Cellular and Animal Models for the Neuronal Degeneration

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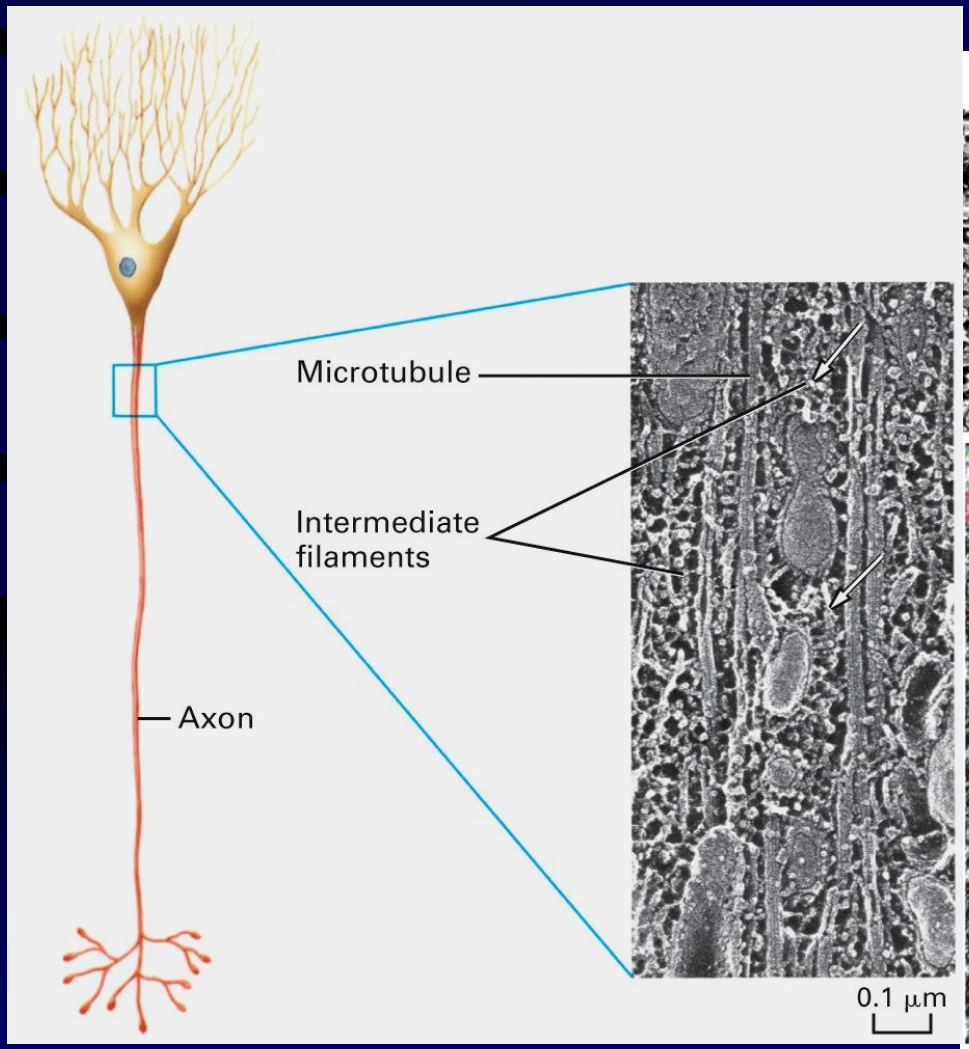
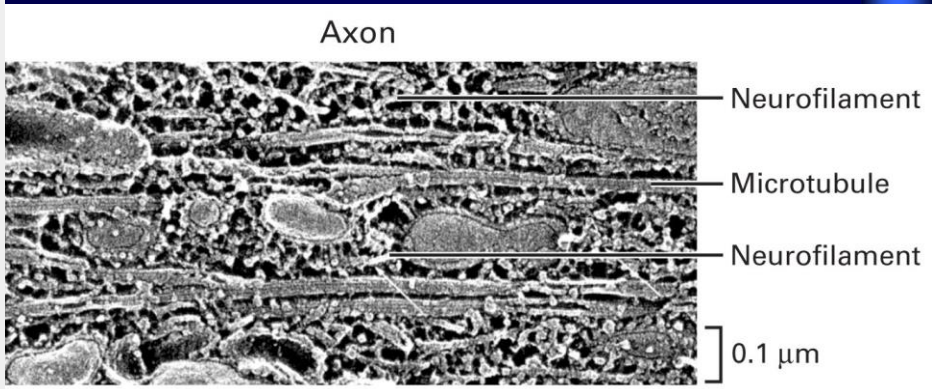
錢宗良

Neuronal Cytoskeletons





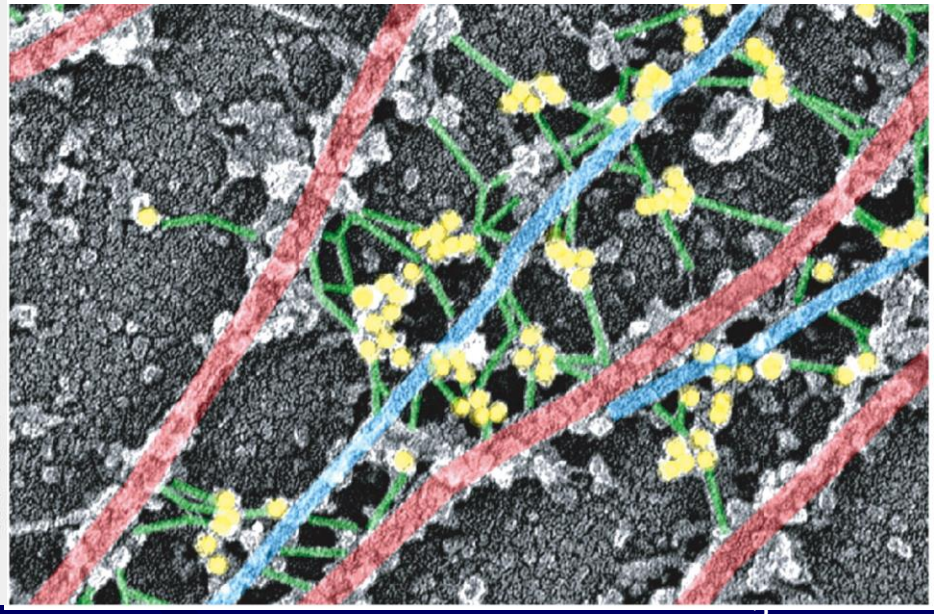
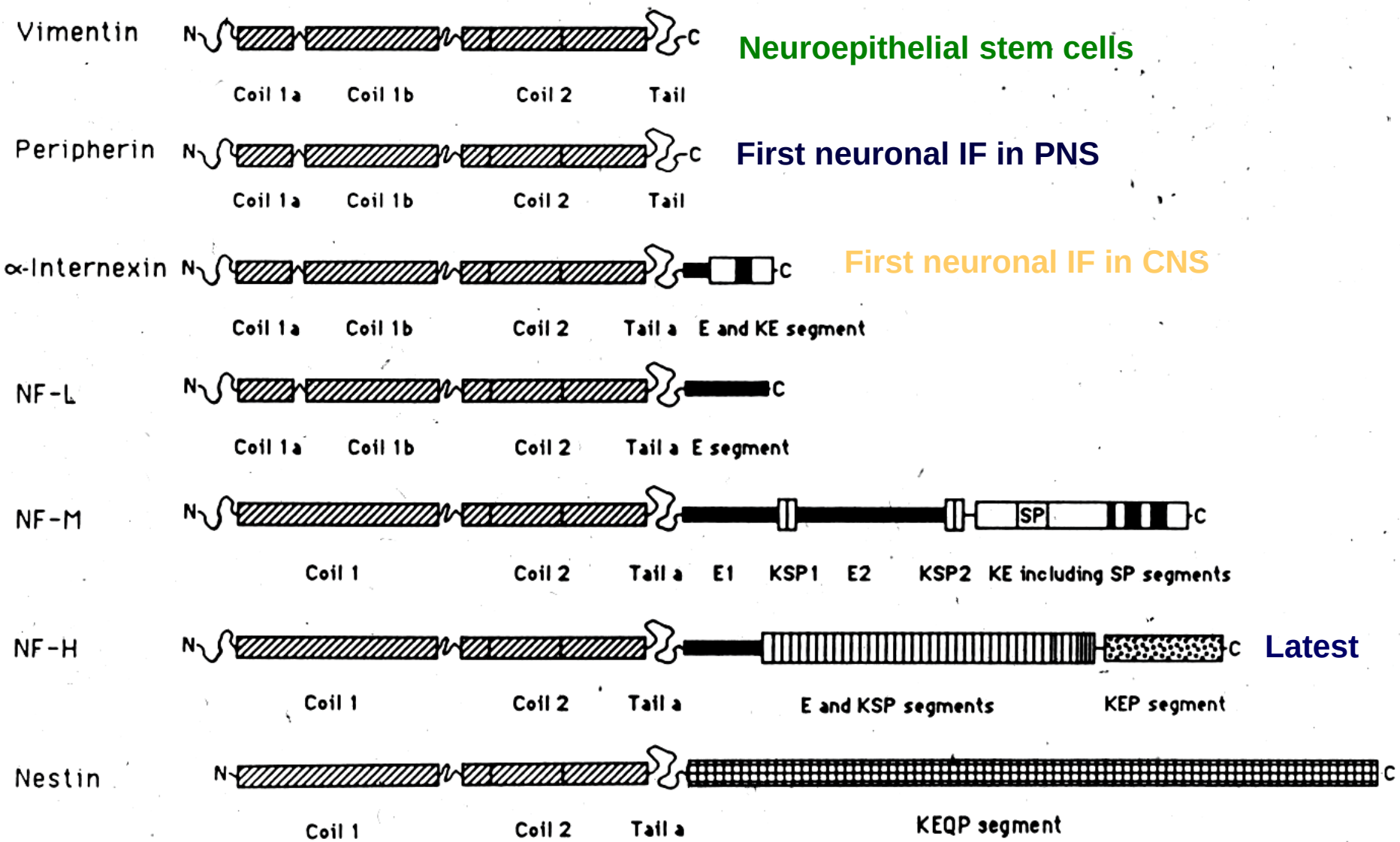


TABLE 19-4 Primary Intermediate Filaments in Mammals

IF Protein	MW (10^{-3})*	Filament Form	Tissue Distribution
NUCLEAR LAMINS			
Lamin A	70	Homopolymer	Nucleus
Lamin B	67	Homopolymer	Nucleus
Lamin C	67	Homopolymer	Nucleus
KERATINS†			
Acidic keratins	40–57	Heteropolymers	Epithelia
Basic keratins	53–67	Heteropolymers	Epithelia
TYPE III INTERMEDIATE FILAMENTS			
Vimentin	57	Homo- and heteropolymers	Mesenchyme (fibroblasts)
Desmin	53	Homo- and heteropolymers	Muscle
Glial fibrillary acidic protein	50	Homo- and heteropolymers	Glial cells, astrocytes
Peripherin	57	Homo- and heteropolymers	Peripheral and central neurons
NEUROFILAMENTS			
NF-L	62	Homopolymers	Mature neurons
NF-M	102	Heteropolymers	Mature neurons
NF-H	110	Heteropolymers	Mature neurons
Internexin	66	—	Developing CNS

*Intermediate filaments show species-dependent variations in molecular weight (MW).
†More than 15 isoforms of both acidic and basic keratins are known.

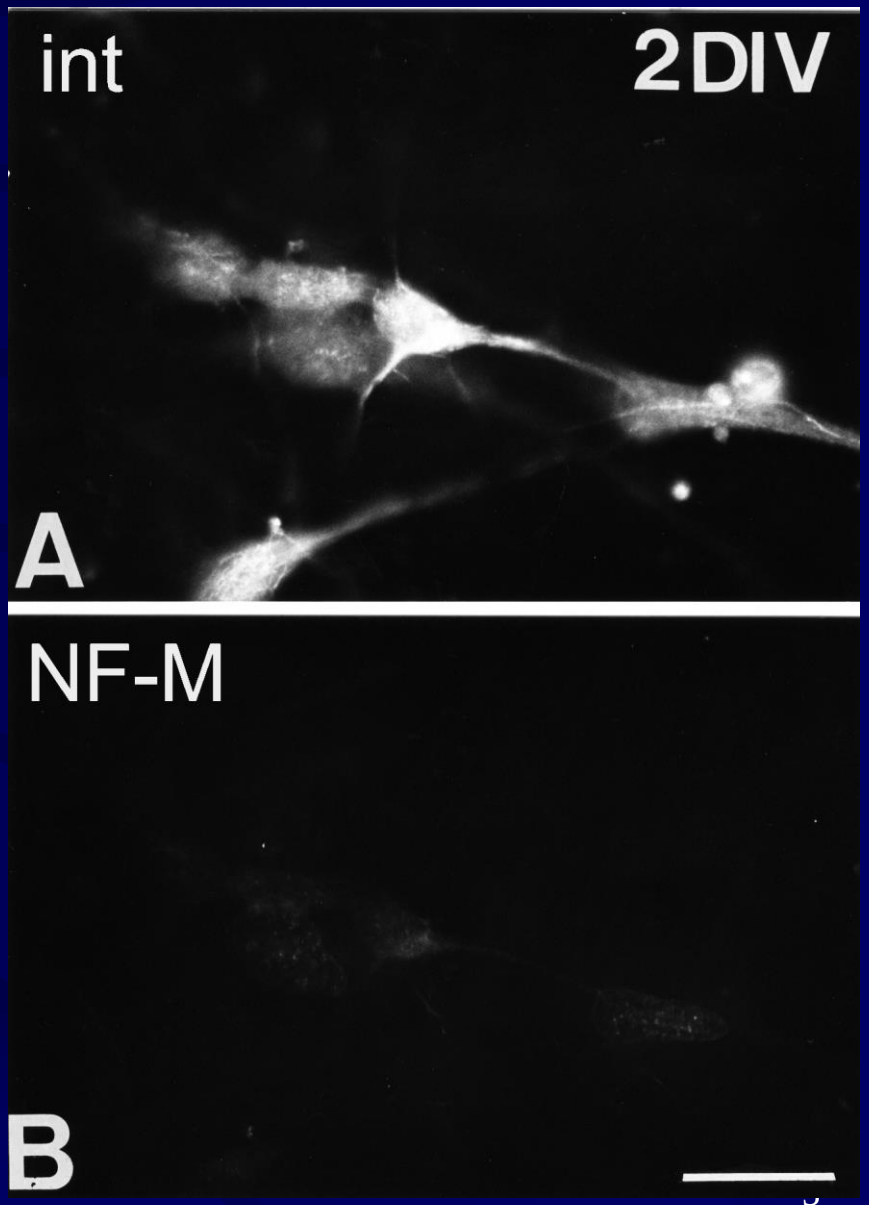
Seven Intermediate Filament Proteins in Neural Differentiation



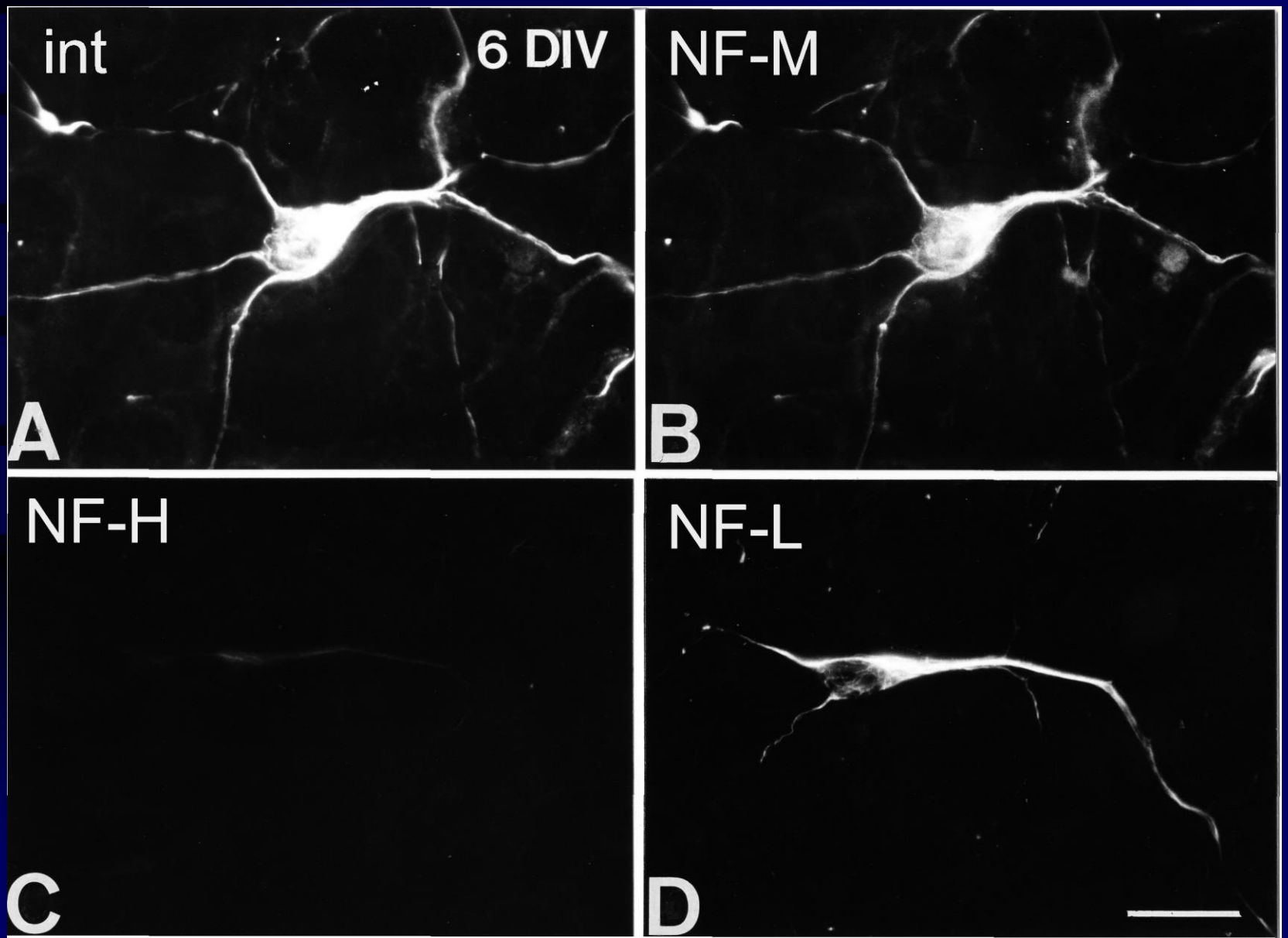
Primary culture of embryonic (E15) hippocampal cells

α-internexin: a 66 kD protein,

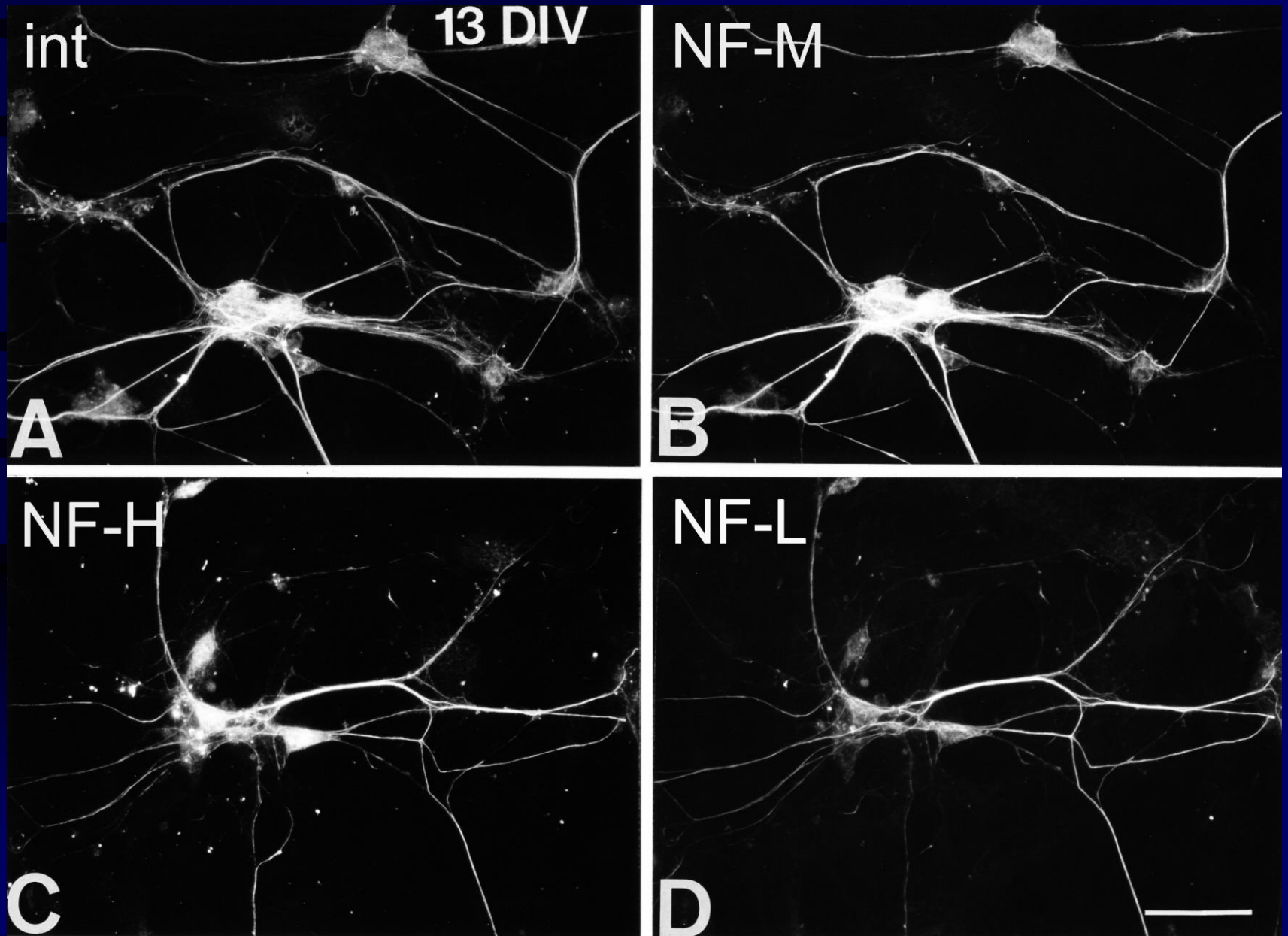
the first neuronal intermediate filament protein expressed in the post-mitotic neurons of developing mammalian central nervous system



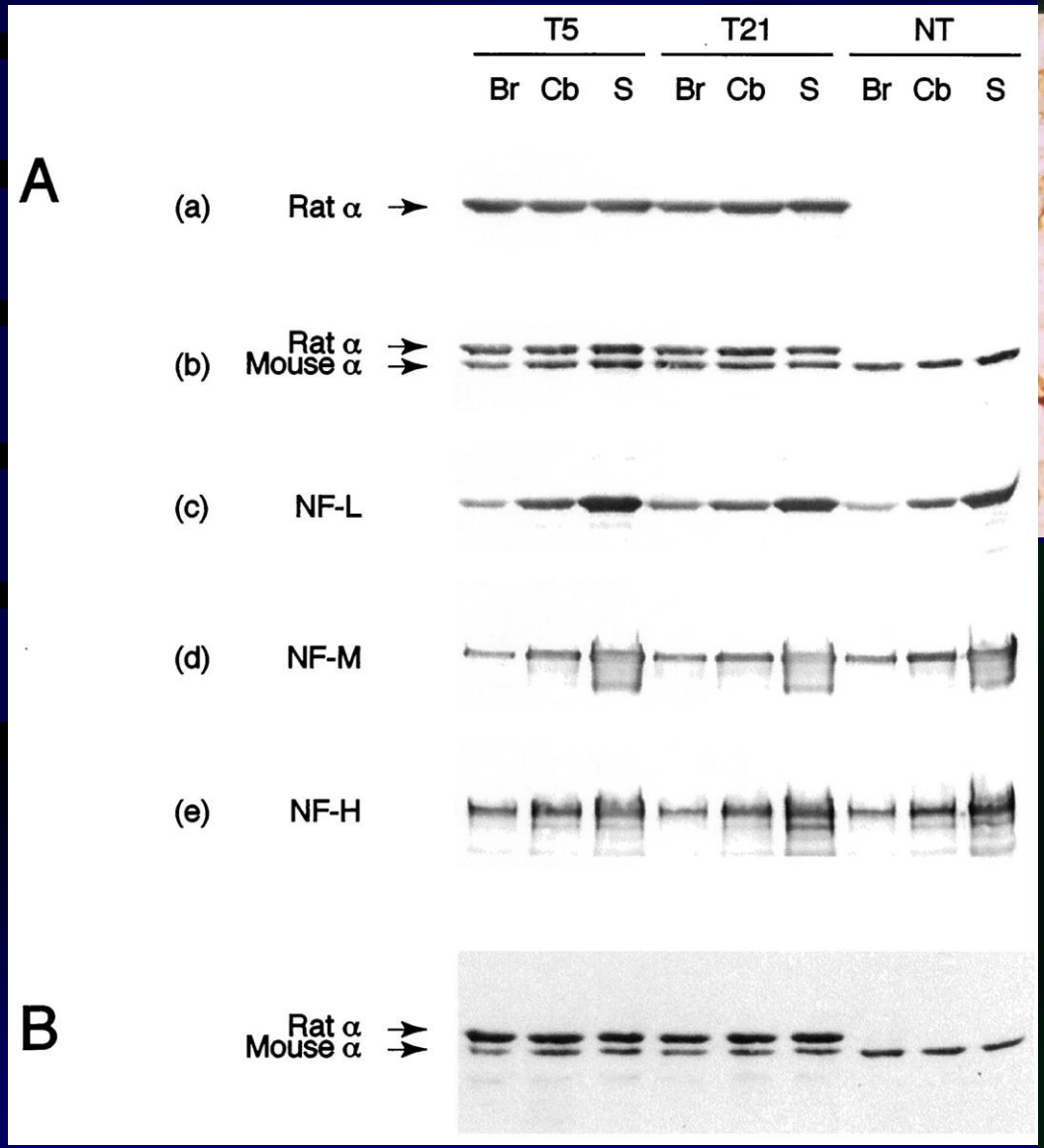
Internexin, NF-M, NF-L but not NF-H expressed in the 6 days *in vitro* (DIV) culture of hippocampal neurons

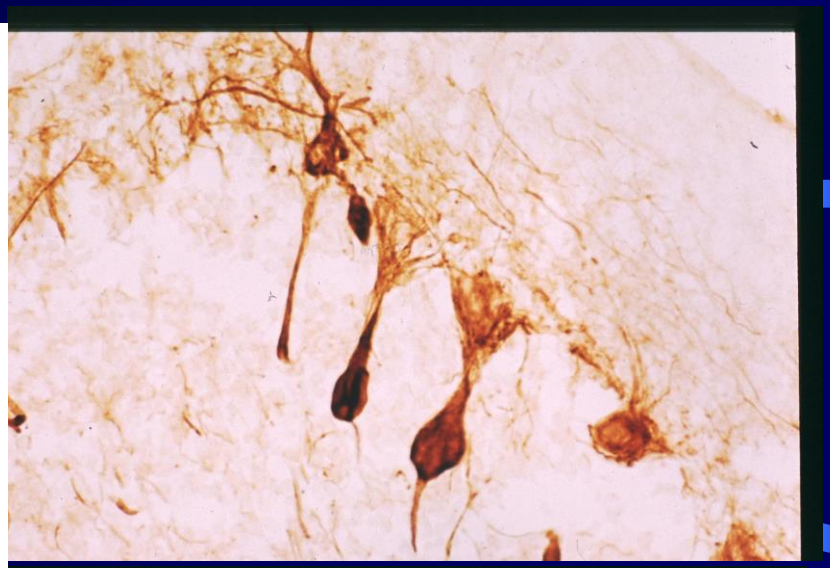


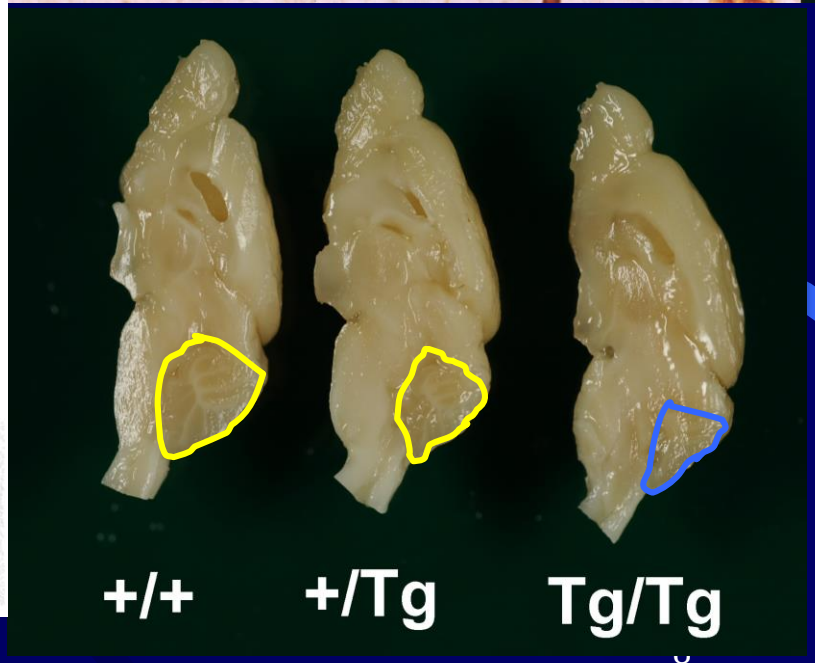
Internexin and Neurofilament Triplet Proteins (NF-L, NF-M and NF-H) all expressed in the 13 DIV hippocampal neurons

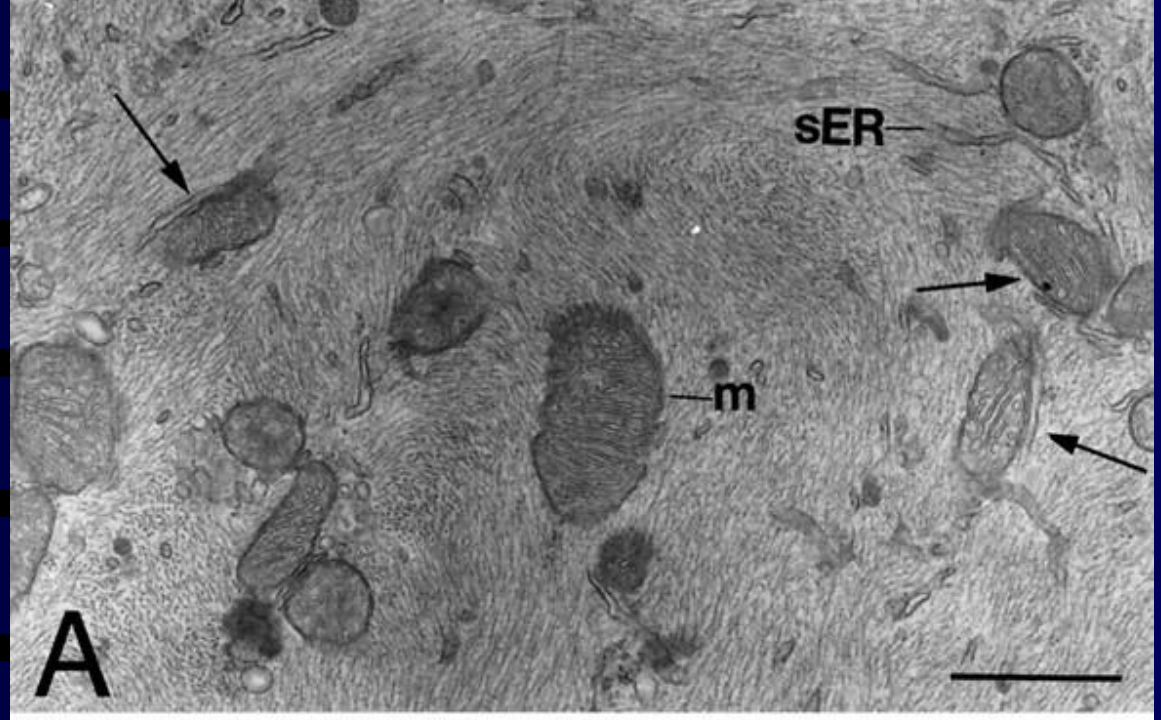


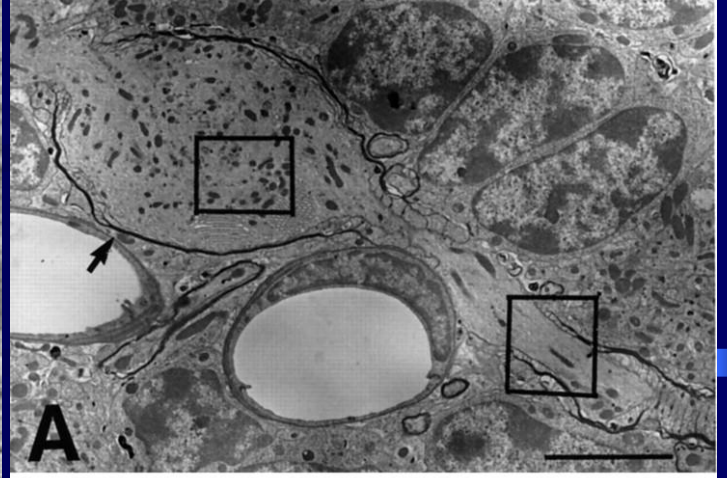
Animal model for cerebellar atrophy (J. Neurosci. 19:2974-2986, 1999)

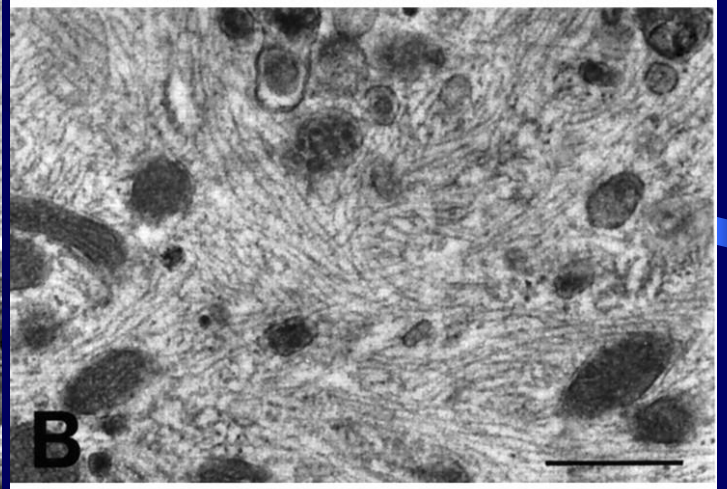


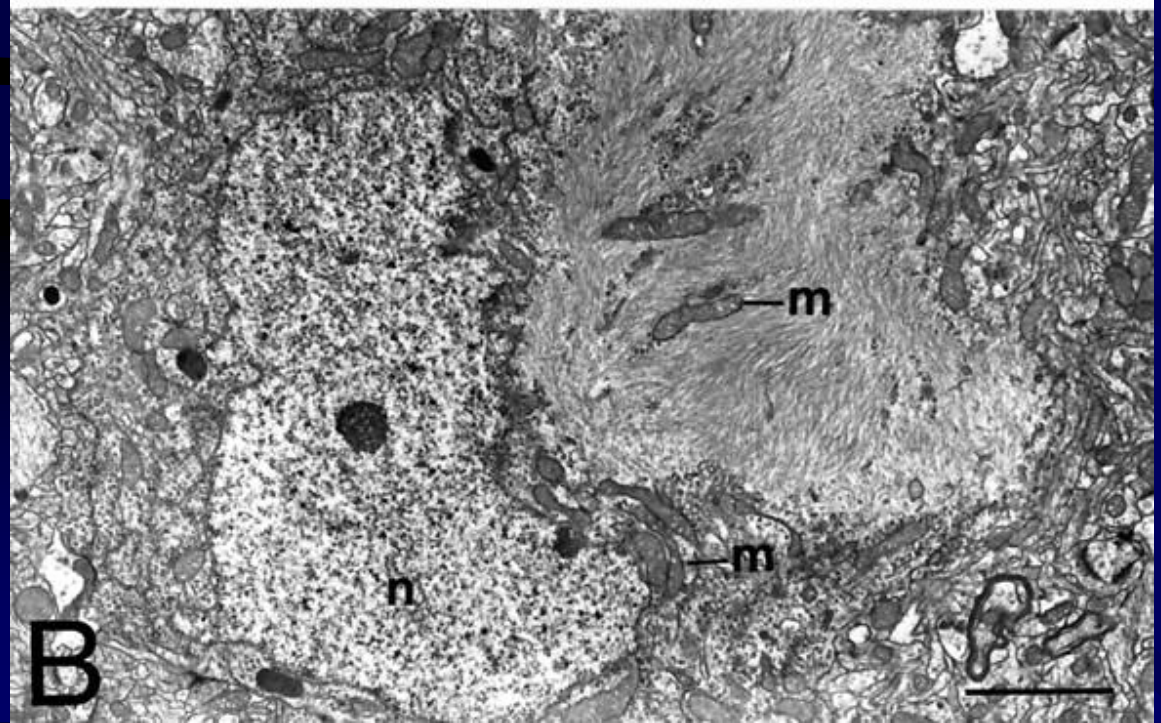





sER
m
A


A


B

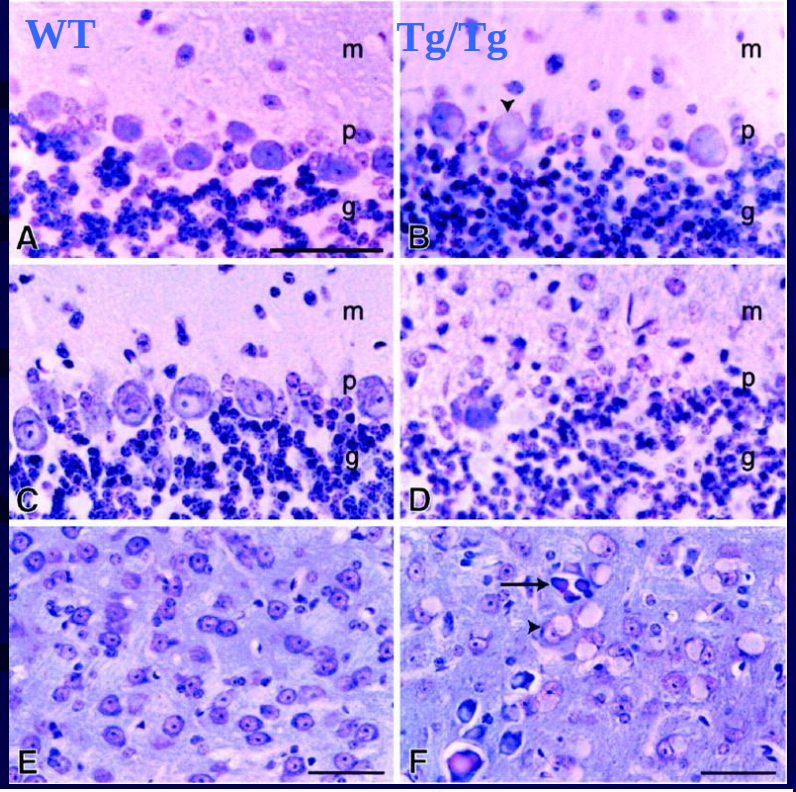

m
m
n
B

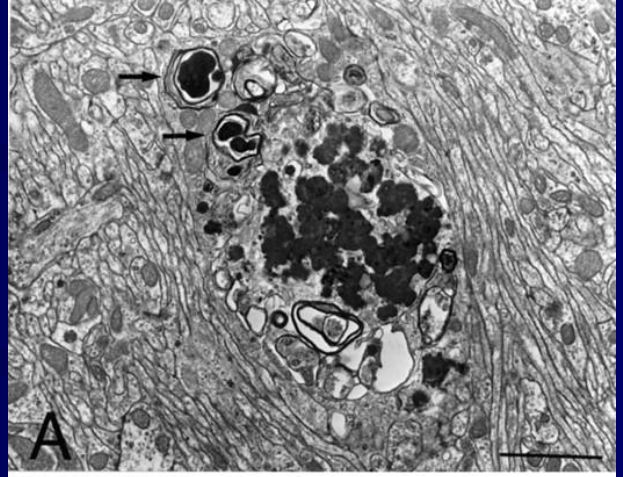

C

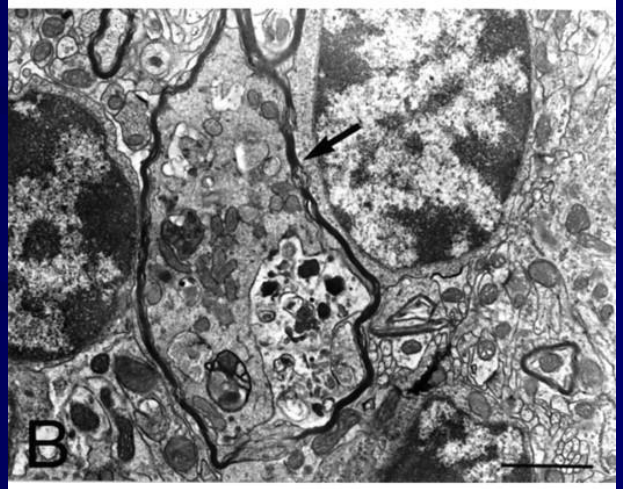
12 m cerebella

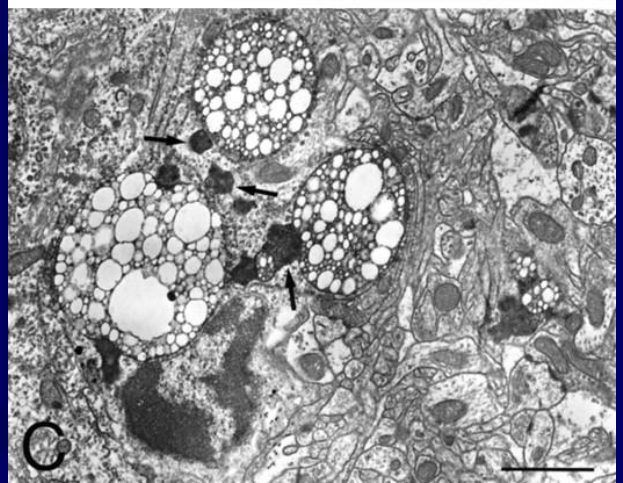
18 m cerebella

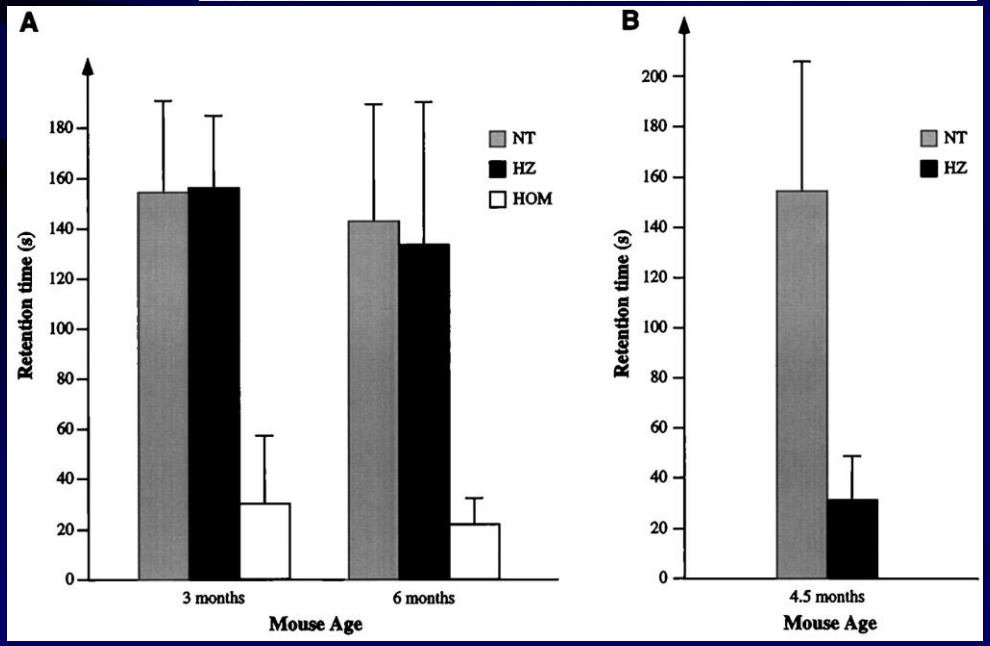
18 m thalamus

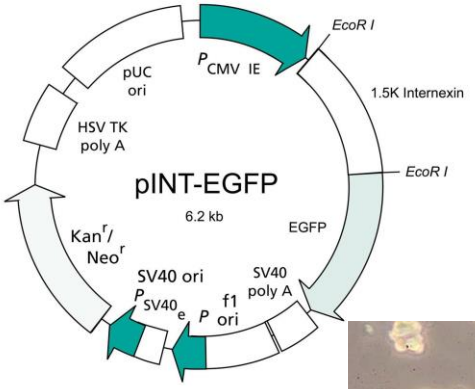








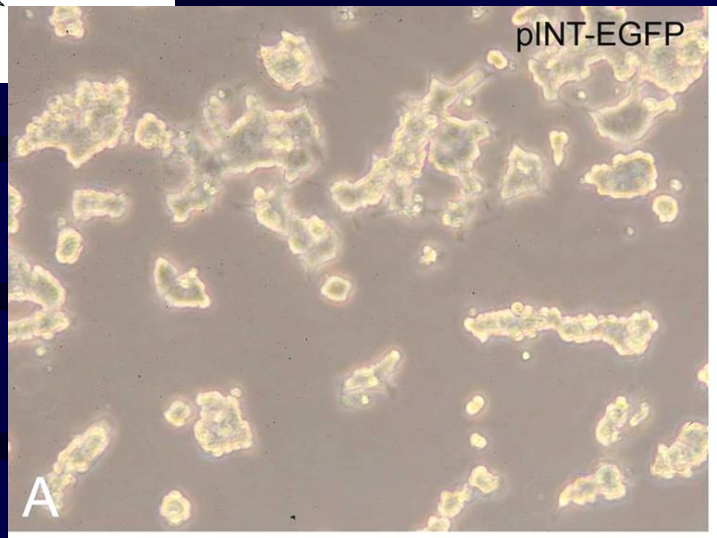


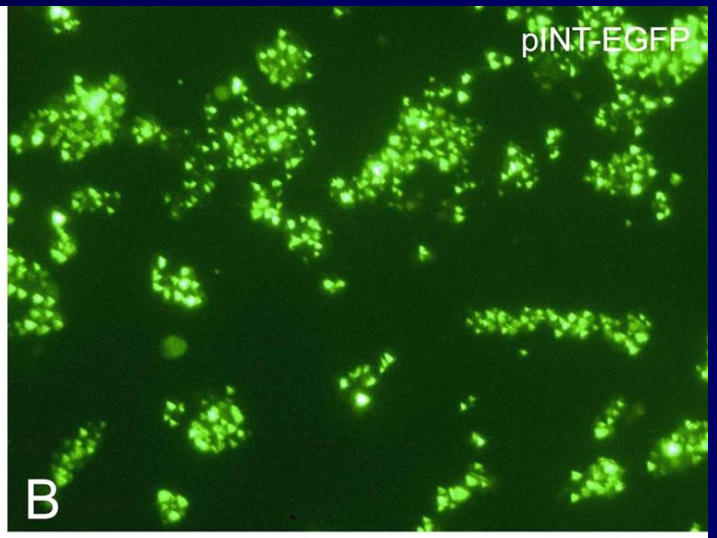


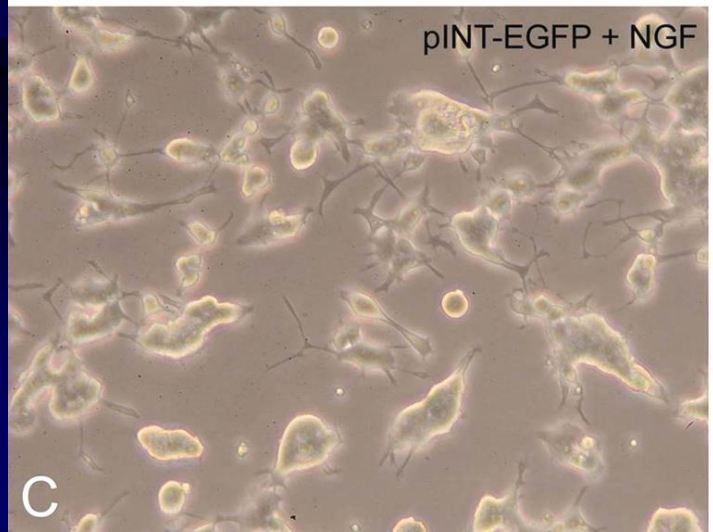
Overexpression of neuronal intermediate filament internexin in the PC-12 cell line

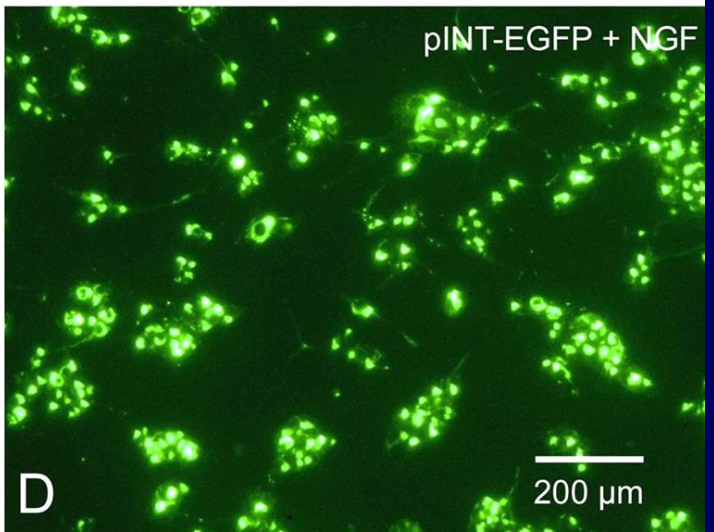
pINT-EGFP transfected cells

Day 3



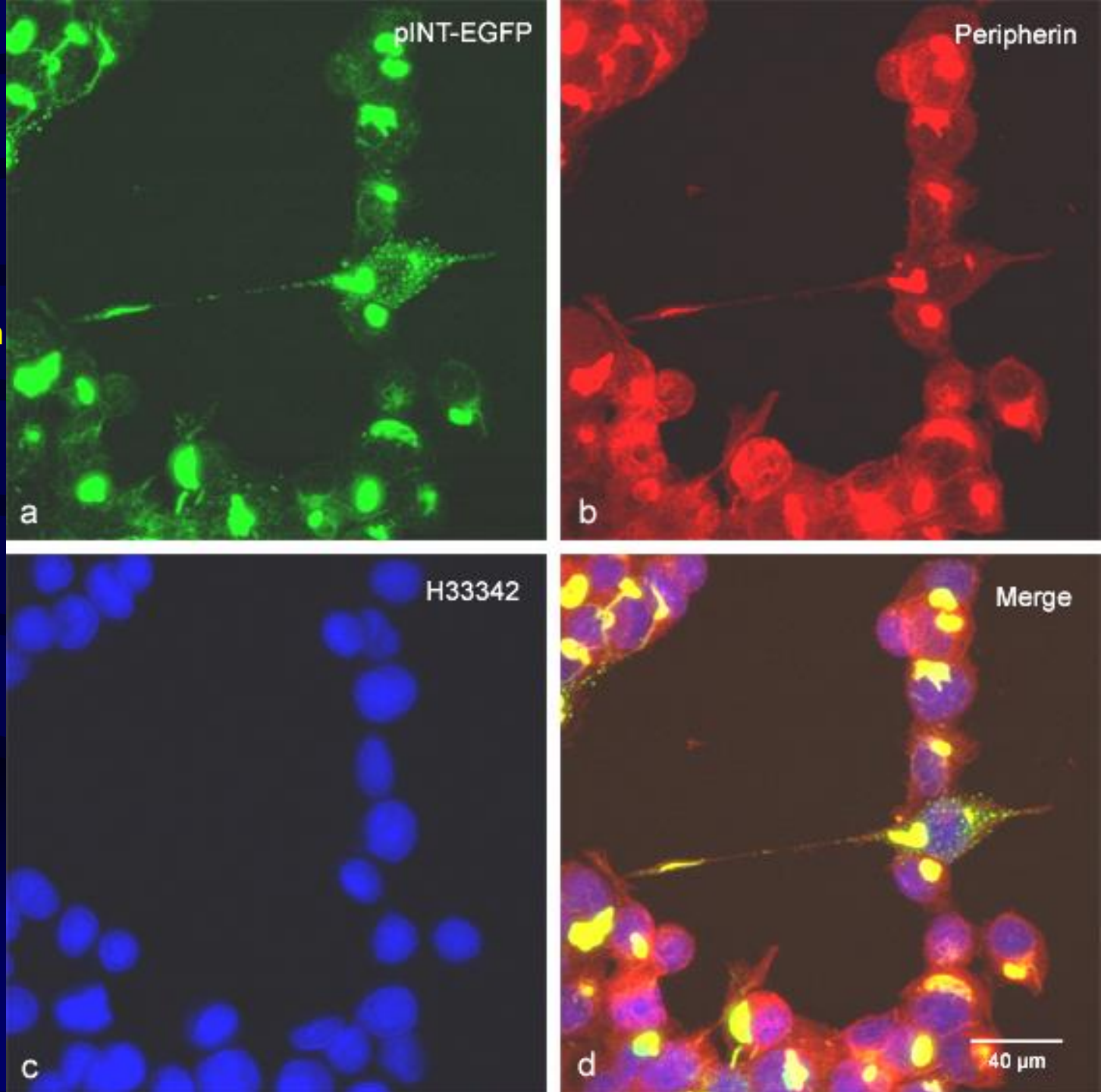




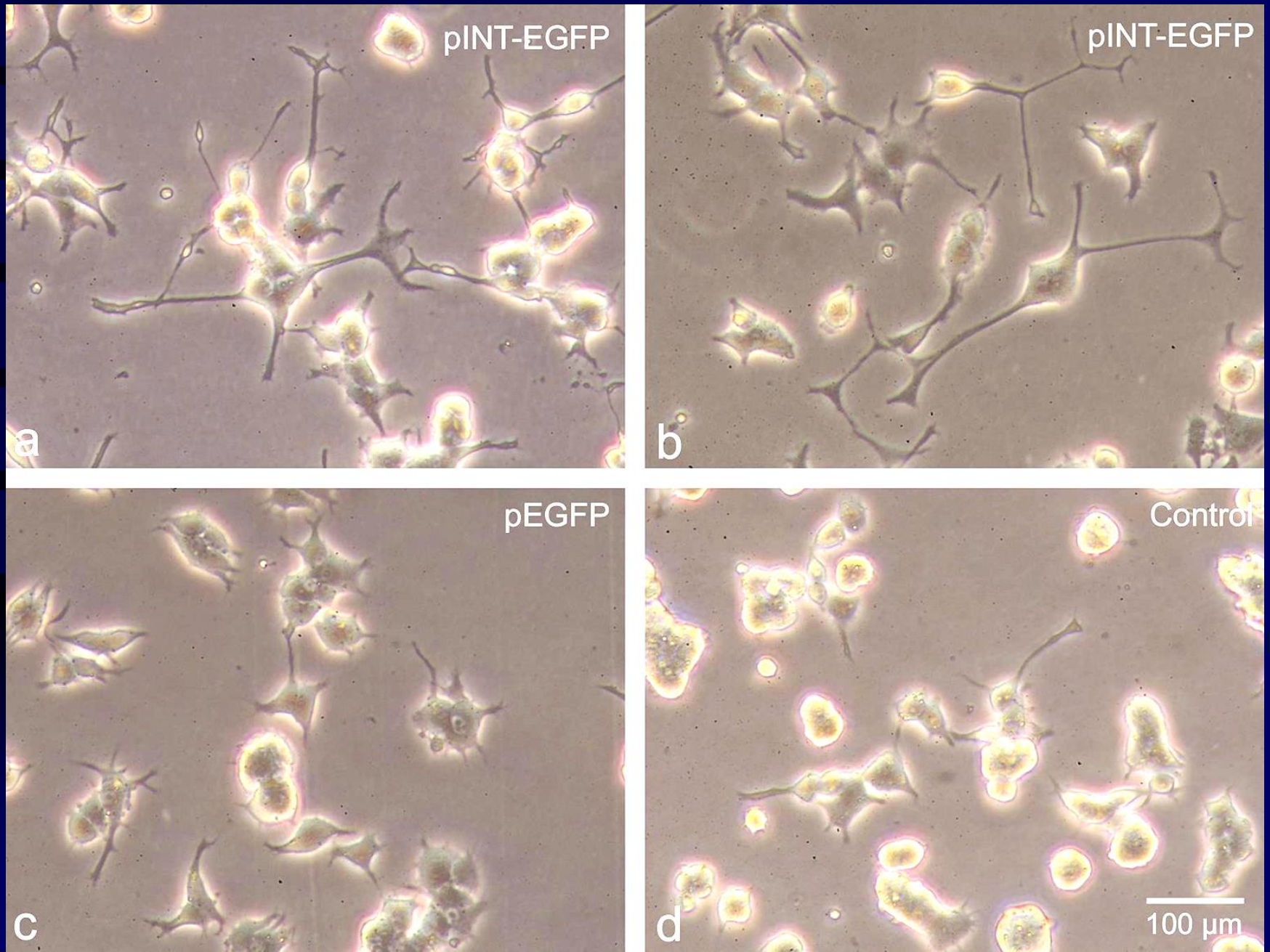


Confocal Pattern

3-day NGF induction

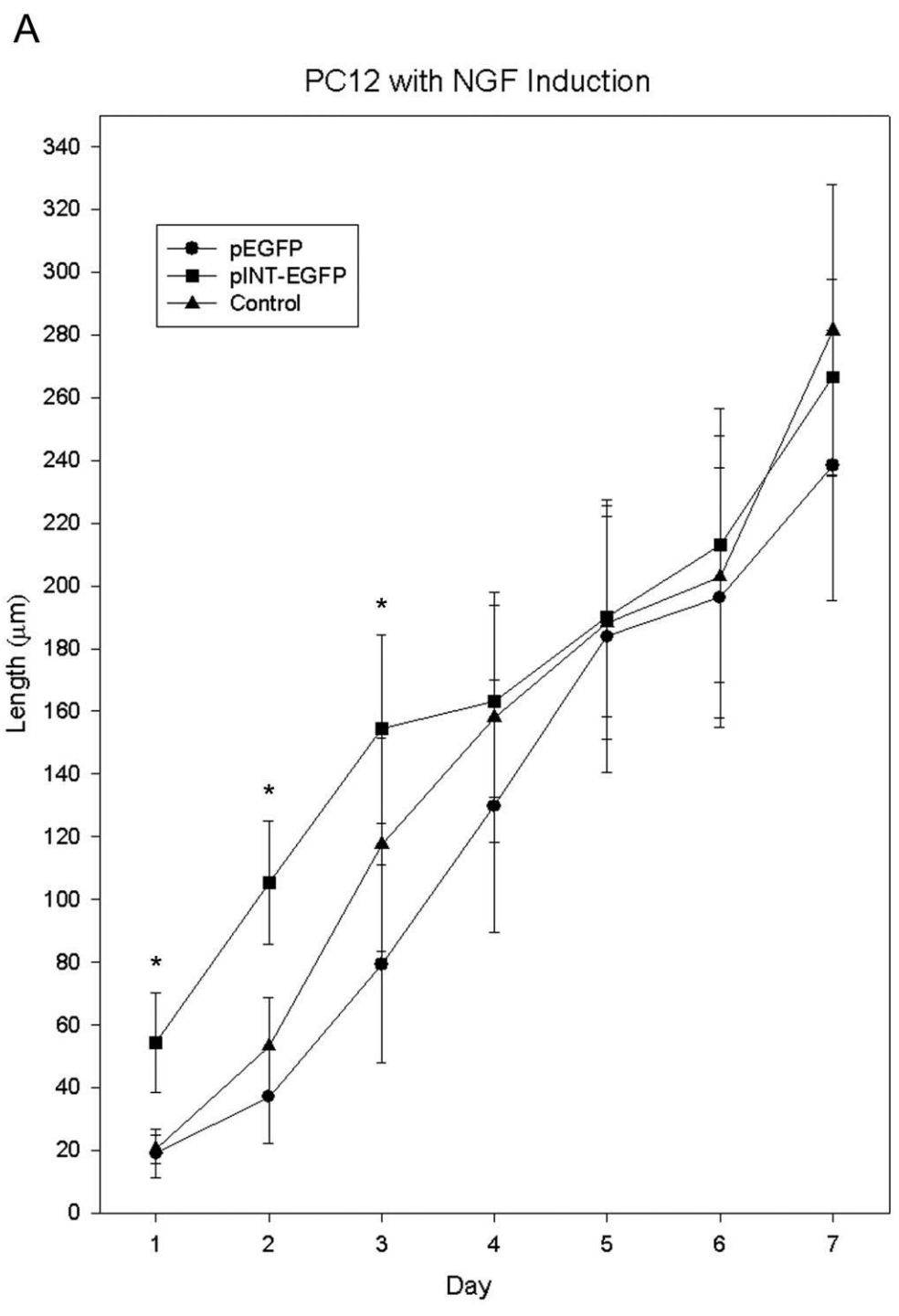


Cells after 2-day NGF induction

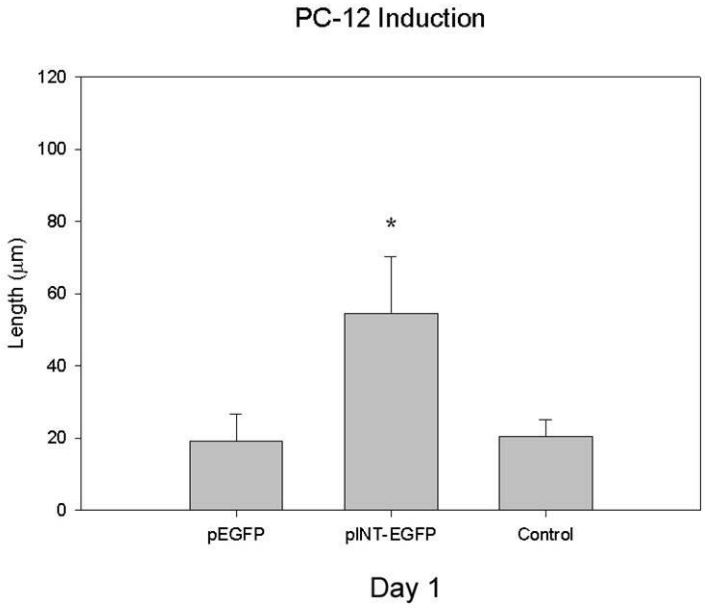


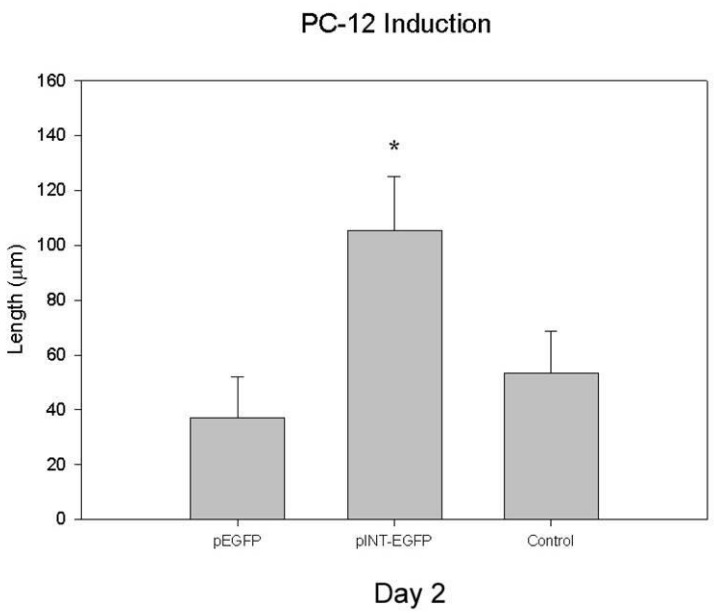
PC-12 Neurite outgrowth after NGF induction

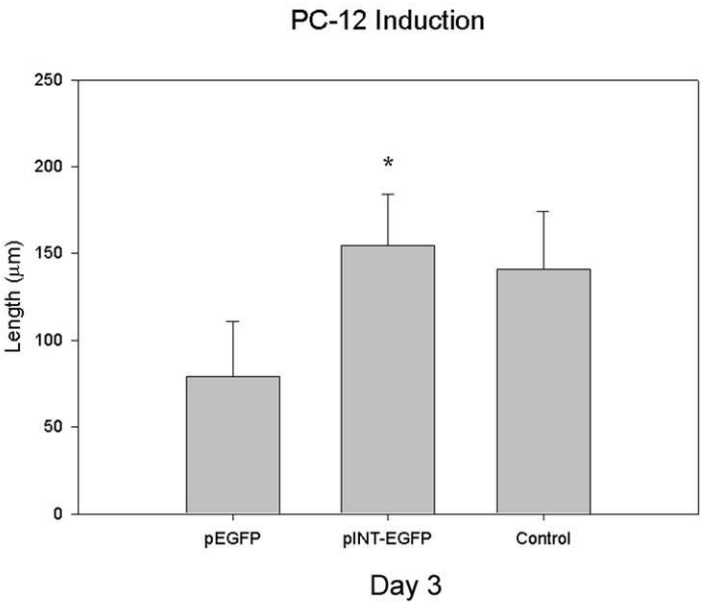
The longest neurite from each single cell was measured at different time points. (n=25)

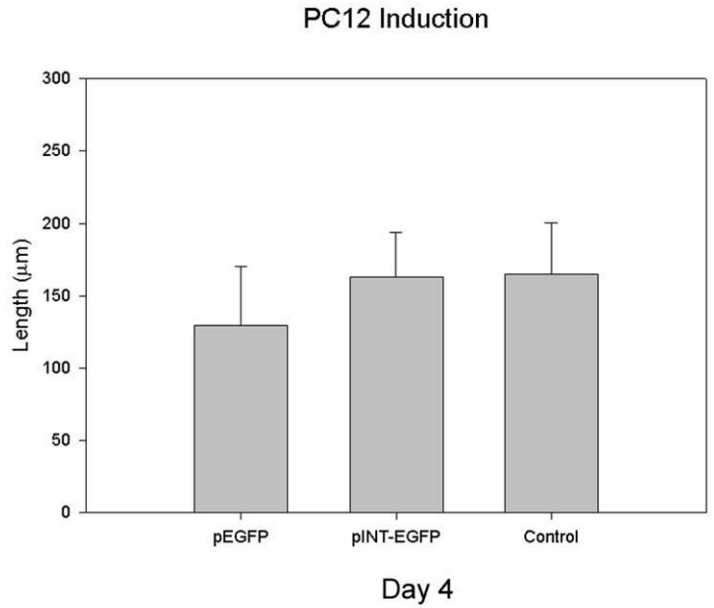


The Neurite outgrowth at the first 4 days

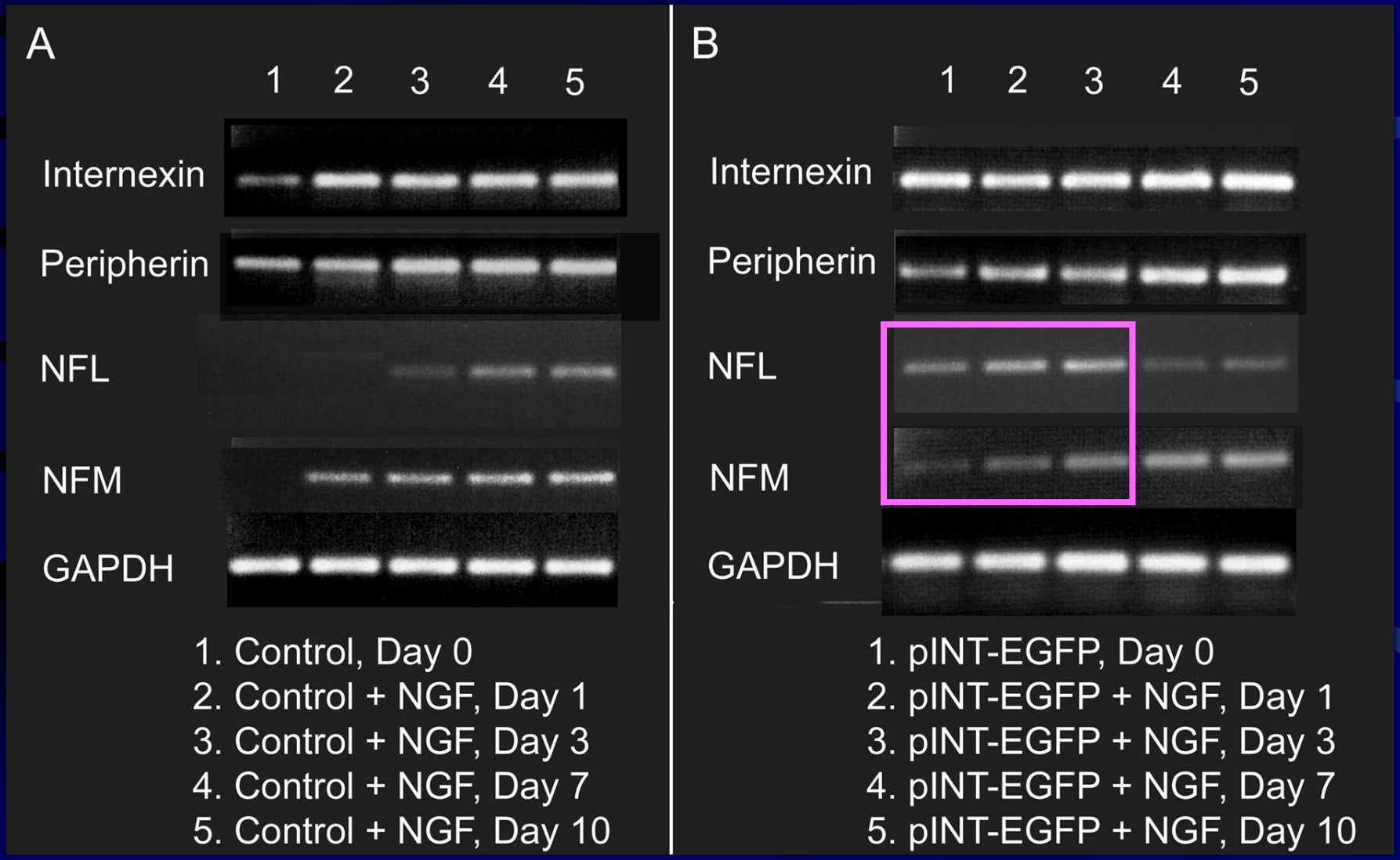




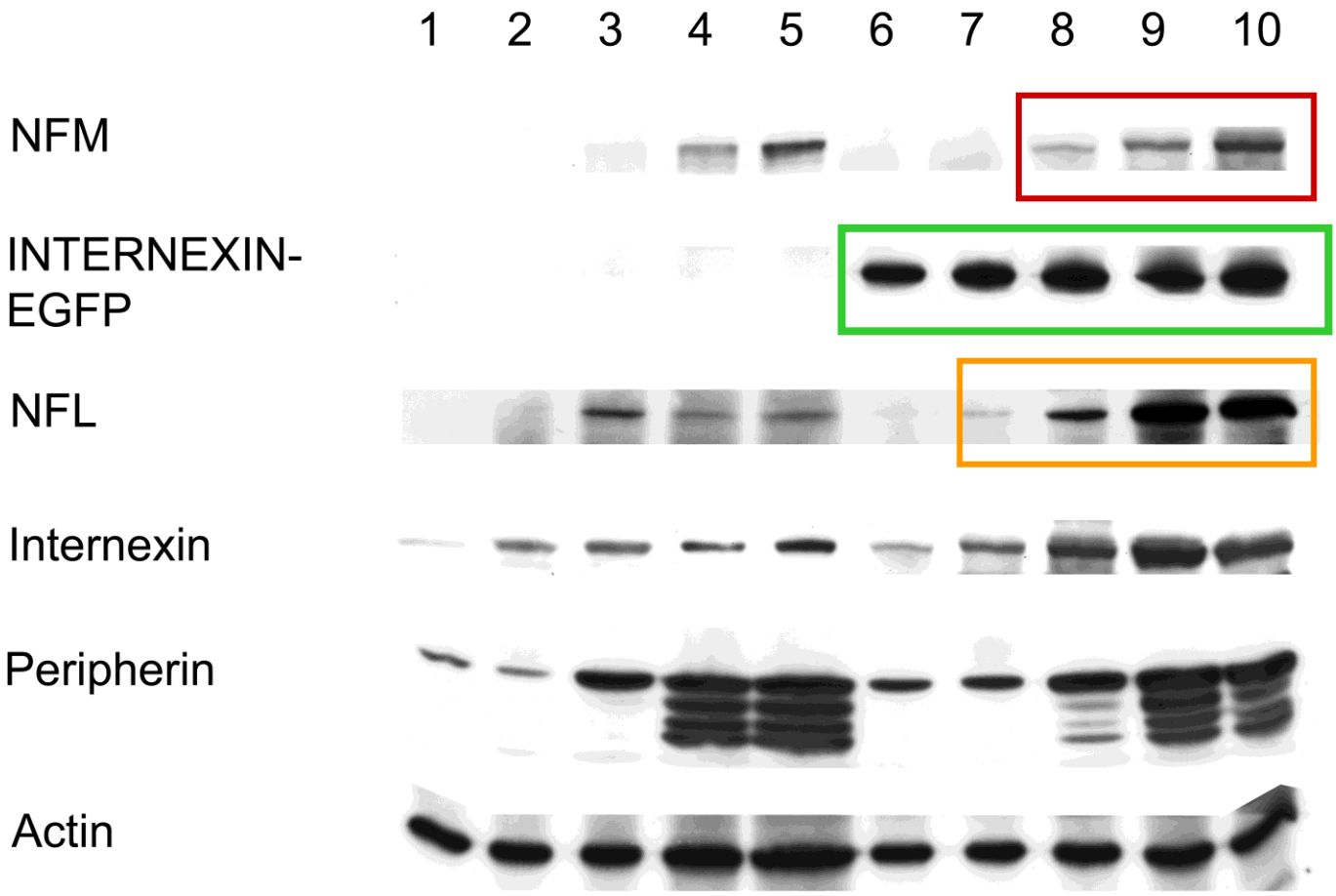




RT-PCR


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 I

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.

The cell death in internexin-EGFP transfected cells

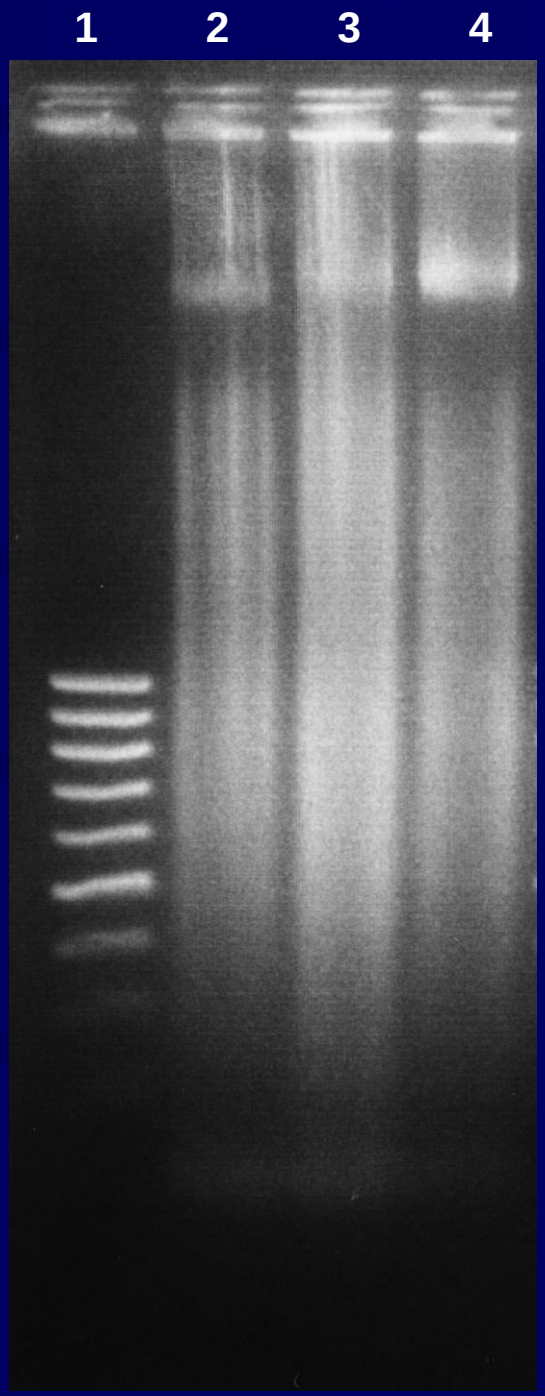
- From our observations, cells transfected with pINT-EGFP were found obviously deattached from the culture plates after 5-day NGF induction.

- 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).

No significant DNA Ladders (7-day NGF induction)

Genomic DNAs of both deattached and attached cells were extracted by the phenol-chloroform method.

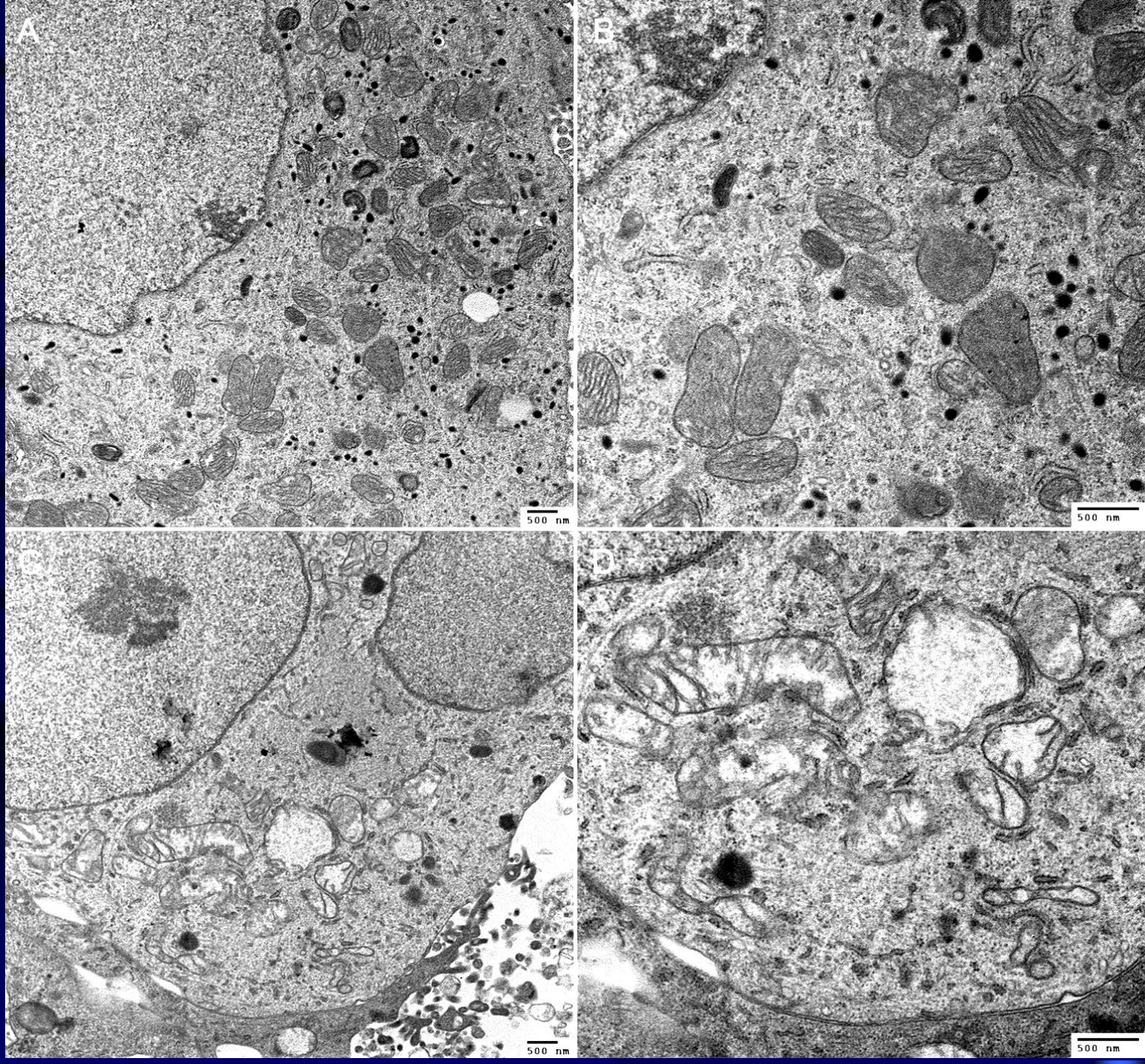
1. **Marker**
2. **pINT-EGFP, 7 day**
3. **pEGFP, 7 day**
4. **Control, 7 day**



Ultrastructure patterns (5-day NGF induction)

Control cells

pINT-EGFP tranfected cells

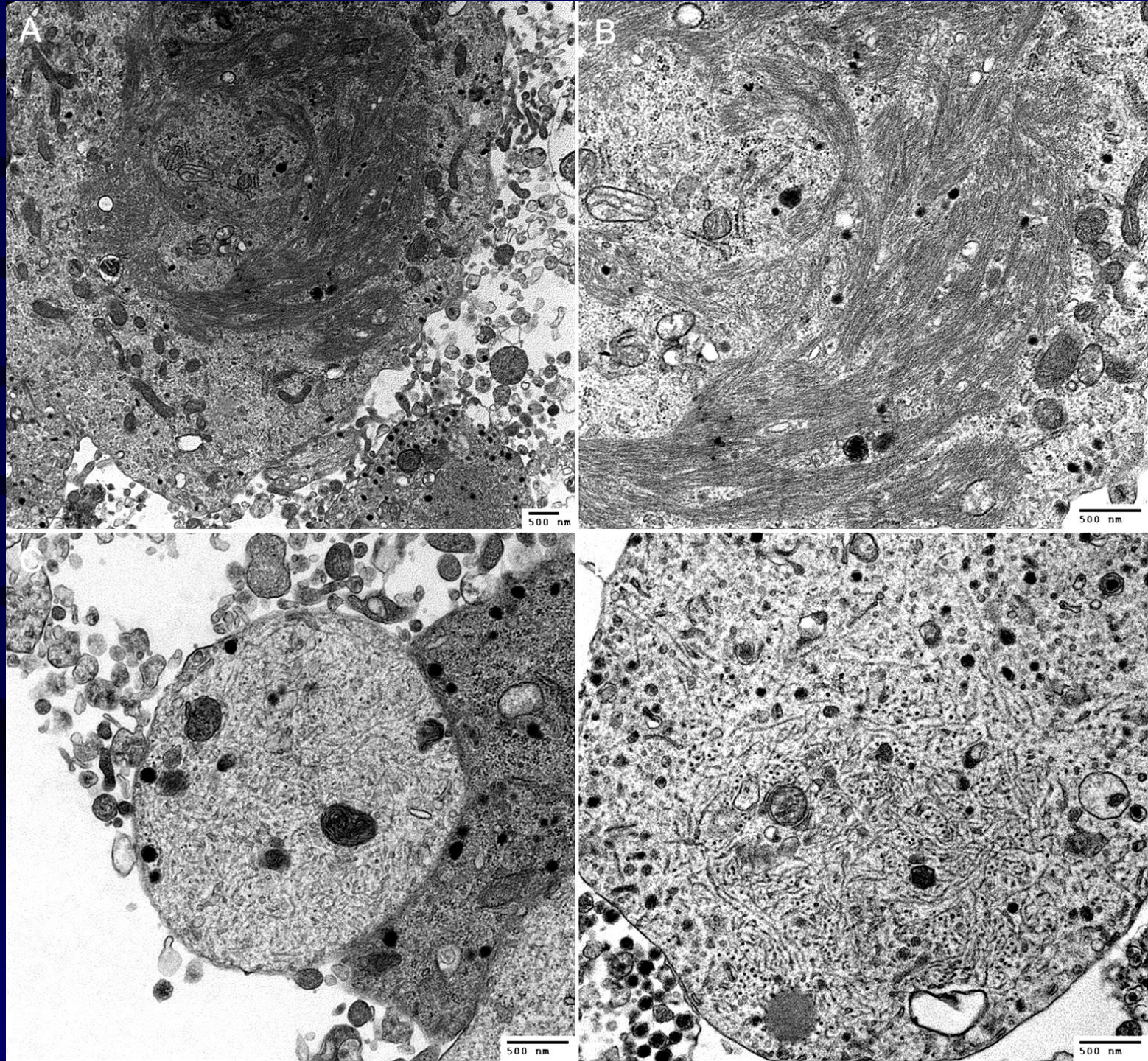


Ultrastructure patterns (5-day NGF)

pINT-EGFP tranfected cells

Accumulations in cell body

Misaccumulations of IFs in neurites

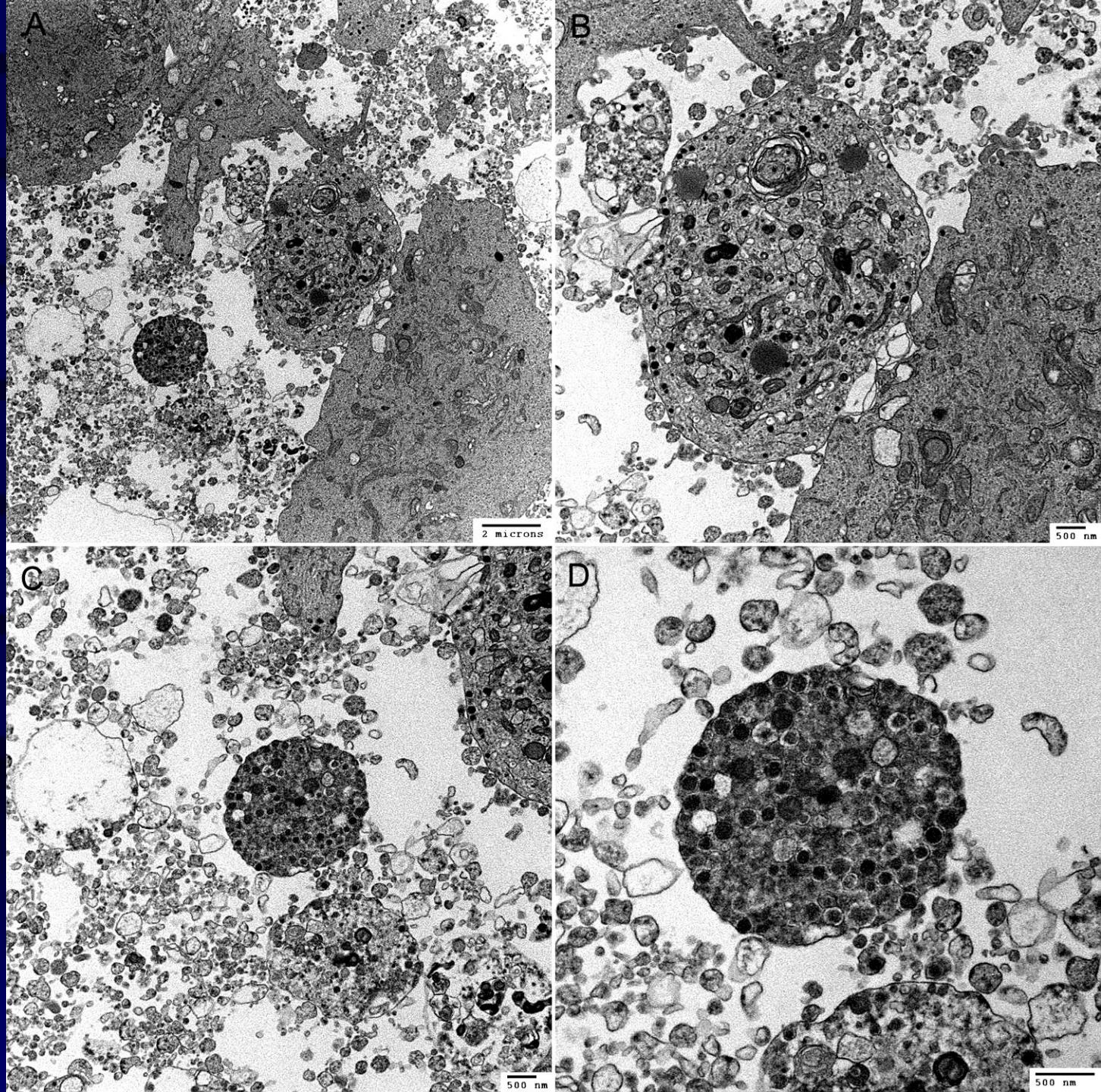


Ultrastructure patterns (5-day NGF)

pINT-EGFP tranfected cells

Degenerating neurite

Degenerated neurite



Caspase activities

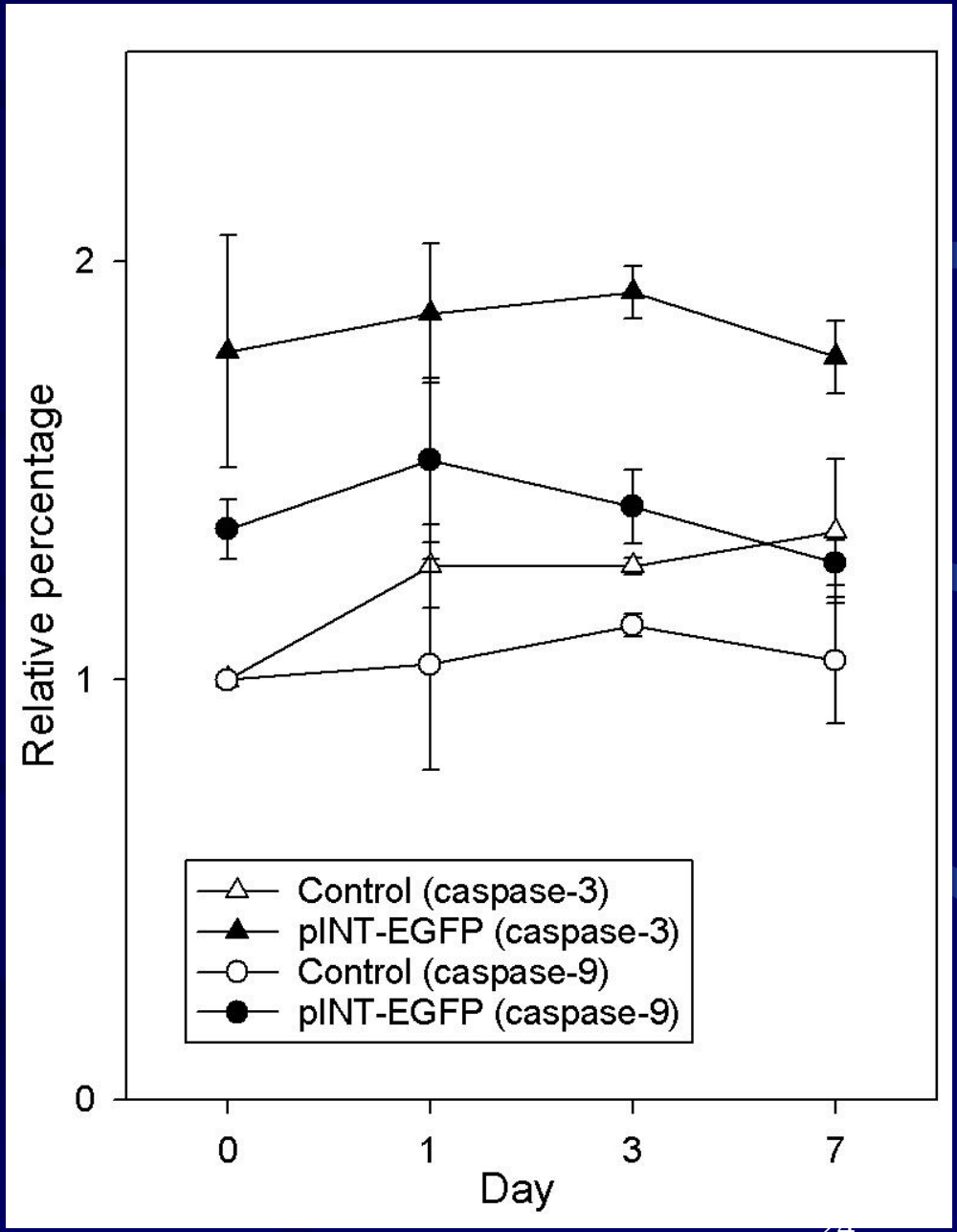
Caspase-3 substrate
Ac-DEVD-pNA

Cell lysates (50 µg protein) + or

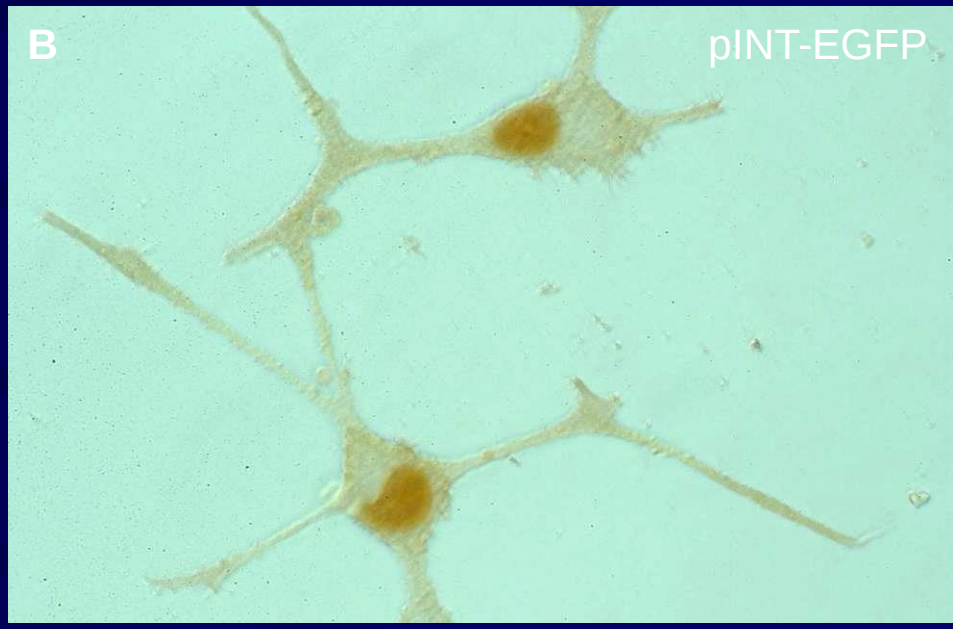
Caspase-9 substrate
Ac-LEHD-pNA

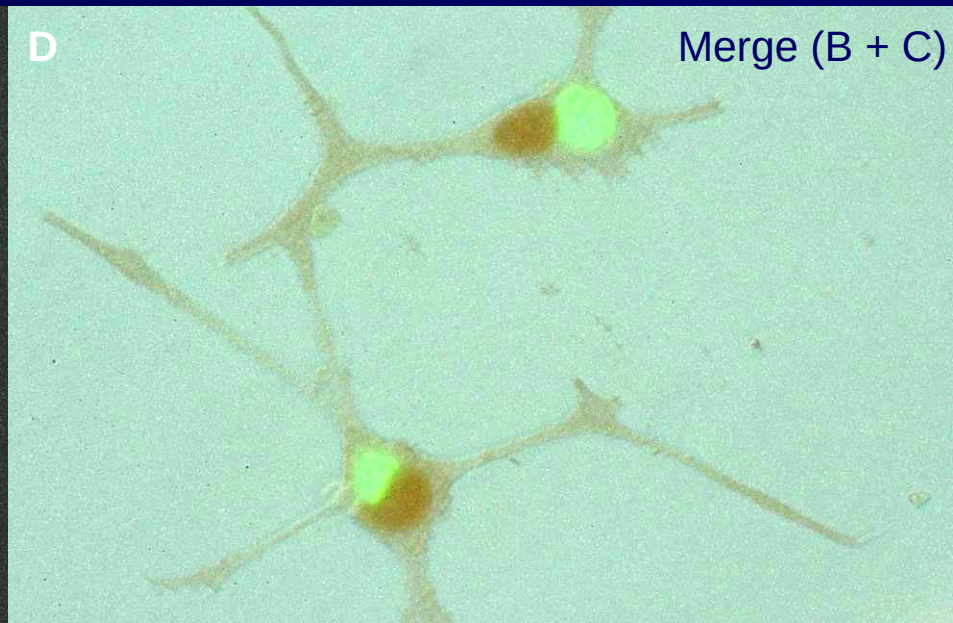
pNA released

measured by spectrophotometry at 405 nm



TUNEL assay at the 5th day of NGF induction



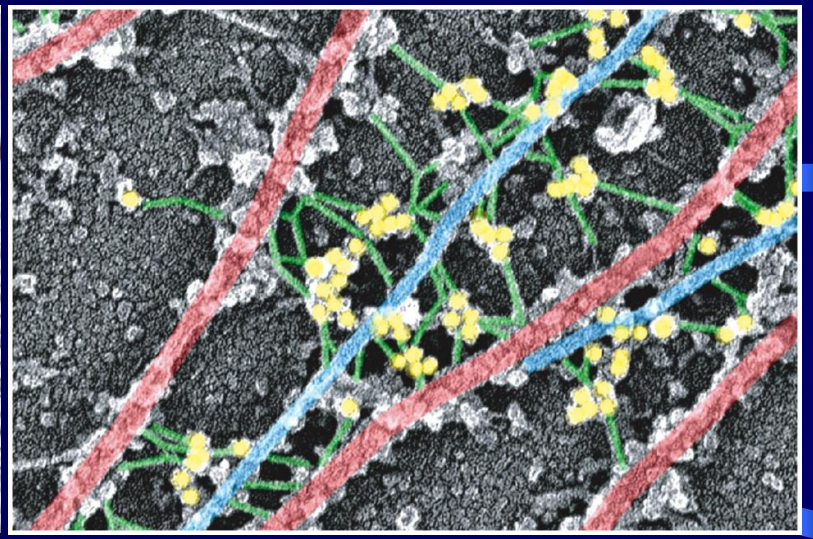


Summary II

1. Overexpression of pINT-EGFP may also cause 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 caspase activities and TUNEL positive patterns.

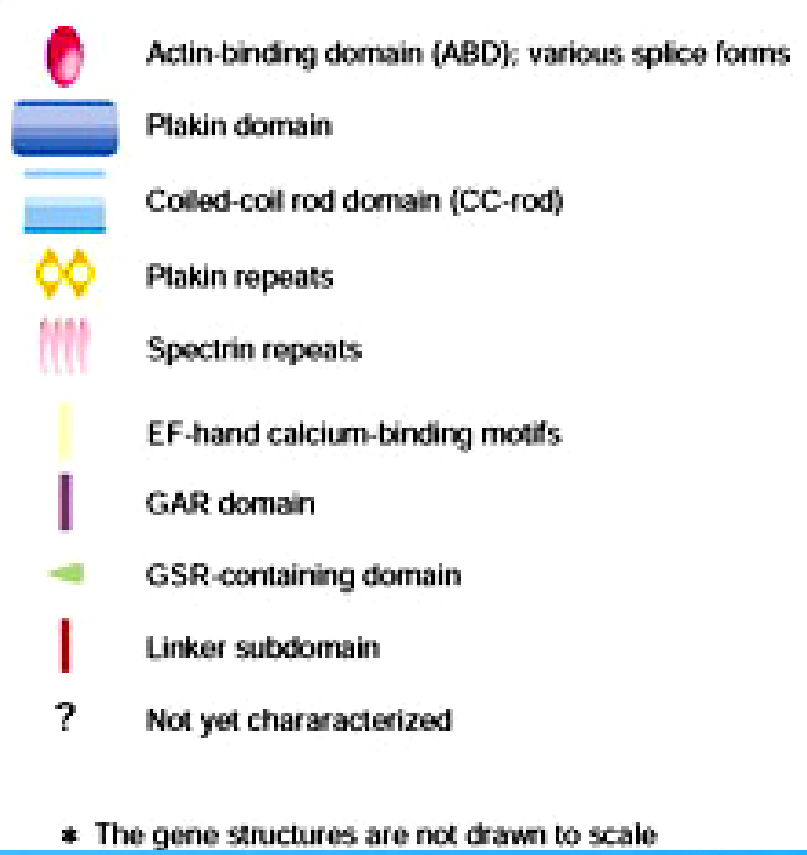
Nature Mutant for Neuronal Degeneration

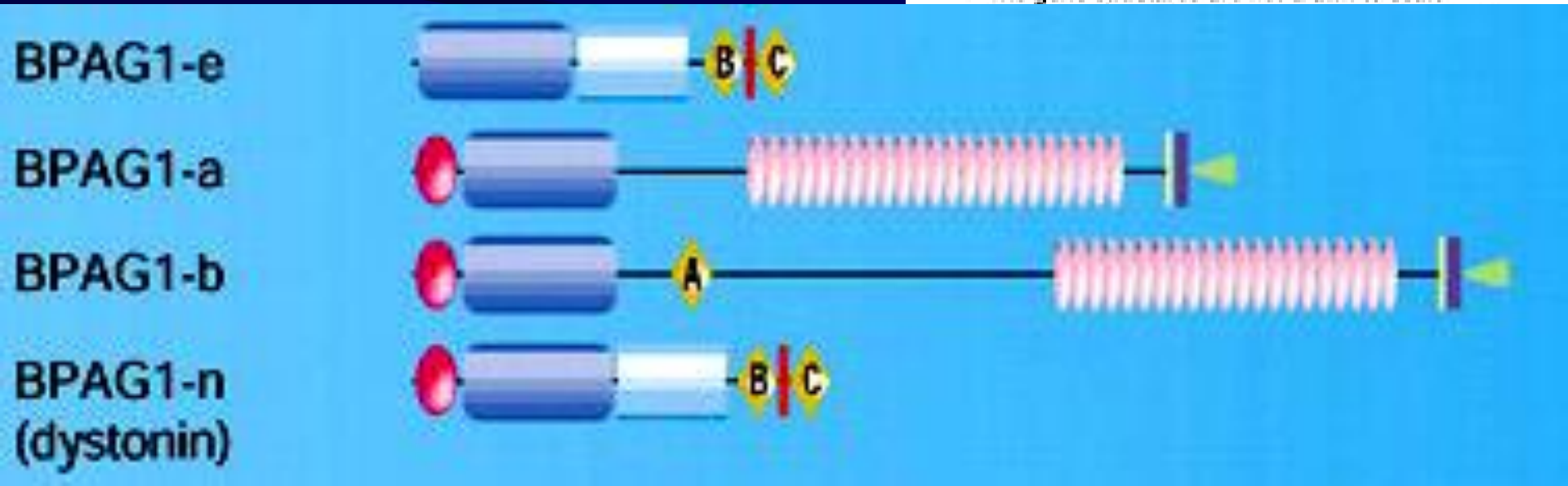


- *Dystonia musculorum (dt)* mouse is a recessive hereditary sensory neuropathy of the mutant mouse, which is defective in *BPAG1* gene.
- It is a very interesting neurological mutant, first discovered as a spontaneously occurring, autosomal recessive variant (Duchen et al., 1963).
- Mice affected with *dt* are seemingly normal at birth, but by 10–12 days they begin twitching, writing, and exhibiting uncoordinated movements.

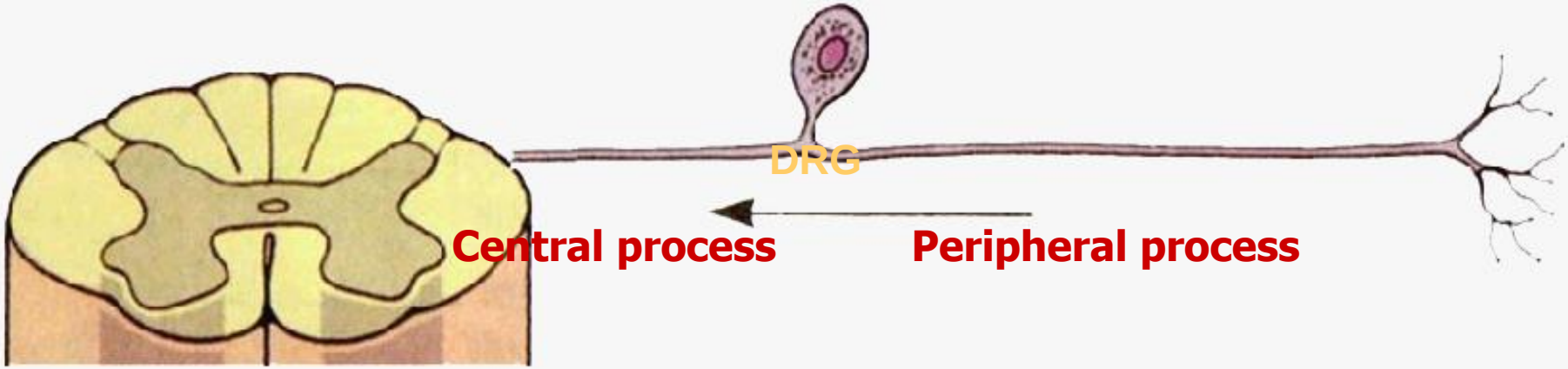
BPAG1 is known as ***dystonin*** and ***MACF2.***

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

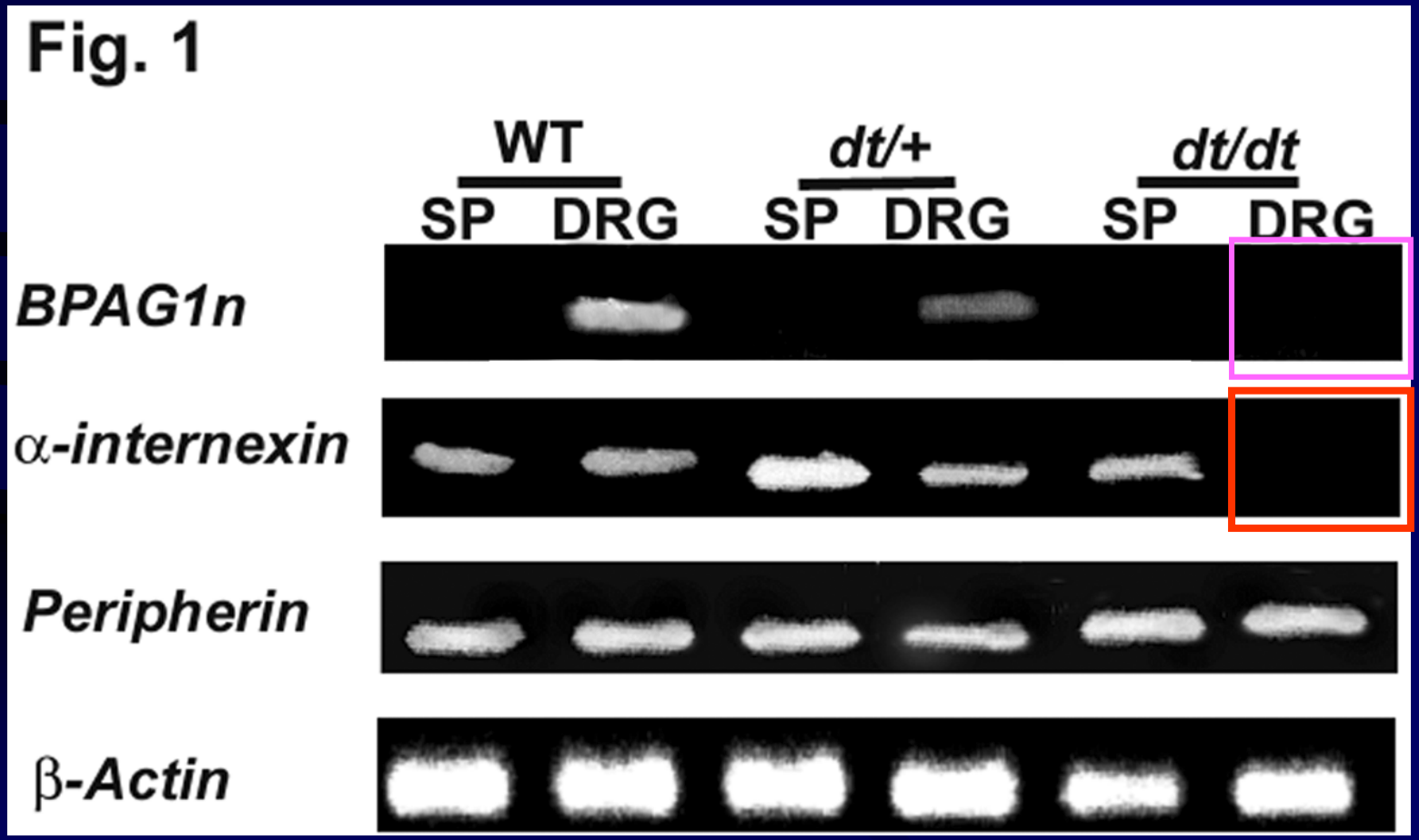




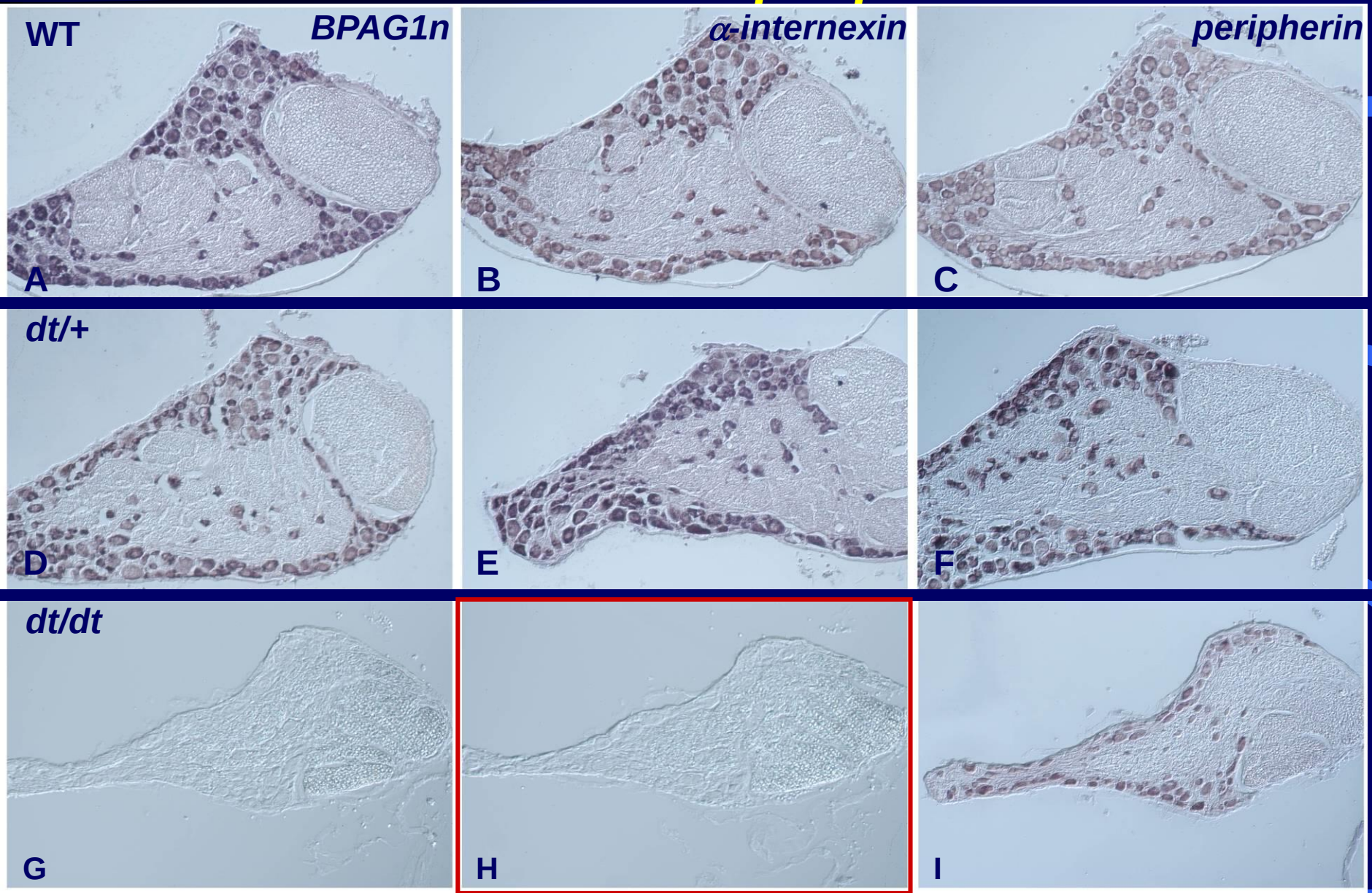
To study the neural dysfunction and degeneration of primary sensory neurons in dorsal root ganglia in *dt* mice.



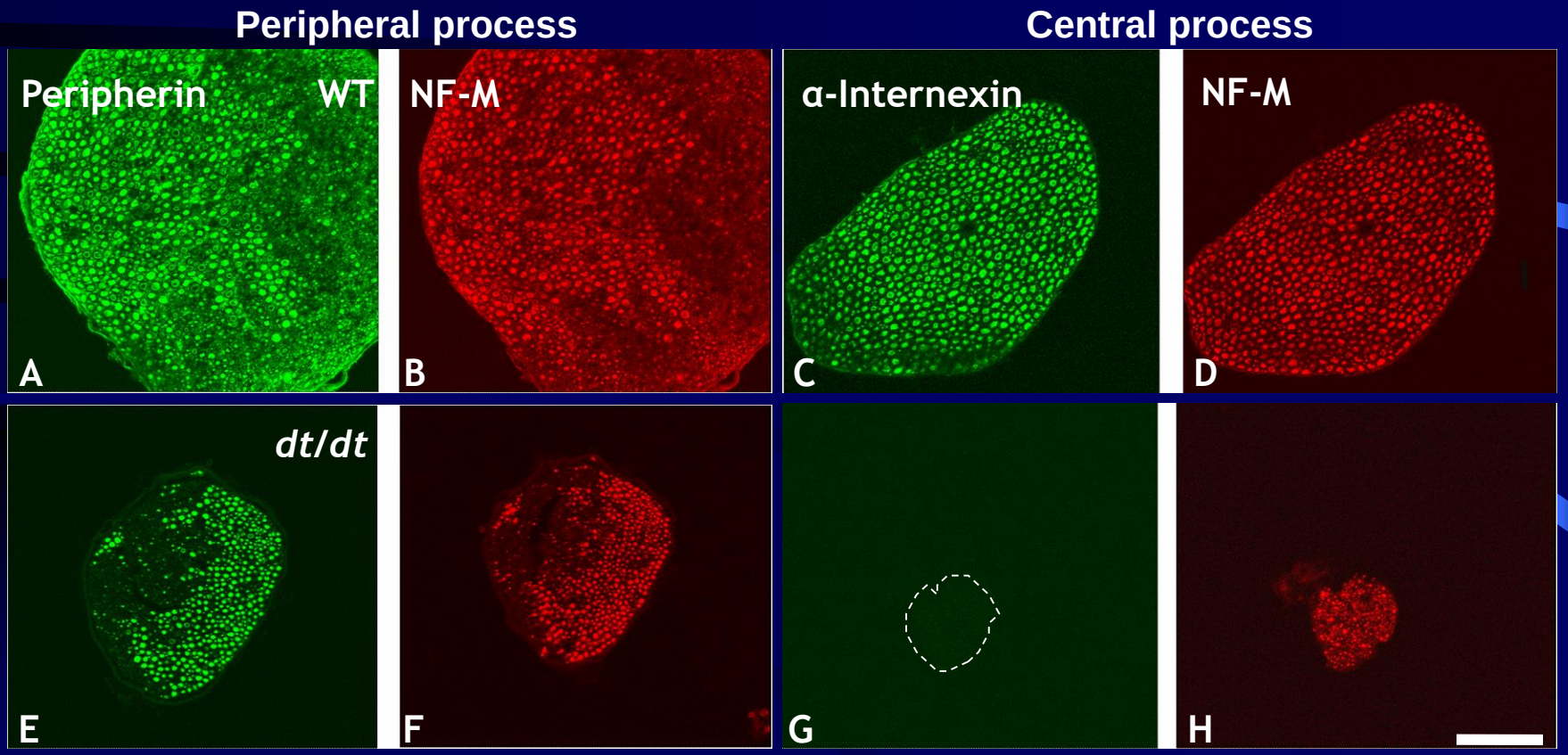
RT-PCR analysis the expression of mRNA



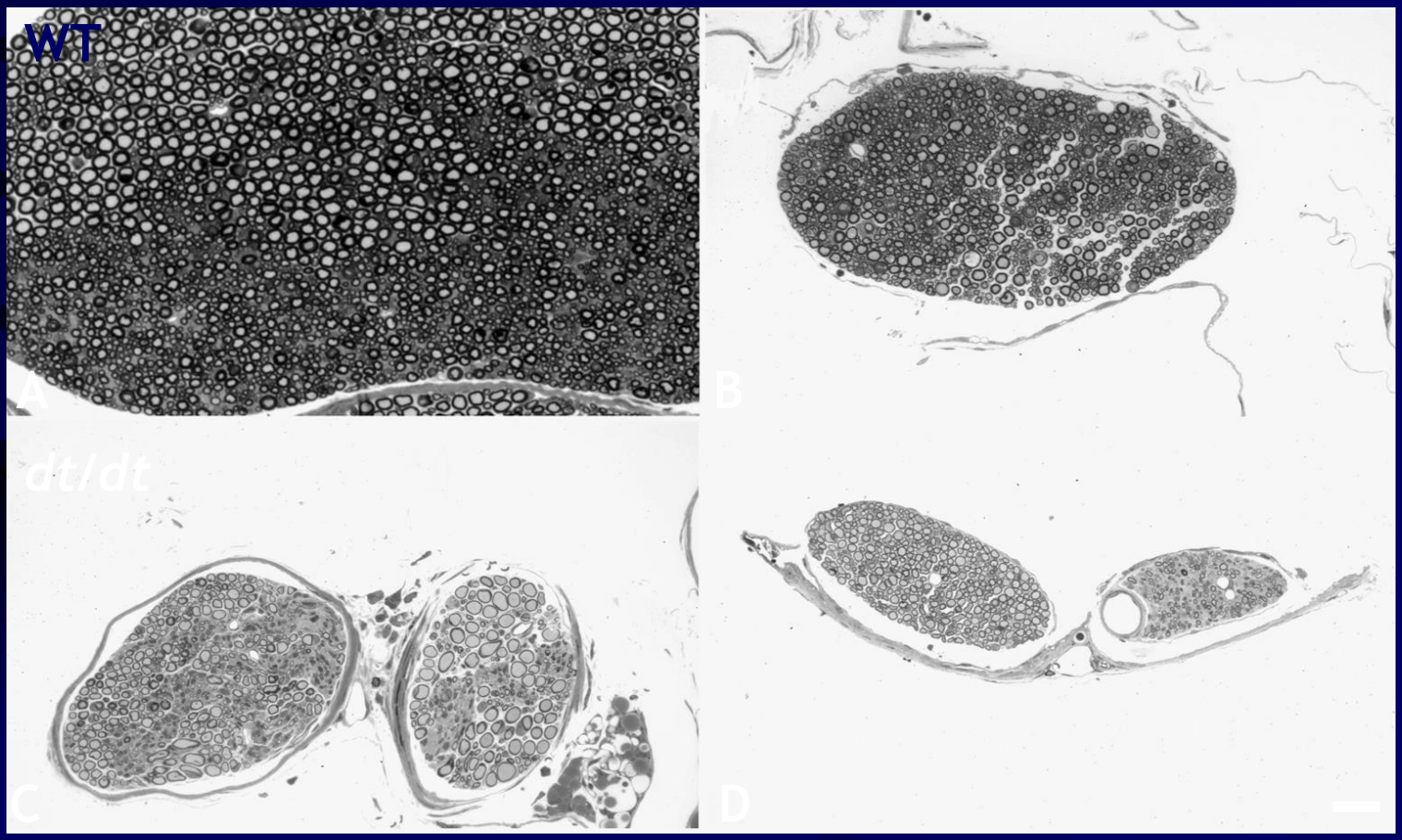
In situ hybridization analysis the *BPAG1n*, *α-internexin* and *peripherin*



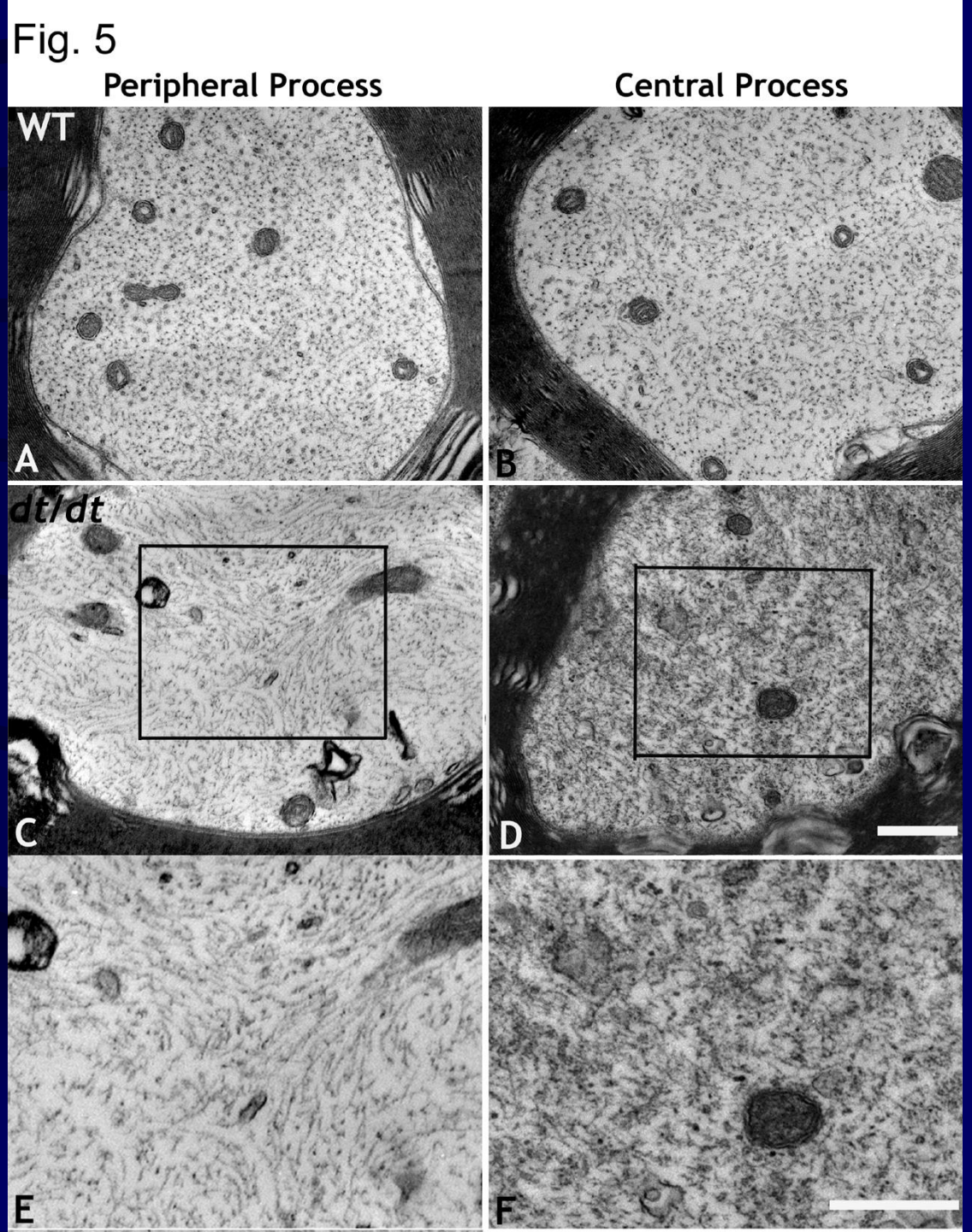
Expression of neurofilaments in WT and *dt/dt* mice



Peripheral and central process form wild type and *dt/dt* mice



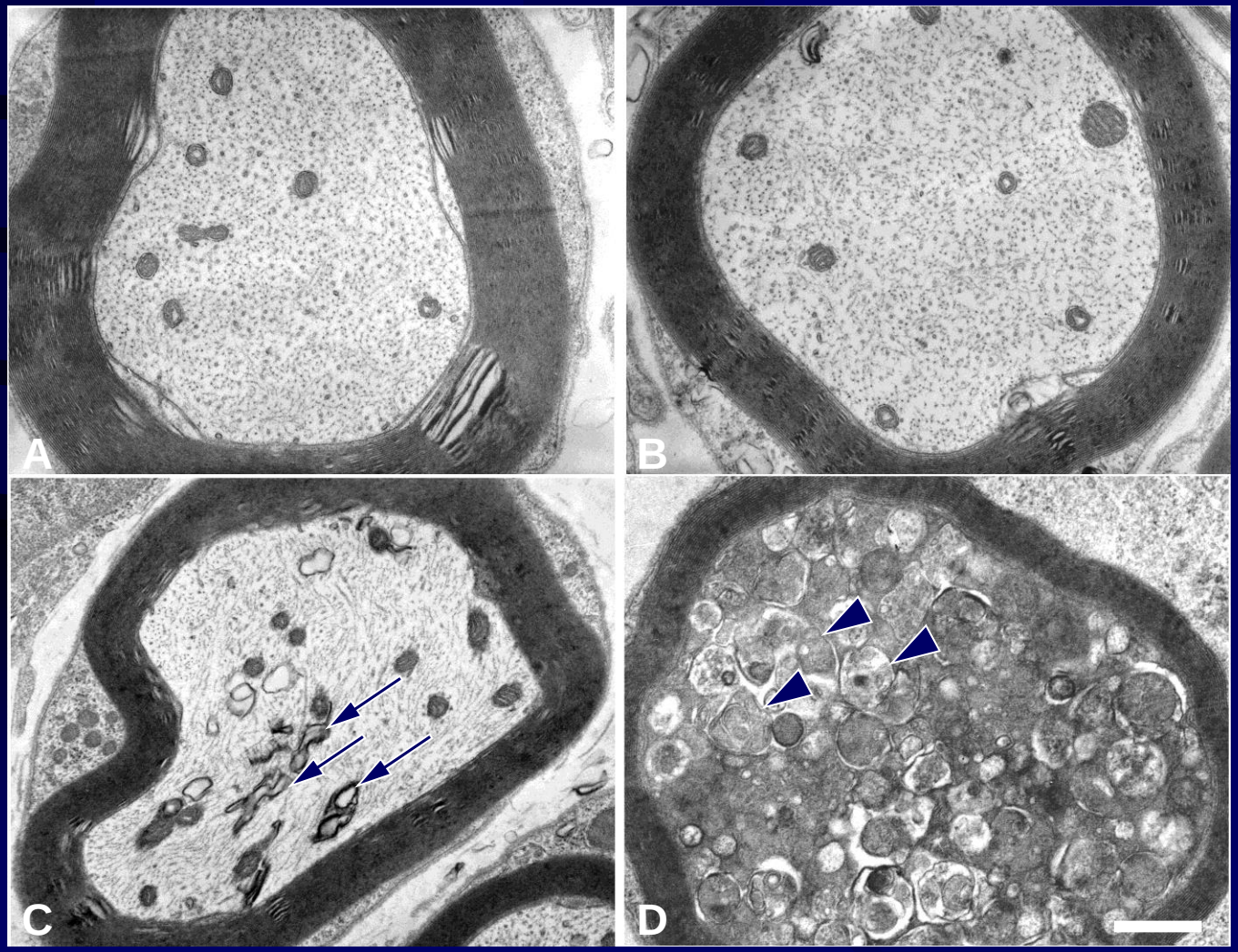
Ultrastructure of peripheral and central process from WT and *dt/dt* mice



Ultrastructure of peripheral and central process from WT and *dt/dt* mice

Peripheral process

Central process



Sensory and autonomic nerve degeneration in the skin of *dt* mutant

Fig. 6

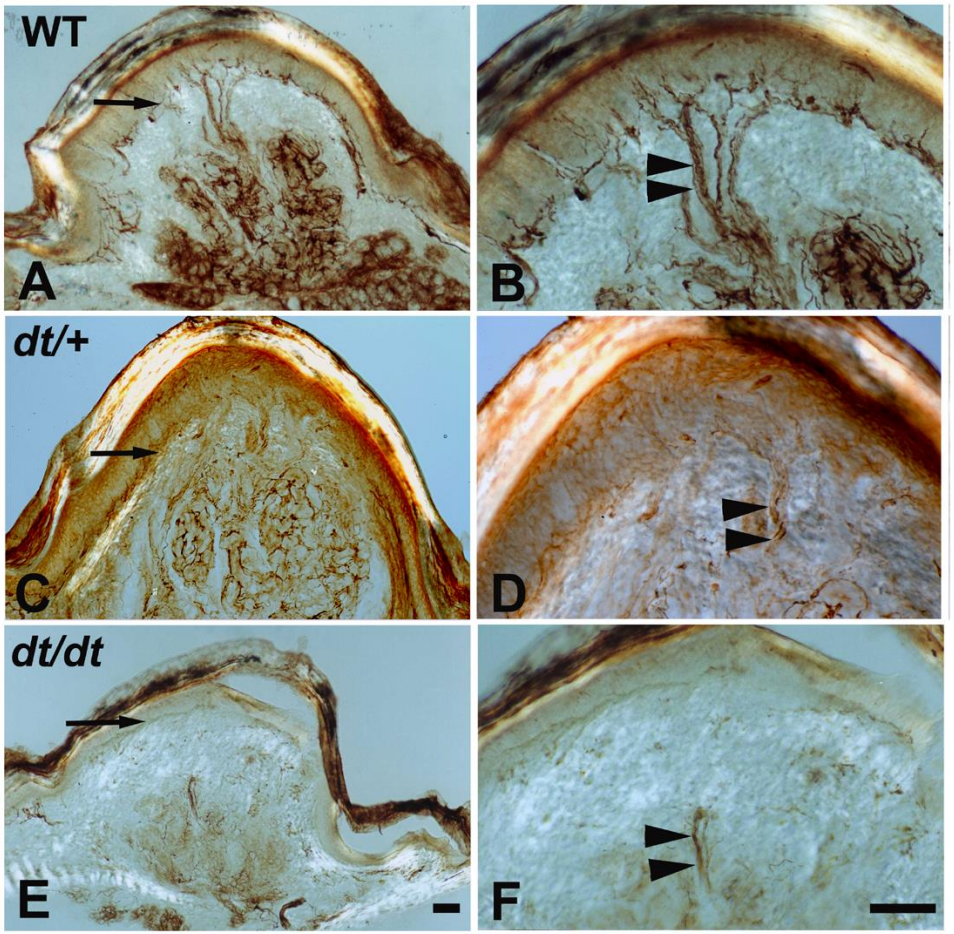
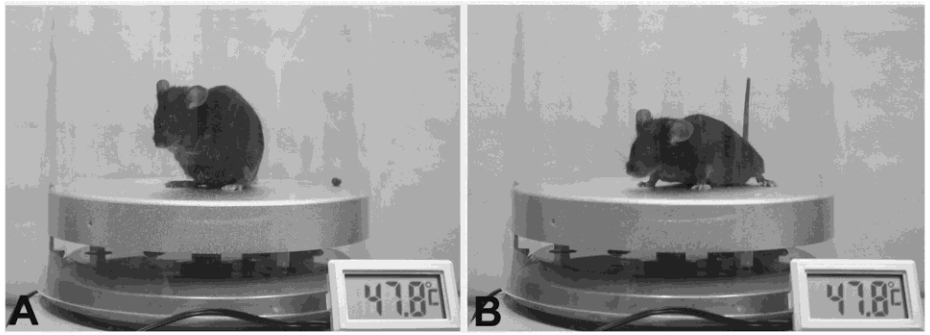
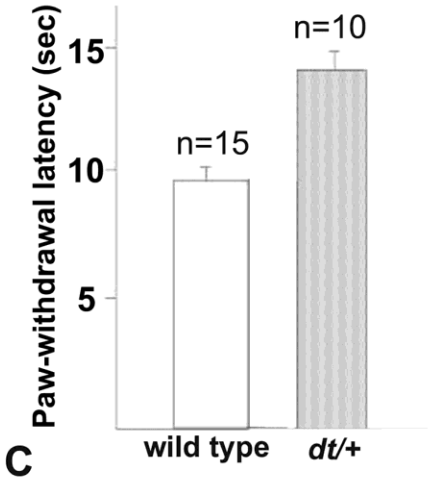
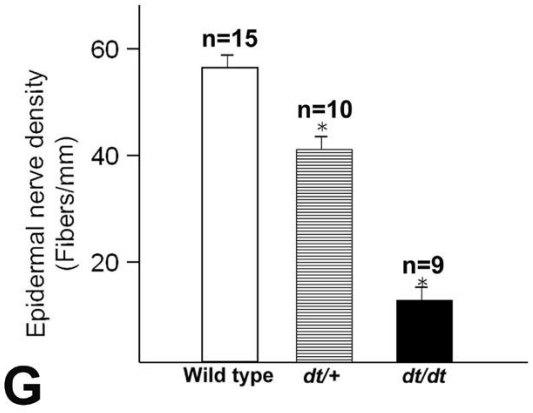


Fig. 7





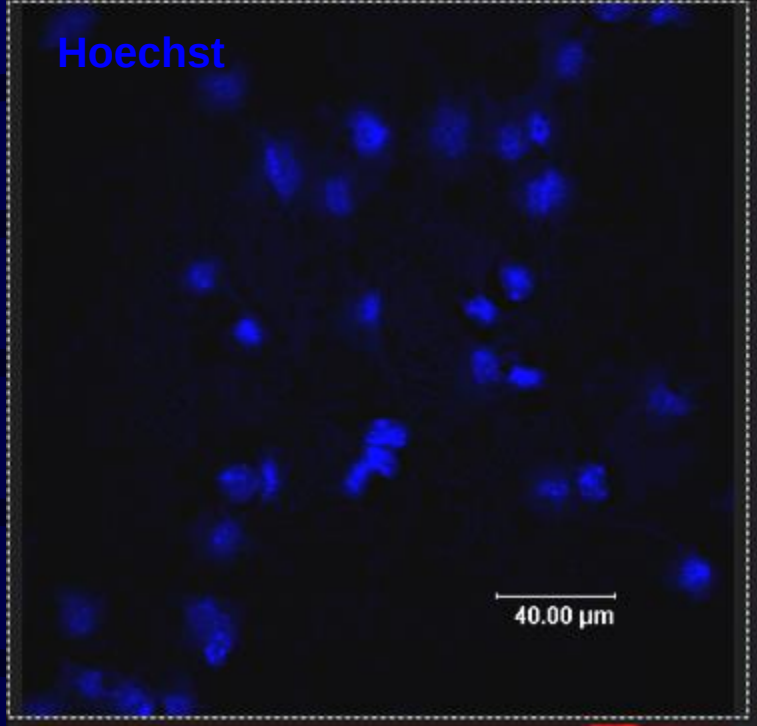


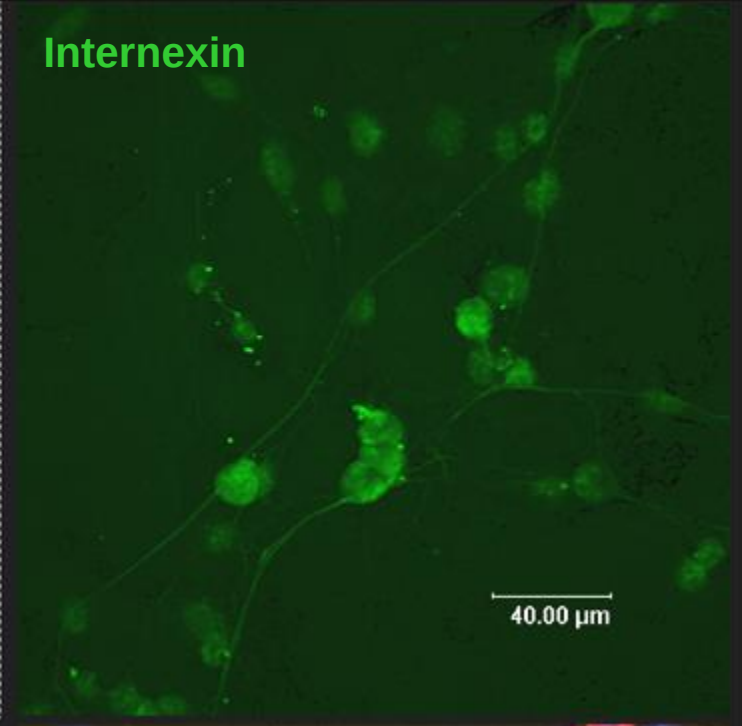
Primary culture DRG neurons

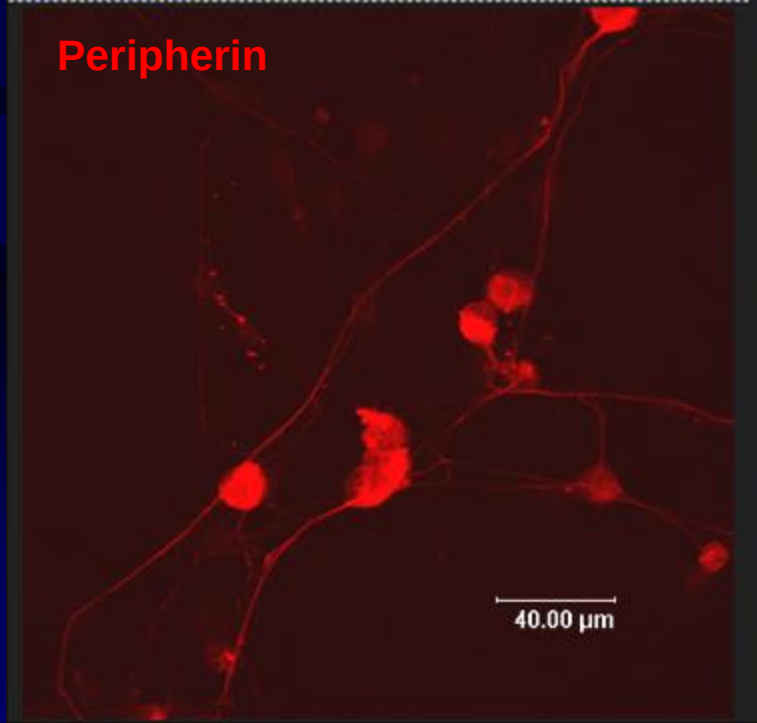
1. Take DRGs and transfer DRGs to a fresh epondroff tube with 0.5 ml HBSS (CMF) on ice.
2. Add 0.5 ml 0.25% Trypsin-EDTA and incubate in rotating incubator at 37℃ for 15 min.
3. Resuspend with 40% FBS L15
4. Spin for 5 min at 1500 rpm, remove supernatant..
5. Resuspend with 1.5 ml 40% FBS L15 in incubator 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.

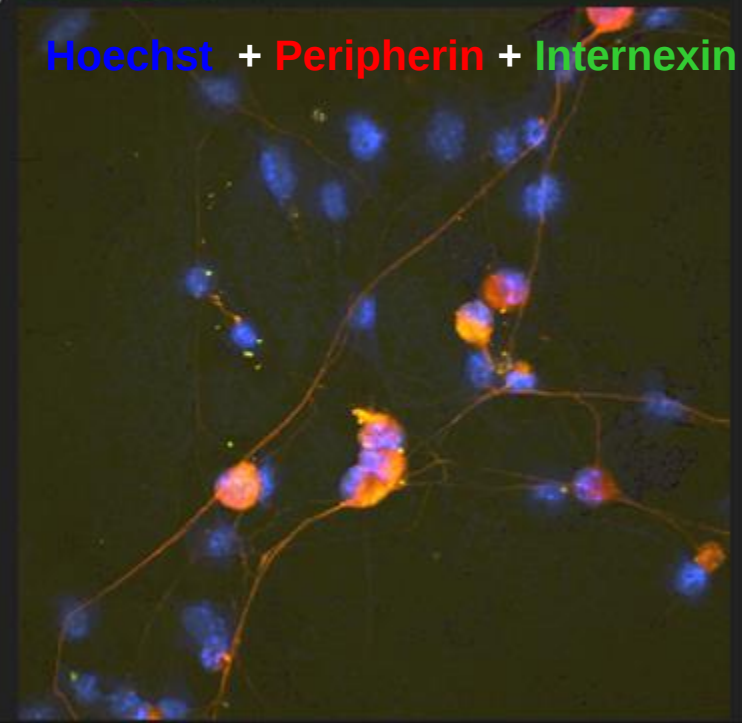
WT DRG

3 DIV


Hoechst
40.00 µm

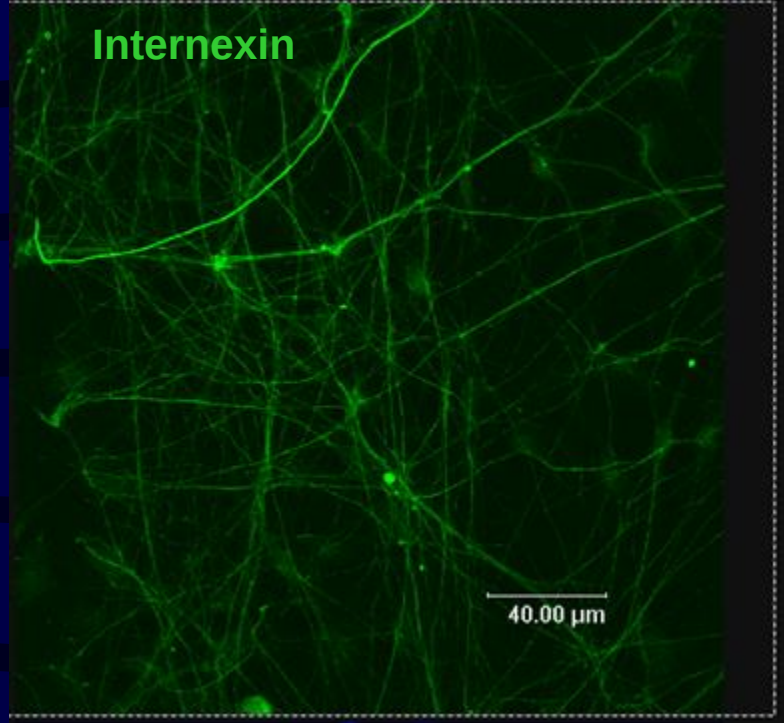

Internexin
40.00 µm

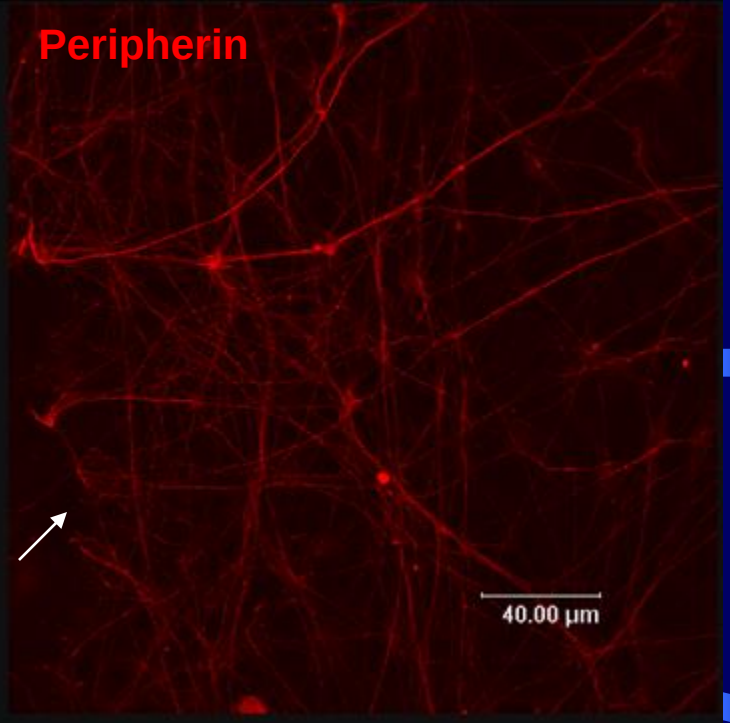

Peripherin
40.00 µm

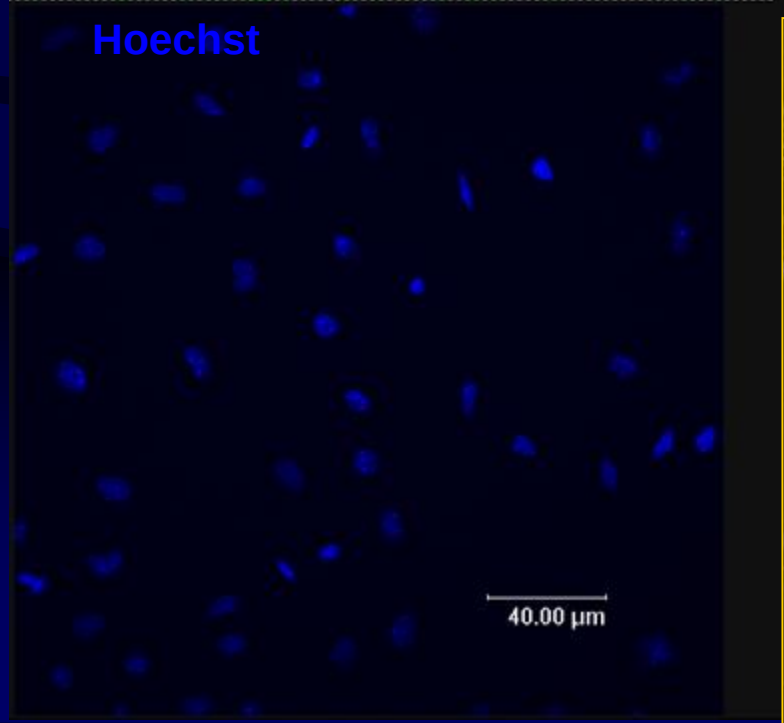

Hoechst + Peripherin + Internexin

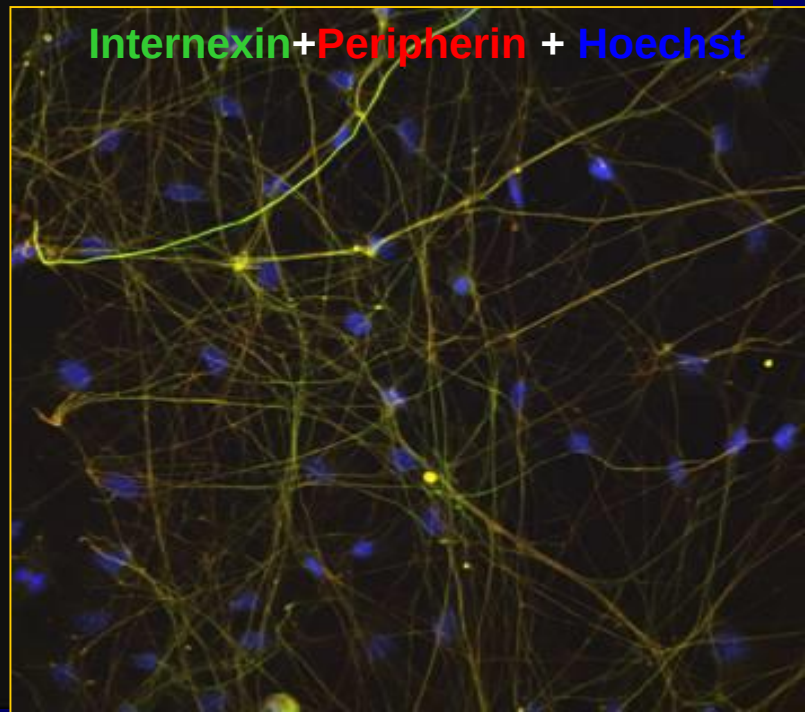
WT DRG

5 DIV


Internexin
40.00 µm

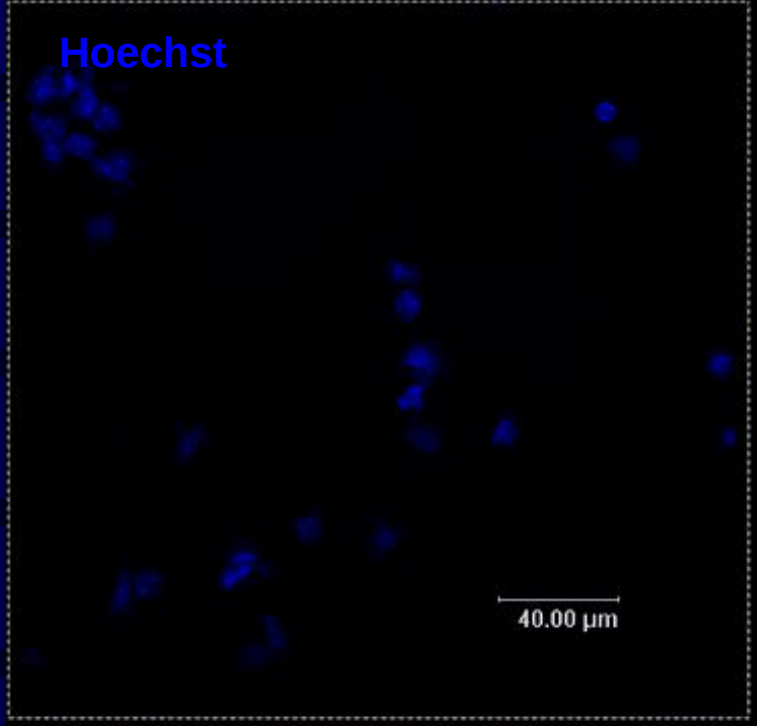

Peripherin
40.00 µm

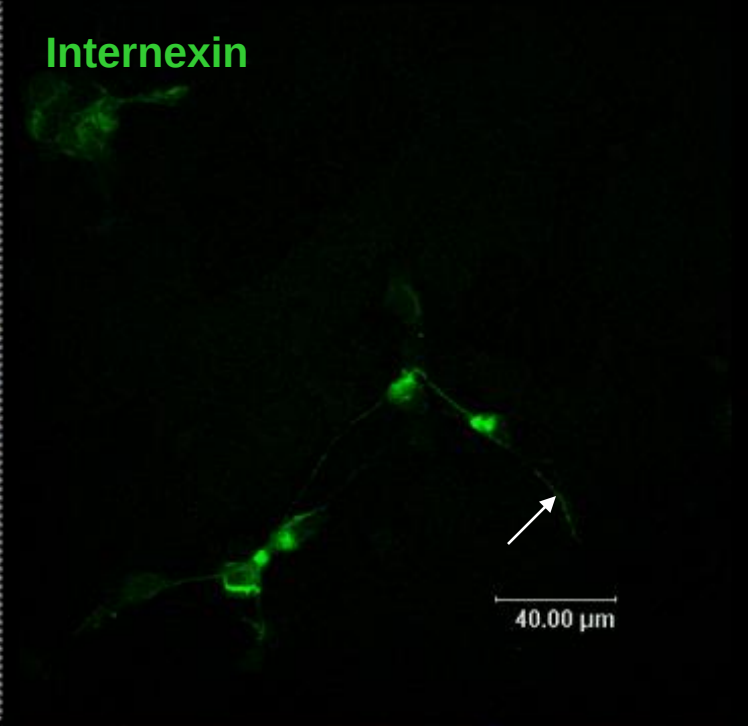

Hoechst
40.00 µm

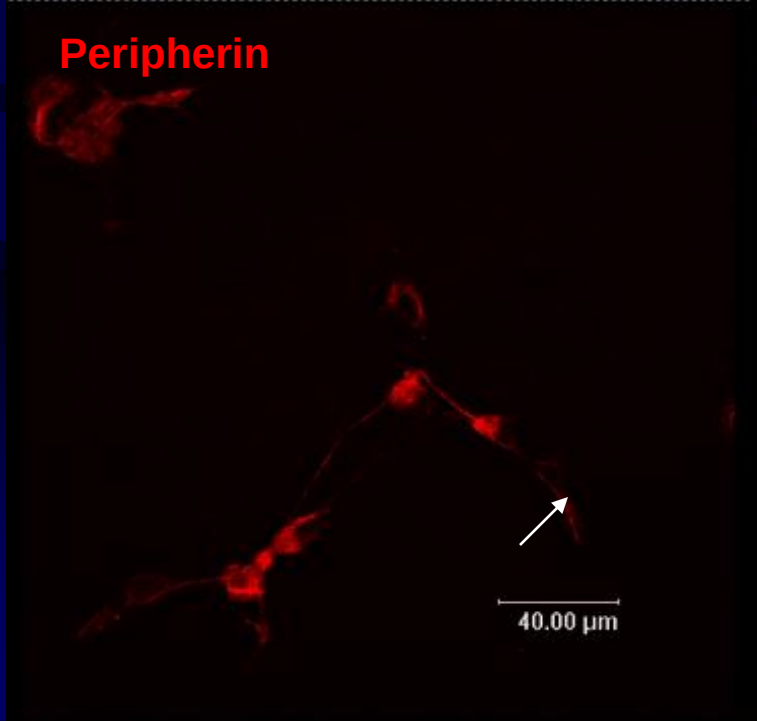

Internexin+Peripherin + Hoechst

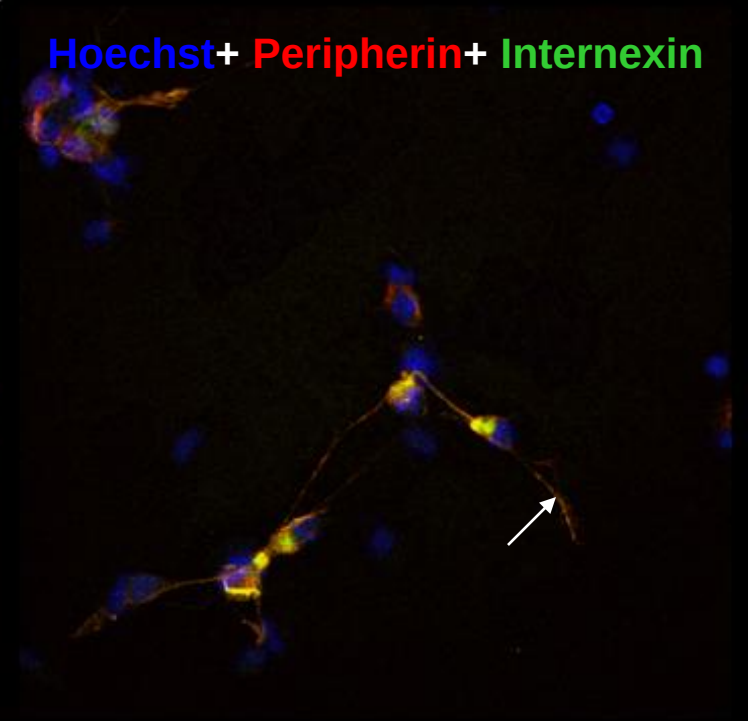
dt/dt DRG

3 DIV



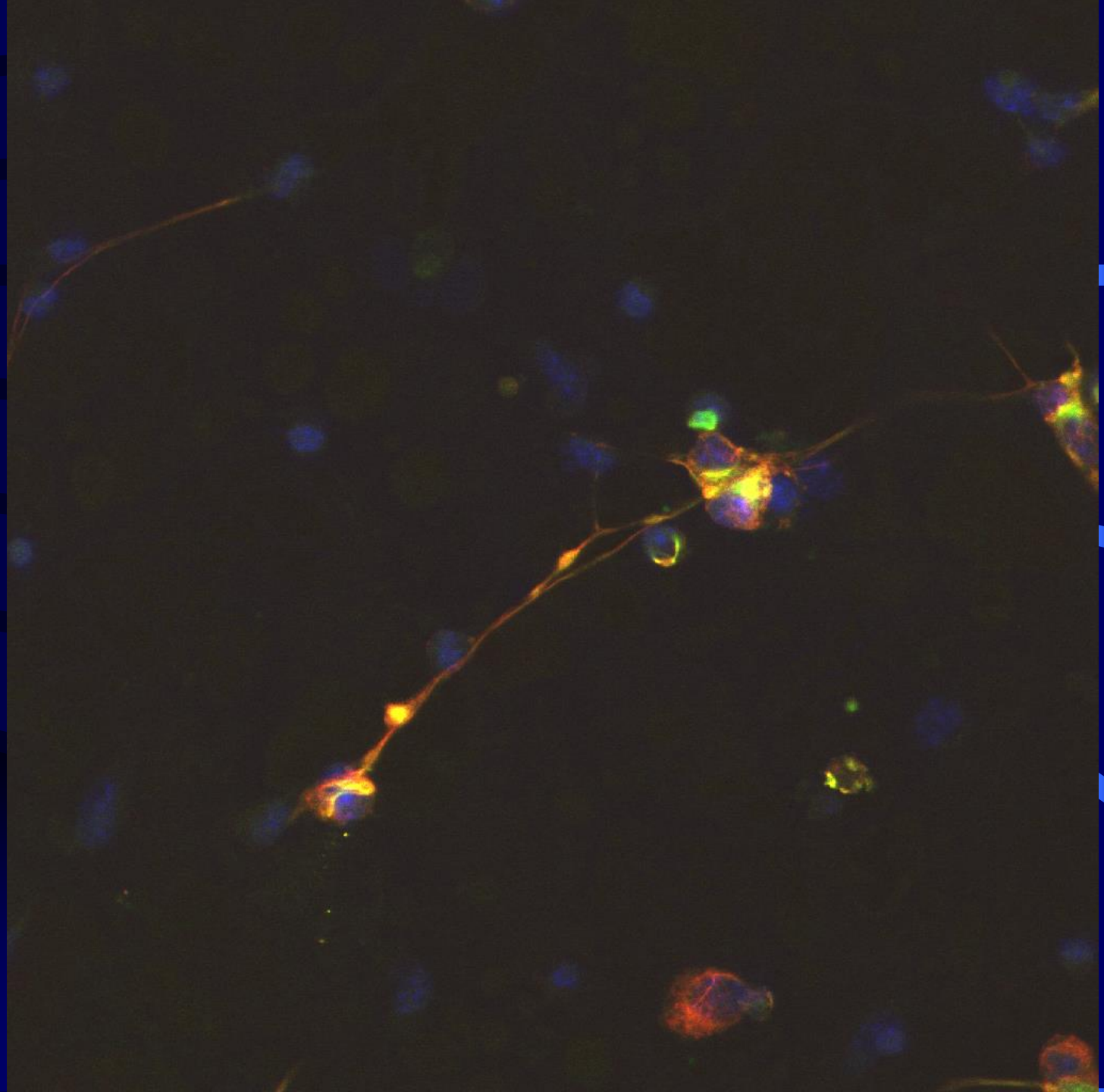






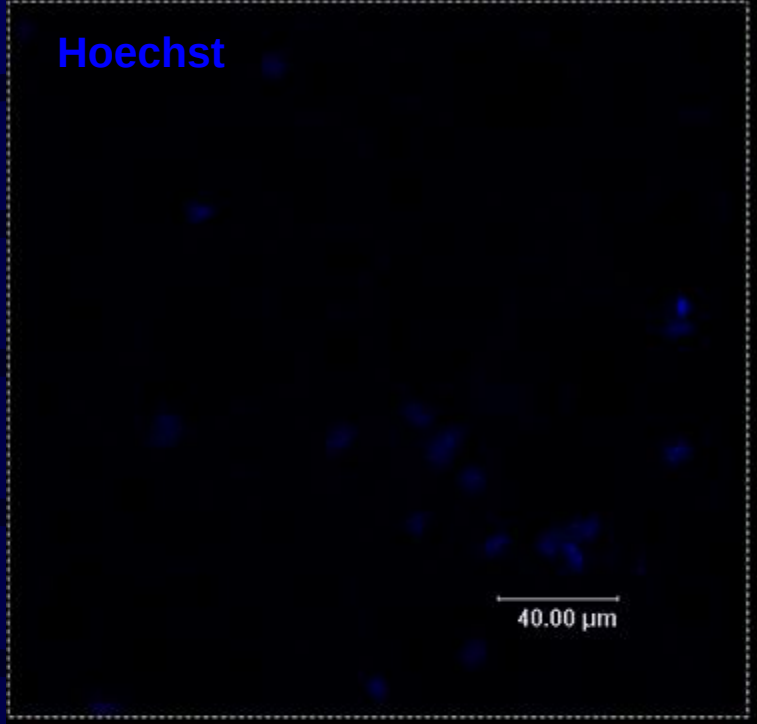
dt/dt DRG

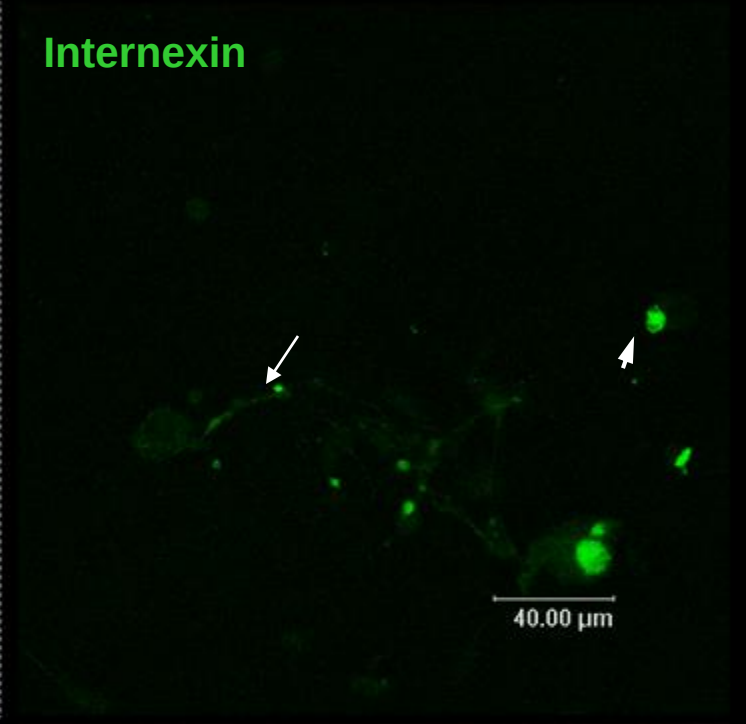
3 DIV

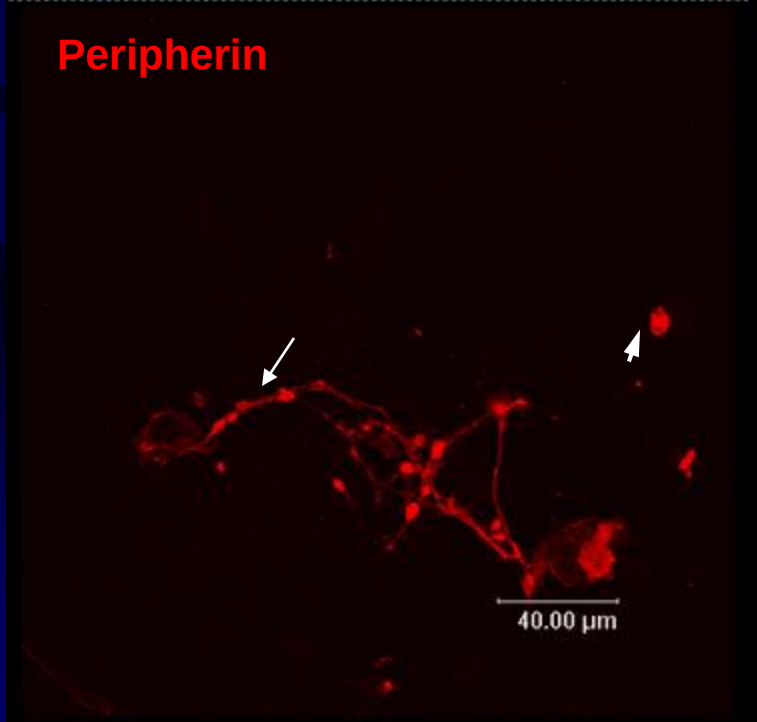


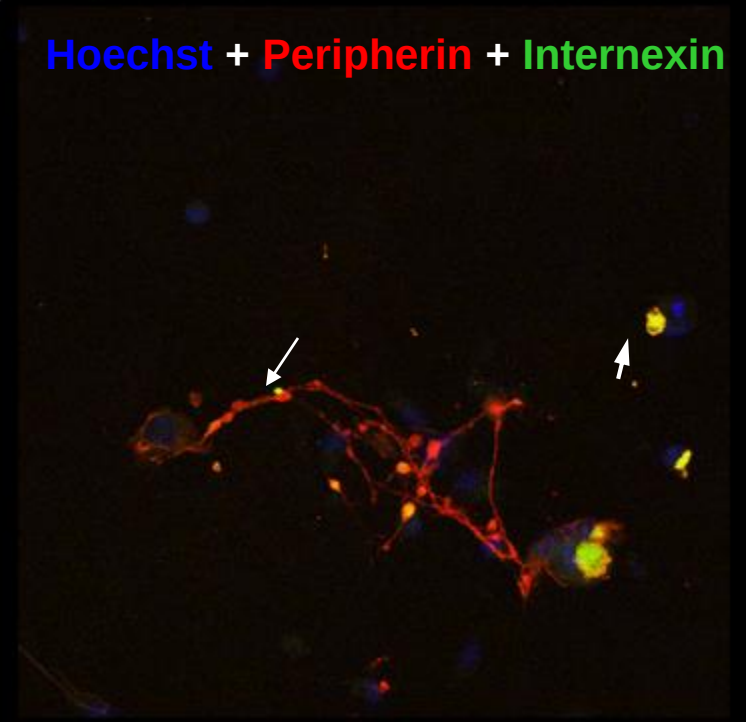
dt/dt DRG

5 DIV



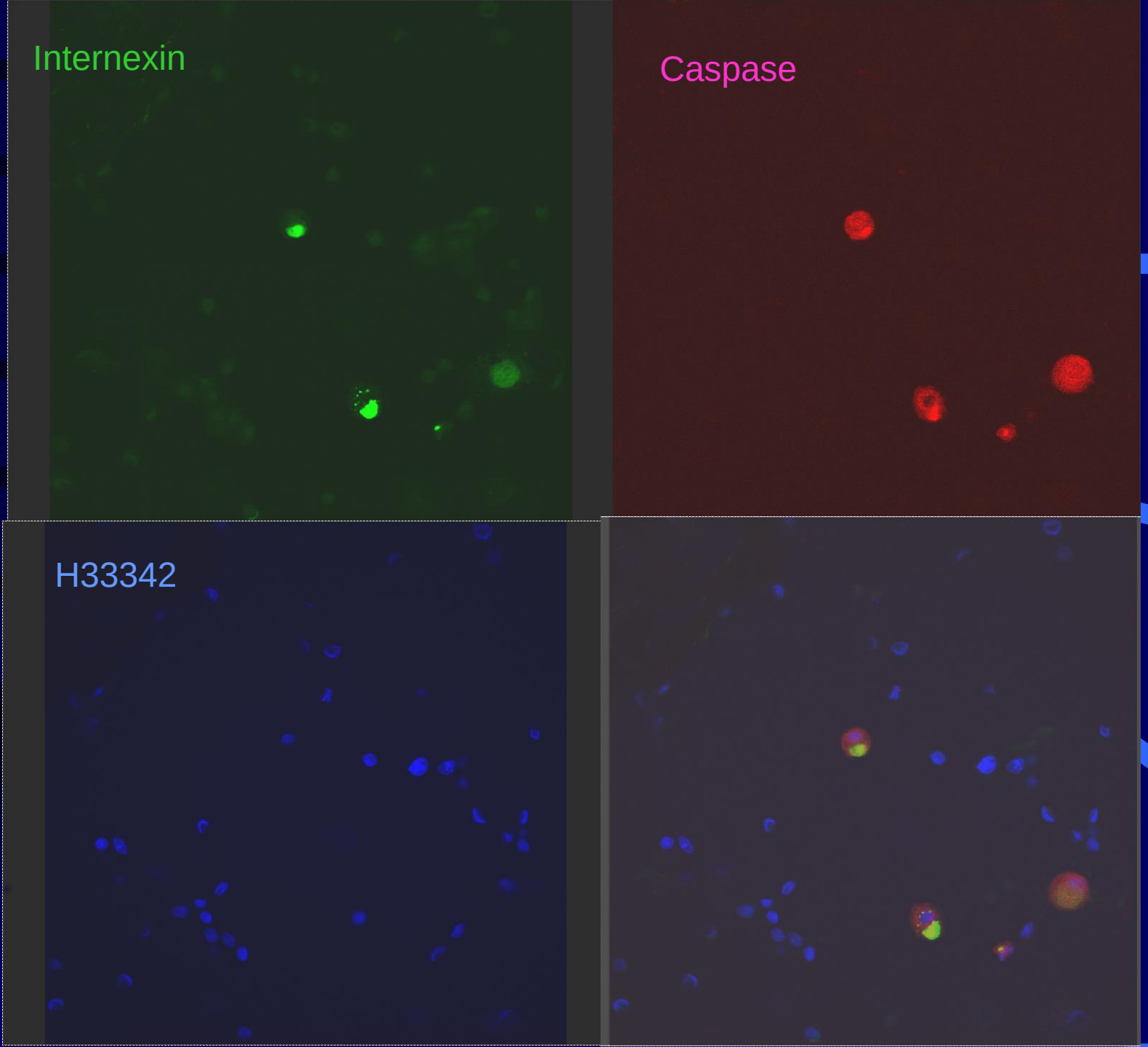






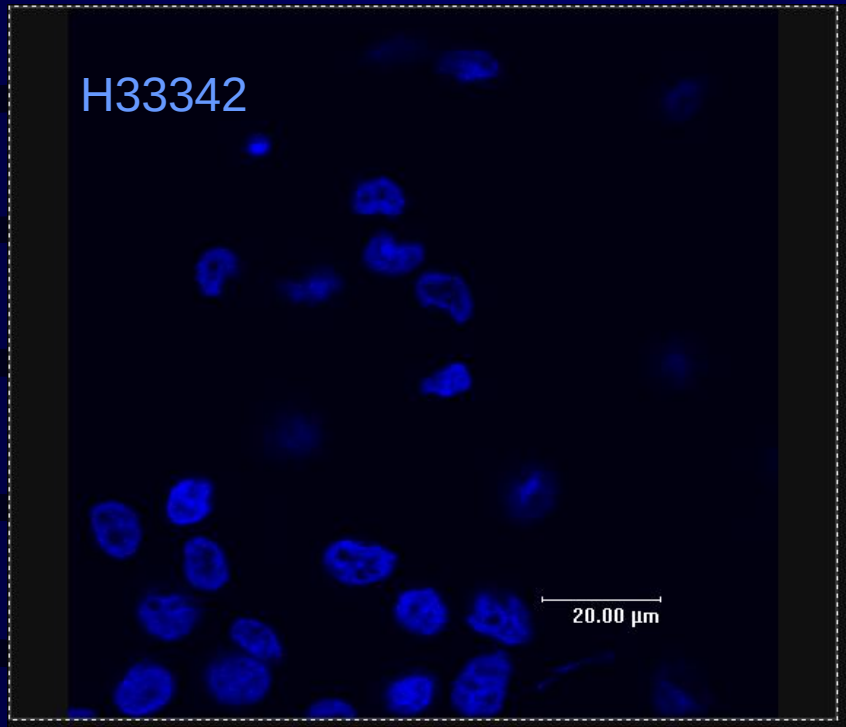
dt/dt
DRG
7 DIV

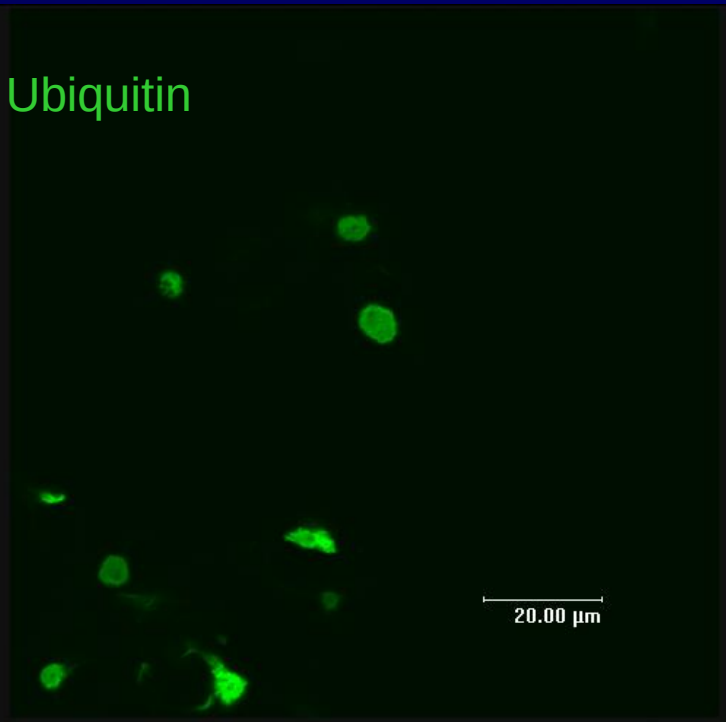
Activated Caspase

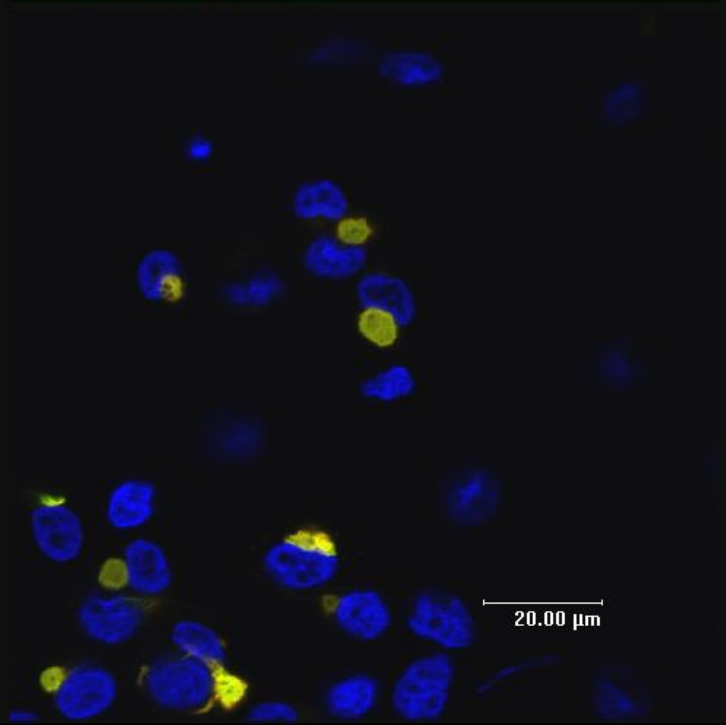


dt/dt
DRG
7 DIV

Ubiquitin
Pathway







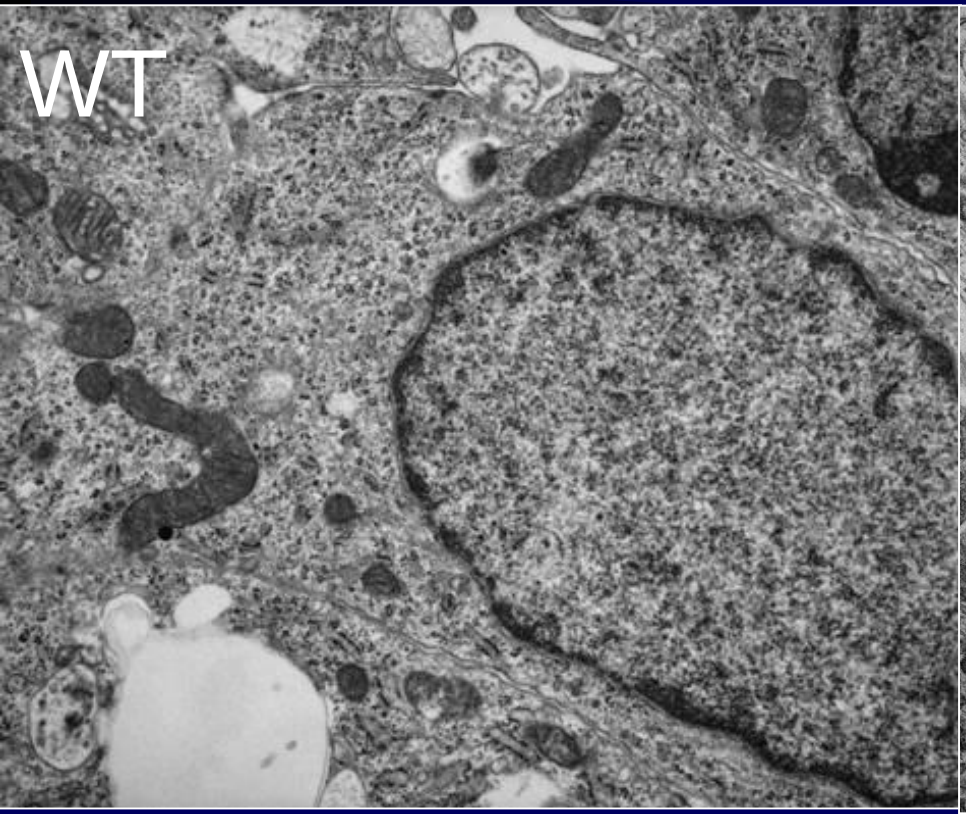
Primary culture of DRG

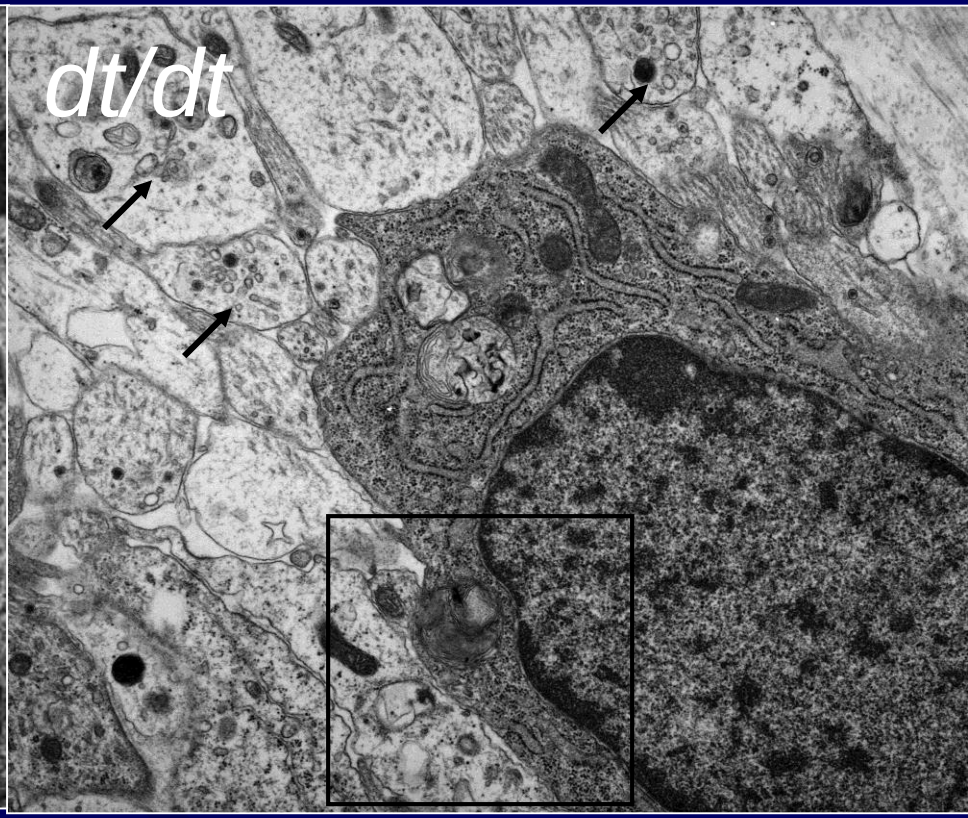
Immunostaining Patterns

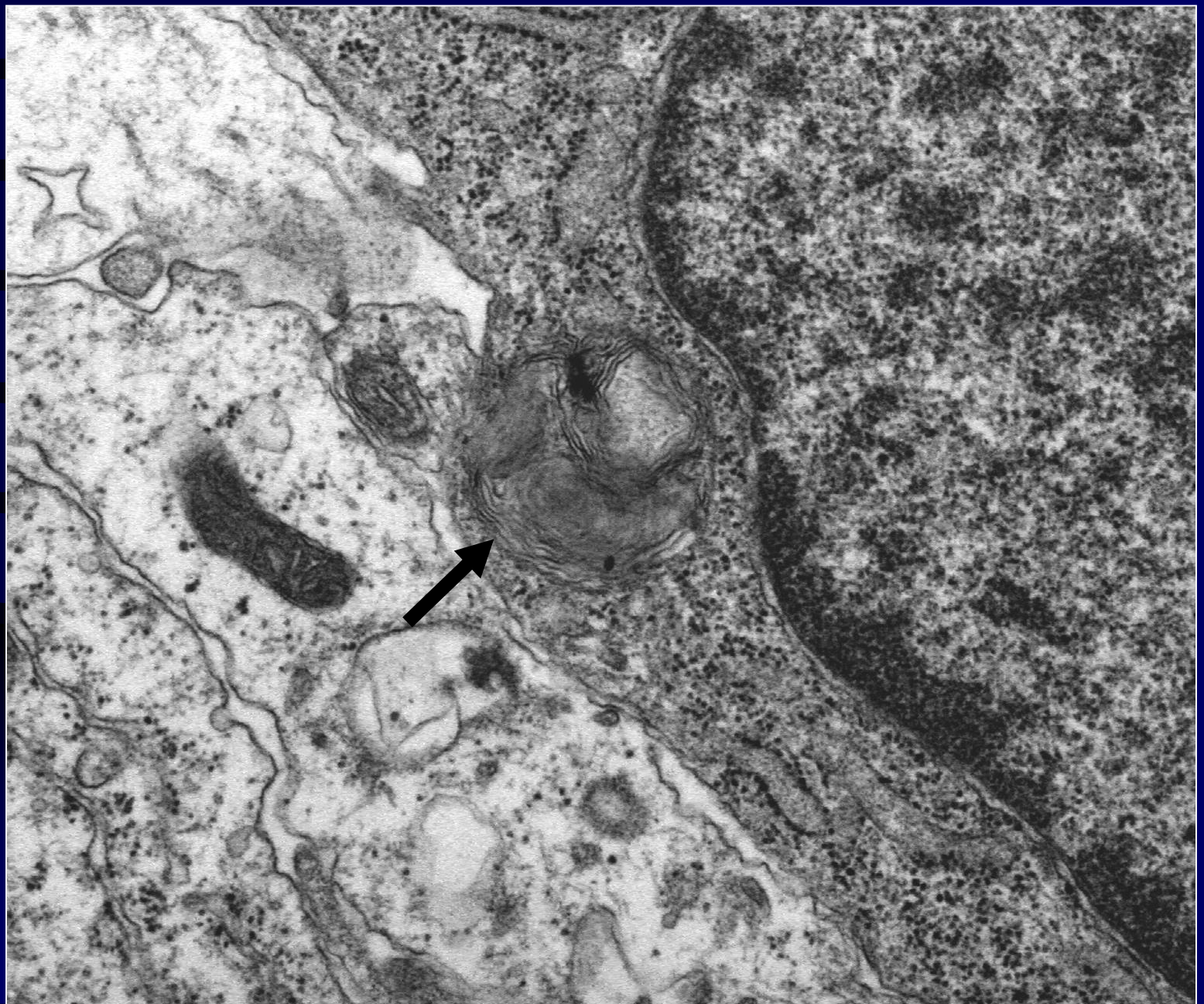
	WT	*dt/dt*
Internexin	+	++ **Aggregations**
Peripherin	+	++ **Aggregations**
Hoechst 33342	round	Apoptosis?

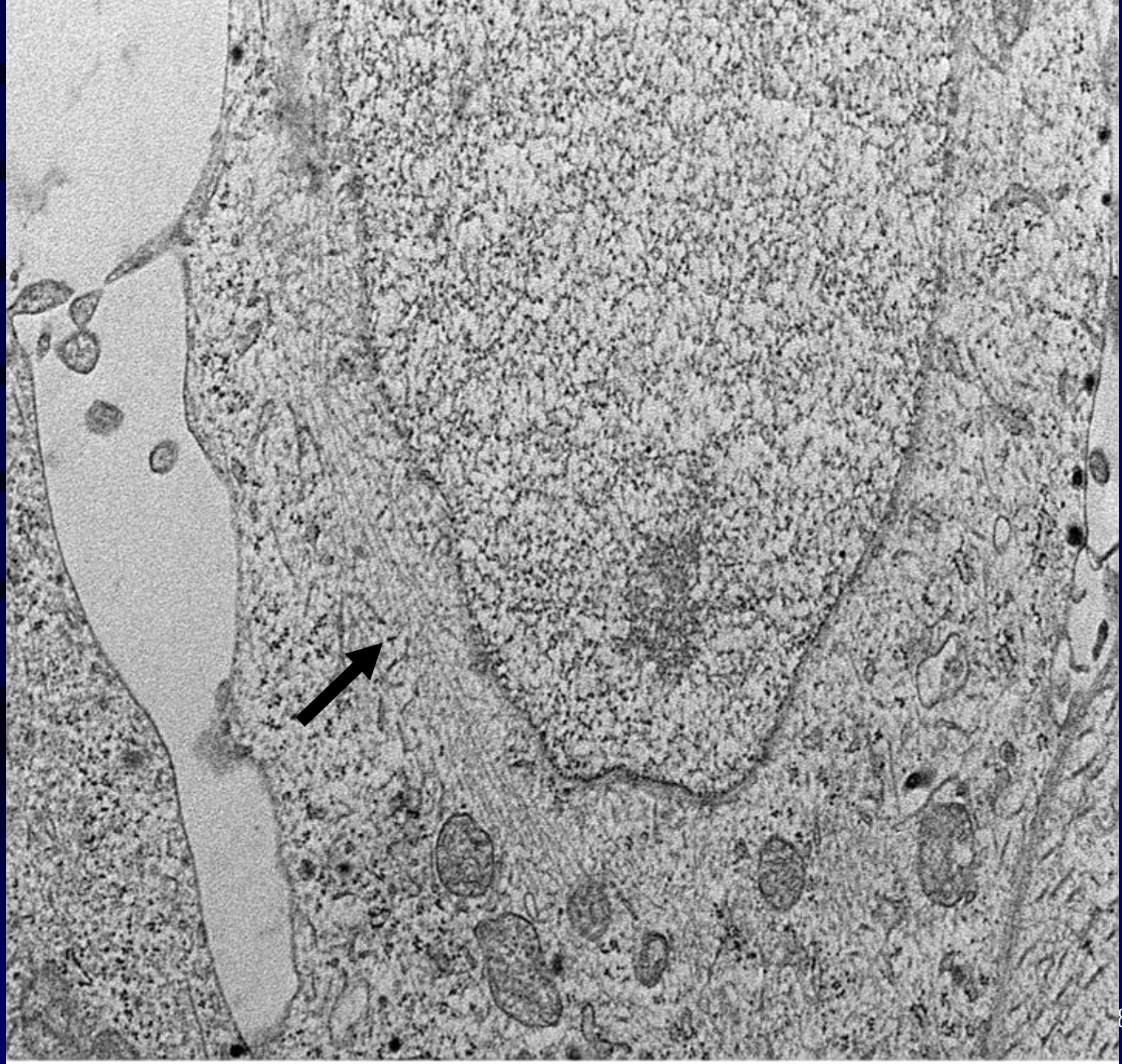
	WT	*dt/dt*
Internexin	+	++ **Aggregations**
Activated Caspase	-	+
Ubiquitin	-	+

NFs accumulation in cytoplasm

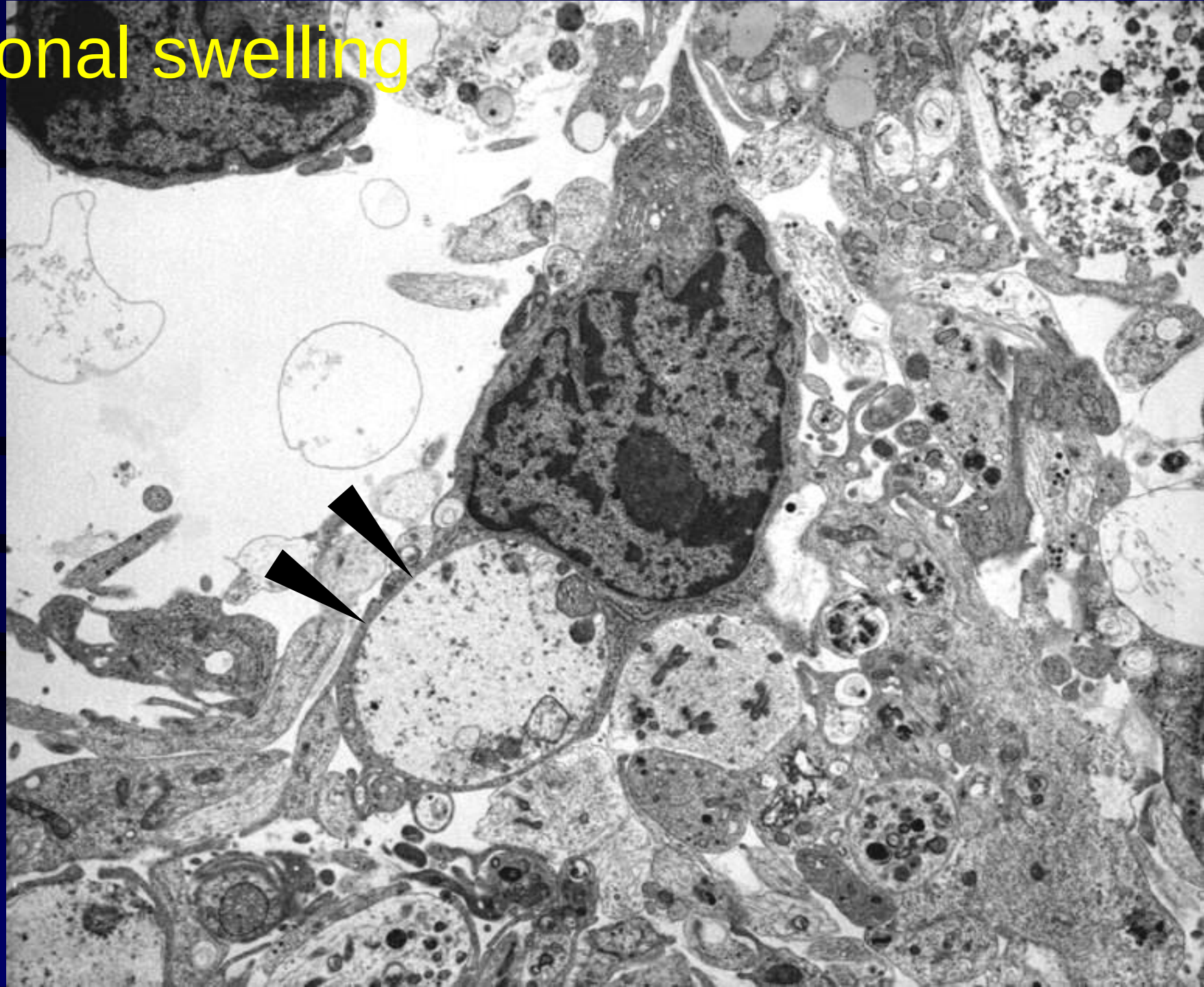


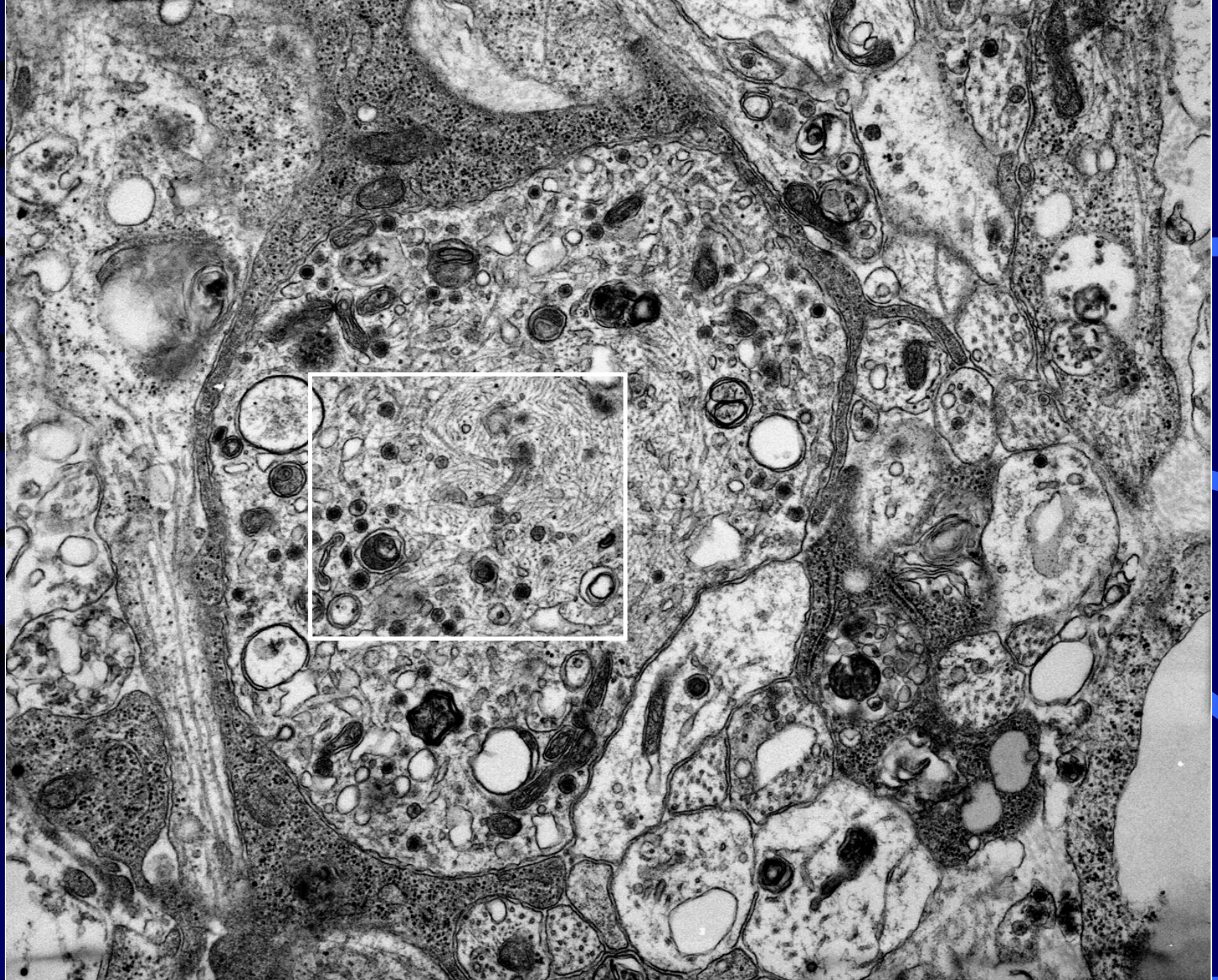


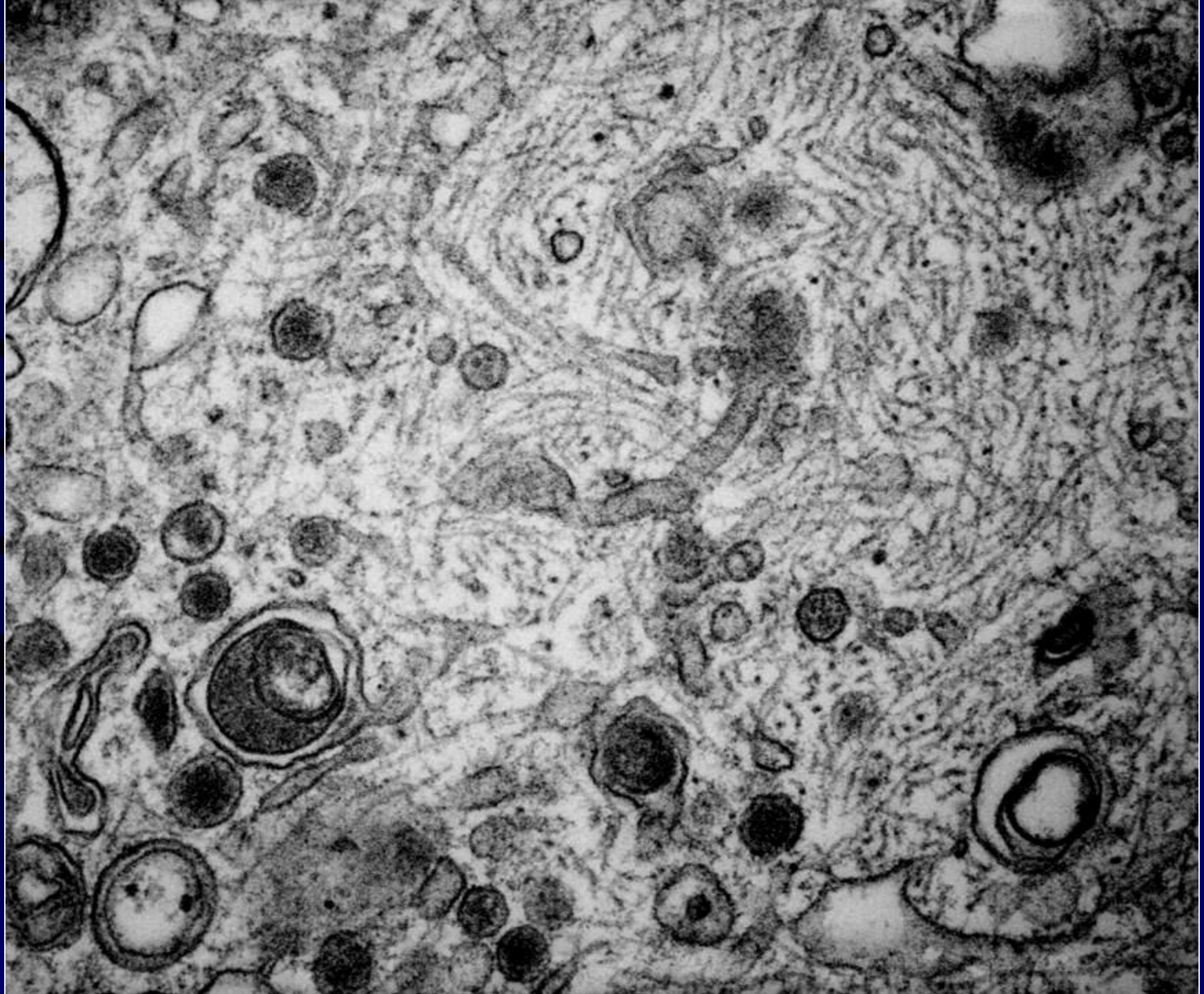




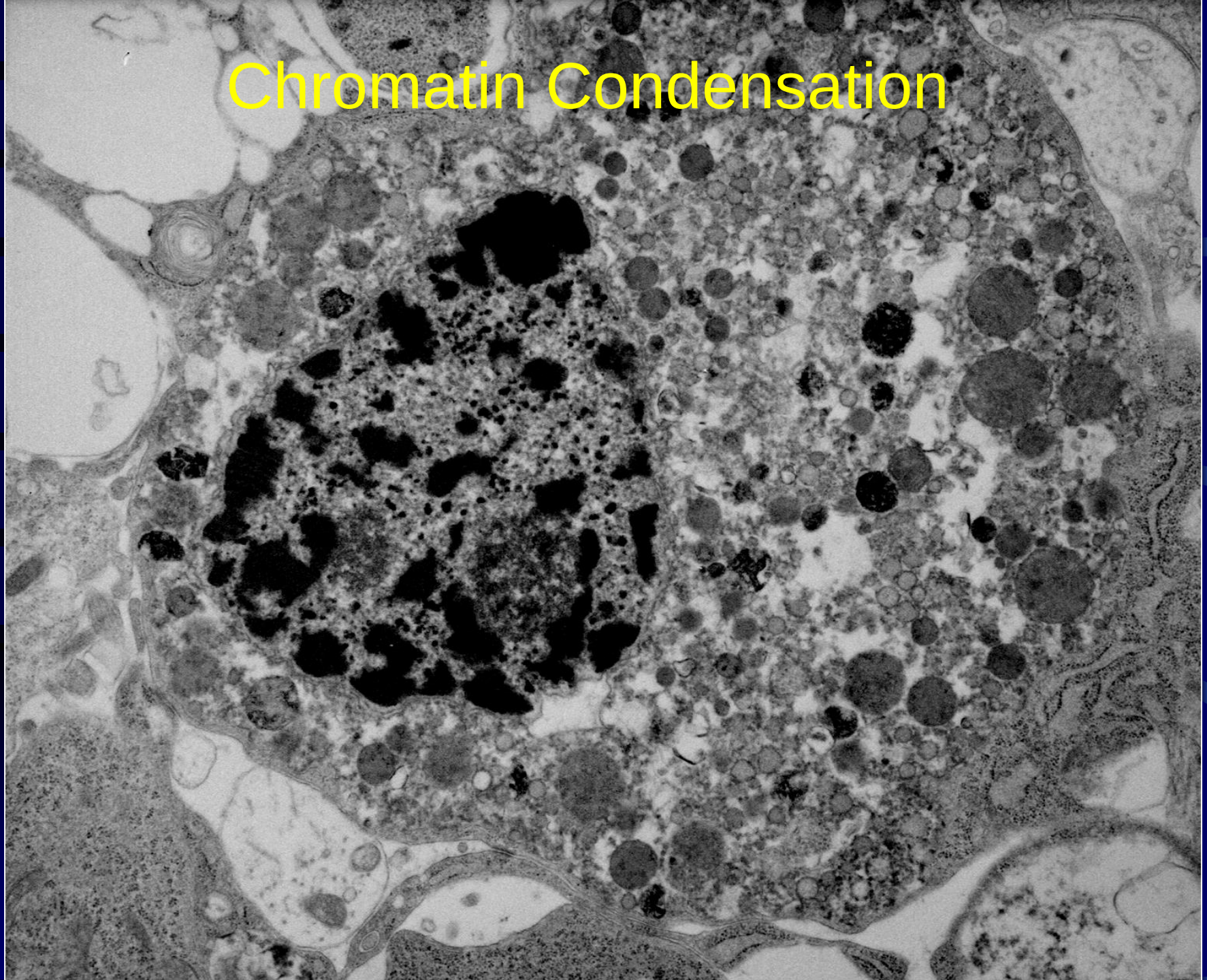
Axonal swelling







Chromatin Condensation

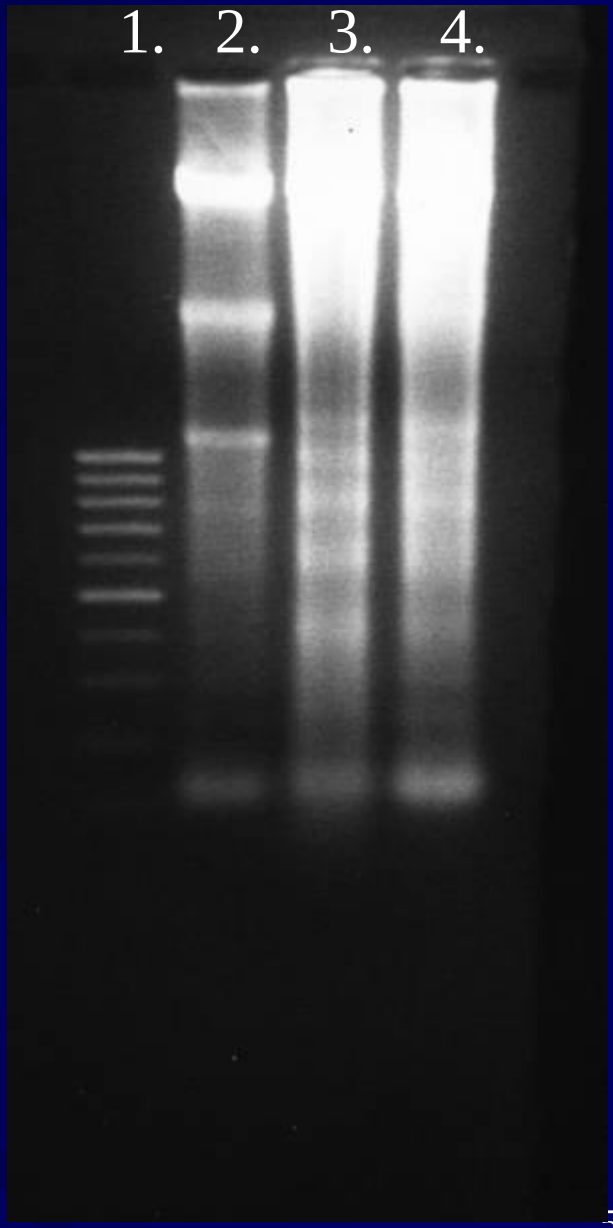


DNA Ladder

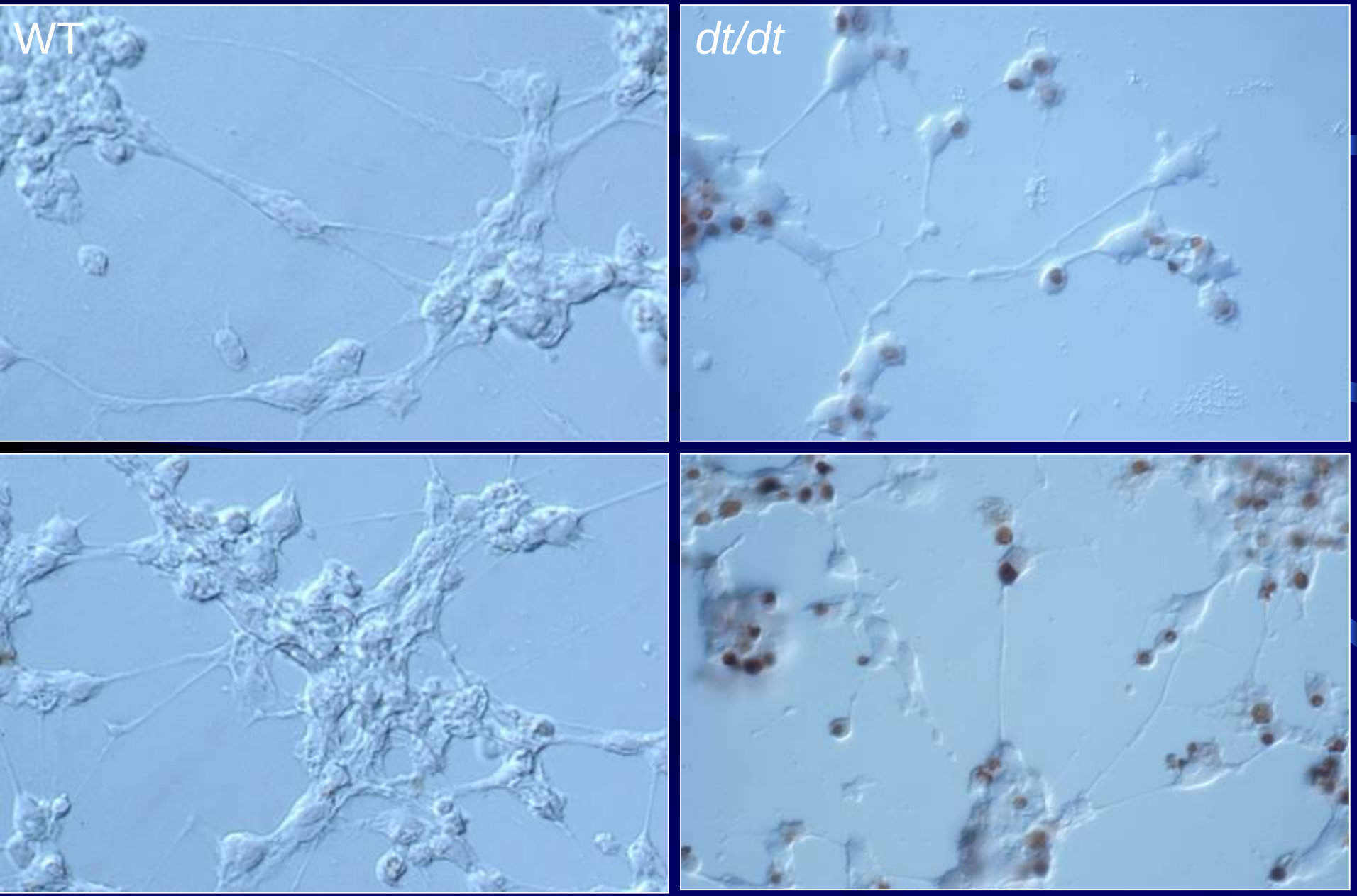
1. Marker: 100 bp marker

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

500 bp



TUNEL Assays

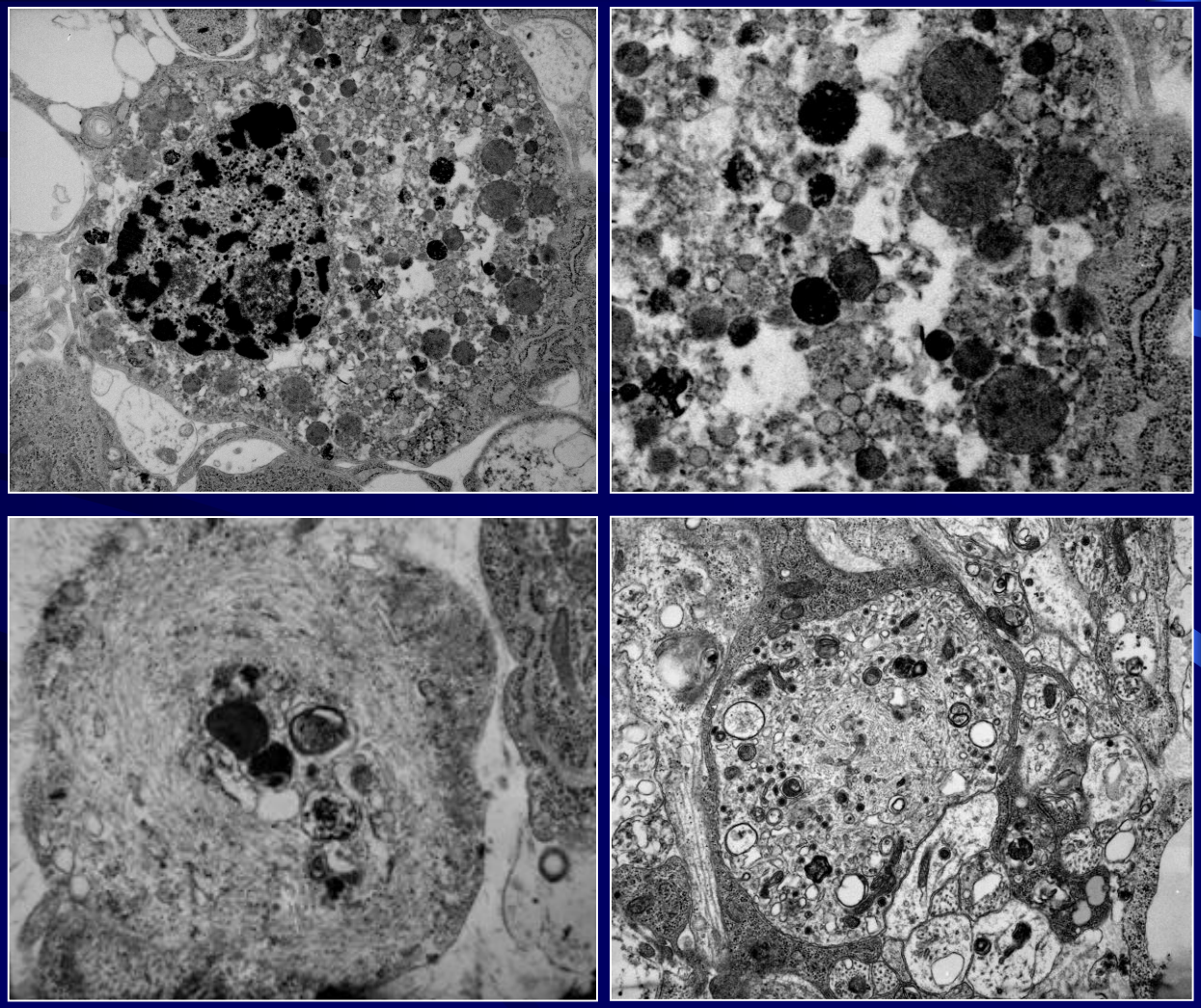


Primary culture of DRG neurons

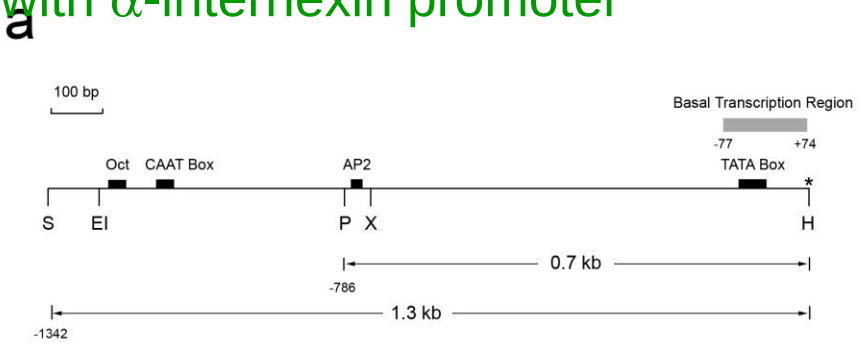
DRG neurons of *dt/dt* mice observation in Electron microscope

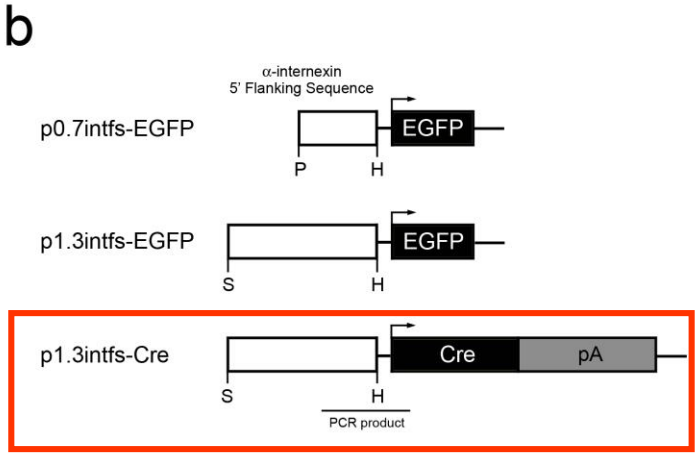
- Chromatin condensation
- NFs accumulation
- Axonal swelling
- DNA fragmentation
- TUNEL-positive neurons

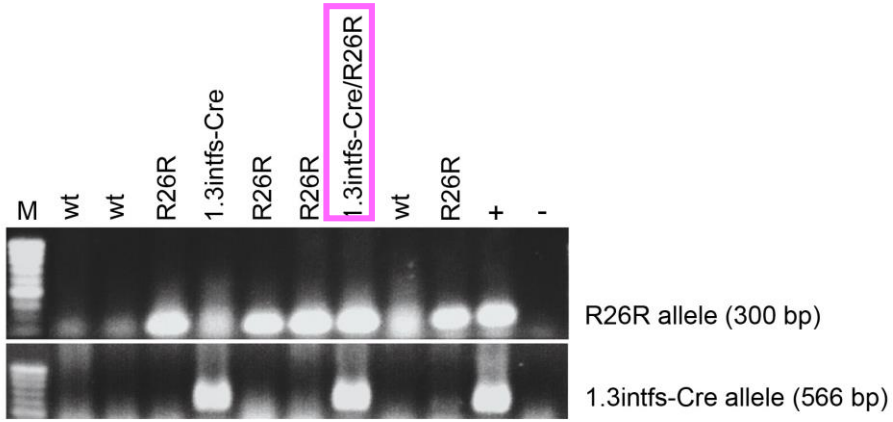
➢ Cell death in apoptosis pathway

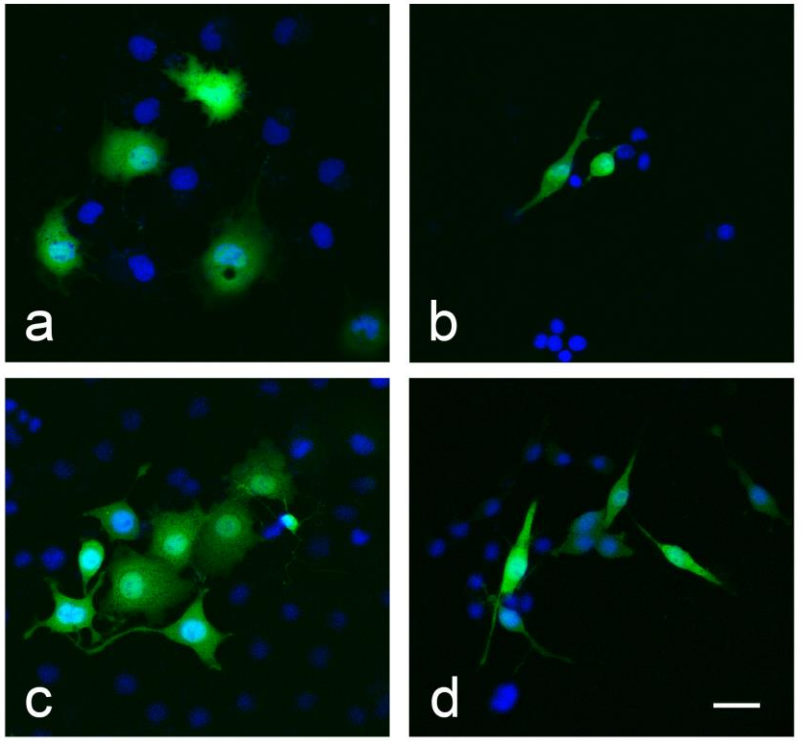


Neural Specific Cre transgenic Mouse with α-internexin promoter









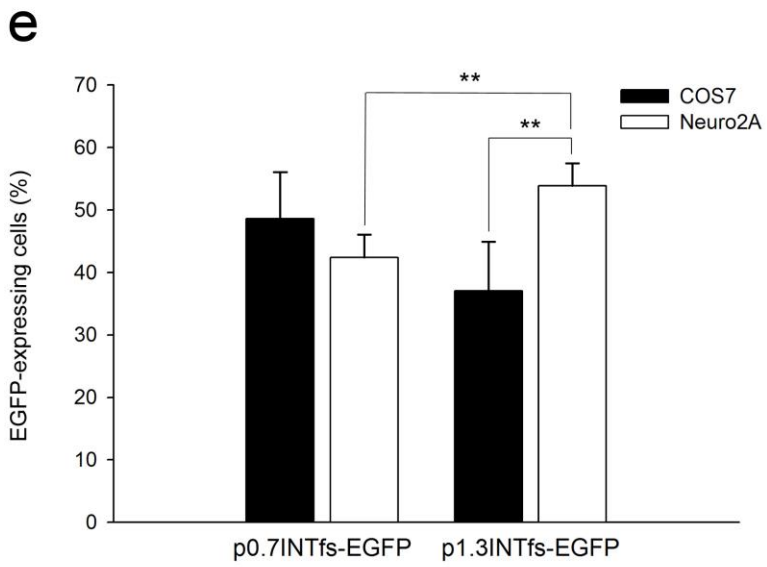


Fig 2

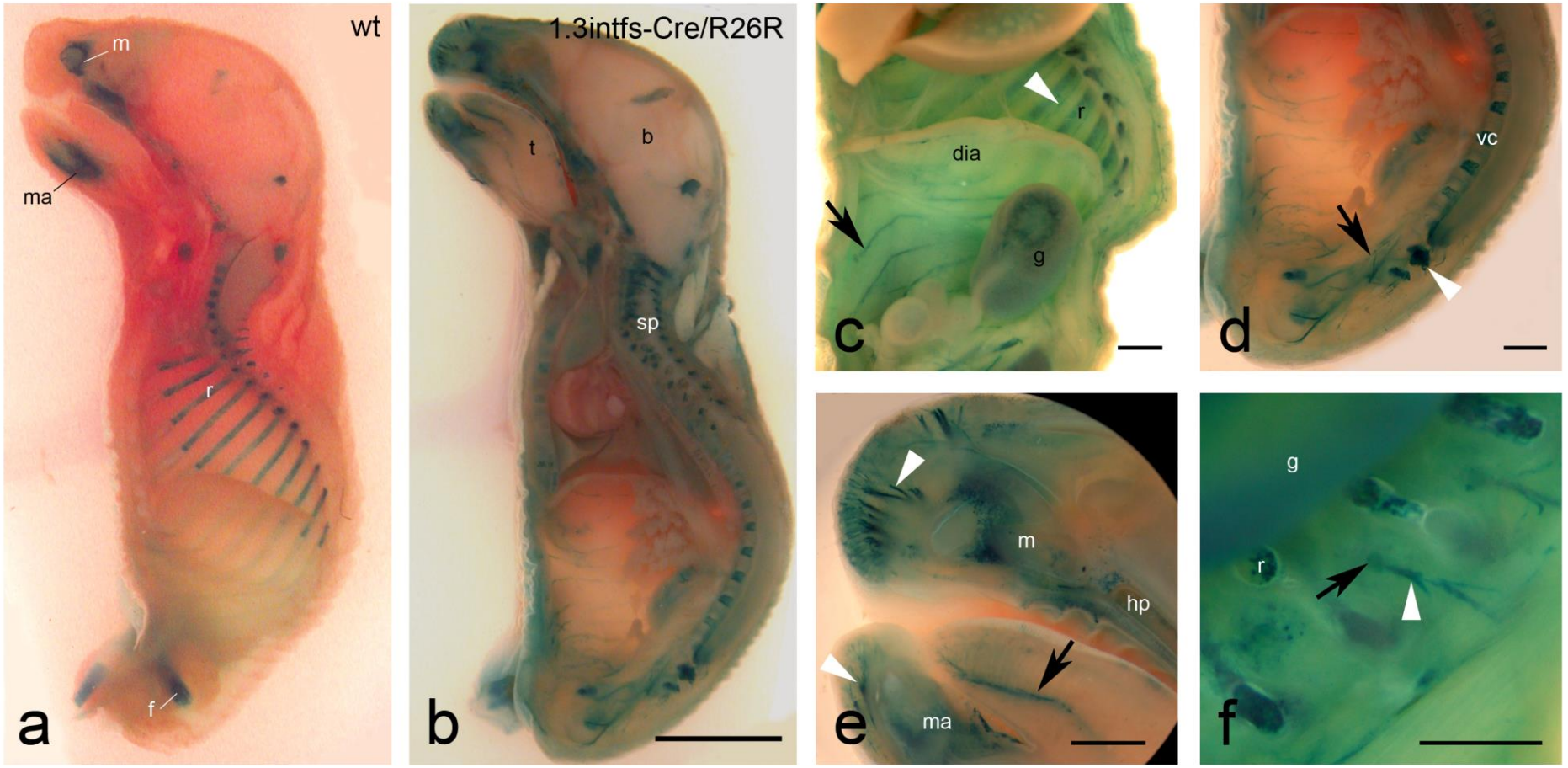


Fig 5