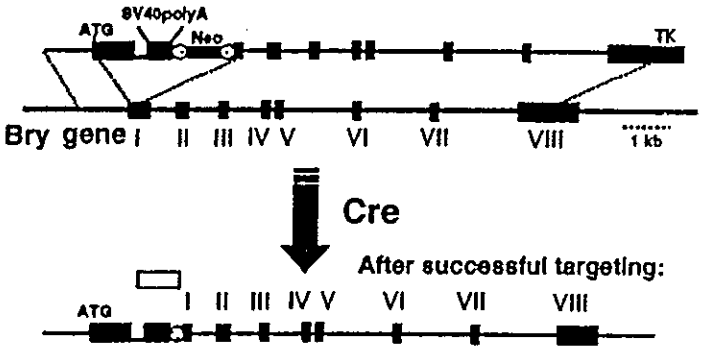

ATG
SV40polyA
Neo
TK
Bry gene
I
II
III
IV
V
VI
VII
VIII
1 kb
Cre
After successful targeting:
ATG
I
II
III
IV
V
VI
VII
VIII

Fig. 1

Fig. 2A

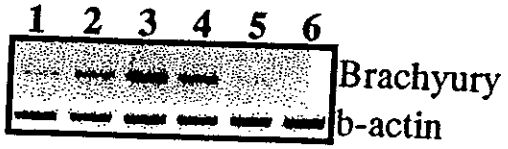

1 2 3 4 5 6
Brachyury
b-actin

Fig. 2B

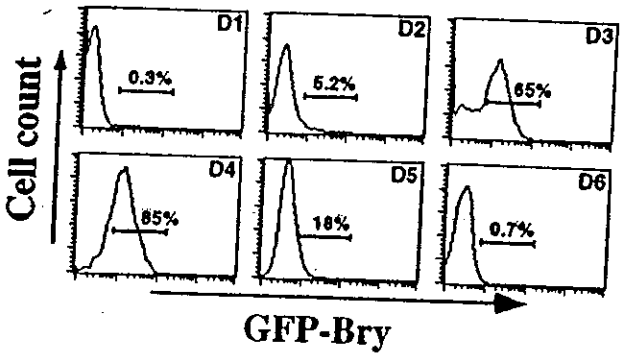

D1
0.3%
D2
5.2%
D3
65%
D4
65%
D5
16%
D6
0.7%
Cell count
GFP-Bry

Fig. 3A

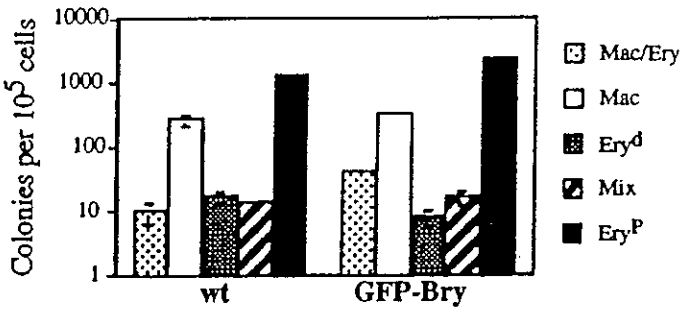


Fig. 3B

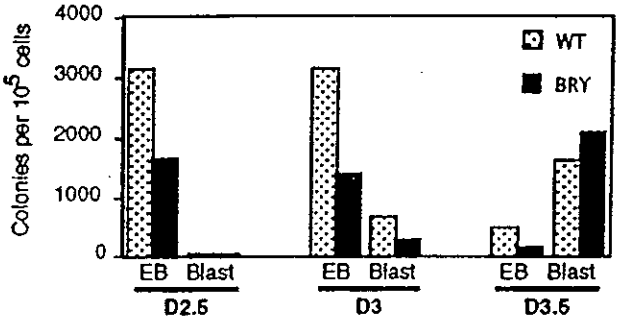


Fig. 3C

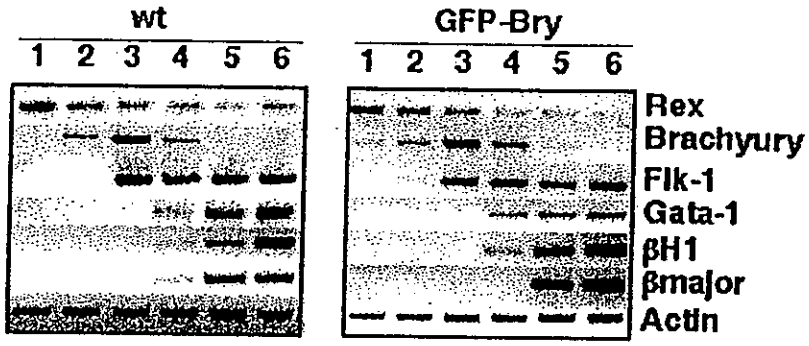


Fig. 4A

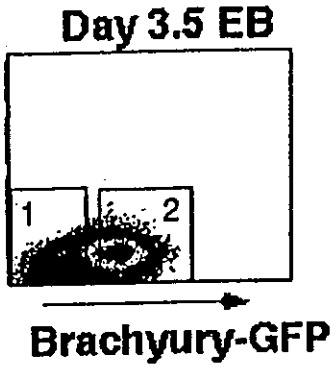

Day 3.5 EB
1
2
Brachyury-GFP

Fig. 4B

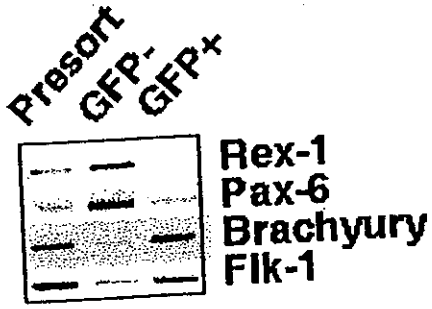

Presort
GFP-
GFP+
Rex-1
Pax-6
Brachyury
Flk-1

Fig. 5A

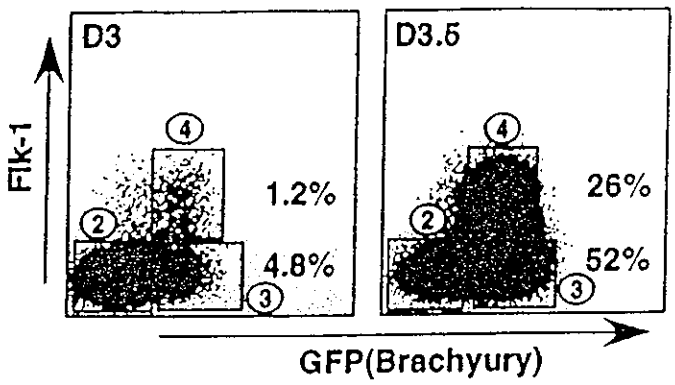


Fig. 5B

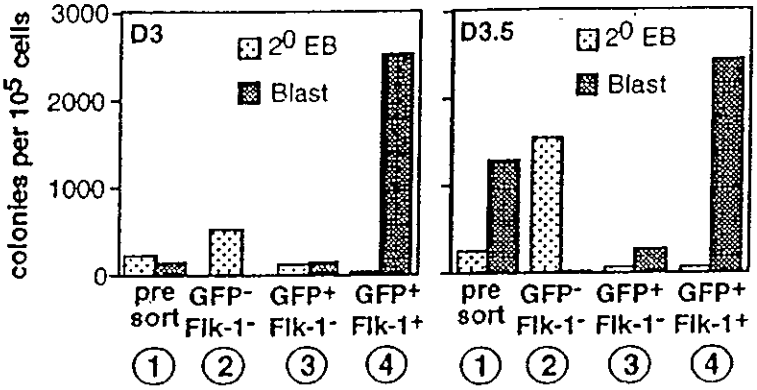
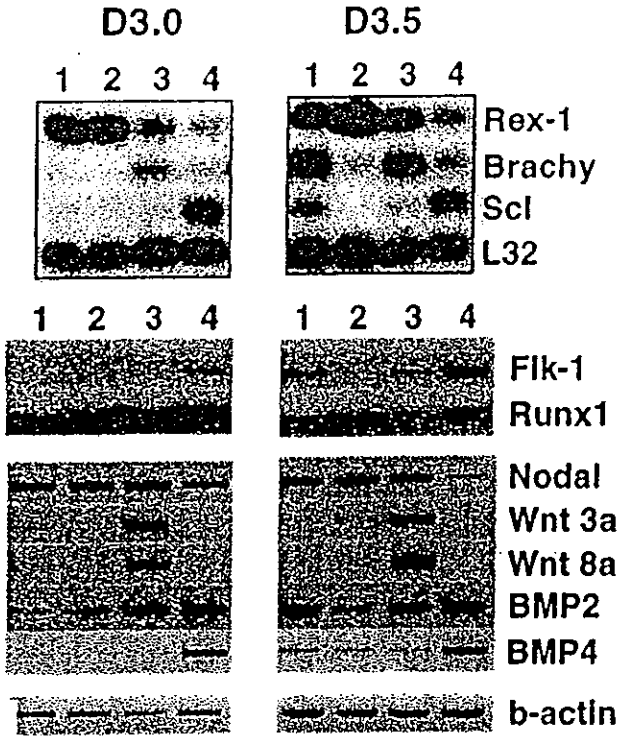


Fig. 5C



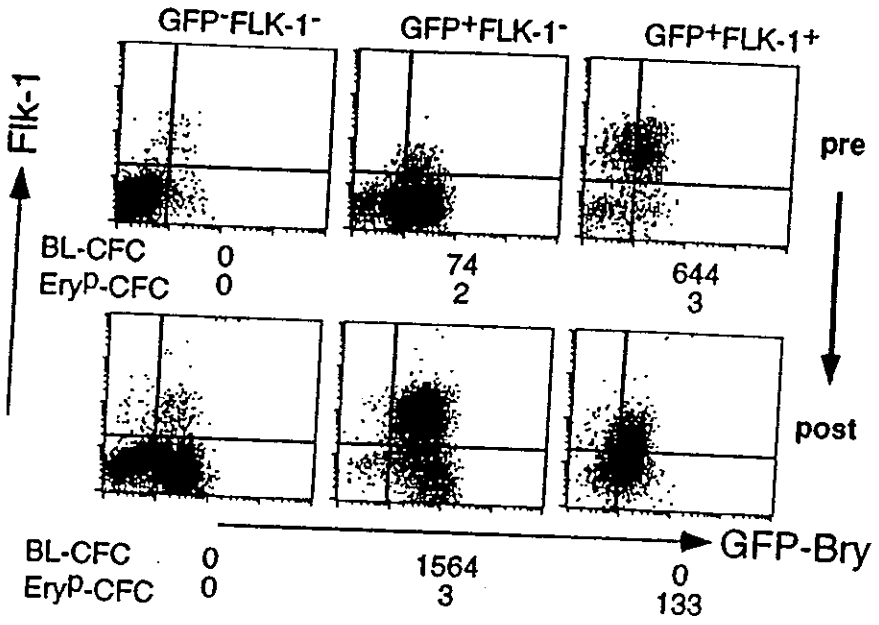

GFP-FLK-1-
GFP+FLK-1-
GFP+FLK-1+
Flk-1
pre
post
BL-CFC 0 74 644
EryP-CFC 0 2 3
BL-CFC 0 1564 0
EryP-CFC 0 3 133
GFP-Bry

Fig. 6

Fig. 7A

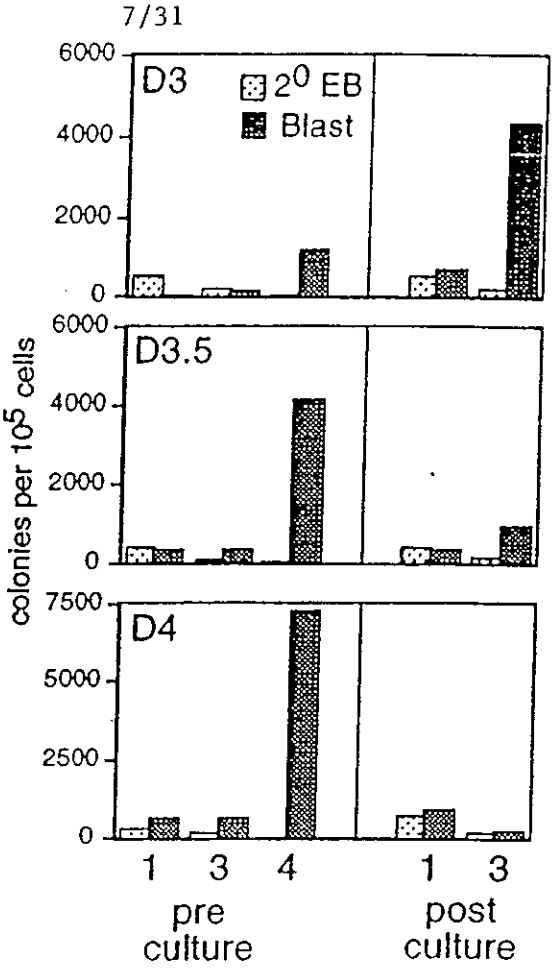

D3
2^0 EB
Blast
D3.5
D4
colonies per 10^5 cells
6000
4000
2000
0
7500
5000
2500
1 3 4
pre culture
1 3
post culture

Fig. 7 B

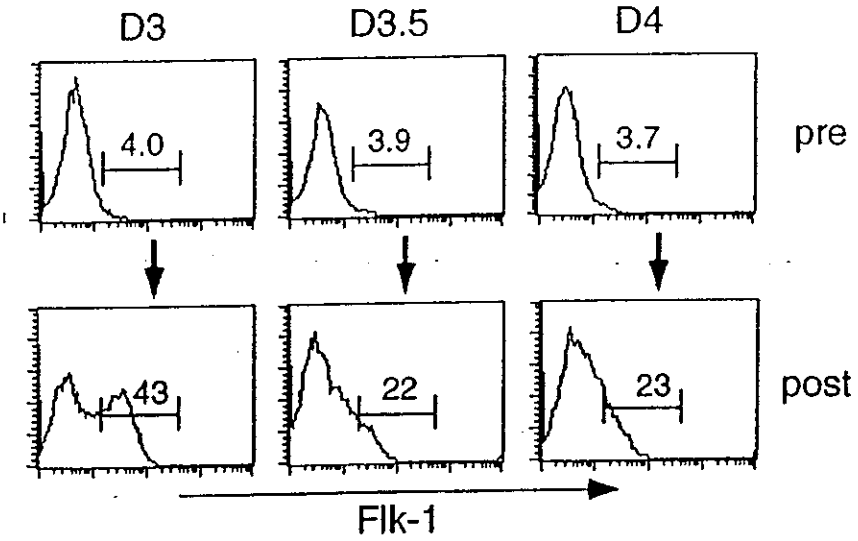
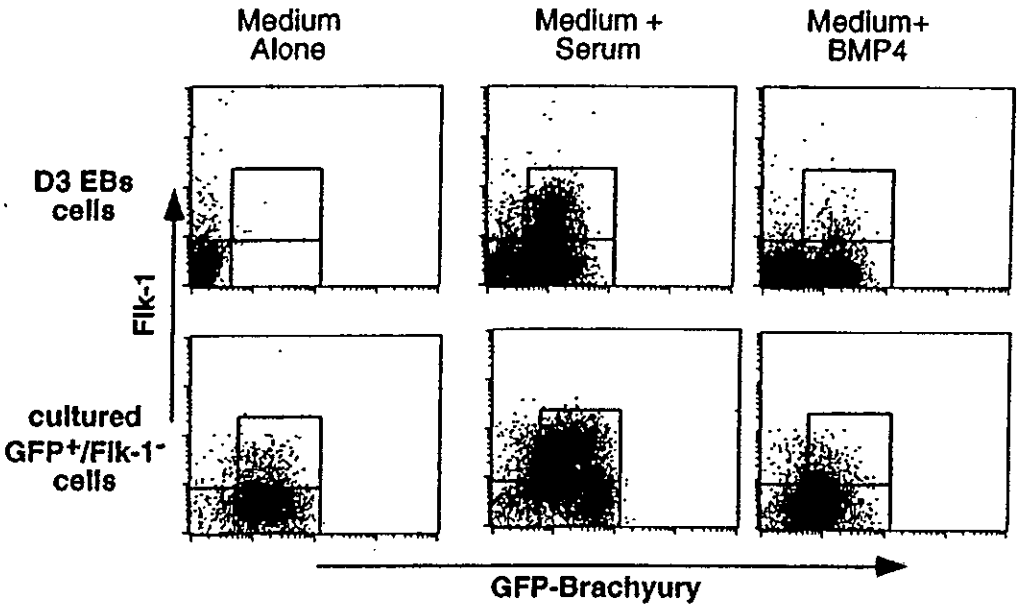

D3
D3.5
D4
4.0
3.9
3.7
pre
43
22
23
post
Flk-1

Fig. 8A

Fig. 8B


Medium
Alone
Medium +
Serum
Medium+
BMP4
D3 EBs
cells
Flk-1
cultured
GFP+/Flk-1-
cells
GFP-Brachyury

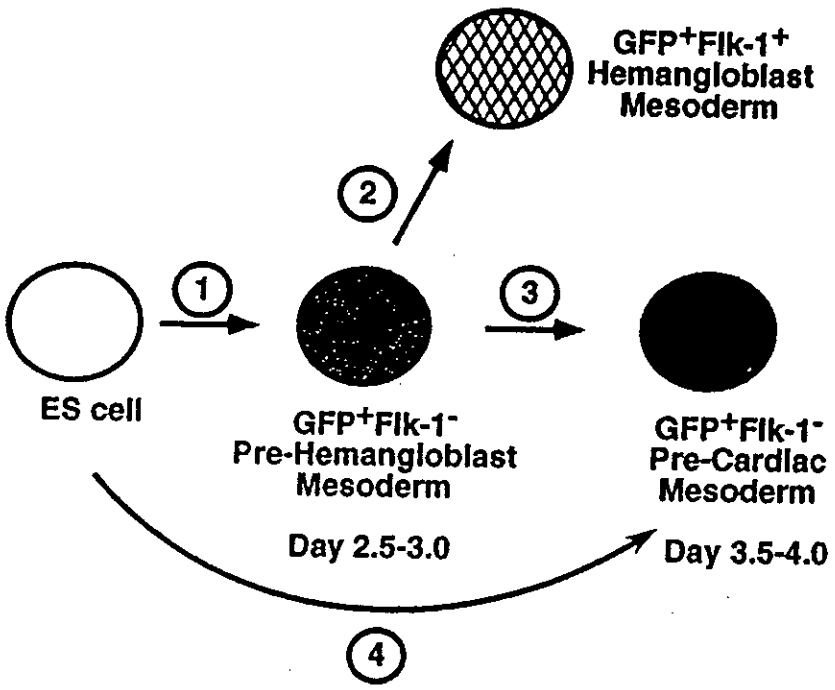
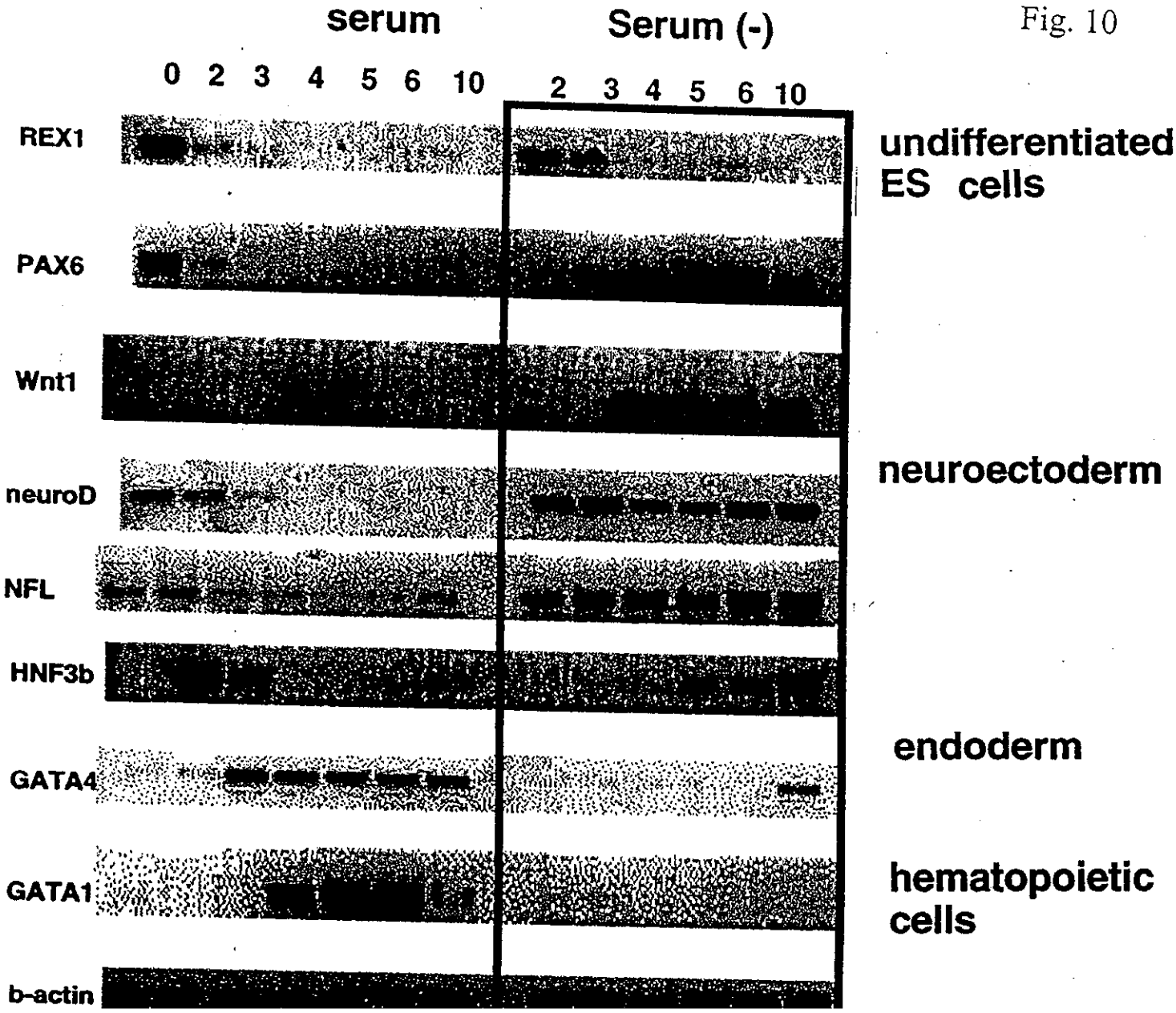

GFP+Flk-1+
Hemangioblast
Mesoderm
2
1
3
ES cell
GFP+Flk-1-
Pre-Hemangioblast
Mesoderm
GFP+Flk-1-
Pre-Cardiac
Mesoderm
Day 2.5-3.0
Day 3.5-4.0
4

Fig. 9

Fig. 10


serum
Serum (-)
0 2 3 4 5 6 10
2 3 4 5 6 10
REX1
PAX6
Wnt1
neuroD
NFL
HNF3b
GATA4
GATA1
b-actin
undifferentiated ES cells
neuroectoderm
endoderm
hematopoietic cells

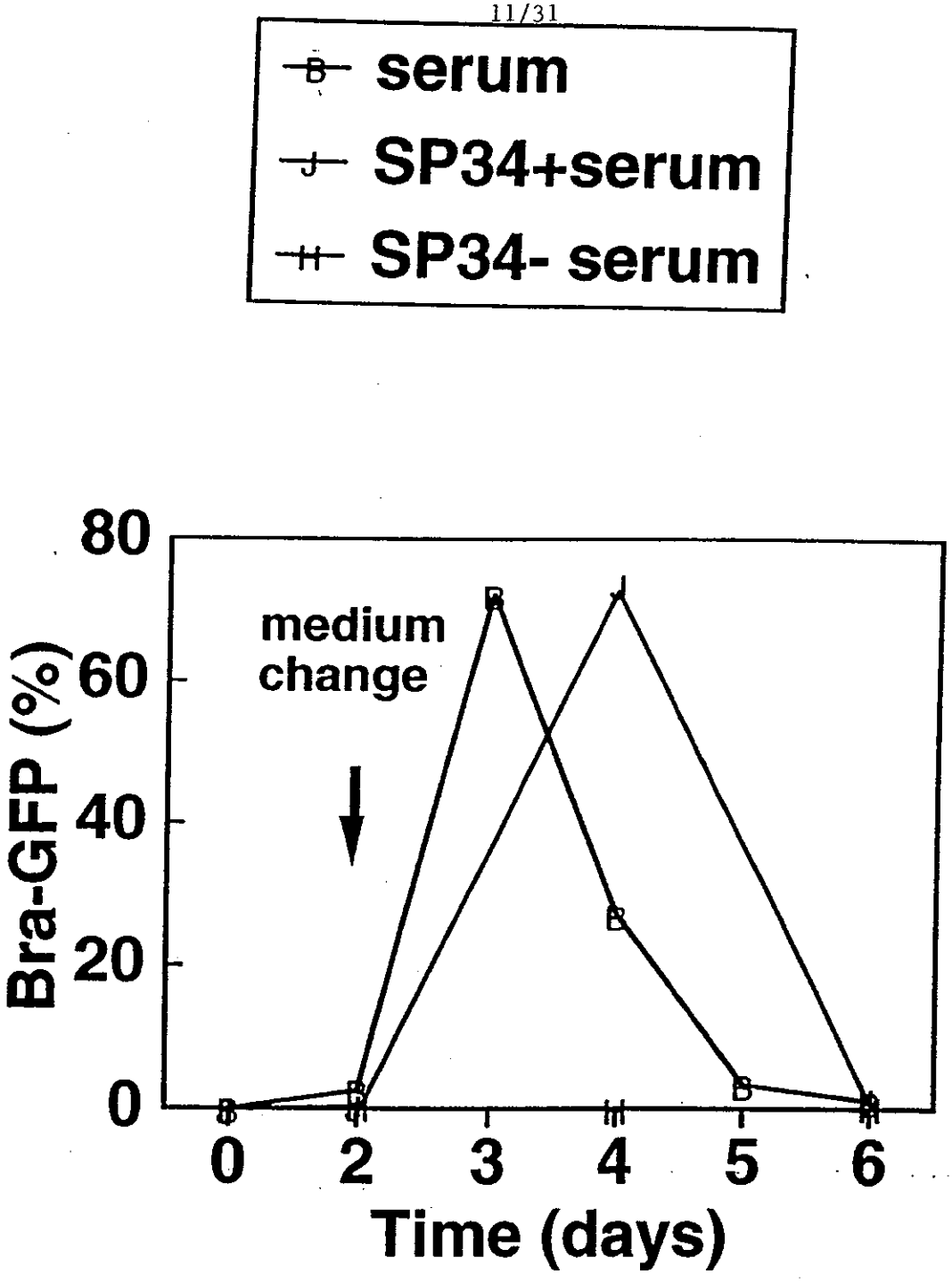
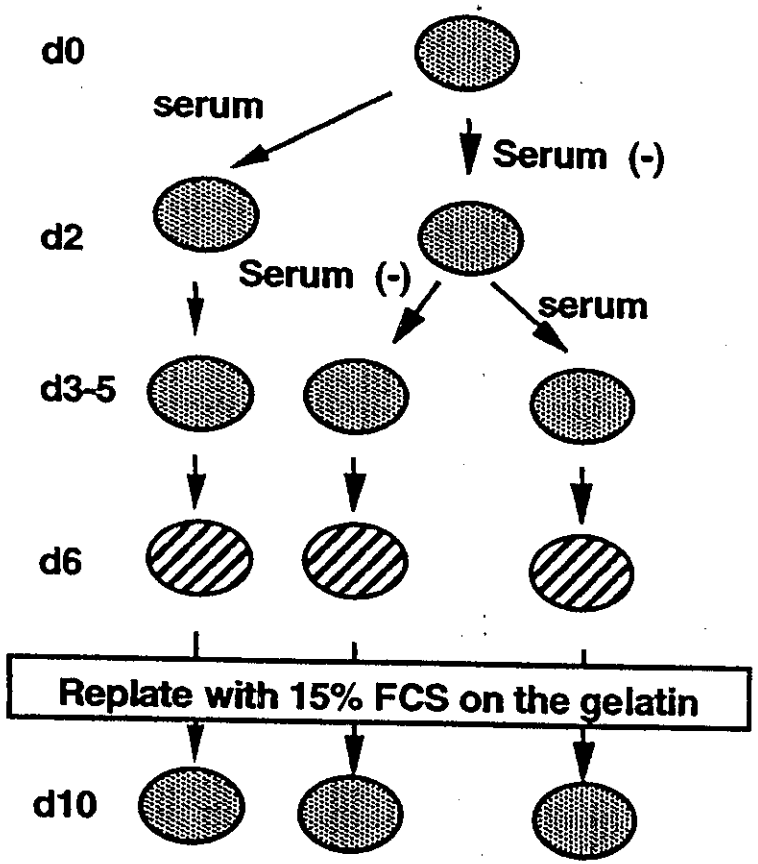
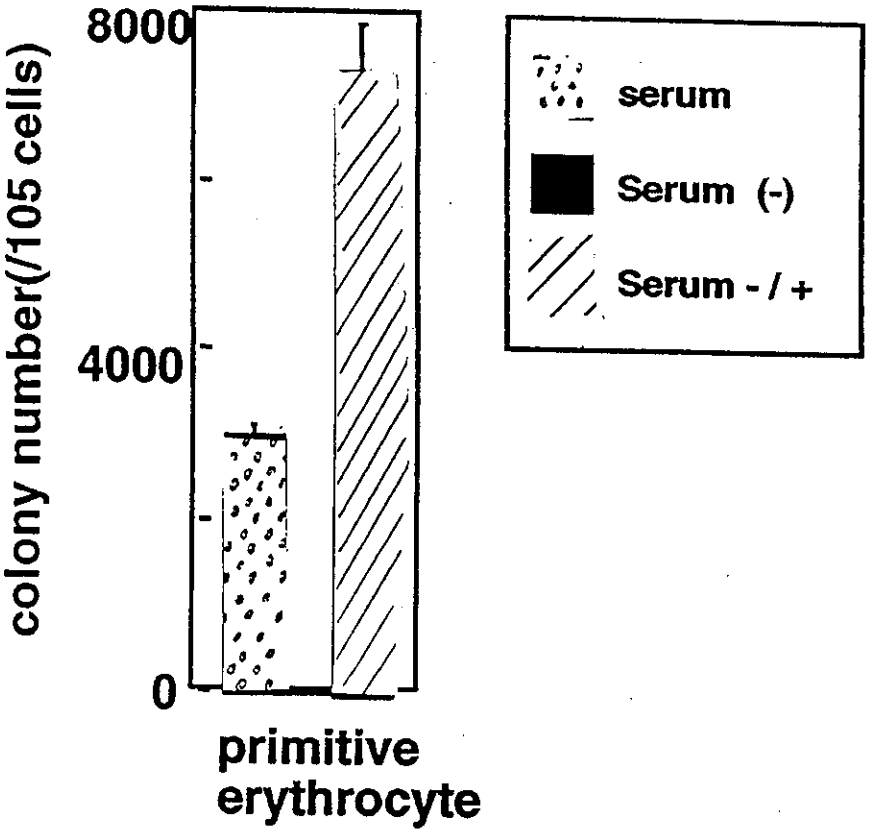

serum
SP34+serum
SP34- serum
80
60
40
20
0
Bra-GFP (%)
medium
change
0
2
3
4
5
6
Time (days)

Fig. 11

Neuronal differentiation



d6 colony assay



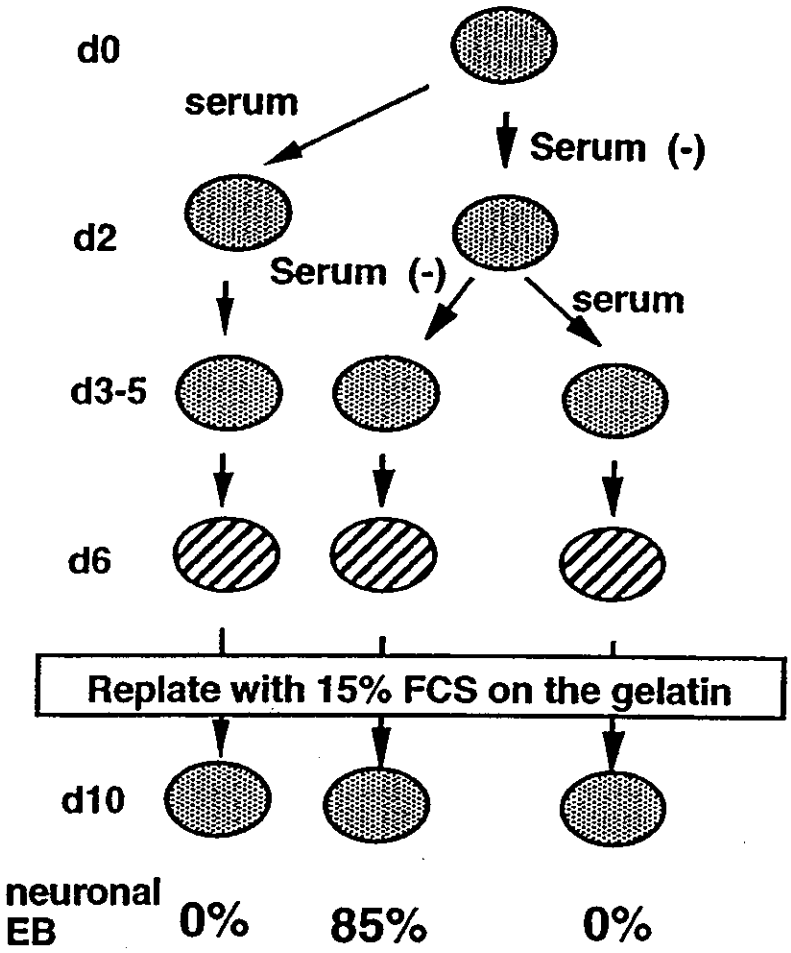

d0
serum
Serum (-)
d2
Serum (-)
serum
d3-5
d6
Replate with 15% FCS on the gelatin
d10
neuronal EB
0%
85%
0%

Fig. 13

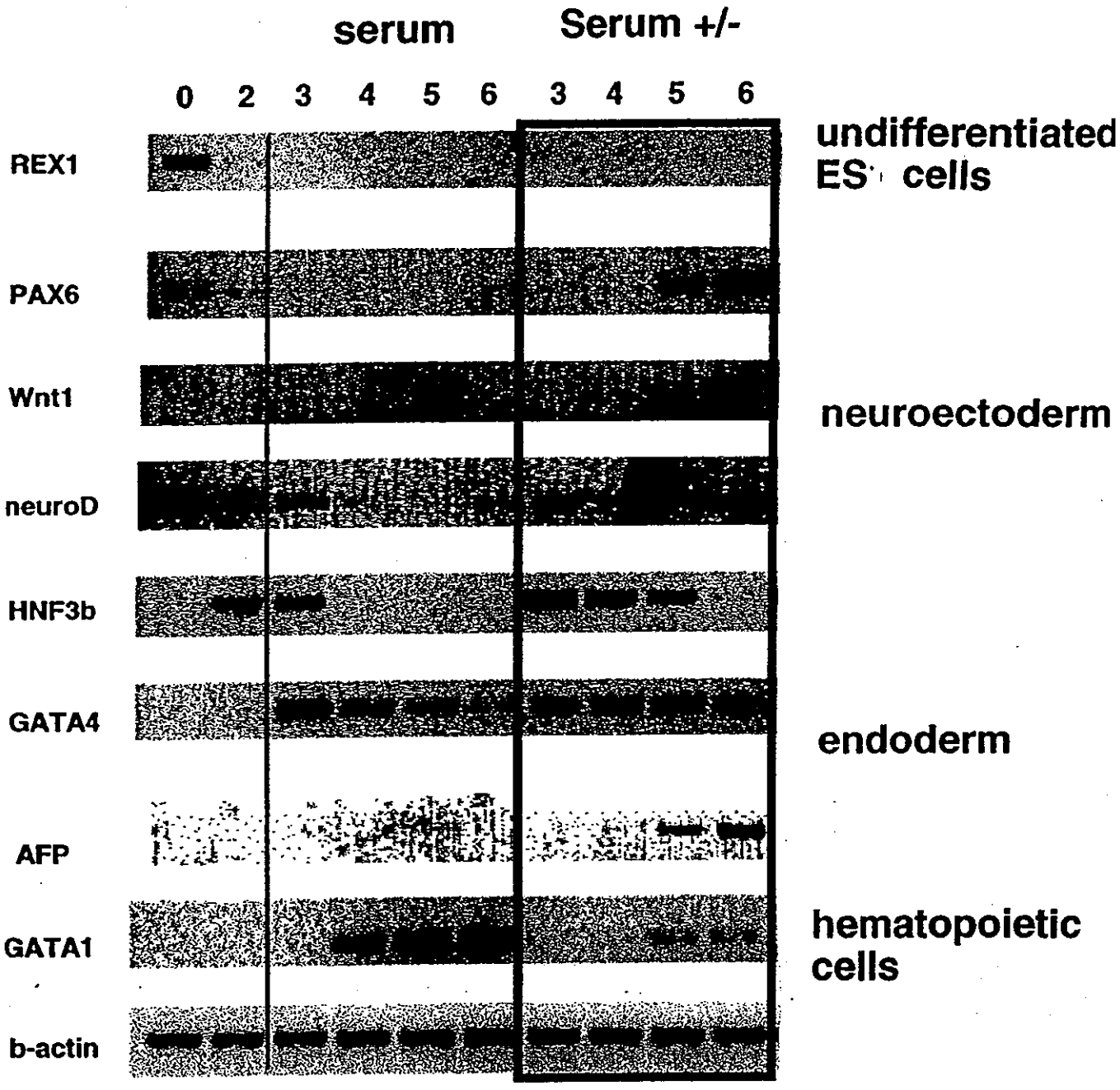
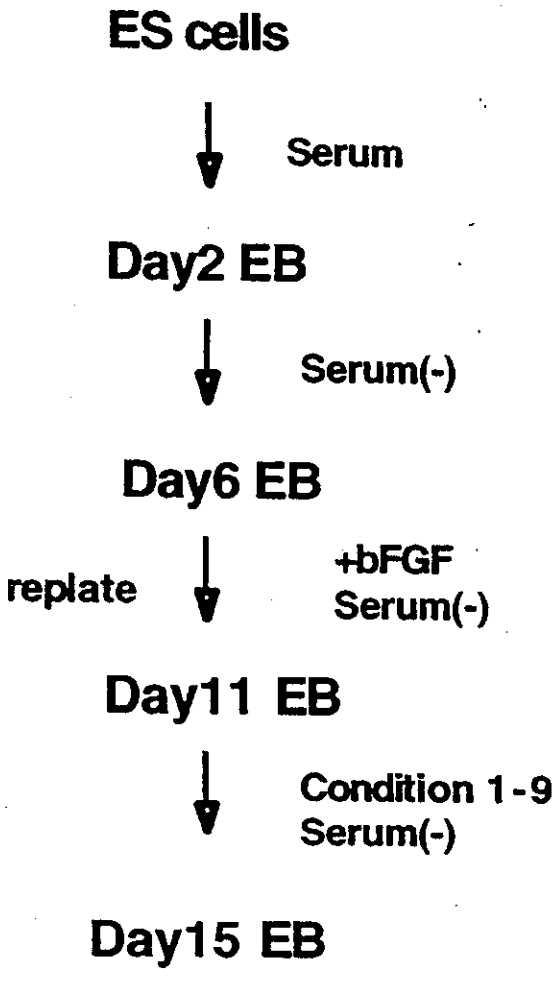
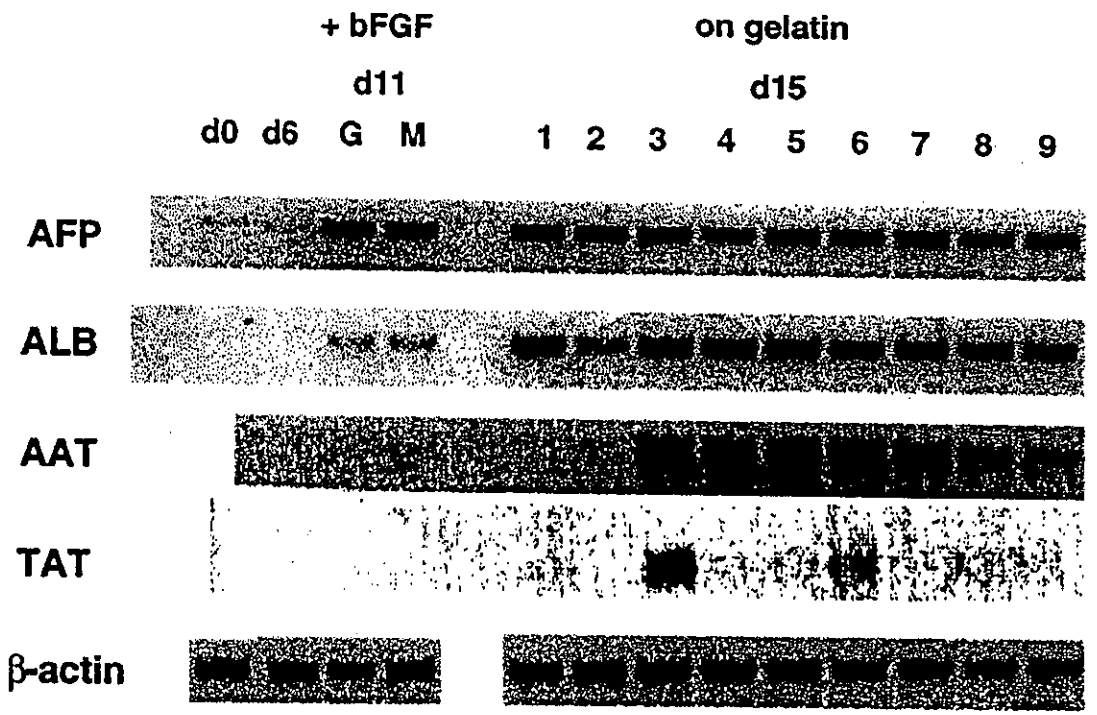

serum
Serum +/-
0 2 3 4 5 6 3 4 5 6
REX1
PAX6
Wnt1
neuroD
HNF3b
GATA4
AFP
GATA1
b-actin
undifferentiated ES cells
neuroectoderm
endoderm
hematopoietic cells

Fig. 14





1. no factor
2. bFGF
3. bFGF + Dex
4. bFGF + OSM
5. bFGF + Dex + OSM
6. nicotinamide
7. bFGF + nicotinamide
8. HGF + bFGF
9. HGH + bFGF + Dex + OSM

AFP; α-fetoprotein
ALB; alubumin
AAT; α1-antitrypsin
TAT; tyrosine aminotransferase

Fig. 15

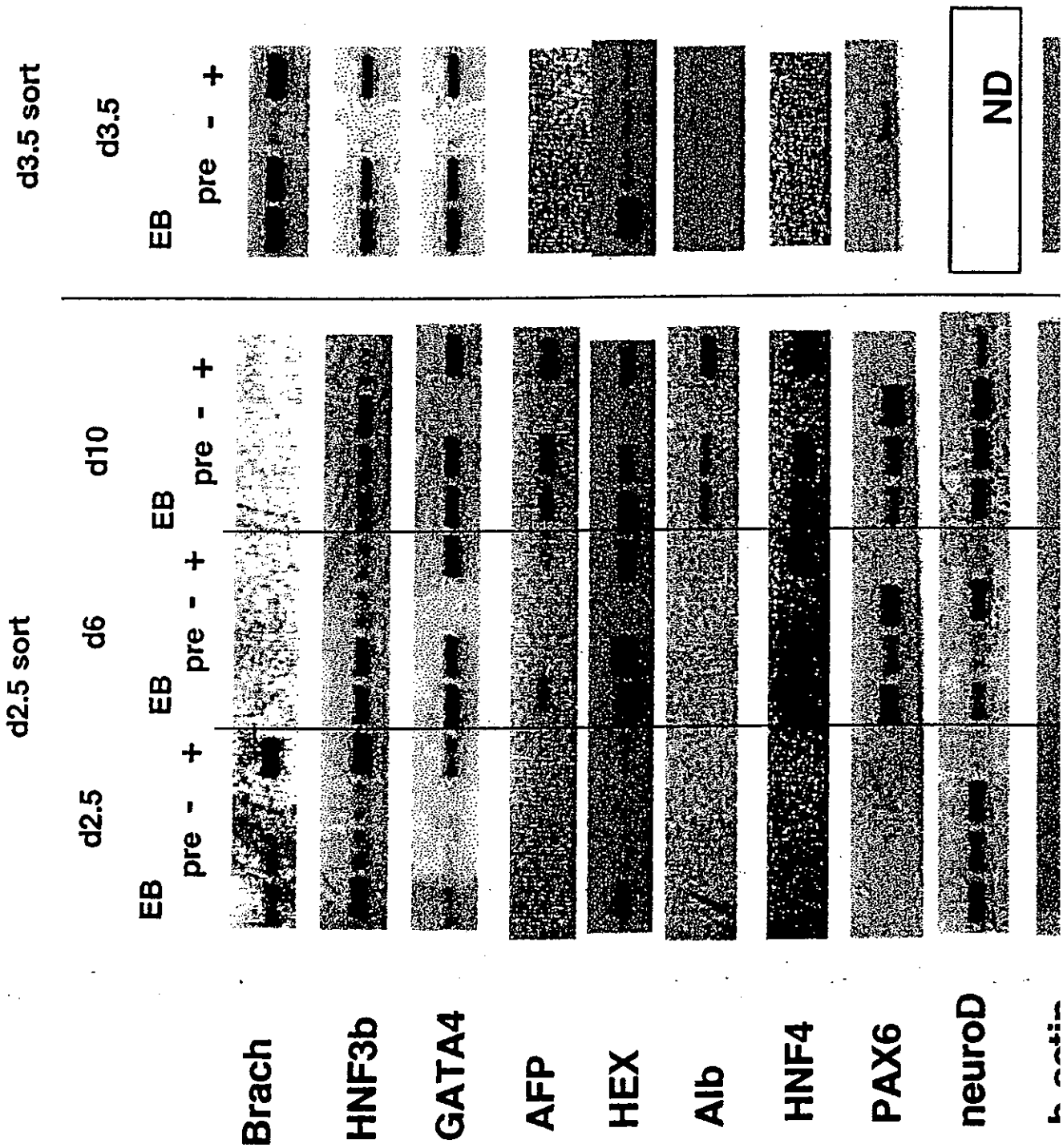

d2.5 sort
d6
d10
d3.5 sort
d3.5
EB
pre - +
EB
pre - +
EB
pre - +
EB
pre - +
Brach
HNF3b
GATA4
AFP
HEX
Alb
HNF4
PAX6
neuroD
ND

Fig. 16

ES Cell
Epiblast brach+Flk-1-
D2.0
D2.5
D3.0
D3.5
Mesendoderm
brach+Flk-1-
Mesoderm (1)
brach+Flk-1-
Mesoderm (2)
brach+Flk-1-
ectoderm
brach-Flk-1-
Endoderm
brach-Flk-1-
Hemangioblast
brach+Flk-1+
neuronal
pancreas
liver
endothelium
hematopoiesis
cardiac
skeletal
adipocyte

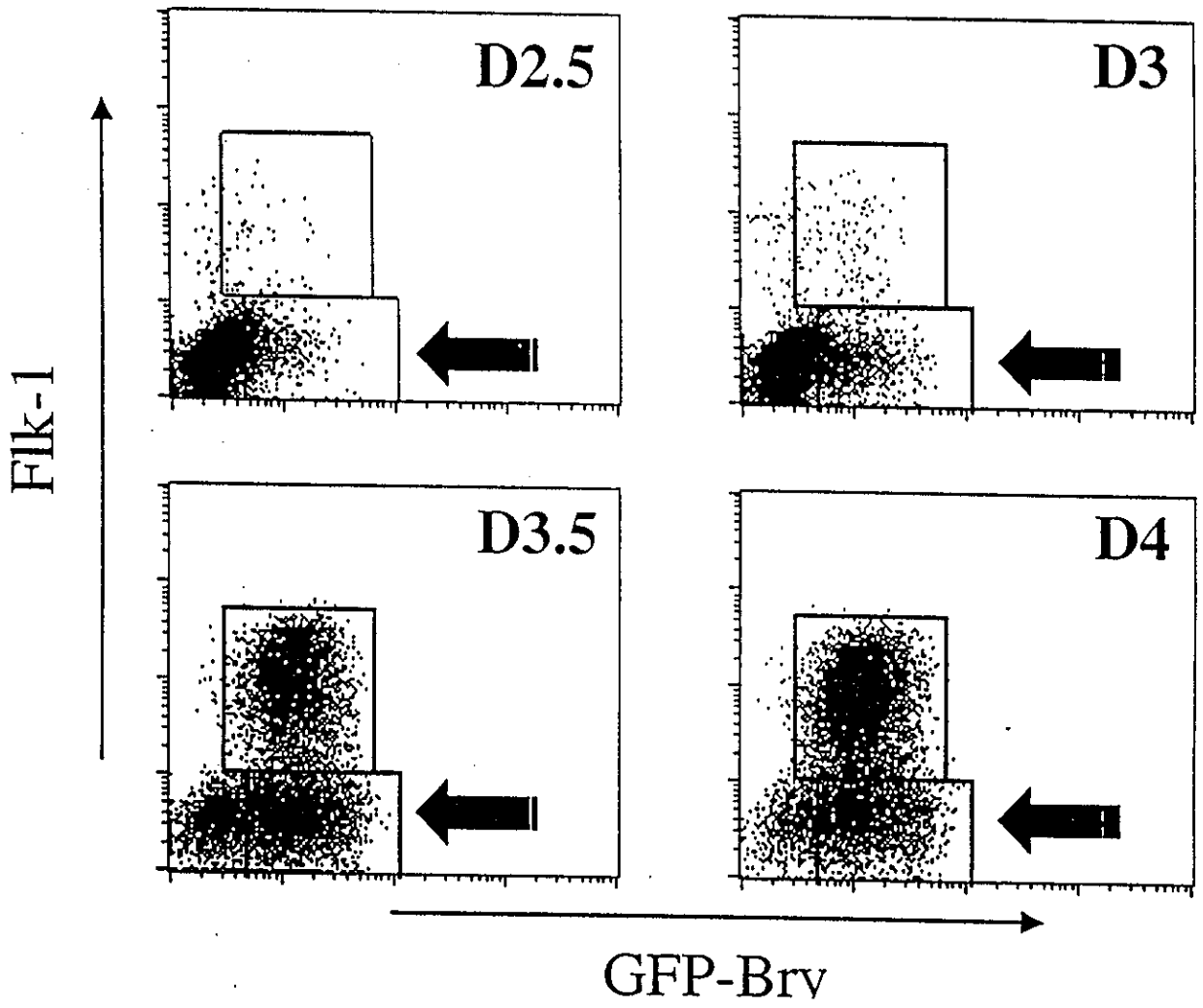


Figure 18

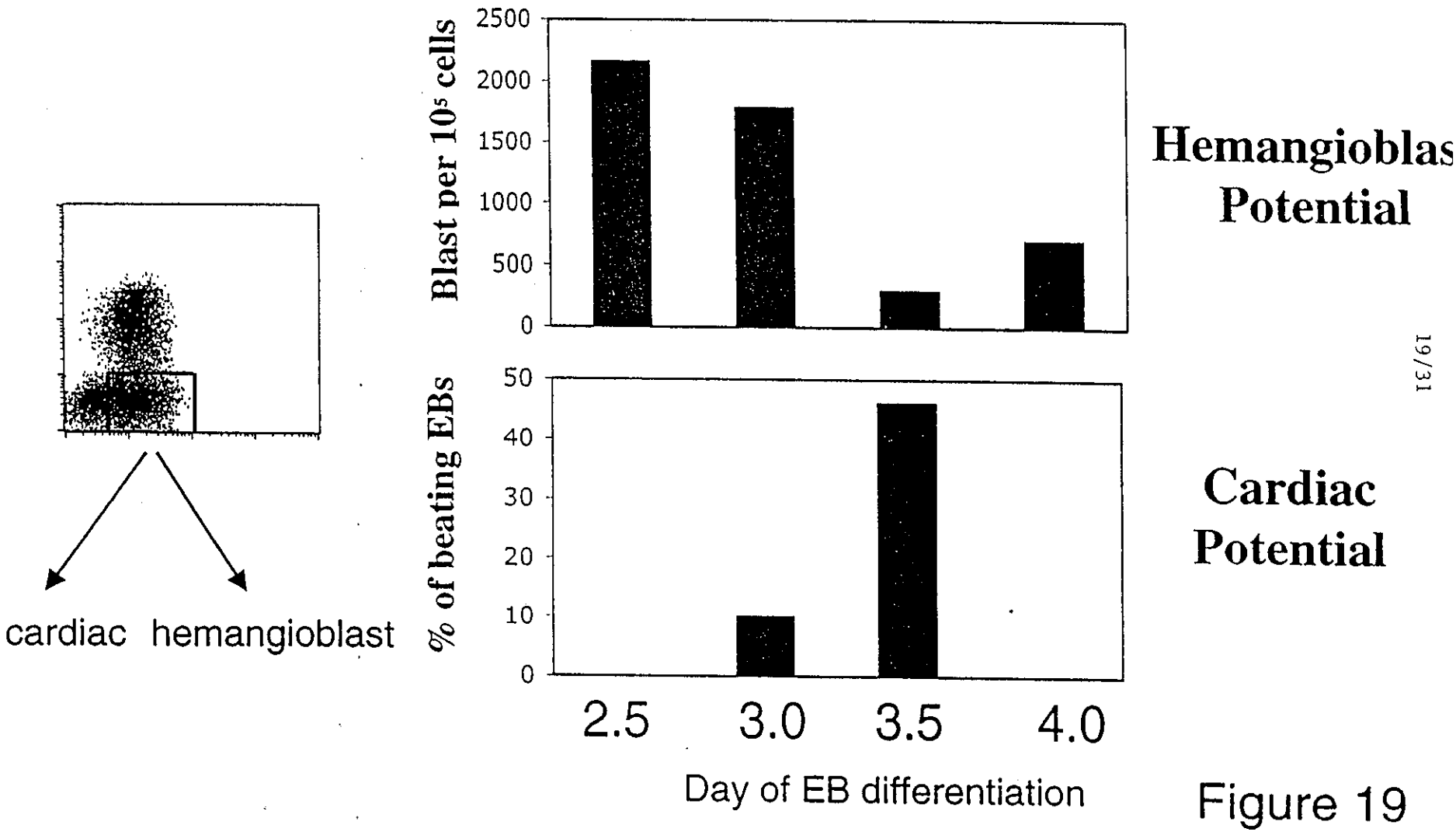
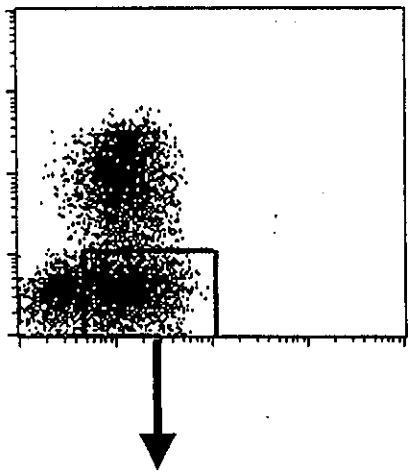

cardiac
hemangioblast
Blast per 10⁵ cells
2500
2000
1500
1000
500
0
% of beating EBs
50
40
30
20
10
0
2.5
3.0
3.5
4.0
Day of EB differentiation
Hemangioblas
Potential
Cardiac
Potential

Figure 19



GFP+ cells:
PCR

Time of Differentiation

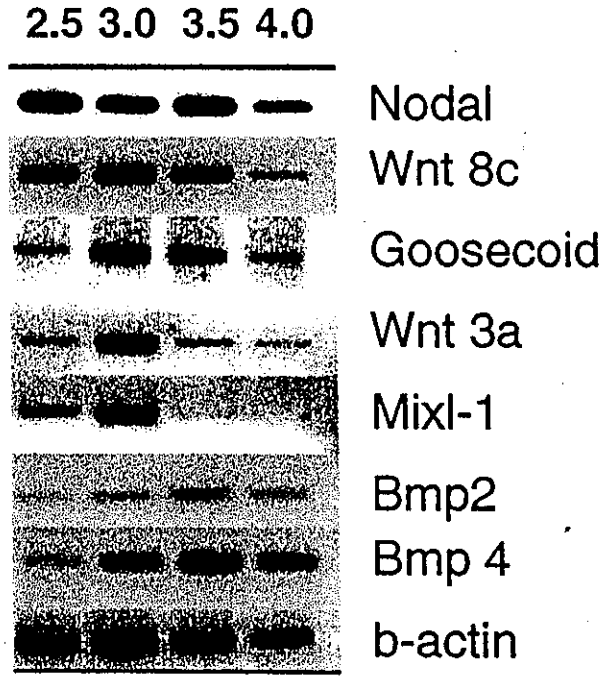


Figure 20

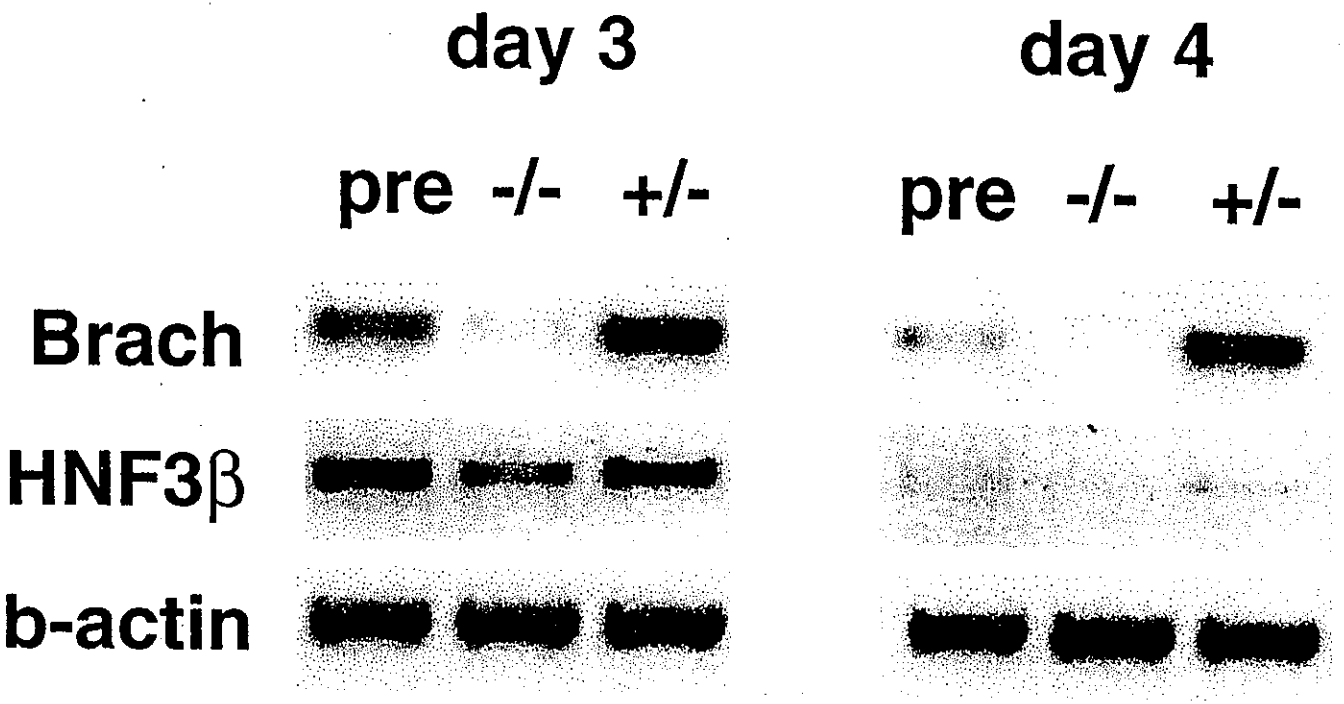


Figure 21

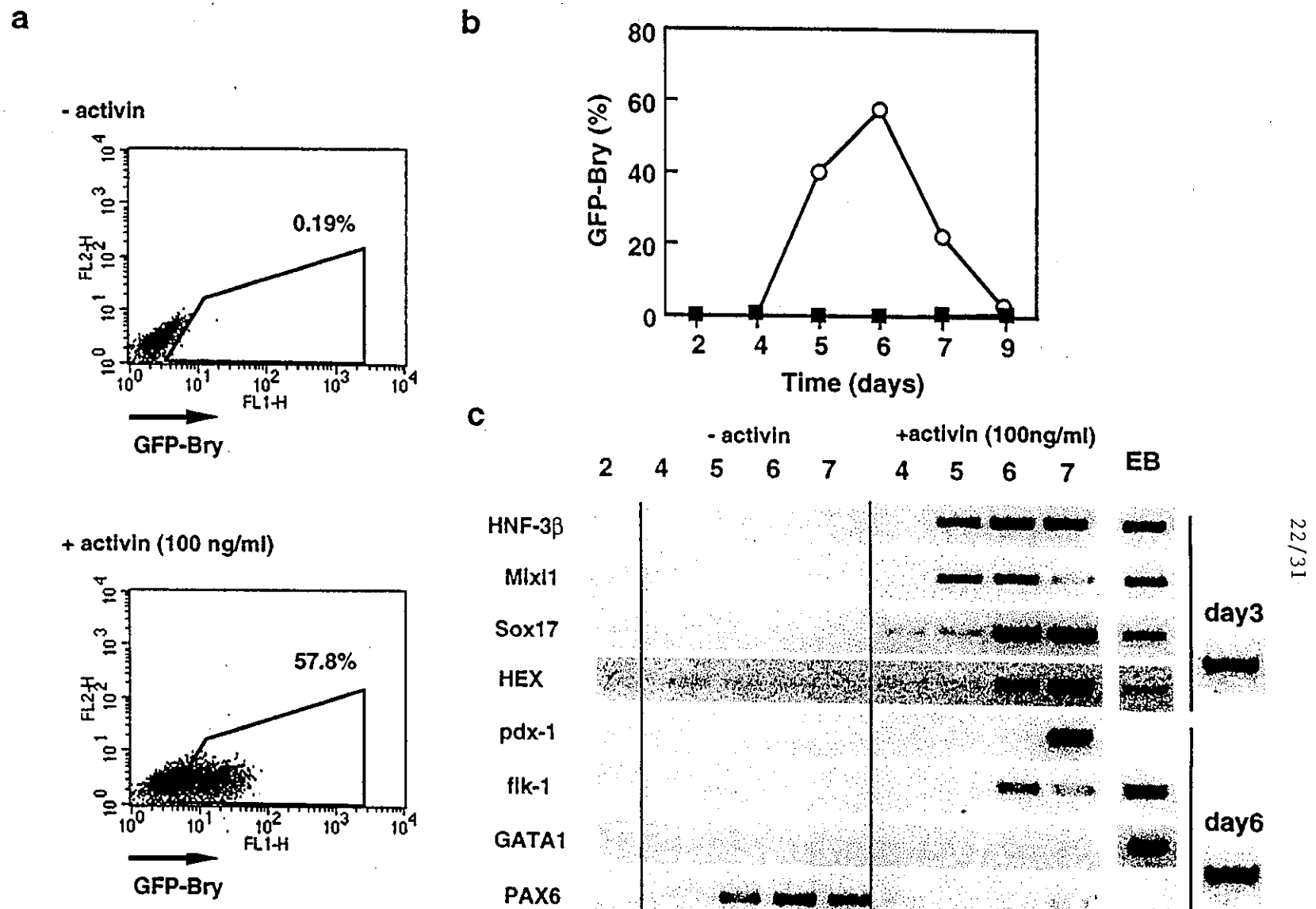

a
- activin
0.19%
FL2-H
FL1-H
GFP-Bry
+ activin (100 ng/ml)
57.8%
FL2-H
FL1-H
GFP-Bry
b
GFP-Bry (%)
80
60
40
20
0
2
4
5
6
7
9
Time (days)
c
- activin
+activin (100ng/ml)
2
4
5
6
7
4
5
6
7
EB
HNF-3β
Mixl1
Sox17
HEX
pdx-1
flk-1
GATA1
PAX6
β-actin
day3
day6

Figure 22

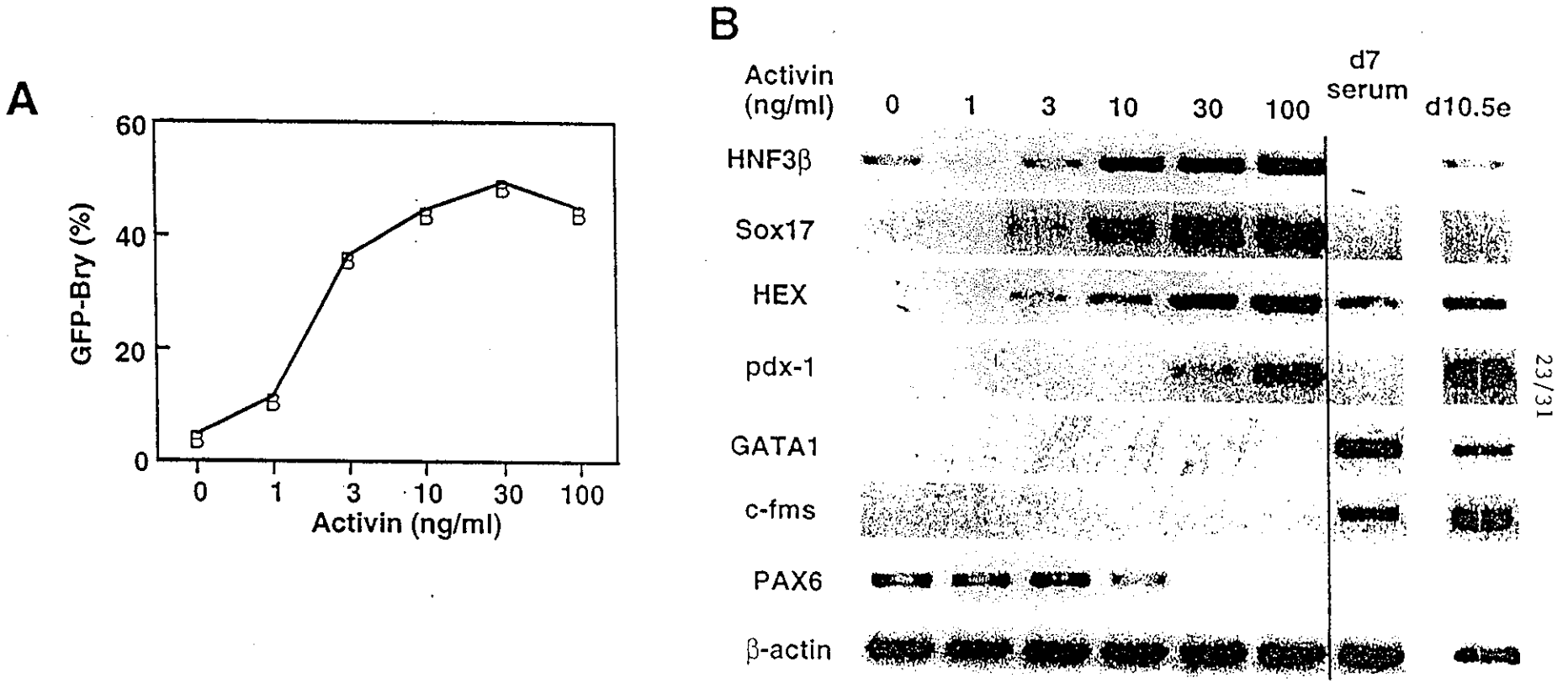
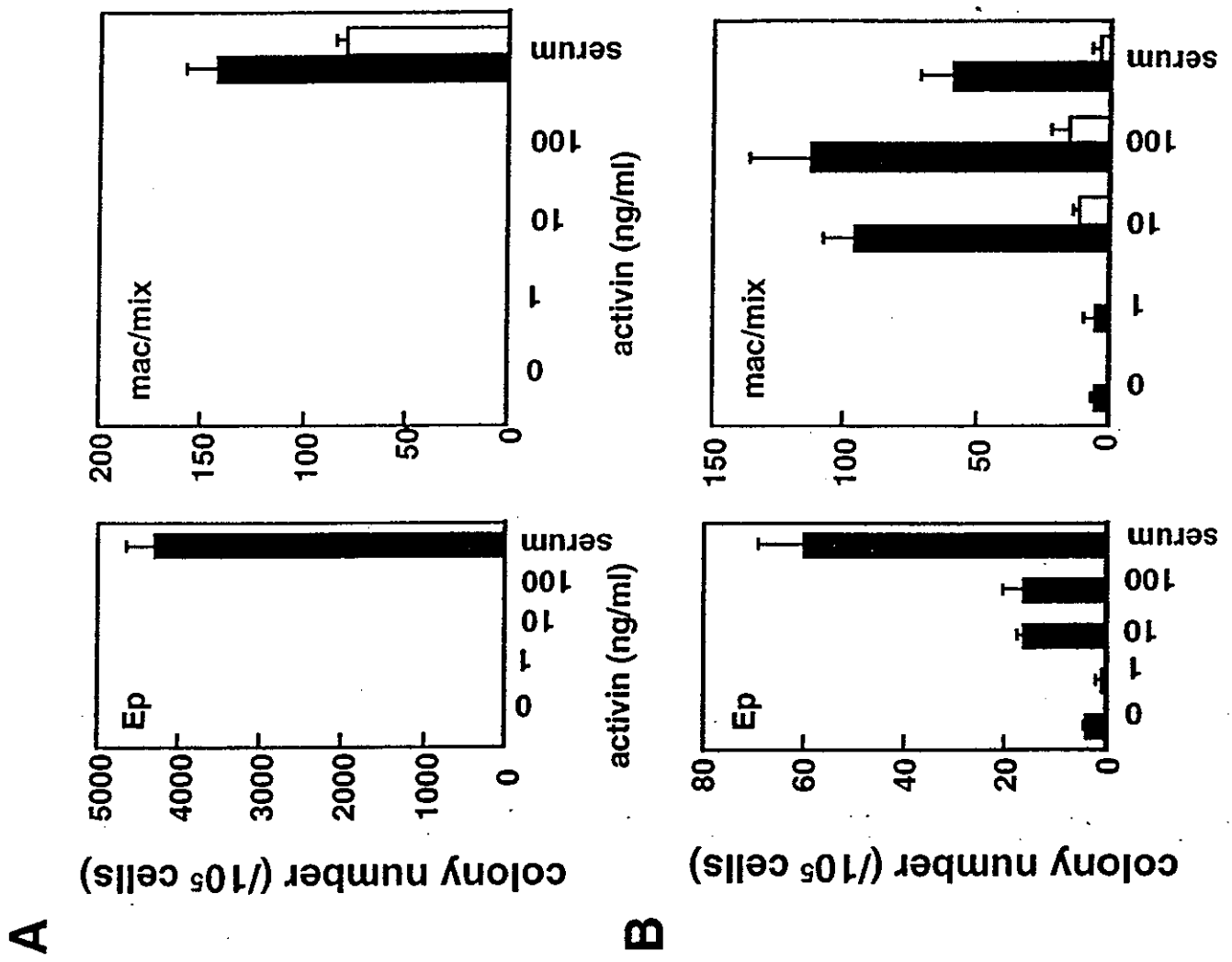

A
GFP-Bry (%)
60
40
20
0
0
1
3
10
30
100
Activin (ng/ml)
B
Activin (ng/ml)
0
1
3
10
30
100
d7 serum
d10.5e
HNF3β
Sox17
HEX
pdx-1
GATA1
c-fms
PAX6
β-actin

Figure 23

Figure 24


A
colony number (/10⁵ cells)
Ep
mac/mix
activin (ng/ml)
0
1
10
100
serum
B
colony number (/10⁵ cells)
Ep
mac/mix
activin (ng/ml)
0
1
10
100
serum

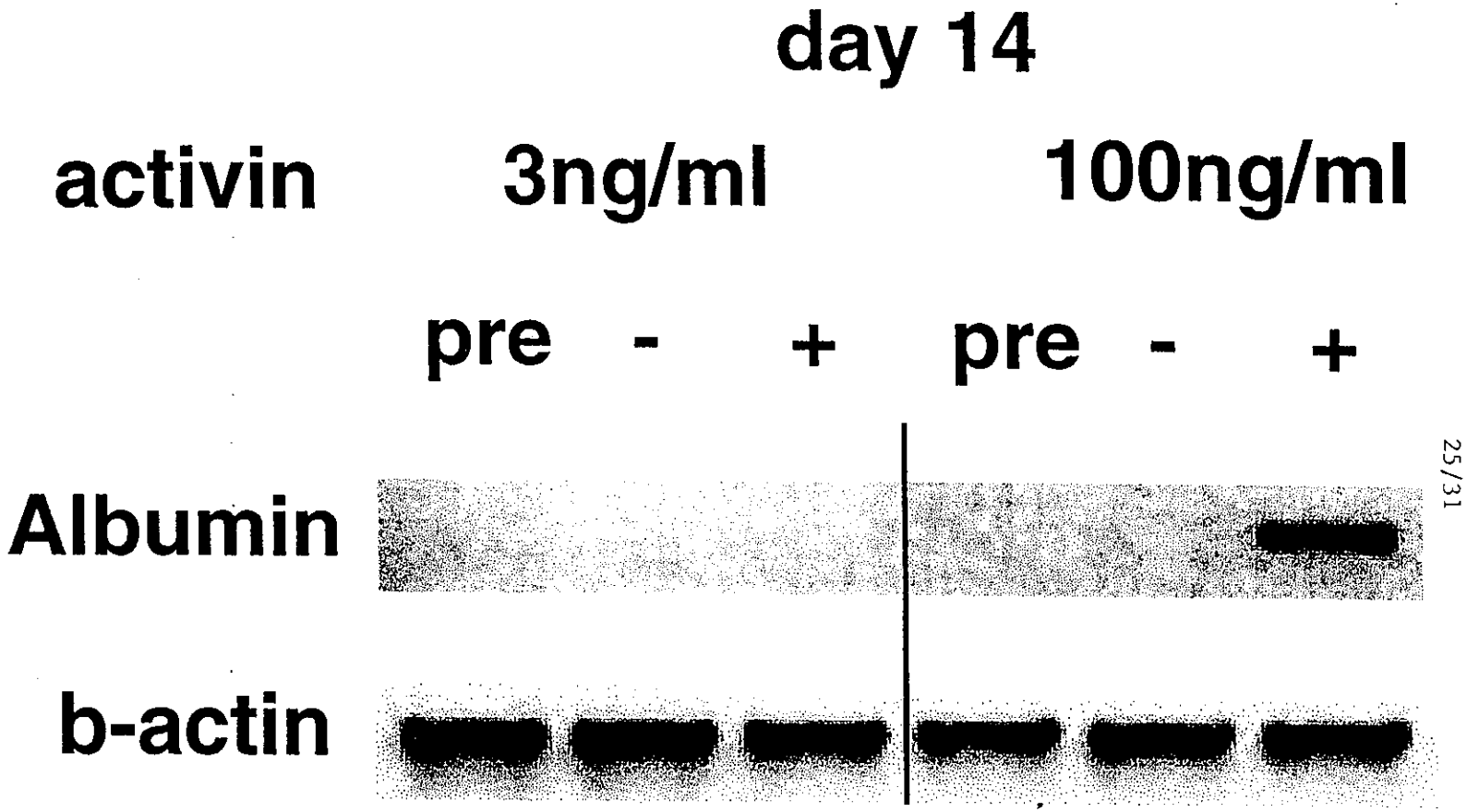


Figure 25

GFP-Bry
(+)



Fig. 26A

GFP-Bry
(-)

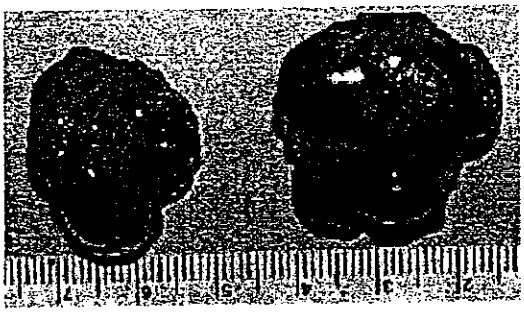


Fig. 26B

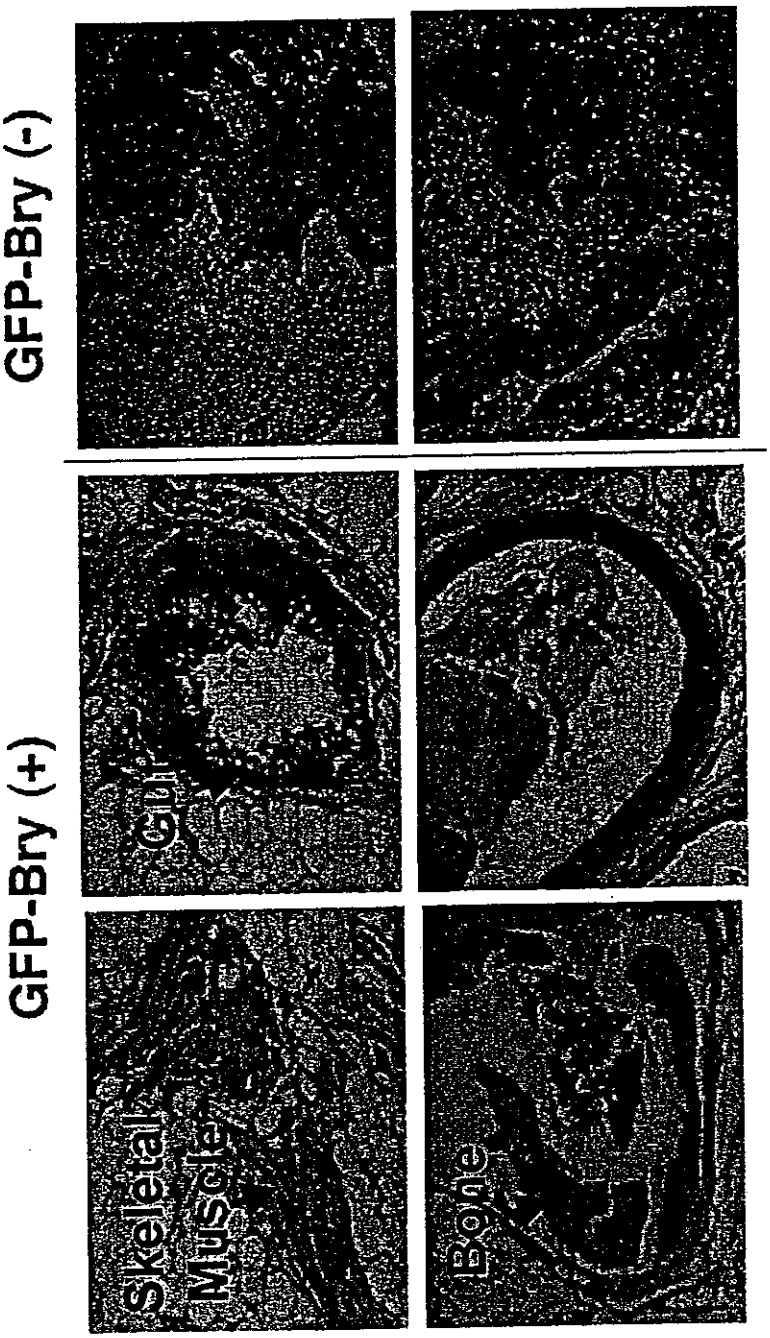


Fig. 26C

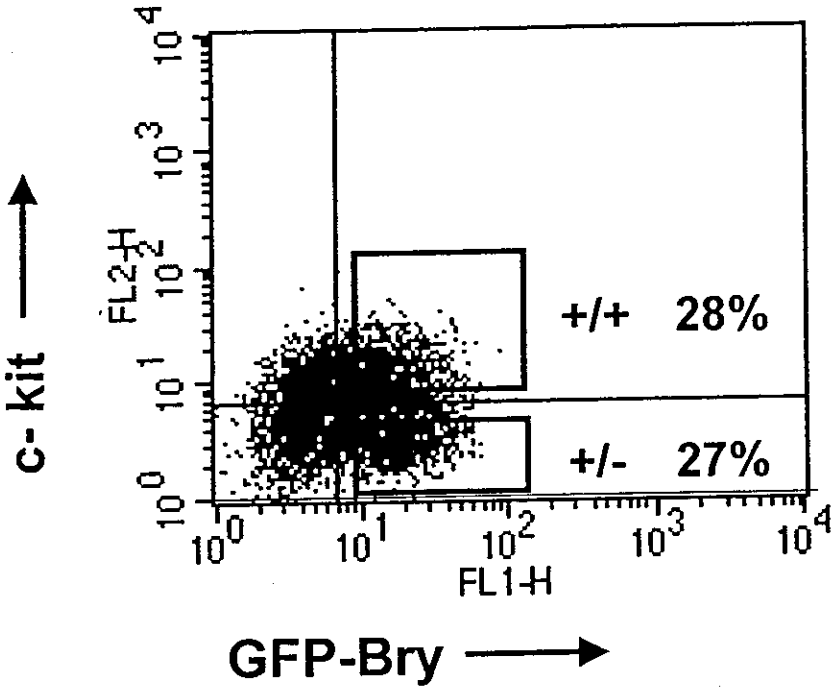


Fig. 27A

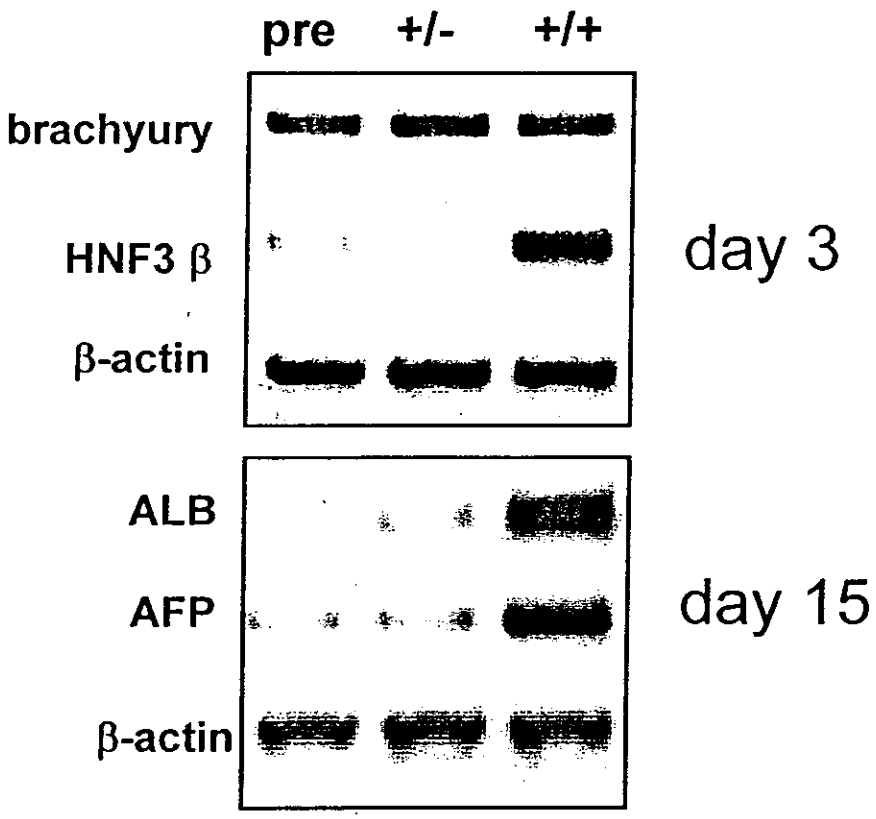


Fig. 27B

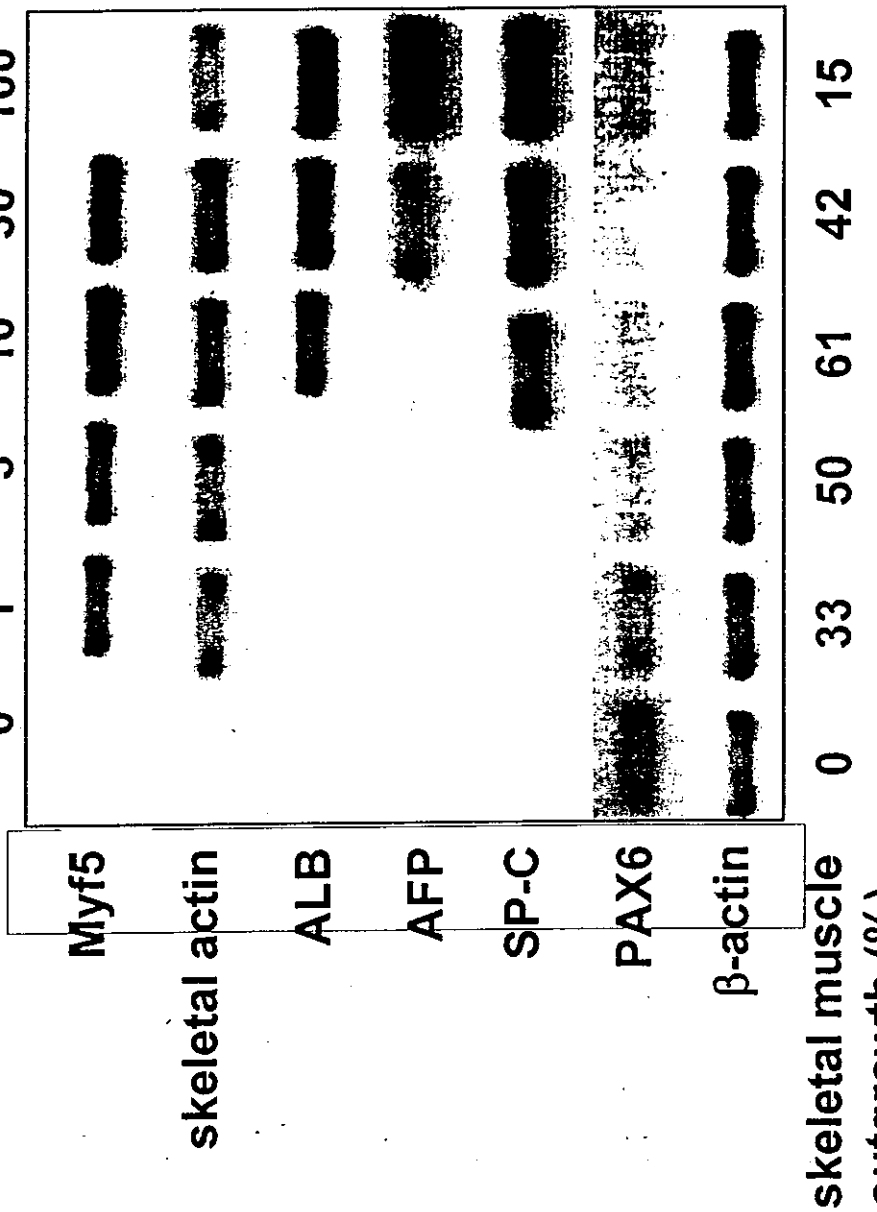

Myf5
skeletal actin
ALB
AFP
SP-C
PAX6
β-actin
0 1 3 10 30 100
skeletal muscle outgrowth (%)
0 33 50 61 42 15

Fig. 28

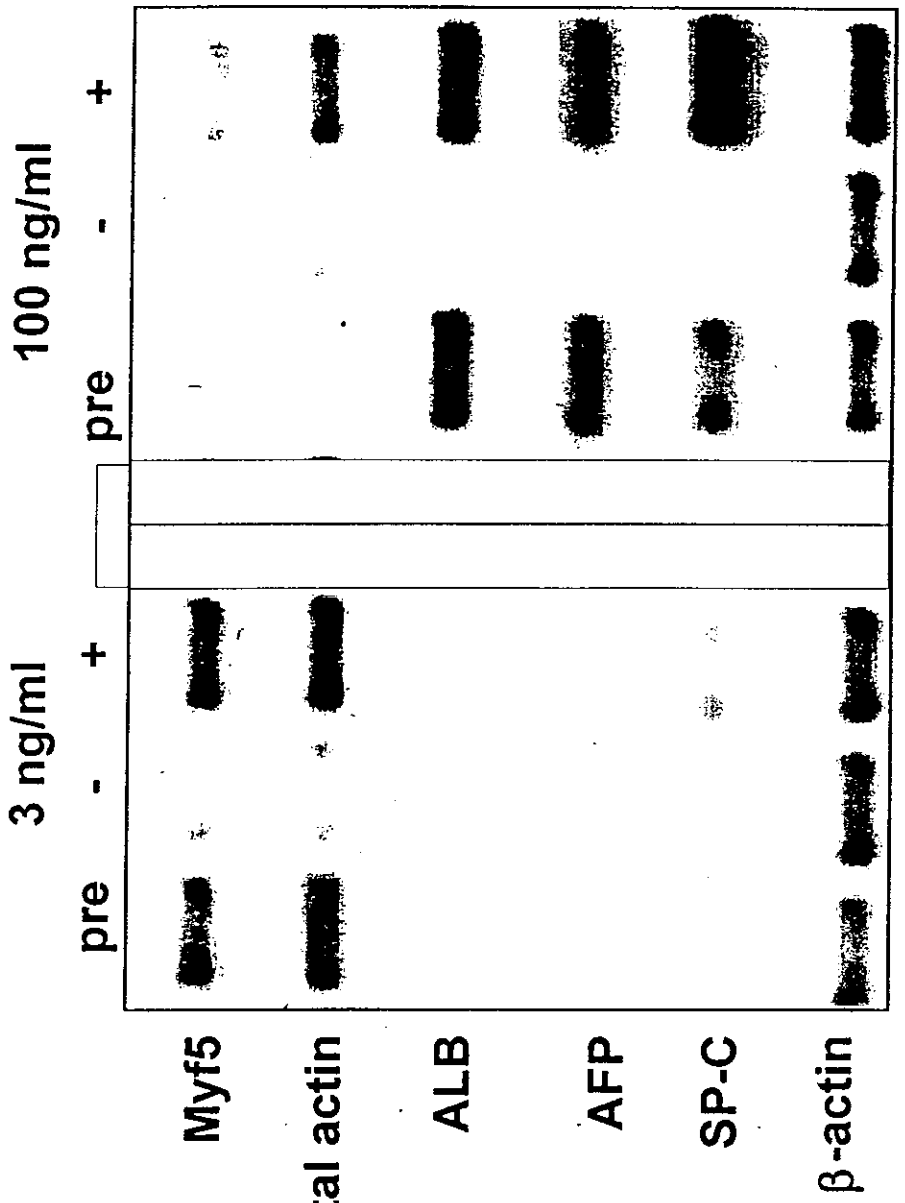

3 ng/ml
100 ng/ml
pre
-
+
pre
-
+
Myf5
skeletal actin
ALB
AFP
SP-C
β-actin

Fig. 29