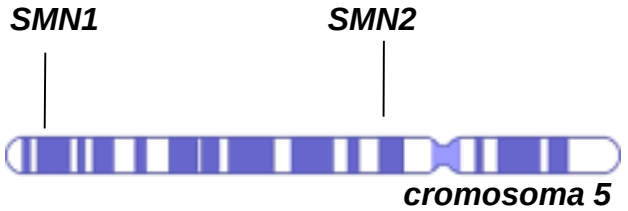
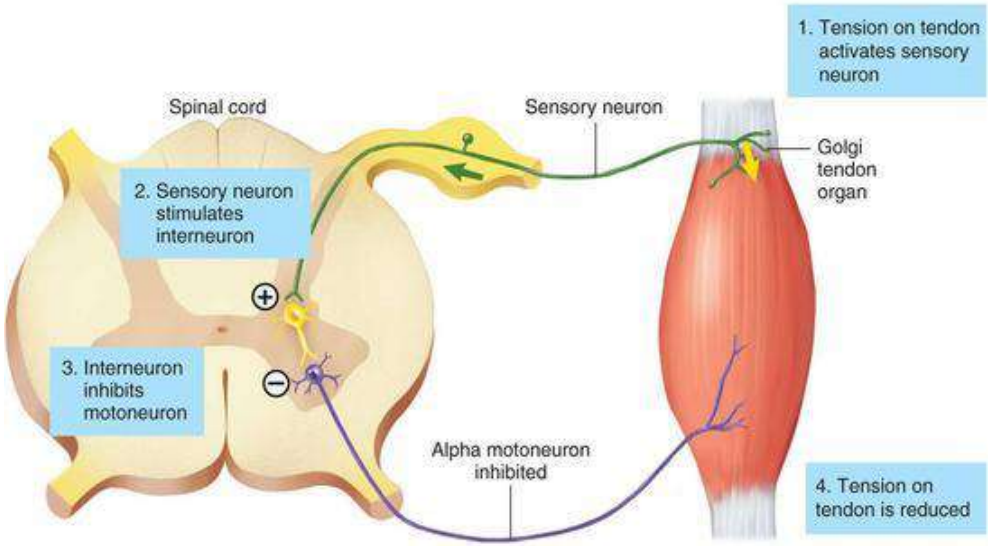
Spinal Muscular Atrophy (SMA)

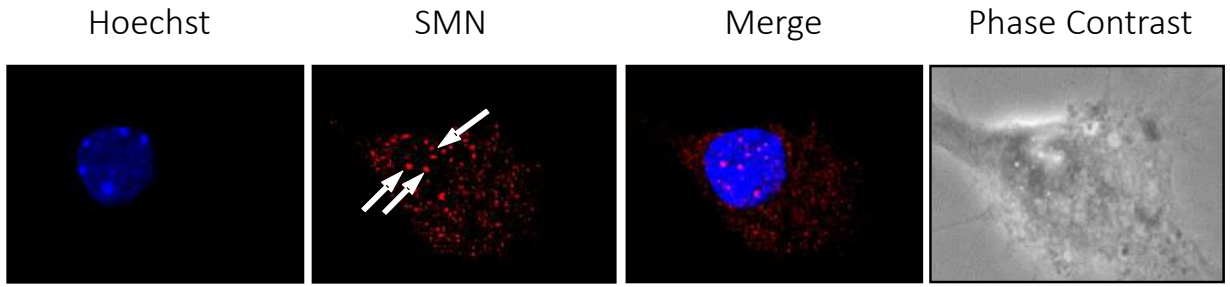
Cultius de Motoneurones (MN) i la seva utilitat per a l'estudi de malalties de la MN

- Hereditary disease
- Degeneration of α-MNs
- Prevalence of 1 in 6000 live births
- Symmetrical muscular weakness
- Caused by deletion or mutation of the Survival Motor Neuron gene (SMN)
- Disease severity varies, based on copy number and expression of SMN2

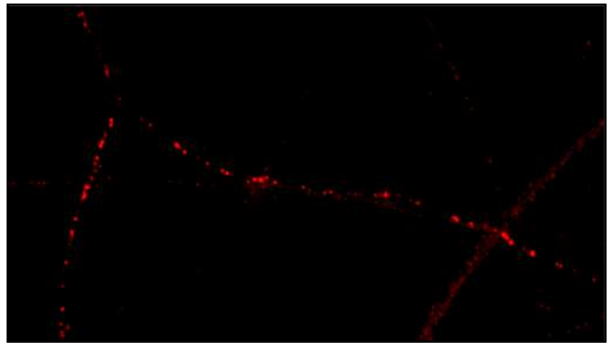




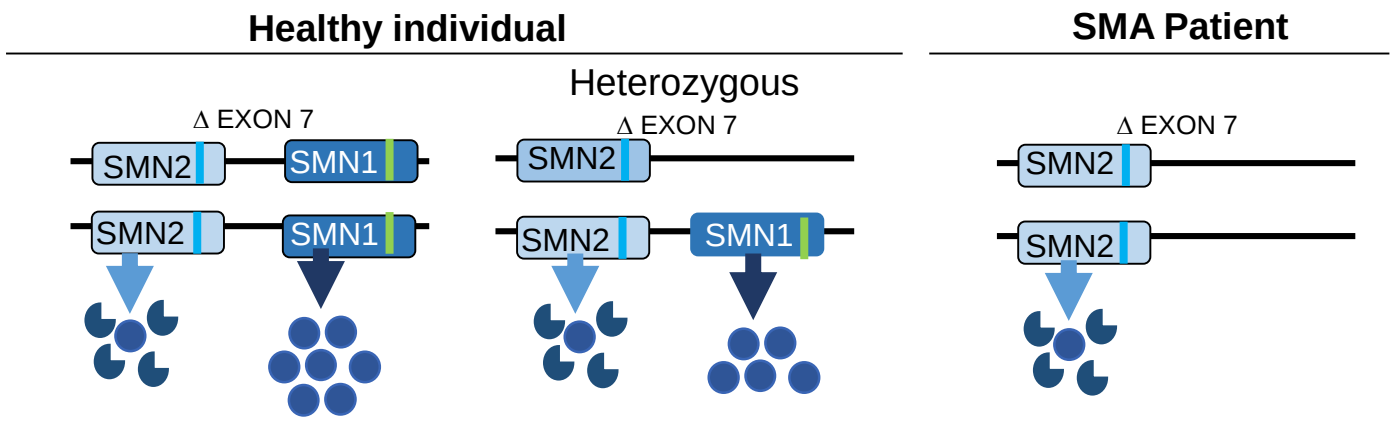
http://www.krigolsonteaching.com/afferent--efferent-systems.html



Neurites

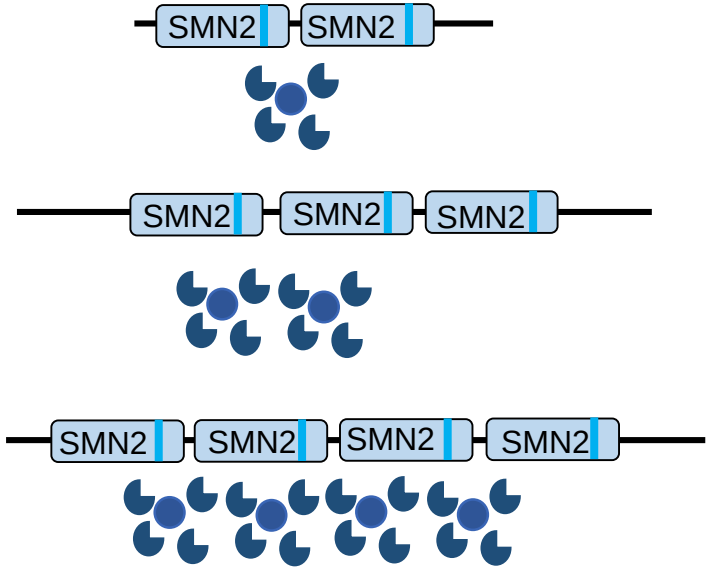


Survival Motor Neuron gene (*SMN*)

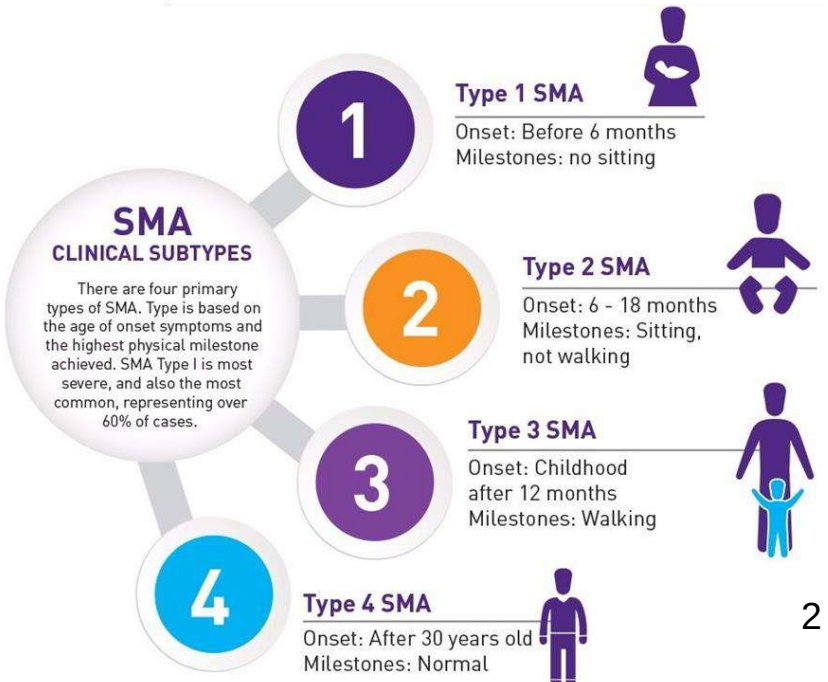


Monani U., Neuron 2005

Disease severity depends on *SMN2* copy number and *SMN2* expression



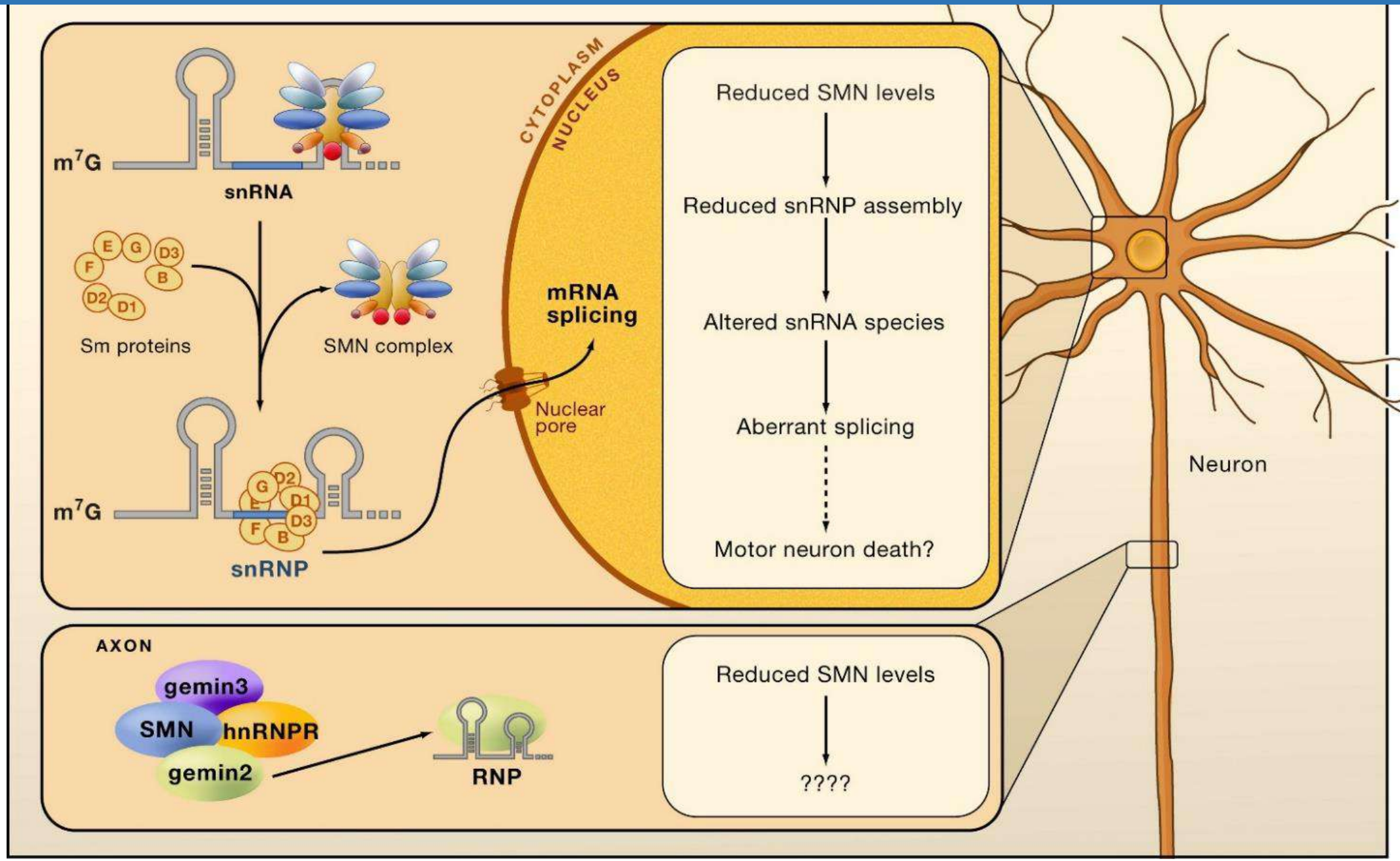
SMA classification



Survival Motor Neuron gene (SMN) Functions

snRNP assembly
Aberrant splicing

mRNA transport

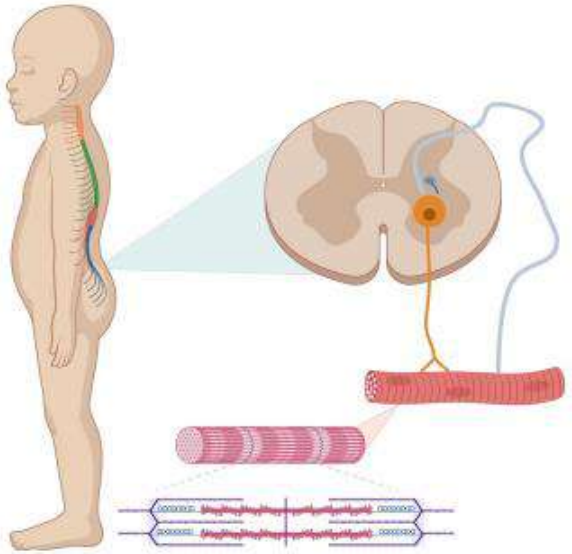


Cell 2008 133, 572-574DOI: (10.1016/j.cell.2008.05.002)

SMA THERAPIES

MOTOR NEURON DEGENERATION

MUSCULAR ATROPHY



IMPAIRED ENS SIGNALS

- Masticatory muscle weakness
- Dysphagia
- Gastroesophageal reflux
- Delayed gastric emptying
- Constipation

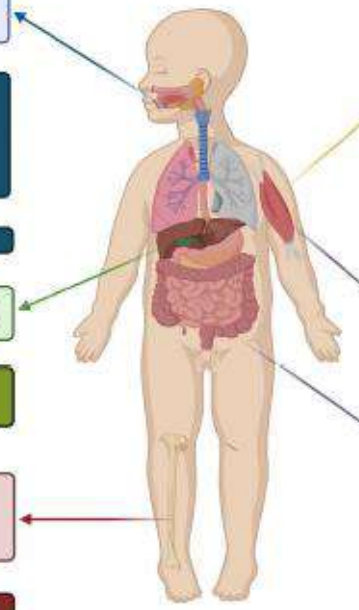
- Risk of aspiration

IMPAIRED FATTY ACID METABOLISM

- Dyslipidemia
- NAFLD

VITAMIN D DEFICIENCY
HYPERCALCIURIA
HYPERCALCEMIA

- Osteopenia
- Bone fractures



SARCOPENIA

- Impaired glucose tolerance
- Fasting hypoglycemia
- Insulin resistance

SARCOPENIA
INSULIN RESISTANCE

- Precocious pubarche

ABDOMINAL MUSCLE WEAKNESS

- Bilateral cryptorchidism

Corsello et al., 2021

NUEVA TERAPIA

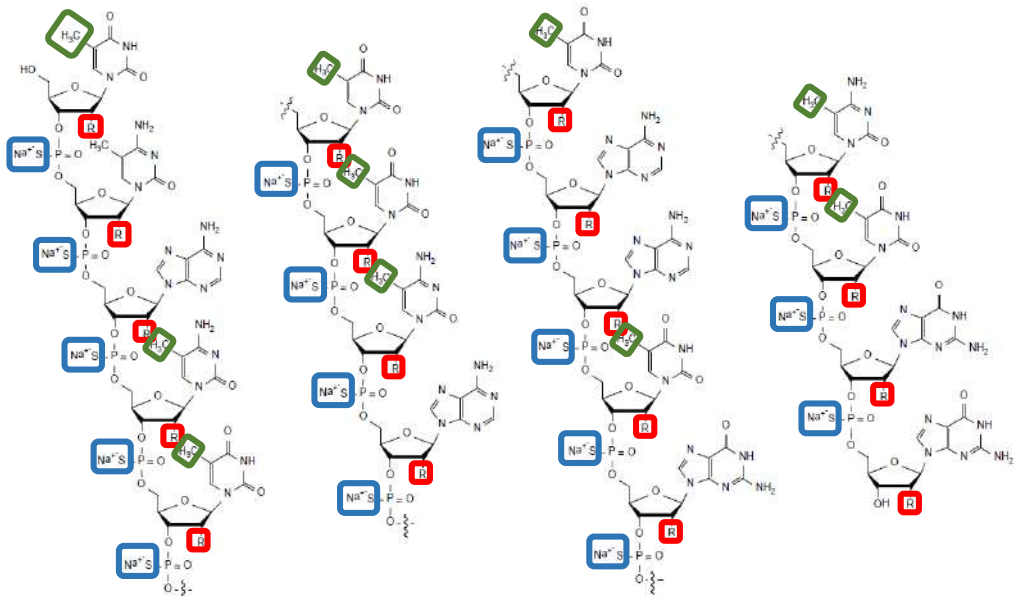
El medicamento más caro del mundo, pero no el más costoso

En España hay unas 400 personas con atrofia muscular espinal, un trastorno para el que se ha aprobado en EEUU una terapia génica que cura la enfermedad con un coste de casi dos millones de euros

ISIS 396443

5′-^{Me}U^{Me}CA^{Me}C^{Me}U^{Me}U^{Me}U^{Me}CA^{Me}UAA^{Me}UG^{Me}C^{Me}UGG-3′

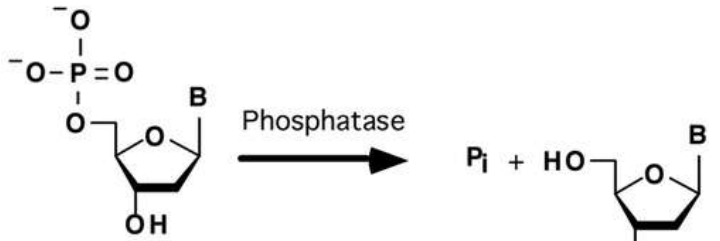
5-methy | 2-metoxietileno | Phosphorothioate

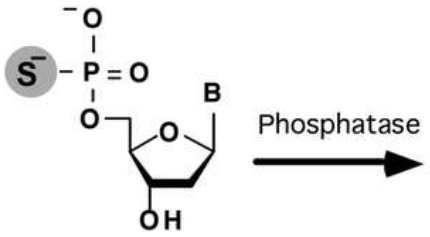


Sequence - idival.2 — 100 nmole DNA Oligo, 1 bases

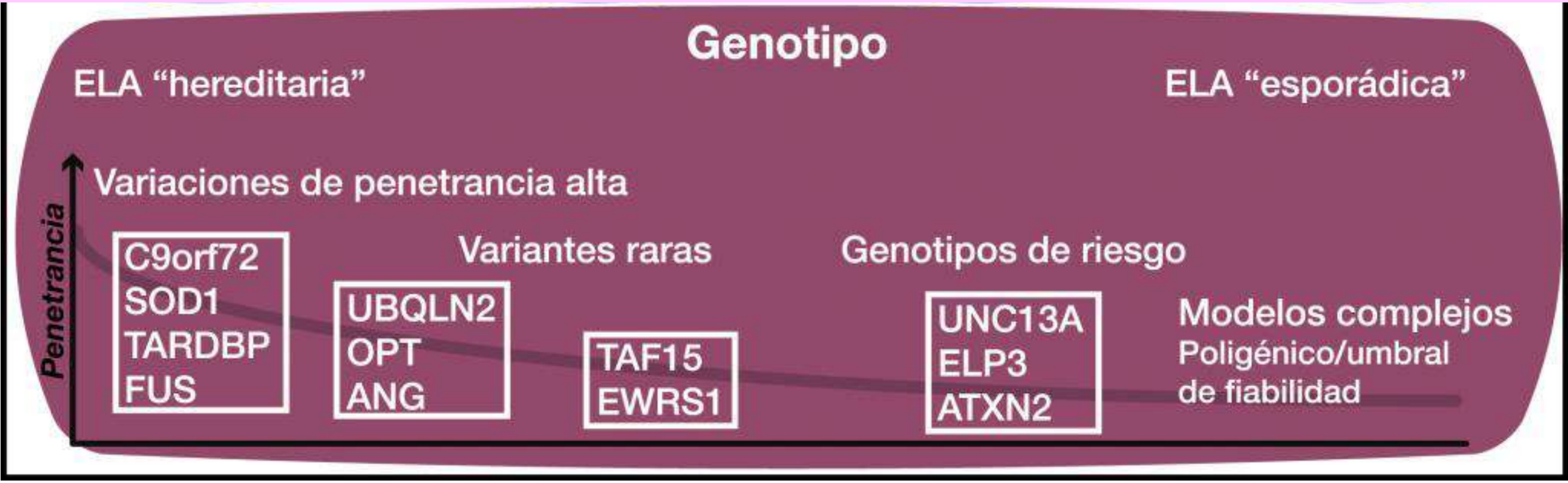
5'- /5Phos/T/iMe-dC//i2MOErA/ /iMe-dC//i2MOErT//i2MOErT/ /i2MOErT//iMe-dC//i2MOErA/ /i2MOErT//i2MOErA//i2MOErA/ /i2MOErT//i2MOErG//iMe-dC/ /i2MOErT//i2MOErG//32MOErG/ -3'

Oligo Base Types	Quantity
DNA Bases	1
Modifications and Services	**Quantity**
Standard Desalting	1
3' 2-MethoxyEthoxy G	1
5' Phosphorylation	1
Int 2-MethoxyEthoxy A	4
Int 2-MethoxyEthoxy G	2
Int 2-MethoxyEthoxy T	6
Int 5-Methyl dC	4





Amyotrophic Lateral Sclerosis (ALS)



10% are genetics

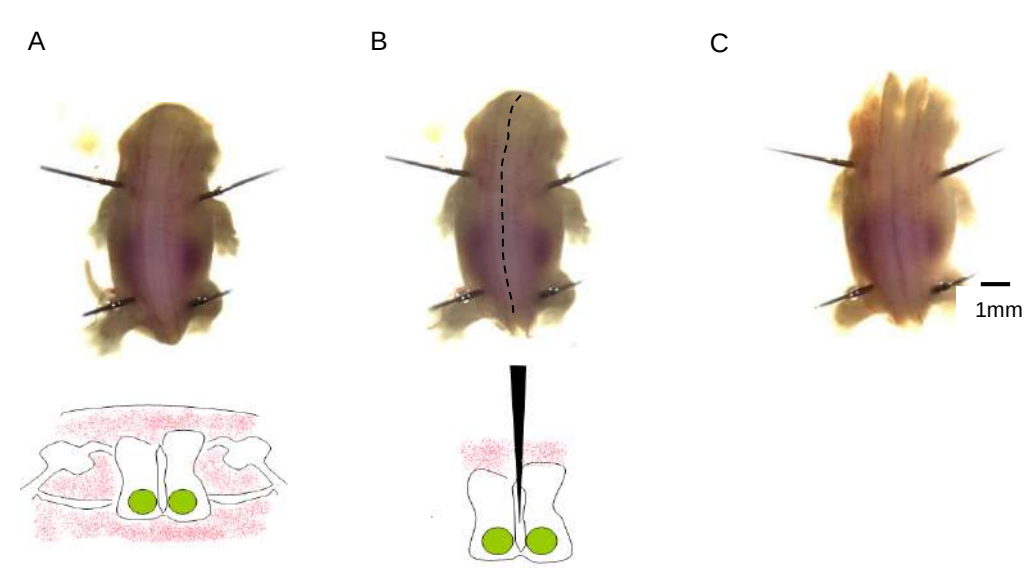
C9ORF72 gene
is the most common inherited cause for both ALS and FTD, in the first intron has expanded GGGGCC repeats, it form inclusions throughout the brain of patients. nuclear RNA aggregates, which recruit specific RNAbinding proteins, thereby potentially inhibiting their functions

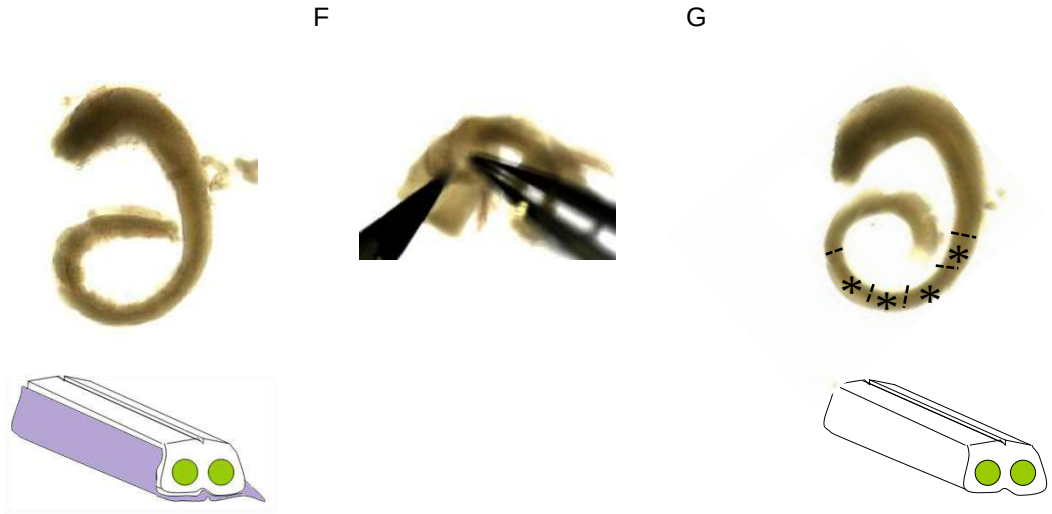
SOD1

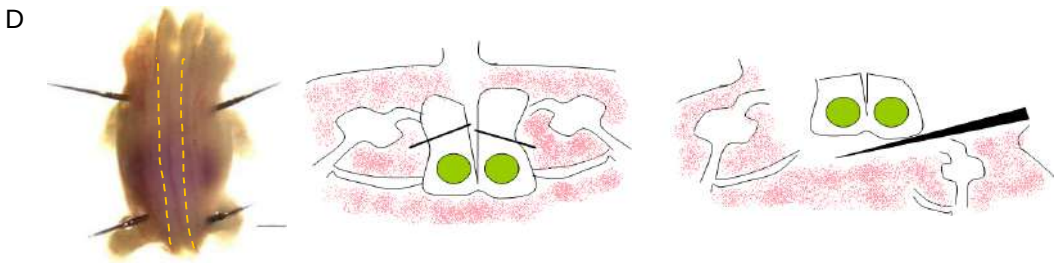
20% are associated with dominant mutations in Cu/Zn superoxide dismutase (SOD1) genes

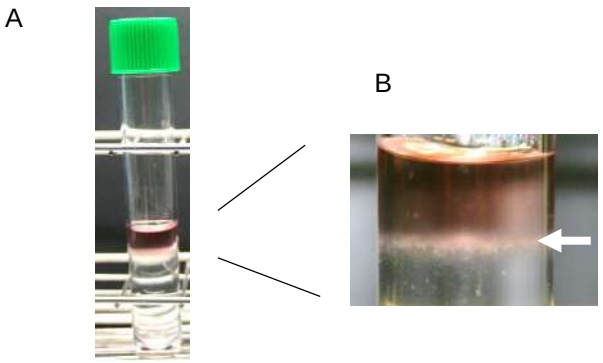
TARDBP (TDP43)

Cultivo primari de MNs

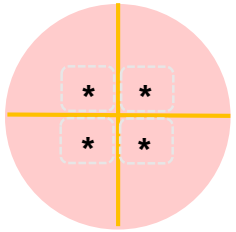






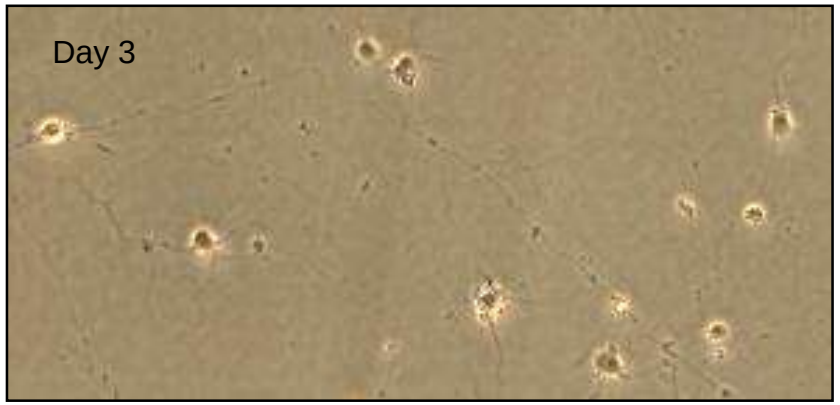


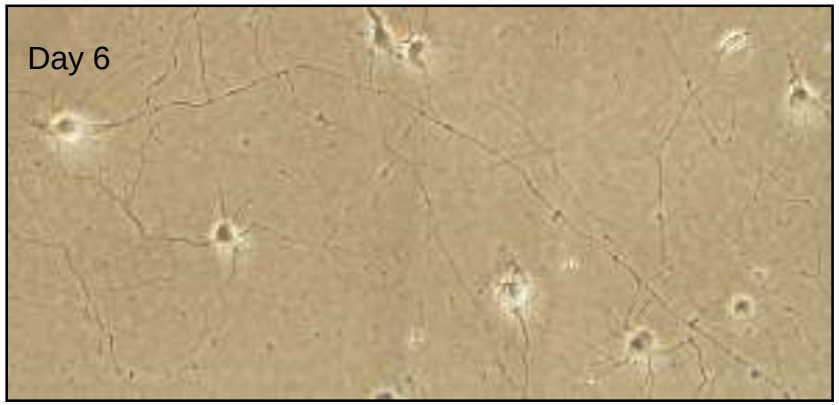
A

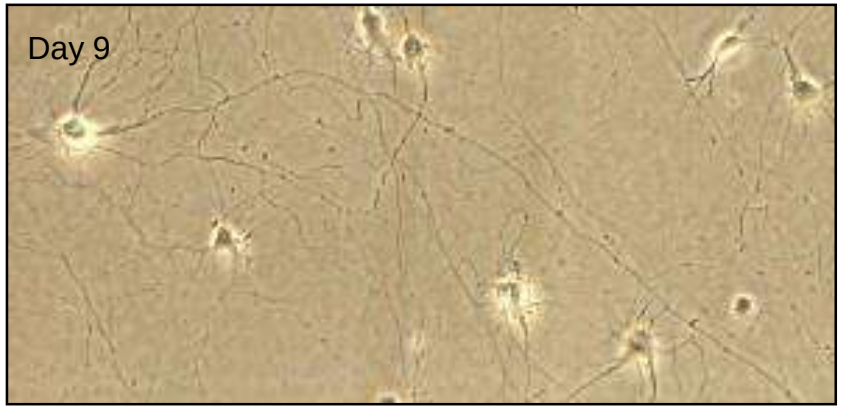


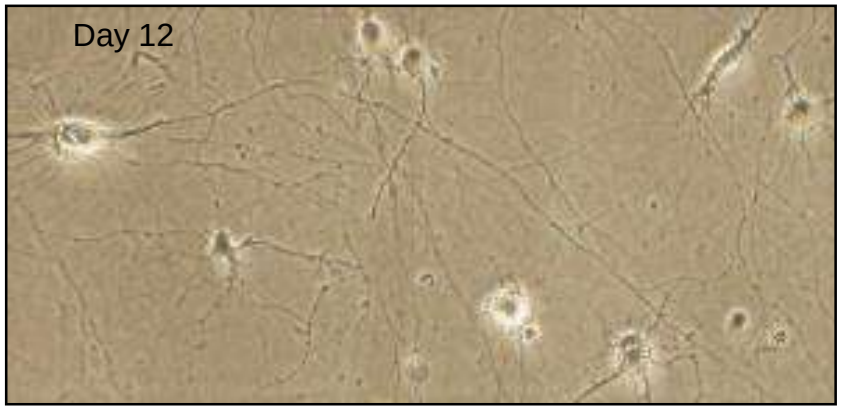
B

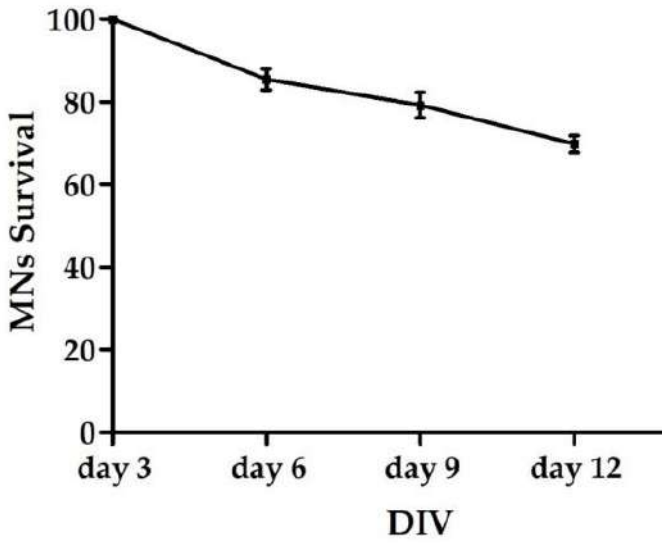

Seeding
Medium change
DIV 0
Viral transduction
3
6
9

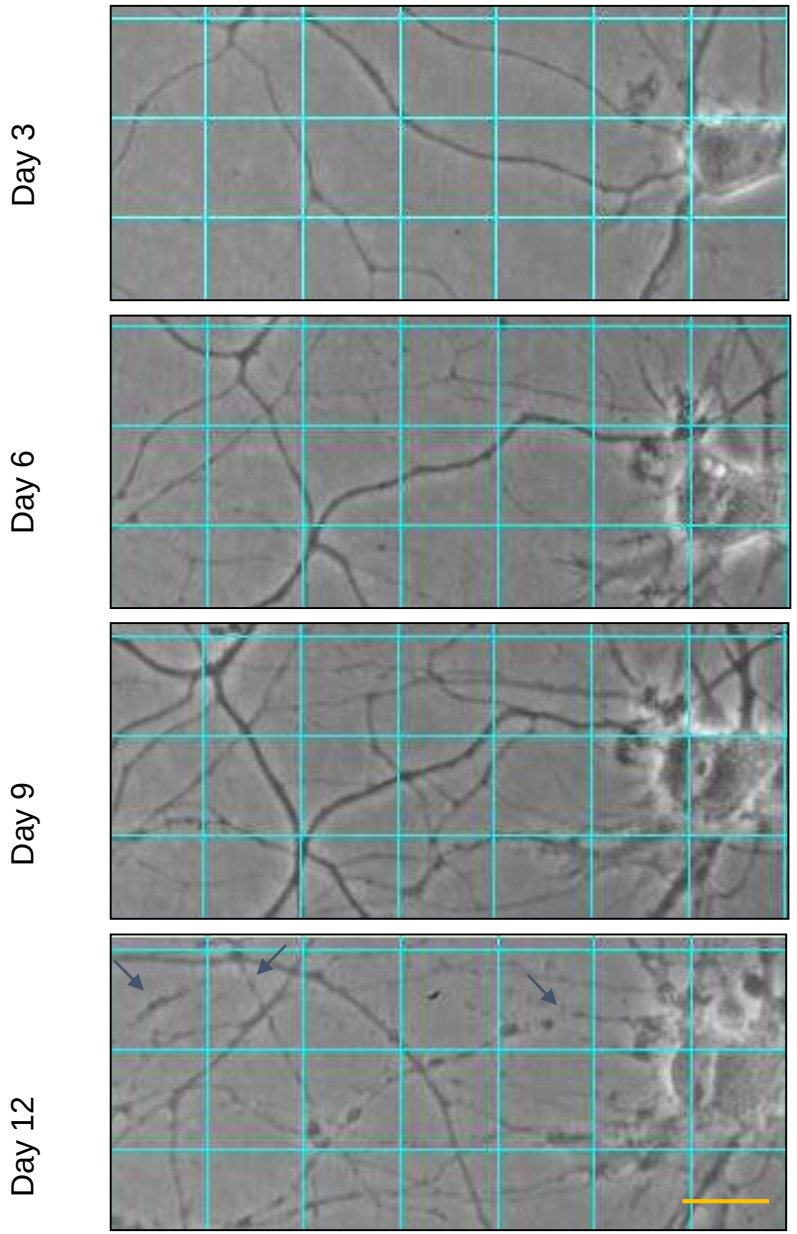

Day 3

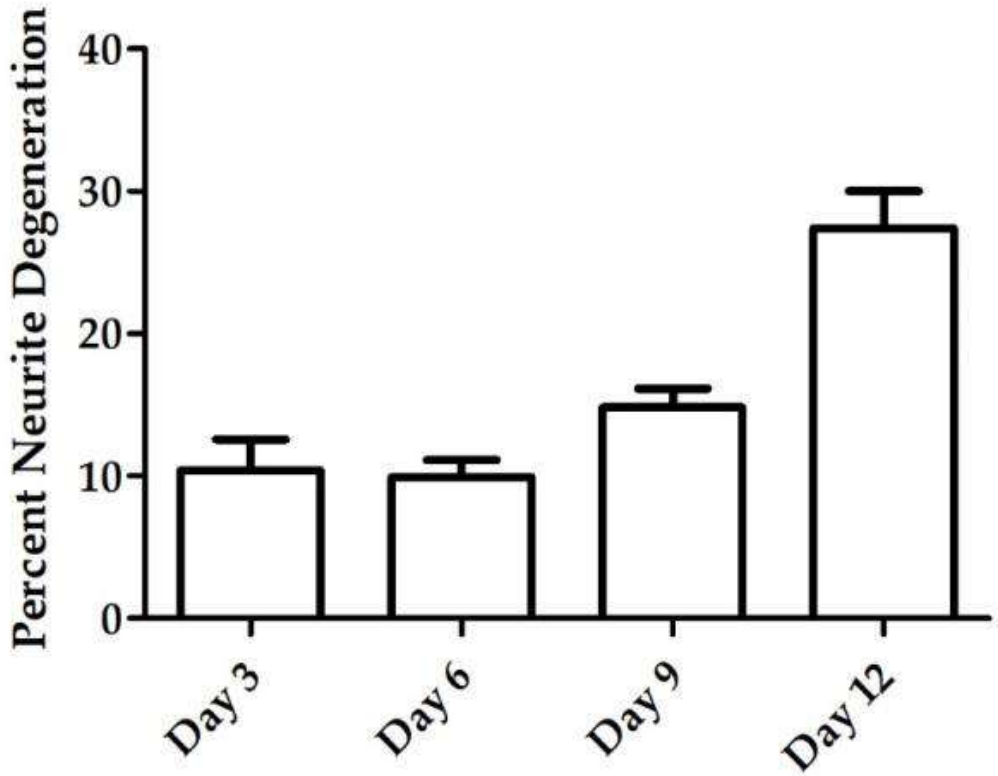

Day 6

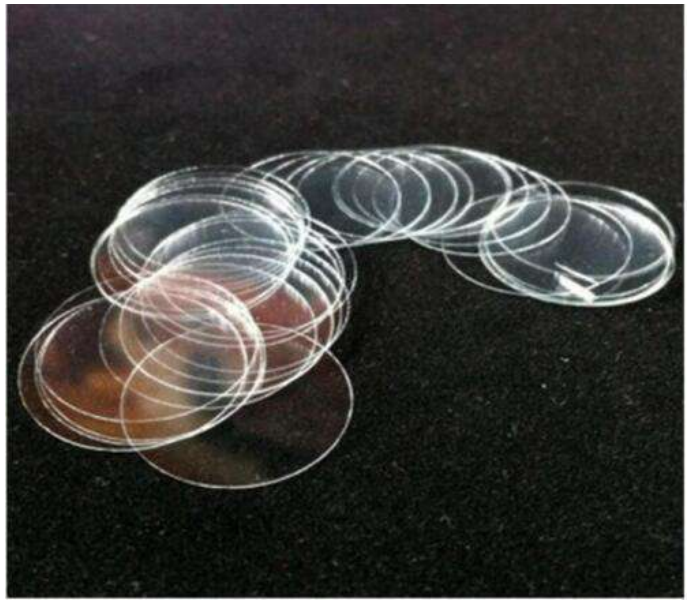

Day 9

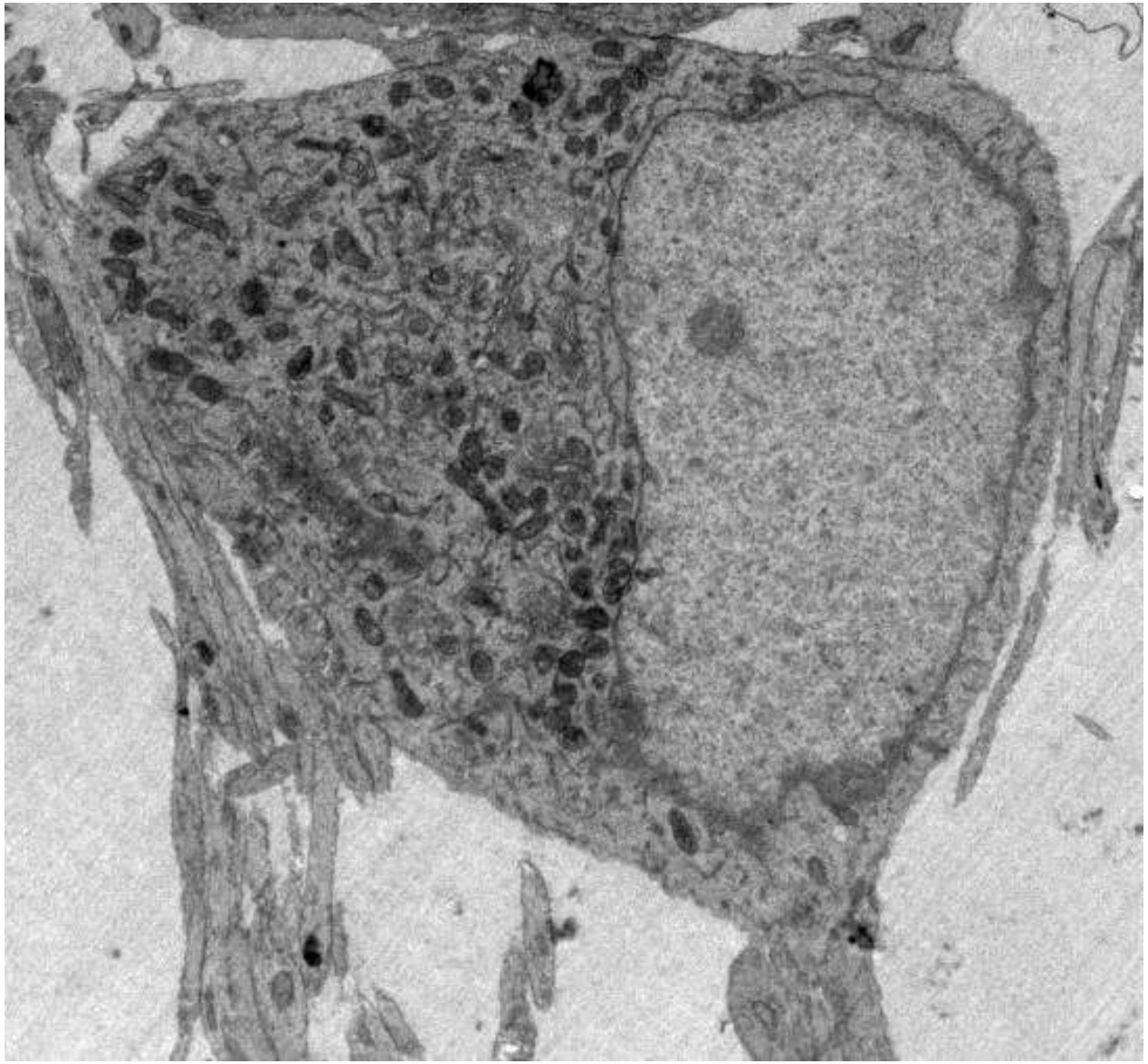

Day 12


MNs Survival
100
80
60
40
20
0
day 3
day 6
day 9
day 12
DIV

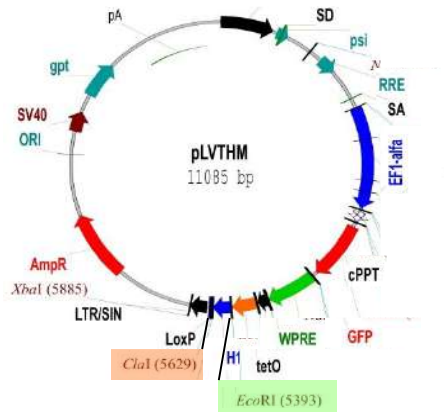

Day 3
Day 6
Day 9
Day 12

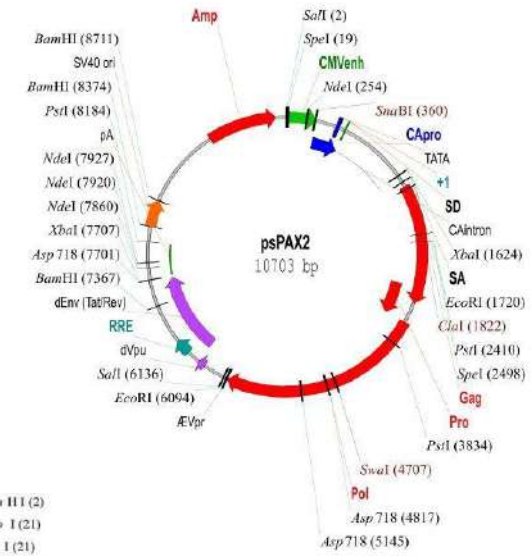

Percent Neurite Degeneration
40
30
20
10
0
Day 3
Day 6
Day 9
Day 12

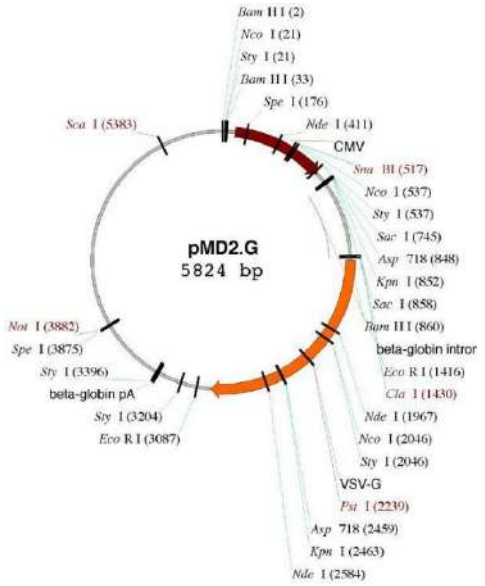


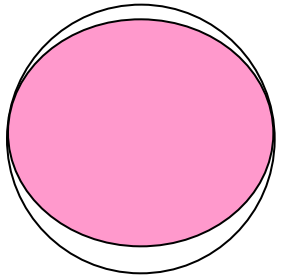


Obtenció de Lentivirus





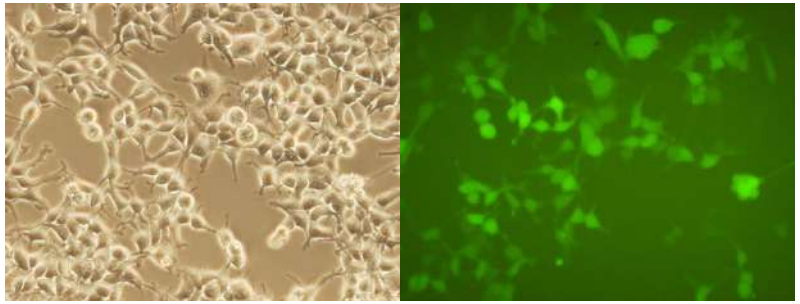




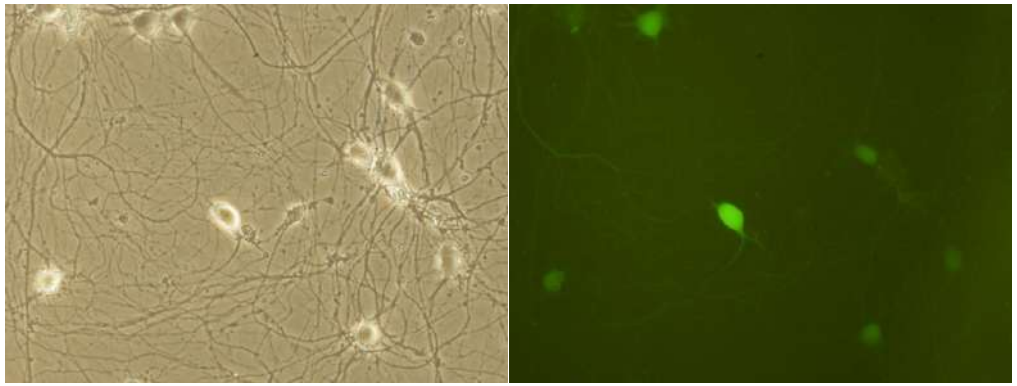
Transfección con PEI

(Polyethylemin)

HEK293 cell line

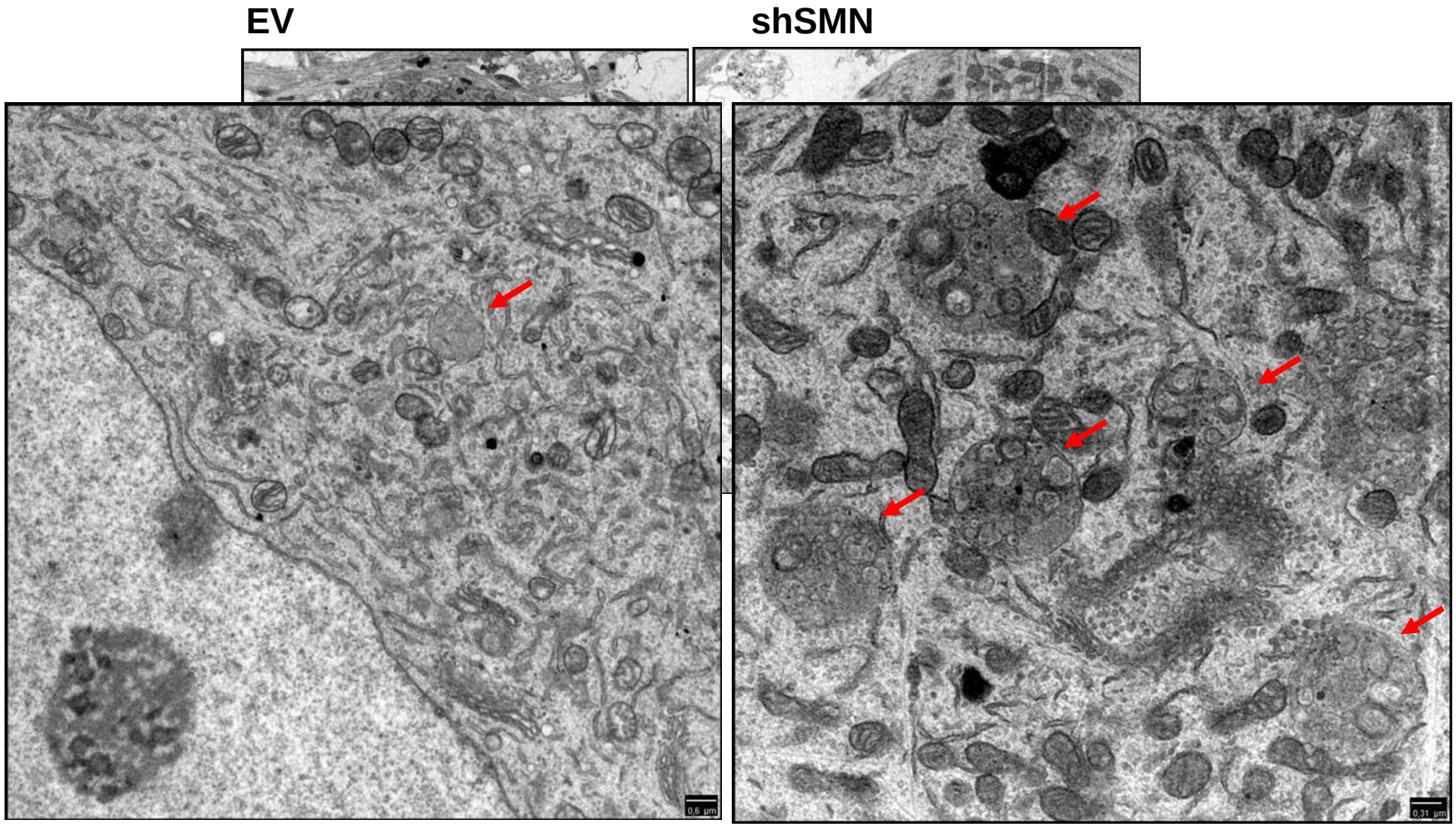


- Recollir sobrenedant 3-4 dies
- Centrifuguem a1000 rpm/ 5min
- Filtrem amb filtres 0.45-0,22 μm diàmetre
- Sobrenedats a 4ºC (1 mes) a -80ºC



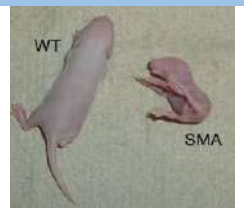
Autophagy in SMA

SMA MN cell culture-knockdown approach



Garcera A *et al.*, Cell Death Dis. 2013

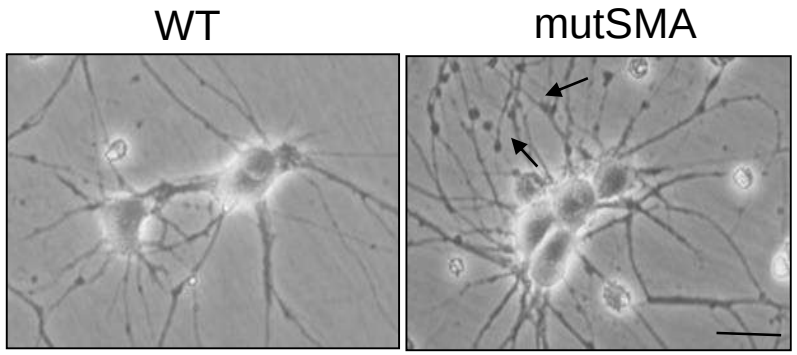
SMA mouse model

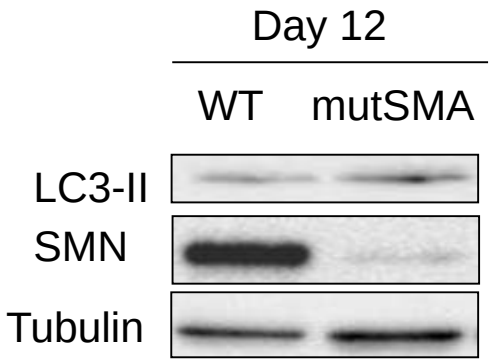


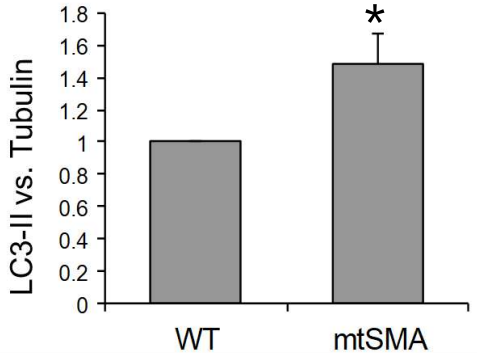
FVB.Cg-Tg(SMN2)^{89Ahmb}Smn1^{tm1Msd}/J

mutSMA

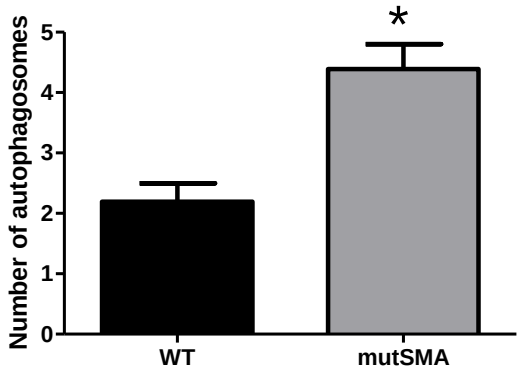
SMA mouse model spinal cord



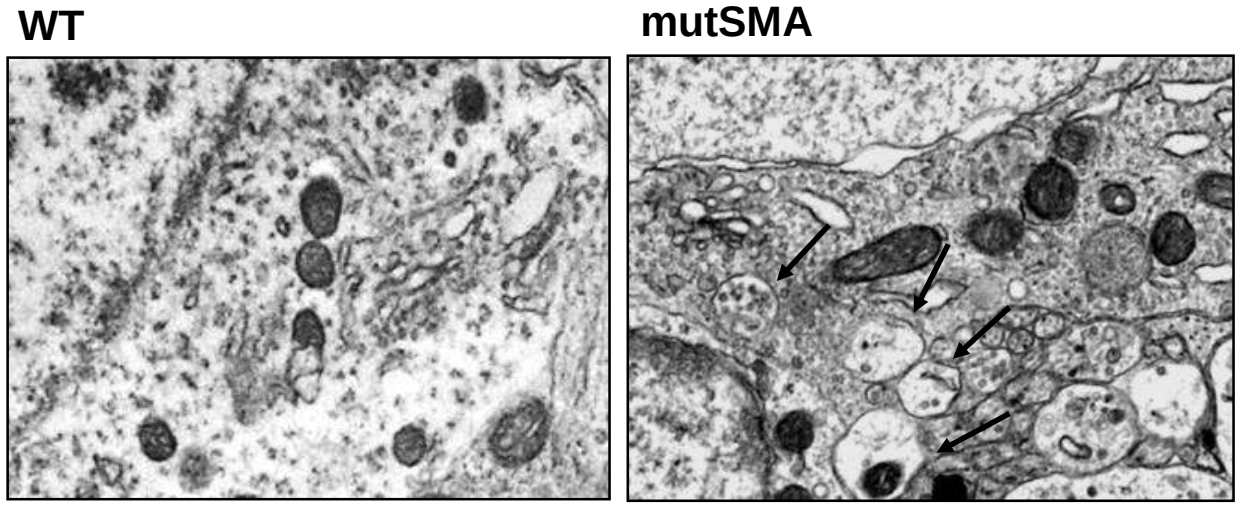




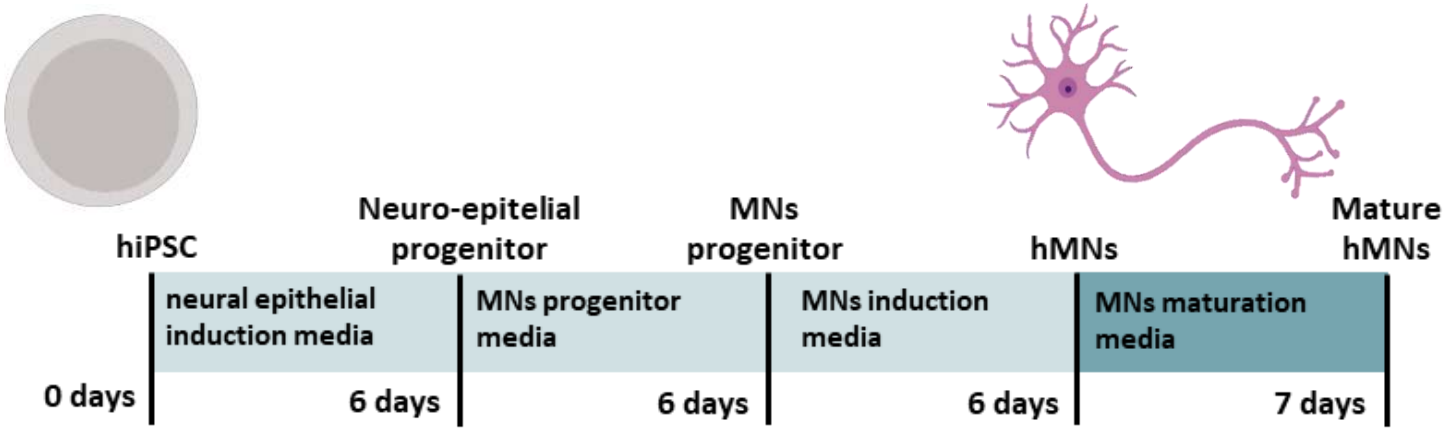
SMA mouse model spinal cord



Periyakaruppiah A *et al*., Exp Neurol. 2016

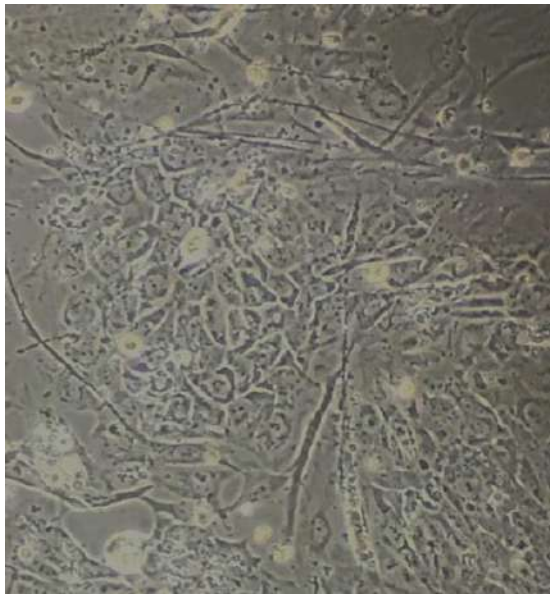


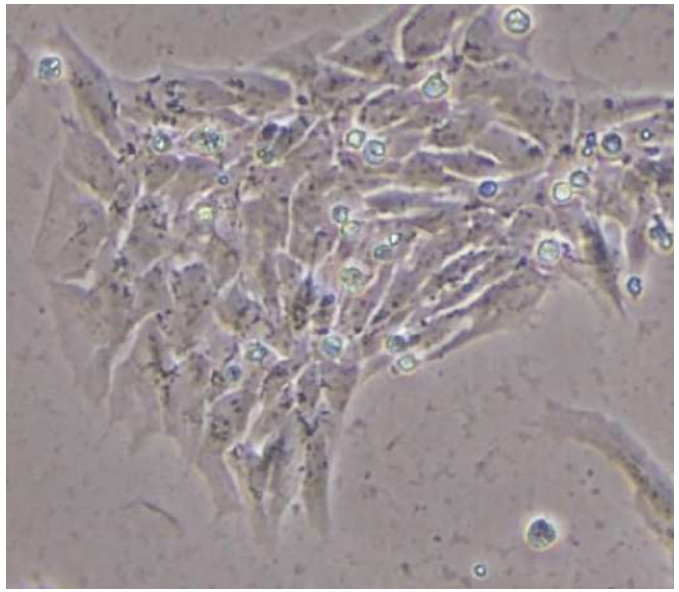
SMA Human induced Pluripotent Stem Cells

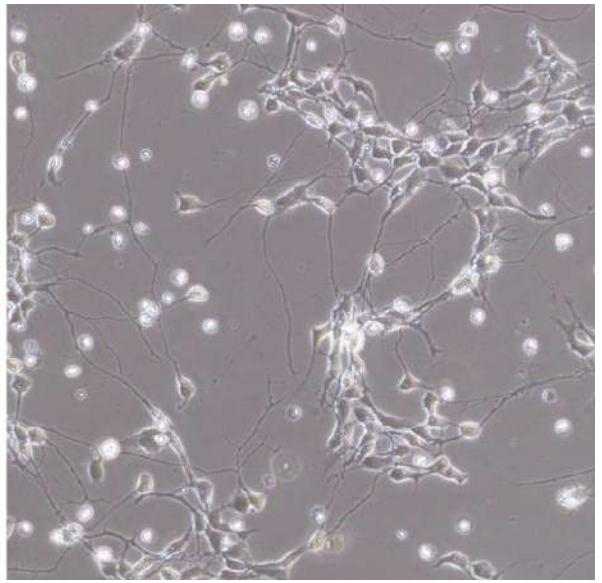


GM11: control
GM23240*B: SMA II (SMN2 2 copies, Δ exon7-8 in SMN1)

Z.W. Du et al., 2015, modified in Portero's lab



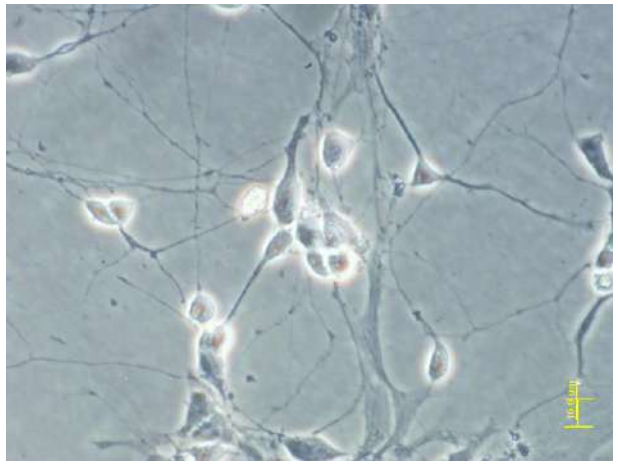


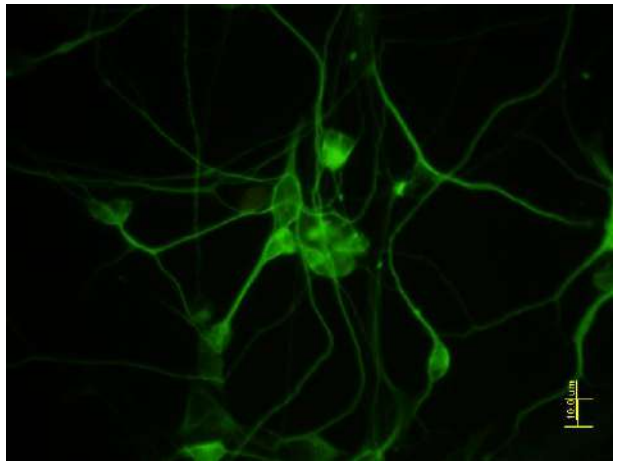


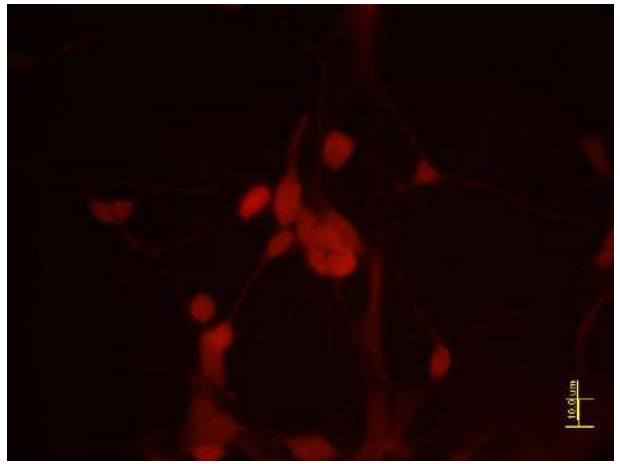
Diferenciació de hiPSC a MNs

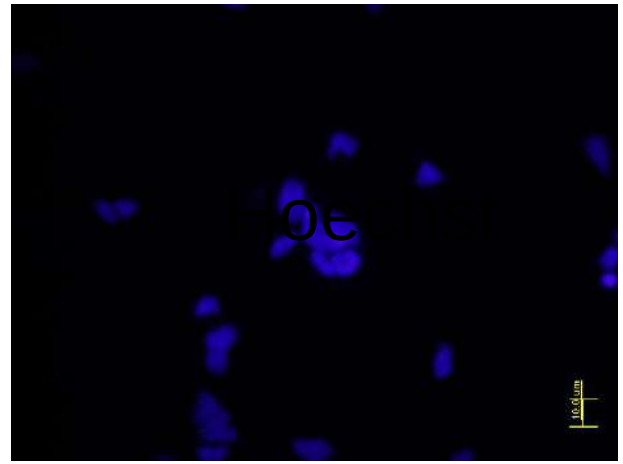
Beta-3-Tub

Islet1-2









de la Fuente et al. *Cell Death and Disease* (2020)11:487
https://doi.org/10.1038/s41419-020-2688-5

Cell Death & Disease

ARTICLE Open Access

Calpain system is altered in survival motor neuron-reduced cells from in vitro and in vivo spinal muscular atrophy models

Sandra de la Fuente[1], Alba Sansa[1], Iván Hidalgo[1], Nuria Vivancos[1], Ricardo Romero-Guevara[2], Ana Garcera[1] and Rosa M. Soler[1]

Neurobiology of Disease 155 (2021) 105366

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journal homepage: www.elsevier.com/locate/ynbdi

Intracellular pathways involved in cell survival are deregulated in mouse and human spinal muscular atrophy motoneurons

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RESEARCH Open Access

Spinal Muscular Atrophy autophagy profile is tissue-dependent: differential regulation between muscle and motoneurons

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ARTICLE OPEN

ERK MAPK signaling pathway inhibition as a potential target to prevent autophagy alterations in Spinal Muscular Atrophy motoneurons

Alba Sansa[1], Maria P. Miralles[1], Maria Beltran[1], Ferran Celma-Nos[1], Jordi Calderó[2], Ana Garcera[1,3] and Rosa M. Soler[1,3]

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Survival motor neuron protein and neurite degeneration are regulated by Gemin3 in spinal muscular atrophy motoneurons

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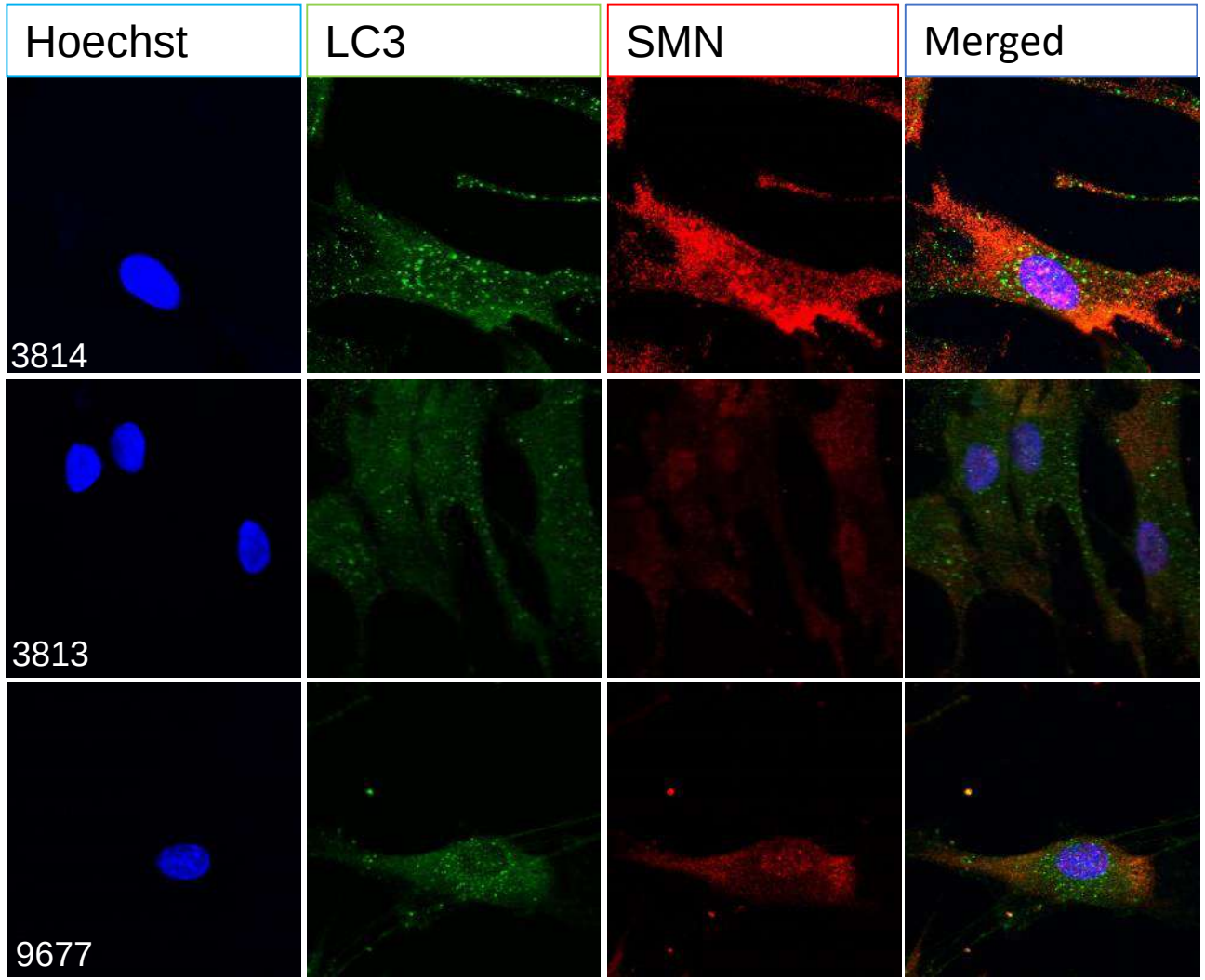
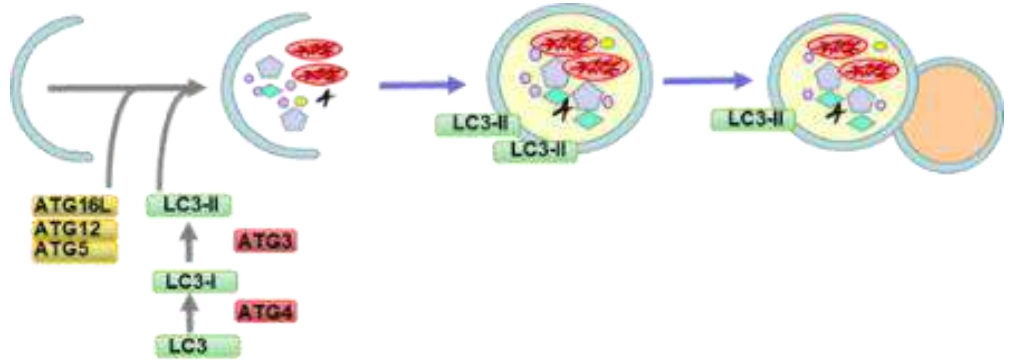
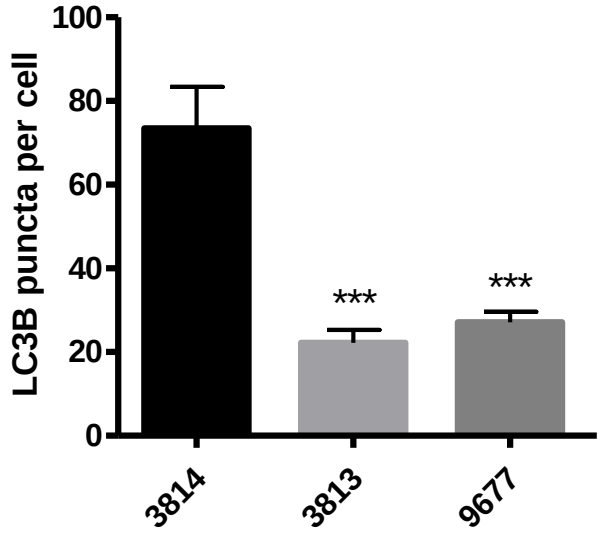
AUTOPHAGY IN SMA FIBROBLASTS

3814: unaffected 3813 mother
3813: SMA II (SMN2 2 copies, Δ exon7-8 in SMN1)
9677: SMA I (SMN2 3 copies, Δ exon7-8 in SMN1)



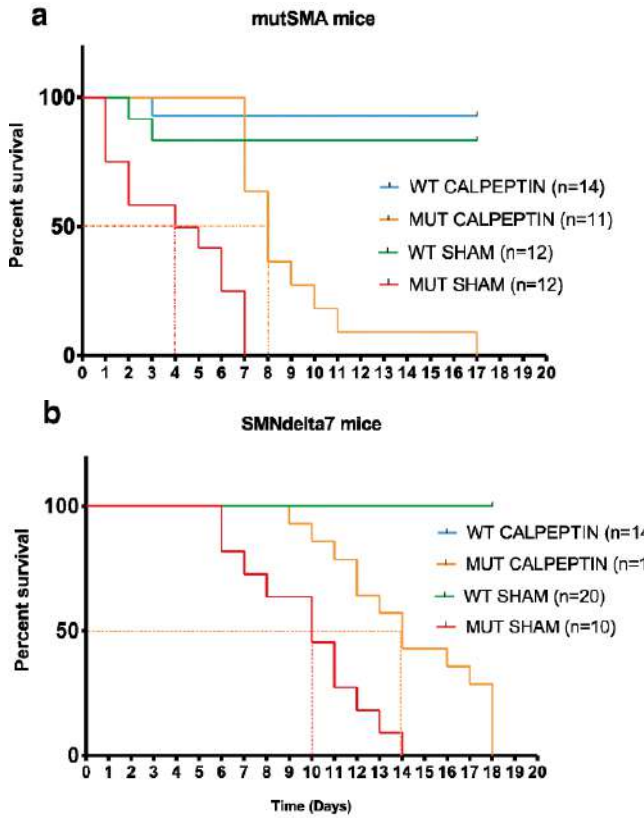


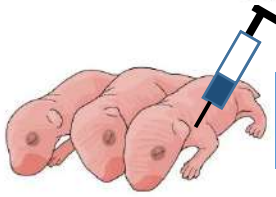


Calpain

Dr. Sandra de la Fuente Ruiz

Calpeptin administration extends survival of SMA mice models

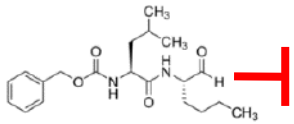




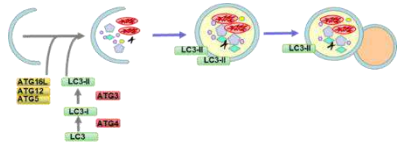
PATENT

Treatment for the
Spinal Muscular Atrophy

PCT/ES2018/070177

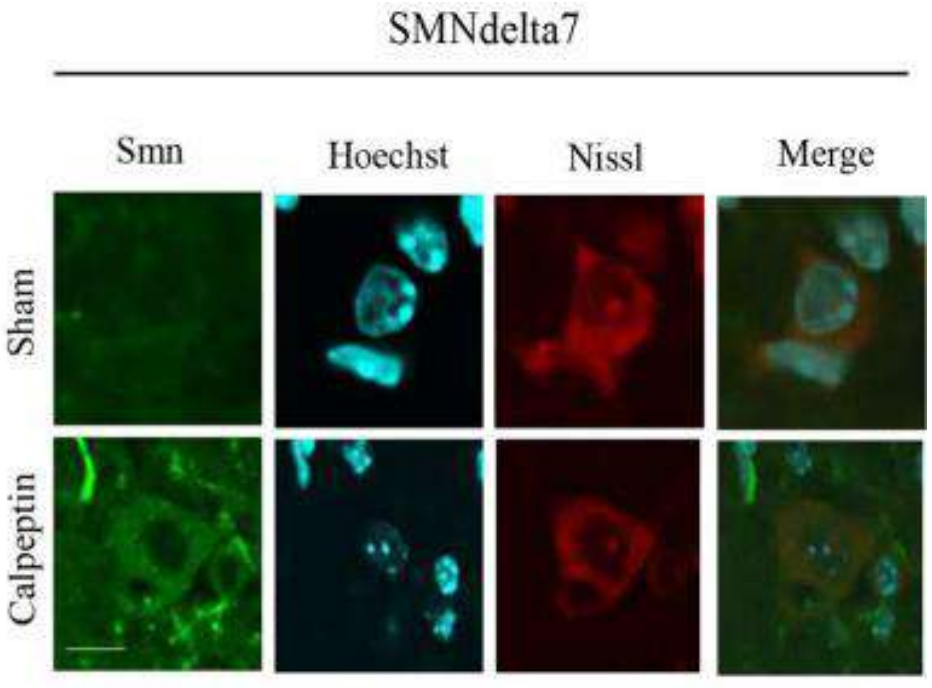


Calpain



Calpeptin

Calpeptin treatment increases Smn protein level



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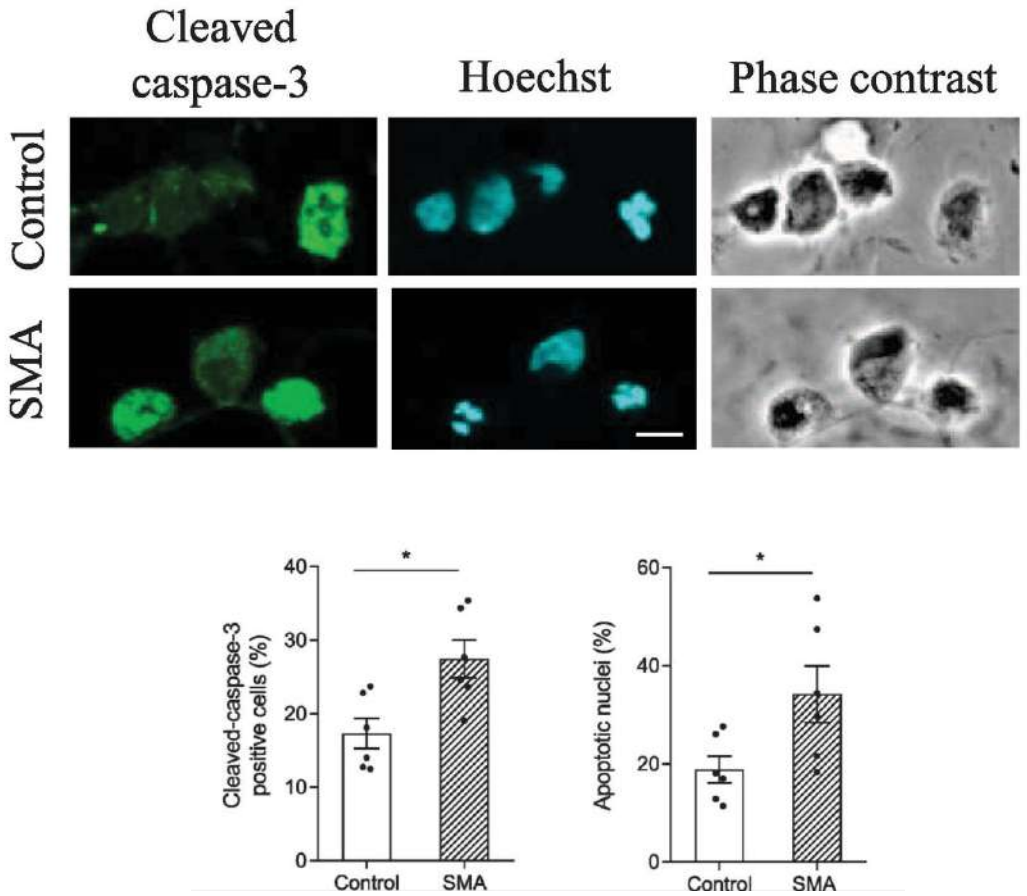
SMA: Signaling pathways altered in the Motoneurons

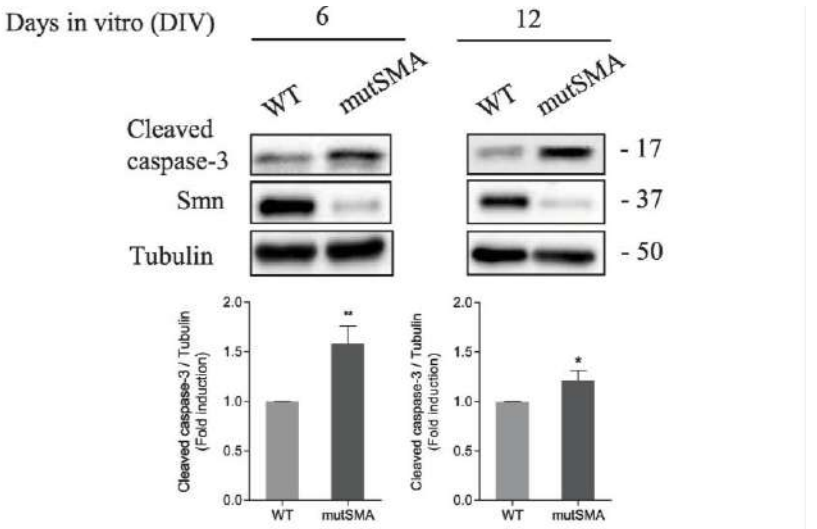
Apoptosis

Alba Sansa Zaragoza

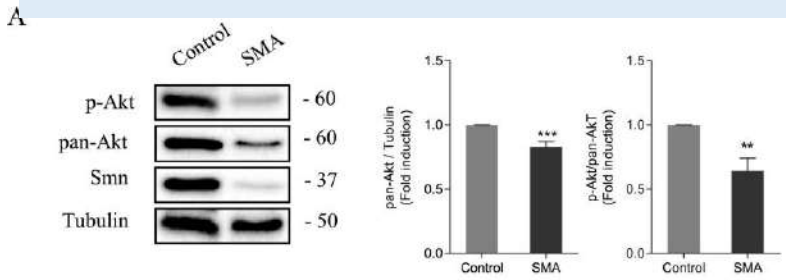


cleaved-caspase-3 proteins are increased in human differentiated SMA MNs

cleaved-caspase-3 proteins are increased in SMA MNs



Akt phosphorylation is reduced in mice and human cultured SMA MNs



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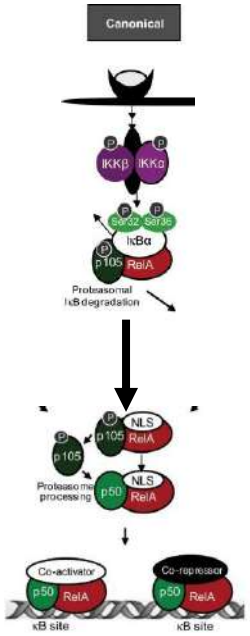
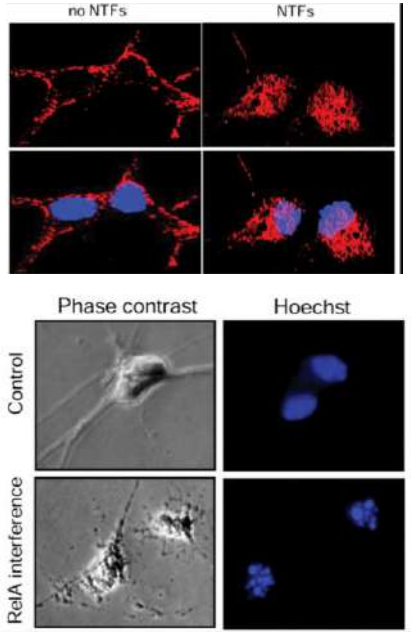
SMA: Signaling pathways altered in the Motoneurons

Apoptosis

María Miralles Expósito





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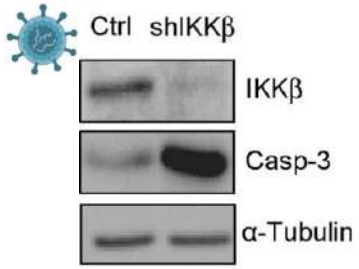

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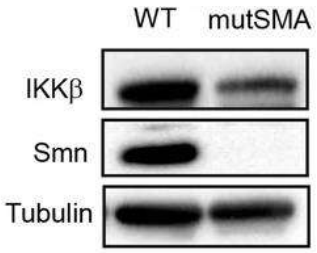

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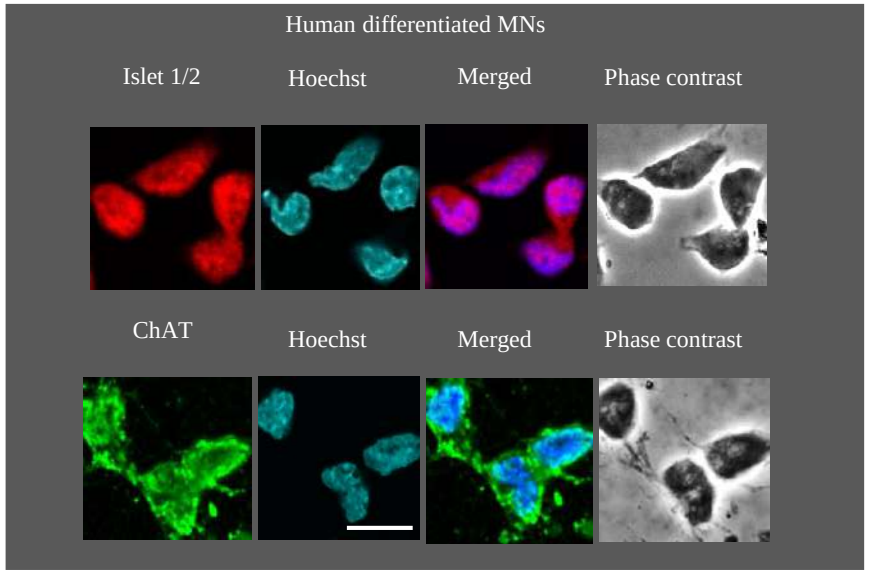
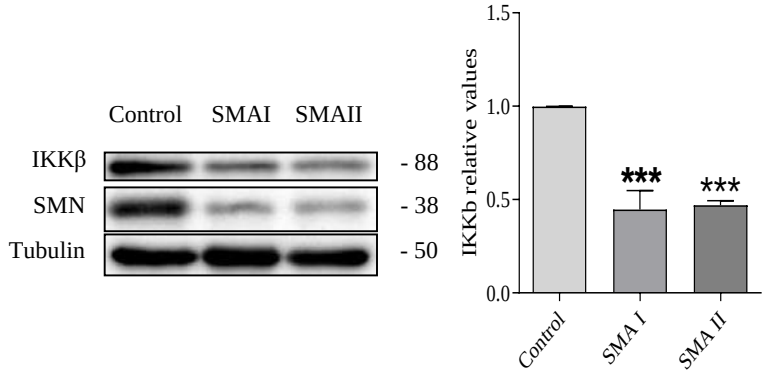
IKKbeta is reduced in SMA MNs





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Analysis of the beneficial effects of calpain inhibitors treatment and combined therapies on Spinal Muscular Atrophy

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