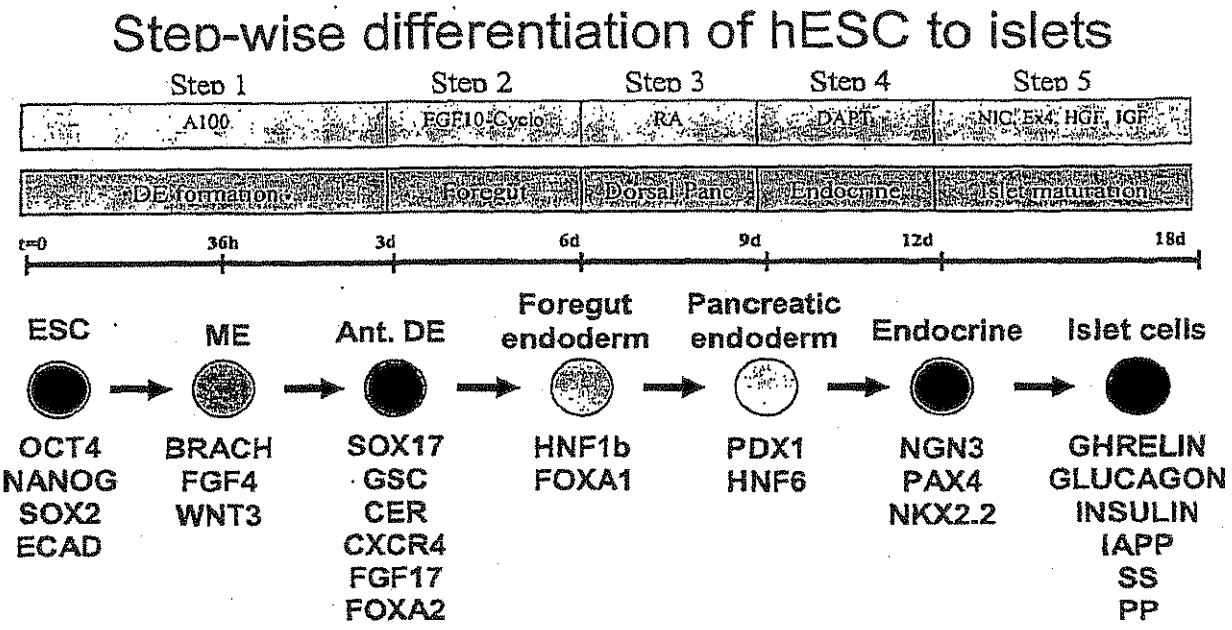
Figure 1


Step-wise differentiation of hESC to islets
Step 1
Step 2
Step 3
Step 4
Step 5
A100
FGF10 Cyclo
RA
DAPT
NIC, ER4, HGF, IGF
DE formation
Foregut
Dorsal Panc
Endocrine
Islet maturation
t=0
36h
3d
6d
9d
12d
18d
ESC
ME
Ant. DE
Foregut endoderm
Pancreatic endoderm
Endocrine
Islet cells
OCT4
NANOG
SOX2
ECAD
BRACH
FGF4
WNT3
SOX17
GSC
CER
CXCR4
FGF17
FOXA2
HNF1b
FOXA1
PDX1
HNF6
NGN3
PAX4
NKX2.2
GHRELIN
GLUCAGON
INSULIN
IAPP
SS
PP

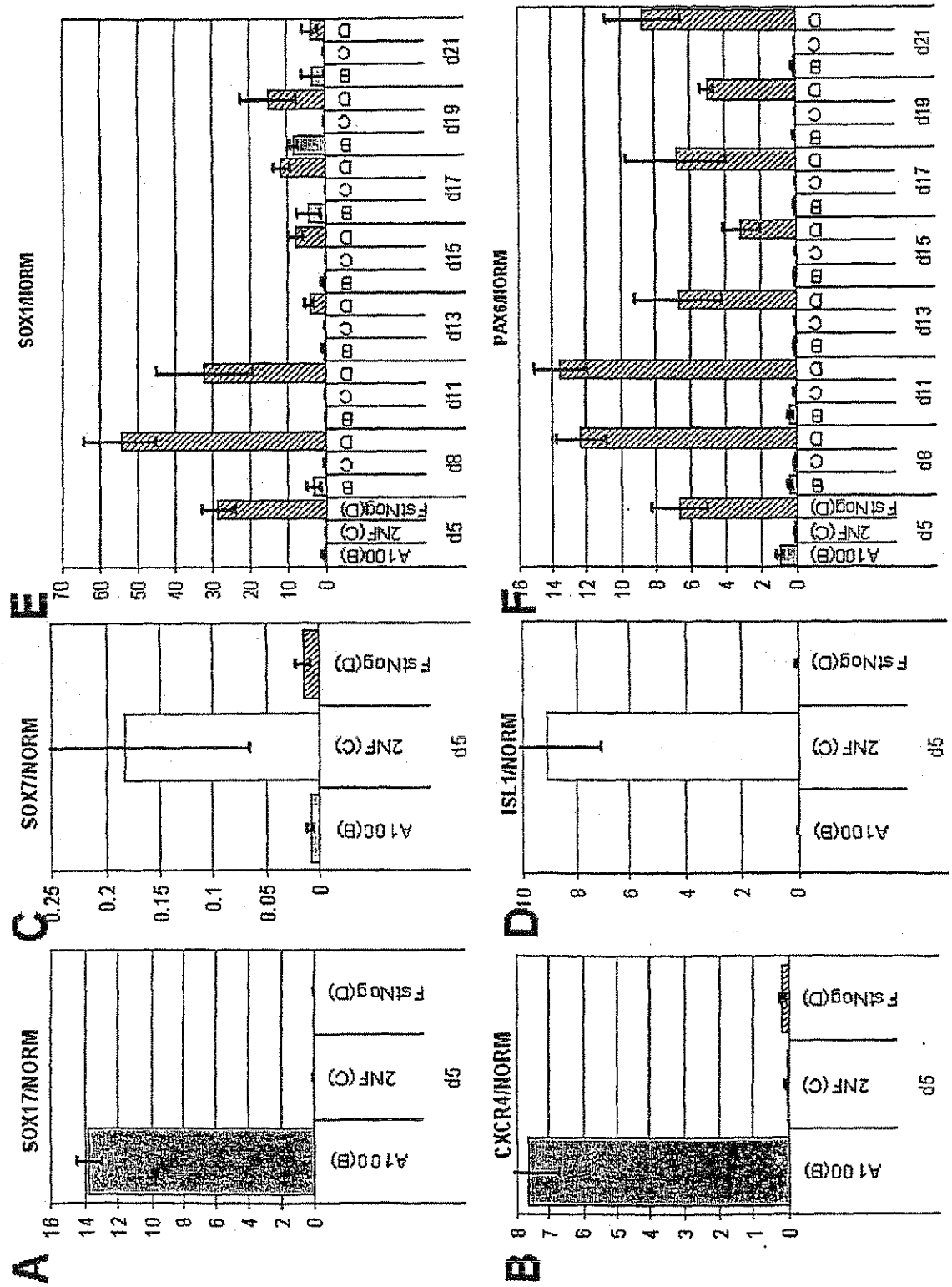
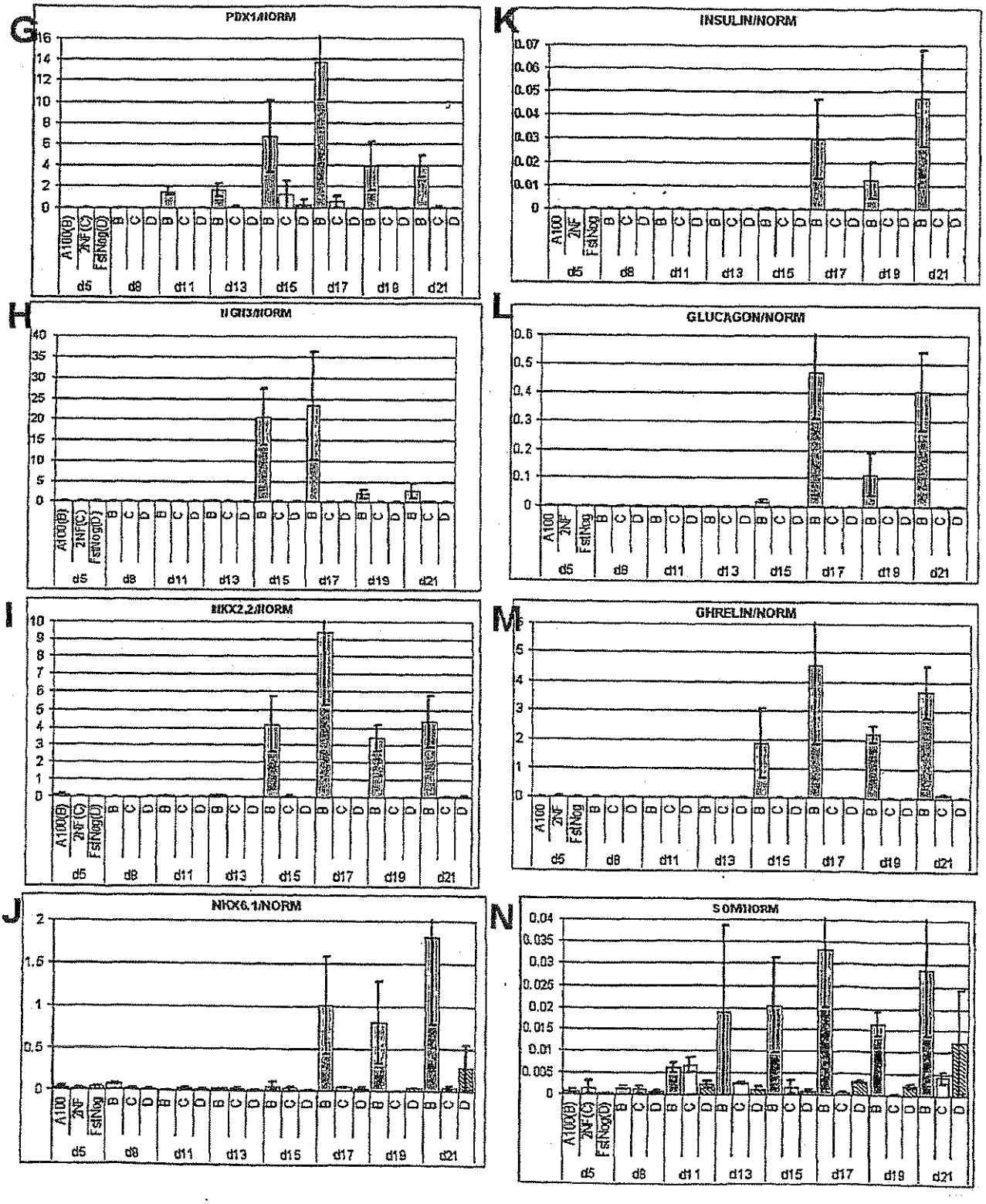

A
SOX17/NORM
A100(B)
2NF(C)
FstNog(D)
d5
B
CXCR4/NORM
C
SOX7/NORM
D
ISL1/NORM
E
SOX1/NORM
d8
d11
d13
d15
d17
d19
d21
F
PAX6/NORM

FIGURE. 2


G
PDX1/NORM
H
NGN3/NORM
I
NKX2.2/NORM
J
NKX6.1/NORM
K
INSULIN/NORM
L
GLUCAGON/NORM
M
GHRELIN/NORM
N
SOM/NORM
A100(B)
2NF(C)
FstNog(D)
d5
d8
d11
d13
d15
d17
d19
d21

(con't)

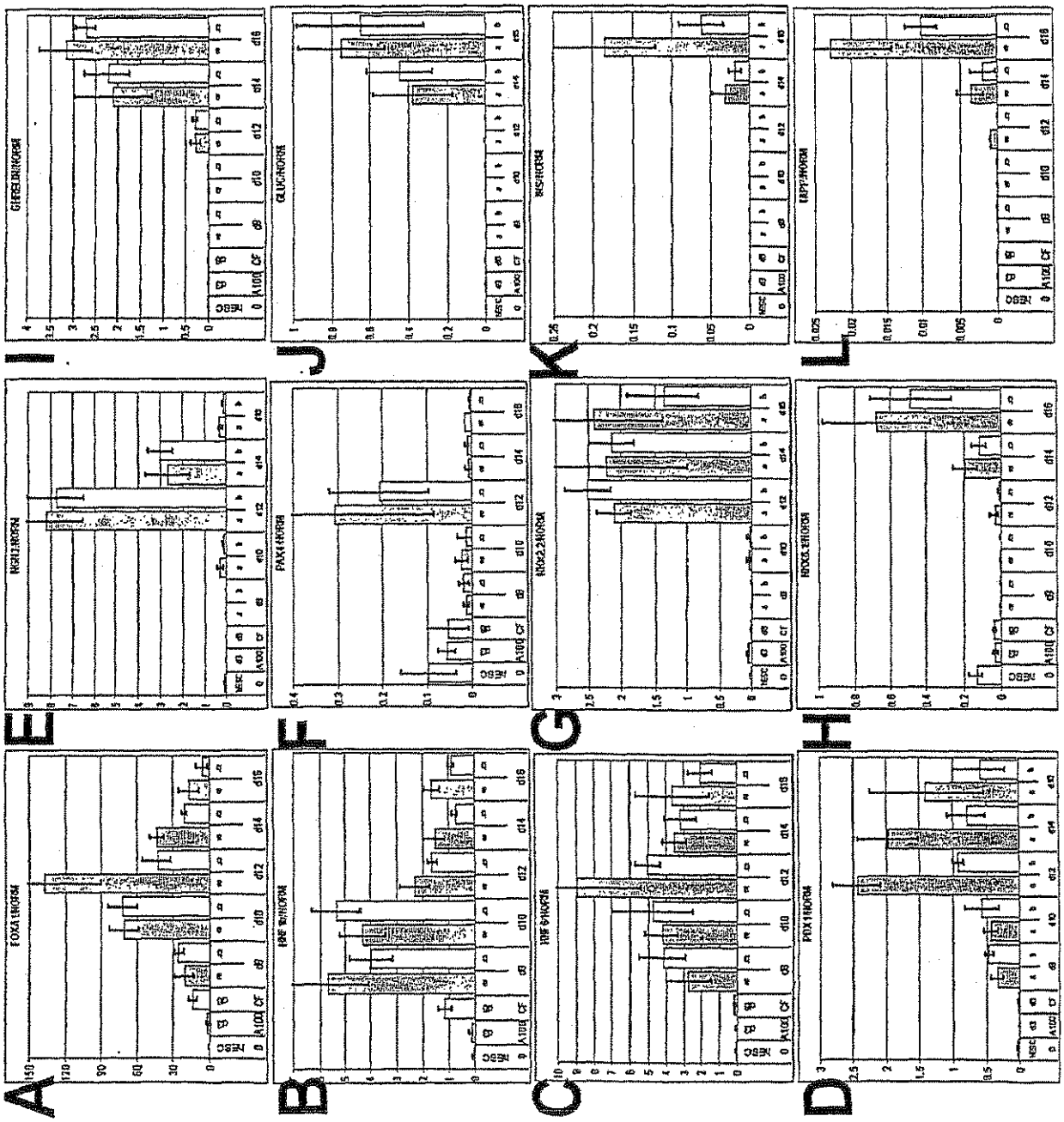


FIGURE. 3

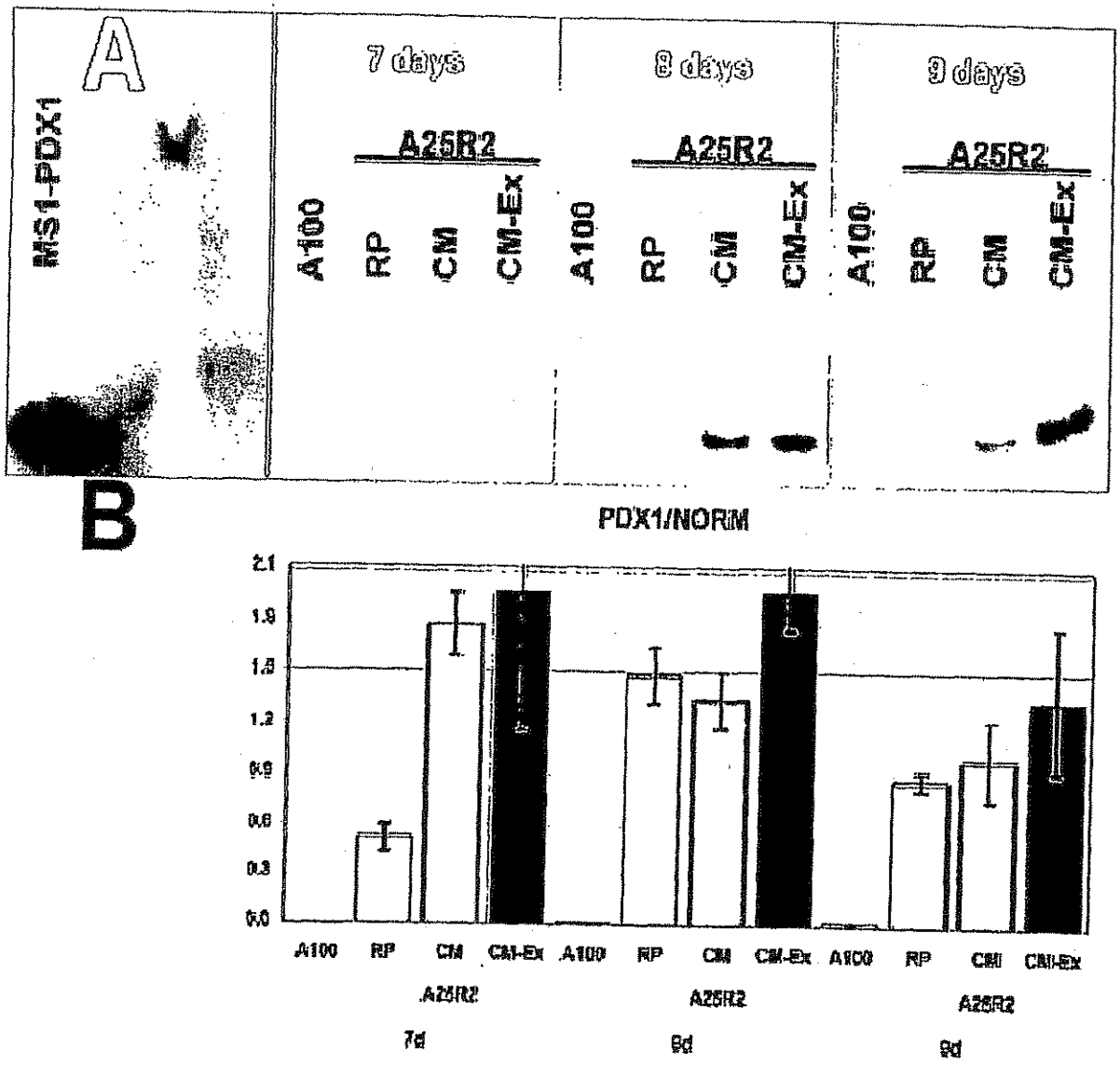

A
MS1-PDX1
7 days
8 days
9 days
A25R2
A100
RP
CM
CM-Ex
B
PDX1/NORM
2.1
1.8
1.2
0.9
0.6
0.3
0.0
A25R2
7d
8d
9d

FIGURE. 4

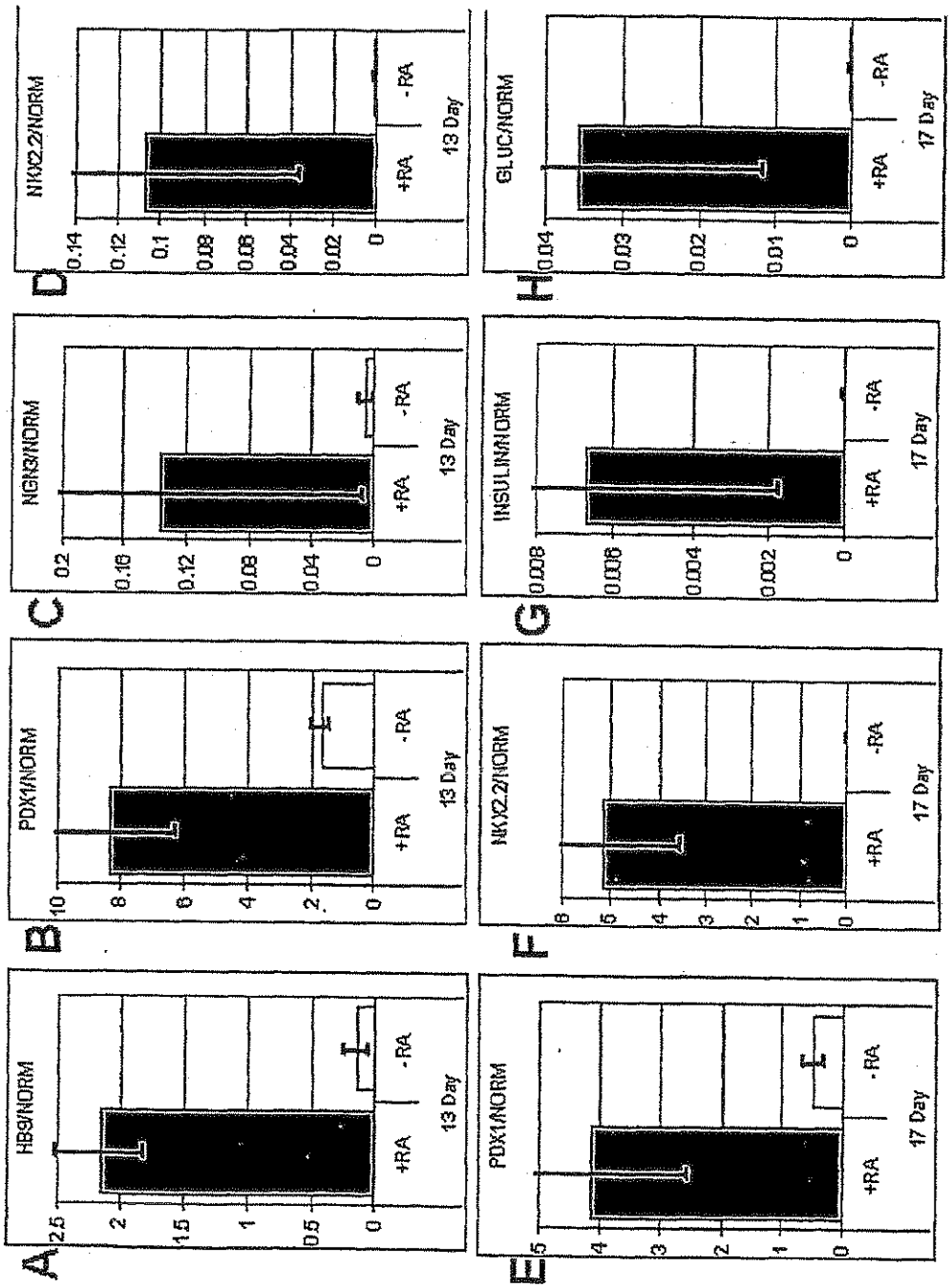

A
HB9/NORM
2.5
2
1.5
1
0.5
0
+RA
-RA
13 Day
B
PDX1/NORM
10
8
6
4
2
0
+RA
-RA
13 Day
C
NGN3/NORM
0.2
0.16
0.12
0.08
0.04
0
+RA
-RA
13 Day
D
NKX2.2/NORM
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
+RA
-RA
13 Day
E
PDX1/NORM
5
4
3
2
1
0
+RA
-RA
17 Day
F
NKX2.2/NORM
6
5
4
3
2
1
0
+RA
-RA
17 Day
G
INSULIN/NORM
0.008
0.006
0.004
0.002
0
+RA
-RA
17 Day
H
GLUC/NORM
0.04
0.03
0.02
0.01
0
+RA
-RA
17 Day

FIGURE. 5

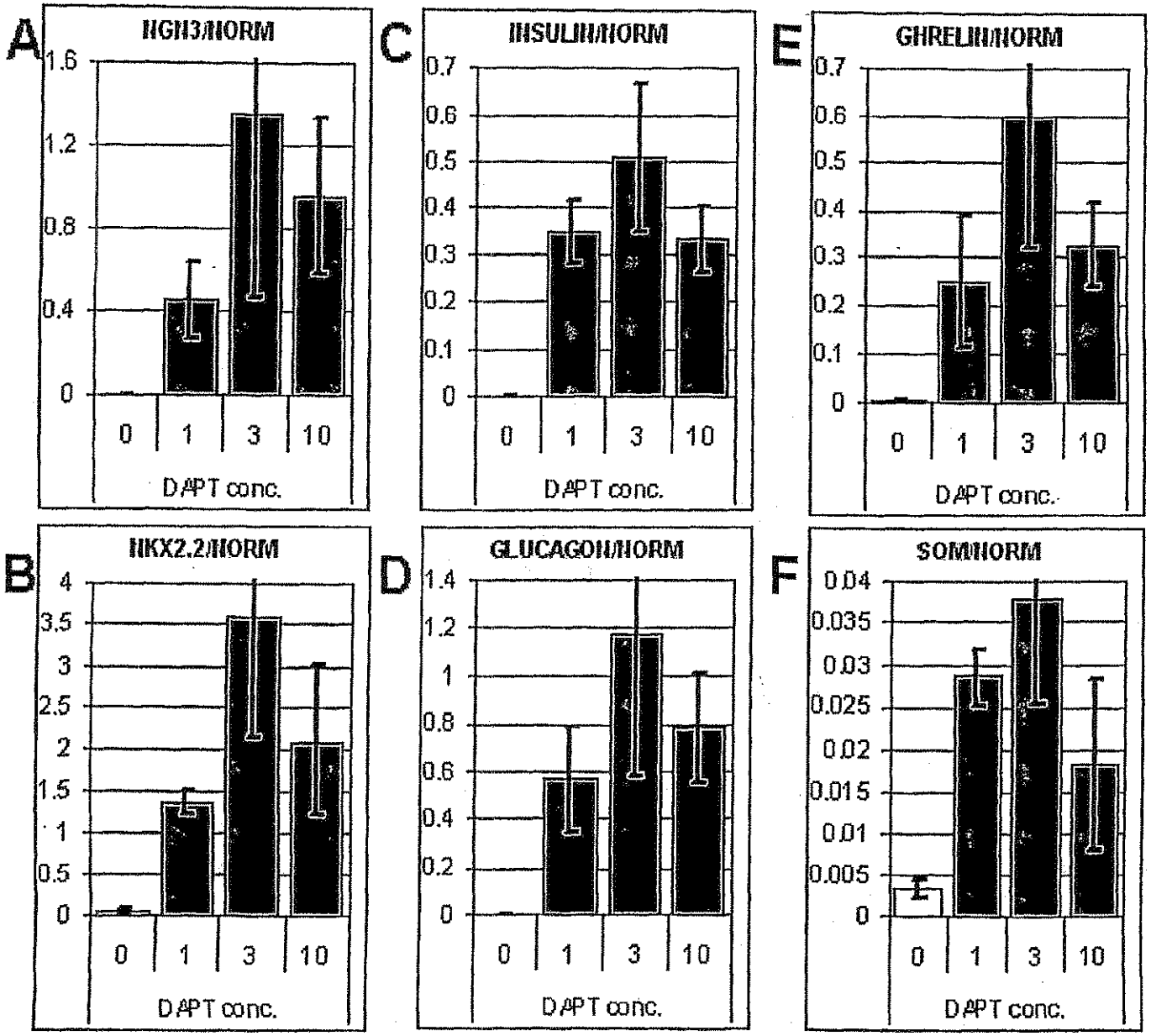

A
HGH3/NORM
1.6
1.2
0.8
0.4
0
0
1
3
10
DAPT conc.
C
INSULIN/NORM
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
1
3
10
DAPT conc.
E
GHRELIN/NORM
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
1
3
10
DAPT conc.
B
NKX2.2/NORM
4
3.5
3
2.5
2
1.5
1
0.5
0
0
1
3
10
DAPT conc.
D
GLUCAGON/NORM
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
1
3
10
DAPT conc.
F
SOM/NORM
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
0
1
3
10
DAPT conc.

FIGURE. 6

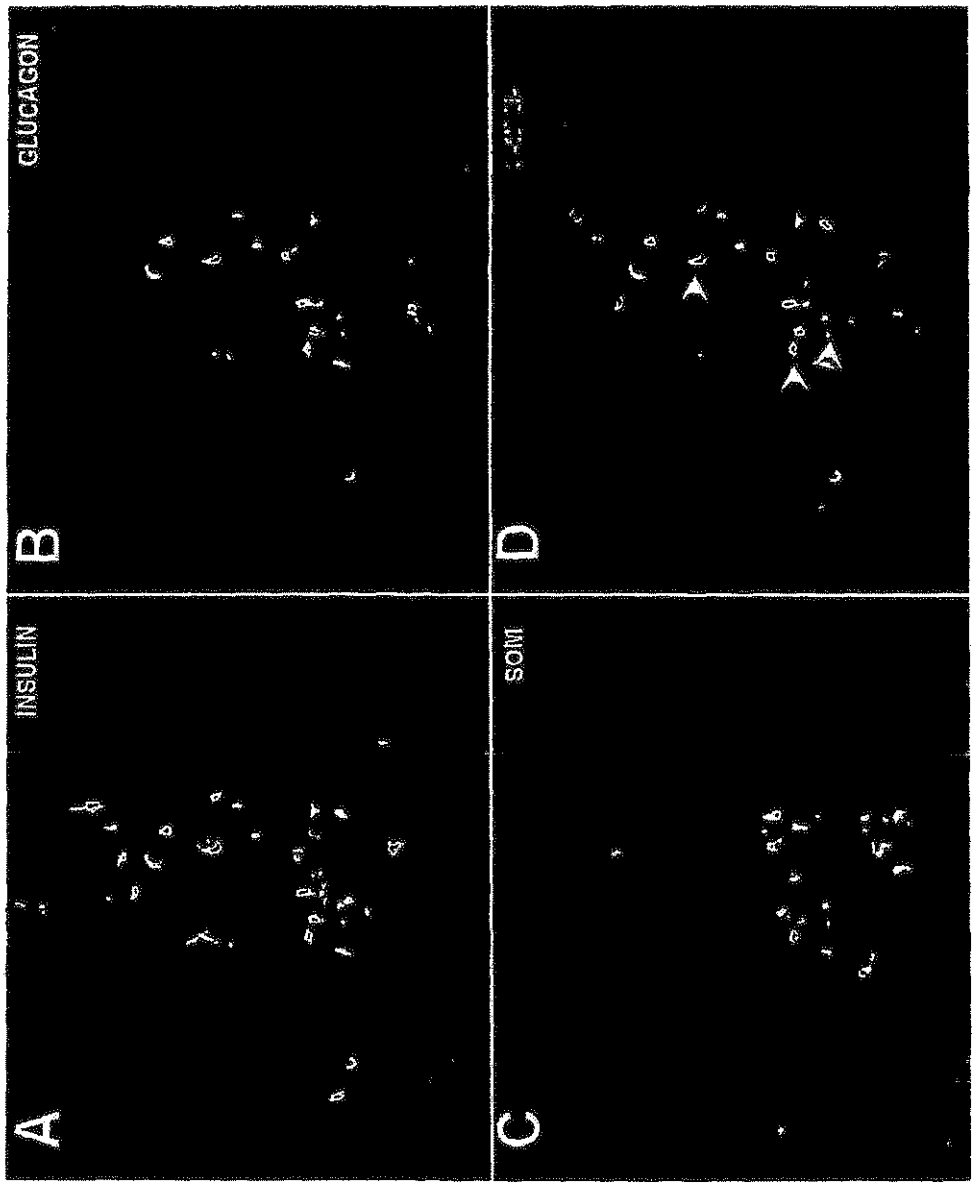


FIGURE. 7

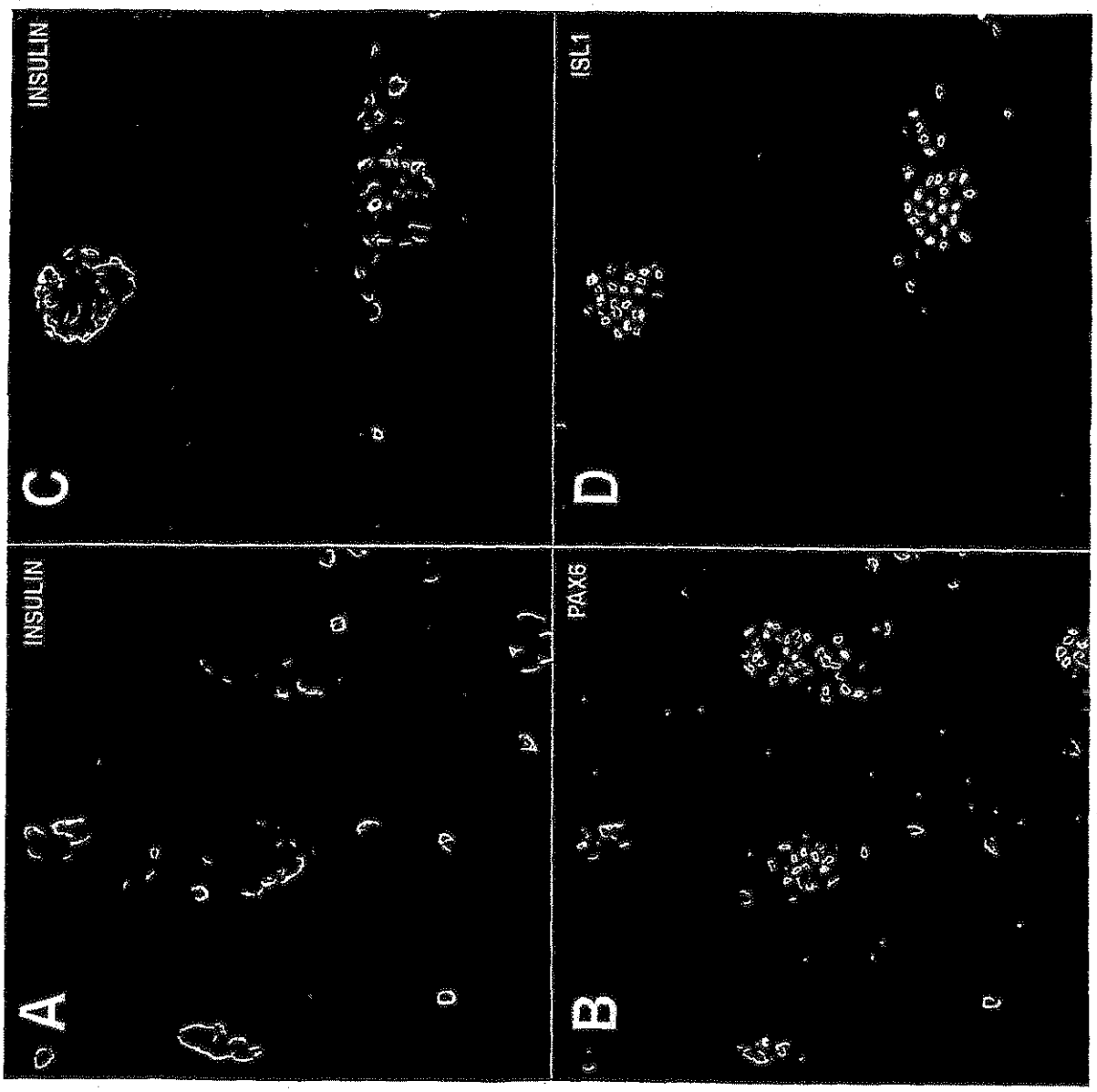


FIGURE. 8

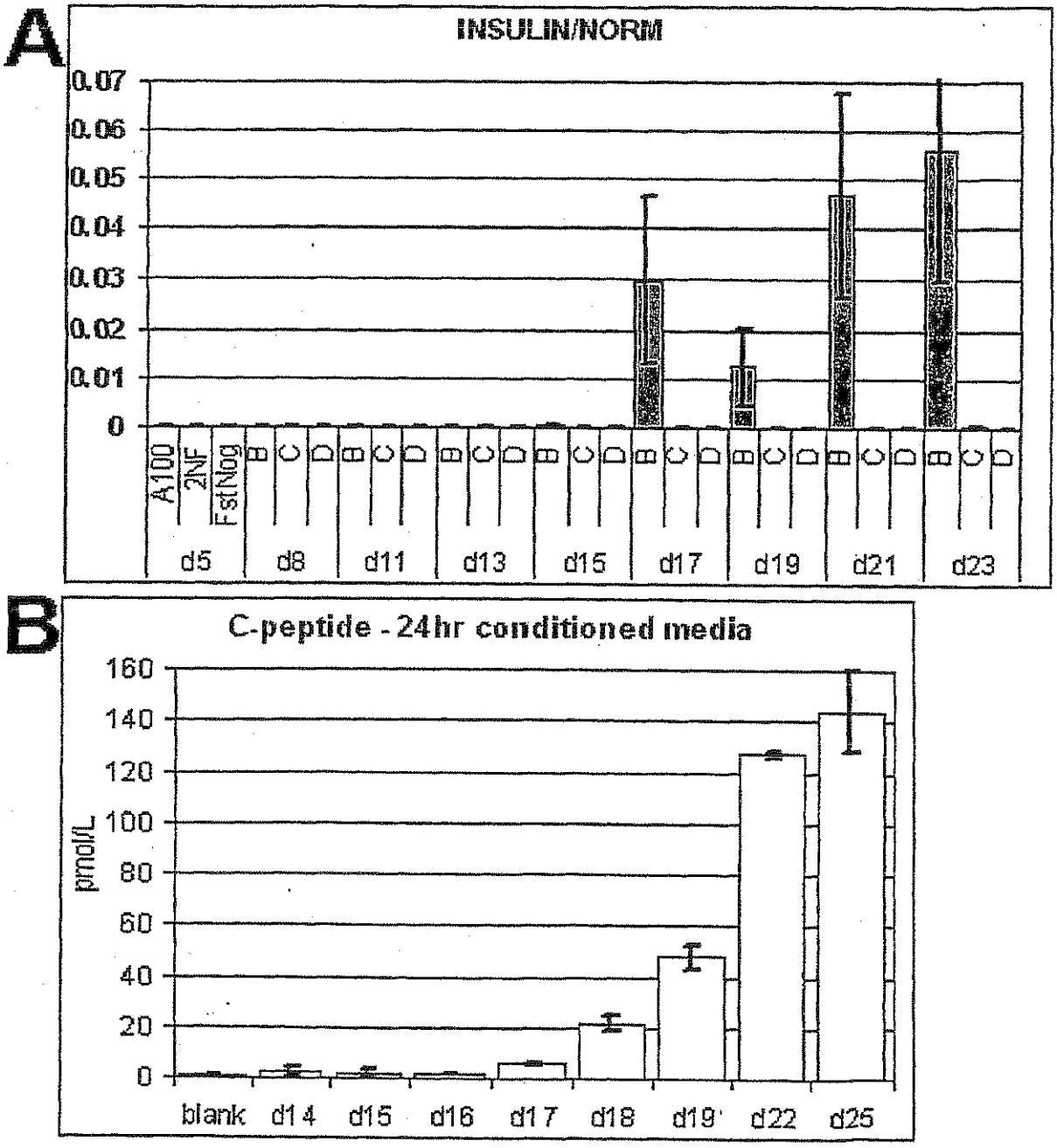

A
INSULIN/NORM
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
A100
2NF
FstNog
B
C
D
d5
d8
d11
d13
d15
d17
d19
d21
d23
B
C-peptide - 24hr conditioned media
pmol/L
160
140
120
100
80
60
40
20
0
blank
d14
d15
d16
d17
d18
d19
d22
d25

FIGURE. 9

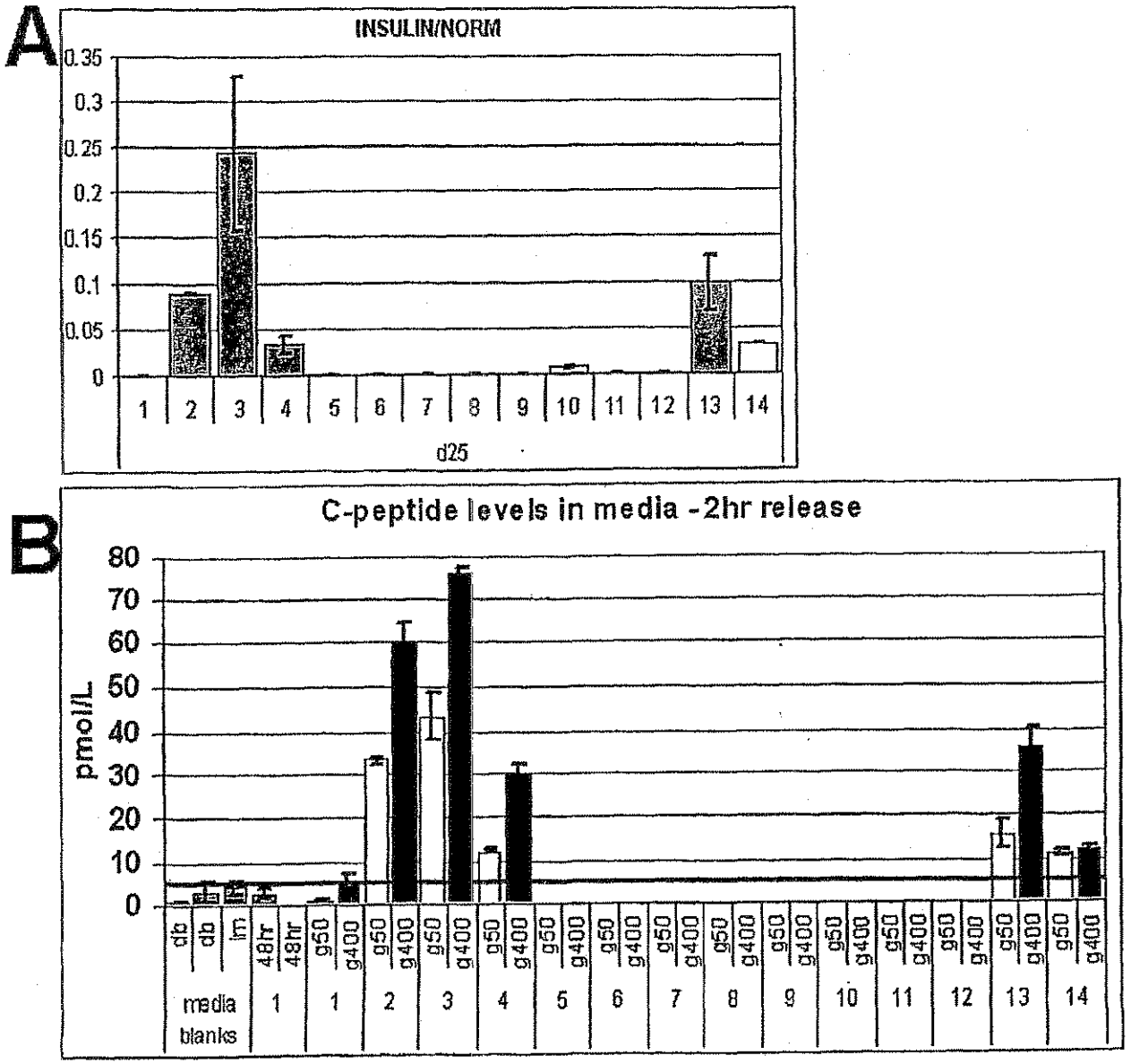

A
INSULIN/NORM
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14
d25
B
C-peptide levels in media -2hr release
pmol/L
80
70
60
50
40
30
20
10
0
db db im 48hr 48hr g50 g400 g50 g400 g50 g400 g50 g400 g50 g400 g50 g400 g50 g400 g50 g400 g50 g400 g50 g400 g50 g400 g50 g400 g50 g400
media 1 1 2 3 4 5 6 7 8 9 10 11 12 13 14
blanks

FIGURE. 10

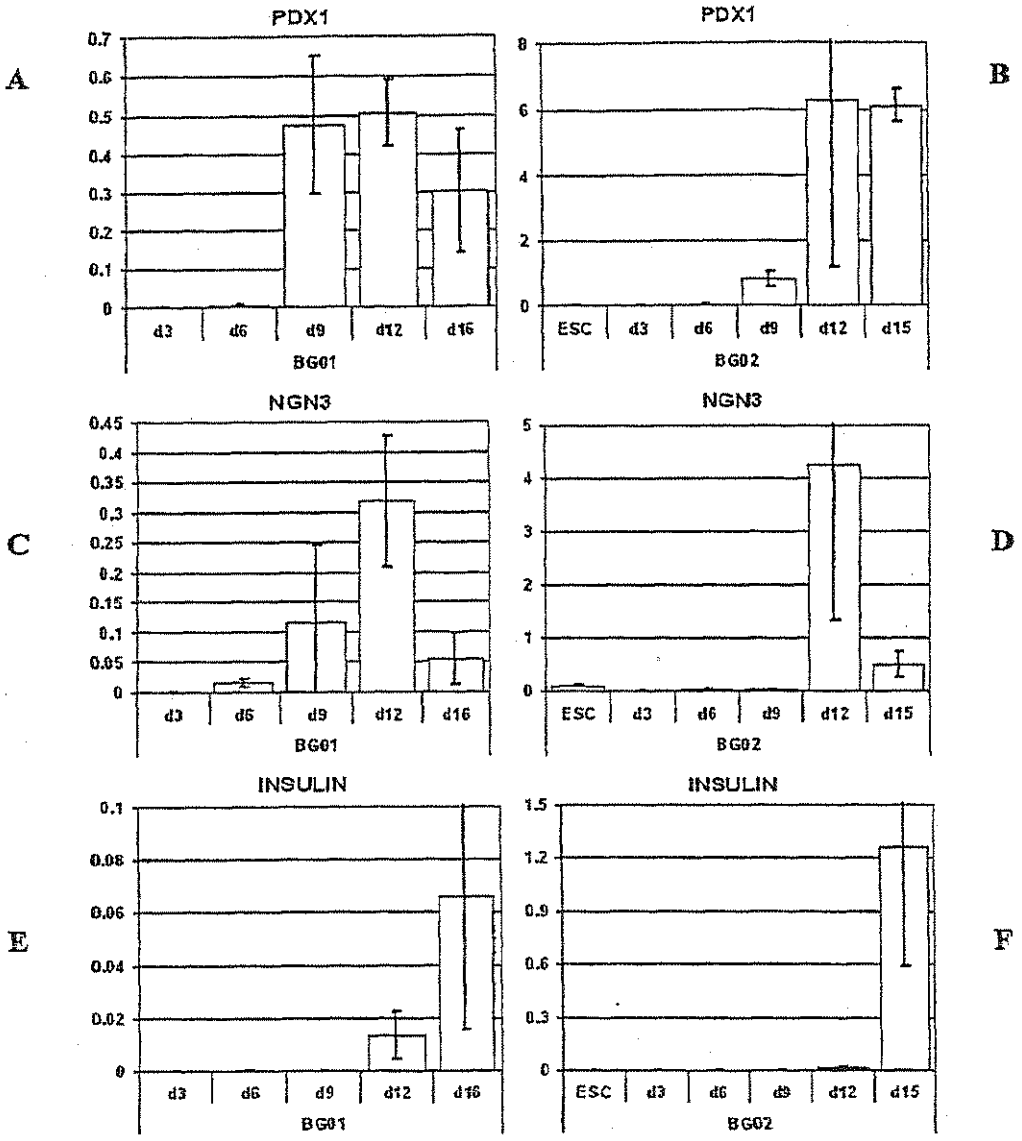

A
PDX1
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
d3
d6
d9
d12
d16
BG01
B
PDX1
8
6
4
2
0
ESC
d3
d6
d9
d12
d15
BG02
C
NGN3
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
d3
d6
d9
d12
d16
BG01
D
NGN3
5
4
3
2
1
0
ESC
d3
d6
d9
d12
d15
BG02
E
INSULIN
0.1
0.08
0.06
0.04
0.02
0
d3
d6
d9
d12
d16
BG01
F
INSULIN
1.5
1.2
0.9
0.6
0.3
0
ESC
d3
d6
d9
d12
d15
BG02

Figure 11

Figure 12

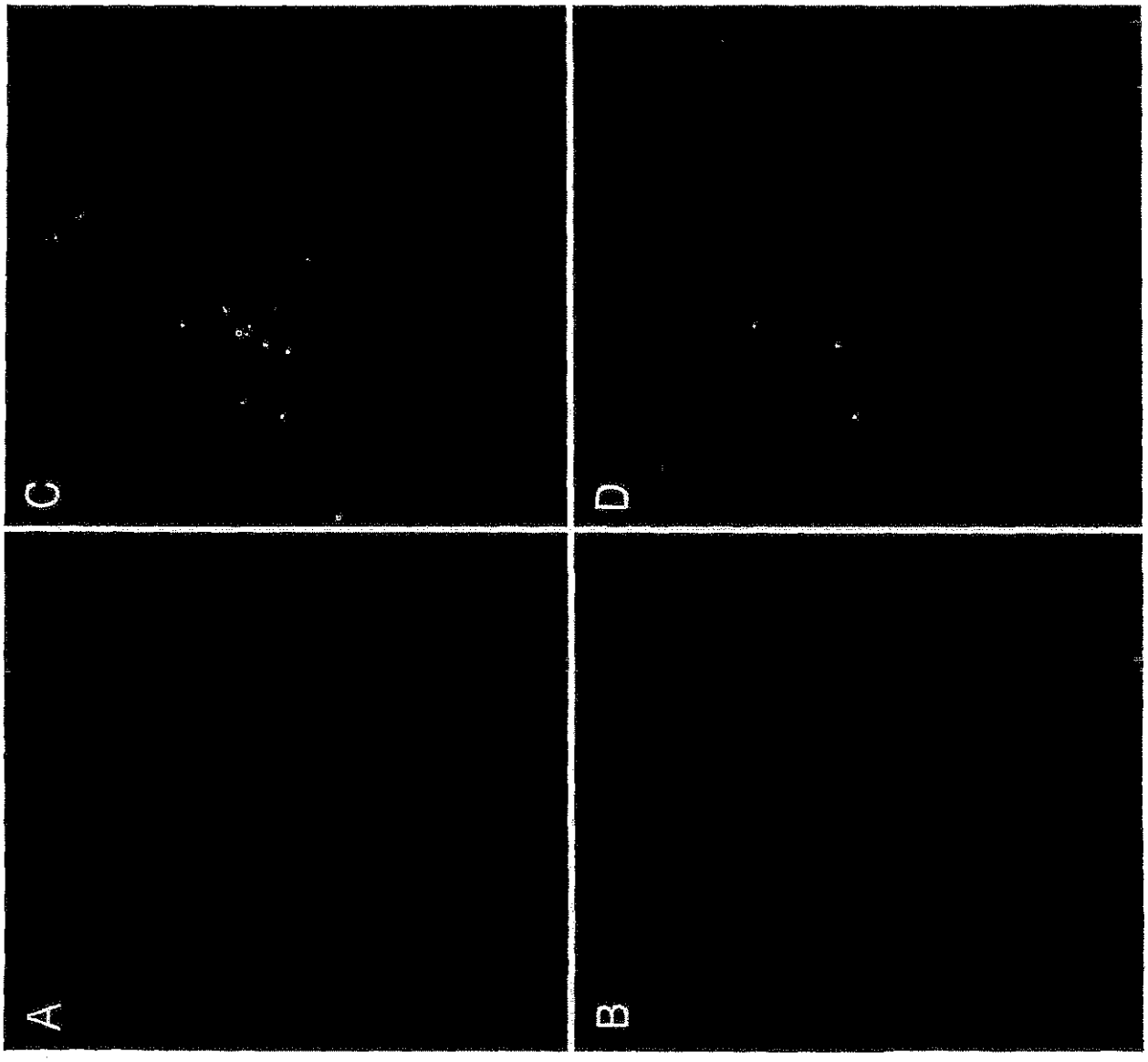


Figure 13

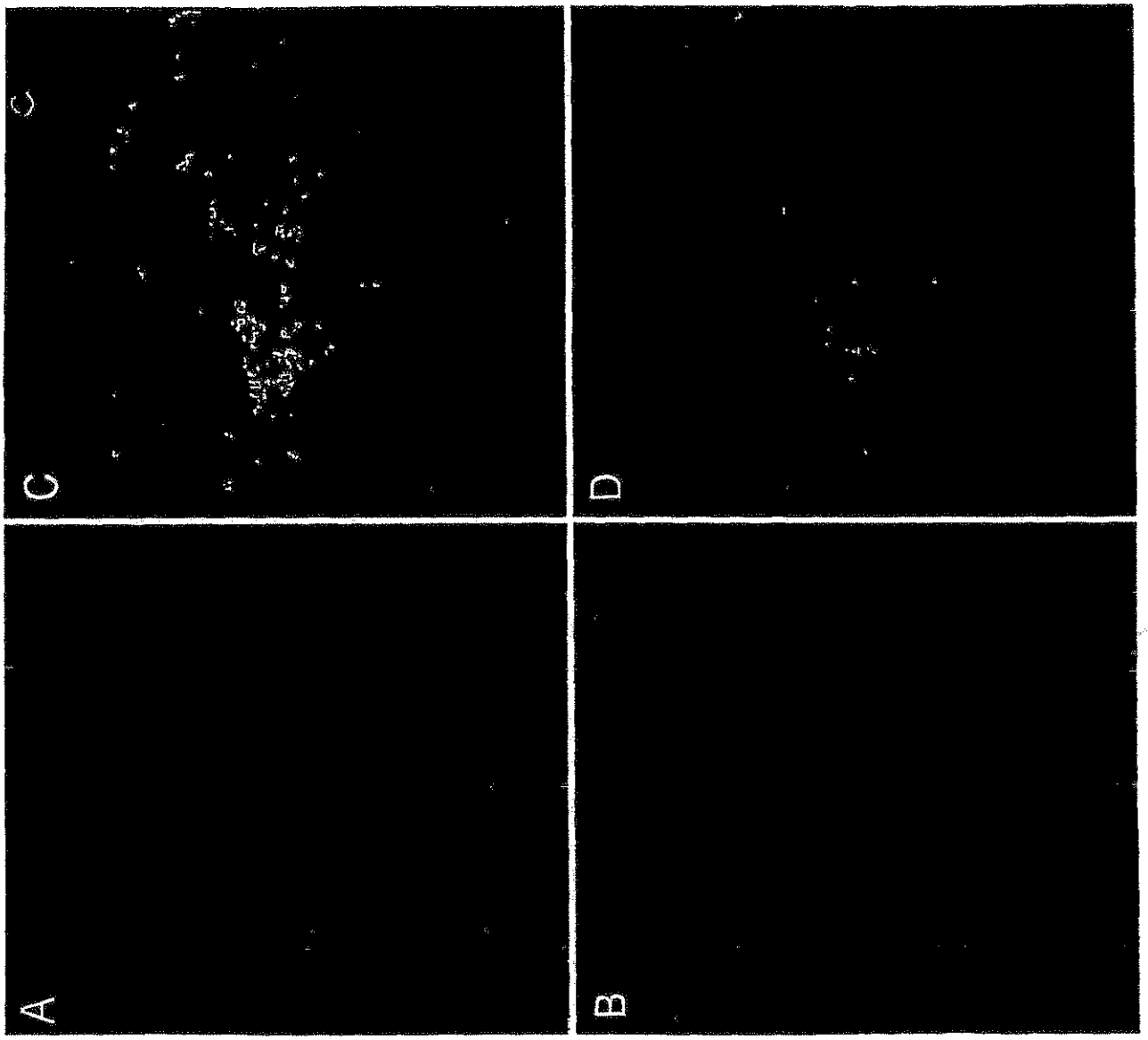


Figure 14

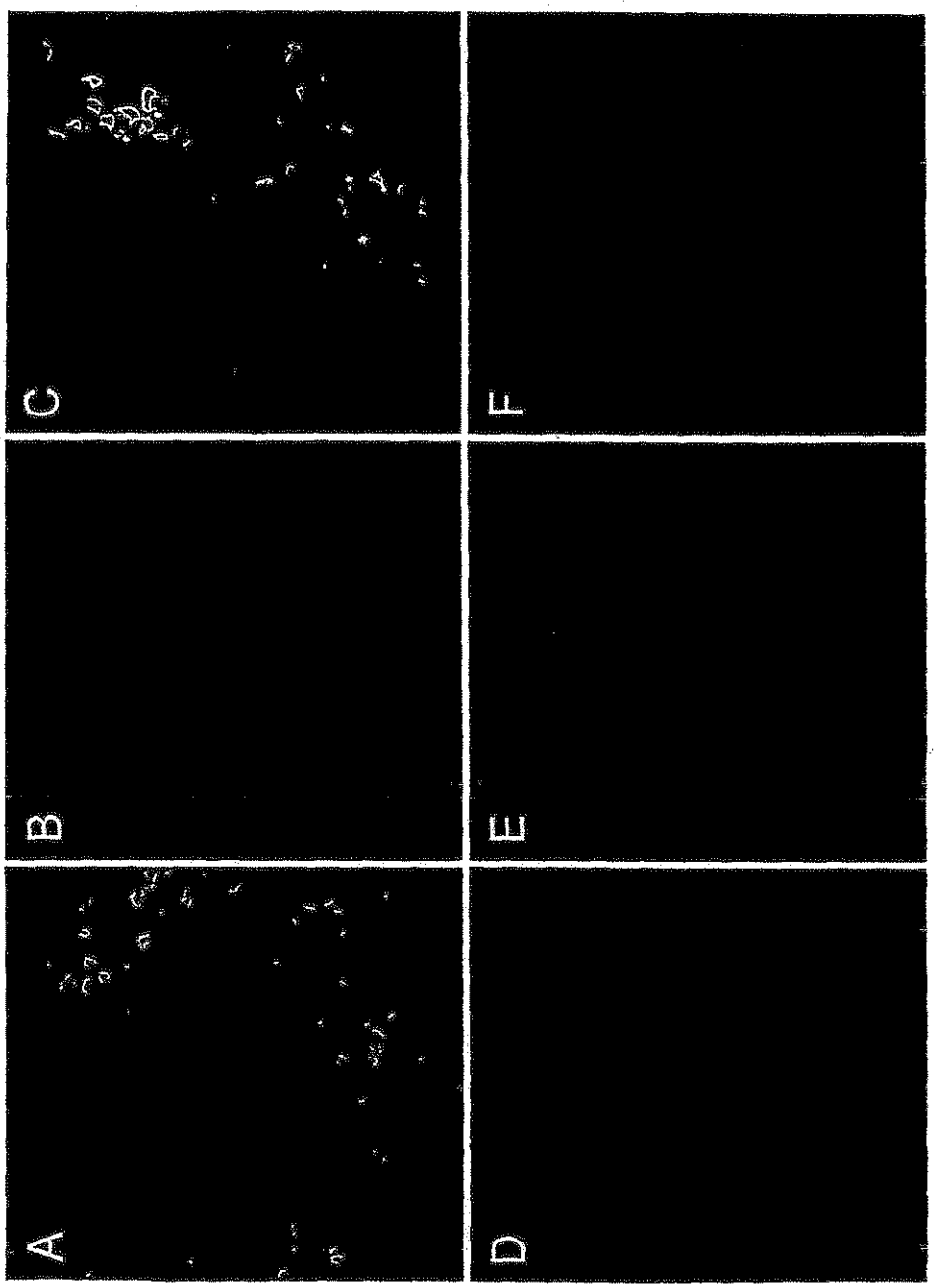


Figure 15

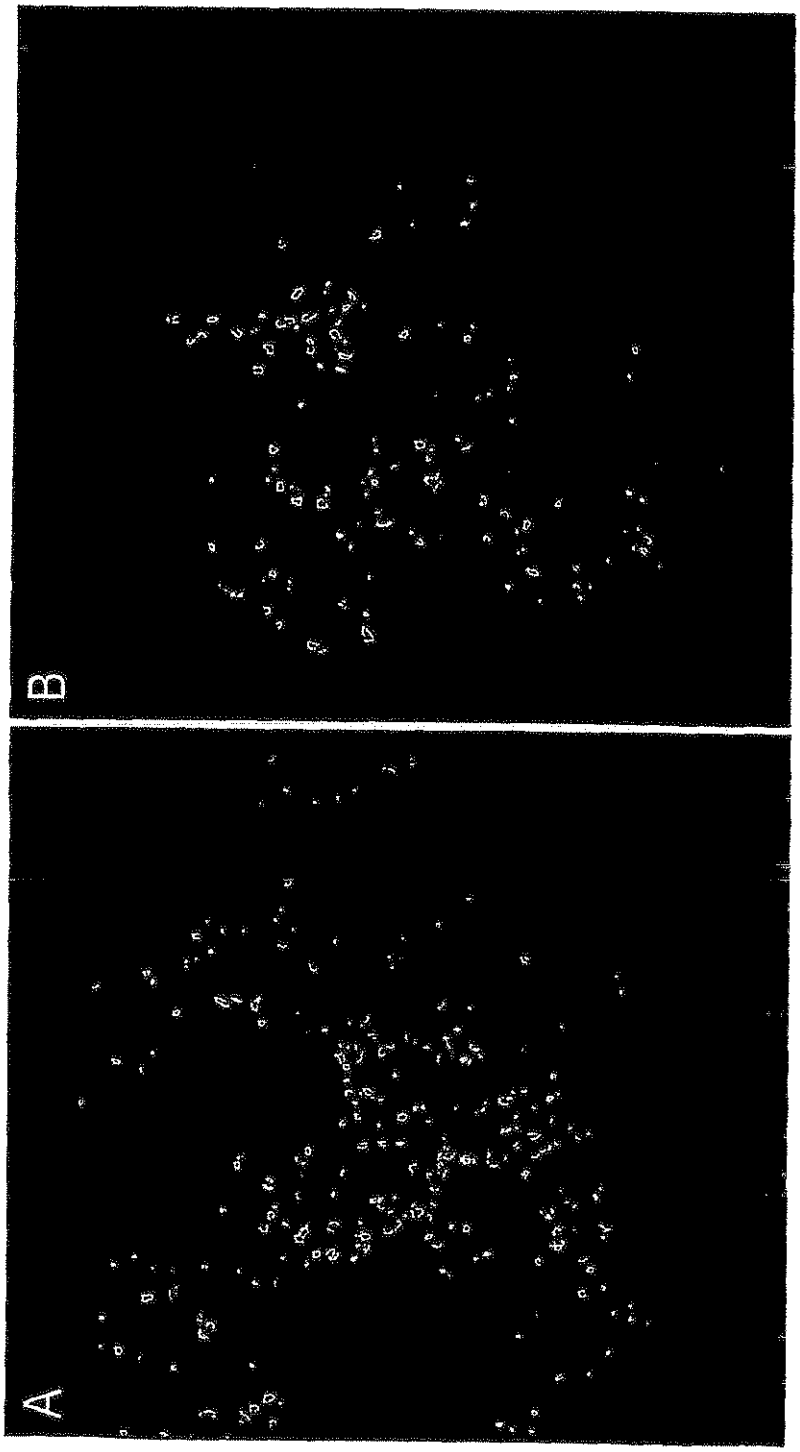


Figure 16

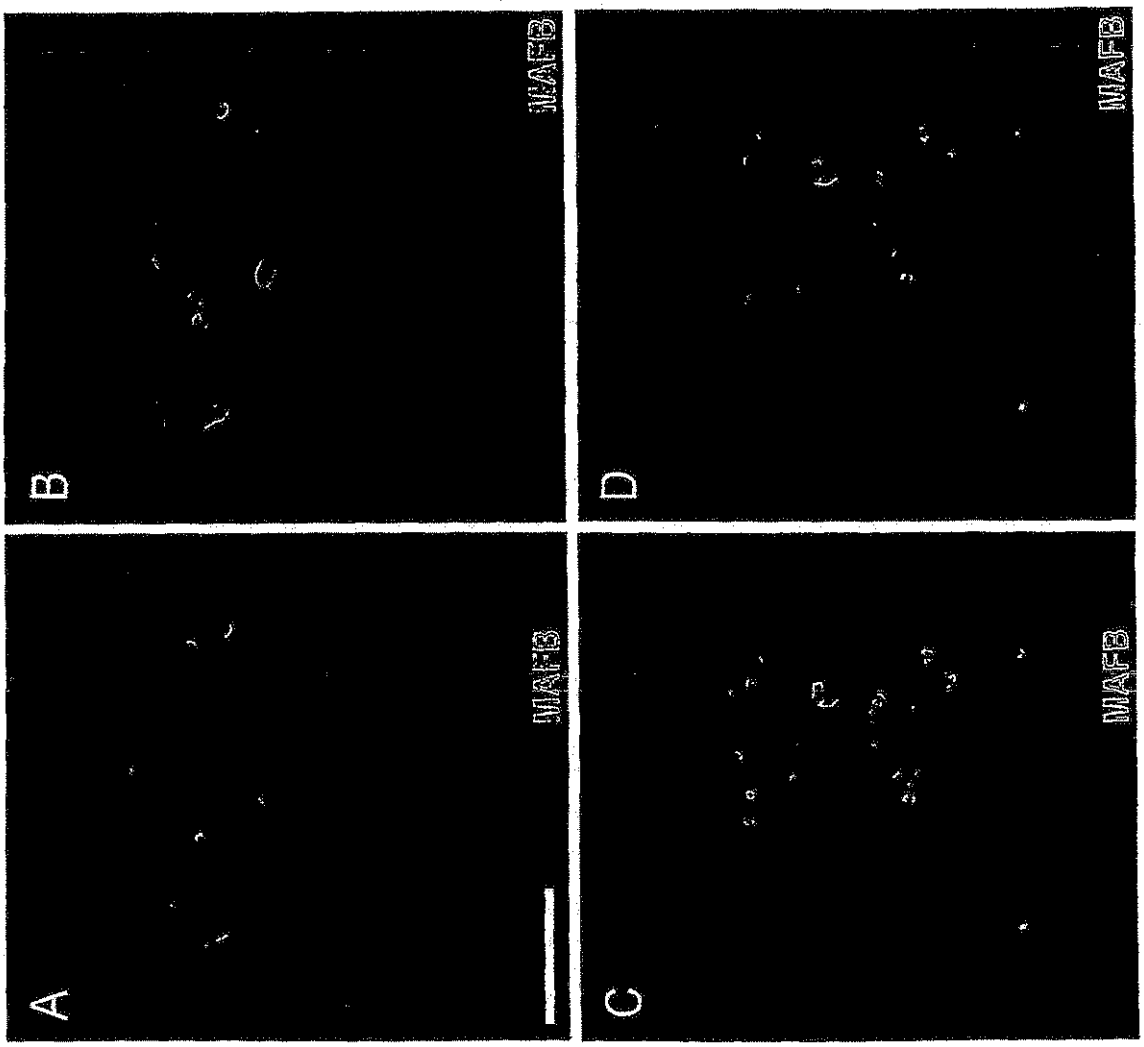


Figure 17

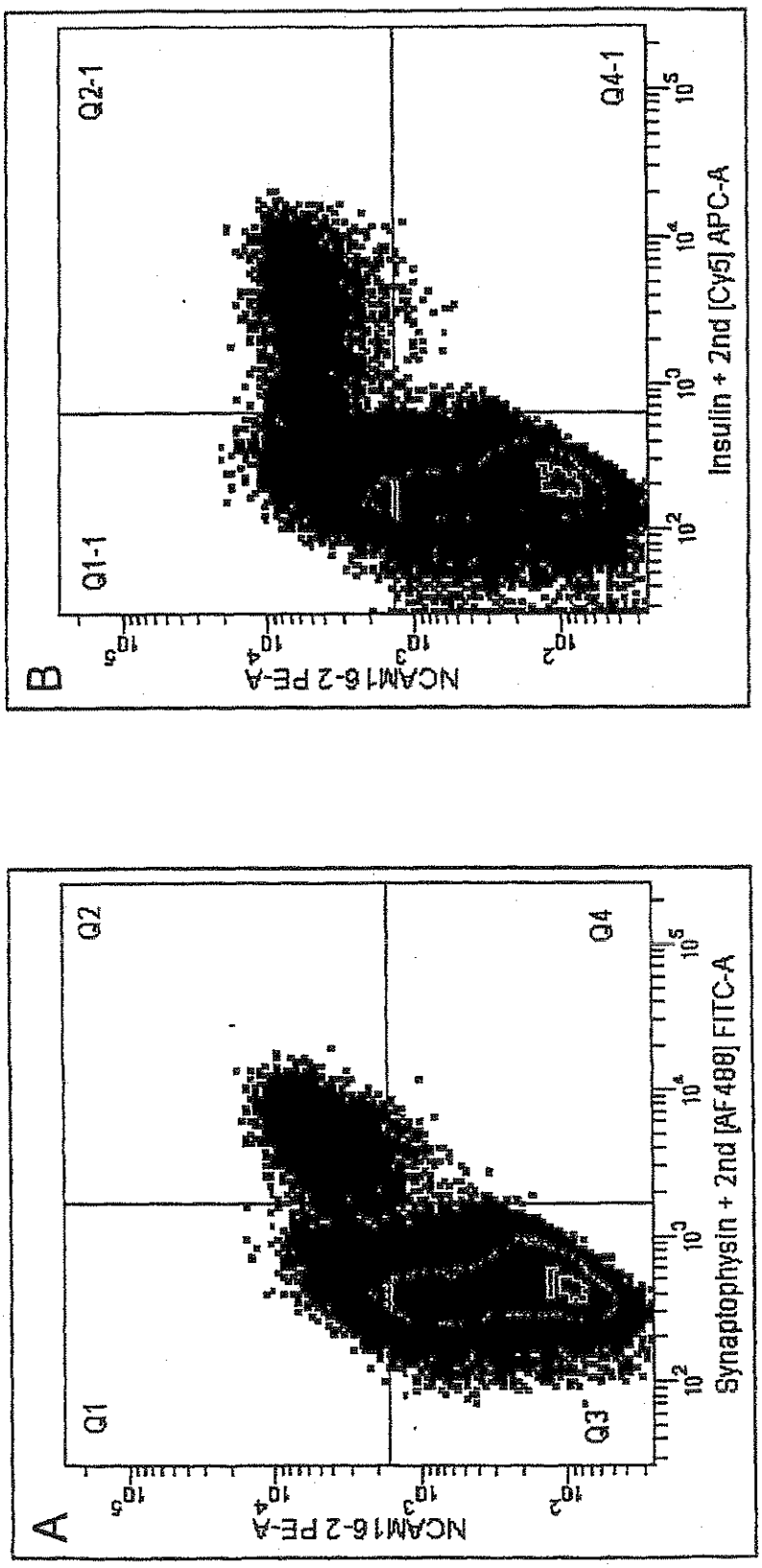

A
Q1
Q2
Q3
Q4
NCAM16-2 PE-A
Synaptophysin + 2nd [AF488] FITC-A
B
Q1-1
Q2-1
Q4-1
NCAM16-2 PE-A
Insulin + 2nd [Cy5] APC-A

Figure 18

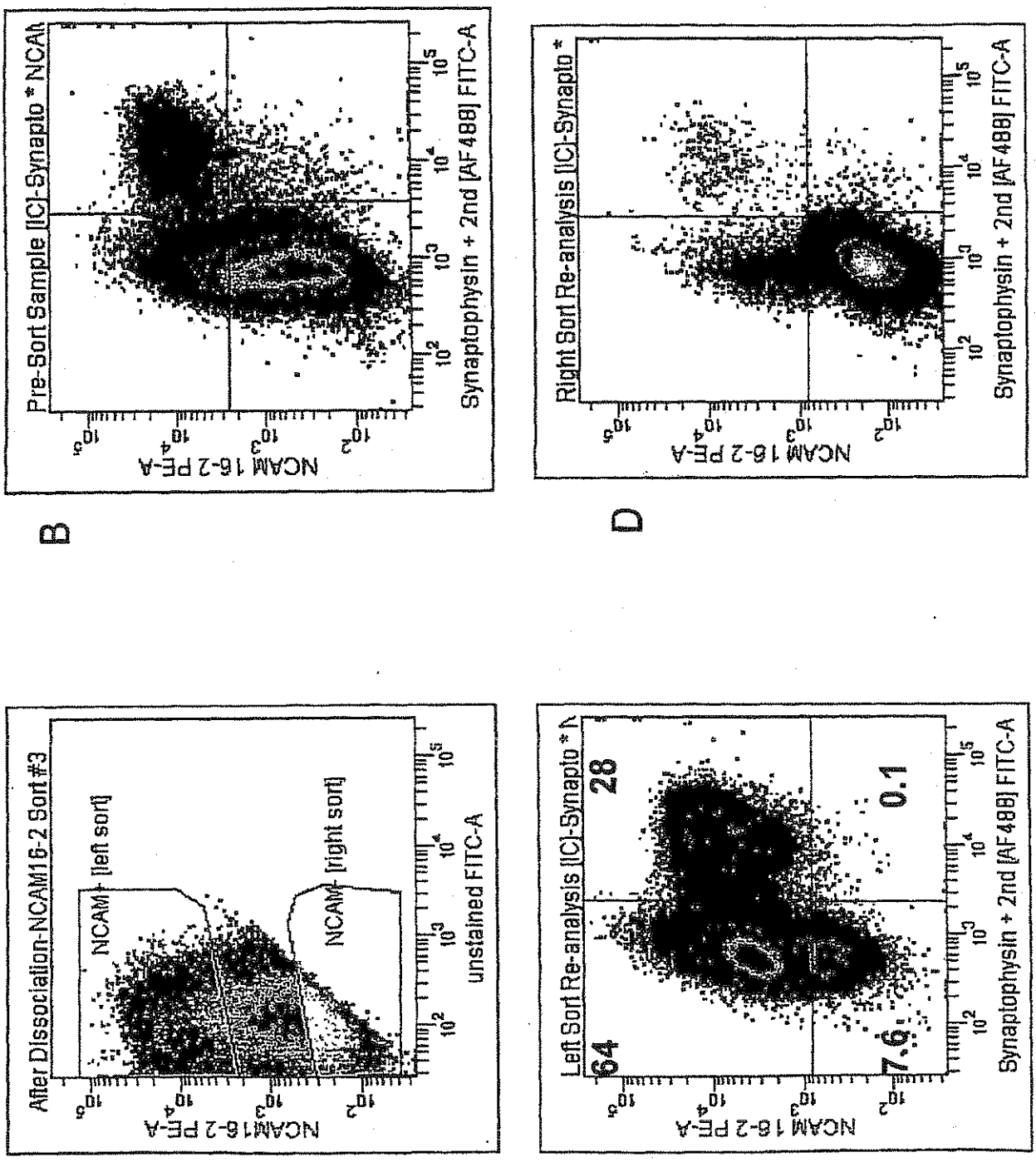

A
After Dissociation-NCAM16-2 Sort #3
NCAM+ [left sort]
NCAM- [right sort]
NCAM 16-2 PE-A
unstained FITC-A
B
Pre-Sort Sample [IC]-Synapto * NCAM
NCAM 16-2 PE-A
Synaptophysin + 2nd [AF488] FITC-A
C
Left Sort Re-analysis [IC]-Synapto * N
64
28
7.9
0.1
NCAM 16-2 PE-A
Synaptophysin + 2nd [AF488] FITC-A
D
Right Sort Re-analysis [IC]-Synapto *
NCAM 16-2 PE-A
Synaptophysin + 2nd [AF488] FITC-A

Figure 19

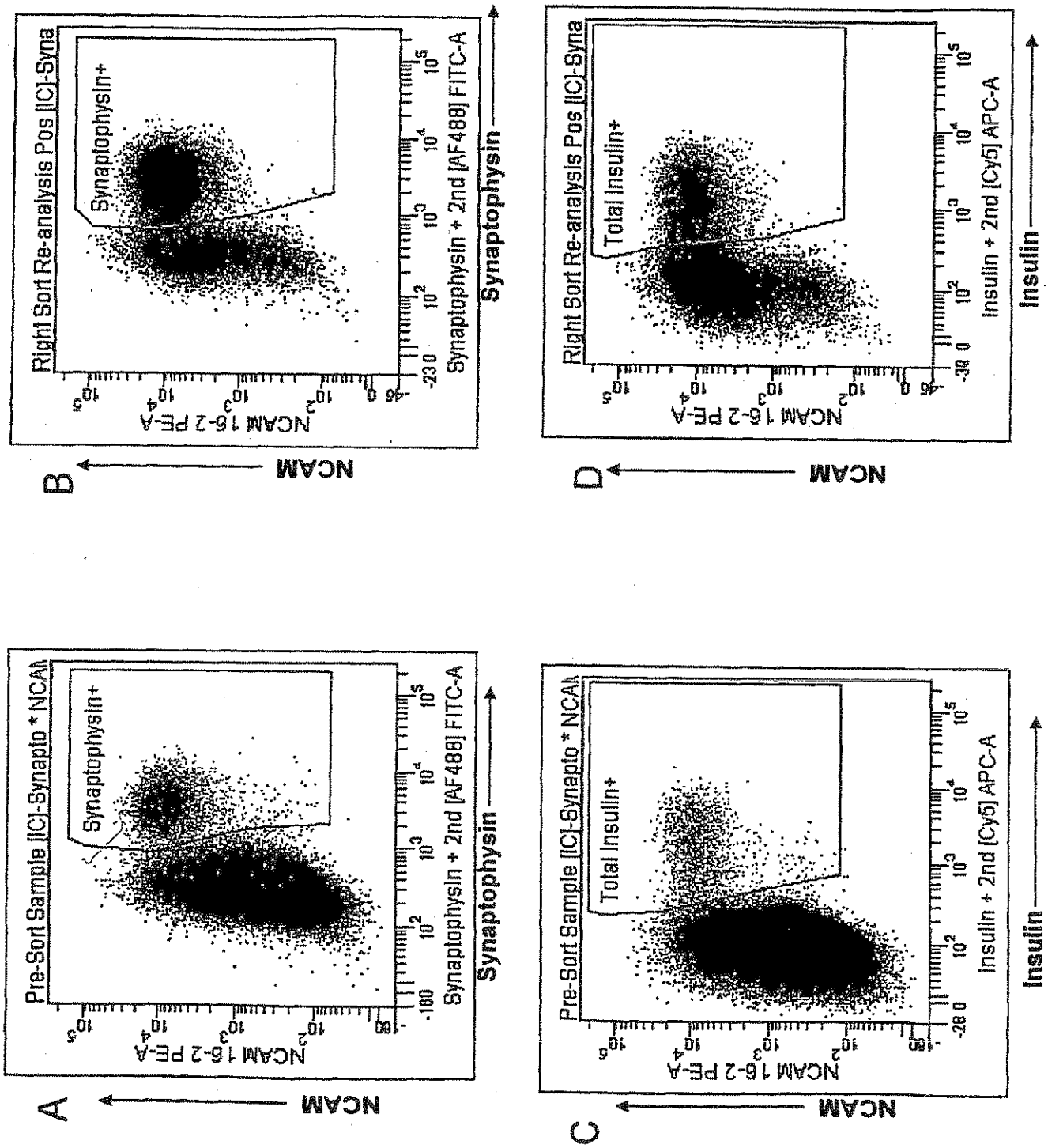

A
Pre-Sort Sample [IC]-Synapto * NCAM
Synaptophysin+
NCAM 16-2 PE-A
Synaptophysin + 2nd [AF488] FITC-A
NCAM
Synaptophysin
B
Right Sort Re-analysis Pos [IC]-Syna
Synaptophysin+
NCAM 16-2 PE-A
Synaptophysin + 2nd [AF488] FITC-A
NCAM
Synaptophysin
C
Pre-Sort Sample [IC]-Synapto * NCAM
Total Insulin+
NCAM 16-2 PE-A
Insulin + 2nd [Cy5] APC-A
NCAM
Insulin
D
Right Sort Re-analysis Pos [IC]-Syna
Total Insulin+
NCAM 16-2 PE-A
Insulin + 2nd [Cy5] APC-A
NCAM
Insulin

Figure 20

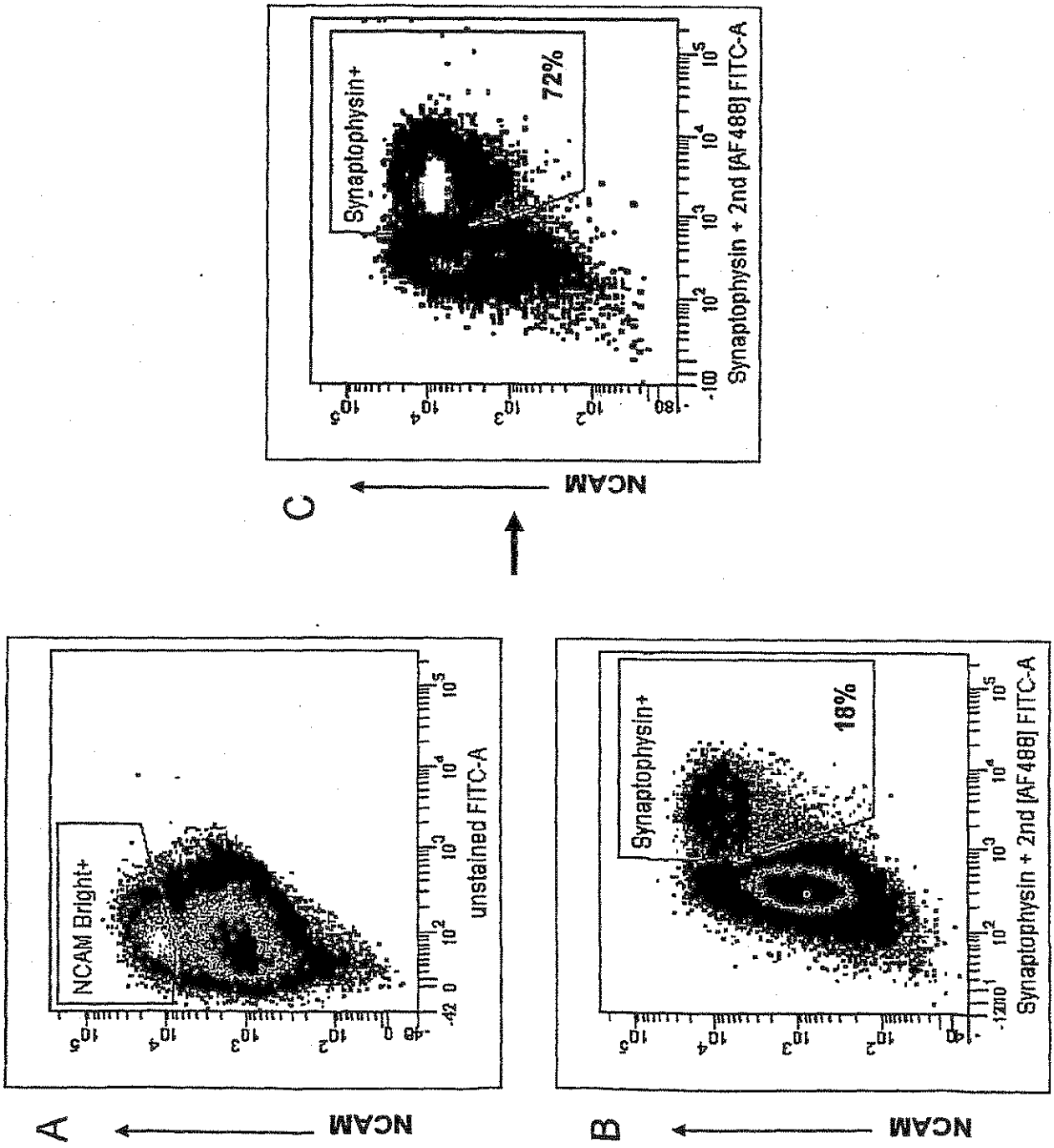

A
NCAM
NCAM Bright+
unstained FITC-A
B
NCAM
Synaptophysin+
18%
Synaptophysin + 2nd [AF488] FITC-A
C
NCAM
Synaptophysin+
72%
Synaptophysin + 2nd [AF488] FITC-A

Figure 21

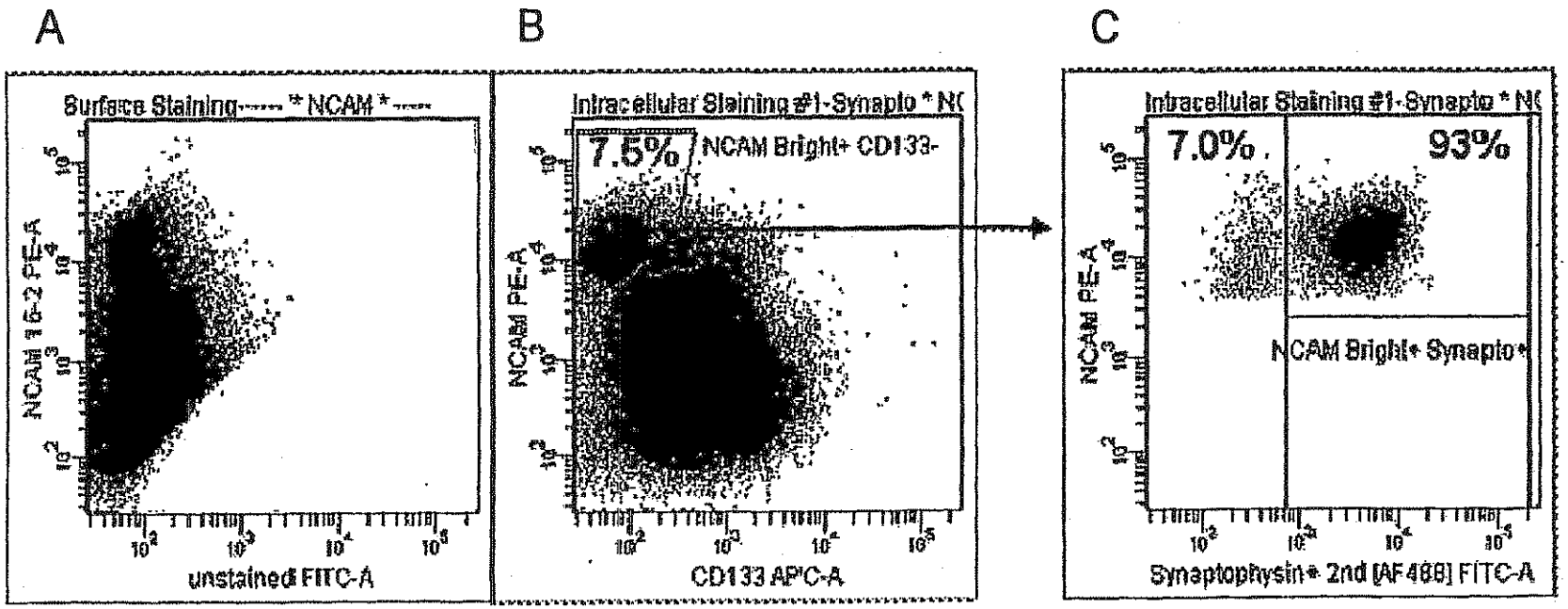

A
B
C
Surface Staining------ * NCAM *------
NCAM 16-2 PE-A
unstained FITC-A
Intracellular Staining #1-Synapto * NC
7.5%
NCAM Bright+ CD133-
NCAM PE-A
CD133 APC-A
Intracellular Staining #1-Synapto * NC
7.0%
93%
NCAM Bright+ Synapto+
NCAM PE-A
Synaptophysin + 2nd [AF488] FITC-A

Figure 22 (1 of 2)

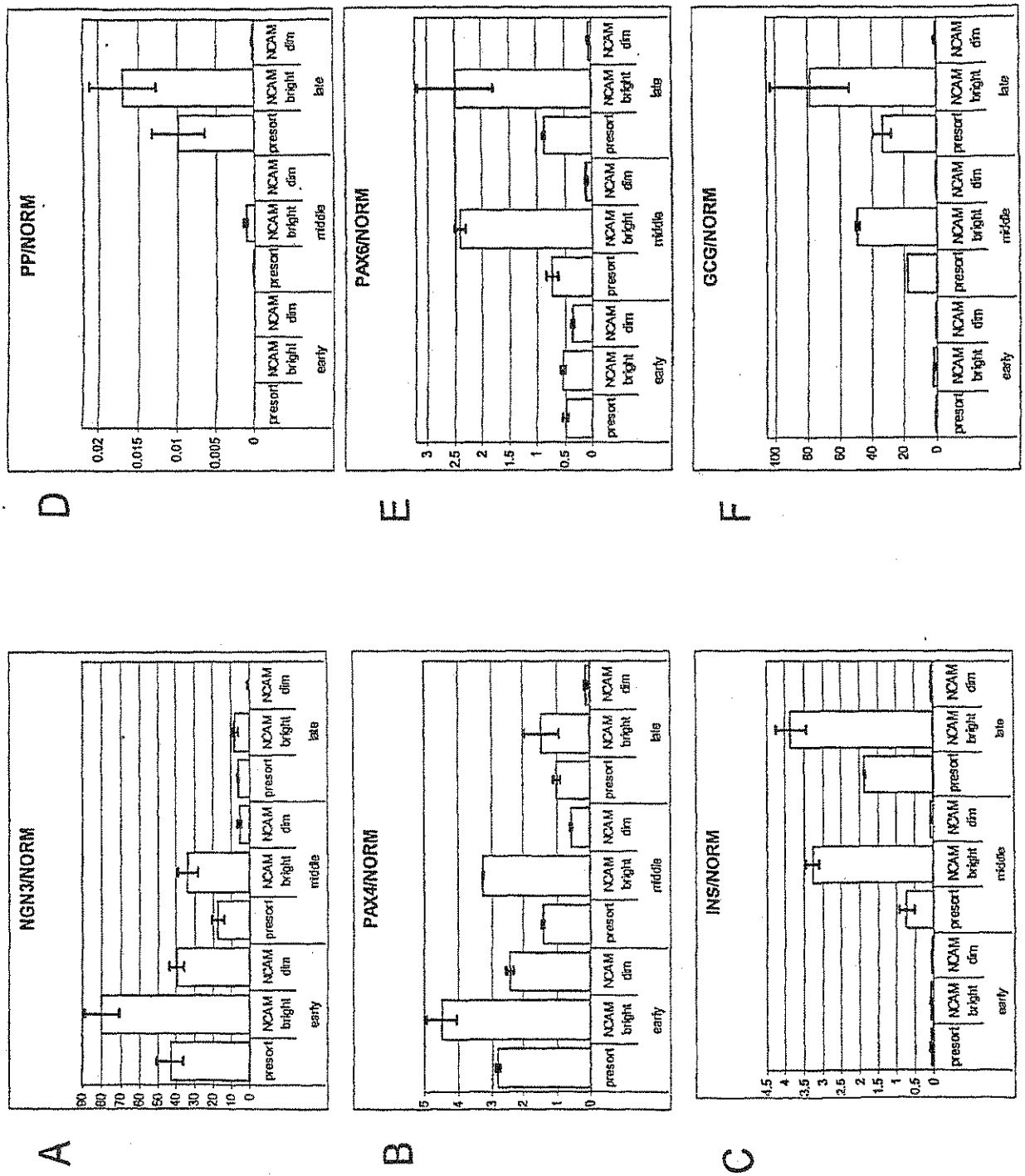
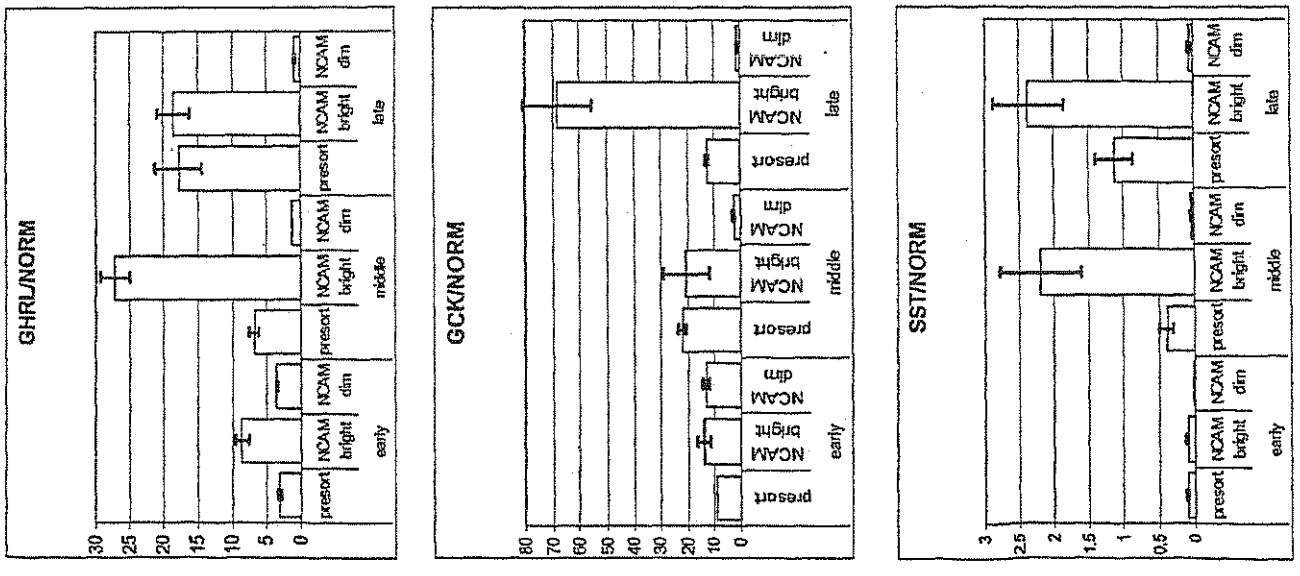


Figure 22 (2 of 2)



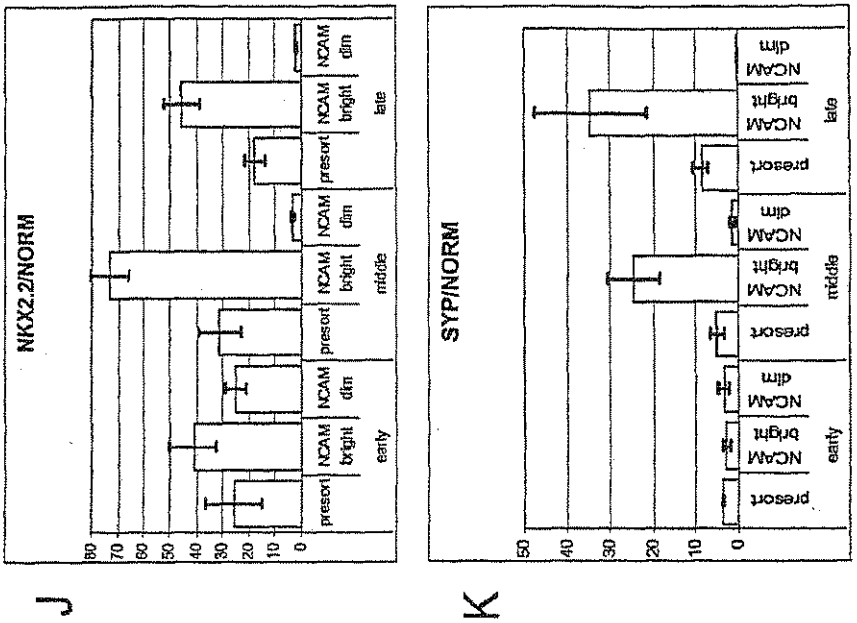
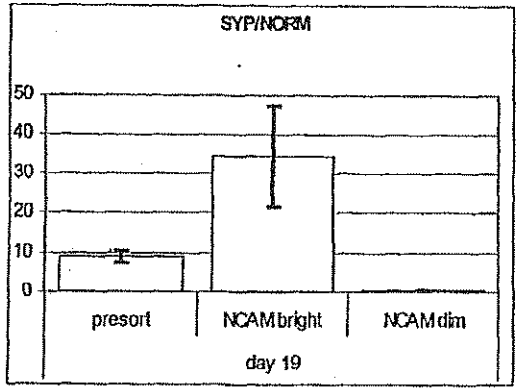
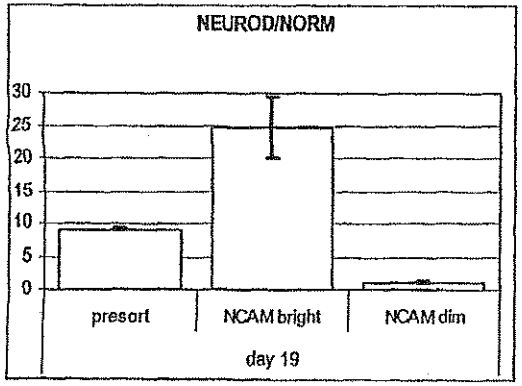


Figure 23

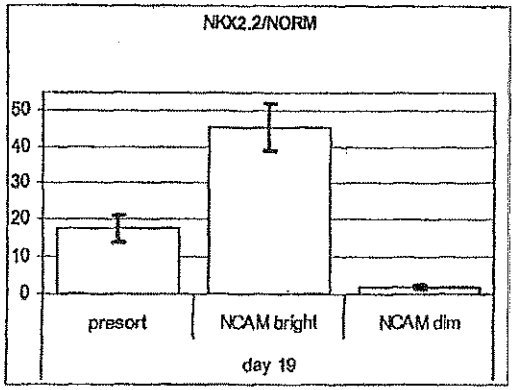
A



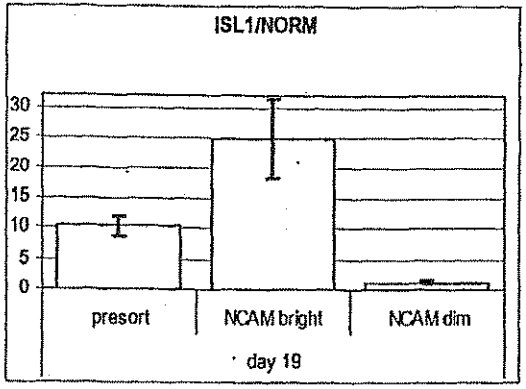
B



C



D



E

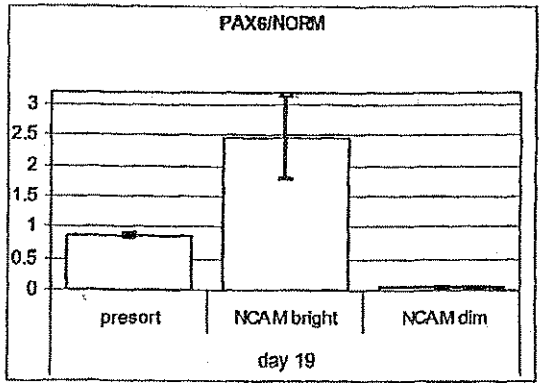


Figure 24 (1 of 2)

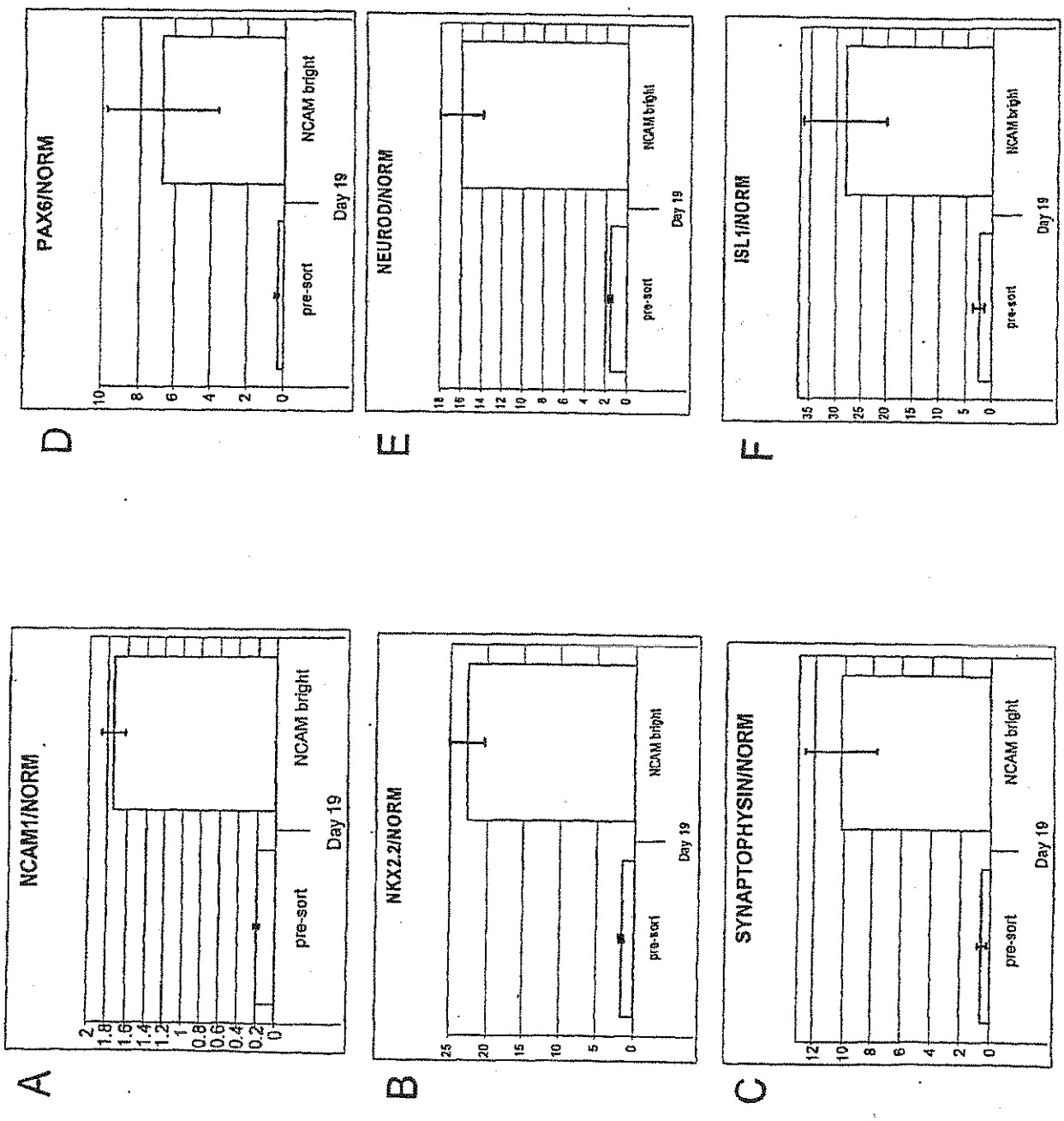


Figure 24 (2 of 2)

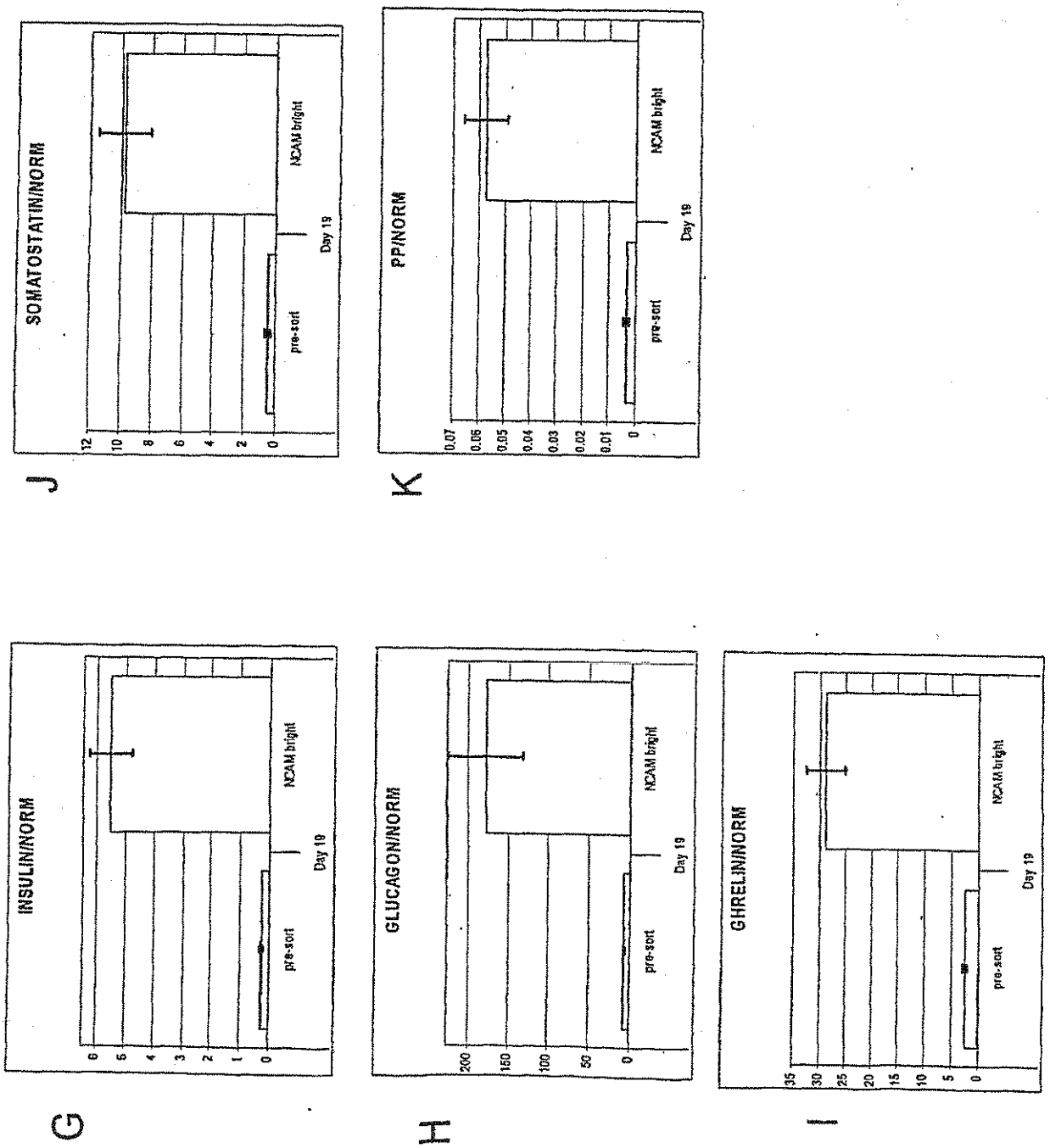


Figure 25

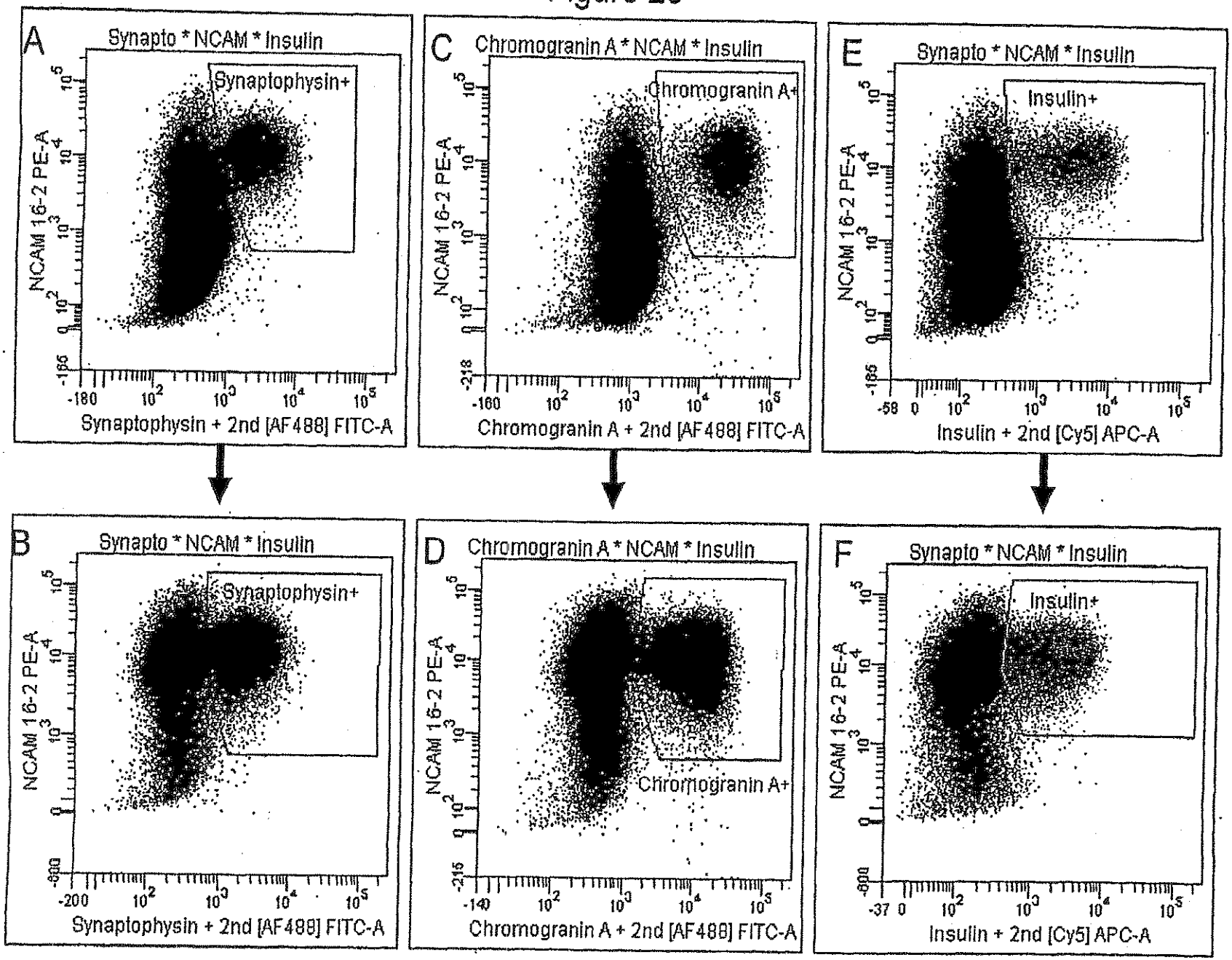
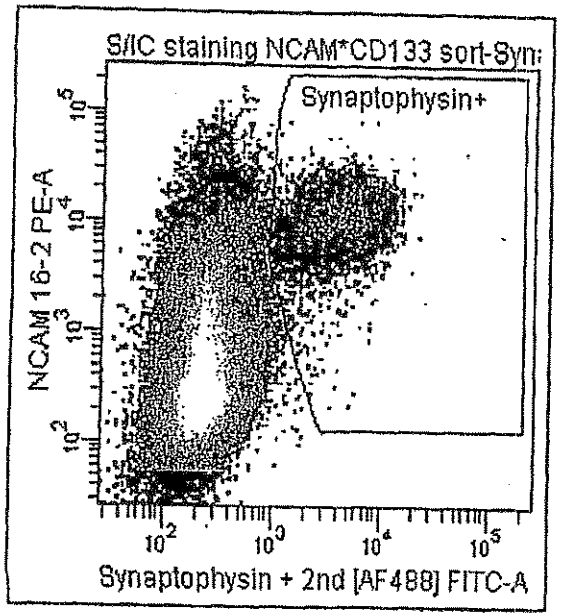
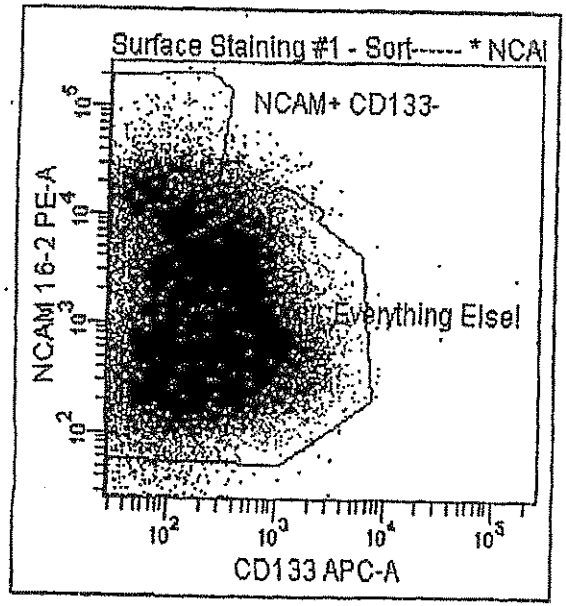

A
Synapto * NCAM * Insulin
Synaptophysin+
NCAM 16-2 PE-A
Synaptophysin + 2nd [AF488] FITC-A
C
Chromogranin A * NCAM * Insulin
Chromogranin A+
NCAM 16-2 PE-A
Chromogranin A + 2nd [AF488] FITC-A
E
Synapto * NCAM * Insulin
Insulin+
NCAM 16-2 PE-A
Insulin + 2nd [Cy5] APC-A
B
Synapto * NCAM * Insulin
Synaptophysin+
NCAM 16-2 PE-A
Synaptophysin + 2nd [AF488] FITC-A
D
Chromogranin A * NCAM * Insulin
Chromogranin A+
NCAM 16-2 PE-A
Chromogranin A + 2nd [AF488] FITC-A
F
Synapto * NCAM * Insulin
Insulin+
NCAM 16-2 PE-A
Insulin + 2nd [Cy5] APC-A

Figure 26

A



B



C

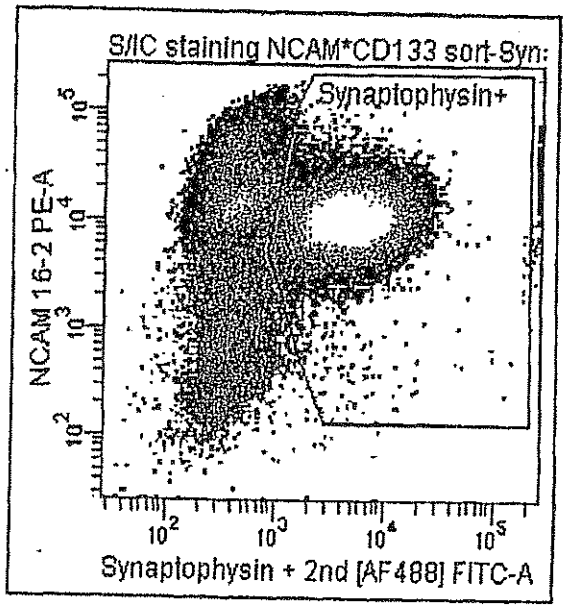


Figure 27

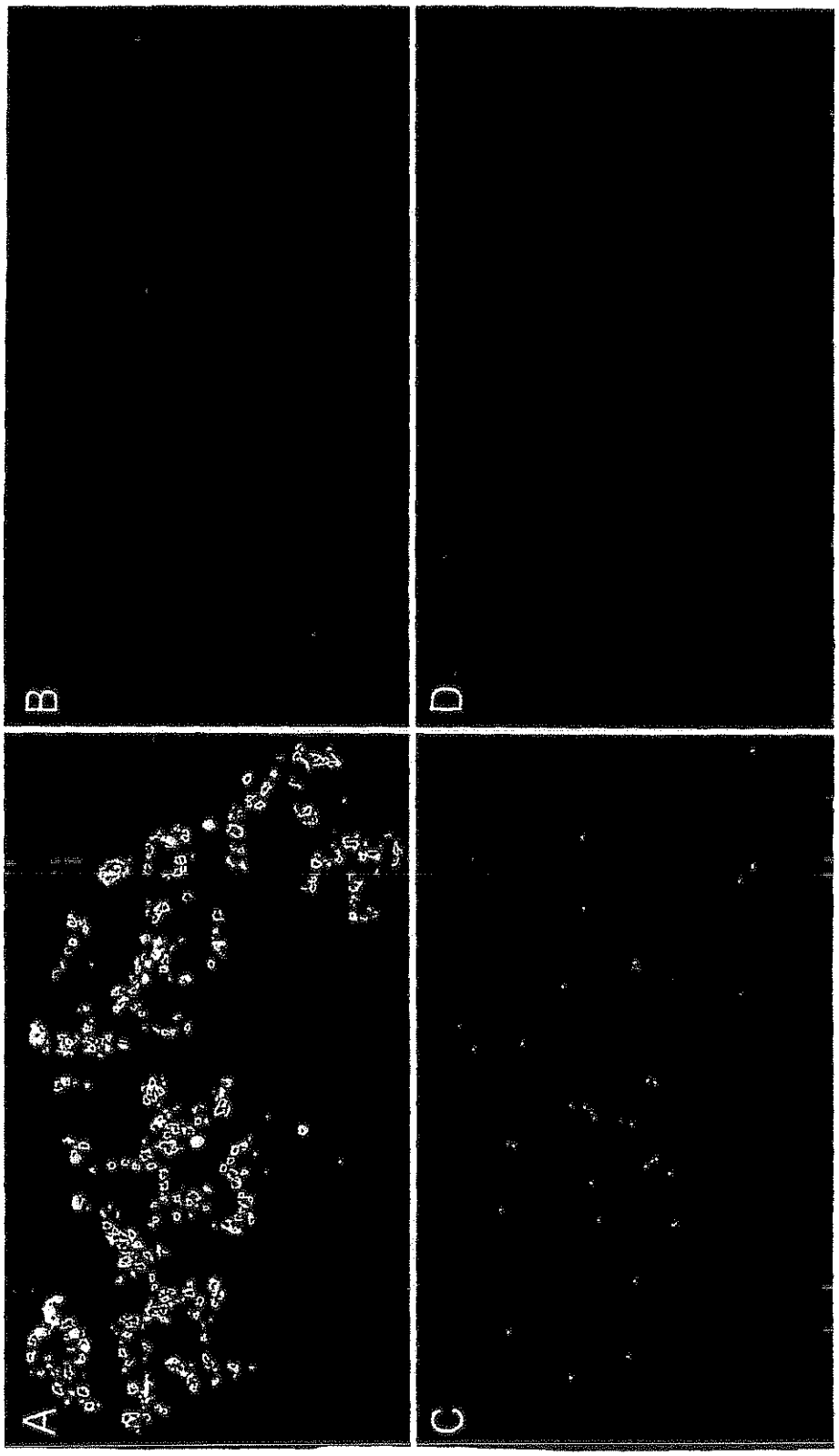
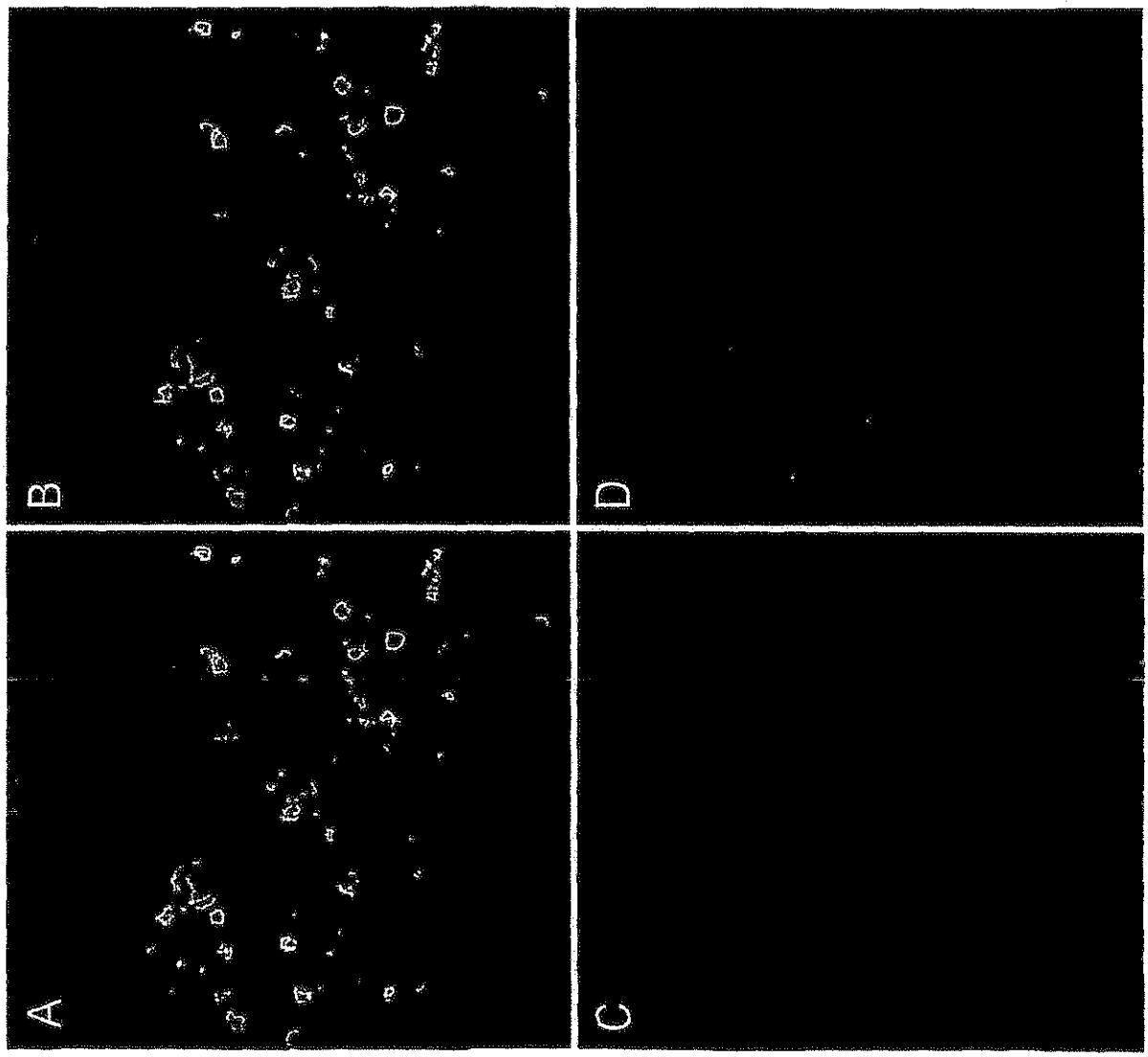


Figure 28



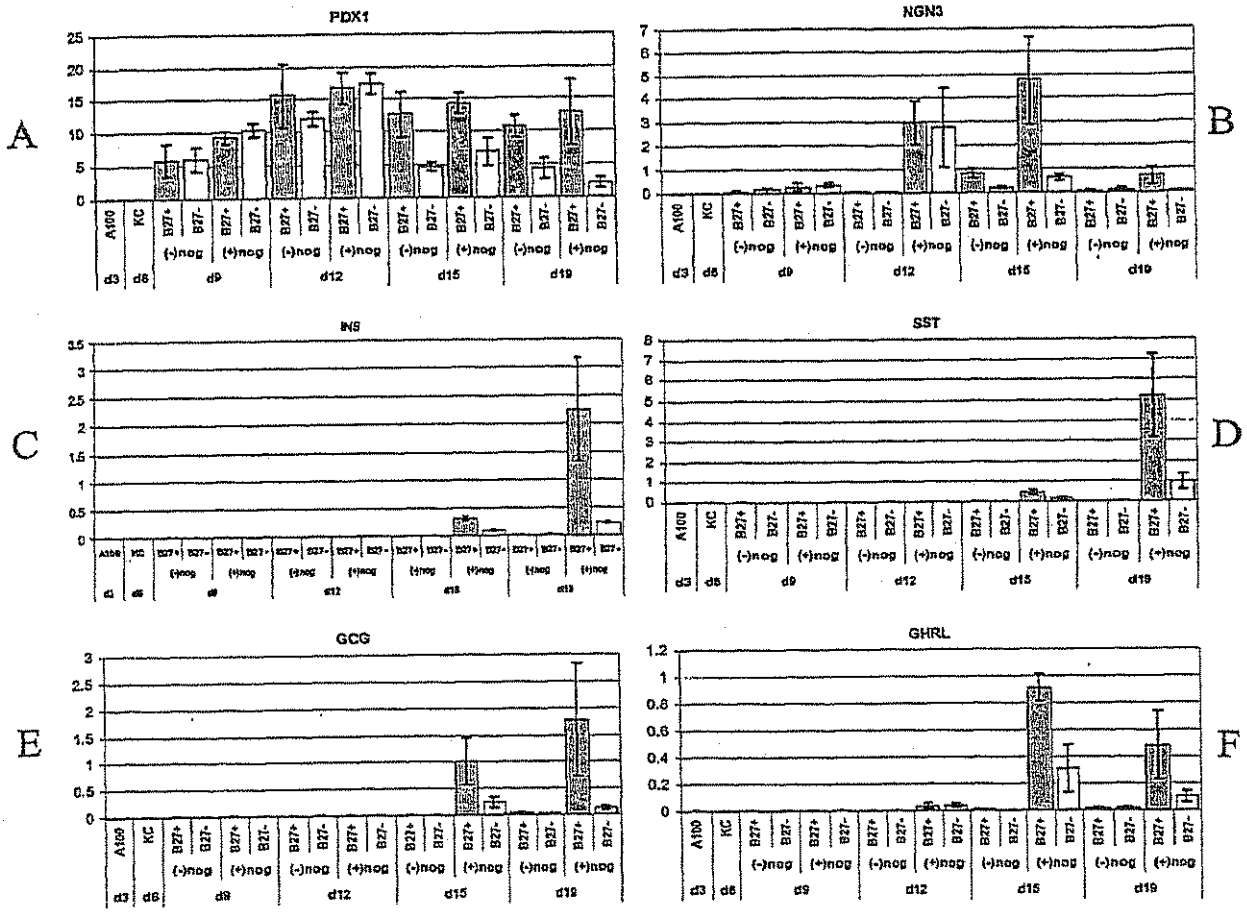

A
PDX1
B
NGN3
C
INS
D
SST
E
GCG
F
GHRL
A100
KC
B27+
B27-
(-)nog
(+)nog
d3
d6
d9
d12
d15
d19

FIGURE 29

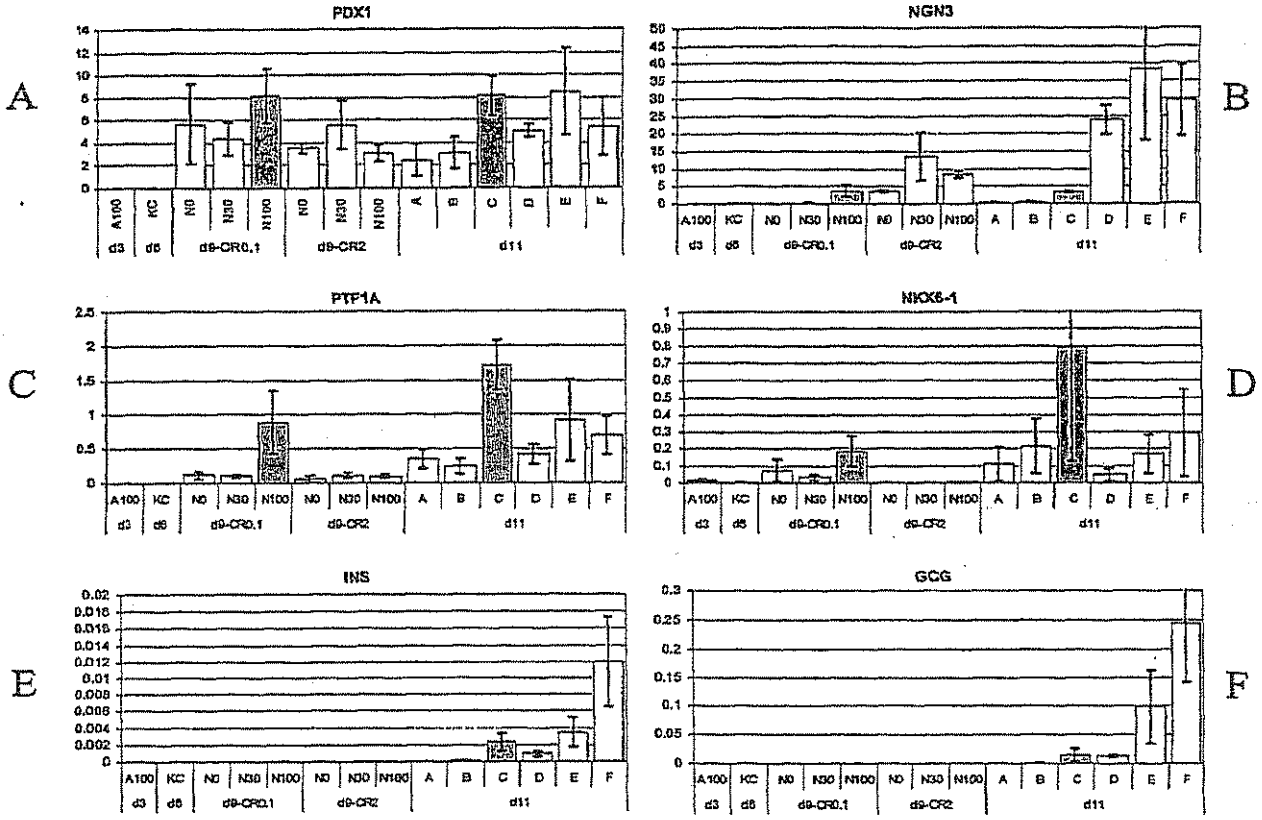

A
PDX1
B
NGN3
C
PTF1A
D
NKX6-1
E
INS
F
GCG
A100
KC
N0
N30
N100
A
B
C
D
E
F
d3
d5
d9-CR0.1
d9-CR2
d11

FIGURE 30