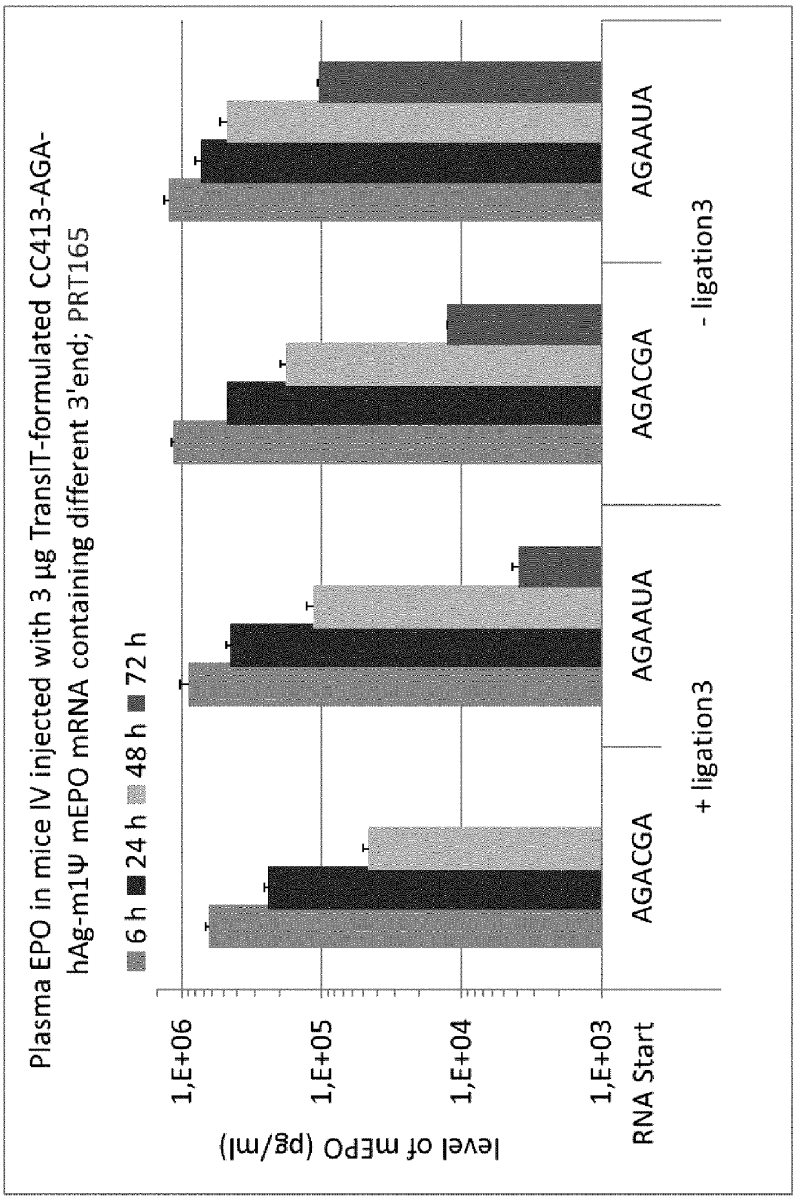
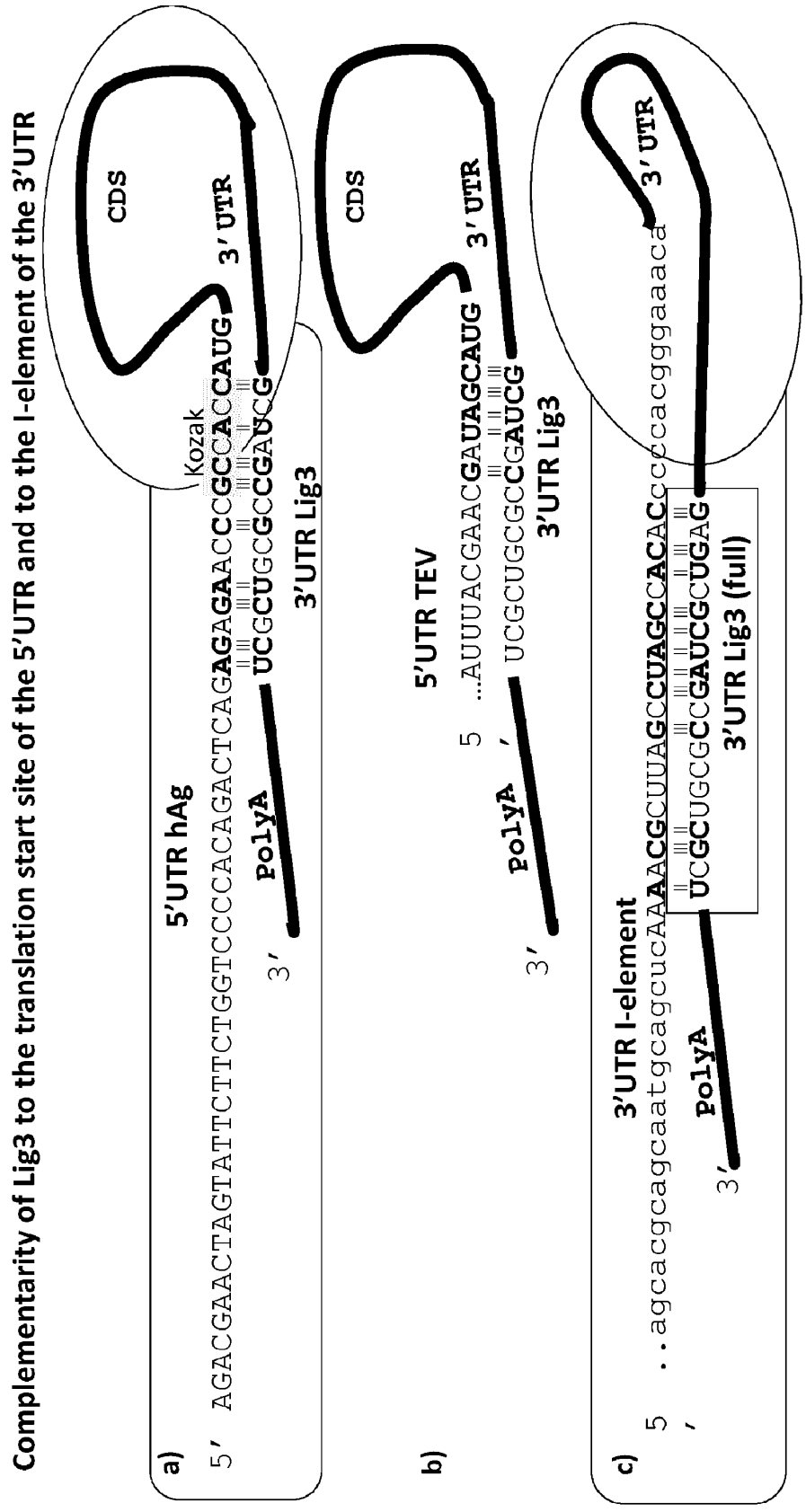

Plasma EPO in mice IV injected with 3 µg TransIT-formulated CC413-AGA-hAg-m1Ψ mEPO mRNA containing different 3'end; PRT165
6 h
24 h
48 h
72 h
level of mEPO (pg/ml)
1,E+06
1,E+05
1,E+04
1,E+03
RNA Start
AGACGA
AGAAUA
AGACGA
AGAAUA
+ ligation3
- ligation3

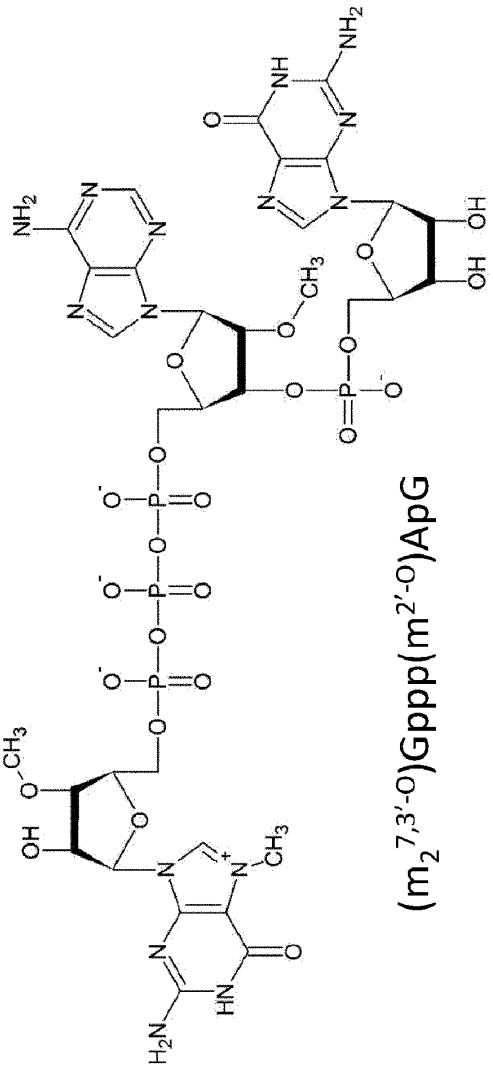
Figure 1

# Figure 2A-2C


Complementarity of Lig3 to the translation start site of the 5'UTR and to the I-element of the 3'UTR
a)
5'UTR hAg
5′ AGACGAACTAGTATTCTTCTGGTCCCCACAGACTCAGAGAGAACCCGCCACCATG
3′ UCGCUGCGCCGAUCG
PolyA
3'UTR Lig3
CDS
Kozak
3'UTR
b)
5'UTR TEV
5 ...AUUUACGAACGAUAGCAUG
3′ UCGCUGCGCCGAUCG
PolyA
3'UTR Lig3
CDS
3'UTR
c)
3'UTR I-element
5 ..agcacgcagcaatgcagcucAAAACGCUUAGCCUAGCCACACccccacgggaaaca
3′ UCGCUGCGCCGAUCGCUGAG
PolyA
3'UTR Lig3 (full)
3'UTR

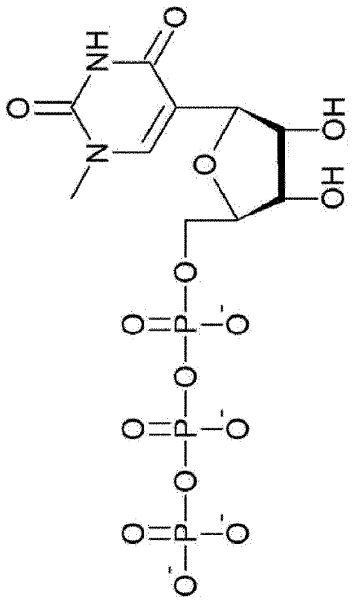
# Figures 3A-3B

A)



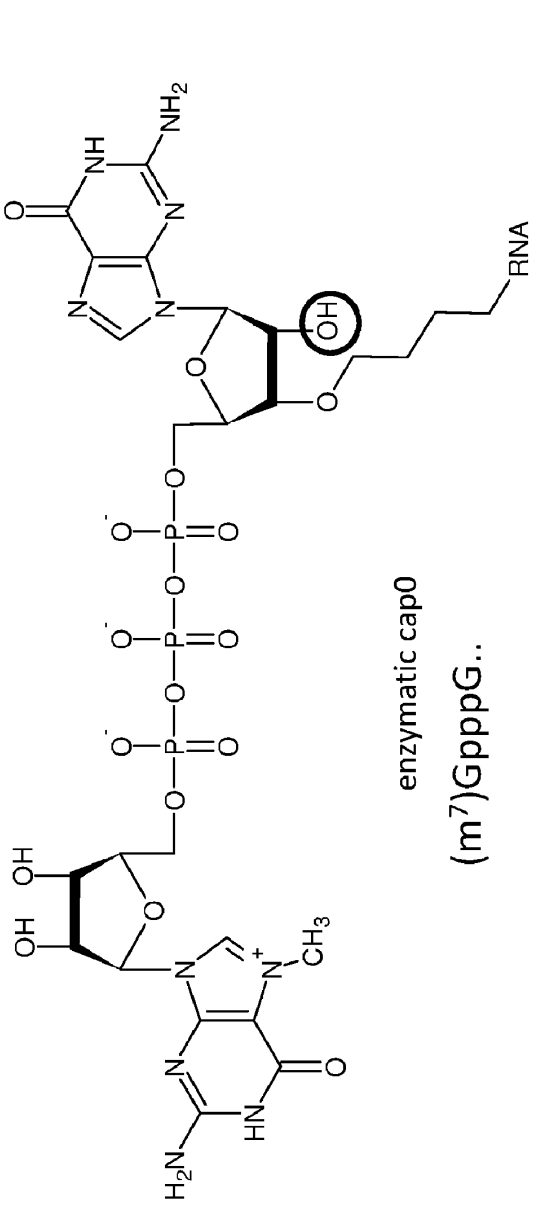
$(m_2^{7,3'\text{-O}})Gppp(m^{2'\text{-O}})ApG$

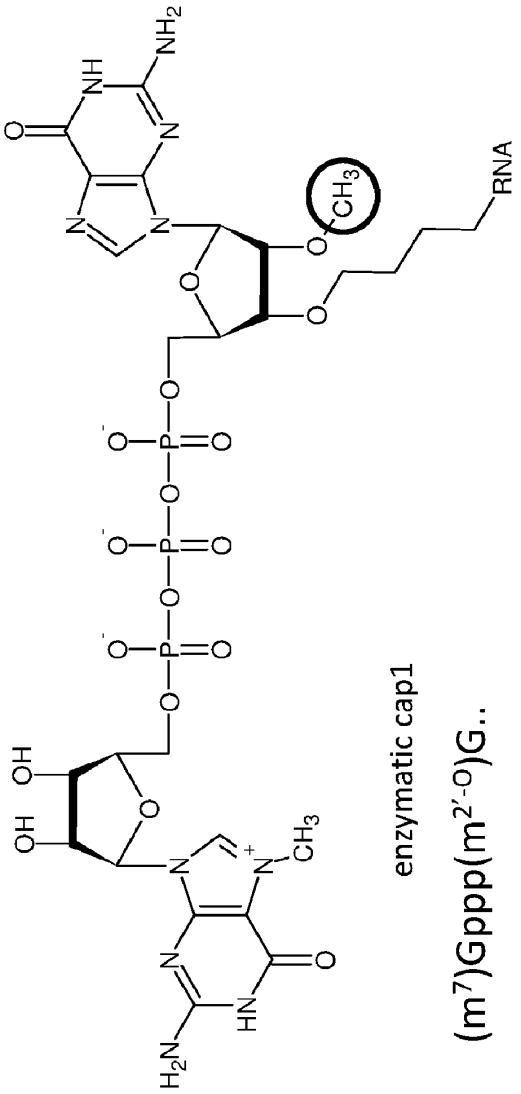
b)



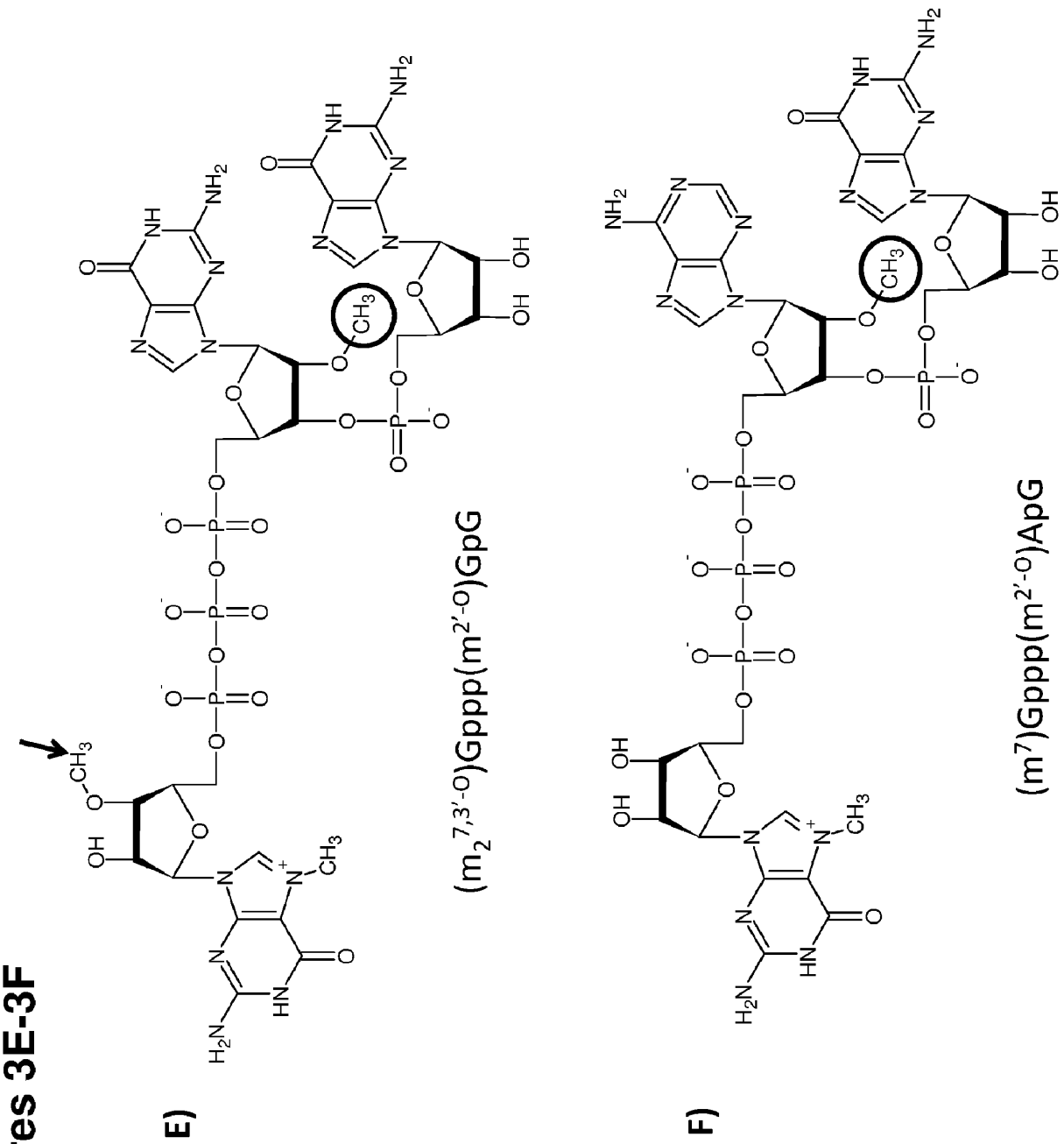
m1-pseudouridine triphosphate

**Figures 3C-3D**



Figures 3E-3F



Figures 3G-3H

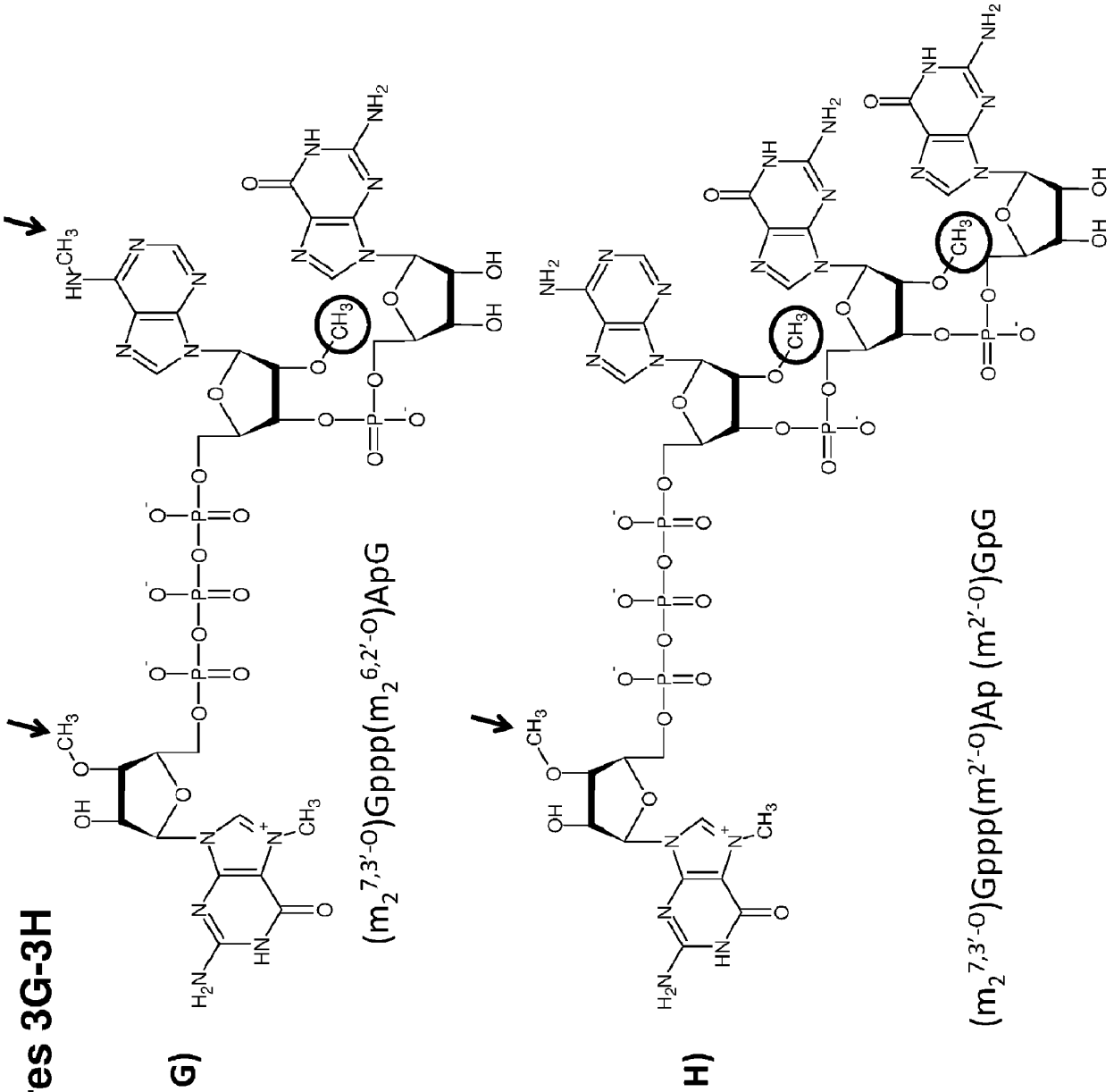
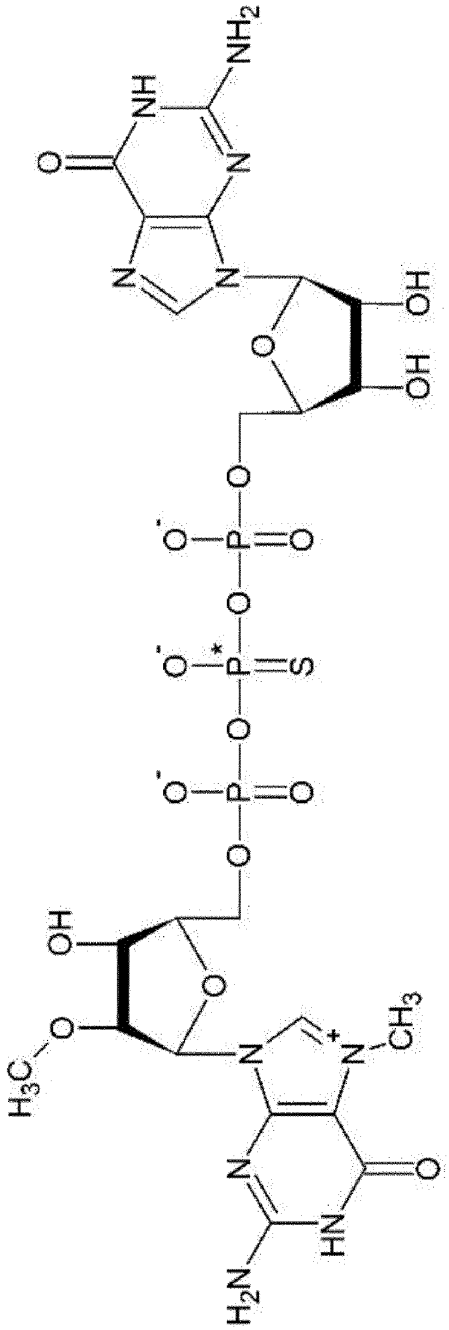


Figure 3l

I)



$(m_2^{7,2'\text{-}O})GppSpG$

Figure 4

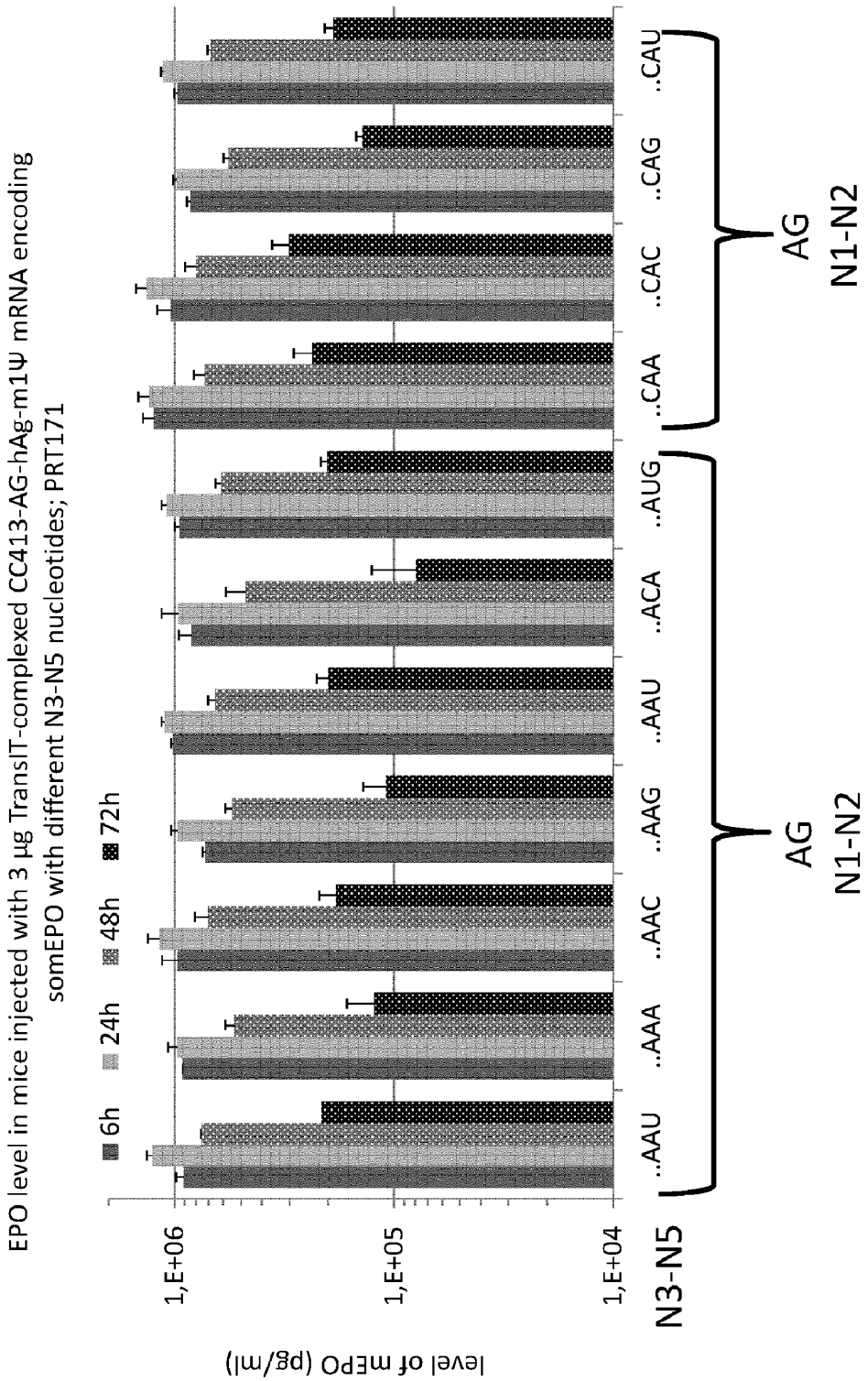

EPO level in mice injected with 3 µg TransIT-complexed CC413-AG-hAg-m1Ψ mRNA encoding somEPO with different N3-N5 nucleotides; PRT171
6h
24h
48h
72h
level of mEPO (pg/ml)
1,E+06
1,E+05
1,E+04
N3-N5
..AAU
..AAA
..AAC
..AAG
..AAU
..ACA
..AUG
..CAA
..CAC
..CAG
..CAU
AG
N1-N2
AG
N1-N2

Figure 5

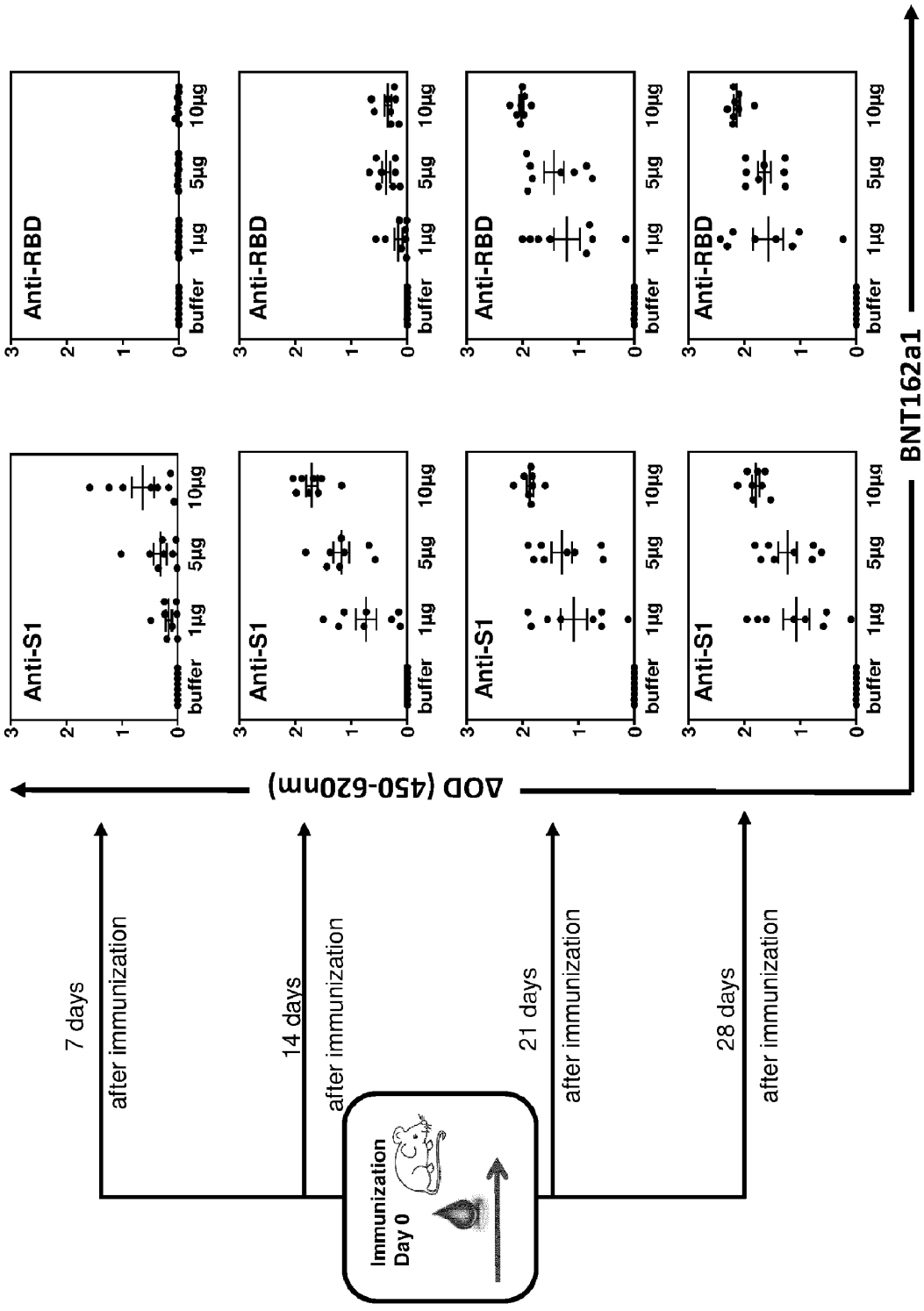

BNT162a1
ΔOD (450-620nm)
Anti-S1
Anti-RBD
buffer
1µg
5µg
10µg
7 days
after immunization
14 days
after immunization
21 days
after immunization
28 days
after immunization
Immunization
Day 0

Figure 6

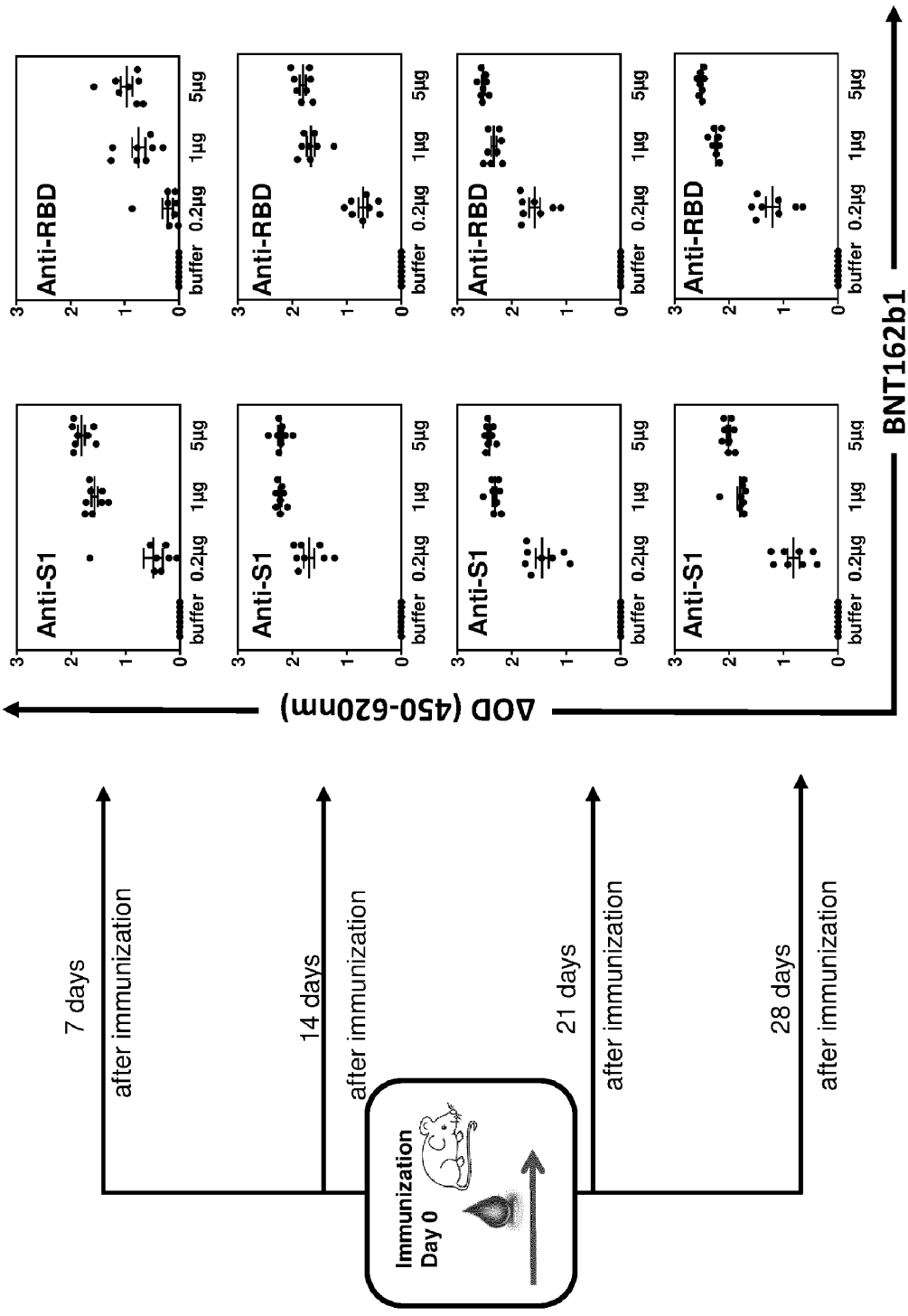

BNT162b1
ΔOD (450-620nm)
Anti-S1
Anti-RBD
buffer
0.2µg
1µg
5µg
Immunization
Day 0
7 days
after immunization
14 days
after immunization
21 days
after immunization
28 days
after immunization

Figure 7

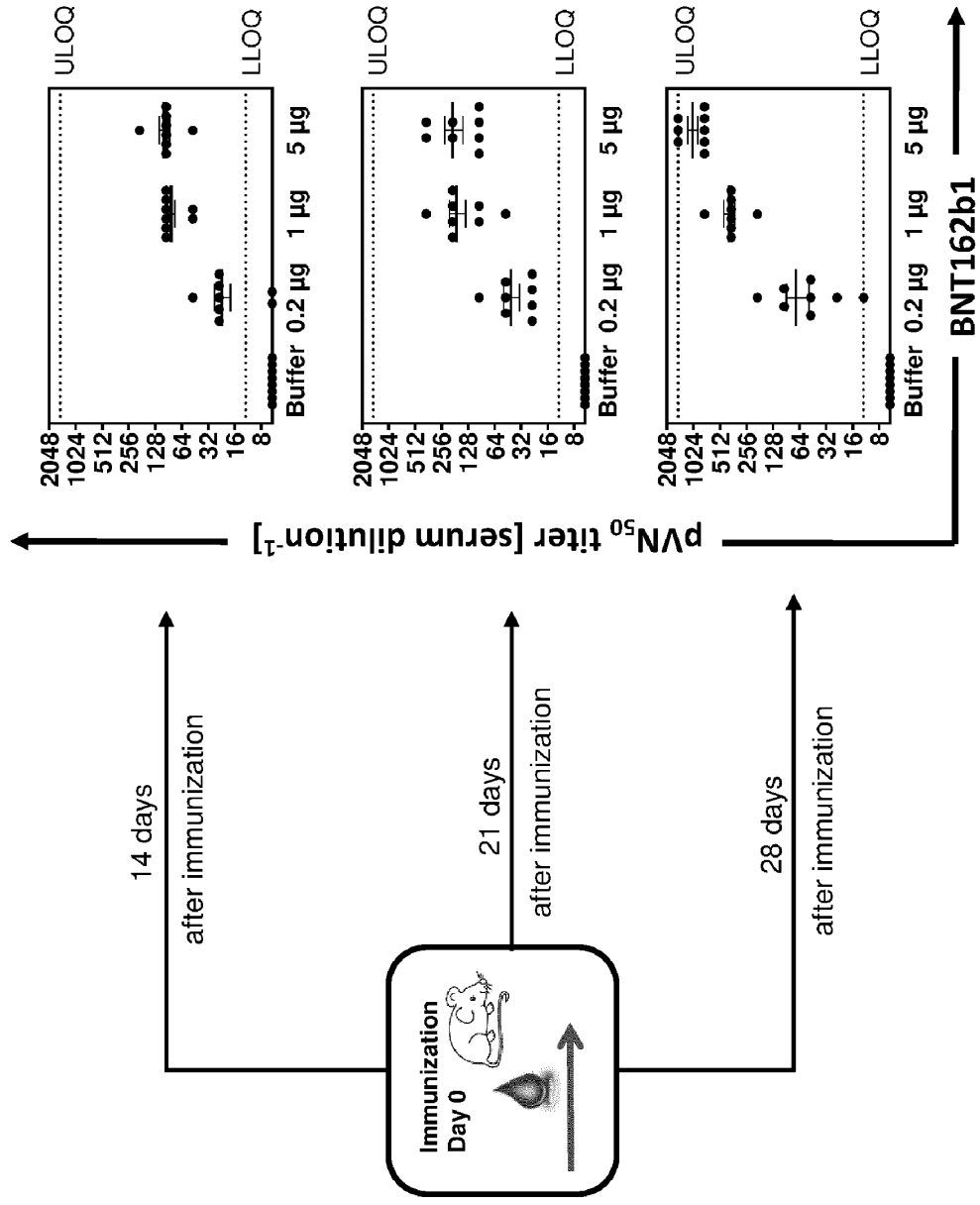

pVN50 titer [serum dilution-1]
ULOQ
LLOQ
2048
1024
512
256
128
64
32
16
8
Buffer
0.2 µg
1 µg
5 µg
BNT162b1
14 days
after immunization
21 days
after immunization
28 days
after immunization
Immunization
Day 0

Figure 8

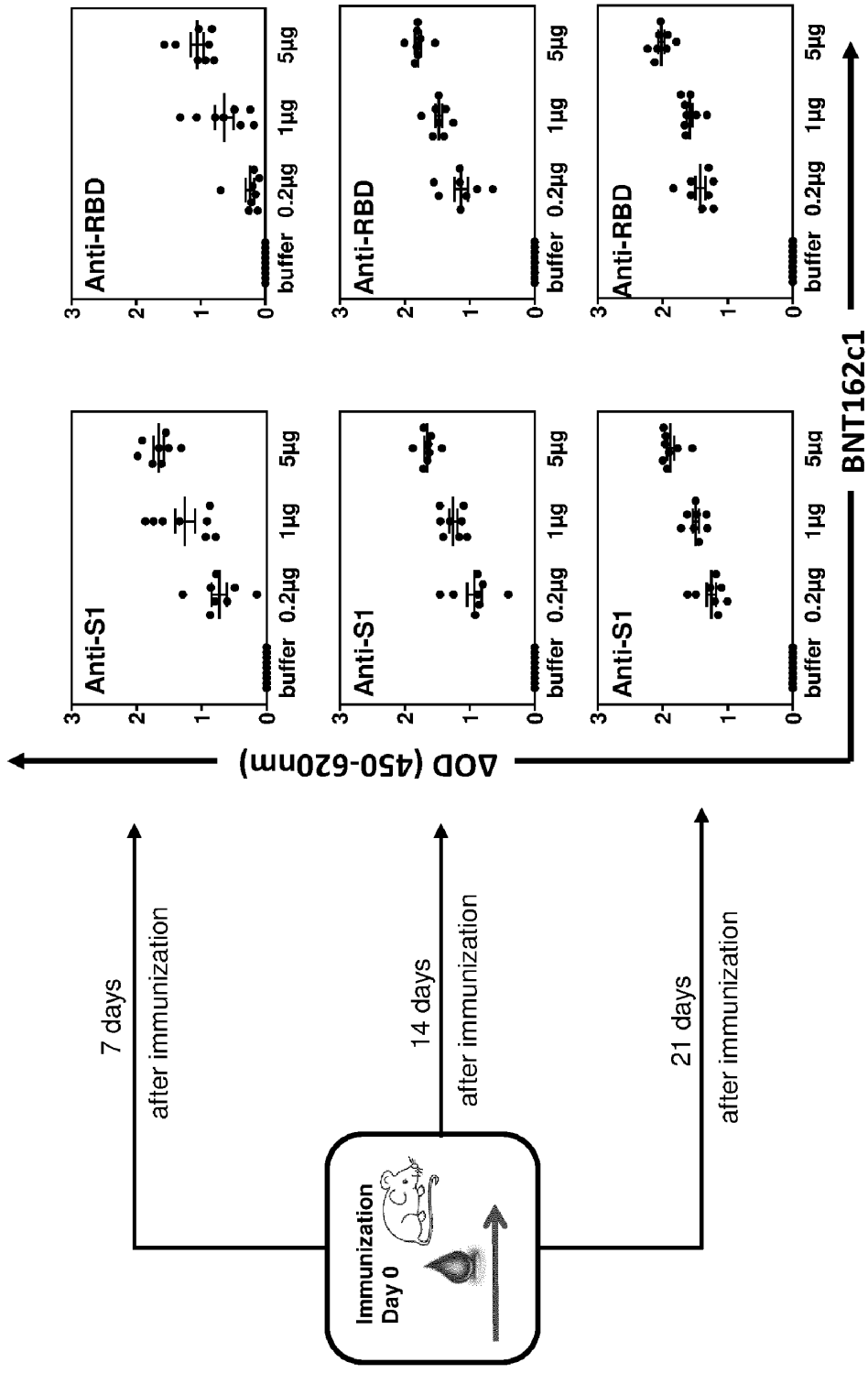

Immunization
Day 0
7 days
after immunization
14 days
after immunization
21 days
after immunization
ΔOD (450-620nm)
Anti-S1
Anti-RBD
buffer
0.2µg
1µg
5µg
BNT162c1

Figure 9

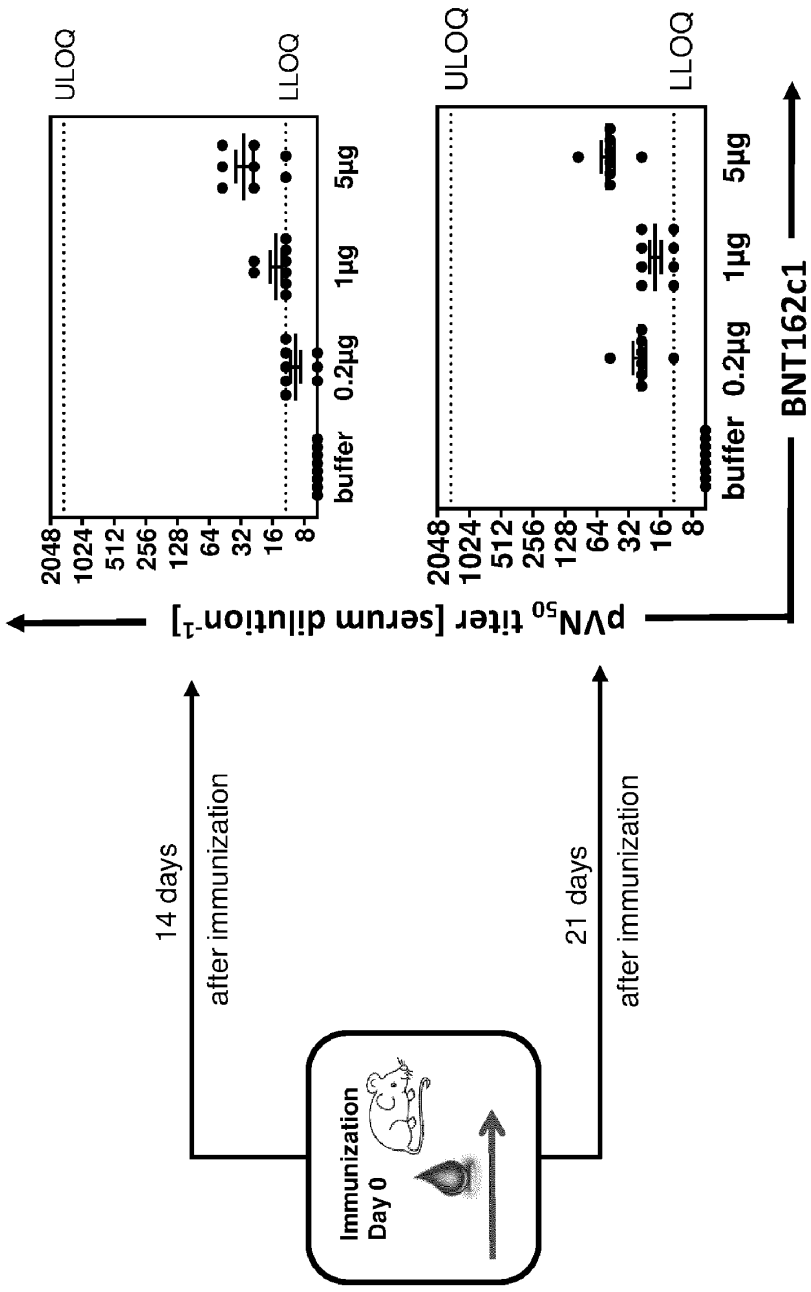

Immunization
Day 0
14 days
after immunization
21 days
after immunization
pVN50 titer [serum dilution-1]
2048
1024
512
256
128
64
32
16
8
ULOQ
LLOQ
buffer
0.2µg
1µg
5µg
BNT162c1

Figure 10

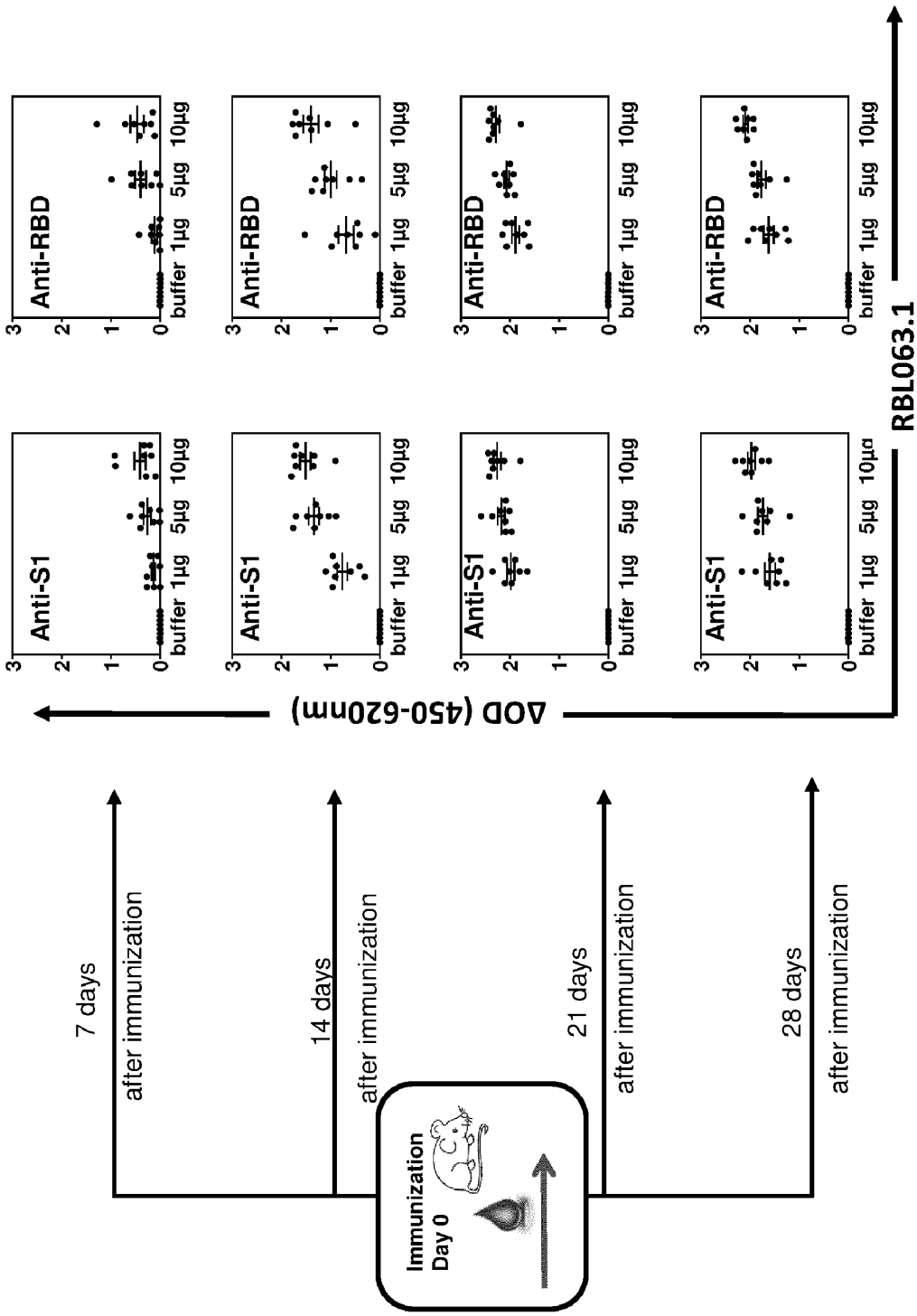

Immunization
Day 0
7 days
after immunization
14 days
after immunization
21 days
after immunization
28 days
after immunization
ΔOD (450-620nm)
Anti-S1
Anti-RBD
buffer 1µg 5µg 10µg
RBL063.1

Figure 11

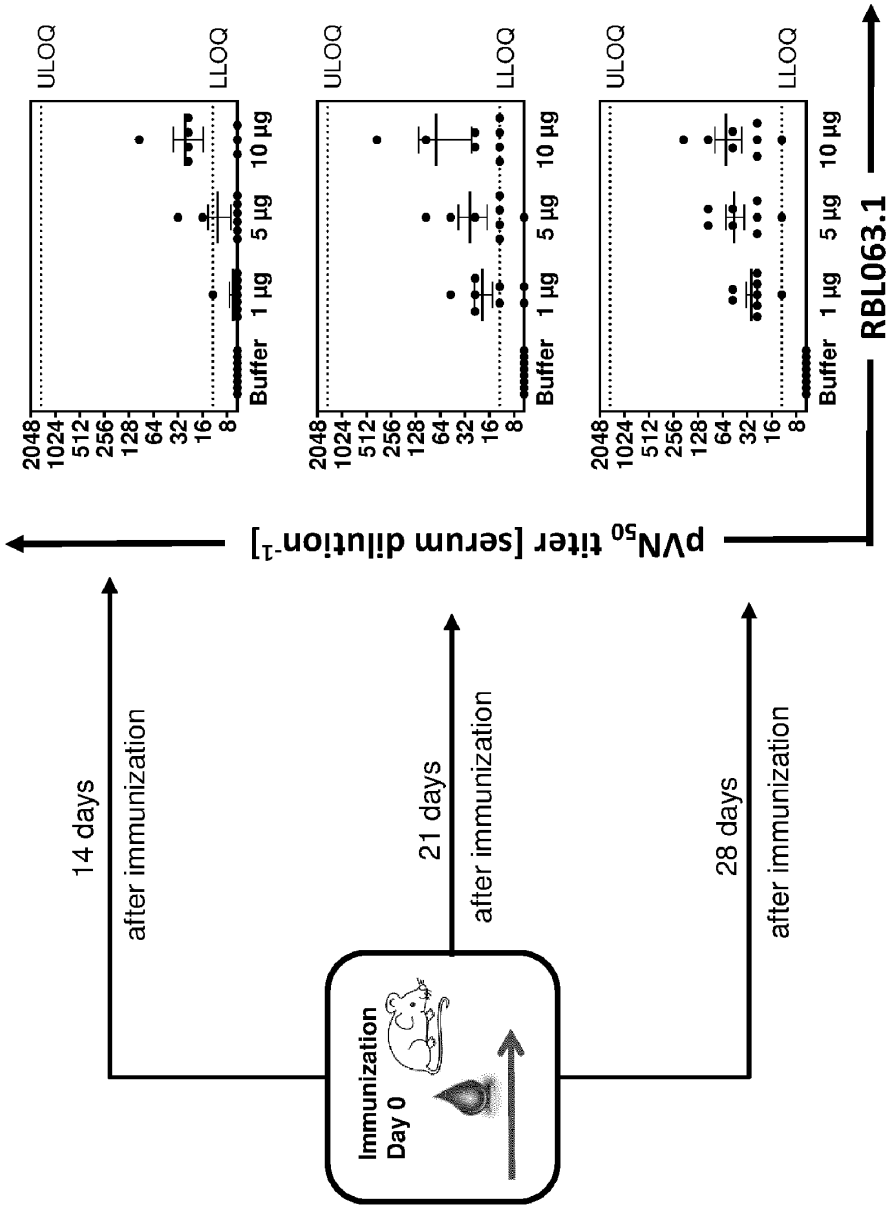

Immunization
Day 0
14 days
after immunization
21 days
after immunization
28 days
after immunization
pVN50 titer [serum dilution-1]
ULOQ
LLOQ
2048
1024
512
256
128
64
32
16
8
Buffer
1 µg
5 µg
10 µg
RBL063.1

Figure 12

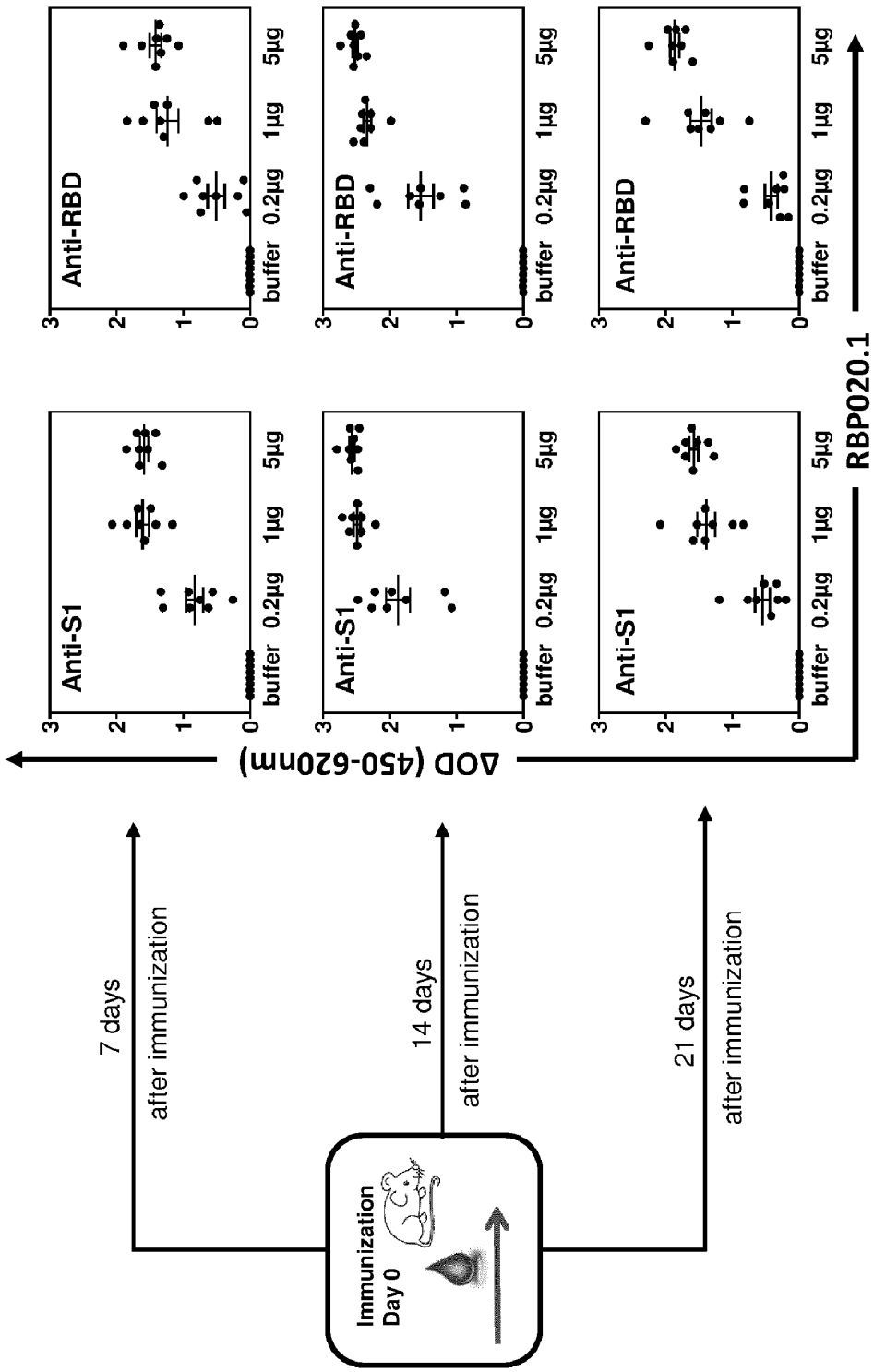

Immunization
Day 0
7 days
after immunization
14 days
after immunization
21 days
after immunization
ΔOD (450-620nm)
Anti-S1
Anti-RBD
buffer
0.2µg
1µg
5µg
RBP020.1

Figure 13

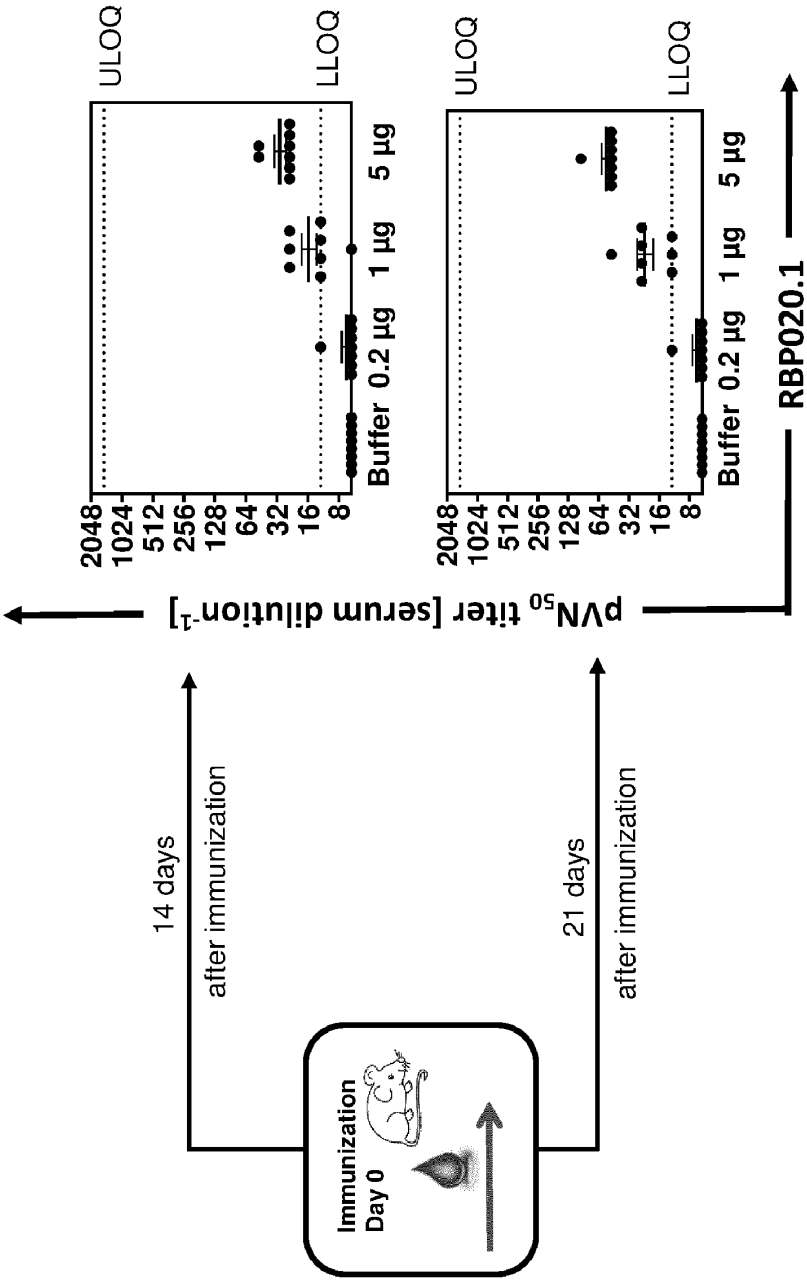

Immunization
Day 0
14 days
after immunization
21 days
after immunization
pVN50 titer [serum dilution-1]
2048
1024
512
256
128
64
32
16
8
ULOQ
LLOQ
Buffer
0.2 µg
1 µg
5 µg
RBP020.1

Figure 14

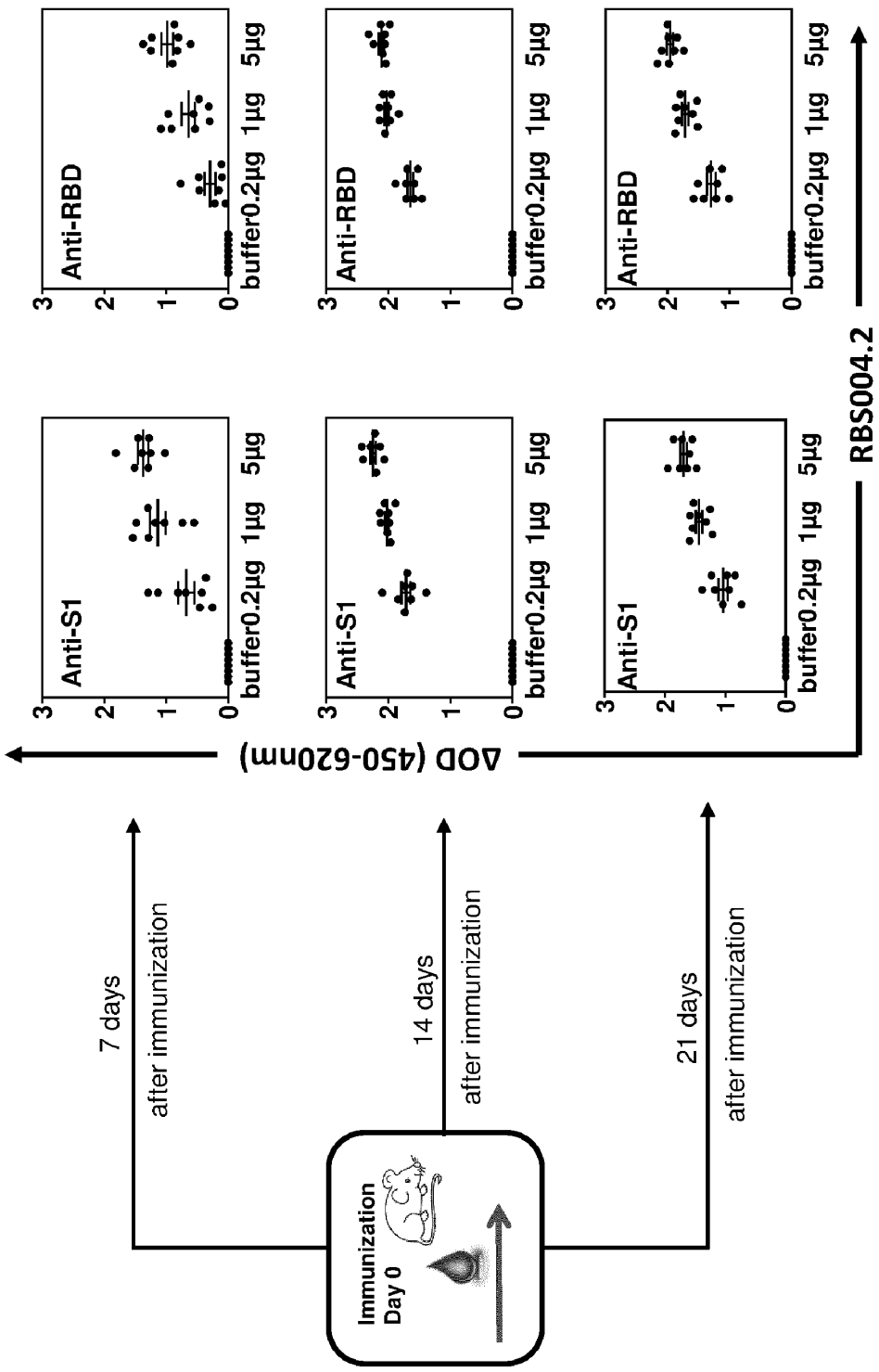

Immunization
Day 0
7 days
after immunization
14 days
after immunization
21 days
after immunization
ΔOD (450-620nm)
RBS004.2
Anti-S1
Anti-RBD
buffer
0.2μg
1μg
5μg
0
1
2
3

Figure 15

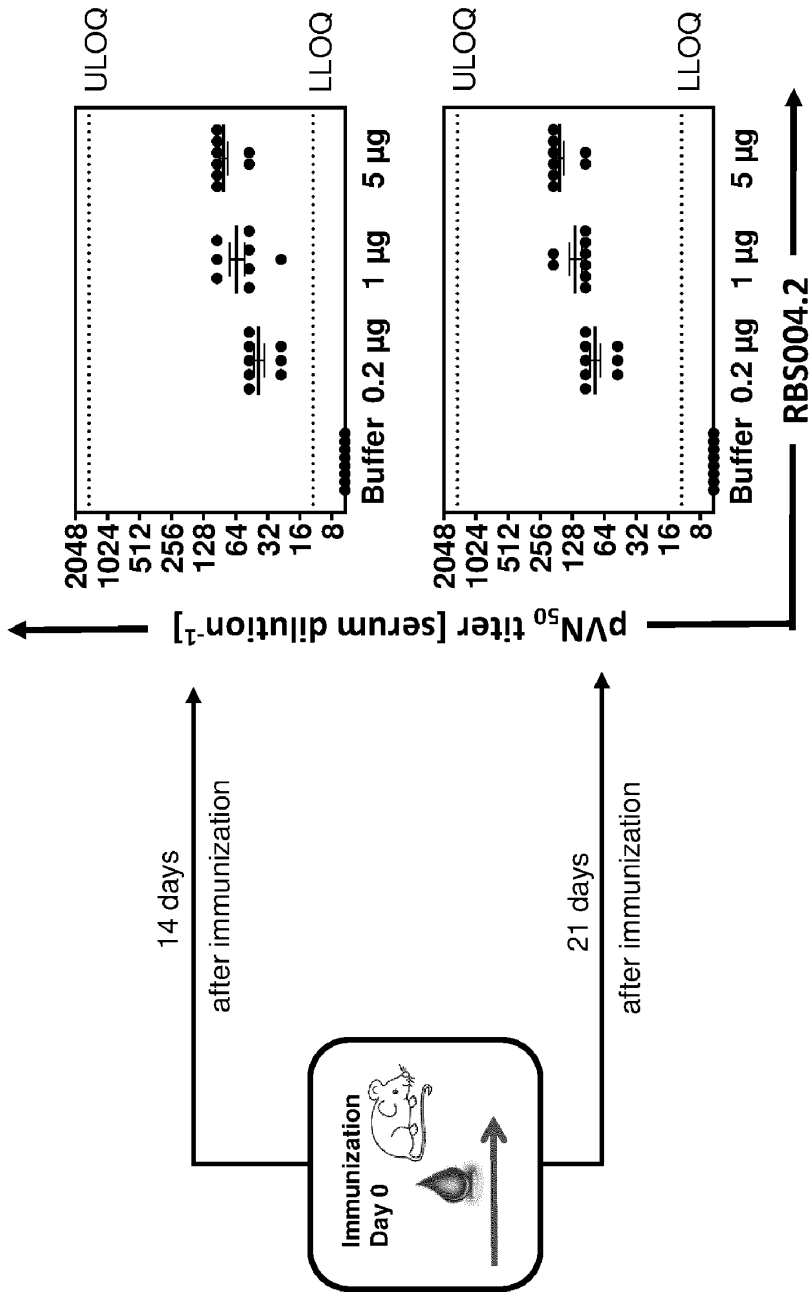

Immunization
Day 0
14 days
after immunization
21 days
after immunization
pVN50 titer [serum dilution-1]
2048
1024
512
256
128
64
32
16
8
ULOQ
LLOQ
Buffer
0.2 µg
1 µg
5 µg
RBS004.2

Figure 16

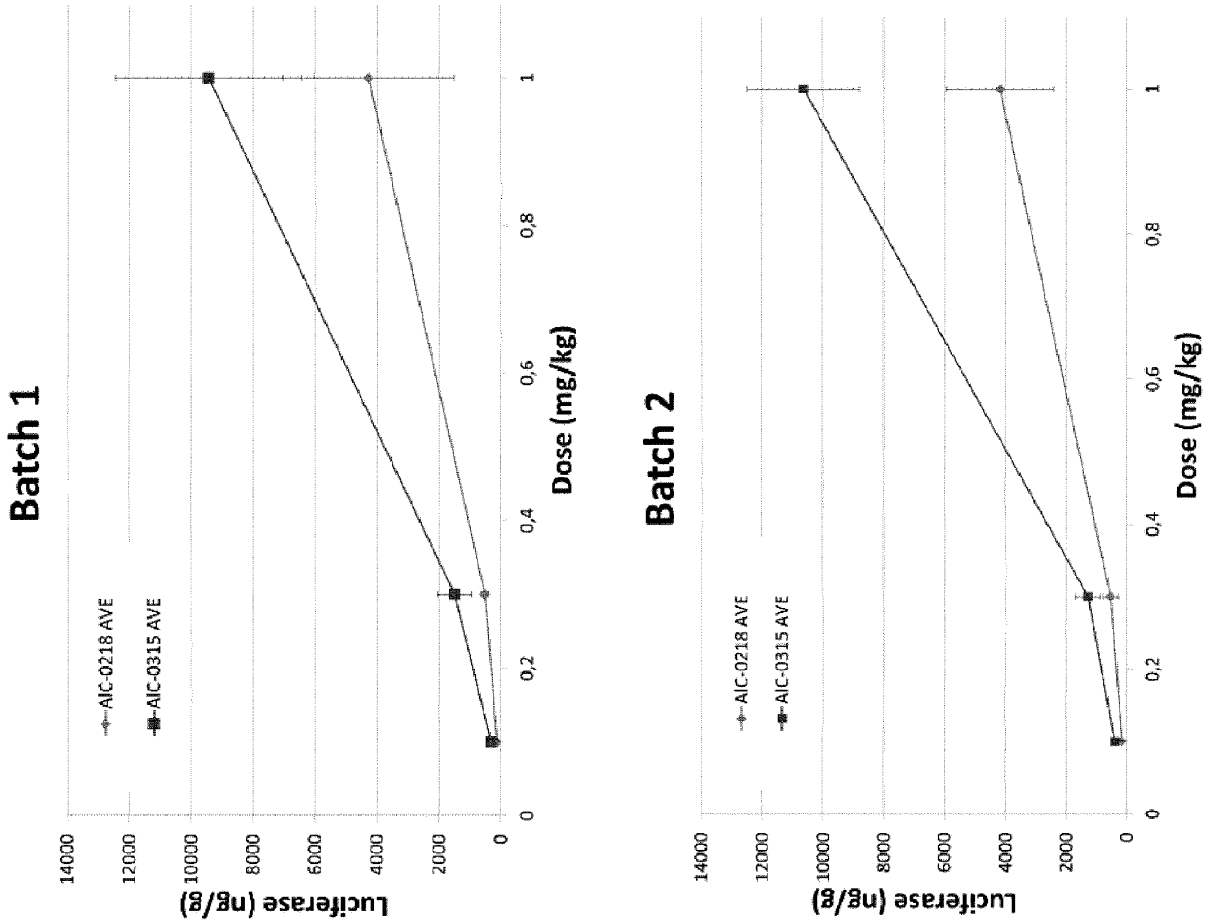

Batch 1
Batch 2
AIC-0218 AVE
AIC-0315 AVE
Luciferase (ng/g)
Dose (mg/kg)
0
2000
4000
6000
8000
10000
12000
14000
0,2
0,4
0,6
0,8
1

Figure 17A

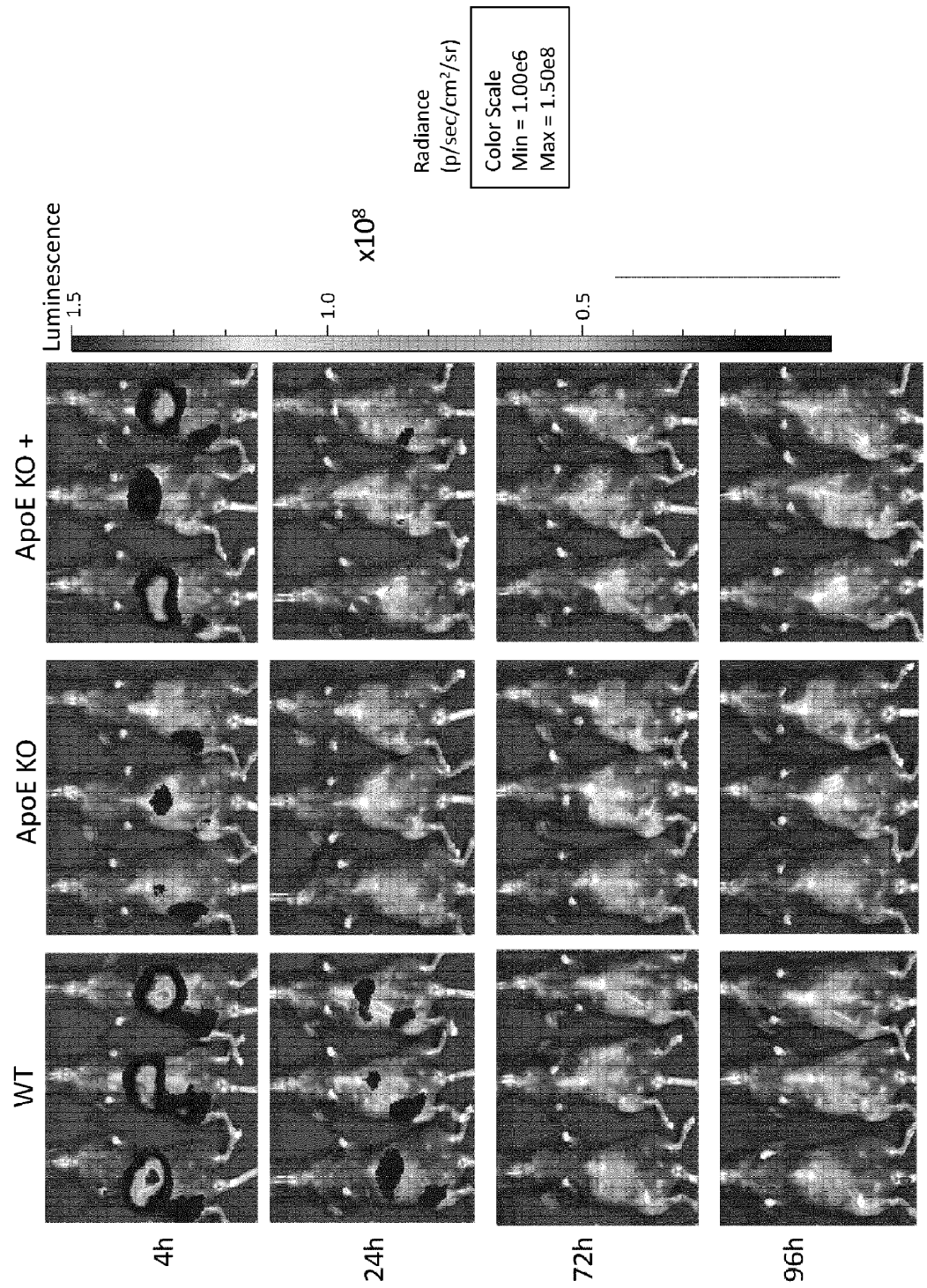

VENTRAL
WT
ApoE KO
ApoE KO +
4h
24h
72h
96h
Luminescence
1.5
1.0
0.5
x10^8
Radiance
(p/sec/cm²/sr)
Color Scale
Min = 1.00e6
Max = 1.50e8

Figure 17B

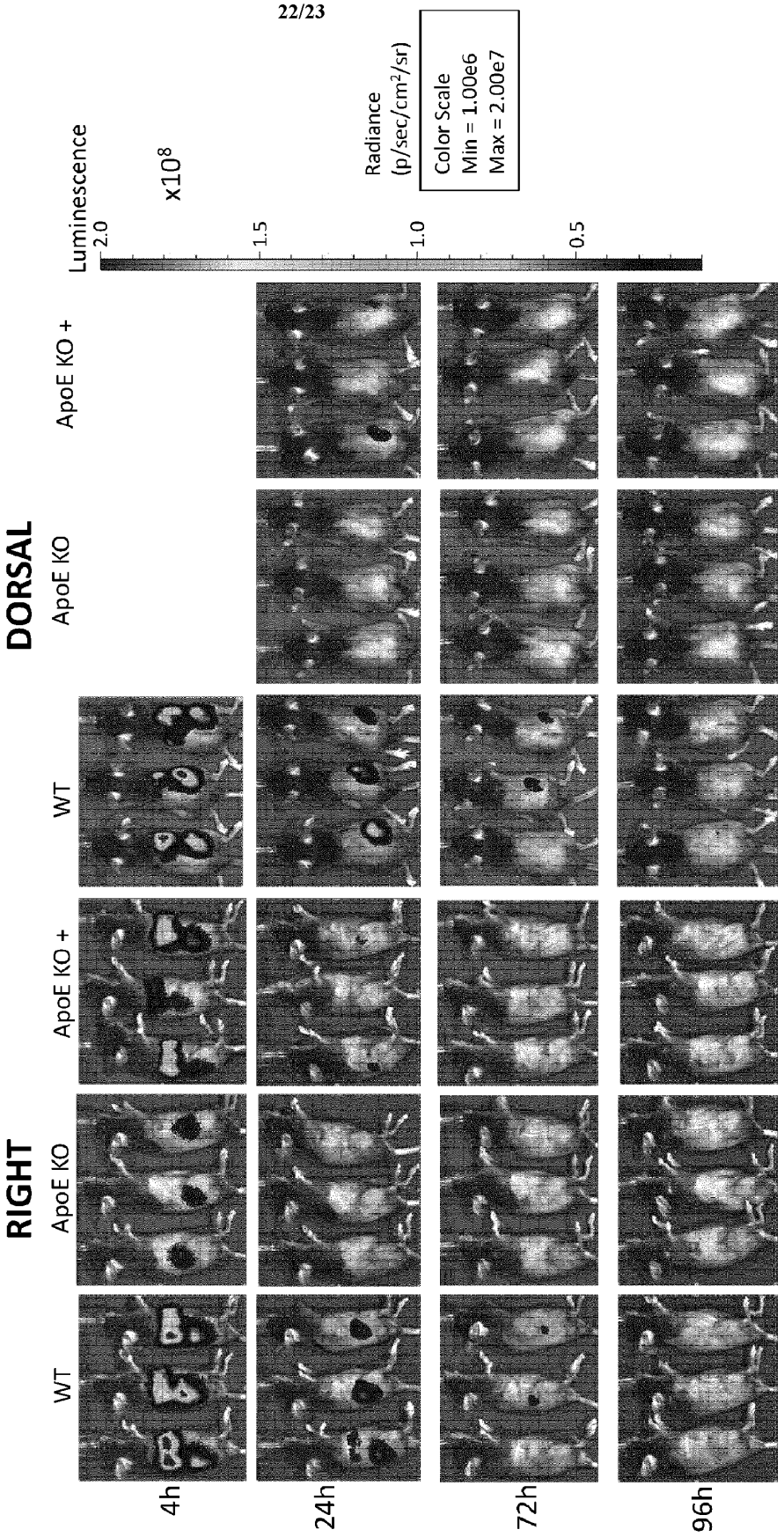
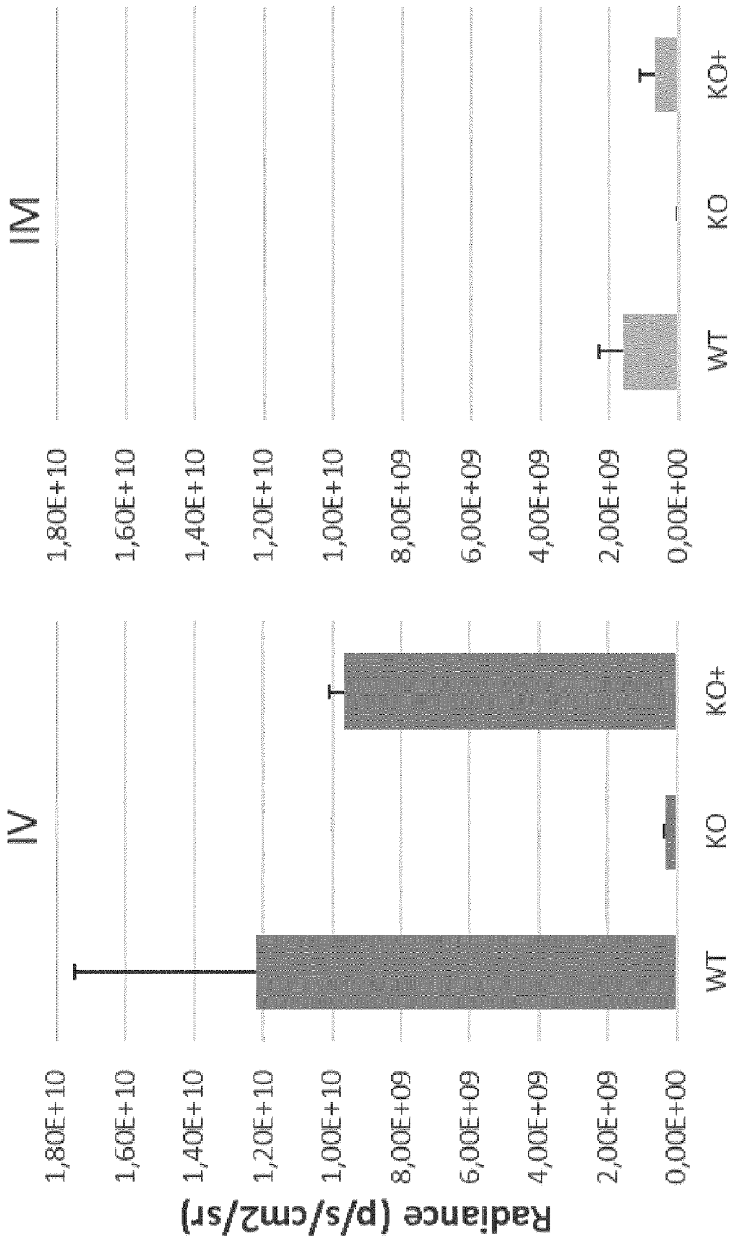

Luminescence
x10^8
2.0
1.5
1.0
0.5
Radiance (p/sec/cm²/sr)
Color Scale
Min = 1.00e6
Max = 2.00e7
DORSAL
RIGHT
WT
ApoE KO
ApoE KO +
WT
ApoE KO
ApoE KO +
4h
24h
72h
96h

Figure 18


IV
IM
Radiance (p/s/cm2/sr)
1,80E+10
1,60E+10
1,40E+10
1,20E+10
1,00E+10
8,00E+09
6,00E+09
4,00E+09
2,00E+09
0,00E+00
WT
KO
KO+