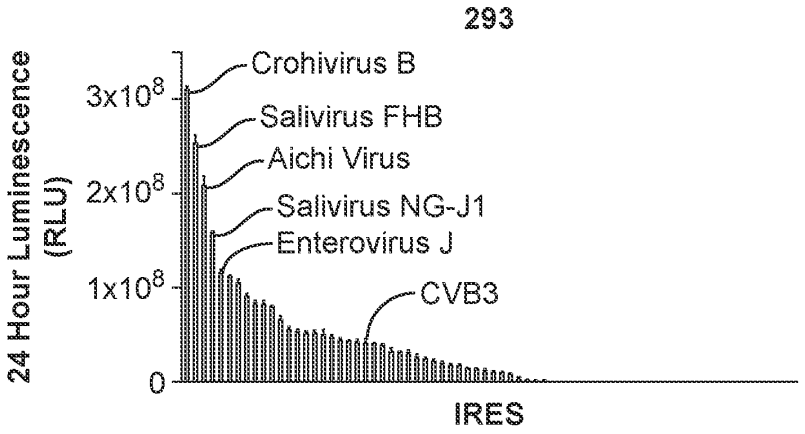

FIG. 1A

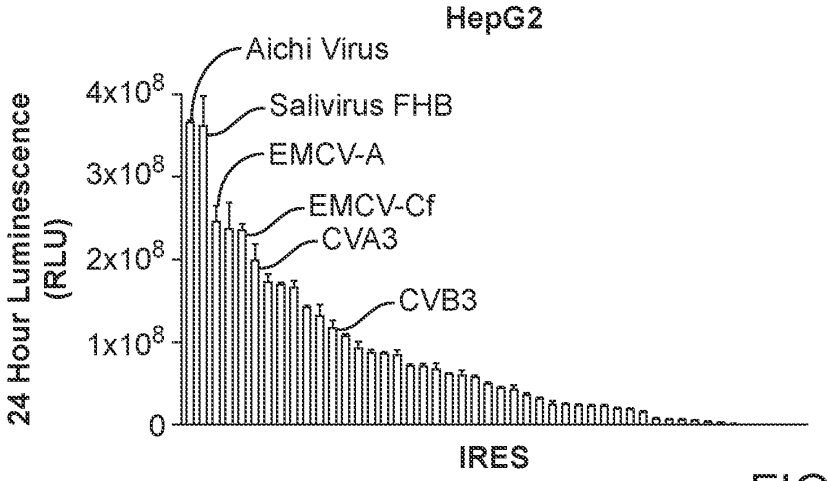

FIG. 1B

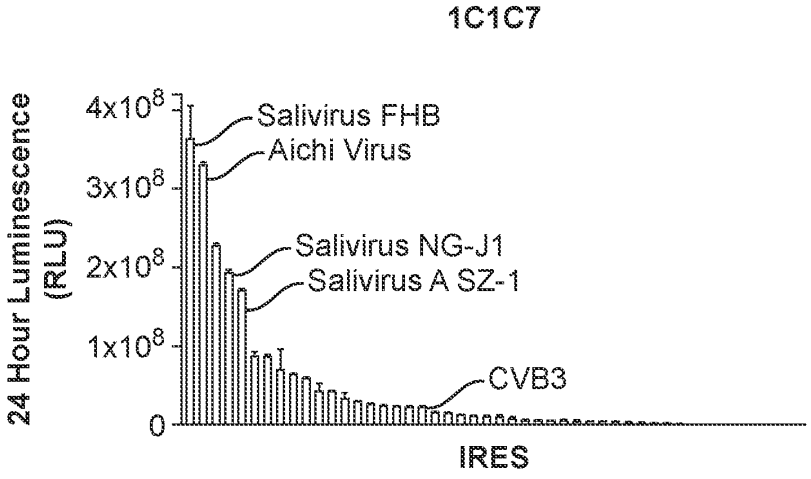

FIG. 1C

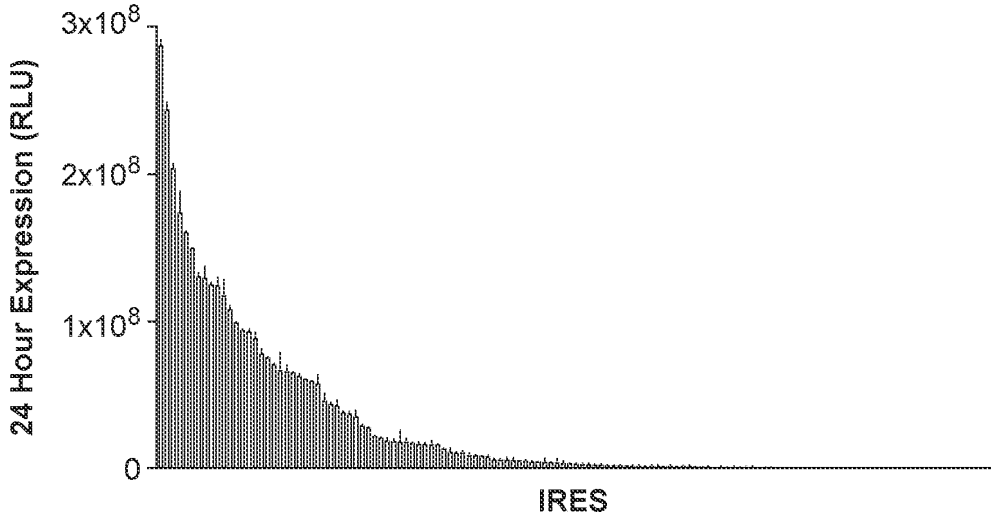
3x10^8
2x10^8
1x10^8
0
24 Hour Expression (RLU)
IRES

FIG. 1D

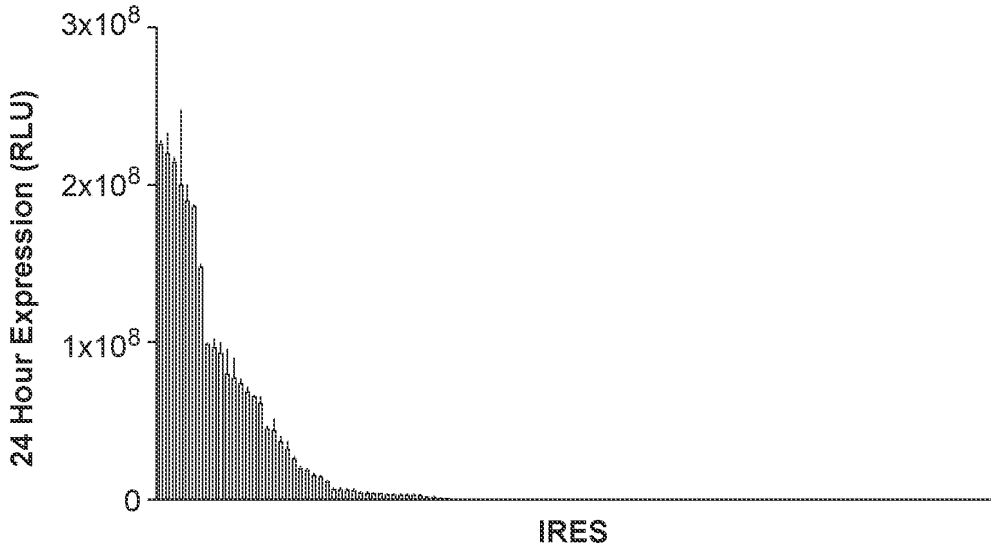
3x10^8
2x10^8
1x10^8
0
24 Hour Expression (RLU)
IRES

FIG. 1E

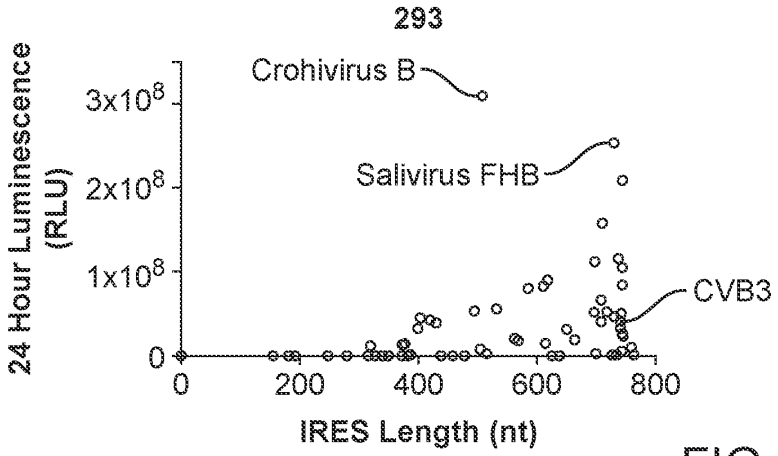

FIG. 2A

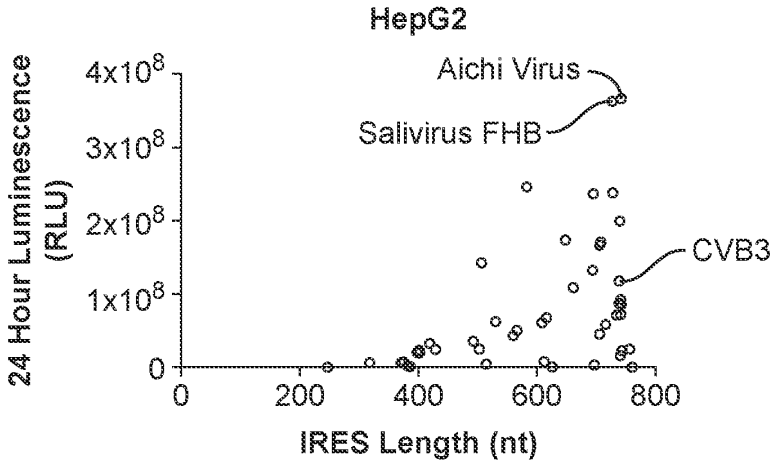

FIG. 2B

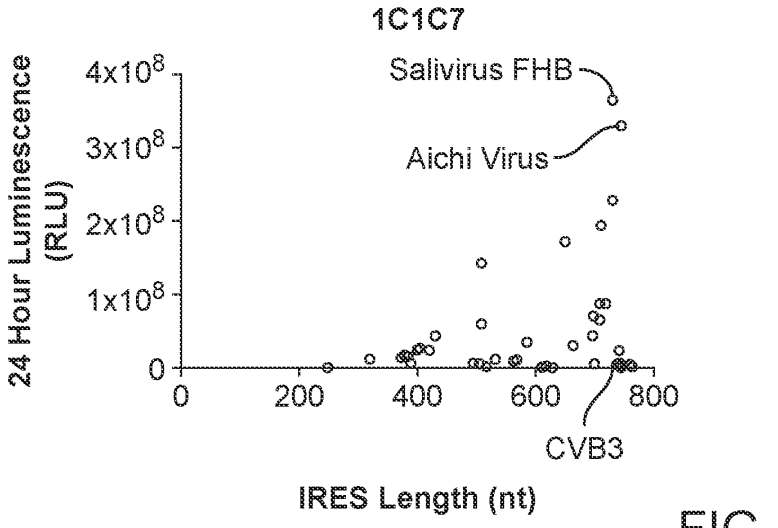

FIG. 2C

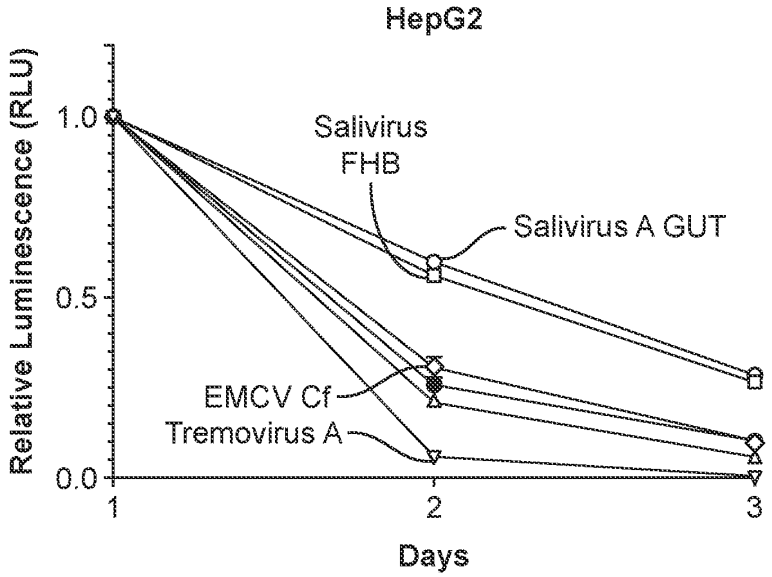
HepG2
Relative Luminescence (RLU)
1.0
0.5
0.0
Salivirus
FHB
Salivirus A GUT
EMCV Cf
Tremovirus A
1
2
3
Days

FIG. 3A

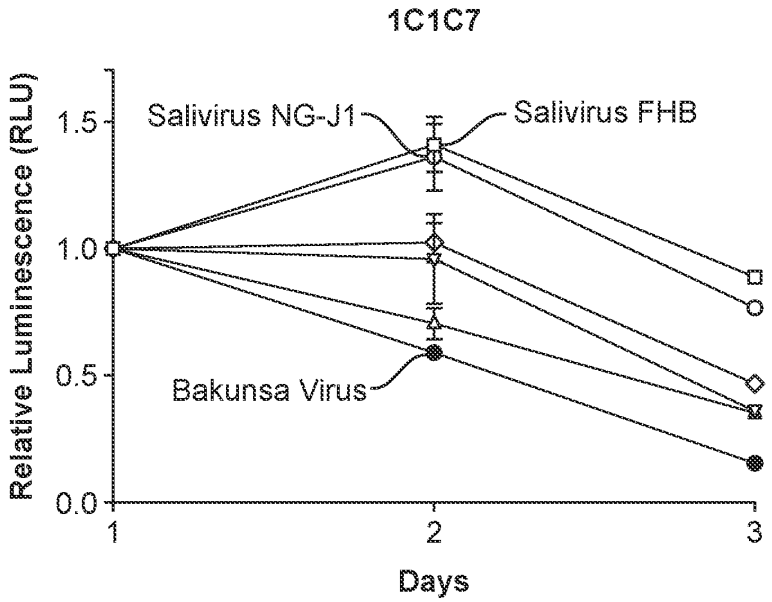
1C1C7
Relative Luminescence (RLU)
1.5
1.0
0.5
0.0
Salivirus NG-J1
Salivirus FHB
Bakunsa Virus
1
2
3
Days

FIG. 3B

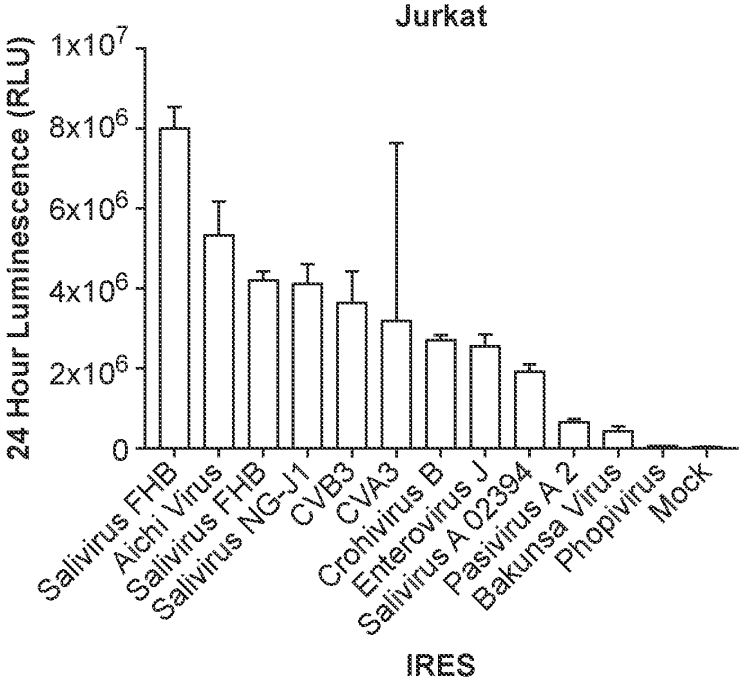
Jurkat
24 Hour Luminescence (RLU)
1x10^7
8x10^6
6x10^6
4x10^6
2x10^6
0
Salivirus FHB
Aichi Virus
Salivirus FHB
Salivirus NG-J1
CVB3
CVA3
Crohivirus B
Enterovirus J
Salivirus A 02394
Pasivirus A 2
Bakunsa Virus
Phopivirus
Mock
IRES

FIG. 4A

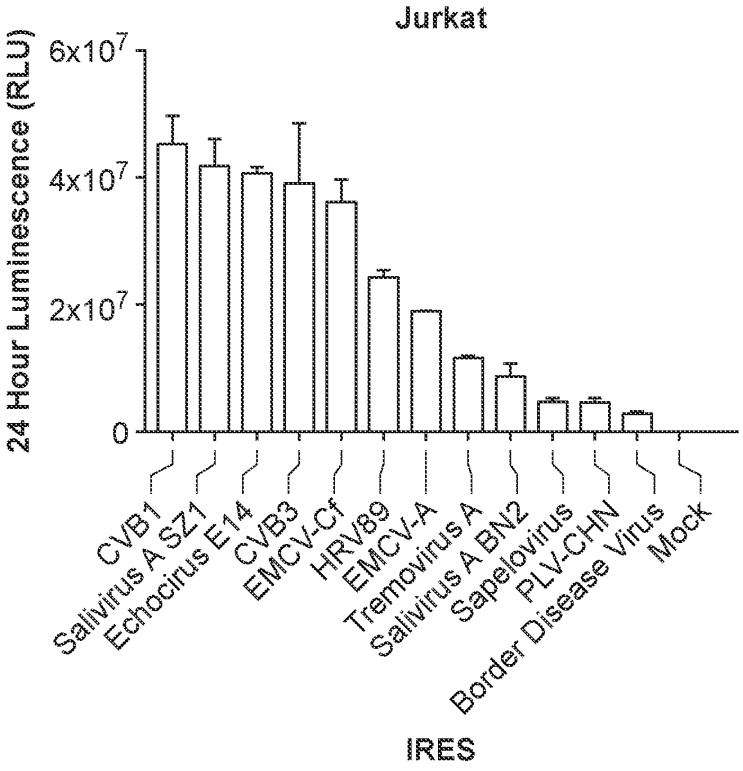
Jurkat
24 Hour Luminescence (RLU)
6x10^7
4x10^7
2x10^7
0
CVB1
Salivirus A SZ1
Echocirus E14
CVB3
EMCV-Cf
HRV89
EMCV-A
Tremovirus A
Salivirus A BN2
Sapelovirus
PLV-CHN
Border Disease Virus
Mock
IRES

FIG. 4B

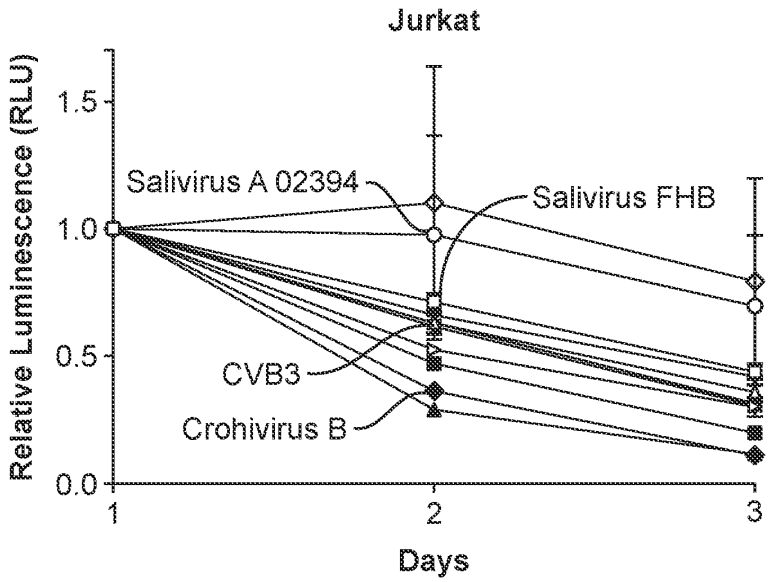
Jurkat
Relative Luminescence (RLU)
1.5
1.0
0.5
0.0
Salivirus A 02394
Salivirus FHB
CVB3
Crohivirus B
1
2
3
Days

FIG. 5A

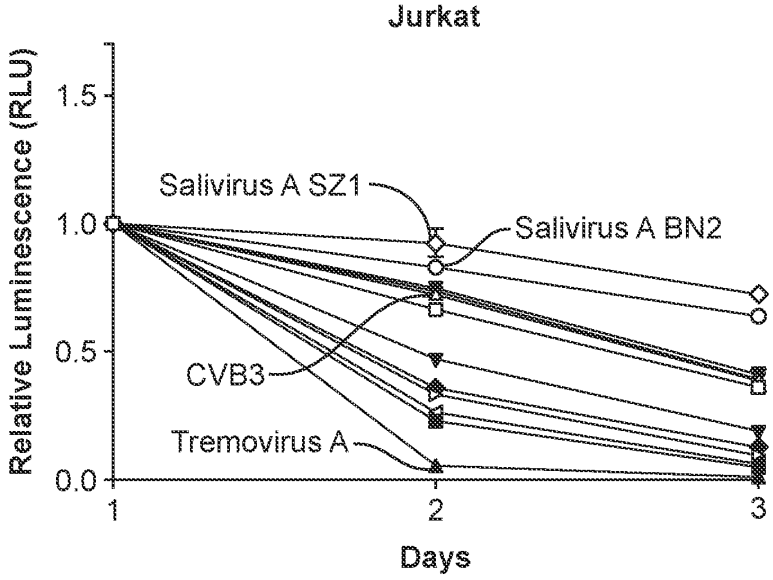
Jurkat
Relative Luminescence (RLU)
1.5
1.0
0.5
0.0
Salivirus A SZ1
Salivirus A BN2
CVB3
Tremovirus A
1
2
3
Days

FIG. 5B

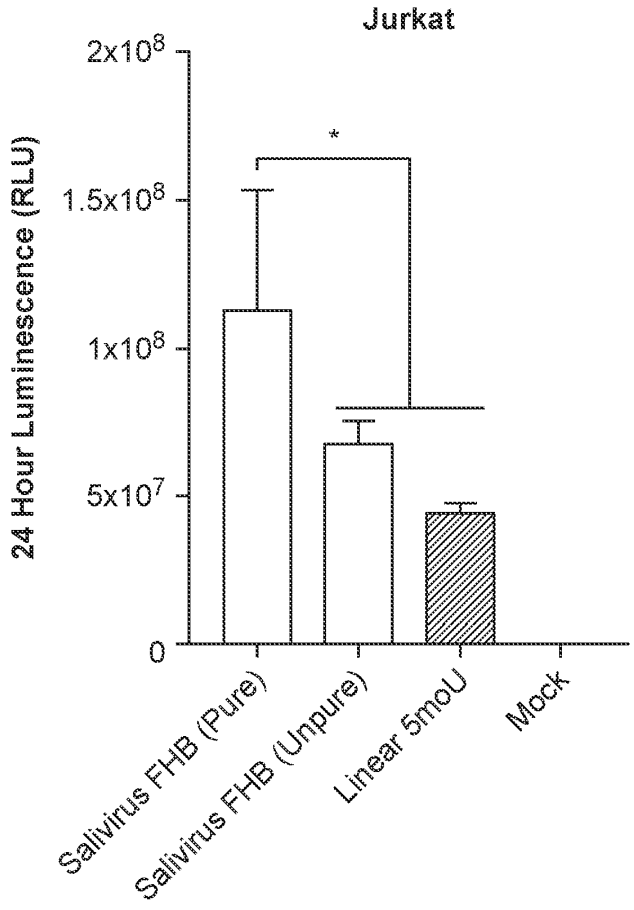
Jurkat
24 Hour Luminescence (RLU)
2x10^8
1.5x10^8
1x10^8
5x10^7
0
*
Salivirus FHB (Pure)
Salivirus FHB (Unpure)
Linear 5moU
Mock

FIG. 6A

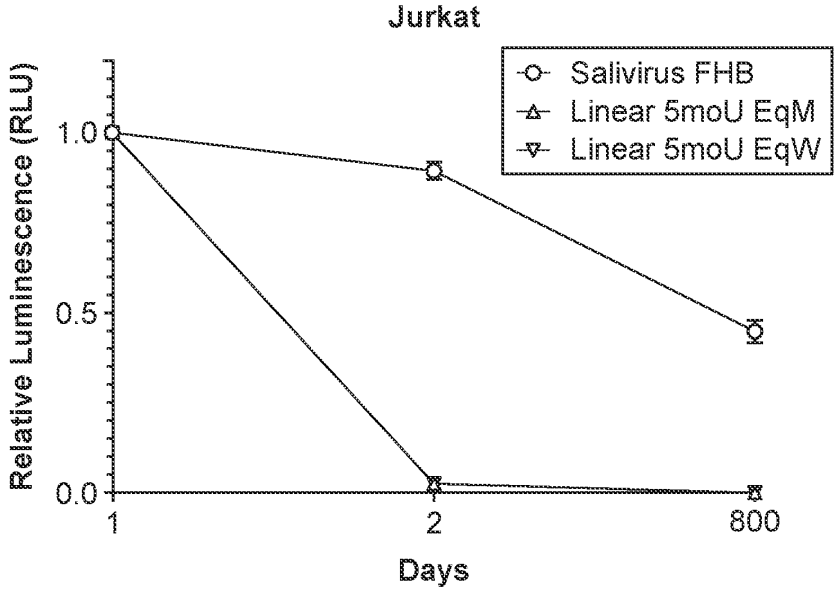
Jurkat
Salivirus FHB
Linear 5moU EqM
Linear 5moU EqW
Relative Luminescence (RLU)
1.0
0.5
0.0
1
2
800
Days

FIG. 6B

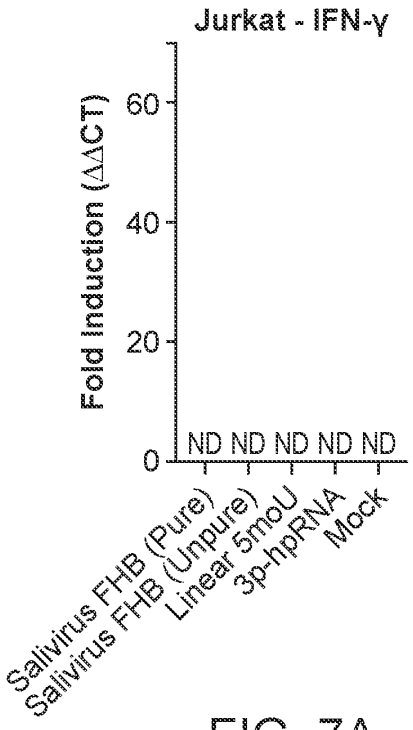
Jurkat - IFN-γ
Fold Induction (ΔΔCT)
60
40
20
0
ND ND ND ND ND
Salivirus FHB (Pure)
Salivirus FHB (Unpure)
Linear 5moU
3p-hpRNA
Mock

FIG. 7A

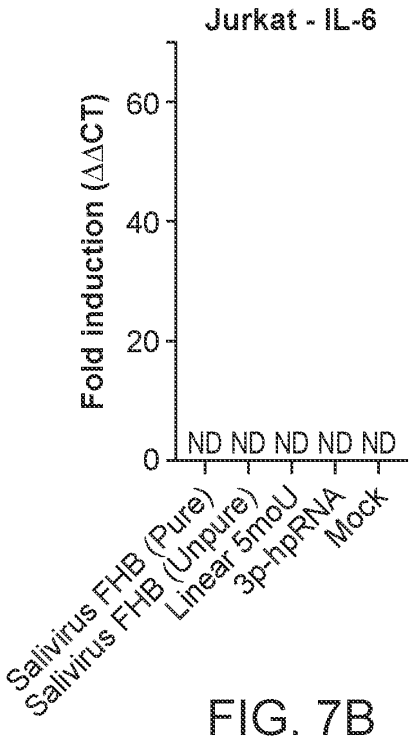
Jurkat - IL-6
Fold induction (ΔΔCT)
60
40
20
0
ND ND ND ND ND
Salivirus FHB (Pure)
Salivirus FHB (Unpure)
Linear 5moU
3p-hpRNA
Mock

FIG. 7B

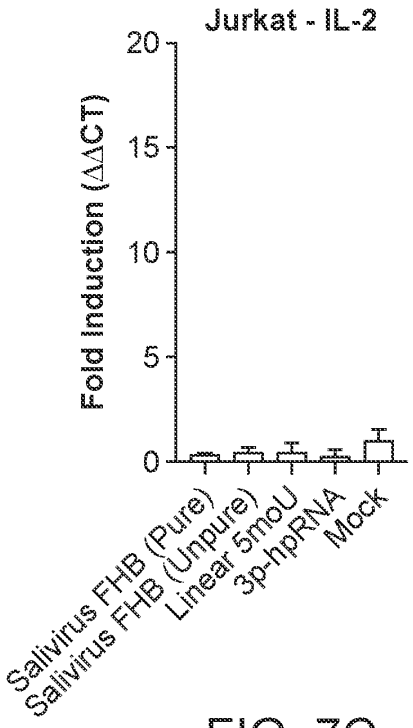
Jurkat - IL-2
Fold Induction (ΔΔCT)
20
15
10
5
0
Salivirus FHB (Pure)
Salivirus FHB (Unpure)
Linear 5moU
3p-hpRNA
Mock

FIG. 7C

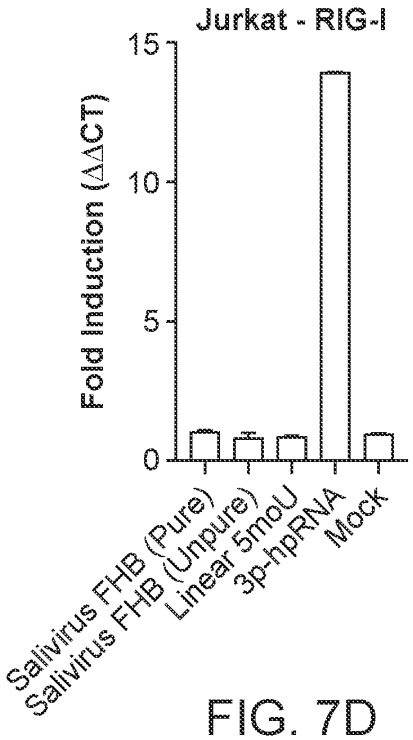
Jurkat - RIG-I
Fold Induction (ΔΔCT)
15
10
5
0
Salivirus FHB (Pure)
Salivirus FHB (Unpure)
Linear 5moU
3p-hpRNA
Mock

FIG. 7D

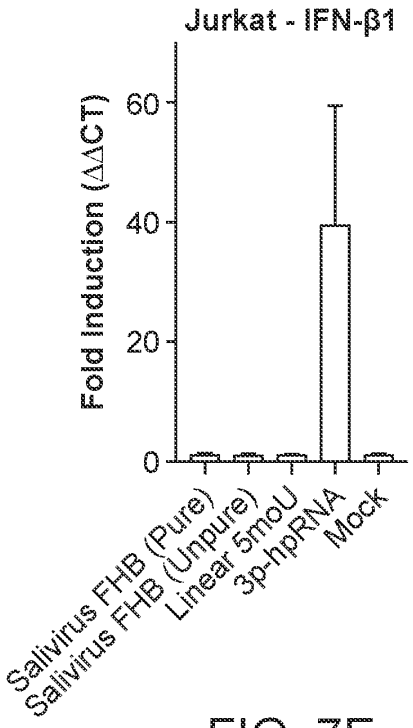
Jurkat - IFN-β1
Fold Induction (ΔΔCT)
60
40
20
0
Salivirus FHB (Pure)
Salivirus FHB (Unpure)
Linear 5moU
3p-hpRNA
Mock

FIG. 7E

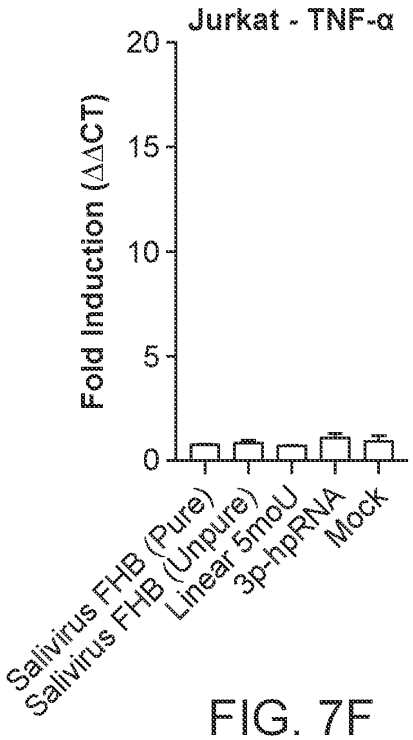
Jurkat - TNF-α
Fold Induction (ΔΔCT)
20
15
10
5
0
Salivirus FHB (Pure)
Salivirus FHB (Unpure)
Linear 5moU
3p-hpRNA
Mock

FIG. 7F

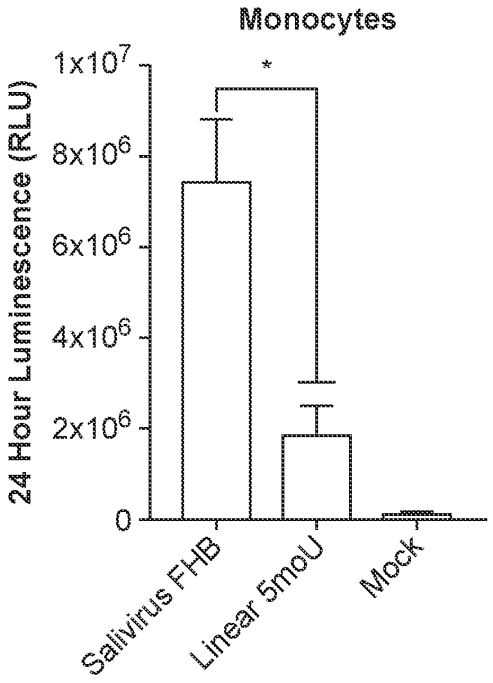
Monocytes
24 Hour Luminescence (RLU)
1x10^7
8x10^6
6x10^6
4x10^6
2x10^6
0
*
Salivirus FHB
Linear 5moU
Mock

FIG. 8A

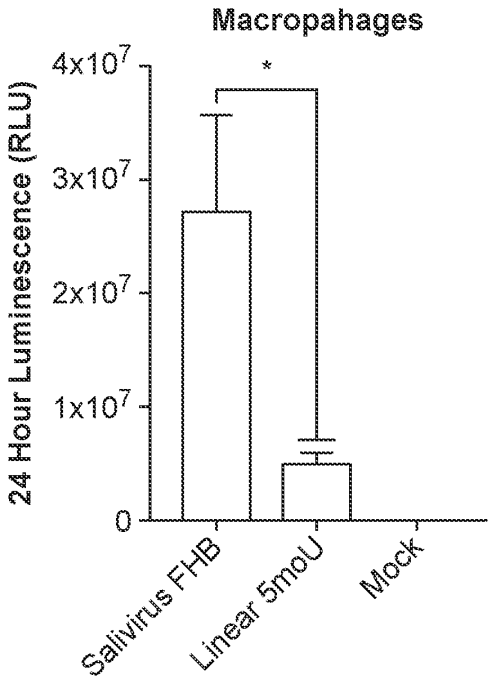
Macropahages
24 Hour Luminescence (RLU)
4x10^7
3x10^7
2x10^7
1x10^7
0
*
Salivirus FHB
Linear 5moU
Mock

FIG. 8B

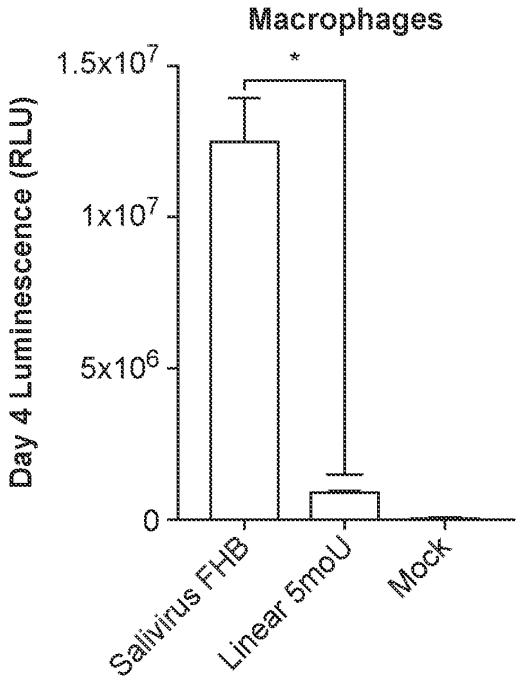
Macrophages
*
1.5x10^7
1x10^7
5x10^6
0
Day 4 Luminescence (RLU)
Salivirus FHB
Linear 5moU
Mock

FIG. 8C

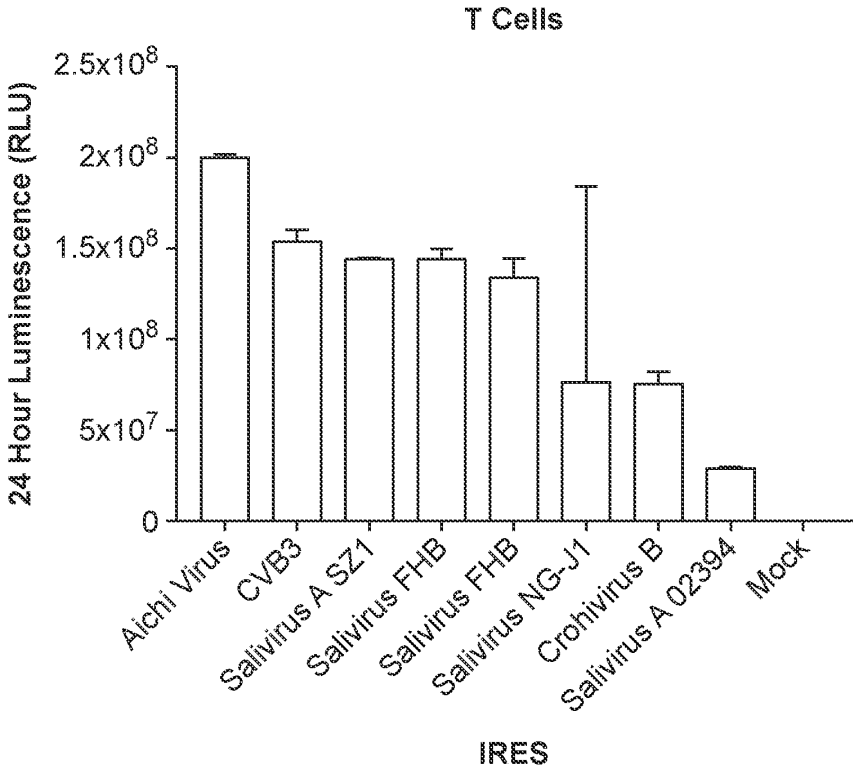
T Cells
24 Hour Luminescence (RLU)
2.5x10^8
2x10^8
1.5x10^8
1x10^8
5x10^7
0
Aichi Virus
CVB3
Salivirus A SZ1
Salivirus FHB
Salivirus FHB
Salivirus NG-J1
Crohivirus B
Salivirus A 02394
Mock
IRES

FIG. 9A

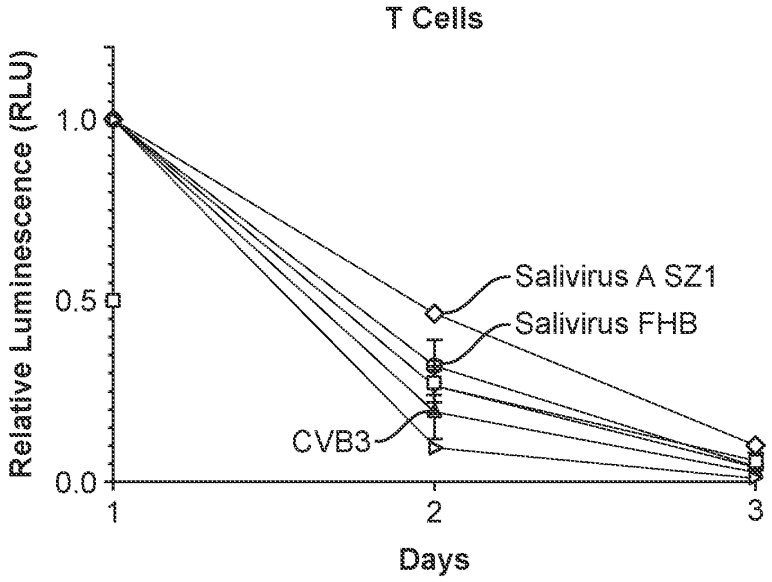
T Cells
Relative Luminescence (RLU)
1.0
0.5
0.0
Salivirus A SZ1
Salivirus FHB
CVB3
1
2
3
Days

FIG. 9B

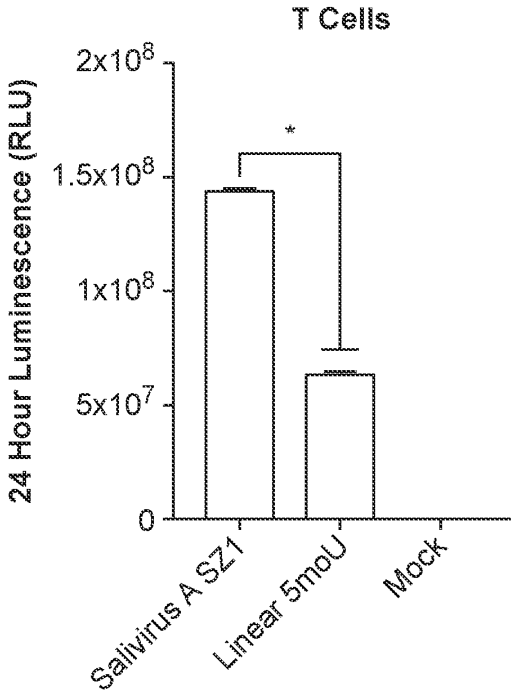

FIG. 10A

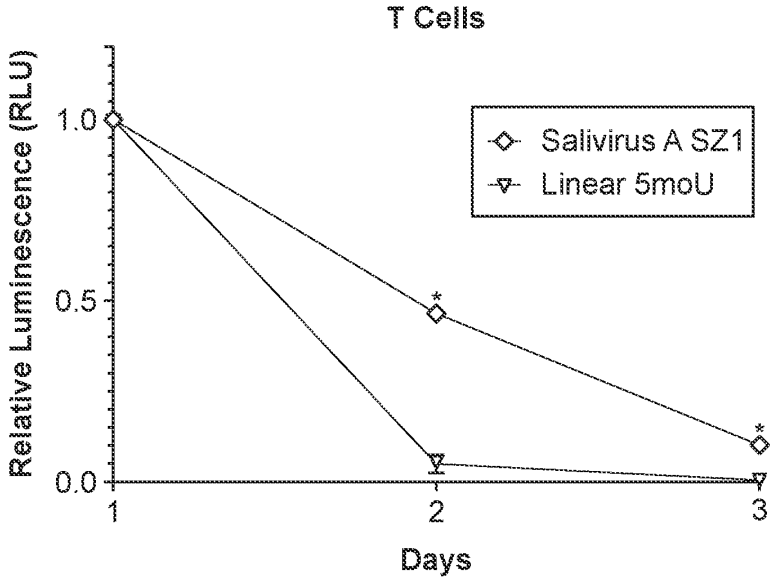

FIG. 10B

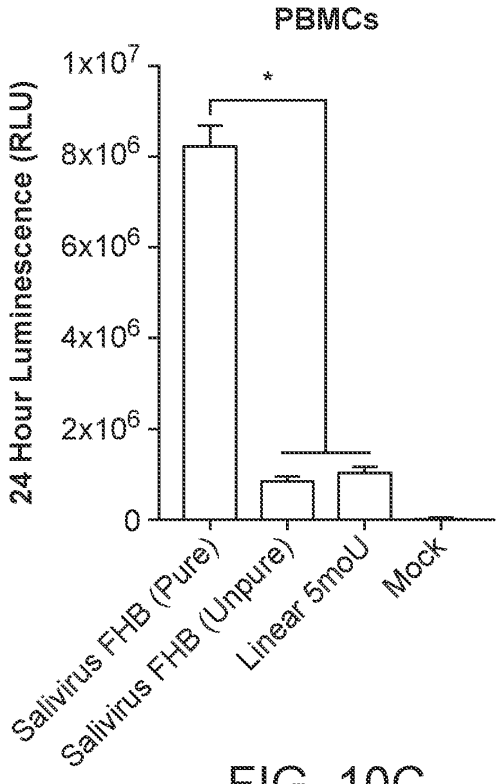
PBMCs
24 Hour Luminescence (RLU)
1x10^7
8x10^6
6x10^6
4x10^6
2x10^6
0
*
Salivirus FHB (Pure)
Salivirus FHB (Unpure)
Linear 5moU
Mock

FIG. 10C

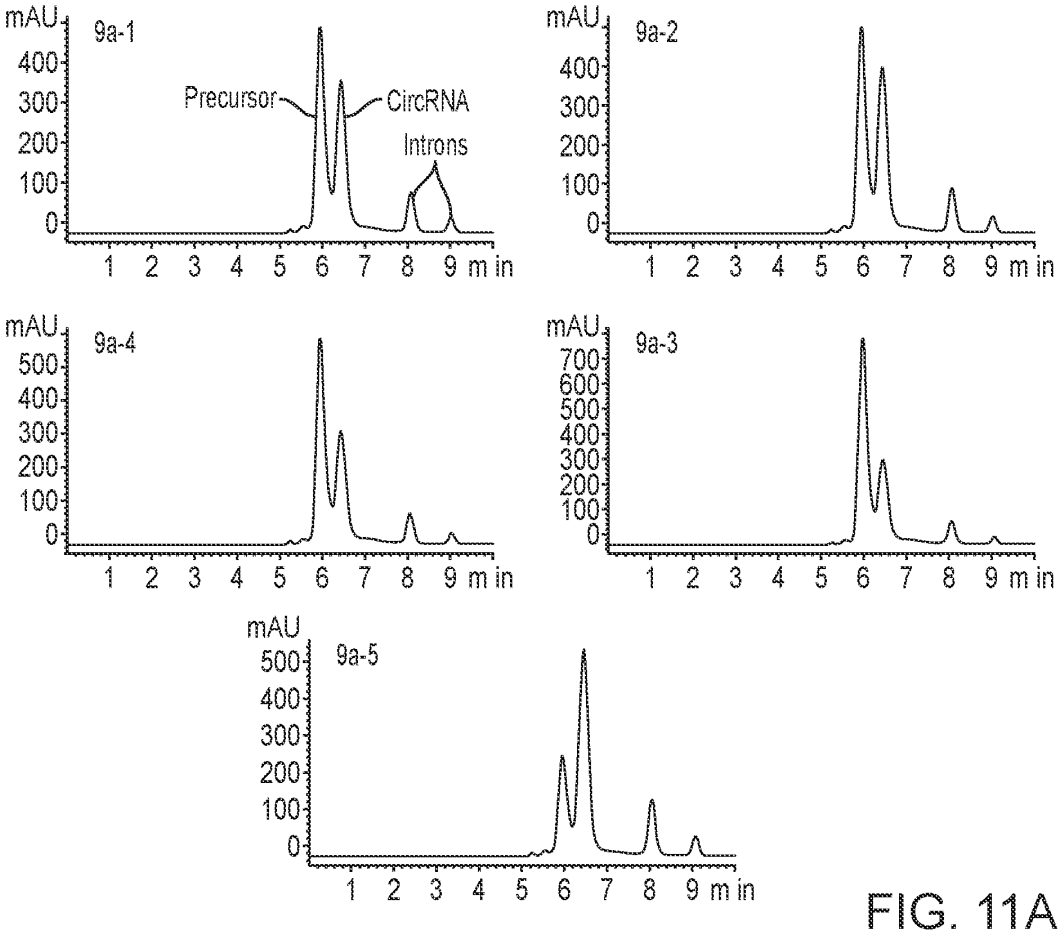

FIG. 11A

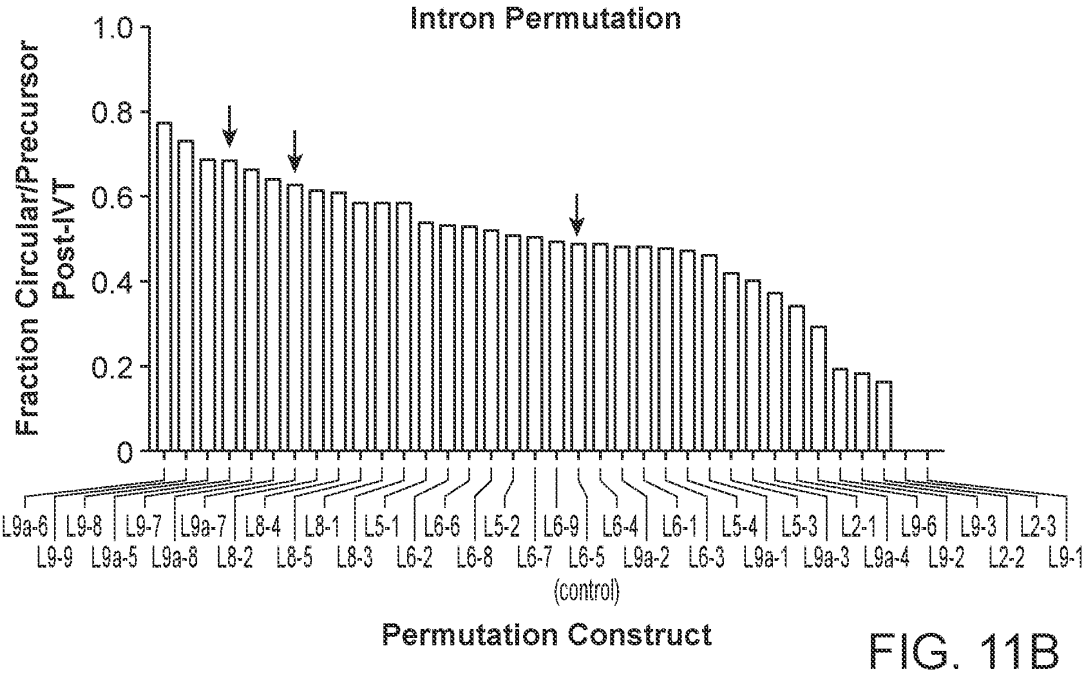

FIG. 11B

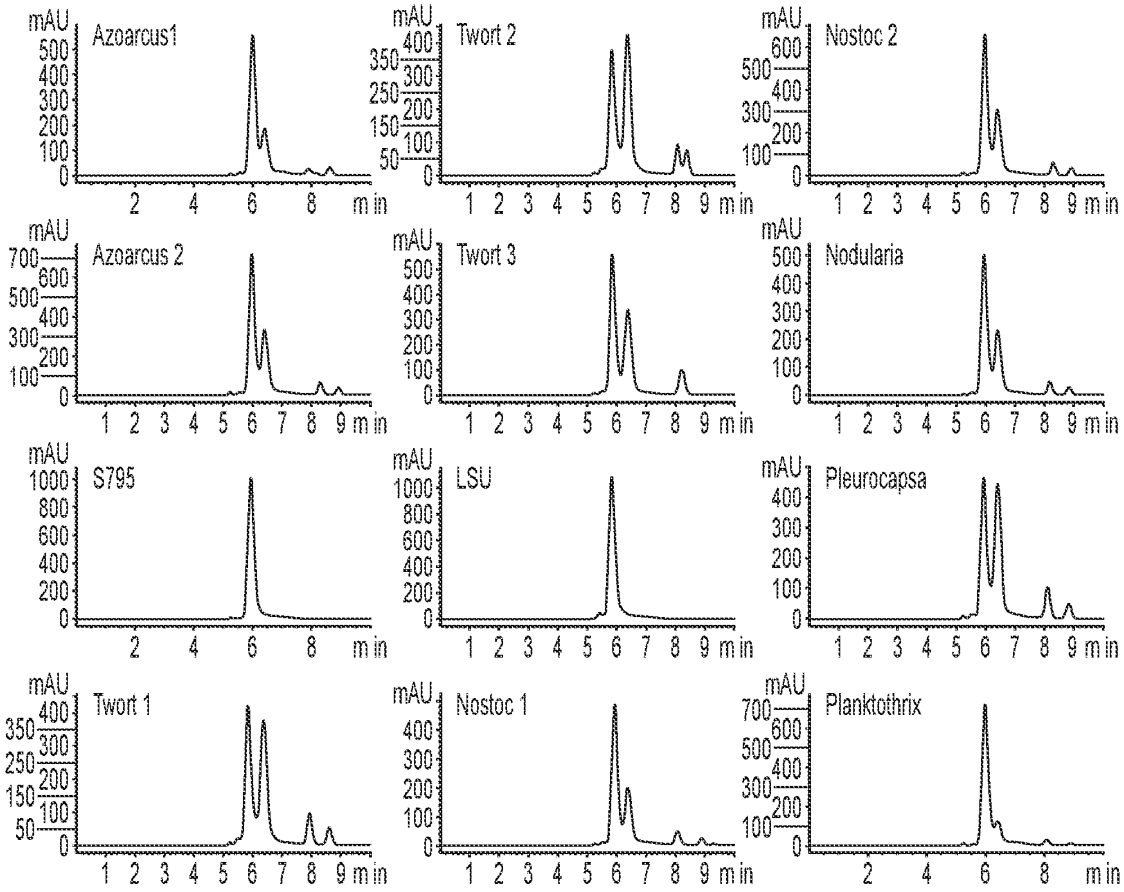

FIG. 12A

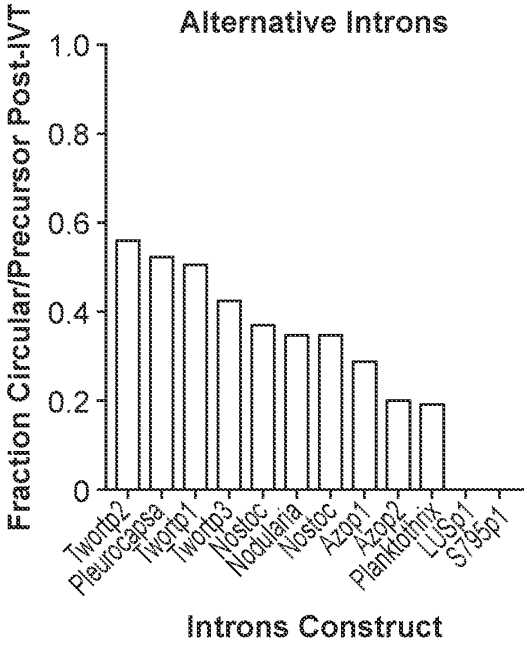

FIG. 12B

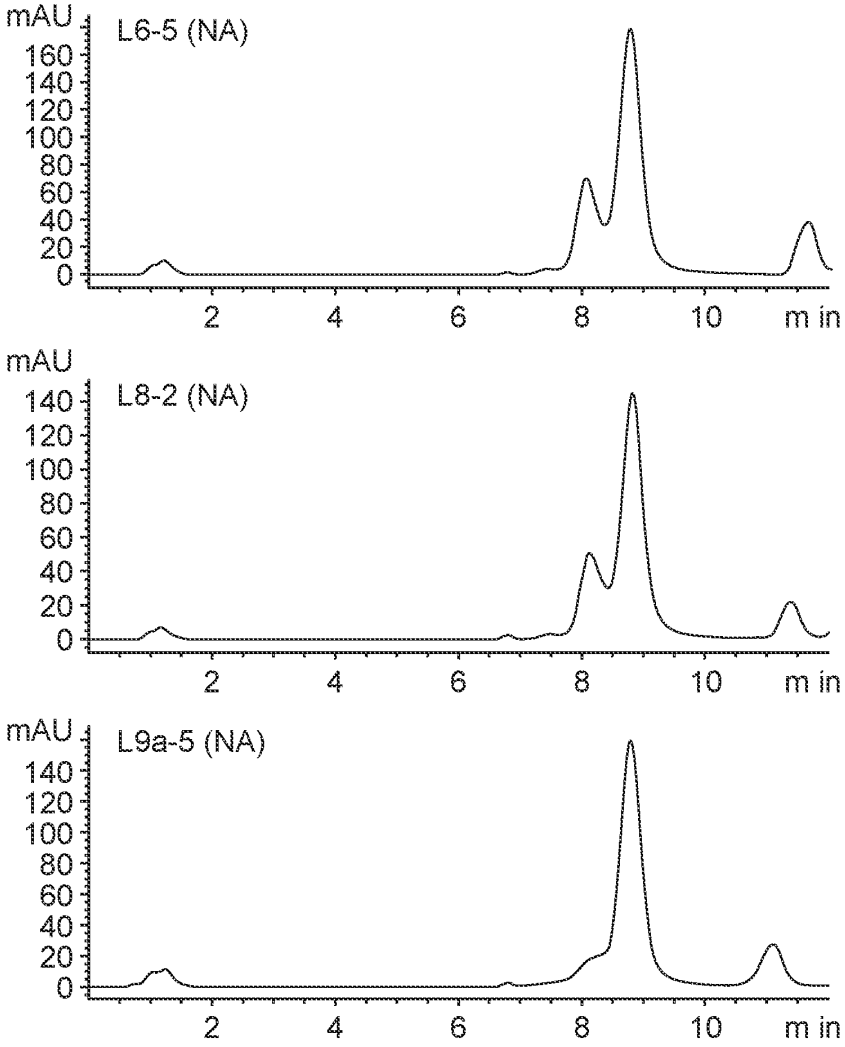
mAU
L6-5 (NA)
160
140
120
100
80
60
40
20
0
2
4
6
8
10
m in
mAU
L8-2 (NA)
140
120
100
80
60
40
20
0
2
4
6
8
10
m in
mAU
L9a-5 (NA)
140
120
100
80
60
40
20
0
2
4
6
8
10
m in

FIG. 13A

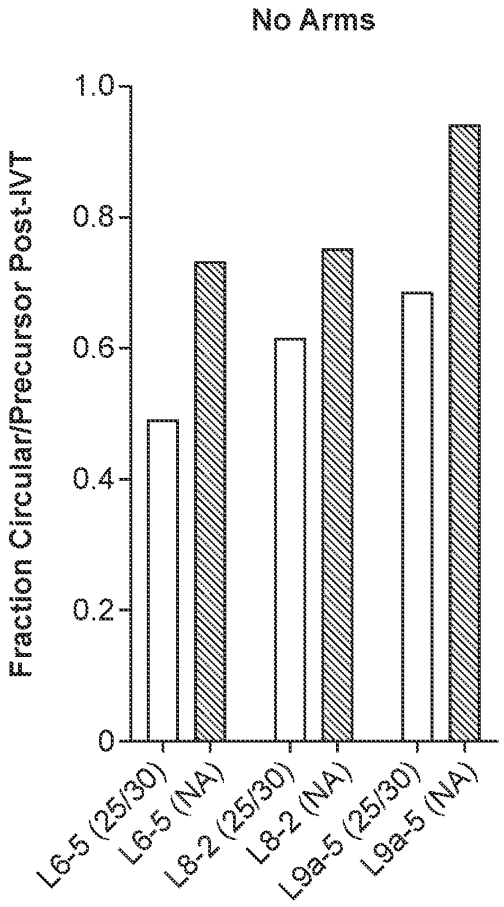
No Arms
Fraction Circular/Precursor Post-IVT
1.0
0.8
0.6
0.4
0.2
0
L6-5 (25/30)
L6-5 (NA)
L8-2 (25/30)
L8-2 (NA)
L9a-5 (25/30)
L9a-5 (NA)

FIG. 13B

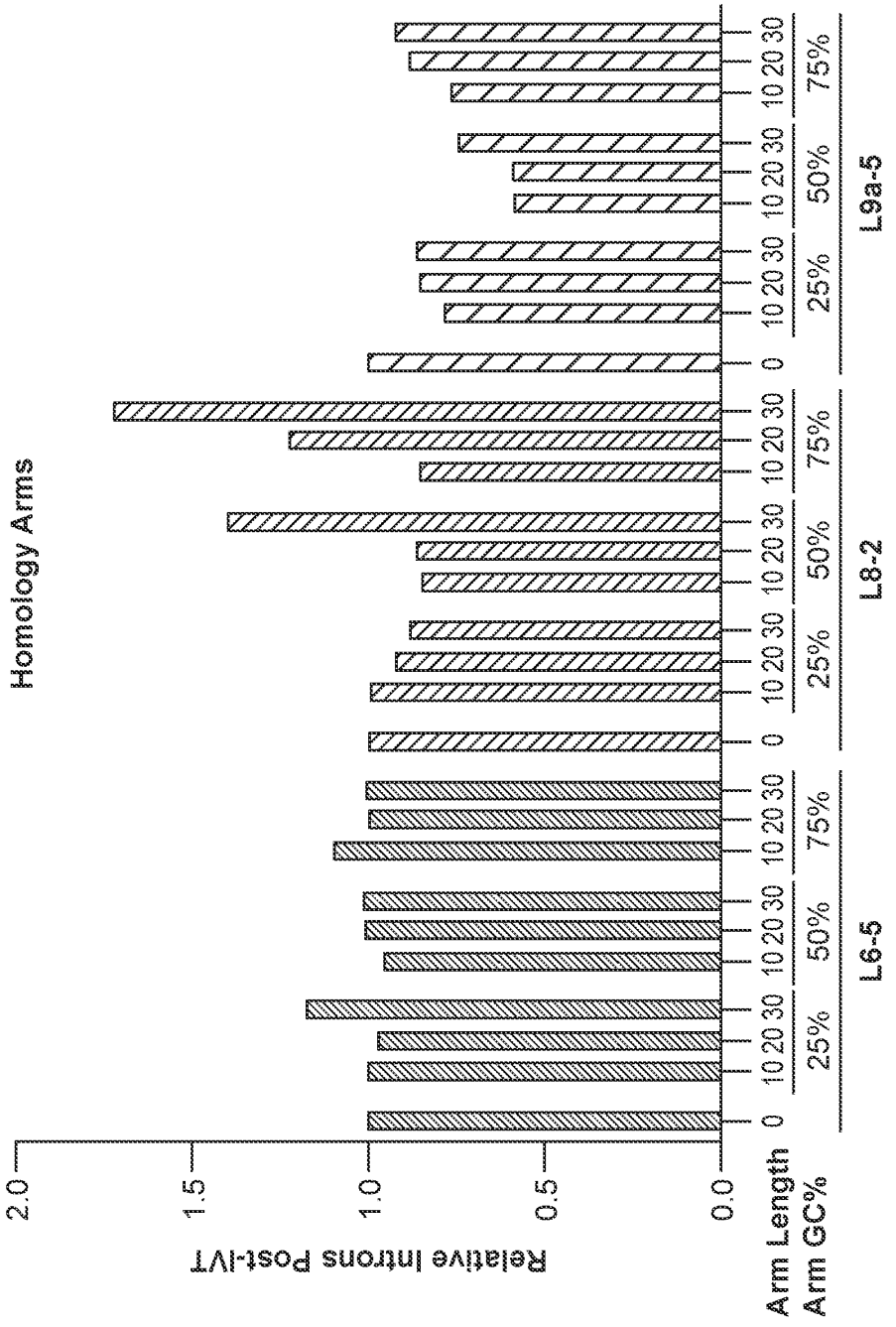
Homology Arms
Relative Introns Post-IVT
2.0
1.5
1.0
0.5
0.0
Arm Length
Arm GC%
0 10 20 30 10 20 30 10 20 30
25% 50% 75%
L6-5
0 10 20 30 10 20 30 10 20 30
25% 50% 75%
L8-2
0 10 20 30 10 20 30 10 20 30
25% 50% 75%
L9a-5
Permutation

FIG. 14

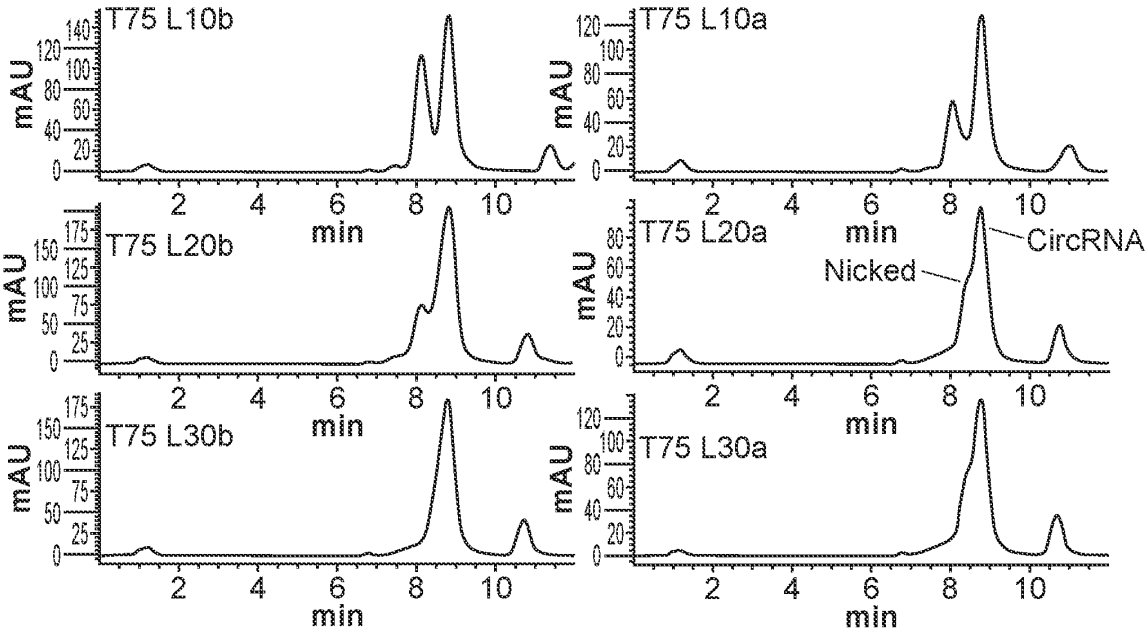

FIG. 15A

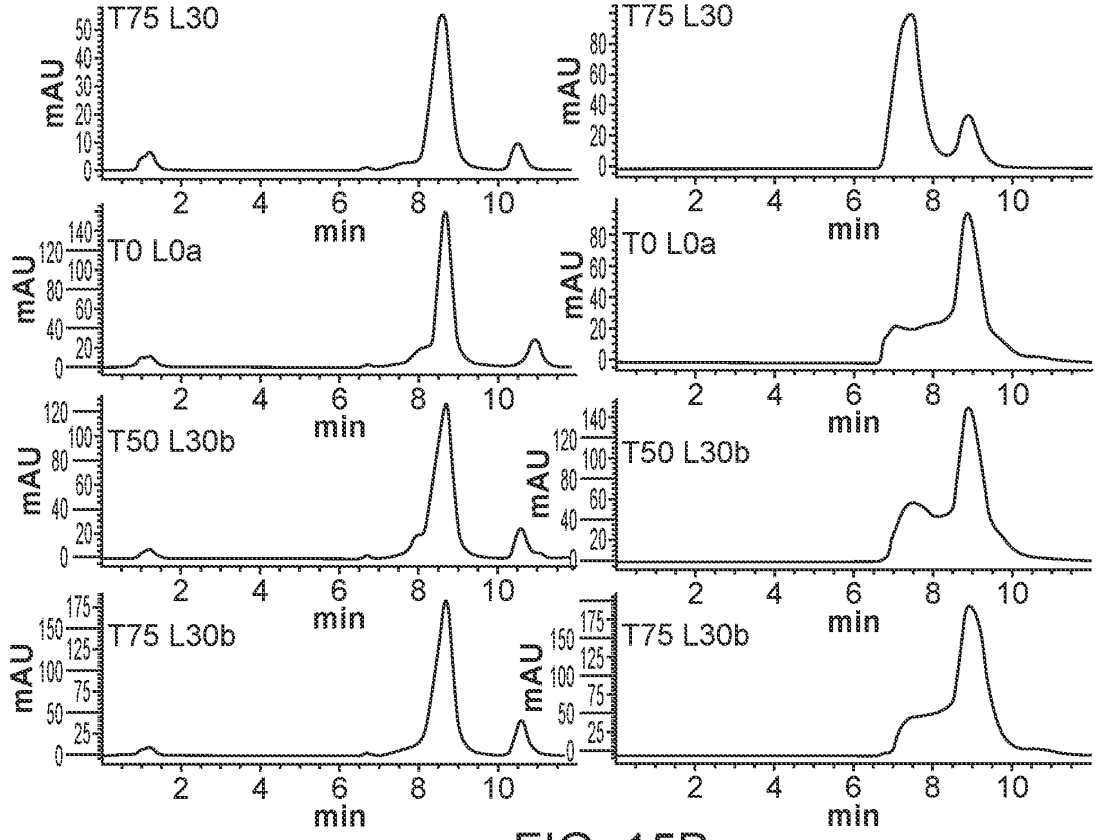

FIG. 15B

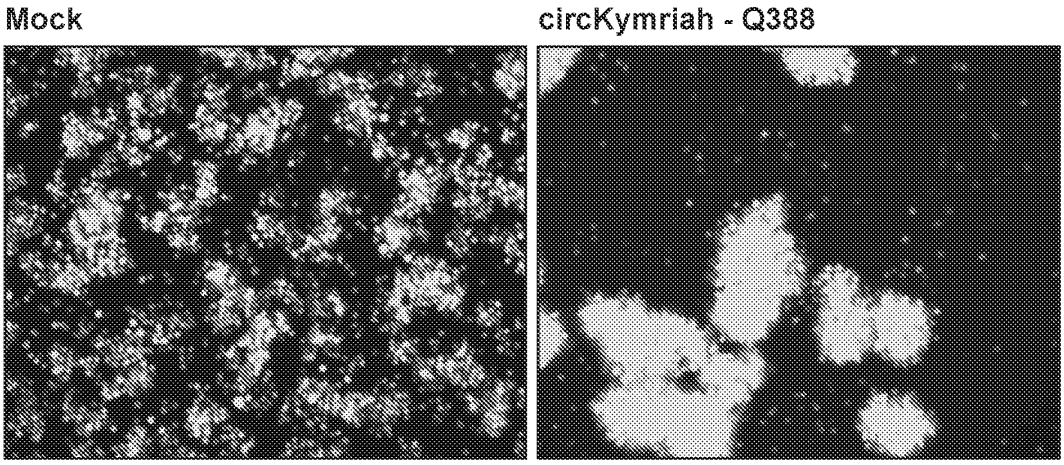

FIG. 16

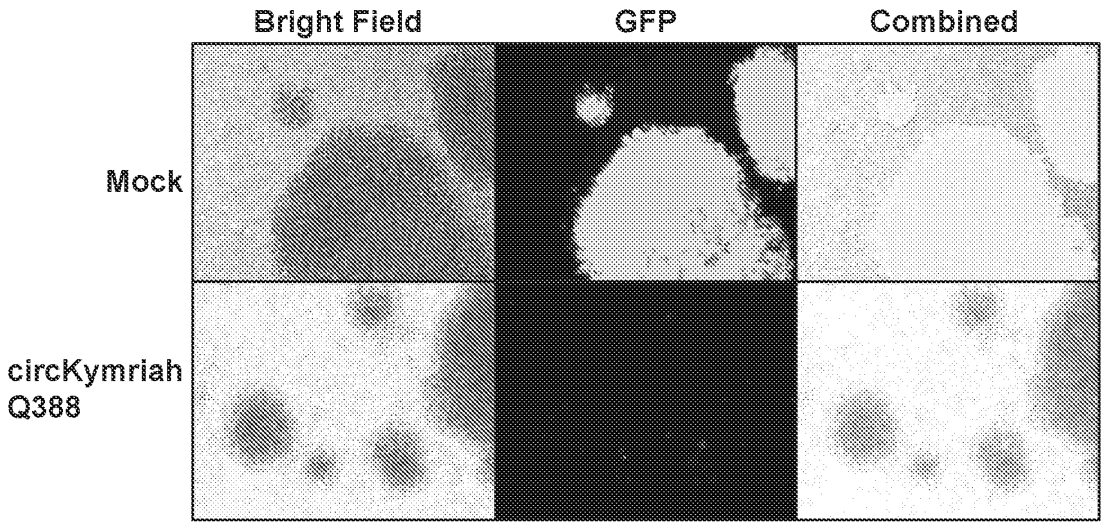

FIG. 17

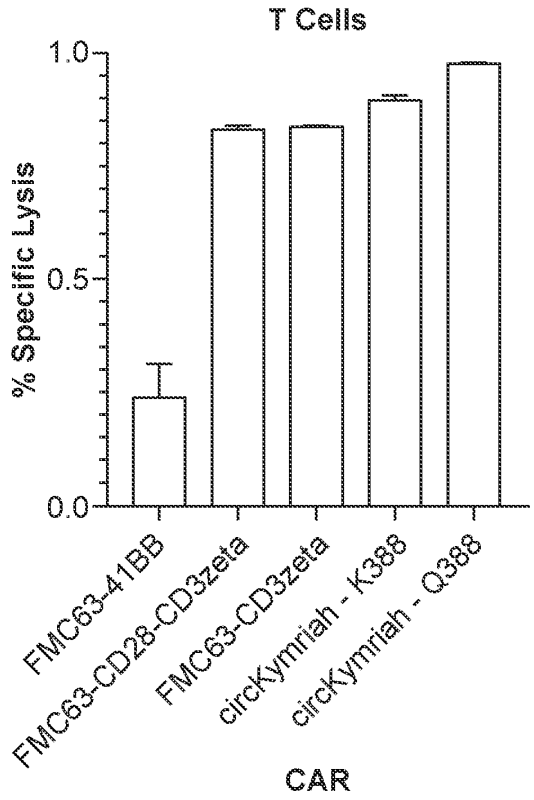
T Cells
1.0
0.5
0.0
% Specific Lysis
FMC63-41BB
FMC63-CD28-CD3zeta
FMC63-CD3zeta
circKymriah - K388
circKymriah - Q388
CAR

FIG. 18

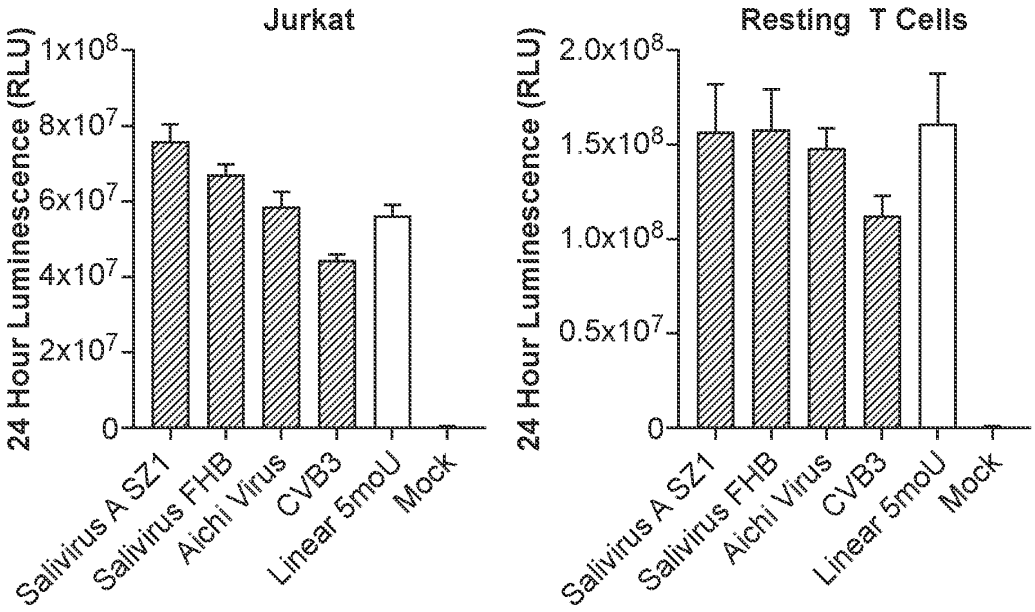

FIG. 19A

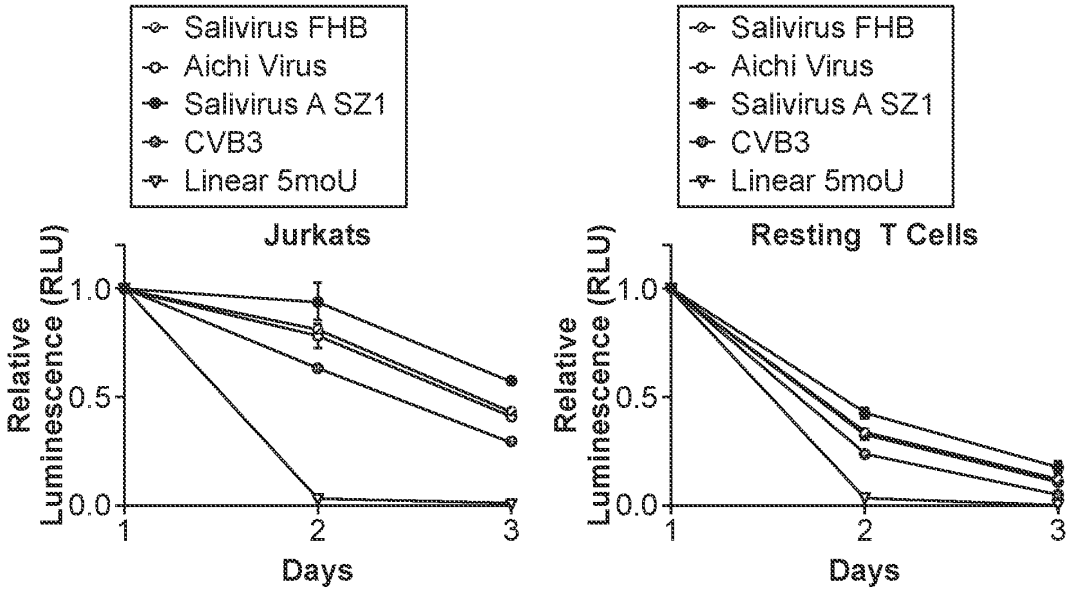

FIG. 19B

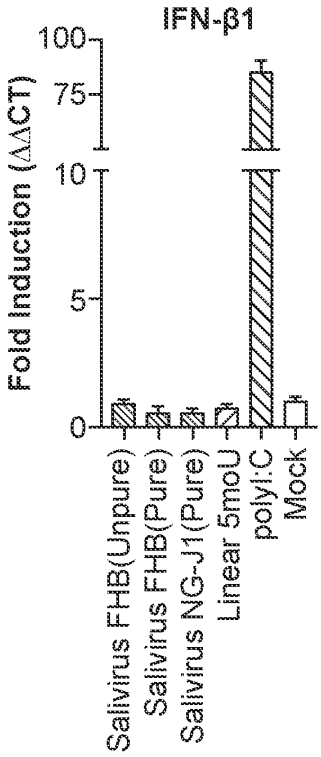

FIG. 20A

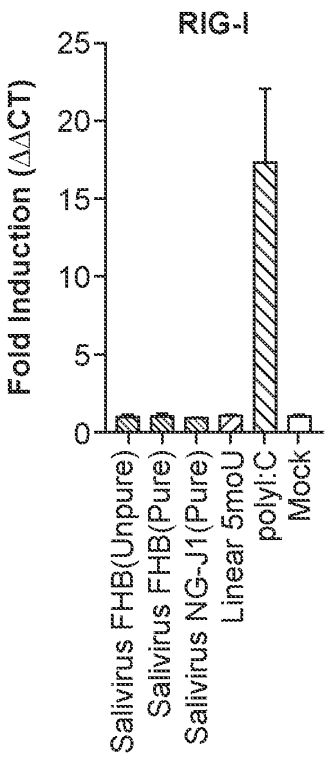

FIG. 20B

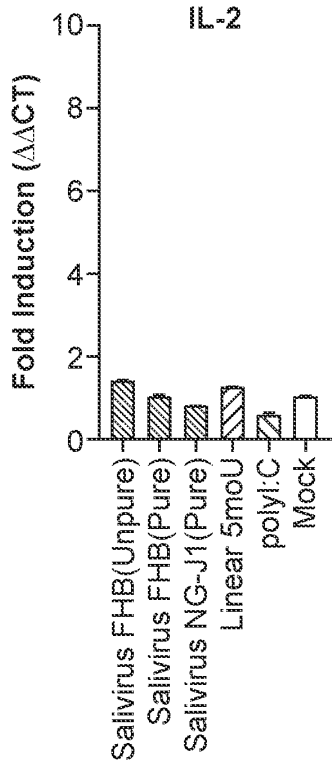

FIG. 20C

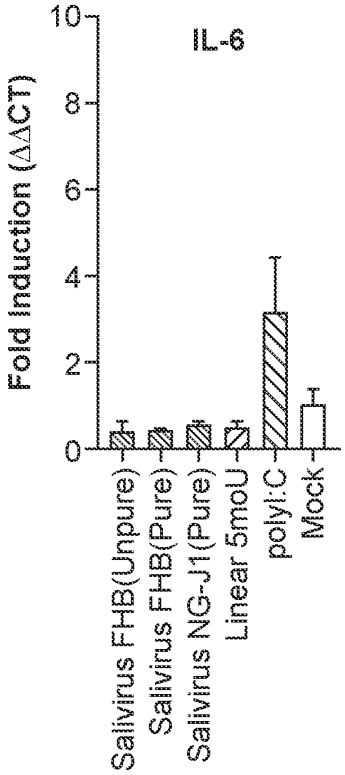

FIG. 20D

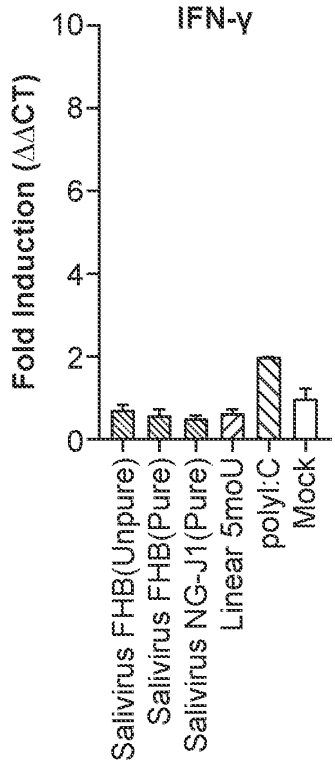

FIG. 20E

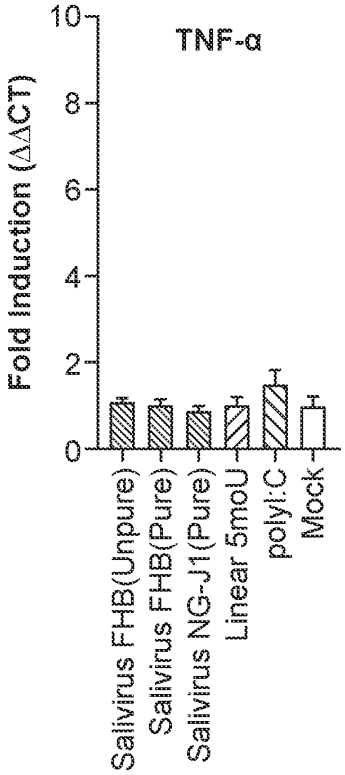

FIG. 20F

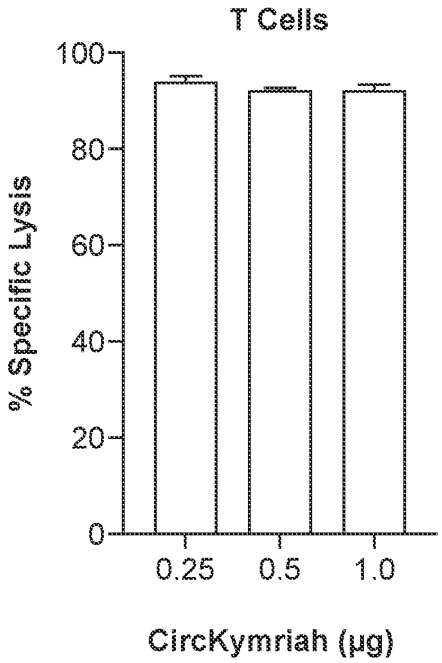
T Cells
100
80
60
40
20
0
% Specific Lysis
0.25
0.5
1.0
CircKymriah (µg)

FIG. 21A

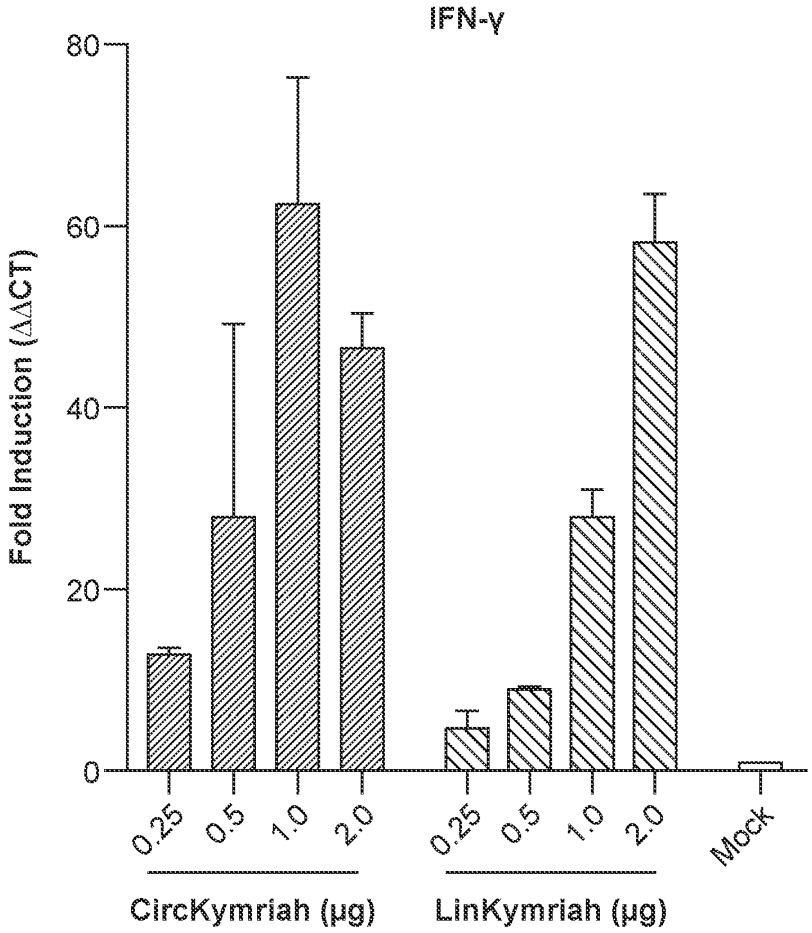
IFN-γ
Fold Induction (ΔΔCT)
80
60
40
20
0
0.25
0.5
1.0
2.0
0.25
0.5
1.0
2.0
Mock
CircKymriah (µg)
LinKymriah (µg)

FIG. 21B

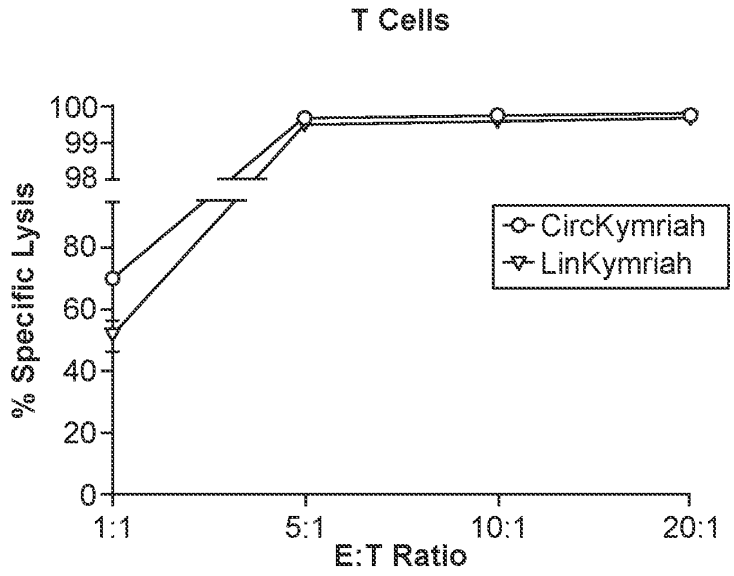

FIG. 22A

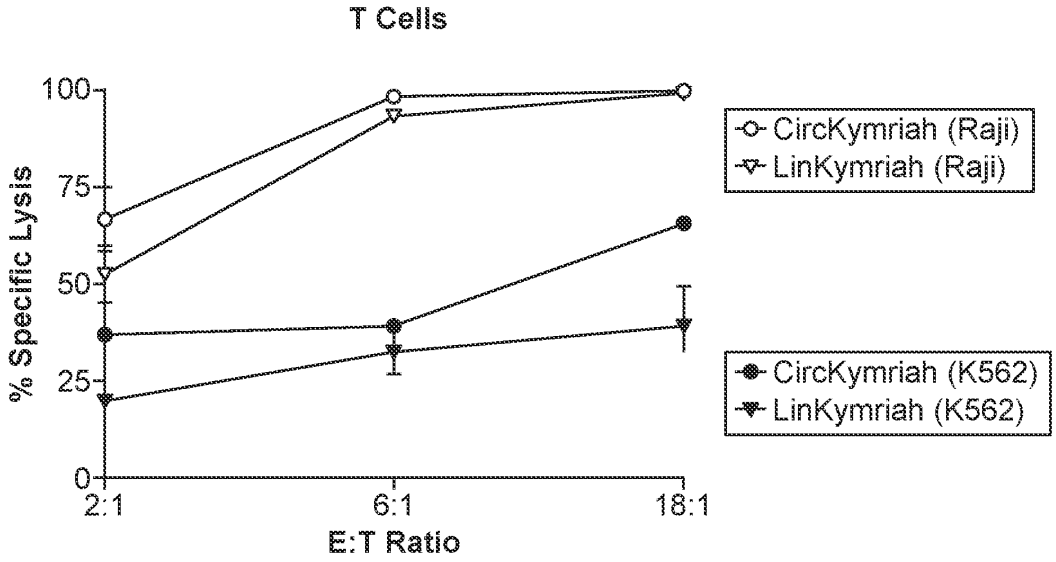

FIG. 22B

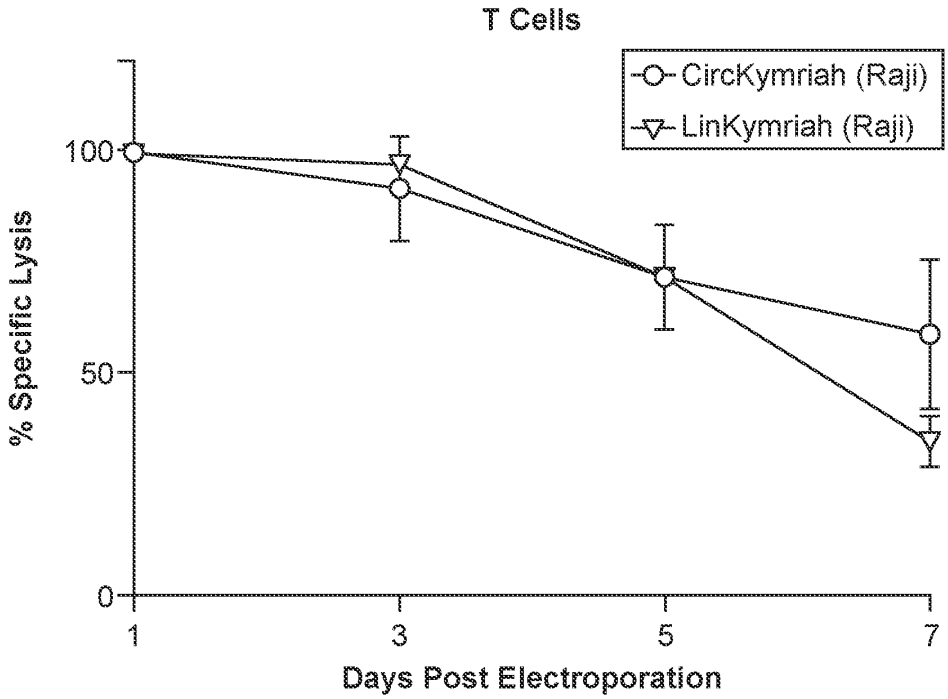
T Cells
CircKymriah (Raji)
LinKymriah (Raji)
% Specific Lysis
100
50
0
1
3
5
7
Days Post Electroporation

FIG. 23

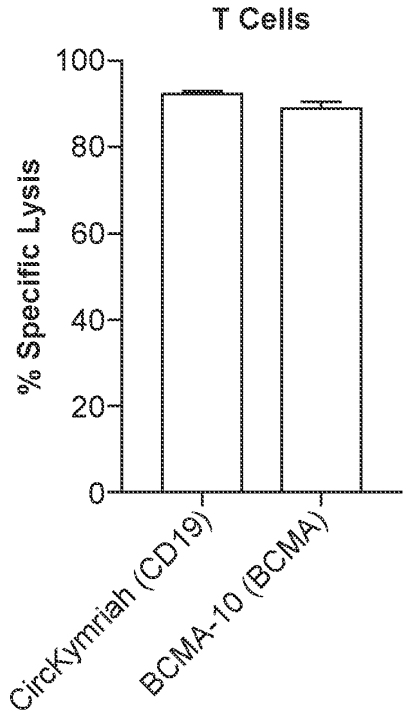
T Cells
% Specific Lysis
100
80
60
40
20
0
CircKymriah (CD19)
BCMA-10 (BCMA)

FIG. 24

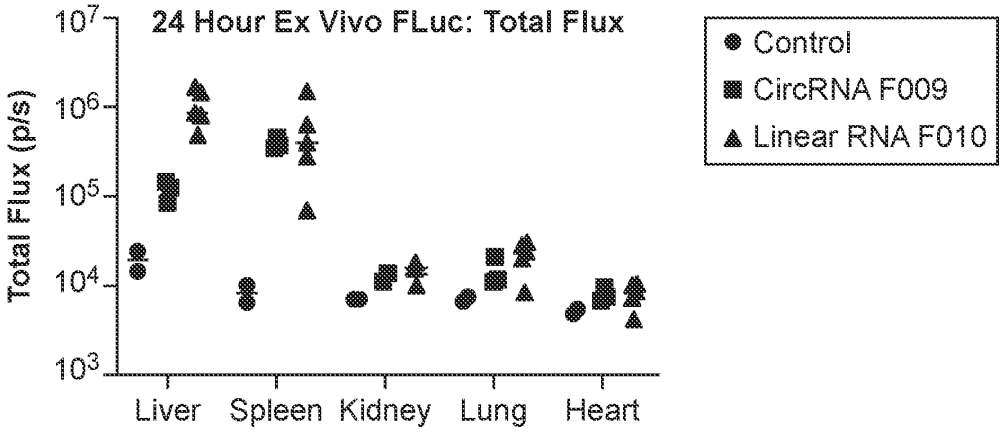
24 Hour Ex Vivo FLuc: Total Flux
Total Flux (p/s)
10^7
10^6
10^5
10^4
10^3
Liver
Spleen
Kidney
Lung
Heart
Control
CircRNA F009
Linear RNA F010

FIG. 25

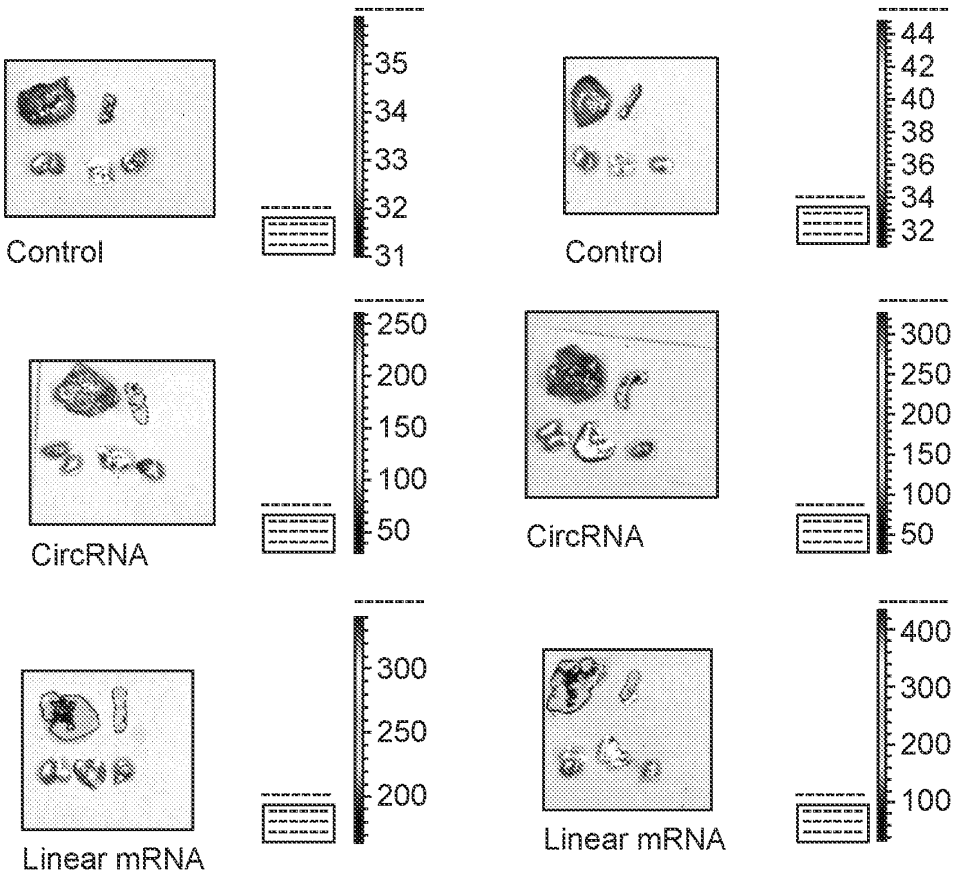
Control
35
34
33
32
31
Control
44
42
40
38
36
34
32
CircRNA
250
200
150
100
50
CircRNA
300
250
200
150
100
50
Linear mRNA
300
250
200
Linear mRNA
400
300
200
100

FIG. 26

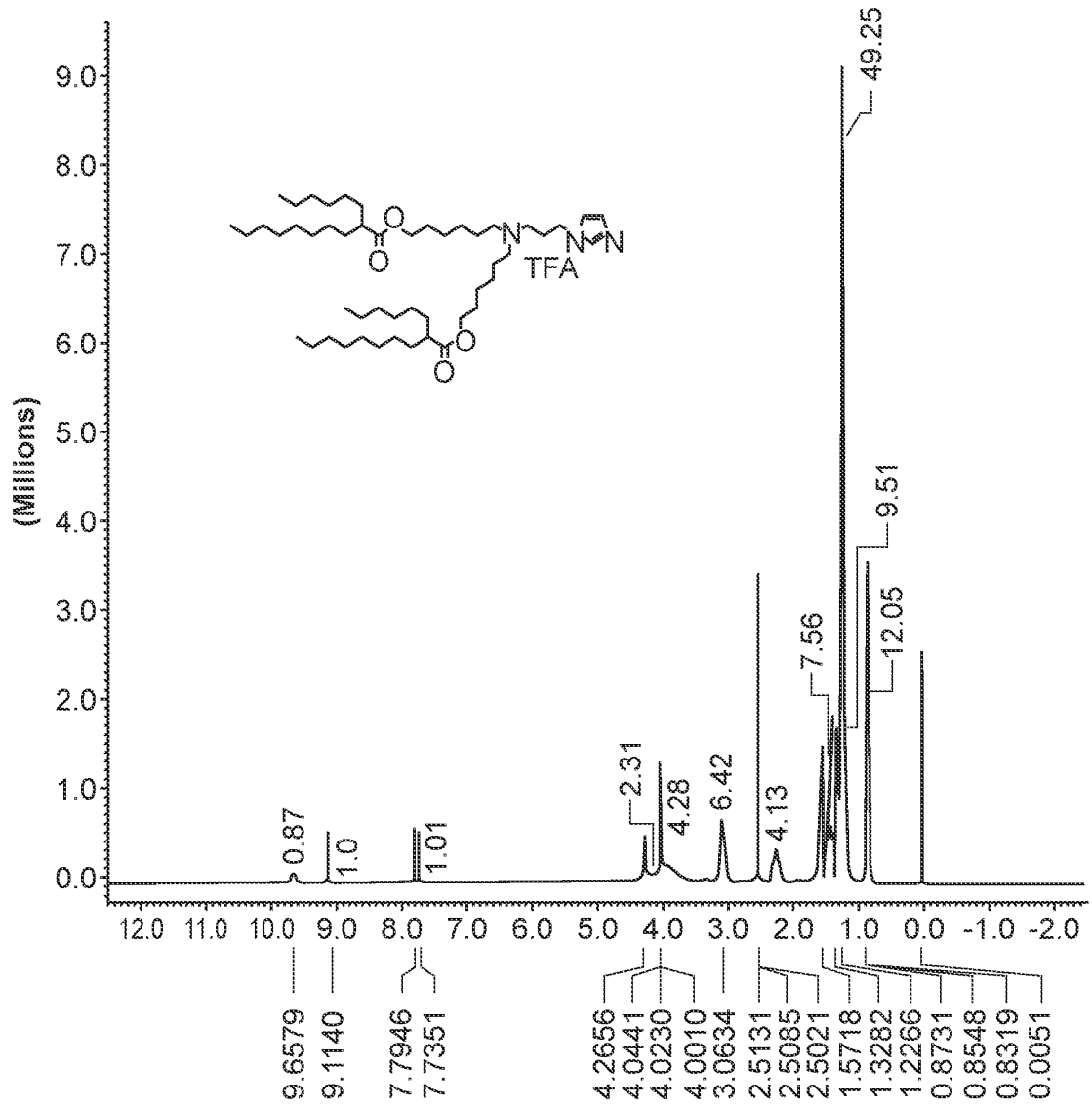
(Millions)
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
TFA
49.25
9.51
12.05
7.56
2.31
4.28
6.42
4.13
0.87
1.0
1.01
12.0 11.0 10.0 9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0.0 -1.0 -2.0
9.6579
9.1140
7.7946
7.7351
4.2656
4.0441
4.0230
4.0010
3.0634
2.5131
2.5085
2.5021
1.5718
1.3282
1.2266
0.8731
0.8548
0.8319
0.0051

FIG. 27A

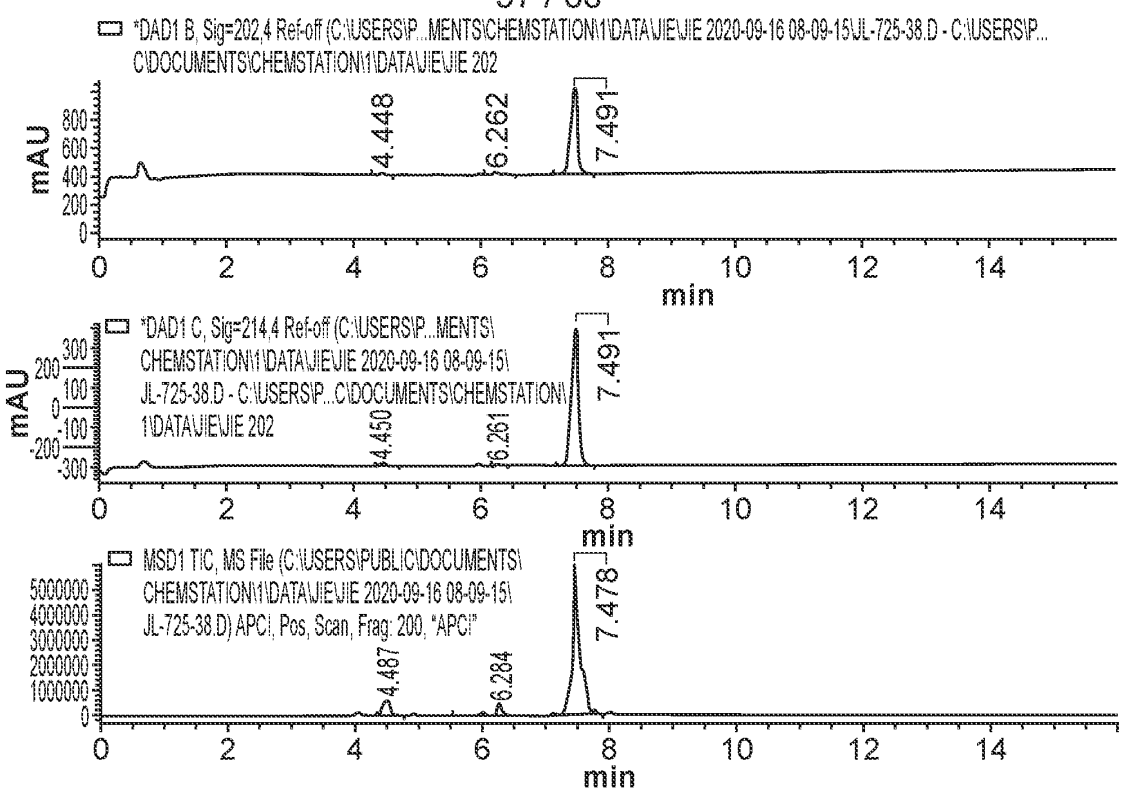

FIG. 27B

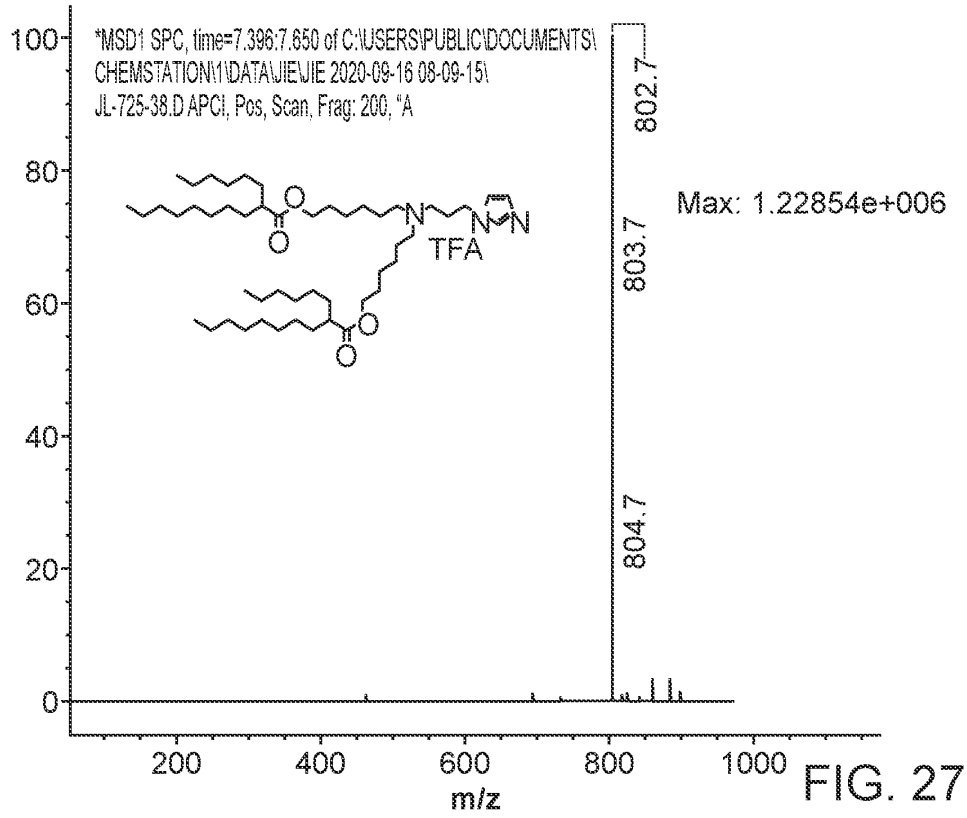

FIG. 27C

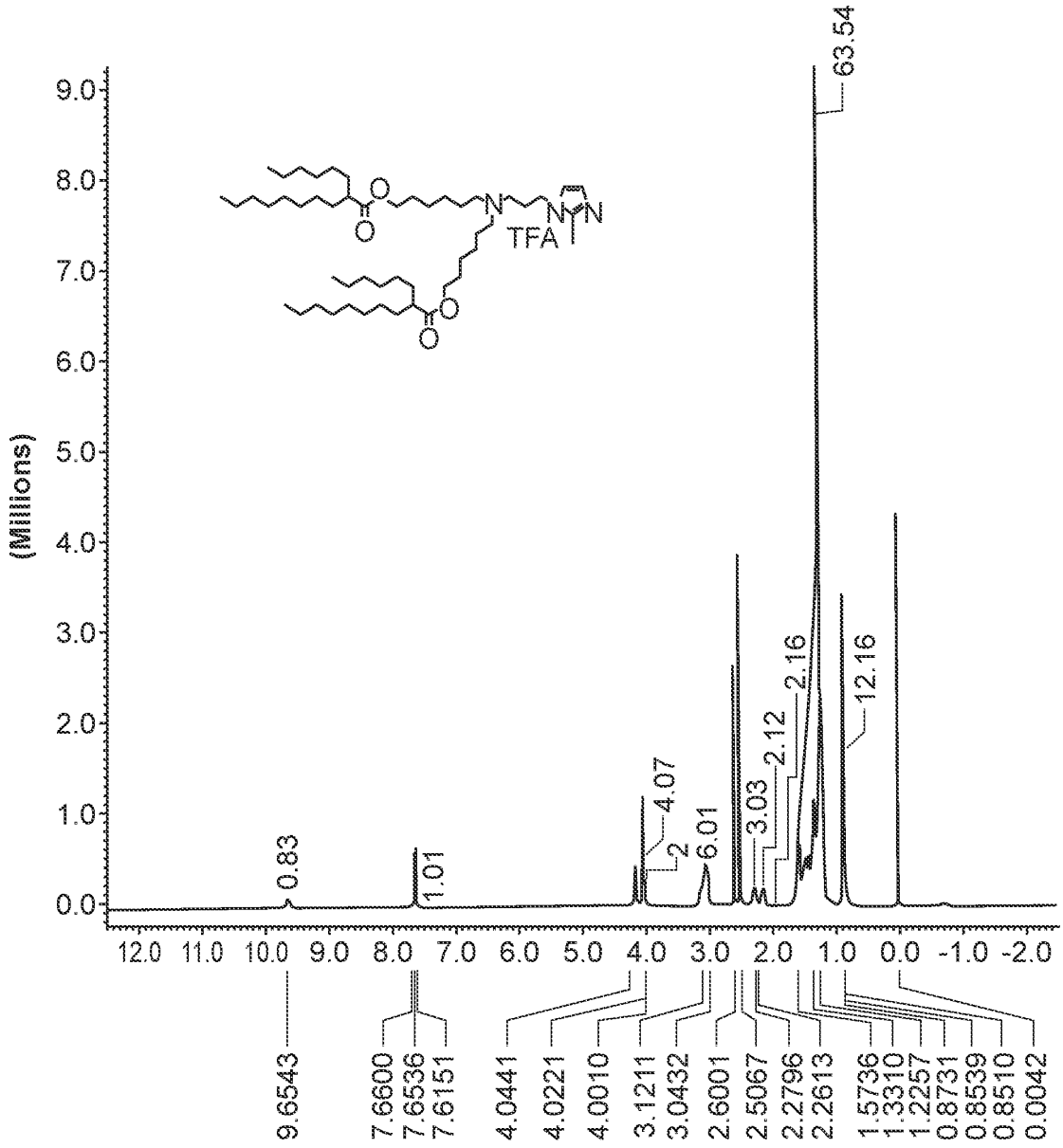
(Millions)
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
TFA
63.54
12.16
2.16
2.12
4.07
3.03
6.01
2
0.83
1.01
12.0 11.0 10.0 9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0.0 -1.0 -2.0
9.6543
7.6600
7.6536
7.6151
4.0441
4.0221
4.0010
3.1211
3.0432
2.6001
2.5067
2.2796
2.2613
1.5736
1.3310
1.2257
0.8731
0.8539
0.8510
0.0042

FIG. 27D

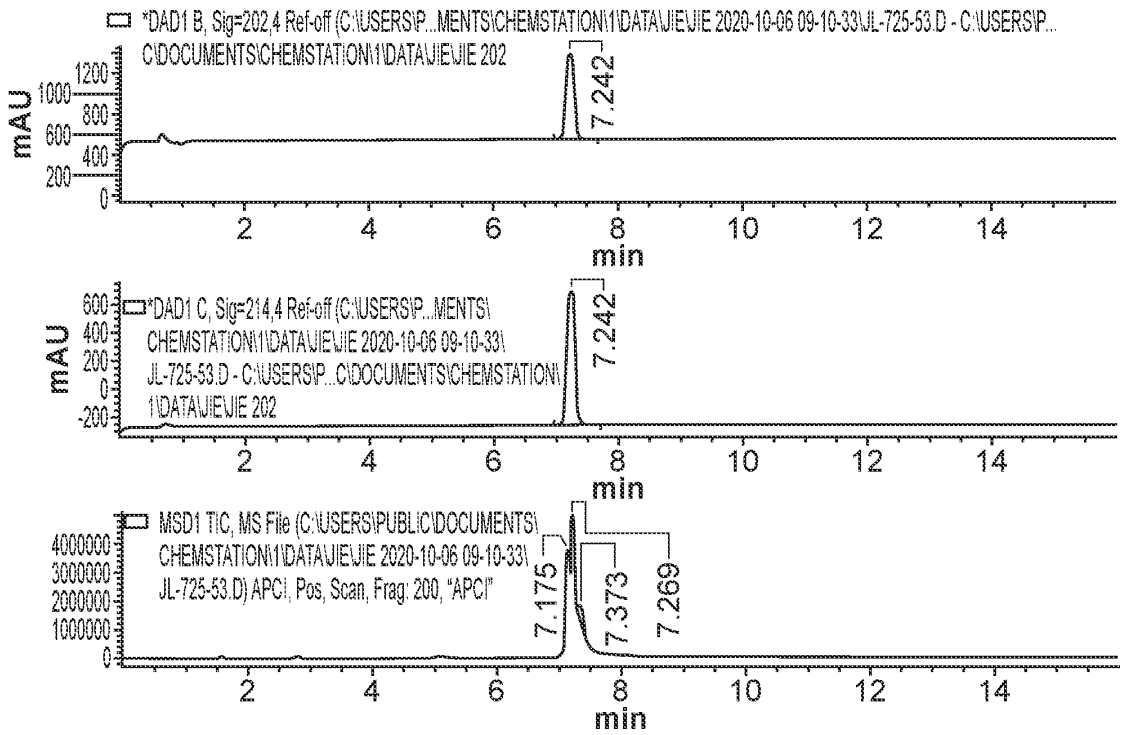

FIG. 27E

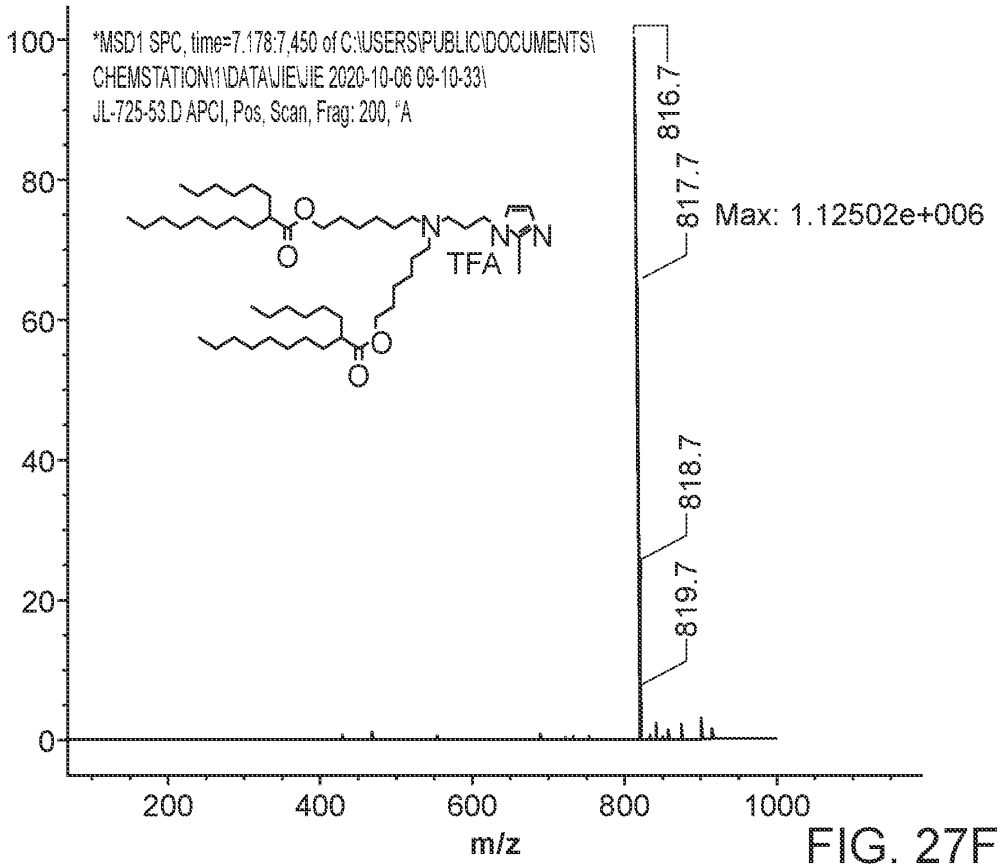

FIG. 27F

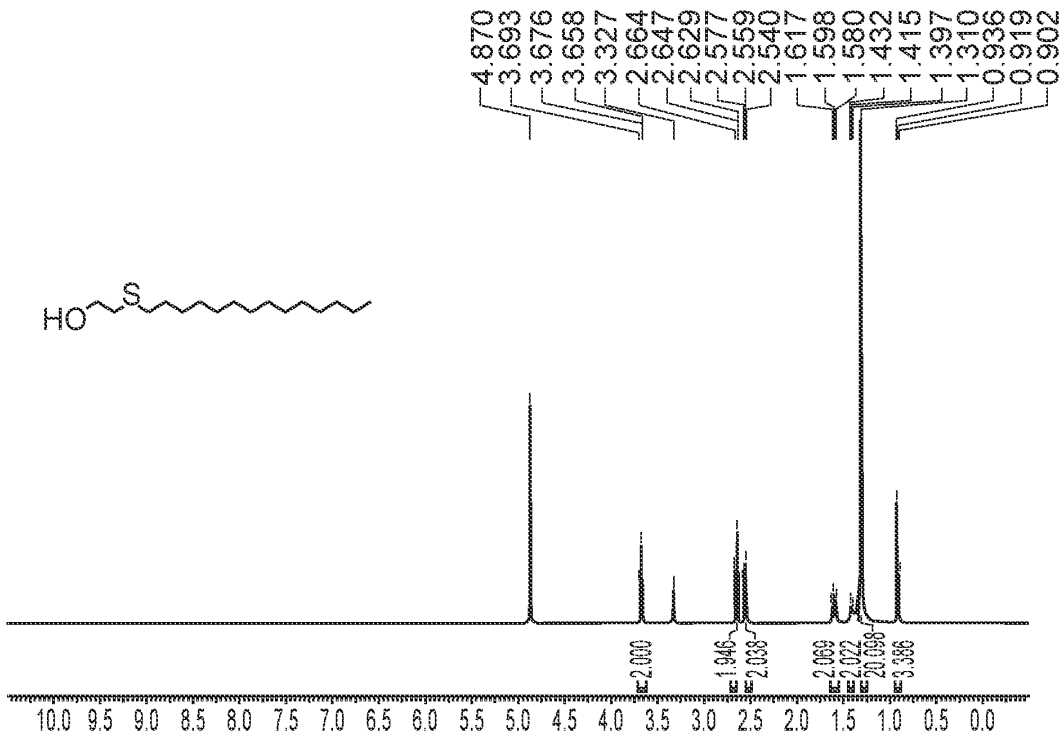

FIG. 28A

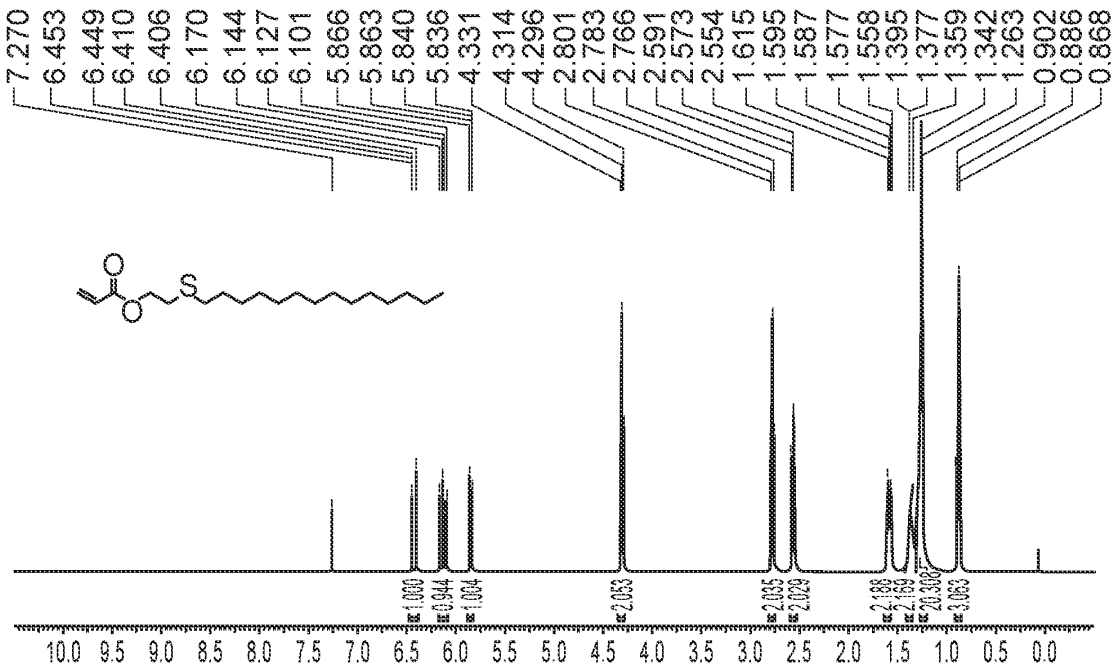

FIG. 28B

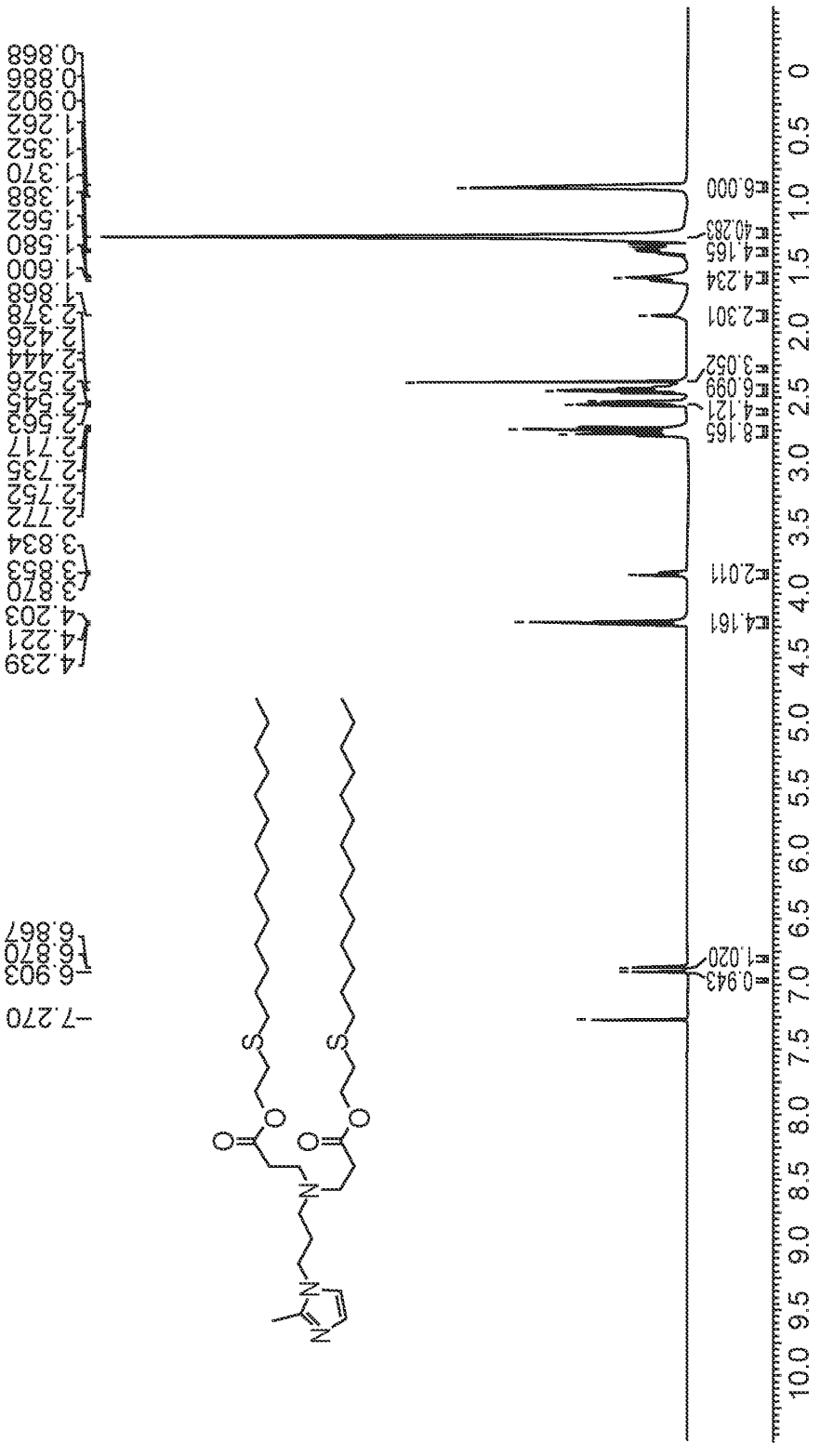
6.000
4.165
4.234
2.301
3.052
6.099
4.121
8.165
2.011
4.161
1.020
0.943
7.270
6.903
6.870
6.867
4.239
4.221
4.203
3.870
3.853
3.834
2.772
2.752
2.735
2.717
2.643
2.626
2.444
2.426
2.378
1.868
1.600
1.580
1.562
1.388
1.370
1.352
1.262
0.902
0.886
0.868
S
S
O
O
O
O
N
N
N

FIG. 28C

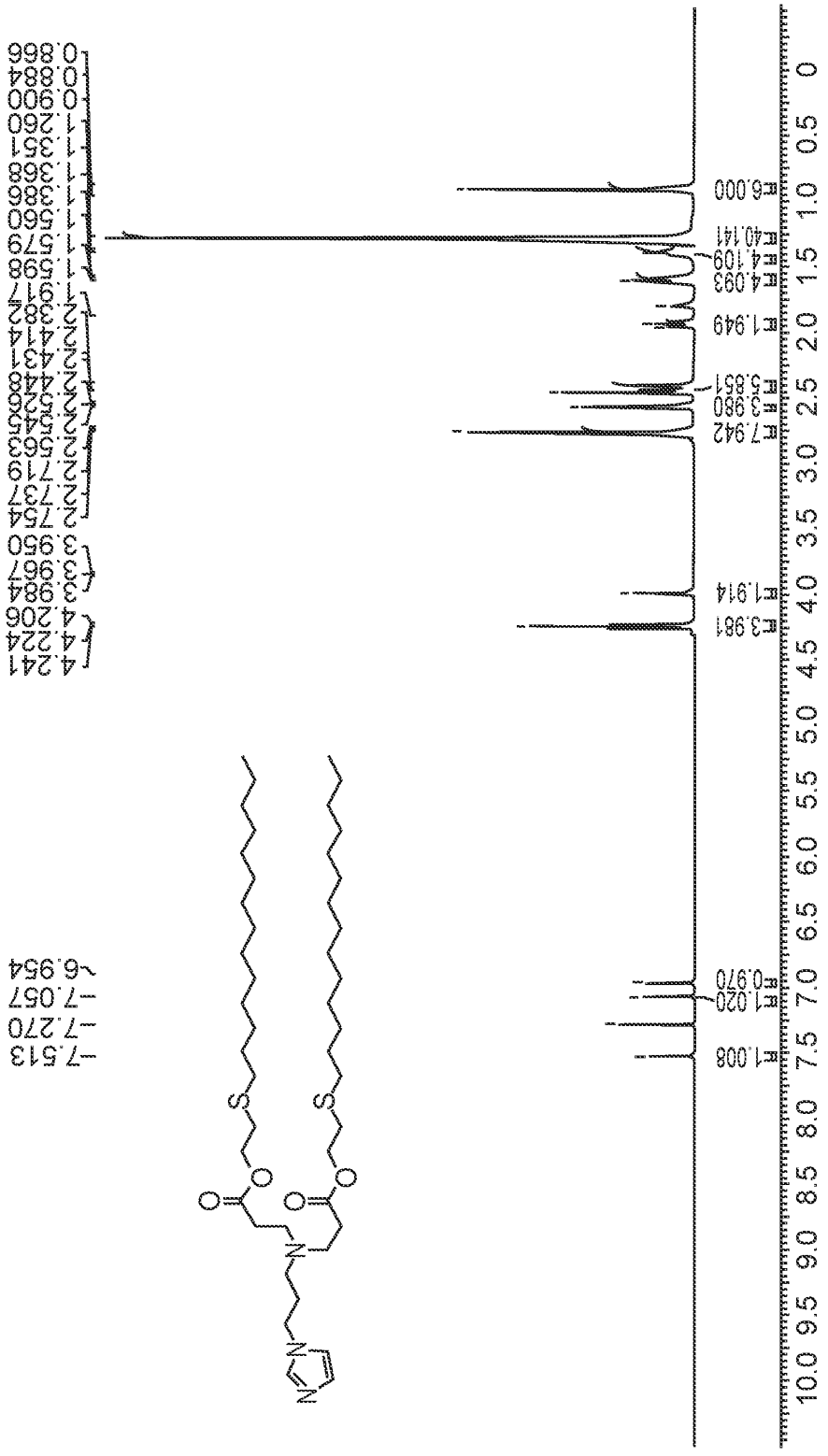
6.000
40.141
4.109
4.093
1.949
5.851
3.980
7.942
1.914
3.981
1.008
1.020
0.970
7.513
7.270
7.057
6.954
4.241
4.224
4.206
3.984
3.967
3.950
2.754
2.737
2.719
2.563
2.545
2.528
2.431
2.414
2.382
1.917
1.598
1.579
1.560
1.386
1.368
1.351
1.260
0.900
0.884
0.866
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
10.0

FIG. 29

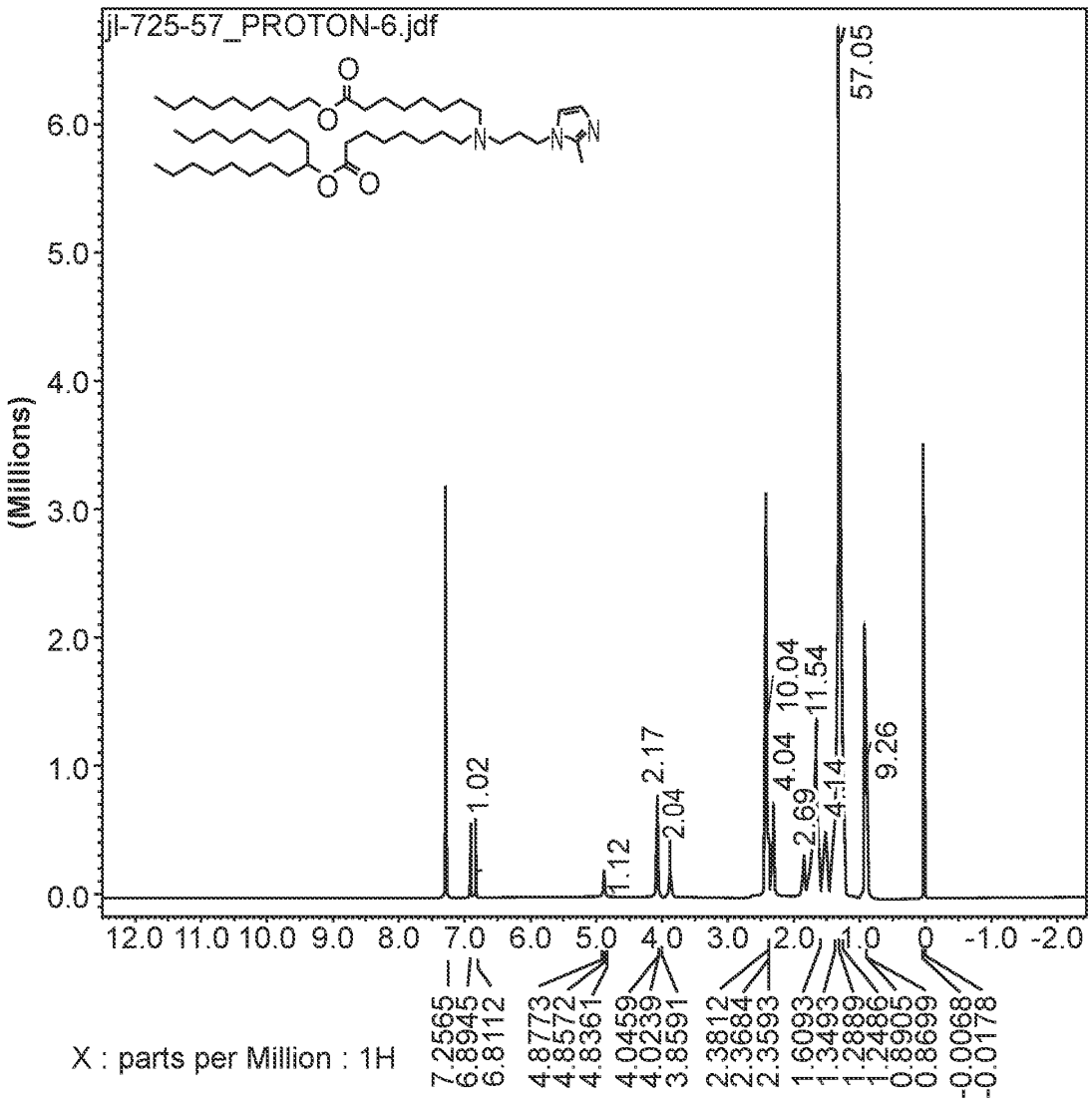
jl-725-57_PROTON-6.jdf
(Millions)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
57.05
1.02
1.12
2.17
2.04
4.04
10.04
11.54
2.69
4.14
9.26
12.0 11.0 10.0 9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0 -1.0 -2.0
X : parts per Million : 1H
7.2565
6.8945
6.8112
4.8773
4.8572
4.8361
4.0459
4.0239
3.8591
2.3812
2.3684
2.3593
1.6093
1.3493
1.2889
1.2486
0.8905
0.8699
-0.0068
-0.0178

FIG. 30A

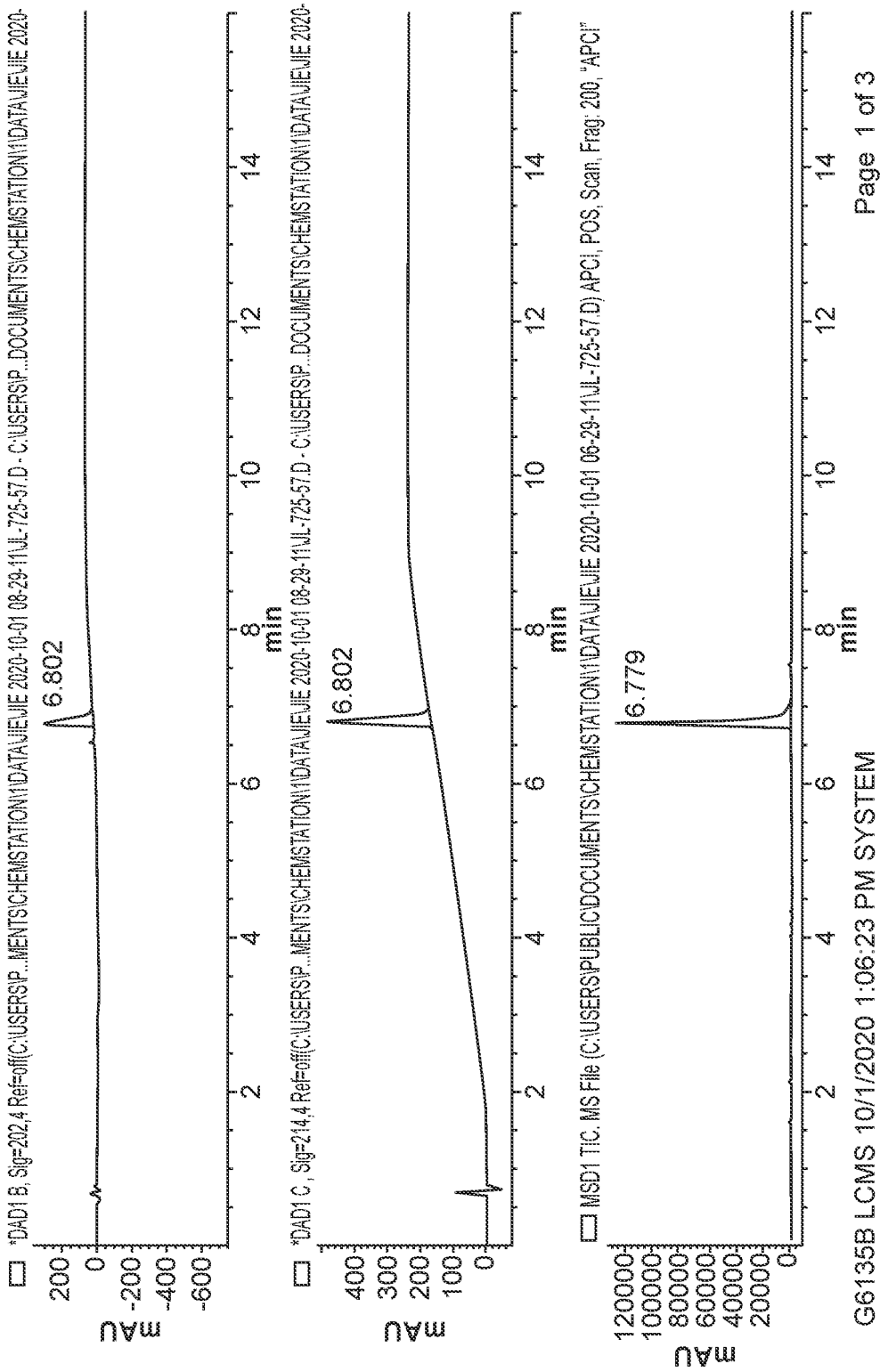
*DAD1 B, Sig=202,4 Ref=off(C:\USERS\P...MENTS\CHEMSTATION\1\DATA\JIEJIE 2020-10-01 08-29-11\JL-725-57.D - C:\USERS\P...DOCUMENTS\CHEMSTATION\1\DATA\JIEJIE 2020-
6.802
mAU
min
*DAD1 C, Sig=214,4 Ref=off(C:\USERS\P...MENTS\CHEMSTATION\1\DATA\JIEJIE 2020-10-01 08-29-11\JL-725-57.D - C:\USERS\P...DOCUMENTS\CHEMSTATION\1\DATA\JIEJIE 2020-
6.802
mAU
min
MSD1 TIC, MS File (C:\USERS\PUBLIC\DOCUMENTS\CHEMSTATION\1\DATA\JIEJIE 2020-10-01 08-29-11\JL-725-57.D) APCI, POS, Scan, Frag: 200, "APCI"
6.779
mAU
min
G6135B LCMS 10/1/2020 1:06:23 PM SYSTEM
Page 1 of 3

FIG. 30B

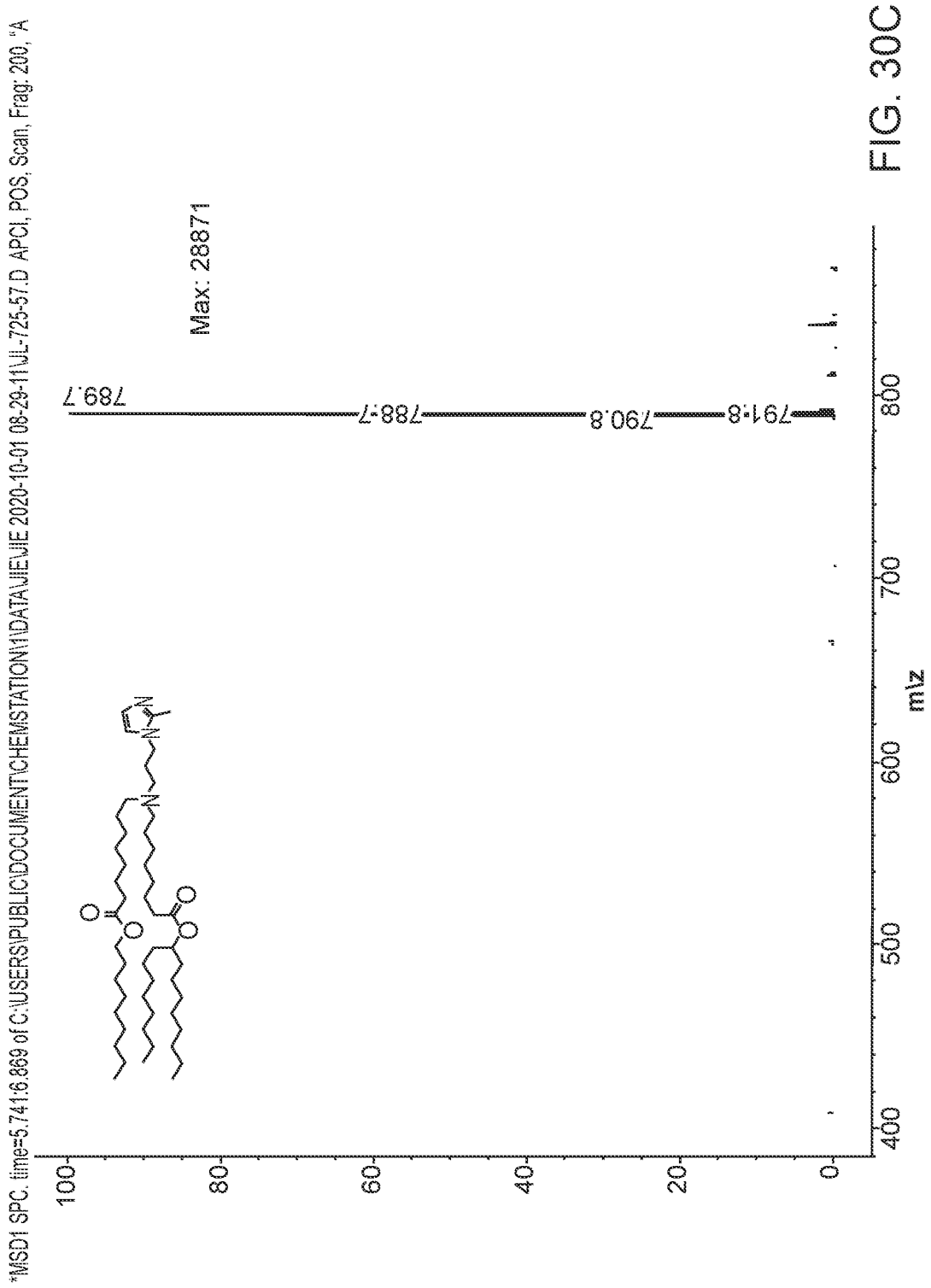
*MSD1 SPC, time=5.741:6.869 of C:\USERS\PUBLIC\DOCUMENTS\CHEMSTATION\1\DATA\JIEWJIE 2020-10-01 08-29-11\JL-725-57.D APCI, POS, Scan, Frag: 200, "A
Max: 28871
789.7
788.7
790.8
791.8
100
80
60
40
20
0
400
500
600
700
800
m/z

FIG. 30C

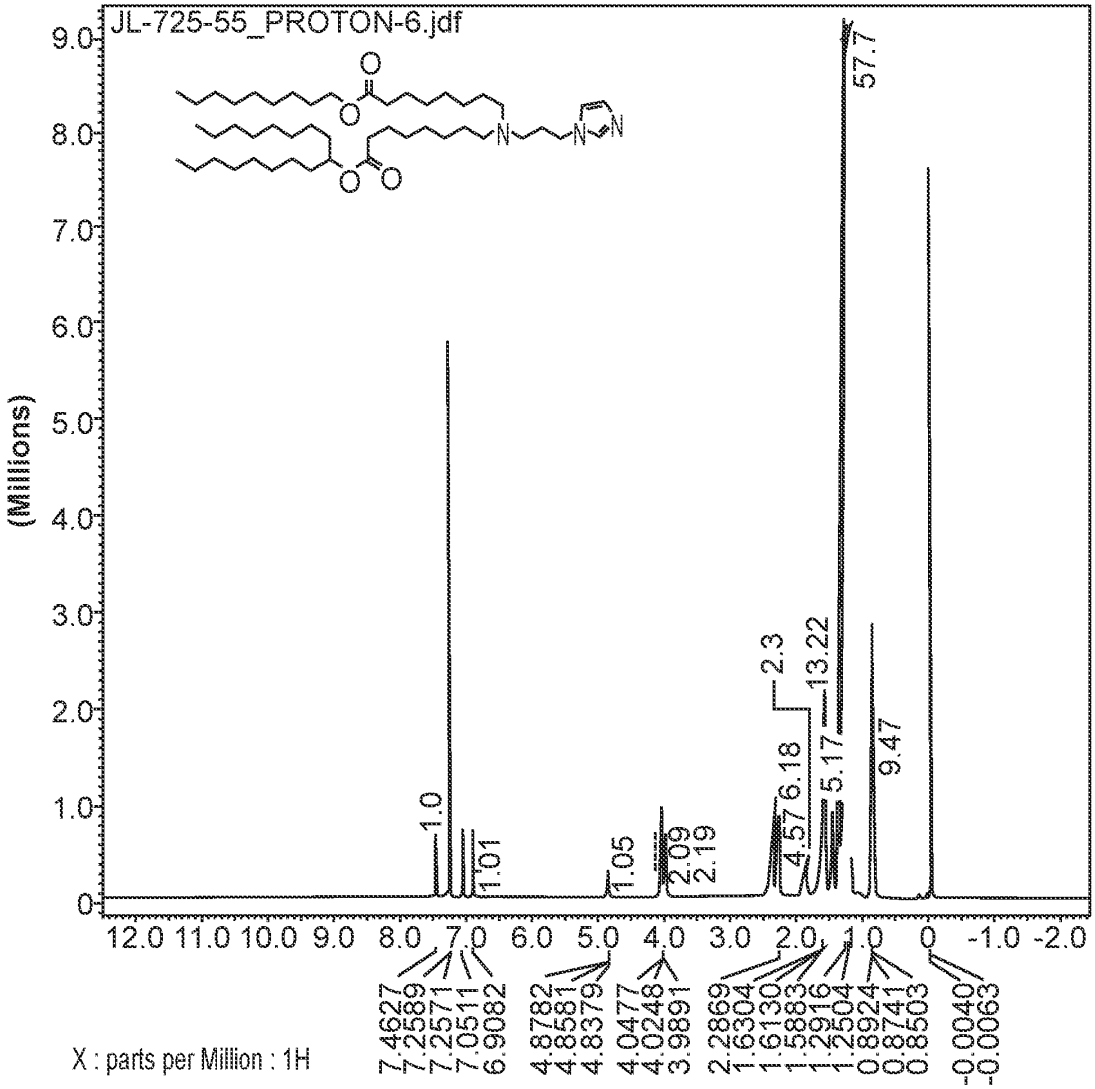
JL-725-55_PROTON-6.jdf
(Millions)
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
1.0
1.01
1.05
2.09
2.19
4.57
6.18
2.3
5.17
13.22
9.47
57.7
12.0 11.0 10.0 9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0 -1.0 -2.0
7.4627
7.2589
7.2571
7.0511
6.9082
4.8782
4.8581
4.8379
4.0477
4.0248
3.9891
2.2869
2.1304
1.6130
1.5883
1.2916
1.2504
0.8924
0.8741
0.8503
-0.0040
-0.0063
X : parts per Million : 1H

FIG. 31A

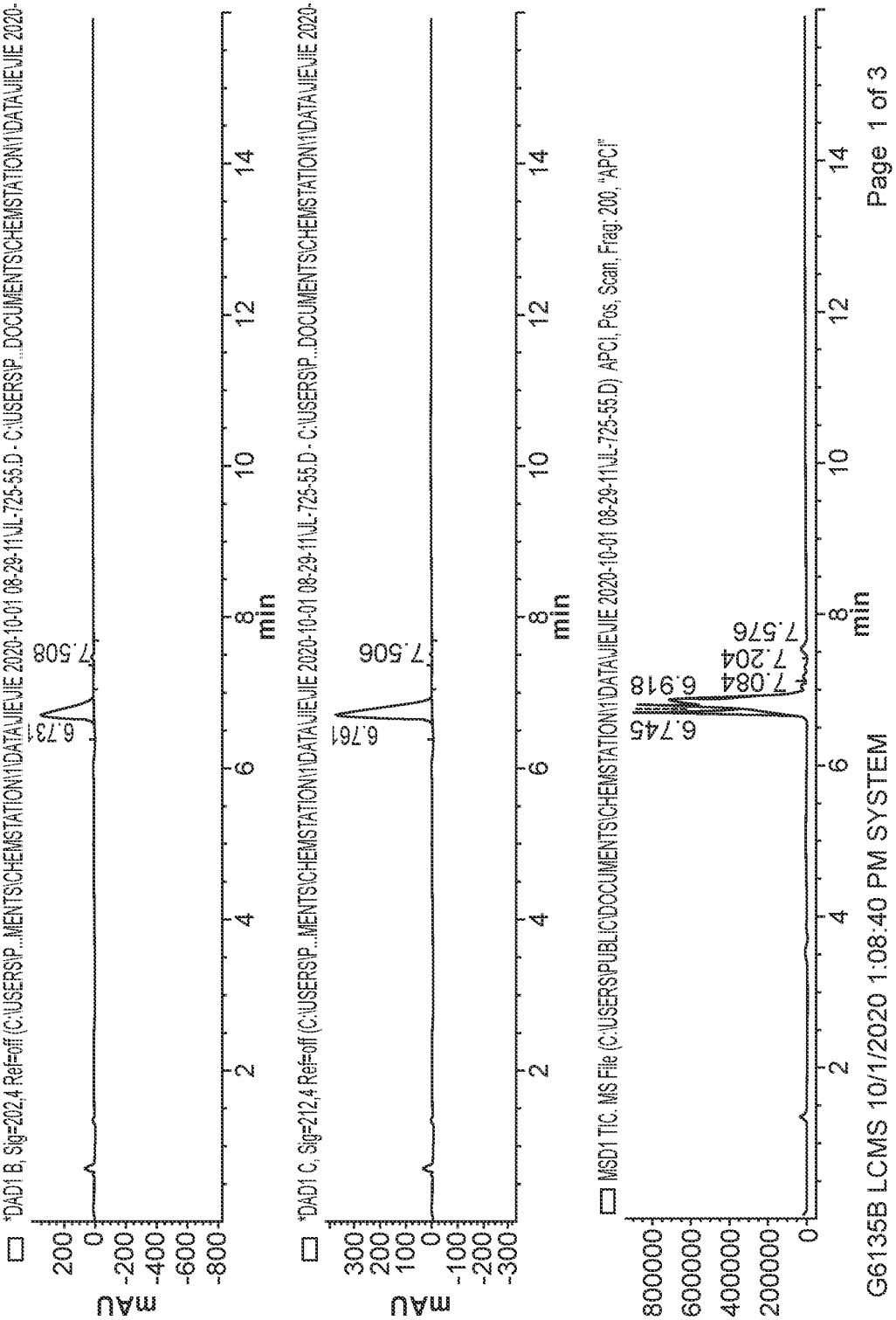
*DAD1 B, Sig=202,4 Ref=off (C:\USERS\P...MENTS\CHEMSTATION\1\DATA\JIEJIE 2020-10-01 08-29-11\JL-725-55.D - C:\USERS\P...DOCUMENTS\CHEMSTATION\1\DATA\JIEJIE 2020-
mAU
6.731
7.508
min
*DAD1 C, Sig=212,4 Ref=off (C:\USERS\P...MENTS\CHEMSTATION\1\DATA\JIEJIE 2020-10-01 08-29-11\JL-725-55.D - C:\USERS\P...DOCUMENTS\CHEMSTATION\1\DATA\JIEJIE 2020-
mAU
6.761
7.506
min
MSD1 TIC, MS File (C:\USERS\PUBLIC\DOCUMENTS\CHEMSTATION\1\DATA\JIEJIE 2020-10-01 08-29-11\JL-725-55.D) APCI, Pos, Scan, Frag: 200, "APCI"
6.745
6.918
7.084
7.204
7.576
min
G6135B LCMS 10/1/2020 1:08:40 PM SYSTEM
Page 1 of 3

FIG. 31B

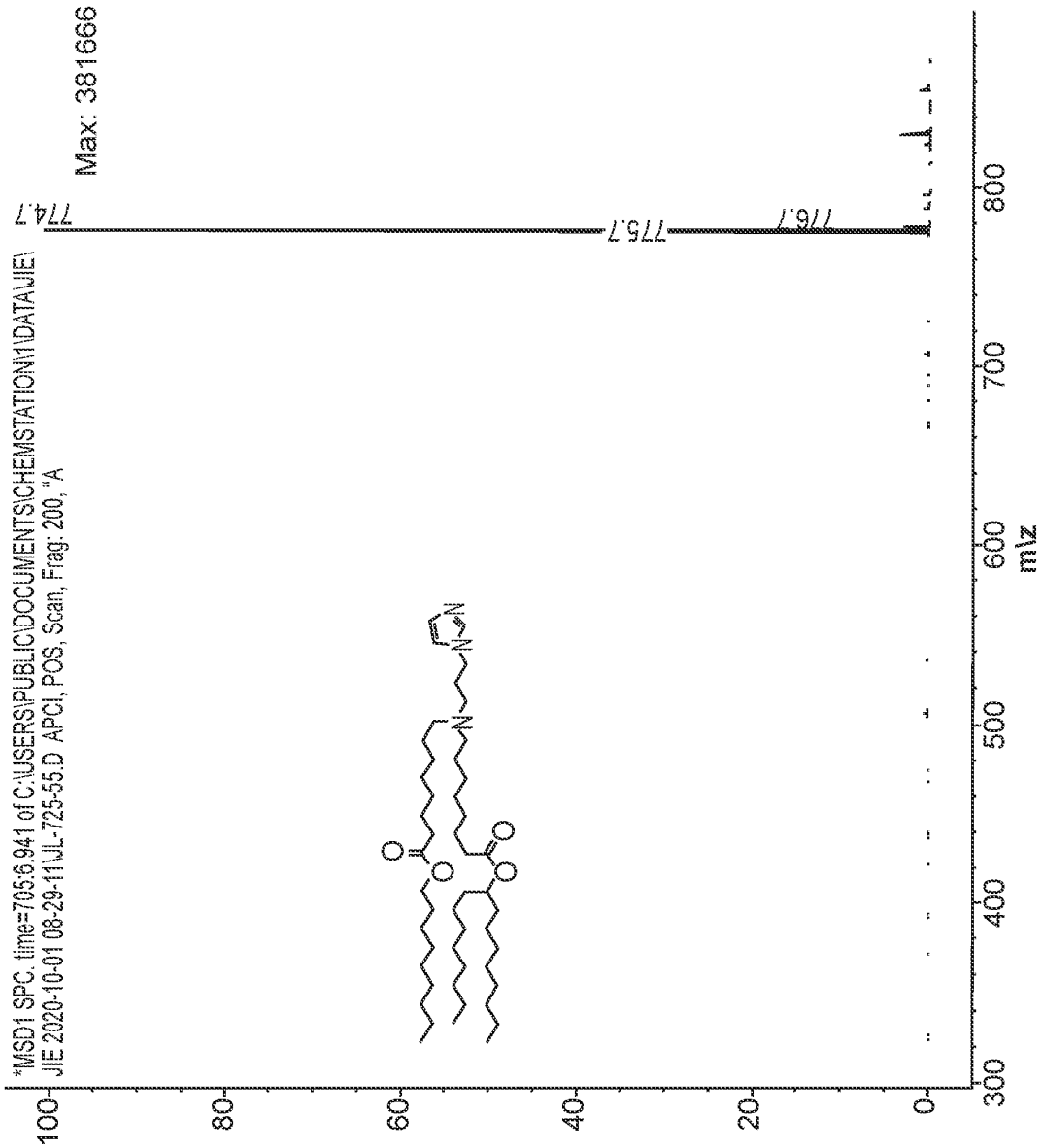
*MSD1 SPC, time=705:6.941 of C:\USERS\PUBLIC\DOCUMENTS\CHEMSTATION\1\DATA\JIE\
JIE 2020-10-01 08-29-11\JL-725-55.D APCI, POS, Scan, Frag: 200, "A
Max: 381666
774.7
775.7
776.7
100
80
60
40
20
0
300
400
500
600
700
800
m/z

FIG. 31C

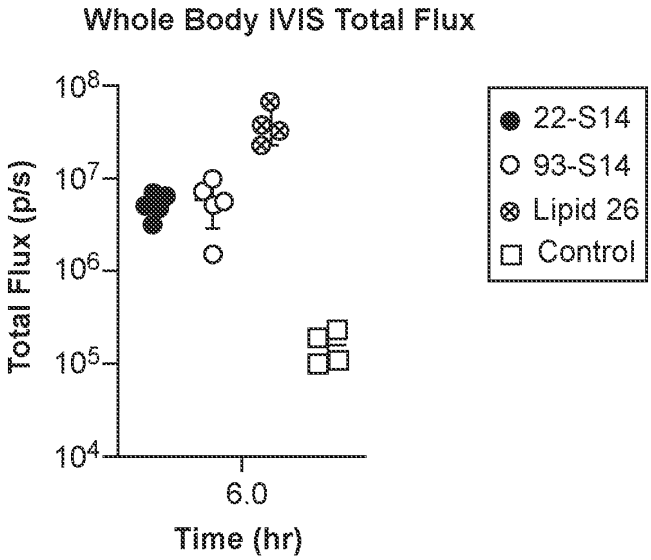

FIG. 32A

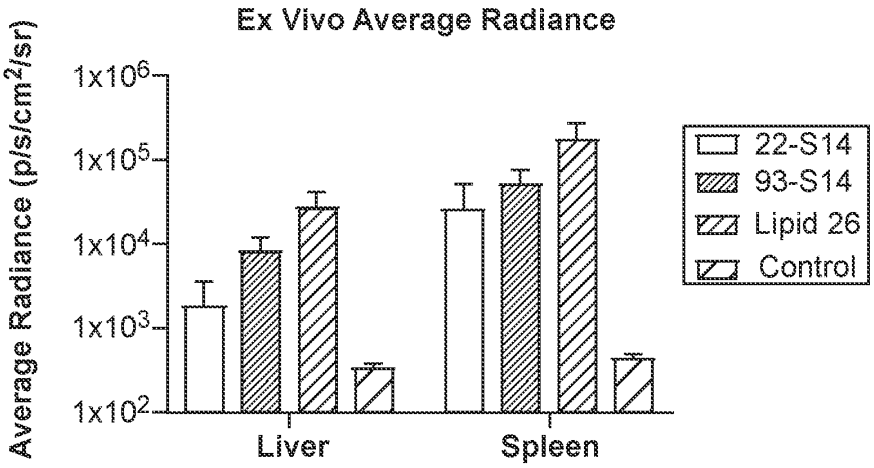

FIG. 32B

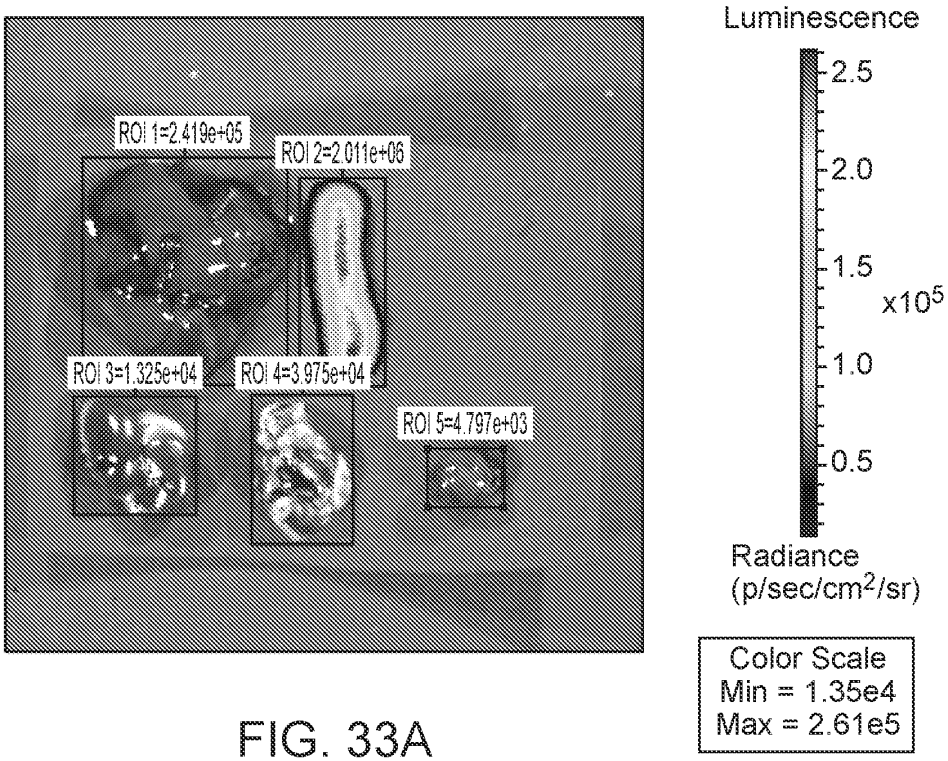

FIG. 33A

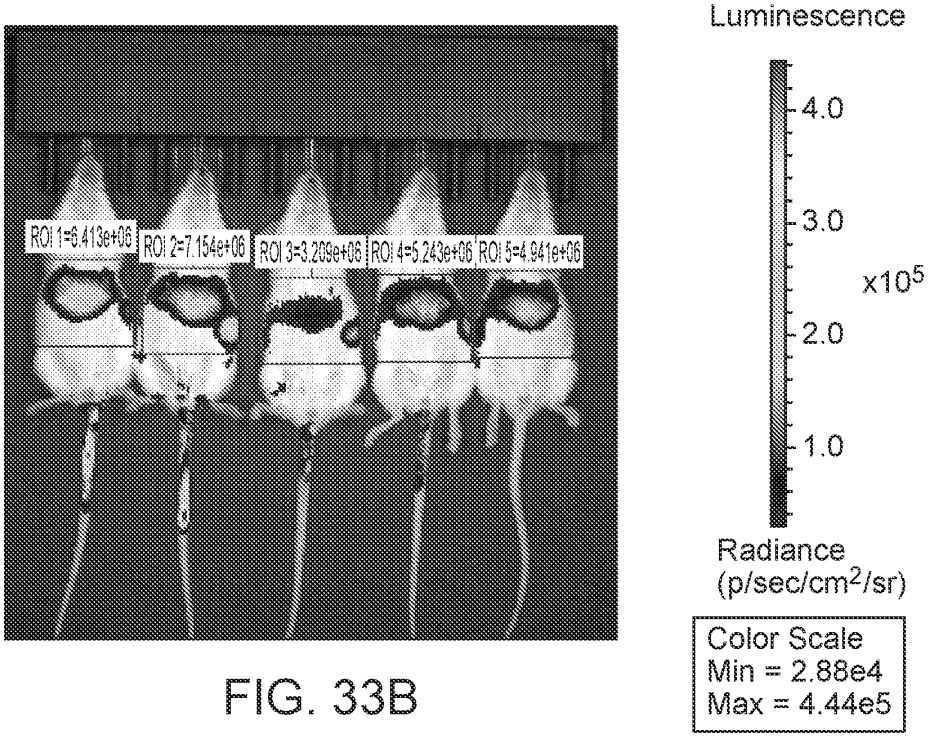

FIG. 33B

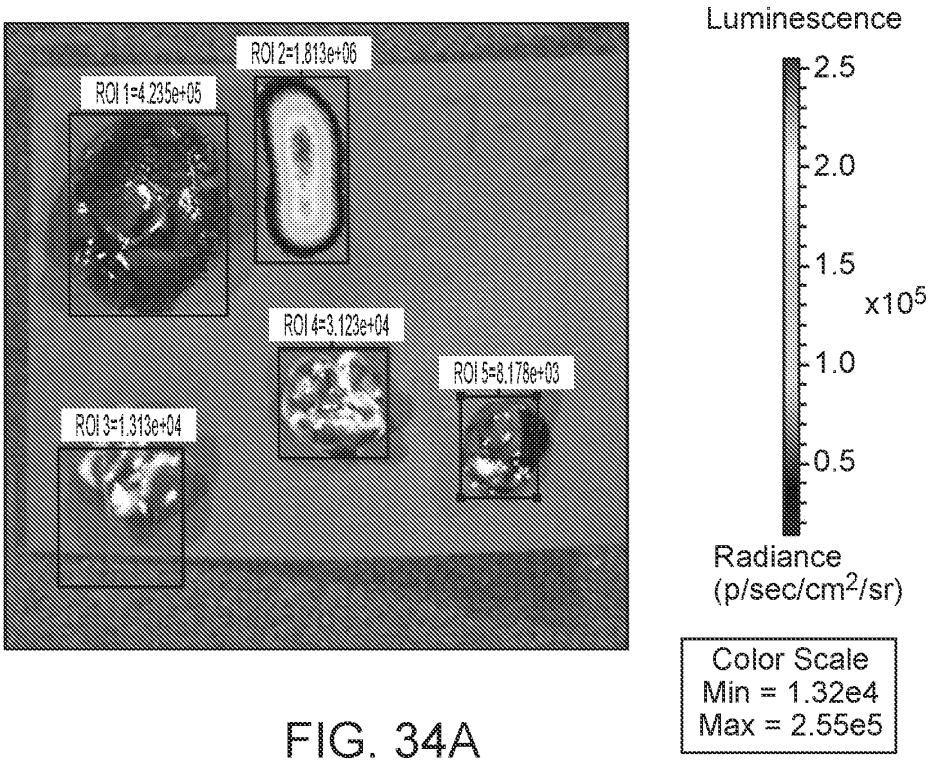

FIG. 34A

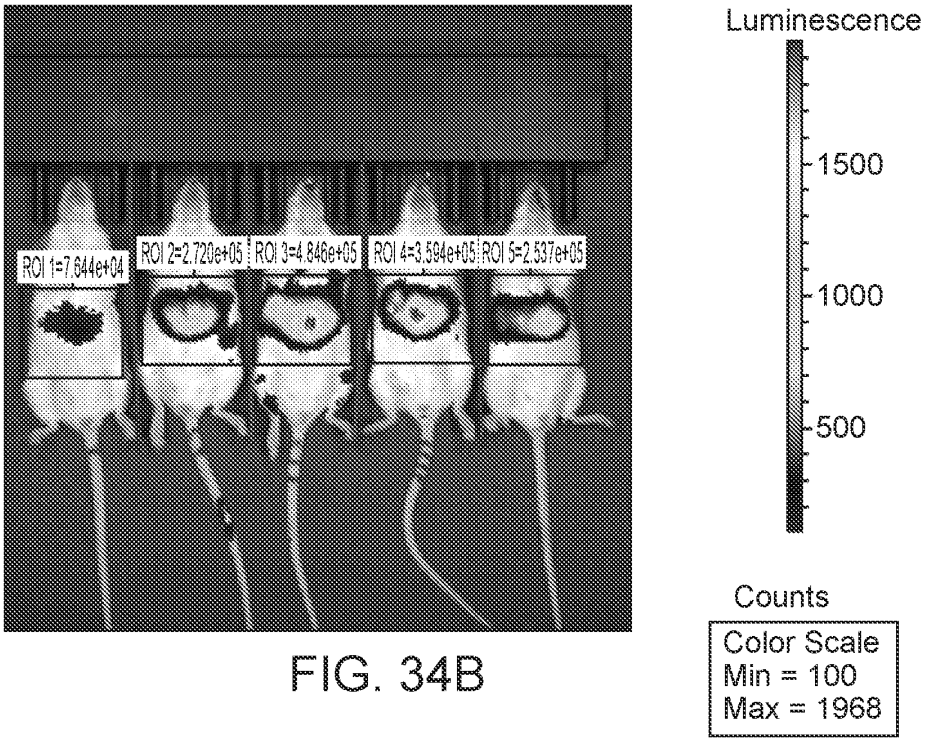

FIG. 34B

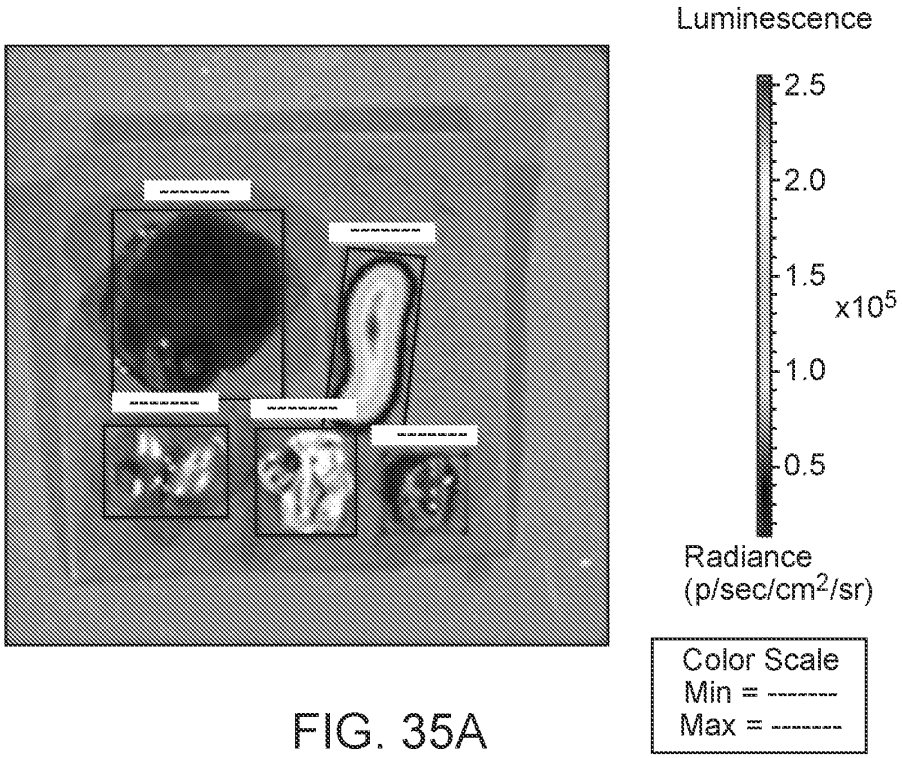

FIG. 35A

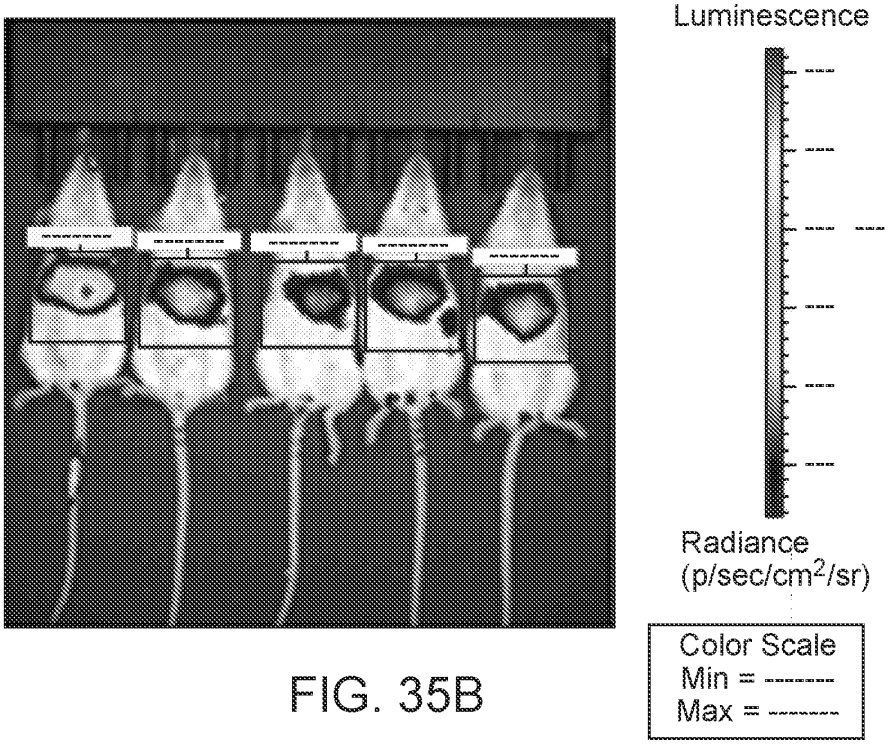

FIG. 35B

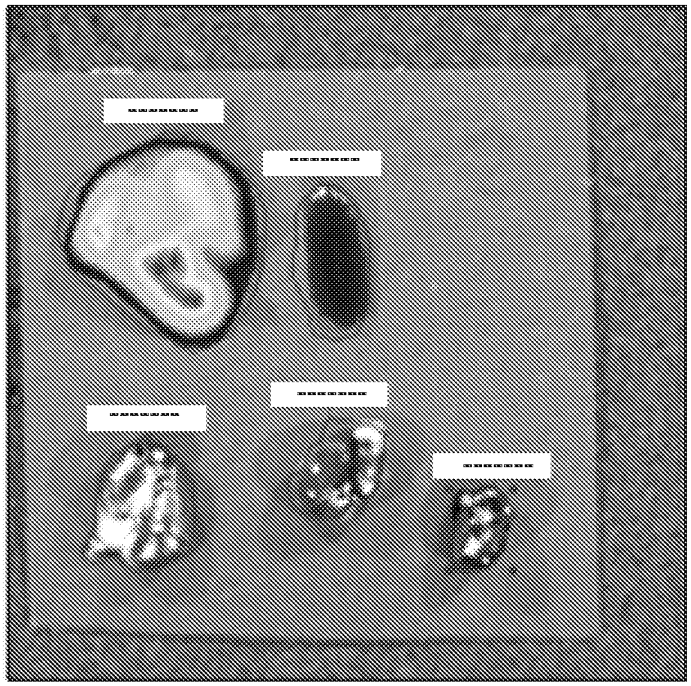

FIG. 36A

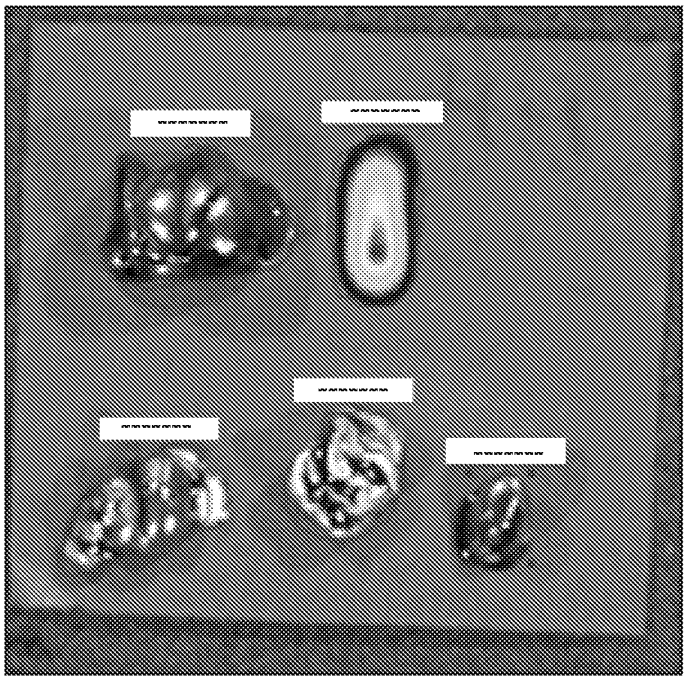

FIG. 36B

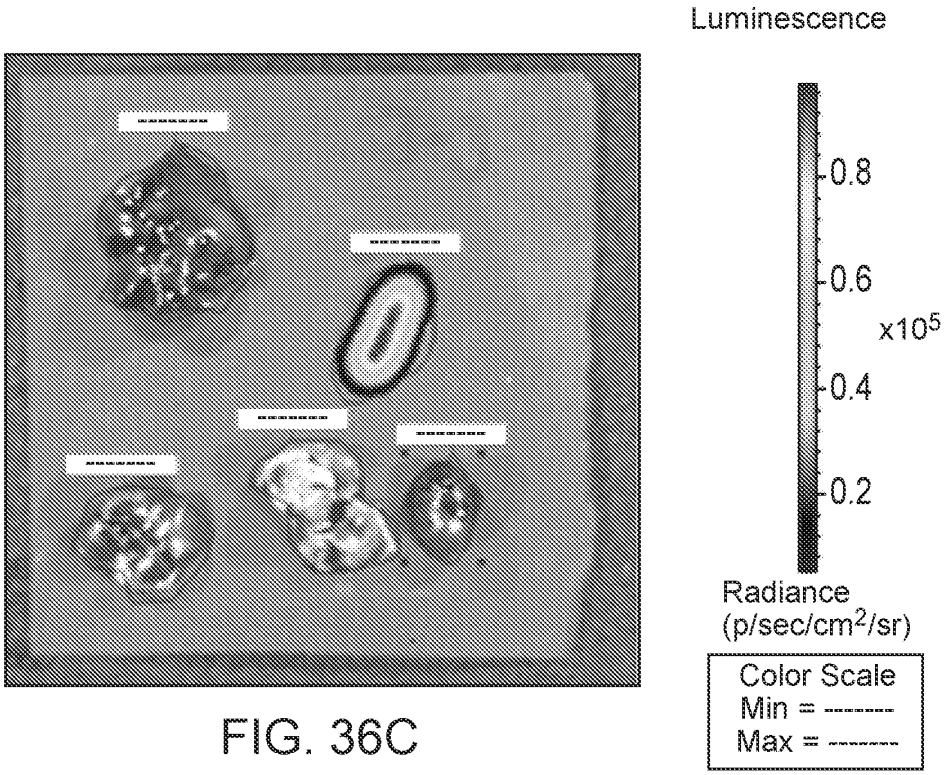

FIG. 36C

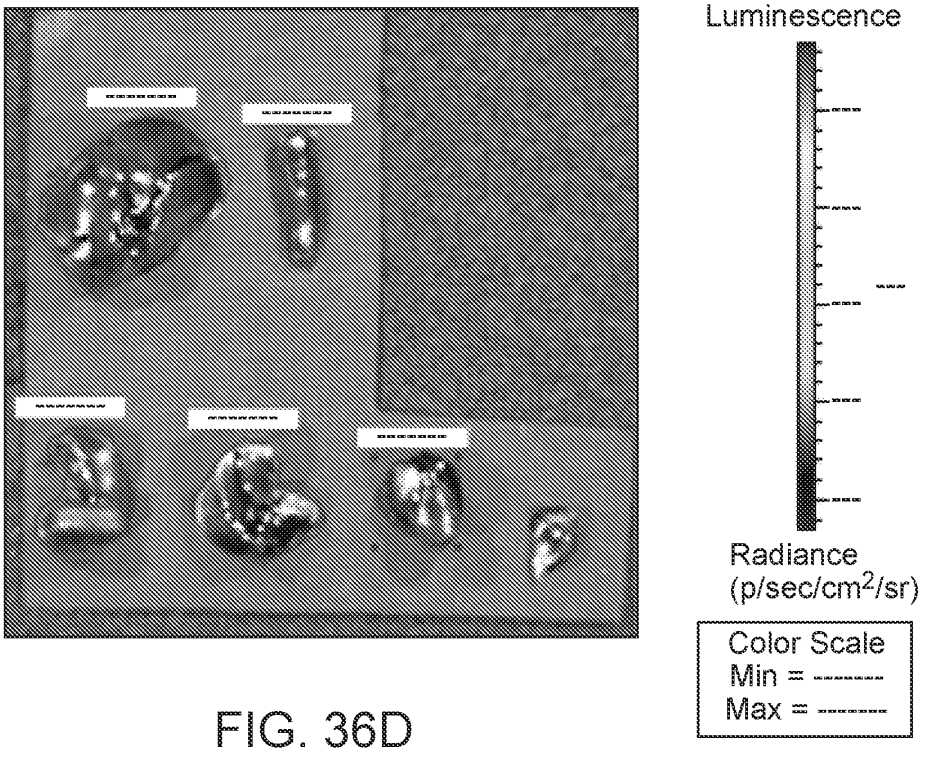

FIG. 36D

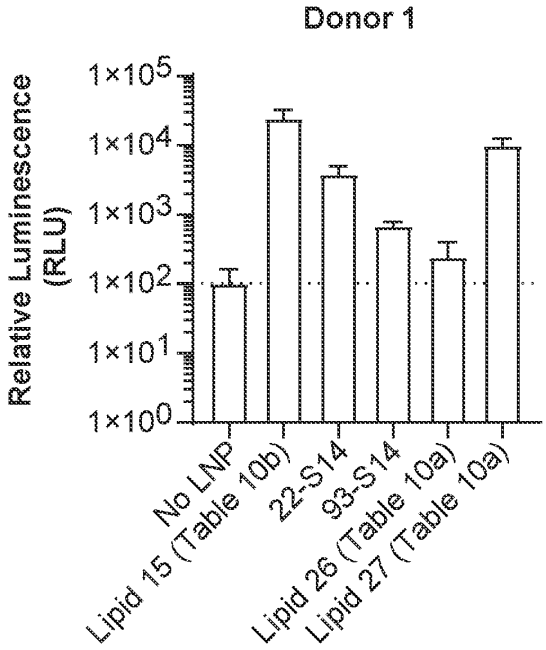

FIG. 37A

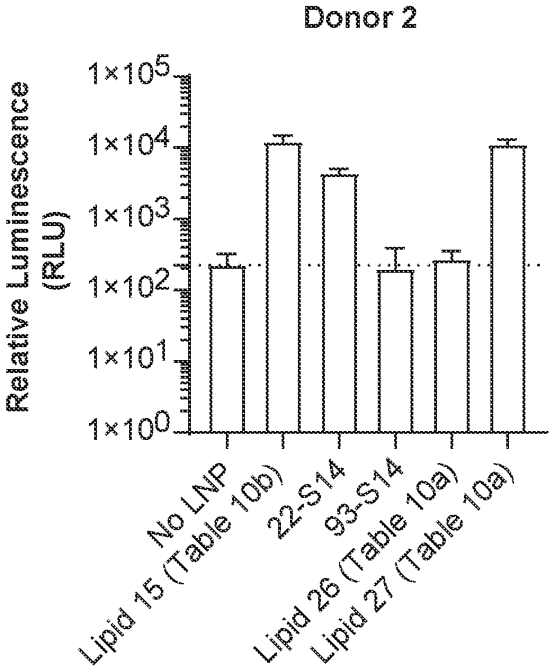

FIG. 37B

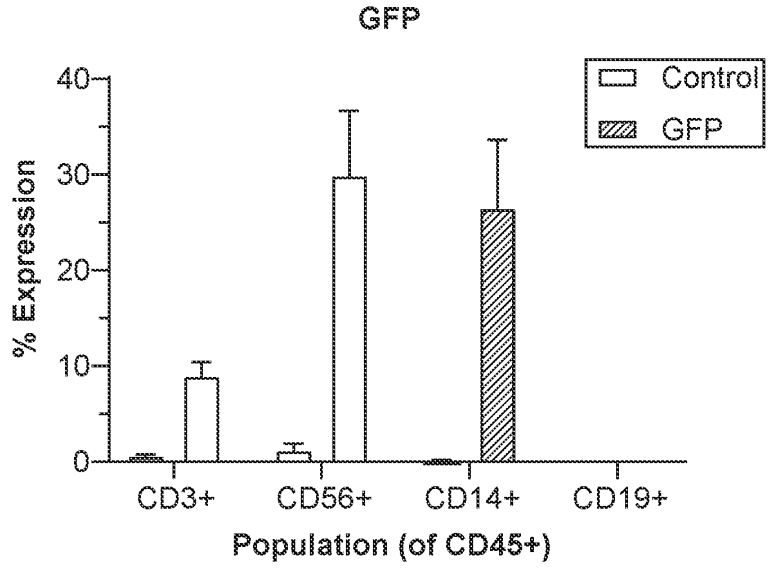

FIG. 38A

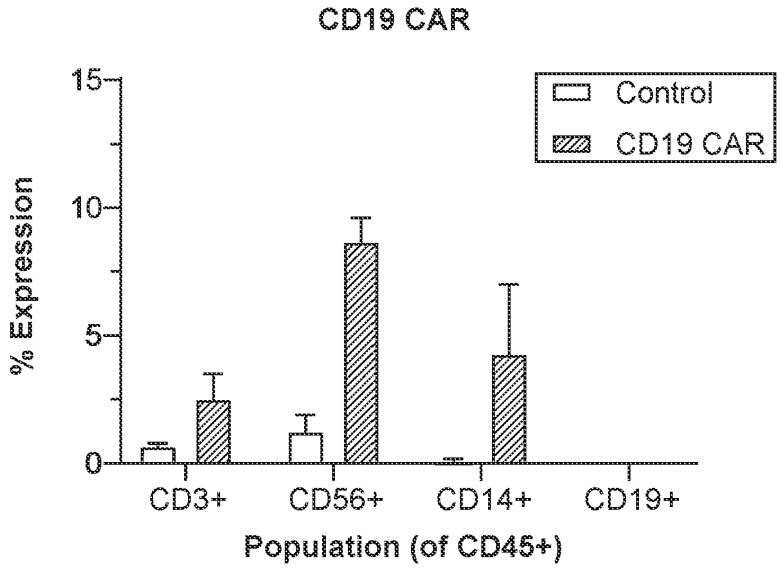

FIG. 38B

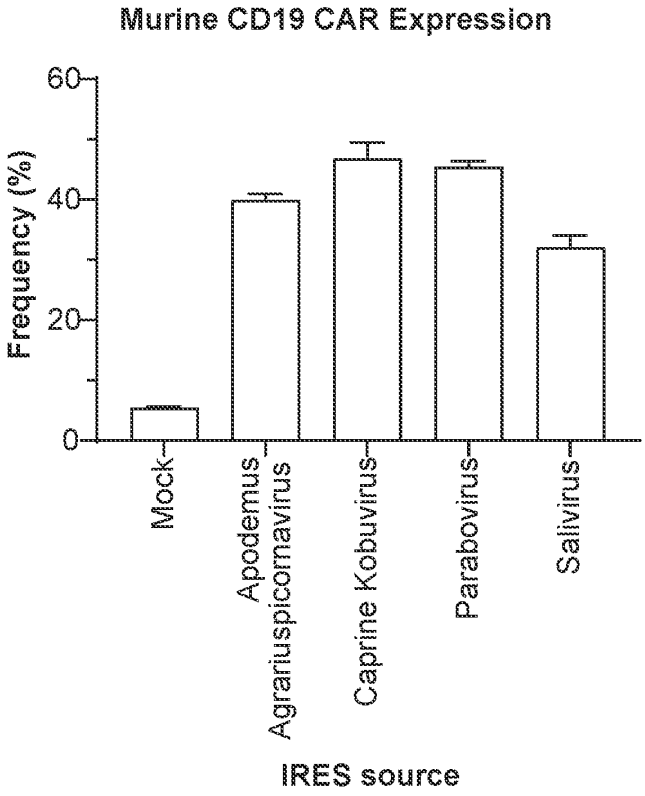
Murine CD19 CAR Expression
Frequency (%)
60
40
20
0
Mock
Apodemus Agrariuspicornavirus
Caprine Kobuvirus
Parabovirus
Salivirus
IRES source

FIG. 39

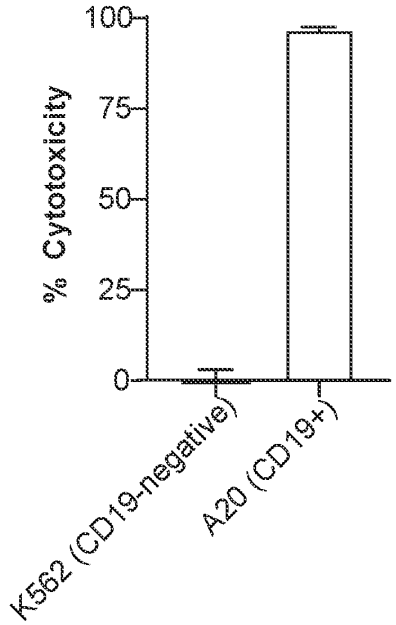
% Cytotoxicity
100
75
50
25
0
K562 (CD19-negative)
A20 (CD19+)

FIG. 40

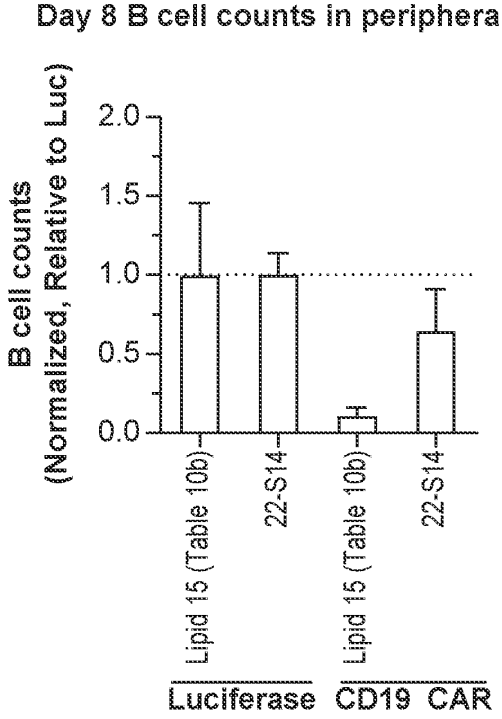

FIG. 41A

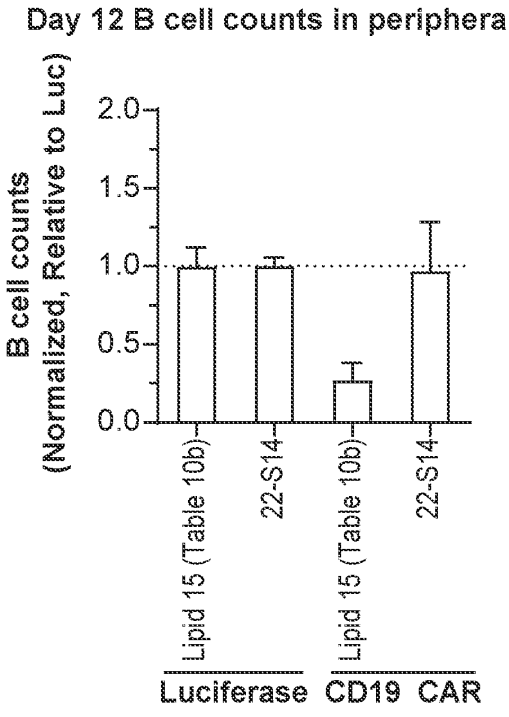

FIG. 41B

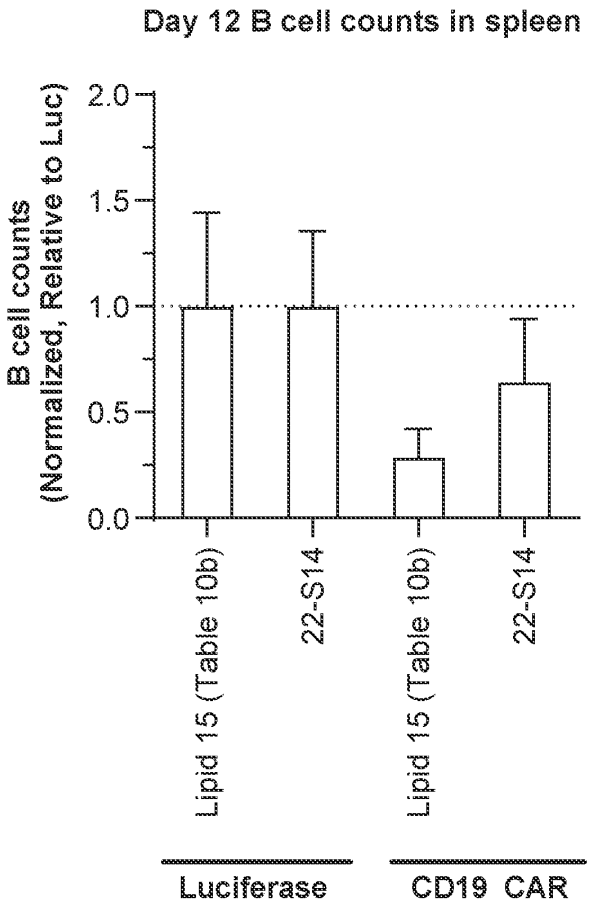
Day 12 B cell counts in spleen
B cell counts
(Normalized, Relative to Luc)
2.0
1.5
1.0
0.5
0.0
Lipid 15 (Table 10b)
22-S14
Lipid 15 (Table 10b)
22-S14
Luciferase
CD19 CAR

FIG. 41C

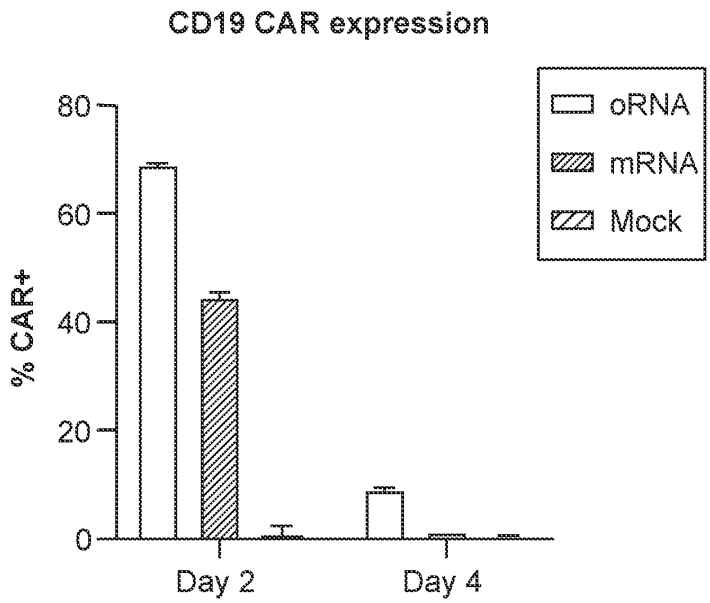

FIG. 42A

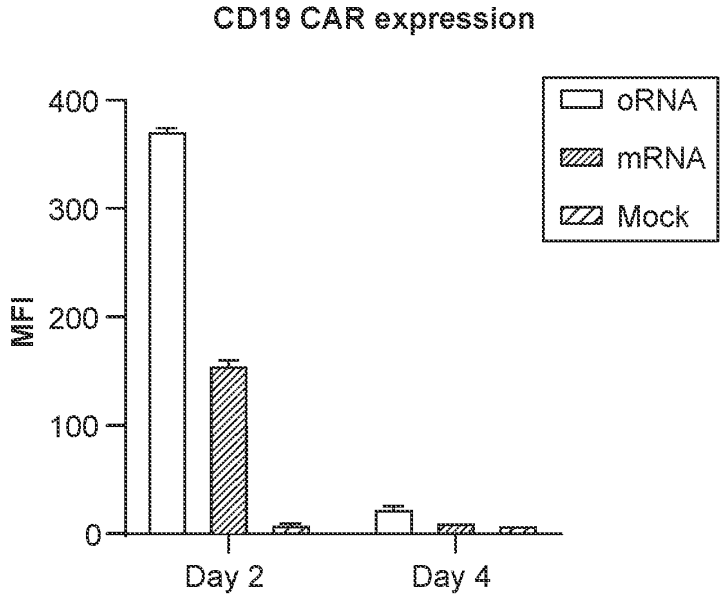

FIG. 42B

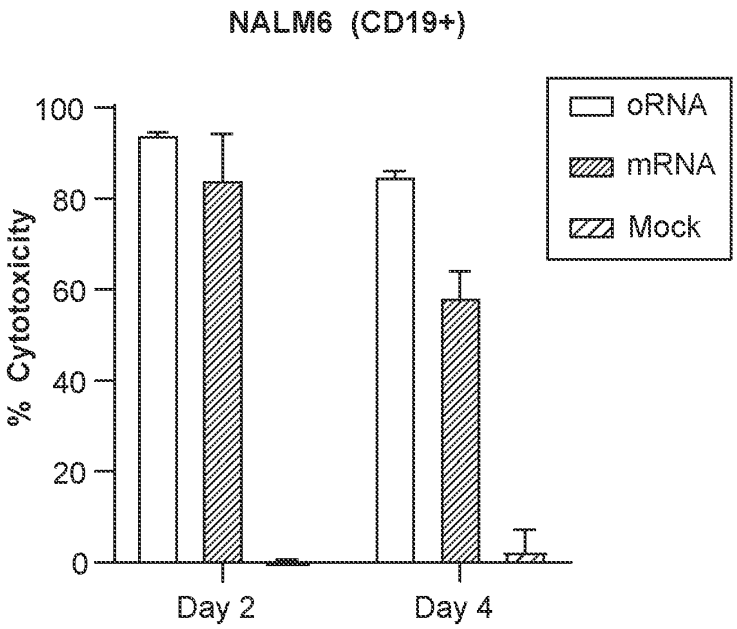

FIG. 43A

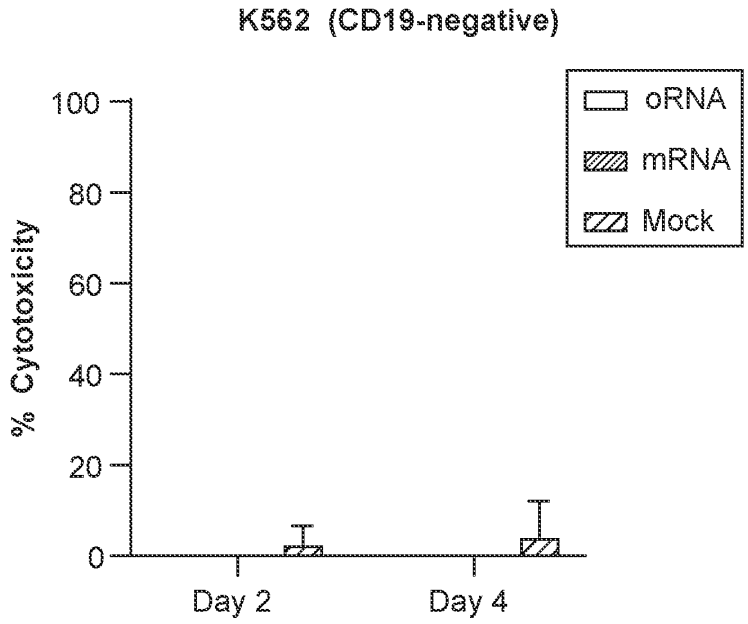

FIG. 43B

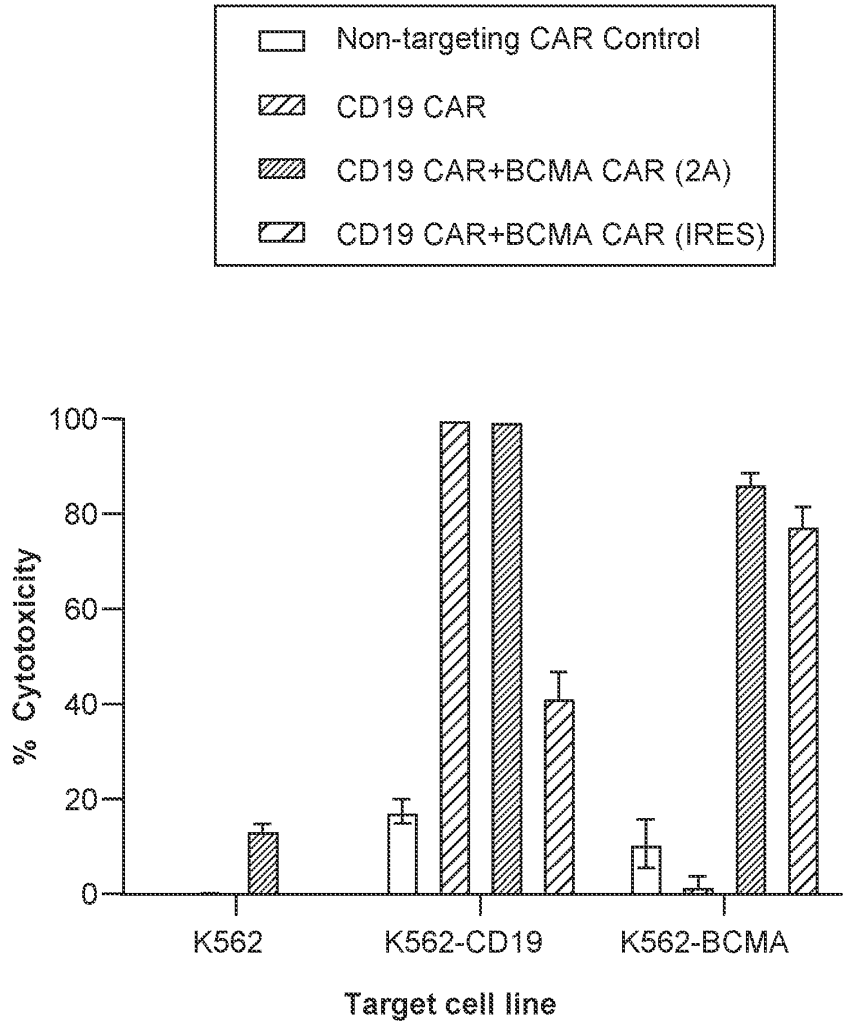
Non-targeting CAR Control
CD19 CAR
CD19 CAR+BCMA CAR (2A)
CD19 CAR+BCMA CAR (IRES)
% Cytotoxicity
100
80
60
40
20
0
K562
K562-CD19
K562-BCMA
Target cell line

FIG. 44

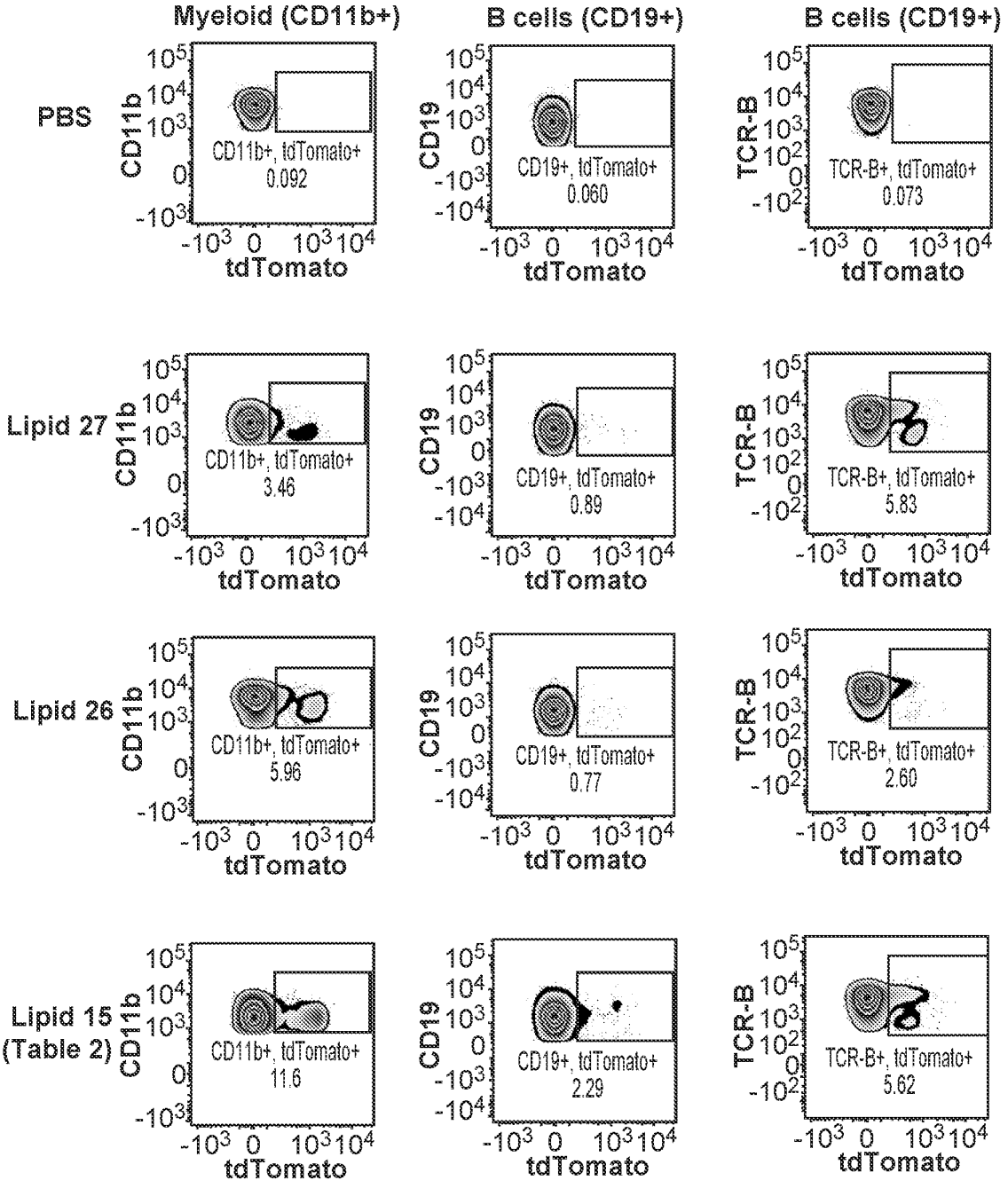
Myeloid (CD11b+)
B cells (CD19+)
B cells (CD19+)
PBS
Lipid 27
Lipid 26
Lipid 15
(Table 2)
CD11b
CD19
TCR-B
tdTomato
CD11b+, tdTomato+
0.092
CD19+, tdTomato+
0.060
TCR-B+, tdTomato+
0.073
CD11b+, tdTomato+
3.46
CD19+, tdTomato+
0.89
TCR-B+, tdTomato+
5.83
CD11b+, tdTomato+
5.96
CD19+, tdTomato+
0.77
TCR-B+, tdTomato+
2.60
CD11b+, tdTomato+
11.6
CD19+, tdTomato+
2.29
TCR-B+, tdTomato+
5.62

FIG. 45A

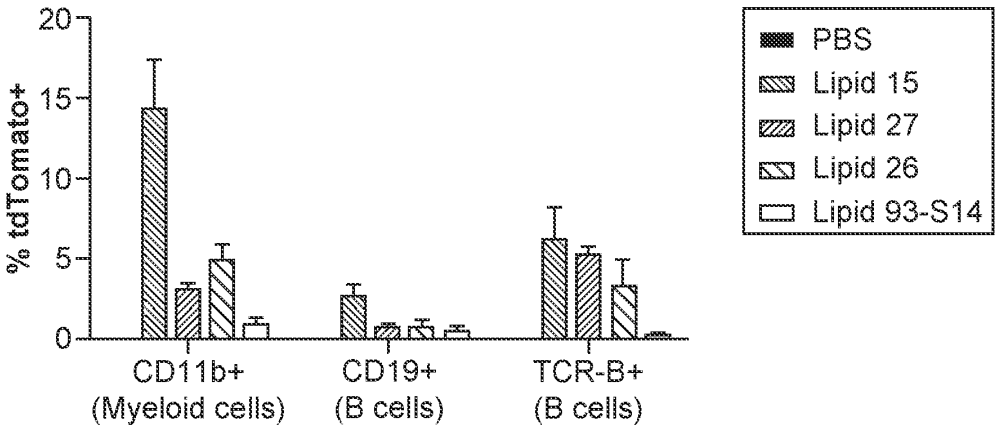

FIG. 45B

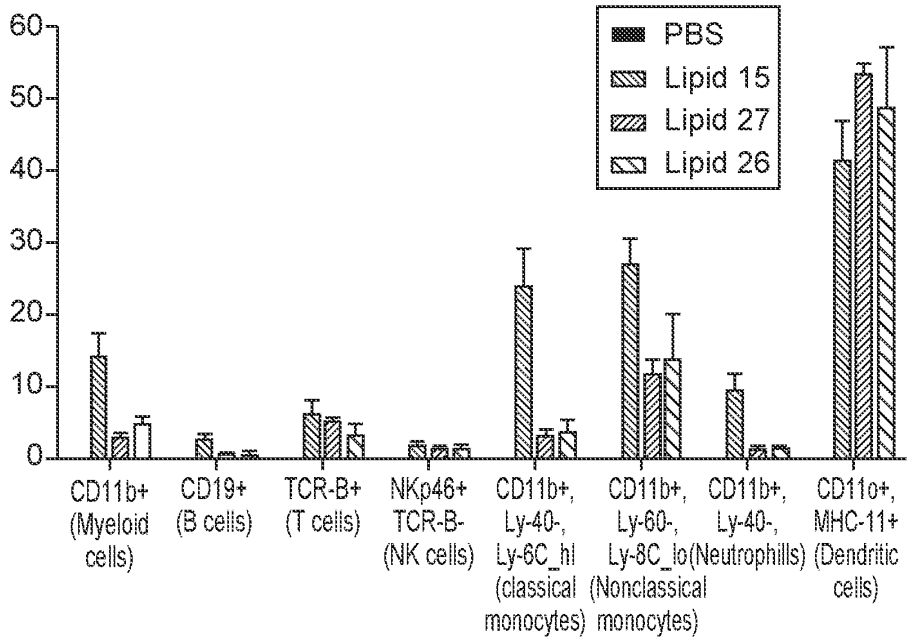

FIG. 45C

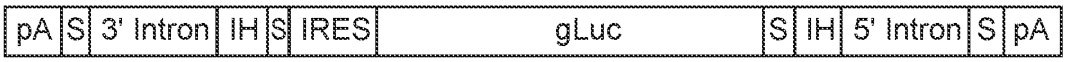

FIG. 46A

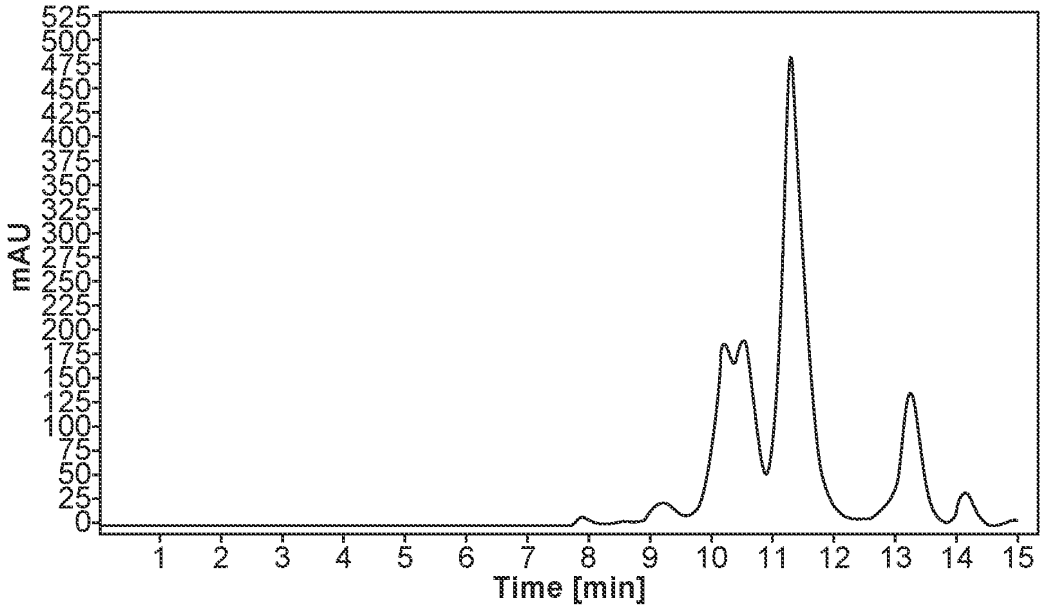

FIG. 46B

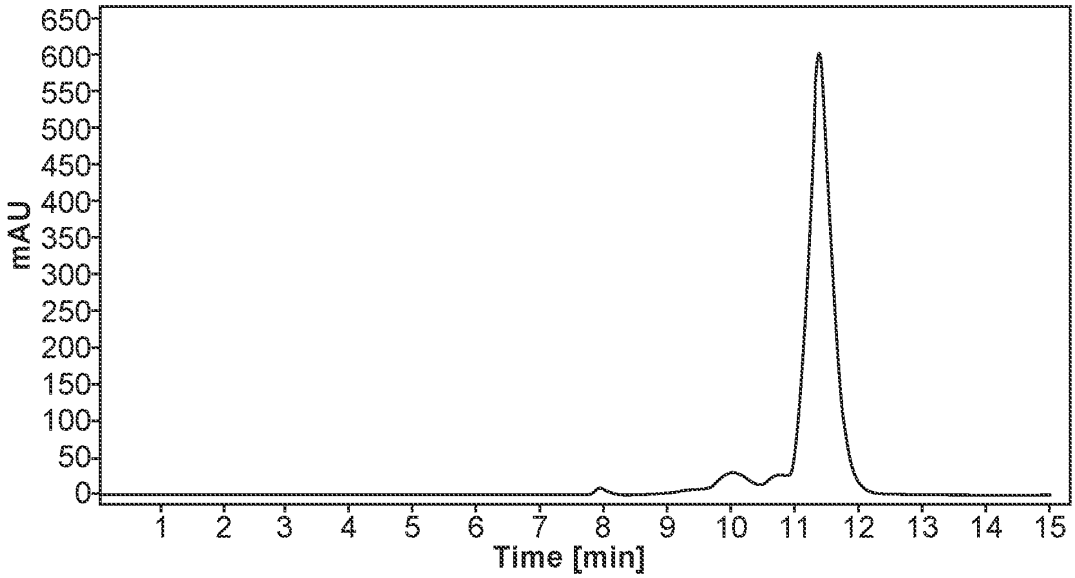

FIG. 46C

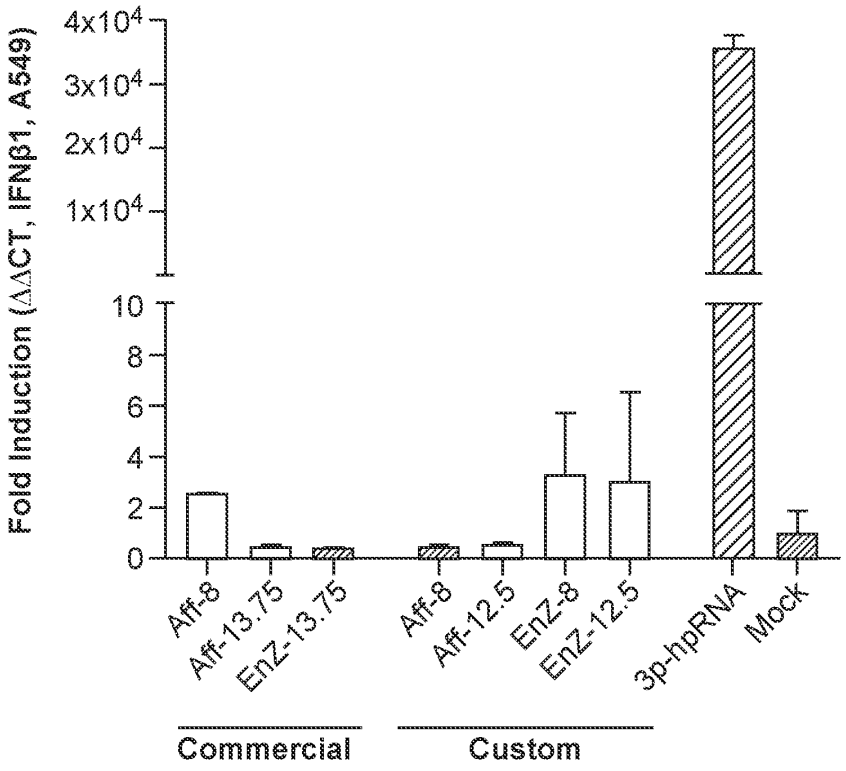
Fold Induction (ΔΔCT, IFNβ1, A549)
4x10^4
3x10^4
2x10^4
1x10^4
10
8
6
4
2
0
Aff-8
Aff-13.75
EnZ-13.75
Aff-8
Aff-12.5
EnZ-8
EnZ-12.5
3p-hpRNA
Mock
Commercial
Custom

FIG. 46D

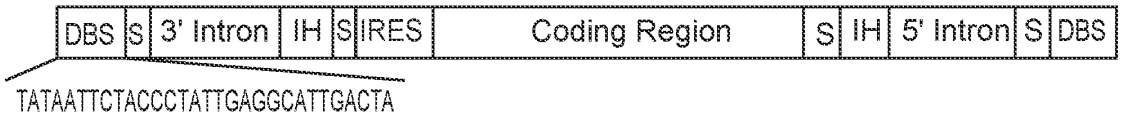

FIG. 47A

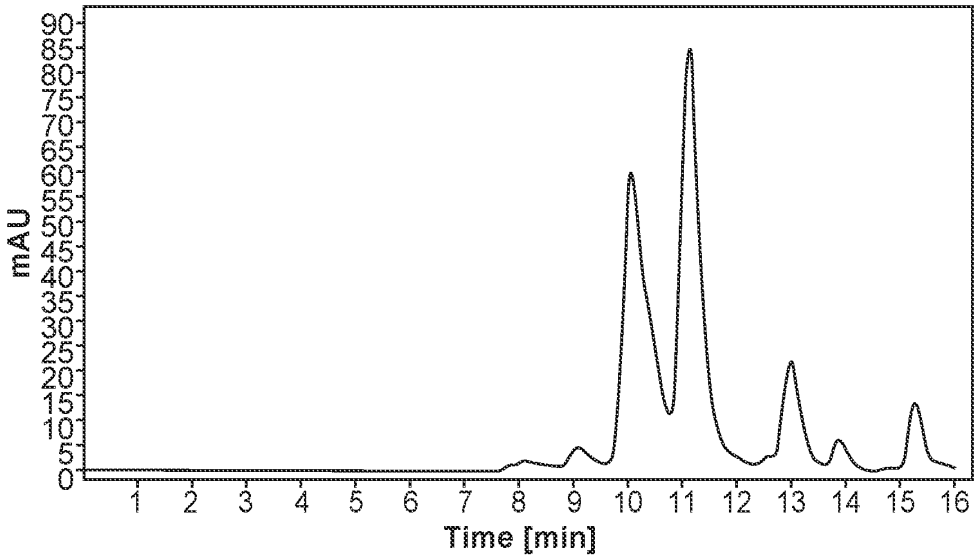

FIG. 47B

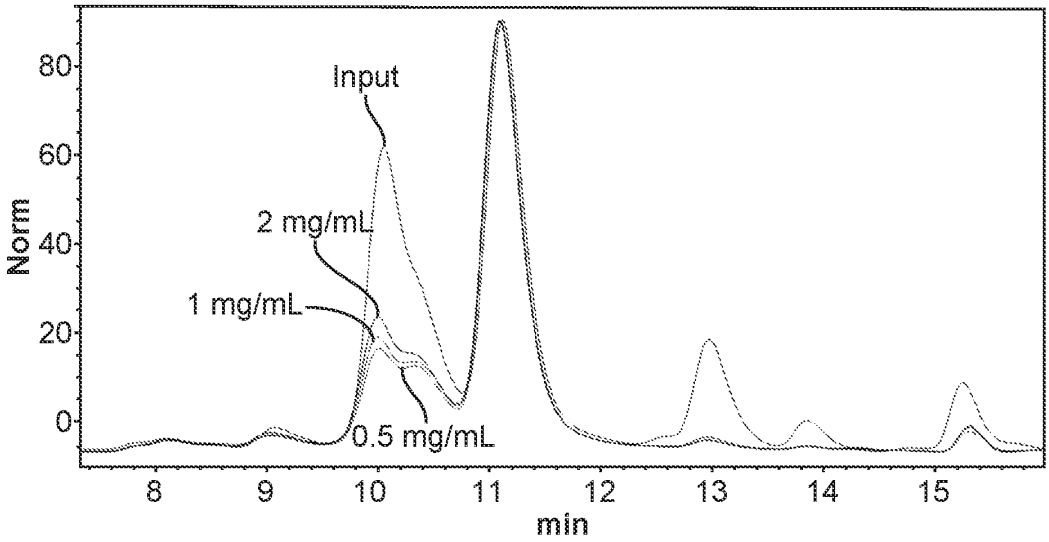

FIG. 47C

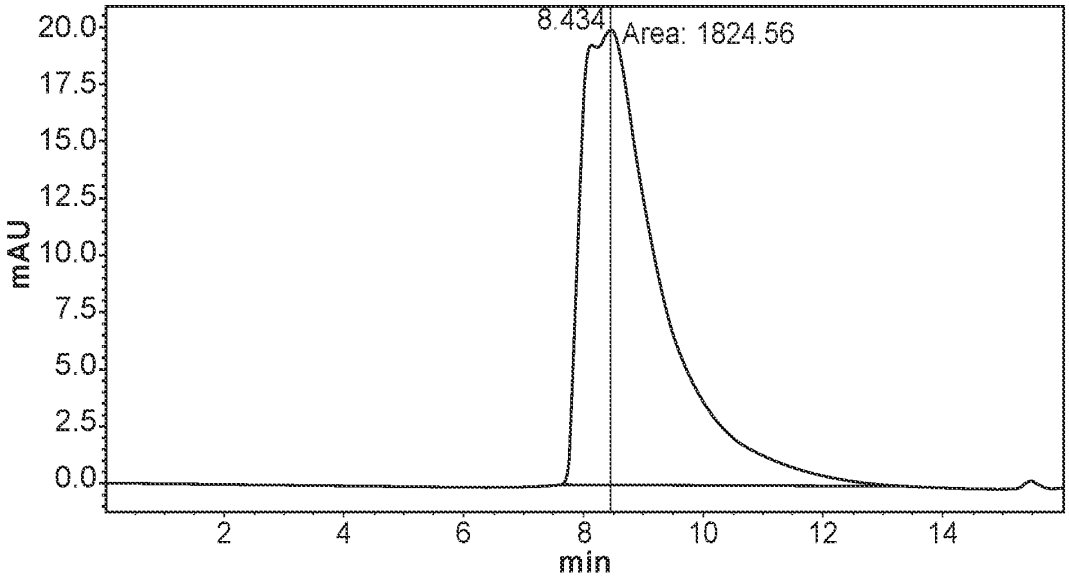

FIG. 48A

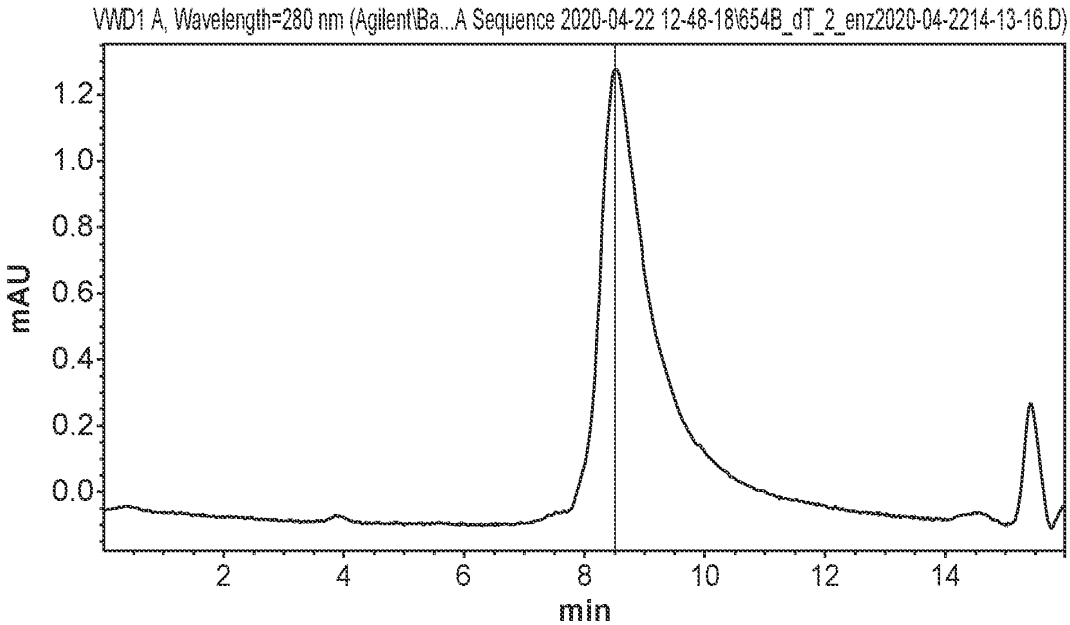

FIG. 48B

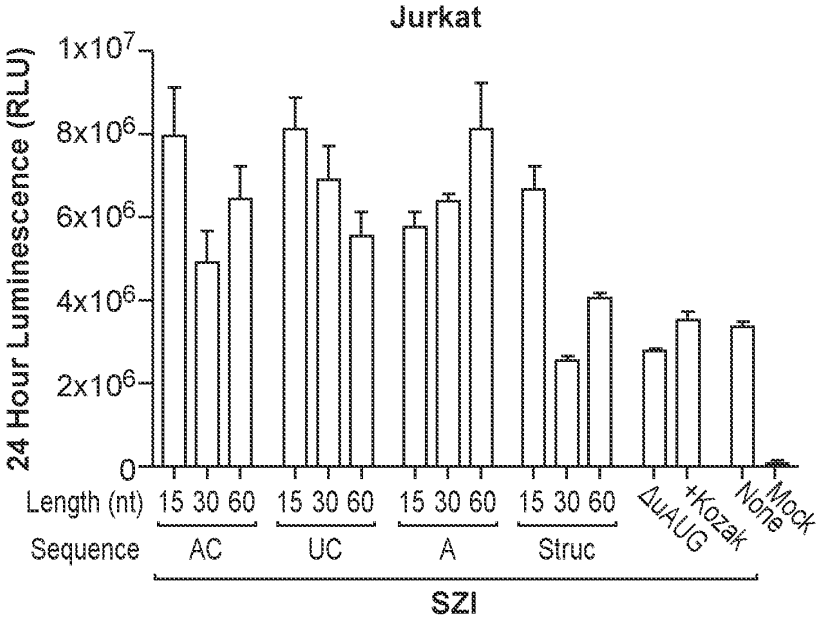

FIG. 49A

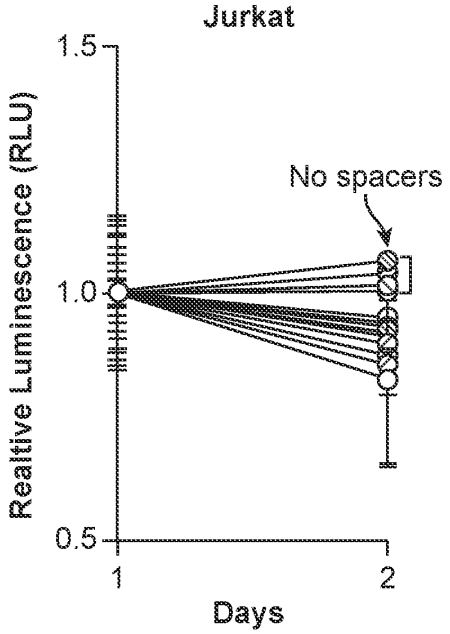

FIG. 49B

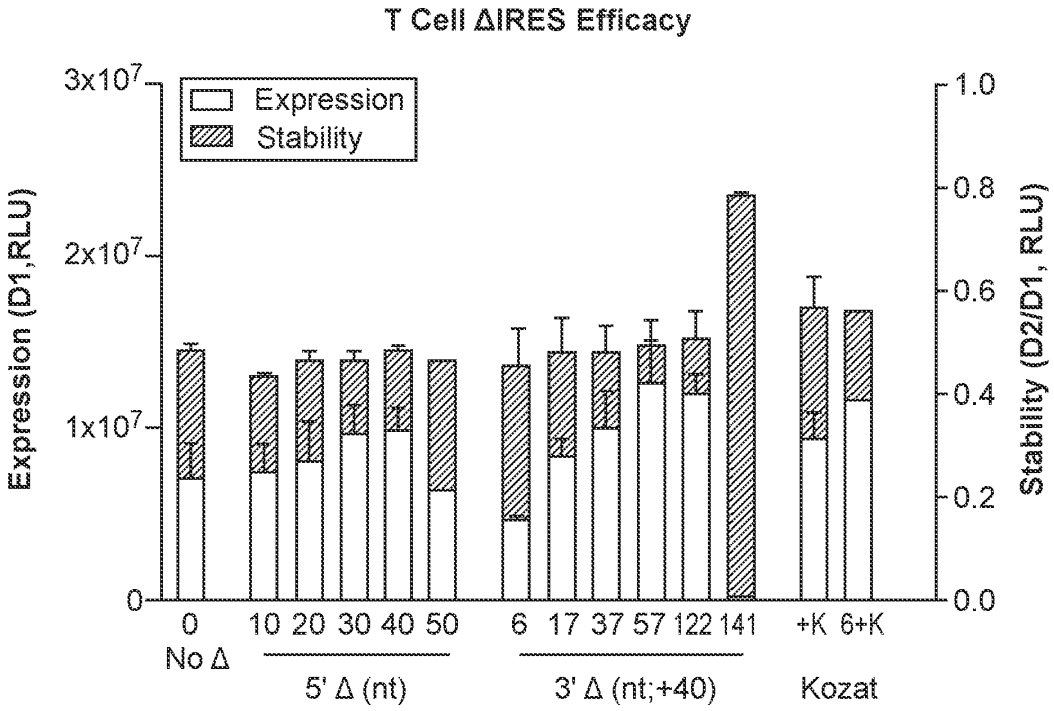
T Cell ΔIRES Efficacy
Expression
Stability
Expression (D1,RLU)
Stability (D2/D1, RLU)
3x10^7
2x10^7
1x10^7
0
1.0
0.8
0.6
0.4
0.2
0.0
0
10 20 30 40 50
6 17 37 57 122 141
+K 6+K
No Δ
5' Δ (nt)
3' Δ (nt;+40)
Kozat

FIG. 50

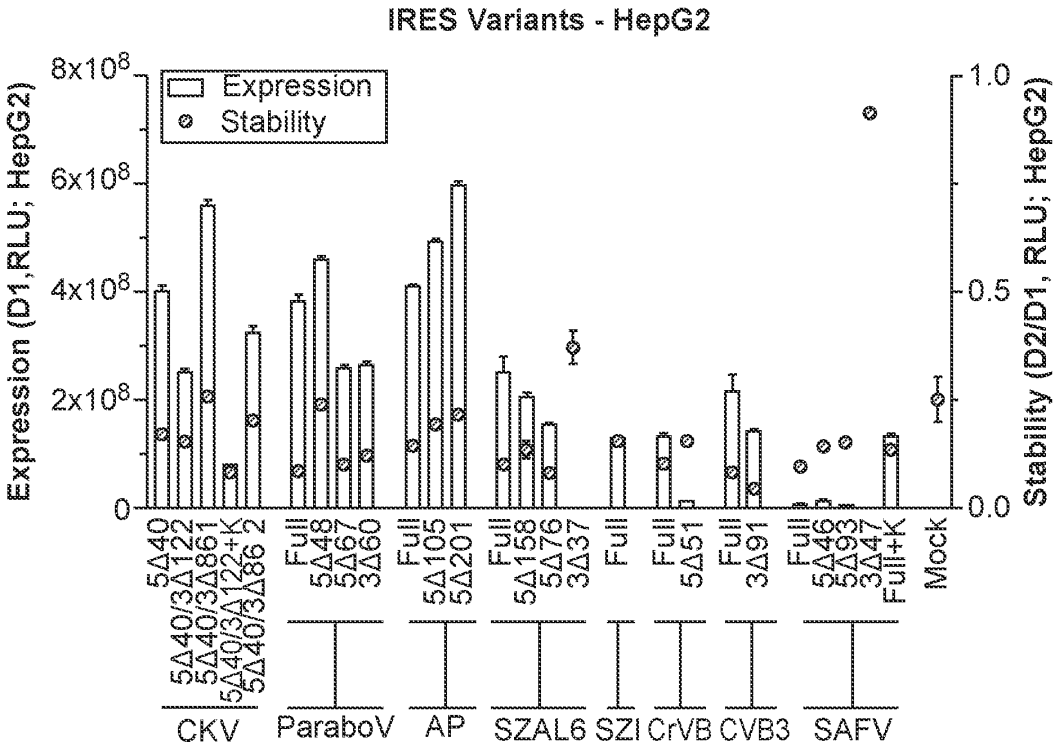
IRES Variants - HepG2
Expression
Stability
Expression (D1,RLU; HepG2)
Stability (D2/D1, RLU; HepG2)
8x10^8
6x10^8
4x10^8
2x10^8
0
1.0
0.5
0.0
5Δ40
5Δ40/3Δ122
5Δ40/3Δ861
5Δ40/3Δ122+K
5Δ40/3Δ86 2
Full
5Δ48
5Δ67
3Δ60
Full
5Δ105
5Δ201
Full
5Δ158
5Δ76
3Δ37
Full
Full
5Δ51
Full
3Δ91
Full
5Δ46
5Δ93
3Δ47
Full+K
Mock
CKV
ParaboV
AP
SZAL6
SZI
CrVB
CVB3
SAFV

FIG. 51

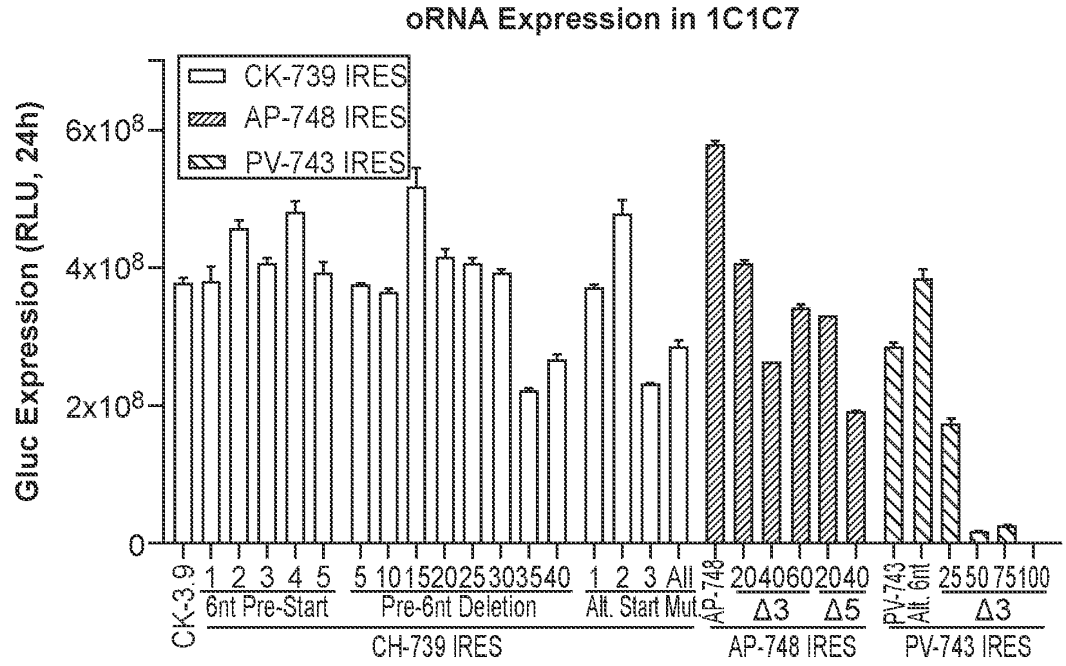
oRNA Expression in 1C1C7
CK-739 IRES
AP-748 IRES
PV-743 IRES
Gluc Expression (RLU, 24h)
6x10^8
4x10^8
2x10^8
0
CK-3.9
1 2 3 4 5
6nt Pre-Start
5 10 15 20 25 30 35 40
Pre-6nt Deletion
1 2 3 All
Alt. Start Mut
AP-748
20 40 60
Δ3
20 40
Δ5
PV-743
Alt. 6nt
25 50 75 100
Δ3
CH-739 IRES
AP-748 IRES
PV-743 IRES

FIG. 52

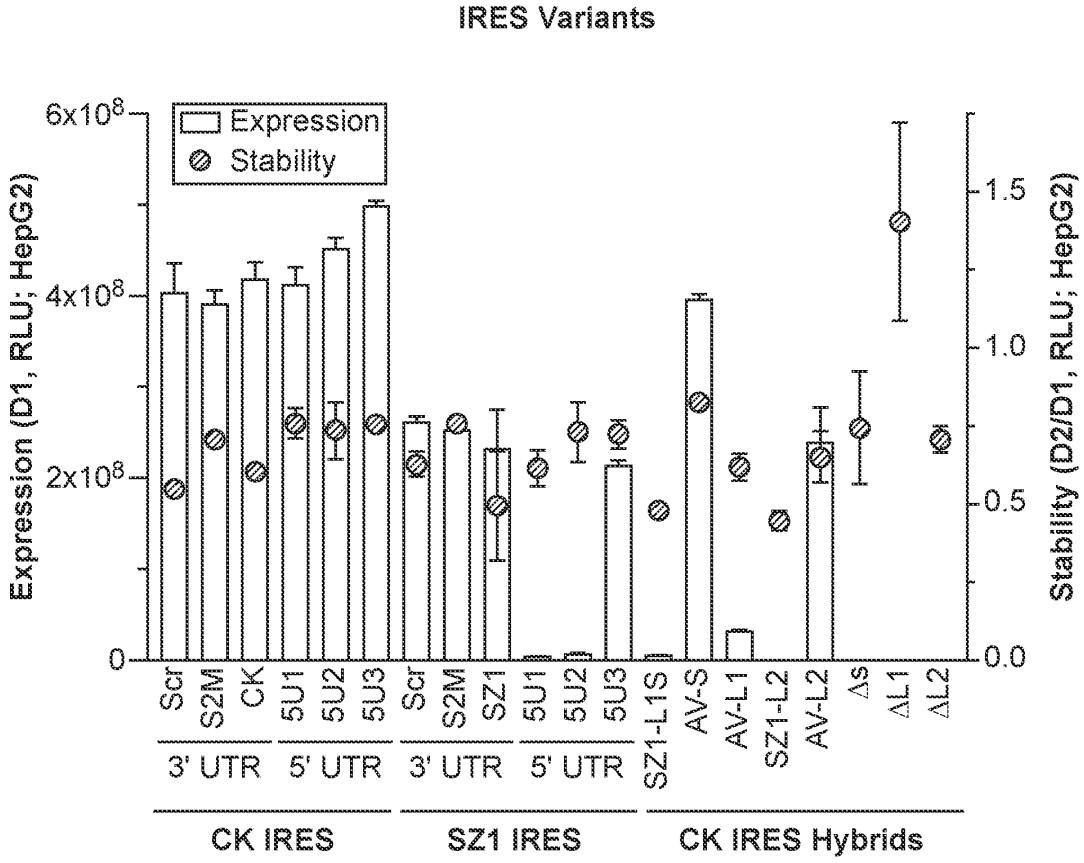
IRES Variants
Expression
Stability
Expression (D1, RLU; HepG2)
Stability (D2/D1, RLU; HepG2)
6x10^8
4x10^8
2x10^8
0
1.5
1.0
0.5
0.0
Scr
S2M
CK
5U1
5U2
5U3
Scr
S2M
SZ1
5U1
5U2
5U3
SZ1-L1S
AV-S
AV-L1
SZ1-L2
AV-L2
Δs
ΔL1
ΔL2
3' UTR
5' UTR
3' UTR
5' UTR
CK IRES
SZ1 IRES
CK IRES Hybrids

FIG. 53

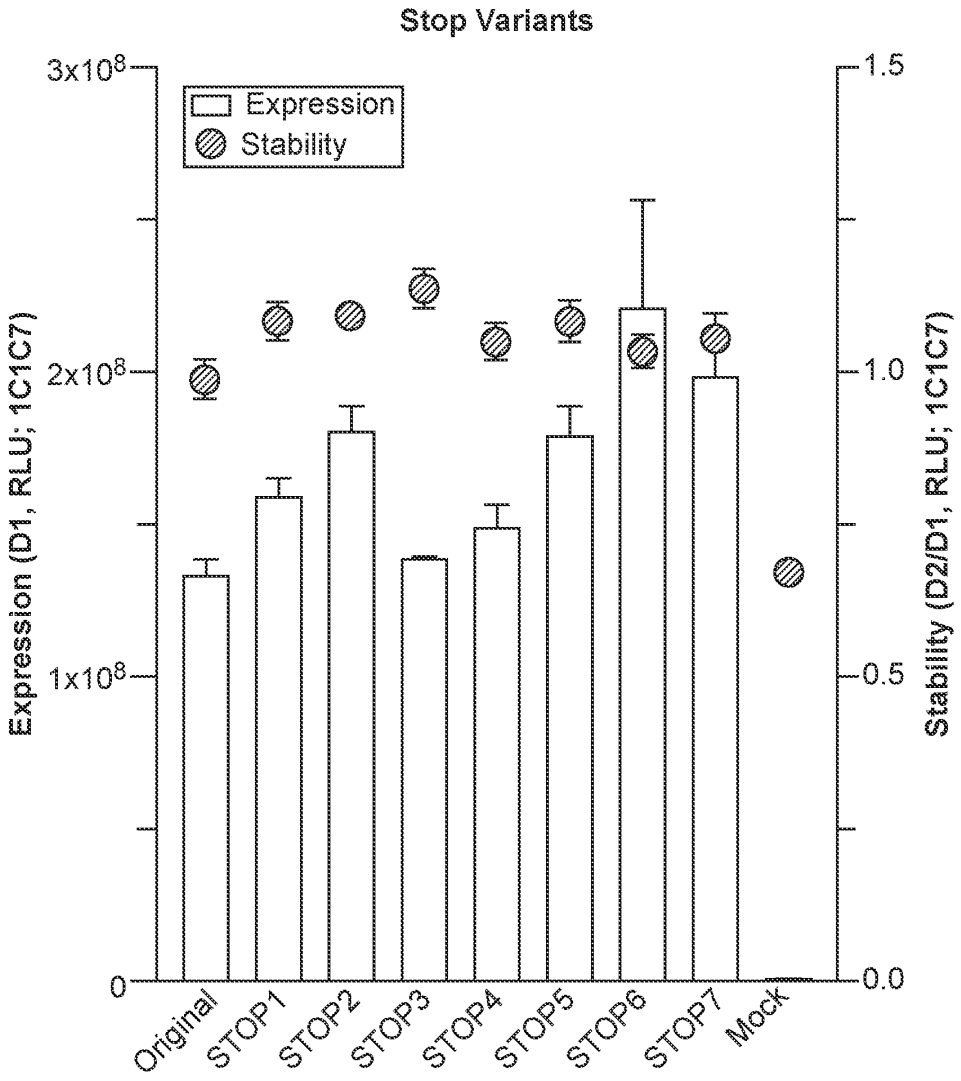
Stop Variants
Expression
Stability
Expression (D1, RLU; 1C1C7)
Stability (D2/D1, RLU; 1C1C7)
3x10^8
2x10^8
1x10^8
0
1.5
1.0
0.5
0.0
Original
STOP1
STOP2
STOP3
STOP4
STOP5
STOP6
STOP7
Mock

FIG. 54

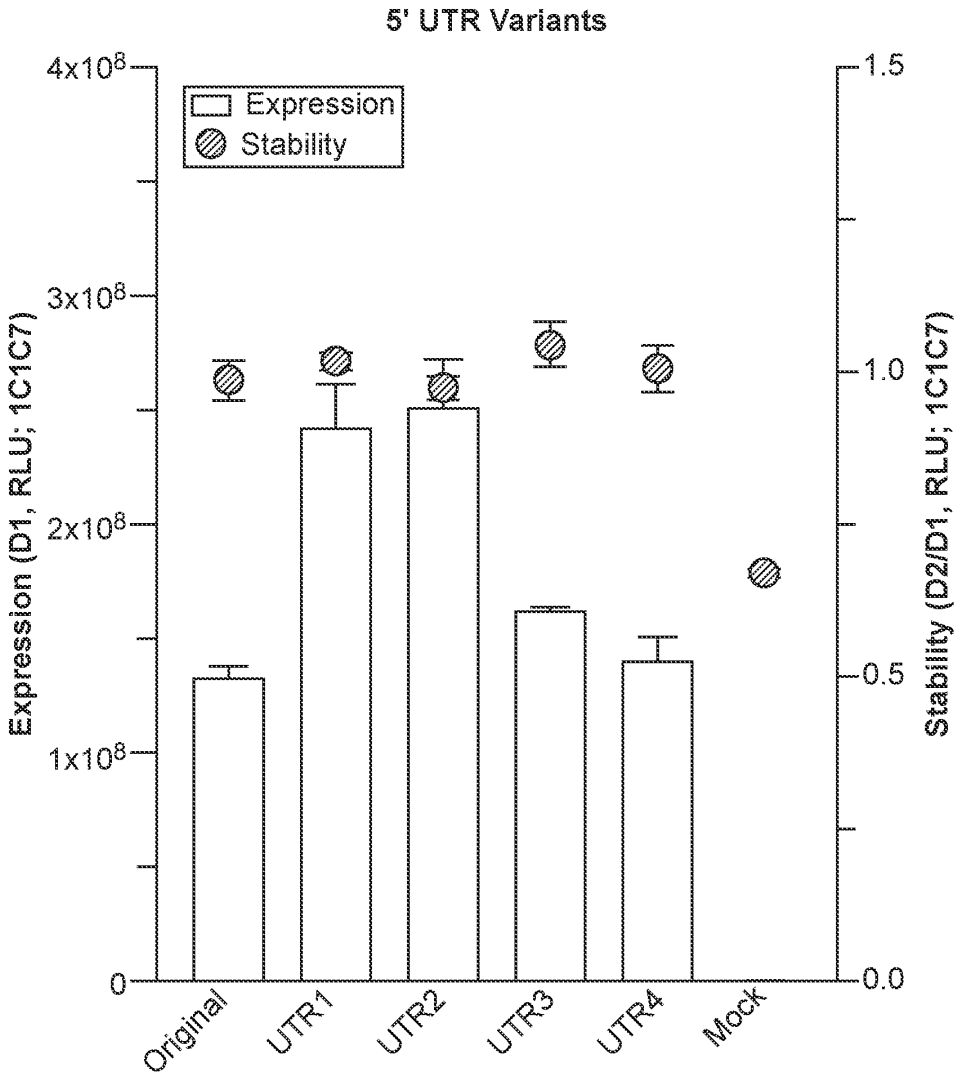
5' UTR Variants
Expression
Stability
Expression (D1, RLU; 1C1C7)
Stability (D2/D1, RLU; 1C1C7)
4x10^8
3x10^8
2x10^8
1x10^8
0
1.5
1.0
0.5
0.0
Original
UTR1
UTR2
UTR3
UTR4
Mock

FIG. 55

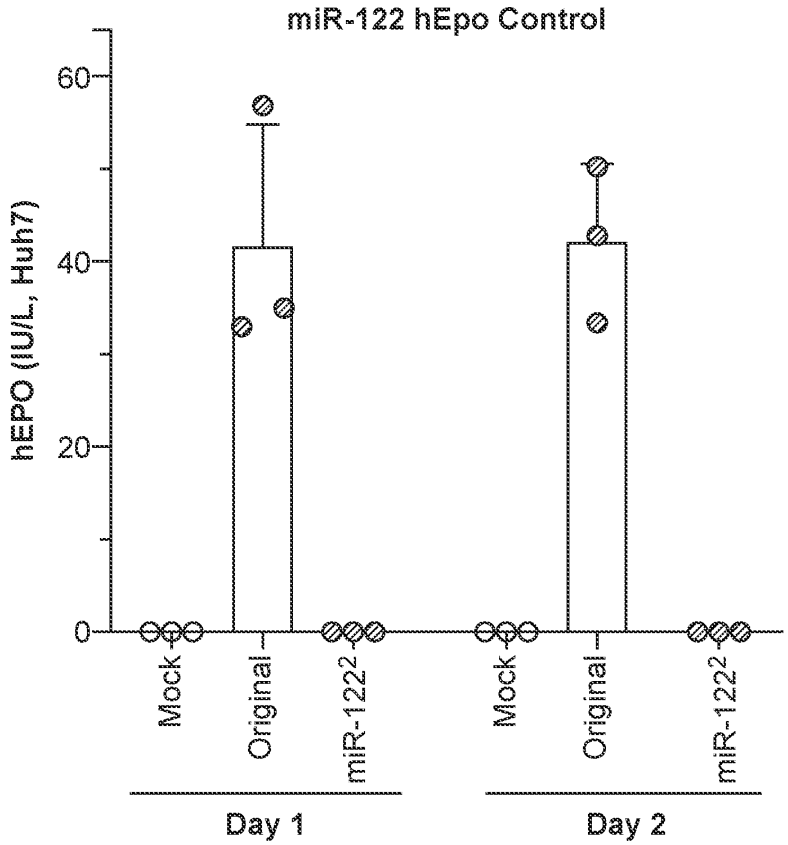
miR-122 hEpo Control
hEPO (IU/L, Huh7)
60
40
20
0
Mock
Original
miR-122²
Mock
Original
miR-122²
Day 1
Day 2

FIG. 56

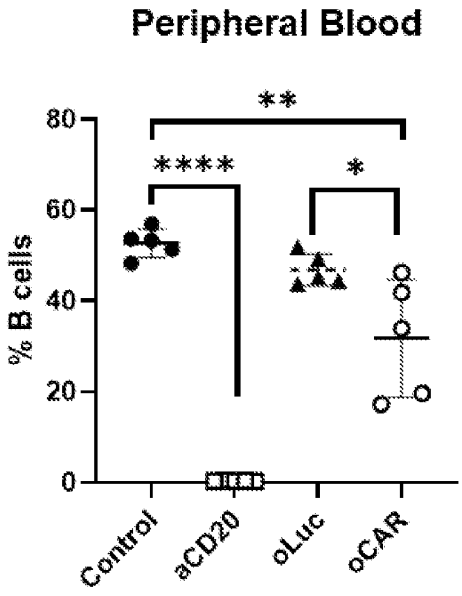

FIG. 57A

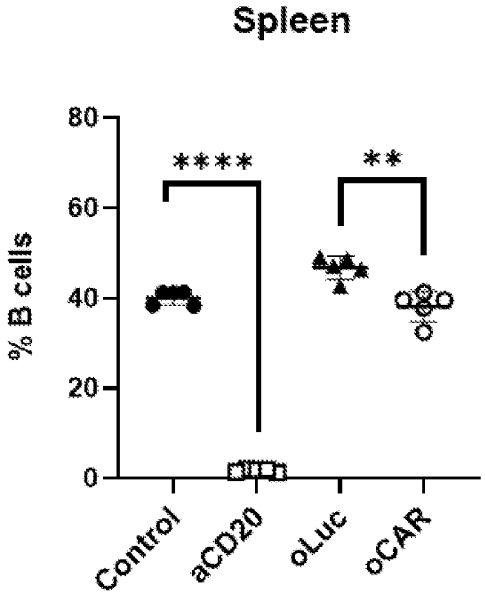

FIG. 57B

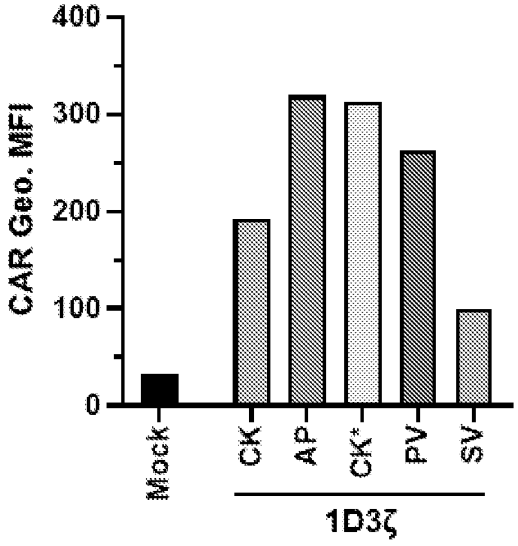

FIG. 58A

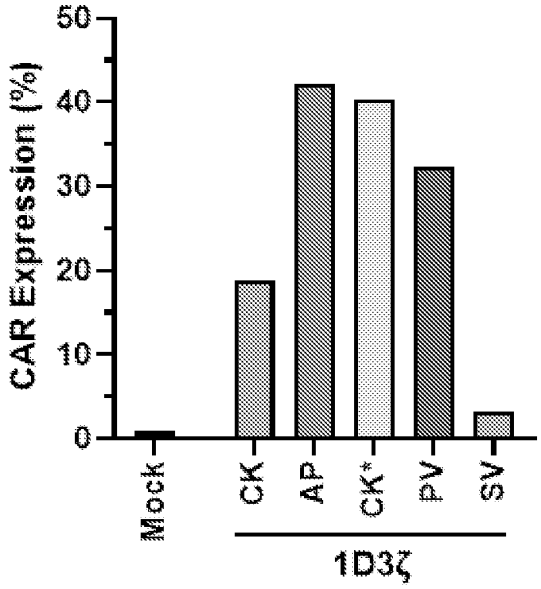

FIG. 58B

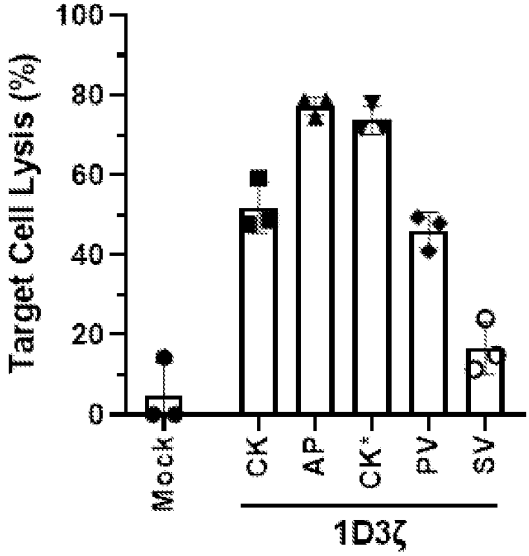

FIG. 58C

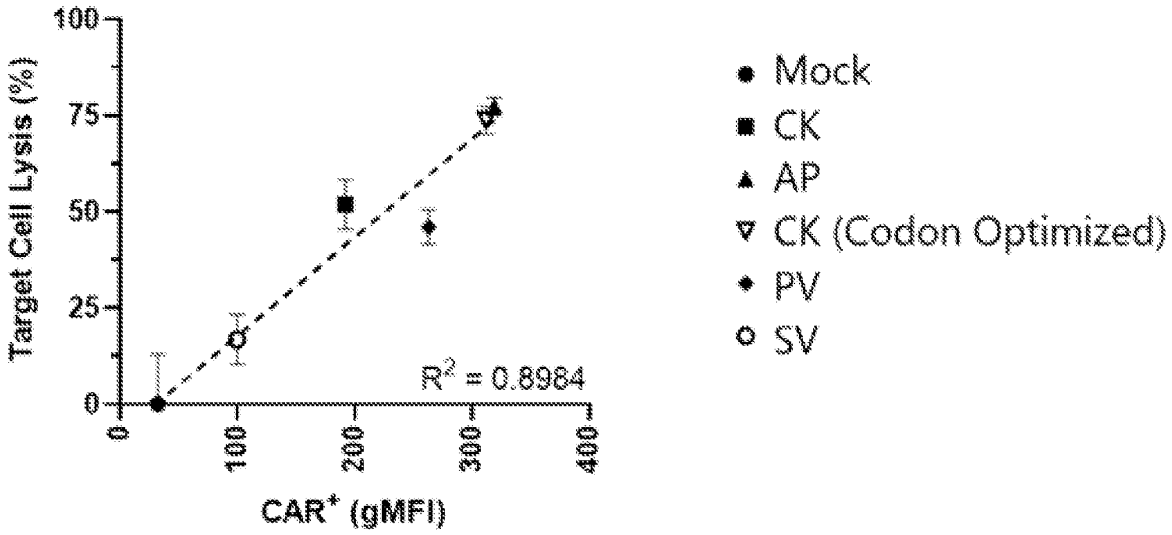
Target Cell Lysis (%)
100
75
50
25
0
0
100
200
300
400
CAR+ (gMFI)
R2 = 0.8984
Mock
CK
AP
CK (Codon Optimized)
PV
SV

FIG. 59

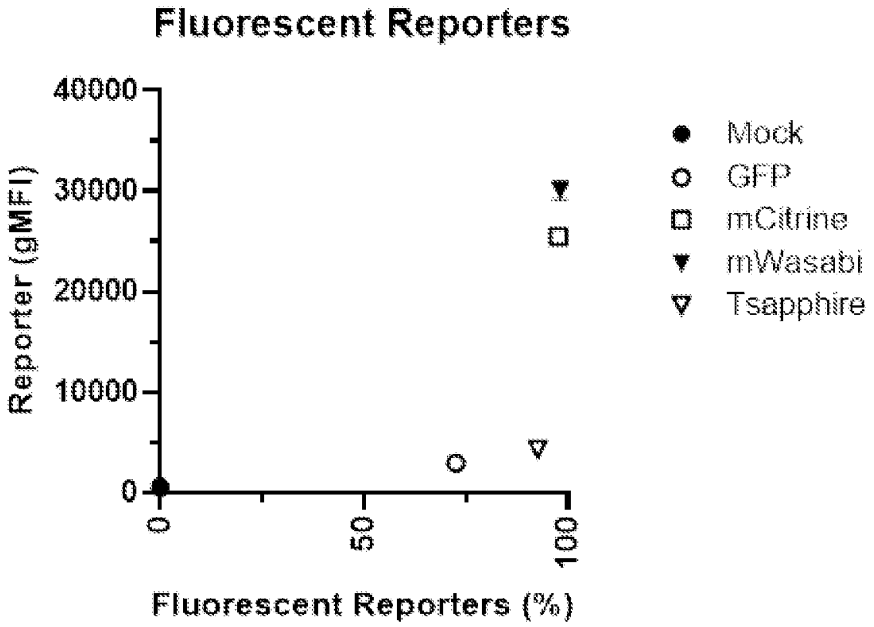

FIG. 60A

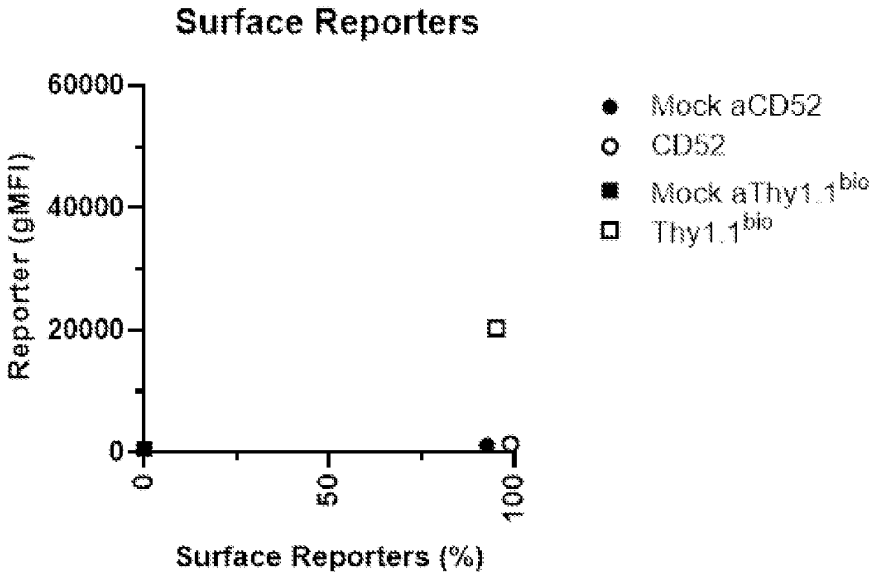

FIG. 60B

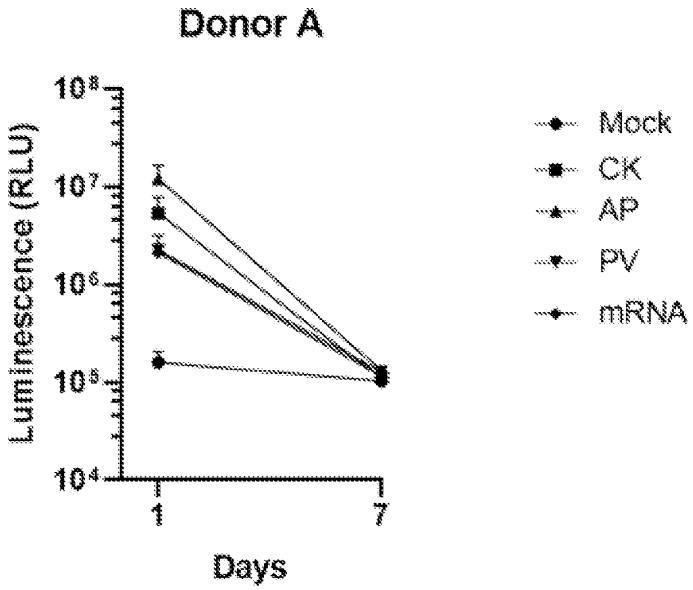

FIG. 61A

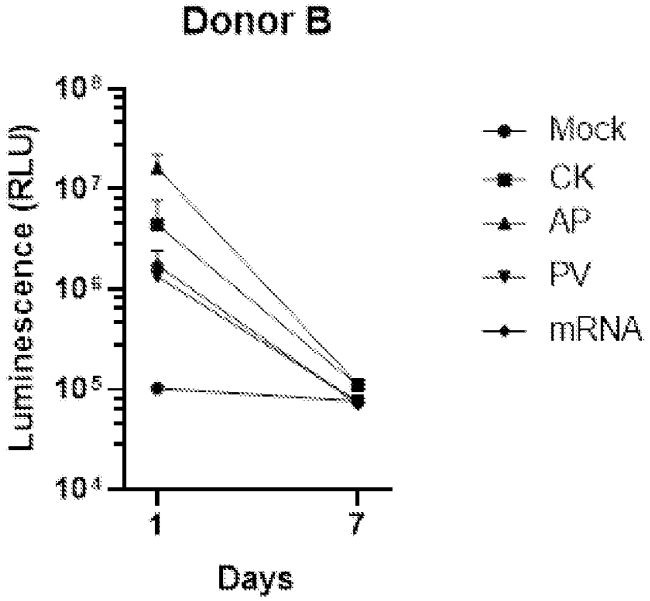

FIG. 61B

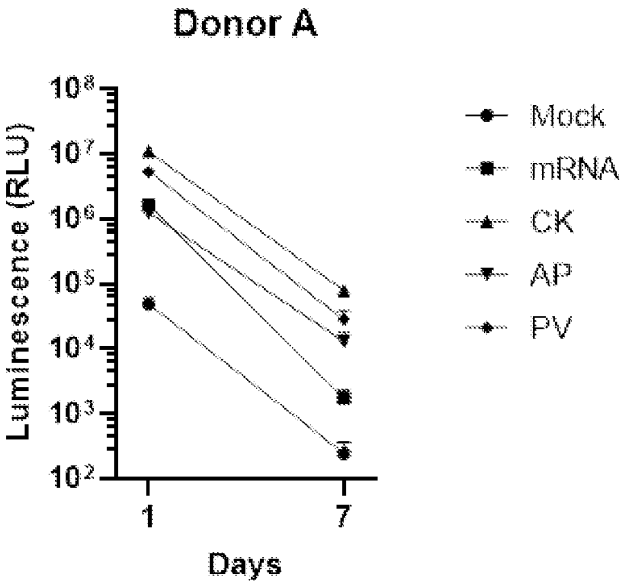

FIG. 61C

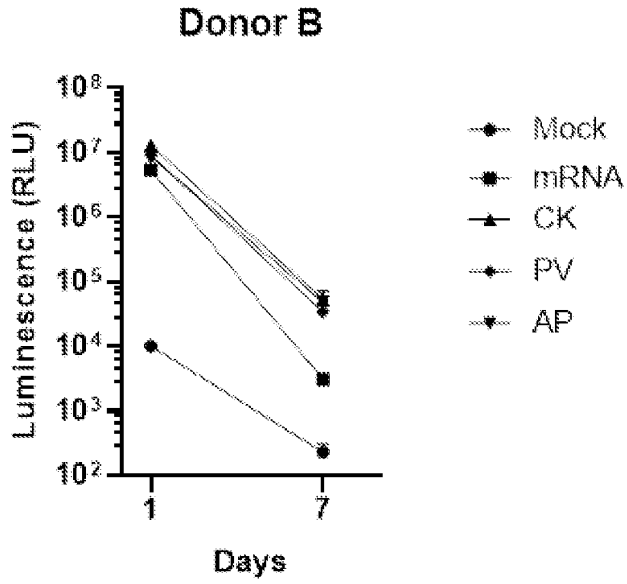

FIG. 61D

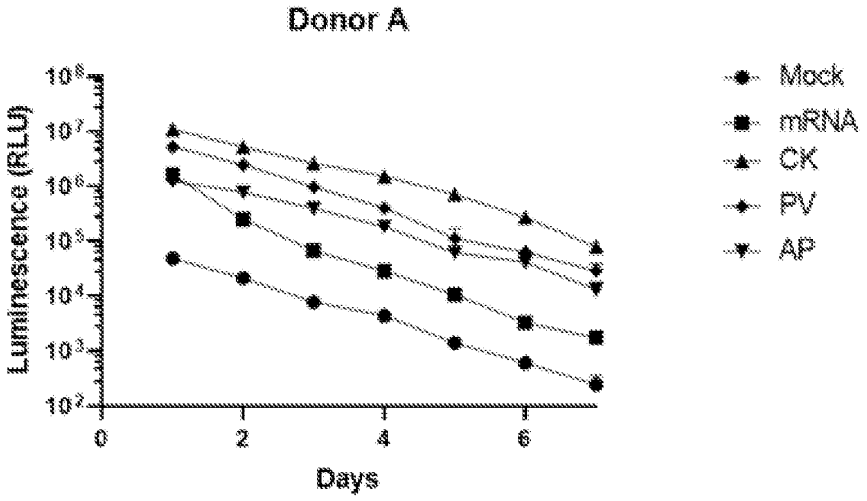

FIG. 61E

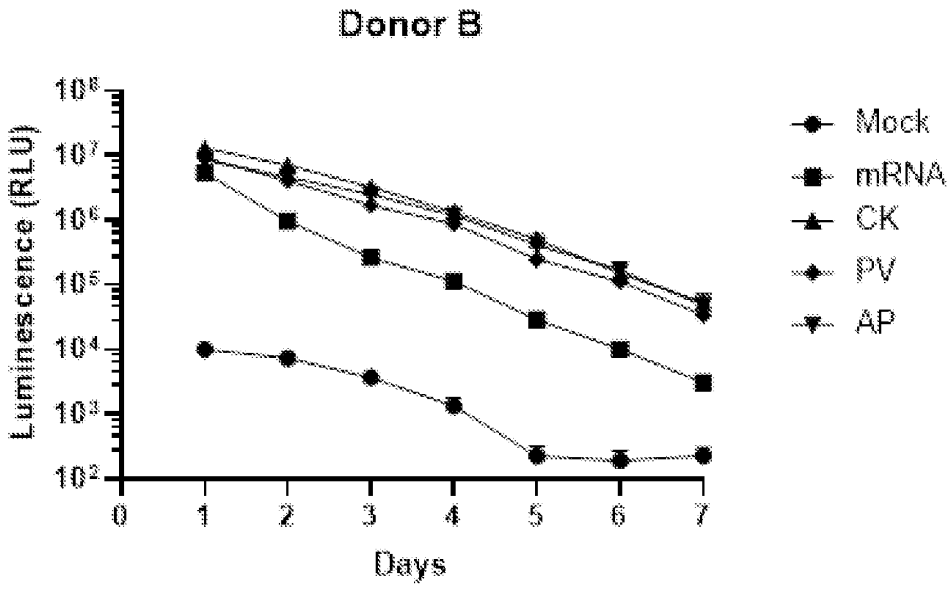

FIG. 61F

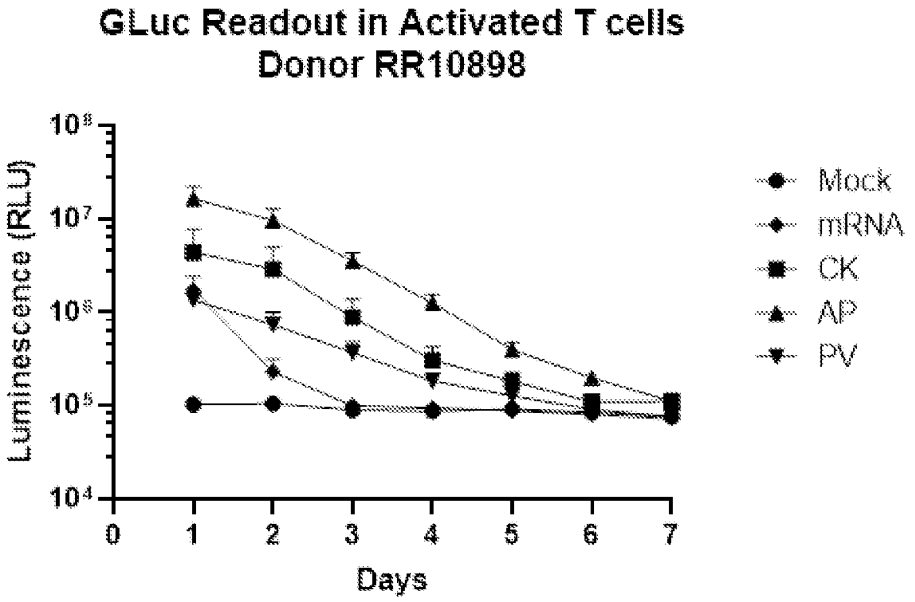
GLuc Readout in Activated T cells
Donor RR10898
Luminescence (RLU)
10^8
10^7
10^6
10^5
10^4
0
1
2
3
4
5
6
7
Days
Mock
mRNA
CK
AP
PV

FIG. 61G

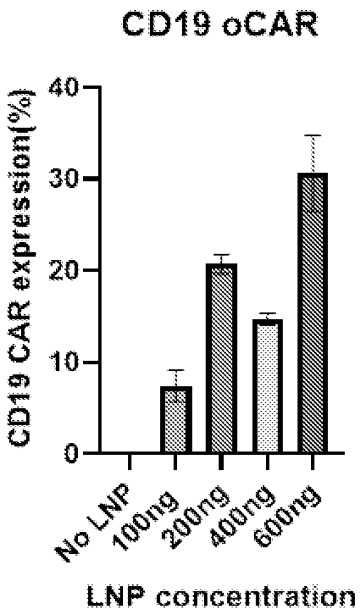

FIG. 62A

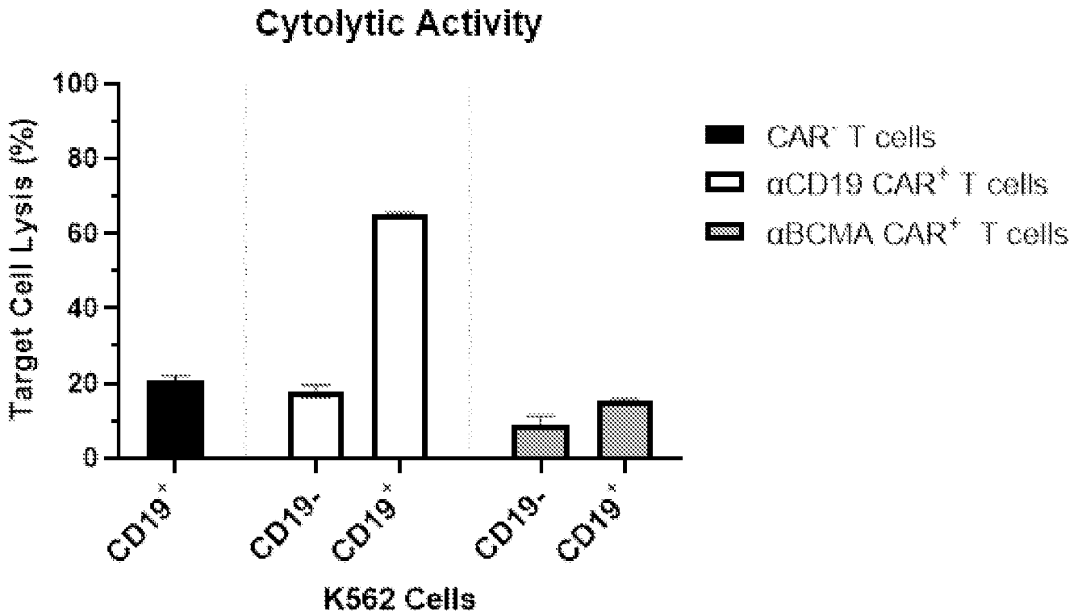

FIG. 62B

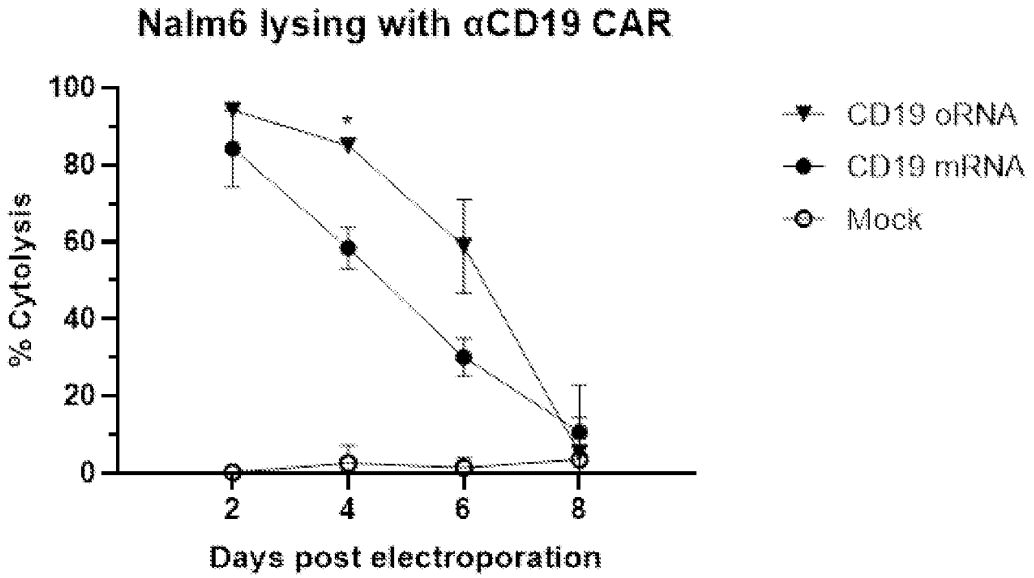

FIG. 63A

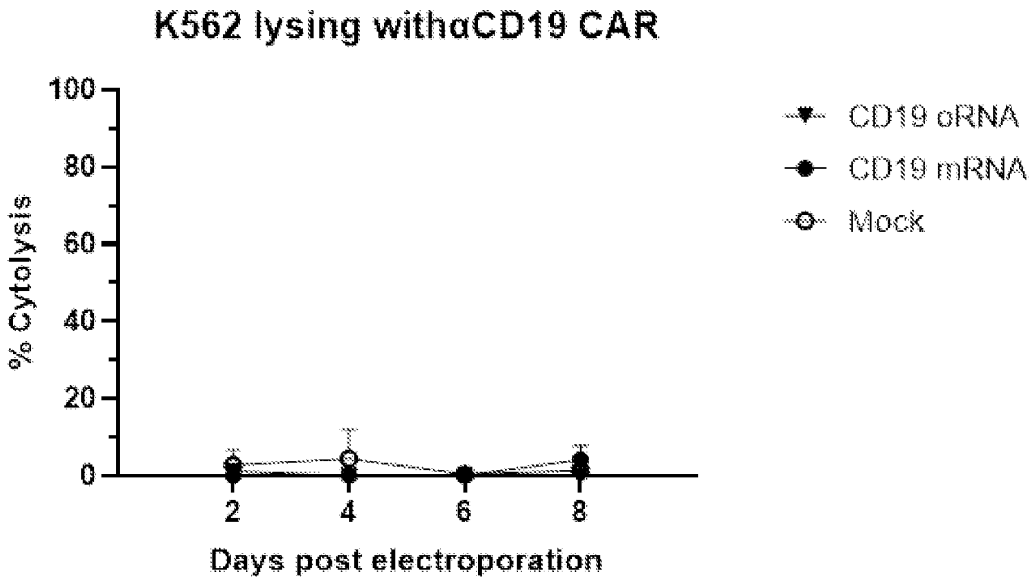

FIG. 63B

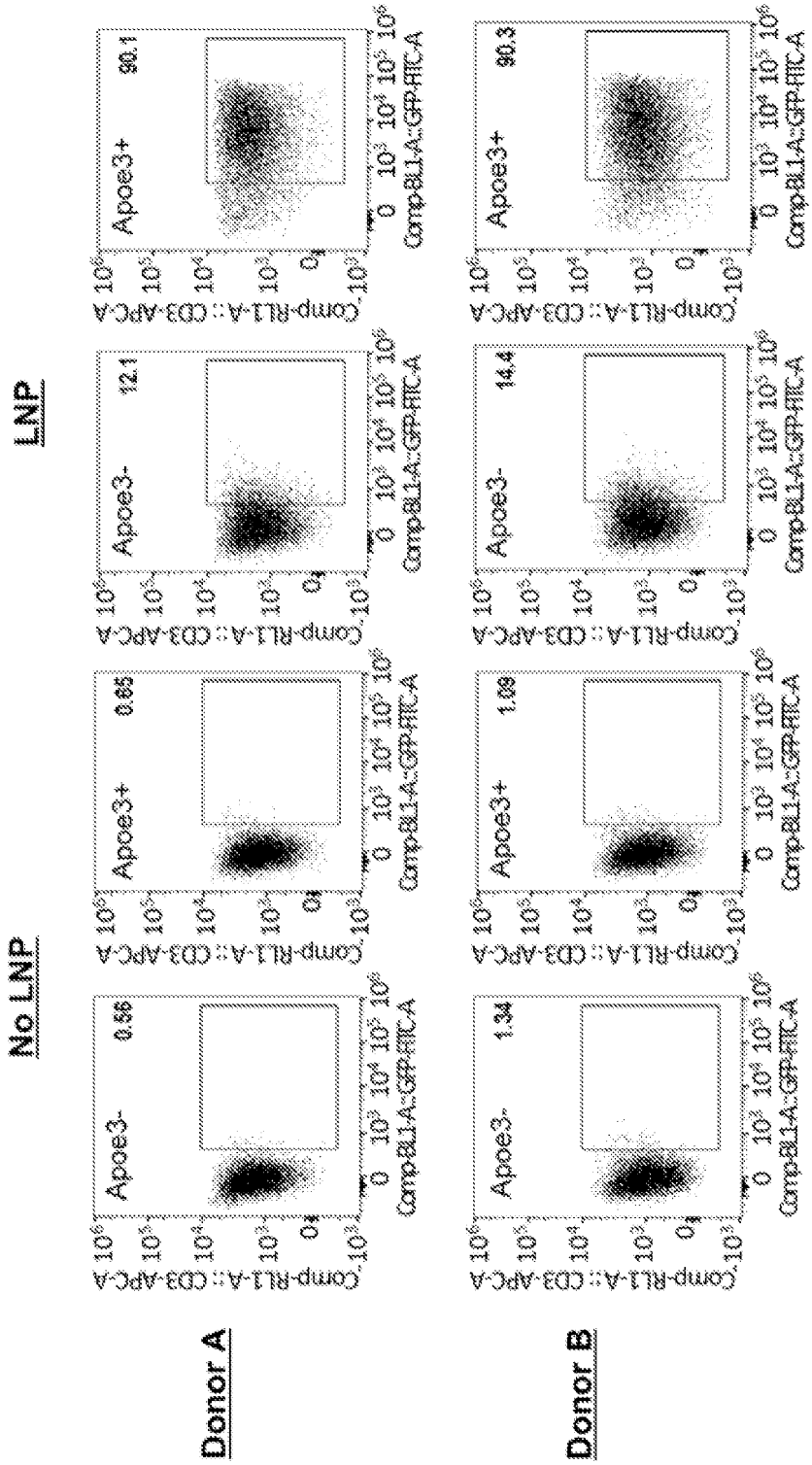
No LNP
LNP
Donor A
Donor B
Apoe3-
Apoe3+
0.56
0.65
1.34
1.09
12.1
14.4
90.1
90.3
Comp-RL1-A :: CD3-APC-A
Comp-BL1-A :: GFP-FITC-A

FIG. 64A

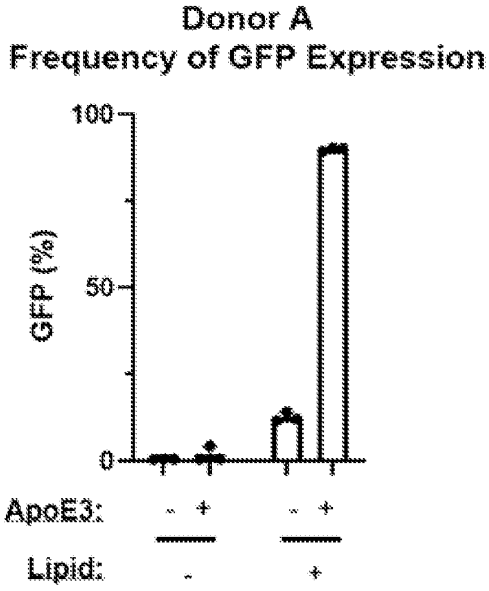

FIG. 64B

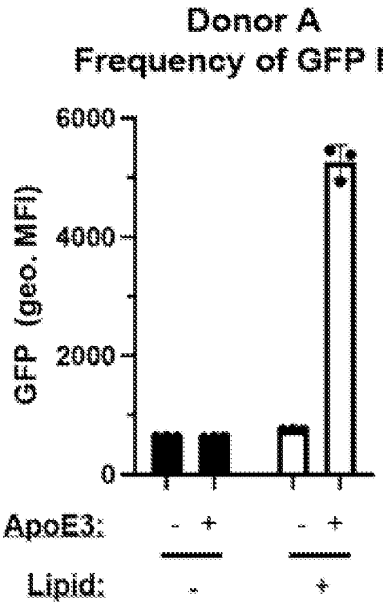

FIG. 64C

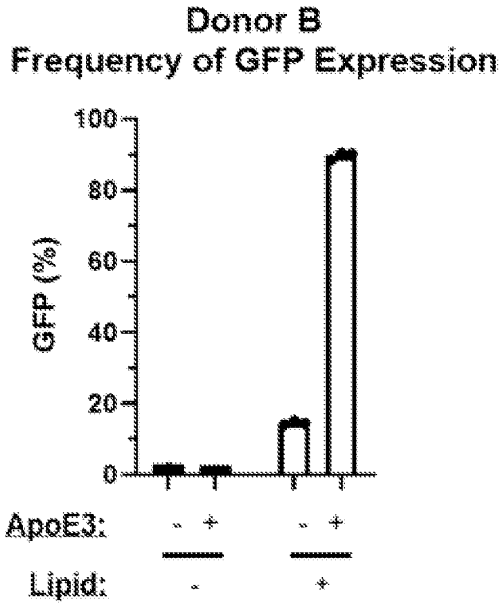

FIG. 64D

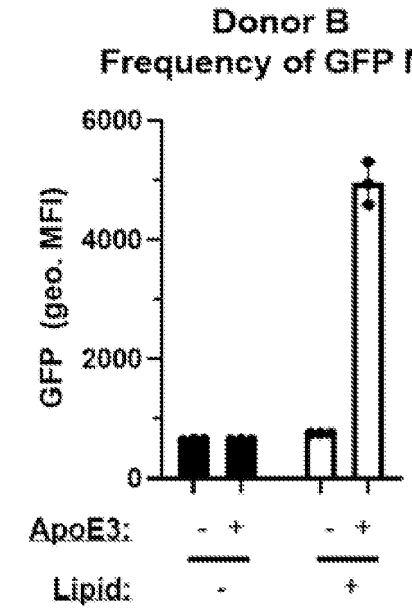

FIG. 64E