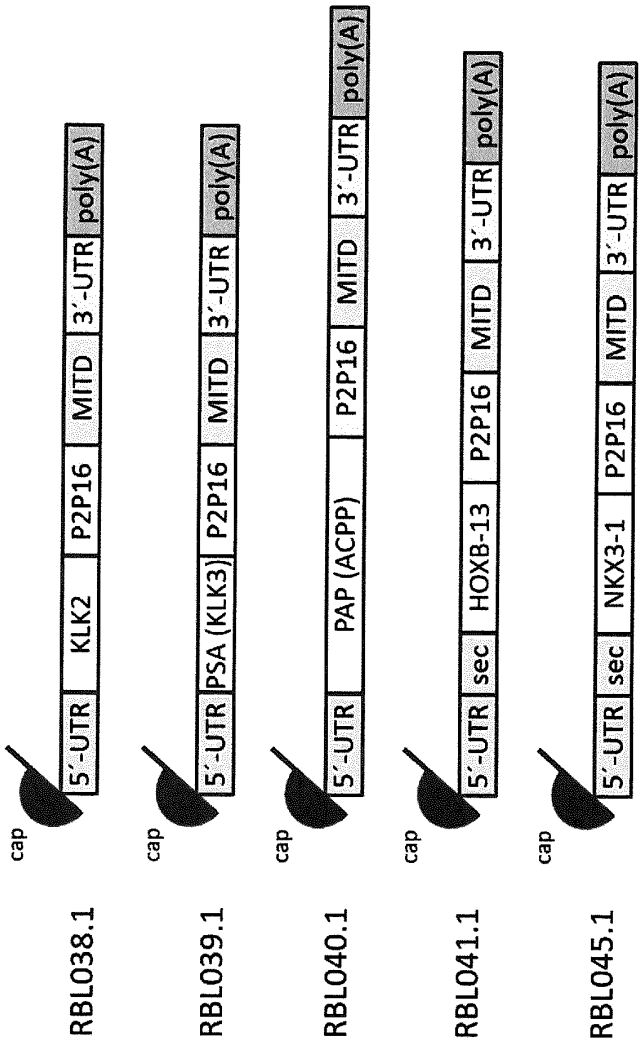
Figure 1


cap
5´-UTR
KLK2
P2P16
MITD
3´-UTR
poly(A)
RBL038.1
cap
5´-UTR
PSA (KLK3)
P2P16
MITD
3´-UTR
poly(A)
RBL039.1
cap
5´-UTR
PAP (ACPP)
P2P16
MITD
3´-UTR
poly(A)
RBL040.1
cap
5´-UTR
sec
HOXB-13
P2P16
MITD
3´-UTR
poly(A)
RBL041.1
cap
5´-UTR
sec
NKX3-1
P2P16
MITD
3´-UTR
poly(A)
RBL045.1

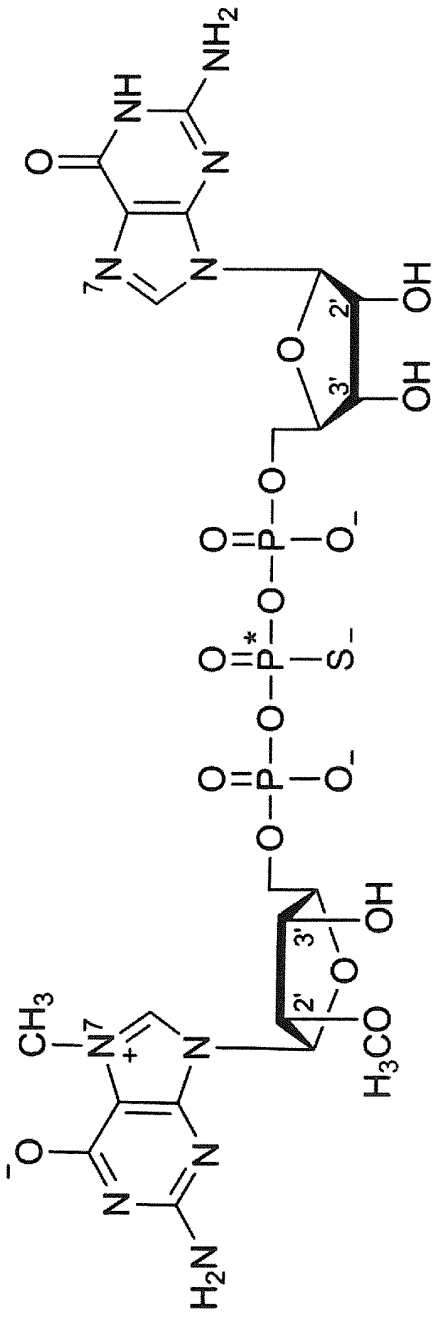


Figure 2

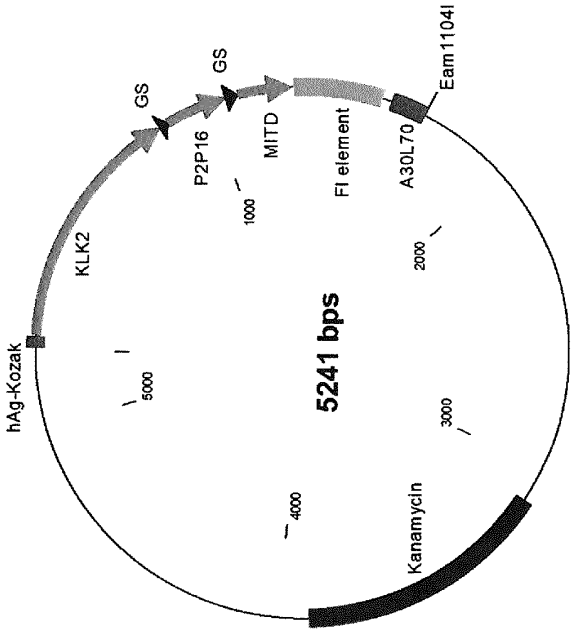

GS
GS
Eam1104I
P2P16
MITD
FI element
A30L70
1000
2000
KLK2
5241 bps
hAg-Kozak
5000
3000
4000
Kanamycin

Figure 3

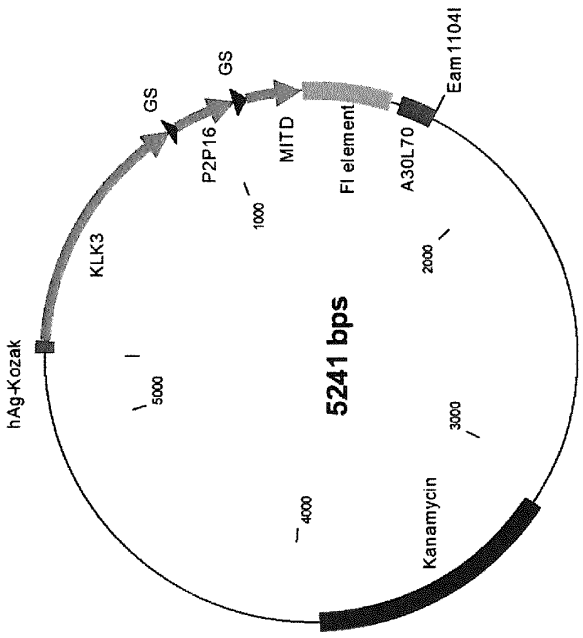

GS
GS
Eam1104I
P2P16
MITD
FI element
A30L70
1000
2000
KLK3
5241 bps
hAg-Kozak
5000
3000
4000
Kanamycin

Figure 4

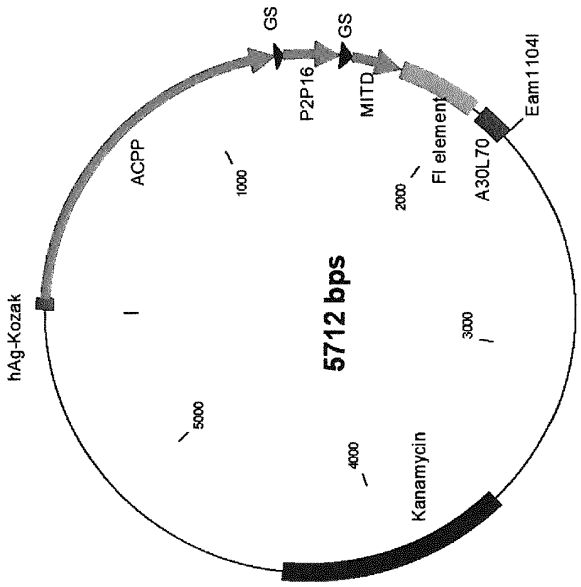

GS
GS
P2P16
MITD
FI element
Eam1104I
A30L70
ACPP
hAg-Kozak
1000
2000
3000
4000
5000
5712 bps
Kanamycin

Figure 5

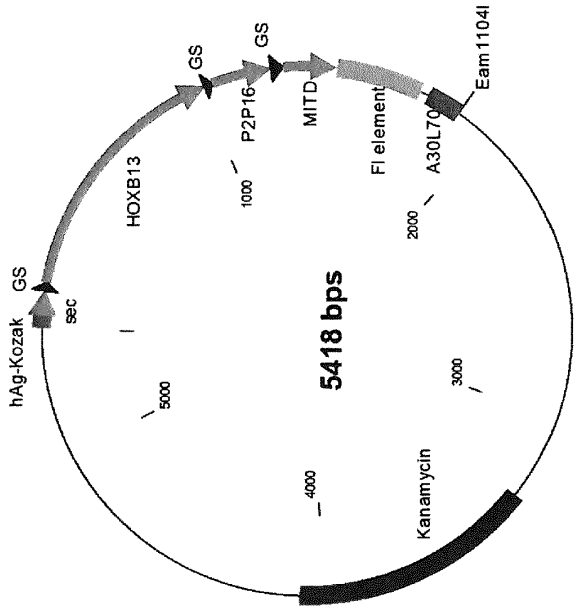

GS
GS
GS
P2P16
MITD
Fl element
A30L70
Eam1104l
HOXB13
1000
2000
hAg-Kozak
sec
5418 bps
5000
3000
4000
Kanamycin

Figure 6

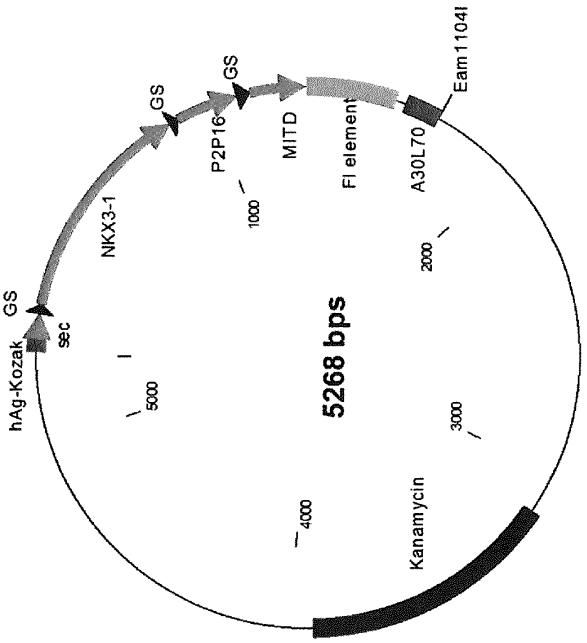
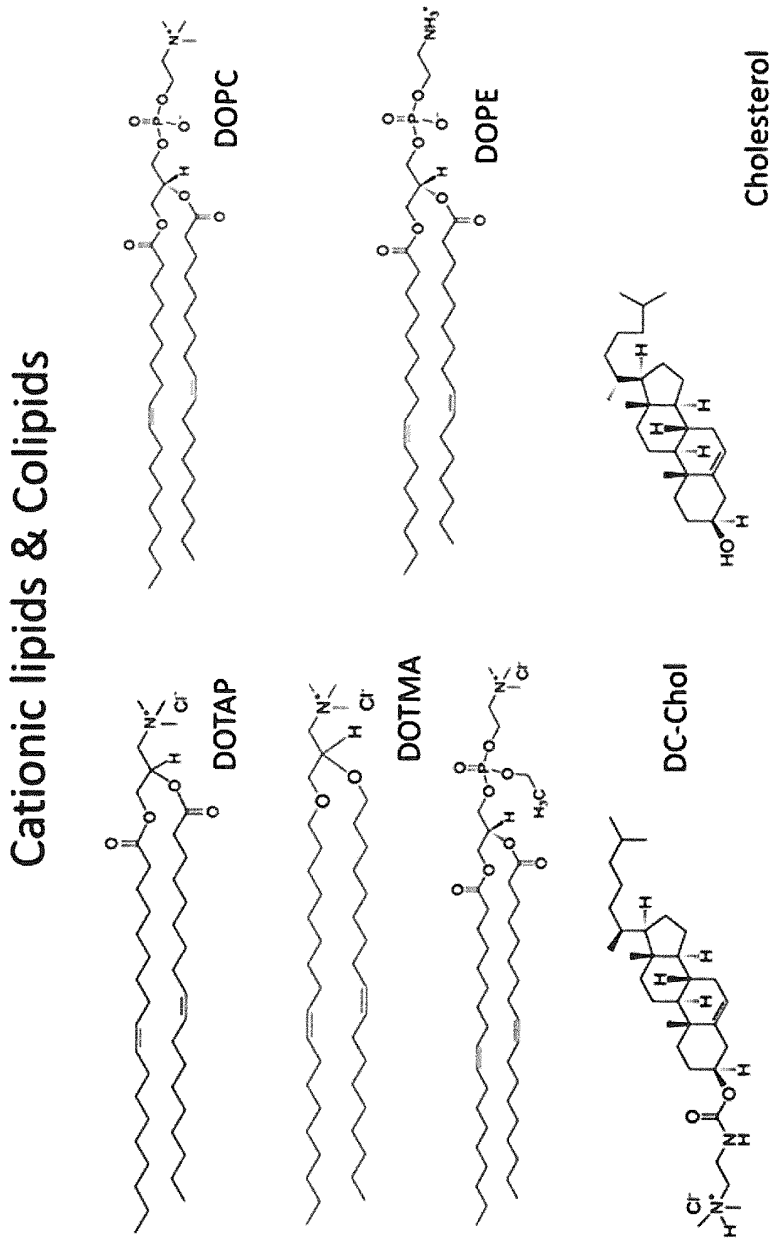
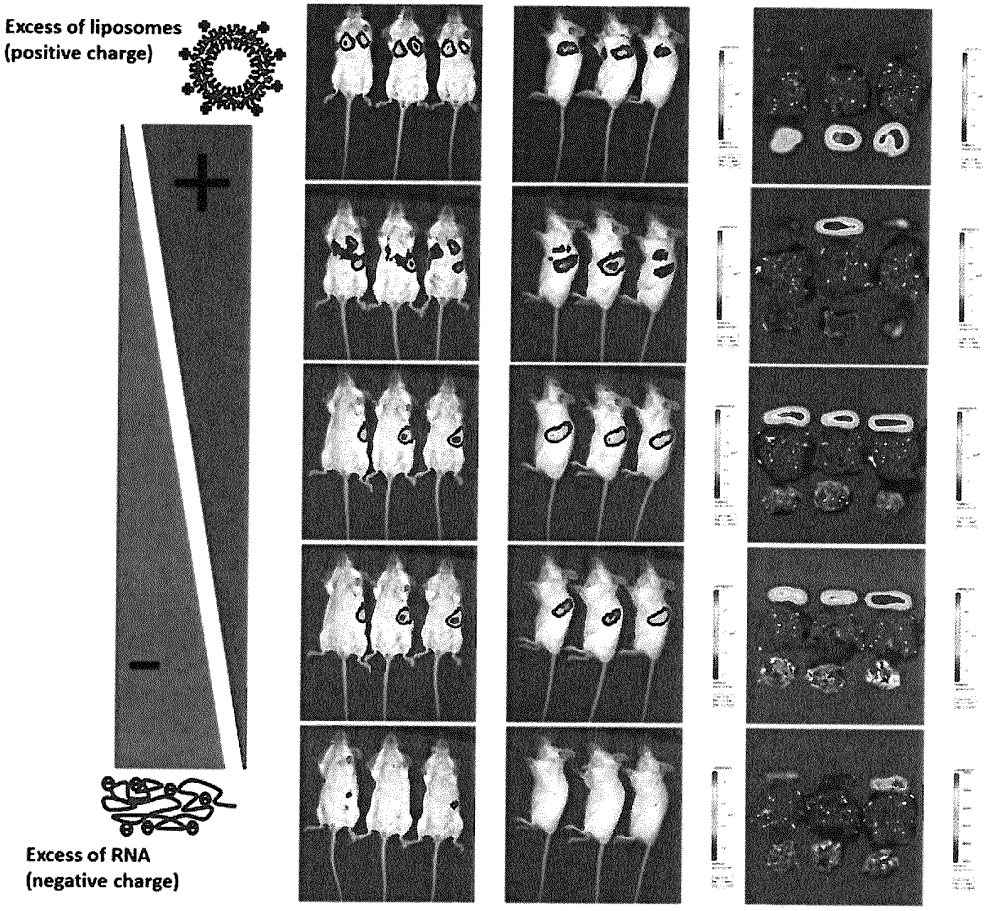

GS
hAg-Kozak
sec
NKX3-1
GS
P2P16
GS
MITD
FI element
A30L70
Eam1104I
5268 bps
1000
2000
3000
4000
5000
Kanamycin

Figure 7

Figure 8


Cationic lipids & Colipids
DOPC
DOPE
Cholesterol
DOTAP
DOTMA
DC-Chol


Excess of liposomes
(positive charge)
+
−
Excess of RNA
(negative charge)

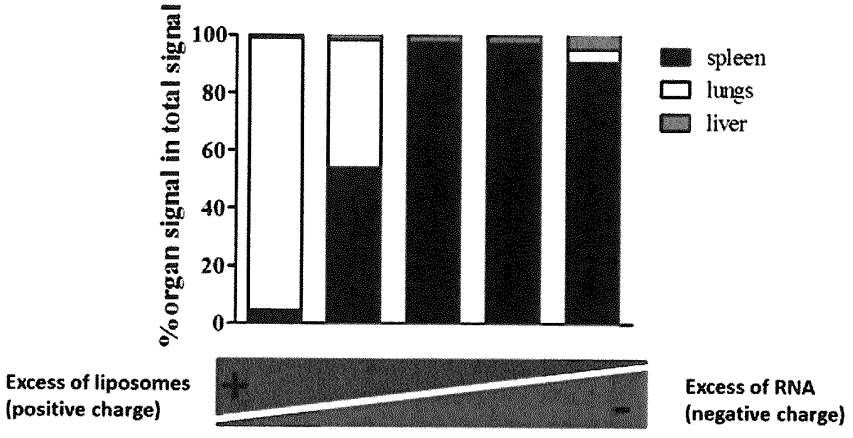

% organ signal in total signal
100
80
60
40
20
0
spleen
lungs
liver
Excess of liposomes
(positive charge)
+
−
Excess of RNA
(negative charge)

Figure 9

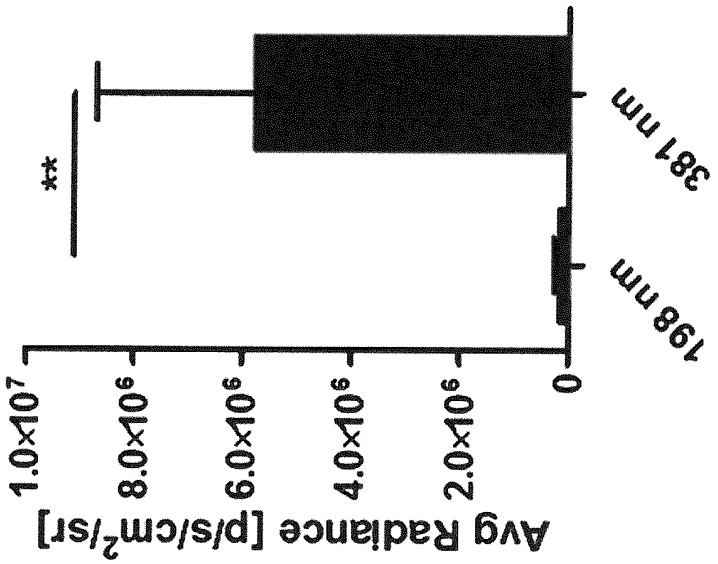
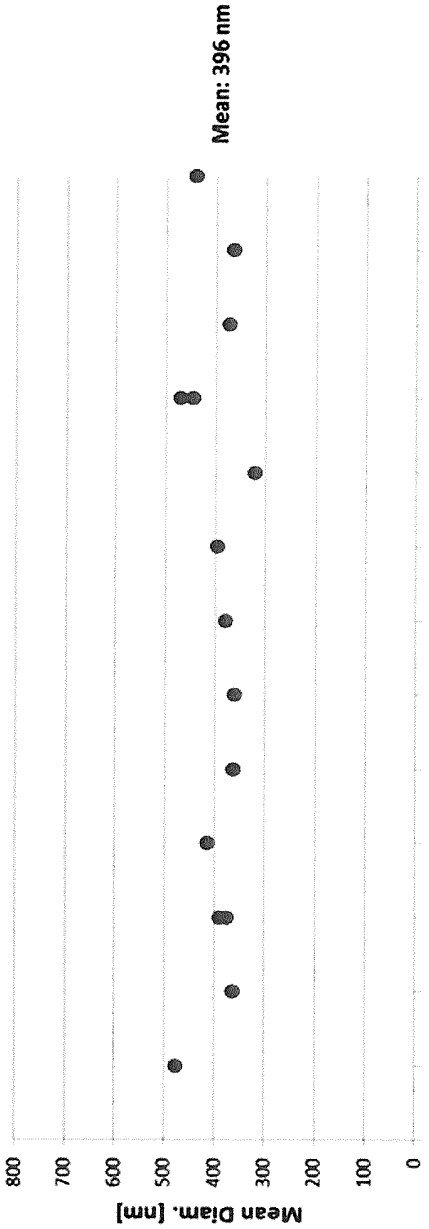

Avg Radiance [p/s/cm²/sr]
1.0×10⁷
8.0×10⁶
6.0×10⁶
4.0×10⁶
2.0×10⁶
0
**
381 nm
198 nm

Figure 10

Figure 11



Experiment #	1	2	3	4	5	6	7	8	9	10	11	12	13
Experimenter	1	1	2	1	1	1	1	1	3	4	1	4	1
Lab	A	A	A	A	A	A	A	A	A	B	A	B	A
RNA	luc	luc	luc	luc	NY-ESO-1	tyrosinase	TPTE	MAGE-A3	luc	luc	luc	luc	luc

Figure 12

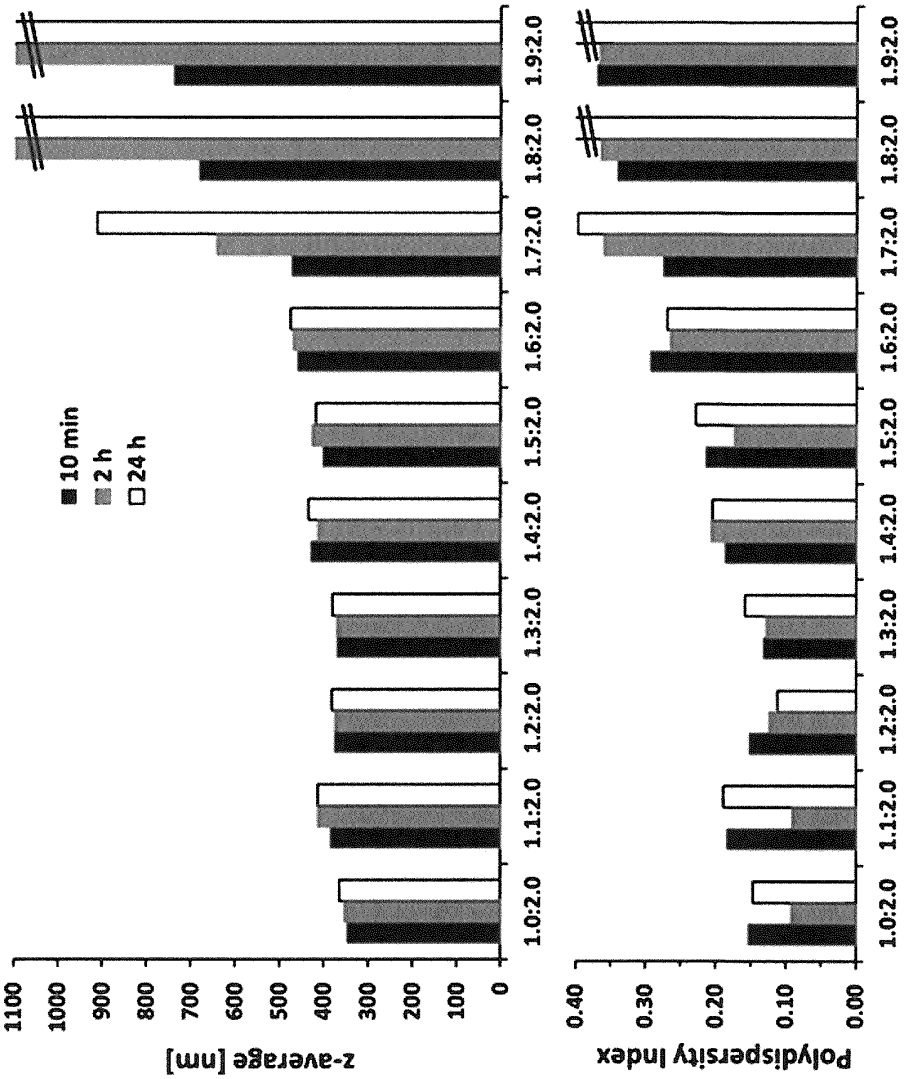
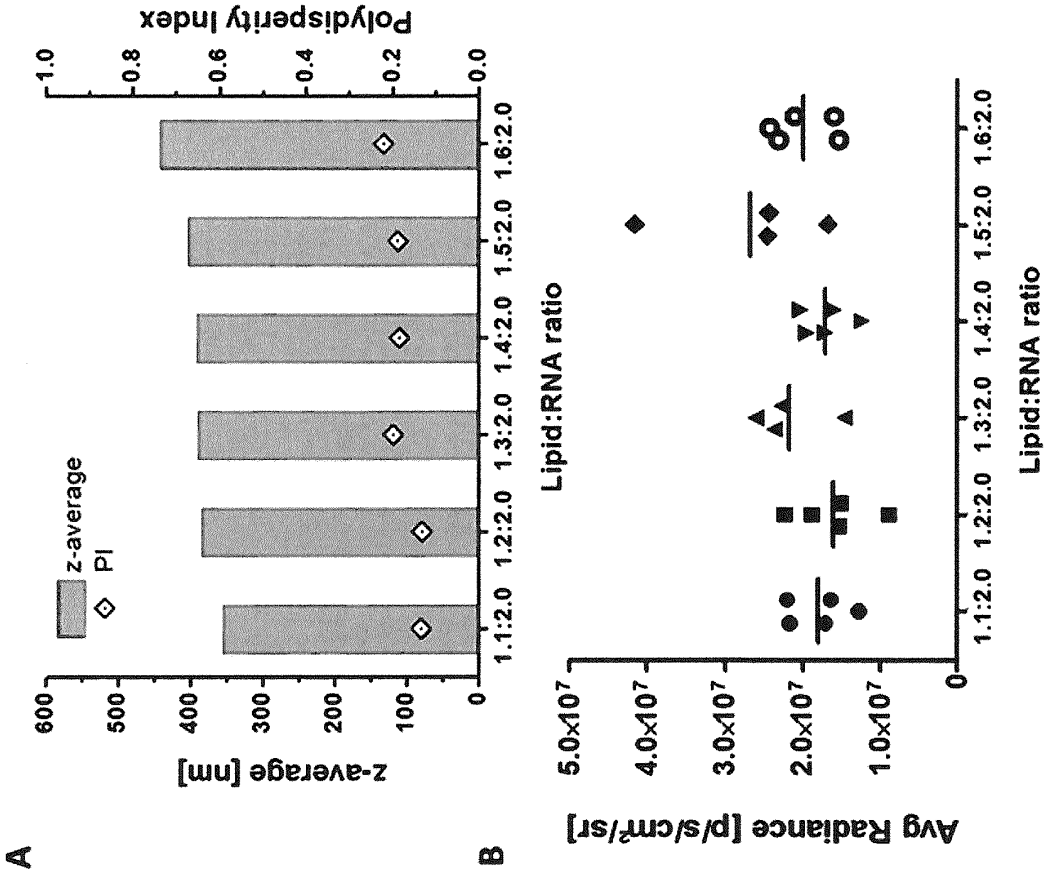

z-average [nm]
1100
1000
900
800
700
600
500
400
300
200
100
0
10 min
2 h
24 h
1.0:2.0
1.1:2.0
1.2:2.0
1.3:2.0
1.4:2.0
1.5:2.0
1.6:2.0
1.7:2.0
1.8:2.0
1.9:2.0
Polydispersity Index
0.40
0.30
0.20
0.10
0.00

Figure 13


A
z-average [nm]
Polydispersity Index
z-average
PI
0
100
200
300
400
500
600
0.0
0.2
0.4
0.6
0.8
1.0
1.1:2.0
1.2:2.0
1.3:2.0
1.4:2.0
1.5:2.0
1.6:2.0
Lipid:RNA ratio
B
Avg Radiance [p/s/cm²/sr]
1.0x10⁷
2.0x10⁷
3.0x10⁷
4.0x10⁷
5.0x10⁷
Lipid:RNA ratio

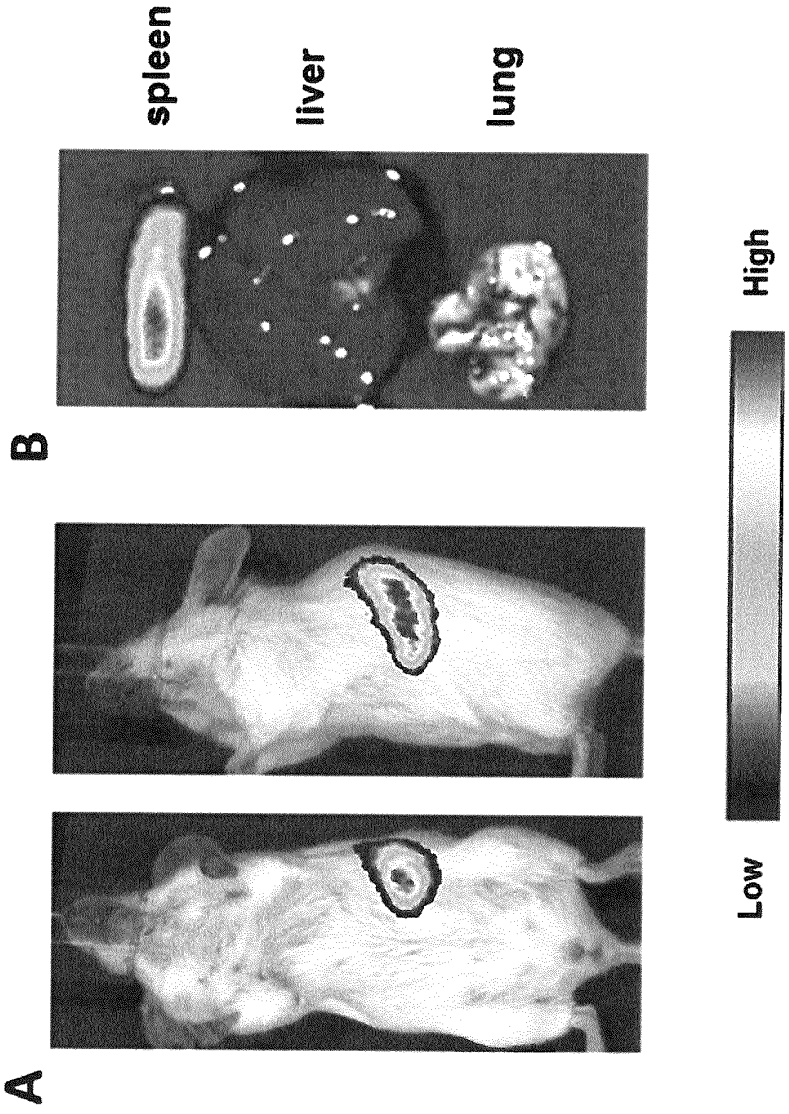


Figure 14

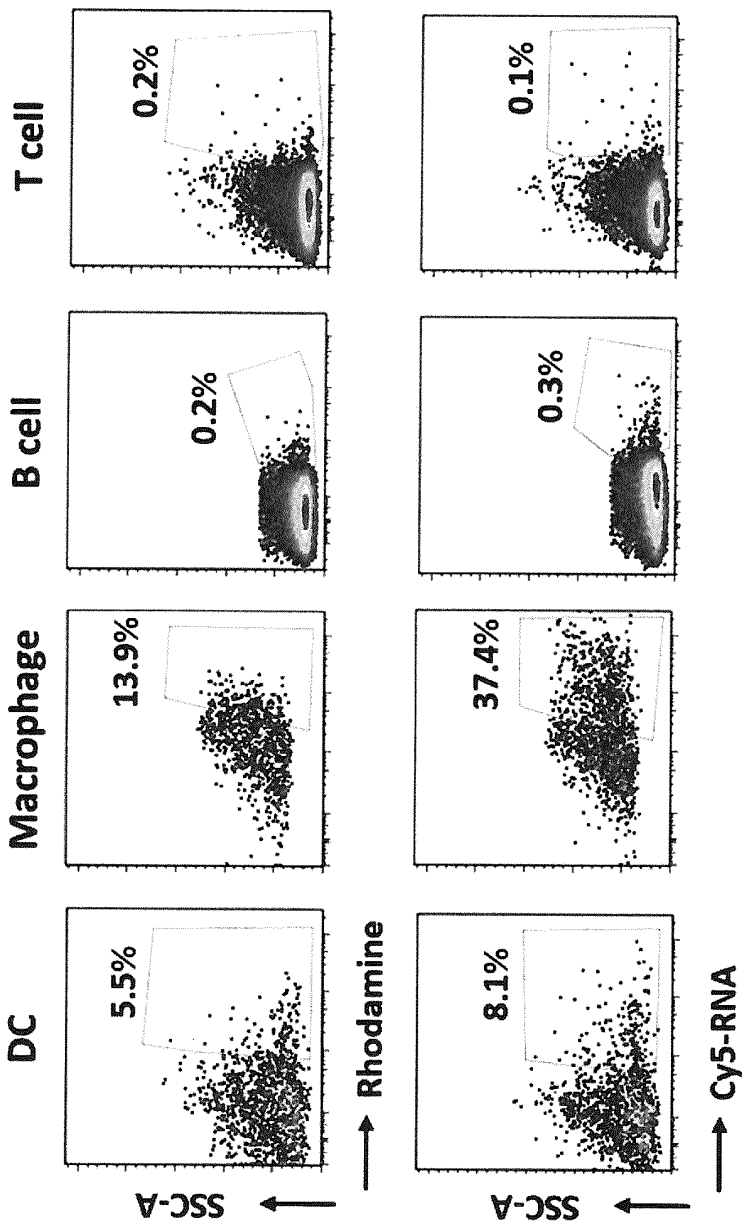

DC
Macrophage
B cell
T cell
5.5%
13.9%
0.2%
0.2%
8.1%
37.4%
0.3%
0.1%
SSC-A
Rhodamine
Cy5-RNA

Figure 15

Figure 16

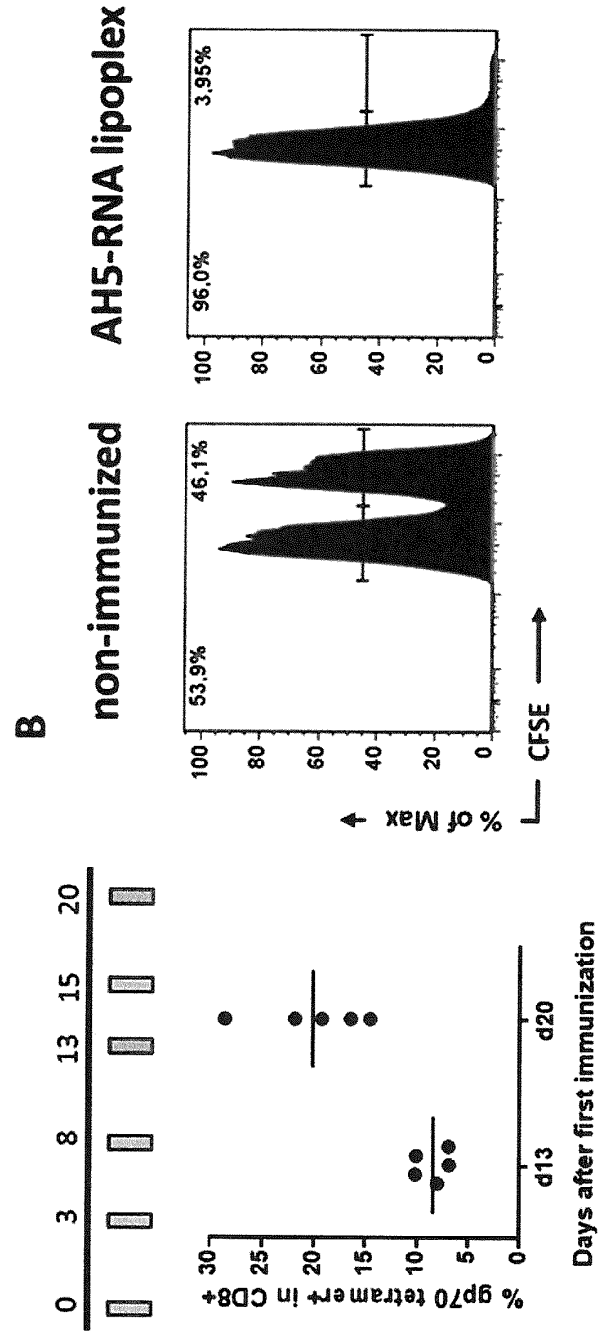
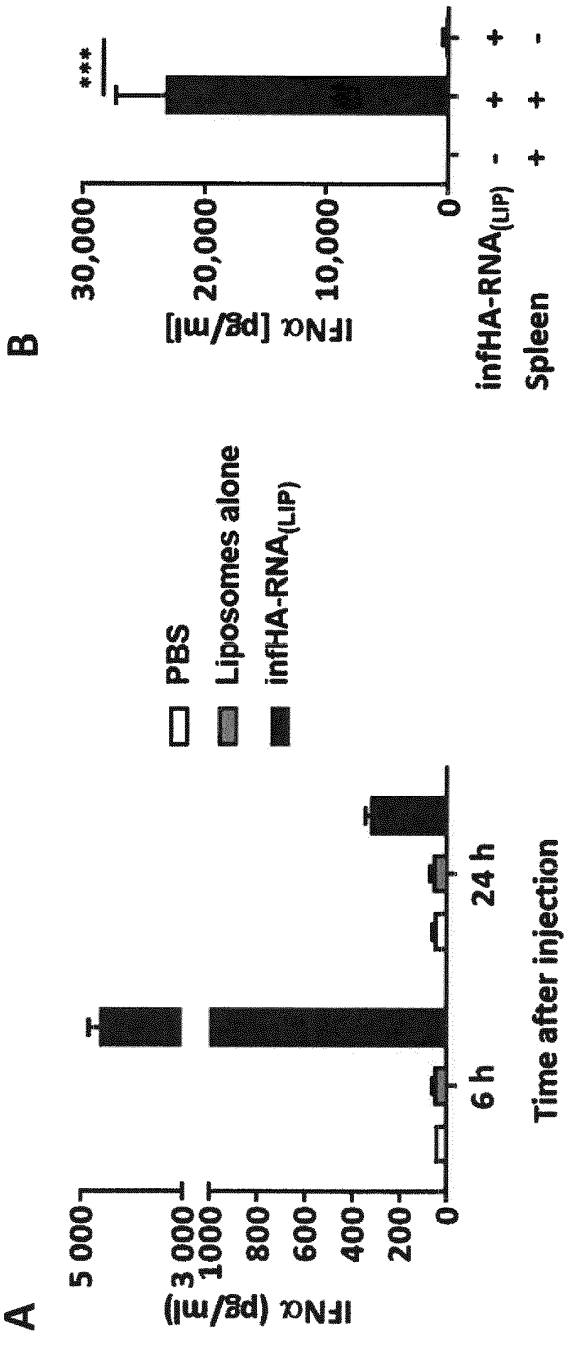

A
0
3
8
13
15
20
% gp70 tetramer+ in CD8+
30
25
20
15
10
5
0
d13
d20
Days after first immunization
B
non-immunized
AH5-RNA lipoplex
53.9%
46.1%
96.0%
3.95%
% of Max
100
80
60
40
20
0
CFSE

Figure 17


A
IFNα (pg/ml)
5 000
3 000
1000
800
600
400
200
0
6 h
24 h
Time after injection
PBS
Liposomes alone
infHA-RNA(LIP)
B
IFNα [pg/ml]
30,000
20,000
10,000
0
***
infHA-RNA(LIP)
Spleen

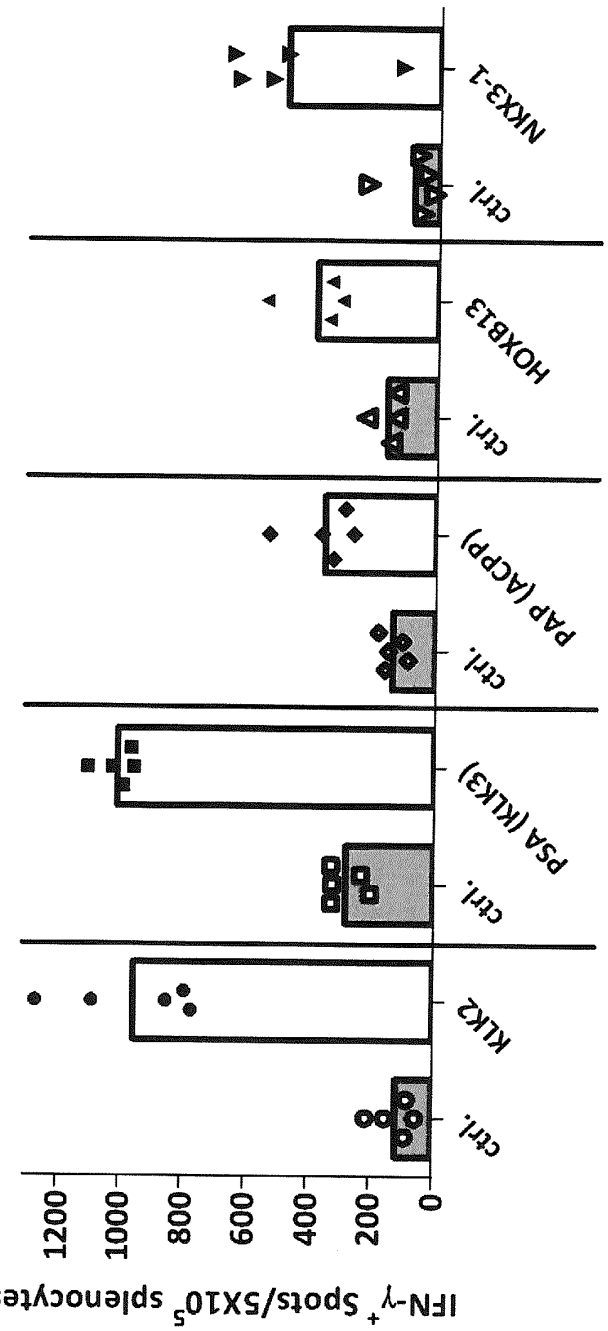

IFN-γ+ Spots/5X10^5 splenocytes
0
200
400
600
800
1000
1200
ctrl.
KLK2
ctrl.
PSA (KLK3)
ctrl.
PAP (ACPP)
ctrl.
HOXB13
ctrl.
NKX3-1

Figure 18

Figure 19

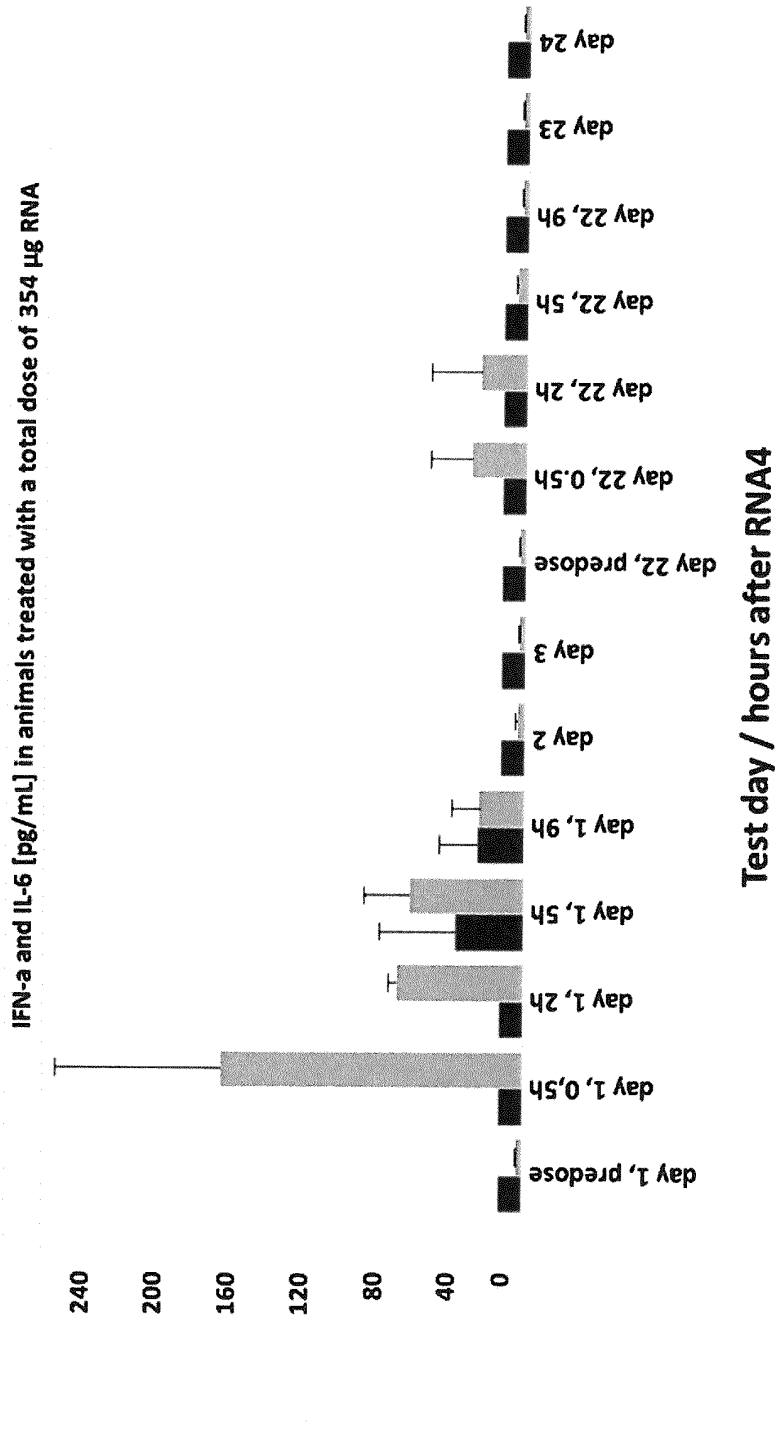
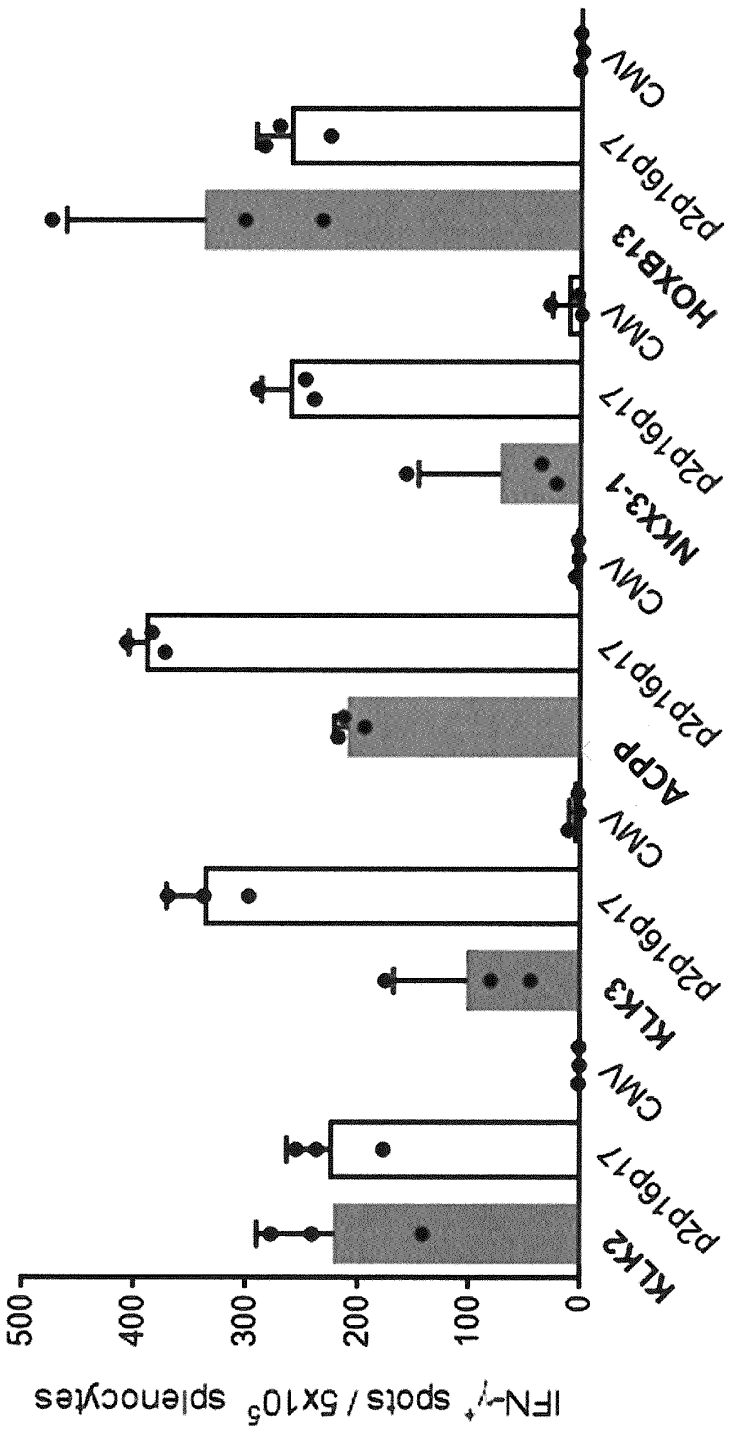

IFN-a and IL-6 [pg/mL] in animals treated with a total dose of 354 µg RNA
0
40
80
120
160
200
240
day 1, predose
day 1, 0,5h
day 1, 2h
day 1, 5h
day 1, 9h
day 2
day 3
day 22, predose
day 22, 0.5h
day 22, 2h
day 22, 5h
day 22, 9h
day 23
day 24
Test day / hours after RNA4

Figure 20


IFN-γ+ spots / 5x10^5 splenocytes
0
100
200
300
400
500
KLK2
p2P16P17
CMV
KLK3
p2P16P17
CMV
ACPP
p2P16P17
CMV
NKX3-1
p2P16P17
CMV
HOXB13
p2P16P17
CMV