



# NUTRAGE

Consiglio Nazionale delle Ricerche

Cestra Gianluca

Di Salvio Michela

Giansanti Mariagrazia

Giorgi Corinna

Guidi Alessandra

Mannironi Cecilia

Martone Julie

Mozzetta Chiara

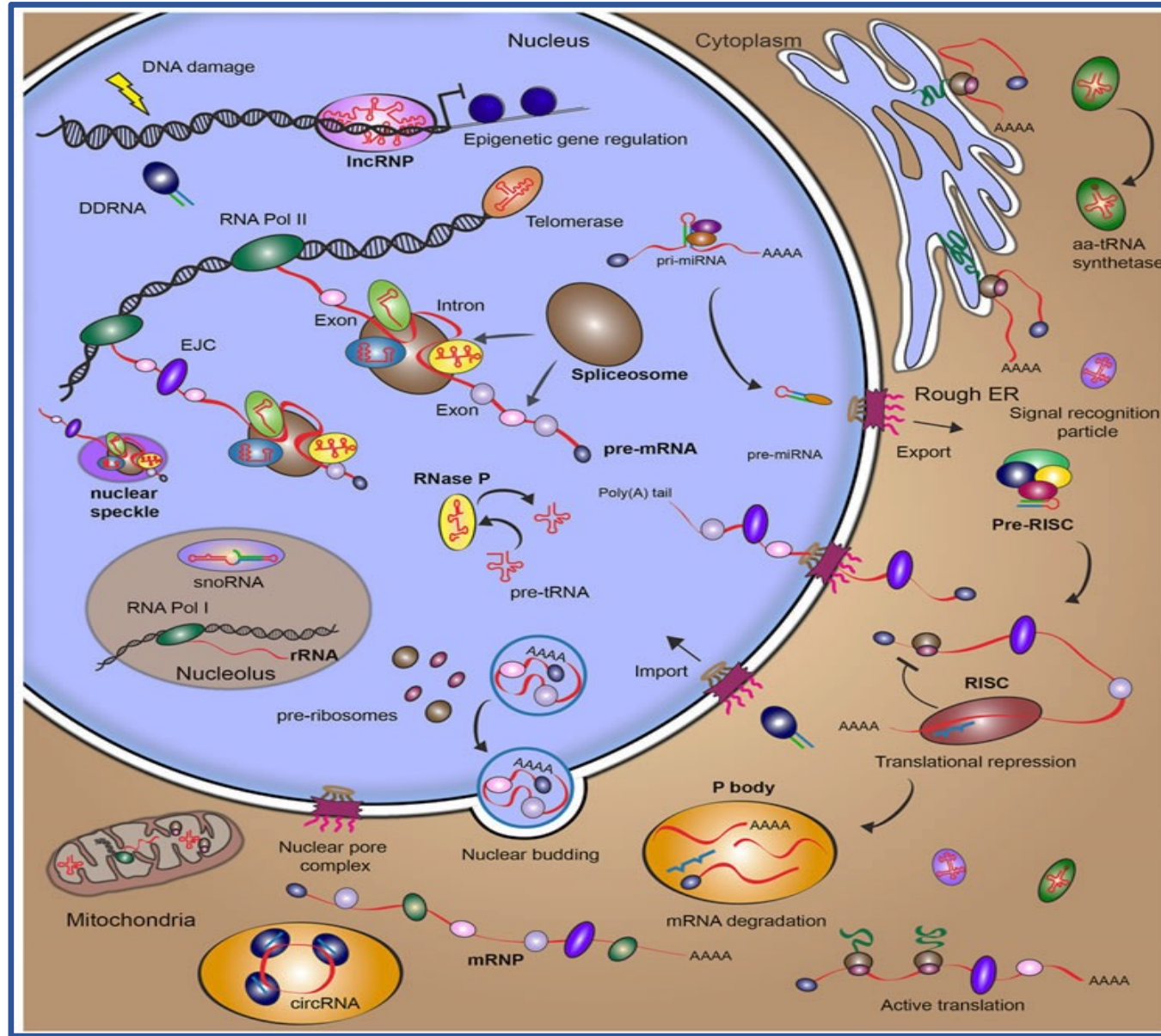
Sechi Stefano

Laneve Pietro

*pietro.laneve@cnr.it*

Circuiti molecolari RNA-dipendenti nella fisiopatologia di neuroni e muscoli

# The RNA world: from life origins to cell interpretations...



<https://rna.umich.edu/about/about/>

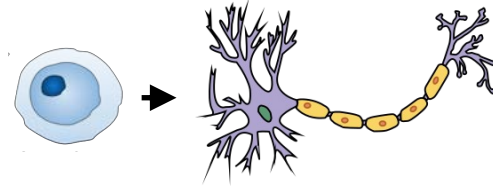
**NUTRAGE PROJECT**  
**SUBTASK 4.2.3**  
**@IBPM-Rome**

**RNA METABOLISM**

**Coding and noncoding RNAs**  
**Ribonucleoprotein complexes**  
**Proteins for RNA expression**

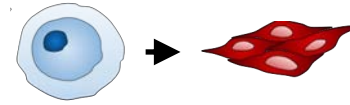
**RNA-dependent mechanisms for gene functions**

## Neuronal differentiation and activity



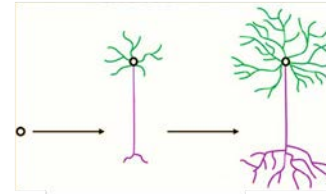
**Dr. Laneve, Dr. Martone**

## Muscle differentiation and activity



**Prof. Mozzetta, Dr. Guidi**

## Axonogenesis



**Dr. Giorgi**

## Memory, Plasticity



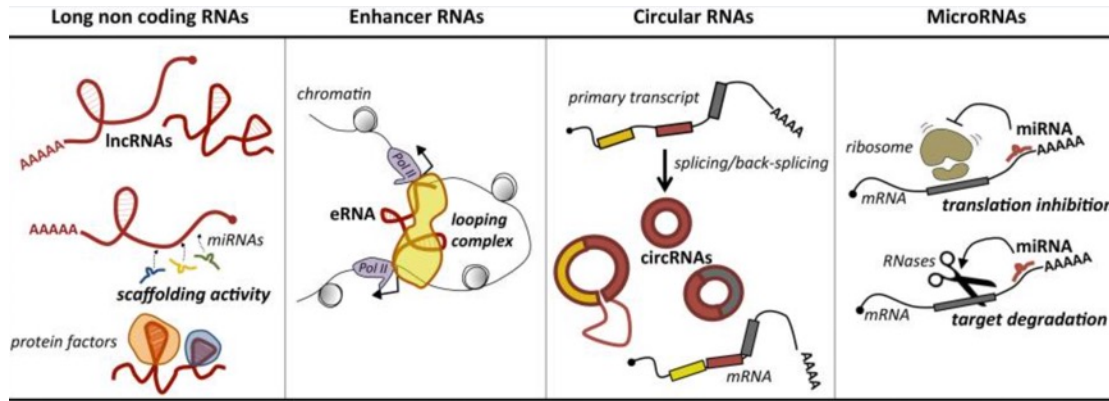
**Dr. Mannironi, Dr. Giansanti, Dr. Sechi**

## Neurodegeneration

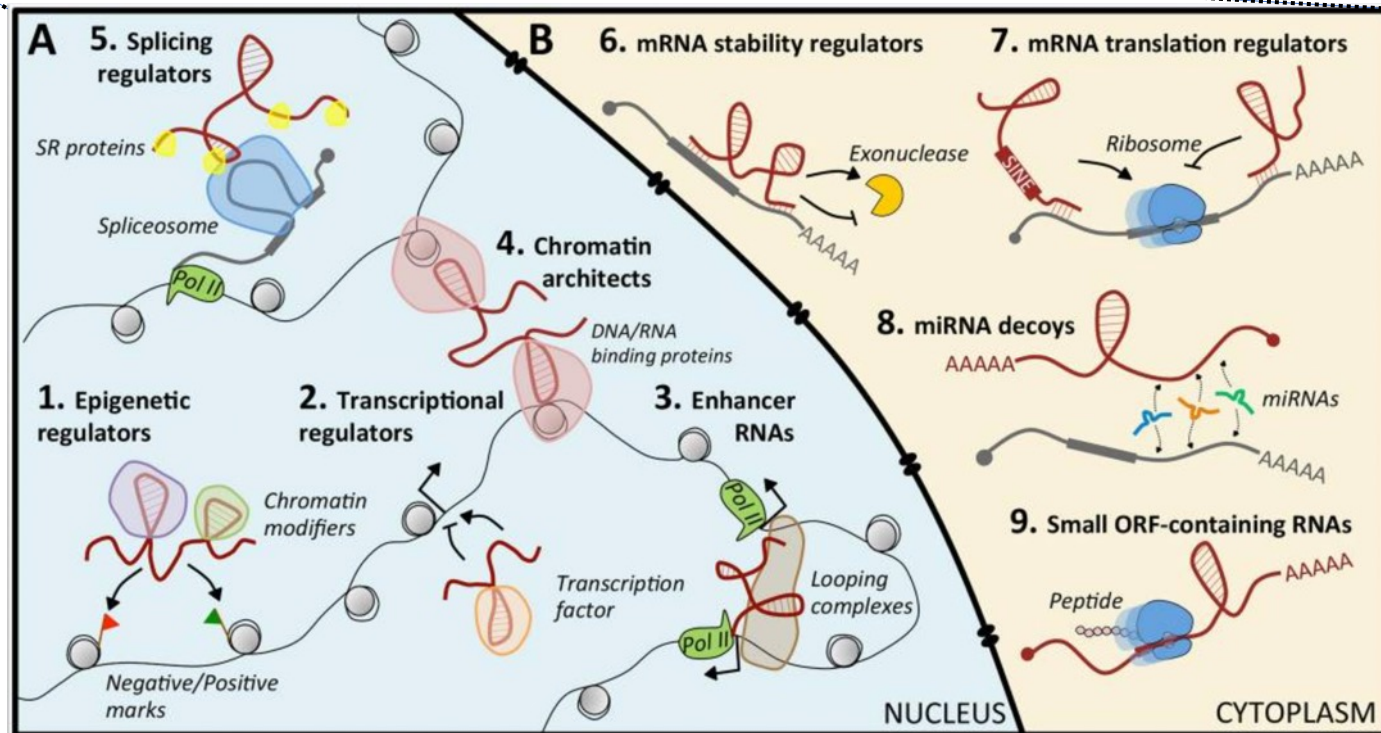


**Dr. Cestra, Dr. Di Salvio**

# Long noncoding RNAs



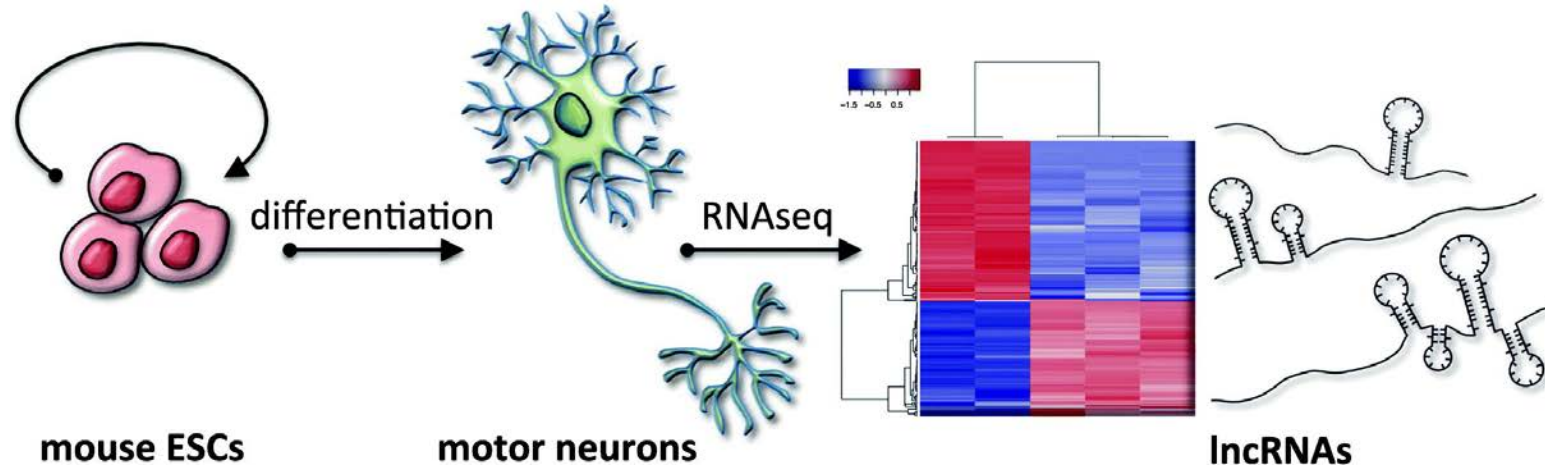
from Laneve and Caffarelli, Front Cell Dev Biol. (2020)



- non-protein-coding RNAs > 500 nucleotides
- untranslated or containing short ORFs
- tissue specific expression
- localised in the nucleus or cytoplasm (or both)
- a variety of molecular mechanisms
- linked to pathologies

Laneve et al., Front Pediatr. (2019)

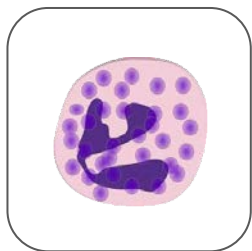
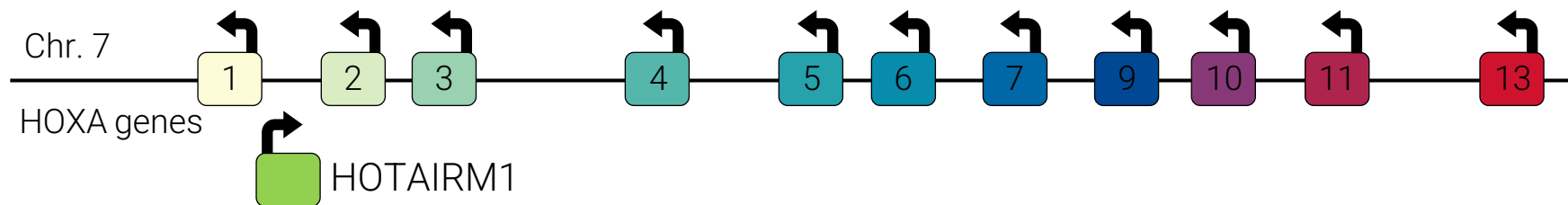
# Identification of lncRNAs in differentiated mouse motor neurons (MNs)



Biscarini et al., Stem Cells Res. (2018)

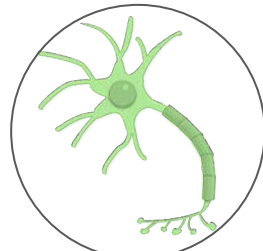
**HOTAIRM1 was the most expressed lncRNA**

# HOTAIRM1 (HOXA transcript antisense RNA, myeloid-specific 1)



Myelopoiesis

- HOXA genes expression
- cell cycle  
(Zhang X et al. 2009; Zhang X et al. 2014)
- autophagy  
(Chen ZH et al. 2017)
- monocytes identity maintenance  
(Xin J et al. 2017)



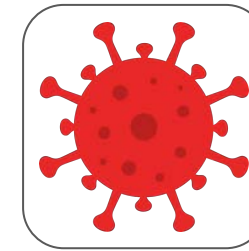
Neurogenesis

- induced more than 50 times in iPSC-derived neurons  
(Lin M et al. 2011)



Cancer

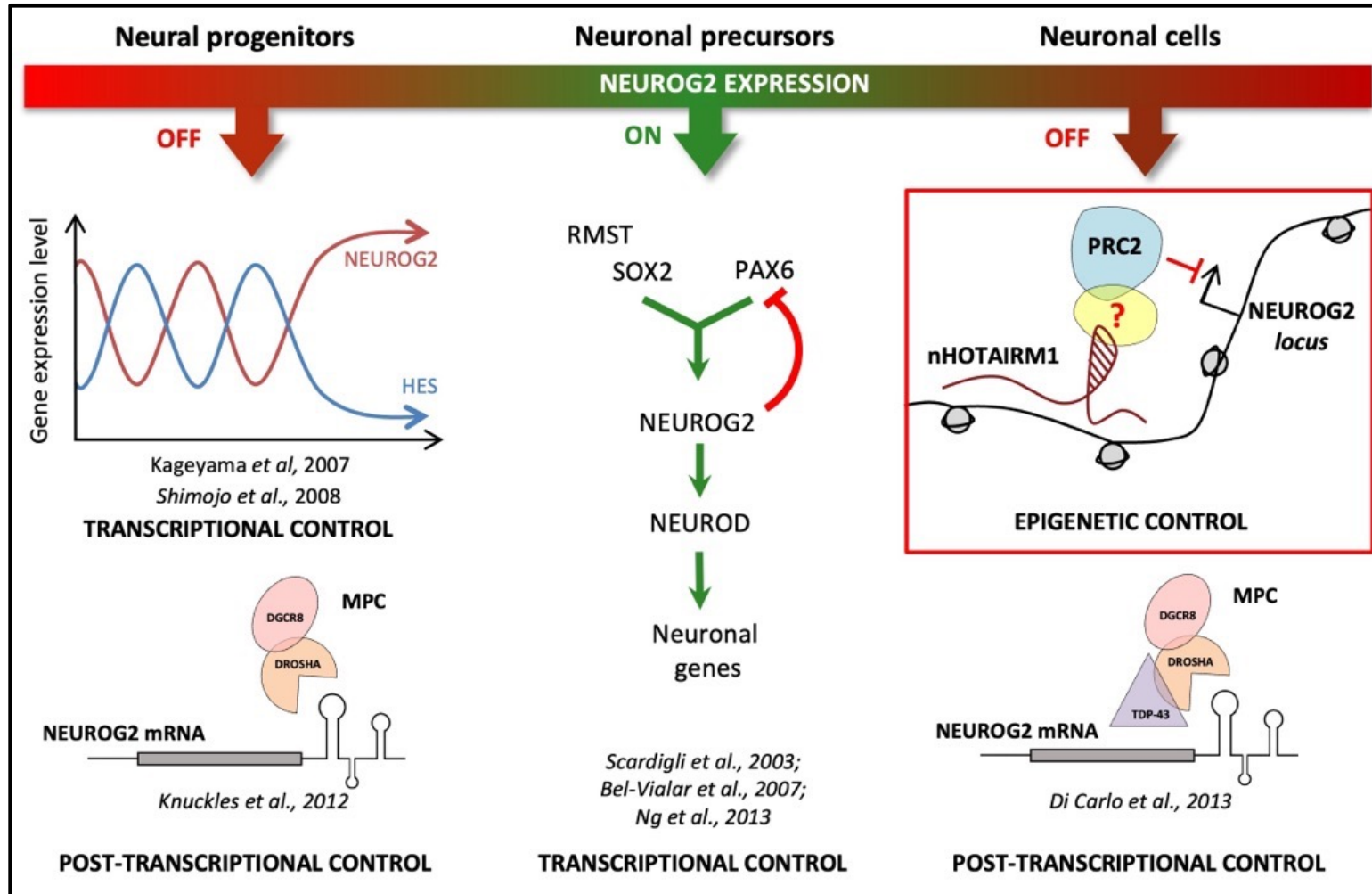
- breast cancer  
(Su X et al. 2014)
- colorectal cancer  
(Wan L et al. 2016)
- ovary cancer  
(Yang K et al. 2017)
- glioma  
(Chen Y et al. 2017)



COVID-19

- one of the 3 main *trans*-acting-lncRNAs that modulate the expression of protein-coding genes during SARS-CoV-2 infection in peripheral blood mononuclear cells (PBMC)  
(Daneshpour M et al. 2021)

# HOTAIRM1: from neurons...



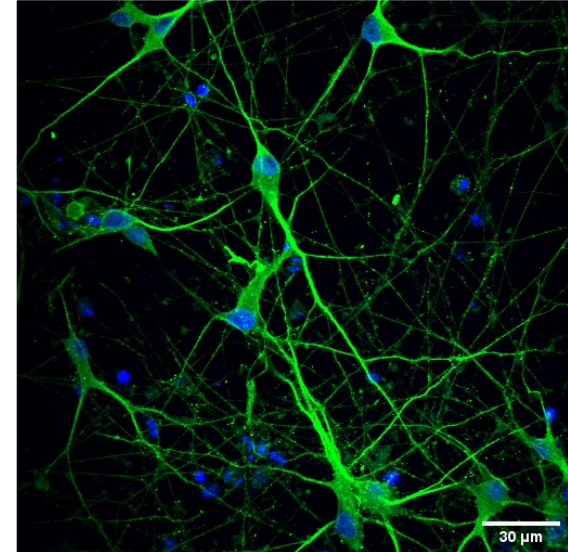
Rea et al., Cell Death & Dis. (2020)

HOTAIRM1 regulates neuronal differentiation by modulating NEUROGENIN 2 and the downstream neurogenic cascade

# to spinal motor neurons (spMNs)...

Laneve, Caffarelli, Tollis, Rocchegiani, Grandioso

GAP43/DAPI



Abundant and homogeneous population of spMNs

Electrophysiologically active spinal MNs

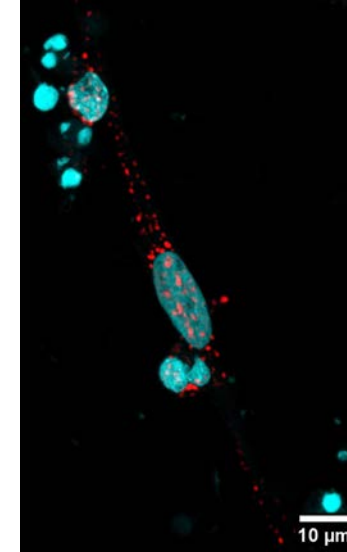
Expression of mature MN markers ChAT and Islet1

Short differentiation timelines

WT spinal MNs (DIV7)

(collaboration Dr. Vitiello, IIT)

nHOTAIRM1/TUBB3/DAPI

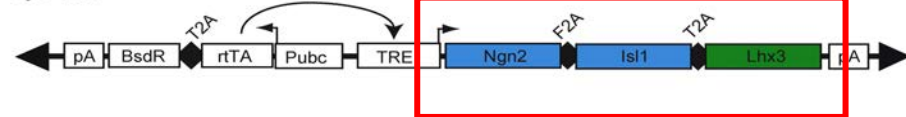


HOTAIRM1 is induced during spMN generation

HOTAIRM1 expressed in spMN soma and neurites

HOTAIRM1 ISH

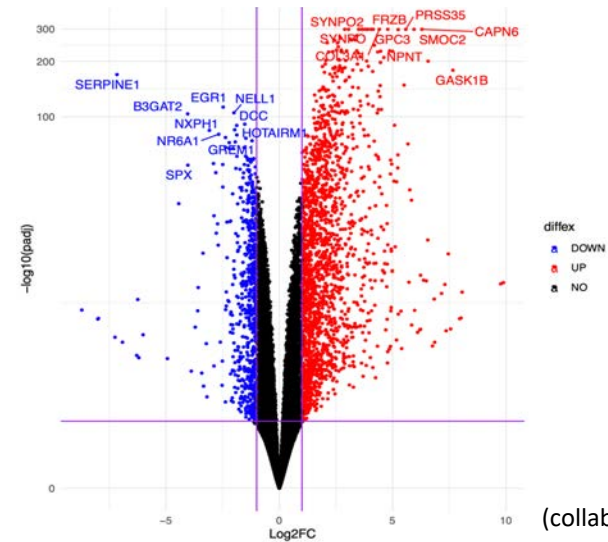
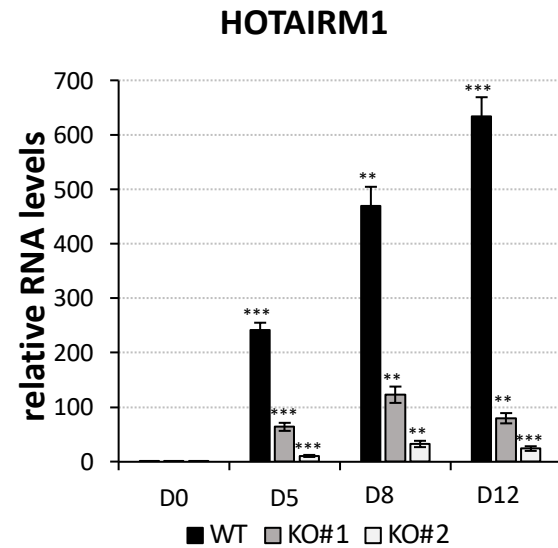
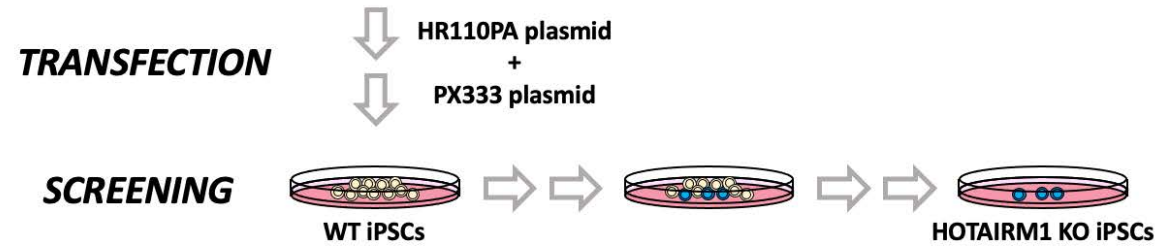
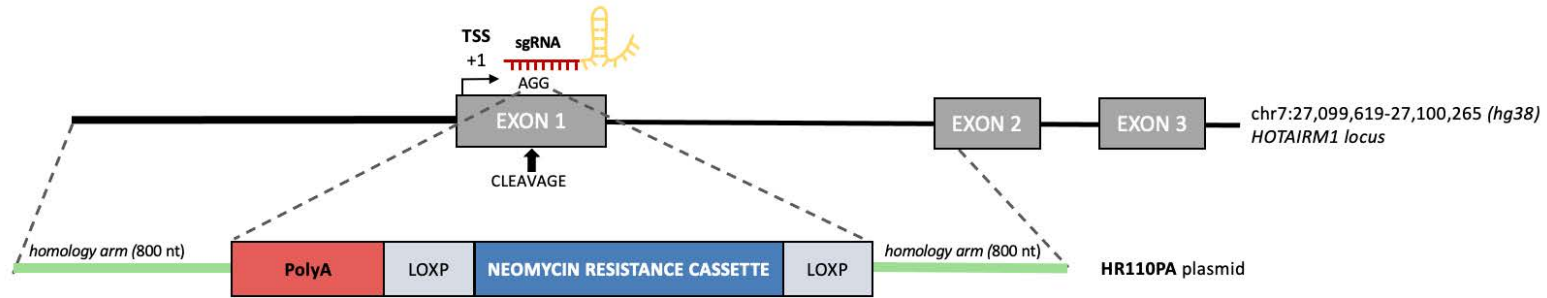
epB-NIL



Inducible vector epB-NIL

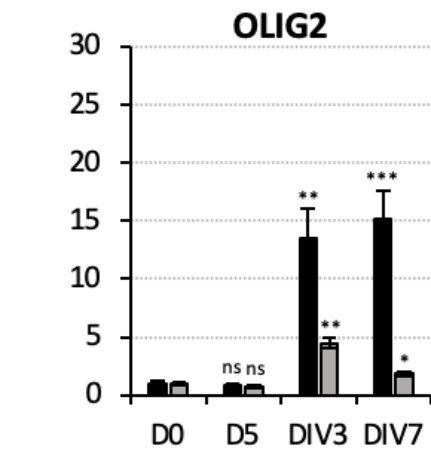
Garone et al., J Vis Exp (2019)

# HOTAIRM1 KO in spMNs

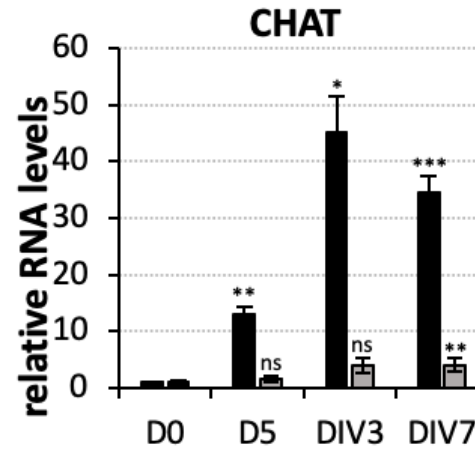


(collaboration Dr. Carissimo, CNR)

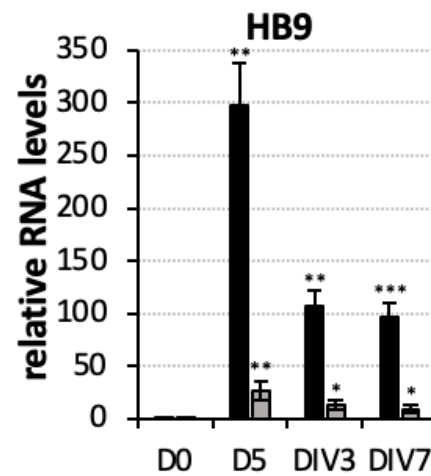
# HOTAIRM1 in spMN differentiation...



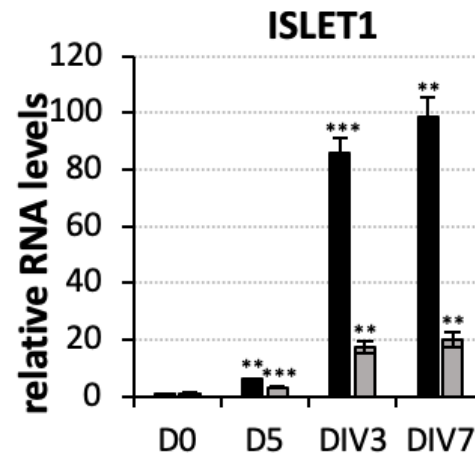
■ WT □ KO#2



■ WT □ KO#2

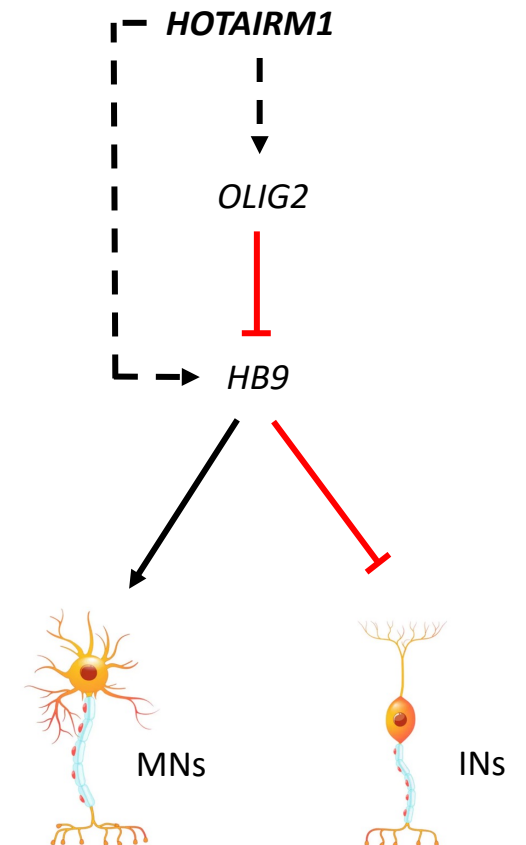


■ WT □ KO#2



■ WT □ KO#2

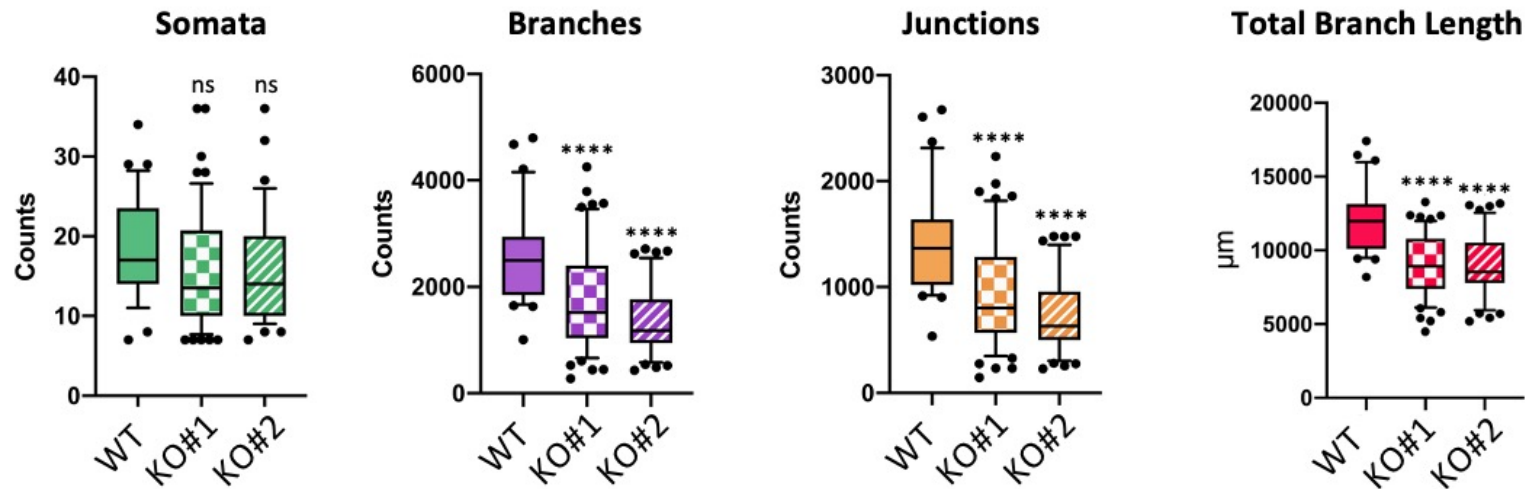
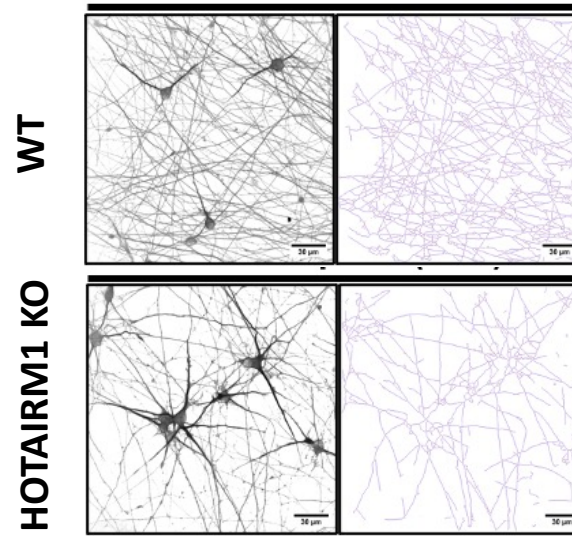
## Cell choice



HOTAIRM1 is a pro-MN molecule, at the crossroad of MN and interneuron (IN) fate

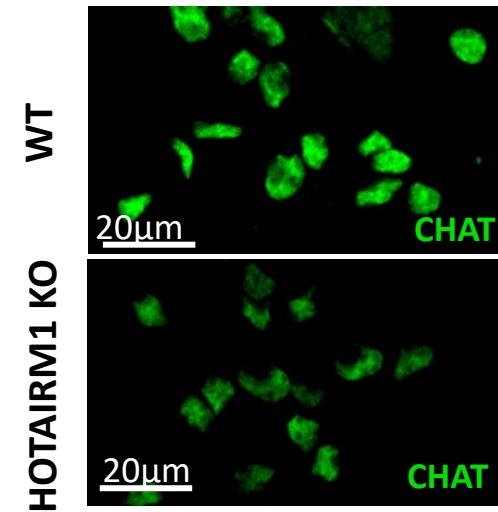
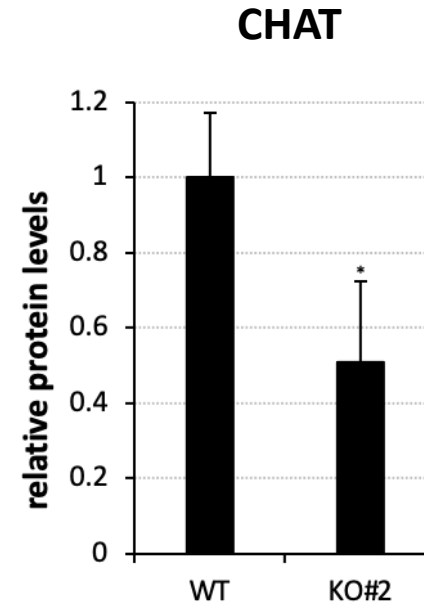
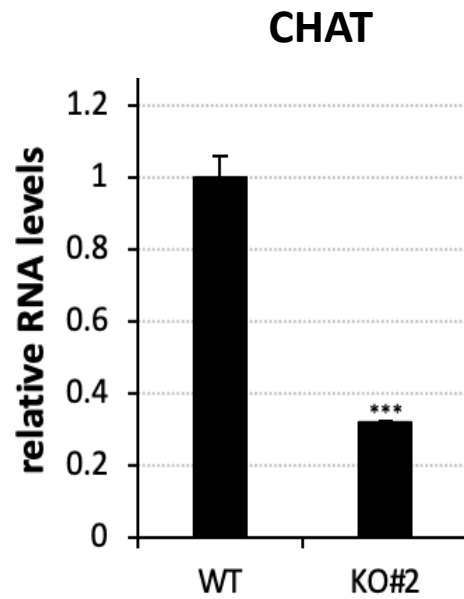
# ...neurite branching...

## Neurites



**HOTAIRM1 is required for proper MN morphology**

## ...and synaptic gene expression



HOTAIRM1 is required for the expression of target genes encoding modulators of synaptic activity

**Tollis et al.**, *The long noncoding RNA HOTAIRM1 is necessary for differentiation and activity of iPSC-derived spinal motor neurons*. Cell Death & Disease (2023)

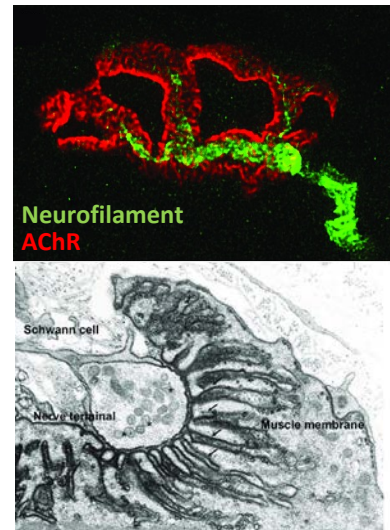
- 1) HOTAIRM1 is involved in spMN generation, maturation and activity
- 2) A mechanisms involving lncRNA-mRNA interactions contributes to these functions
- 3) Studying the bases of cell differentiation and activity contributes to understand cell specificity

## Directions

HOTAIRM1:

- 1) Is expressed in MNs
- 2) Is implicated in synaptic activity

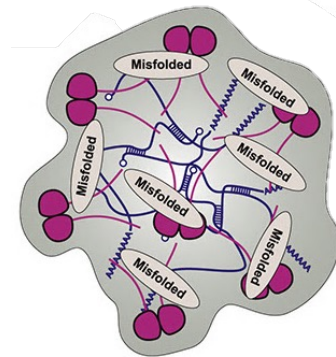
Role in neuromuscular junction



from **Rodriguez Cruz et al.**, Front Mol Neurosci. (2020)

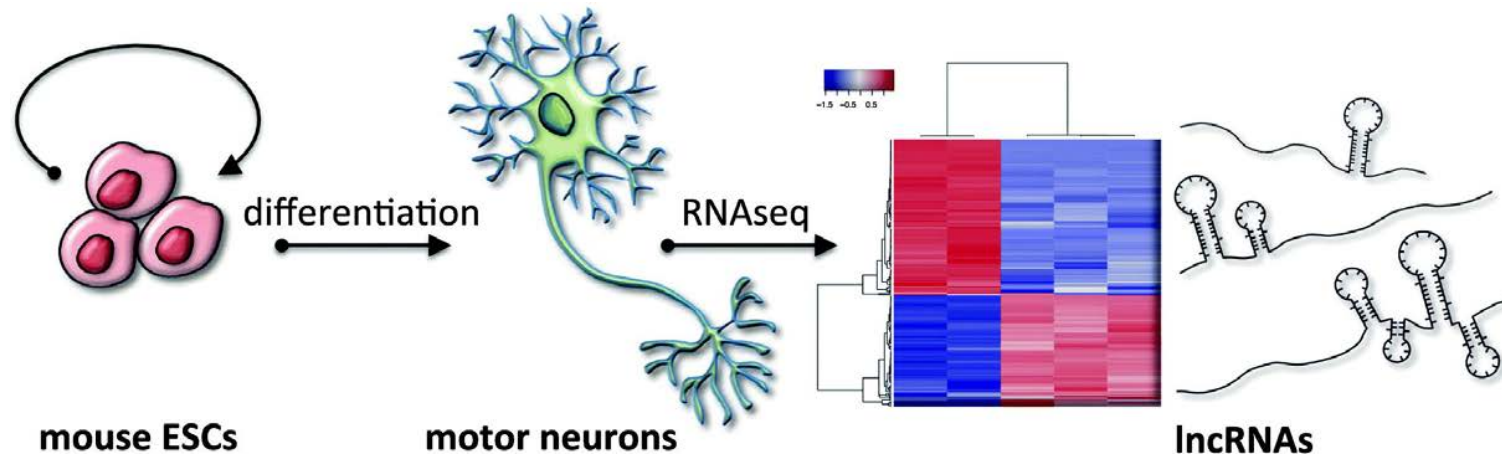
- HOTAIRM1:
- 1) Interacts with ALS factors
  - 2) Promotes the formation of aggregates and

Role of nHOTAIRM1 in neurodegeneration

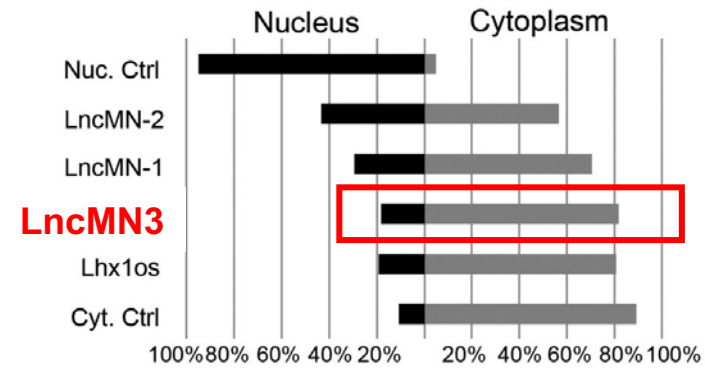
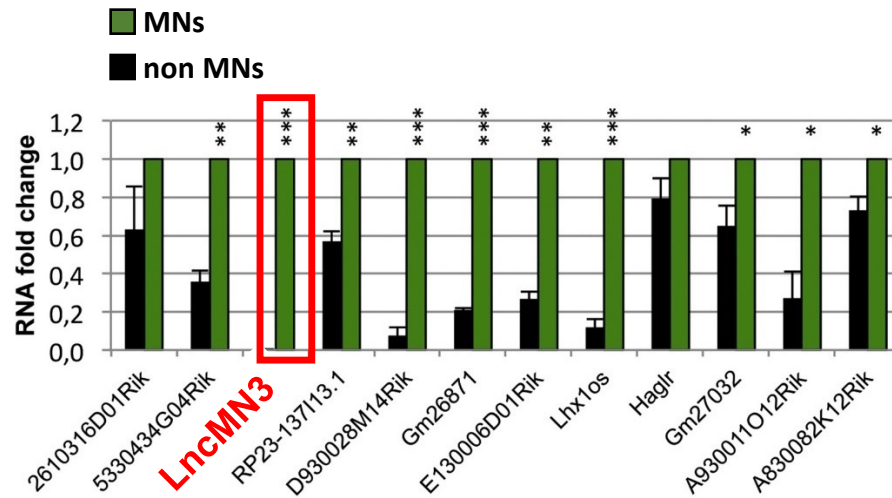


from **Guillén-Boixet et al.**, Cell (2022)

# Identification of lncRNAs in differentiated mouse MNs



Biscarini et al., Stem Cells Res. (2018)



Biscarini et al., Stem Cells Res. (2018)

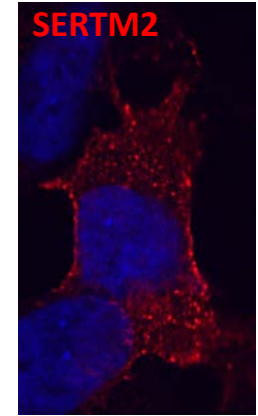
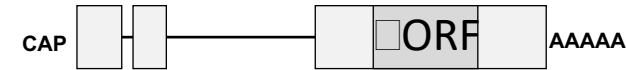
## LncMN3, the most enriched lncRNA

# ...to code or not to code?

Martone, Bozzoni, Lisi, Setti, Santini, Nutarelli

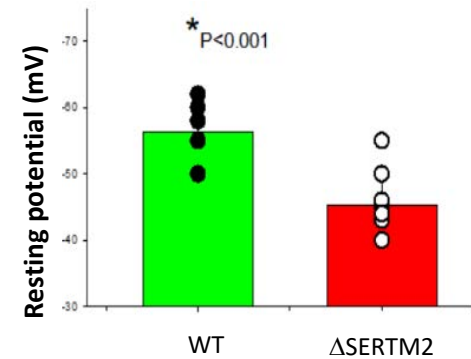
**IncMN3** translates for **SERTM2**

- a transmembrane micropeptide
- conserved in Vertebrates
- expressed in motor neurons



**patch clamp experiments**

SERTM2 is involved in the MN resting potential

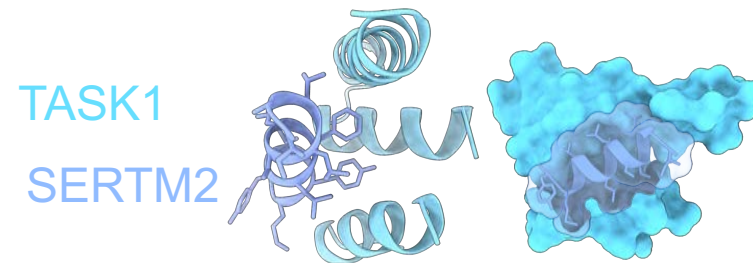


(collaboration Prof. Fucile, Sapienza-Rome)

(collaboration Dott. Nicoletti, Sapienza-Rome)

**homology sequence analyses and co-IP experiments**

SERTM2 interacts with TASK1 and may modulate K<sup>+</sup> channels



(collaboration Prof. Paiardini, Sapienza-Rome)

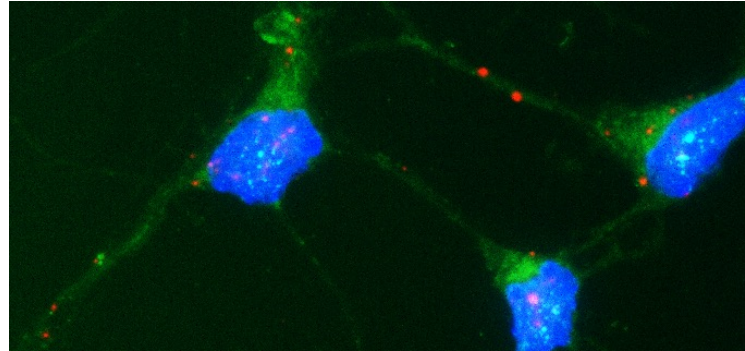
Lisi et al., in preparation

**SERTM2, one of the few micropeptides in CNS, modulates MN excitability**

# Identification and function of AS-p75

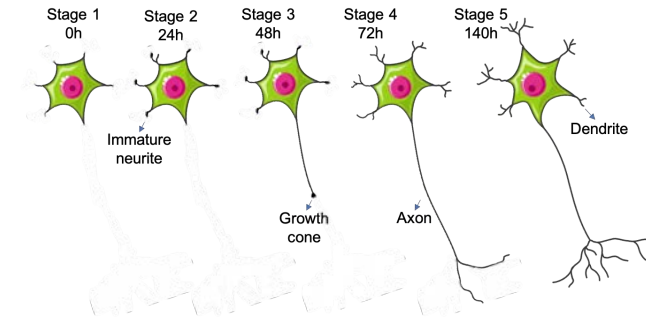
Giorgi, De Paolis, Paolillo, Siri, Spina, Caporali, La Regina, Canossa, Vignoli

AS-p75 / p75 / DAPI

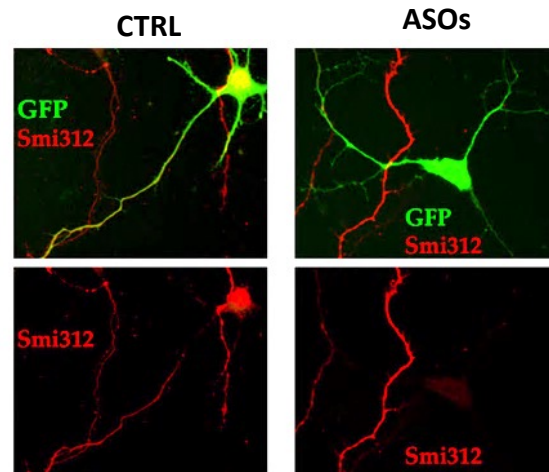


AS-p75, ISH

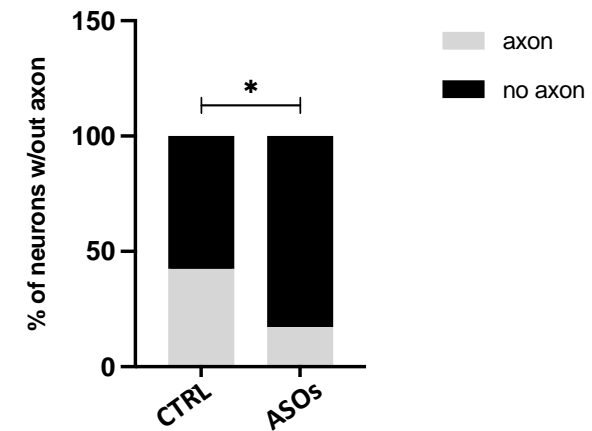
## Modeling axogenesis



Cortical neurons



Neurofilament IF in targeted neurons

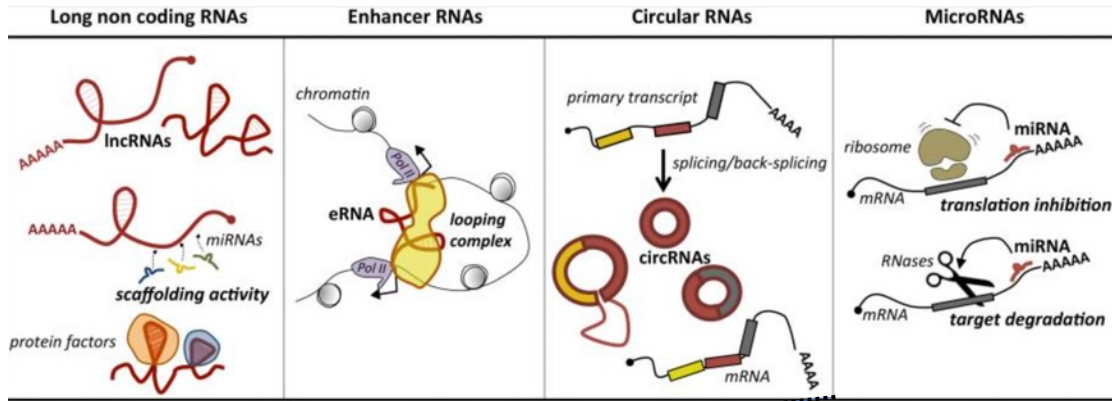


Targeting AS-p75 impairs # of neurons with axons

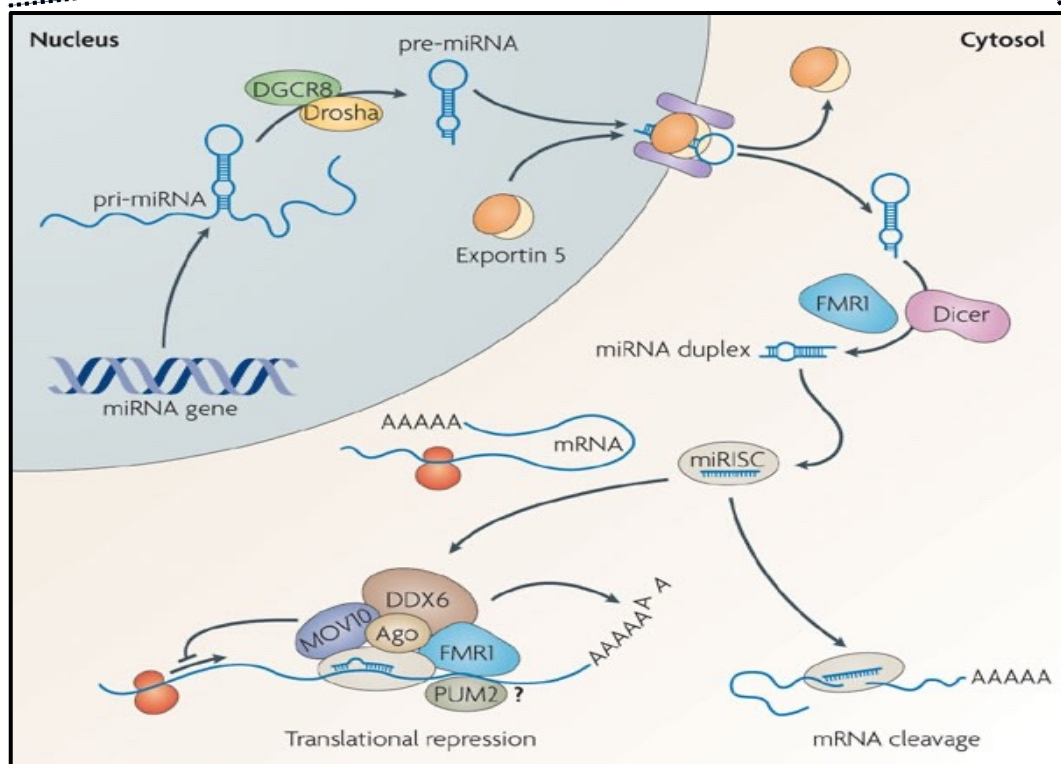
De Paolis et al., submitted

A novel lncRNA antisense to p75<sup>NTR</sup> mRNA is required for axon specification

# microRNAs



from Laneve and Caffarelli, Front Cell Dev Biol. (2020)



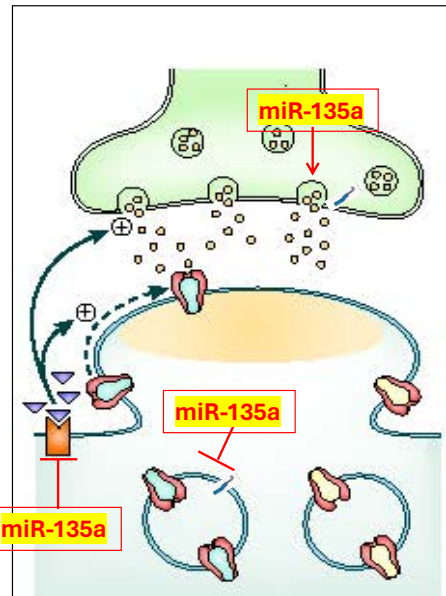
- 20-23 nucleotides
- tissue specific expression
- basically localised in the cytoplasm
- a few post-transcriptional mechanisms
- pleiotropic and combinatorial
- linked to pathologies

Schratt, Nature Rev Neurosci. (2009)

# microRNAs in neuronal plasticity

Mannironi, Gasparini, Pericaroli, Franco

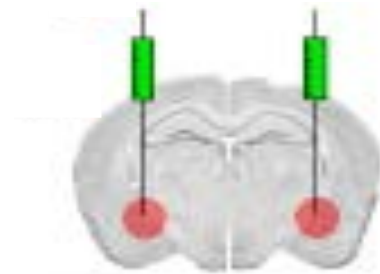
## miR-135a and synaptic functions



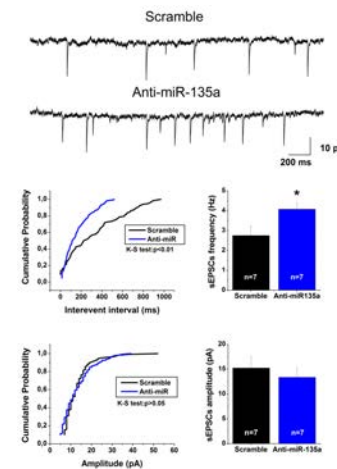
GluA1/2 AMPAR  
 GluA2/3 AMPAR  
 Corticosterone  
 MR  
 Complexin 1/2

**Mannironi et al., PlosOne (2013)**  
**Mannironi et al., Mol Neurobiol (2013)**  
**Gasparini et al., Mol Neurobiol (2020)**

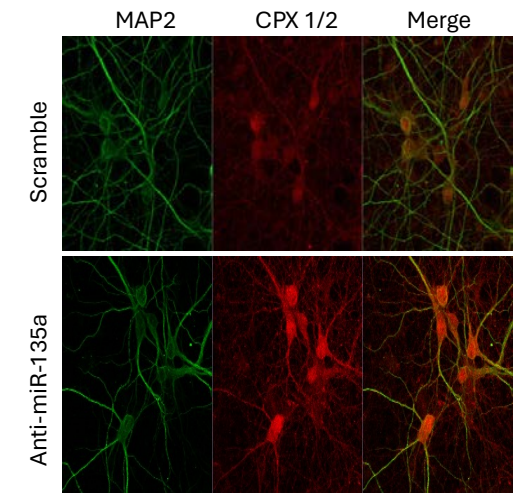
## Anti-miR-135a focal injection in mouse brain



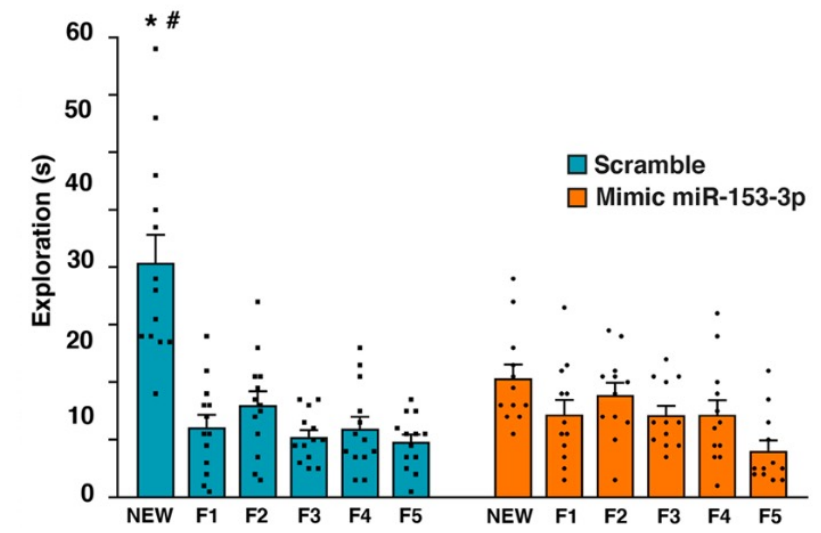
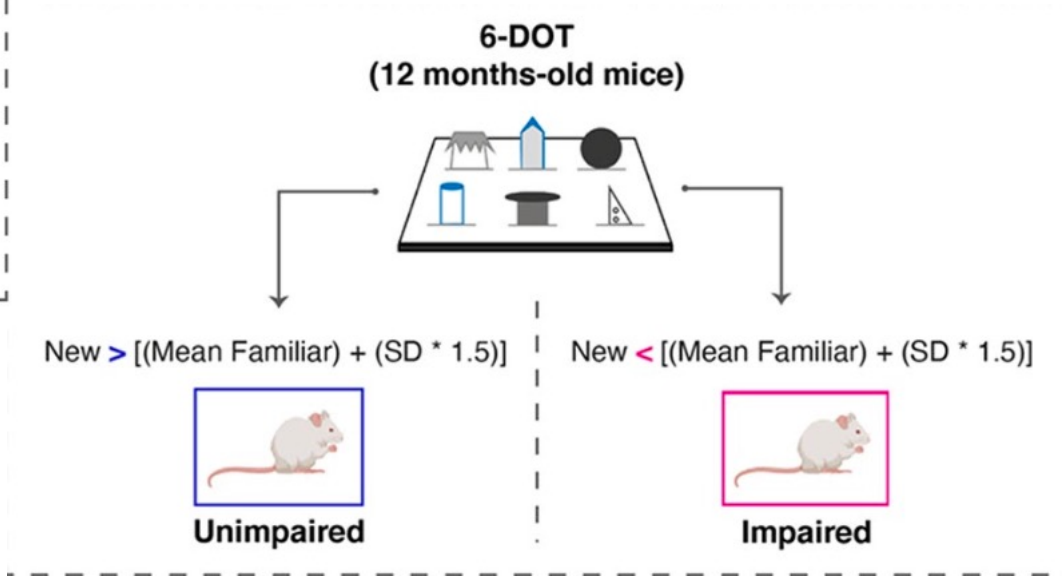
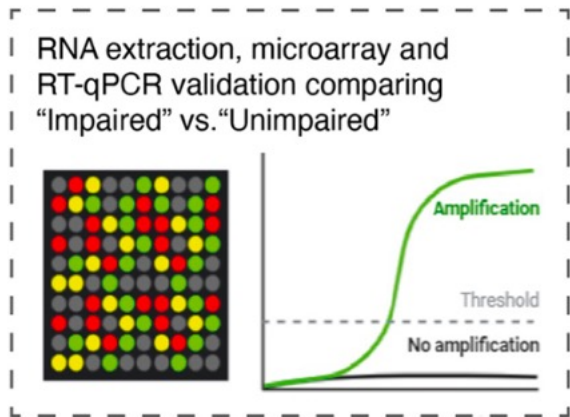
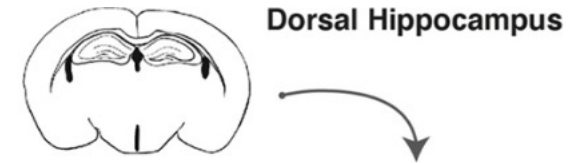
## Electrophysiological recording ex-vivo and in cells



## micro-RNA target analysis



# miR-153-3p and short-term memory



Stabile et al., Mol Neurobiol (2023)

Short-Term Memory deficit associates with miR-153-3p upregulation in the hippocampus of middle-aged mice



## SUBTASK 4.2.3

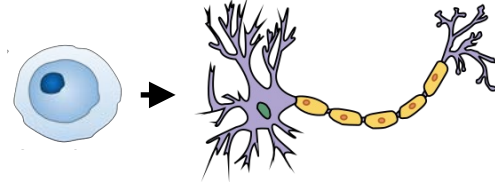
Identificazione di RNA codificanti e non codificanti, di complessi ribonucleoproteici implicati nel metabolismo dell'RNA e di modulatori epigenetici che controllano i circuiti molecolari RNA-dipendenti alla base della fisiopatologia neuromuscolare

@



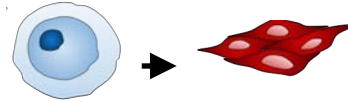
Istituto di Biologia e  
Patologia Molecolari

### Neuronal differentiation and activity



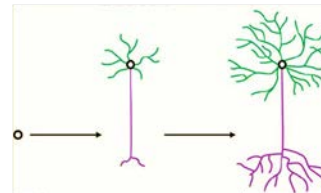
Dr. Laneve  
Dr. Martone

### Muscle differentiation and activity



Prof. Mozzetta  
Dr. Guidi

### Axonogenesis



Dr. Giorgi

### Memory, Plasticity



Dr. Mannironi  
Dr. Giansanti  
Dr. Sechi

### Neurodegeneration



Dr. Cestra,  
Dr. Di Salvio



THANKS

