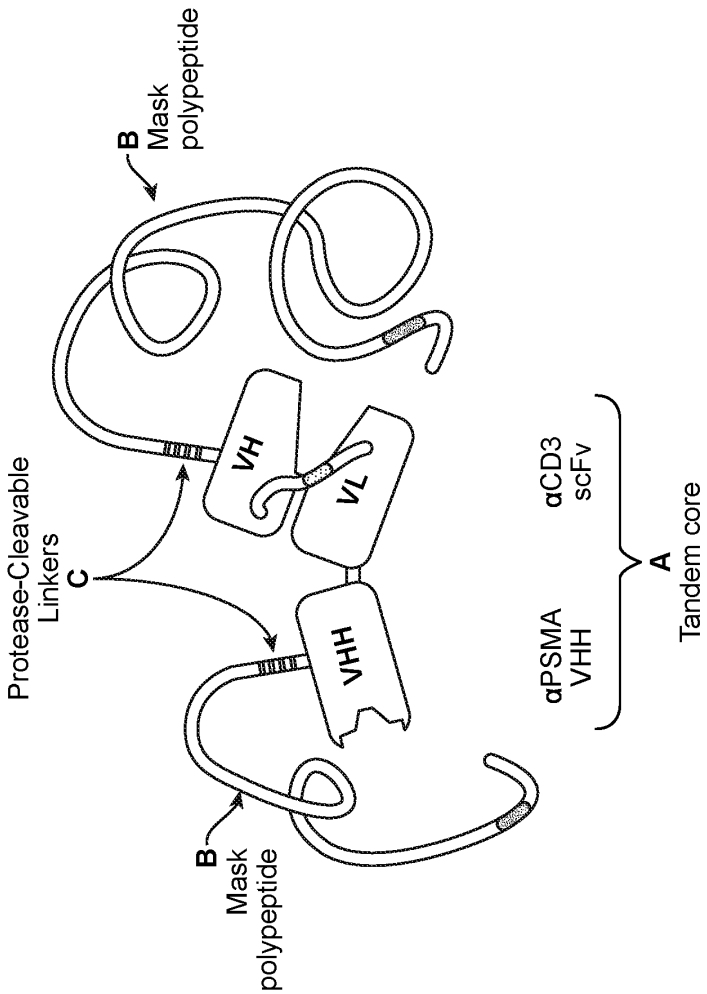

B
Mask
polypeptide
Protease-Cleavable
Linkers
C
VH
VL
VHH
αCD3
scFv
αPSMA
VHH
A
Tandem core
B
Mask
polypeptide

FIG. 1A

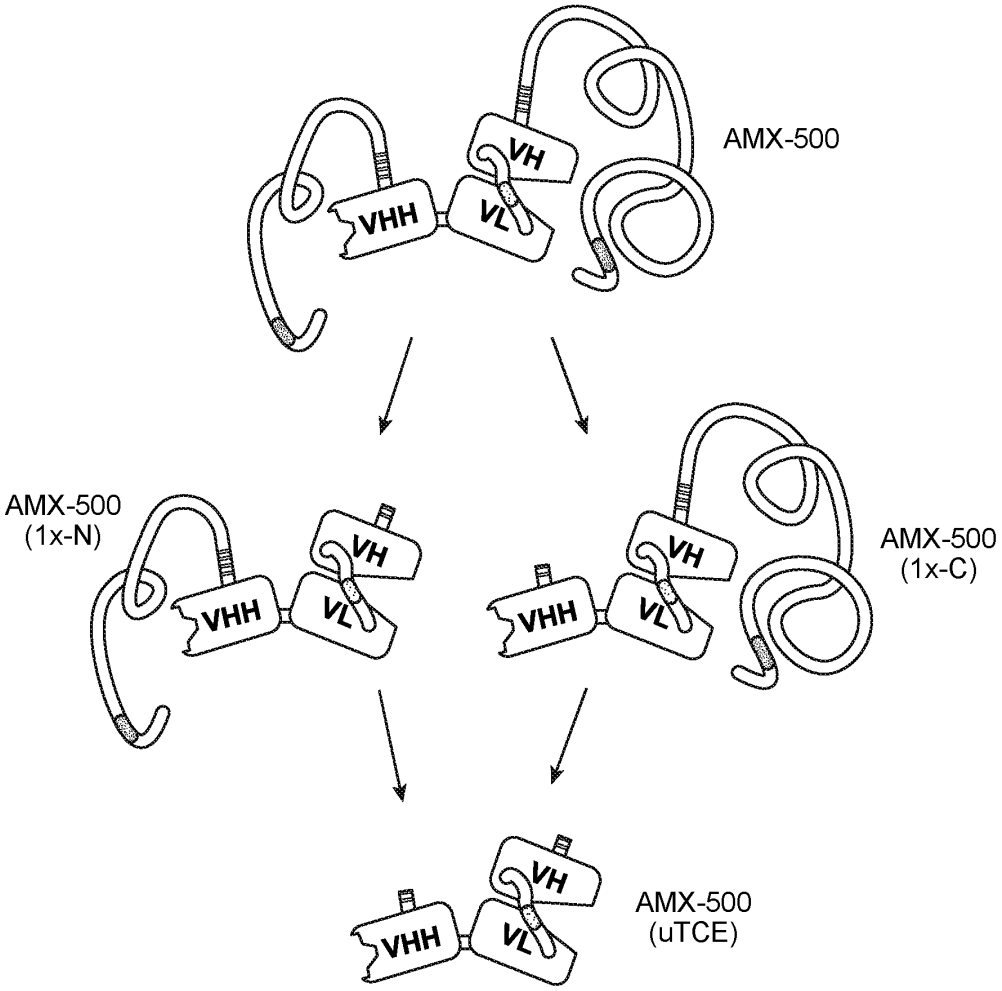

AMX-500
VH
VHH
VL
AMX-500
(1x-N)
VH
VHH
VL
AMX-500
(1x-C)
VH
VHH
VL
AMX-500
(uTCE)
VH
VHH
VL

FIG. 1B

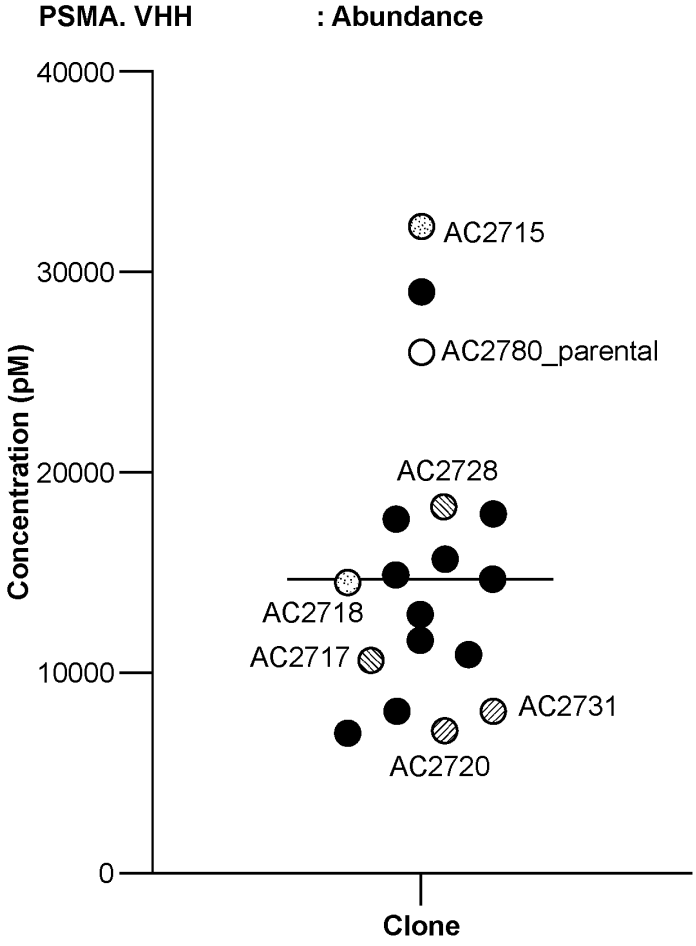

PSMA. VHH : Abundance
40000
30000
20000
10000
0
Concentration (pM)
AC2715
AC2780_parental
AC2728
AC2718
AC2717
AC2731
AC2720
Clone

FIG. 2A

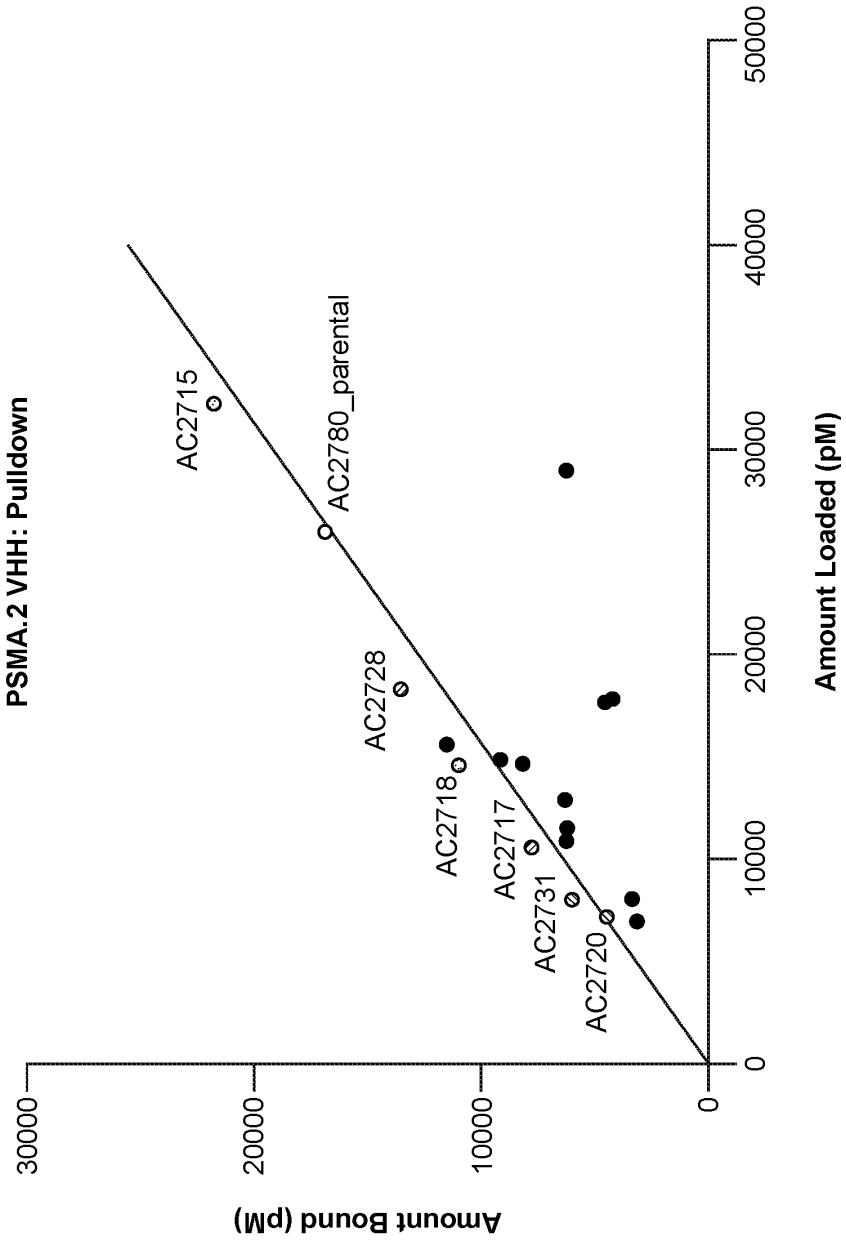

PSMA.2 VHH: Pulldown
Amount Bound (pM)
Amount Loaded (pM)
0
10000
20000
30000
0
10000
20000
30000
40000
50000
AC2715
AC2780_parental
AC2728
AC2718
AC2717
AC2731
AC2720

FIG. 2B

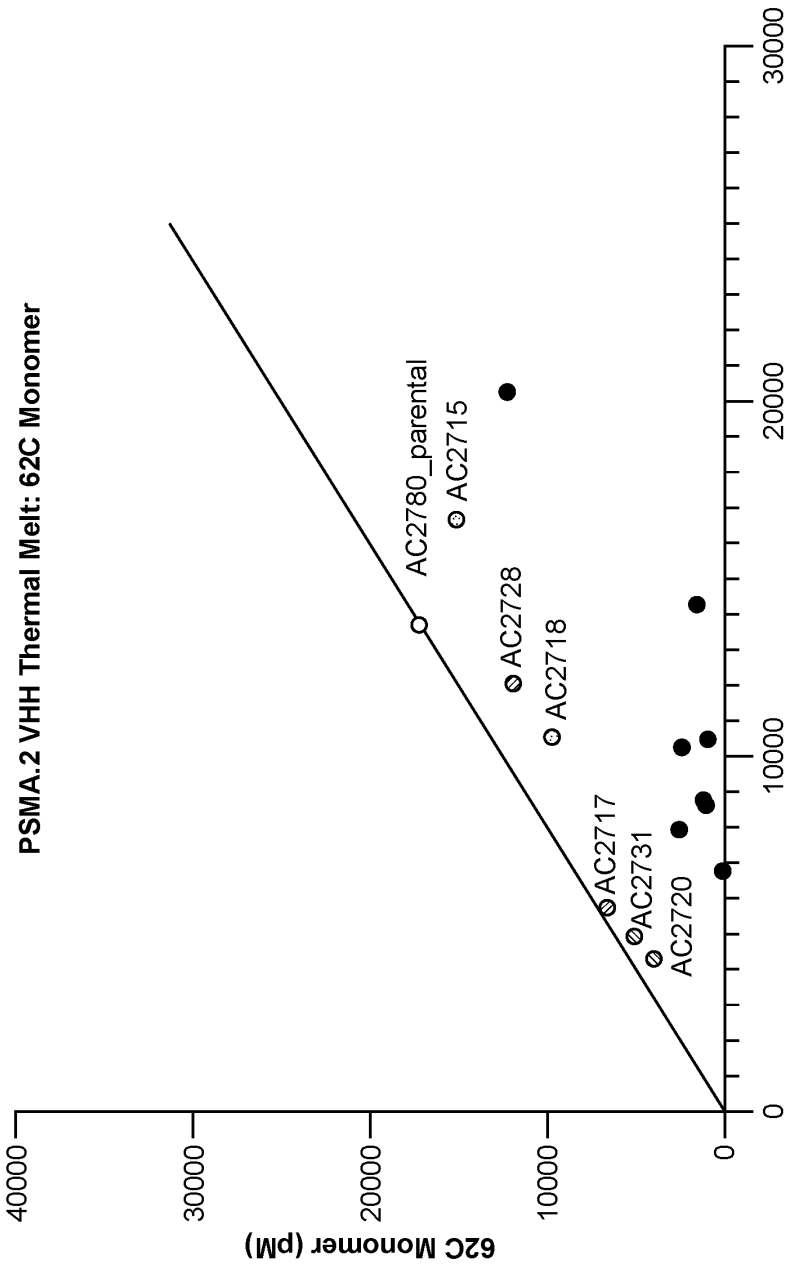

PSMA.2 VHH Thermal Melt: 62C Monomer
62C Monomer (pM)
RT Monomer (pM)
0
10000
20000
30000
40000
AC2780_parental
AC2715
AC2728
AC2718
AC2717
AC2731
AC2720

FIG. 2C

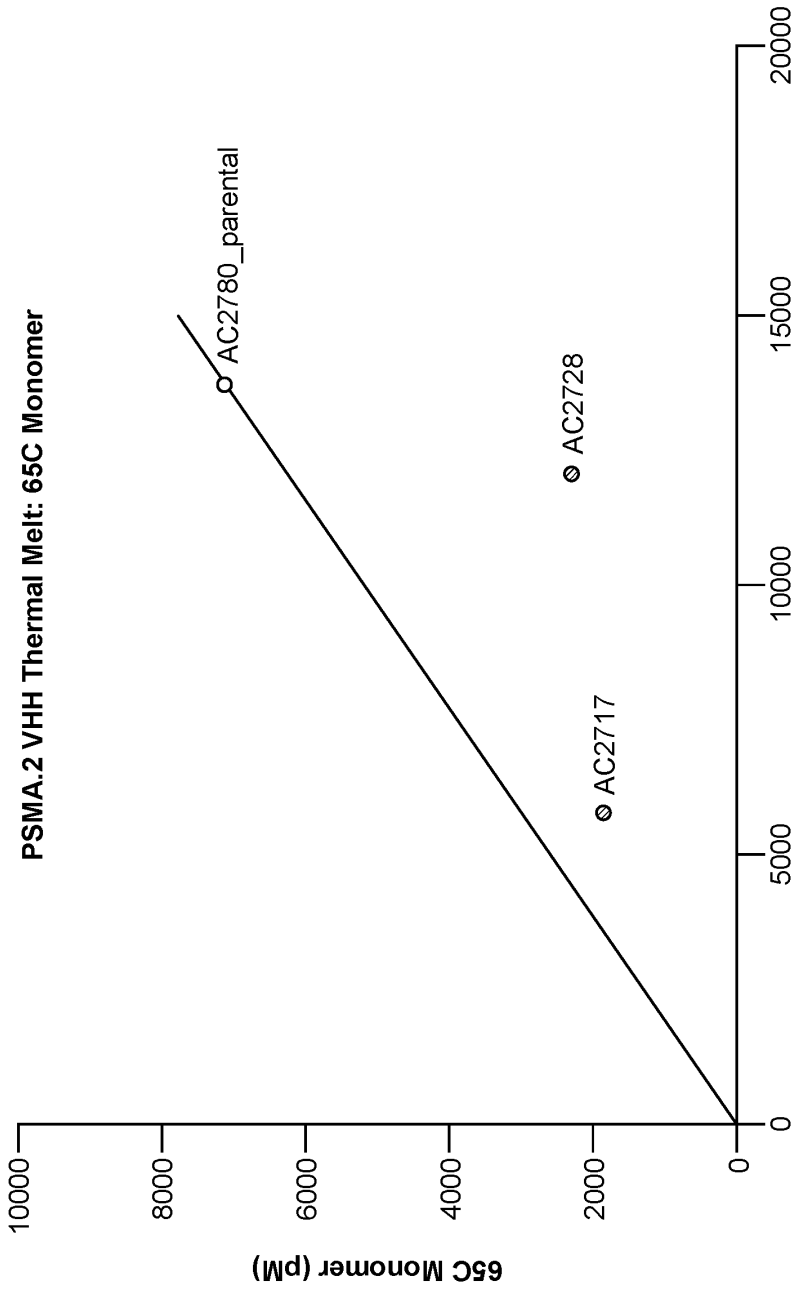

PSMA.2 VHH Thermal Melt: 65C Monomer
AC2780_parental
AC2728
AC2717
65C Monomer (pM)
RT Monomer (pM)
0
2000
4000
6000
8000
10000
0
5000
10000
15000
20000

FIG. 2D

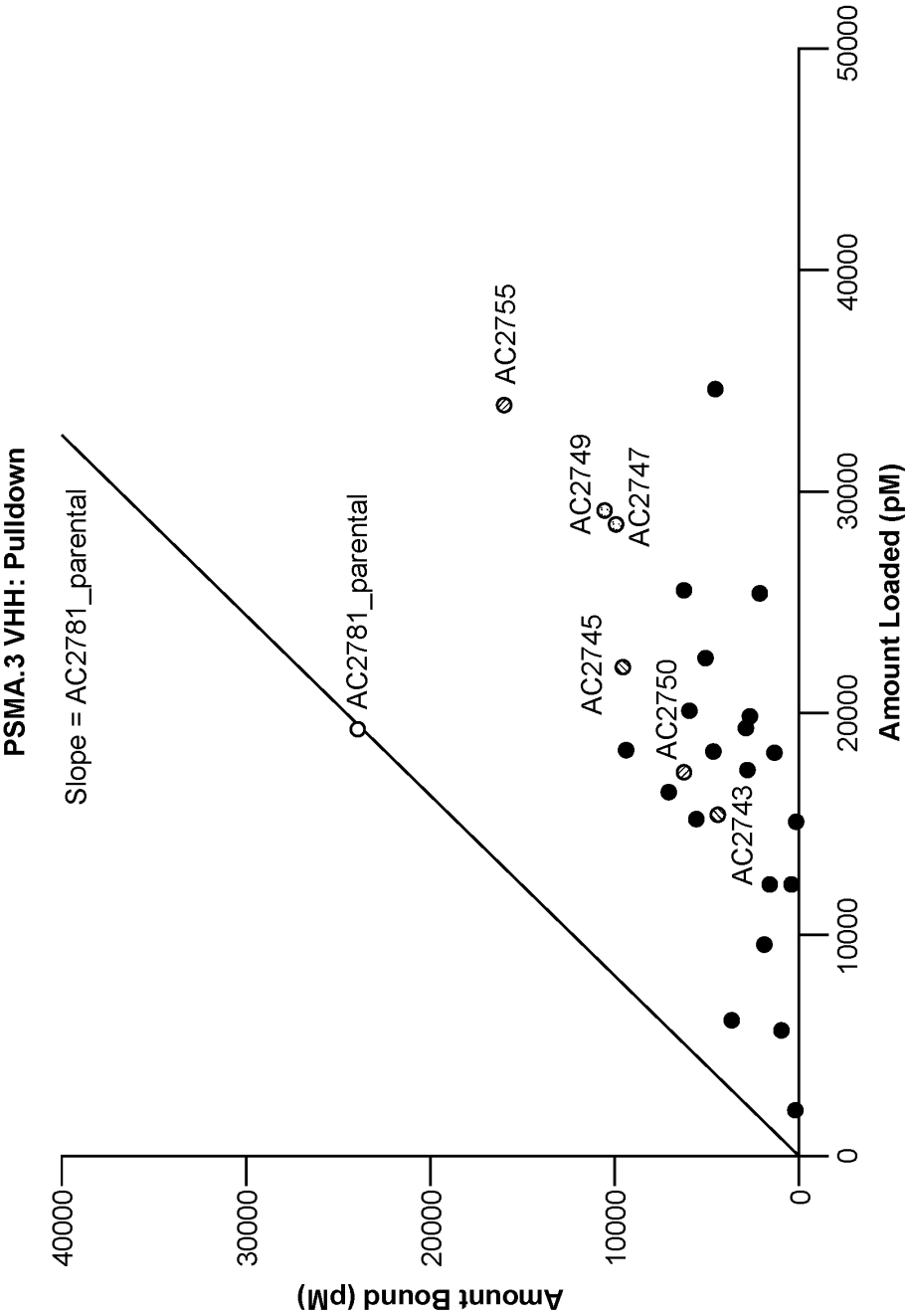

PSMA.3 VHH: Pulldown
Slope = AC2781_parental
AC2781_parental
AC2755
AC2749
AC2747
AC2745
AC2750
AC2743
Amount Bound (pM)
Amount Loaded (pM)
0
10000
20000
30000
40000
50000

FIG. 3A

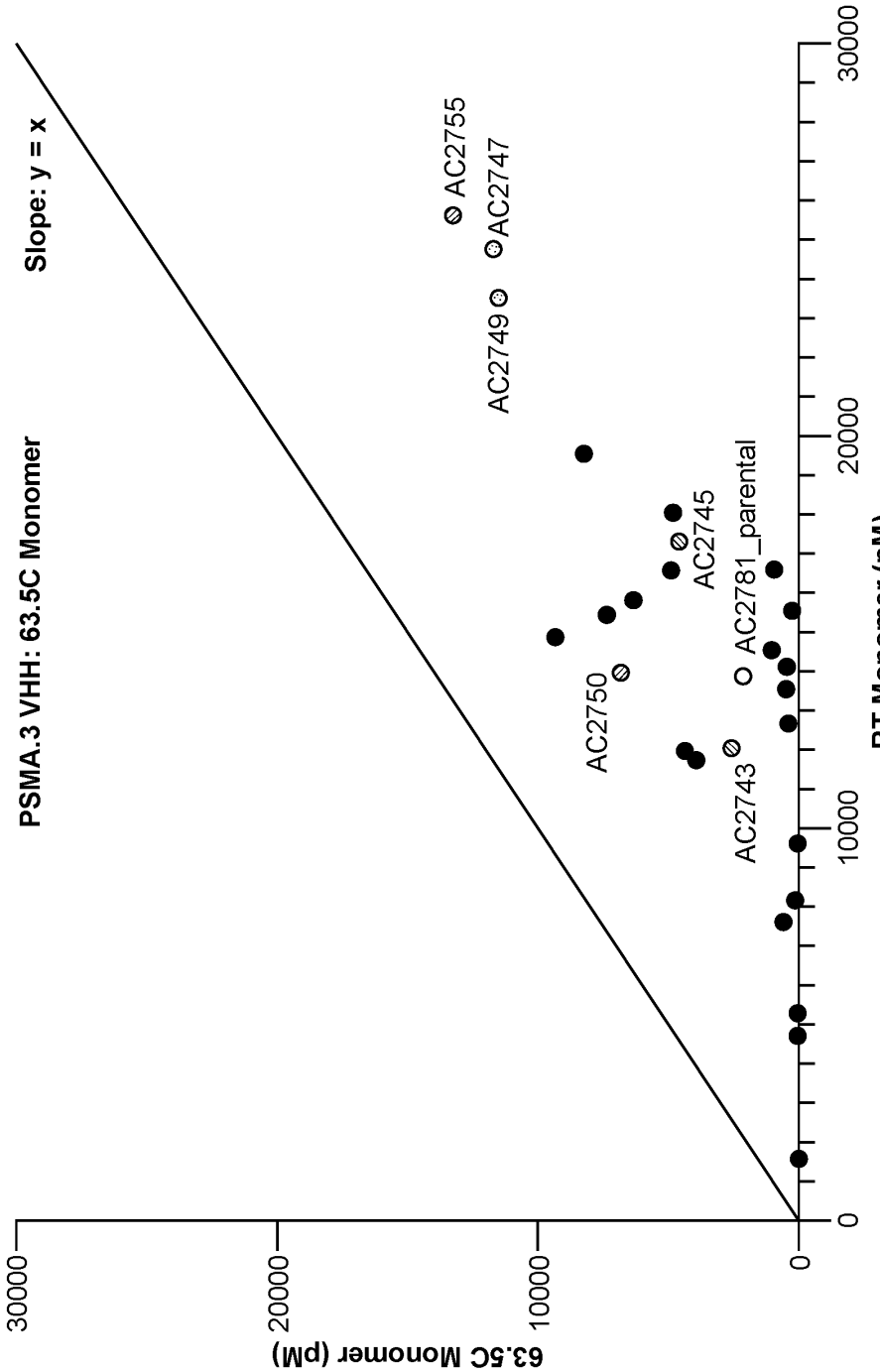

PSMA.3 VHH: 63.5C Monomer
Slope: y = x
63.5C Monomer (pM)
RT Monomer (pM)
0
10000
20000
30000
AC2755
AC2747
AC2749
AC2745
AC2781_parental
AC2750
AC2743

FIG. 3B

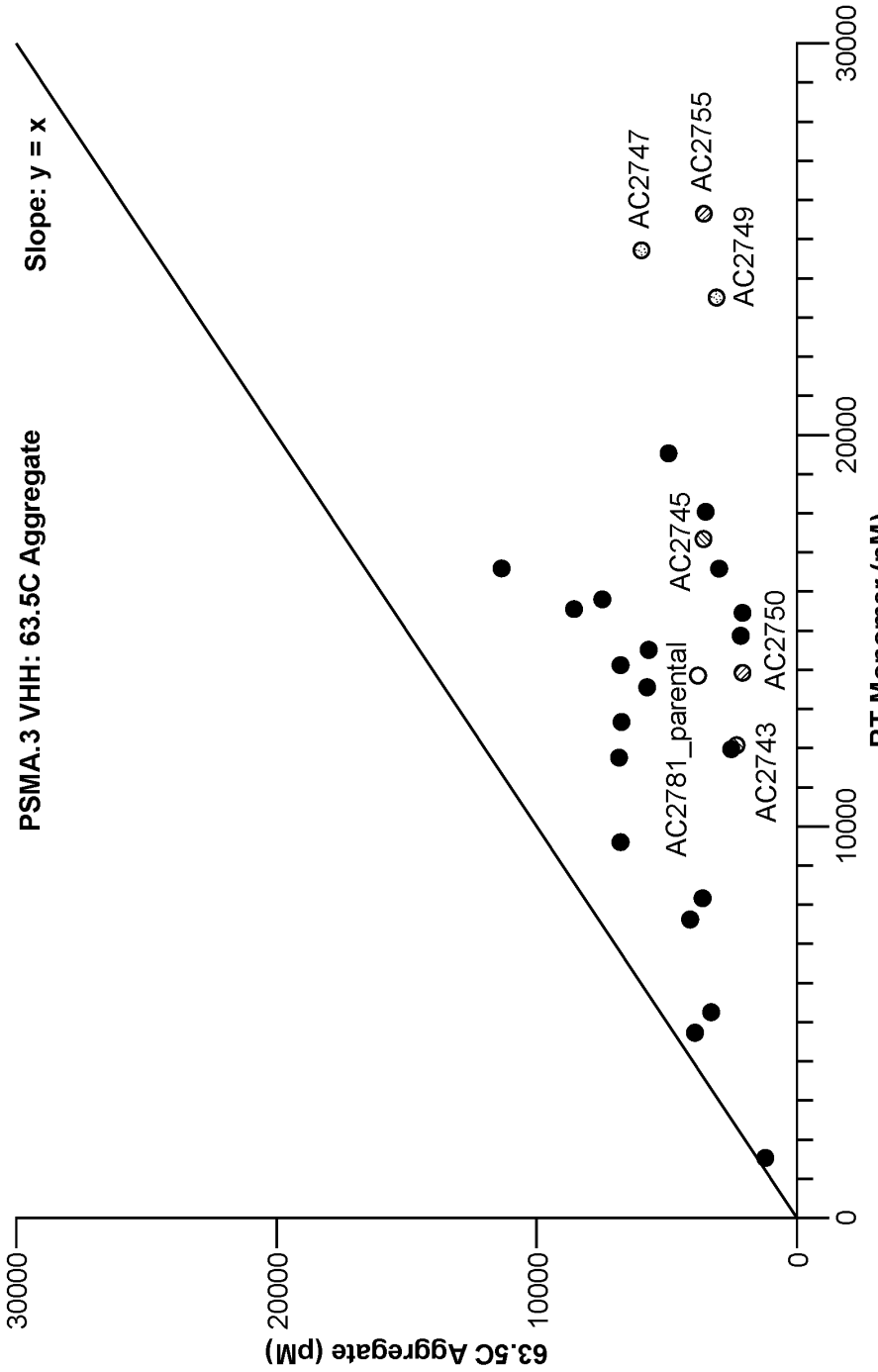

PSMA.3 VHH: 63.5C Aggregate
Slope: y = x
63.5C Aggregate (pM)
RT Monomer (pM)
0
10000
20000
30000
AC2747
AC2755
AC2749
AC2745
AC2781_parental
AC2750
AC2743

FIG. 3C

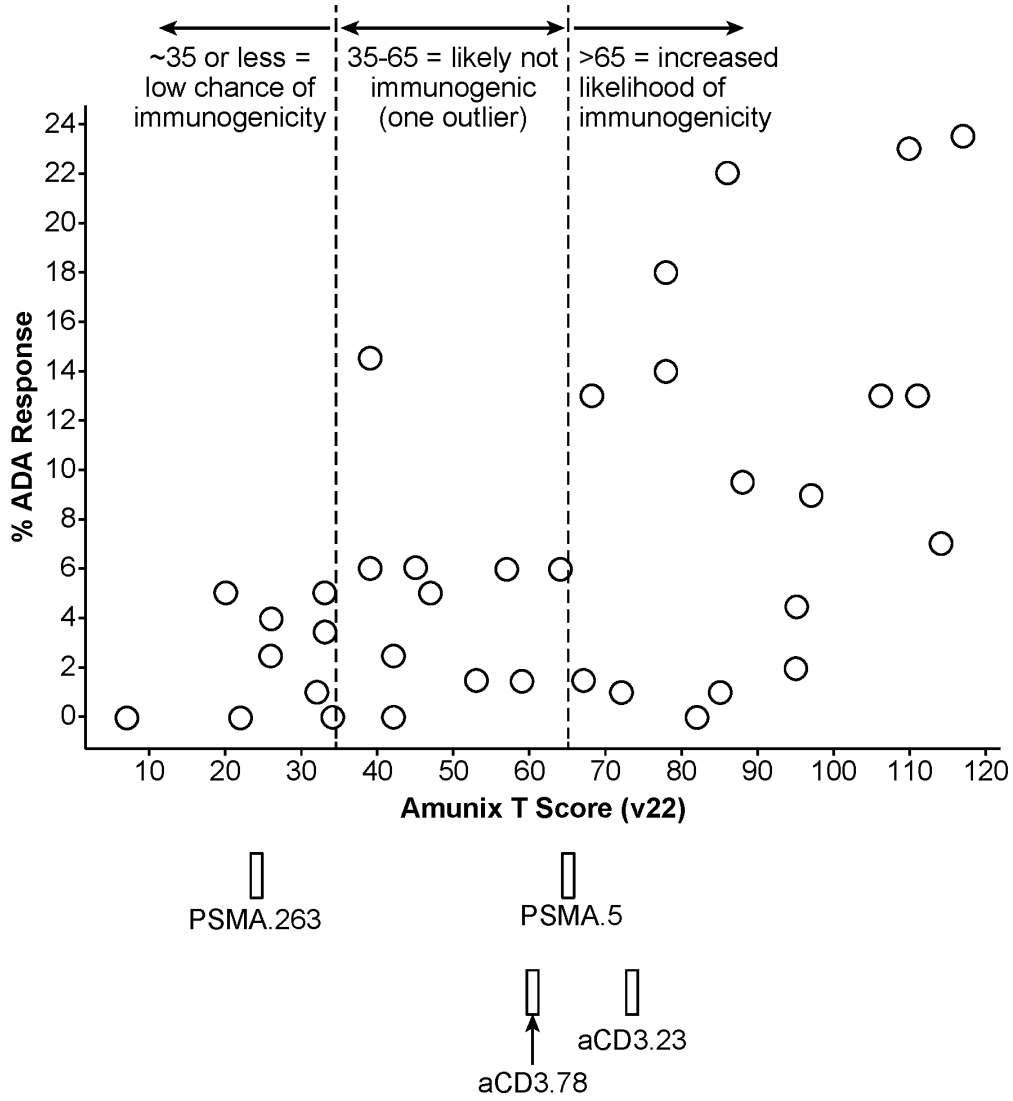

~35 or less = low chance of immunogenicity
35-65 = likely not immunogenic (one outlier)
>65 = increased likelihood of immunogenicity
% ADA Response
0
2
4
6
8
10
12
14
16
18
20
22
24
10
20
30
40
50
60
70
80
90
100
110
120
Amunix T Score (v22)
PSMA.263
PSMA.5
aCD3.23
aCD3.78

FIG. 4

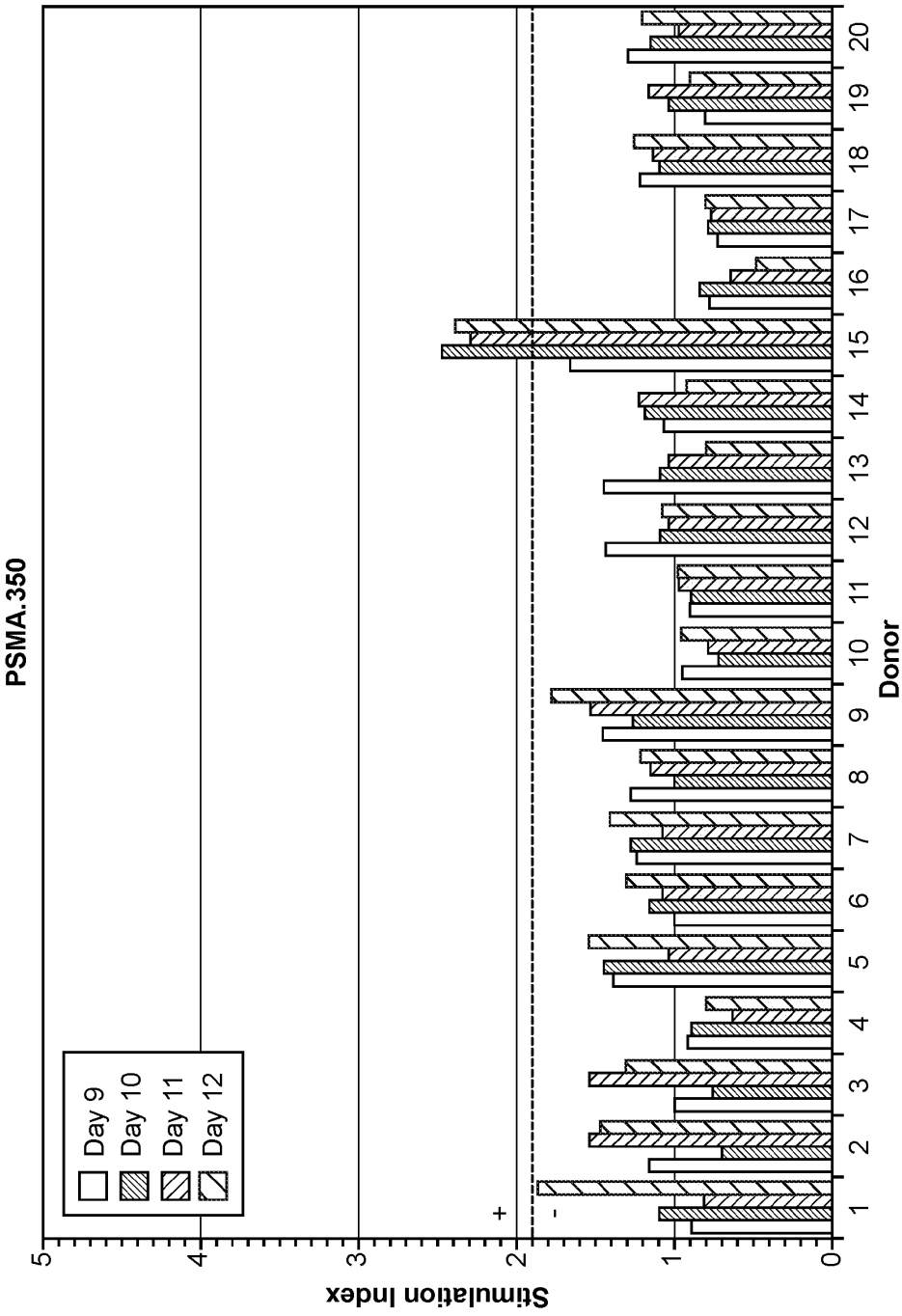

PSMA.350
Day 9
Day 10
Day 11
Day 12
Stimulation Index
Donor
+
-
0
1
2
3
4
5
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20

FIG. 5

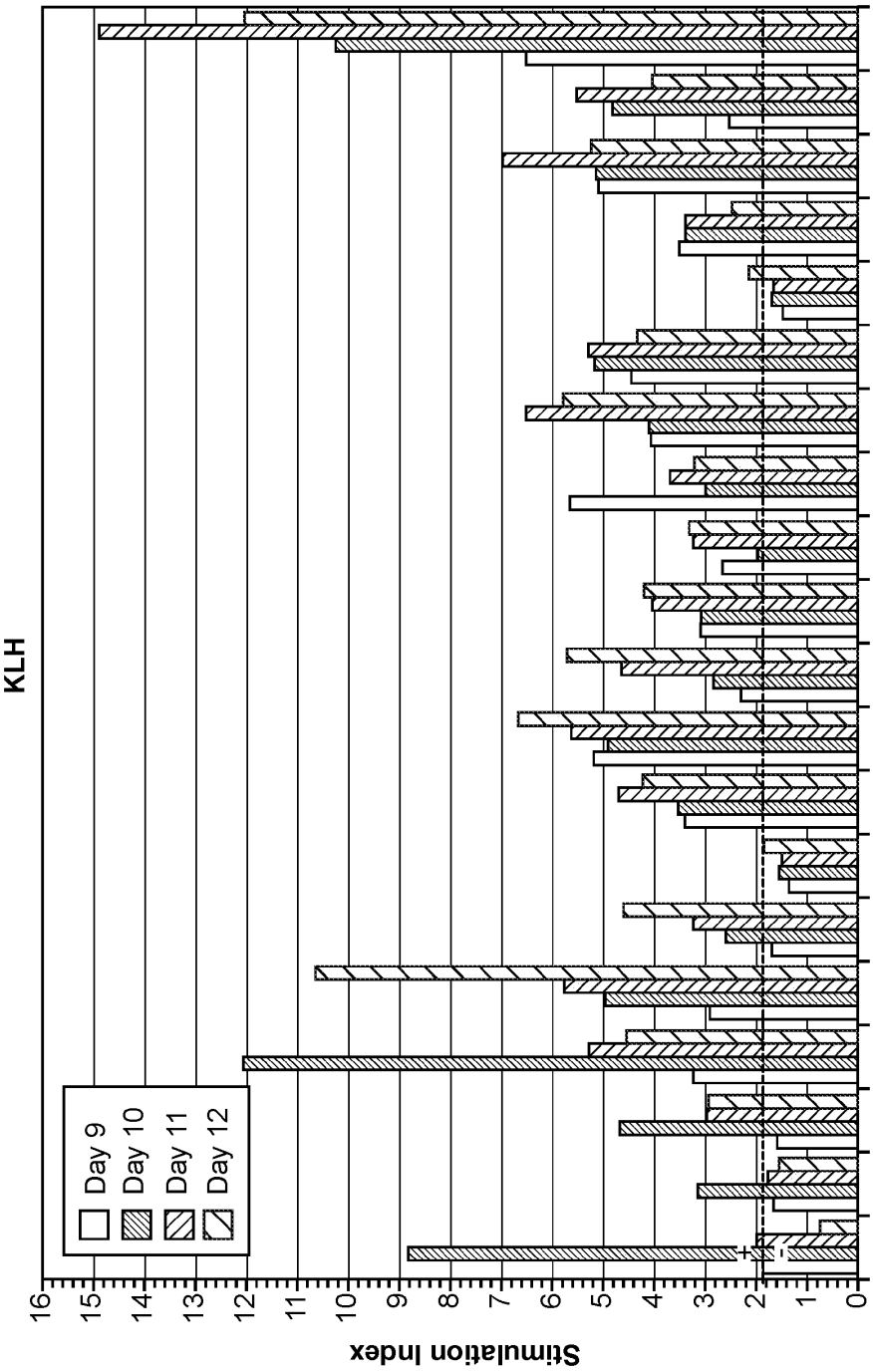

KLH
Day 9
Day 10
Day 11
Day 12
Stimulation Index
Donor
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
+
-

FIG. 5 (Cont.)

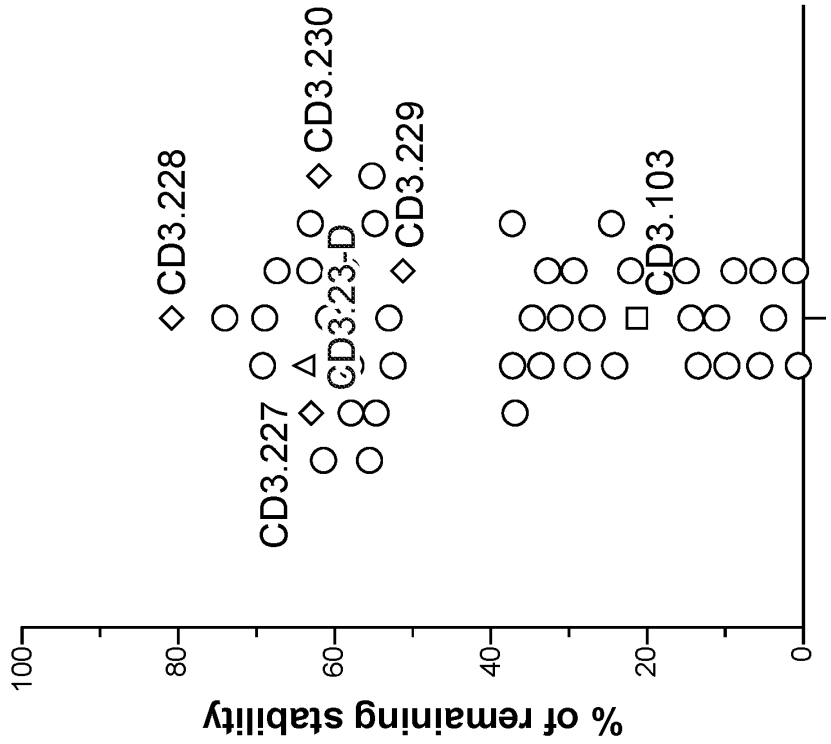

% of remaining stability
100
80
60
40
20
0
CD3.228
CD3.230
CD3.229
CD3.227
CD3.23-D
CD3.103

FIG. 6B

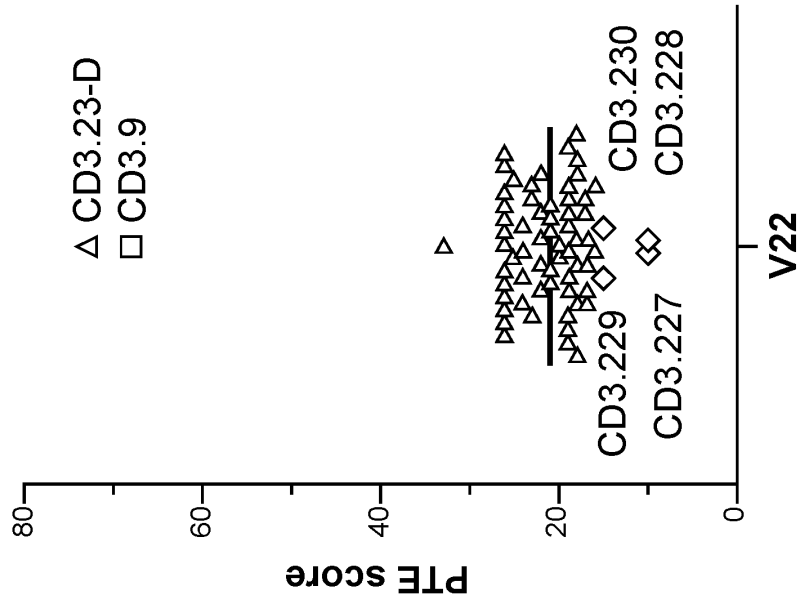

PTE score
80
60
40
20
0
CD3.23-D
CD3.9
CD3.230
CD3.228
CD3.229
CD3.227
V22

FIG. 6A

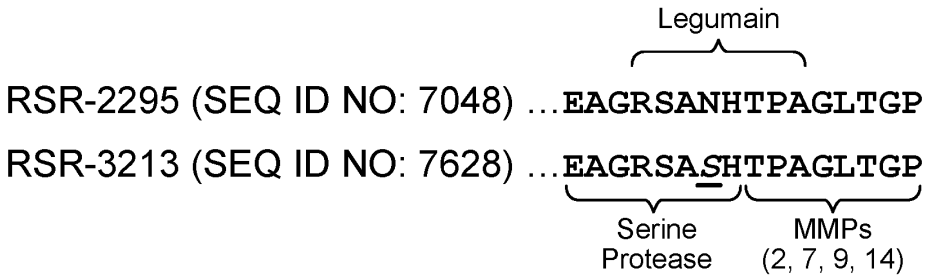

Legumain
RSR-2295 (SEQ ID NO: 7048) …EAGRSANHTPAGLTGP
RSR-3213 (SEQ ID NO: 7628) …EAGRSASHTPAGLTGP
Serine Protease
MMPs (2, 7, 9, 14)

FIG. 7A

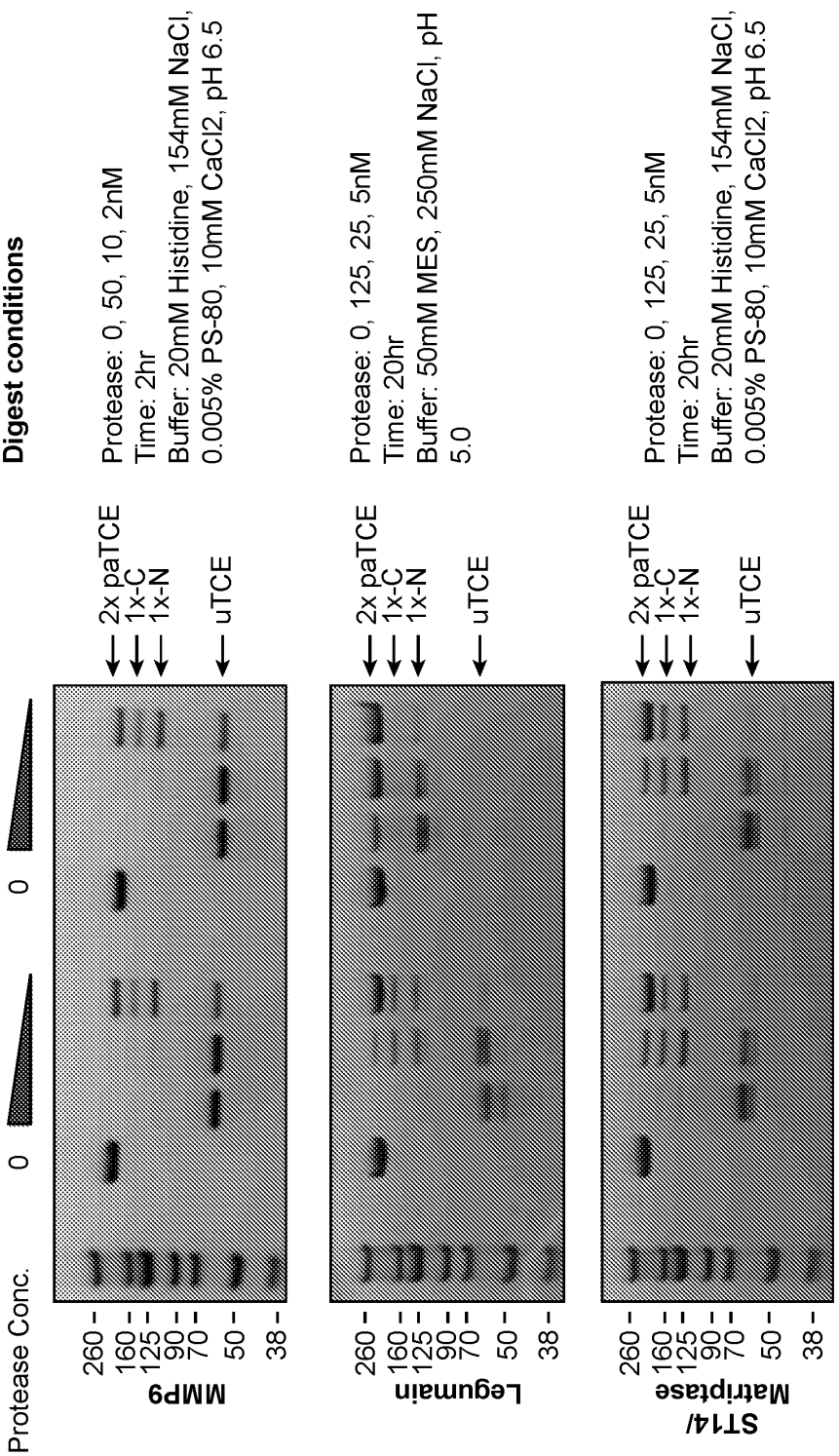

Digest conditions
Protease: 0, 50, 10, 2nM
Time: 2hr
Buffer: 20mM Histidine, 154mM NaCl, 0.005% PS-80, 10mM CaCl2, pH 6.5
Protease: 0, 125, 25, 5nM
Time: 20hr
Buffer: 50mM MES, 250mM NaCl, pH 5.0
Protease: 0, 125, 25, 5nM
Time: 20hr
Buffer: 20mM Histidine, 154mM NaCl, 0.005% PS-80, 10mM CaCl2, pH 6.5
RSR-2295
RSR-3213
Protease Conc.
0
0
2x paTCE
1x-C
1x-N
uTCE
MMP9
Legumain
ST14/ Matriptase
260
160
125
90
70
50
38

FIG. 7B

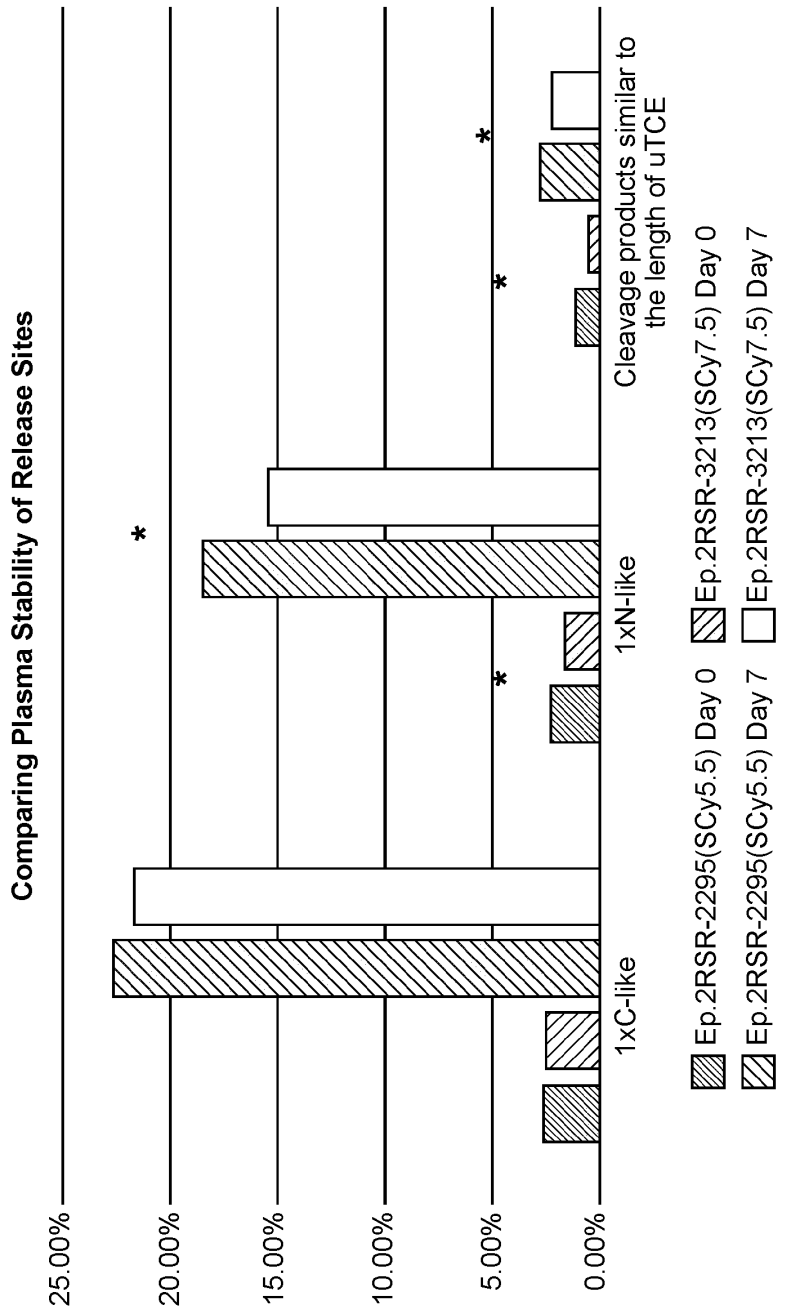

Comparing Plasma Stability of Release Sites
25.00%
20.00%
15.00%
10.00%
5.00%
0.00%
*
*
*
*
1xC-like
1xN-like
Cleavage products similar to the length of uTCE
Ep.2RSR-2295(SCy5.5) Day 0
Ep.2RSR-3213(SCy7.5) Day 0
Ep.2RSR-2295(SCy5.5) Day 7
Ep.2RSR-3213(SCy7.5) Day 7
*p < 0.05

FIG. 8A

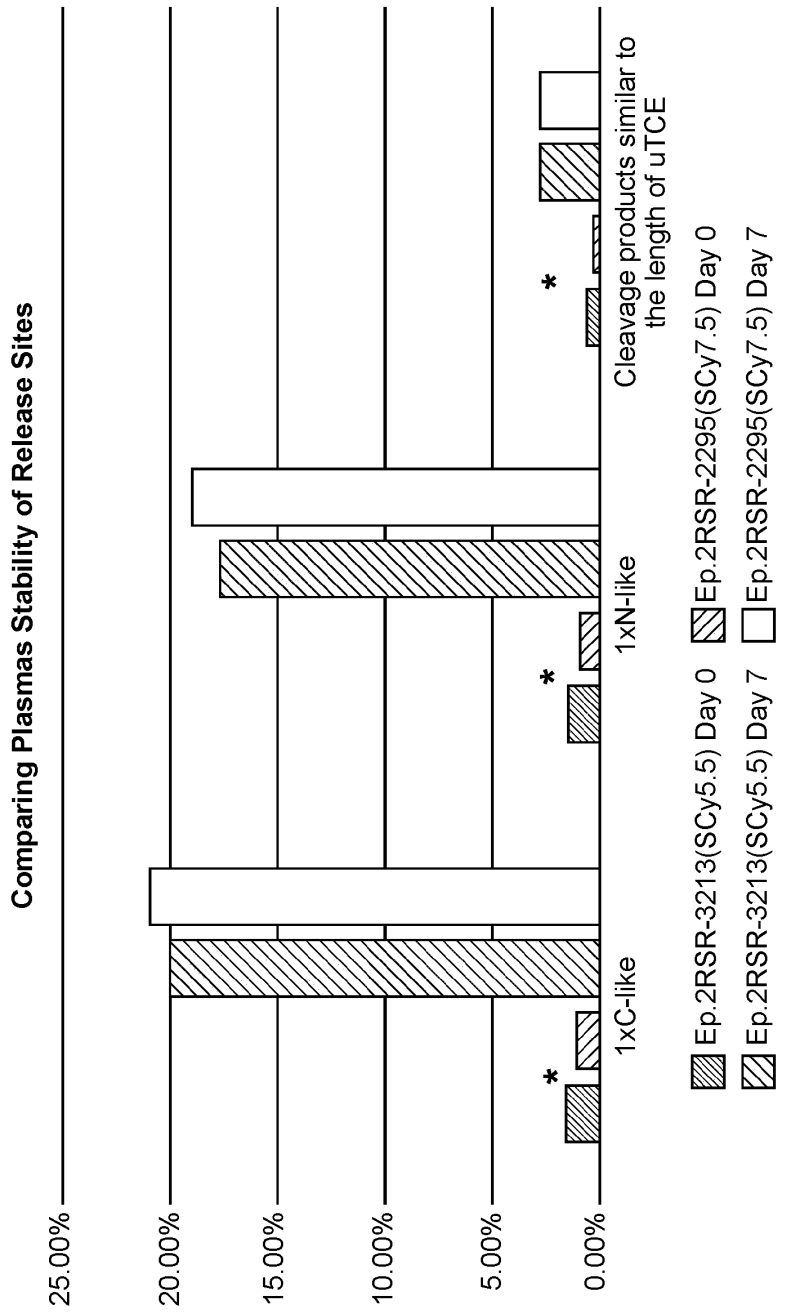

Comparing Plasmas Stability of Release Sites
25.00%
20.00%
15.00%
10.00%
5.00%
0.00%
1xC-like
1xN-like
Cleavage products similar to the length of uTCE
*
*
*
Ep.2RSR-3213(SCy5.5) Day 0
Ep.2RSR-2295(SCy7.5) Day 0
Ep.2RSR-3213(SCy5.5) Day 7
Ep.2RSR-2295(SCy7.5) Day 7
*p < 0.05

FIG. 8B

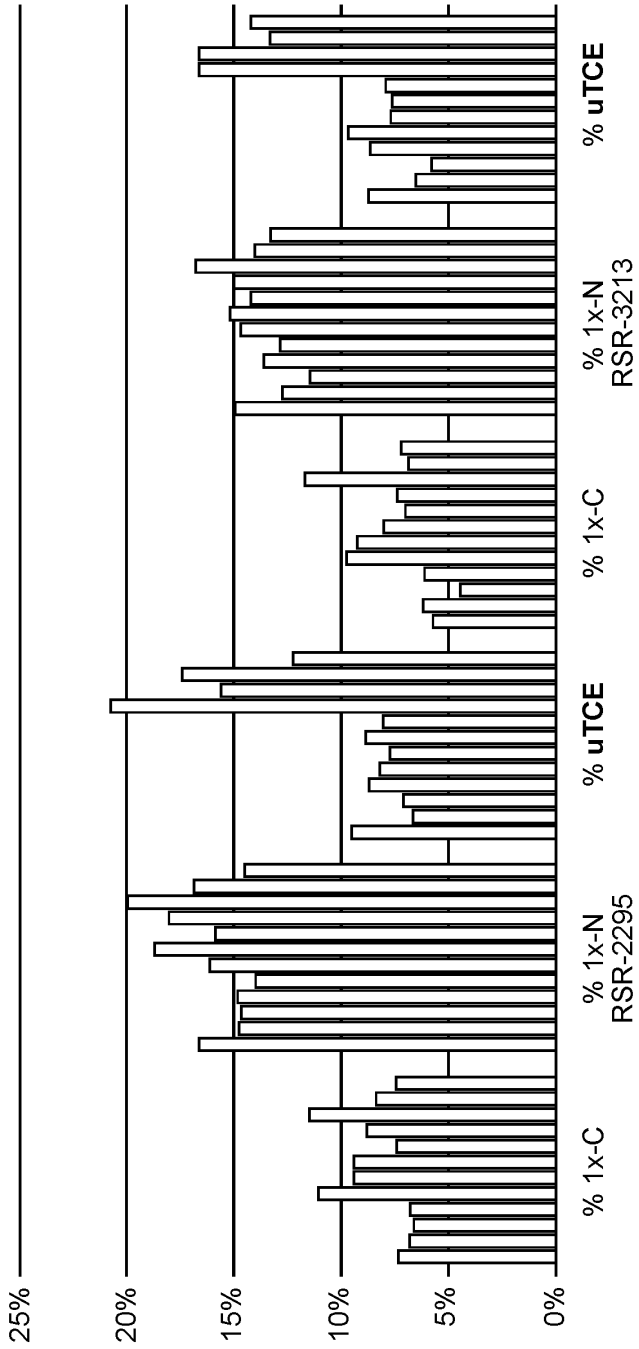


FIG. 8C

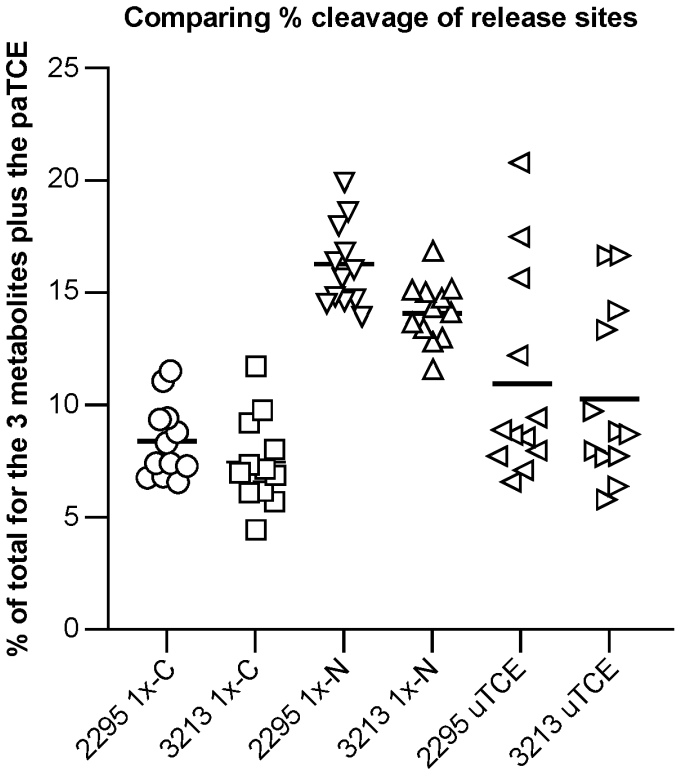

Comparing % cleavage of release sites
% of total for the 3 metabolites plus the paTCE
25
20
15
10
5
0
2295 1x-C
3213 1x-C
2295 1x-N
3213 1x-N
2295 uTCE
3213 uTCE

FIG. 8D

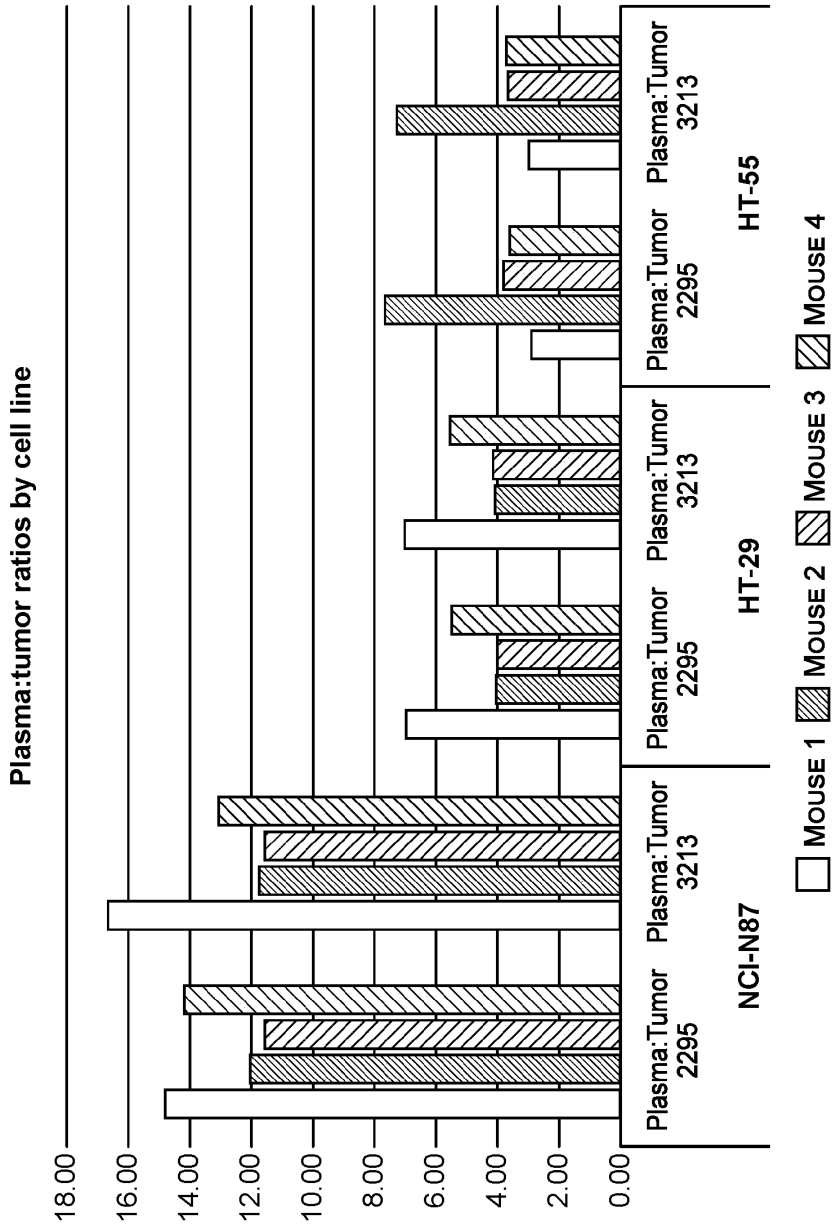

Plasma:tumor ratios by cell line
18.00
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
Plasma:Tumor 2295
Plasma:Tumor 3213
NCI-N87
Plasma:Tumor 2295
Plasma:Tumor 3213
HT-29
Plasma:Tumor 2295
Plasma:Tumor 3213
HT-55
Mouse 1
Mouse 2
Mouse 3
Mouse 4

FIG. 9

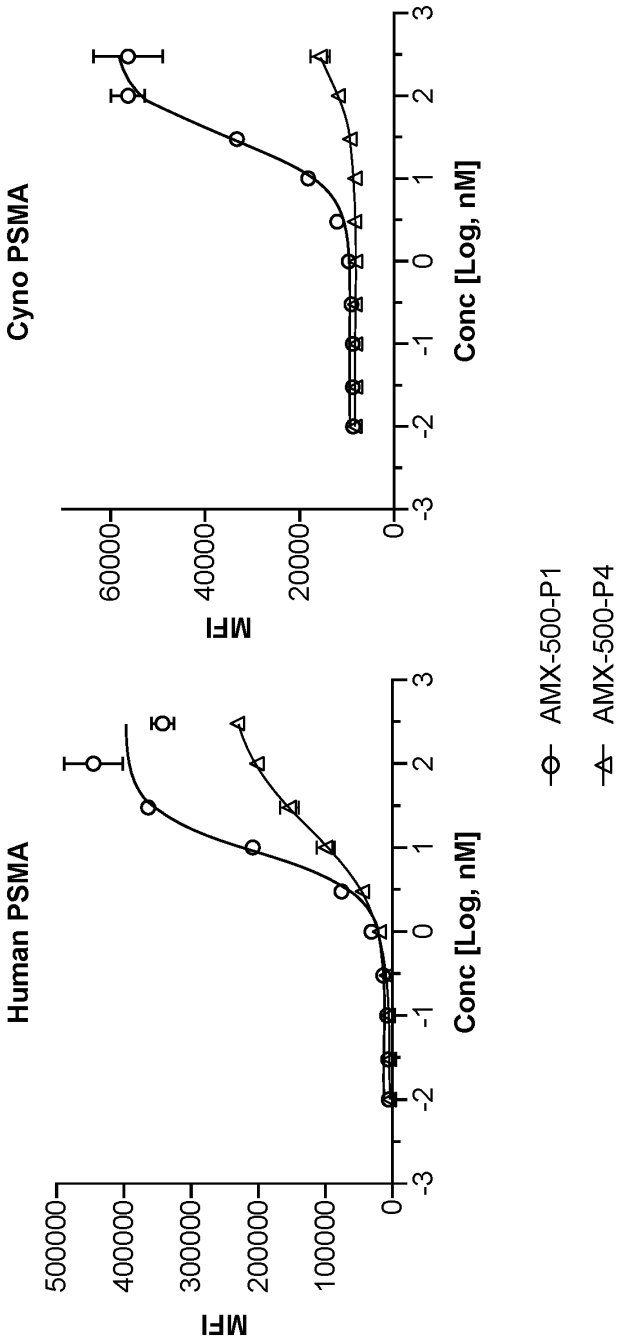

Cyno PSMA
MFI
60000
40000
20000
0
-3
-2
-1
0
1
2
3
Conc [Log, nM]
Human PSMA
MFI
500000
400000
300000
200000
100000
0
-3
-2
-1
0
1
2
3
Conc [Log, nM]
AMX-500-P1
AMX-500-P4

FIG. 10

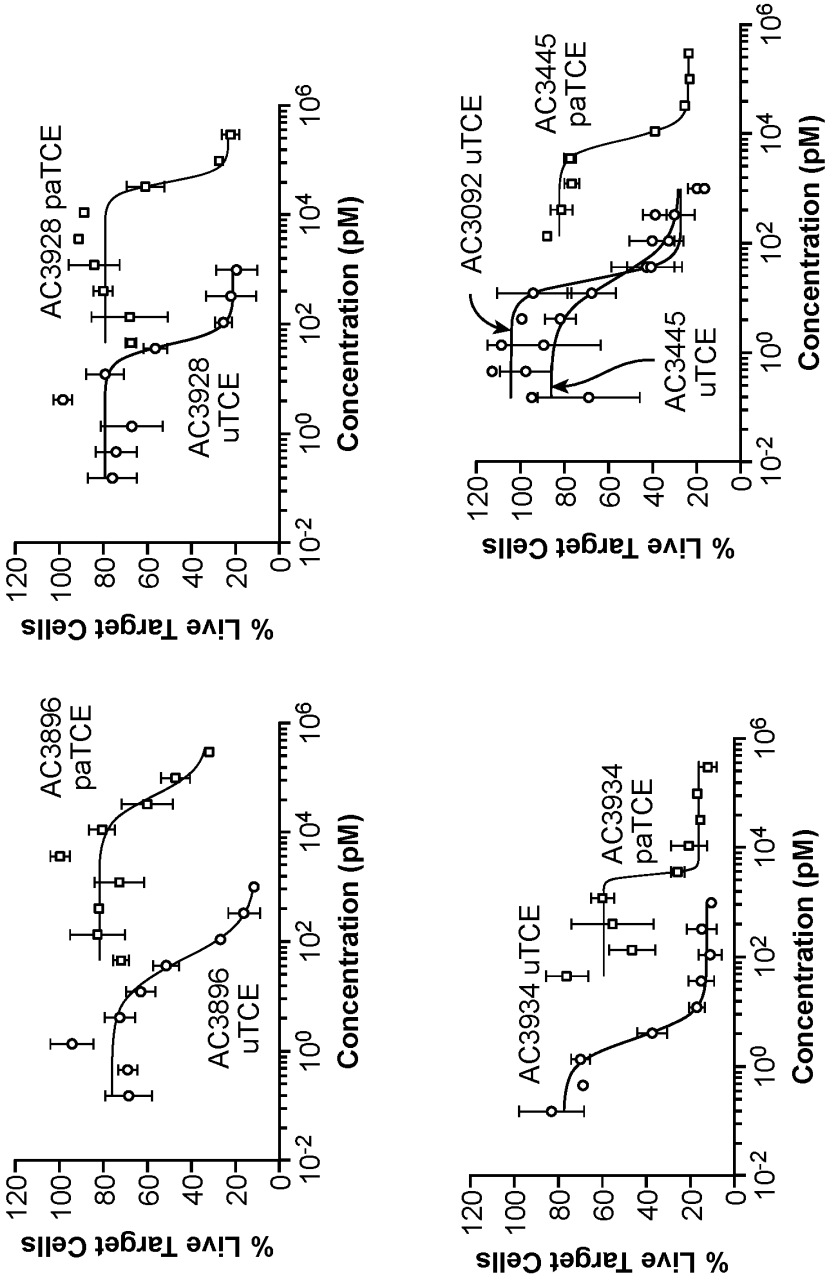

AC3928 paTCE
AC3928 uTCE
AC3092 uTCE
AC3445 paTCE
AC3445 uTCE
AC3896 paTCE
AC3896 uTCE
AC3934 uTCE
AC3934 paTCE
% Live Target Cells
Concentration (pM)

FIG. 11A

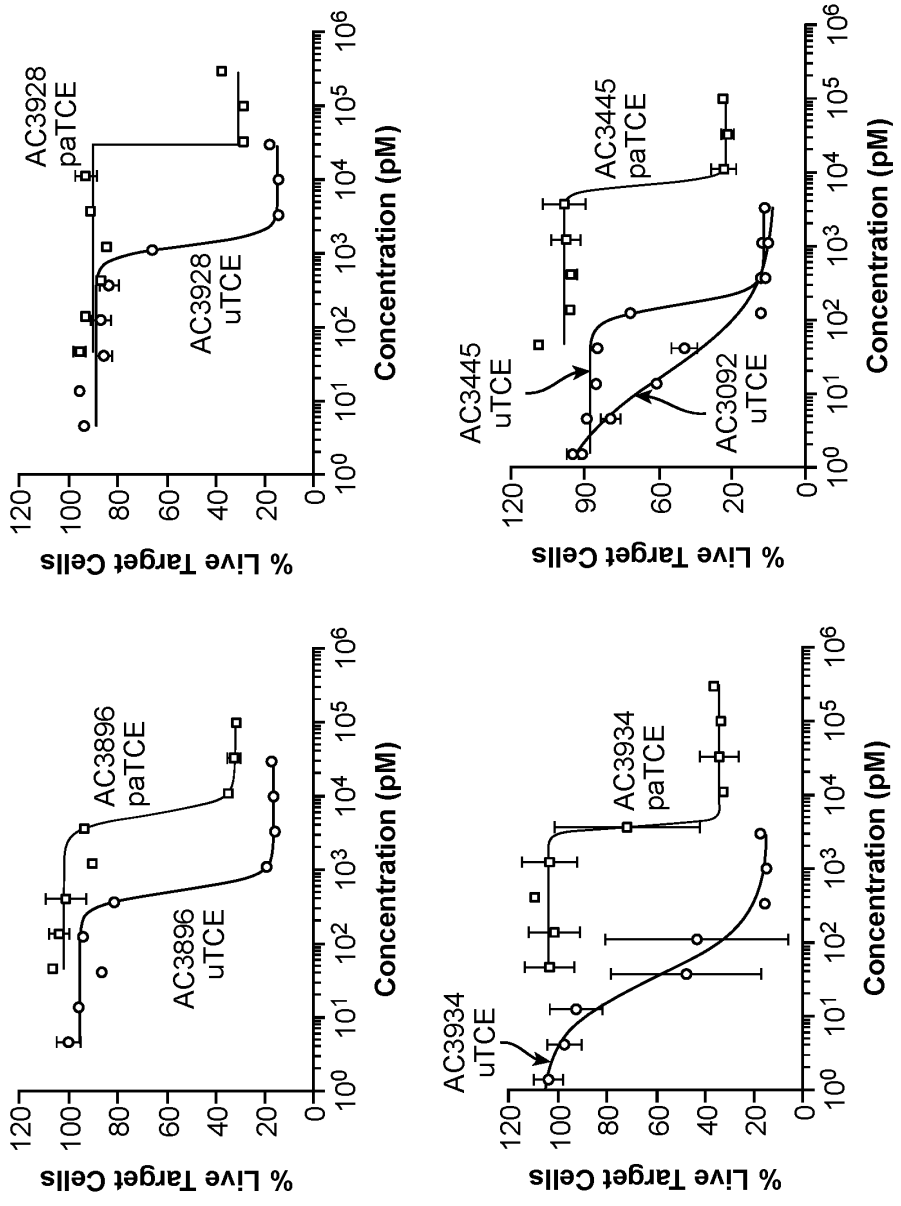

AC3928 paTCE
AC3928 uTCE
AC3445 paTCE
AC3445 uTCE
AC3092 uTCE
AC3896 paTCE
AC3896 uTCE
AC3934 paTCE
AC3934 uTCE
% Live Target Cells
Concentration (pM)

FIG. 11B

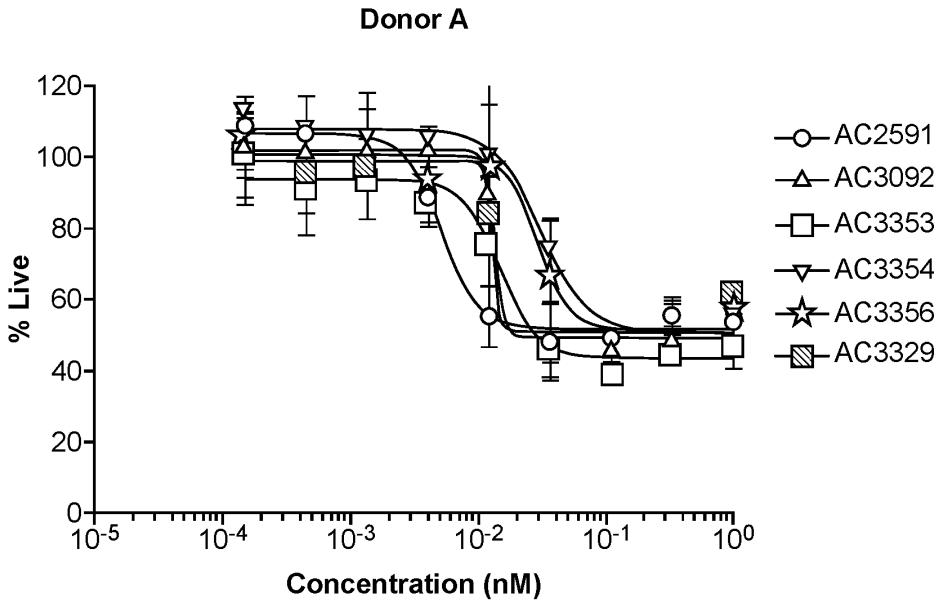

Donor A
% Live
120
100
80
60
40
20
0
10-5
10-4
10-3
10-2
10-1
100
Concentration (nM)
AC2591
AC3092
AC3353
AC3354
AC3356
AC3329

FIG. 12A

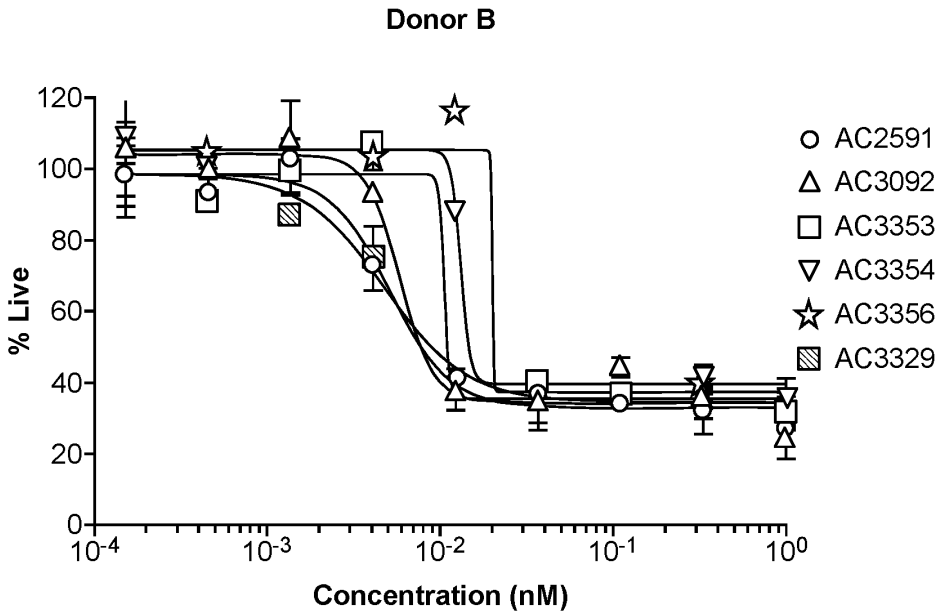

Donor B
% Live
120
100
80
60
40
20
0
10-4
10-3
10-2
10-1
100
Concentration (nM)
AC2591
AC3092
AC3353
AC3354
AC3356
AC3329

FIG. 12B

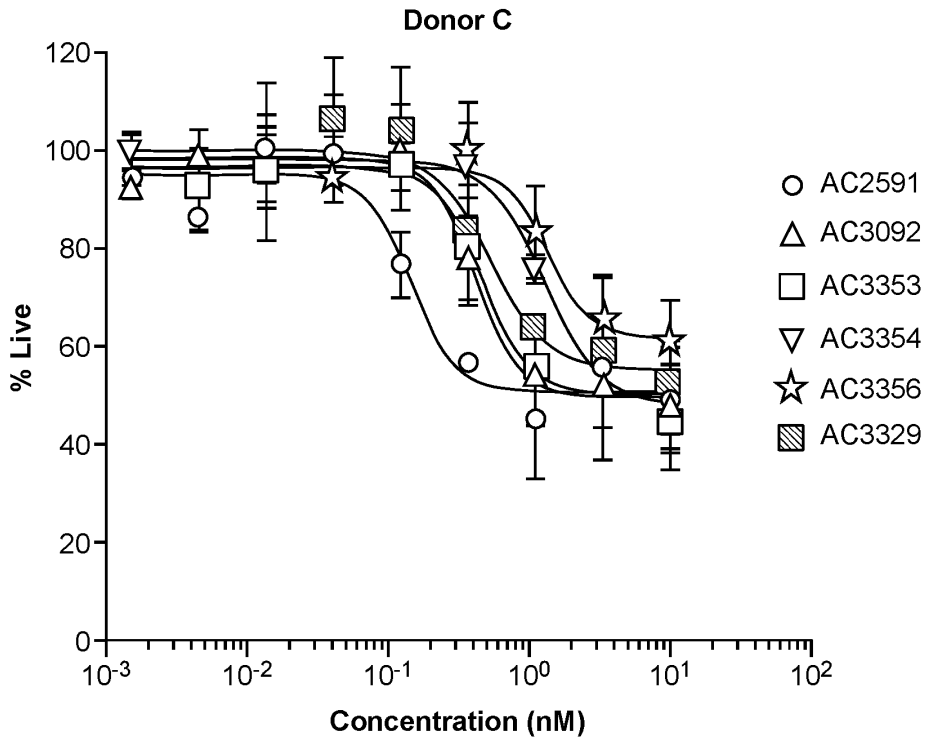

Donor C
% Live
120
100
80
60
40
20
0
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
Concentration (nM)
AC2591
AC3092
AC3353
AC3354
AC3356
AC3329

FIG. 12C

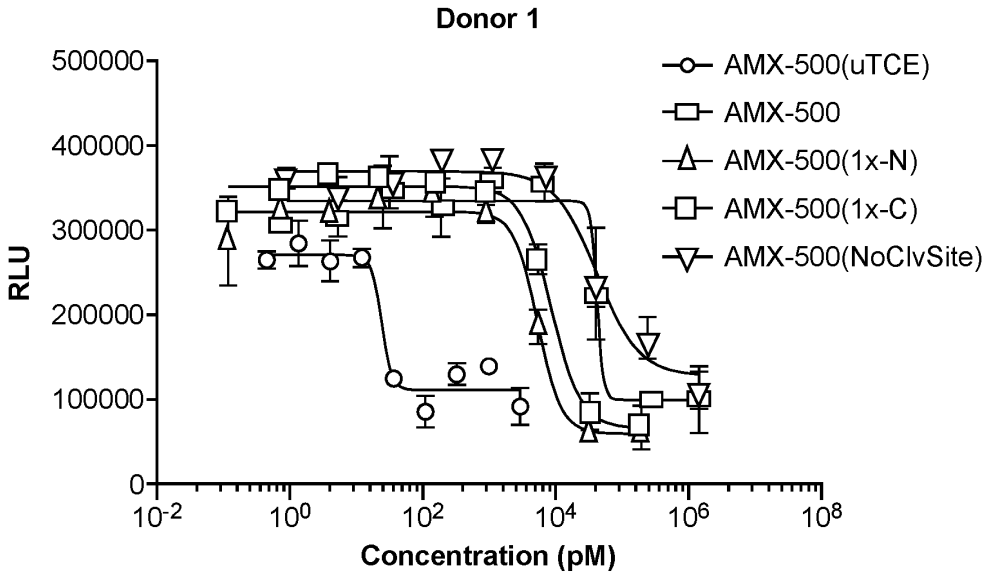

Donor 1
AMX-500(uTCE)
AMX-500
AMX-500(1x-N)
AMX-500(1x-C)
AMX-500(NoClvSite)
RLU
500000
400000
300000
200000
100000
0
10-2
100
102
104
106
108
Concentration (pM)

FIG. 13A

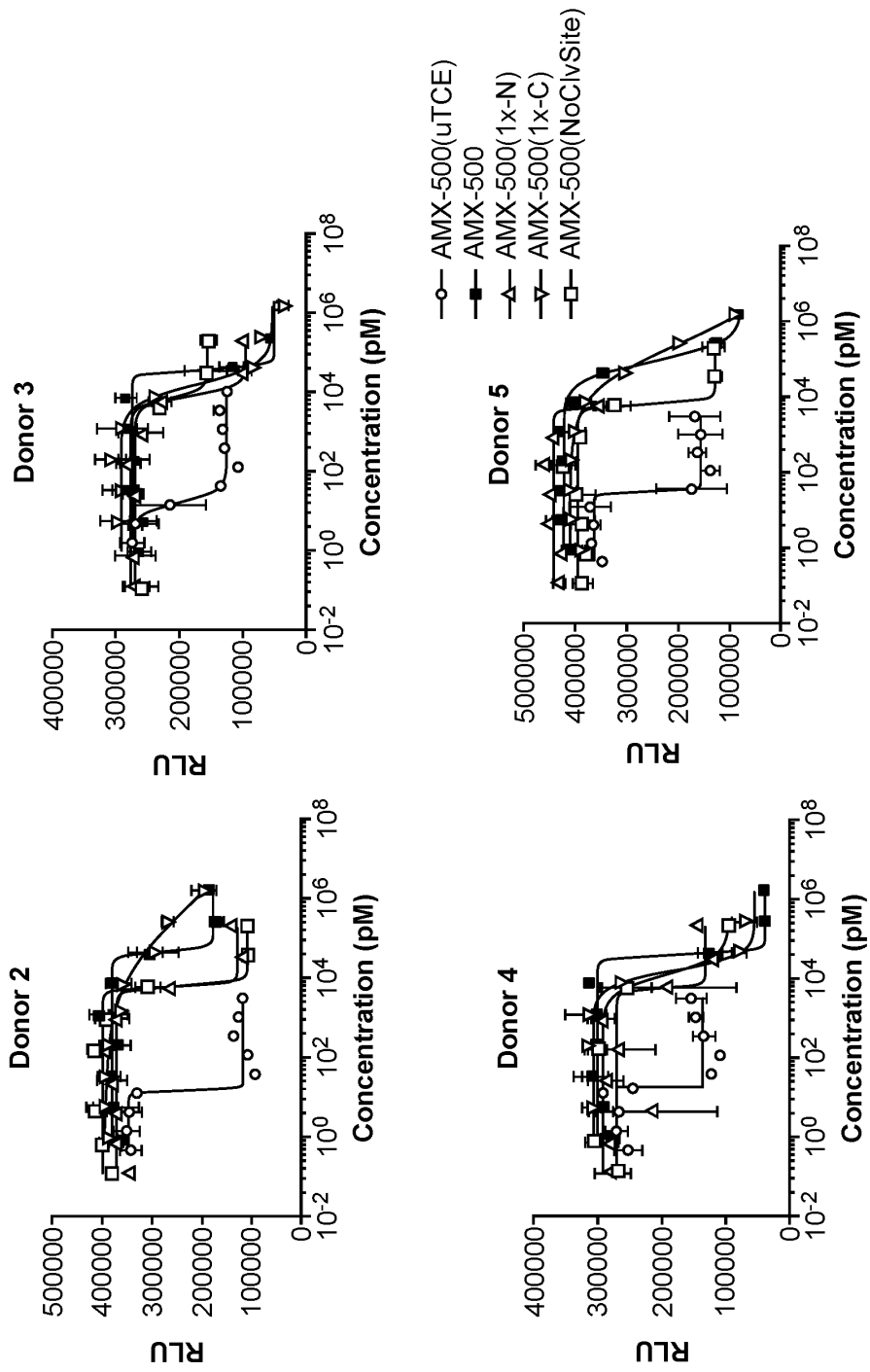

Donor 2
Donor 3
Donor 4
Donor 5
RLU
Concentration (pM)
AMX-500(uTCE)
AMX-500
AMX-500(1x-N)
AMX-500(1x-C)
AMX-500(NoClvSite)

FIG. 13B

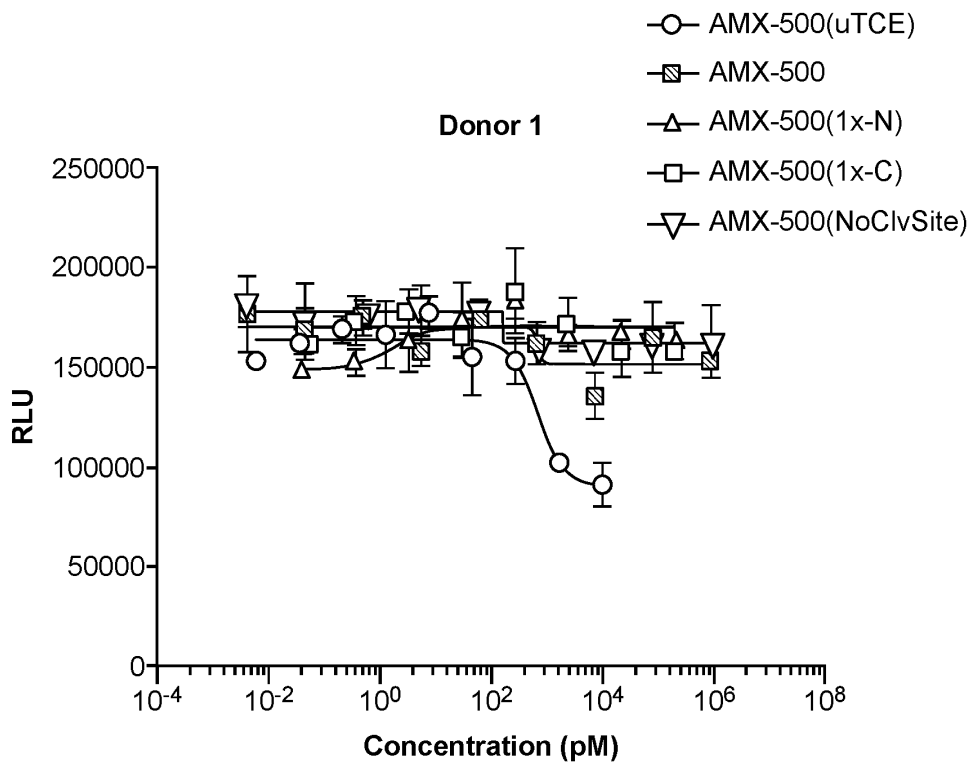

AMX-500(uTCE)
AMX-500
AMX-500(1x-N)
AMX-500(1x-C)
AMX-500(NoClvSite)
Donor 1
RLU
250000
200000
150000
100000
50000
0
10-4
10-2
100
102
104
106
108
Concentration (pM)

FIG. 14A

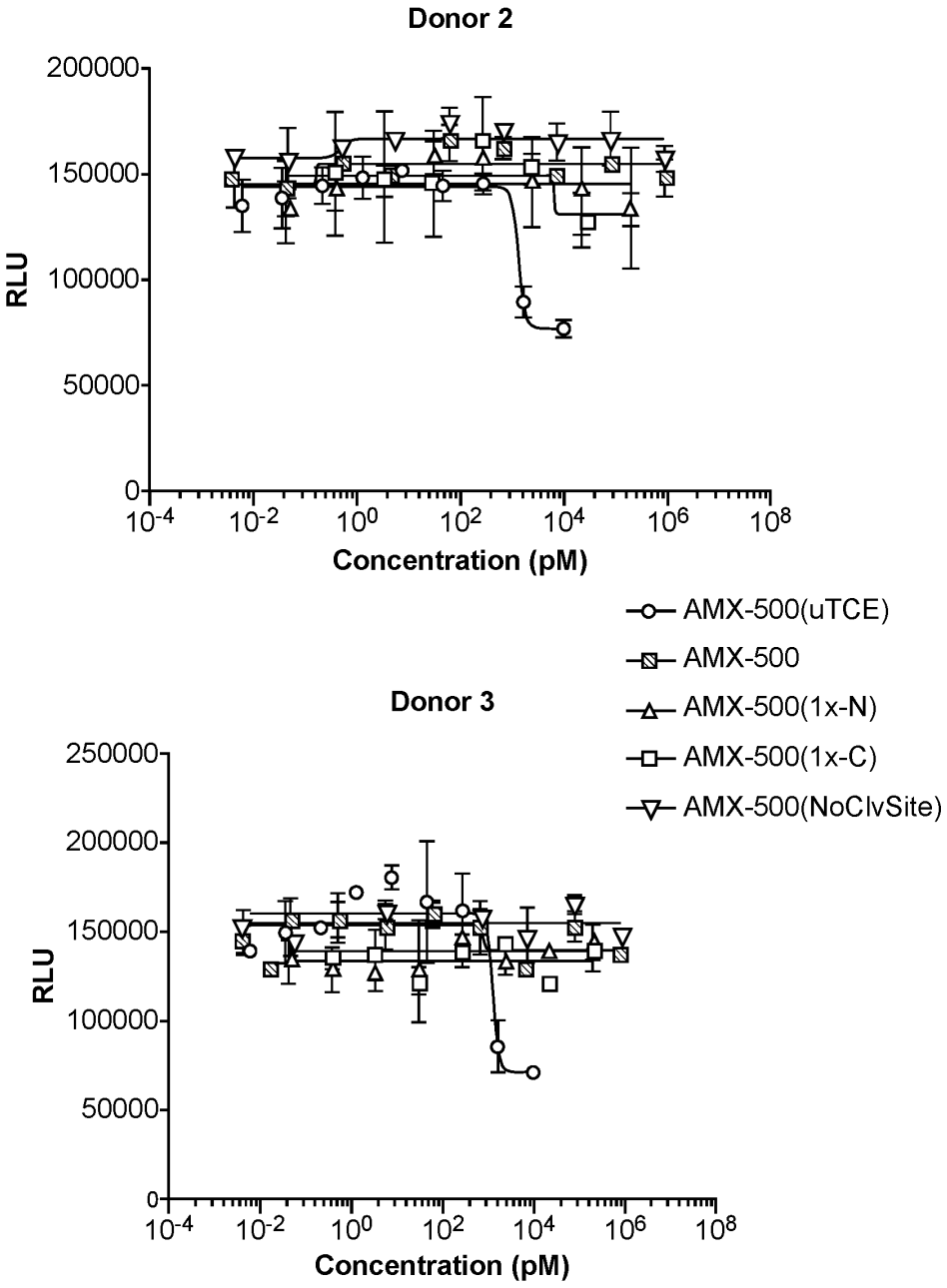

Donor 2
RLU
200000
150000
100000
50000
0
10^{-4} 10^{-2} 10^{0} 10^{2} 10^{4} 10^{6} 10^{8}
Concentration (pM)
AMX-500(uTCE)
AMX-500
AMX-500(1x-N)
AMX-500(1x-C)
AMX-500(NoClvSite)
Donor 3
RLU
250000
200000
150000
100000
50000
0
10^{-4} 10^{-2} 10^{0} 10^{2} 10^{4} 10^{6} 10^{8}
Concentration (pM)

FIG. 14B

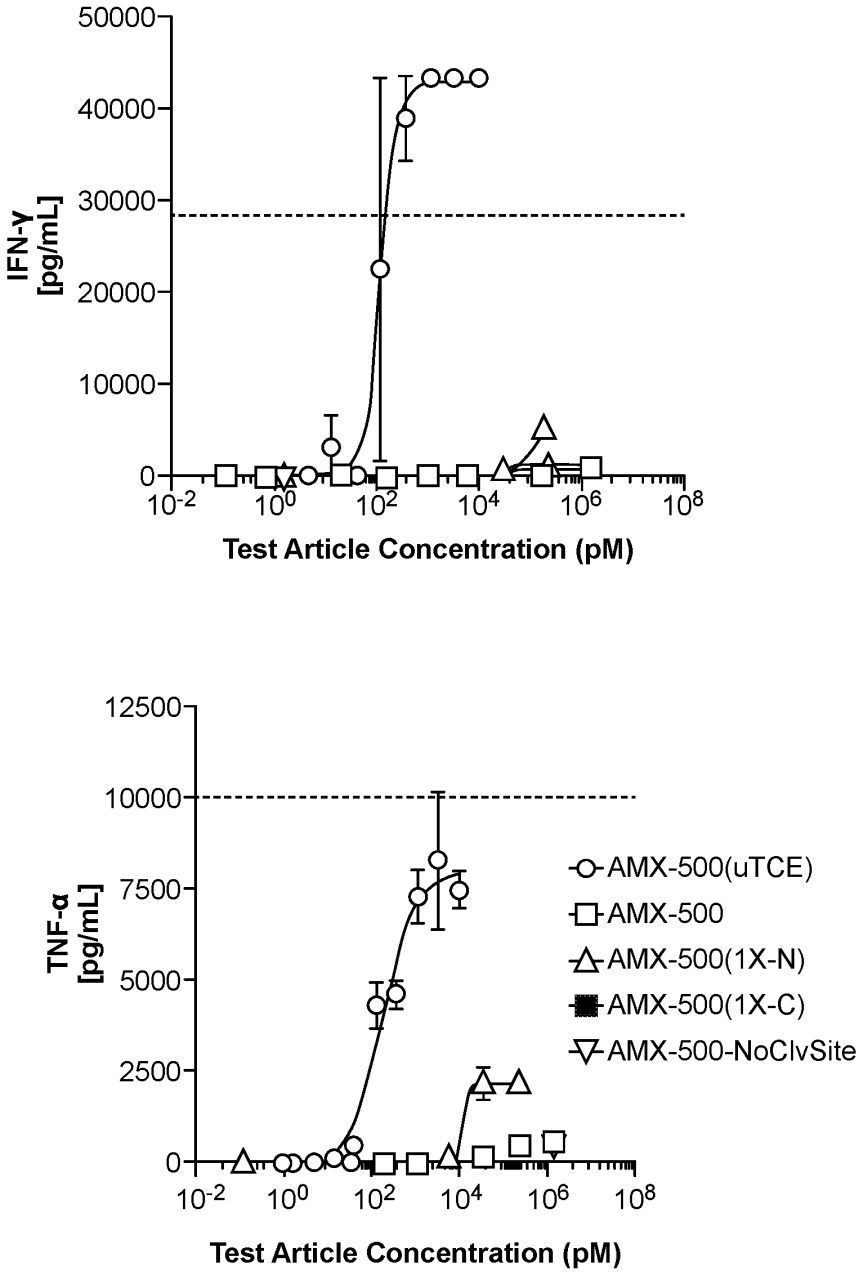

50000
40000
30000
20000
10000
0
IFN-γ [pg/mL]
10-2
100
102
104
106
108
Test Article Concentration (pM)
12500
10000
7500
5000
2500
0
TNF-α [pg/mL]
AMX-500(uTCE)
AMX-500
AMX-500(1X-N)
AMX-500(1X-C)
AMX-500-NoClvSite
Test Article Concentration (pM)

FIG. 15

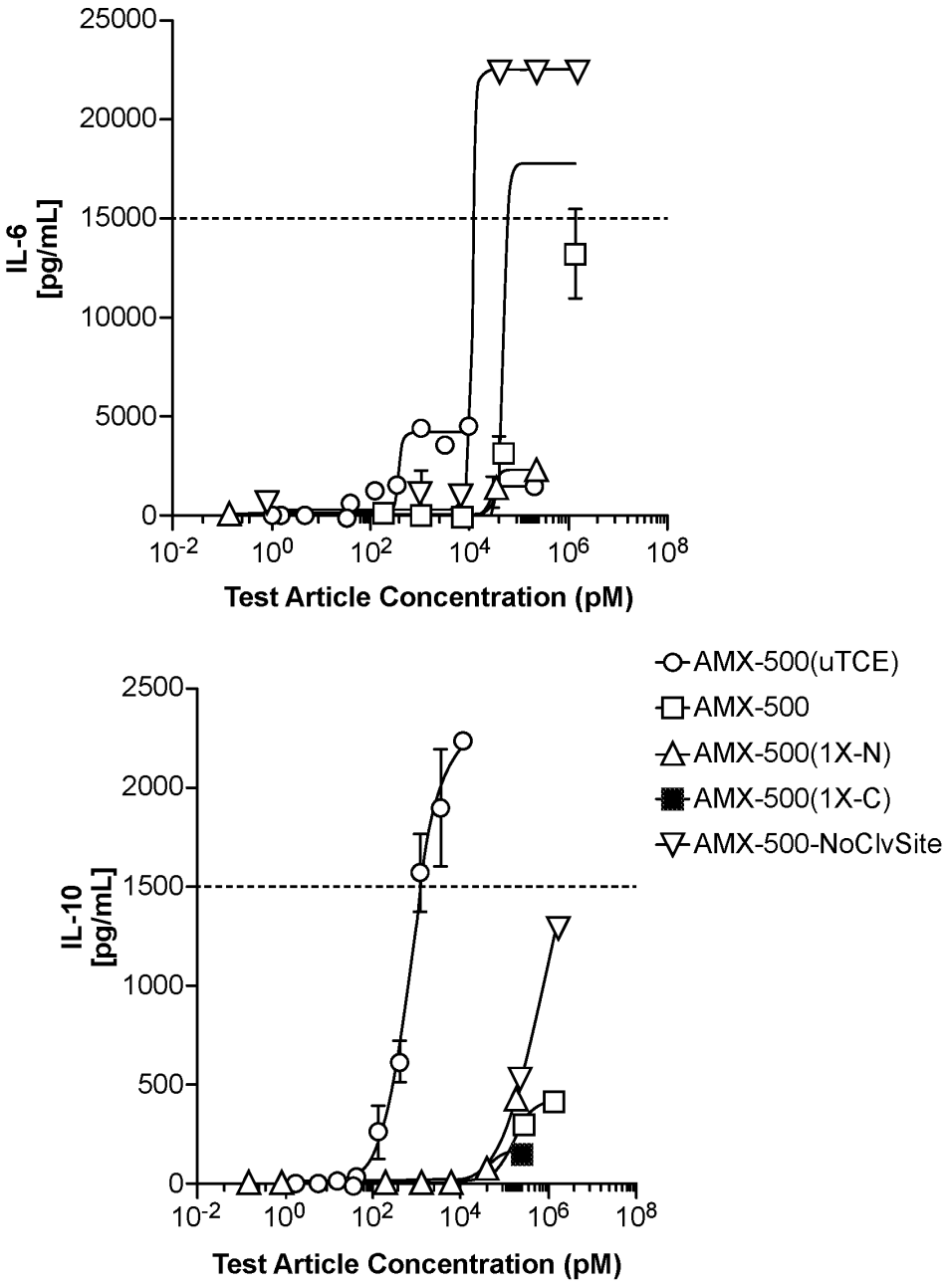

IL-6 [pg/mL]
25000
20000
15000
10000
5000
0
10-2
100
102
104
106
108
Test Article Concentration (pM)
IL-10 [pg/mL]
2500
2000
1500
1000
500
0
Test Article Concentration (pM)
AMX-500(uTCE)
AMX-500
AMX-500(1X-N)
AMX-500(1X-C)
AMX-500-NoClvSite

FIG. 15 (Cont. 1)

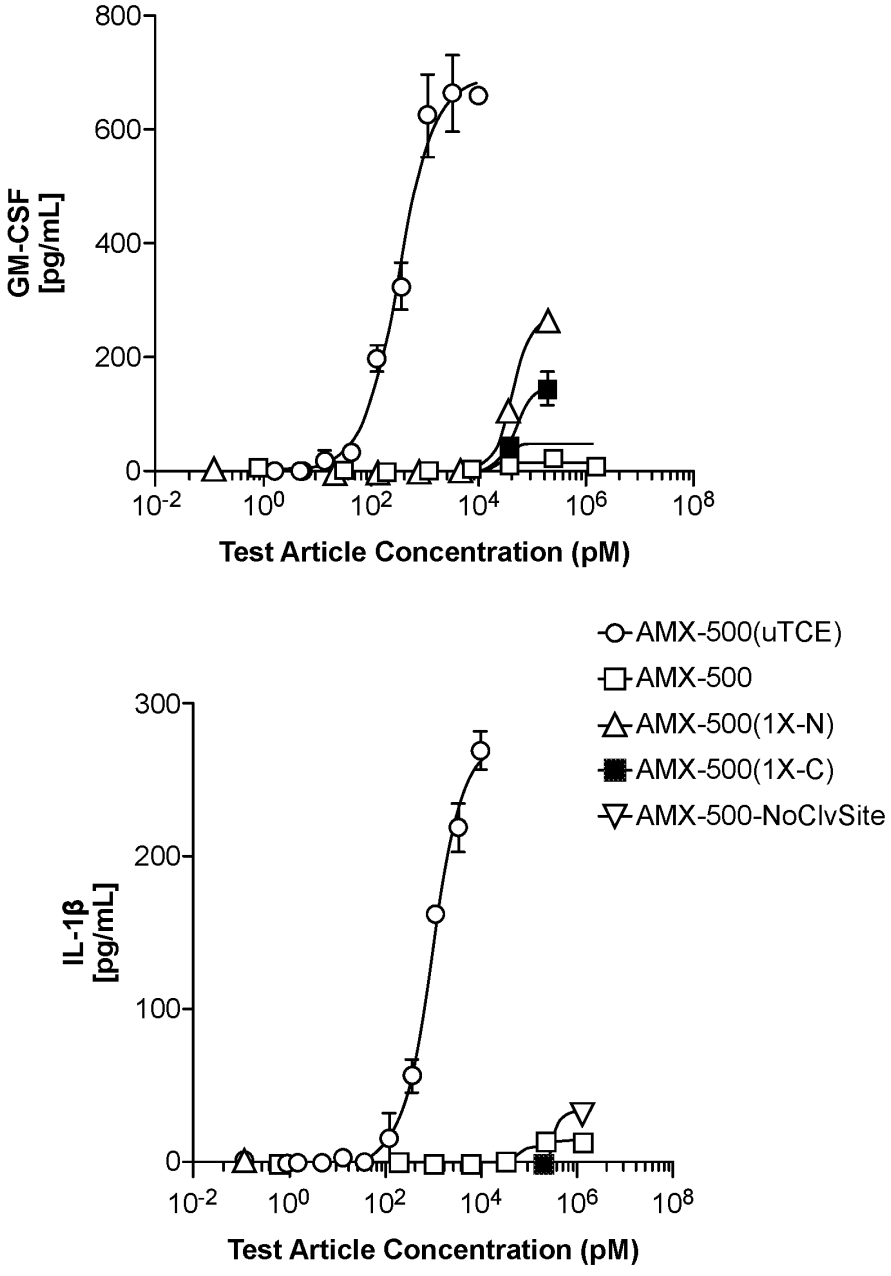

GM-CSF [pg/mL]
800
600
400
200
0
10^{-2}
10^{0}
10^{2}
10^{4}
10^{6}
10^{8}
Test Article Concentration (pM)
AMX-500(uTCE)
AMX-500
AMX-500(1X-N)
AMX-500(1X-C)
AMX-500-NoClvSite
IL-1β [pg/mL]
300
200
100
0
10^{-2}
10^{0}
10^{2}
10^{4}
10^{6}
10^{8}
Test Article Concentration (pM)

FIG. 15 (Cont. 2)

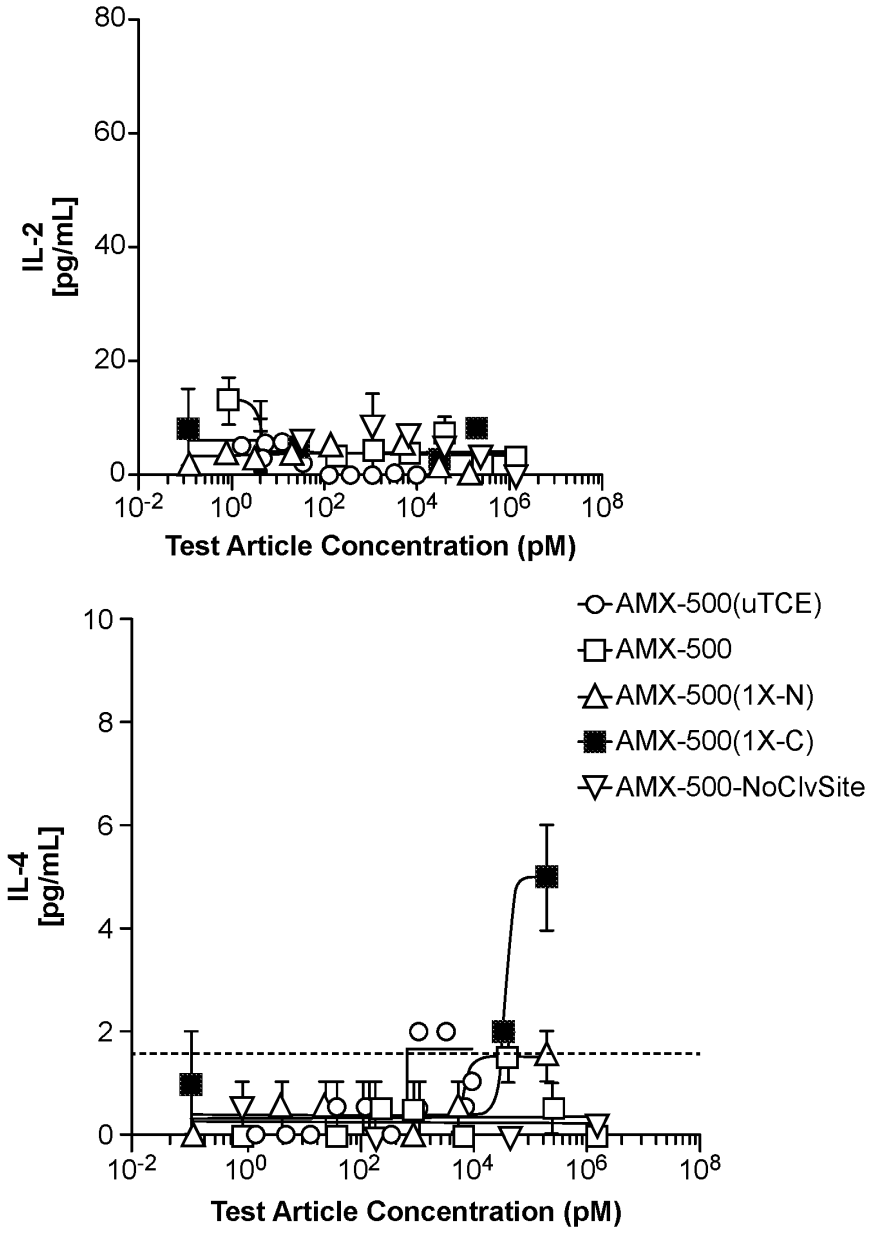

IL-2 [pg/mL]
80
60
40
20
0
10^{-2}
10^{0}
10^{2}
10^{4}
10^{6}
10^{8}
Test Article Concentration (pM)
AMX-500(uTCE)
AMX-500
AMX-500(1X-N)
AMX-500(1X-C)
AMX-500-NoClvSite
IL-4 [pg/mL]
10
8
6
4
2
0
10^{-2}
10^{0}
10^{2}
10^{4}
10^{6}
10^{8}
Test Article Concentration (pM)

FIG. 15 (Cont. 3)

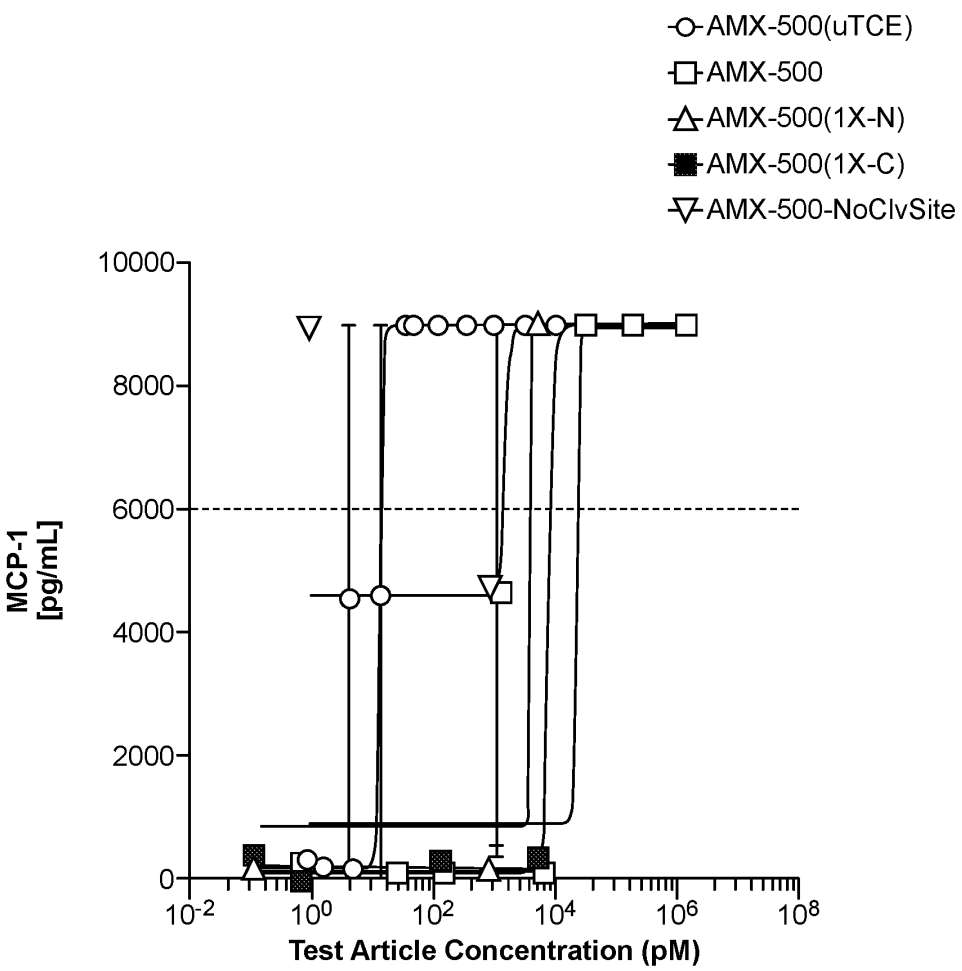

AMX-500(uTCE)
AMX-500
AMX-500(1X-N)
AMX-500(1X-C)
AMX-500-NoClvSite
10000
8000
6000
4000
2000
0
MCP-1 [pg/mL]
10-2
100
102
104
106
108
Test Article Concentration (pM)

FIG. 15 (Cont. 4)

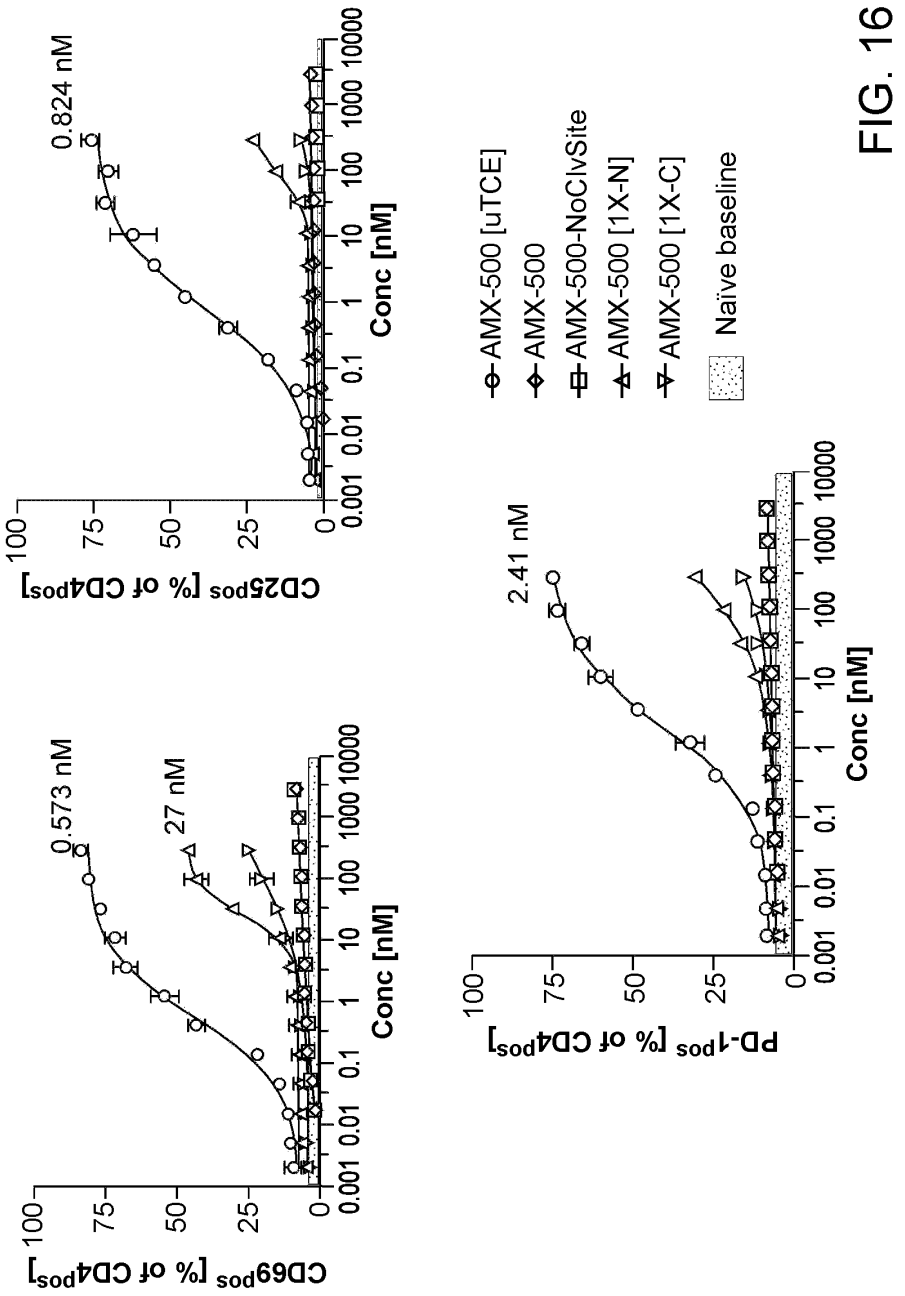

AMX-500 [uTCE]
AMX-500
AMX-500-NoClvSite
AMX-500 [1X-N]
AMX-500 [1X-C]
Naïve baseline
0.824 nM
0.573 nM
27 nM
2.41 nM
CD25pos [% of CD4pos]
CD69pos [% of CD4pos]
PD-1pos [% of CD4pos]
Conc [nM]
0.001 0.01 0.1 1 10 100 1000 10000
0 25 50 75 100

FIG. 16

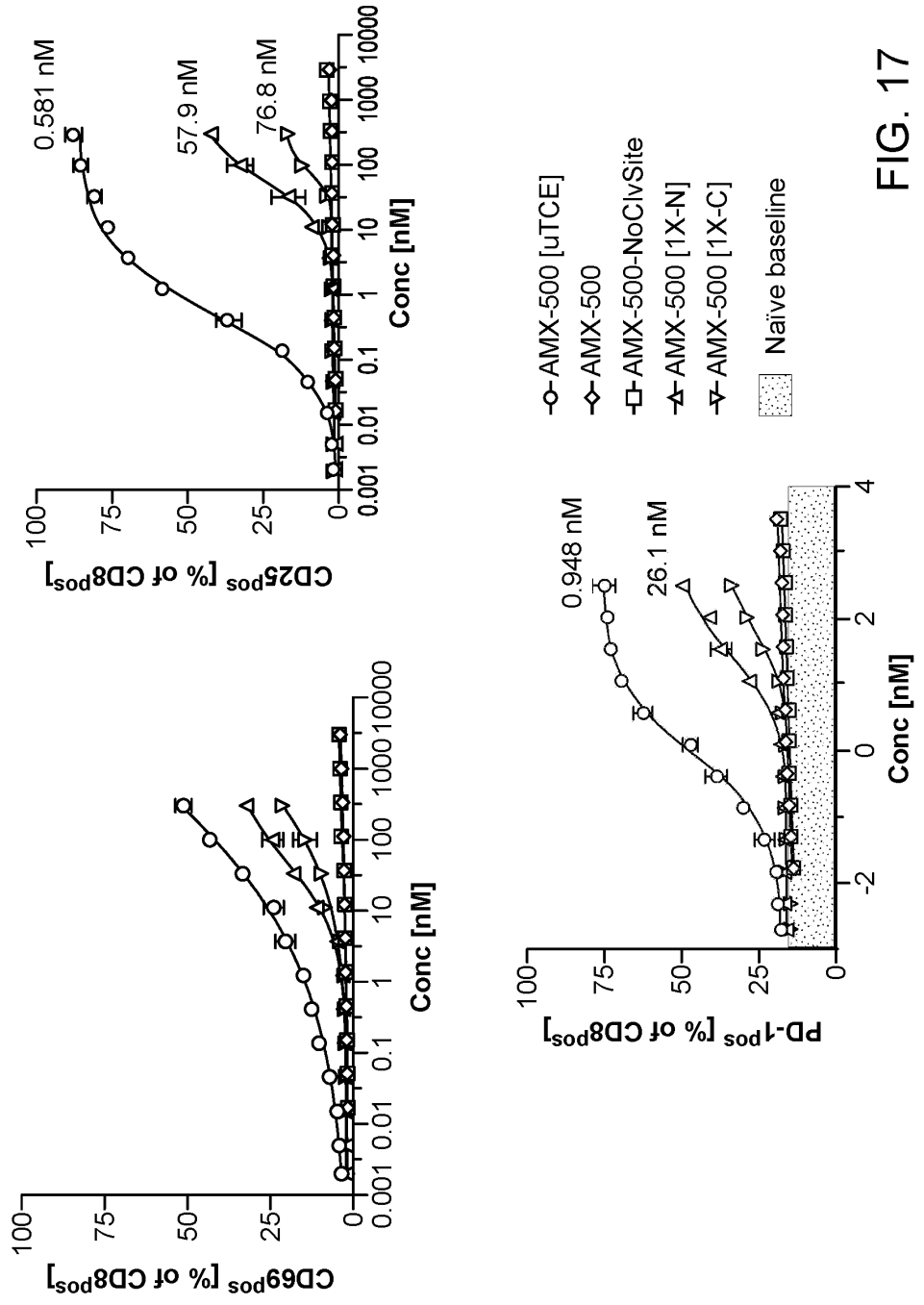

AMX-500 [uTCE]
AMX-500
AMX-500-NoClvSite
AMX-500 [1X-N]
AMX-500 [1X-C]
Naïve baseline
0.581 nM
57.9 nM
76.8 nM
0.948 nM
26.1 nM
CD25pos [% of CD8pos]
CD69pos [% of CD8pos]
PD-1pos [% of CD8pos]
Conc [nM]

FIG. 17

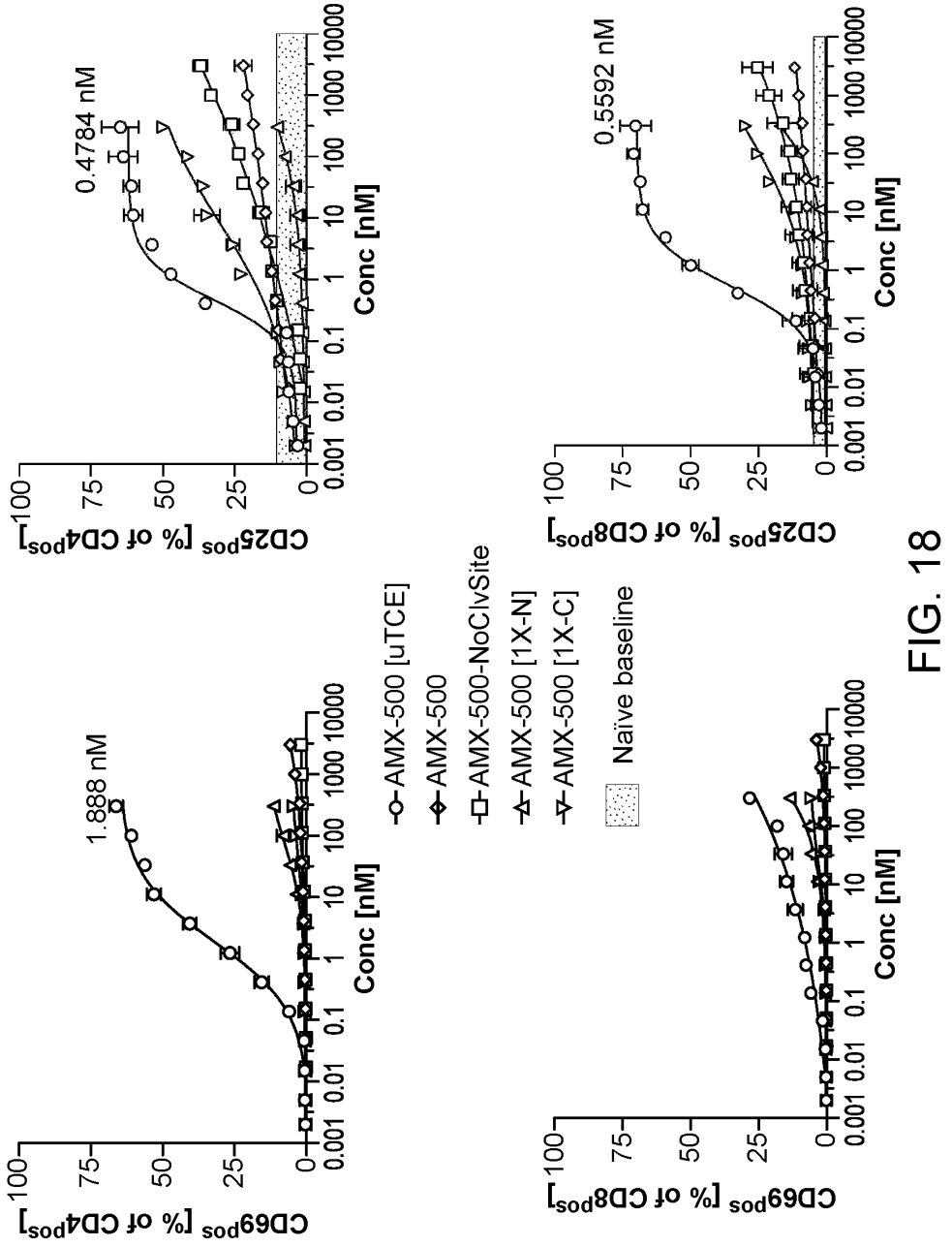

0.4784 nM
0.5592 nM
1.888 nM
CD25pos [% of CD4pos]
CD25pos [% of CD8pos]
CD69pos [% of CD4pos]
CD69pos [% of CD8pos]
Conc [nM]
0.001 0.01 0.1 1 10 100 1000 10000
0 25 50 75 100
AMX-500 [uTCE]
AMX-500
AMX-500-NoClvSite
AMX-500 [1X-N]
AMX-500 [1X-C]
Naïve baseline

FIG. 18

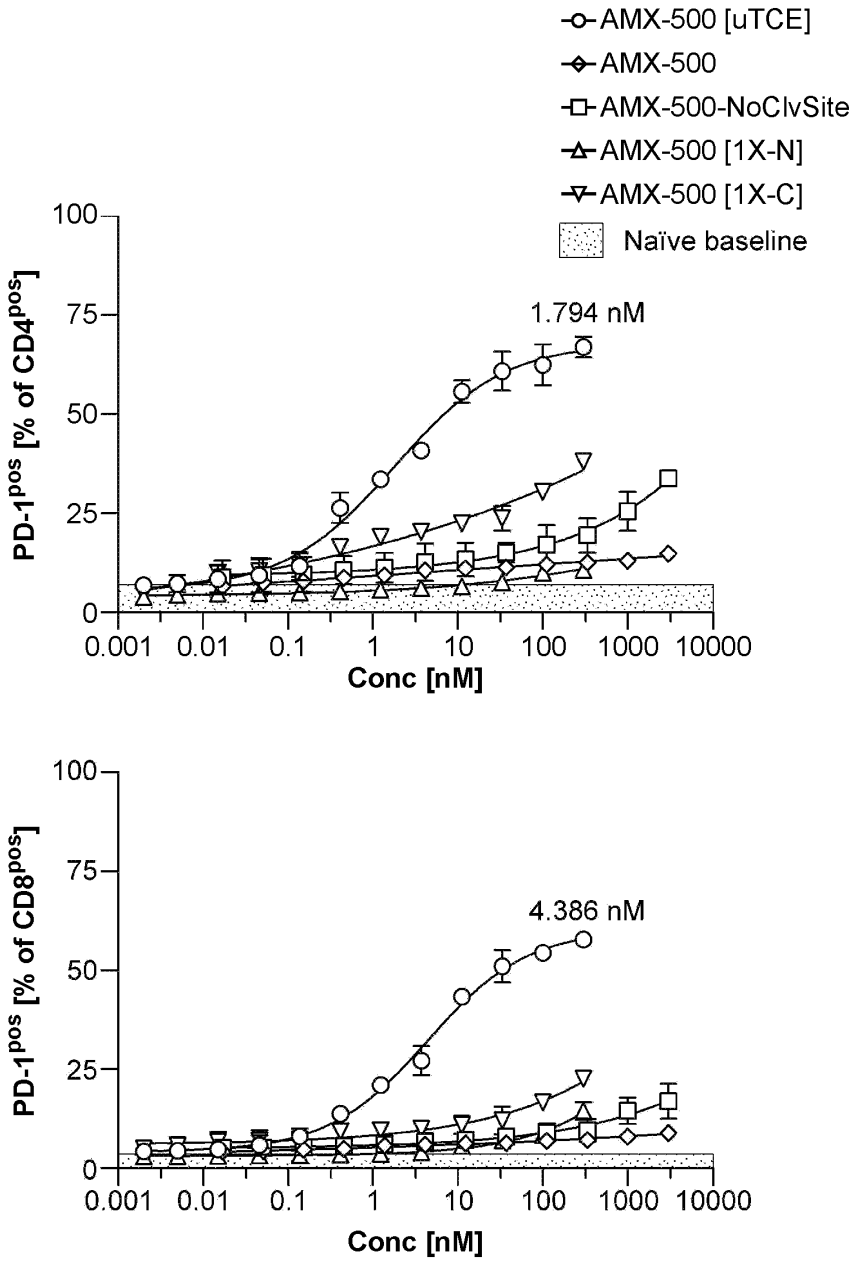

AMX-500 [uTCE]
AMX-500
AMX-500-NoClvSite
AMX-500 [1X-N]
AMX-500 [1X-C]
Naïve baseline
1.794 nM
PD-1pos [% of CD4pos]
100
75
50
25
0
0.001 0.01 0.1 1 10 100 1000 10000
Conc [nM]
4.386 nM
PD-1pos [% of CD8pos]
100
75
50
25
0
0.001 0.01 0.1 1 10 100 1000 10000
Conc [nM]

FIG. 18 (Cont.)

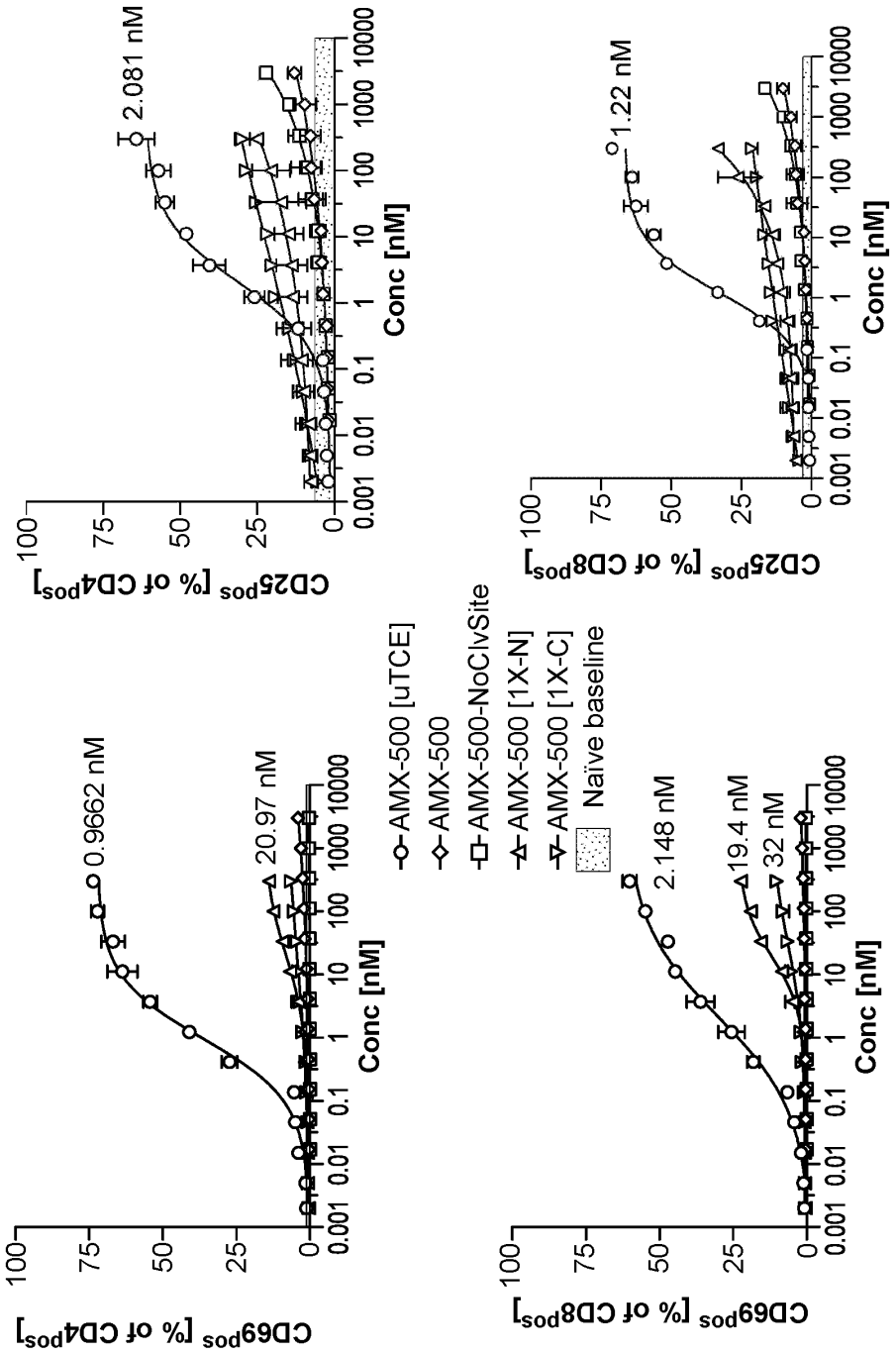

CD69pos [% of CD4pos]
CD25pos [% of CD4pos]
CD69pos [% of CD8pos]
CD25pos [% of CD8pos]
Conc [nM]
0.9662 nM
20.97 nM
2.081 nM
2.148 nM
19.4 nM
32 nM
1.22 nM
AMX-500 [uTCE]
AMX-500
AMX-500-NoClvSite
AMX-500 [1X-N]
AMX-500 [1X-C]
Naïve baseline

FIG. 19

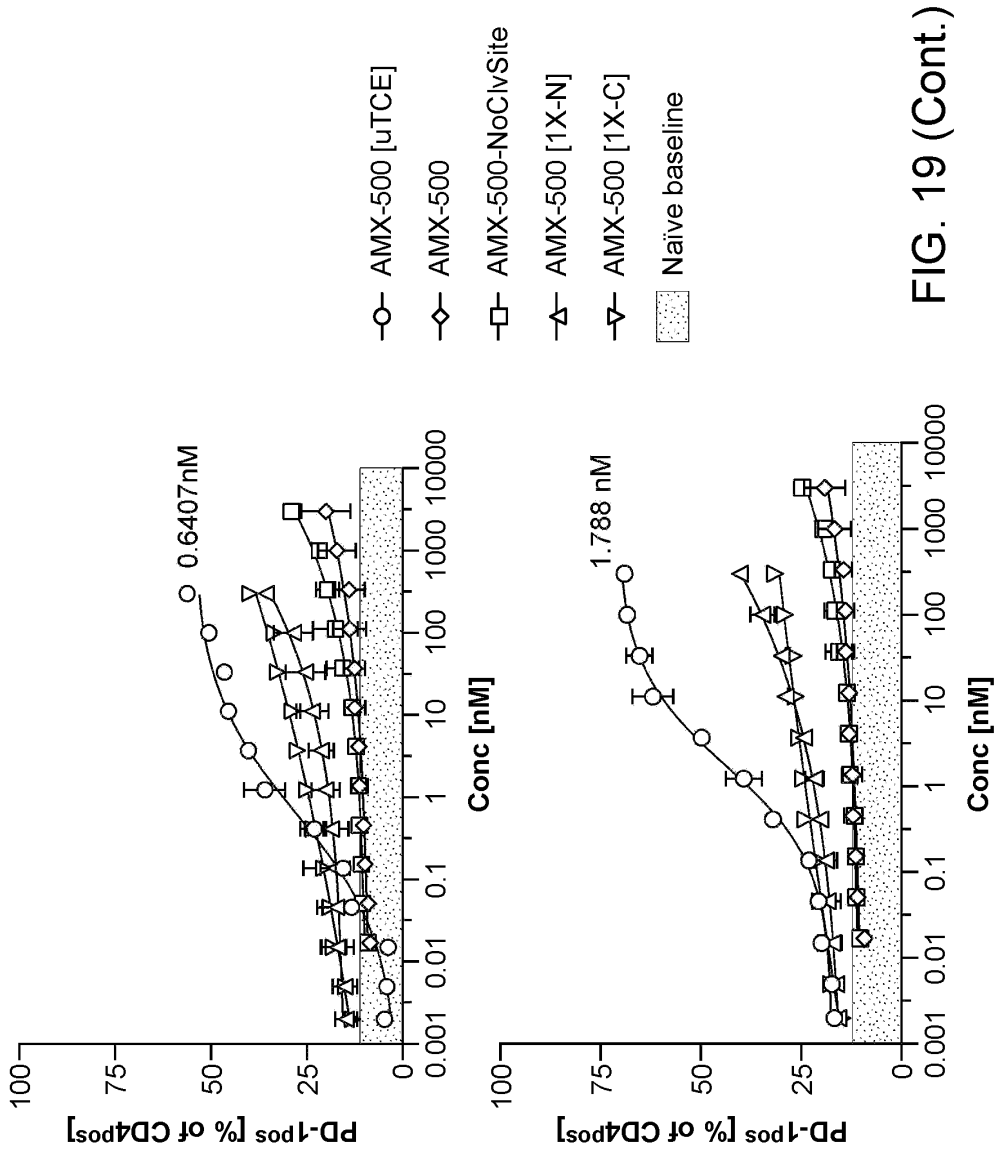

AMX-500 [uTCE]
AMX-500
AMX-500-NoClvSite
AMX-500 [1X-N]
AMX-500 [1X-C]
Naïve baseline
0.6407nM
1.788 nM
PD-1pos [% of CD4pos]
Conc [nM]
0
25
50
75
100
0.001
0.01
0.1
1
10
100
1000
10000

FIG. 19 (Cont.)

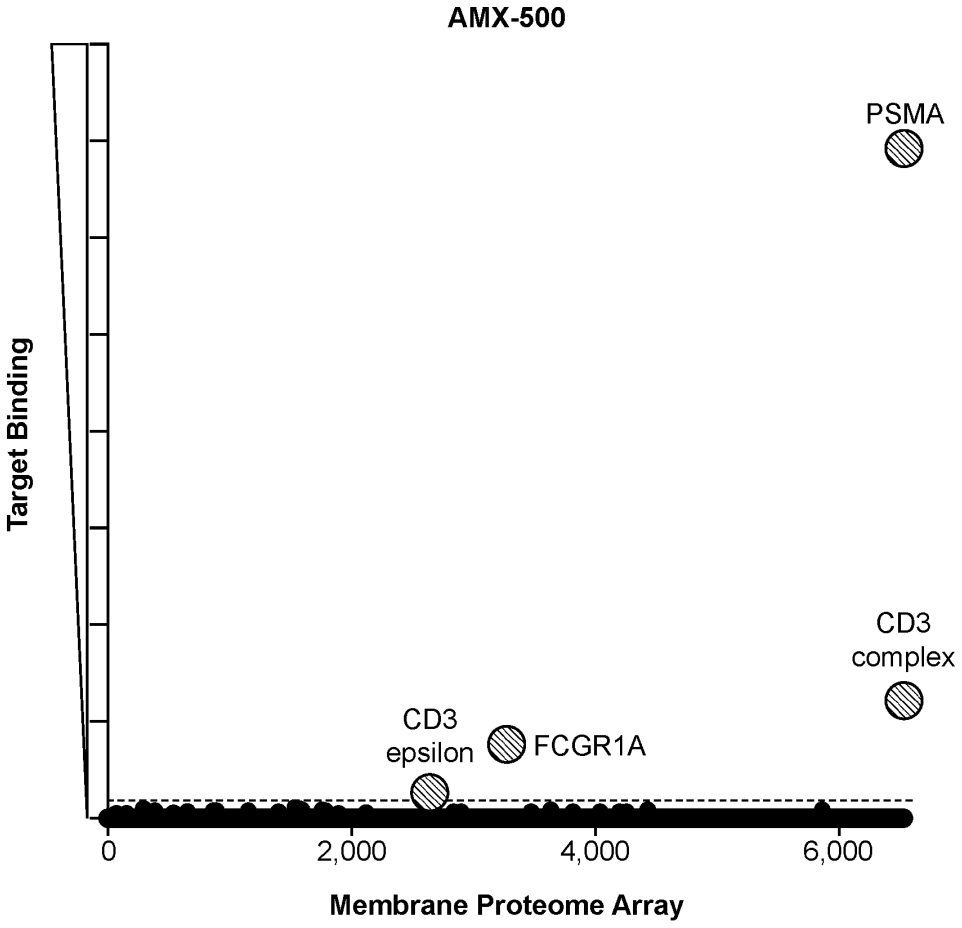

AMX-500
PSMA
CD3
complex
CD3
epsilon
FCGR1A
Target Binding
0
2,000
4,000
6,000
Membrane Proteome Array

FIG. 20A

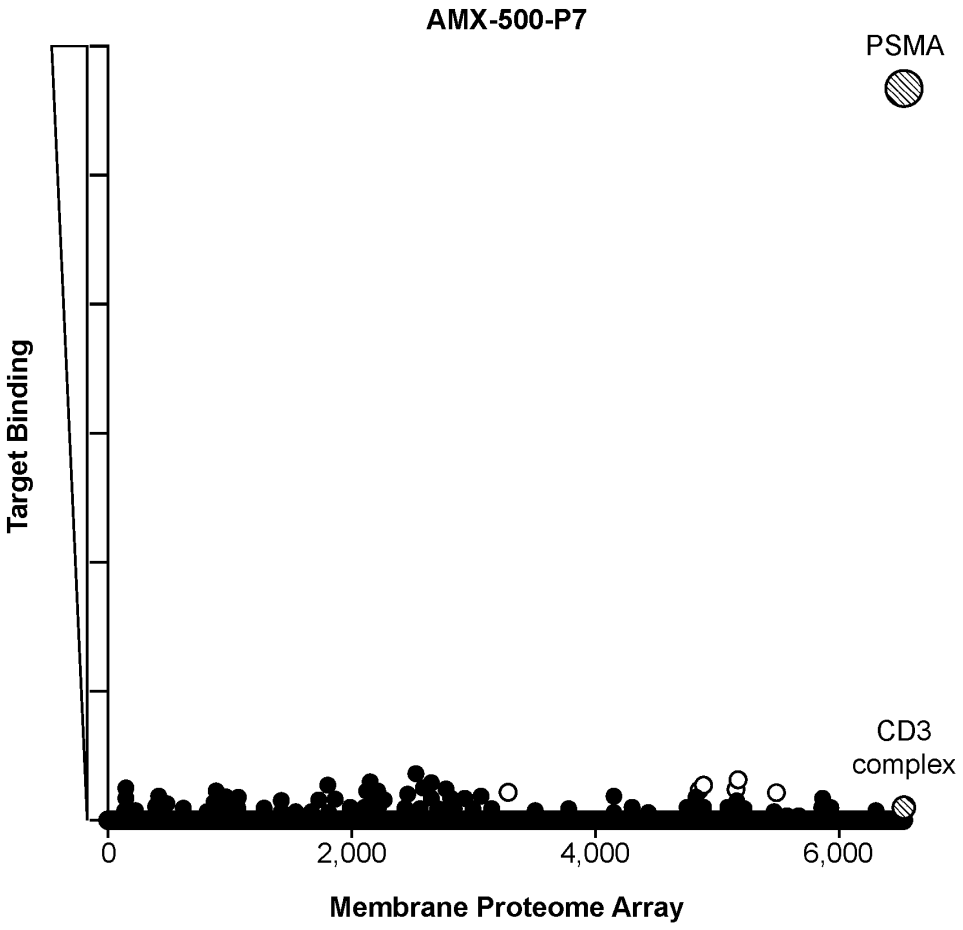

AMX-500-P7
PSMA
CD3
complex
Target Binding
0
2,000
4,000
6,000
Membrane Proteome Array

FIG. 20B

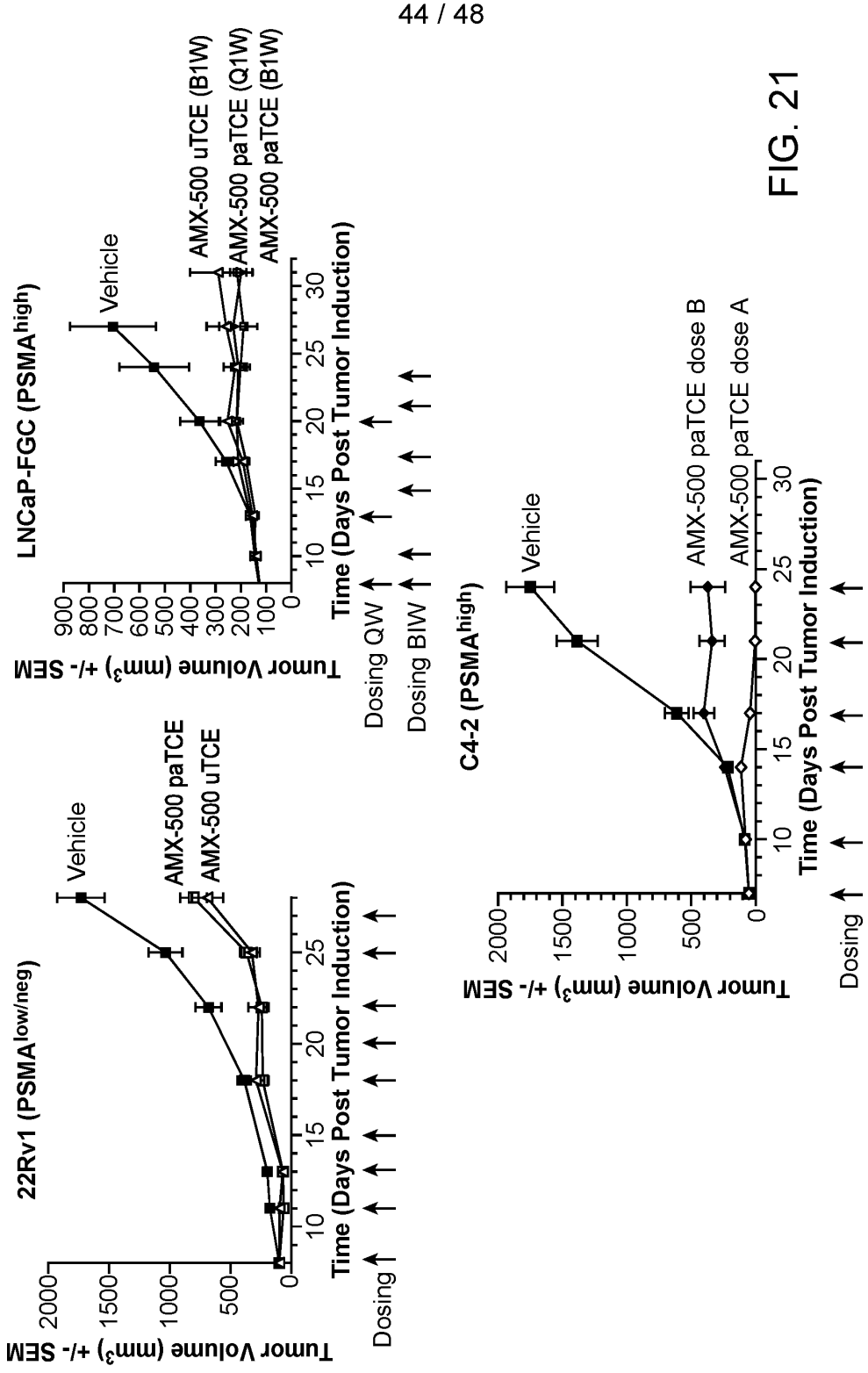

22Rv1 (PSMA low/neg)
Vehicle
AMX-500 paTCE
AMX-500 uTCE
Tumor Volume (mm3) +/- SEM
Time (Days Post Tumor Induction)
Dosing
LNCaP-FGC (PSMA high)
Vehicle
AMX-500 uTCE (B1W)
AMX-500 paTCE (Q1W)
AMX-500 paTCE (B1W)
Tumor Volume (mm3) +/- SEM
Time (Days Post Tumor Induction)
Dosing QW
Dosing BIW
C4-2 (PSMA high)
Vehicle
AMX-500 paTCE dose B
AMX-500 paTCE dose A
Tumor Volume (mm3) +/- SEM
Time (Days Post Tumor Induction)
Dosing

FIG. 21

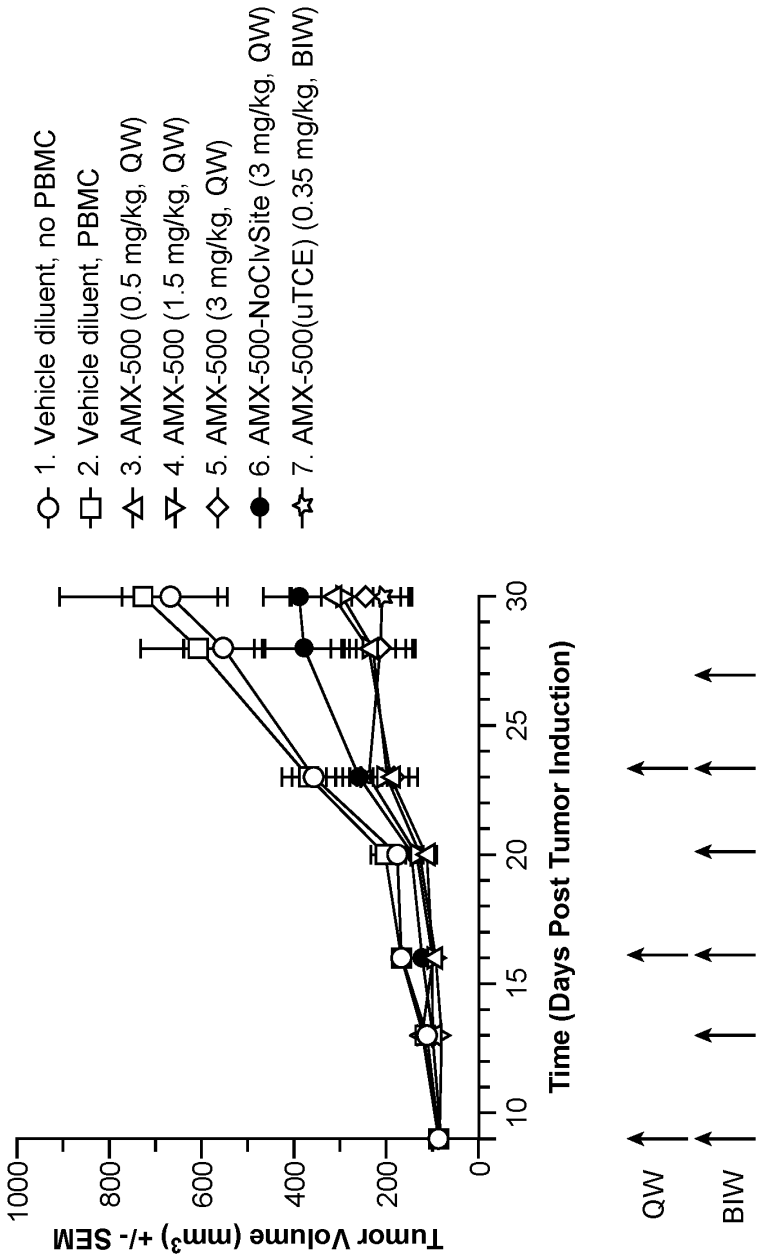

1. Vehicle diluent, no PBMC
2. Vehicle diluent, PBMC
3. AMX-500 (0.5 mg/kg, QW)
4. AMX-500 (1.5 mg/kg, QW)
5. AMX-500 (3 mg/kg, QW)
6. AMX-500-NoClvSite (3 mg/kg, QW)
7. AMX-500(uTCE) (0.35 mg/kg, BIW)
Tumor Volume (mm3) +/- SEM
1000
800
600
400
200
0
10
15
20
25
30
Time (Days Post Tumor Induction)
QW
BIW

FIG. 22

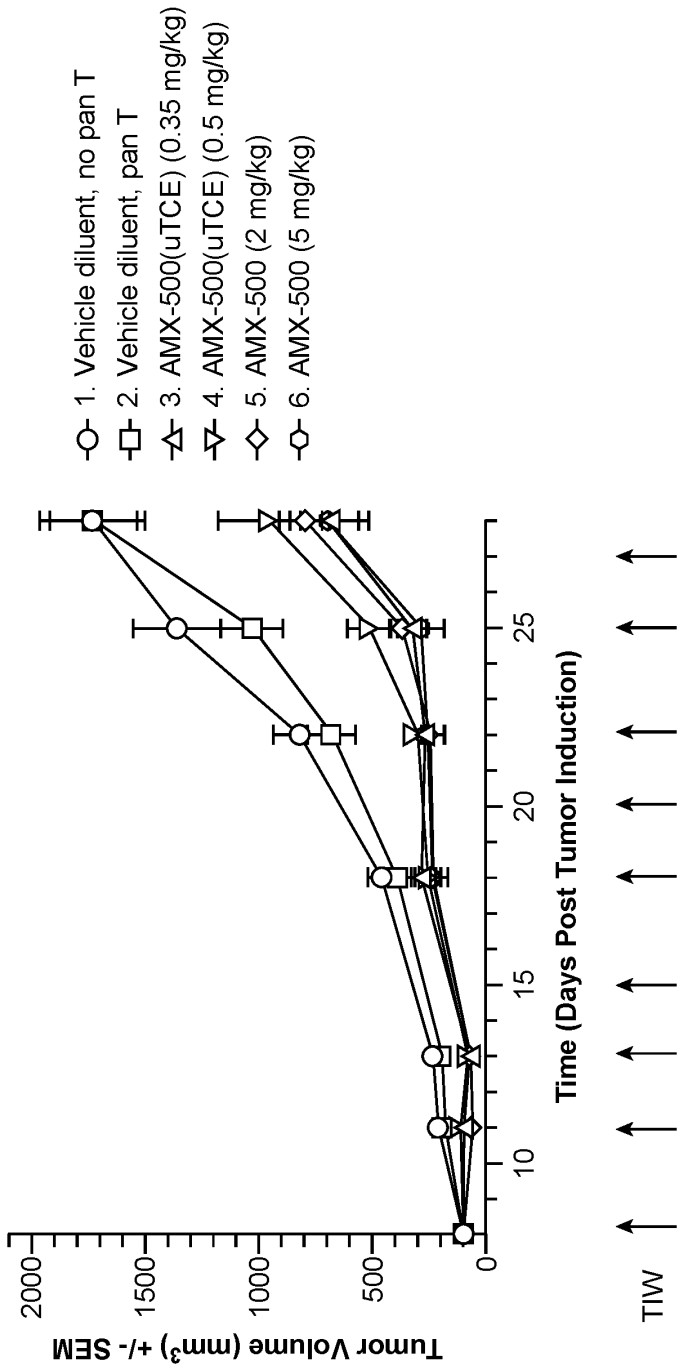
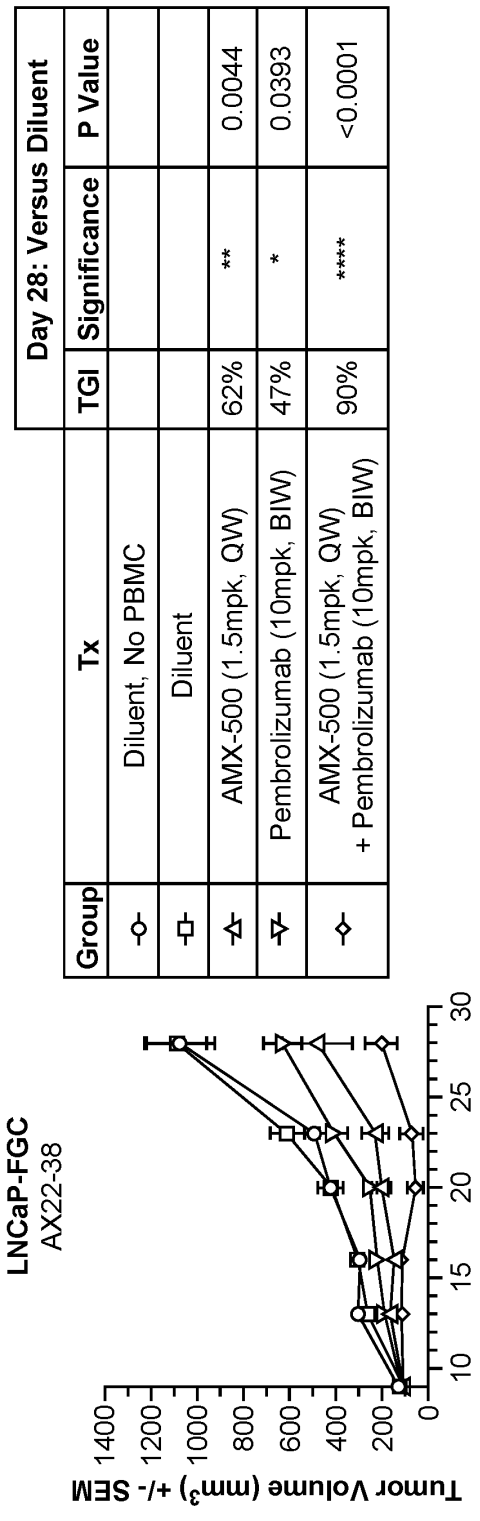

1. Vehicle diluent, no pan T
2. Vehicle diluent, pan T
3. AMX-500(uTCE) (0.35 mg/kg)
4. AMX-500(uTCE) (0.5 mg/kg)
5. AMX-500 (2 mg/kg)
6. AMX-500 (5 mg/kg)
Tumor Volume (mm3) +/- SEM
2000
1500
1000
500
0
10
15
20
25
Time (Days Post Tumor Induction)
TIW

FIG. 23



Group	Tx	Day 28: Versus Diluent		
		TGI	Significance	P Value
	Diluent, No PBMC			
	Diluent			
	AMX-500 (1.5mpk, QW)	62%	**	0.0044
	Pembrolizumab (10mpk, BIW)	47%	*	0.0393
	AMX-500 (1.5mpk, QW) + Pembrolizumab (10mpk, BIW)	90%	****	<0.0001

FIG. 24

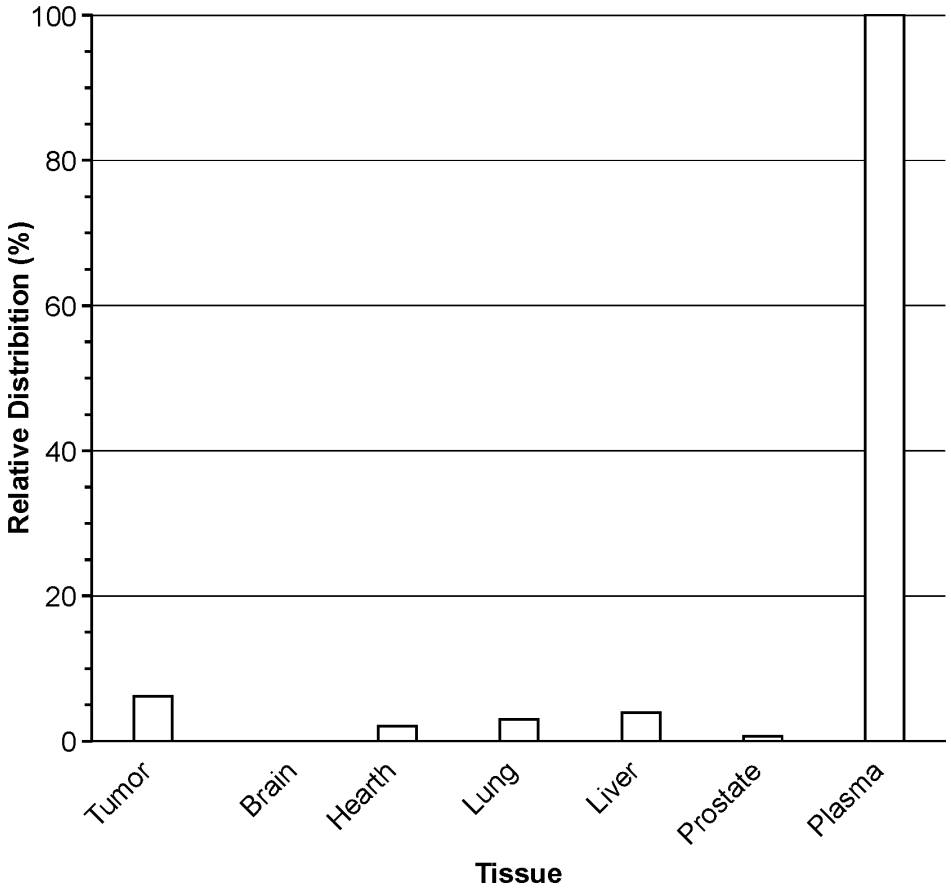

100
80
60
40
20
0
Relative Distribition (%)
Tumor
Brain
Hearth
Lung
Liver
Prostate
Plasma
Tissue

FIG. 25