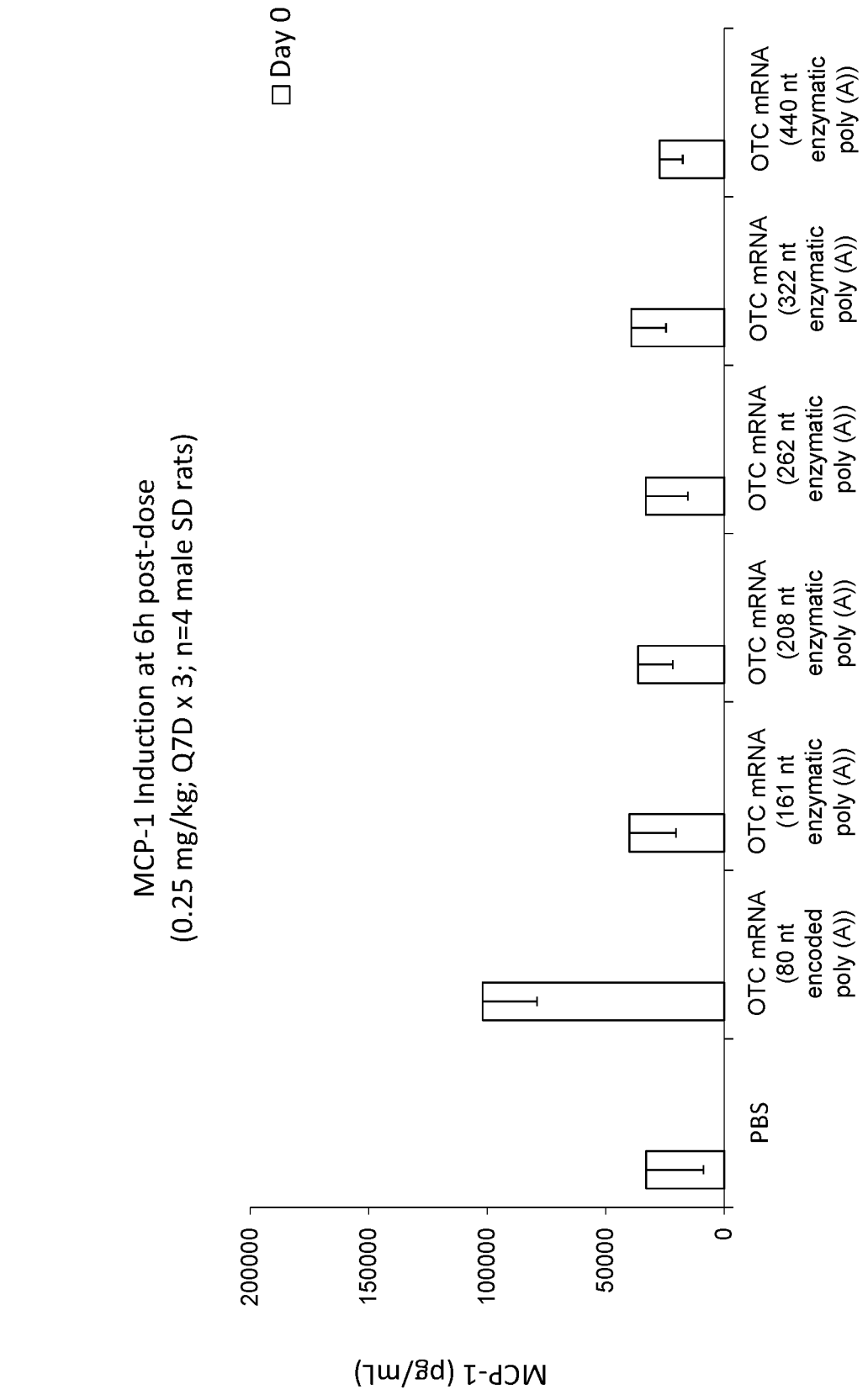
MCP-1 Induction at 6h post-dose
(0.25 mg/kg; Q7D x 3; n=4 male SD rats)
Day 0
MCP-1 (pg/mL)
200000
150000
100000
50000
0
PBS
OTC mRNA (80 nt encoded poly (A))
OTC mRNA (161 nt enzymatic poly (A))
OTC mRNA (208 nt enzymatic poly (A))
OTC mRNA (262 nt enzymatic poly (A))
OTC mRNA (322 nt enzymatic poly (A))
OTC mRNA (440 nt enzymatic poly (A))

FIG. 1

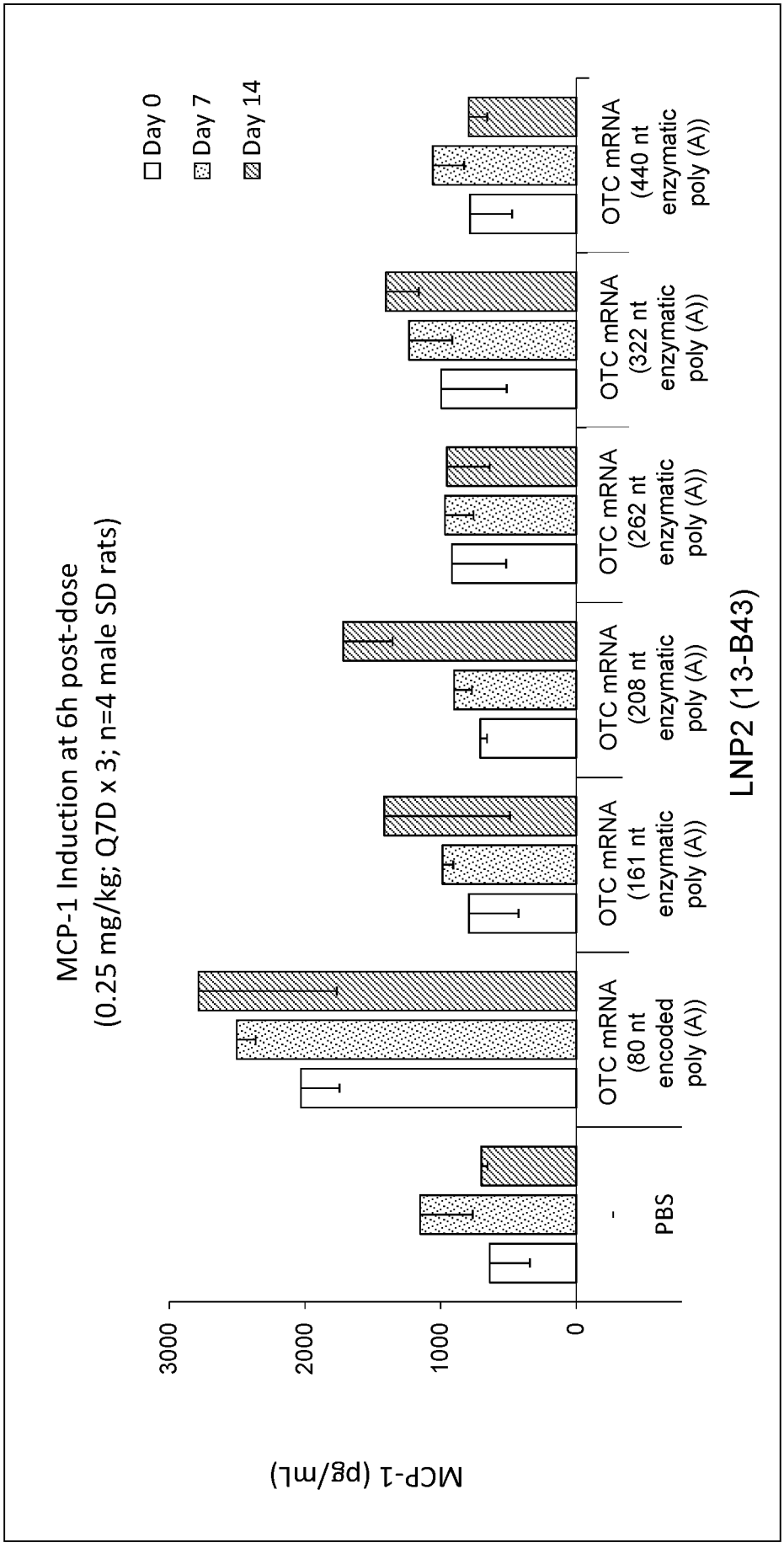
MCP-1 Induction at 6h post-dose
(0.25 mg/kg; Q7D x 3; n=4 male SD rats)
Day 0
Day 7
Day 14
MCP-1 (pg/mL)
3000
2000
1000
0
PBS
-
OTC mRNA (80 nt encoded poly (A))
OTC mRNA (161 nt enzymatic poly (A))
OTC mRNA (208 nt enzymatic poly (A))
OTC mRNA (262 nt enzymatic poly (A))
OTC mRNA (322 nt enzymatic poly (A))
OTC mRNA (440 nt enzymatic poly (A))
LNP2 (13-B43)

FIG. 2A

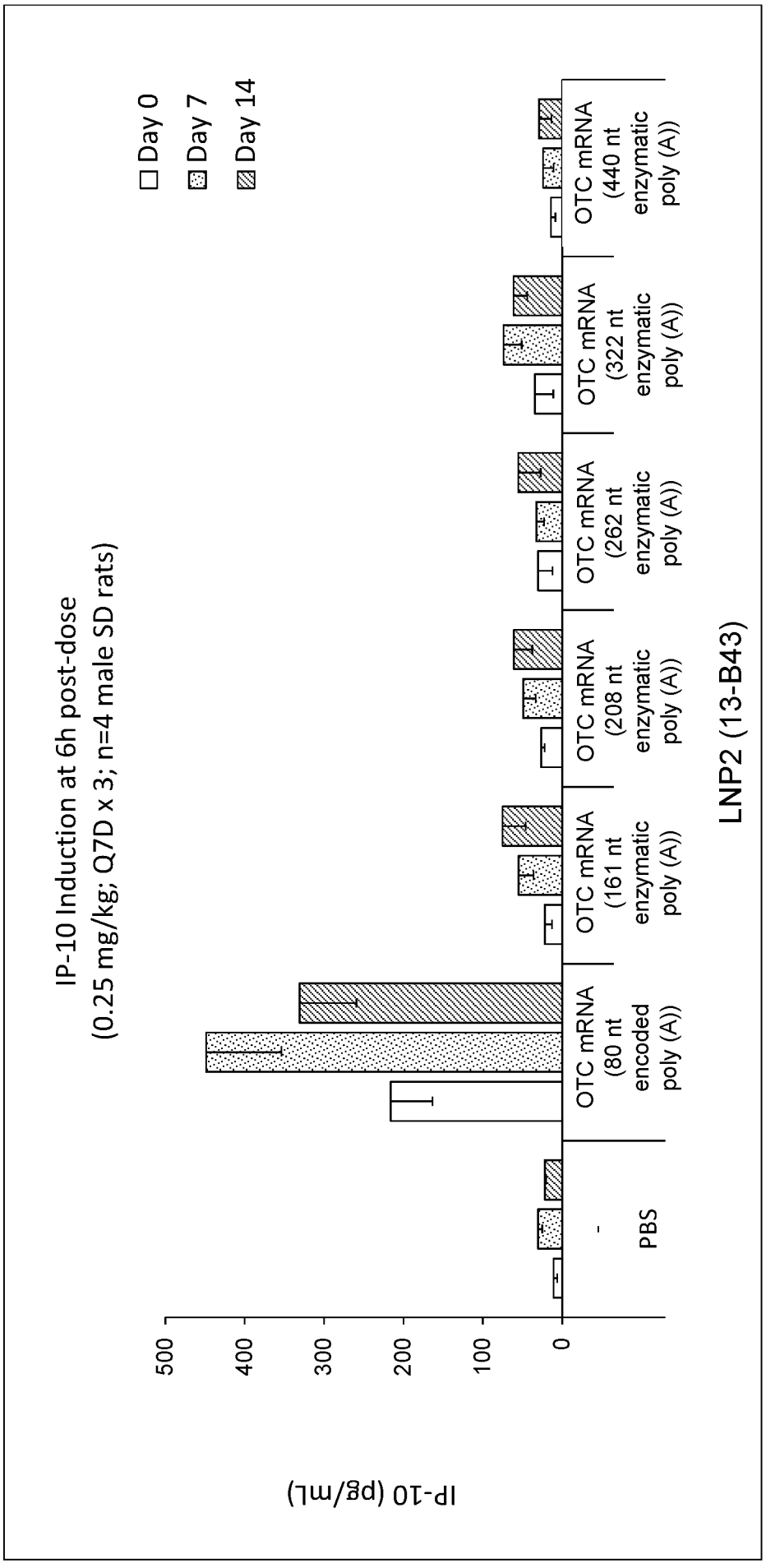
IP-10 Induction at 6h post-dose
(0.25 mg/kg; Q7D x 3; n=4 male SD rats)
Day 0
Day 7
Day 14
IP-10 (pg/mL)
500
400
300
200
100
0
-
PBS
OTC mRNA (80 nt encoded poly (A))
OTC mRNA (161 nt enzymatic poly (A))
OTC mRNA (208 nt enzymatic poly (A))
OTC mRNA (262 nt enzymatic poly (A))
OTC mRNA (322 nt enzymatic poly (A))
OTC mRNA (440 nt enzymatic poly (A))
LNP2 (13-B43)

FIG. 2B

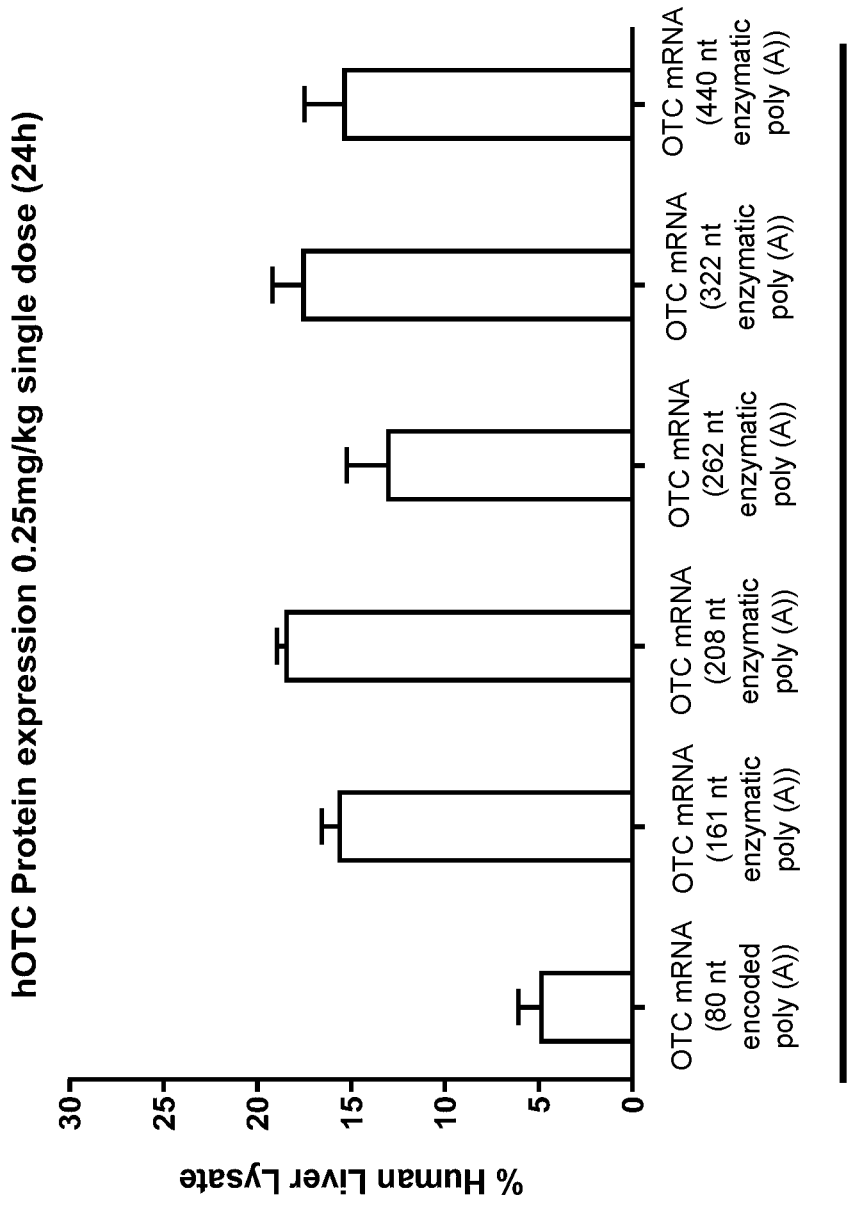
hOTC Protein expression 0.25mg/kg single dose (24h)
% Human Liver Lysate
30
25
20
15
10
5
0
OTC mRNA (80 nt encoded poly (A))
OTC mRNA (161 nt enzymatic poly (A))
OTC mRNA (208 nt enzymatic poly (A))
OTC mRNA (262 nt enzymatic poly (A))
OTC mRNA (322 nt enzymatic poly (A))
OTC mRNA (440 nt enzymatic poly (A))
LNP 2

FIG. 3A

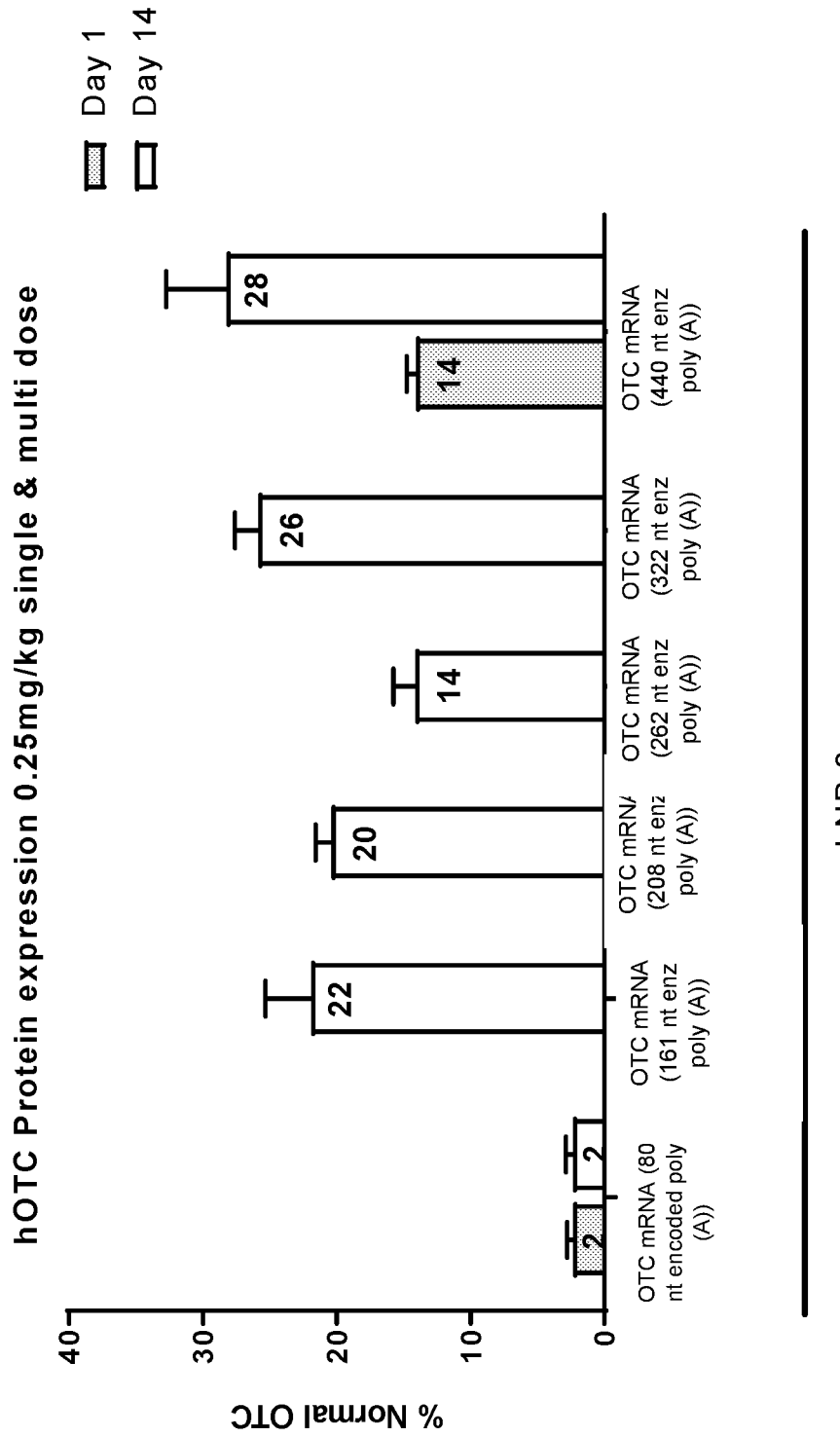
hOTC Protein expression 0.25mg/kg single & multi dose
Day 1
Day 14
% Normal OTC
0
10
20
30
40
2
2
22
20
14
26
14
28
OTC mRNA (80 nt encoded poly (A))
OTC mRNA (161 nt enz poly (A))
OTC mRNA (208 nt enz poly (A))
OTC mRNA (262 nt enz poly (A))
OTC mRNA (322 nt enz poly (A))
OTC mRNA (440 nt enz poly (A))
LNP 2

FIG. 3B

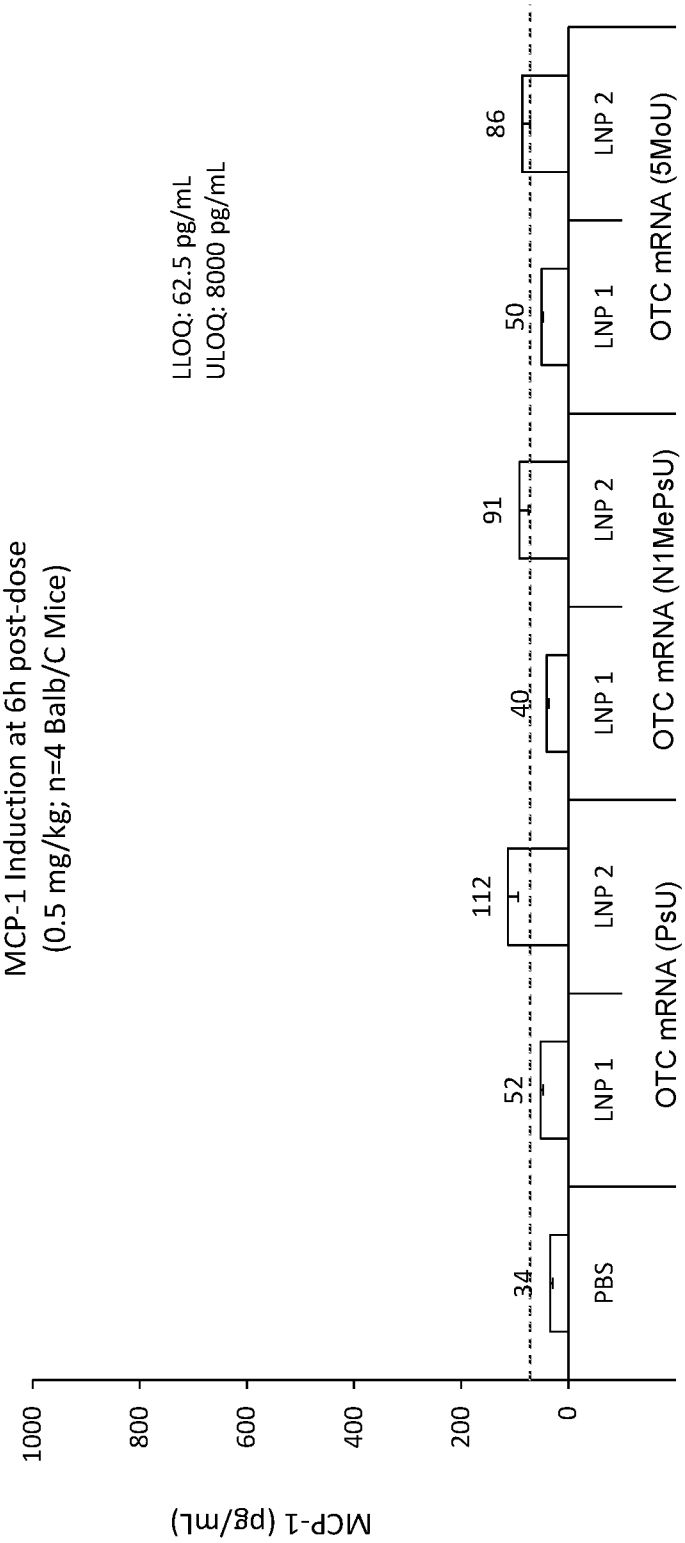
MCP-1 Induction at 6h post-dose
(0.5 mg/kg; n=4 Balb/C Mice)
LLOQ: 62.5 pg/mL
ULOQ: 8000 pg/mL
MCP-1 (pg/mL)
1000
800
600
400
200
0
34
52
112
40
91
50
86
PBS
LNP 1
LNP 2
LNP 1
LNP 2
LNP 1
LNP 2
OTC mRNA (PsU)
OTC mRNA (N1MePsU)
OTC mRNA (5MoU)

FIG. 4

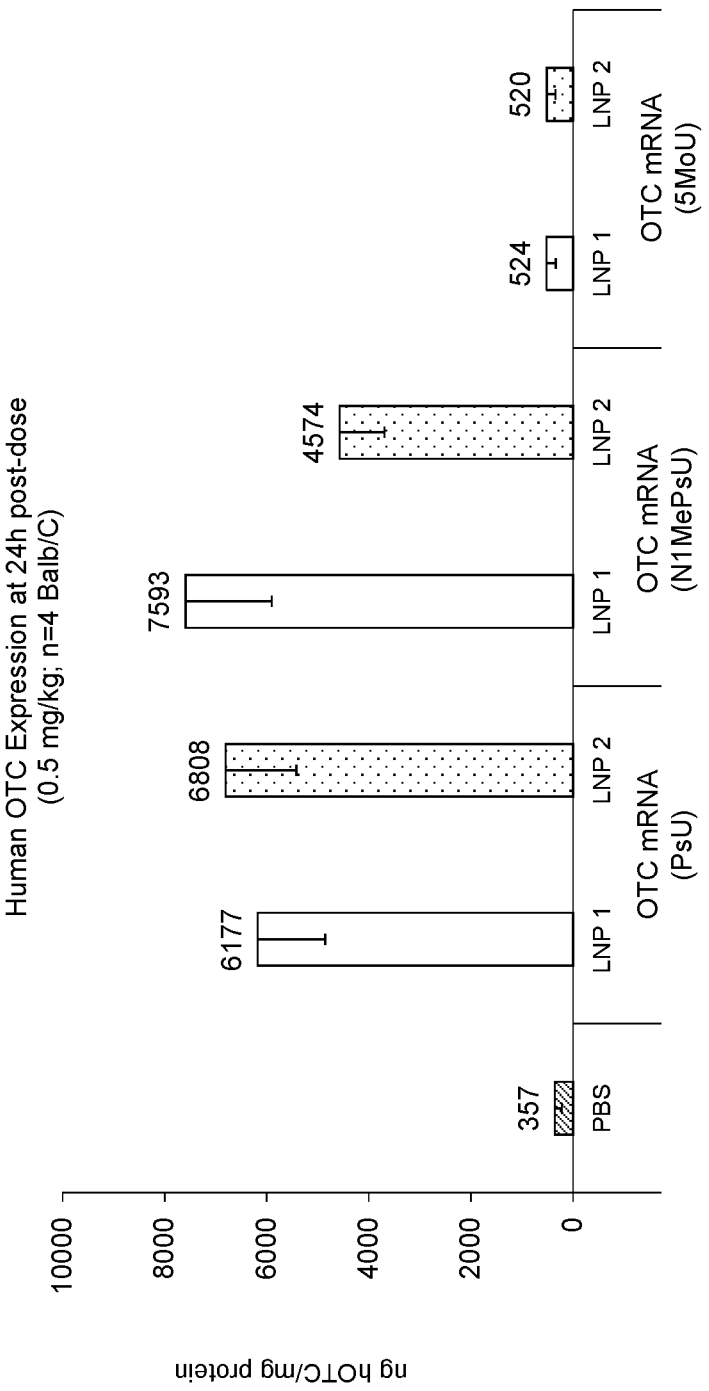
Human OTC Expression at 24h post-dose
(0.5 mg/kg; n=4 Balb/C)
ng hOTC/mg protein
10000
8000
6000
4000
2000
0
357
PBS
6177
6808
LNP 1
LNP 2
OTC mRNA
(PsU)
7593
4574
LNP 1
LNP 2
OTC mRNA
(N1MePsU)
524
520
LNP 1
LNP 2
OTC mRNA
(5MoU)

FIG. 5

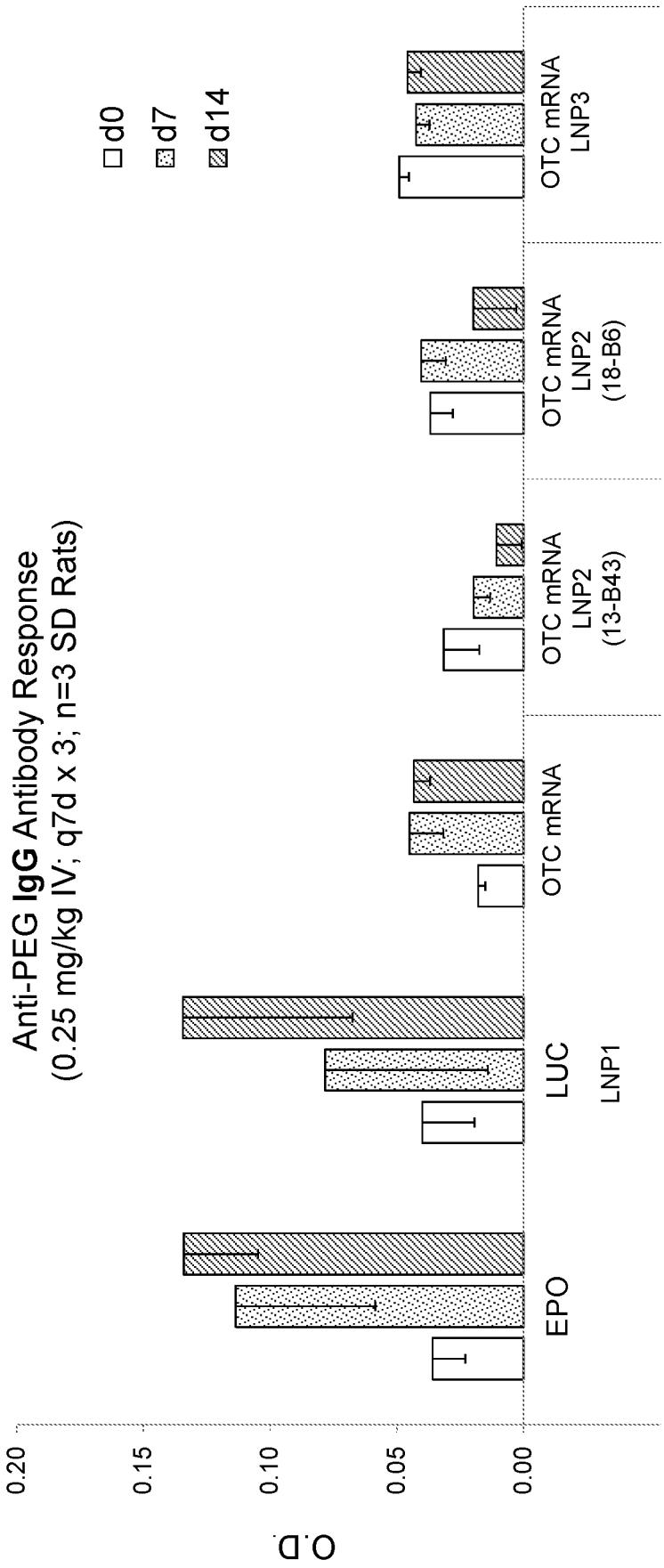
Anti-PEG IgG Antibody Response
(0.25 mg/kg IV; q7d x 3; n=3 SD Rats)
O.D.
0.20
0.15
0.10
0.05
0.00
d0
d7
d14
EPO
LUC
OTC mRNA
LNP1
OTC mRNA
LNP2
(13-B43)
OTC mRNA
LNP2
(18-B6)
OTC mRNA
LNP3

FIG. 6A

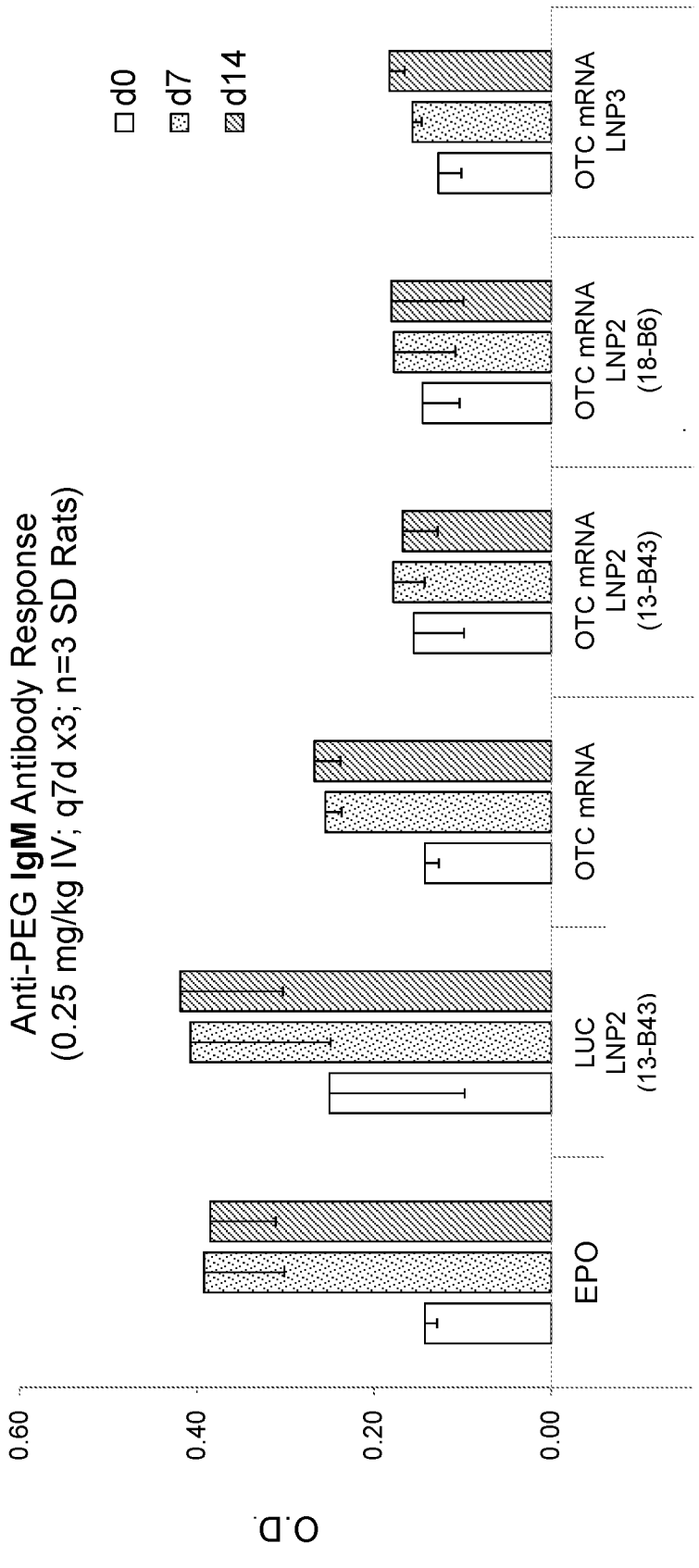
Anti-PEG IgM Antibody Response
(0.25 mg/kg IV; q7d x3; n=3 SD Rats)
O.D.
0.60
0.40
0.20
0.00
d0
d7
d14
EPO
LUC LNP2 (13-B43)
OTC mRNA
OTC mRNA LNP2 (13-B43)
OTC mRNA LNP2 (18-B6)
OTC mRNA LNP3

FIG. 6B

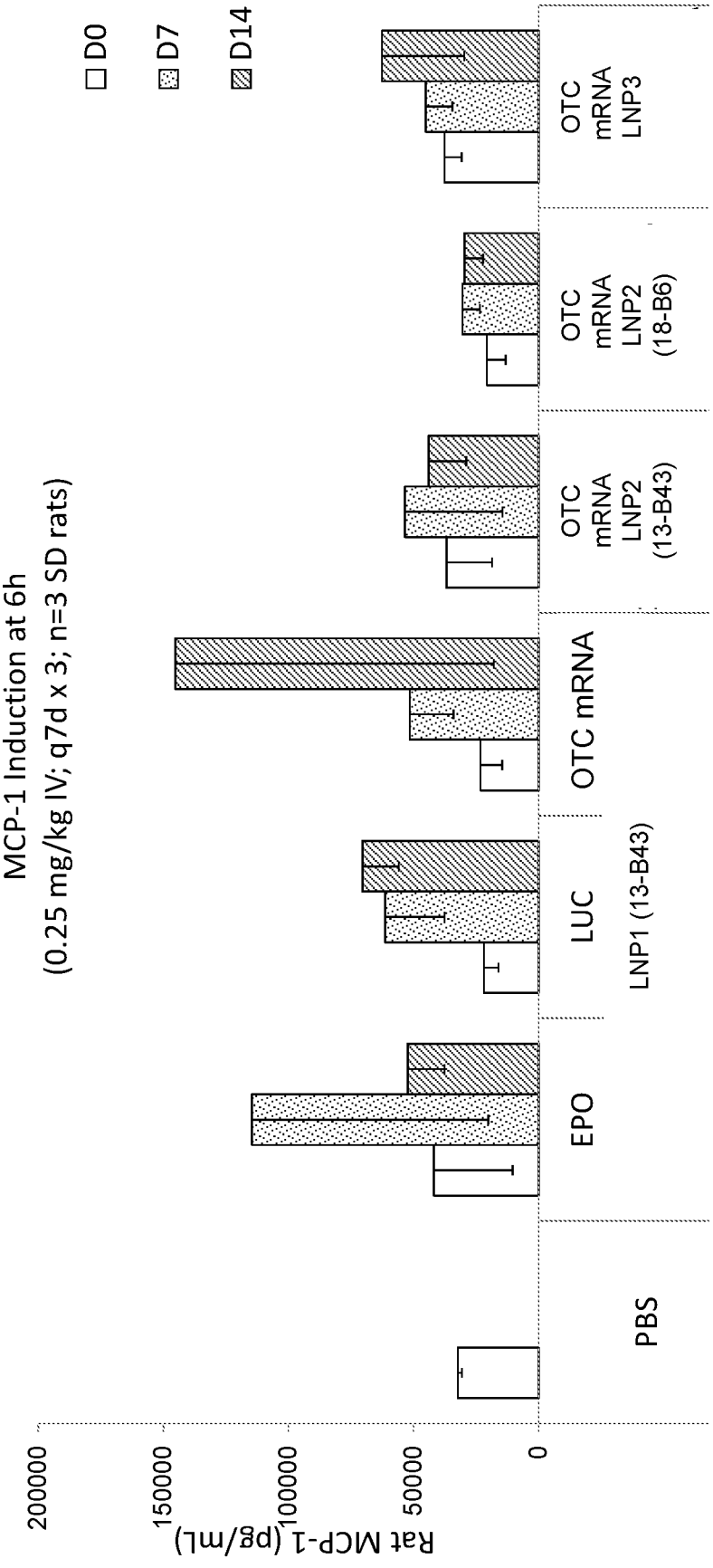
MCP-1 Induction at 6h
(0.25 mg/kg IV; q7d x 3; n=3 SD rats)
D0
D7
D14
Rat MCP-1 (pg/mL)
200000
150000
100000
50000
0
PBS
EPO
LUC
OTC mRNA
OTC mRNA LNP2 (13-B43)
OTC mRNA LNP2 (18-B6)
OTC mRNA LNP3
LNP1 (13-B43)

FIG. 7

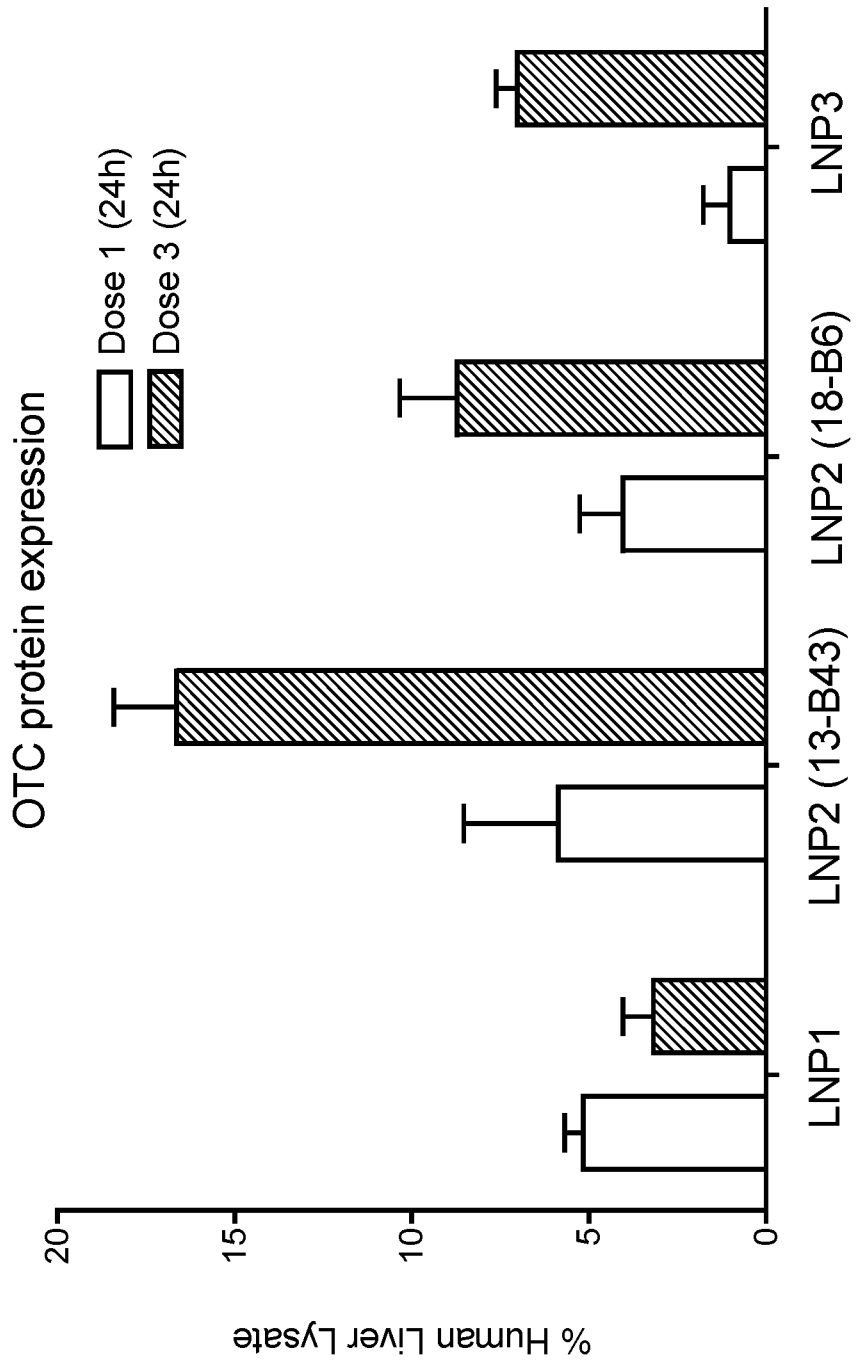
OTC protein expression
Dose 1 (24h)
Dose 3 (24h)
% Human Liver Lysate
0
5
10
15
20
LNP1
LNP2 (13-B43)
LNP2 (18-B6)
LNP3

FIG. 8

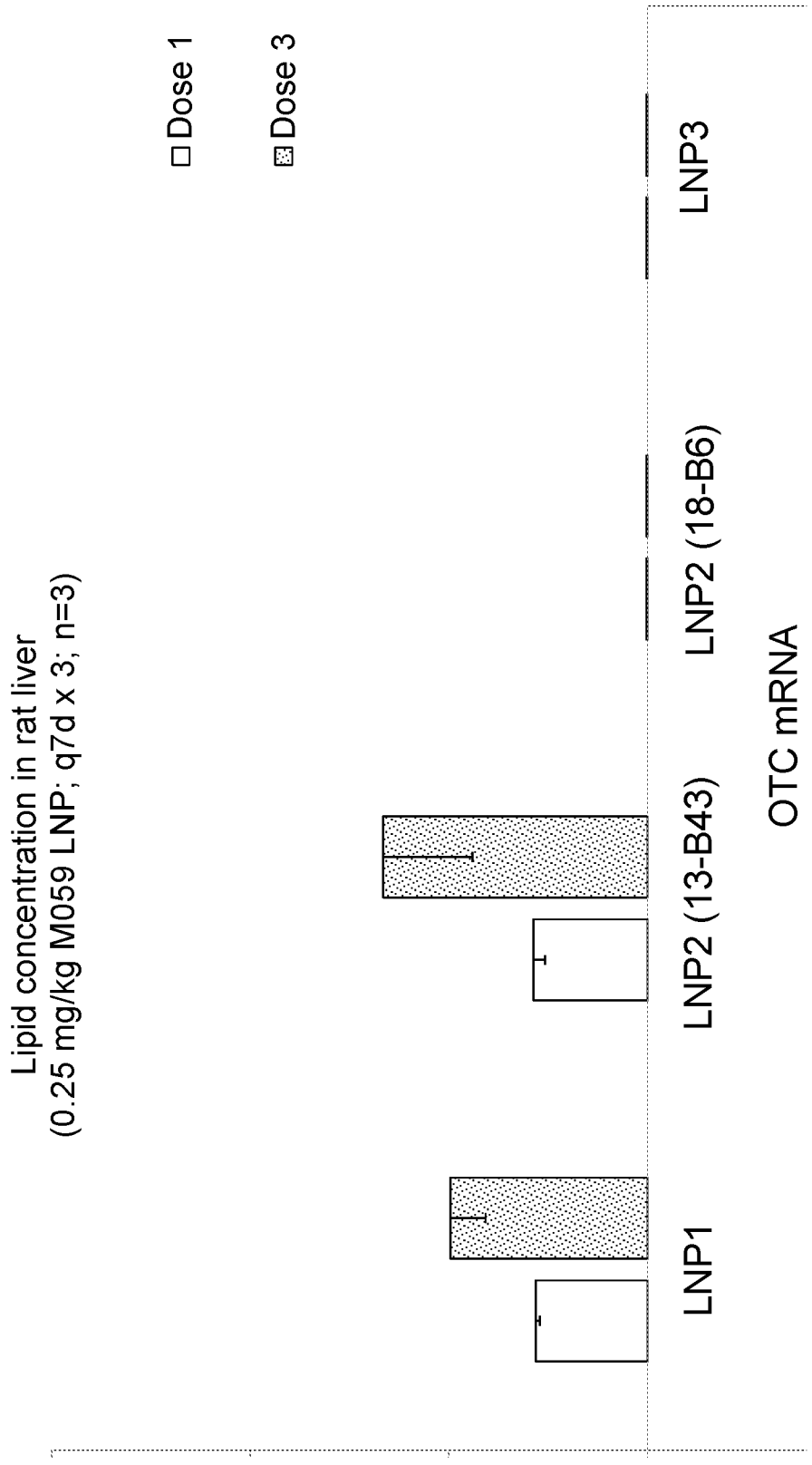
Lipid concentration in rat liver
(0.25 mg/kg M059 LNP; q7d x 3; n=3)
Dose 1
Dose 3
Lipid (ng/g liver)
150000
100000
50000
0
LNP1
LNP2 (13-B43)
LNP2 (18-B6)
LNP3
OTC mRNA

FIG. 9

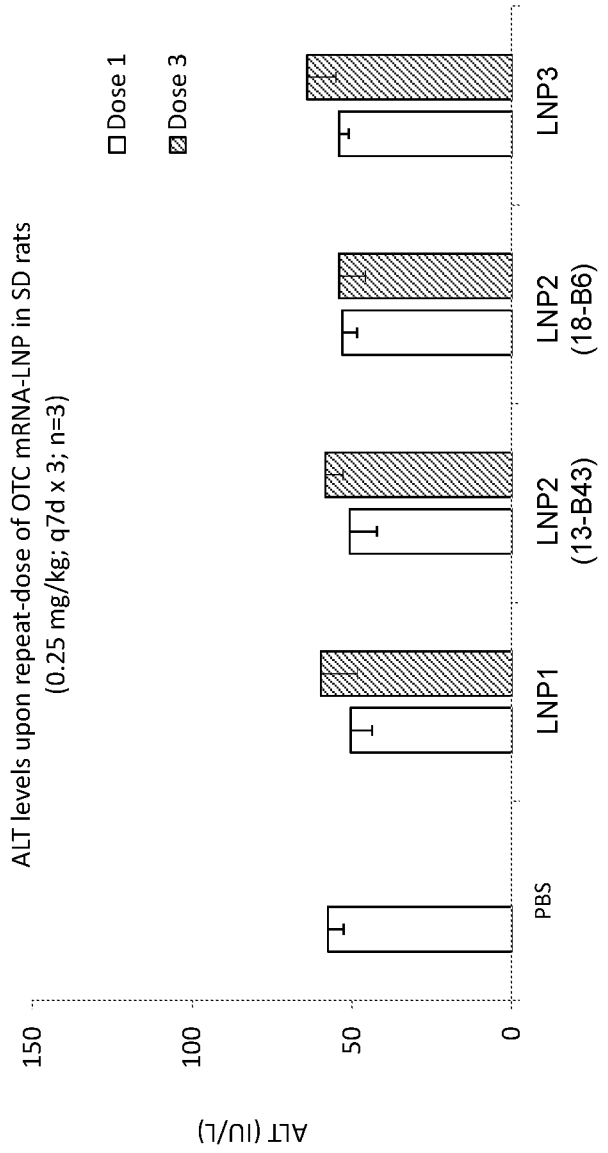
ALT levels upon repeat-dose of OTC mRNA-LNP in SD rats
(0.25 mg/kg; q7d x 3; n=3)
Dose 1
Dose 3
ALT (IU/L)
150
100
50
0
PBS
LNP1
LNP2
(13-B43)
LNP2
(18-B6)
LNP3

FIG. 10A

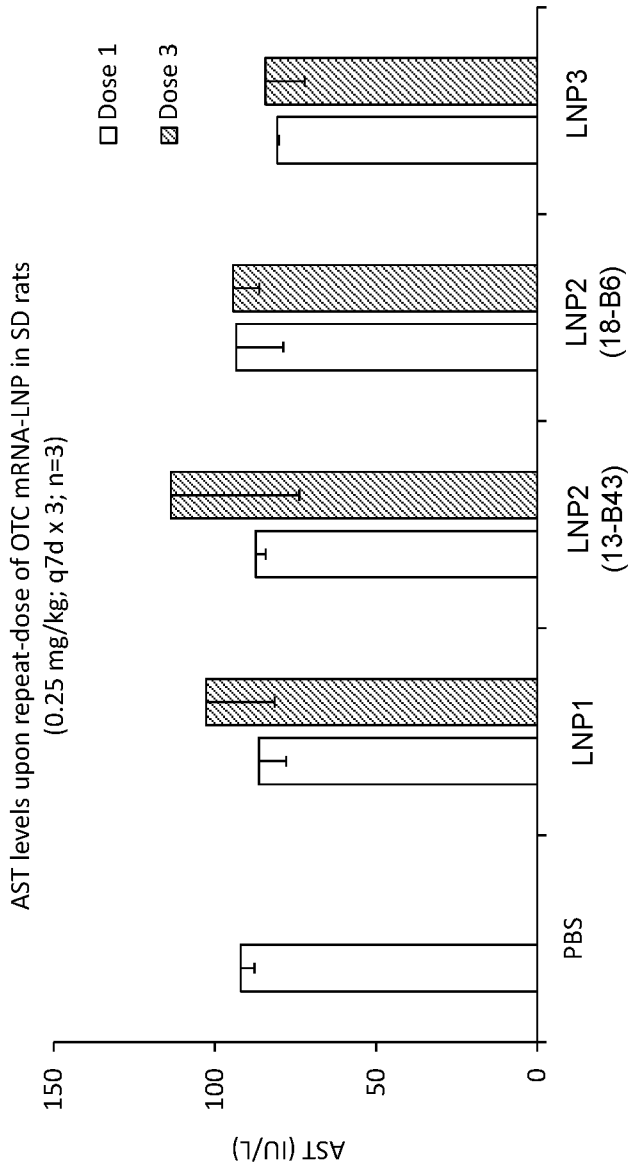
AST levels upon repeat-dose of OTC mRNA-LNP in SD rats
(0.25 mg/kg; q7d x 3; n=3)
Dose 1
Dose 3
AST (IU/L)
150
100
50
0
PBS
LNP1
LNP2
(13-B43)
LNP2
(18-B6)
LNP3

FIG. 10B

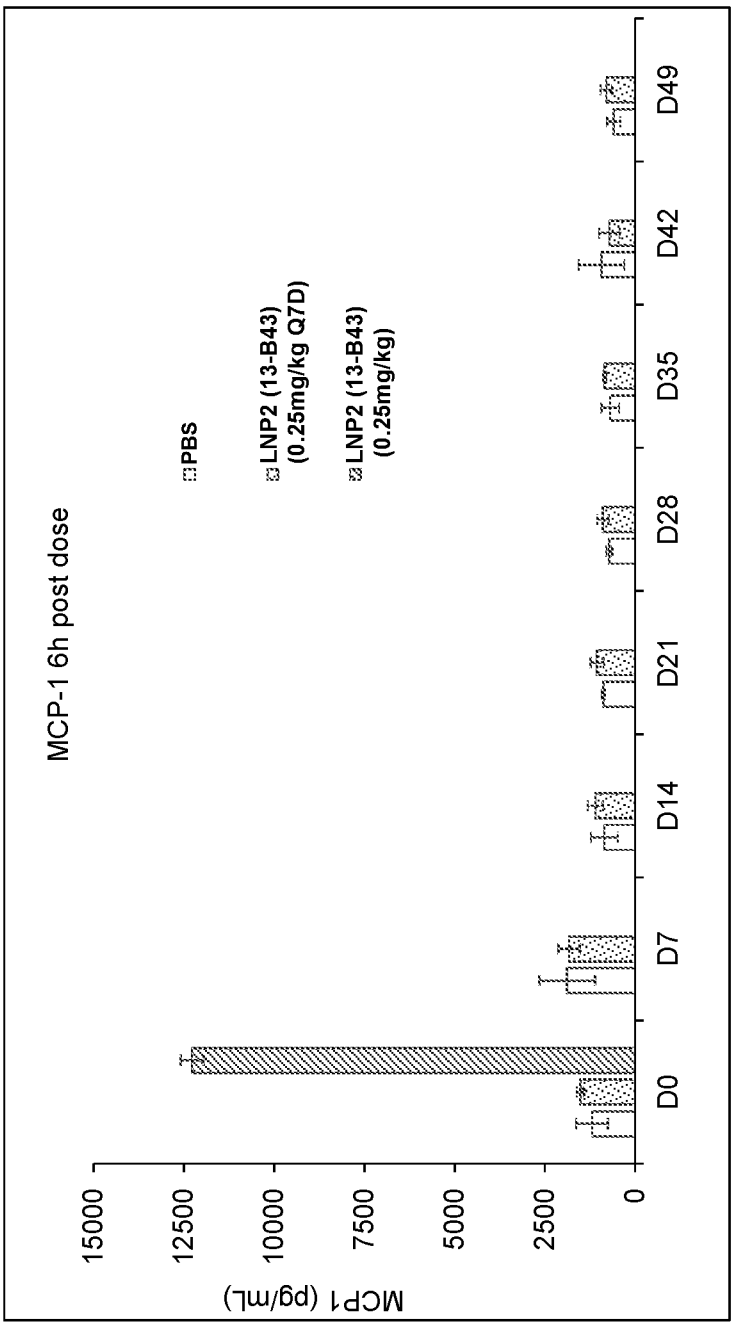
MCP-1 6h post dose
PBS
LNP2 (13-B43) (0.25mg/kg Q7D)
LNP2 (13-B43) (0.25mg/kg)
MCP1 (pg/mL)
15000
12500
10000
7500
5000
2500
0
D0
D7
D14
D21
D28
D35
D42
D49

FIG. 11A

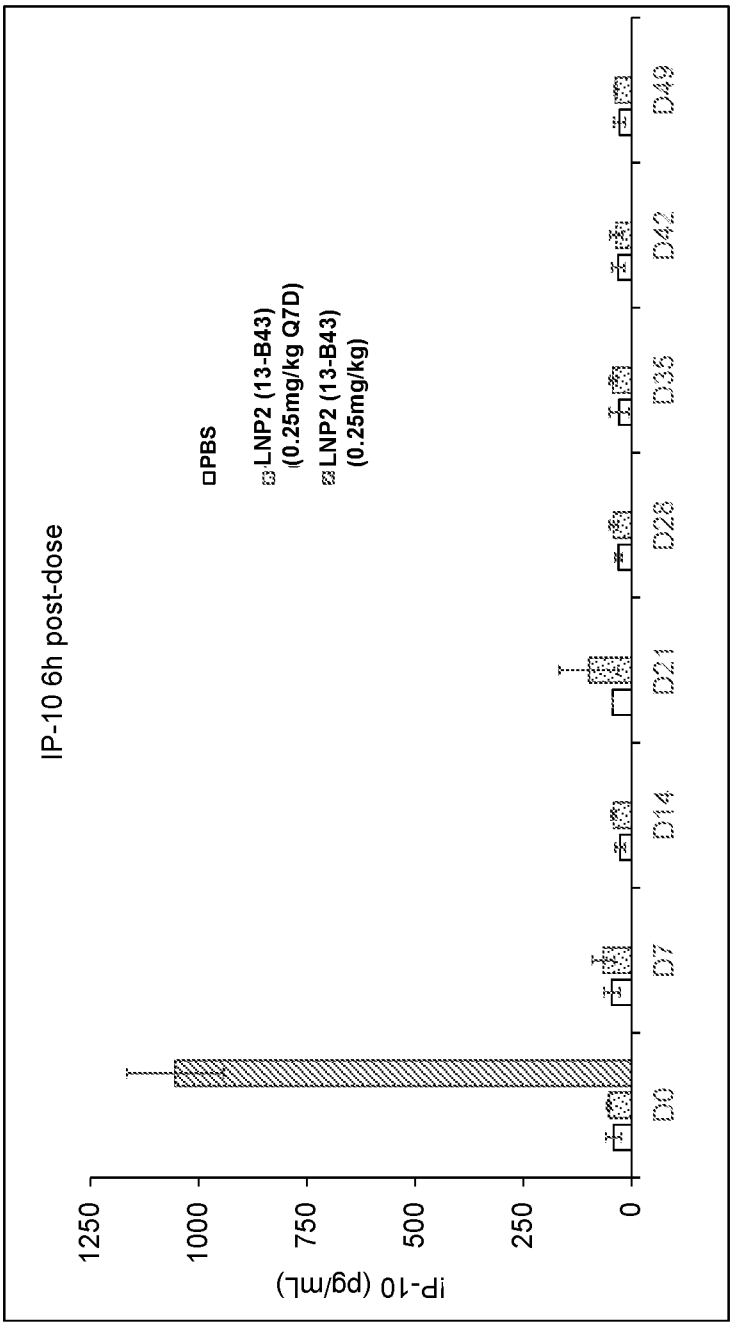
IP-10 6h post-dose
PBS
LNP2 (13-B43) (0.25mg/kg Q7D)
LNP2 (13-B43) (0.25mg/kg)
IP-10 (pg/mL)
1250
1000
750
500
250
0
D0
D7
D14
D21
D28
D35
D42
D49

FIG. 11B

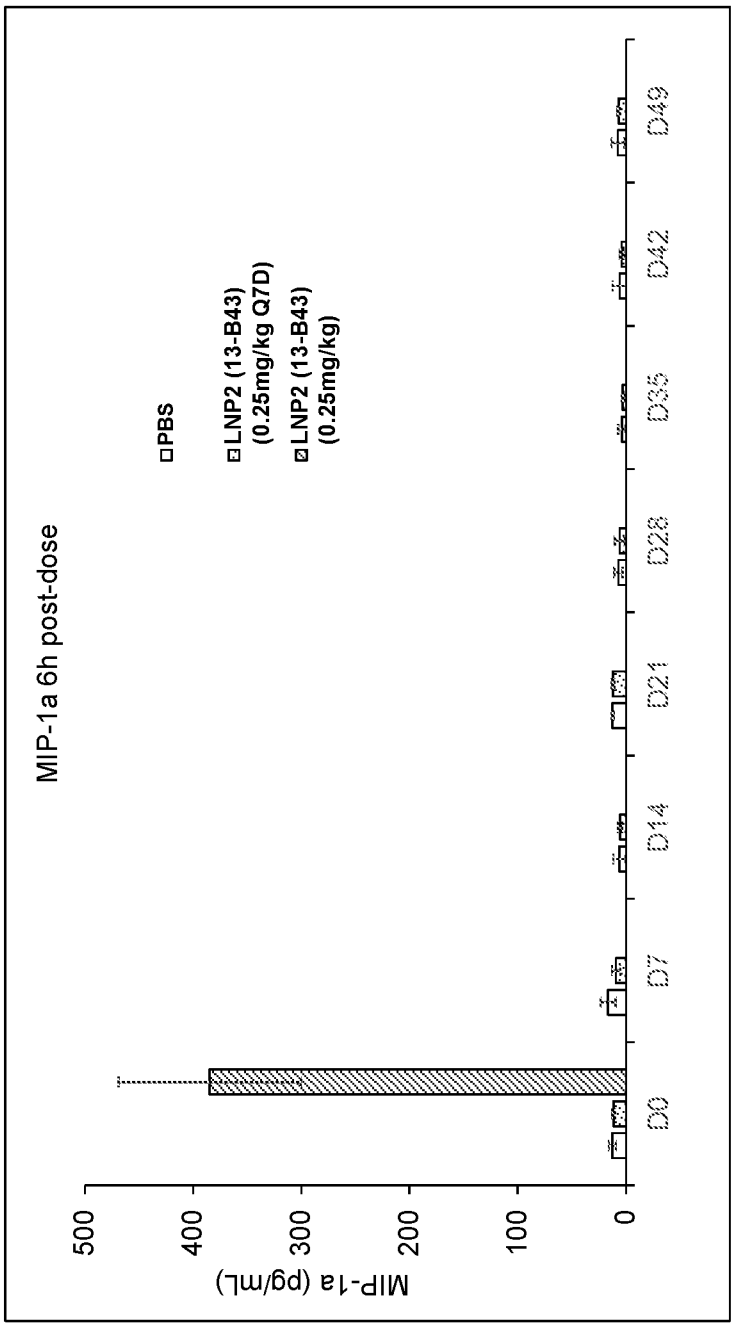
MIP-1a 6h post-dose
PBS
LNP2 (13-B43) (0.25mg/kg Q7D)
LNP2 (13-B43) (0.25mg/kg)
MIP-1a (pg/mL)
500
400
300
200
100
0
D0
D7
D14
D21
D28
D35
D42
D49

FIG. 11C

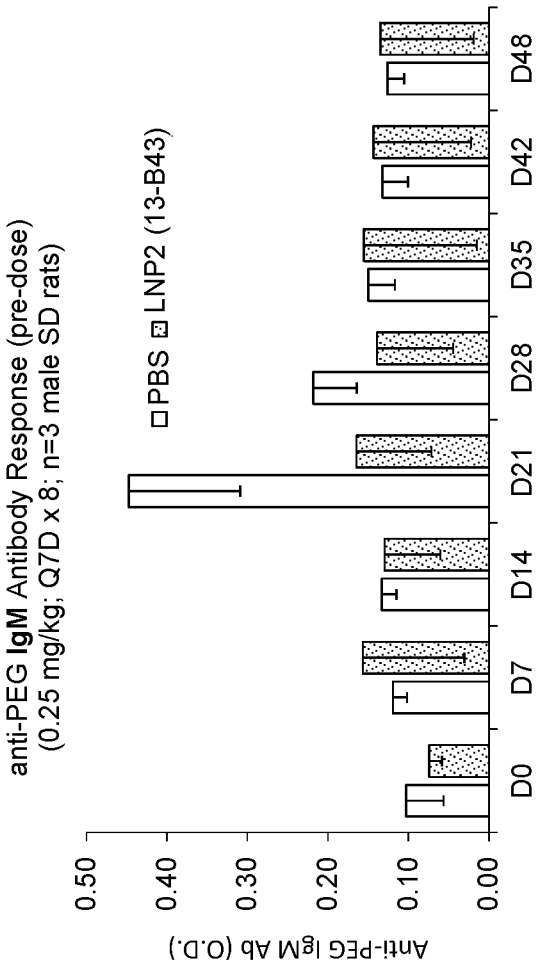
anti-PEG IgM Antibody Response (pre-dose)
(0.25 mg/kg; Q7D x 8; n=3 male SD rats)
PBS
LNP2 (13-B43)
Anti-PEG IgM Ab (O.D.)
0.00
0.10
0.20
0.30
0.40
0.50
D0
D7
D14
D21
D28
D35
D42
D48

FIG. 12

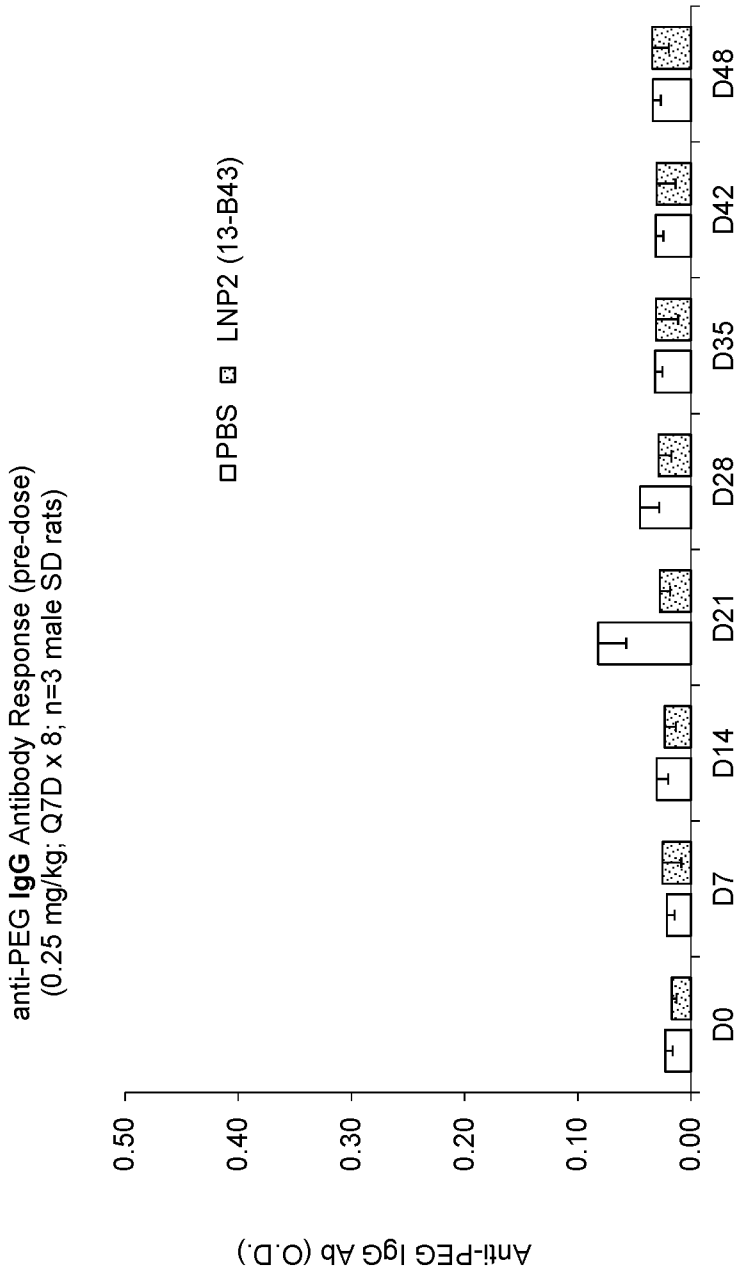
anti-PEG IgG Antibody Response (pre-dose)
(0.25 mg/kg; Q7D x 8; n=3 male SD rats)
PBS
LNP2 (13-B43)
Anti-PEG IgG Ab (O.D.)
0.00
0.10
0.20
0.30
0.40
0.50
D0
D7
D14
D21
D28
D35
D42
D48

FIG. 13

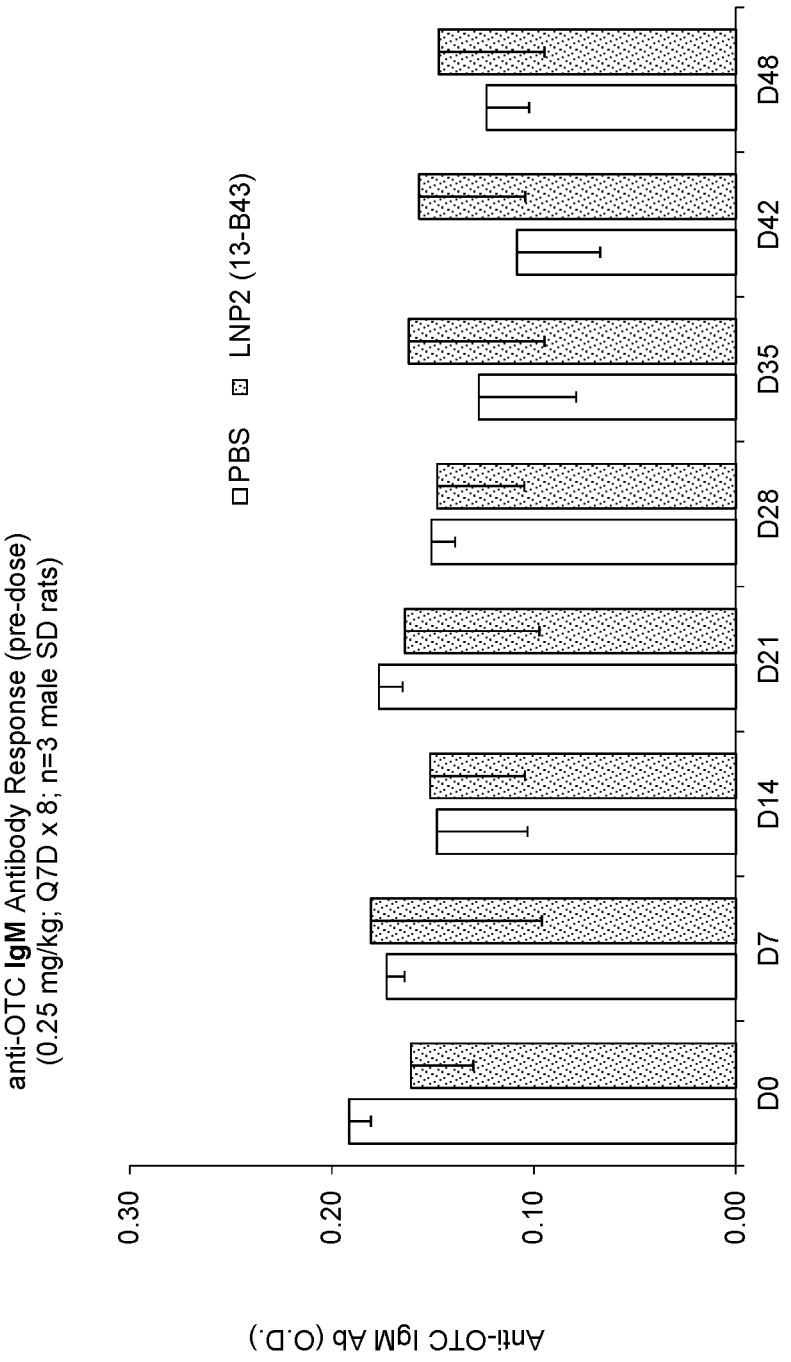
anti-OTC IgM Antibody Response (pre-dose)
(0.25 mg/kg; Q7D x 8; n=3 male SD rats)
PBS
LNP2 (13-B43)
Anti-OTC IgM Ab (O.D.)
0.30
0.20
0.10
0.00
D0
D7
D14
D21
D28
D35
D42
D48

FIG. 14

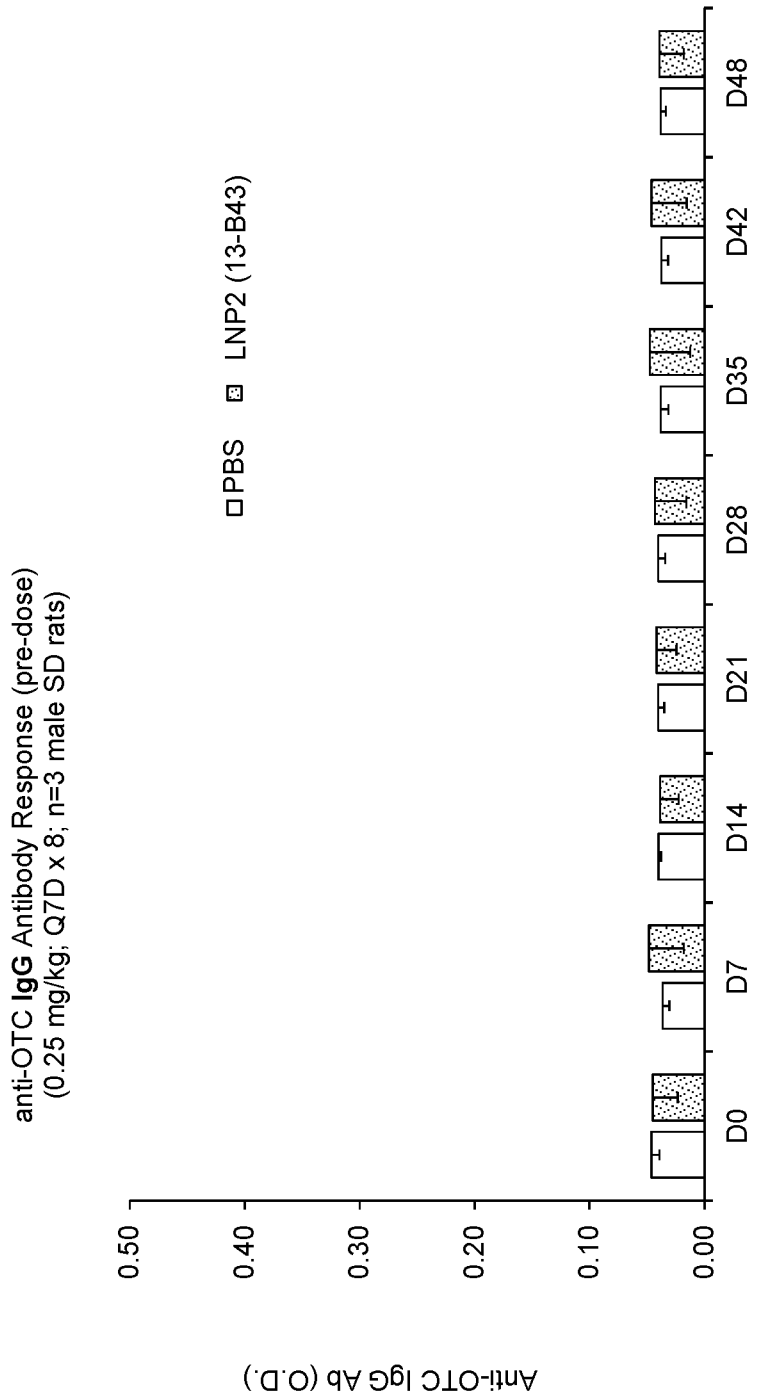
anti-OTC IgG Antibody Response (pre-dose)
(0.25 mg/kg; Q7D x 8; n=3 male SD rats)
PBS
LNP2 (13-B43)
Anti-OTC IgG Ab (O.D.)
0.00
0.10
0.20
0.30
0.40
0.50
D0
D7
D14
D21
D28
D35
D42
D48

FIG. 15

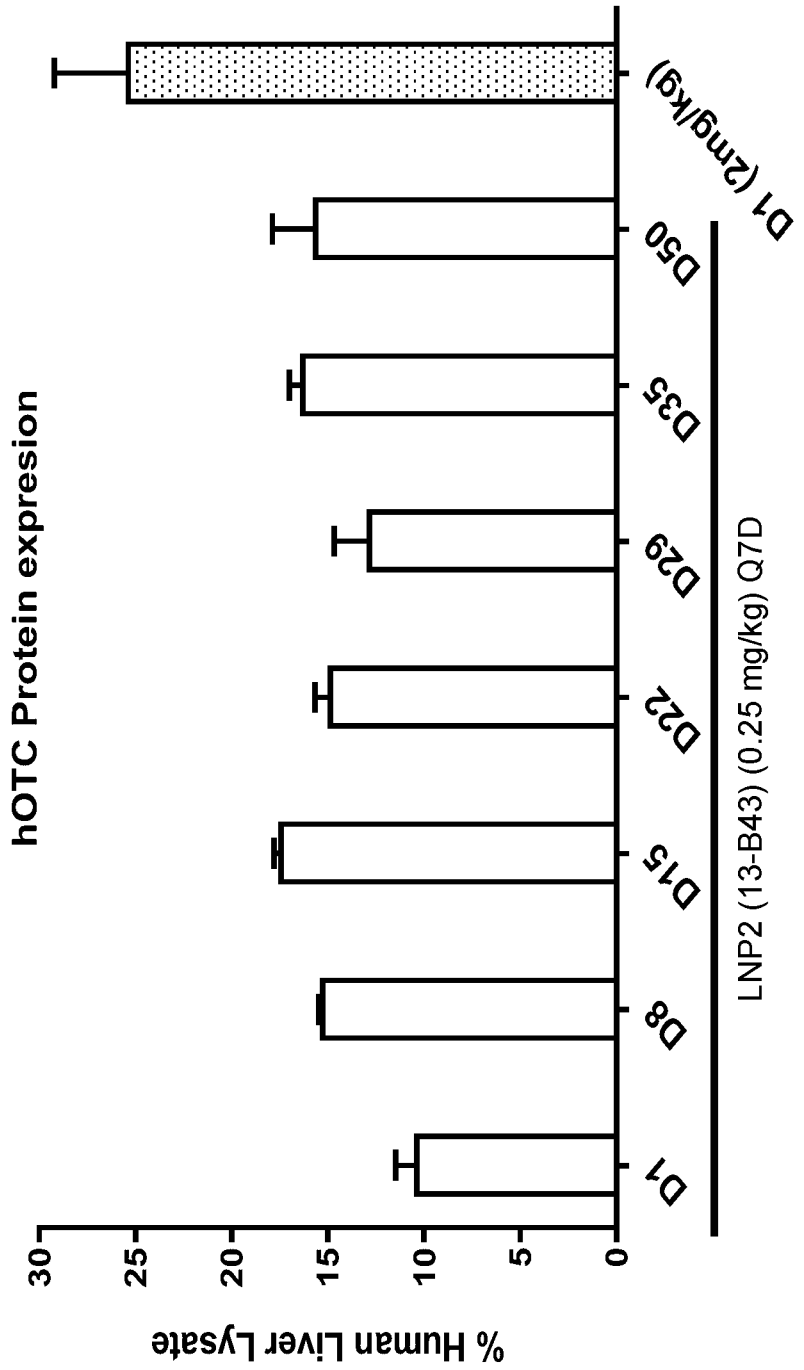
hOTC Protein expresion
% Human Liver Lysate
30
25
20
15
10
5
0
D1
D8
D15
D22
D29
D35
D50
D1 (2mg/kg)
LNP2 (13-B43) (0.25 mg/kg) Q7D

FIG. 16

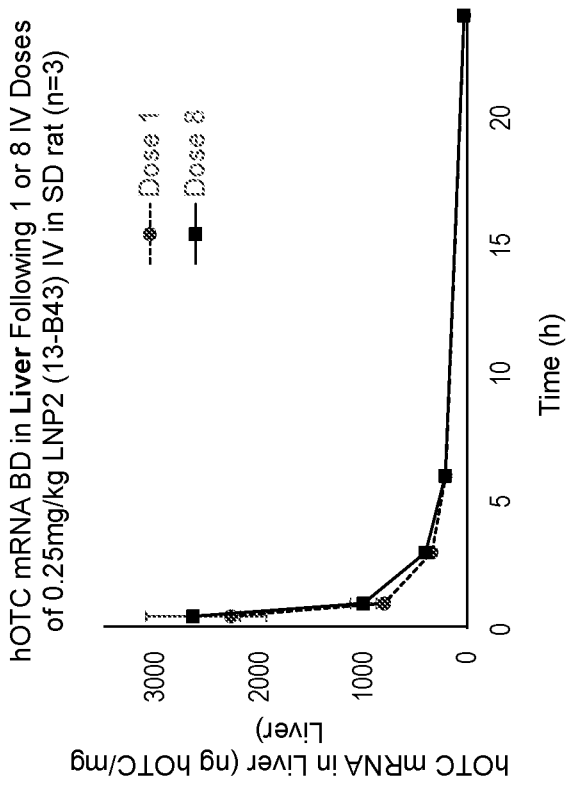

FIG. 17A

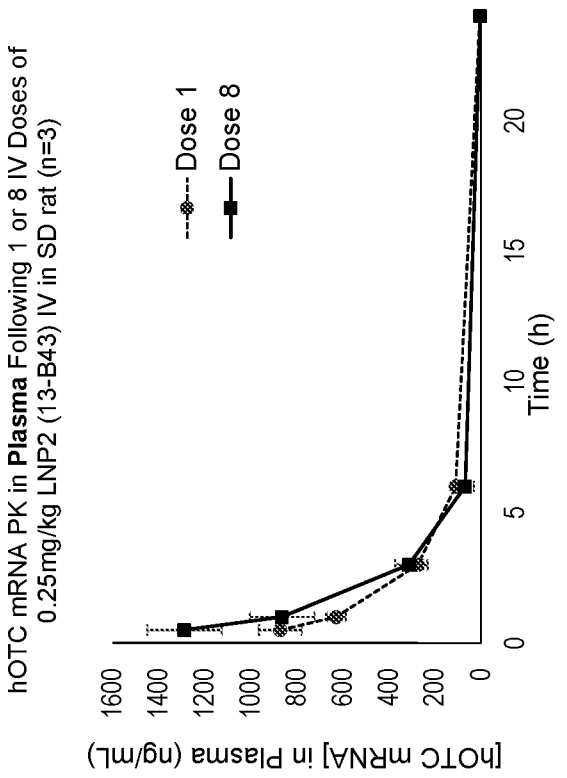

FIG. 17B

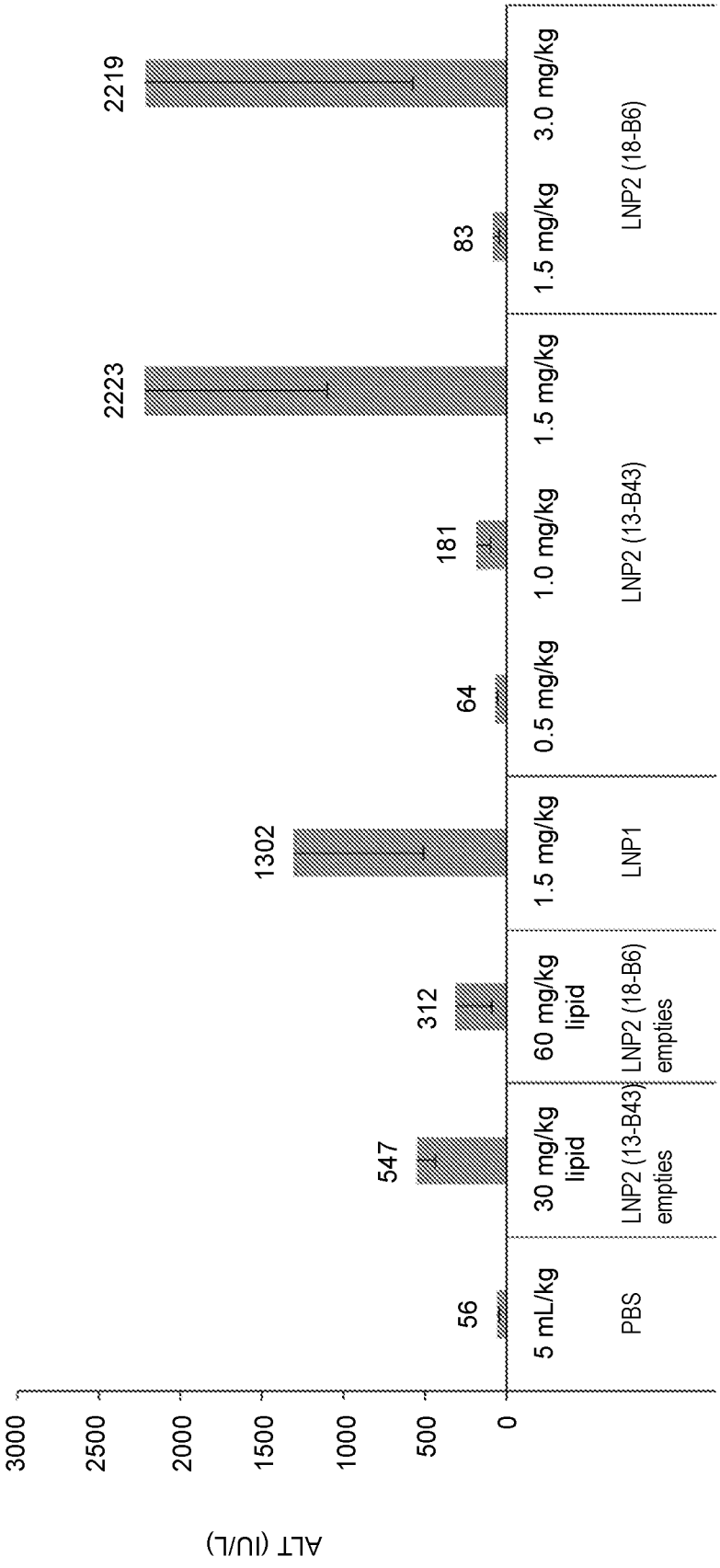
ALT at 24h post-dose
ALT (IU/L)
3000
2500
2000
1500
1000
500
0
56
547
312
1302
64
181
2223
83
2219
5 mL/kg
30 mg/kg lipid
60 mg/kg lipid
1.5 mg/kg
0.5 mg/kg
1.0 mg/kg
1.5 mg/kg
1.5 mg/kg
3.0 mg/kg
PBS
LNP2 (13-B43) empties
LNP2 (18-B6) empties
LNP1
LNP2 (13-B43)
LNP2 (18-B6)

FIG. 18A

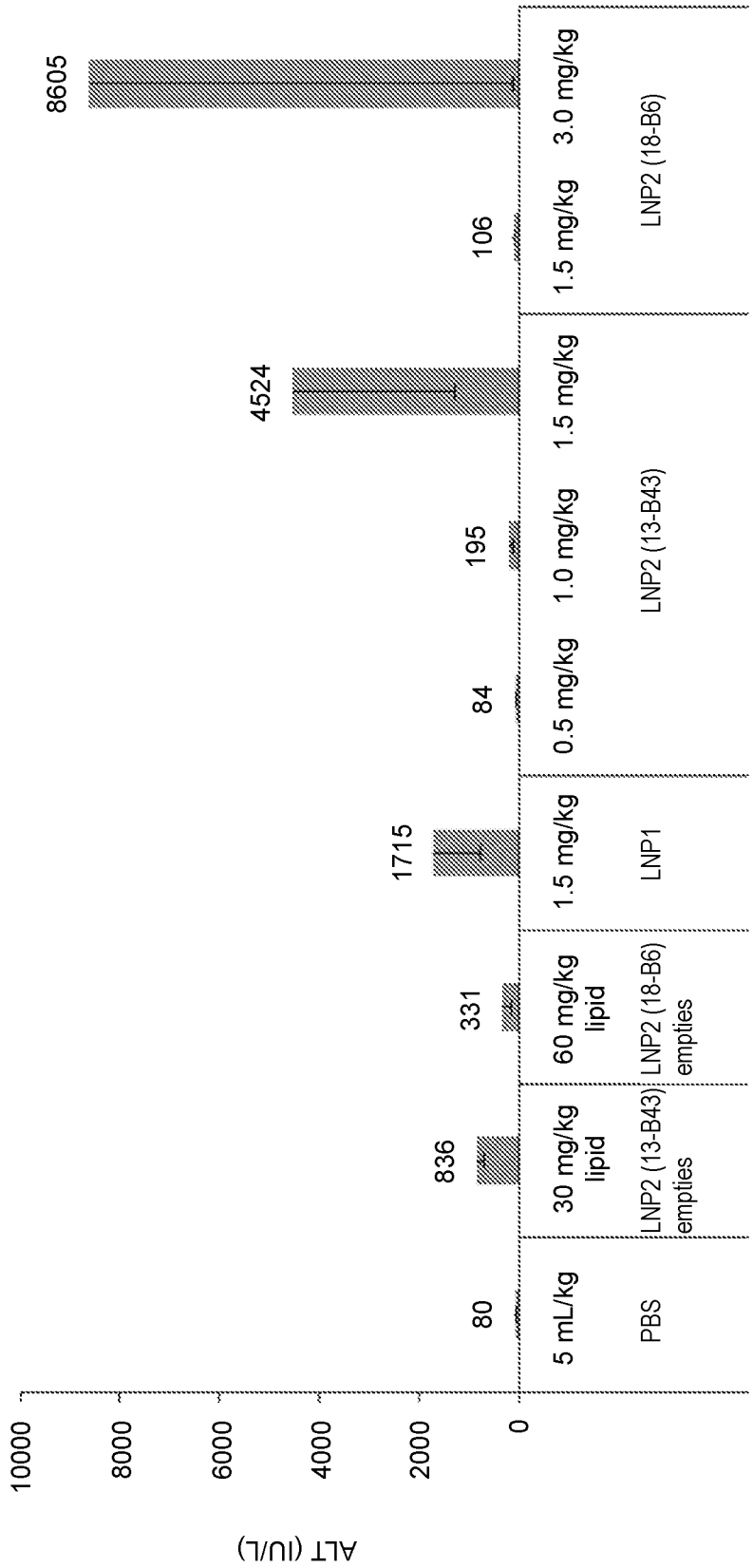
ALT at 24h post-dose
ALT (IU/L)
10000
8000
6000
4000
2000
0
80
836
331
1715
84
195
4524
106
8605
5 mL/kg
PBS
30 mg/kg lipid
LNP2 (13-B43) empties
60 mg/kg lipid
LNP2 (18-B6) empties
1.5 mg/kg
LNP1
0.5 mg/kg
1.0 mg/kg
1.5 mg/kg
LNP2 (13-B43)
1.5 mg/kg
3.0 mg/kg
LNP2 (18-B6)

FIG. 18B

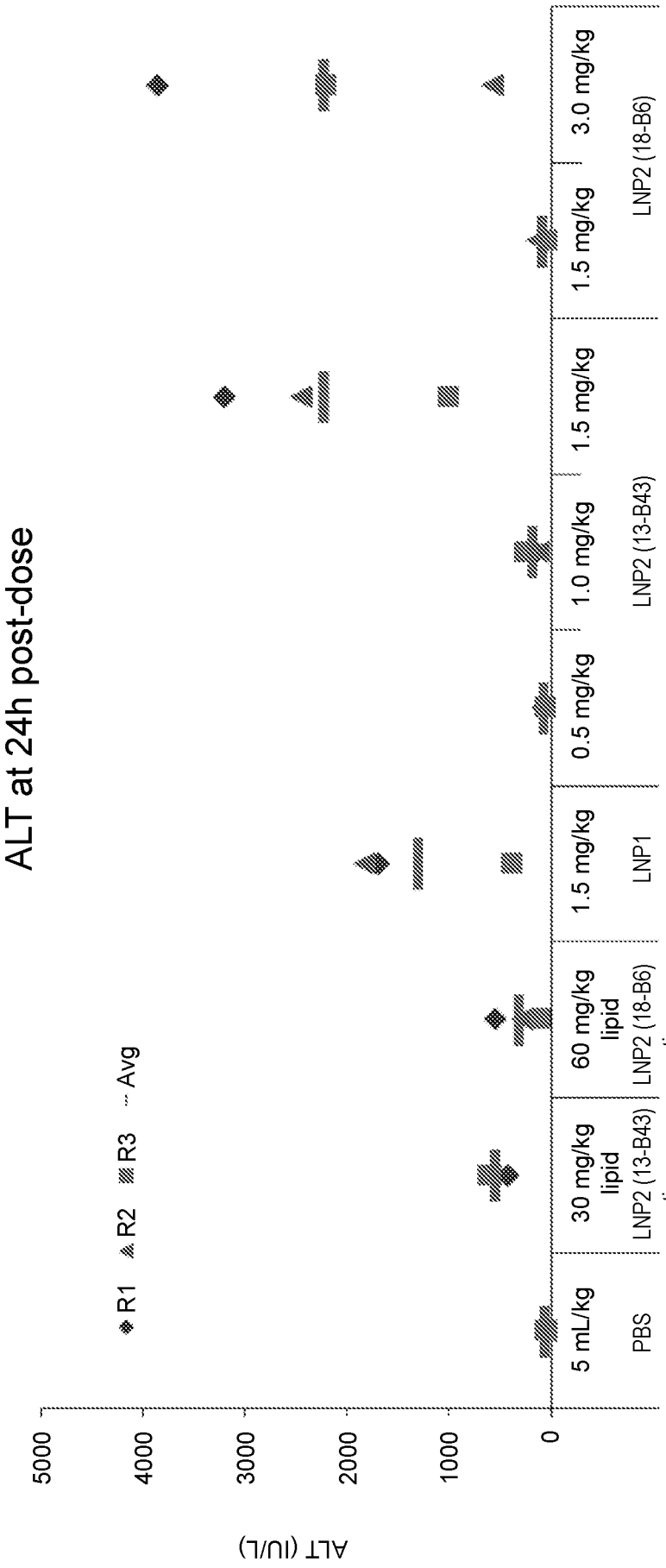
ALT at 24h post-dose
ALT (IU/L)
5000
4000
3000
2000
1000
0
R1
R2
R3
Avg
5 mL/kg
PBS
30 mg/kg lipid
LNP2 (13-B43) empties
60 mg/kg lipid
LNP2 (18-B6) empties
1.5 mg/kg
LNP1
0.5 mg/kg
1.0 mg/kg
1.5 mg/kg
LNP2 (13-B43)
1.5 mg/kg
3.0 mg/kg
LNP2 (18-B6)

FIG. 18C

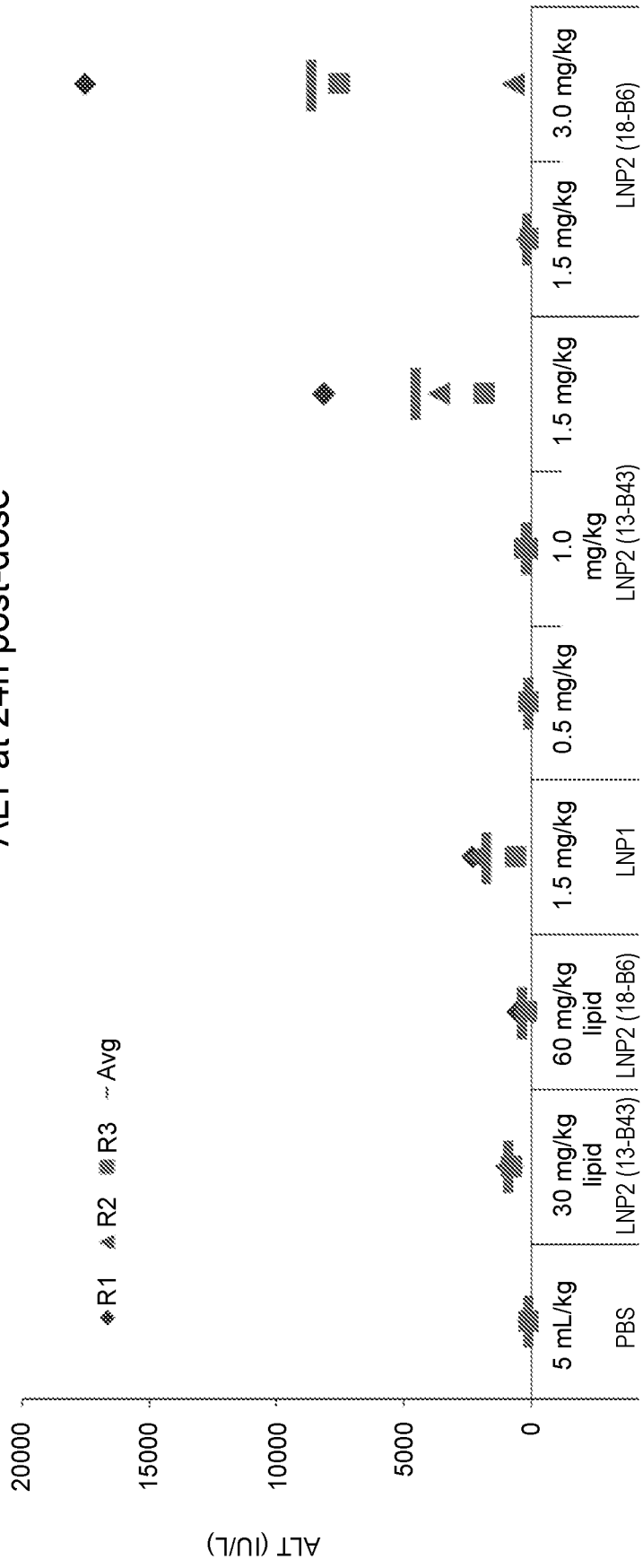
ALT at 24h post-dose
R1
R2
R3
Avg
ALT (IU/L)
20000
15000
10000
5000
0
5 mL/kg
PBS
30 mg/kg lipid
LNP2 (13-B43) empties
60 mg/kg lipid
LNP2 (18-B6) empties
1.5 mg/kg
LNP1
0.5 mg/kg
1.0 mg/kg
LNP2 (13-B43)
1.5 mg/kg
1.5 mg/kg
3.0 mg/kg
LNP2 (18-B6)

FIG. 18D

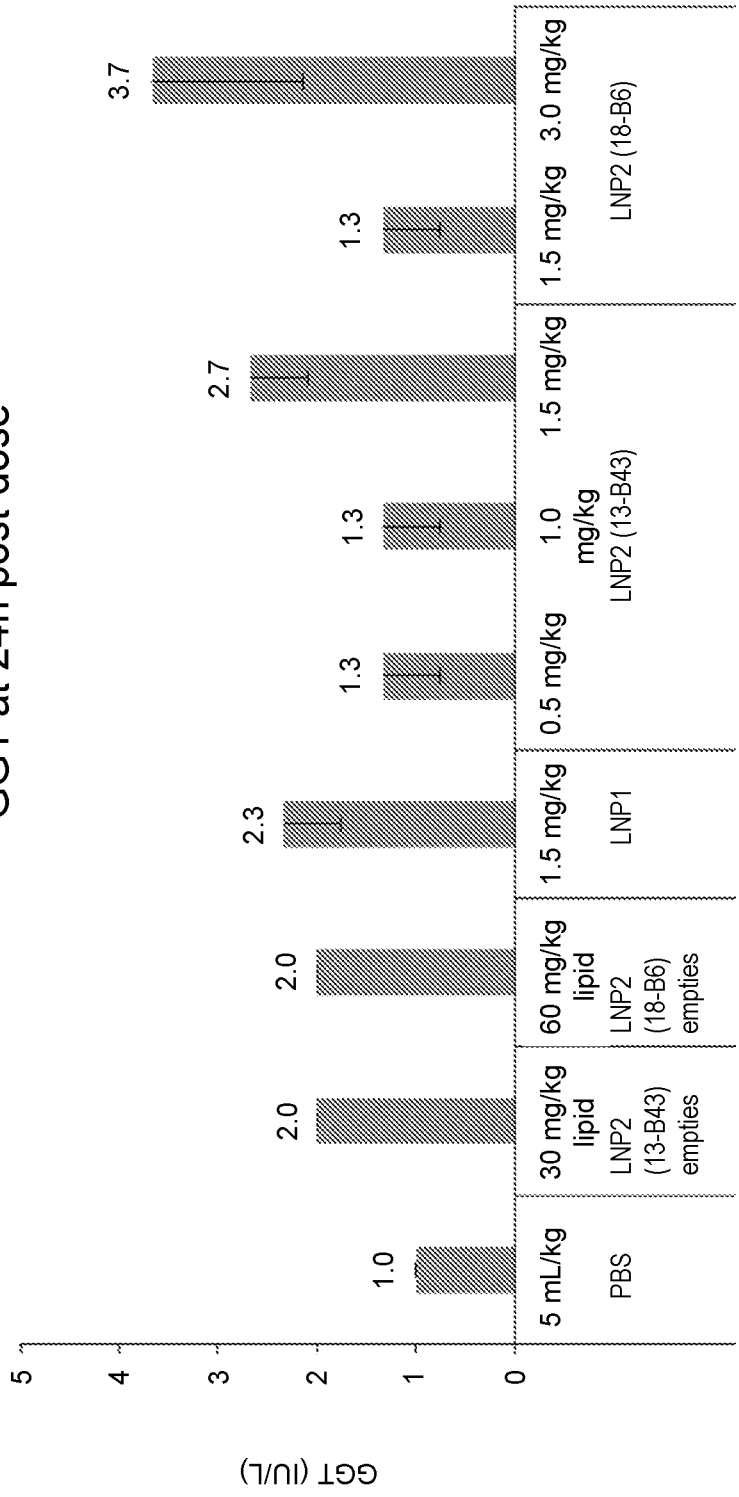
GGT at 24h post-dose
GGT (IU/L)
0
1
2
3
4
5
1.0
2.0
2.0
2.3
1.3
1.3
2.7
1.3
3.7
5 mL/kg
PBS
30 mg/kg lipid
LNP2 (13-B43) empties
60 mg/kg lipid
LNP2 (18-B6) empties
1.5 mg/kg
LNP1
0.5 mg/kg
1.0 mg/kg
1.5 mg/kg
LNP2 (13-B43)
1.5 mg/kg
3.0 mg/kg
LNP2 (18-B6)

FIG. 19A

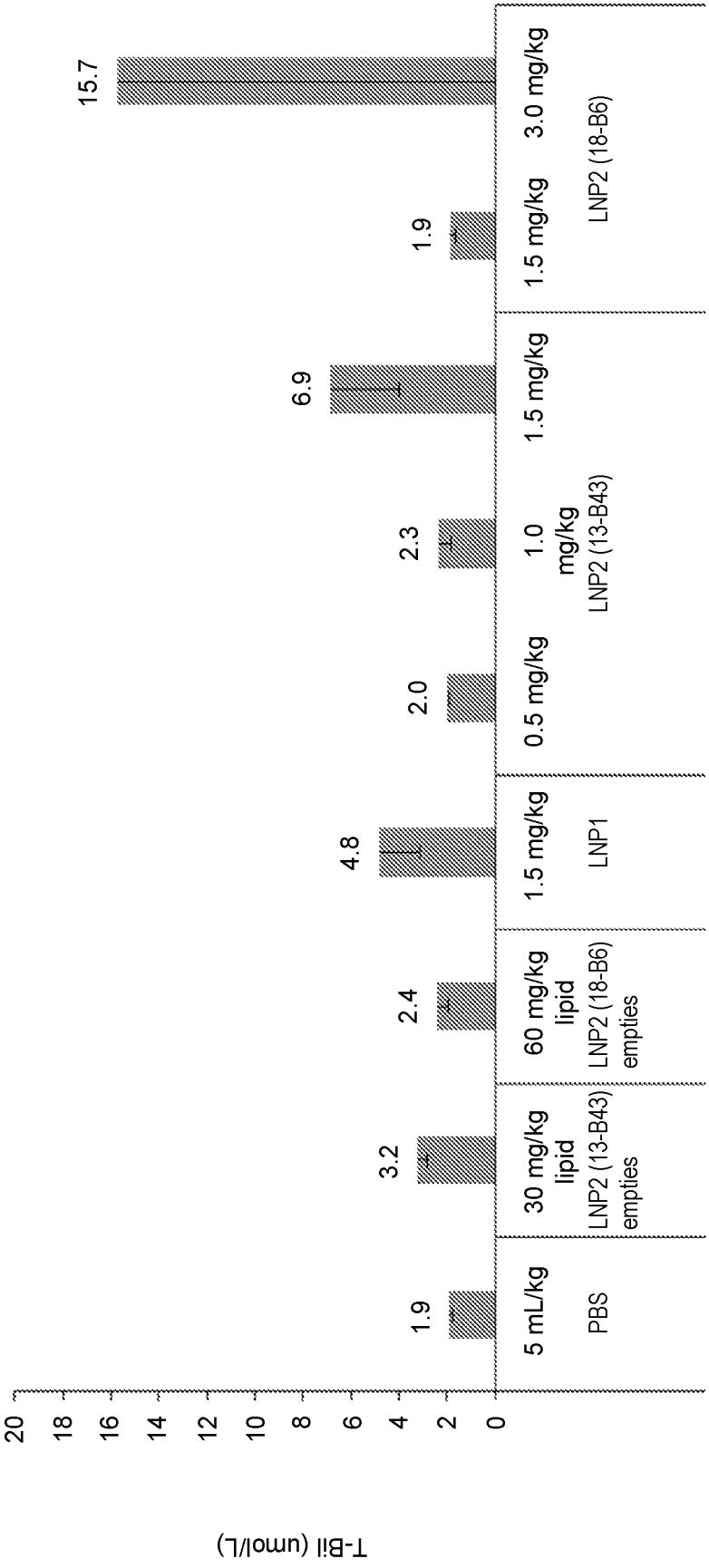
Total Bilirubin at 24h post-dose
T-Bil (umol/L)
0
2
4
6
8
10
12
14
16
18
20
1.9
3.2
2.4
4.8
2.0
2.3
6.9
1.9
15.7
5 mL/kg
PBS
30 mg/kg lipid
LNP2 (13-B43) empties
60 mg/kg lipid
LNP2 (18-B6) empties
1.5 mg/kg
LNP1
0.5 mg/kg
1.0 mg/kg
LNP2 (13-B43)
1.5 mg/kg
1.5 mg/kg
LNP2 (18-B6)
3.0 mg/kg

FIG. 19B

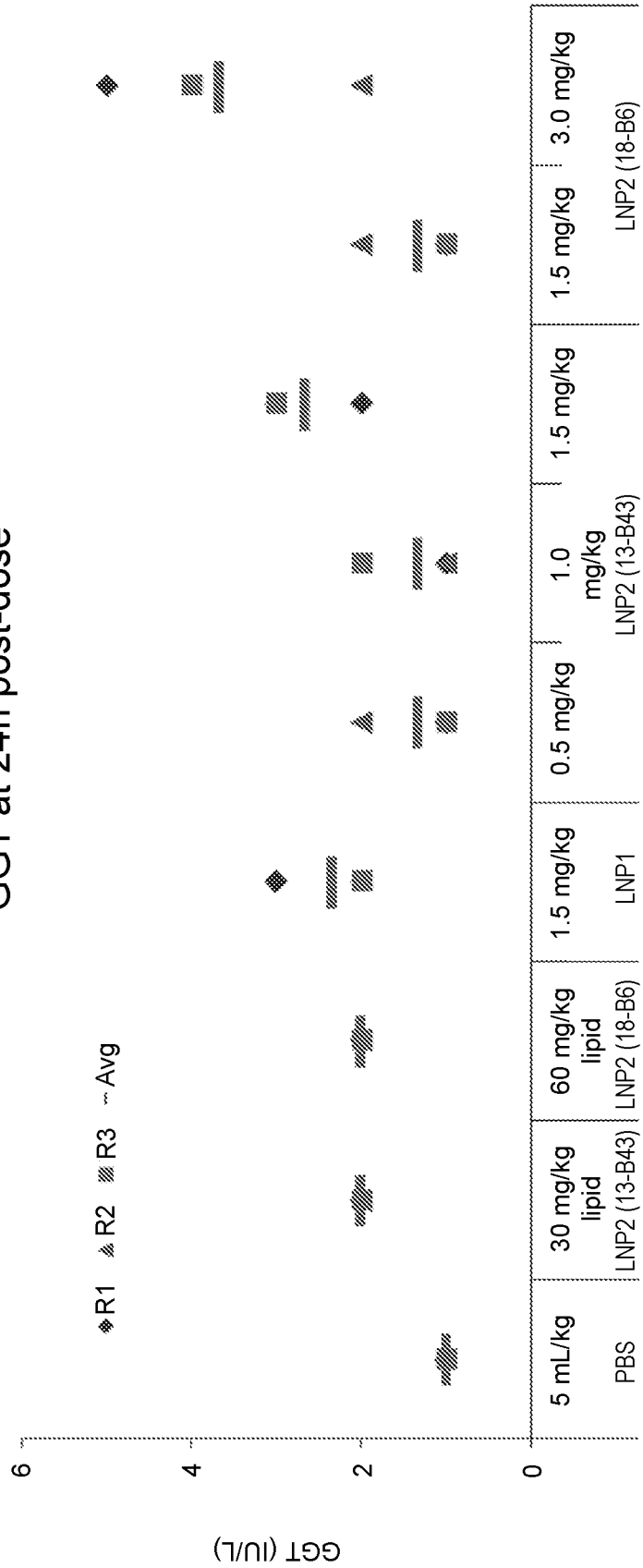
GGT at 24h post-dose
R1
R2
R3
Avg
GGT (IU/L)
0
2
4
6
5 mL/kg
PBS
30 mg/kg lipid
LNP2 (13-B43) empties
60 mg/kg lipid
LNP2 (18-B6) empties
1.5 mg/kg
LNP1
0.5 mg/kg
1.0 mg/kg
LNP2 (13-B43)
1.5 mg/kg
1.5 mg/kg
3.0 mg/kg
LNP2 (18-B6)

FIG. 19C

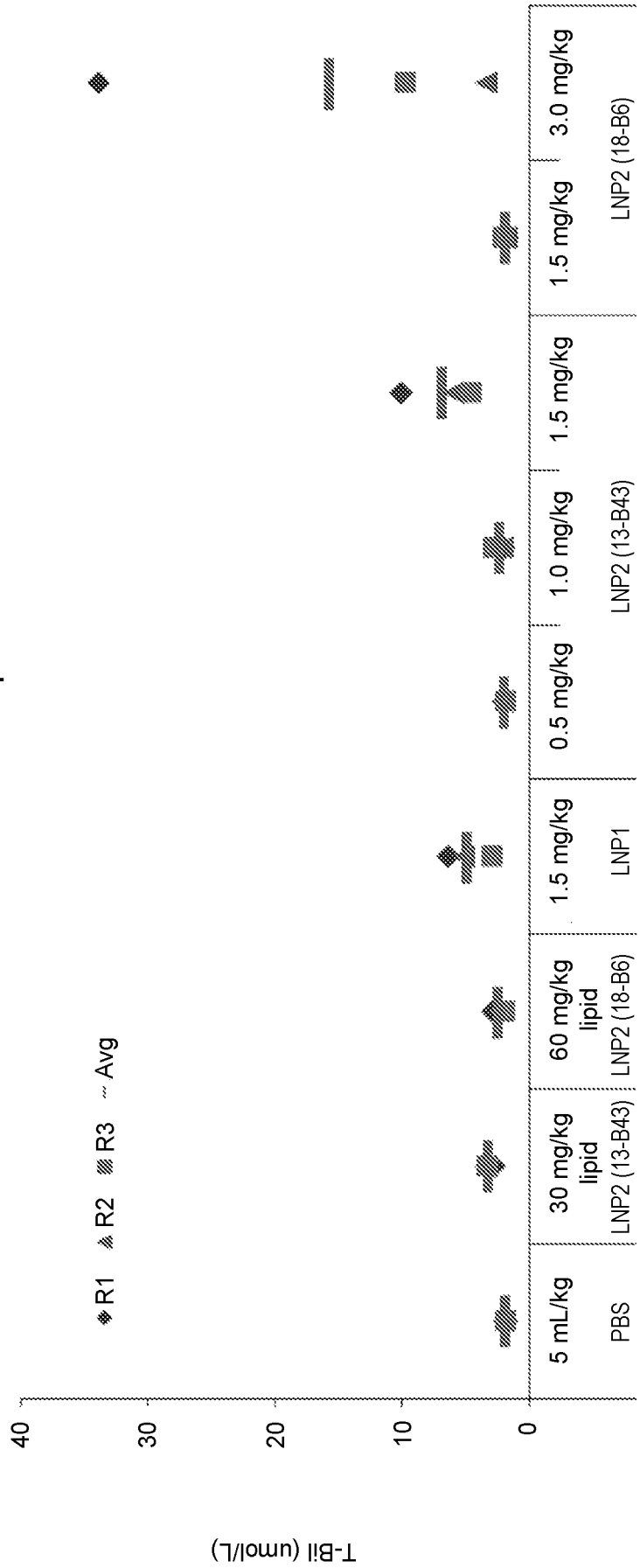
T-Bill at 24h post-dose
R1
R2
R3
Avg
T-Bil (umol/L)
40
30
20
10
0
5 mL/kg
PBS
30 mg/kg lipid
LNP2 (13-B43) empties
60 mg/kg lipid
LNP2 (18-B6) empties
1.5 mg/kg
LNP1
0.5 mg/kg
1.0 mg/kg
LNP2 (13-B43)
1.5 mg/kg
1.5 mg/kg
3.0 mg/kg
LNP2 (18-B6)

FIG. 19D

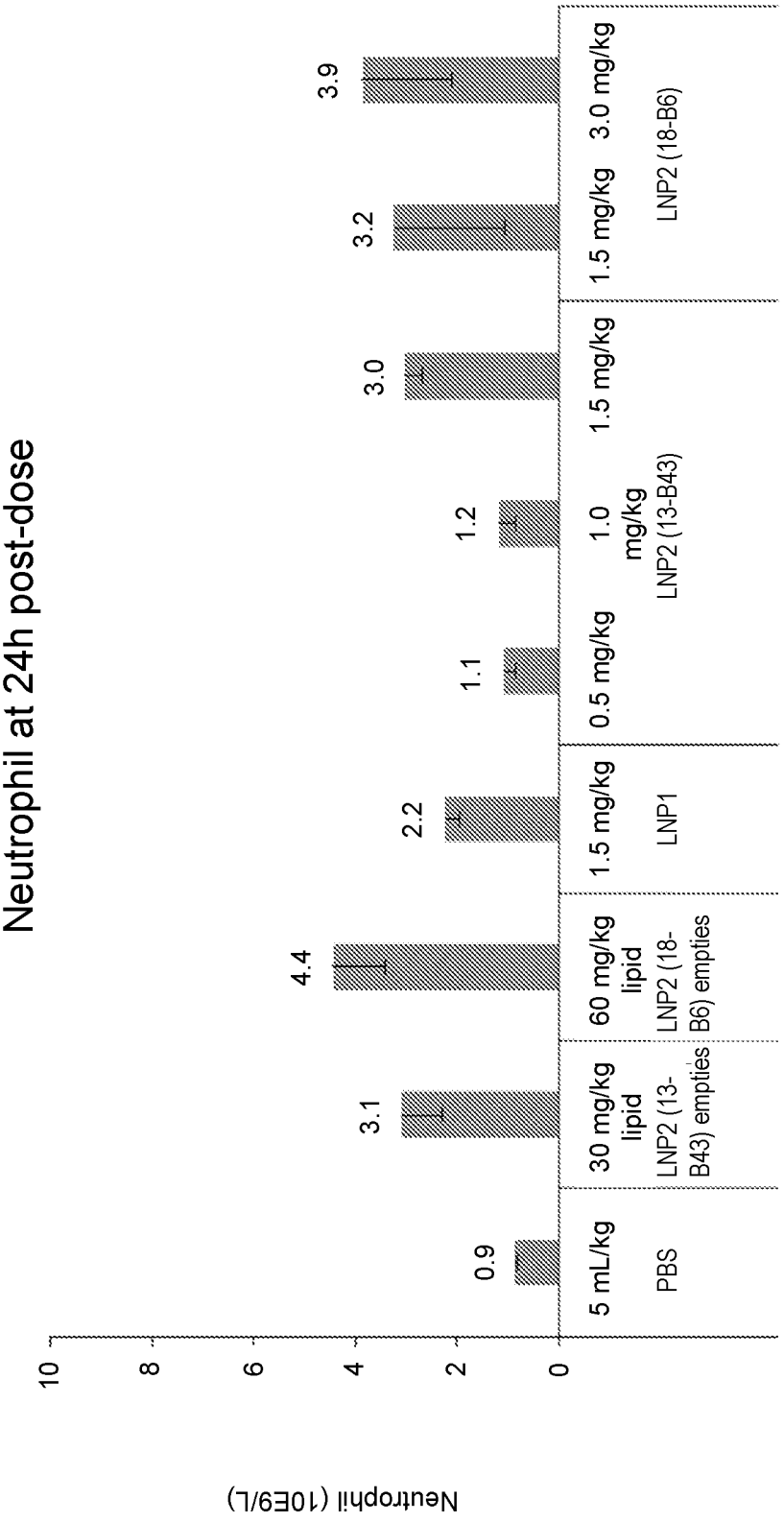
Neutrophil at 24h post-dose
Neutrophil (10E9/L)
10
8
6
4
2
0
0.9
3.1
4.4
2.2
1.1
1.2
3.0
3.2
3.9
5 mL/kg
PBS
30 mg/kg lipid
LNP2 (13-B43) empties
60 mg/kg lipid
LNP2 (18-B6) empties
1.5 mg/kg
LNP1
0.5 mg/kg
1.0 mg/kg
1.5 mg/kg
LNP2 (13-B43)
1.5 mg/kg
3.0 mg/kg
LNP2 (18-B6)

FIG. 20A

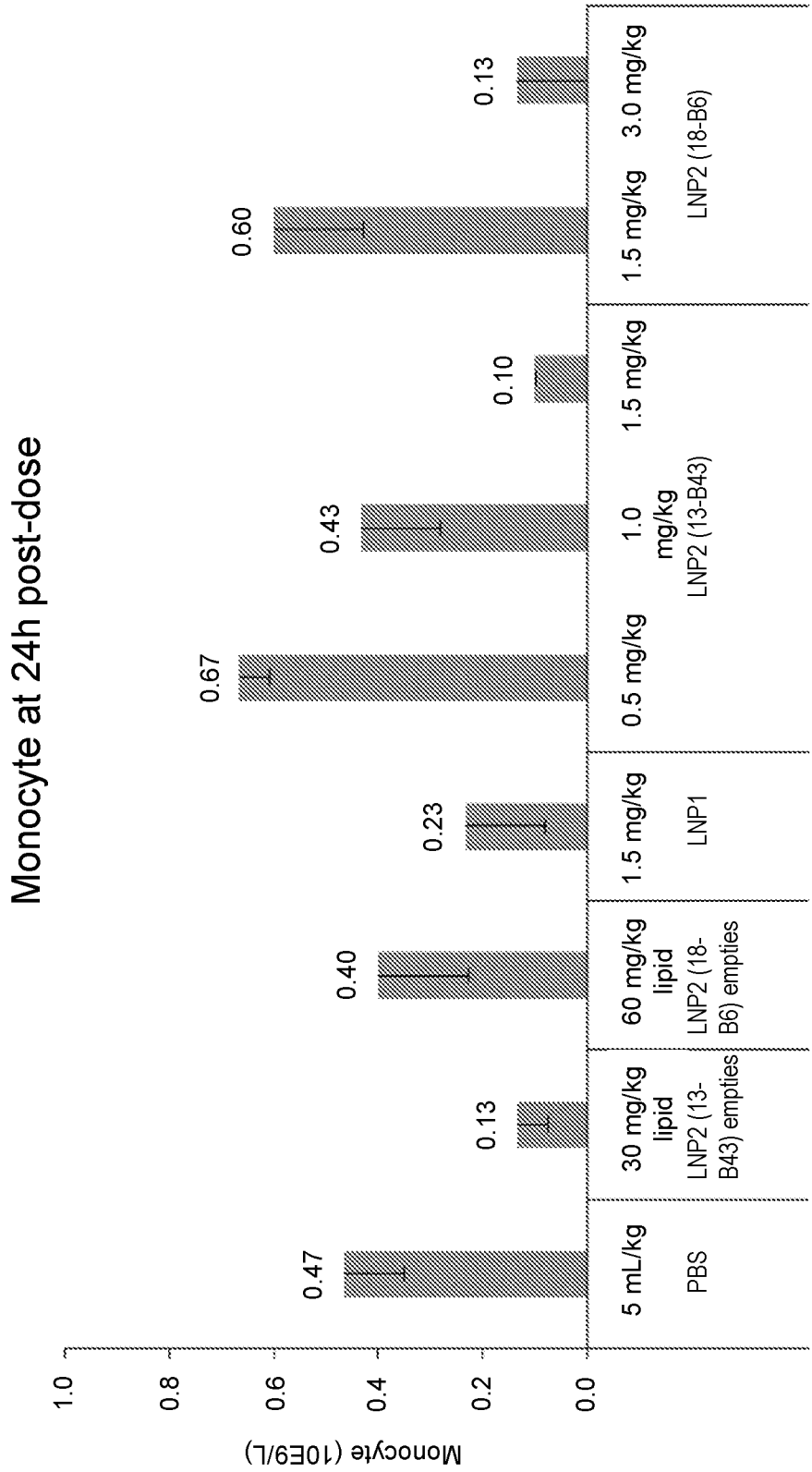
Monocyte at 24h post-dose
Monocyte (10E9/L)
1.0
0.8
0.6
0.4
0.2
0.0
0.47
0.13
0.40
0.23
0.67
0.43
0.10
0.60
0.13
5 mL/kg
PBS
30 mg/kg lipid
LNP2 (13-B43) empties
60 mg/kg lipid
LNP2 (18-B6) empties
1.5 mg/kg
LNP1
0.5 mg/kg
1.0 mg/kg
LNP2 (13-B43)
1.5 mg/kg
1.5 mg/kg
3.0 mg/kg
LNP2 (18-B6)

FIG. 20B

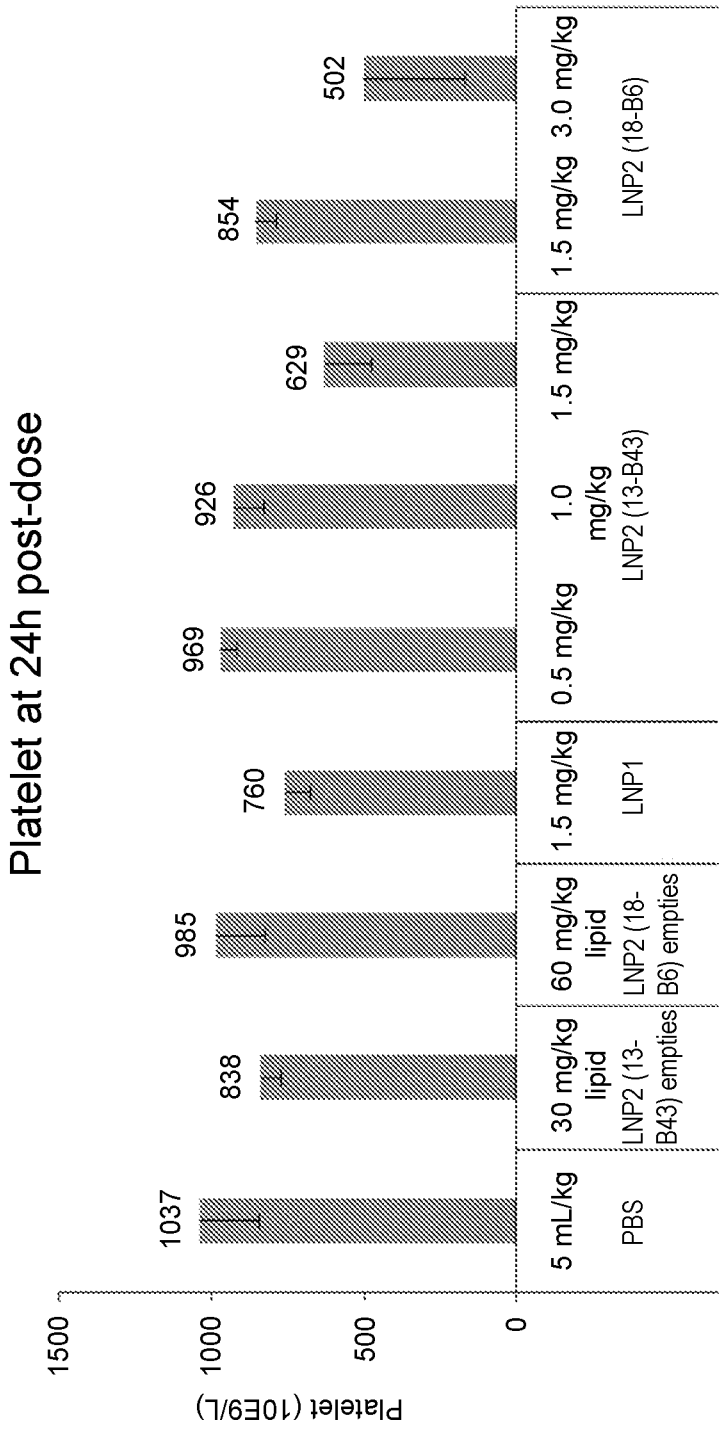
Platelet at 24h post-dose
Platelet (10E9/L)
1500
1000
500
0
1037
838
985
760
969
926
629
854
502
5 mL/kg
PBS
30 mg/kg lipid
LNP2 (13-B43) empties
60 mg/kg lipid
LNP2 (18-B6) empties
1.5 mg/kg
LNP1
0.5 mg/kg
1.0 mg/kg
1.5 mg/kg
LNP2 (13-B43)
1.5 mg/kg
3.0 mg/kg
LNP2 (18-B6)

FIG. 20C

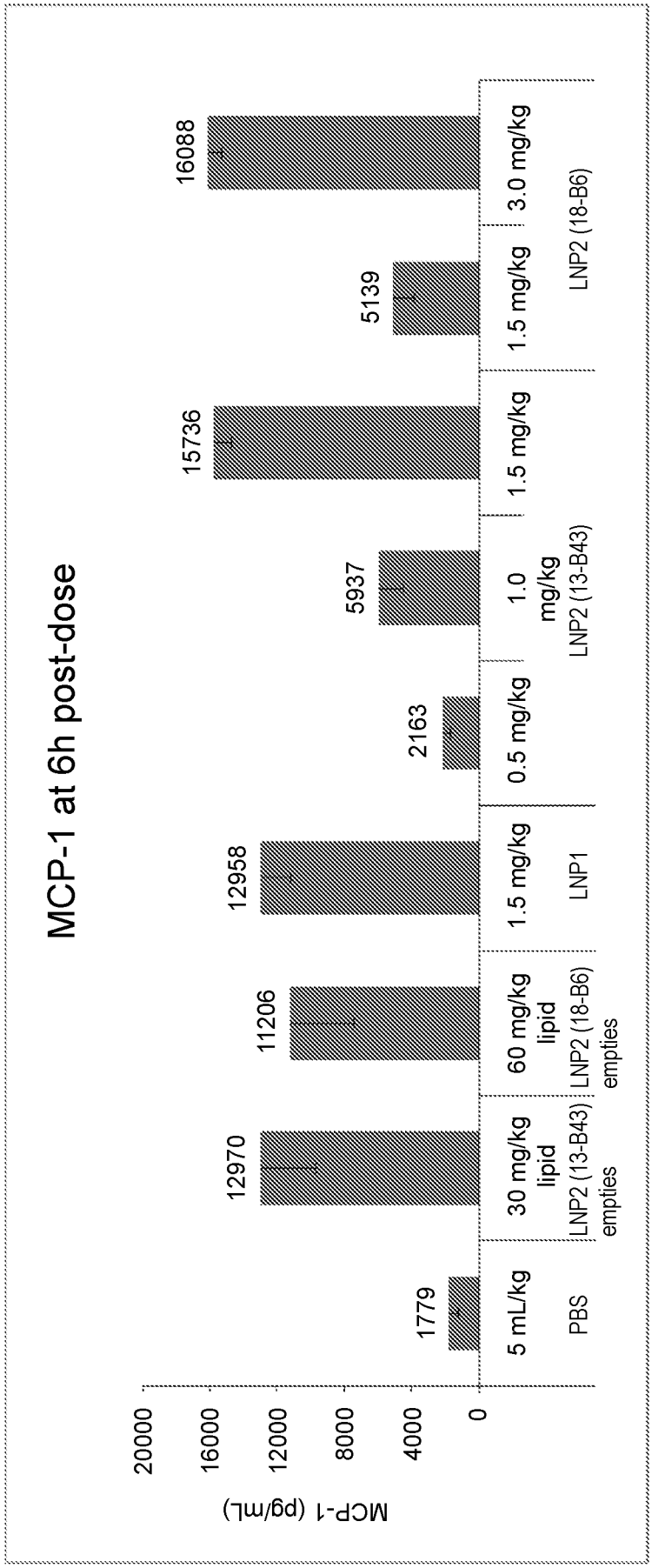
MCP-1 at 6h post-dose
MCP-1 (pg/mL)
20000
16000
12000
8000
4000
0
1779
12970
11206
12958
2163
5937
15736
5139
16088
5 mL/kg
30 mg/kg lipid
60 mg/kg lipid
1.5 mg/kg
0.5 mg/kg
1.0 mg/kg
1.5 mg/kg
1.5 mg/kg
3.0 mg/kg
PBS
LNP2 (13-B43) empties
LNP2 (18-B6) empties
LNP1
LNP2 (13-B43)
LNP2 (18-B6)

FIG. 21A

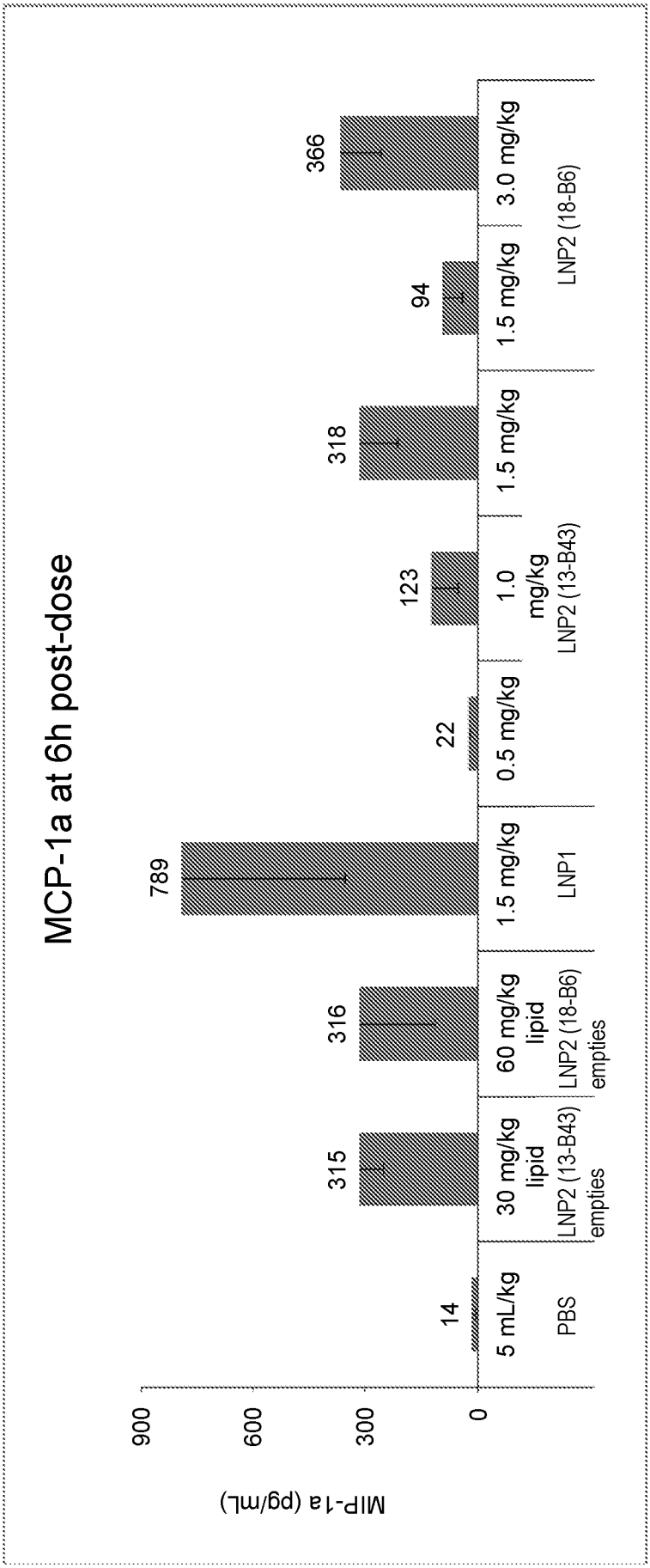
MCP-1a at 6h post-dose
MIP-1a (pg/mL)
900
600
300
0
14
315
316
789
22
123
318
94
366
5 mL/kg
PBS
30 mg/kg lipid
LNP2 (13-B43) empties
60 mg/kg lipid
LNP2 (18-B6) empties
1.5 mg/kg
LNP1
0.5 mg/kg
1.0 mg/kg
LNP2 (13-B43)
1.5 mg/kg
1.5 mg/kg
3.0 mg/kg
LNP2 (18-B6)

FIG. 21B

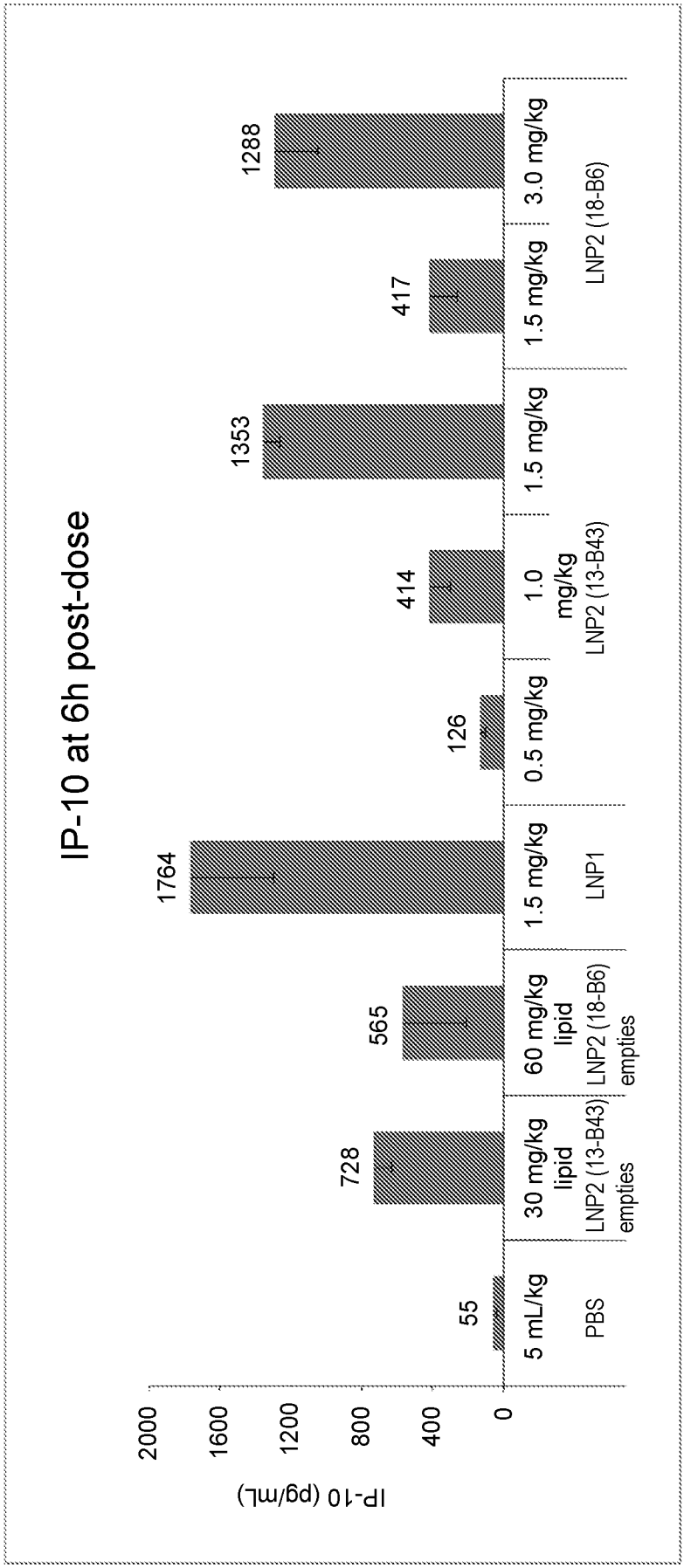
IP-10 at 6h post-dose
IP-10 (pg/mL)
2000
1600
1200
800
400
0
55
728
565
1764
126
414
1353
417
1288
5 mL/kg
PBS
30 mg/kg lipid
LNP2 (13-B43) empties
60 mg/kg lipid
LNP2 (18-B6) empties
1.5 mg/kg
LNP1
0.5 mg/kg
1.0 mg/kg
LNP2 (13-B43)
1.5 mg/kg
1.5 mg/kg
3.0 mg/kg
LNP2 (18-B6)

FIG. 21C

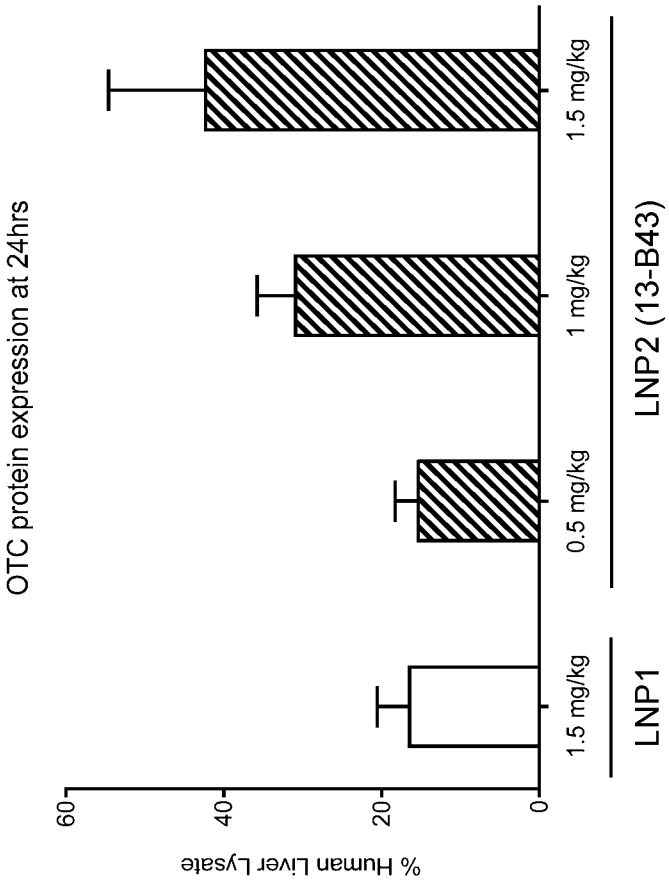
OTC protein expression at 24hrs
% Human Liver Lysate
60
40
20
0
1.5 mg/kg
0.5 mg/kg
1 mg/kg
1.5 mg/kg
LNP1
LNP2 (13-B43)

FIG. 22

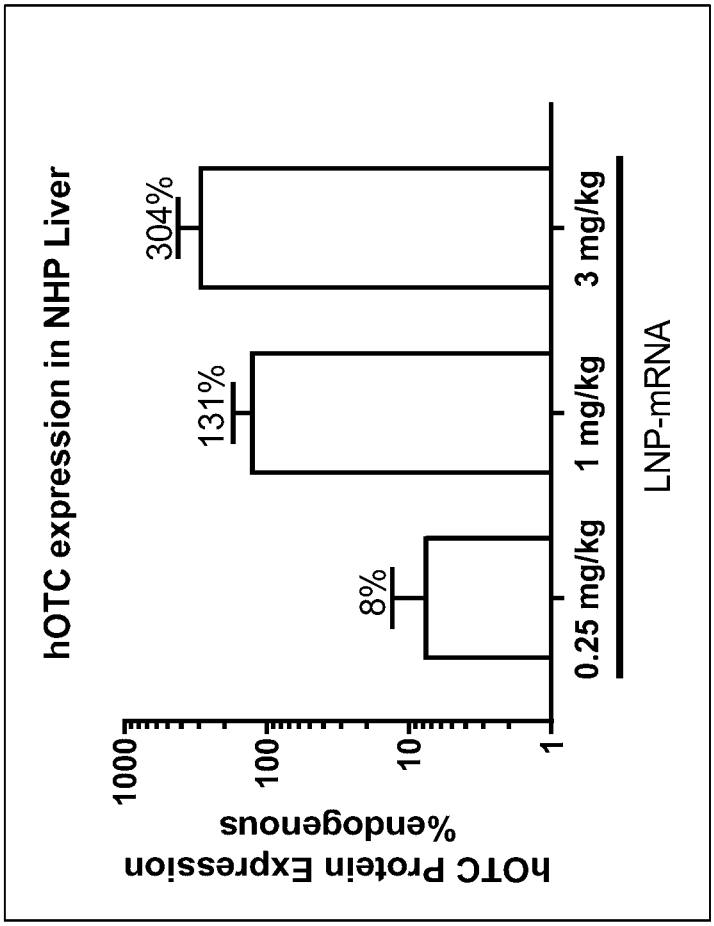
hOTC expression in NHP Liver
hOTC Protein Expression
%endogenous
1000
100
10
1
8%
131%
304%
0.25 mg/kg
1 mg/kg
3 mg/kg
LNP-mRNA

FIG. 23A

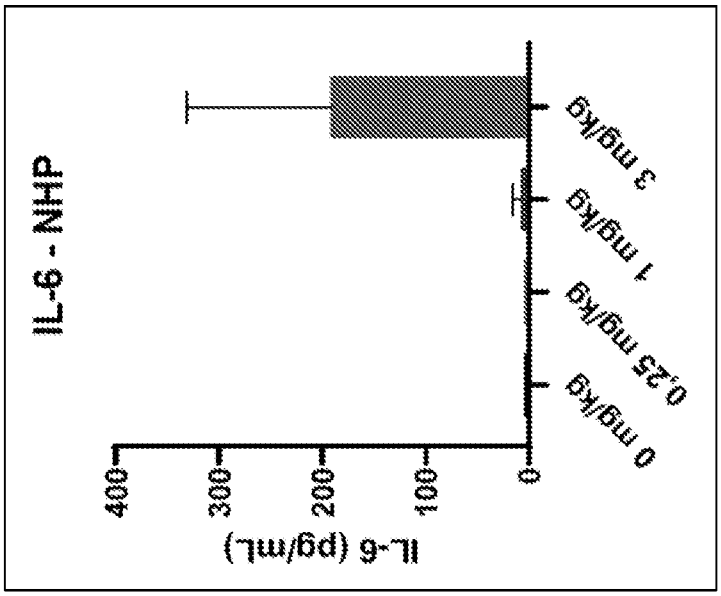

FIG. 23C

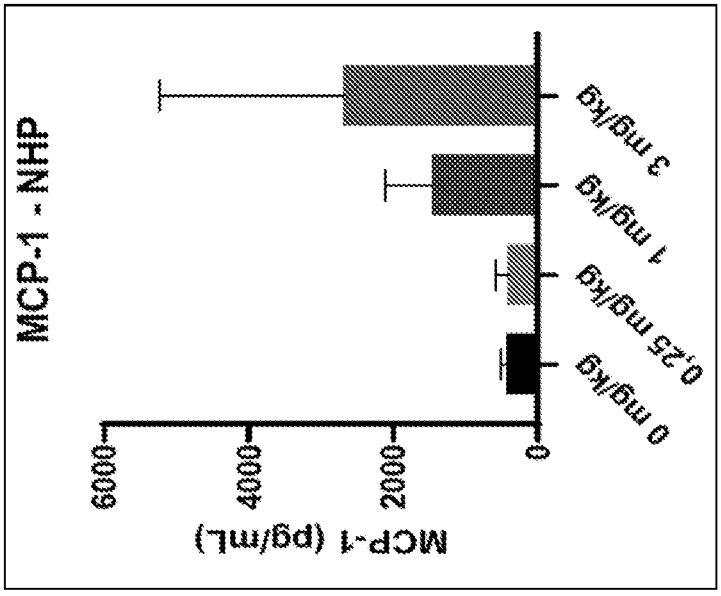

FIG. 23B