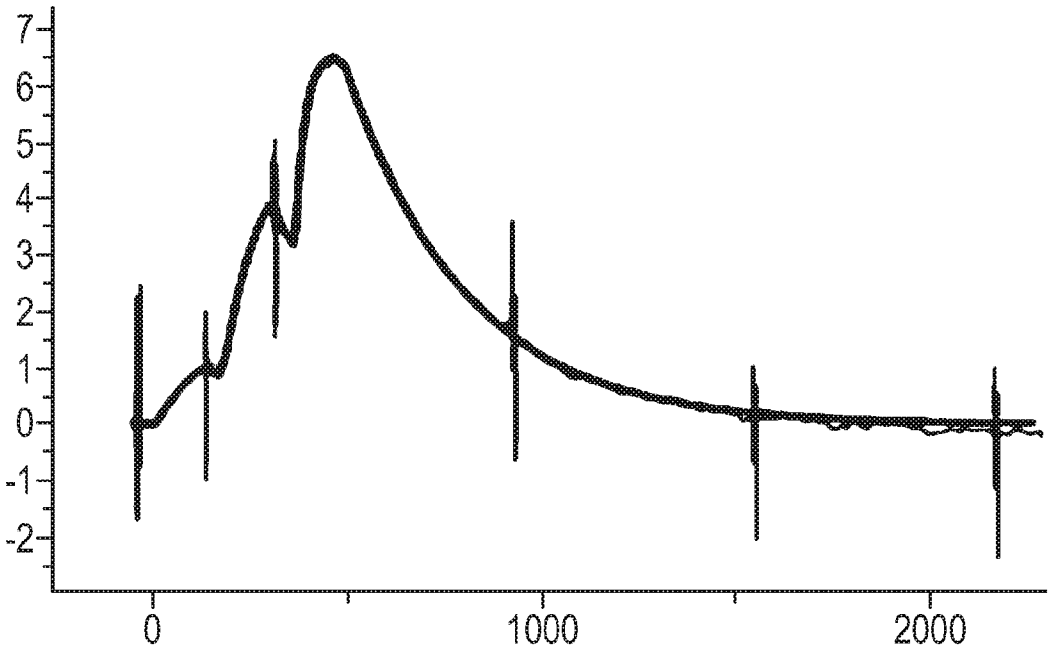
FIG. 1

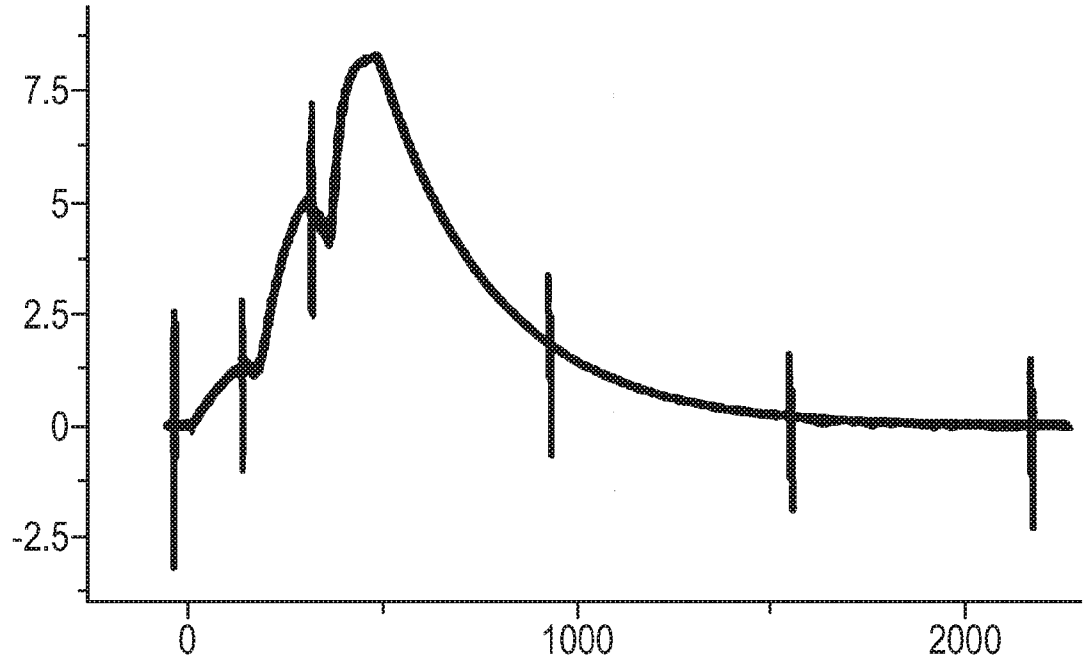
Ligand	CL002405648
Analyte	CD33-hu-domain
Sensogram	Ch 1, Fc 2-1
Temperature	25



Model	Group 1 1:1 binding		
ka{1/Ms}	9.62e+05	kd{1/s}	3.25e+03
Rmax{RU}	7.1	KD {M}	3.38e-09

FIG. 1(contd)

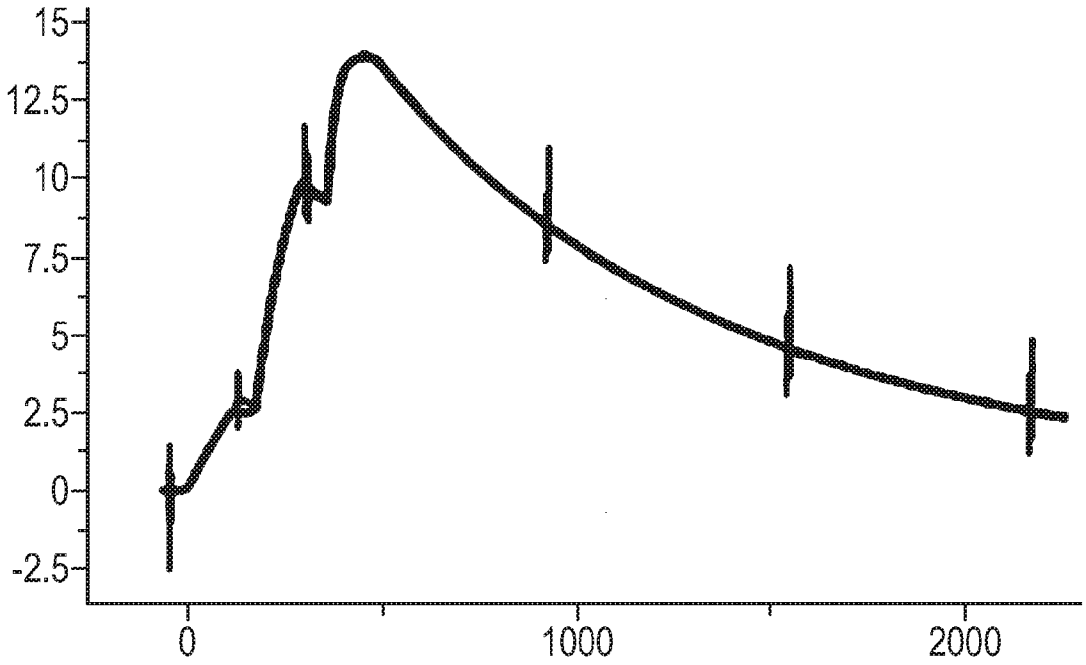
Ligand	CL002405784
Analyte	CD33-hu-domain
Sensogram	Ch 6, Fc 2-1
Temperature	25



Model	Group 6 1:1 binding		
ka{1/Ms}	1.03e+06	kd{1/s}	3.40e-03
Rmax{RU}	9.0	KD {M}	3.31e-09

FIG. 1(contd)

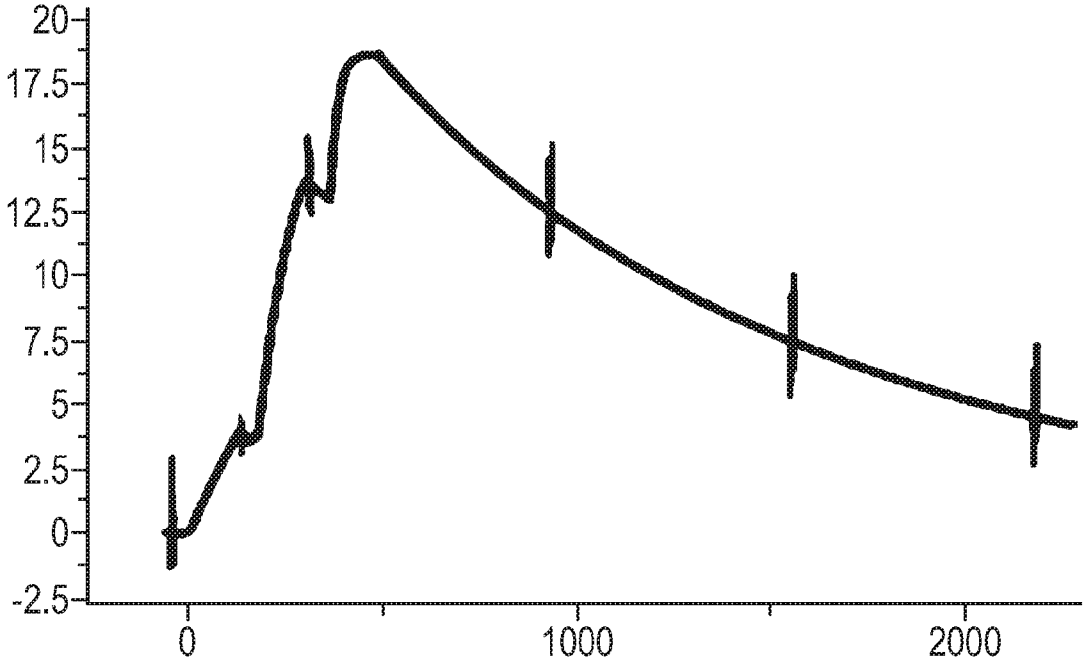
Ligand	CL002405648
Analyte	CD33-hu-FL
Sensogram	Ch 1, Fc 2-1
Temperature	25



Model	Group 9 1:1 binding		
ka{1/Ms}	1.38e+06	kd{1/s}	1.17e-03
Rmax{RU}	14.2	KD {M}	8.47e-10

FIG. 1(contd)

Ligand	CL002405784
Analyte	CD33-hu-FL
Sensogram	Ch 6, Fc 2-1
Temperature	25



Model	Group 19 1:1 binding		
ka{1/Ms}	1.40e+06	kd{1/s}	9.28e-04
Rmax{RU}	18.9	KD {M}	6.61e-10

FIG. 2

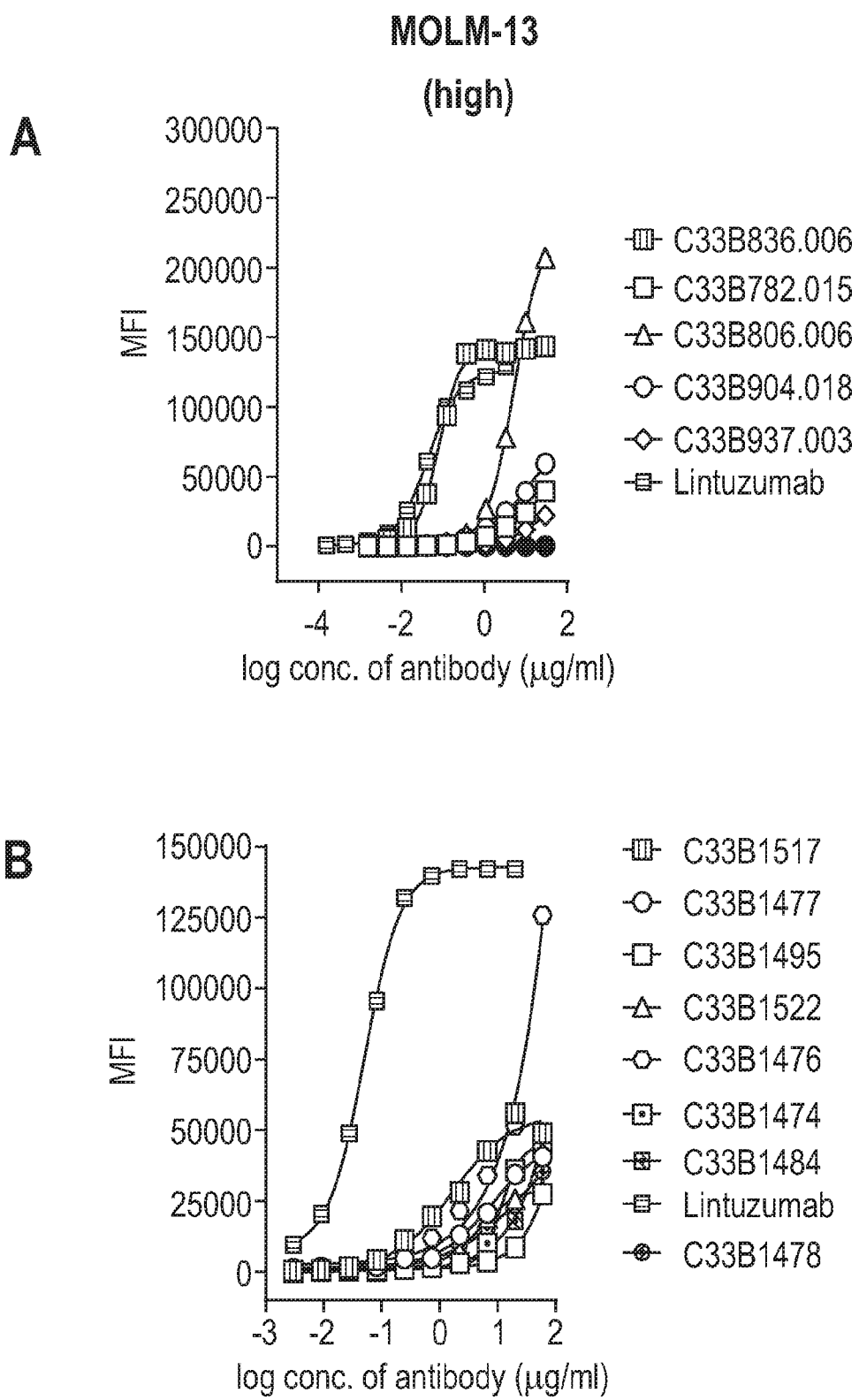

MOLM-13
(high)
A
MFI
300000
250000
200000
150000
100000
50000
0
-4
-2
0
2
log conc. of antibody (µg/ml)
C33B836.006
C33B782.015
C33B806.006
C33B904.018
C33B937.003
Lintuzumab
B
MFI
150000
125000
100000
75000
50000
25000
0
-3
-2
-1
0
1
2
log conc. of antibody (µg/ml)
C33B1517
C33B1477
C33B1495
C33B1522
C33B1476
C33B1474
C33B1484
Lintuzumab
C33B1478

FIG. 2(contd)

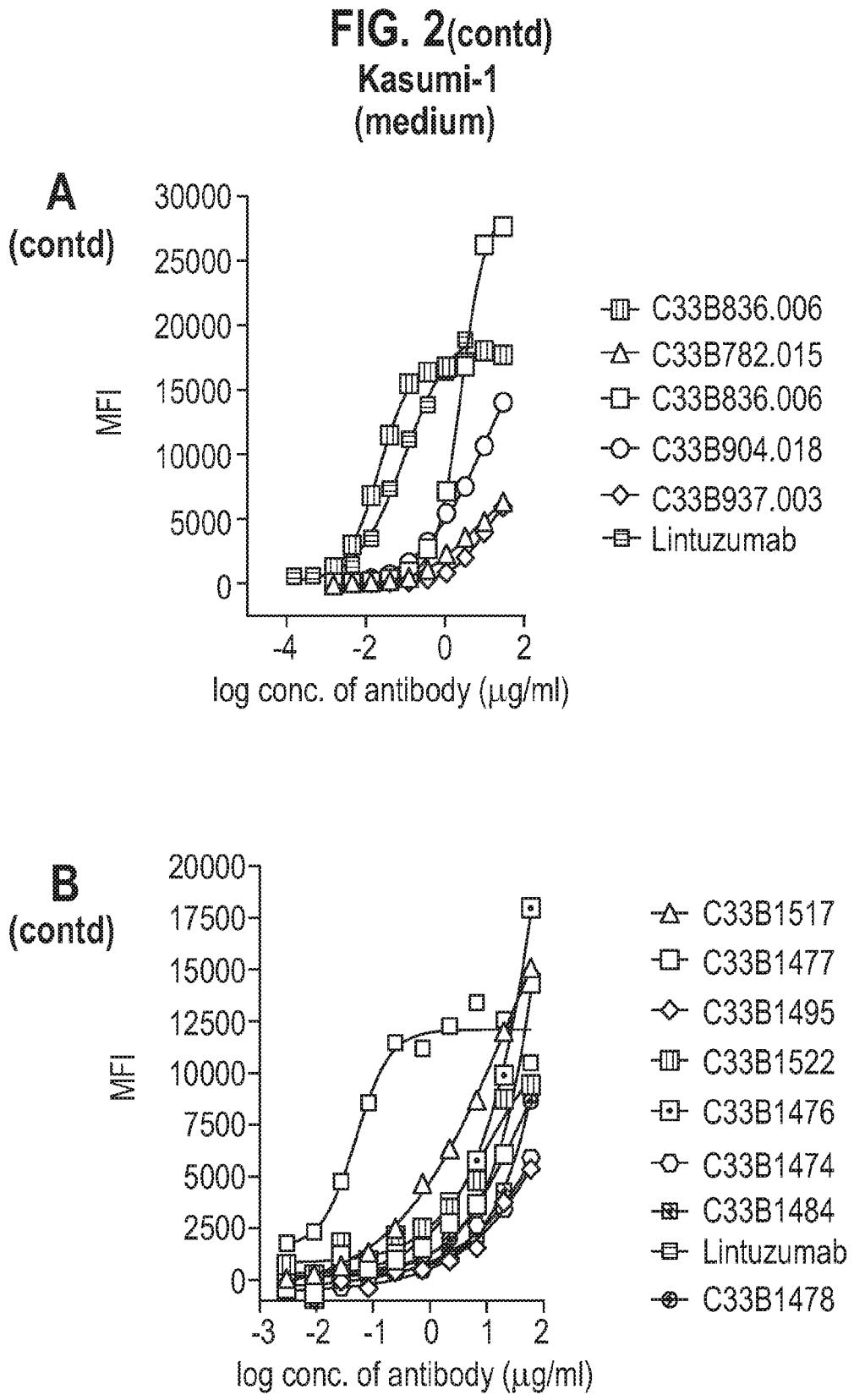

Kasumi-1
(medium)
A
(contd)
MFI
30000
25000
20000
15000
10000
5000
0
-4
-2
0
2
log conc. of antibody (µg/ml)
C33B836.006
C33B782.015
C33B836.006
C33B904.018
C33B937.003
Lintuzumab
B
(contd)
MFI
20000
17500
15000
12500
10000
7500
5000
2500
0
-3
-2
-1
0
1
2
log conc. of antibody (µg/ml)
C33B1517
C33B1477
C33B1495
C33B1522
C33B1476
C33B1474
C33B1484
Lintuzumab
C33B1478

FIG. 2(contd)

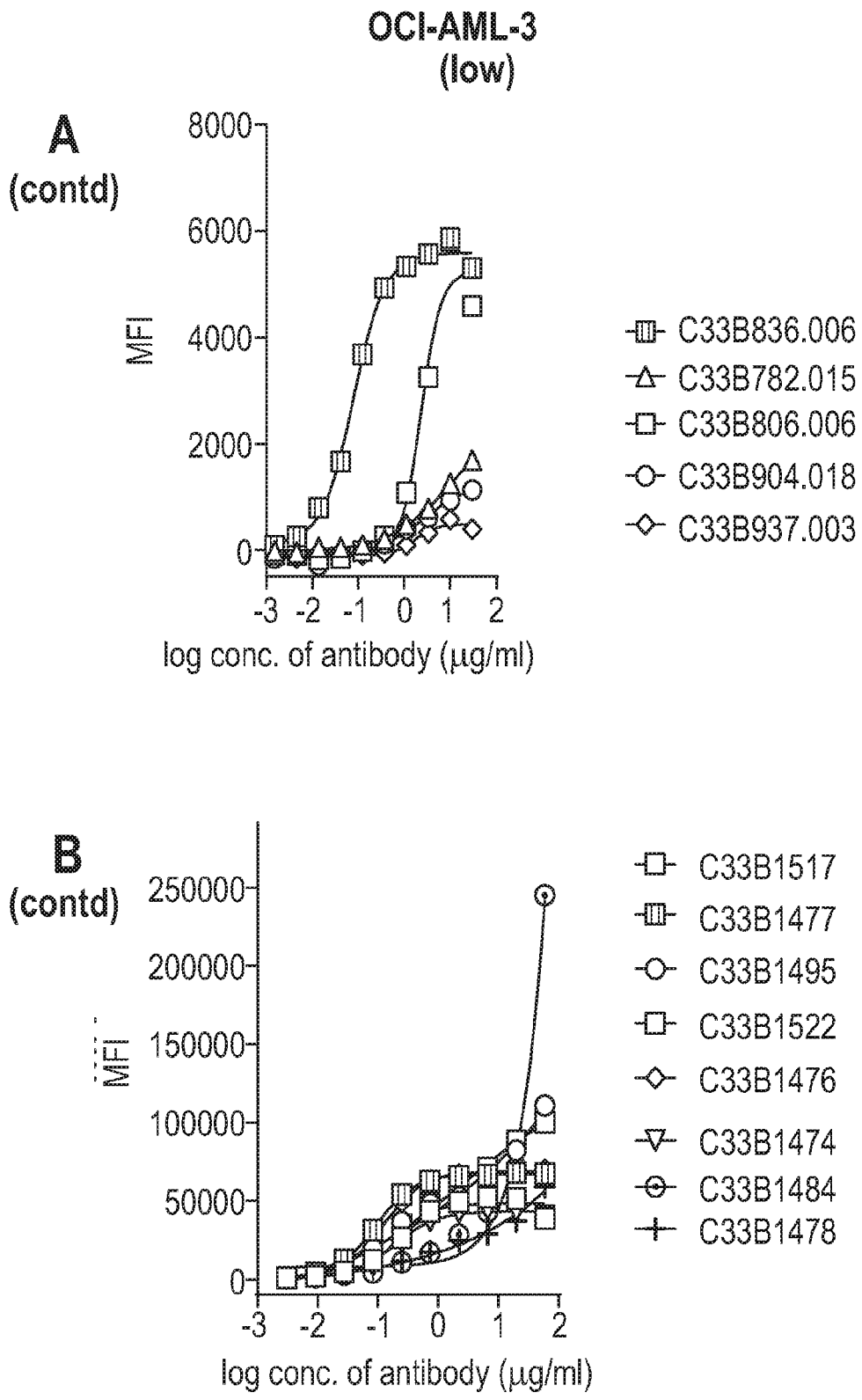
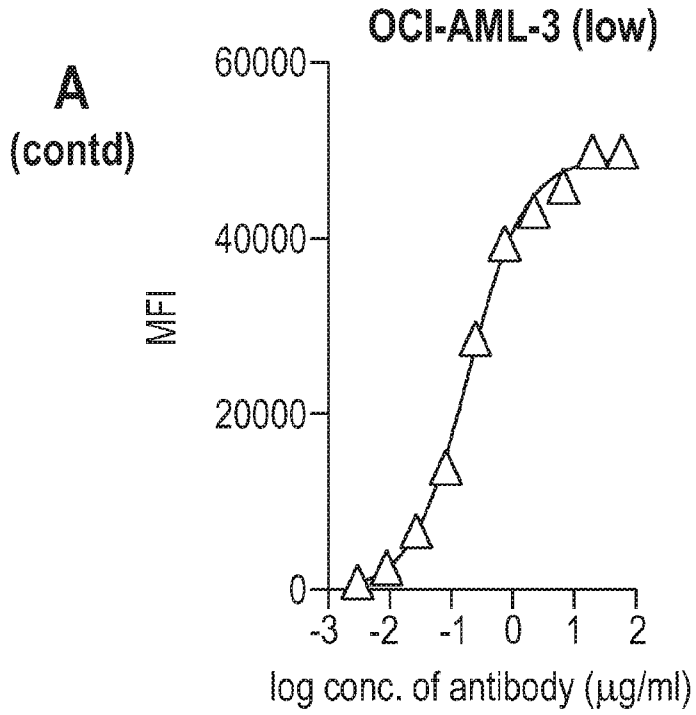

OCI-AML-3
(low)
A
(contd)
MFI
8000
6000
4000
2000
0
-3 -2 -1 0 1 2
log conc. of antibody (µg/ml)
C33B836.006
C33B782.015
C33B806.006
C33B904.018
C33B937.003
B
(contd)
MFI
250000
200000
150000
100000
50000
0
-3 -2 -1 0 1 2
log conc. of antibody (µg/ml)
C33B1517
C33B1477
C33B1495
C33B1522
C33B1476
C33B1474
C33B1484
C33B1478

FIG. 2(contd)


A
(contd)
OCI-AML-3 (low)
60000
40000
20000
0
MFI
-3 -2 -1 0 1 2
log conc. of antibody (µg/ml)

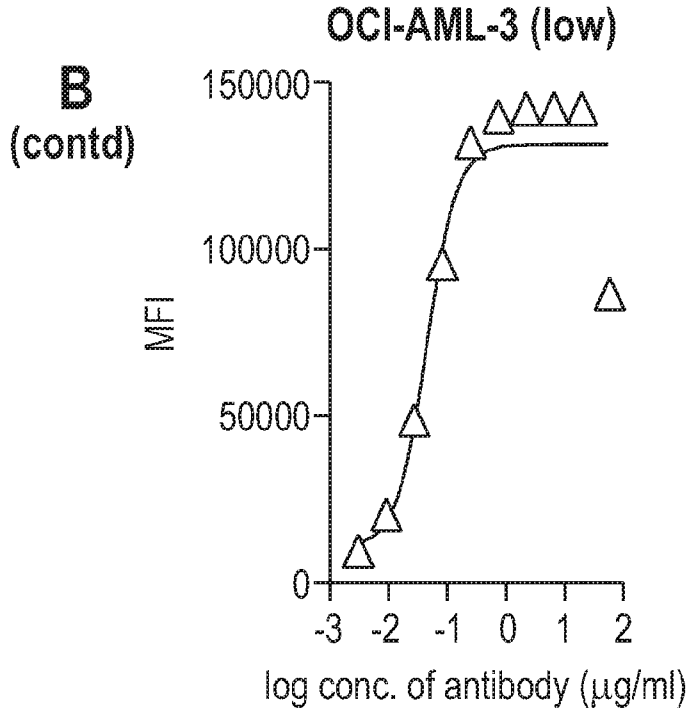

B
(contd)
OCI-AML-3 (low)
150000
100000
50000
0
MFI
-3 -2 -1 0 1 2
log conc. of antibody (µg/ml)

FIG. 3

C33B806.006

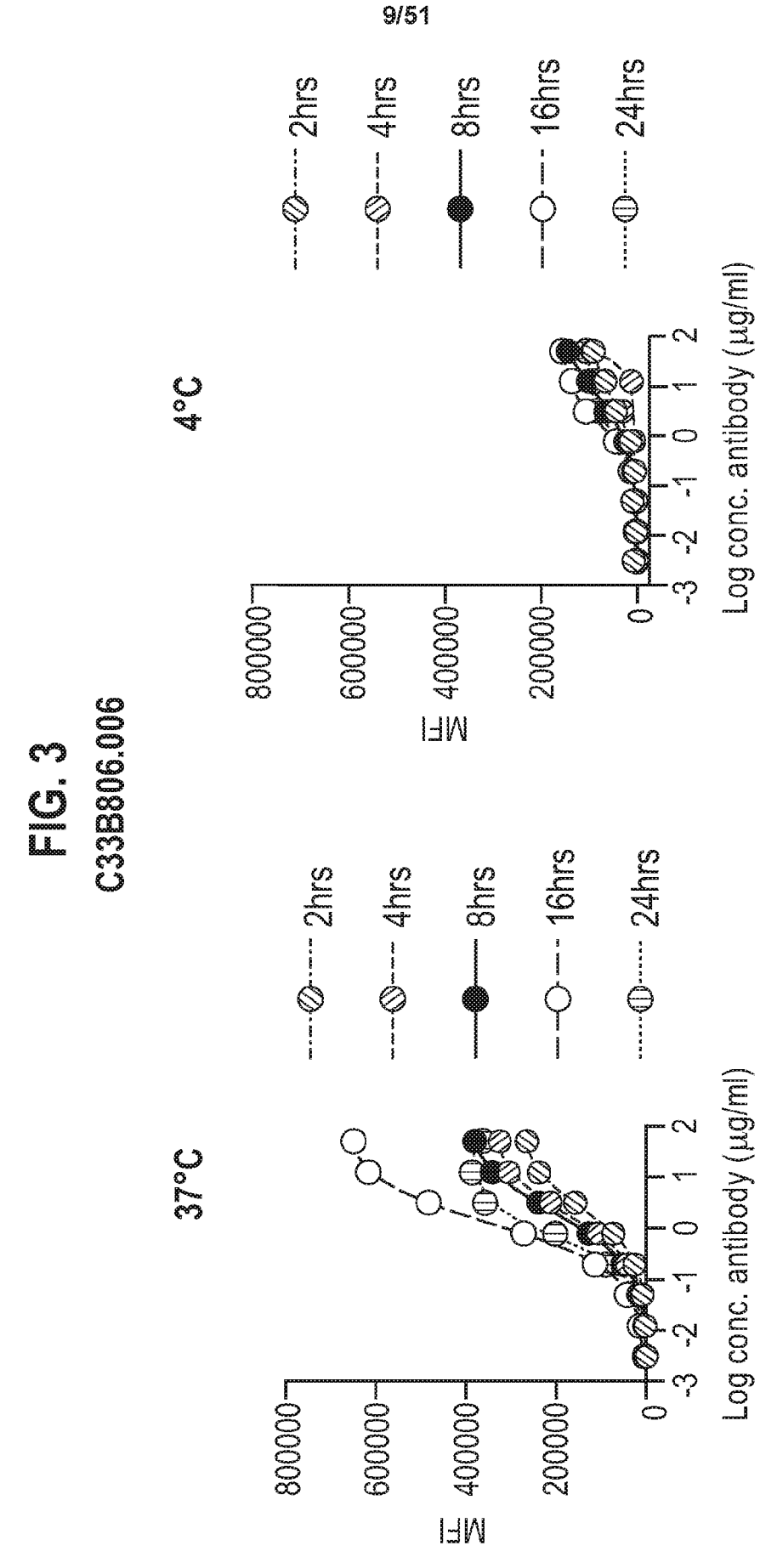


FIG. 3(contd)
C33B782.015

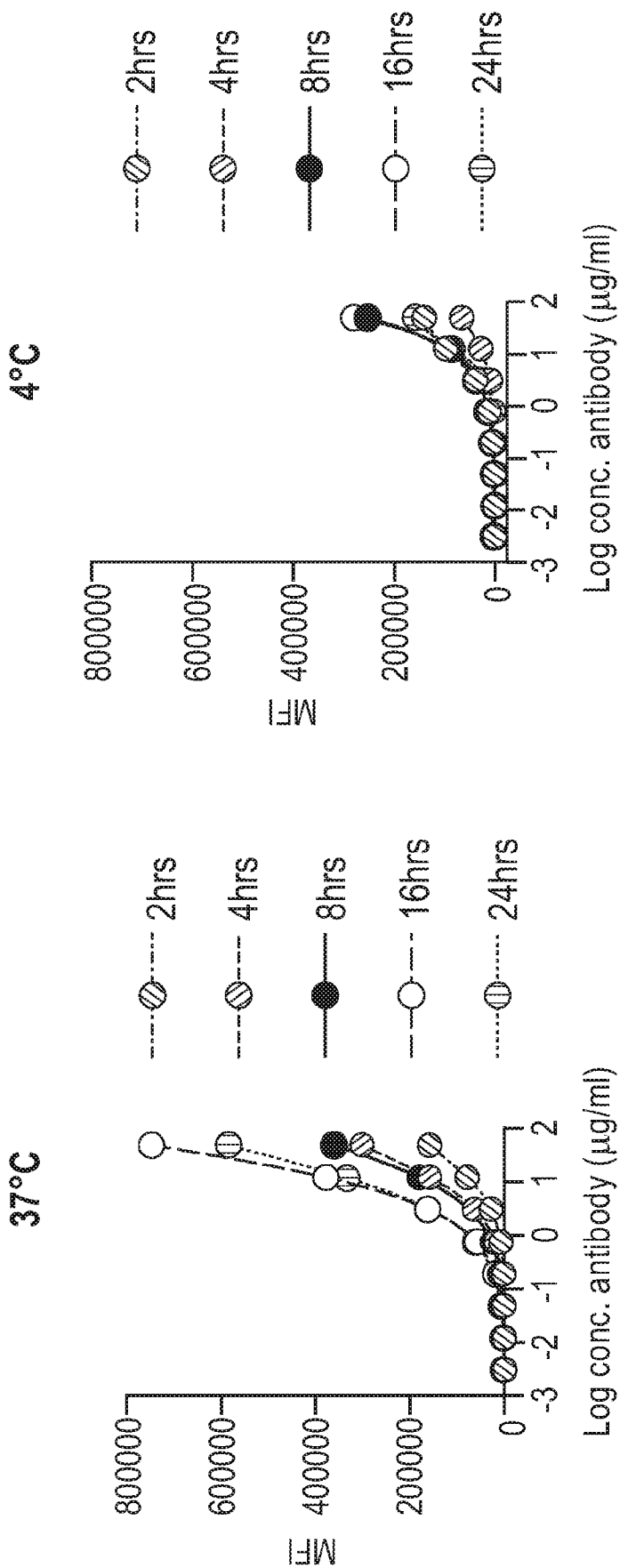


FIG. 3(contd)

C33B904.01

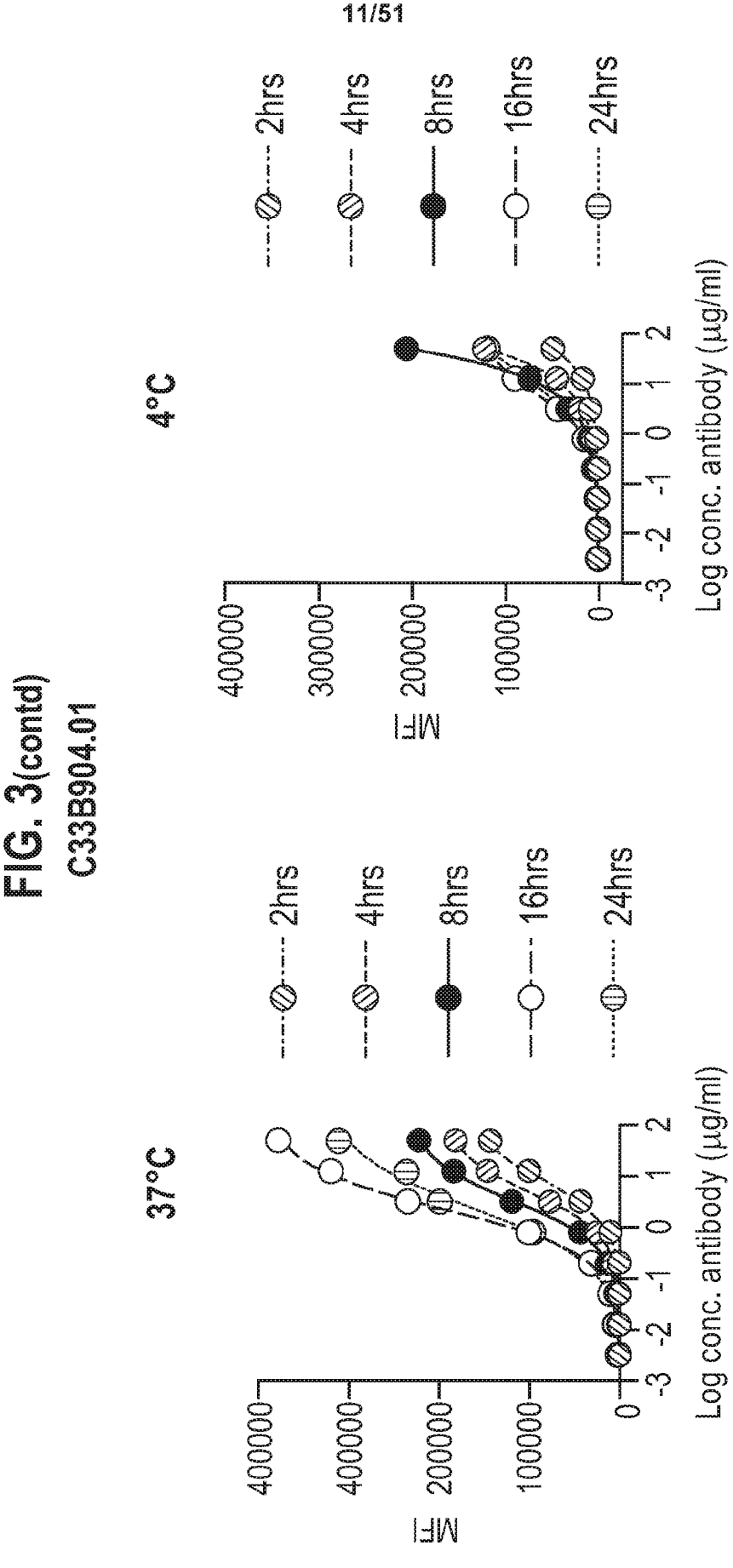


FIG. 3(contd)

C33B937.003

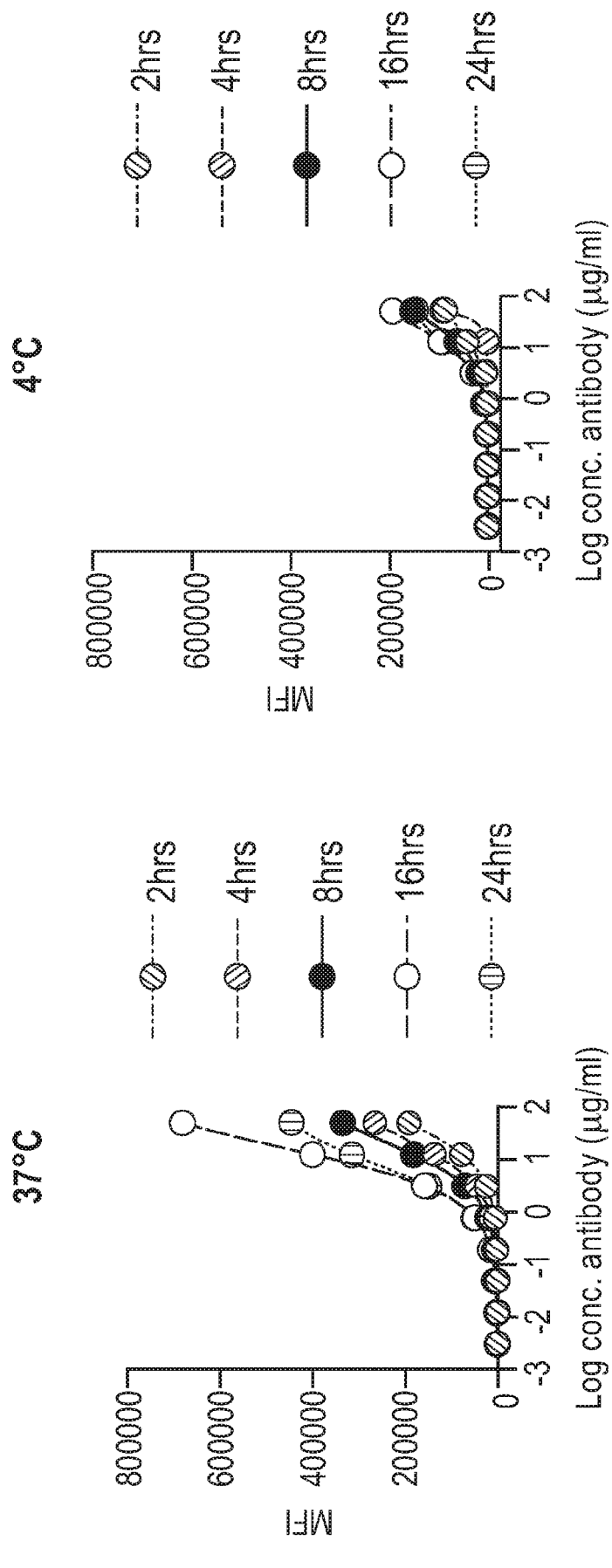


FIG. 3(contd)
C33B836.006

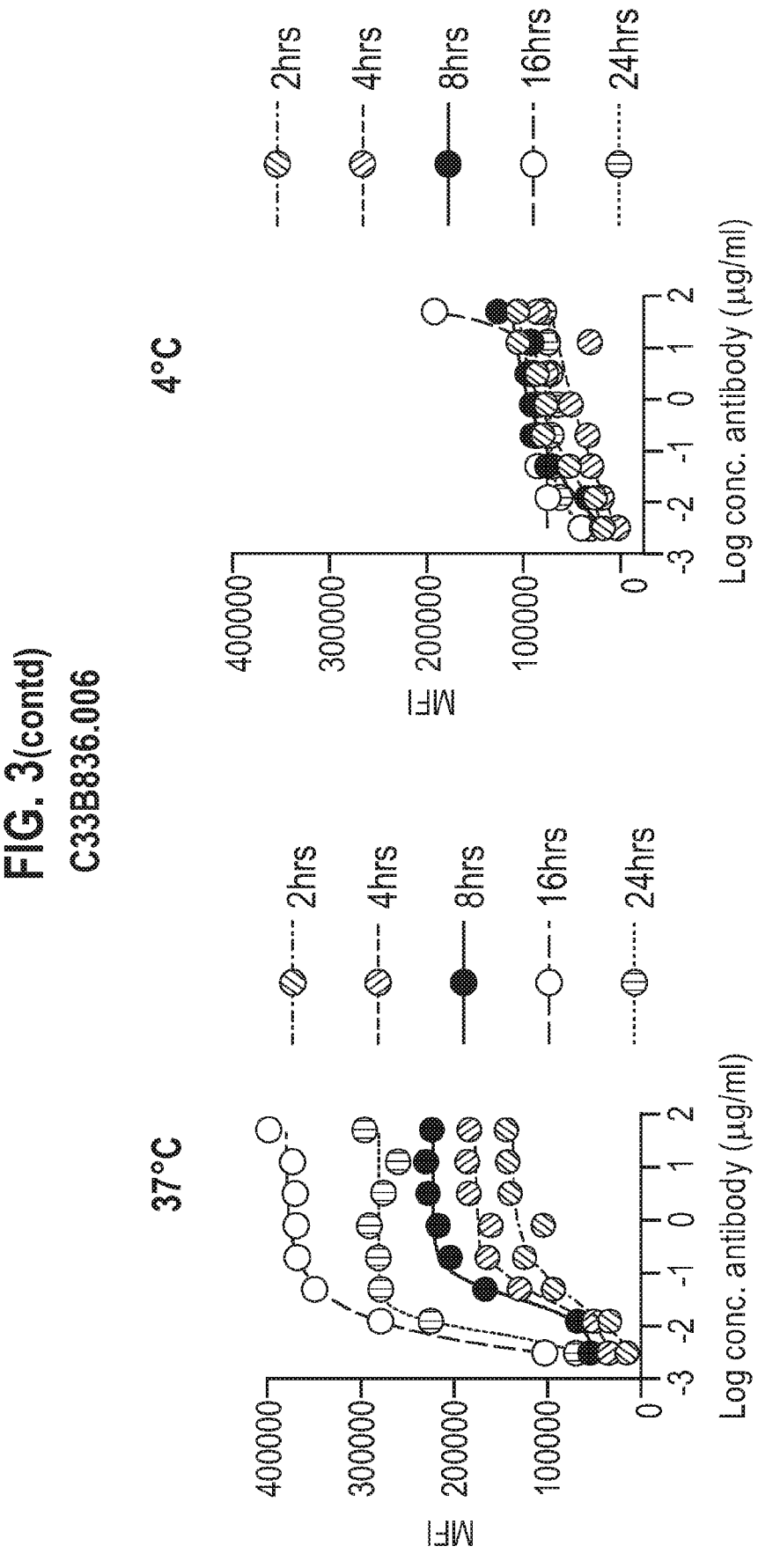


FIG. 3(contd)

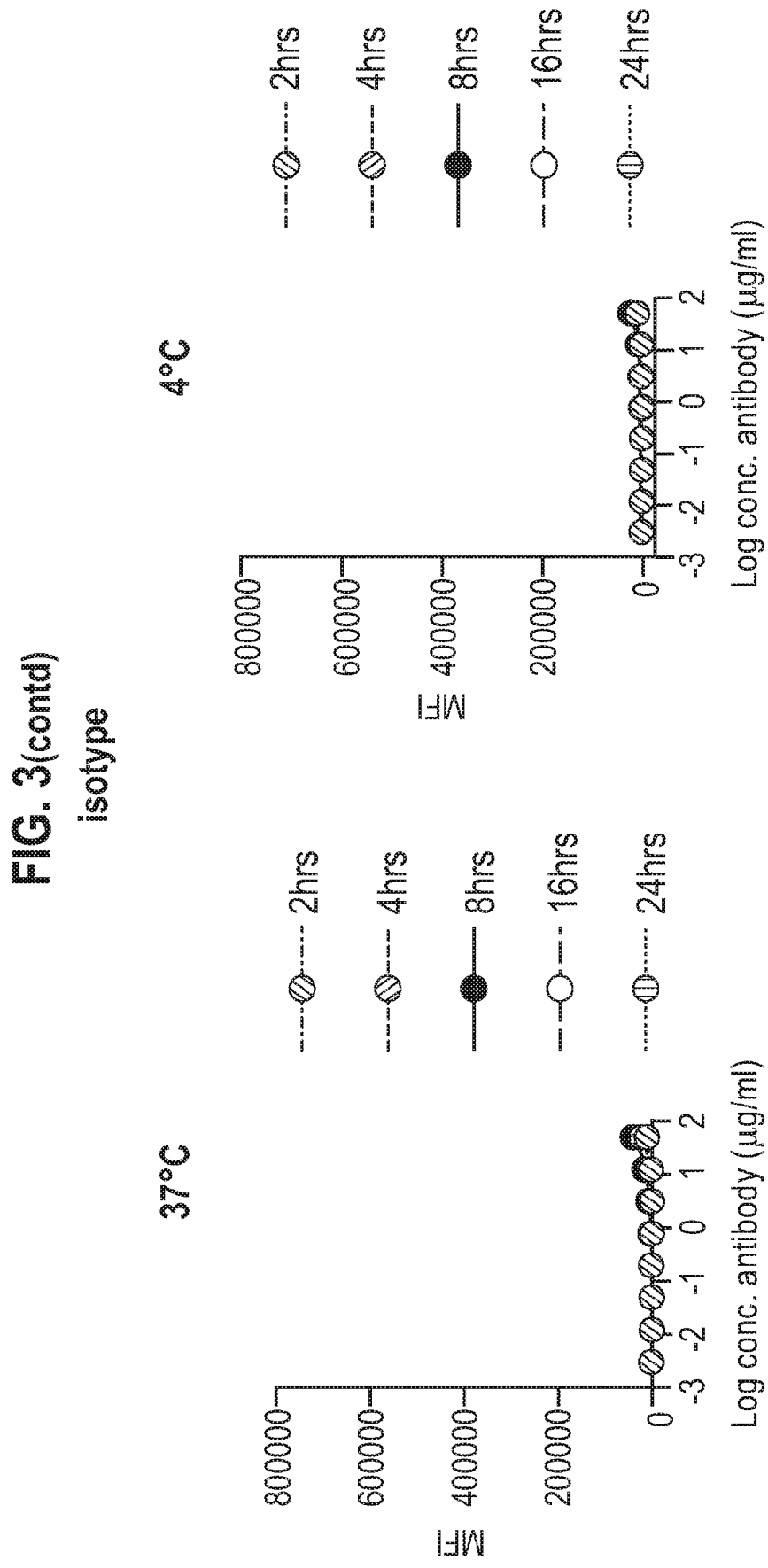

isotype
37°C
4°C
MFI
800000
600000
400000
200000
0
-3
-2
-1
0
1
2
Log conc. antibody (µg/ml)
2hrs
4hrs
8hrs
16hrs
24hrs

FIG. 3(contd)

sCD33B1474

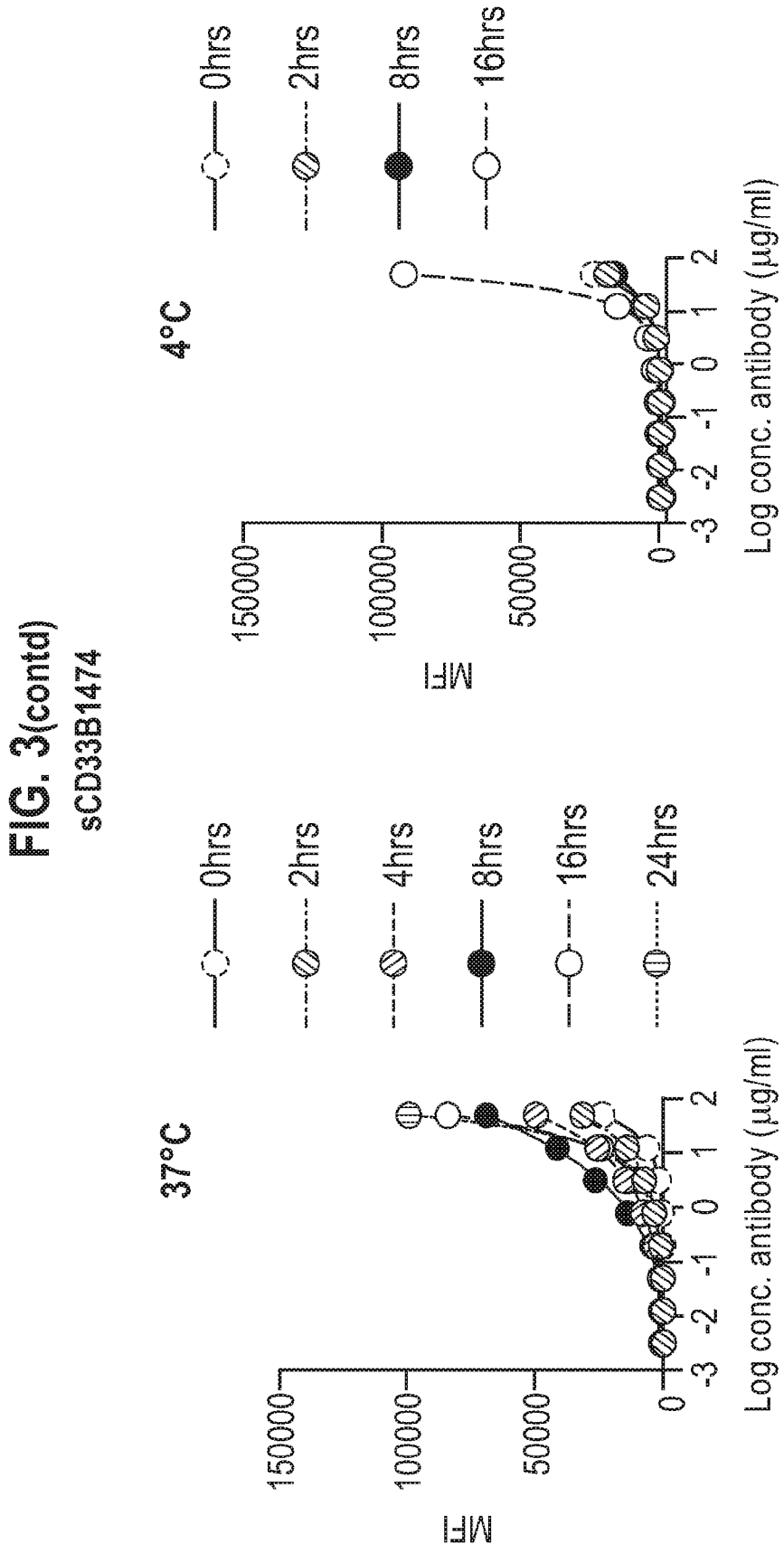


FIG. 3(contd)
sCD33B1478

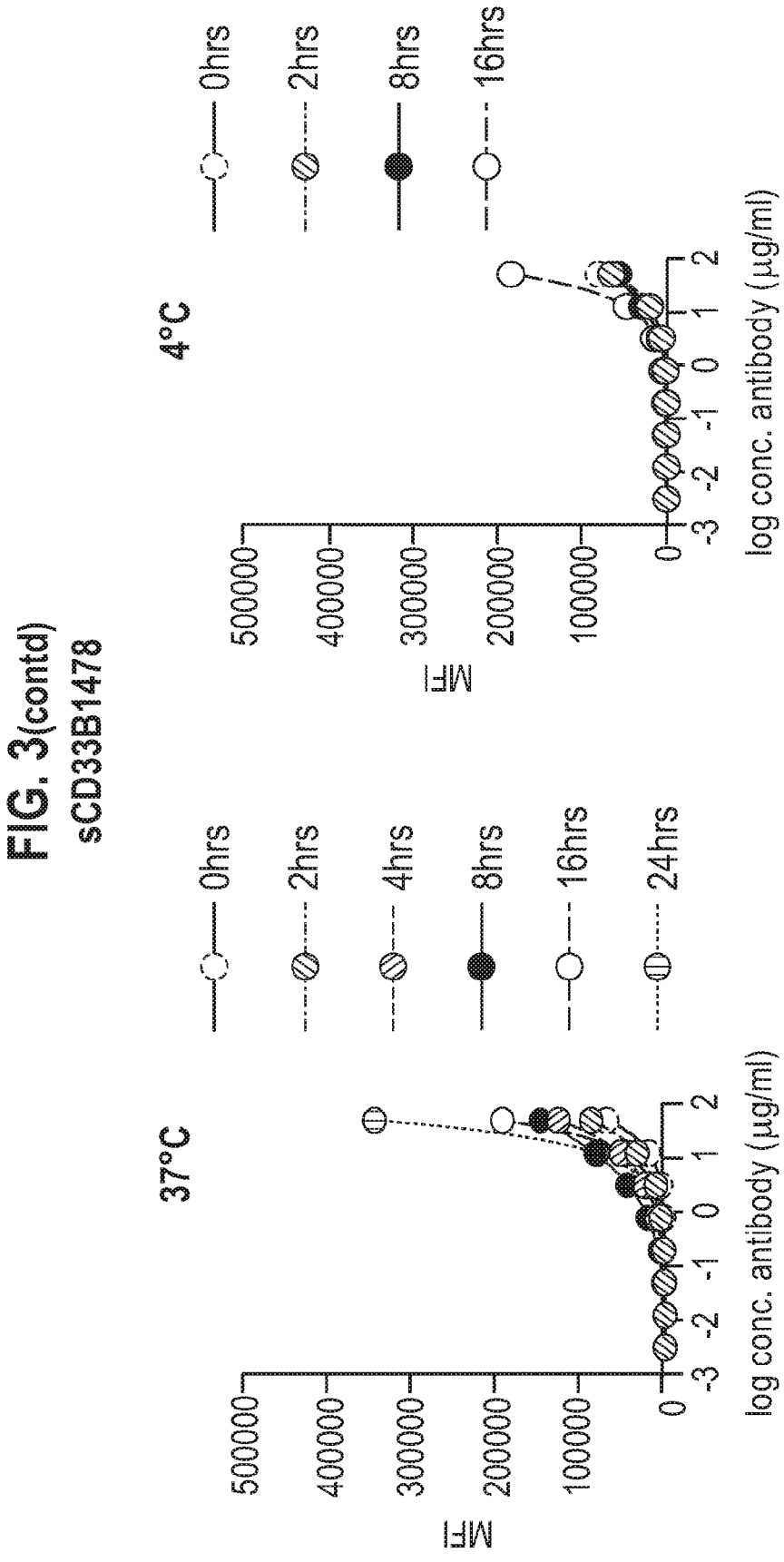


FIG. 3(contd)
sCD33B1517

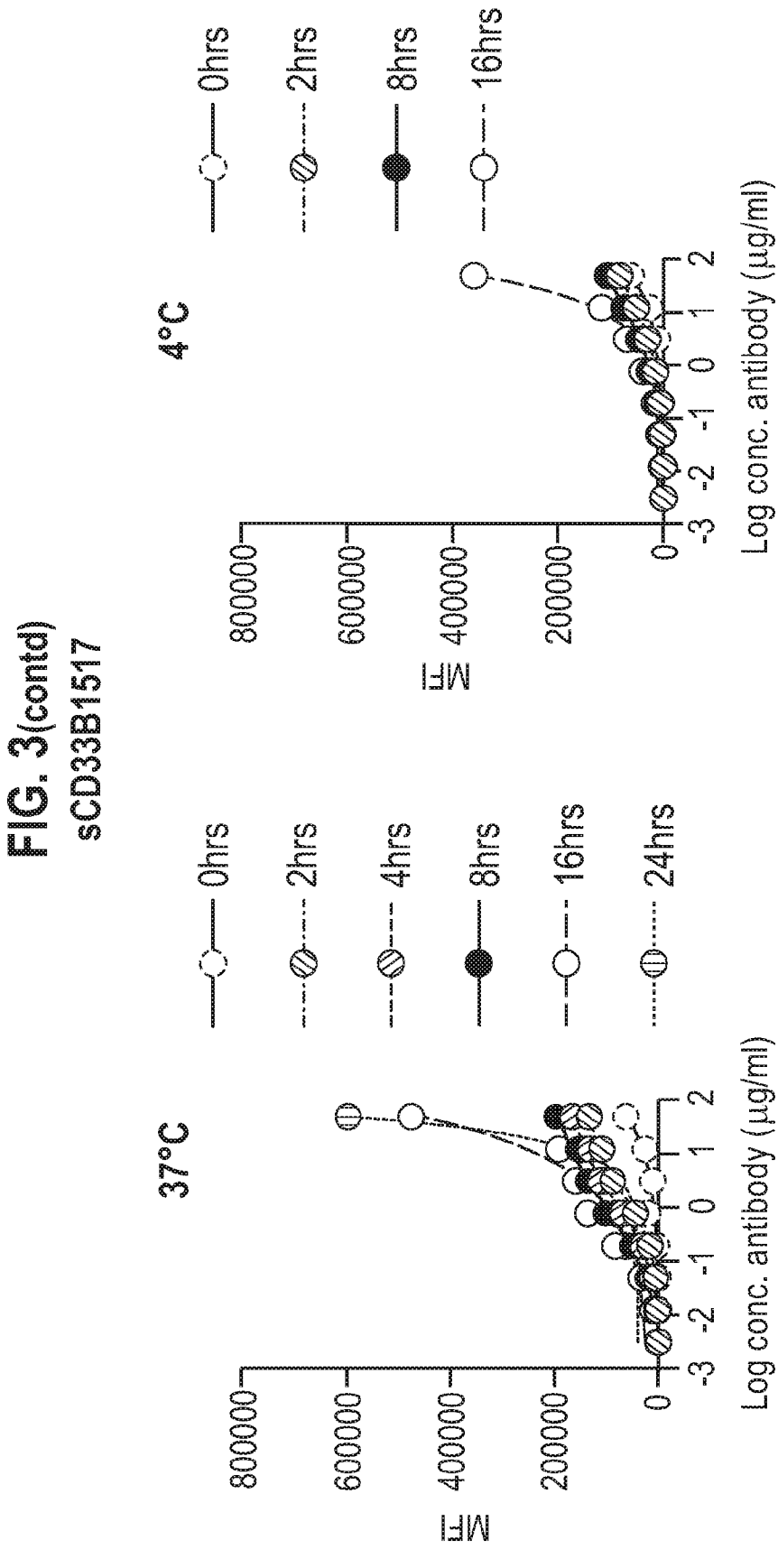


FIG. 3(contd)
sCD33B1476

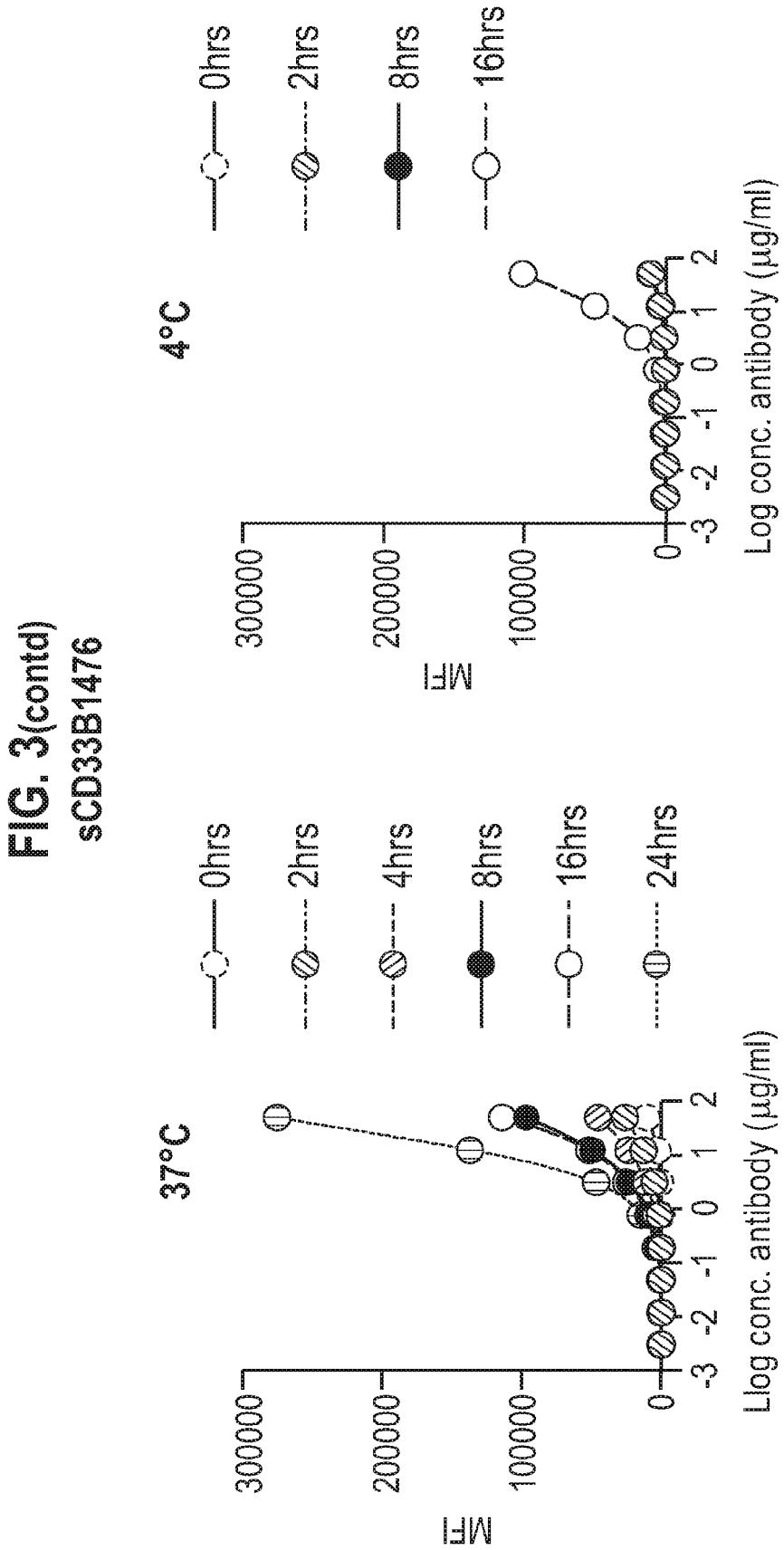


FIG. 3(contd)
sCD33B1484

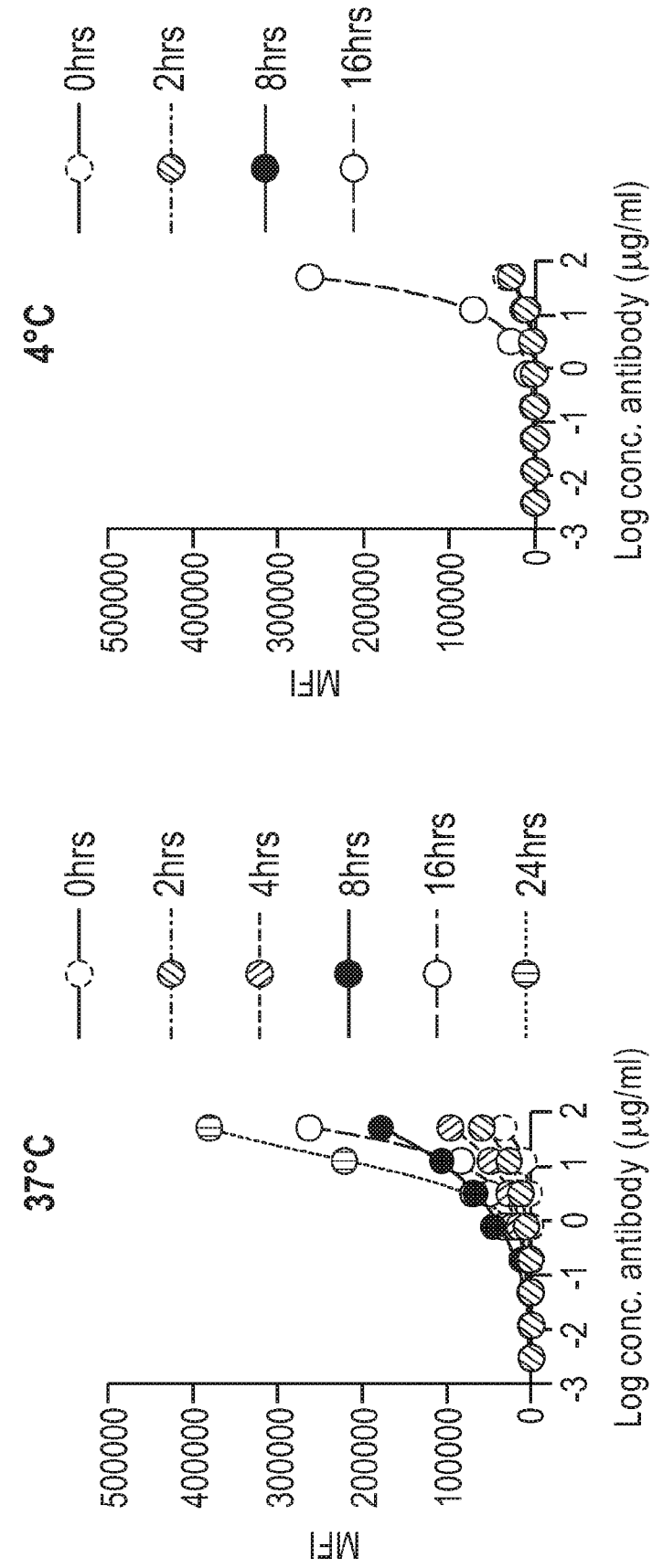


FIG. 3(contd)
sCD33B1522

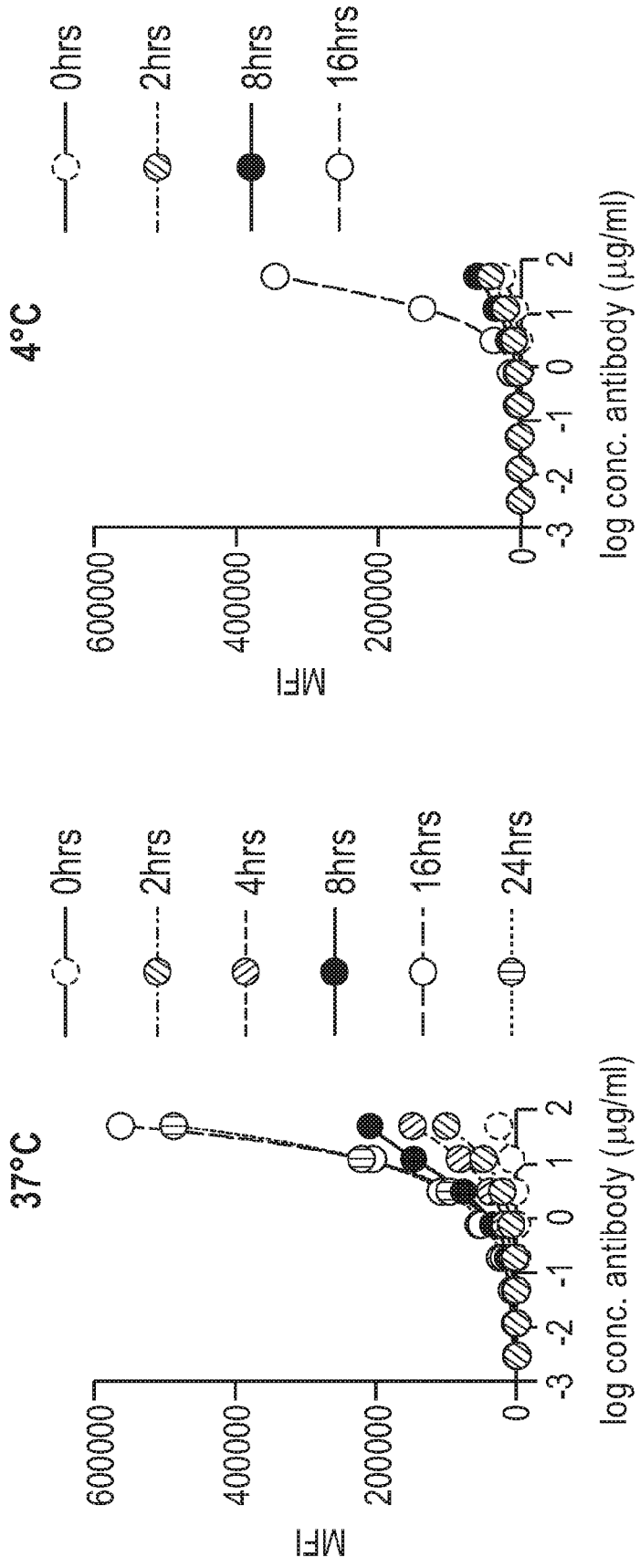


FIG. 3(contd)
sCD33B1477

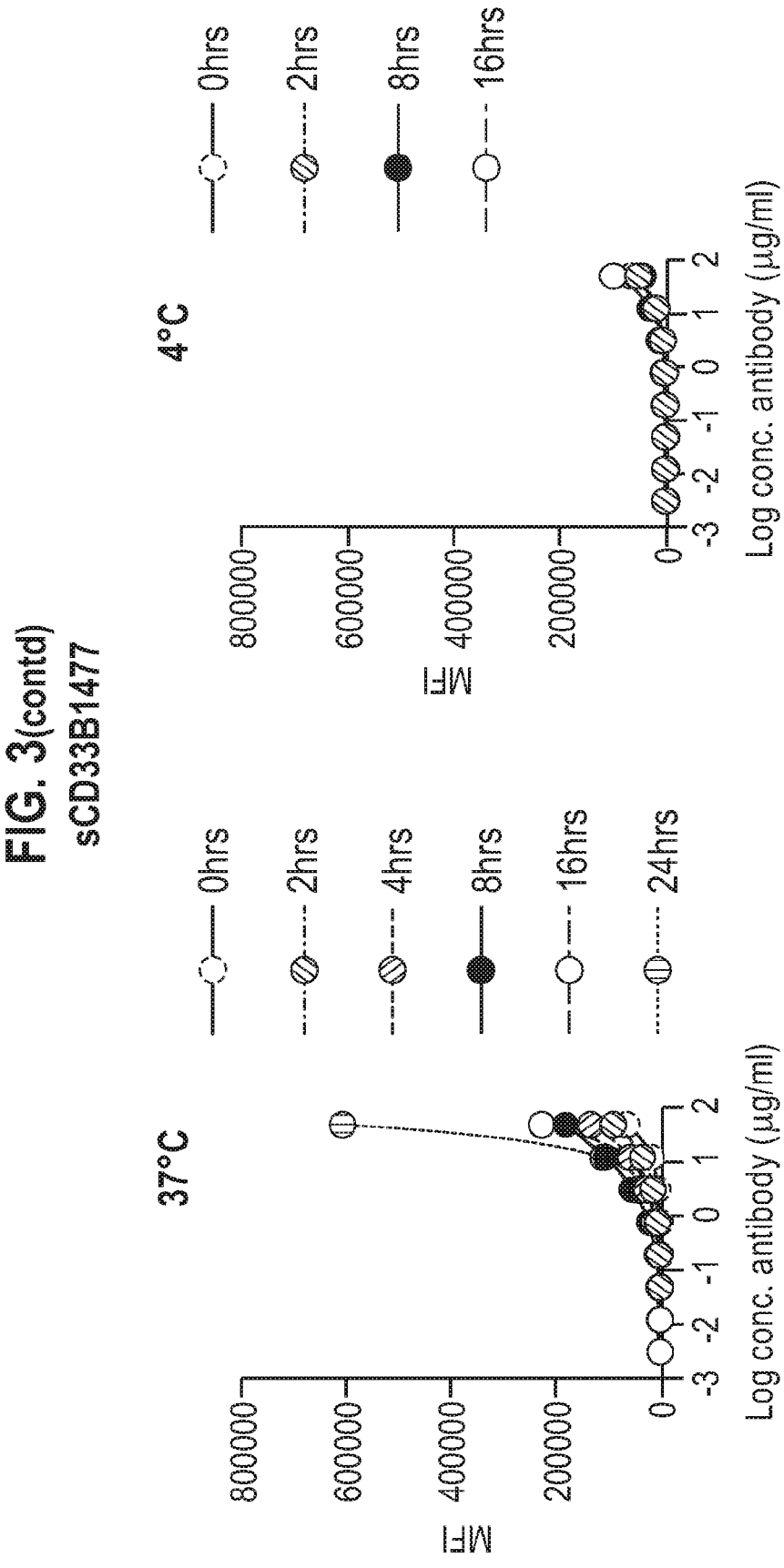


FIG. 3(contd)
sCD33B1495

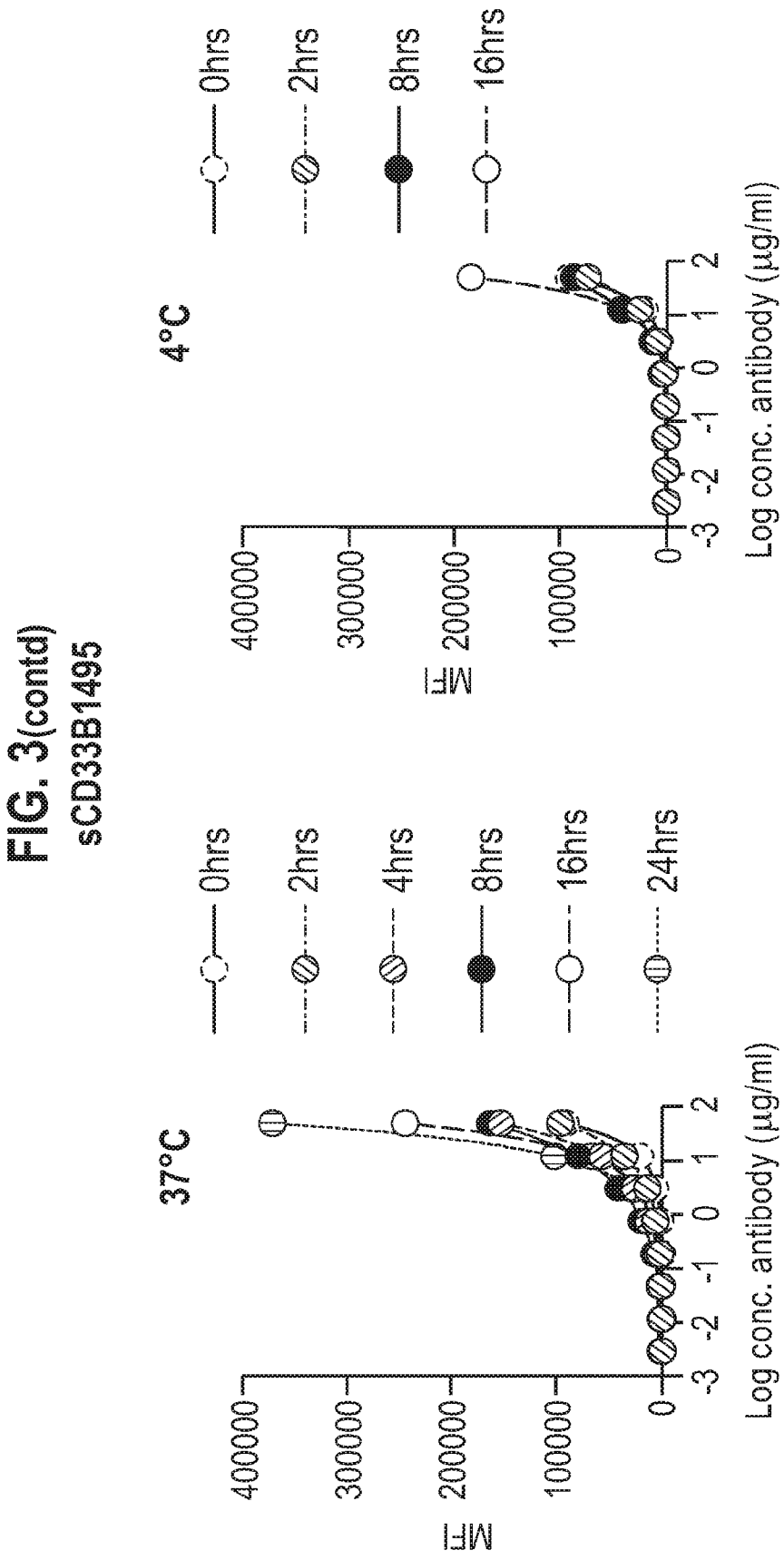


FIG. 3(contd)
Lintuzumab

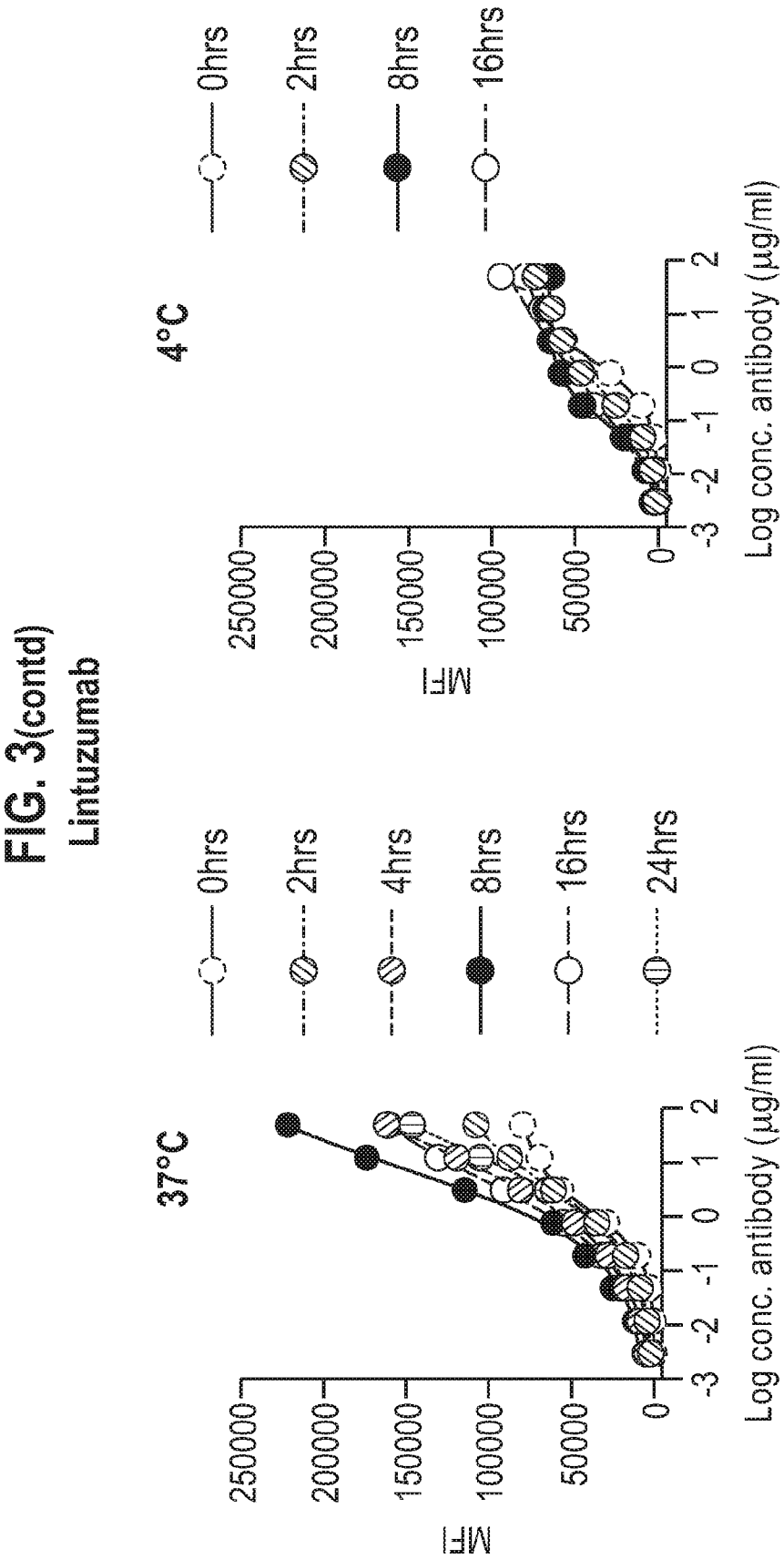


FIG. 4A

MOLM-13

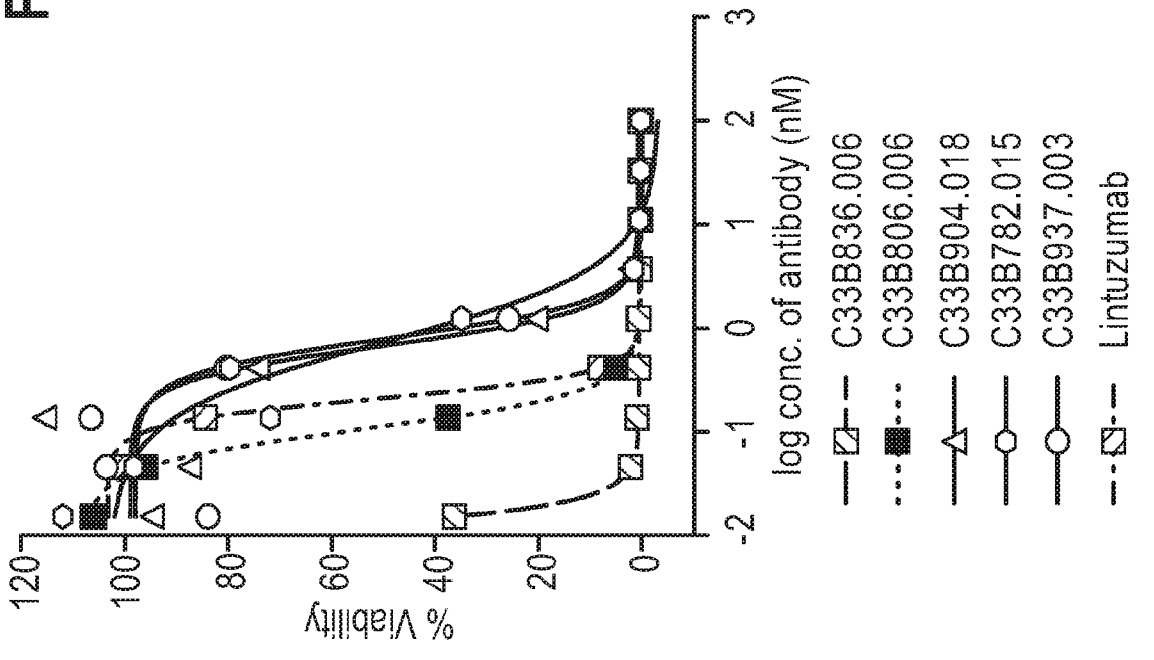


FIG. 4B Kasumi-1

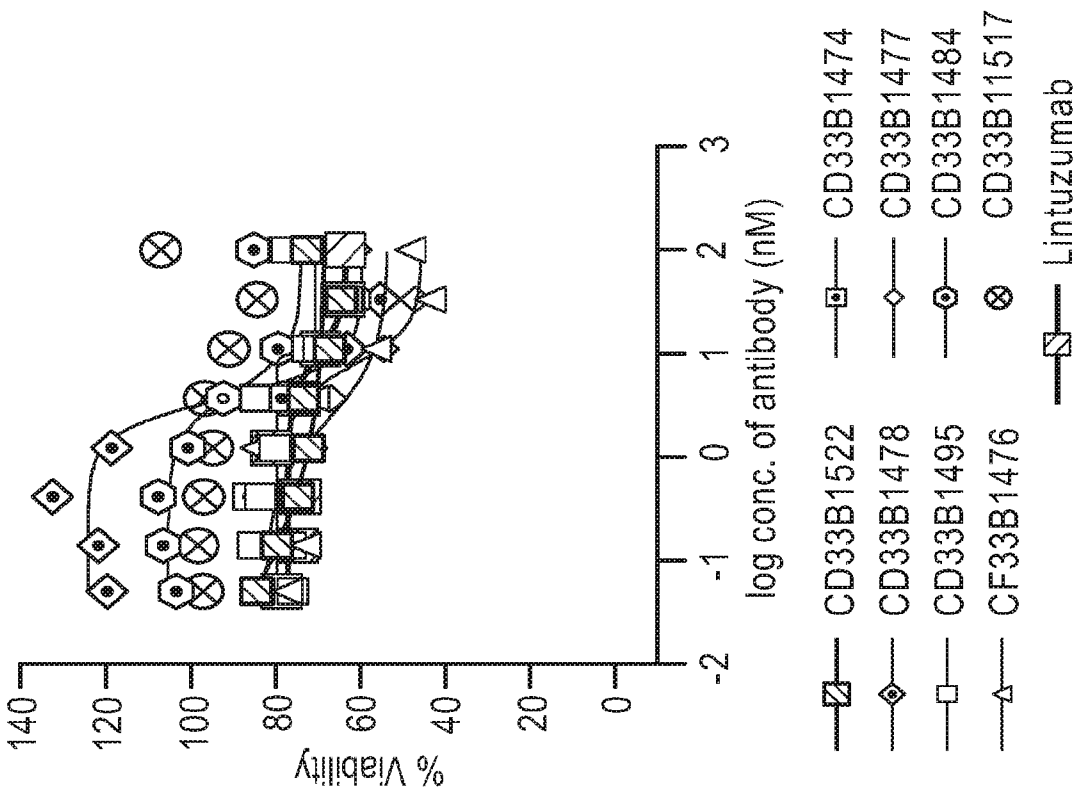


FIG. 4C OCI-AML-3

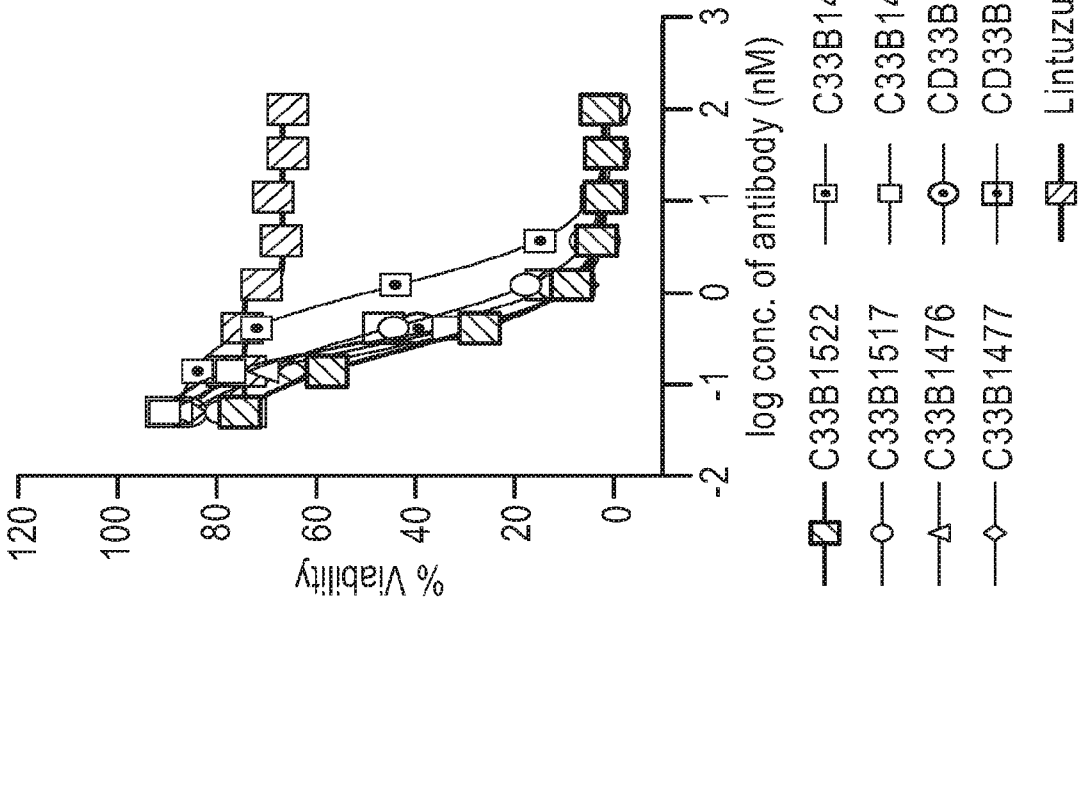


FIG. 4D

HDLM-2

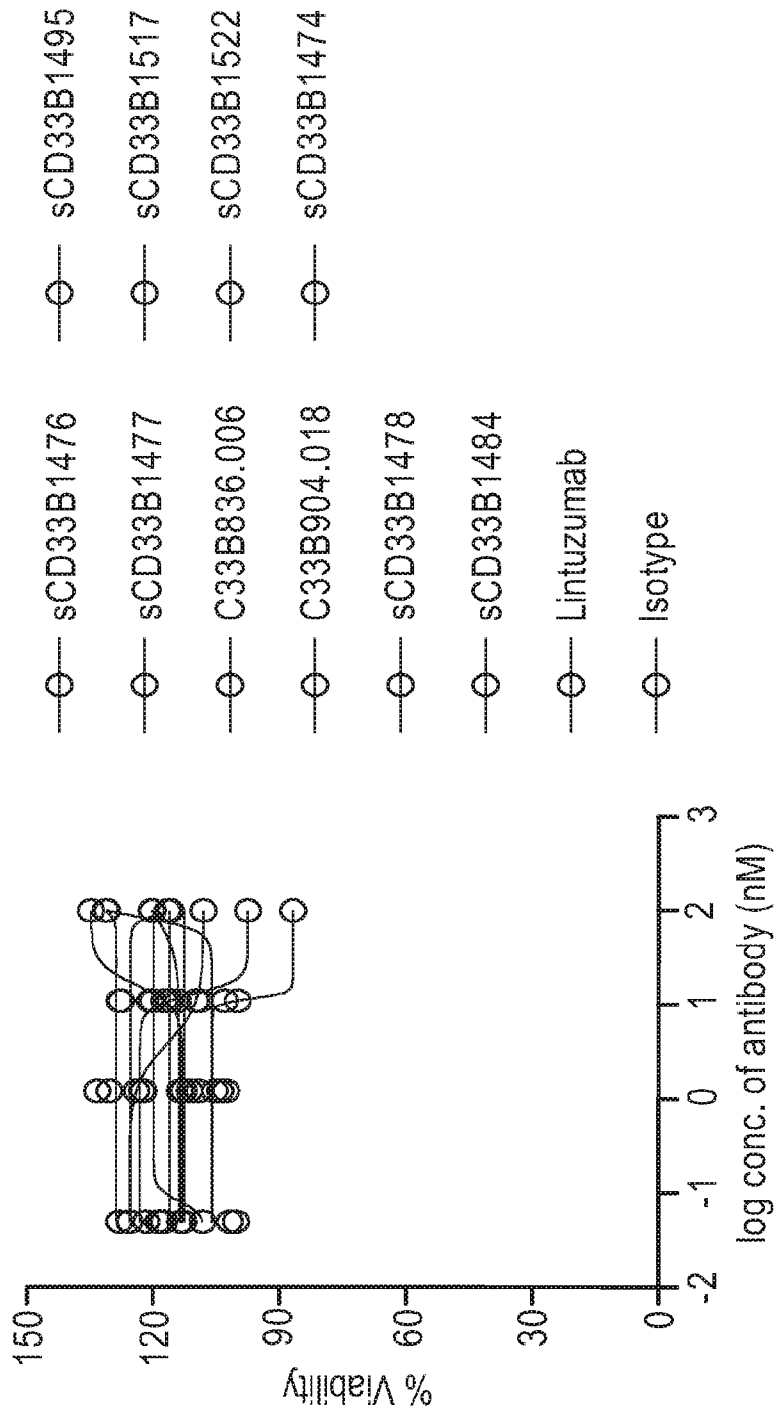

sCD33B1495
sCD33B1517
sCD33B1522
sCD33B1474
sCD33B1476
sCD33B1477
C33B836.006
C33B904.018
sCD33B1478
sCD33B1484
Lintuzumab
Isotype
% Viability
150
120
90
60
30
0
-2
-1
0
1
2
3
log conc. of antibody (nM)

FIG. 5

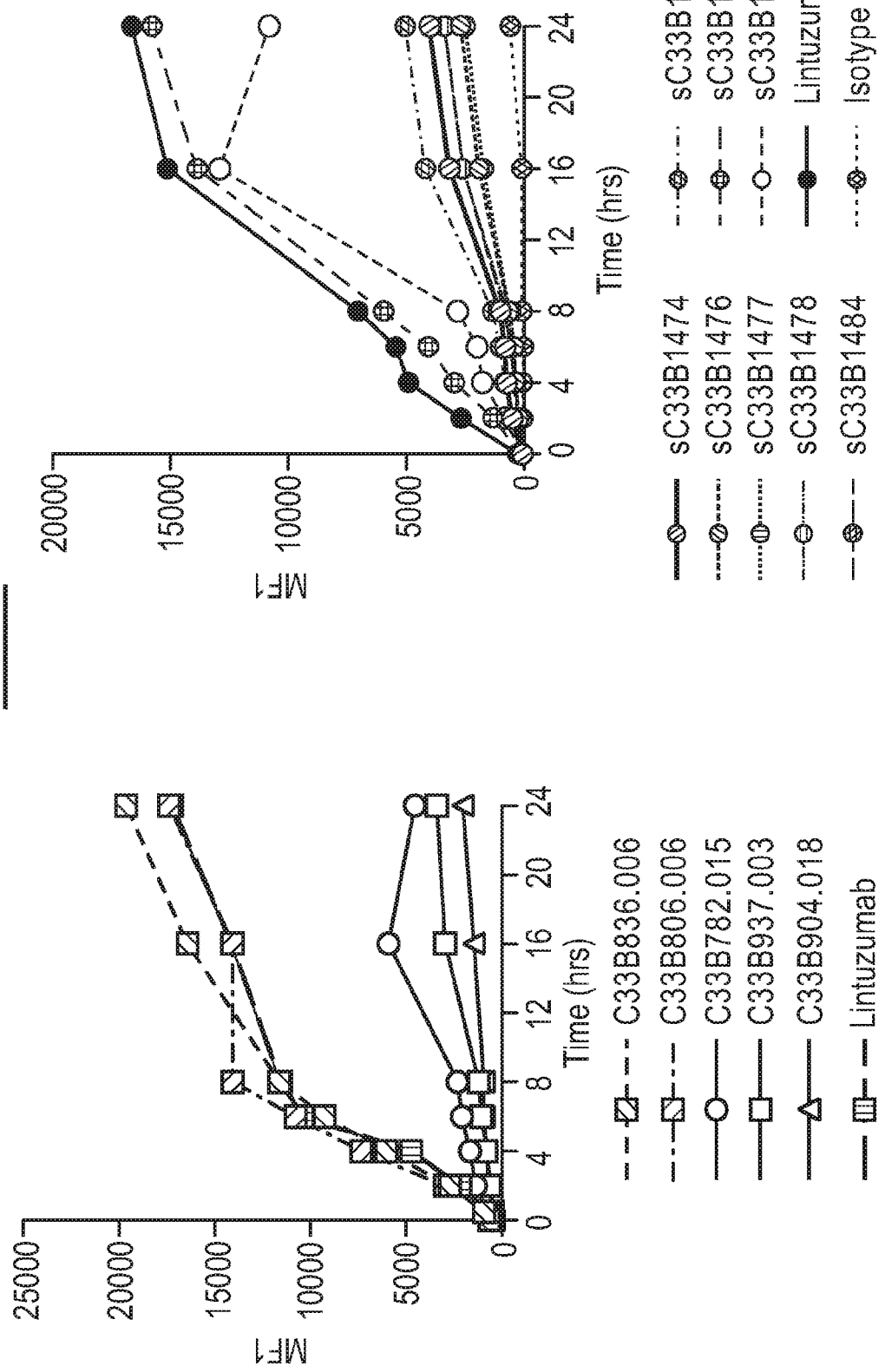

MOLM-13
MFI
Time (hrs)
0
5000
10000
15000
20000
25000
0
4
8
12
16
20
24
C33B836.006
C33B806.006
C33B782.015
C33B937.003
C33B904.018
Lintuzumab
sC33B1474
sC33B1476
sC33B1477
sC33B1478
sC33B1484
sC33B1495
sC33B1517
sC33B1522
Lintuzumab
Isotype

FIG. 5(contd)

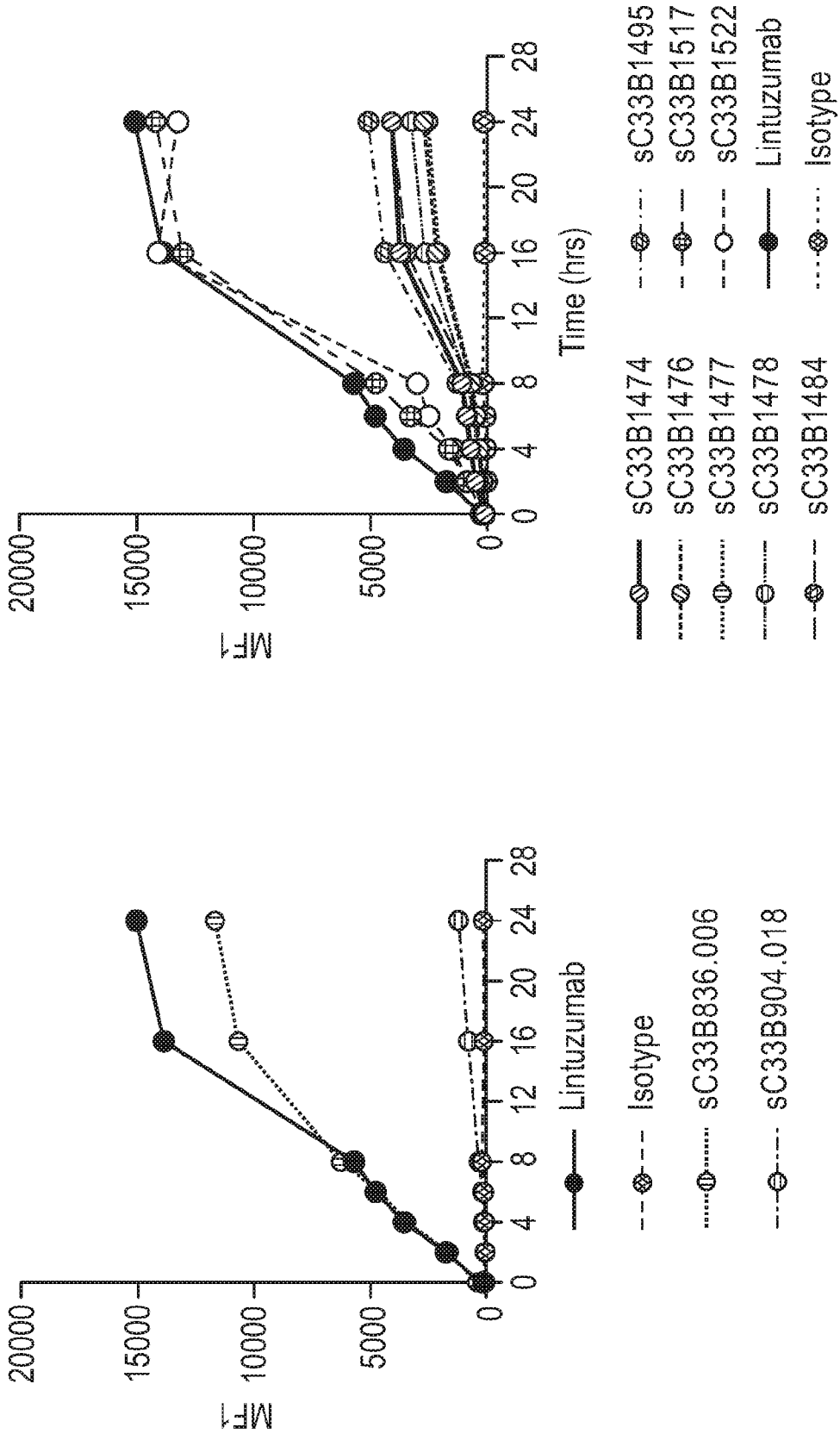

Kasumi-1
MFI
20000
15000
10000
5000
0
0
4
8
12
16
20
24
28
Time (hrs)
Lintuzumab
Isotype
sC33B836.006
sC33B904.018
sC33B1474
sC33B1476
sC33B1477
sC33B1478
sC33B1484
sC33B1495
sC33B1517
sC33B1522
Lintuzumab
Isotype

FIG. 5(contd)

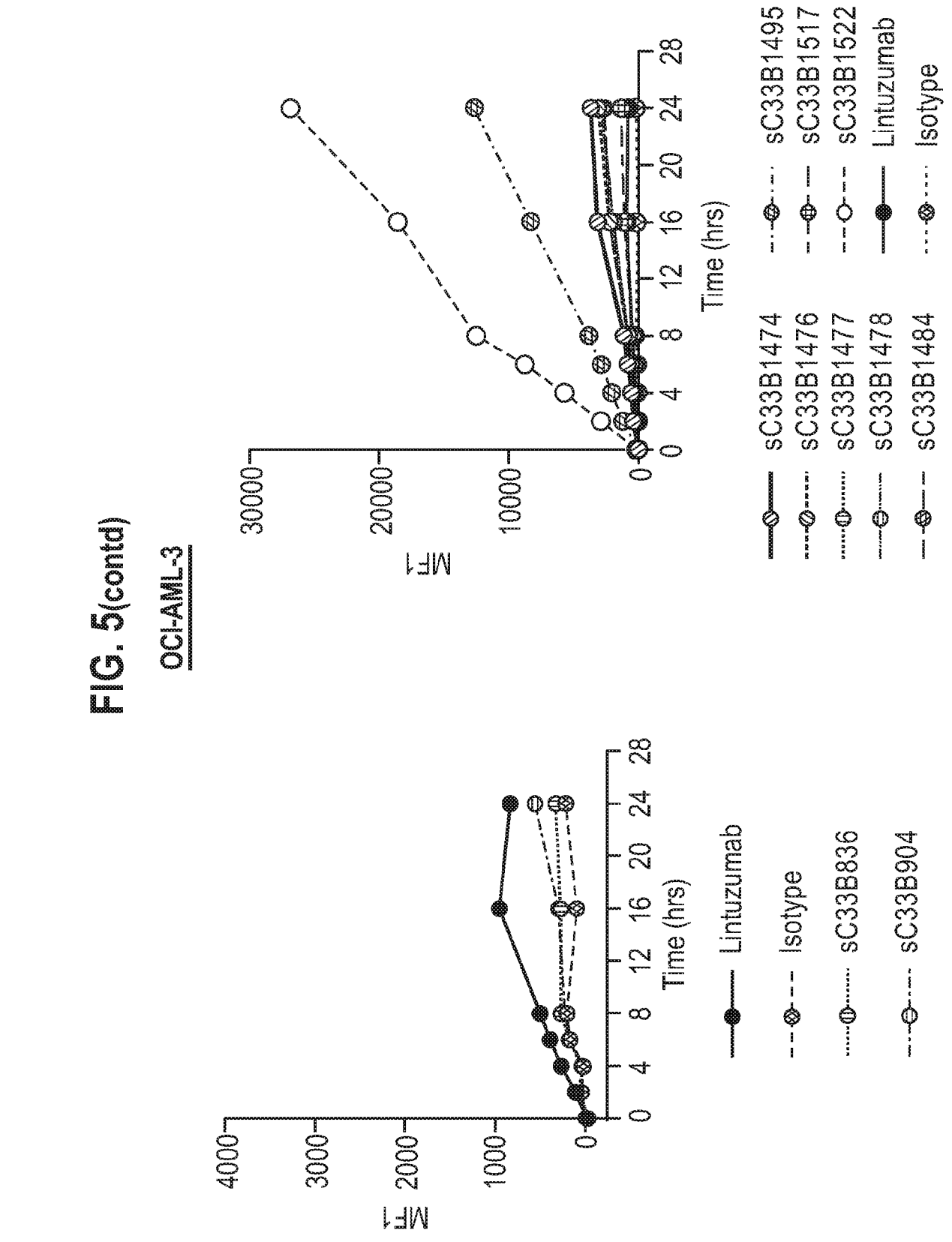

OCI-AML-3
MFI
Time (hrs)
0
4
8
12
16
20
24
28
1000
2000
3000
4000
10000
20000
30000
Lintuzumab
Isotype
sC33B836
sC33B904
sC33B1474
sC33B1476
sC33B1477
sC33B1478
sC33B1484
sC33B1495
sC33B1517
sC33B1522
Lintuzumab
Isotype

FIG. 6

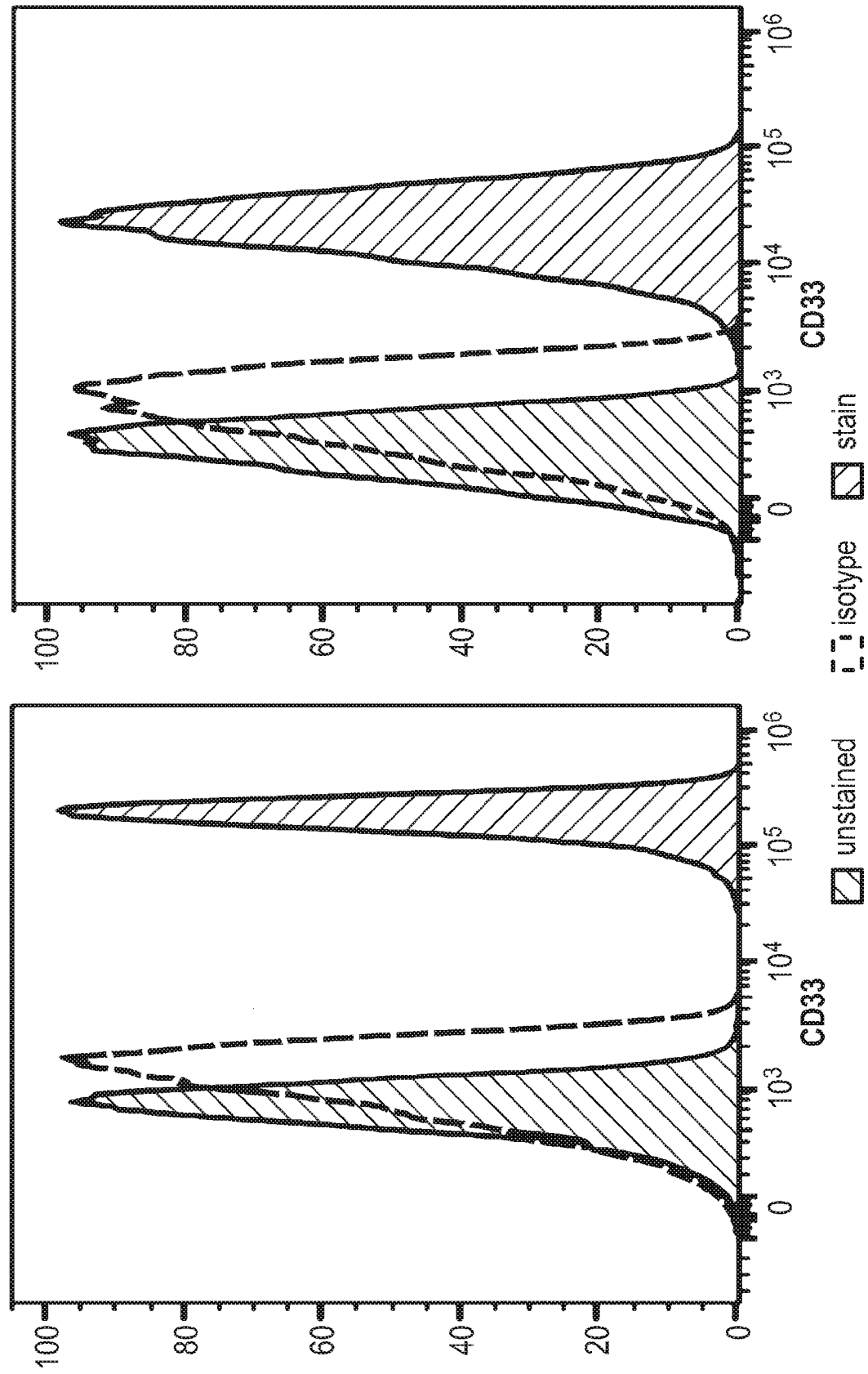

MOLM-13 (high)
Kasumi-1 (med)
CD33
CD33
0
20
40
60
80
100
0
10^3
10^4
10^5
10^6
unstained
isotype
stain

FIG. 6(contd)

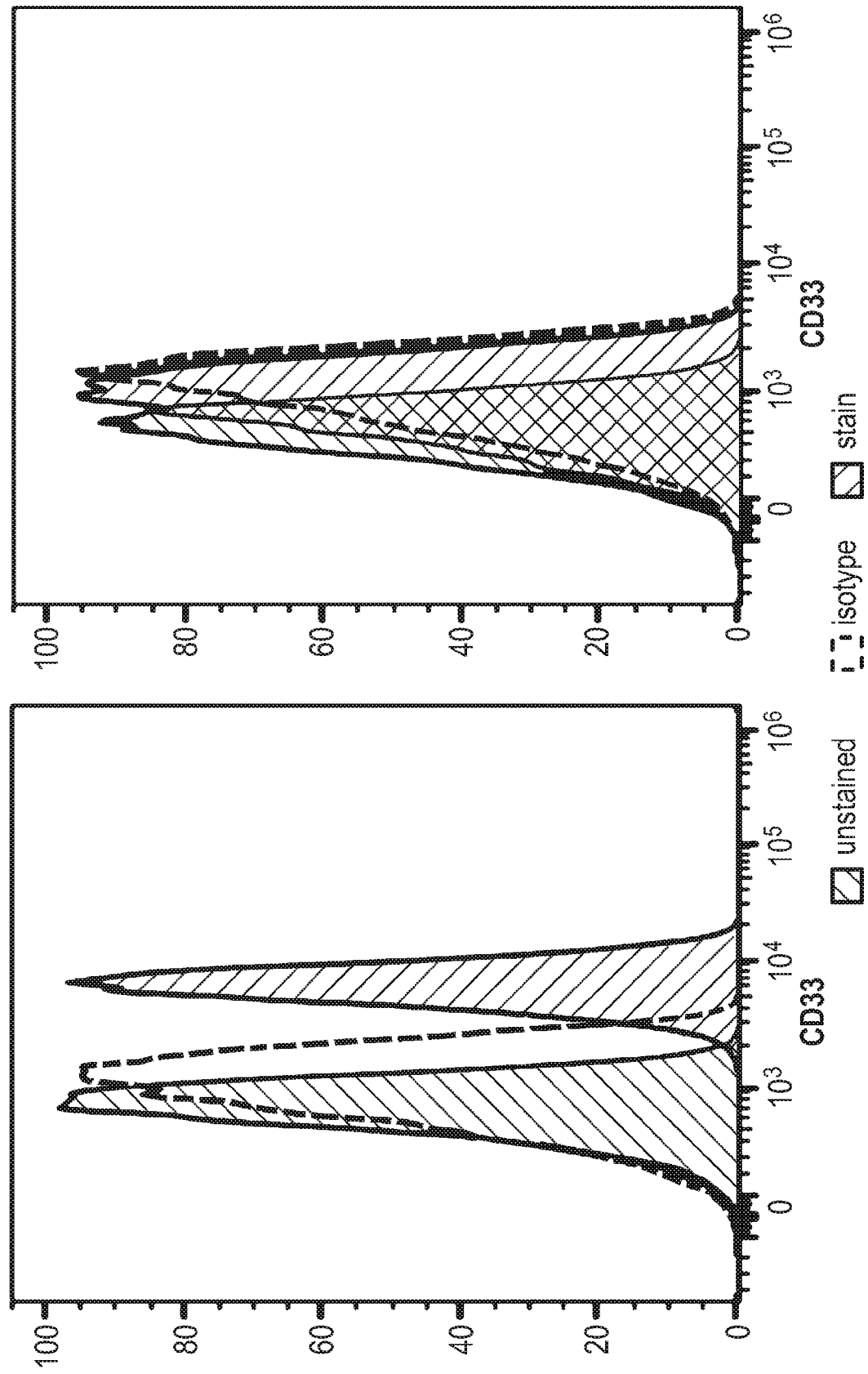

HDLM-2 (negative)
OCI-AML-3 (low)
100
80
60
40
20
0
0
10^3
10^4
10^5
10^6
CD33
unstained
isotype
stain

FIG. 6(contd)

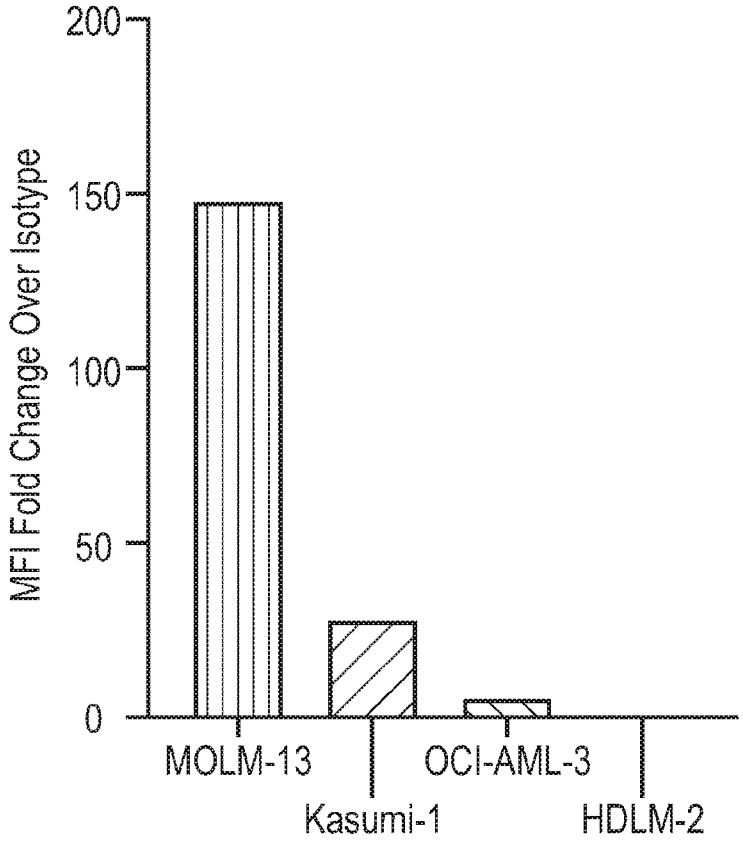

200
150
100
50
0
MFI Fold Change Over Isotype
MOLM-13
Kasumi-1
OCI-AML-3
HDLM-2

FIG. 7

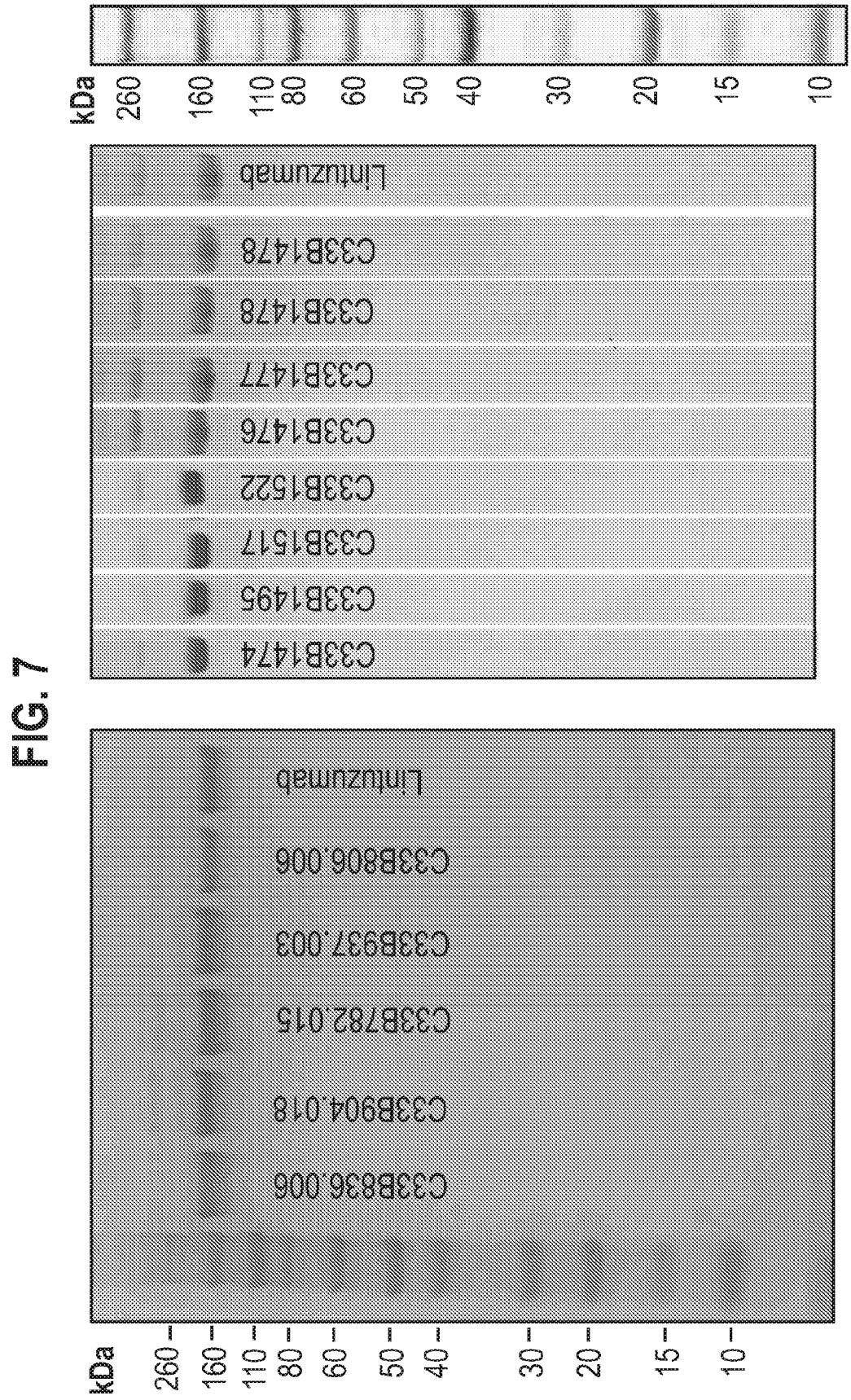

kDa
260
160
110
80
60
50
40
30
20
15
10
C33B836.006
C33B904.018
C33B782.015
C33B937.003
C33B806.006
Lintuzumab
C33B1474
C33B1495
C33B1517
C33B1522
C33B1476
C33B1477
C33B1478
C33B1478
Lintuzumab

FIG. 7(contd)

Antibody	DAR
sC331474	4.5
sC331476	2.7
sC331477	2.6
sC331478	3.7
sC331484	5.2
sC331495	4.3
sC331517	2.1
sC331522	2.1
C33B836.006	3.2
C33B782.015	2.8
C33B904.018	3.5
C33B806.006	2.4
C33B937.003	3.2
Lintuzumab	3.6

FIG. 8A

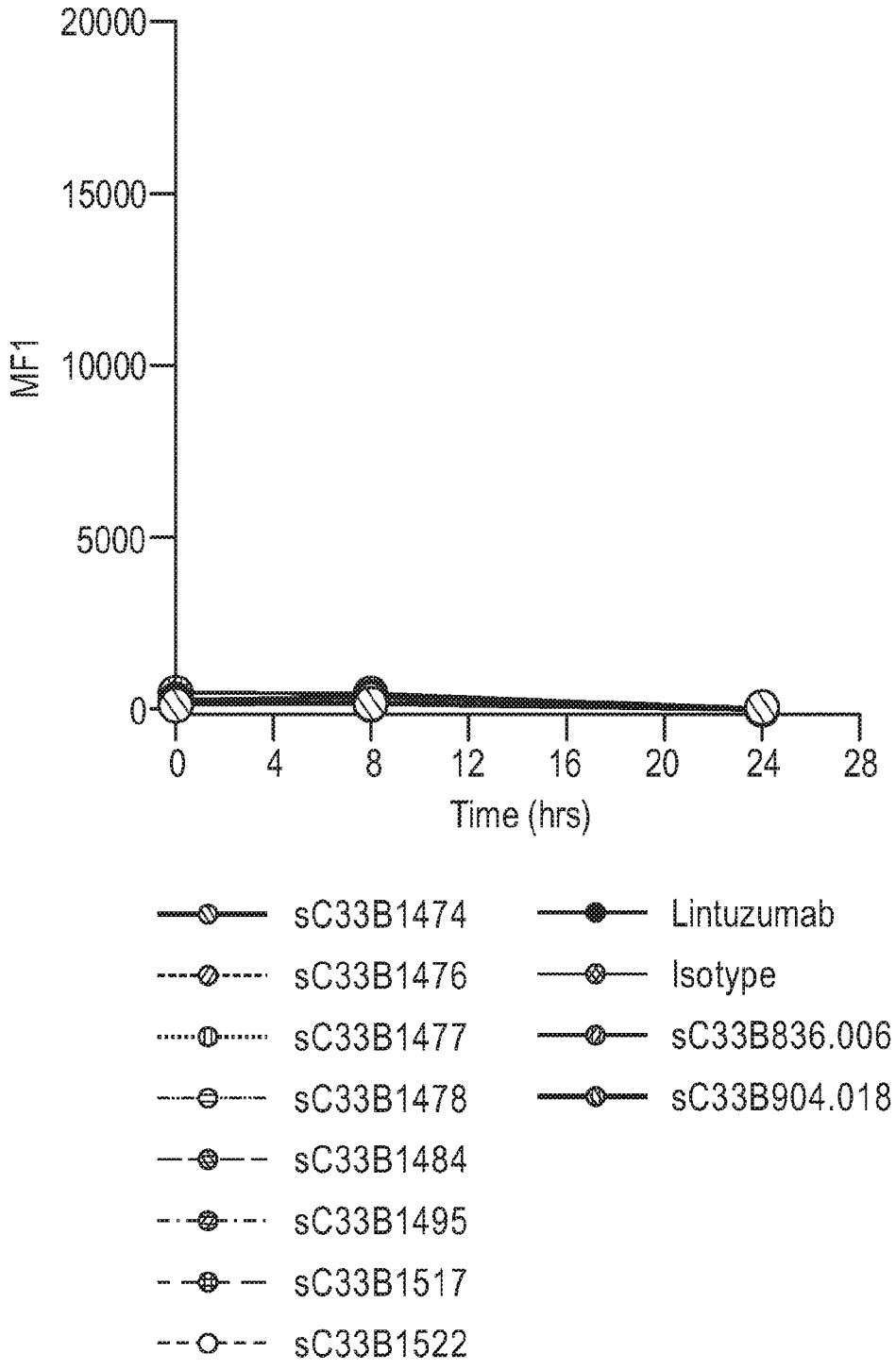

Kasumi-1
20000
15000
10000
5000
0
MFI
0
4
8
12
16
20
24
28
Time (hrs)
sC33B1474
sC33B1476
sC33B1477
sC33B1478
sC33B1484
sC33B1495
sC33B1517
sC33B1522
Lintuzumab
Isotype
sC33B836.006
sC33B904.018

FIG. 8A(contd)

OCI-AML-3

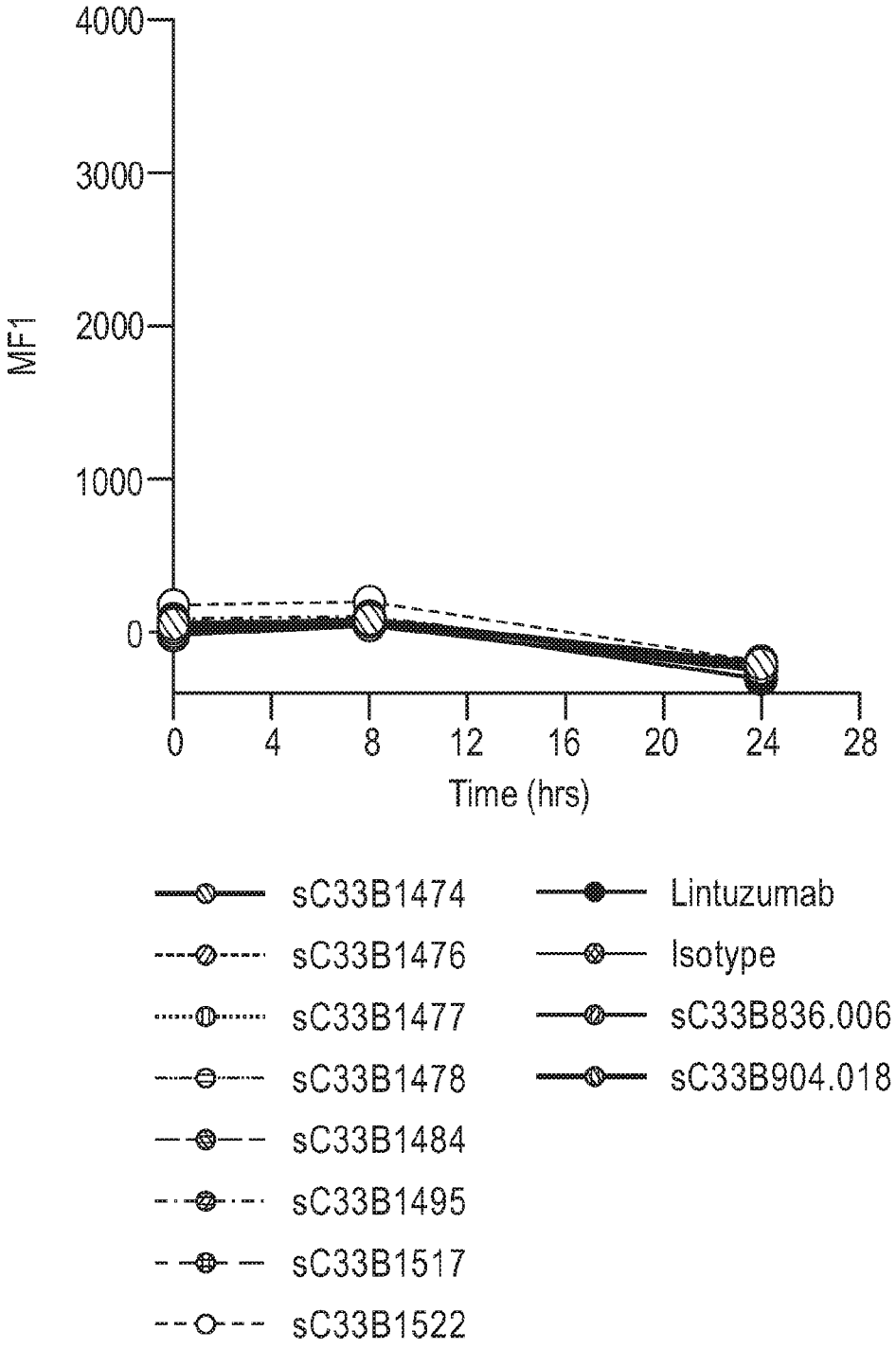


FIG. 8B

HDLM-2

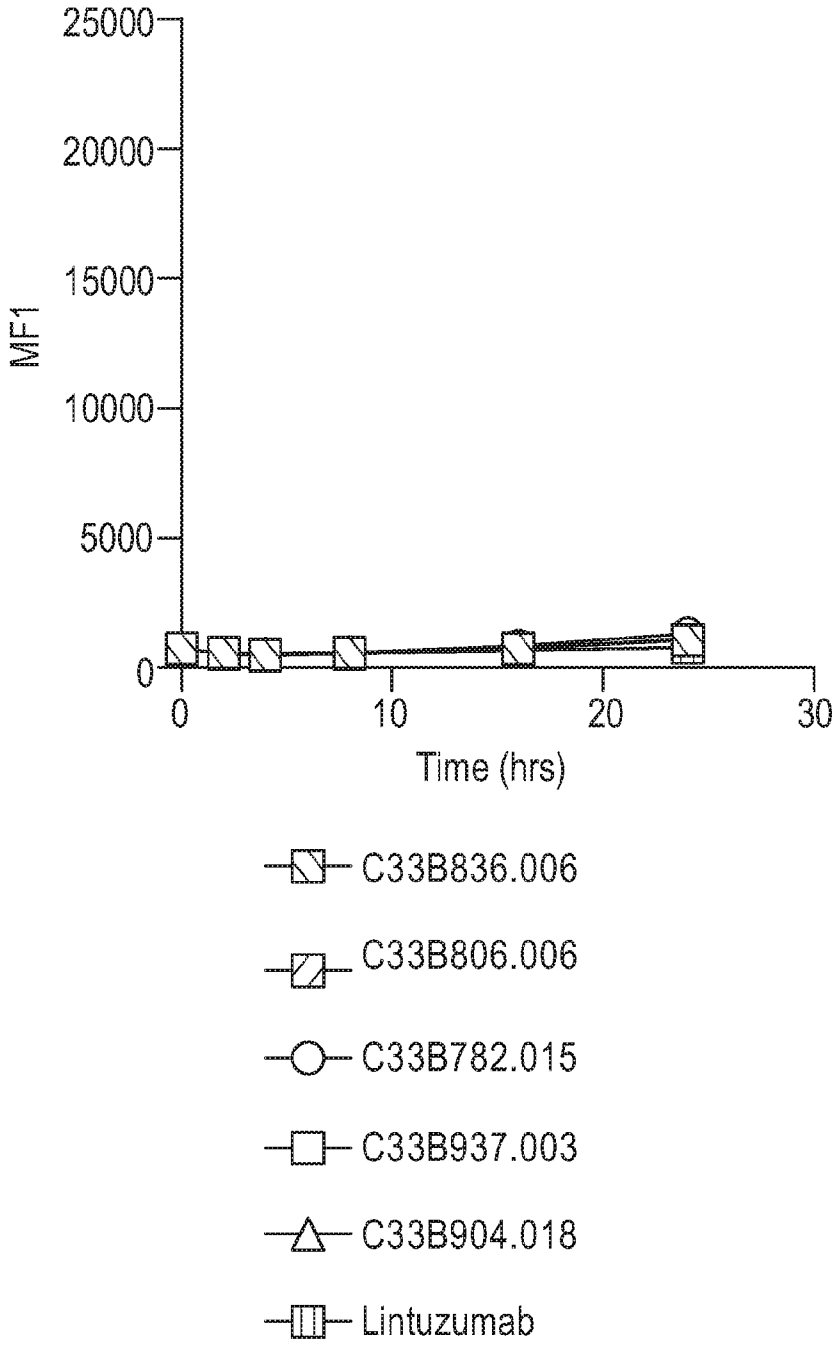
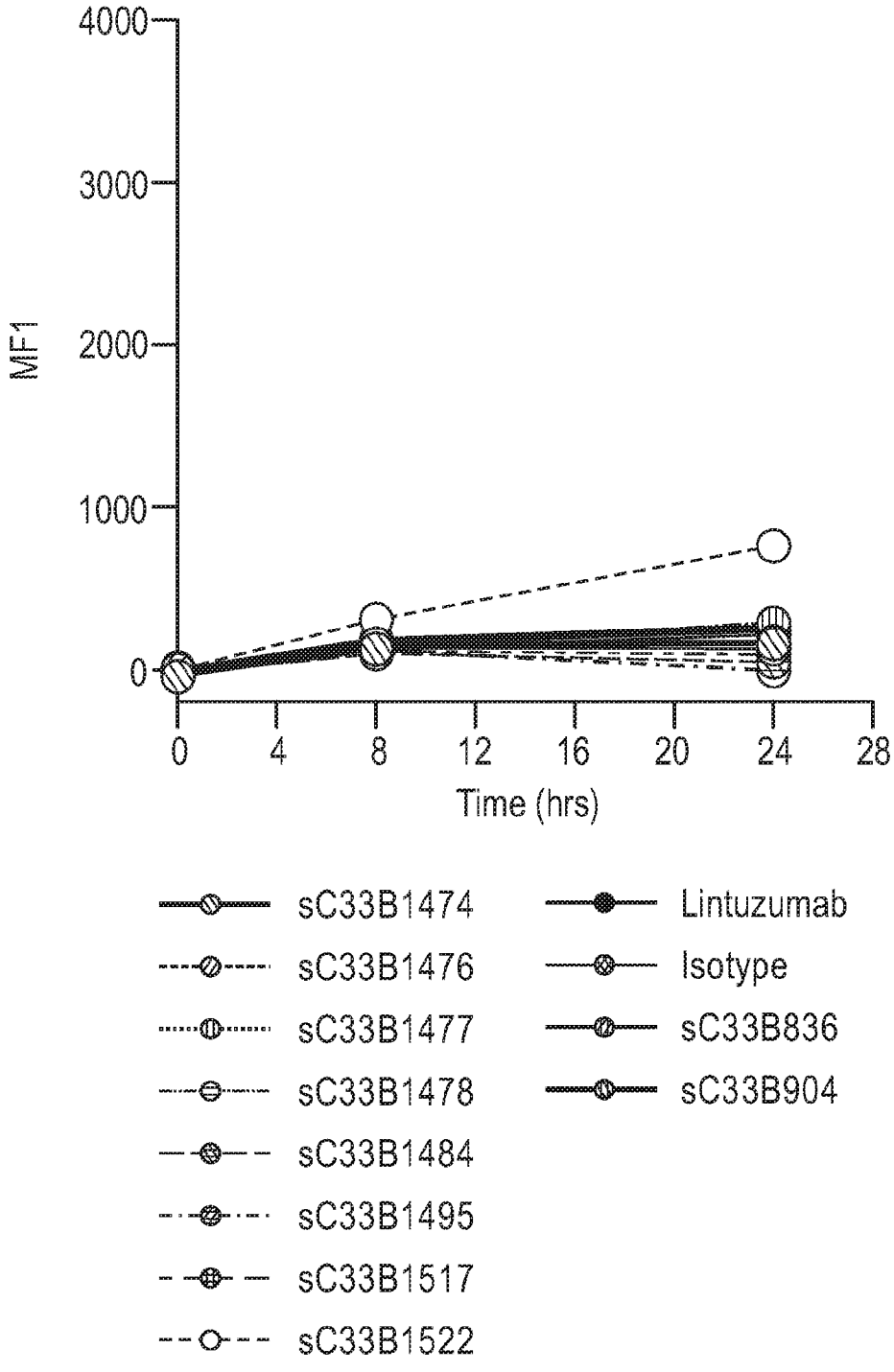

25000
20000
15000
10000
5000
0
MF1
0
10
20
30
Time (hrs)
C33B836.006
C33B806.006
C33B782.015
C33B937.003
C33B904.018
Lintuzumab

FIG. 8B(contd)

HDLM-2



	MOLM-13 (high)	Kasumi-1 (med)	OCI-AML-3 (low)
Antibody	EC_{50} (nM)	EC_{50} (nM)	EC_{50} (nM)
C33B836.006	0.5	17.06	0.46
C33B806.006	35.6	0.13	15.3
C33B904.018	ND	ND	ND
C33B937.003	ND	ND	ND
C33B782.015	ND	ND	ND
C33B1517	10.6	ND	1.4
C33B1522	32.66	73.4	ND
C33B1477	56	ND	0.6
C33B1474	78.4	ND	1.26
C33B1478	ND	ND	ND
C33B1476	ND	ND	0.8
C33B1495	ND	ND	ND
C33B1484	ND	ND	ND
Lintuzumab	0.31	0.34	0.9

FIG. 9

FIG. 10

	MOLM-13 (high)	Kasumi-1 (med)	OCI-AML-3 (low)
Antibody	EC_{50} (nM)	% viability	EC_{50} (nM)
C33B836.006	***~ 0.002***	Not tested	Not tested
C33B806.006	0.106	Not tested	Not tested
C33B904.018	0.744	Not tested	Not tested
C33B937.003	0.840	Not tested	Not tested
C33B782.015	1.062	Not tested	Not tested
C33B1517	***0.09***	106	0.45
C33B1522	0.9	73.4	0.24
C33B1476	1.09	48.1	0.27
C33B1477	1.76	60.9	0.24
C33B1478	2.5	64.6	0.34
C33B1484	3.5	85.01	0.39
C33B1474	5.93	78.1	1.18
C33B1495	14.44	77.4	0.30
Lintuzumab	0.04	63.5	0.9

FIG. 12

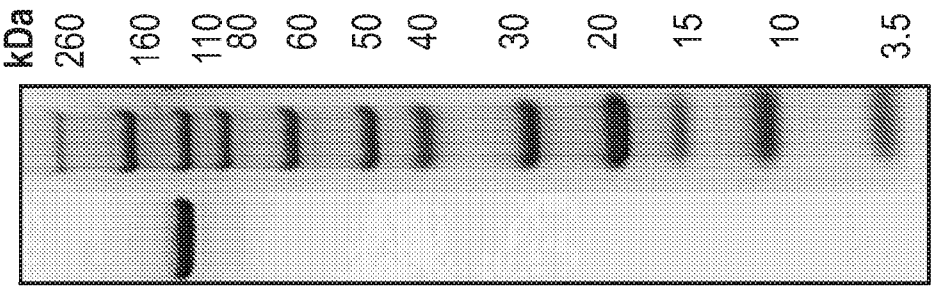

kDa
260
160
110
80
60
50
40
30
20
15
10
3.5

FIG. 11

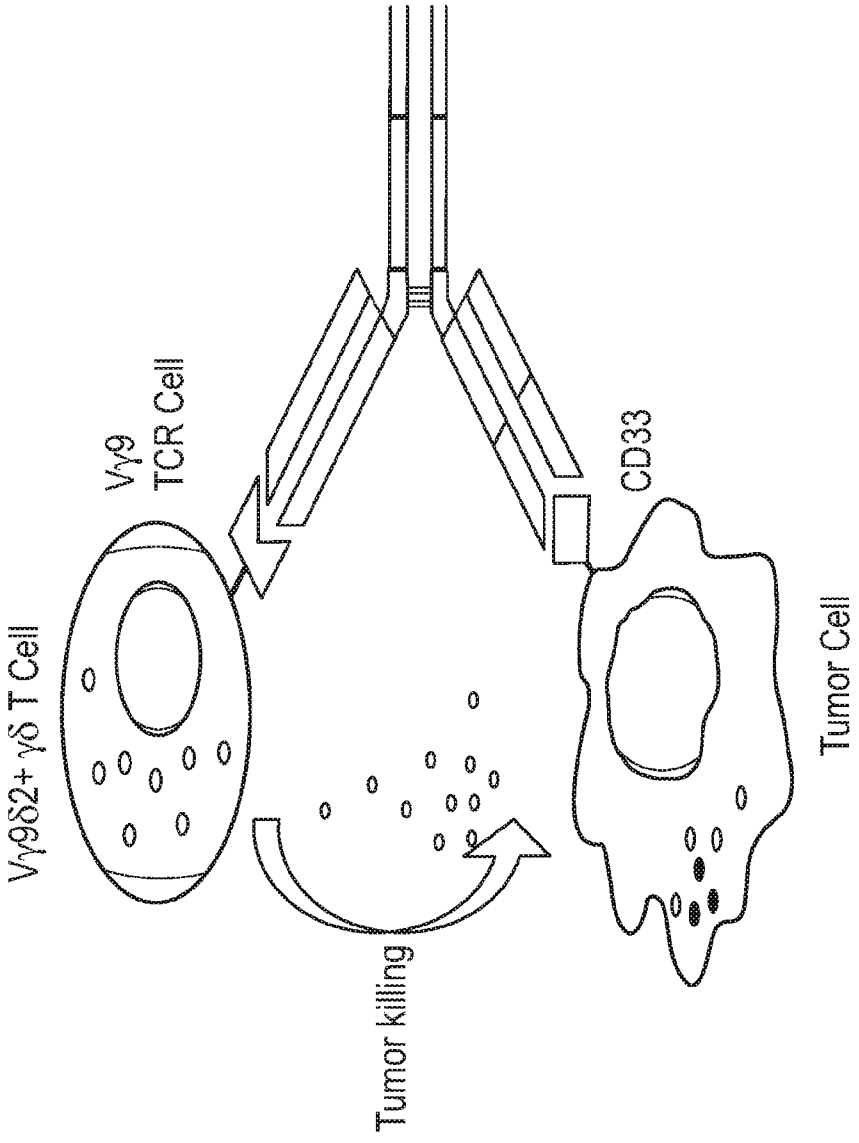
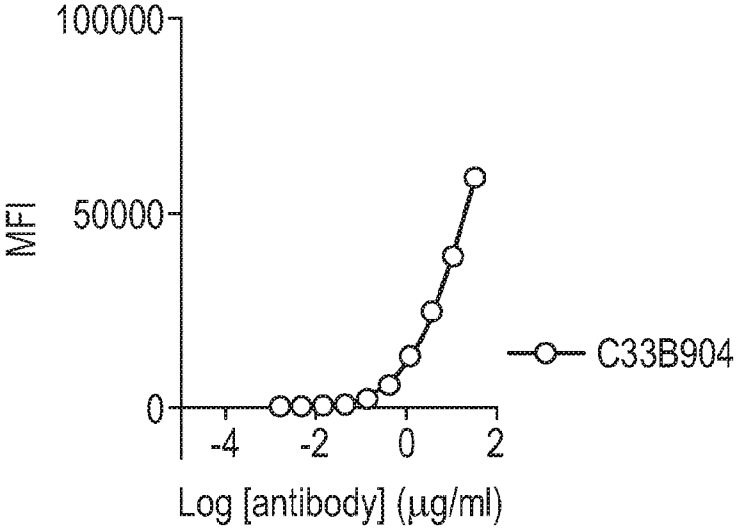
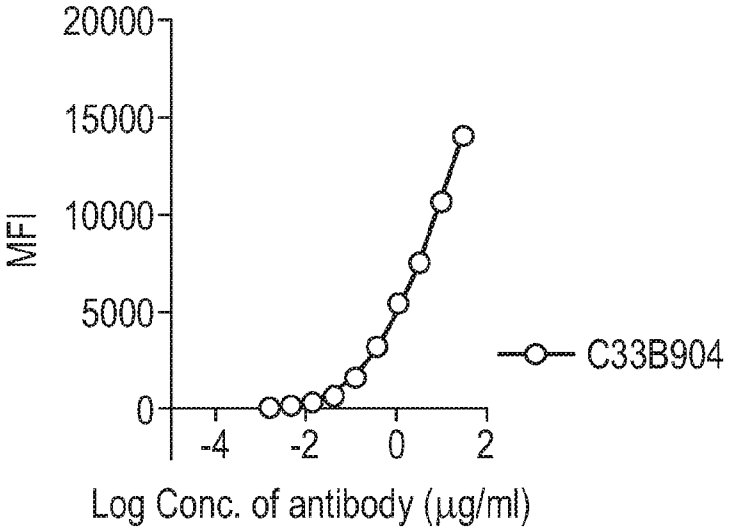

Vγ9δ2+ γδ T Cell
Vγ9
TCR Cell
CD33
Tumor Cell
Tumor killing

FIG. 13
MOLM-13
(high)



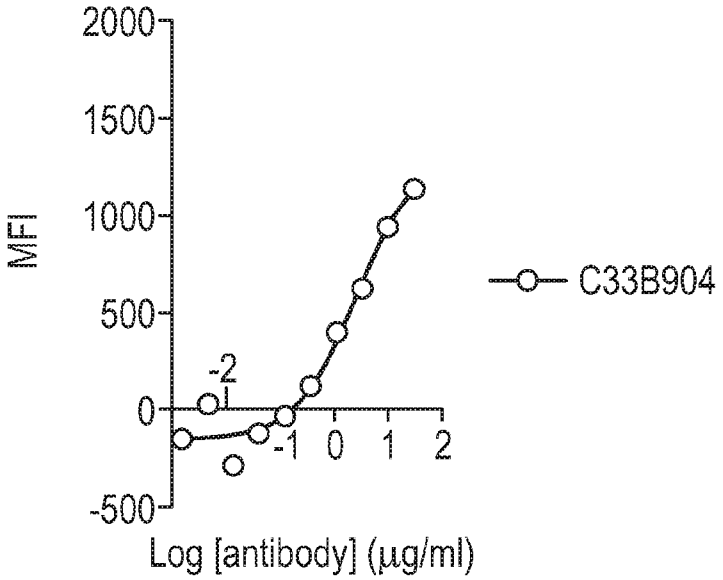
Antibody	EC_{50} (nM)	EC_{90} (nM)
C33B904	~134.33	3748

FIG. 14
Kasumi-1
(med)



Antibody	EC_{50} (nM)	EC_{90} (nM)
C33B904	82.2	6660

FIG. 15
OCI-AML-3
(low)



Antibody	EC_{50} (nM)	EC_{90} (nM)
C33B904	16.4	274

FIG. 16
ET ratio 1:1

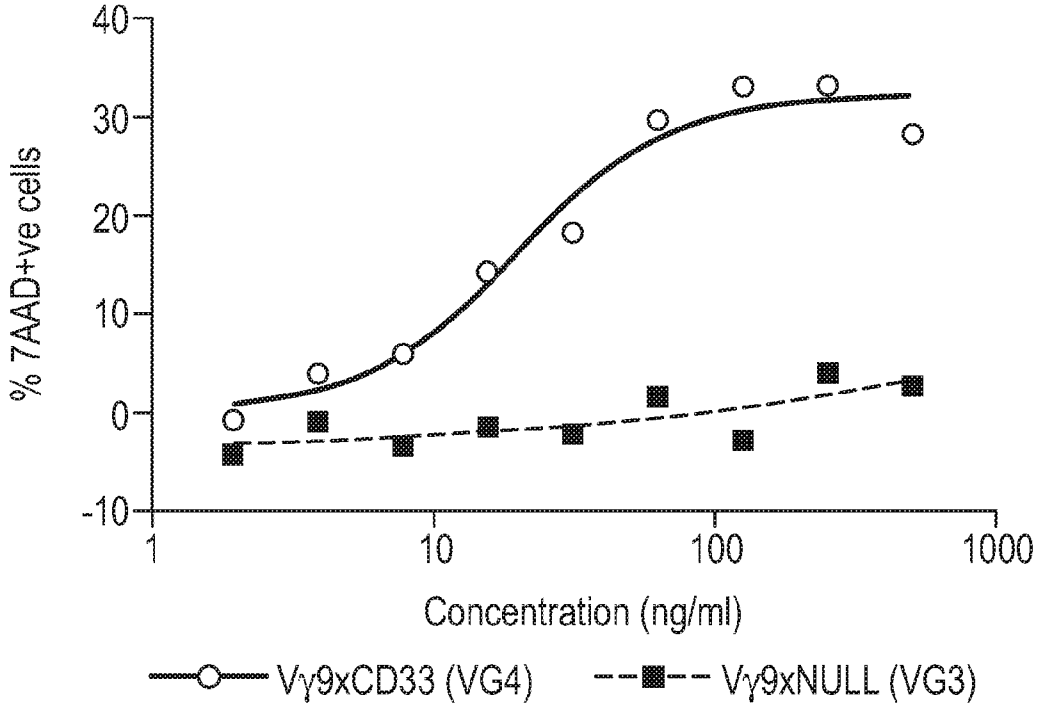


FIG. 17

ET ratio 5:1

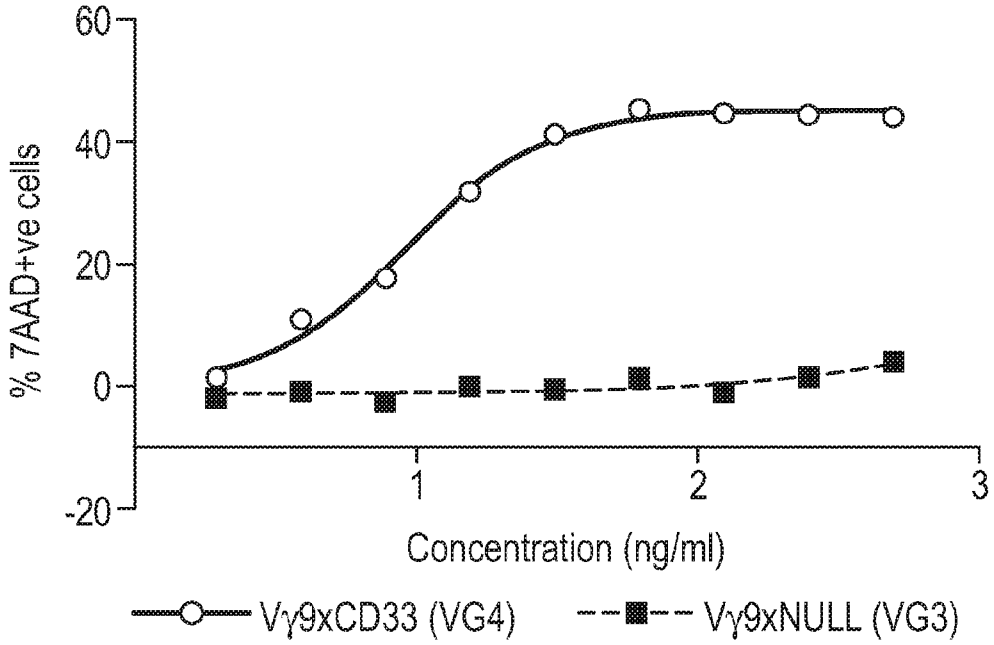


FIG. 18

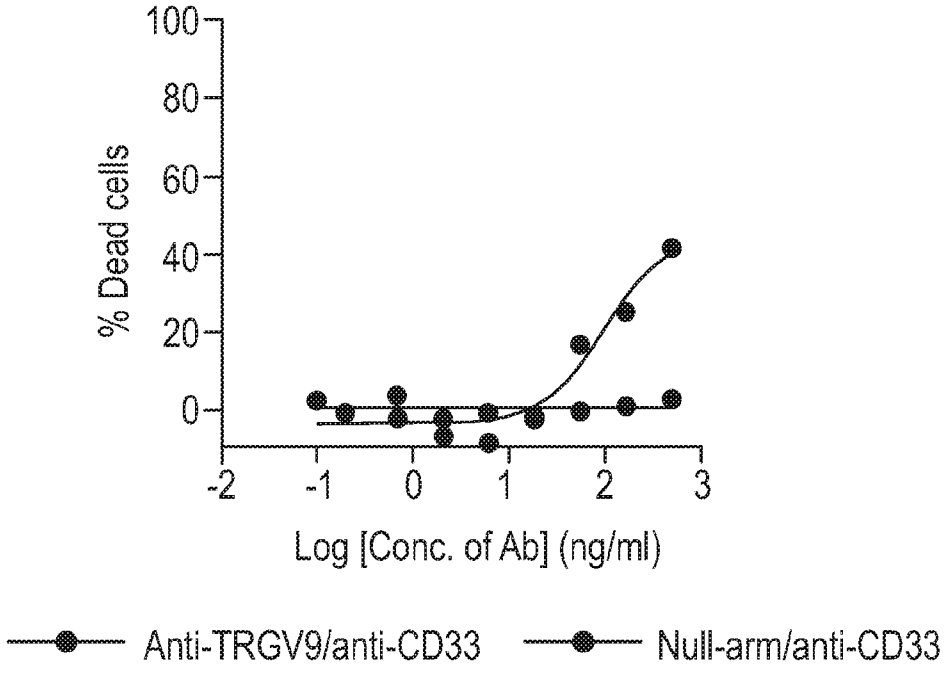
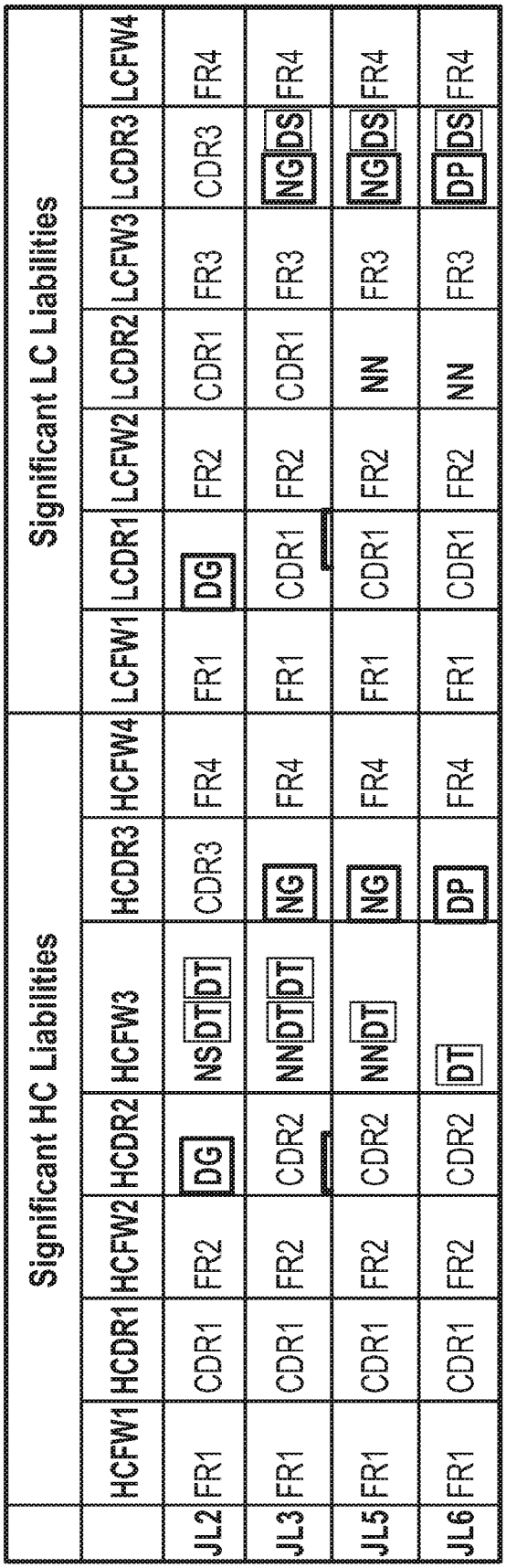


FIG. 19



	Significant HC Liabilities								Significant LC Liabilities							
	HCFW1	HCDR1	HCFW2	HCDR2	HCFW3	HCDR3	HCFW4	LCFW1	LCDR1	LCFW2	LCDR2	LCFW3	LCDR3	LCFW4
JL2	FR1	CDR1	FR2	DG	NSDTDT	CDR3	FR4	FR1	DG	FR2	CDR1	FR3	CDR3	FR4
JL3	FR1	CDR1	FR2	CDR2	NNDTDT	NG	FR4	FR1	CDR1	FR2	CDR1	FR3	NGDS	FR4
JL5	FR1	CDR1	FR2	CDR2	NNDT	NG	FR4	FR1	CDR1	FR2	NN	FR3	NGDS	FR4
JL6	FR1	CDR1	FR2	CDR2	DT	DP	FR4	FR1	CDR1	FR2	NN	FR3	DPDS	FR4

FIG. 20

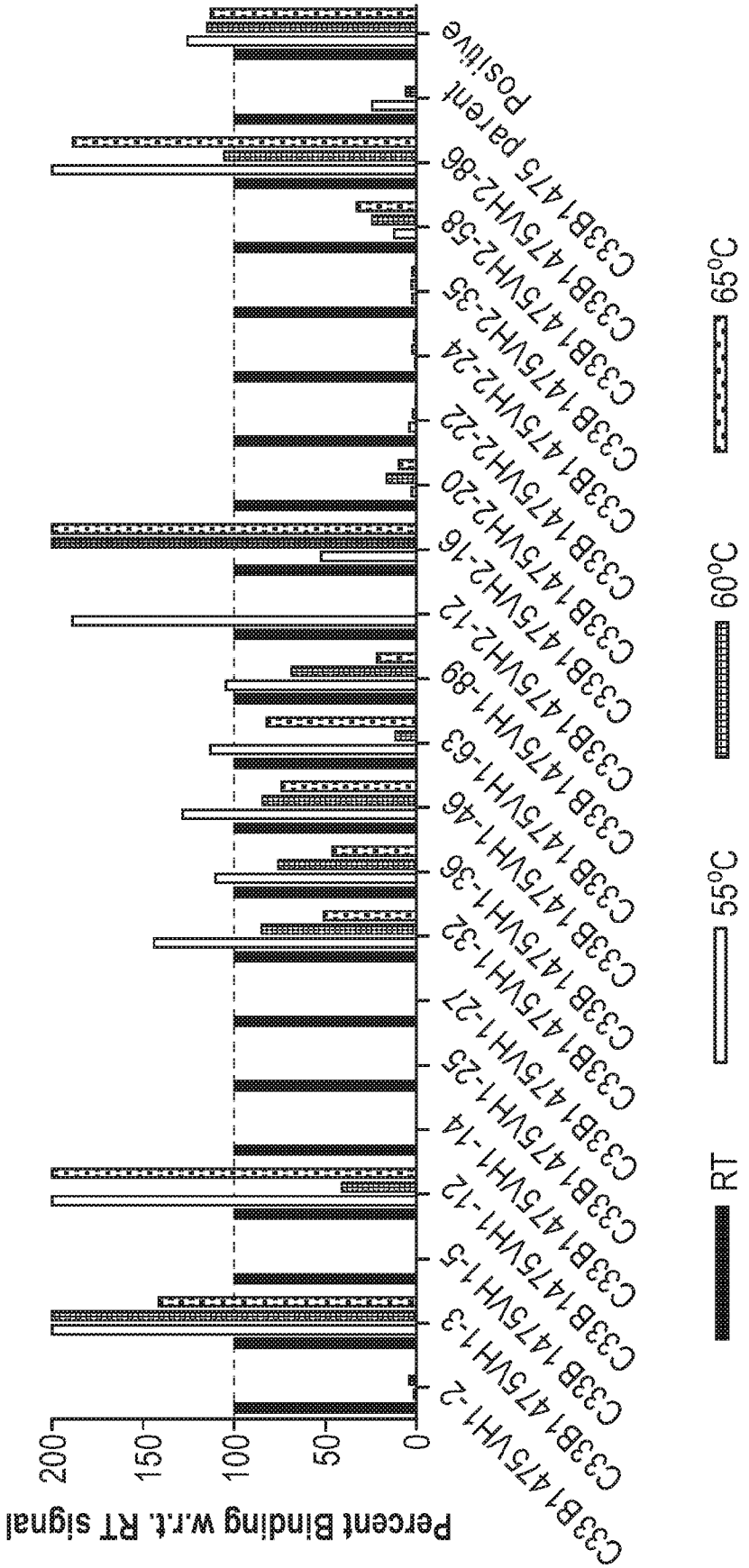
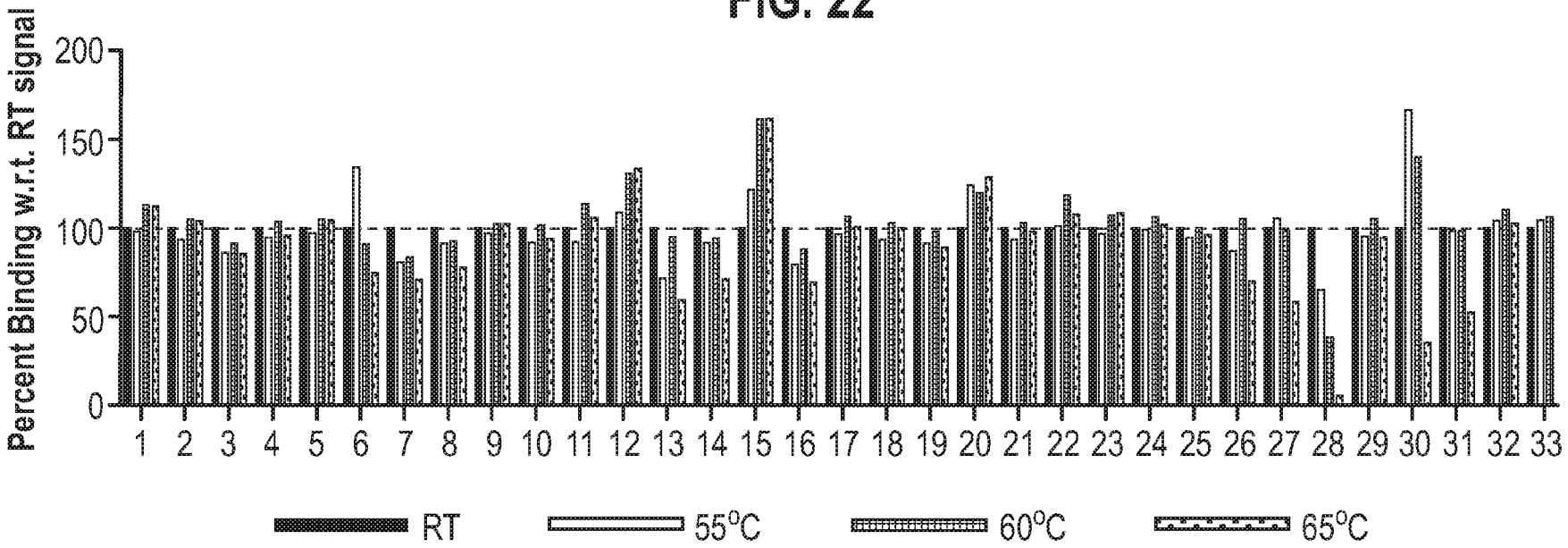

Percent Binding w.r.t. RT signal
200
150
100
50
0
RT
55°C
60°C
65°C
C33B1475VH1-2
C33B1475VH1-3
C33B1475VH1-5
C33B1475VH1-12
C33B1475VH1-14
C33B1475VH1-25
C33B1475VH1-27
C33B1475VH1-32
C33B1475VH1-36
C33B1475VH1-46
C33B1475VH1-63
C33B1475VH1-89
C33B1475VH2-12
C33B1475VH2-16
C33B1475VH2-20
C33B1475VH2-22
C33B1475VH2-24
C33B1475VH2-35
C33B1475VH2-58
C33B1475VH2-86
C33B1475 parent
Positive

FIG. 21

1	C33B1516VL1-1	**2**	3B1516VL1-10	**3**	C33B1516VL1-11	**4**	C33B1516VL1-14	**5**	C33B1516VL1-16
6	C33B1516VL1-2	**7**	C33B1516VL1-20	**8**	C33B1516VL1-22	**9**	C33B1516VL1-23	**10**	C33B1516VL1-30
11	C33B1516VL1-4	**12**	C33B1516VL2-10	**13**	C33B1516VL2-11	**14**	C33B1516VL2-29	**15**	C33B1516VL2-3
16	C33B1516VL2-35	**17**	C33B1516VL2-4	**18**	C33B1516VL2-7	**19**	C33B1516VL2-8	**20**	C33B1516VH1-1
21	C33B1516VH1-10	**22**	C33B1516VH1-13	**23**	C33B1516VH1-17	**24**	C33B1516VH1-20	**25**	C33B1516VH1-21
26	C33B1516VH1-27	**27**	C33B1516VH1-3	**28**	C33B1516VH1-38	**29**	C33B1516VH1-4	**30**	C33B1516VH1-56
31	C33B1516VH1-6	**32**	C33B1516VH1-7	**33**	C33B1516VH1-8	**34**	C33B1516VH2-8	**35**	C33B1516VH2-9
36	C33B1516VL1-39	**37**	C33B1516VL1-41	**38**	C33B1516VL1-63	**39**	C33B1516VL1-90	**40**	C33B1516VL2-37
41	C33B1516VL2-38	**42**	C33B1516VL2-90	**43**	C33B1516Parent	**44**	Positive		**KEY**

FIG. 22



1	C33B1517VL1-1	**2**	C33B1517VL1-7	**3**	C33B1517VL1-12	**4**	C33B1517VL1-8	**5**	C33B1517VL1-13
6	C33B1517VL1-16	**7**	C33B1517VL1-22	**8**	C33B1517VL1-35	**9**	C33B1517VL1-24	**10**	C33B1517VL1-27
11	C33B1517VL1-49	**12**	C33B1517VL1-66	**13**	C33B1517VL1-69	**14**	C33B1517VL1-71	**15**	C33B1517VH1-1
16	C33B1517VH1-3	**17**	C33B1517VH1-6	**18**	C33B1517VH1-4	**19**	C33B1517VH1-8	**20**	C33B1517VH1-11
21	C33B1517VH1-17	**22**	C33B1517VH1-27	**23**	C33B1517VH1-33	**24**	C33B1517VH1-60	**25**	C33B1517VH1-71
26	C33B1517VH2-7	**27**	C33B1517VH2-30	**28**	C33B1517VH2-31	**29**	C33B1517VH2-60	**30**	C33B1517VH2-68
31	C33B1517VH2-76	**32**	Positive	**33**	C33B1517 Parent	**KEY**			

FIG. 23

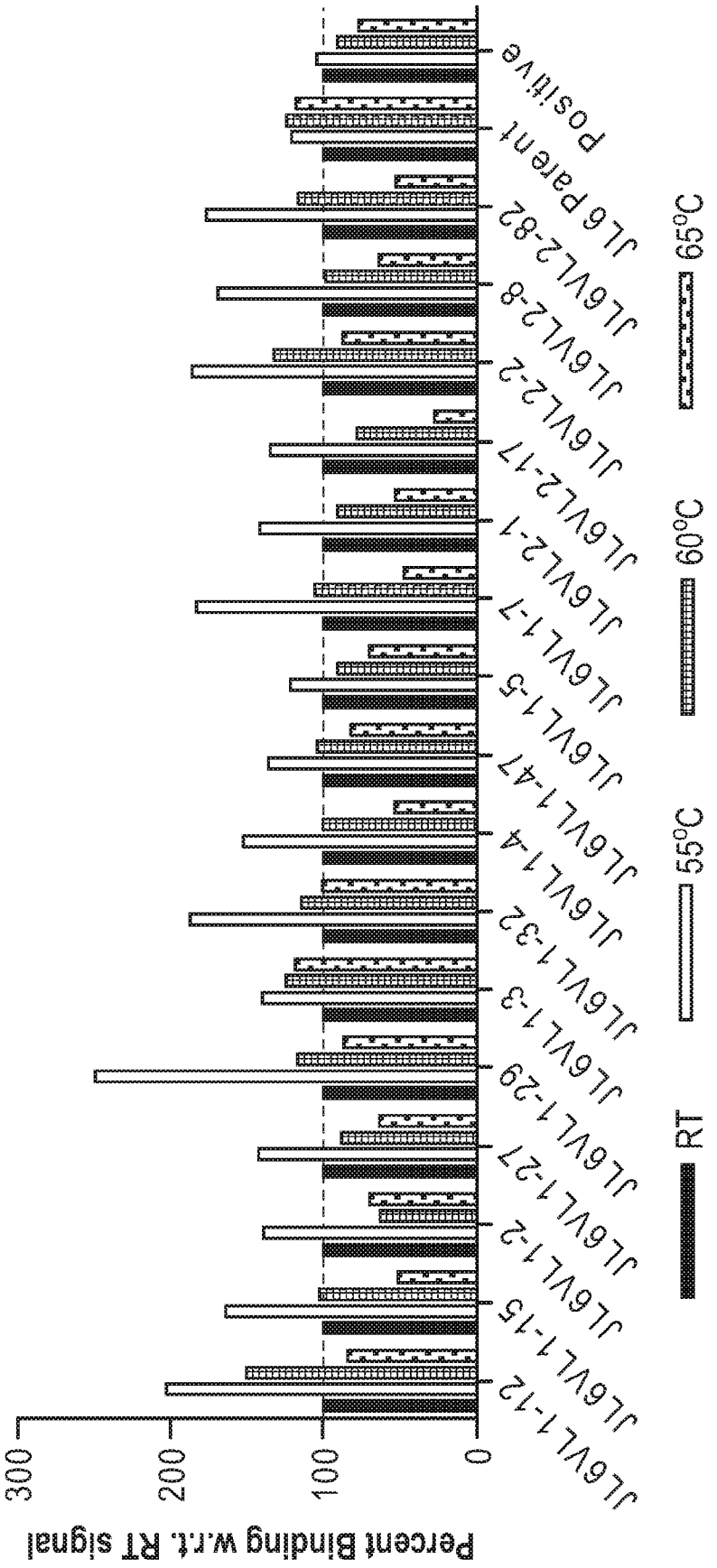

Percent Binding w.r.t. RT signal
300
200
100
0
RT
55°C
60°C
65°C
JL6VL1-12
JL6VL1-15
JL6VL1-2
JL6VL1-27
JL6VL1-29
JL6VL1-3
JL6VL1-32
JL6VL1-4
JL6VL1-47
JL6VL1-5
JL6VL1-7
JL6VL2-1
JL6VL2-17
JL6VL2-2
JL6VL2-8
JL6VL2-82
JL6 Parent
Positive

FIG. 24

JL2

Clone ID	AA
C33B1475VH1-2	G
C33B1475VH1-3	A
C33B1475VH1-5	Y
C33B1475VH1-12	P
C33B1475VH1-14	N
C33B1475VH1-22	S
C33B1475VH1-27	R
C33B1475VH1-32	L
C33B1475VH1-36	V
C33B1475VH1-46	T
C33B1475VH1-63	F
C33B1475VH1-89	W
C33B1475VH2-12	V
C33B1475VH2-16	P
C33B1475VH2-20	R
C33B1475VH2-22	A
C33B1475VH2-24	L
C33B1475VH2-35	Q
C33B1475VH2-58	T
C33B1475VH2-86	H

JL3

Clone ID	AA	Clone ID	AA
C33B1516 VL1-1	V	C33B1516VH1-1	V
C33B1516 VL1-2	R	C33B1516VH1-3	S
C33B1516 VL1-4	P	C33B1516VH1-4	E
C33B1516 VL1-10	T	C33B1516VH1-6	H
C33B1516 VL1-11	G	C33B1516VH1-7	A
C33B1516 VL1-14	Q	C33B1516VH1-8	G
C33B1516 VL1-16	S	C33B1516VH1-10	K
C33B1516 VL1-20	A	C33B1516VH1-13	T
C33B1516 VL1-22	L	C33B1516VH1-17	F
C33B1516 VL1-23	Y	C33B1516VH1-20	V
C33B1516 VL1-30	E	C33B1516VH1-21	R
C33B1516VL1-39	F	C33B1516VH1-27	L
C33B1516VL1-41	D	C33B1516VH1-38	H
C33B1516VL1-63	I	C33B1516VH1-56	I
C33B1516VL1-90	H	C33B1516VH2-8	S
C33B1516 VL2-3	V	C33B1516VH2-9	A
C33B1516 VL2-4	P		
C33B1516 VL2-7	R		
C33B1516 VL2-8	D		
C33B1516 VL2-10	E		
C33B1516 VL2-11	W		
C33B1516 VL2-29	Y		
C33B1516 VL2-35	A		
C33B1516VL2-37	N		
C33B1516VL2-38	K		
C33B1516VL2-90	L		

FIG. 24(contd)

JL5

Clone ID	AA	Clone ID	AA
C33B1517VL1-1	G	C33B1517VH1-1	R
C33B1517VL1-7	D	C33B1517VH1-3	P
C33B1517VL1-8	P	C33B1517VH1-4	G
C33B1517VL1-12	K	C33B1517VH1-6	V
C33B1517VL1-13	E	C33B1517VH1-8	L
C33B1517VL1-16	A	C33B1517VH1-11	D
C33B1517VL1-22	R	C33B1517VH1-17	E
C33B1517VL1-24	Q	C33B1517VH1-33	A
C33B1517VL1-27	I	C33B1517VH1-60	Q
C33B1517VL1-35	V	C33B1517VH1-71	T
C33B1517VL1-49	H	C33B1517VH2-7	S
C33B1517VL1-66	K	C33B1517VH2-30	A
C33B1517VL1-69	W	C33B1517VH2-31	L
C33B1517VL1-71	S	C33B1517VH2-60	P
		C33B1517VH2-68	T
		C33B1517VH2-76	Q

JL6

Clone ID	AA
C33B1522VL1-2	S
C33B1522VL1-3	Q
C33B1522VL1-4	V
C33B1522VL1-5	G
C33B1522VL1-7	W
C33B1522VL1-12	T
C33B1522VL1-15	I
C33B1522VL1-27	E
C33B1522VL1-29	A
C33B1522VL1-32	R
C33B1522VL1-47	L
C33B1522VL2-82	V
C33B1522VL2-1	A
C33B1522VL2-2	P
C33B1522VL2-8	L
C33B1522VL2-17	T