Inserm
Institut national
de la santé et de la recherche médicale

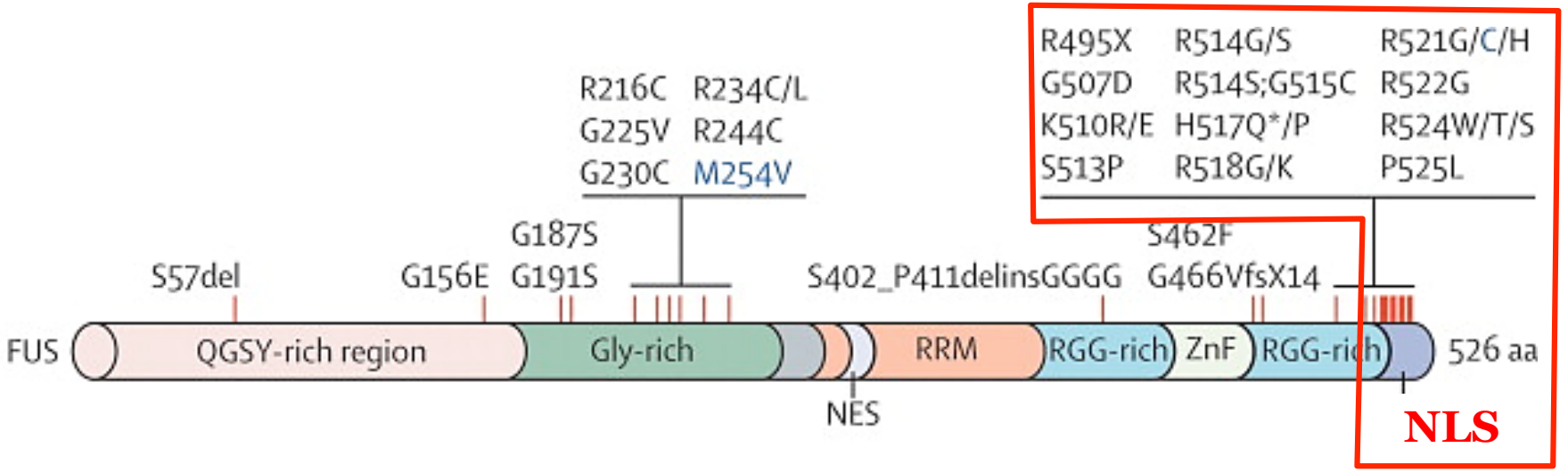
UNIVERSITÉ DE STRASBOURG

Role of Fus in post synaptic differentiation of neuromuscular junction : involvement in Amyotrophic Lateral Sclerosis

Gina Picchiarelli

Inserm U1118 Peripheral and central mechanisms of neurodegeneration
Strasbourg, France

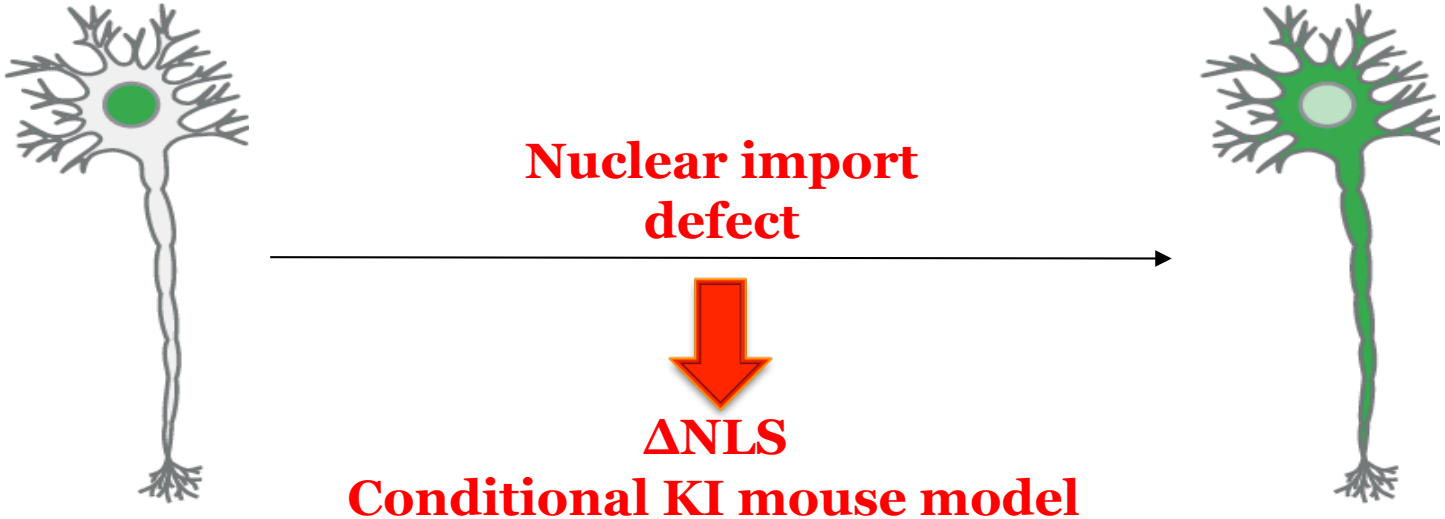
Fus mutations cluster in PY-NLS



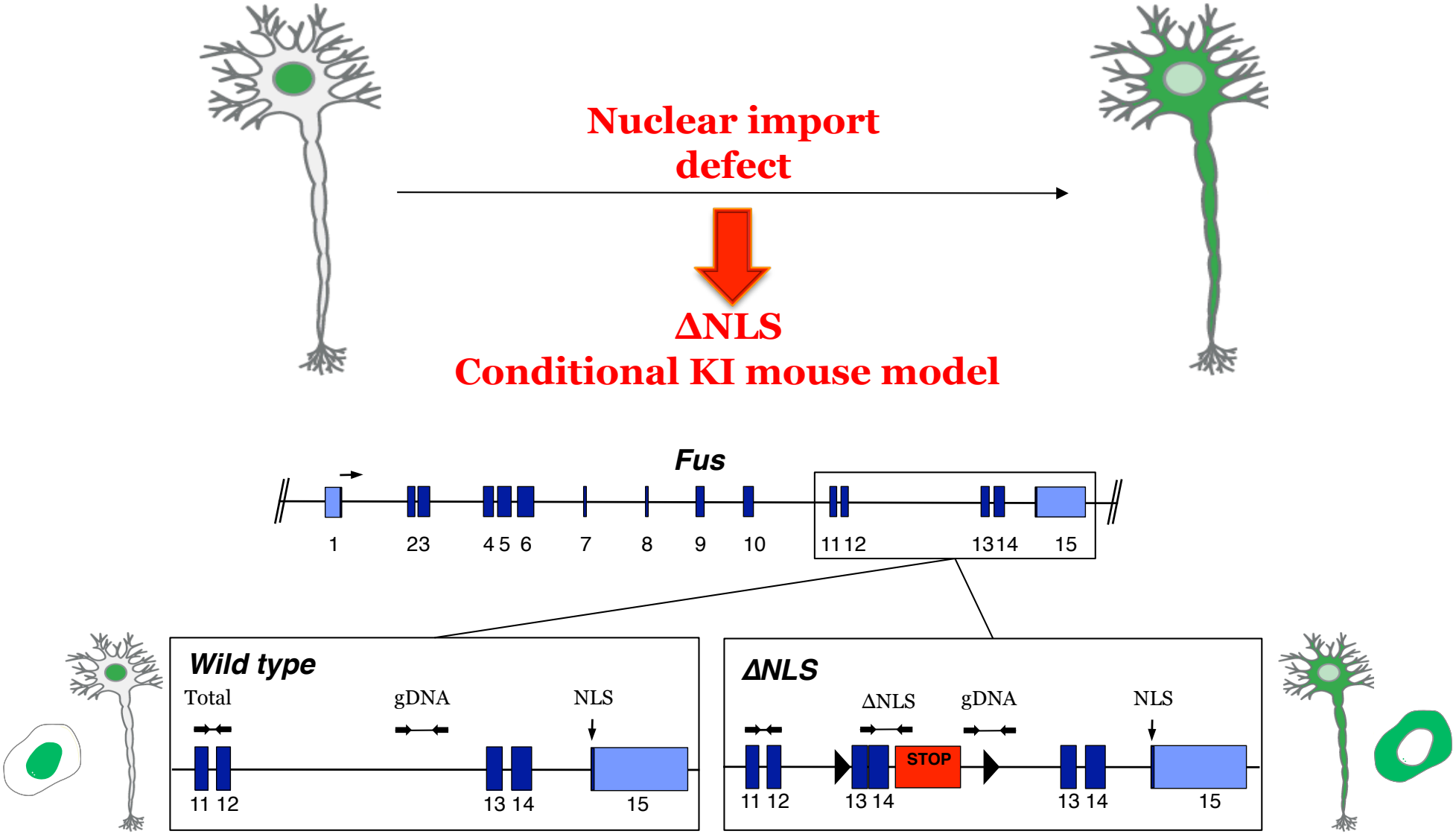
- Truncating mutations:
  - Deletion of Nuclear Localisation Signal (NLS)
  - Young onset
  - Rapid disease progression
  - Impairment of Fus nuclear import

MacKenzie, Lancet Neurol, 2010

Fus ΔNLS model



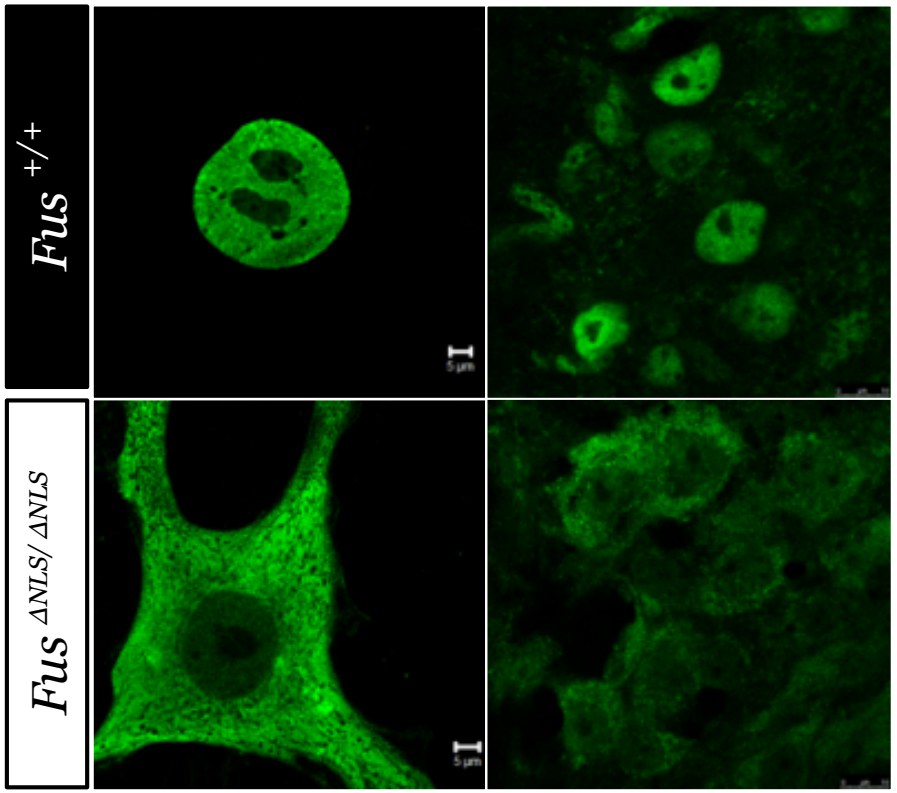
Fus ΔNLS model



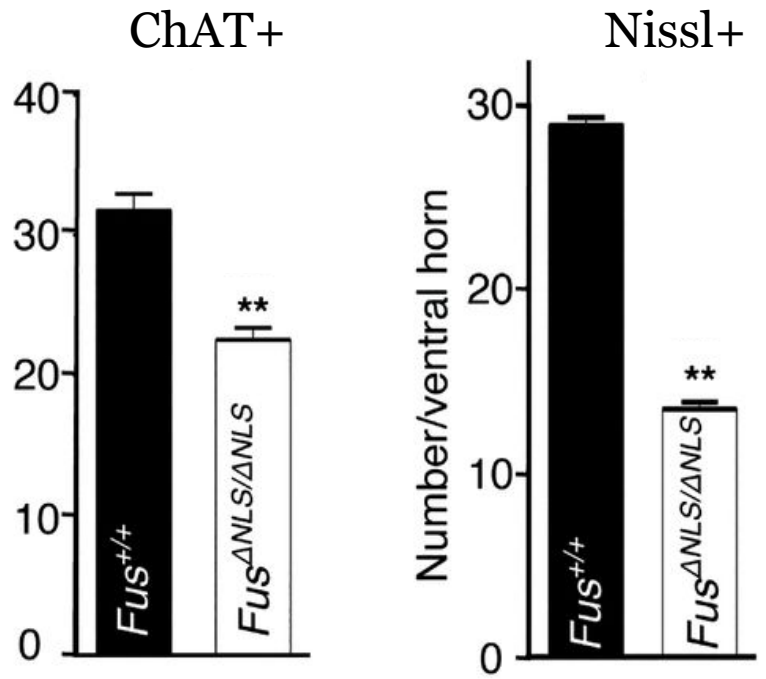
SCEKIC-ZAHIROVIC et al, EMBO J, 2016

Complete Fus mislocalization leads to MN degeneration

FUS localization



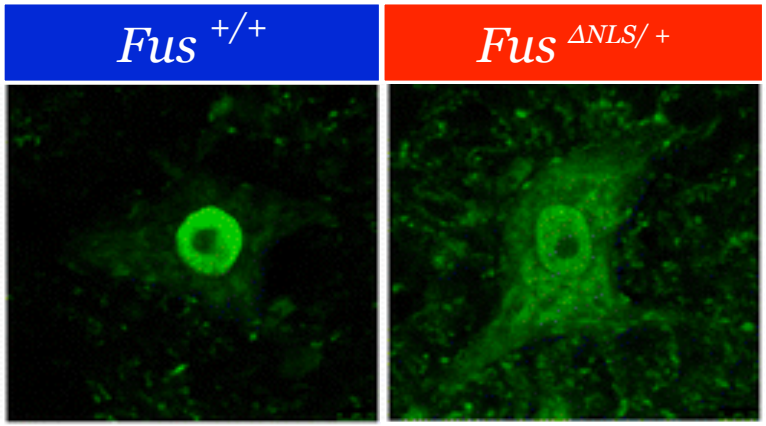
Number of Motoneurons



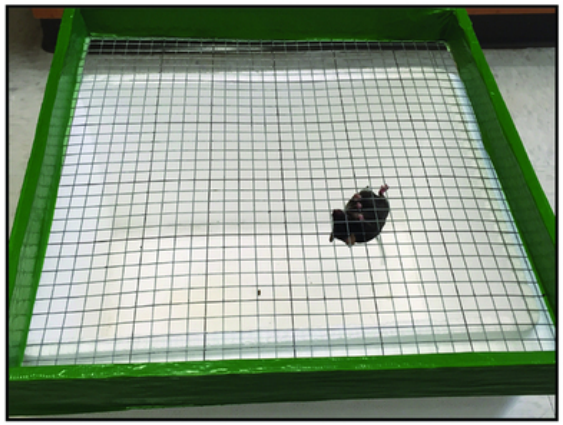
SCEKIC-ZAHIROVIC et al, EMBO J, 2016

Partial Fus mislocalization leads to MN degeneration and motor deficit

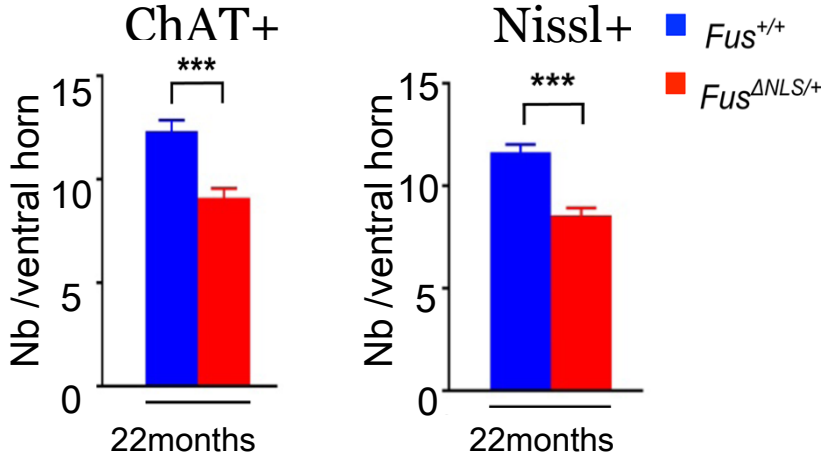
FUS localization in SC



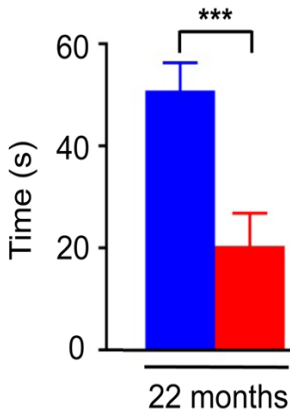
Motor Function



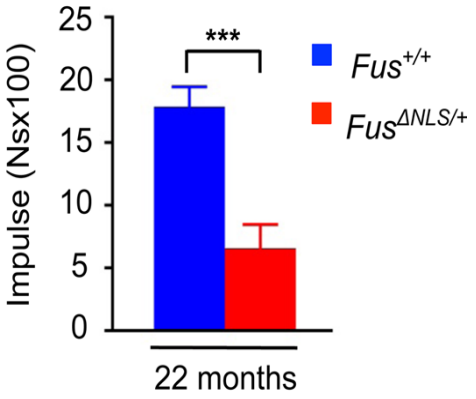
Number of Motoneurons in SC



Hanging time

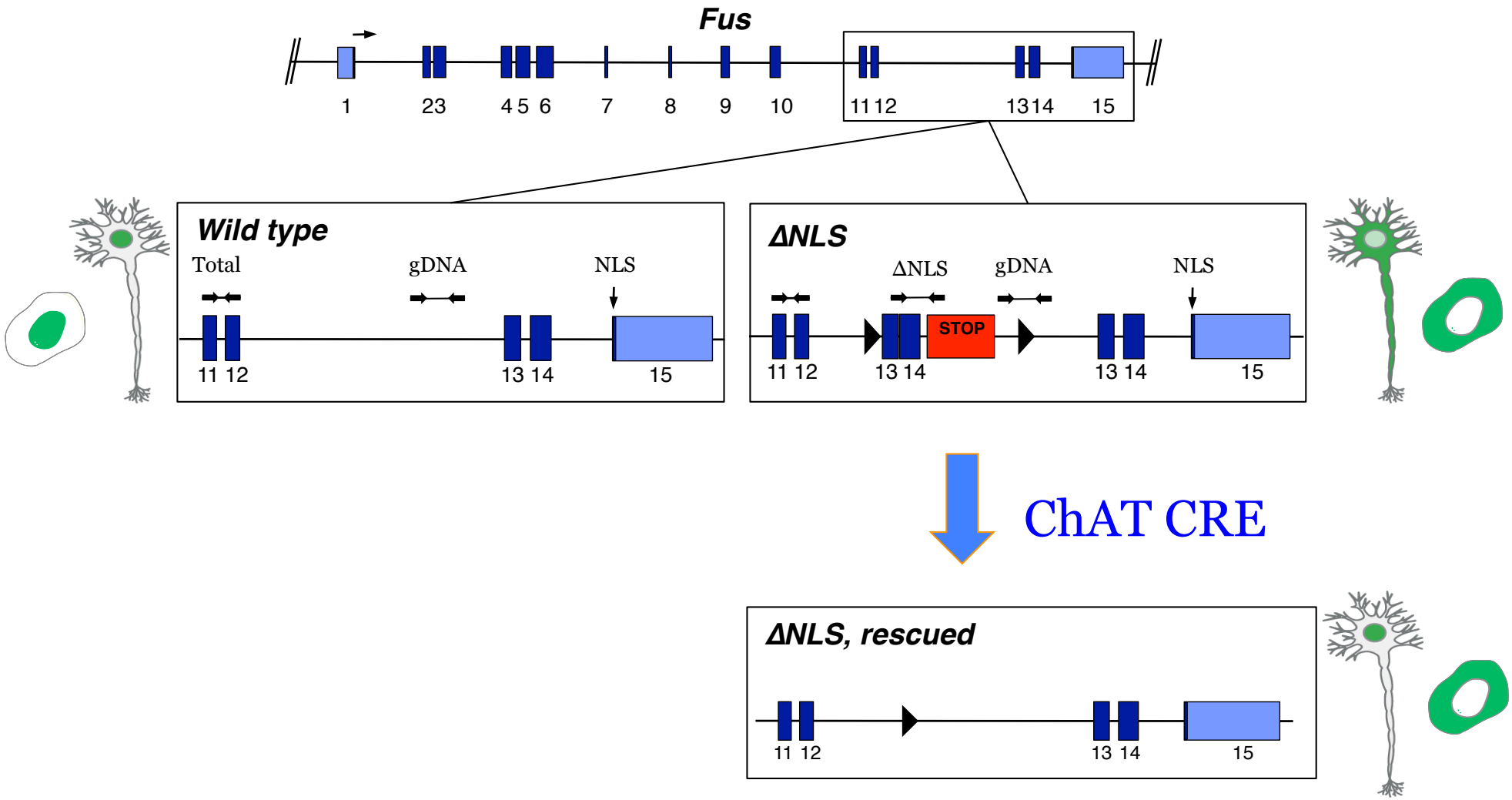


Holding impulse
=Hanging time/weight



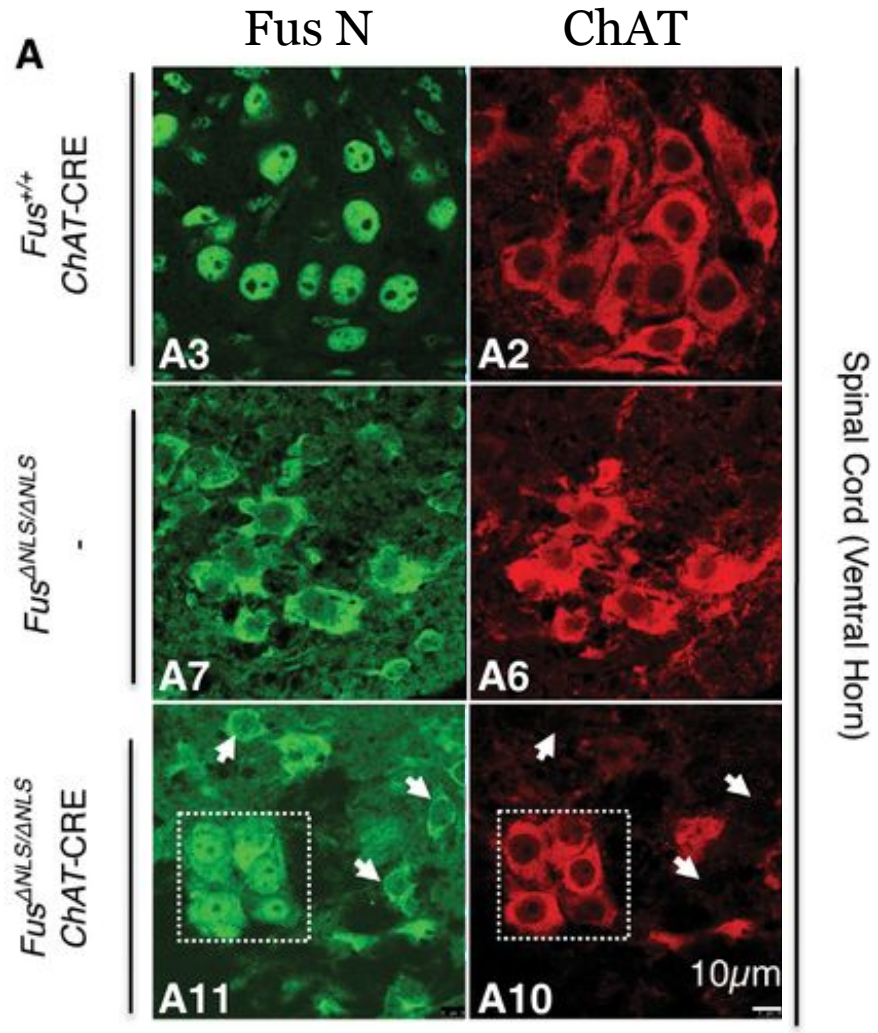
Jelena Scekic-Zahirovic et al, Acta neuropathologica, 2017

Fus ΔNLS rescue in motoneurons



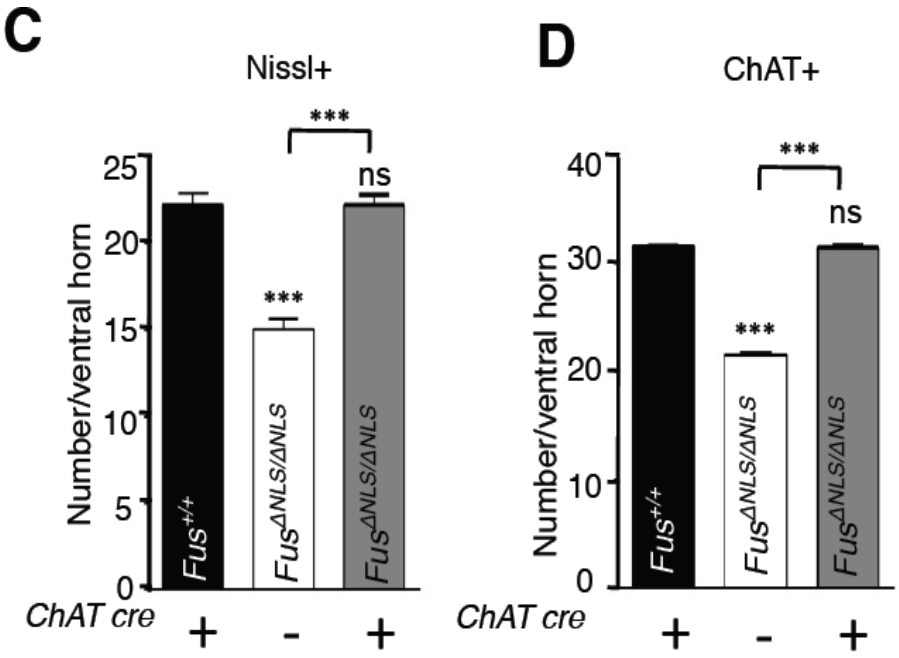
Fus is restored in the nucleus of MN and rescues MN degeneration in new born mice

Fus localization



Number of Motoneurons

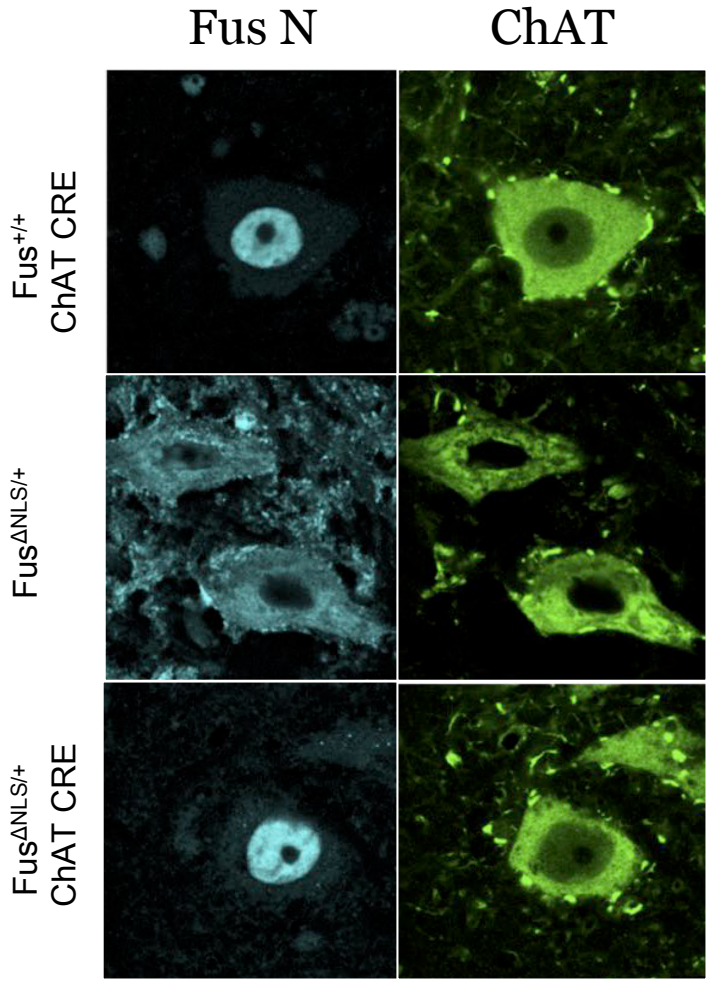
Po SC



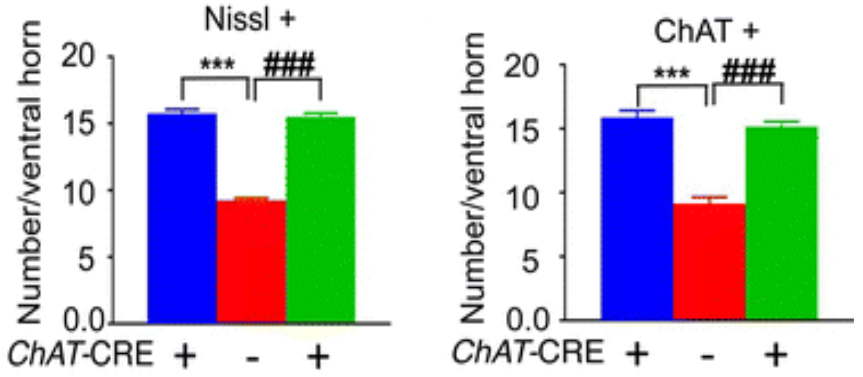
SCEKIC-ZAHIROVIC et al, EMBO J, 2016

Fus is restored in the nucleus of MN and rescues MN loss but not motor function in adult mice

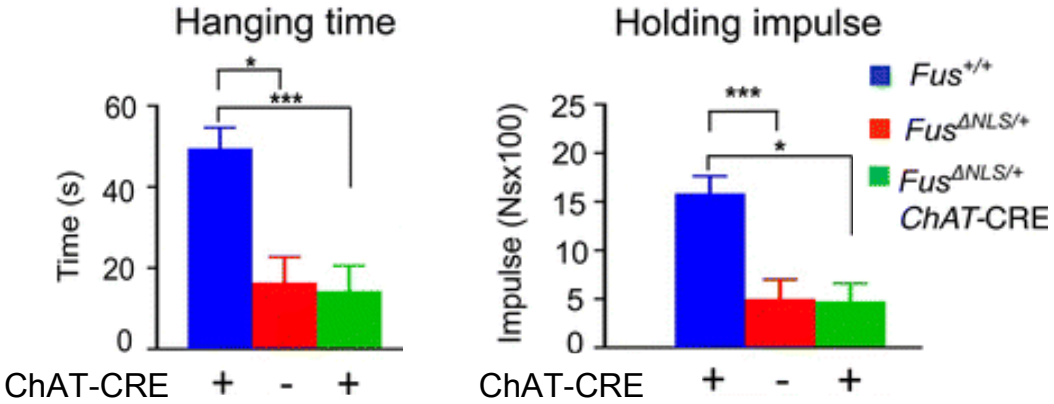
Fus localization



Number of Motoneurons

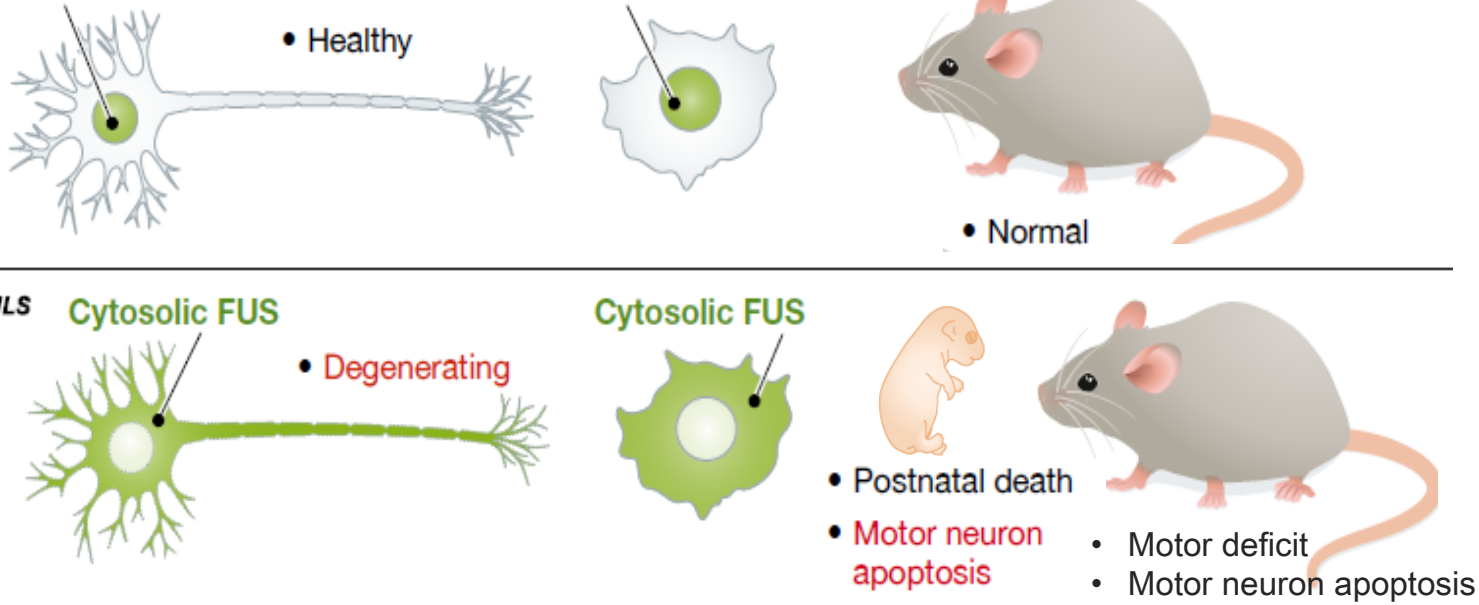


Motor Function



Summary

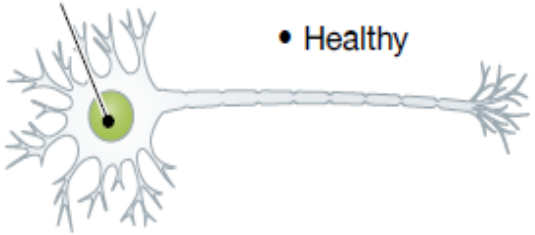
	CELLULAR FUS LOCALIZATION		PHENOTYPE
	Motor neurons	Other cells	
Fus$^{+/+}$	Nuclear FUS • Healthy	Nuclear FUS	• Normal
Fus$^{\Delta NLS/\Delta NLS}$	Cytosolic FUS • Degenerating	Cytosolic FUS	• Postnatal death • Motor neuron apoptosis • Motor deficit • Motor neuron apoptosis

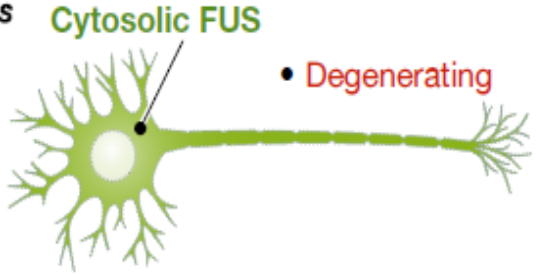


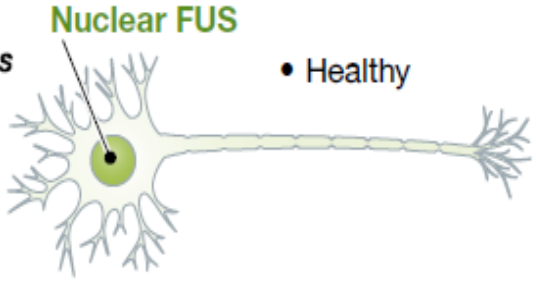
DORMANN, EMBO Journal, 2016

Summary

	CELLULAR FUS LOCALIZATION		PHENOTYPE
	Motor neurons	Other cells	
Fus$^{+/+}$	Nuclear FUS • Healthy	Nuclear FUS	• Normal
Fus$^{\Delta NLS/\Delta NLS}$	Cytosolic FUS • Degenerating	Cytosolic FUS	• Postnatal death • Motor neuron apoptosis • Motor deficit • Motor neuron apoptosis
ChAT-Cre *Fus*$^{\Delta NLS/\Delta NLS}$	Nuclear FUS • Healthy	Cytosolic FUS	• Postnatal death • Normal motor • Motor deficit • Normal motor neuron

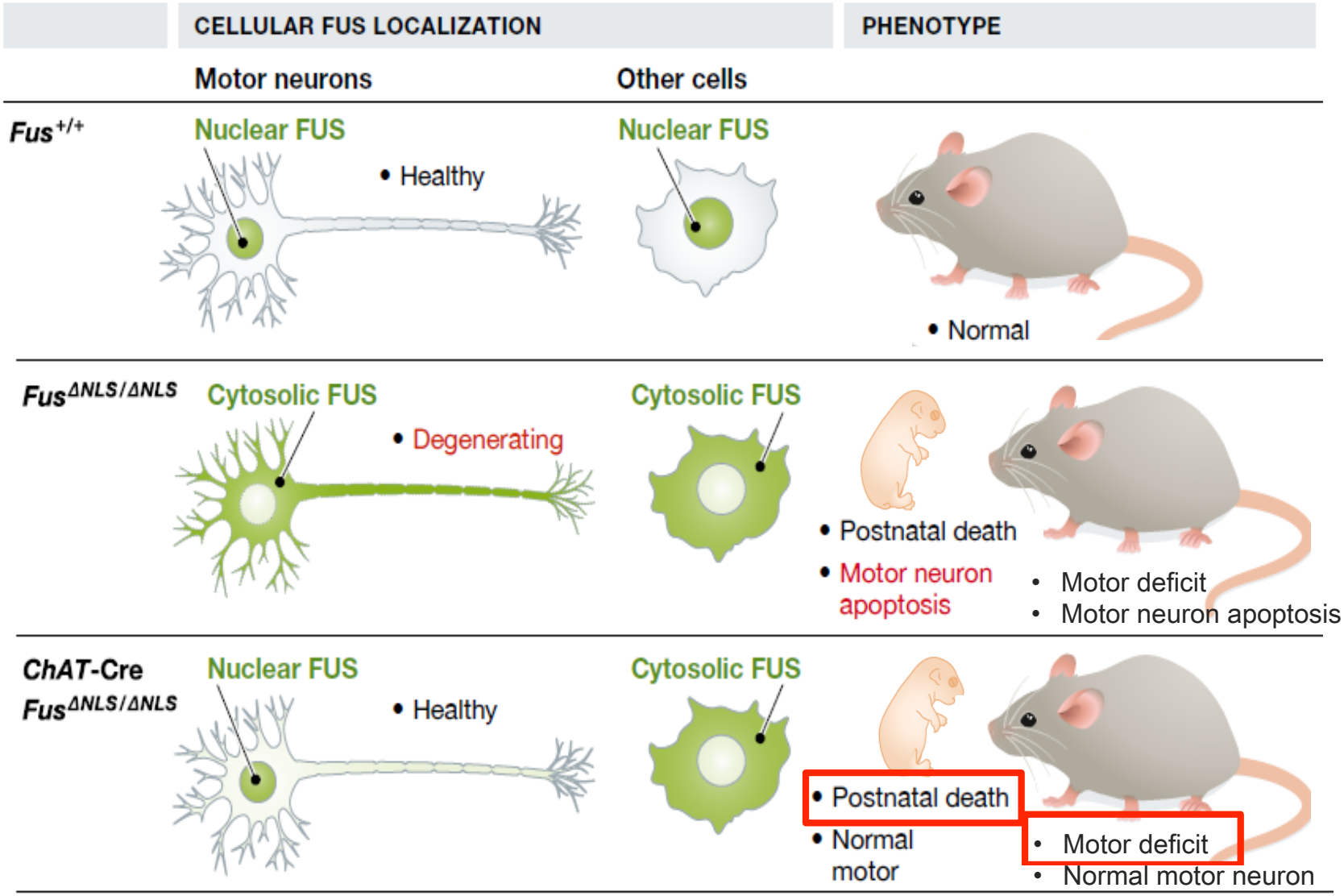






DORMANN, EMBO Journal, 2016

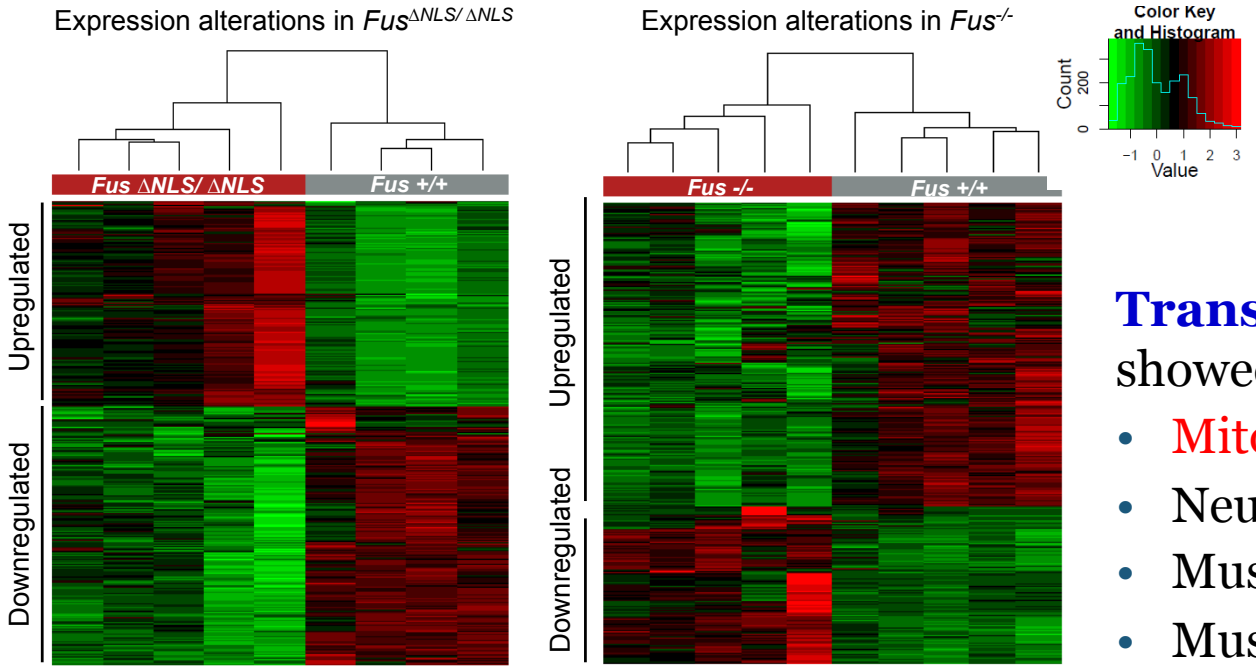
Summary



DORMANN, EMBO Journal, 2016

Is cytoplasmic FUS mislocalization pathogenic in muscle?

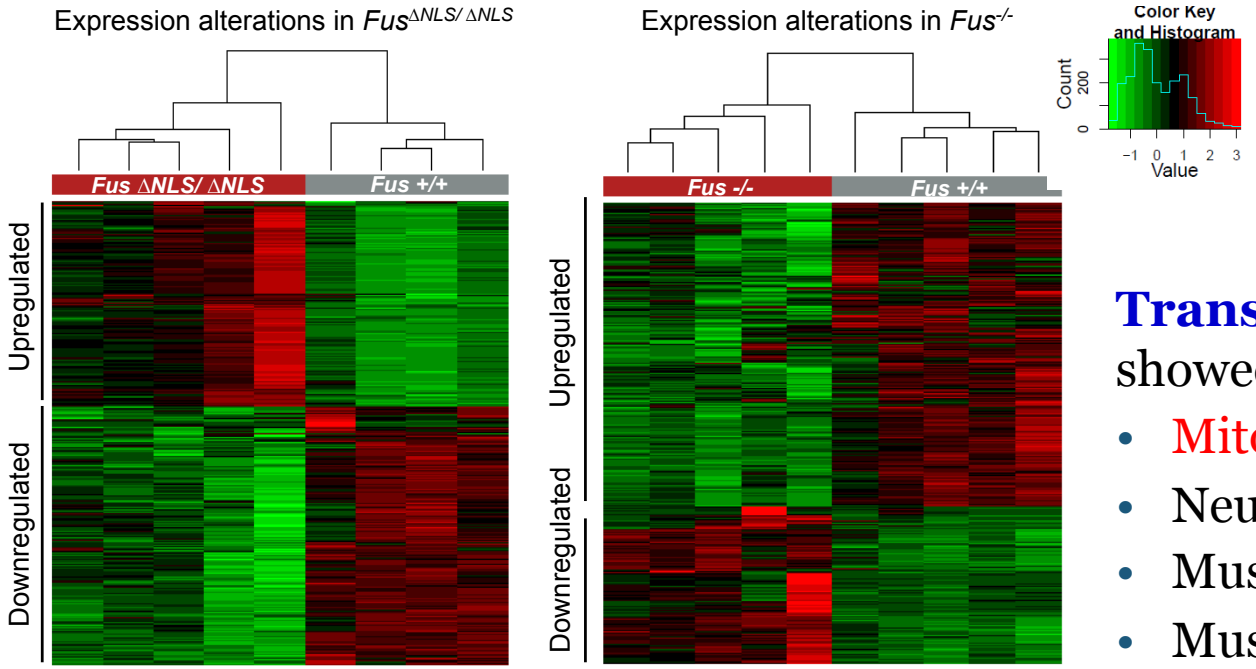
Transcriptomic analysis reveals Mitochondrial dysfunction in muscle of Fus ΔNLS and KO mice



Transcriptomics analysis showed deregulation in :

- Mitochondrial function
- Neuromuscular junction
- Muscle contraction
- Muscle development

Transcriptomic analysis reveals Mitochondrial dysfunction in muscle of Fus ΔNLS and KO mice



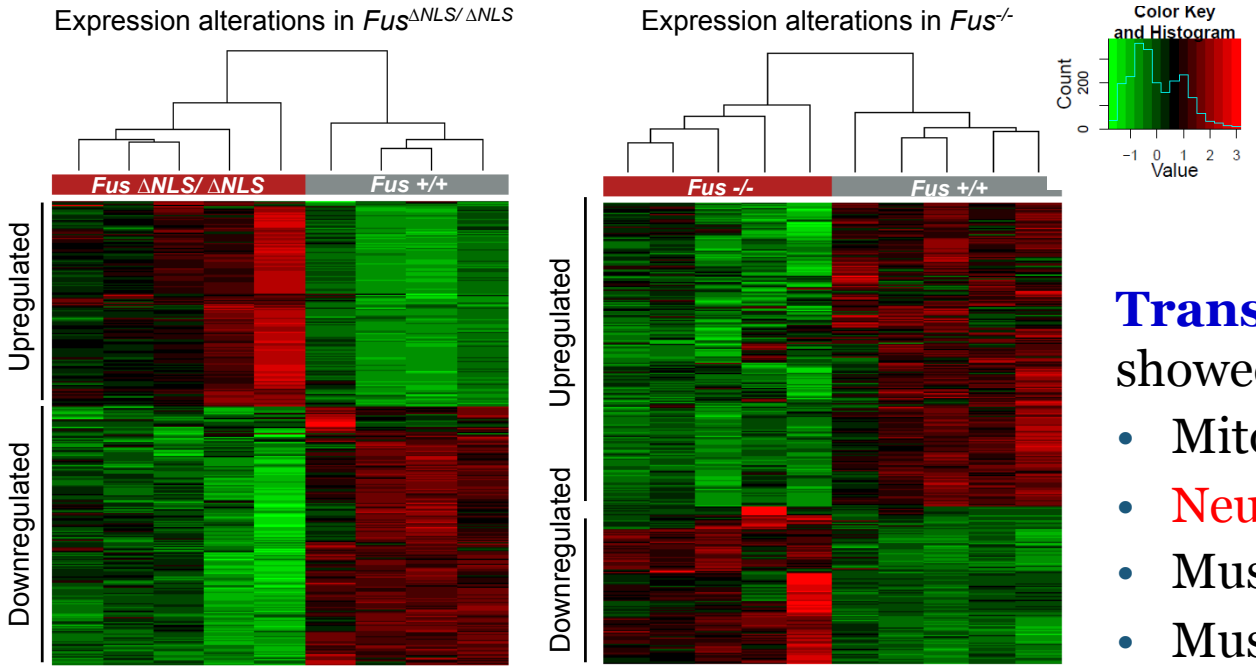
Transcriptomics analysis showed deregulation in :

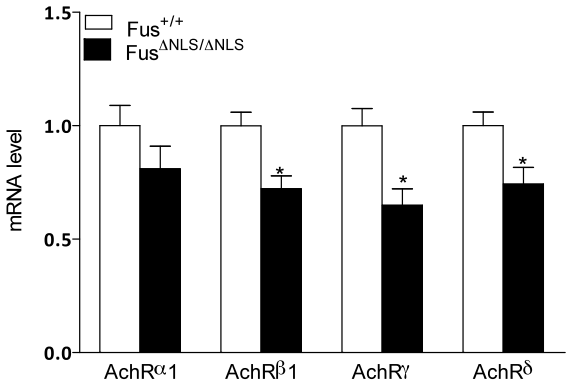
- Mitochondrial function
- Neuromuscular junction
- Muscle contraction
- Muscle development

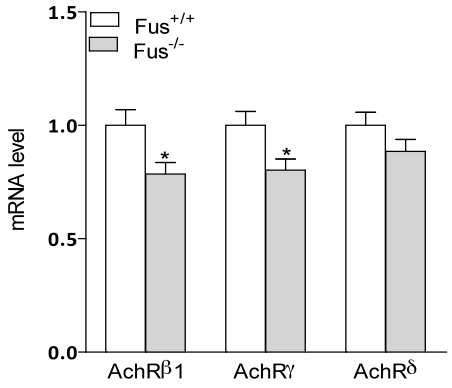
Gene ontology analysis showed deregulations in :

- Respiratory electron transport
- Mitochondrial biogenesis
- Mitophagy
- Citric acid cycle
- Mitochondrial calcium ion transport
- Mitochondrial translation
- Programmed cell death

Transcriptomic analysis reveals NMJ dysfunction in muscle of Fus ΔNLS and KO mice



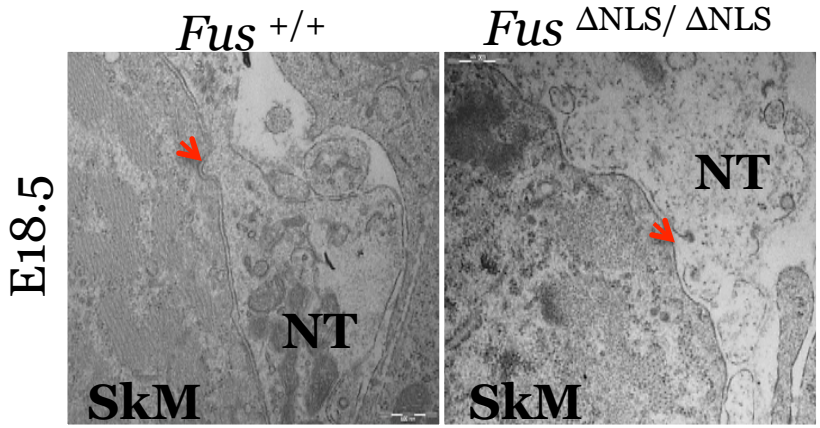




Transcriptomics analysis showed deregulation in :

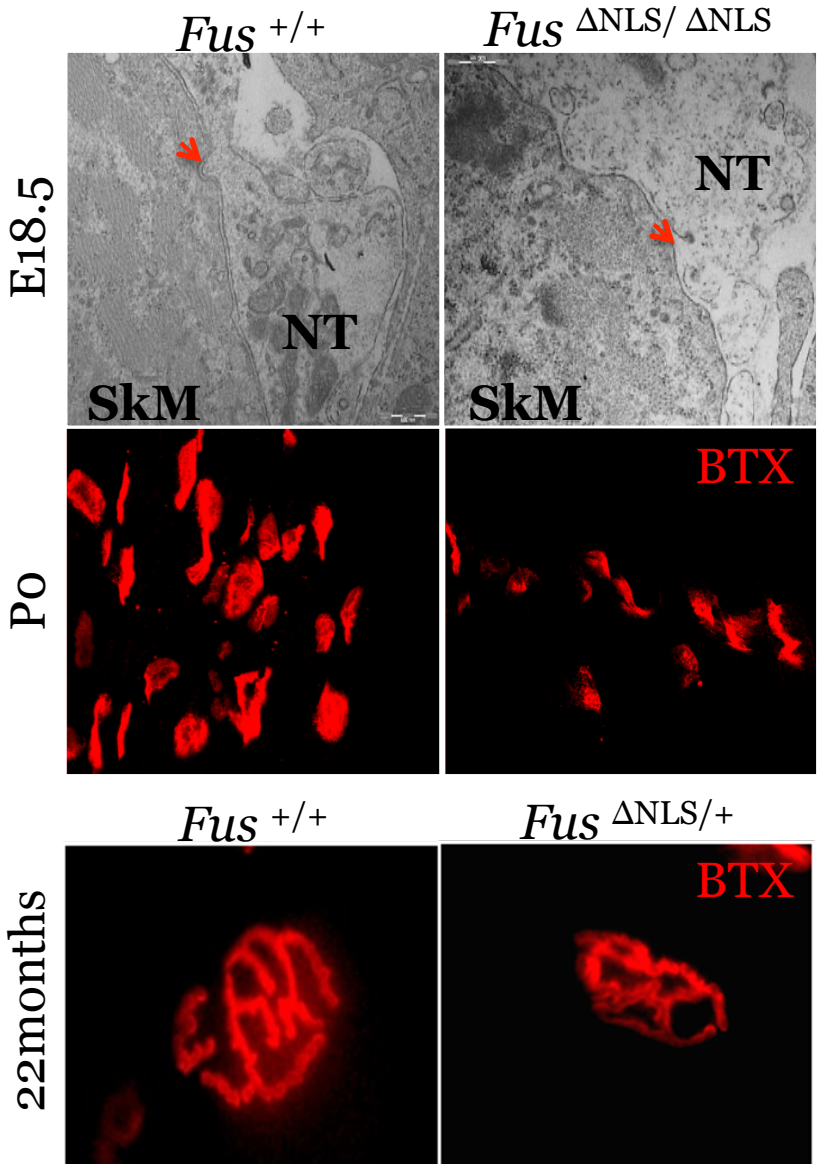
- Mitochondrial functions
- Neuromuscular junction
- Muscle contraction
- Muscle development

Fus is required for the normal morphology of the neuromuscular junction



Ultrastructural abnormalities in post synaptic part of NMJ in ΔNLS/ΔNLS mice at E18,5

Fus is required for the normal morphology of the neuromuscular junction



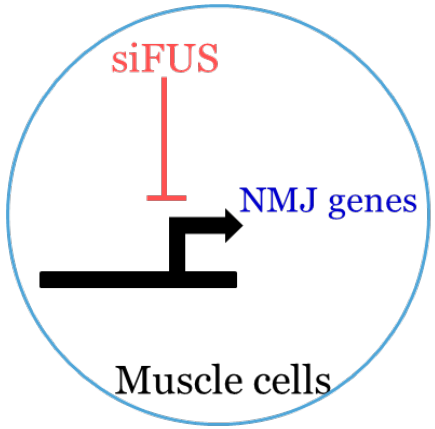
Ultrastructural abnormalities in post synaptic part of NMJ in ΔNLS/ΔNLS mice at E18,5

Smaller and less endplates in ΔNLS/ΔNLS mice

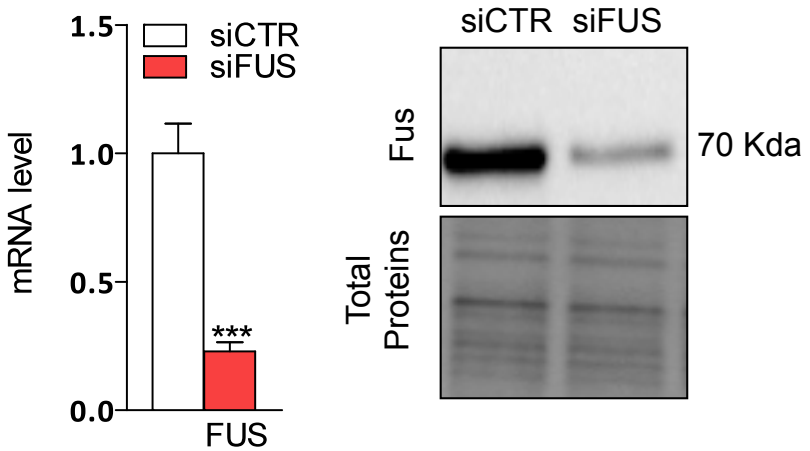
Smaller endplates in ΔNLS/+mice

Fus is necessary for NMJ genes Expression in muscle cells

Experimental design

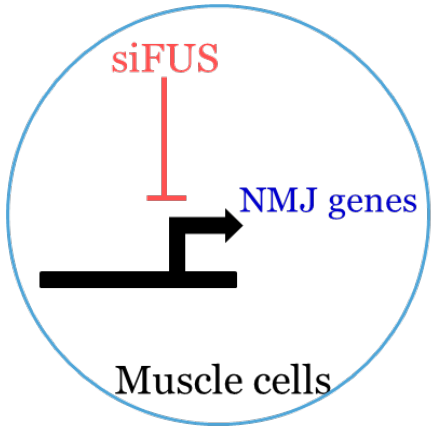


Experimental controls

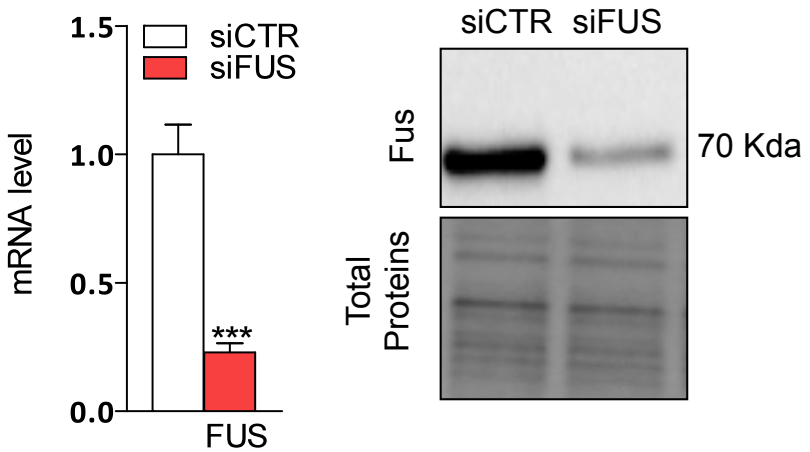


Fus is necessary for NMJ genes Expression in muscle cells

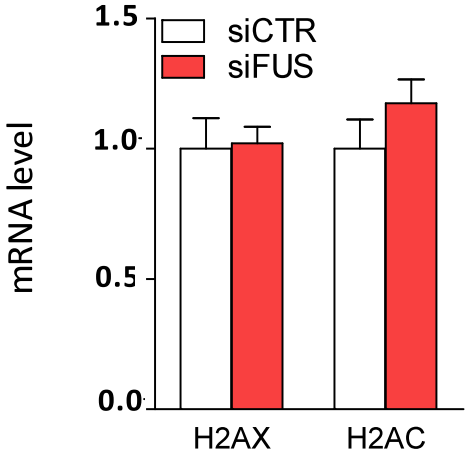
Experimental design



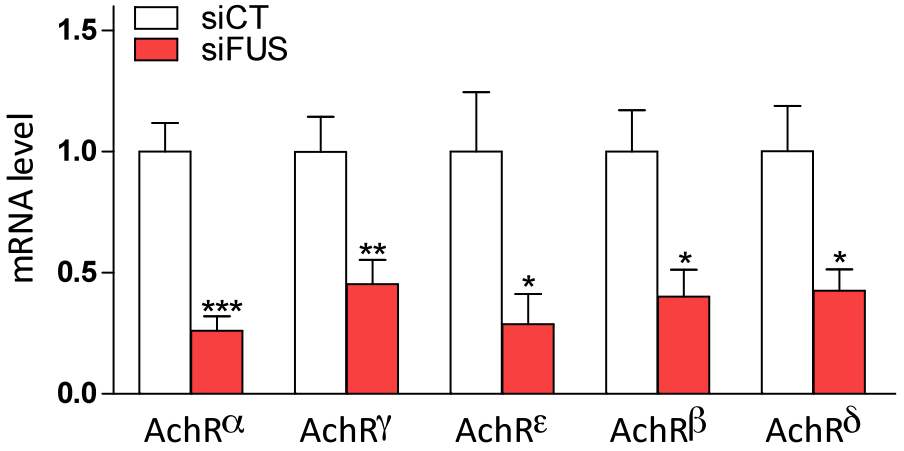
Experimental controls



Histone genes expression

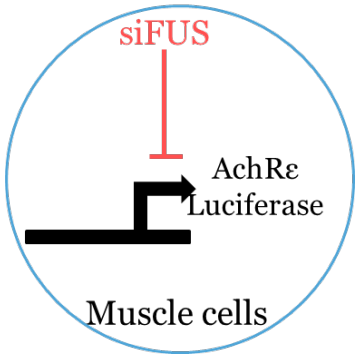


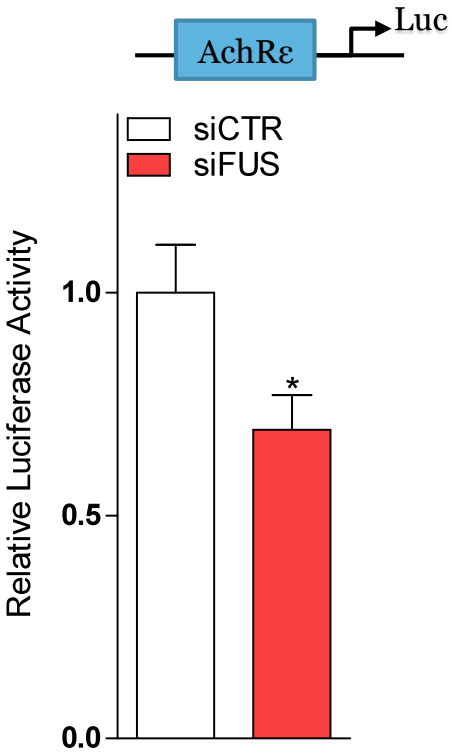
NMJ genes expression



Fus is critical for transcription of NMJ Genes in muscle cells

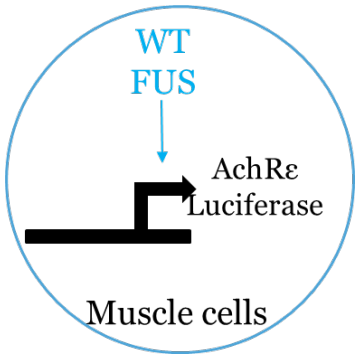
Experimental design

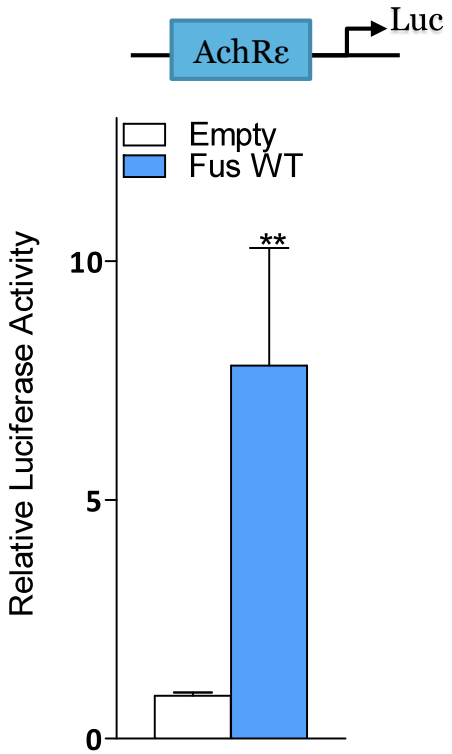




Fus is necessary for NMJ genes expression

Experimental design

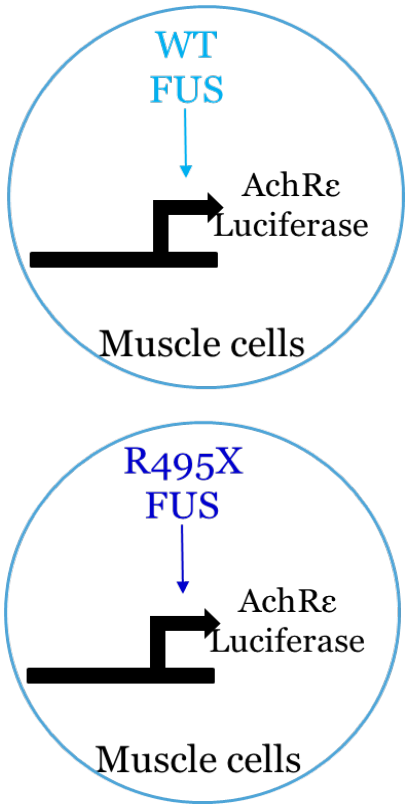




Fus is sufficient for NMJ genes expression

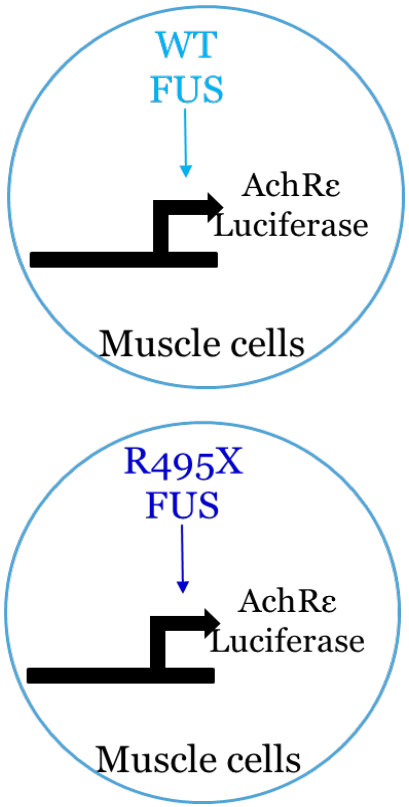
What is the effect of mutant Fus on NMJ genes expression?

Experimental design

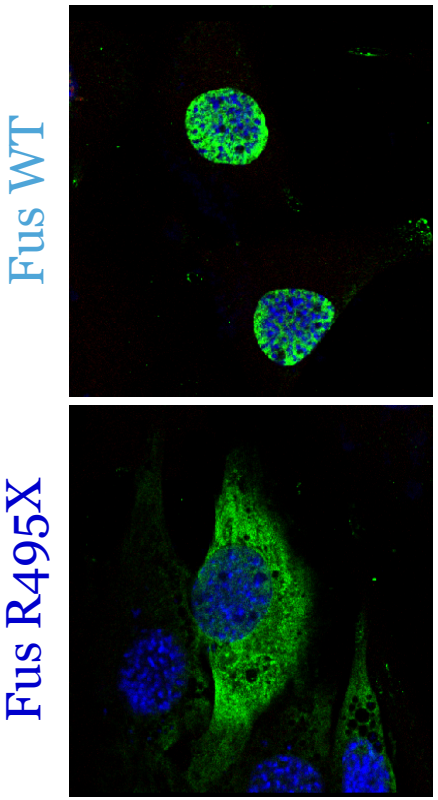


What is the effect of mutant Fus on NMJ genes expression?

Experimental design



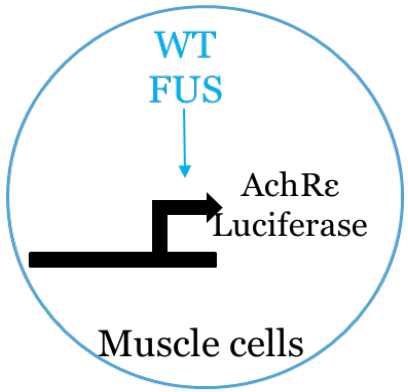
FUS - DAPI

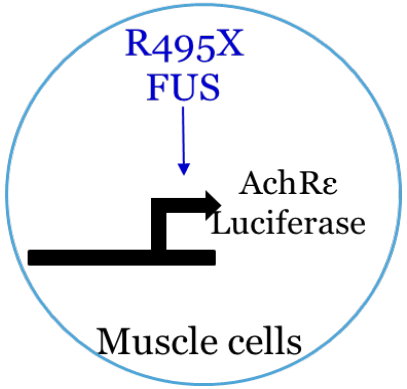


Mutant Fus is cytoplasmic

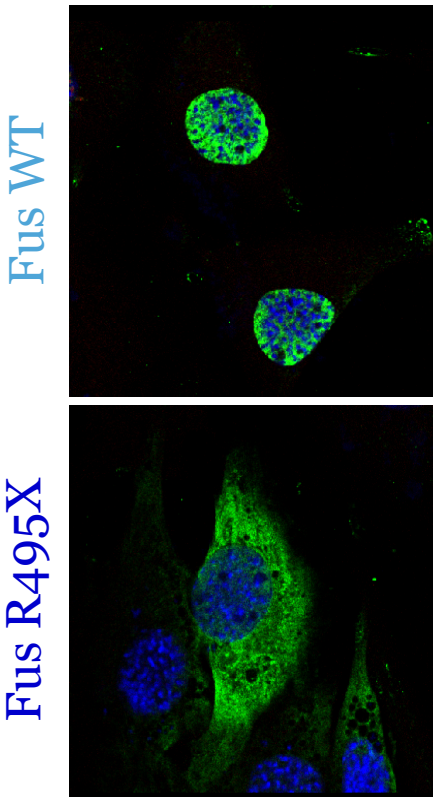
Fus WT but not R495X is sufficient to increase NMJ genes expression in muscle cells

Experimental design

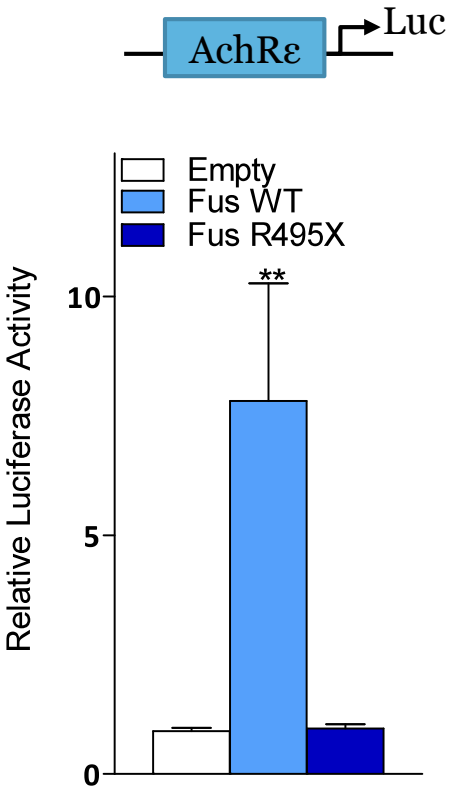




FUS - DAPI



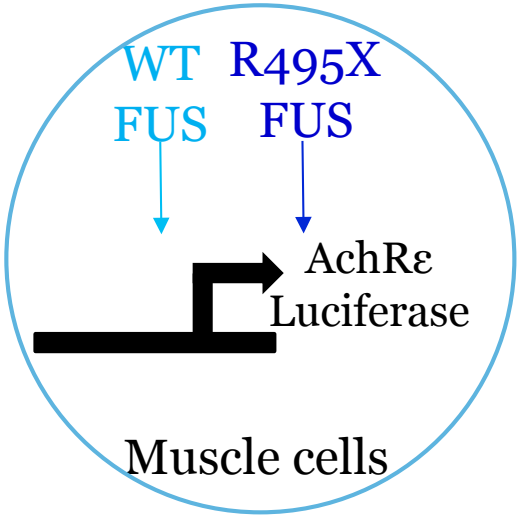
Mutant Fus is cytoplasmic



Mutant Fus presents a loss of function

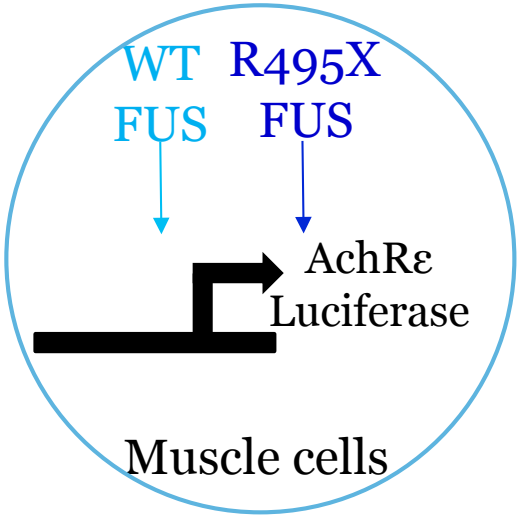
Does Mutant Fus inhibit Fus WT effect on NMJ genes expression?

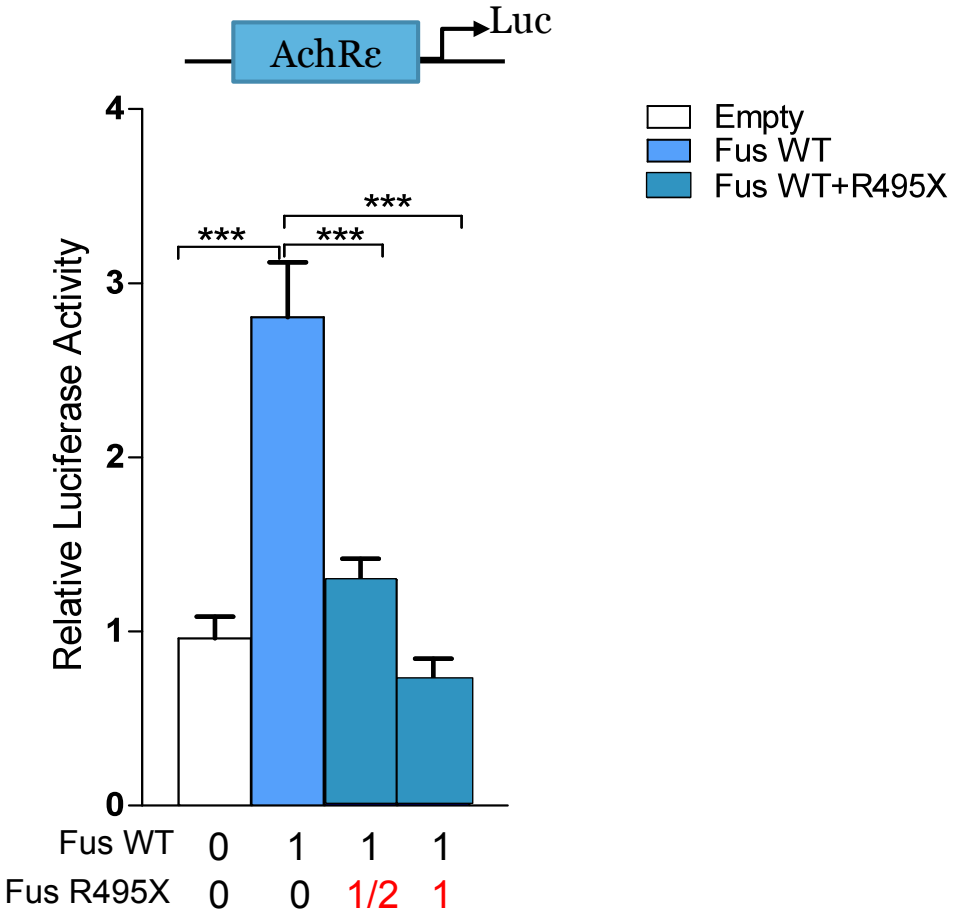
Experimental design



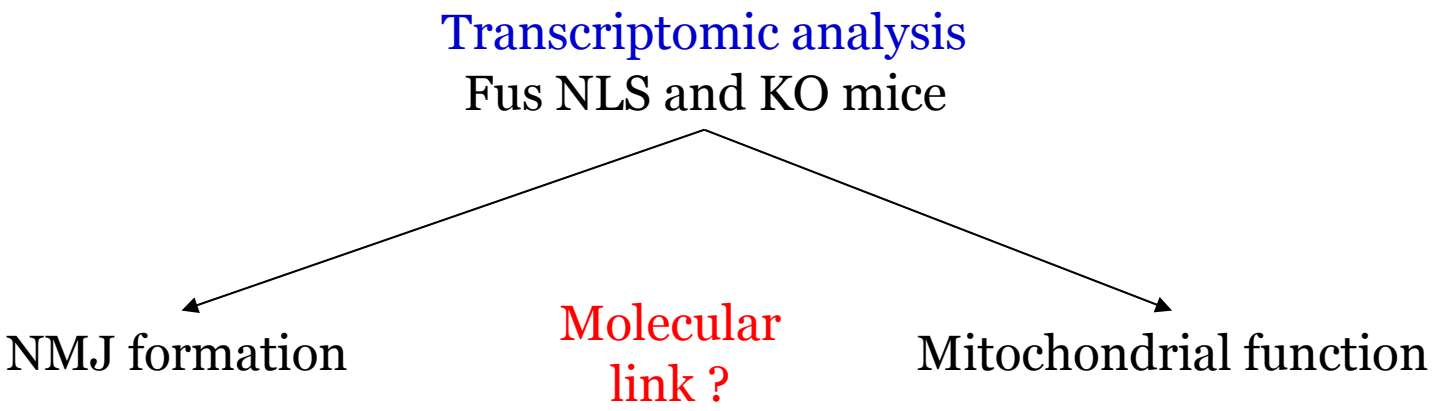
Mutant Fus has a dominant negative effect on Fus WT that prevents increase of NMJ genes expression

Experimental design

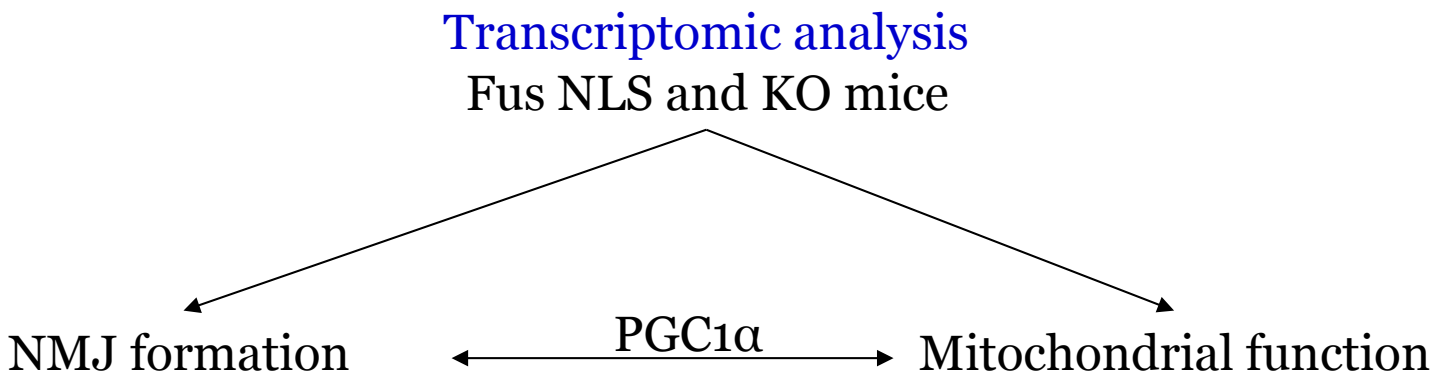




What could be the molecular link between NMJ formation and Mitochondrial function?



PGC1α a commun factor involved in NMJ and Mitochondrial functions

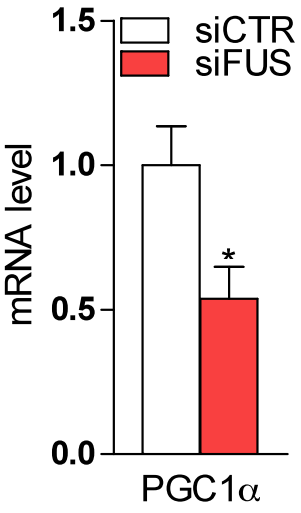


PGC1a and FUS:

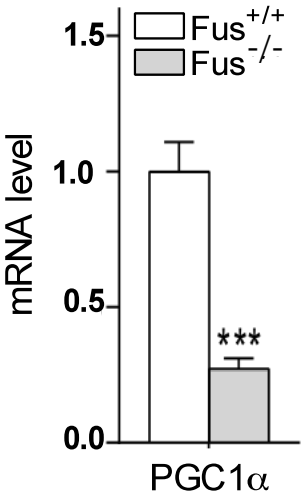
→PGC1a regulates TLS (FUS): Role in oxidative stress gene expression
Sanchez-Ramos et al, original research communication, 2011

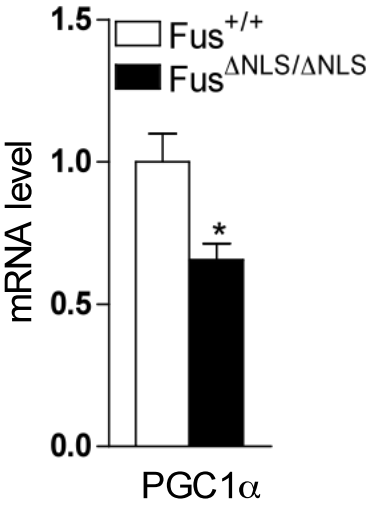
Fus impacts PGC1α expression, a key player in mitochondrial metabolism and NMJ formation

Muscle cells

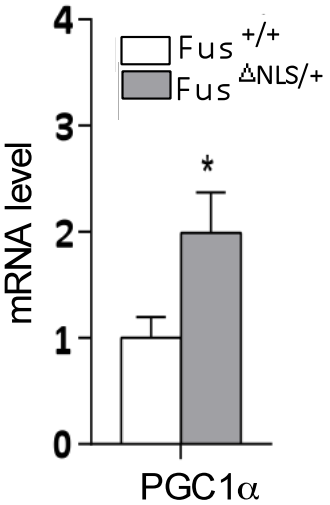


Newborn mice





Adult mice



Conclusion

- Fus is required for the normal morphology of the neuromuscular junction
- Fus is necessary and sufficient to increase NMJ genes expression
- Mutant Fus has a dominant negative effect on Fus WT that blocks NMJ gene expression
- Fus impacts PGC1α a key player in NMJ and mitochondrial function

→ Fus plays a key role in the development and maintenance of the post synaptic part of the NMJ

Acknowledgements

- U1118
  - Luc DUPUIS
  - Jean-Philippe LOEFFLER
  - Stéphane DIETERLE
  - Jelena SCEKIC-ZAHIROVIC
  - All the team of U1118
- Max Planck Munster
  - Erik STORKEBAUM
  - Sina MERSMANN
- UCSD
  - Clotilde LAGIER TOURENNE
  - Sun YING
  - Mélanie JAMBEAU
- Ulm university
  - Albert LUDOLPH
  - Tobias BOECKERS
- IGBMC
  - Nicolas CHARLET
  - Chantal SELLIER

ALS Association
Fighting Lou Gehrig's Disease
Create a world without ALS.

Frick foundation for ALS research

AFMTÉLÉTHON
INNOVER POUR GUERIR
4ET5 DECEMBRE 2015

mnda
motor neurone disease association

ARSLA
Association pour la Recherche sur la Sclérose Latérale Amyotrophique et autres maladies du motoneurone

Fondation Thierry Latran

Université de Strasbourg