Figure 1

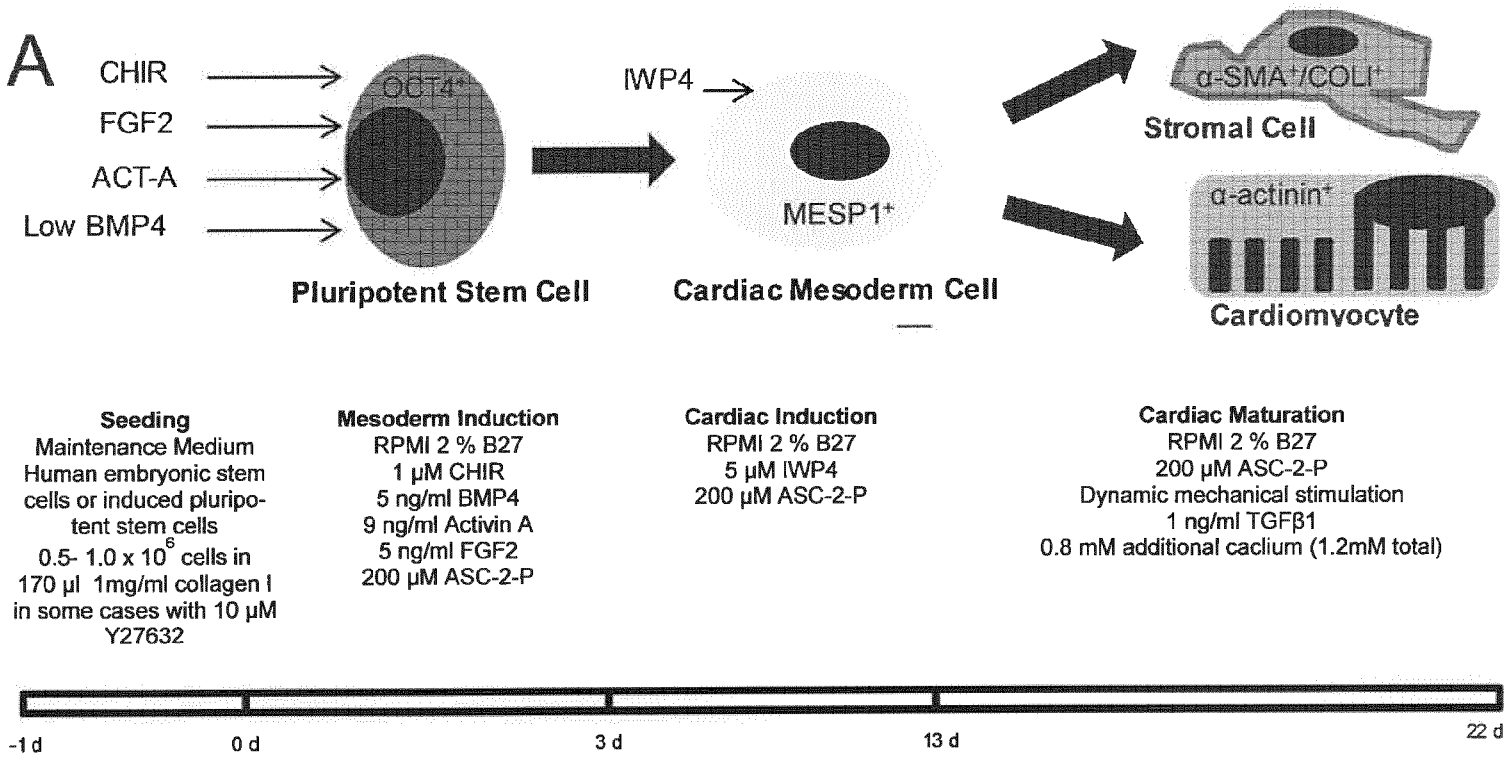

A
CHIR
FGF2
ACT-A
Low BMP4
OCT4+
Pluripotent Stem Cell
IWP4
MESP1+
Cardiac Mesoderm Cell
α-SMA+/COLI+
Stromal Cell
α-actinin+
Cardiomyocyte
Seeding
Maintenance Medium
Human embryonic stem cells or induced pluripotent stem cells
0.5- 1.0 x 10^6 cells in 170 µl 1mg/ml collagen I in some cases with 10 µM Y27632
Mesoderm Induction
RPMI 2 % B27
1 µM CHIR
5 ng/ml BMP4
9 ng/ml Activin A
5 ng/ml FGF2
200 µM ASC-2-P
Cardiac Induction
RPMI 2 % B27
5 µM IWP4
200 µM ASC-2-P
Cardiac Maturation
RPMI 2 % B27
200 µM ASC-2-P
Dynamic mechanical stimulation
1 ng/ml TGFβ1
0.8 mM additional caclium (1.2mM total)
-1 d
0 d
3 d
13 d
22 d

Figure 1 (continued)

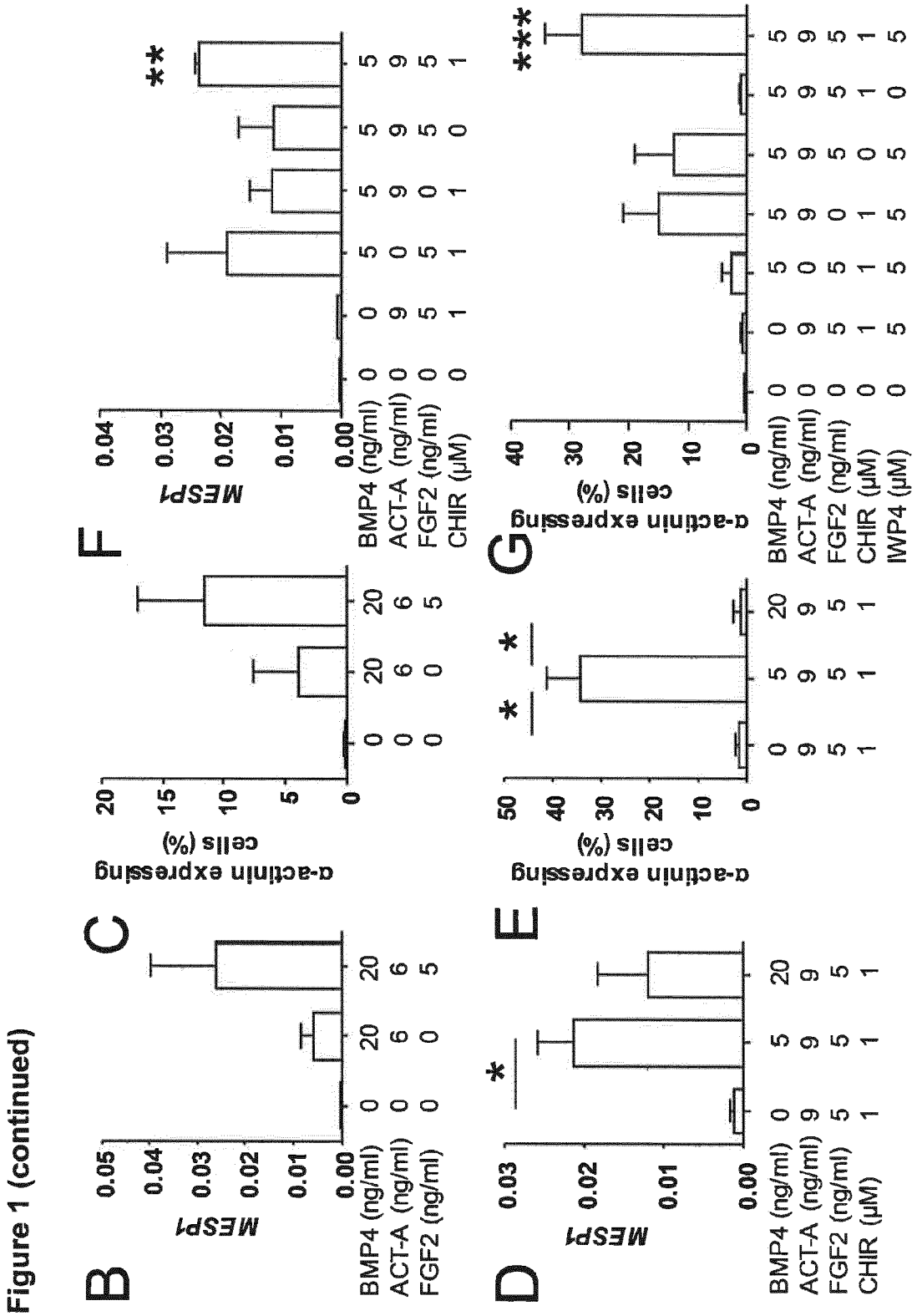
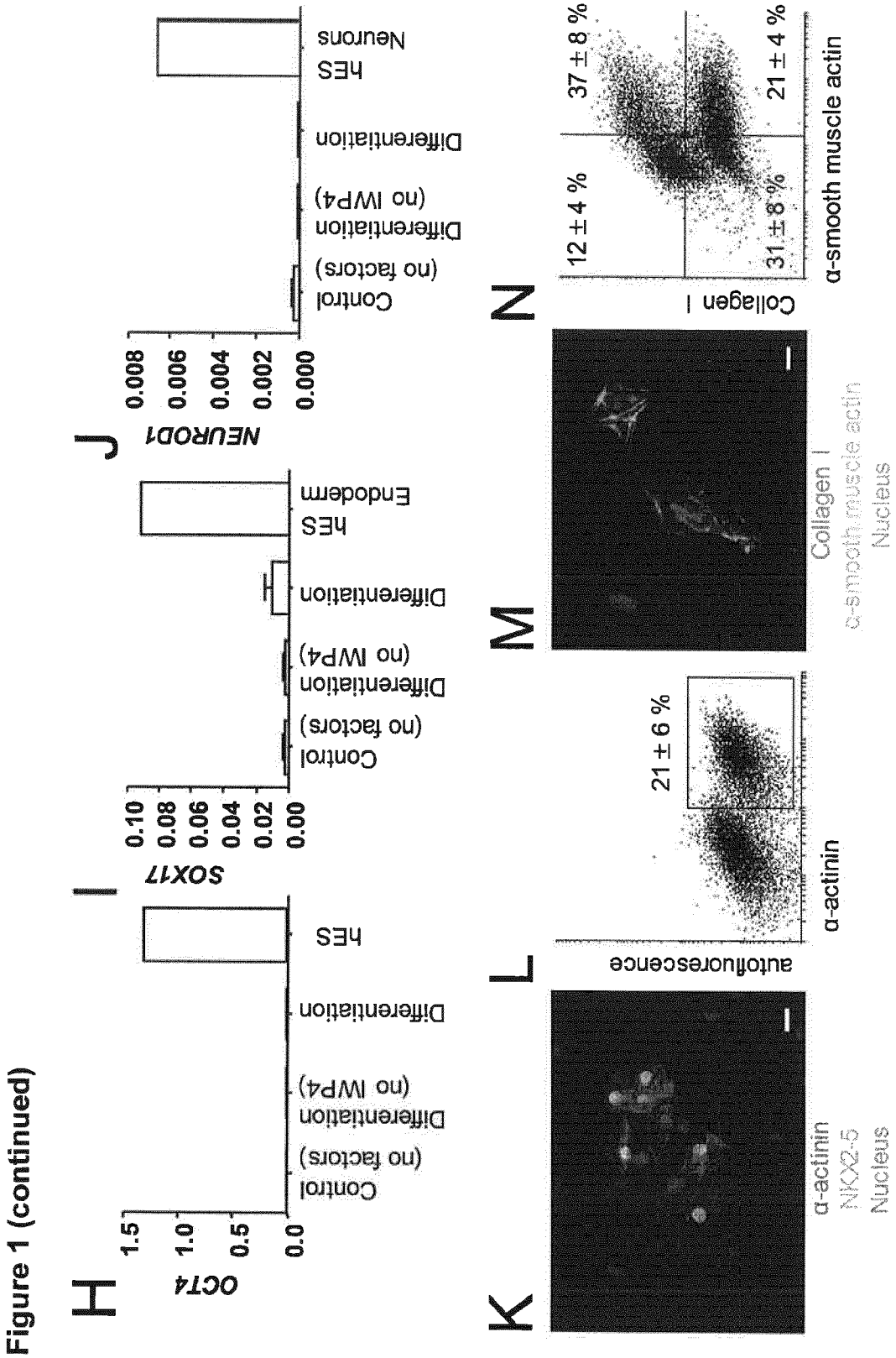


Figure 1 (continued)



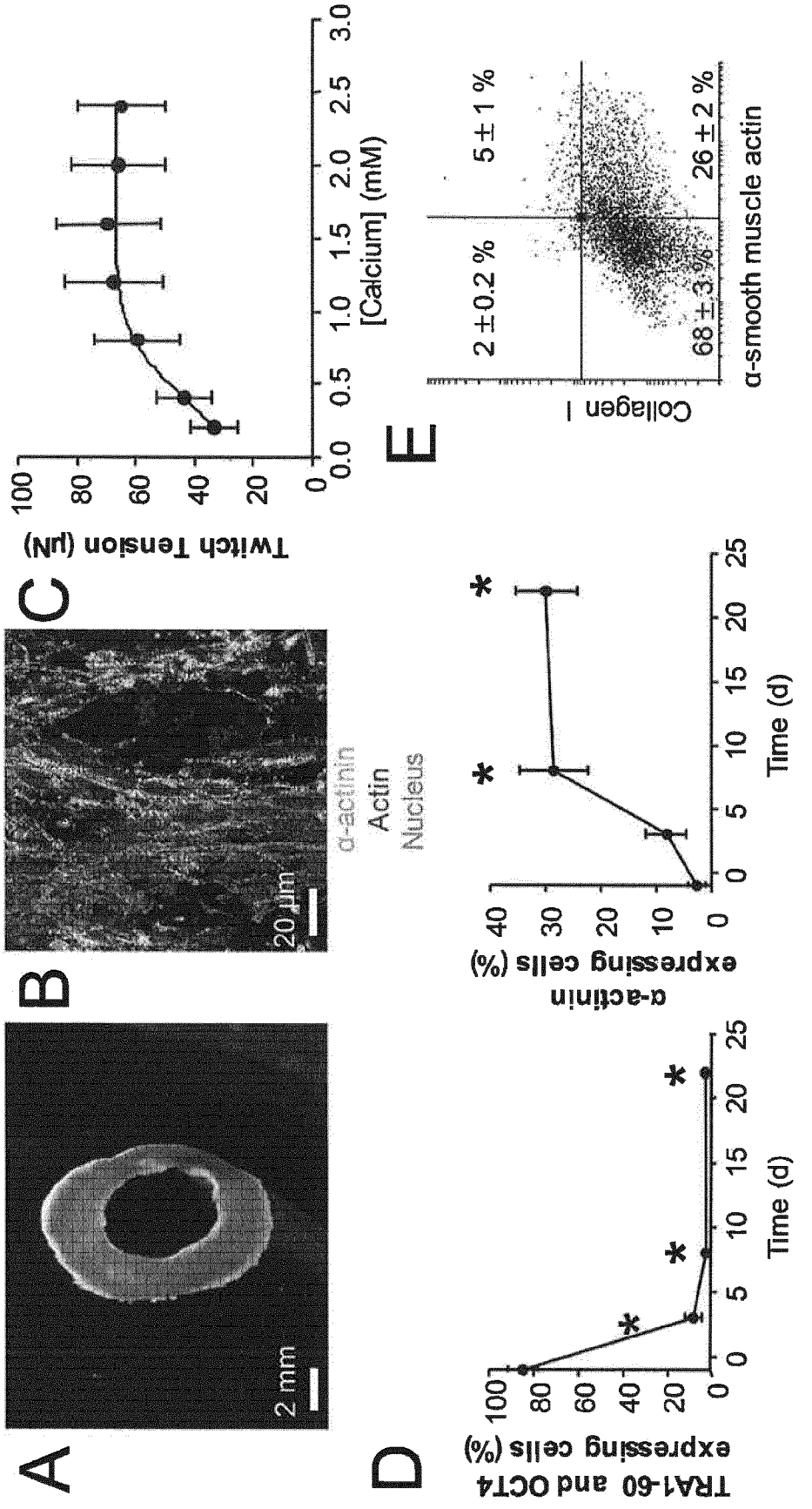

A
2 mm
B
20 µm
α-actinin
Actin
Nucleus
C
Twitch Tension (µN)
[Calcium] (mM)
D
TRA1-60 and OCT4 expressing cells (%)
Time (d)
α-actinin expressing cells (%)
Time (d)
E
Collagen I
α-smooth muscle actin
2 ± 0.2 %
5 ± 1 %
68 ± 3 %
26 ± 2 %

Figure 2

Figure 2 (Continued)

F

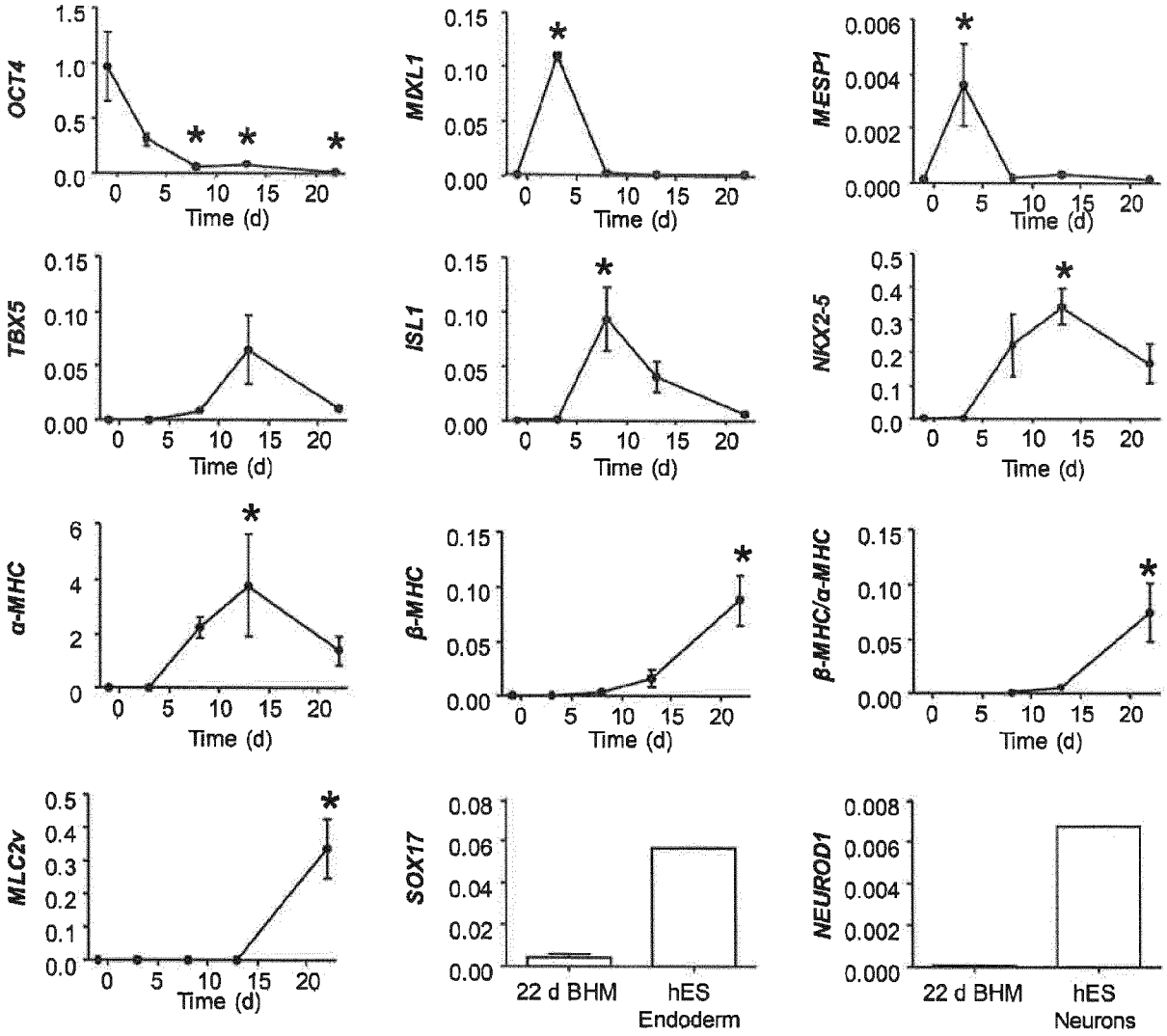


Figure 3

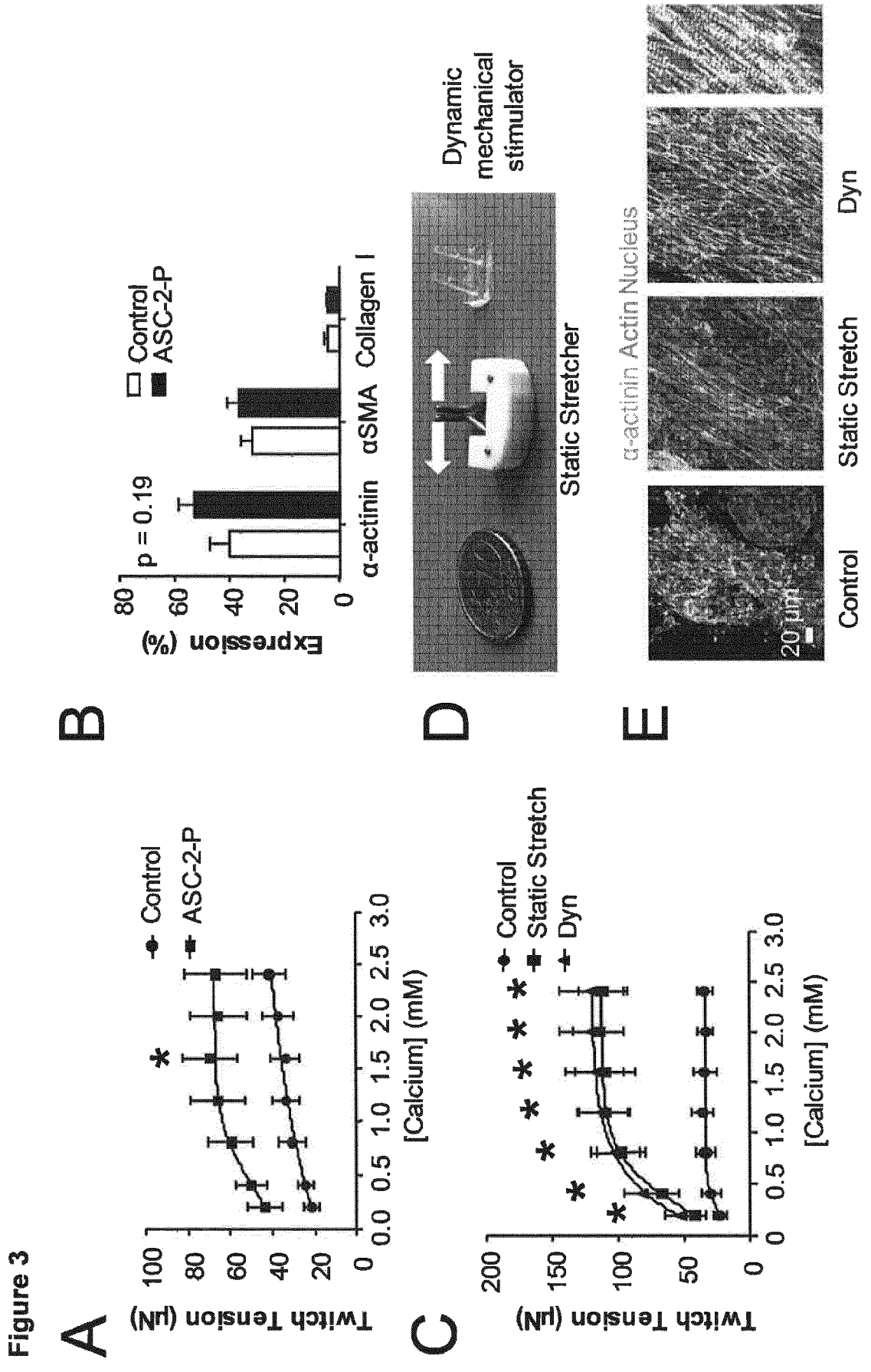

A
Twitch Tension (µN)
Control
ASC-2-P
[Calcium] (mM)
B
Expression (%)
Control
ASC-2-P
p = 0.19
α-actinin
αSMA
Collagen I
C
Twitch Tension (µN)
Control
Static Stretch
Dyn
[Calcium] (mM)
D
Dynamic mechanical stimulator
Static Stretcher
E
α-actinin Actin Nucleus
Control
Static Stretch
Dyn
20 µm

Figure 3
(Continued)

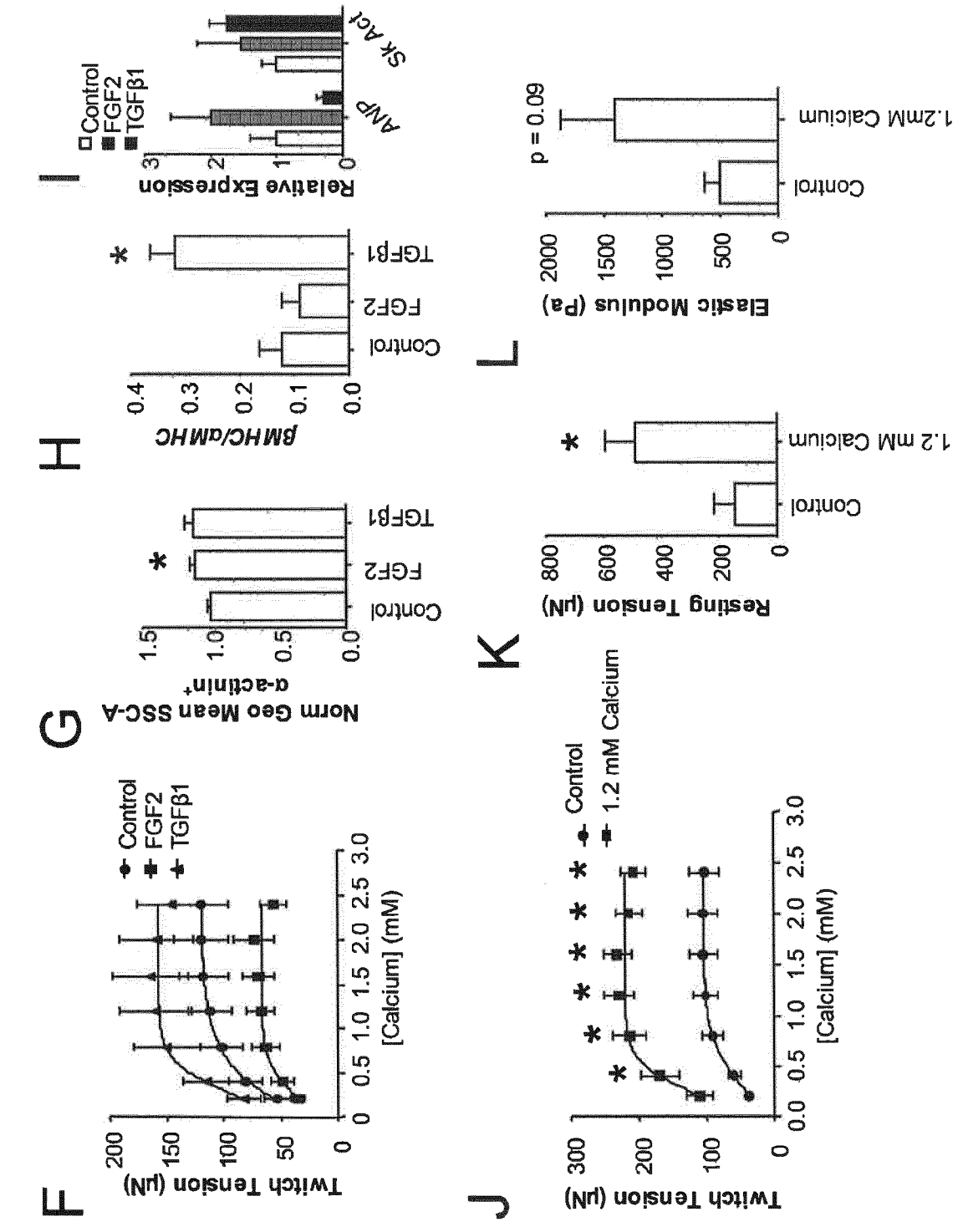


Figure 4

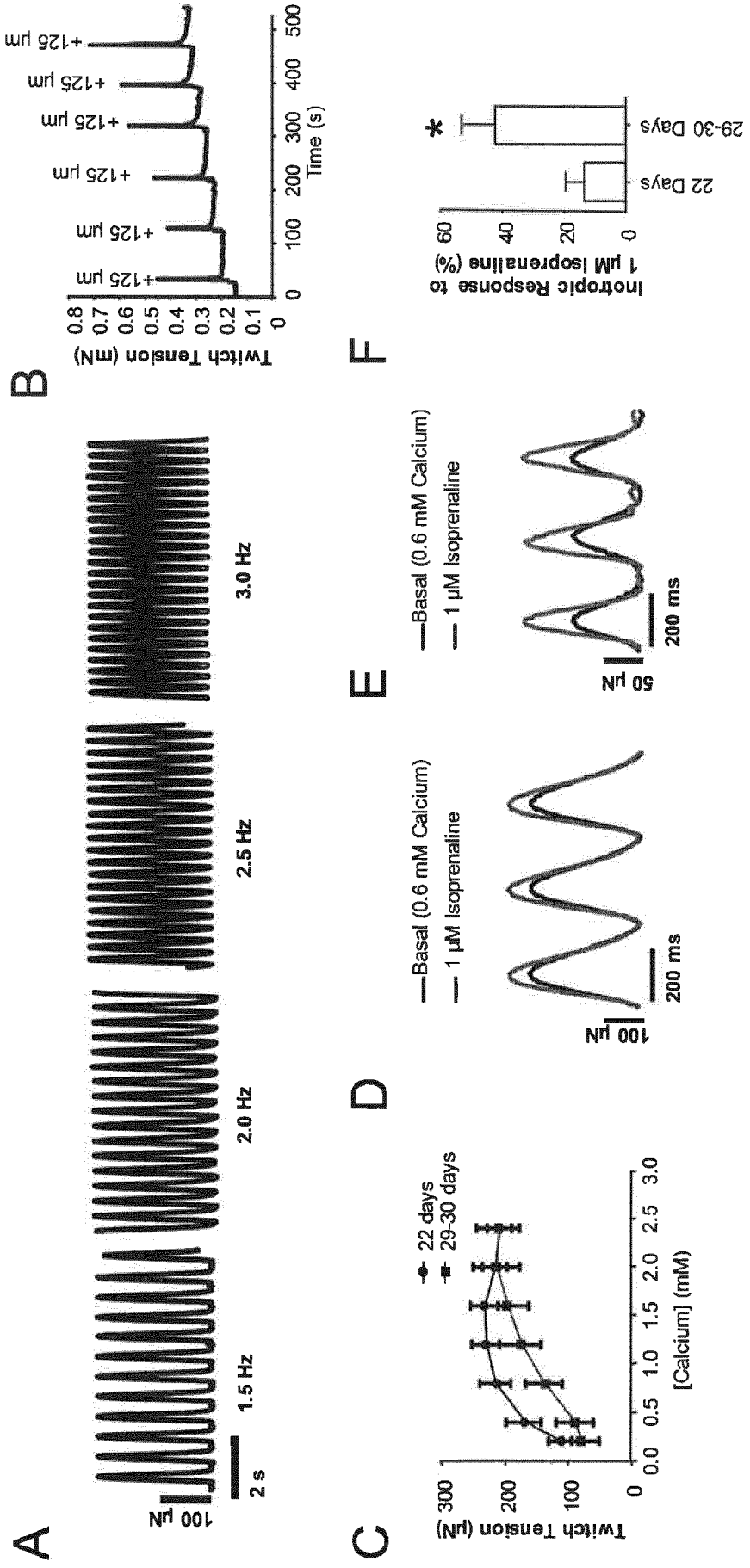

A
100 µN
2 s
1.5 Hz
2.0 Hz
2.5 Hz
3.0 Hz
B
Twitch Tension (mN)
Time (s)
+125 µm
C
22 days
29-30 days
Twitch Tension (µN)
[Calcium] (mM)
D
Basal (0.6 mM Calcium)
1 µM Isoprenaline
100 µN
200 ms
E
Basal (0.6 mM Calcium)
1 µM Isoprenaline
50 µN
200 ms
F
Inotropic Response to 1 µM Isoprenaline (%)
22 Days
29-30 Days

Figure 5

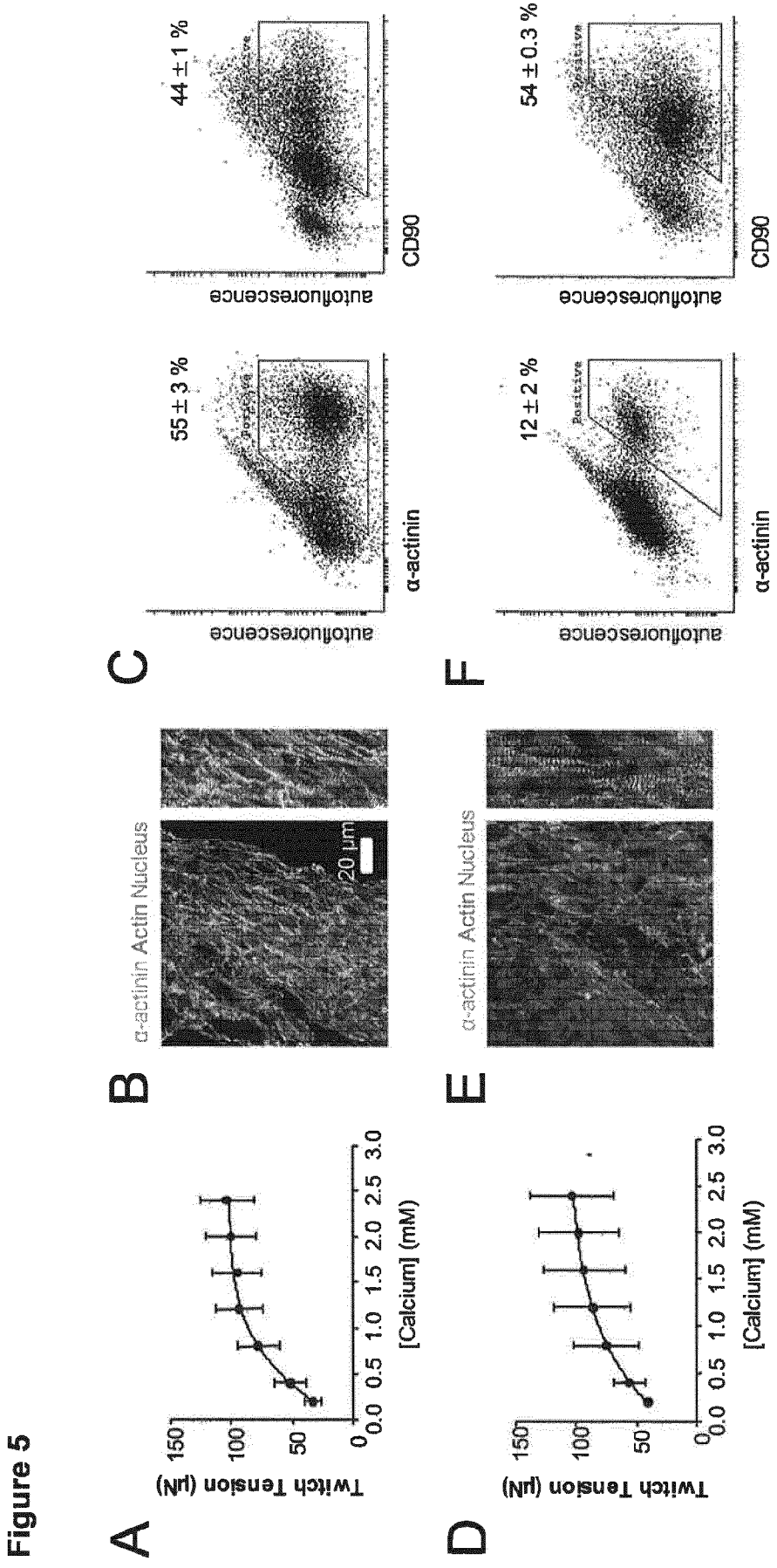
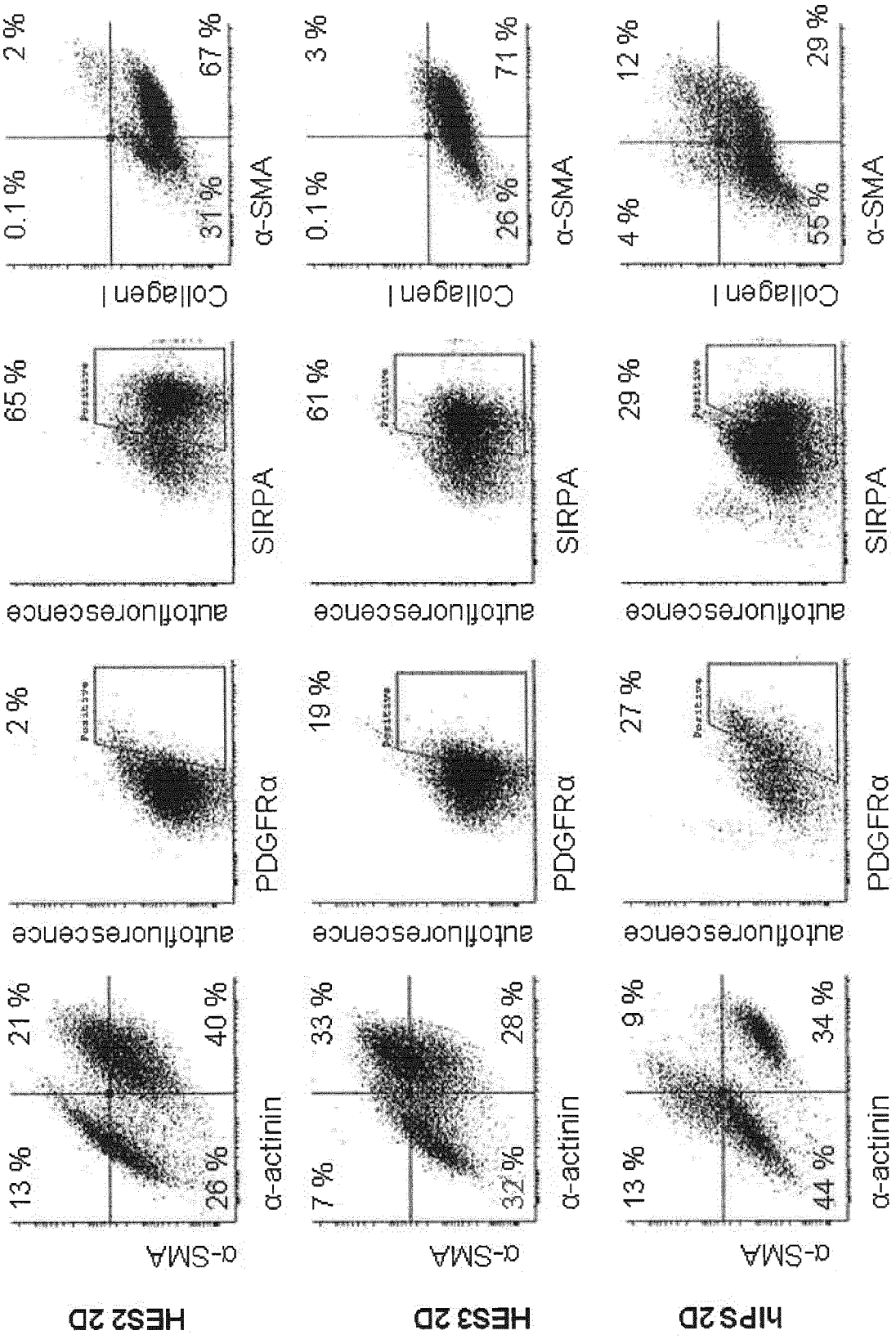

A
Twitch Tension (µN)
150
100
50
0
0.0 0.5 1.0 1.5 2.0 2.5 3.0
[Calcium] (mM)
B
α-actinin Actin Nucleus
20 µm
C
55 ± 3 %
Positive
autofluorescence
α-actinin
44 ± 1 %
Positive
autofluorescence
CD90
D
Twitch Tension (µN)
150
100
50
0
0.0 0.5 1.0 1.5 2.0 2.5 3.0
[Calcium] (mM)
E
α-actinin Actin Nucleus
F
12 ± 2 %
Positive
autofluorescence
α-actinin
54 ± 0.3 %
Positive
autofluorescence
CD90

Figure 6


HES2 2D
HES3 2D
hIPS 2D
α-SMA
α-actinin
PDGFRα
SIRPA
Collagen I
autofluorescence
13 %
21 %
26 %
40 %
2 %
65 %
0.1 %
2 %
31 %
67 %
7 %
33 %
32 %
28 %
19 %
61 %
0.1 %
3 %
26 %
71 %
9 %
27 %
29 %
13 %
34 %
44 %
4 %
12 %
55 %
29 %

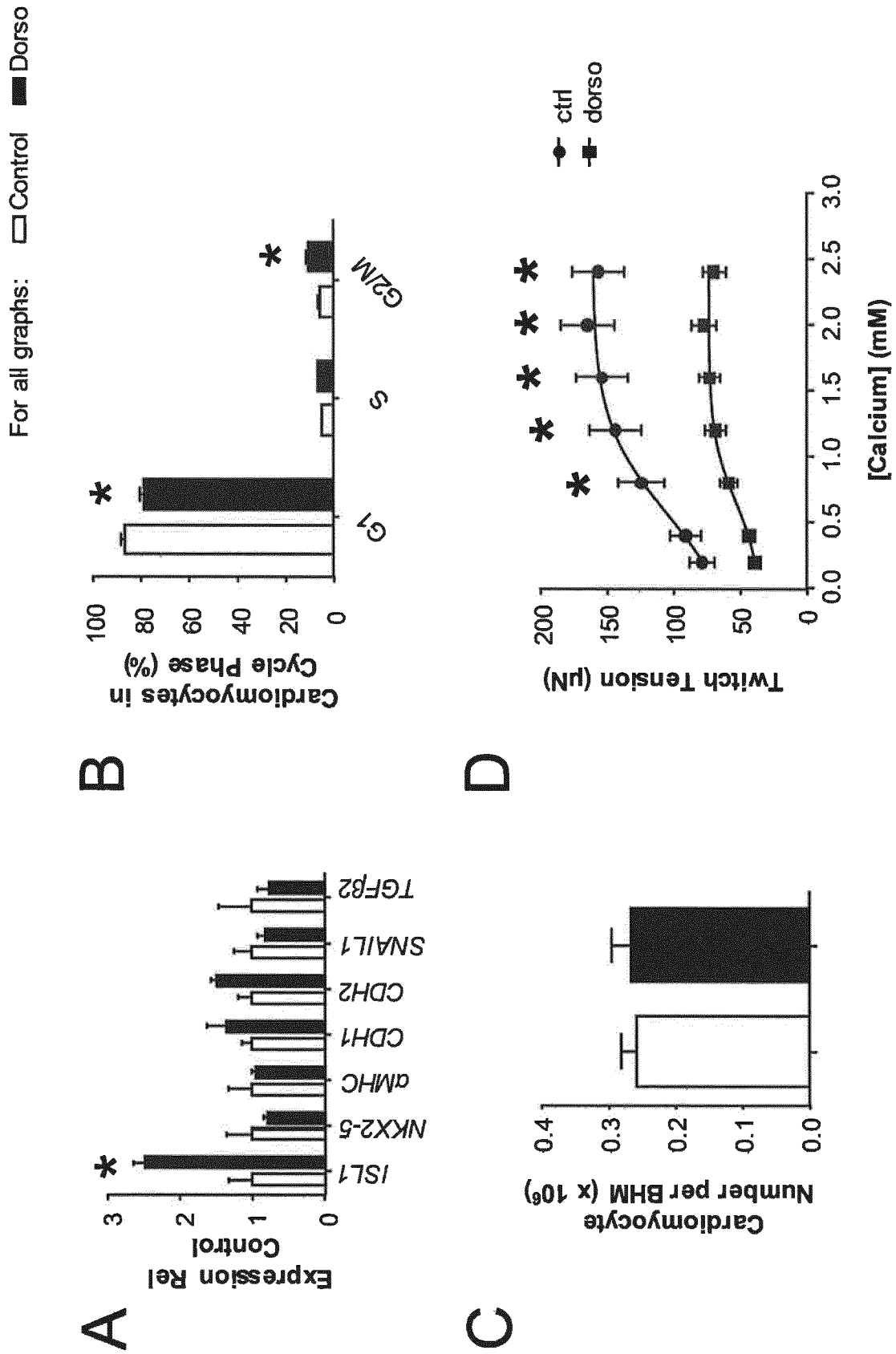

For all graphs: Control Dorso
A
Expression Rel Control
ISL1
NKX2-5
αMHC
CDH1
CDH2
SNAIL1
TGFβ2
B
Cardiomyocytes in Cycle Phase (%)
G1
S
G2M
C
Cardiomyocyte Number per BHM (x 10⁶)
D
Twitch Tension (μN)
ctrl
dorso
[Calcium] (mM)

Figure 7

Figure 8

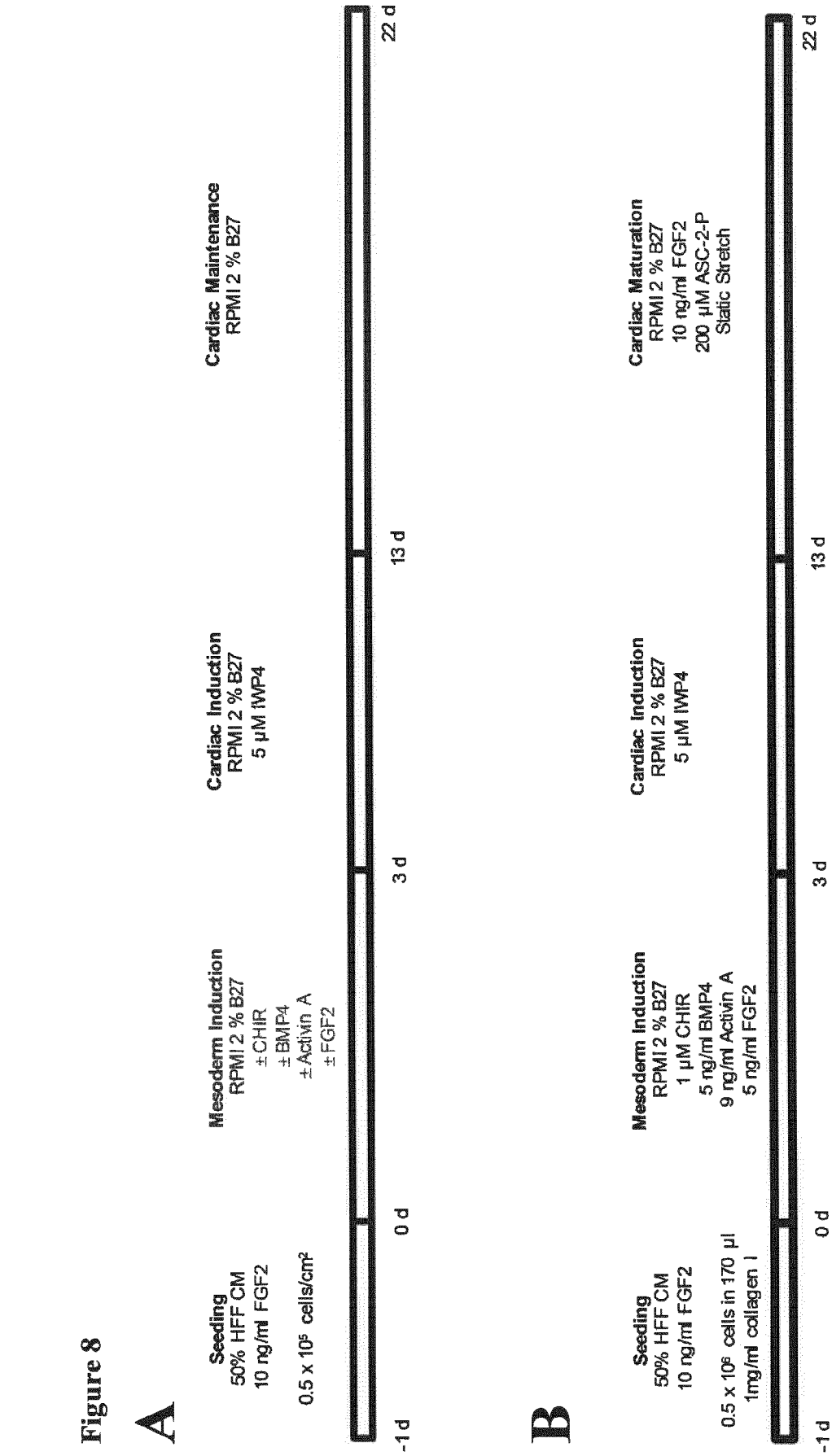
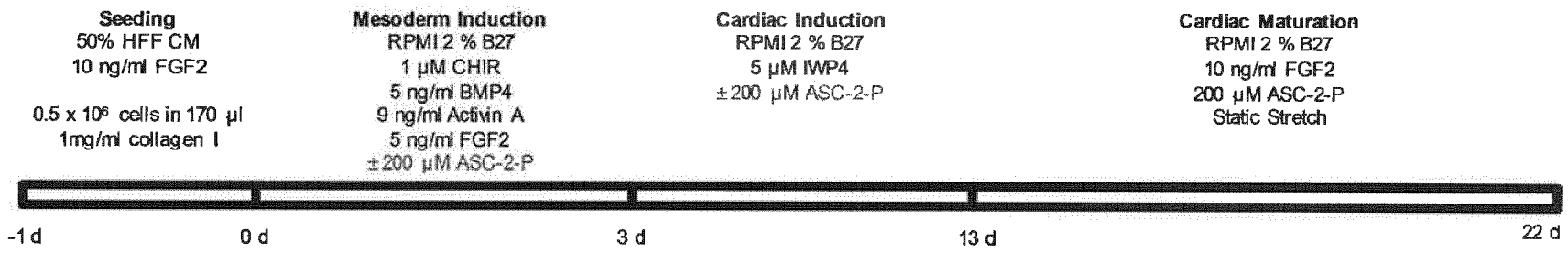
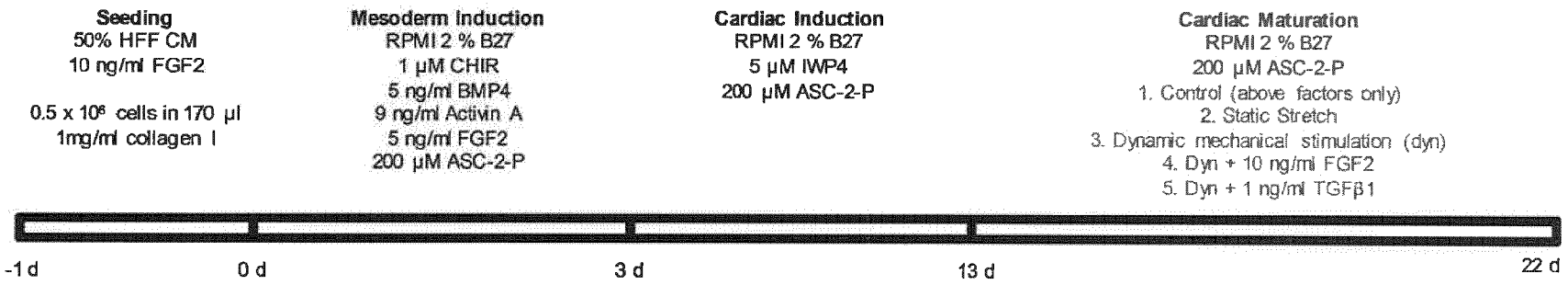

A
Seeding
50% HFF CM
10 ng/ml FGF2
0.5 x 10⁵ cells/cm²
Mesoderm Induction
RPMI 2 % B27
±CHIR
± BMP4
±Activin A
±FGF2
Cardiac Induction
RPMI 2 % B27
5 µM IWP4
Cardiac Maintenance
RPMI 2 % B27
-1 d
0 d
3 d
13 d
22 d
B
Seeding
50% HFF CM
10 ng/ml FGF2
0.5 x 10⁶ cells in 170 µl
1mg/ml collagen I
Mesoderm Induction
RPMI 2 % B27
1 µM CHIR
5 ng/ml BMP4
9 ng/ml Activin A
5 ng/ml FGF2
Cardiac Induction
RPMI 2 % B27
5 µM IWP4
Cardiac Maturation
RPMI 2 % B27
10 ng/ml FGF2
200 µM ASC-2-P
Static Stretch
-1 d
0 d
3 d
13 d
22 d

Figure 8 (Continued)

C



D



E

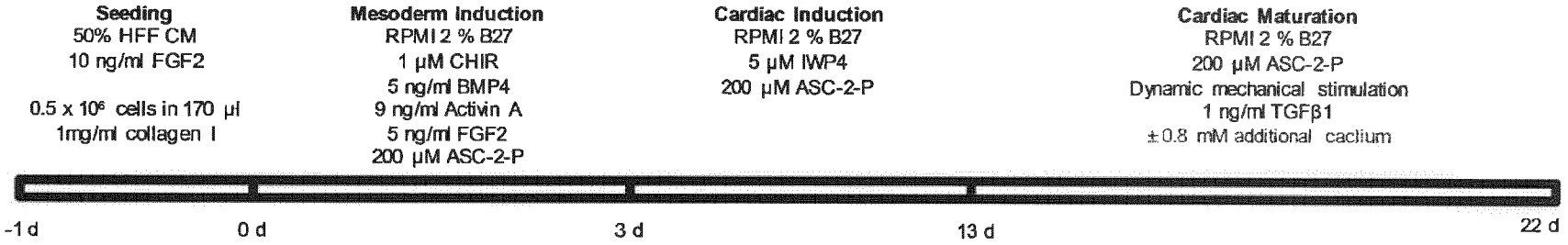
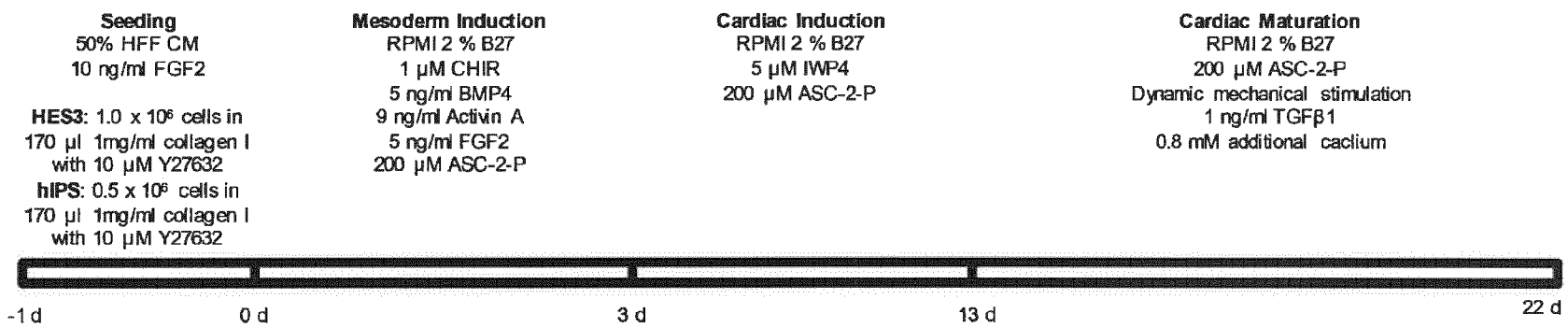
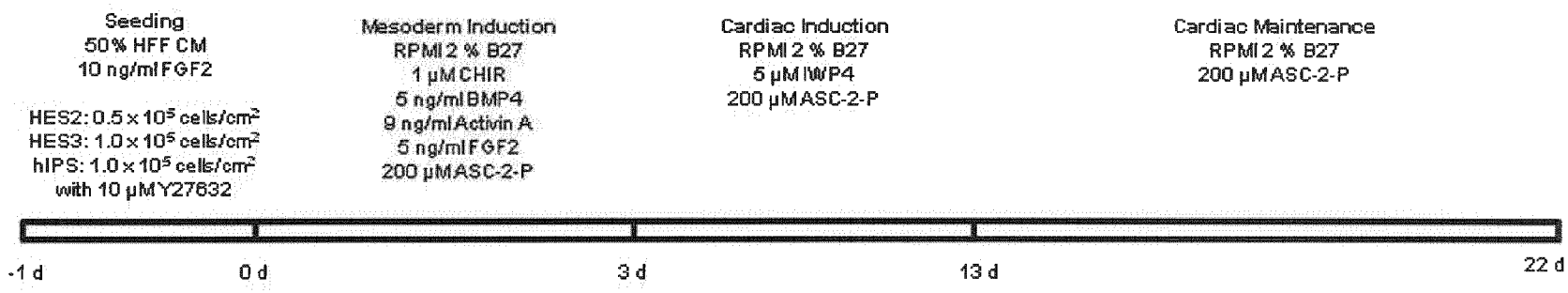


Figure 8 (Continued)

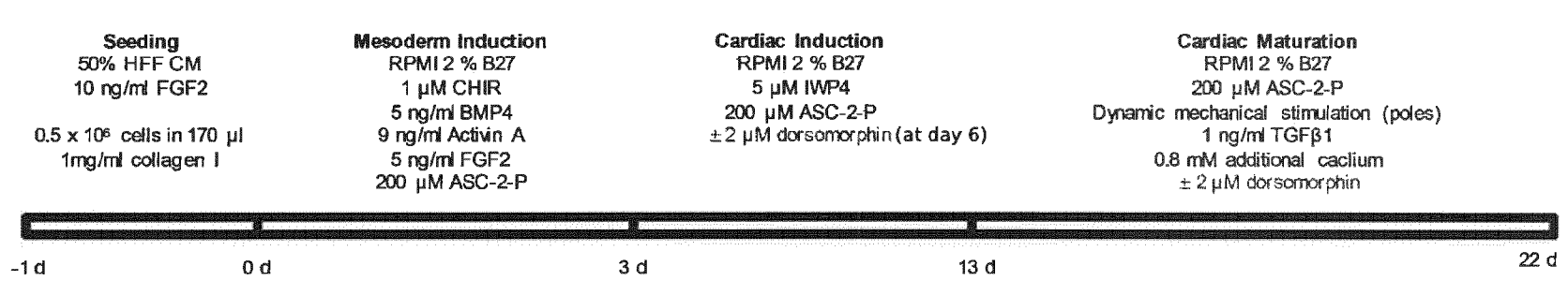
F

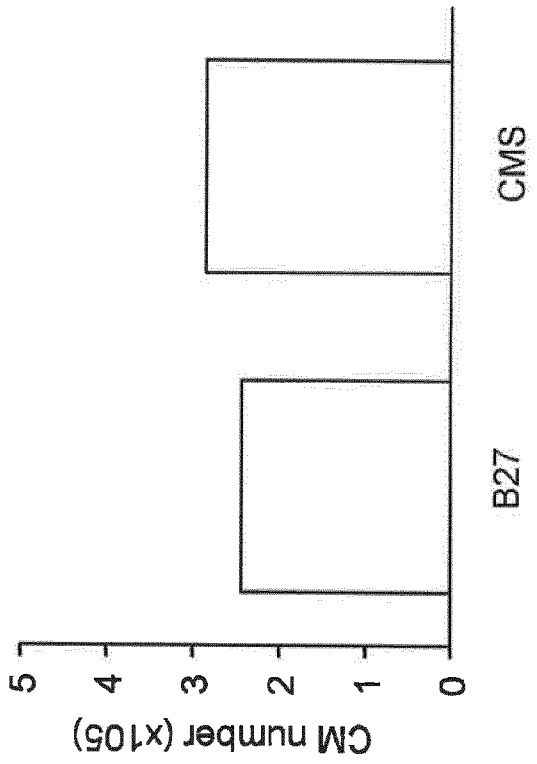


G



H




Total cardiomyocyte number
CM number (x105)
0
1
2
3
4
5
B27
CMS

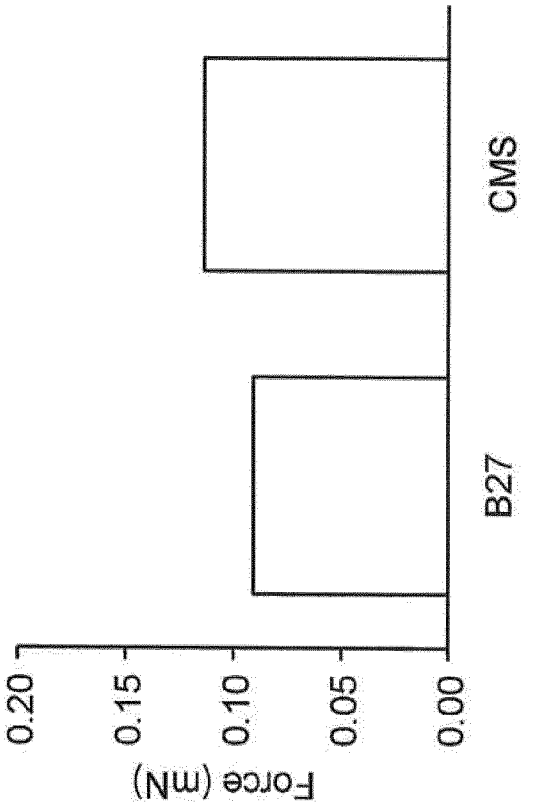

Force at 2 mM Ca
Force (mN)
0.00
0.05
0.10
0.15
0.20
B27
CMS

Figure 9

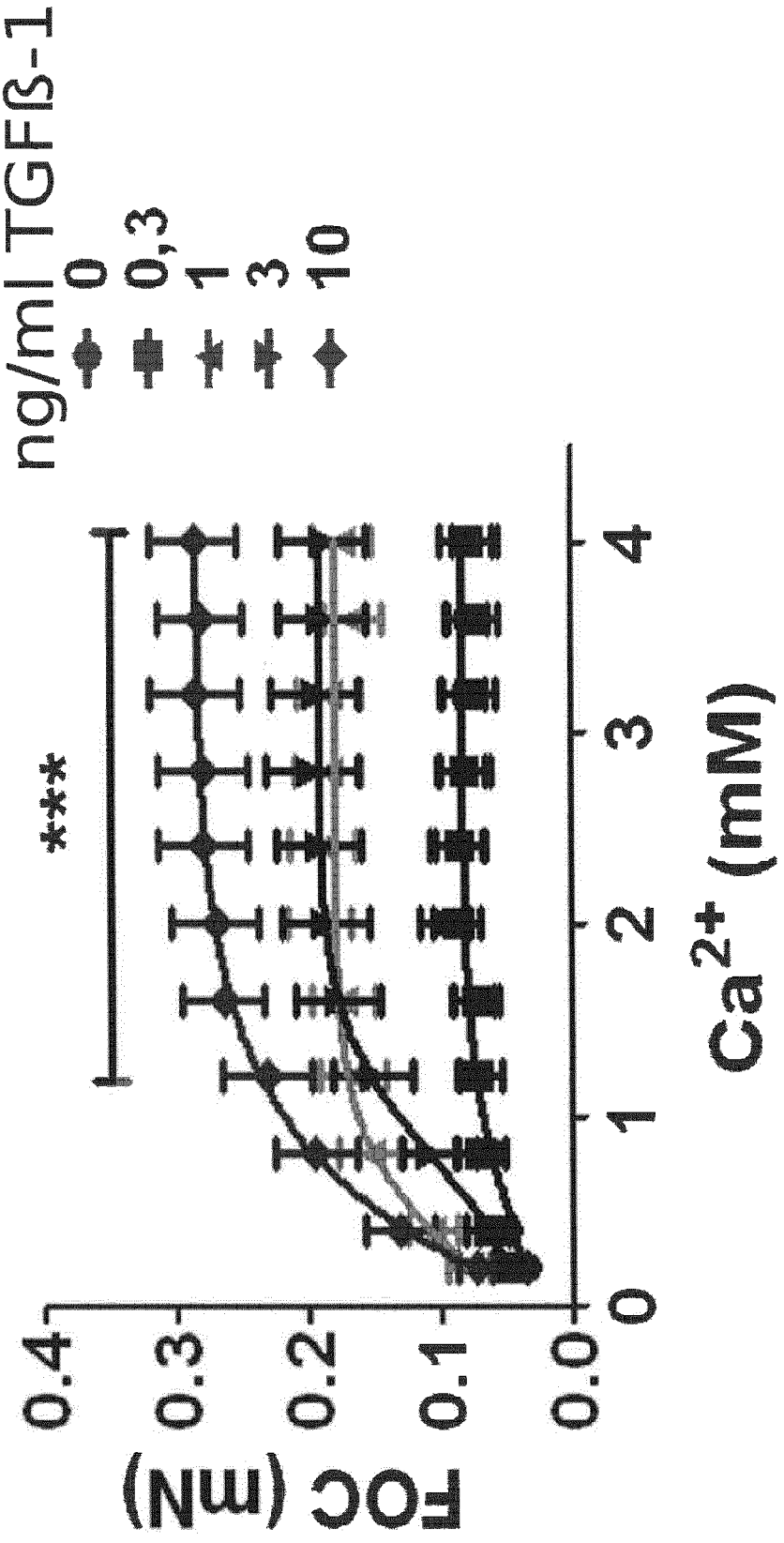


Figure 10