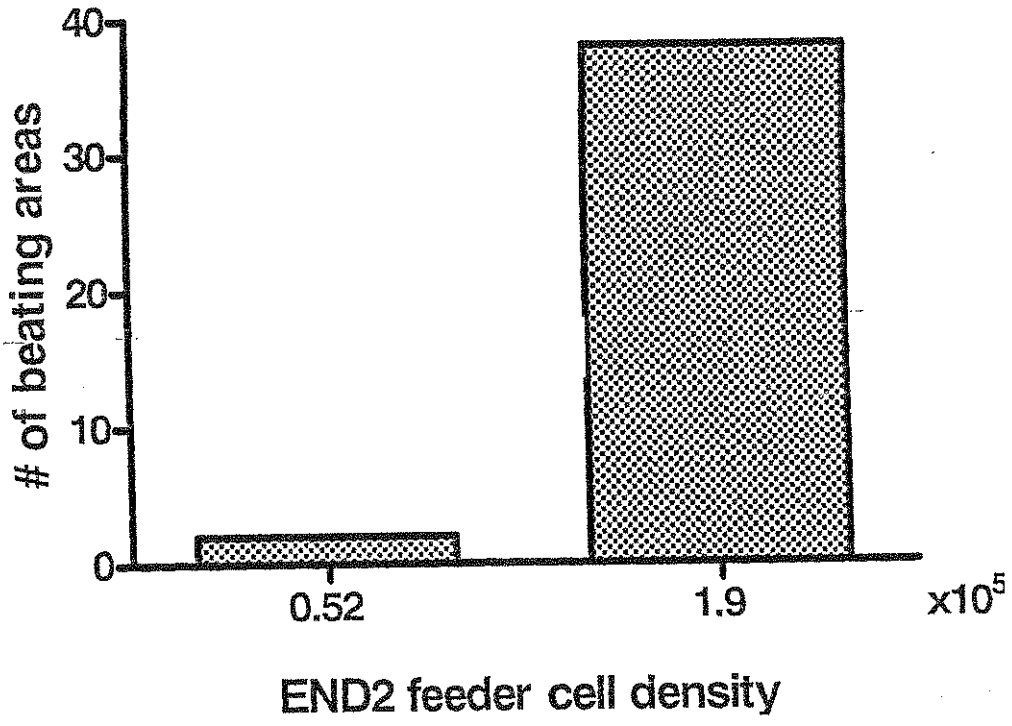

# of beating areas
40
30
20
10
0
0.52
1.9
x10^5
END2 feeder cell density

FIG 1

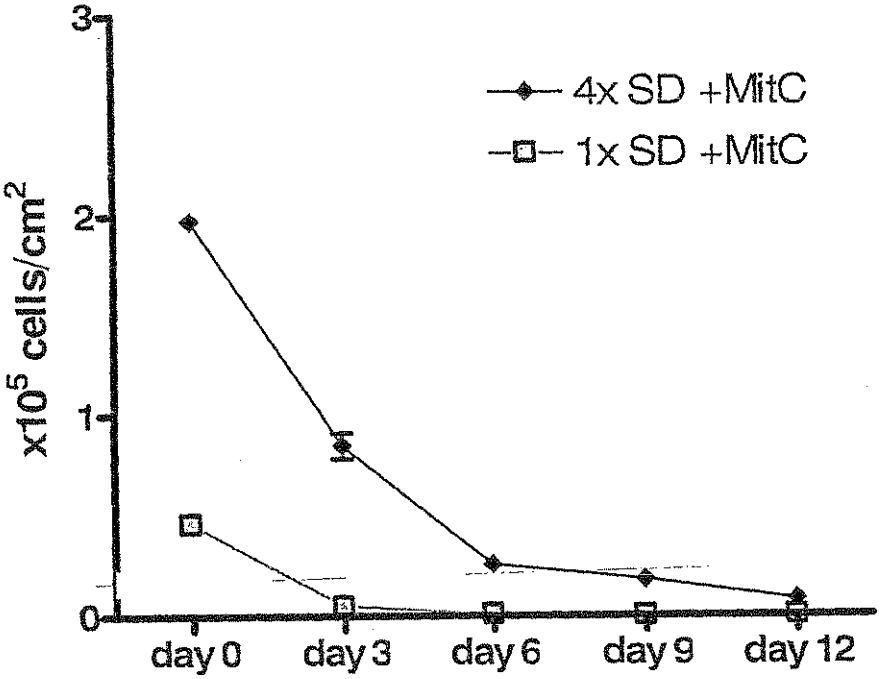

4x SD +MitC
1x SD +MitC
x10^5 cells/cm^2
3
2
1
0
day 0
day 3
day 6
day 9
day 12

FIG 2

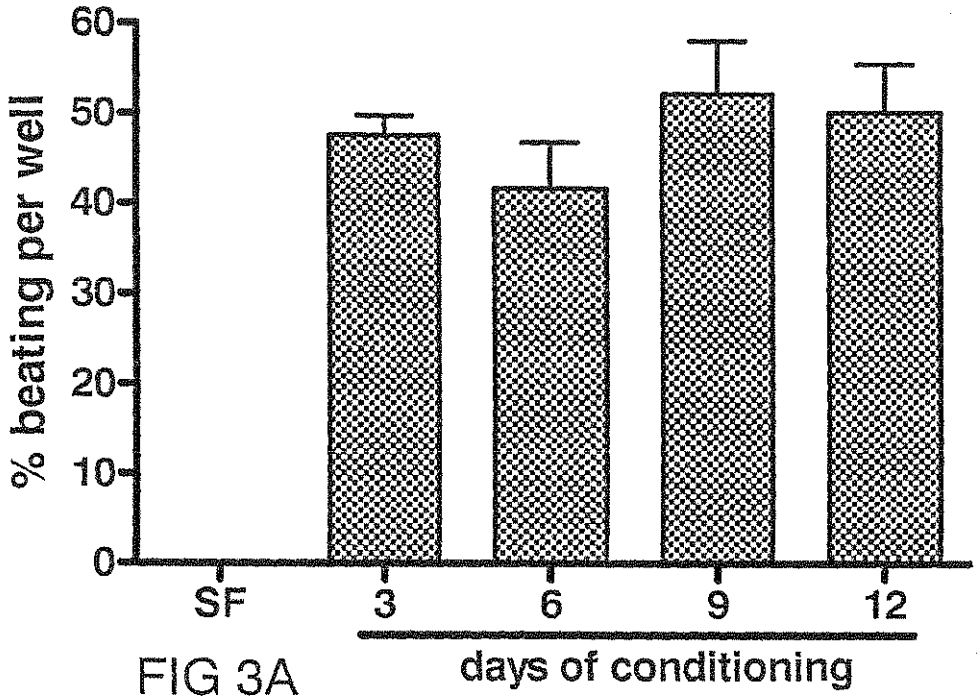

% beating per well
60
50
40
30
20
10
0
SF
3
6
9
12
days of conditioning

FIG 3A

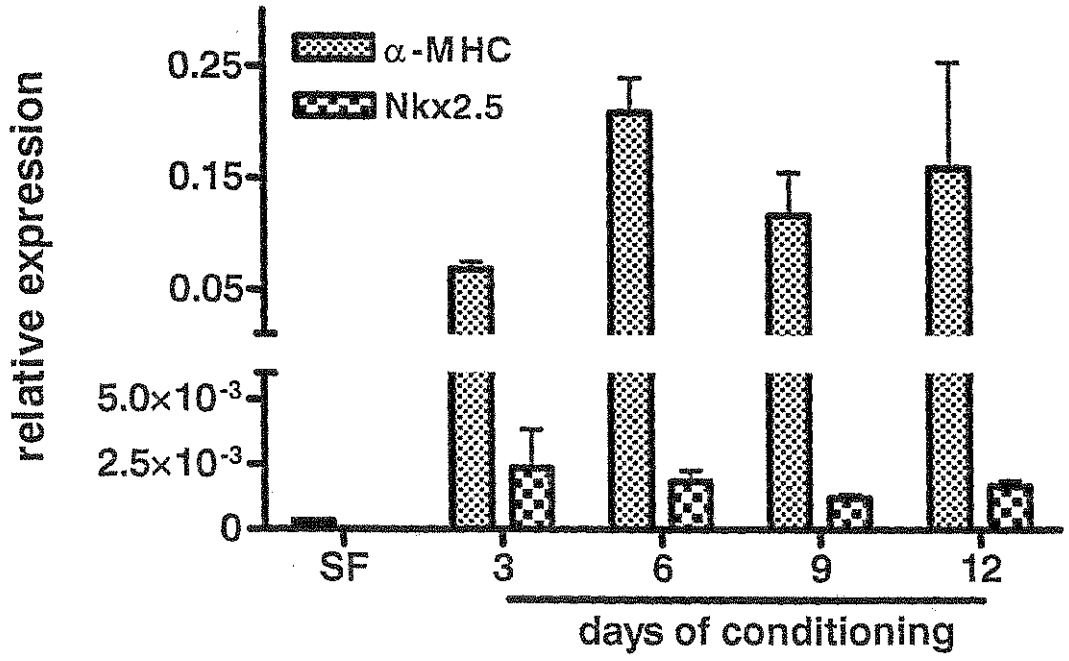

relative expression
α-MHC
Nkx2.5
0.25
0.15
0.05
5.0x10-3
2.5x10-3
0
SF
3
6
9
12
days of conditioning

FIG 3B

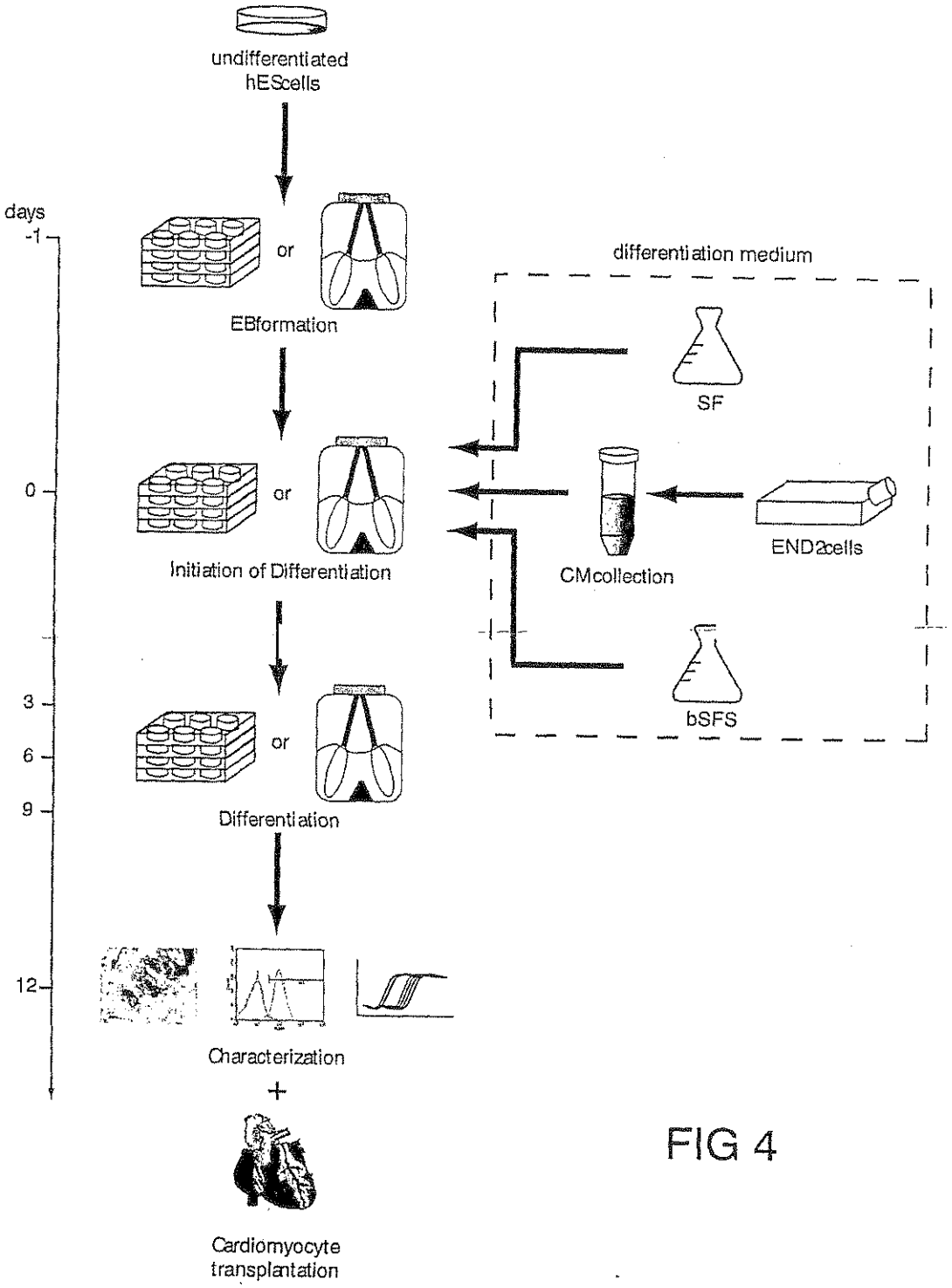

undifferentiated
hEScells
days
-1
or
EBformation
differentiation medium
SF
0
or
Initiation of Differentiation
CMcollection
END2cells
3
6
9
or
Differentiation
bSFS
12
Characterization
+
Cardiomyocyte
transplantation

FIG 4

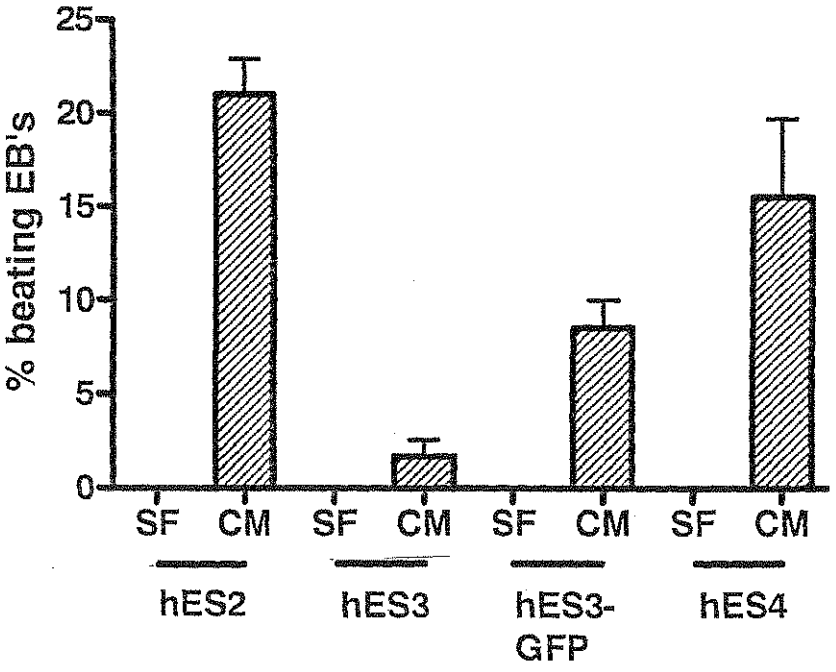

% beating EB's
25
20
15
10
5
0
SF
CM
SF
CM
SF
CM
SF
CM
hES2
hES3
hES3-GFP
hES4

FIG 5

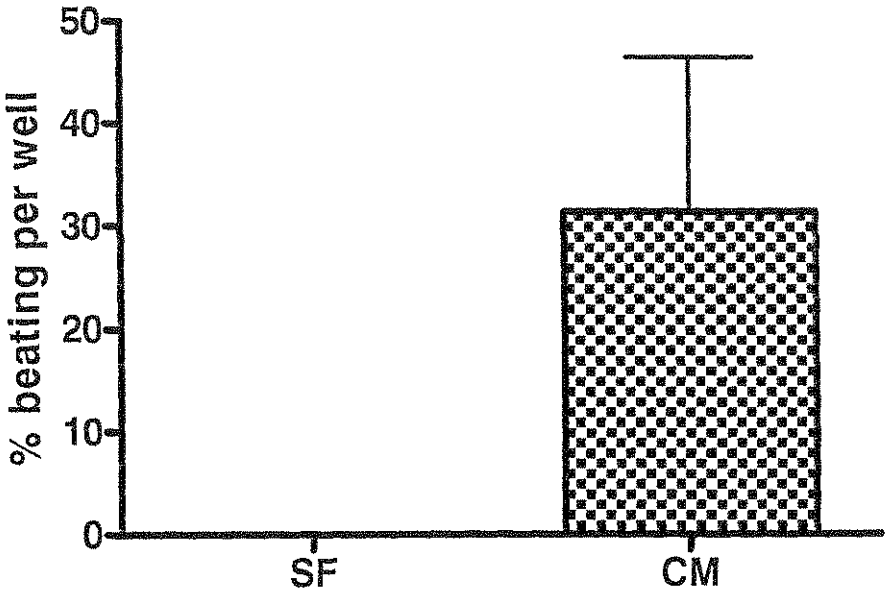

% beating per well
50
40
30
20
10
0
SF
CM

FIG 6A

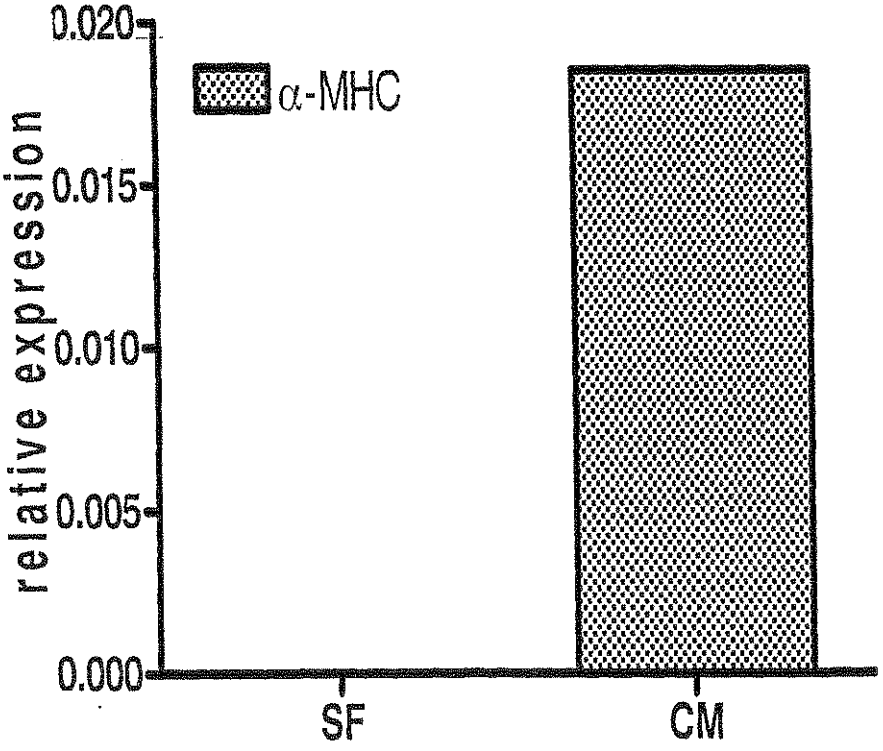

relative expression
0.020
0.015
0.010
0.005
0.000
α-MHC
SF
CM

FIG 6B

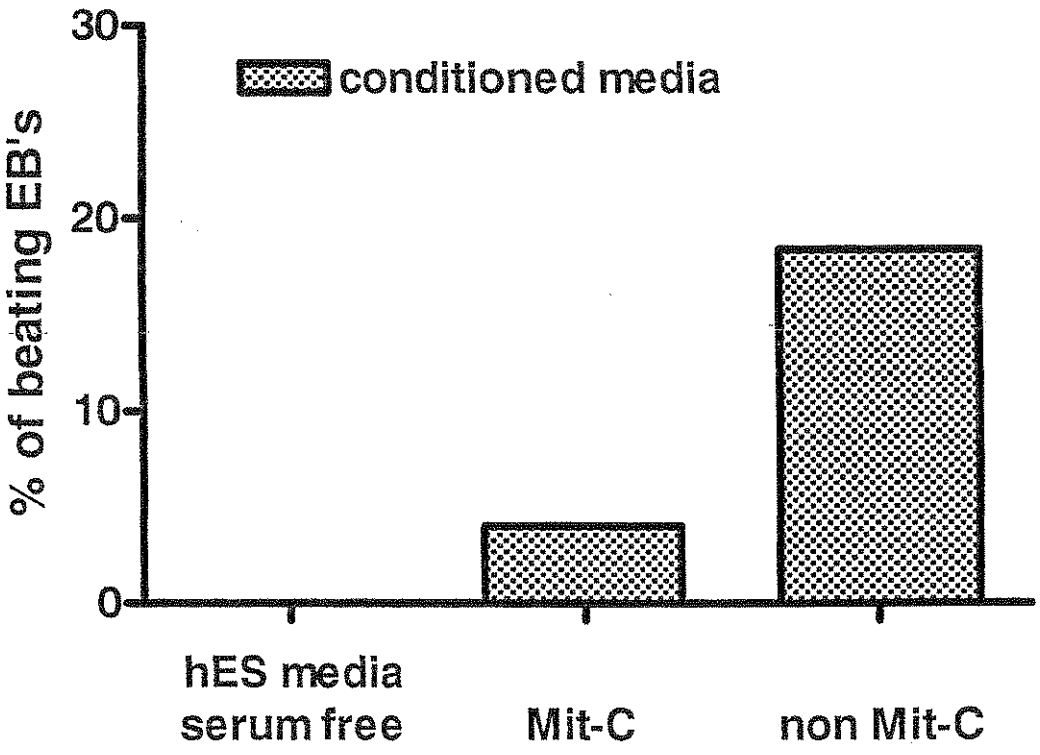

30
20
10
0
% of beating EB's
conditioned media
hES media
serum free
Mit-C
non Mit-C

FIG 7

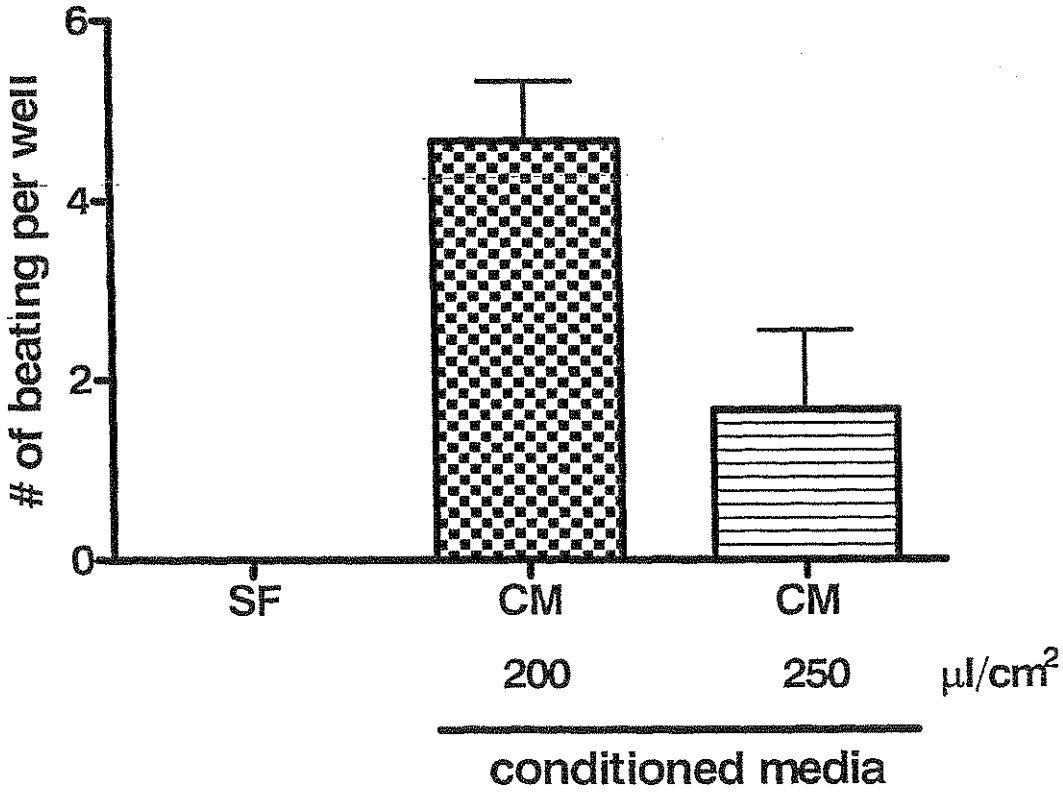

# of beating per well
6
4
2
0
SF
CM
200
CM
250
µl/cm²
conditioned media

FIG 8

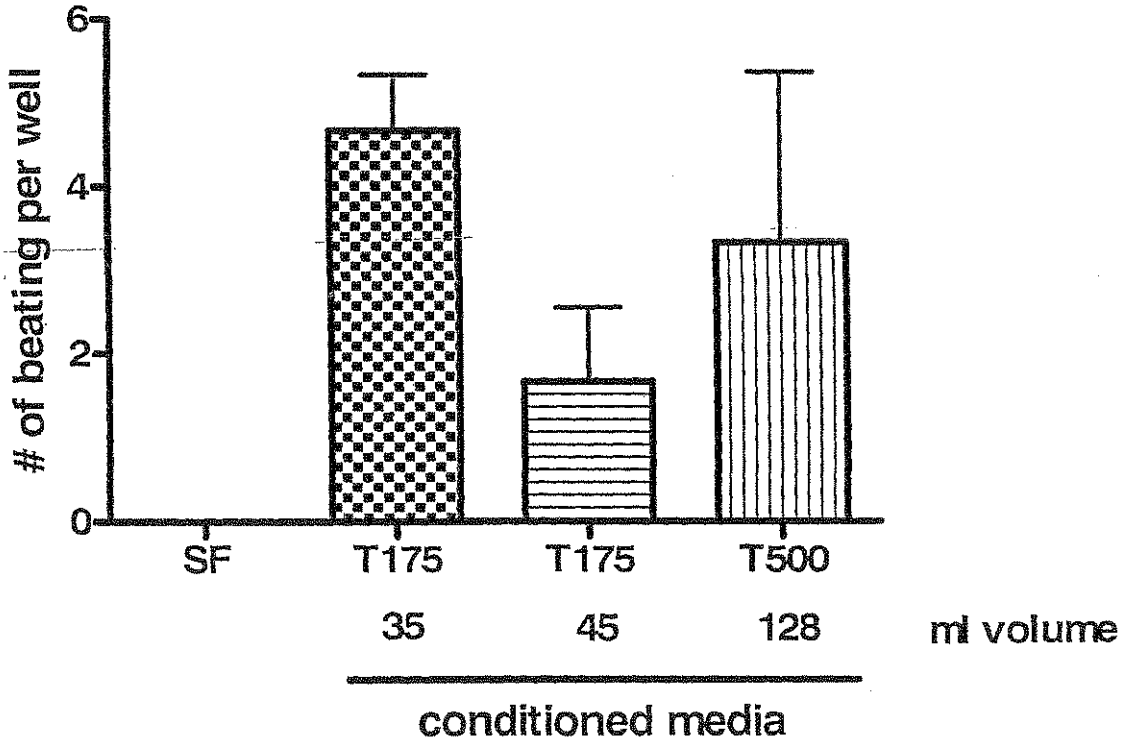

# of beating per well
6
4
2
0
SF
T175
T175
T500
35
45
128
ml volume
conditioned media

FIG 9

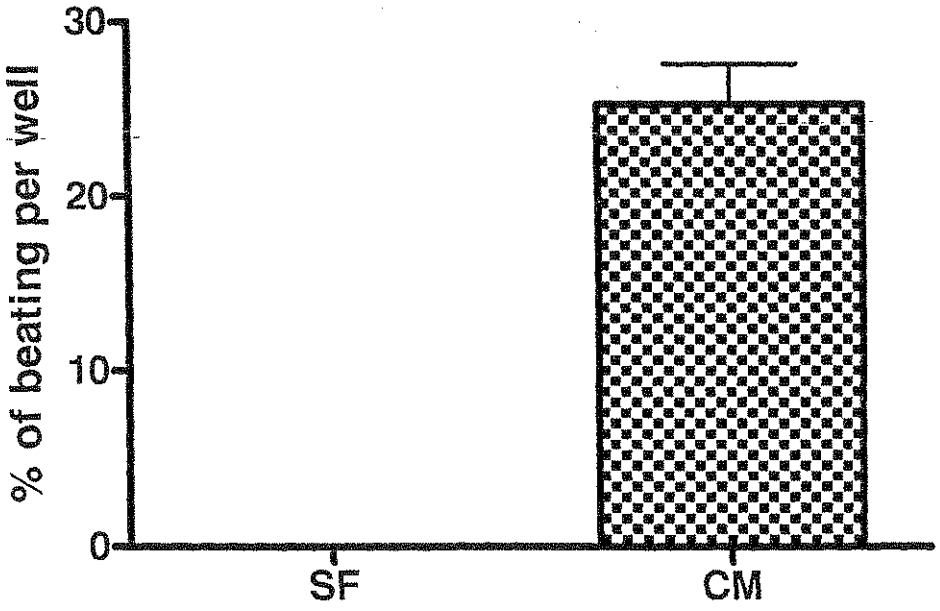


FIG 10

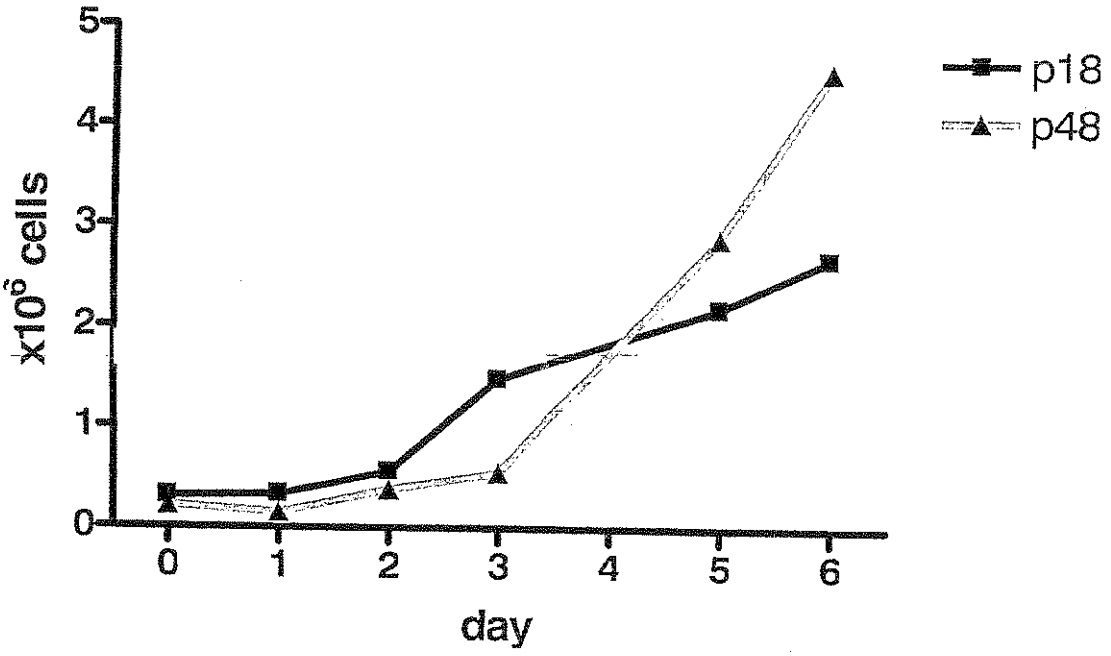


FIG 11

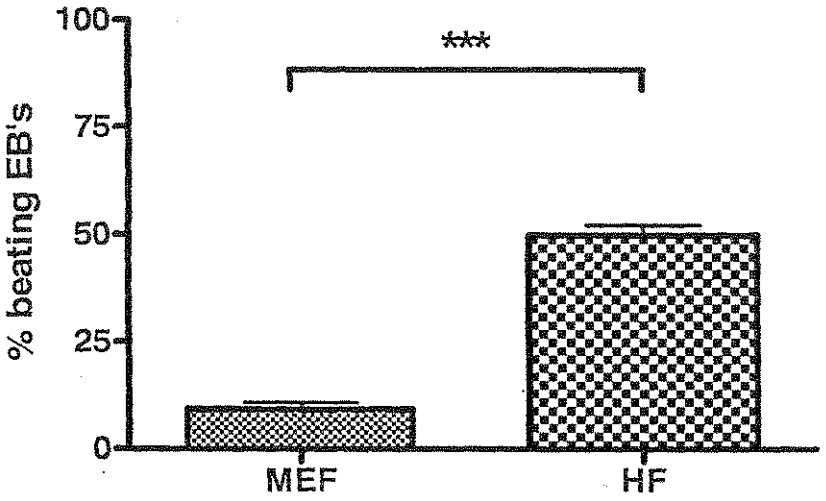


FIG 12A

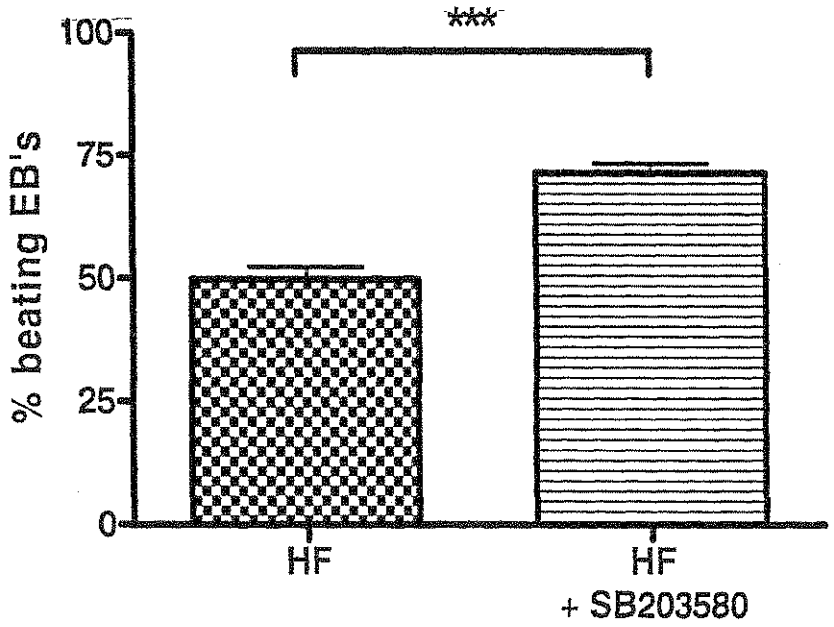


FIG 12B

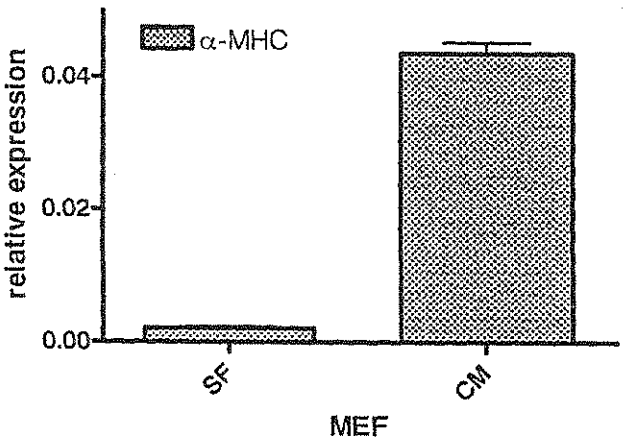


FIG 13A

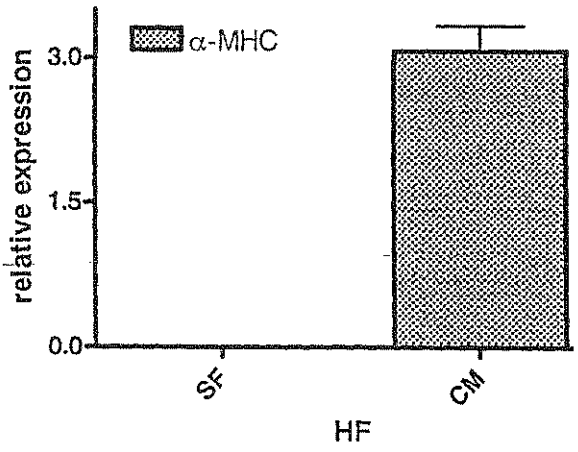


FIG 13B

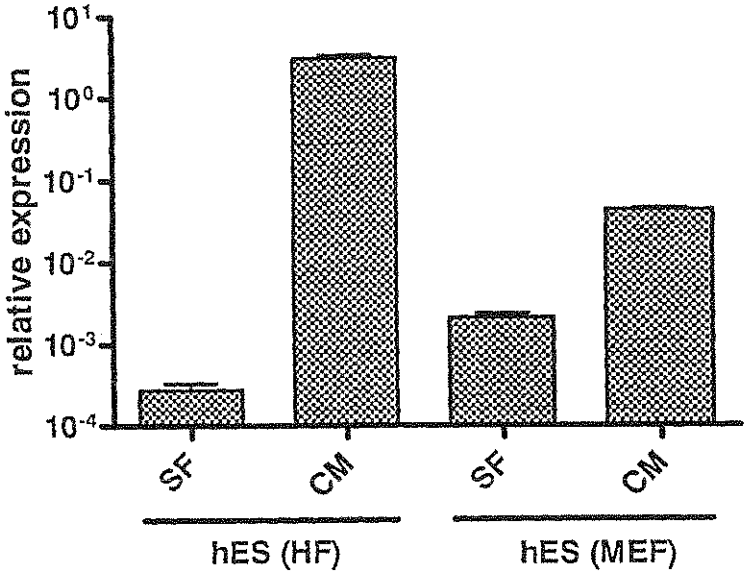

relative expression
10^{1}
10^{0}
10^{-1}
10^{-2}
10^{-3}
10^{-4}
SF
CM
SF
CM
hES (HF)
hES (MEF)

FIG 13C

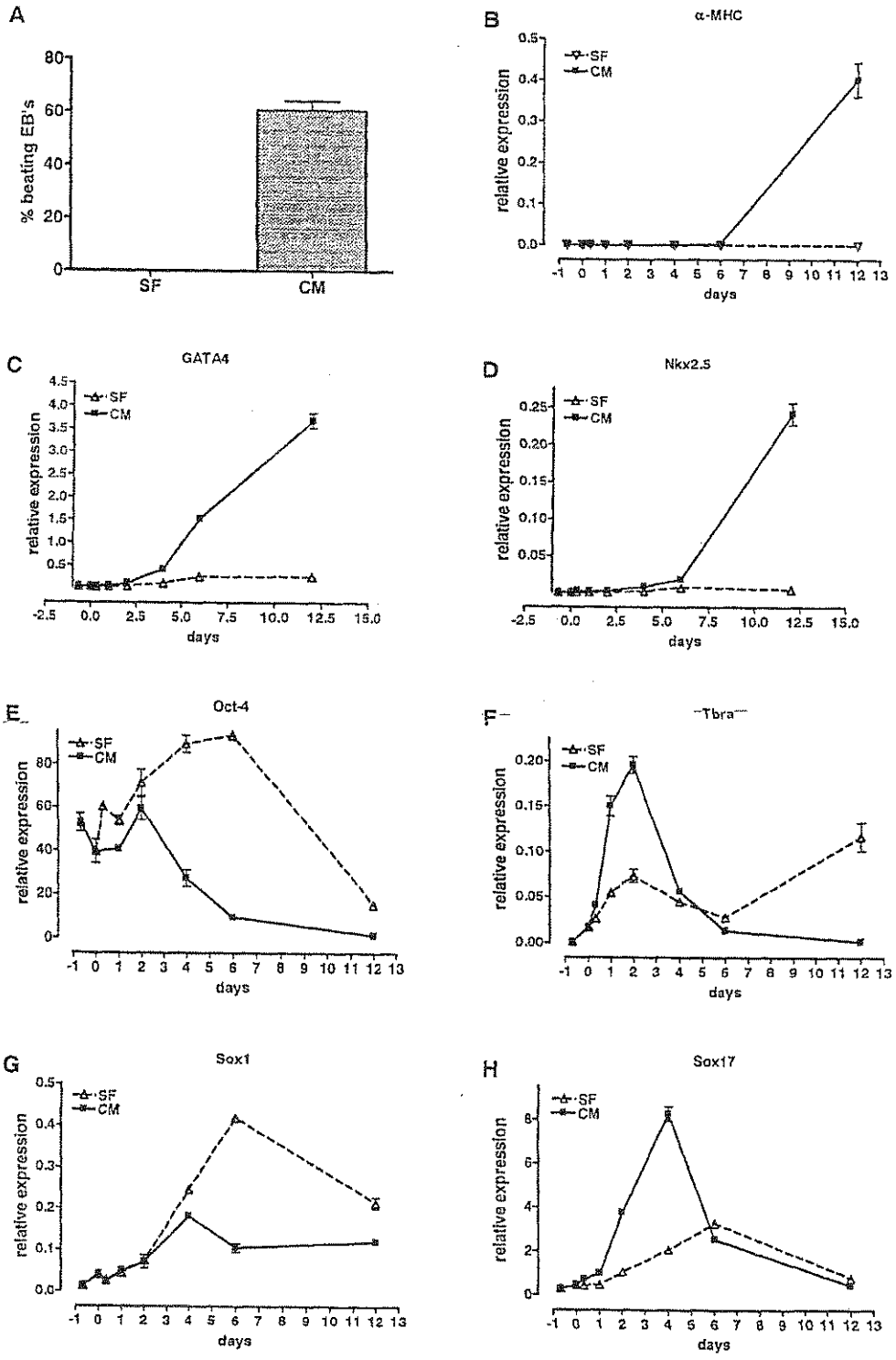

A
% beating EB's
SF
CM
B
α-MHC
relative expression
days
C
GATA4
D
Nkx2.5
E
Oct-4
F
Tbra
G
Sox1
H
Sox17

FIG 14

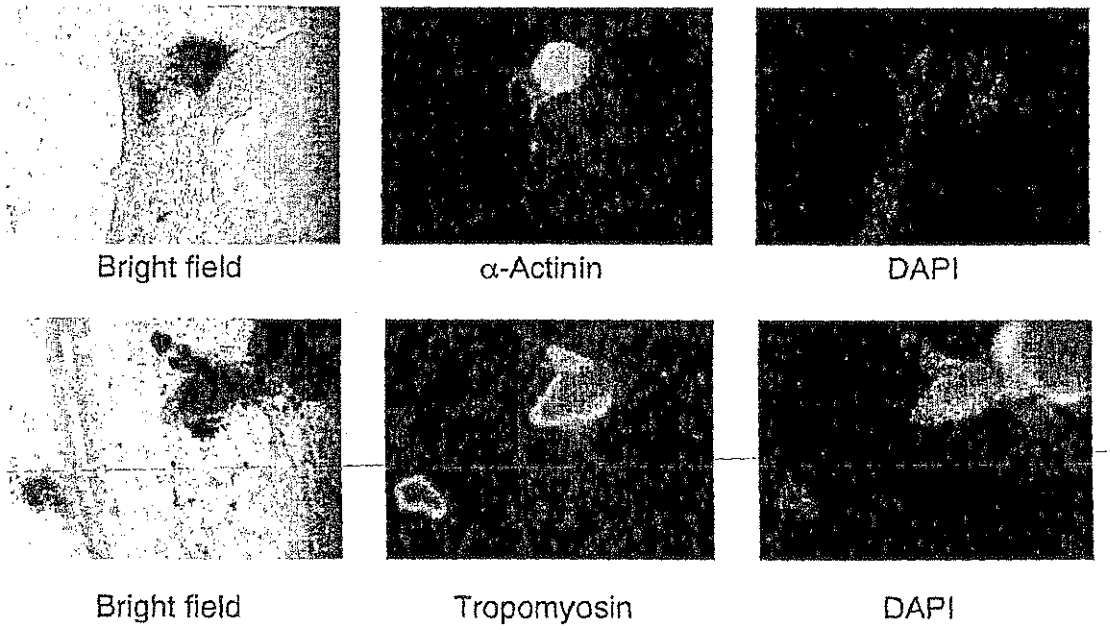


FIG 15

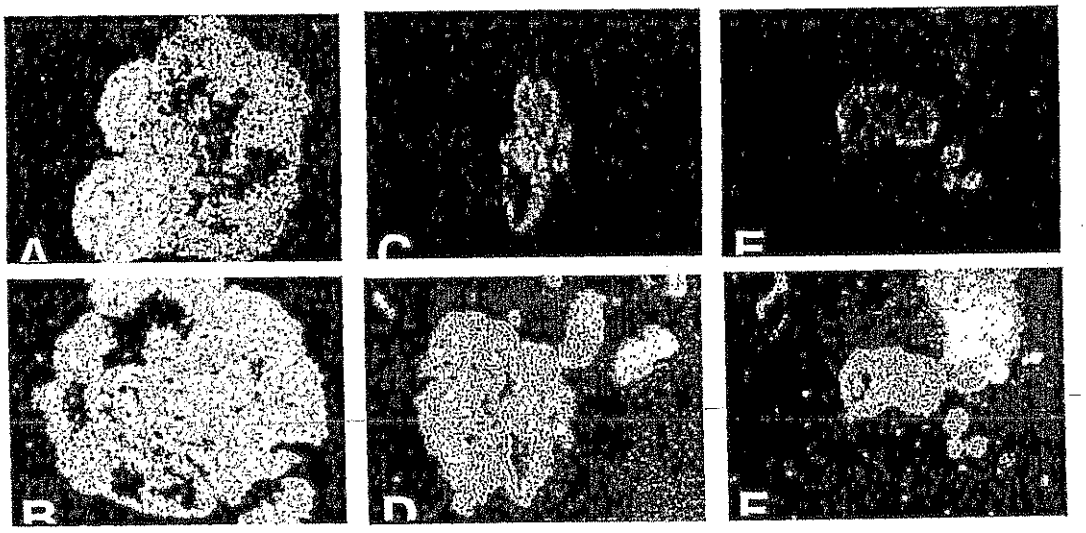
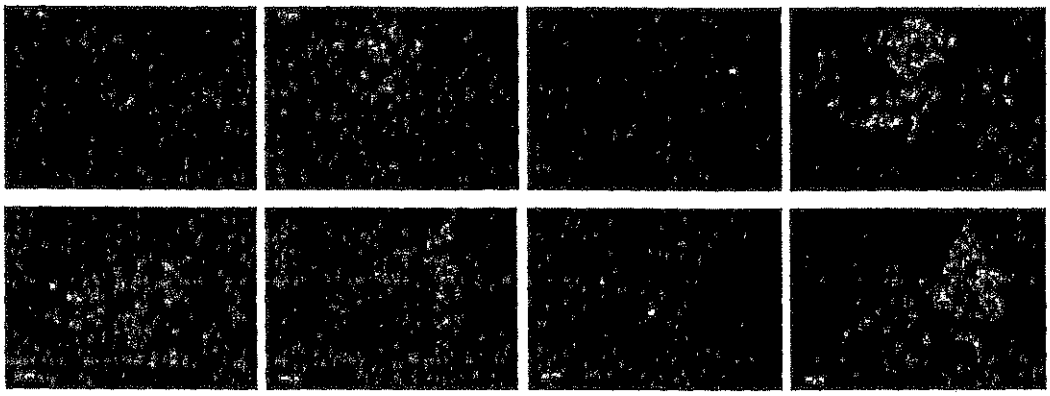
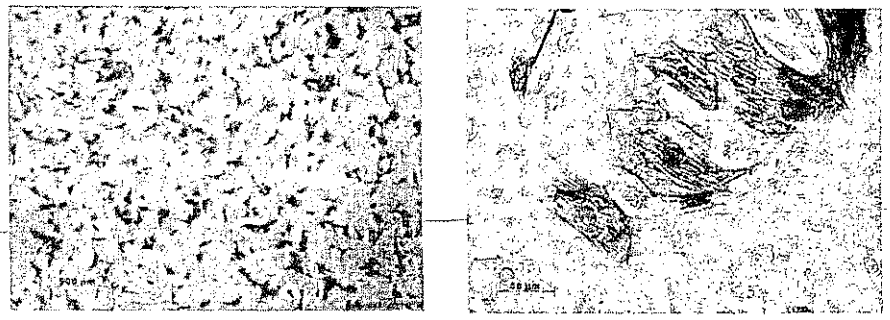


FIG 16

A



B



C

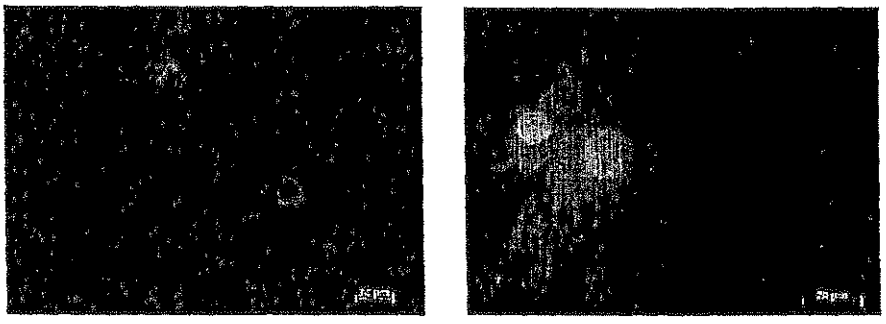


FIG 17

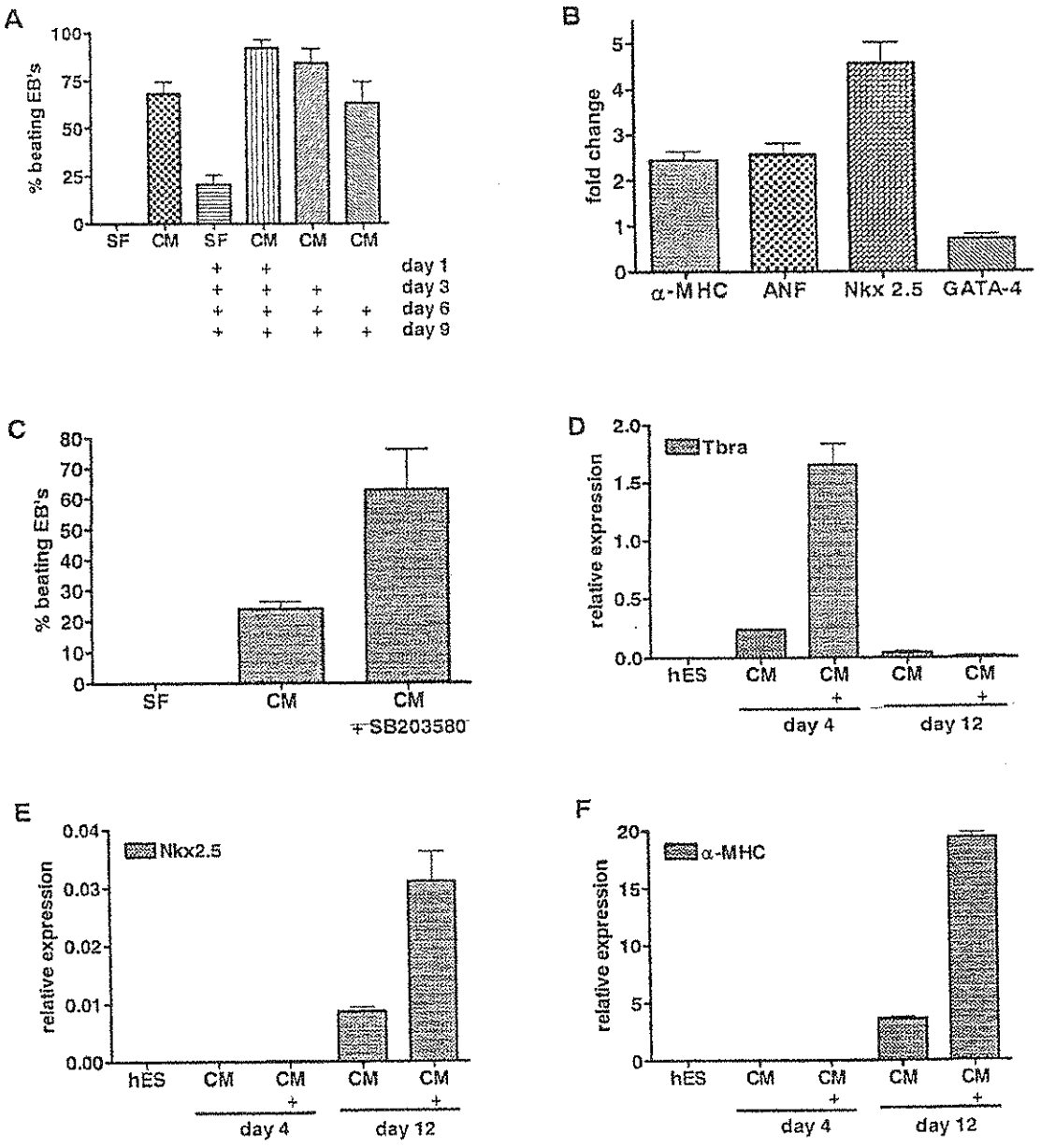

A
% beating EB's
100
75
50
25
0
SF
CM
SF
CM
CM
CM
day 1
day 3
day 6
day 9
B
fold change
5
4
3
2
1
0
α-MHC
ANF
Nkx 2.5
GATA-4
C
% beating EB's
80
70
60
50
40
30
20
10
0
SF
CM
CM
+SB203580
D
relative expression
2.0
1.5
1.0
0.5
0.0
Tbra
hES
CM
CM
CM
CM
day 4
day 12
E
relative expression
0.04
0.03
0.02
0.01
0.00
Nkx2.5
hES
CM
CM
CM
CM
day 4
day 12
F
relative expression
20
15
10
5
0
α-MHC
hES
CM
CM
CM
CM
day 4
day 12

FIG 18

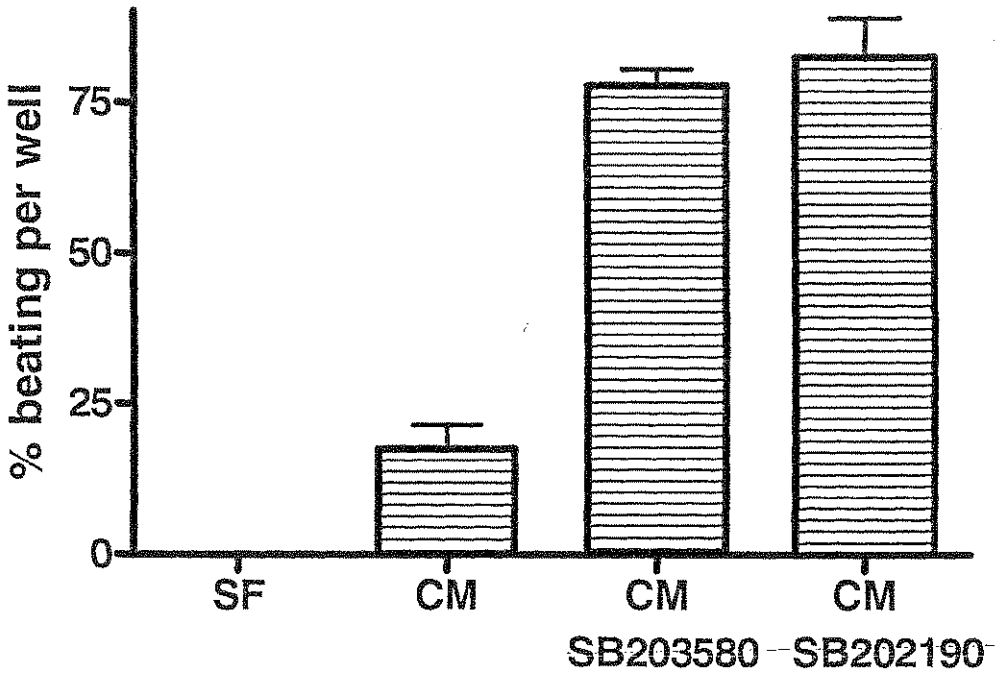


FIG 19

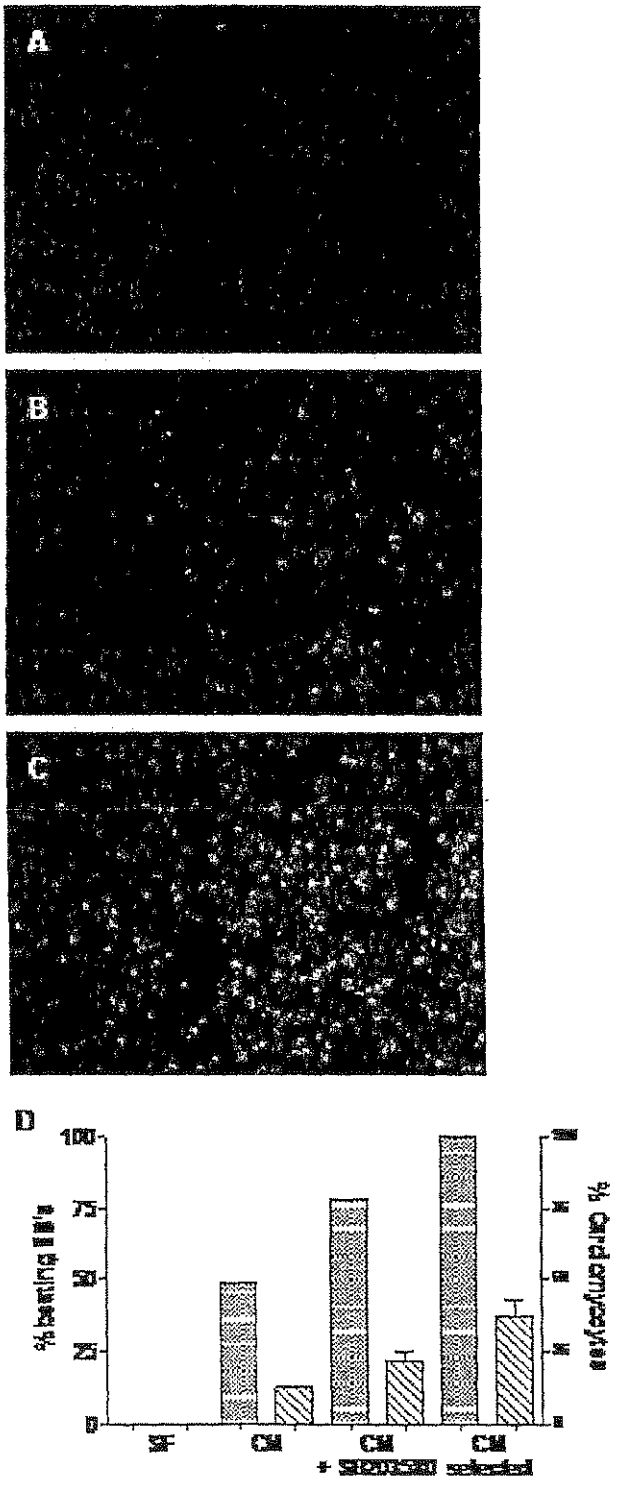
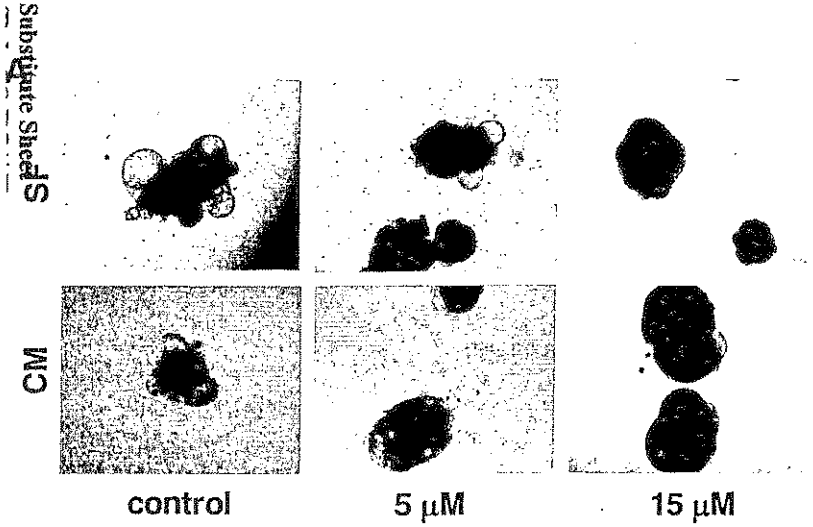

A
B
C
D
100
75
50
25
0
% beating EBs
% Card.myocytes
MF
CM
CM
+ SB203580
CM
selected

FIG 20

Substitute Sheet (Rule 26)



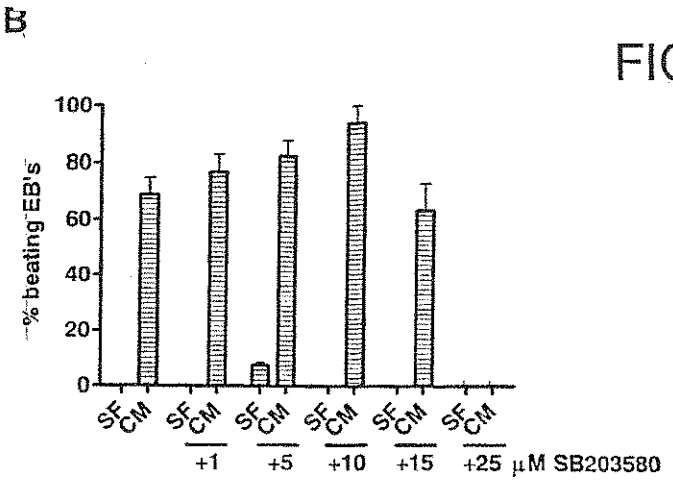
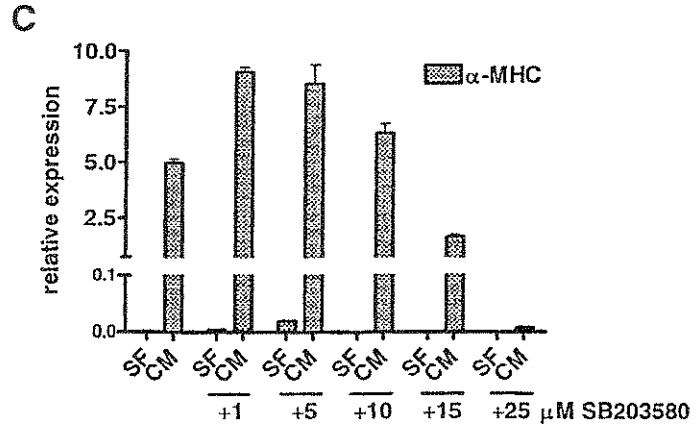
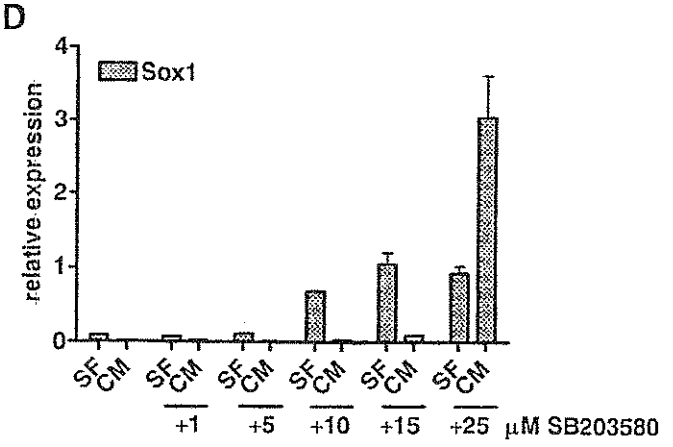
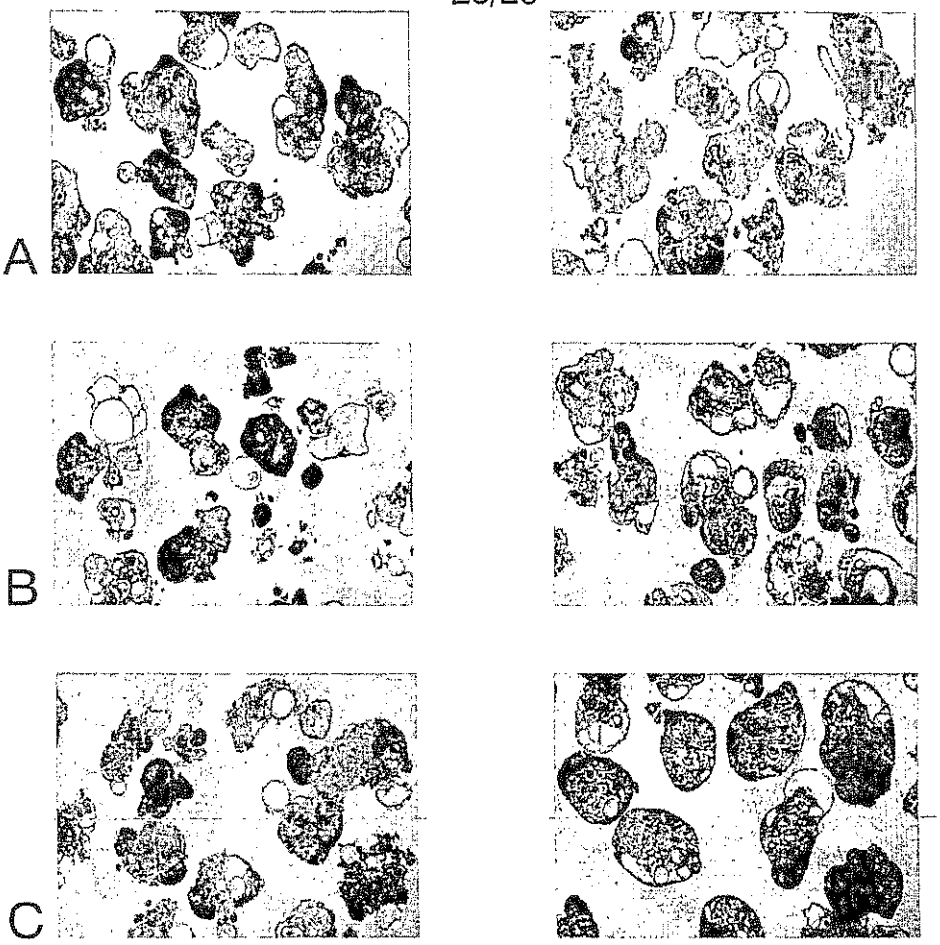


FIG 21







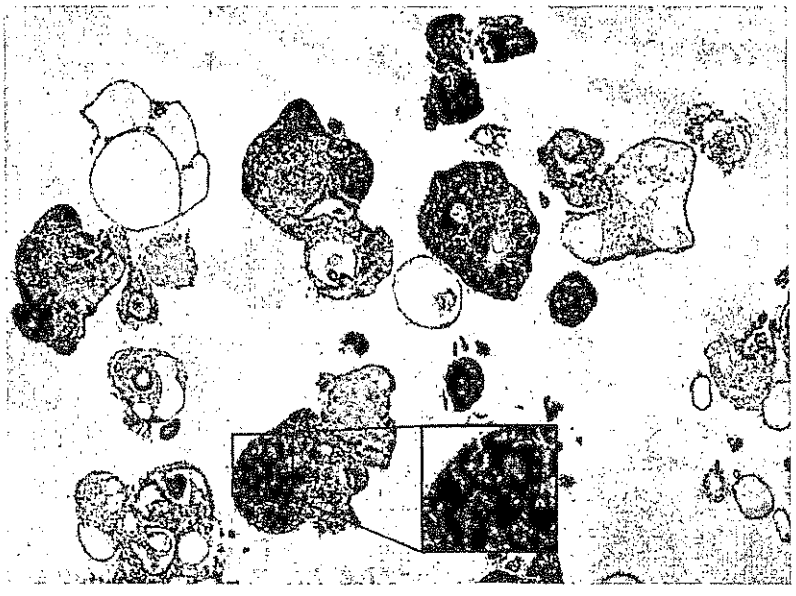
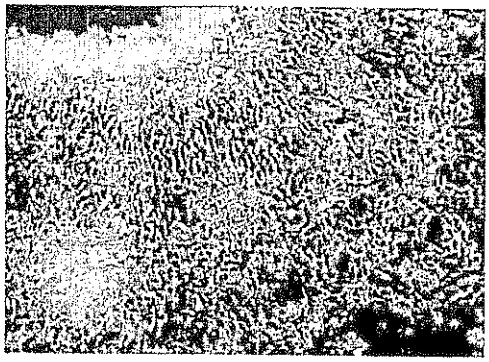


FIG 22

A



B



FIG 23

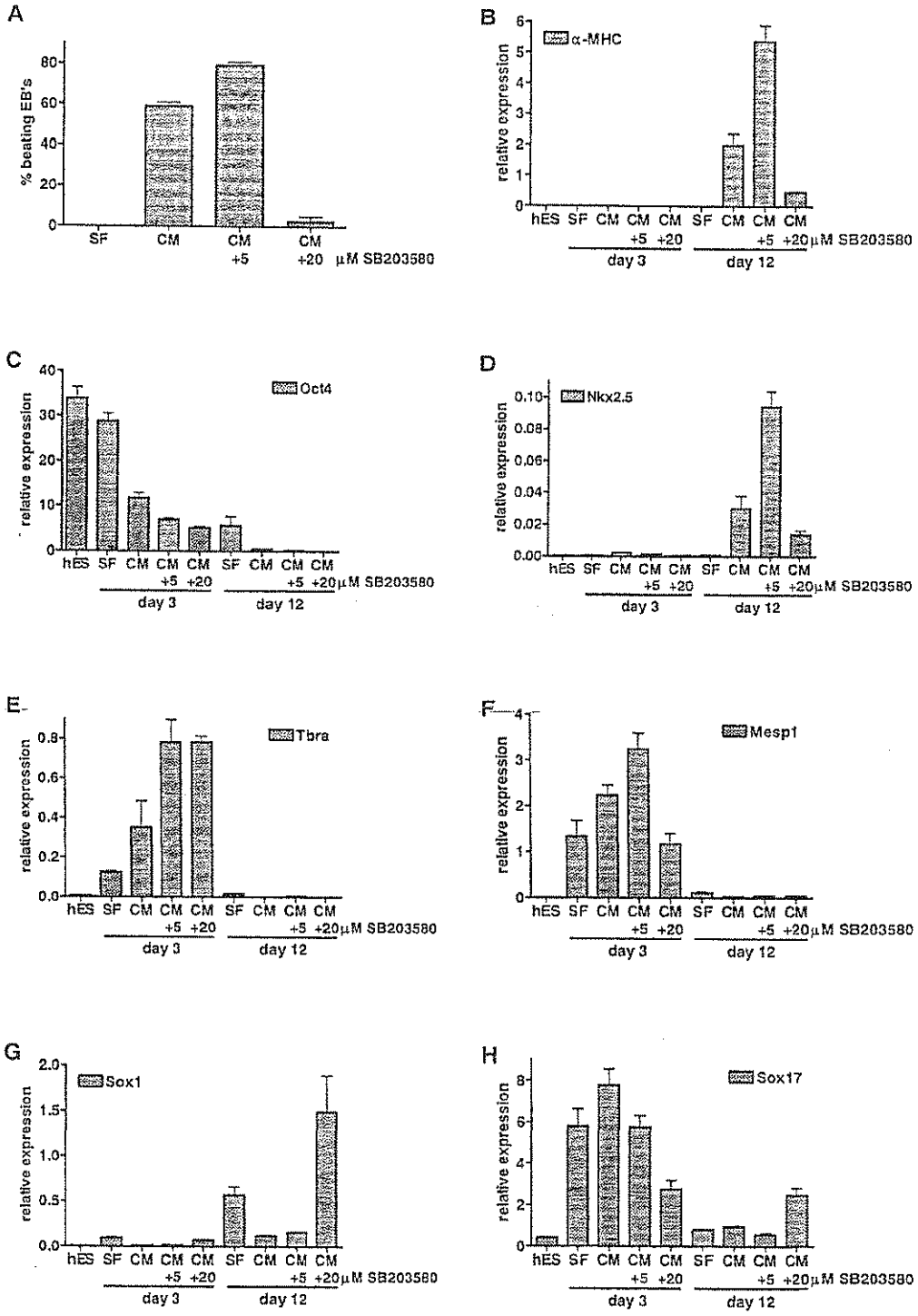
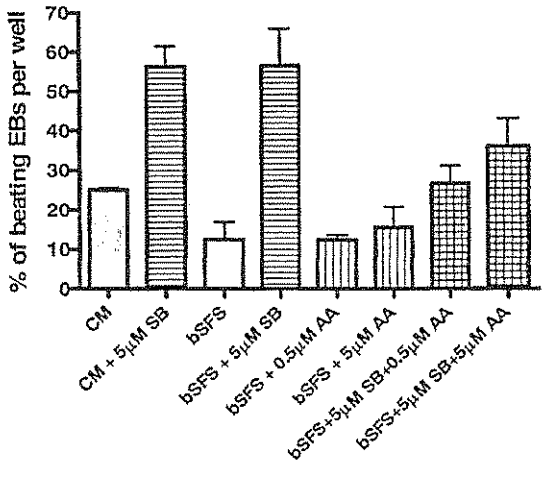
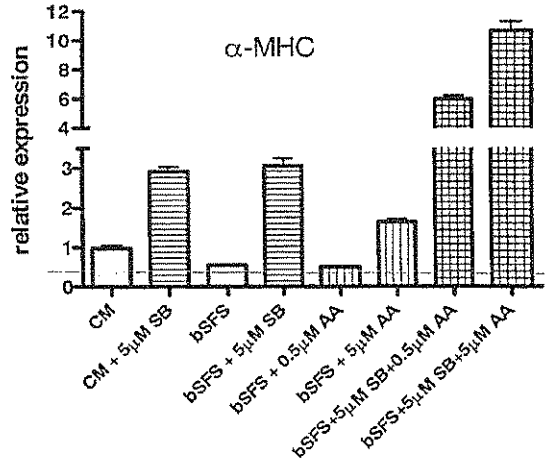

A
% beating EB's
SF
CM
CM +5
CM +20
μM SB203580
B
α-MHC
relative expression
hES SF CM CM CM SF CM CM CM
+5 +20 +5 +20 μM SB203580
day 3
day 12
C
Oct4
relative expression
D
Nkx2.5
relative expression
E
Tbra
relative expression
F
Mesp1
relative expression
G
Sox1
relative expression
H
Sox17
relative expression

FIG 24

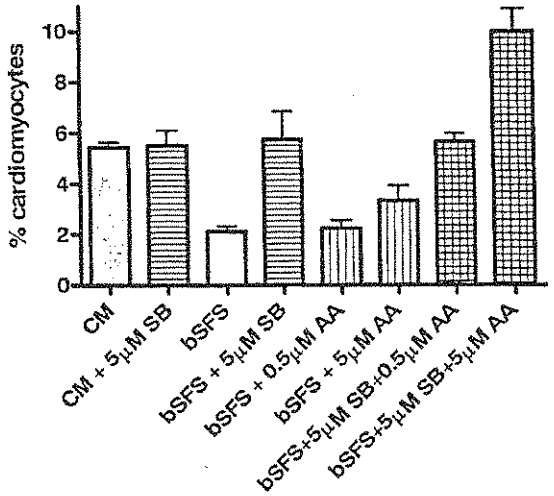

% of beating EBs per well
70
60
50
40
30
20
10
0
CM
CM + 5μM SB
bSFS
bSFS + 5μM SB
bSFS + 0.5μM AA
bSFS + 5μM AA
bSFS+5μM SB+0.5μM AA
bSFS+5μM SB+5μM AA

A


α-MHC
relative expression
12
10
8
6
4
3
2
1
0
CM
CM + 5μM SB
bSFS
bSFS + 5μM SB
bSFS + 0.5μM AA
bSFS + 5μM AA
bSFS+5μM SB+0.5μM AA
bSFS+5μM SB+5μM AA

B

FIG 25


% cardiomyocytes
10
8
6
4
2
0
CM
CM + 5μM SB
bSFS
bSFS + 5μM SB
bSFS + 0.5μM AA
bSFS + 5μM AA
bSFS+5μM SB+0.5μM AA
bSFS+5μM SB+5μM AA

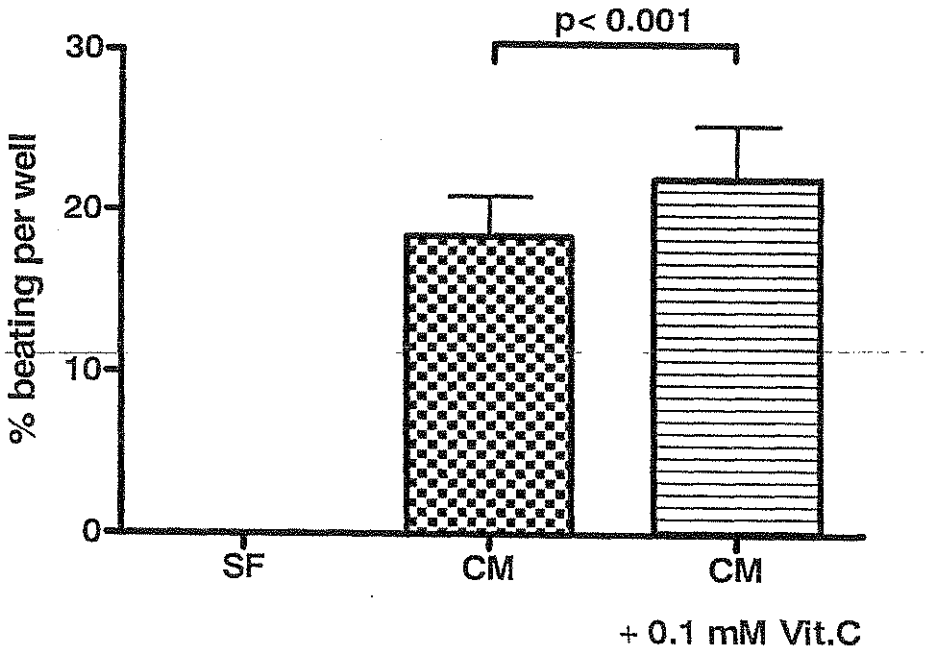


FIG 26

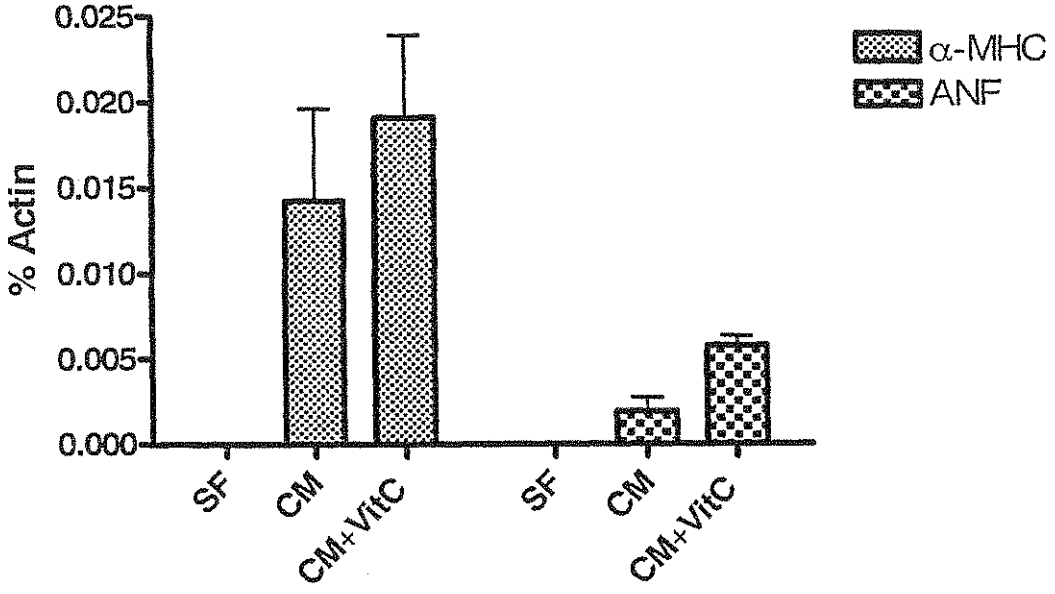
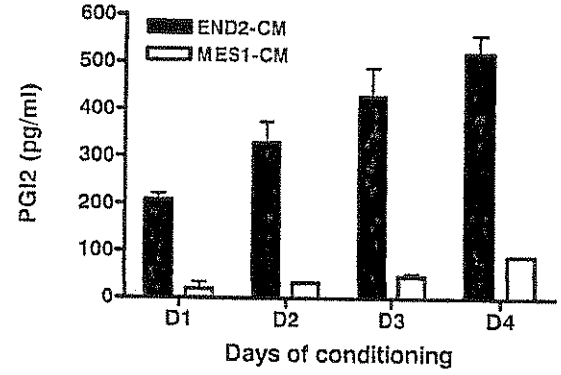
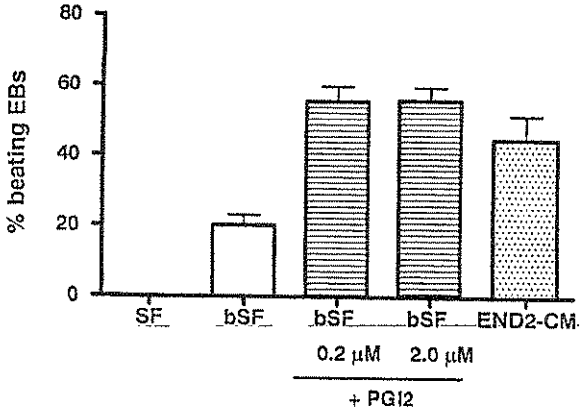

0.025
0.020
0.015
0.010
0.005
0.000
% Actin
α-MHC
ANF
SF
CM
CM+VitC
SF
CM
CM+VitC

FIG 27

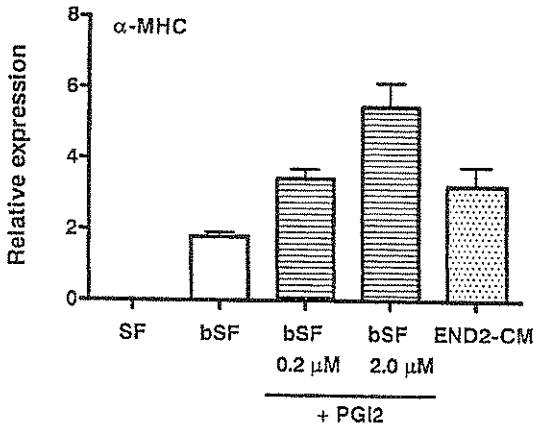

END2-CM
MES1-CM
PGI2 (pg/ml)
600
500
400
300
200
100
0
D1
D2
D3
D4
Days of conditioning

A


% beating EBs
80
60
40
20
0
SF
bSF
bSF
bSF
END2-CM
0.2 μM
2.0 μM
+ PGI2

B

FIG 28


α-MHC
Relative expression
8
6
4
2
0
SF
bSF
bSF
bSF
END2-CM
0.2 μM
2.0 μM
+ PGI2

C