Animal and Cellular Models for the Neuronal Degeneration

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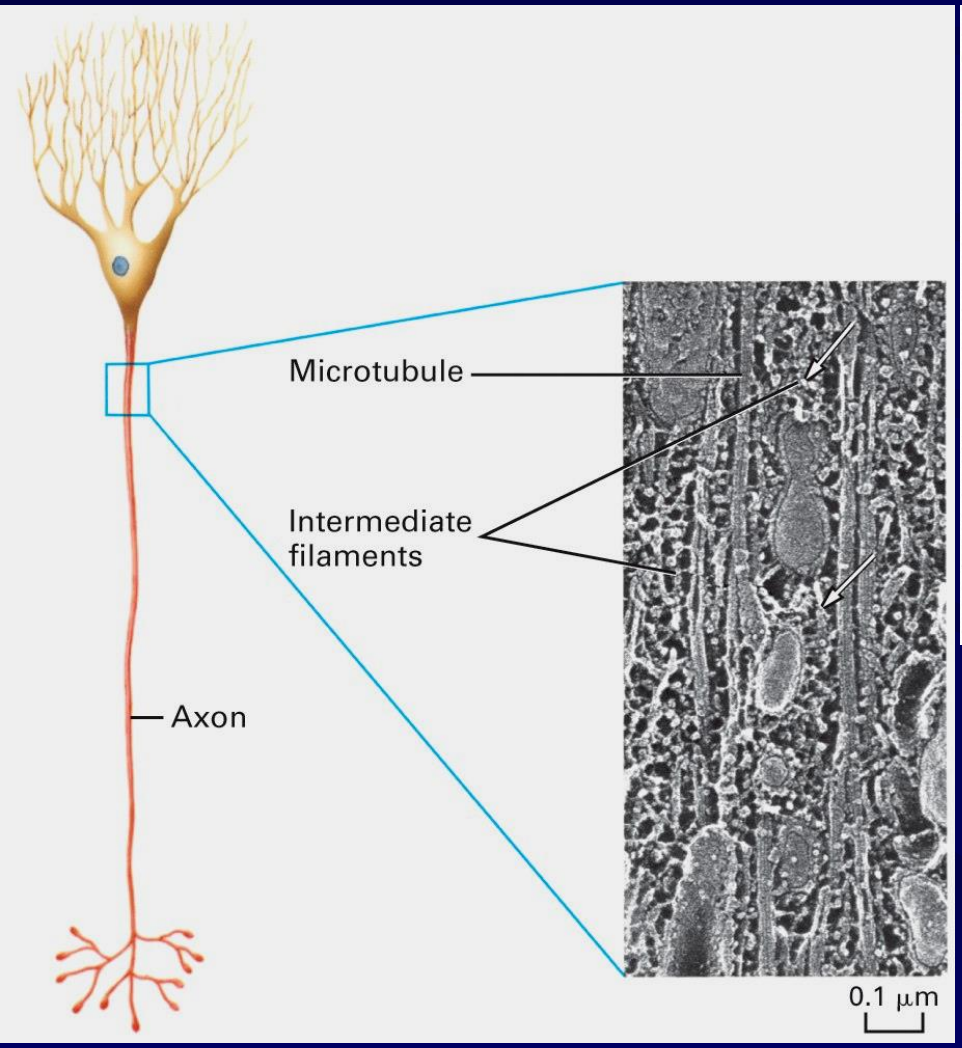
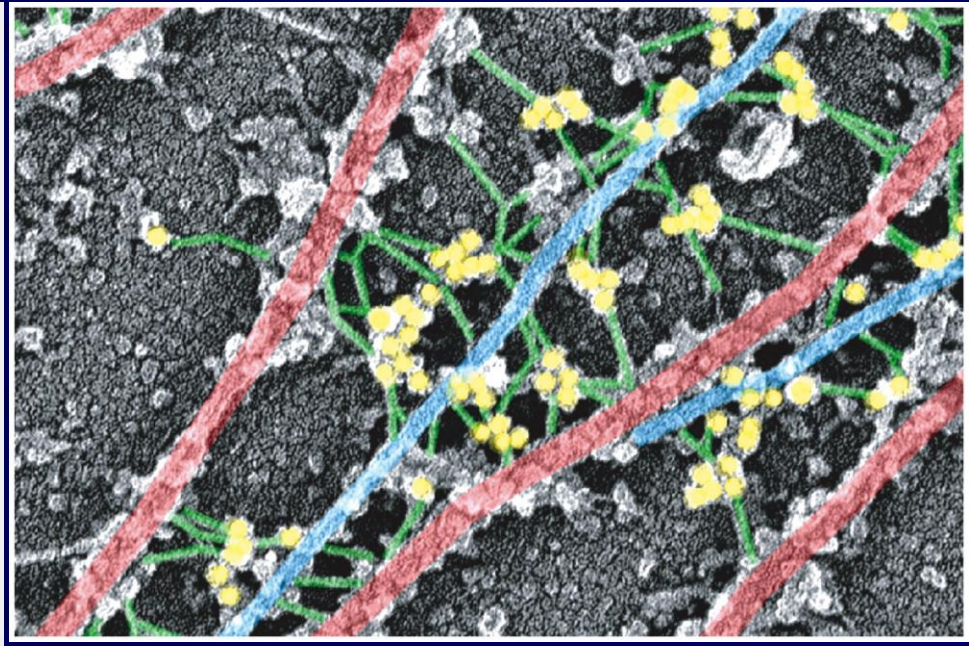
Neuronal Cytoskeletons



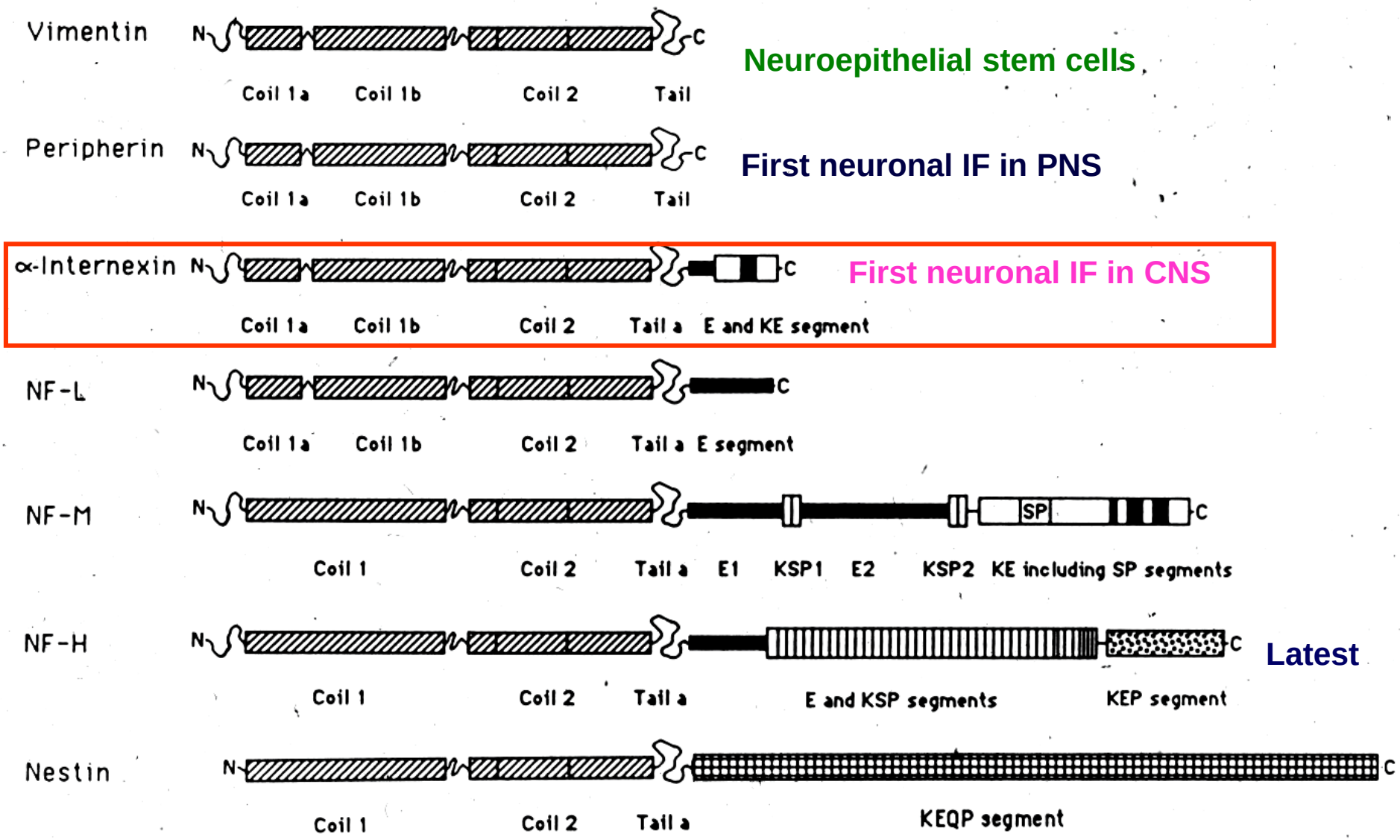
Microtubule



Intermediate filament:
Neurofilaments

Plakin family:
cytoskeleton linker proteins

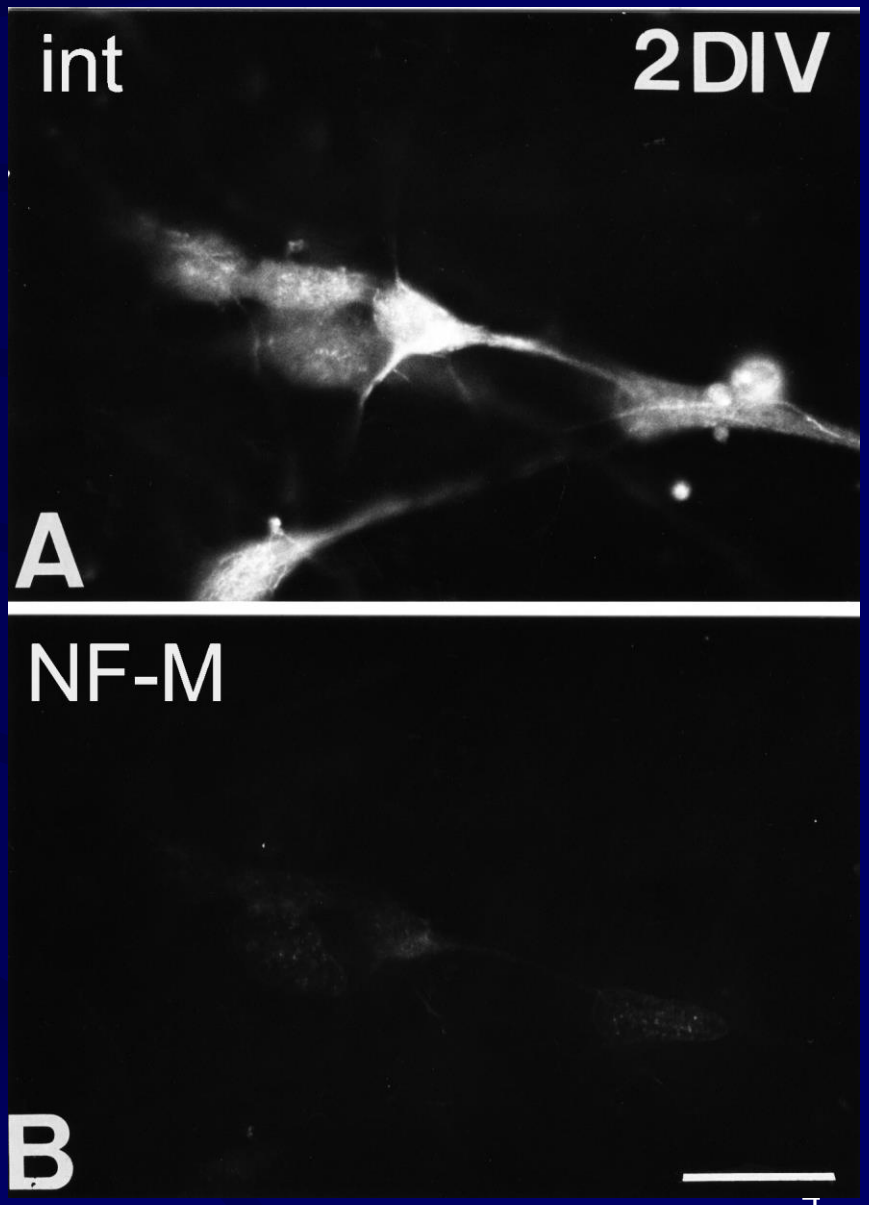
Seven Intermediate Filament Proteins in Neural Differentiation



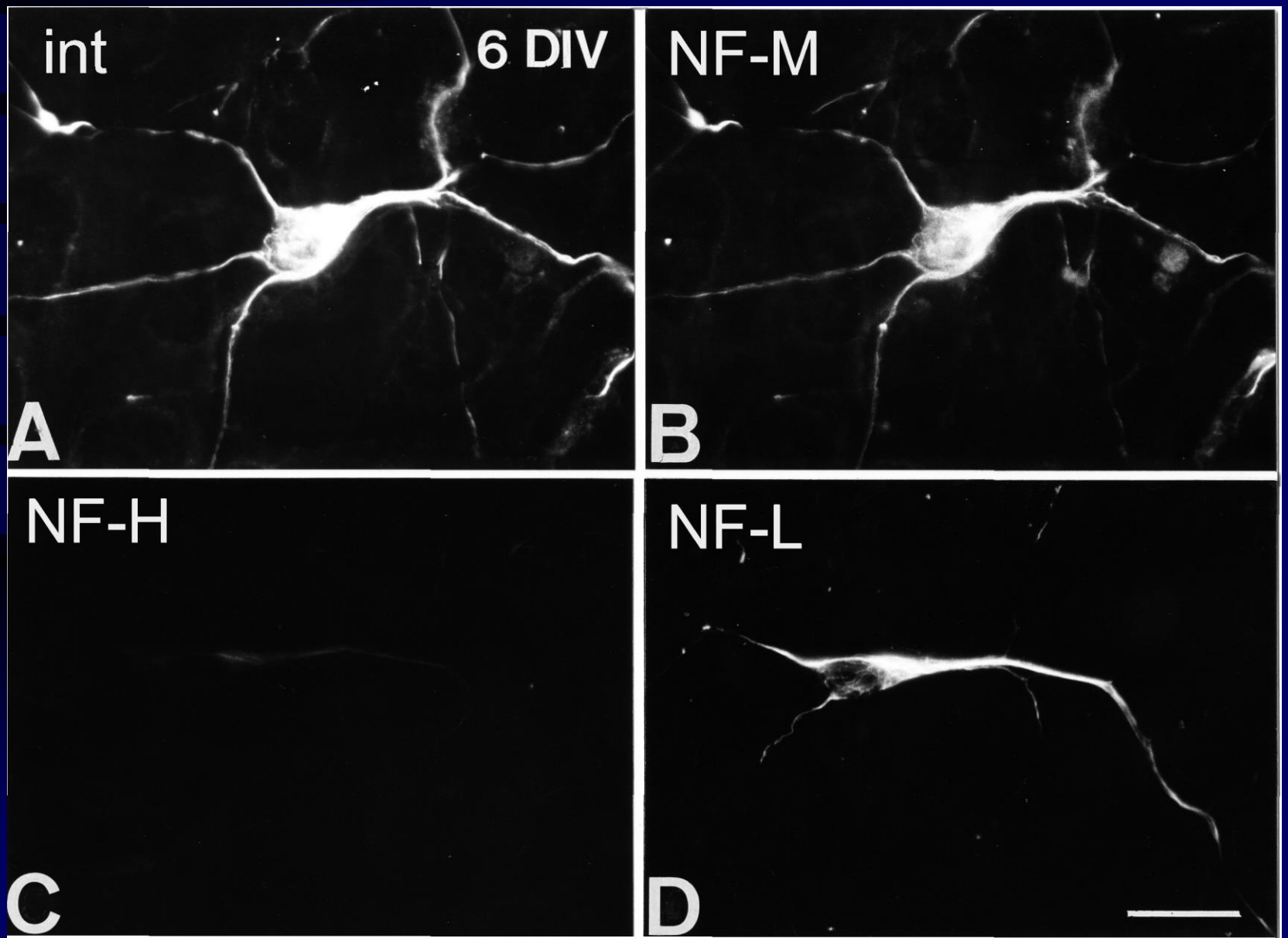
Primary culture of embryonic (E15) hippocampal cells

α-internexin: a 66 kD protein,

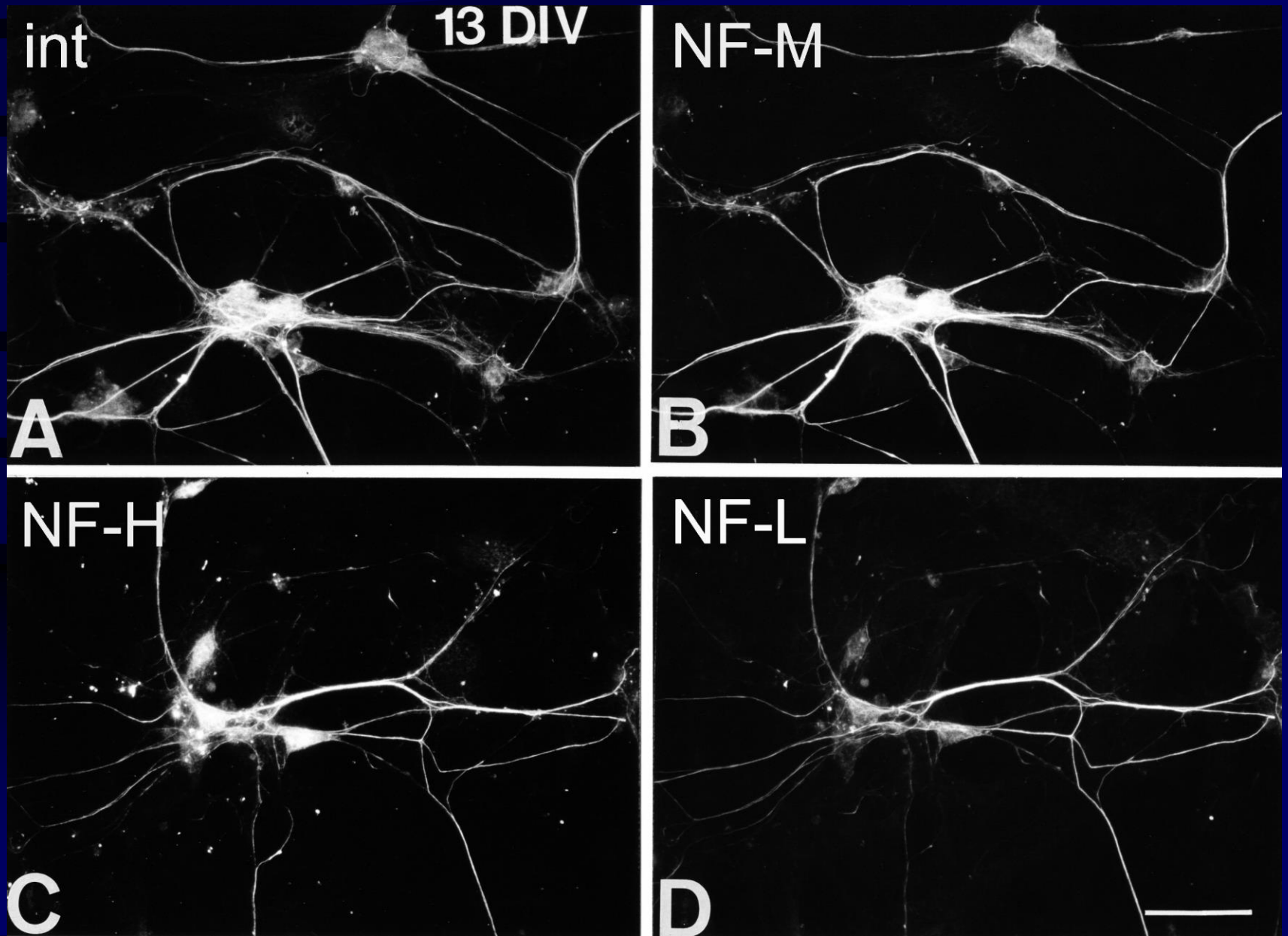
the first neuronal intermediate filament protein expressed in the post-mitotic neurons of developing mammalian central nervous system



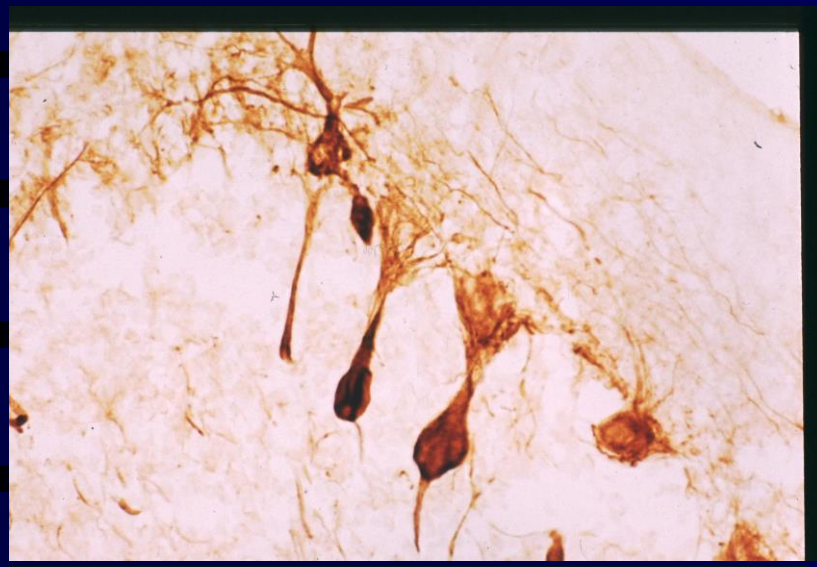
Internexin, NF-M, NF-L but not NF-H expressed in the 6 days *in vitro* (DIV) culture of hippocampal neurons

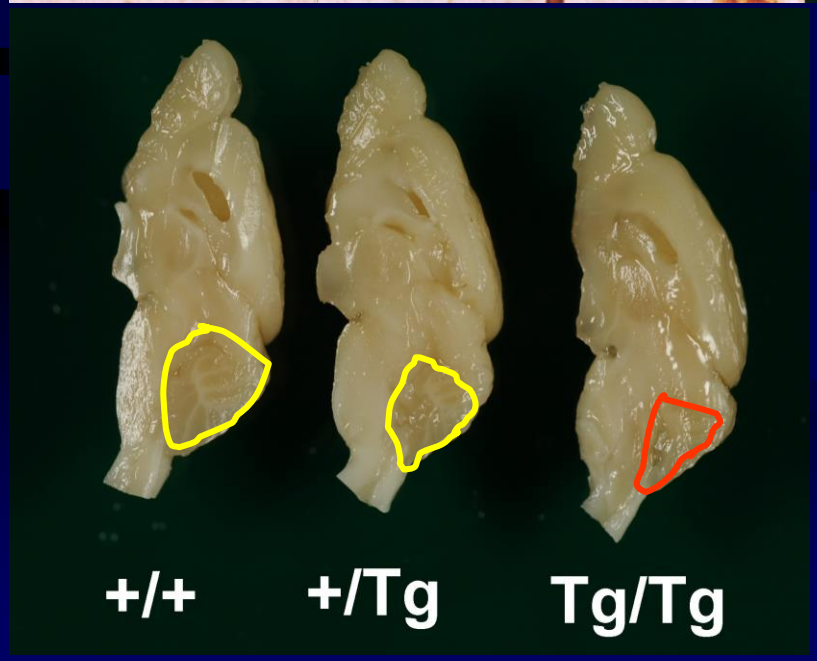


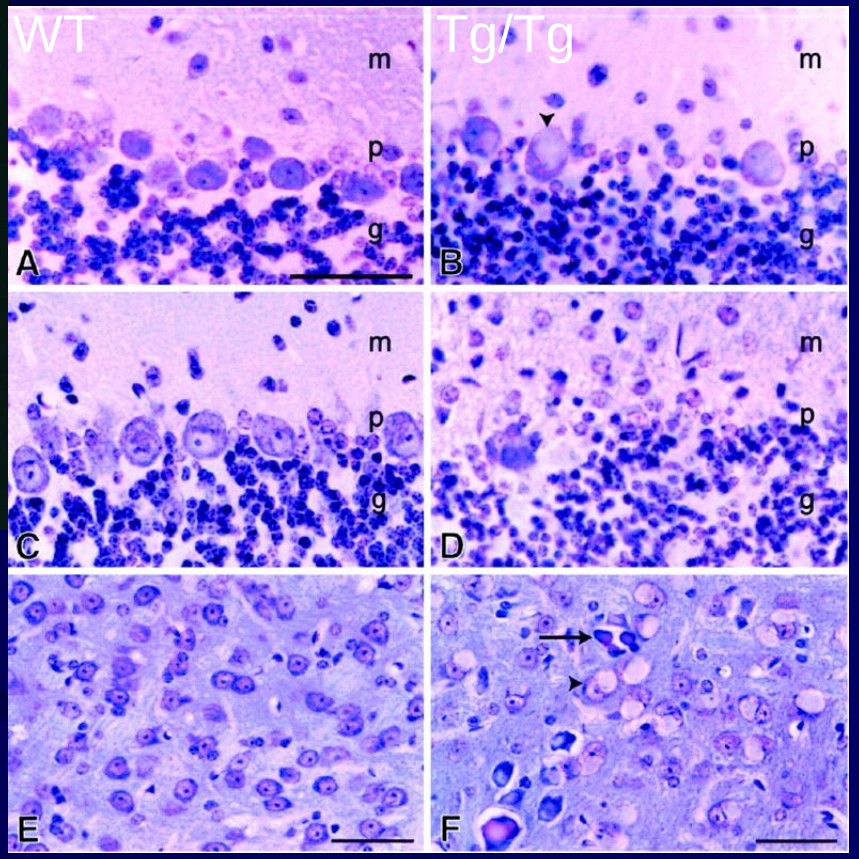
Internexin and Neurofilament Triplet Proteins (NF-L, NF-M and NF-H) all expressed in the 13 DIV hippocampal neurons



Animal model for cerebellar atrophy (J. Neurosci. 19:2974-2986, 1999)







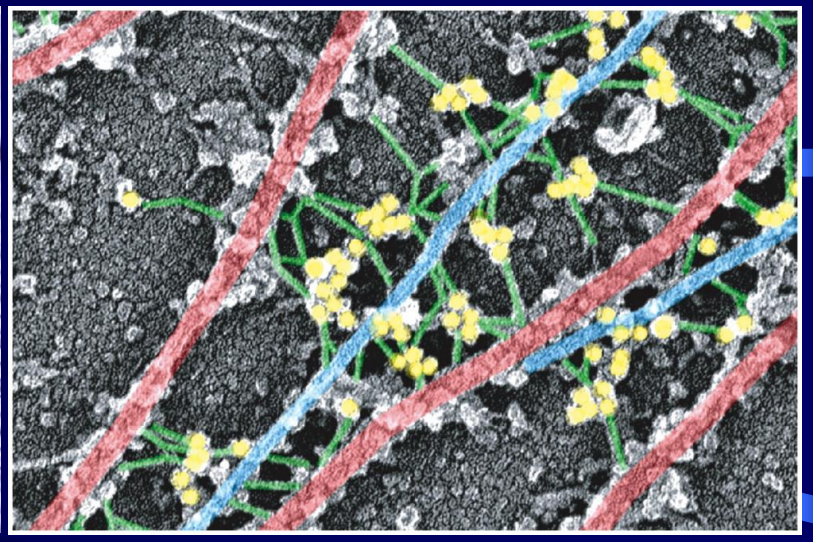
12 m cerebella

18 m cerebella

18 m thalamus

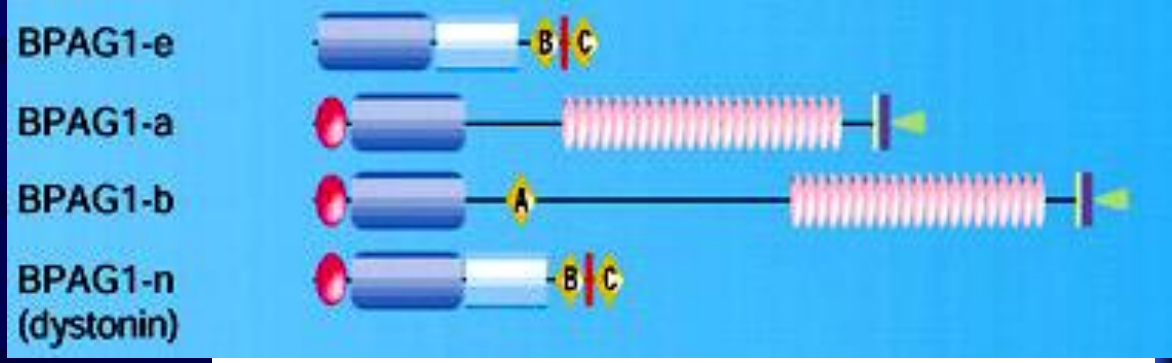
Neuronal loss in the cerebella and thalamus of transgenic mice

Nature Mutant for Neuronal Degeneration

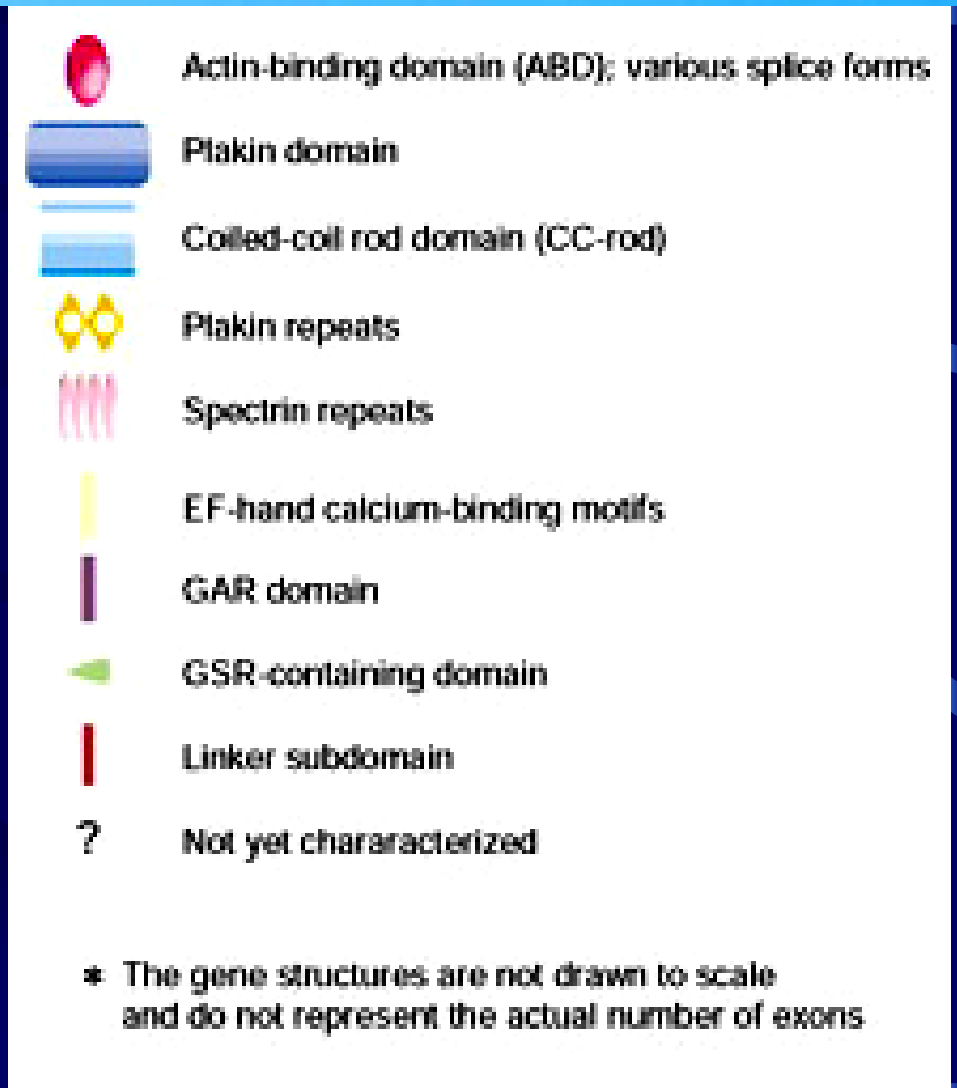


- *Dystonia musculorum (dt)* mouse is a recessive hereditary sensory neuropathy of the mutant mouse, which is defective in *BPAG1* gene.
- Mice affected with *dt* are seemingly normal at birth, but by 10–12 days they begin twitching, writhing, and exhibiting uncoordinated movements.
- **BPAG1** cross-links the intermediate filaments and other cytoskeletons.

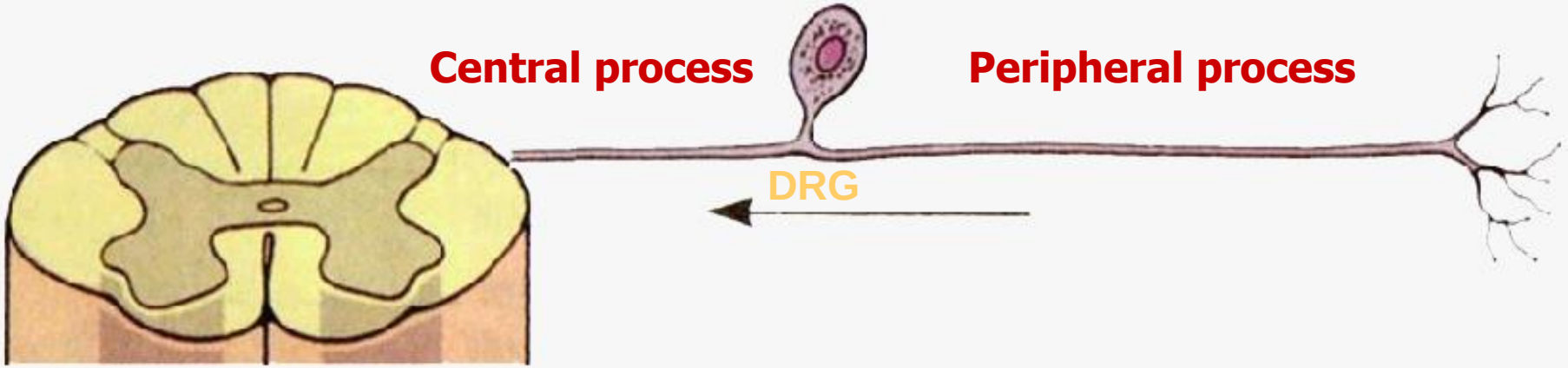
BPAG1 is known as ***dystonin.***



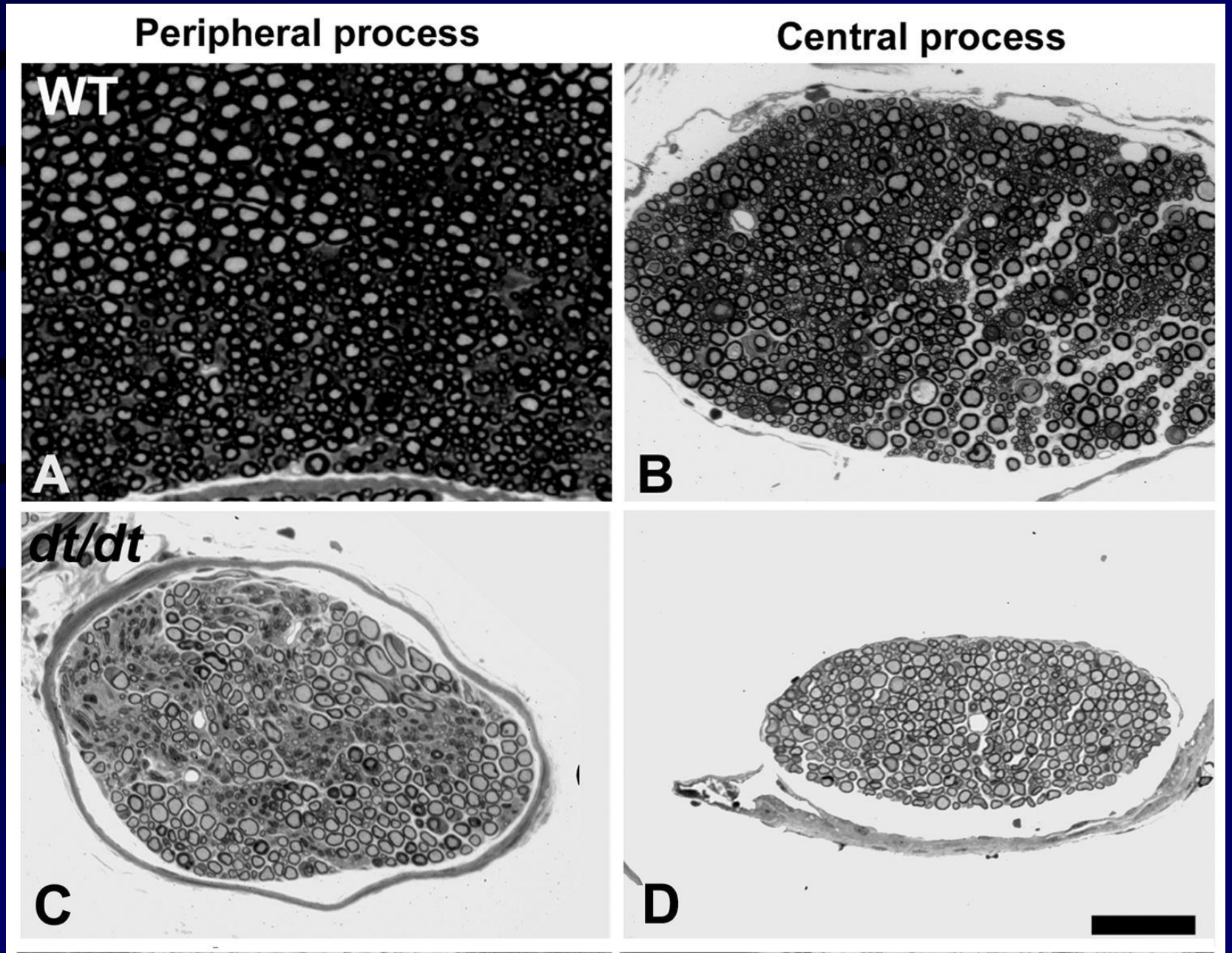
Dystonin, a neural isoform of BPAG1, contains actin-binding domain (ABD) at N-terminus, and is a cytoskeletal crosslinker protein.



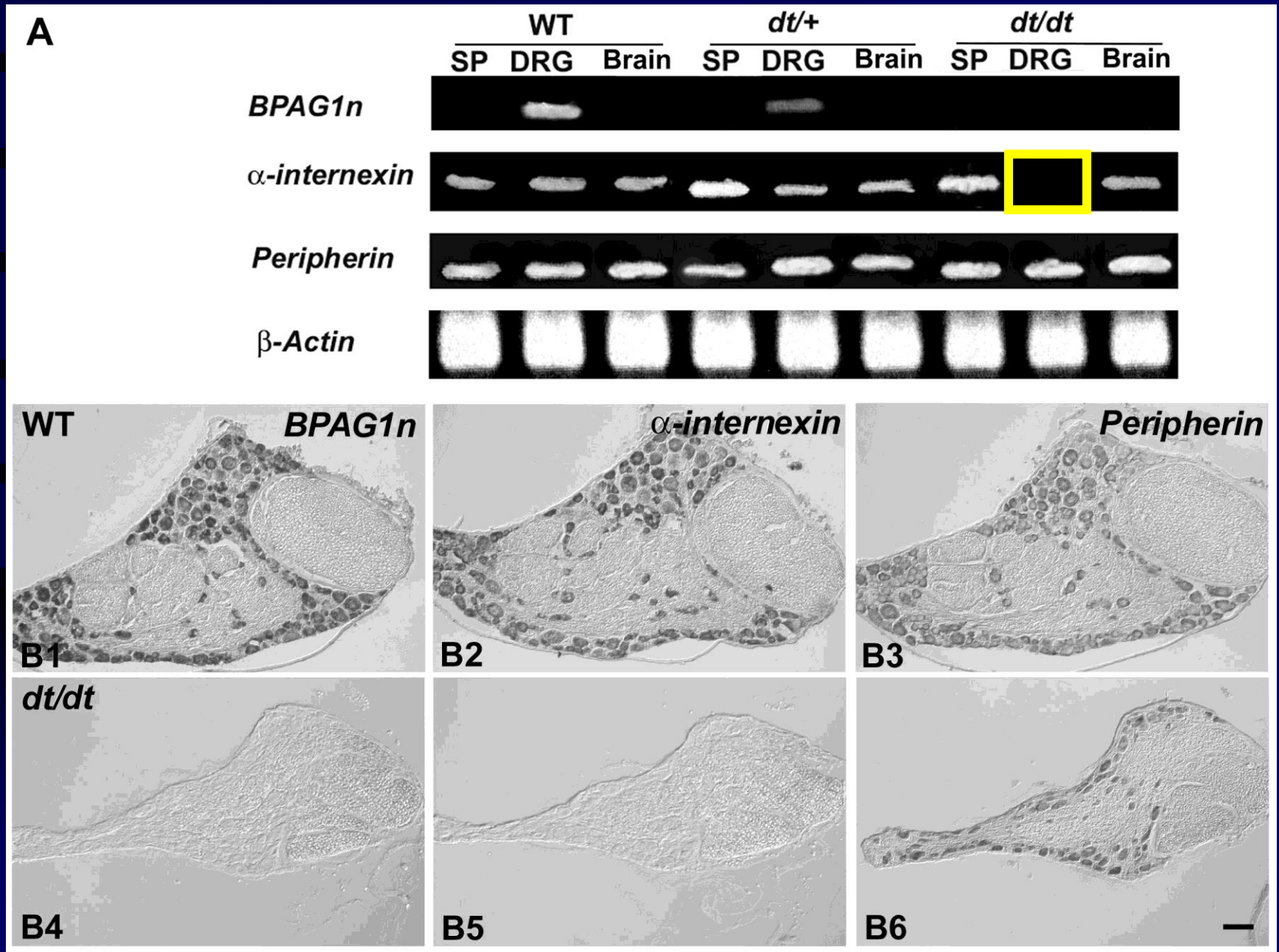
To study the neural dysfunction and degeneration of primary sensory neurons in dorsal root ganglia in *dt* mice.



Peripheral and central processes from WT and *dt/dt* mice

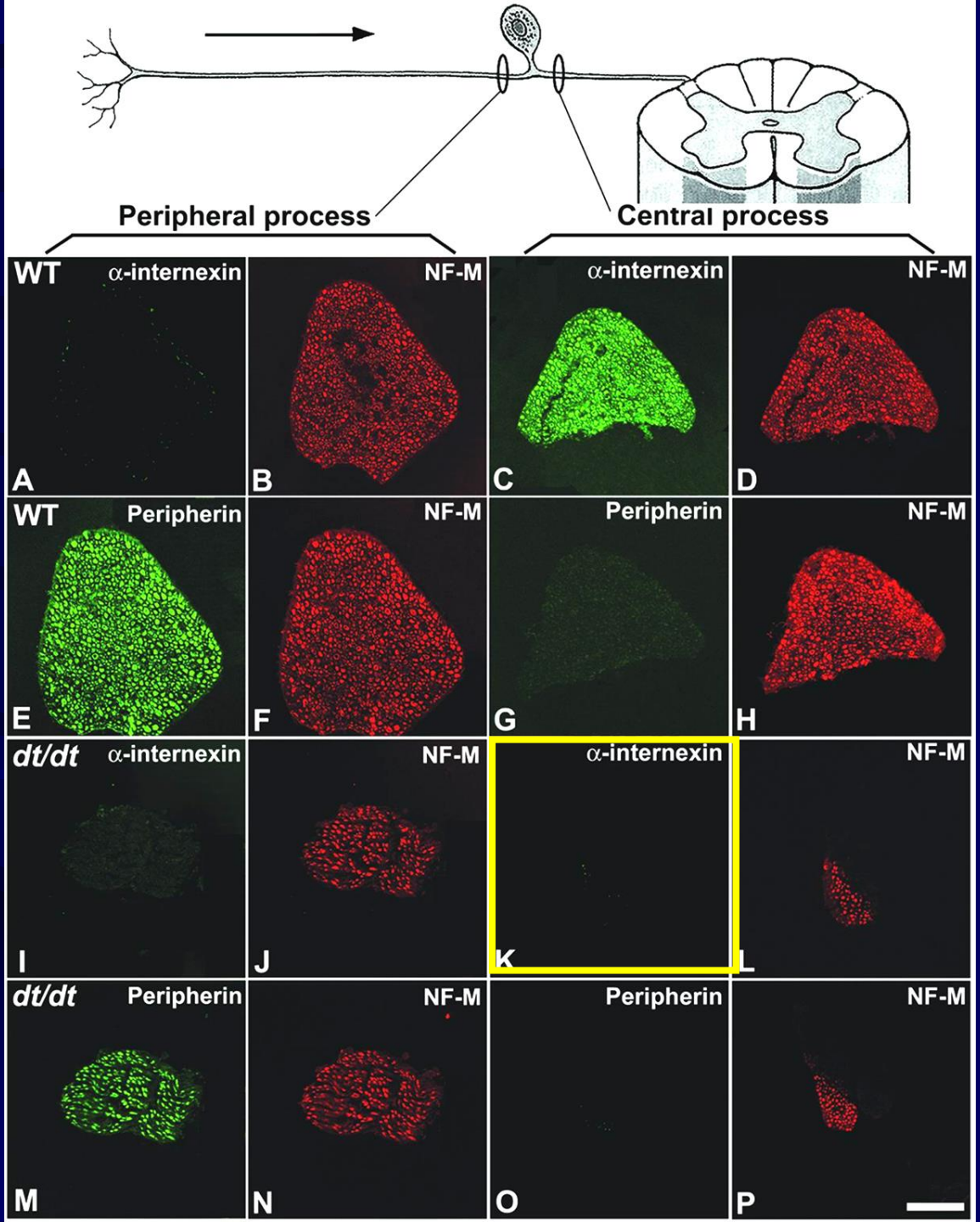


RT-PCR and in situ hybridization analysis



Expression of neuronal intermediate filaments in WT and *dt/dt* mice

α-interenxin is absent in the central process of adult *dt/dt* mice



Sensory and autonomic nerves degenerated in the skin of *dt* mutant

Fig. 6

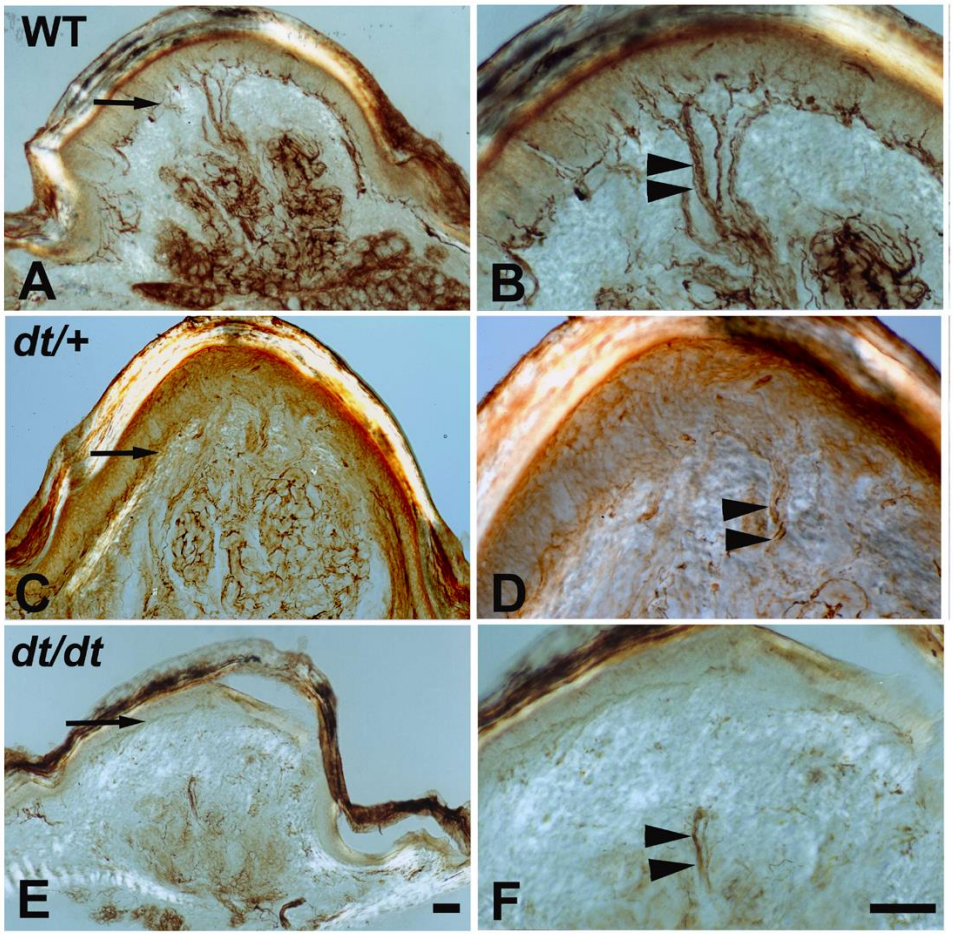
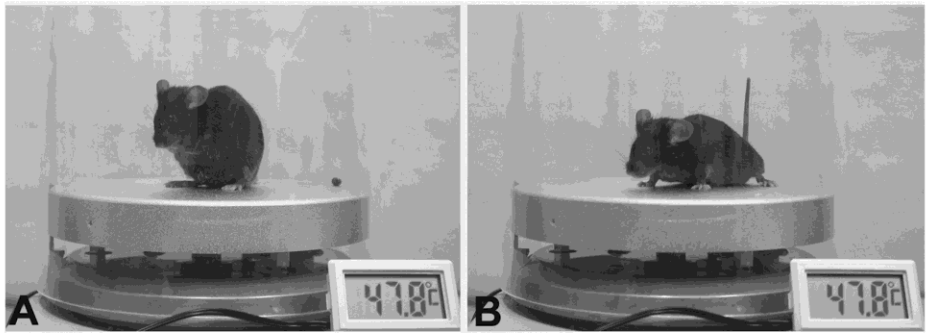
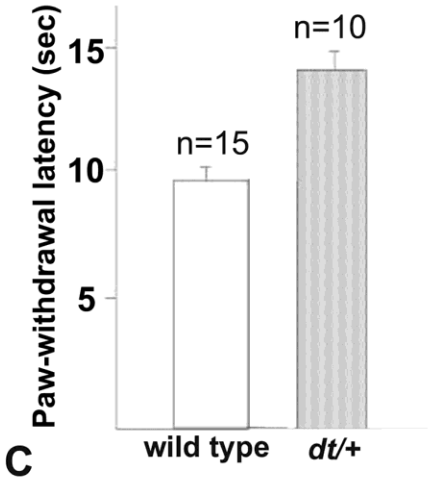
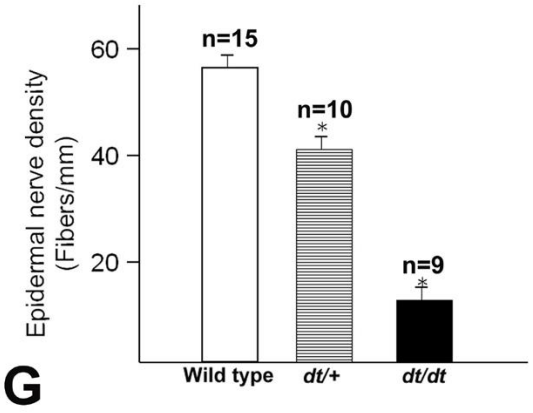


Fig. 7





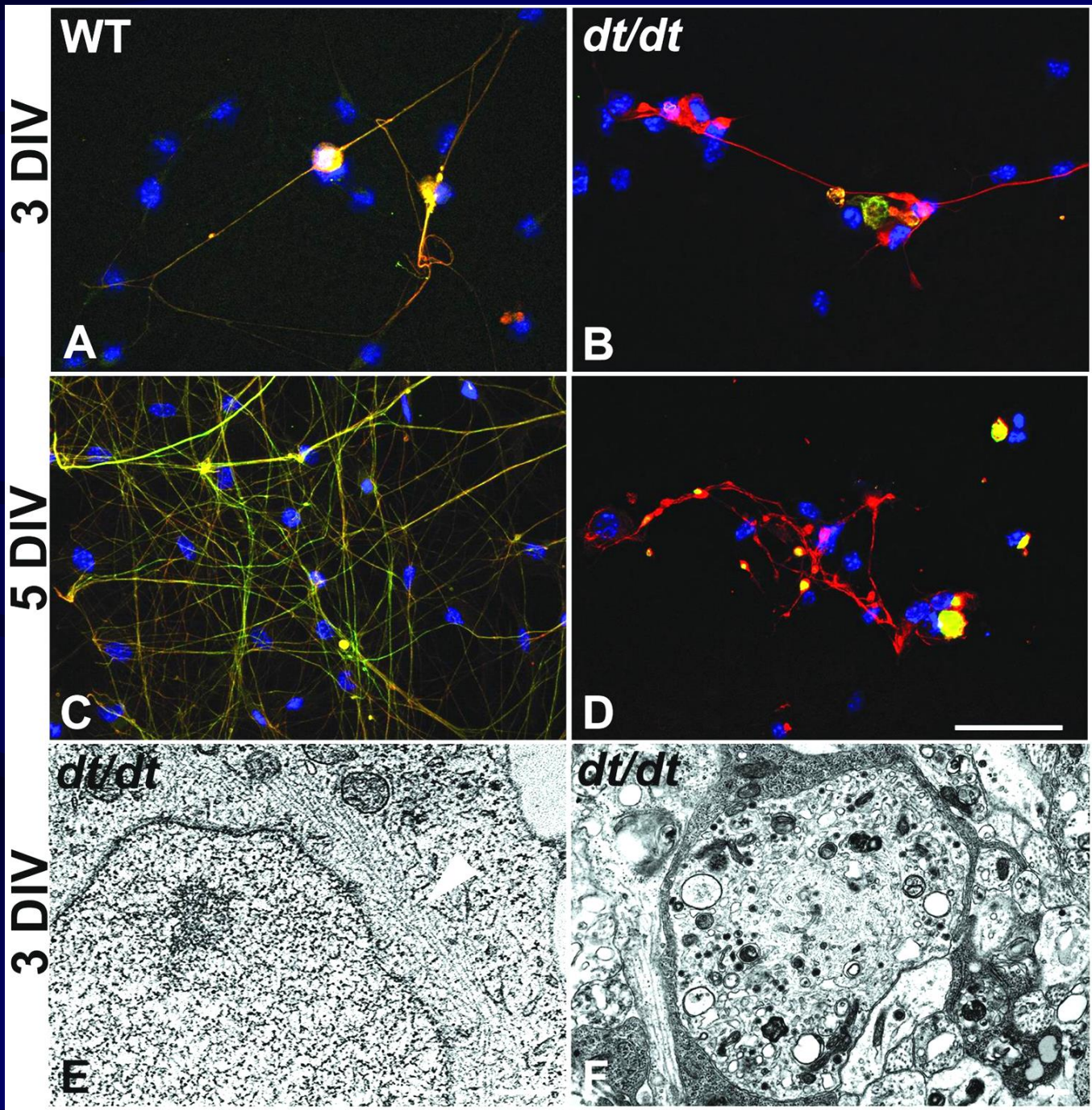


Primary culture DRG neurons

1. Take DRGs and transfer DRGs to a fresh epondroff tube with 0.5 ml HBSS (CMF) on ice.
2. Add 0.5 ml 0.25% Trypsin-EDTA and incubate in rotating incubator at 37℃ for 15 min.
3. Resuspend with 40% FBS L15
4. Spin for 5 min at 1500 rpm, remove supernatant..
5. Resuspend with 1.5 ml 40% FBS L15 in incubator at 37℃ for 15 min.
6. Spin for 5 min at 1500 rpm.
7. Resuspend in 2 ml NB1 with FBS, glucose, 100ng/ml NGF.
8. Transfer containing neurons medium to 30 mm poly-L-lysine coated Petri dish and then incubate 10-20 min (preplating).
9. Transfer the medium to 35 mm Petri dish containing poly-L-lysine coated slide.

Cultured DRG neurons from E15.5 embryos

α-interenxin proteins are accumulated in the cell bodies as well as in the processes of *dt/dt* neurons.

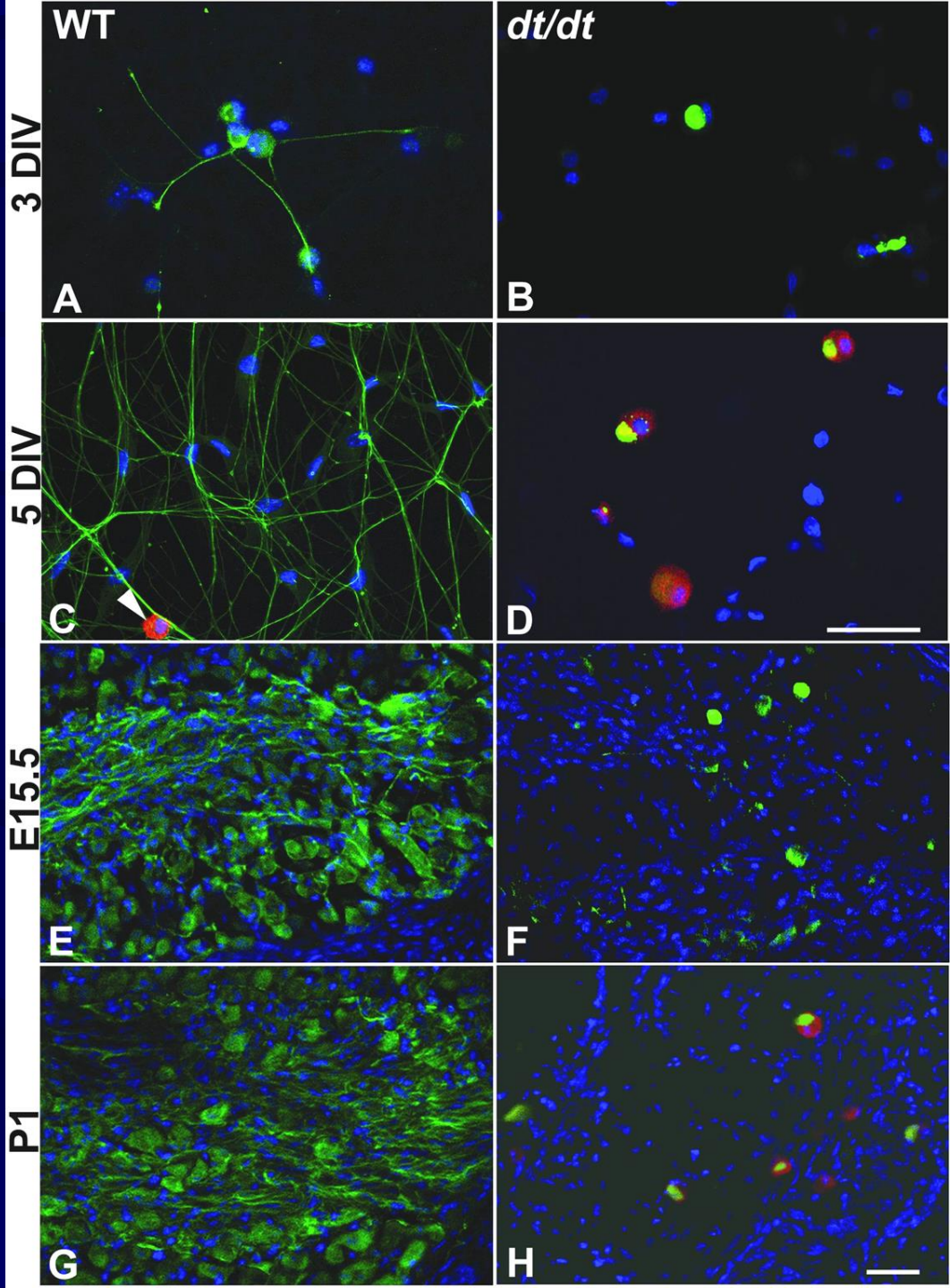


Primary culture of DRG neurons

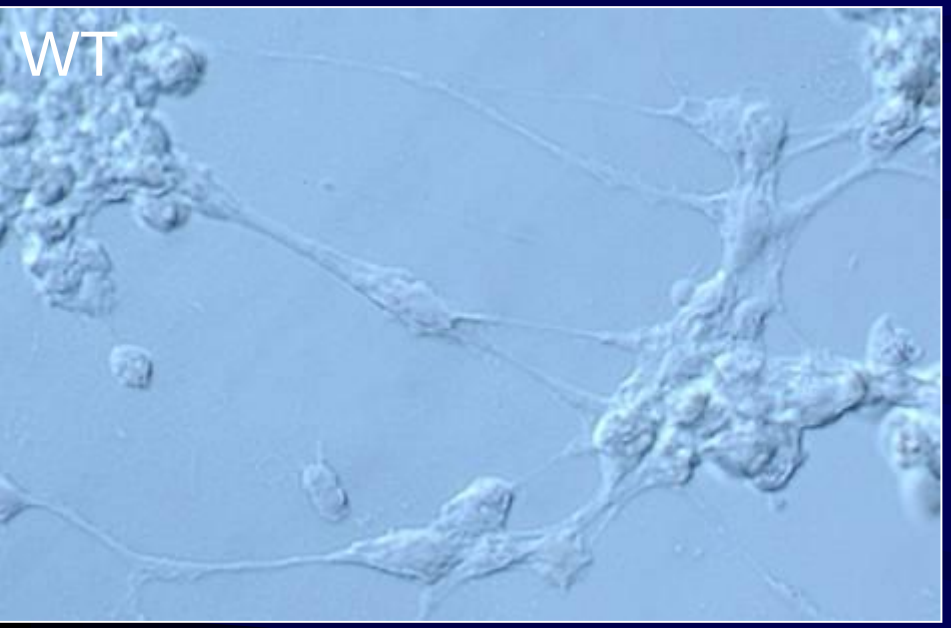
	WT	*dt/dt*
Internexin	+	++ **Aggregations**
Activated Caspase	-	+

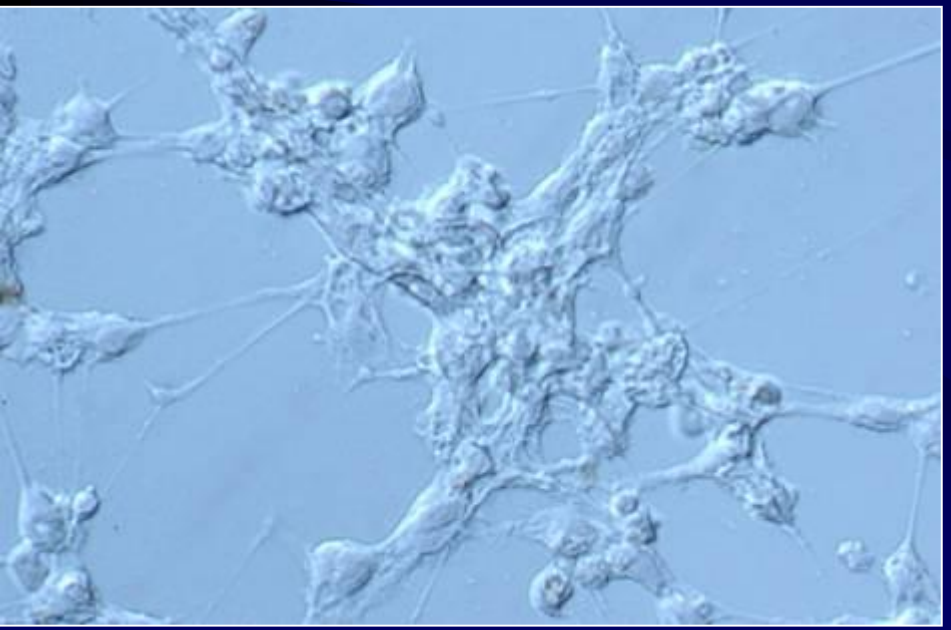
Perinatal development

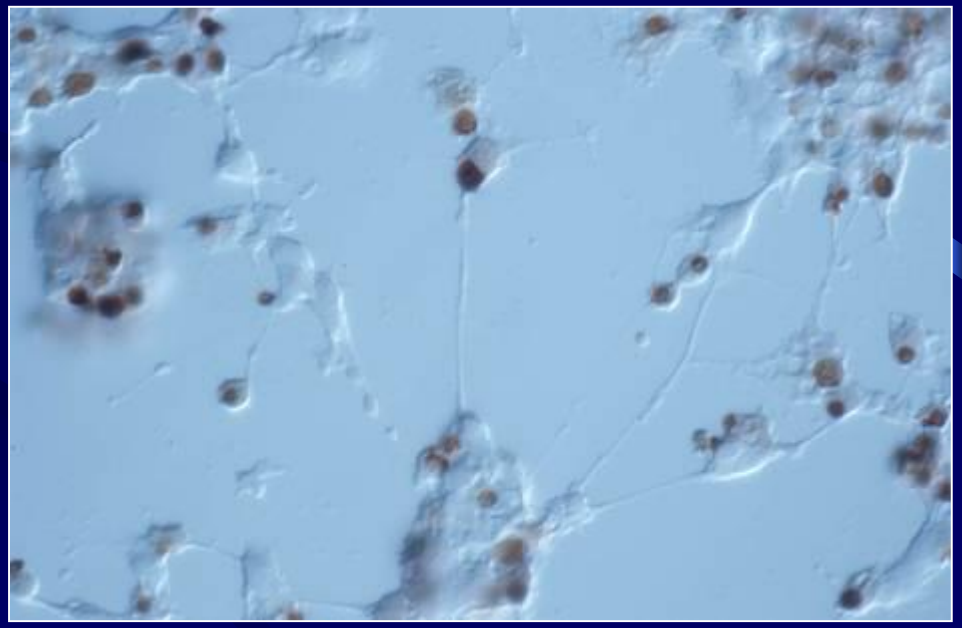
	WT	*dt/dt*
Internexin	+	++ **Aggregations**
Activated Caspase	-	+



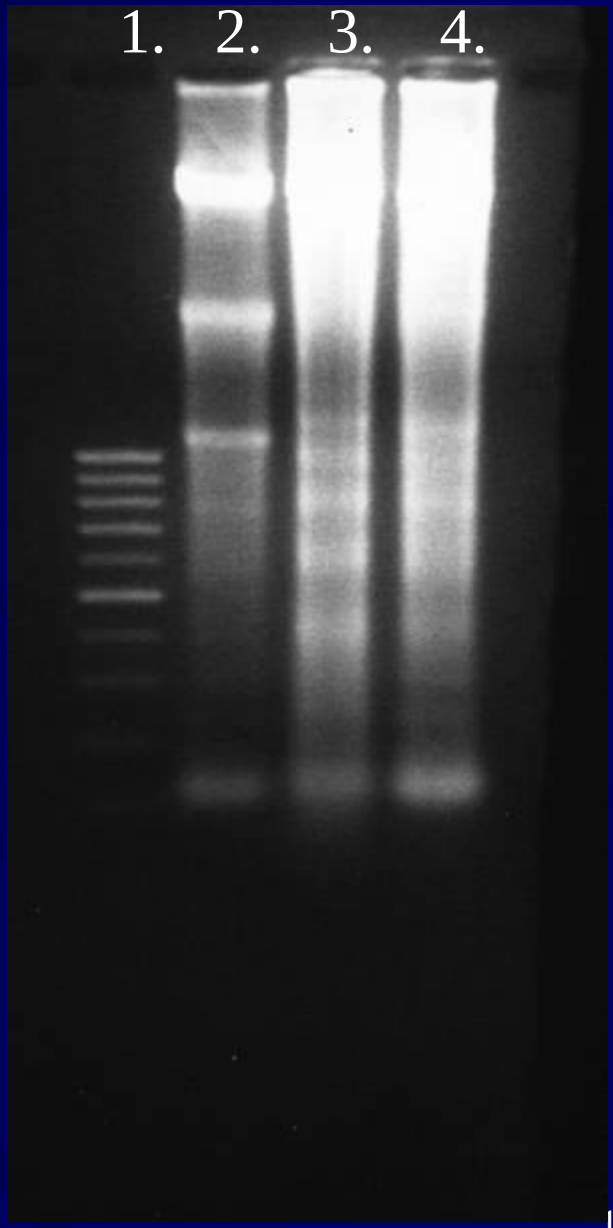
TUNEL Assays







DNA ladder pattern from cultured DRG of 5DIV



1. Marker: 100 bp marker

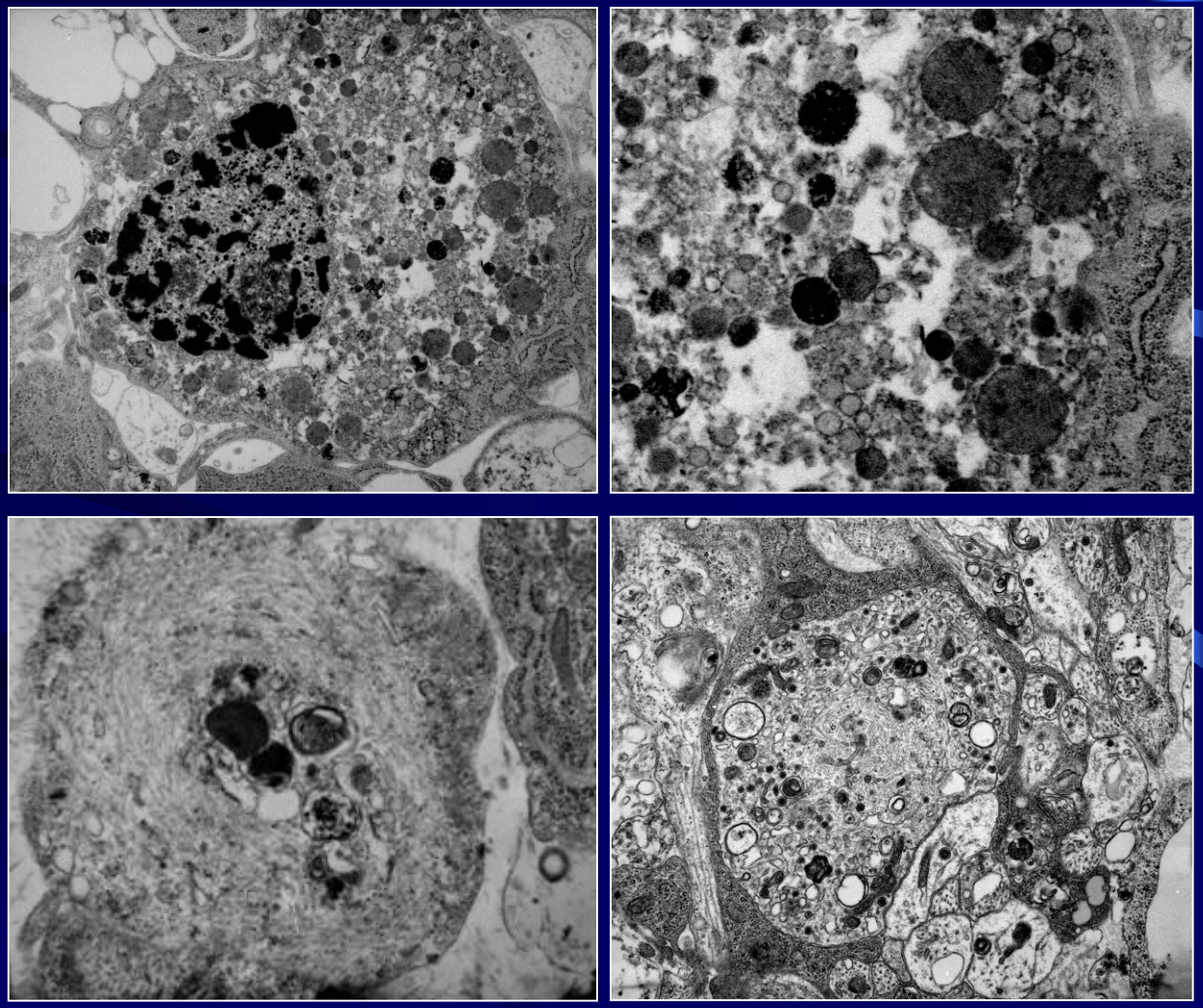
2.3.4. DNA extraction from DRG neurons of *dt/dt*

Primary culture of DRG neurons

DRG neurons of *dt/dt* mice observation by Electron microscope

- Chromatin condensation
- IFs accumulation
- Axonal swelling

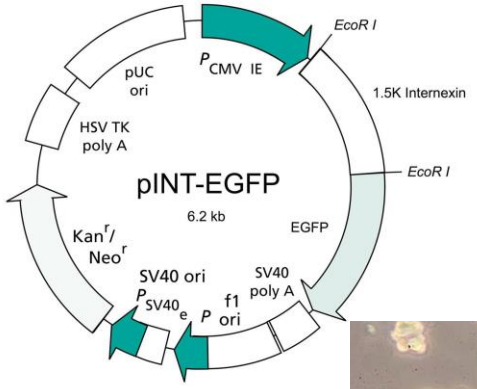
➢ Cell apoptosis



Summary I

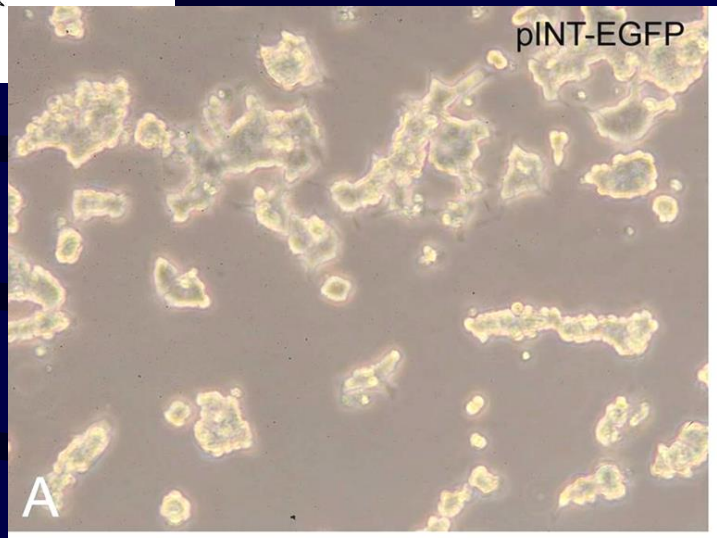
- The interaction between BPAG1 and α-internexin may be one of the key factors involved in the neuronal degeneration of DRG in the *dt* mutant.
- Abnormal accumulation of α-internexin and other cytoskeletal components may impair the axonal transport and subsequently turn on the cascade of neuronal apoptosis during development.

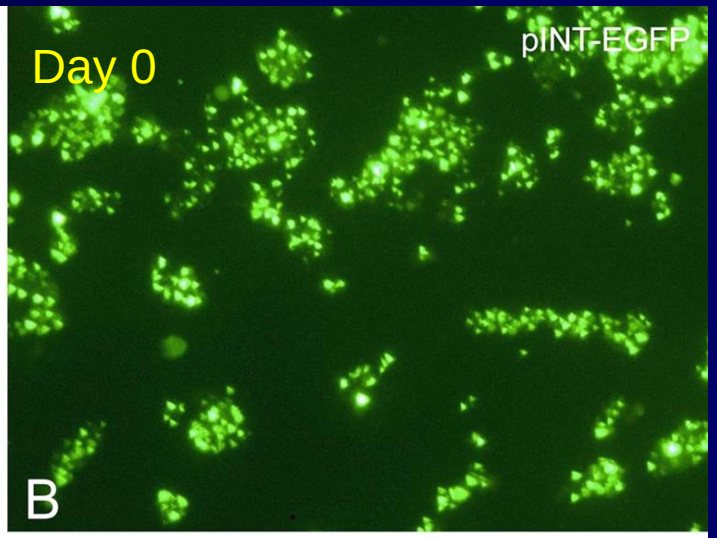
(J. Neuropathol. Exp. Neurol. 65:336-347 , 2006)

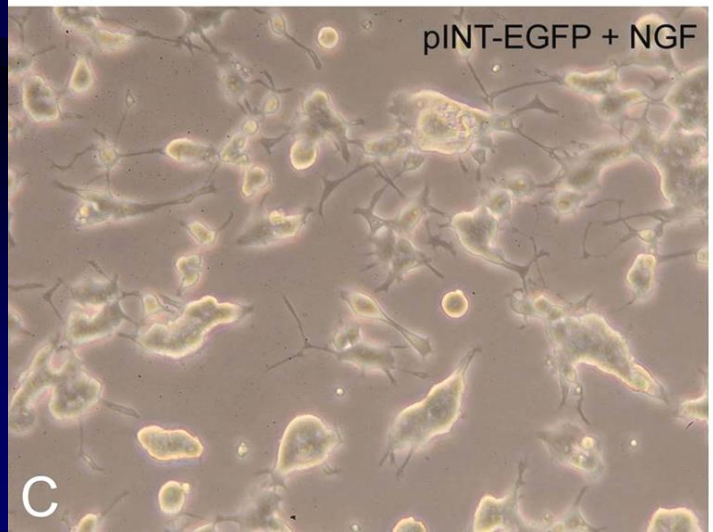


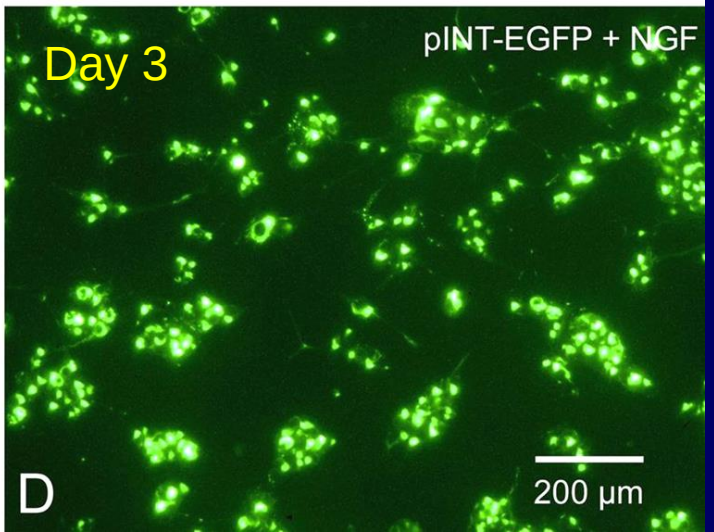
Overexpression of neuronal intermediate filament α-internexin in the PC-12 cell line

(J. Neurosci. Res. 80:693-706, 2005)



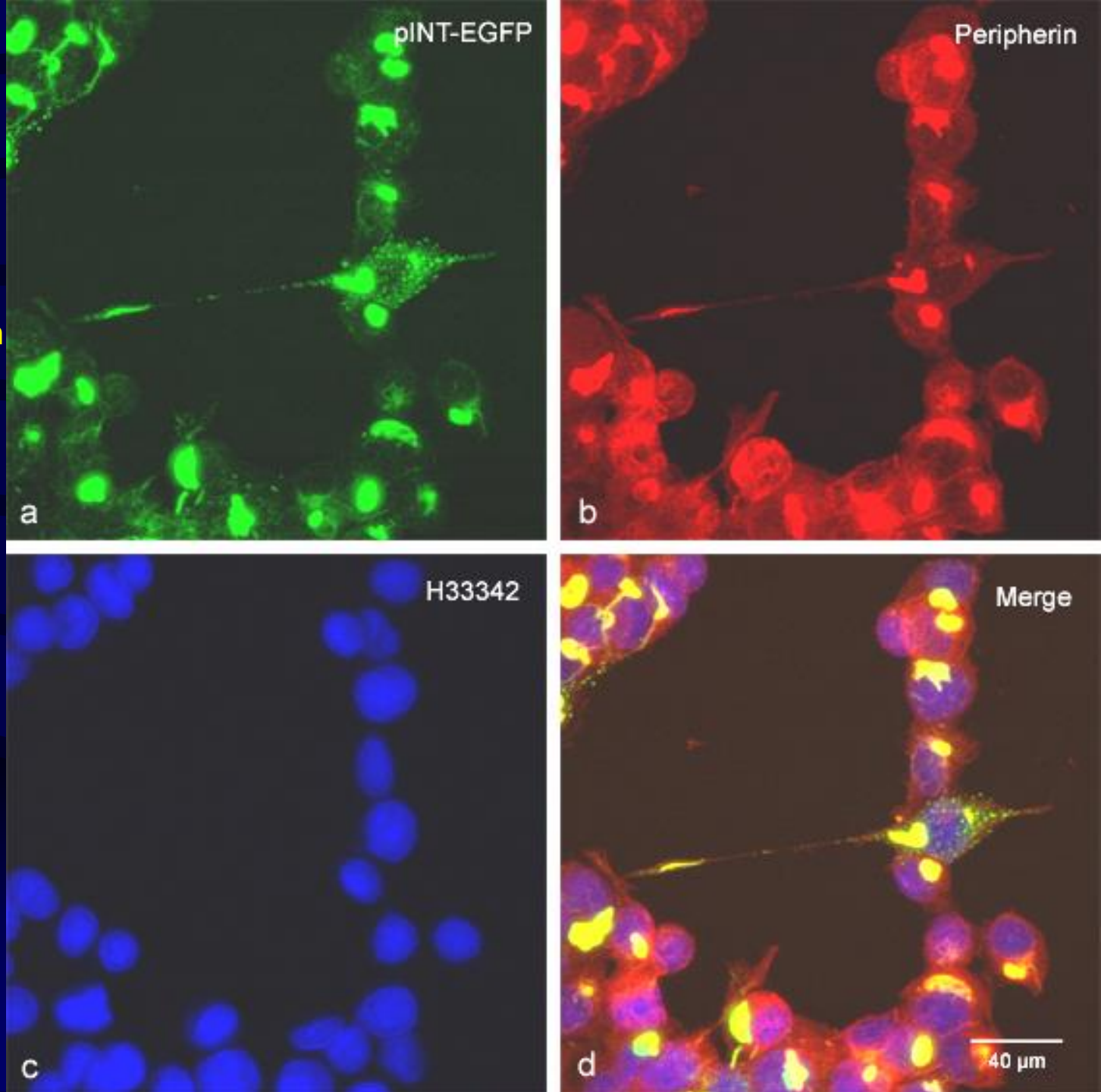




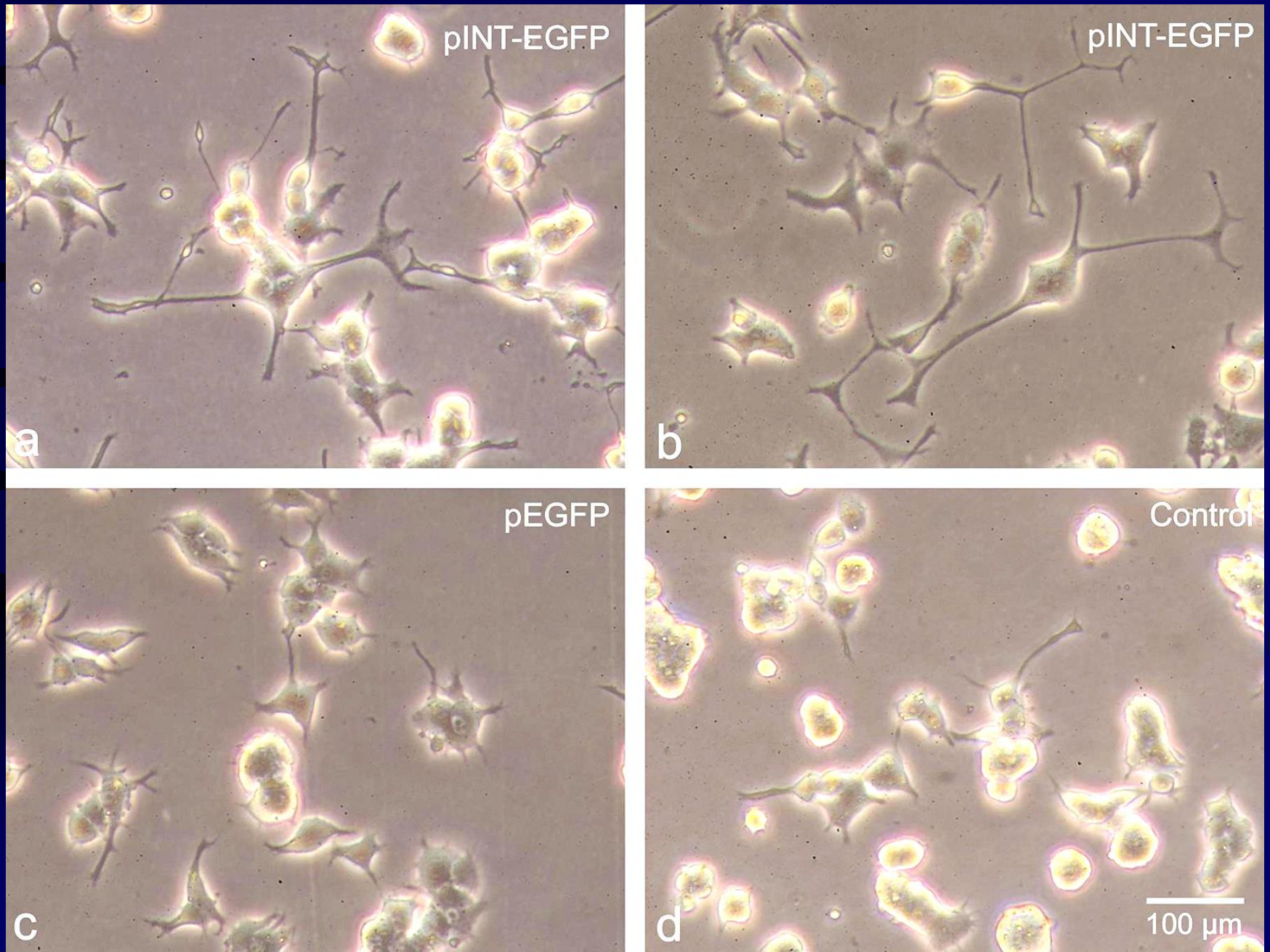


Confocal Patterns

3-day NGF induction

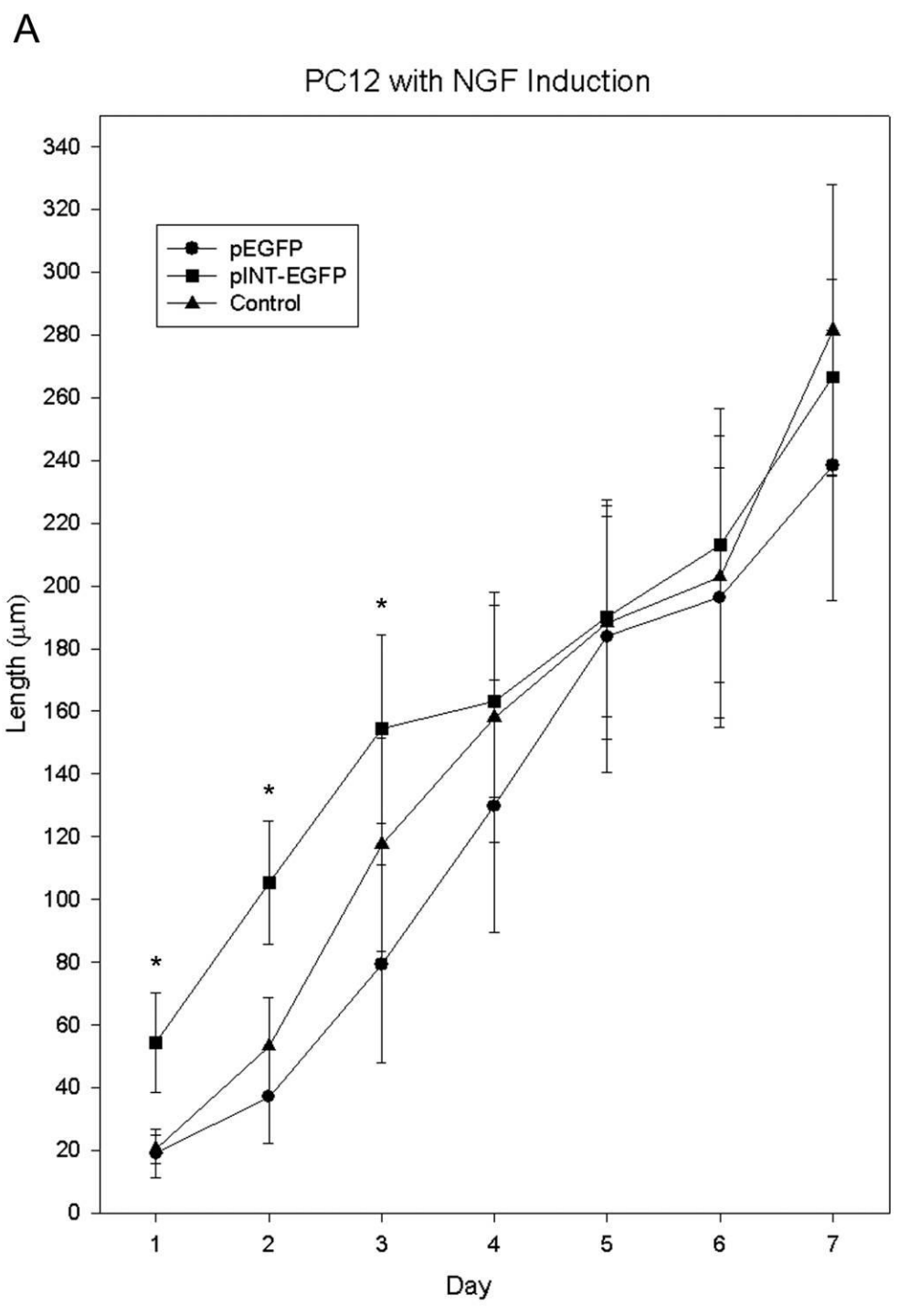


Cells after 2-day NGF induction

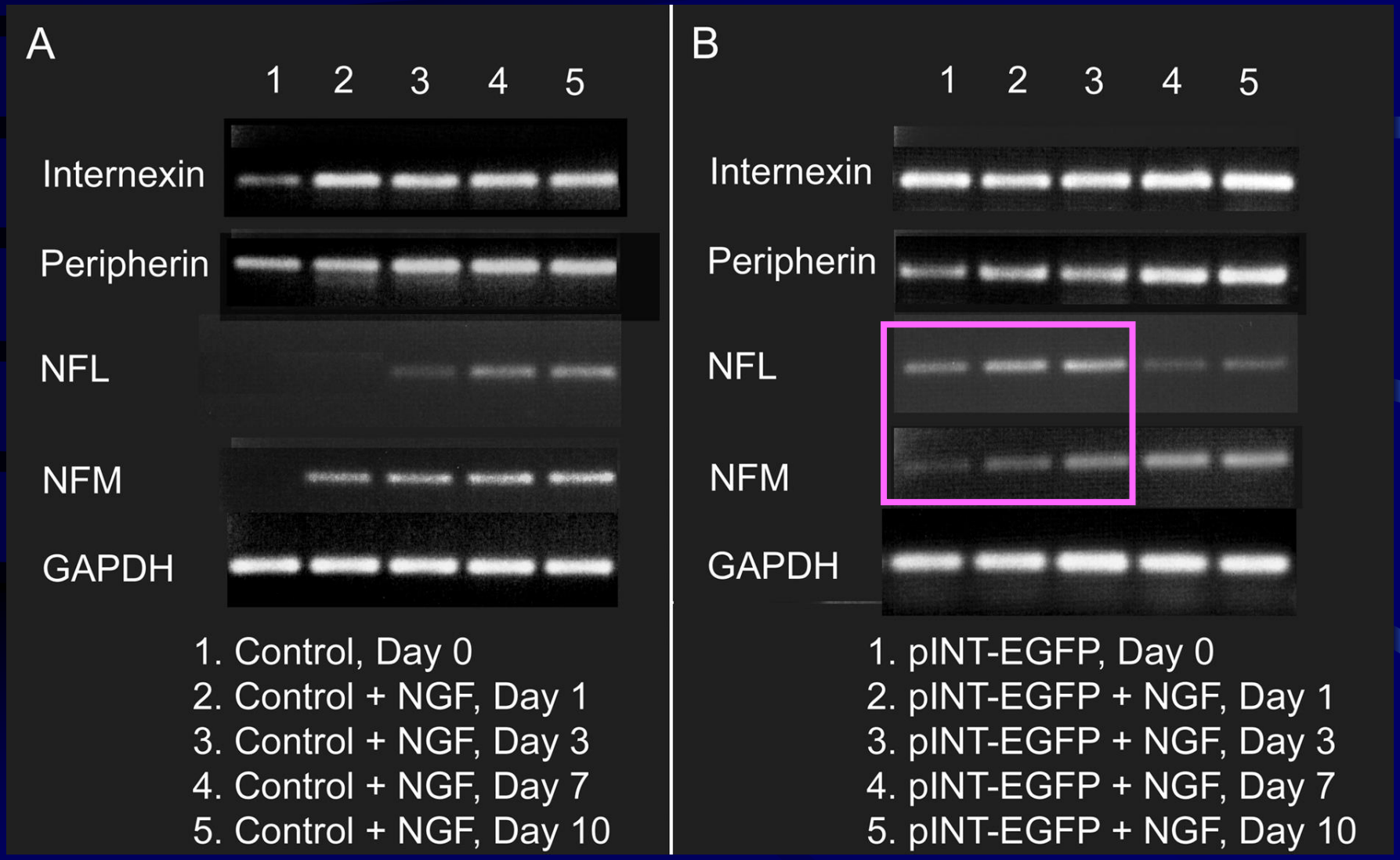


PC-12 Neurite outgrowth after NGF induction

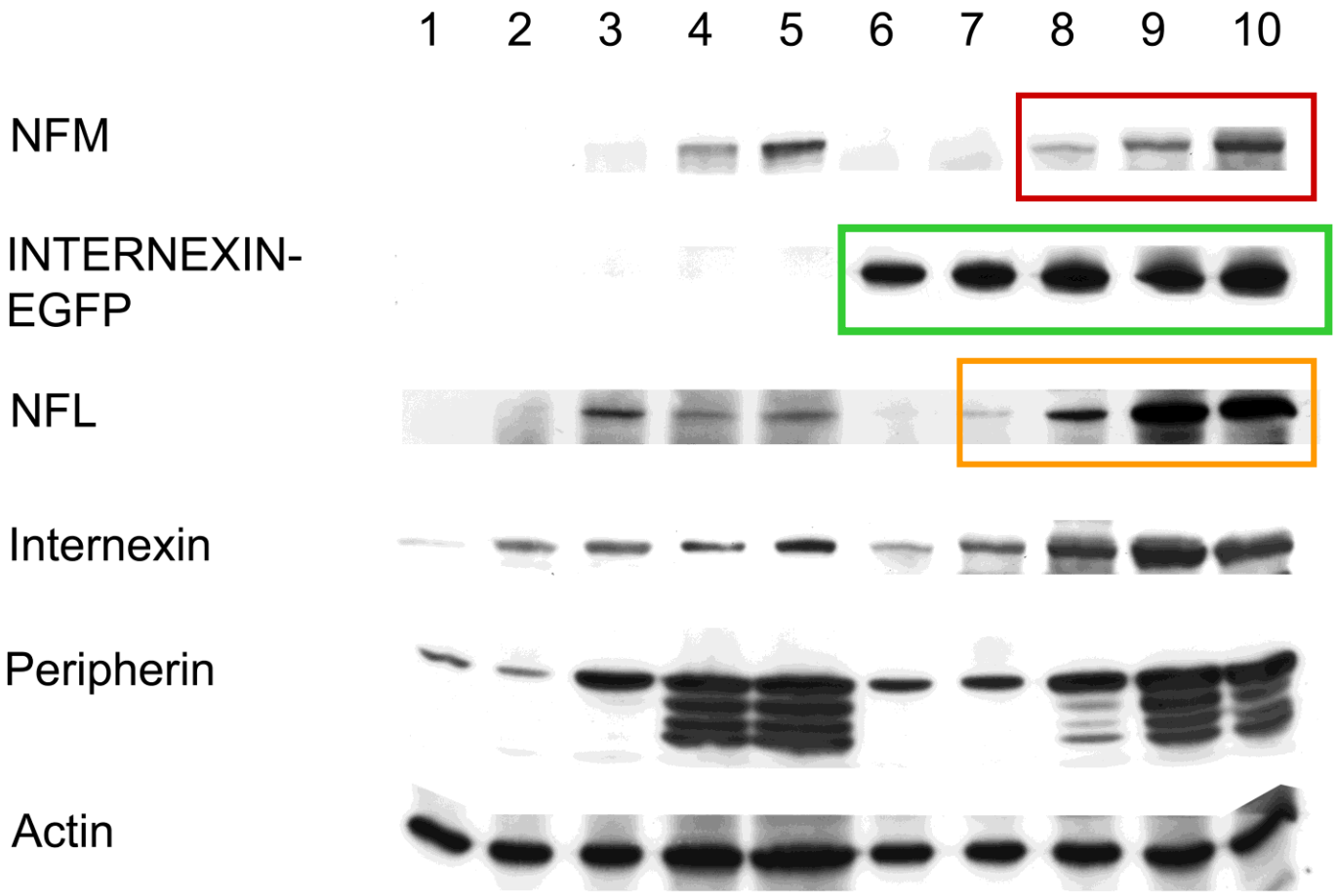
The longest neurite from each single cell was measured at different time points (n=25).



RT-PCR


A
1 2 3 4 5
Internexin
Peripherin
NFL
NFM
GAPDH
1. Control, Day 0
2. Control + NGF, Day 1
3. Control + NGF, Day 3
4. Control + NGF, Day 7
5. Control + NGF, Day 10
B
1 2 3 4 5
Internexin
Peripherin
NFL
NFM
GAPDH
1. pINT-EGFP, Day 0
2. pINT-EGFP + NGF, Day 1
3. pINT-EGFP + NGF, Day 3
4. pINT-EGFP + NGF, Day 7
5. pINT-EGFP + NGF, Day 10

Western Blot



1. Control, Day 0
2. Control + NGF, Day 1
3. Control + NGF, Day 3
4. Control + NGF, Day 7
5. Control + NGF, Day 10

6. pINT-EGFP, Day 0
7. pINT-EGFP + NGF, Day 1
8. pINT-EGFP + NGF, Day 3
9. pINT-EGFP + NGF, Day 7
10. pINT-EGFP + NGF, Day 10

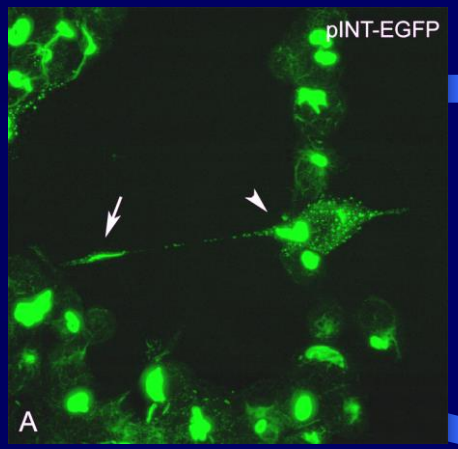
Summary II

1. Overexpression of pINT-EGFP enhances neurite outgrowth, it could be suggested that internexin may play an important role in early neuronal differentiation.

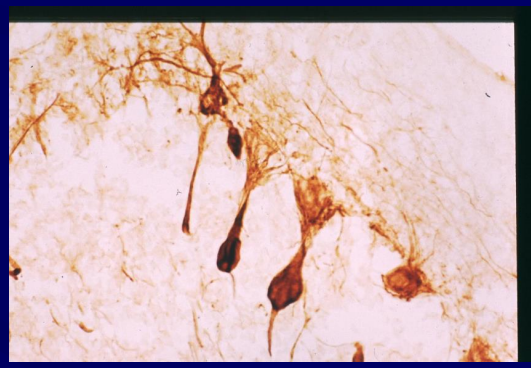
2. Internexin may regulate the expression of other neurofilaments during neuronal development, since overexpressed internexin-EGFP enhanced the expression of NF-L and NF-M.

Cell Death vs. α- internexin Overexpression

- From our observations, cells transfected with pINT-EGFP were found obviously detached from the culture plates after 5-day NGF induction.



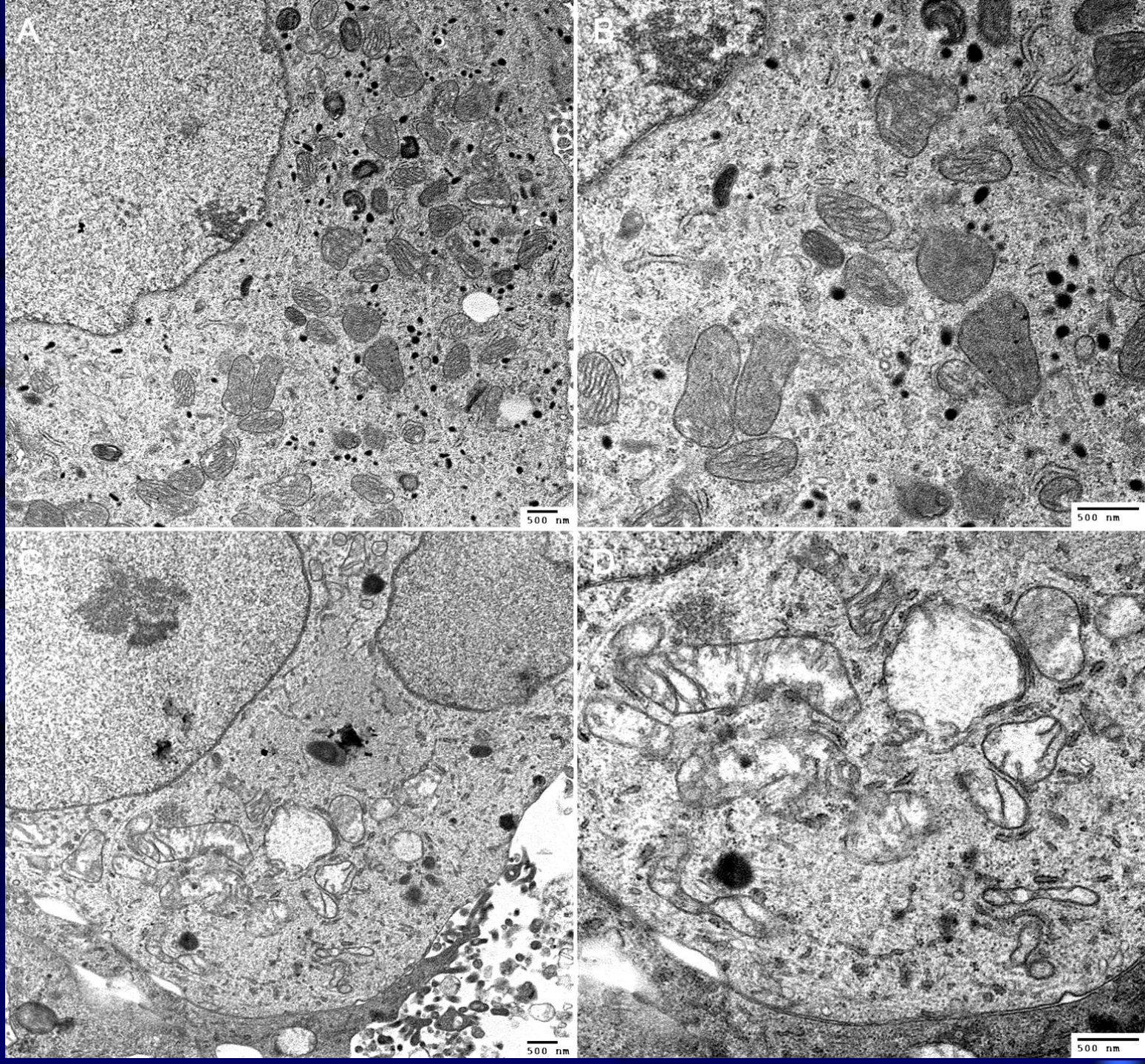
- α-internexin-overexpressing transgenic mice show neuronal dysfunction, progressive neurodegeneration and loss of neurons in the neocortex, thalamus, and cerebellum of aged transgenic mice (Ching et al., 1999).



Ultrastructure patterns (5-day NGF induction)

Control cells

pINT-EGFP tranfected cells

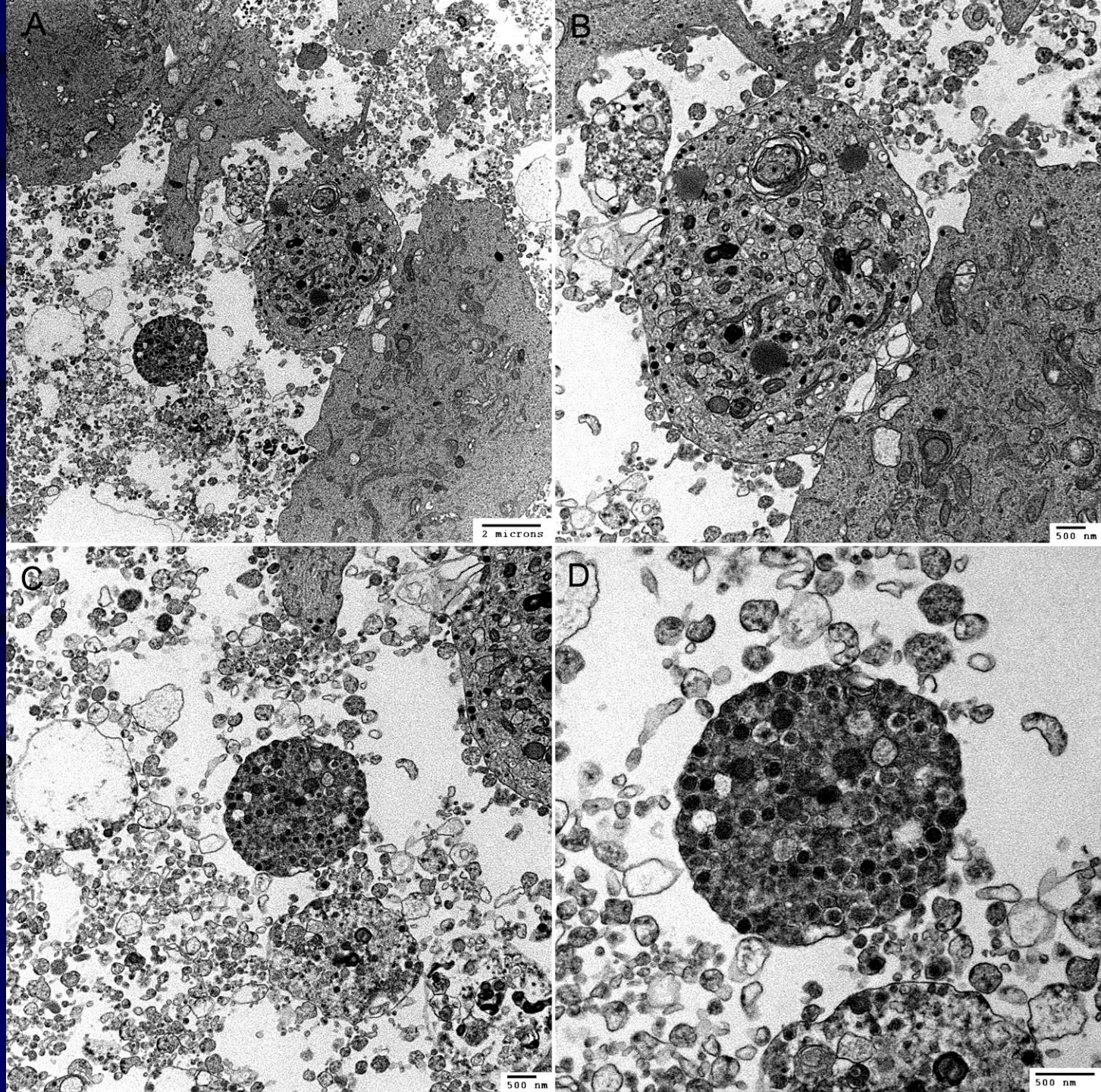


Ultrastructural patterns (5-day NGF)

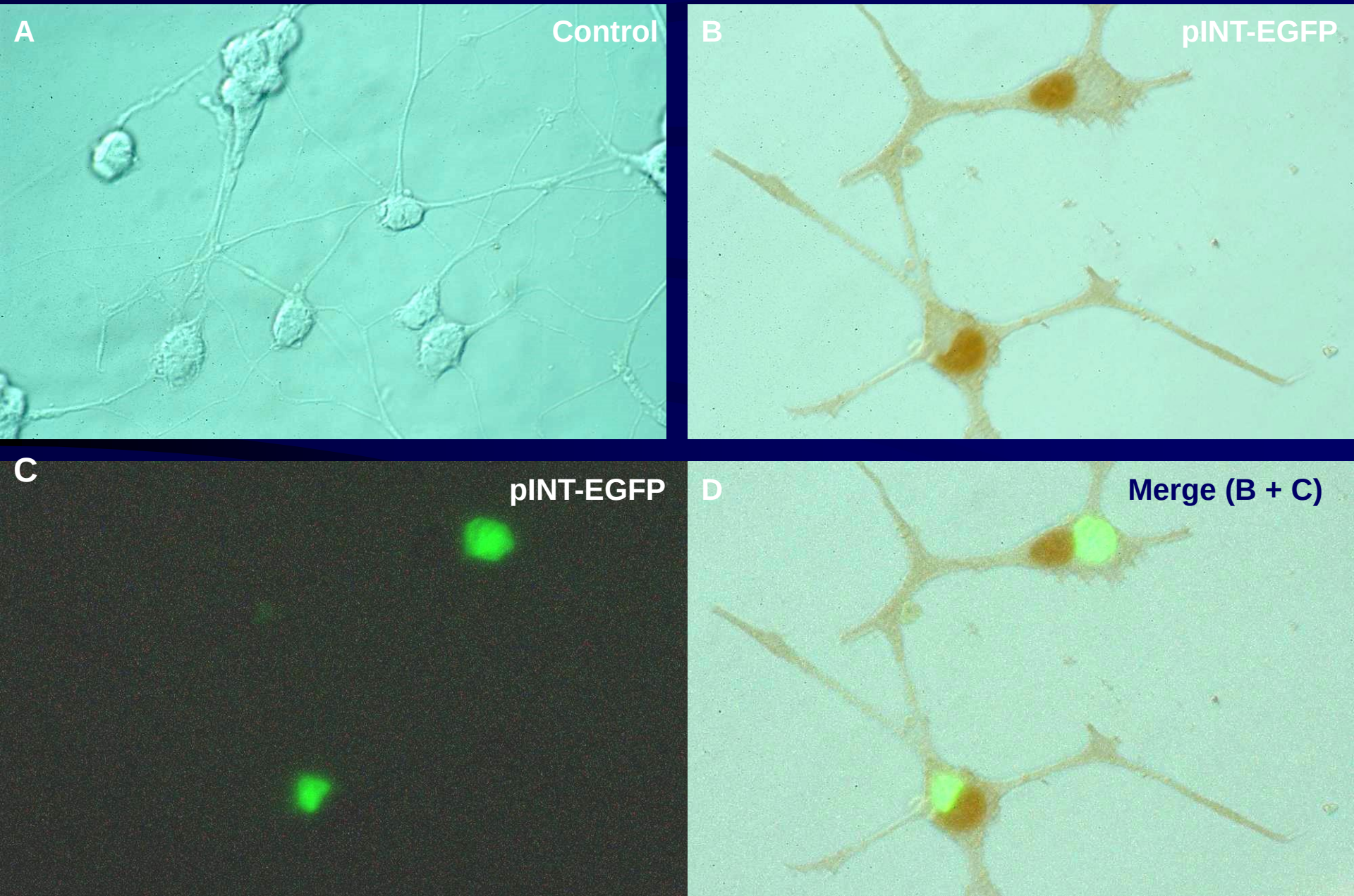
pINT-EGFP transfected cells

Degenerating neurite

Degenerated neurite



TUNEL assay at the 5th day of NGF induction



Summary III

1. Overexpression of pINT-EGFP may induce swelling mitochondria and massive intermediate filament accumulations in cell bodies and processes.

2. Early events of apoptosis could be characterized in the pINT-EGFP transfected cells by the caspase activity and TUNEL positive patterns.

Microarray: pINT-EGFP Day 6 vs. PC12 Day 6

Neuronal proteins	Regulation	Fold
internexin, alpha	UP	414.961
neurofilament 3, medium	UP	4.85568
neurofilament, light polypeptide	UP	6.28745
nestin	UP	3.34591
peripherin 1	UP	2.25822
microtubule-associated protein 1 light chain 3 alpha	UP	2.48439
Microtubule-associated proteins 1A/1B light chain 3	DOWN	3.38617
synapsin II	DOWN	3.02512

Microarray core,
NTU Research Center for Medical Excellence

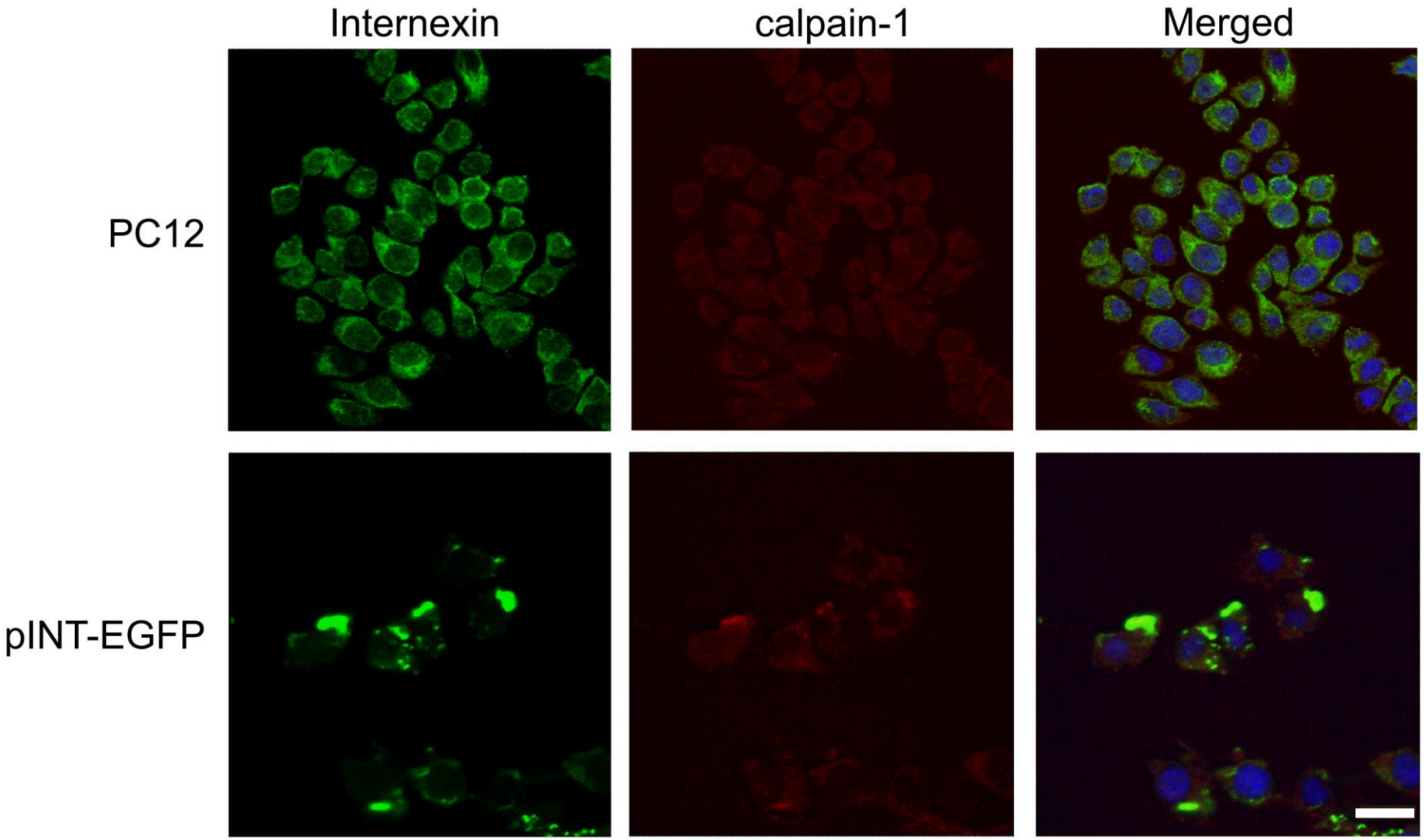
Microarray: pINT-EGFP Day 6 Vs. PC12 Day 6

Calpain family of proteases	Regulation	Fold
calpain 1	UP	2.55472
calpain 2	UP	2.31951
Calpastatin (Inhibitor of calpain)	UP	2.50921

Caspase family of proteases	Regulation	Fold
caspase 1	UP	2.40321
caspase 6	UP	2.19194
caspase 9	UP	3.8618
caspase 12	UP	7.17128
caspase 8 associated protein 2 (predicted)	UP	3.13442
apoptosis, caspase activation inhibitor (predicted)	UP	3.00528
caspase recruitment domain protein 9	UP	3.88923

Calpain Pattern

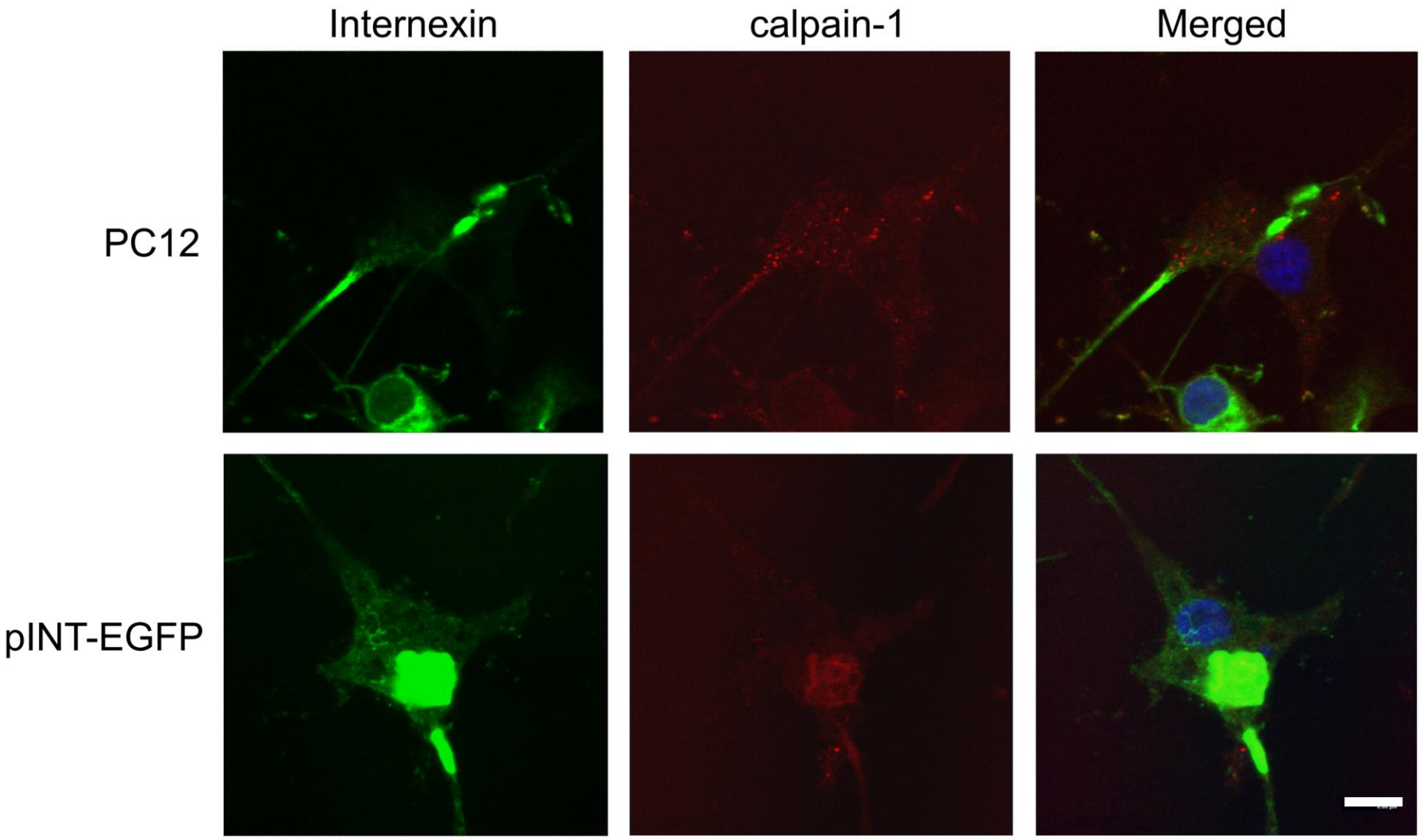
Day 0



Scale bar= 8μm

Calpain Pattern

NGF induction, Day 6



Scale bar= 8μm

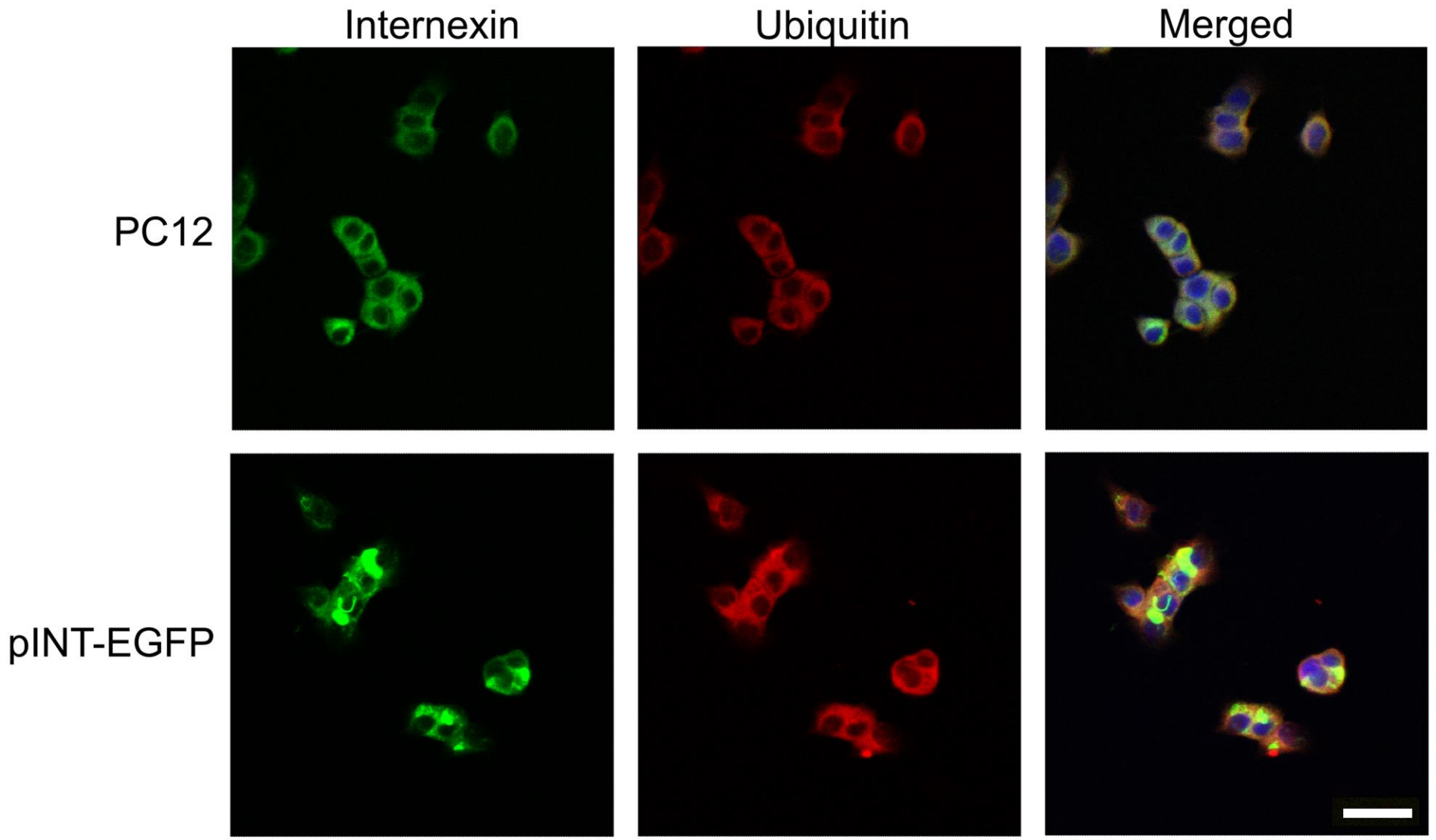
Microarray: pINT-EGFP Day 6 vs. PC12 Day 6

Ubiquitin proteasome system	Regulation	Fold
ubiquitin specific protease 13 (isopeptidase T-3) (predicted)	DOWN	16.9253
ubiquitin specific protease 13 (isopeptidase T-3) (predicted)	DOWN	4.14307
Ubiquitin-conjugating enzyme E2G 2 (predicted)	DOWN	2.93784
Itchy homolog E3 ubiquitin protein ligase	DOWN	2.88891
proteosome (prosome, macropain) subunit, beta type 9	DOWN	3.29785

Heat shock proteins and molecular chaperones	Regulation	Fold
Heat shock 70kD protein 1A	UP	5.56855
heat shock 70kD protein 1A /// heat shock 70kD protein 1B	UP	2.86266
heat shock protein, alpha-crystallin-related, B6	DOWN	2.82089
heat shock protein 1 (chaperonin)	DOWN	2.17408

Ubiquitin pattern

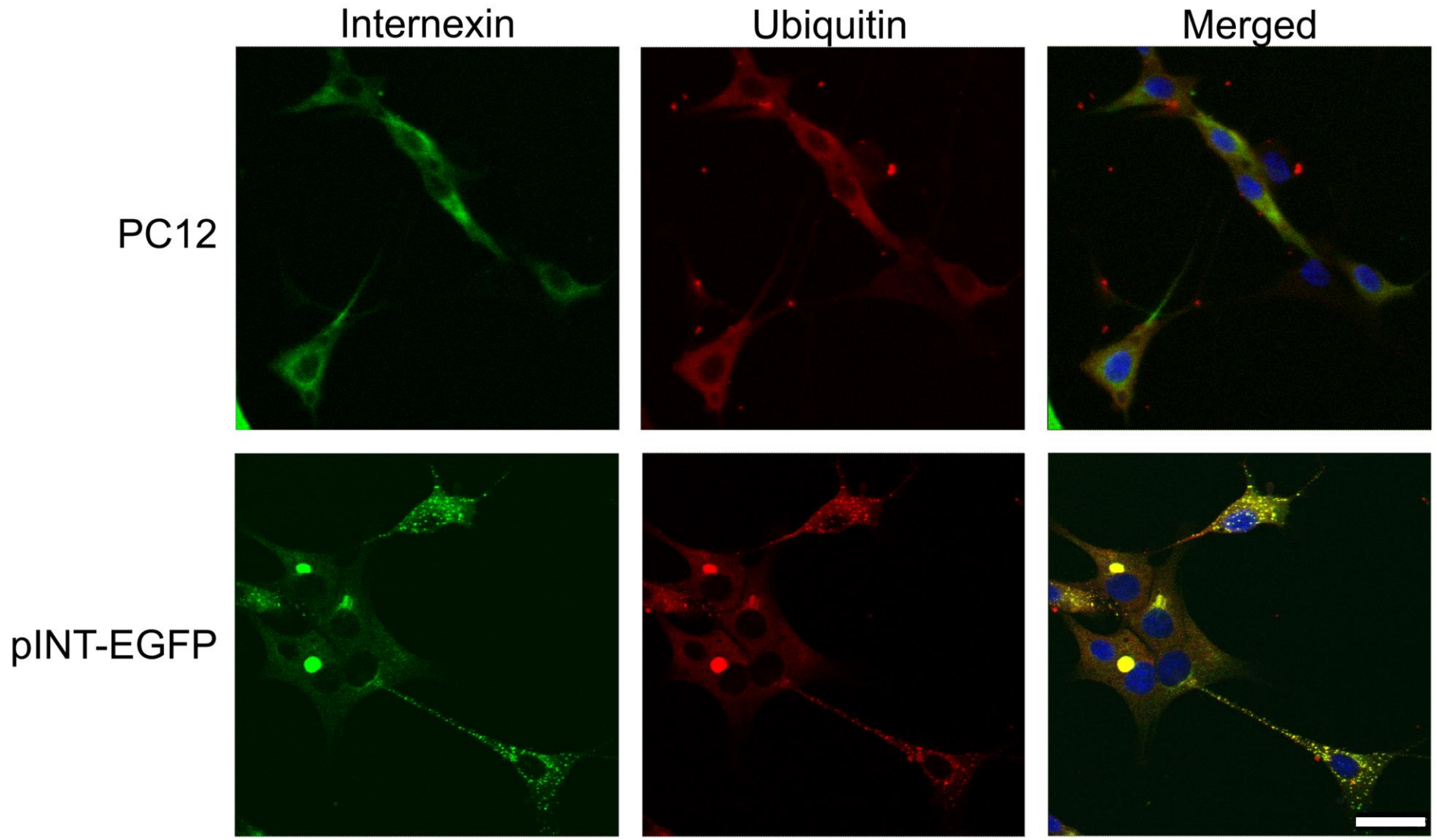
Day 0



Scale bar= 20μm

Ubiquitin pattern

NGF induction, Day 6

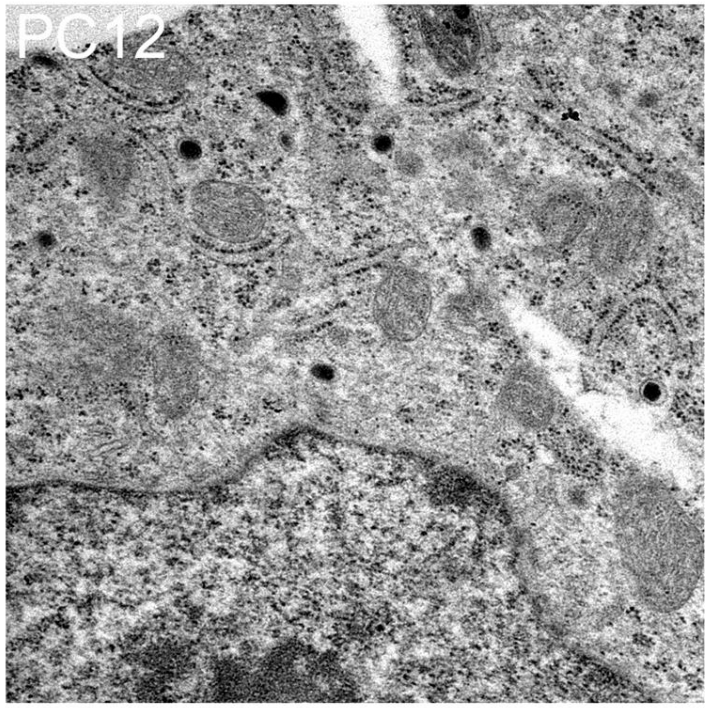


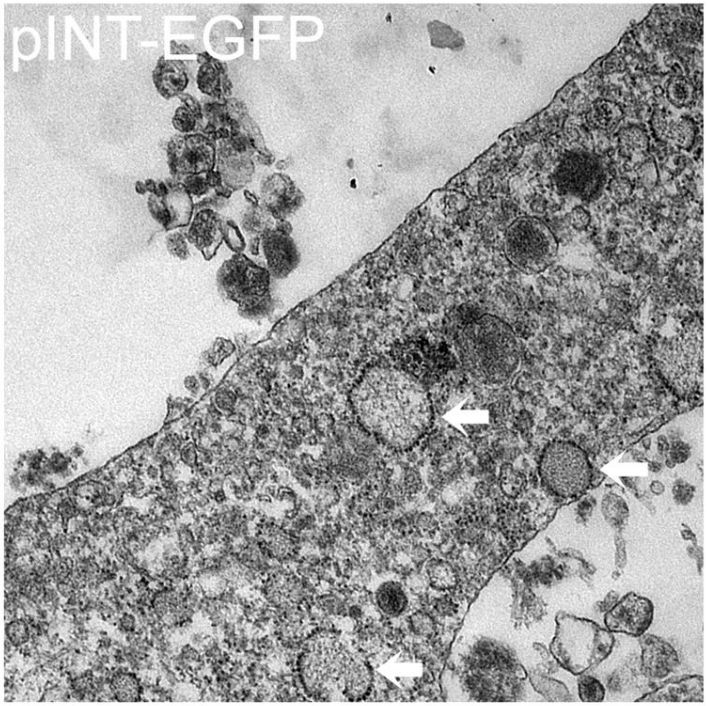
Scale bar= 20µm

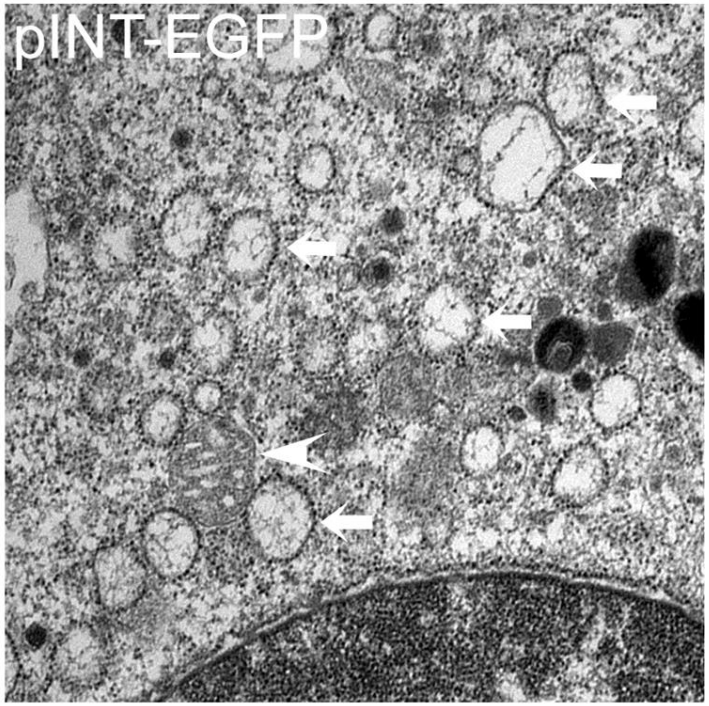
Ultrastructural patterns :

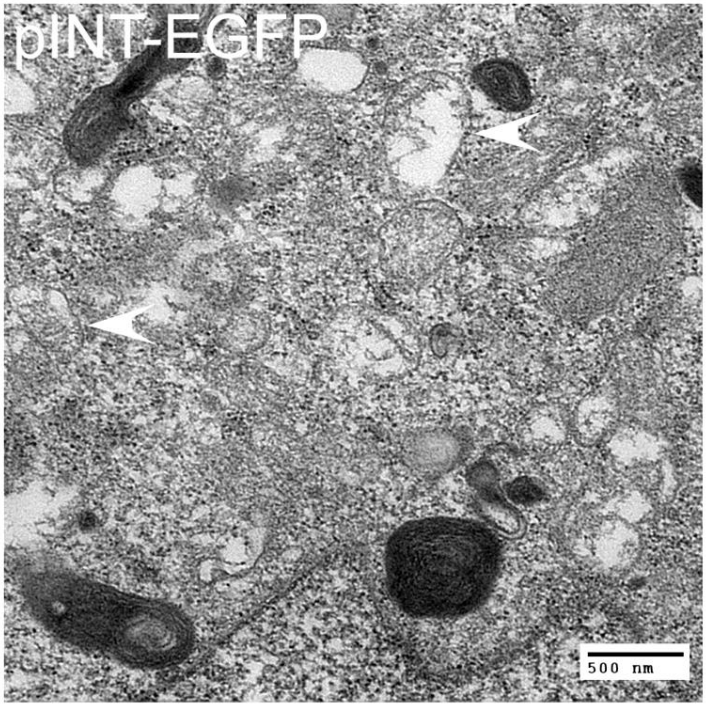
nontransfected PC12 cells and pINT-EGFP-transfected cells after NGF induction for 8 days

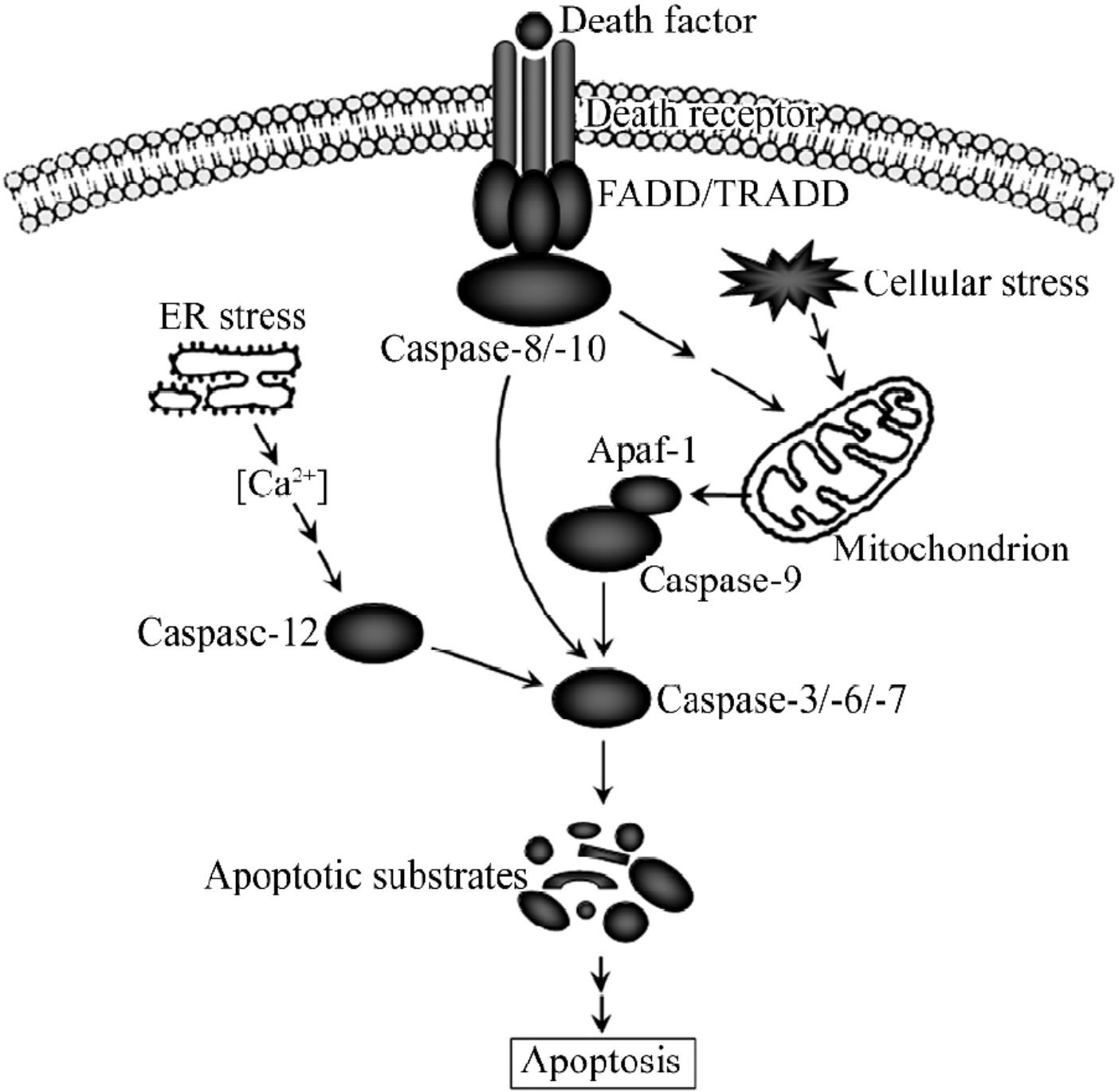
ER stress?

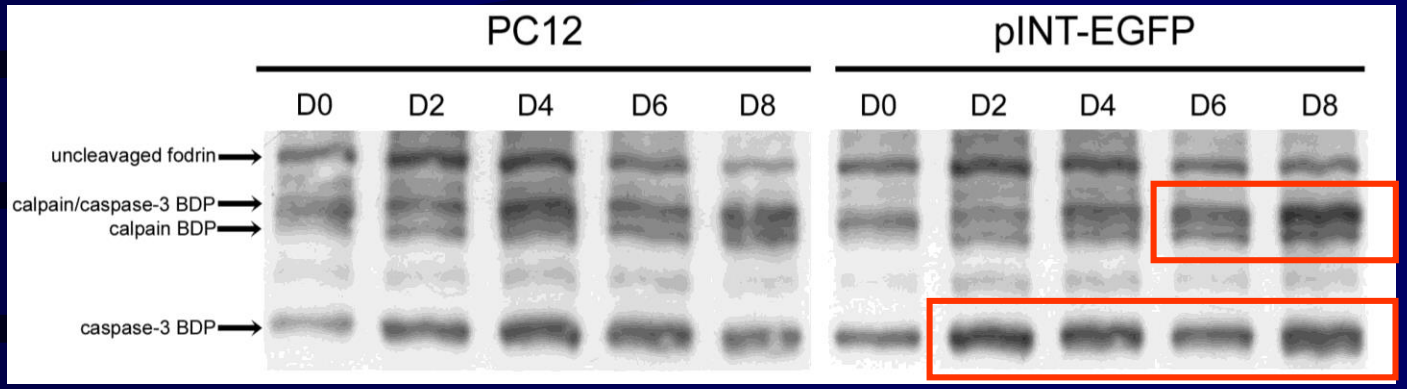


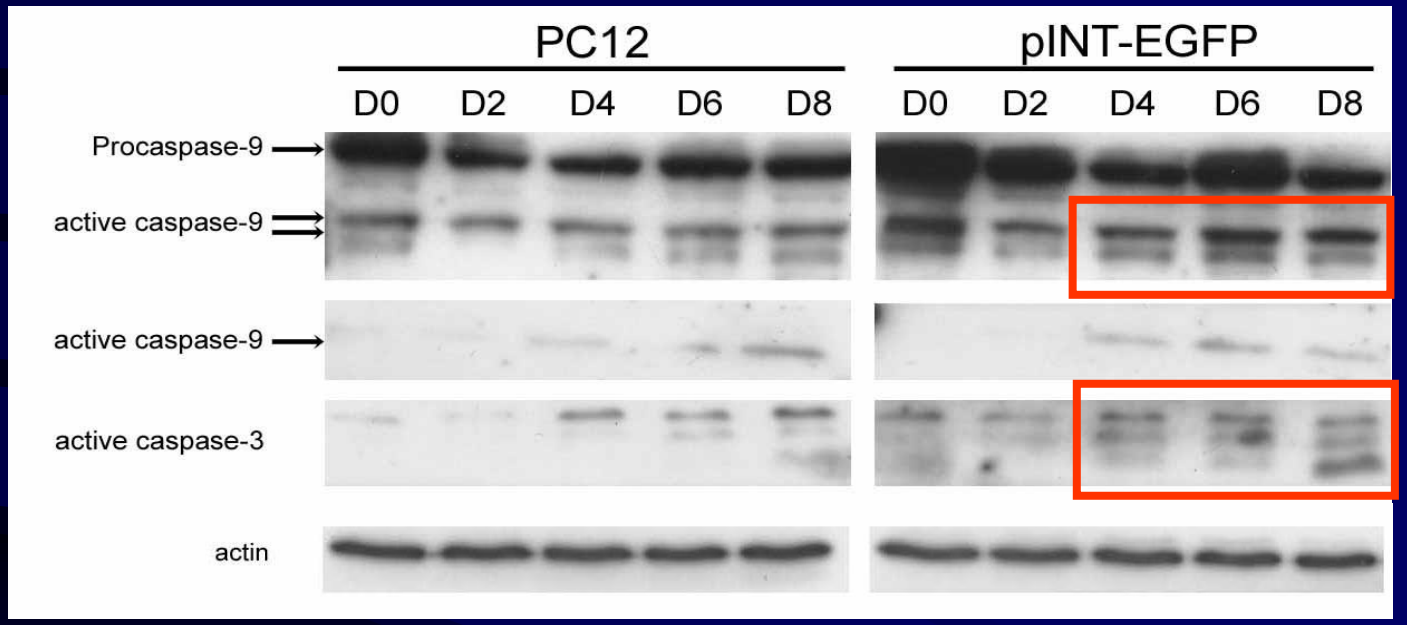


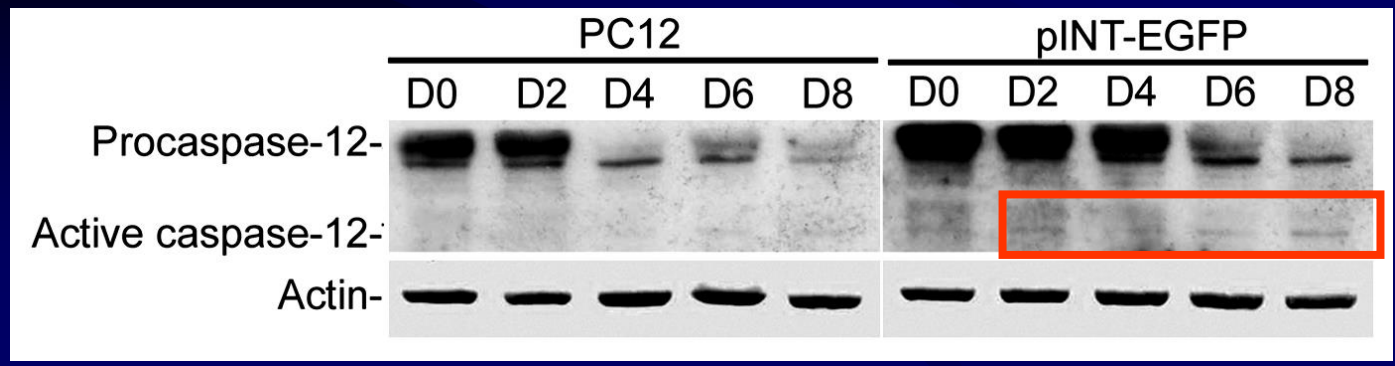




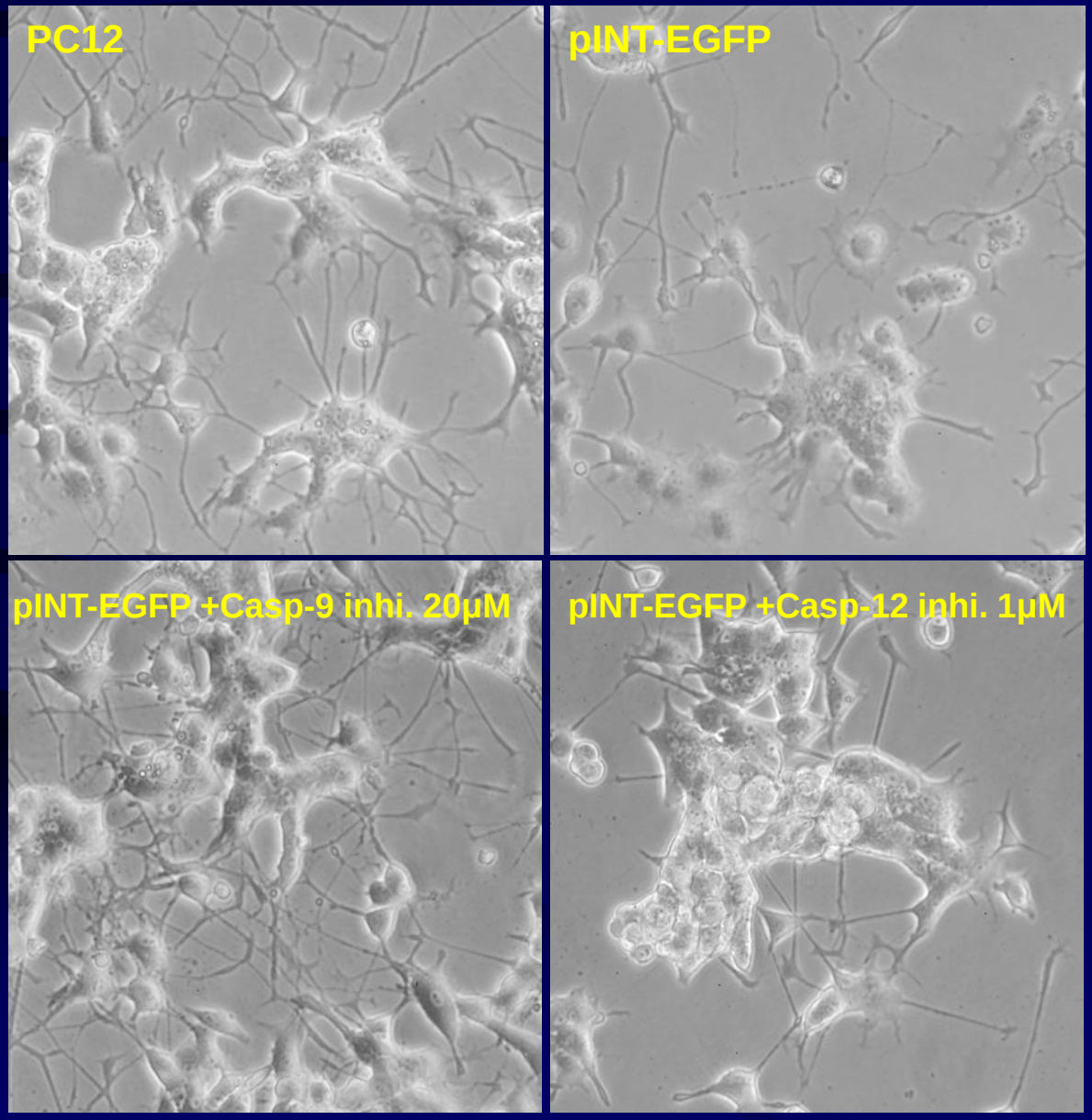

Death factor
Death receptor
FADD/TRADD
Cellular stress
ER stress
Caspase-8/-10
Apaf-1
[Ca2+]
Mitochondrion
Caspase-9
Caspase-12
Caspase-3/-6/-7
Apoptotic substrates
Apoptosis


PC12
pINT-EGFP
D0
D2
D4
D6
D8
uncleavaged fodrin
calpain/caspase-3 BDP
calpain BDP
caspase-3 BDP

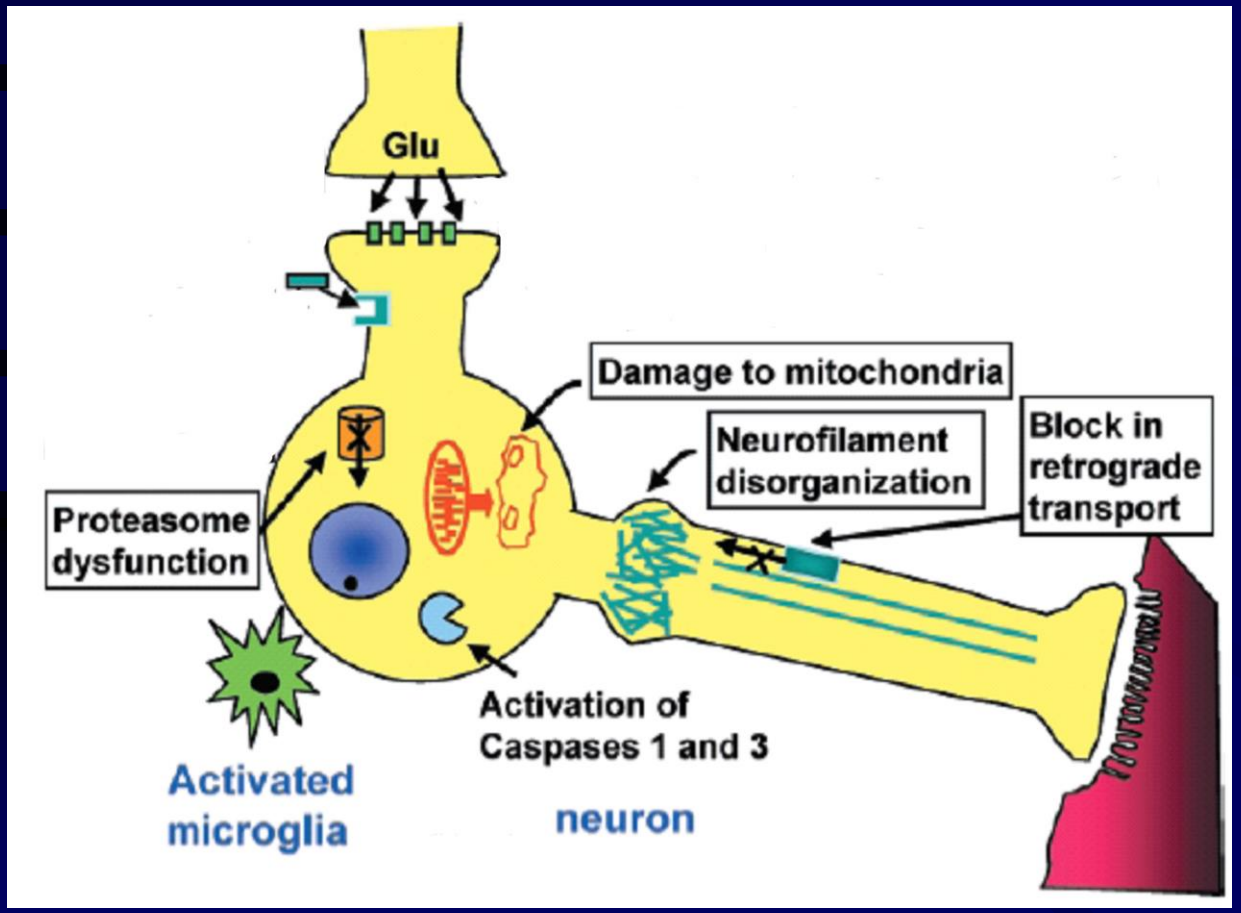

PC12
pINT-EGFP
D0
D2
D4
D6
D8
Procaspase-9
active caspase-9
active caspase-9
active caspase-3
actin

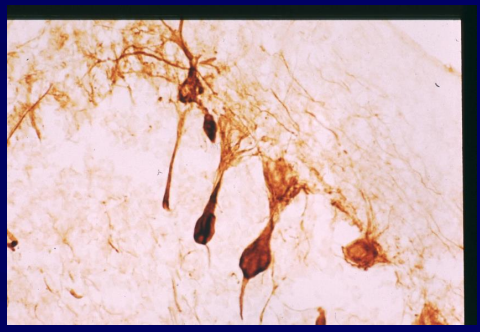

PC12
pINT-EGFP
D0
D2
D4
D6
D8
Procaspase-12-
Active caspase-12-
Actin-

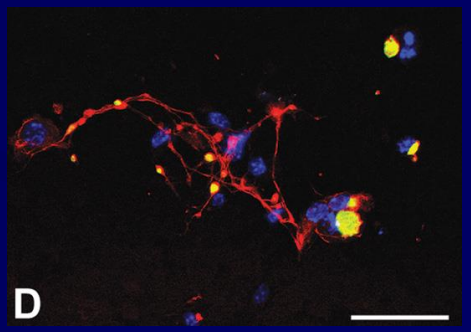
Rescue Effect of Caspase-9 and Caspase-12 inhibitors
(8-day NGF induction)

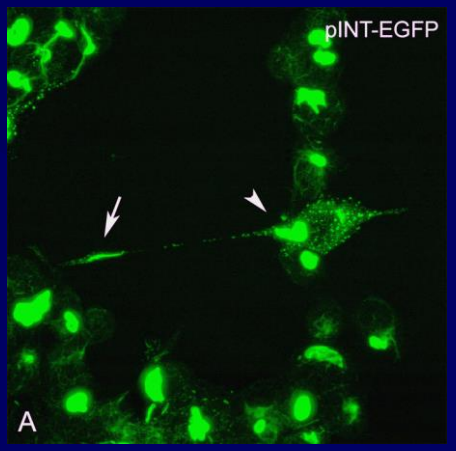


Abnormal accumulation of α-internexin and other cytoskeletal components may impair the axonal transport and subsequently turn on the cascade of neuronal apoptosis during development.



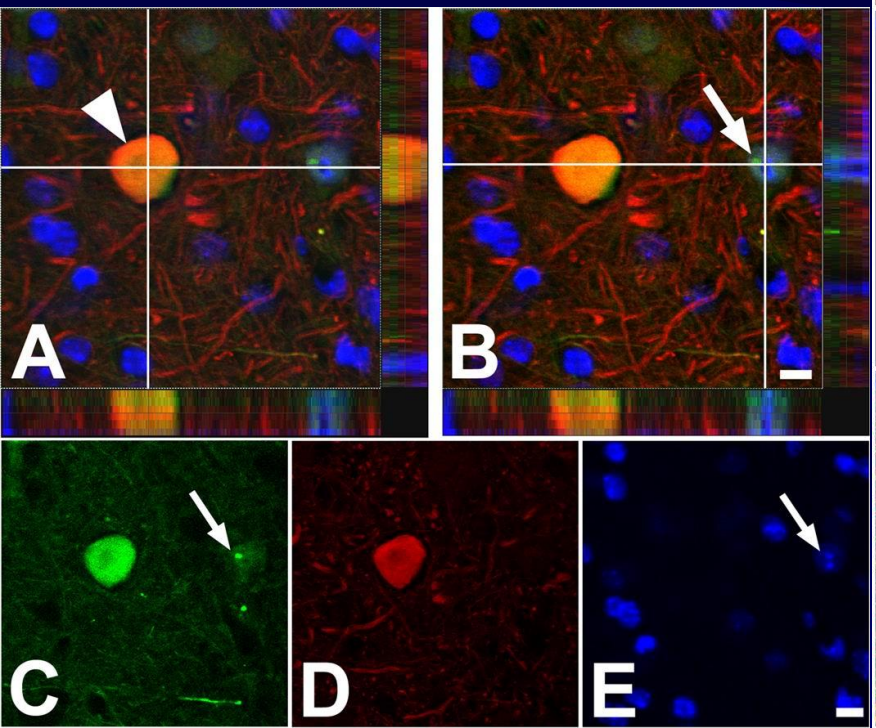


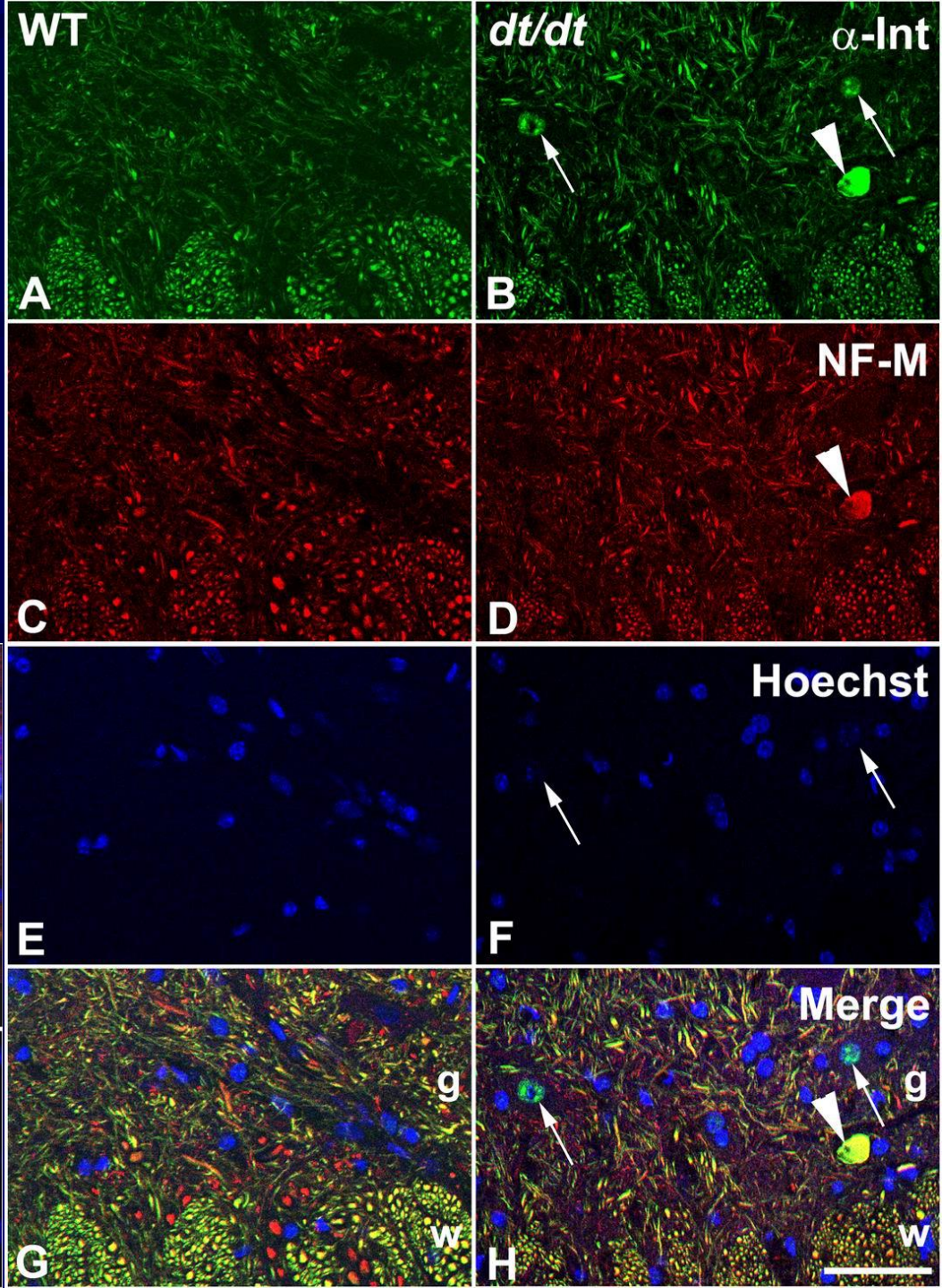




Thank you for your attention!

Intermediate filaments mis-accumulated within motor neurons in the spinal cord of *dt* mice





Motor neurons could survive in the spinal cord of *dt* mice

Why?

Neuronal type differences?

DRG neuron vs. motor neuron