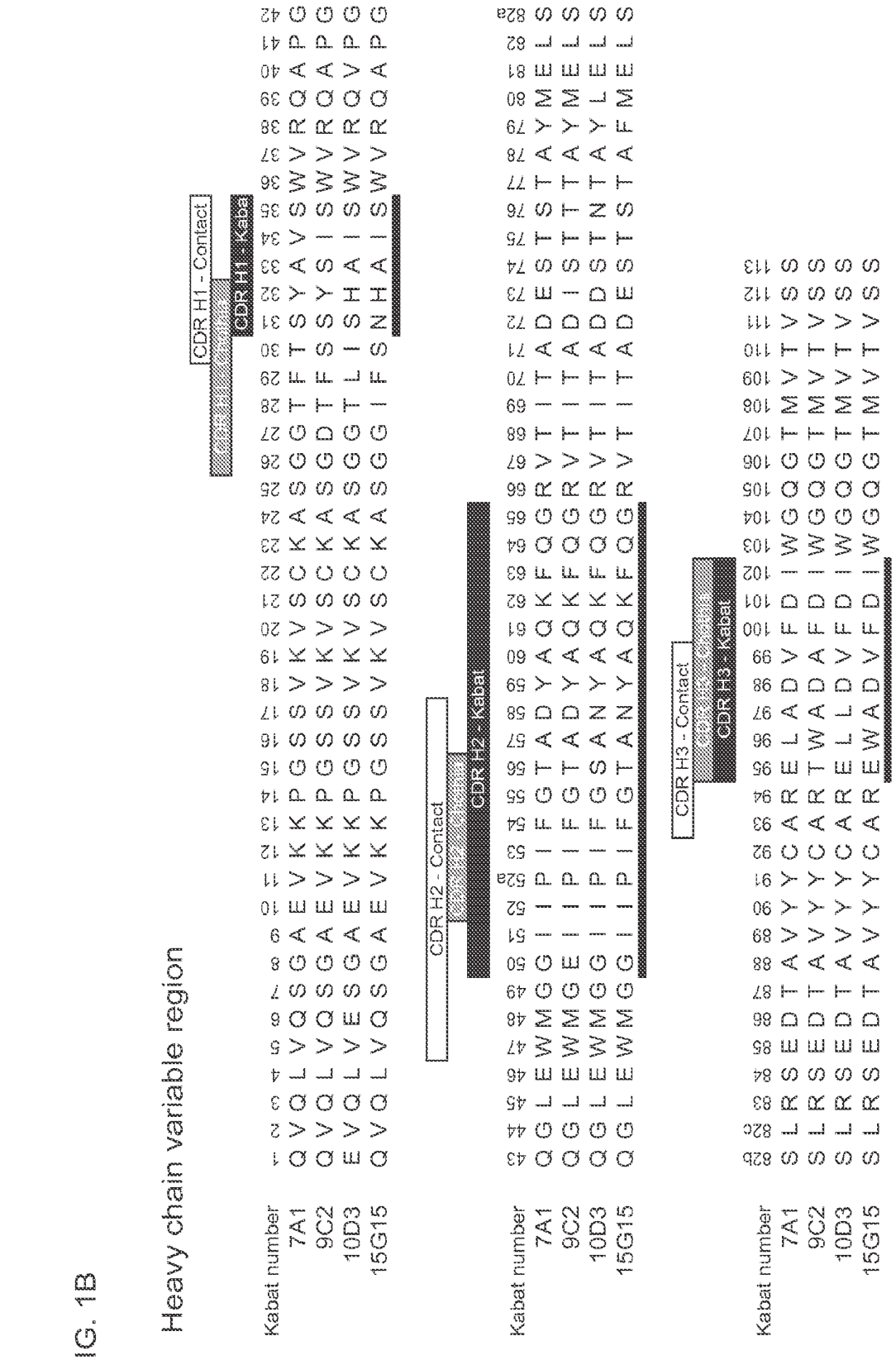


FIG. 2A

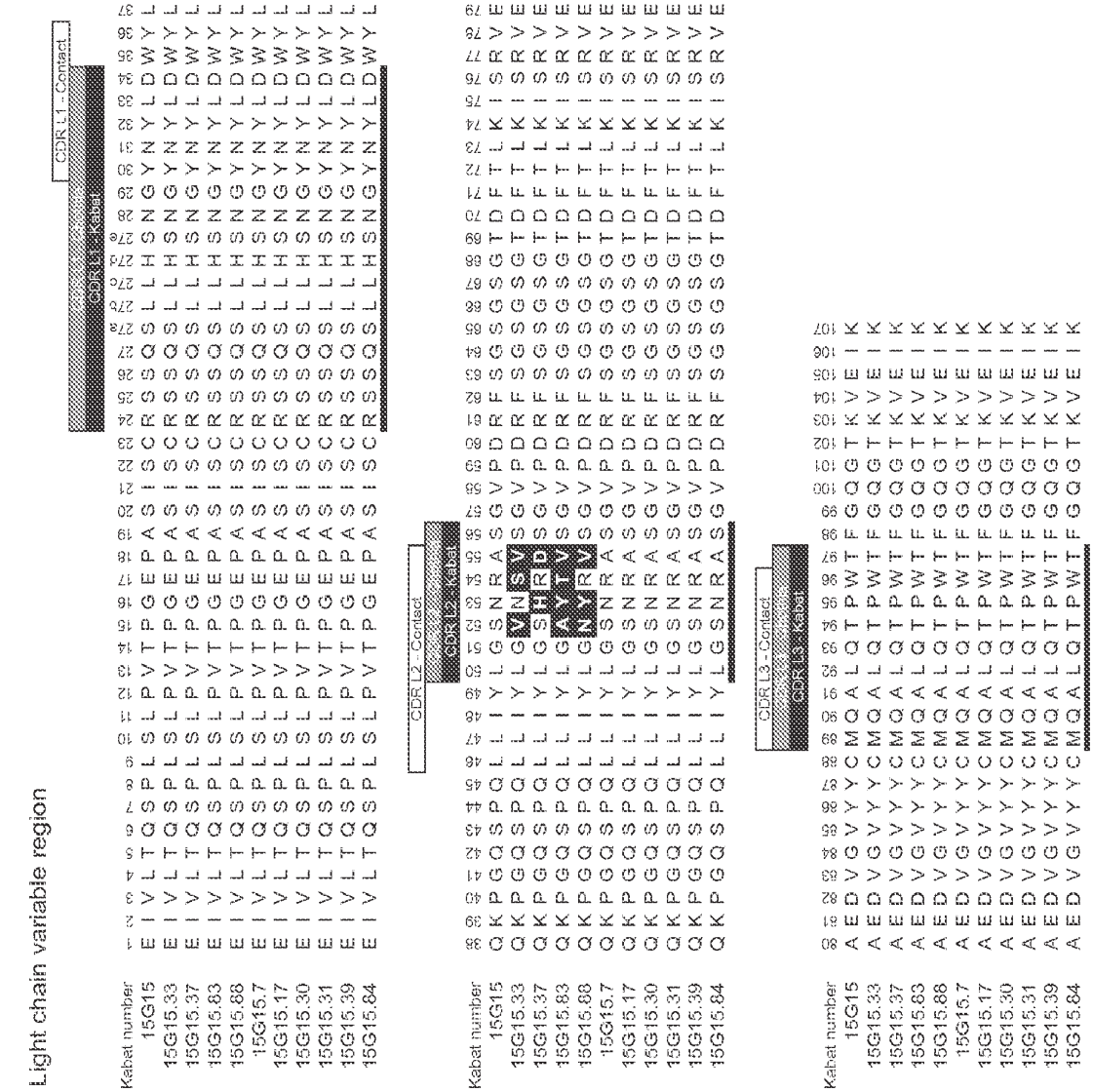

Light chain variable region
CDR L1 - Contact
CDR L1 - Kabat
CDR L2 - Contact
CDR L2 - Kabat
CDR L3 - Contact
CDR L3 - Kabat
Kabat number
15G15
15G15.33
15G15.37
15G15.83
15G15.88
15G15.7
15G15.17
15G15.30
15G15.31
15G15.39
15G15.84

FIG. 2B

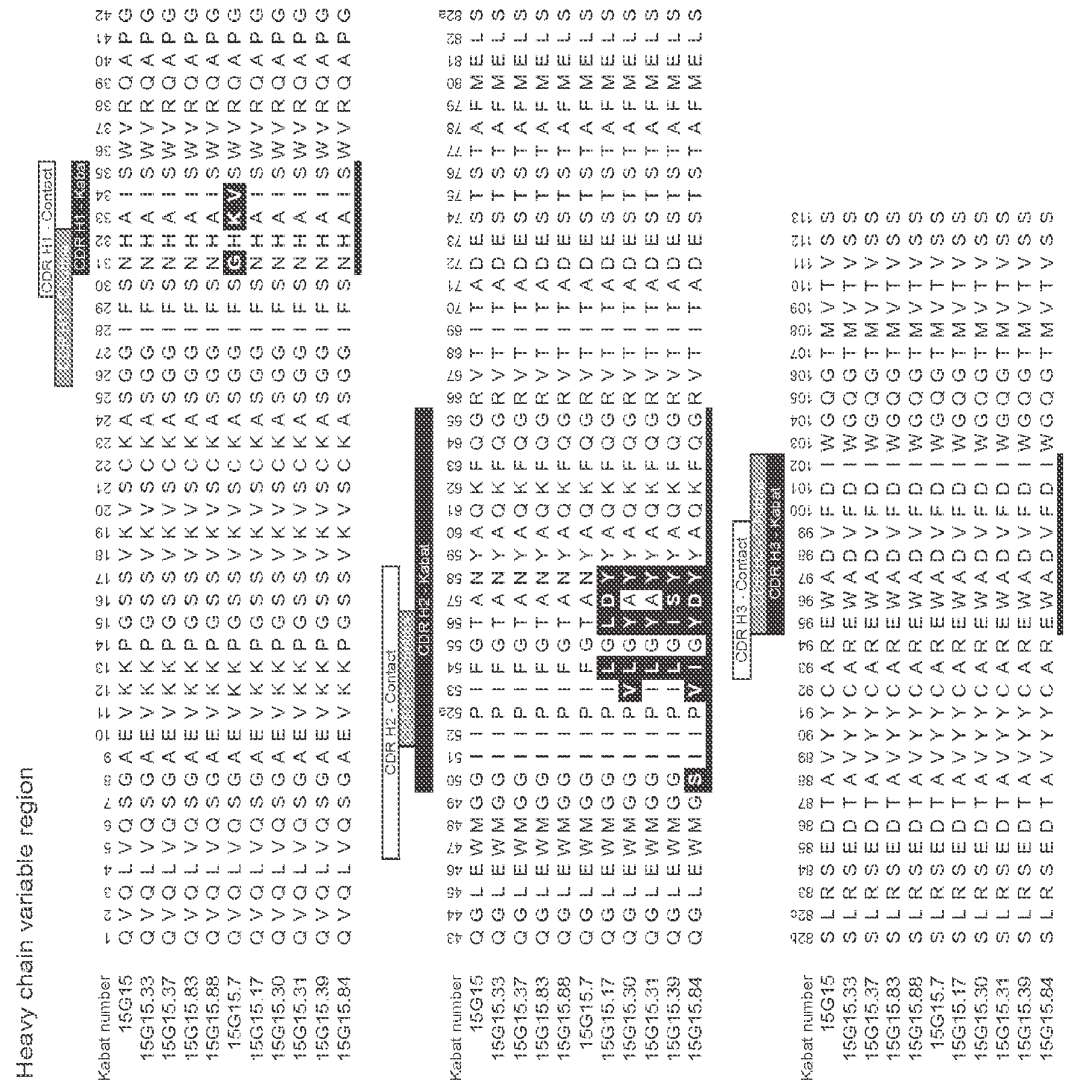

Heavy chain variable region
CDR H1 - Contact
CDR H1 - Kabat
CDR H2 - Contact
CDR H2 - Kabat
CDR H3 - Contact
CDR H3 - Kabat
Kabat number
15G15
15G15.33
15G15.37
15G15.83
15G15.88
15G15.7
15G15.17
15G15.30
15G15.31
15G15.39
15G15.84

FIG. 3A

Light chain variable region

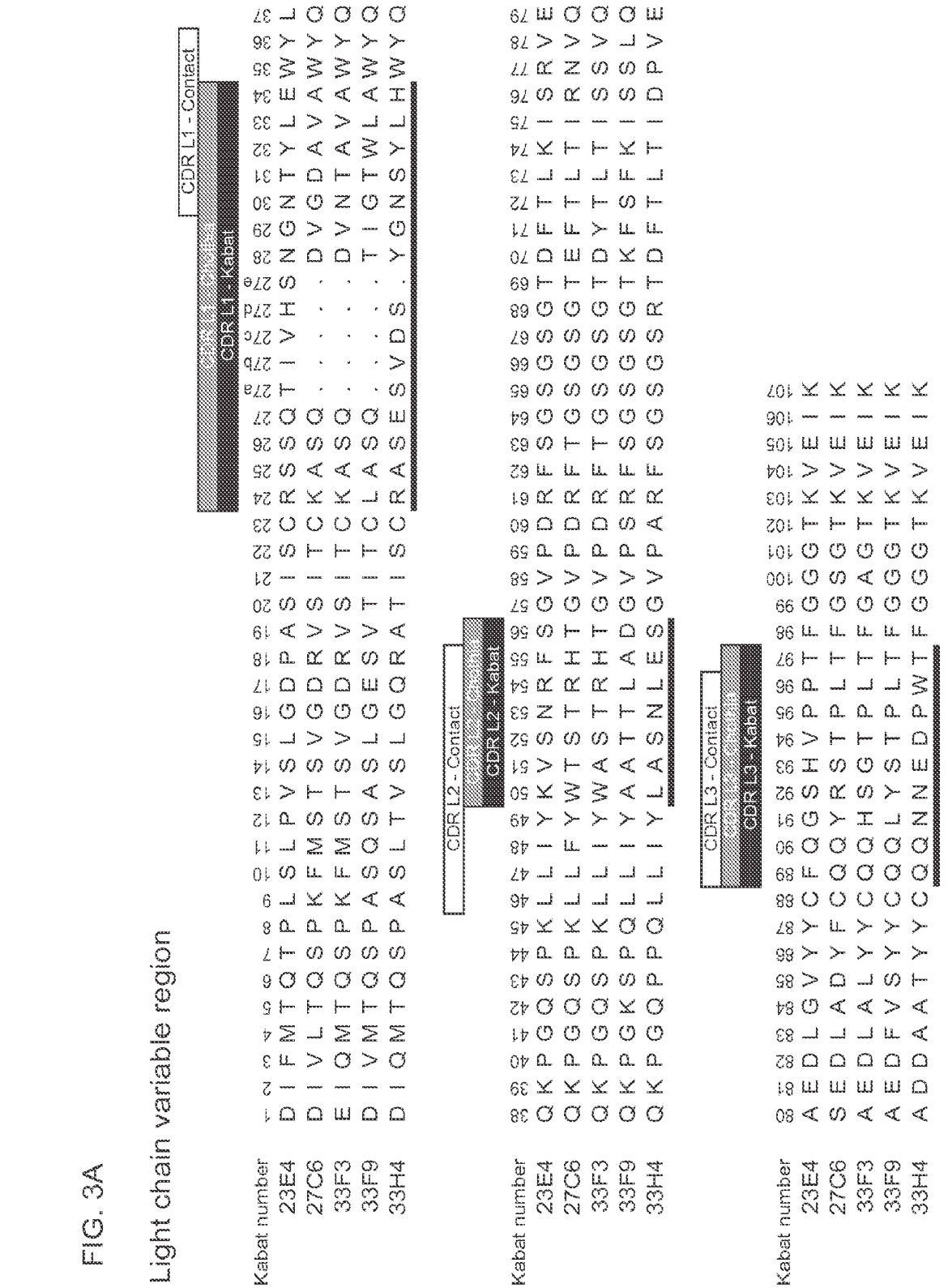


FIG. 3B

Heavy chain variable region

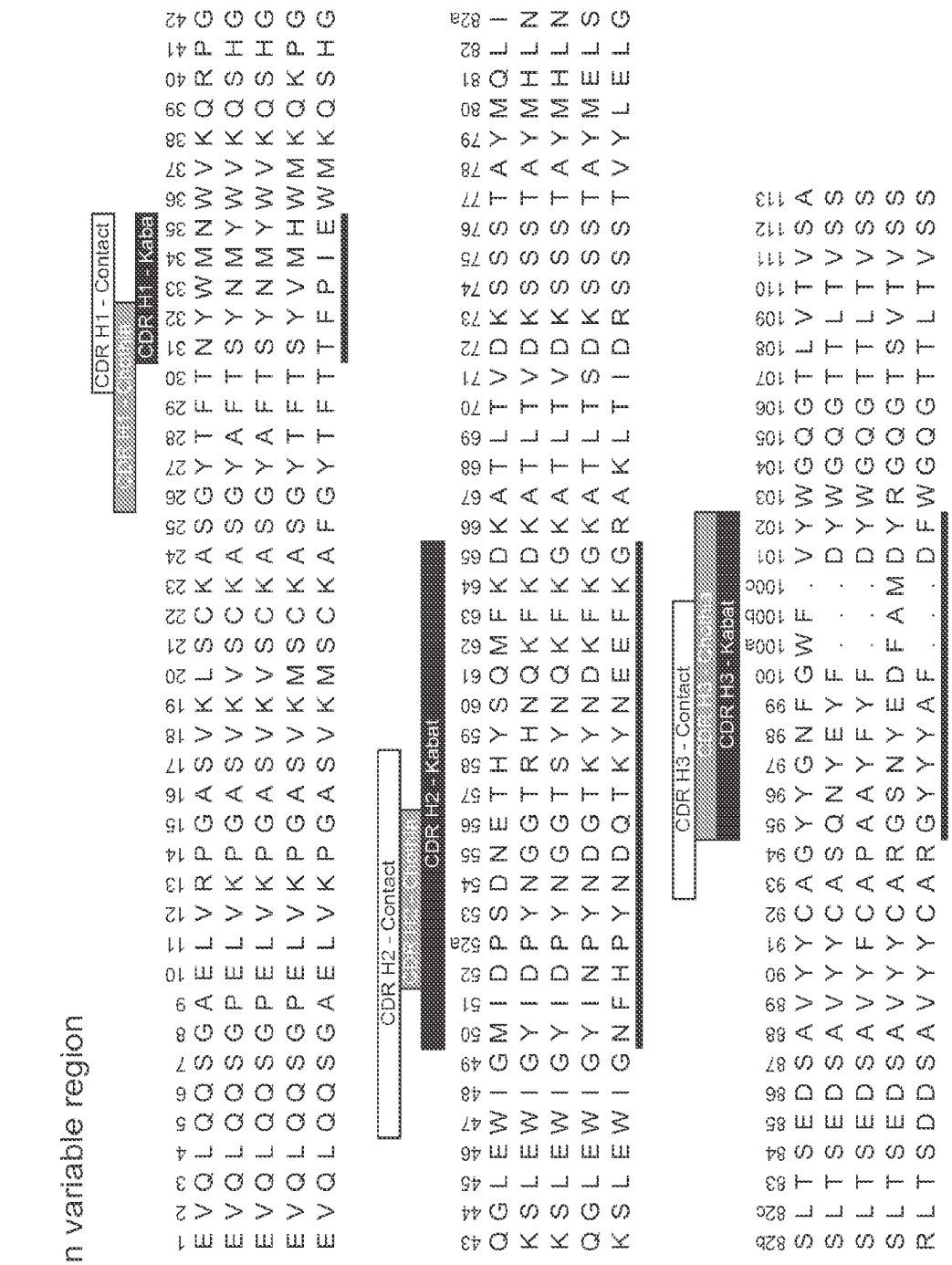


FIG. 4A

Light chain variable region

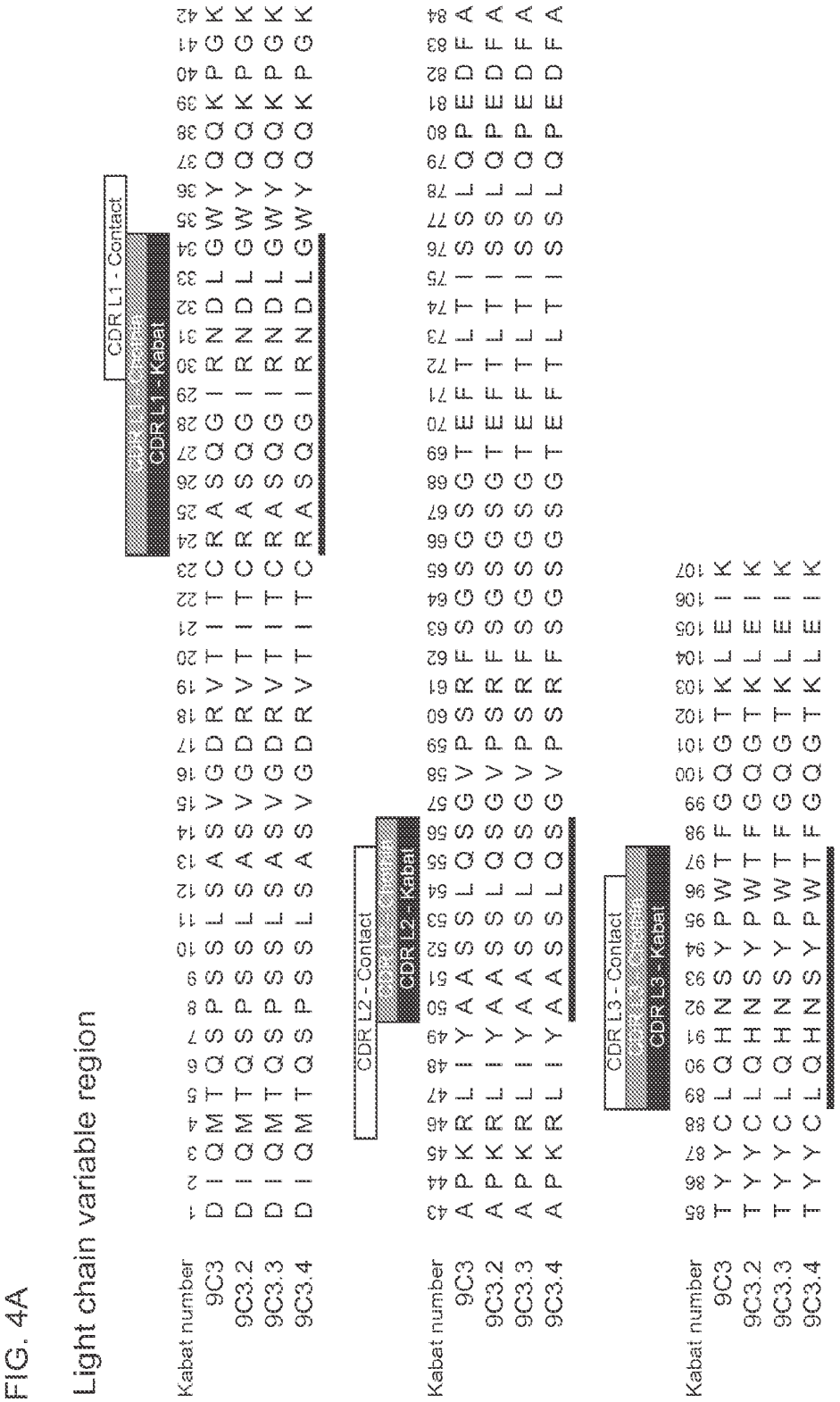


FIG. 4B

Heavy chain variable region

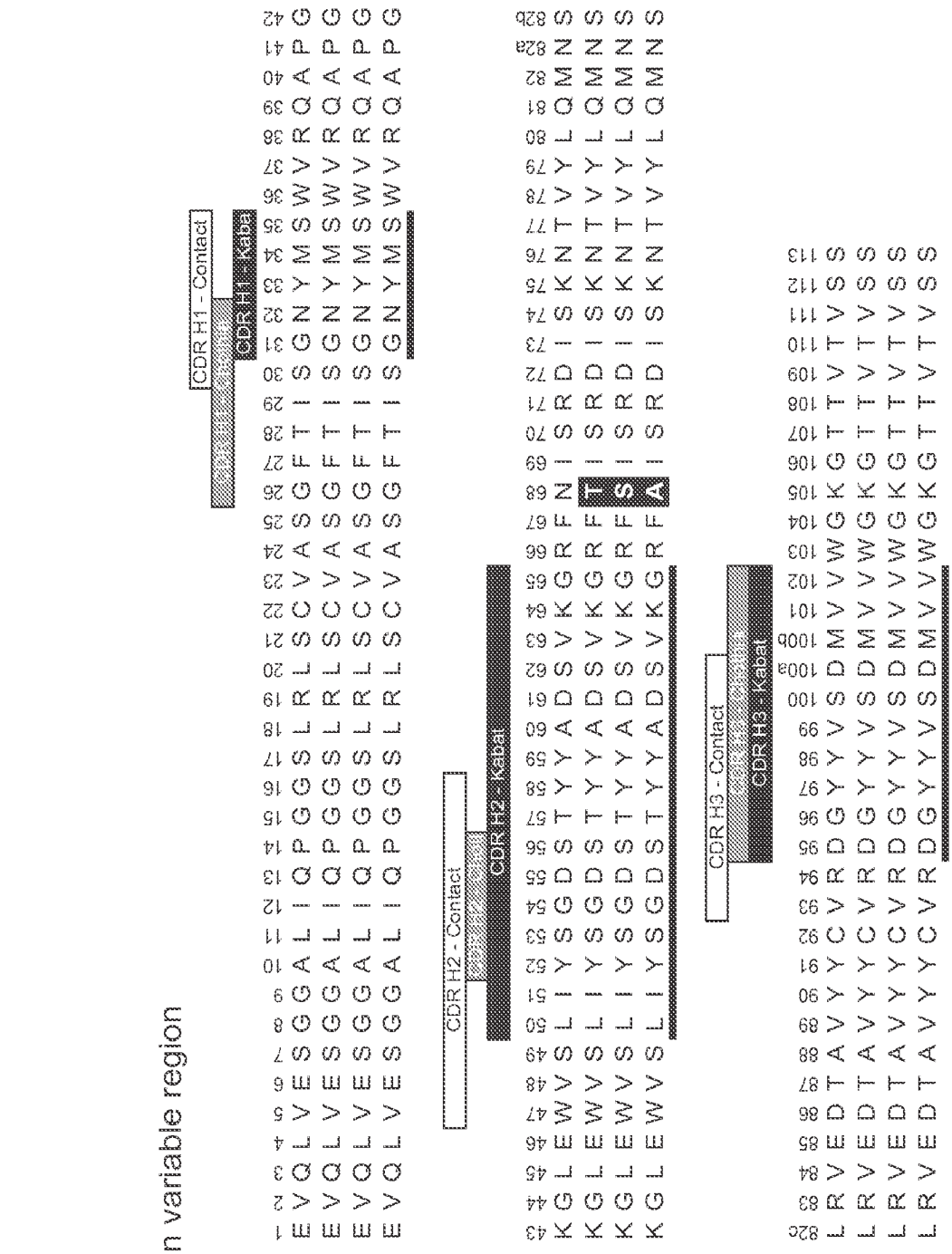


FIG. 5

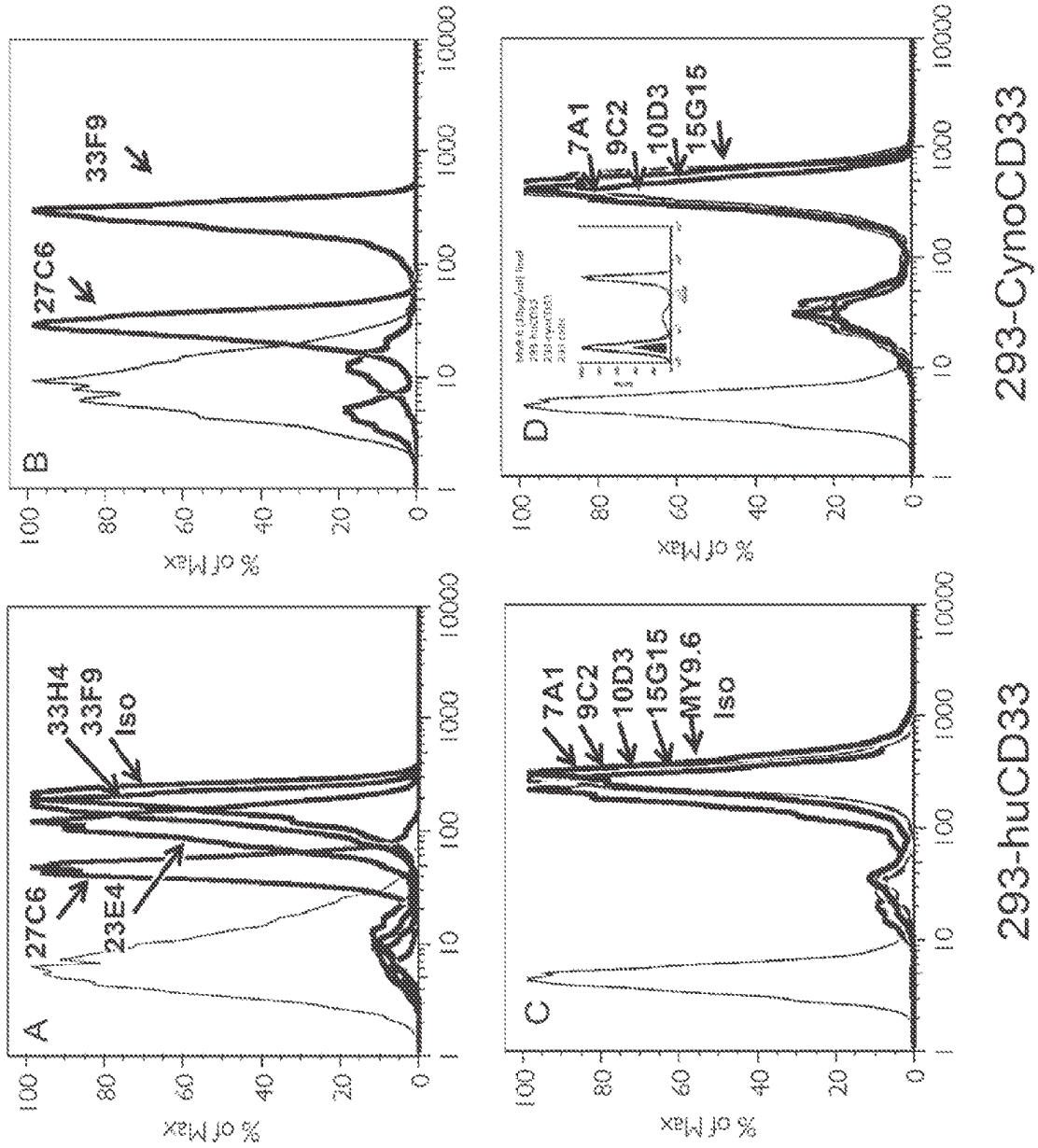

A
27C6
33H4
33F9
Iso
23E4
B
27C6
33F9
C
7A1
9C2
10D3
15G15
MY9.6
Iso
D
7A1
9C2
10D3
15G15
% of Max
293-huCD33
293-CynoCD33

FIG. 6

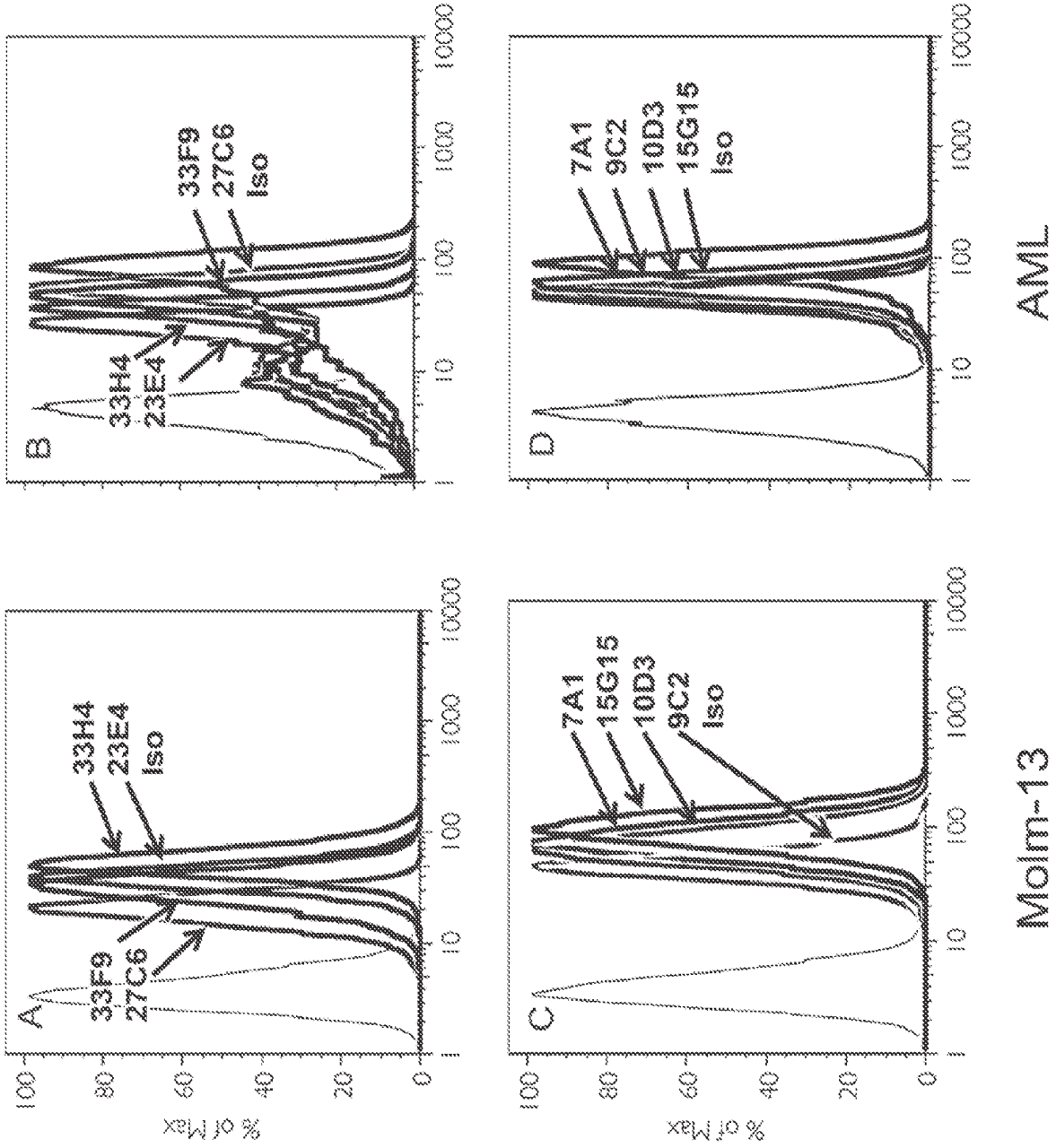

A
33F9
27C6
33H4
23E4
Iso
B
33H4
23E4
33F9
27C6
Iso
C
7A1
15G15
10D3
9C2
Iso
D
7A1
9C2
10D3
15G15
Iso
% of Max
100
80
60
40
20
0
1
10
100
1000
10000
Molm-13
AML

FIG. 7

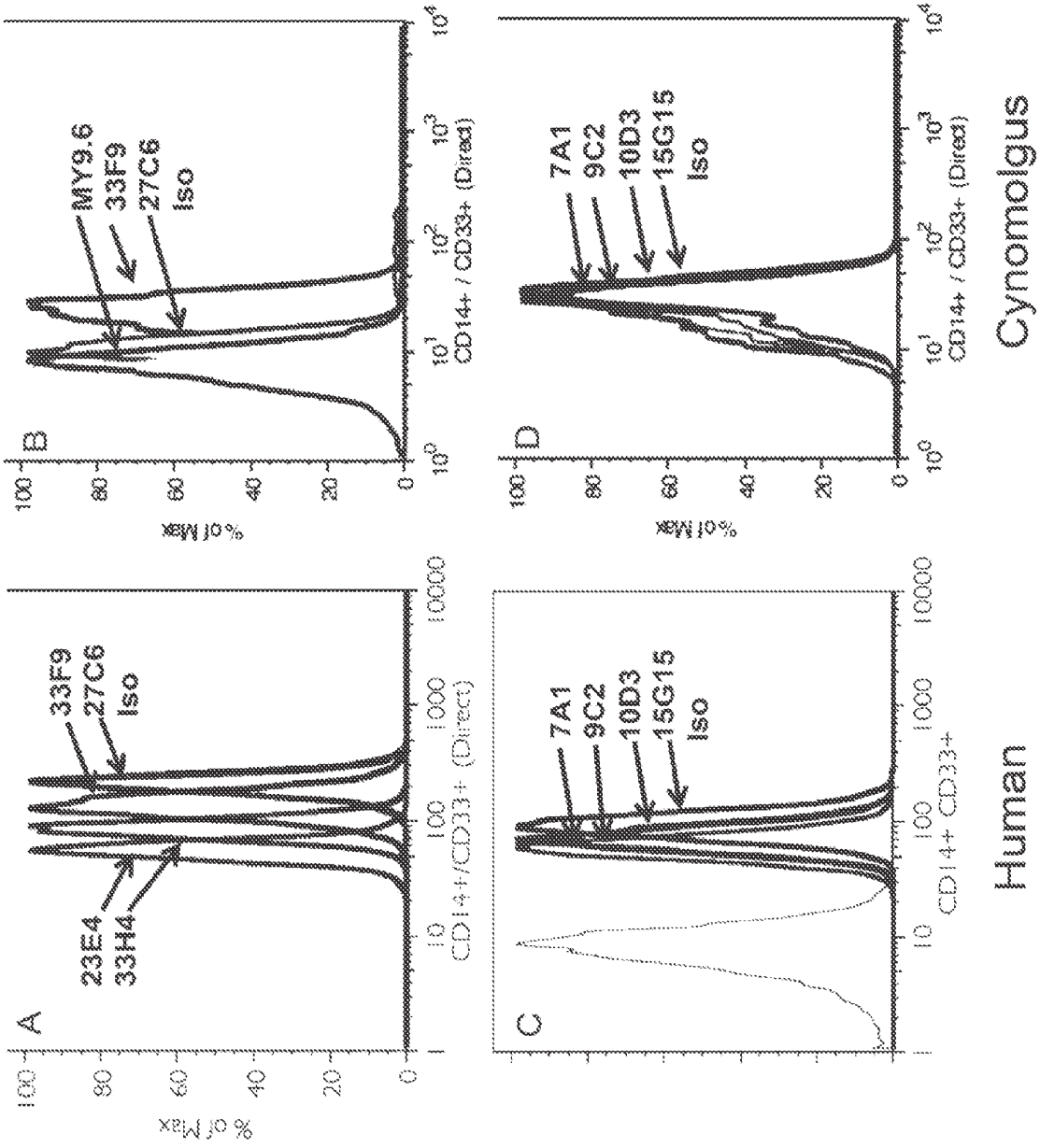
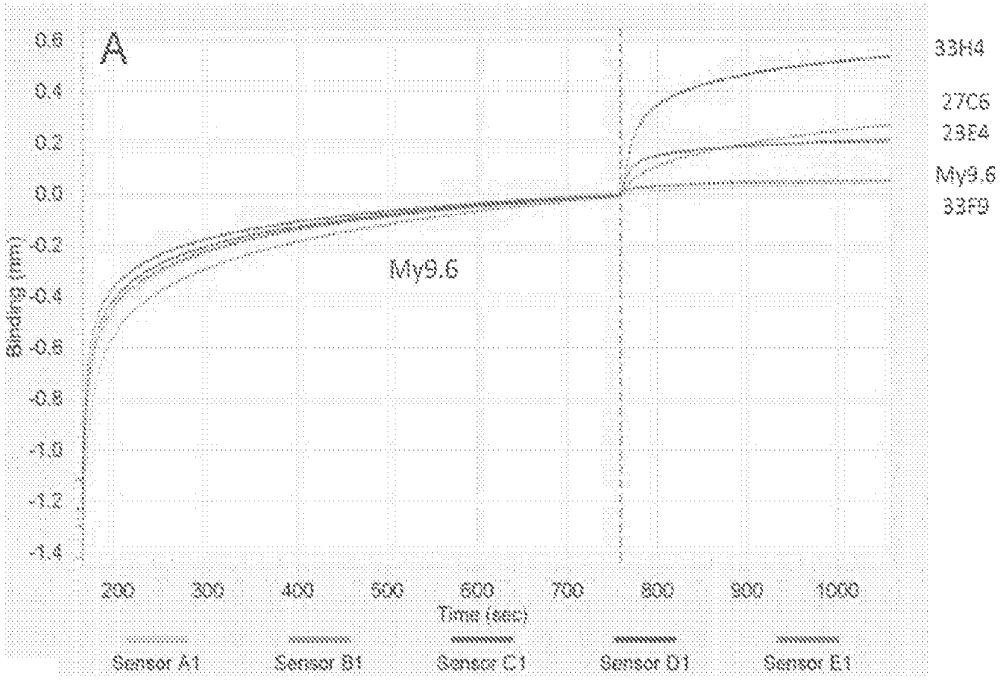

A
B
C
D
% of Max
CD14+/CD33+ (Direct)
CD14+ / CD33+ (Direct)
CD14+ CD33+
MY9.6
33F9
27C6
Iso
23E4
33H4
7A1
9C2
10D3
15G15
Human
Cynomolgus

FIG. 8


A
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
Binding (nm)
My9.6
200
300
400
500
600
700
800
900
1000
Time (sec)
Sensor A1
Sensor B1
Sensor C1
Sensor D1
Sensor E1
33H4
27C6
23E4
My9.6
33F9

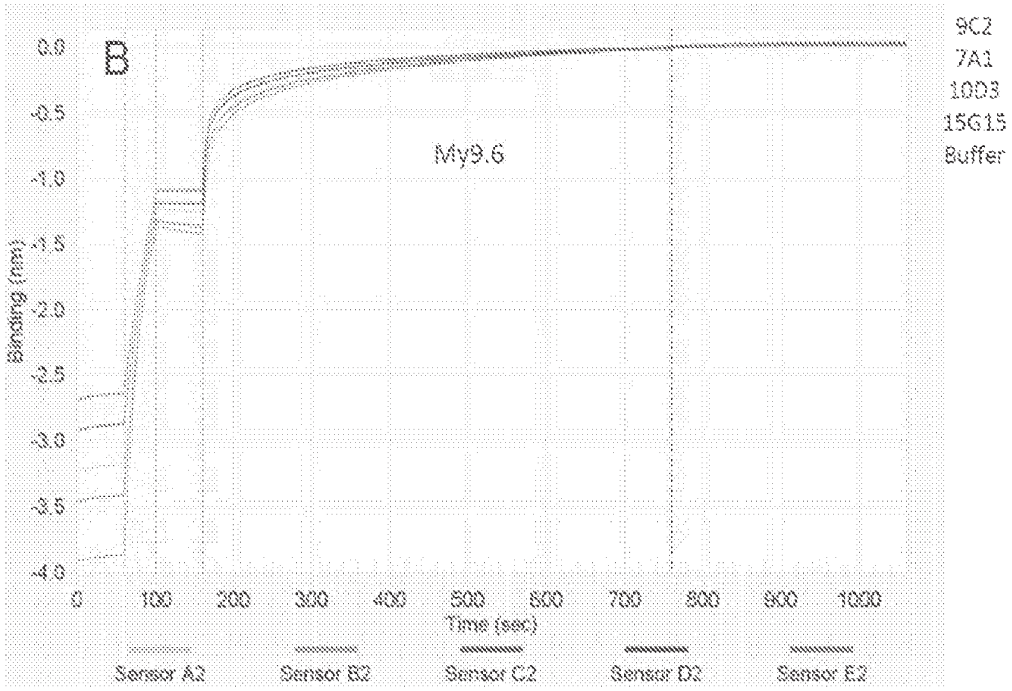

B
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.5
-4.0
Binding (nm)
My9.6
0
100
200
300
400
500
600
700
800
900
1000
Time (sec)
Sensor A2
Sensor B2
Sensor C2
Sensor D2
Sensor E2
9C2
7A1
10D3
15G15
Buffer

FIG. 8

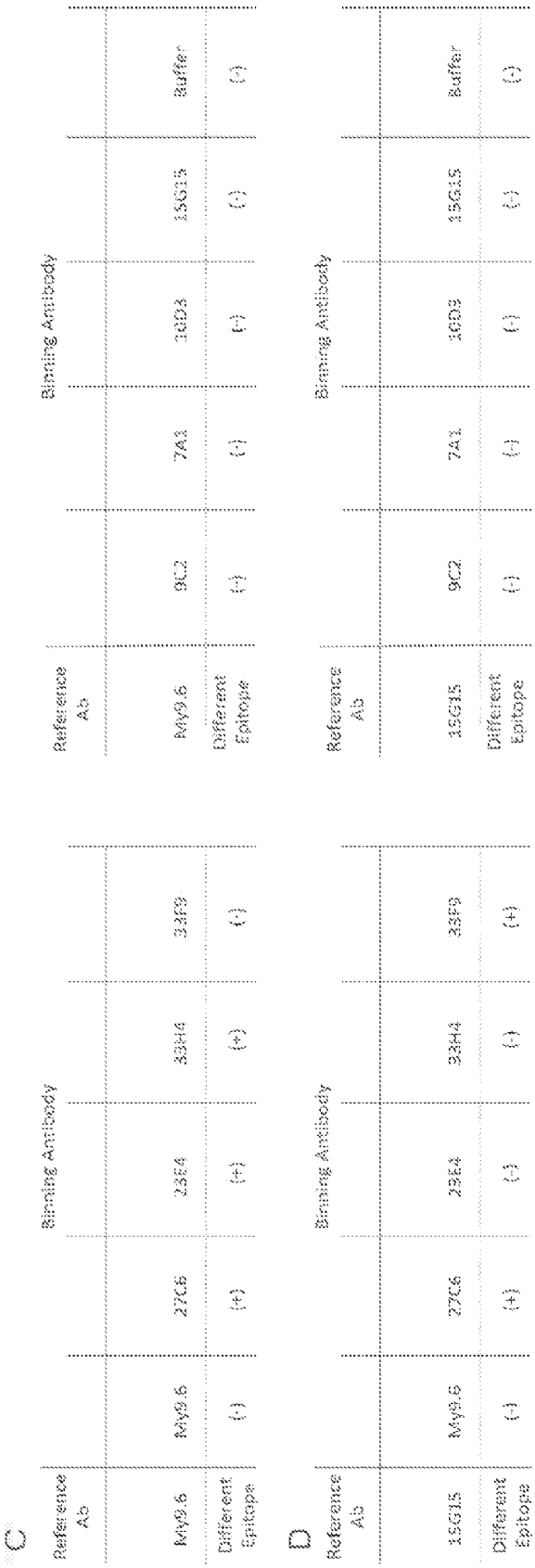

C
Reference Ab
Binding Antibody
My9.6
27C6
28E4
33H4
33F9
My9.6
(-)
(+)
(+)
(+)
(-)
Different Epitope
(-)
(+)
(+)
(-)
(-)
D
Reference Ab
Binding Antibody
15G15
27C6
28E4
33H4
33F9
My9.6
(-)
(+)
(-)
(-)
(+)
Different Epitope
(-)
(+)
(-)
(-)
(+)
Reference Ab
Binding Antibody
My9.6
9C2
7A1
30D3
15G15
Buffer
Different Epitope
(-)
(-)
(-)
(-)
(-)
(-)
Reference Ab
Binding Antibody
15G15
9C2
7A1
30D3
15G15
Buffer
Different Epitope
(-)
(-)
(-)
(-)
(-)
(-)

Figure 9

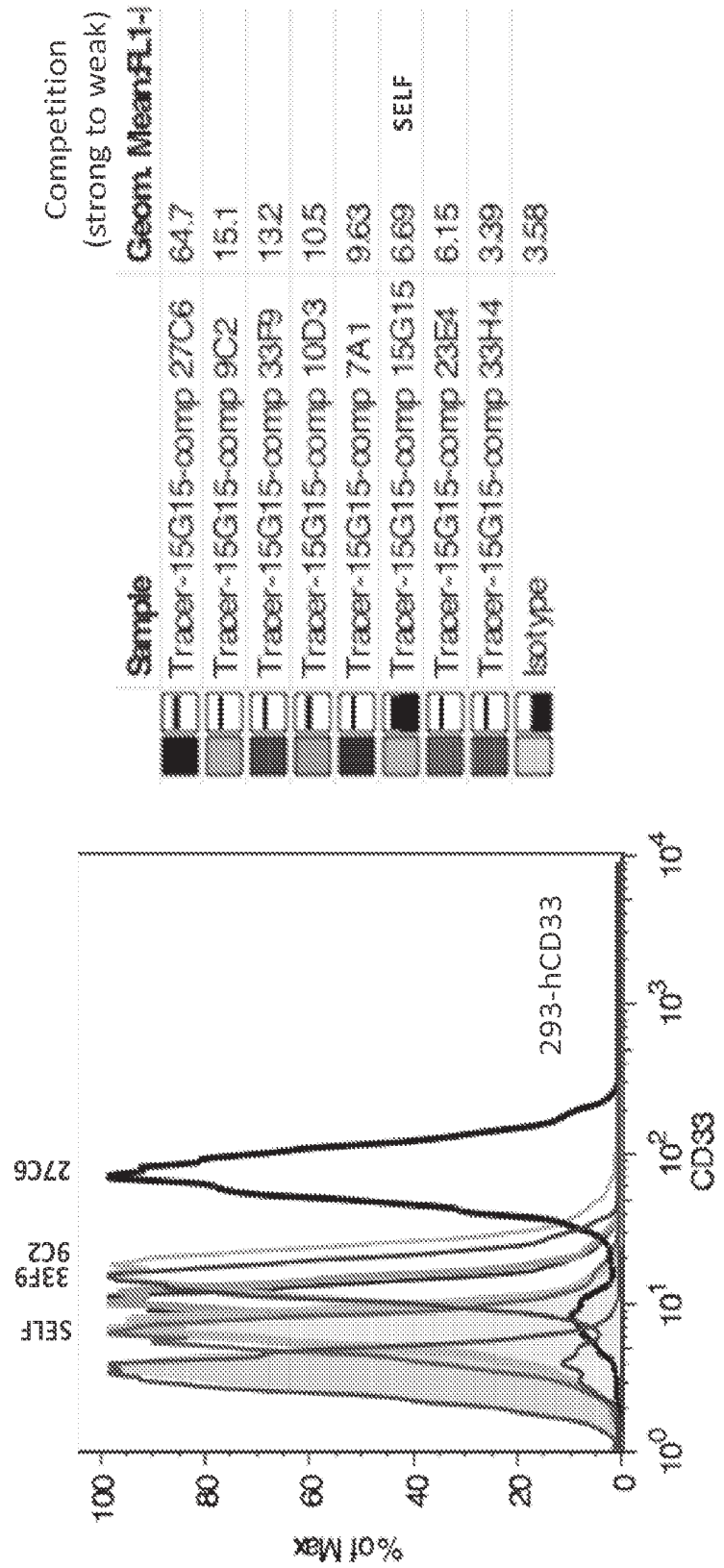

Competition
(strong to weak)
Sample
Geom. Mean(FL1-H
Tracer-15G15-comp 27C6
64.7
Tracer-15G15-comp 9C2
15.1
Tracer-15G15-comp 33F9
13.2
Tracer-15G15-comp 10D3
10.5
Tracer-15G15-comp 7A1
9.63
Tracer-15G15-comp 15G15
6.69
SELF
Tracer-15G15-comp 23E4
6.15
Tracer-15G15-comp 33H4
3.39
Isotype
3.58
293-hCD33
SELF
33F9
9C2
27C6
% of Max
CD33
0
20
40
60
80
100
10^0
10^1
10^2
10^3
10^4

Figure 10

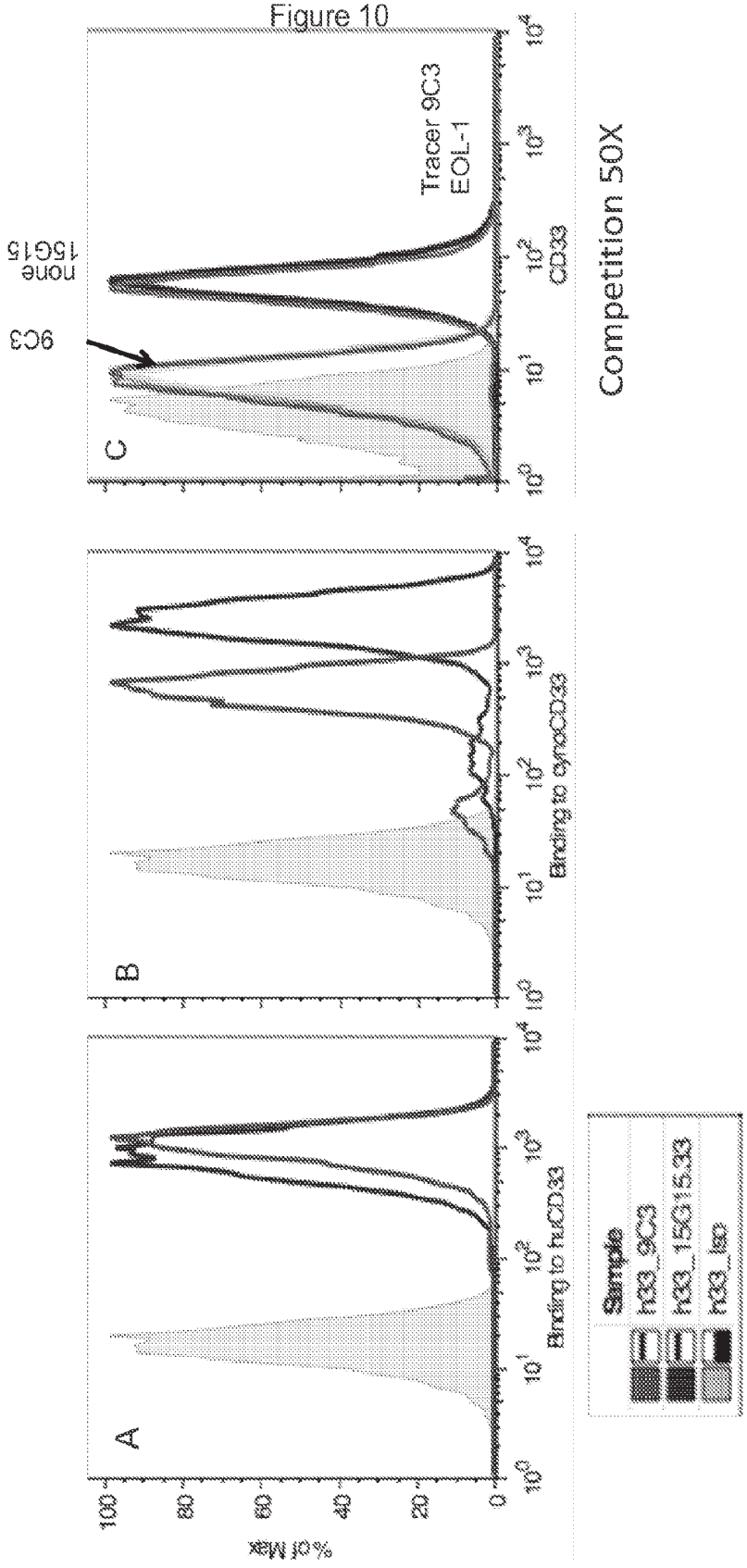

Competition 50X
C
Tracer 9C3
EOL-1
CD33
none
15G15
9C3
B
Binding to cynoCD33
A
Binding to huCD33
% of Max
Sample
h33_9C3
h33_15G15.33
h33_Iso

Figure 11

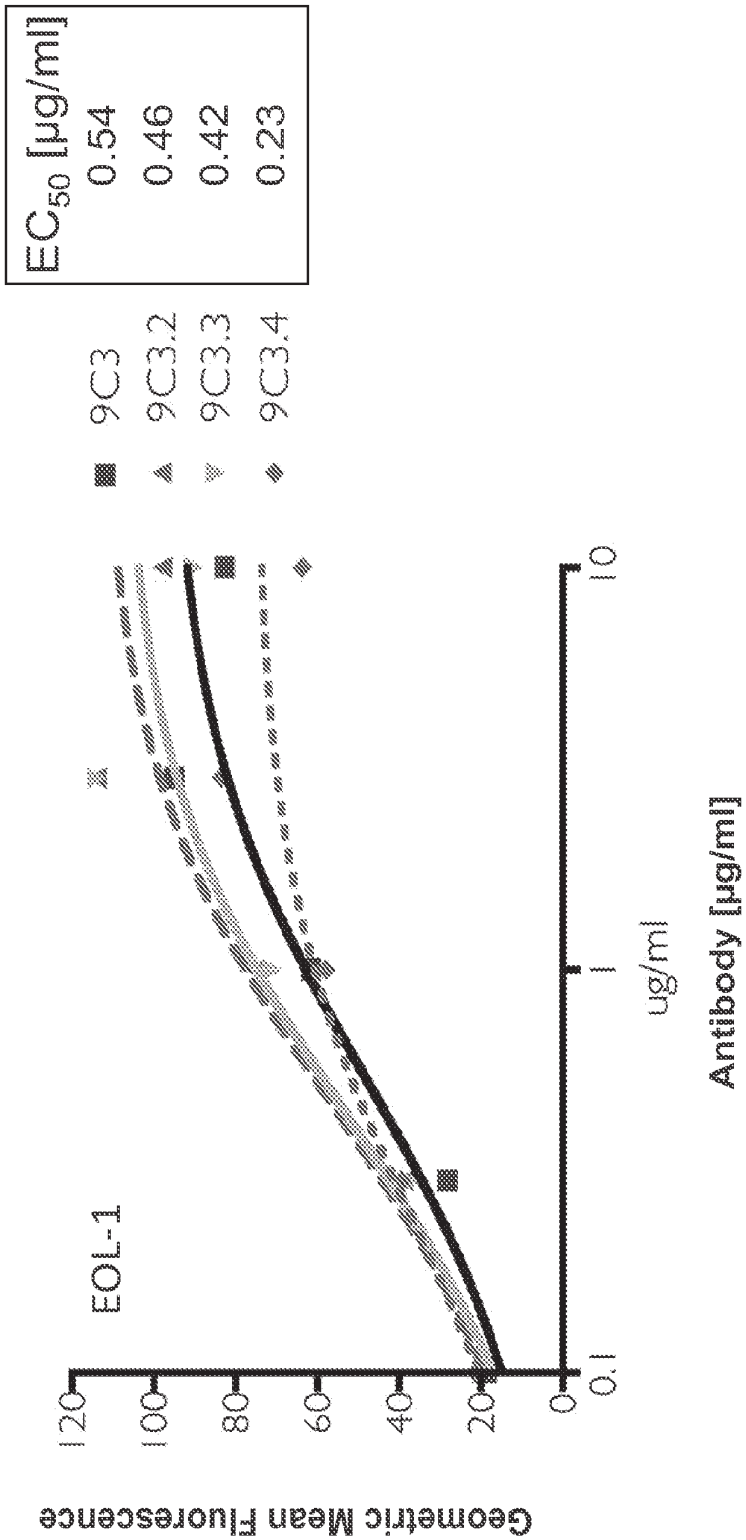

EC50 [µg/ml]
0.54
0.46
0.42
0.23
9C3
9C3.2
9C3.3
9C3.4
EOL-1
Geometric Mean Fluorescence
120
100
80
60
40
20
0
0.1
1
10
ug/ml
Antibody [µg/ml]

Figure 12

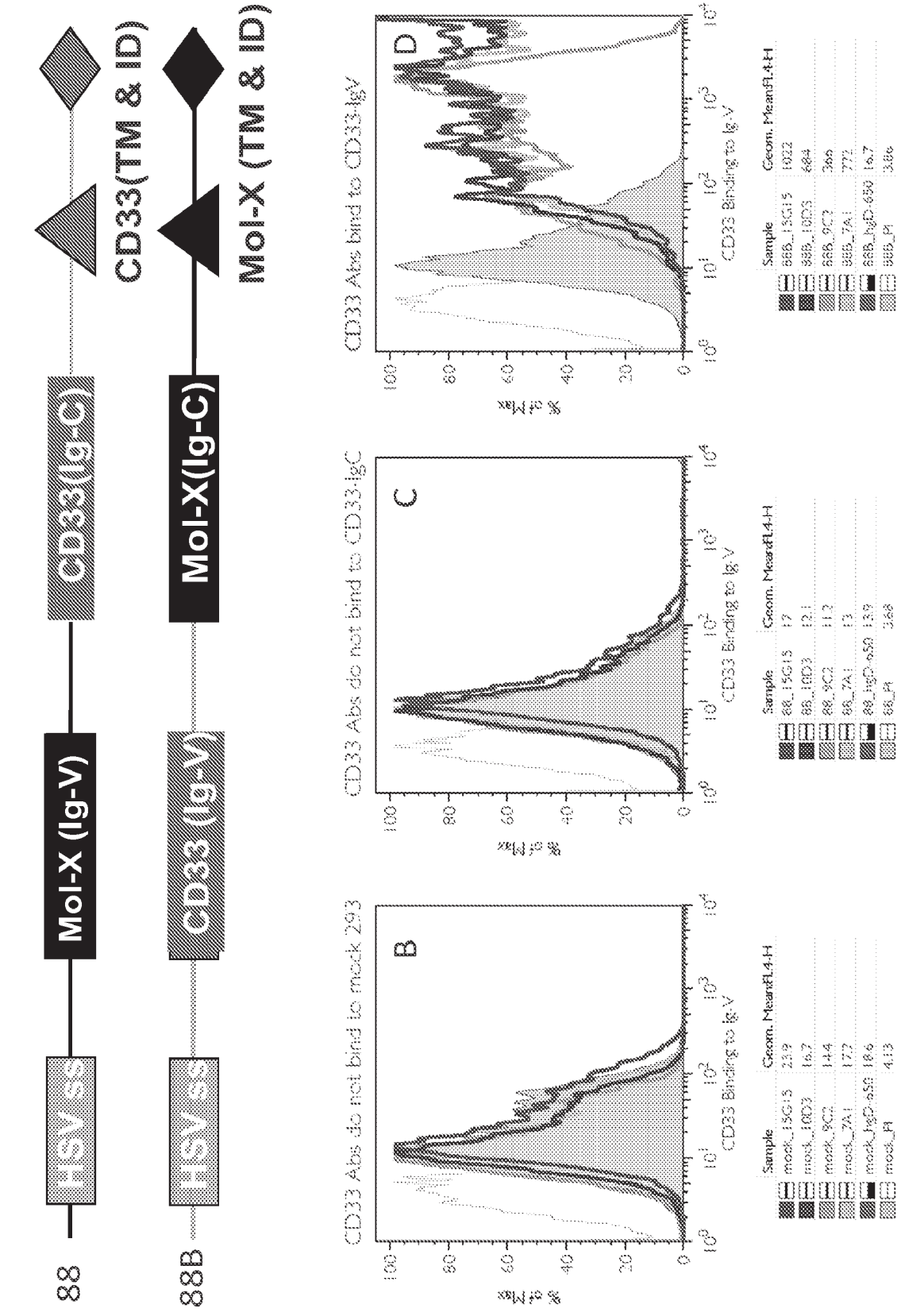
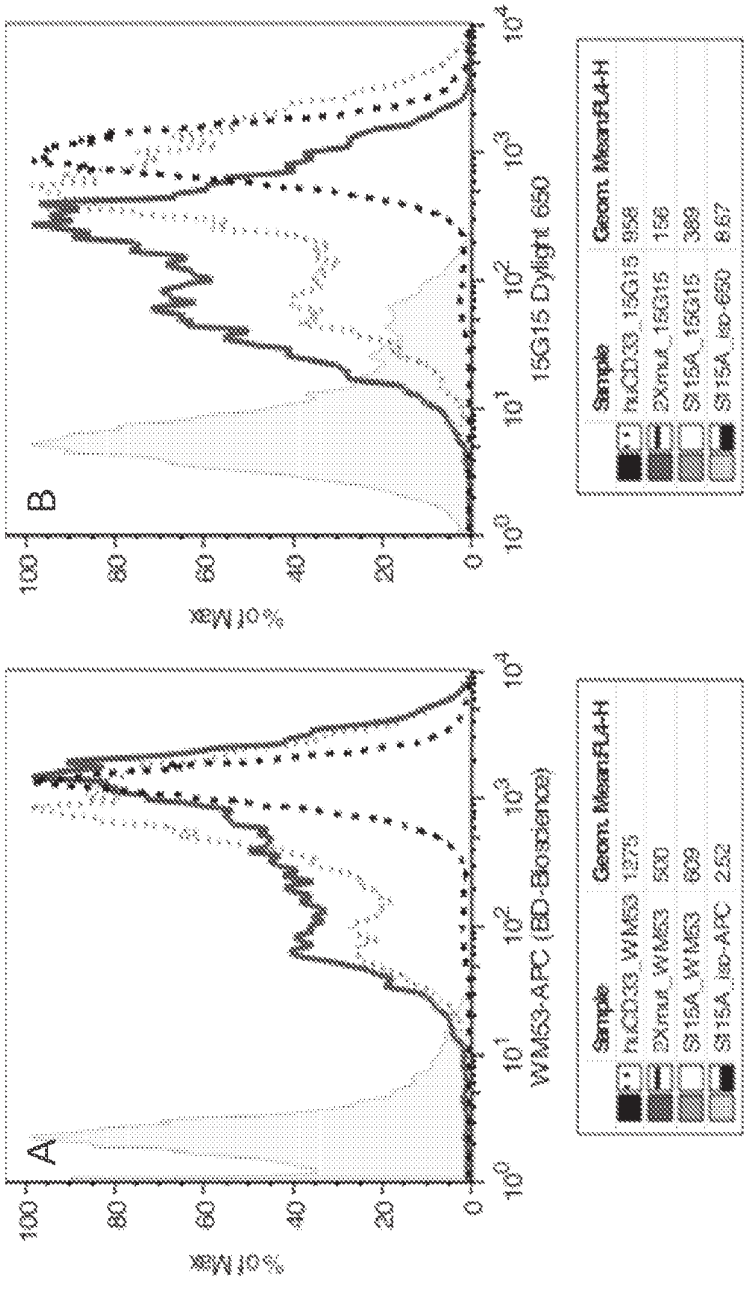

A
88
HSV ss
MoI-X (Ig-V)
CD33(Ig-C)
CD33(TM & ID)
88B
HSV ss
CD33 (Ig-V)
MoI-X(Ig-C)
MoI-X (TM & ID)
B
CD33 Abs do not bind to mock 293
C
CD33 Abs do not bind to CD33-IgC
D
CD33 Abs bind to CD33-IgV
% of Max
CD33 Binding to Ig-V
Sample
Geom. MeanFL4-H
mock_15G15 219
mock_18D3 162
mock_9C2 144
mock_7A1 172
mock_IgD-a50 186
mock_PI 413
88_15G15 17
88_18D3 12.1
88_9C2 11.2
88_7A1 13
88_IgD-a50 13.9
88_PI 3.68
88B_15G15 1922
88B_18D3 684
88B_9C2 365
88B_7A1 772
88B_IgD-650 16.7
88B_PI 3.86

Figure 13


A
B
% of Max
WM53-APC (BD-Biosciences)
15G15 Dylight 650
Sample
Geom. Mean:FL4-H
huCD33_15G15 956
2Xmut_15G15 156
S15A_15G15 389
S15A_iso-650 867
huCD33_WM53 1275
2Xmut_WM53 500
S15A_WM53 809
S15A_iso-APC 252

C

huCD33_0-99
2_huCD33_R69
1 MDPNFWLQVQESVTVQEGLCVLVPCTFFHPIPYYDKNSPVHGYWFREGAI 50
1 MDPNFWLQVQESVTVQEGLCVLVPCTFFHPIPYYDKNSPVHGYWFREGAI 50
(S102A)
(S115A)
huCD33_0-99
2_huCD33_R69
51 ISGDSPVATNKLDQEVQEETQGRFRLLGDPSRNNCSLSIVDARRRDNGSY 100
51 ISRDSPVATNKLDQEVQEETQGRFRLLGDPSRNNCSLSIVDARRRDNGSY 100
huCD33_0-99
2_huCD33_R69
101 FFRMERGSTKYSYKSPQLS 119
101 FFRMERGSIKYSYKSPQLS 119
Ig-V Like Domain

Figure 14

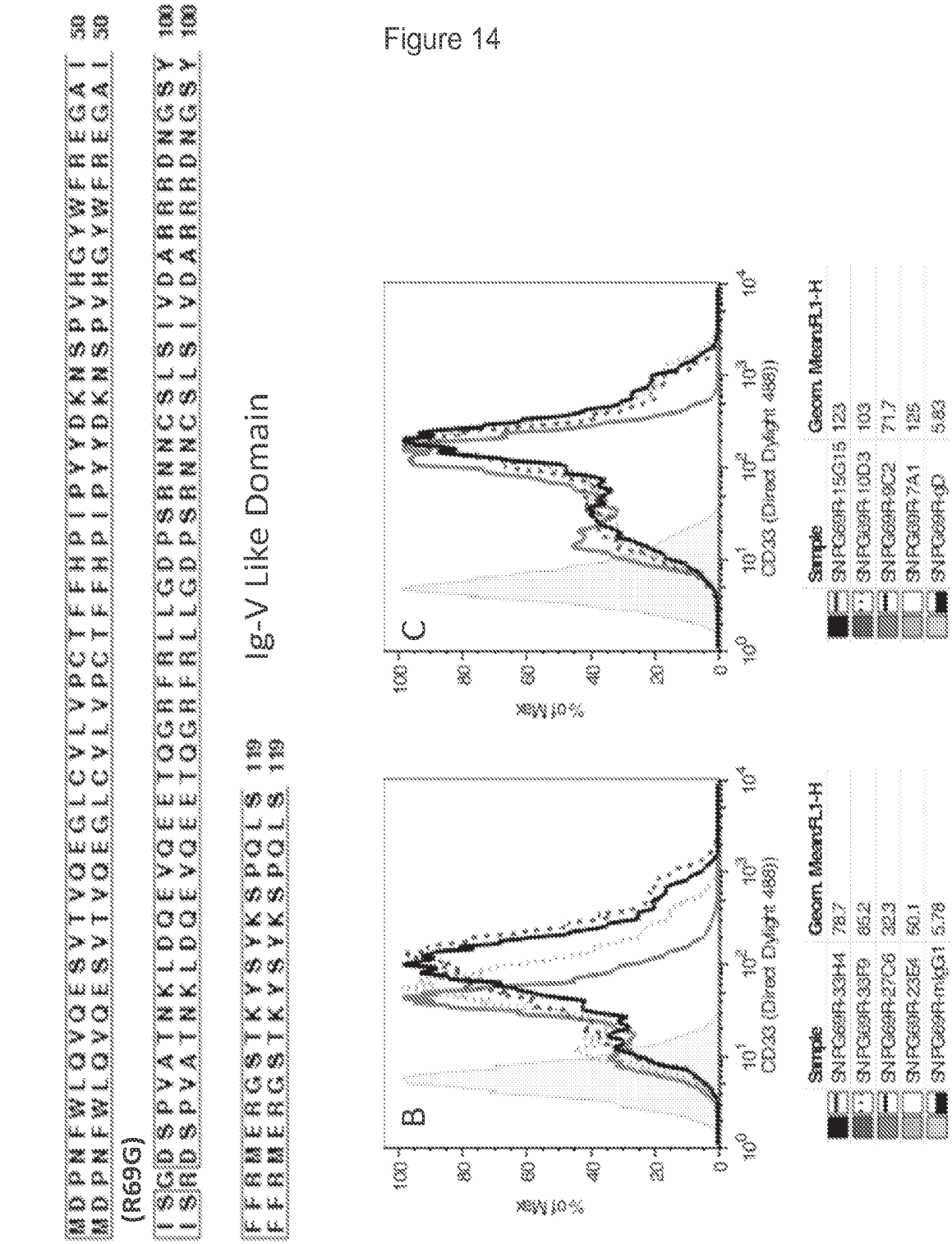
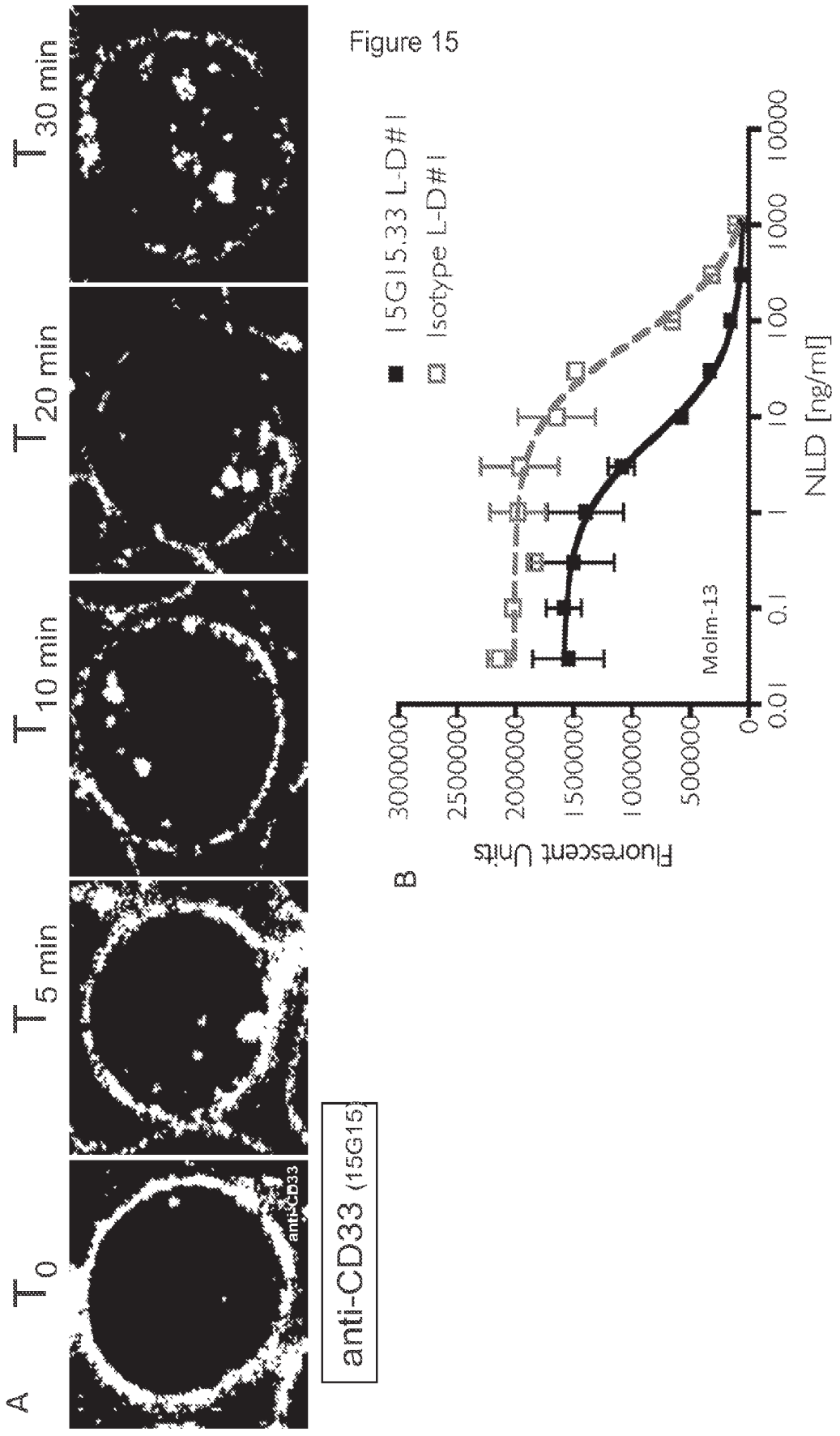

A
Ig-V Like Domain
(R69G)
B
C
% of Max
CD33 (Direct Dylight 488))
Sample
Geom. Mean(RL1-H
SNPG69R-33H4 78.7
SNPG69R-33F9 65.2
SNPG69R-27C6 32.9
SNPG69R-23E4 50.1
SNPG69R-mIgG1 5.78
SNPG69R-15G15 123
SNPG69R-10D3 103
SNPG69R-9C2 71.7
SNPG69R-7A1 125
SNPG69R-gD 5.83

Figure 15


A
T0
T5 min
T10 min
T20 min
T30 min
anti-CD33
anti-CD33 (15G15)
B
15G15.33 L-D#1
Isotype L-D#1
Fluorescent Units
3000000
2500000
2000000
1500000
1000000
500000
0
Molm-13
0.01
0.1
1
10
100
1000
10000
NLD [ng/ml]

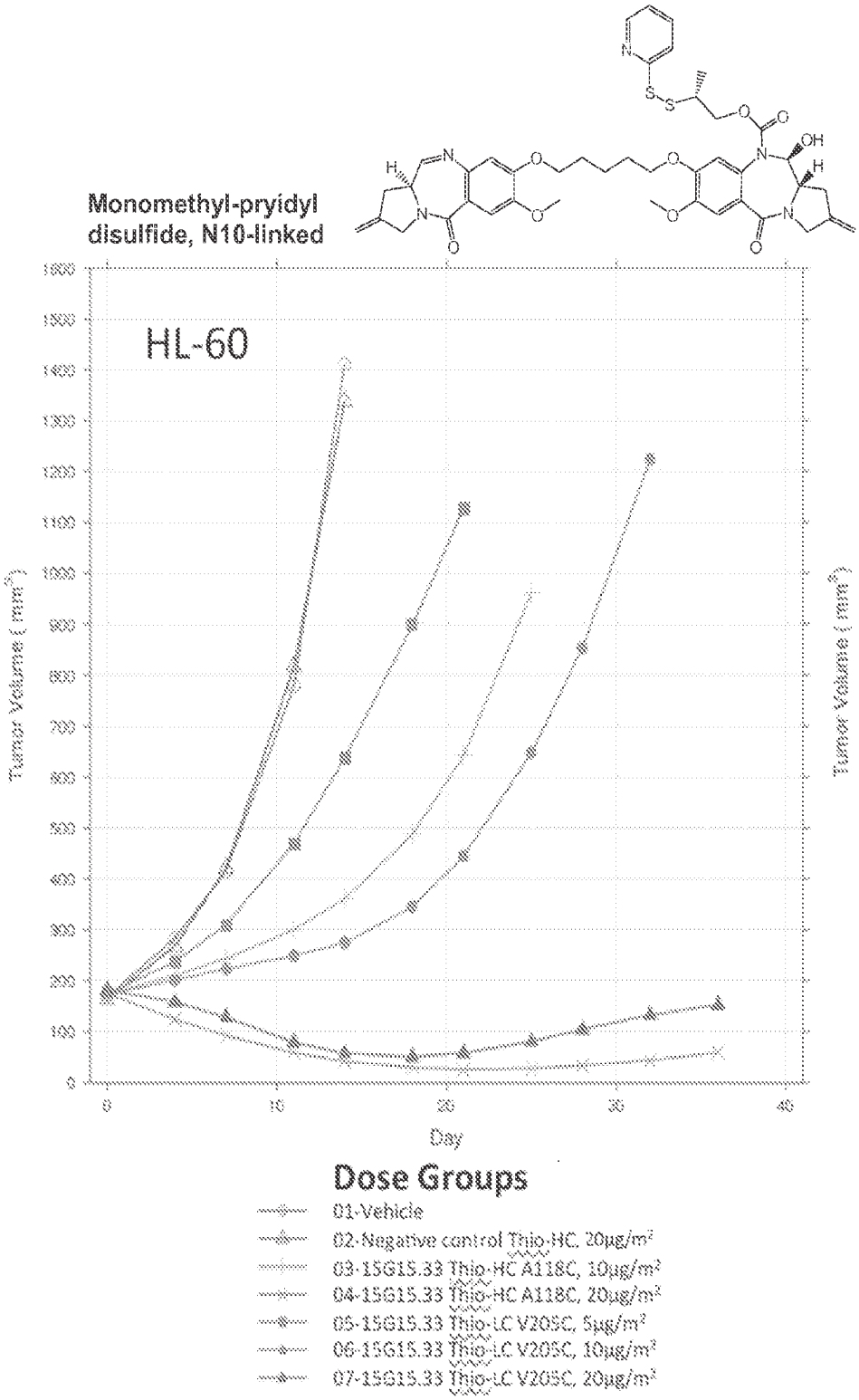

Monomethyl-pryidyl disulfide, N10-linked
HL-60
Tumor Volume (mm³)
Day
Dose Groups
01-Vehicle
02-Negative control Thio-HC, 20µg/m²
03-15G15.33 Thio-HC A118C, 10µg/m²
04-15G15.33 Thio-HC A118C, 20µg/m²
05-15G15.33 Thio-LC V205C, 5µg/m²
06-15G15.33 Thio-LC V205C, 10µg/m²
07-15G15.33 Thio-LC V205C, 20µg/m²

FIG. 16A

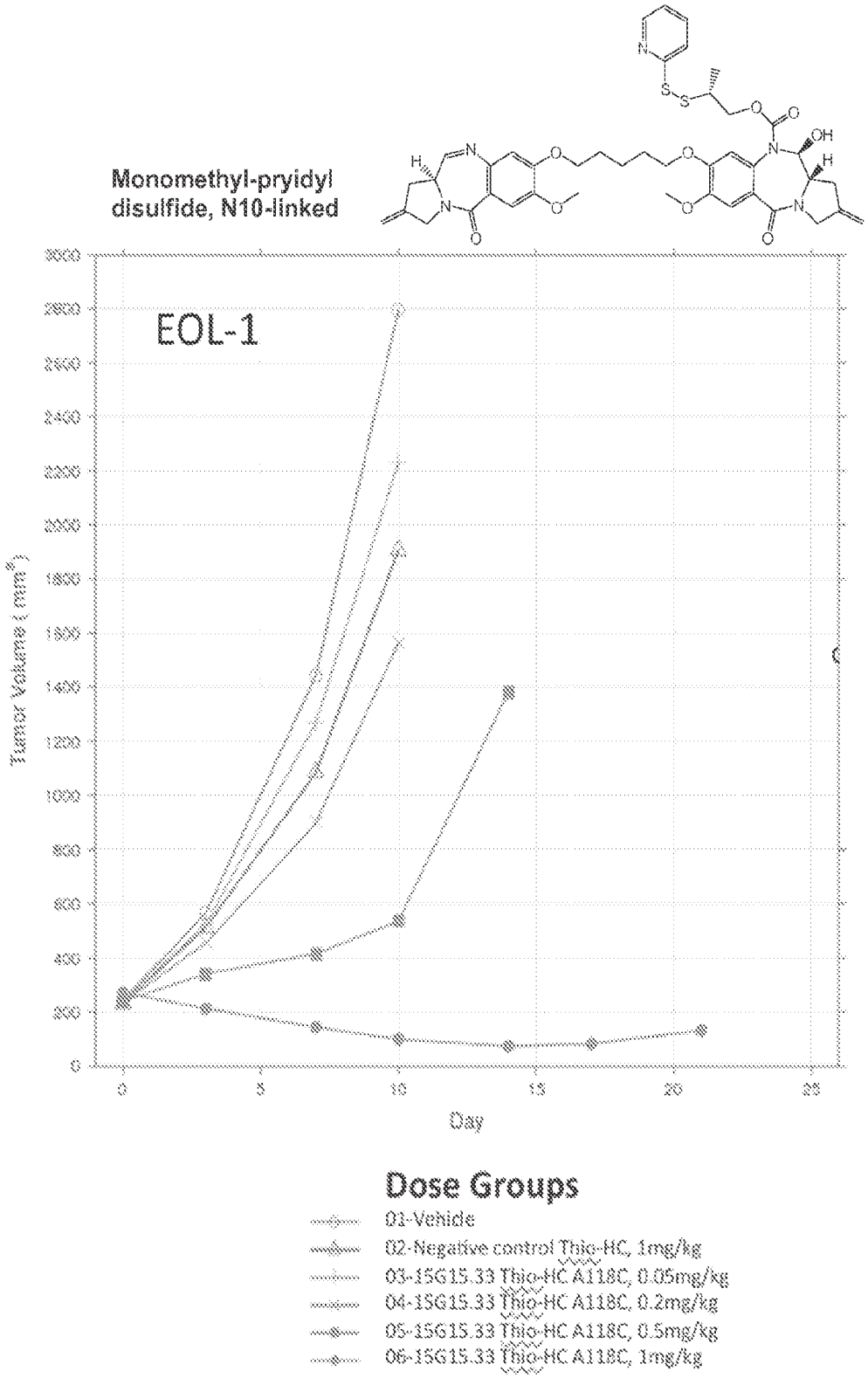
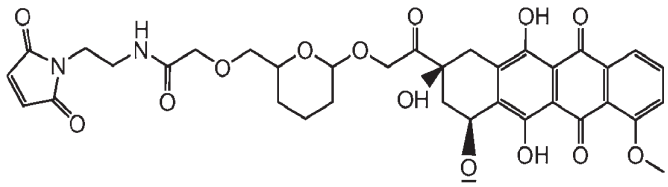
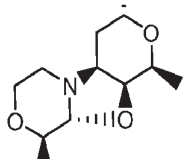
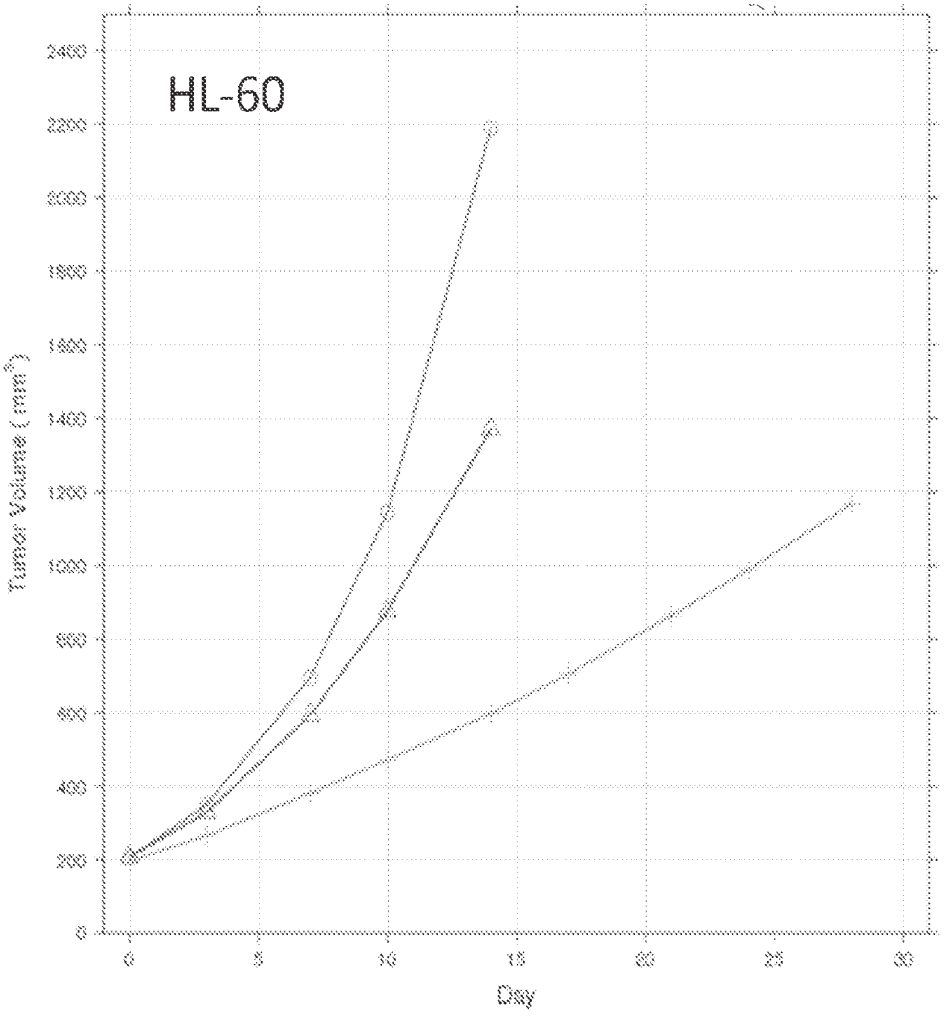

Monomethyl-pryidyl disulfide, N10-linked
EOL-1
Tumor Volume (mm³)
Day
Dose Groups
01-Vehicle
02-Negative control Thio-HC, 1mg/kg
03-15G15.33 Thio-HC A118C, 0.05mg/kg
04-15G15.33 Thio-HC A118C, 0.2mg/kg
05-15G15.33 Thio-HC A118C, 0.5mg/kg
06-15G15.33 Thio-HC A118C, 1mg/kg

FIG. 16B



Maleimide with acetal linker-PNU





Dose Groups

01-Vehicle Histidine Buffer #8
05-Negative control Thio-HC, A118C, 200μg/m² (~8.6mg/kg)
08-15G15 Thio-HC A118C, 200μg/m² (~11.9mg/kg)

FIG. 17