Figure 1

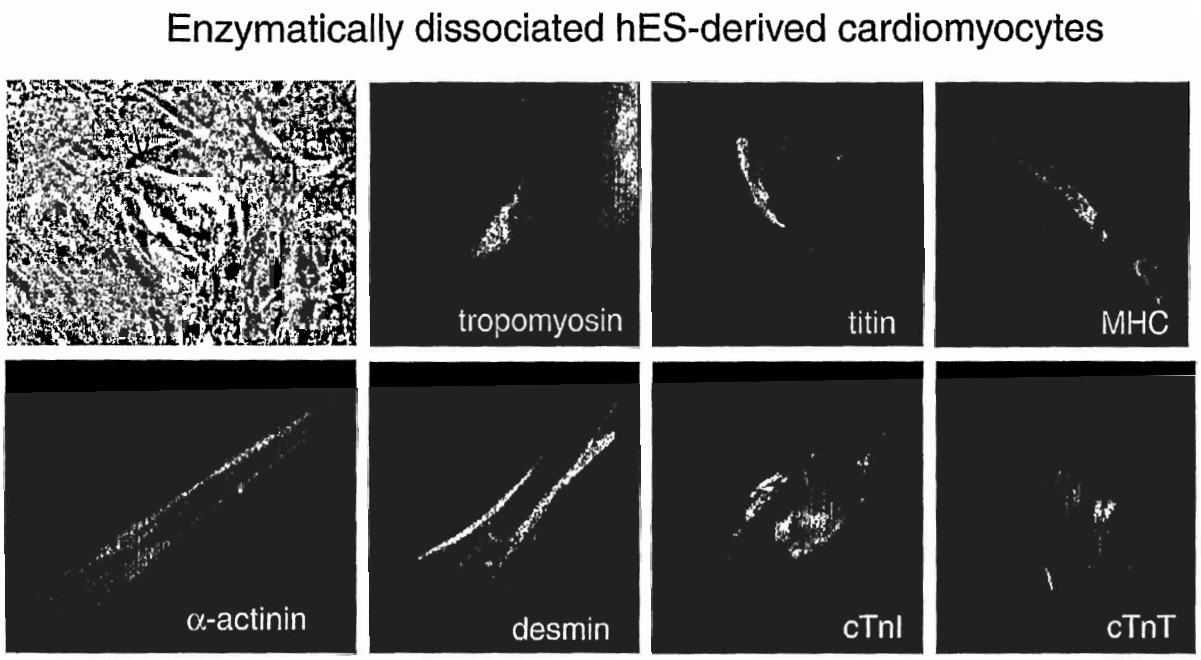

Enzymatically dissociated hES-derived cardiomyocytes
tropomyosin
titin
MHC
α-actinin
desmin
cTnI
cTnT

Figure 2

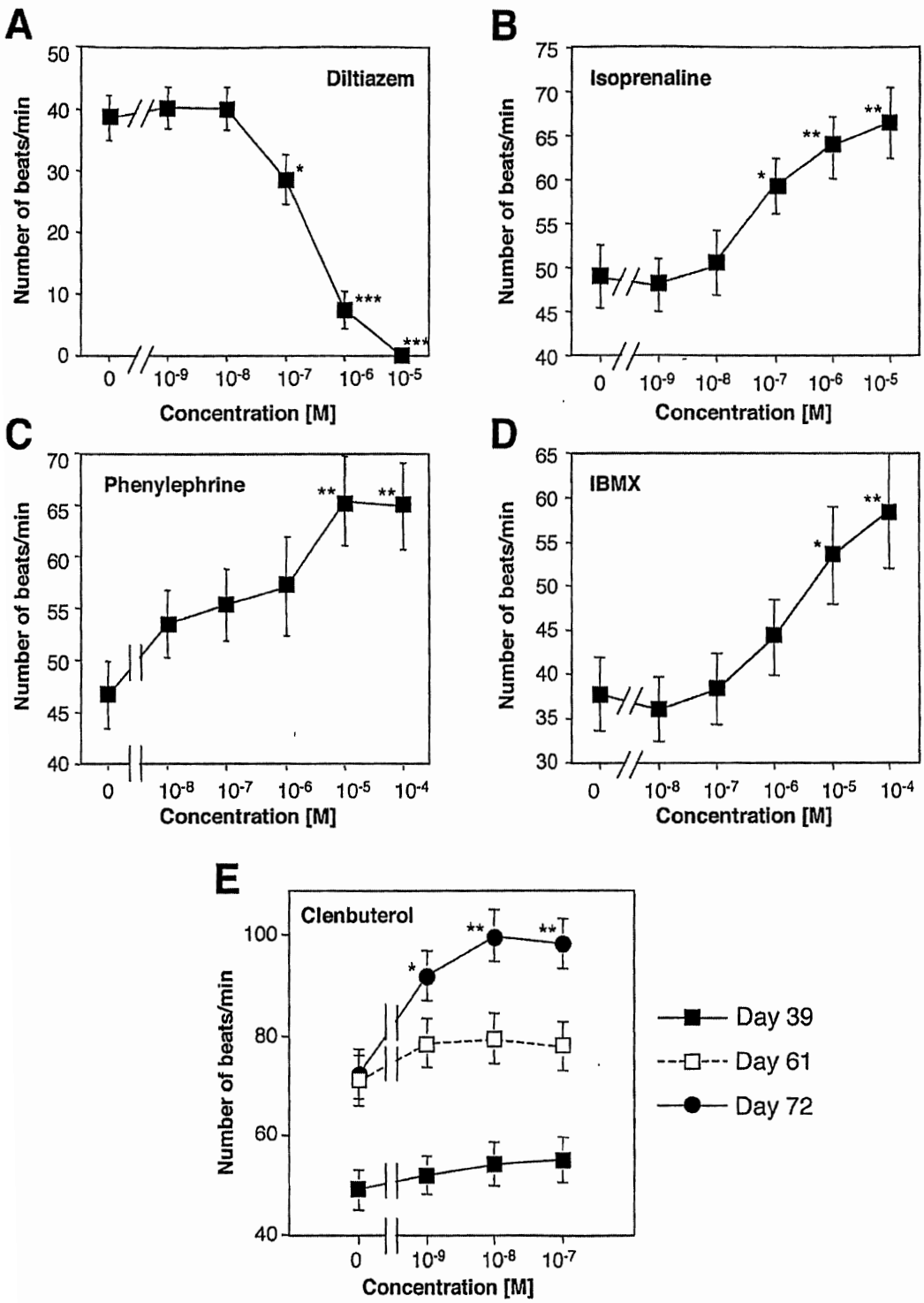
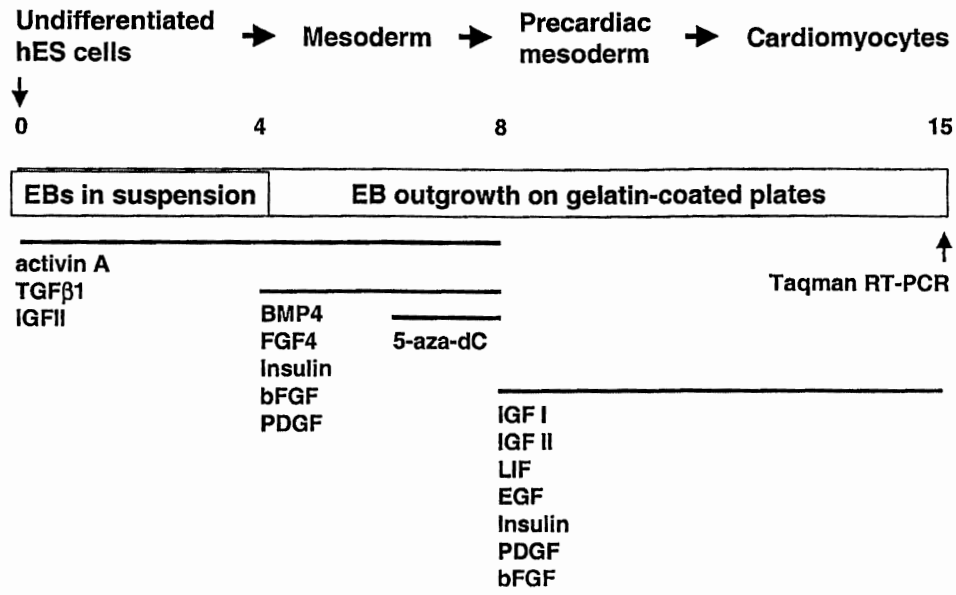

A
Diltiazem
Number of beats/min
Concentration [M]
B
Isoprenaline
Number of beats/min
Concentration [M]
C
Phenylephrine
Number of beats/min
Concentration [M]
D
IBMX
Number of beats/min
Concentration [M]
E
Clenbuterol
Number of beats/min
Concentration [M]
Day 39
Day 61
Day 72

Figure 3


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
IGF I
IGF II
LIF
EGF
Insulin
PDGF
bFGF
Taqman RT-PCR

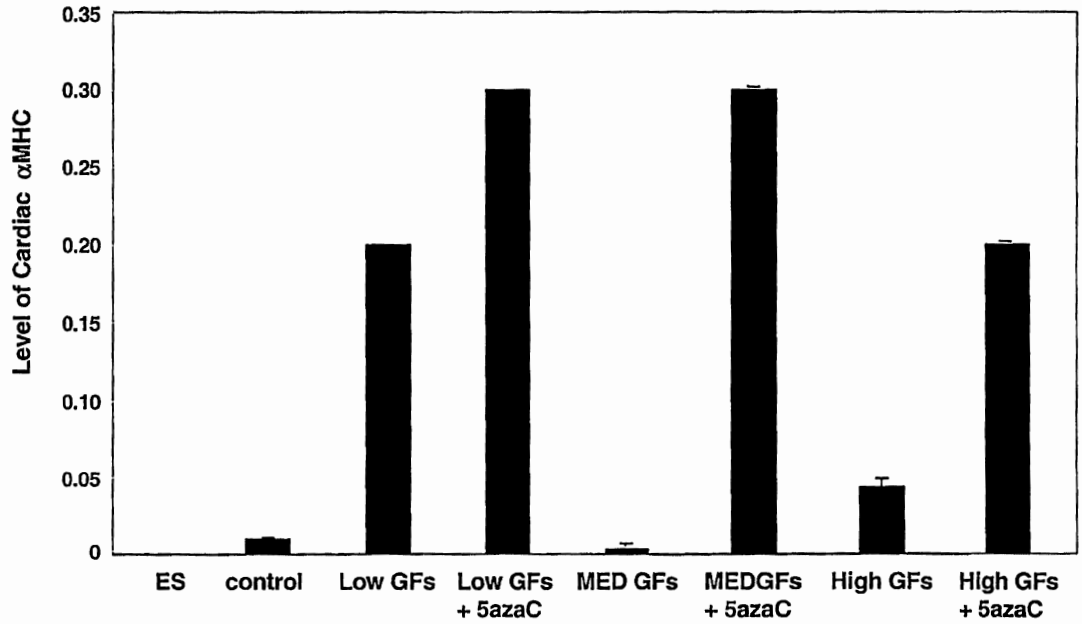
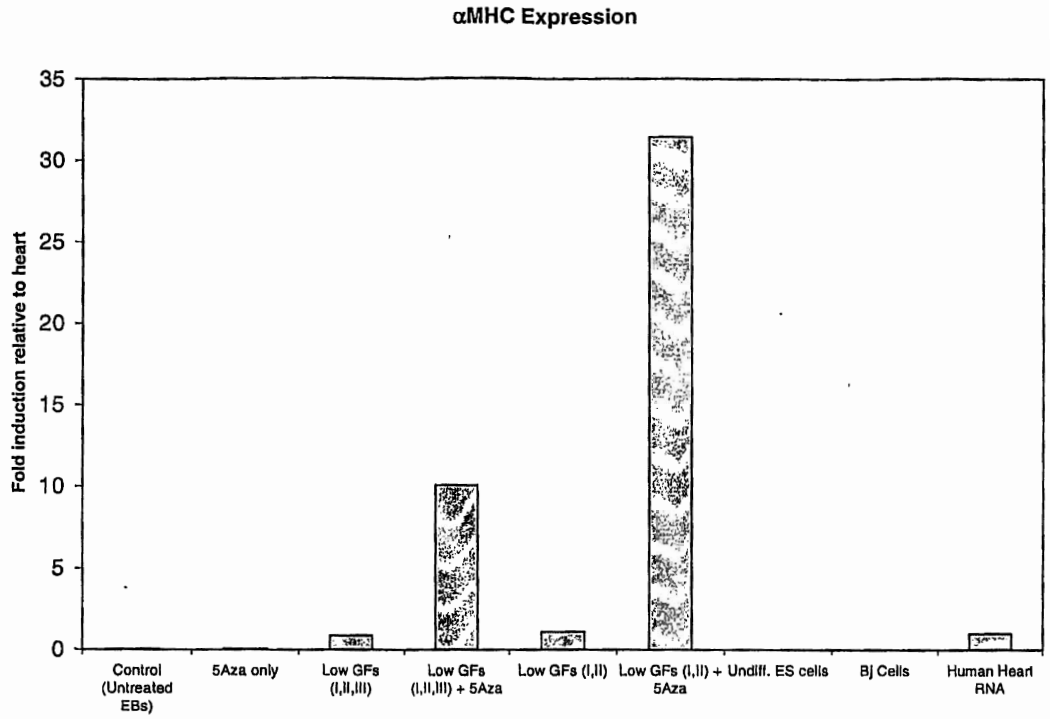

Level of Cardiac αMHC
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
ES
control
Low GFs
Low GFs + 5azaC
MED GFs
MEDGFs + 5azaC
High GFs
High GFs + 5azaC

Figure 4(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

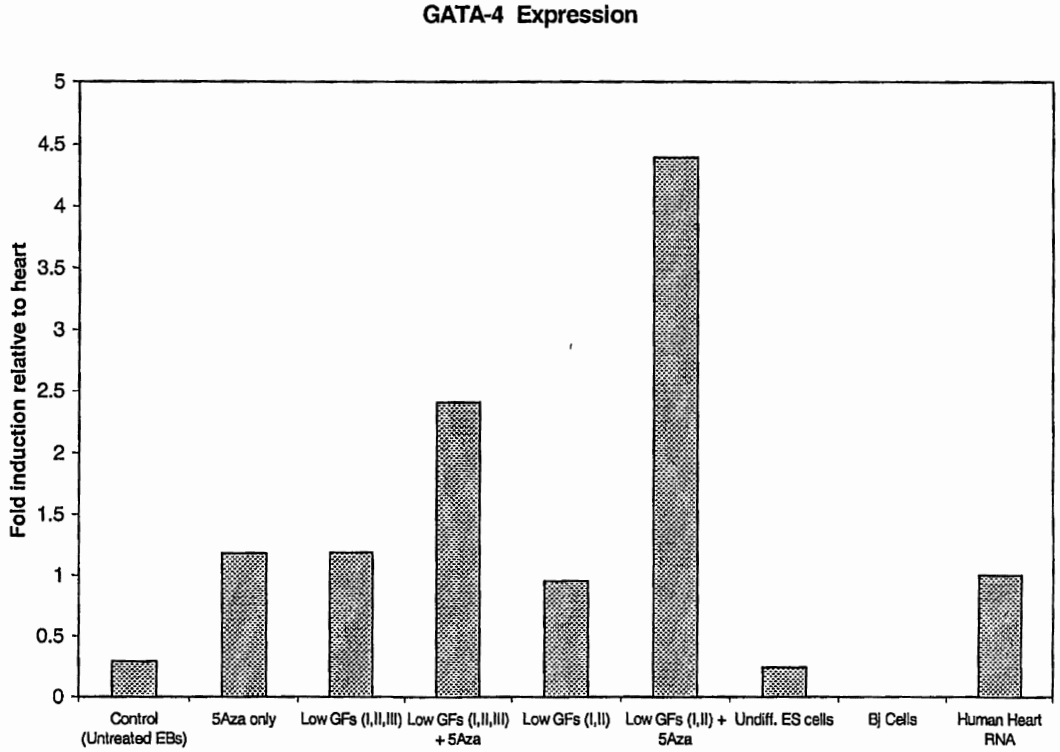

GATA-4 Expression
Fold induction relative to heart
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Control (Untreated EBs)
5Aza only
Low GFs (I,II,III)
Low GFs (I,II,III) + 5Aza
Low GFs (I,II)
Low GFs (I,II) + 5Aza
Undiff. ES cells
Bj Cells
Human Heart RNA

Figure 4(B)

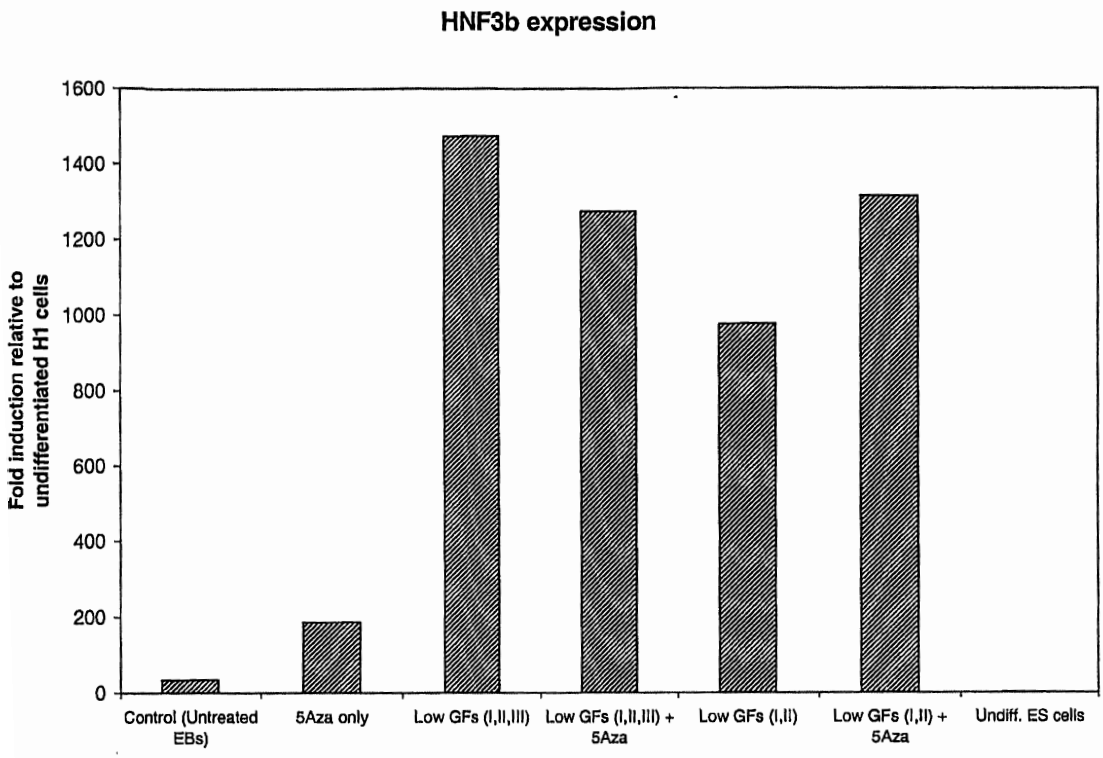

HNF3b expression
Fold induction relative to undifferentiated H1 cells
1600
1400
1200
1000
800
600
400
200
0
Control (Untreated EBs)
5Aza only
Low GFs (I,II,III)
Low GFs (I,II,III) + 5Aza
Low GFs (I,II)
Low GFs (I,II) + 5Aza
Undiff. ES cells

Figure 5(A)

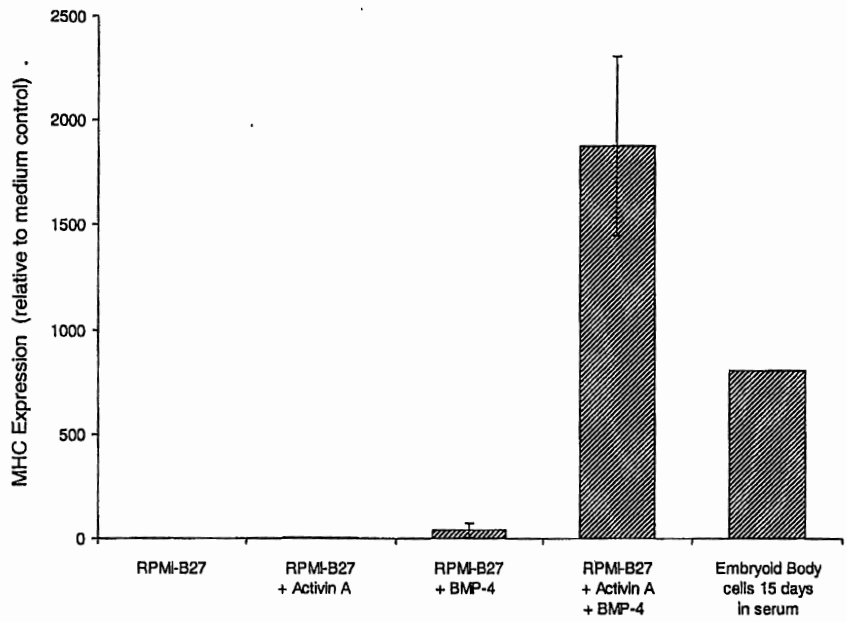


Figure 5(B)

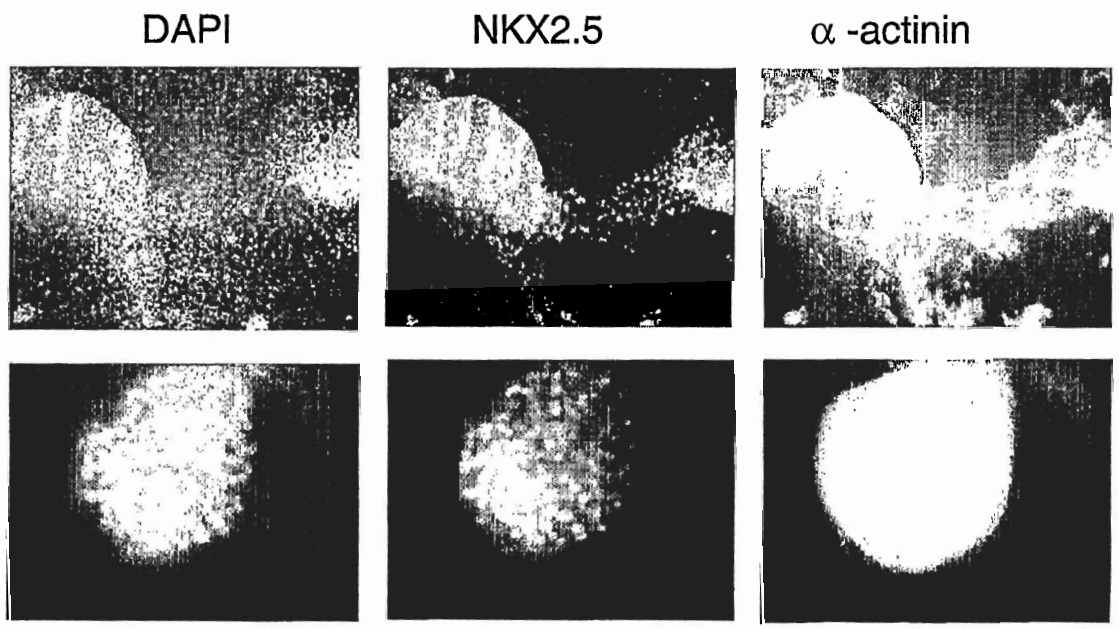


Figure 6

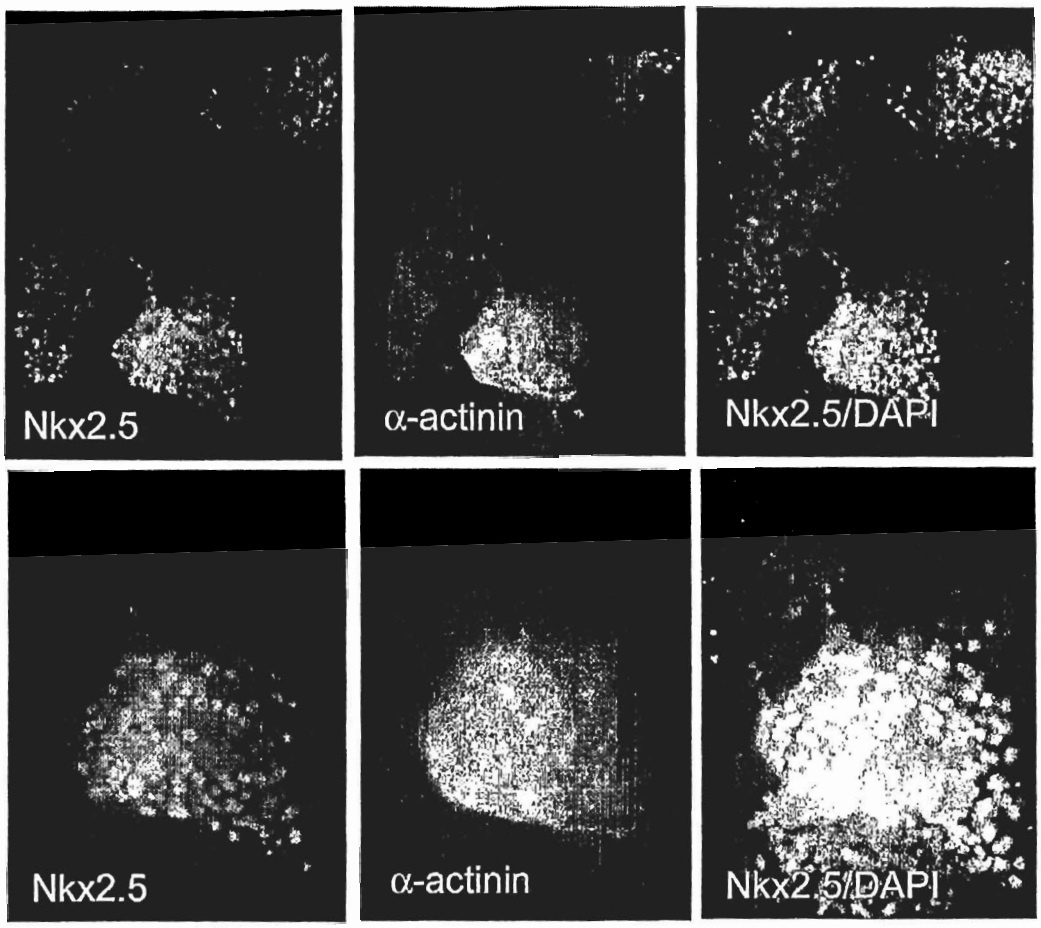
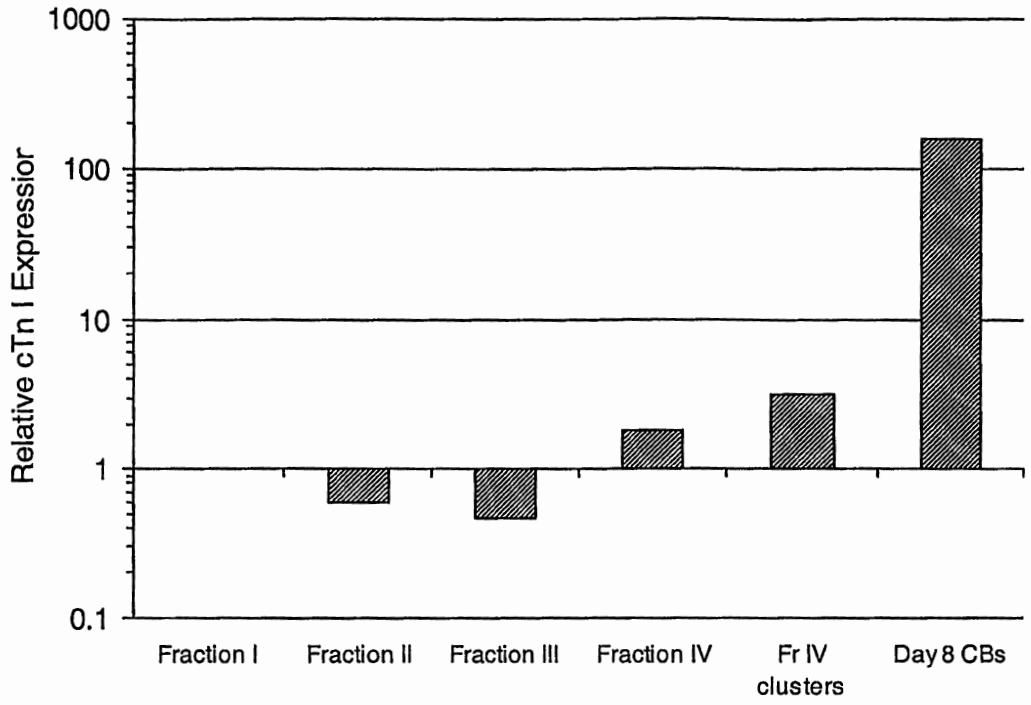


Figure 7


1000
100
10
1
0.1
Relative cTn I Expressior
Fraction I
Fraction II
Fraction III
Fraction IV
Fr IV clusters
Day 8 CBs

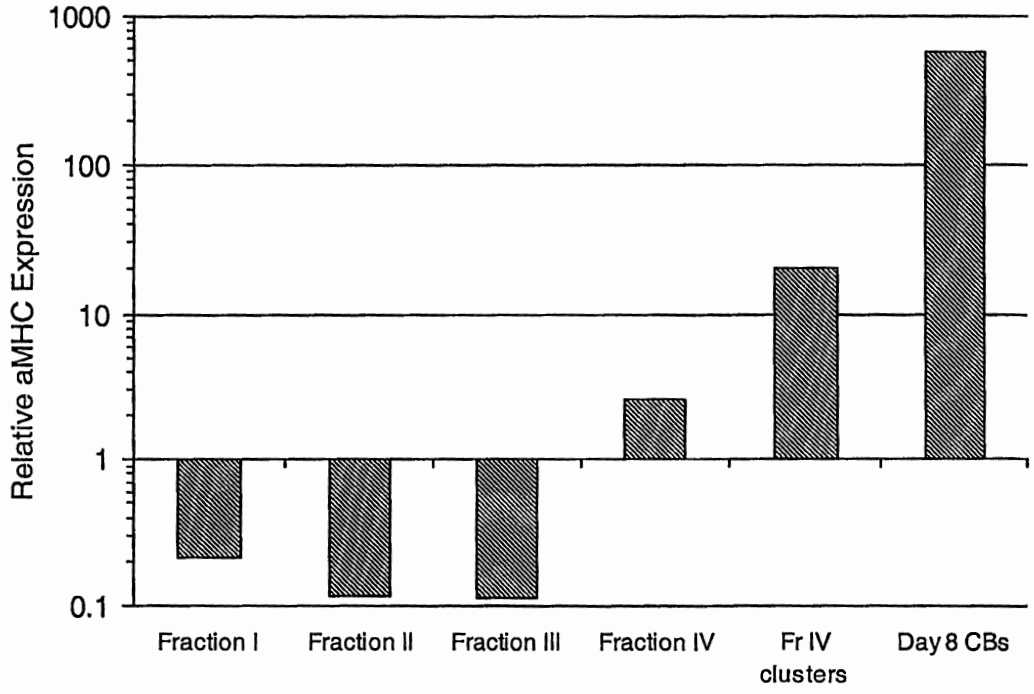

1000
100
10
1
0.1
Relative aMHC Expression
Fraction I
Fraction II
Fraction III
Fraction IV
Fr IV clusters
Day 8 CBs

Figure 8(A)

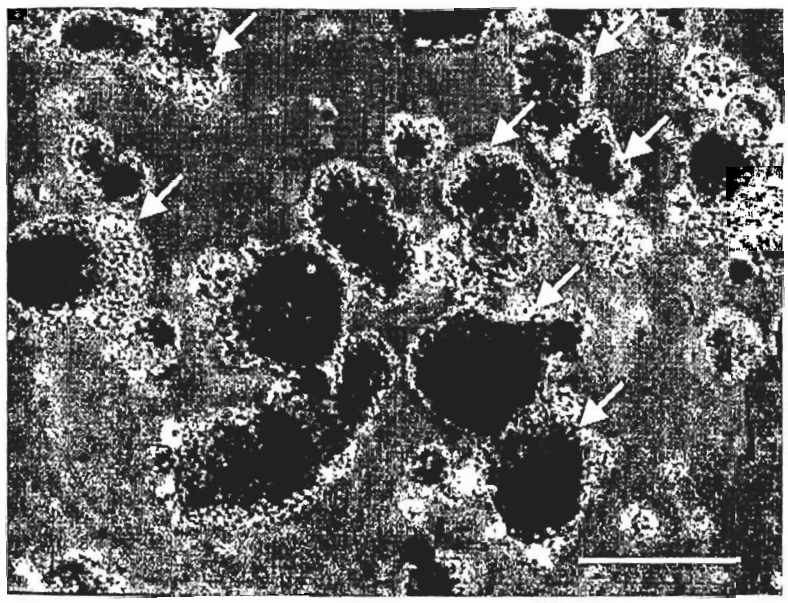


Figure 8(B)

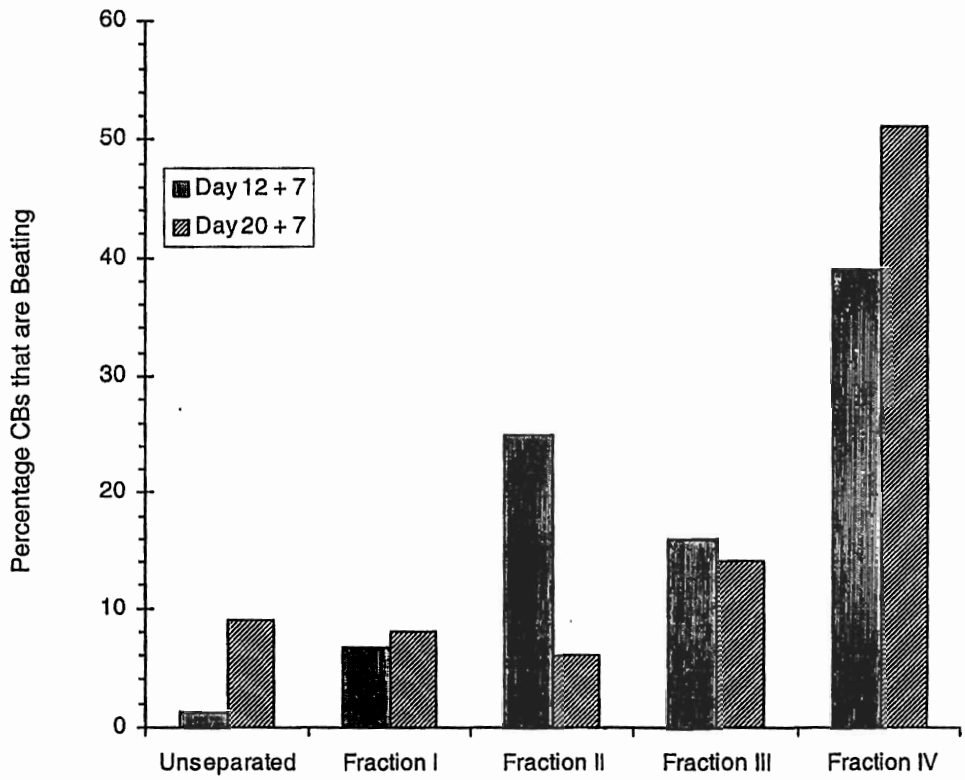


Figure 9

