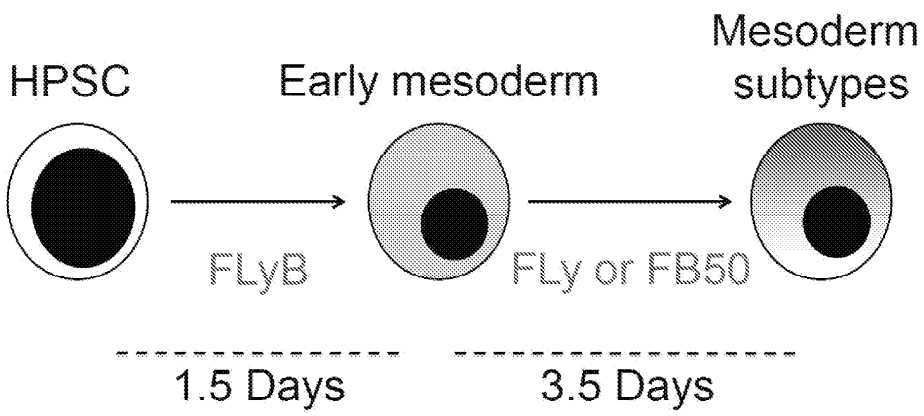


Figure 1A

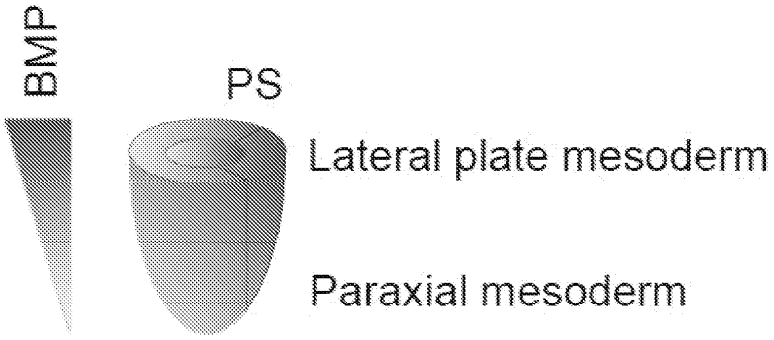


Figure 1B

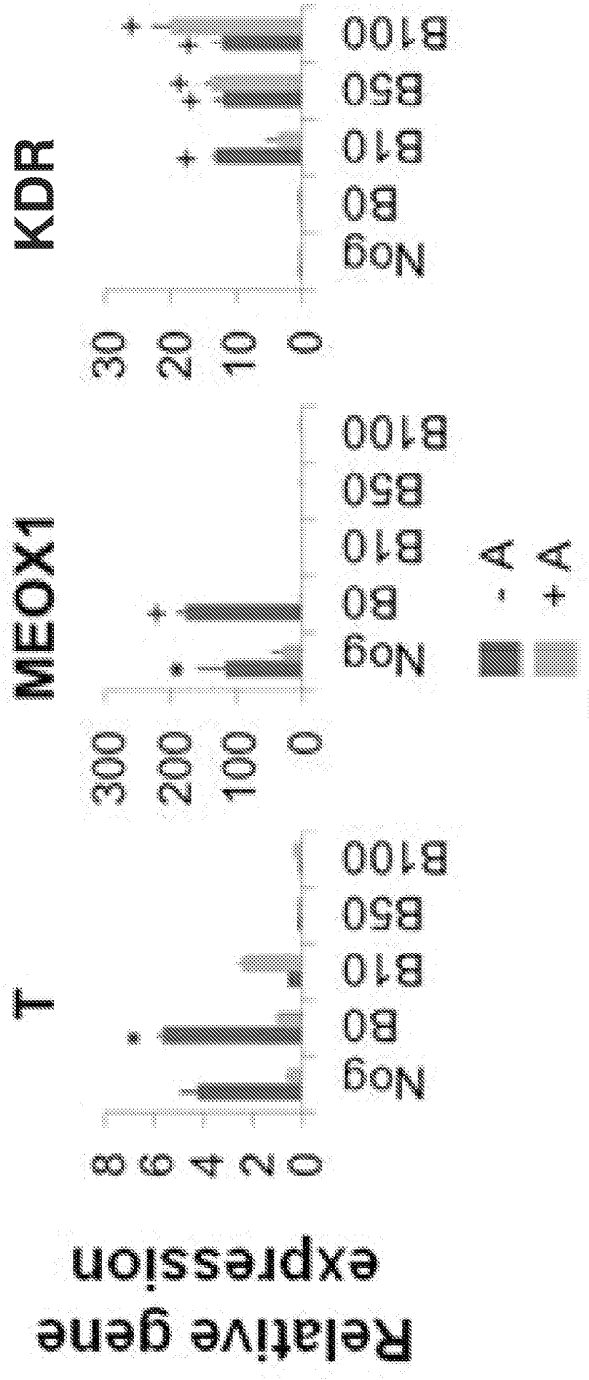


Figure 2

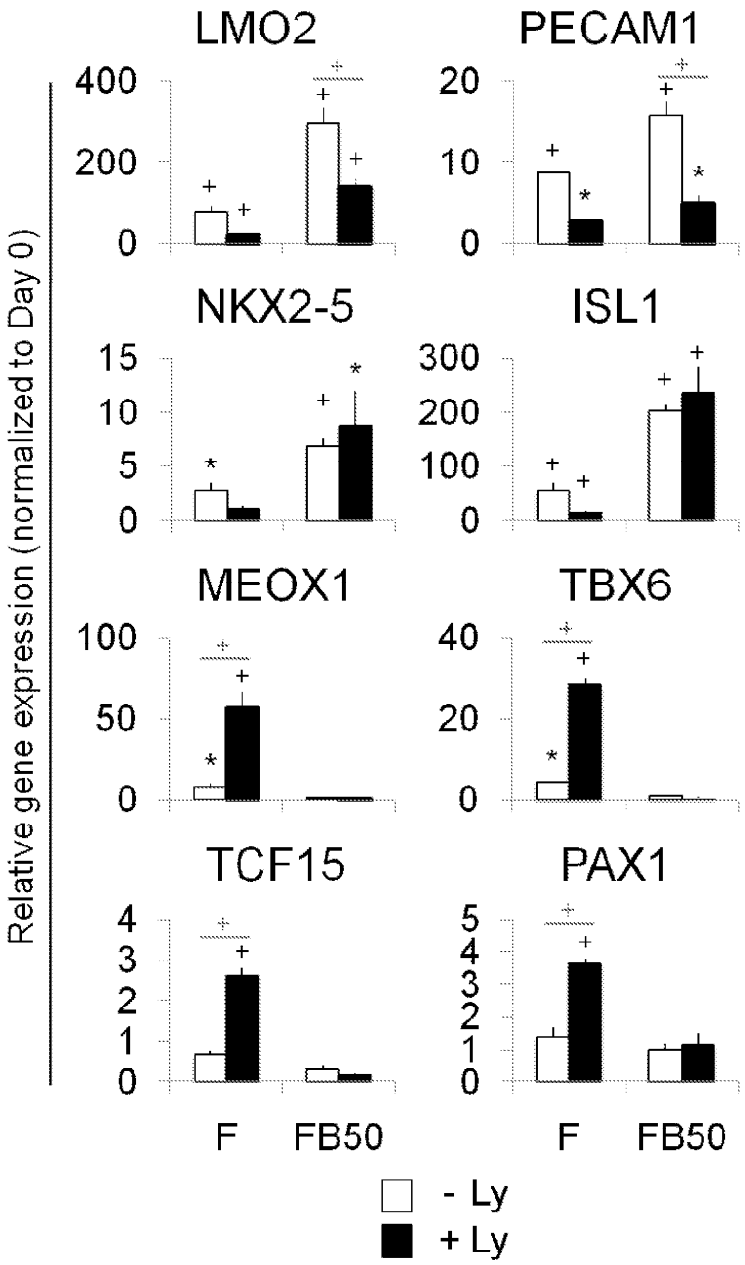

LMO2
PECAM1
NKX2-5
ISL1
MEOX1
TBX6
TCF15
PAX1
Relative gene expression (normalized to Day 0)
400
200
0
20
10
15
5
300
100
40
4
3
2
1
5
F
FB50
- Ly
+ Ly

Figure 3

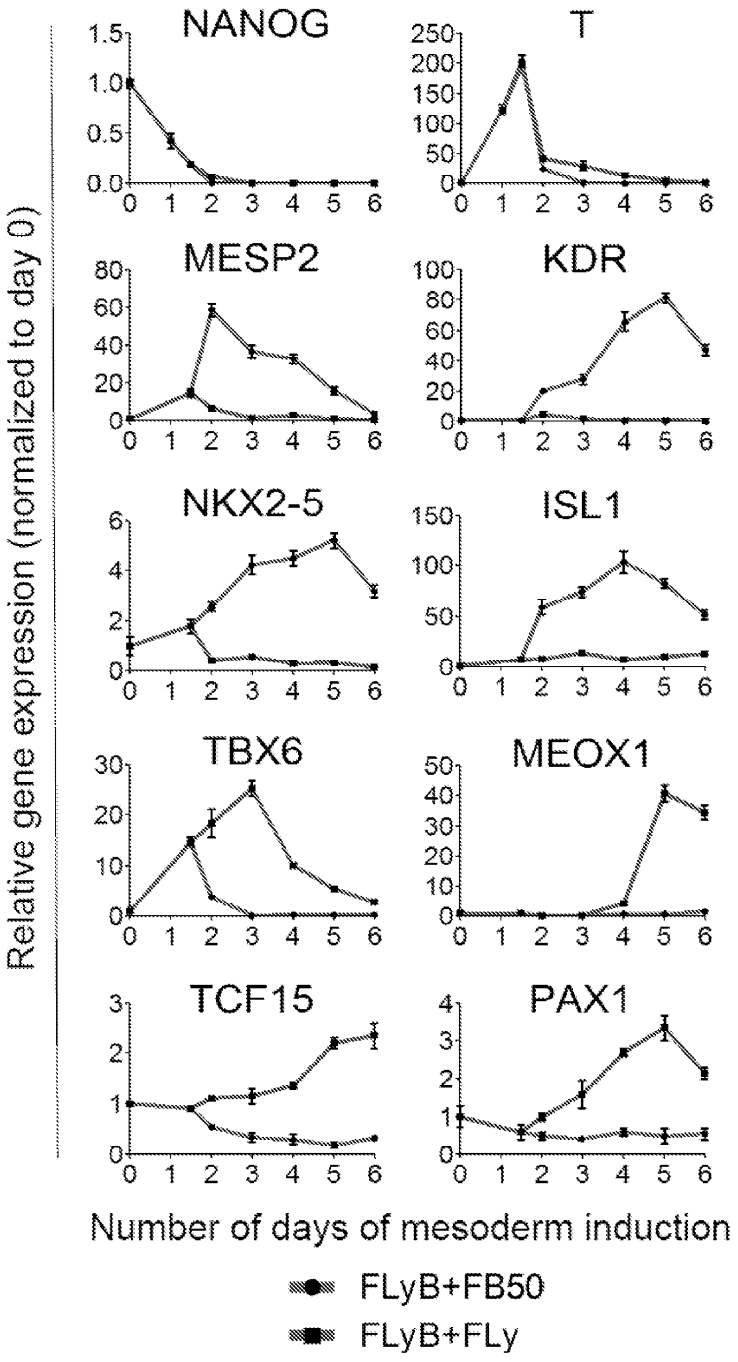

NANOG
T
MESP2
KDR
NKX2-5
ISL1
TBX6
MEOX1
TCF15
PAX1
Relative gene expression (normalized to day 0)
Number of days of mesoderm induction
FLyB+FB50
FLyB+FLy

Figure 4

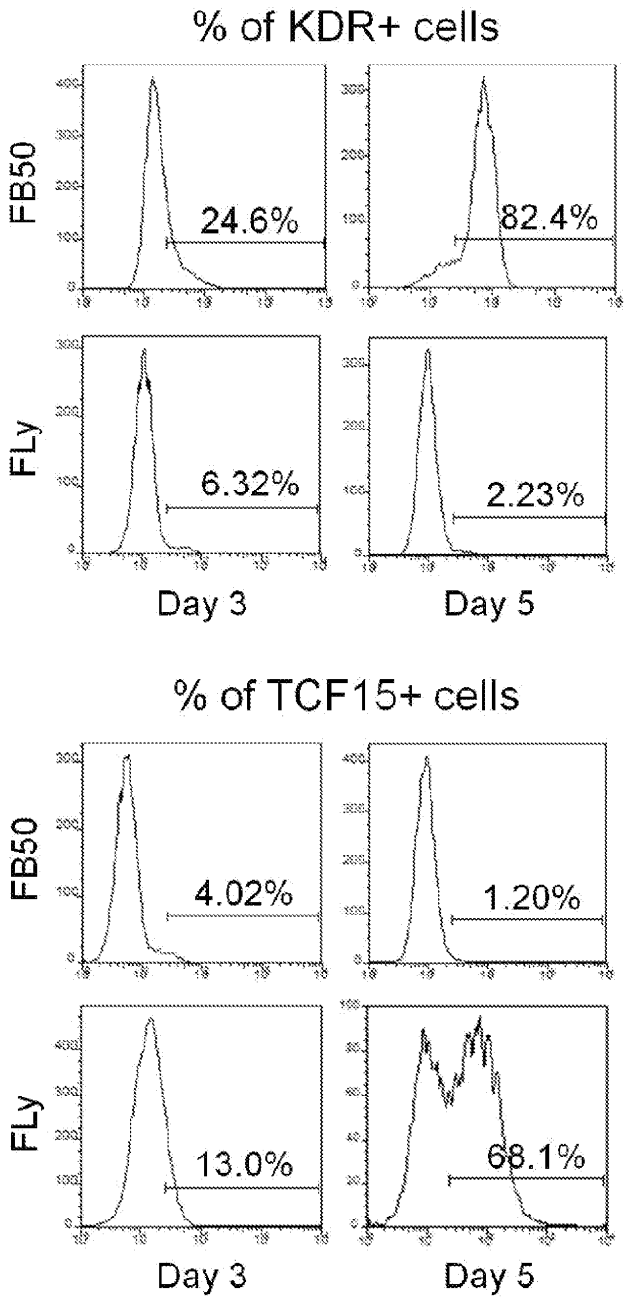

% of KDR+ cells
FB50
24.6%
82.4%
FLy
6.32%
2.23%
Day 3
Day 5
% of TCF15+ cells
FB50
4.02%
1.20%
FLy
13.0%
68.1%
Day 3
Day 5

Figure 5

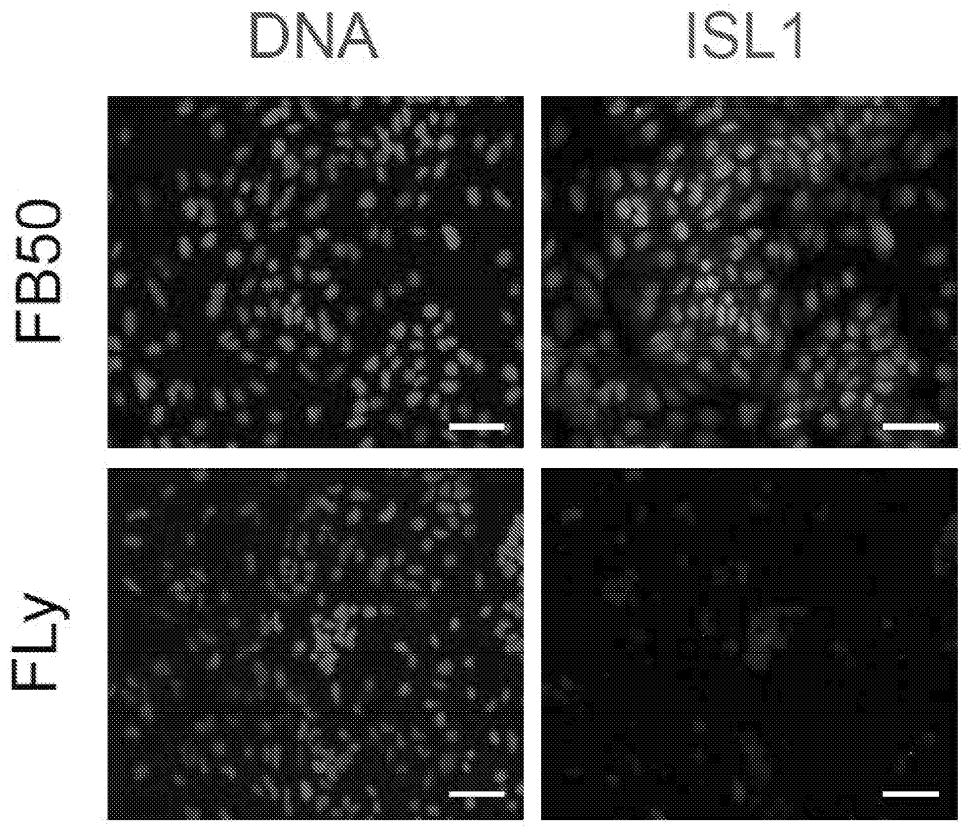


Figure 6A

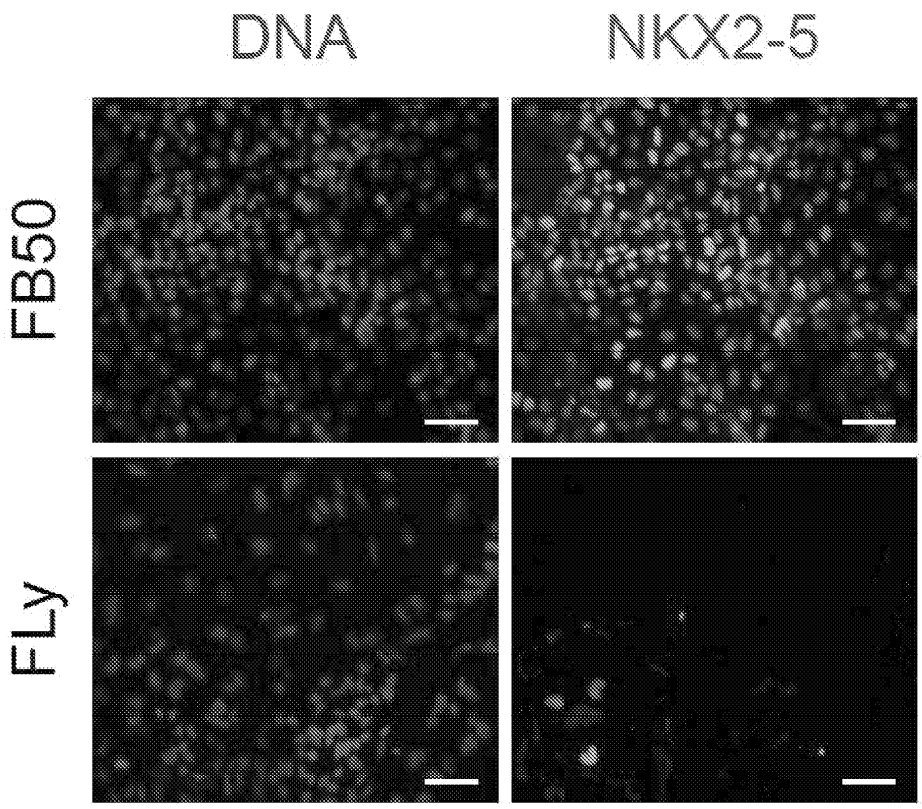


Figure 6B

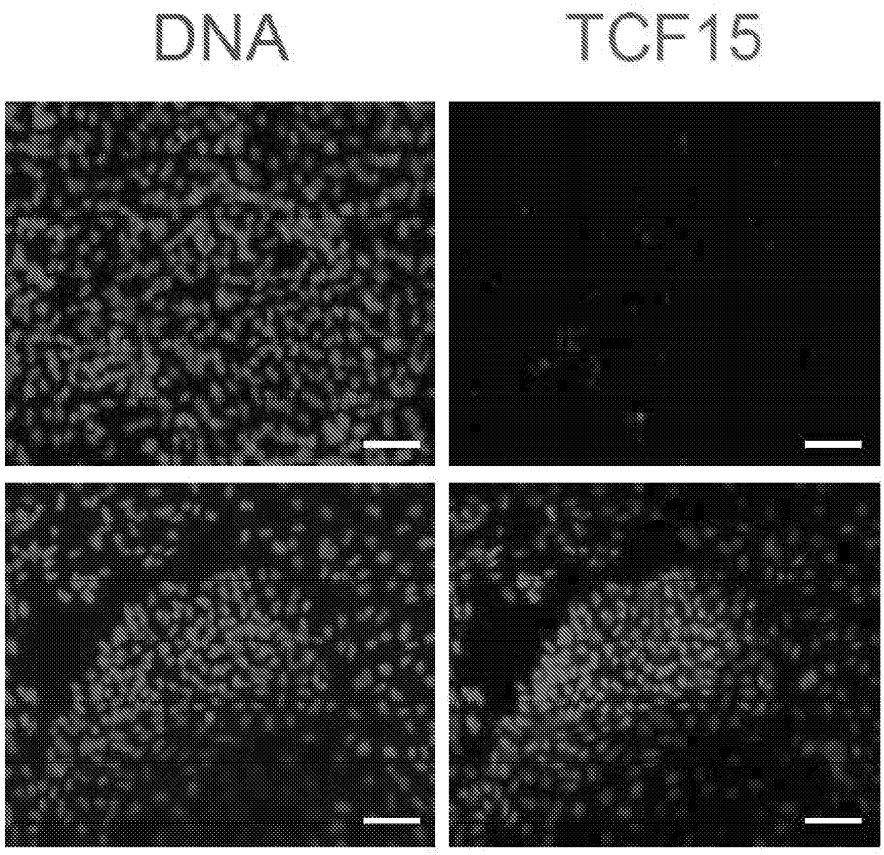


Figure 6C

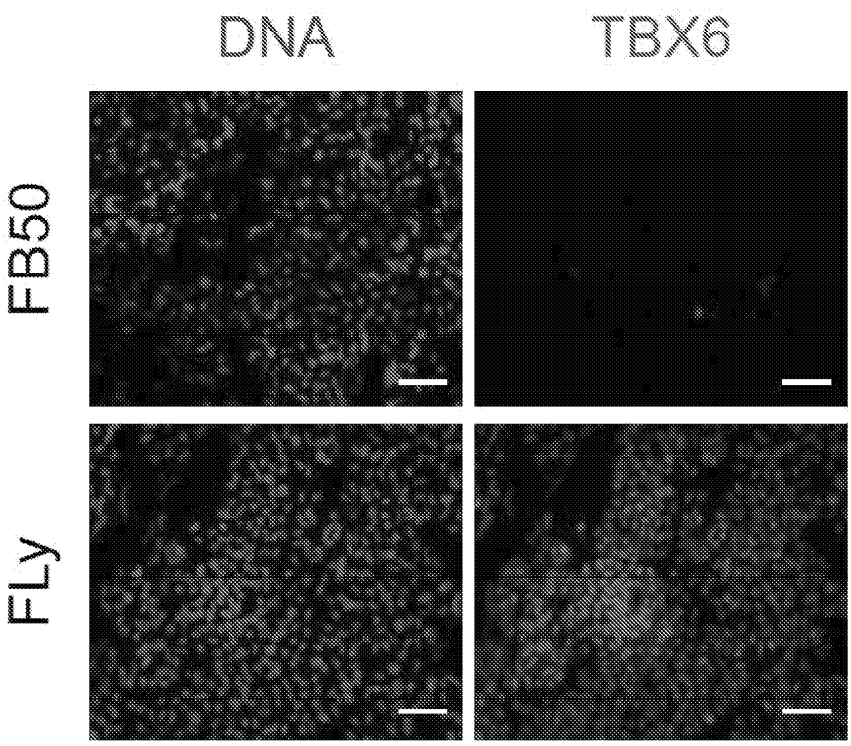


Figure 6D

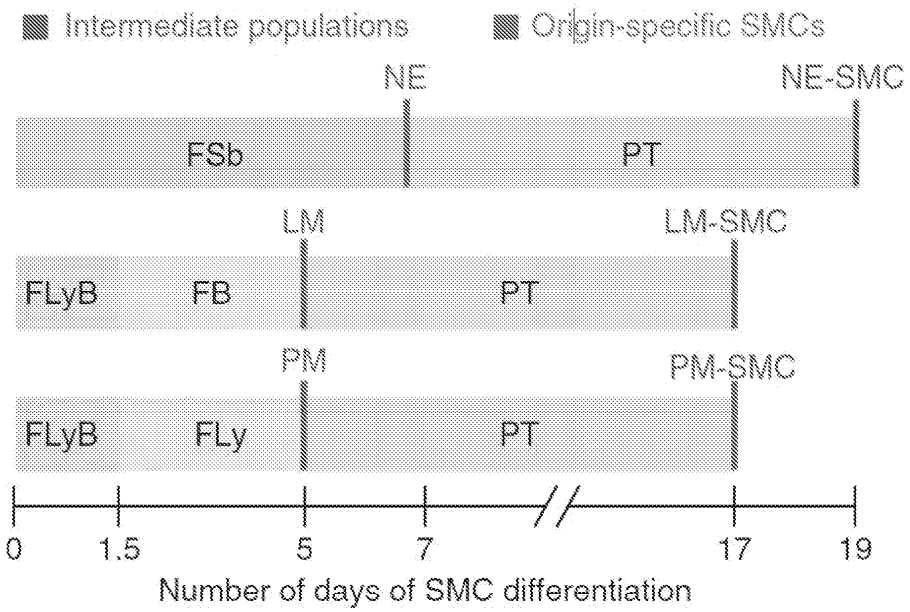

Intermediate populations
Origin-specific SMCs
NE
NE-SMC
FSb
PT
LM
LM-SMC
FLyB
FB
PT
PM
PM-SMC
FLyB
FLy
PT
0
1.5
5
7
17
19
Number of days of SMC differentiation

Figure 7

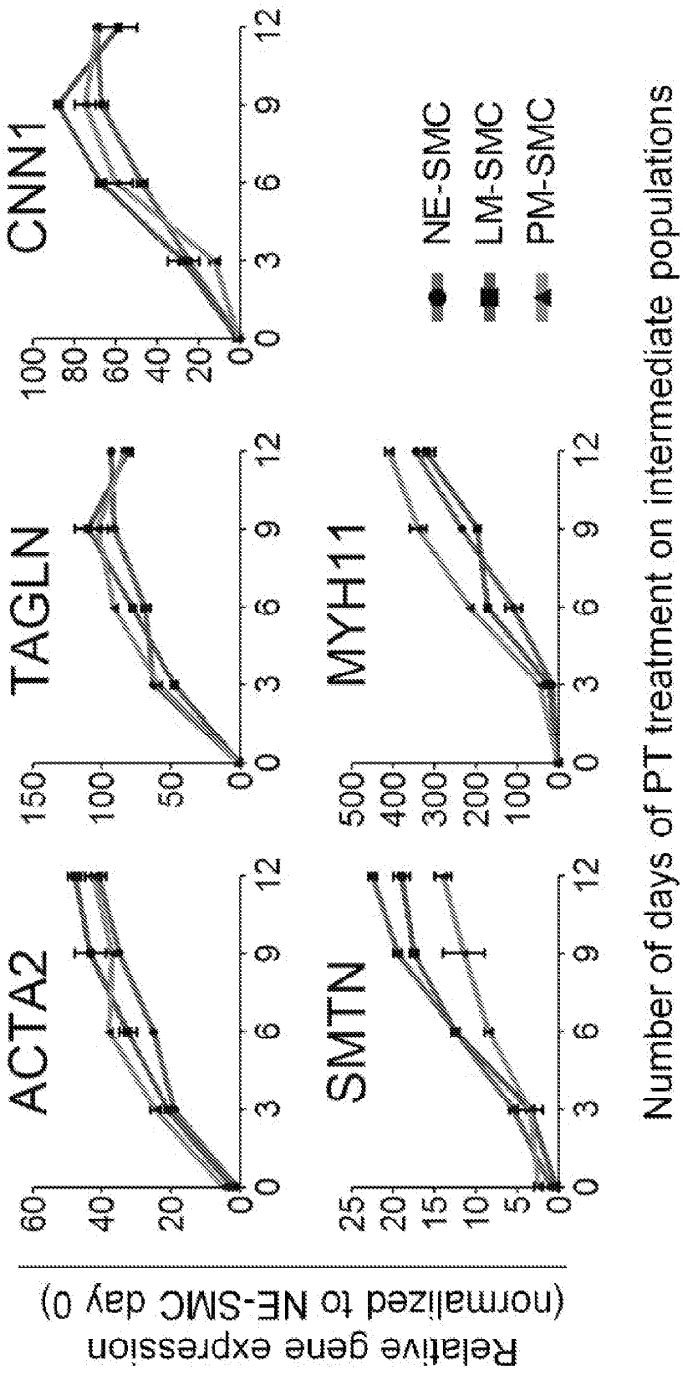

ACTA2
TAGLN
CNN1
SMTN
MYH11
NE-SMC
LM-SMC
PM-SMC
Relative gene expression
(normalized to NE-SMC day 0)
Number of days of PT treatment on intermediate populations

Figure 8

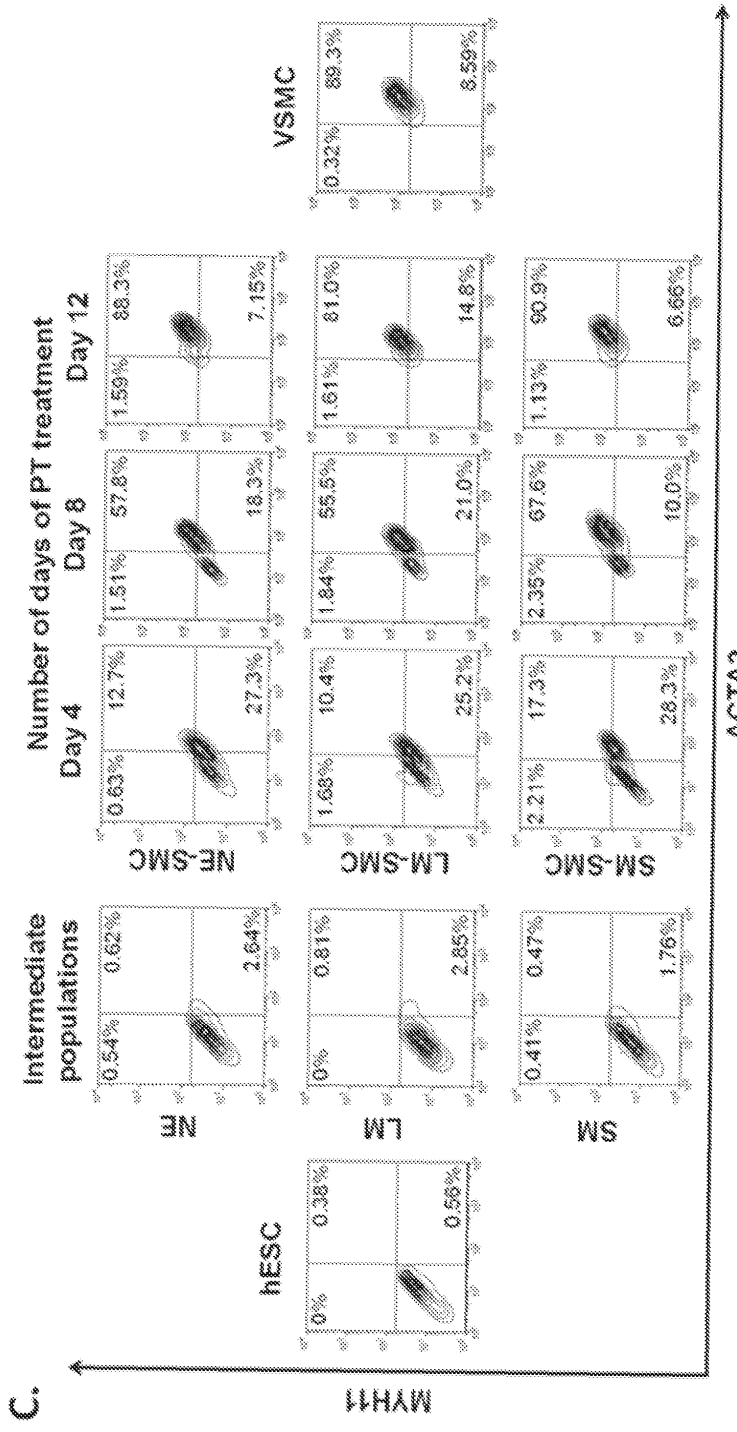

C.
MYH11
ACTA2
hESC
Intermediate populations
NE
LM
SM
Number of days of PT treatment
Day 4
Day 8
Day 12
NE-SMC
LM-SMC
SM-SMC
VSMC
0%
0.38%
0.66%
0.54%
0.62%
2.64%
0%
0.81%
2.85%
0.41%
0.47%
1.76%
0.63%
12.7%
27.3%
1.51%
57.8%
18.3%
1.59%
88.3%
7.15%
1.68%
10.4%
25.2%
1.84%
55.5%
21.0%
1.61%
81.0%
14.6%
2.21%
17.3%
28.3%
2.35%
67.6%
10.0%
1.13%
90.9%
6.66%
0.32%
89.3%
8.59%

Figure 9

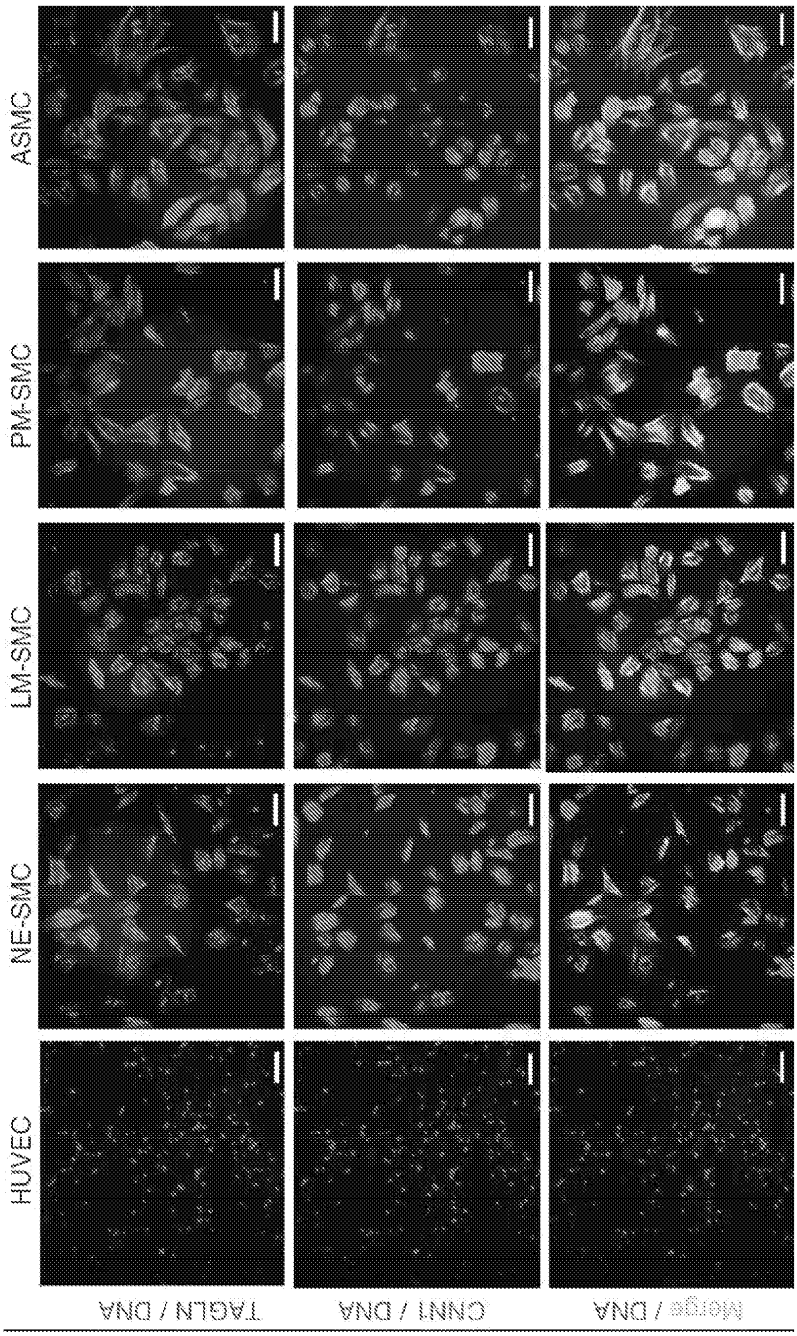


Figure 10

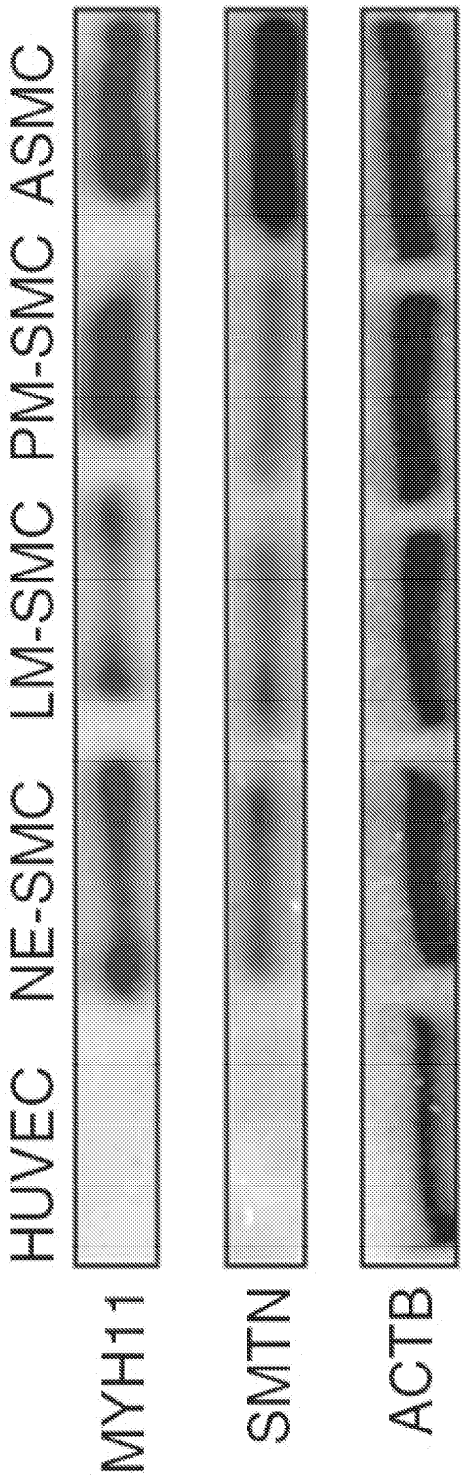
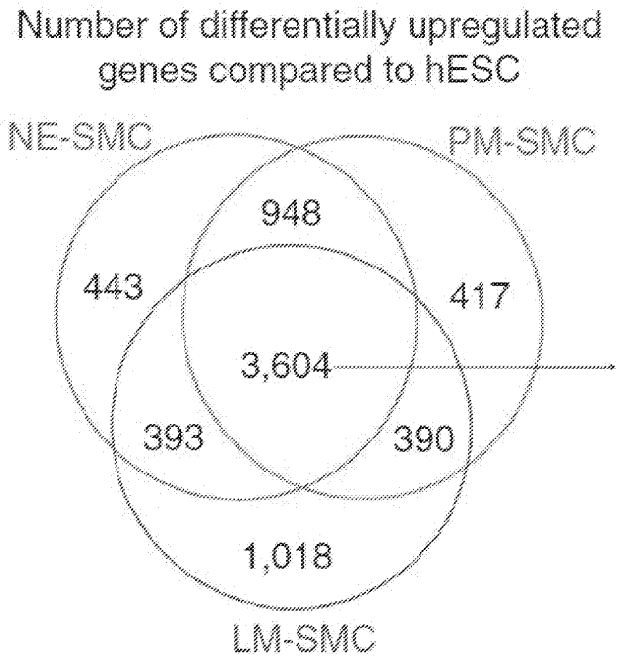


Figure 11



Top 20 enriched functional classification	Number of genes	*P* value
Endoplasmic reticulum parts	282	3.8E-30
Golgi apparatus and vesicle coating	248	3.2E-24
Protein transport	208	1.9E-20
Lysosome	67	4.7E-09
Identical protein binding	145	2.6E-08
Blood vessel development and morphogenesis	68	1.8E-07
Glycosylation	62	1.3E-08
Cell death	150	5.2E-06
Actin cytoskeleton binding and organization	113	1.8E-06
Extracellular matrix and receptor interaction	82	4.8E-05
Regulation of protein kinase cascade	72	4.7E-04
Cell fraction	213	7.8E-05
GTPase mediated signal transduction	82	4.1E-08
Calcium ion binding site	21	2.8E-05
Cell migration	102	6.1E-05
Cell-cell and cell-substrate adhesion	131	2.4E-04
PDGFR signaling pathway	9	3.5E-04
Regulation of cell size	51	3.7E-05
Collagen fibril organization	13	4.5E-04
Cellular response to nutrient levels	17	4.1E-05

Figure 12

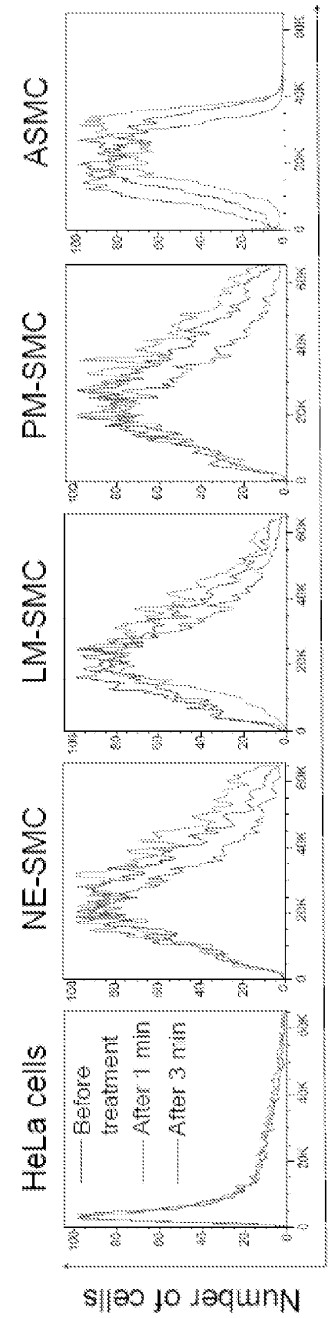

HeLa cells
NE-SMC
LM-SMC
PM-SMC
ASMC
Before treatment
After 1 min
After 3 min
Number of cells
Fluo-4 intensity

Figure 13

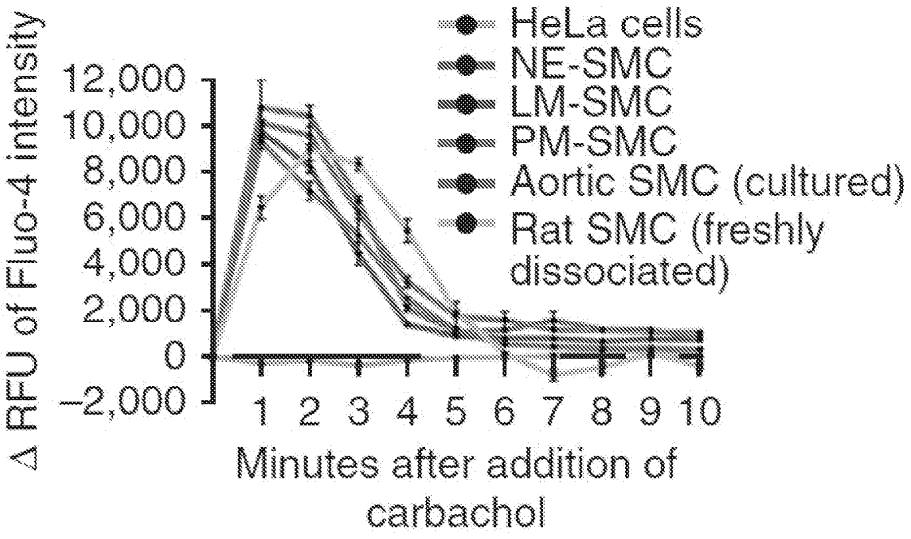


Figure 14

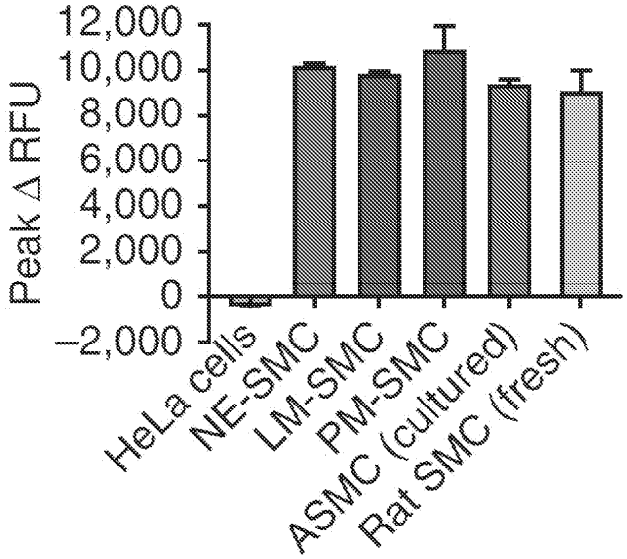


Figure 15

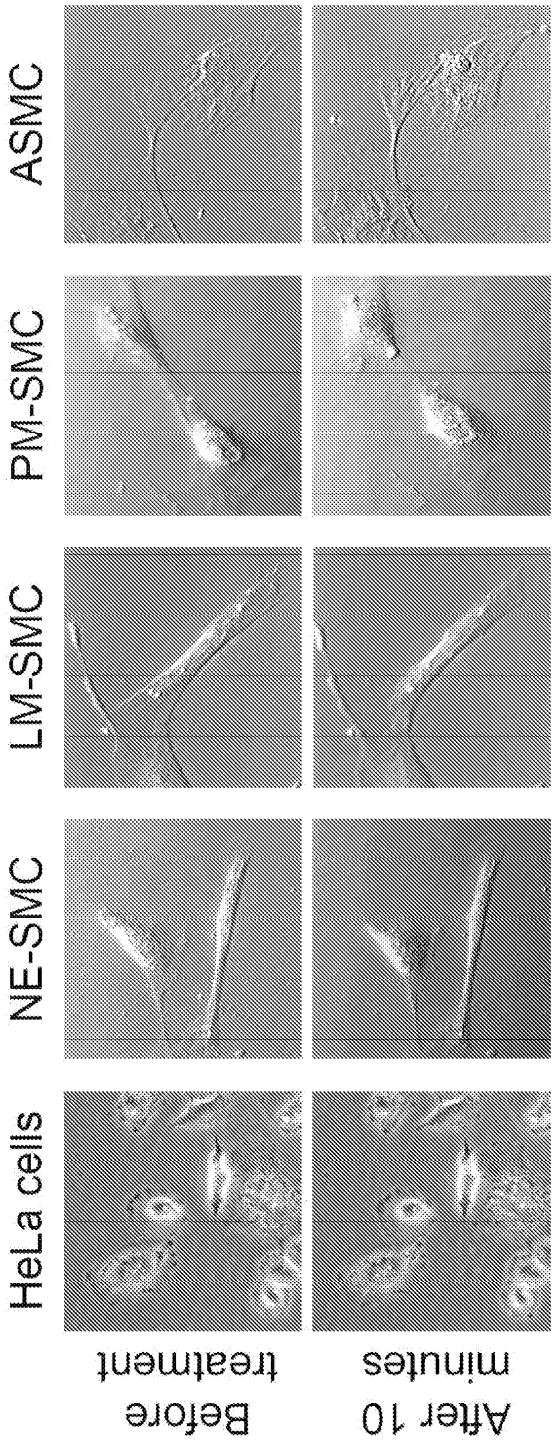

HeLa cells
NE-SMC
LM-SMC
PM-SMC
ASMC
Before treatment
After 10 minutes

Figure 16A

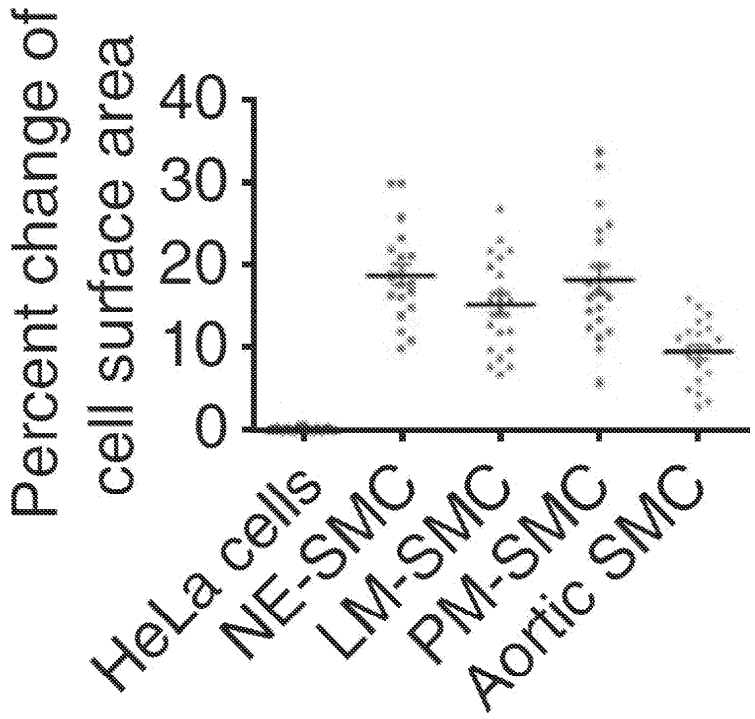

Percent change of cell surface area
40
30
20
10
0
HeLa cells
NE-SMC
LM-SMC
PM-SMC
Aortic SMC

Figure 16B

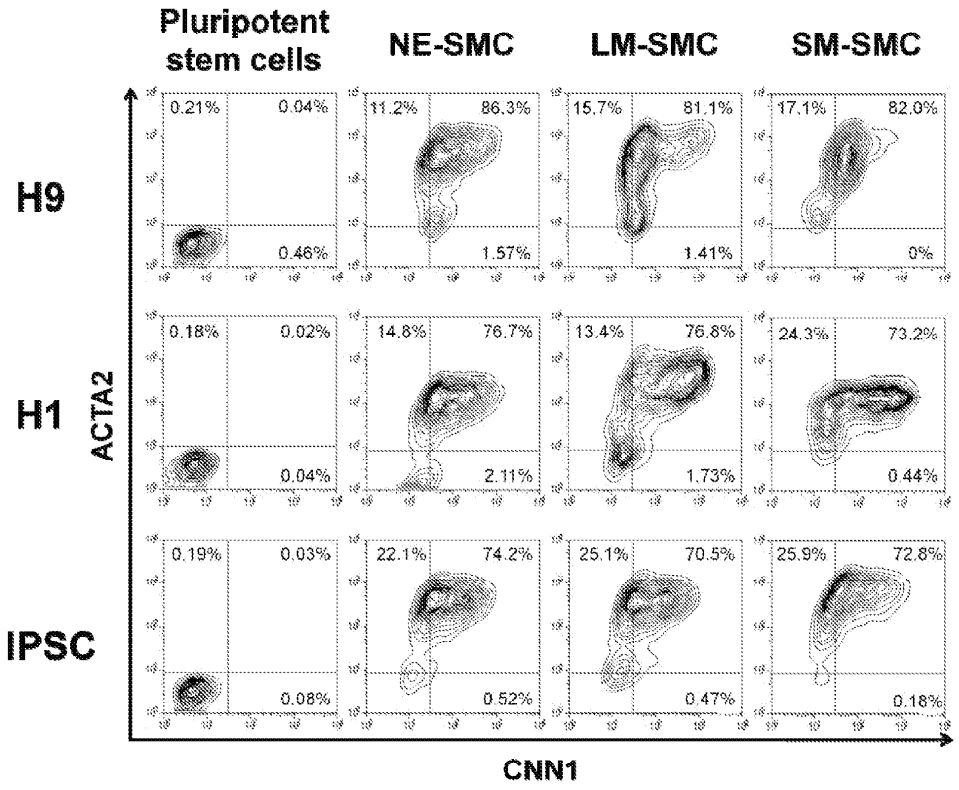

Pluripotent stem cells
NE-SMC
LM-SMC
SM-SMC
H9
H1
IPSC
ACTA2
CNN1
0.21%
0.04%
0.46%
11.2%
86.3%
1.57%
15.7%
81.1%
1.41%
17.1%
82.0%
0%
0.18%
0.02%
0.04%
14.8%
76.7%
2.11%
13.4%
76.8%
1.73%
24.3%
73.2%
0.44%
0.19%
0.03%
0.08%
22.1%
74.2%
0.52%
25.1%
70.5%
0.47%
25.9%
72.8%
0.18%

Figure 17

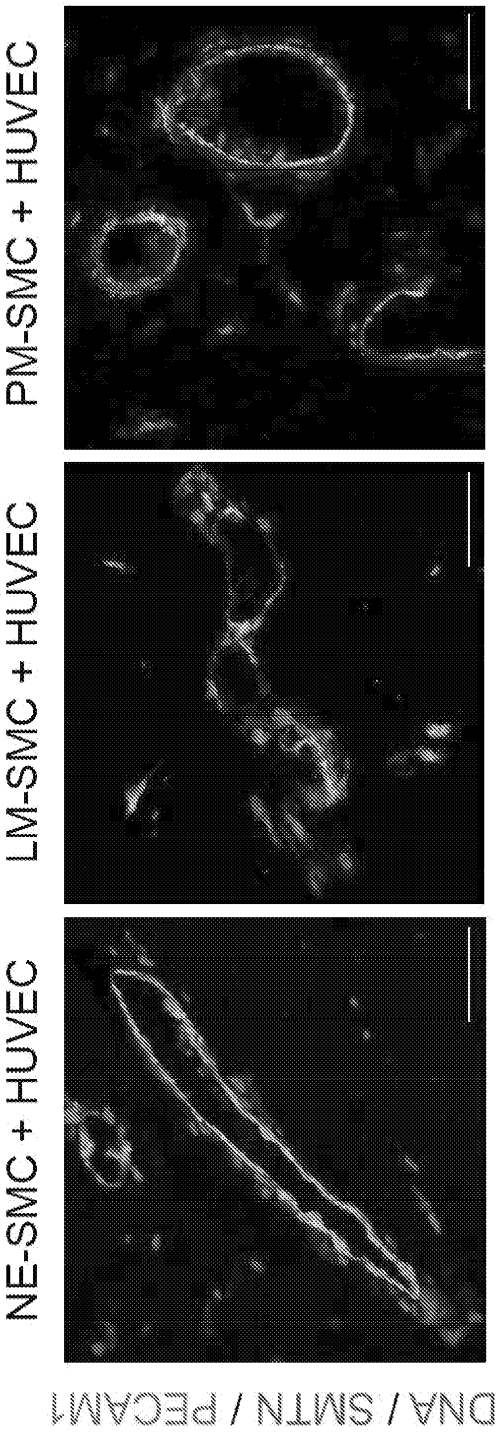


Figure 18

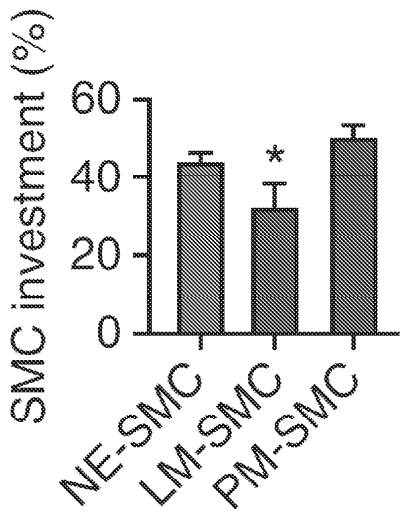


Figure 19

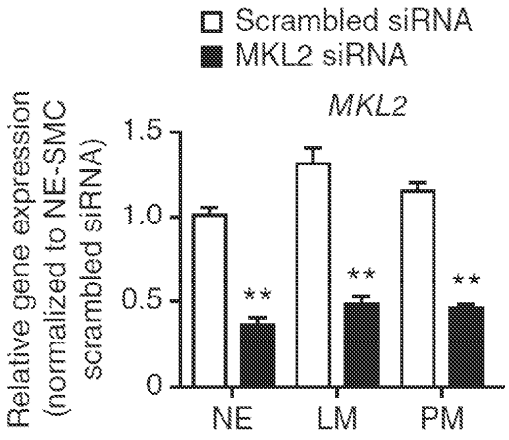


Figure 20

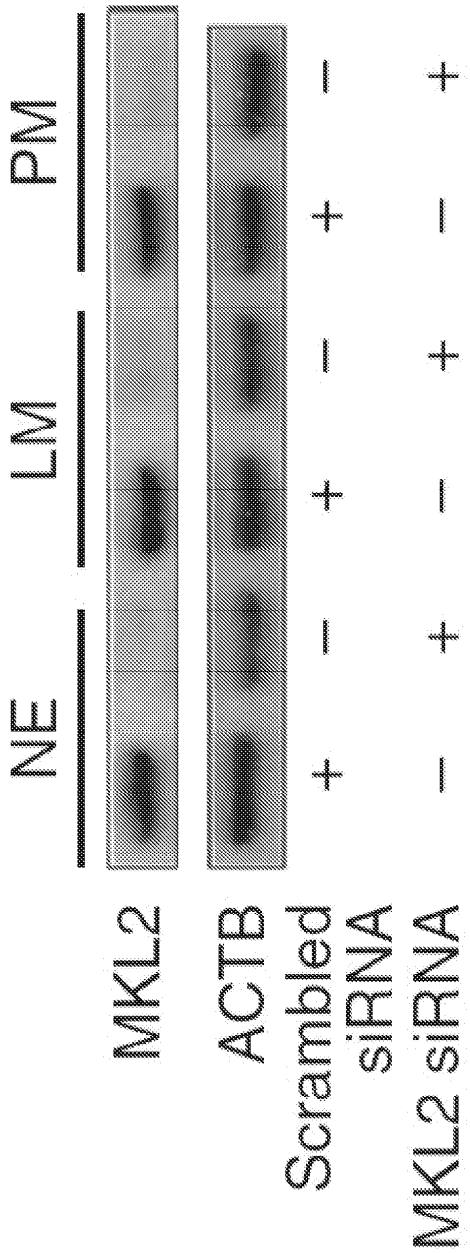


Figure 21

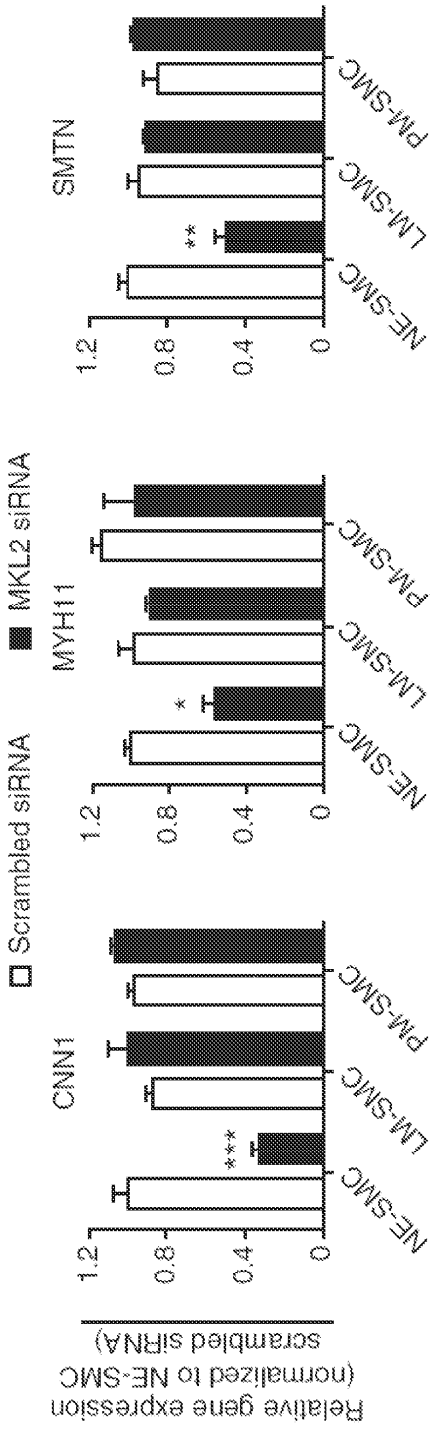

Scrambled siRNA
MKL2 siRNA
CNN1
MYH11
SMTN
Relative gene expression
(normalized to NE-SMC
scrambled siRNA)
1.2
0.8
0.4
0
***
*
**
NE-SMC
LM-SMC
PM-SMC

Figure 22

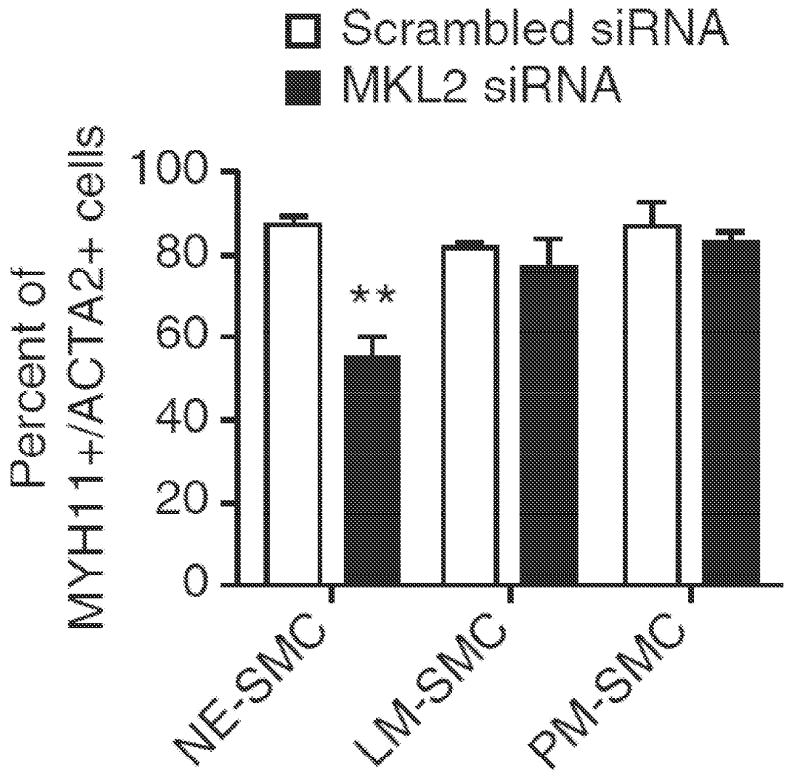

Scrambled siRNA
MKL2 siRNA
Percent of MYH11+/ACTA2+ cells
100
80
60
40
20
0
**
NE-SMC
LM-SMC
PM-SMC

Figure 23

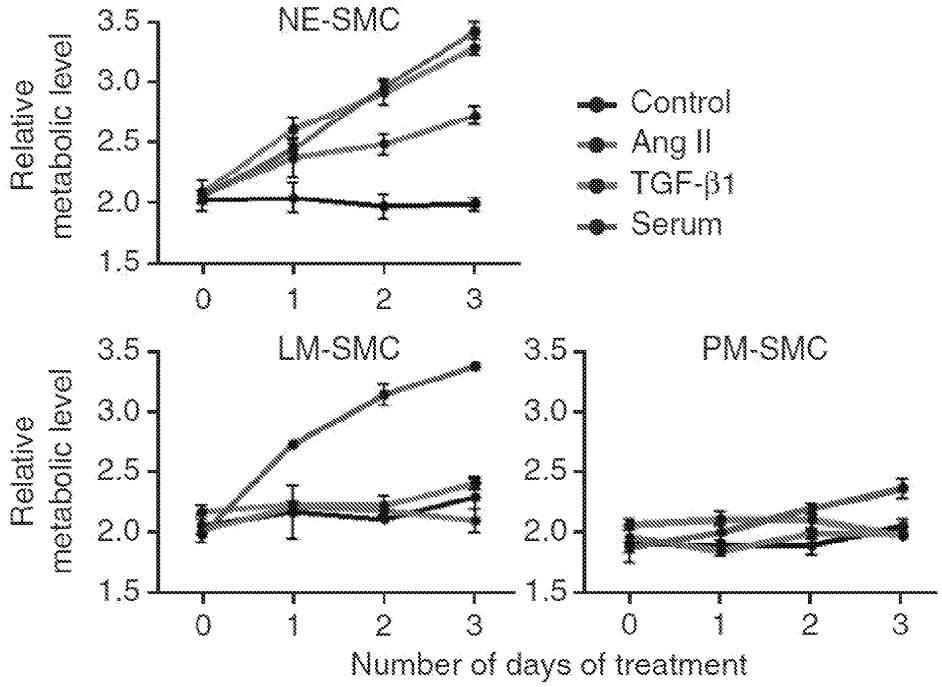

NE-SMC
LM-SMC
PM-SMC
Relative metabolic level
Number of days of treatment
Control
Ang II
TGF-β1
Serum
3.5
3.0
2.5
2.0
1.5
0
1
2
3

Figure 24

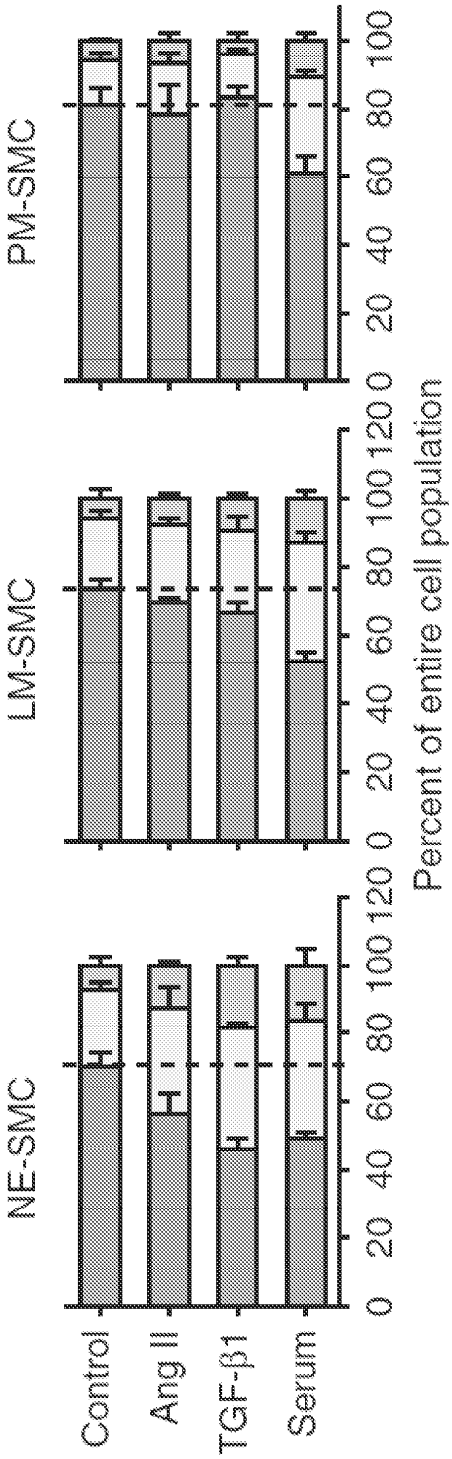

NE-SMC
LM-SMC
PM-SMC
Control
Ang II
TGF-β1
Serum
0 20 40 60 80 100 120
Percent of entire cell population

Figure 25A

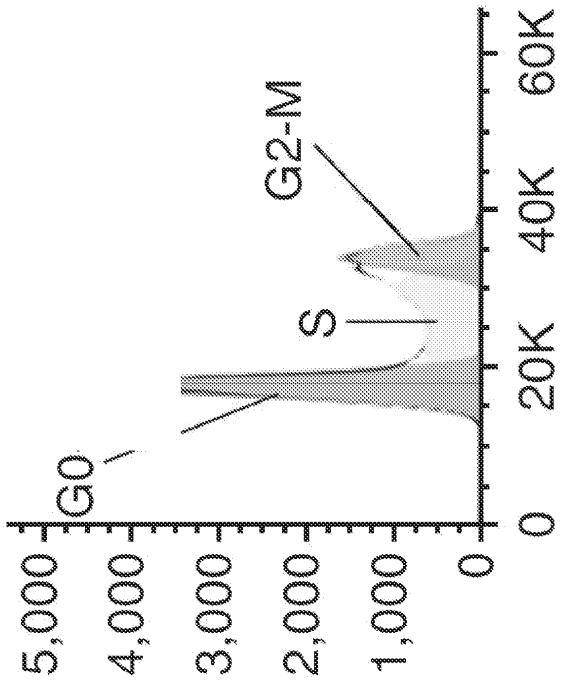


Figure 25B

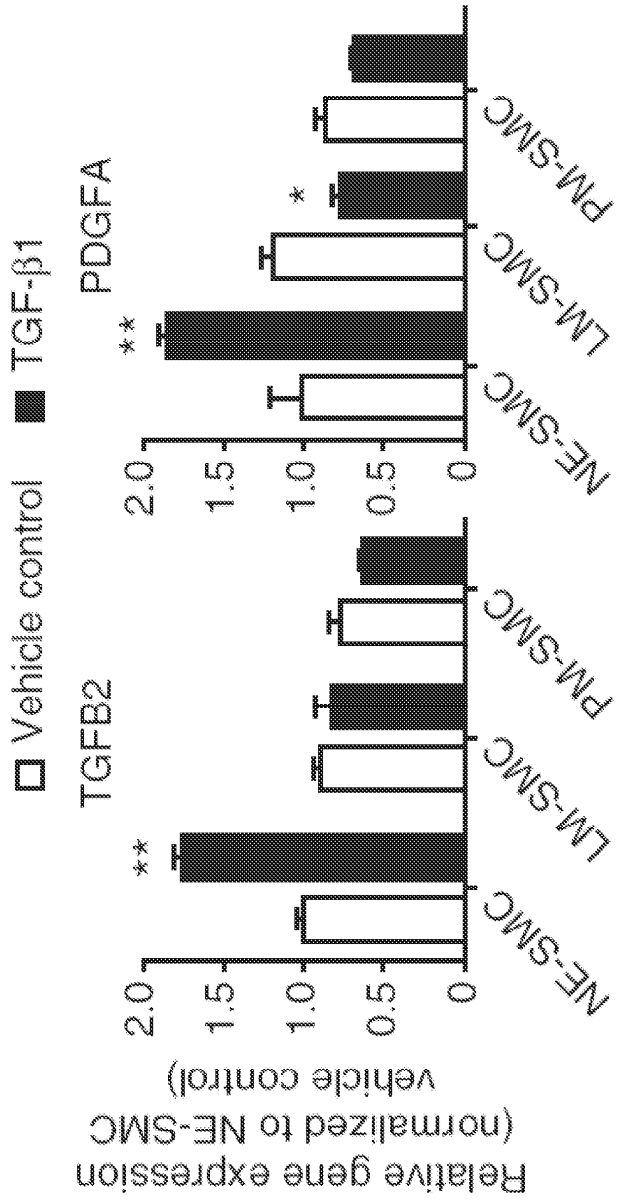

Vehicle control
TGF-β1
TGFB2
PDGFA
Relative gene expression
(normalized to NE-SMC
vehicle control)
2.0
1.5
1.0
0.5
0
**
*
NE-SMC
LM-SMC
PM-SMC

Figure 26

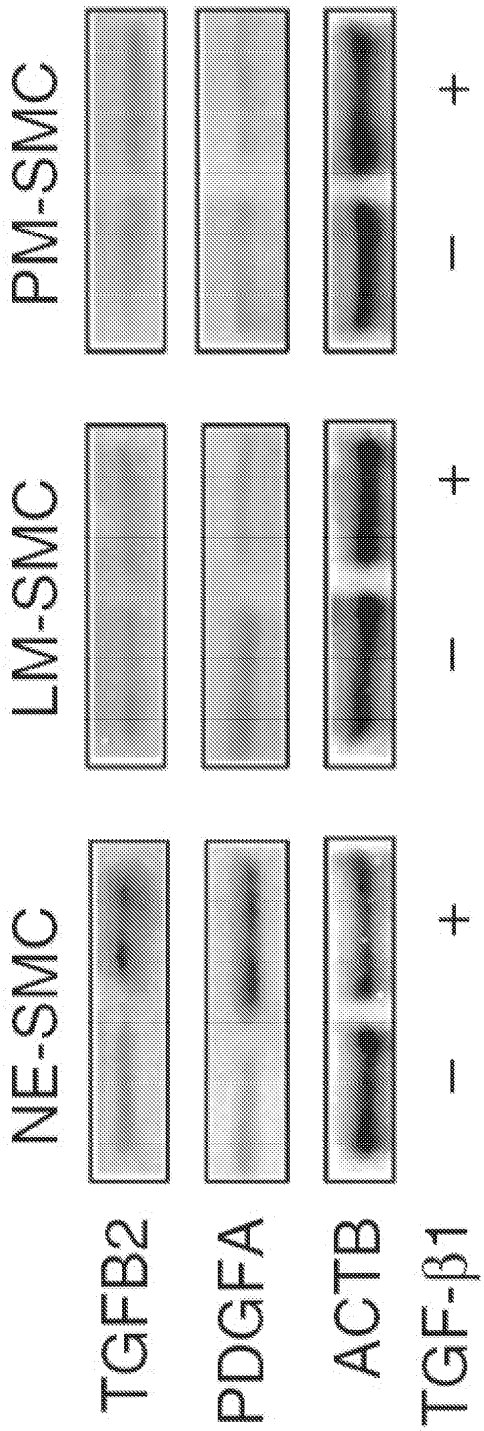


Figure 27

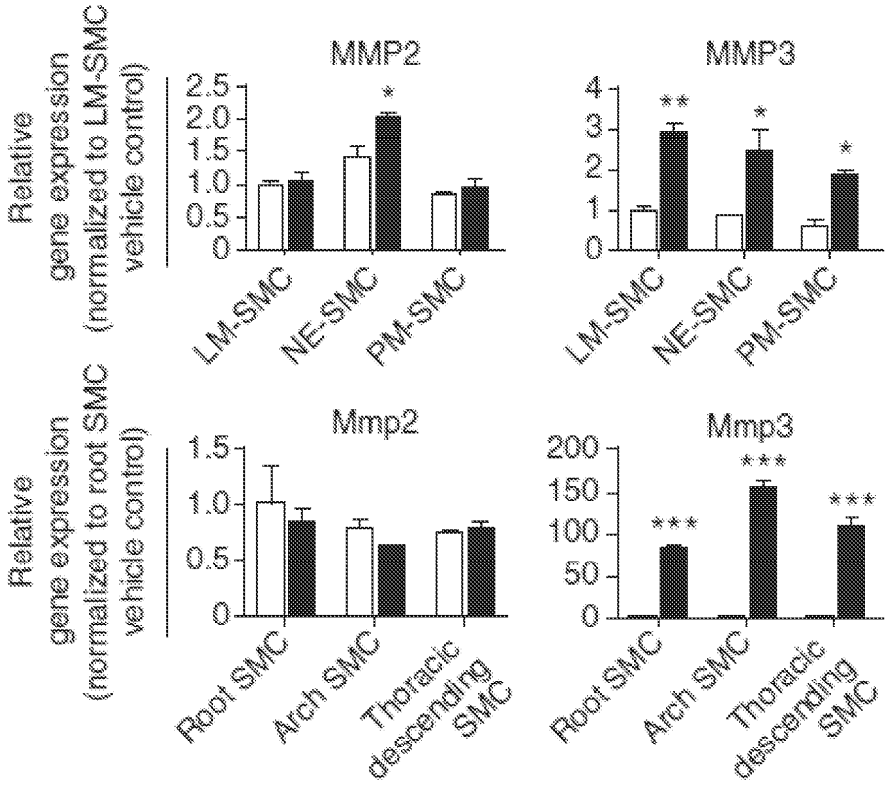

Relative gene expression (normalized to LM-SMC vehicle control)
MMP2
MMP3
LM-SMC
NE-SMC
PM-SMC
Relative gene expression (normalized to root SMC vehicle control)
Mmp2
Mmp3
Root SMC
Arch SMC
Thoracic descending SMC

Figure 28A

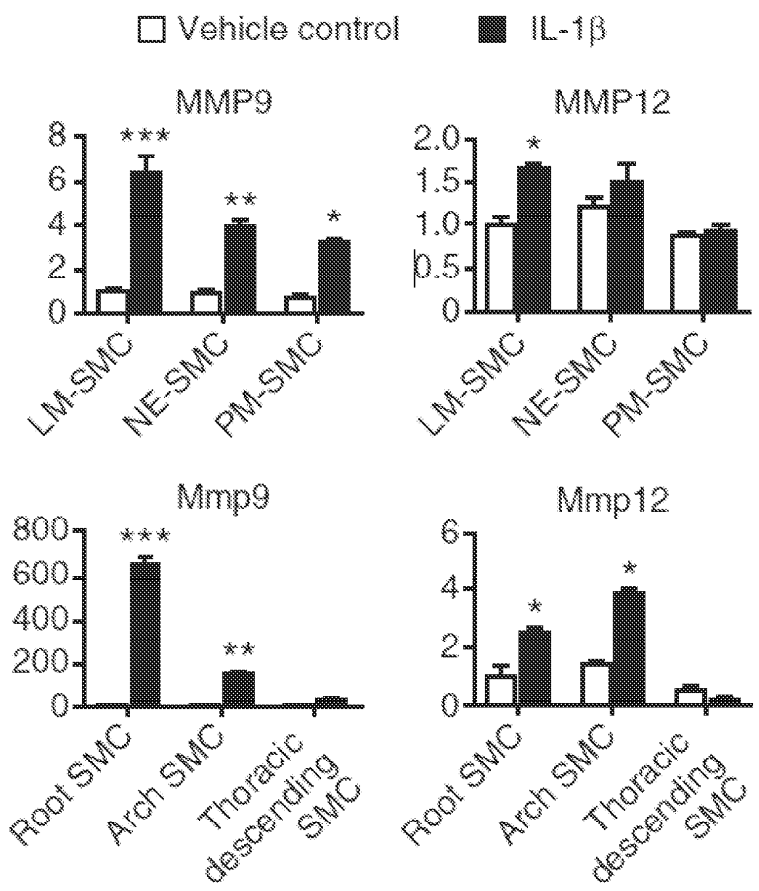

Vehicle control
IL-1β
MMP9
MMP12
Mmp9
Mmp12
LM-SMC
NE-SMC
PM-SMC
Root SMC
Arch SMC
Thoracic descending SMC

Figure 28B

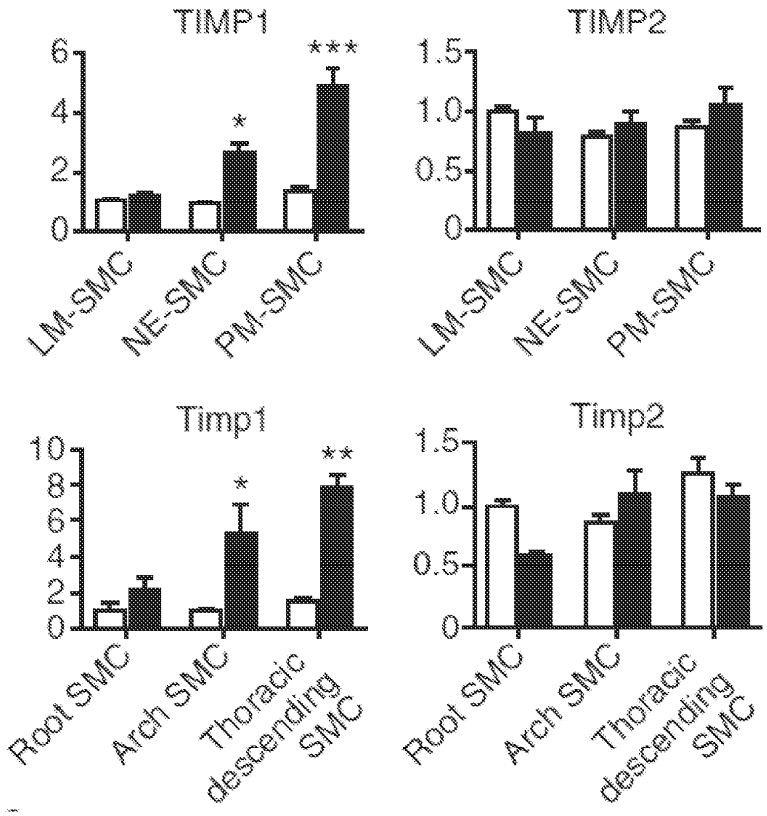

TIMP1
6
4
2
0
*
***
LM-SMC
NE-SMC
PM-SMC
TIMP2
1.5
1.0
0.5
0
LM-SMC
NE-SMC
PM-SMC
Timp1
10
8
6
4
2
0
*
**
Root SMC
Arch SMC
Thoracic descending SMC
Timp2
1.5
1.0
0.5
0
Root SMC
Arch SMC
Thoracic descending SMC

Figure 28C

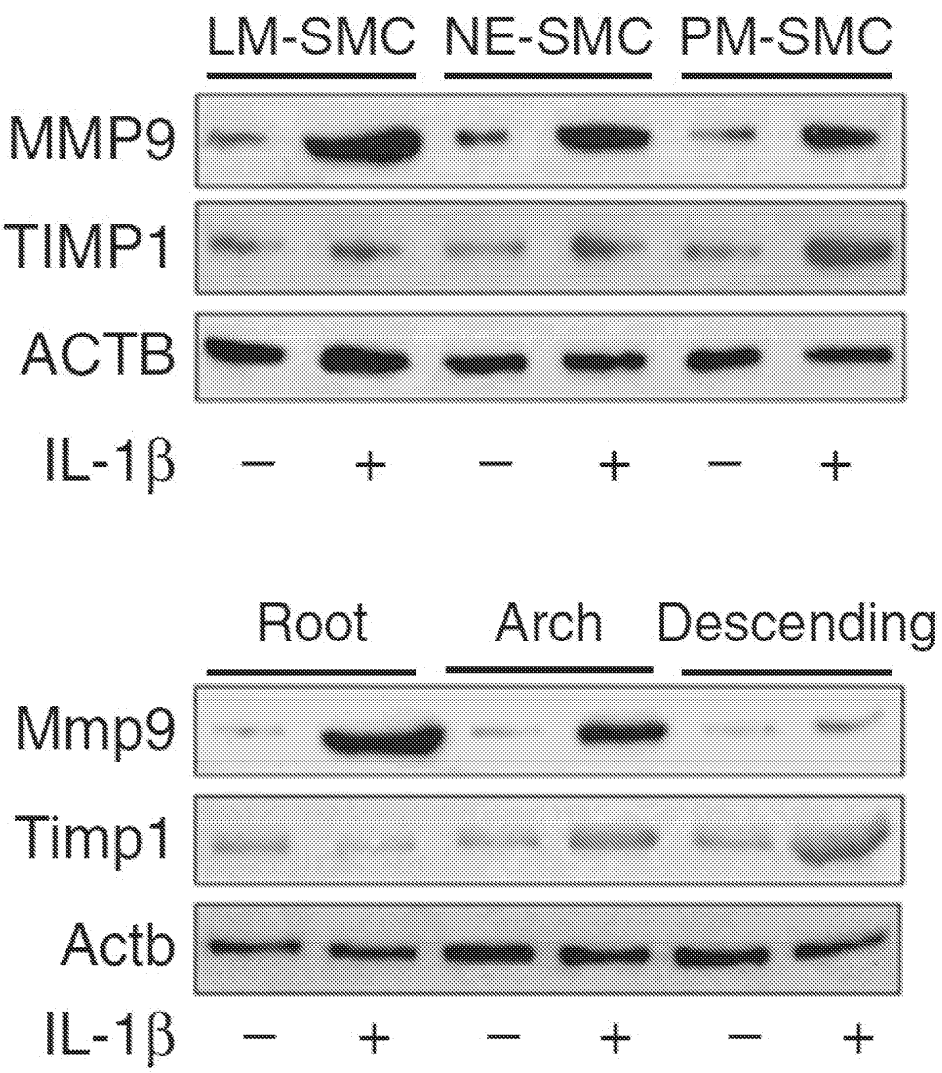

LM-SMC
NE-SMC
PM-SMC
MMP9
TIMP1
ACTB
IL-1β
– + – + – +
Root
Arch
Descending
Mmp9
Timp1
Actb
IL-1β
– + – + – +

Figure 29

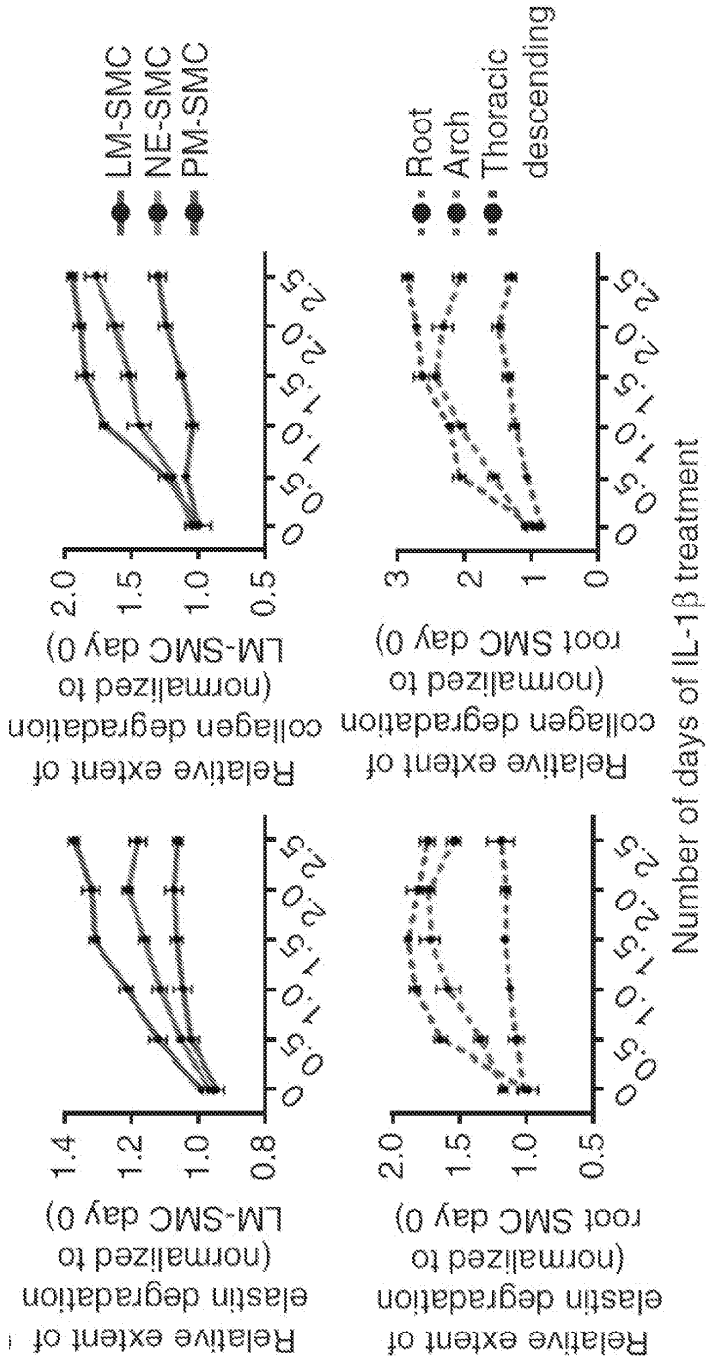

Relative extent of elastin degradation (normalized to LM-SMC day 0)
Relative extent of collagen degradation (normalized to LM-SMC day 0)
Relative extent of elastin degradation (normalized to root SMC day 0)
Relative extent of collagen degradation (normalized to root SMC day 0)
Number of days of IL-1β treatment
LM-SMC
NE-SMC
PM-SMC
Root
Arch
Thoracic descending

Figure 30

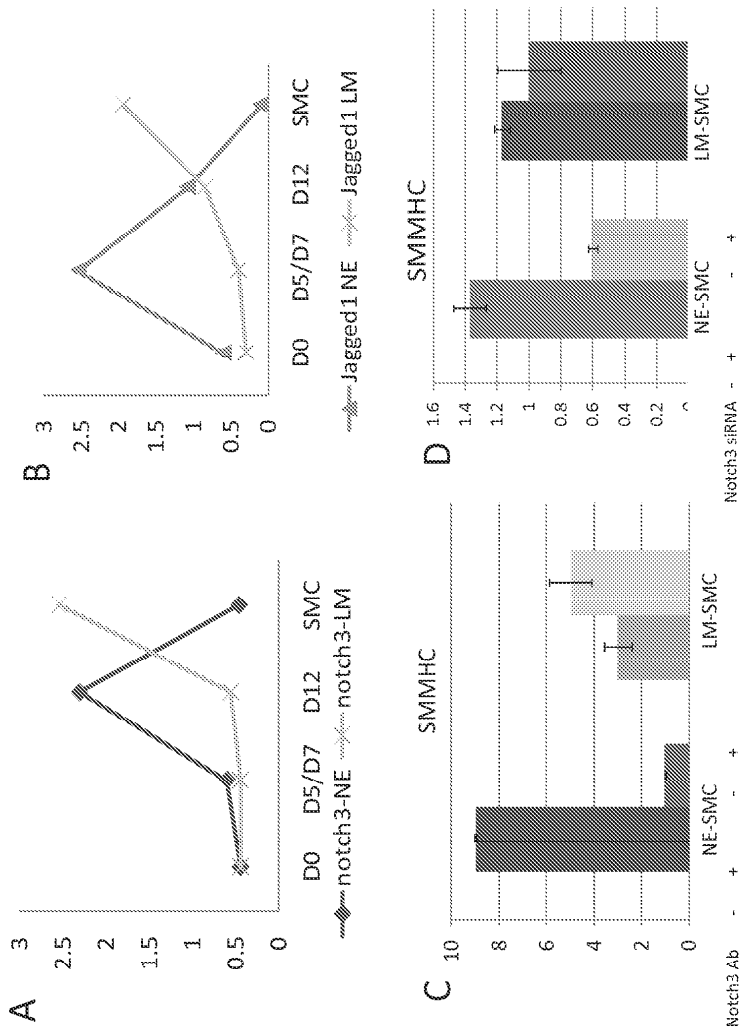

A
B
C
D
D0
D5/D7
D12
SMC
notch3-NE
notch3-LM
Jagged1 NE
Jagged1 LM
SMMHC
NE-SMC
LM-SMC
Notch3 Ab
Notch3 siRNA

Figure 31

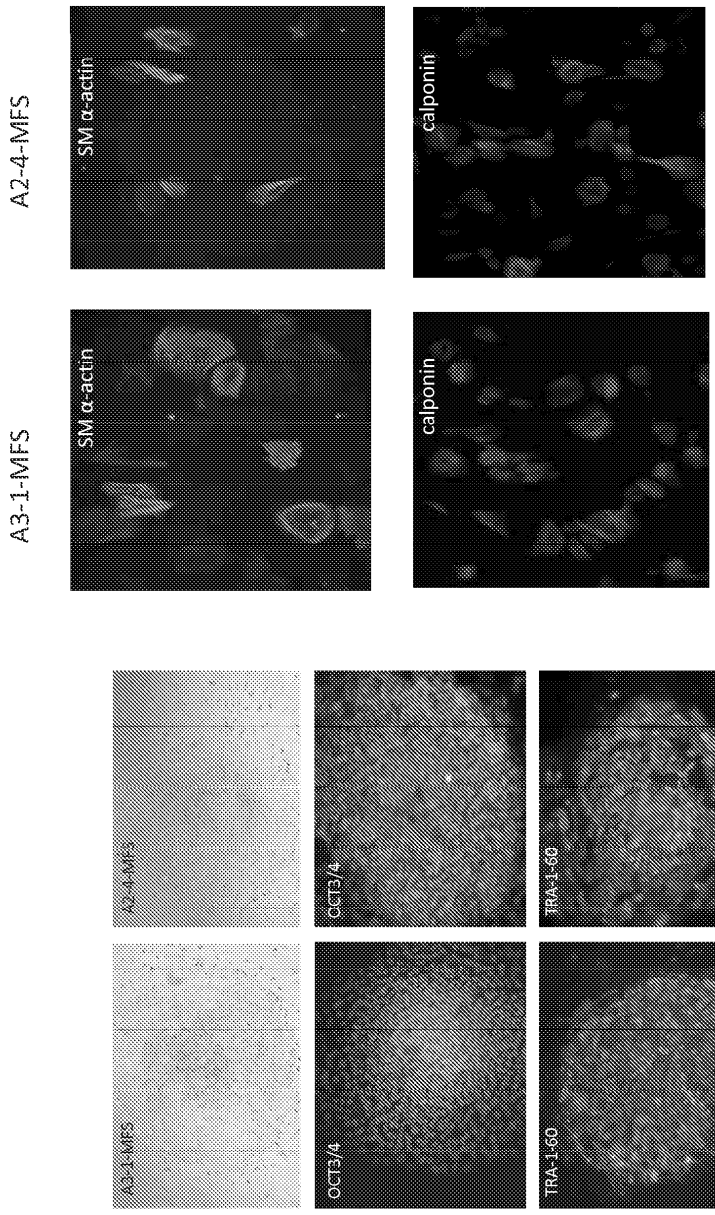

A2-4-MFS
SM α-actin
calponin
A3-1-MFS
SM α-actin
calponin
A3-1-MFS
OCT3/4
TRA-1-60
A3-1-MFS
OCT3/4
TRA-1-60

Figure 32

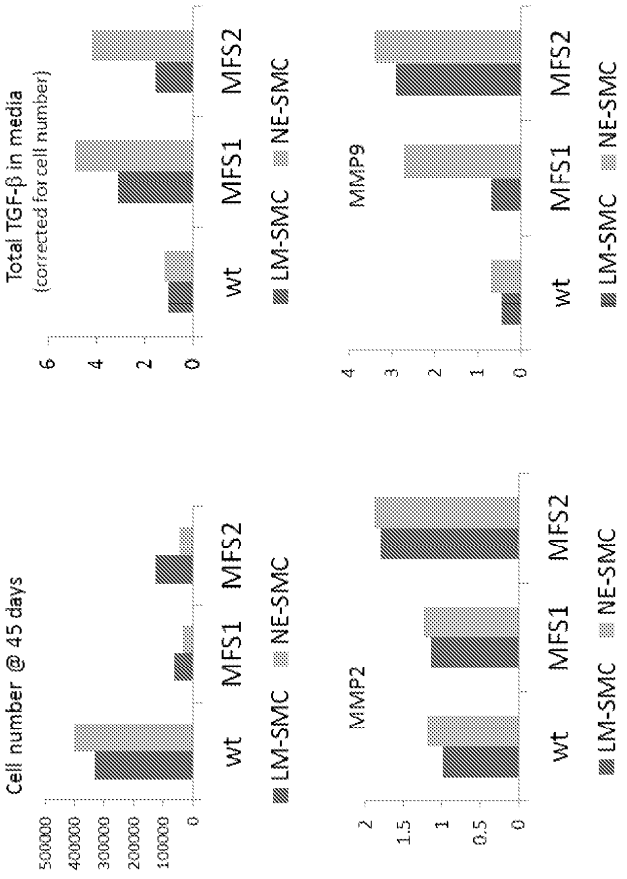

Cell number @ 45 days
500000
400000
300000
200000
100000
0
wt
MFS1
MFS2
LM-SMC
NE-SMC
Total TGF-β in media
(corrected for cell number)
6
4
2
0
wt
MFS1
MFS2
LM-SMC
NE-SMC
MMP2
2
1.5
1
0.5
0
wt
MFS1
MFS2
LM-SMC
NE-SMC
MMP9
4
3
2
1
0
wt
MFS1
MFS2
LM-SMC
NE-SMC

Figure 33

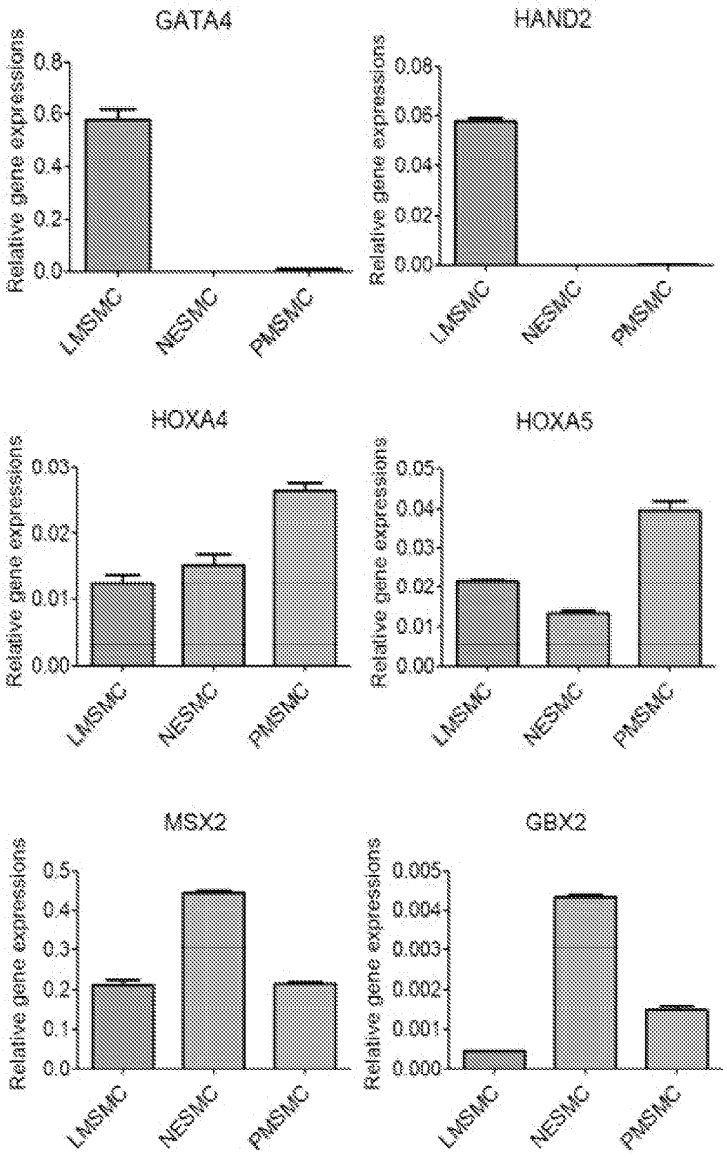

GATA4
HAND2
HOXA4
HOXA5
MSX2
GBX2
Relative gene expressions
LMSMC
NESMC
PMSMC

Figure 34

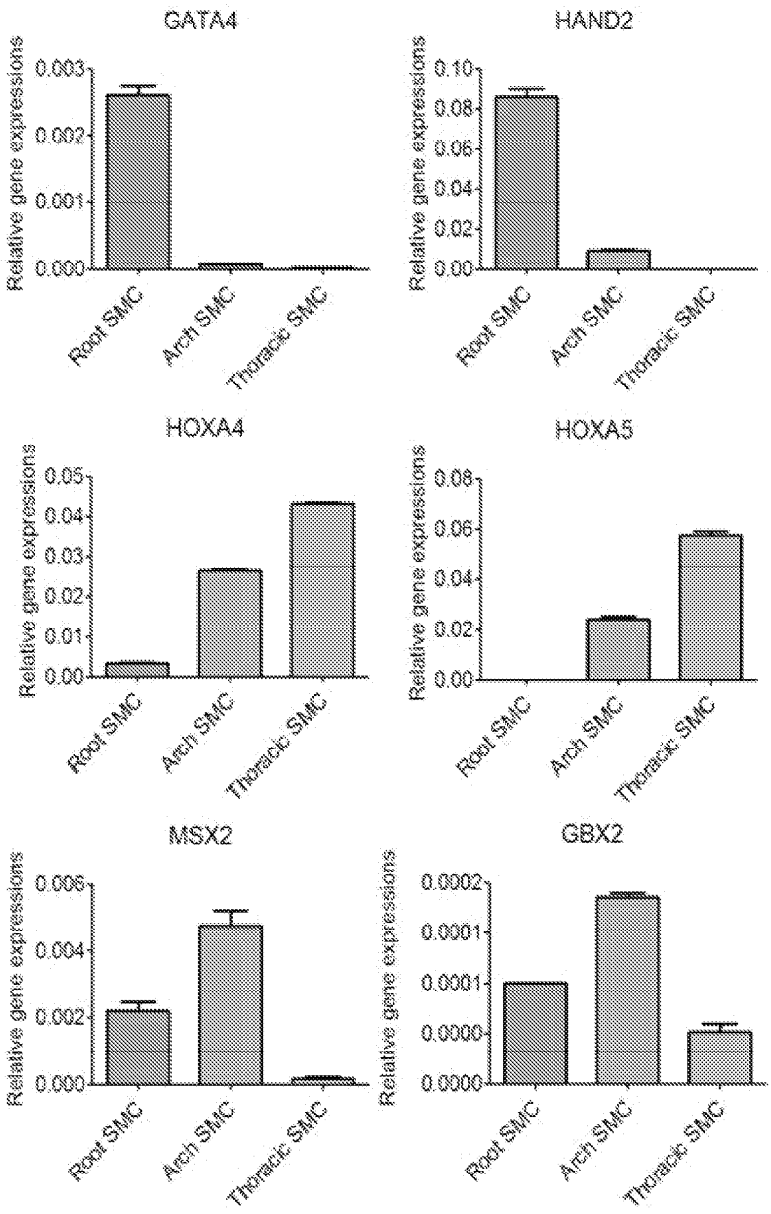

GATA4
Relative gene expressions
0.003
0.002
0.001
0.000
Root SMC
Arch SMC
Thoracic SMC
HAND2
Relative gene expressions
0.10
0.08
0.06
0.04
0.02
0.00
Root SMC
Arch SMC
Thoracic SMC
HOXA4
Relative gene expressions
0.05
0.04
0.03
0.02
0.01
0.00
Root SMC
Arch SMC
Thoracic SMC
HOXA5
Relative gene expressions
0.08
0.06
0.04
0.02
0.00
Root SMC
Arch SMC
Thoracic SMC
MSX2
Relative gene expressions
0.006
0.004
0.002
0.000
Root SMC
Arch SMC
Thoracic SMC
GBX2
Relative gene expressions
0.0002
0.0001
0.0001
0.0000
0.0000
Root SMC
Arch SMC
Thoracic SMC

Figure 35