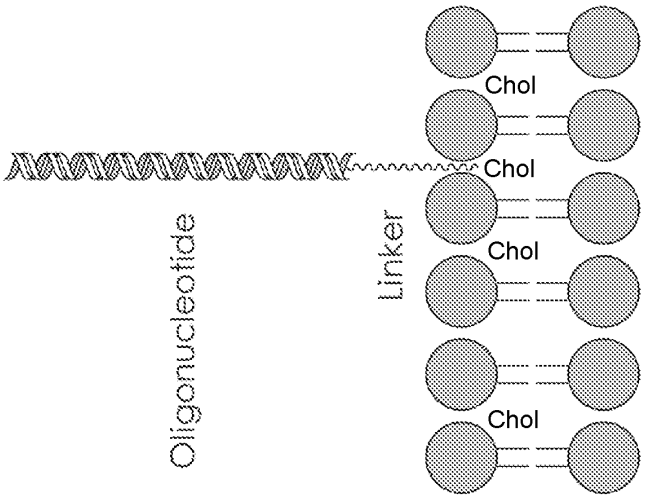

Chol
Chol
Chol
Chol
Linker
Oligonucleotide

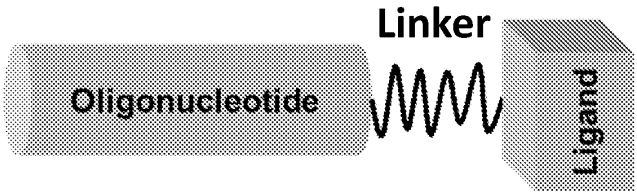
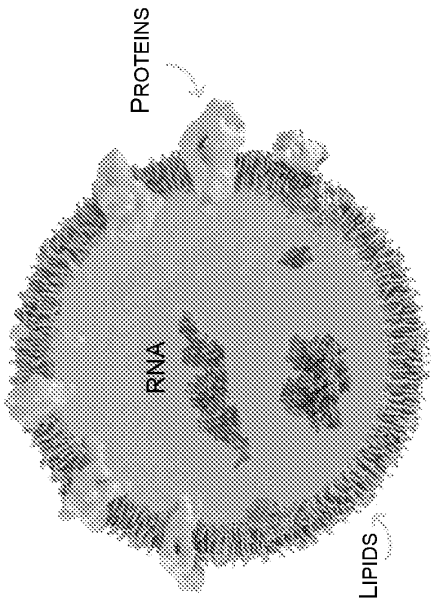

Linker
Oligonucleotide
Ligand

FIG. 1


PROTEINS
RNA
LIPIDS

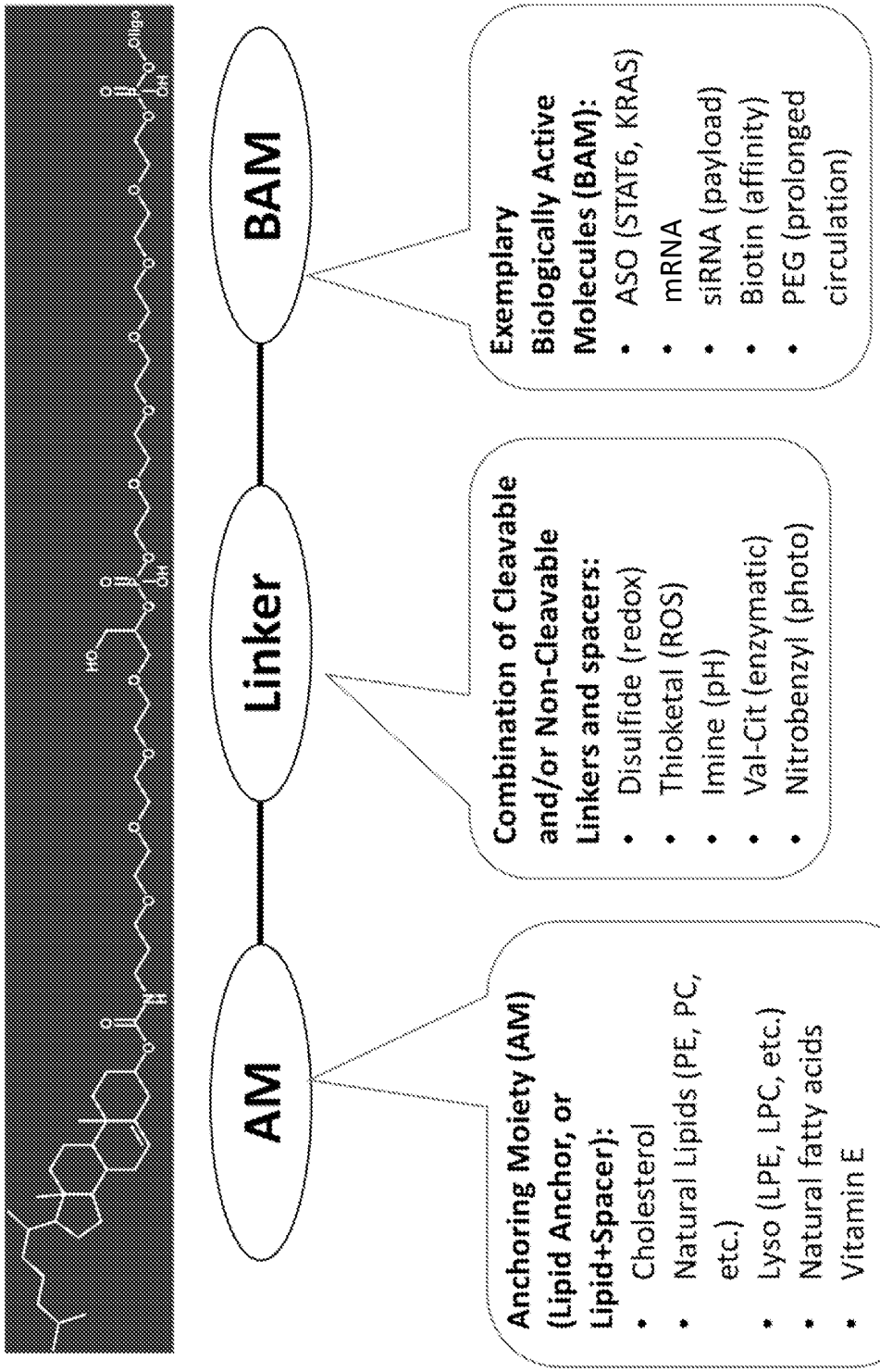
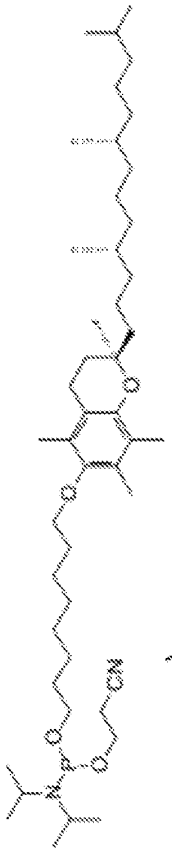

AM
Linker
BAM
Anchoring Moiety (AM) (Lipid Anchor, or Lipid+Spacer):
Cholesterol
Natural Lipids (PE, PC, etc.)
Lyso (LPE, LPC, etc.)
Natural fatty acids
Vitamin E
Pyridoxone, niacin
Combination of Cleavable and/or Non-Cleavable Linkers and spacers:
Disulfide (redox)
Thioketal (ROS)
Imine (pH)
Val-Cit (enzymatic)
Nitrobenzyl (photo)
Exemplary Biologically Active Molecules (BAM):
ASO (STAT6, KRAS)
mRNA
siRNA (payload)
Biotin (affinity)
PEG (prolonged circulation)

FIG. 2

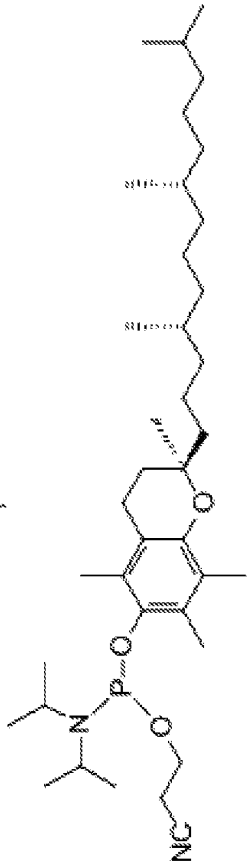
Exemplary Key Building Blocks for Linkers

Membrane Anchors (Lipid)

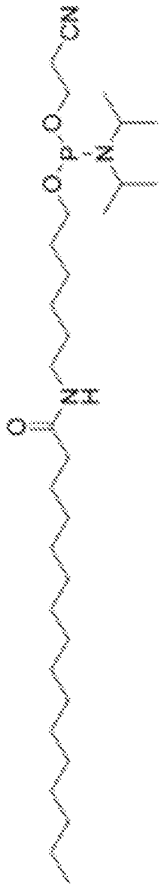
Tocopherol-Octyl(C8) phosphoramidite



Tocopherol phosphoramidite



Palmitate-Hexyl(C6) phosphoramidite



Cholesterol-TEG phosphoramidite



Cholesterol-Hexyl(C6) phosphoramidite

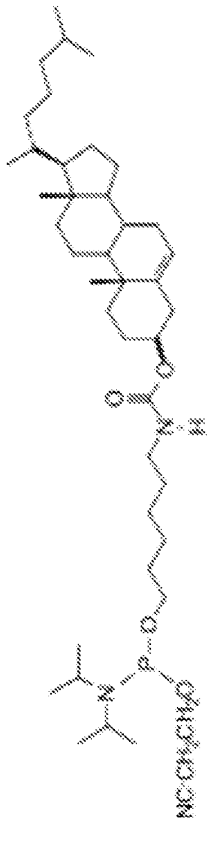


FIG. 3

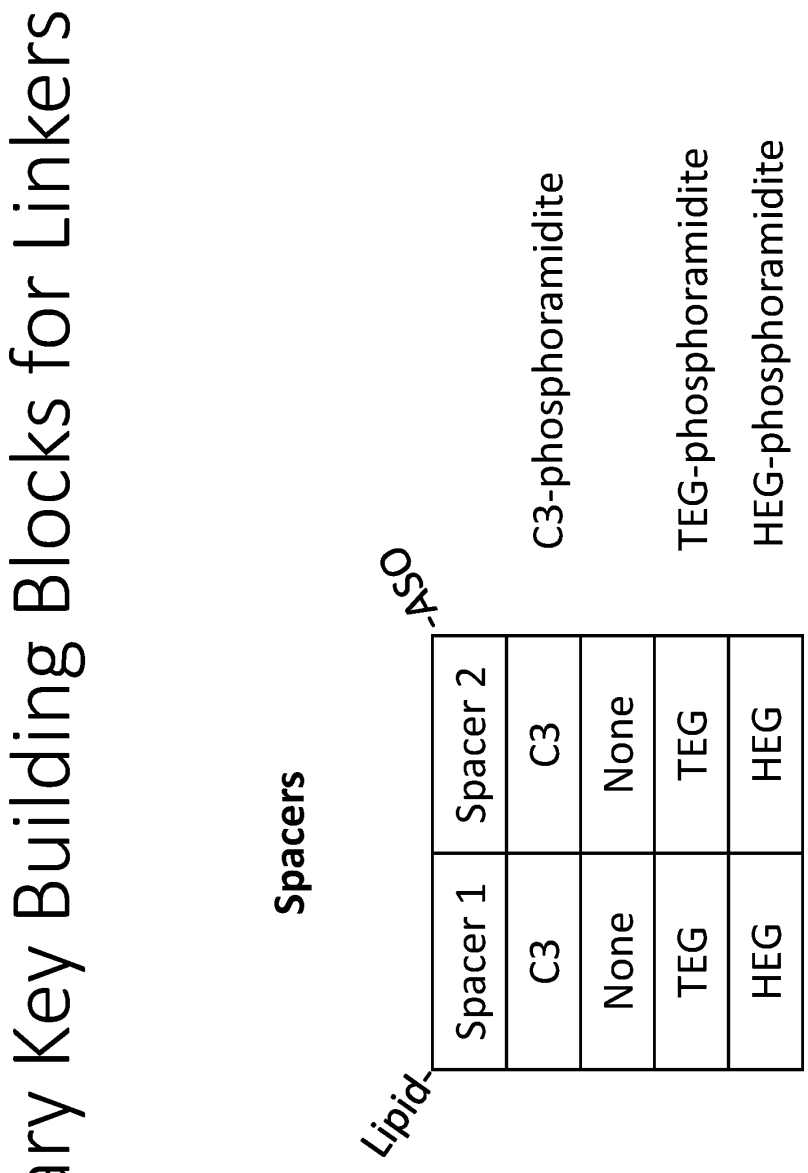

Exemplary Key Building Blocks for Linkers
Spacers
Lipid-
-ASO
Spacer 1
Spacer 2
C3
C3
None
None
TEG
TEG
HEG
HEG
C3-phosphoramidite
TEG-phosphoramidite
HEG-phosphoramidite

FIG. 4

No.	Lipid Anchor	Link	Spacer 1	Link	Spacer 2	Link	ASO	Loading Efficiency	ASO Per Exo.	Average
C1	Cholesterol-**C6**	PO	C3	PS	No	No	FFLuc	80.39%	4824	5032+/-386
C2					TEG	PS		93.60%	5616	
C3					HEG	PS		90.67%	5440	
C4			TEG		No	No		88.08%	5285	
C5					C3	PS		86.74%	5204	
C6					HEG	PS		78.99%	4740	
C7			HEG		No	No		80.85%	4851	
C8					C3	PS		82.39%	4943	
C9			No	No	No	No		73.06%	4384	
T1	Cholesterol-**TEG**	PO	C3	PS	No	No		76.65%	4599	3991+/-490
T2					TEG	PS		79.67%	4780	
T3					HEG	PS		70.89%	4253	
T4			TEG		No	No		68.17%	4090	
T5					C3	PS		67.03%	4022	
T6					HEG	PS		58.82%	3529	
T7			HEG		No	No		61.11%	3667	
T8					C3	PS		60.21%	3612	
T9			No	No	No	No		56.14%	3368	
L1	Tocopherol-C8	PO	TEG	PS	HEG	PS		57.55%	3453	4241+/-722
L2	Tocopherol							81.17%	4870	
L3	Palmitate-C6							73.33%	4400	

PO: Phosphodiester PS: Phosphorothioate C6: Hexamethylene C3: trimethylene TEG: Triethylene glycol HEG: Hexaethylene glycol

FIG. 5

SUBSTITUTE SHEET (RULE 26)

SUBSTITUTE SHEET (RULE 26)

No.	Lipid Anchor	Link	Spacer 1	Link	Spacer 2	Link	ASO	Loading Efficiency	ASO Per Exo.	Average
C1	Cholesterol-**C6**	PO	C3	PS	No	No	FFLuc	43.44%	2606	2442+/-339
C2					**TEG**	PS		41.07%	2464	
C3					HEG	PS		46.47%	2788	
C4			TEG		No	No		43.85%	2631	
C5					C3	PS		44.46%	2668	
C6					HEG	PS		41.04%	2462	
C7			HEG		No	No		40.07%	2404	
C8					C3	PS		38.93%	2336	
C9			No	No	No	No		27.04%	1623	
T1	Cholesterol-**TEG**	PO	C3	PS	No	No		29.24%	1754	1728+/-264
T2					TEG	PS		27.65%	1659	
T3					HEG	PS		33.22%	1993	
T4			TEG		No	No		31.50%	1890	
T5					C3	PS		32.34%	1940	
T6					HEG	PS		29.35%	1761	
T7			HEG		No	No		31.79%	1907	
T8					C3	PS		24.86%	1491	
T9			No	No	No	No		19.34%	1161	
L1	Tocopherol-C8	PO	TEG	PS	HEG	PS		68.78%	4127	2979+/-1006
L2	Tocopherol							42.73%	2564	
L3	Palmitate-C6							37.45%	2247	

PO: Phosphodiester PS: Phosphorothioate C6: Hexamethylene C3: trimethylene TEG: Triethylene glycol HEG: Hexaethylene glycol

FIG. 6

Statistical Analysis of Linker Effect on Loading Density:
Lumped Native and PrX EVs

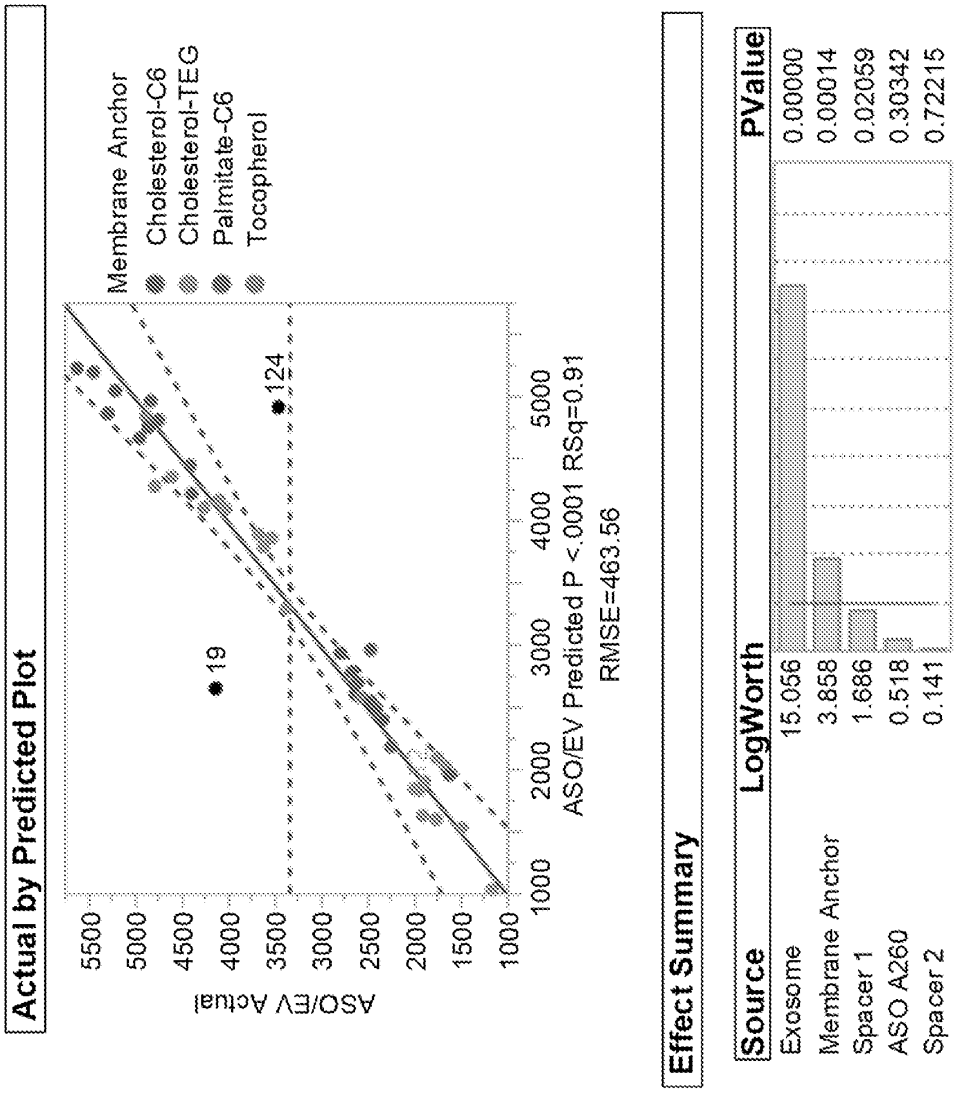
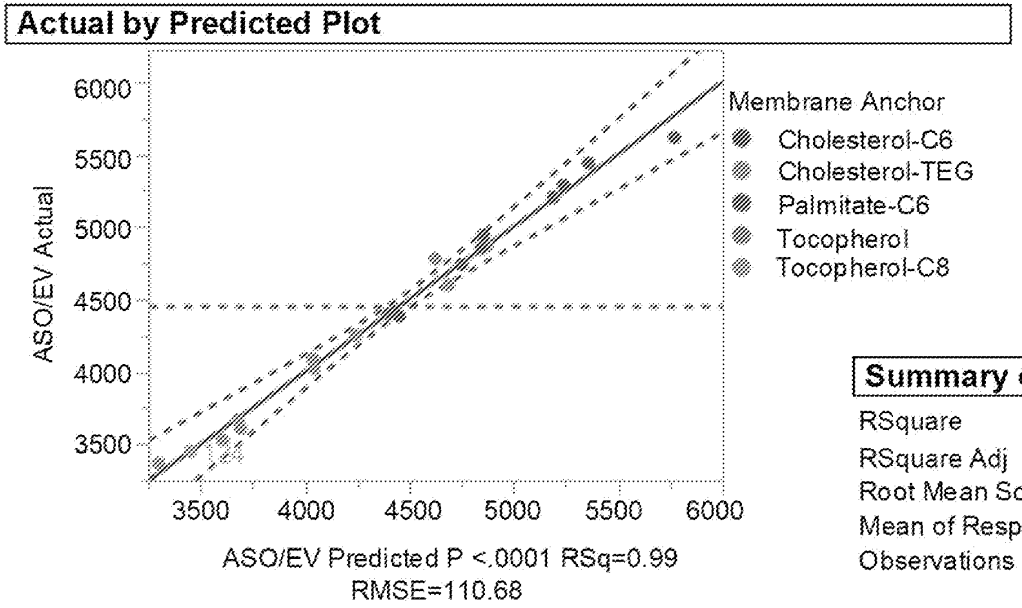


FIG. 7

Individual Statistical Models for Native and PrX are Highly Significant

Native



Summary of Fit	
RSquare	0.954633
RSquare Adj	0.899184
Root Mean Square Error	215.543
Mean of Response	4472.857
Observations (or Sum Wgts)	21

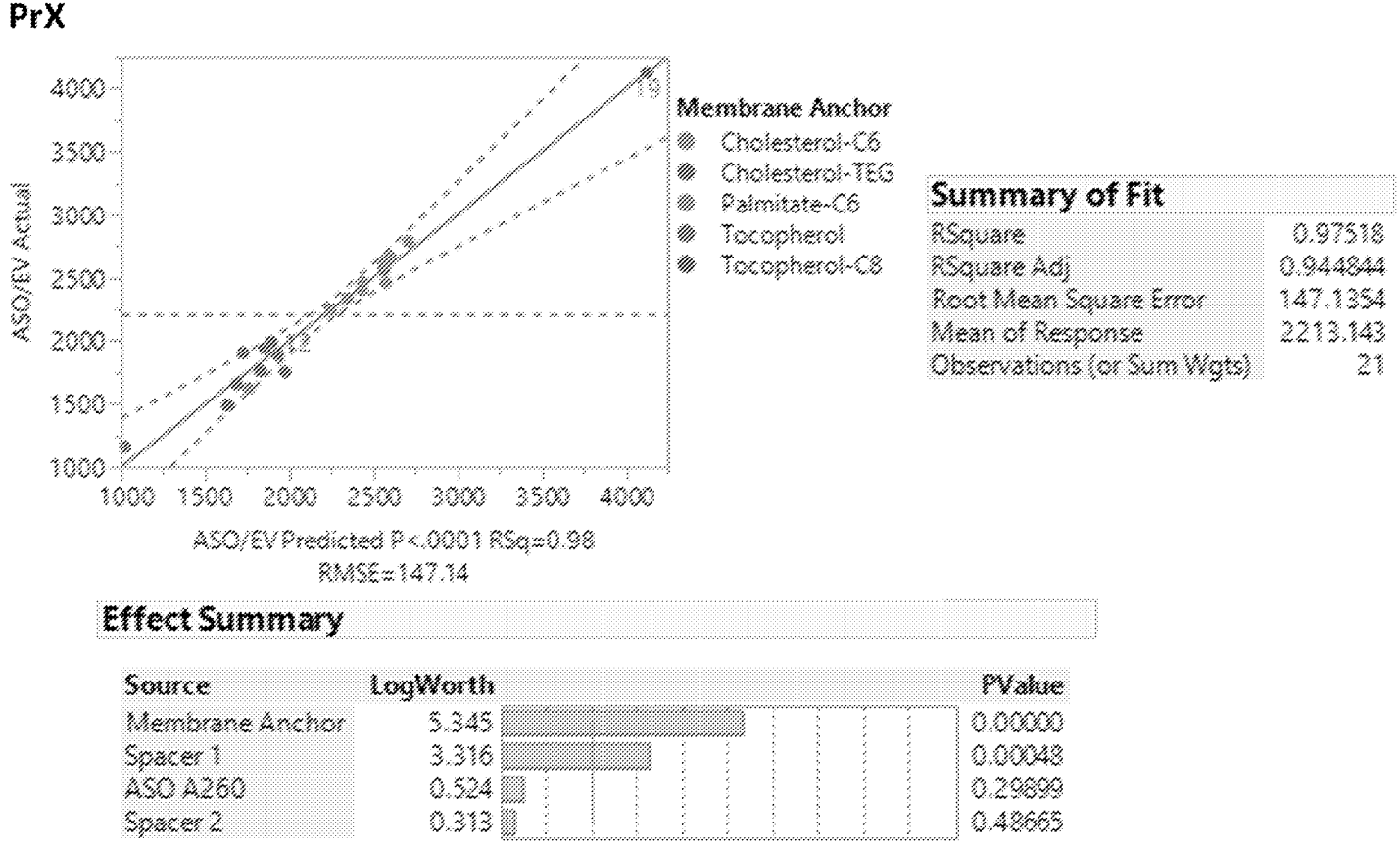
FIG. 8A

Effect Summary

Source	LogWorth	PValue
Membrane Anchor	4.676	0.00002
Spacer 1	2.673	0.00212
ASO A260	1.485	0.03275
Spacer 2	1.423	0.03772

Residuals show no skew and appear to be normally distributed

Individual Statistical Models for Native and PrX are Highly Significant



Residuals show no skew and appear to be normally distributed

FIG. 8B

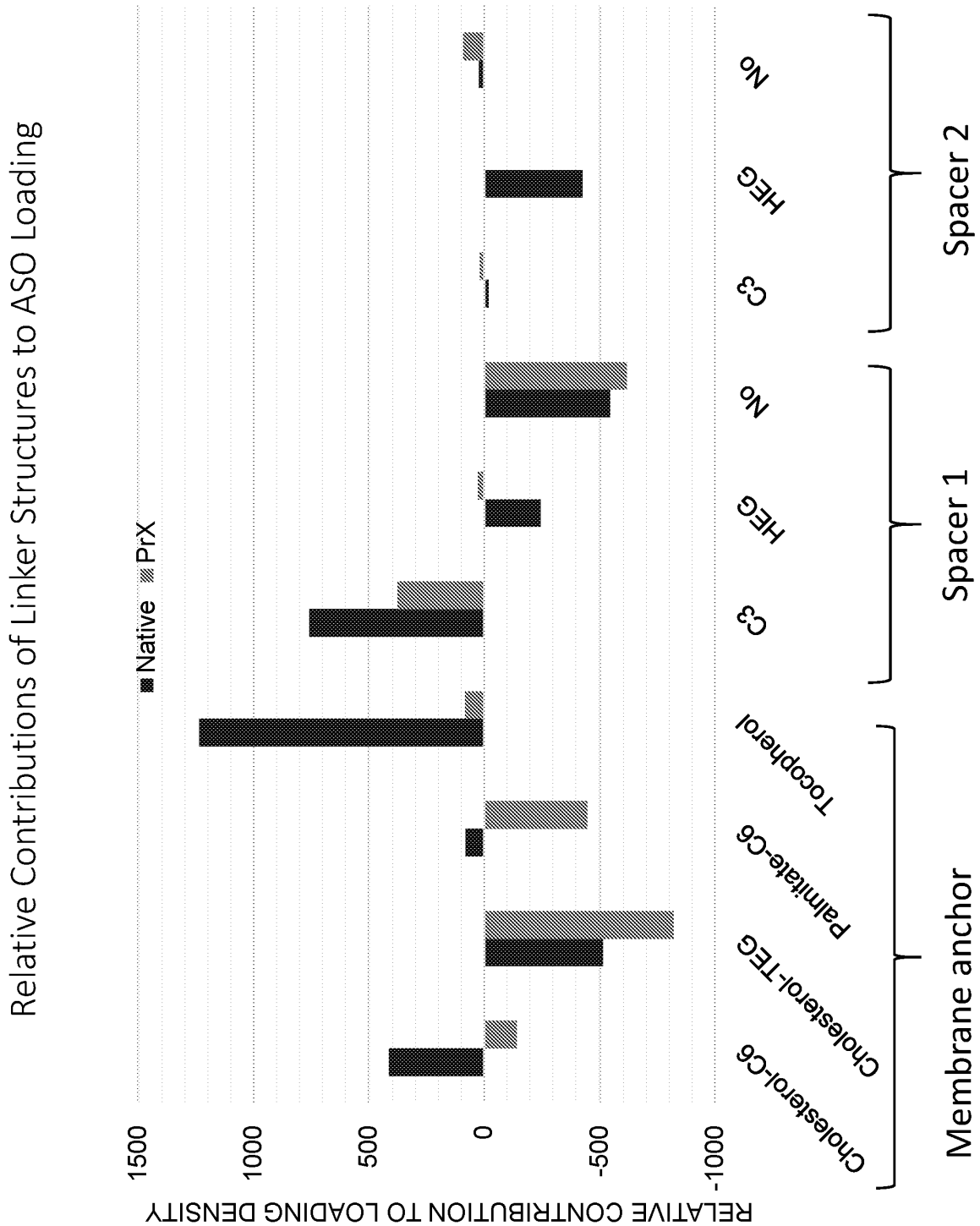

Relative Contributions of Linker Structures to ASO Loading
Native
PrX
RELATIVE CONTRIBUTION TO LOADING DENSITY
1500
1000
500
0
-500
-1000
Cholesterol-C6
Cholesterol-TEG
Palmitate-C6
Tocopherol
C3
HEG
No
C3
HEG
No
Membrane anchor
Spacer 1
Spacer 2

FIG. 8C

- Highest activity observed with constructs 2 and 4
- Greater differences in activity across constructs with Chol2 lipid
- Highest ASO loading efficiencies in L1-L3

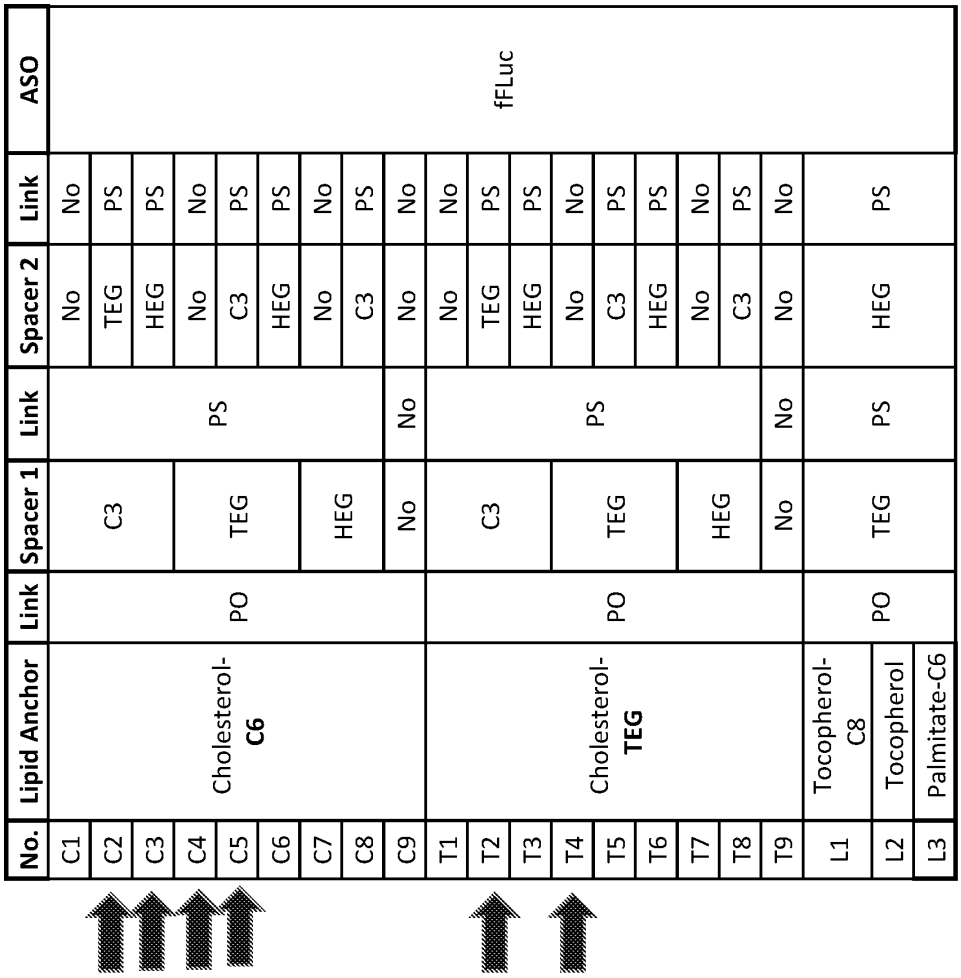


FIG. 9A

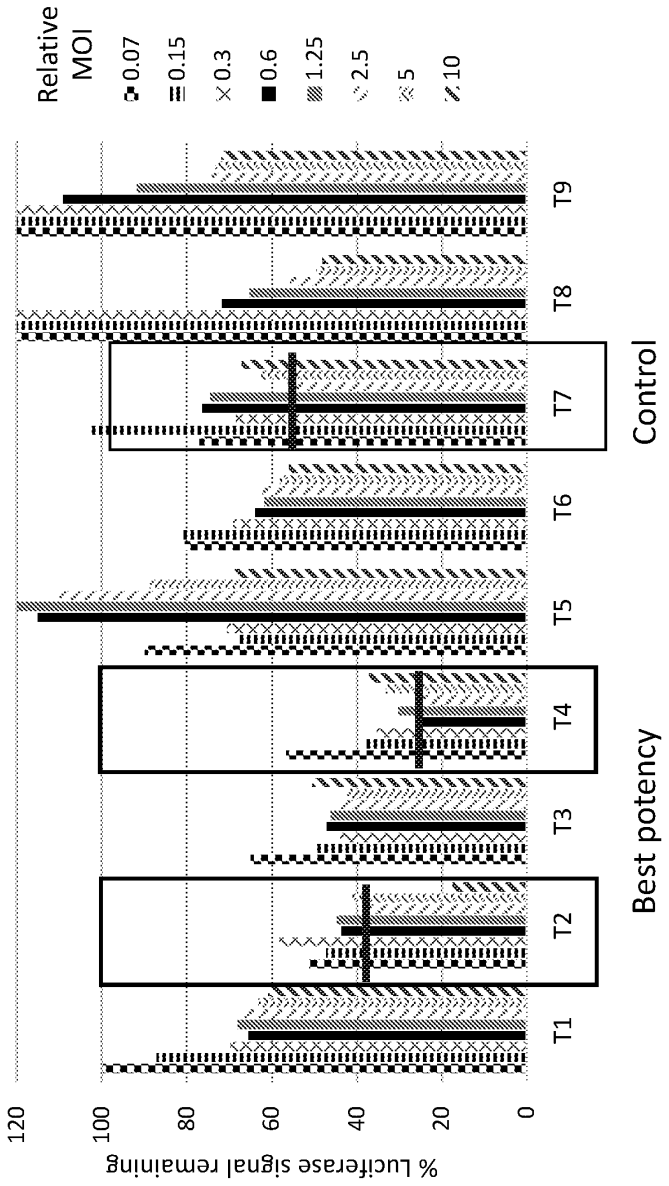

Relative MOI
0.07
0.15
0.3
0.6
1.25
2.5
5
10
% Luciferase signal remaining
120
100
80
60
40
20
0
T1
T2
T3
T4
T5
T6
T7
T8
T9
Best potency
Control

FIG. 9B

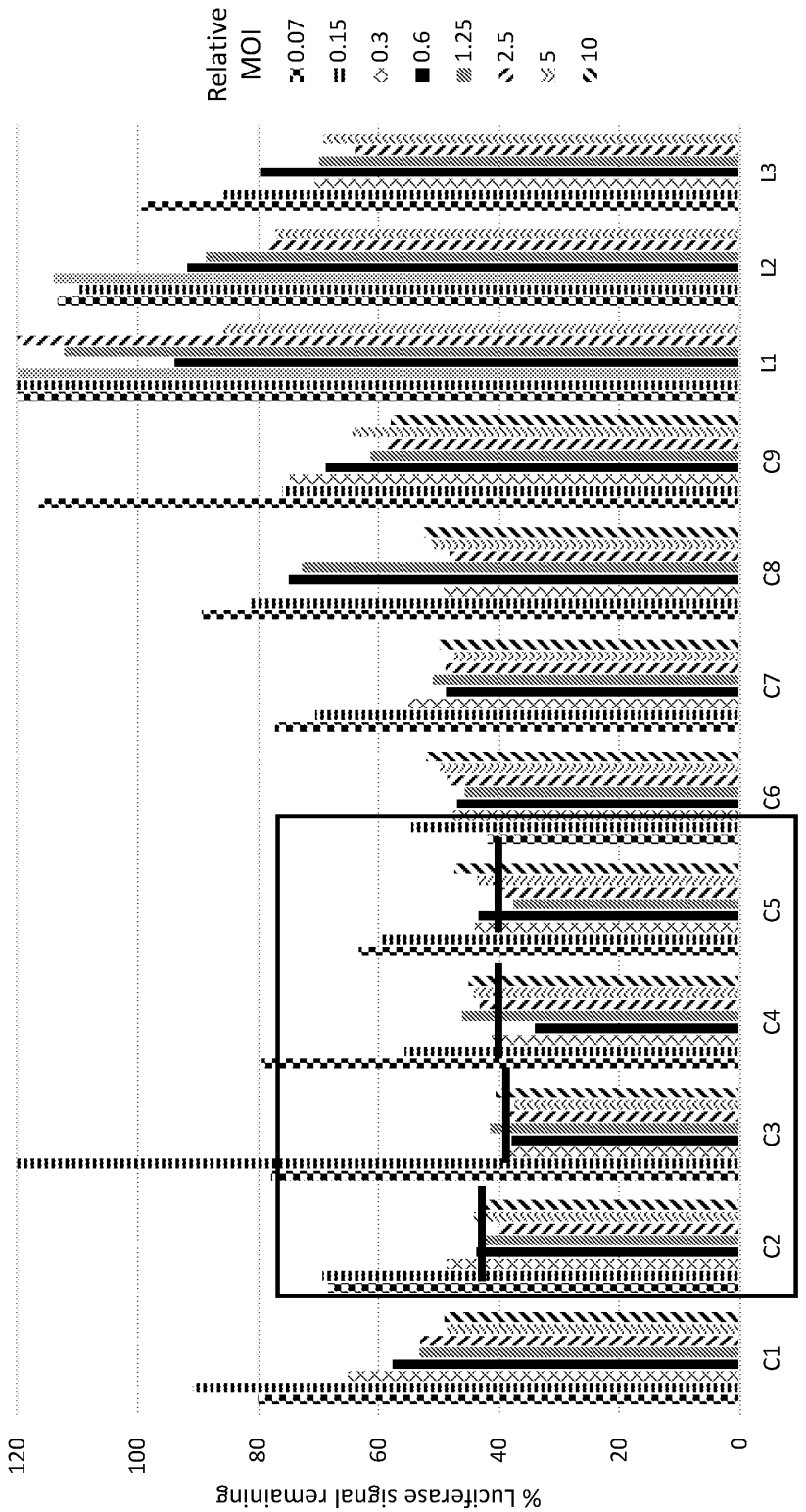

Relative MOI
0.07
0.15
0.3
0.6
1.25
2.5
5
10
% Luciferase signal remaining
0
20
40
60
80
100
120
C1
C2
C3
C4
C5
C6
C7
C8
C9
L1
L2
L3

FIG. 9C

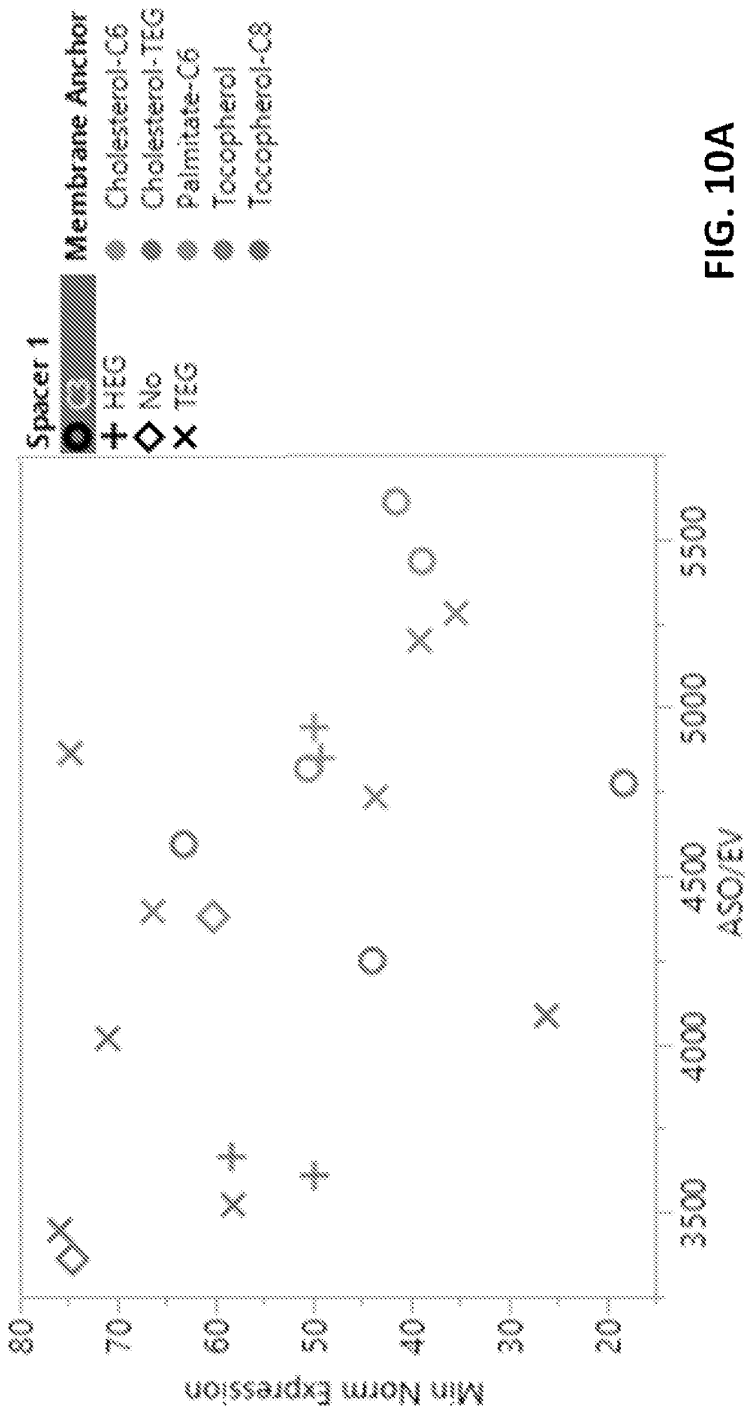

Membrane Anchor
Cholesterol-C6
Cholesterol-TEG
Palmitate-C6
Tocopherol
Tocopherol-C8
Spacer 1
HEG
No
TEG
Min Norm Expression
80
70
60
50
40
30
20
3500
4000
4500
5000
5500
ASO/EV

FIG. 10A

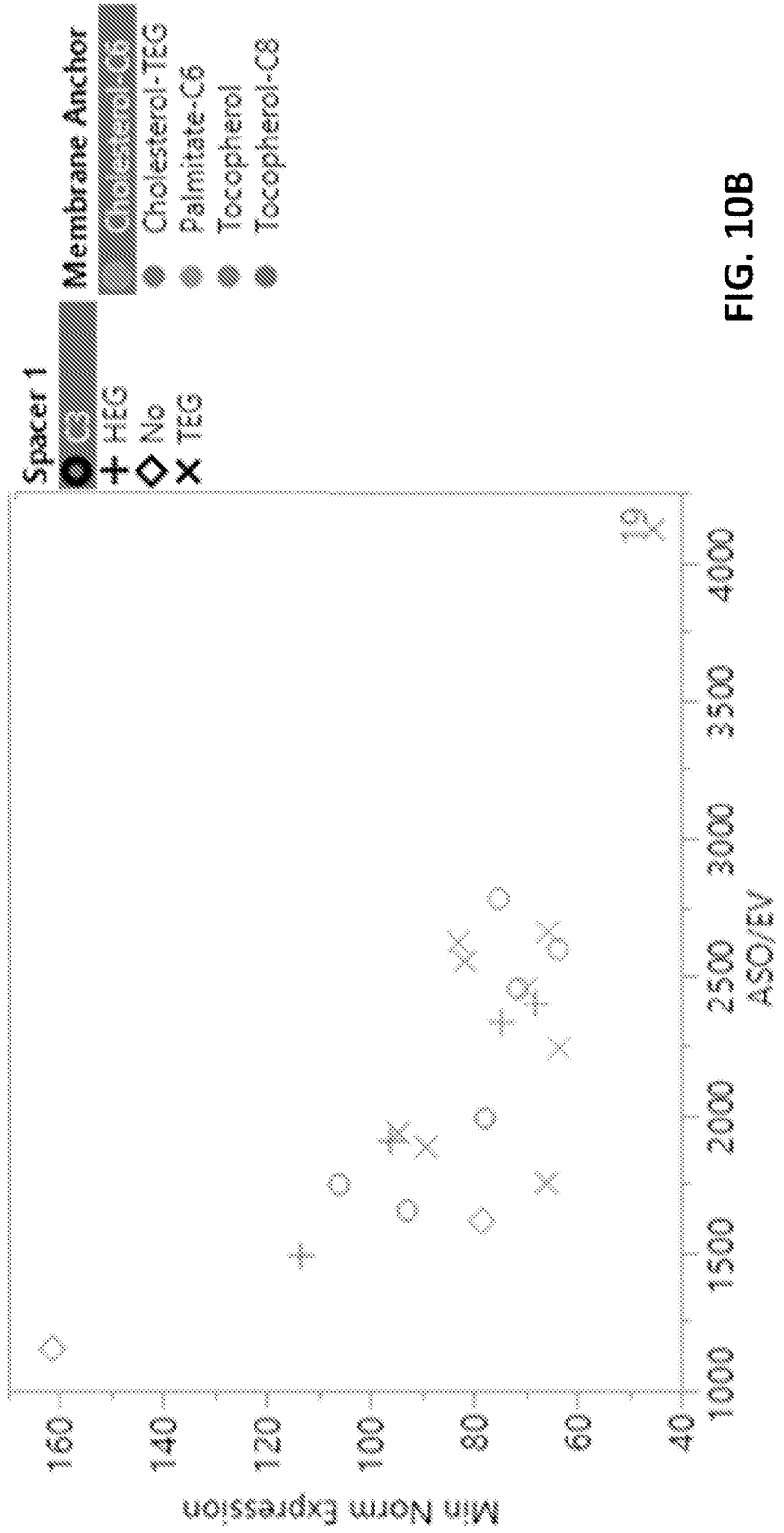

Spacer 1
C3
HEG
No
TEG
Membrane Anchor
Cholesterol-C6
Cholesterol-TEG
Palmitate-C6
Tocopherol
Tocopherol-C8
Min Norm Expression
160
140
120
100
80
60
40
1000
1500
2000
2500
3000
3500
4000
ASO/EV

FIG. 10B

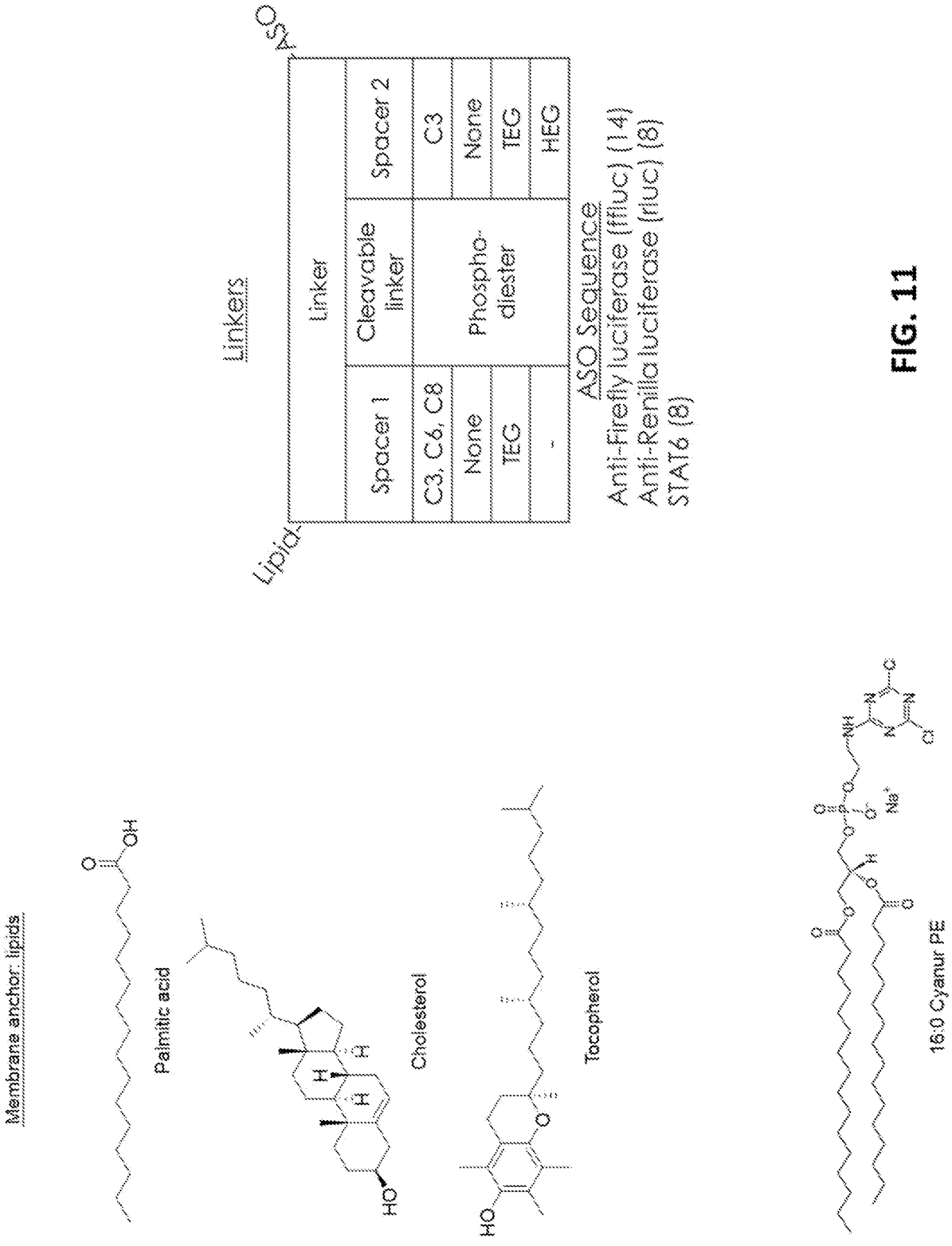
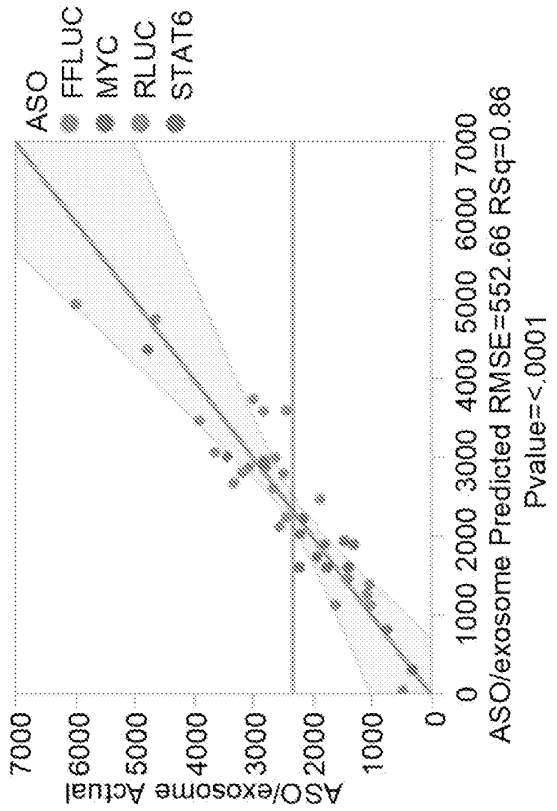

Linkers
Linker
Cleavable linker
Phospho-diester
Spacer 1
Spacer 2
C3, C6, C8
C3
None
None
TEG
TEG
-
HEG
Lipid
S
O
ASO Sequence
Anti-Firefly luciferase (ffluc) (14)
Anti-Renilla luciferase (rluc) (8)
STAT6 (8)
Membrane anchor: lipids
Palmitic acid
Cholesterol
Tocopherol
16:0 Cyanur PE

FIG. 11

ASO name	Sequence
FFLUC	5'-TbsCbsGbsAmsAmsdGsdTsdAsdCsdTsdCsdGsdGsdCsdGsTmsAmsGbsGbsTb-3'
RLUC	5'-AbsAbsCbsCmsCmsdAsdGsdGsdTsdCsdGsdGsdAsdCsTmsCmsGbsAbsTb-3'
STAT6	5'-GbsCbsAbsdAsdGsdAsdTs(5MdC)s(5MdC)s(5MdC) sdGsdGsdAsdTsdTs(5MdC)sdGsGbsTbsCb-3'
MYC	5'-GbsAbsCbsGmsTmsdGsdGs(5MdC)sdAs(5MdC)s(5MdC)sdTs(5MdC)sdTsdTsGmsAmsGbsGbsAb-3'

FIG. 12

FIG. 13A



Source	LogWorth	PValue
ASO	3.493	0.00032
Spacer 1	2.846	0.00143
Membrane Anchor	1.774	0.01683
Spacer 2	1.199	0.06319

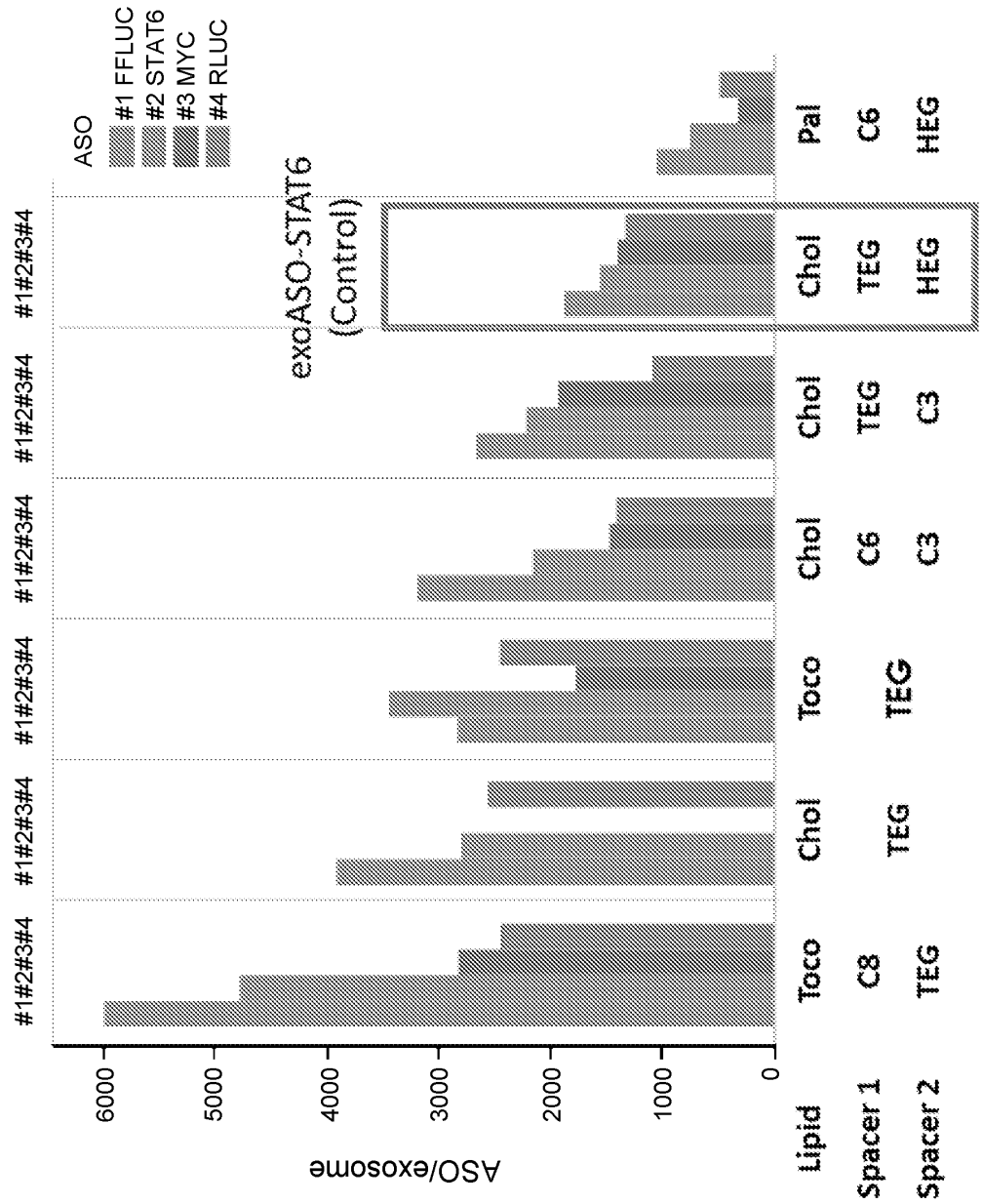

ASO
#1 FFLUC
#2 STAT6
#3 MYC
#4 RLUC
exoASO-STAT6
(Control)
ASO/exosome
6000
5000
4000
3000
2000
1000
0
#1#2#3#4
#1#2#3#4
#1#2#3#4
#1#2#3#4
#1#2#3#4
#1#2#3#4
Lipid
Spacer 1
Spacer 2
Toco
C8
TEG
Chol
TEG
Toco
TEG
Chol
C6
C3
Chol
TEG
C3
Chol
TEG
HEG
Pal
C6
HEG

FIG. 13B

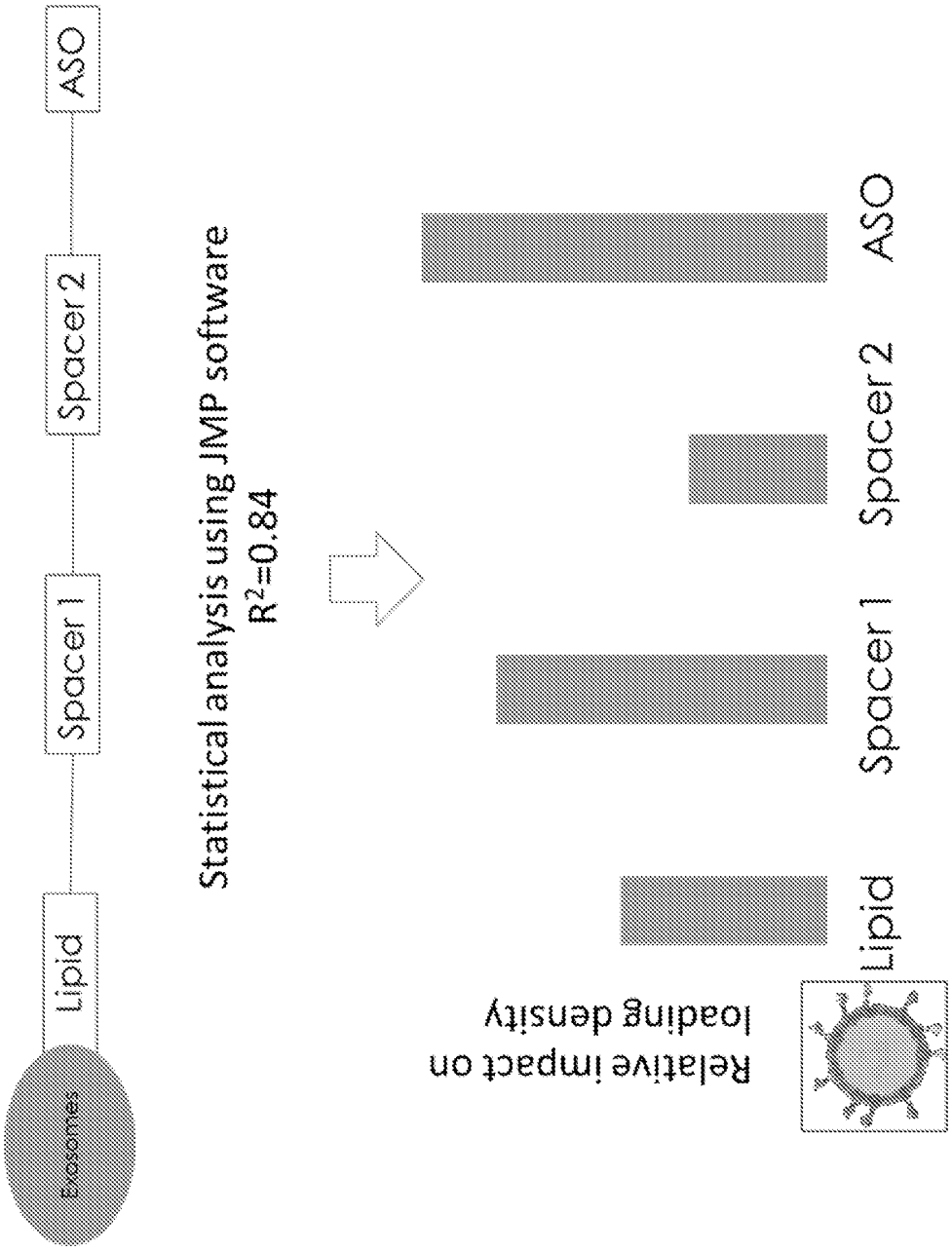

Exosomes
Lipid
Spacer 1
Spacer 2
ASO
Statistical analysis using JMP software
R^2=0.84
Relative impact on loading density
Lipid
Spacer 1
Spacer 2
ASO

FIG. 13C

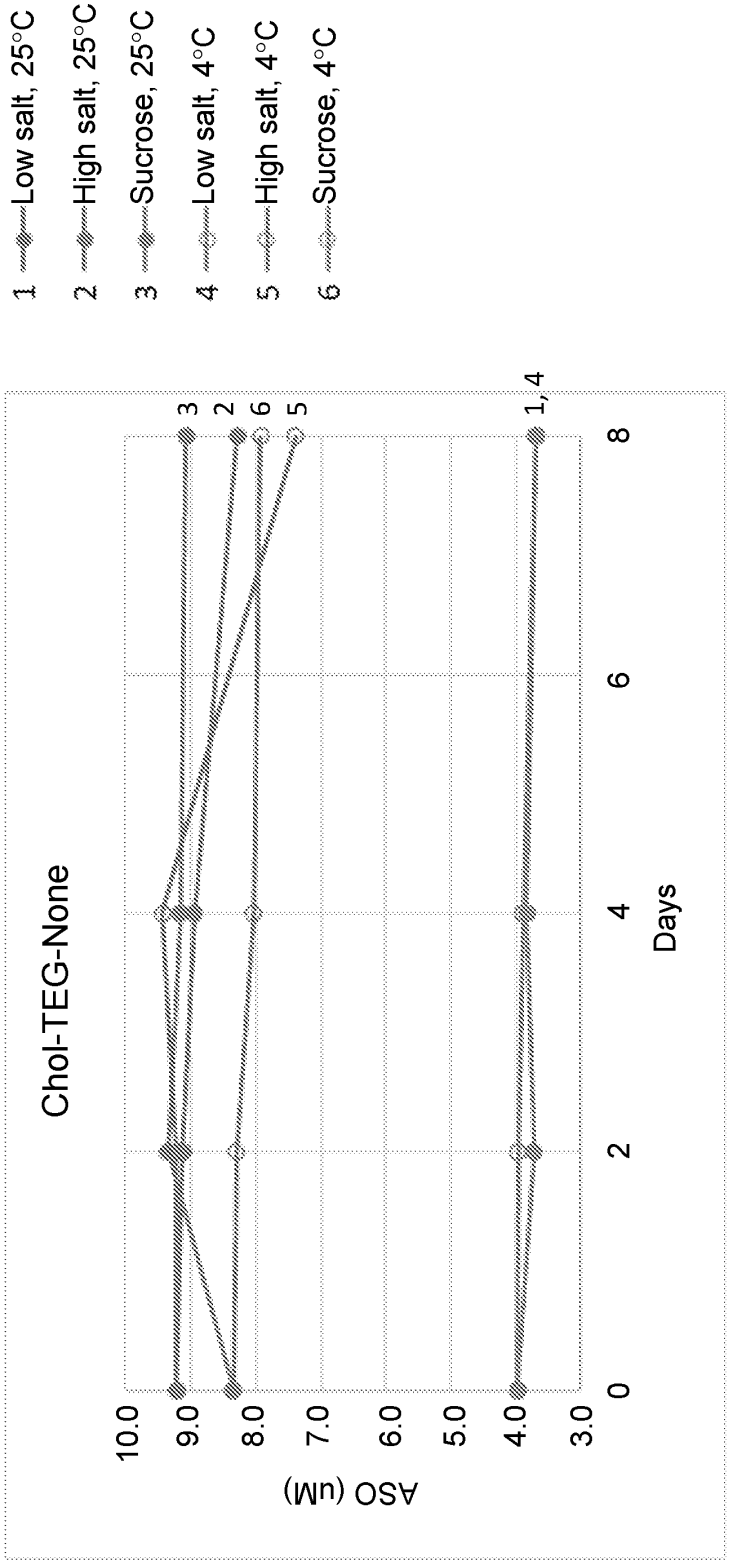

1 Low salt, 25°C
2 High salt, 25°C
3 Sucrose, 25°C
4 Low salt, 4°C
5 High salt, 4°C
6 Sucrose, 4°C
Chol-TEG-None
ASO (uM)
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
0
2
4
6
8
Days
3
2
6
5
1, 4

FIG. 14A

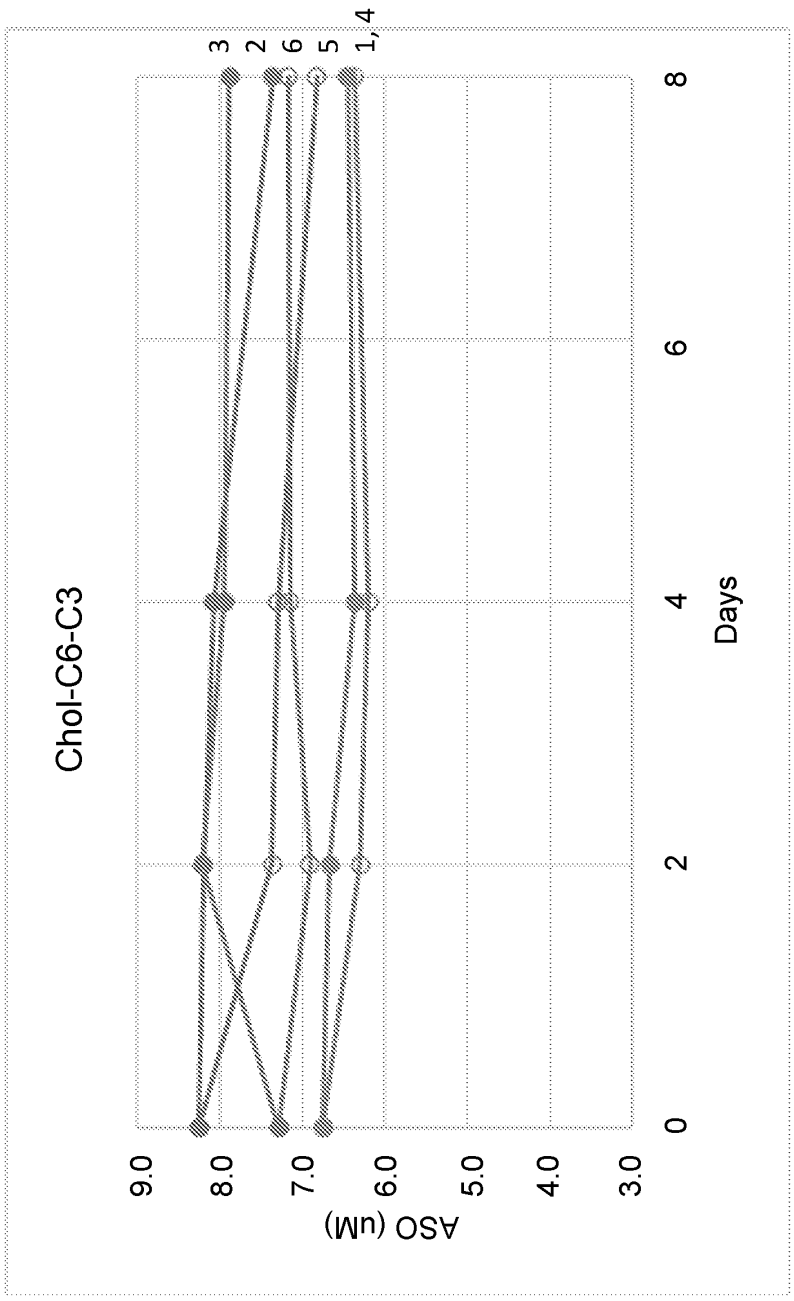

1 Low salt, 25°C
2 High salt, 25°C
3 Sucrose, 25°C
4 Low salt, 4°C
5 High salt, 4°C
6 Sucrose, 4°C
Chol-C6-C3
ASO (uM)
9.0
8.0
7.0
6.0
5.0
4.0
3.0
0
2
4
6
8
Days
3
2
6
5
1, 4

FIG. 14B

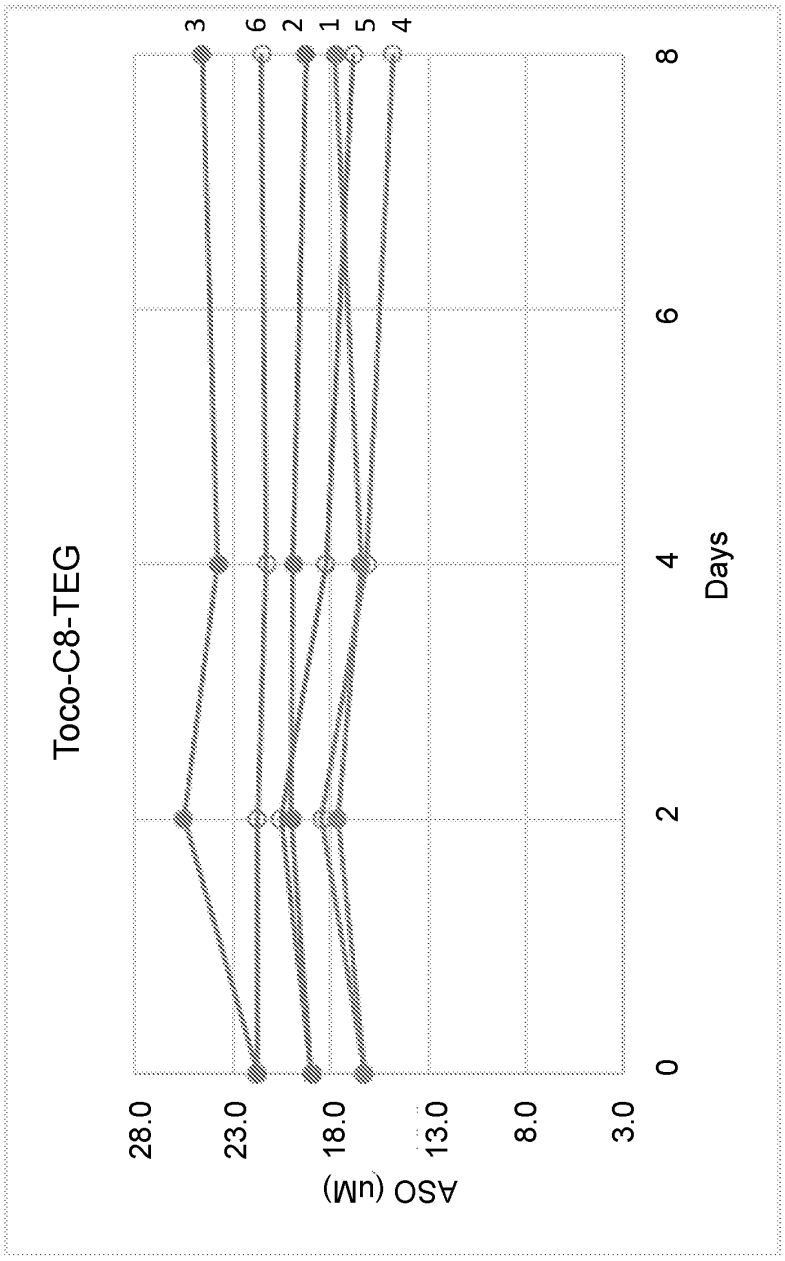

1 Low salt, 25°C
2 High salt, 25°C
3 Sucrose, 25°C
4 Low salt, 4°C
5 High salt, 4°C
6 Sucrose, 4°C
Toco-C8-TEG
ASO (uM)
28.0
23.0
18.0
13.0
8.0
3.0
0
2
4
6
8
Days

FIG. 14C

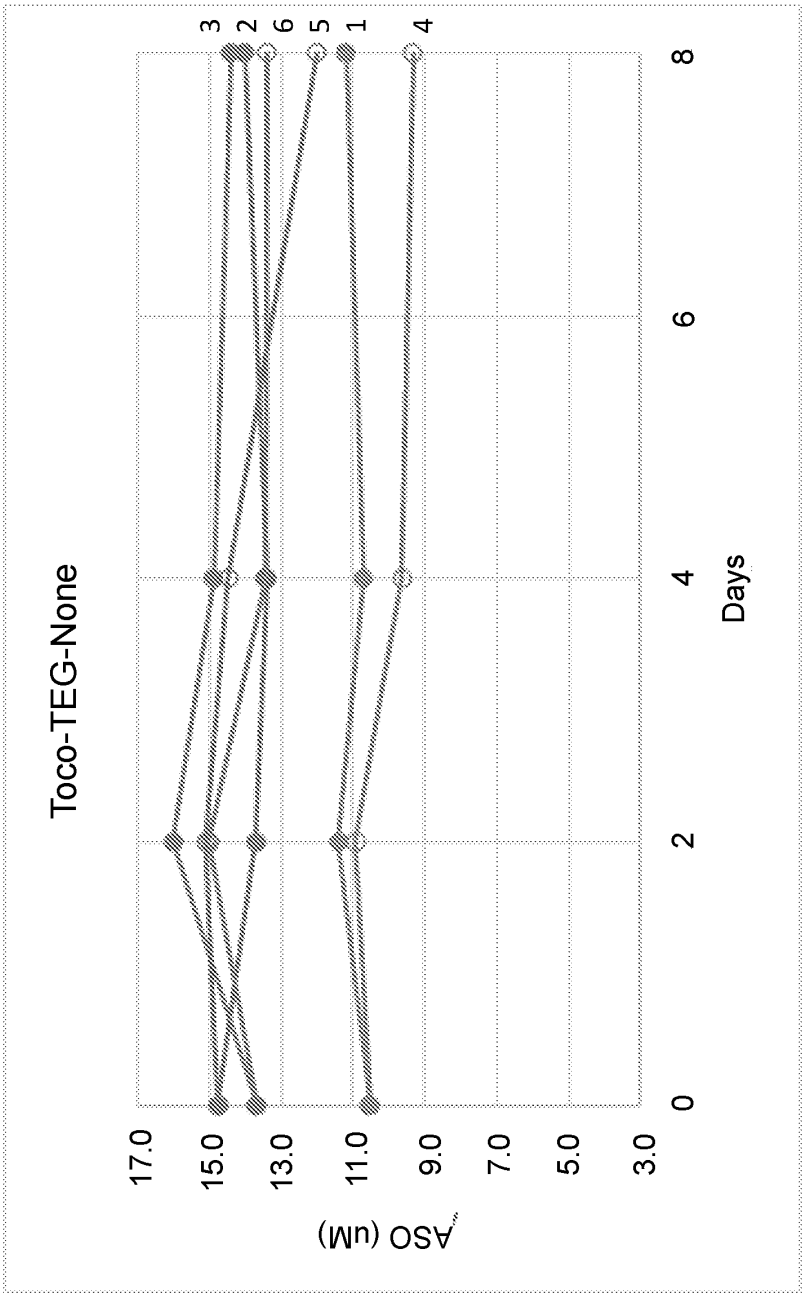

1 Low salt, 25°C
2 High salt, 25°C
3 Sucrose, 25°C
4 Low salt, 4°C
5 High salt, 4°C
6 Sucrose, 4°C
Toco-TEG-None
ASO (uM)
17.0
15.0
13.0
11.0
9.0
7.0
5.0
3.0
0
2
4
6
8
Days

FIG. 14D

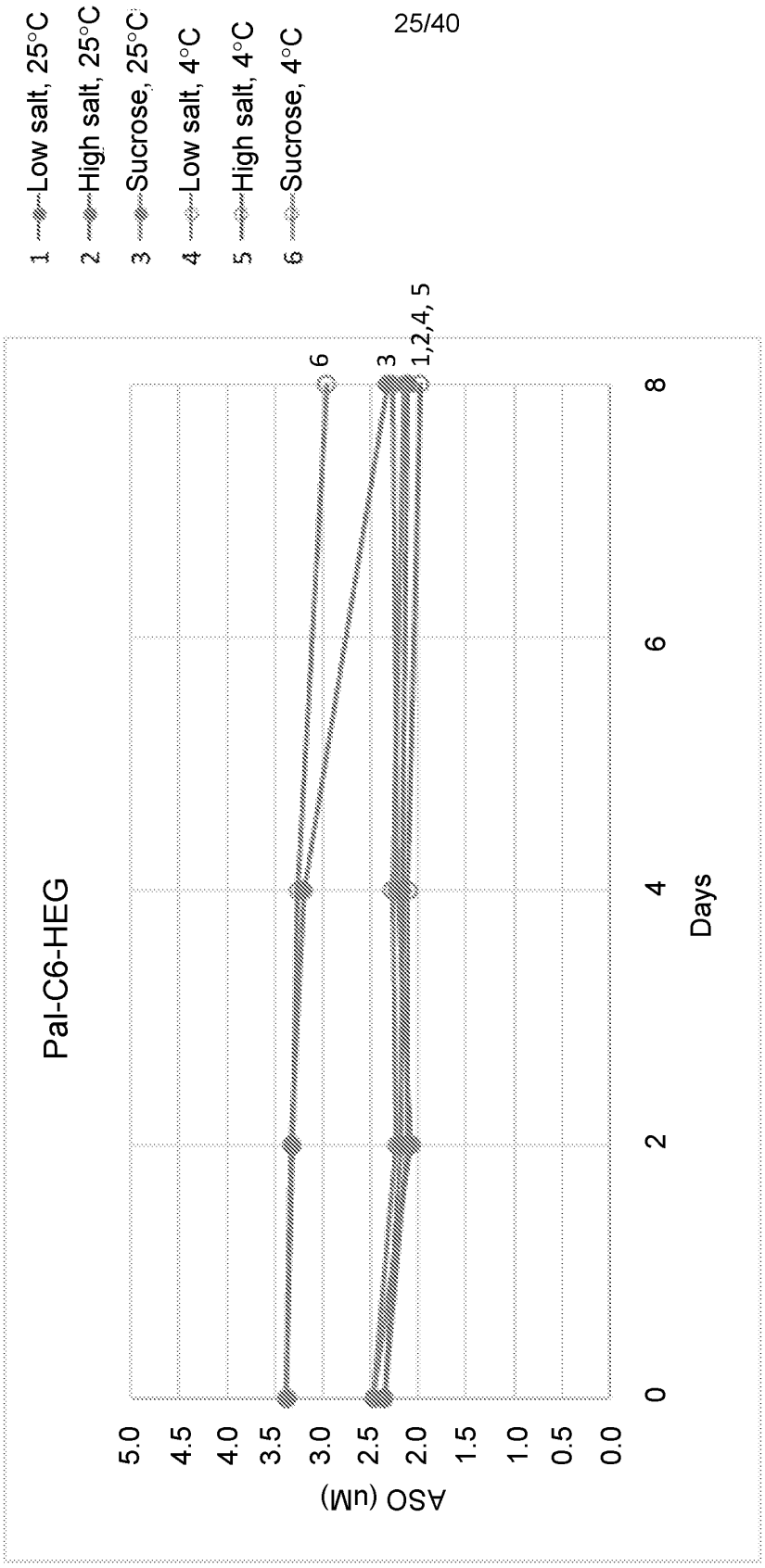

1 Low salt, 25°C
2 High salt, 25°C
3 Sucrose, 25°C
4 Low salt, 4°C
5 High salt, 4°C
6 Sucrose, 4°C
Pal-C6-HEG
ASO (uM)
Days
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
0
2
4
6
8
6
3
1,2,4, 5

FIG. 14E

ASO sequence	Conditions					
	Low salt 25 °C	High salt 25 °C	High salt + Sucrose 25 °C	Low salt 4 °C	High salt 4 °C	High salt + Sucrose 4 °C
Chol-TEG-None	+	+++	+++	+	+++	+++
Chol-C6-C3	++	+++	+++	++	+++	+++
Toco-C8-TEG	+	++	+++	+	++	+++
Toco-TEG-None	+	+++	+++	+	+++	+++
Pal-C6-HEG	+	+	++	+	+	++

+++ Loading density

Stable over 8 days

Decrease in stability after 4 days

FIG. 15

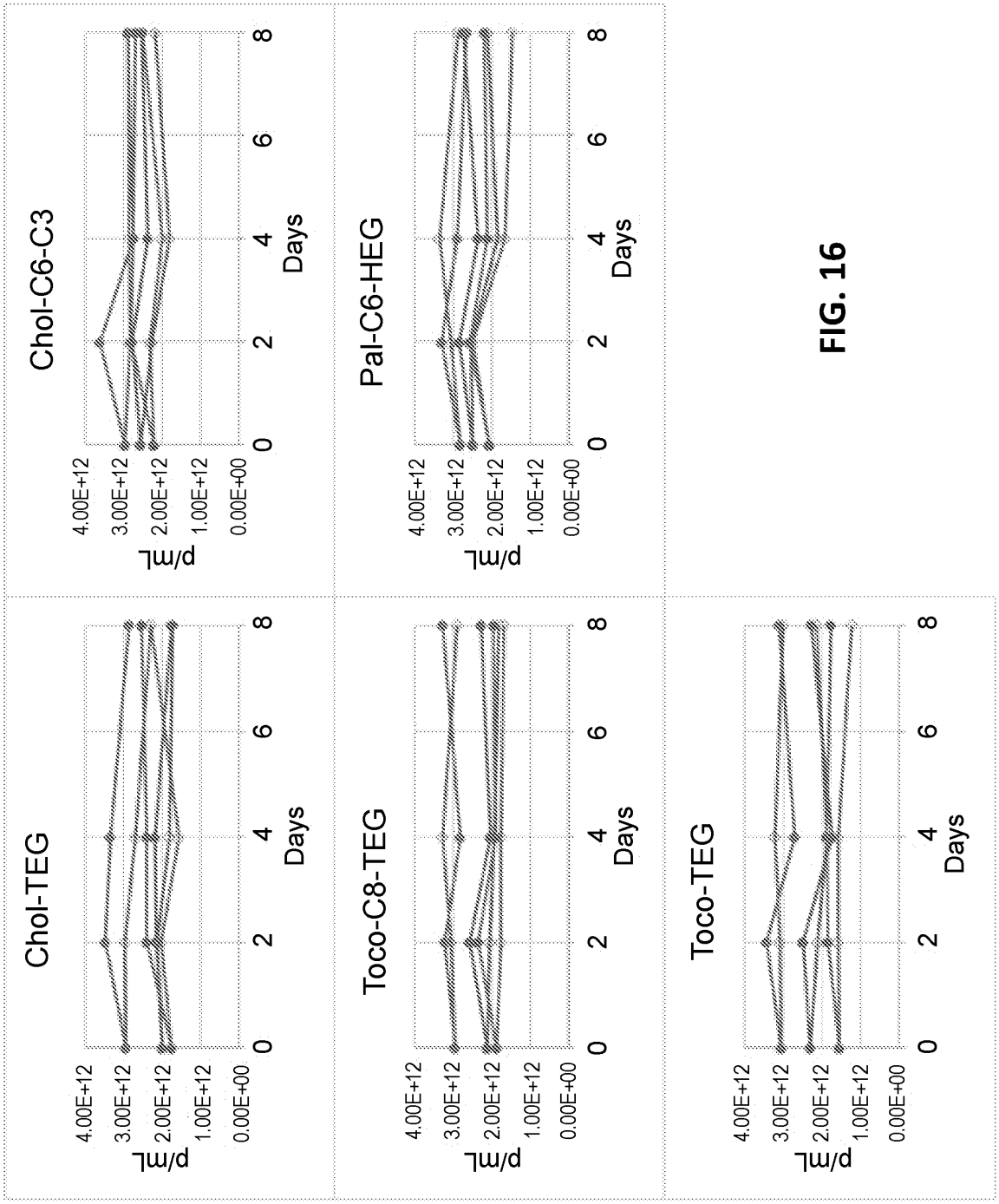

Chol-C6-C3
Pal-C6-HEG
Chol-TEG
Toco-C8-TEG
Toco-TEG
p/mL
Days
4.00E+12
3.00E+12
2.00E+12
1.00E+12
0.00E+00
0
2
4
6
8

FIG. 16

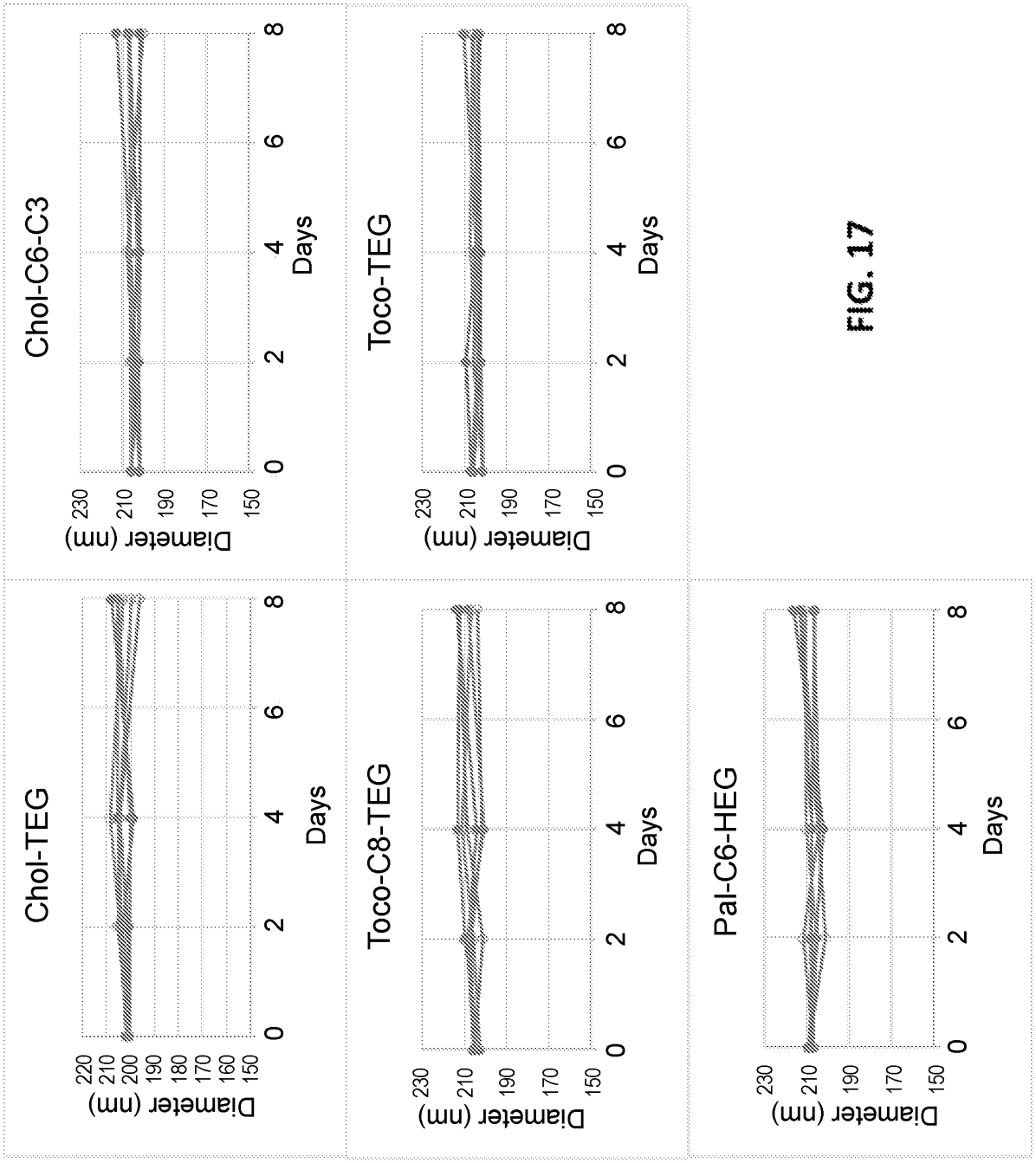

Chol-C6-C3
Diameter (nm)
230
210
190
170
150
0
2
4
6
8
Days
Toco-TEG
Diameter (nm)
230
210
190
170
150
0
2
4
6
8
Days
Chol-TEG
Diameter (nm)
220
210
200
190
180
170
160
150
0
2
4
6
8
Days
Toco-C8-TEG
Diameter (nm)
230
210
190
170
150
0
2
4
6
8
Days
Pal-C6-HEG
Diameter (nm)
230
210
190
170
150
0
2
4
6
8
Days

FIG. 17

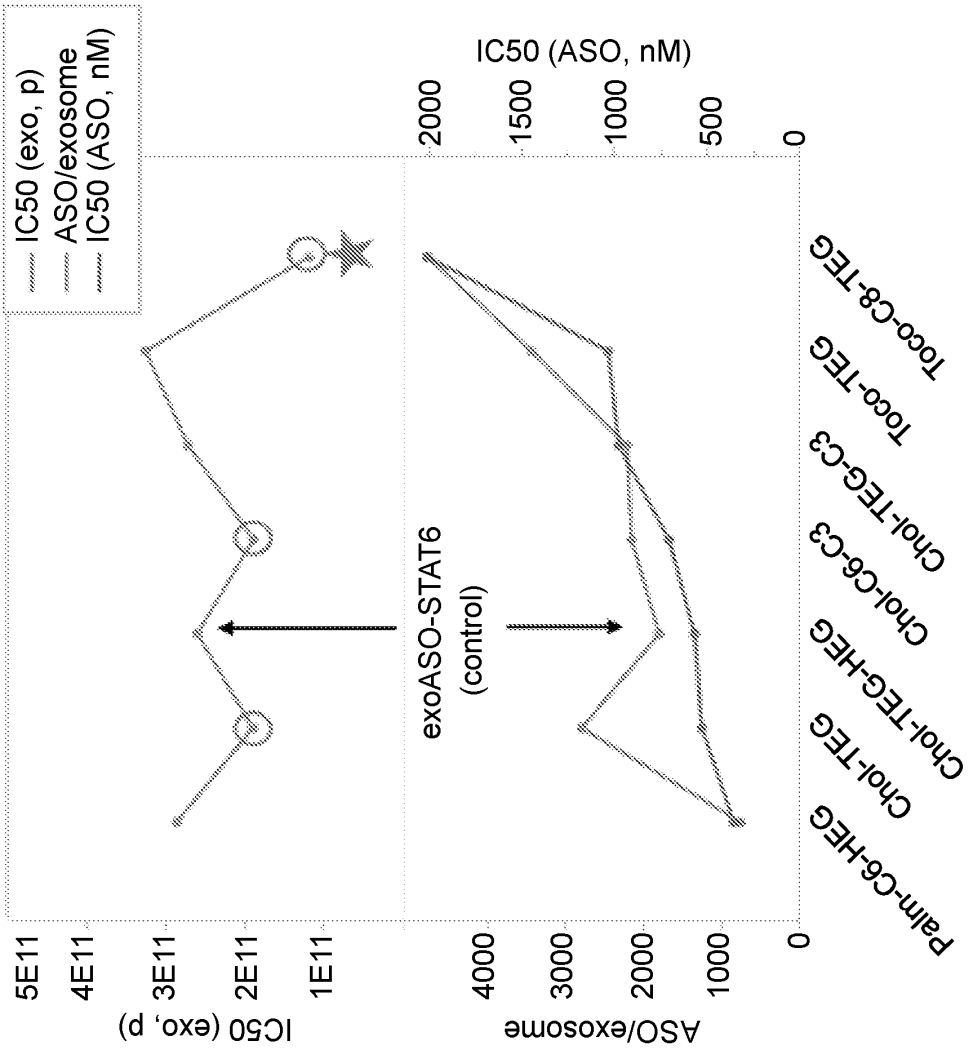

IC50 (exo, p)
ASO/exosome
IC50 (ASO, nM)
5E11
4E11
3E11
2E11
1E11
IC50 (exo, p)
4000
3000
2000
1000
0
ASO/exosome
2000
1500
1000
500
0
IC50 (ASO, nM)
exoASO-STAT6
(control)
Palm-C6-HEG
Chol-TEG
Chol-TEG-HEG
Chol-C6-C3
Chol-TEG-C3
Toco-TEG
Toco-C8-TEG

FIG. 18

ASO#	5' modification		Oligonucleotide
	Membrane Anchor	Linker	
1	Cholesterol-TEG (PS)	HEG (PS)	STAT6
2	Cholesterol-TEG (PO)	HEG (PS)	
3	Cholesterol-TEG (PO)	HEG (PO)	
4	Cholesterol-TEG (PS)	HEG (PO)	

FIG. 19A

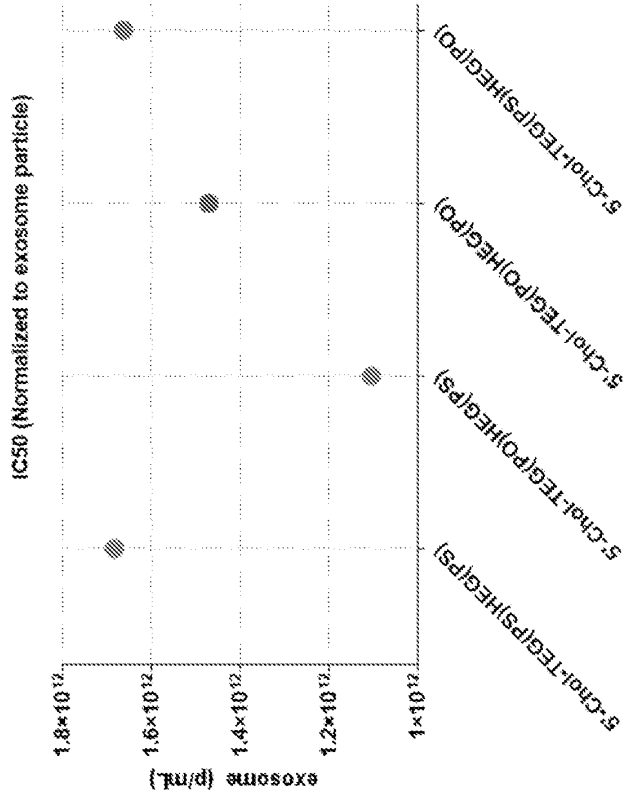


FIG. 19C

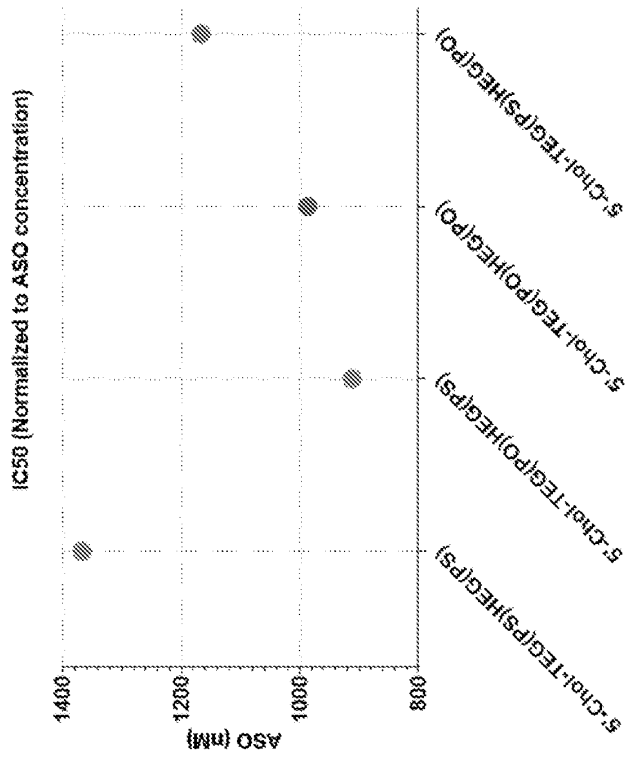


FIG. 19B

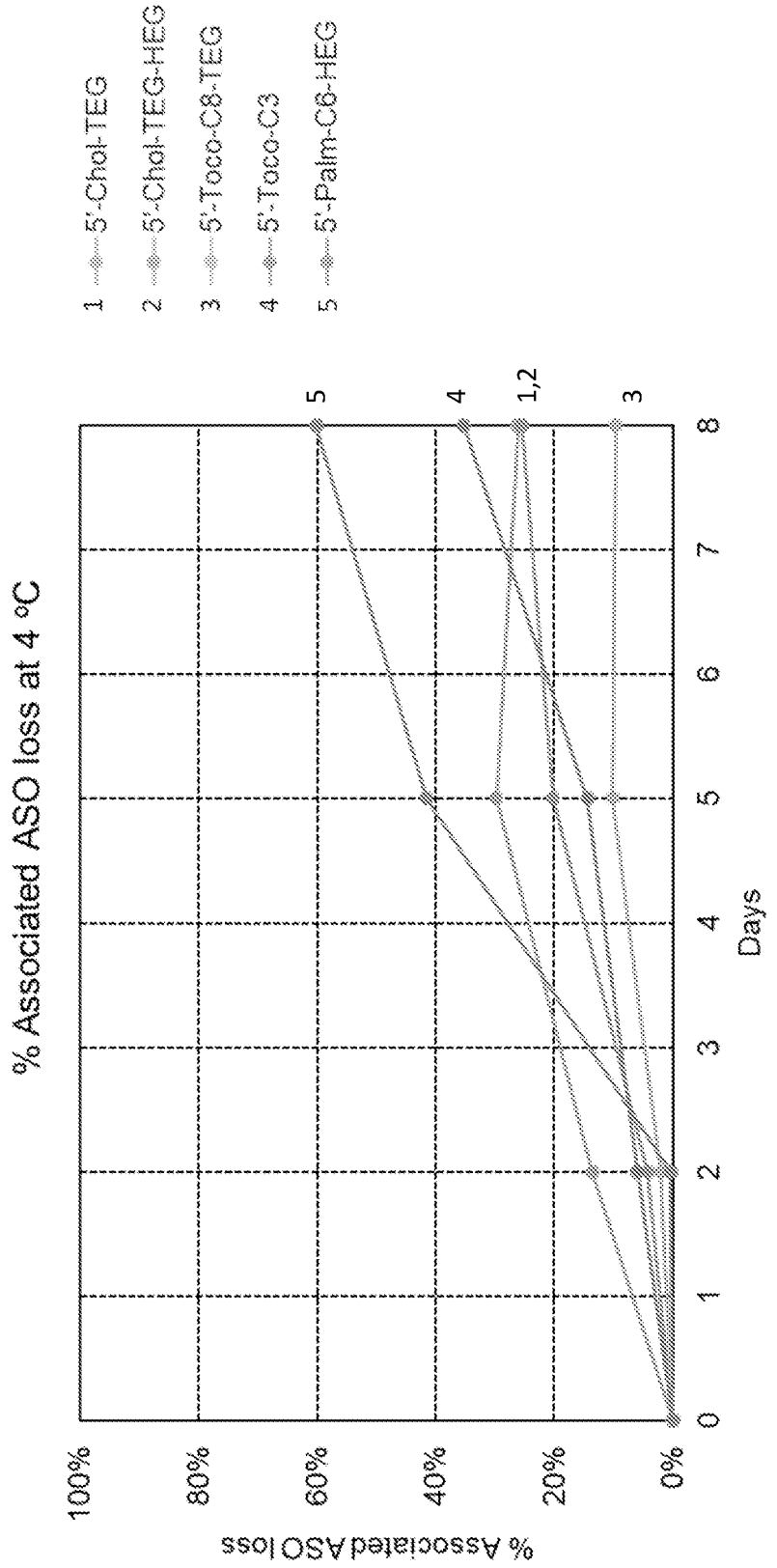

% Associated ASO loss at 4 °C
1 5'-Chol-TEG
2 5'-Chol-TEG-HEG
3 5'-Toco-C8-TEG
4 5'-Toco-C3
5 5'-Palm-C6-HEG
5
4
1,2
3
100%
80%
60%
40%
20%
0%
% Associated ASO loss
0
1
2
3
4
5
6
7
8
Days

FIG. 20

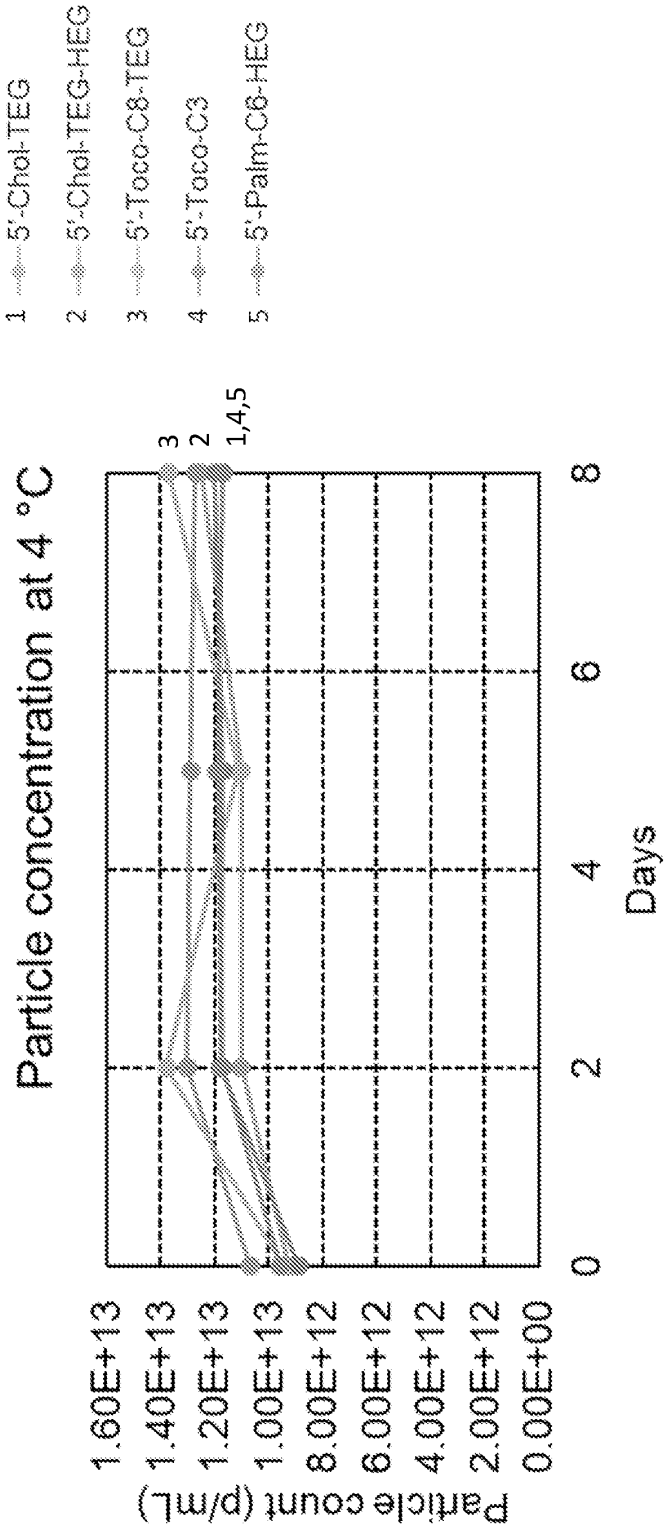

1 5'-Chol-TEG
2 5'-Chol-TEG-HEG
3 5'-Toco-C8-TEG
4 5'-Toco-C3
5 5'-Palm-C6-HEG
Particle concentration at 4 °C
Particle count (p/mL)
1.60E+13
1.40E+13
1.20E+13
1.00E+13
8.00E+12
6.00E+12
4.00E+12
2.00E+12
0.00E+00
0
2
4
6
8
Days
3
2
1,4,5

FIG. 21

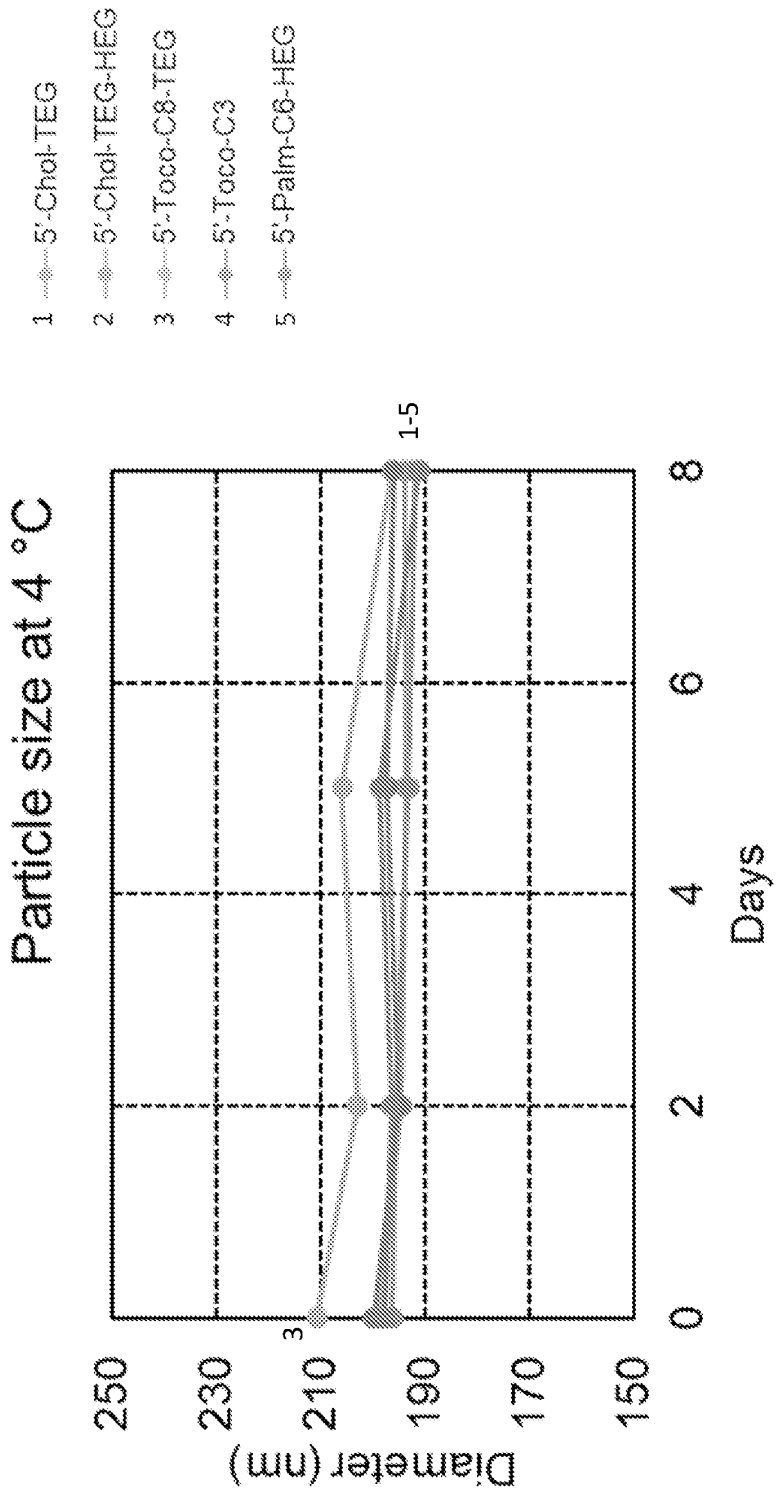

1 5'-Chol-TEG
2 5'-Chol-TEG-HEG
3 5'-Toco-C8-TEG
4 5'-Toco-C3
5 5'-Palm-C6-HEG
Particle size at 4 °C
Diameter (nm)
250
230
210
190
170
150
0
2
4
6
8
Days
1-5
3

FIG. 22

Entry	5' modification			Oligonucleotide	Chemistry	Drug release mechanism
	Membrane Anchor	Linker				
1	Cholesterol-TEG-N3	DBCO-PEG4-Val-Cit-PAB-PNP	C6-NH2	ASO (STAT6)	Valine-Citrullin-p-aminobenzoyl	Cathepsin B
2	Cholesterol-TEG-N3	BCN-PEG3-VC-PFP Ester	C6-NH2		Valine-Citrullin	Cathepsin B

FIG. 23A

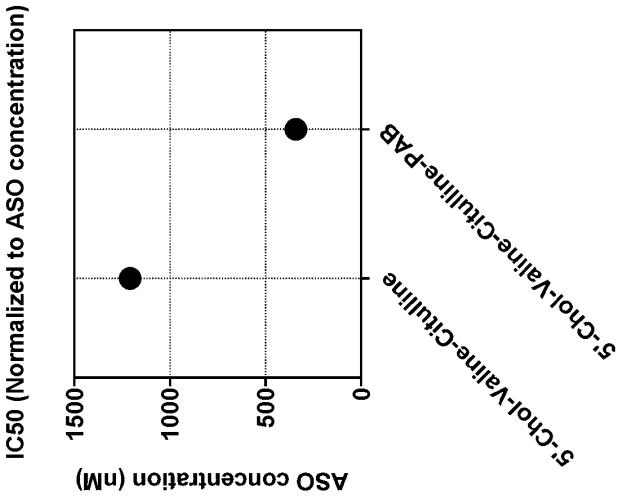


FIG. 23B

Entry	5' Modification		Oligonucleotide	Chemistry	Drug release mechanism
	Lipid	Linker			
1	Cholesterol-TEG (PO)	-	EGFP ASO	phosphodiester	phospodiesterase
2	Cholesterol-TEG (PO)	HEG (PO)		phosphodiester	phospodiesterase
3	Tocopherol-C8 (PO)	TEG (PO)		phosphodiester	phospodiesterase
4	Tocopherol (PO)	C3 (PO)		phosphodiester	phospodiesterase
5	Palmitate-C6 (PO)	HEG (PO)		phosphodiester	phosphodiesterase
6	Cholesterol-TEG-N3	BCN-PEG4-PFP ester	EGFP ASO-C6-NH2	non-cleavable	-
7	Cholesterol-chloroformate + ethylene diamine	Acid-PEG3-SS-PEG3-acid		disulfide	redox
8	Cholesterol-TEG-N3	DBCO-PEG13-COOH		non-cleavable	-
9	Cholesterol-TEG-N3	DBCO-PEG4-Val-Cit-PAB-PNP		Val-Cit-PAB	cathepsin

FIG. 24

Entry	5' modification		Oligonucleotide	Chemistry	Drug release mechanism
	Membrane Anchor	Linker			
1	Cholesterol-TEG (PS)	C6-SS-C6 (PS)	ASO (STAT6)	disulfide	redox

FIG. 25

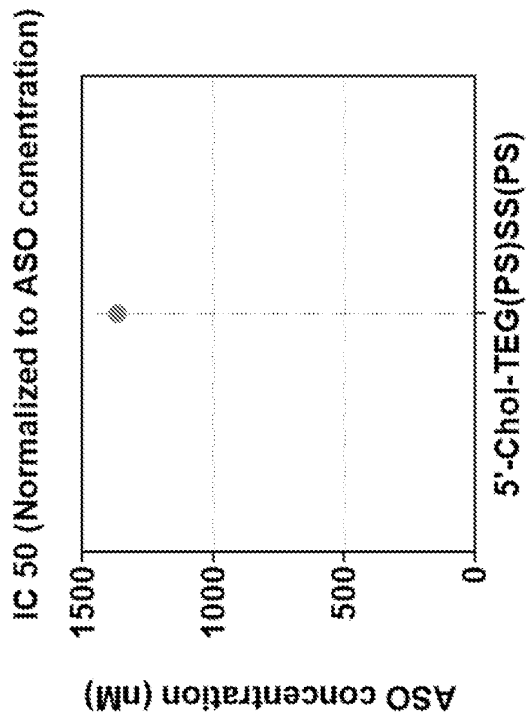


FIG. 26

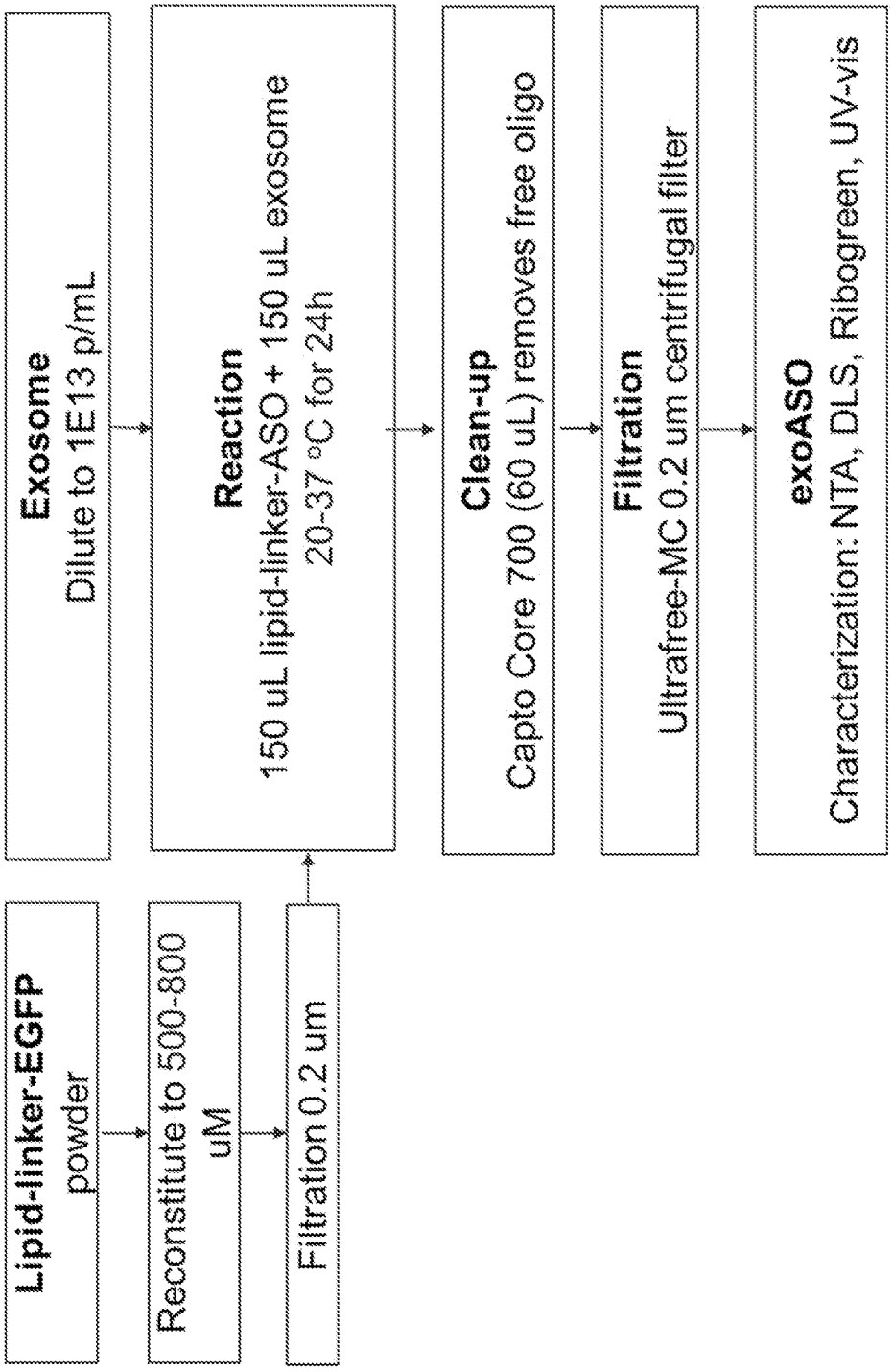

Lipid-linker-EGFP
powder
Reconstitute to 500-800 uM
Filtration 0.2 um
Exosome
Dilute to 1E13 p/mL
Reaction
150 uL lipid-linker-ASO + 150 uL exosome
20-37 °C for 24h
Clean-up
Capto Core 700 (60 uL) removes free oligo
Filtration
Ultrafree-MC 0.2 um centrifugal filter
exoASO
Characterization: NTA, DLS, Ribogreen, UV-vis

FIG. 27

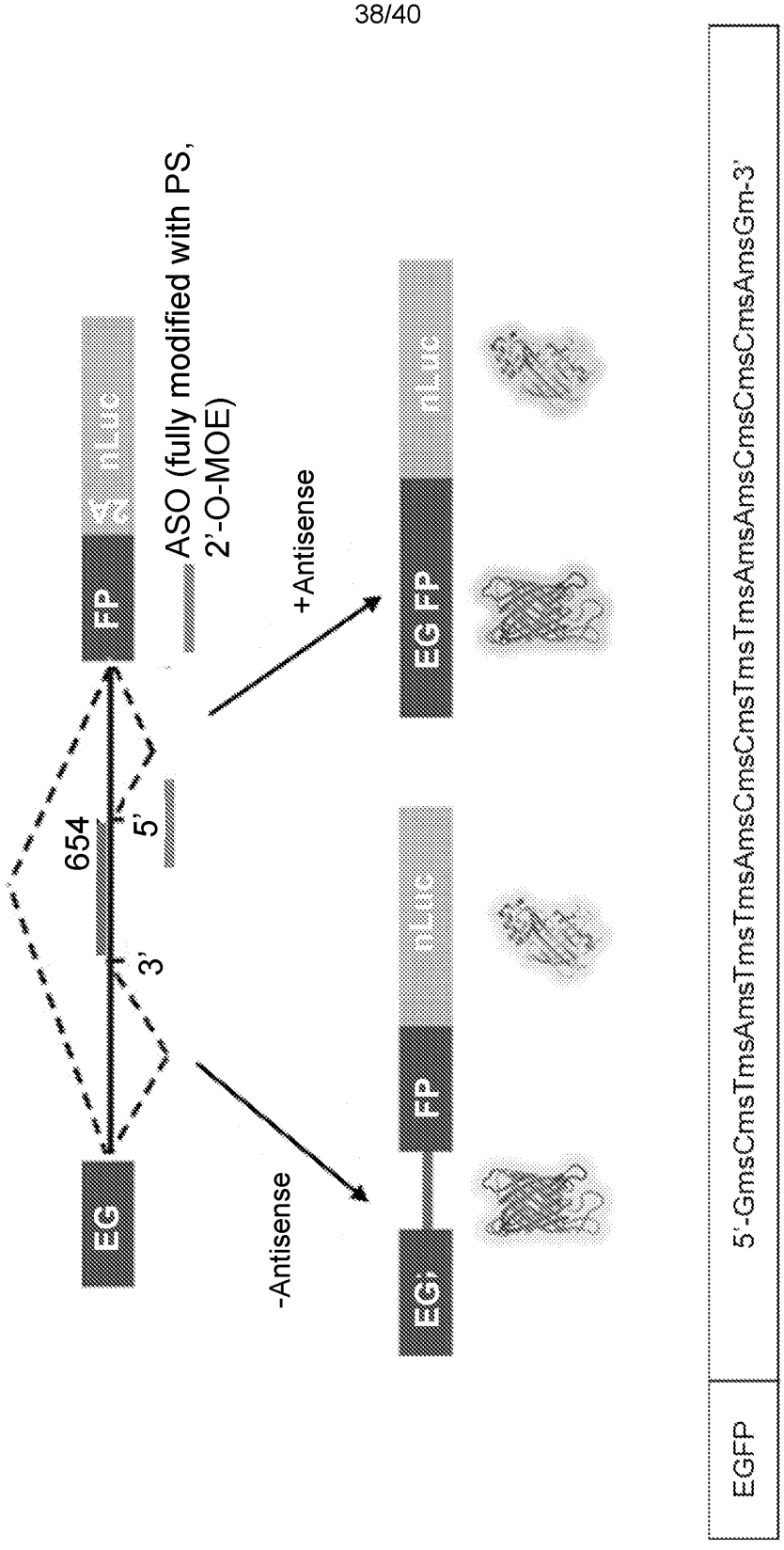

EGFP
5'-GmsCmsTmsAmsTmsTmsAmsCmsCmsTmsTmsAmsAmsCmsCmsCmsAmsGm-3'
EG
FP
654
5'
3'
ASO (fully modified with PS, 2'-O-MOE)
+Antisense
-Antisense
EG FP
EG

FIG. 28

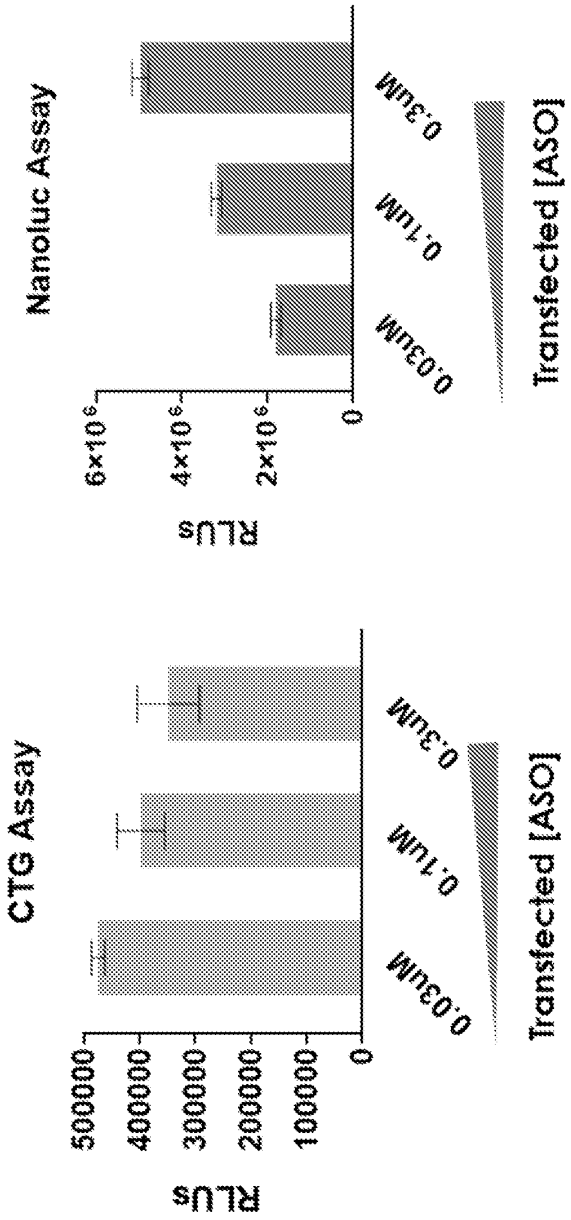

Nanoluc Assay
RLUs
6×10^6
4×10^6
2×10^6
0
0.03uM
0.1uM
0.3uM
Transfected [ASO]
CTG Assay
RLUs
500000
400000
300000
200000
100000
0
0.03uM
0.1uM
0.3uM
Transfected [ASO]

FIG. 29

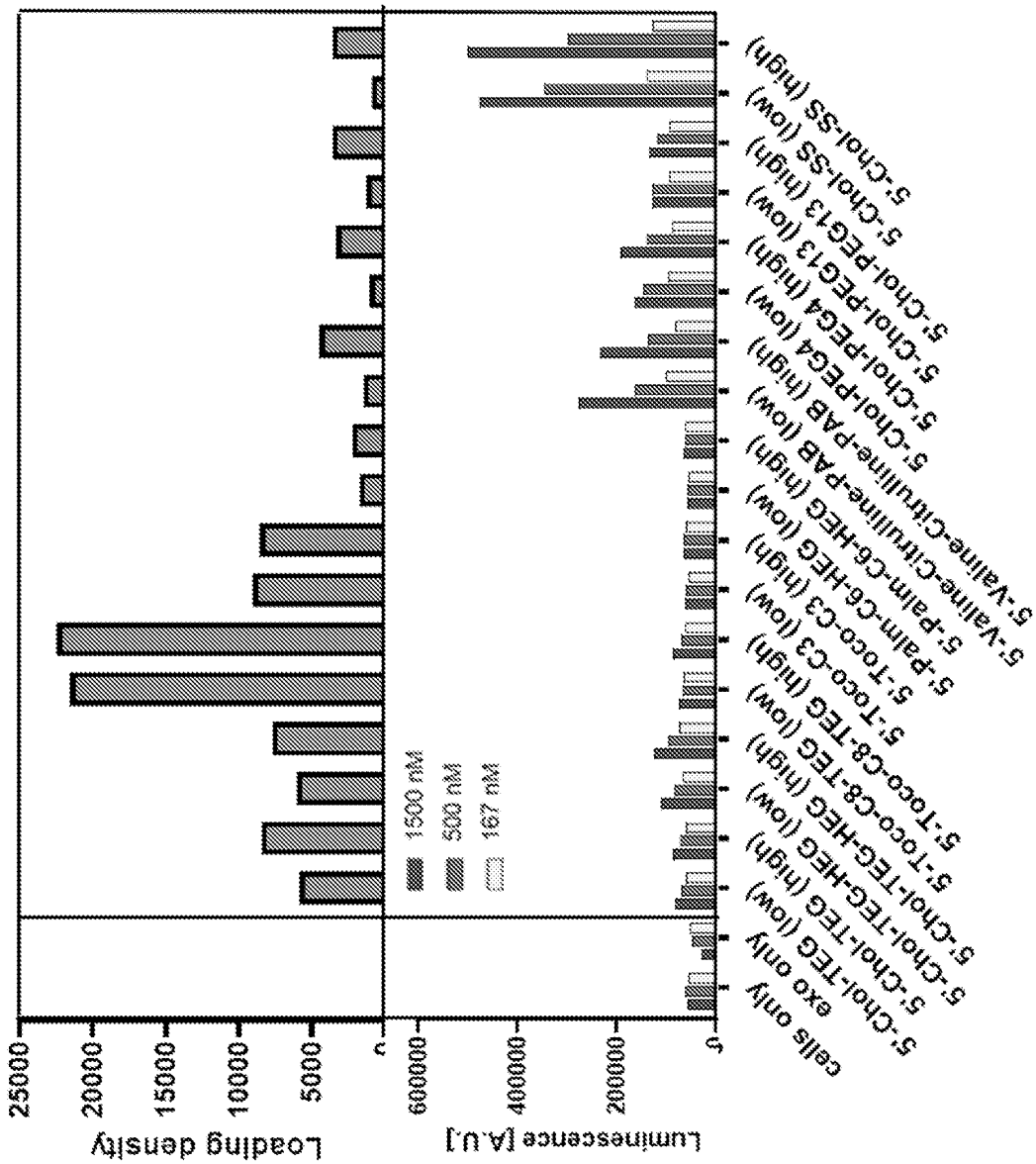

Loading density
25000
20000
15000
10000
5000
0
1500 nM
500 nM
167 nM
Luminescence [A.U.]
600000
400000
200000
0
cells only
exo only
5-Chol-TEG (low)
5-Chol-TEG (high)
5-Chol-TEG-HEG (low)
5-Chol-TEG-HEG (high)
5-Toco-C8-TEG (low)
5-Toco-C8-TEG (high)
5-Toco-C3 (low)
5-Toco-C3 (high)
5-Palm-C6-HEG (low)
5-Palm-C6-HEG (high)
5-Valine-Citrulline-PAB (low)
5-Valine-Citrulline-PAB (high)
5-Chol-PEG4 (low)
5-Chol-PEG4 (high)
5-Chol-PEG13 (low)
5-Chol-PEG13 (high)
5-Chol-SS (low)
5-Chol-SS (high)

FIG. 30