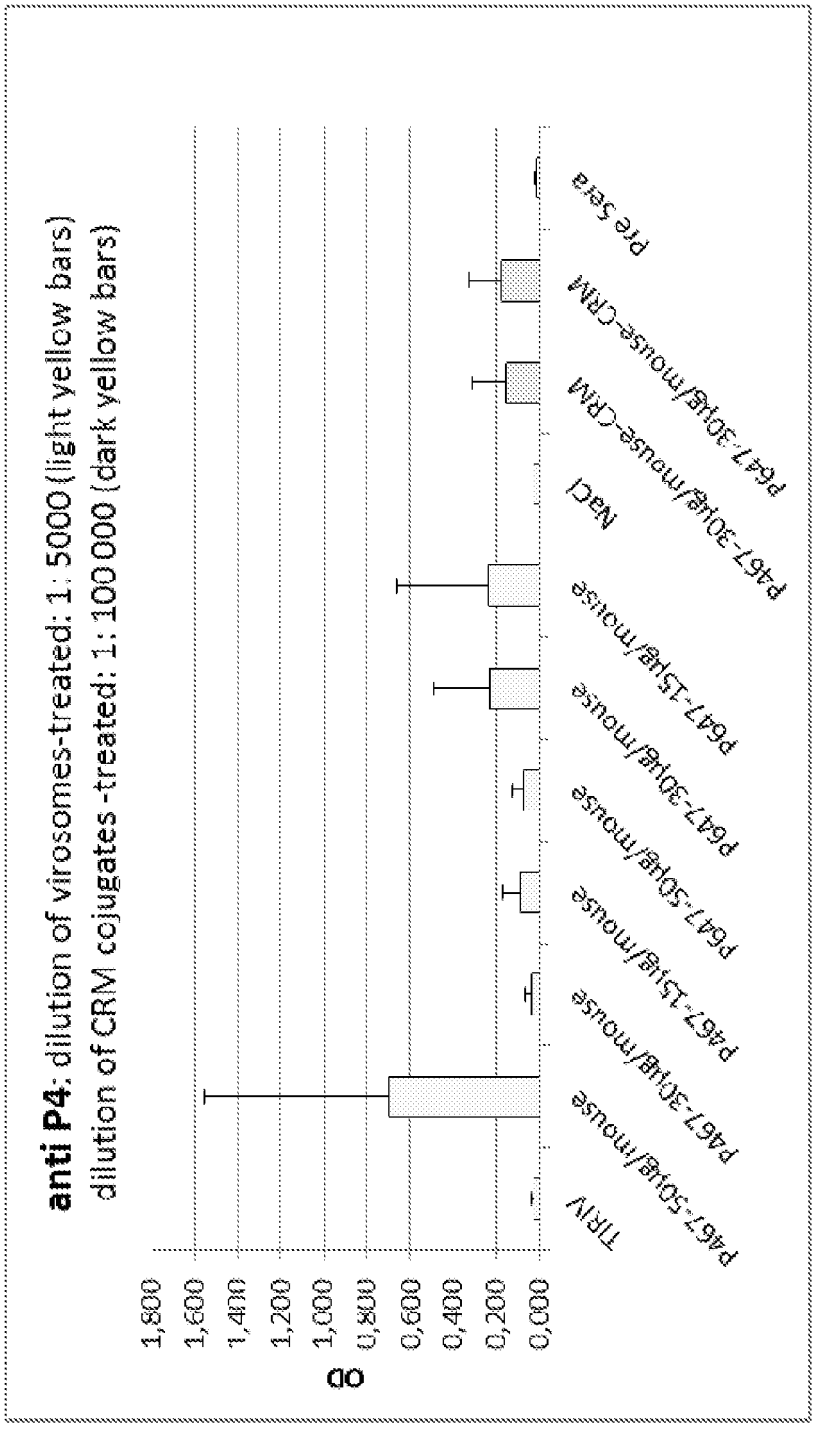
FIGURE 1


anti P4: dilution of virosomes-treated: 1: 5000 (light yellow bars)
dilution of CRM cojugates -treated: 1: 100 000 (dark yellow bars)
OD
1,800
1,600
1,400
1,200
1,000
0,800
0,600
0,400
0,200
0,000
TRIV
P467-50µg/mouse
P467-30µg/mouse
P467-15µg/mouse
P647-50µg/mouse
P647-30µg/mouse
P647-15µg/mouse
NaCl
P467-30µg/mouse-CRM
P647-30µg/mouse-CRM
Pre Sera

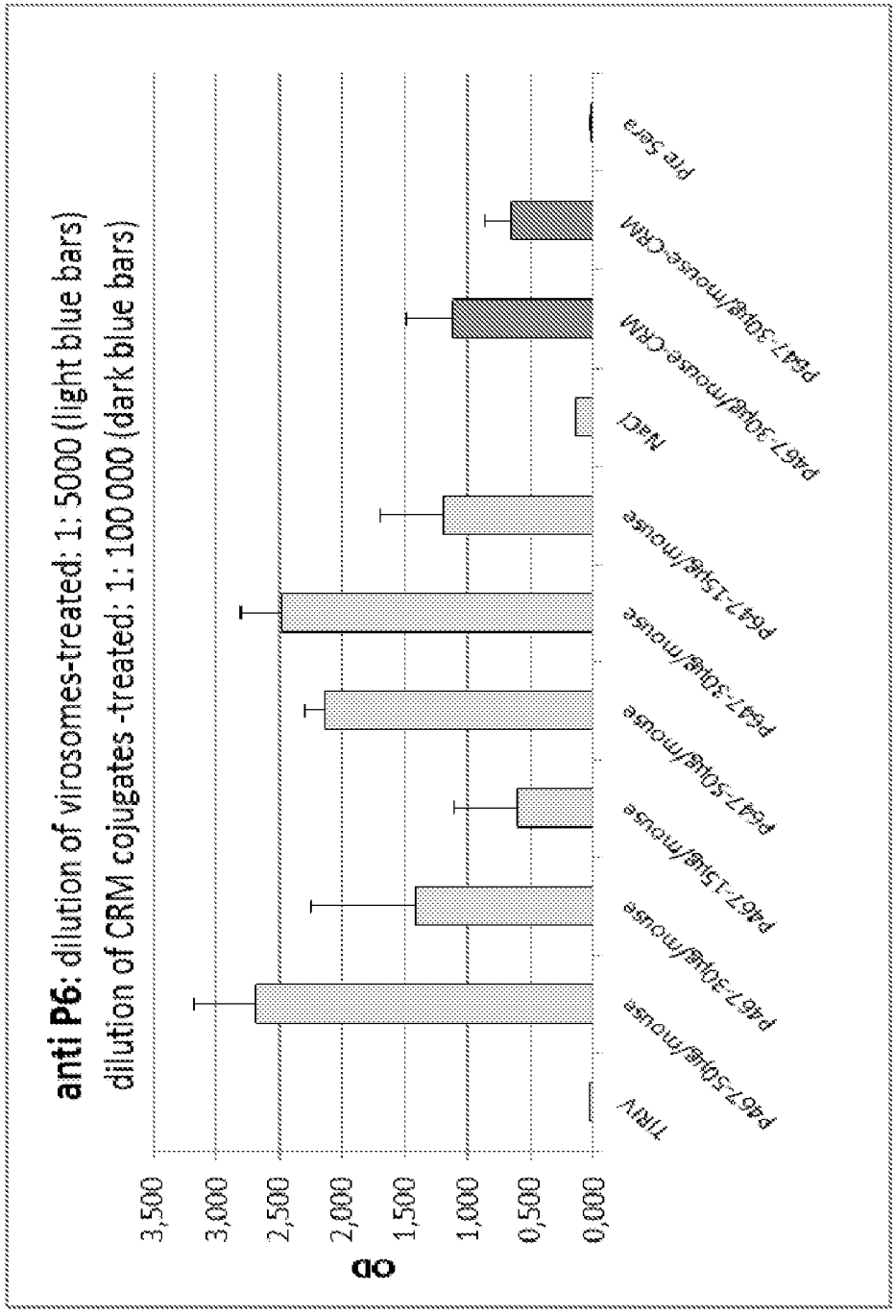
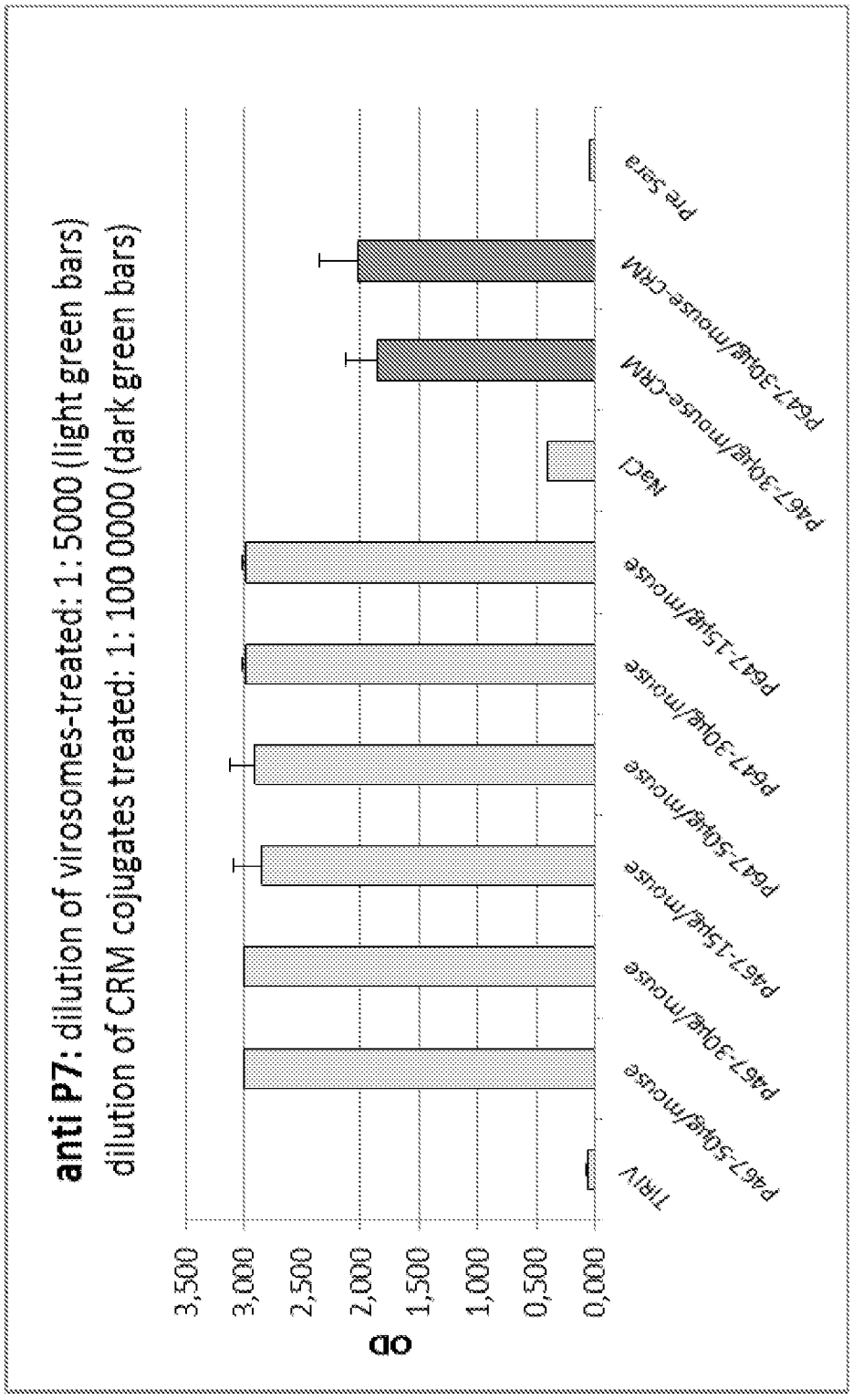

anti P6: dilution of virosomes-treated: 1: 5000 (light blue bars)
dilution of CRM cojugates -treated: 1: 100 000 (dark blue bars)
OD
3,500
3,000
2,500
2,000
1,500
1,000
0,500
0,000
TRIV
p467-50ug/mouse
p467-30ug/mouse
p467-15ug/mouse
p647-50ug/mouse
p647-30ug/mouse
p647-15ug/mouse
NaCl
p467-30ug/mouse-CRM
p647-30ug/mouse-CRM
pre sera

FIGURE 2

FIGURE 3


anti P7: dilution of virosomes-treated: 1: 5000 (light green bars)
dilution of CRM cojugates treated: 1: 100 0000 (dark green bars)
OD
3,500
3,000
2,500
2,000
1,500
1,000
0,500
0,000
TRIV
P467-50µg/mouse
P467-30µg/mouse
P467-15µg/mouse
P647-50µg/mouse
P647-30µg/mouse
P647-15µg/mouse
NaCl
P467-30µg/mouse-CRM
P647-30µg/mouse-CRM
Pre Sera

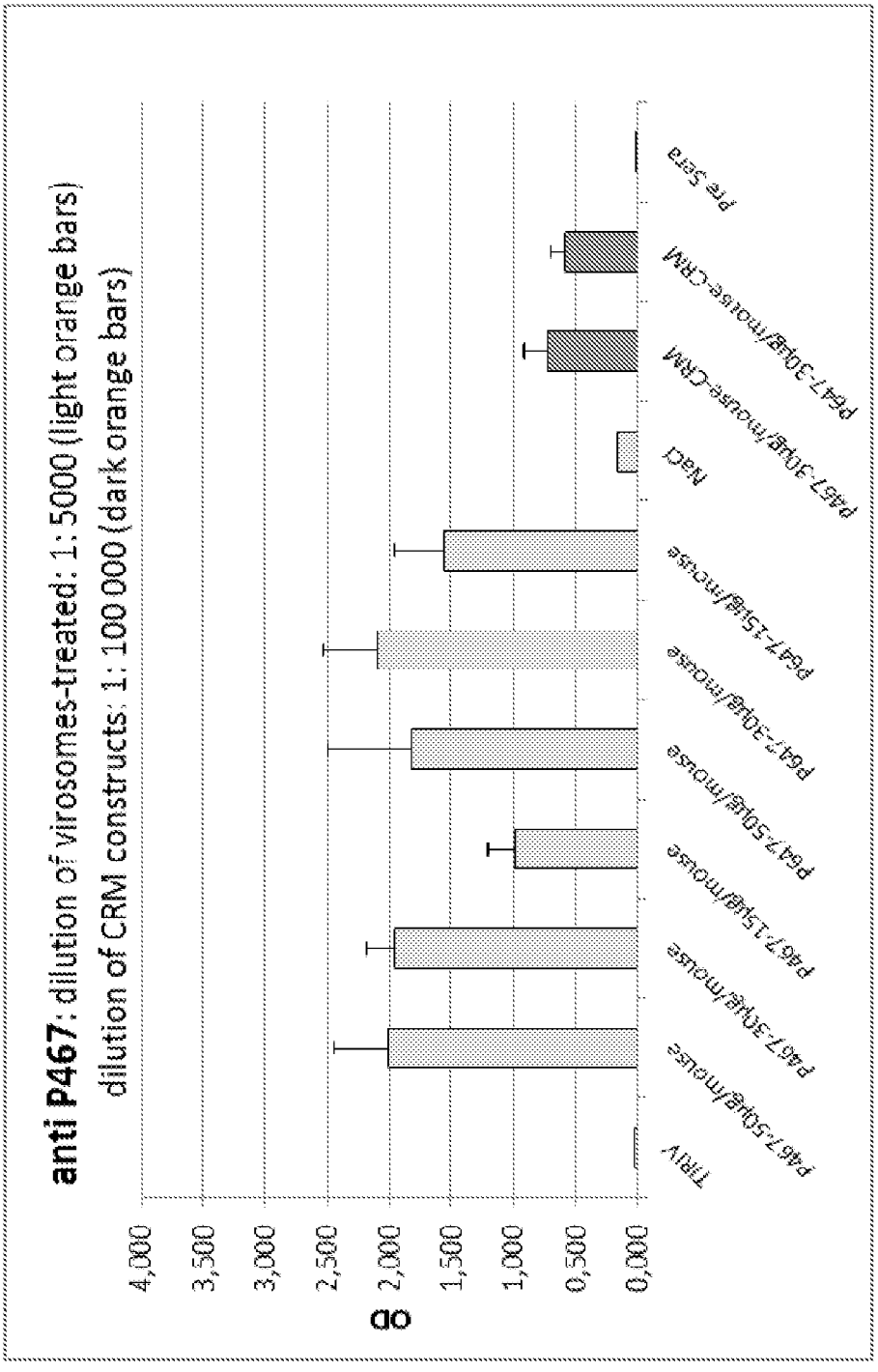
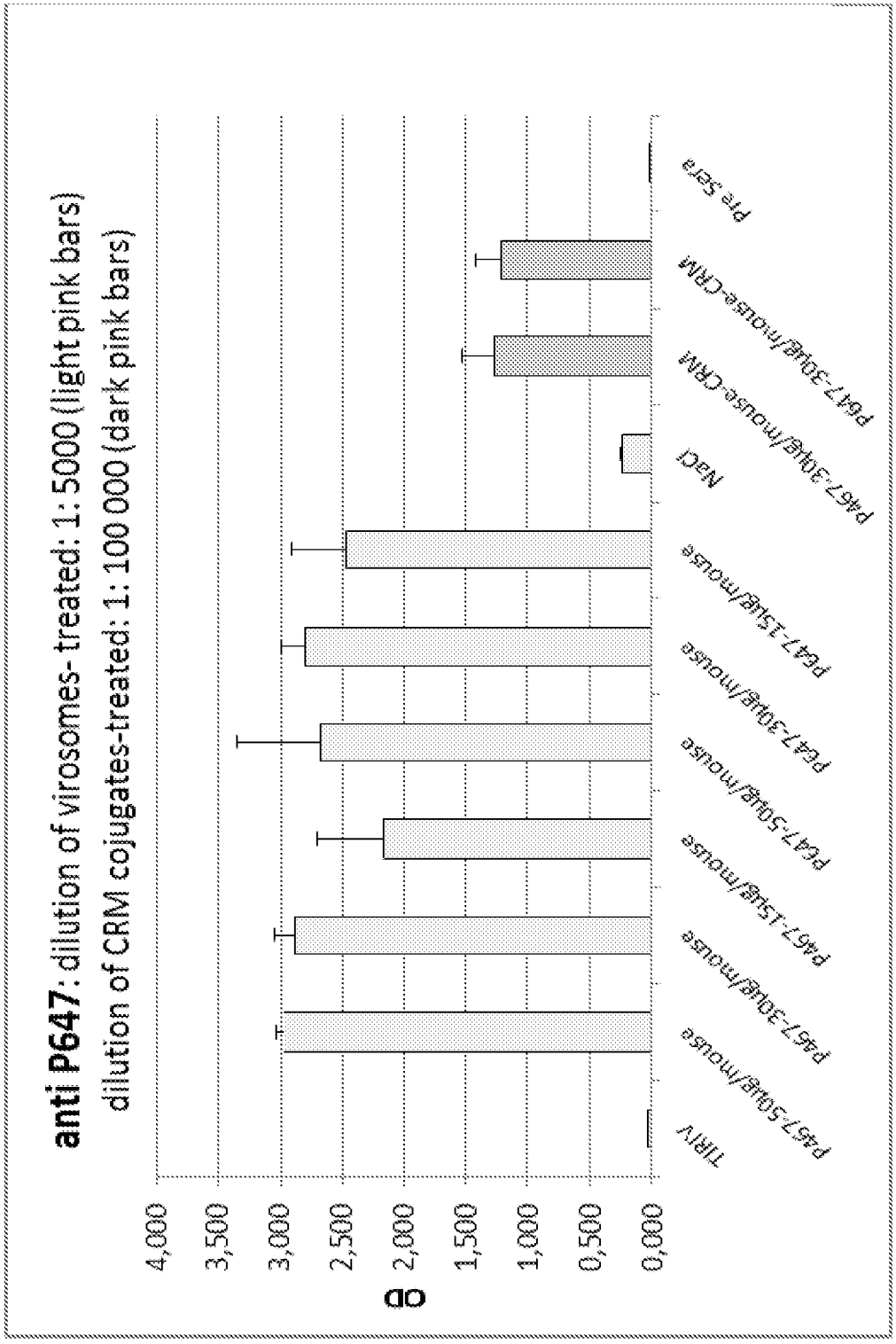

anti P467: dilution of virosomes-treated: 1: 5000 (light orange bars)
dilution of CRM constructs: 1: 100 000 (dark orange bars)
OD
4,000
3,500
3,000
2,500
2,000
1,500
1,000
0,500
0,000
TRIV
P467-5Oug/mouse
P467-30ug/mouse
P467-15ug/mouse
P647-50ug/mouse
P647-30ug/mouse
P647-15ug/mouse
NaCl
P467-30ug/mouse-CRM
P647-30ug/mouse-CRM
Pre Sera

FIGURE 4

FIGURE 5


anti P647: dilution of virosomes- treated: 1: 5000 (light pink bars)
dilution of CRM cojugates-treated: 1: 100 000 (dark pink bars)
OD
4,000
3,500
3,000
2,500
2,000
1,500
1,000
0,500
0,000
TIRIV
P647-50µg/mouse
P647-30µg/mouse
P647-15µg/mouse
P647-5µg/mouse
P647-3µg/mouse
P647-1,5µg/mouse
NaCl
P647-30µg/mouse-CRM
P647-30µg/mouse-CRM
Pre Sera

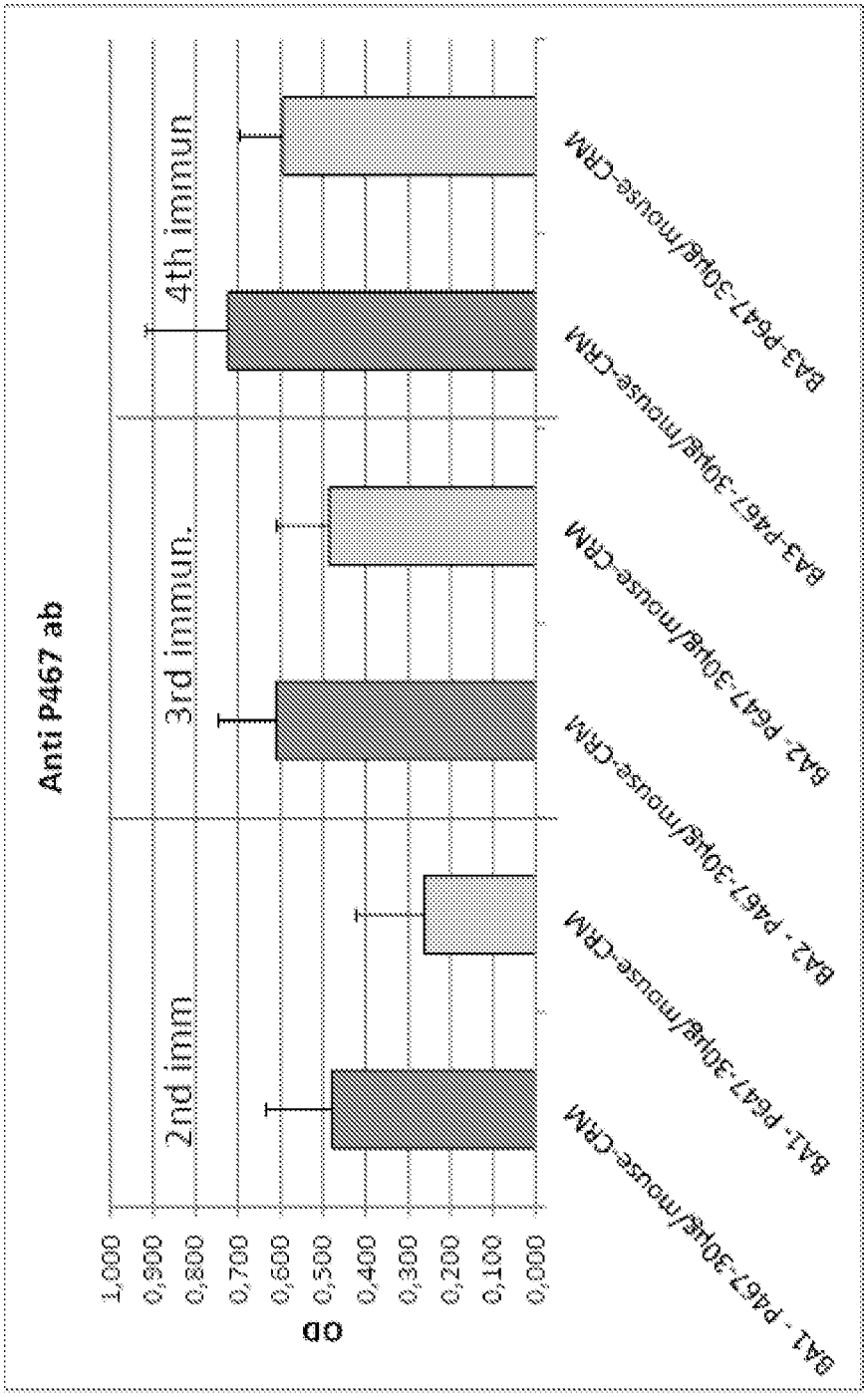

Anti P467 ab
OD
1,000
0,900
0,800
0,700
0,600
0,500
0,400
0,300
0,200
0,100
0,000
2nd imm
3rd immun.
4th immun
BA1 - p467-30ug/mouse-CPM
BA1- p647-30ug/mouse-CPM
BA2 - p467-30ug/mouse-CPM
BA2- p647-30ug/mouse-CPM
BA3-p467-30ug/mouse-CPM
BA3-p647-30ug/mouse-CPM

FIGURE 6

FIGURE 7

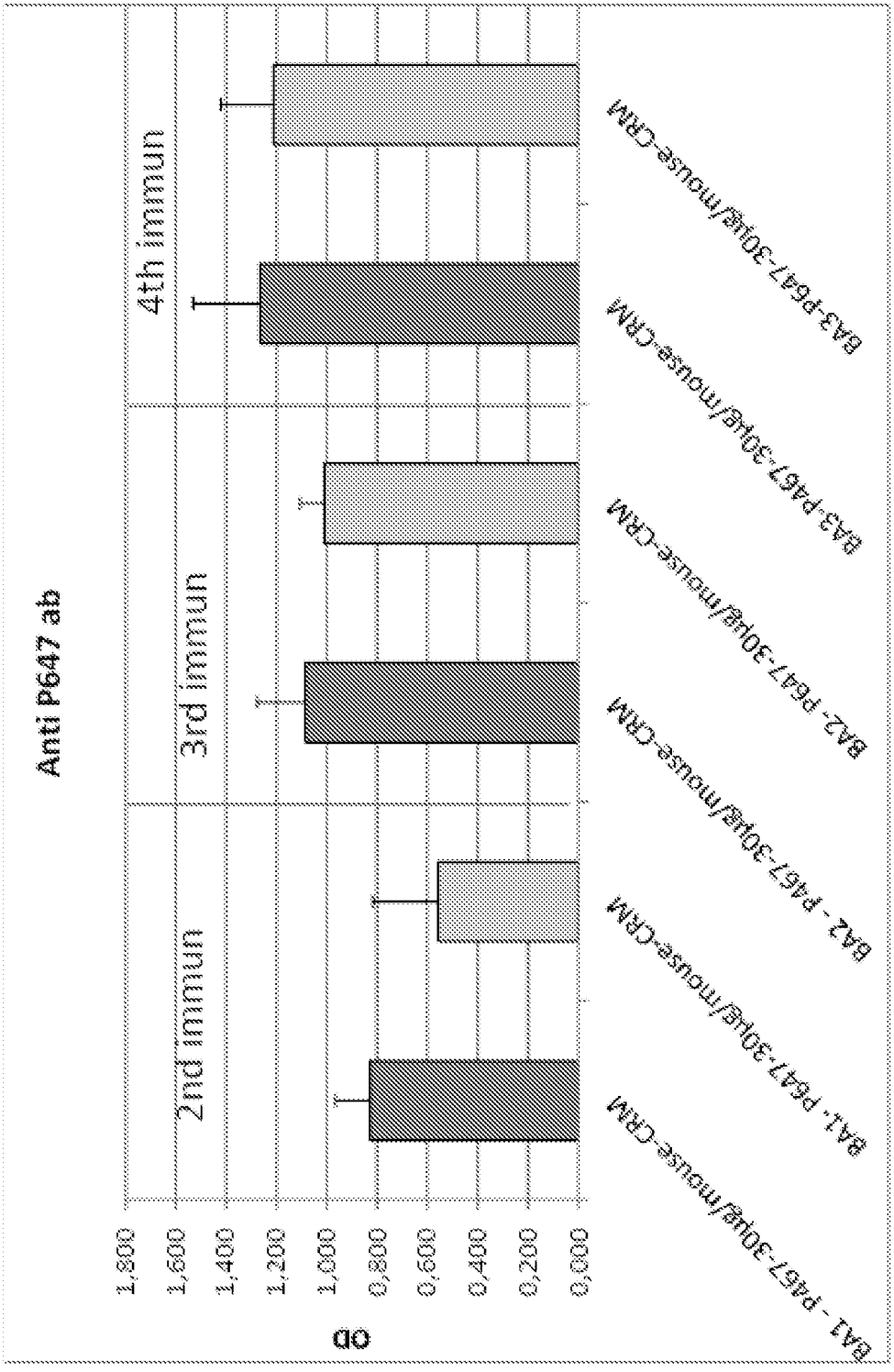

Anti P647 ab
OD
1,800
1,600
1,400
1,200
1,000
0,800
0,600
0,400
0,200
0,000
2nd immun
3rd immun
4th immun
BA1 - P647-30ug/mouse-CRM
BA1- P647-30ug/mouse-CRM
BA2 - P647-30ug/mouse-CRM
BA2- P647-30ug/mouse-CRM
BA3-P647-30ug/mouse-CRM
BA3-P647-30ug/mouse-CRM

FIGURE 8

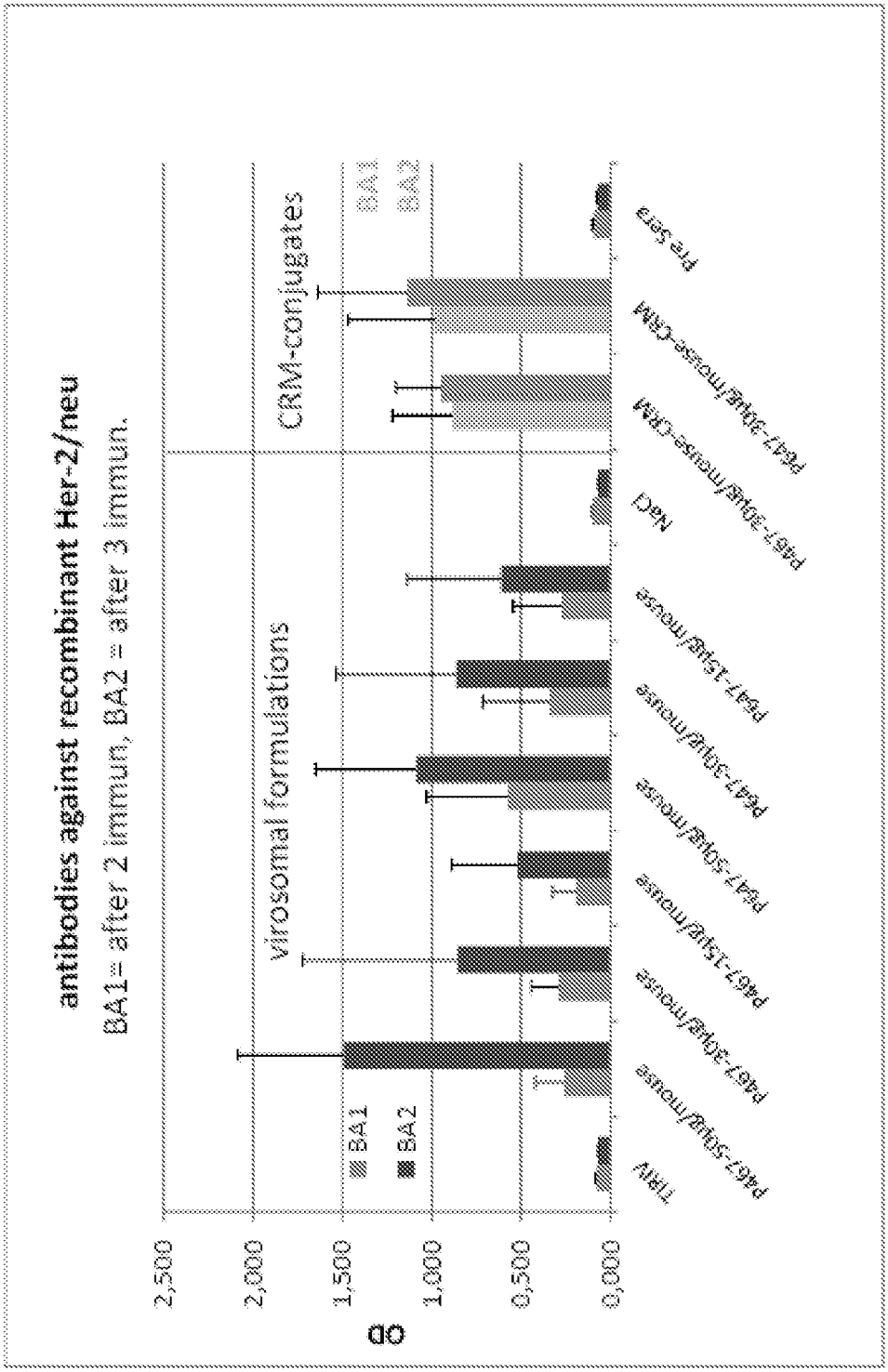

antibodies against recombinant Her-2/neu
BA1= after 2 immun, BA2 = after 3 immun.
virosomal formulations
CRM-conjugates
BA1
BA2
OD
2,500
2,000
1,500
1,000
0,500
0,000
naive/mouse
pAd7-50µg/mouse
pAd7-30µg/mouse
pAd7-15µg/mouse
pAd7-50µg/mouse
pAd7-30µg/mouse
pAd7-15µg/mouse
NaCl
pAd7-30µg/mouse-CRM
pAd7-30µg/mouse-CRM
Pre Sera

FIGURE 9

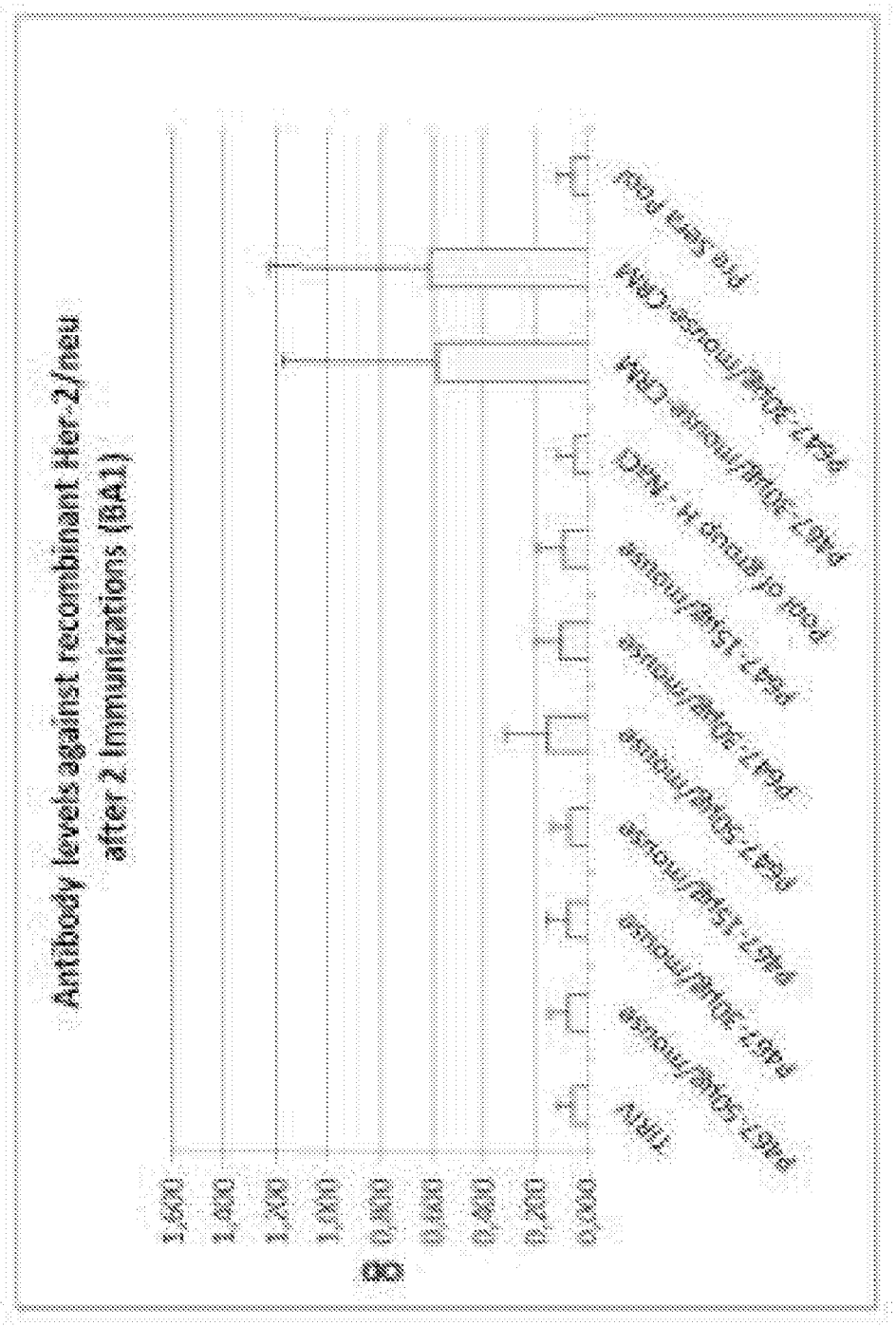

Antibody levels against recombinant Her-2/neu
after 2 Immunizations (BA1)
OD
1,600
1,400
1,200
1,000
0,800
0,600
0,400
0,200
0,000
naïve
pAd37-Spike/mouse
pAd37-Spike/mouse
pAd37-Spike/mouse

FIGURE 10

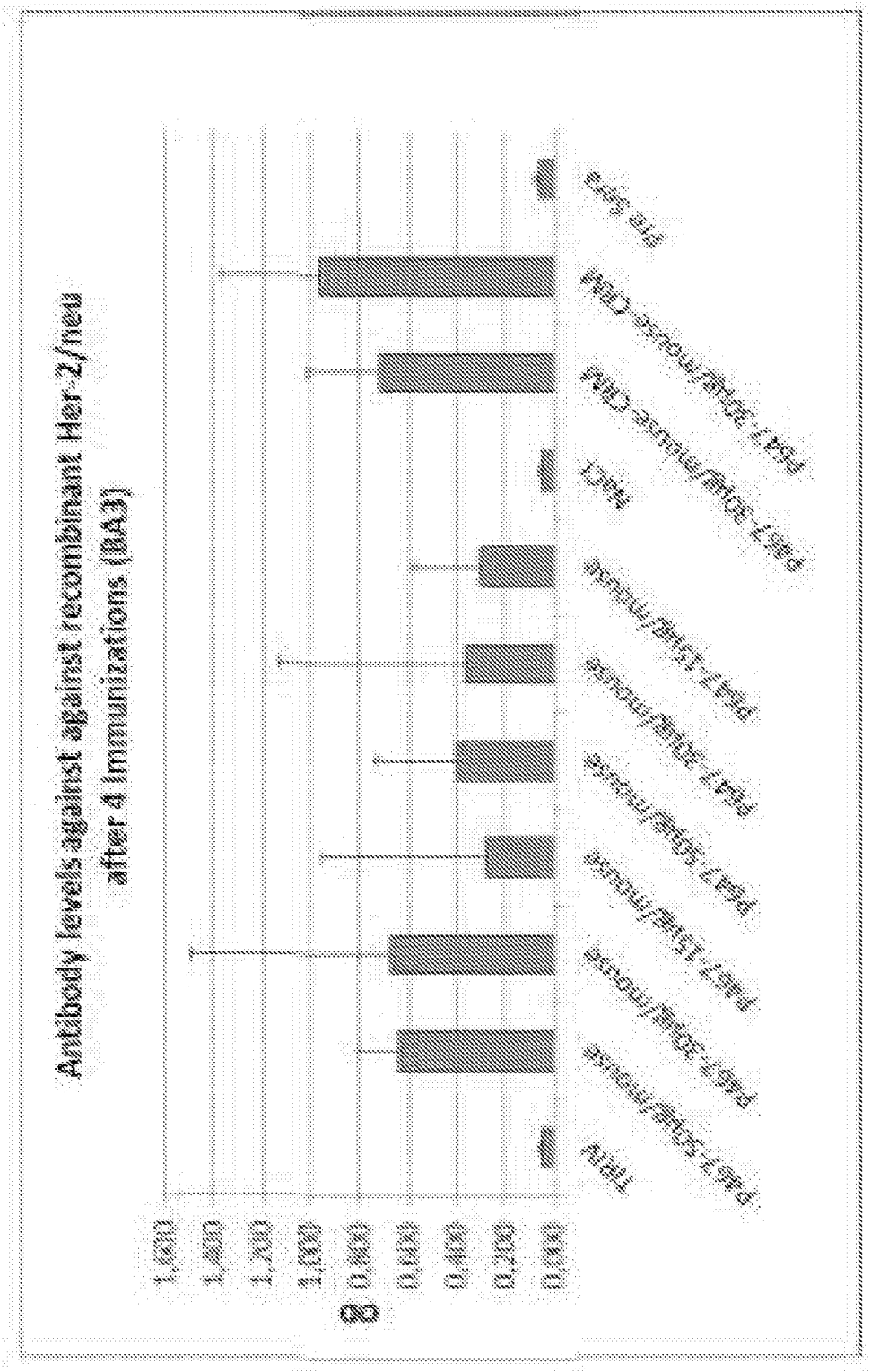

Antibody levels against against recombinant Her-2/neu
after 4 immunizations (BA3)
OD
1,600
1,400
1,200
1,000
0,800
0,600
0,400
0,200
0,000
pNGVL4a-Sig/E7(detox)/HPV16/HPV18
pNGVL4a-Sig/E7(detox)/HPV16/HPV18-CRM
pNGVL4a-Sig/E7(detox)/HPV16/HPV18-CRM
pNGVL4a-Sig/E7(detox)/HPV16/HPV18
pNGVL4a-Sig/E7(detox)/HPV16/HPV18
pNGVL4a-Sig/E7(detox)/HPV16/HPV18
pNGVL4a-Sig/E7(detox)/HPV16/HPV18
pNGVL4a-Sig/E7(detox)/HPV16/HPV18
PBS
Naïve

FIGURE 11

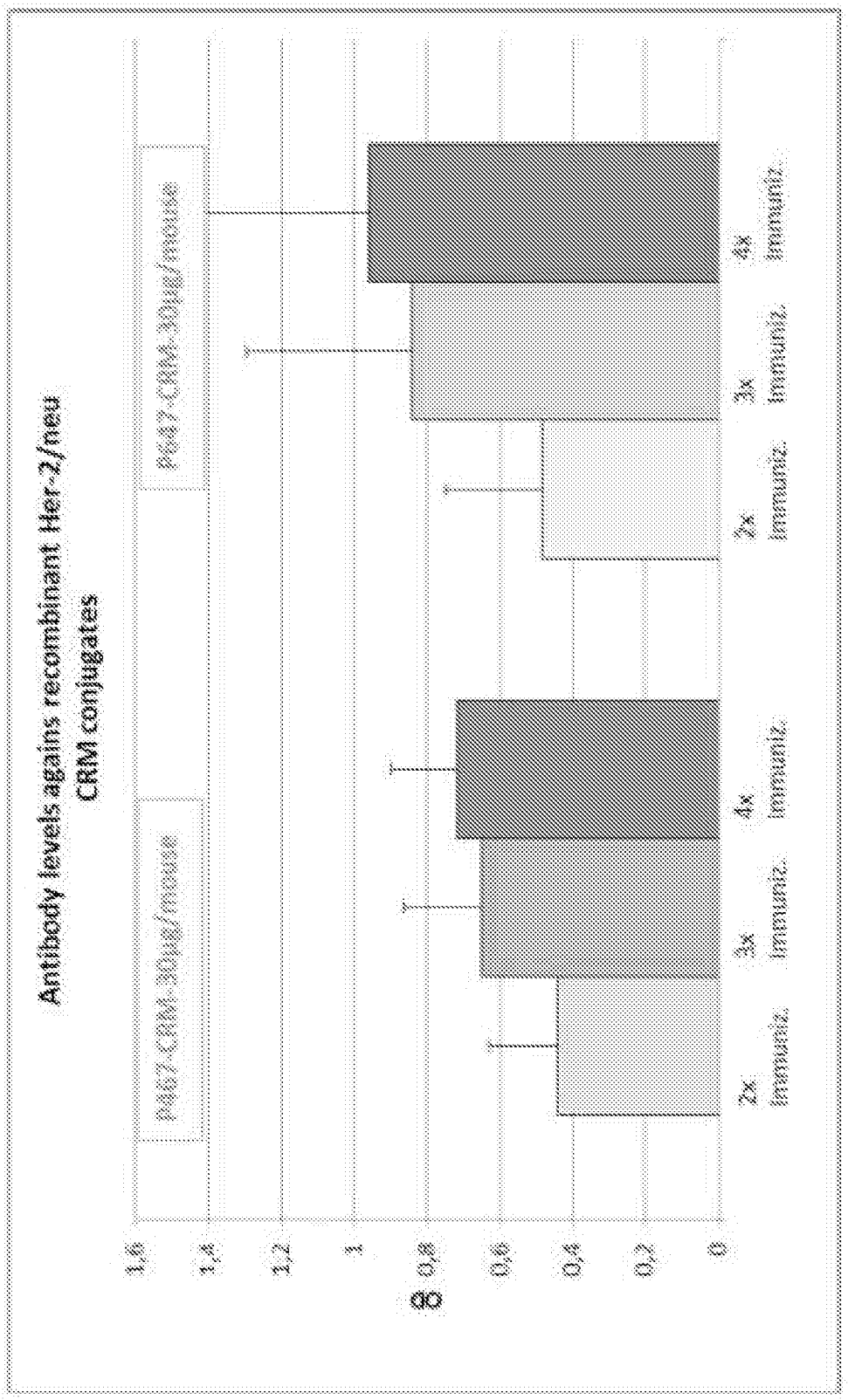

Antibody levels agains recombinant Her-2/neu
CRM conjugates
P467-CRM- 30ug/mouse
P647-CRM- 30ug/mouse
OD
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
2x immuniz.
3x immuniz.
4x immuniz.
2x immuniz.
3x immuniz.
4x immuniz.

FIGURE 12

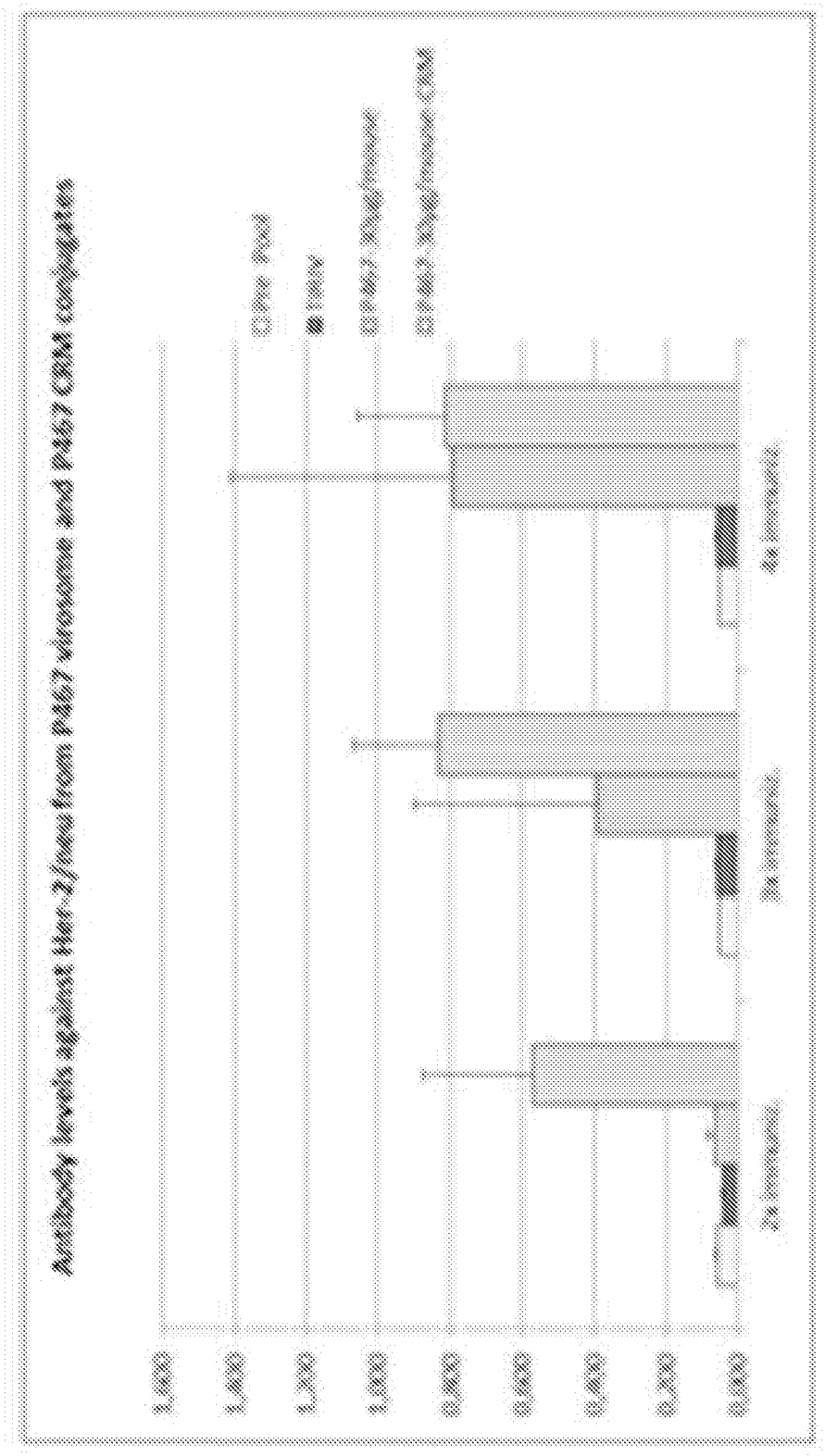
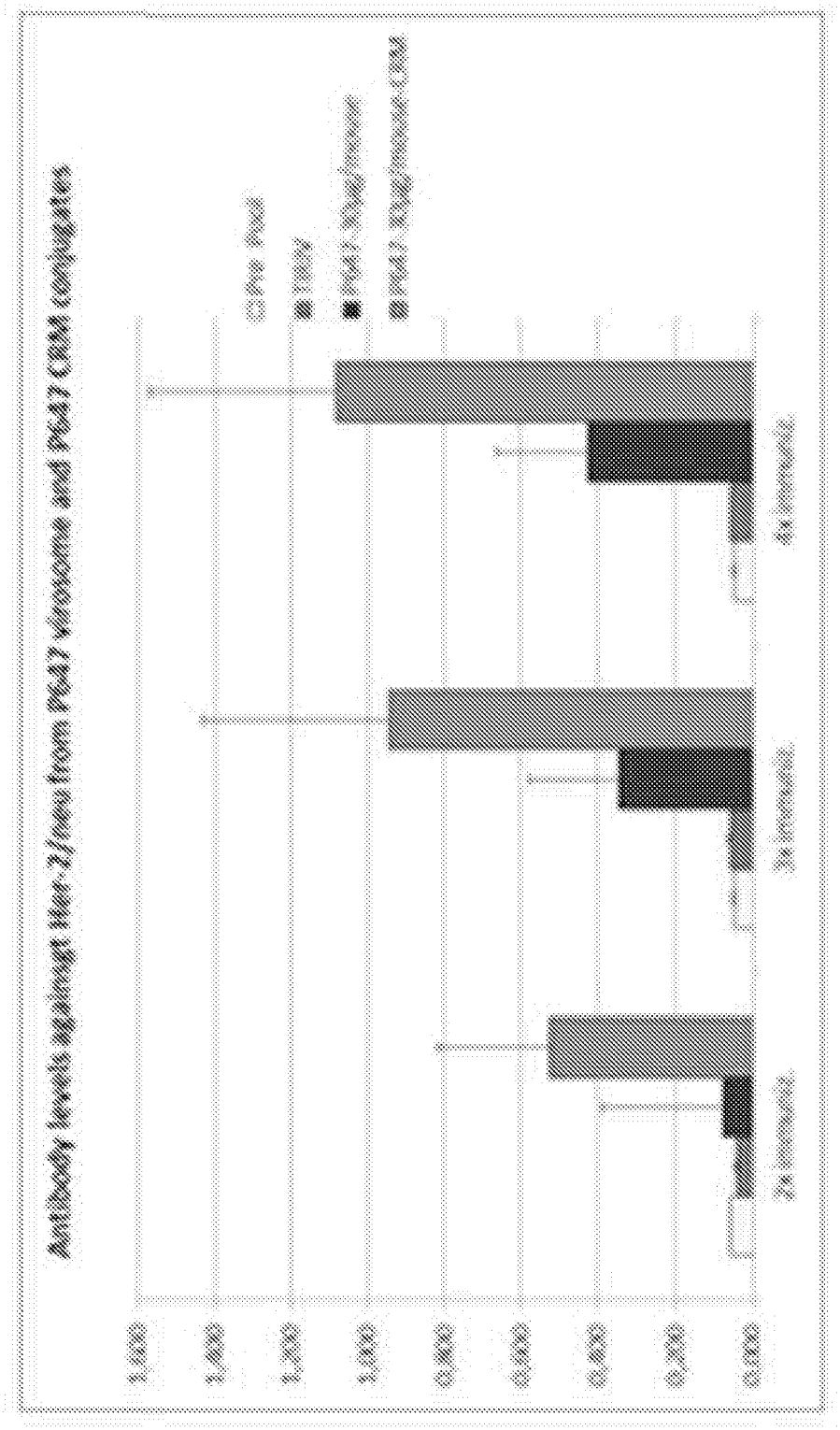

Antibody levels against Hsv-2/mu from PA67 virosome and PA67 CBM conjugates
CBM PA67
CBM2 Polyhistidine
CBM2 Polyhistidine CBM
1:400
1:800
1:1600
1:3200
1:6400
1:12800
1:25600
1:51200
2x immunis.
3x immunis.
4x immunis.

FIGURE 13


Antibody levels against Her-2/neu from PG17 vaccine and PG17 GM4 conjugates
Pre bleed
Day
PG17 alone/mouse
PG17 conjugate GM4
1,600
1,400
1,200
1,000
0,800
0,600
0,400
0,200
0,000
2o immunis
3o immunis
4o immunis

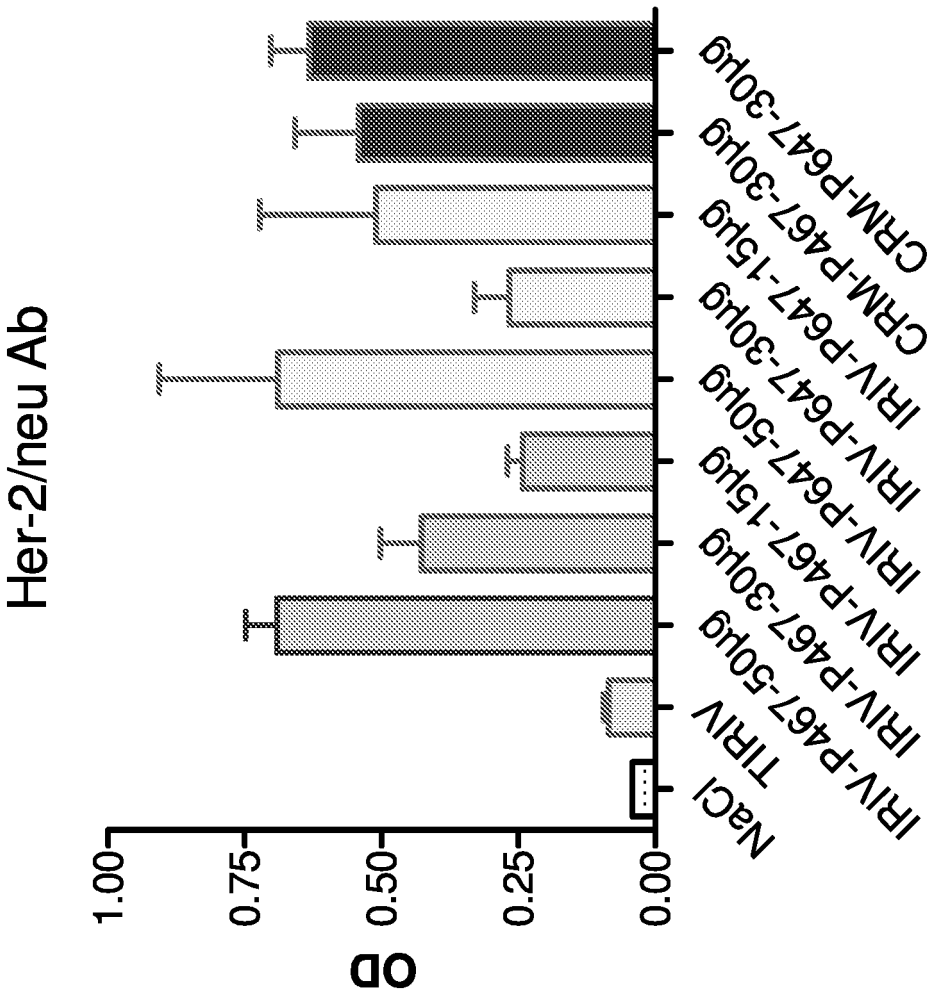

Her-2/neu Ab
OD
1.00
0.75
0.50
0.25
0.00
NaCl
TIRIV
IRIV-P467-50µg
IRIV-P467-30µg
IRIV-P467-15µg
IRIV-P647-50µg
IRIV-P647-30µg
IRIV-P647-15µg
CRM-P467-30µg
CRM-P647-30µg

FIGURE 14

FIGURE 15A

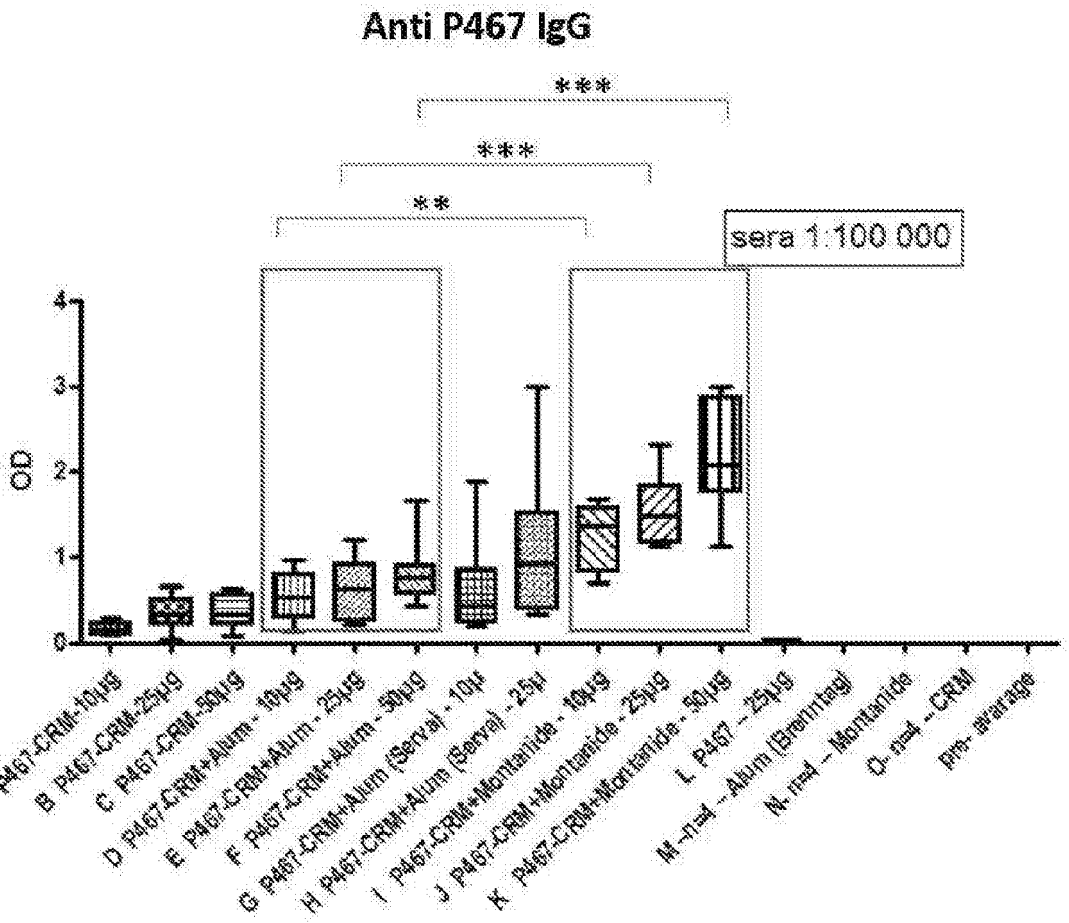

Anti P467 IgG
***
***
**
sera 1:100 000
OD
0
1
2
3
4
A P467-CRM-10µg
B P467-CRM-25µg
C P467-CRM-50µg
D P467-CRM+Alum - 10µg
E P467-CRM+Alum - 25µg
F P467-CRM+Alum - 50µg
G P467-CRM+Alum (Serval) - 10µ
H P467-CRM+Alum (Serval) - 25µ
I P467-CRM+Montanide - 10µg
J P467-CRM+Montanide - 25µg
K P467-CRM+Montanide - 50µg
L P467 – 25µg
M –n=4 – Alum (Brenntag)
N- n=4 – Montanide
O- n=4 – CRM
pre- sera

FIGURE 15B

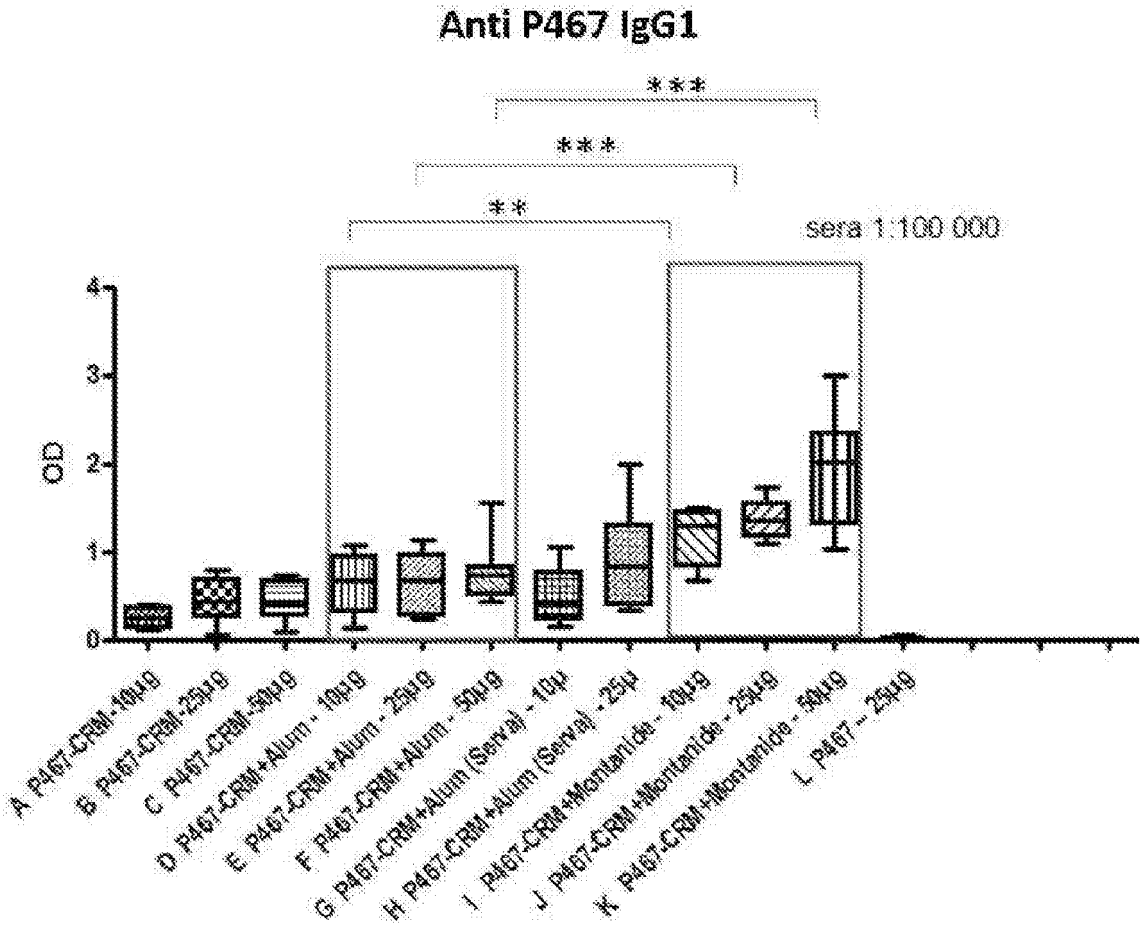

Anti P467 IgG1
***
***
**
sera 1:100 000
OD
0
1
2
3
4
A P467-CRM-10µg
B P467-CRM-25µg
C P467-CRM-50µg
D P467-CRM+Alum - 10µg
E P467-CRM+Alum - 25µg
F P467-CRM+Alum - 50µg
G P467-CRM+Alum (Serva) - 10µ
H P467-CRM+Alum (Serva) - 25µ
I P467-CRM+Montanide - 10µg
J P467-CRM+Montanide - 25µg
K P467-CRM+Montanide - 50µg
L P467 - 25µg

FIGURE 15C

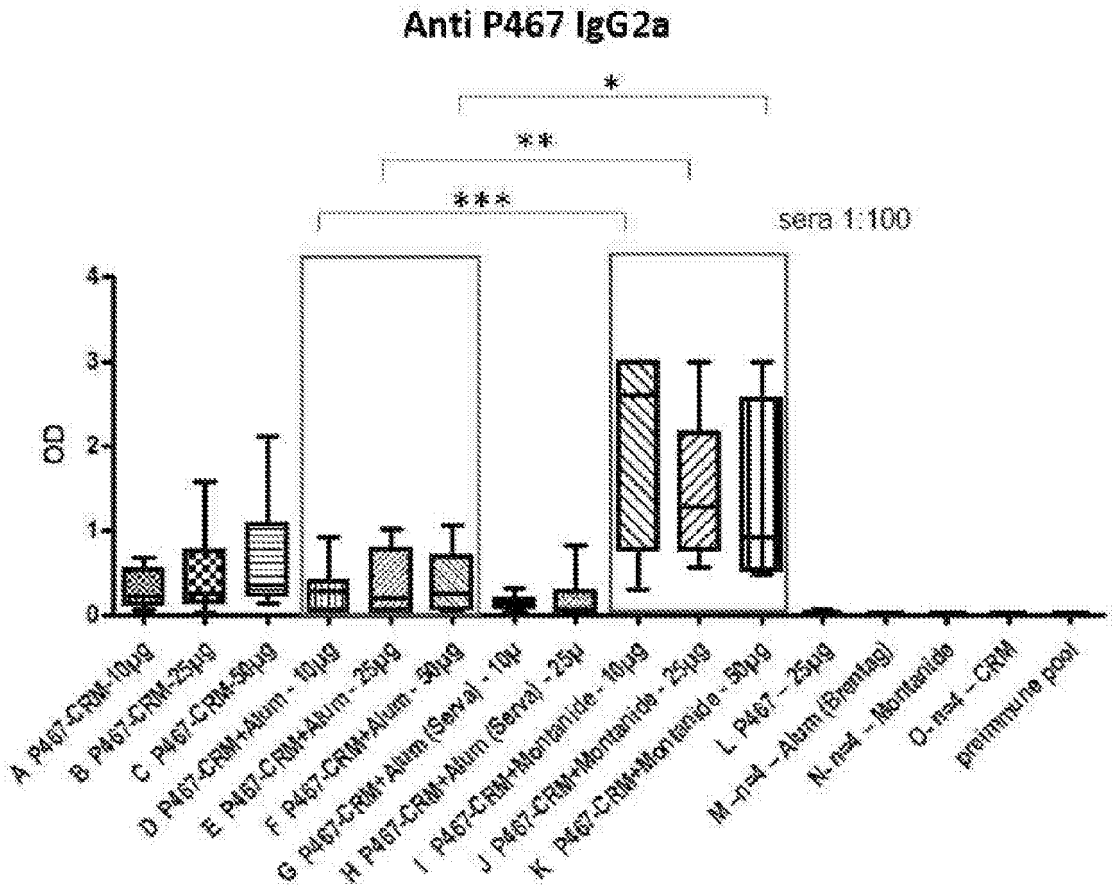

Anti P467 IgG2a
*
**
***
sera 1:100
OD
0
1
2
3
4
A P467-CRM-10µg
B P467-CRM-25µg
C P467-CRM-50µg
D P467-CRM+Alum - 10µg
E P467-CRM+Alum - 25µg
F P467-CRM+Alum - 50µg
G P467-CRM+Alum (Serva) - 10µ
H P467-CRM+Alum (Serva) - 25µ
I P467-CRM+Montanide - 10µg
J P467-CRM+Montanide - 25µg
K P467-CRM+Montanide - 50µg
L P467 - 25µg
M -n=4 – Alum (Brenntag)
N- n=4 – Montanide
O- n=4 – CRM
premmune pool

FIGURE 16A

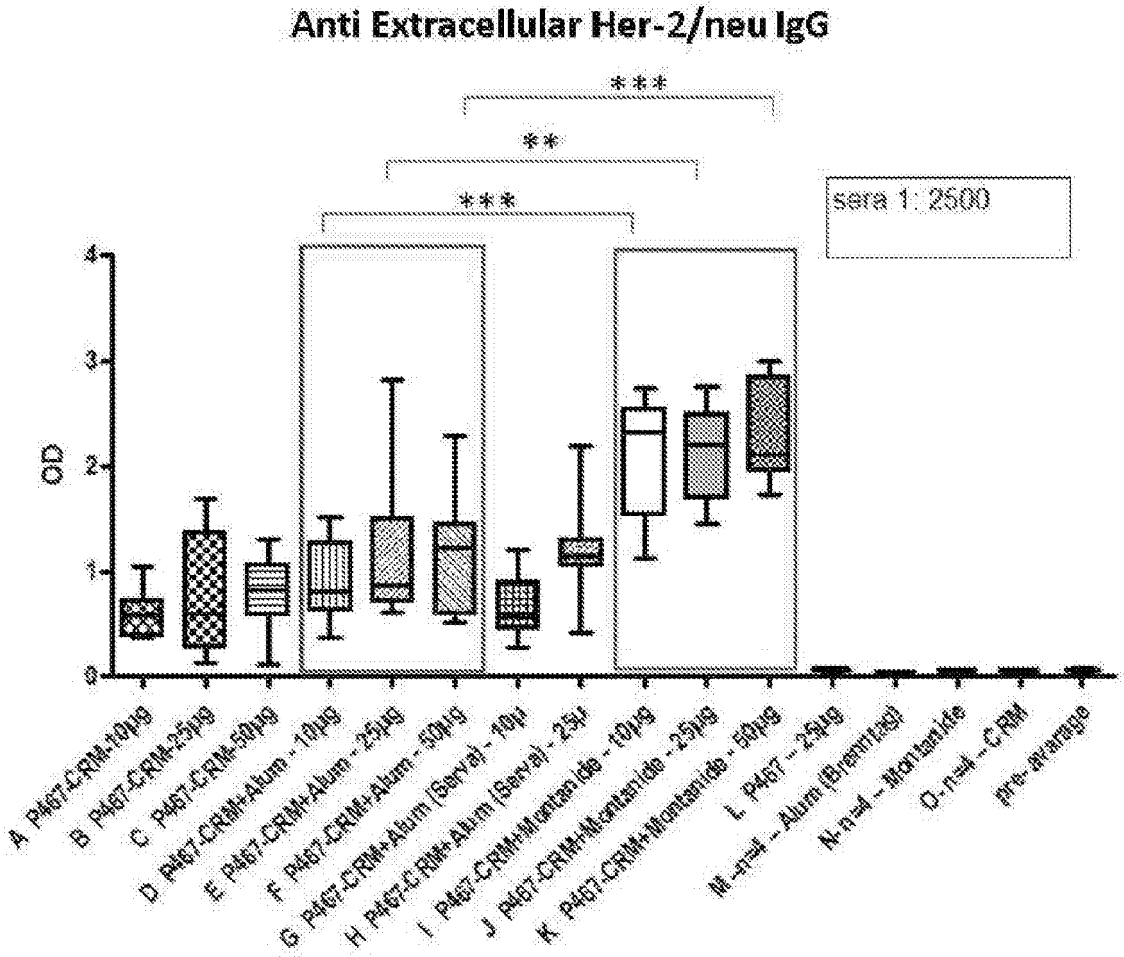

Anti Extracellular Her-2/neu IgG
***
**
***
sera 1: 2500
OD
0
1
2
3
4
A P467-CRM-10µg
B P467-CRM-25µg
C P467-CRM-50µg
D P467-CRM+Alum - 10µg
E P467-CRM+Alum - 25µg
F P467-CRM+Alum - 50µg
G P467-CRM+Alum (Serval) - 10µ
H P467-CRM+Alum (Serval) - 25µ
I P467-CRM+Montanide - 10µg
J P467-CRM+Montanide - 25µg
K P467-CRM+Montanide - 50µg
L P467 - 25µg
M n=4 - Alum (Brenntag)
N n=4 - Montanide
O n=4 - CRM
pre- arrange

FIGURE 16B

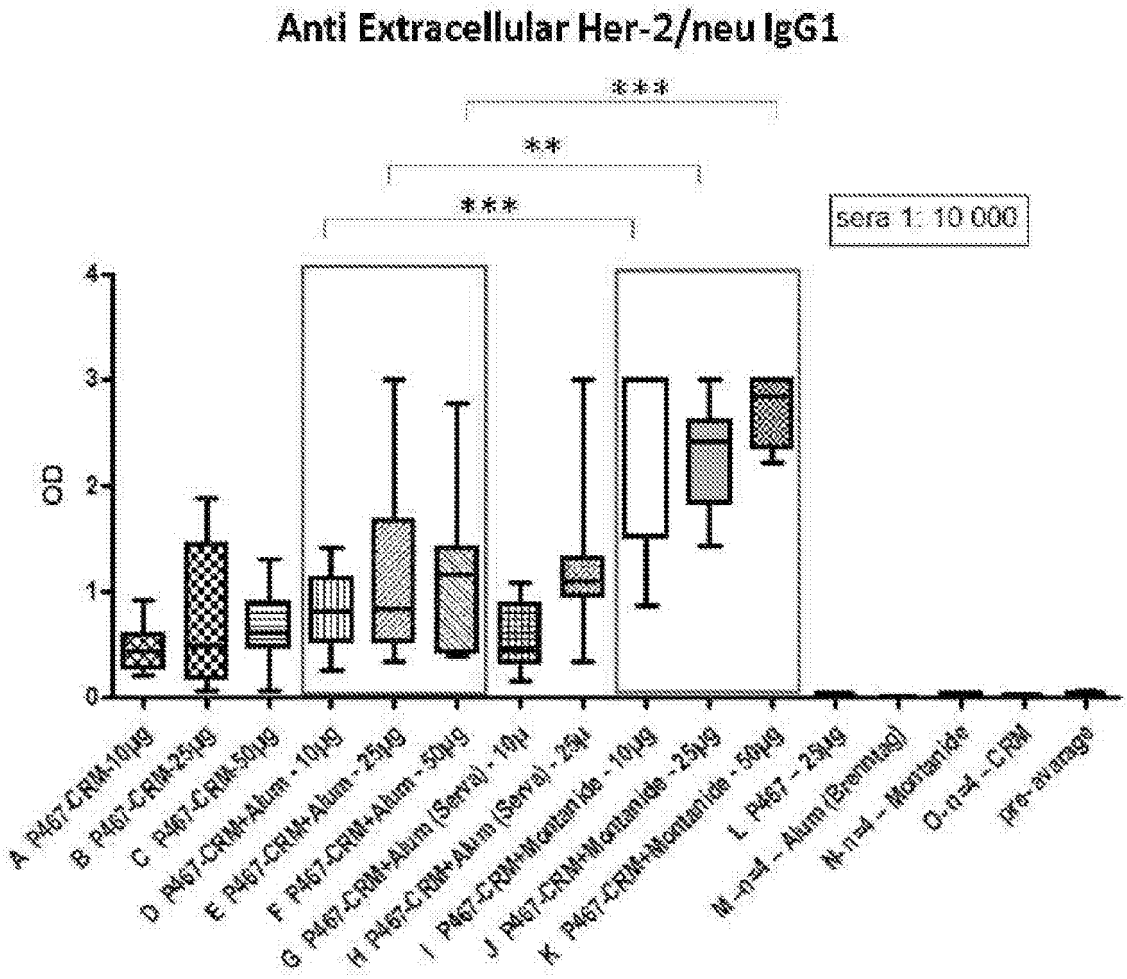

Anti Extracellular Her-2/neu IgG1
***
**
***
sera 1: 10 000
OD
0
1
2
3
4
A P467-CRM-10µg
B P467-CRM-25µg
C P467-CRM-50µg
D P467-CRM+Alum - 10µg
E P467-CRM+Alum - 25µg
F P467-CRM+Alum - 50µg
G P467-CRM+Alum (Serva) - 10µ
H P467-CRM+Alum (Serva) - 25µ
I P467-CRM+Montanide - 10µg
J P467-CRM+Montanide - 25µg
K P467-CRM+Montanide - 50µg
L P467 - 25µg
M n=4 – Alum (Brenntag)
N n=4 – Montanide
O n=4 – CRM
pre-animage

FIGURE 17A

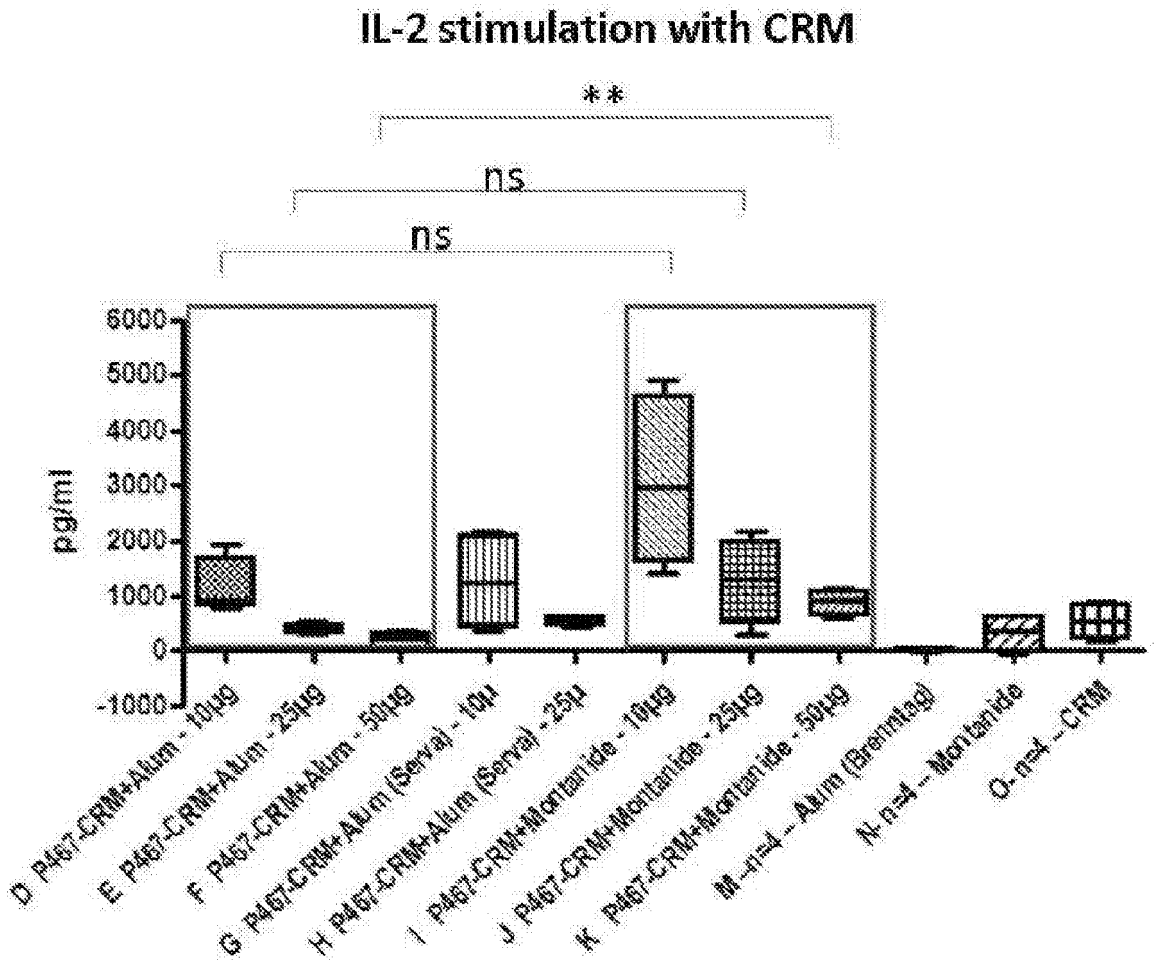

IL-2 stimulation with CRM
**
ns
ns
pg/ml
6000
5000
4000
3000
2000
1000
0
-1000
D P467-CRM+Alum - 10µg
E P467-CRM+Alum - 25µg
F P467-CRM+Alum - 50µg
G P467-CRM+Alum (Serva) - 10µ
H P467-CRM+Alum (Serva) - 25µ
I P467-CRM+Montanide - 10µg
J P467-CRM+Montanide - 25µg
K P467-CRM+Montanide - 50µg
M - n=4 -- Alum (Brenntag)
N- n=4 -- Montanide
O- n=4 -- CRM

FIGURE 17B

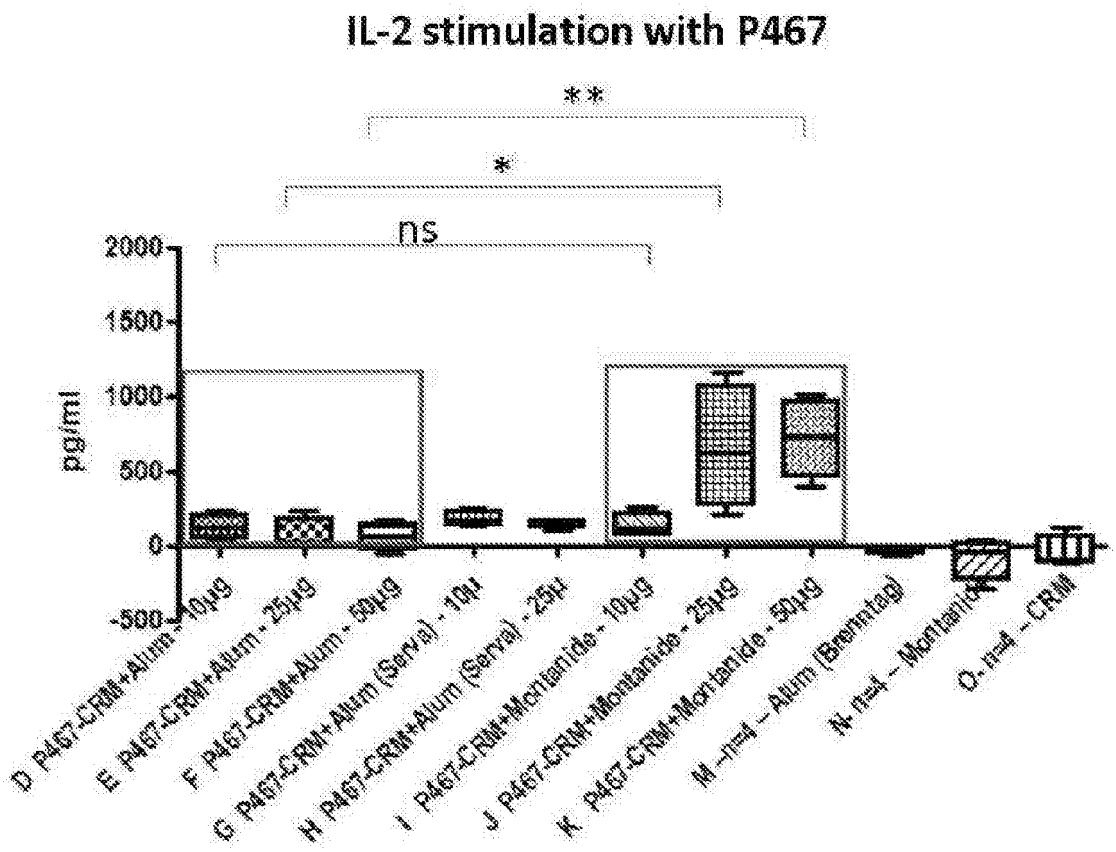

IL-2 stimulation with P467
**
*
ns
2000
1500
1000
500
0
-500
pg/ml
D P467-CRM+Alum - 10µg
E P467-CRM+Alum - 25µg
F P467-CRM+Alum - 50µg
G P467-CRM+Alum (Serva) - 10µ
H P467-CRM+Alum (Serva) - 25µ
I P467-CRM+Montanide - 10µg
J P467-CRM+Montanide - 25µg
K P467-CRM+Montanide - 50µg
M –n=4 – Alum (Brenntag)
N- n=4 – Montanide
O- n=4 – CRM

FIGURE 18A

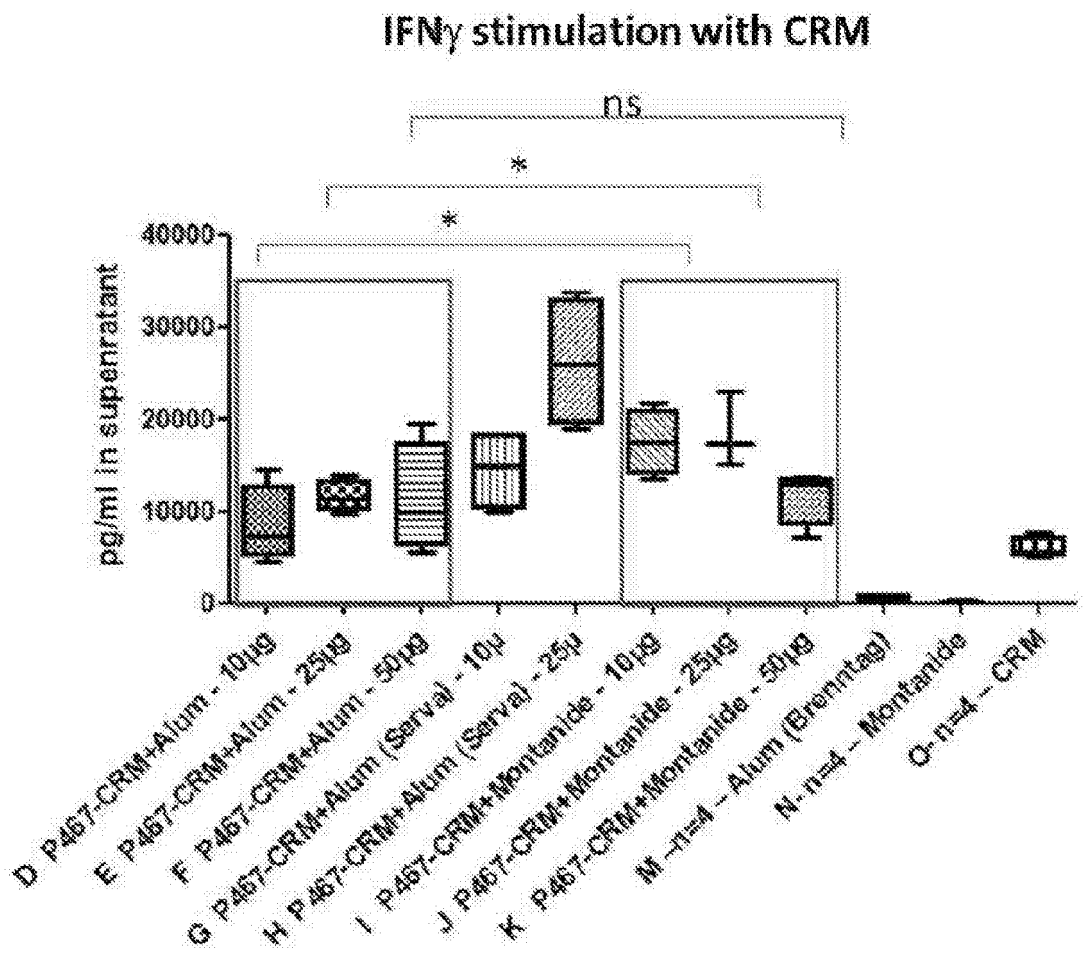

IFNγ stimulation with CRM
ns
*
*
40000
30000
20000
10000
0
pg/ml in supenratant
D P467-CRM+Alum - 10µg
E P467-CRM+Alum - 25µg
F P467-CRM+Alum - 50µg
G P467-CRM+Alum (Serva) - 10µ
H P467-CRM+Alum (Serva) - 25µ
I P467-CRM+Montanide - 10µg
J P467-CRM+Montanide - 25µg
K P467-CRM+Montanide - 50µg
M -n=4 – Alum (Brenntag)
N- n=4 – Montanide
O- n=4 – CRM

FIGURE 18B

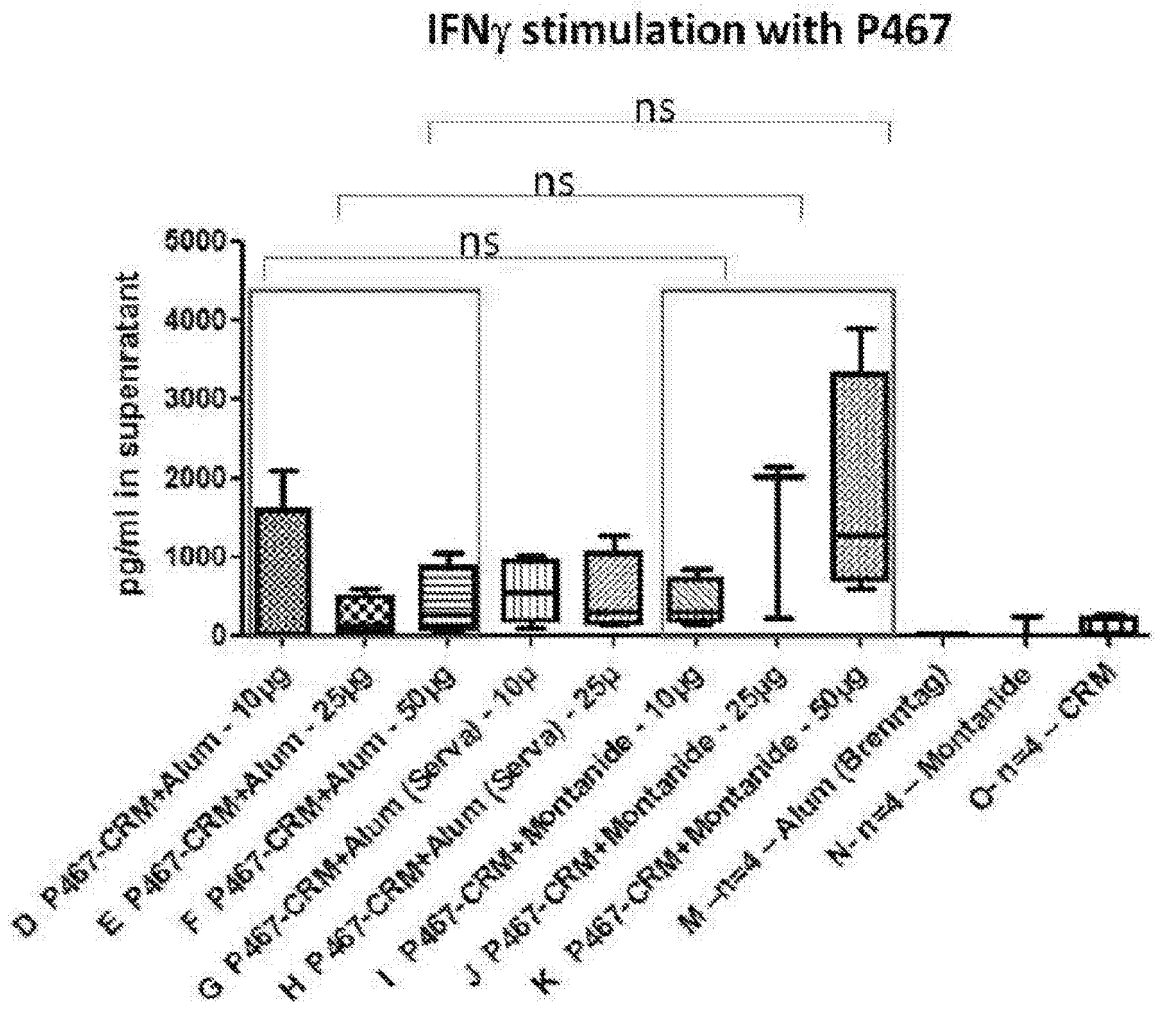

IFNγ stimulation with P467
ns
ns
ns
pg/ml in supernatant
5000
4000
3000
2000
1000
0
D P467-CRM+Alum - 10µg
E P467-CRM+Alum - 25µg
F P467-CRM+Alum - 50µg
G P467-CRM+Alum (Serva) - 10µ
H P467-CRM+Alum (Serva) - 25µ
I P467-CRM+Montanide - 10µg
J P467-CRM+Montanide - 25µg
K P467-CRM+Montanide - 50µg
M –n=4 – Alum (Brenntag)
N- n=4 – Montanide
O- n=4 – CRM

FIGURE 19A

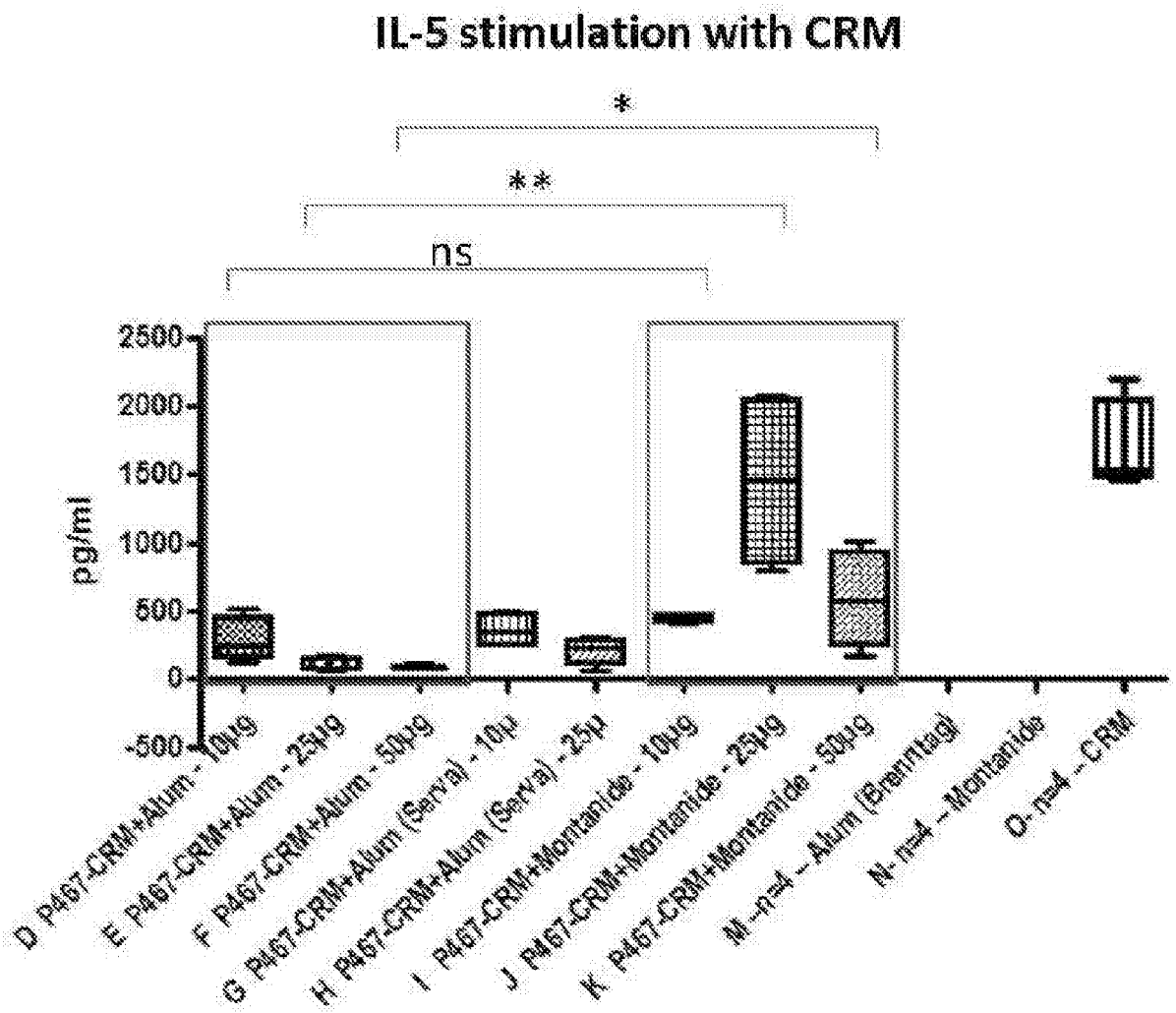

IL-5 stimulation with CRM
*
**
ns
2500
2000
1500
1000
500
0
-500
pg/ml
D P467-CRM+Alum - 10µg
E P467-CRM+Alum - 25µg
F P467-CRM+Alum - 50µg
G P467-CRM+Alum (Serva) - 10µ
H P467-CRM+Alum (Serva) - 25µ
I P467-CRM+Montanide - 10µg
J P467-CRM+Montanide - 25µg
K P467-CRM+Montanide - 50µg
M -n=4 – Alum (Brenntag)
N- n=4 – Montanide
O- n=4 – CRM

FIGURE 19B

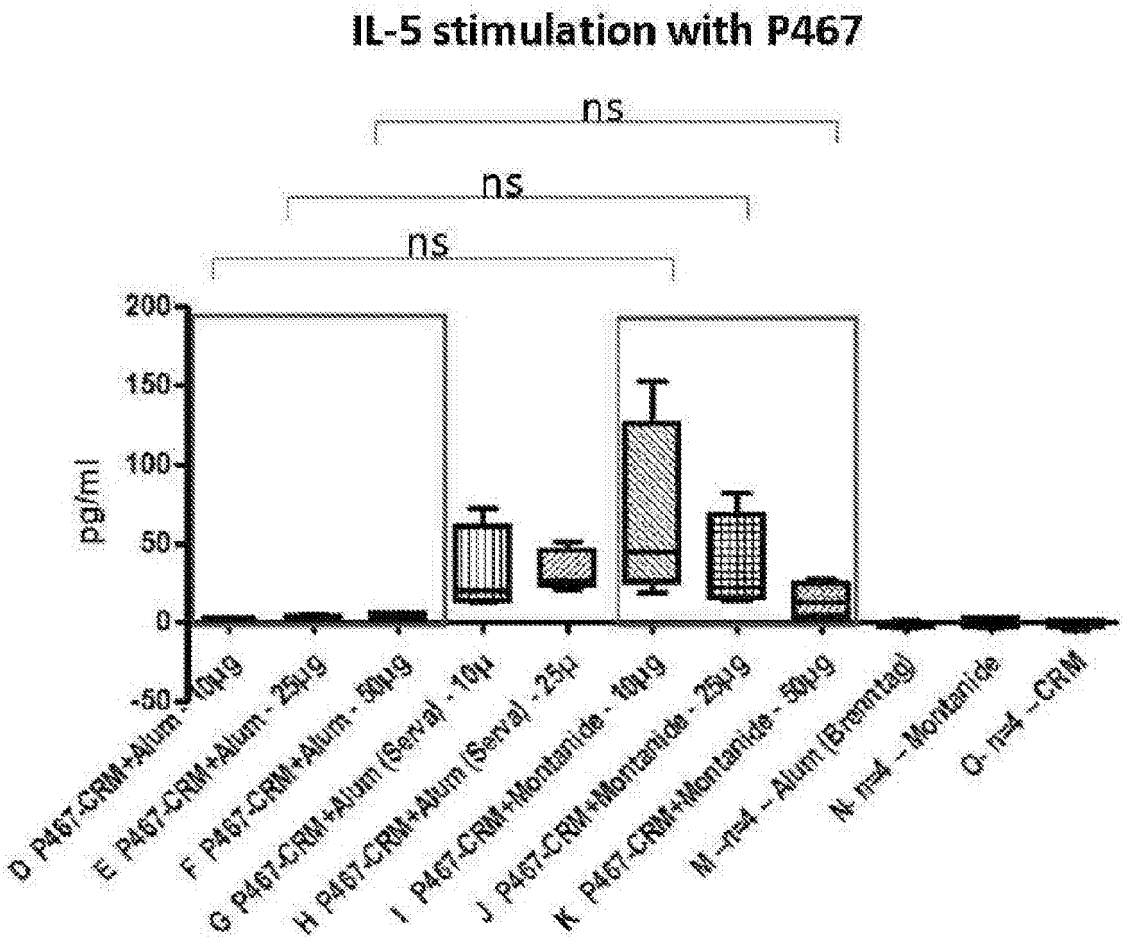

IL-5 stimulation with P467
ns
ns
ns
200
150
100
50
0
-50
pg/ml
D P467-CRM+Alum - 10µg
E P467-CRM+Alum - 25µg
F P467-CRM+Alum - 50µg
G P467-CRM+Alum (Serva) - 10µ
H P467-CRM+Alum (Serva) - 25µ
I P467-CRM+Montanide - 10µg
J P467-CRM+Montanide - 25µg
K P467-CRM+Montanide - 50µg
M –n=4 – Alum (Brenntag)
N- n=4 – Montanide
O- n=4 – CRM

FIGURE 20A

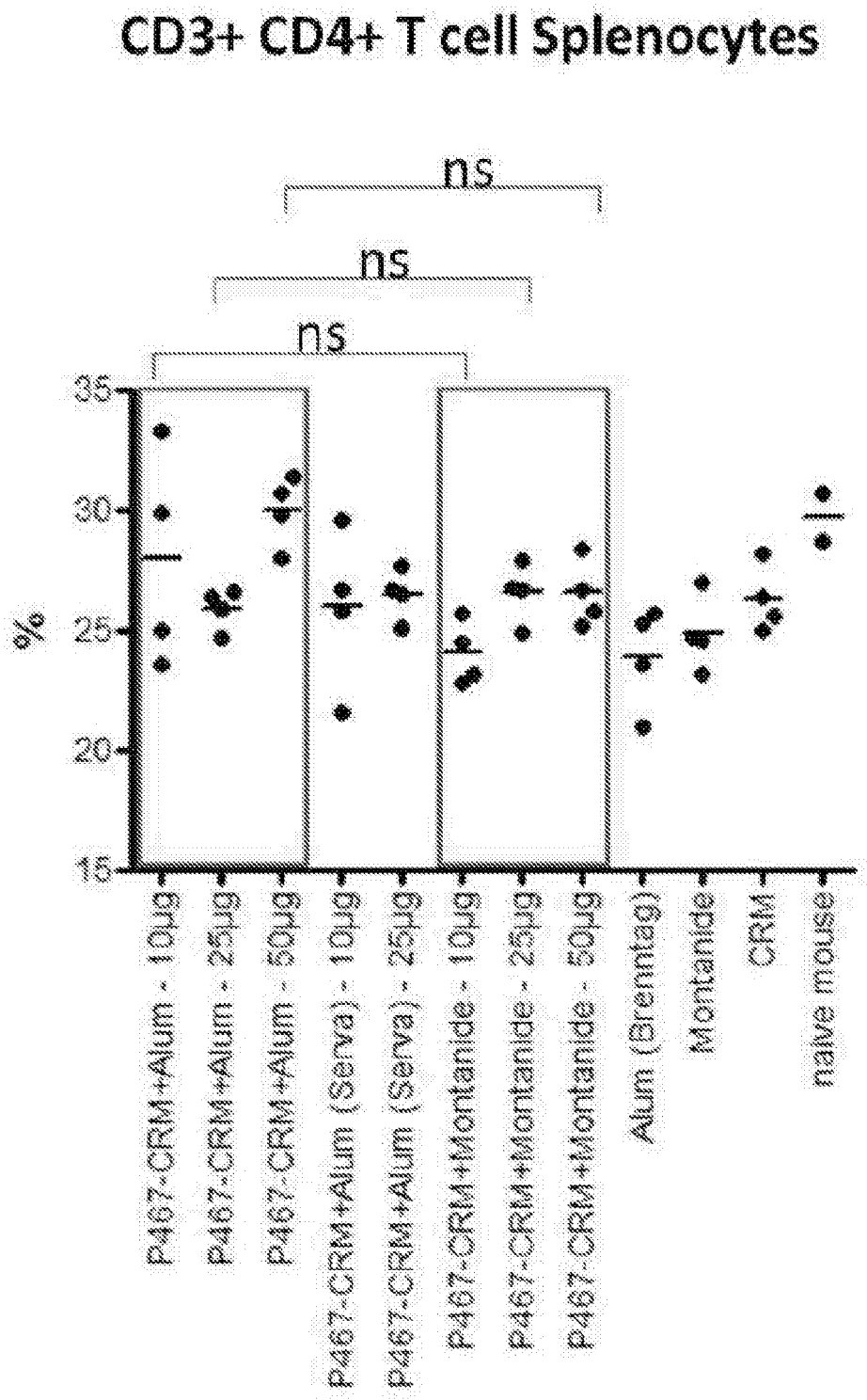

CD3+ CD4+ T cell Splenocytes
ns
ns
ns
35
30
25
20
15
%
P467-CRM+Alum - 10µg
P467-CRM+Alum - 25µg
P467-CRM+Alum - 50µg
P467-CRM+Alum (Serva) - 10µg
P467-CRM+Alum (Serva) - 25µg
P467-CRM+Montanide - 10µg
P467-CRM+Montanide - 25µg
P467-CRM+Montanide - 50µg
Alum (Brenntag)
Montanide
CRM
naive mouse

FIGURE 20B

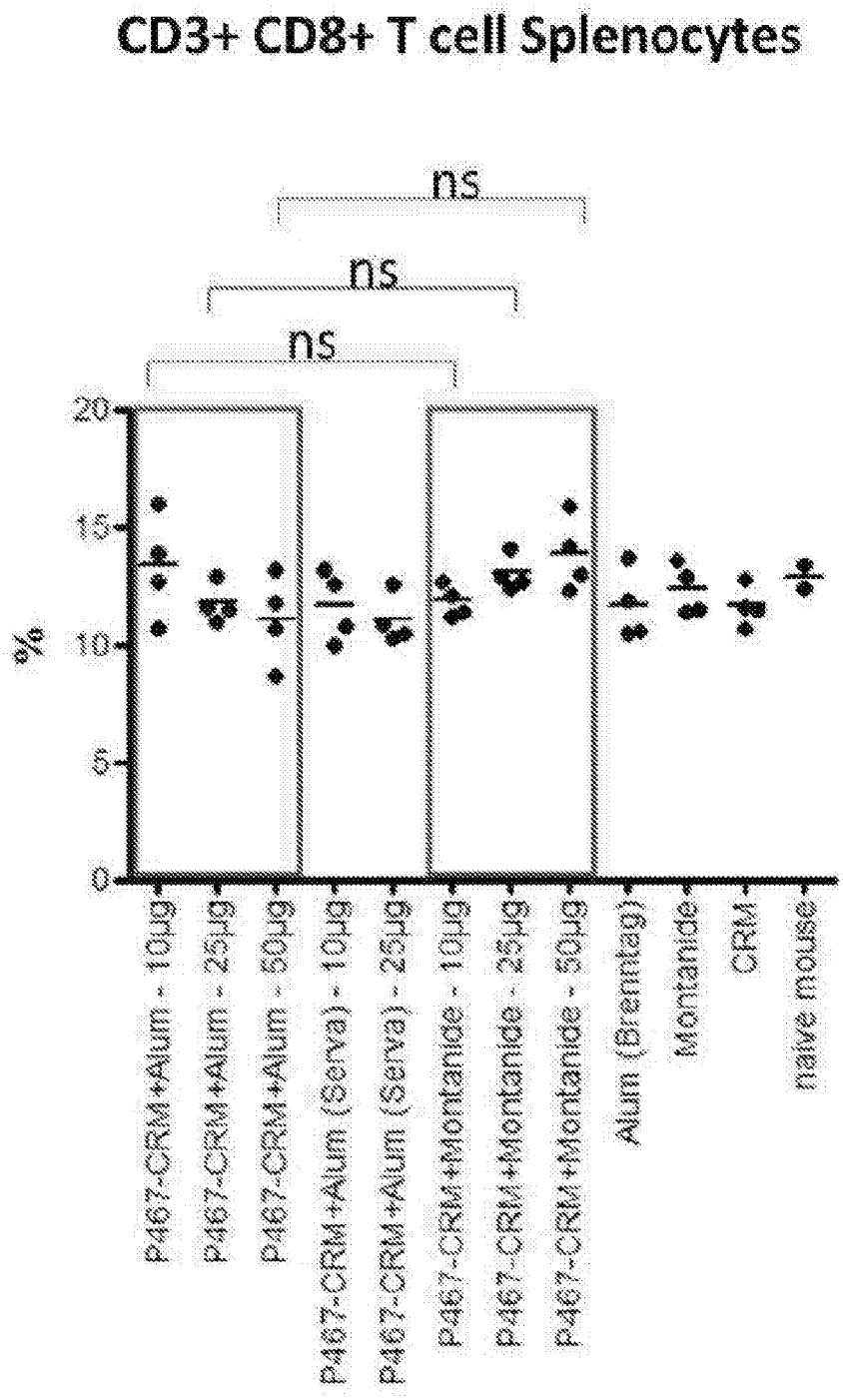

CD3+ CD8+ T cell Splenocytes
ns
ns
ns
%
0
5
10
15
20
P467-CRM+Alum - 10µg
P467-CRM+Alum - 25µg
P467-CRM+Alum - 50µg
P467-CRM+Alum (Serva) - 10µg
P467-CRM+Alum (Serva) - 25µg
P467-CRM+Montanide - 10µg
P467-CRM+Montanide - 25µg
P467-CRM+Montanide - 50µg
Alum (Brenntag)
Montanide
CRM
naive mouse

FIGURE 20C

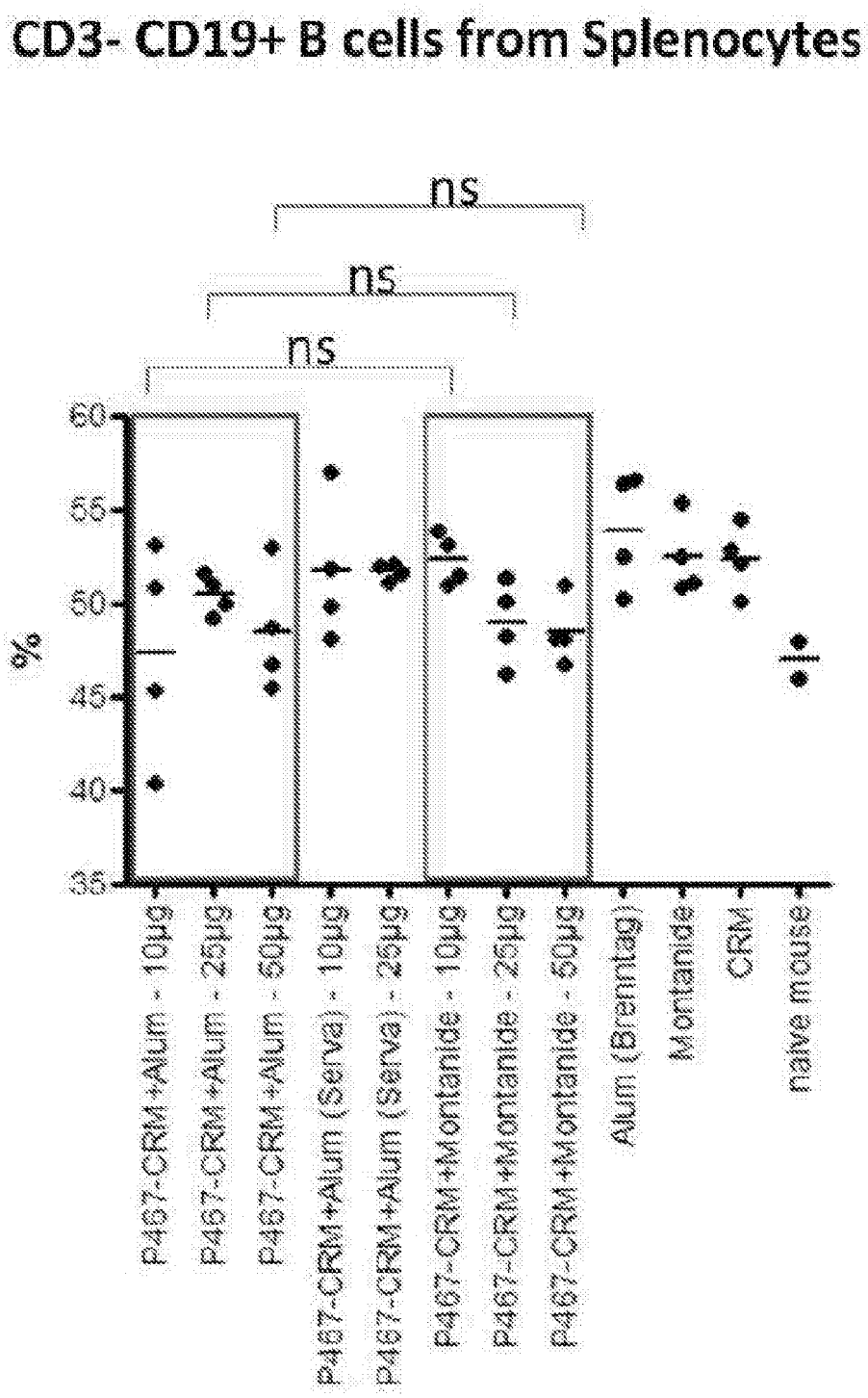

CD3- CD19+ B cells from Splenocytes
ns
ns
ns
%
60
55
50
45
40
35
P467-CRM+Alum - 10µg
P467-CRM+Alum - 25µg
P467-CRM+Alum - 50µg
P467-CRM+Alum (Serva) - 10µg
P467-CRM+Alum (Serva) - 25µg
P467-CRM+Montanide - 10µg
P467-CRM+Montanide - 25µg
P467-CRM+Montanide - 50µg
Alum (Brenntag)
Montanide
CRM
naive mouse

FIGURE 20D

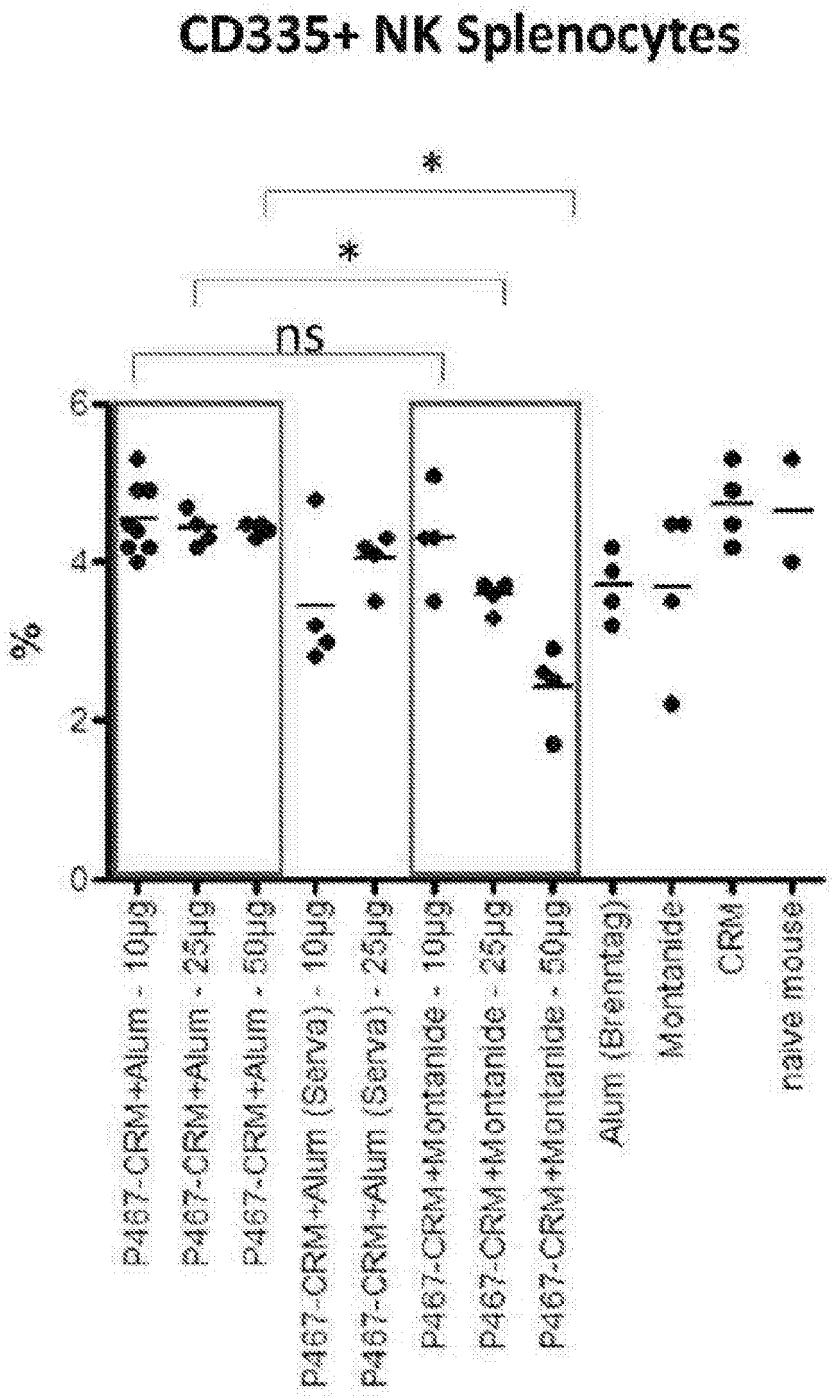

CD335+ NK Splenocytes
*
*
ns
%
0
2
4
6
P467-CRM+Alum - 10µg
P467-CRM+Alum - 25µg
P467-CRM+Alum - 50µg
P467-CRM+Alum (Serva) - 10µg
P467-CRM+Alum (Serva) - 25µg
P467-CRM+Montanide - 10µg
P467-CRM+Montanide - 25µg
P467-CRM+Montanide - 50µg
Alum (Brenntag)
Montanide
CRM
naive mouse

FIGURE 21A

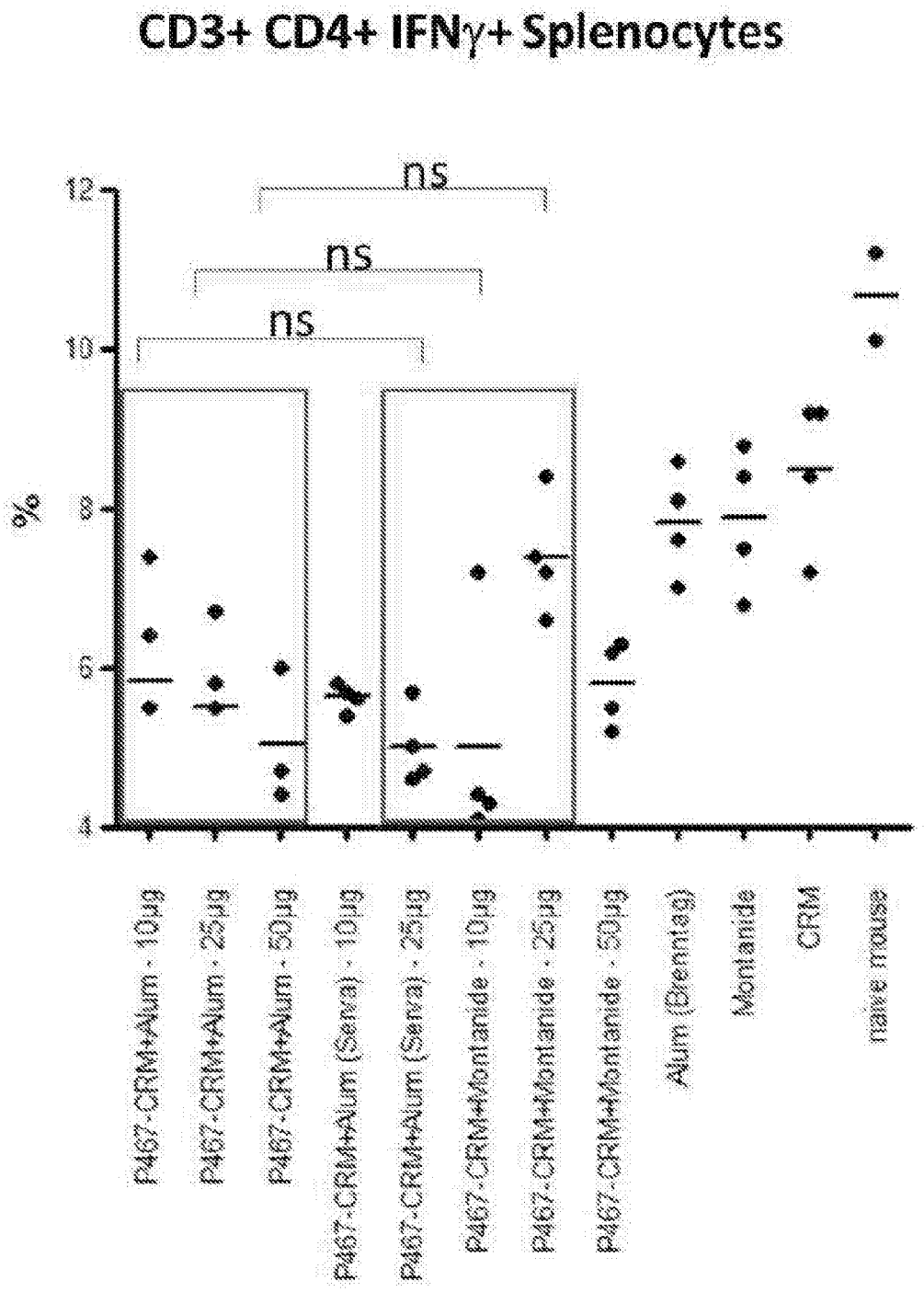

CD3+ CD4+ IFNγ+ Splenocytes
%
12
10
8
6
4
ns
ns
ns
P467-CRM+Alum - 10µg
P467-CRM+Alum - 25µg
P467-CRM+Alum - 50µg
P467-CRM+Alum (Senza) - 10µg
P467-CRM+Alum (Senza) - 25µg
P467-CRM+Montanide - 10µg
P467-CRM+Montanide - 25µg
P467-CRM+Montanide - 50µg
Alum (Brenntag)
Montanide
CRM
naive mouse

FIGURE 21B

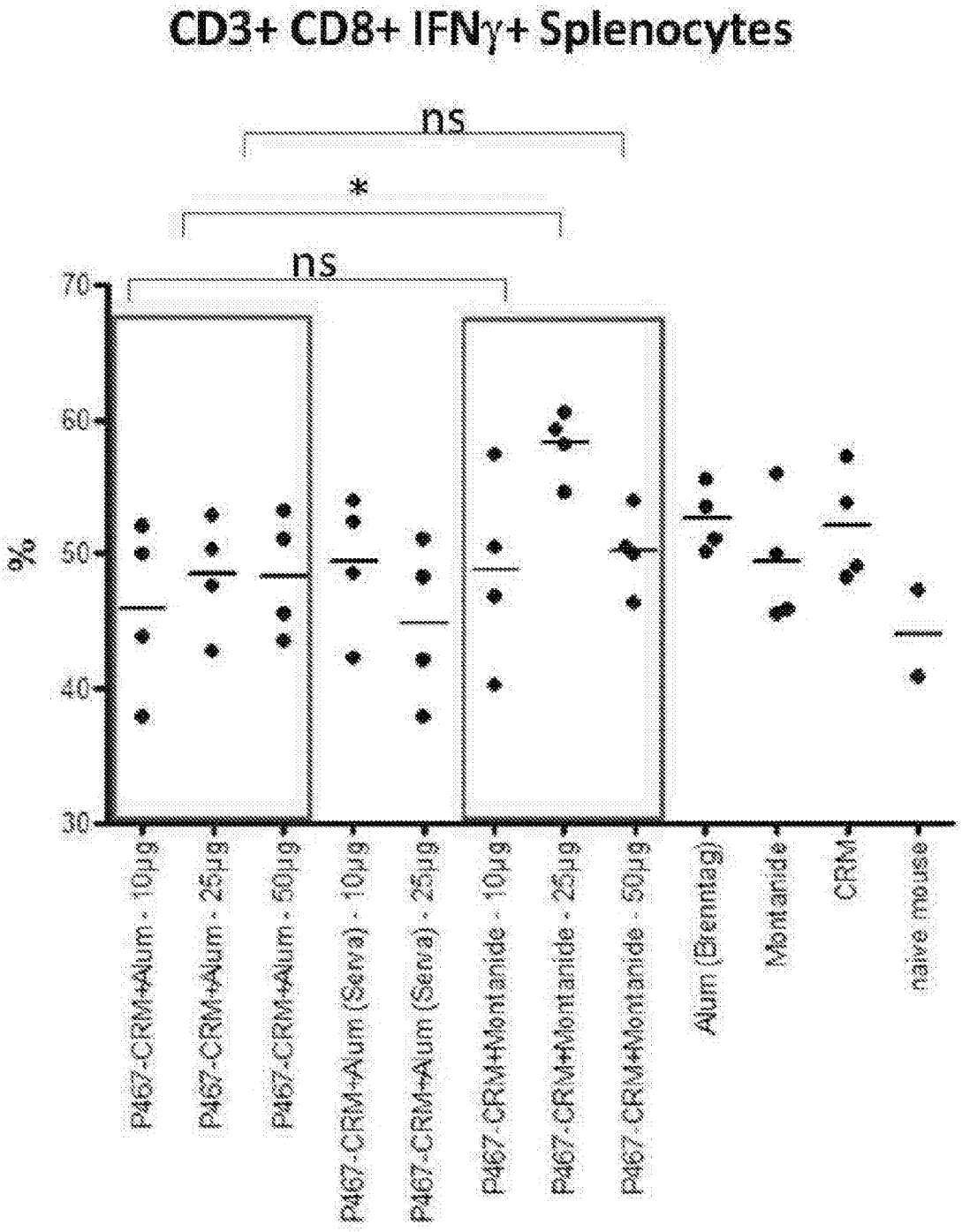

CD3+ CD8+ IFNγ+ Splenocytes
ns
*
ns
%
70
60
50
40
30
P467-CRM+Alum - 10µg
P467-CRM+Alum - 25µg
P467-CRM+Alum - 50µg
P467-CRM+Alum (Serva) - 10µg
P467-CRM+Alum (Serva) - 25µg
P467-CRM+Montanide - 10µg
P467-CRM+Montanide - 25µg
P467-CRM+Montanide - 50µg
Alum (Brenntag)
Montanide
CRM
naive mouse

FIGURE 21C

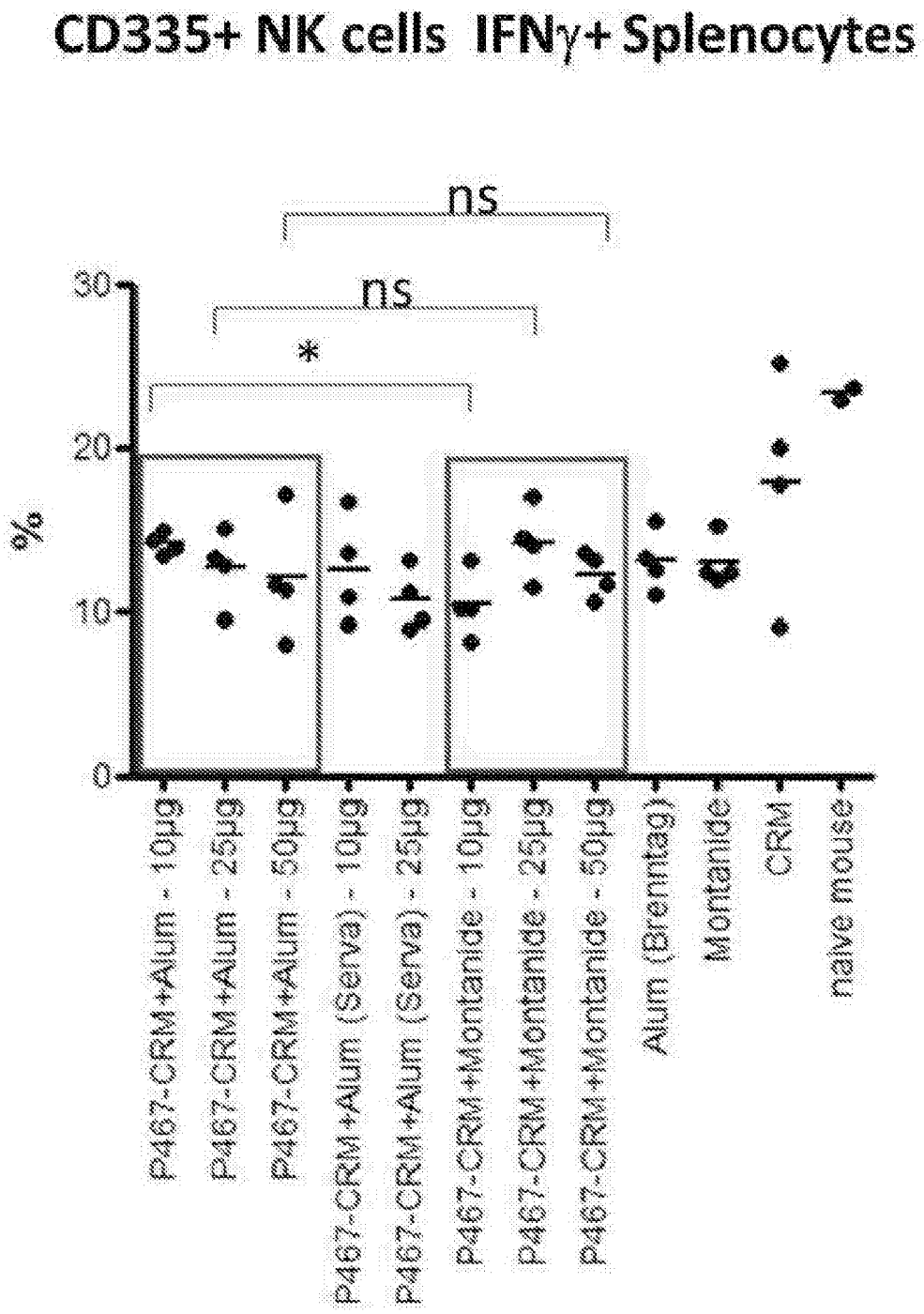

CD335+ NK cells IFNγ+ Splenocytes
ns
ns
*
%
30
20
10
0
P467-CRM+Alum - 10µg
P467-CRM+Alum - 25µg
P467-CRM+Alum - 50µg
P467-CRM+Alum (Serva) - 10µg
P467-CRM+Alum (Serva) - 25µg
P467-CRM+Montanide - 10µg
P467-CRM+Montanide - 25µg
P467-CRM+Montanide - 50µg
Alum (Bremtag)
Montanide
CRM
naive mouse

FIGURE 22

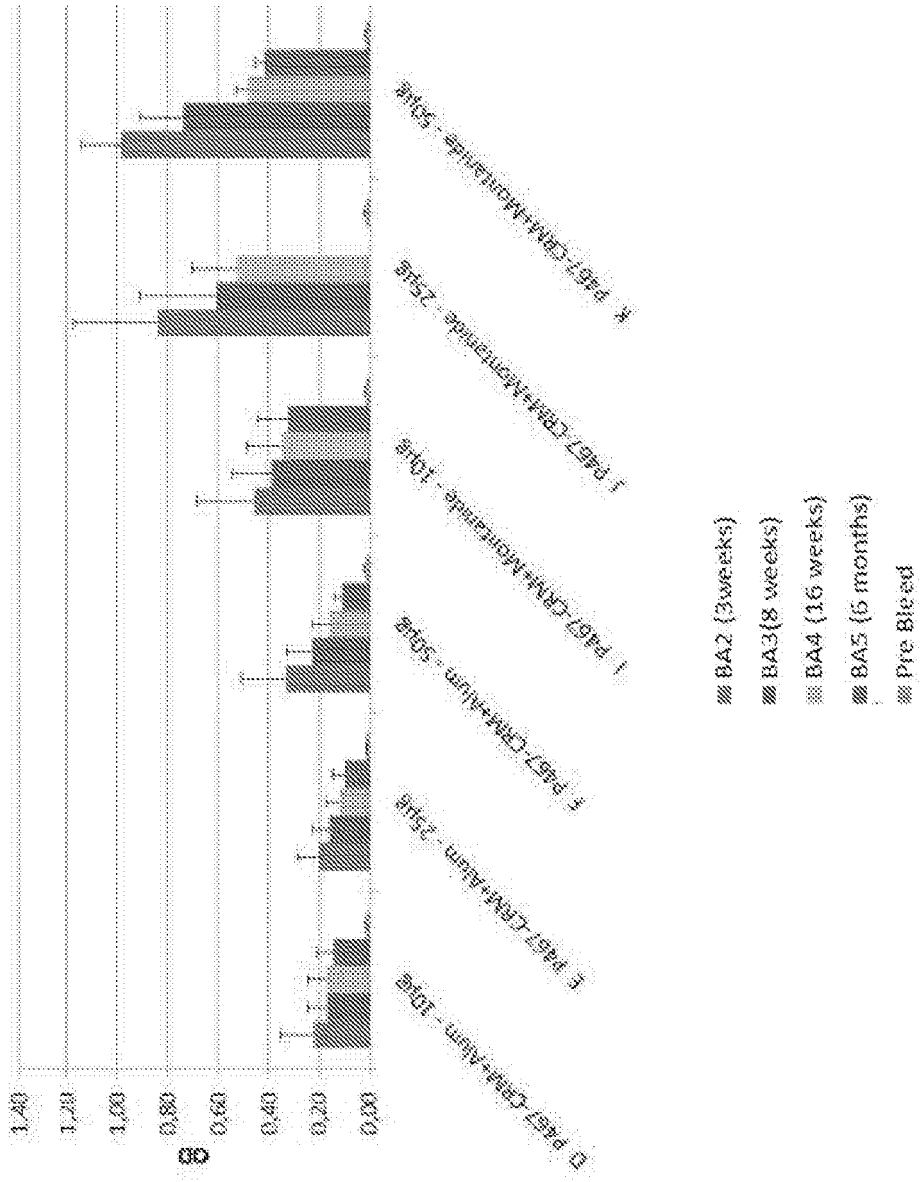
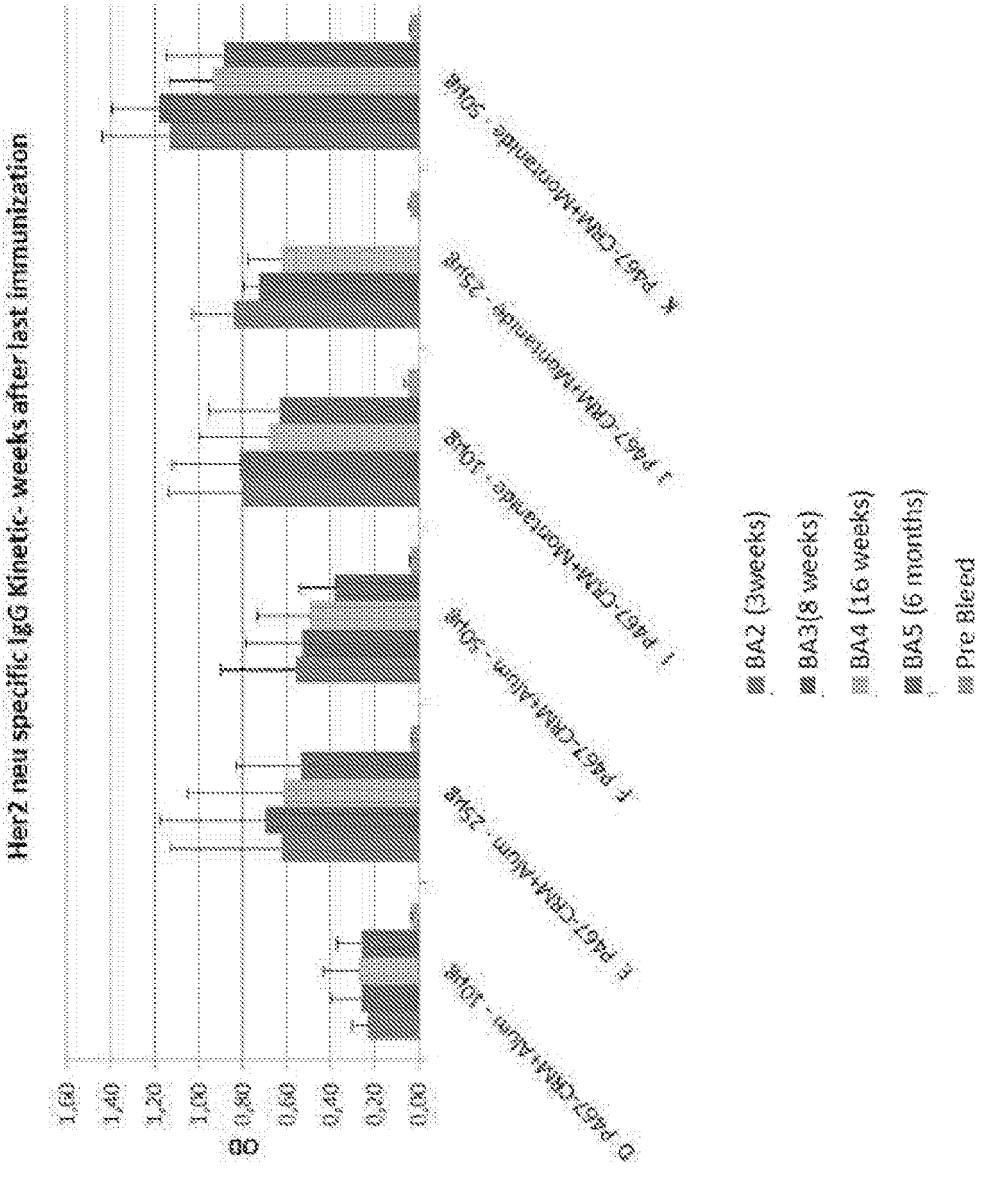

P467 IgG Kinetic- weeks after last immunization
OD
1,40
1,20
1,00
0,80
0,60
0,40
0,20
0,00
D P467-CpG+Alum - 10µg
E P467-CpG+Alum - 25µg
F P467-CpG+Alum - 50µg
I P467-CpG+Montanide - 10µg
J P467-CpG+Montanide - 25µg
K P467-CpG+Montanide - 50µg
BA2 (3weeks)
BA3(8 weeks)
BA4 (16 weeks)
BA5 (6 months)
Pre Bleed

FIGURE 23


Her2 neu specific IgG Kinetic- weeks after last immunization
OD
1,60
1,40
1,20
1,00
0,80
0,60
0,40
0,20
0,00
D pMG7-CMV-HA-Alum - 10ug
E pMG7-CMV-HA-Alum - 25ug
F pMG7-CMV-HA-Alum - 50ug
I pMG7-CMV-Montanide - 10ug
J pMG7-CMV-Montanide - 25ug
K pMG7-CMV-Montanide - 50ug
BA2 (3weeks)
BA3(8 weeks)
BA4 (16 weeks)
BA5 (6 months)
Pre Bleed