**FIGURES**

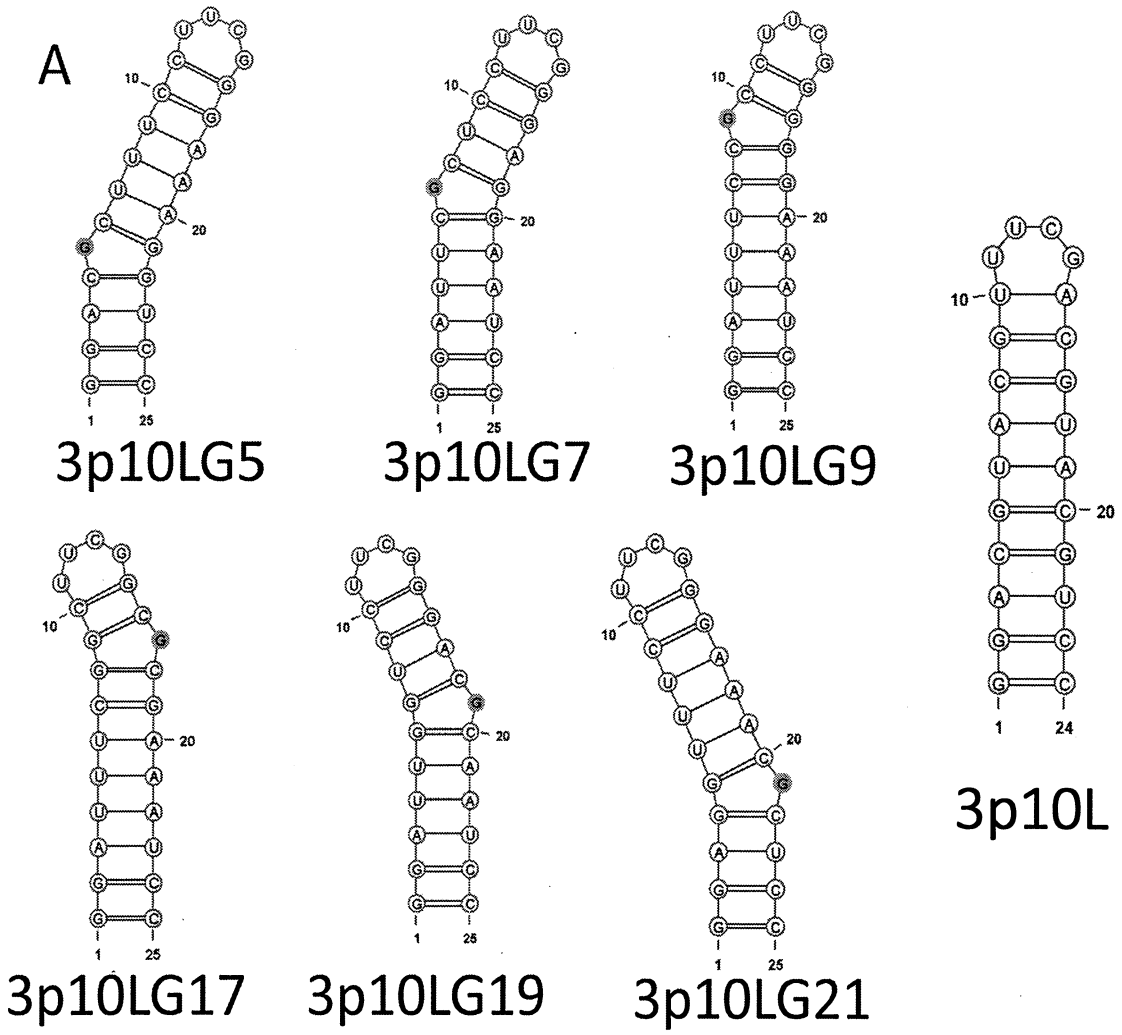
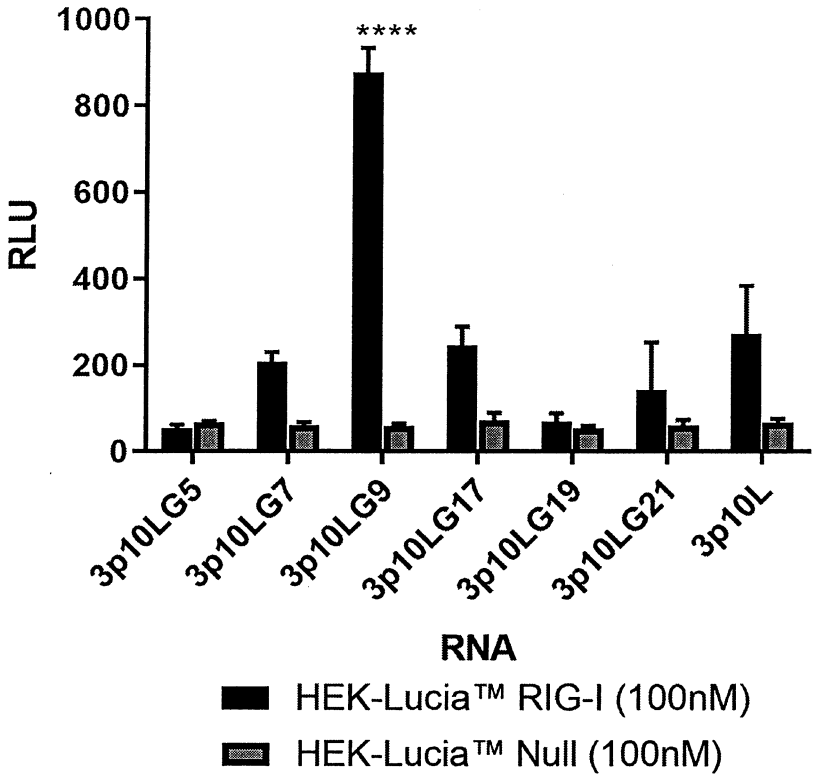
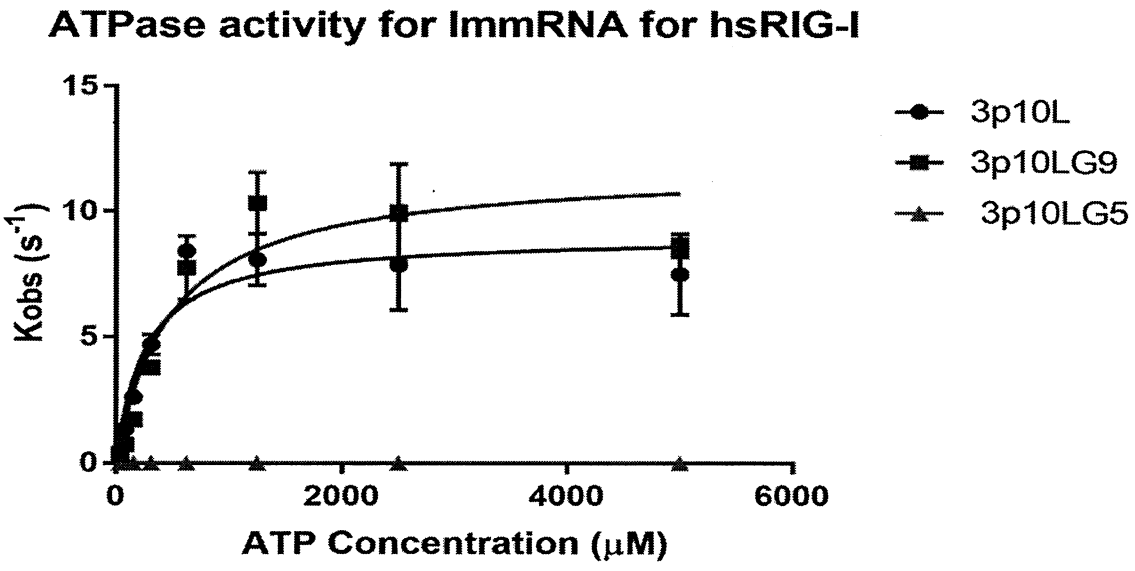



**Figure 1**

B



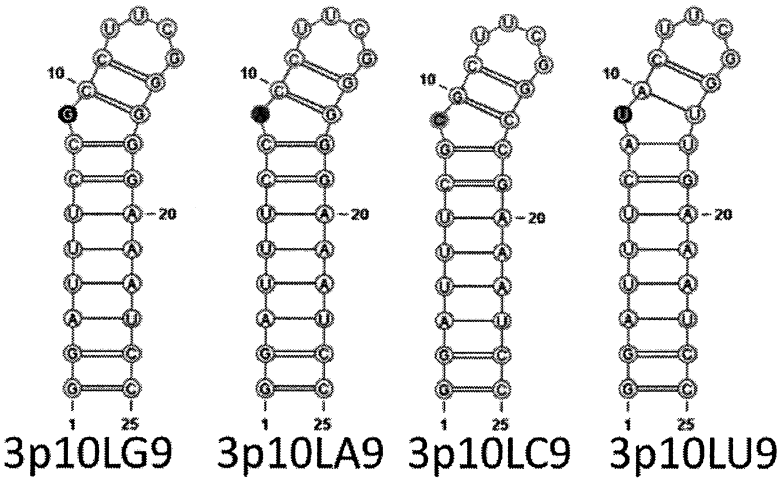

C




| RNA | Kcat($s^{-1}$) | Km (μM) | Kcat/Km ($s^{-1}$ $mM^{-1}$) |
|---|---|---|---|
| 3p10L | 9.0±0.6 | 261.8±67.1 | 34.4±8.9 |
| 3p10LG9 | 11.7±1 | 484±129.5 | 25.3±7.8 |

**Figure 1 (continued)**

A


10
20
1
25
3p10LG9
3p10LA9
3p10LC9
3p10LU9

B

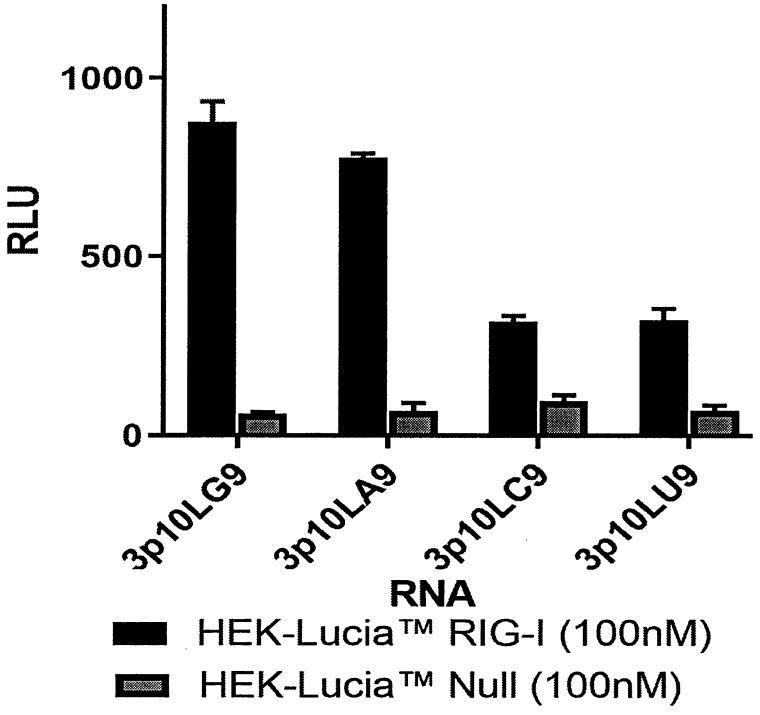
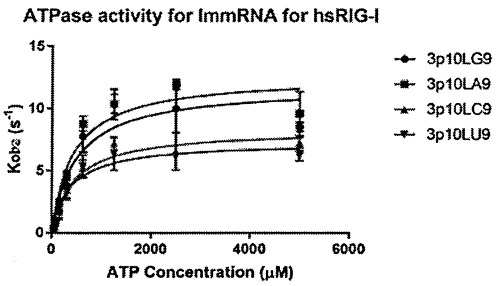

1000
500
0
RLU
3p10LG9
3p10LA9
3p10LC9
3p10LU9
RNA
HEK-Lucia™ RIG-I (100nM)
HEK-Lucia™ Null (100nM)

**Figure 2**

C




| RNA | Kcat($s^{-1}$) | Km (μM) | Kcat/Km ($s^{-1}$ $mM^{-1}$) |
|---|---|---|---|
| 3p10LG9 | 11.7±1 | 484±129.5 | 25.3±7.8 |
| 3p10LA9 | 12.6±0.8 | 429.6±91.8 | 29.3±8.6 |
| 3p10LC9 | 8.3±0.5 | 439.8±88.6 | 18.9±5.6 |
| 3p10LU9 | 7.3±0.4 | 377.6±76.3 | 19.3±5.5 |

**Figure 2 (continued)**

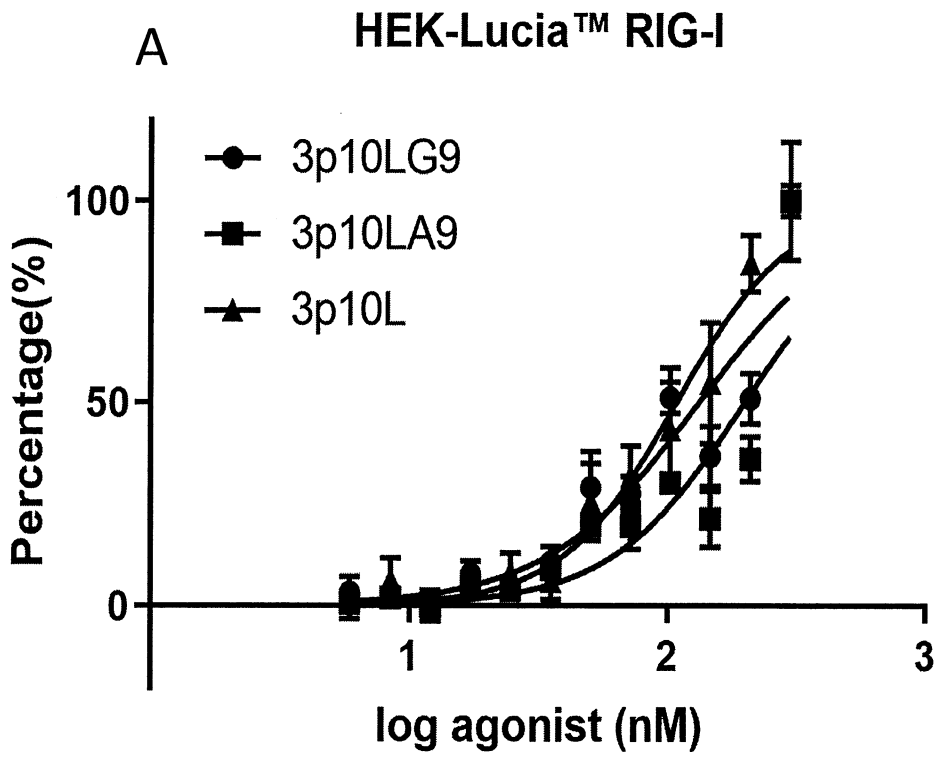
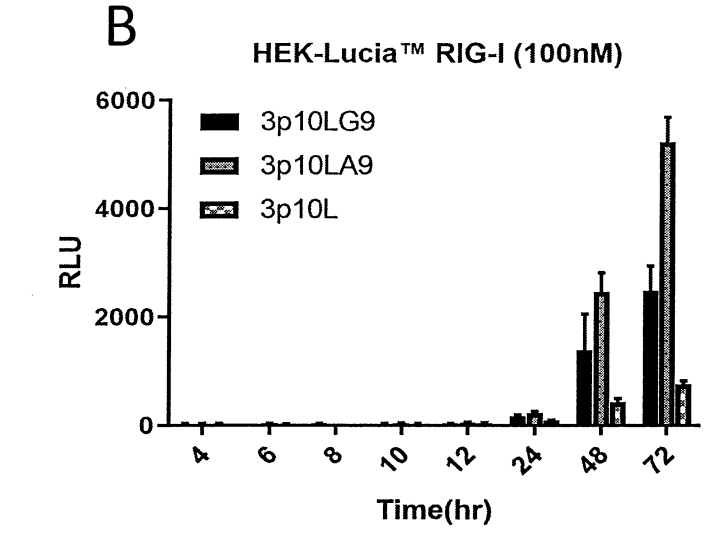
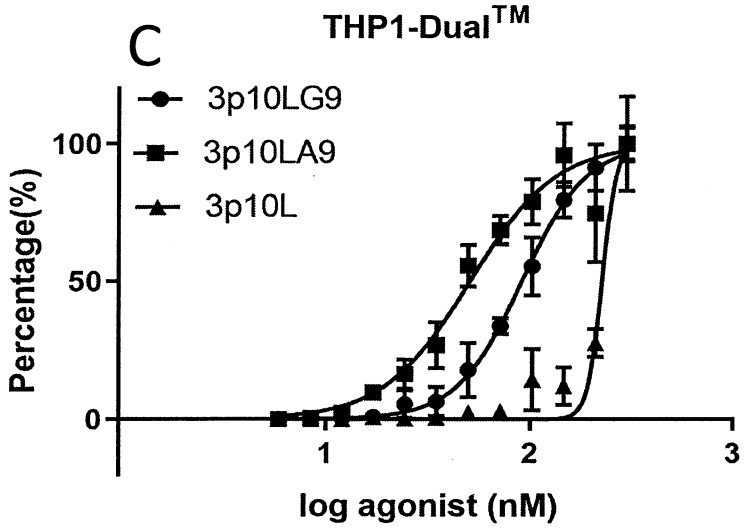



**Figure 3**



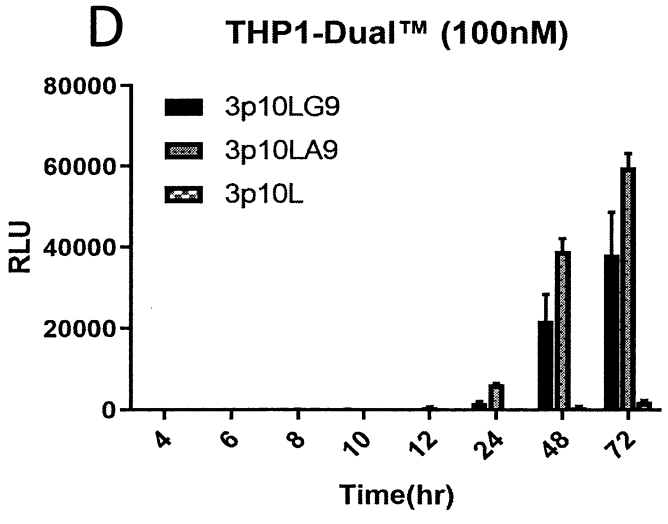
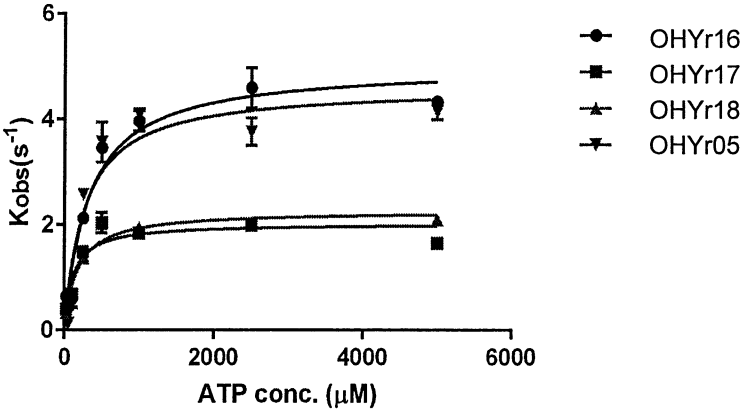




**Figure 3 (continued)**

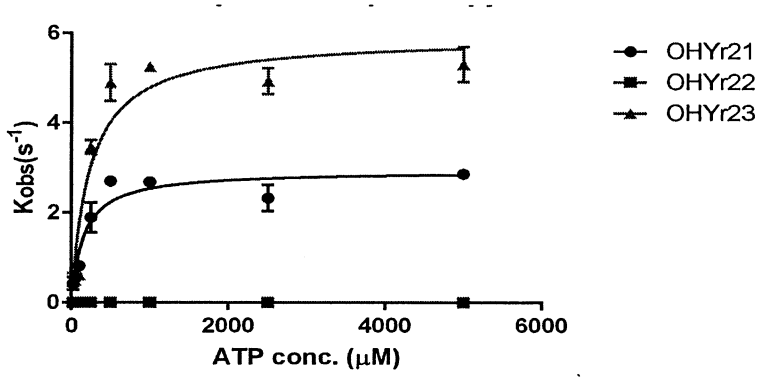
A

ATPase activity for ImmRNA stem modification




B

ATPase activity for ImmRNA with different base composition




C

ATPase rate vs Substrate concentration for mmRIG-I with RNA of different length

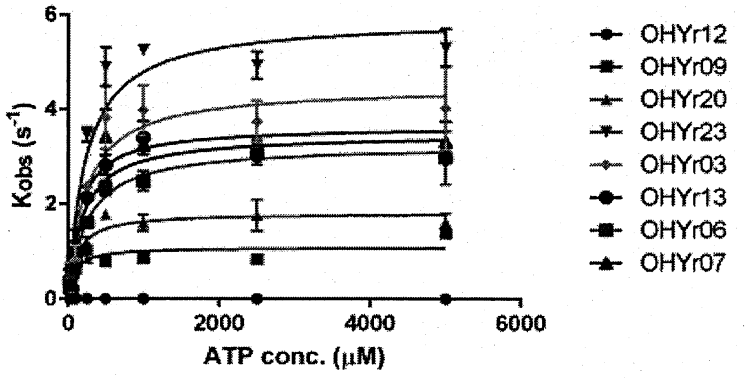
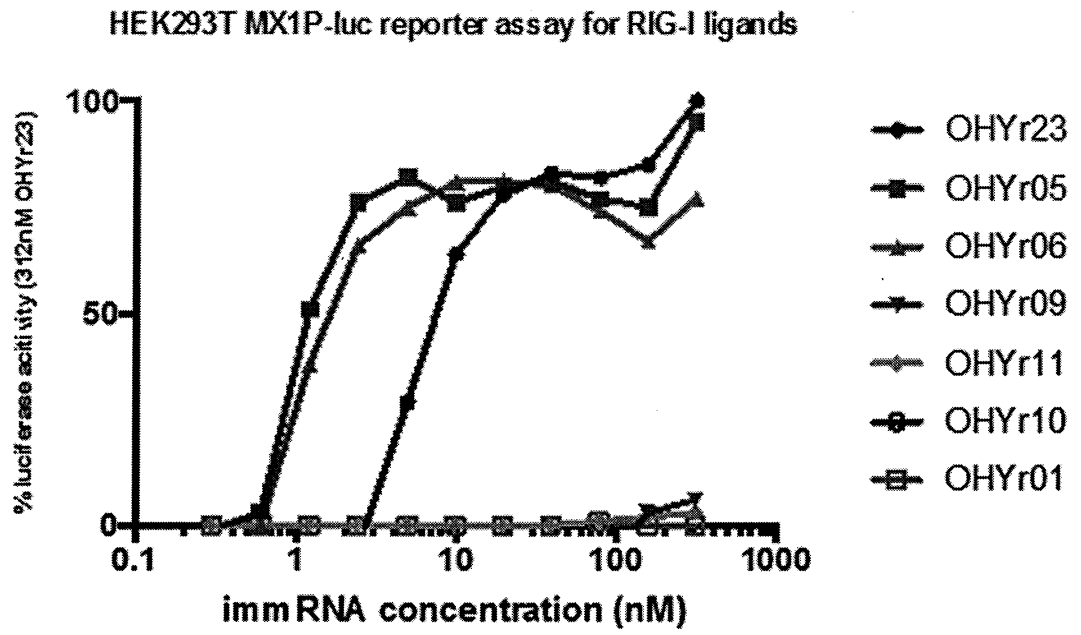



Figure 4

A


HEK293T MX1P-luc reporter assay for RIG-I ligands
% luciferase activity (312nM OHYr23)
100
50
0
0.1
1
10
100
1000
imm RNA concentration (nM)
OHYr23
OHYr05
OHYr06
OHYr09
OHYr11
OHYr10
OHYr01

B

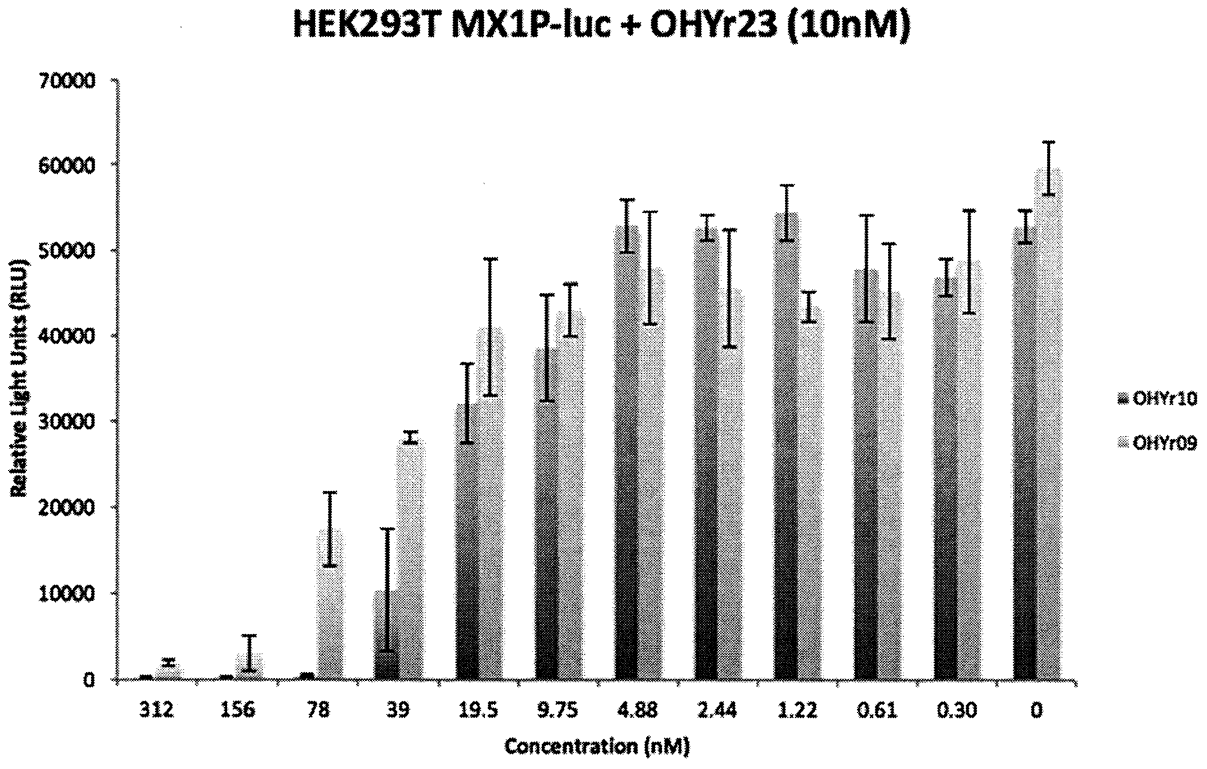
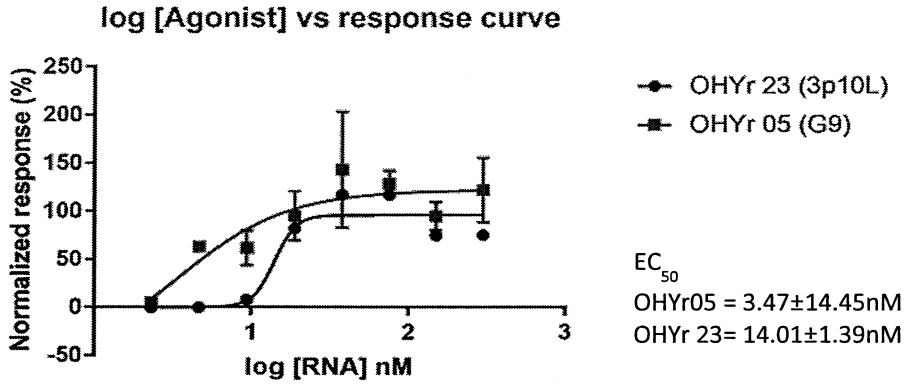
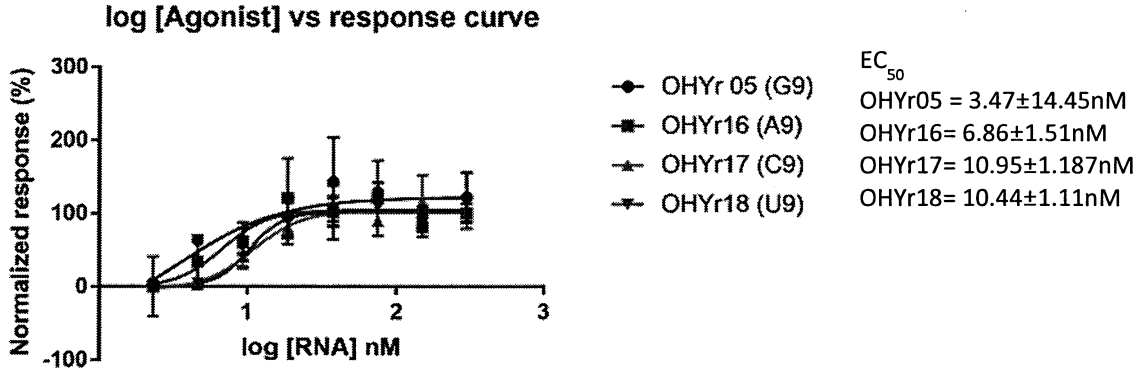

HEK293T MX1P-luc + OHYr23 (10nM)
Relative Light Units (RLU)
70000
60000
50000
40000
30000
20000
10000
0
312
156
78
39
19.5
9.75
4.88
2.44
1.22
0.61
0.30
0
Concentration (nM)
OHYr10
OHYr09

Figure 5

A


log [Agonist] vs response curve
Normalized response (%)
250
200
150
100
50
0
-50
1
2
3
log [RNA] nM
OHYr 23 (3p10L)
OHYr 05 (G9)
EC50
OHYr05 = 3.47±14.45nM
OHYr 23= 14.01±1.39nM

B


log [Agonist] vs response curve
Normalized response (%)
300
200
100
0
-100
1
2
3
log [RNA] nM
OHYr 05 (G9)
OHYr16 (A9)
OHYr17 (C9)
OHYr18 (U9)
EC50
OHYr05 = 3.47±14.45nM
OHYr16= 6.86±1.51nM
OHYr17= 10.95±1.187nM
OHYr18= 10.44±1.11nM

C

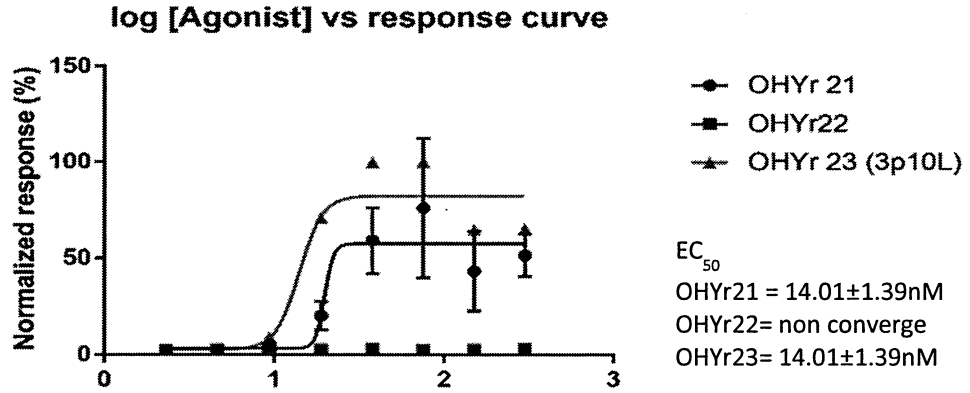
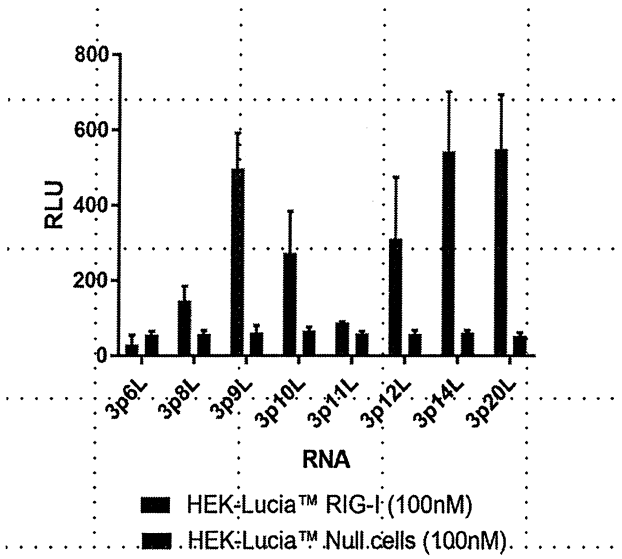
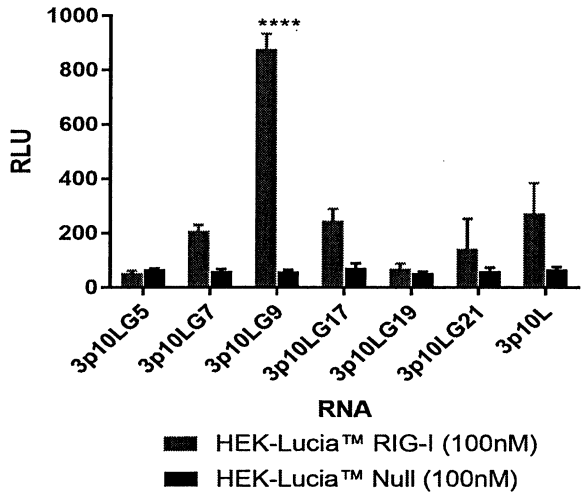

log [Agonist] vs response curve
Normalized response (%)
150
100
50
0
0
1
2
3
OHYr 21
OHYr22
OHYr 23 (3p10L)
EC50
OHYr21 = 14.01±1.39nM
OHYr22= non converge
OHYr23= 14.01±1.39nM

**Figure 6**

A


800
600
400
200
0
RLU
3p6L
3p8L
3p9L
3p10L
3p11L
3p12L
3p14L
3p20L
RNA
HEK-Lucia™ RIG-I (100nM)
HEK-Lucia™ Null cells (100nM)

B


1000
800
600
400
200
0
RLU
****
3p10LG5
3p10LG7
3p10LG9
3p10LG17
3p10LG19
3p10LG21
3p10L
RNA
HEK-Lucia™ RIG-I (100nM)
HEK-Lucia™ Null (100nM)

C

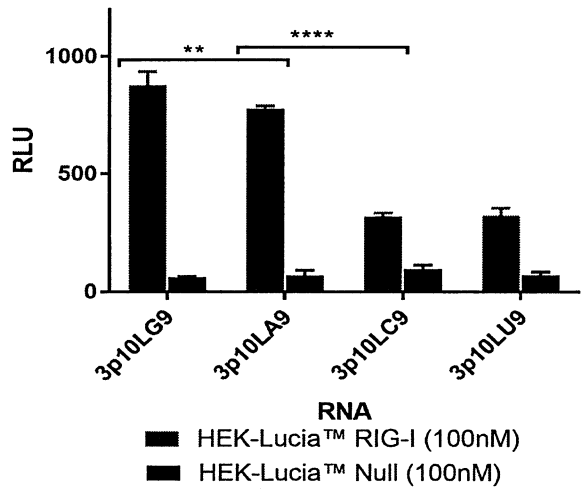
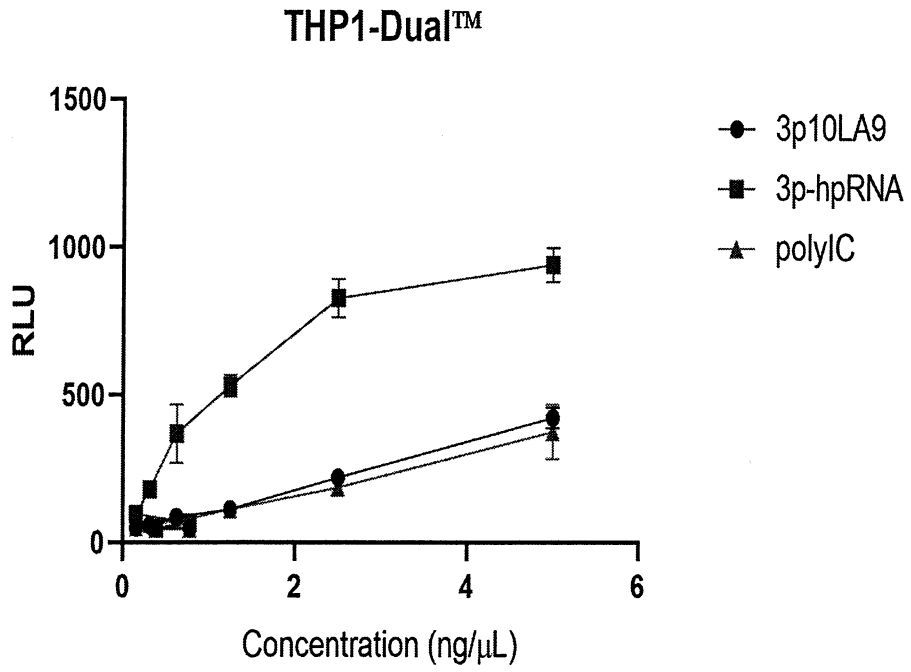

1000
500
0
RLU
**
****
3p10LG9
3p10LA9
3p10LC9
3p10LU9
RNA
HEK-Lucia™ RIG-I (100nM)
HEK-Lucia™ Null (100nM)

**Figure 7**

A


THP1-Dual™
1500
1000
500
0
RLU
0
2
4
6
Concentration (ng/µL)
3p10LA9
3p-hpRNA
polyIC

B

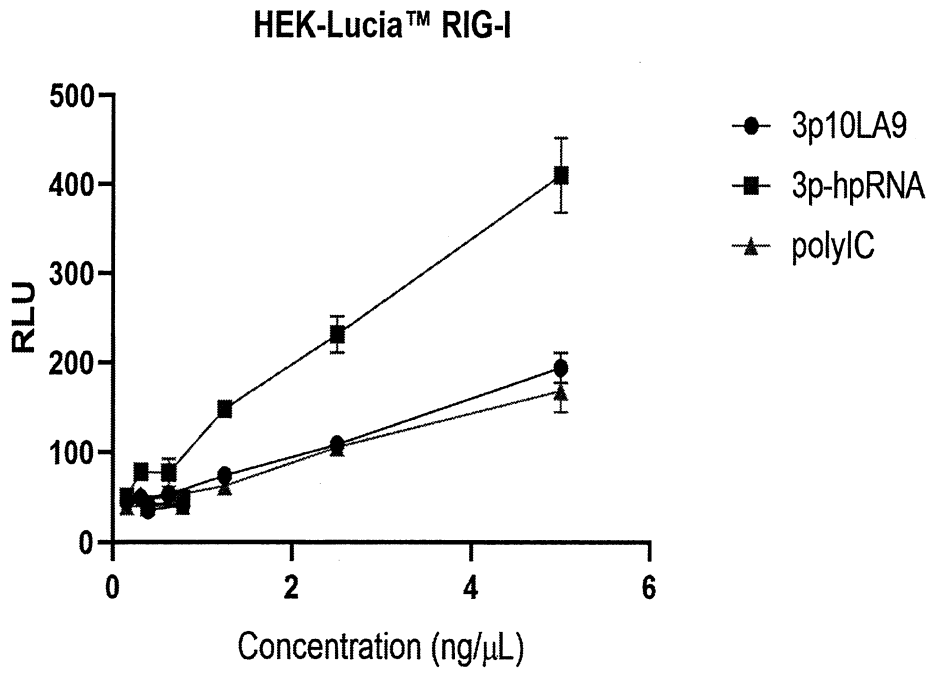
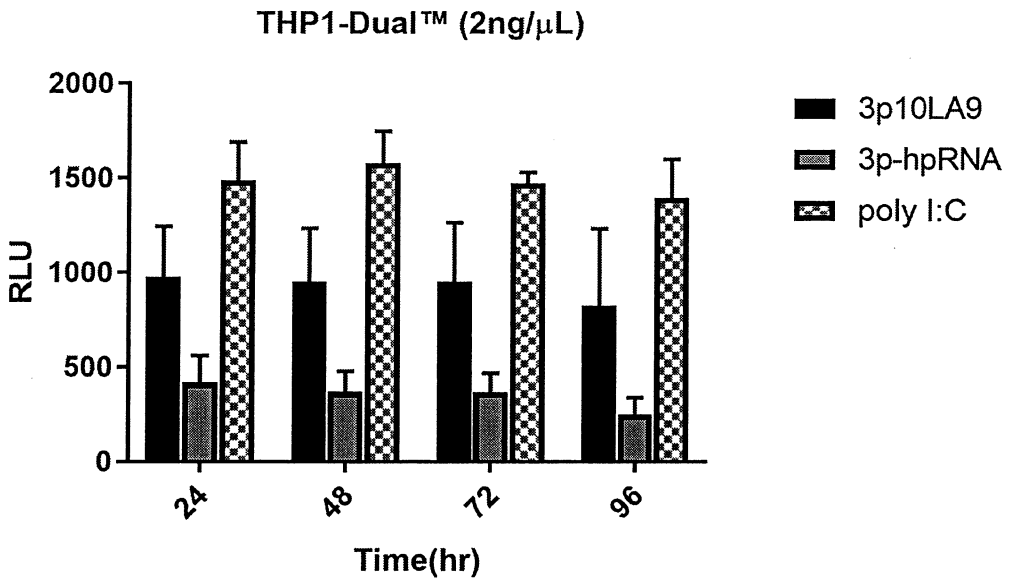

HEK-Lucia™ RIG-I
500
400
300
200
100
0
RLU
0
2
4
6
Concentration (ng/µL)
3p10LA9
3p-hpRNA
polyIC

**Figure 8**

A


THP1-Dual™ (2ng/μL)
RLU
2000
1500
1000
500
0
24
48
72
96
Time(hr)
3p10LA9
3p-hpRNA
poly I:C

B

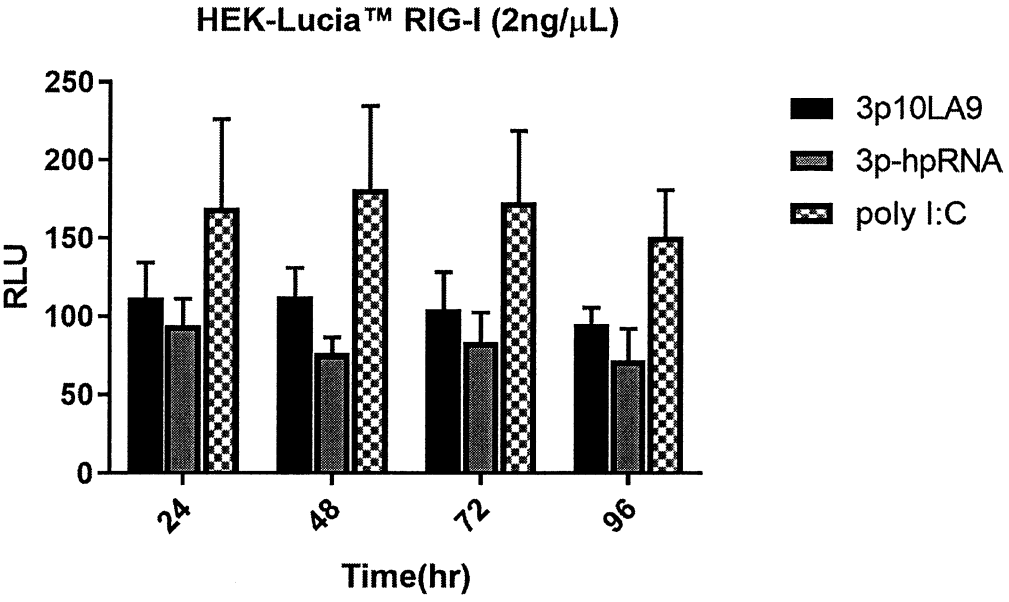
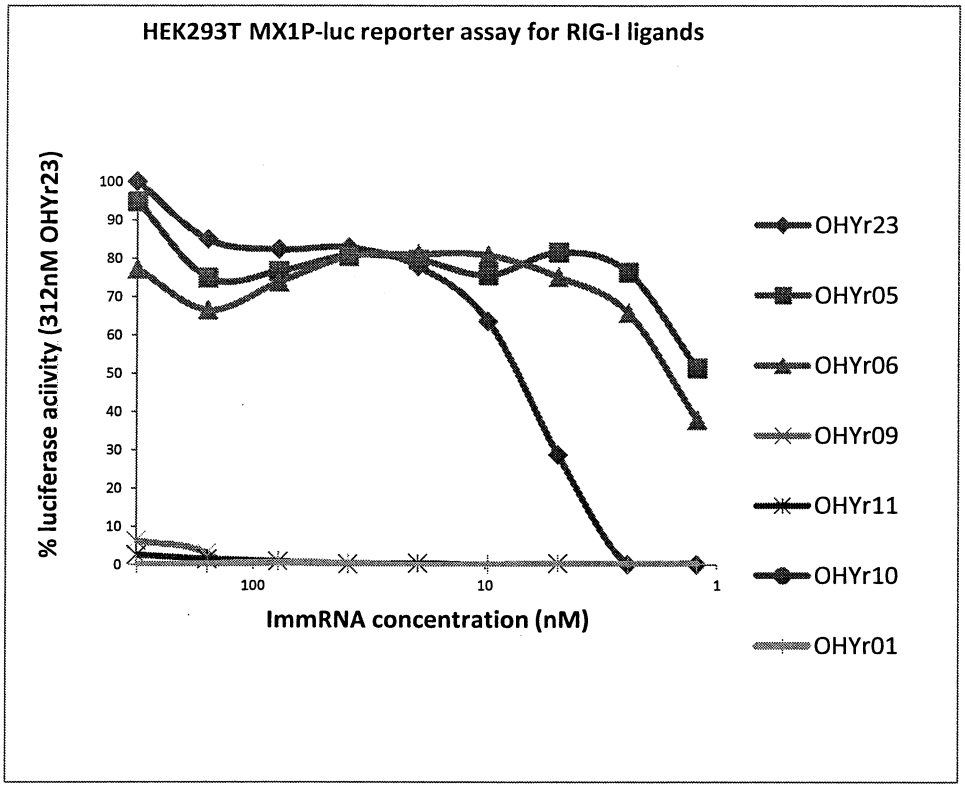

HEK-Lucia™ RIG-I (2ng/μL)
RLU
250
200
150
100
50
0
24
48
72
96
Time(hr)
3p10LA9
3p-hpRNA
poly I:C

**Figure 9**

A


HEK293T MX1P-luc reporter assay for RIG-I ligands
% luciferase aciivity (312nM OHYr23)
100
90
80
70
60
50
40
30
20
10
0
100
10
1
ImmRNA concentration (nM)
OHYr23
OHYr05
OHYr06
OHYr09
OHYr11
OHYr10
OHYr01

B

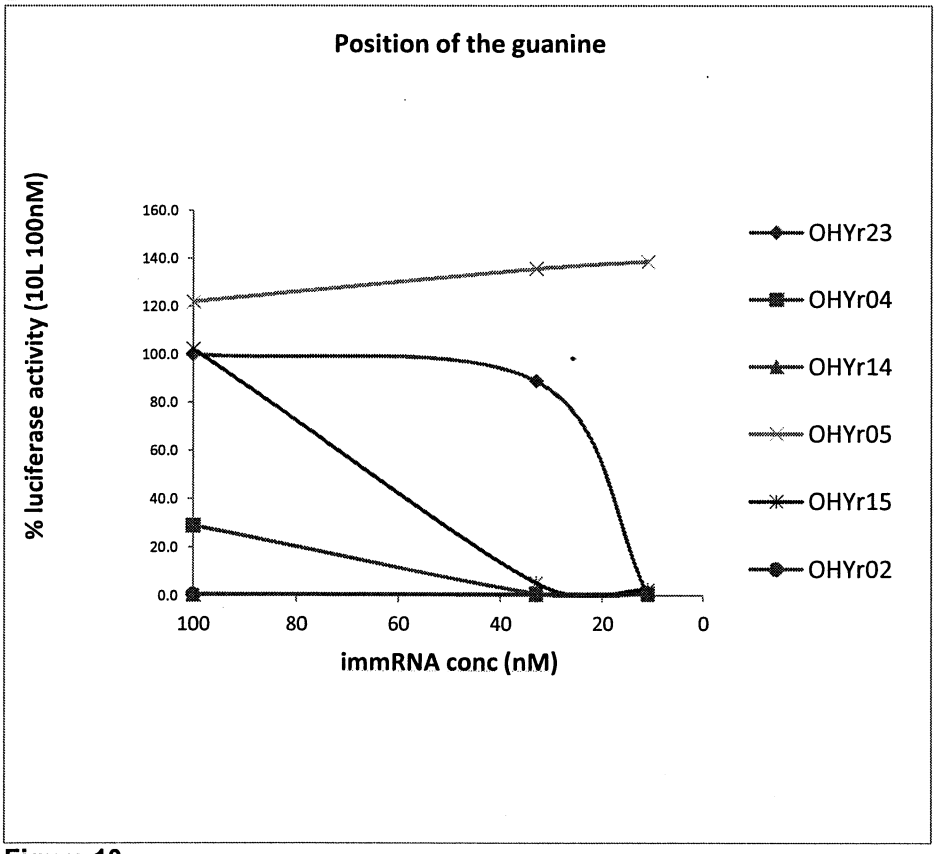
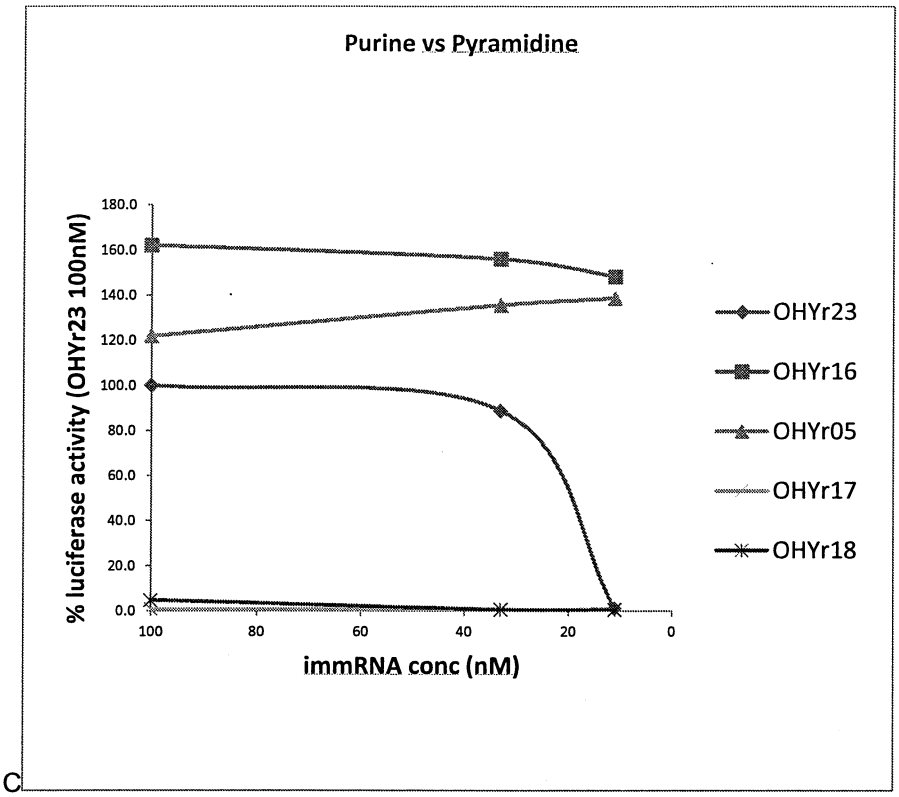

Position of the guanine
% luciferase activity (10L 100nM)
160.0
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
100
80
60
40
20
0
immRNA conc (nM)
OHYr23
OHYr04
OHYr14
OHYr05
OHYr15
OHYr02

**Figure 10**




C

**Figure 10 (continued)**

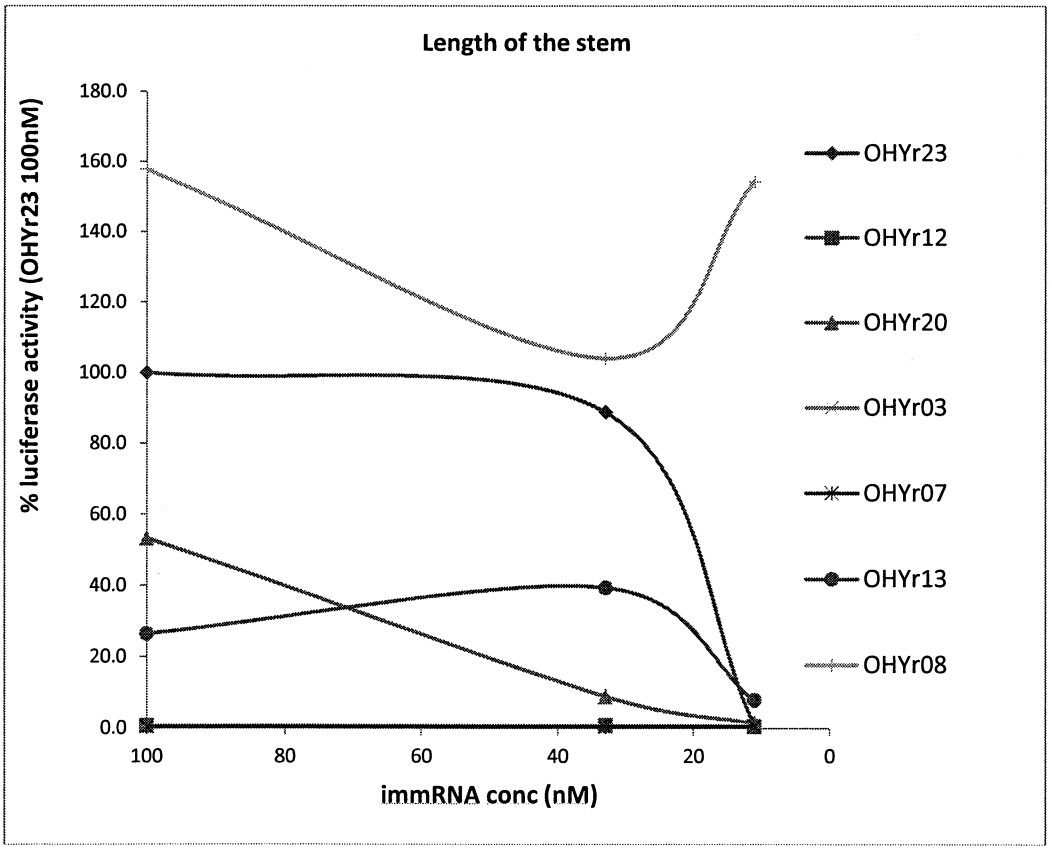



**Figure 11**

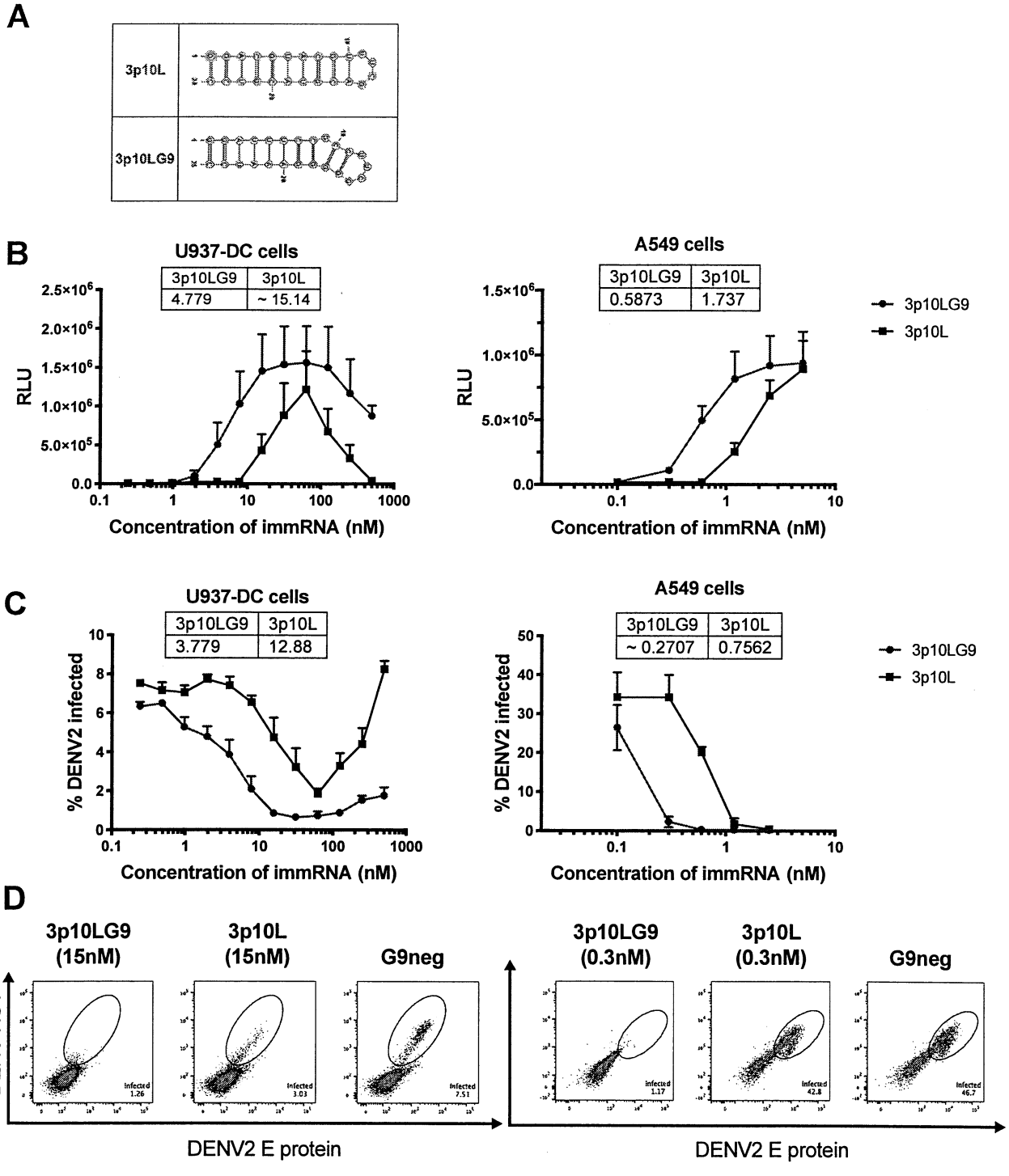
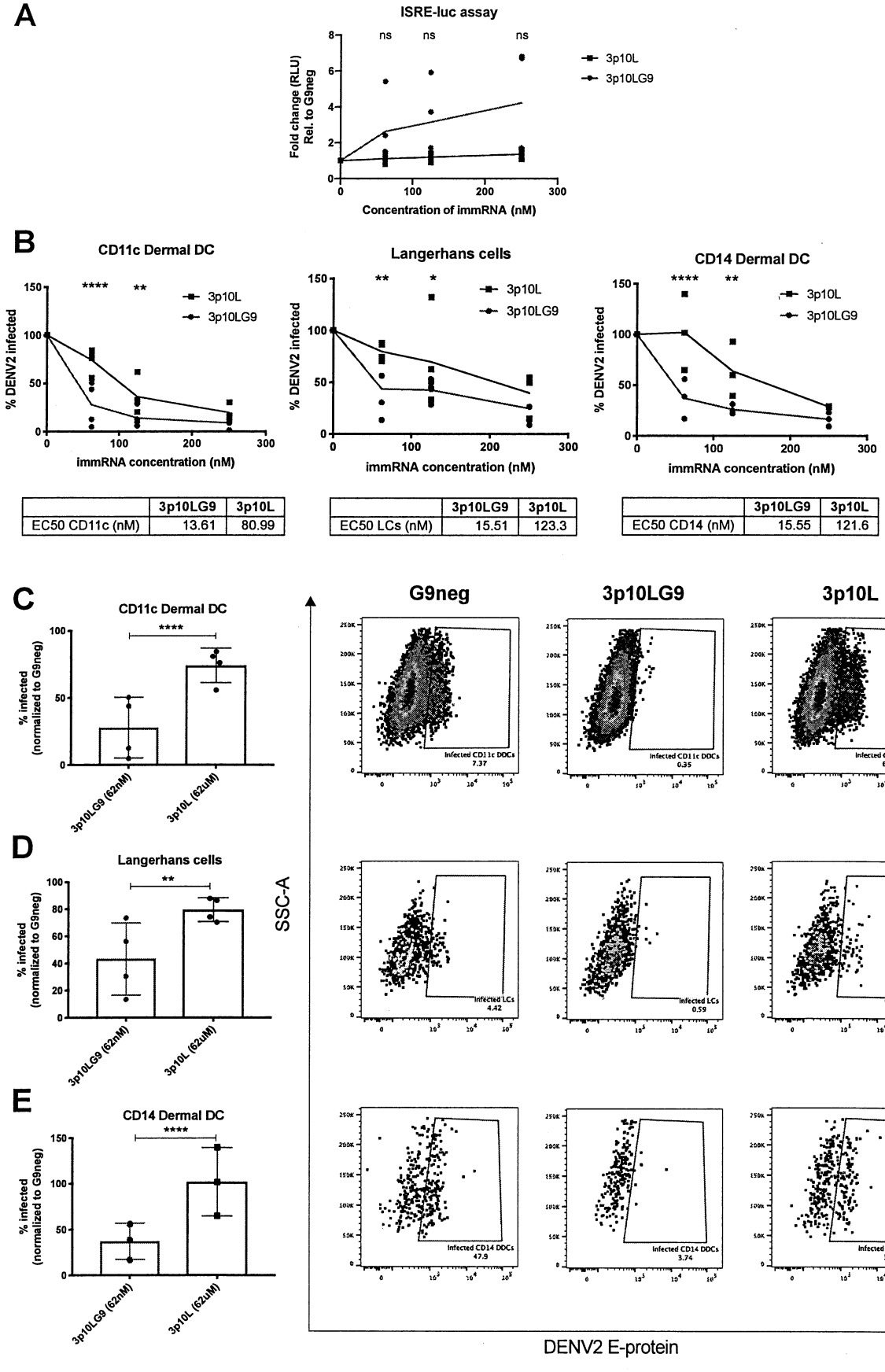

A
3p10L
3p10LG9
B
U937-DC cells
3p10LG9 | 3p10L
4.779 | ~ 15.14
RLU
Concentration of immRNA (nM)
A549 cells
3p10LG9 | 3p10L
0.5873 | 1.737
RLU
Concentration of immRNA (nM)
3p10LG9
3p10L
C
U937-DC cells
3p10LG9 | 3p10L
3.779 | 12.88
% DENV2 infected
Concentration of immRNA (nM)
A549 cells
3p10LG9 | 3p10L
~ 0.2707 | 0.7562
% DENV2 infected
Concentration of immRNA (nM)
3p10LG9
3p10L
D
3p10LG9 (15nM)
3p10L (15nM)
G9neg
3p10LG9 (0.3nM)
3p10L (0.3nM)
G9neg
DENV NS1
DENV2 E protein
DENV2 E protein

**Figure 12**




| | 3p10LG9 | 3p10L |
|---|---|---|
| EC50 CD11c (nM) | 13.61 | 80.99 |

| | 3p10LG9 | 3p10L |
|---|---|---|
| EC50 LCs (nM) | 15.51 | 123.3 |

| | 3p10LG9 | 3p10L |
|---|---|---|
| EC50 CD14 (nM) | 15.55 | 121.6 |

**Figure 13**

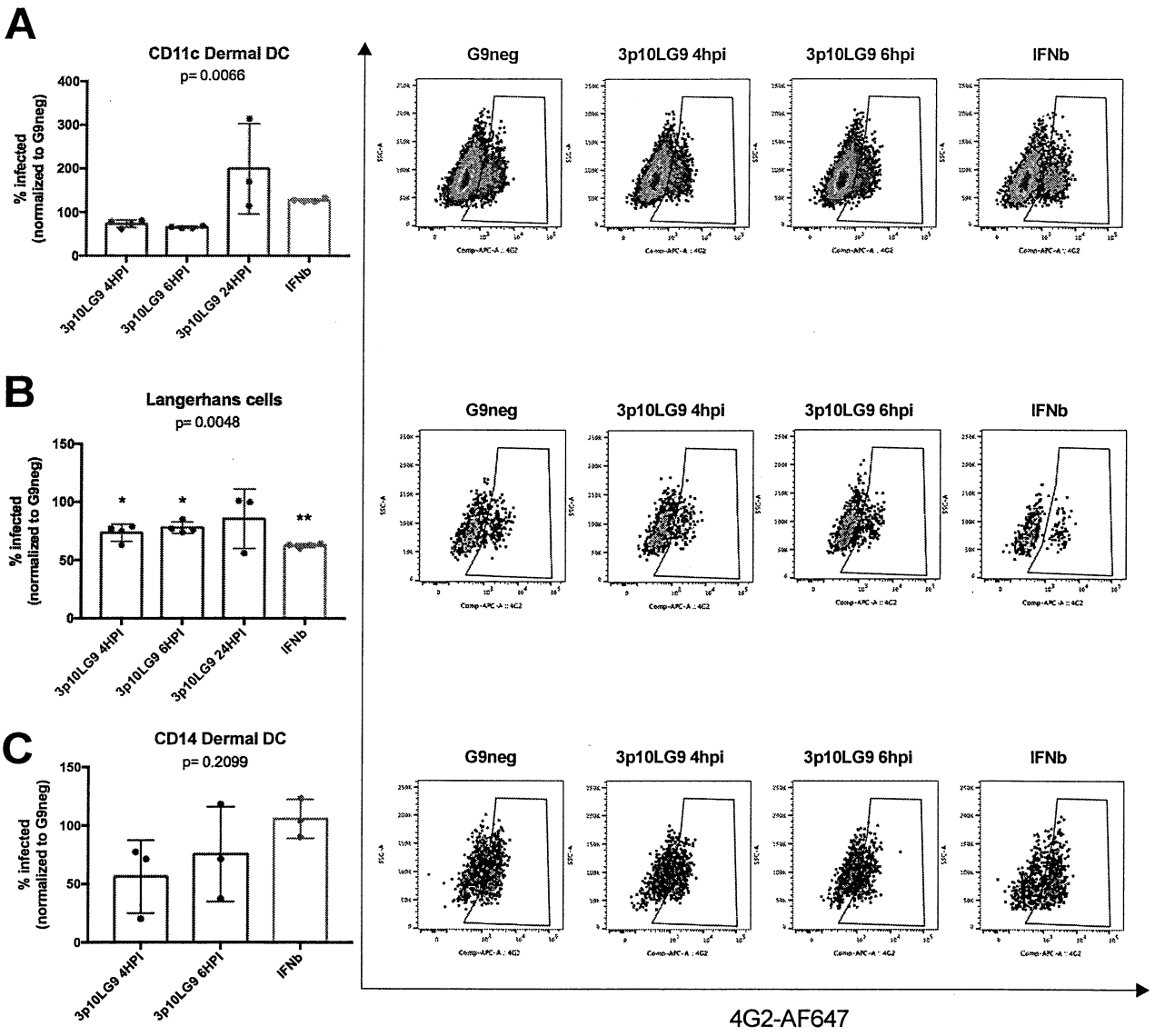

A
CD11c Dermal DC
p= 0.0066
% infected (normalized to G9neg)
400
300
200
100
0
3p10LG9 4HPI
3p10LG9 6HPI
3p10LG9 24HPI
IFNb
B
Langerhans cells
p= 0.0048
% infected (normalized to G9neg)
150
100
50
0
*
*
**
3p10LG9 4HPI
3p10LG9 6HPI
3p10LG9 24HPI
IFNb
C
CD14 Dermal DC
p= 0.2099
% infected (normalized to G9neg)
150
100
50
0
3p10LG9 4HPI
3p10LG9 6HPI
IFNb
G9neg
3p10LG9 4hpi
3p10LG9 6hpi
IFNb
SSC-A
Comp-APC-A :: 4G2
4G2-AF647

**Figure 14**

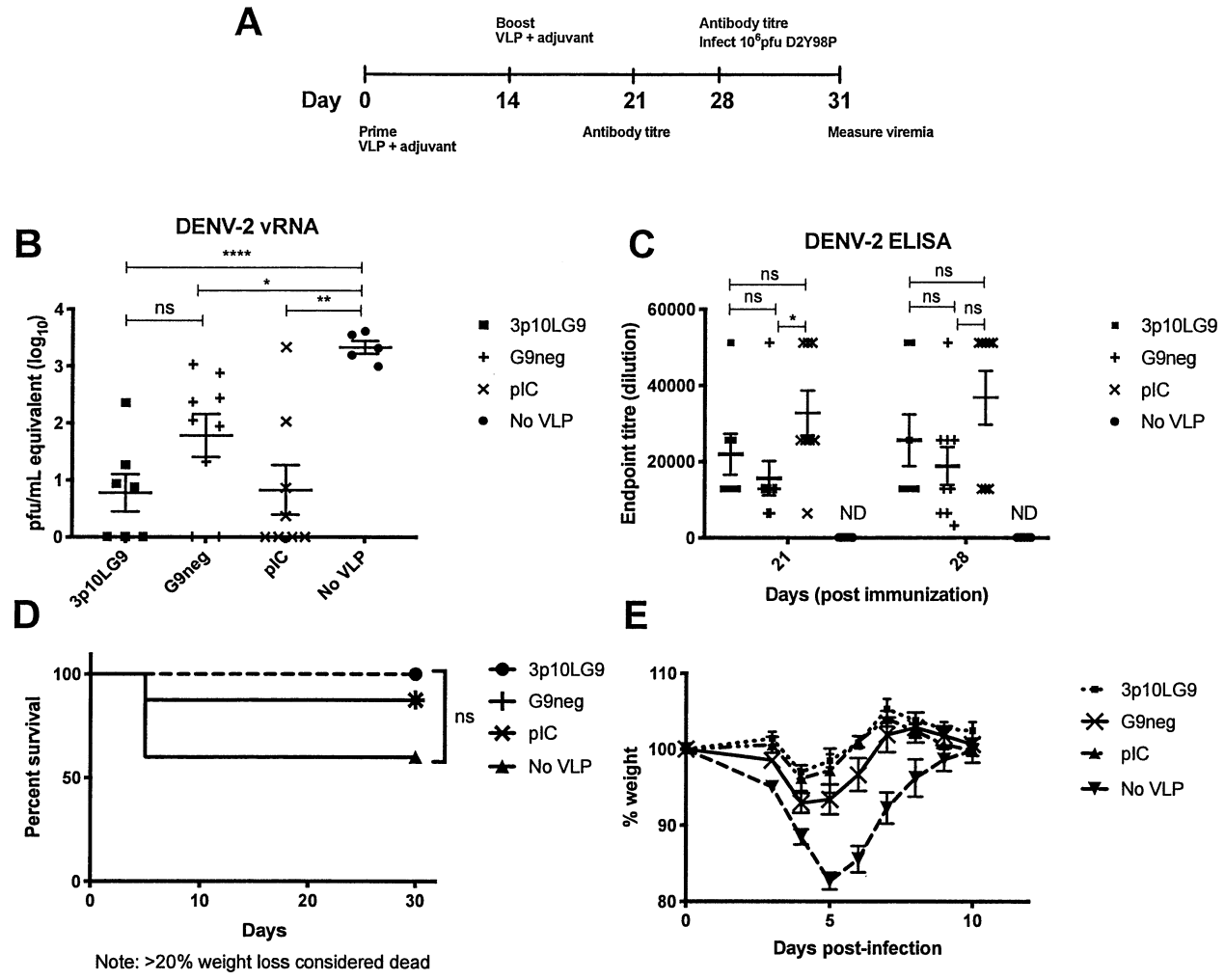



Figure 15

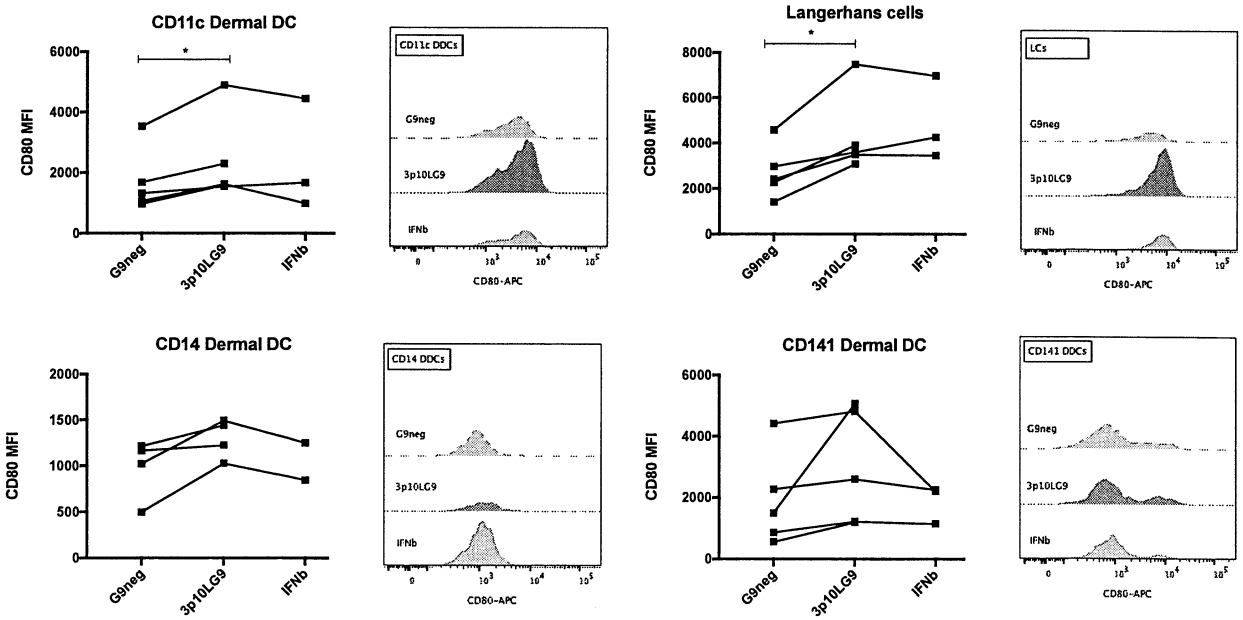



Figure 16

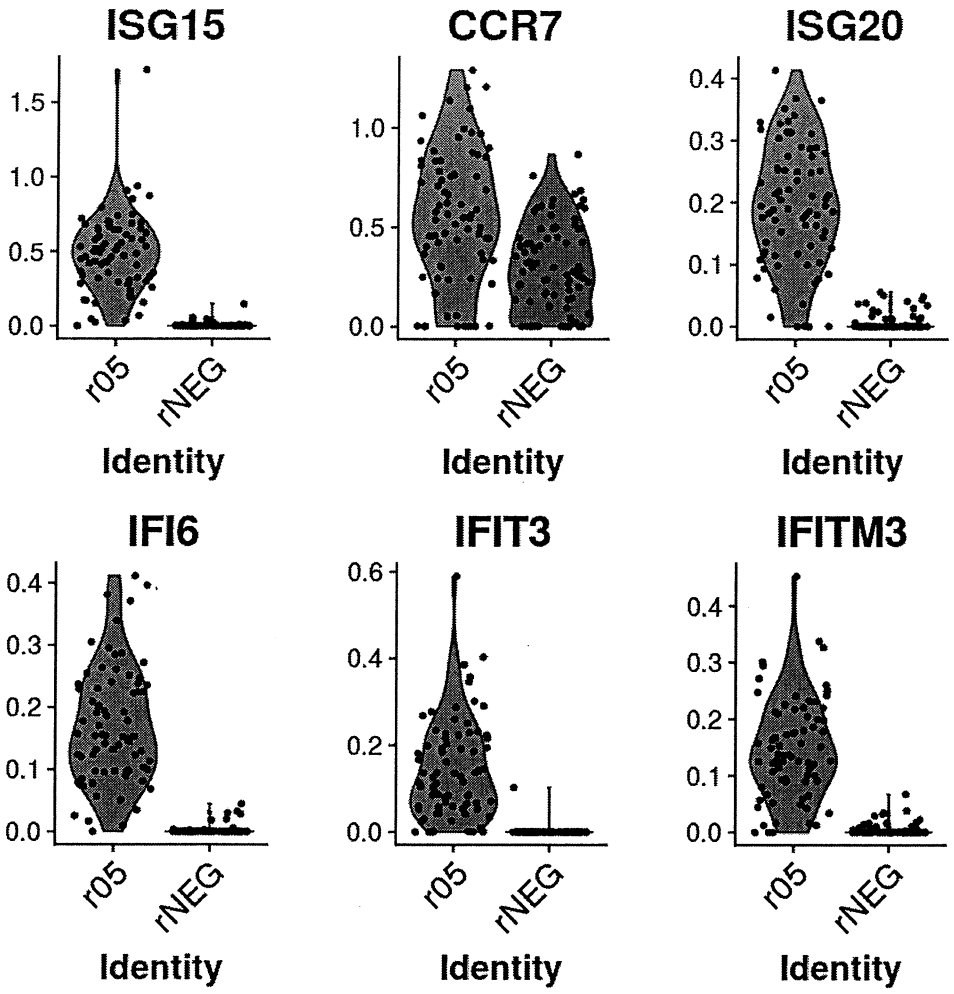



**Figure 17**

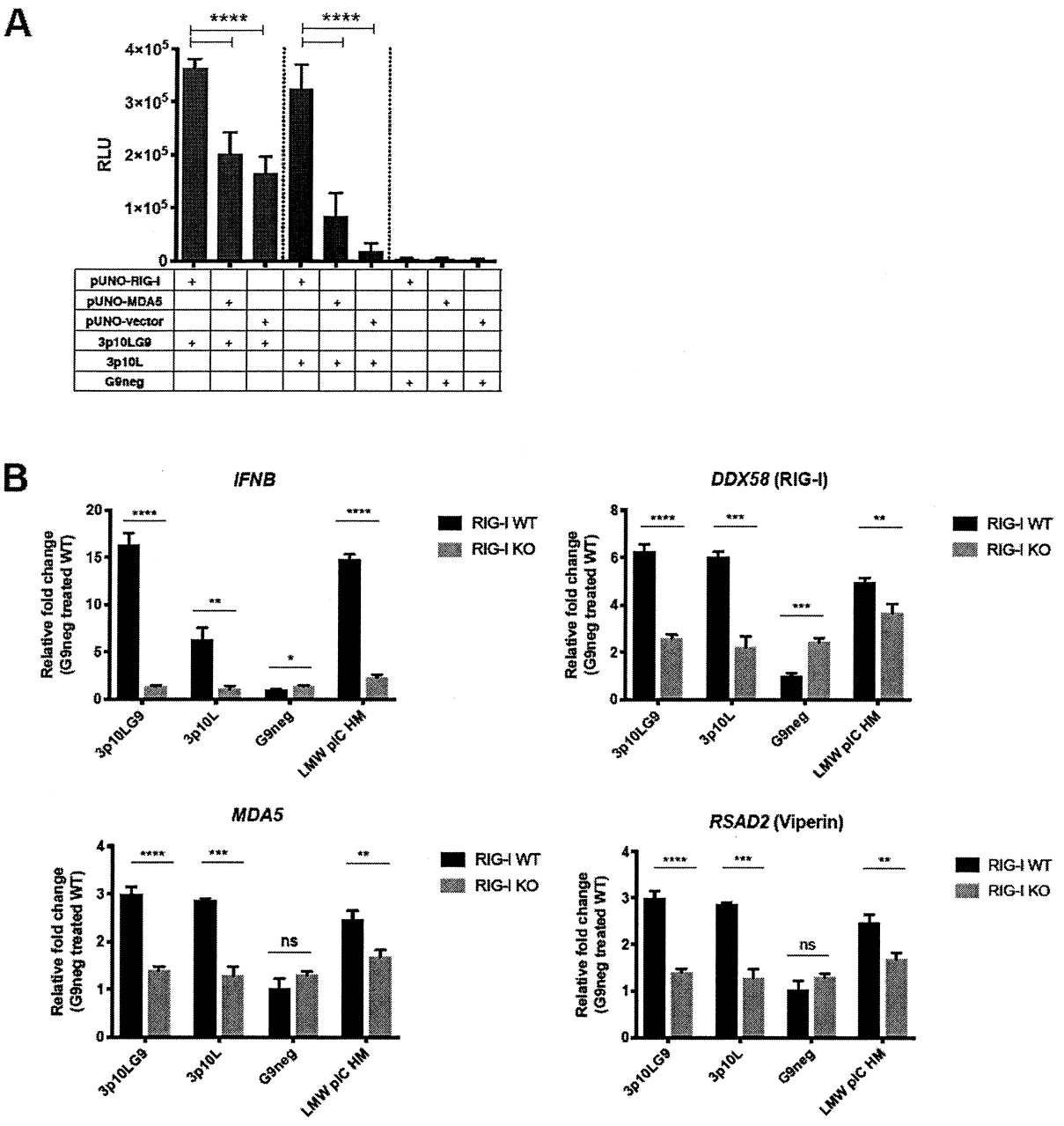

A
****
****
4×10^5
3×10^5
2×10^5
1×10^5
0
RLU
pUNO-RIG-I
pUNO-MDA5
pUNO-vector
3p10LG9
3p10L
G9neg
B
IFNB
DDX58 (RIG-I)
MDA5
RSAD2 (Viperin)
Relative fold change (G9neg treated WT)
RIG-I WT
RIG-I KO
3p10LG9
3p10L
G9neg
LMW pIC HM
ns

**Figure 18**

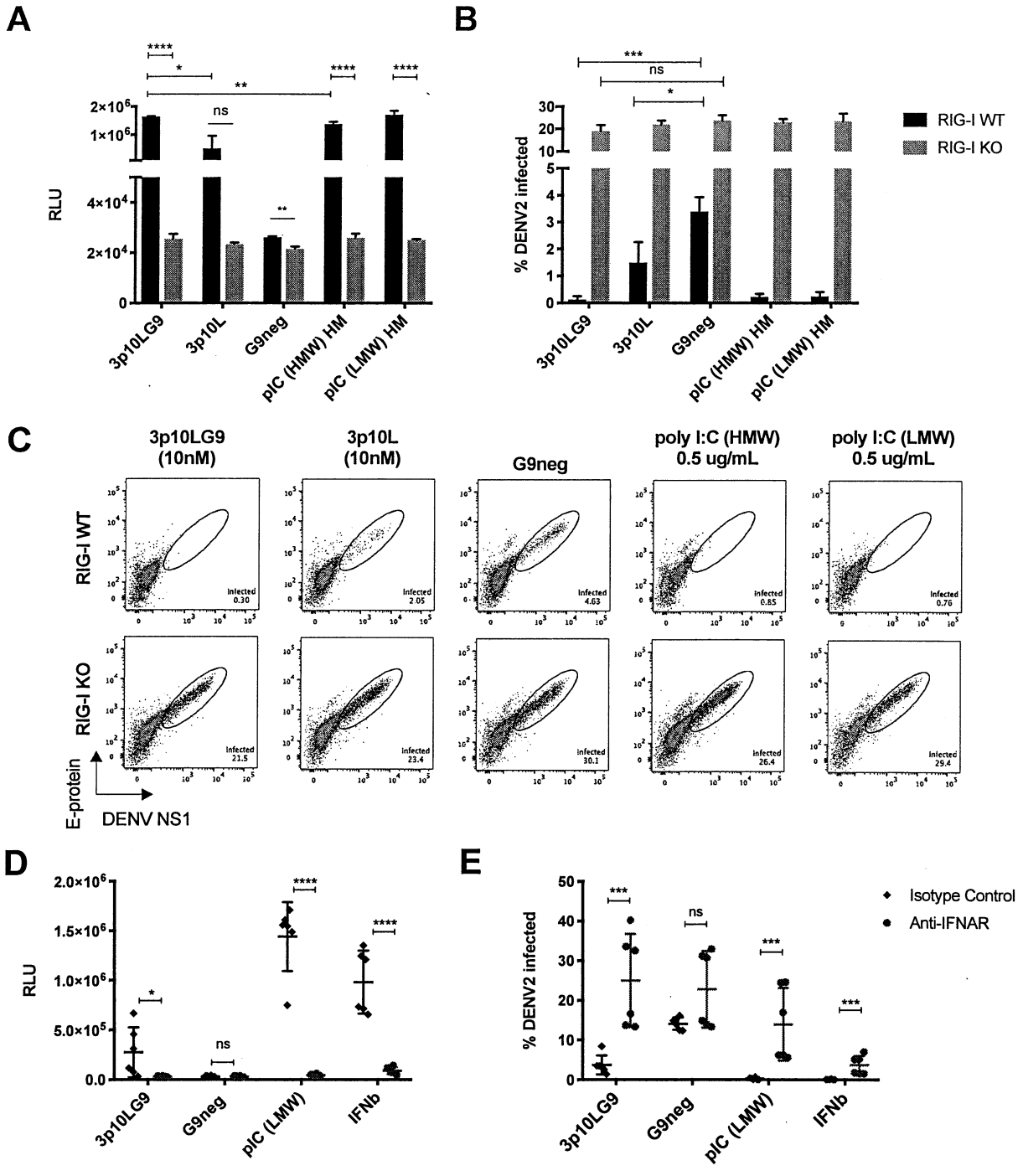

A
B
RLU
% DENV2 infected
RIG-I WT
RIG-I KO
3p10LG9
3p10L
G9neg
pIC (HMW) HM
pIC (LMW) HM
C
3p10LG9 (10nM)
3p10L (10nM)
G9neg
poly I:C (HMW) 0.5 ug/mL
poly I:C (LMW) 0.5 ug/mL
RIG-I WT
RIG-I KO
E-protein
DENV NS1
D
E
Isotype Control
Anti-IFNAR
IFNb

**Figure 19**

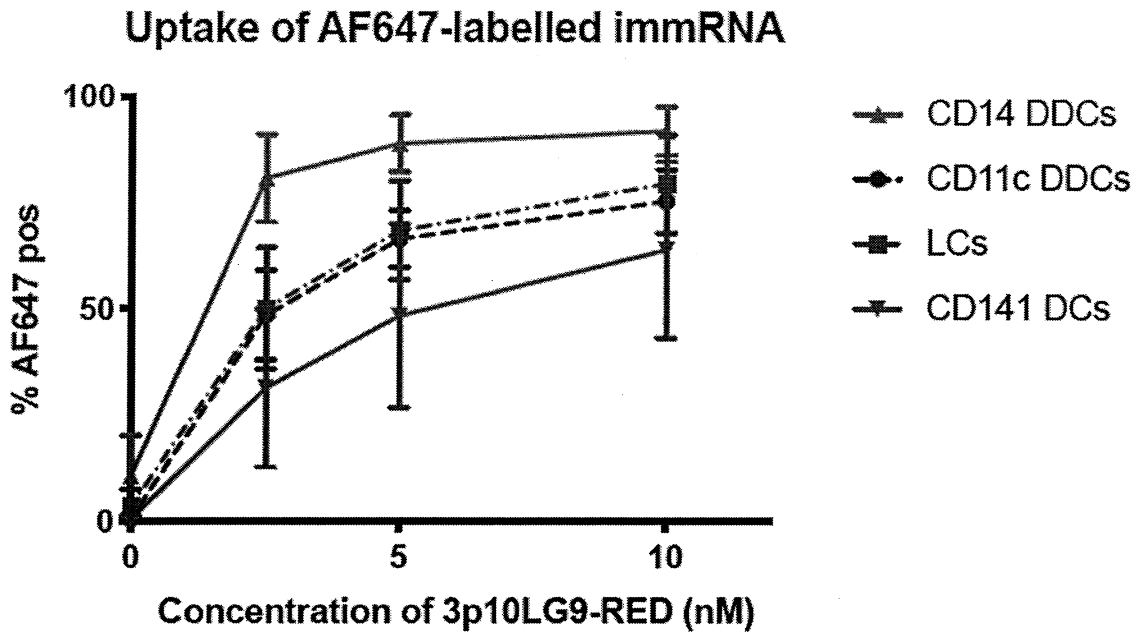



Figure 20

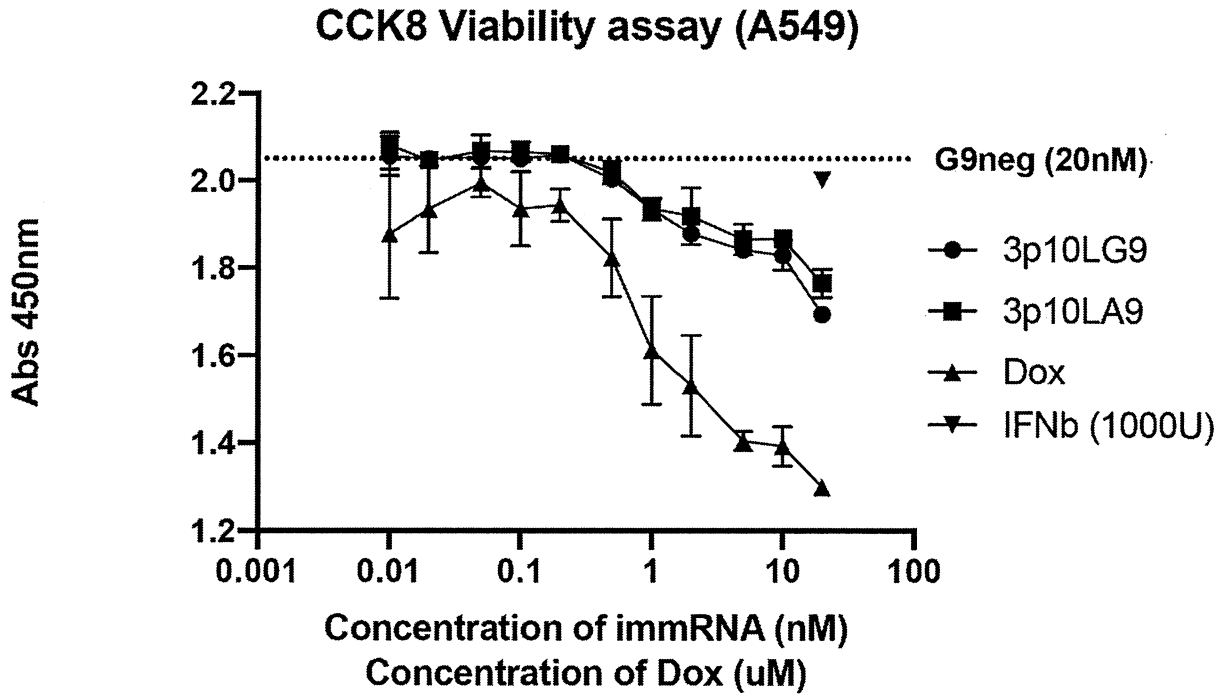



Figure 21