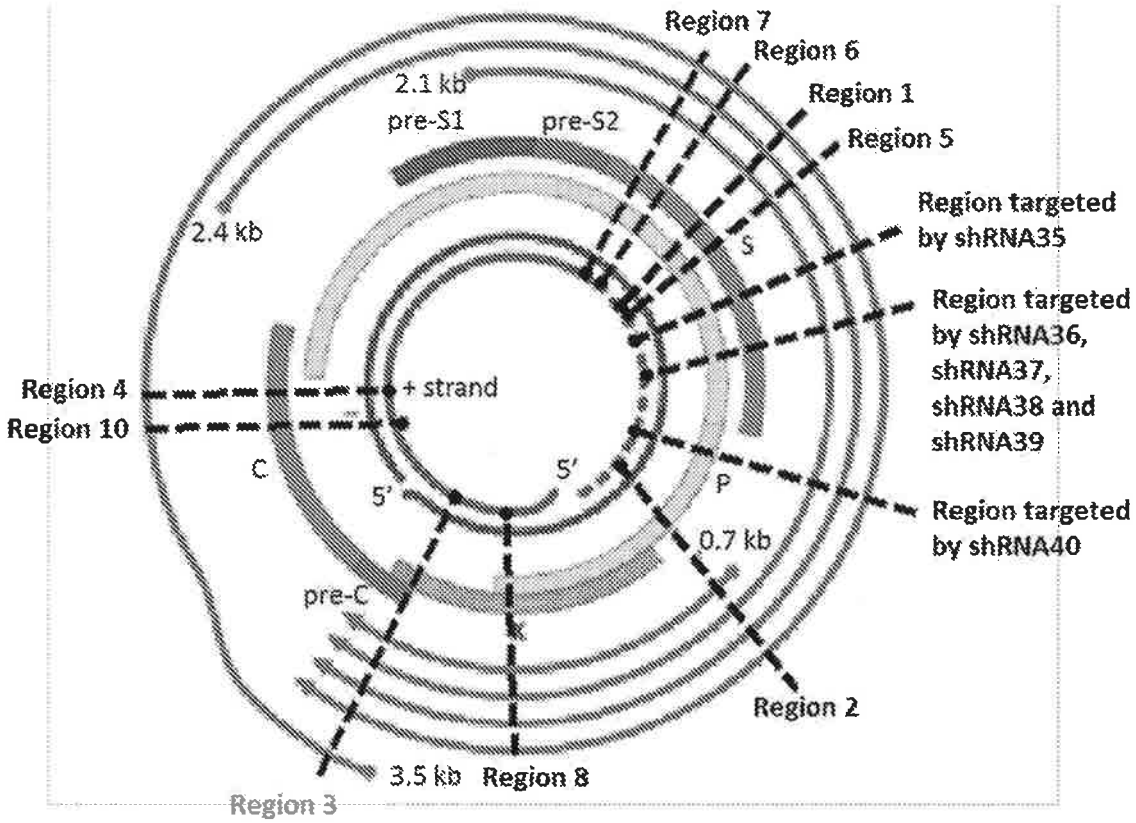
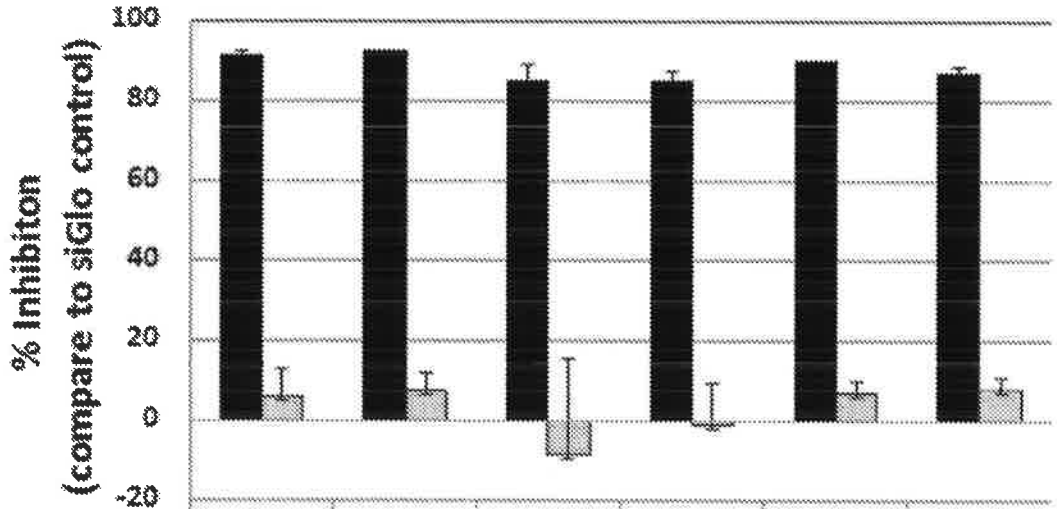
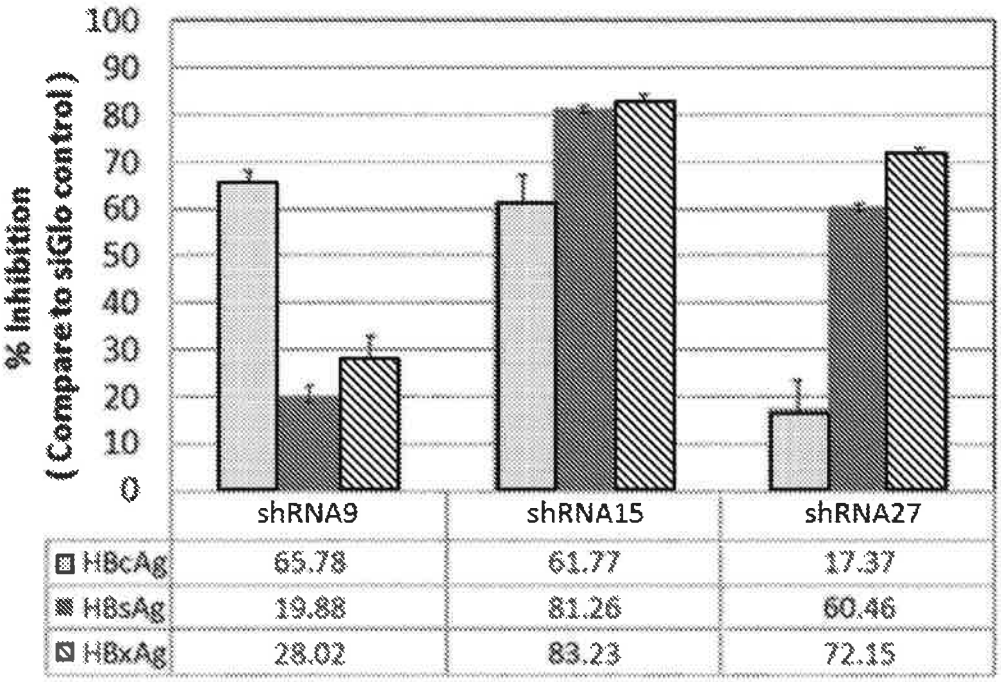

Region 7
Region 6
Region 1
Region 5
2.1 kb
pre-S1
pre-S2
2.4 kb
Region targeted by shRNA35
S
Region targeted by shRNA36, shRNA37, shRNA38 and shRNA39
Region 4
Region 10
+ strand
C
5'
5'
P
Region targeted by shRNA40
0.7 kb
pre-C
Region 2
3.5 kb
Region 8
Region 3

Fig. 1



	Region 5 1pmol	Region 5 2pmol	Region 9 1pmol	Region 9 2pmol	Region 4 1pmol	Region 4 2pmol
■ Anti-Sense	91.47	92.27	84.61	84.75	89.71	86.87
▨ Sense	6.03	7.40	-8.73	-1.29	6.57	7.98

Fig. 2



	shRNA9	shRNA15	shRNA27
HBcAg	65.78	61.77	17.37
HBsAg	19.88	81.26	60.46
HBxAg	28.02	83.23	72.15

Fig. 3

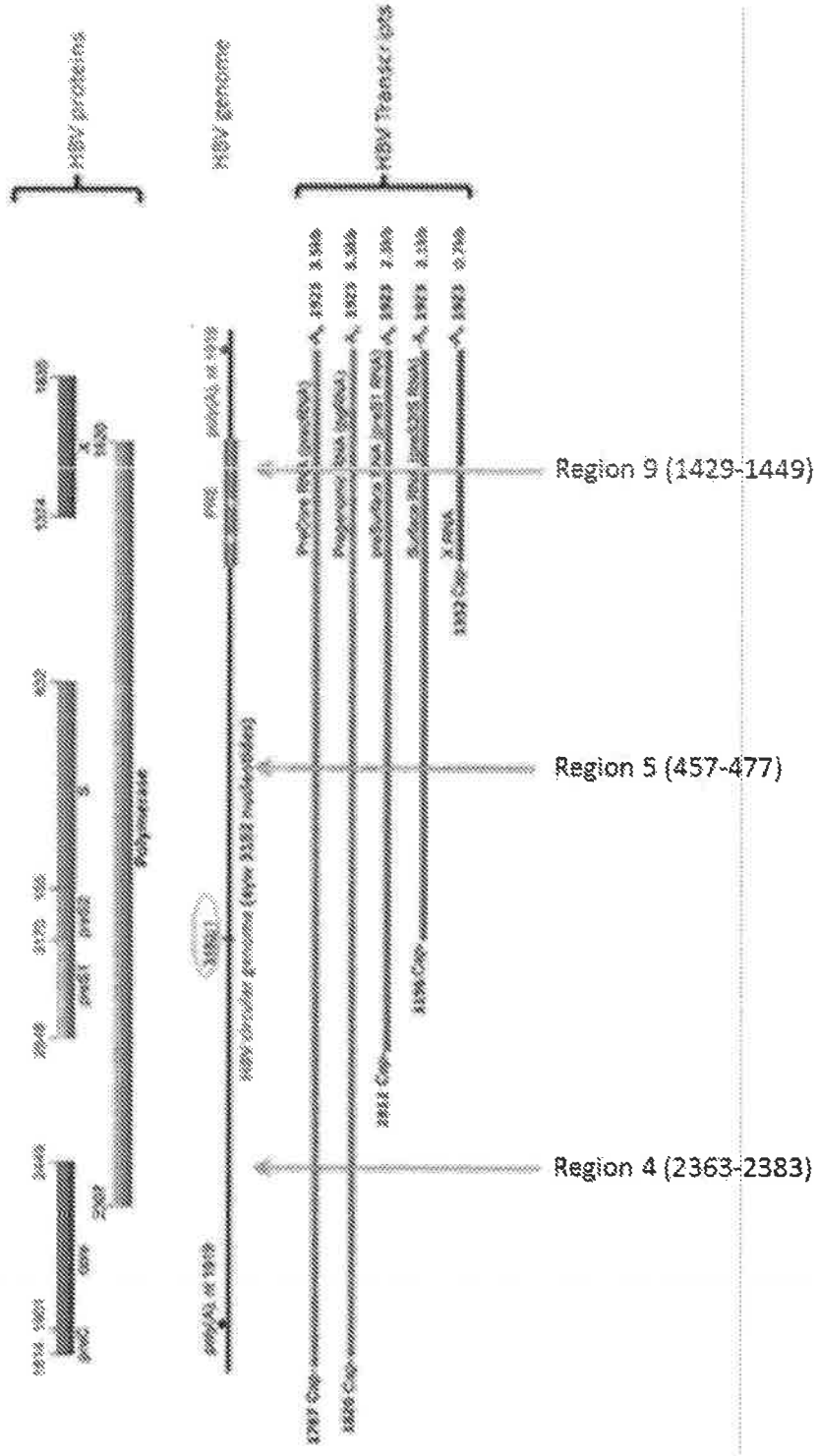


Fig. 4

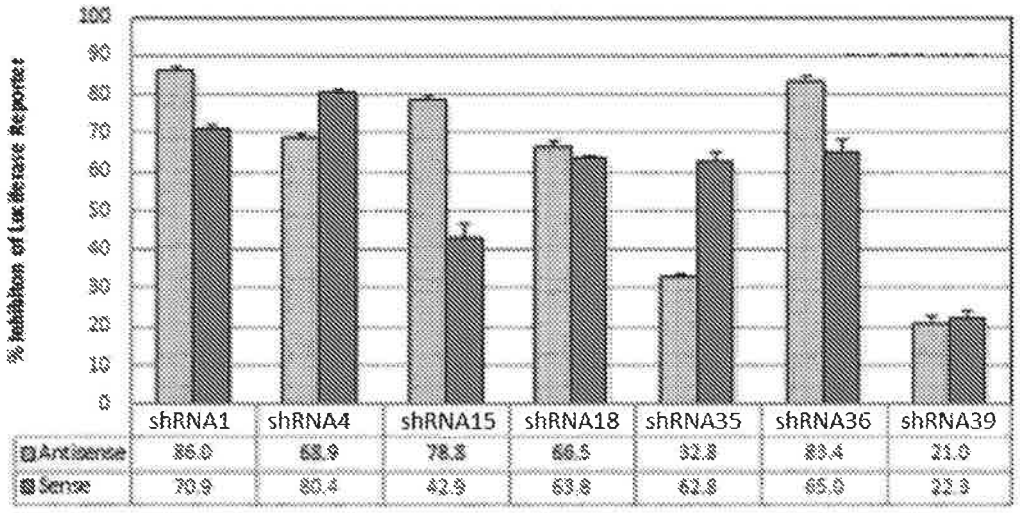

% Inhibition of Luciferase Reporter
100
90
80
70
60
50
40
30
20
10
0
shRNA1
shRNA4
shRNA15
shRNA18
shRNA35
shRNA36
shRNA39
Antisense
86.0
68.9
78.8
66.5
32.8
83.4
21.0
Sense
70.9
80.4
42.9
63.8
62.8
65.0
22.3

Fig. 5a

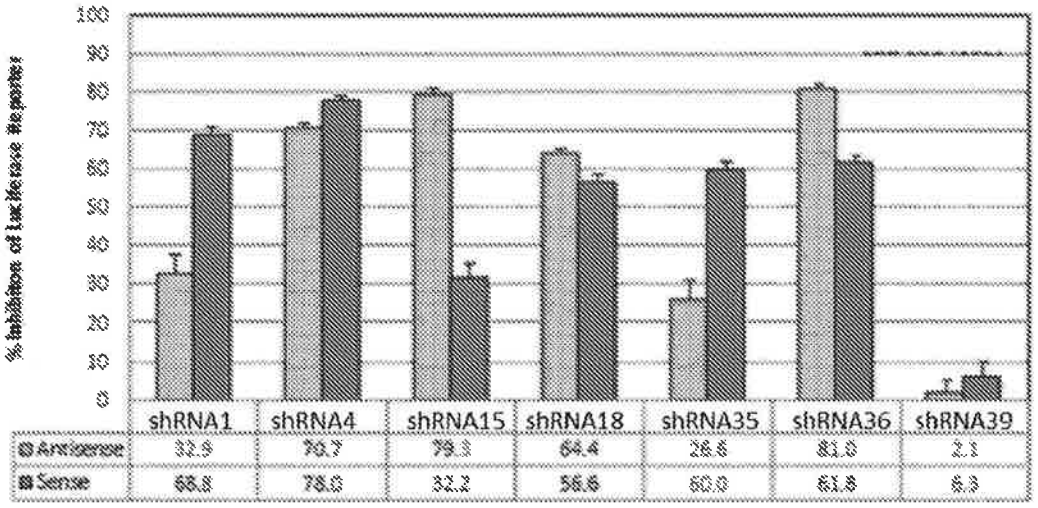

% Inhibition of Luciferase Reporter
100
90
80
70
60
50
40
30
20
10
0
shRNA1
shRNA4
shRNA15
shRNA18
shRNA35
shRNA36
shRNA39
Antisense
32.9
70.7
79.3
64.4
26.6
81.0
2.1
Sense
68.8
78.0
32.2
56.6
60.0
61.8
6.3

Fig. 5b

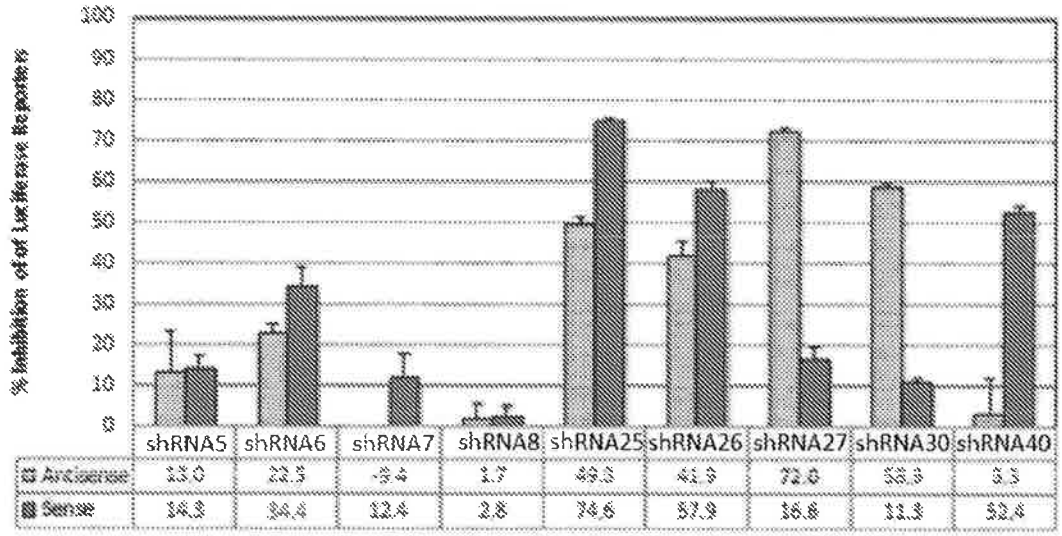

% Inhibition of of Luciferase Reporters
100
90
80
70
60
50
40
30
20
10
0
shRNA5 shRNA6 shRNA7 shRNA8 shRNA25 shRNA26 shRNA27 shRNA30 shRNA40
Antisense 23.0 22.5 -9.4 1.7 49.3 41.9 72.0 58.9 5.5
Sense 14.3 34.4 12.4 2.8 74.6 57.9 16.8 11.3 52.4

Fig. 5c

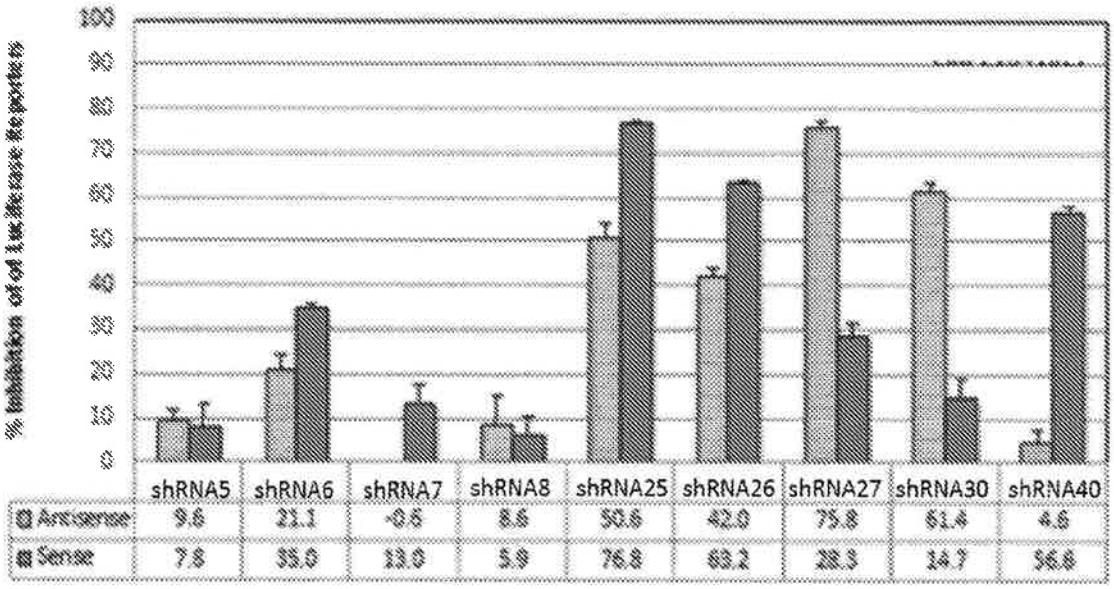

% Inhibition of of Luciferase Reporters
100
90
80
70
60
50
40
30
20
10
0
shRNA5 shRNA6 shRNA7 shRNA8 shRNA25 shRNA26 shRNA27 shRNA30 shRNA40
Antisense 9.6 21.1 -0.6 8.6 50.6 42.0 75.8 61.4 4.6
Sense 7.8 35.0 13.0 5.9 76.8 63.2 28.3 14.7 56.8

Fig. 5d

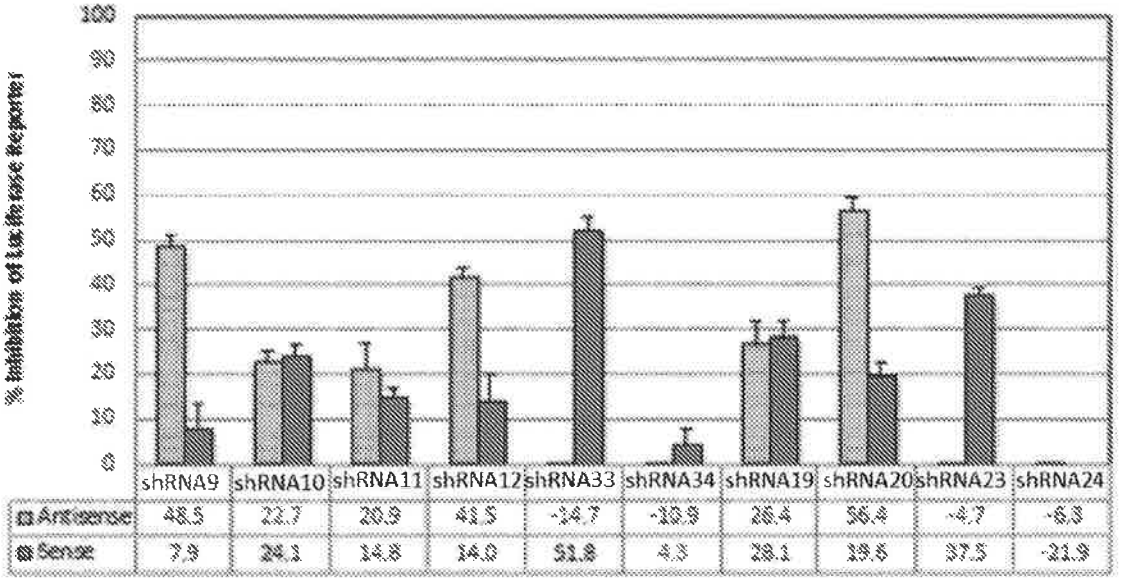

% inhibition of luciferase reporter
100
90
80
70
60
50
40
30
20
10
0
shRNA9 shRNA10 shRNA11 shRNA12 shRNA33 shRNA34 shRNA19 shRNA20 shRNA23 shRNA24
Antisense 48.5 22.7 20.9 41.5 -24.7 -10.9 28.4 56.4 -4.7 -6.3
Sense 7.9 24.1 14.8 14.0 51.8 4.3 28.1 19.6 37.5 -21.9

Fig. 5e

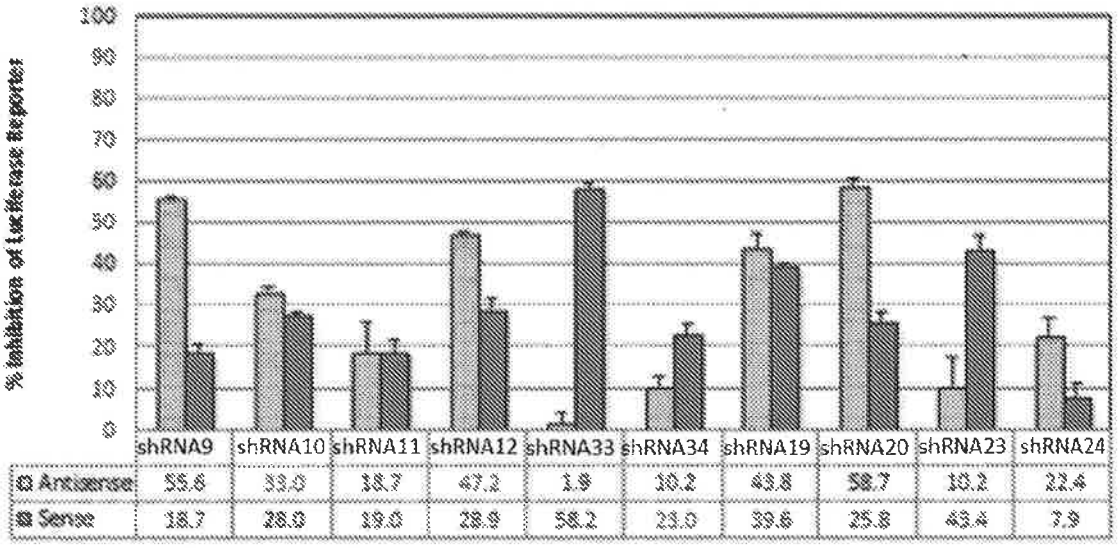

% inhibition of luciferase reporter
100
90
80
70
60
50
40
30
20
10
0
shRNA9 shRNA10 shRNA11 shRNA12 shRNA33 shRNA34 shRNA19 shRNA20 shRNA23 shRNA24
Antisense 55.6 33.0 18.7 47.2 1.9 10.2 43.8 58.7 10.2 22.4
Sense 18.7 28.0 19.0 28.9 58.2 23.0 39.6 25.8 43.4 7.9

Fig. 5f

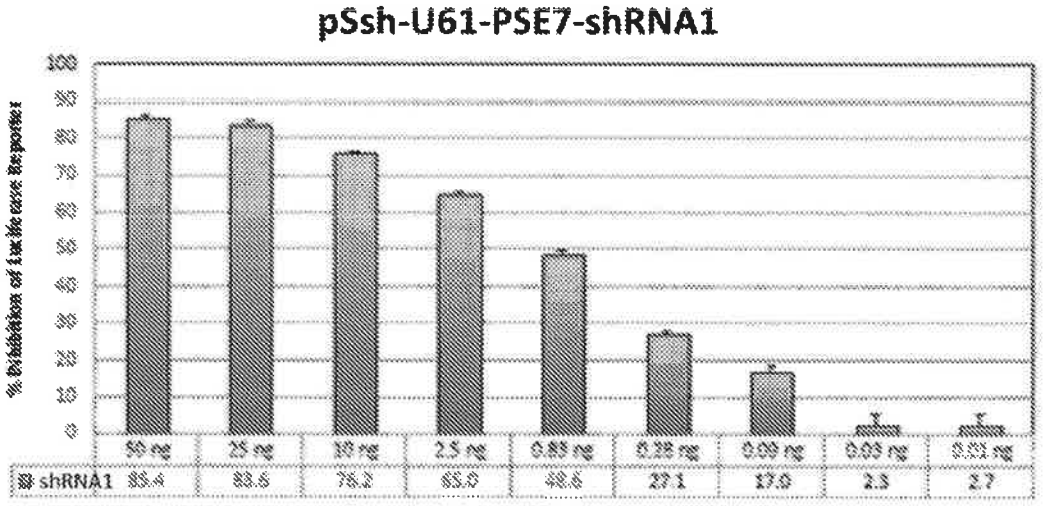

pSsh-U61-PSE7-shRNA1
% Inhibition of Luciferase Reporter
100
90
80
70
60
50
40
30
20
10
0
50 ng
25 ng
10 ng
2.5 ng
0.83 ng
0.28 ng
0.09 ng
0.03 ng
0.01 ng
shRNA1 85.4 83.6 76.2 65.0 40.6 27.1 17.0 2.3 2.7

Fig. 6a

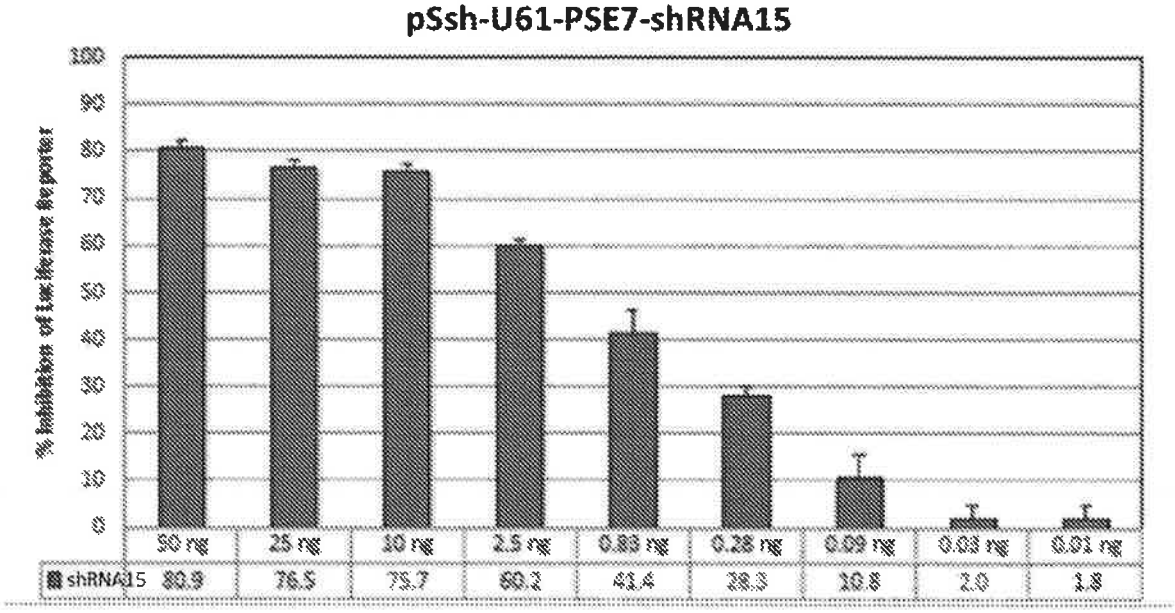

pSsh-U61-PSE7-shRNA15
% Inhibition of Luciferase Reporter
100
90
80
70
60
50
40
30
20
10
0
50 ng
25 ng
10 ng
2.5 ng
0.83 ng
0.28 ng
0.09 ng
0.03 ng
0.01 ng
shRNA15 80.9 76.5 75.7 60.2 41.4 28.3 10.8 2.0 1.8

Fig. 6b

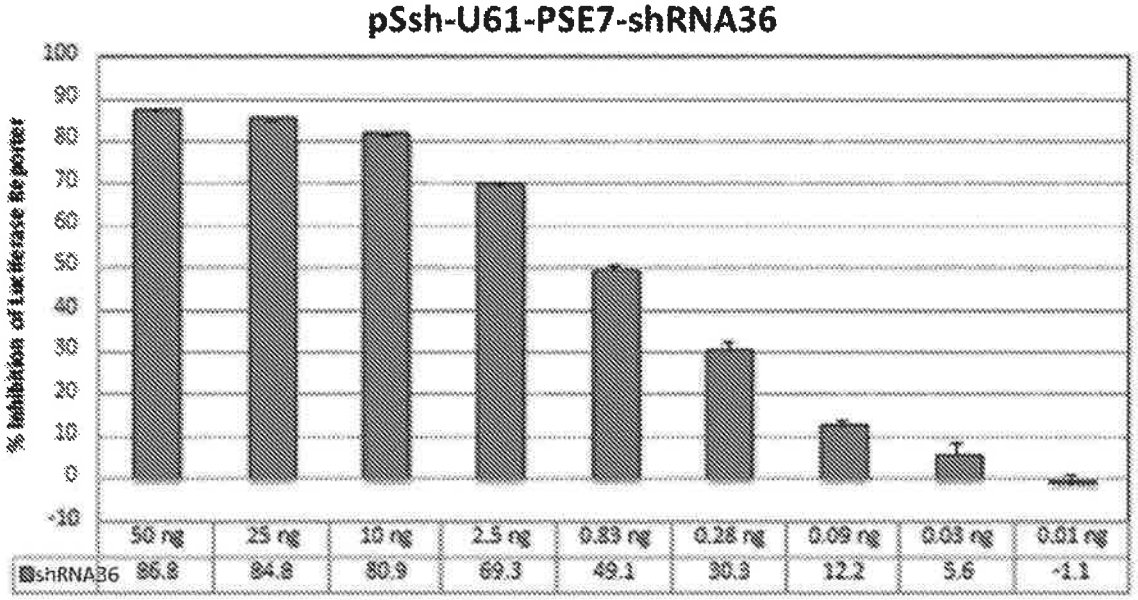

pSsh-U61-PSE7-shRNA36
% Inhibition of Luciferase Reporter
100
90
80
70
60
50
40
30
20
10
0
-10
50 nM
25 nM
10 nM
2.5 nM
0.83 nM
0.28 nM
0.09 nM
0.03 nM
0.01 nM
shRNA36
86.8
84.8
80.9
69.3
49.1
30.3
12.2
5.6
-1.1

Fig. 6c

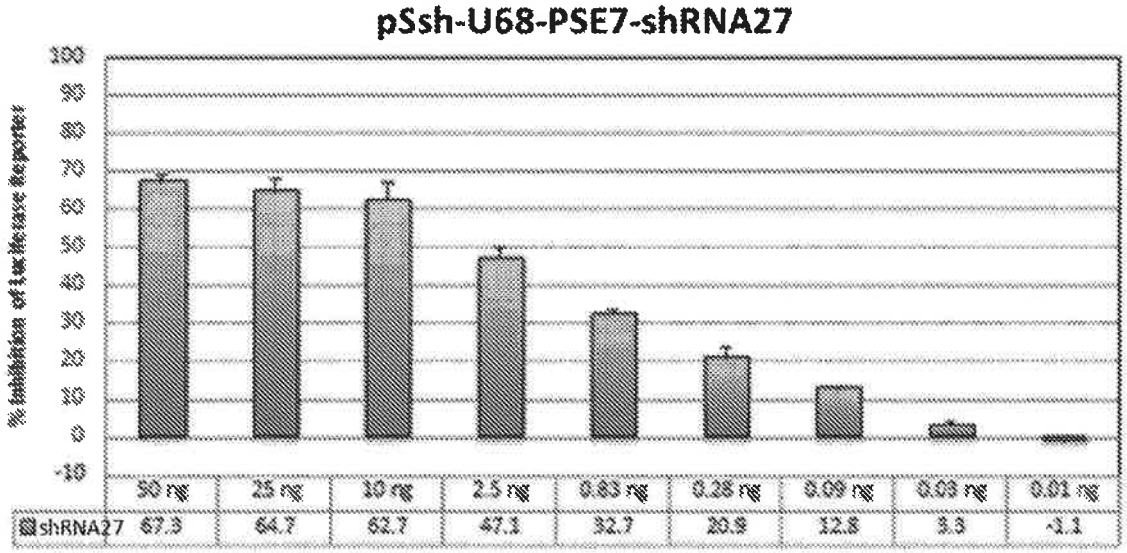

pSsh-U68-PSE7-shRNA27
% Inhibition of Luciferase Reporter
100
90
80
70
60
50
40
30
20
10
0
-10
50 nM
25 nM
10 nM
2.5 nM
0.83 nM
0.28 nM
0.09 nM
0.03 nM
0.01 nM
shRNA27
67.3
64.7
62.7
47.1
32.7
20.9
12.8
3.3
-1.1

Fig. 6d

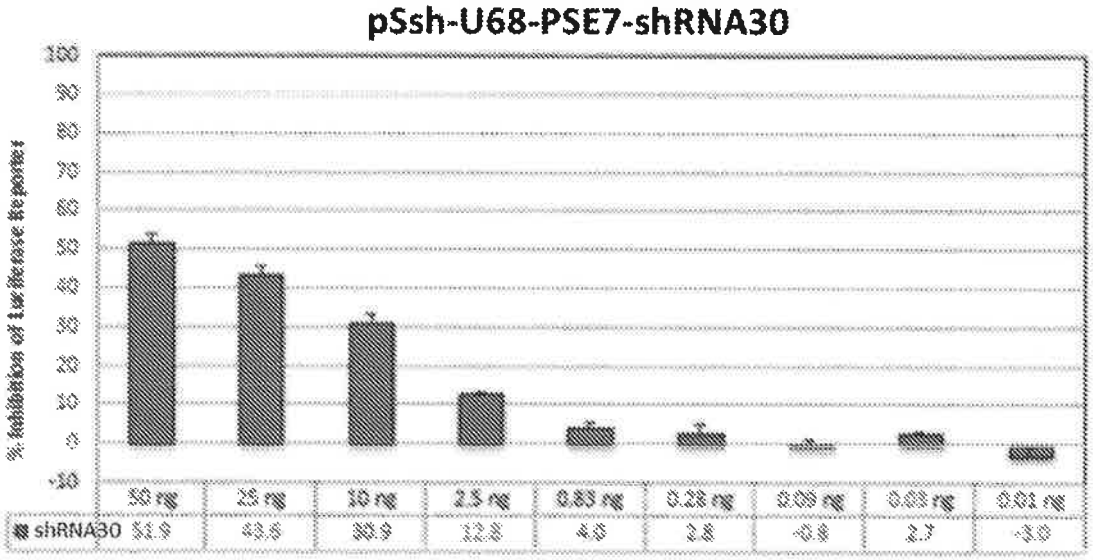

pSsh-U68-PSE7-shRNA30
% inhibition of Luciferase Reporter
100
90
80
70
60
50
40
30
20
10
0
-10
50 nM
25 nM
10 nM
2.5 nM
0.83 nM
0.28 nM
0.09 nM
0.03 nM
0.01 nM
shRNA30 51.9 43.6 30.9 12.8 4.0 2.8 -0.8 2.7 -3.0

Fig. 6e

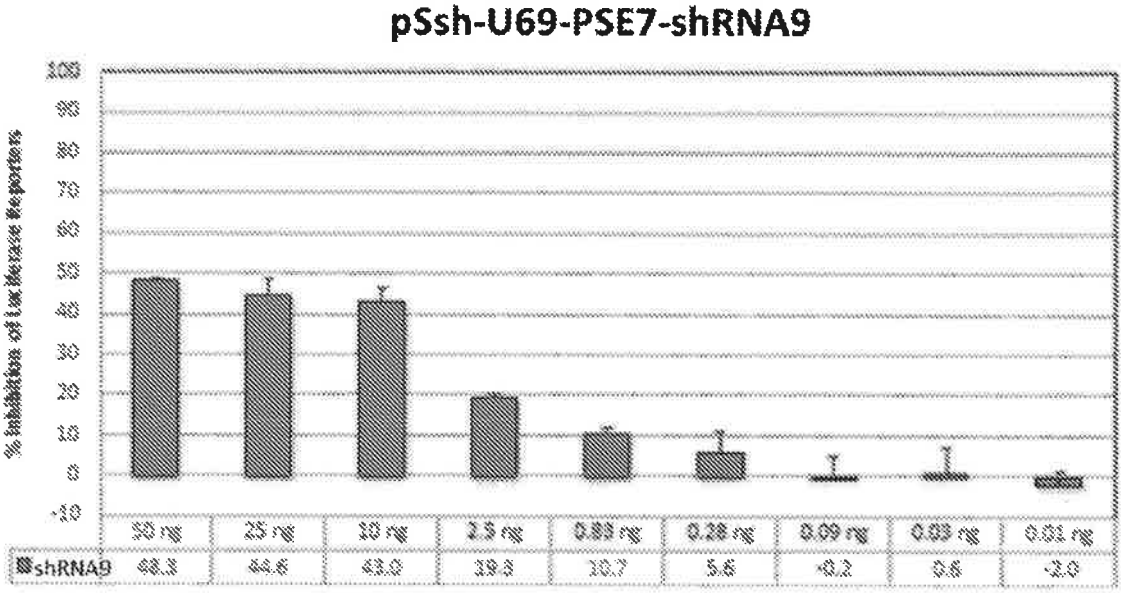

pSsh-U69-PSE7-shRNA9
% inhibition of Luciferase Reporters
100
90
80
70
60
50
40
30
20
10
0
-10
50 nM
25 nM
10 nM
2.5 nM
0.83 nM
0.28 nM
0.09 nM
0.03 nM
0.01 nM
shRNA9 48.3 44.6 43.0 19.3 10.7 5.6 -0.2 0.6 -2.0

Fig. 6f

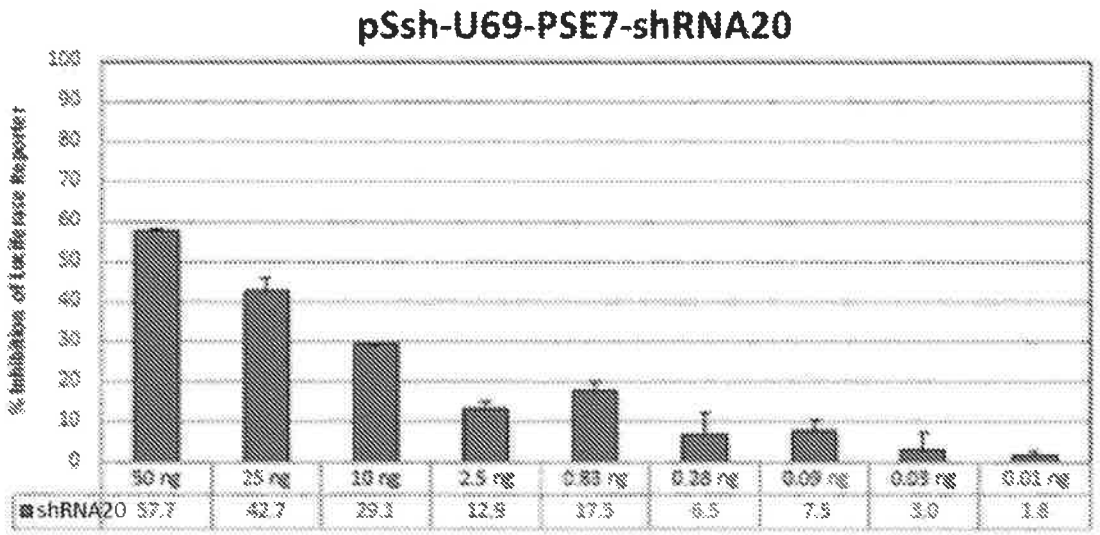

pSsh-U69-PSE7-shRNA20
% Inhibition of Luciferase Reporter
100
90
80
70
60
50
40
30
20
10
0
50 nM
25 nM
10 nM
2.5 nM
0.83 nM
0.28 nM
0.09 nM
0.03 nM
0.01 nM
shRNA20 57.7 42.7 29.3 12.9 17.5 6.5 7.9 3.0 1.8

Fig. 6g

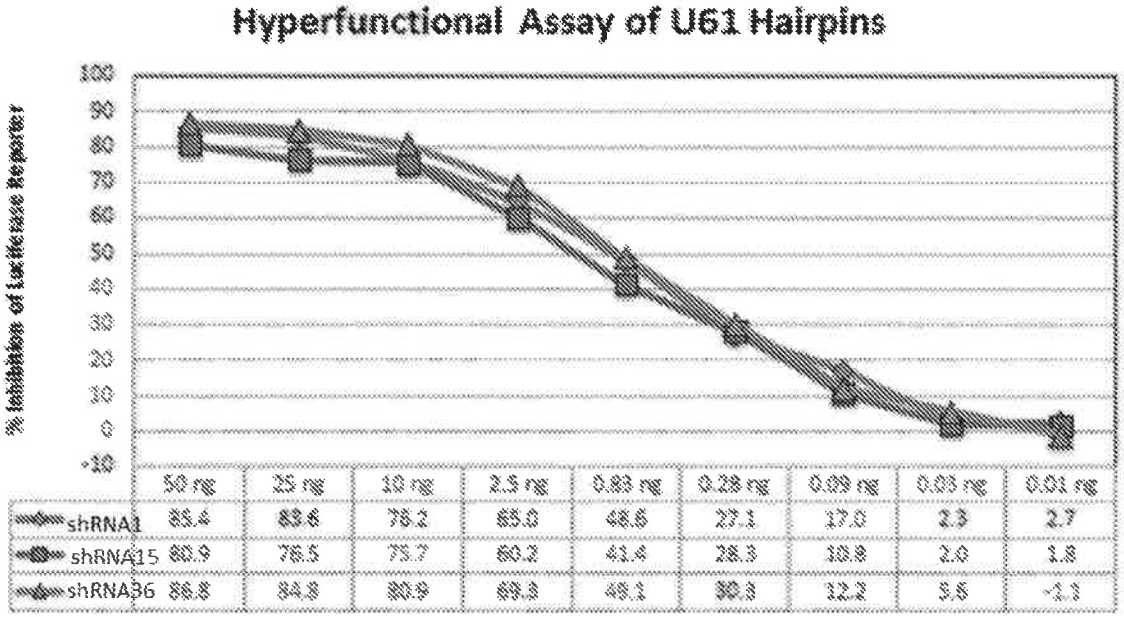

Hyperfunctional Assay of U61 Hairpins
% Inhibition of Luciferase Reporter
100
90
80
70
60
50
40
30
20
10
0
-10
50 nM
25 nM
10 nM
2.5 nM
0.83 nM
0.28 nM
0.09 nM
0.03 nM
0.01 nM
shRNA1 85.4 83.6 76.2 65.0 48.6 27.1 17.0 2.3 2.7
shRNA15 80.9 78.5 75.7 60.2 41.4 28.3 10.8 2.0 1.8
shRNA36 86.8 84.8 80.9 69.3 48.1 30.3 12.2 5.6 -1.3

Fig. 6h

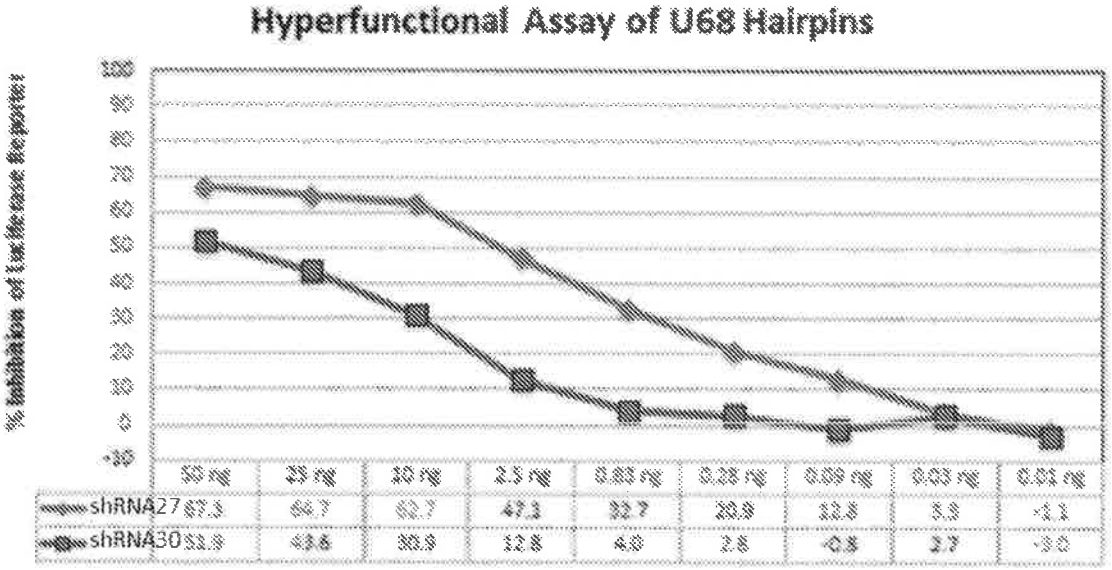

Hyperfunctional Assay of U68 Hairpins
% Inhibition of Luciferase Reporter
50 nM
25 nM
10 nM
2.5 nM
0.83 nM
0.28 nM
0.09 nM
0.03 nM
0.01 nM
shRNA27 67.3 64.7 62.7 47.3 32.7 20.9 12.8 5.9 -1.1
shRNA30 53.9 43.6 30.9 12.8 4.0 2.6 -0.8 2.7 -3.0

Fig. 6i

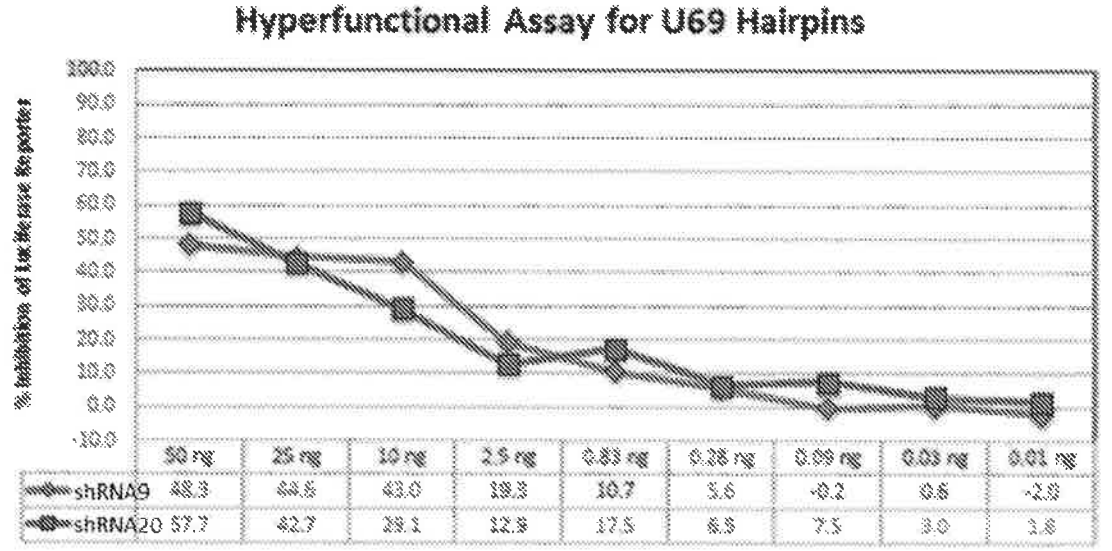

Hyperfunctional Assay for U69 Hairpins
% Inhibition of Luciferase Reporter
50 nM
25 nM
10 nM
2.5 nM
0.83 nM
0.28 nM
0.09 nM
0.03 nM
0.01 nM
shRNA9 48.3 44.6 43.0 18.3 10.7 5.6 -0.2 0.6 -2.0
shRNA20 37.7 42.7 29.1 12.9 17.5 6.5 7.5 3.0 1.6

Fig. 6j

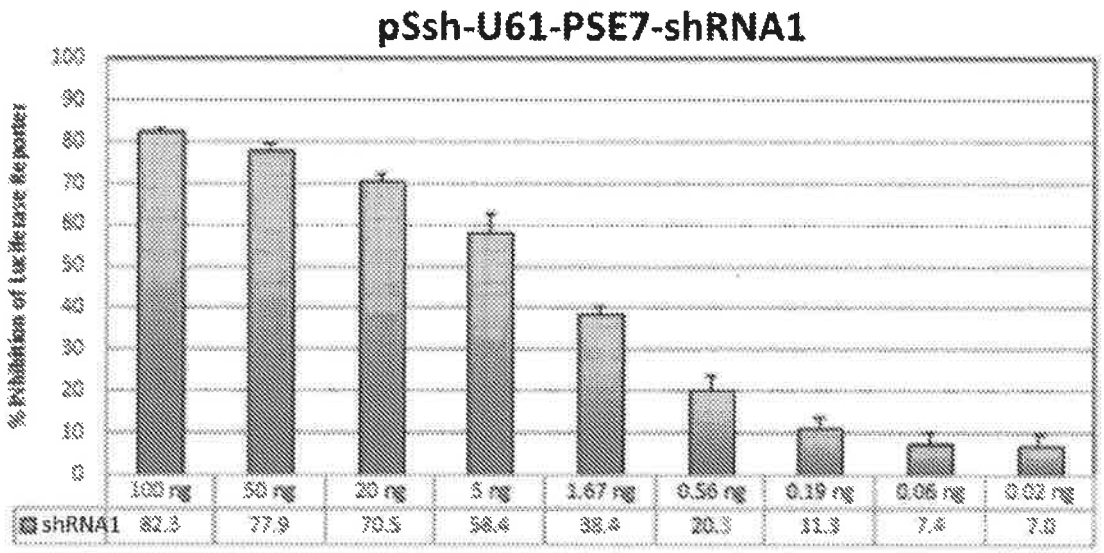

pSsh-U61-PSE7-shRNA1
% Inhibition of Luciferase Reporter
100 ng
50 ng
20 ng
5 ng
1.67 ng
0.56 ng
0.19 ng
0.06 ng
0.02 ng
shRNA1 82.3 77.9 70.5 58.4 38.8 20.3 11.3 7.4 7.8

Fig. 7a

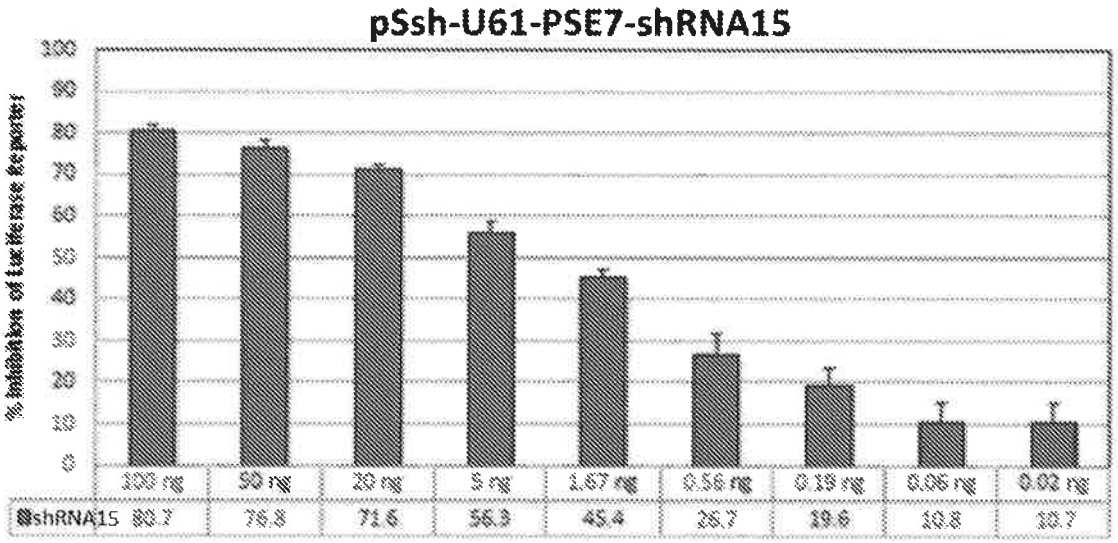

pSsh-U61-PSE7-shRNA15
% Inhibition of Luciferase Reporter
100 ng
50 ng
20 ng
5 ng
1.67 ng
0.56 ng
0.19 ng
0.06 ng
0.02 ng
shRNA15 80.7 76.8 71.6 56.3 45.4 26.7 19.6 10.8 10.7

Fig. 7b

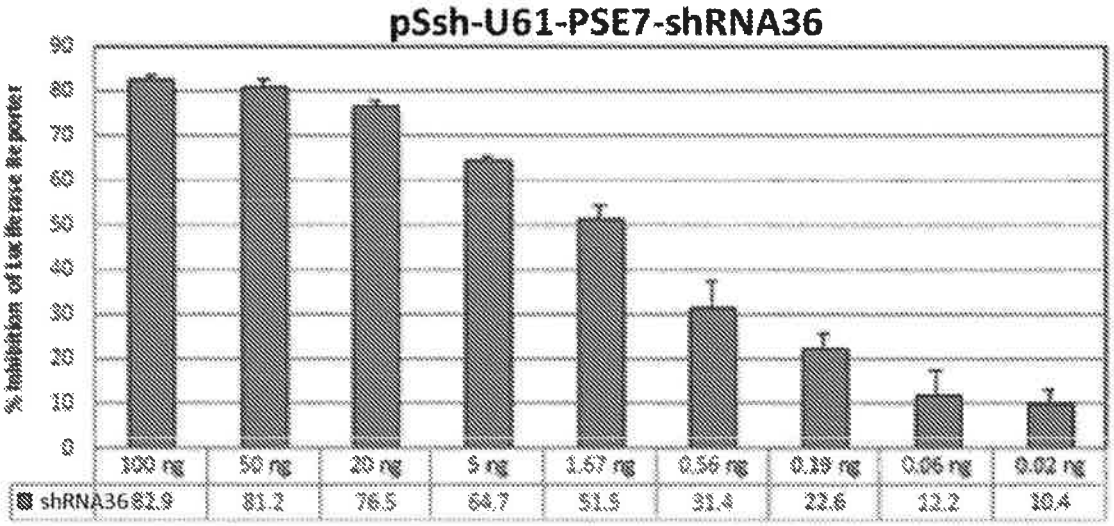

pSsh-U61-PSE7-shRNA36
% inhibition of Luciferase Reporter
100 ng
50 ng
20 ng
5 ng
1.67 ng
0.56 ng
0.19 ng
0.06 ng
0.02 ng
shRNA36 82.9
81.2
76.5
64.7
51.5
31.4
22.6
12.2
10.4

Fig. 7c

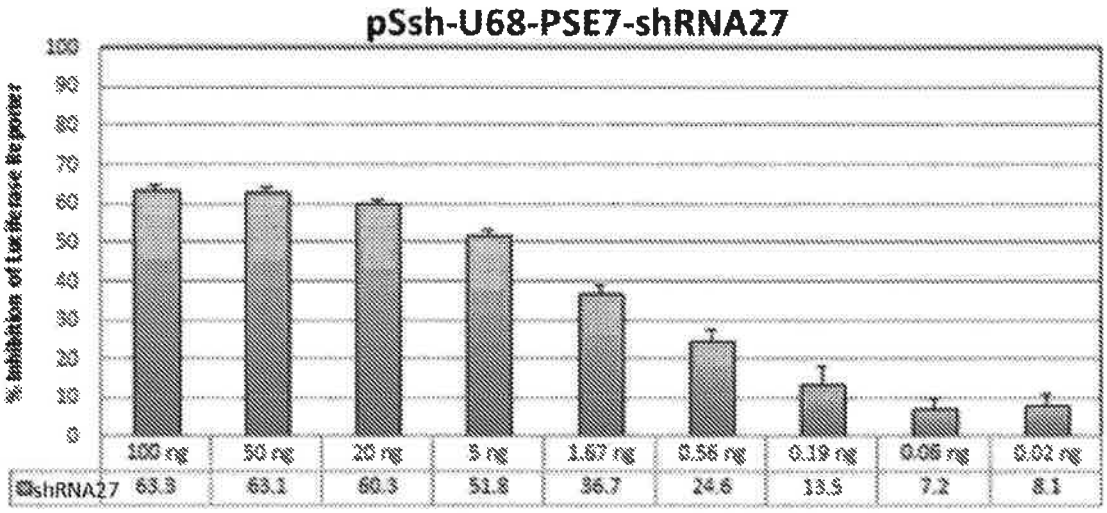

pSsh-U68-PSE7-shRNA27
% inhibition of Luciferase Reporter
100 ng
50 ng
20 ng
5 ng
1.67 ng
0.56 ng
0.19 ng
0.06 ng
0.02 ng
shRNA27 63.9
63.1
60.3
51.8
36.7
24.6
13.5
7.2
8.1

Fig. 7d

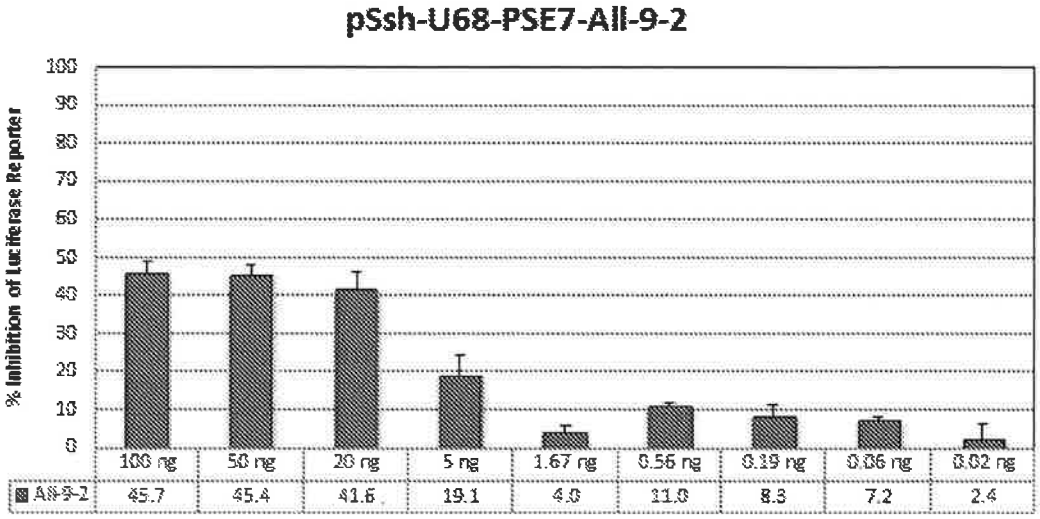

pSsh-U68-PSE7-All-9-2
% Inhibition of Luciferase Reporter
100
90
80
70
60
50
40
30
20
10
0
100 ng
50 ng
20 ng
5 ng
1.67 ng
0.56 ng
0.19 ng
0.06 ng
0.02 ng
All-9-2
45.7
45.4
41.6
19.1
4.0
11.0
8.3
7.2
2.4

Fig. 7e

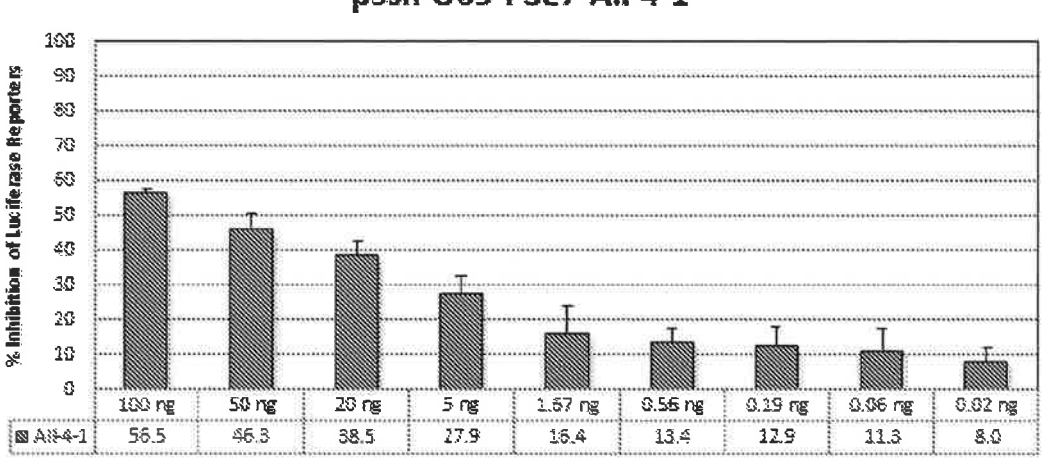

pSsh-U69-PSE7-All-4-1
% Inhibition of Luciferase Reporters
100
90
80
70
60
50
40
30
20
10
0
100 ng
50 ng
20 ng
5 ng
1.67 ng
0.56 ng
0.19 ng
0.06 ng
0.02 ng
All-4-1
56.5
46.3
38.5
27.9
16.4
13.4
12.9
11.3
8.0

Fig. 7f

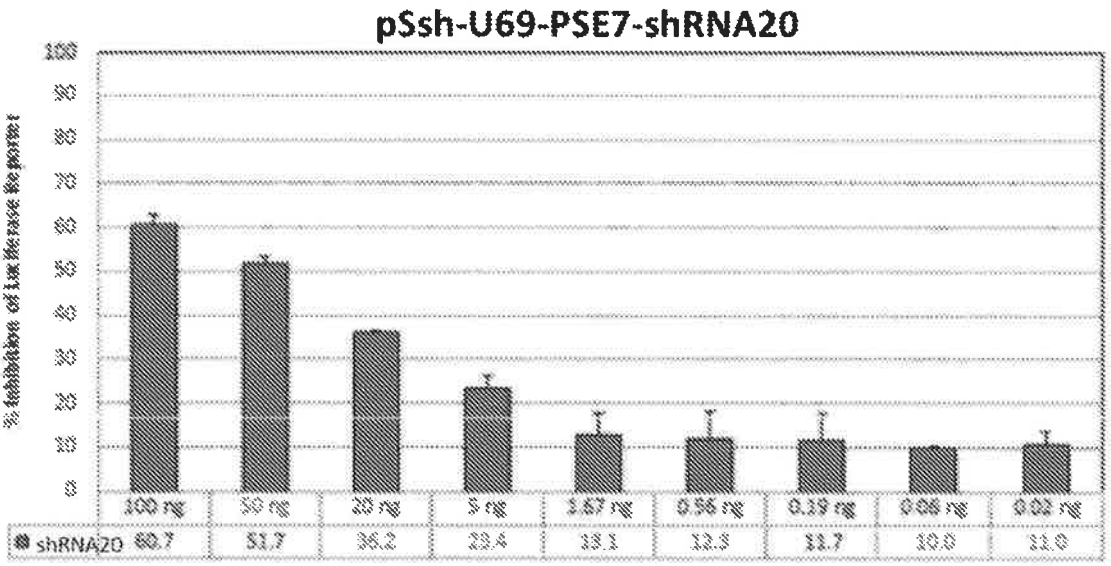

pSsh-U69-PSE7-shRNA20
% Inhibition of Luciferase Reporter
100 ng
50 ng
20 ng
5 ng
1.67 ng
0.56 ng
0.19 ng
0.06 ng
0.02 ng
shRNA20 60.7 51.7 36.2 23.4 13.1 12.3 11.7 10.0 11.0

Fig. 7g

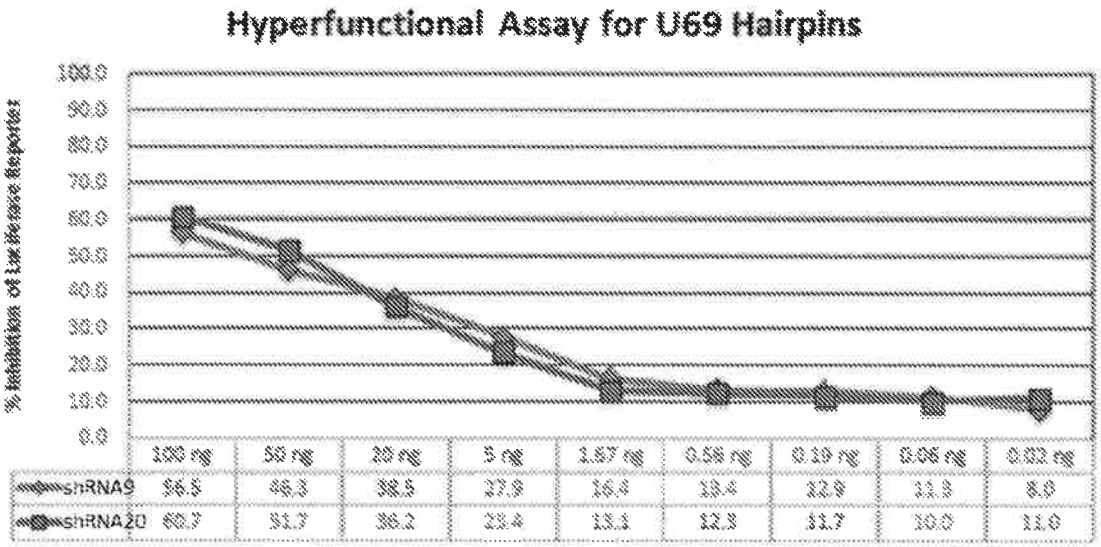

Hyperfunctional Assay for U69 Hairpins
% Inhibition of Luciferase Reporter
100 ng
50 ng
20 ng
5 ng
1.67 ng
0.56 ng
0.19 ng
0.06 ng
0.02 ng
shRNA9 56.5 46.3 38.5 27.9 16.4 13.4 12.9 11.3 8.0
shRNA20 60.7 51.7 36.2 23.4 13.1 12.3 11.7 10.0 11.0

Fig. 7h

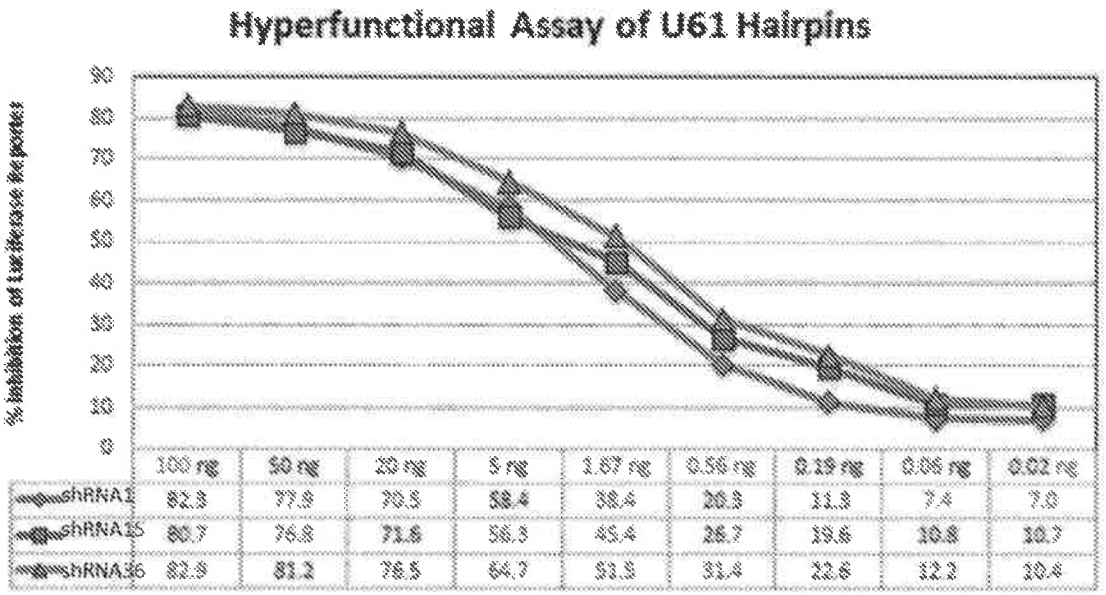

Hyperfunctional Assay of U61 Hairpins
% Inhibition of Luciferase Reporter
100 ng 50 ng 20 ng 5 ng 1.67 ng 0.56 ng 0.19 ng 0.06 ng 0.02 ng
shRNA1 82.3 77.9 70.5 58.4 38.4 20.3 11.3 7.4 7.0
shRNA35 80.7 76.8 71.6 56.3 45.4 26.7 19.6 10.8 10.7
shRNA36 82.9 81.2 76.5 64.7 51.5 31.4 22.6 12.2 10.4

Fig. 7i

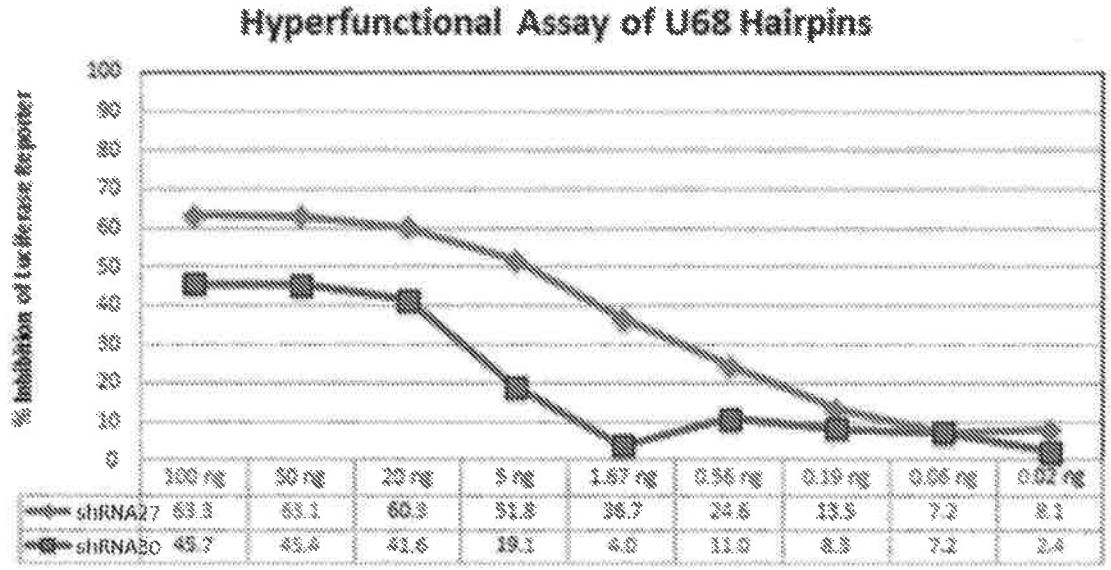

Hyperfunctional Assay of U68 Hairpins
% Inhibition of Luciferase Reporter
100 ng 50 ng 20 ng 5 ng 1.67 ng 0.56 ng 0.19 ng 0.06 ng 0.02 ng
shRNA27 63.3 63.1 60.3 51.8 36.7 24.6 13.5 7.2 8.1
shRNA30 45.7 45.4 41.6 39.1 6.0 11.0 8.3 7.2 2.4

Fig. 7j

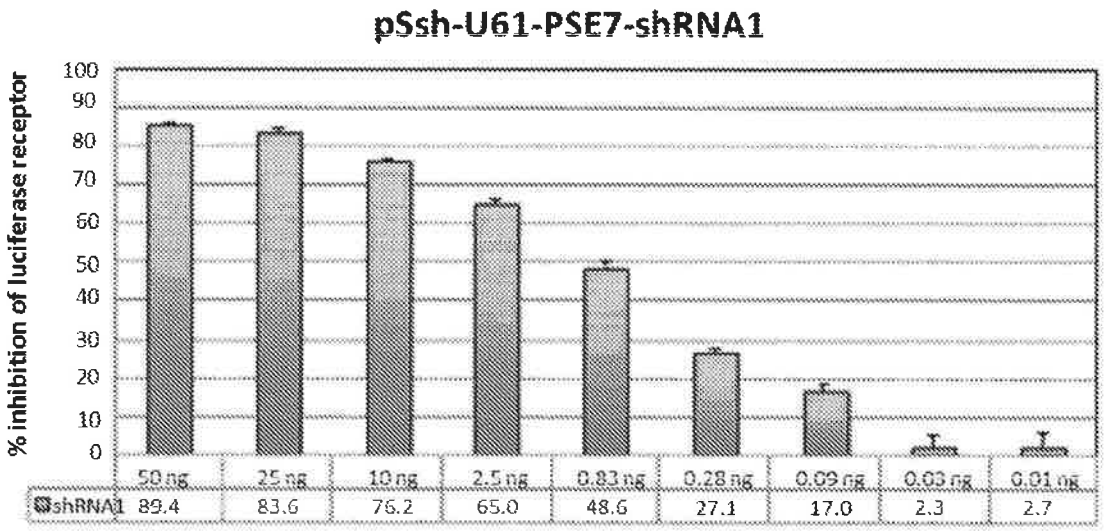

pSsh-U61-PSE7-shRNA1
% inhibition of luciferase receptor
100
90
80
70
60
50
40
30
20
10
0
50 ng
25 ng
10 ng
2.5 ng
0.83 ng
0.28 ng
0.09 ng
0.03 ng
0.01 ng
shRNA1 89.4
83.6
76.2
65.0
48.6
27.1
17.0
2.3
2.7

Fig. 8a

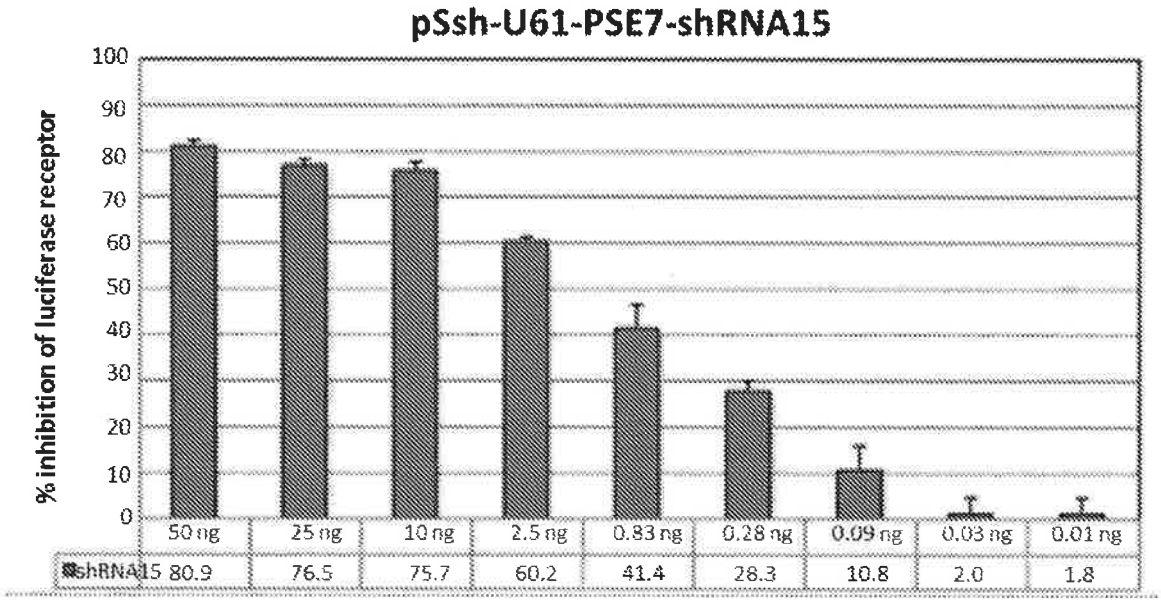

pSsh-U61-PSE7-shRNA15
% inhibition of luciferase receptor
100
90
80
70
60
50
40
30
20
10
0
50 ng
25 ng
10 ng
2.5 ng
0.83 ng
0.28 ng
0.09 ng
0.03 ng
0.01 ng
shRNA15 80.9
76.5
75.7
60.2
41.4
28.3
10.8
2.0
1.8

Fig. 8b

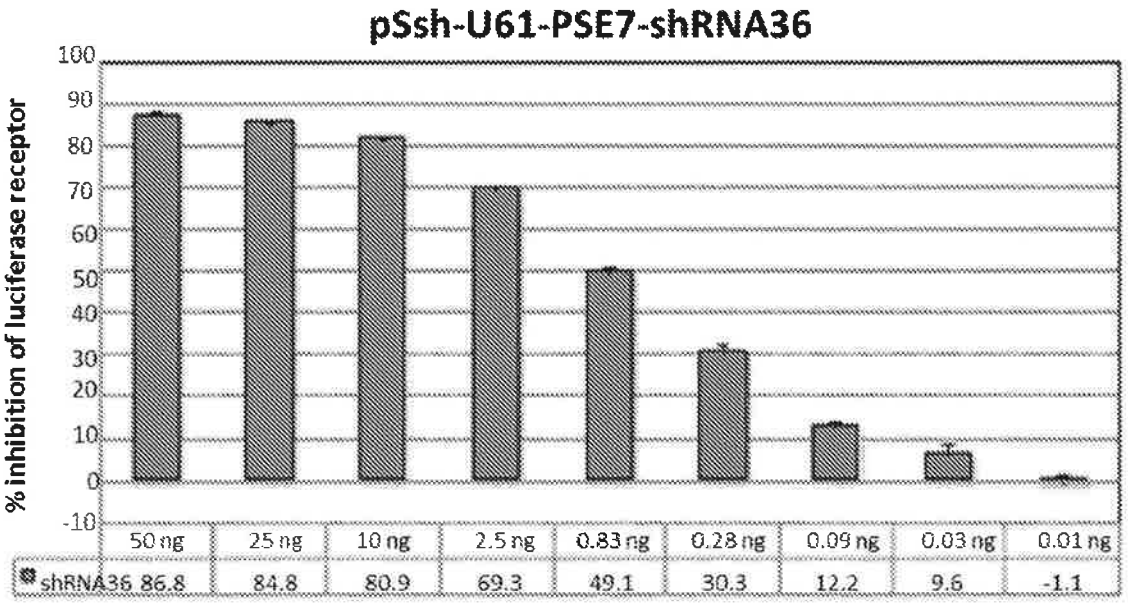

pSsh-U61-PSE7-shRNA36
% inhibition of luciferase receptor
100
90
80
70
60
50
40
30
20
10
0
-10
50 ng
25 ng
10 ng
2.5 ng
0.83 ng
0.28 ng
0.09 ng
0.03 ng
0.01 ng
shRNA36 86.8
84.8
80.9
69.3
49.1
30.3
12.2
9.6
-1.1

Fig. 8c

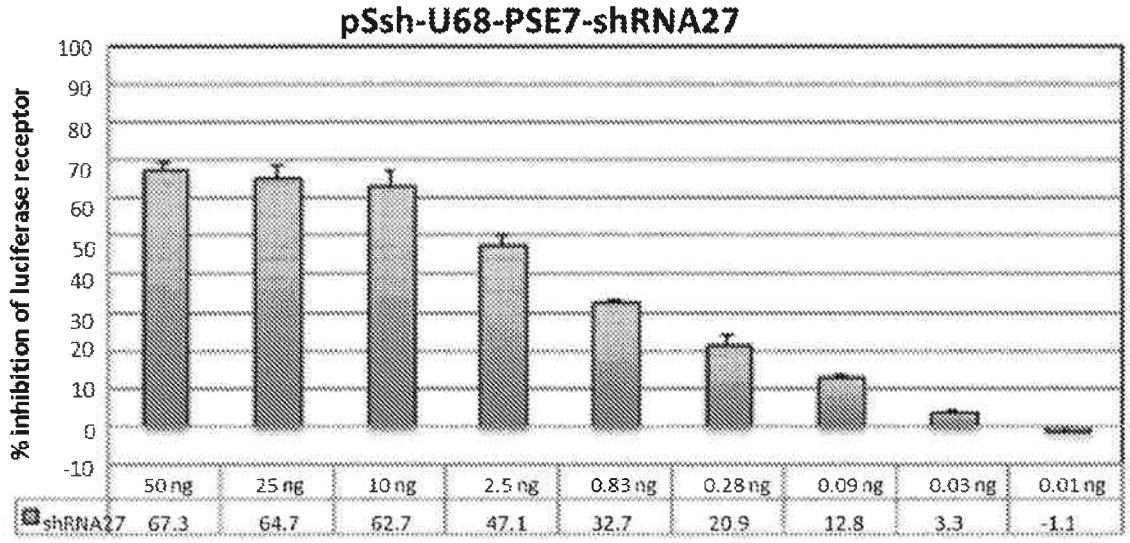

pSsh-U68-PSE7-shRNA27
% inhibition of luciferase receptor
100
90
80
70
60
50
40
30
20
10
0
-10
50 ng
25 ng
10 ng
2.5 ng
0.83 ng
0.28 ng
0.09 ng
0.03 ng
0.01 ng
shRNA27 67.3
64.7
62.7
47.1
32.7
20.9
12.8
3.3
-1.1

Fig. 8d

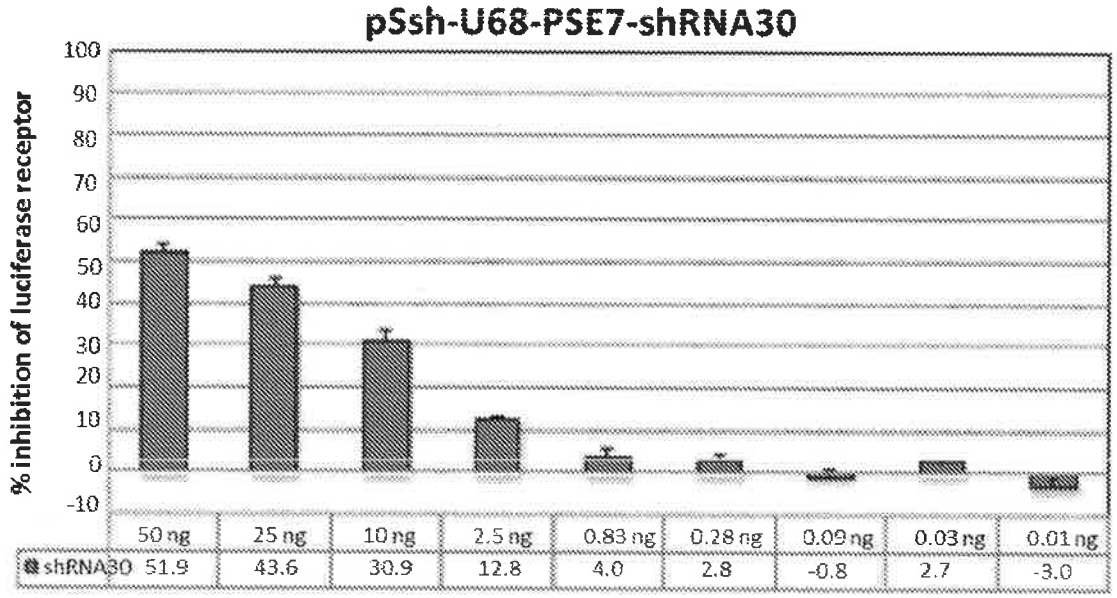

pSsh-U68-PSE7-shRNA30
% inhibition of luciferase receptor
100
90
80
70
60
50
40
30
20
10
0
-10
50 ng
25 ng
10 ng
2.5 ng
0.83 ng
0.28 ng
0.09 ng
0.03 ng
0.01 ng
shRNA30
51.9
43.6
30.9
12.8
4.0
2.8
-0.8
2.7
-3.0

Fig. 8e

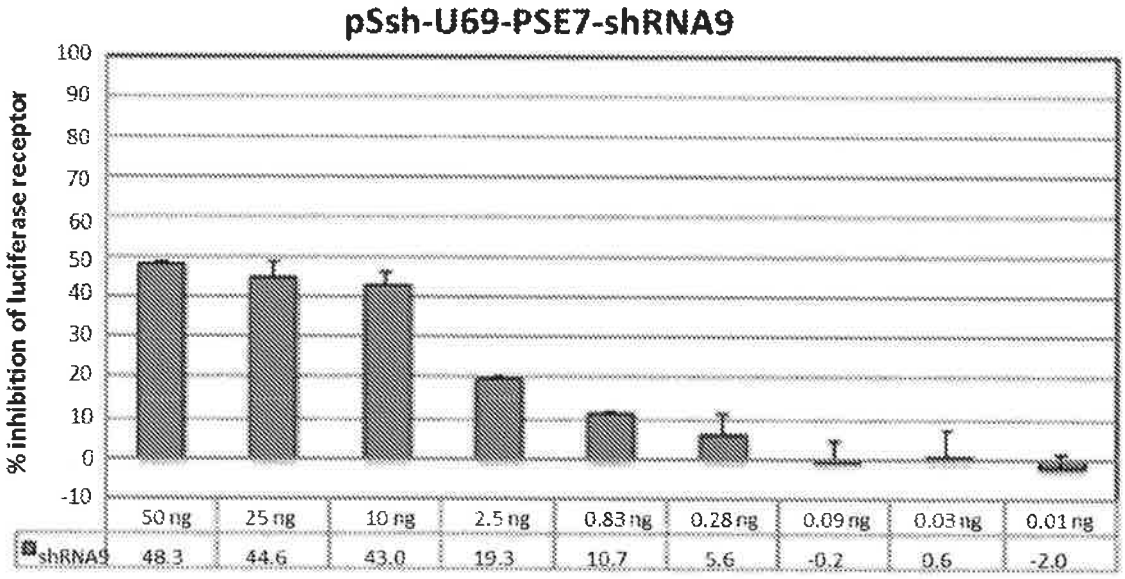

pSsh-U69-PSE7-shRNA9
% inhibition of luciferase receptor
100
90
80
70
60
50
40
30
20
10
0
-10
50 ng
25 ng
10 ng
2.5 ng
0.83 ng
0.28 ng
0.09 ng
0.03 ng
0.01 ng
shRNA9
48.3
44.6
43.0
19.3
10.7
5.6
-0.2
0.6
-2.0

Fig. 8f

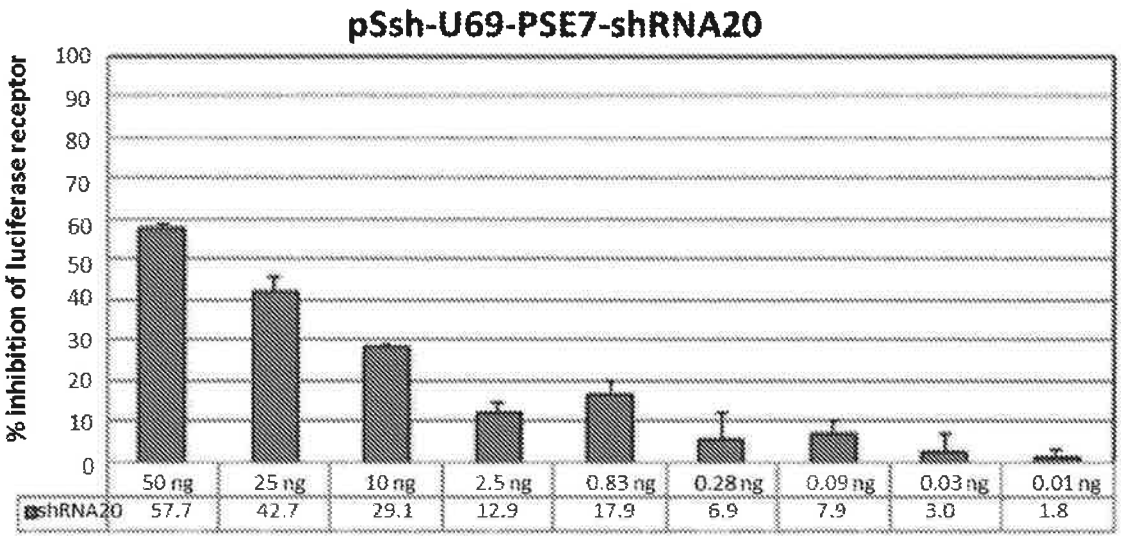

pSsh-U69-PSE7-shRNA20
% inhibition of luciferase receptor
100
90
80
70
60
50
40
30
20
10
0
50 ng
25 ng
10 ng
2.5 ng
0.83 ng
0.28 ng
0.09 ng
0.03 ng
0.01 ng
shRNA20
57.7
42.7
29.1
12.9
17.9
6.9
7.9
3.0
1.8

Fig. 8g

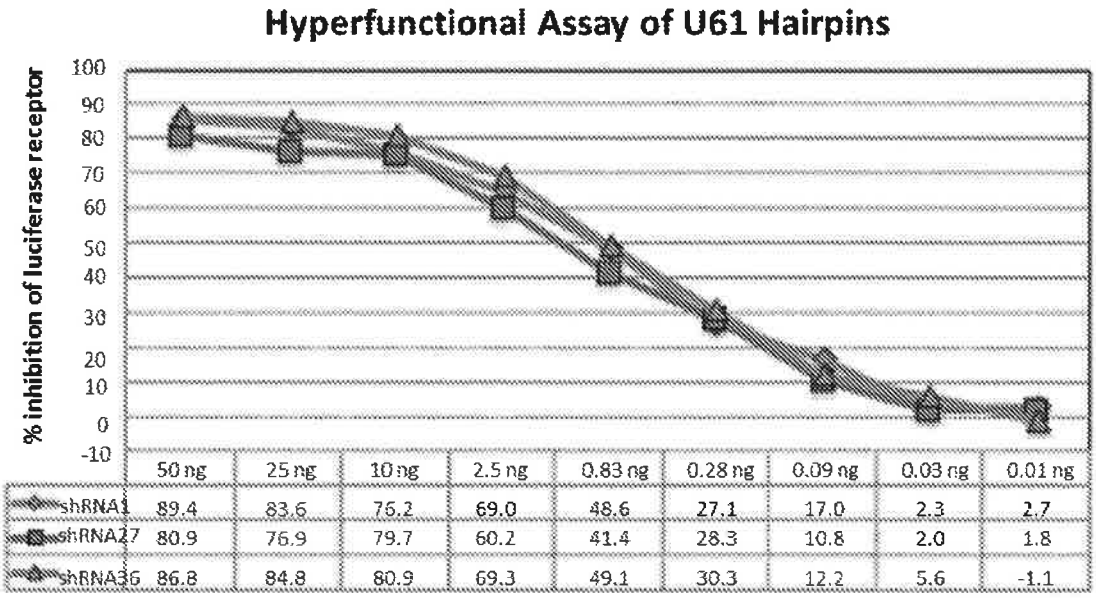

Hyperfunctional Assay of U61 Hairpins
% inhibition of luciferase receptor
100
90
80
70
60
50
40
30
20
10
0
-10
50 ng
25 ng
10 ng
2.5 ng
0.83 ng
0.28 ng
0.09 ng
0.03 ng
0.01 ng
shRNA1
89.4
83.6
76.2
69.0
48.6
27.1
17.0
2.3
2.7
shRNA27
80.9
76.9
79.7
60.2
41.4
28.3
10.8
2.0
1.8
shRNA36
86.8
84.8
80.9
69.3
49.1
30.3
12.2
5.6
-1.1

Fig. 8h

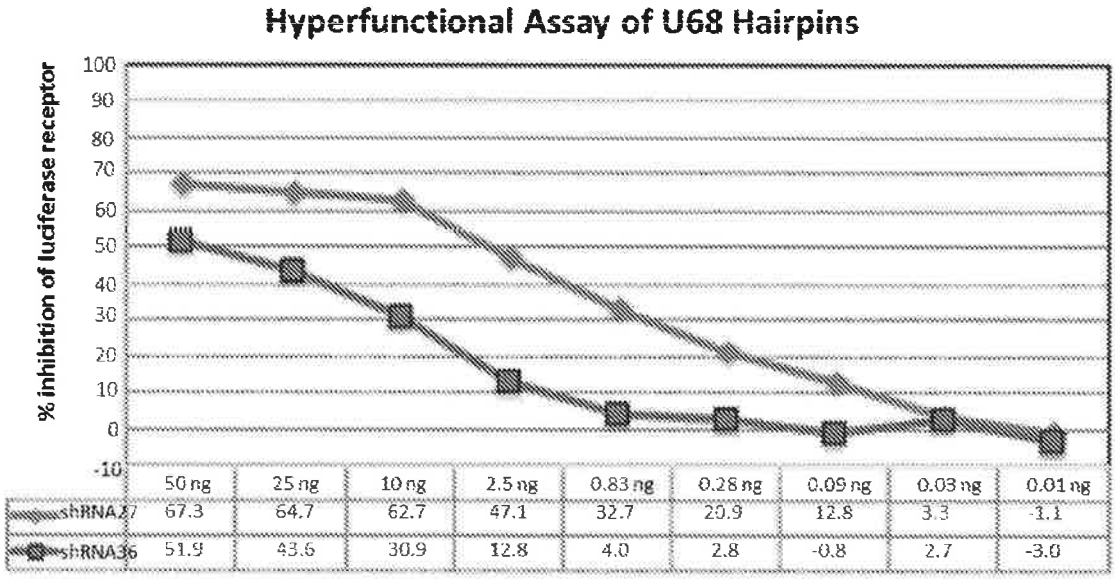

Hyperfunctional Assay of U68 Hairpins
% inhibition of luciferase receptor
100
90
80
70
60
50
40
30
20
10
0
-10
50 ng 25 ng 10 ng 2.5 ng 0.83 ng 0.28 ng 0.09 ng 0.03 ng 0.01 ng
shRNA37 67.3 64.7 62.7 47.1 32.7 20.9 12.8 3.3 -1.1
shRNA36 51.9 43.6 30.9 12.8 4.0 2.8 -0.8 2.7 -3.0

Fig. 8i

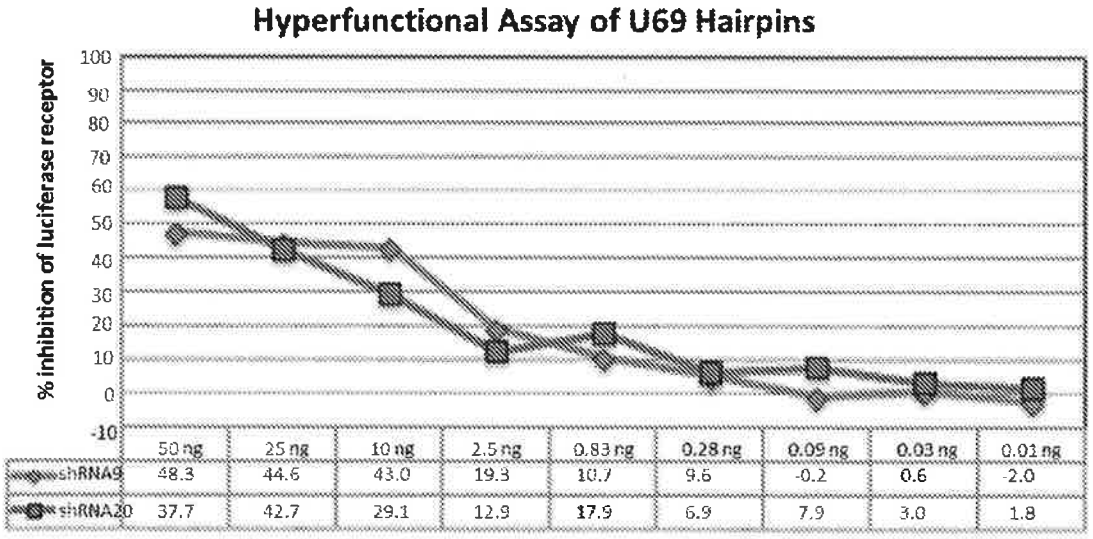

Hyperfunctional Assay of U69 Hairpins
% inhibition of luciferase receptor
100
90
80
70
60
50
40
30
20
10
0
-10
50 ng 25 ng 10 ng 2.5 ng 0.83 ng 0.28 ng 0.09 ng 0.03 ng 0.01 ng
shRNA9 48.3 44.6 43.0 19.3 10.7 9.6 -0.2 0.6 -2.0
shRNA20 37.7 42.7 29.1 12.9 17.9 6.9 7.9 3.0 1.8

Fig. 8j

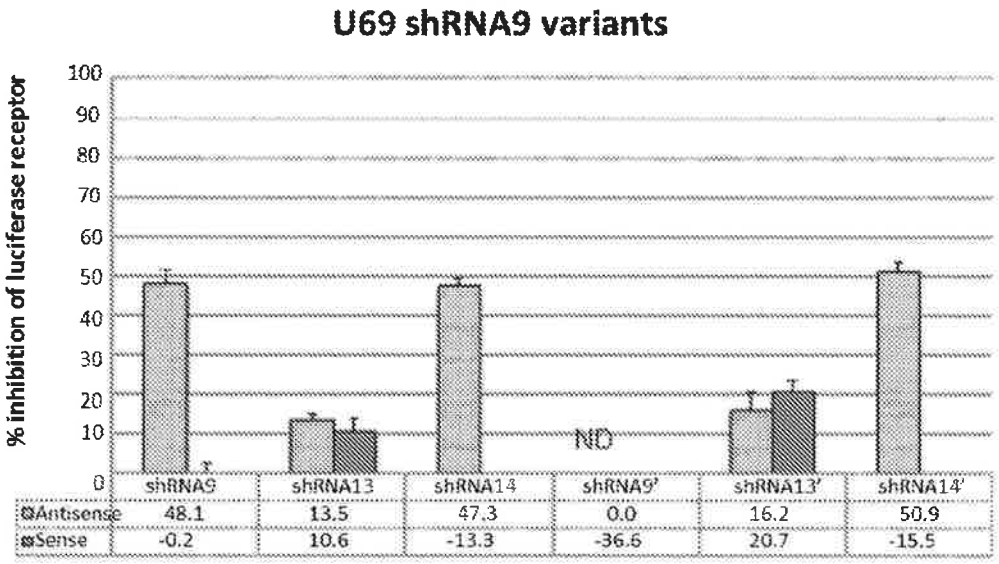

U69 shRNA9 variants
% inhibition of luciferase receptor
100
90
80
70
60
50
40
30
20
10
0
ND
shRNA9
shRNA13
shRNA14
shRNA9'
shRNA13'
shRNA14'
Antisense 48.1 13.5 47.3 0.0 16.2 50.9
Sense -0.2 10.6 -13.3 -36.6 20.7 -15.5

Fig. 9a

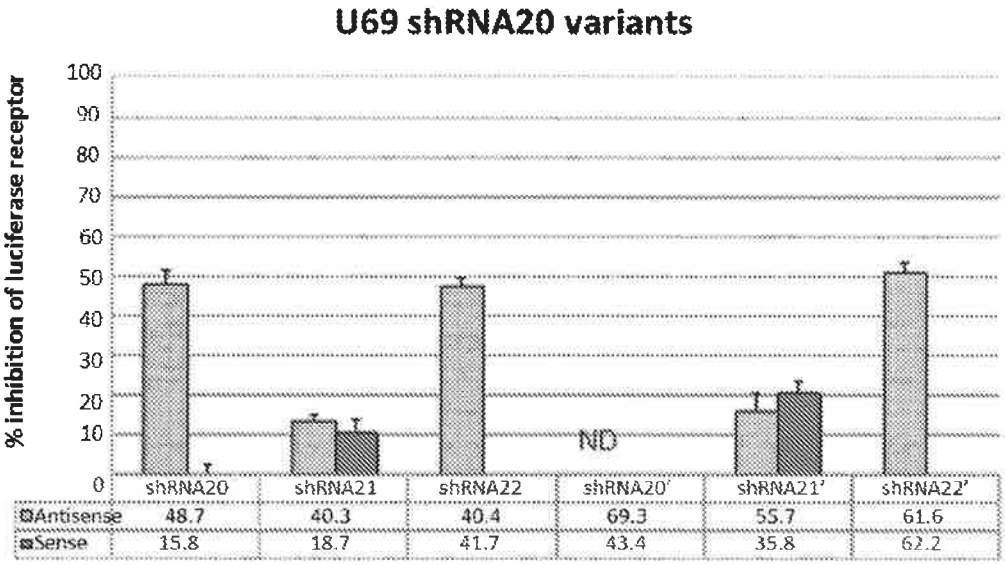

U69 shRNA20 variants
% inhibition of luciferase receptor
100
90
80
70
60
50
40
30
20
10
0
ND
shRNA20
shRNA21
shRNA22
shRNA20'
shRNA21'
shRNA22'
Antisense 48.7 40.3 40.4 69.3 55.7 61.6
Sense 15.8 18.7 41.7 43.4 35.8 62.2

Fig. 9b

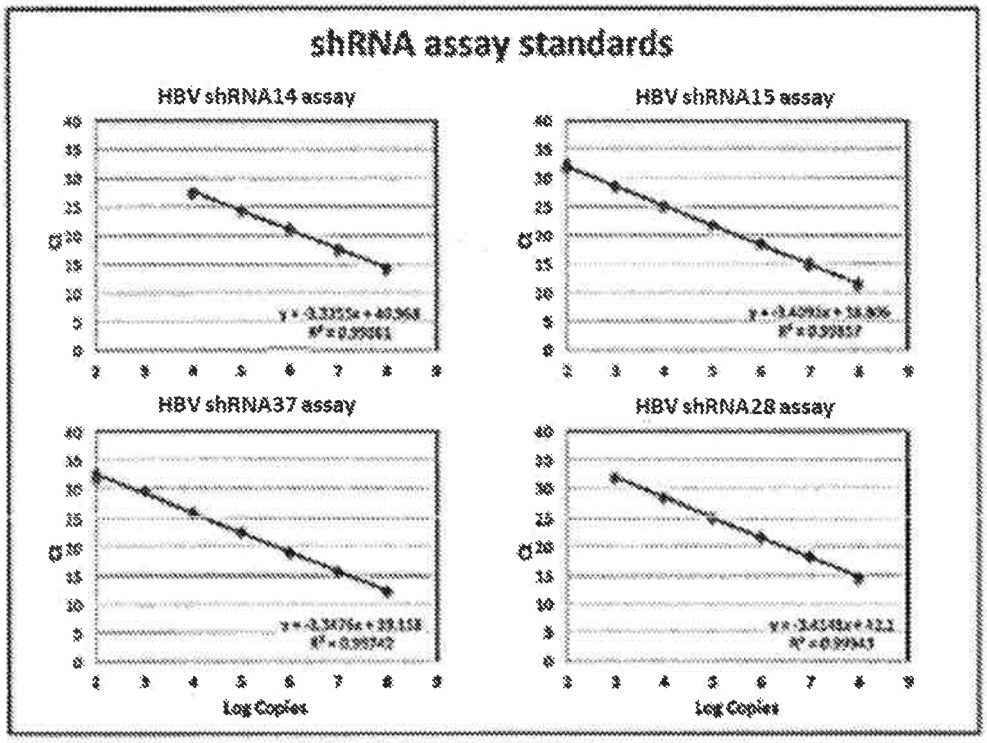


Fig. 10

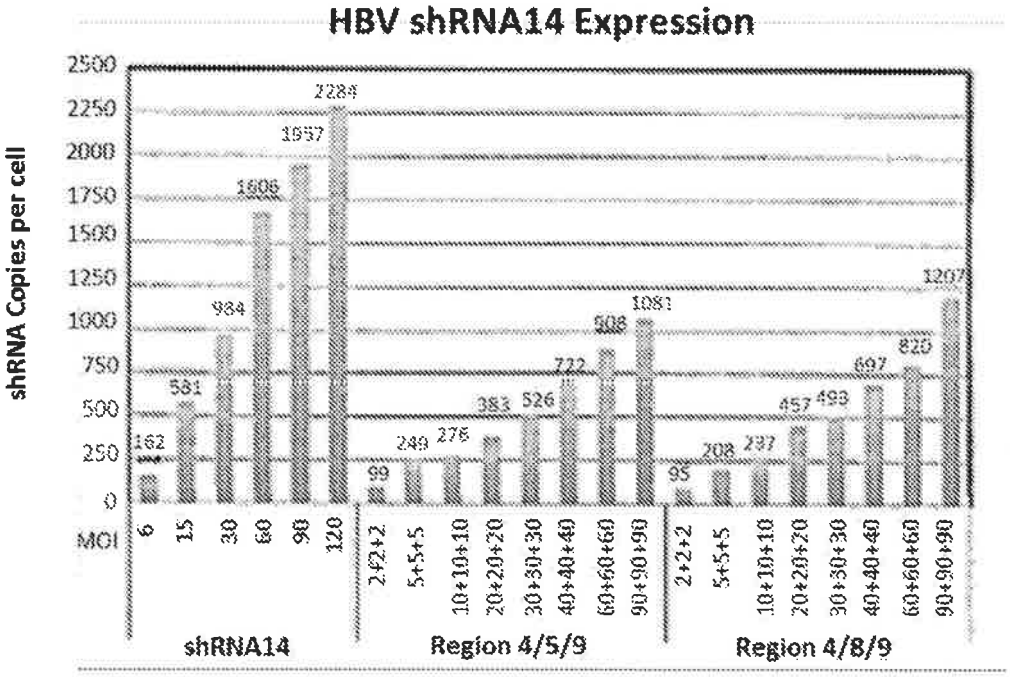


Fig. 11a

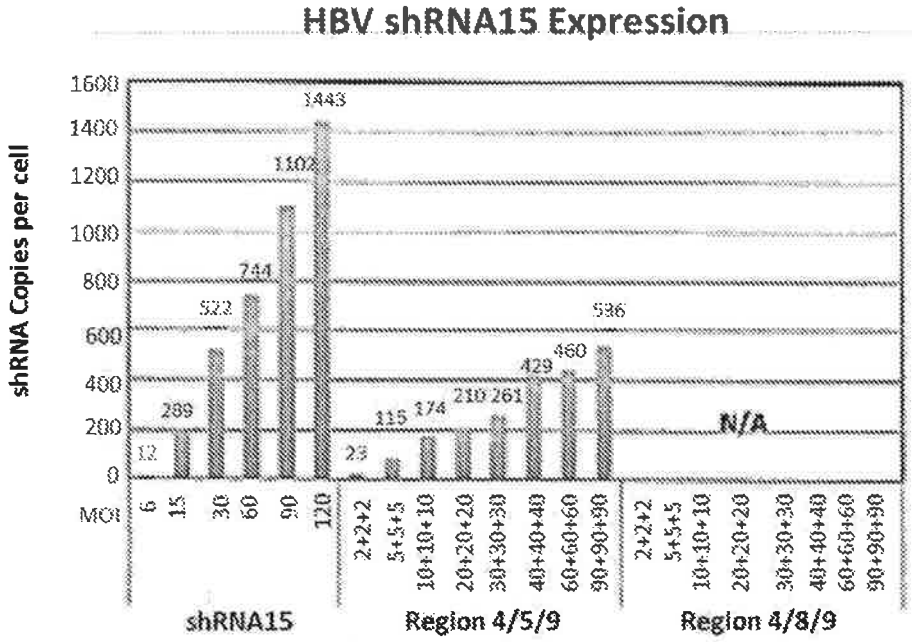


Fig. 11b

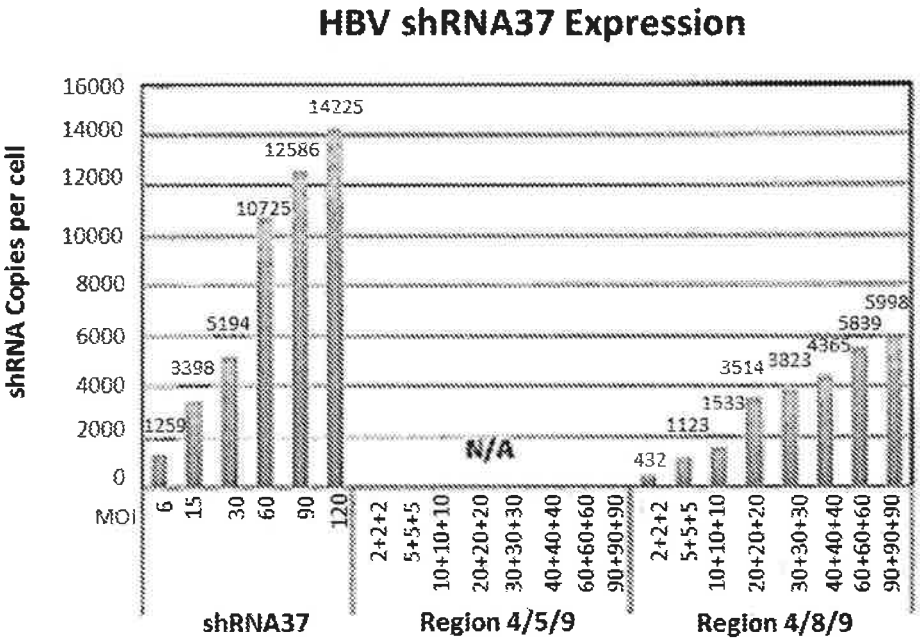


Fig. 11c

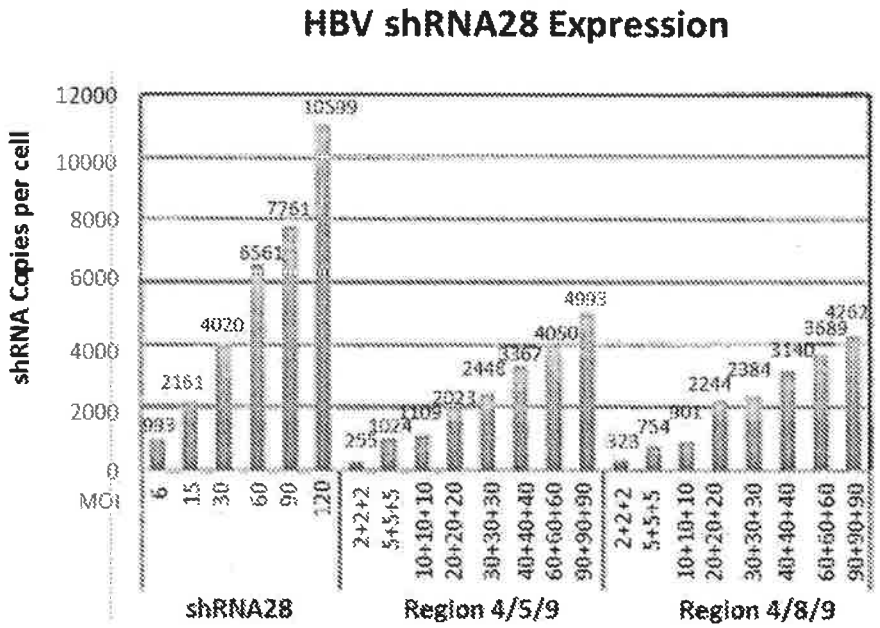


Fig. 11d

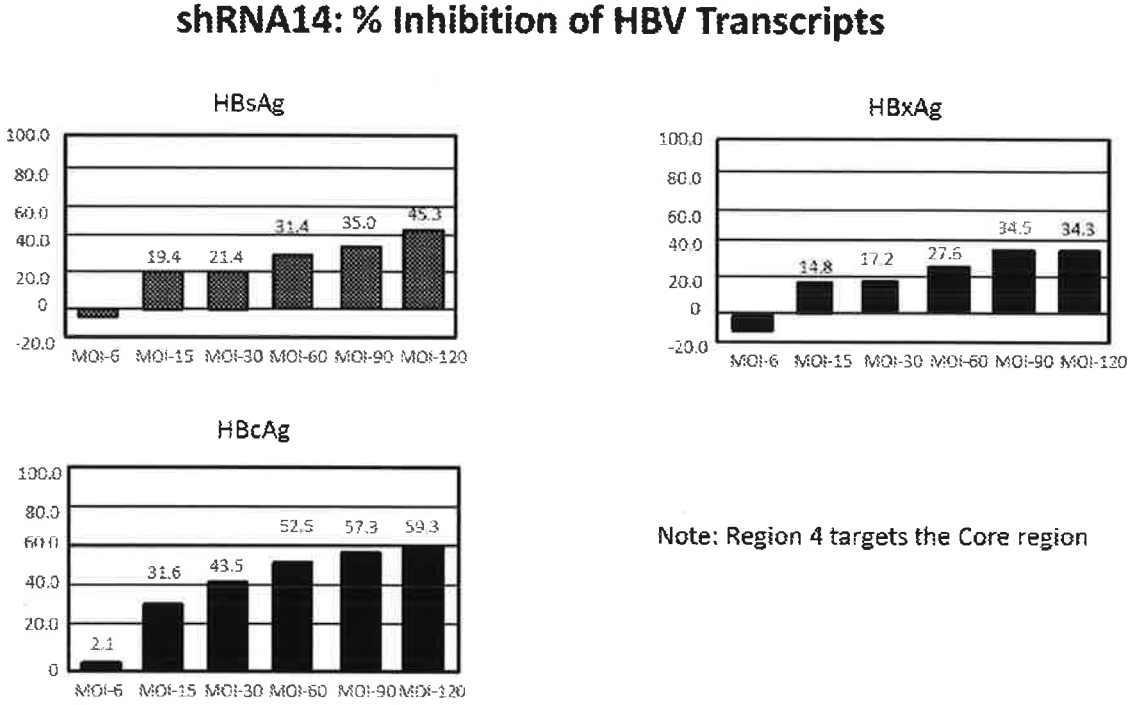


Fig. 12a

shRNA37: % Inhibition of HBV Transcripts

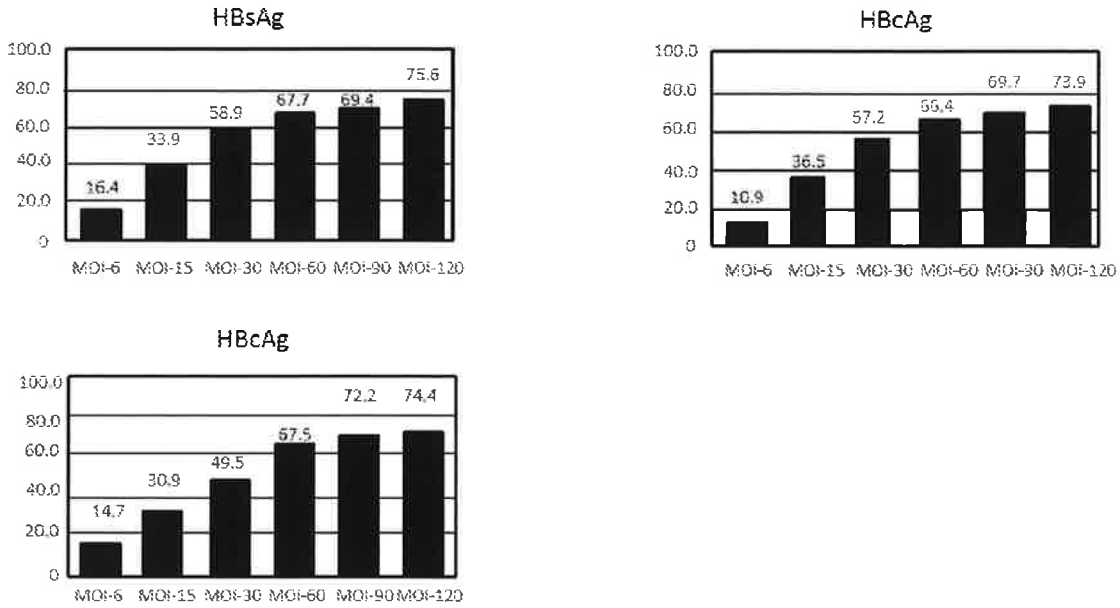


Fig. 12b

shRNA15: % Inhibition of HBV Transcripts

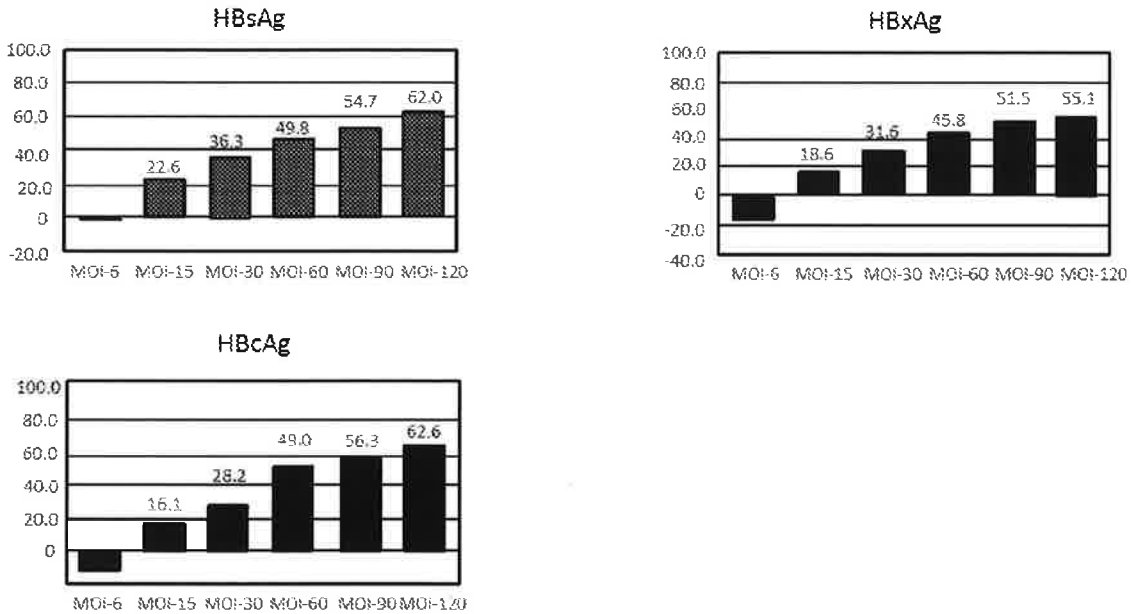


Fig. 12c

shRNA28: % Inhibition of HBV Transcripts

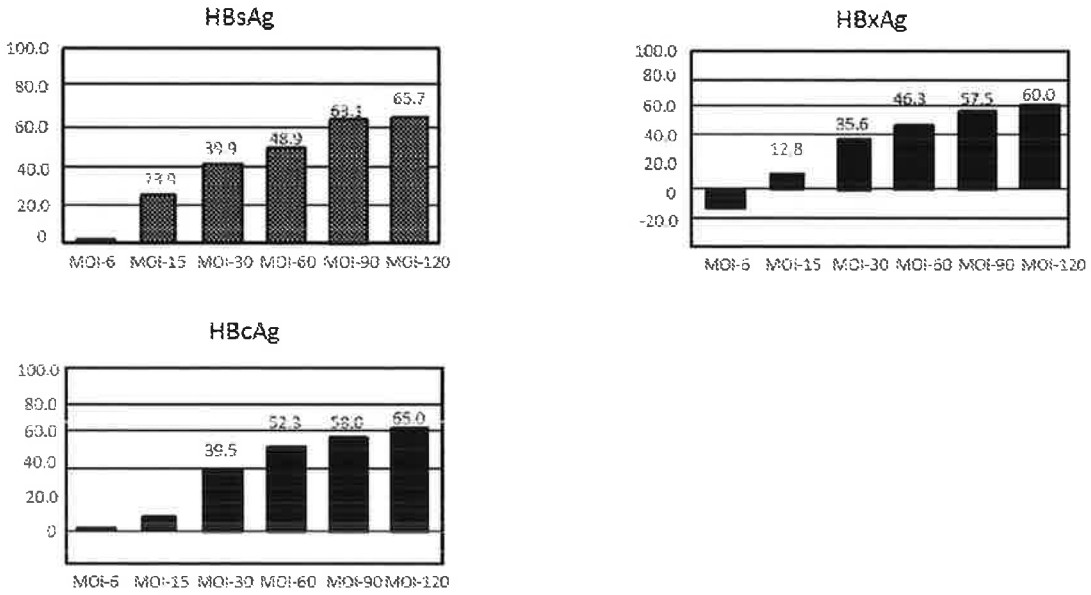


Fig. 12d

Region 4/8/9 Triple: % Inhibition of HBV Transcripts

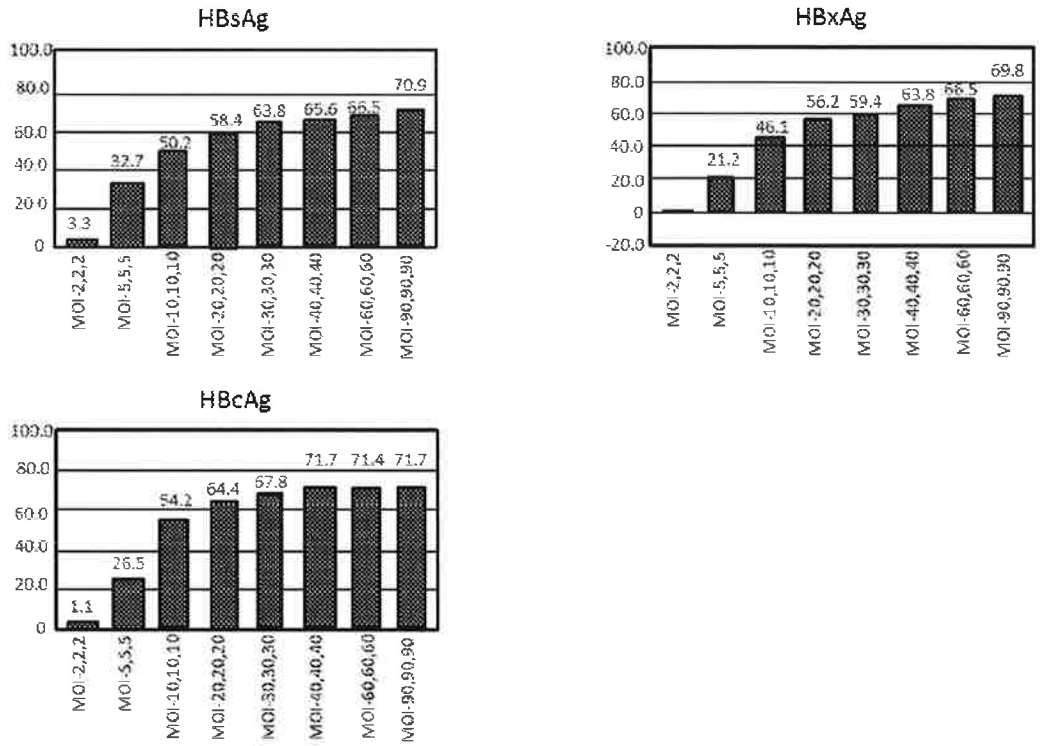


Fig. 12e

Region 4/5/9 Triple: % Inhibition of HBV Transcripts

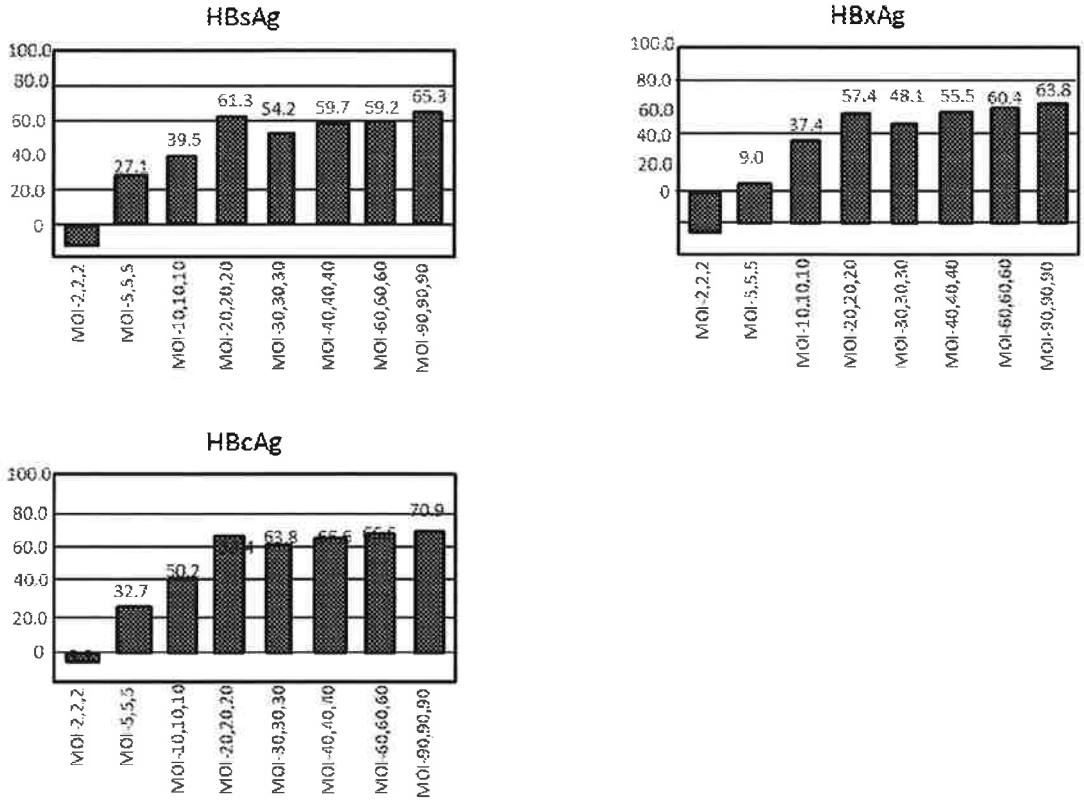


Fig. 12f

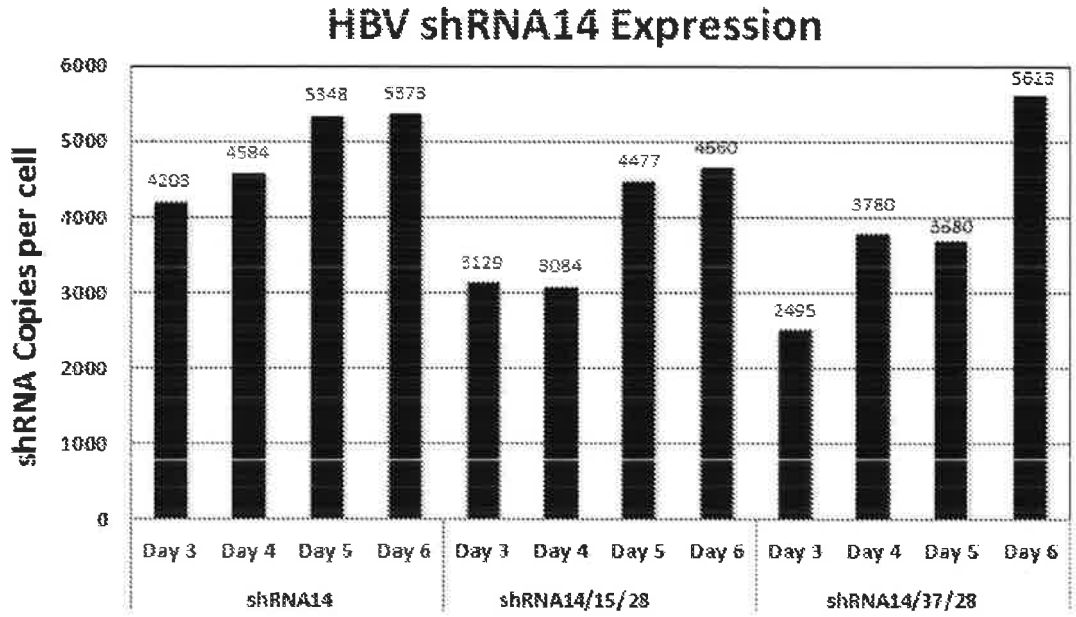


Fig. 13a

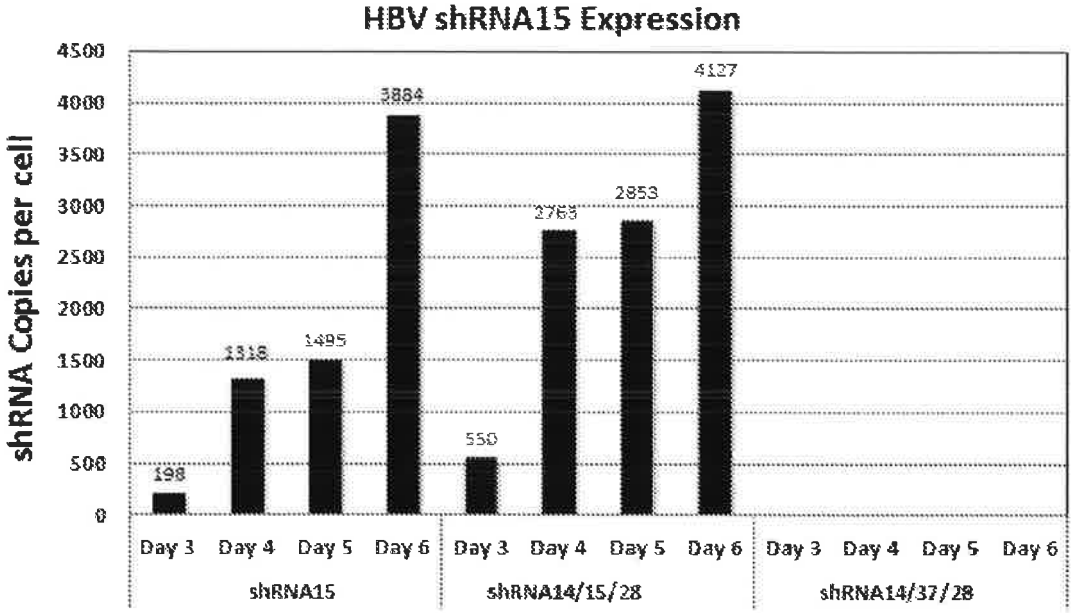


Fig. 13b

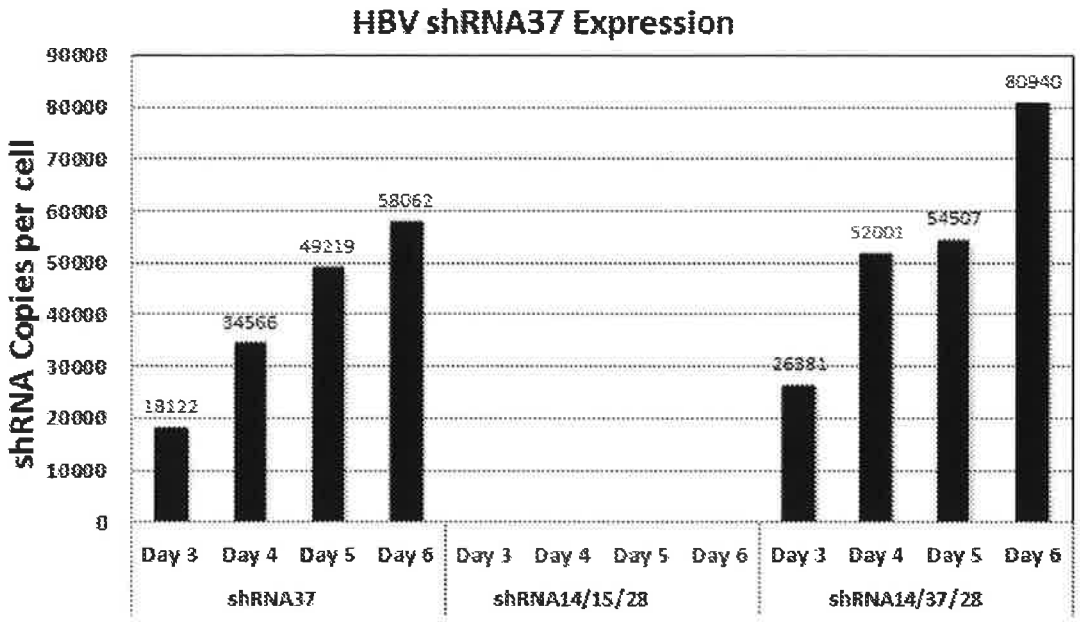


Fig. 13c

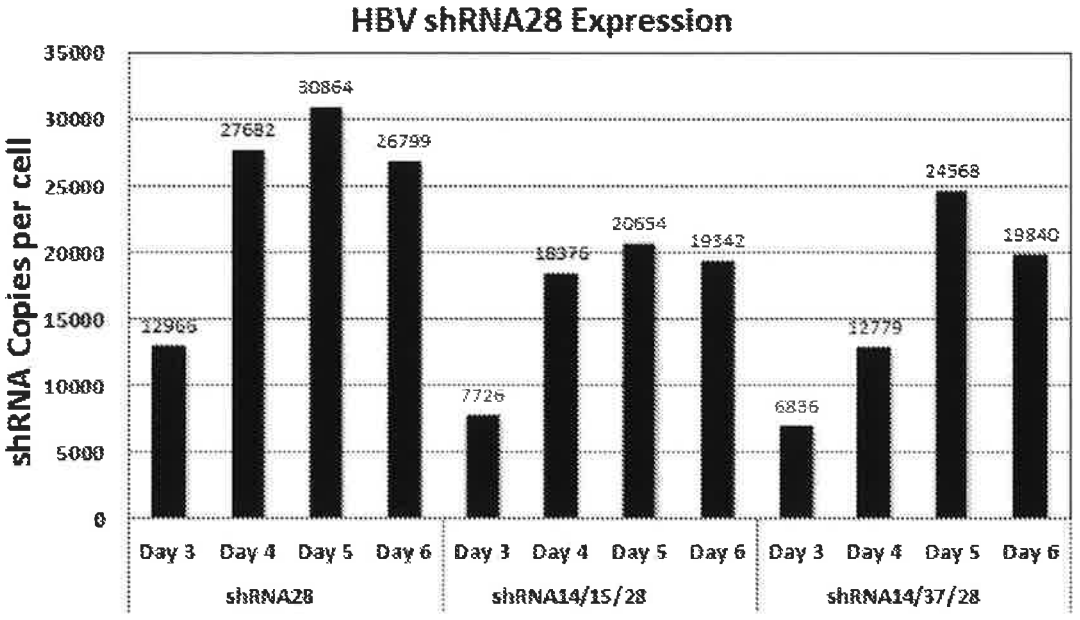


Fig. 13d

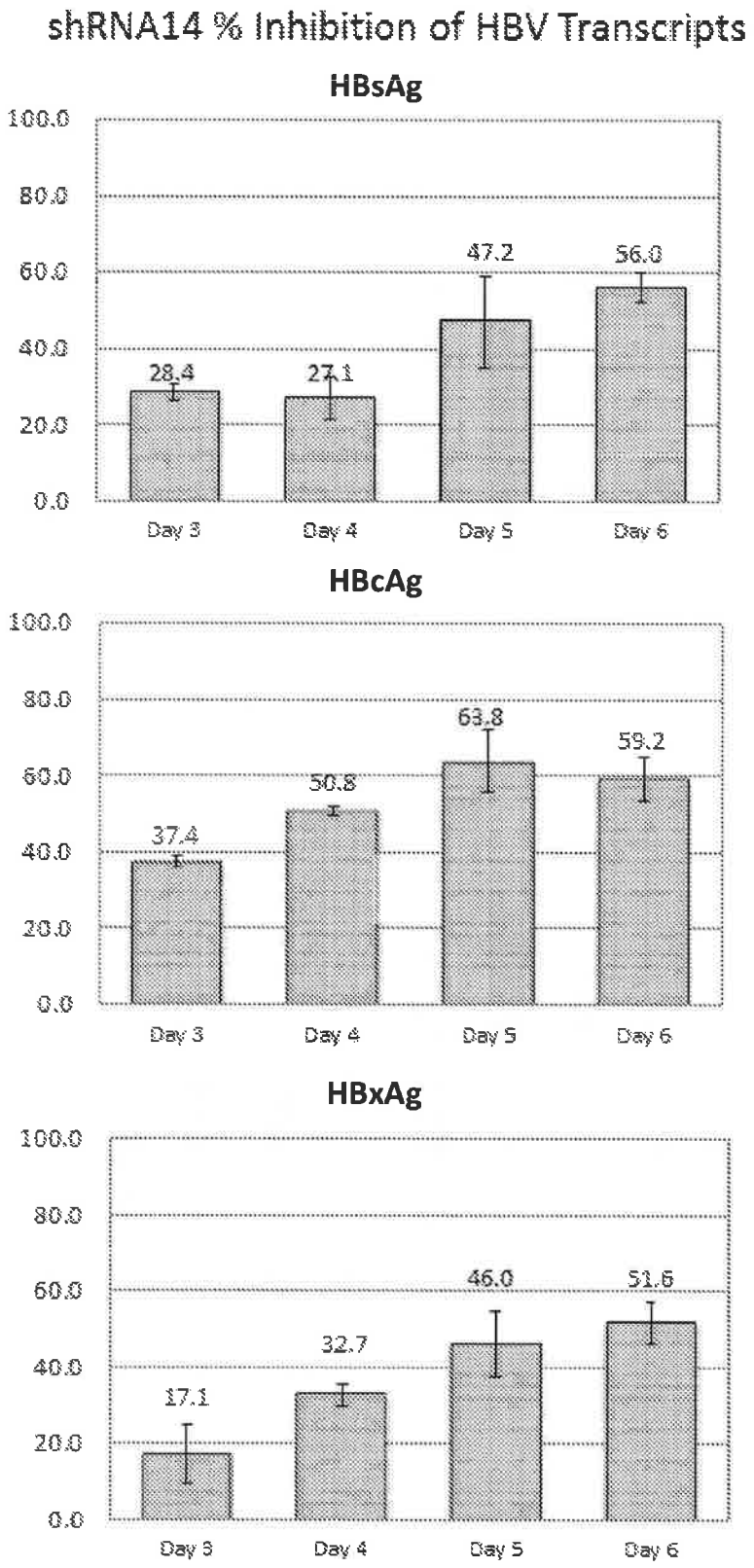

shRNA14 % Inhibition of HBV Transcripts
HBsAg
100.0
80.0
60.0
40.0
20.0
0.0
28.4
27.1
47.2
56.0
Day 3
Day 4
Day 5
Day 6
HBcAg
100.0
80.0
60.0
40.0
20.0
0.0
37.4
50.8
63.8
59.2
Day 3
Day 4
Day 5
Day 6
HBxAg
100.0
80.0
60.0
40.0
20.0
0.0
17.1
32.7
46.0
51.6
Day 3
Day 4
Day 5
Day 6

Fig. 14a

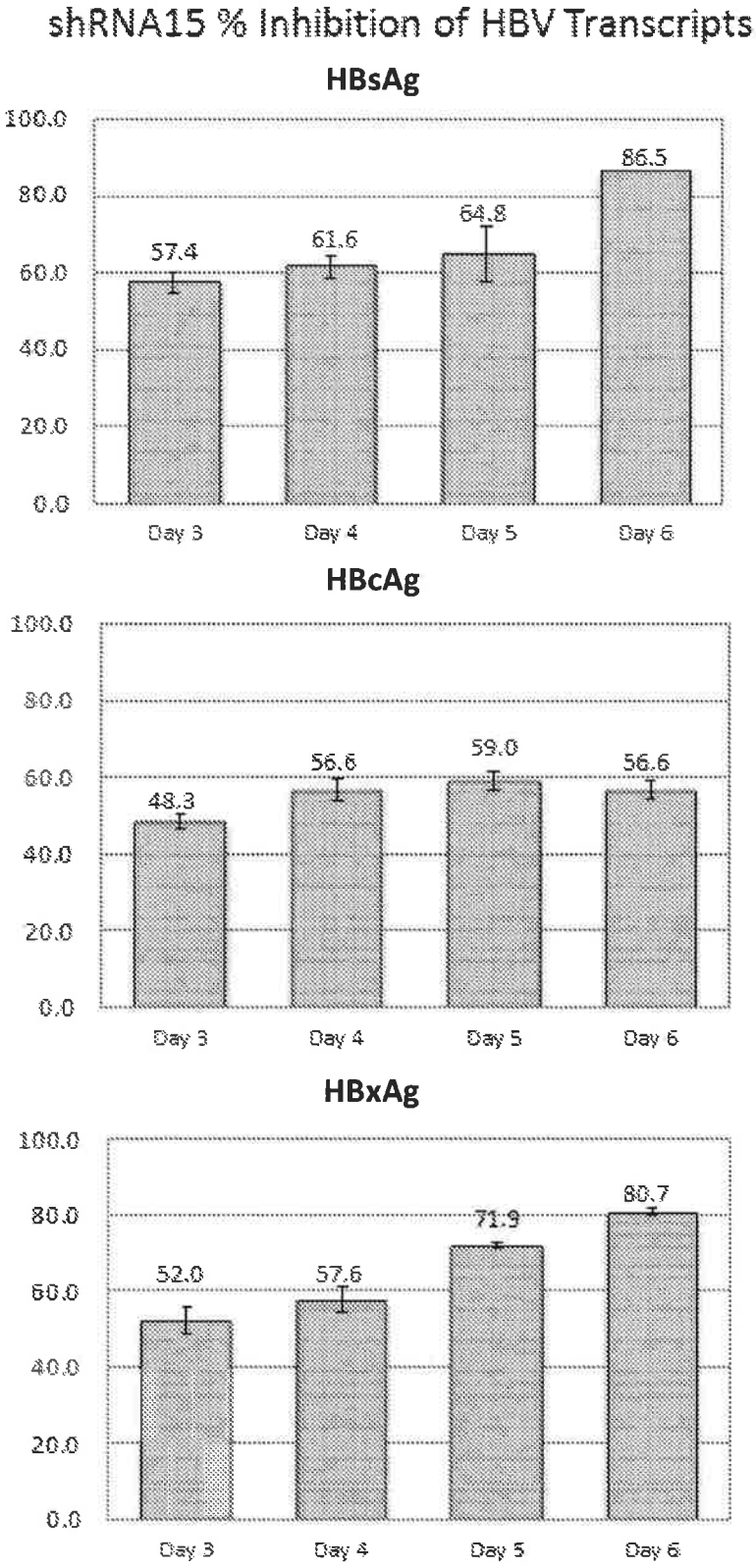

shRNA15 % Inhibition of HBV Transcripts
HBsAg
100.0
80.0
60.0
40.0
20.0
0.0
57.4
61.6
64.8
86.5
Day 3
Day 4
Day 5
Day 6
HBcAg
100.0
80.0
60.0
40.0
20.0
0.0
48.3
56.6
59.0
56.6
Day 3
Day 4
Day 5
Day 6
HBxAg
100.0
80.0
60.0
40.0
20.0
0.0
52.0
57.6
71.9
80.7
Day 3
Day 4
Day 5
Day 6

Fig. 14b

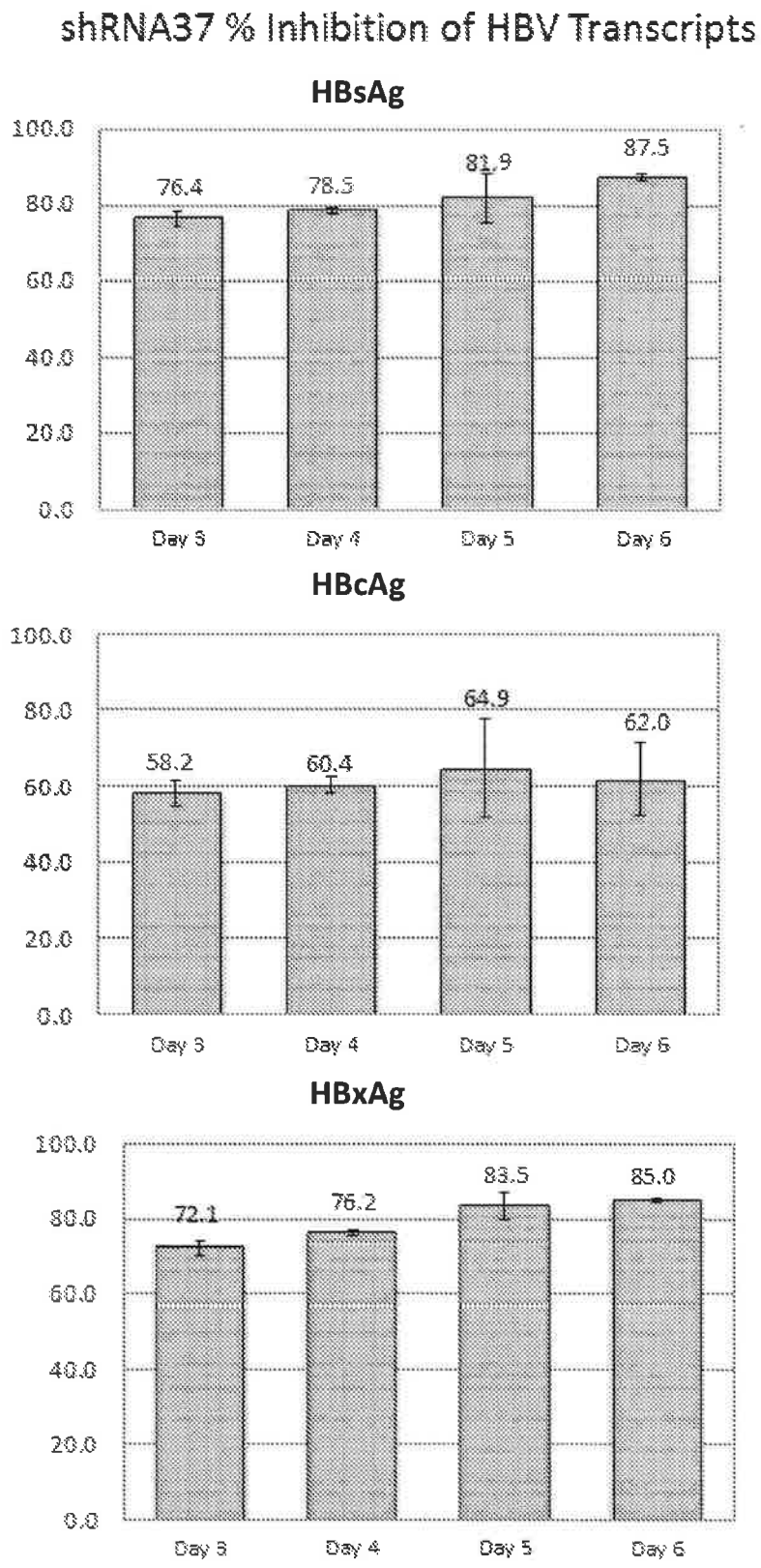

shRNA37 % Inhibition of HBV Transcripts
HBsAg
100.0
80.0
60.0
40.0
20.0
0.0
76.4
78.5
81.9
87.5
Day 3
Day 4
Day 5
Day 6
HBcAg
100.0
80.0
60.0
40.0
20.0
0.0
58.2
60.4
64.9
62.0
Day 3
Day 4
Day 5
Day 6
HBxAg
100.0
80.0
60.0
40.0
20.0
0.0
72.1
76.2
83.5
85.0
Day 3
Day 4
Day 5
Day 6

Fig. 14c

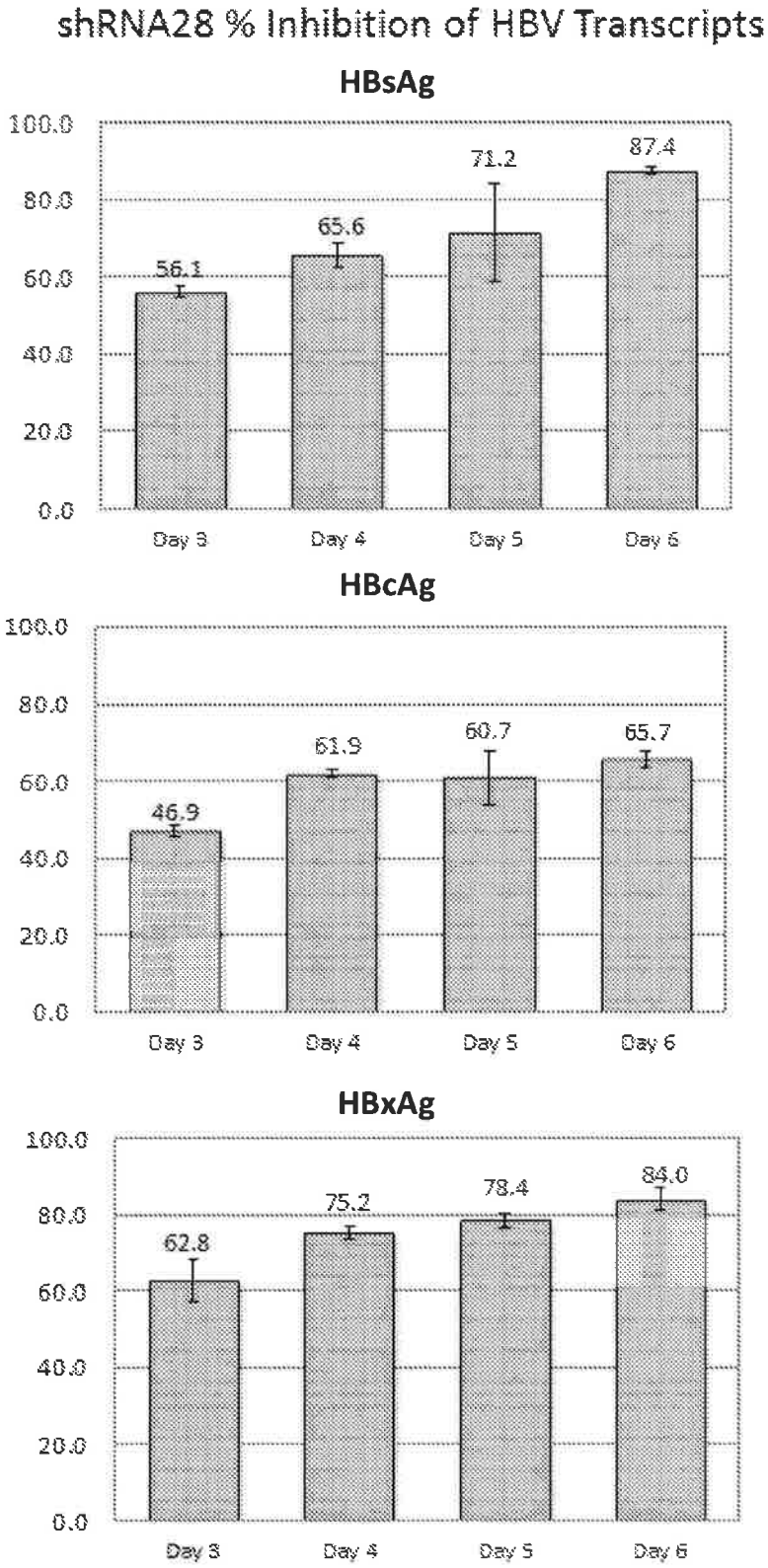

shRNA28 % Inhibition of HBV Transcripts
HBsAg
100.0
80.0
60.0
40.0
20.0
0.0
56.1
65.6
71.2
87.4
Day 3
Day 4
Day 5
Day 6
HBcAg
100.0
80.0
60.0
40.0
20.0
0.0
46.9
61.9
60.7
65.7
Day 3
Day 4
Day 5
Day 6
HBxAg
100.0
80.0
60.0
40.0
20.0
0.0
62.8
75.2
78.4
84.0
Day 3
Day 4
Day 5
Day 6

Fig. 14d

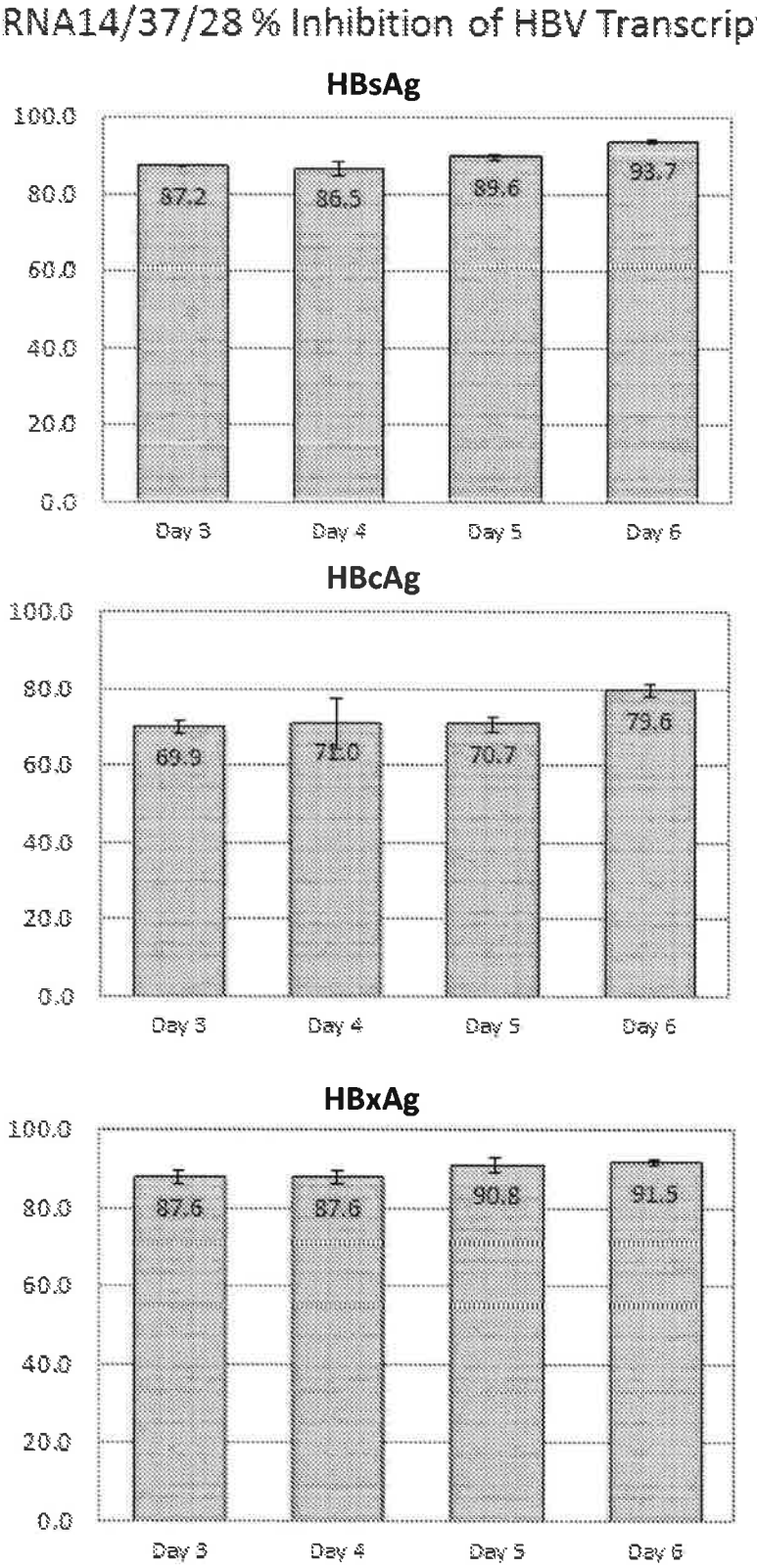

shRNA14/37/28 % Inhibition of HBV Transcripts
HBsAg
100.0
80.0
60.0
40.0
20.0
0.0
87.2
86.5
89.6
93.7
Day 3
Day 4
Day 5
Day 6
HBcAg
100.0
80.0
60.0
40.0
20.0
0.0
69.9
71.0
70.7
79.6
Day 3
Day 4
Day 5
Day 6
HBxAg
100.0
80.0
60.0
40.0
20.0
0.0
87.6
87.6
90.8
91.5
Day 3
Day 4
Day 5
Day 6

Fig. 14e

shRNA14/15/28 % Inhibition of HBV Transcripts

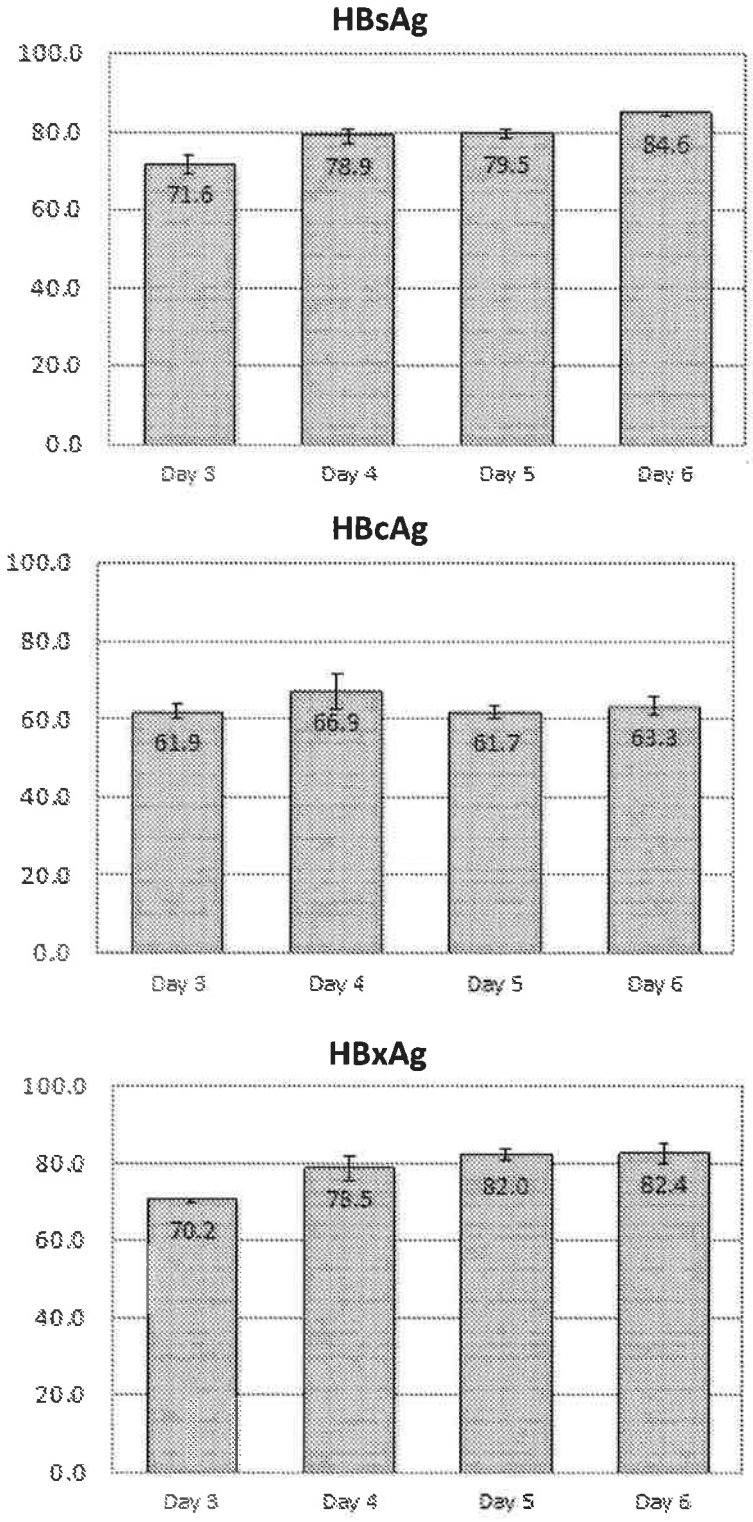
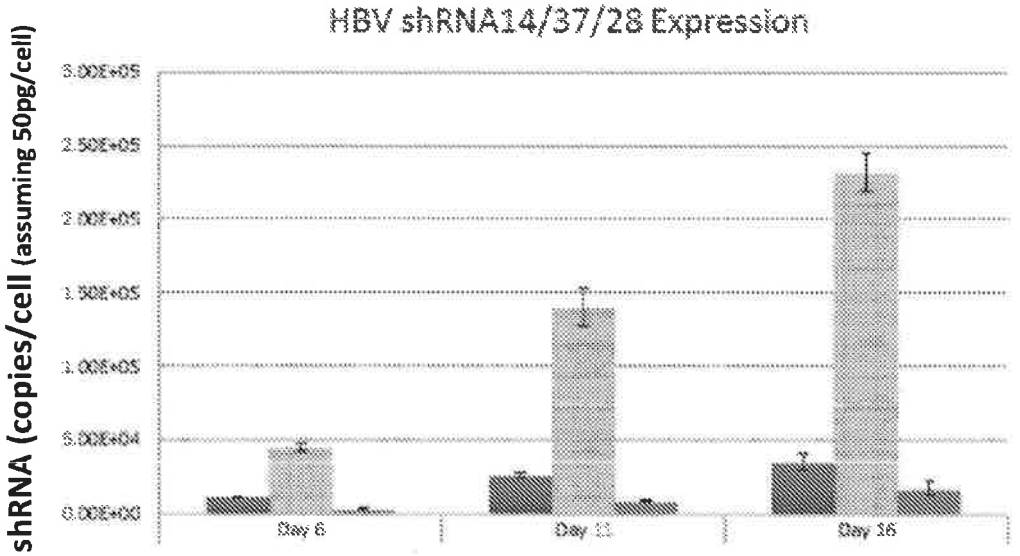


Fig. 14f



shRNA14	1.15E+05	2.63E+04	3.53E+04
shRNA37	4.47E+04	1.40E+05	2.32E+05
shRNA28	3.14E+03	9.26E+03	1.76E+04

Fig. 15a

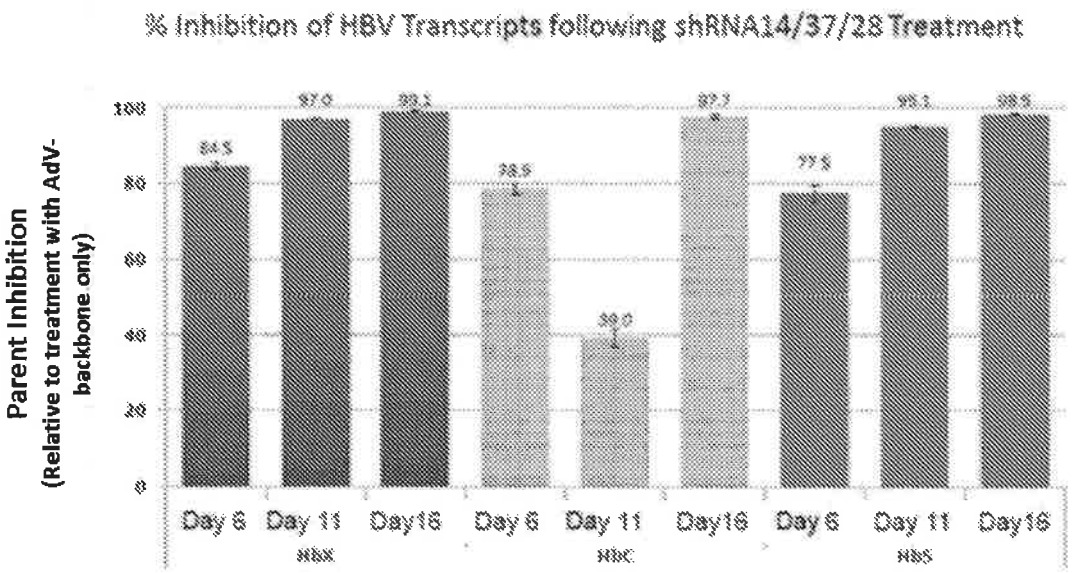


Fig. 15b

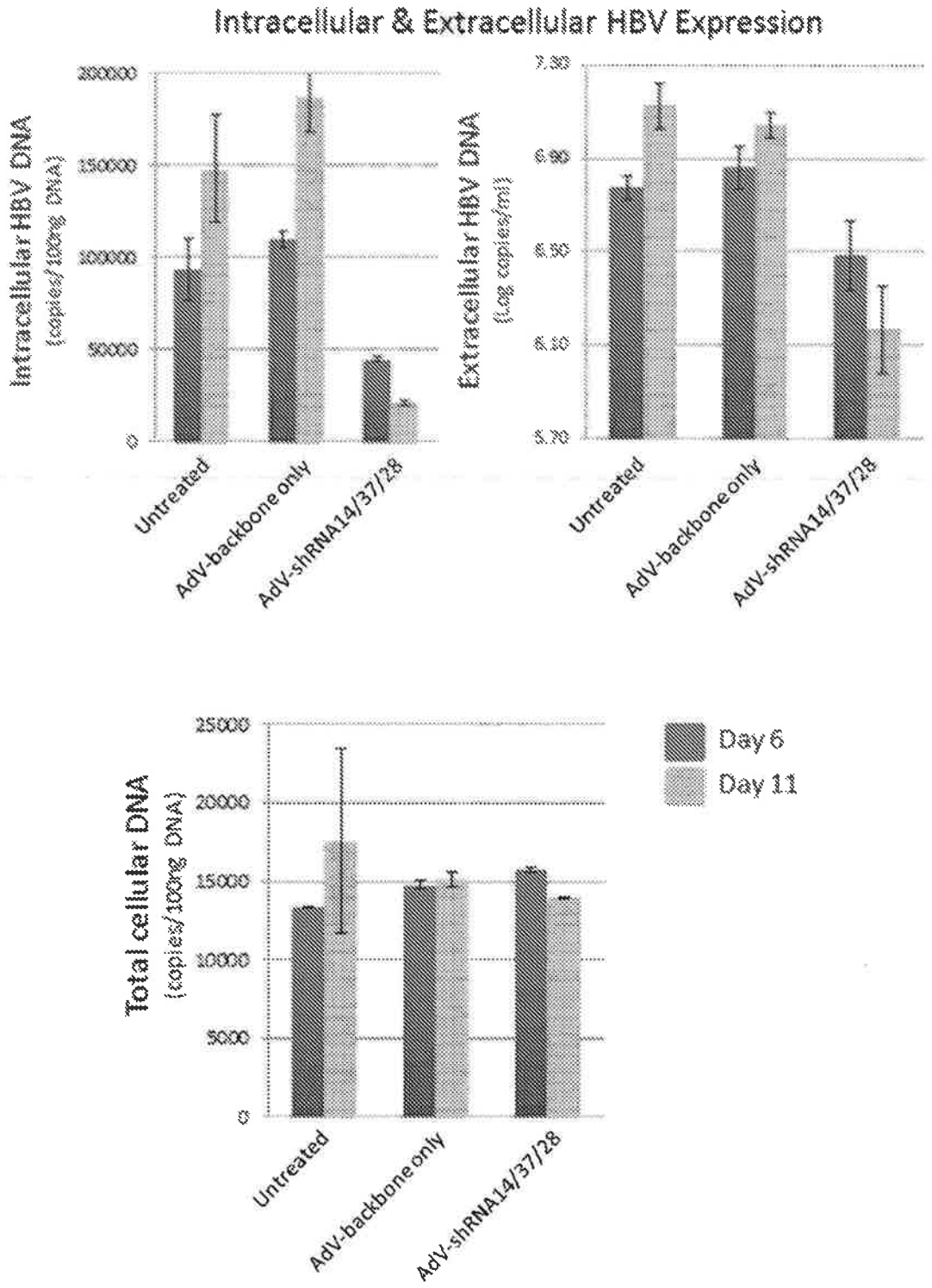

Intracellular & Extracellular HBV Expression
Intracellular HBV DNA
(copies/100ng DNA)
200000
150000
100000
50000
0
Untreated
AdV-backbone only
AdV-shRNA14/37/28
Extracellular HBV DNA
(Log copies/ml)
7.30
6.90
6.50
6.10
5.70
Untreated
AdV-backbone only
AdV-shRNA14/37/28
Total cellular DNA
(copies/100ng DNA)
25000
20000
15000
10000
5000
0
Untreated
AdV-backbone only
AdV-shRNA14/37/28
Day 6
Day 11

Fig. 15c

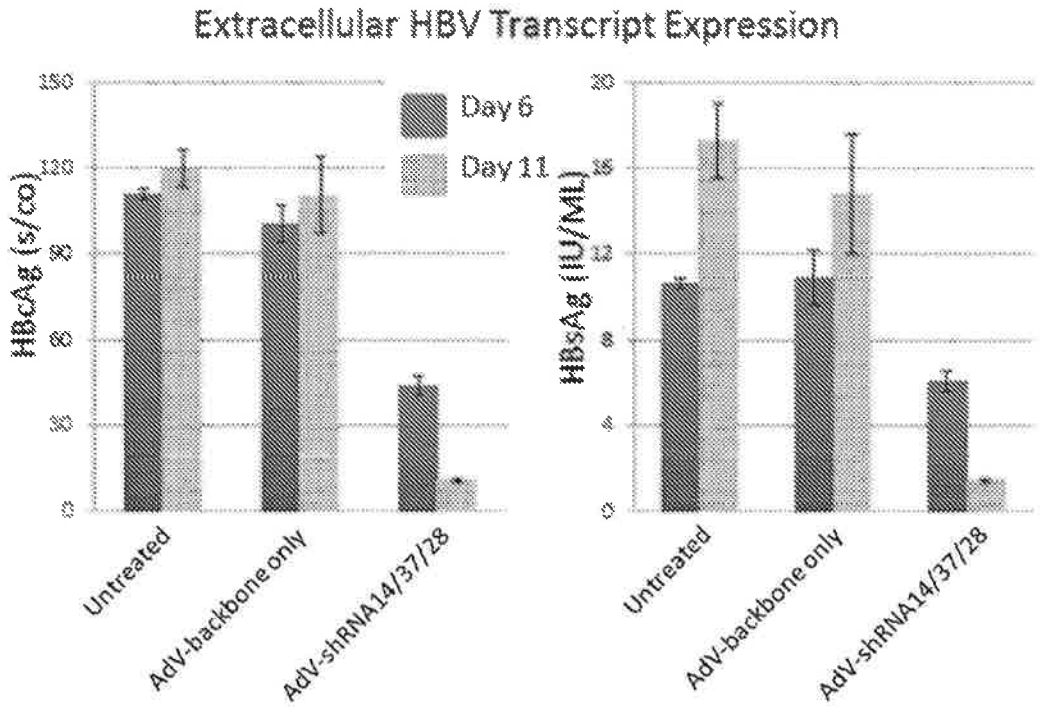


Fig. 15d

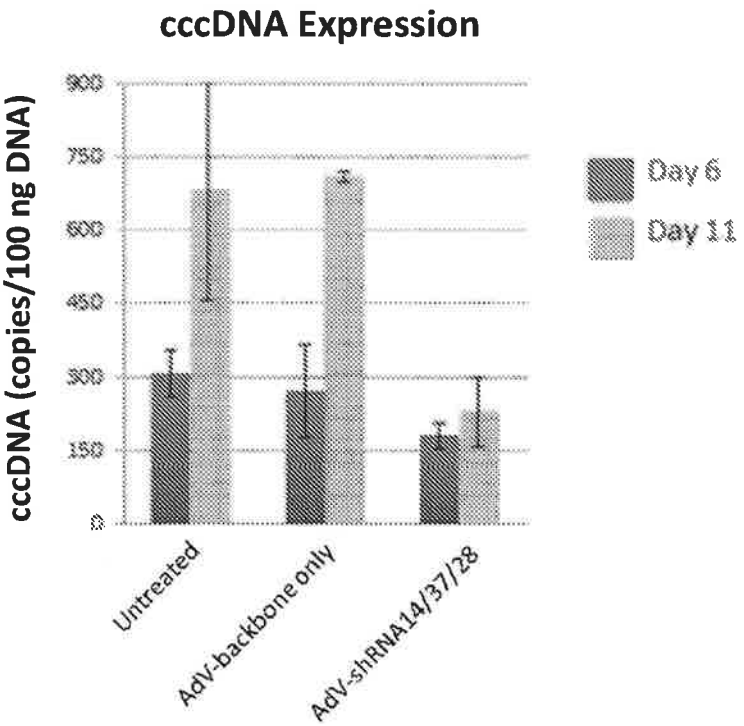


Fig. 15e

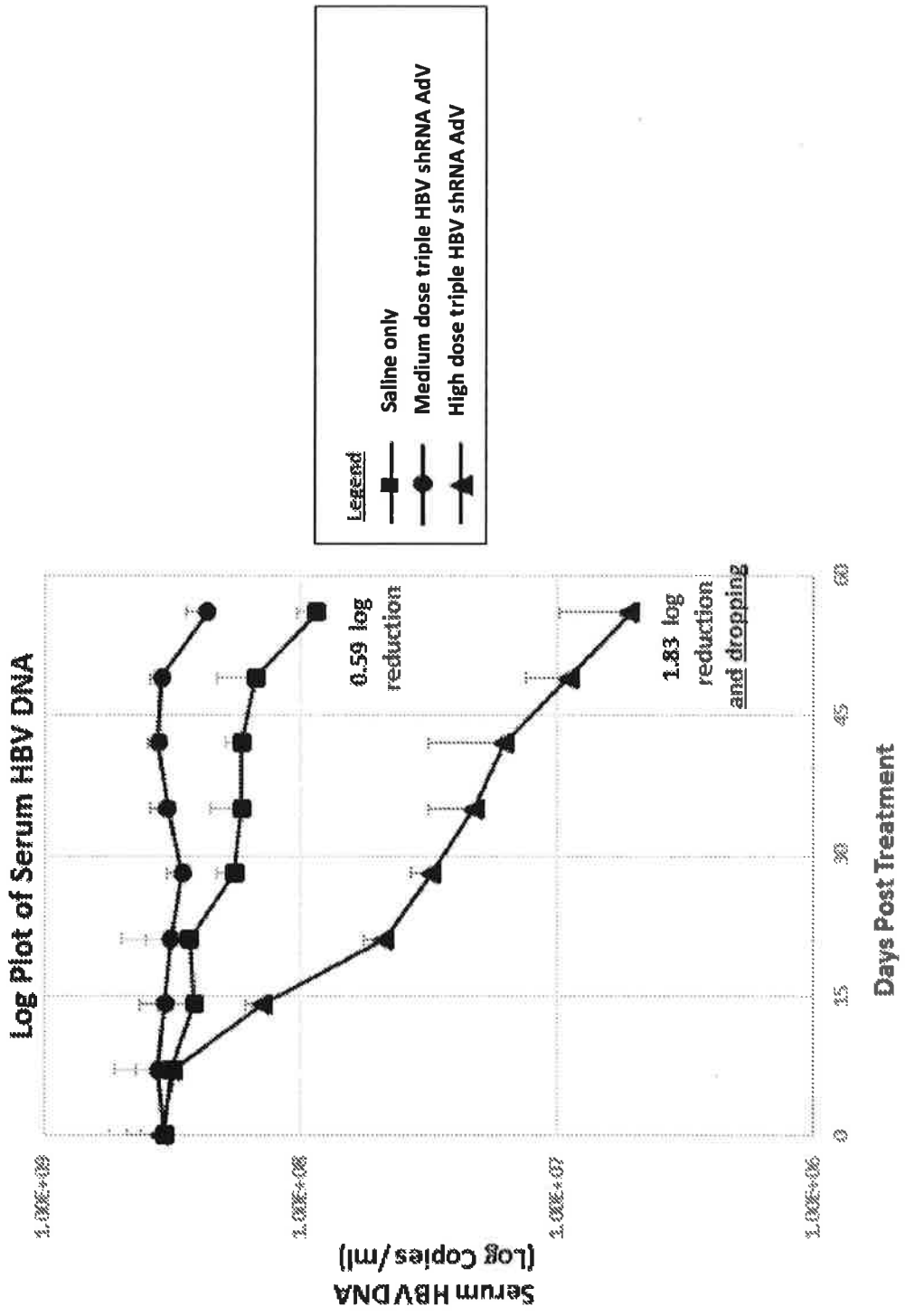

Log Plot of Serum HBV DNA
Legend
Saline only
Medium dose triple HBV shRNA AdV
High dose triple HBV shRNA AdV
0.59 log reduction
1.83 log reduction and dropping
Serum HBV DNA (Log Copies/ml)
Days Post Treatment
0
15
30
45
60

Fig. 16

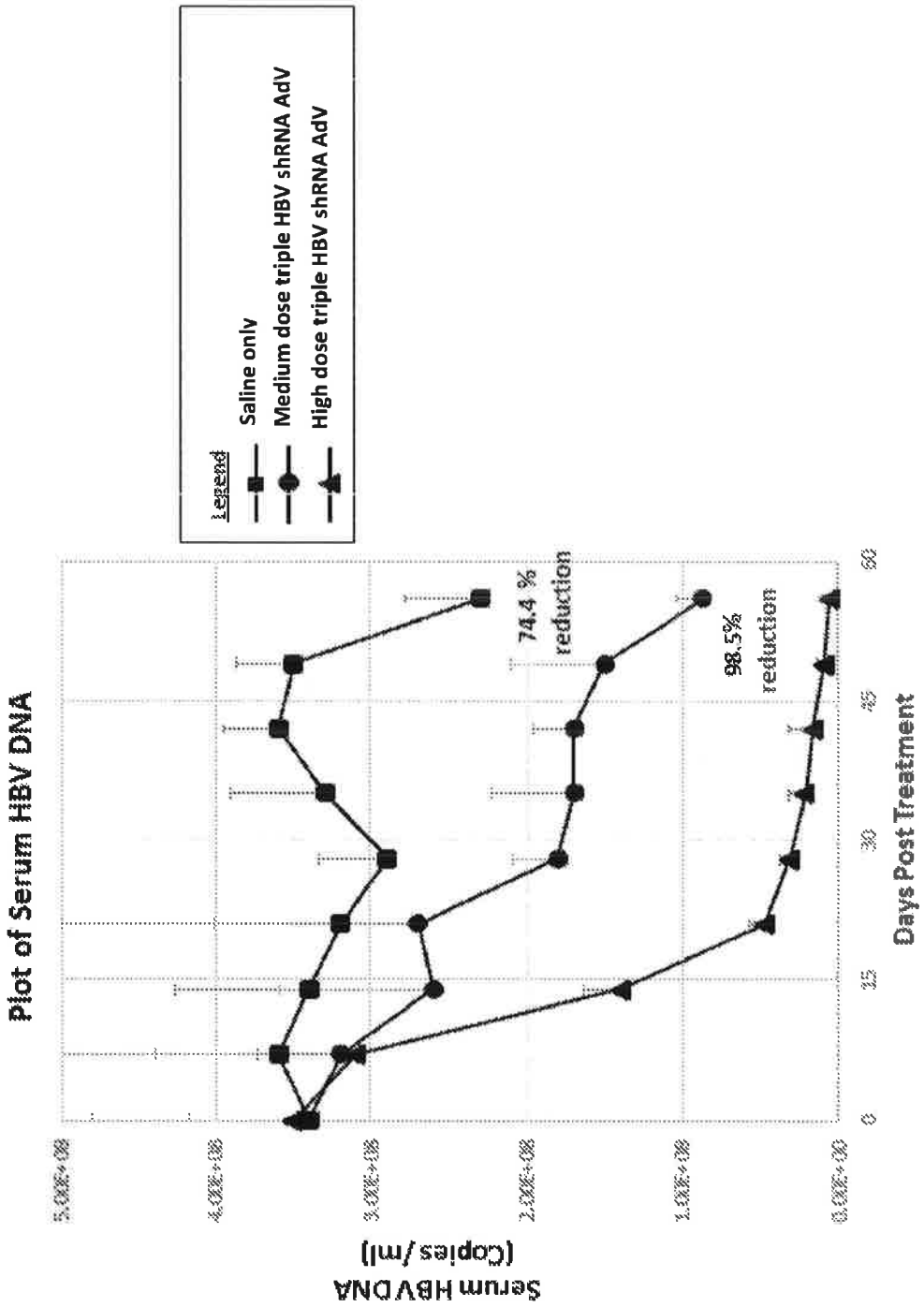

Plot of Serum HBV DNA
Legend
Saline only
Medium dose triple HBV shRNA AdV
High dose triple HBV shRNA AdV
74.4 % reduction
98.5% reduction
Serum HBV DNA (Copies/ml)
Days Post Treatment
0
15
30
45
60

Fig. 17

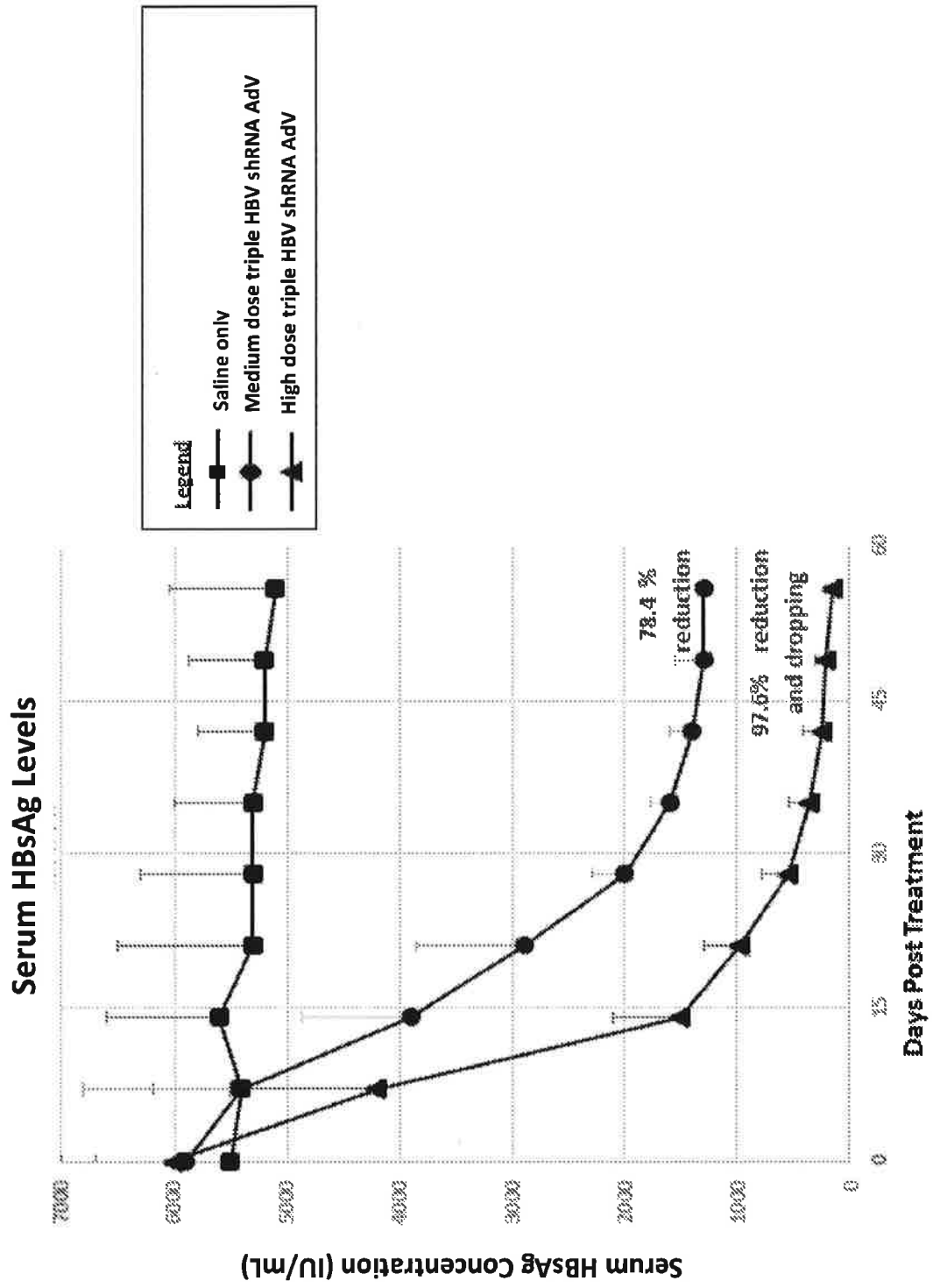

Serum HBsAg Levels
Legend
Saline only
Medium dose triple HBV shRNA AdV
High dose triple HBV shRNA AdV
78.4 % reduction
97.6% reduction and dropping
Serum HBsAg Concentration (IU/mL)
Days Post Treatment
0
15
30
45
60
1000
2000
3000
4000
5000
6000
7000

Fig. 18

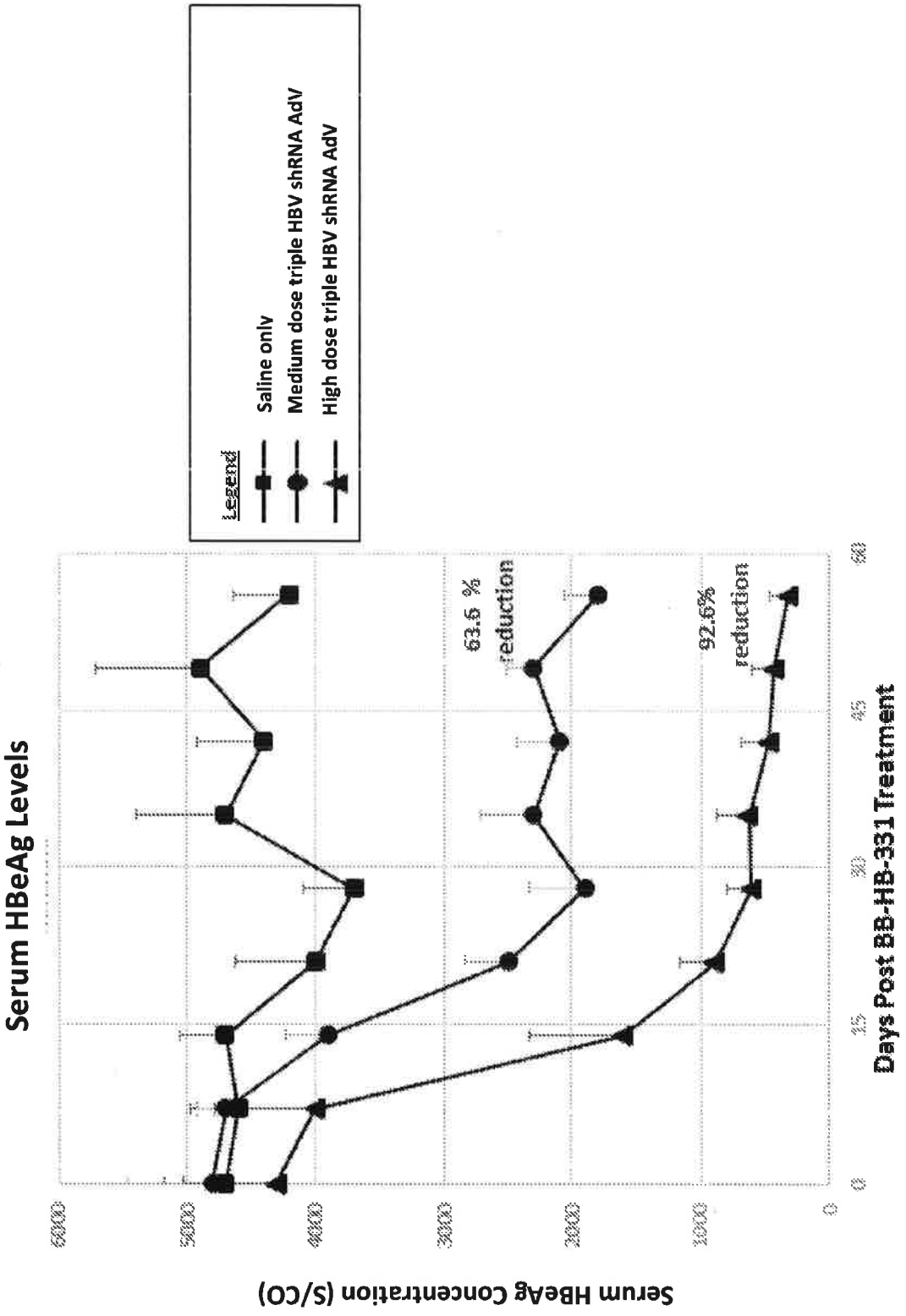

Serum HBeAg Levels
Legend
Saline only
Medium dose triple HBV shRNA AdV
High dose triple HBV shRNA AdV
63.6 % reduction
92.6% reduction
Serum HBeAg Concentration (S/CO)
Days Post BB-HB-331 Treatment
6000
5000
4000
3000
2000
1000
0
0
15
30
45
60

Fig. 19

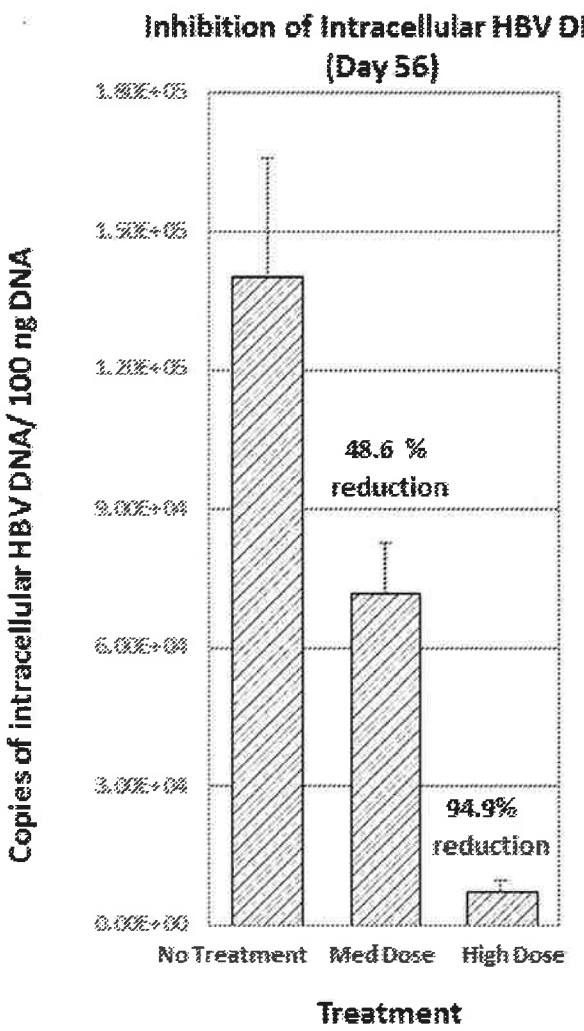

Inhibition of Intracellular HBV DNA
(Day 56)
Copies of intracellular HBV DNA/ 100 ng DNA
48.6 %
reduction
94.9%
reduction
No Treatment
Med Dose
High Dose
Treatment

Fig. 20

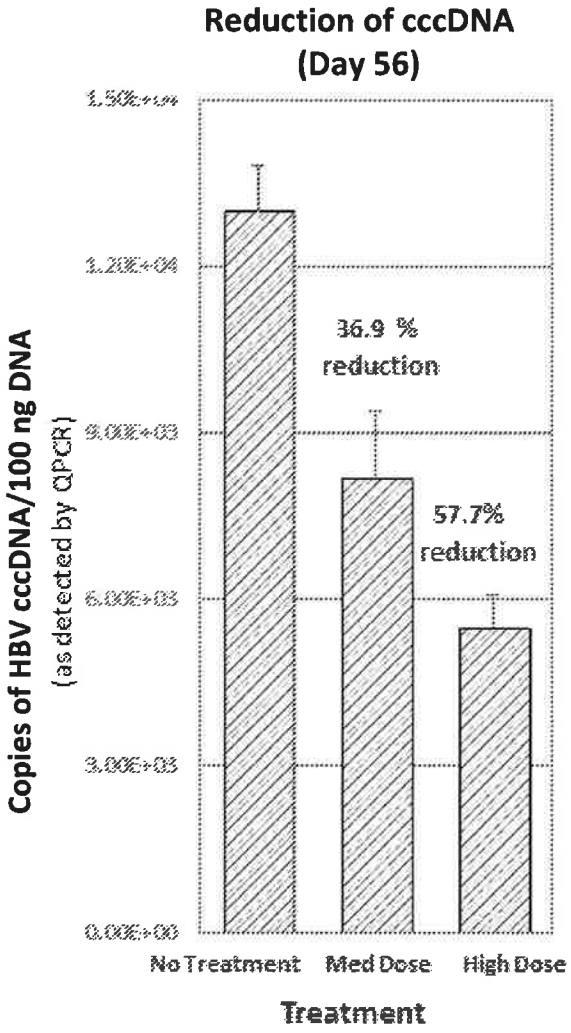

Reduction of cccDNA
(Day 56)
Copies of HBV cccDNA/100 ng DNA
(as detected by QPCR)
36.9 %
reduction
57.7%
reduction
No Treatment
Med Dose
High Dose
Treatment

Fig. 21

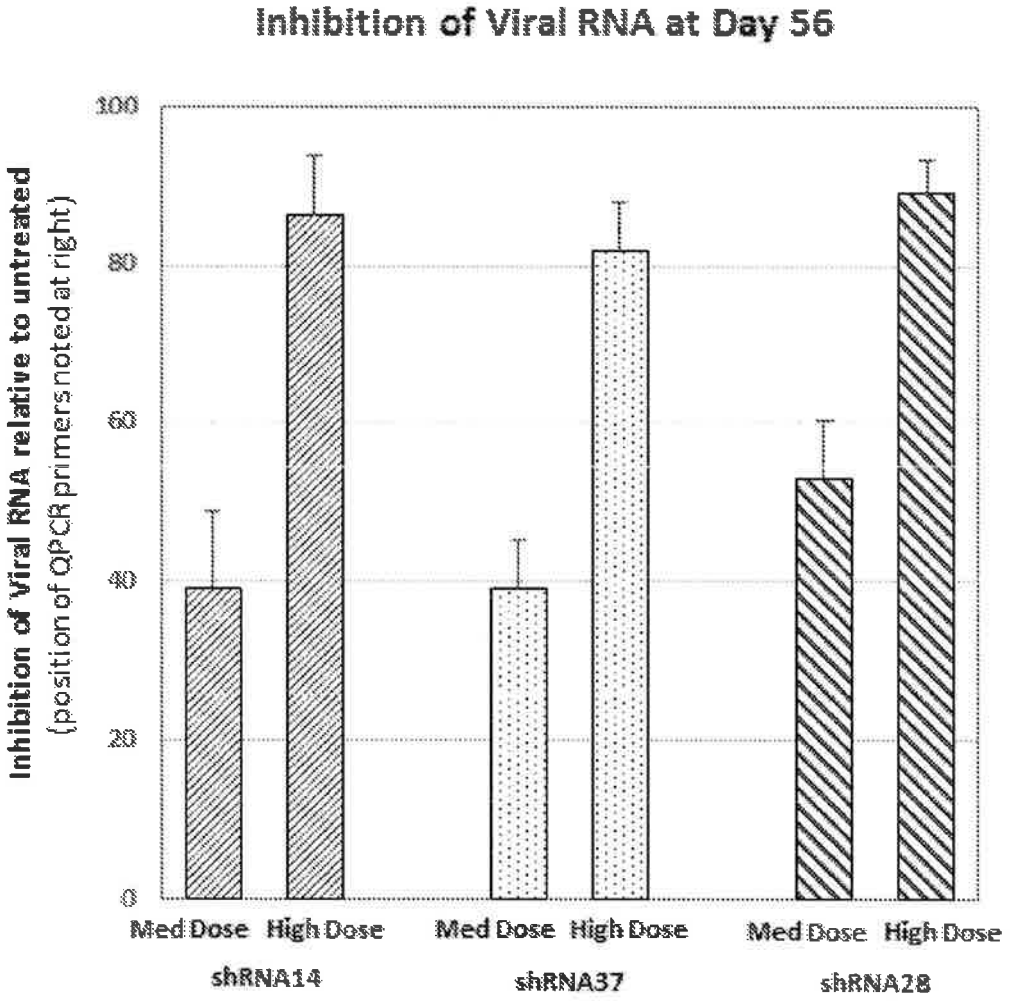

Inhibition of Viral RNA at Day 56
Inhibition of Viral RNA relative to untreated
(position of QPCR primers noted at right)
100
80
60
40
20
0
Med Dose
High Dose
Med Dose
High Dose
Med Dose
High Dose
shRNA14
shRNA37
shRNA28

Fig. 22