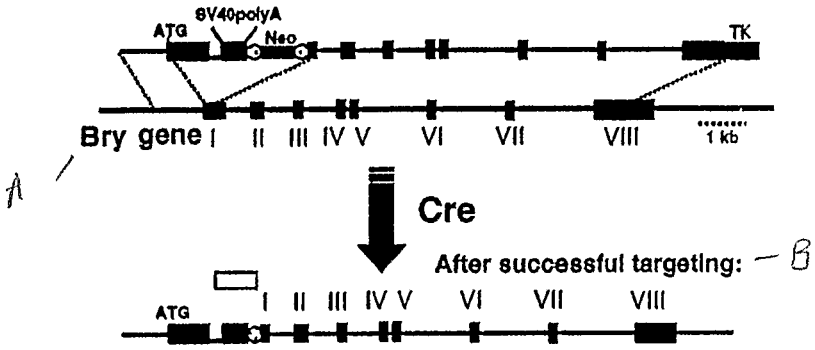

SV40polyA
ATG
Neo
TK
Bry gene
I II III IV V VI VII VIII
1 kb
A
Cre
After successful targeting:
B
ATG
I II III IV V VI VII VIII

Fig. 1

Fig. 2A

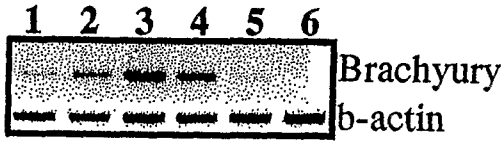

1 2 3 4 5 6
Brachyury
b-actin

Fig. 2B

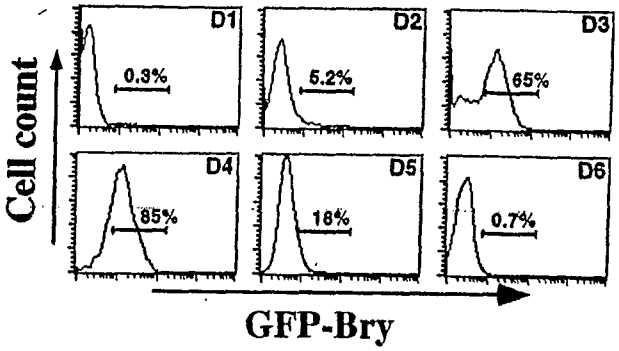

D1
0.3%
D2
5.2%
D3
65%
D4
85%
D5
18%
D6
0.7%
Cell count
GFP-Bry

Fig. 3A

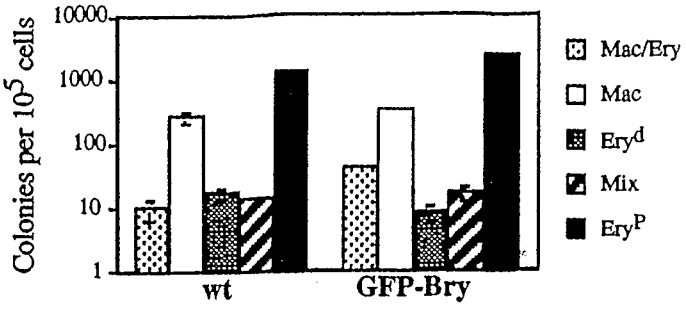


Fig. 3B

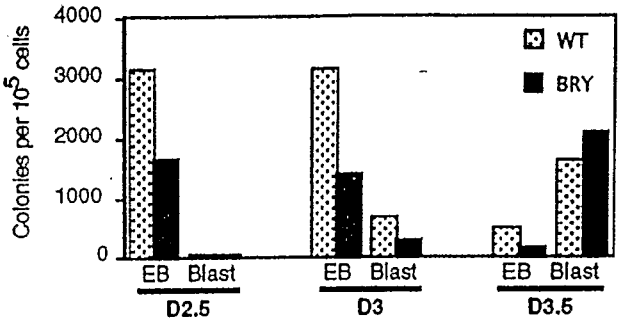


Fig. 3C

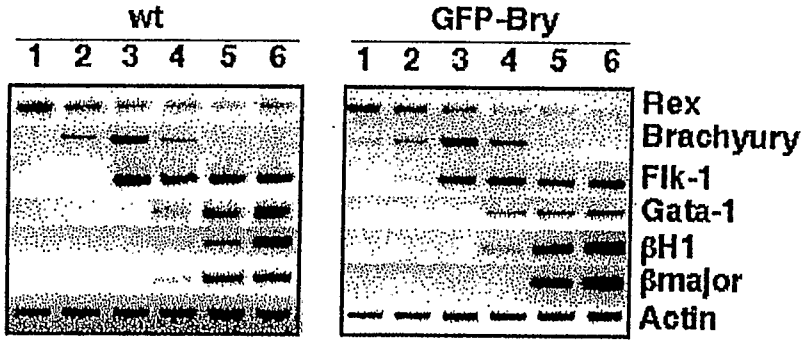


Fig. 4A

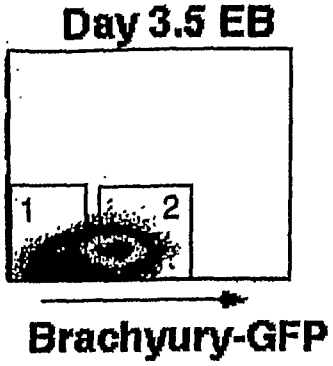

Day 3.5 EB
1
2
Brachyury-GFP

Fig. 4B

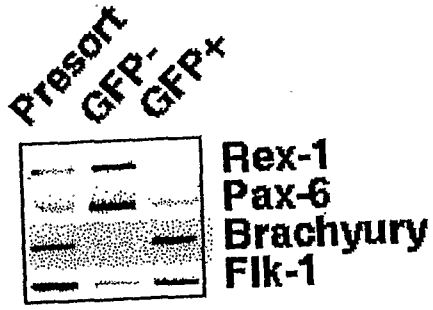

Presort
GFP-
GFP+
Rex-1
Pax-6
Brachyury
Flk-1

Fig. 5A

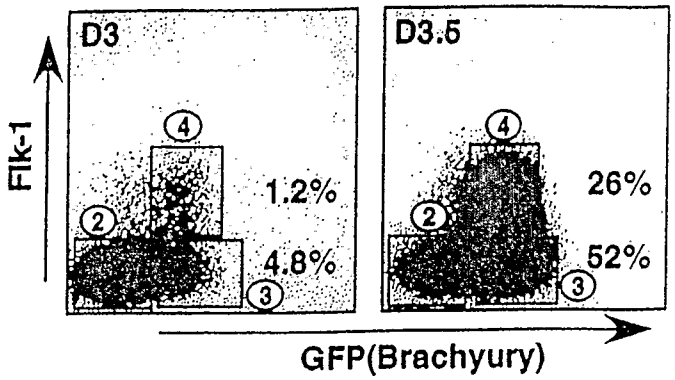


Fig. 5B

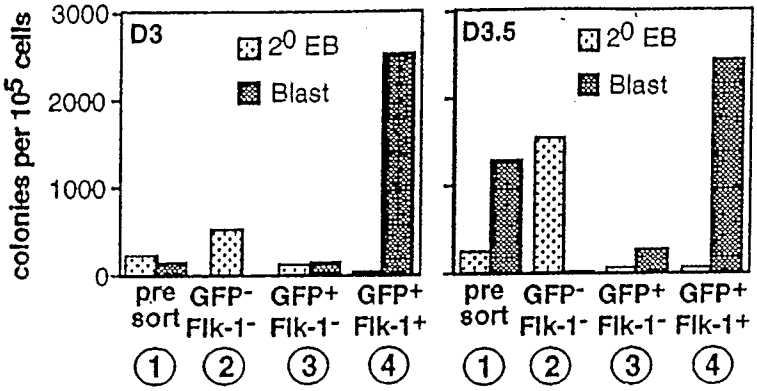
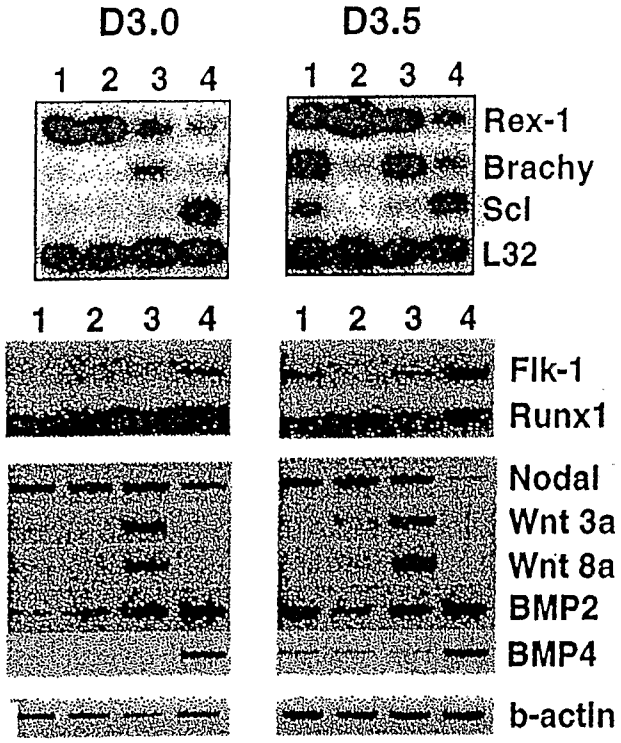


Fig. 5C



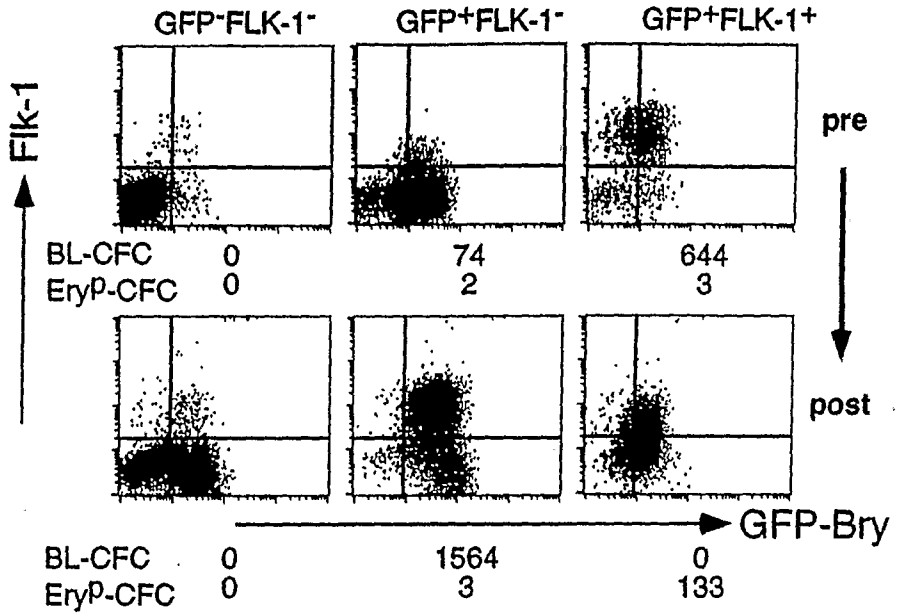

GFP-FLK-1-
GFP+FLK-1-
GFP+FLK-1+
Flk-1
pre
post
BL-CFC 0 74 644
EryP-CFC 0 2 3
BL-CFC 0 1564 0
EryP-CFC 0 3 133
GFP-Bry

Fig. 6

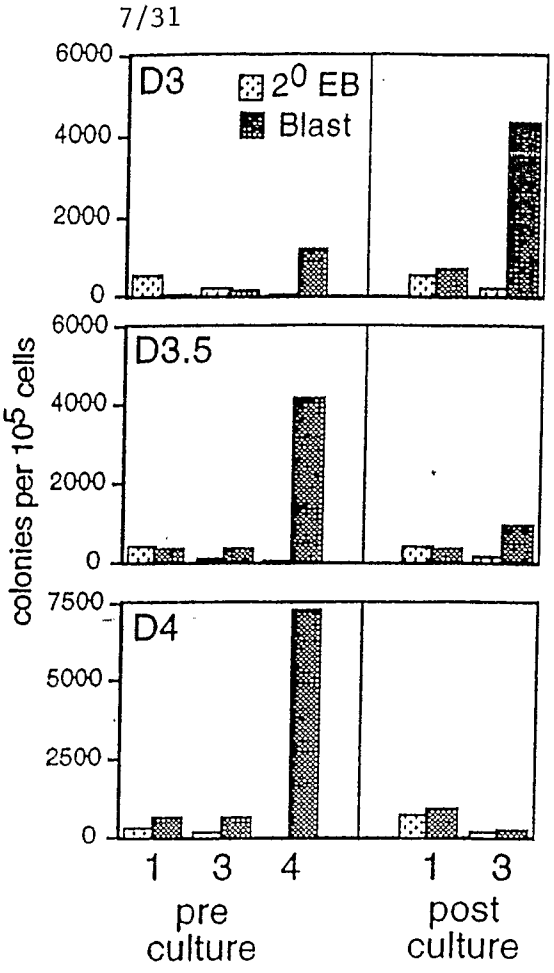

D3
2^0 EB
Blast
D3.5
D4
colonies per 10^5 cells
6000
4000
2000
0
7500
5000
2500
1 3 4
pre culture
1 3
post culture

Fig. 7A

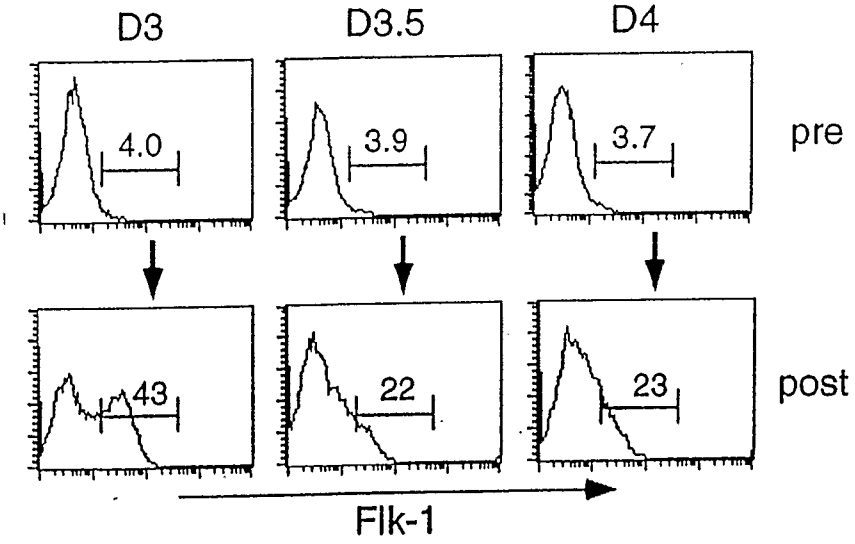
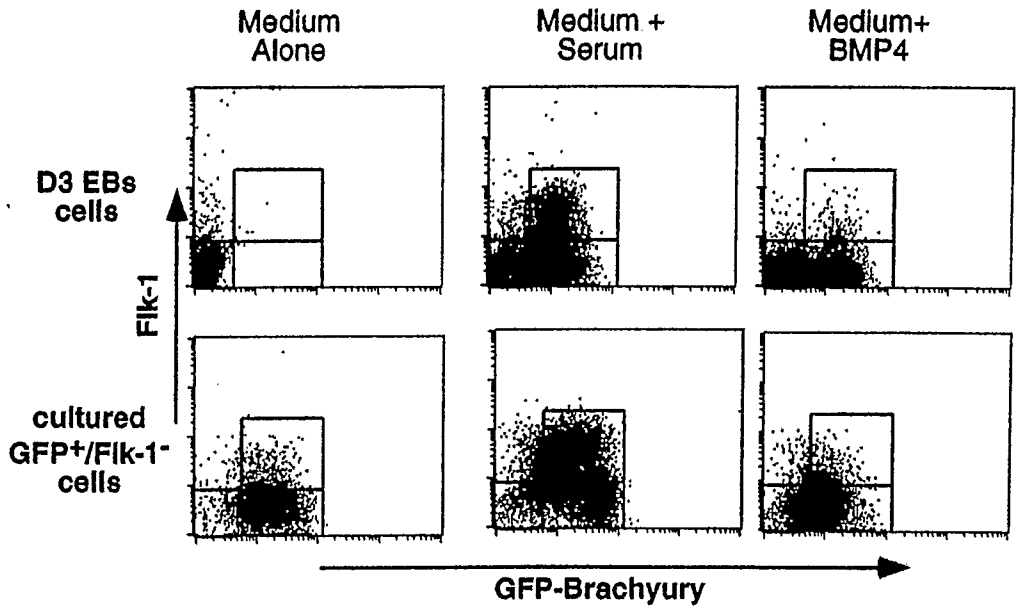

D3
D3.5
D4
4.0
3.9
3.7
pre
43
22
23
post
Flk-1

Fig. 7 B

Fig. 8A

Fig. 8B


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

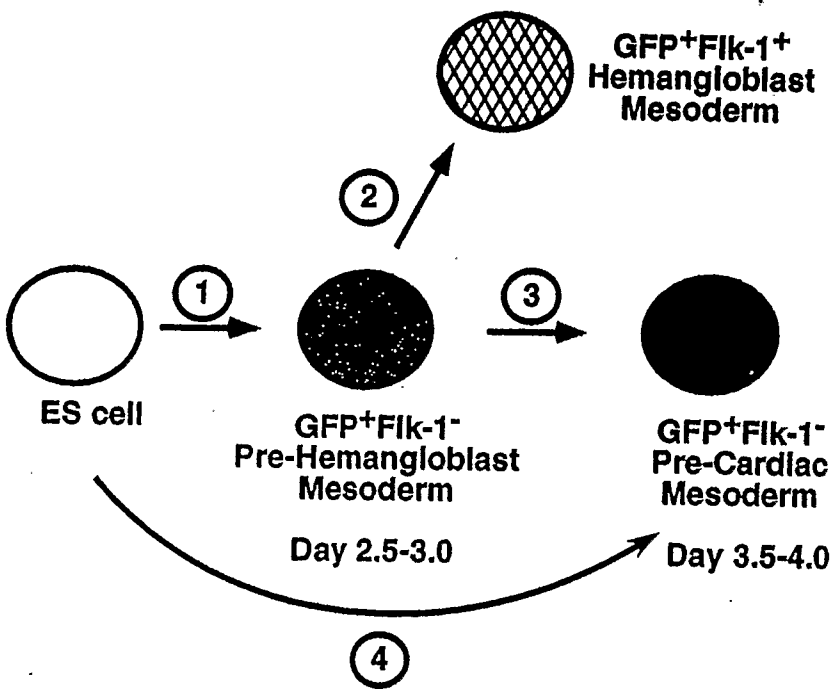
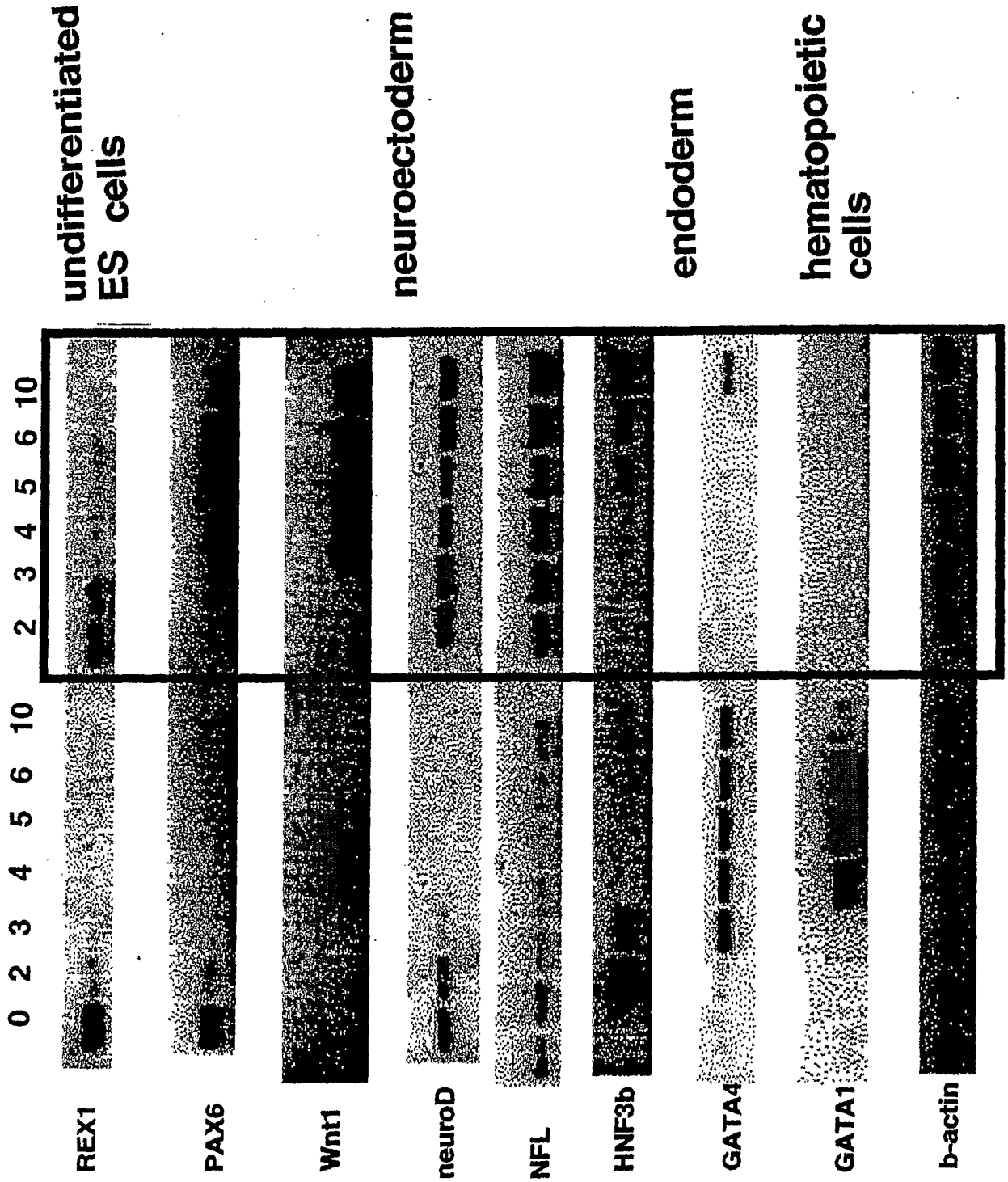

GFP+Flk-1+
Hemangioblast
Mesoderm
2
1
3
ES cell
GFP+Flk-1-
Pre-Hemangioblast
Mesoderm
GFP+Flk-1-
Pre-Cardiac
Mesoderm
Day 2.5-3.0
Day 3.5-4.0
4

Fig. 9

Fig. 10


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

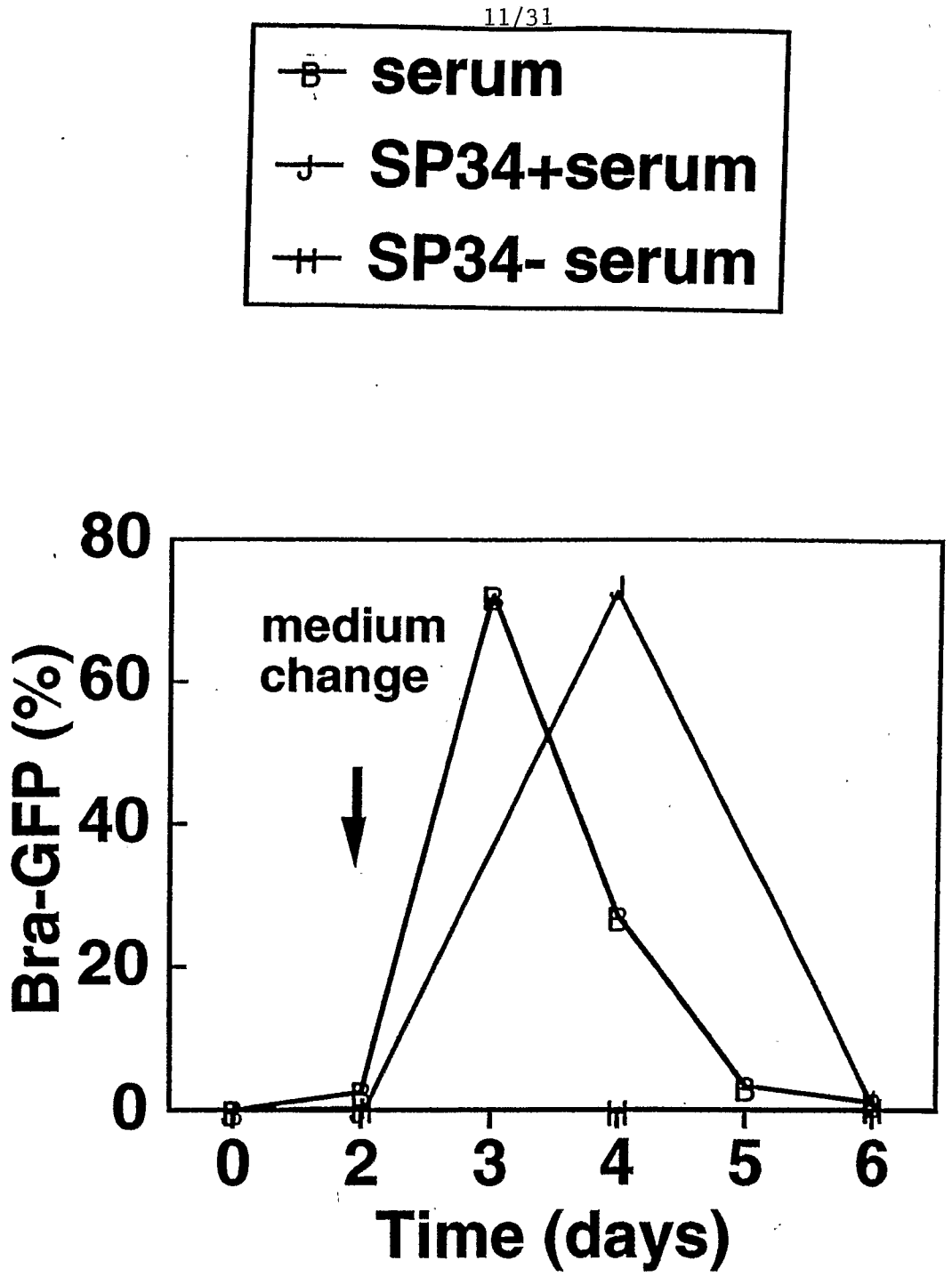

serum
SP34+serum
SP34- serum
80
60
40
20
0
Bra-GFP (%)
medium
change
0
2
3
4
5
6
Time (days)

Fig. 11

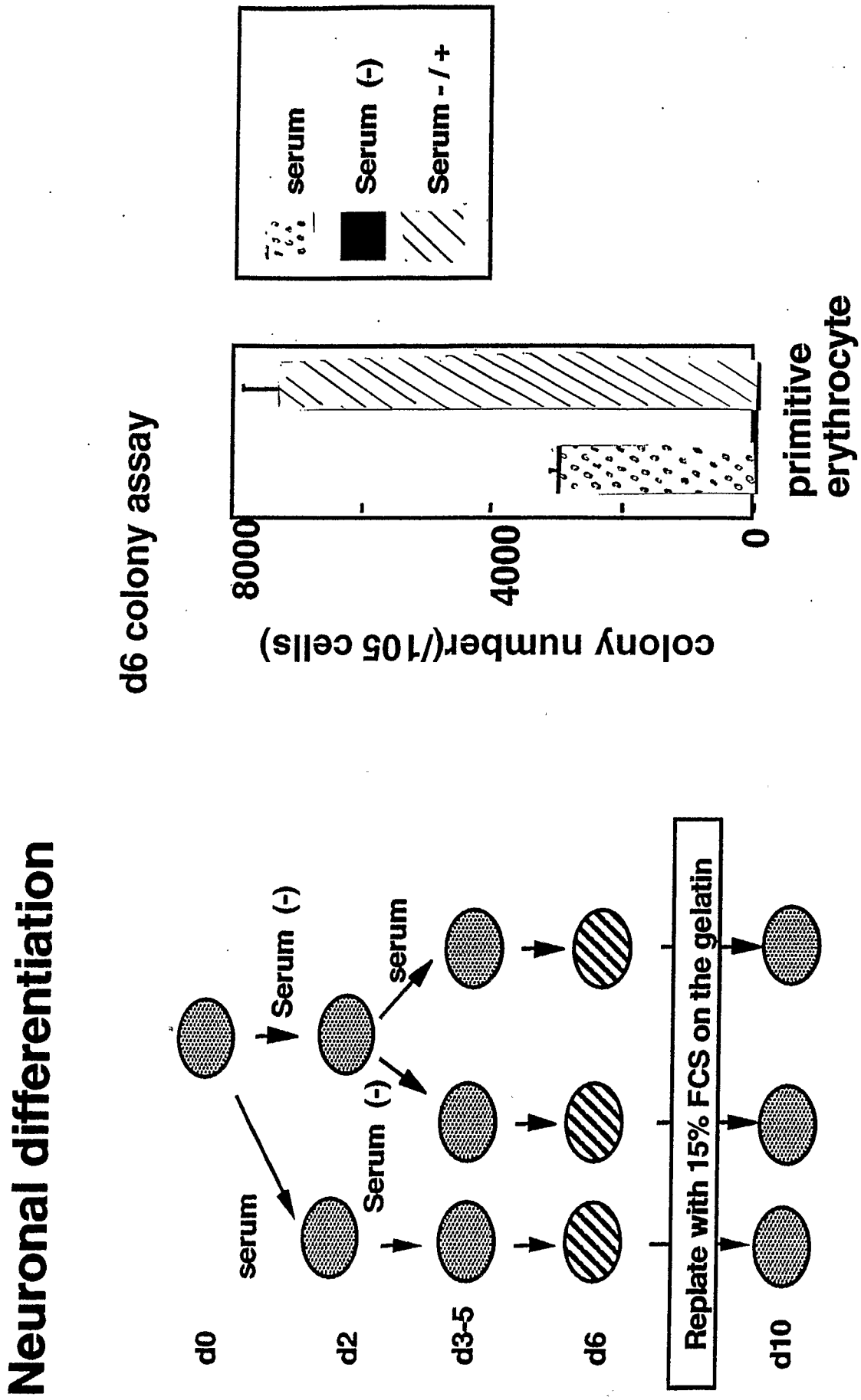

Neuronal differentiation
d0
serum
Serum (-)
d2
Serum (-)
serum
d3-5
d6
Replate with 15% FCS on the gelatin
d10
d6 colony assay
colony number(/10⁵ cells)
8000
4000
0
primitive erythrocyte
serum
Serum (-)
Serum - / +

Fig. 12

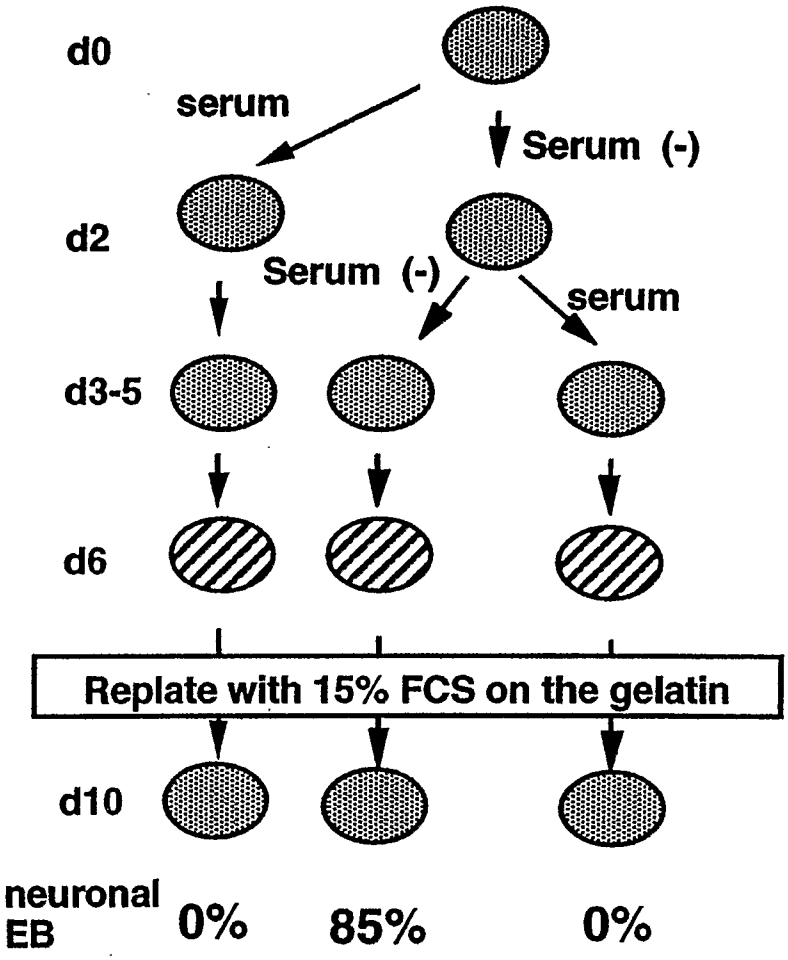

d0
serum
Serum (-)
d2
Serum (-)
serum
d3-5
d6
Replate with 15% FCS on the gelatin
d10
neuronal EB
0%
85%
0%

Fig. 13

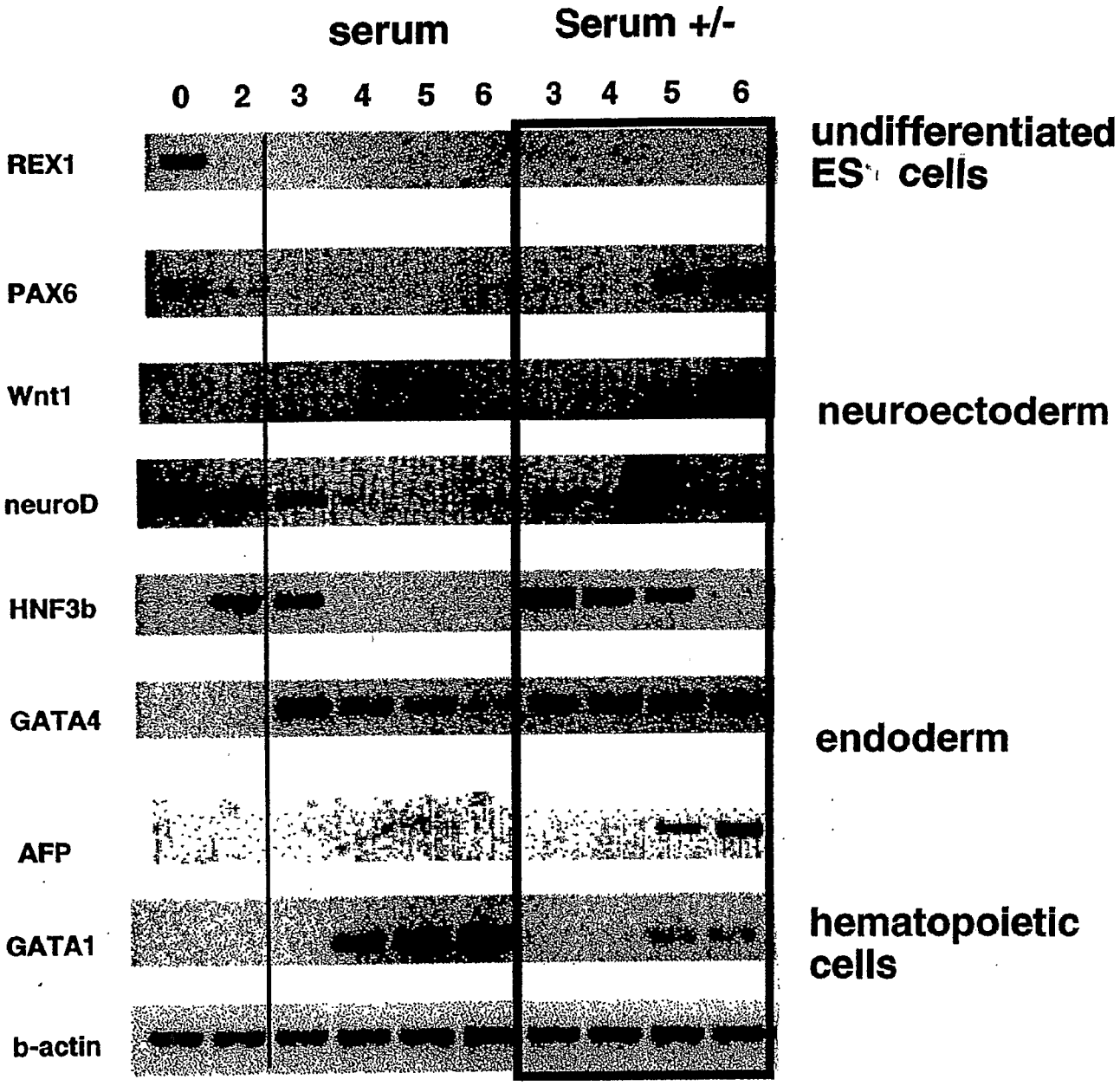
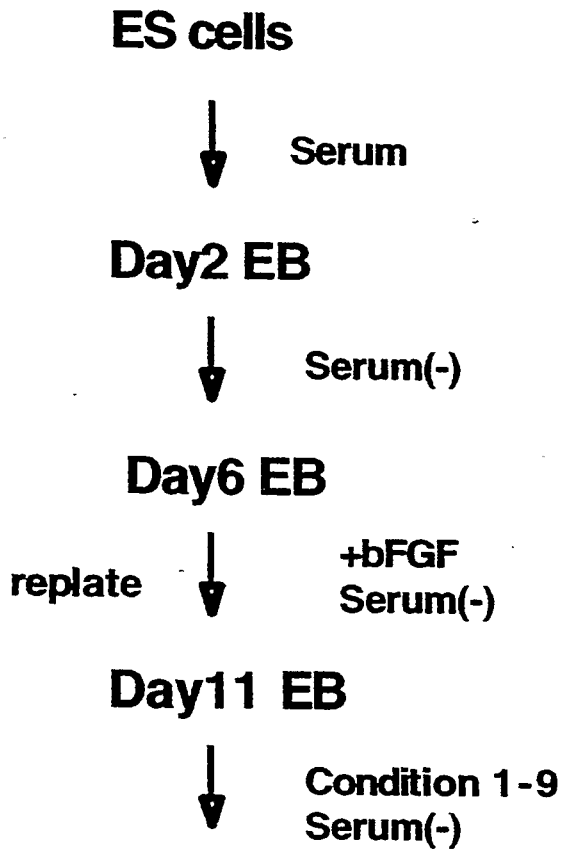
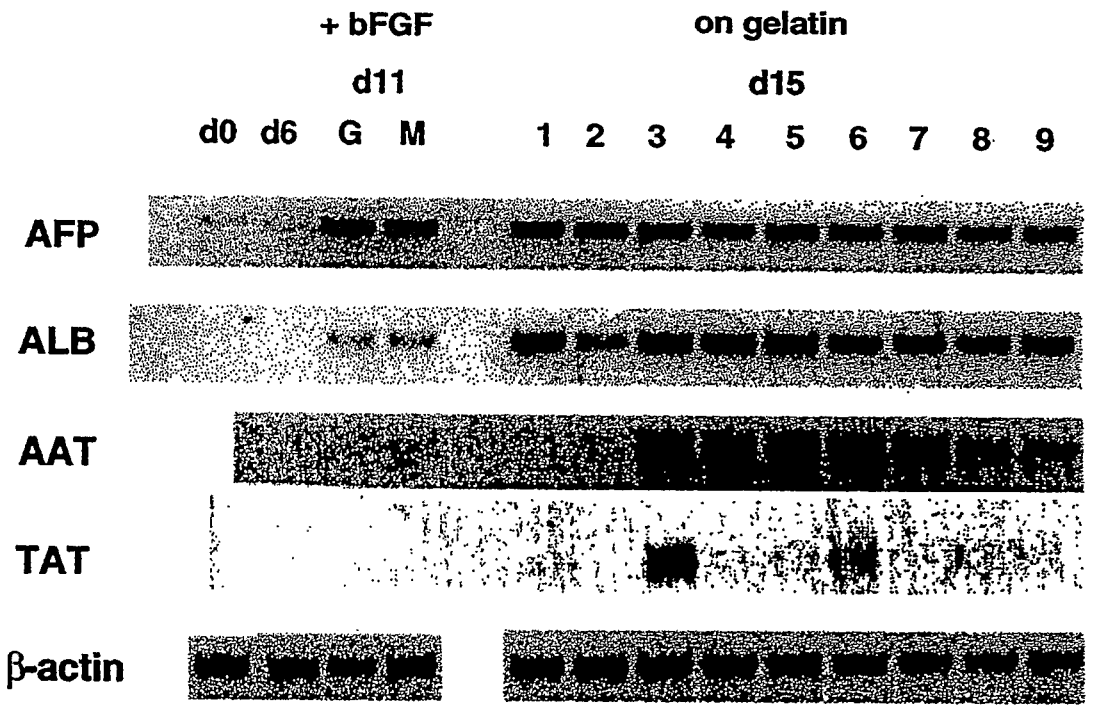

serum
Serum +/-
0 2 3 4 5 6 3 4 5 6
REX1
PAX6
Wnt1
neuroD
HNF3b
GATA4
AFP
GATA1
b-actin
undifferentiated ES cells
neuroectoderm
endoderm
hematopoietic cells

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

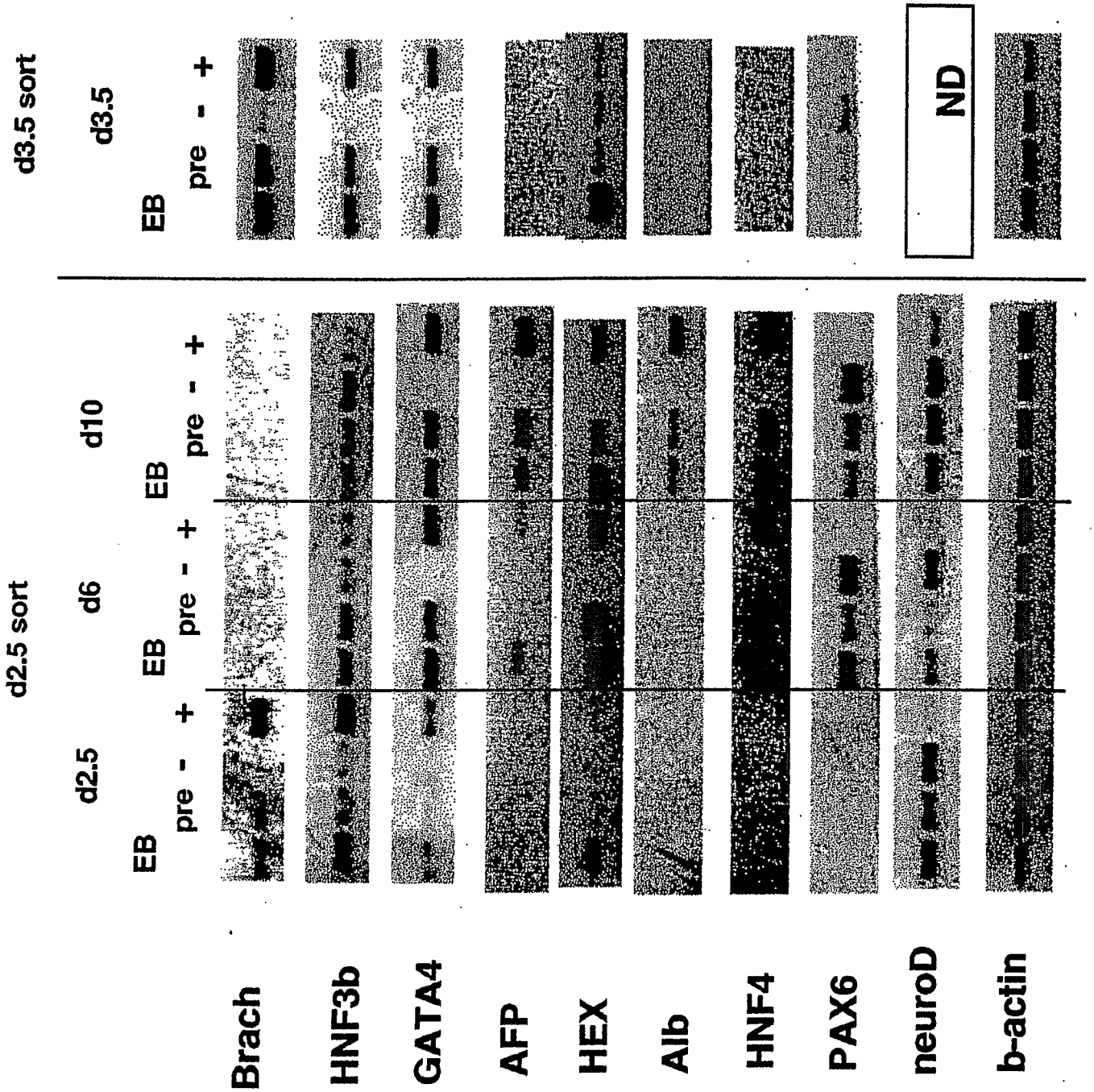

d2.5 sort
d3.5 sort
EB
pre
d2.5
d6
d10
d3.5
Brach
HNF3b
GATA4
AFP
HEX
Alb
HNF4
PAX6
neuroD
b-actin
ND

Fig. 16

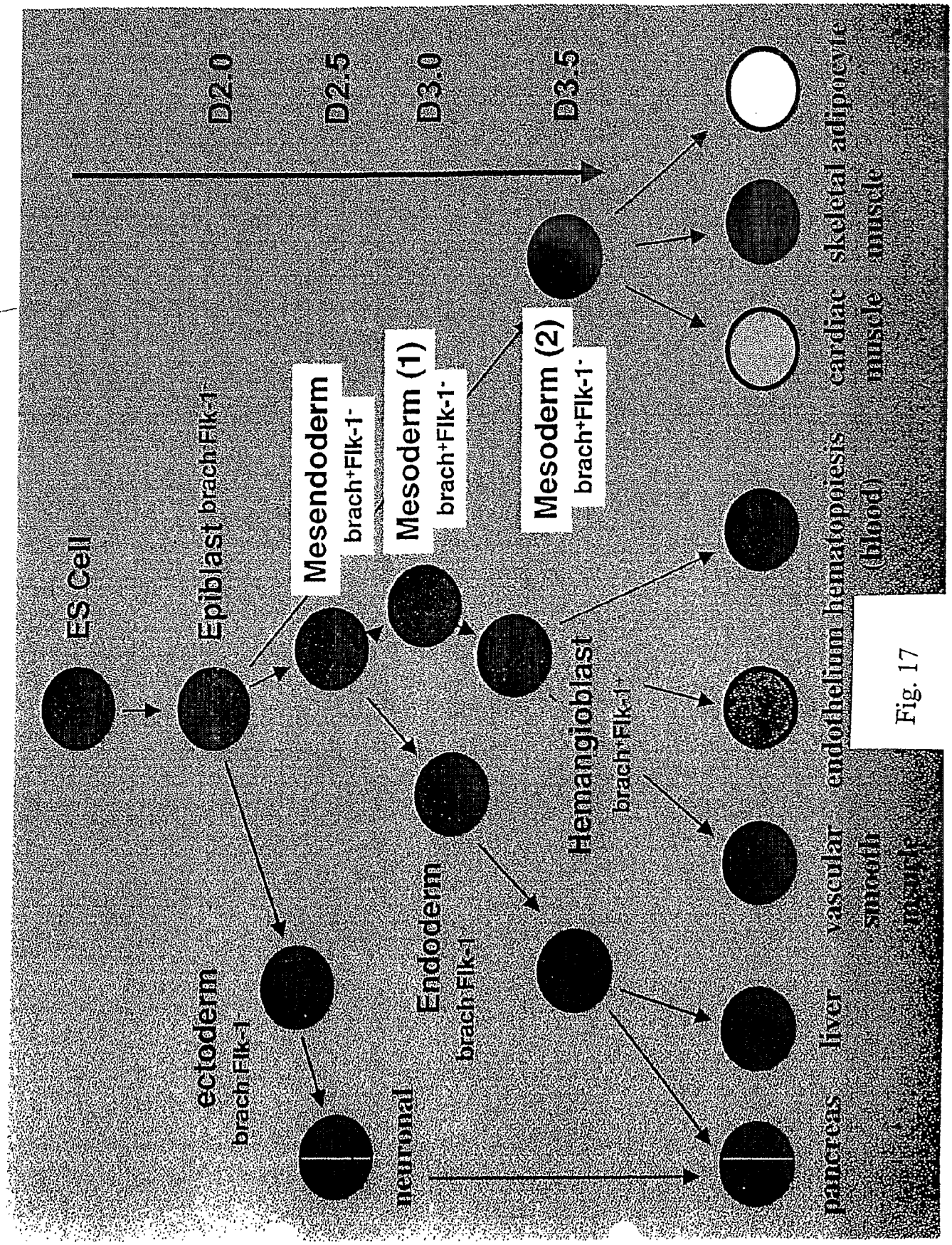
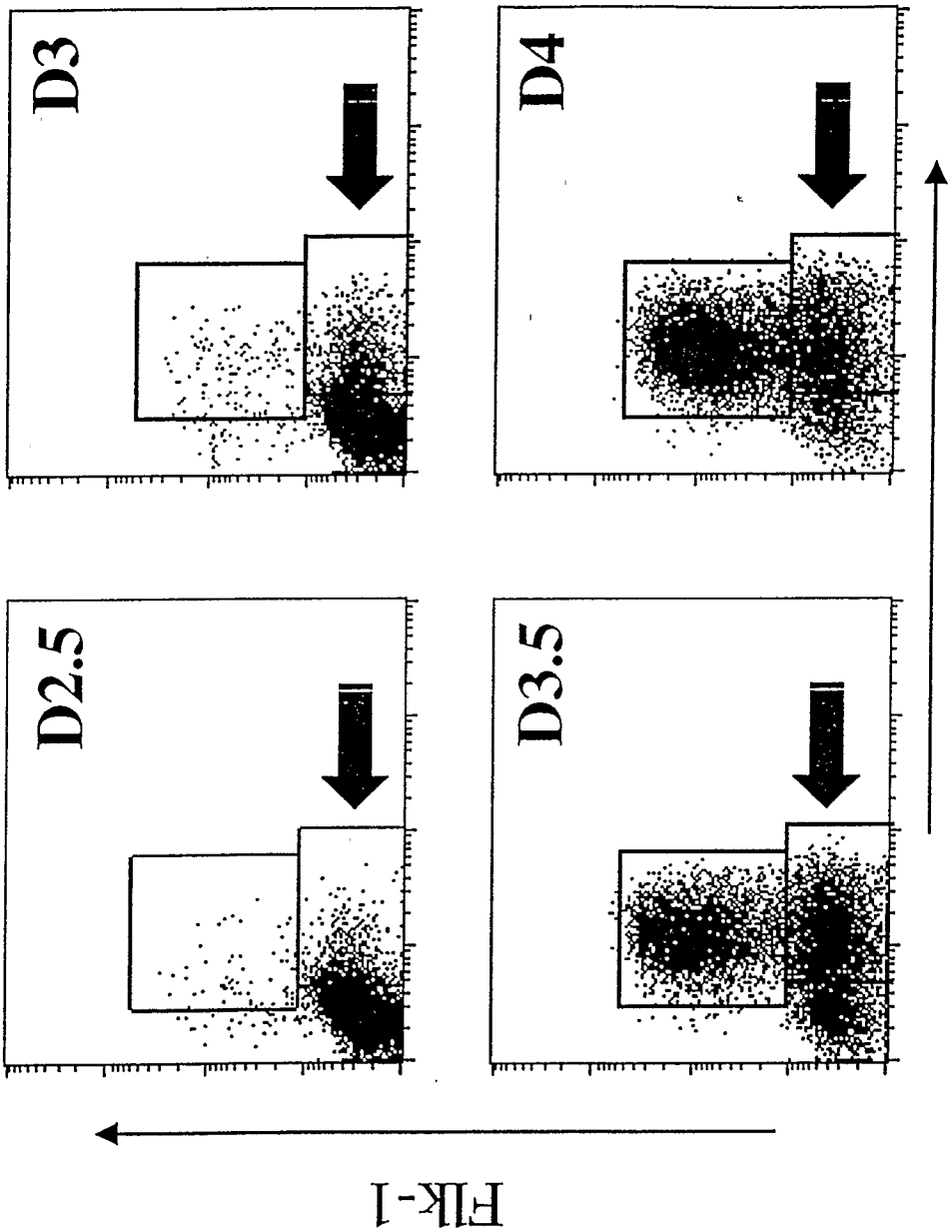
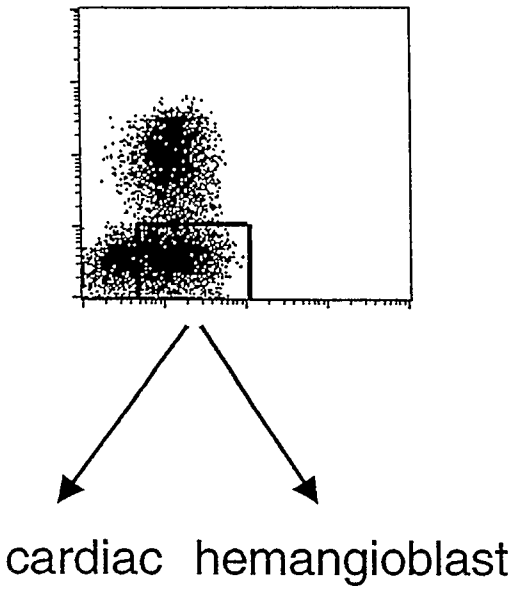
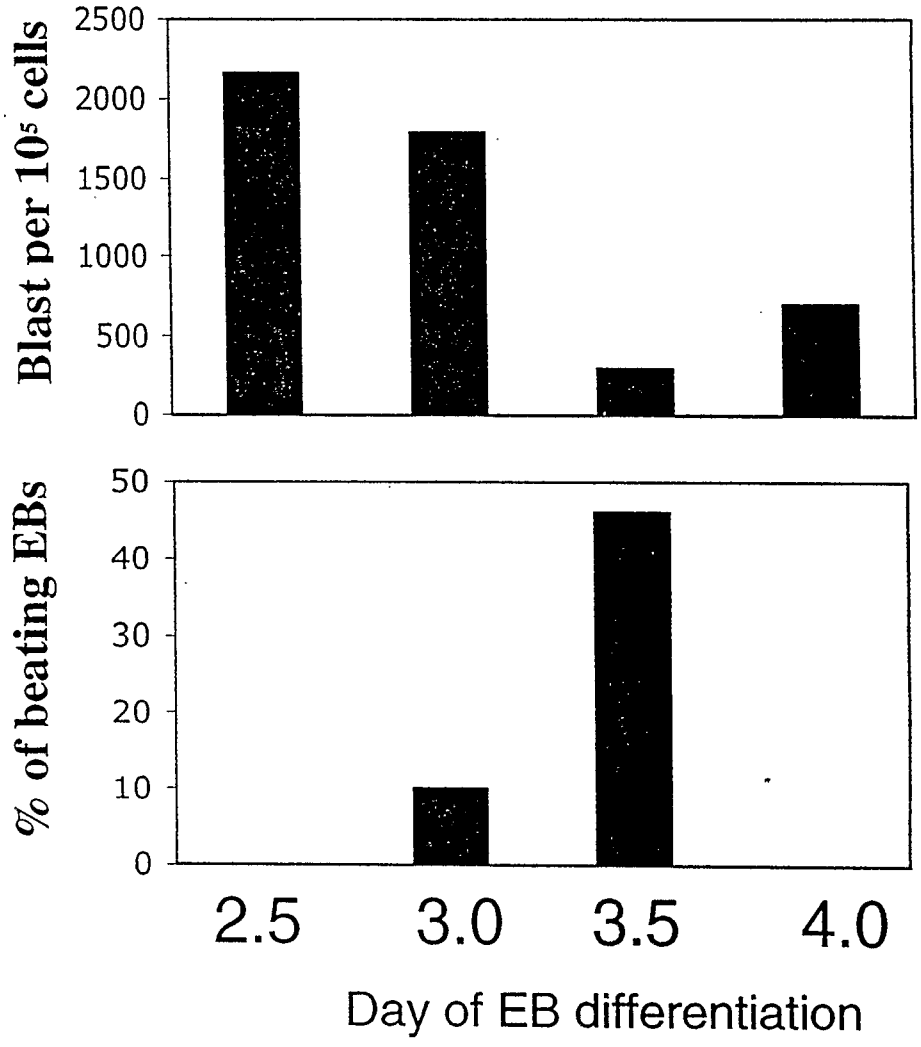

D2.0
D2.5
D3.0
D3.5
ES Cell
Epiblast brach+Flk-1-
Mesendoderm
brach+Flk-1-
Mesoderm (1)
brach+Flk-1-
Mesoderm (2)
brach+Flk-1-
ectoderm
brach-Flk-1-
neuronal
Endoderm
brach-Flk-1-
Hemangioblast
brach+Flk-1+
pancreas
liver
vascular smooth muscle
endothelium hematopoiesis (blood)
cardiac muscle
skeletal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

Fig. 17

Figure 18







Hemangioblas Potential

Cardiac Potential

Figure 19

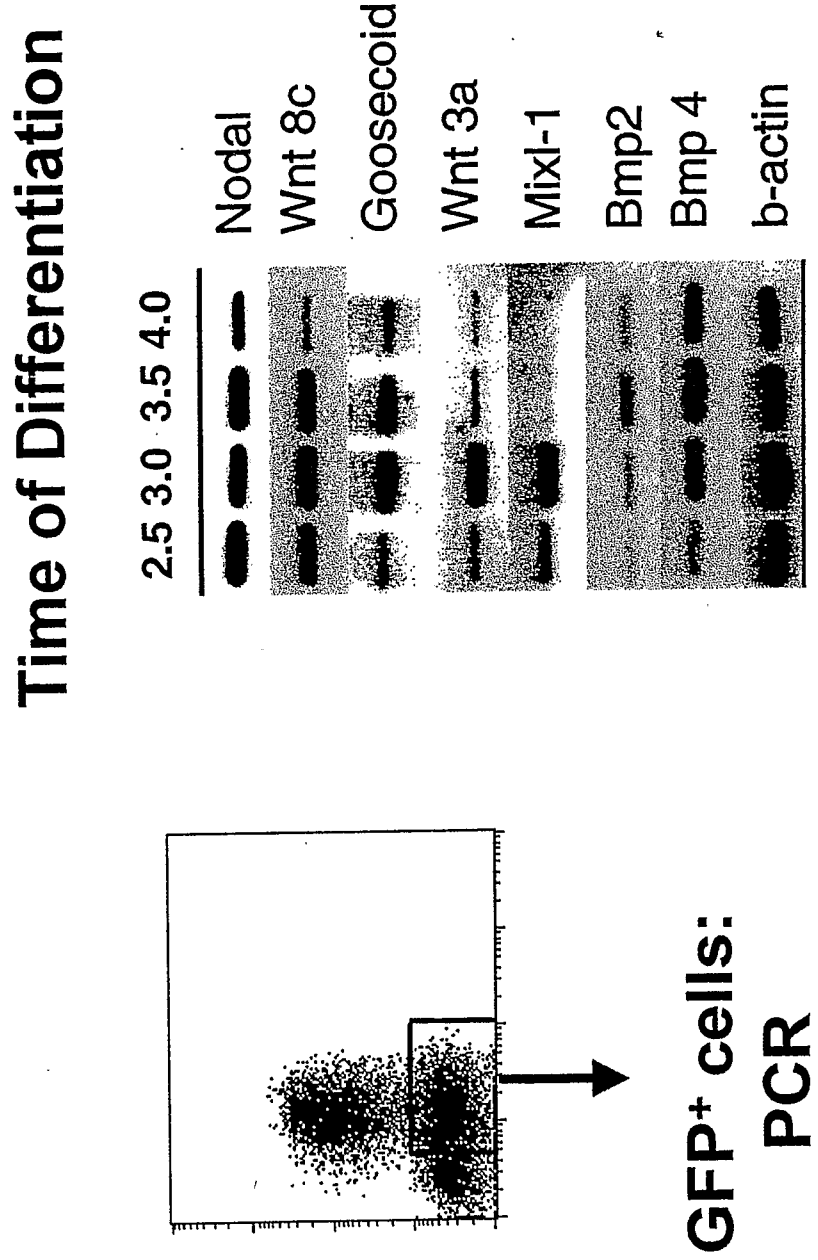

Time of Differentiation
2.5 3.0 3.5 4.0
Nodal
Wnt 8c
Goosecoid
Wnt 3a
Mixl-1
Bmp2
Bmp 4
b-actin
GFP+ cells:
PCR

Figure 20

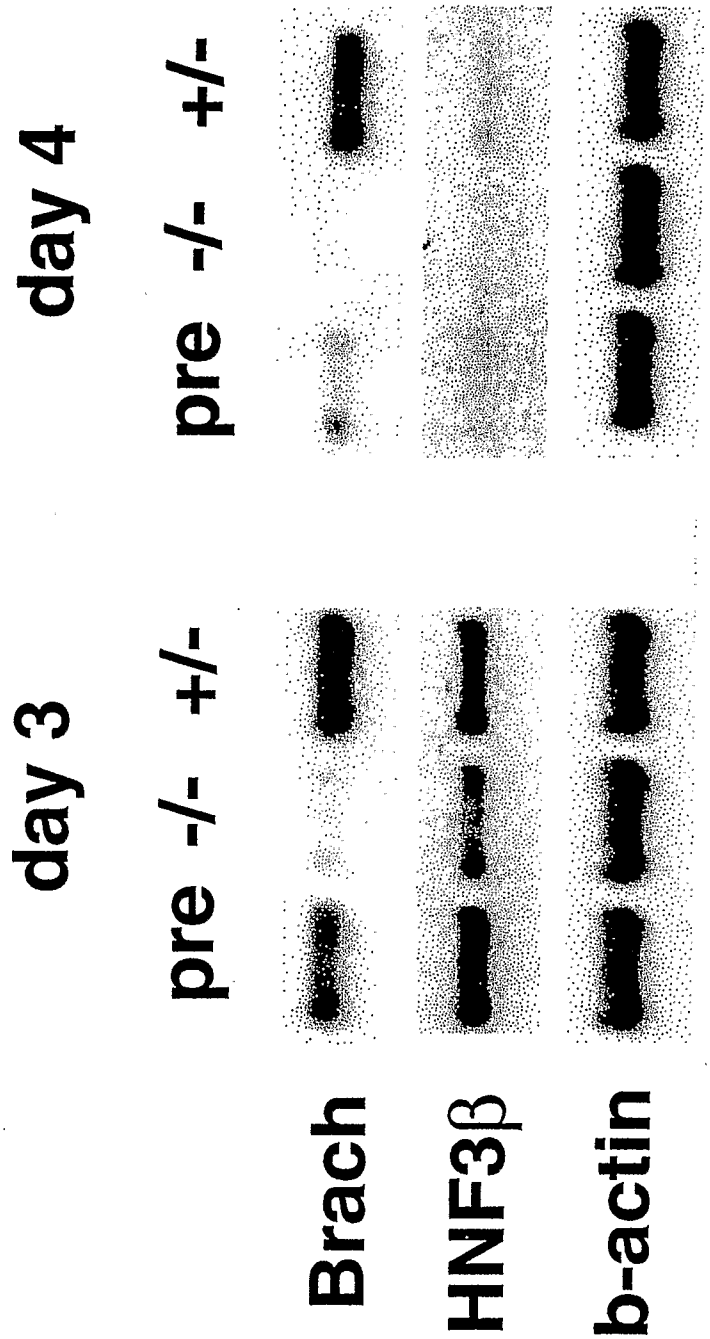


Figure 21

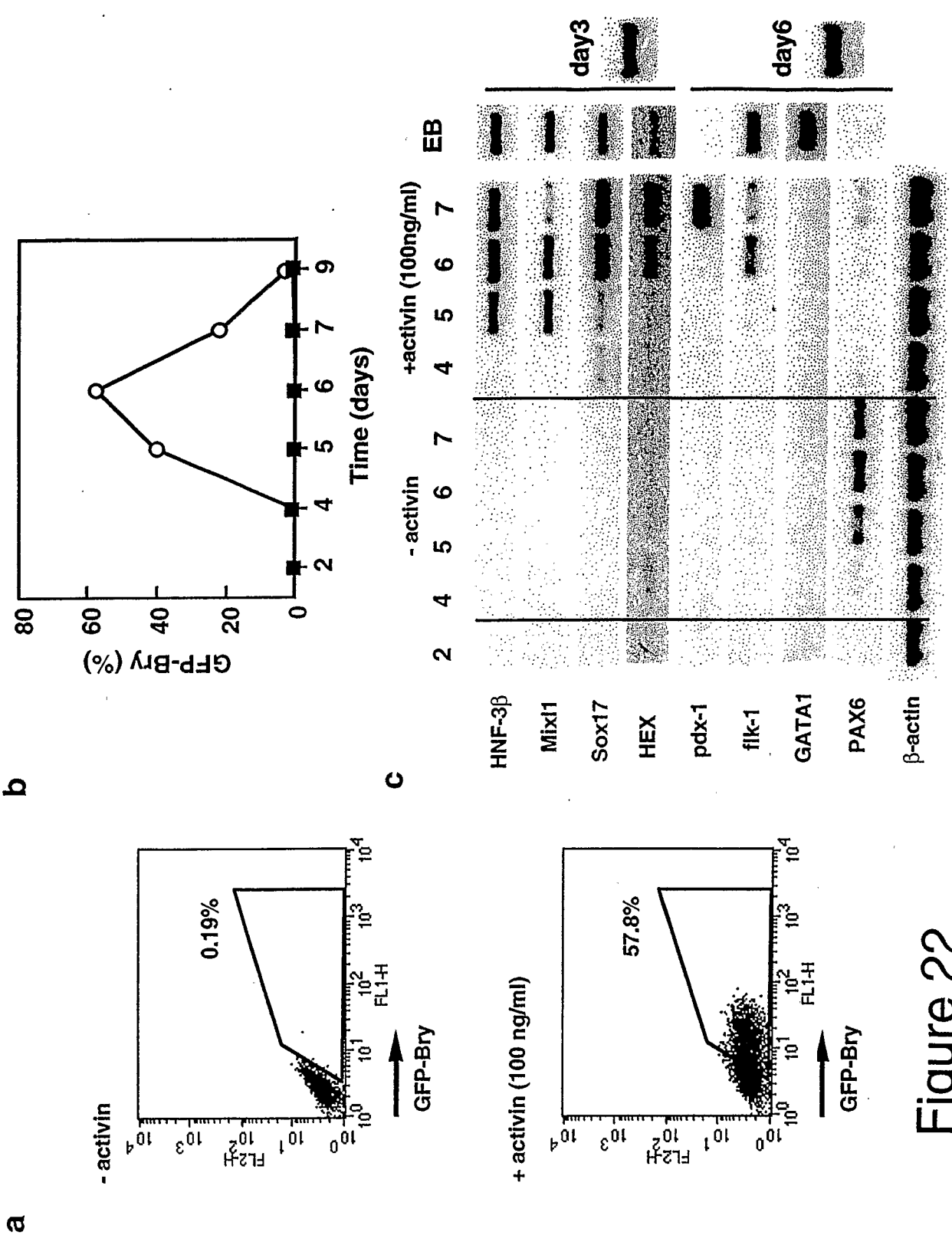

a
- activin
0.19%
FL2-H
FL1-H
GFP-Bry
+ activin (100 ng/ml)
57.8%
b
GFP-Bry (%)
Time (days)
c
- activin
+activin (100ng/ml)
EB
day3
day6
HNF-3β
Mixl1
Sox17
HEX
pdx-1
flk-1
GATA1
PAX6
β-actin

Figure 22

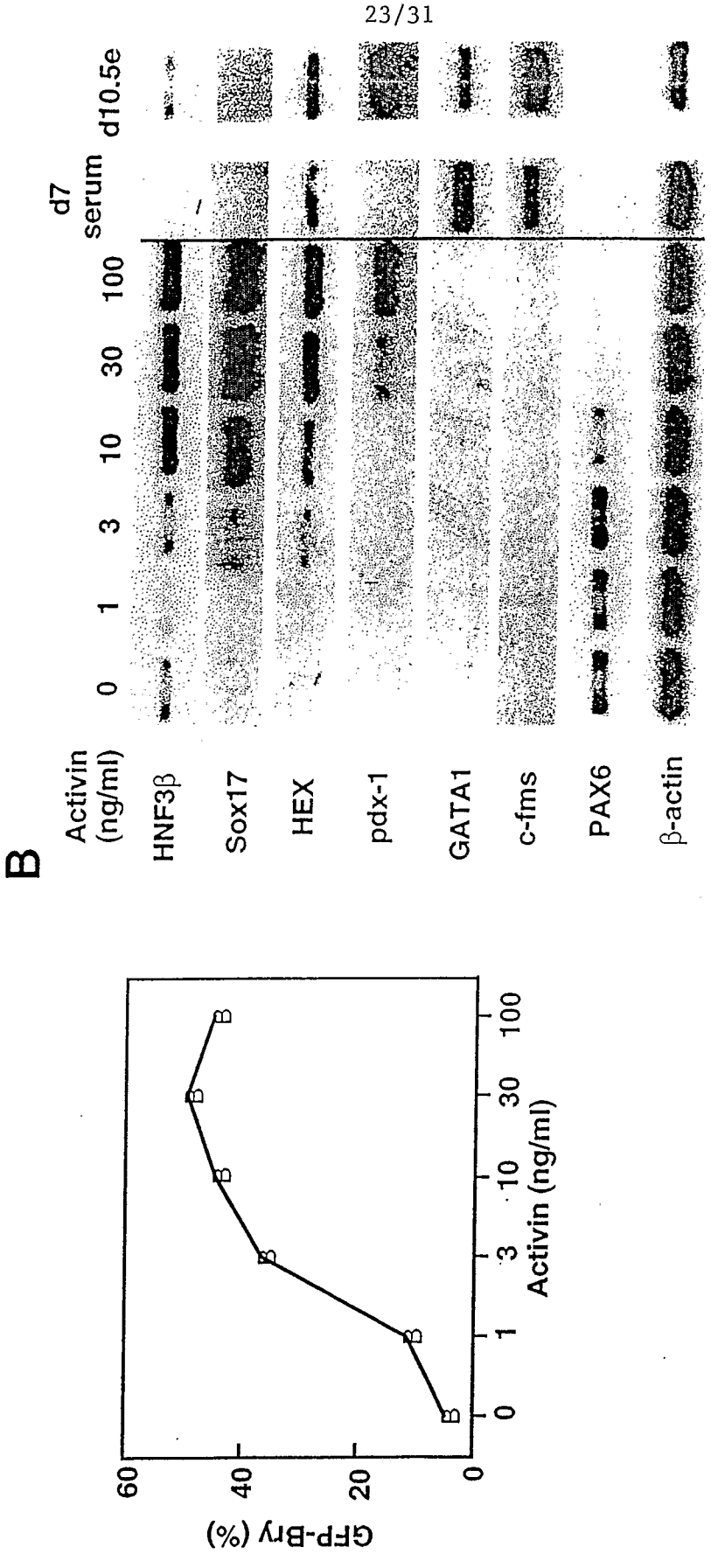
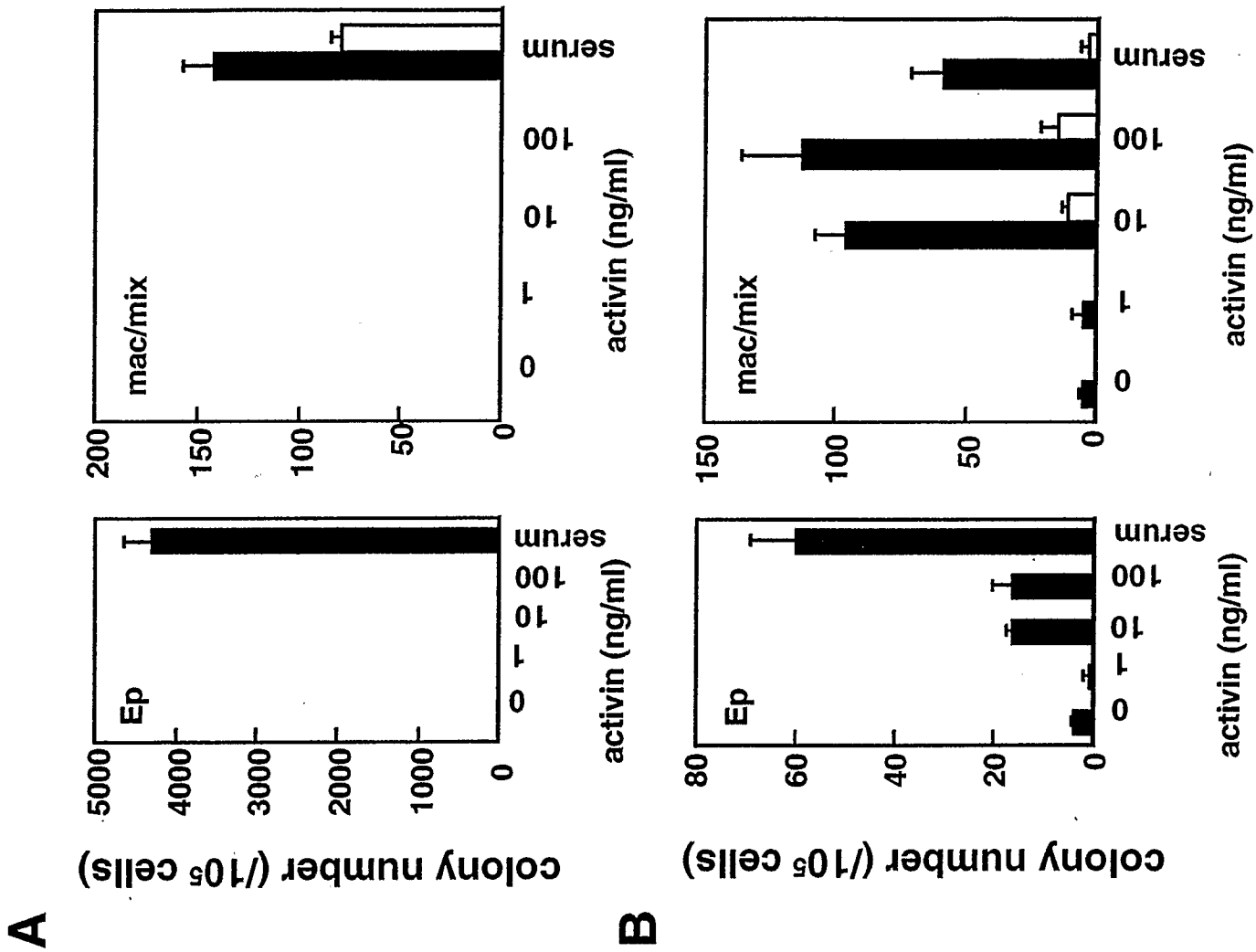

A
GFP-Bry (%)
60
40
20
0
0
1
3
10
30
100
Activin (ng/ml)
B
Activin (ng/ml)
0
1
3
10
30
100
d7 serum
d10.5e
HNF3β
Sox17
HEX
pdx-1
GATA1
c-fms
PAX6
β-actin

Figure 23

Figure 24


A
colony number (/10⁵ cells)
Ep
mac/mix
activin (ng/ml)
0
1
10
100
serum
0
1000
2000
3000
4000
5000
50
100
150
200
B
colony number (/10⁵ cells)
Ep
mac/mix
activin (ng/ml)
0
1
10
100
serum
0
20
40
60
80
50
100
150

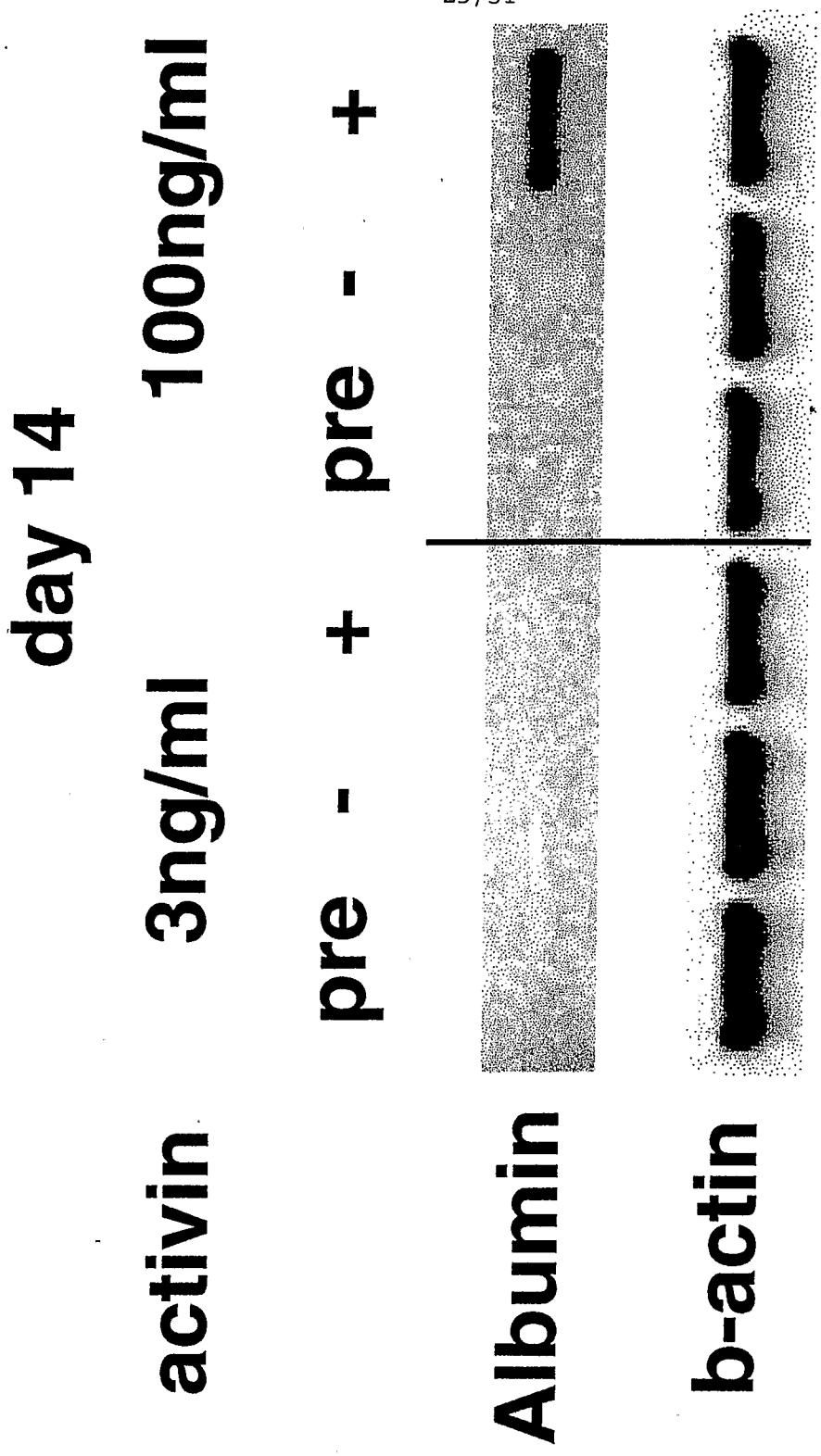


Figure 25

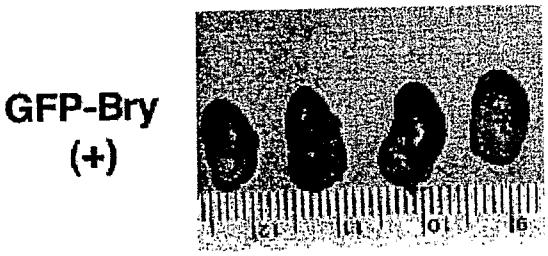


Fig. 26A

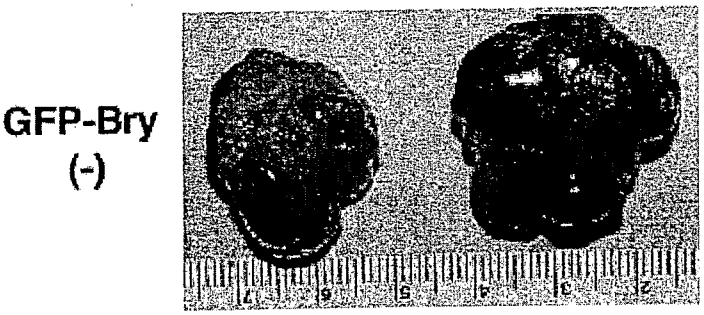


Fig. 26B

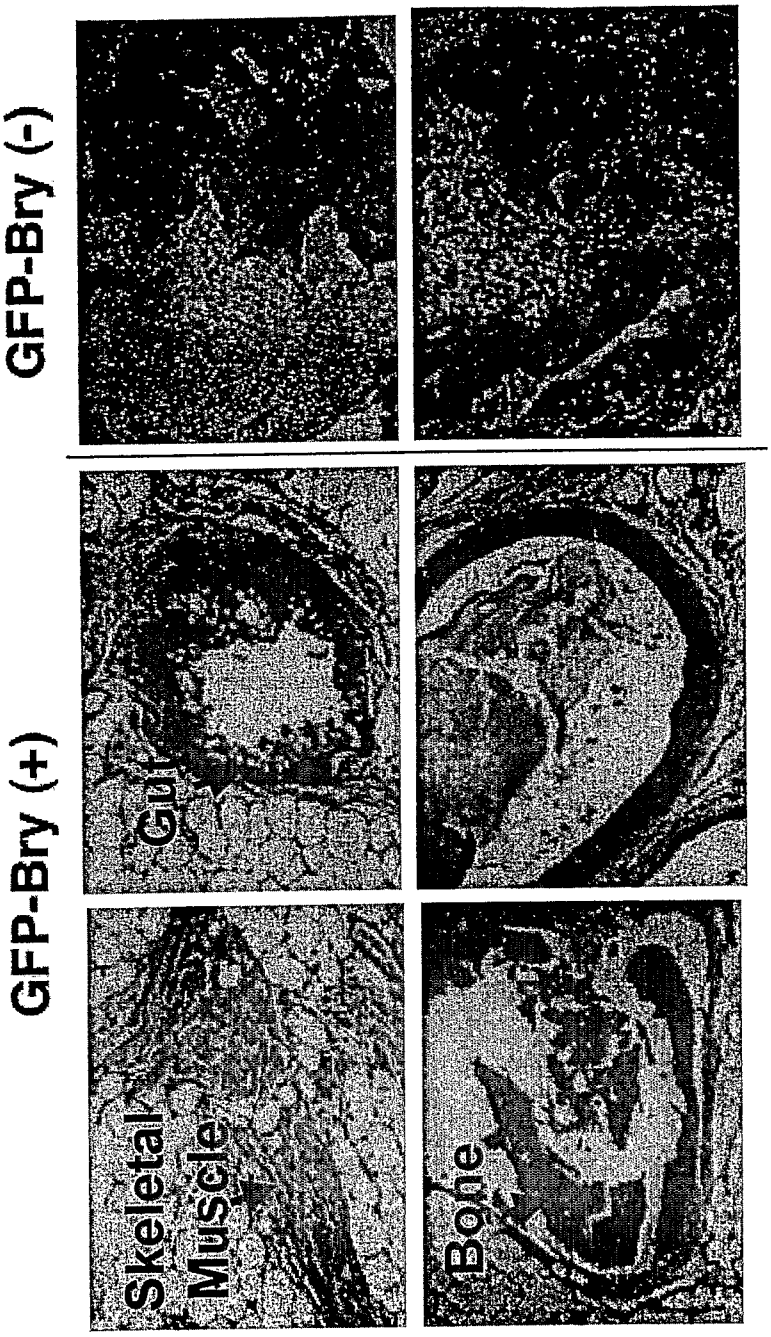


Fig. 26C

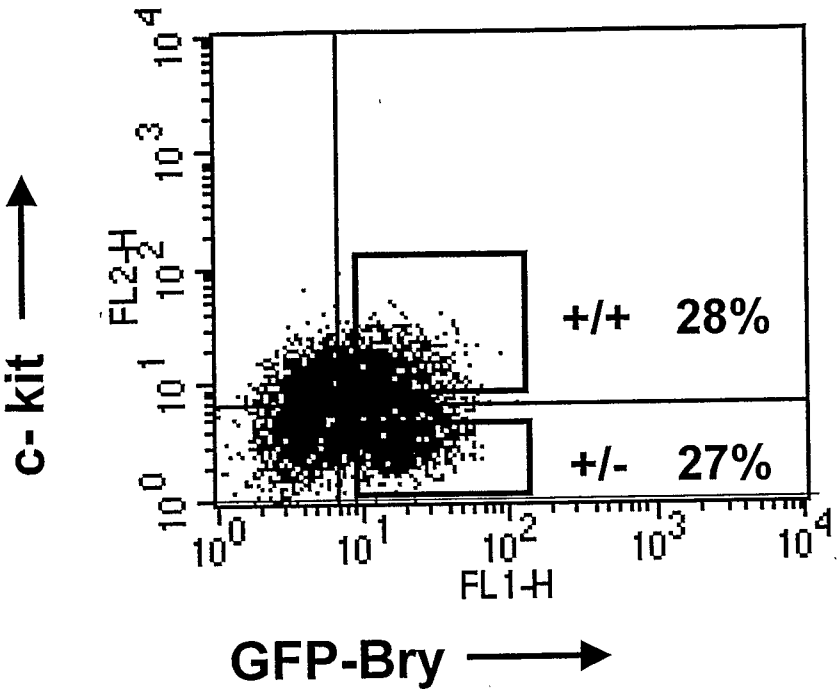


Fig. 27A

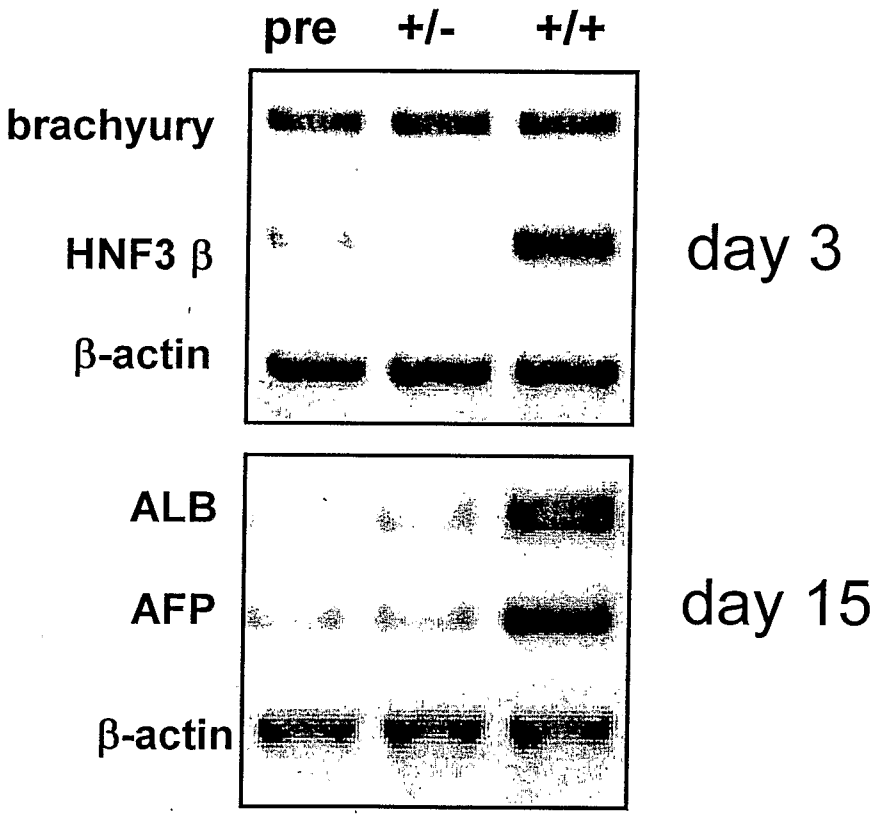


Fig. 27B

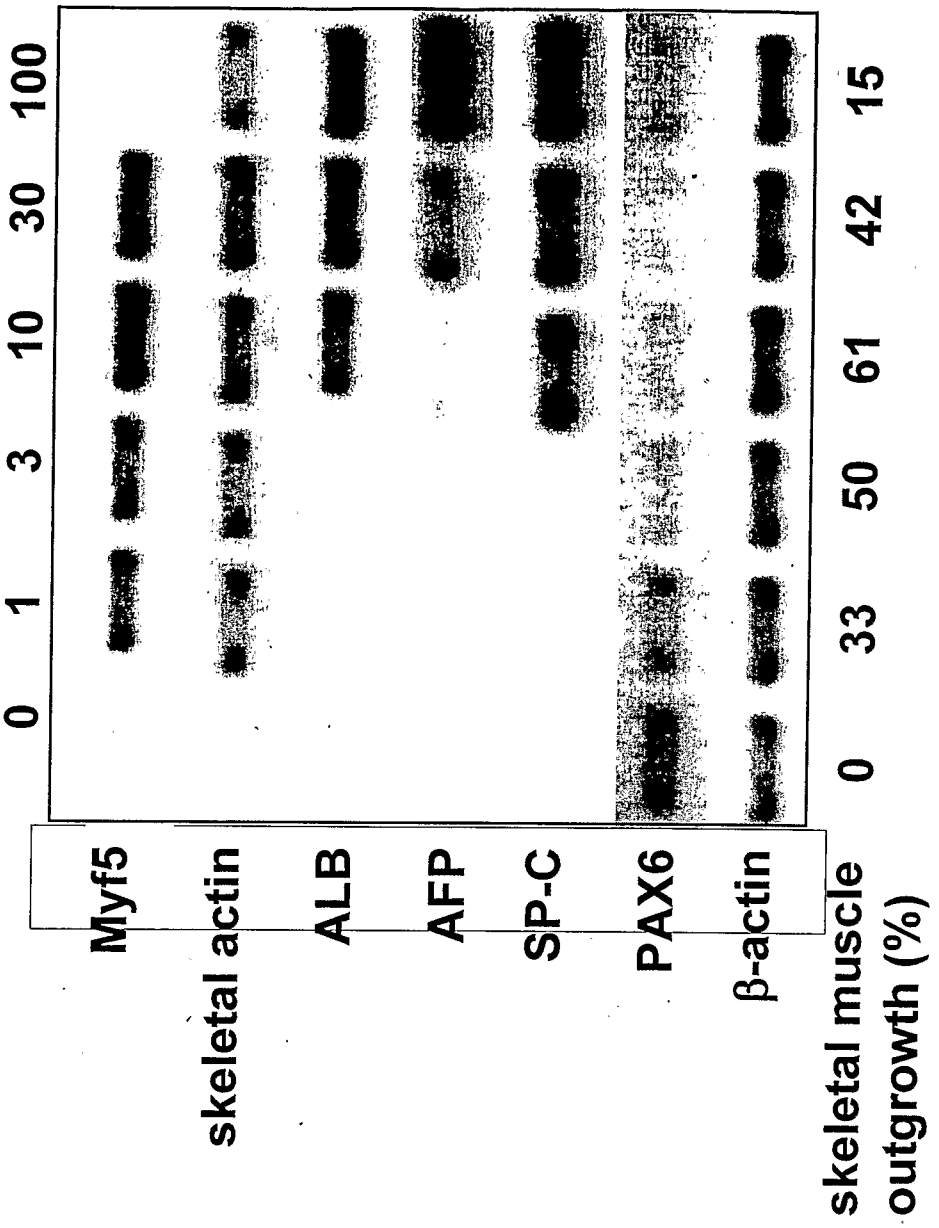

0 1 3 10 30 100
0 33 50 61 42 15
Myf5
skeletal actin
ALB
AFP
SP-C
PAX6
β-actin
skeletal muscle outgrowth (%)

Fig. 28

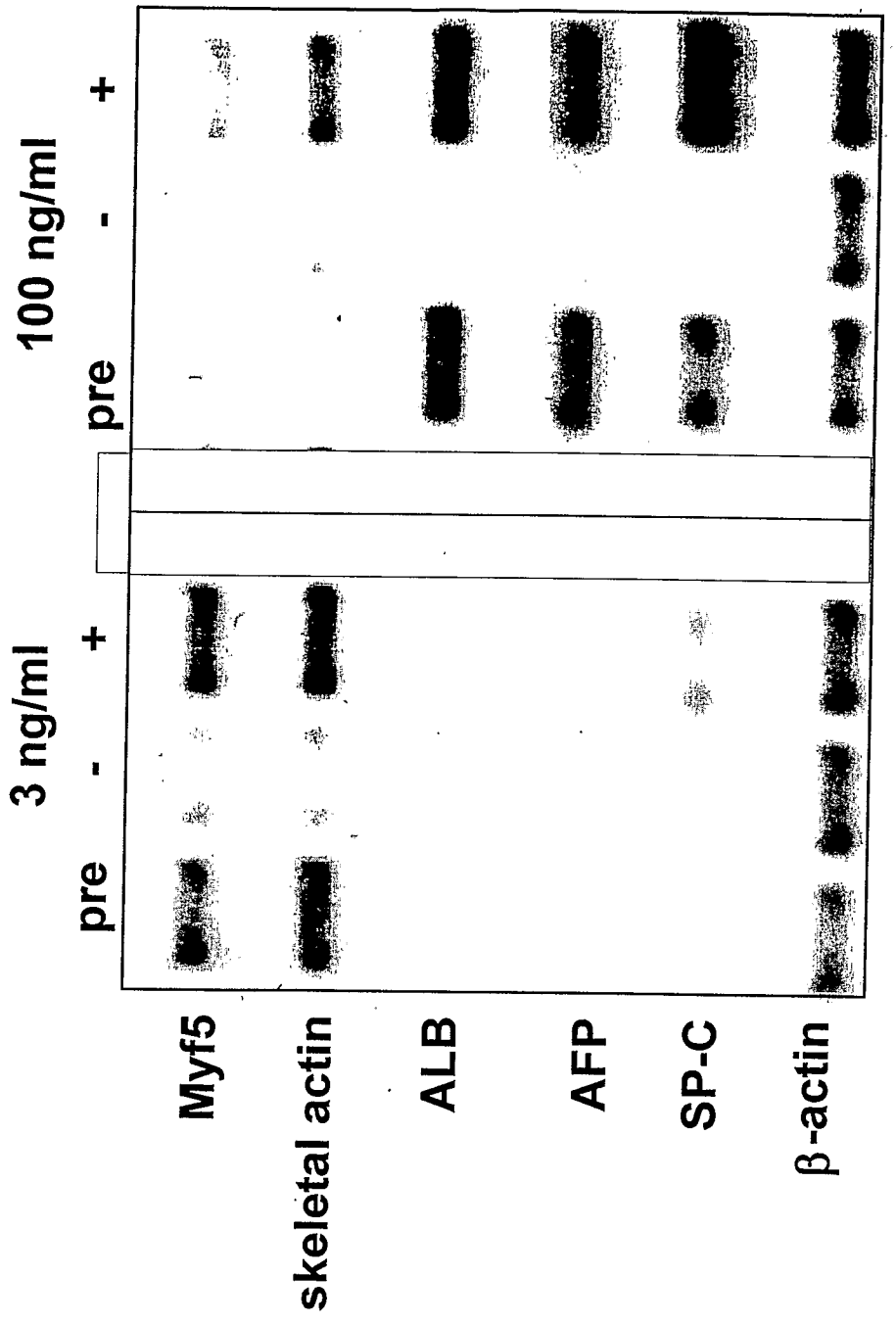

3 ng/ml
100 ng/ml
pre
-
+
pre
-
+
Myf5
skeletal actin
ALB
AFP
SP-C
β-actin

Fig. 29