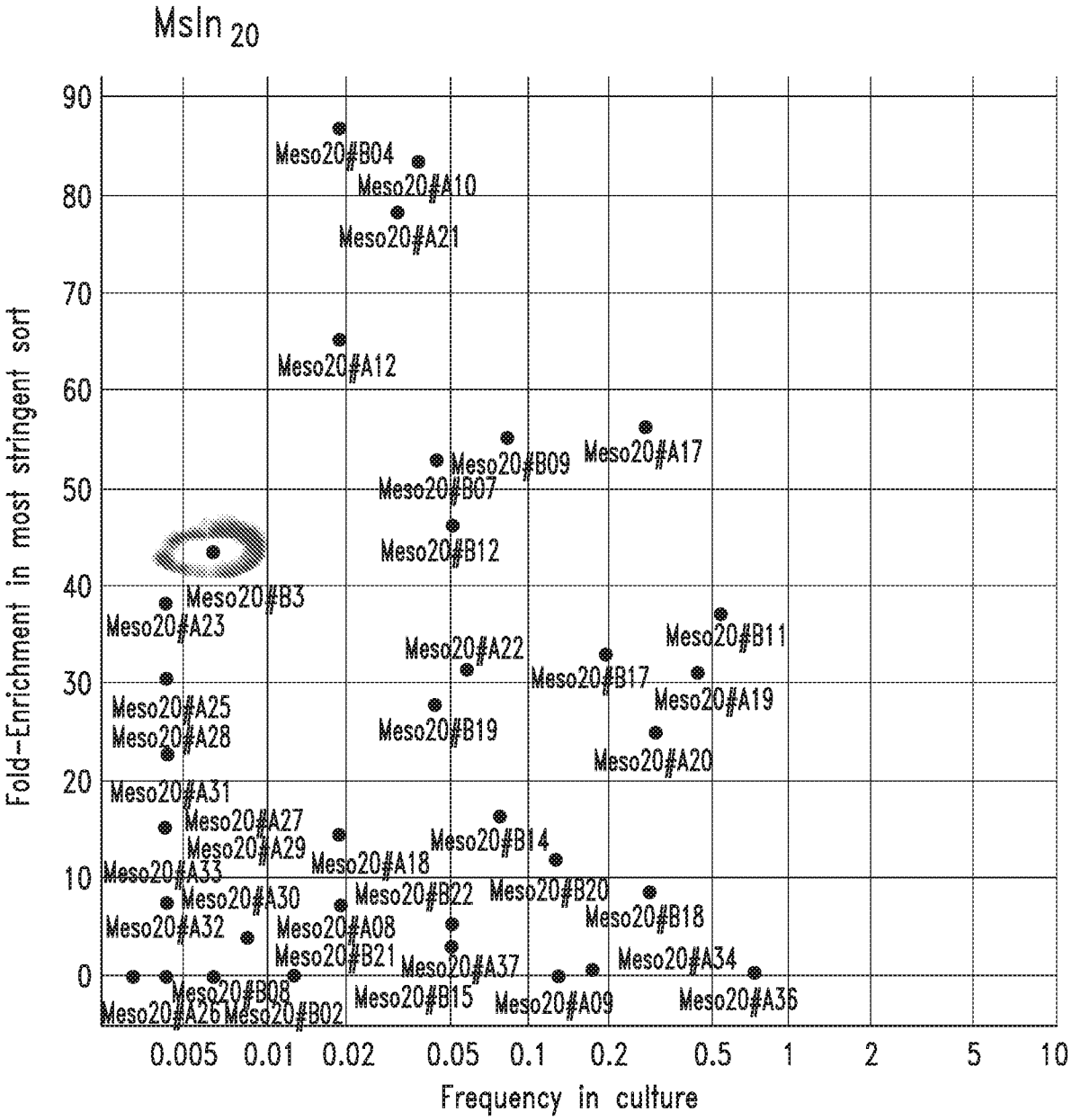

MsIn 20
Fold-Enrichment in most stringent sort
Frequency in culture
90
80
70
60
50
40
30
20
10
0
0.005
0.01
0.02
0.05
0.1
0.2
0.5
1
2
5
10
Meso20#B04
Meso20#A10
Meso20#A21
Meso20#A12
Meso20#B09
Meso20#A17
Meso20#B07
Meso20#B12
Meso20#B3
Meso20#A23
Meso20#A22
Meso20#B11
Meso20#B17
Meso20#A19
Meso20#A25
Meso20#A28
Meso20#B19
Meso20#A20
Meso20#A31
Meso20#A27
Meso20#A29
Meso20#B14
Meso20#A18
Meso20#A33
Meso20#A30
Meso20#B22
Meso20#B20
Meso20#B18
Meso20#A32
Meso20#A08
Meso20#B21
Meso20#A37
Meso20#A34
Meso20#B08
Meso20#B15
Meso20#A09
Meso20#A36
Meso20#A26
Meso20#B02

FIG. 1A

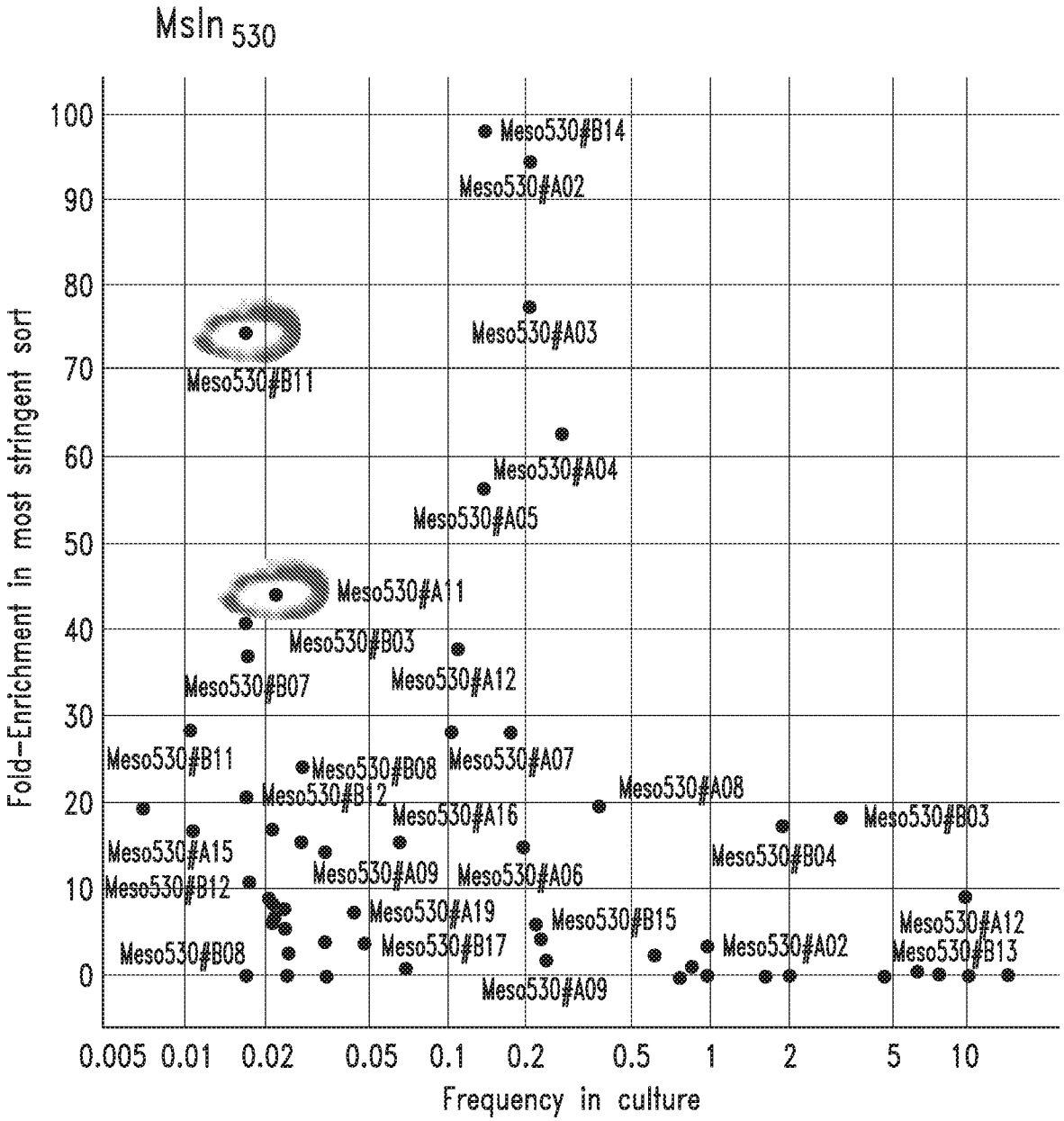

MsIn 530
Fold-Enrichment in most stringent sort
Frequency in culture
100
90
80
70
60
50
40
30
20
10
0
0.005
0.01
0.02
0.05
0.1
0.2
0.5
1
2
5
10
Meso530#B14
Meso530#A02
Meso530#A03
Meso530#B11
Meso530#A04
Meso530#A05
Meso530#A11
Meso530#B03
Meso530#B07
Meso530#A12
Meso530#B11
Meso530#B08
Meso530#A07
Meso530#A08
Meso530#B12
Meso530#A16
Meso530#B03
Meso530#A15
Meso530#B04
Meso530#A09
Meso530#A06
Meso530#B12
Meso530#A19
Meso530#B15
Meso530#A12
Meso530#B08
Meso530#B17
Meso530#A02
Meso530#B13
Meso530#A09

FIG. 1B

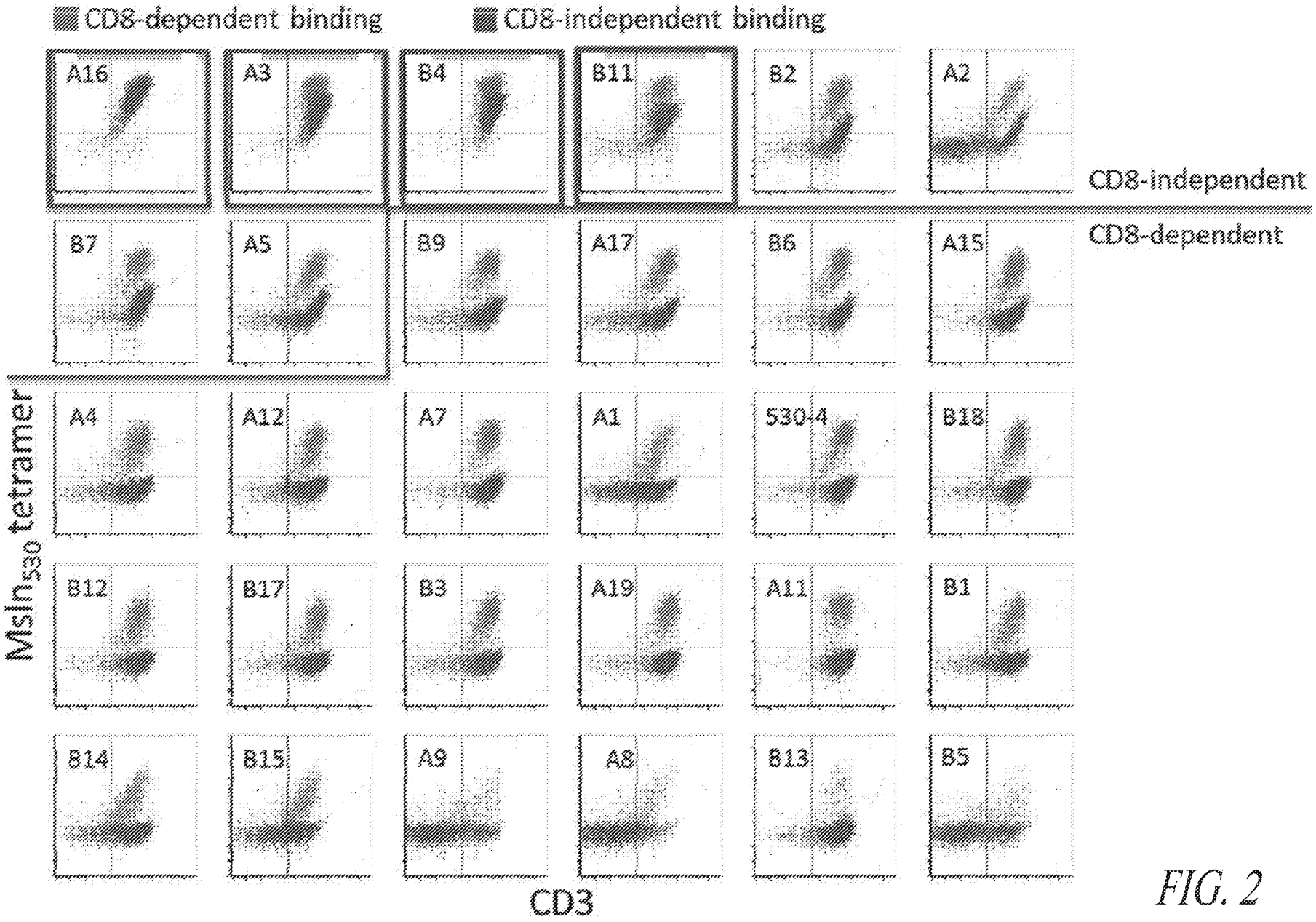

CD8-dependent binding
CD8-independent binding
A16
A3
B4
B11
B2
A2
CD8-independent
B7
A5
B9
A17
B6
A15
CD8-dependent
A4
A12
A7
A1
S30-4
B18
B12
B17
B3
A19
A11
B1
B14
B15
A9
A8
B13
B5
MsIn530 tetramer
CD3

FIG. 2

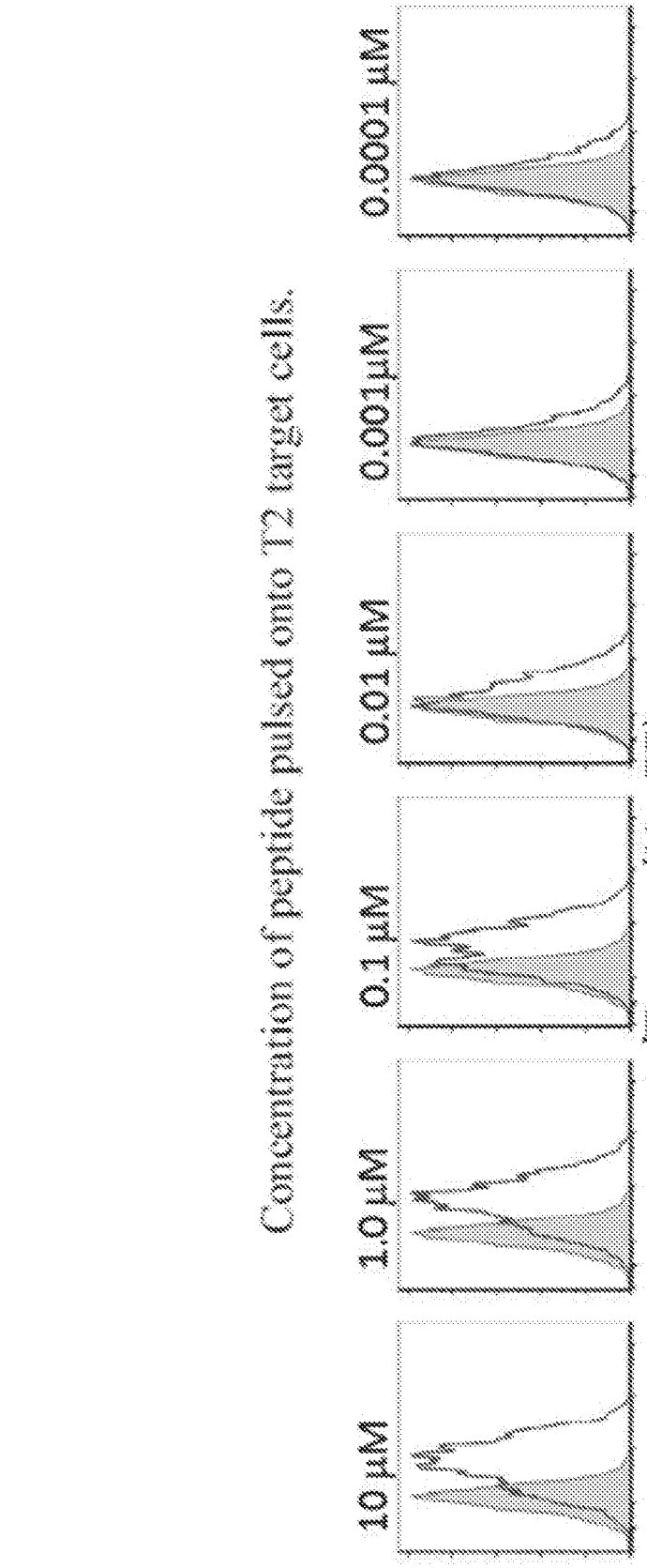

Concentration of peptide pulsed onto T2 target cells.
10 µM
1.0 µM
0.1 µM
0.01 µM
0.001µM
0.0001 µM
tdTomato (Nur77)

FIG. 3A

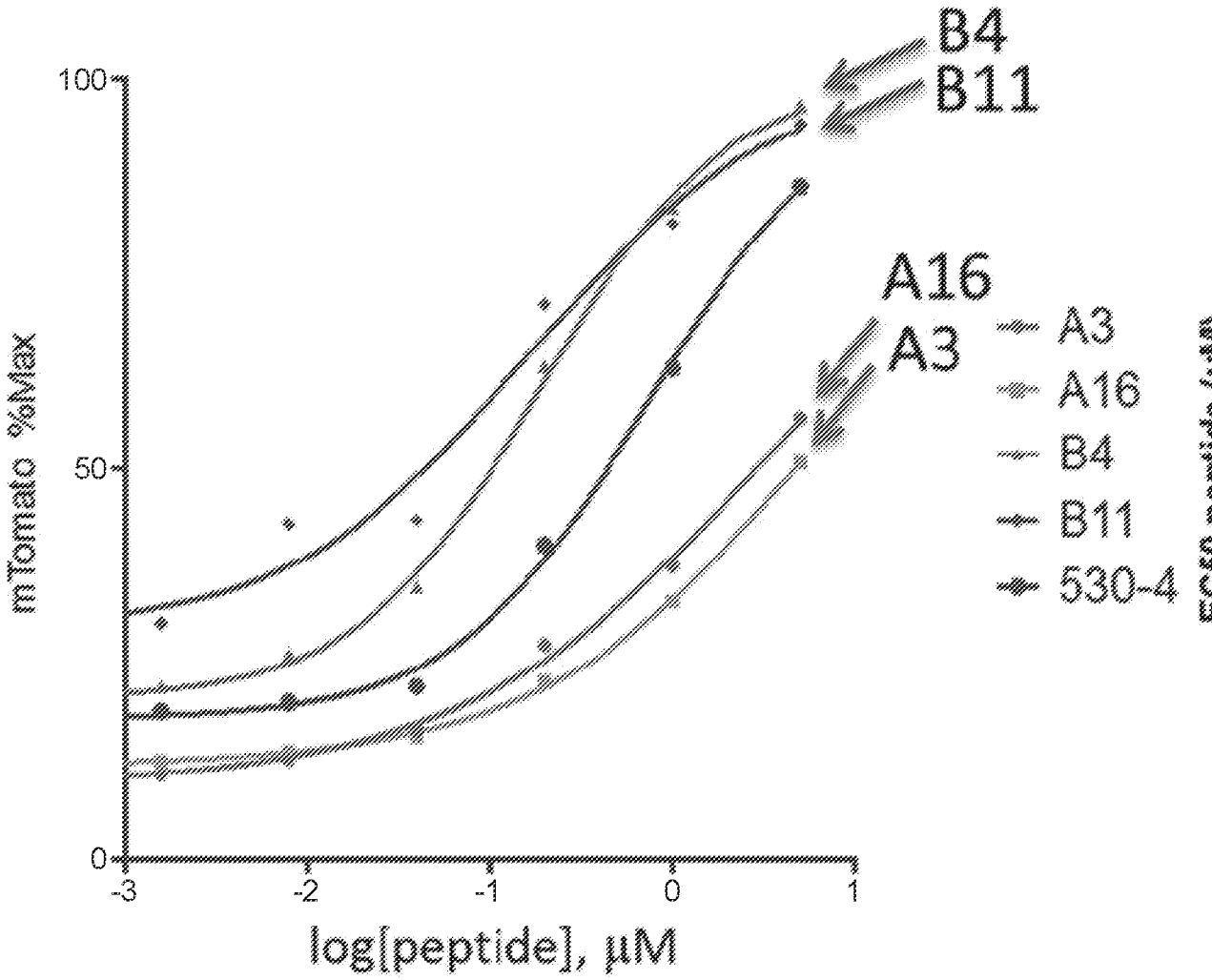

B4
B11
A16
A3
A3
A16
B4
B11
530-4
mTomato %Max
100
50
0
-3
-2
-1
0
1
log[peptide], µM

FIG. 3B

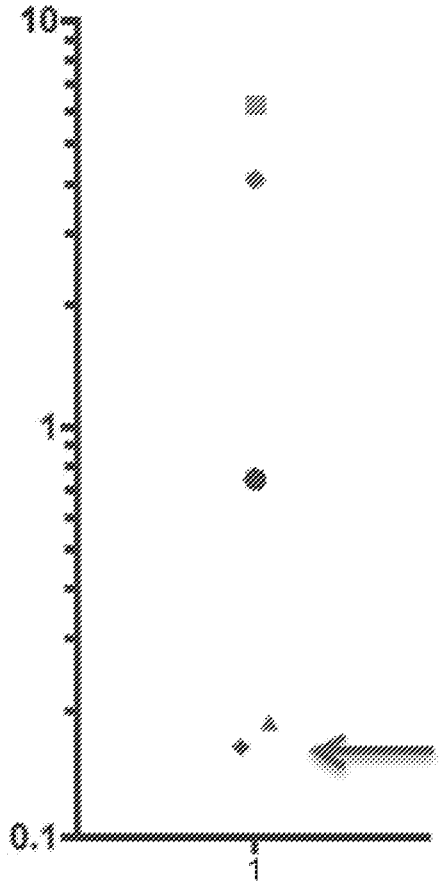

EC50 peptide (uM)
10
1
0.1
1

FIG. 3C

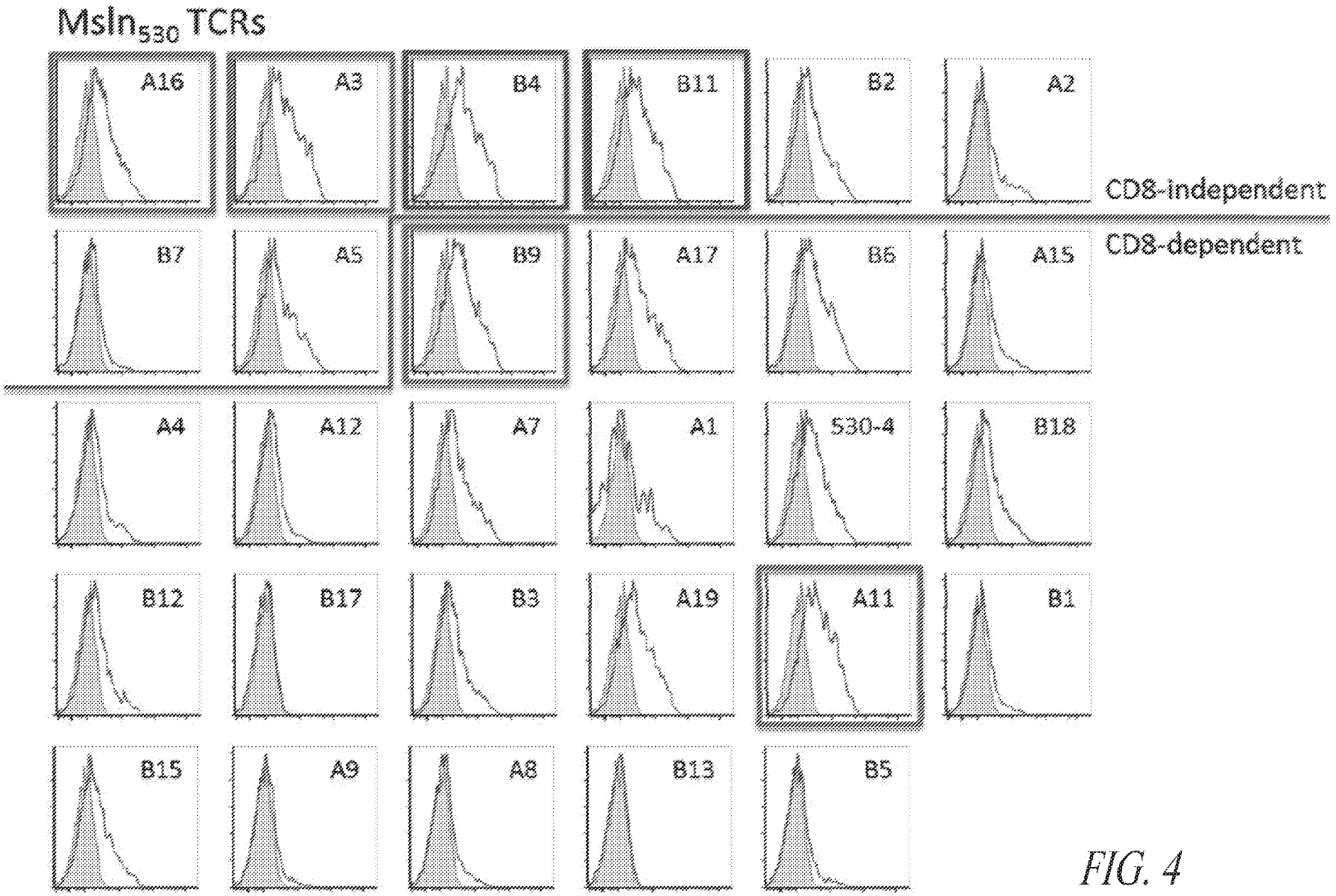

MsIn530 TCRs
A16
A3
B4
B11
B2
A2
CD8-independent
B7
A5
B9
A17
B6
A15
CD8-dependent
A4
A12
A7
A1
530-4
B18
B12
B17
B3
A19
A11
B1
B15
A9
A8
B13
B5

FIG. 4

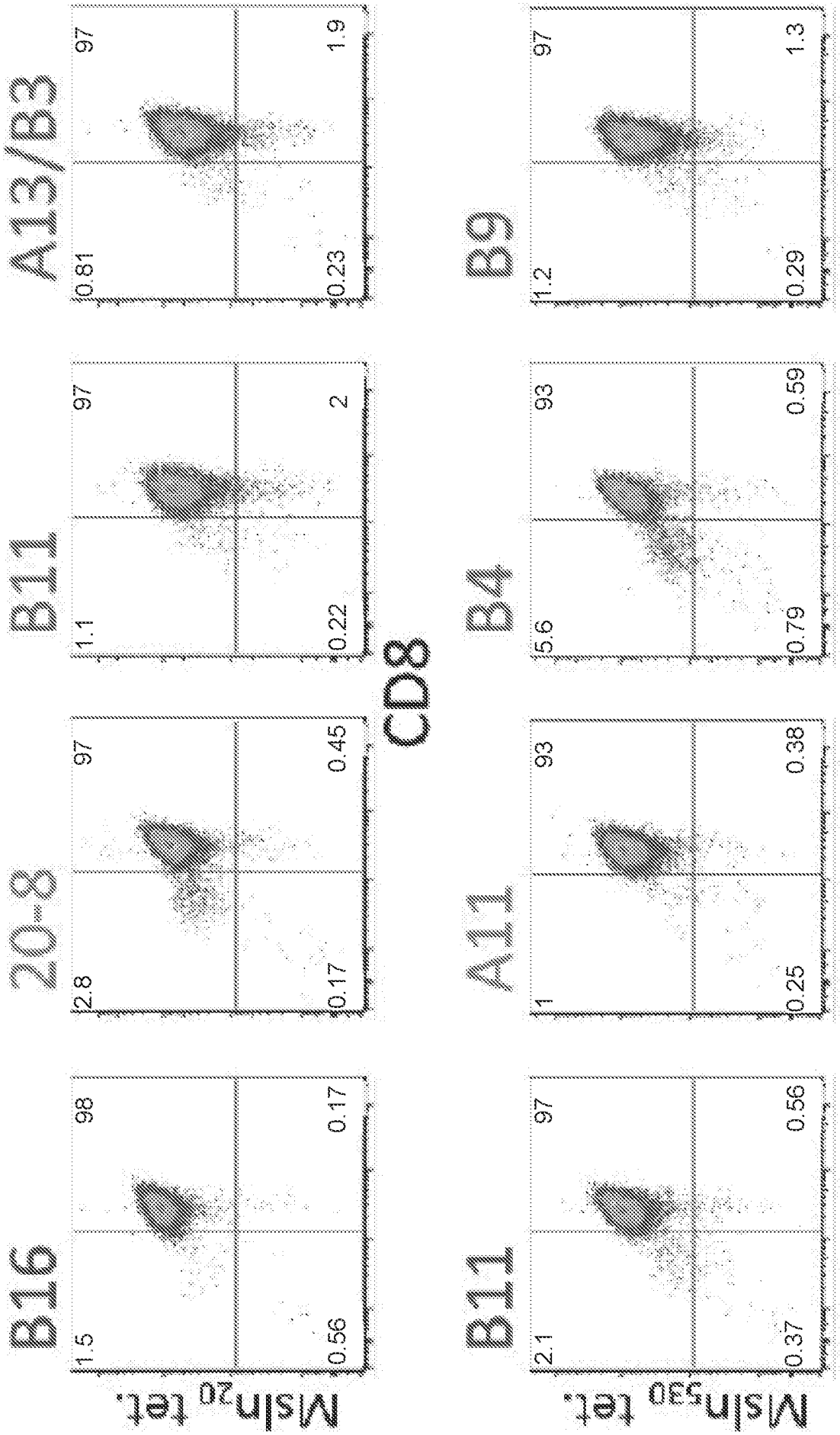

B16
20-8
B11
A13/B3
B4
A11
B9
B11
Msln20 tet.
Msln530 tet.
CD8
CD8
1.5
98
0.17
0.56
2.8
97
0.45
0.17
1.1
97
2
0.22
0.81
97
1.9
0.23
15.6
93
0.59
0.79
1
93
0.38
0.25
1.2
97
1.3
0.29
2.1
97
0.56
0.37

FIG. 5A

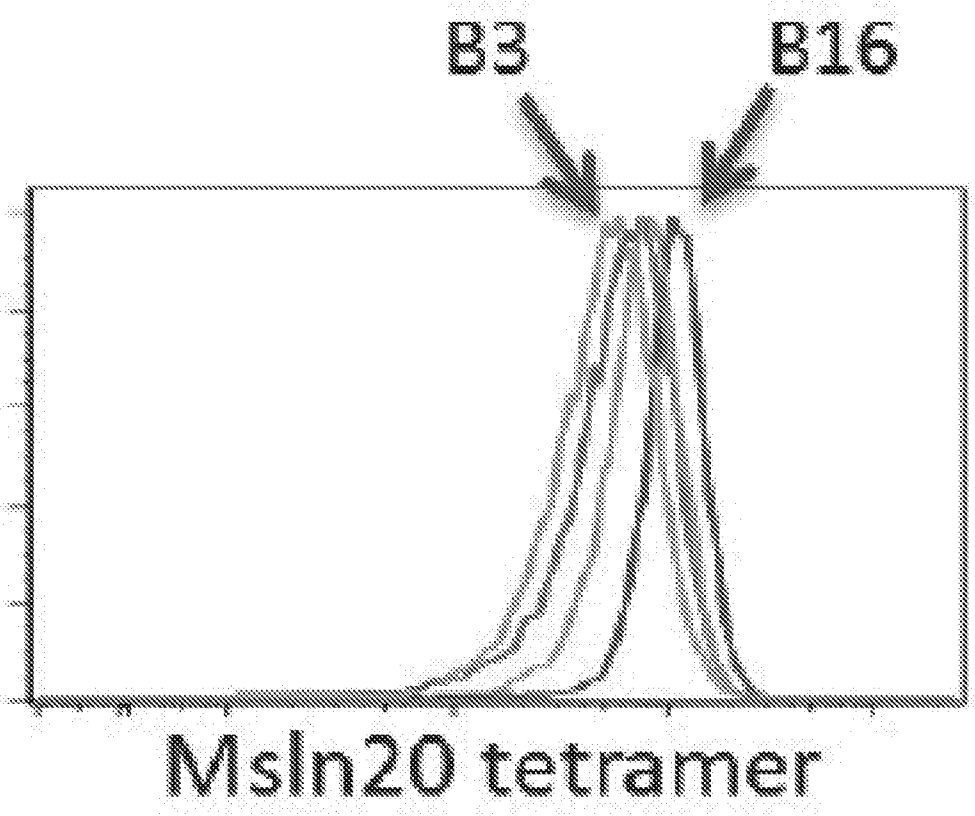


FIG. 5B

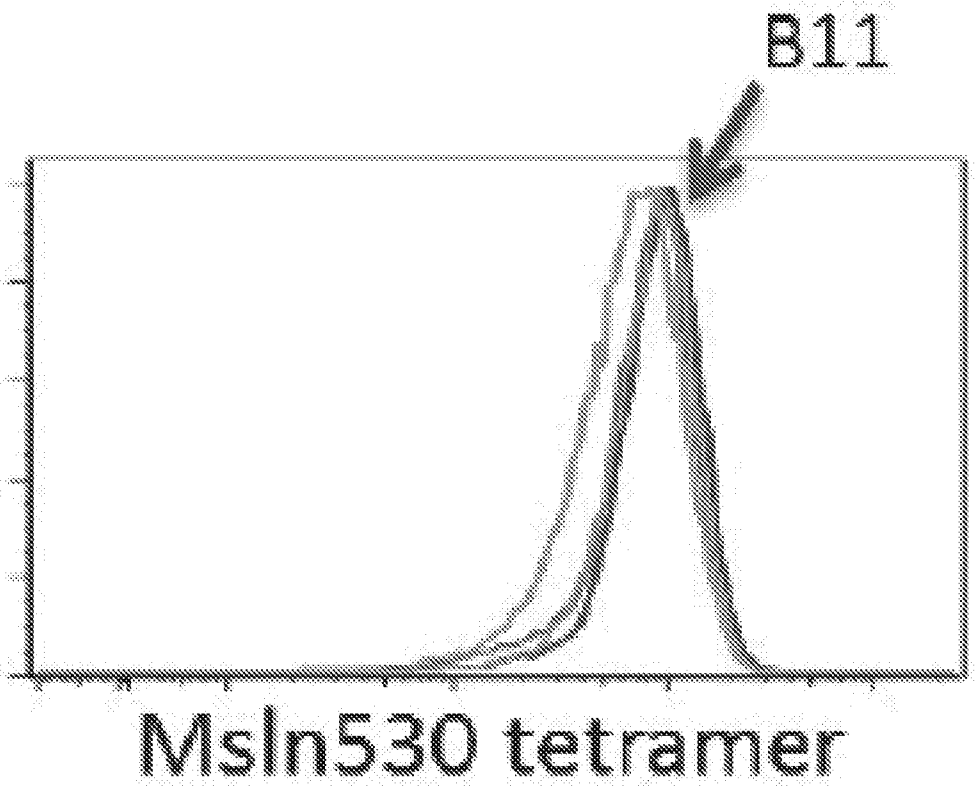


FIG. 5C

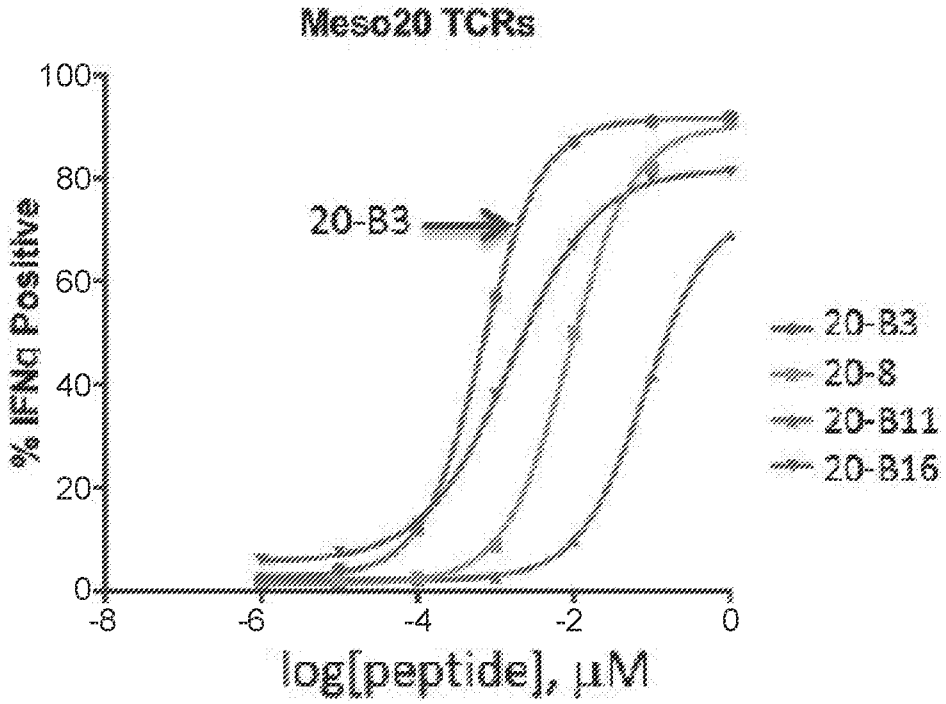

Meso20 TCRs
% IFNg Positive
100
80
60
40
20
0
-8
-6
-4
-2
0
log[peptide], µM
20-B3
20-B3
20-8
20-B11
20-B16

FIG. 6A

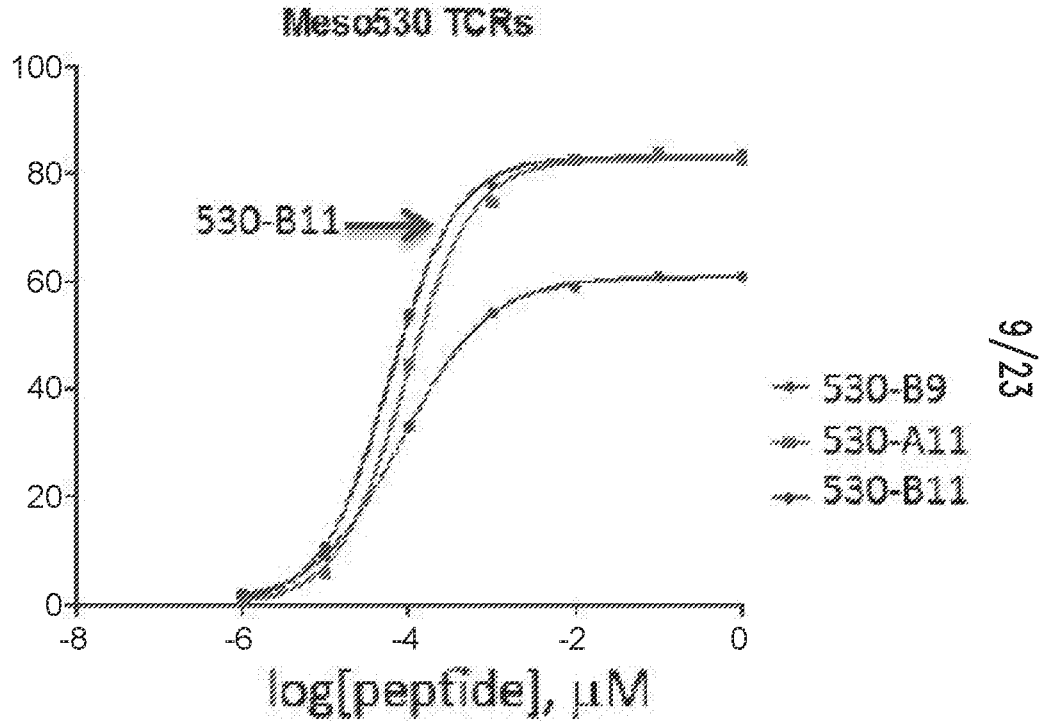

Meso530 TCRs
100
80
60
40
20
0
-8
-6
-4
-2
0
log[peptide], µM
530-B11
530-B9
530-A11
530-B11

FIG. 6B

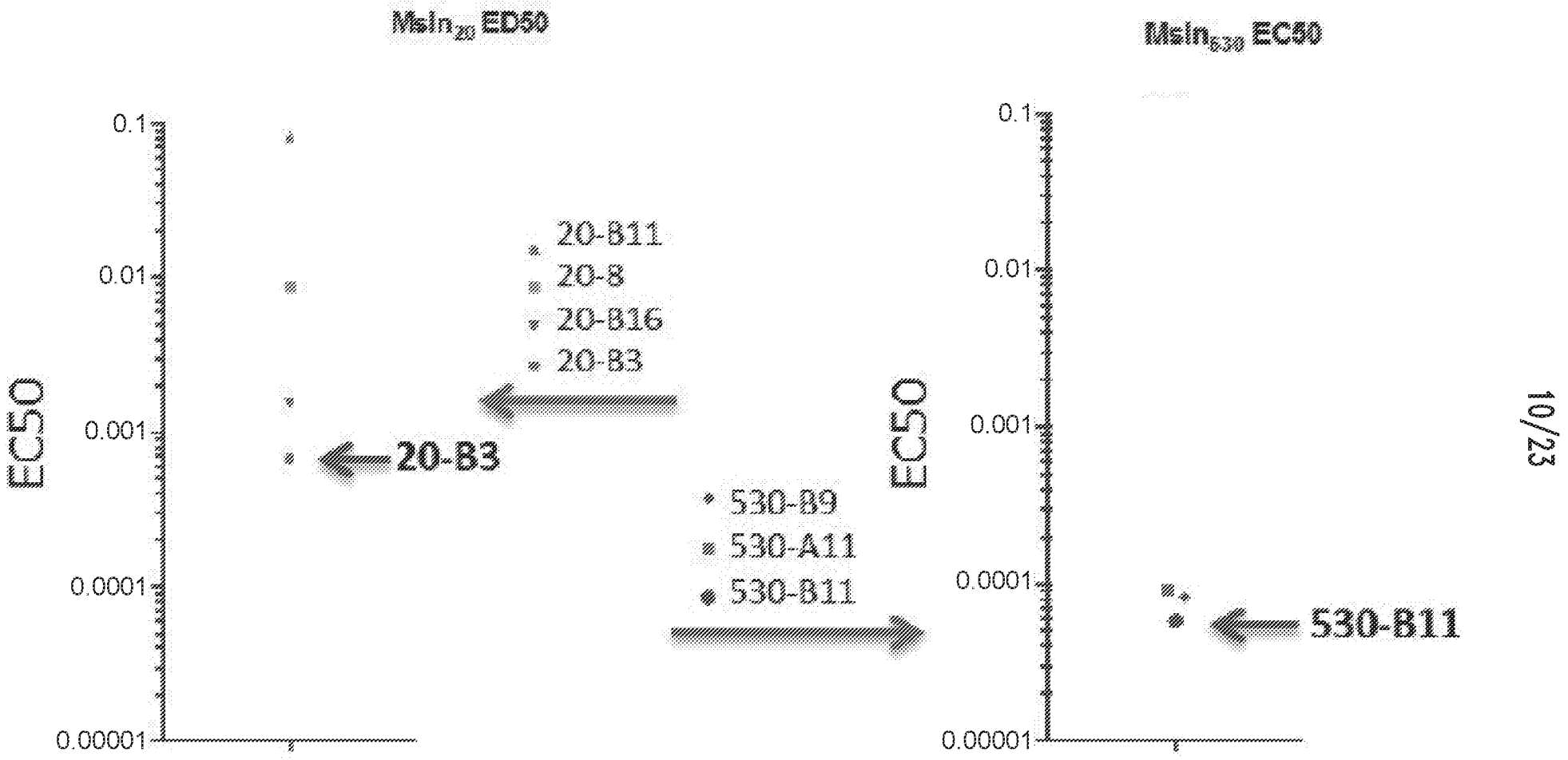

MsIn20 ED50
MsIn530 EC50
EC50
EC50
0.1
0.01
0.001
0.0001
0.00001
20-B11
20-8
20-B16
20-B3
20-B3
530-B9
530-A11
530-B11
530-B11

FIG. 6C

MDA-MB-468

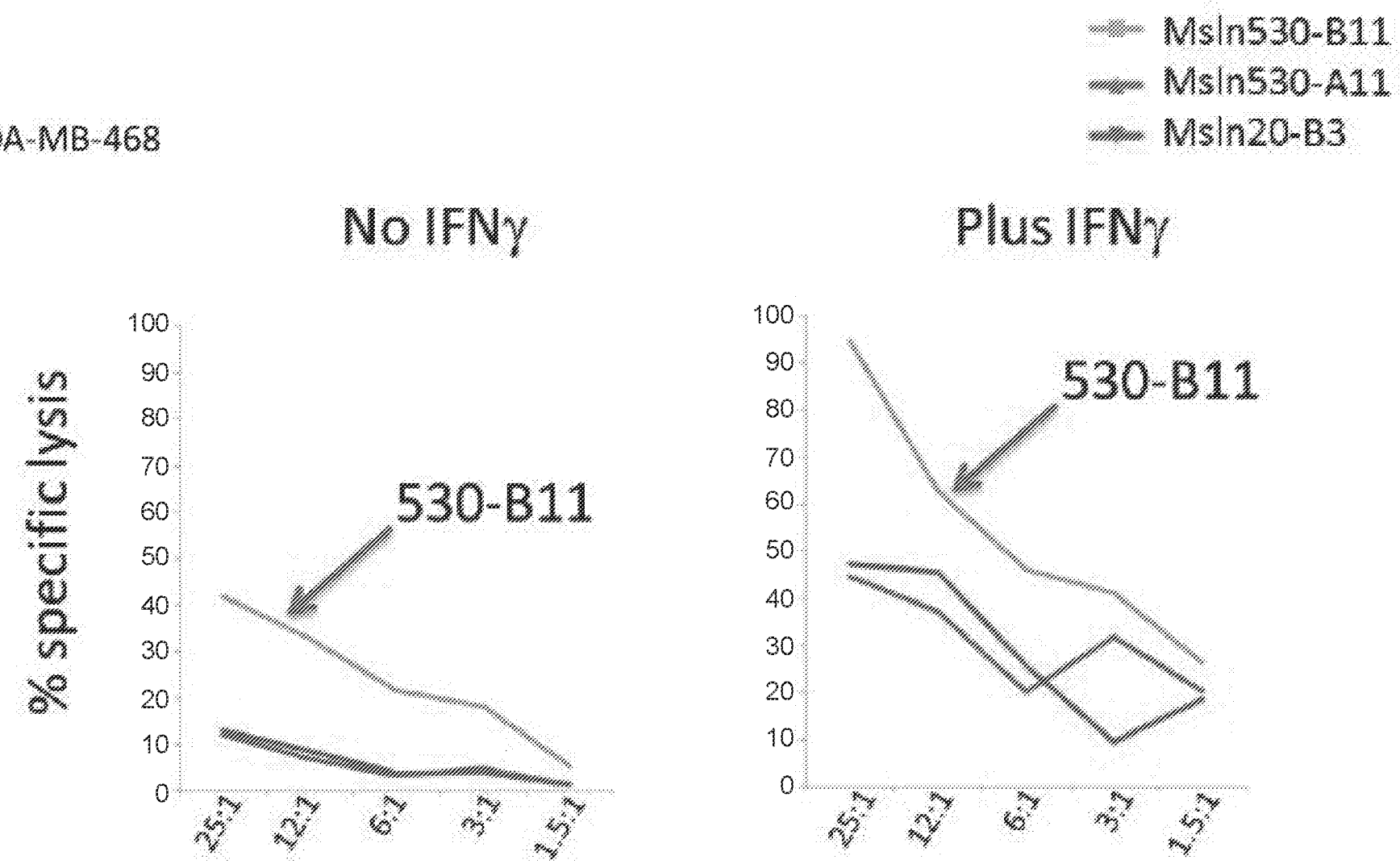


FIG. 7A

FIG. 7B

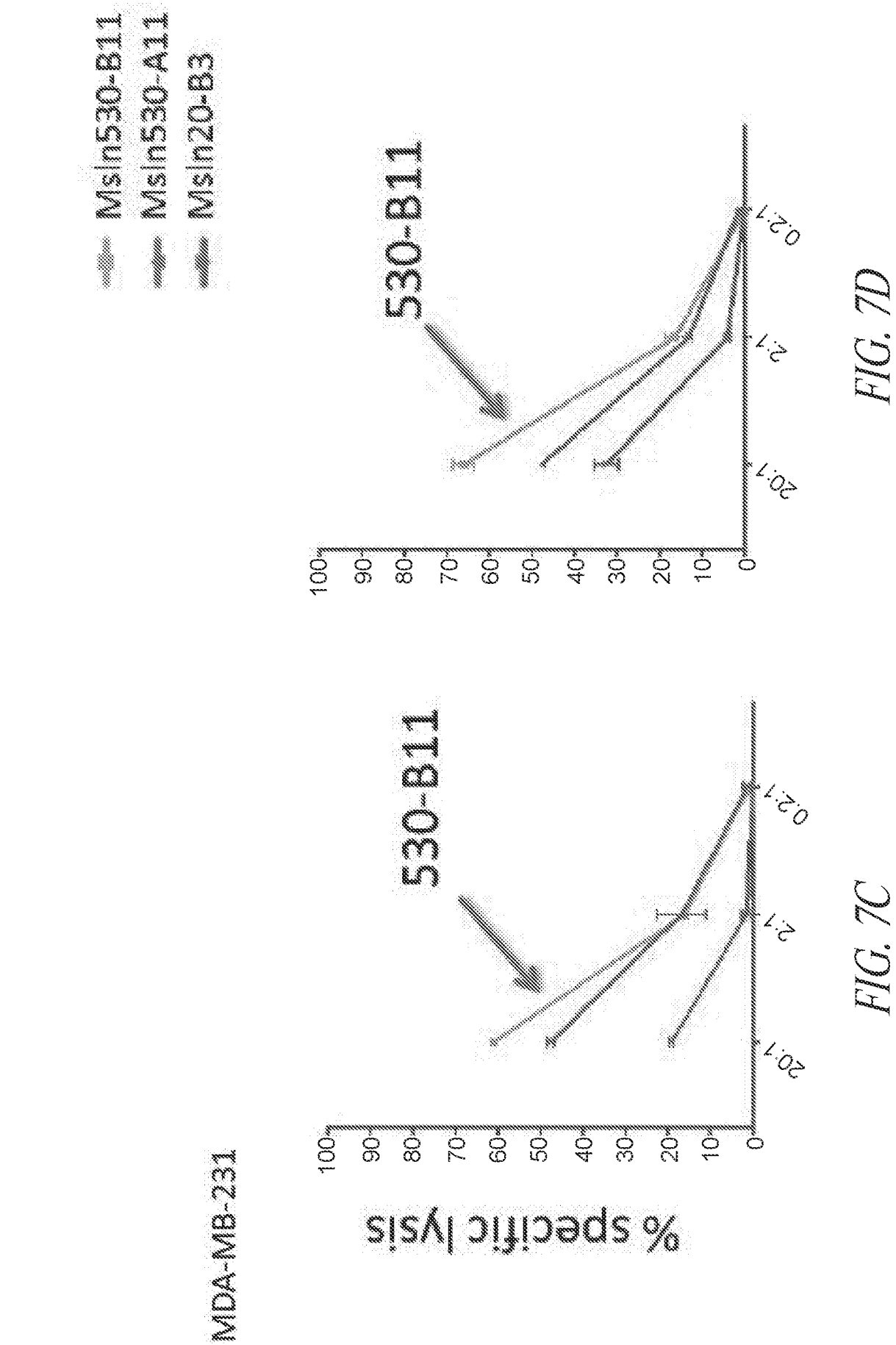

MDA-MB-231
% specific lysis
100
90
80
70
60
50
40
30
20
10
0
530-B11
0.2:1
2:1
20:1
530-B11
Ms|n530-B11
Ms|n530-A11
Ms|n20-B3

FIG. 7C

FIG. 7D

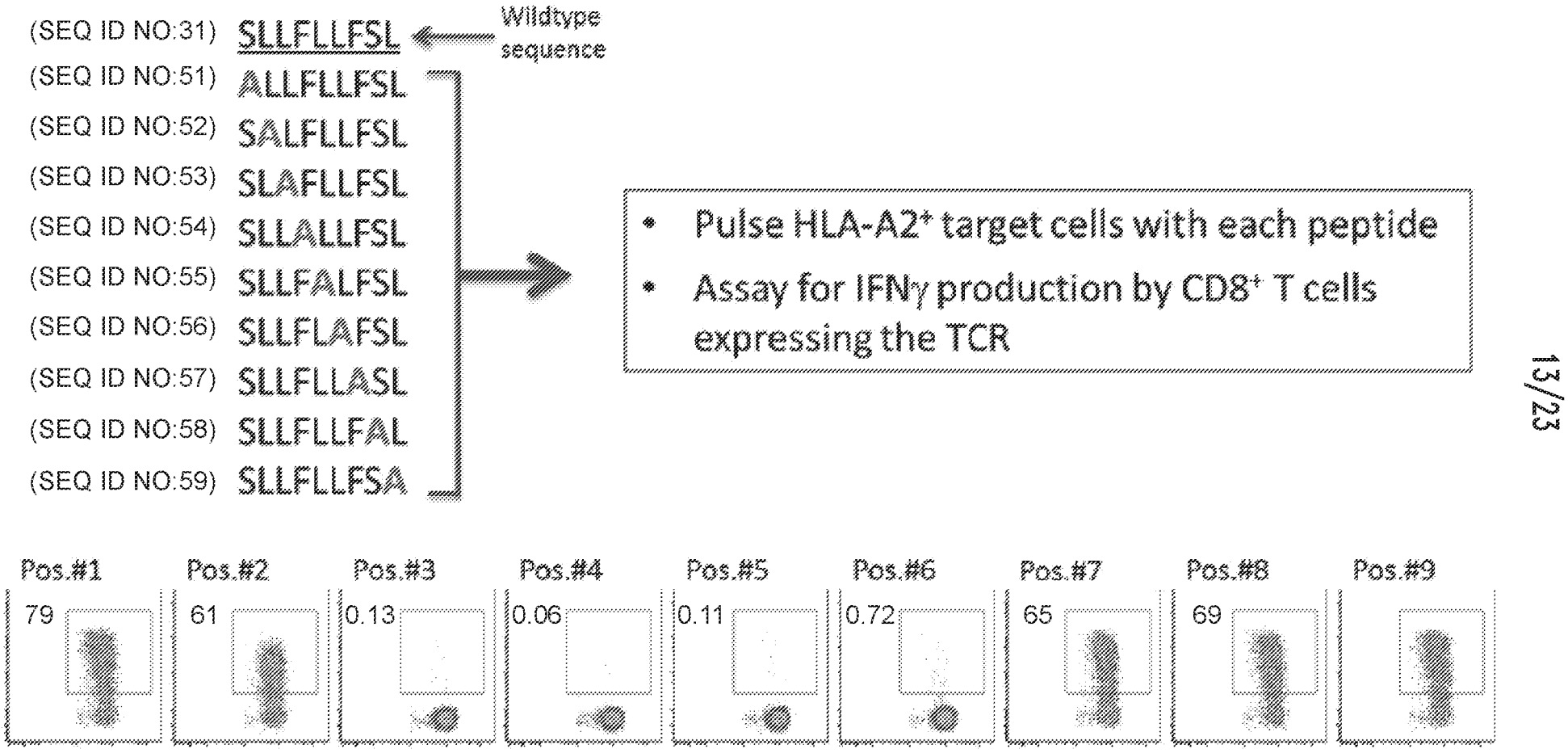

(SEQ ID NO:31) SLLFLLFSL
Wildtype sequence
(SEQ ID NO:51) ALLFLLFSL
(SEQ ID NO:52) SALFLLFSL
(SEQ ID NO:53) SLAFLLFSL
(SEQ ID NO:54) SLLALLFSL
(SEQ ID NO:55) SLLFALFSL
(SEQ ID NO:56) SLLFLAFSL
(SEQ ID NO:57) SLLFLLASL
(SEQ ID NO:58) SLLFLLFAL
(SEQ ID NO:59) SLLFLLFSA
• Pulse HLA-A2+ target cells with each peptide
• Assay for IFNγ production by CD8+ T cells expressing the TCR
Pos.#1
Pos.#2
Pos.#3
Pos.#4
Pos.#5
Pos.#6
Pos.#7
Pos.#8
Pos.#9
%IFNγ+
79
61
0.13
0.06
0.11
0.72
65
69

FIG. 8

$MSLN_{20}$ TCRs

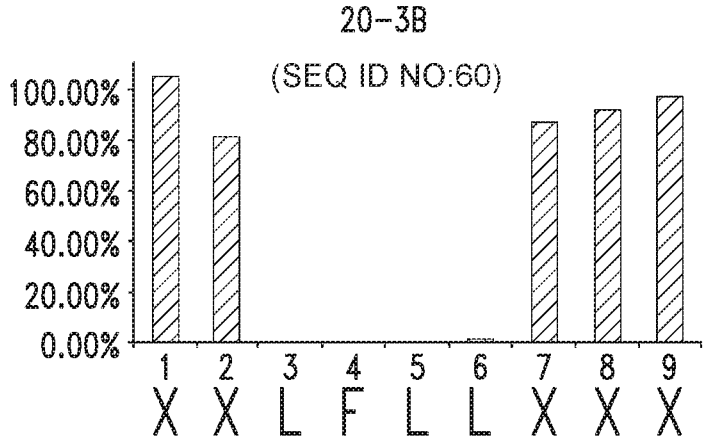


FIG. 9A

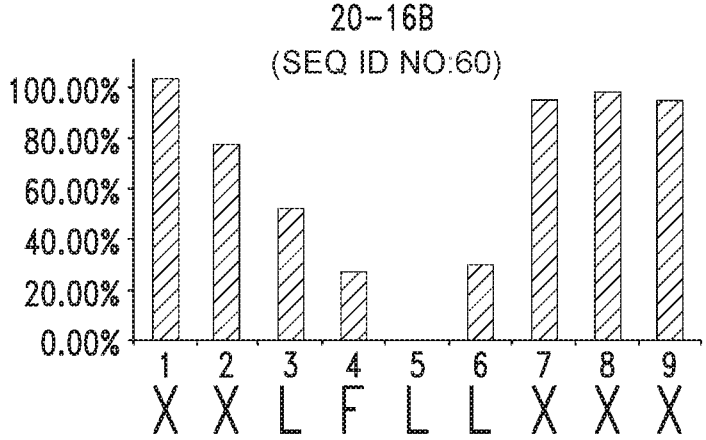


FIG. 9C

$MSLN_{530}$ TCRs

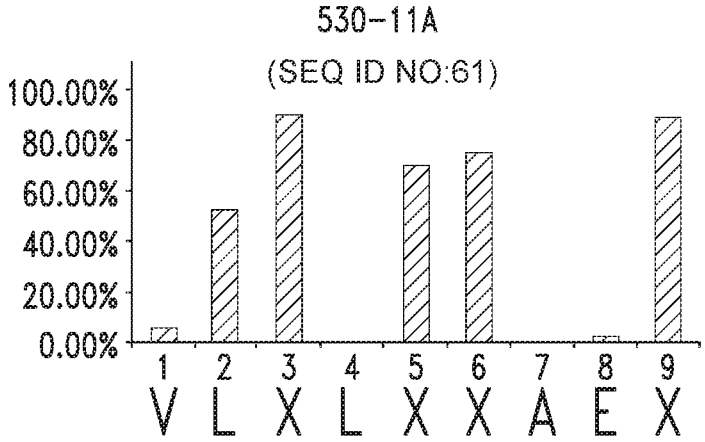


FIG. 9B

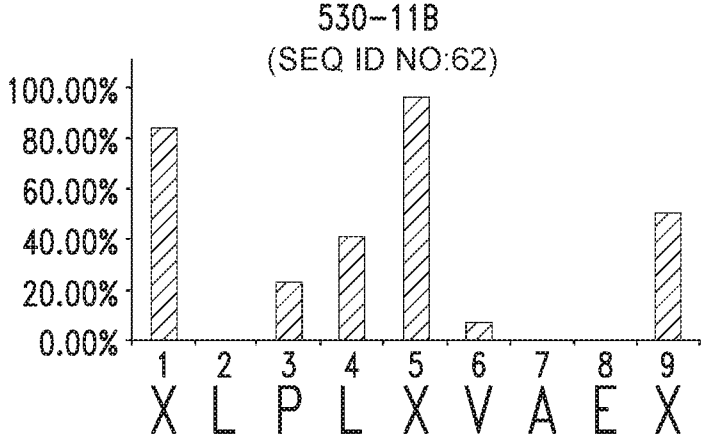


FIG. 9D

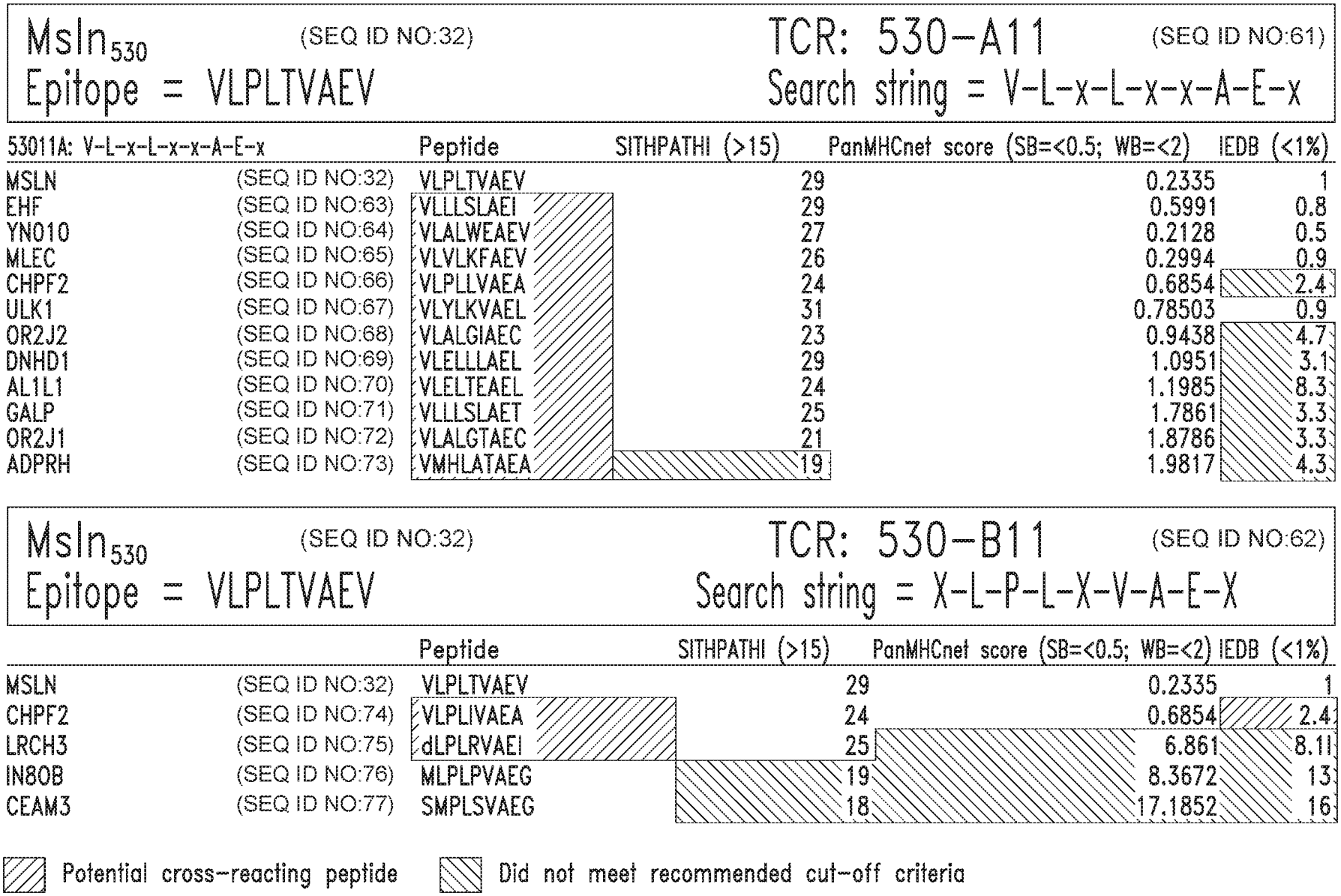
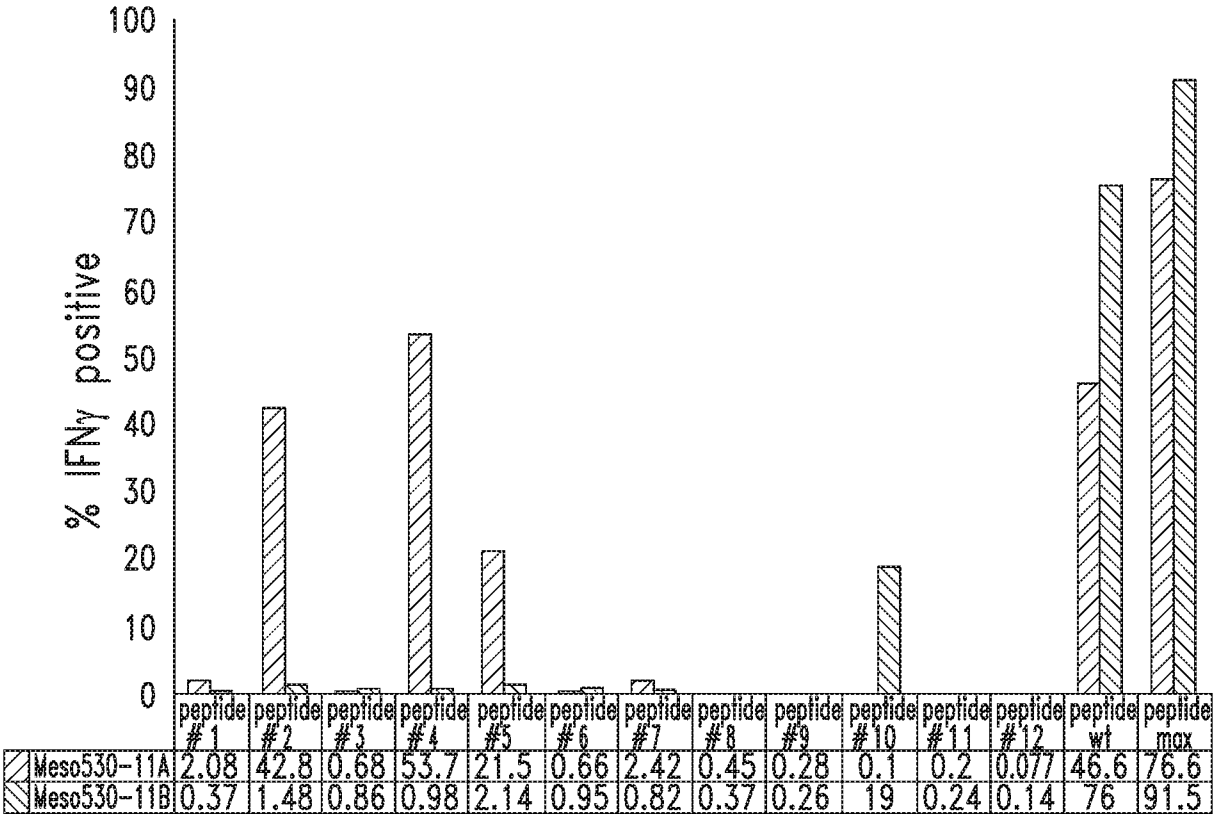

Msln530 (SEQ ID NO:32)
Epitope = VLPLTVAEV
TCR: 530-A11 (SEQ ID NO:61)
Search string = V-L-x-L-x-x-A-E-x
53011A: V-L-x-L-x-x-A-E-x
Peptide
SITHPATHI (>15)
PanMHCnet score (SB=<0.5; WB=<2)
IEDB (<1%)
MSLN (SEQ ID NO:32) VLPLTVAEV 29 0.2335 1
EHF (SEQ ID NO:63) VLLLSLAEI 29 0.5991 0.8
YN010 (SEQ ID NO:64) VLALWEAEV 27 0.2128 0.5
MLEC (SEQ ID NO:65) VLVLKFAEV 26 0.2994 0.9
CHPF2 (SEQ ID NO:66) VLPLLVAEA 24 0.6854 2.4
ULK1 (SEQ ID NO:67) VLYLKVAEL 31 0.78503 0.9
OR2J2 (SEQ ID NO:68) VLALGIAEC 23 0.9438 4.7
DNHD1 (SEQ ID NO:69) VLELLLAEL 29 1.0951 3.1
AL1L1 (SEQ ID NO:70) VLELTEAEL 24 1.1985 8.3
GALP (SEQ ID NO:71) VLLLSLAET 25 1.7861 3.3
OR2J1 (SEQ ID NO:72) VLALGTAEC 21 1.8786 3.3
ADPRH (SEQ ID NO:73) VMHLATAEA 19 1.9817 4.3
Msln530 (SEQ ID NO:32)
Epitope = VLPLTVAEV
TCR: 530-B11 (SEQ ID NO:62)
Search string = X-L-P-L-X-V-A-E-X
Peptide
SITHPATHI (>15)
PanMHCnet score (SB=<0.5; WB=<2)
IEDB (<1%)
MSLN (SEQ ID NO:32) VLPLTVAEV 29 0.2335 1
CHPF2 (SEQ ID NO:74) VLPLIVAEA 24 0.6854 2.4
LRCH3 (SEQ ID NO:75) dLPLRVAEI 25 6.861 8.11
IN80B (SEQ ID NO:76) MLPLPVAEG 19 8.3672 13
CEAM3 (SEQ ID NO:77) SMPLSVAEG 18 17.1852 16
Potential cross-reacting peptide
Did not meet recommended cut-off criteria

FIG. 10



	peptide #1	peptide #2	peptide #3	peptide #4	peptide #5	peptide #6	peptide #7	peptide #8	peptide #9	peptide #10	peptide #11	peptide #12	peptide wt	peptide max
Meso530-11A	2.08	42.8	0.68	53.7	21.5	0.66	2.42	0.45	0.28	0.1	0.2	0.077	46.6	76.6
Meso530-11B	0.37	1.48	0.86	0.98	2.14	0.95	0.82	0.37	0.26	19	0.24	0.14	76	91.5

FIG. 11A

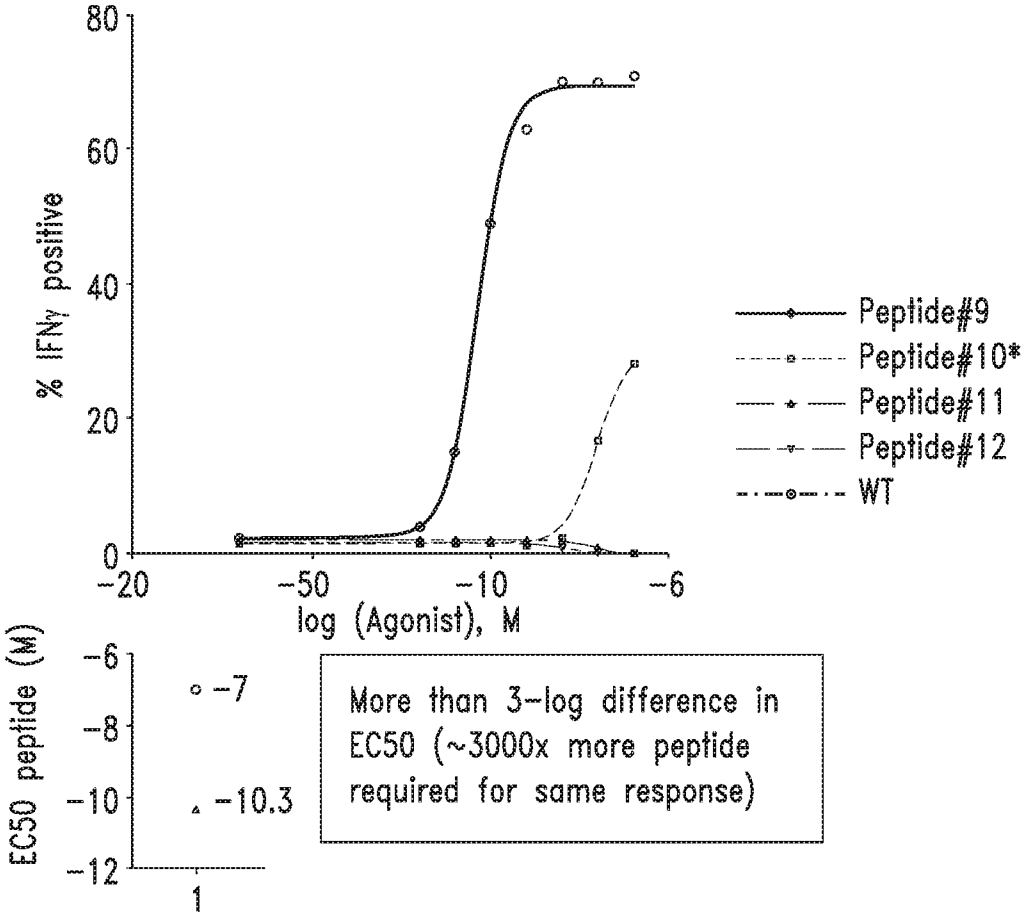
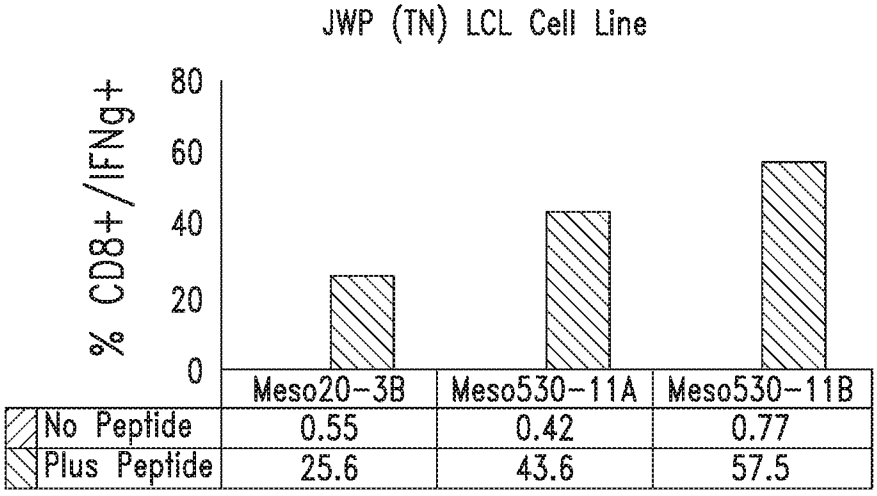


FIG. 11B

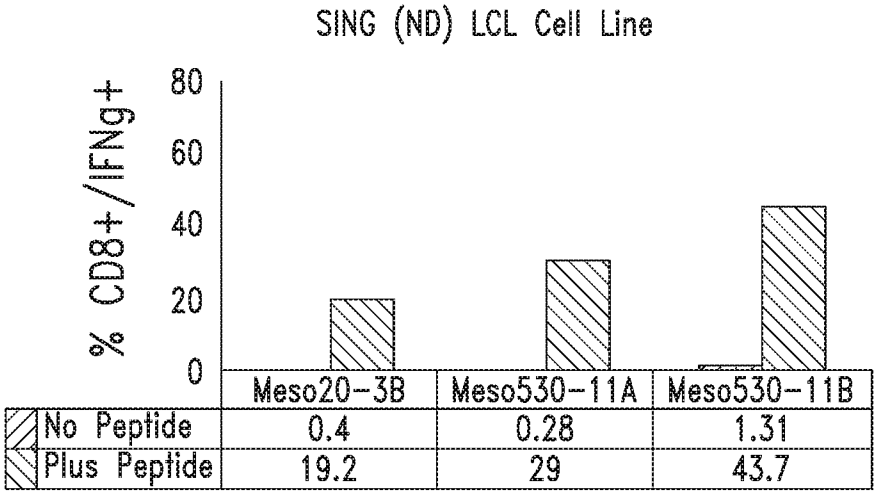
Initials (Prot.)	HLA-A	HLA-B	HLA-Cw
DAH #2 (956)	A1,A2	B7,B15	C1,C7
DMJ (1334)	A2,A2	B44,B44	C5,C7
FGA (1334)	A2,A30	B7,B44	C7,C7
GWB (956)	A2,A11	B15,B44	C3,C5
JAS (956)	A1,A68	B44,B55	C3,C5
JRL #2 (956)	A1,A2	B8,B55	C3,C7
JWP (TN)	A2,A33	B7,B15	C3,C7
SING (ND)	A24,A32	B35,B40	C2,C4

FIG. 12A



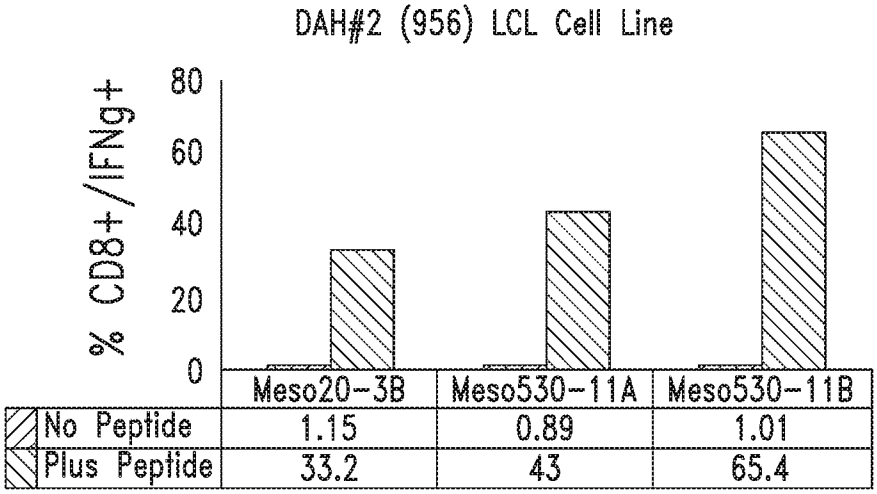
	Meso20-3B	Meso530-11A	Meso530-11B
No Peptide	0.55	0.42	0.77
Plus Peptide	25.6	43.6	57.5

FIG. 12B



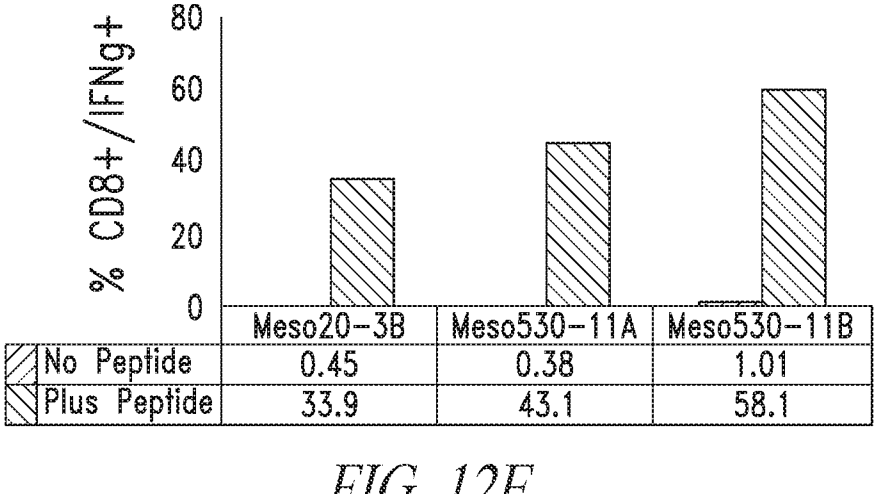
	Meso20-3B	Meso530-11A	Meso530-11B
No Peptide	0.4	0.28	1.31
Plus Peptide	19.2	29	43.7

FIG. 12C



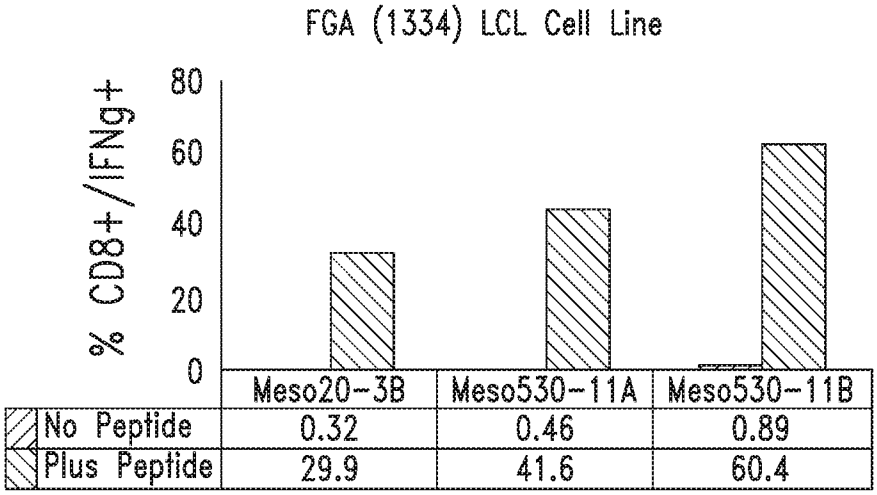
	Meso20-3B	Meso530-11A	Meso530-11B
No Peptide	1.15	0.89	1.01
Plus Peptide	33.2	43	65.4

FIG. 12D



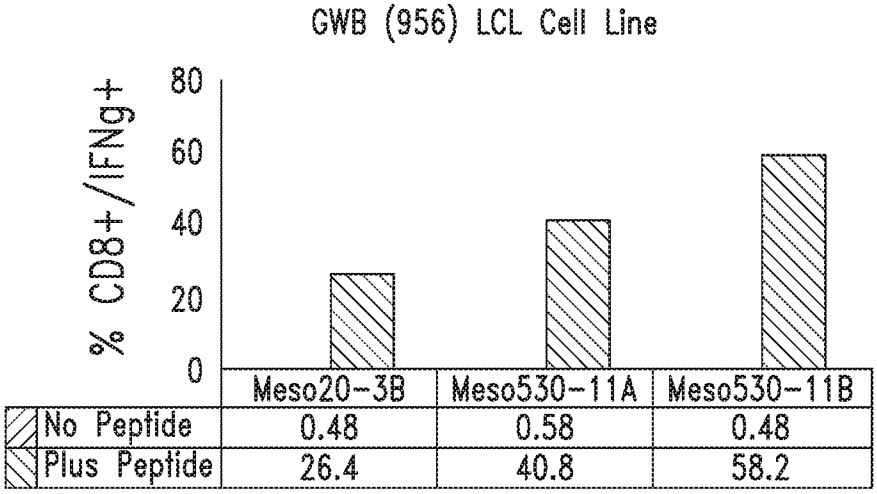
	Meso20-3B	Meso530-11A	Meso530-11B
No Peptide	0.45	0.38	1.01
Plus Peptide	33.9	43.1	58.1

FIG. 12E



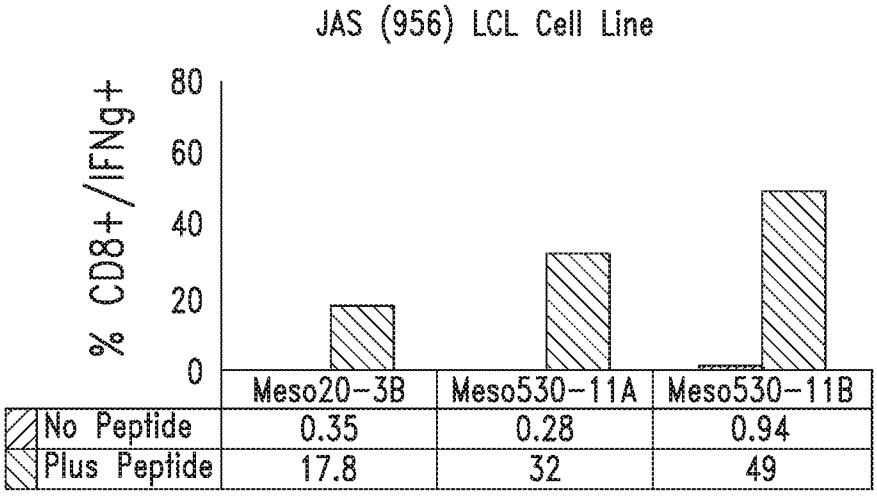
	Meso20-3B	Meso530-11A	Meso530-11B
No Peptide	0.32	0.46	0.89
Plus Peptide	29.9	41.6	60.4

FIG. 12F



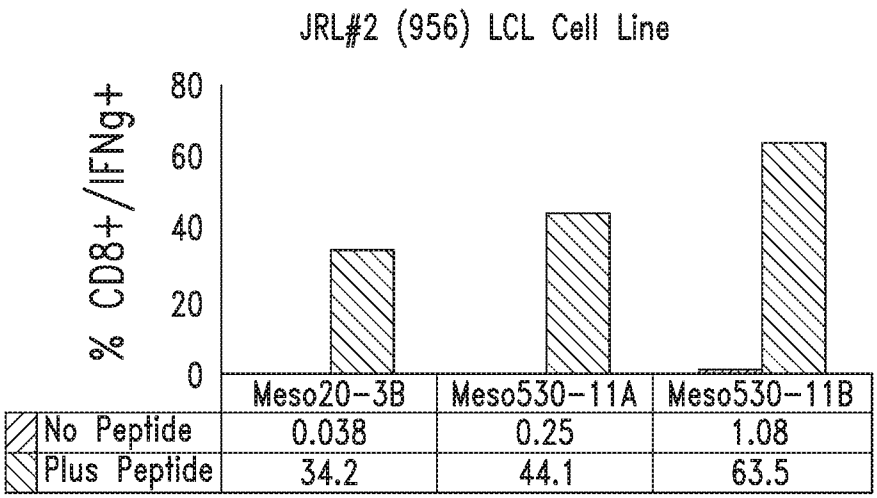
	Meso20-3B	Meso530-11A	Meso530-11B
No Peptide	0.48	0.58	0.48
Plus Peptide	26.4	40.8	58.2

FIG. 12G



	Meso20-3B	Meso530-11A	Meso530-11B
No Peptide	0.35	0.28	0.94
Plus Peptide	17.8	32	49

FIG. 12H

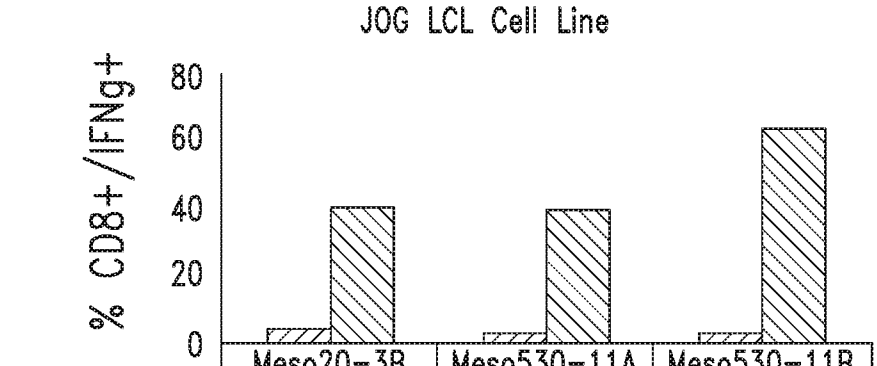


	Meso20-3B	Meso530-11A	Meso530-11B
No Peptide	0.038	0.25	1.08
Plus Peptide	34.2	44.1	63.5

FIG. 12I

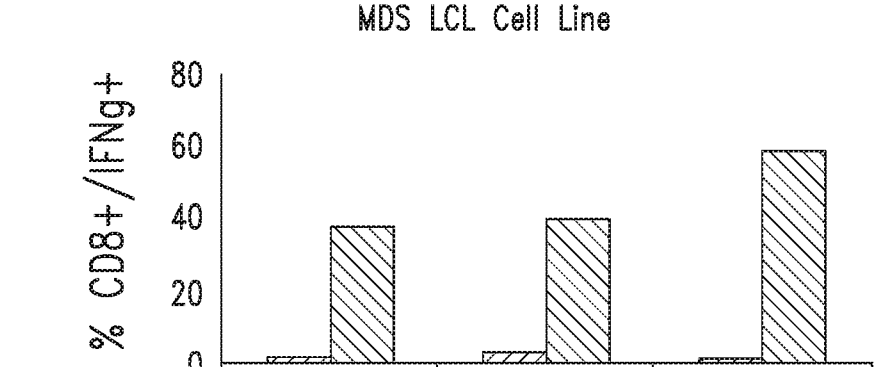
Initials (Prot.)	HLA-A	HLA-B	HLA-Cw
FAH	A3, A68	B13:02:01, B40	C2, C6:02:01
CLP	A2, A3	B40, B51	C3, C15
JOG	A3, A29	B40, B44	C3, C16
MS	A11, A11	B13:01:01, B15	C4, C12
GIM	A2, A30	B7, B13:02:01	C6:02:01, C7
MDS	A1, A24	B7, B57	C6:02:01, C7
PAJ	A1, A3	B8, B49	C7, C7

FIG. 13A



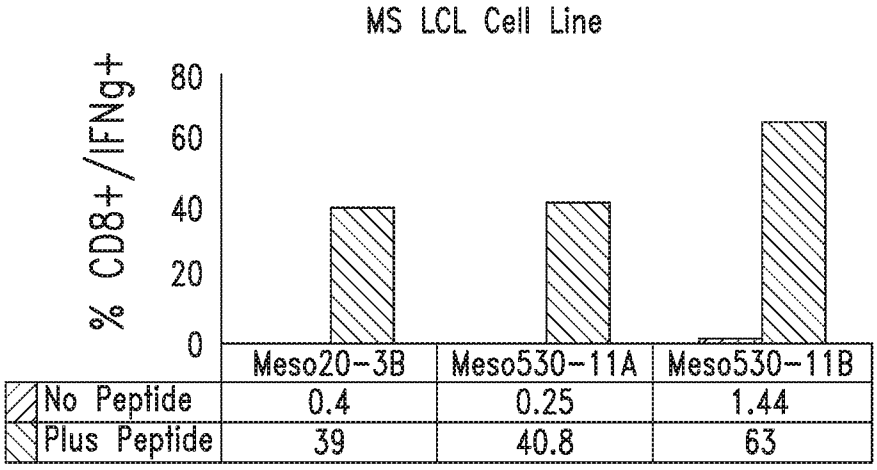
	Meso20-3B	Meso530-11A	Meso530-11B
No Peptide	2.68	1.91	1.53
Plus Peptide	40.8	39.9	64.2

FIG. 13B



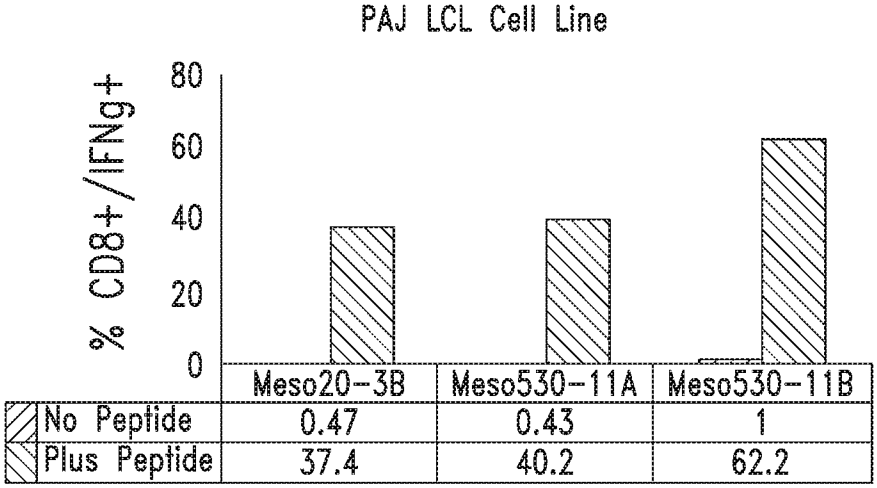
	Meso20-3B	Meso530-11A	Meso530-11B
No Peptide	1.75	3.13	1.42
Plus Peptide	41	43.5	64.3

FIG. 13C



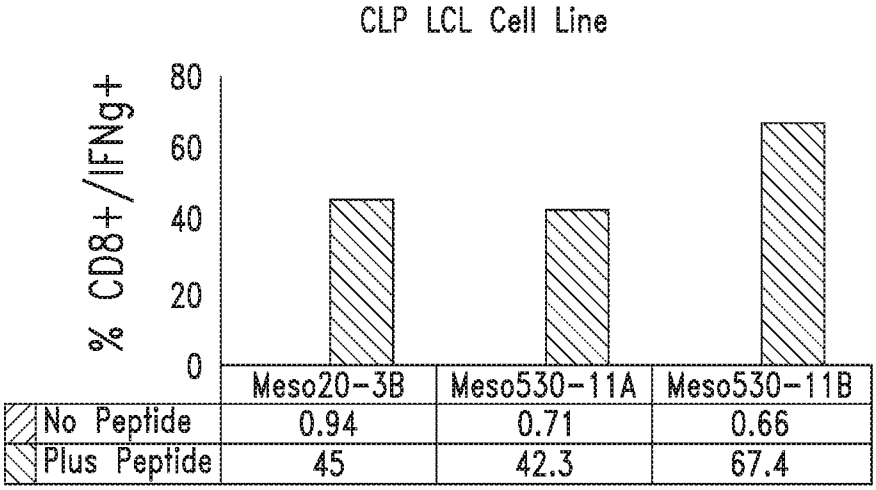
	Meso20-3B	Meso530-11A	Meso530-11B
No Peptide	0.4	0.25	1.44
Plus Peptide	39	40.8	63

FIG. 13D



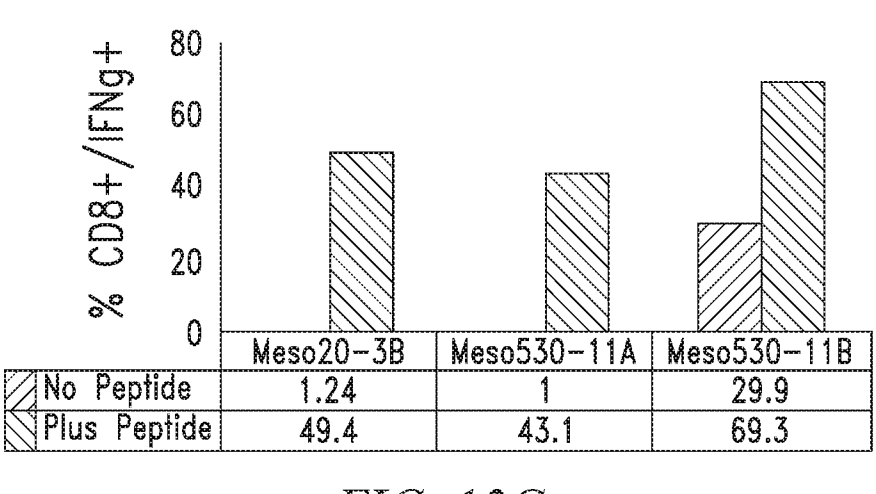
	Meso20-3B	Meso530-11A	Meso530-11B
No Peptide	0.47	0.43	1
Plus Peptide	37.4	40.2	62.2

FIG. 13E



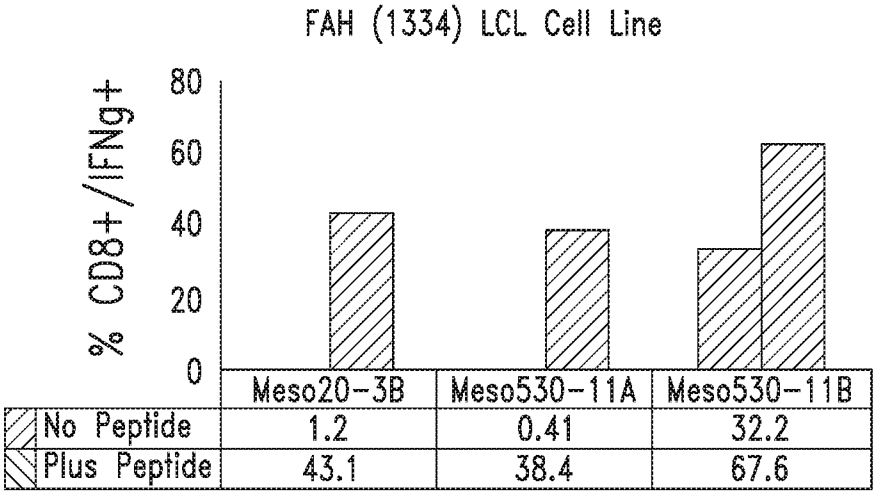
	Meso20-3B	Meso530-11A	Meso530-11B
No Peptide	0.94	0.71	0.66
Plus Peptide	45	42.3	67.4

FIG. 13F



	Meso20-3B	Meso530-11A	Meso530-11B
No Peptide	1.24	1	29.9
Plus Peptide	49.4	43.1	69.3

FIG. 13G



	Meso20-3B	Meso530-11A	Meso530-11B
No Peptide	1.2	0.41	32.2
Plus Peptide	43.1	38.4	67.6

FIG. 13H