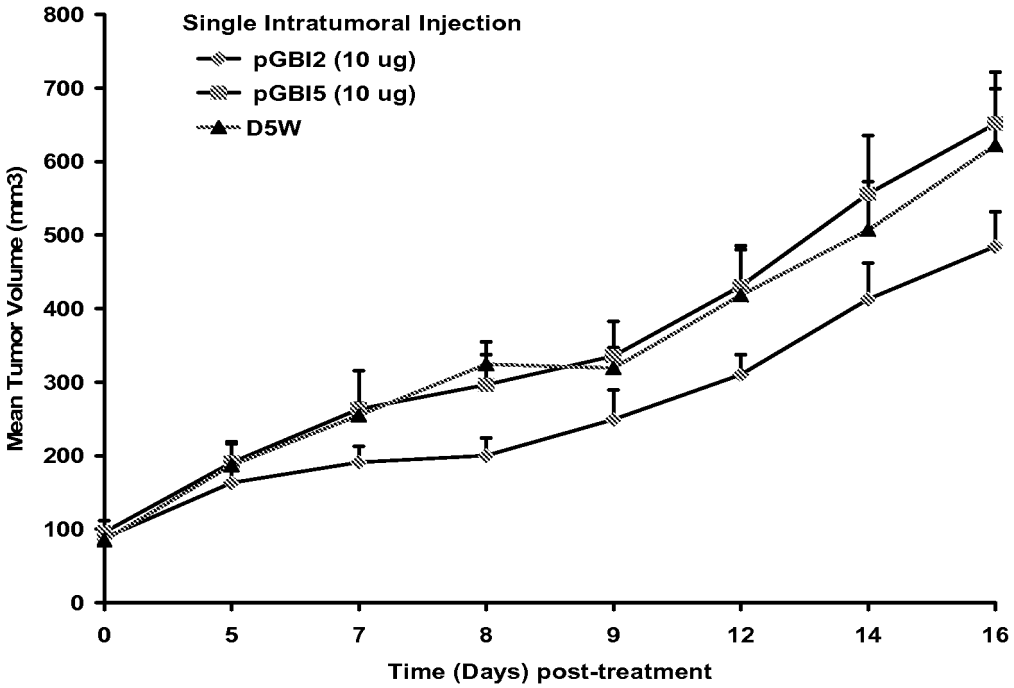

Single Intratumoral Injection
pGBI2 (10 ug)
pGBI5 (10 ug)
D5W
Mean Tumor Volume (mm3)
800
700
600
500
400
300
200
100
0
0
5
7
8
9
12
14
16
Time (Days) post-treatment

FIG. 1A

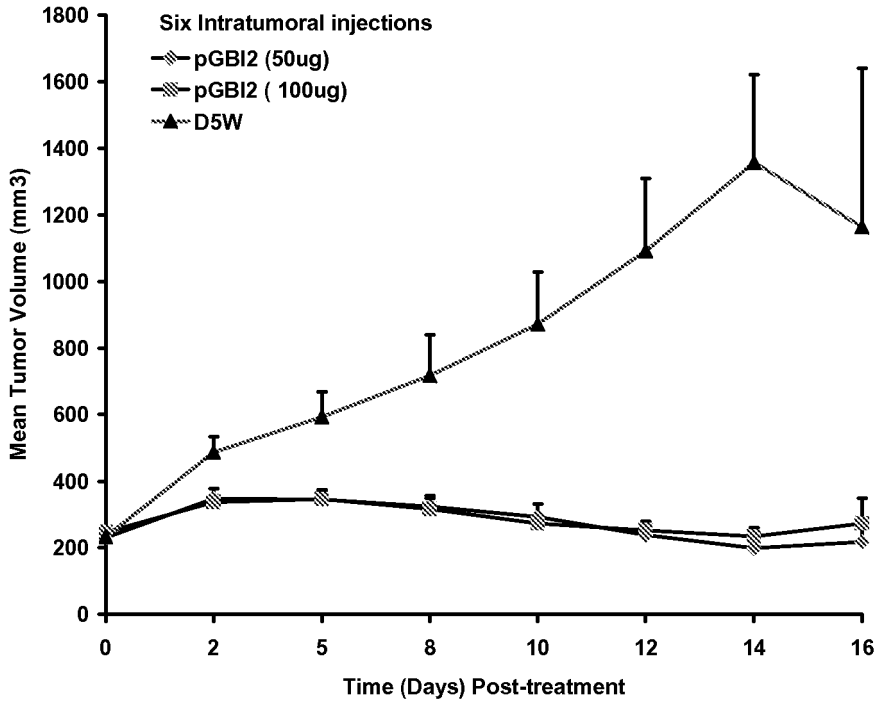

Six Intratumoral injections
pGBI2 (50ug)
pGBI2 (100ug)
D5W
Mean Tumor Volume (mm3)
1800
1600
1400
1200
1000
800
600
400
200
0
0
2
5
8
10
12
14
16
Time (Days) Post-treatment

FIG. 1B

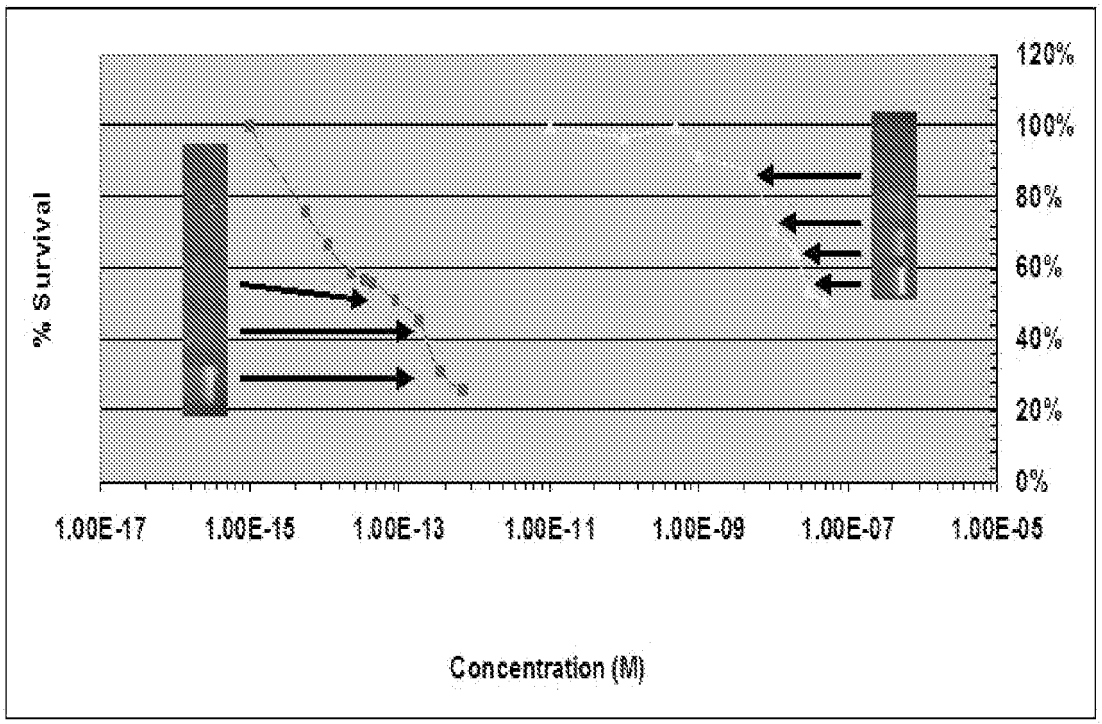

% Survival
120%
100%
80%
60%
40%
20%
0%
1.00E-17
1.00E-15
1.00E-13
1.00E-11
1.00E-09
1.00E-07
1.00E-05
Concentration (M)

FIG. 2

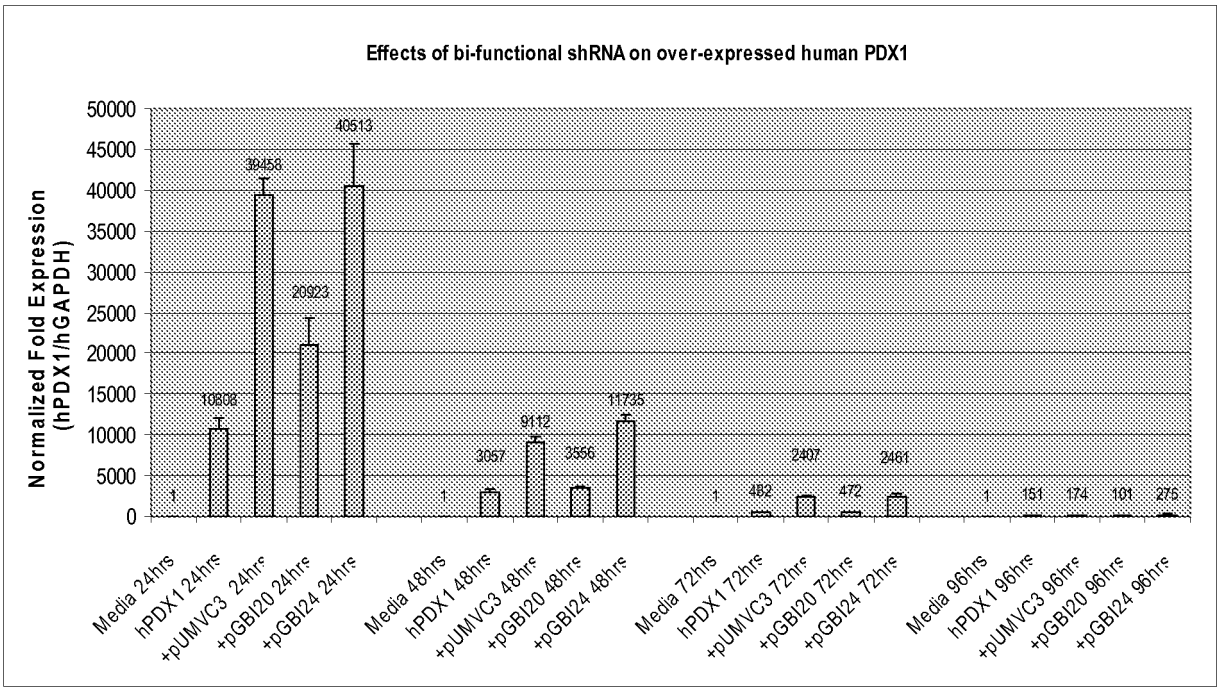

Effects of bi-functional shRNA on over-expressed human PDX1
Normalized Fold Expression (hPDX1/hGAPDH)
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
1
10808
39458
20923
40513
1
3057
9112
3556
11735
1
482
2407
472
2461
1
151
174
101
275
Media 24hrs
hPDX1 24hrs
+pUMVC3 24hrs
+pGBI20 24hrs
+pGBI24 24hrs
Media 48hrs
hPDX1 48hrs
+pUMVC3 48hrs
+pGBI20 48hrs
+pGBI24 48hrs
Media 72hrs
hPDX1 72hrs
+pUMVC3 72hrs
+pGBI20 72hrs
+pGBI24 72hrs
Media 96hrs
hPDX1 96hrs
+pUMVC3 96hrs
+pGBI20 96hrs
+pGBI24 96hrs

FIG. 3A

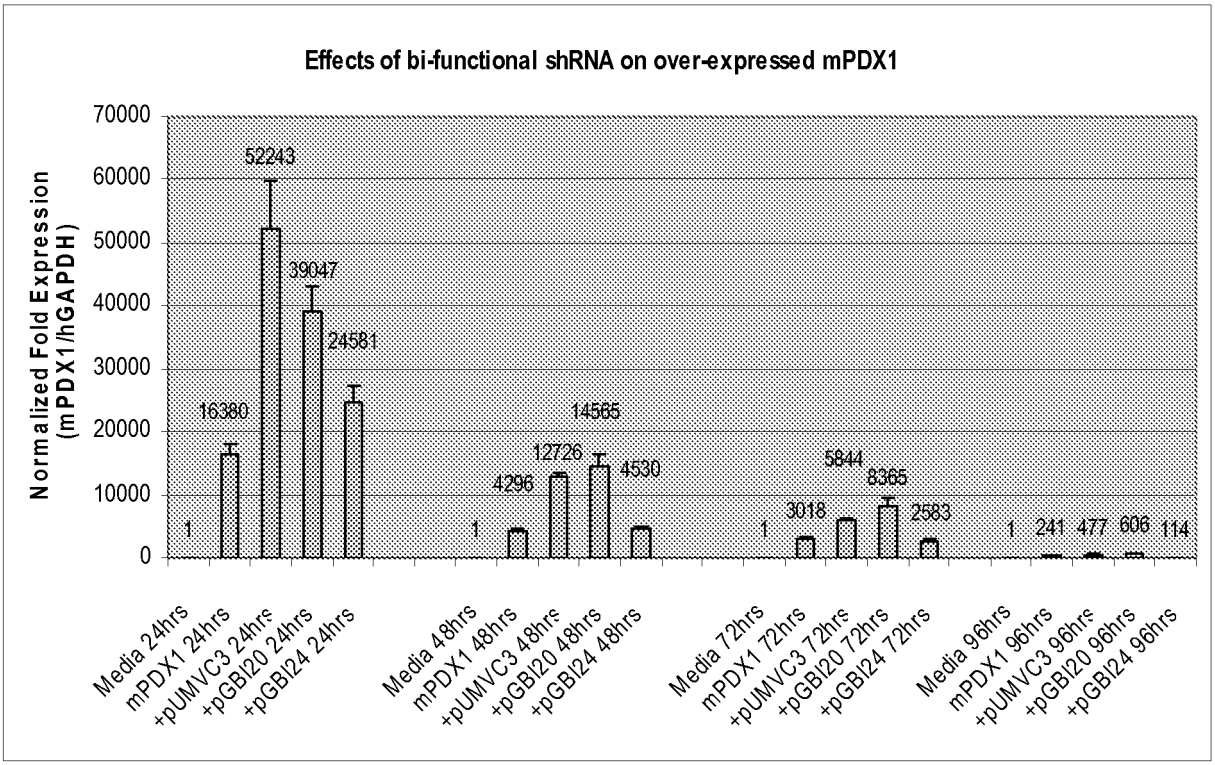

Effects of bi-functional shRNA on over-expressed mPDX1
Normalized Fold Expression (mPDX1/hGAPDH)
70000
60000
50000
40000
30000
20000
10000
0
1
16380
52243
39047
24581
1
4296
12726
14565
4530
1
3018
5844
8365
2583
1
241
477
606
114
Media 24hrs
mPDX1 24hrs
+pUMVC3 24hrs
+pGBI20 24hrs
+pGBI24 24hrs
Media 48hrs
mPDX1 48hrs
+pUMVC3 48hrs
+pGBI20 48hrs
+pGBI24 48hrs
Media 72hrs
mPDX1 72hrs
+pUMVC3 72hrs
+pGBI20 72hrs
+pGBI24 72hrs
Media 96hrs
mPDX1 96hrs
+pUMVC3 96hrs
+pGBI20 96hrs
+pGBI24 96hrs

FIG. 3B

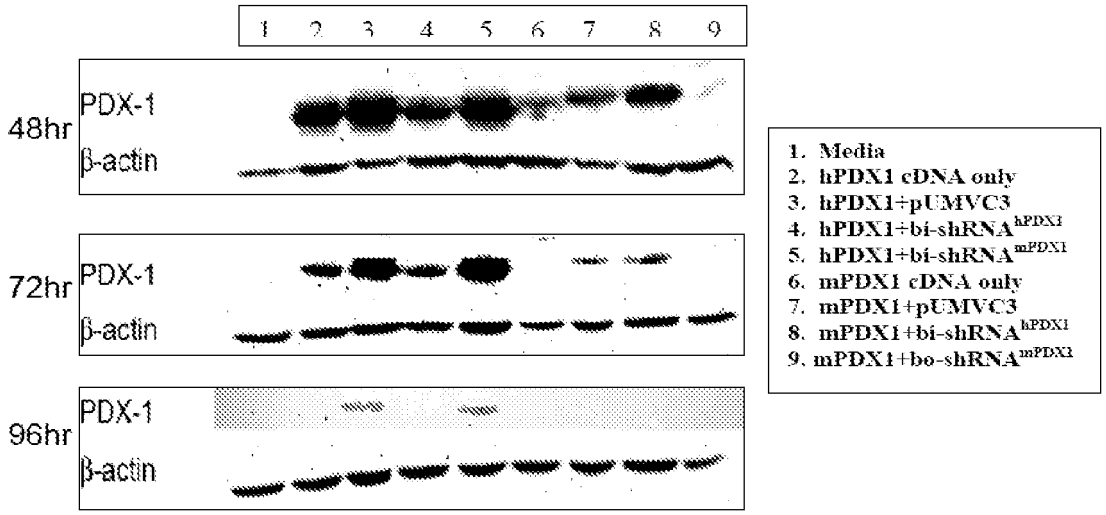

1 2 3 4 5 6 7 8 9
48hr
PDX-1
β-actin
72hr
PDX-1
β-actin
96hr
PDX-1
β-actin
1. Media
2. hPDX1 cDNA only
3. hPDX1+pUMVC3
4. hPDX1+bi-shRNA hPDX1
5. hPDX1+bi-shRNA mPDX1
6. mPDX1 cDNA only
7. mPDX1+pUMVC3
8. mPDX1+bi-shRNA hPDX1
9. mPDX1+bo-shRNA mPDX1

FIG. 4

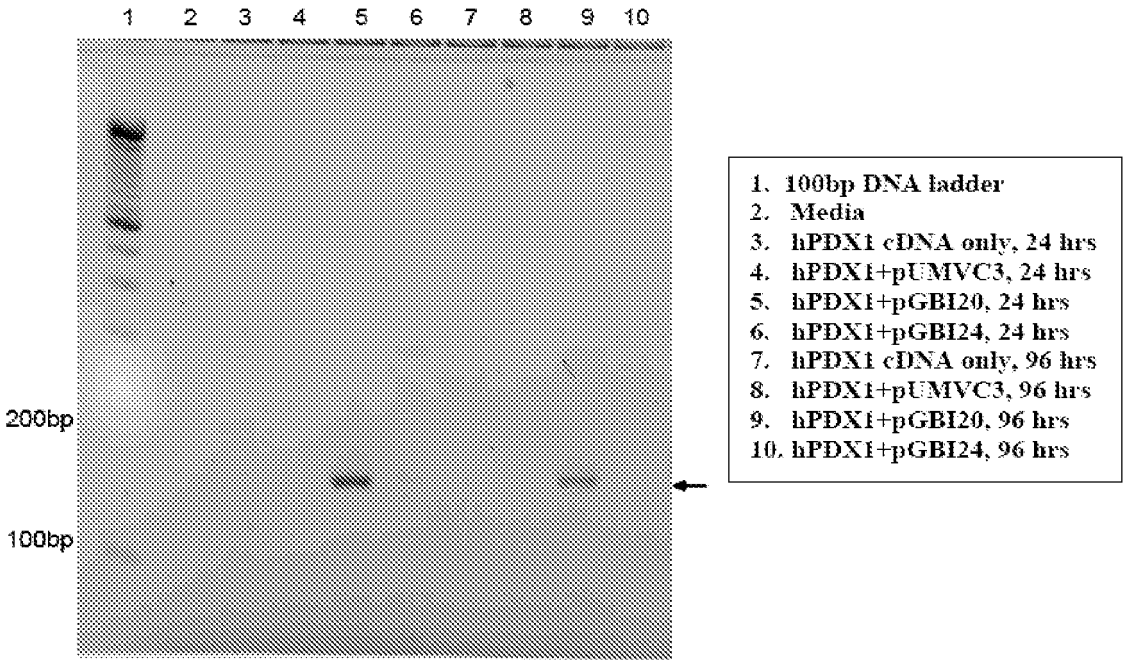

1 2 3 4 5 6 7 8 9 10
200bp
100bp
1. 100bp DNA ladder
2. Media
3. hPDX1 cDNA only, 24 hrs
4. hPDX1+pUMVC3, 24 hrs
5. hPDX1+pGBI20, 24 hrs
6. hPDX1+pGBI24, 24 hrs
7. hPDX1 cDNA only, 96 hrs
8. hPDX1+pUMVC3, 96 hrs
9. hPDX1+pGBI20, 96 hrs
10. hPDX1+pGBI24, 96 hrs

FIG. 5A

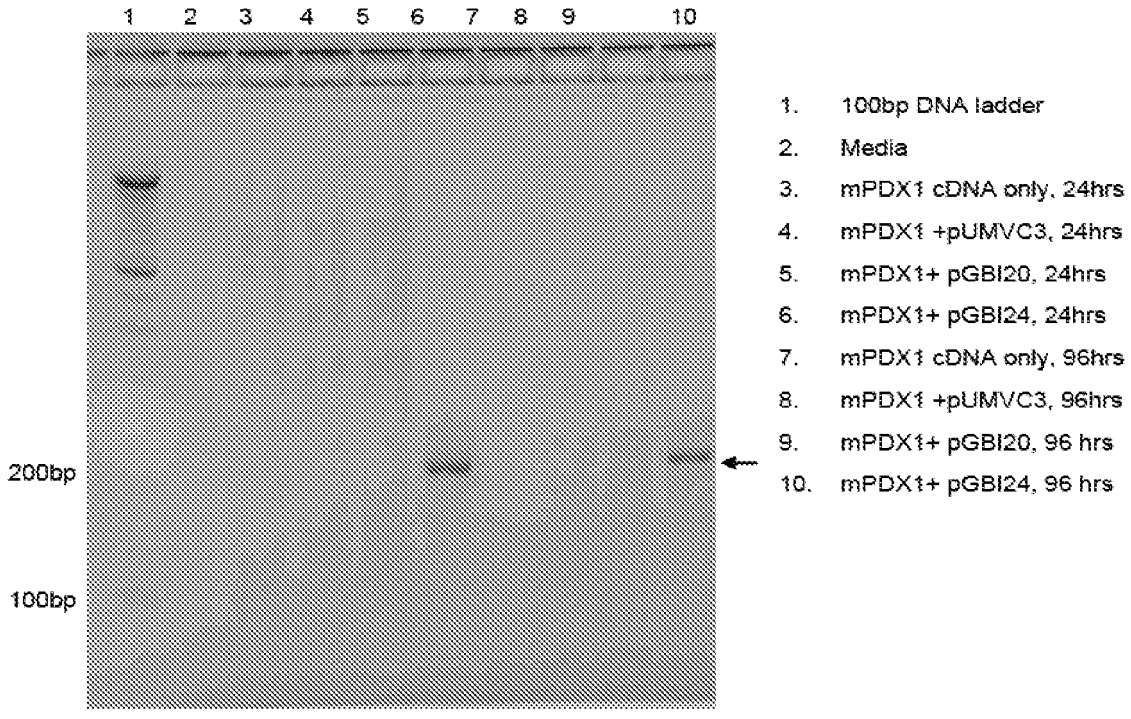

1 2 3 4 5 6 7 8 9 10
200bp
100bp
1. 100bp DNA ladder
2. Media
3. mPDX1 cDNA only, 24hrs
4. mPDX1 +pUMVC3, 24hrs
5. mPDX1+ pGBI20, 24hrs
6. mPDX1+ pGBI24, 24hrs
7. mPDX1 cDNA only, 96hrs
8. mPDX1 +pUMVC3, 96hrs
9. mPDX1+ pGBI20, 96 hrs
10. mPDX1+ pGBI24, 96 hrs

FIG. 5B

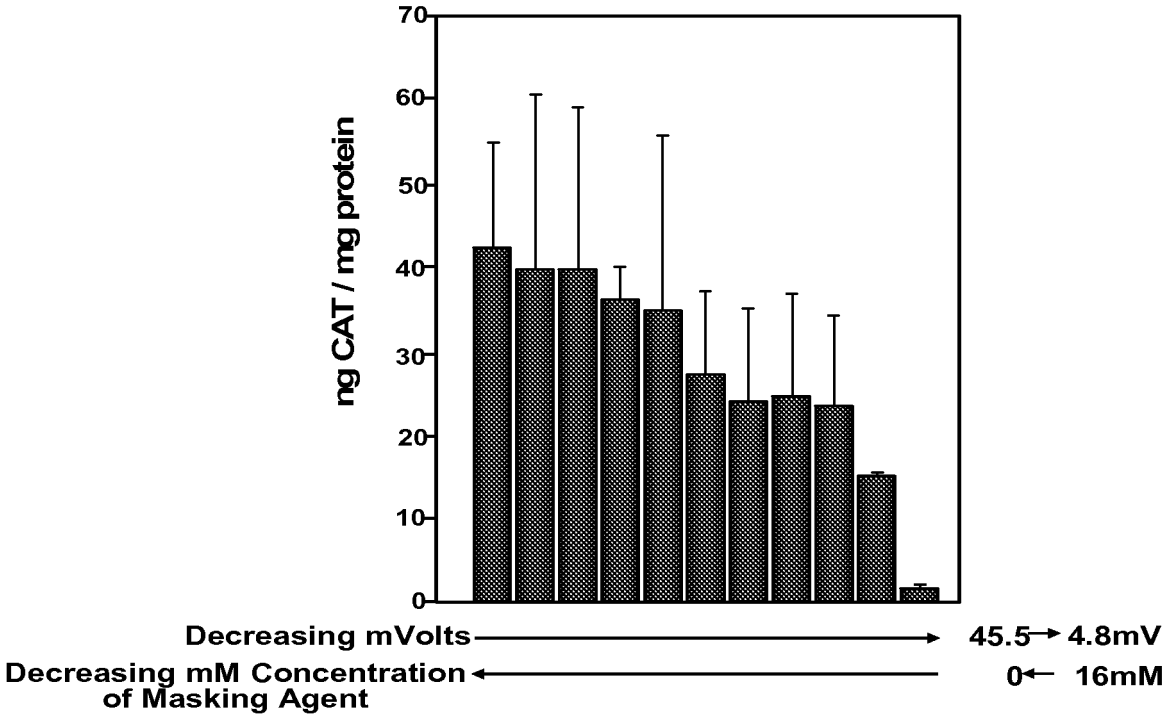

ng CAT / mg protein
70
60
50
40
30
20
10
0
Decreasing mVolts
45.5→4.8mV
Decreasing mM Concentration of Masking Agent
0← 16mM

FIG. 6A

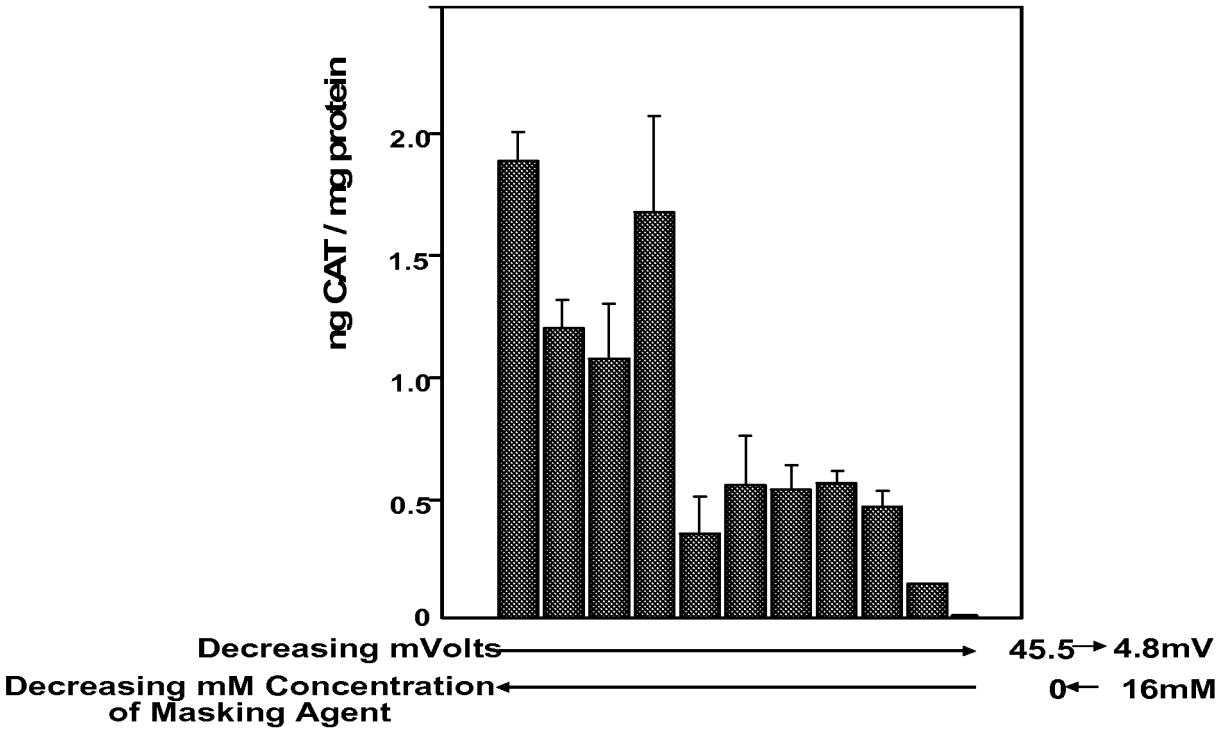

ng CAT / mg protein
2.0
1.5
1.0
0.5
0
Decreasing mVolts
45.5→4.8mV
Decreasing mM Concentration of Masking Agent
0← 16mM

FIG. 6B

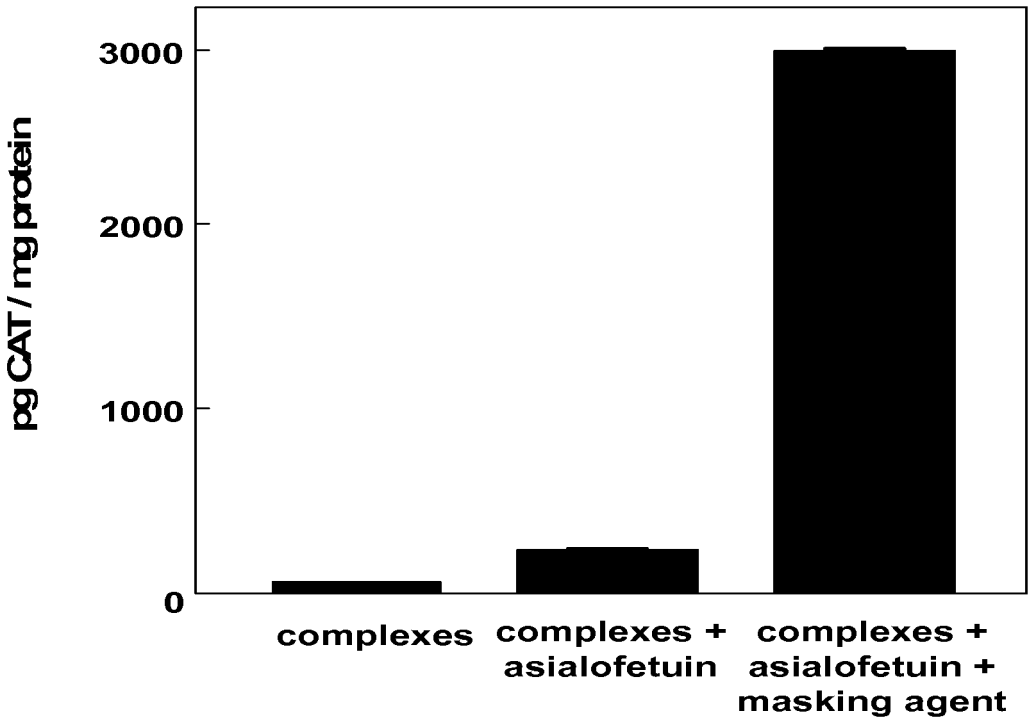


FIG. 6C

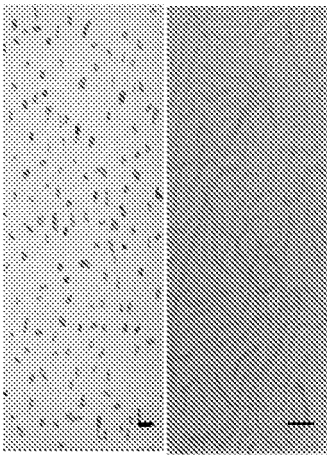


FIG. 7A

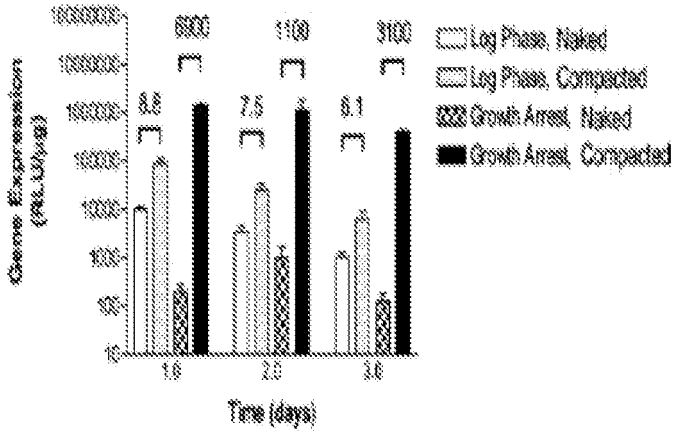


FIG. 7B

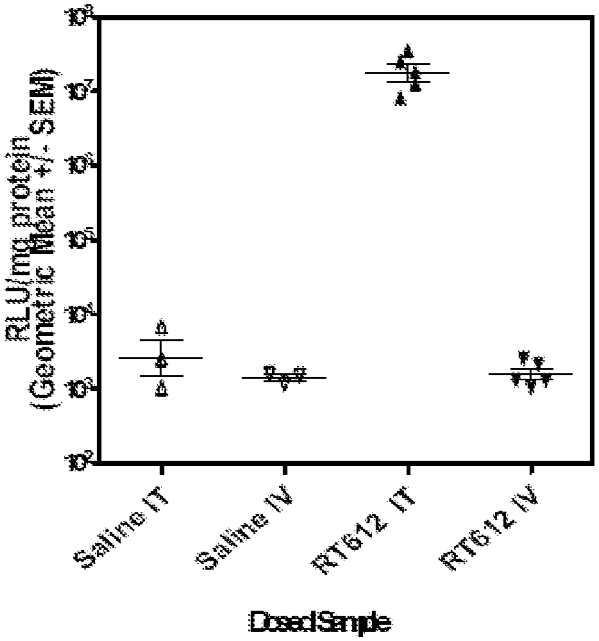
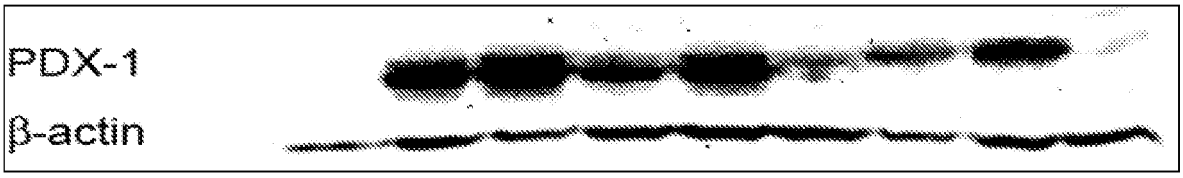


FIG. 7C

Expression plasmids electroporated

Transgene	—	H	H	H	H	M	M	M	M
Bi-shRNA	—	—	E	H	M	–	E	H	M



E=empty vector; H=human PDX-1; M=mouse PDX-1

FIG. 8

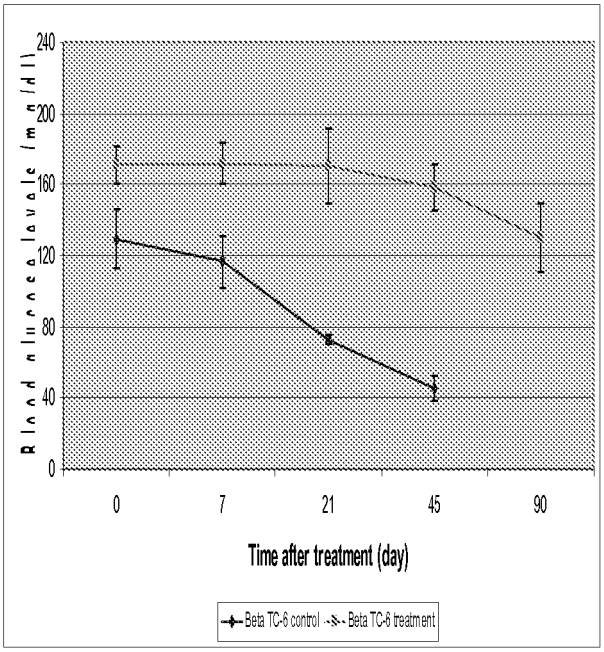


FIG. 9A

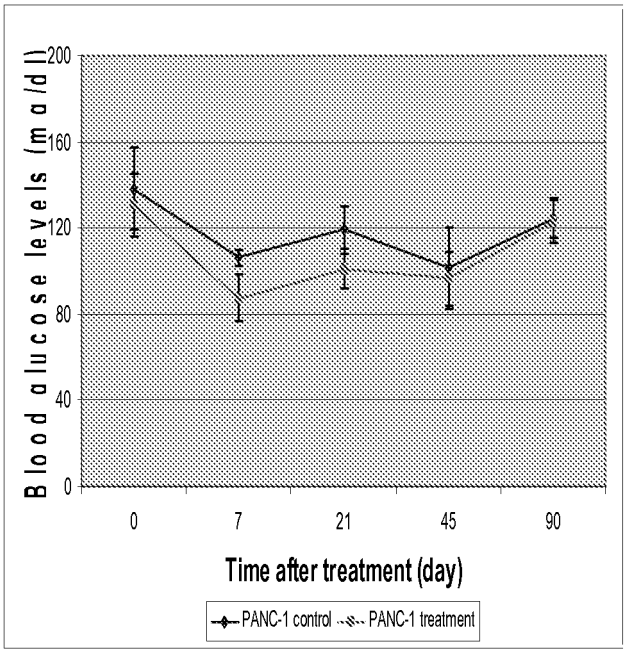
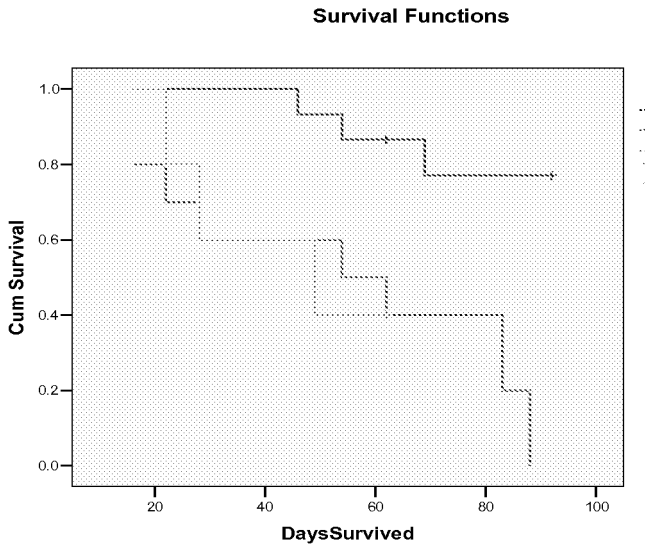


FIG. 9B



Mean Survival in Mice

untreated 44.6 days (3/5 died)
pUMVC3 54.0 days (8/10 died)
pGBI20 84.2 days (3/15 died)

p=0.001 (Agent vs. Vehicle)
p=0.003 (Agent vs. Vehicle vs. Untreated)

FIG. 10A

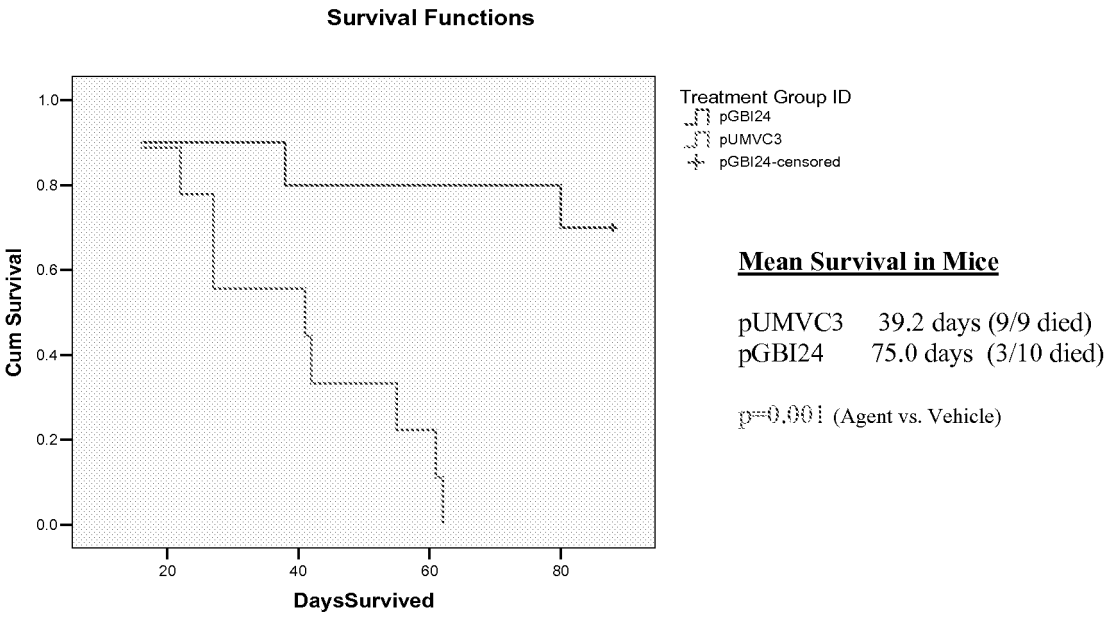


FIG. 10B

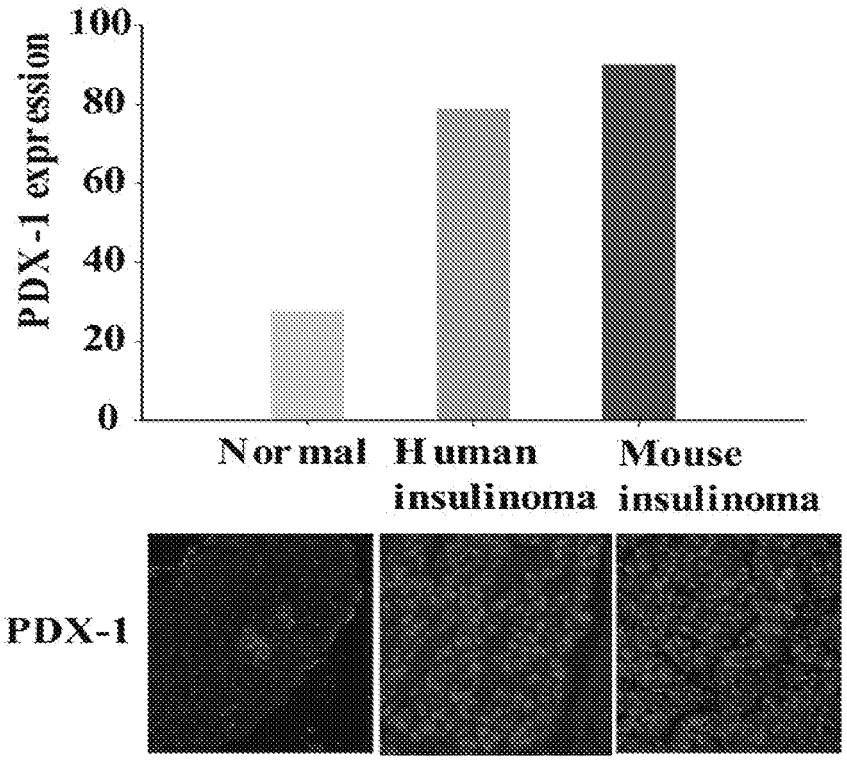


FIG. 11

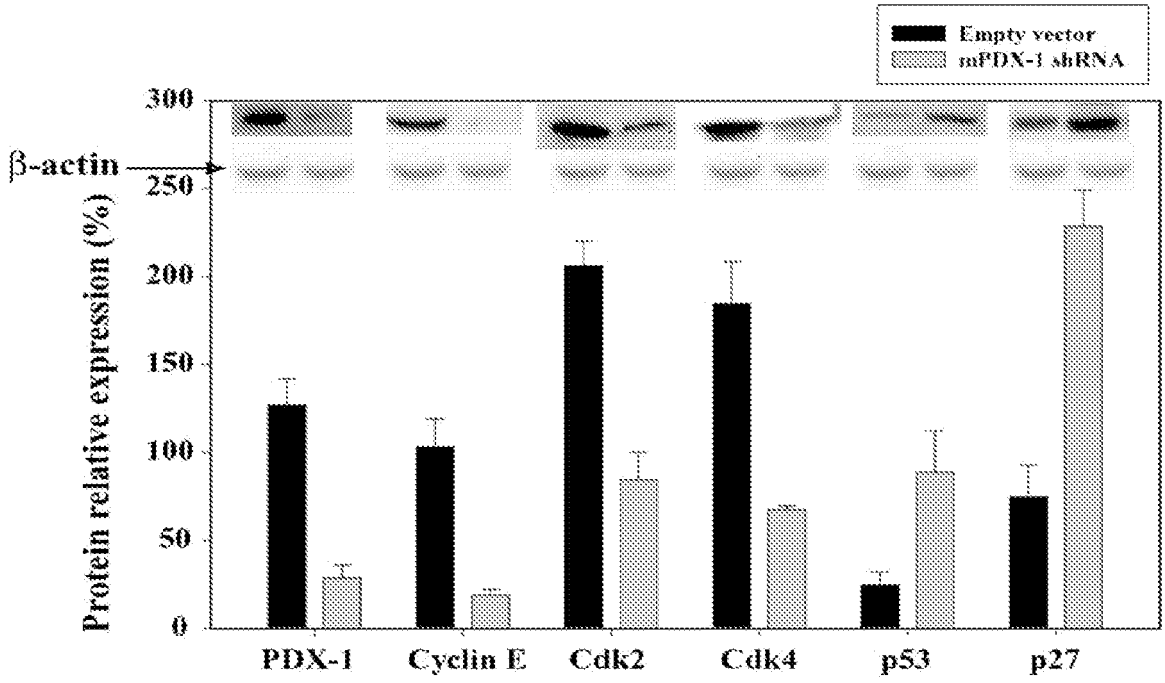

Empty vector
mPDX-1 shRNA
β-actin
Protein relative expression (%)
300
250
200
150
100
50
0
PDX-1
Cyclin E
Cdk2
Cdk4
p53
p27

FIG. 12

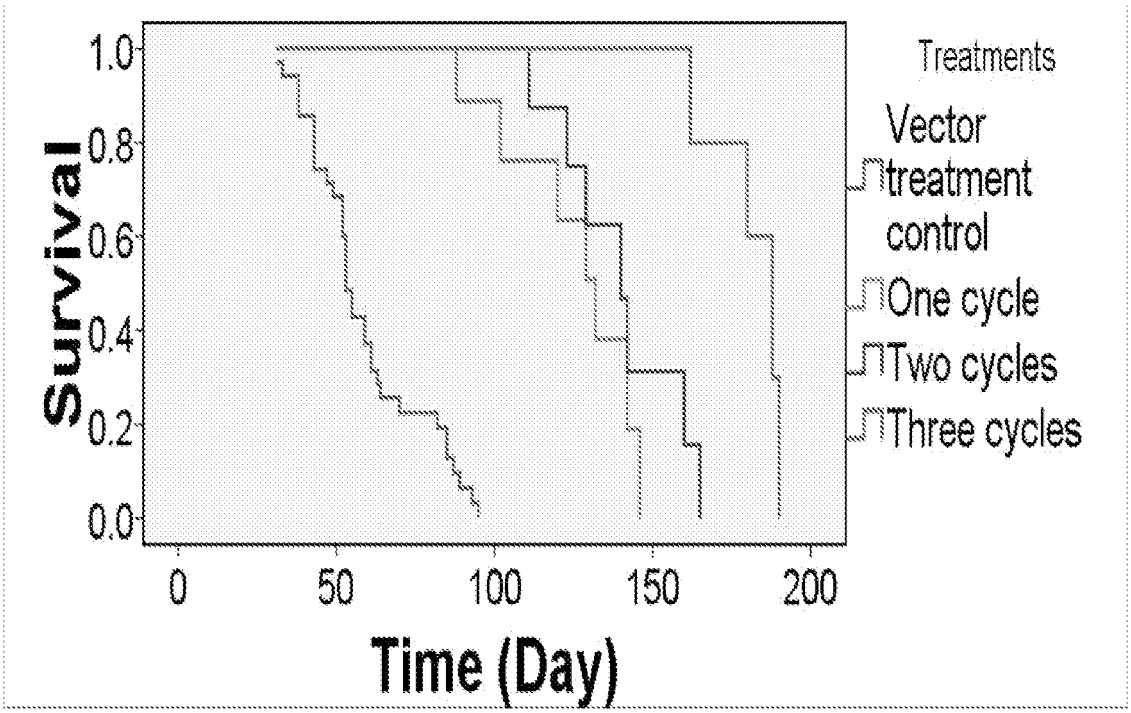

Survival
1.0
0.8
0.6
0.4
0.2
0.0
0
50
100
150
200
Time (Day)
Treatments
Vector treatment control
One cycle
Two cycles
Three cycles

FIG. 13

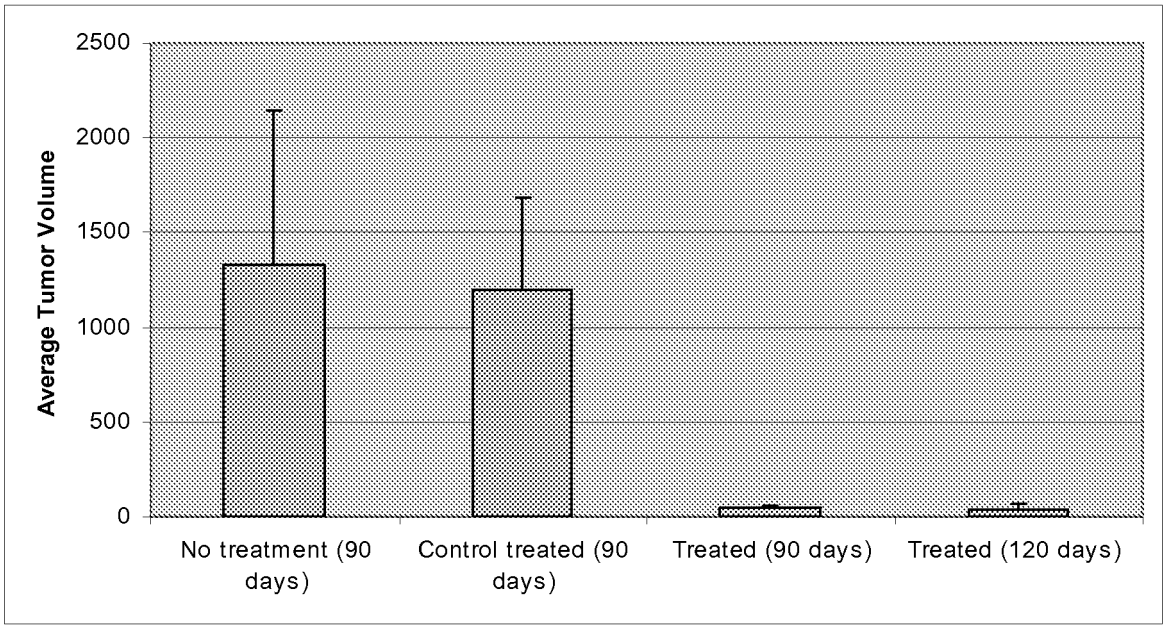

Average Tumor Volume
2500
2000
1500
1000
500
0
No treatment (90 days)
Control treated (90 days)
Treated (90 days)
Treated (120 days)

FIG. 14

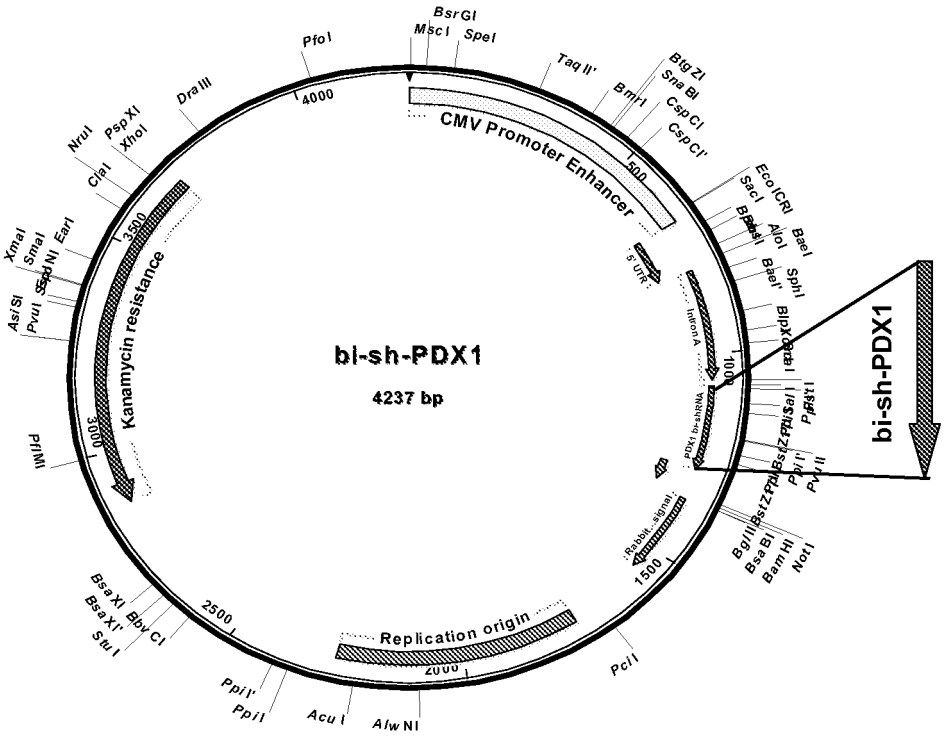

bi-sh-PDX1
4237 bp
CMV Promoter Enhancer
Kanamycin resistance
Replication origin
bi-sh-PDX1
BsrGI
MscI
SpeI
PfoI
TaqII'
BtgZI
SnaBI
BmrI
CspCI
CspCI'
EcoICRI
SacI
AloI
BaeI
SphI
BlpI
PstI
PpiI'
PvuII
BglII
BsaBI
BamHI
NotI
PclI
AlwNI
AcuI
PpiI
PpiI'
BbvCI
BsaXI
BsaXI'
StuI
PflMI
AsiSI
PvuI
SspNI
XmaI
SmaI
EarI
ClaI
NruI
PspXI
XhoI
DraIII

FIG. 15