Figure 1

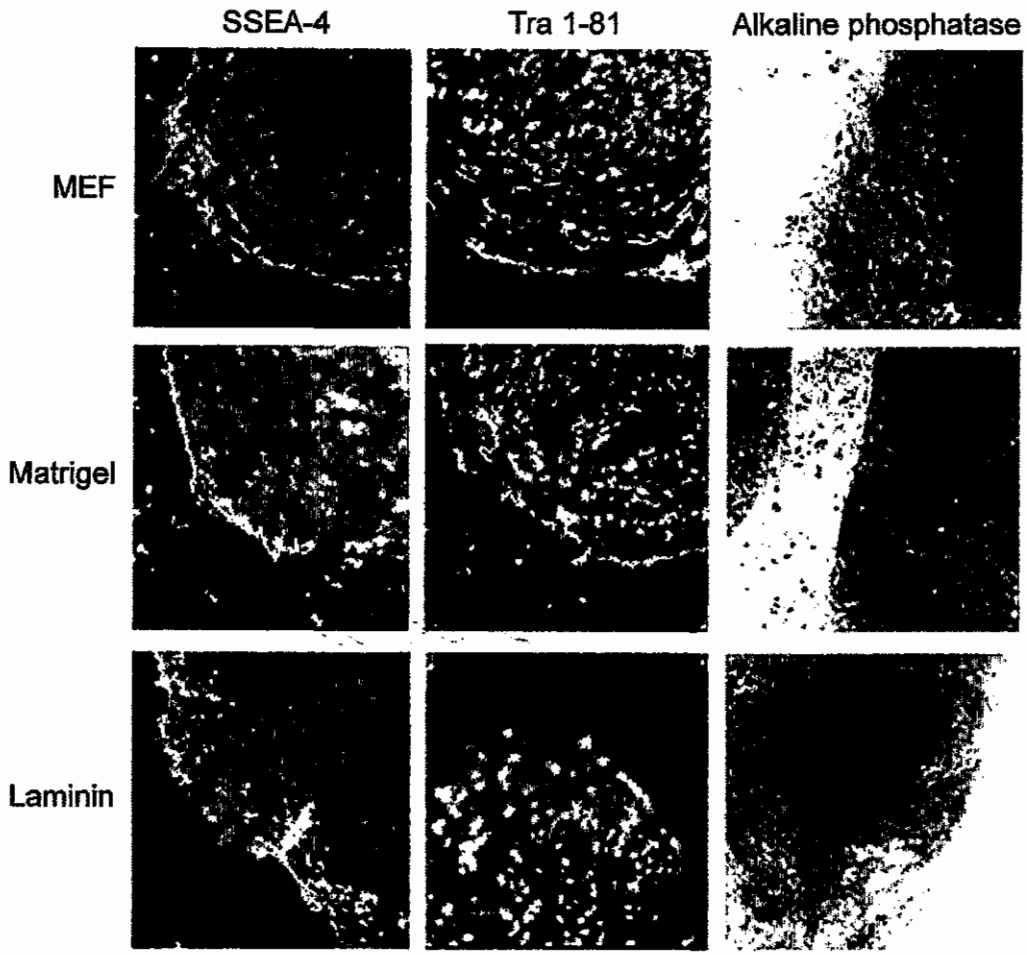


Figure 2

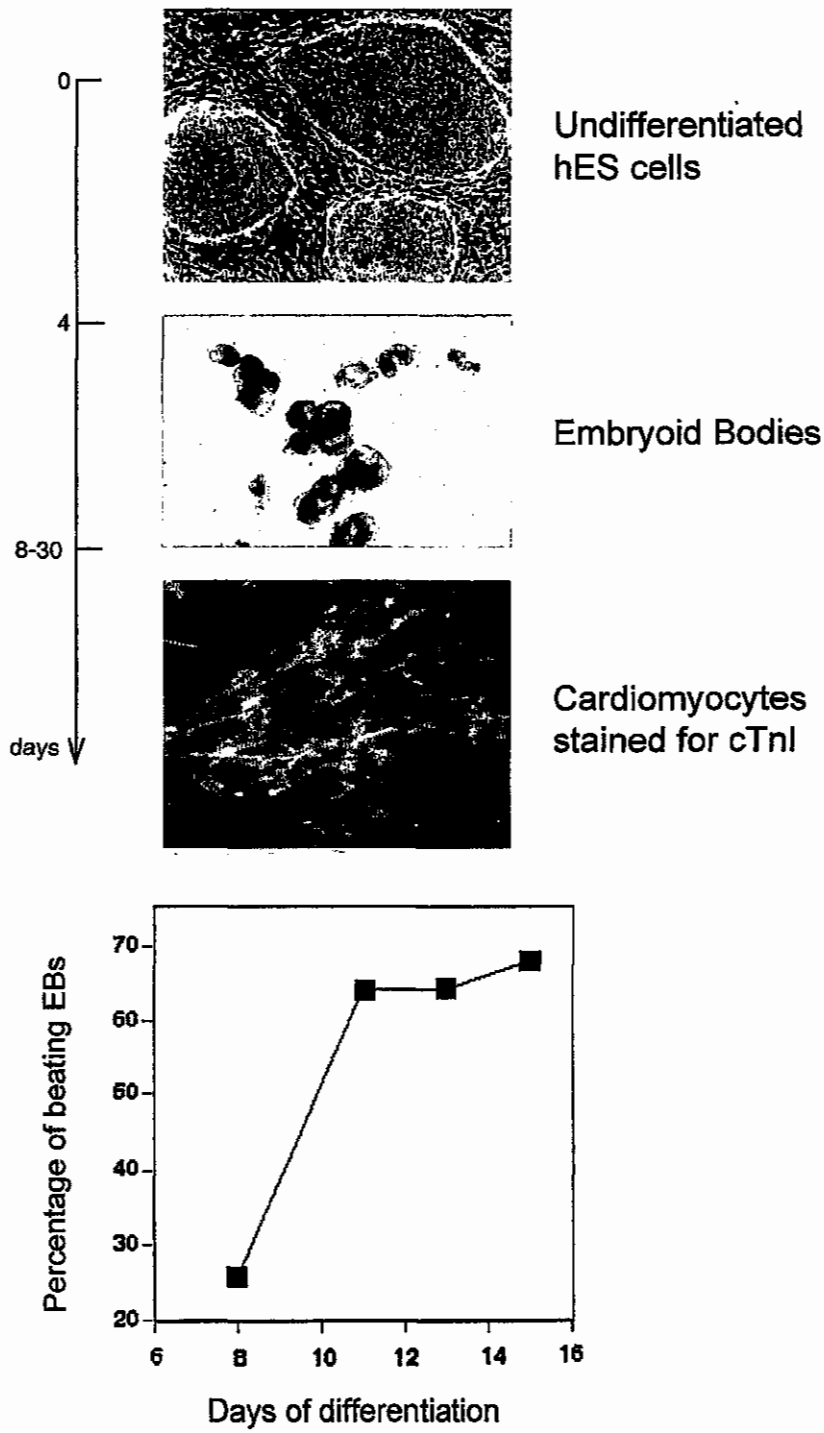
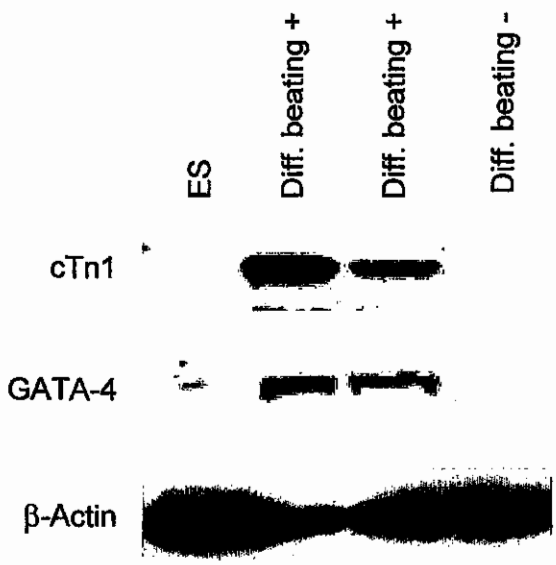

0
4
8-30
days
Undifferentiated hES cells
Embryoid Bodies
Cardiomyocytes stained for cTnI
Percentage of beating EBs
70
60
50
40
30
20
6
8
10
12
14
16
Days of differentiation

Figure 3


ES
Diff. beating +
Diff. beating +
Diff. beating -
cTn1
GATA-4
β-Actin

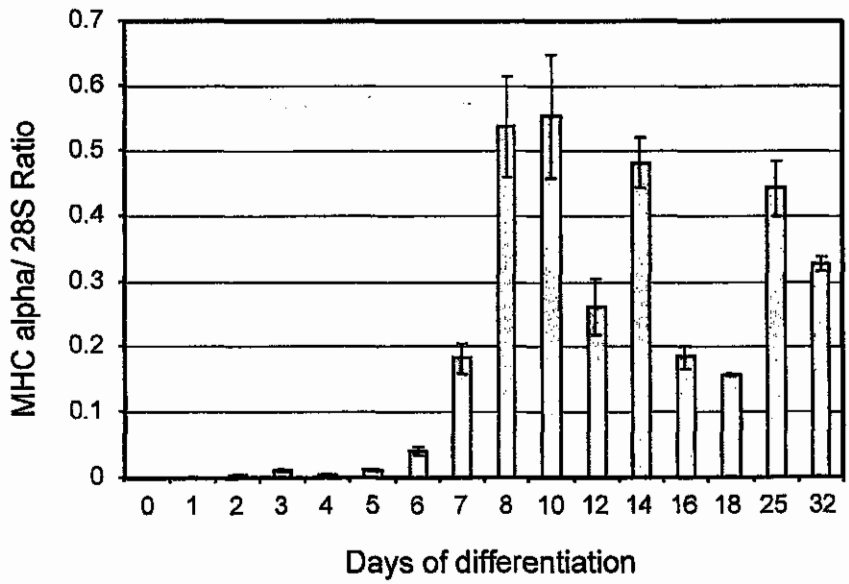

MHC alpha/ 28S Ratio
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0 1 2 3 4 5 6 7 8 10 12 14 16 18 25 32
Days of differentiation

Figure 4

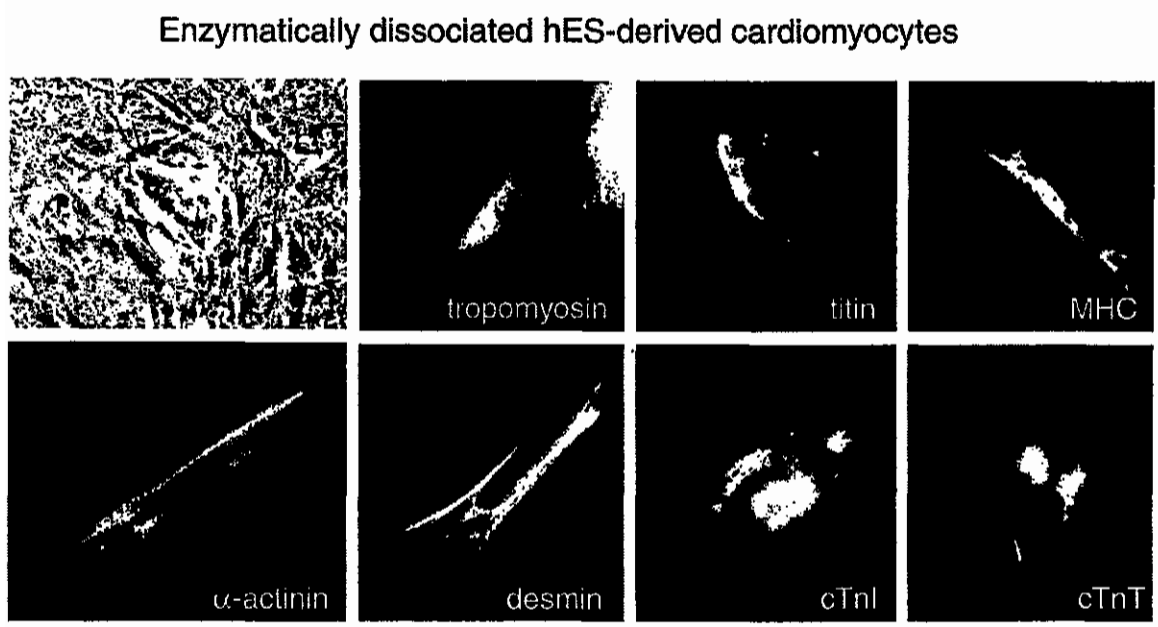

Enzymatically dissociated hES-derived cardiomyocytes
tropomyosin
titin
MHC
α-actinin
desmin
cTnI
cTnT

Figure 5

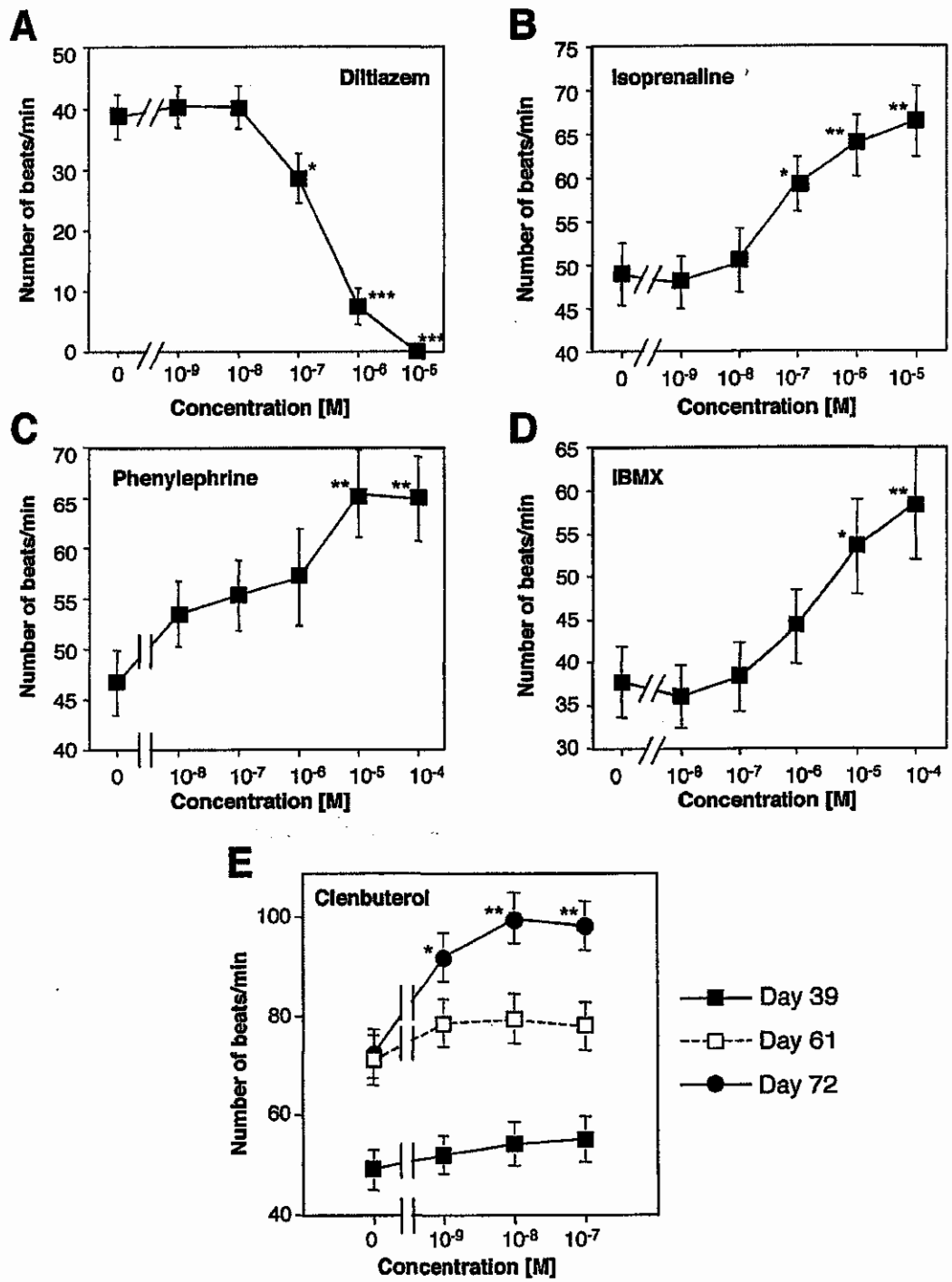

A
Diltiazem
Number of beats/min
Concentration [M]
B
Isoprenaline
Number of beats/min
Concentration [M]
C
Phenylephrine
Number of beats/min
Concentration [M]
D
IBMX
Number of beats/min
Concentration [M]
E
Clenbuterol
Number of beats/min
Concentration [M]
Day 39
Day 61
Day 72

Figure 6

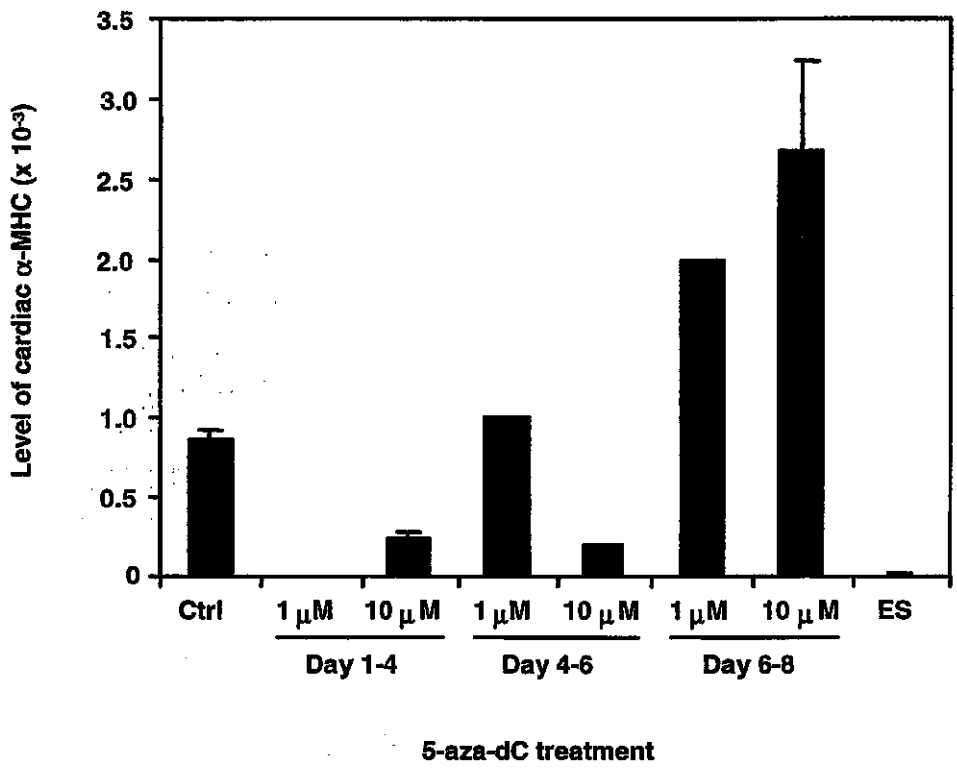
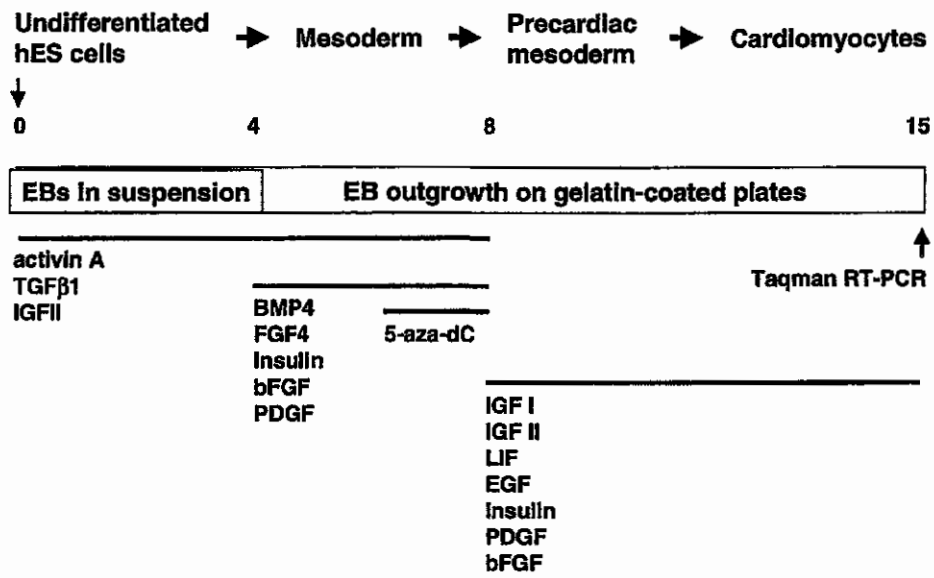

Level of cardiac α-MHC (x 10⁻³)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
Ctrl
1 μM
10 μM
1 μM
10 μM
1 μM
10 μM
ES
Day 1-4
Day 4-6
Day 6-8
5-aza-dC treatment

Figure 7


Undifferentiated hES cells
Mesoderm
Precardiac mesoderm
Cardiomyocytes
0
4
8
15
EBs in suspension
EB outgrowth on gelatin-coated plates
activin A
TGFβ1
IGFII
BMP4
FGF4
Insulin
bFGF
PDGF
5-aza-dC
Taqman RT-PCR
IGF I
IGF II
LIF
EGF
Insulin
PDGF
bFGF

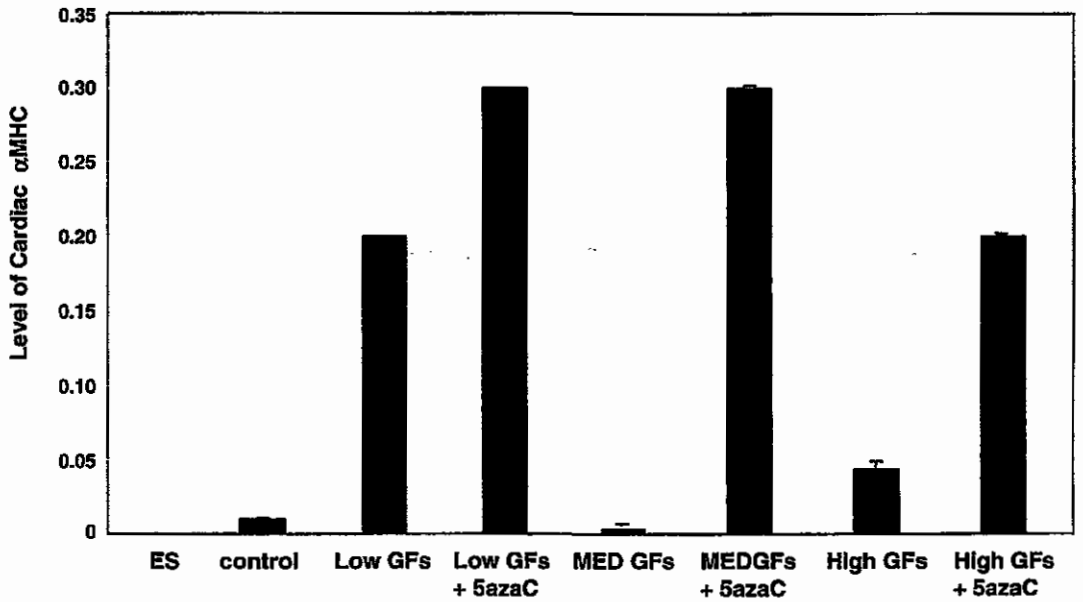
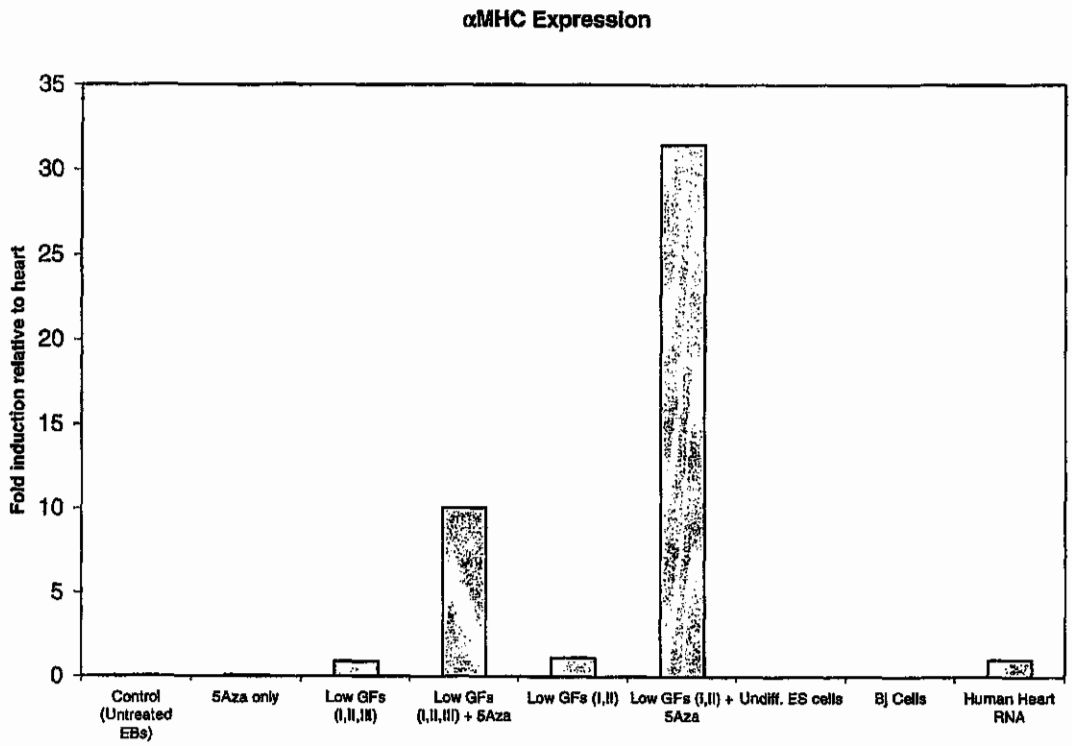

Level of Cardiac αMHC
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
ES
control
Low GFs
Low GFs + 5azaC
MED GFs
MEDGFs + 5azaC
High GFs
High GFs + 5azaC

Figure 8(A)


αMHC Expression
Fold induction relative to heart
35
30
25
20
15
10
5
0
Control (Untreated EBs)
5Aza only
Low GFs (I,II,III)
Low GFs (I,II,III) + 5Aza
Low GFs (I,II)
Low GFs (I,II) + 5Aza
Undiff. ES cells
BJ Cells
Human Heart RNA

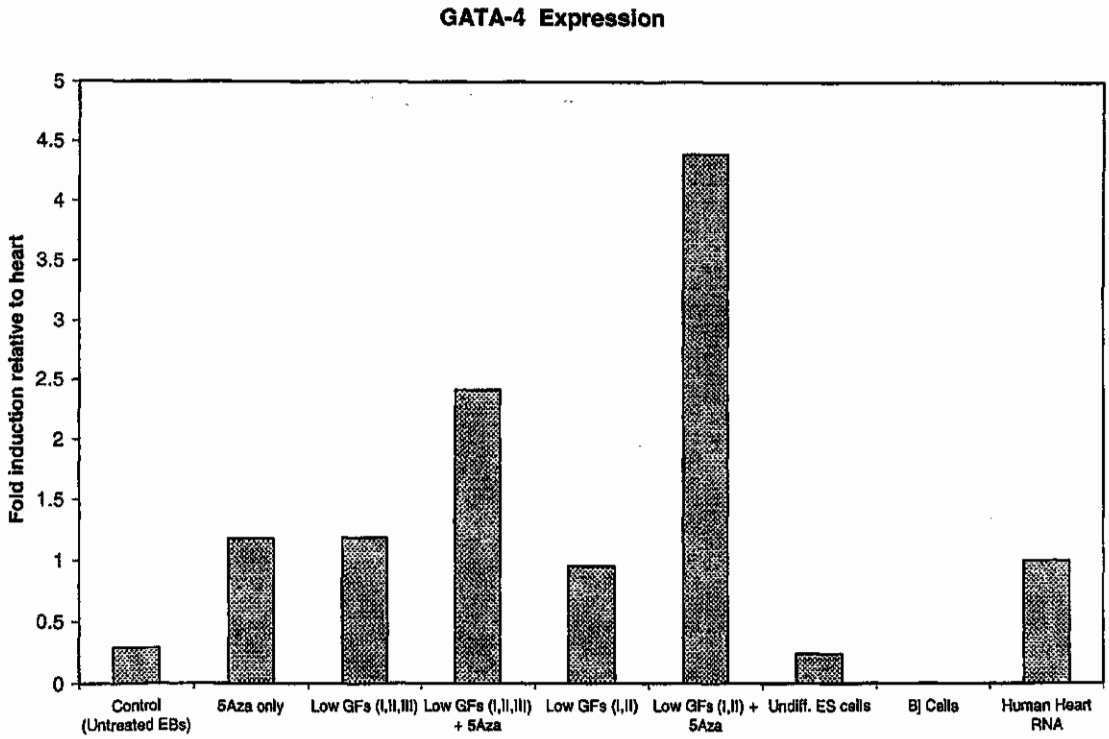

GATA-4 Expression
Fold induction relative to heart
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Control (Untreated EBs)
5Aza only
Low GFs (I,II,III)
Low GFs (I,II,III) + 5Aza
Low GFs (I,II)
Low GFs (I,II) + 5Aza
Undiff. ES cells
BJ Cells
Human Heart RNA

Figure 8(B)

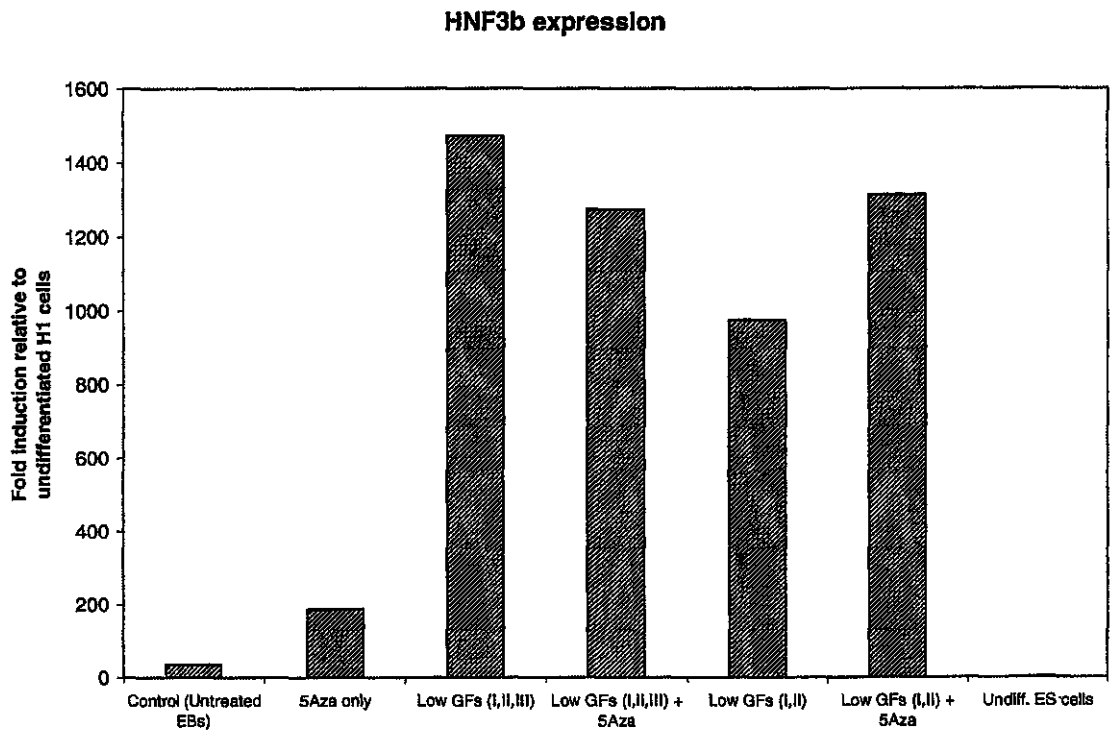
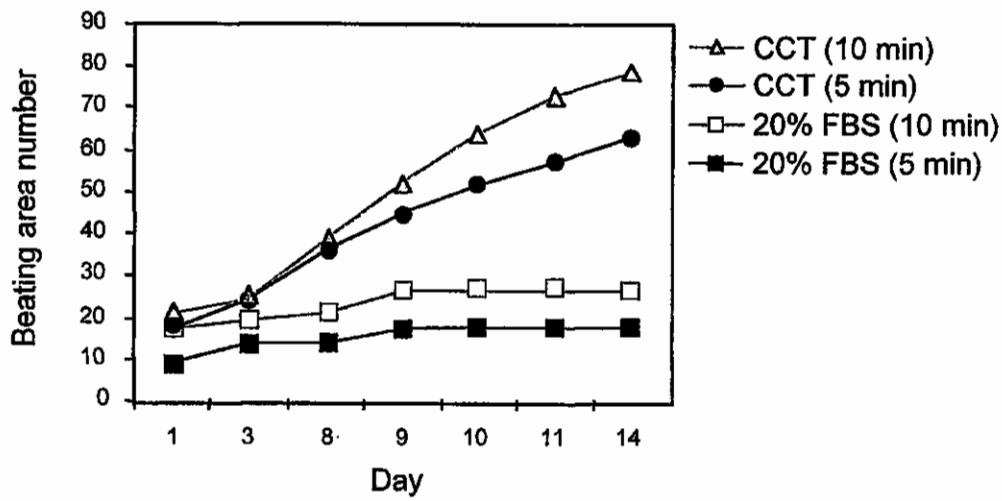
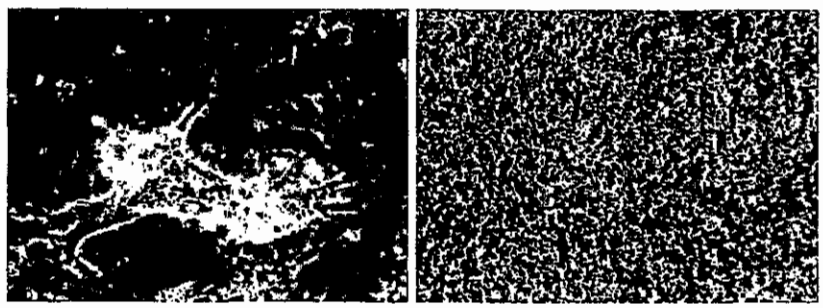

HNF3b expression
Fold induction relative to undifferentiated H1 cells
1600
1400
1200
1000
800
600
400
200
0
Control (Untreated EBs)
5Aza only
Low GFs (I,II,III)
Low GFs (I,II,III) + 5Aza
Low GFs (I,II)
Low GFs (I,II) + 5Aza
Undiff. ES cells

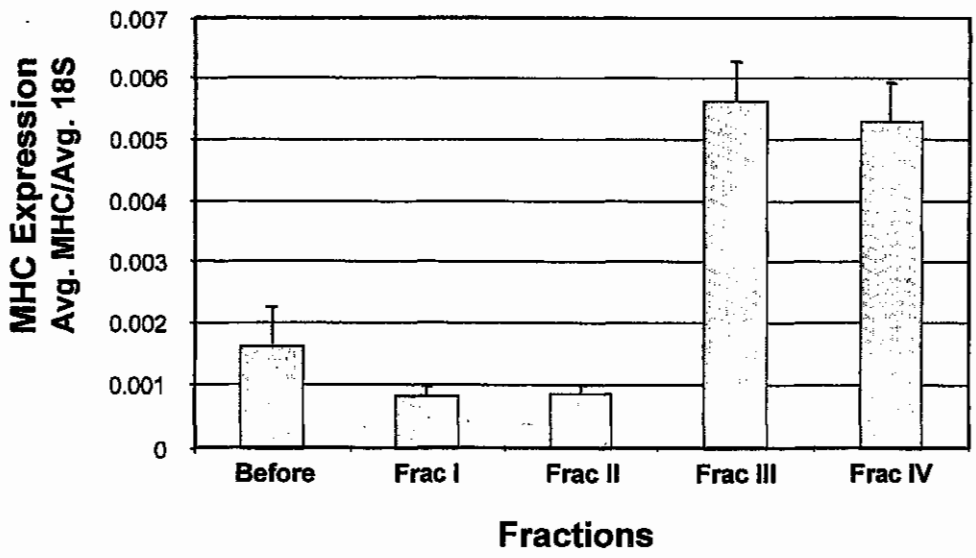
Figure 9





Creatine, carnitine & taurine 20% FBS

Figure 10


MHC Expression
Avg. MHC/Avg. 18S
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0
Before
Frac I
Frac II
Frac III
Frac IV
Fractions