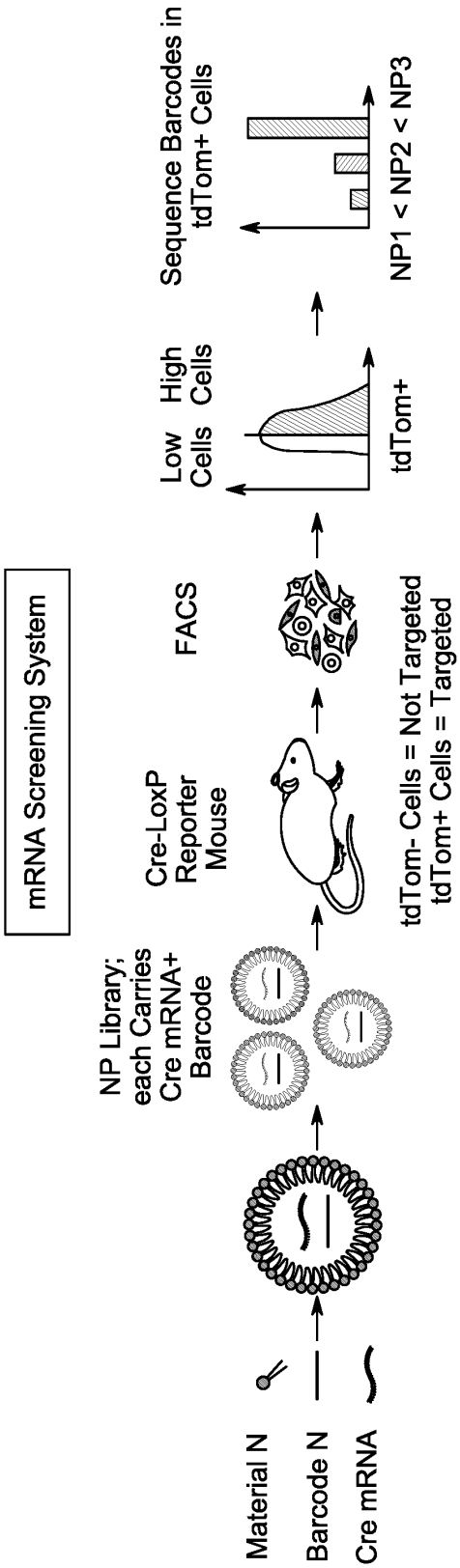

mRNA Screening System
Material N
Barcode N
Cre mRNA
NP Library; each Carries Cre mRNA+ Barcode
Cre-LoxP Reporter Mouse
tdTom- Cells = Not Targeted
tdTom+ Cells = Targeted
FACS
Low Cells
High Cells
tdTom+
Sequence Barcodes in tdTom+ Cells
NP1 < NP2 < NP3

FIG. 1

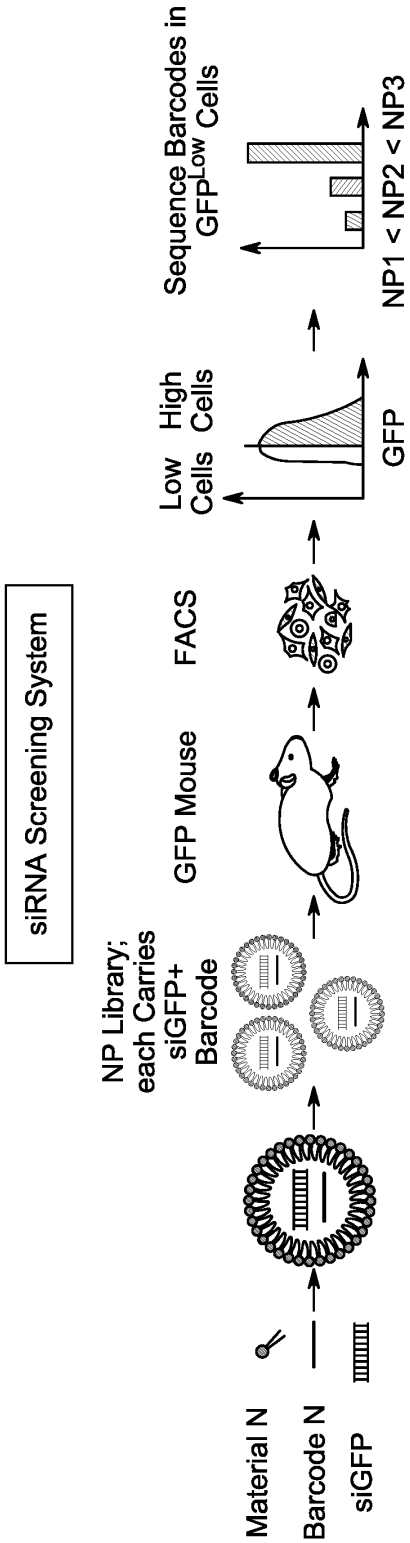

siRNA Screening System
Material N
Barcode N
siGFP
NP Library; each Carries siGFP+ Barcode
GFP Mouse
FACS
Low Cells
High Cells
GFP
GFP^High Cells = Not Targeted
GFP^Low Cells = Targeted
Sequence Barcodes in GFP^Low Cells
NP1 < NP2 < NP3

FIG. 2

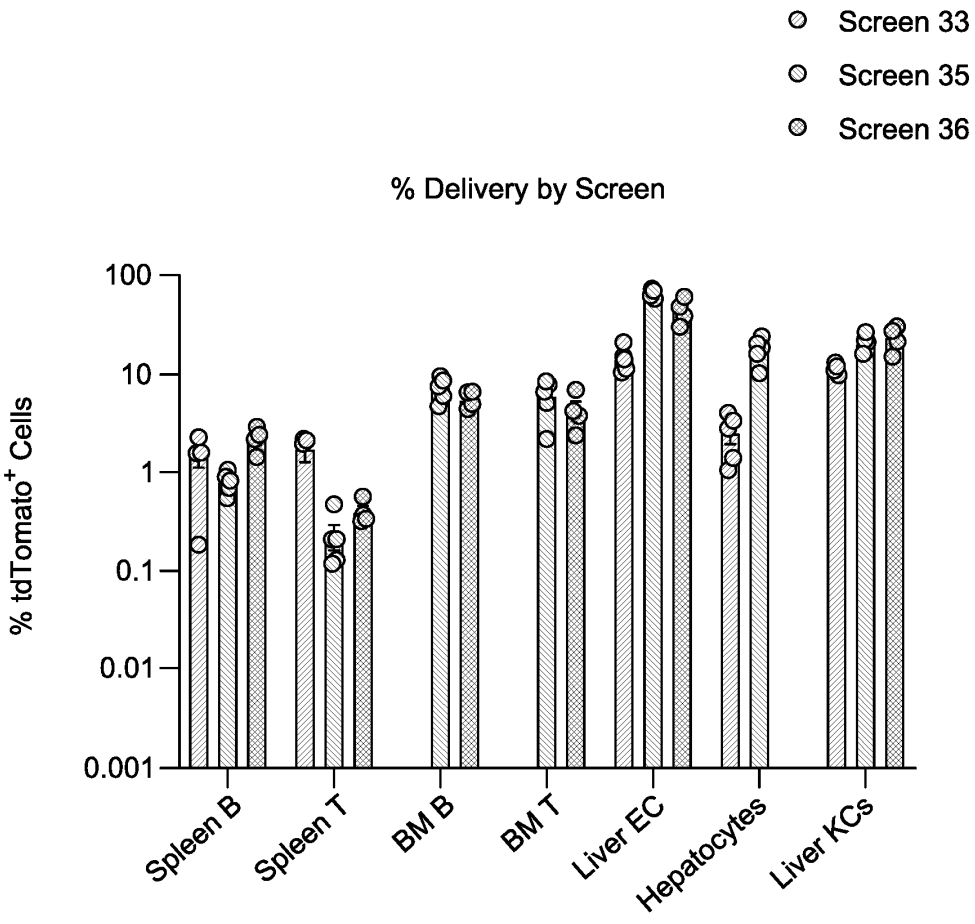

Screen 33
Screen 35
Screen 36
% Delivery by Screen
% tdTomato+ Cells
100
10
1
0.1
0.01
0.001
Spleen B
Spleen T
BM B
BM T
Liver EC
Hepatocytes
Liver KCs

FIG. 3

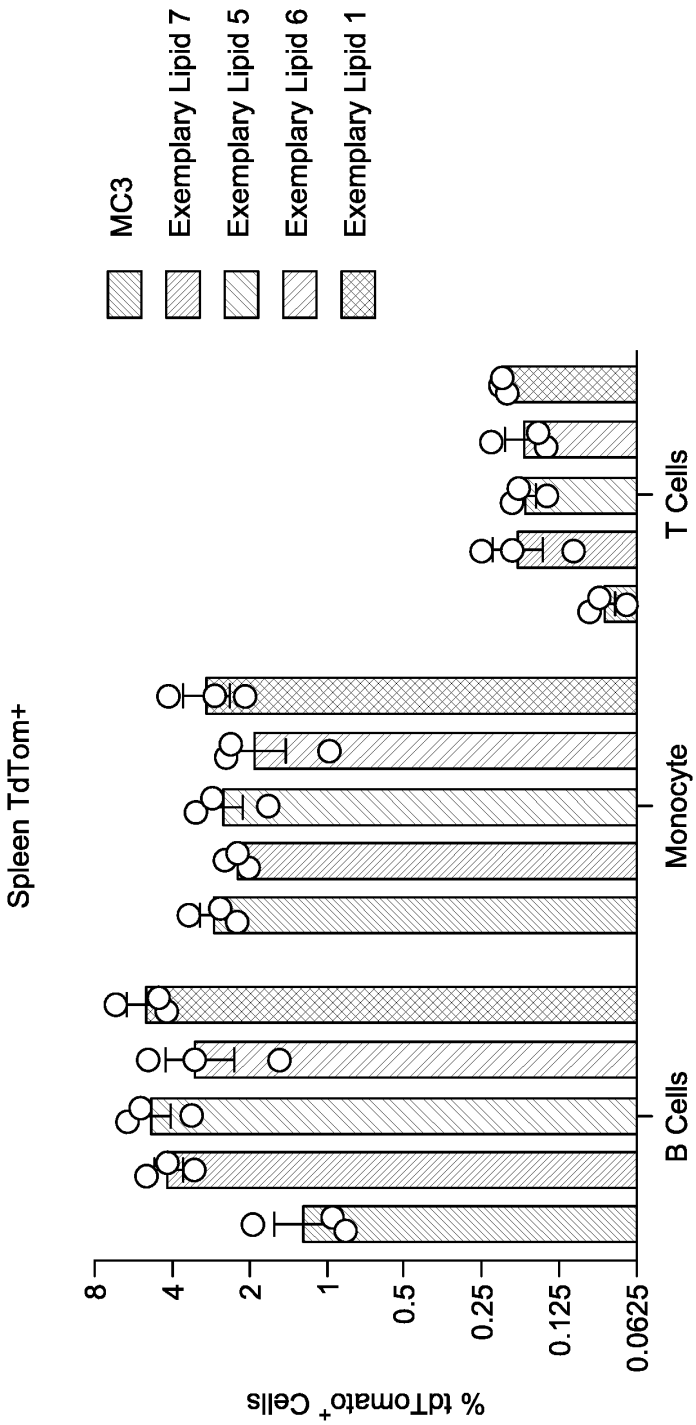

MC3
Exemplary Lipid 7
Exemplary Lipid 5
Exemplary Lipid 6
Exemplary Lipid 1
Spleen TdTom+
% tdTomato+ Cells
8
4
2
1
0.5
0.25
0.125
0.0625
B Cells
Monocyte
T Cells

FIG. 4

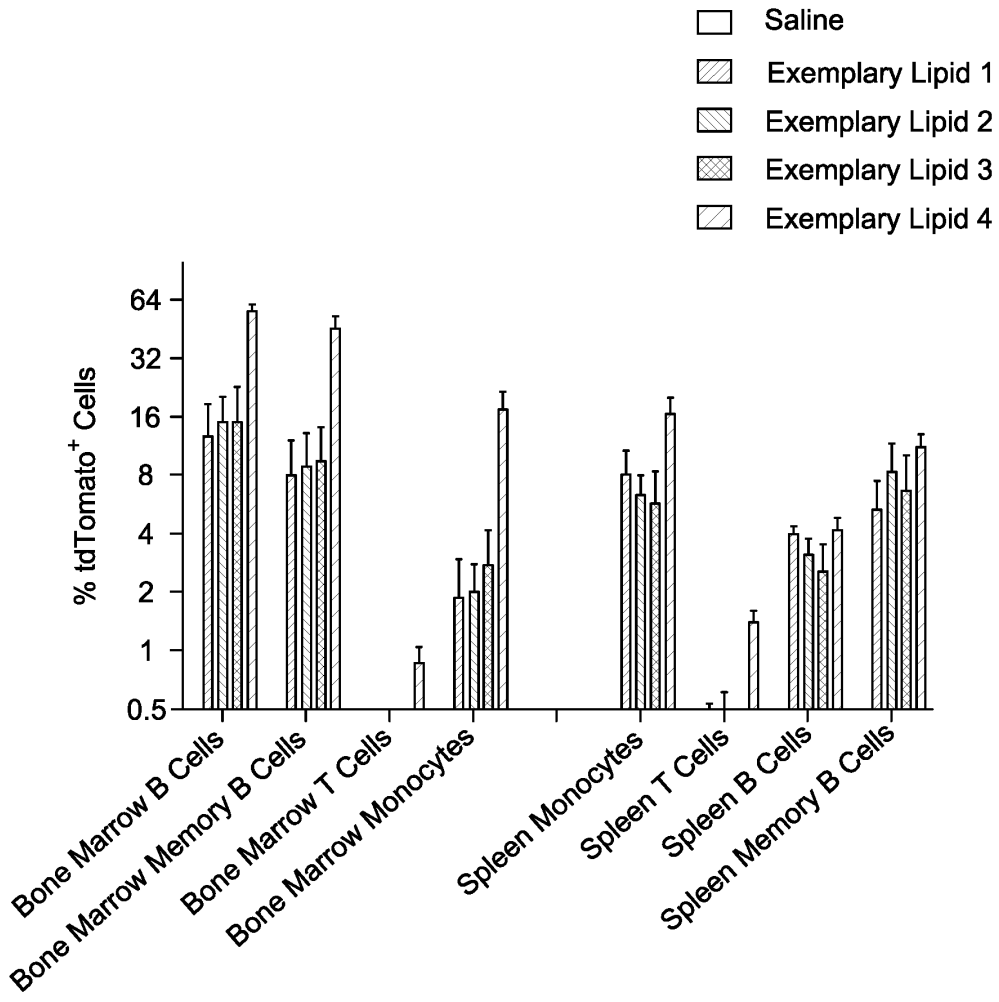

Saline
Exemplary Lipid 1
Exemplary Lipid 2
Exemplary Lipid 3
Exemplary Lipid 4
% tdTomato+ Cells
64
32
16
8
4
2
1
0.5
Bone Marrow B Cells
Bone Marrow Memory B Cells
Bone Marrow T Cells
Bone Marrow Monocytes
Spleen Monocytes
Spleen T Cells
Spleen B Cells
Spleen Memory B Cells

FIG. 5

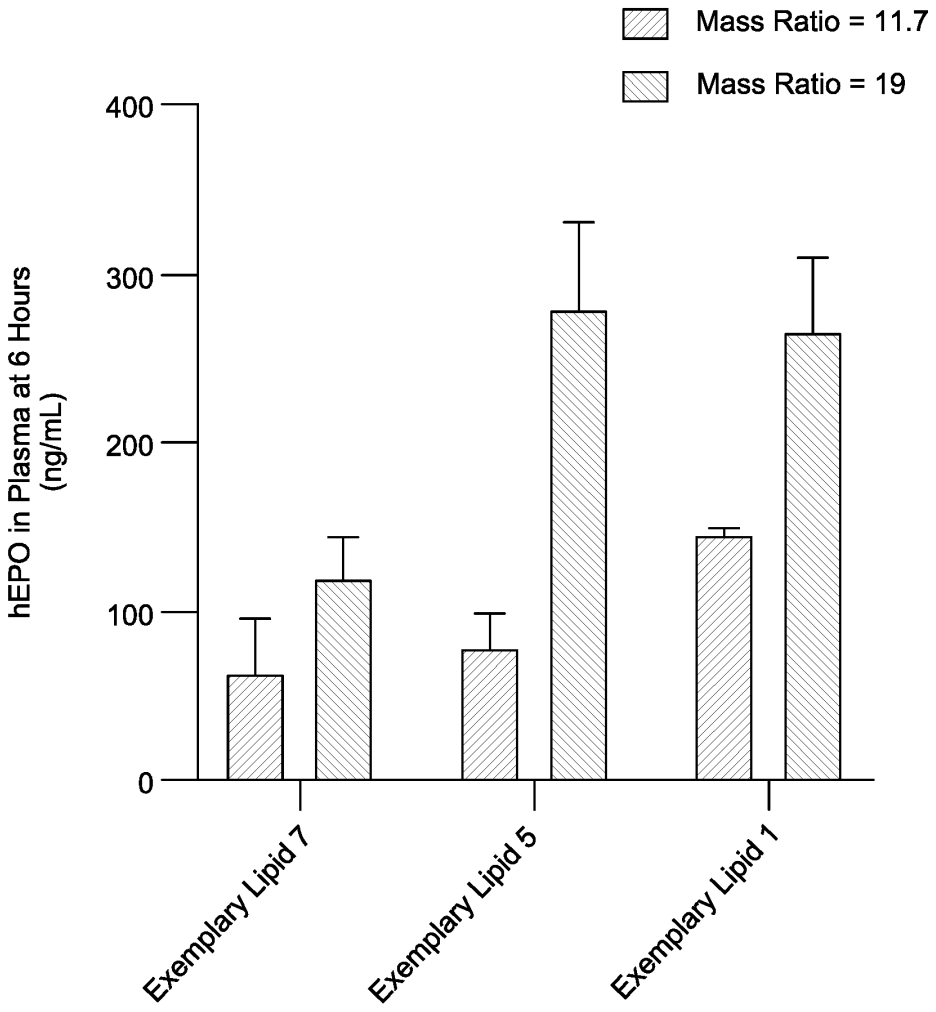

Mass Ratio = 11.7
Mass Ratio = 19
hEPO in Plasma at 6 Hours (ng/mL)
400
300
200
100
0
Exemplary Lipid 7
Exemplary Lipid 5
Exemplary Lipid 1

FIG. 6

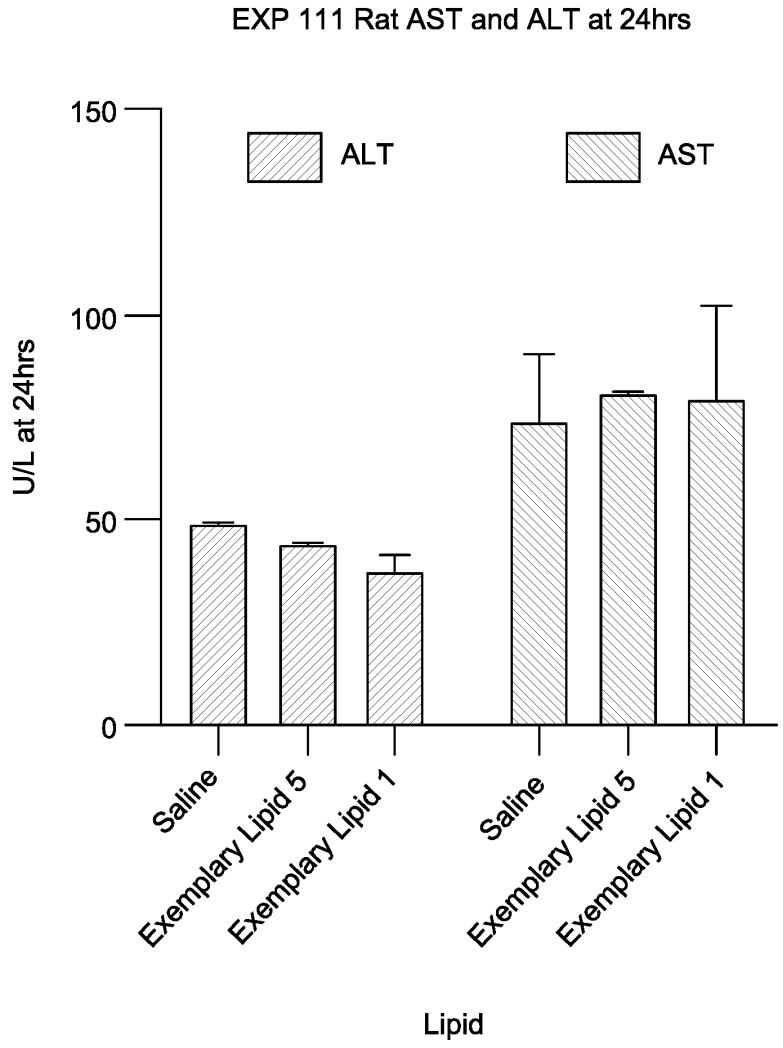

EXP 111 Rat AST and ALT at 24hrs
150
100
50
0
U/L at 24hrs
ALT
AST
Saline
Exemplary Lipid 5
Exemplary Lipid 1
Saline
Exemplary Lipid 5
Exemplary Lipid 1
Lipid

FIG. 7

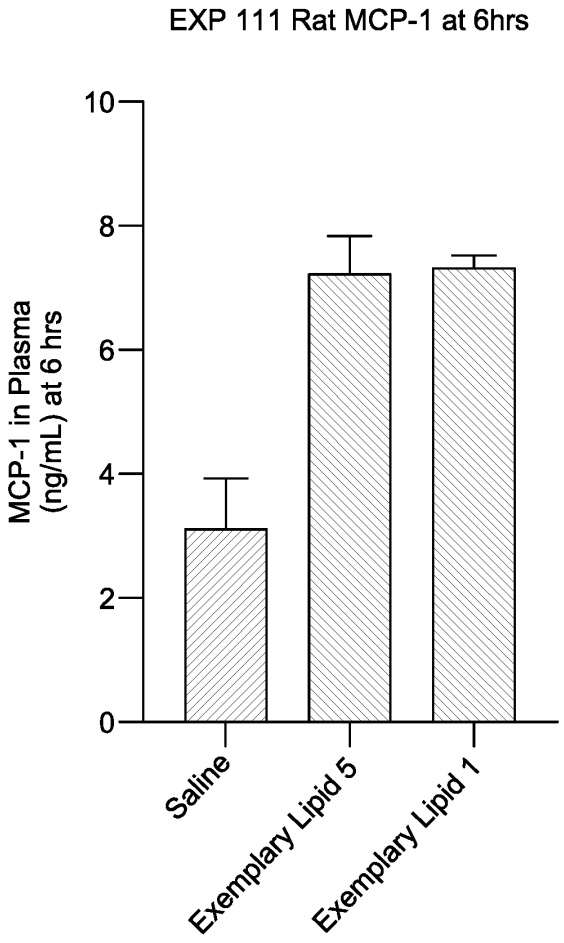

EXP 111 Rat MCP-1 at 6hrs
MCP-1 in Plasma (ng/mL) at 6 hrs
10
8
6
4
2
0
Saline
Exemplary Lipid 5
Exemplary Lipid 1

FIG. 8

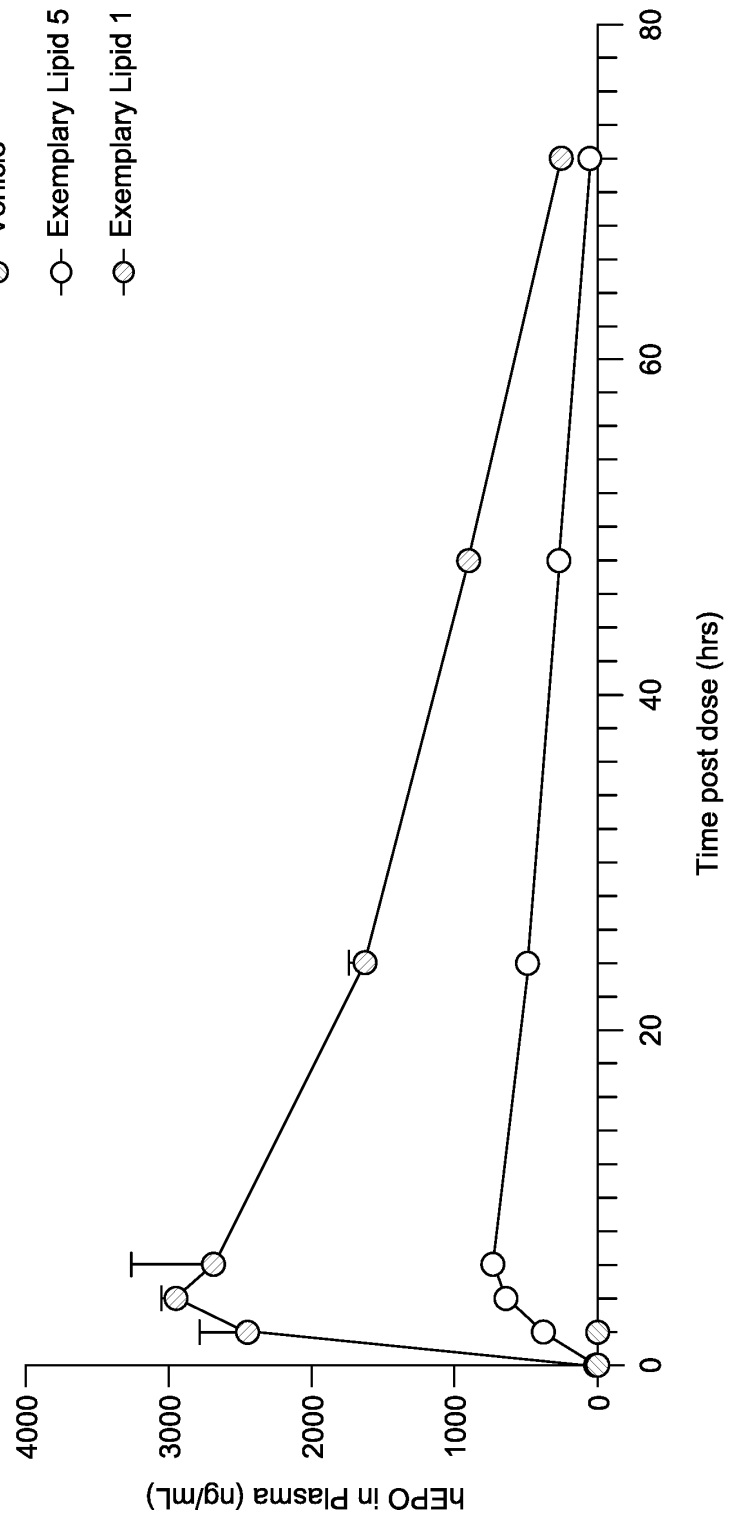

Vehicle
Exemplary Lipid 5
Exemplary Lipid 1
hEPO in Plasma (ng/mL)
4000
3000
2000
1000
0
0
20
40
60
80
Time post dose (hrs)

FIG. 9

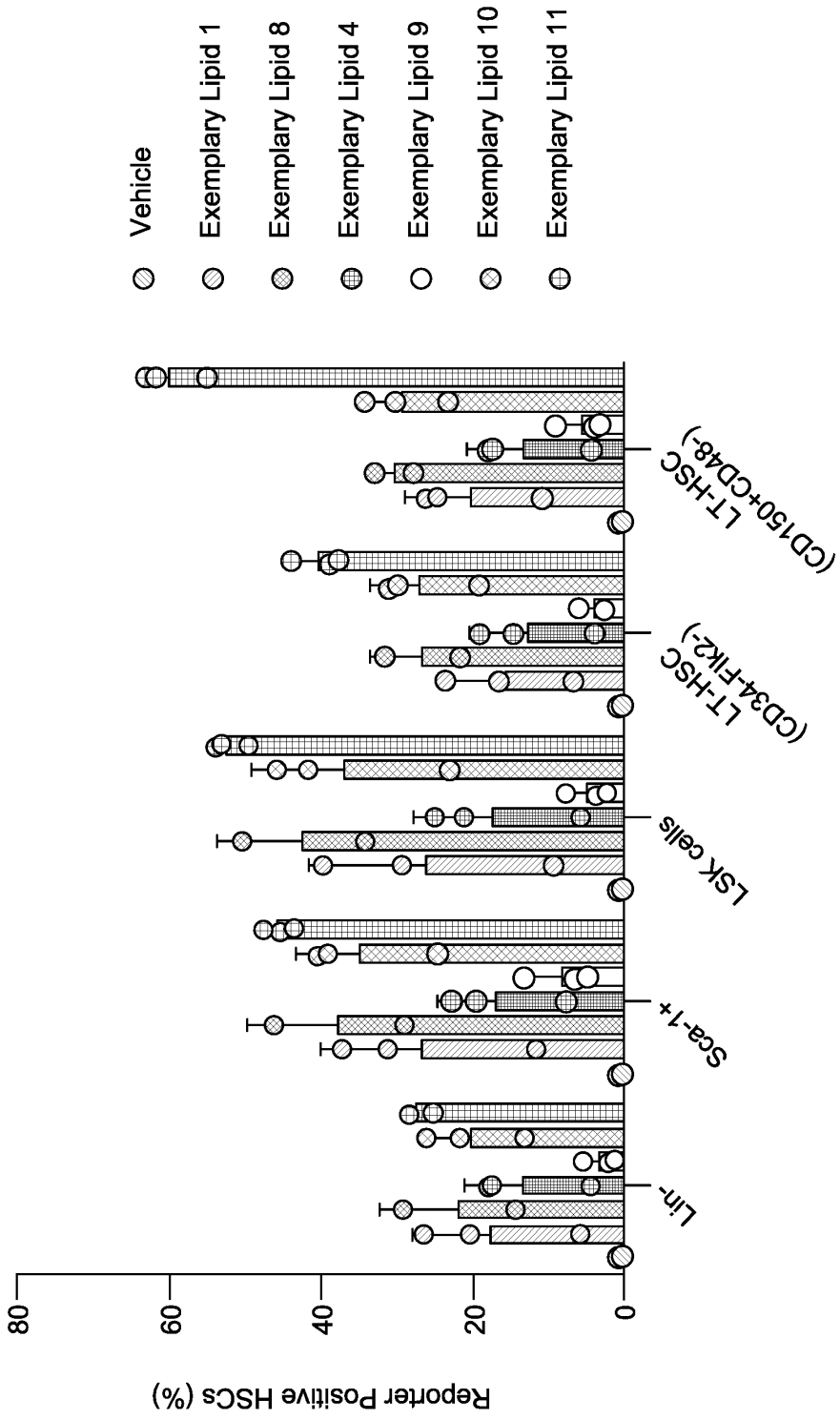

Vehicle
Exemplary Lipid 1
Exemplary Lipid 8
Exemplary Lipid 4
Exemplary Lipid 9
Exemplary Lipid 10
Exemplary Lipid 11
Reporter Positive HSCs (%)
0
20
40
60
80
Lin-
Sca-1+
LSK cells
LT-HSC (CD34-Flk2-)
LT-HSC (CD150+CD48-)

FIG. 10

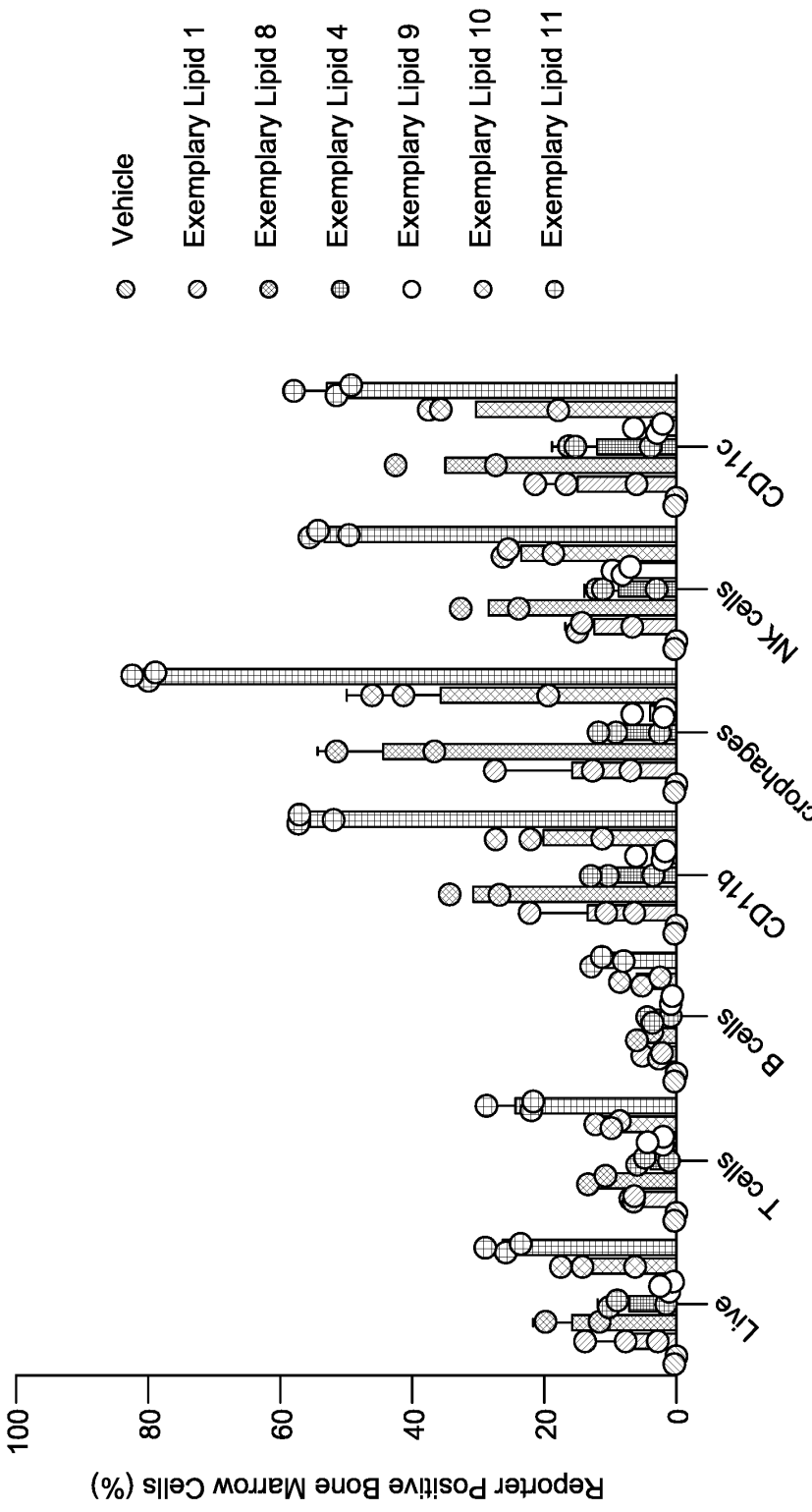

Vehicle
Exemplary Lipid 1
Exemplary Lipid 8
Exemplary Lipid 4
Exemplary Lipid 9
Exemplary Lipid 10
Exemplary Lipid 11
Reporter Positive Bone Marrow Cells (%)
0
20
40
60
80
100
Live
T cells
B cells
CD11b
Macrophages
NK cells
CD11c

FIG. 11

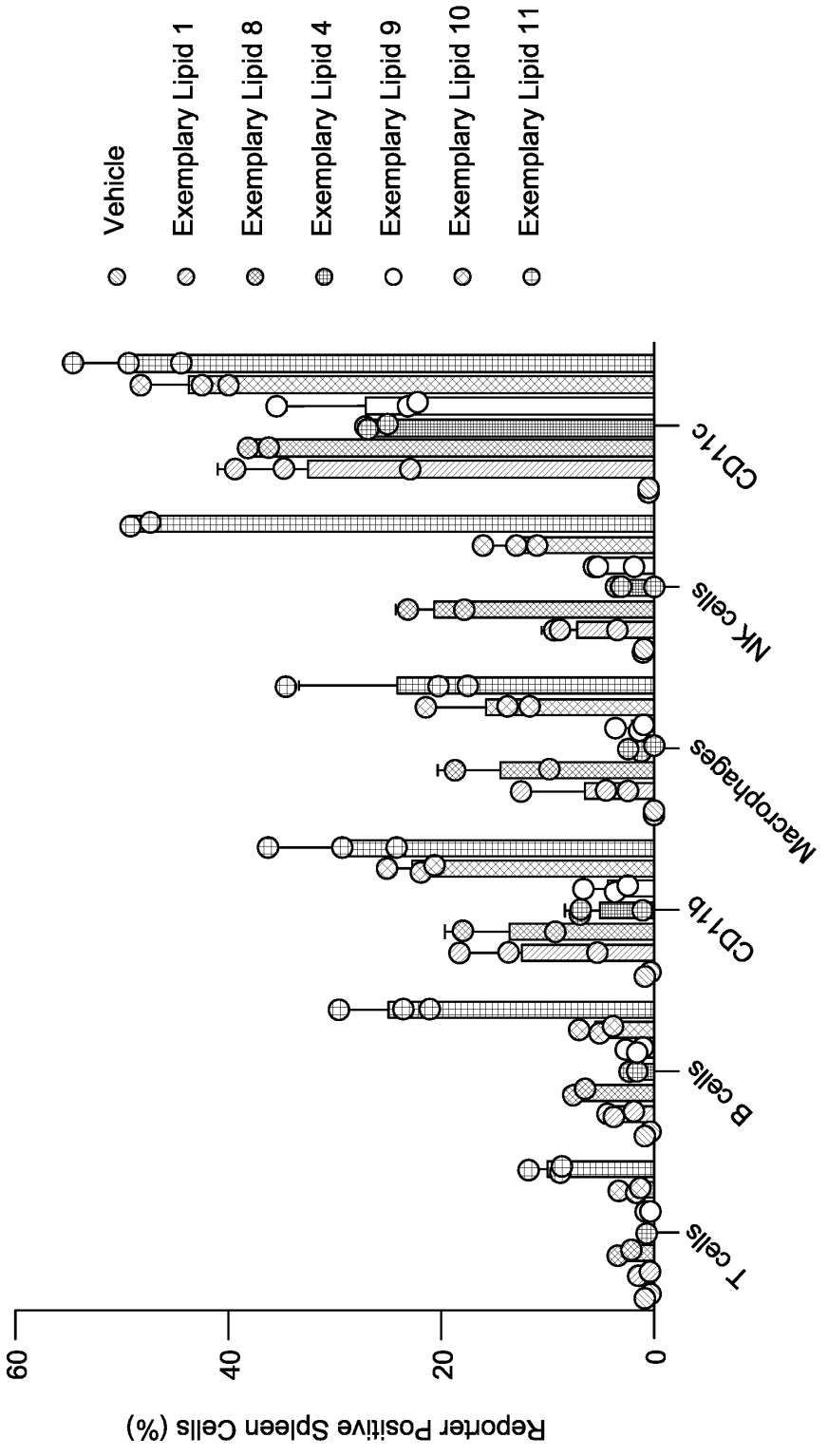

Vehicle
Exemplary Lipid 1
Exemplary Lipid 8
Exemplary Lipid 4
Exemplary Lipid 9
Exemplary Lipid 10
Exemplary Lipid 11
Reporter Positive Spleen Cells (%)
0
20
40
60
T cells
B cells
CD11b
Macrophages
NK cells
CD11c

FIG. 12

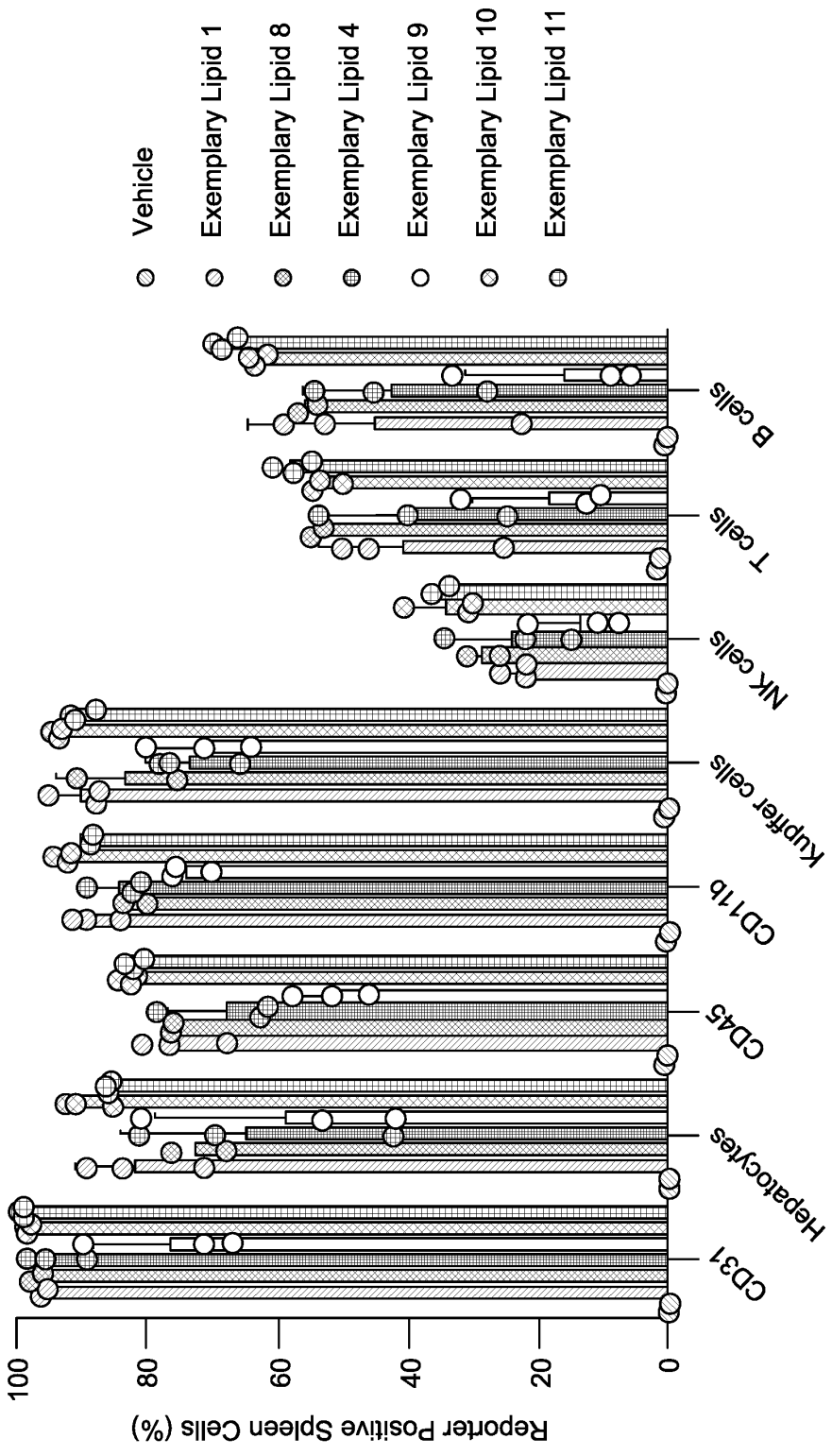
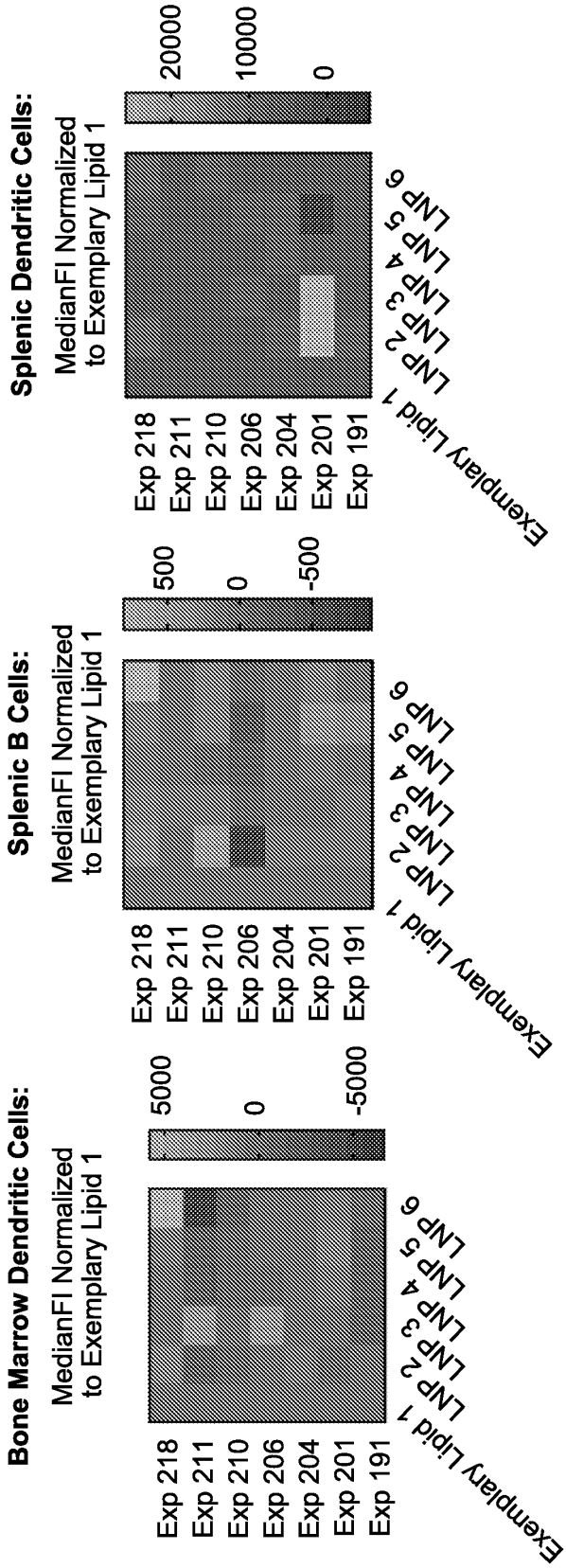

Vehicle
Exemplary Lipid 1
Exemplary Lipid 8
Exemplary Lipid 4
Exemplary Lipid 9
Exemplary Lipid 10
Exemplary Lipid 11
Reporter Positive Spleen Cells (%)
0
20
40
60
80
100
CD31
Hepatocytes
CD45
CD11b
Kupffer cells
NK cells
T cells
B cells

FIG. 13



1	8	x	x	10	11	
1	x	x	x	x	x	
1	x	x	x	x	x	
1	x	x	x	x	x	
1	x	x	x	x	x	
1	15	16	x	x	x	
1	x	x	x	x	x	

FIG. 14

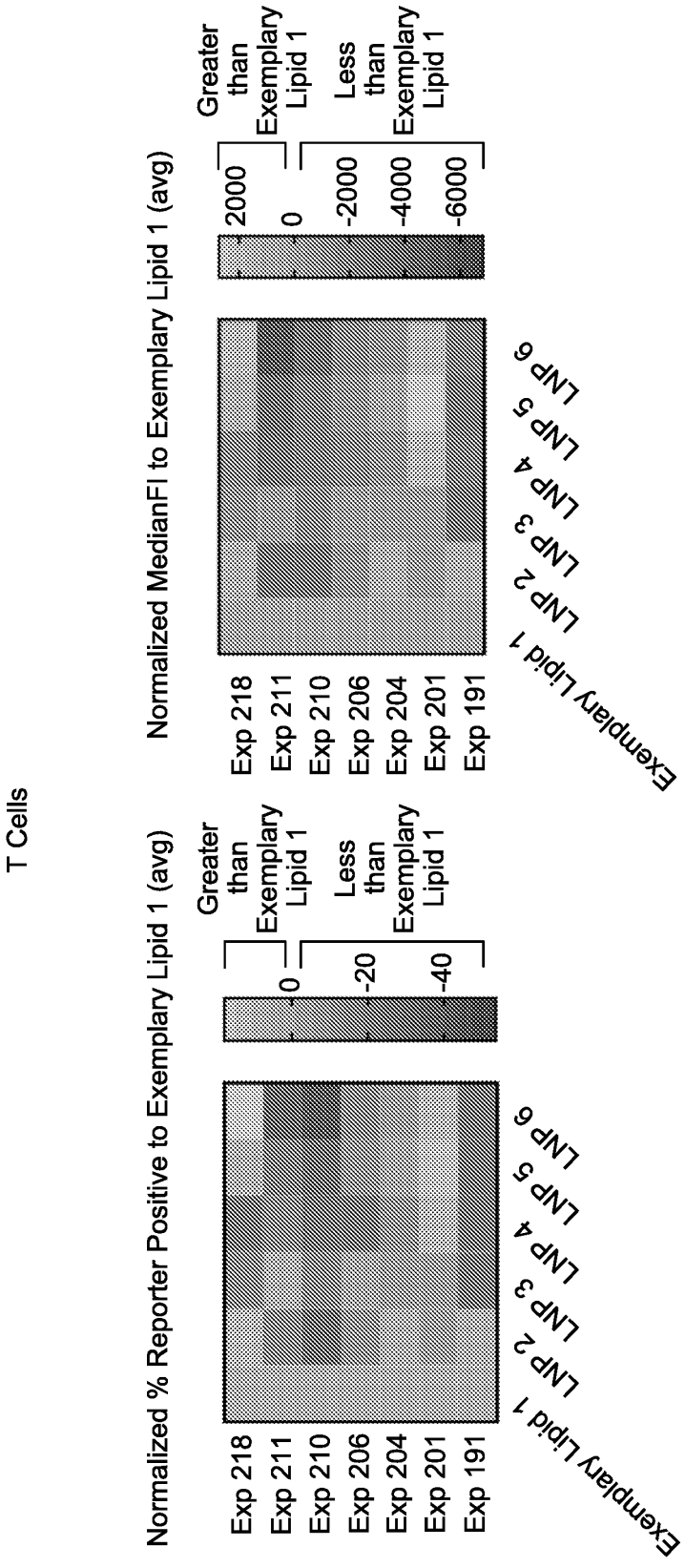

T Cells
Normalized % Reporter Positive to Exemplary Lipid 1 (avg)
Greater than Exemplary Lipid 1
Less than Exemplary Lipid 1
0
-20
-40
Exp 218
Exp 211
Exp 210
Exp 206
Exp 204
Exp 201
Exp 191
Exemplary Lipid 1
LNP 2
LNP 3
LNP 4
LNP 5
LNP 6
Normalized MedianFI to Exemplary Lipid 1 (avg)
Greater than Exemplary Lipid 1
Less than Exemplary Lipid 1
2000
0
-2000
-4000
-6000
Exp 218
Exp 211
Exp 210
Exp 206
Exp 204
Exp 201
Exp 191
Exemplary Lipid 1
LNP 2
LNP 3
LNP 4
LNP 5
LNP 6

FIG. 15

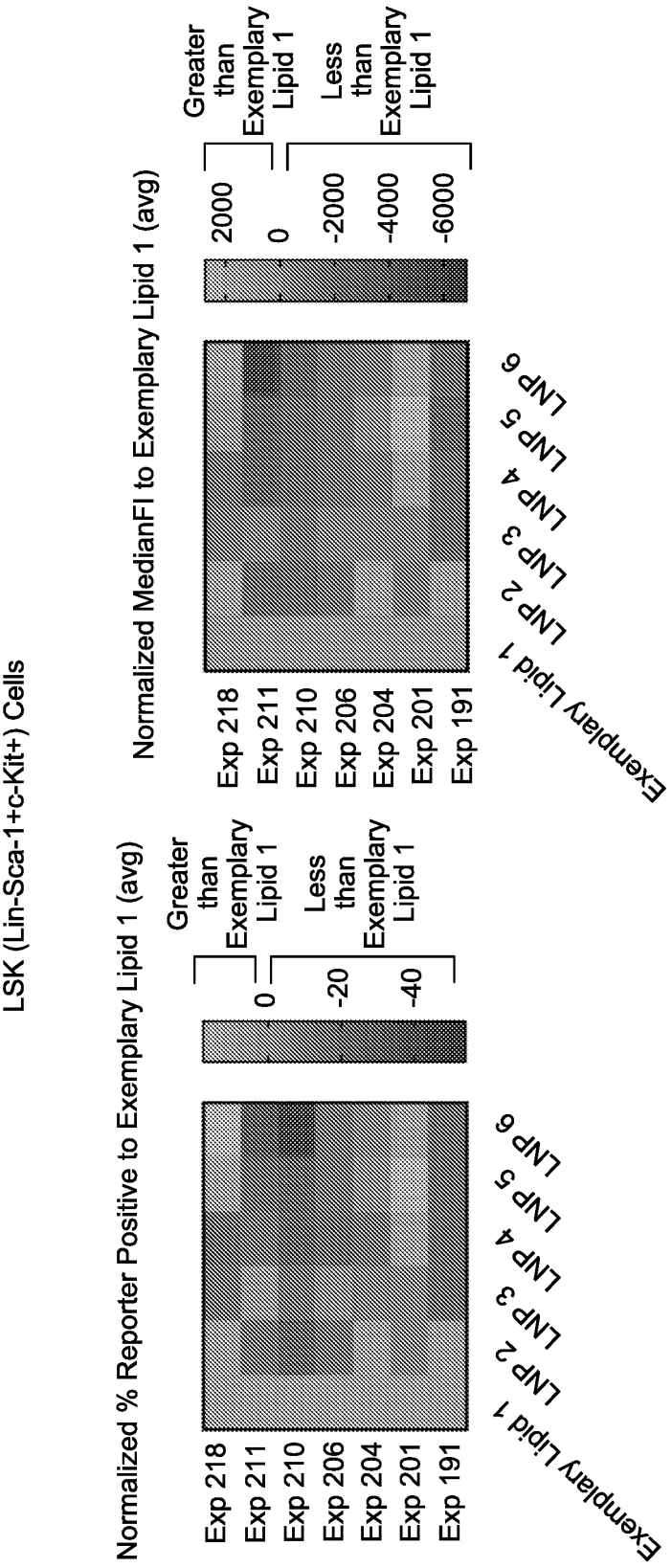

LSK (Lin-Sca-1+c-Kit+) Cells
Normalized % Reporter Positive to Exemplary Lipid 1 (avg)
Greater than Exemplary Lipid 1
Less than Exemplary Lipid 1
0
-20
-40
Exp 218
Exp 211
Exp 210
Exp 206
Exp 204
Exp 201
Exp 191
Exemplary Lipid 1
LNP 2
LNP 3
LNP 4
LNP 5
LNP 6
Normalized MedianFI to Exemplary Lipid 1 (avg)
Greater than Exemplary Lipid 1
Less than Exemplary Lipid 1
2000
0
-2000
-4000
-6000

FIG. 16

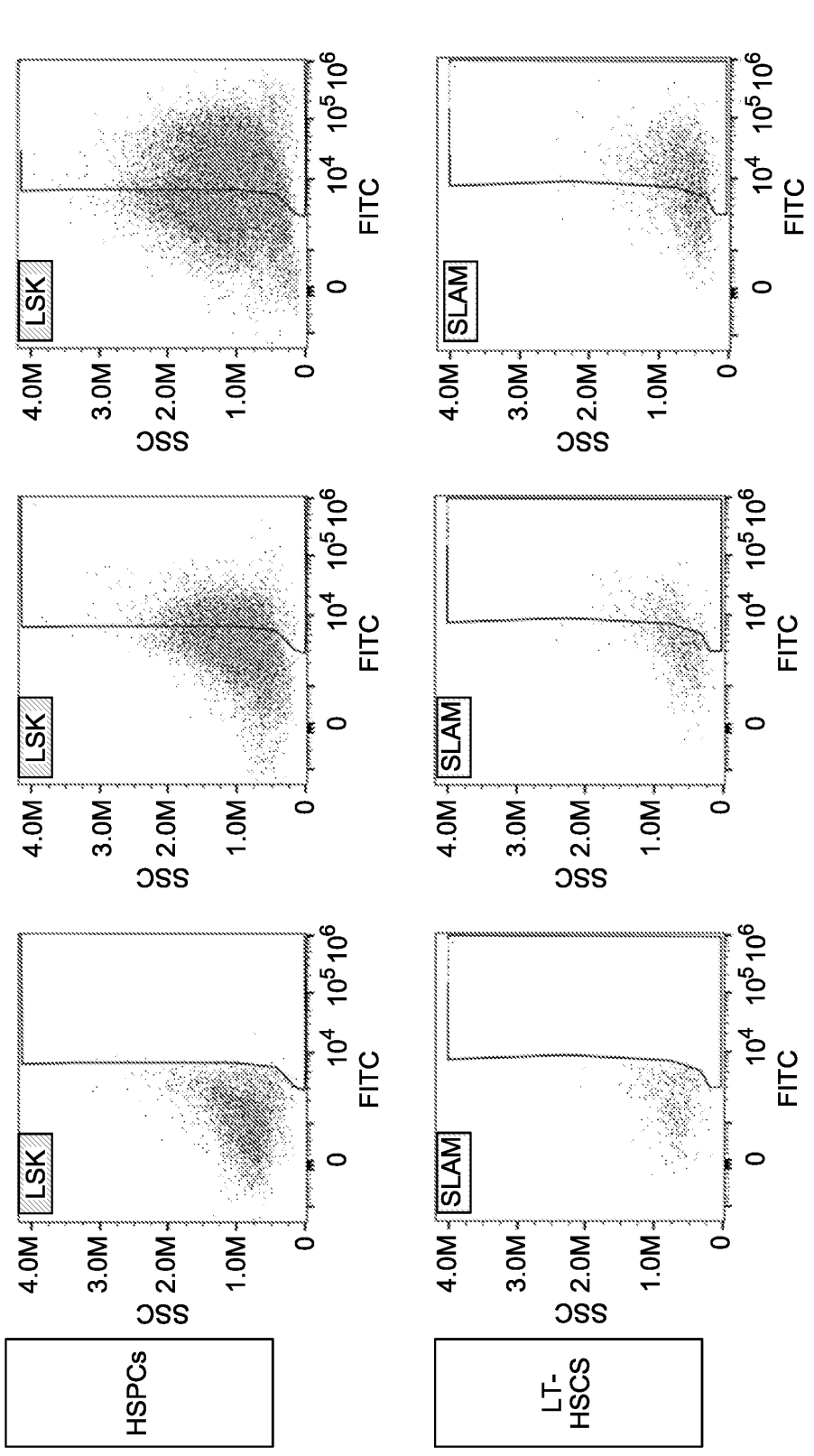

Saline Control
Exemplary Lipid 1
Exemplary Lipid 11
HSPCs
LT-HSCs
LSK
SLAM
SSC
FITC
4.0M
3.0M
2.0M
1.0M
0
10^4
10^5
10^6

FIG. 17

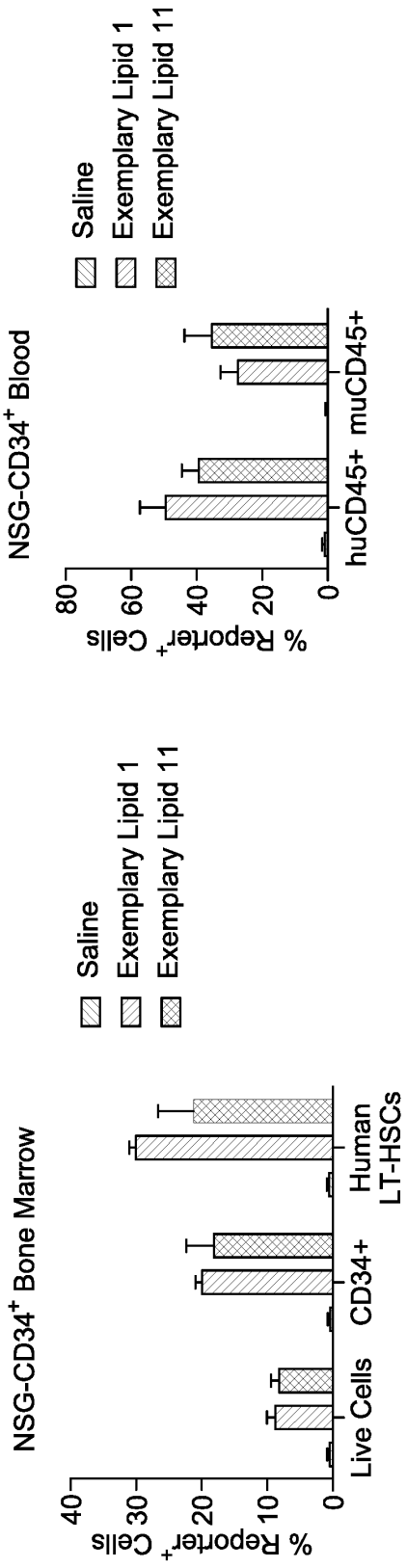
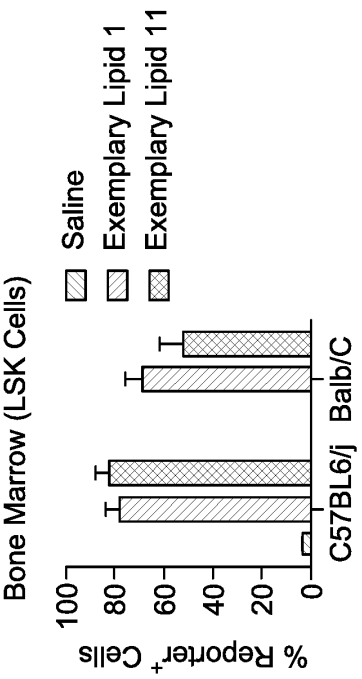
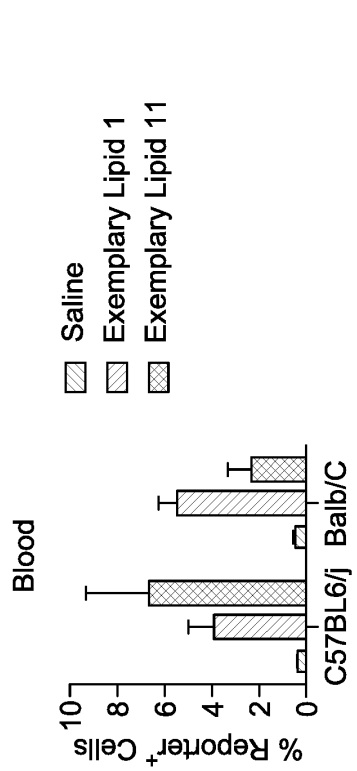

NSG-CD34+ Bone Marrow
% Reporter+ Cells
0
10
20
30
40
Live Cells
CD34+
Human LT-HSCs
Saline
Exemplary Lipid 1
Exemplary Lipid 11
NSG-CD34+ Blood
% Reporter+ Cells
0
20
40
60
80
huCD45+
muCD45+
Saline
Exemplary Lipid 1
Exemplary Lipid 11

FIG. 18


Bone Marrow (LSK Cells)
Saline
Exemplary Lipid 1
Exemplary Lipid 11
% Reporter+ Cells
100
80
60
40
20
0
C57BL6/j
Balb/C


Blood
Saline
Exemplary Lipid 1
Exemplary Lipid 11
% Reporter+ Cells
10
8
6
4
2
0
C57BL6/j
Balb/C

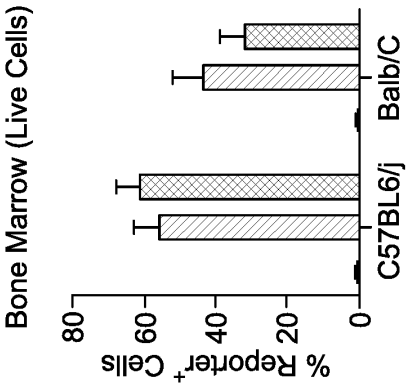

Bone Marrow (Live Cells)
Saline
Exemplary Lipid 1
Exemplary Lipid 11
% Reporter+ Cells
80
60
40
20
0
C57BL6/j
Balb/C

FIG. 19

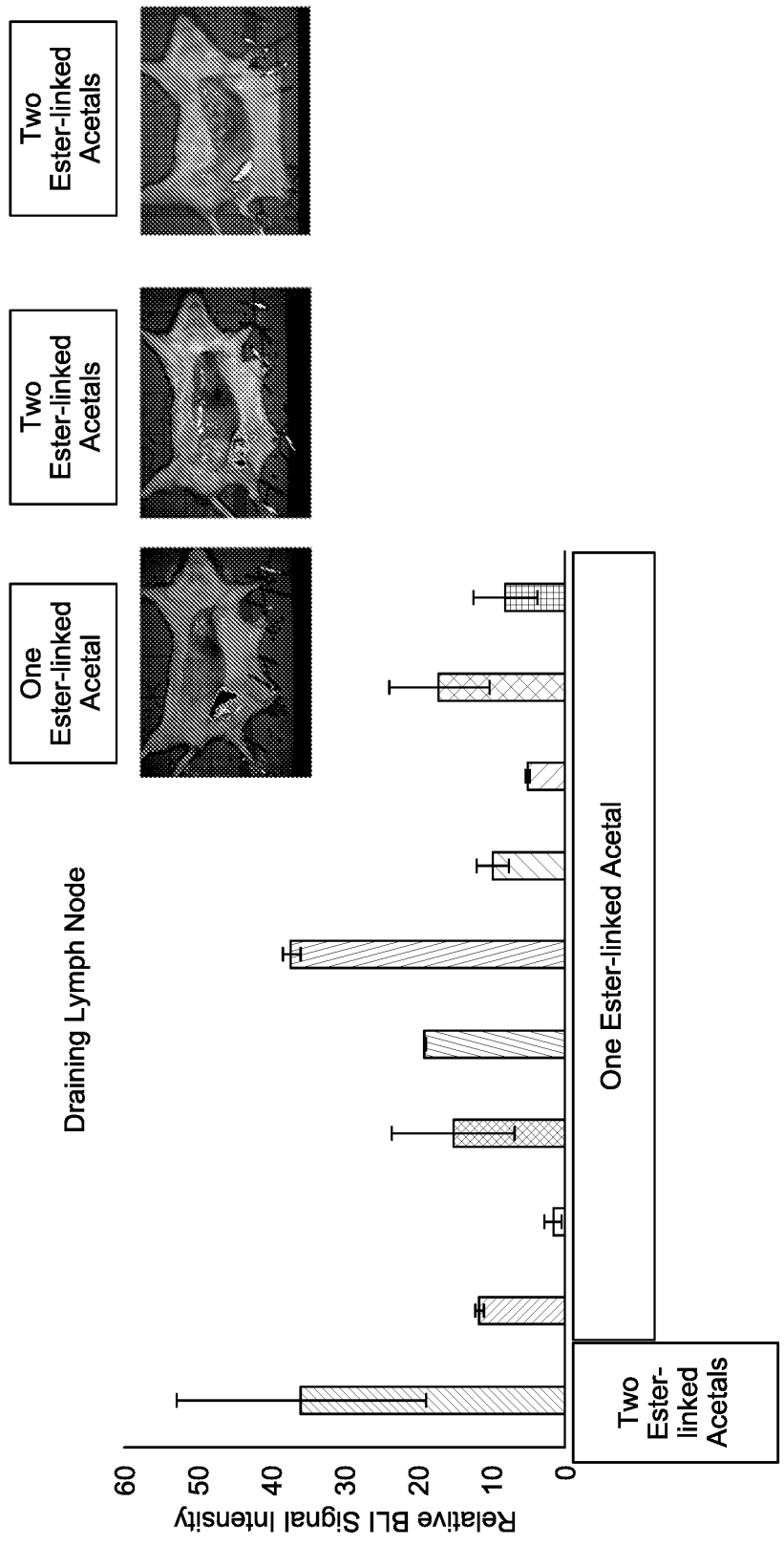

Two Ester-linked Acetals
Two Ester-linked Acetals
One Ester-linked Acetal
Draining Lymph Node
Relative BLI Signal Intensity
60
50
40
30
20
10
0
Two Ester-linked Acetals
One Ester-linked Acetal

FIG. 20

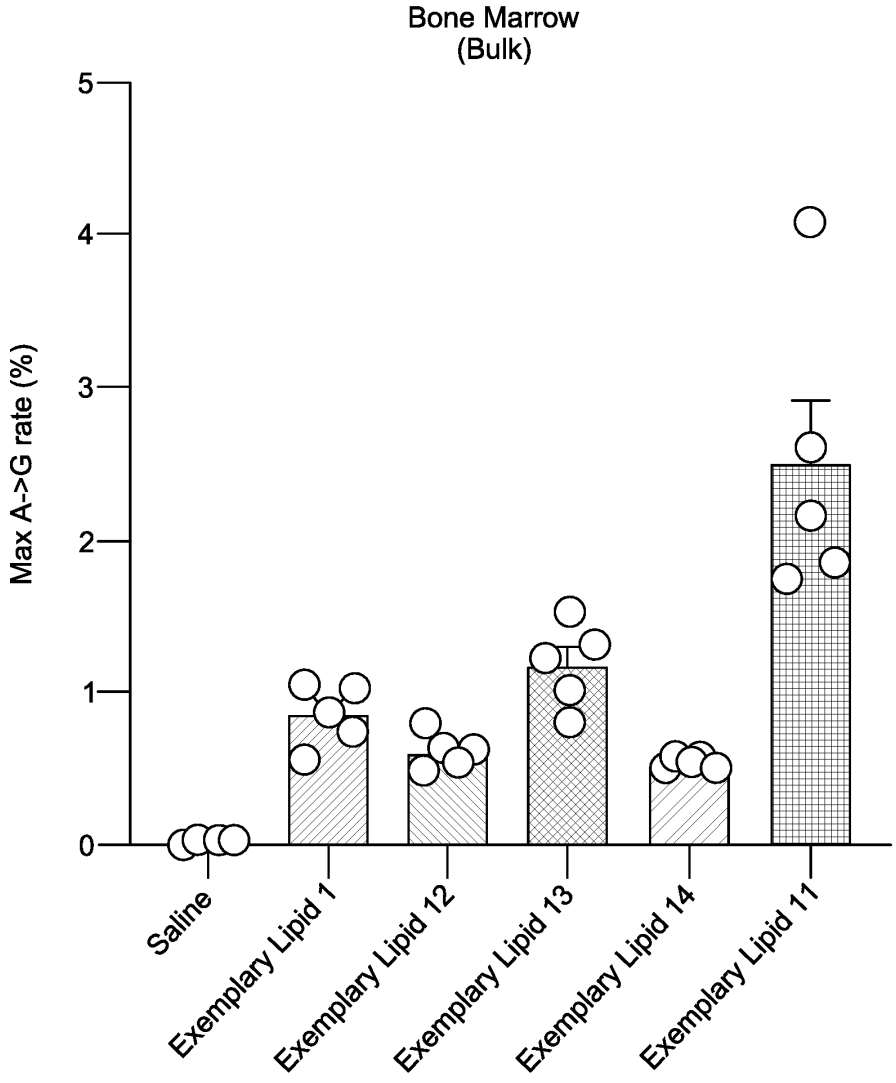

Bone Marrow
(Bulk)
Max A->G rate (%)
5
4
3
2
1
0
Saline
Exemplary Lipid 1
Exemplary Lipid 12
Exemplary Lipid 13
Exemplary Lipid 14
Exemplary Lipid 11

FIG. 21

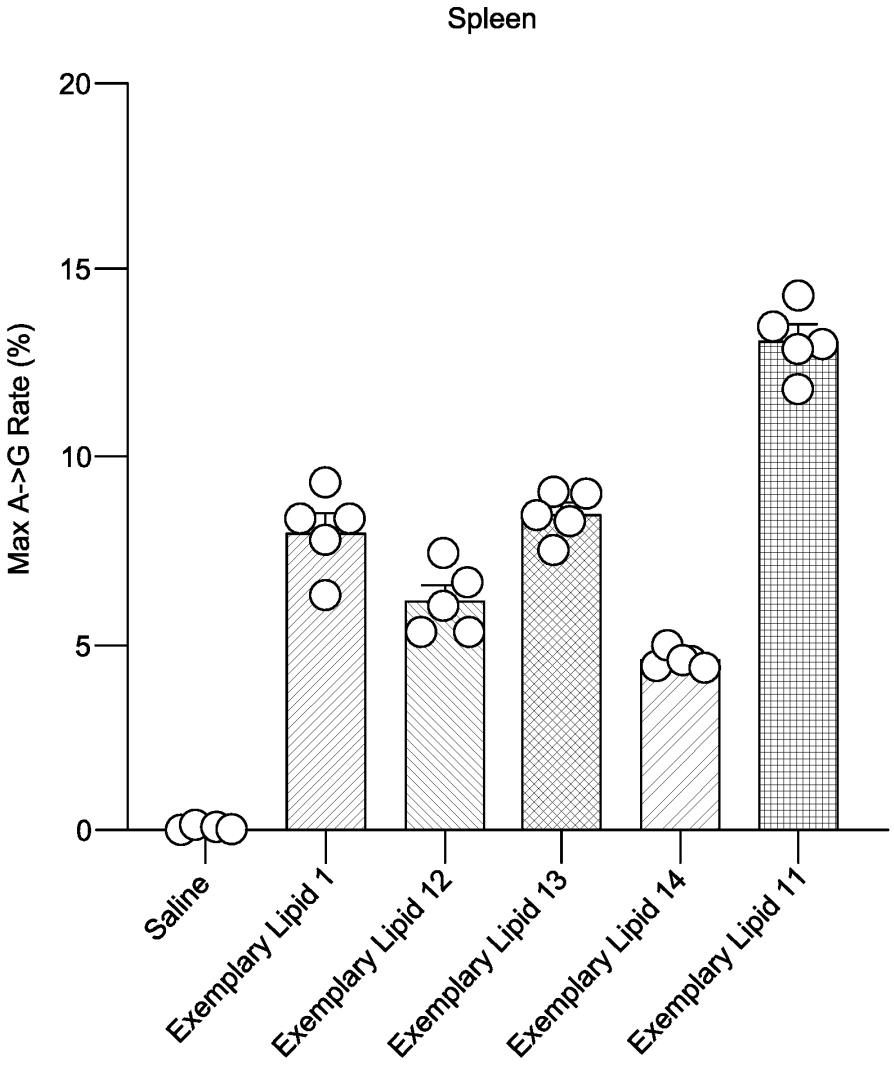

Spleen
Max A->G Rate (%)
20
15
10
5
0
Saline
Exemplary Lipid 1
Exemplary Lipid 12
Exemplary Lipid 13
Exemplary Lipid 14
Exemplary Lipid 11

FIG. 22

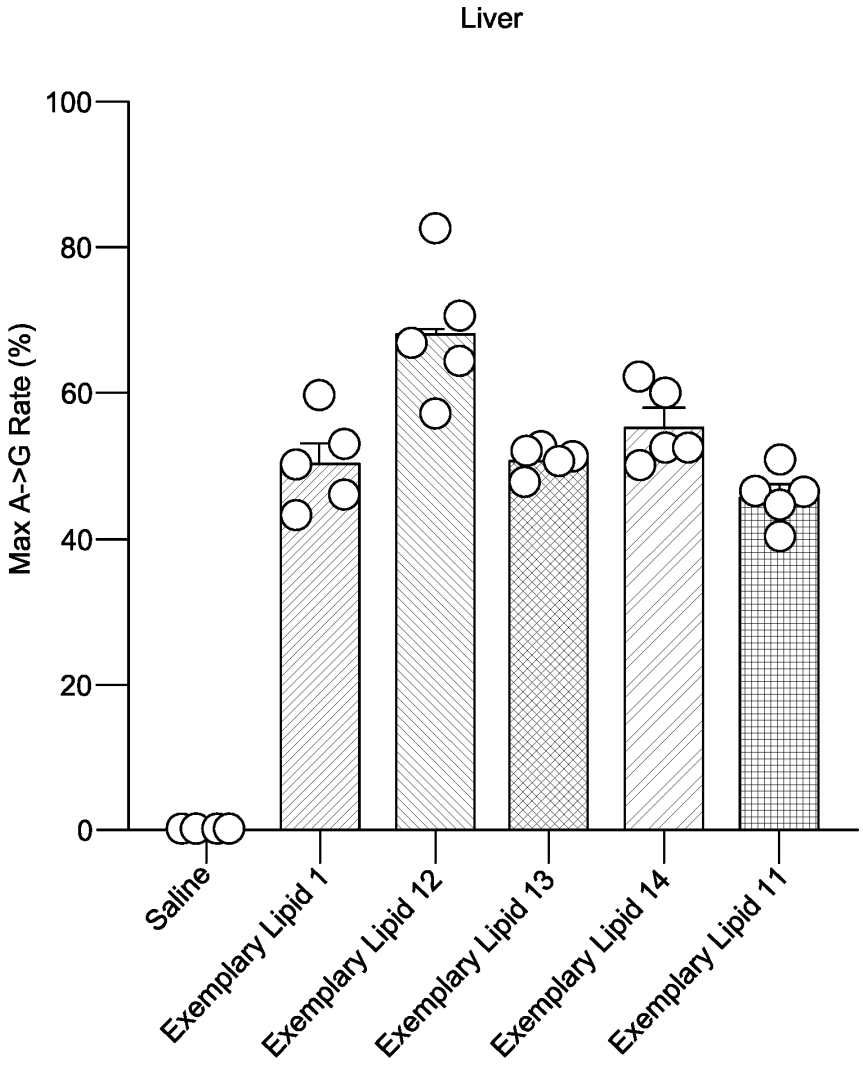

Liver
Max A->G Rate (%)
100
80
60
40
20
0
Saline
Exemplary Lipid 1
Exemplary Lipid 12
Exemplary Lipid 13
Exemplary Lipid 14
Exemplary Lipid 11

FIG. 23

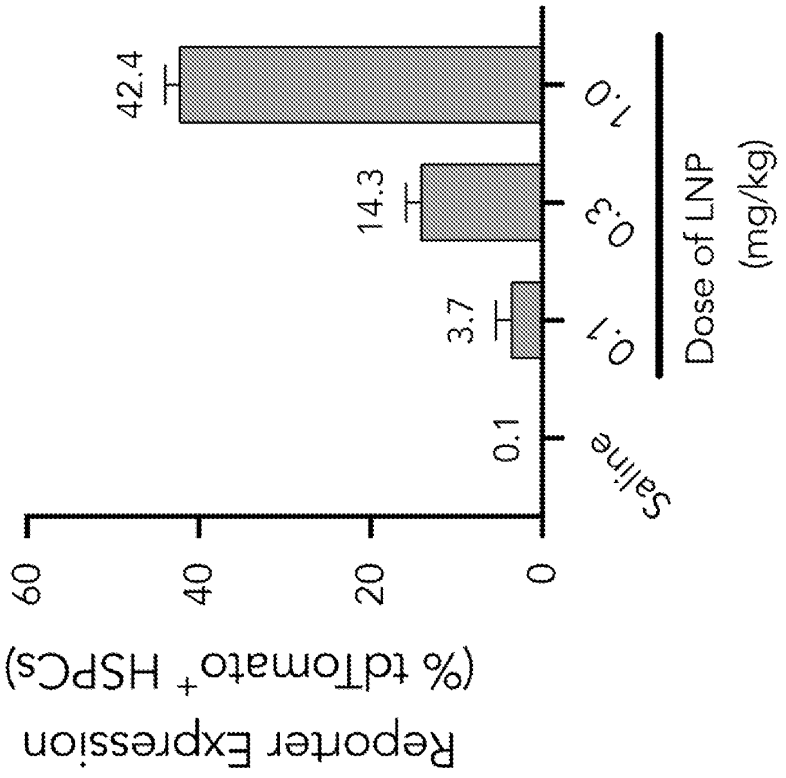

Reporter Expression
(% tdTomato+ HSPCs)
60
40
20
0
42.4
14.3
3.7
0.1
Saline
0.1
0.3
1.0
Dose of LNP
(mg/kg)

FIG. 24

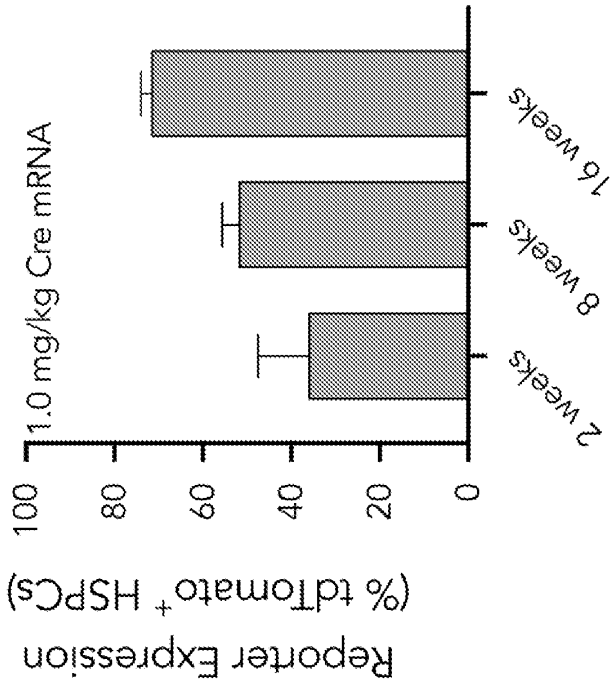


FIG. 25B

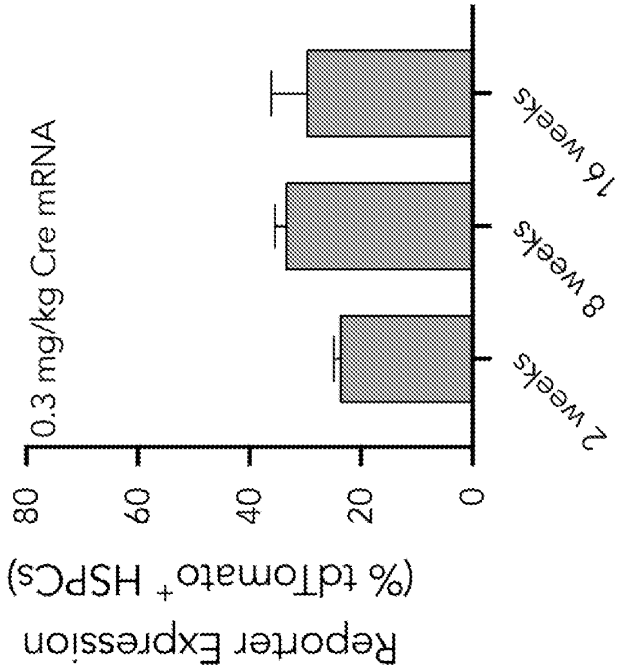


FIG. 25A

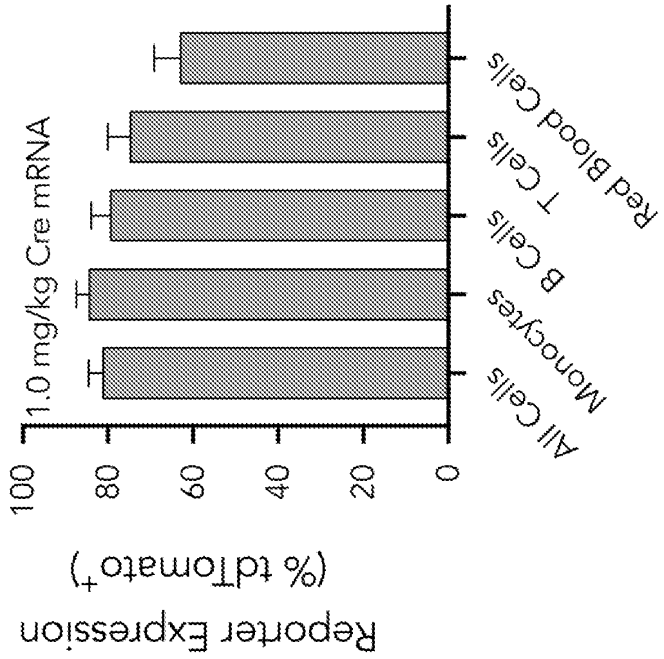


FIG. 25D

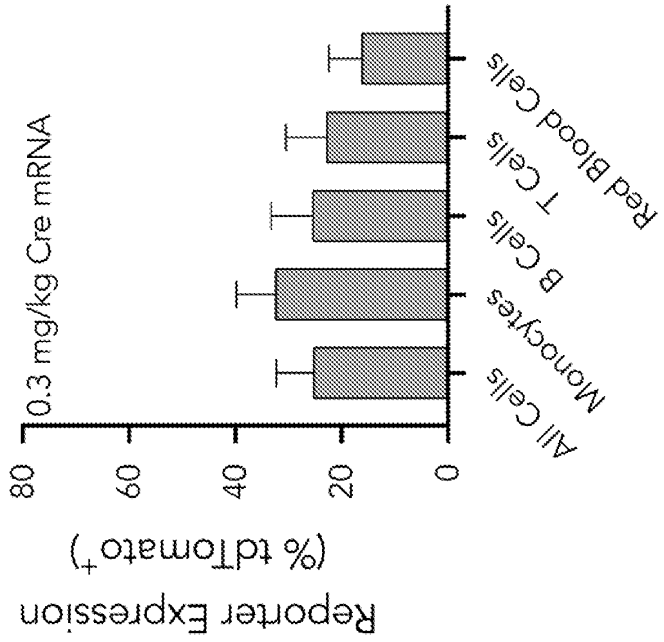


FIG. 25C

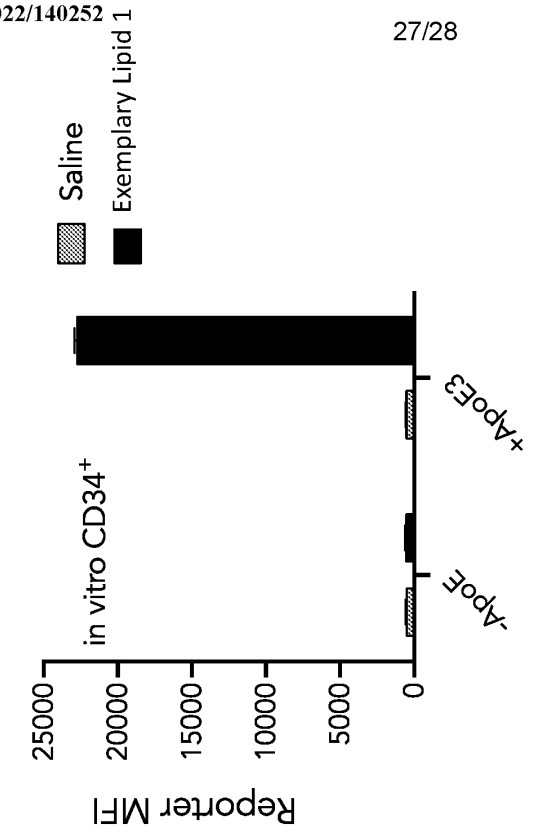


FIG. 26B

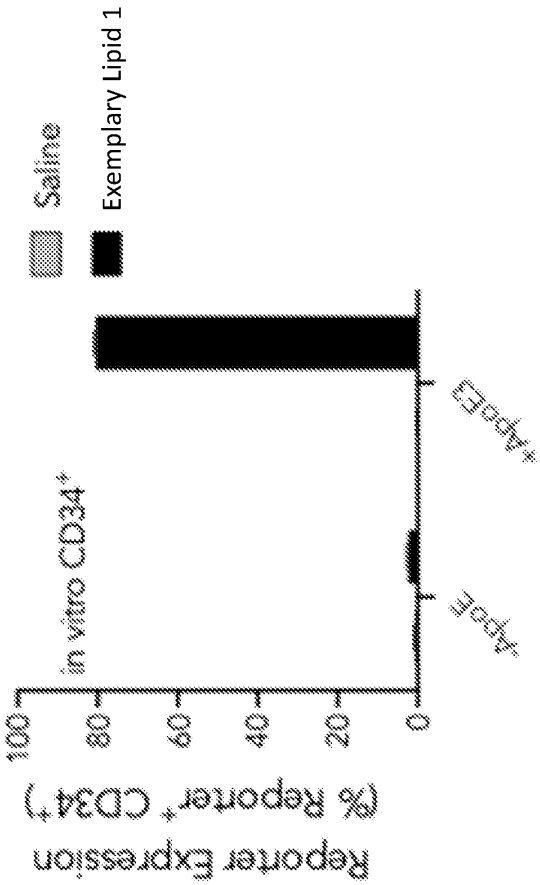


FIG. 26A

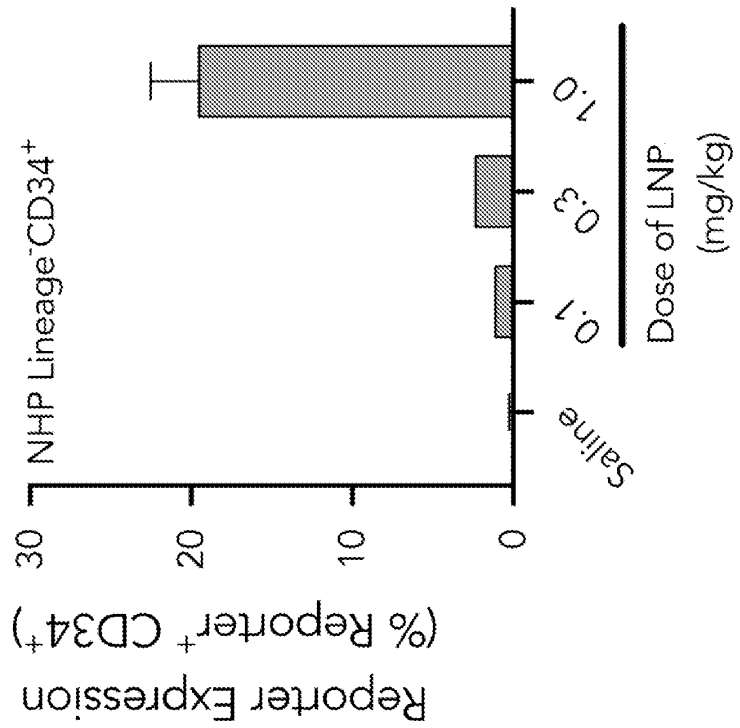

Reporter Expression
(% Reporter+ CD34+)
NHP Lineage-CD34+
30
20
10
0
Saline
0.1
0.3
1.0
Dose of LNP
(mg/kg)

FIG. 27