Intermediate Filaments:
Good Markers for Neural Stem Cell Differentiation

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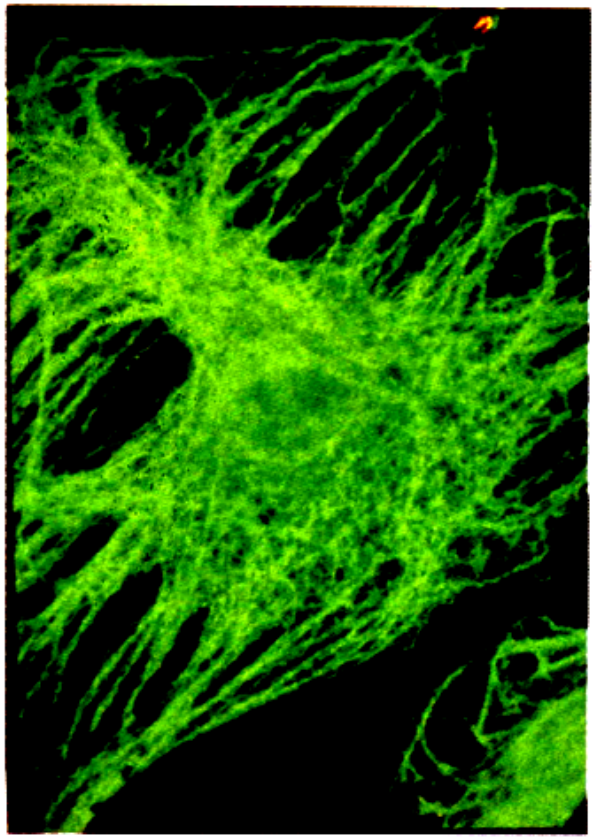
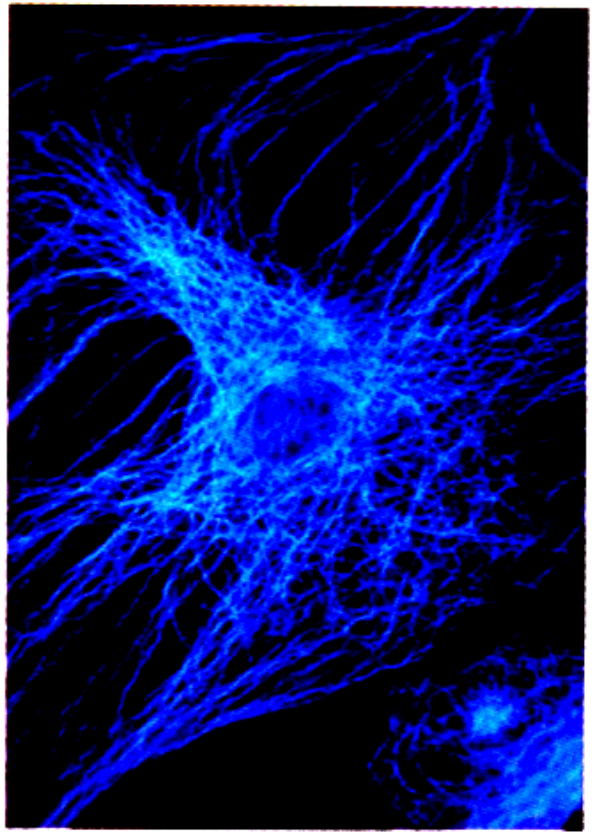
Cytoskeletons in the culture cell

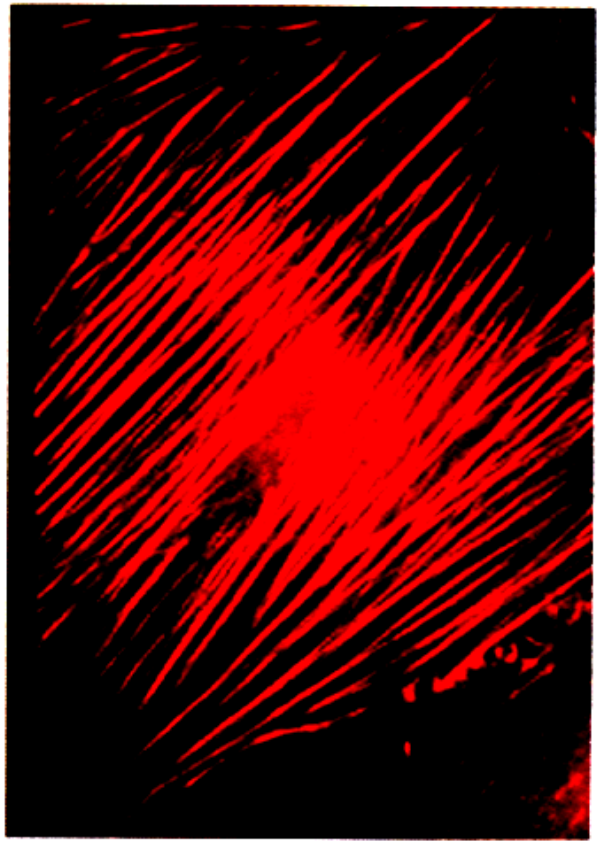
(a) Intermediate filaments (vimentin)



(b) Microtubules (tubulin)

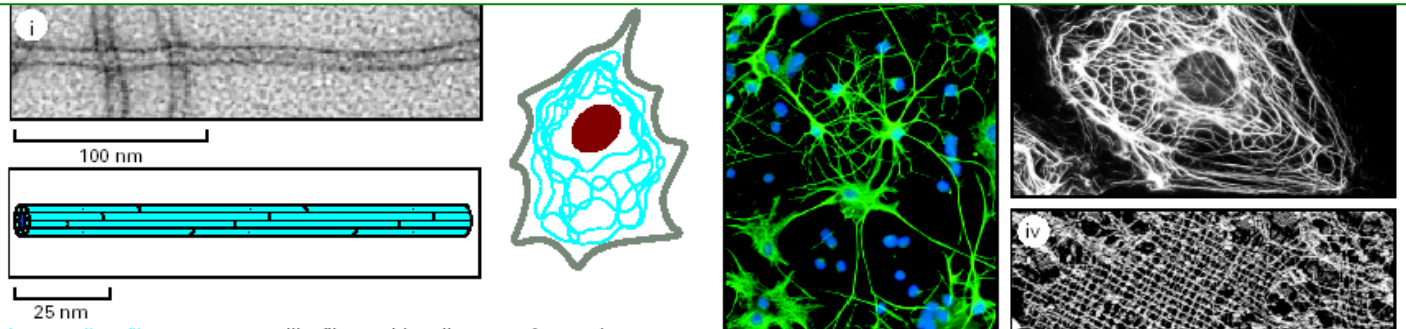


(c) Microfilaments (actin)



Major Types of Intermediate Filament Proteins in Mammalian cells

TYPES OF IF	COMPONENT POLYPEPTIDES	CELLULAR LOCATION
Nuclear	lamins A, B, and C	nuclear lamina (inner lining of nuclear envelope)
Vimentin-like	vimentin	many cells of mesenchymal origin
	desmin	muscle
	glial fibrillary acidic protein	glial cells (astrocytes and some Schwann cells)
	peripherin	some neurons
Epithelial	type I keratins (acidic)	epithelial cells and their derivatives (e.g., hair and nails)
	type II keratins (basic)	
Axonal	neurofilament proteins (NF-L, NF-M, and NF-H)	neurons

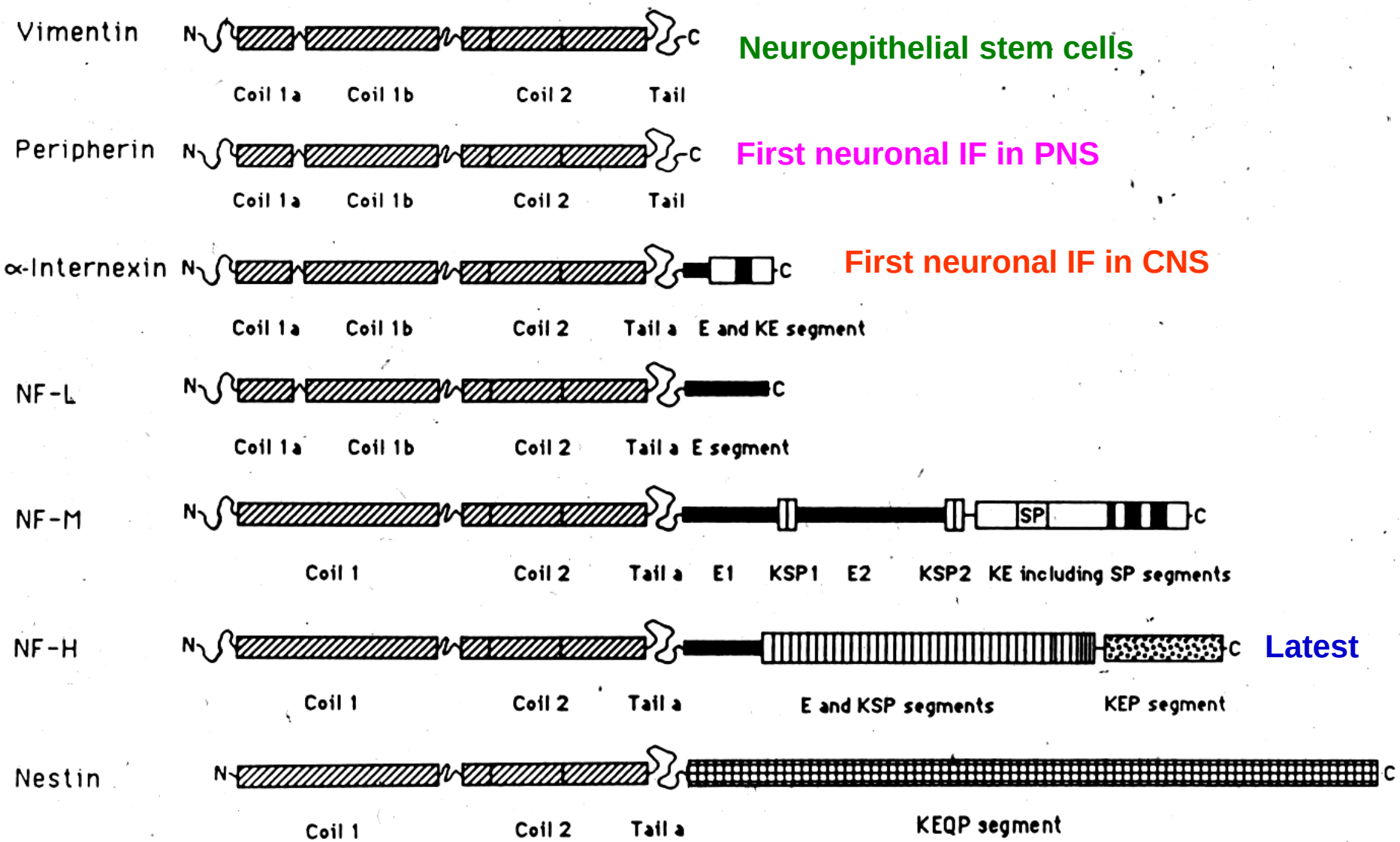


Intermediate filaments are ropelike fibers with a diameter of around 10 nm; they are made of intermediate filament proteins, which constitute a large and heterogeneous family. One type of intermediate filament forms a meshwork called the nuclear lamina just beneath the inner nuclear membrane. Other types extend across the cytoplasm, giving cells mechanical strength. In an epithelial tissue, they span the cytoplasm from one cell-cell junction to another, thereby strengthening the entire epithelium.

Micrographs courtesy of Roy Quinlan (i); Nancy L. Kedersha (ii); Mary Osborn (iii); Ueli Aebi (iv).

Cited from ***Molecular Biology of the Cell***, 4 ed., 2002

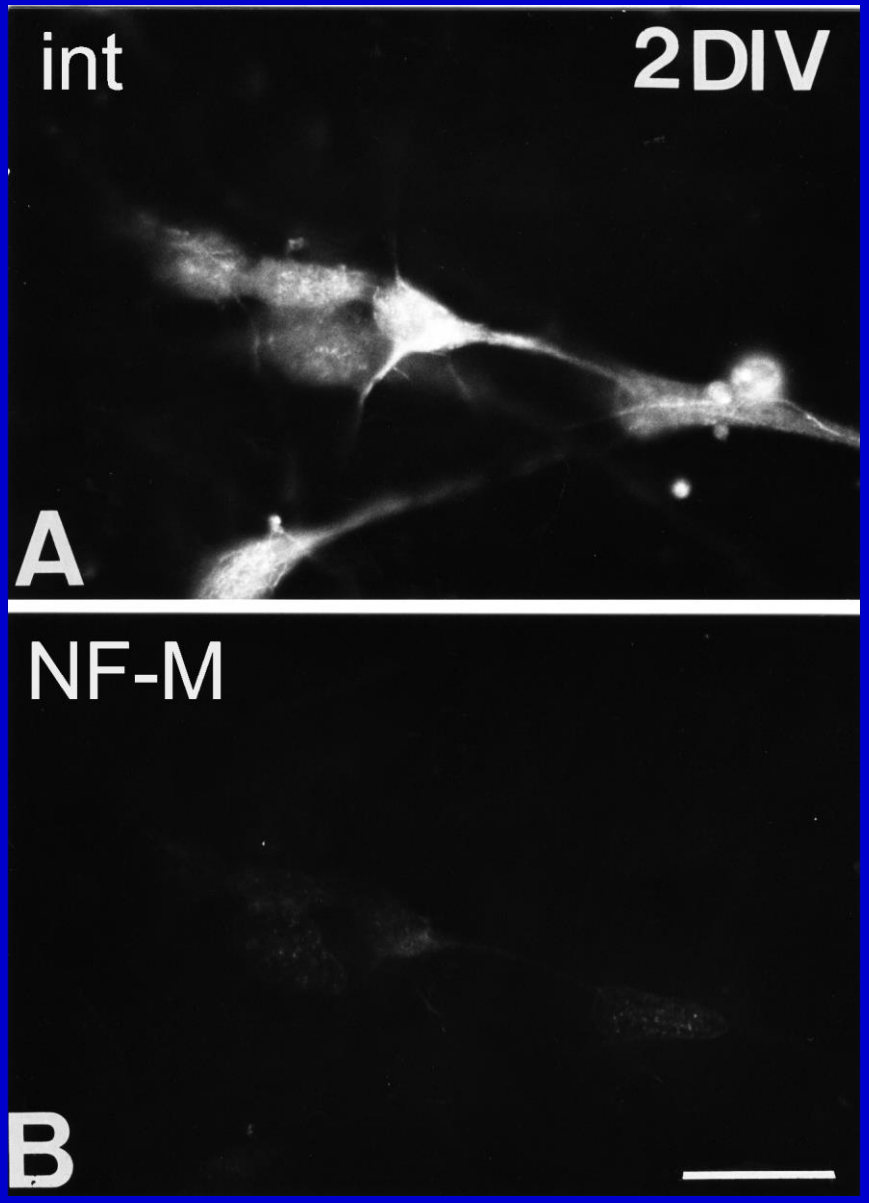
Seven Intermediate Filament Proteins in Neural Differentiation



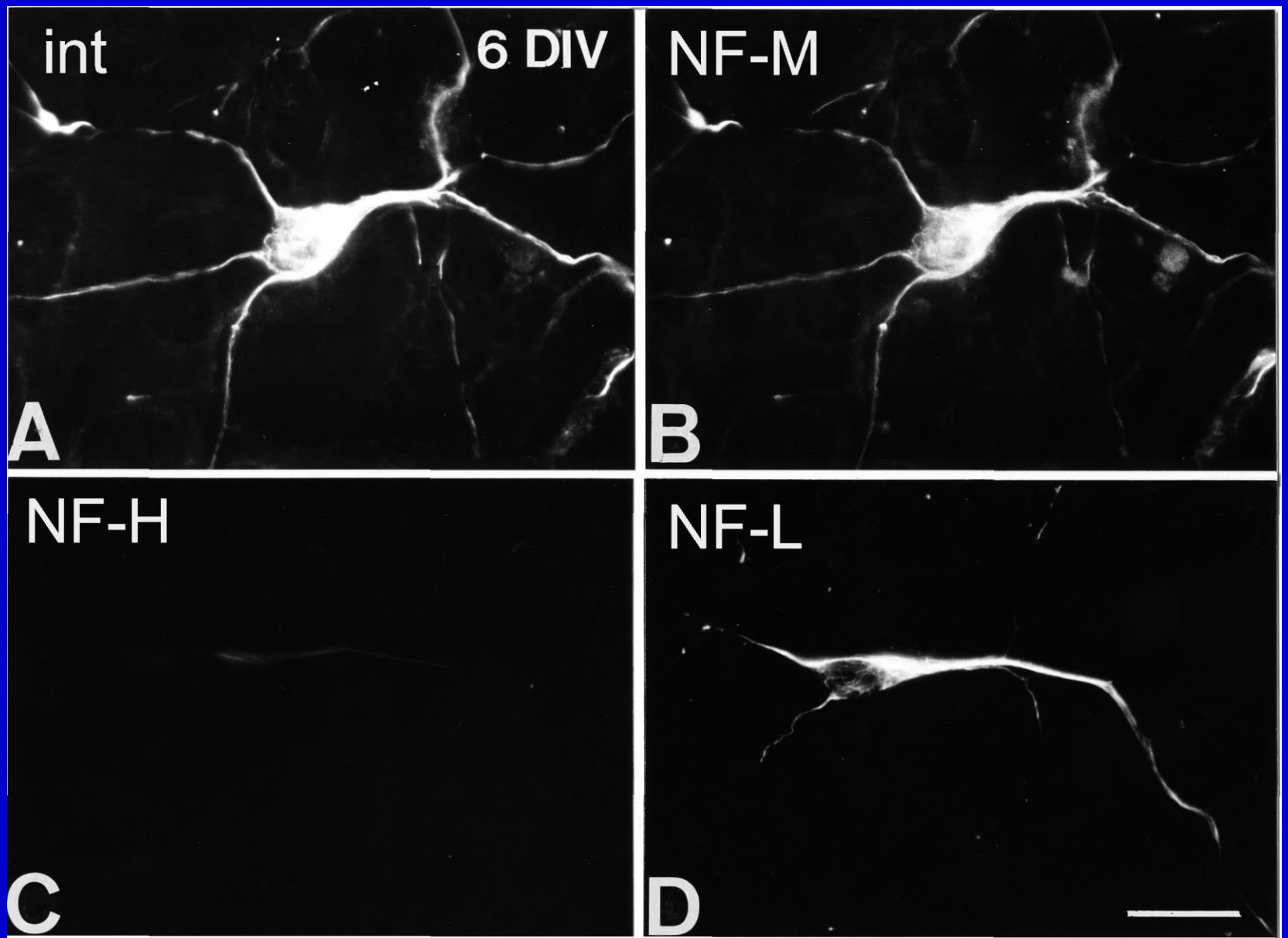
Primary culture of embryonic hippocampal cells

α-internexin: a 66 kD protein,

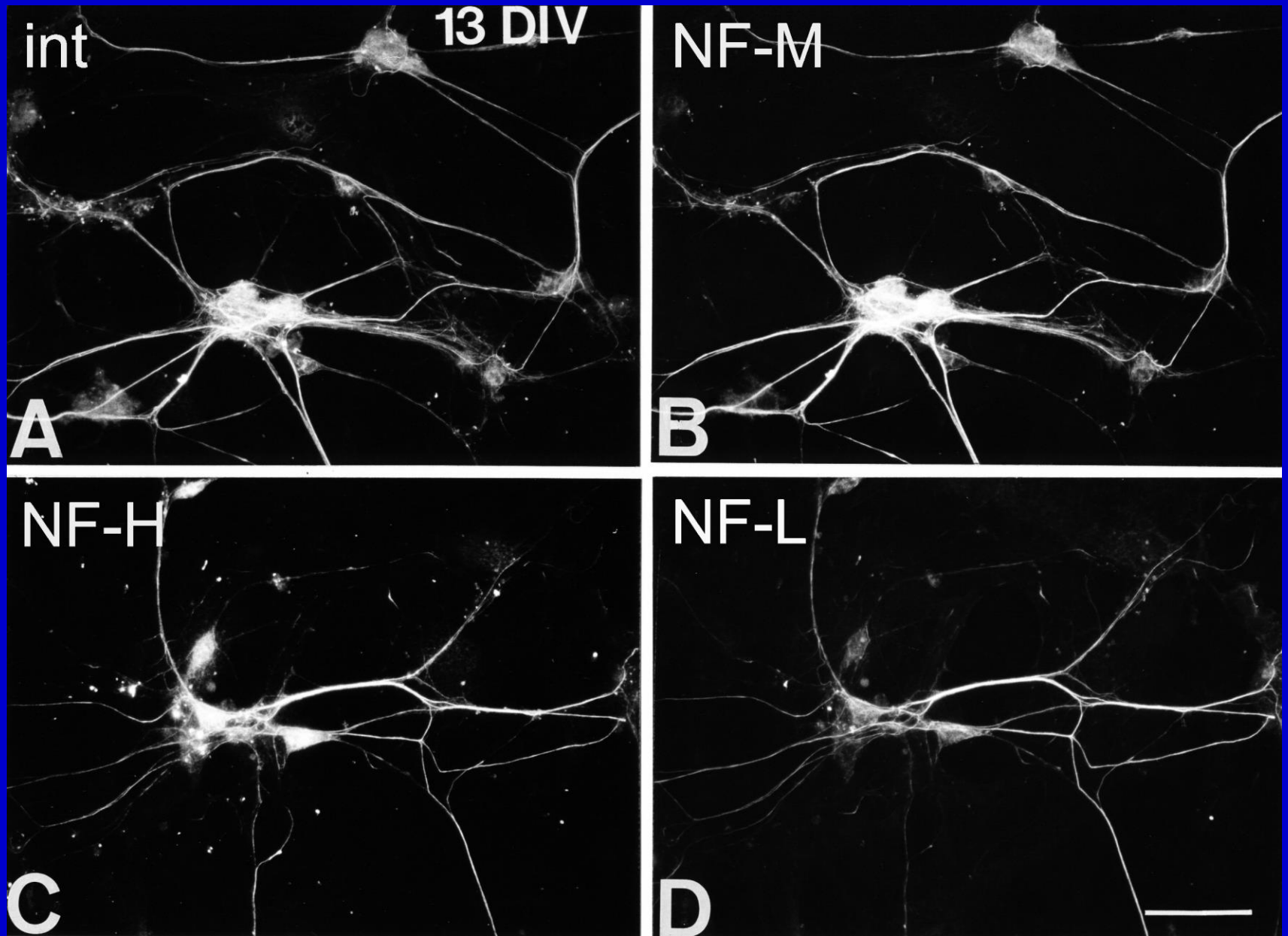
the first neuronal intermediate filament protein expressed in the post-mitotic neurons of developing mammalian central nervous system



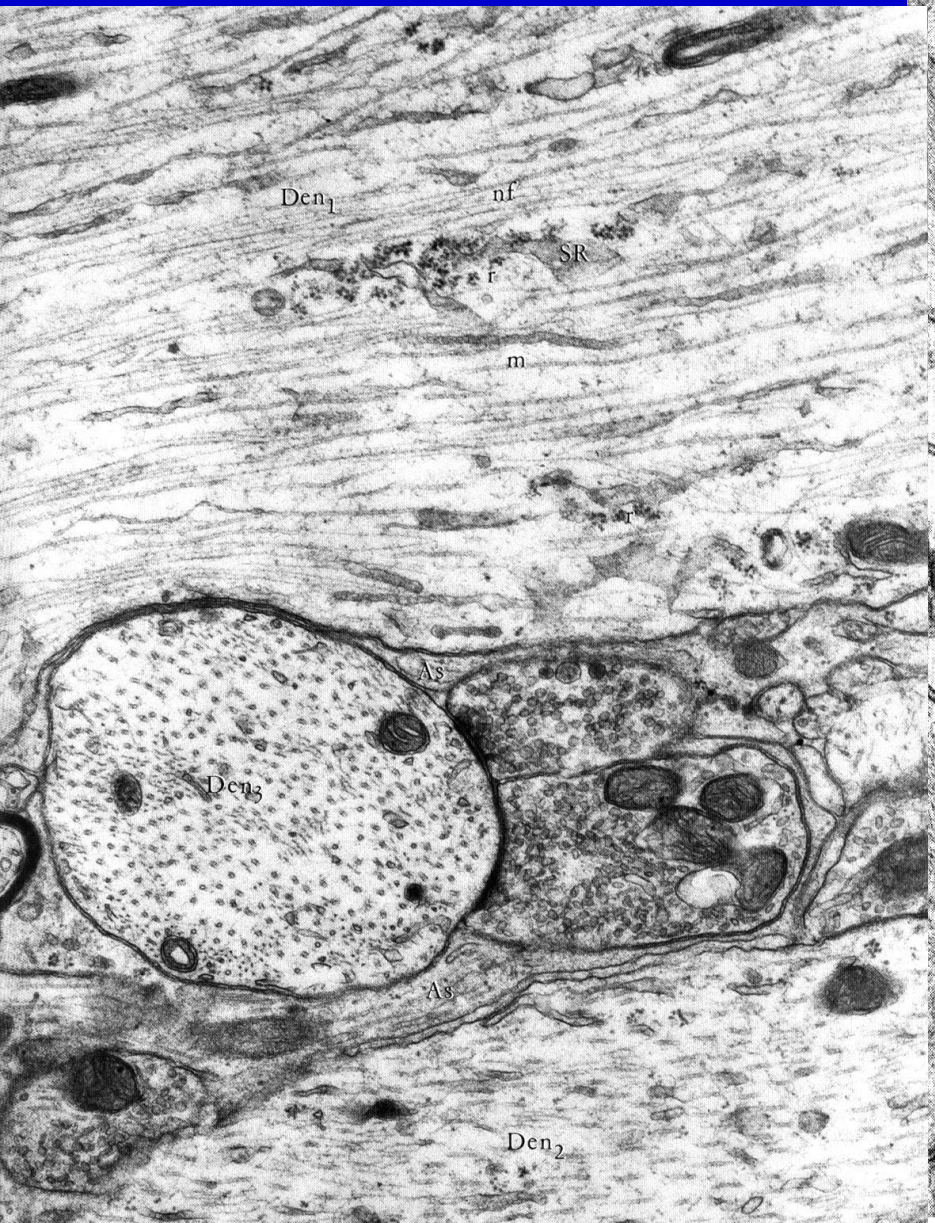
Internexin, NF-M, NF-L but not NF-H expressed in the 6 days *in vitro* (DIV) culture of hippocampal neurons

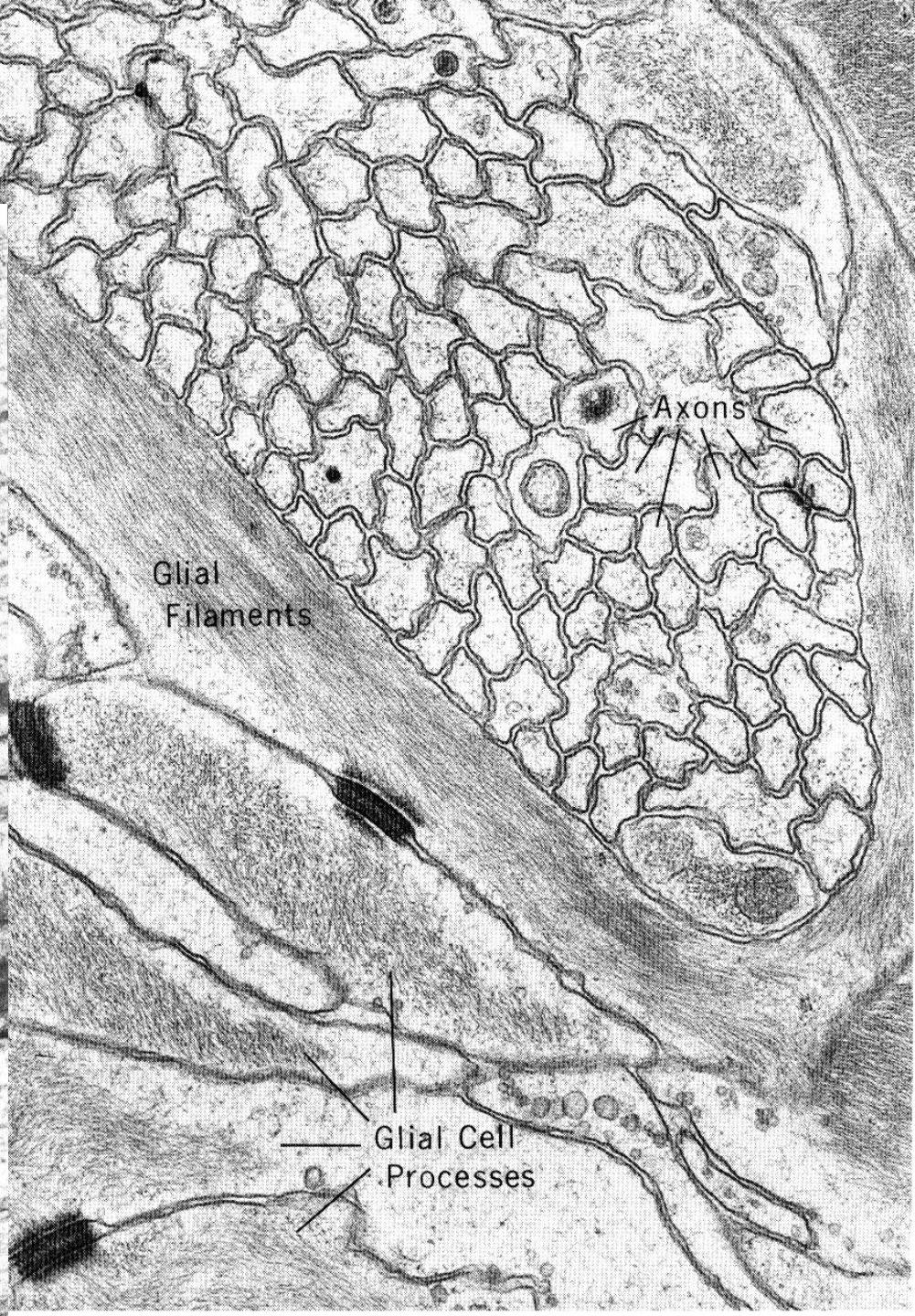


Internexin and Neurofilament Triplet Proteins (NF-L, NF-M and NF-H) all expressed in the 13 DIV hippocampal neurons

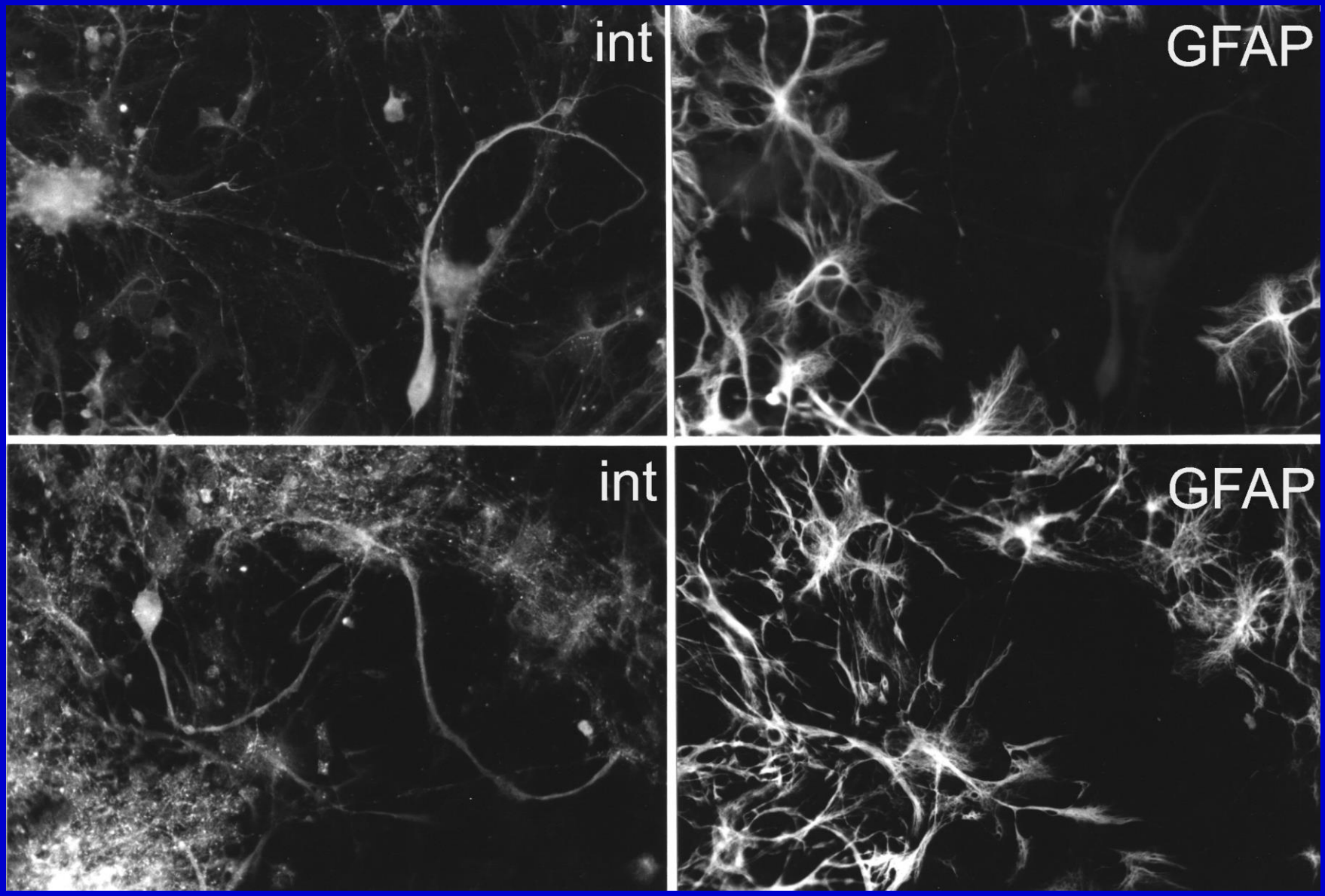


Neurofilaments and GFAP

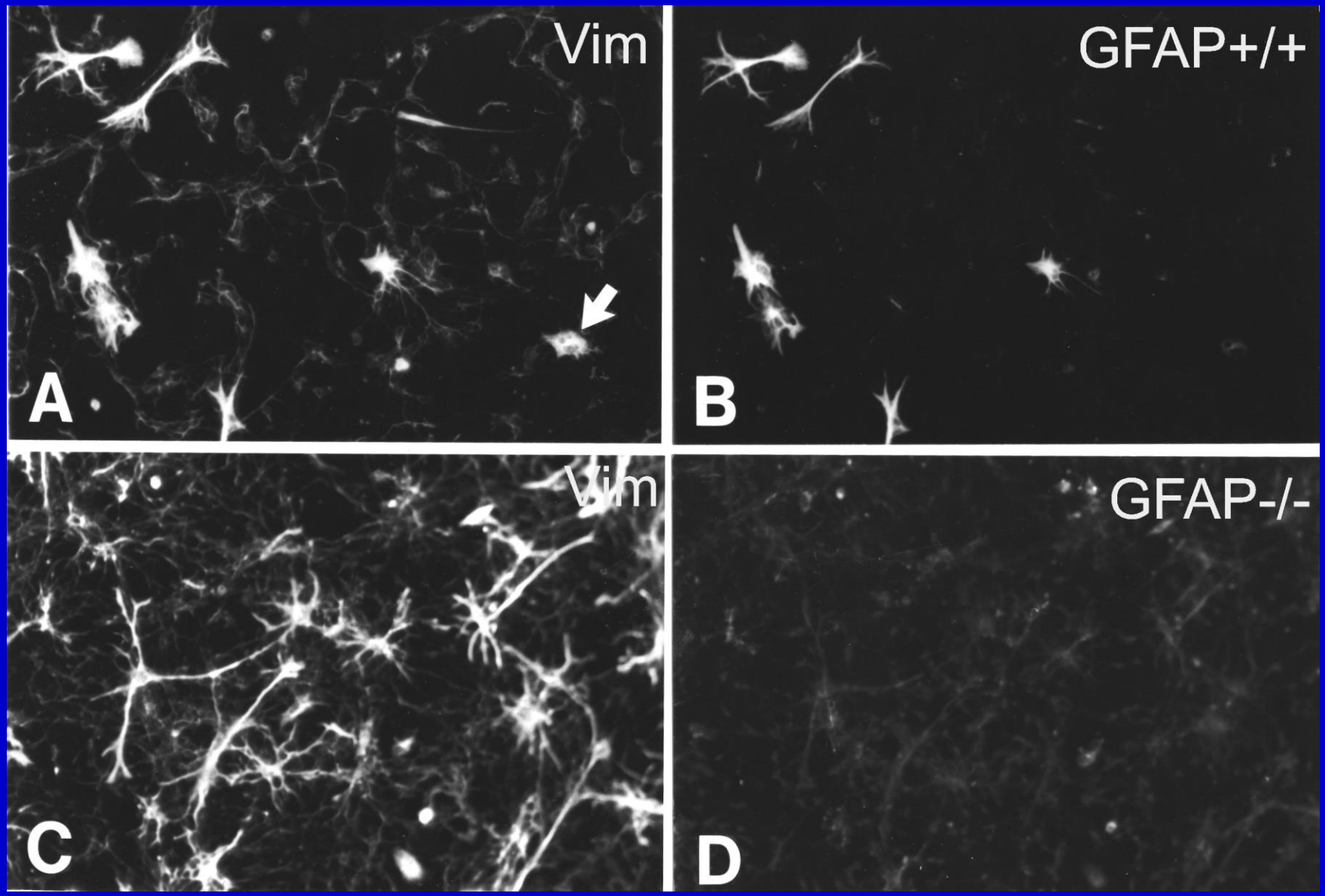




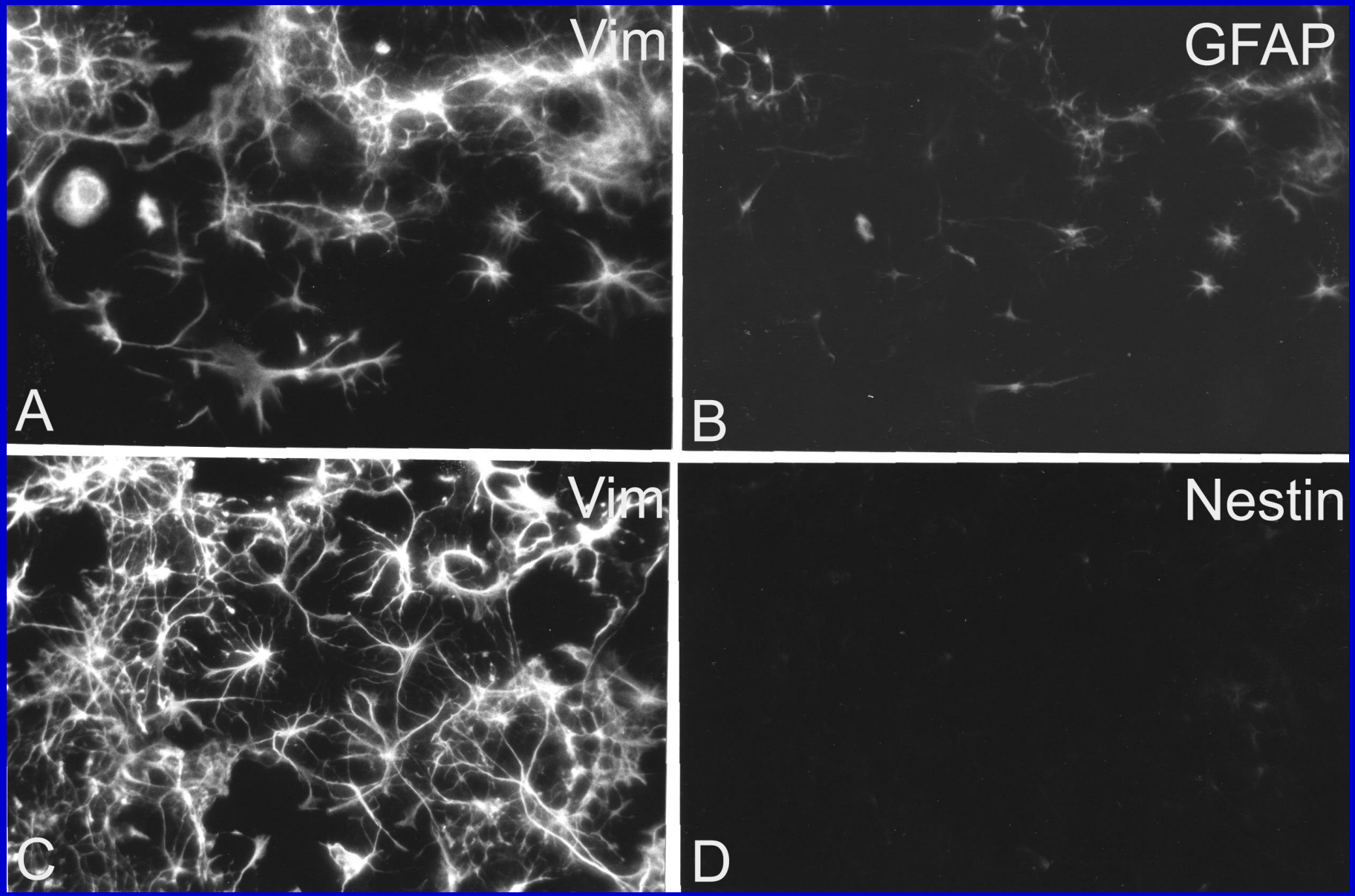
Primary culture of early postnatal cerebellar cells



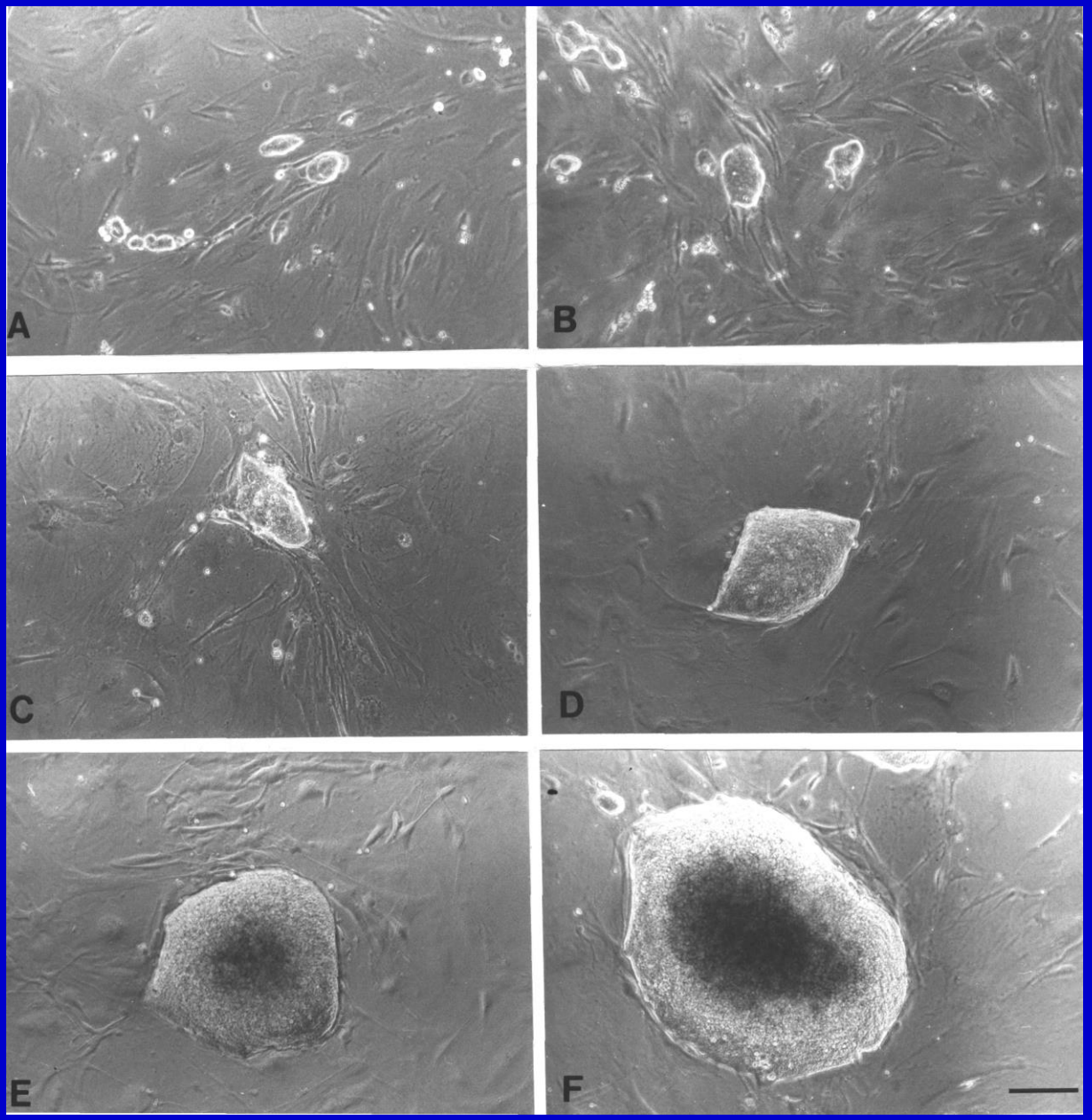
Primary culture of glial cells



Primary culture of glial cells



Neural Differentiation of Mouse Embryonic Stem Cells



Mouse R1 ES cells

plated on a feeder layer of primary mouse embryonic fibroblasts
(in DMEM with 20% FBS supplemented by 10 ng/ml recombinant human LIF)

↓

Embryoid body formation:
Cultivation of 5×10^4/ml ES cells in DMEM+20% FBS in petri dish for 4 days

↓

EBs plated and attached onto gelatin-coated cover-slide

↓

DMEM/F12 media supplemented with ITSF
(5 ug/ml Insulin, 50 ug/ml Transferrin, 30 nm sodium Selenite, 5 ug/ml Fibronectin)

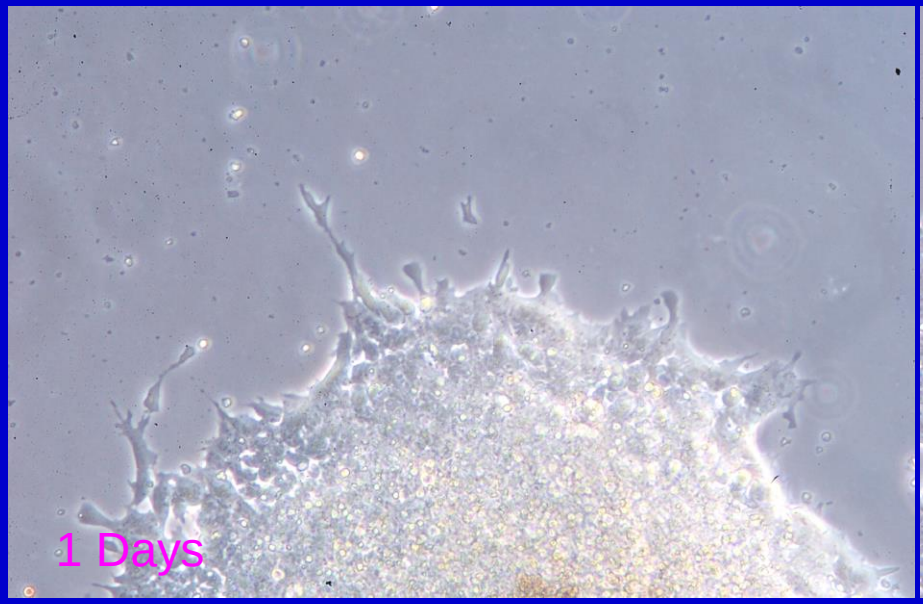
↓

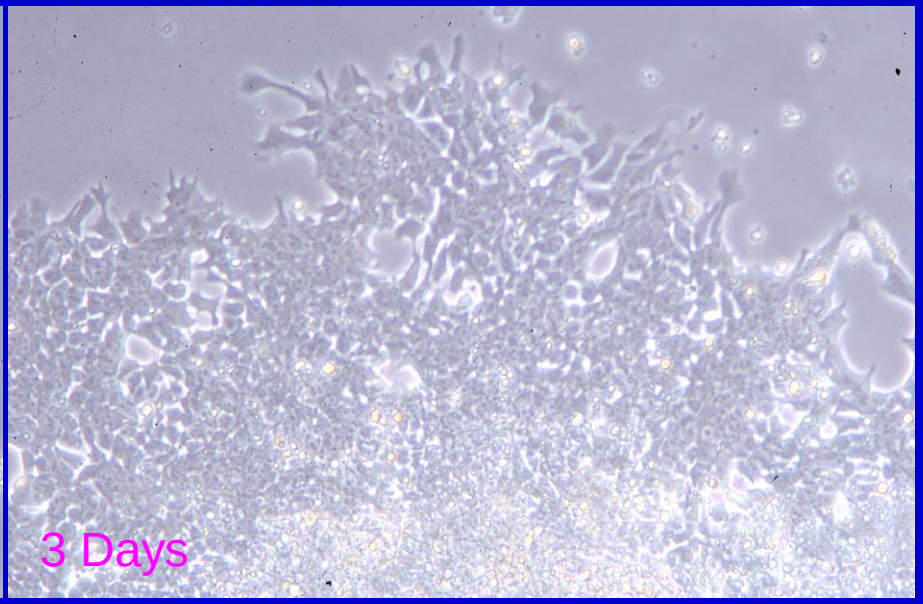
After 7 days, media changed into DMEM/F12 supplemented with N2 (bFGF)

↓

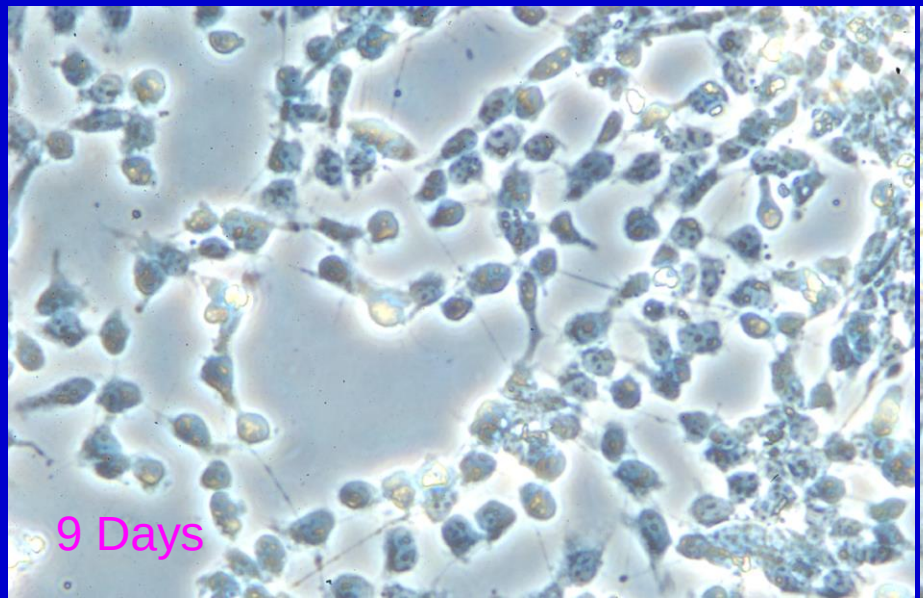
After 7 days, media changed into DMEM/F12 supplemented with N2

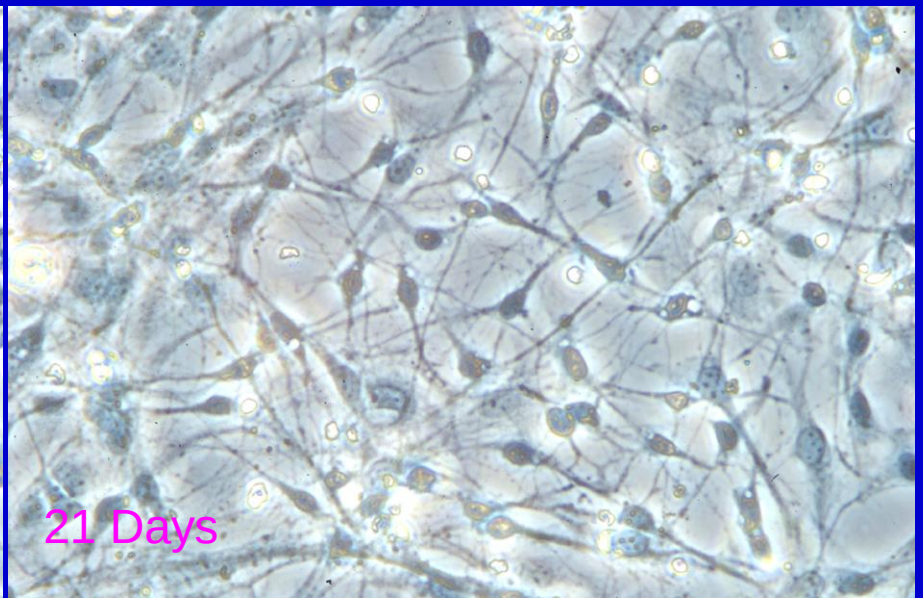
Embryoid body in DMEM/F12 media supplemented with ITSF



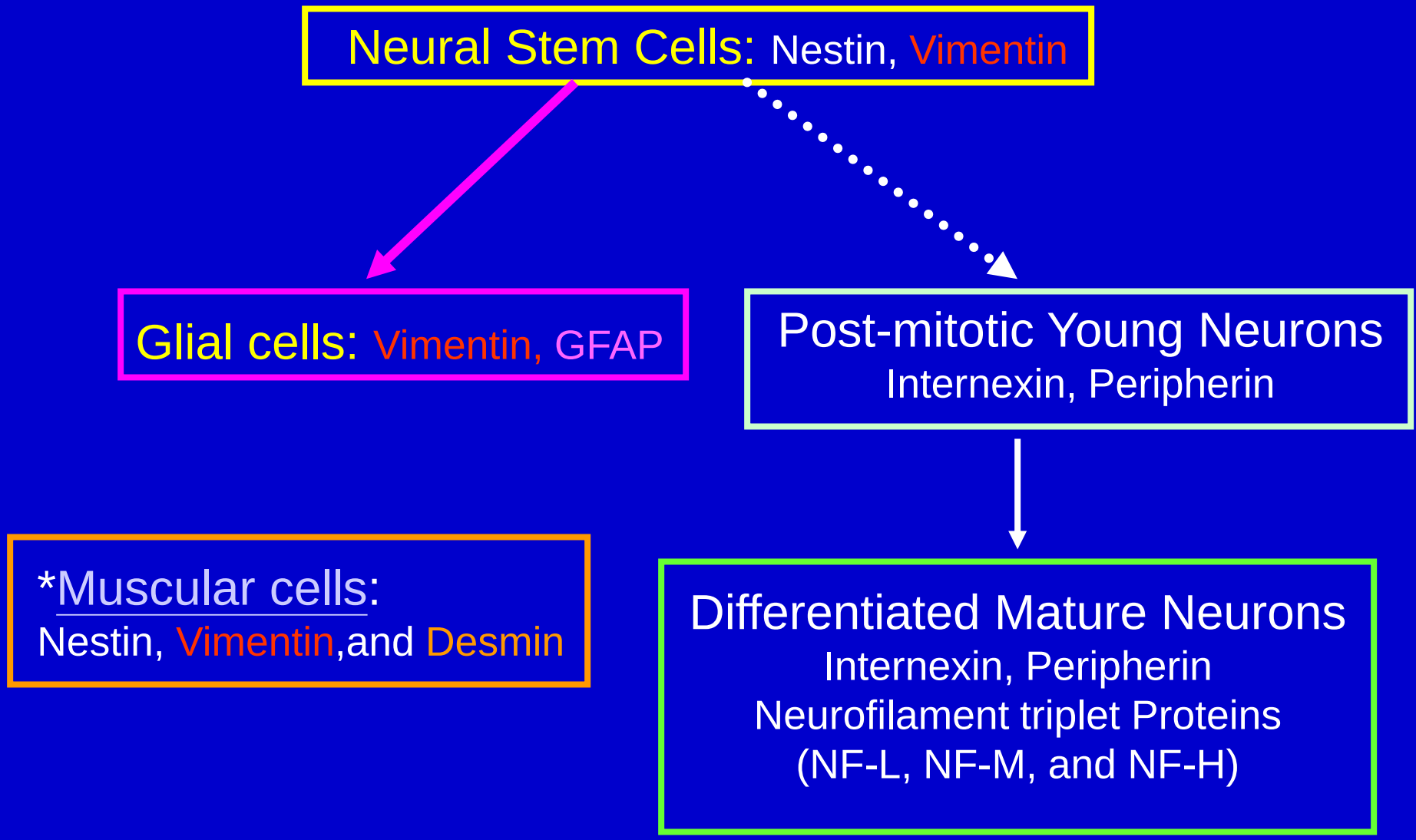


Differentiating cells after neural induction with N2 supplement

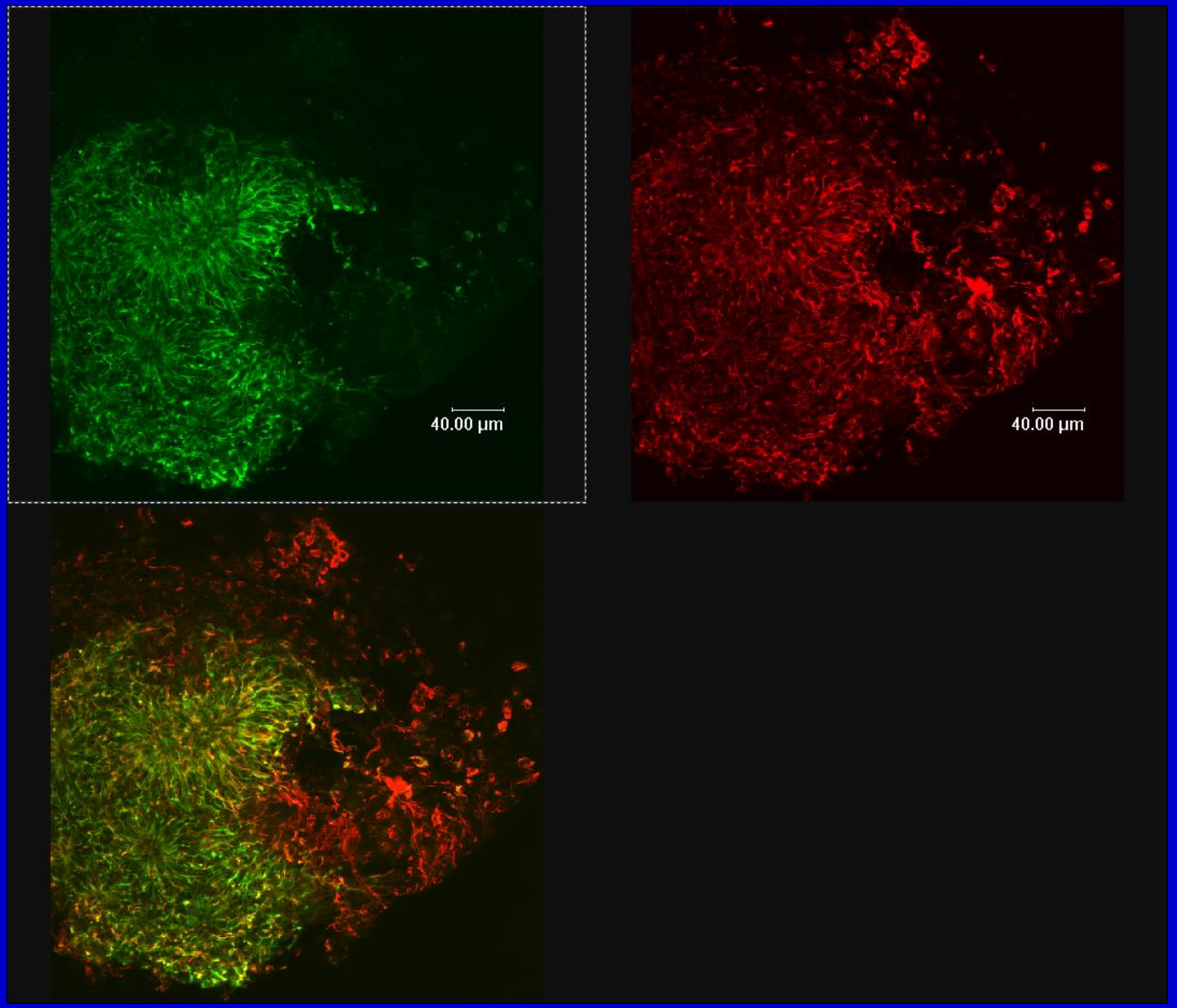




Intermediate Filament Proteins are good markers for determining the differentiation status of neural stem cells

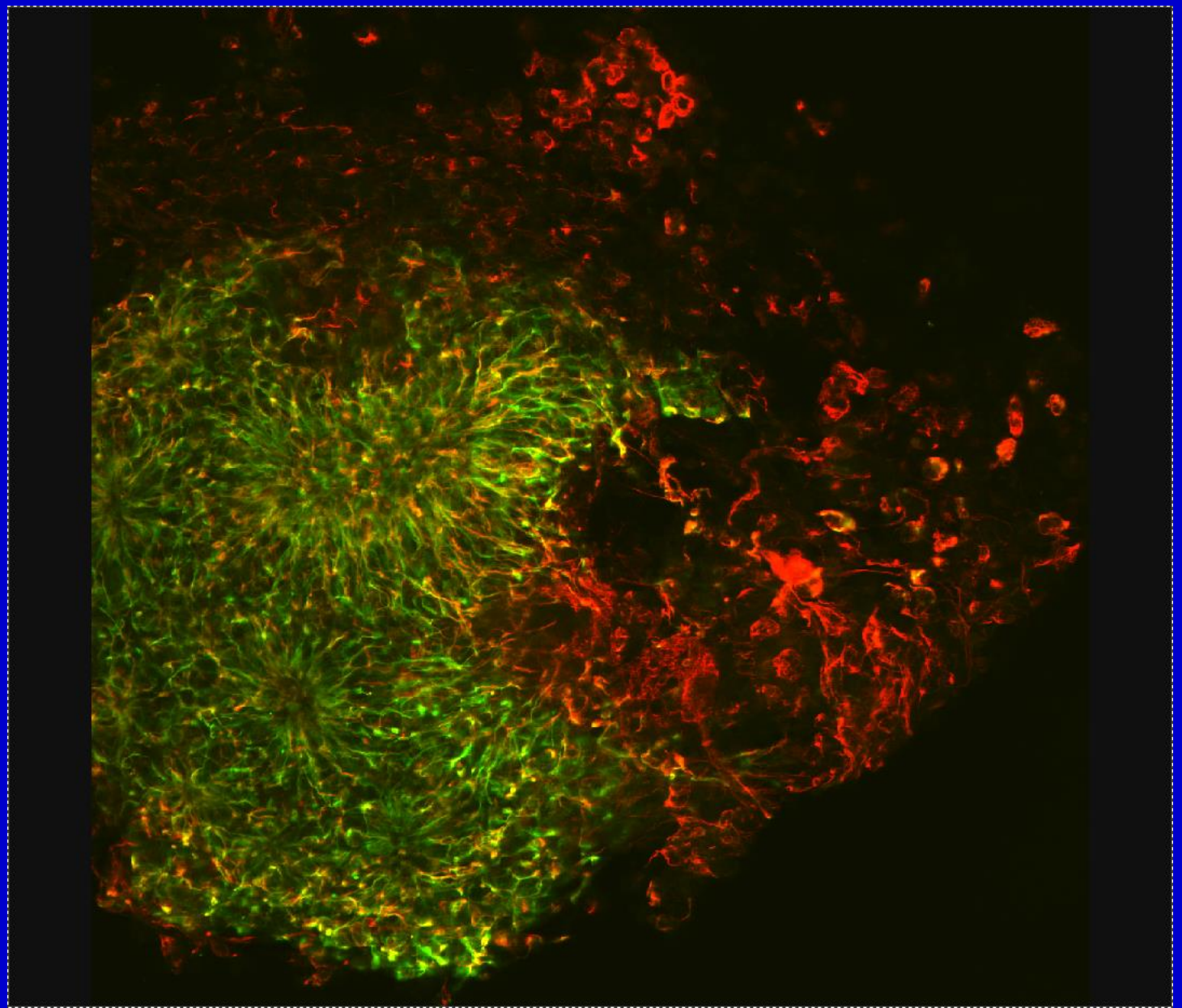


Embryoid body in DMEM/F12 media supplemented with ITSF for 7 days



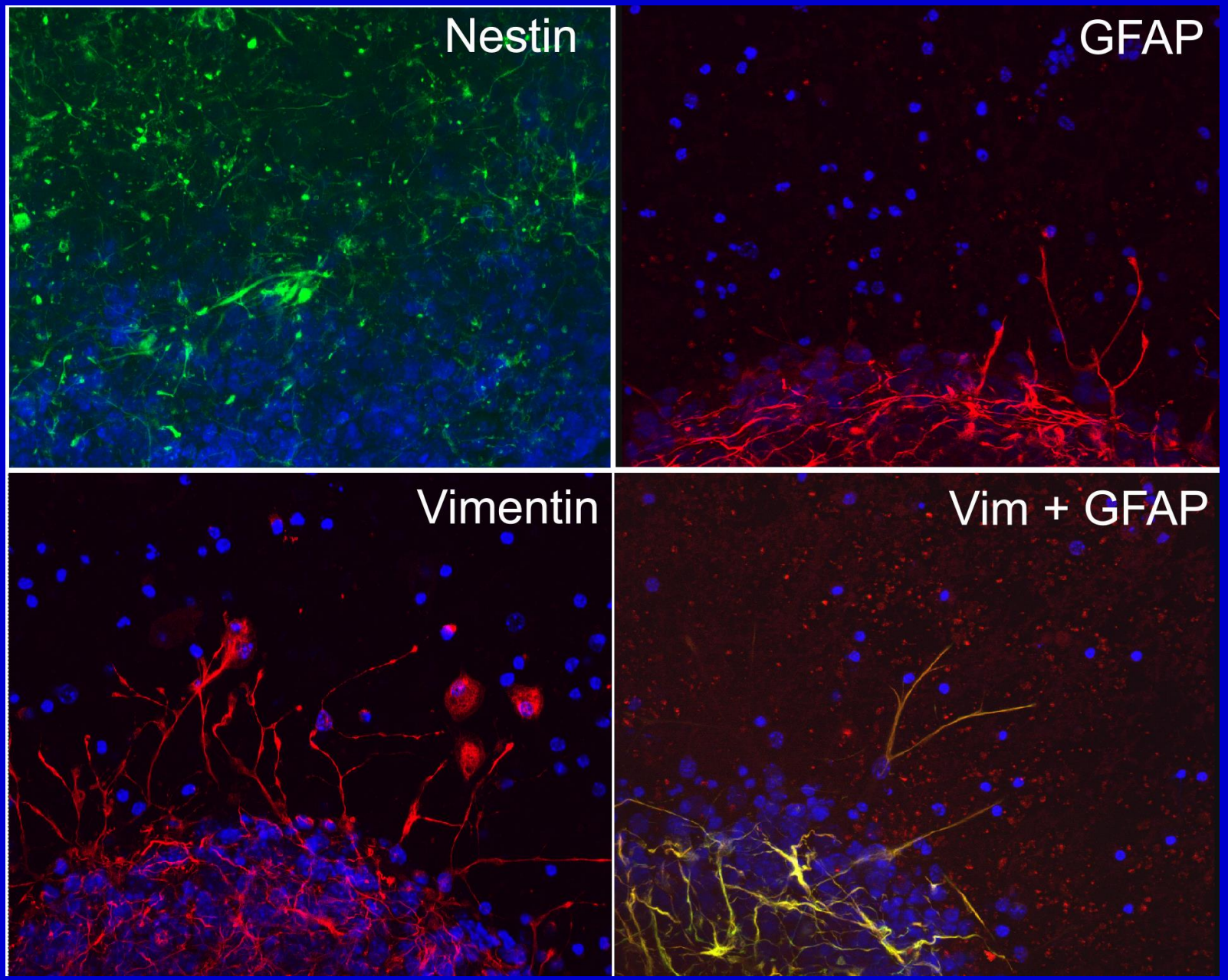
Green: Nestin
Red: Vimentin

Embryoid body in DMEM/F12 media supplemented with ITSF for 7 days

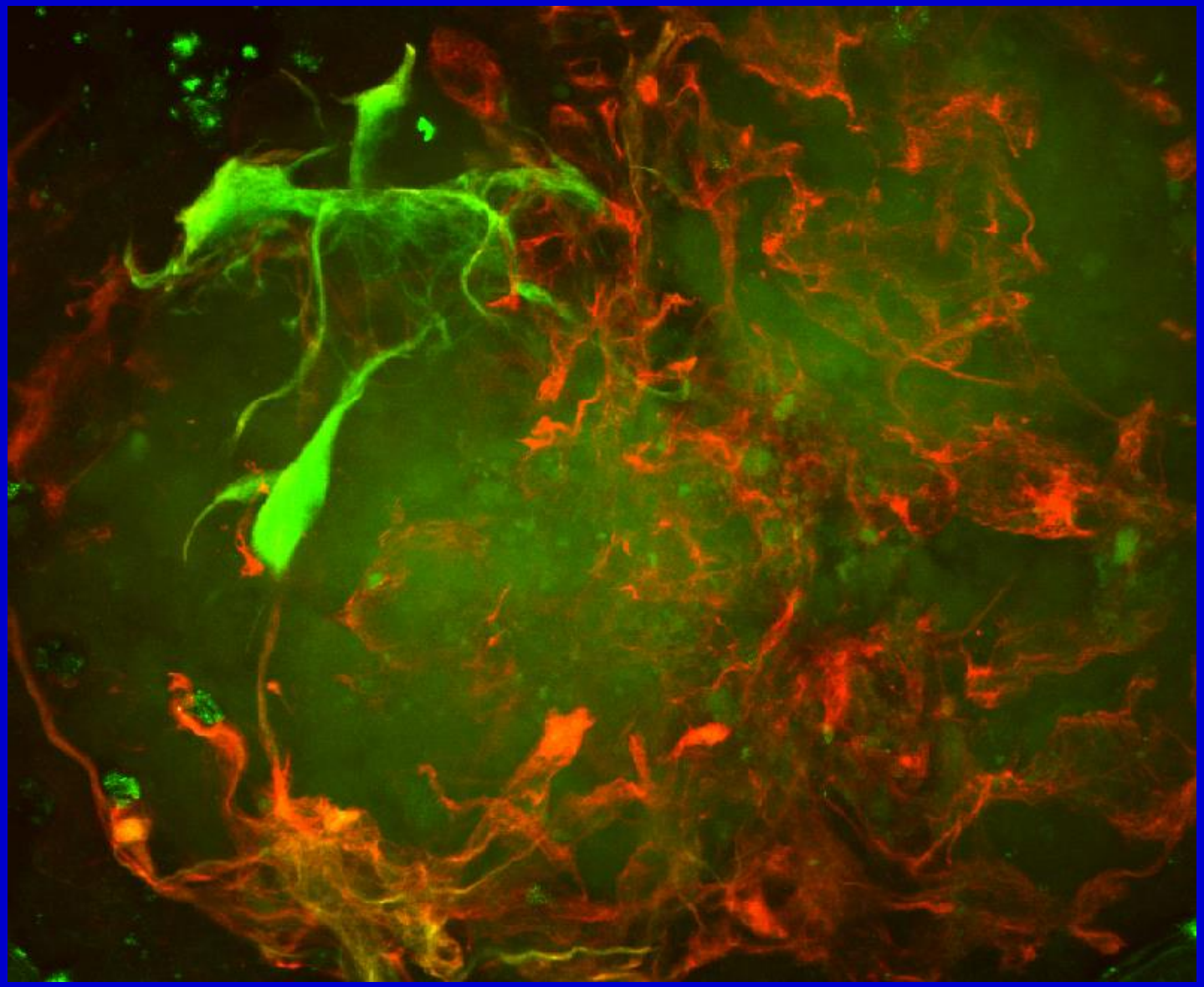


Green: Nestin
Red: Vimentin

Embryoid body in DMEM/F12 media supplemented with ITSF for 7 days

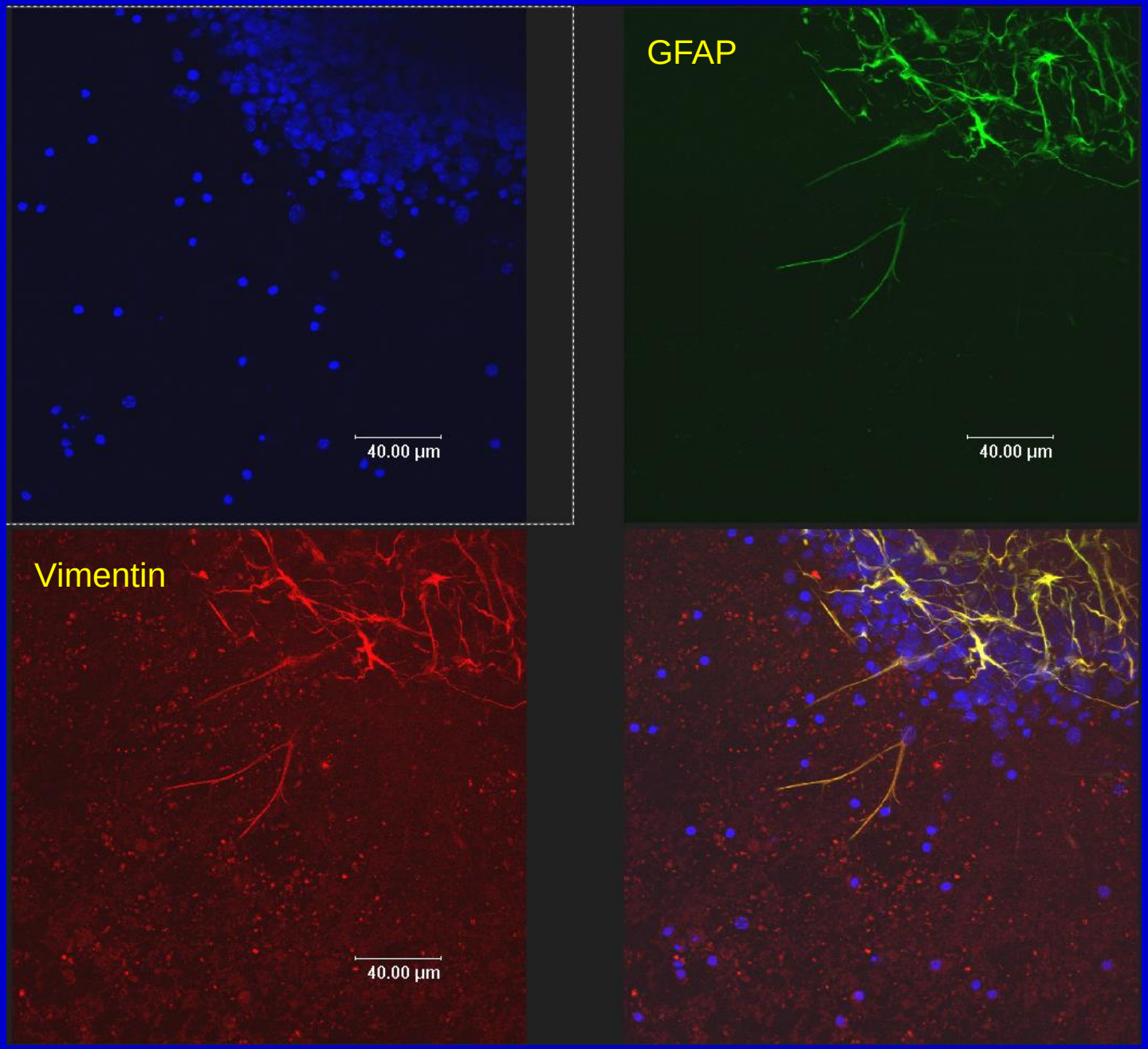


Embryoid body in DMEM/F12 media supplemented with ITSF for 10 days

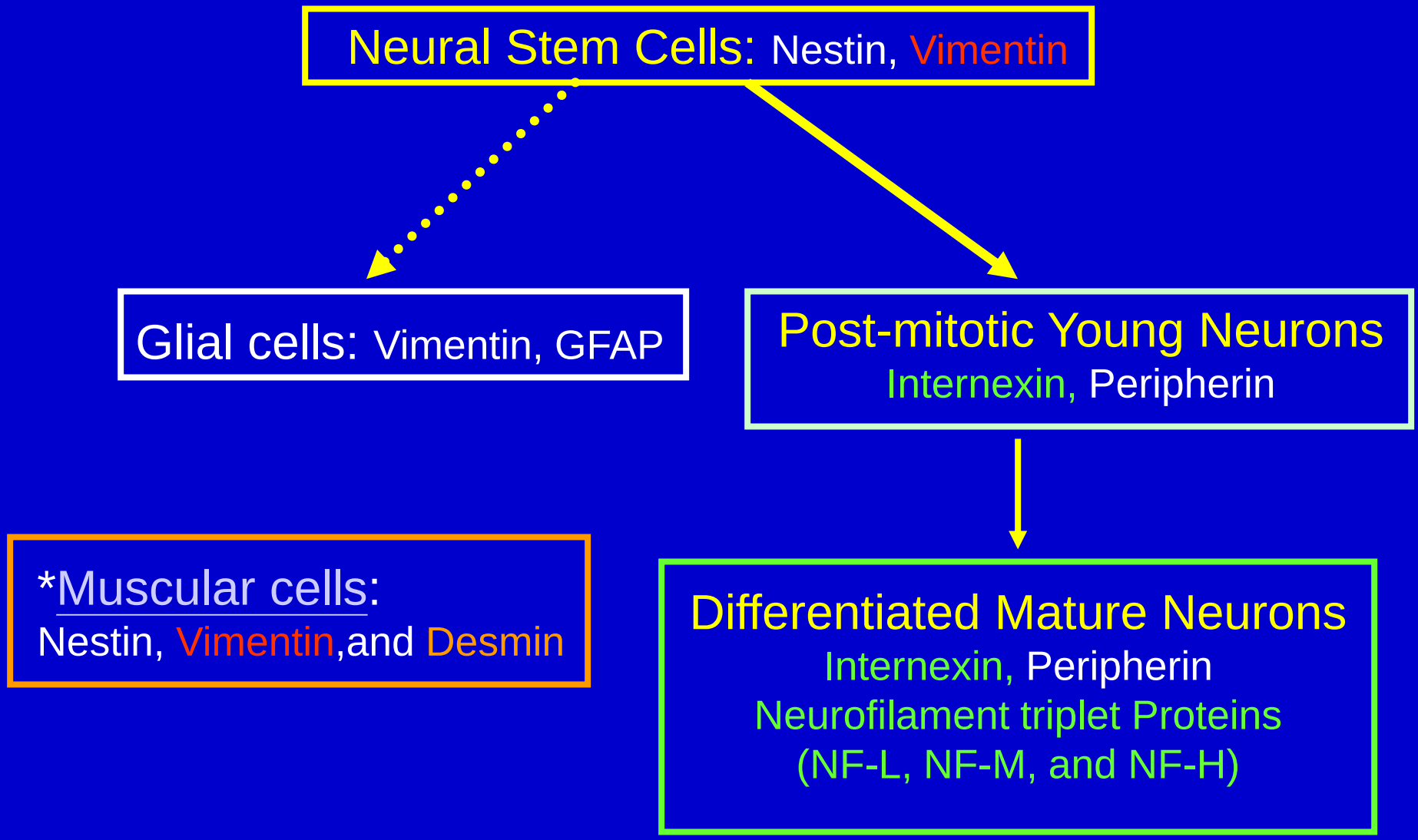


Green: GFAP
Red: Vimentin

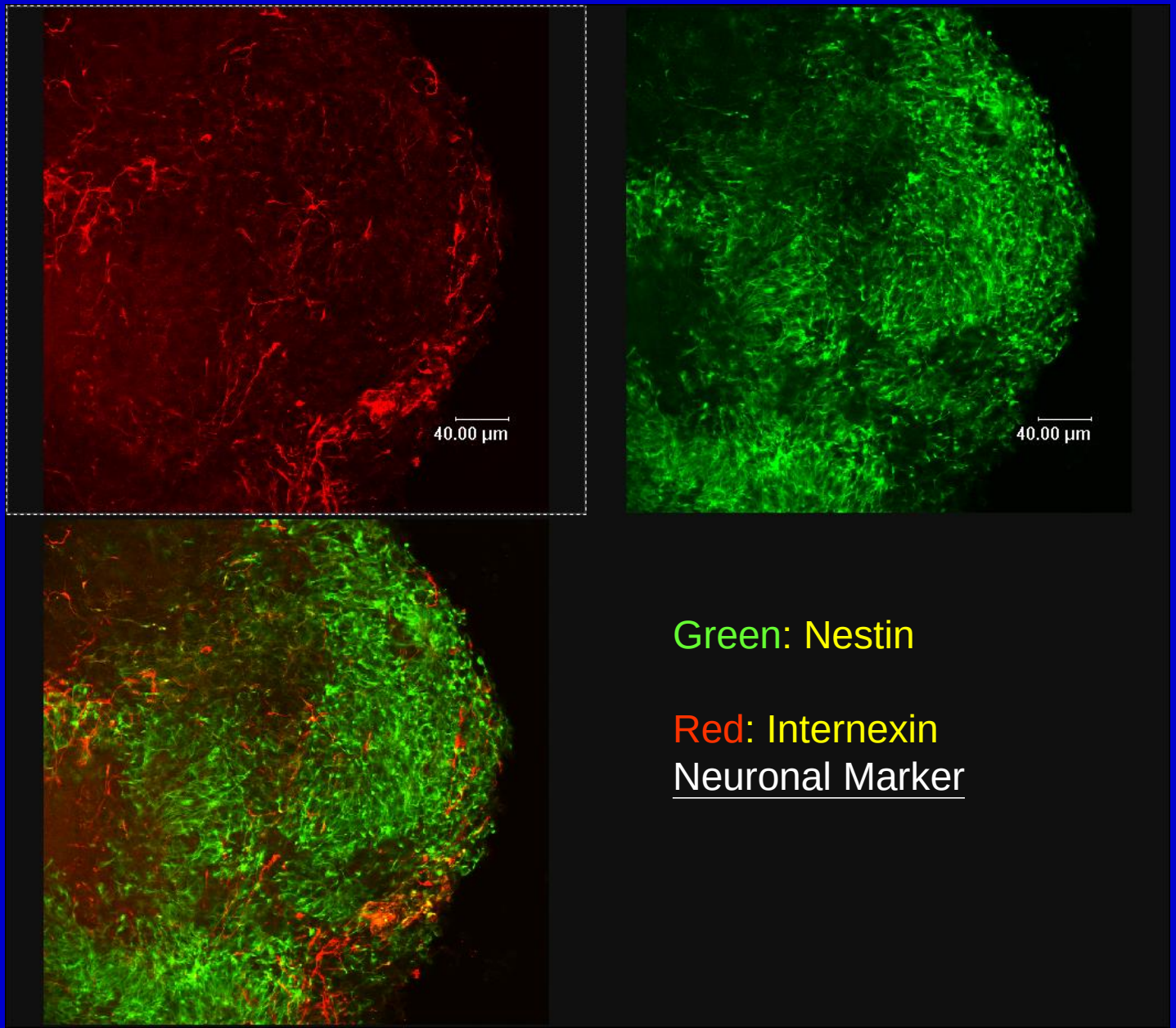
GFAP and Vimentin co-expressed in the differentiating glial cells (17days)



Intermediate Filament Proteins are good markers for determining the differentiation status of neural stem cells



Neural induction of embryoid body for 7 days

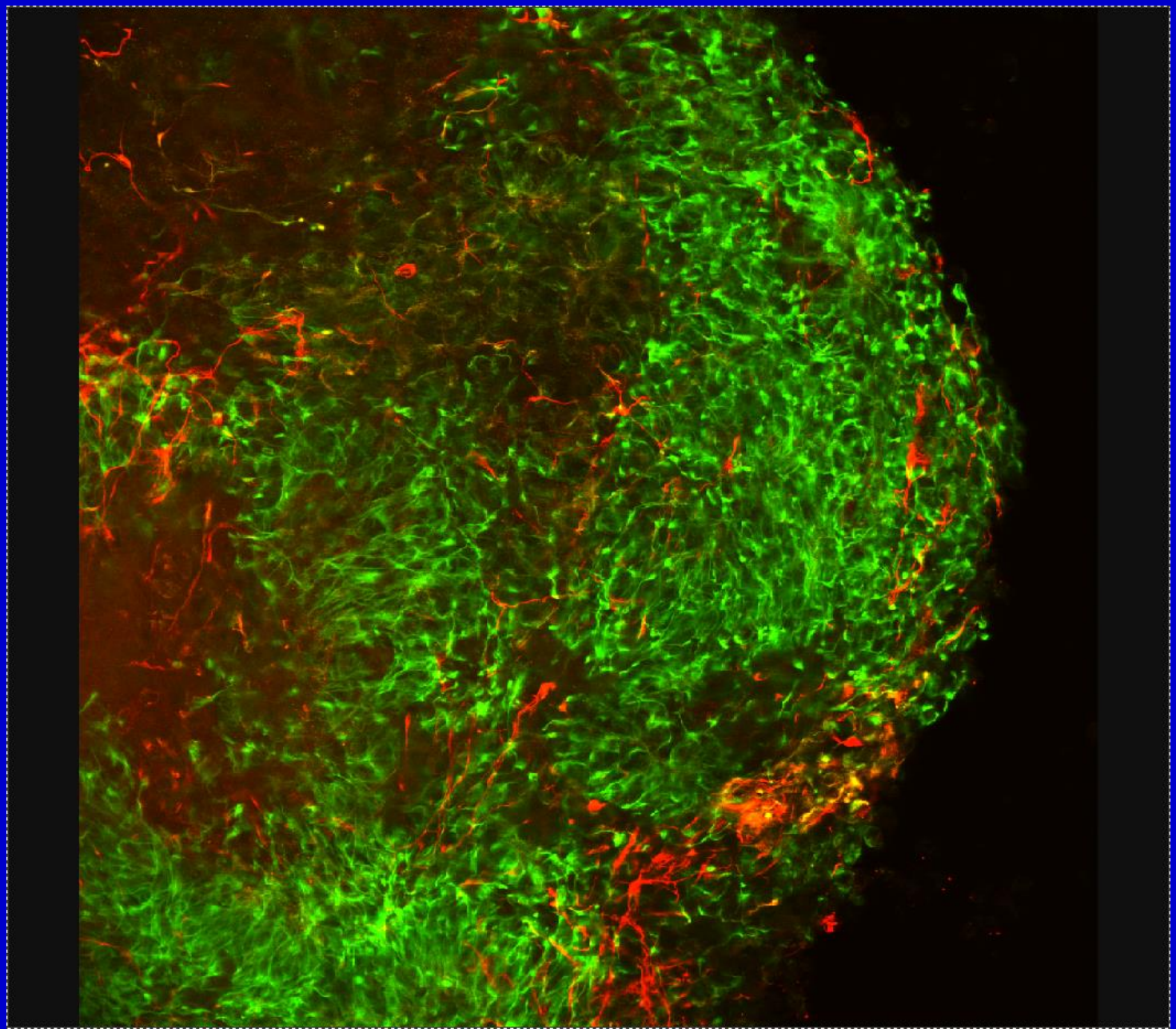


Green: Nestin

Red: Internexin

Neuronal Marker

Neural induction of embryoid body for 7 days

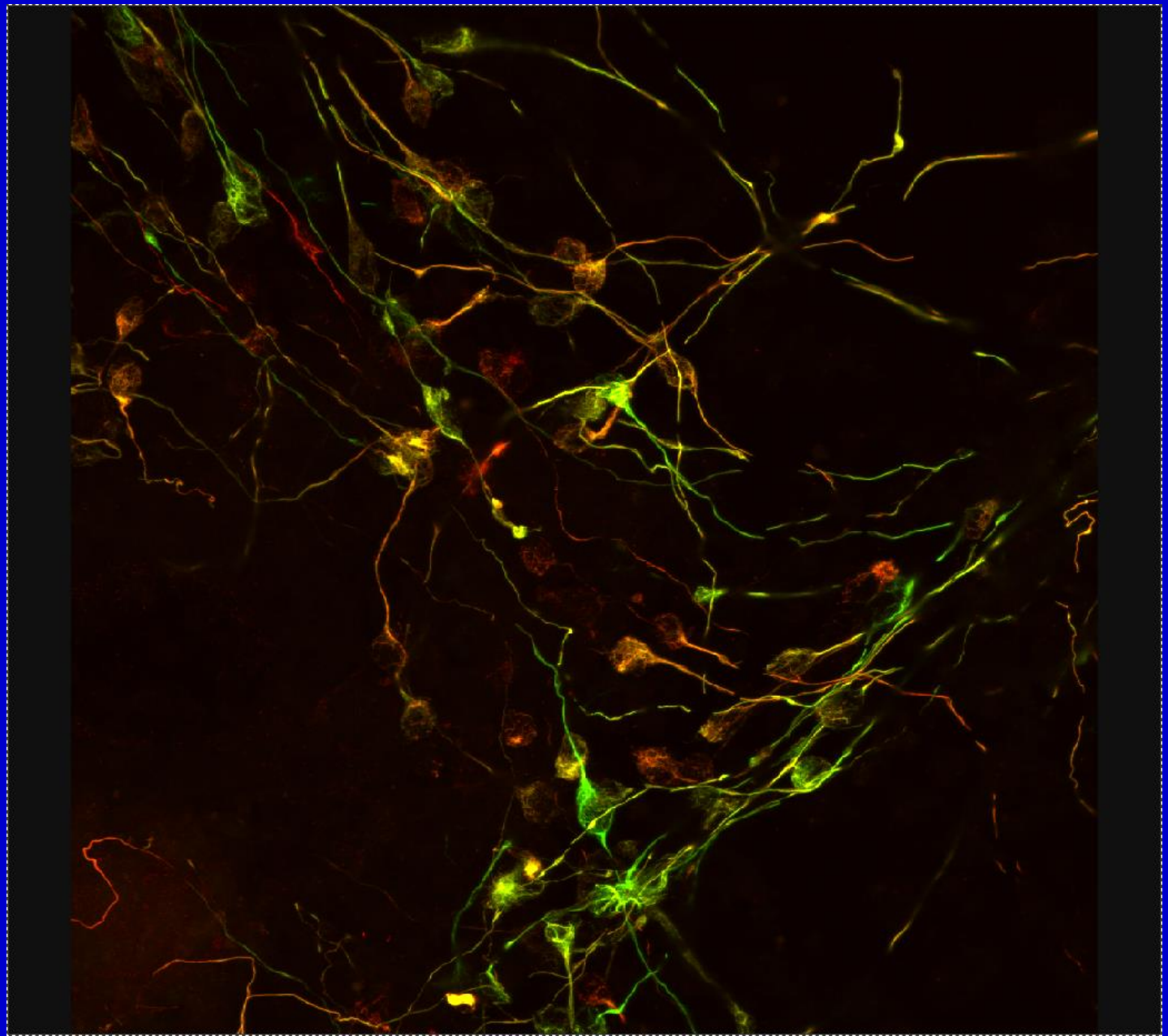


Green: Nestin

Red: Internexin

Neuronal Marker

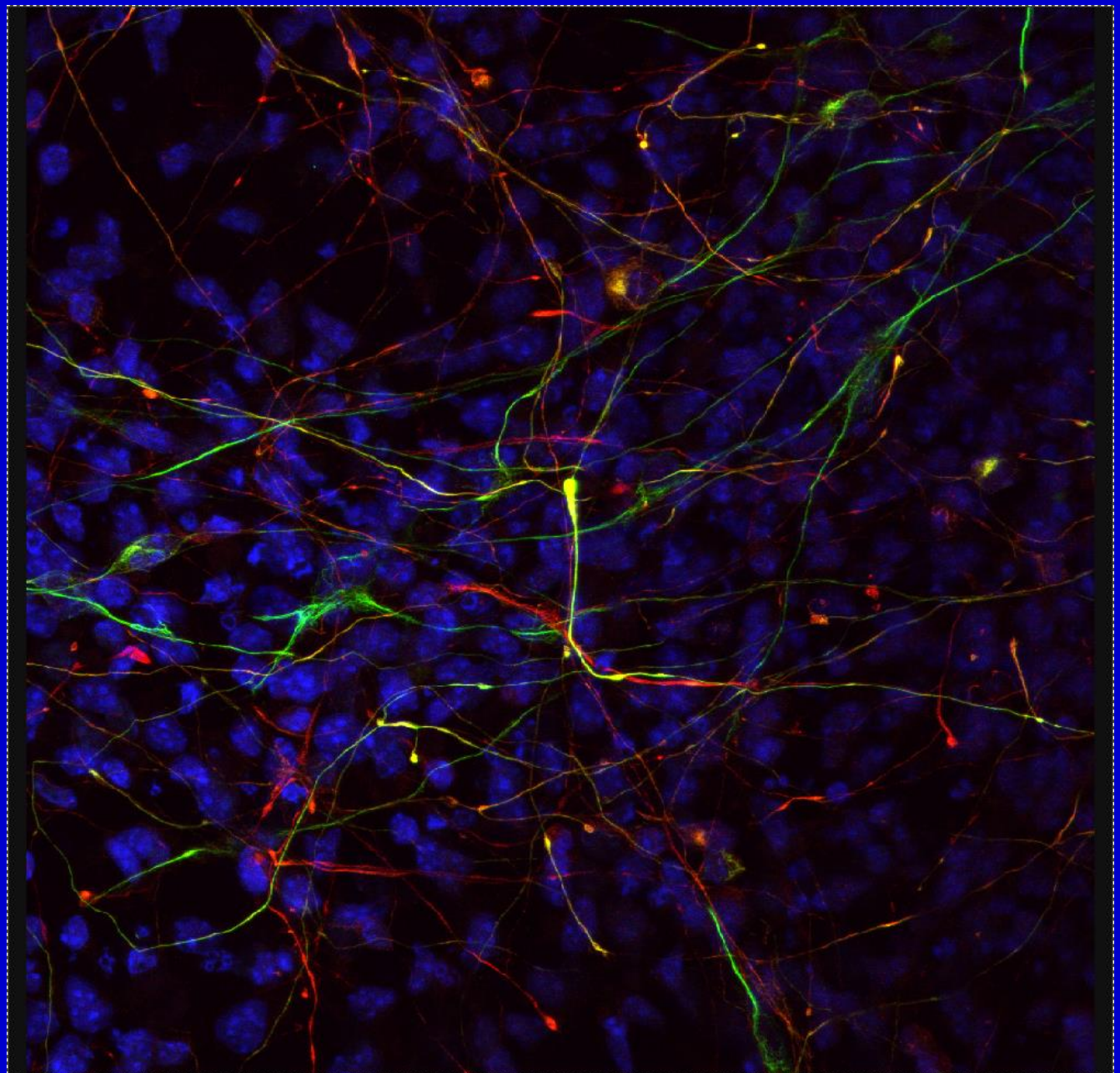
Neural induction of embryoid body for 7 days



Green: NF-L
Red: Internexin

Neuronal Markers

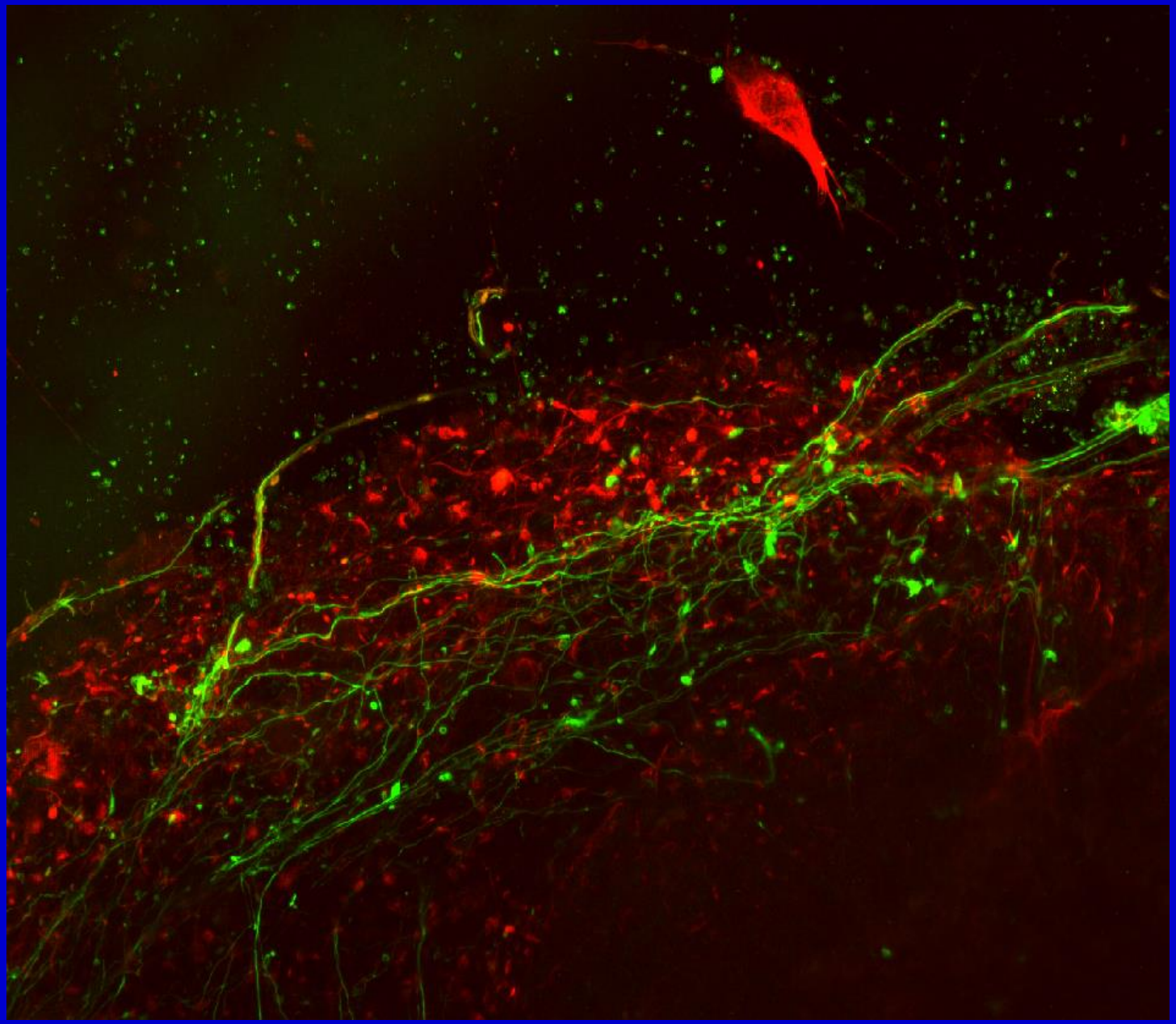
Neural induction of embryoid body for 13 days



Green: NF-L
Red: Internexin

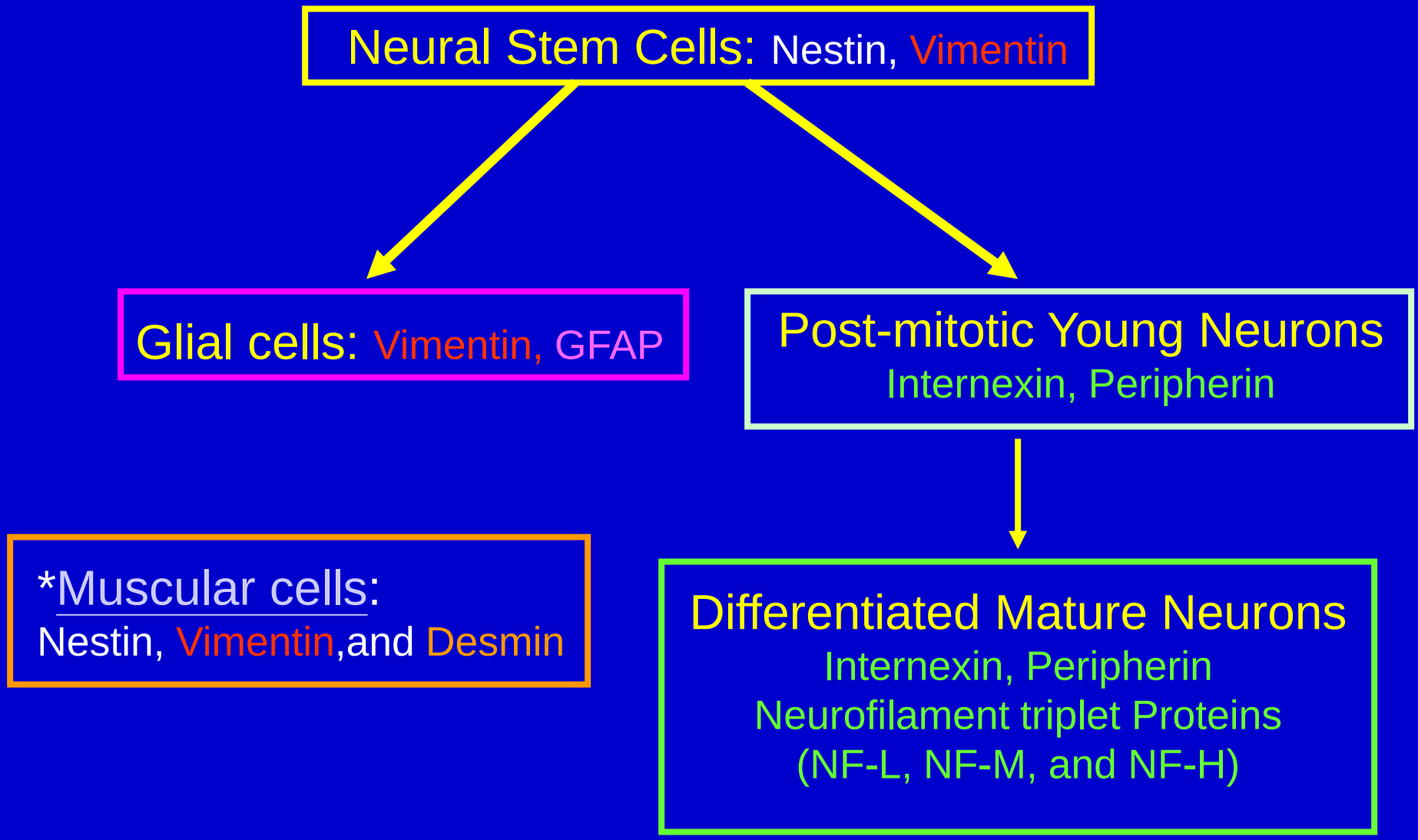
Neuronal Markers

Neural induction of embryoid body for 13 days



Green: NF-M
Red: Vimentin

Intermediate Filament Proteins are good markers for determining the differentiation status of neural stem cells



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MAP2A and Tau

Figure 16–32. Molecular Biology of the Cell, 4th Edition.

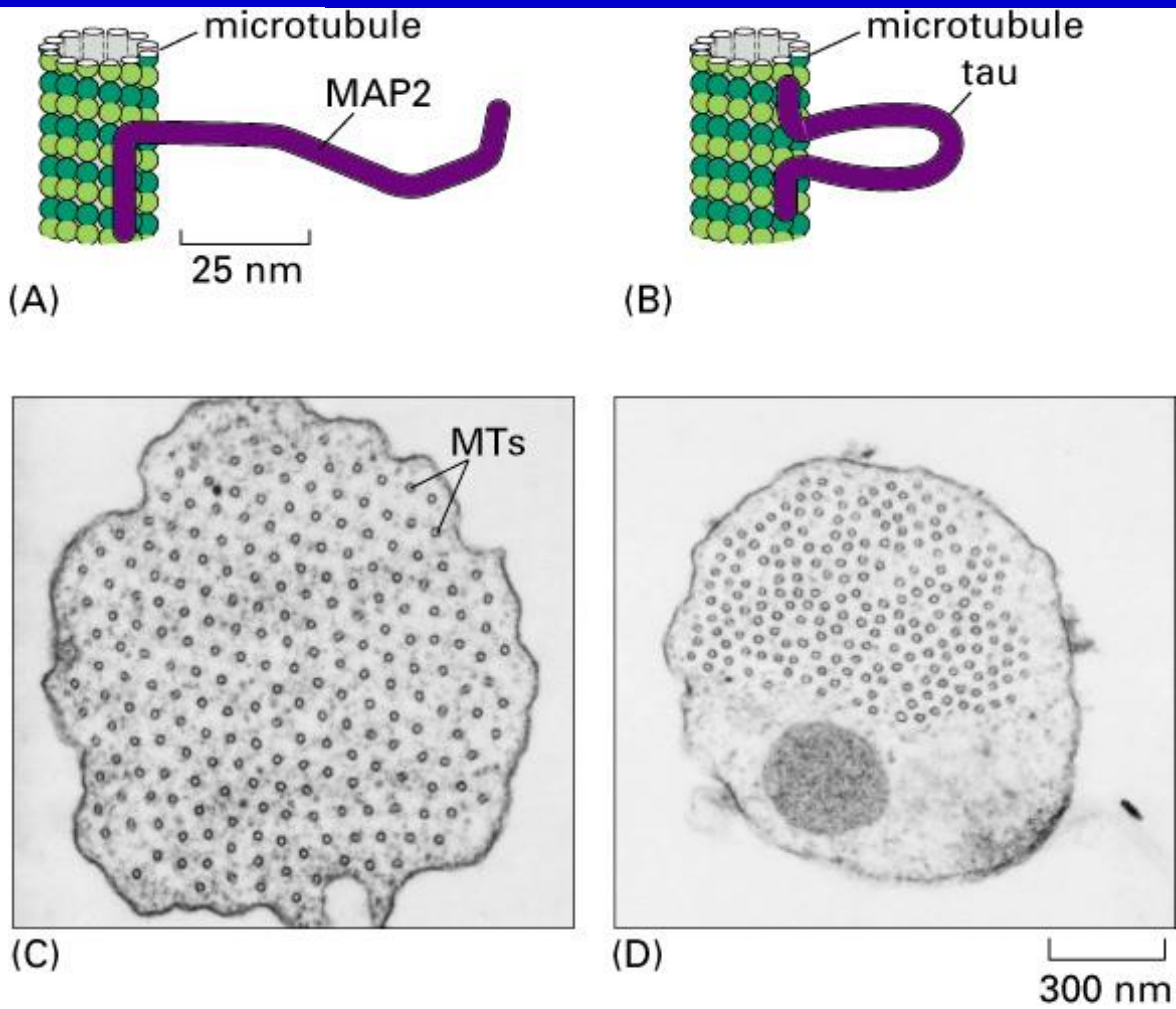
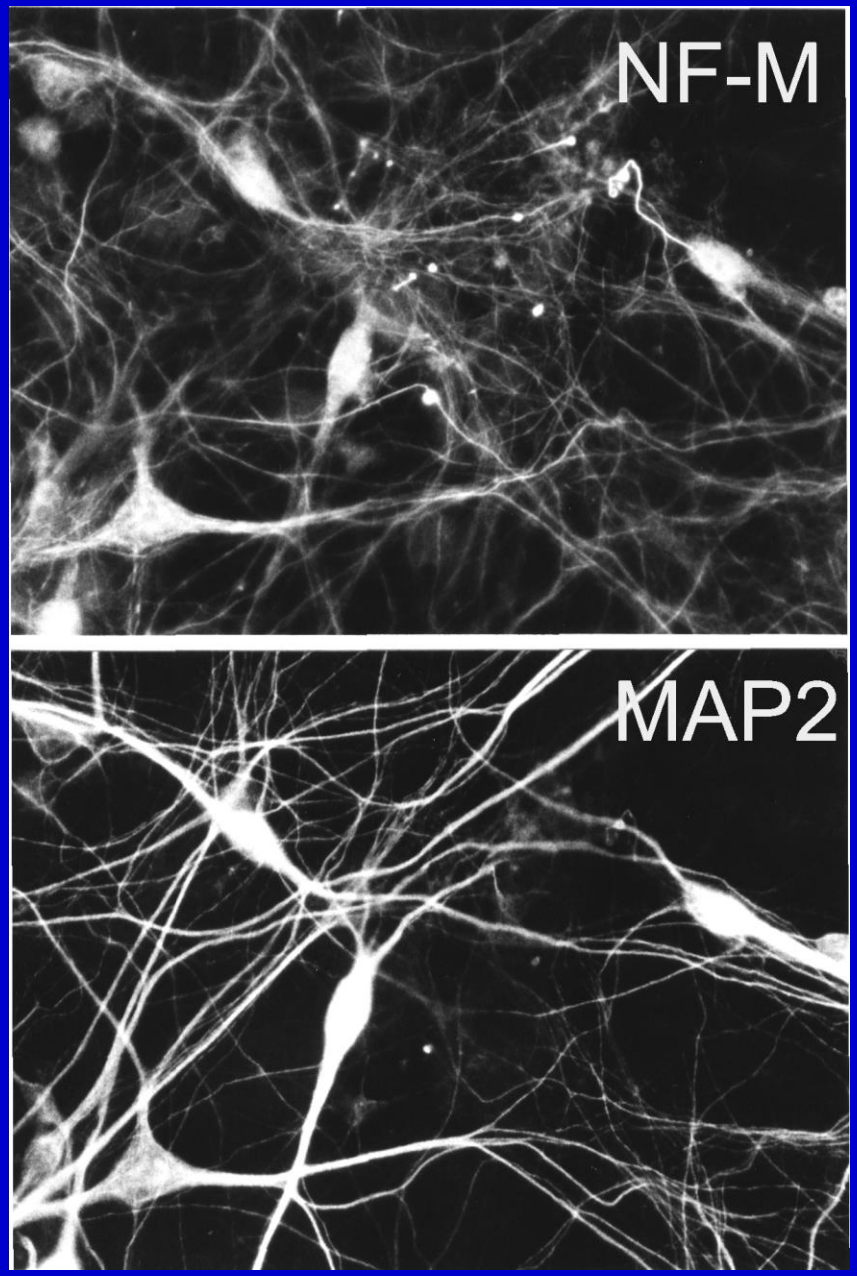
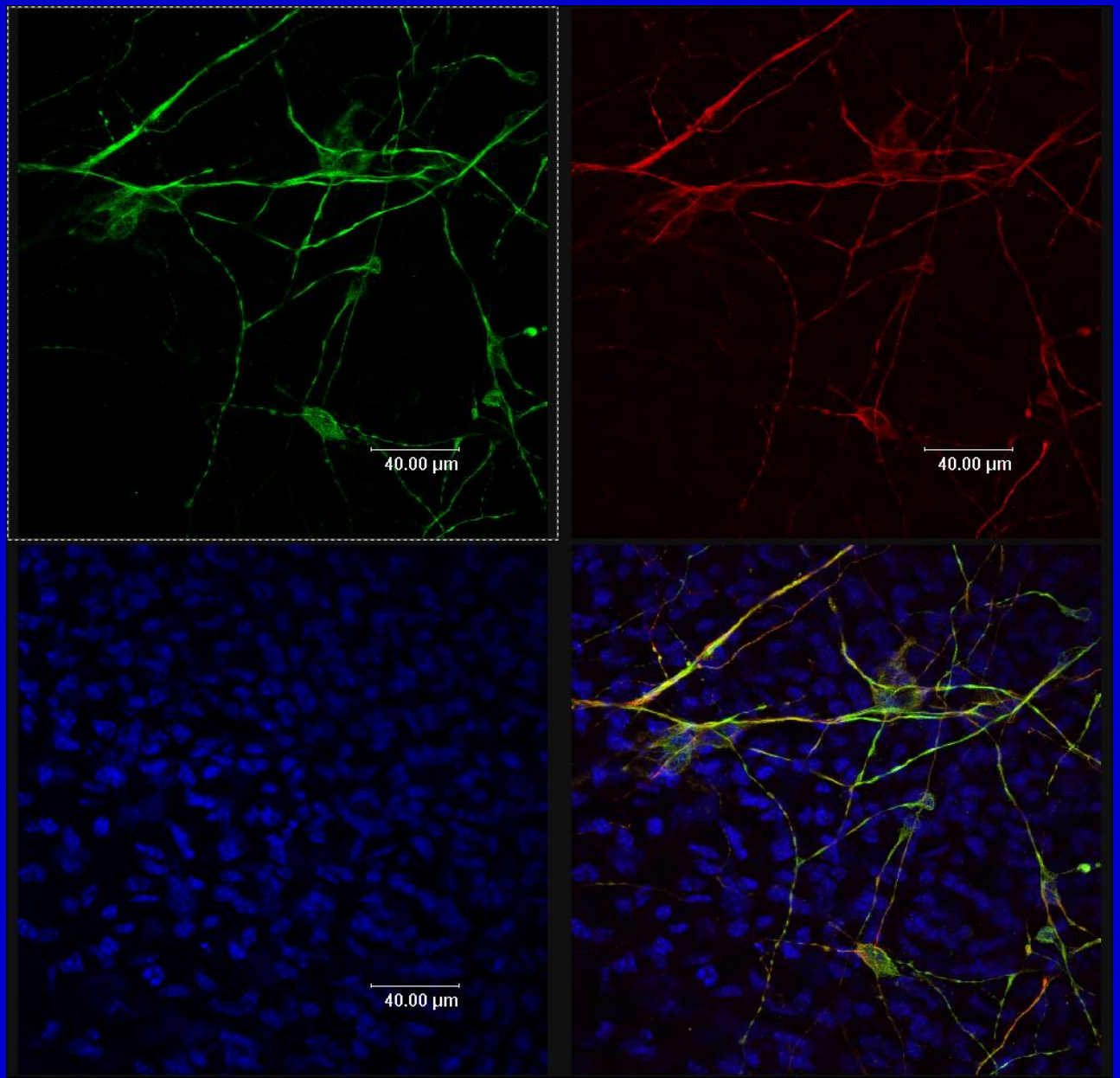


Figure 16–33. Molecular Biology of the Cell, 4th Edition.

Primary culture of embryonic hippocampal Neurons



Neuronal differentiation from Embryoid Body in DMEM/F12 media supplemented with N2 for 9 days

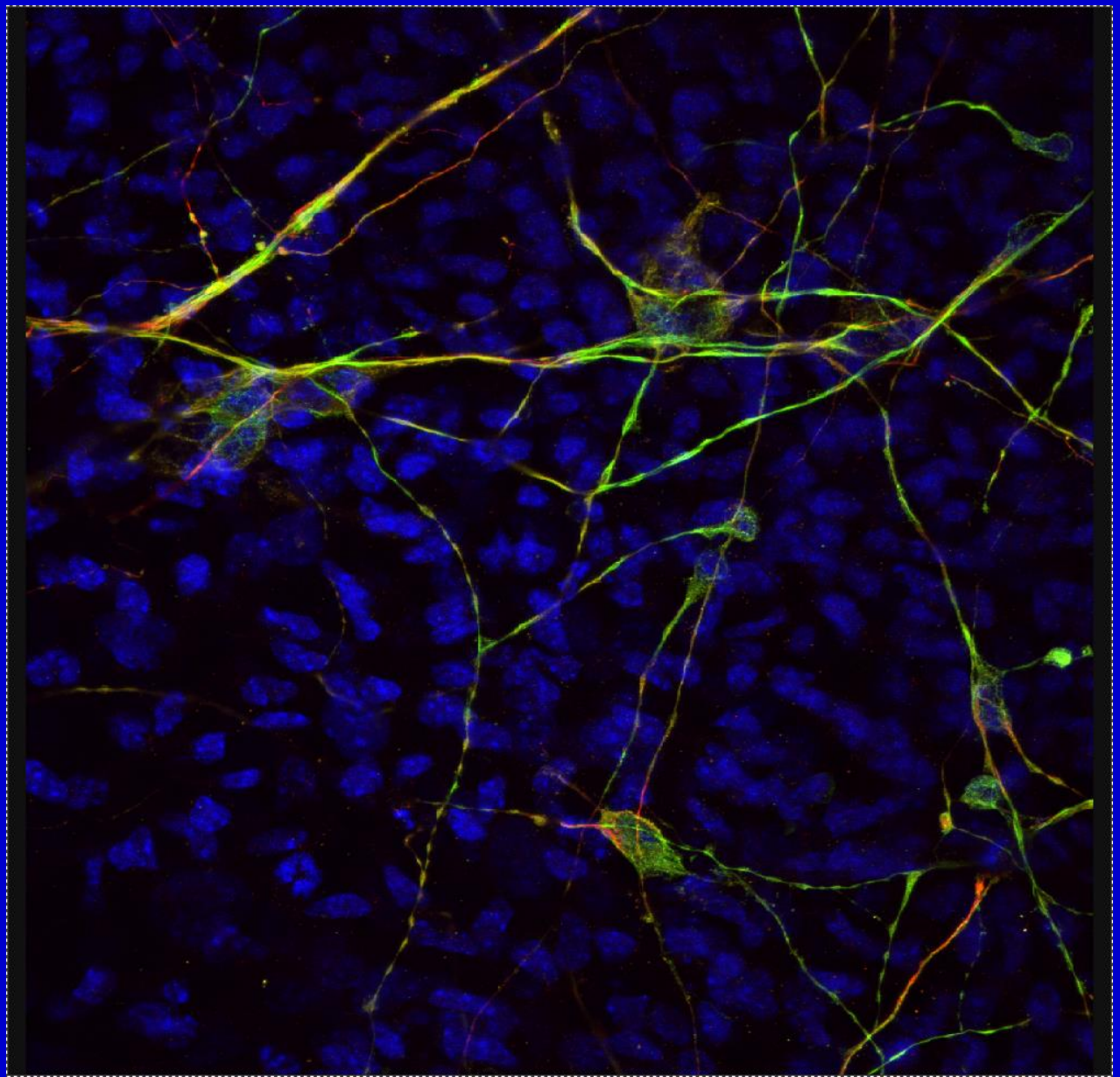


Neuronal Markers

Green:
Tubulin β III (Tu J)

Red: Internexin

Neuronal differentiation from Embryoid Body in DMEM/F12 media supplemented with N2 for 9 days



Neuronal Markers

Green:
Tubulin β III (Tu J)

Red: Internexin

Embryoid body in DMEM/F12 media supplemented with N2 for 5 days
Neuronal marker Tubulin β III (Tu J) and GFAP