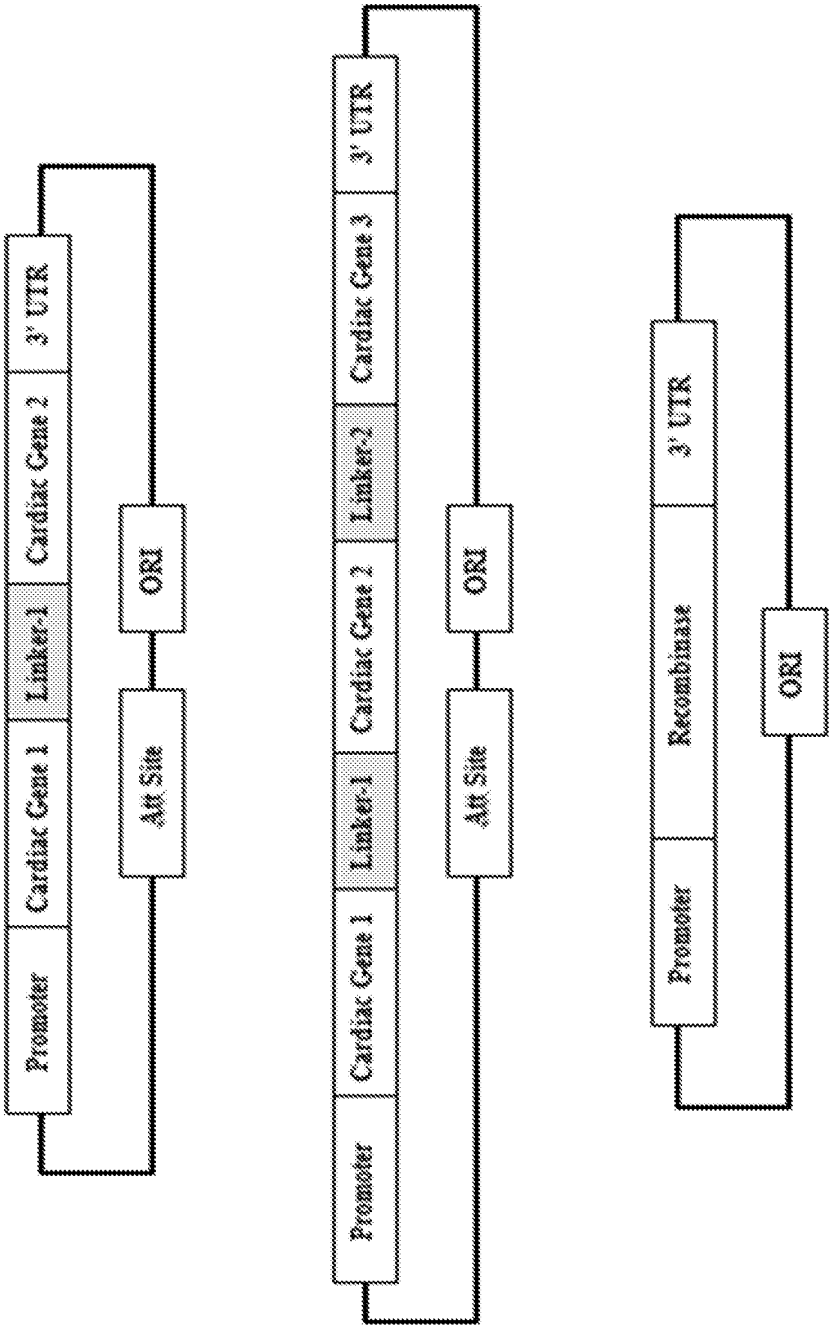

Promoter
Cardiac Gene 1
Linker-1
Cardiac Gene 2
3' UTR
Att Site
ORI
Promoter
Cardiac Gene 1
Linker-1
Cardiac Gene 2
Linker-2
Cardiac Gene 3
3' UTR
Att Site
ORI
Promoter
Recombinase
3' UTR
ORI

FIG. 1

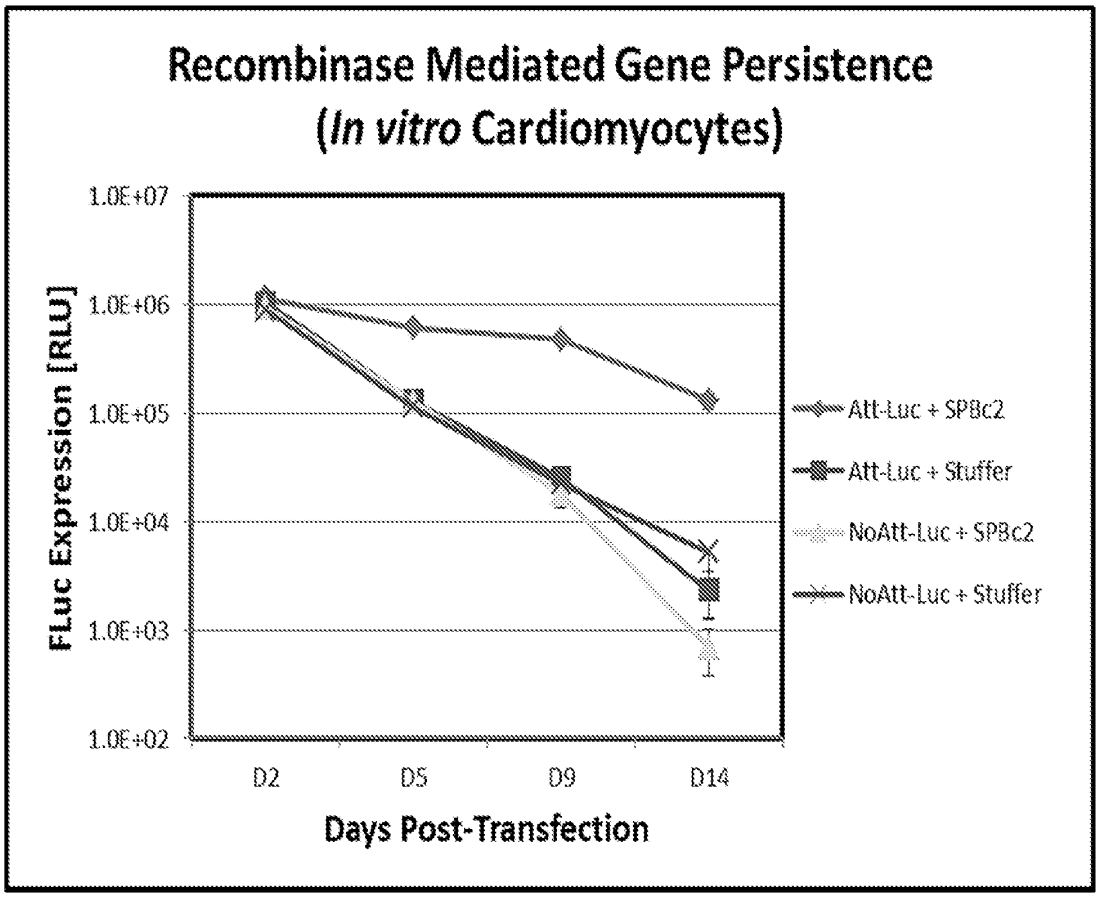
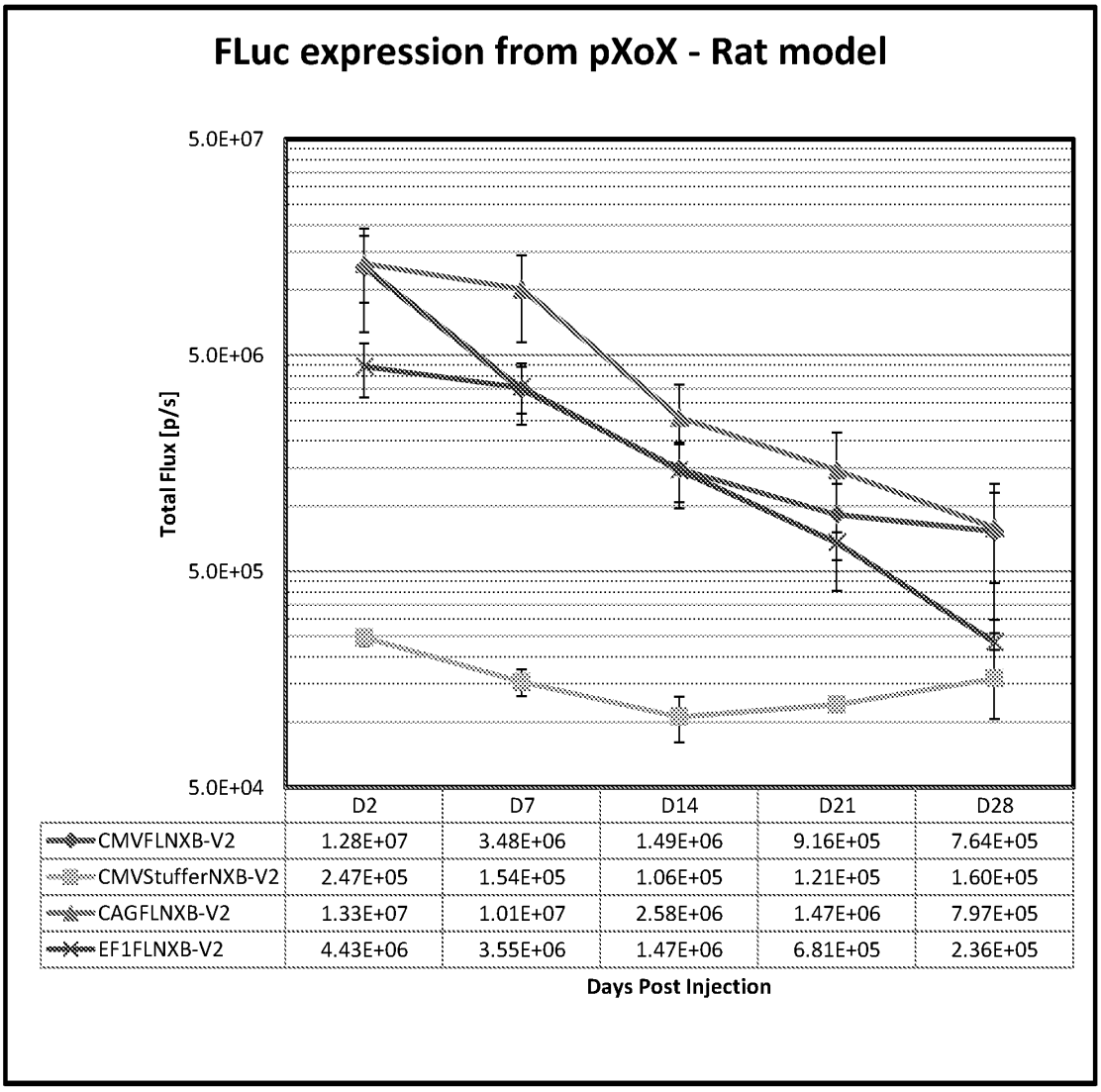

Recombinase Mediated Gene Persistence
(In vitro Cardiomyocytes)
FLuc Expression [RLU]
1.0E+07
1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
D2
D5
D9
D14
Days Post-Transfection
Att-Luc + SPBc2
Att-Luc + Stuffer
NoAtt-Luc + SPBc2
NoAtt-Luc + Stuffer

FIG. 2



	D2	D7	D14	D21	D28
CMVFLNXB-V2	1.28E+07	3.48E+06	1.49E+06	9.16E+05	7.64E+05
CMVStufferNXB-V2	2.47E+05	1.54E+05	1.06E+05	1.21E+05	1.60E+05
CAGFLNXB-V2	1.33E+07	1.01E+07	2.58E+06	1.47E+06	7.97E+05
EF1FLNXB-V2	4.43E+06	3.55E+06	1.47E+06	6.81E+05	2.36E+05

FIG. 3

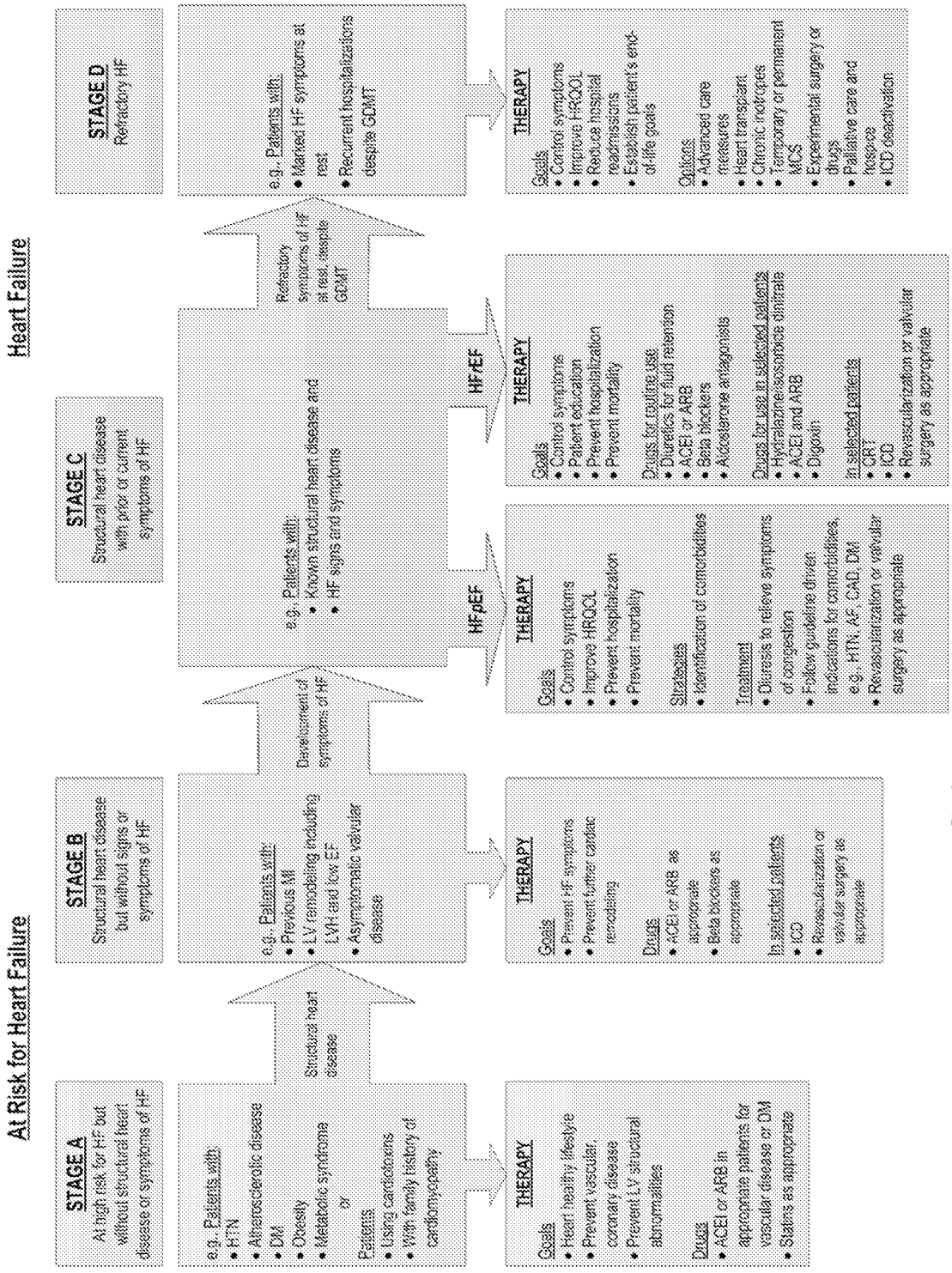

At Risk for Heart Failure
Heart Failure
STAGE A
At high risk for HF but without structural heart disease or symptoms of HF
e.g., Patients with:
• HTN
• Atherosclerotic disease
• DM
• Obesity
• Metabolic syndrome
or
Patients
• Using cardiotoxins
• With family history of cardiomyopathy
Structural heart disease
THERAPY
Goals
• Heart healthy lifestyle
• Prevent vascular, coronary disease
• Prevent LV structural abnormalities
Drugs
• ACEI or ARB in appropriate patients for vascular disease or DM
• Statins as appropriate
STAGE B
Structural heart disease but without signs or symptoms of HF
e.g., Patients with:
• Previous MI
• LV remodeling including LVH and low EF
• Asymptomatic valvular disease
Development of symptoms of HF
THERAPY
Goals
• Prevent HF symptoms
• Prevent further cardiac remodeling
Drugs
• ACEI or ARB as appropriate
• Beta blockers as appropriate
In selected patients
• ICD
• Revascularization or valvular surgery as appropriate
STAGE C
Structural heart disease with prior or current symptoms of HF
e.g. Patients with:
• Known structural heart disease and
• HF signs and symptoms
HFpEF
THERAPY
Goals
• Control symptoms
• Improve HRQOL
• Prevent hospitalization
• Prevent mortality
Strategies
• Identification of comorbidities
Treatment
• Diuresis to relieve symptoms of congestion
• Follow guideline driven indications for comorbidities, e.g., HTN, AF, CAD, DM
• Revascularization or valvular surgery as appropriate
HFrEF
THERAPY
Goals
• Control symptoms
• Patient education
• Prevent hospitalization
• Prevent mortality
Drugs for routine use
• Diuretics for fluid retention
• ACEI or ARB
• Beta blockers
• Aldosterone antagonists
Drugs for use in selected patients
• Hydralazine/isosorbide dinitrate
• ACEI and ARB
• Digoxin
In selected patients
• CRT
• ICD
• Revascularization or valvular surgery as appropriate
Refractory symptoms of HF at rest, despite GDMT
STAGE D
Refractory HF
e.g., Patients with:
• Marked HF symptoms at rest
• Recurrent hospitalizations despite GDMT
THERAPY
Goals
• Control symptoms
• Improve HRQOL
• Reduce hospital readmissions
• Establish patient's end-of-life goals
Options
• Advanced care measures
• Heart transplant
• Chronic inotropes
• Temporary or permanent MCS
• Experimental surgery or drugs
• Palliative care and hospice
• ICD deactivation

FIG. 4

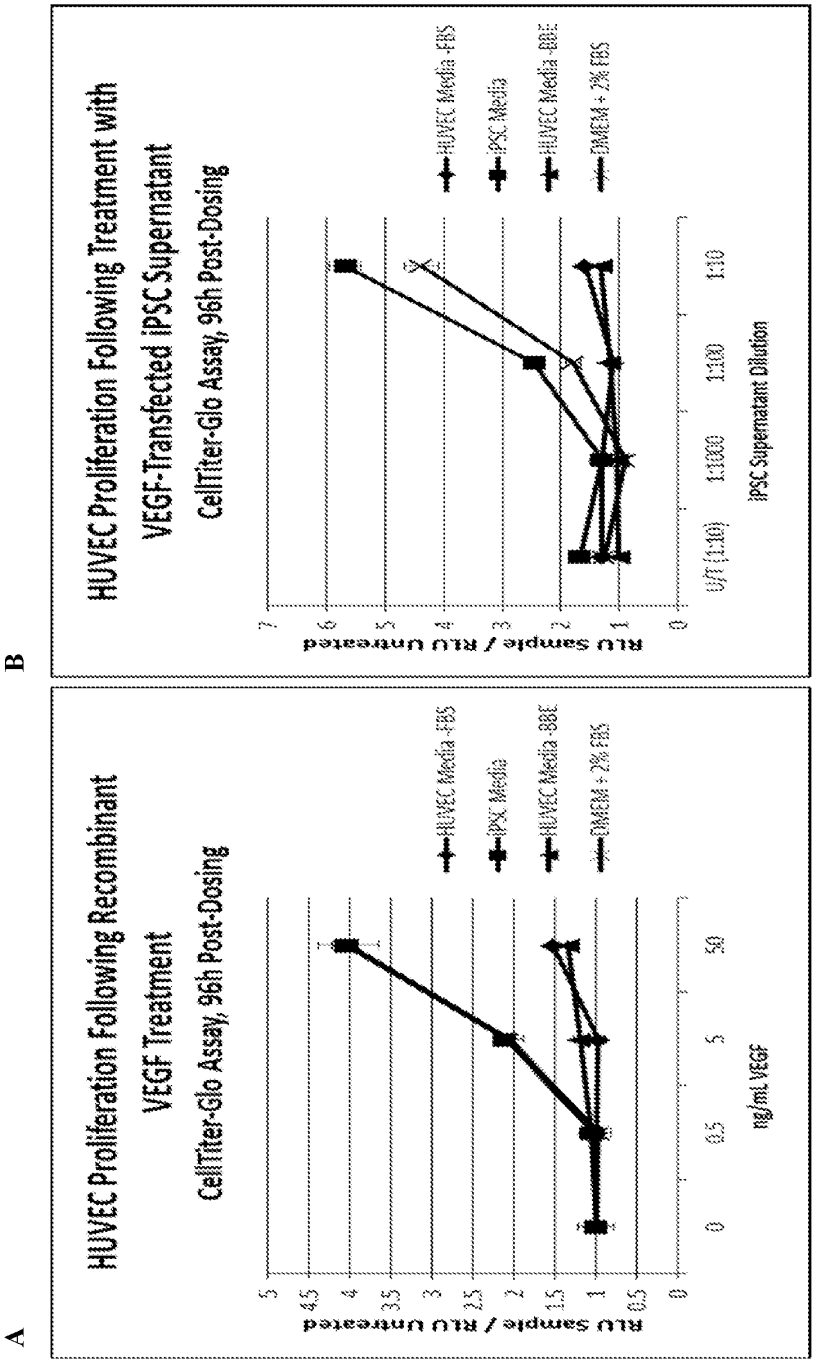
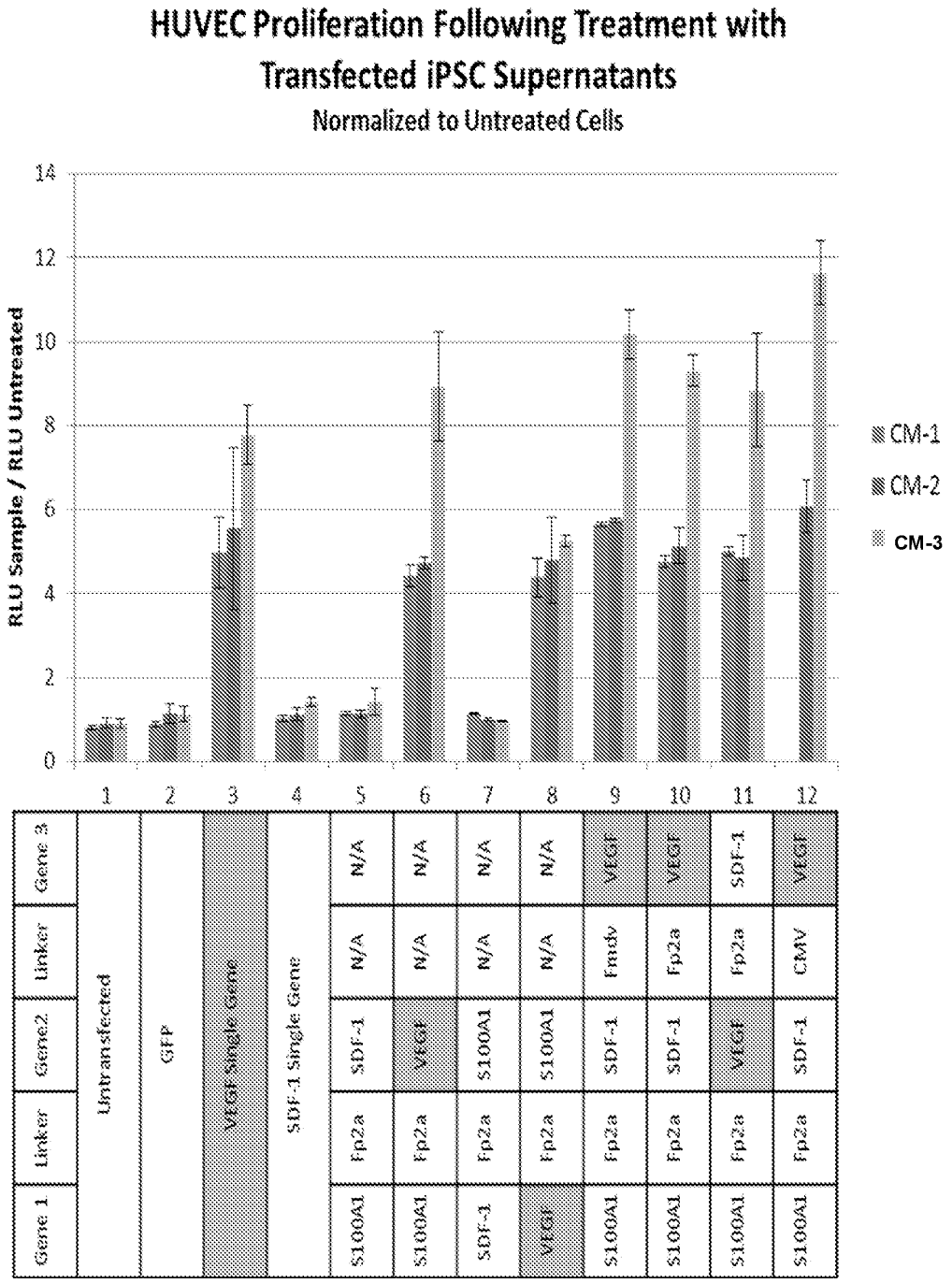

A
HUVEC Proliferation Following Recombinant VEGF Treatment
CellTiter-Glo Assay, 96h Post-Dosing
RLU Sample / RLU Untreated
ng/mL VEGF
HUVEC Media -FBS
iPSC Media
HUVEC Media -BBE
DMEM + 2% FBS
B
HUVEC Proliferation Following Treatment with VEGF-Transfected iPSC Supernatant
CellTiter-Glo Assay, 96h Post-Dosing
RLU Sample / RLU Untreated
UT (1:10)
1:1000
1:100
1:10
iPSC Supernatant Dilution
HUVEC Media -FBS
iPSC Media
HUVEC Media -BBE
DMEM + 2% FBS

FIG. 5



CM-1 = Cardiomyocyte transfection -1
CM-2 = Cardiomyocyte transfection -2
CM-3 = Cardiomyocyte transfection -3

FIG. 6

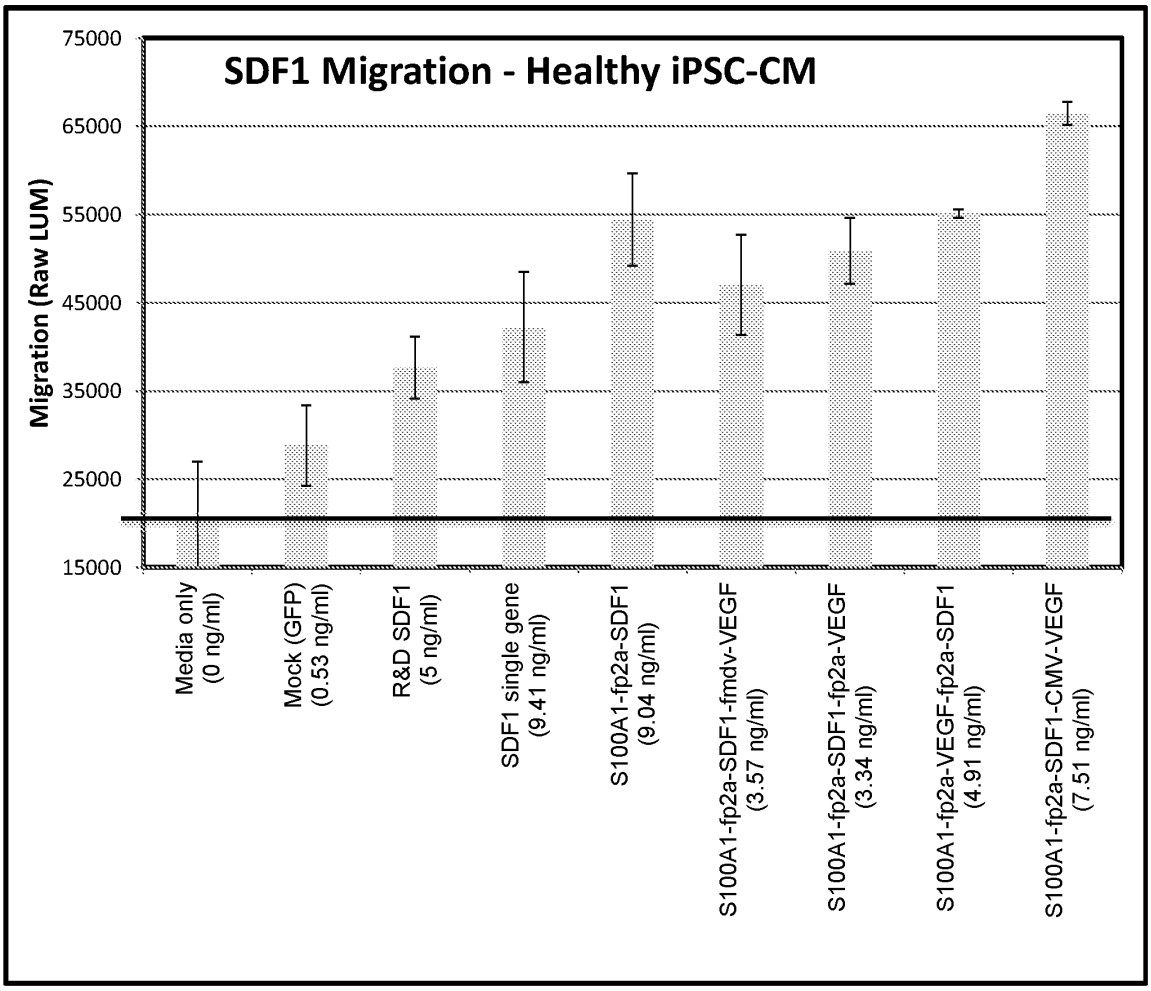
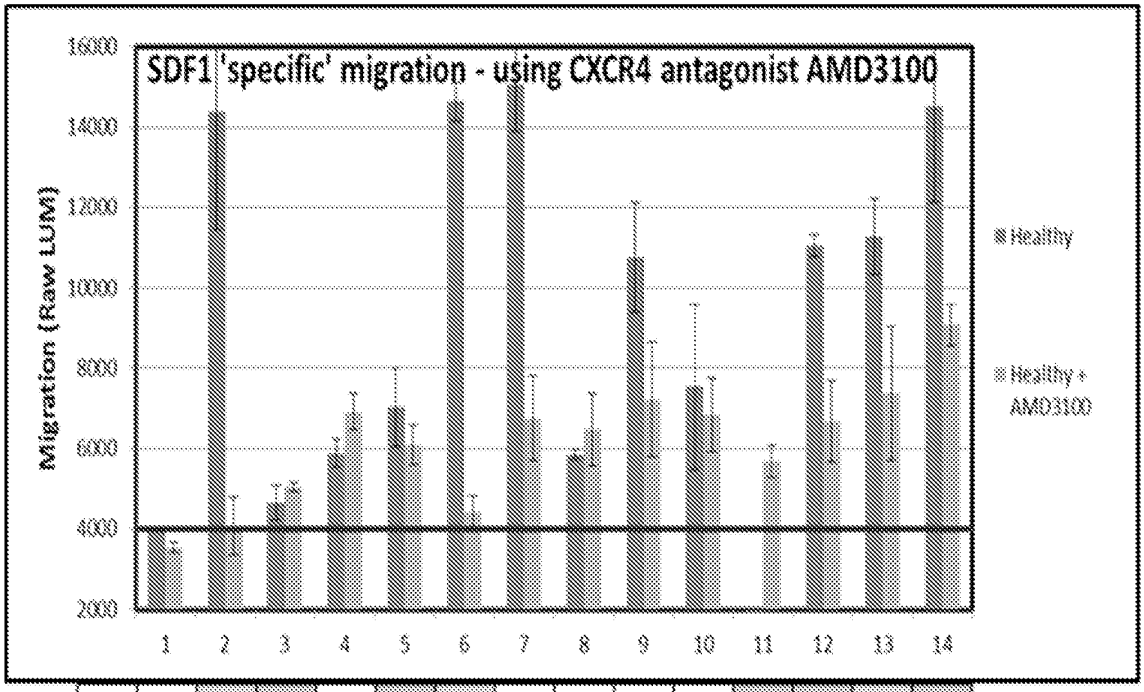

SDF1 Migration - Healthy iPSC-CM
Migration (Raw LUM)
75000
65000
55000
45000
35000
25000
15000
Media only (0 ng/ml)
Mock (GFP) (0.53 ng/ml)
R&D SDF1 (5 ng/ml)
SDF1 single gene (9.41 ng/ml)
S100A1-fp2a-SDF1 (9.04 ng/ml)
S100A1-fp2a-SDF1-fmdv-VEGF (3.57 ng/ml)
S100A1-fp2a-SDF1-fp2a-VEGF (3.34 ng/ml)
S100A1-fp2a-VEGF-fp2a-SDF1 (4.91 ng/ml)
S100A1-fp2a-SDF1-CMV-VEGF (7.51 ng/ml)

FIG. 7



	Gene 1	Linker	Gene2	Linker	Gene 3
Media only					
R&D SDF1 (100ng/ml)					
R&D VEGF (500ng/ml)					
Mock (GFP)					
VEGF Single Gene					
SDF-1 Single Gene					
	S100A1	Fp2a	SDF-1	N/A	N/A
	S100A1	Fp2a	VEGF	N/A	N/A
	SDF-1	Fp2a	S100A1	N/A	N/A
	VEGF	Fp2a	S100A1	N/A	N/A
	S100A1	Fp2a	SDF-1	fmdv	VEGF
	S100A1	Fp2a	SDF-1	Fp2a	VEGF
	S100A1	Fp2a	VEGF	Fp2a	SDF-1
	S100A1	Fp2a	SDF-1	CMV	VEGF

FIG. 8

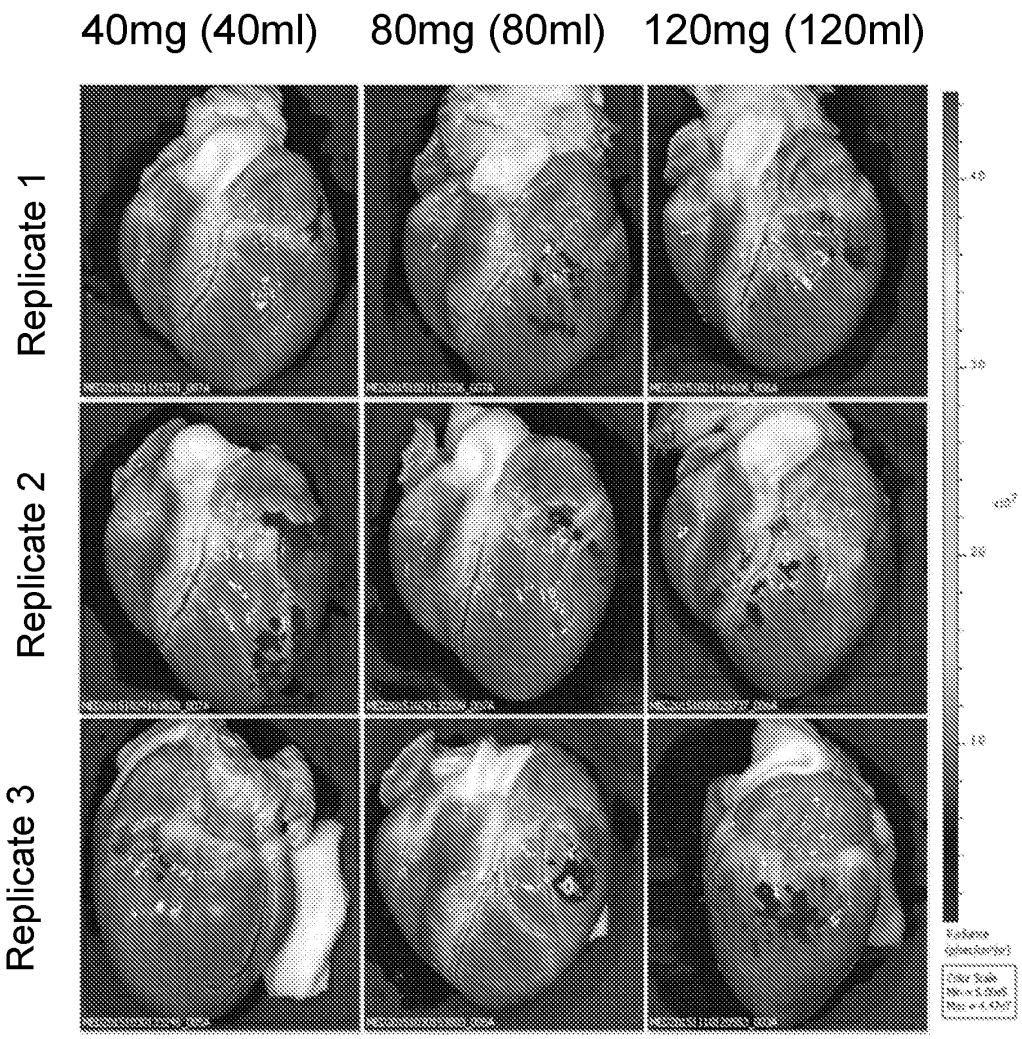

40mg (40ml)
80mg (80ml)
120mg (120ml)
Replicate 1
Replicate 2
Replicate 3

FIG. 9

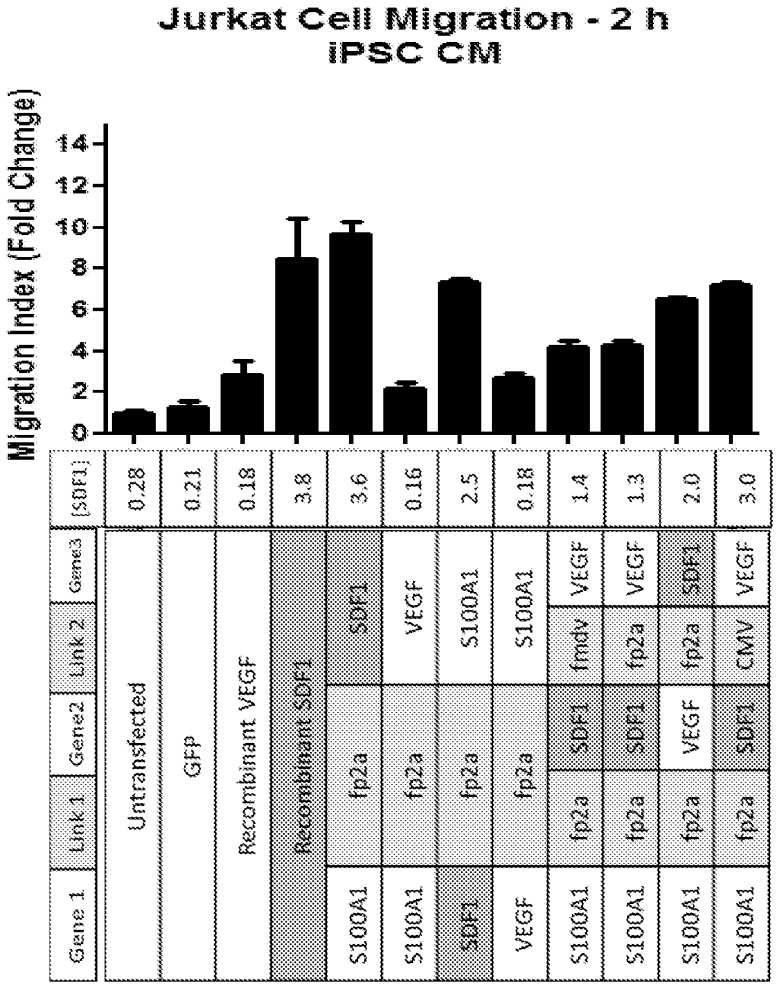

Jurkat Cell Migration - 2 h
iPSC CM
Migration Index (Fold Change)
0
2
4
6
8
10
12
14
[SDF1]
0.28
0.21
0.18
3.8
3.6
0.16
2.5
0.18
1.4
1.3
2.0
3.0
Gene3
VEGF
VEGF
SDF1
VEGF
Link 2
fmdv
fp2a
fp2a
CMV
Gene2
SDF1
SDF1
VEGF
SDF1
Link 1
fp2a
fp2a
fp2a
fp2a
fp2a
fp2a
fp2a
fp2a
fp2a
Gene 1
S100A1
S100A1
SDF1
VEGF
S100A1
S100A1
S100A1
S100A1
S100A1
Untransfected
GFP
Recombinant VEGF
Recombinant SDF1

FIG. 10

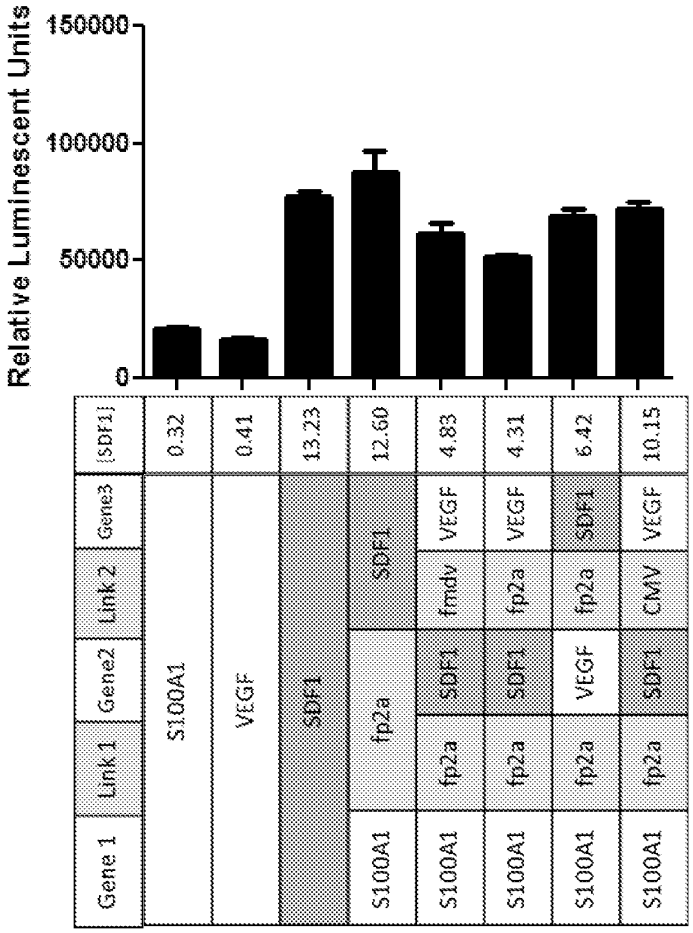

Relative Luminescent Units
150000
100000
50000
0
Gene 1
Link 1
Gene2
Link 2
Gene3
[SDF1]
S100A1
fp2a
SDF1
fmdv
VEGF
CMV
0.32
0.41
13.23
12.60
4.83
4.31
6.42
10.15

FIG. 11

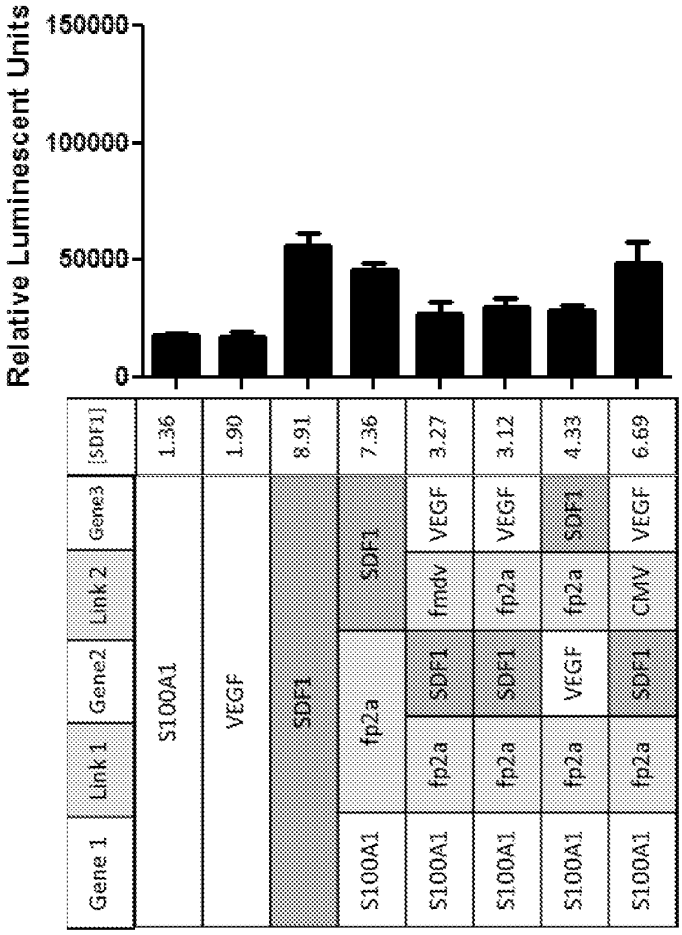

Relative Luminescent Units
150000
100000
50000
0
[SDF1]
Gene3
Link 2
Gene2
Link 1
Gene 1
1.36
1.90
8.91
7.36
3.27
3.12
4.33
6.69
S100A1
VEGF
SDF1
fp2a
VEGF
fmdv
SDF1
fp2a
CMV

FIG. 12

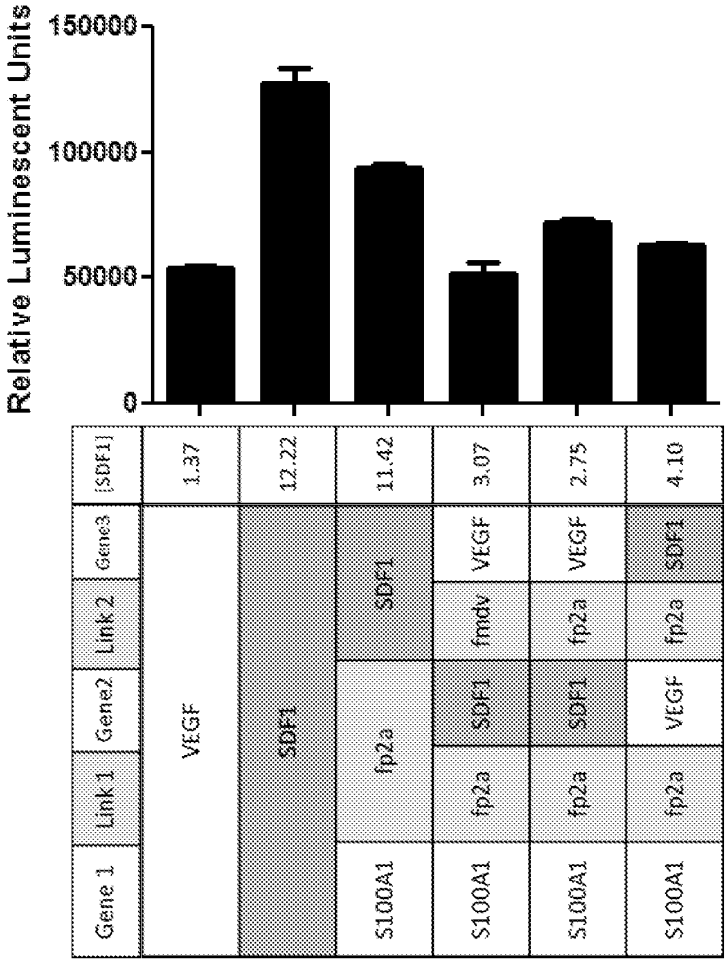
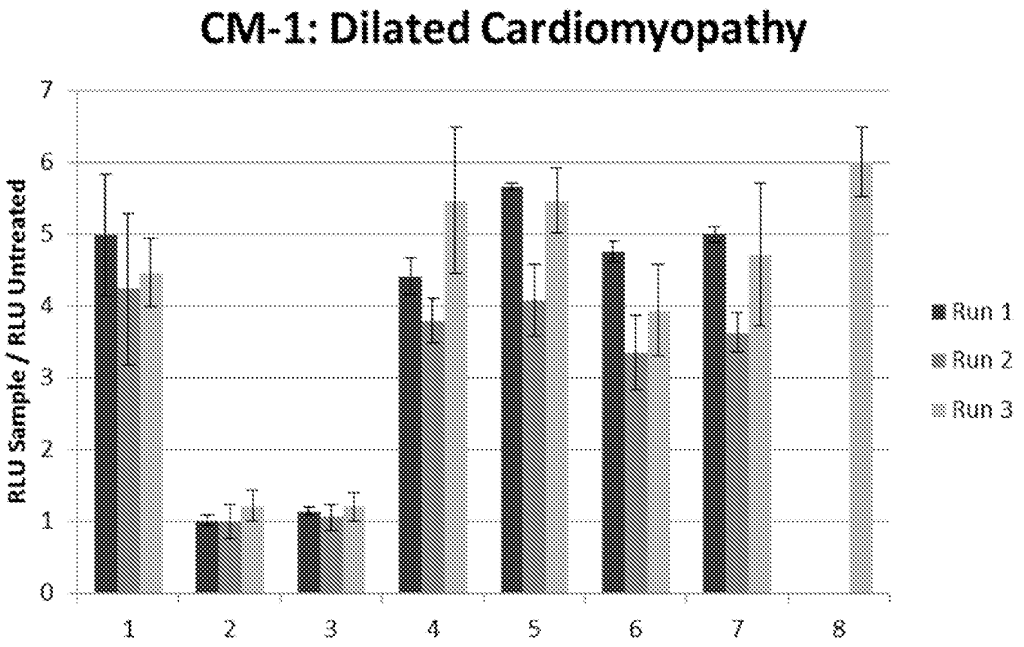

Relative Luminescent Units
150000
100000
50000
0
[SDF1]
1.37
12.22
11.42
3.07
2.75
4.10
Gene3
Link 2
Gene2
Link 1
Gene 1
VEGF
SDF1
fp2a
S100A1
fmdv

FIG. 13



Gene 1	S100A1	S100A1	S100A1	S100A1	S100A1	S100A1	S100A1
Linker	Fp2a	Fp2a	Fp2a	Fp2a	Fp2a	Fp2a	Fp2a
Gene2	SDF-1	VEGF	SDF1	SDF1	VEGF	VEGF	SDF1
Linker	N/A	N/A	Fmdv	Fp2a	Fp2a	Fp2a	CMV
Gene 3	N/A	N/A	VEGF	VEGF	SDF1	VEGF	

VEGF Single Gene

SDF1 Single Gene

FIG. 14 A

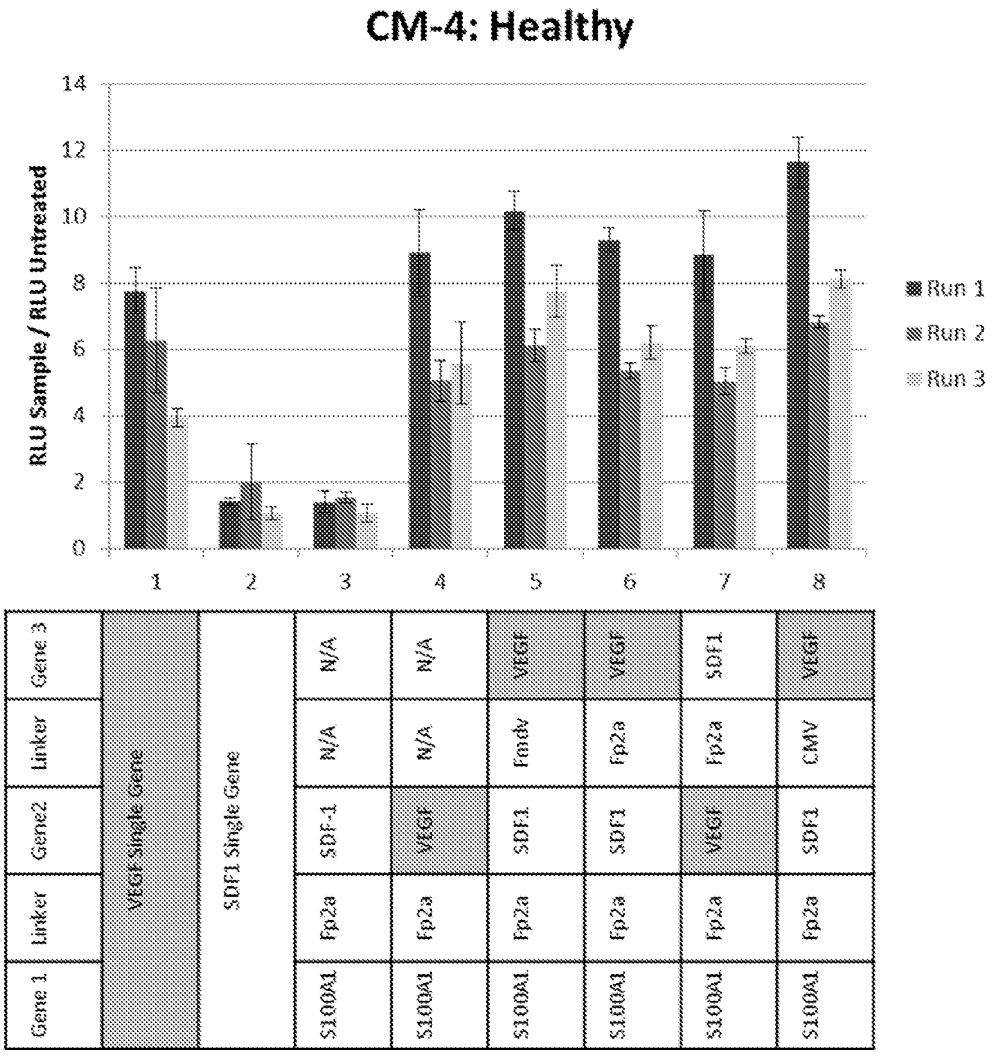
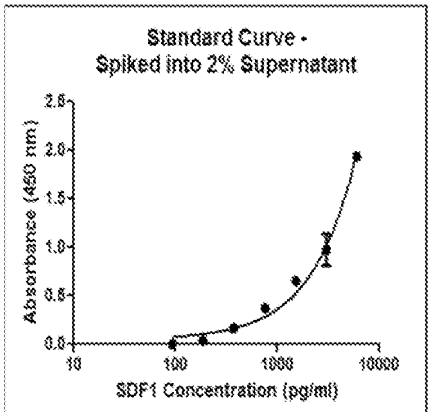
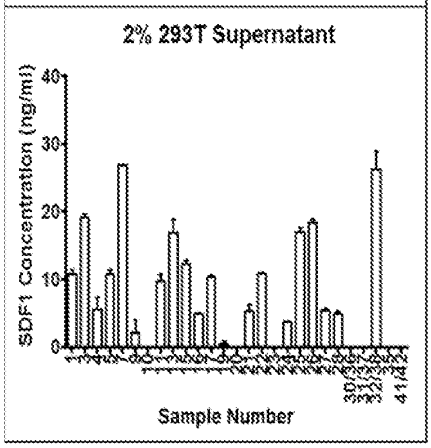

CM-4: Healthy
RLU Sample / RLU Untreated
14
12
10
8
6
4
2
0
1
2
3
4
5
6
7
8
Run 1
Run 2
Run 3
VEGF Single Gene
SDF1 Single Gene
Gene 1
Linker
Gene2
Linker
Gene 3
S100A1
Fp2a
SDF-1
N/A
N/A
S100A1
Fp2a
VEGF
N/A
N/A
S100A1
Fp2a
SDF1
Fmdv
VEGF
S100A1
Fp2a
SDF1
Fp2a
SDF1
S100A1
Fp2a
VEGF
Fp2a
VEGF
S100A1
Fp2a
SDF1
CMV

FIG. 14 B

A.



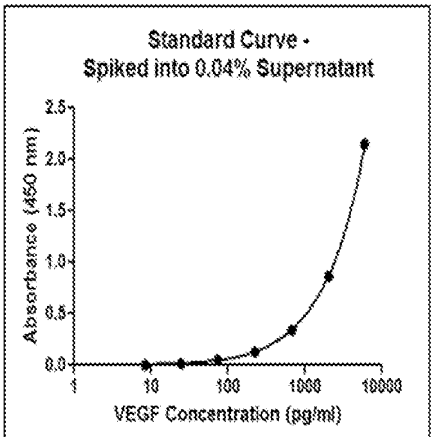
B.



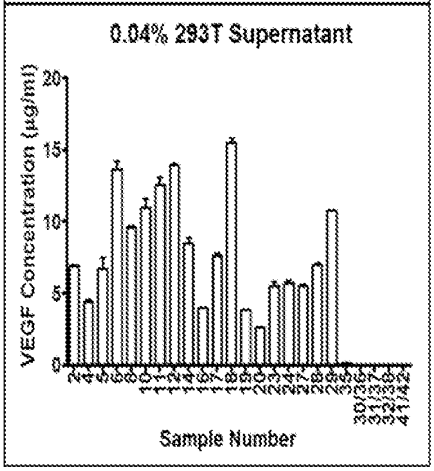
	Kozak	Linker	GOI	[SDF1] (ng/ml)
1	No	Furin-(AP)-F2A	S100A1-SDF1	10.84
3			SDF1-S100A1	19.17
4			SDF1-VEGF191	5.56
5			VEGF191-SDF1	10.77
7		(GSG)-P2A	S100A1-SDF1	26.90
9			SDF1-S100A1	2.09
10			SDF1-VEGF191	0
11			VEGF191-SDF1	9.77
13		Furin-(APVKQGSG)-P2A	S100A1-SDF1	16.88
15			SDF1-S100A1	12.27
16			SDF1-VEGF191	4.99
17			VEGF191-SDF1	10.36
19	Yes	Furin-(AP)-F2A	SDF1-VEGF191	0.43
20			VEGF191-SDF1	0
21		(GSG)-P2A	S100A1-SDF1	5.33
22			SDF1-S100A1	10.94
23			SDF1-VEGF191	0
24			VEGF191-SDF1	3.74
25		Furin-(APVKQGSG)-P2A	S100A1-SDF1	16.95
26			SDF1-S100A1	18.45
27			SDF1-VEGF191	5.42
28			VEGF191-SDF1	4.96
30/36	No	N/A	VEGF191	0
31/37			S100A1	0
32/38			SDF1	26.22
35			Mock	0
41/42			Untransfected	0

FIG. 15

A.



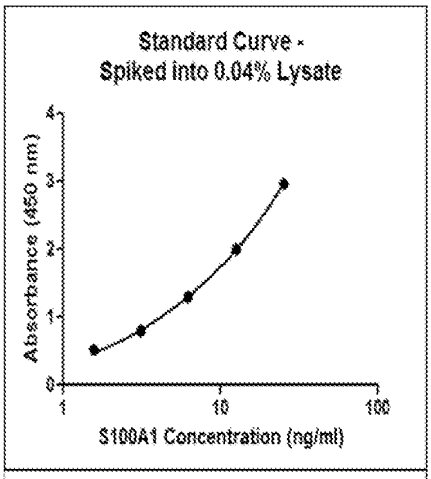
B.



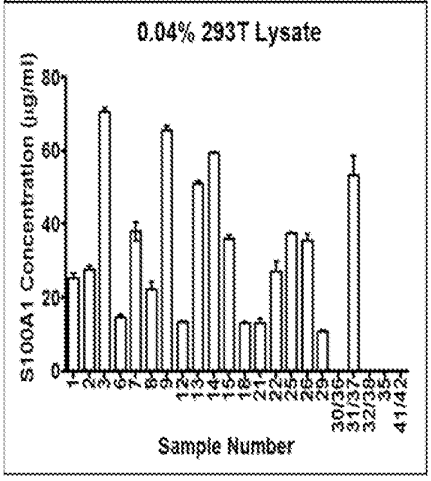
	Kozak	Linker	GOI	[VEGF] (µg/ml)
2	No	Furin-(AP)-F2A	S100A1-VEGF191	6.95
4			SDF1-VEGF191	4.43
5			VEGF191-SDF1	6.72
6			VEGF191-S100A1	13.60
8		(GSG)-P2A	S100A1-VEGF191	9.64
10			SDF1-VEGF191	10.95
11			VEGF191-SDF1	12.57
12			VEGF191-S100A1	13.94
14		Furin-(APVKQGSG)-P2A	S100A1-VEGF191	8.49
16			SDF1-VEGF191	3.97
17			VEGF191-SDF1	7.58
18			VEGF191-S100A1	15.49
19	Yes	Furin-(AP)-F2A	SDF1-VEGF191	3.86
20			VEGF191-SDF1	2.65
23		(GSG)-P2A	SDF1-VEGF191	5.53
24			VEGF191-SDF1	5.69
27		Furin-(APVKQGSG)-P2A	SDF1-VEGF191	5.53
28			VEGF191-SDF1	6.96
29			VEGF191-S100A1	10.78
30/36*	No	N/A	VEGF191	0.18
31/37			S100A1	0
32/38			SDF1	0
35			Mock	0
41/42			Untransfected	0

FIG. 16

A.



B.



	Kozak	Linker	GOI	[S100A1] (µg/ml)
1	No	Furin-(AP)-F2A	S100A1-SDF1	25.4
2			S100A1-VEGF191	27.6
3			SDF1-S100A1	70.7
6			VEGF191-S100A1	14.5
7		(GSG)-P2A	S100A1-SDF1	38.0
8			S100A1-VEGF191	22.2
9			SDF1-S100A1	65.4
12			VEGF191-S100A1	13.4
13		Furin-(APVKQGSG)-P2A	S100A1-SDF1	51.1
14			S100A1-VEGF191	59.3
15			SDF1-S100A1	36.0
18			VEGF191-S100A1	13.0
21	Yes	(GSG)-P2A	S100A1-SDF1	13.1
22			SDF1-S100A1	27.0
25		Furin-(APVKQGSG)-P2A	S100A1-SDF1	37.4
26			SDF1-S100A1	35.5
29			VEGF191-S100A1	10.9
30/36*	No	N/A	VEGF191	0
31/37			S100A1	53.2
32/38			SDF1	0
35			Mock	0
41/42			Untransfected	0

FIG. 17

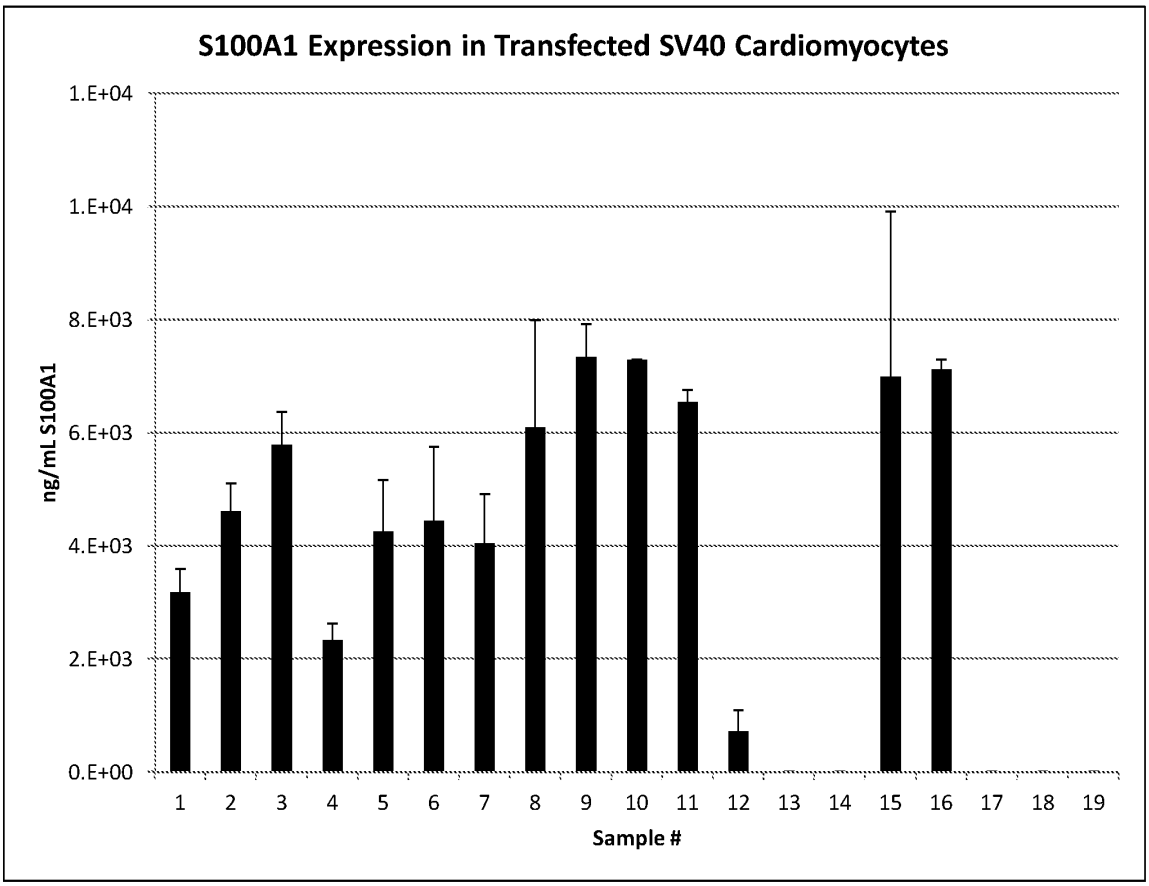

S100A1 Expression in Transfected SV40 Cardiomyocytes
1.E+04
1.E+04
8.E+03
6.E+03
4.E+03
2.E+03
0.E+00
ng/mL S100A1
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Sample #

FIG. 18

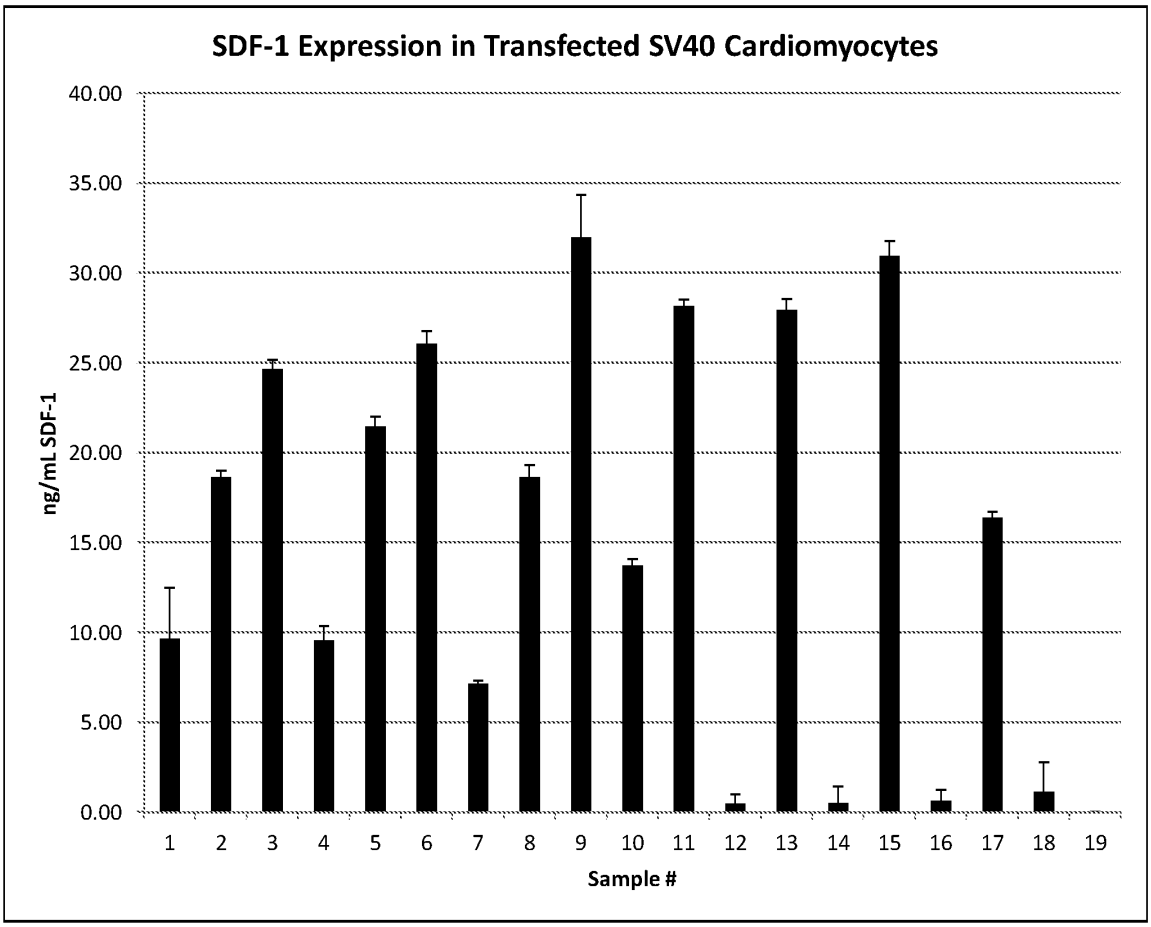

SDF-1 Expression in Transfected SV40 Cardiomyocytes
ng/mL SDF-1
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Sample #

FIG. 19

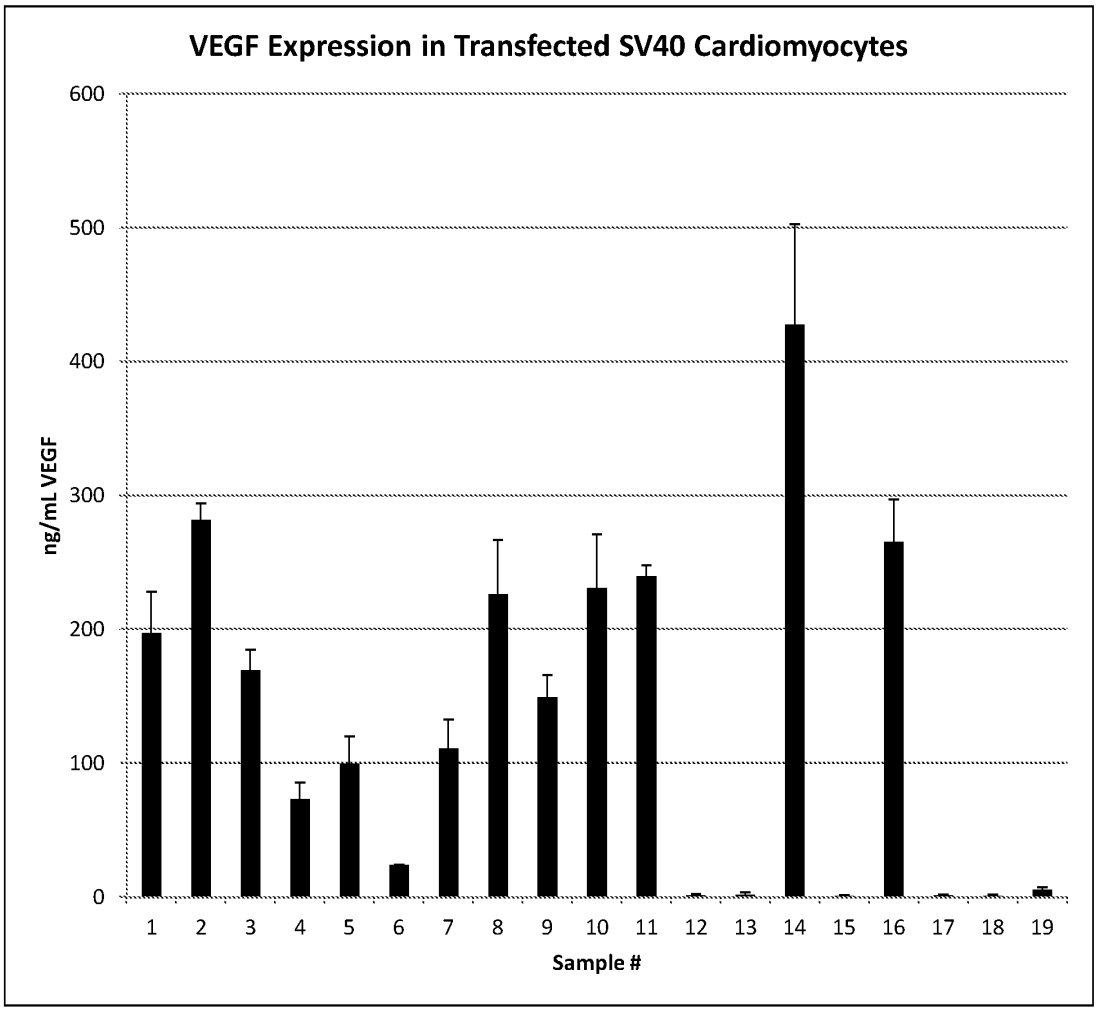

VEGF Expression in Transfected SV40 Cardiomyocytes
ng/mL VEGF
600
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
Sample #

FIG. 20

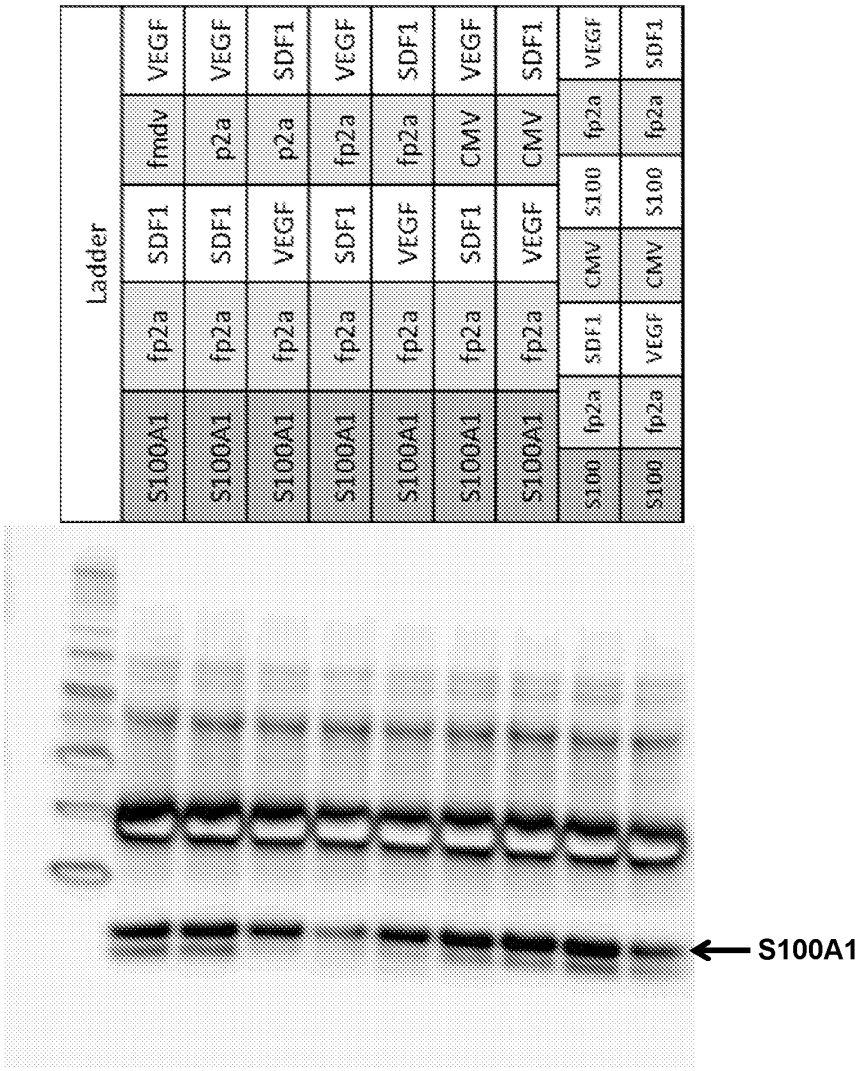

Ladder
S100A1 fp2a SDF1 fmdv VEGF
S100A1 fp2a SDF1 p2a VEGF
S100A1 fp2a VEGF p2a SDF1
S100A1 fp2a SDF1 fp2a VEGF
S100A1 fp2a VEGF fp2a SDF1
S100A1 fp2a SDF1 CMV VEGF
S100A1 fp2a VEGF CMV SDF1
S100 fp2a SDF1 CMV S100 fp2a VEGF
S100 fp2a VEGF CMV S100 fp2a SDF1
S100A1

FIG. 21

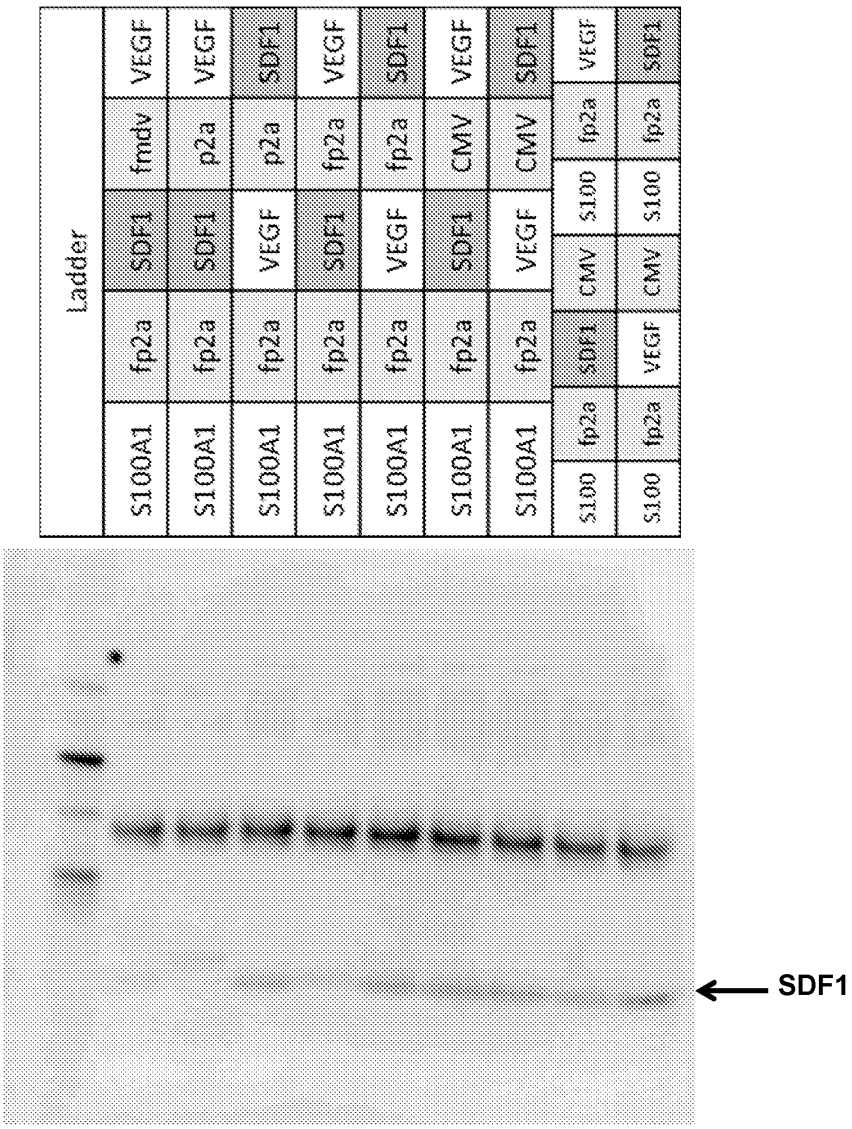

Ladder
S100A1
fp2a
SDF1
fmdv
VEGF
S100A1
fp2a
SDF1
p2a
VEGF
S100A1
fp2a
VEGF
p2a
SDF1
S100A1
fp2a
SDF1
fp2a
VEGF
S100A1
fp2a
VEGF
fp2a
SDF1
S100A1
fp2a
SDF1
CMV
VEGF
S100A1
fp2a
VEGF
CMV
SDF1
S100
fp2a
CMV
S100
VEGF
S100
fp2a
SDF1
S100
fp2a
VEGF
SDF1

FIG. 22

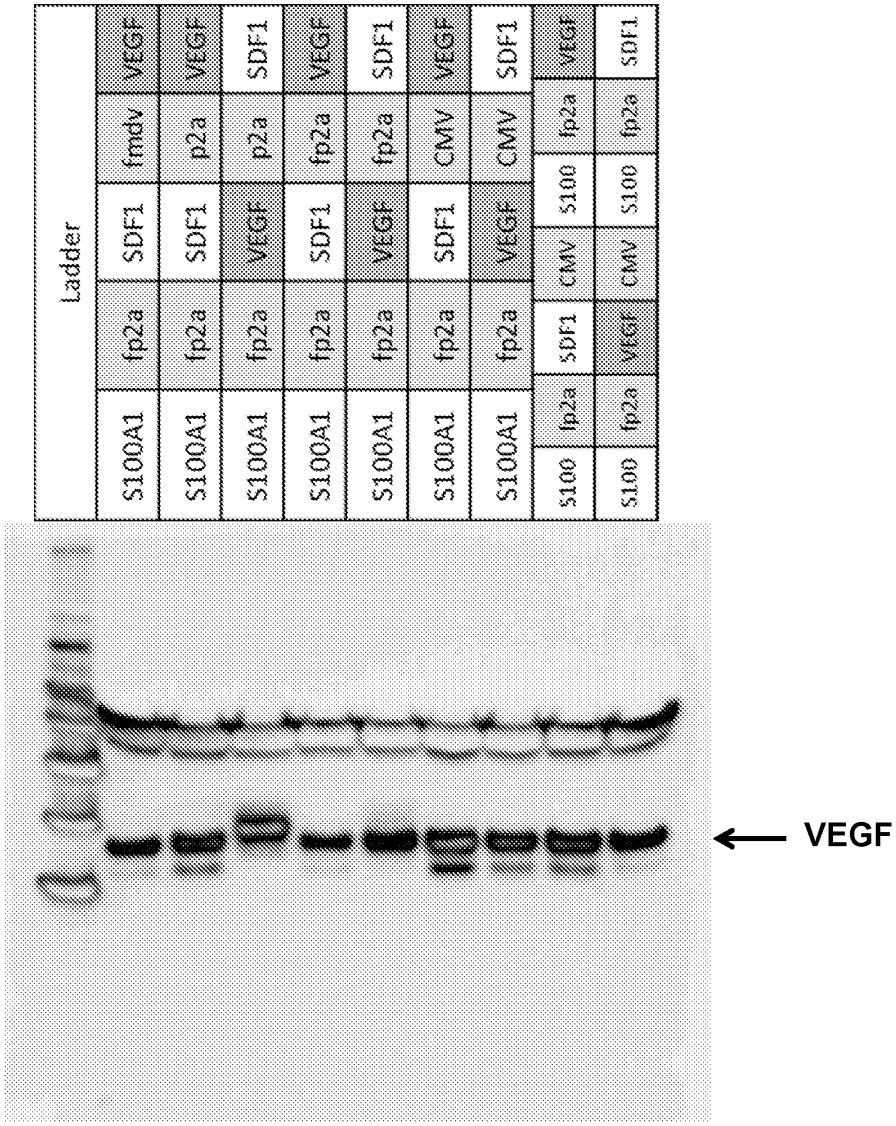

Ladder
S100A1 fp2a SDF1 fmdv VEGF
S100A1 fp2a SDF1 p2a VEGF
S100A1 fp2a VEGF p2a SDF1
S100A1 fp2a SDF1 fp2a VEGF
S100A1 fp2a VEGF fp2a SDF1
S100A1 fp2a SDF1 CMV VEGF
S100A1 fp2a VEGF CMV SDF1
S100 fp2a SDF1 CMV S100 fp2a VEGF
S100 fp2a VEGF CMV S100 fp2a SDF1
VEGF

FIG. 23

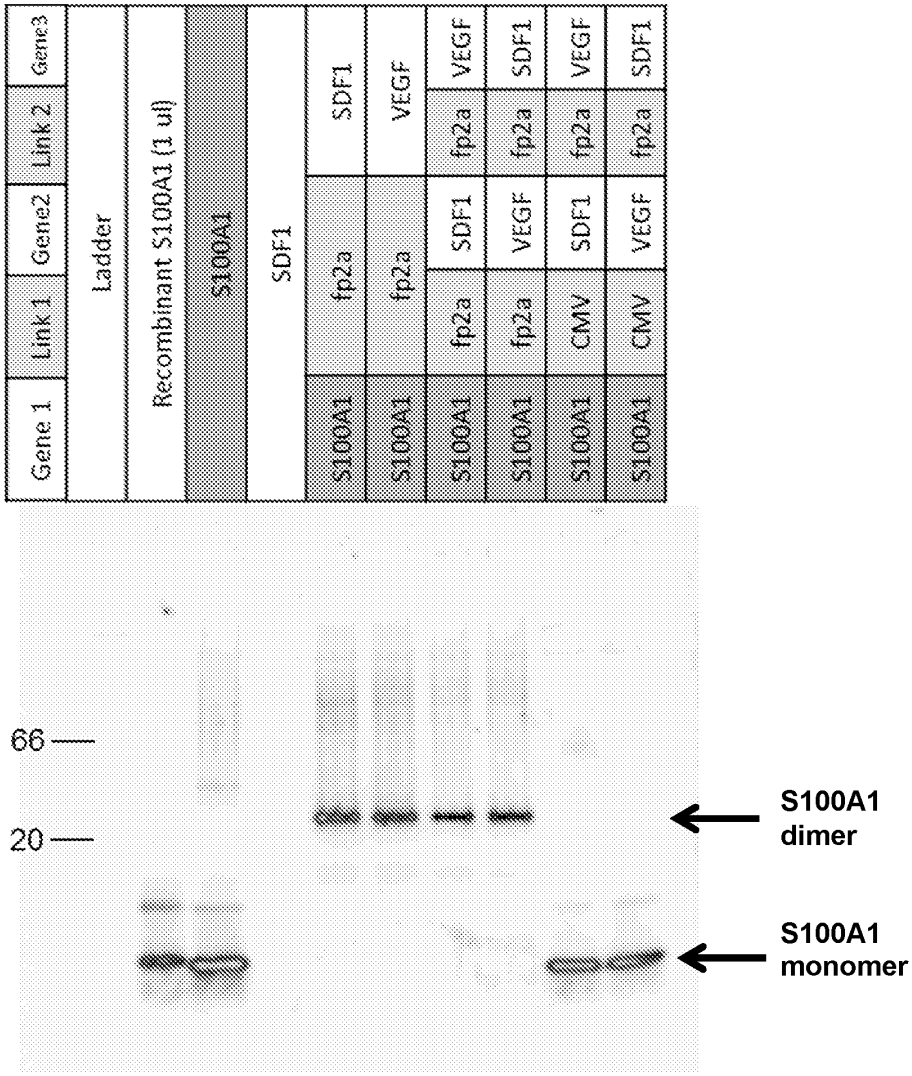
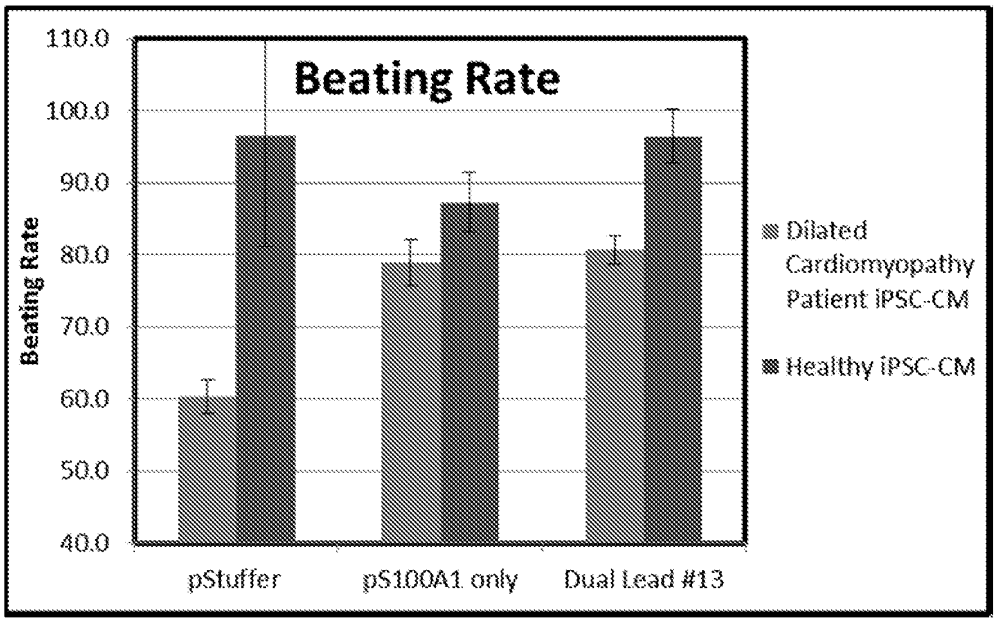

Gene 1
Link 1
Gene2
Link 2
Gene3
Ladder
Recombinant S100A1 (1 ul)
S100A1
SDF1
S100A1
fp2a
SDF1
S100A1
fp2a
VEGF
S100A1
fp2a
SDF1
S100A1
fp2a
VEGF
S100A1
CMV
SDF1
S100A1
CMV
VEGF
S100A1
fp2a
VEGF
fp2a
SDF1
fp2a
VEGF
fp2a
SDF1
66
20
S100A1 dimer
S100A1 monomer

FIG. 24

A.


Beating Rate
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
Beating Rate
pStuffer
pS100A1 only
Dual Lead #13
Dilated Cardiomyopathy Patient iPSC-CM
Healthy iPSC-CM

B.

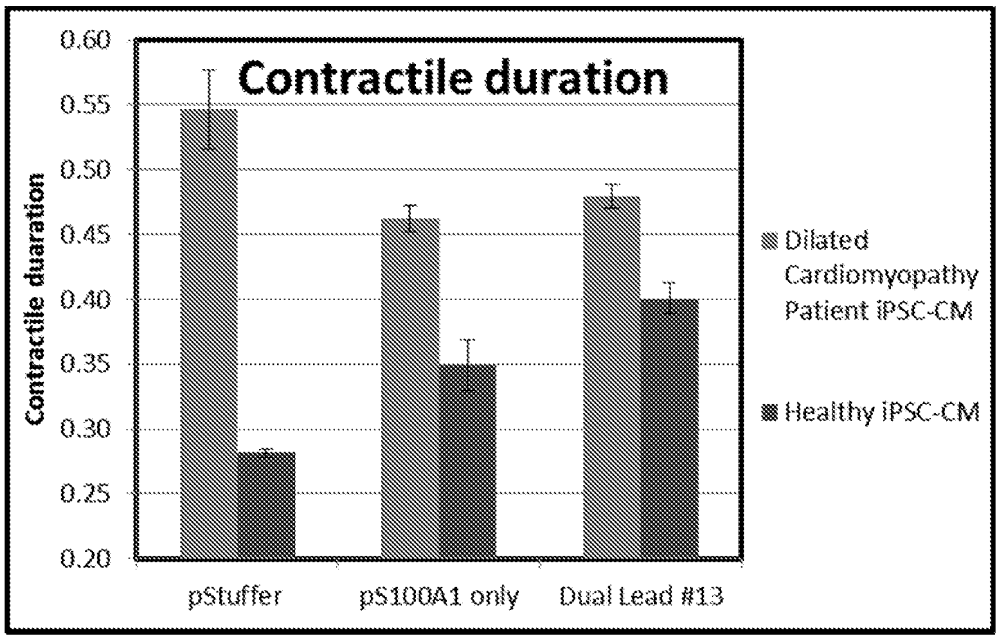
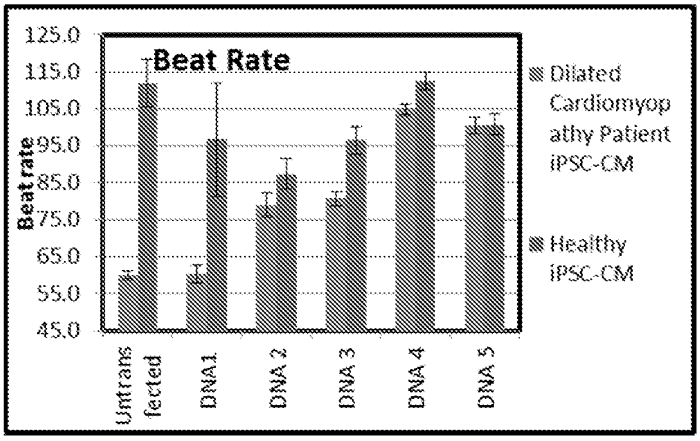
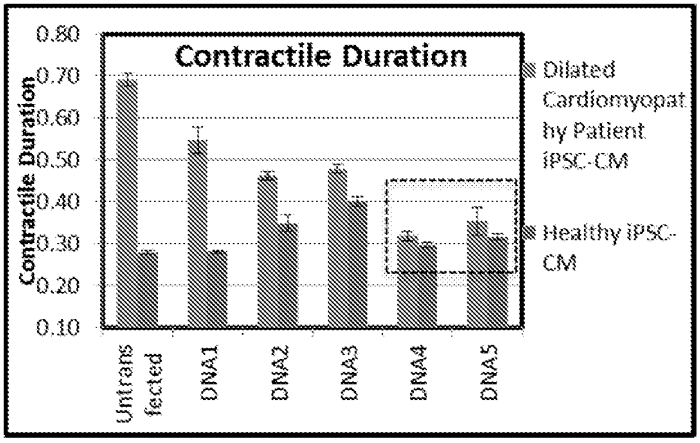

Contractile duration
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
Contractile duaration
pStuffer
pS100A1 only
Dual Lead #13
Dilated Cardiomyopathy Patient iPSC-CM
Healthy iPSC-CM

FIG. 25

A.


Beat Rate
Beat rate
125.0
115.0
105.0
95.0
85.0
75.0
65.0
55.0
45.0
Untransfected
DNA 1
DNA 2
DNA 3
DNA 4
DNA 5
Dilated Cardiomyopathy Patient iPSC-CM
Healthy iPSC-CM

B.


Contractile Duration
Contractile Duration
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
Untransfected
DNA1
DNA2
DNA3
DNA4
DNA5
Dilated Cardiomyopathy Patient iPSC-CM
Healthy iPSC-CM

C.

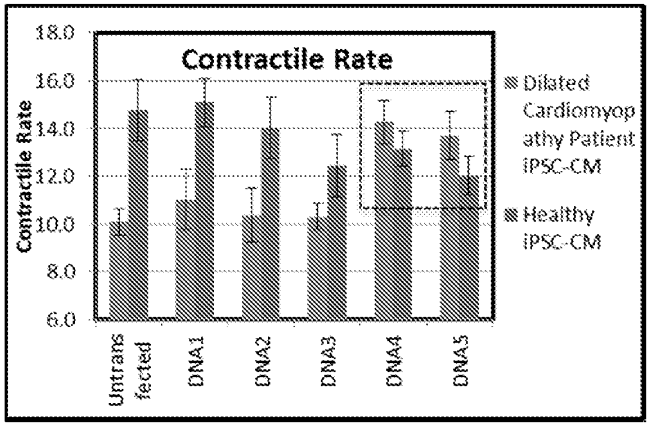
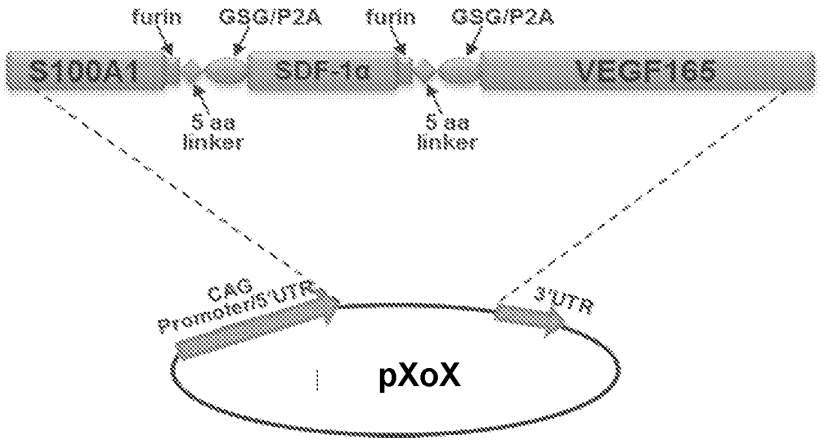

Contractile Rate
Contractile Rate
18.0
16.0
14.0
12.0
10.0
8.0
6.0
Untransfected
DNA1
DNA2
DNA3
DNA4
DNA5
Dilated Cardiomyopathy Patient iPSC-CM
Healthy iPSC-CM

FIG. 26

A.


furin
GSG/P2A
furin
GSG/P2A
S100A1
SDF-1α
VEGF165
5 aa linker
5 aa linker
CAG Promoter
5'UTR
3'UTR
pXoX

B.

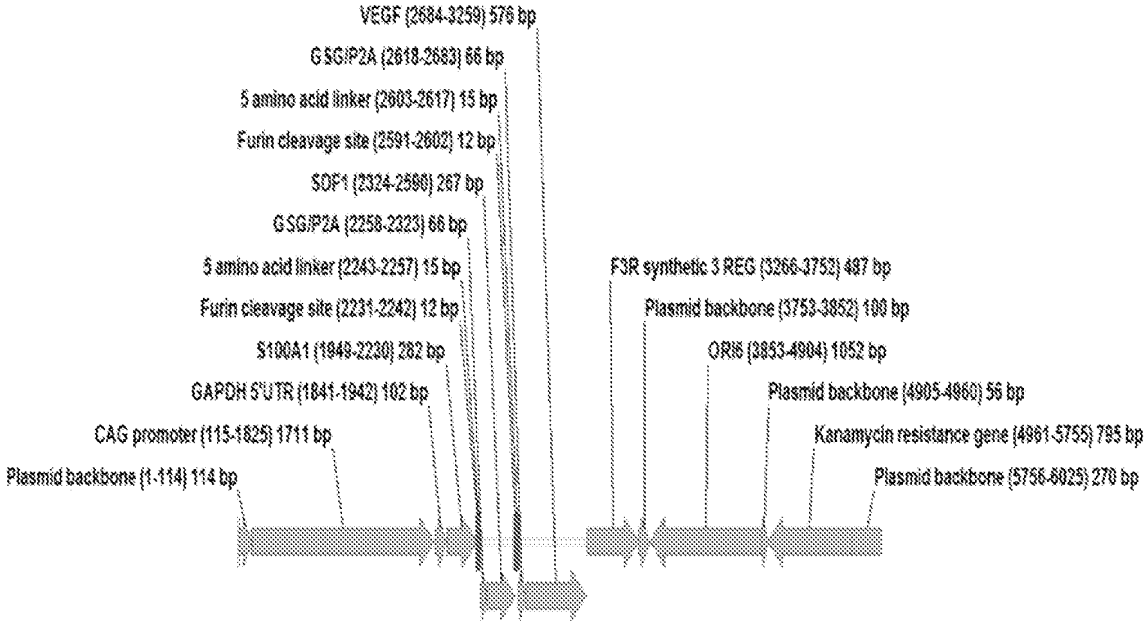

VEGF (2684-3259) 576 bp
GSG/P2A (2618-2683) 66 bp
5 amino acid linker (2603-2617) 15 bp
Furin cleavage site (2591-2602) 12 bp
SDF1 (2324-2566) 267 bp
GSG/P2A (2258-2323) 66 bp
5 amino acid linker (2243-2257) 15 bp
Furin cleavage site (2231-2242) 12 bp
S100A1 (1949-2230) 282 bp
GAPDH 5'UTR (1841-1942) 102 bp
CAG promoter (115-1825) 1711 bp
Plasmid backbone (1-114) 114 bp
F3R synthetic 3 REG (3266-3752) 487 bp
Plasmid backbone (3753-3852) 100 bp
ORI6 (3853-4904) 1052 bp
Plasmid backbone (4905-4960) 56 bp
Kanamycin resistance gene (4961-5755) 795 bp
Plasmid backbone (5756-6025) 270 bp

FIG. 27

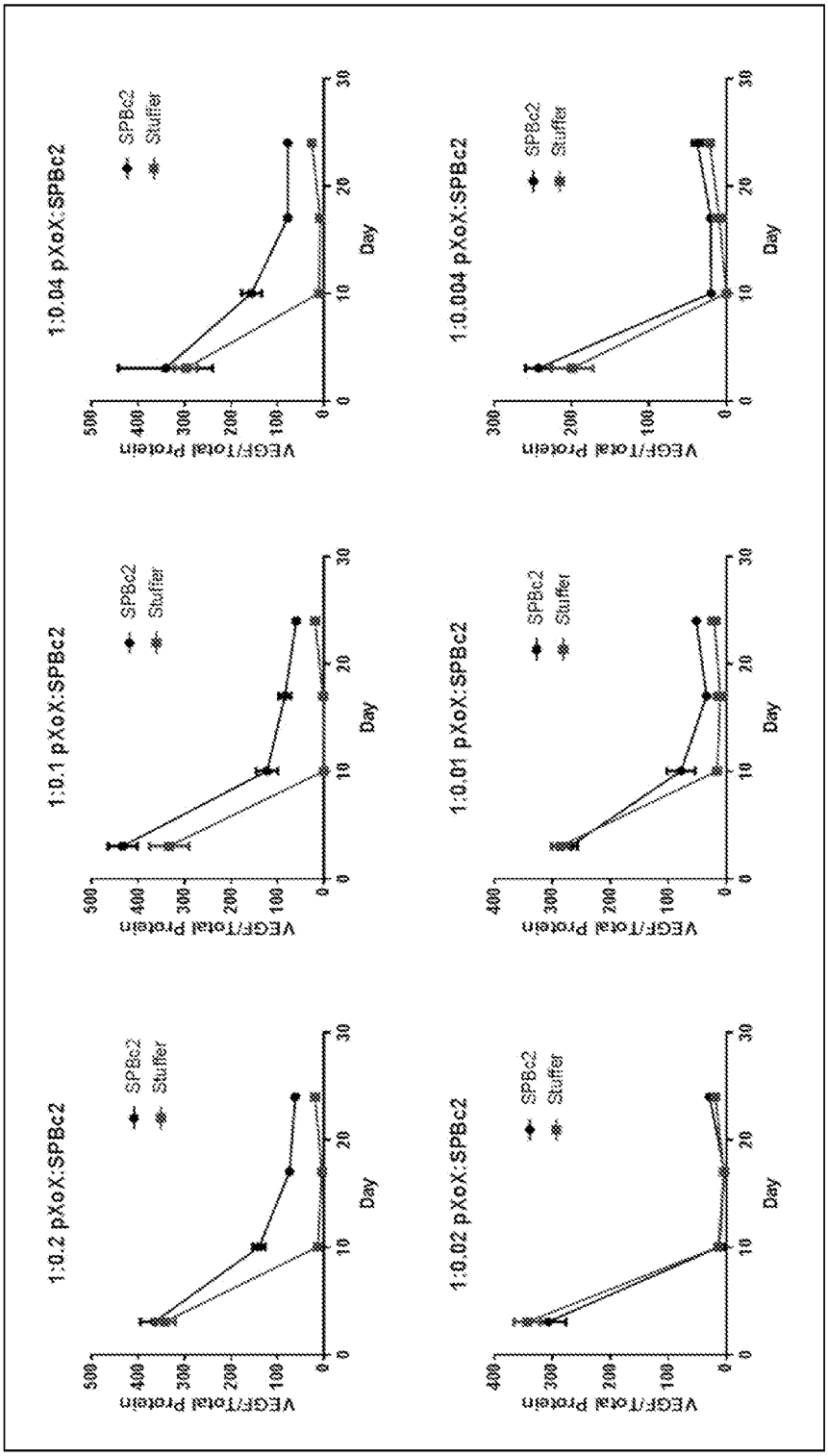

1:0.2 pXoX:SPBc2
1:0.1 pXoX:SPBc2
1:0.04 pXoX:SPBc2
1:0.02 pXoX:SPBc2
1:0.01 pXoX:SPBc2
1:0.004 pXoX:SPBc2
SPBc2
Stuffer
VEGF/Total Protein
Day

FIG. 28

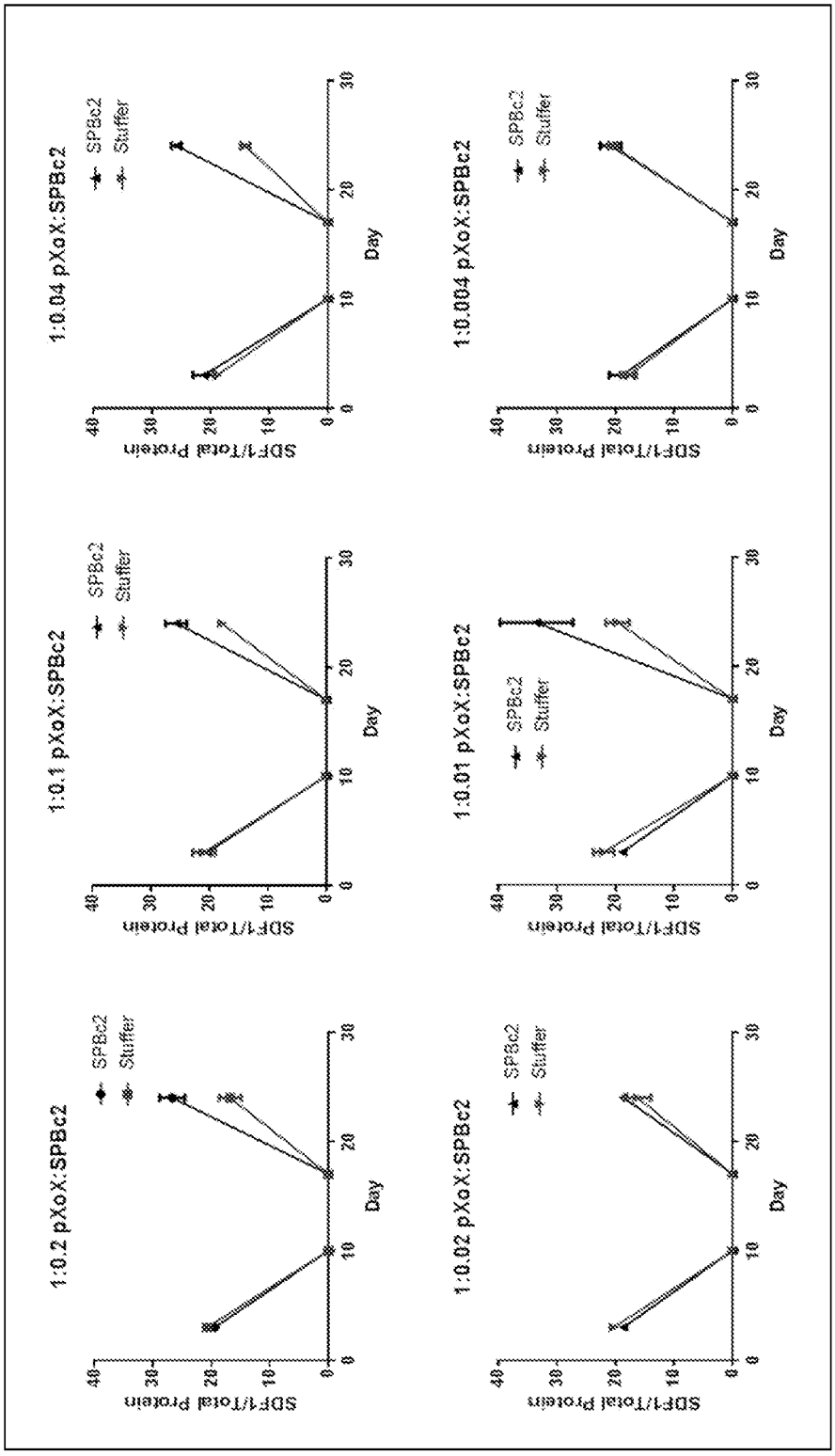

1:0.04 pXoX:SPBc2
1:0.02 pXoX:SPBc2
1:0.01 pXoX:SPBc2
1:0.004 pXoX:SPBc2
1:0.2 pXoX:SPBc2
1:0.1 pXoX:SPBc2
SPBc2
Stuffer
SDF1/Total Protein
Day
0
10
20
30
40

FIG. 29

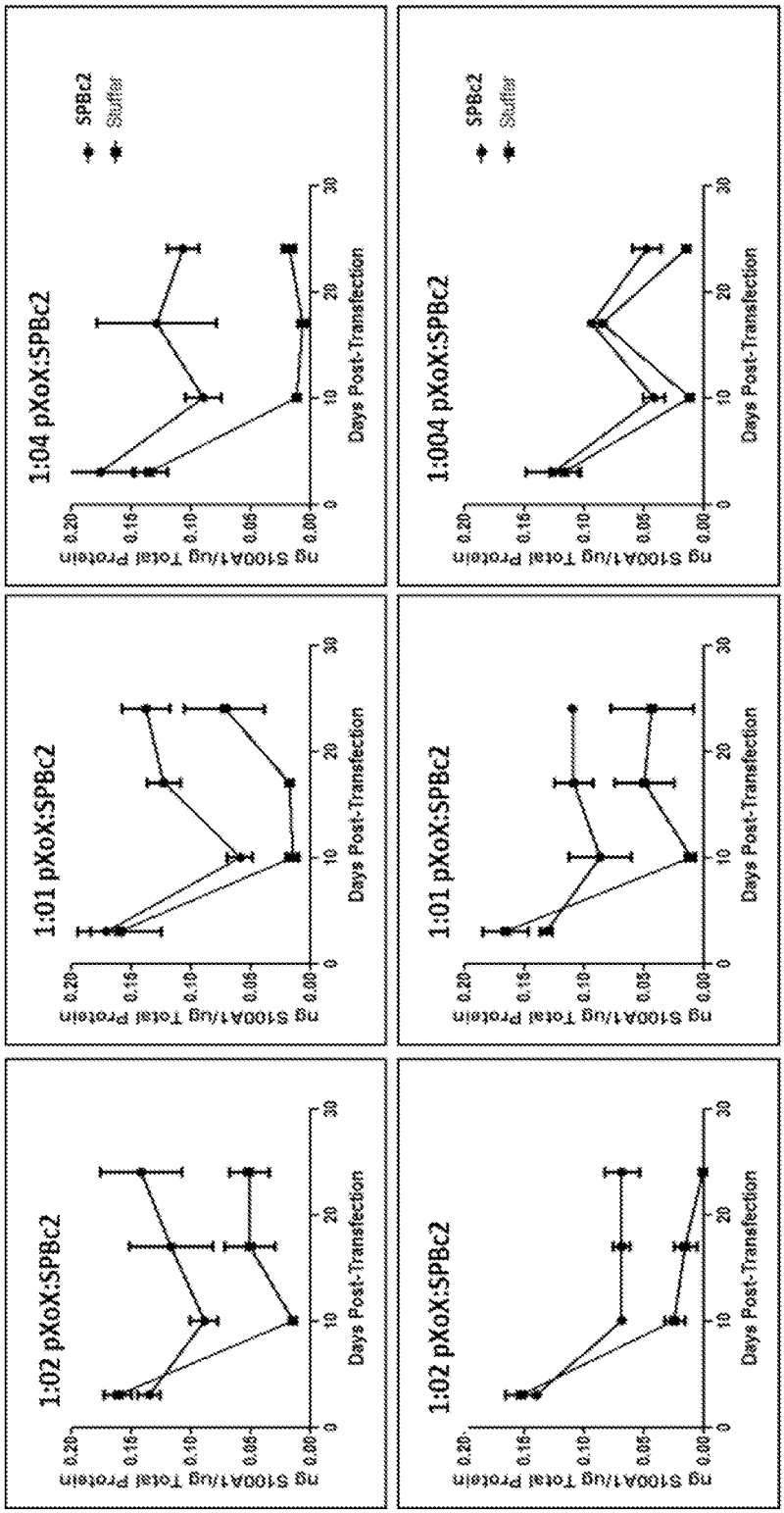
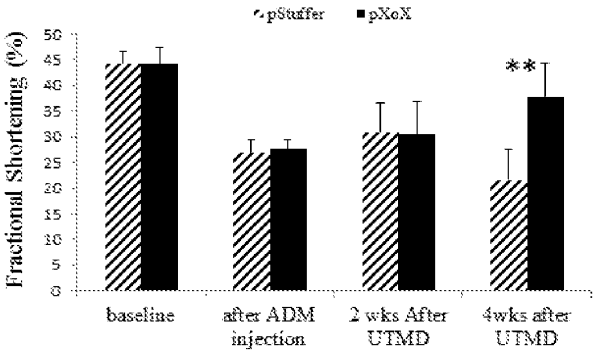
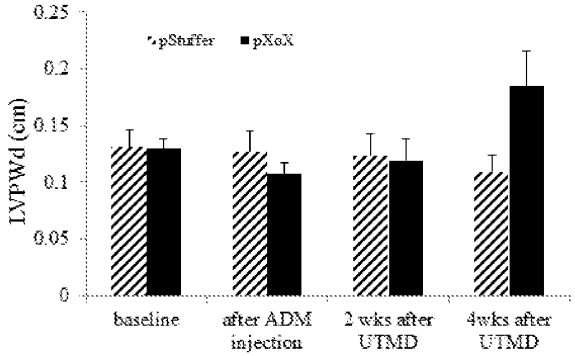

1:02 pXoX:SPBc2
1:01 pXoX:SPBc2
1:04 pXoX:SPBc2
1:004 pXoX:SPBc2
SPBc2
Stuffer
ng S100A1/ug Total Protein
Days Post-Transfection
0.00
0.05
0.10
0.15
0.20
0
10
20
30

FIG. 30

A.


pStuffer
pXoX
Fractional Shortening (%)
50
45
40
35
30
25
20
15
10
5
0
**
baseline
after ADM injection
2 wks After UTMD
4wks after UTMD

B.


pStuffer
pXoX
LVPWd (cm)
0.25
0.2
0.15
0.1
0.05
0
baseline
after ADM injection
2 wks after UTMD
4wks after UTMD

C.

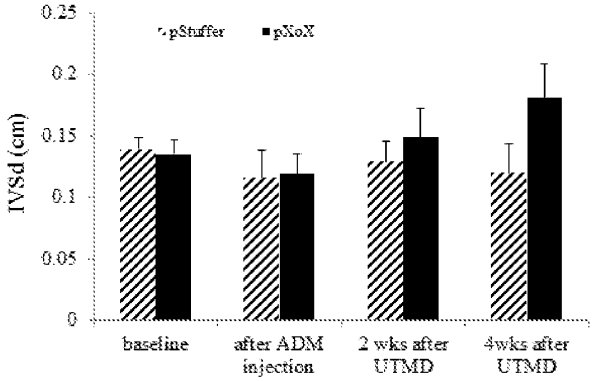

pStuffer
pXoX
IVSd (cm)
0.25
0.2
0.15
0.1
0.05
0
baseline
after ADM injection
2 wks after UTMD
4wks after UTMD

FIG. 31

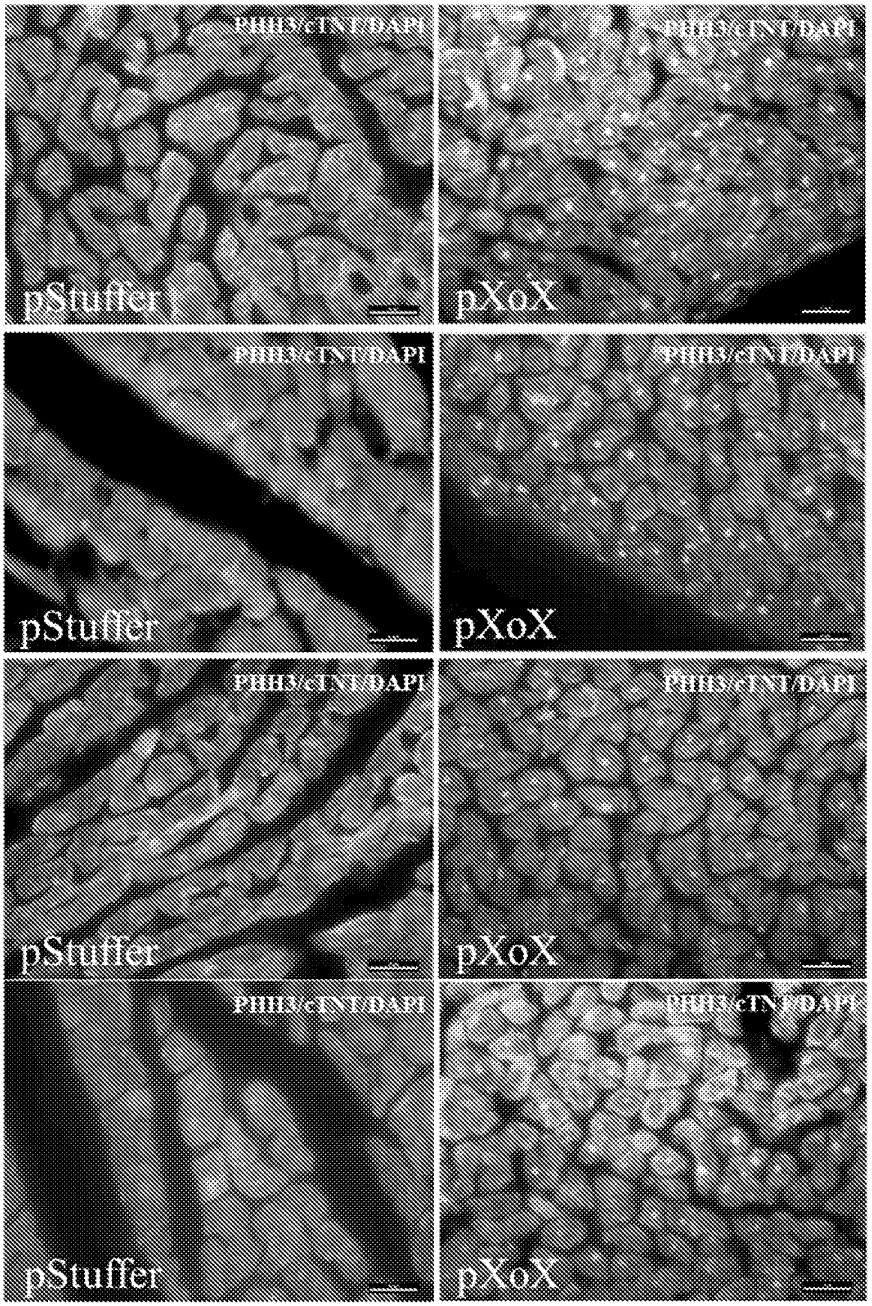

PHH3/cTNT/DAPI
pStuffer
PHH3/cTNT/DAPI
pXoX
PHH3/cTNT/DAPI
pStuffer
PHH3/cTNT/DAPI
pXoX
PHH3/cTNT/DAPI
pStuffer
PHH3/cTNT/DAPI
pXoX
PHH3/cTNT/DAPI
pStuffer
PHH3/cTNT/DAPI
pXoX

FIG. 32

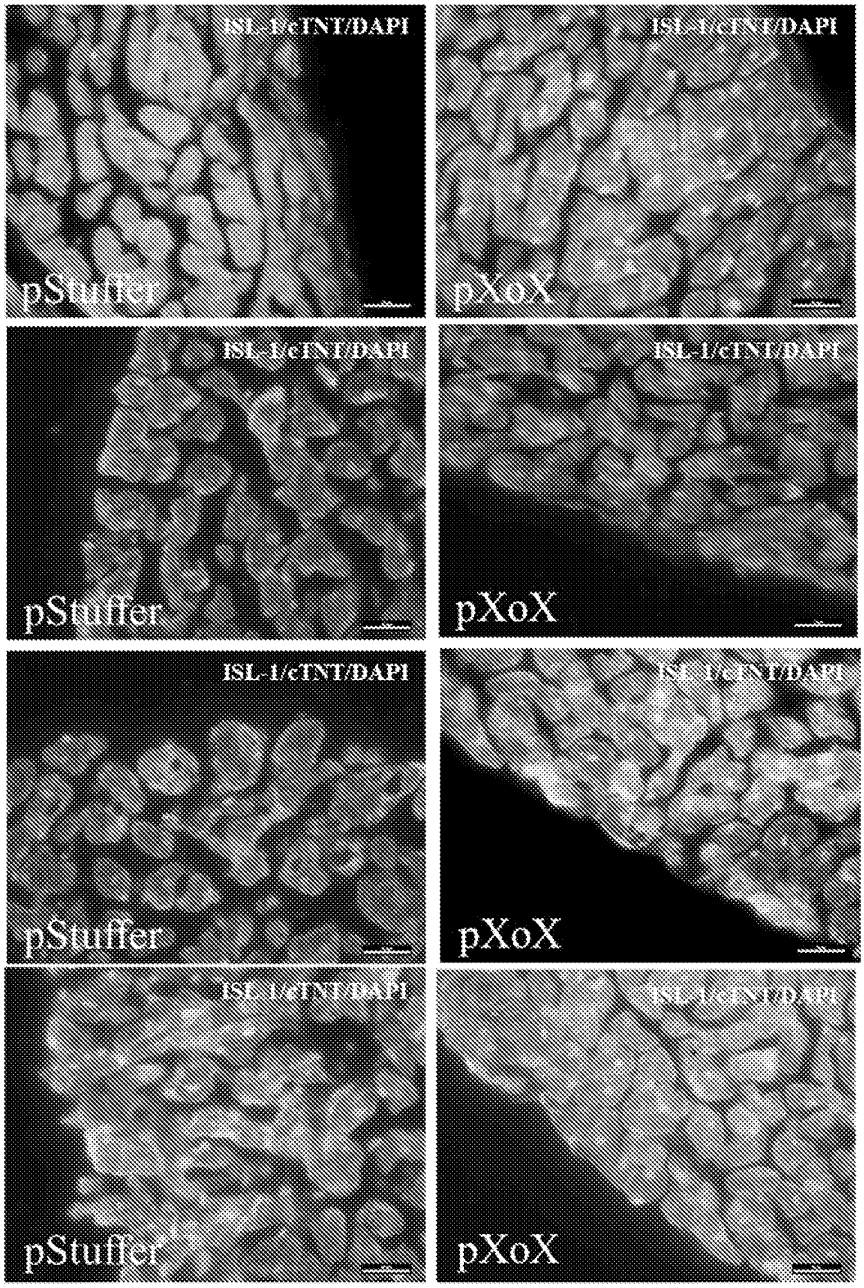

ISL-1/cTNT/DAPI
pStuffer
ISL-1/cTNT/DAPI
pXoX
ISL-1/cTNT/DAPI
pStuffer
ISL-1/cTNT/DAPI
pXoX
ISL-1/cTNT/DAPI
pStuffer
ISL-1/cTNT/DAPI
pXoX
ISL-1/cTNT/DAPI
pStuffer
ISL-1/cTNT/DAPI
pXoX

FIG. 33