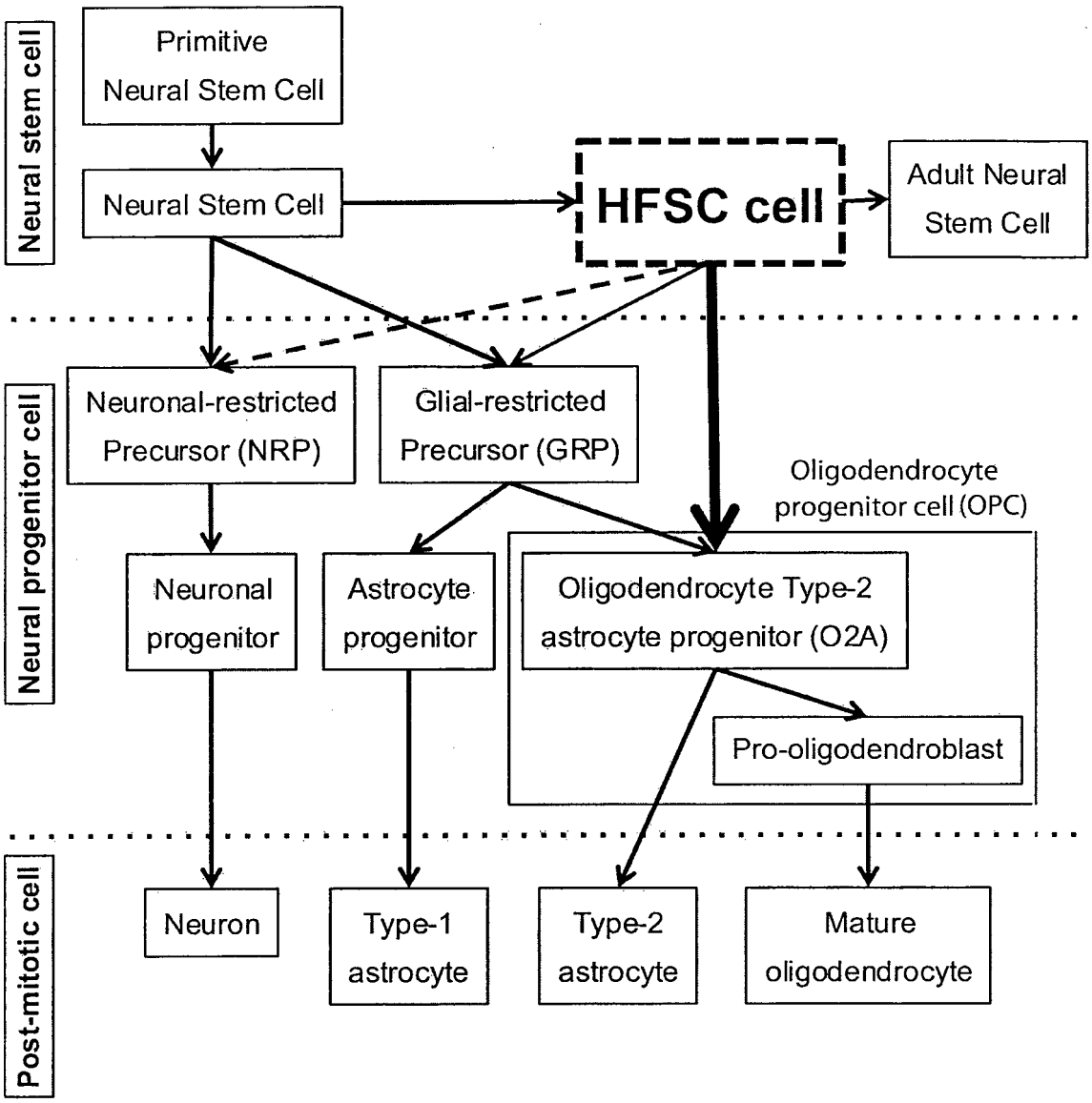

Neural stem cell
Primitive Neural Stem Cell
Neural Stem Cell
HFSC cell
Adult Neural Stem Cell
Neural progenitor cell
Neuronal-restricted Precursor (NRP)
Glial-restricted Precursor (GRP)
Oligodendrocyte progenitor cell (OPC)
Neuronal progenitor
Astrocyte progenitor
Oligodendrocyte Type-2 astrocyte progenitor (O2A)
Pro-oligodendroblast
Post-mitotic cell
Neuron
Type-1 astrocyte
Type-2 astrocyte
Mature oligodendrocyte

Figure 1

Cell type	Positive Markers	Negative Markers
HFSC cell	CD133, Sox2, Nestin, CD9, NG2, CD140a (PDGF-R!), A2B5 (weak), O4 (weak), Olig2, PSA-NCAM	CD44, GFAP, Neurofilament
Neural stem cell	CD133, Nestin, Sox2, Integrin ß 1/CD29, SSEA-1/LEX/CD15, CXCR4/CD184	CD34, CD45, CD44
Radial glia	GLAST, Vimentin, RC2, BLBP	
Neuronal-restricted precursor (NRP)	PSA-NCAM, CD56	A2B5
Glial-restricted precursor (GRP)	A2B5	PSA-NCAM
Neuronal progenitor	PSA-NCAM, Beta III tublin, Nestin	A2B5
Immature neuron	" III tublin, Doublecortin	
Mature neuron	MAP2, Neurofilament, Tau	
Astrocyte progenitor	S100ß, CD44	GFAP
Type-1 astrocyte	GFAP	A2B5
Type-2 astrocyte	GFAP, A2B5	
Oligodendrocyte Type-2 Astrocyte progenitor (O2A)	CD140a (PDGF-R!), Olig2, NG2, A2B5, GD3, O4 (weak),	CD133
Pro-oligodendroblast	NG2, A2B5, GD3 (weak), O4, Sox10	CD133, Sox2, GalC, CNPase, MBP, PLP
Immature oligodendrocyte	A2B5, O4 (strong), GalC, CNPase (2',3'-Cyclic-nucleotide 3'-phosphodiesterase)	MBP
Mature oligodendrocyte	MBP, CNPase, PLP	NG2, GalC

Figure 2

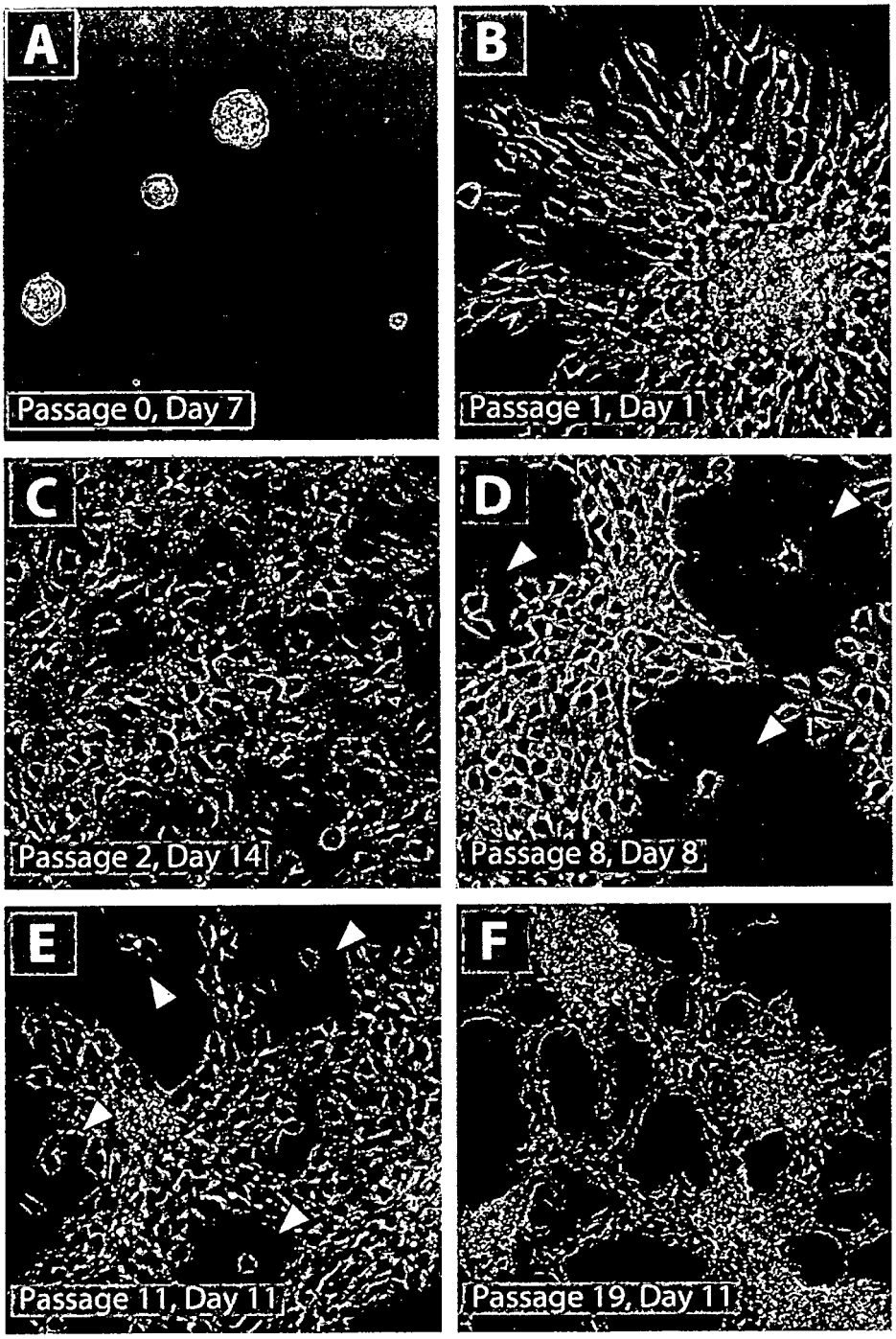
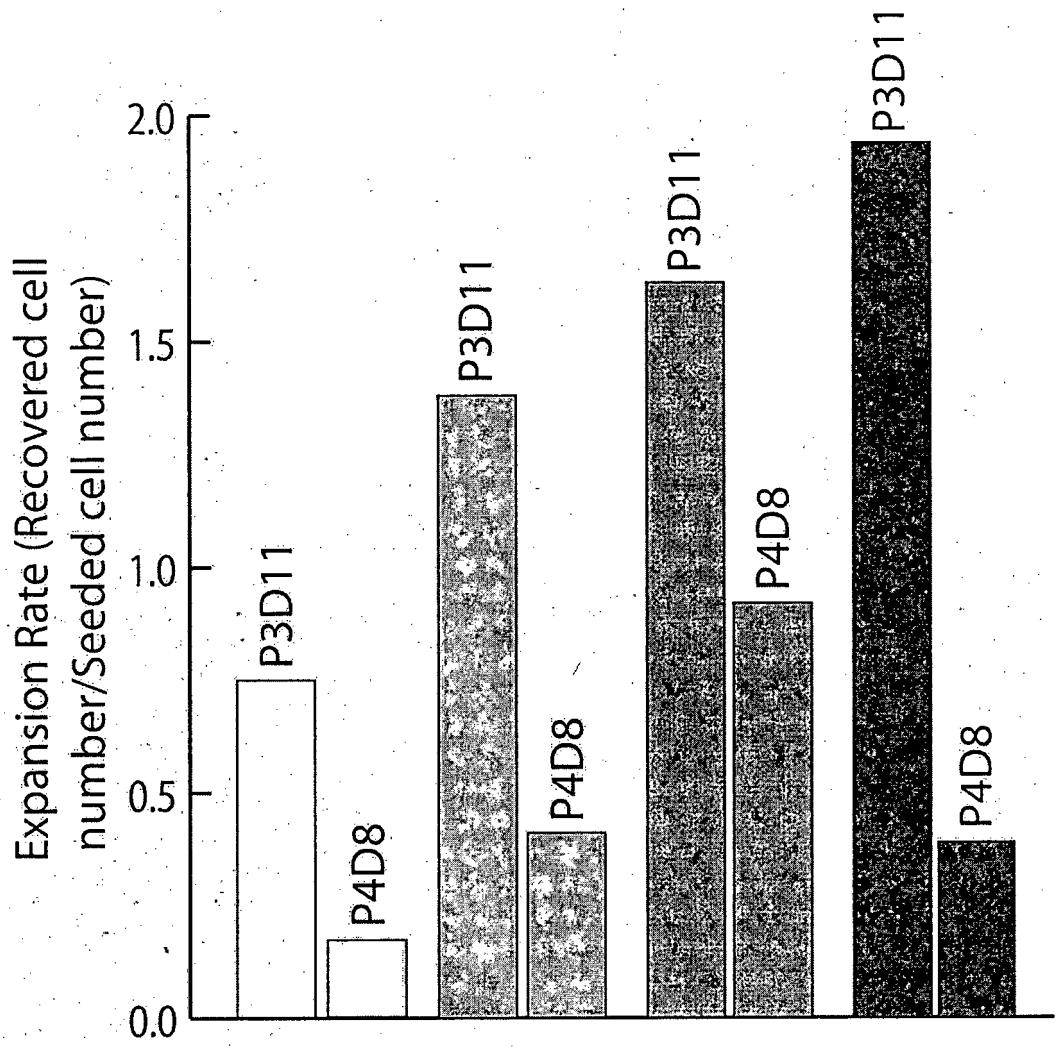

A
Passage 0, Day 7
B
Passage 1, Day 1
C
Passage 2, Day 14
D
Passage 8, Day 8
E
Passage 11, Day 11
F
Passage 19, Day 11

Figure 3



PDGF-AA	20	20	20	20 (ng/ml)
bFGF	10	10	10	10 (ng/ml)
NT-3	0	5	0	5 (ng/ml)
IGF-1	0	0	10	10 (ng/ml)

Figure 4

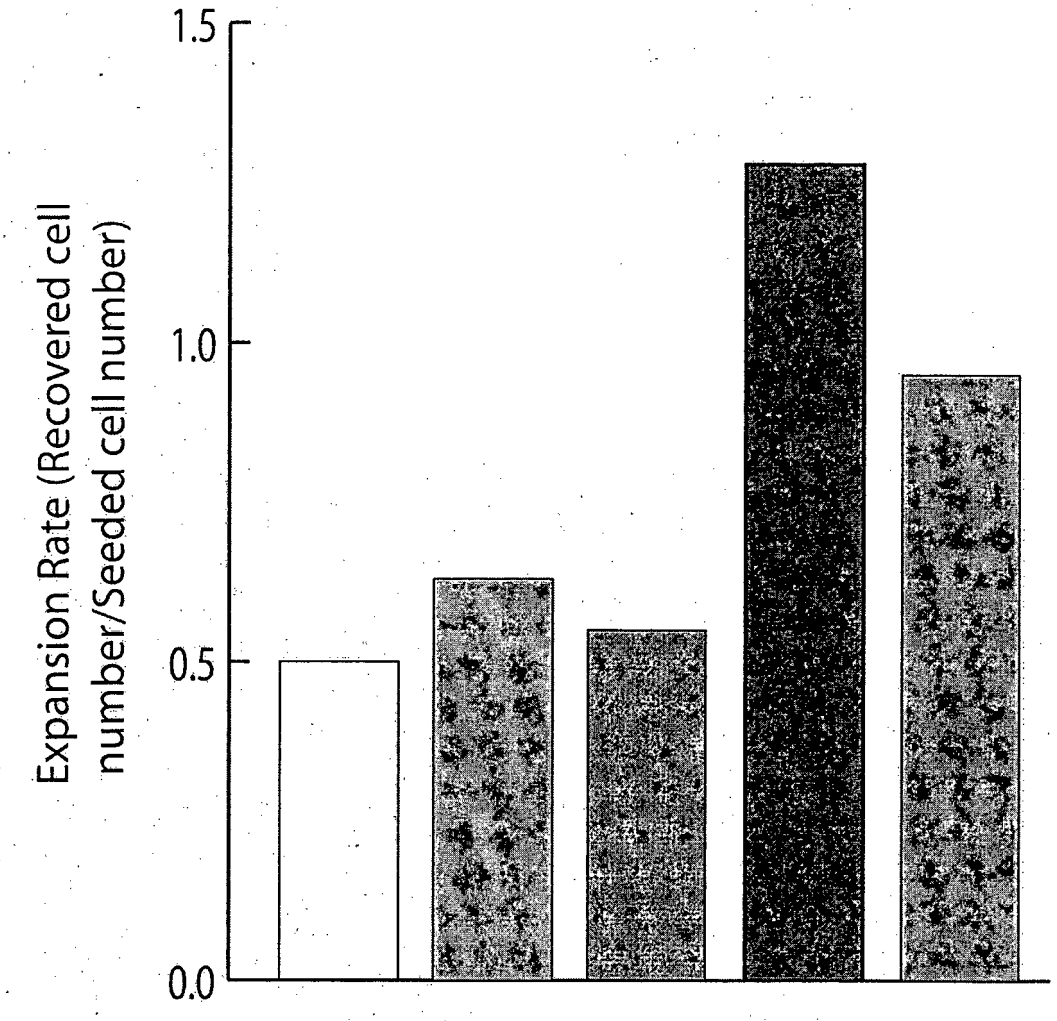

Expansion Rate (Recovered cell number/Seeded cell number)
1.5
1.0
0.5
0.0
PDGF-AA 20 20 100 100 100 (ng/ml)
bFGF 10 10 10 10 10 (ng/ml)
IGF-1 10 10 10 10 0 (ng/ml)
1-thioglycerol 0 50 0 50 50 (μM)

Figure 5

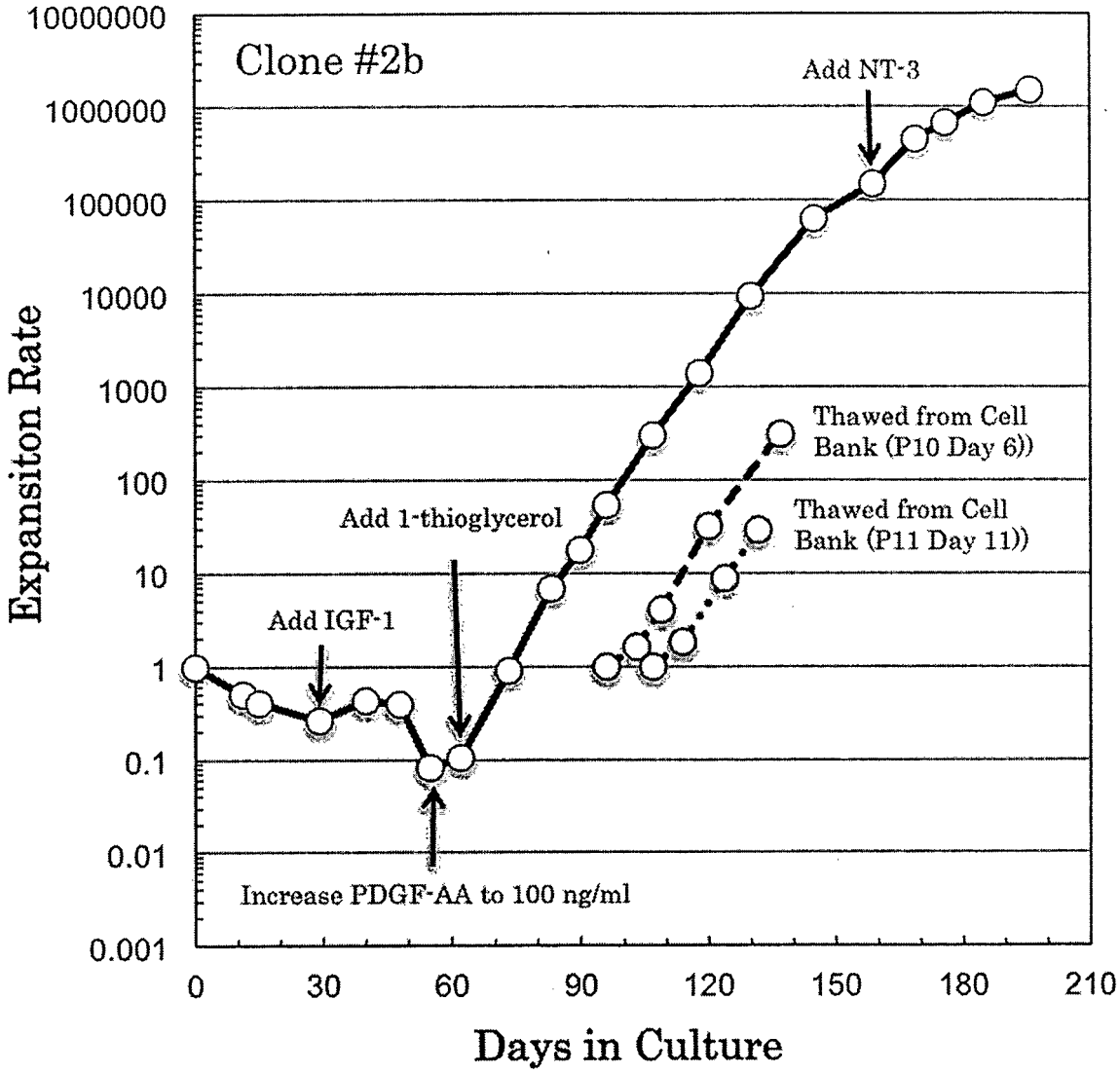

Clone #2b
Add NT-3
Thawed from Cell Bank (P10 Day 6))
Thawed from Cell Bank (P11 Day 11))
Add 1-thioglycerol
Add IGF-1
Increase PDGF-AA to 100 ng/ml
Expansiton Rate
10000000
1000000
100000
10000
1000
100
10
1
0.1
0.01
0.001
0
30
60
90
120
150
180
210
Days in Culture

Figure 6

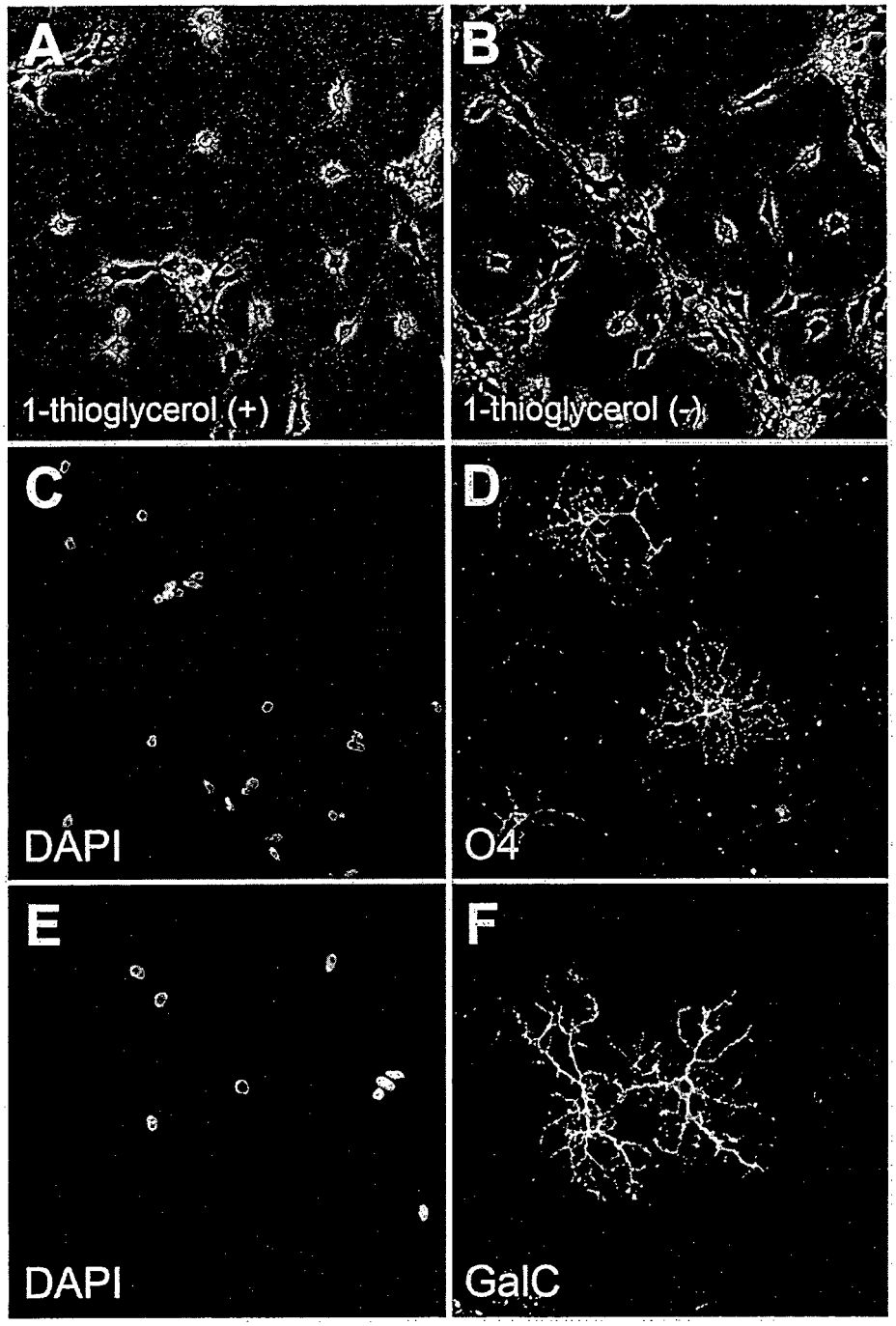


Figure 7

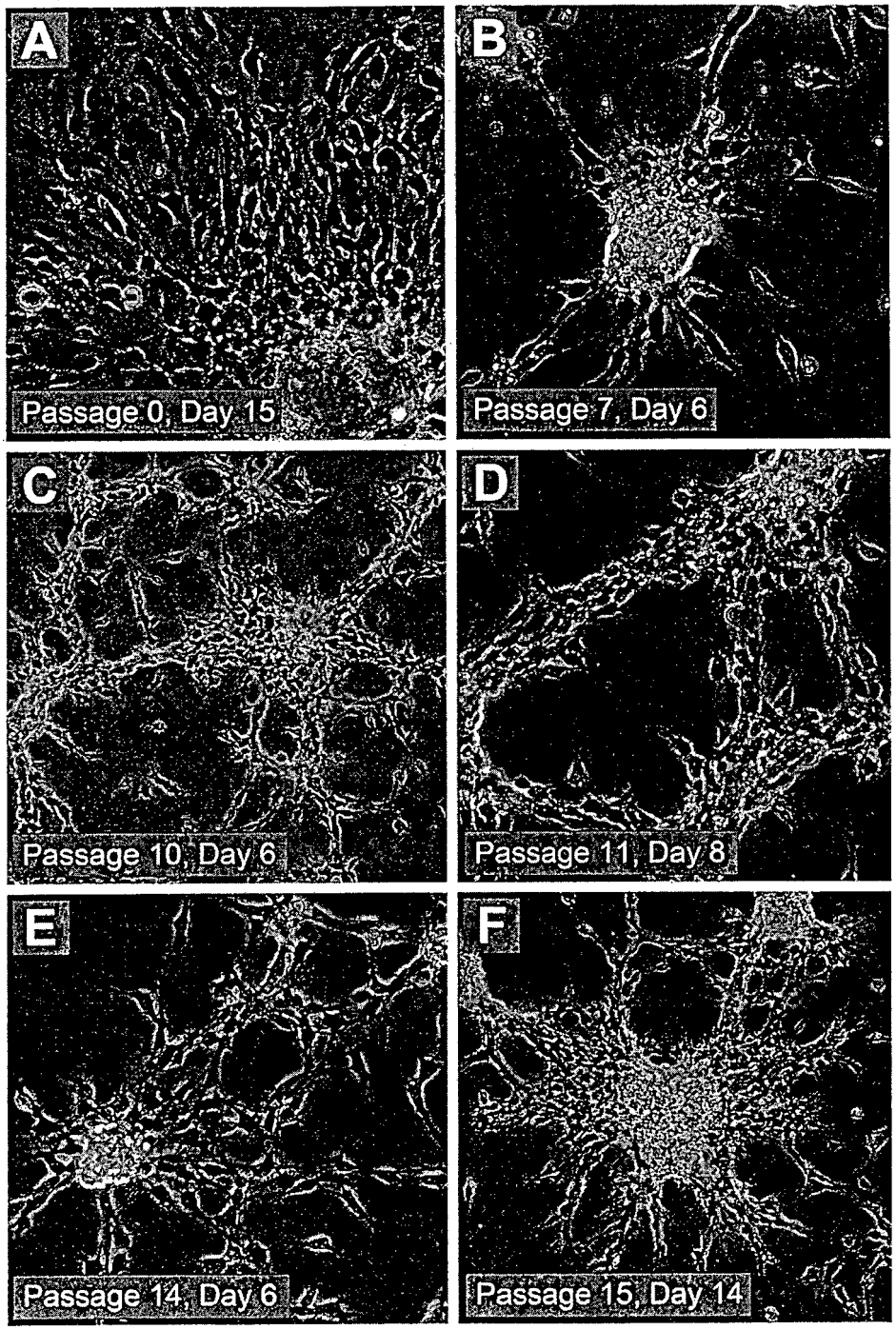

A
Passage 0, Day 15
B
Passage 7, Day 6
C
Passage 10, Day 6
D
Passage 11, Day 8
E
Passage 14, Day 6
F
Passage 15, Day 14

Figure 8

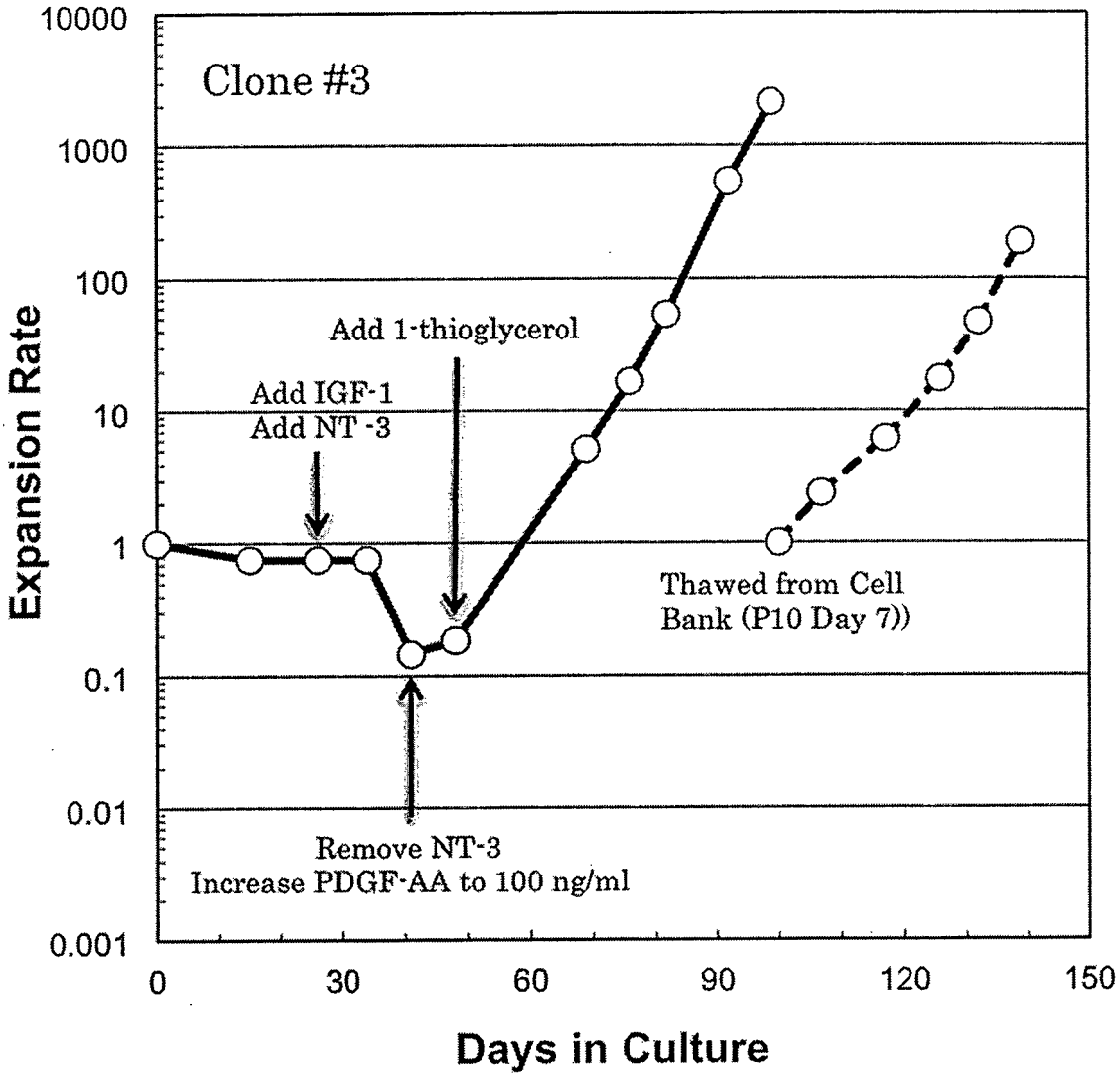

Clone #3
Expansion Rate
10000
1000
100
10
1
0.1
0.01
0.001
Add 1-thioglycerol
Add IGF-1
Add NT -3
Thawed from Cell
Bank (P10 Day 7))
Remove NT-3
Increase PDGF-AA to 100 ng/ml
0
30
60
90
120
150
Days in Culture

Figure 9

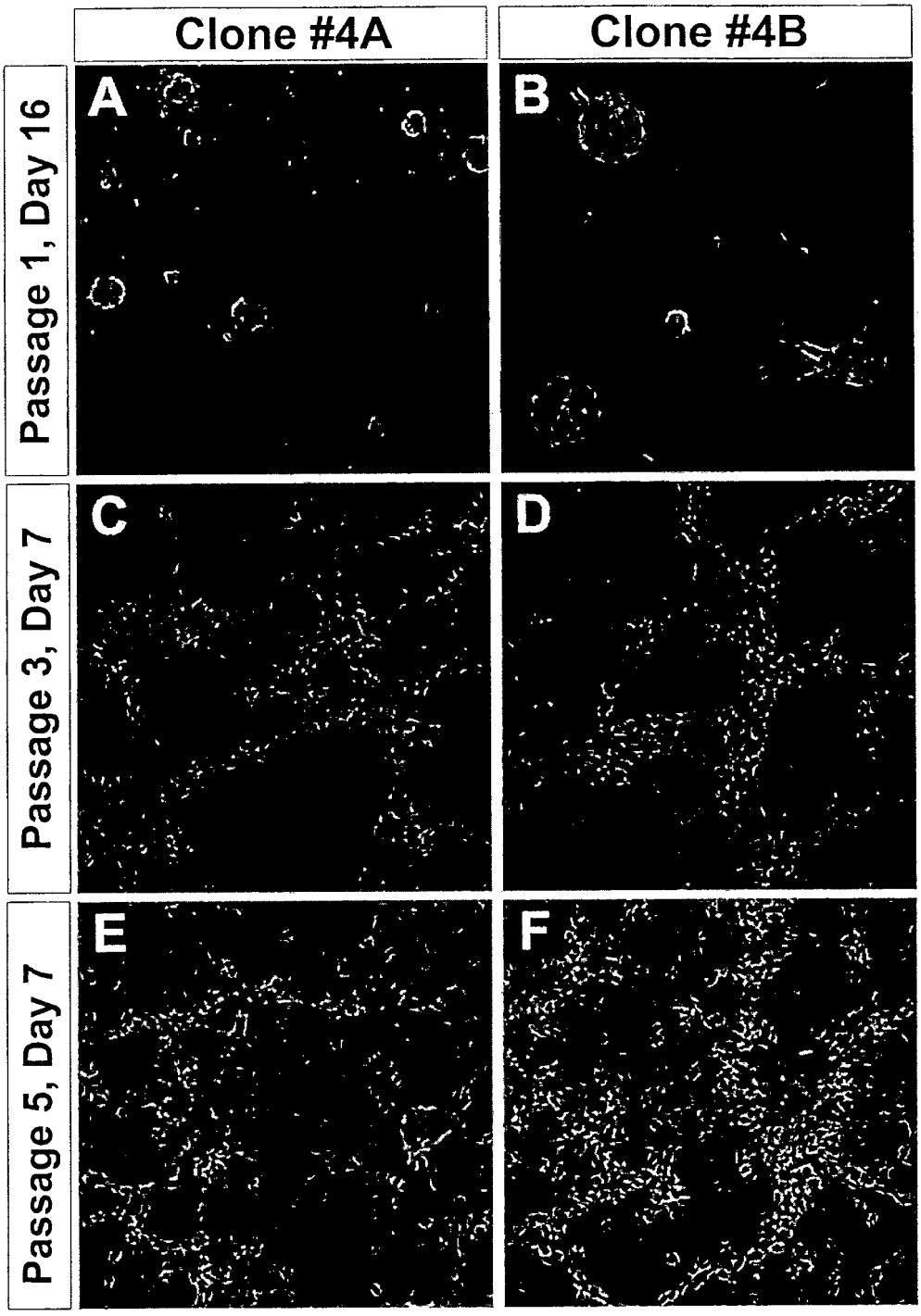

Clone #4A
Clone #4B
Passage 1, Day 16
Passage 3, Day 7
Passage 5, Day 7
A
B
C
D
E
F

Figure 10

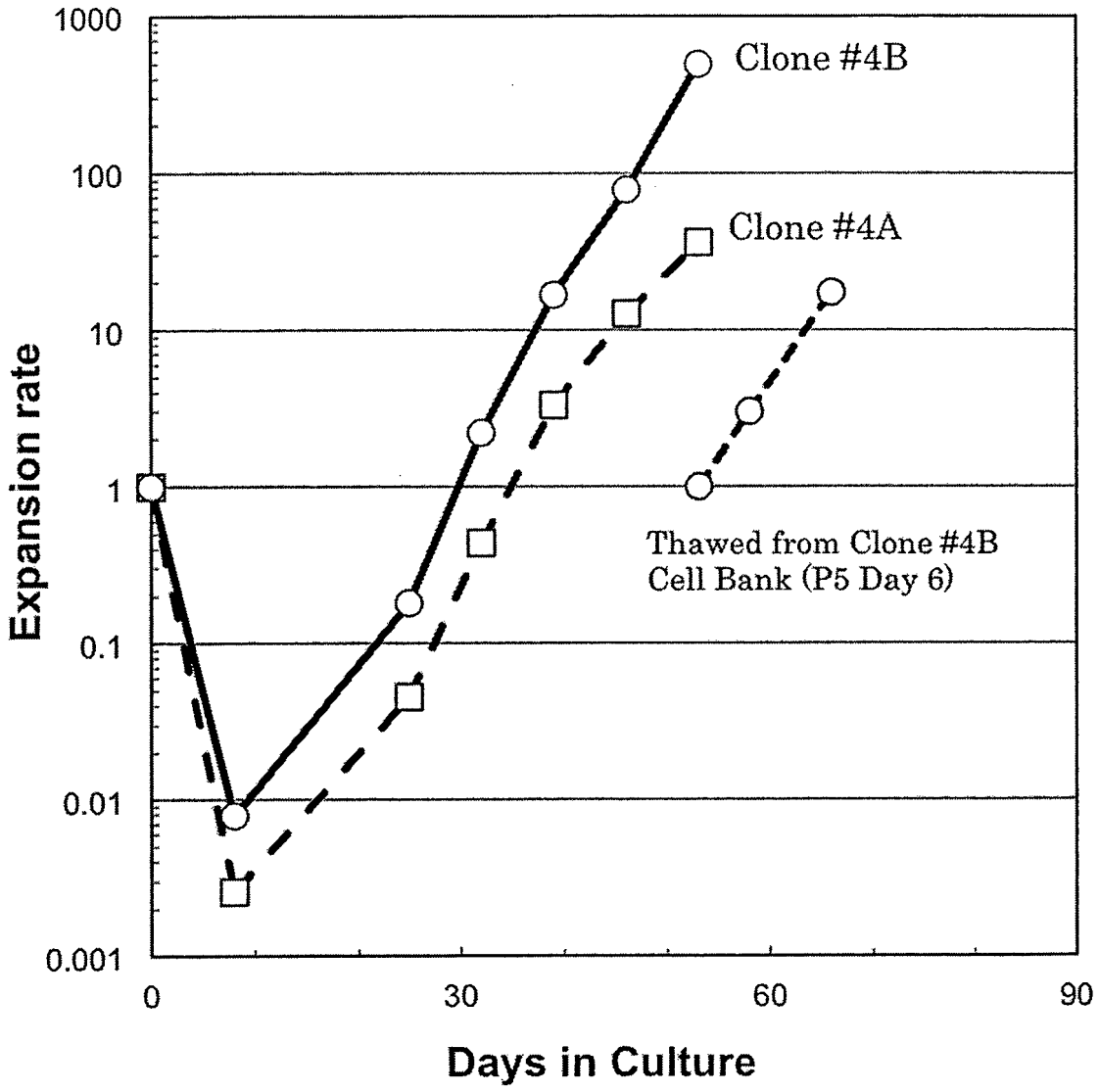

1000
100
10
1
0.1
0.01
0.001
Expansion rate
0
30
60
90
Days in Culture
Clone #4B
Clone #4A
Thawed from Clone #4B
Cell Bank (P5 Day 6)

Figure 11

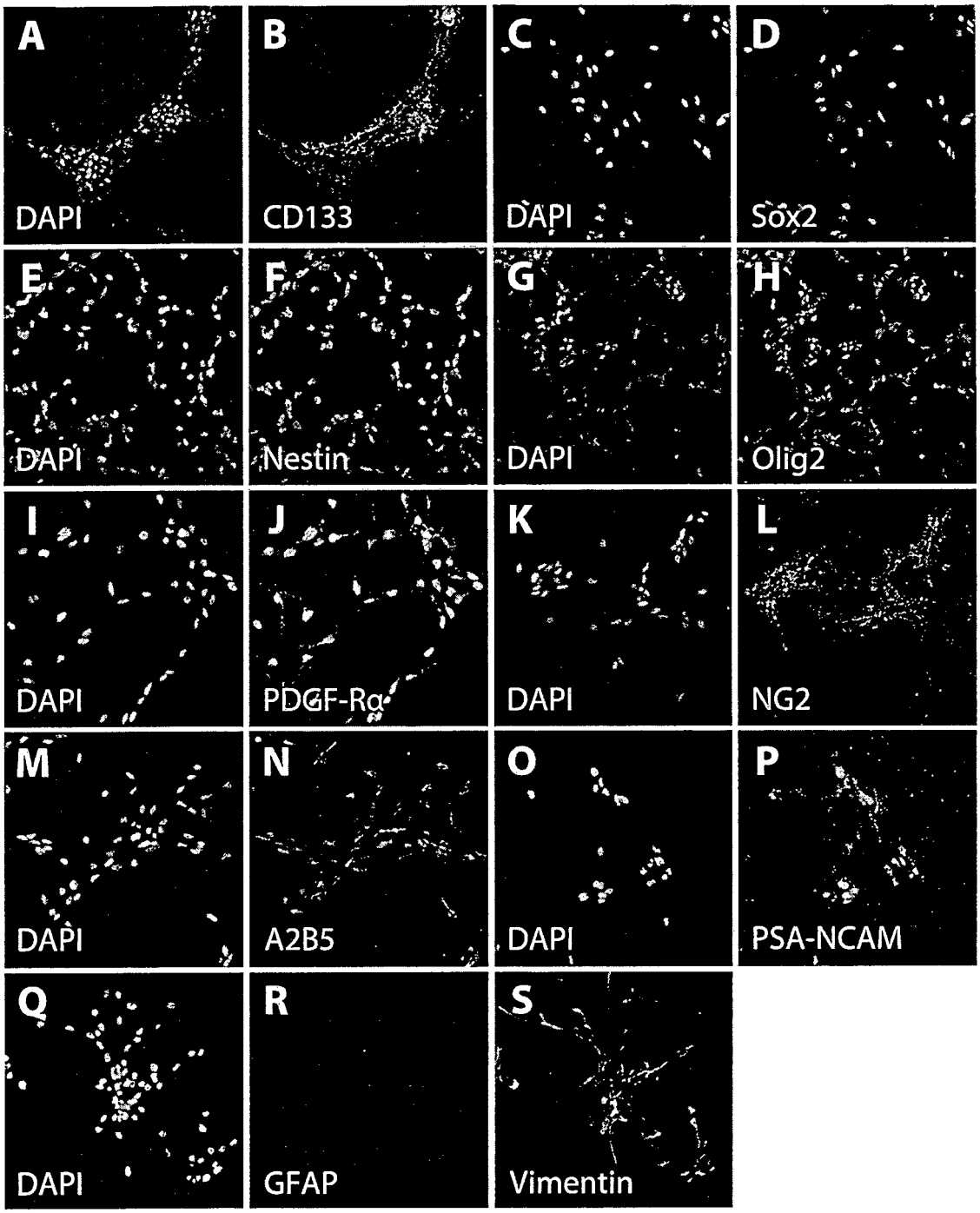

A
DAPI
B
CD133
C
DAPI
D
Sox2
E
DAPI
F
Nestin
G
DAPI
H
Olig2
I
DAPI
J
PDGF-Rα
K
DAPI
L
NG2
M
DAPI
N
A2B5
O
DAPI
P
PSA-NCAM
Q
DAPI
R
GFAP
S
Vimentin

Figure 12

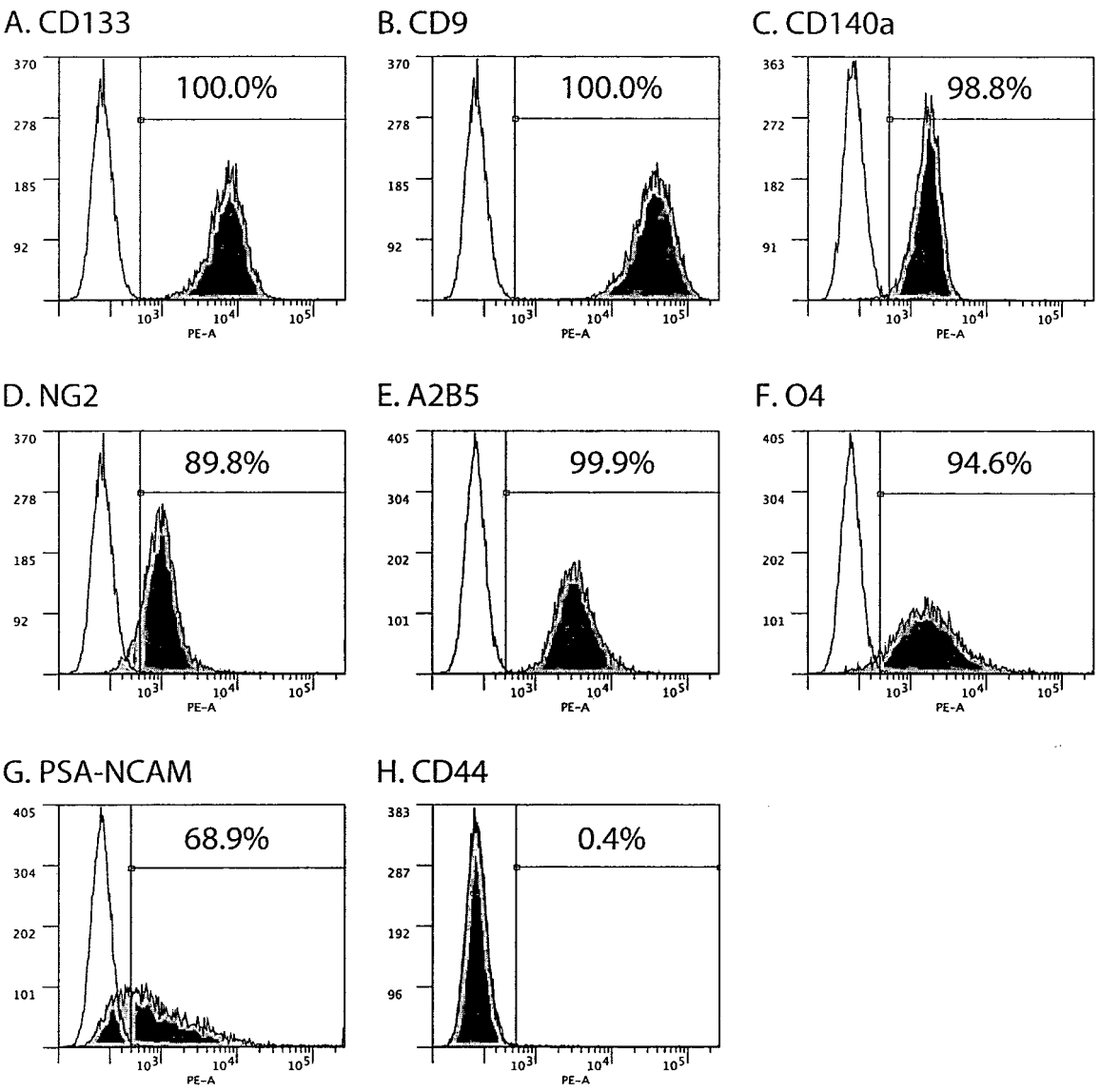

A. CD133
100.0%
B. CD9
100.0%
C. CD140a
98.8%
D. NG2
89.8%
E. A2B5
99.9%
F. O4
94.6%
G. PSA-NCAM
68.9%
H. CD44
0.4%
PE-A

Figure 13

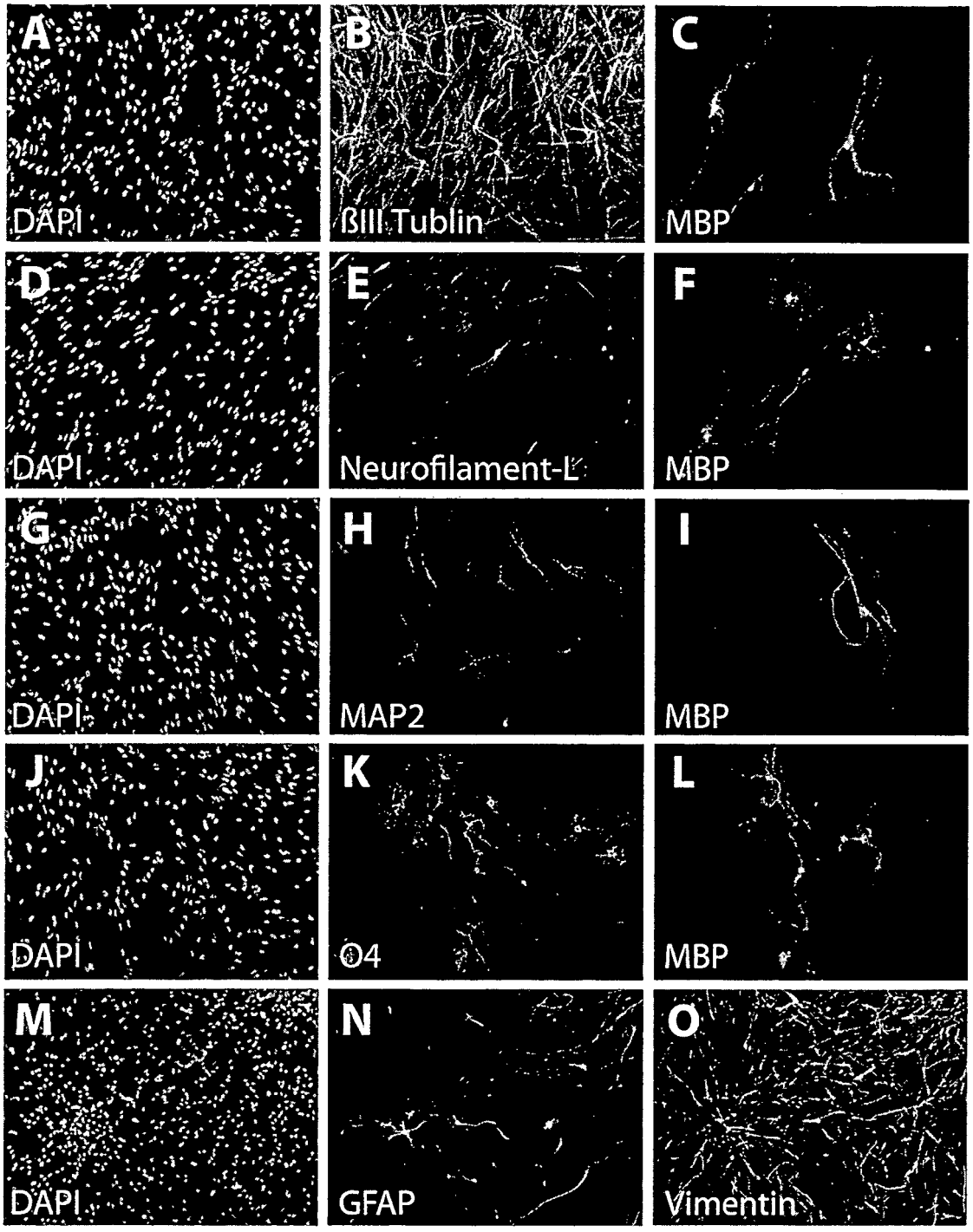

A
DAPI
B
ßIII Tublin
C
MBP
D
DAPI
E
Neurofilament-L
F
MBP
G
DAPI
H
MAP2
I
MBP
J
DAPI
K
O4
L
MBP
M
DAPI
N
GFAP
O
Vimentin

Figure 14

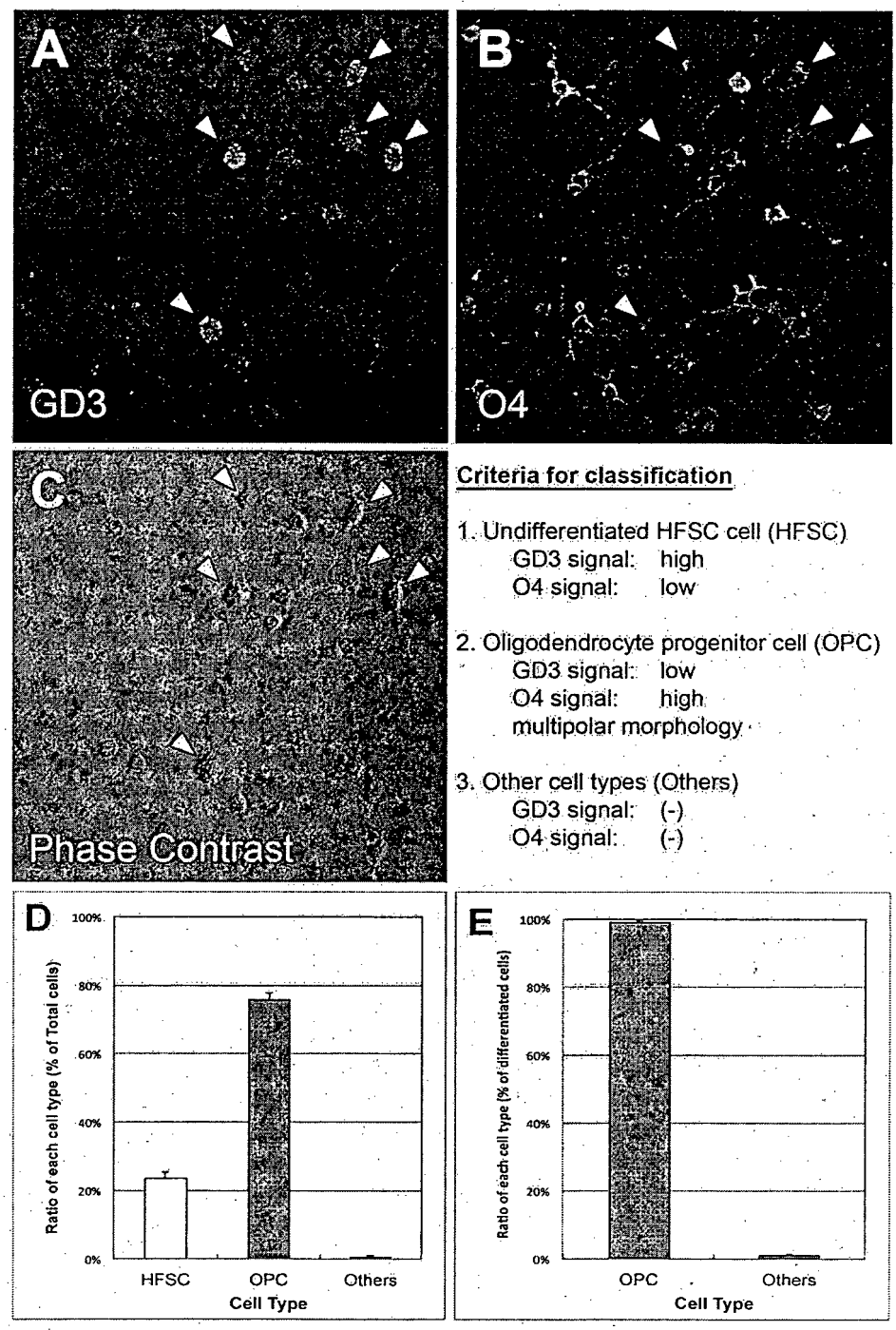

A
GD3
B
O4
C
Phase Contrast
Criteria for classification
1. Undifferentiated HFSC cell (HFSC)
GD3 signal: high
O4 signal: low
2. Oligodendrocyte progenitor cell (OPC)
GD3 signal: low
O4 signal: high
multipolar morphology
3. Other cell types (Others)
GD3 signal: (-)
O4 signal: (-)
D
Ratio of each cell type (% of Total cells)
100%
80%
60%
40%
20%
0%
HFSC
OPC
Others
Cell Type
E
Ratio of each cell type (% of differentiated cells)
100%
80%
60%
40%
20%
0%
OPC
Others
Cell Type

Figure 15