Long non coding RNAs (lncRNAs) in motorneuron (MN) development and disease

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Stanza 30

- LncRNAs show prominent expression in the nervous system and have been implicated in neural development, function and disease.

- Remarkably large number of annotated lncRNAs (approximately 40%) is expressed specifically in the brain (Derrien et al., 2012)

- Ubiquitously expressed lncRNAs are generally expressed at high levels, while cell type- or tissue-specific lncRNAs, such as those in MNs, are often expressed at lower levels (Jiang, Li,, et al., 2016)

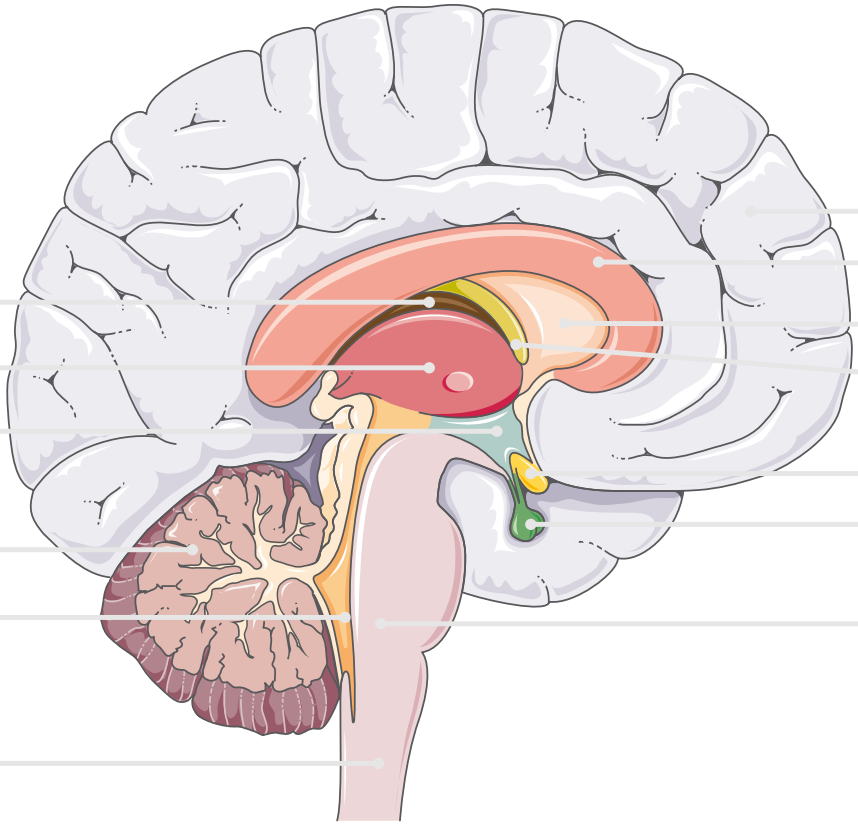
- lncRNAs have been linked to processes such as neuron development, neurite growth, synaptic transmission, memory consolidation and ageing (Mehler & Mattick, 2007; Mercer et al., 2008; Pereira Fernandes et al., 2018; Shi et al., 2017)

Motorneurons (MNs)

MNs are a group of neurons that have their cell bodies:

- in the cortex (upper MNs)
- in the brainstem and spinal cord (lower MNs) and project axons into the brainstem, spinal cord or towards peripheral muscles. These projections control essential functions such as movement, breathing and swallowing.

Brain

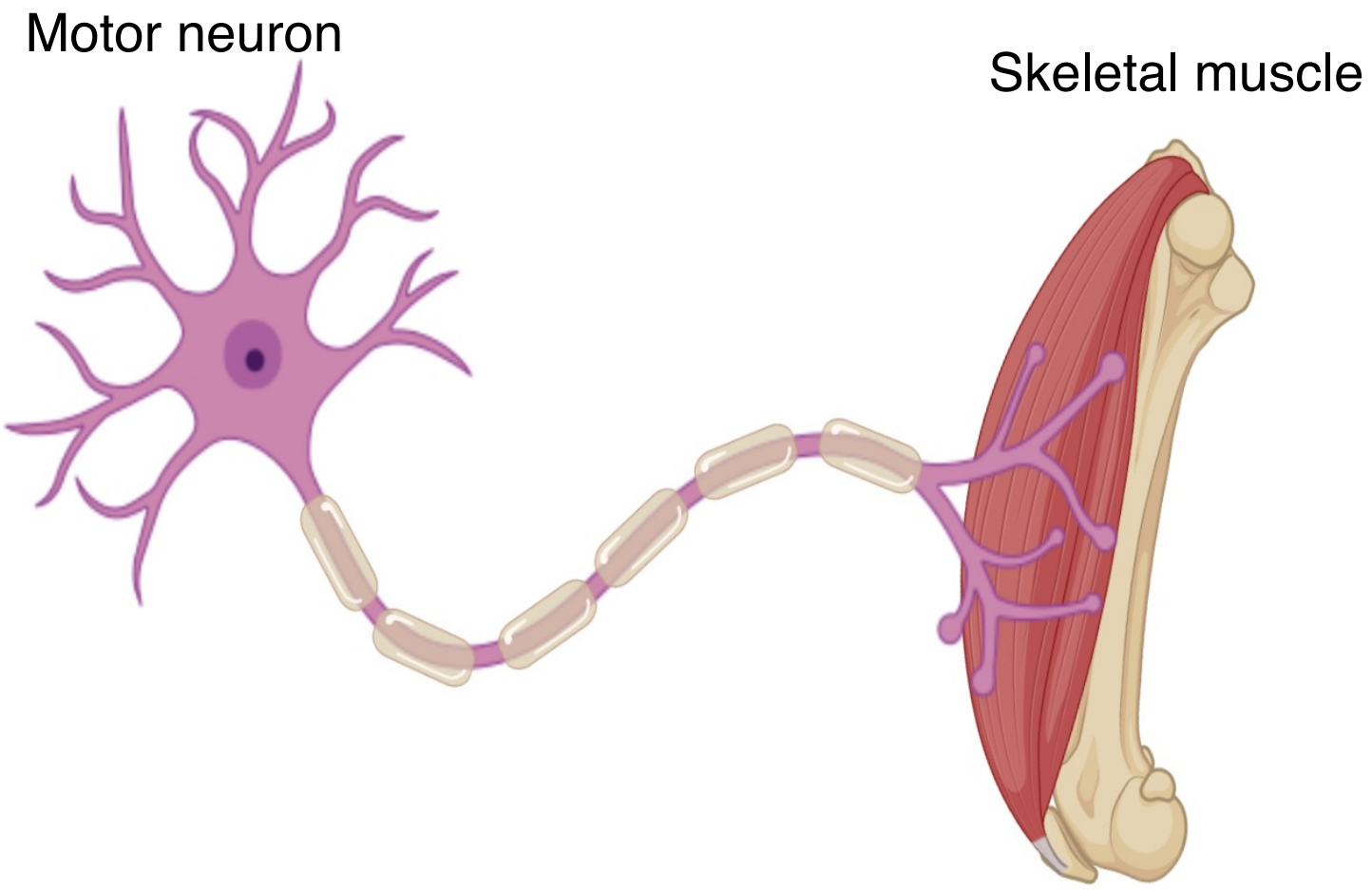


3rd ventricle
Thalamus
Hypothalamus
Cerebellum
4th ventricle
Spinal cord

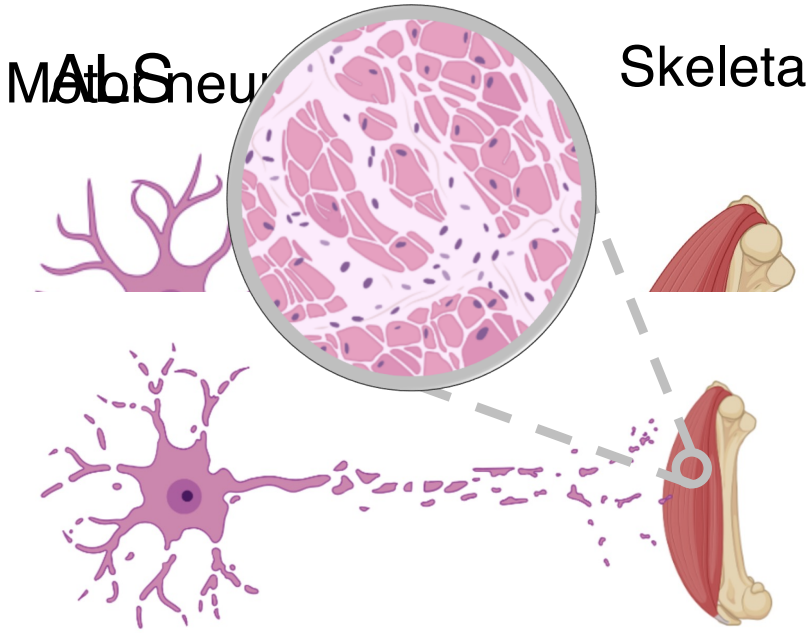
Cortex
Corpus callosum
Septum pellucidum
Fornix
Optic chiasma
Hypophysis
Brain stem

Not surprising given their important functions, selective degeneration of MNs is a hallmark of motor neuron diseases (MNDs) such as:

amyotrophic lateral sclerosis (ALS)
spinal muscular atrophy (SMA)


Motor neuron
Skeletal muscle

MN and ALS



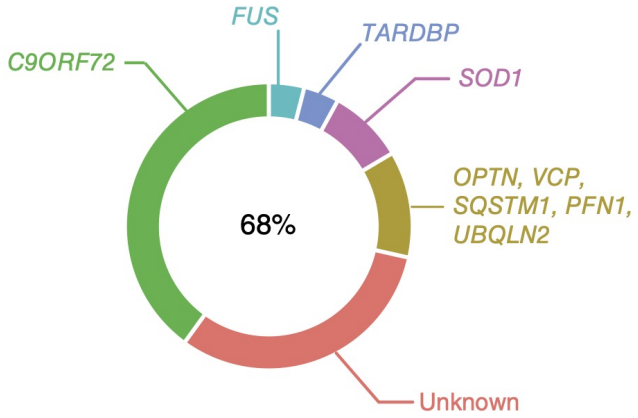
Neurodegenerative disease

Affects MNs

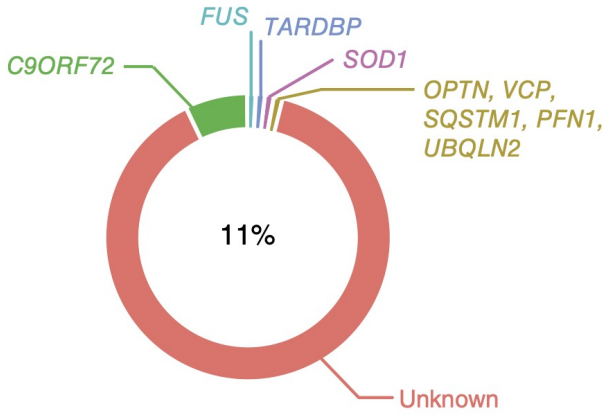
No cure

Da 1 a 2 per 100.000 persone l'anno

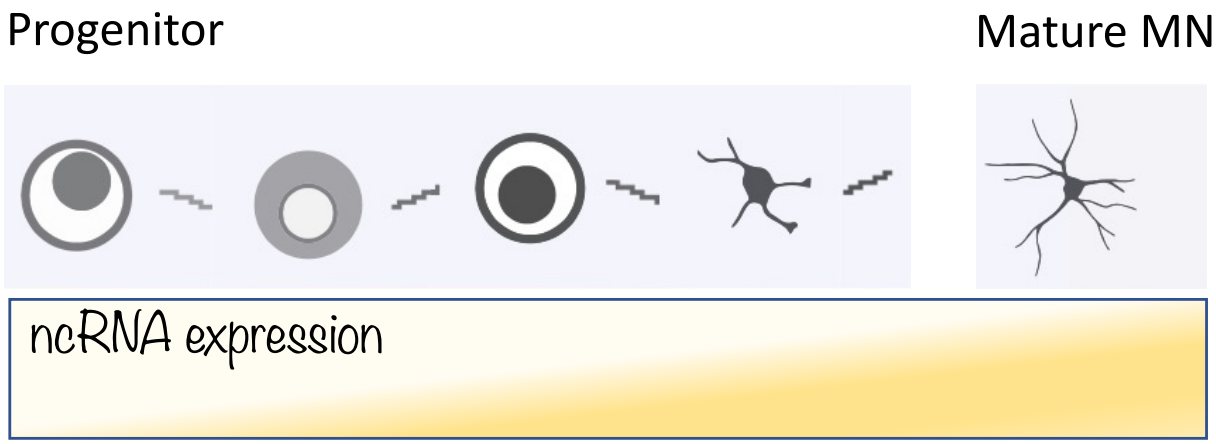
Familial



Sporadic



Motor neuron development



A role for lncRNAs in the specification of neuron subtypes has been proposed.

lncRNAs and motor neuron development

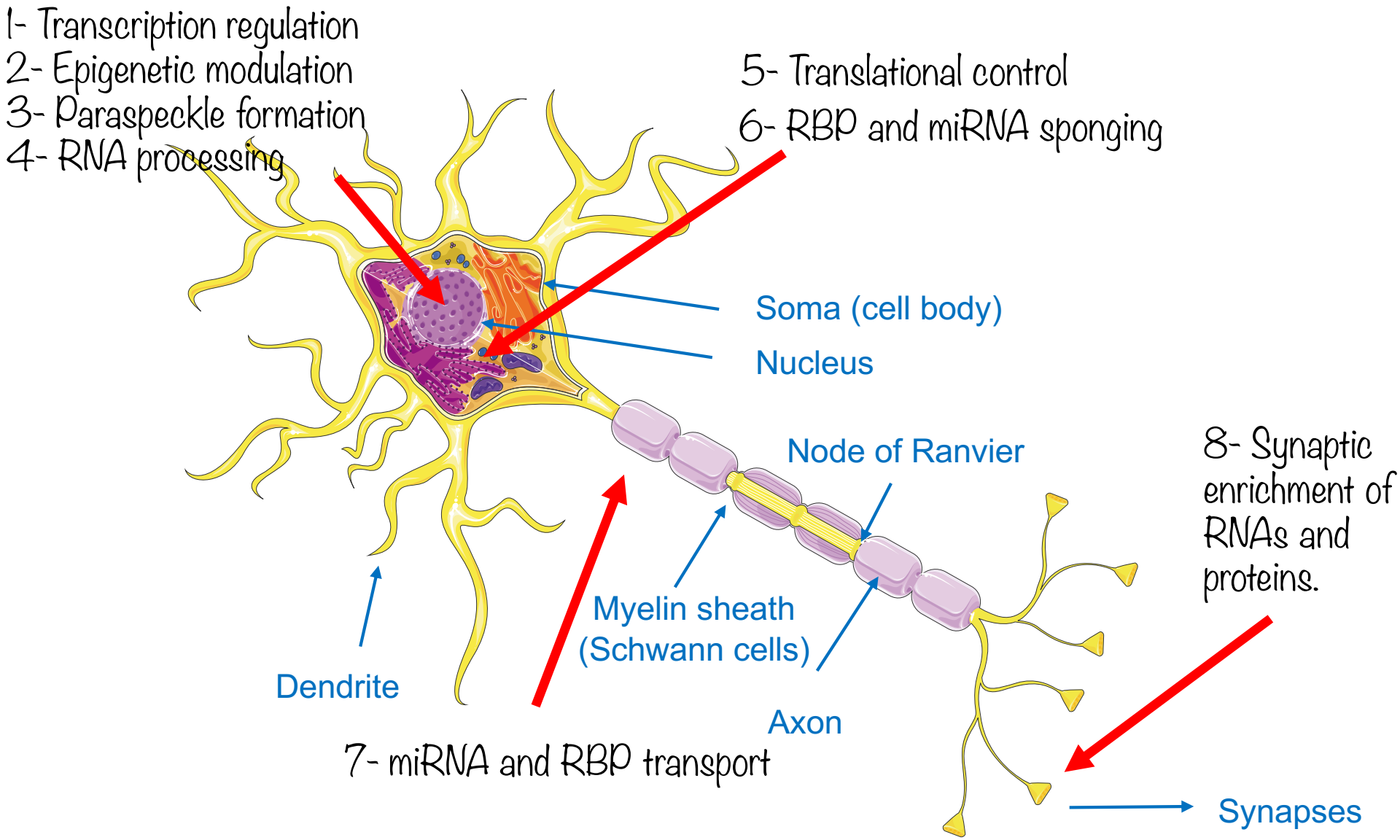
TABLE 2 Overview of the expression and proposed function of lncRNAs in motor neuron development

Name	ncRNA	Regulation	Observation	Mechanism	References
Meg3	lncRNA	Up-regulated; spatial regulation	Regulated throughout embryonic stem cells–motor neuron (ESC-MN) differentiation; enriched in the nucleus	Epigenetic regulation of *Hoxa4:Hoxc5* expression	Yen et al., (2018)
CAT7	lncRNA	Up-regulated	Regulated during early stages of human ESC-MN differentiation	Regulation of polycomb repressive complex 1 (PRC1) associated genes	Ray et al., (2016)
Hoxb5os	lncRNA	Up-regulated	Regulated throughout ESC-MN differentiation	Tbd	Rizvi et al., (2017)
Gm12688/Gm14204	lncRNA	Cell type-specific expression	Uniquely expressed in V1/V1 and V2b GABAergic interneurons	Tbd	Rizvi et al., (2017)
LncMN−1,−2,−3 and *Lhx1os*	lncRNA	Cell type-specific expression; up-regulated	Specifically enriched in MNs; regulated during differentiation of mouse ESC (mESC)/ human-induced pluripotent stem cells (hiPSC)-derived MNs	Tbd	Biscarini et al., (2018)
Lncrps25	lncRNA	Down-regulated	Knockdown reduces swimming activity because of defects in primary MNs	Via *olig2* (Tbd)	Gao et al., (2020)
Malat1, *Meg3*, *Rmst*, *Xist* and *Miat*	lncRNA	Spatial distribution	Specifically enriched in somatodendritic/axonal fractions	Tbd	Briese et al., (2016)
c−1, *c−2*, *c−13*, *c−16*, *c−48*, *c−80*, *c−82*, *c−84*, *c−88*	circRNA	Up-regulated	Regulated during mESC/hiPSC-derived MN differentiation	Tbd	Errichelli et al., (2017)
Human circSMN	circRNA	Multiple isoforms produced	Primate specificity of SMN-derived circRNAs	Tbd	Ottesen et al., (2019)

Abbreviations: hiPSC, human-induced pluripotent stem cells; Meg3, maternally expressed gene 3; mESC, mouse embroynic stem cells; Tbd, to be determined.

Vangoor et al. 2020

LncRNAs have been implicated in a wide range of functions in developing MNs



Adapted from Vangoor et al. 2020; Service medical art

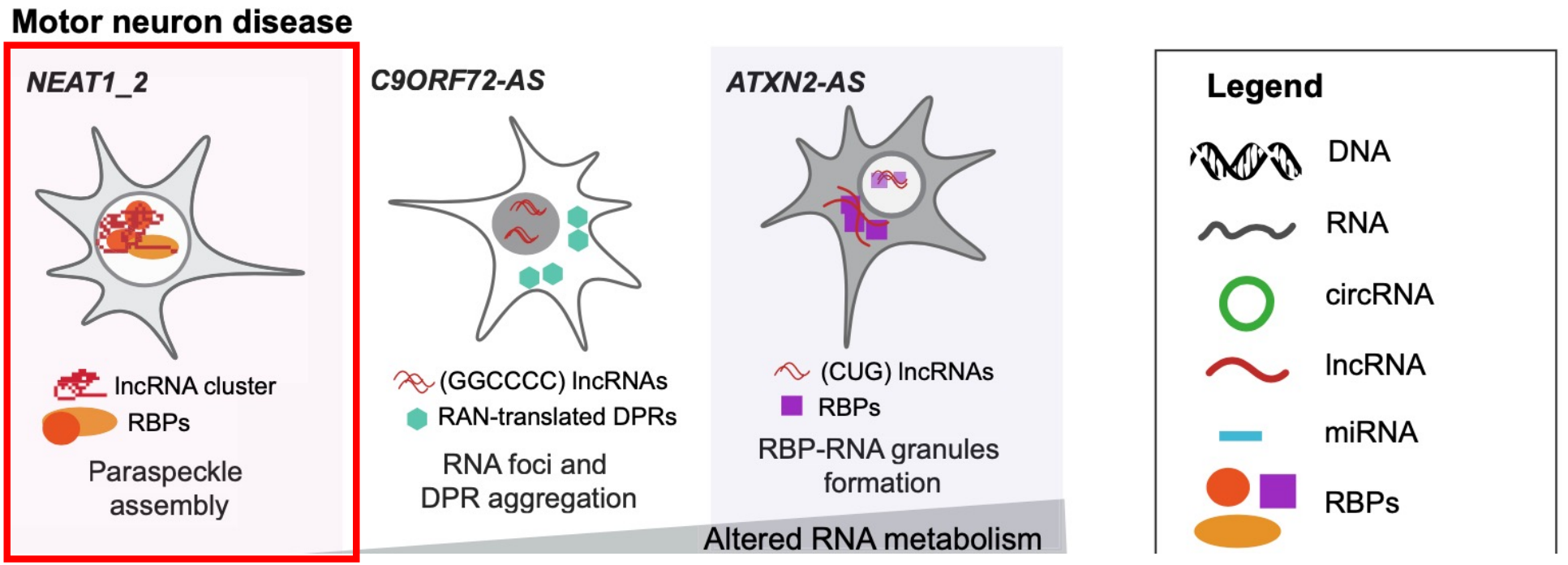
lncRNAs have been linked to MN disease

TABLE 4 Overview of the expression and proposed functions of lncRNAs in motor neuron disease

Name	ncRNA	Disease	Regulation	Function	References
NEAT1	lncRNA	ALS	Up-regulated at early stage	Regulates paraspeckle formation, increased *NEAT1* expression leads to neurotoxicity	Clemson et al., (2009); Nishimoto et al., (2013) and Suzuki et al., (2019)
C9ORF72-AS	antisense RNA	ALS	Up-regulated	Forms RNA foci that recruit RBPs, DPR protein formation via repeat-associated non-ATG-initiated (RAN) translation leading to neurotoxicity	Cheng et al., (2019); Mizielinska et al., (2014); Mori, et al. (2013); Sareen et al., (2013); Swinnen et al., (2018) and Wen et al., (2014)
ATXN2-AS	antisense RNA	ALS	Up-regulated	Repeat expansion RNA induces neurotoxicity	Li, Sun, et al. (2016)
SMN-AS	antisense RNA	SMA	Up-regulated	Recruits polycomb repressive complex 2 (PRC2) complex to the *SMN* gene to suppress SMN expression	d'Ydewalle et al., (2017) and Woo et al., (2017)
ZEB1-AS, *ZBTB11-AS*	antisense RNA	ALS	Up-regulated in blood samples (peripheral blood mononuclear cells [PBMCs])	Tbd	Gagliardi, et al. (2018)
UBXN7-AS, *ATG10-AS*, *ADORA2A-AS*	antisense RNA	ALS	Up-regulated in blood samples (PBMCs)	Tbd	Gagliardi, et al. (2018)
hsa_circ_0001173, *hsa_circ_0043138*, *hsa_circ_0088036*	circRNA	ALS	Up-regulated in blood samples (PBMCs)	Biomarker potential	Dolinar et al., (2019)

Abbreviations: ATXN2, Ataxin-2; NEAT1, nuclear-enriched abundant transcript 1; SMN, survival motor neuron; Tbd, to be determined.

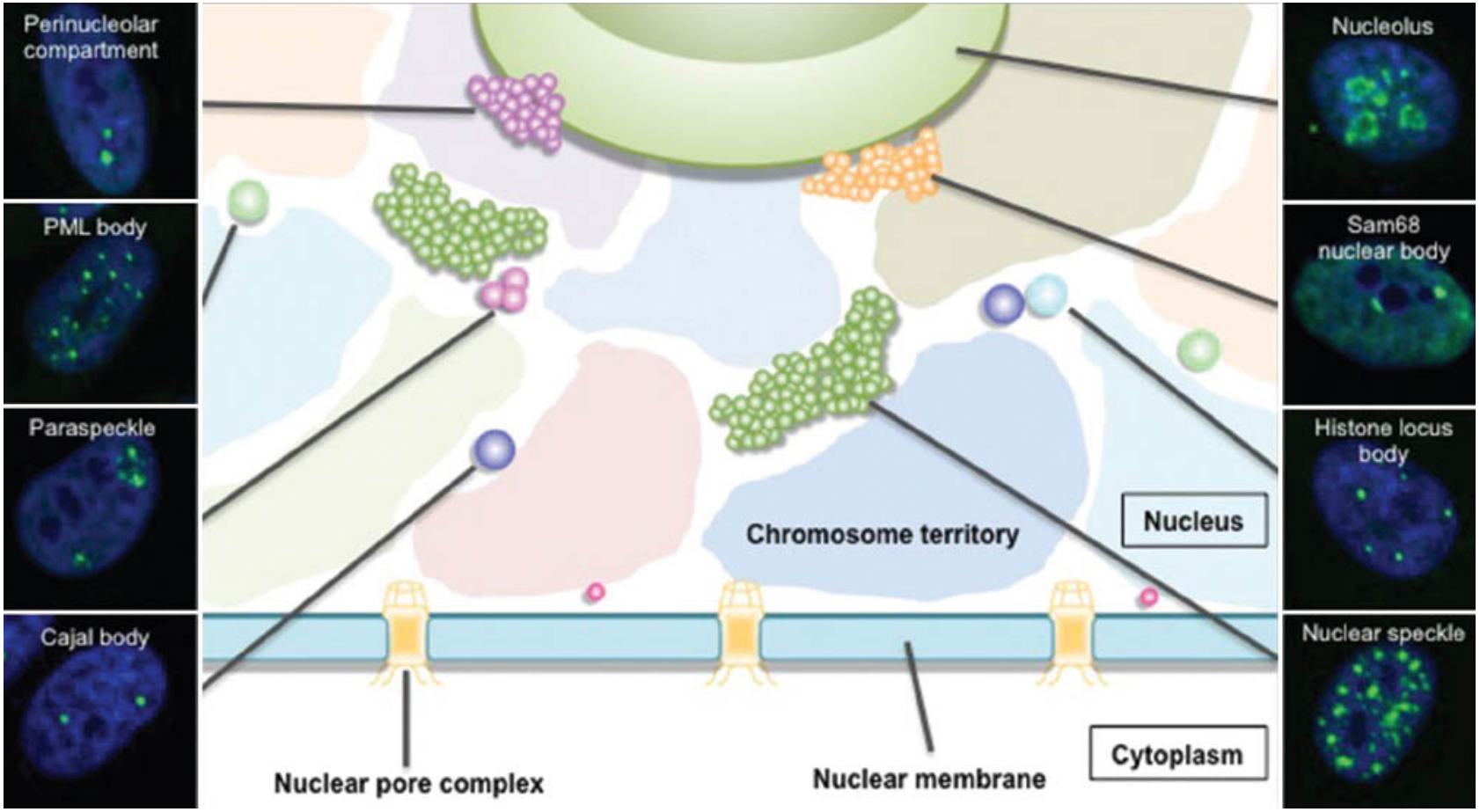
lncRNAs have been linked to MN disease: three examples



Given the prominent role for defects in RNA biology in ALS, it is not surprising that lncRNAs also contribute to the development of ALS and other MNDs.

Adapted from Vangoor et al. 2020; Service medical art

Nuclear bodies (NBs)



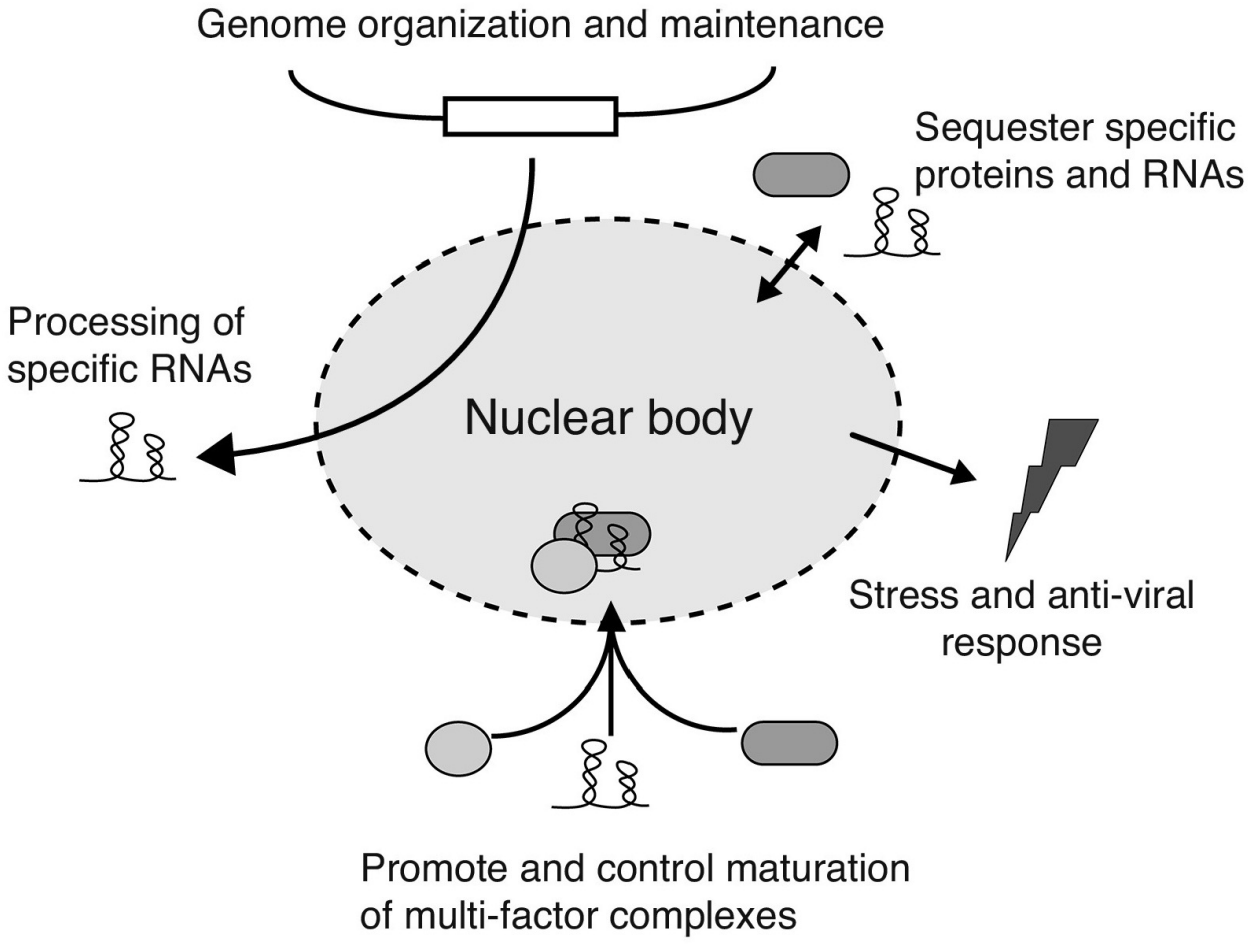
Yamazaki&Hirose, Frontiers in Bioscience, 2015

NBs are non-membrane bound structures in the nucleoplasm that fulfill the following requirements:

1 - they are microscopically visible (at least during some periods of the cell cycle);
2- they concentrate specific nuclear factors, namely proteins and RNAs
3- they constantly exchange their components with the surrounding nucleoplasm.

Different functions of nuclear bodies

Simply forcing a local high concentration of distinct scaffolding proteins is sufficient to seed particular NBs



Current Opinion in Cell Biology

Architectural RNAs that seed NBs

Evolutionary conservation of motor neuron lncRNAs

Name	ncRNA	Reported species	References
Meg3, *Rian* and *Mirg*	lncRNA	All mammals	Ogata and Kagami, (2016) and Yen et al., (2018)
CAT7	lncRNA	All mammals	Ray et al., (2016)
Hoxb5os	lncRNA	Mouse and human	Papaioannou et al., (2019)
Gm12688/ Gm14204	lncRNA	Mouse, human (NA)	Rizvi et al., (2017)
LncMN−1,−2,−3 and *Lhx1os*	lncRNA	Mouse and human	Biscarini et al., (2018)
Lncrps25	lncRNA	*Danio rerio* and human	Gao et al., (2020) and Ulitsky et al., (2011)
Malat1	lncRNA	All mammals	Ulitsky et al., (2011)
Rmst	lncRNA	Birds and mammals	Chodroff et al., (2010)
Xist	lncRNA	All mammals	Johnsson et al., (2014)
Miat (Gomafu)	lncRNA	All mammals	Chodroff et al., (2010); Sone et al., (2007)
FUS-linked circRNAs	circRNA	Mouse and human	Errichelli et al., (2017)
SMN circRNAs	circRNA	Mouse and human	Ottesen et al., (2019)
NEAT1	lncRNA	Mouse and human	Clemson et al., (2009)
BDNFOS	lncRNA	Primates	Lipovich et al., (2012)
TFEBα	lncRNA	Human	Davis et al., (2003)
Myolinc	lncRNA	Mouse	Militello et al., (2018)
SATIII	lncRNA	*Drosophila melanogaster* and human	Chung et al., (2018)

Vangoor et al. 2020

Neat1

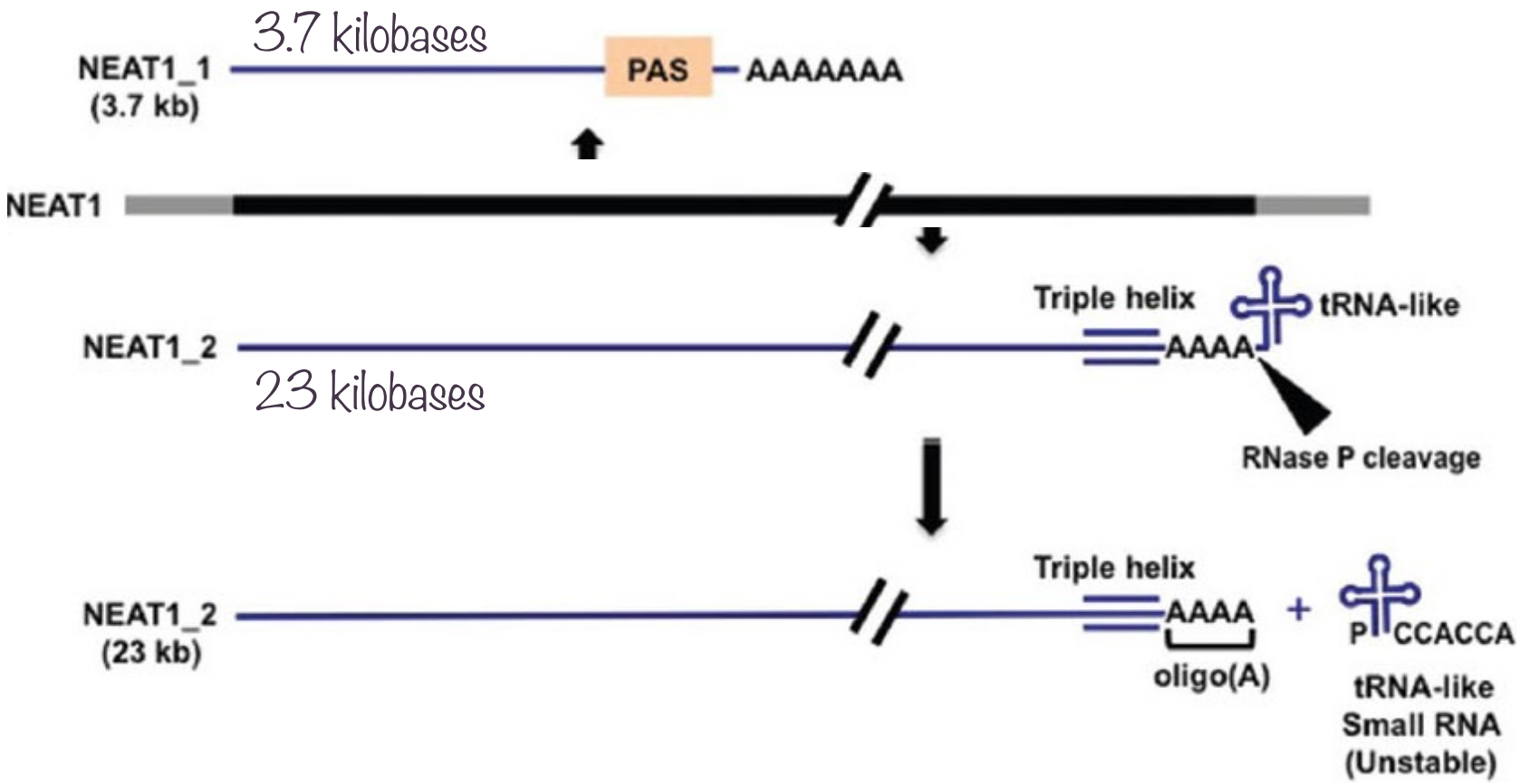
NEAT1 is one of the most abundant lncRNAs in the mammalian nucleus

Unlike other lncRNAs, which commonly lack sequence conservation, NEAT1 is relatively conserved across mammalian species, supporting its important biological function

NEAT1 overexpression, together with an increase in ParaSpeckles (PSs) density, has been found in ALS motor neurons, suggesting a direct contribution of NEAT1 in ALS disease by modulating the functions of ALS-associated RNA-binding proteins

Human structures of NEAT1_1 and NEAT1_2 long non-coding RNAs.

Nuclear paraspeckle assembly transcript 1



Two isoforms of NEAT1 are transcribed from the same locus. NEAT1_1 utilizes a canonical polyadenylation signal (PAS) for 3' end processing. In the case of 3' end processing of NEAT1_2, RNase P cleaves the 3' end of NEAT1_2 by recognizing a tRNA-like structure. NEAT1_2 possesses a genomically encoded oligo(A) sequence and a unique triple helical structure at the 3' end. Cleaved tRNA-like small RNA is unstable and rapidly degraded.

Yamazaki&Hirose, Frontiers in Bioscience, 2015

Neat1_2

NEAT1_2 but not NEAT1_1 is an essential component of paraspeckles

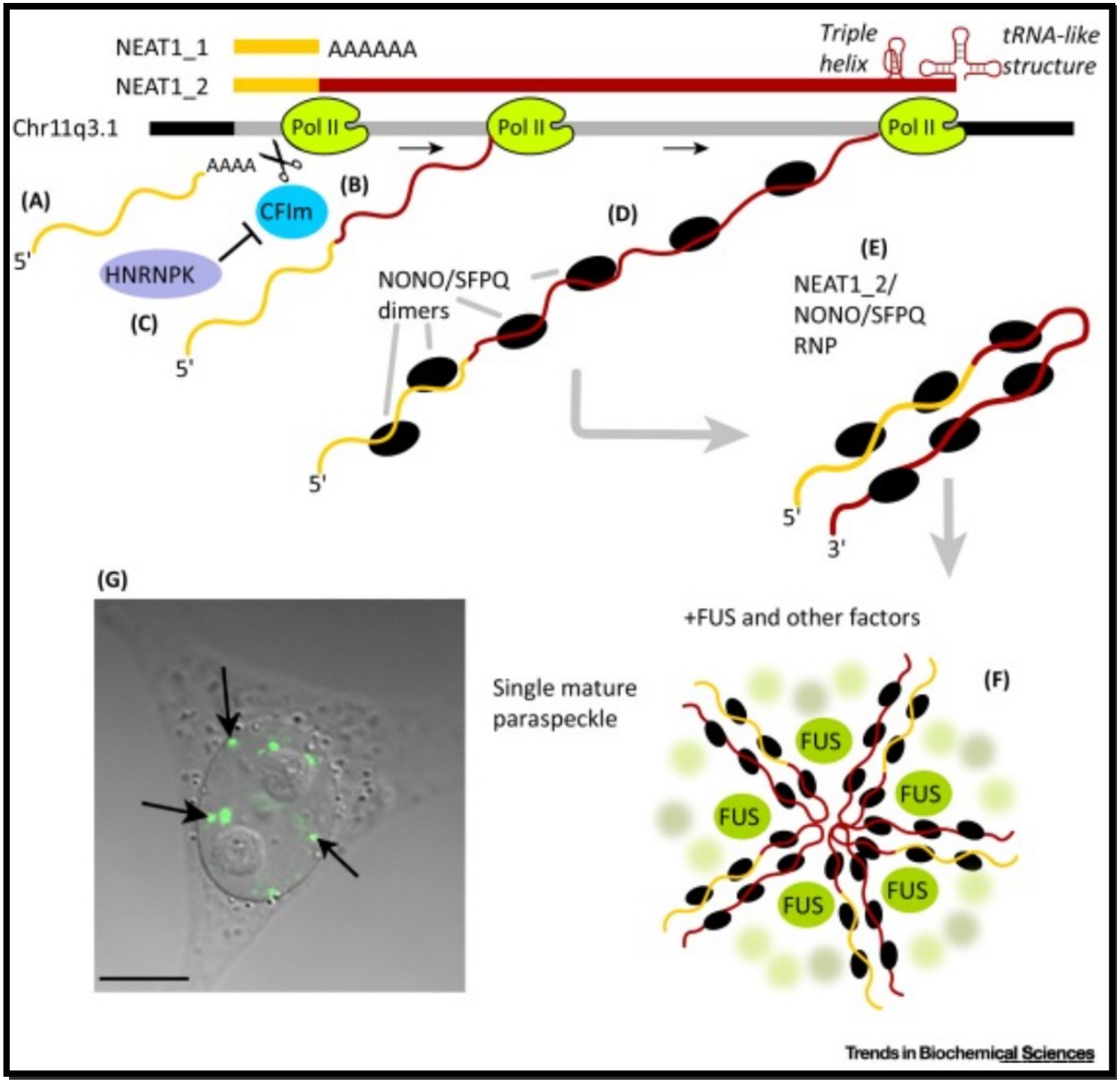
NEAT1_2 provides a scaffold for >60 protein components and likely multiple RNA components including NEAT1_1

It is believed that NEAT1_2/paraspeckles fulfil a number of functions independent of the presence of NEAT1_1. Among them:

i) regulation of translation via nuclear retention;

ii) regulation of transcription via sequestration of transcription factors, such as SFPQ

iii) modulation of pri-miRNA processing.


NEAT1_1
AAAAAA
NEAT1_2
Triple helix
tRNA-like structure
Chr11q3.1
Pol II
Pol II
Pol II
AAAA
(A)
5'
CFIm
HNRNPK
(B)
(C)
5'
(D)
NONO/SFPQ dimers
5'
(E)
NEAT1_2/ NONO/SFPQ RNP
5'
3'
(G)
+FUS and other factors
Single mature paraspeckle
(F)
FUS
FUS
FUS
FUS
FUS
Trends in Biochemical Sciences

PSs cellular functions

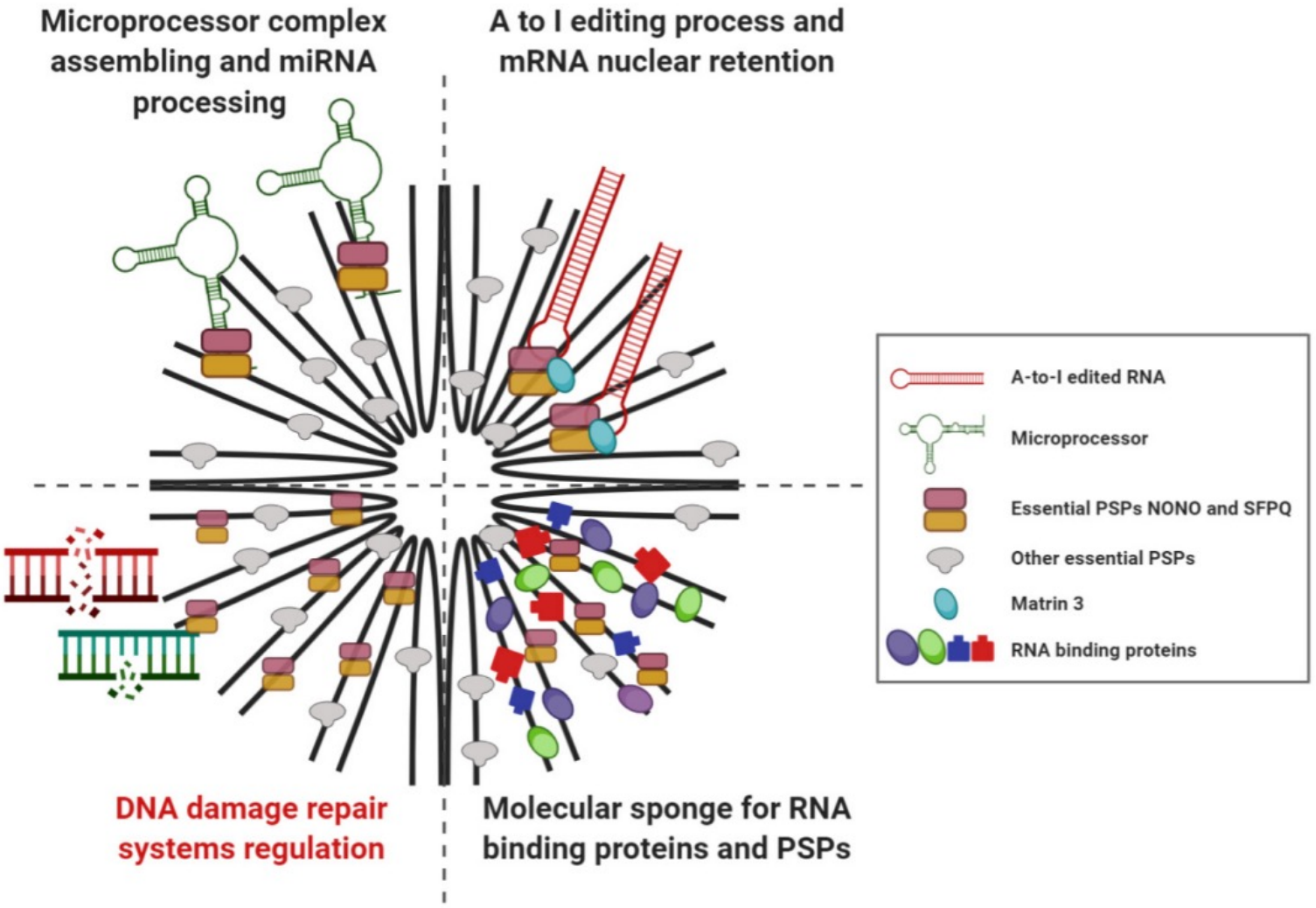
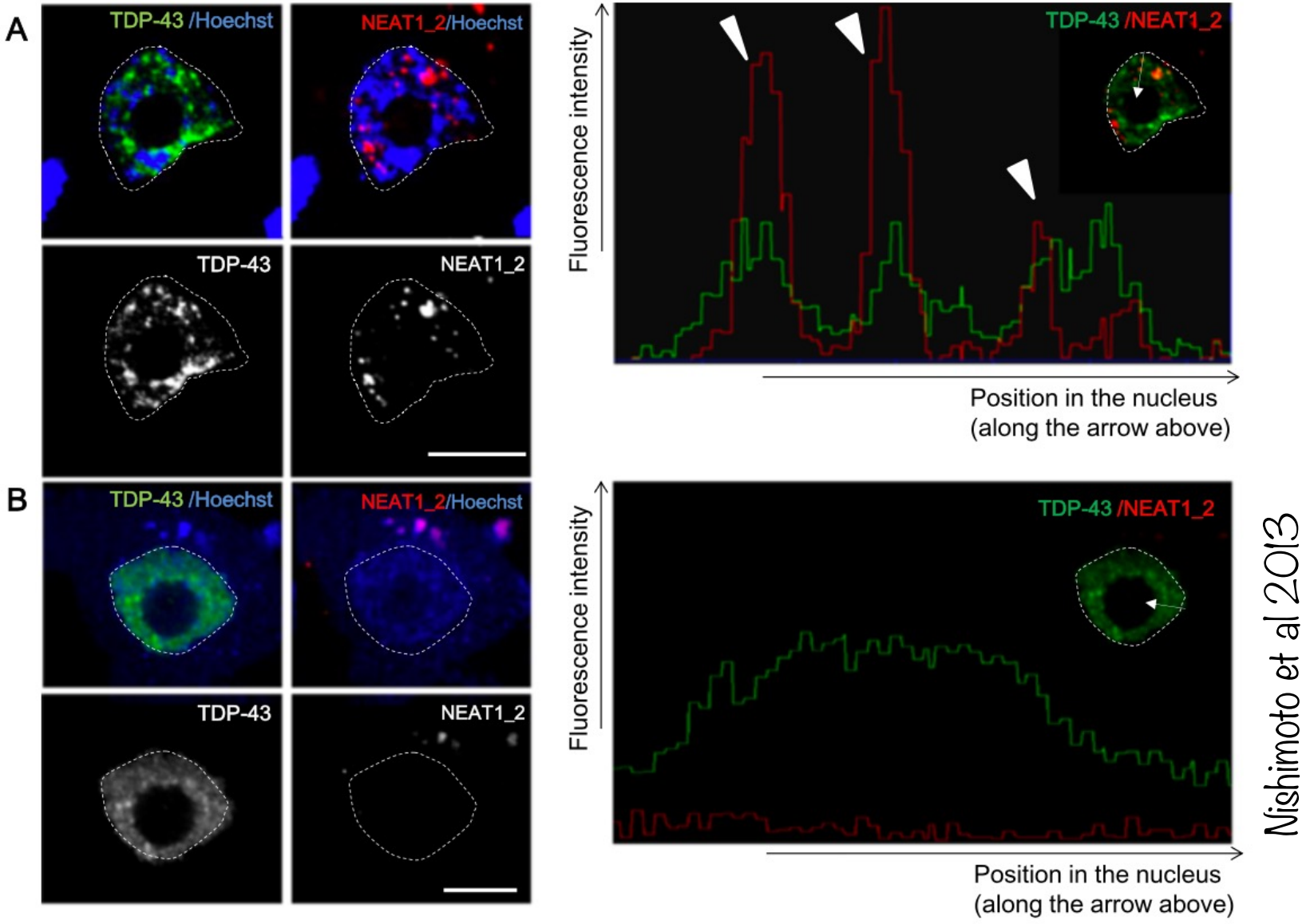


Figure 3. Graphical summary of PSs cellular functions. Upper, right panel: PSs as a gene expression regulator through the A to I editing process and the consequent nuclear retention of different mRNAs. Lower, right: PSs as molecular sponges for RNA binding proteins and PSPs. Upper, left: PSs as regulator of miRNA biogenesis by regulating the assembling of the microprocessor complex involved in processing of miRNA. Lower, left: PSs as possible structural scaffold for cellular DNA damage response systems.

Since NEAT1_1 is expressed in multiple cell types devoid of paraspeckles *in vivo*, including neurons, it is clear that it also has a variety of NEAT1_2/paraspeckle-independent functions.

Paraspeckles are stress-responsive nuclear bodies, in that NEAT1_2 upregulation and the increase in the size/number of paraspeckles accompany a number of physiological and pathological stressful conditions, such as differentiation and inhibited proteasome function

NEAT1 fine-tunes the function of multiple neurodegeneration-associated pathways , including critical ones, such as inflammation and neuronal apoptosis.



RNA-FISH using DIG-labeled NEAT1_2 probe indicates that NEAT1_2 lncRNA often appears in the nuclei of human motor neurons in sporadic ALS cases. Right panel shows the profile image of fluorescence intensity of NEAT1_2 lncRNA and TDP-43 along the arrow in the nucleus. Most NEAT1_2 signals overlapped with parts of aggregated TDP-43 in the nucleus (arrowheads). Dotted lines represent the outline of the nucleus. B. No NEAT1_2 expression is detected in most motor neurons in control cases. Dotted lines represent the outline of the nucleus.

Table 2 Pathological staging of motor neurons in ALS according to TDP-43 distribution

Stage 0	TDP-43 is normally distributed within the well-marginated nucleus.
Stage I	The nucleus degradated, and TDP-43 was also seen in the cytoplasm.
Stage II	The nuclear TDP-43 was so cleared that it was not recognized.The plasma membrane was still retained.
Stage III	The plasma membrane disappeared.

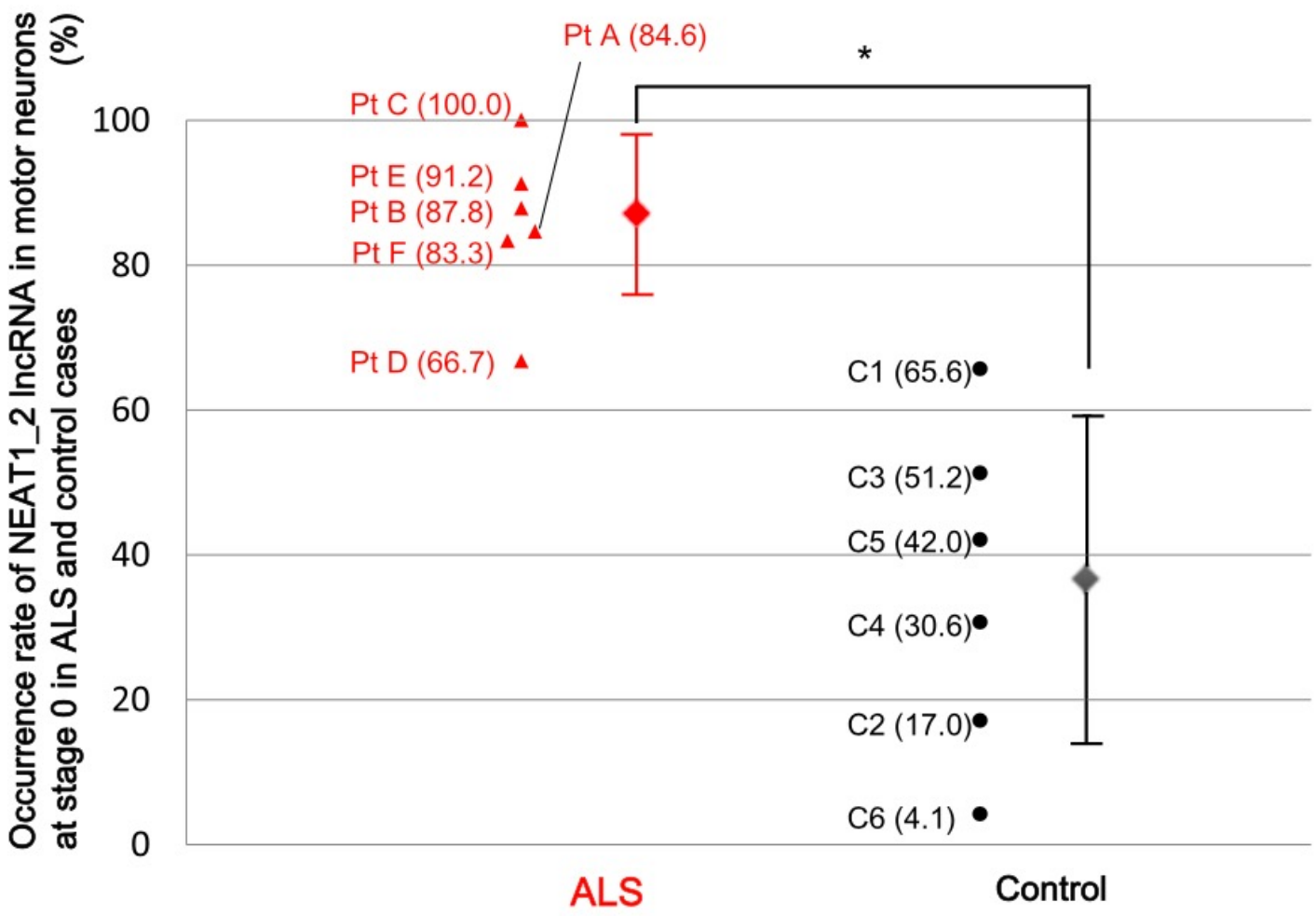


Table 1

NEAT1 expression in the CNS of patients and rodent models of neurodegenerative diseases.

Disease	NEAT levels (and how measured)	Where measured	Proposed role	Proposed mechanism(s)	References
Amyotrophic lateral sclerosis (ALS)	Up (qRT-PCR, in situ hybridisation). Confirmed NEAT1_2 upregulation.	Human: spinal motor neurons and glia Rodent: N/A	Protective	1 Compensatory increase in NEAT1 expression and paraspeckle assembly due to compromised function of TDP-43 in miRNA biogenesis. 2 Inflammatory response (activation of type I interferon signalling in TDP-43 depleted cells).	[30,109]

Protein components of paraspekles (NEAT1 interactors)

Table 2

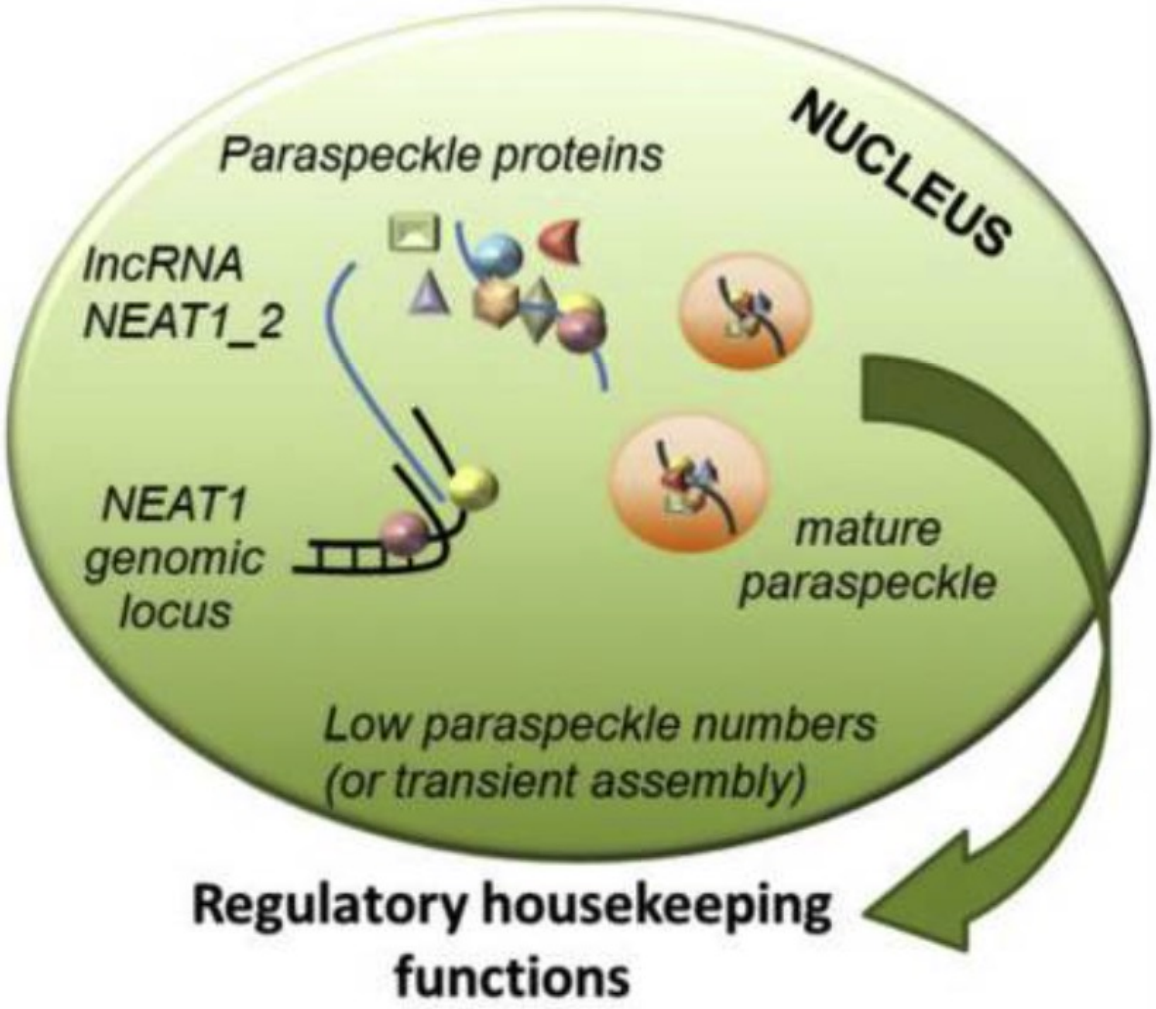
Protein components of paraspeckles (NEAT1 interactors) genetically linked to ALS/FTD.

Protein	Importance for paraspeckle assembly	Regulation of NEAT1_2 levels	Role in ALS	Role in other neurodegenerative diseases
FUS	Essential, >75% loss upon knockdown [37,45]	No or minimal	>50 mutations in fALS and sALS; FUS proteinopathy in these cases [143,144]	FTD (FTLD-FUS) [145]
TDP-43	Depletion enhances paraspeckle assembly [30]	Yes (more NEAT1_2 upon TDP-43 depletion)	>60 mutations in fALS and sALS; TDP-43 proteinopathy in cases with *TARDBP* and *C9ORF72* mutations and in 95% of all sALS cases [110,144,146]	FTD (FTD-TDP) [146]; AD [147]
TAF15	Important, 30–75% loss upon knockdown [37]	No	6 mutations in 6 unrelated sALS cases and 2 mutations – in 2 fALS cases [148,149]	FTD (FTD-FUS) [150]
EWS	Important, 30–75% loss upon knockdown [37]	Yes	2 mutations in 2 unrelated sALS cases [151]	FTD (FTD-FUS) [150]
hnRNPA1	Important, 30–75% loss upon knockdown [37]	No	2 mutations in fALS cases; 2 rare variants [152,153]	Multisystem proteinopathy (MSP) [152]
CREST[a]	ND	ND	4 mutations in 4 unrelated sALS cases [154,155]	N/A
MATR3	Depletion enhances paraspeckle assembly [156]	Yes (more NEAT1_2 upon MATR3 depletion)	~10 mutations in fALS and sALS cases [157,158]	Initially diagnosed myopathy with vocal cord paralysis, diagnosis changed to 'ALS' [159]
SFPQ	Essential, >75% loss upon knockdown [37]	Yes	2 mutations in 2 sALS cases [160]	N/A

[a]neurospecific, effect on paraspeckles in stable cell lines could not be tested.

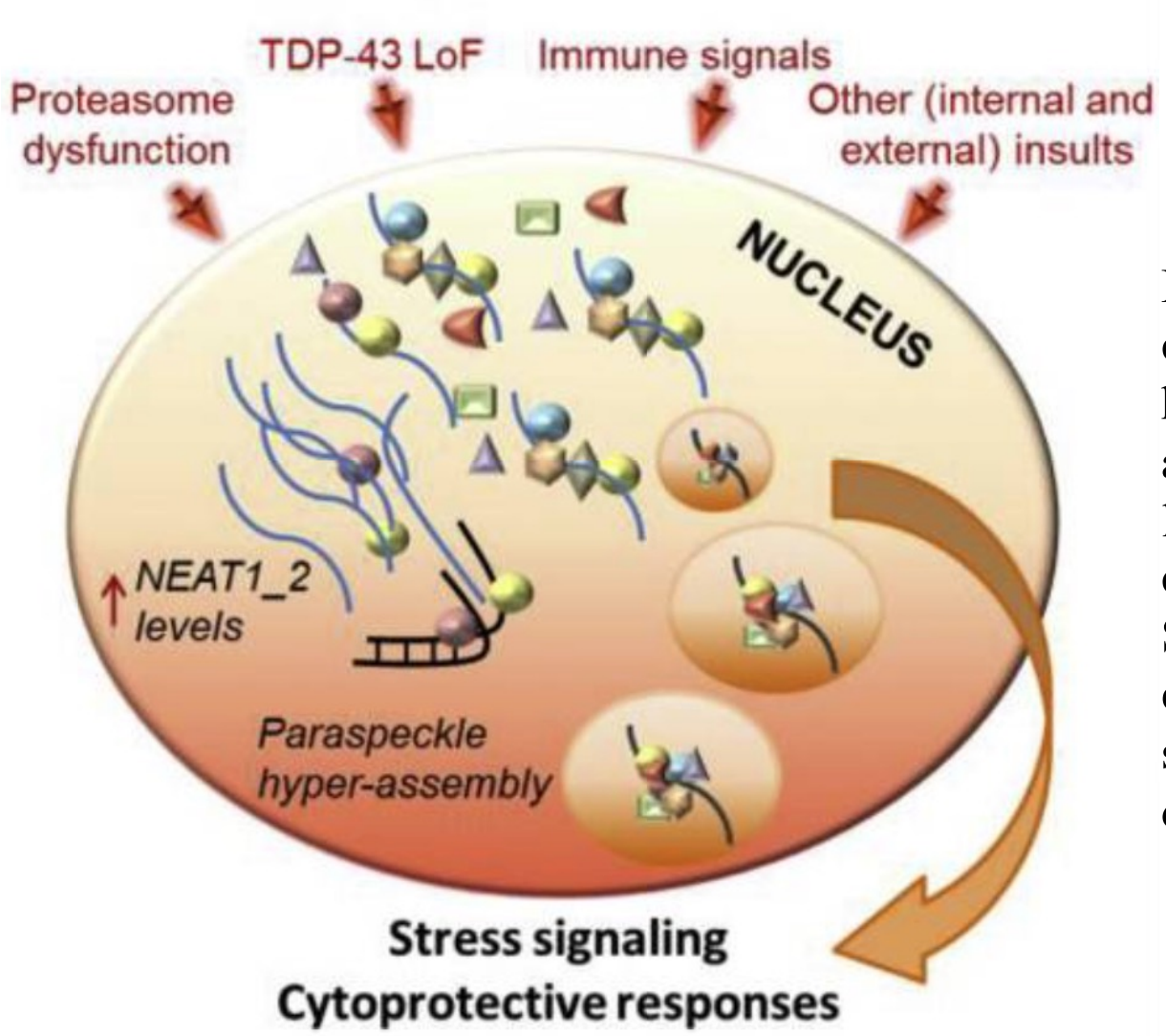
The RBPs TAR DNA-binding protein 43 (TDP-43) and FUS are normally located in the nucleus and influence RNA metabolism. In ALS MNs, an abnormal accumulation of these proteins is observed in the cytoplasm that is thought to contribute to MN degeneration because of effects on RNA processing and other RNA-related mechanisms (Blokhuis et al., 2013).

Normal



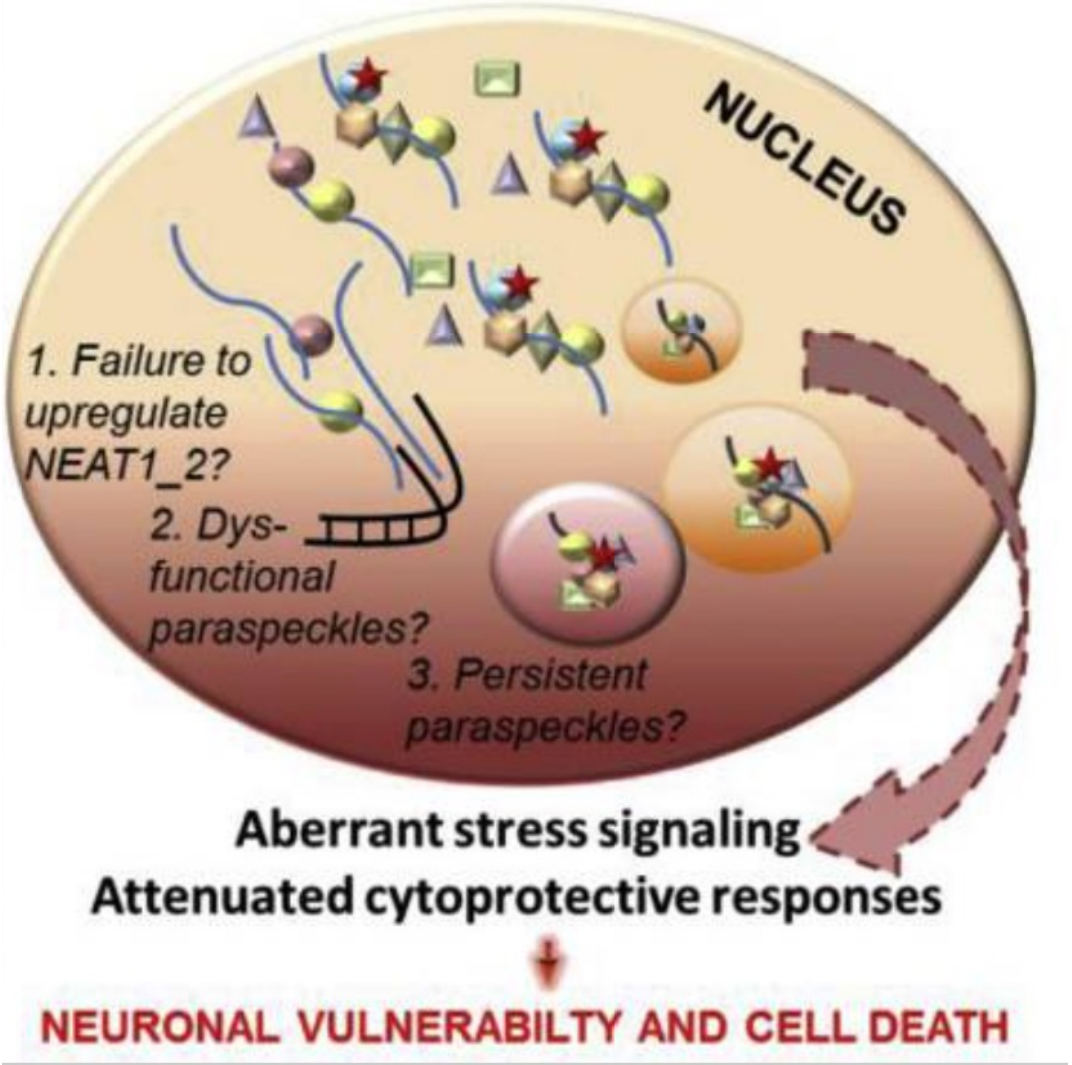
Under basal conditions, levels of NEAT1_2 in motor neurons are low and so are the paraspeckle numbers. Paraspeckle assembly might also be transient ("on demand").

Developing ALS



During development of pathological changes typical for ALS, paraspeckle hyper-assembly is triggered by internal and external insults, such as TDP-43 loss of function (LoF), proteostasis collapse and immune response. Subsequent signalling events would enable protective neuronal response to stress and delay neuronal degeneration.

Developing ALS when a paraspeckle protein is affected



These data support the idea that altered *NEAT1* expression in ALS leads to defects in paraspeckle formation causing cell death and neurodegeneration.

In ALS cases with an essential/important paraspeckle protein affected by a mutation, its mutant isoform might negatively impact on protective paraspeckle hyper-assembly. This can be realised through:

i) failure to upregulate NEAT1_2 (e.g. if proteins regulating NEAT1_2 levels, such as SFPQ and hnRNP K, are mutated or sequestered into abnormal inclusions/RNA foci);

ii) attenuated assembly of paraspeckles or assembly of dysfunctional paraspeckles (e.g. if a structural paraspeckle protein, such as FUS, is mutated);

iii) persistence of paraspeckles (e.g. if a mutation confers abnormal stability). Defective paraspeckle response may expedite the development of molecular pathology and accelerate disease onset and progression. A mutant protein is marked by a red star.

Contents lists available at ScienceDirect

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Characterization of the lncRNA transcriptome in mESC-derived motor neurons: Implications for FUS-ALS

Silvia Biscarini [a], Davide Capauto [a], Giovanna Peruzzi [a], Lei Lu [b], Alessio Colantoni [c], Tiziana Santini [a], Neil A. Shneider [b], Elisa Caffarelli [d], Pietro Laneve [a,*,1], Irene Bozzoni [a,c,d,e,**]

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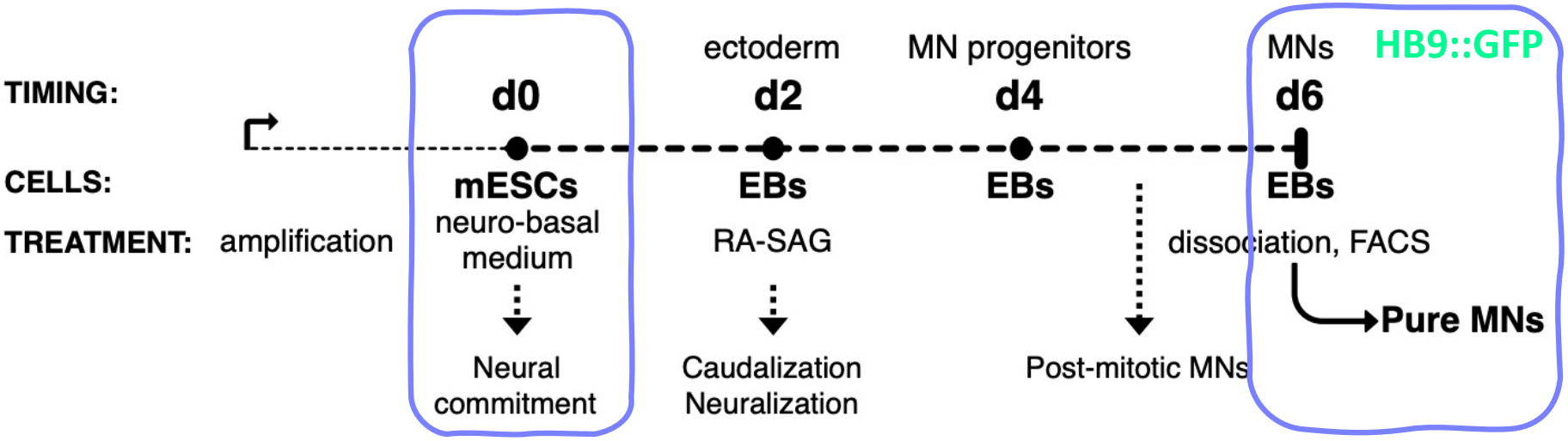
Als...

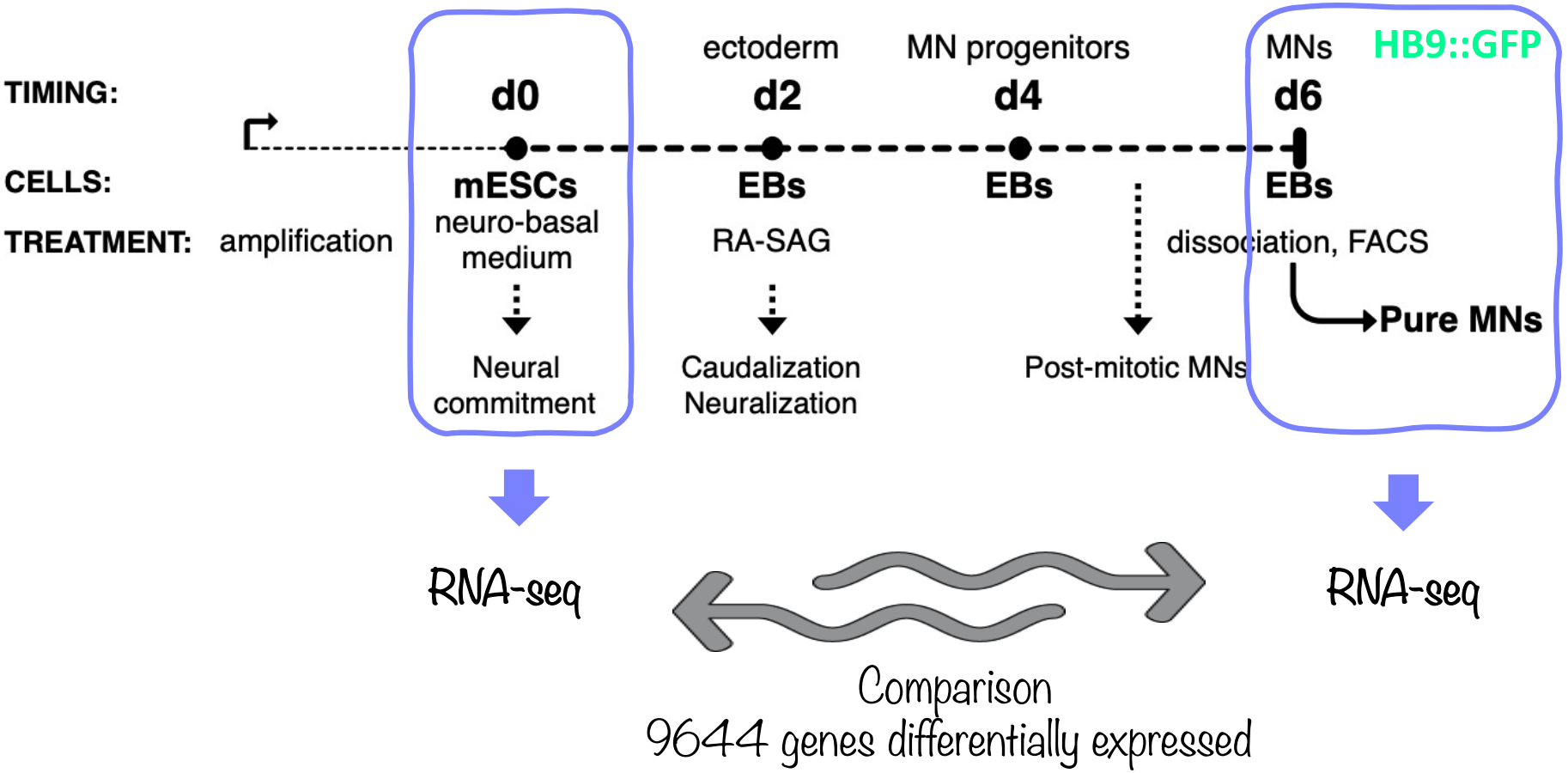
ALS is an incurable adult-onset neurodegenerative disease, which affects upper and lower motor neurons (MNs), and leads to paralysis and death in 3–5 years from diagnosis. Several genetic alterations are associated with ALS, including caus- ative mutations in FUS, TDP-43 and expansions in C9ORF72 point to the essential role of aberrant RNA metabolism in ALS pathogenesis

Starting from the the beginning...



Wichterle H. et al., 2002; Errichelli et al., 2017; Biscarini et al., 2018

Starting from the the beginning...

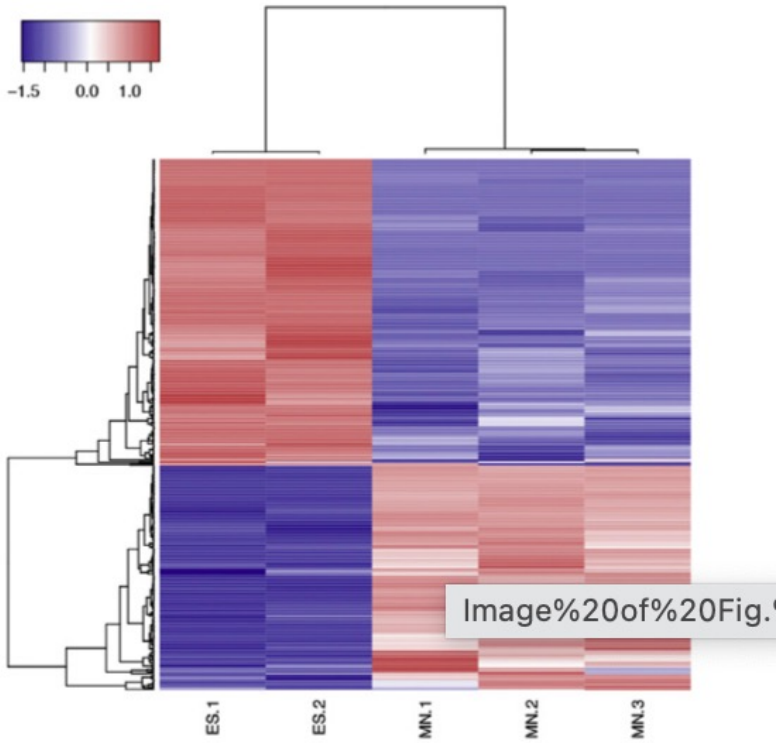


Errichelli et al., 2017; Biscarini et al., 2018

Starting from the the beginning...

RNA-seq ⟷ RNA-seq

Comparison
9644 genes differentially expressed

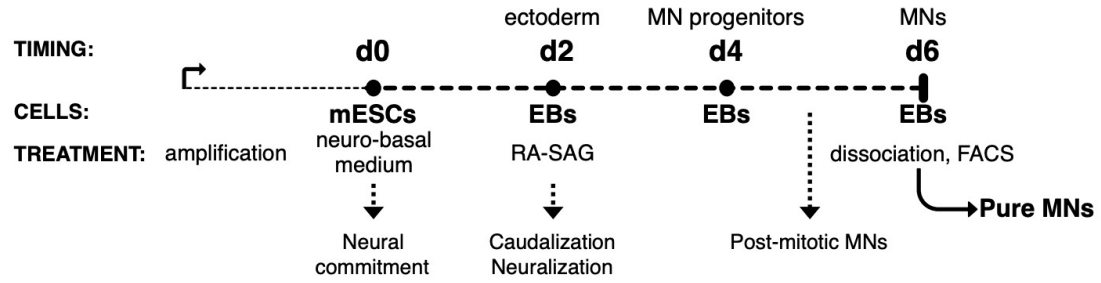


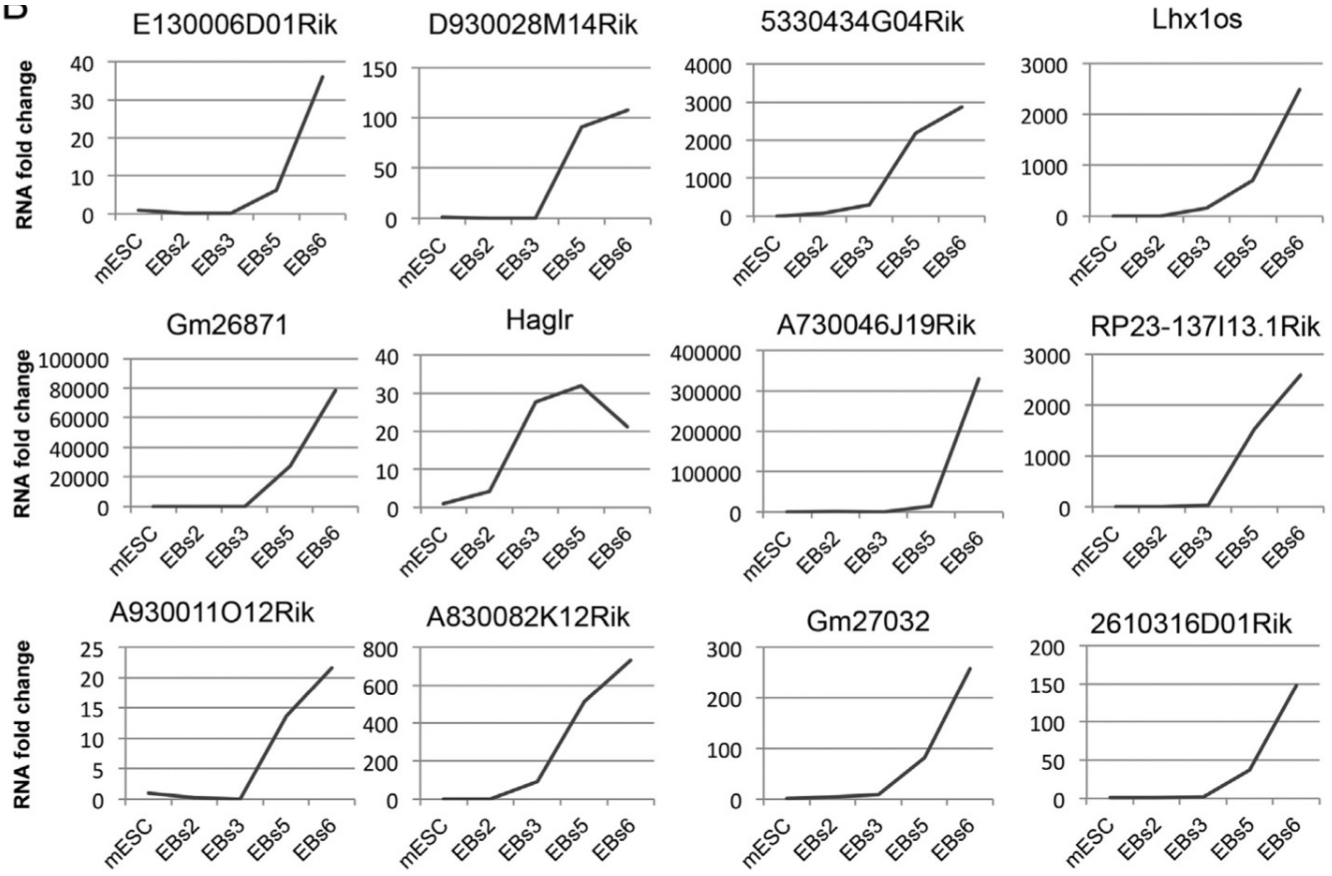
469 encoded for bona fide lncRNAs
The family of lncRNAs up-regulated in MNs derived from 270 loci and includes some species already known to play key roles in neurogenesis; among them, Miat, Rmst, Hotairm1, Meg3, Rian and Mirg.

lncRNAs involved in motor neural differentiation process (12).

Errichelli et al., 2017; Biscarini et al., 2018

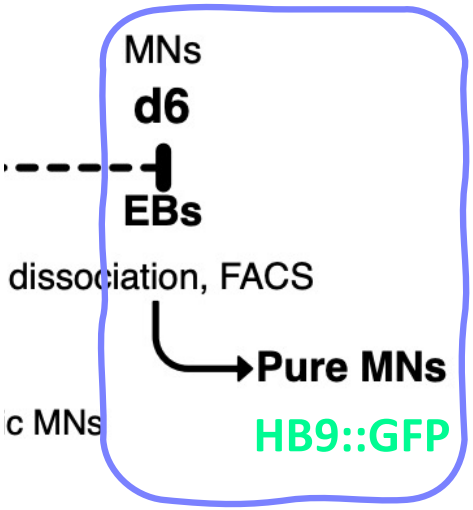
qRT-PCR analysis of the 12 lncRNAs up-regulated during MN differentiation

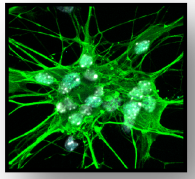




Biscarini et al., 2018

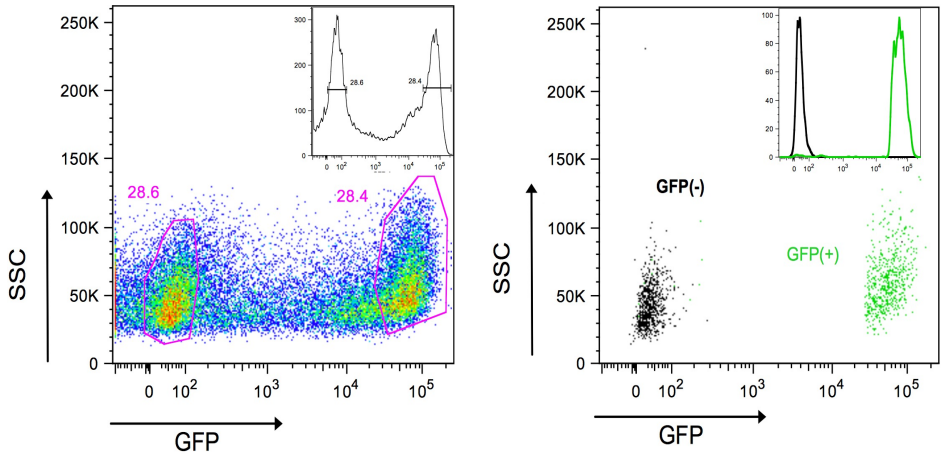
lncRNAs enriched in motor neurons

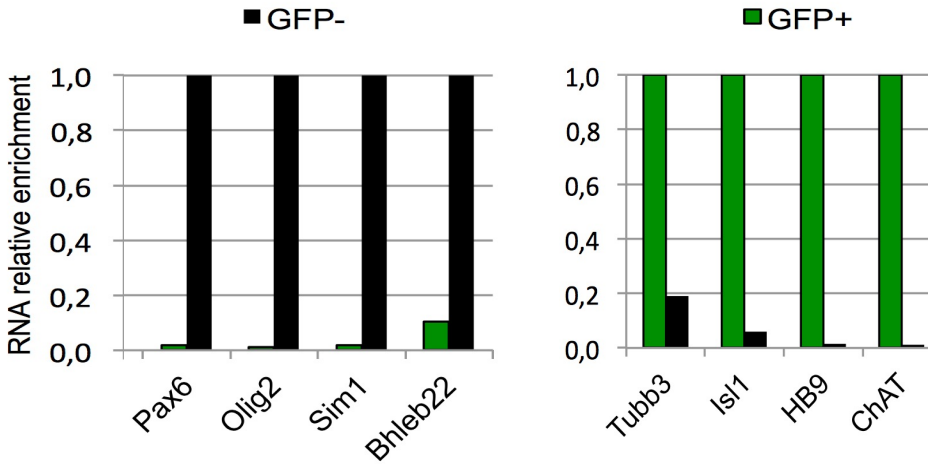




Pax6 and Olig2 transcription factors, responsible for establishing MN progenitors

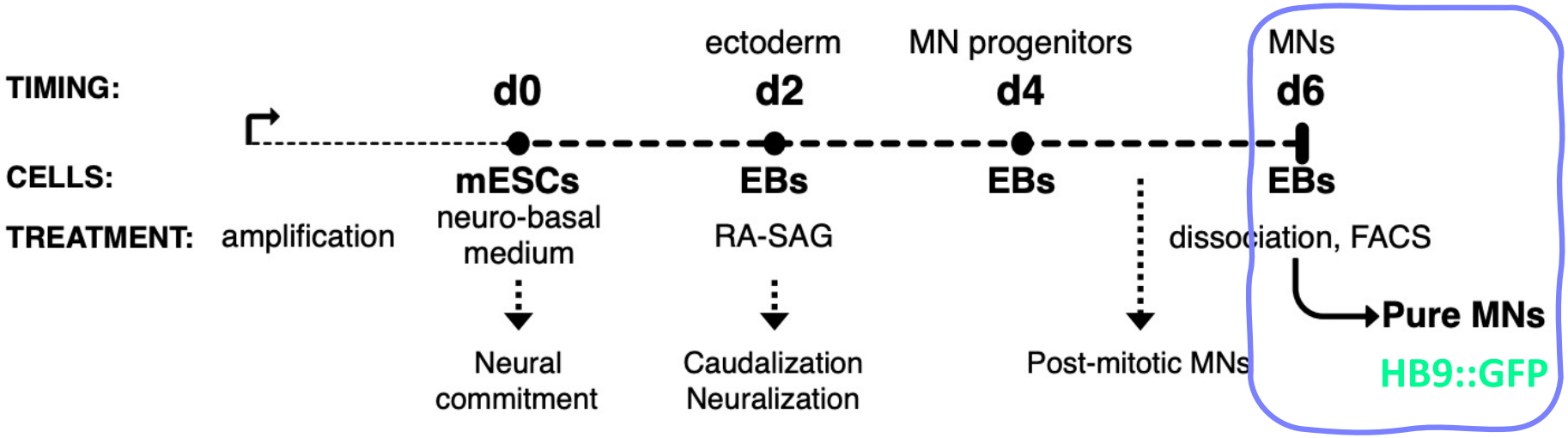
Genes required for consolidation of MN identity (Hb9) and for development (Islet-1) and function (ChAT) of spinal MNs were highly enriched in Hb9::GFP+ cells

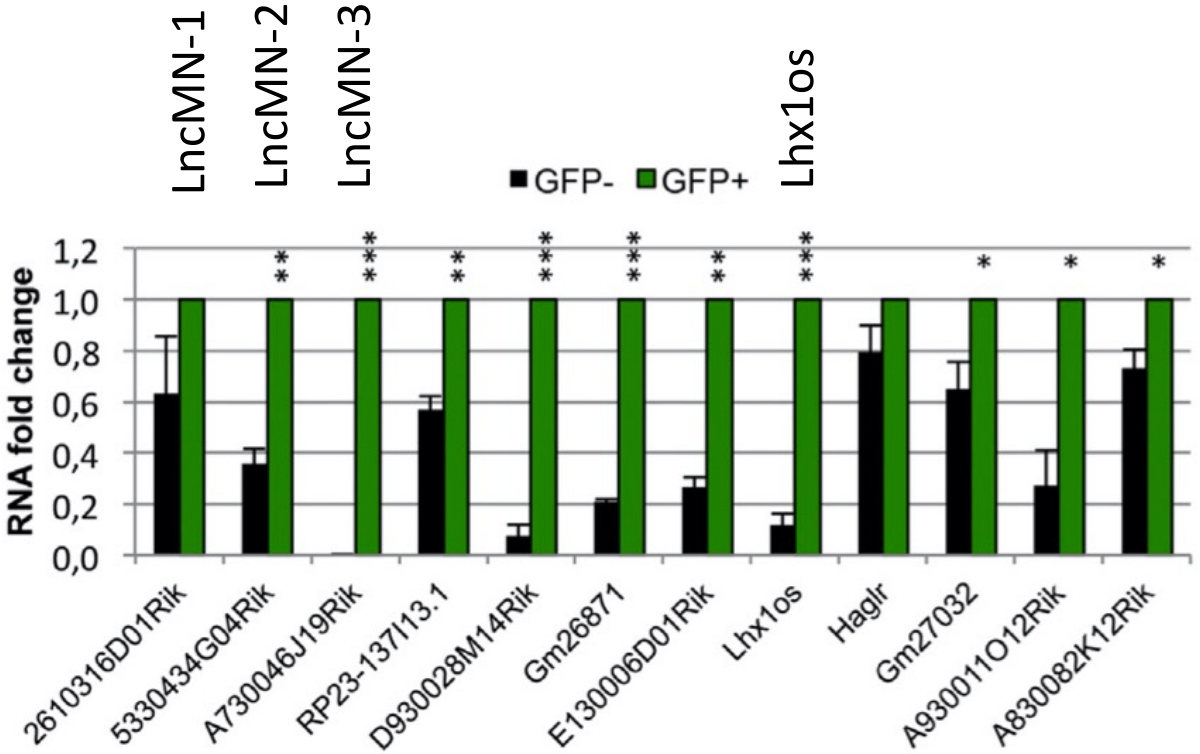


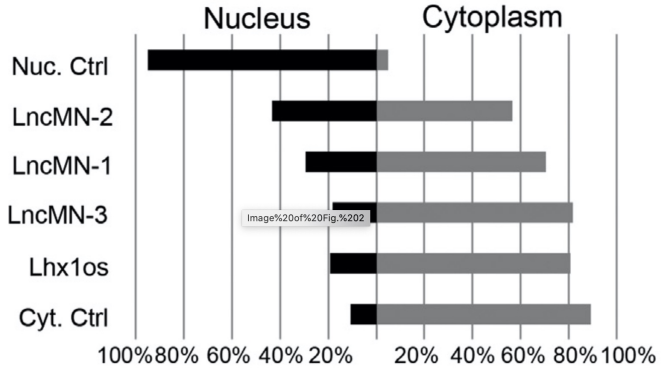


Biscarini S. et al., 2018

lncRNAs enriched in motor neurons

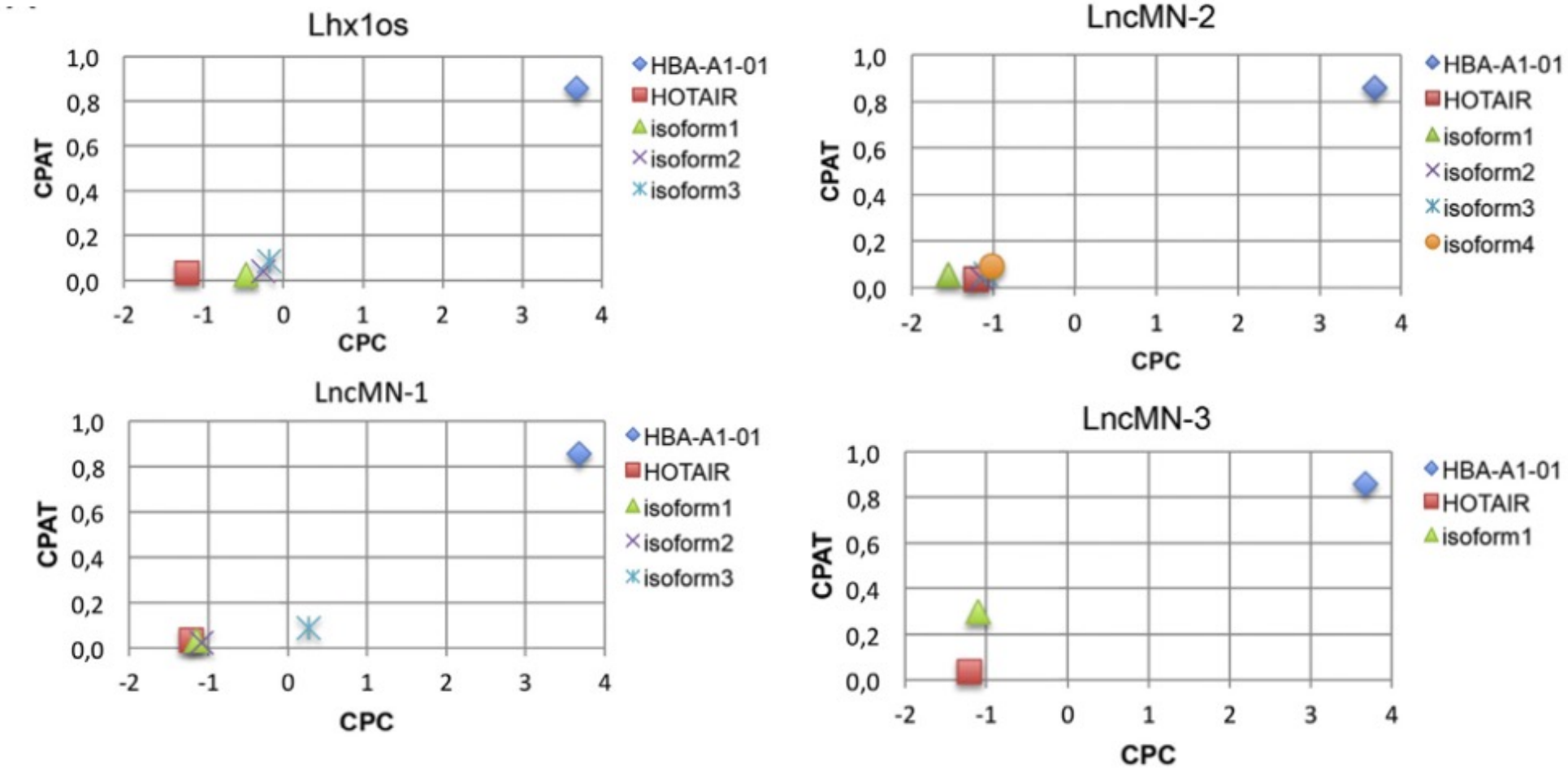






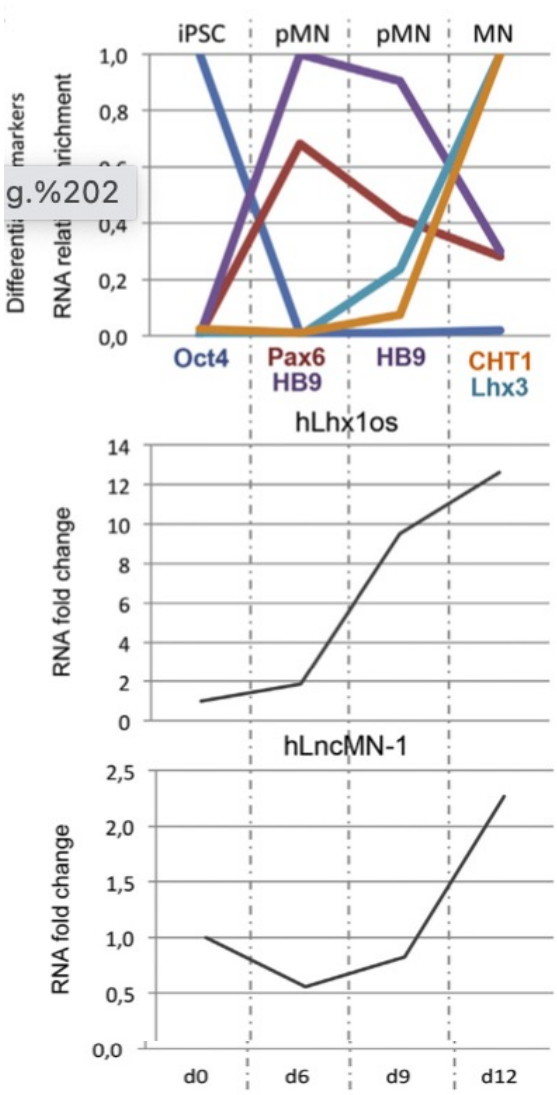
Biscarini et al., 2018

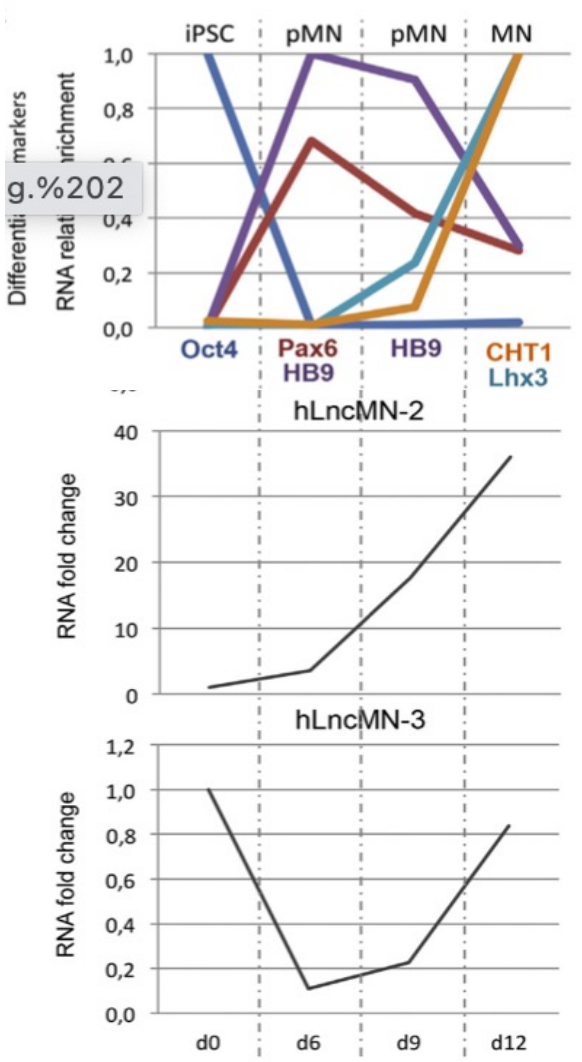
Codogeneity graph



Biscarini et al., 2018

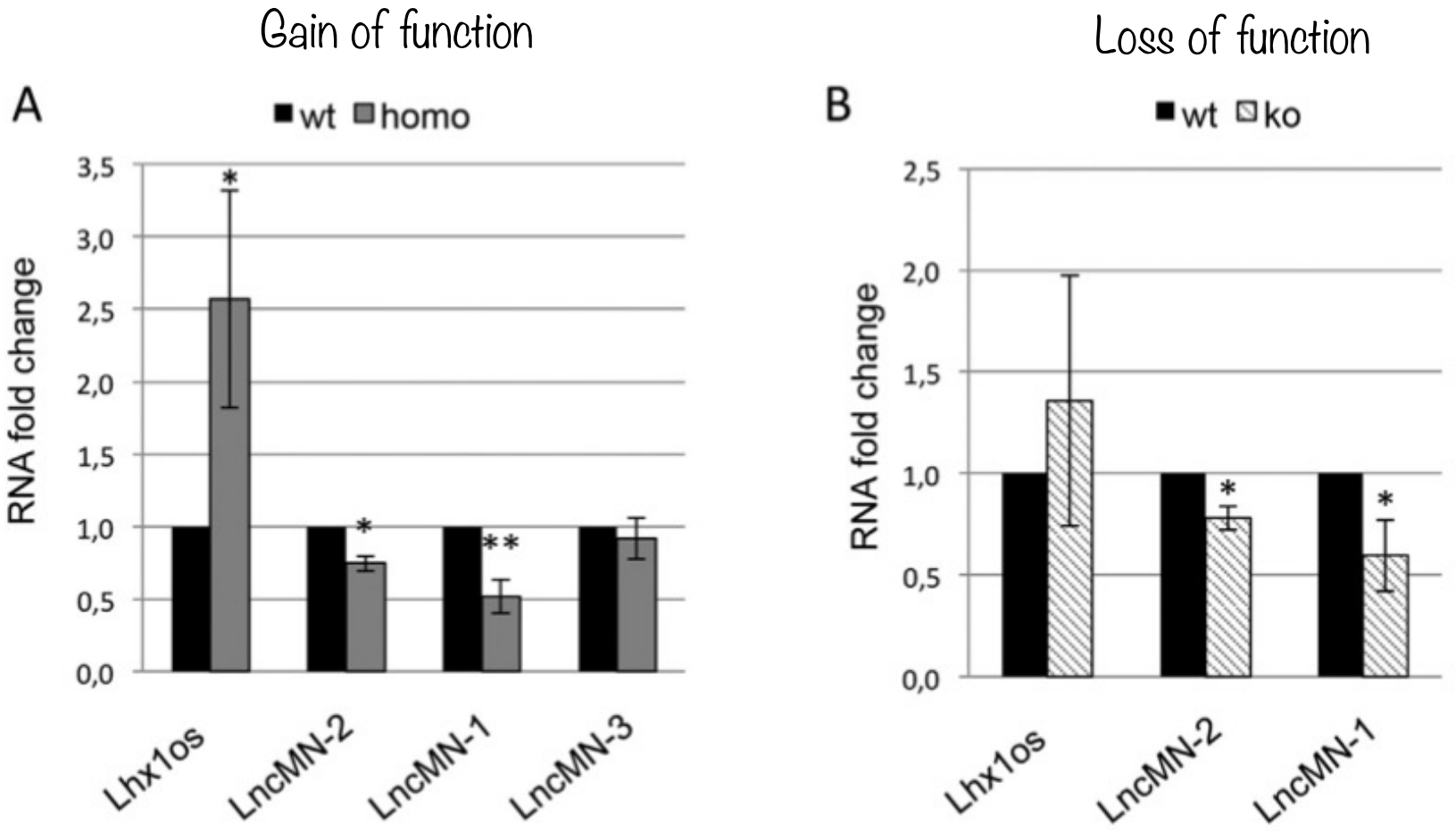
Expression profile of selected lncRNAs during human MN differentiation from iPSCs.





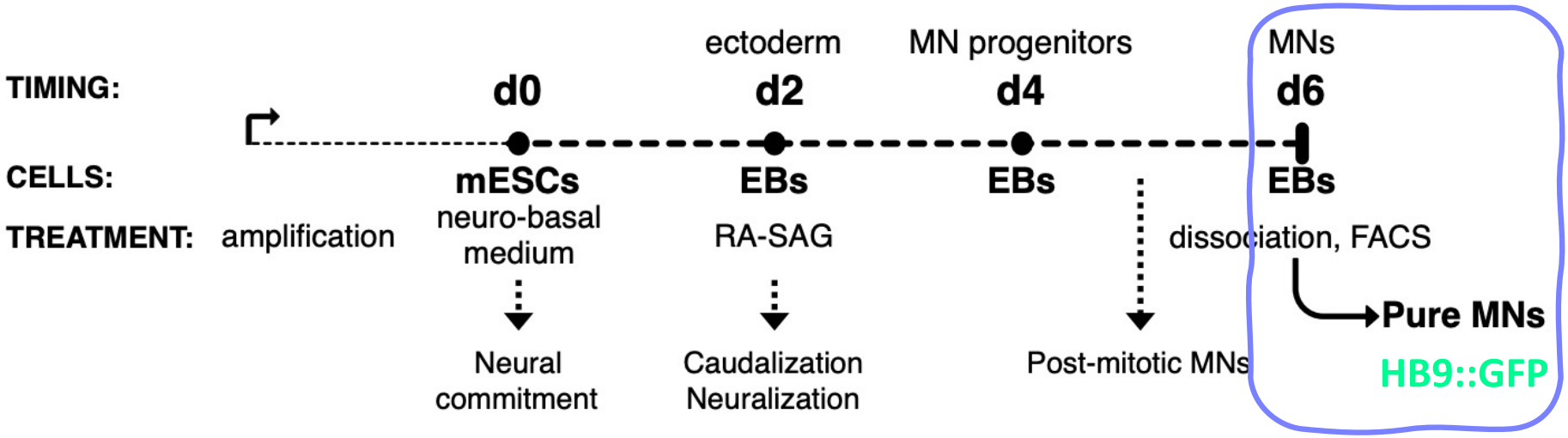
Biscarini et al., 2018

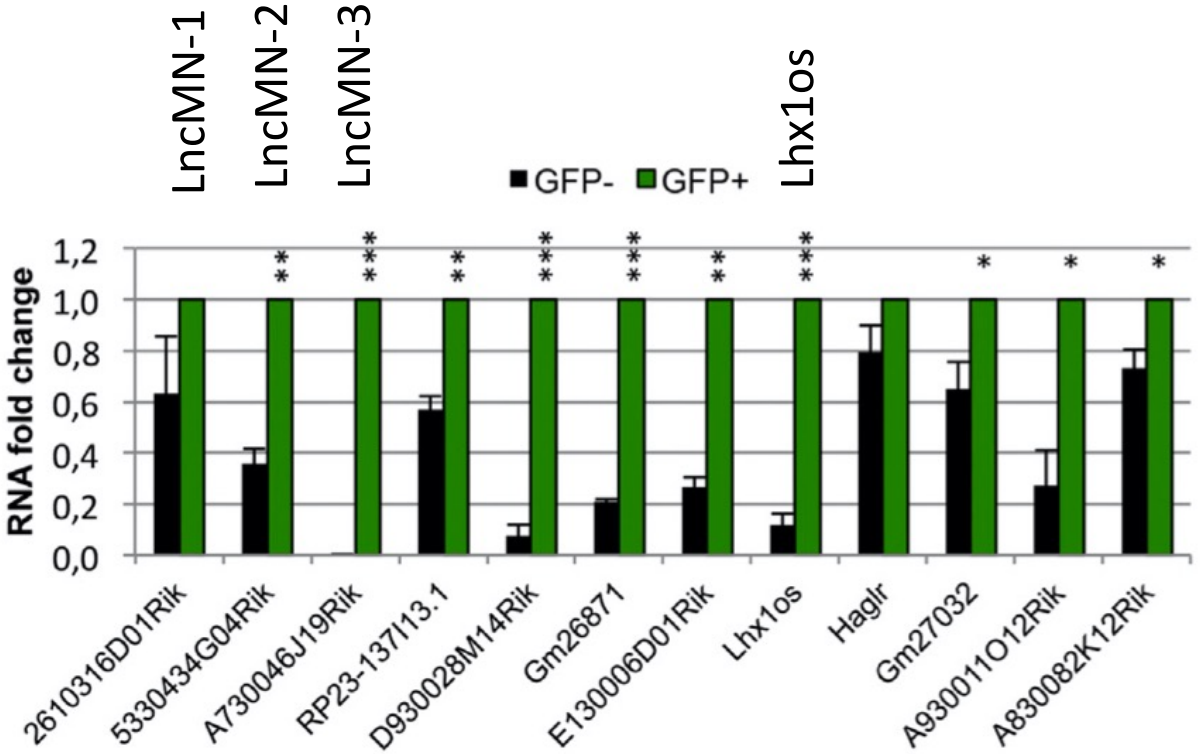
LncRNA expression in FUS-ALS MNs

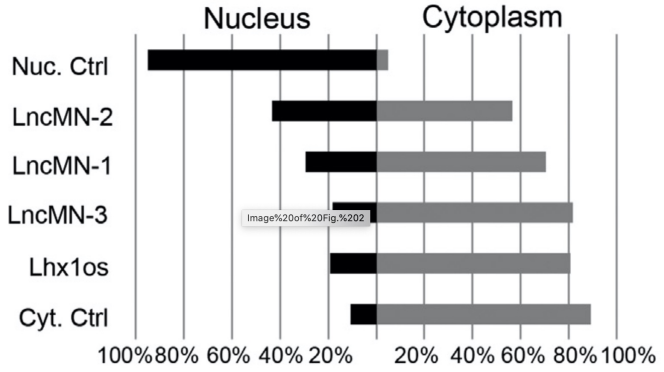


Fus mutant mouse MNs carrying the equivalent of one of the most severe human ALS-associated FUS alleles (P517L) MNs (homo, gray bars), relative to Fus+/+ MNs (wt, black bars). qRT-PCR analysis of specific lncRNAs in Fus−/− MNs (ko, striped bars), relative to Fus+/+ MNs (wt, black bars).

lncRNAs enriched in motor neurons





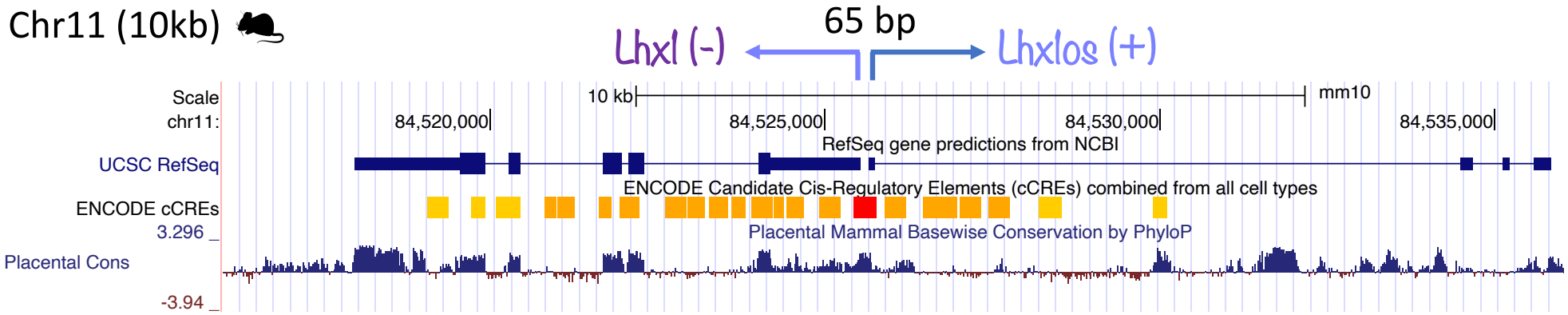


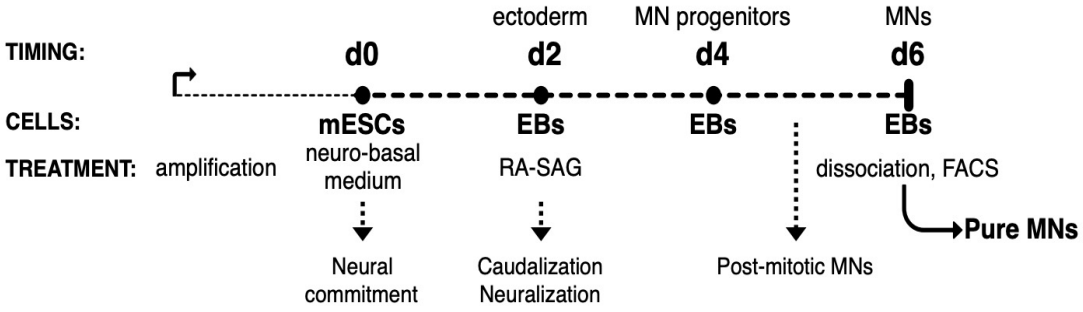
Biscarini et al., 2018

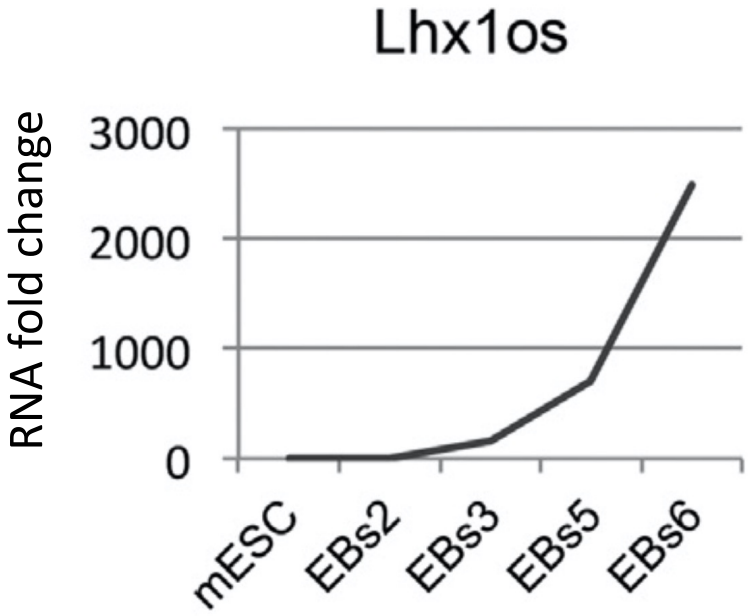
Lhx1os genomic locus

Color	UCSC label	ENCODE classification
red	prom	promoter-like signature
orange	enhP	proximal enhancer-like signature
yellow	enhD	distal enhancer-like signature
pink	K4m3	DNase-H3K4me3
blue	CTCF	CTCF-only

Chr11 (10kb)

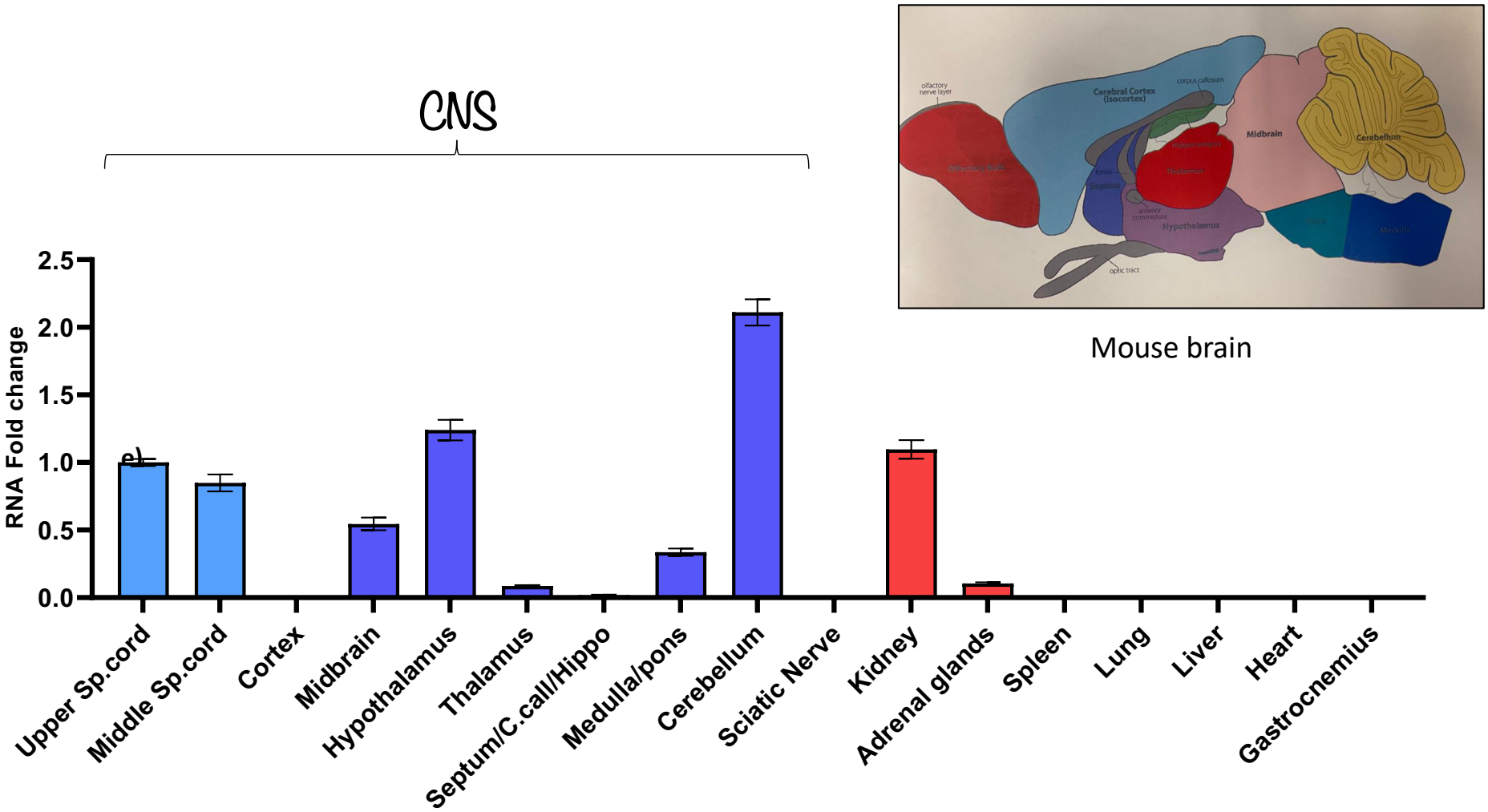






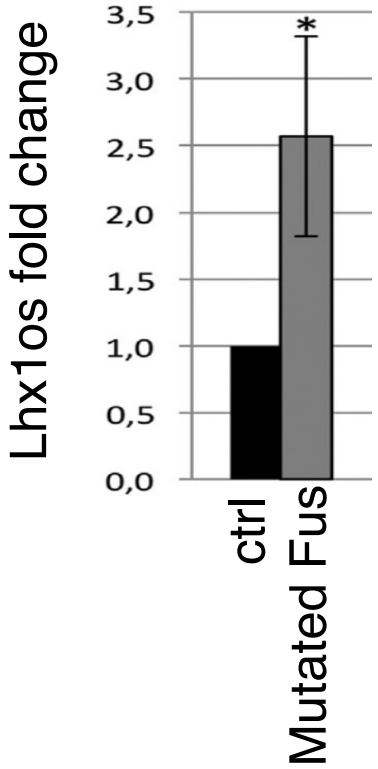
Errichelli et al., 2017; Biscarini et al., 2018

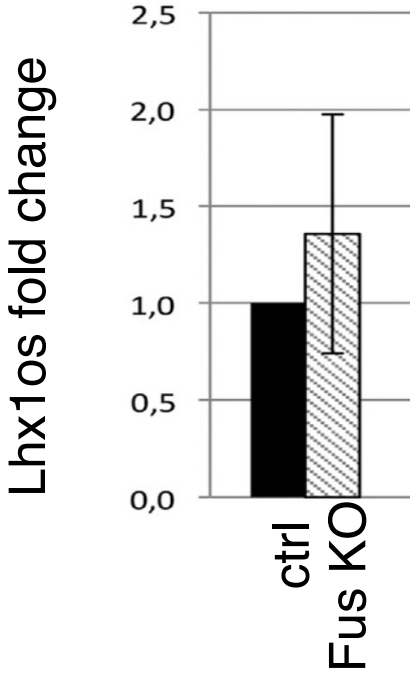
Lhx1os expression in mouse tissues



Mouse brain

Lhx1os is up-regulated in *in vitro* derived MNs from mESCs of FUS-ALS mouse models



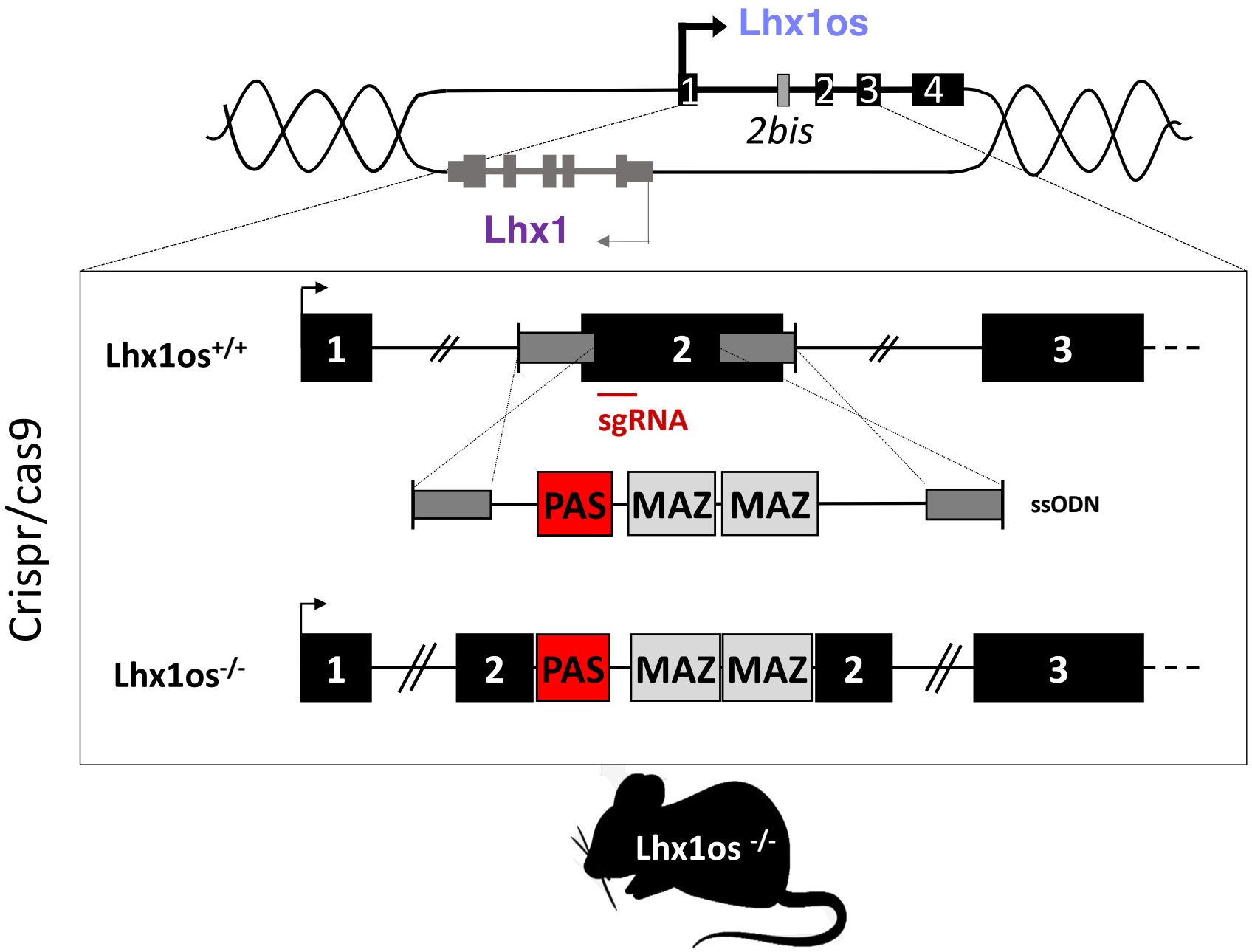


Ctrl: Fus+/+ MNs
Mutated Fus : FusP517L/P517L MNs
Fus KO: Fus−/− MNs

mutant mouse MNs carrying the equivalent of one of the most severe ALS-associated FUS alleles (P517L).

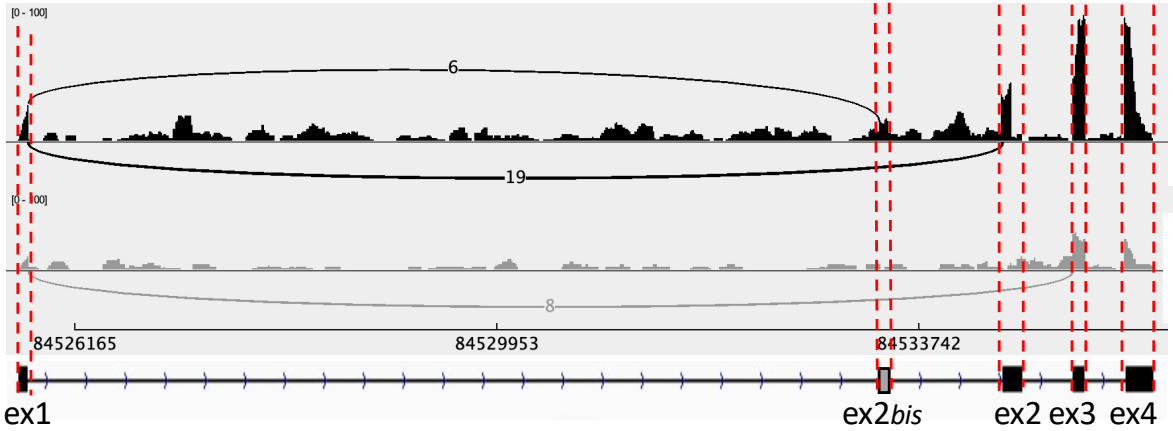
Lhx1os expression analysis in SCs derived from mutant SOD mice in ongoing

Lhx1os mouse KO strategy



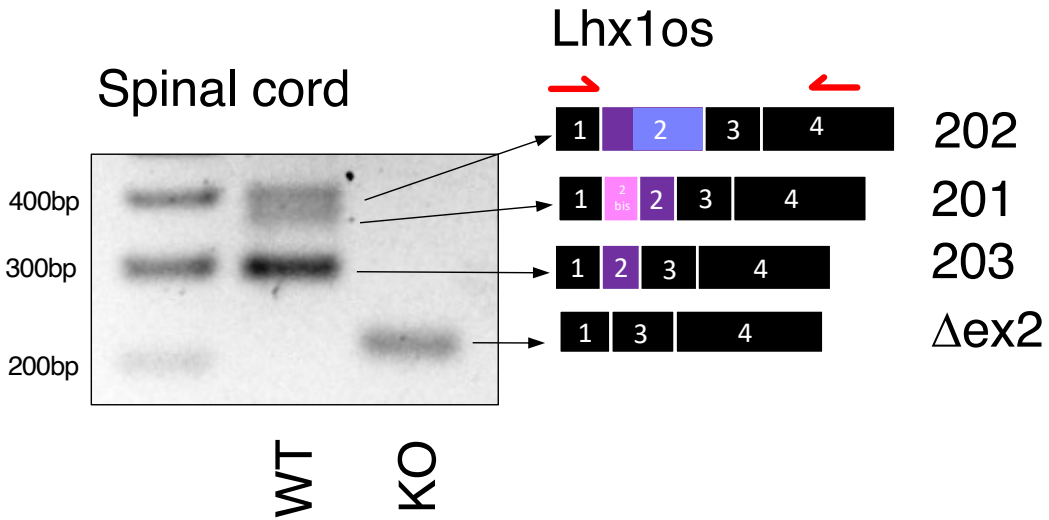
Lhx1os $^{-/-}$ mouse

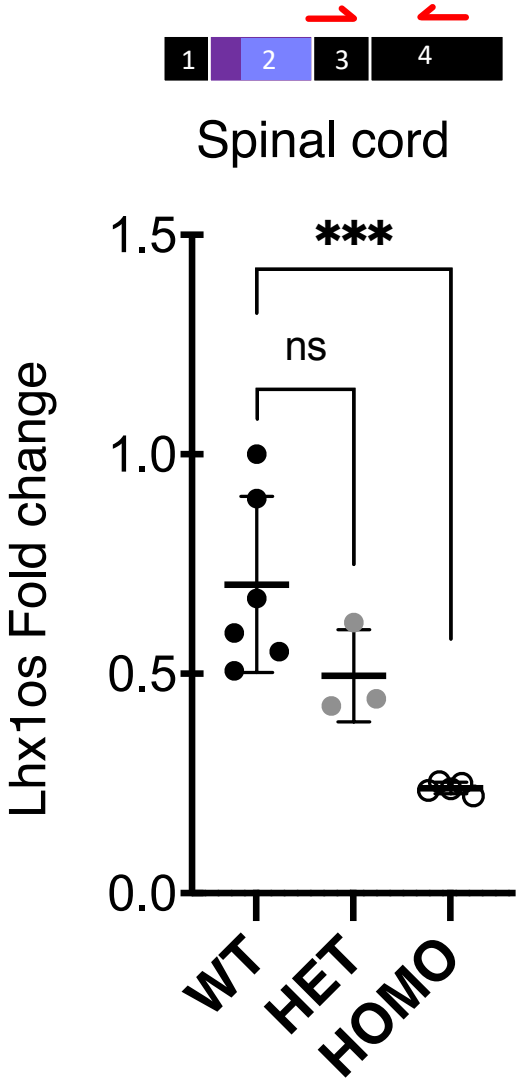
Reads from Lhx1os $^{+/+}$ and Lhx1os $^{-/-}$ spinal cord RNA–seq



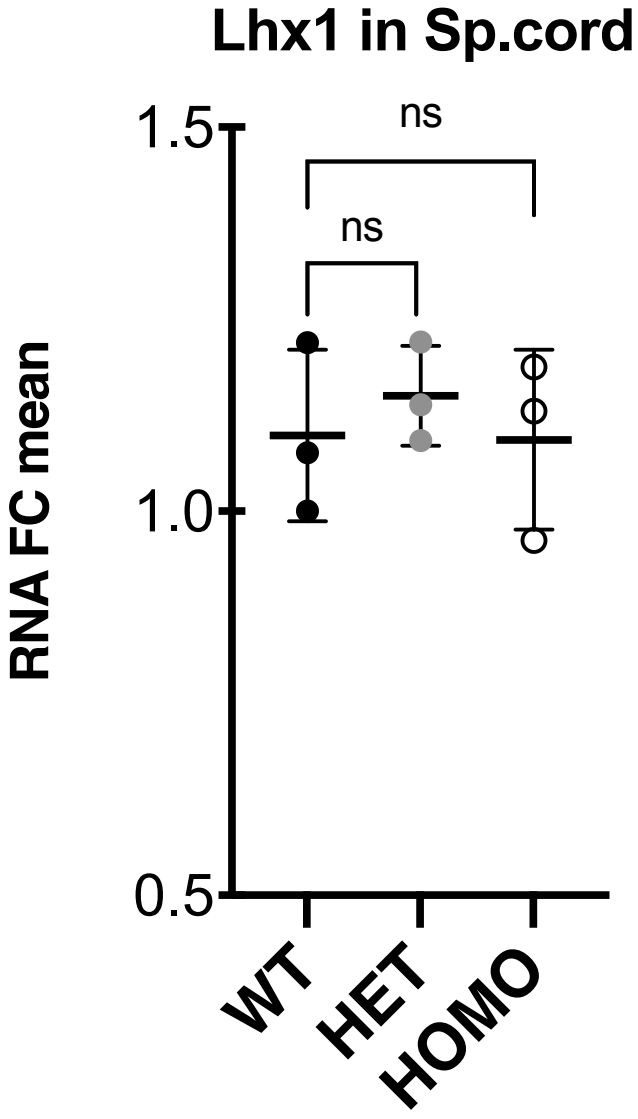
Lhx1os $^{+/+}$

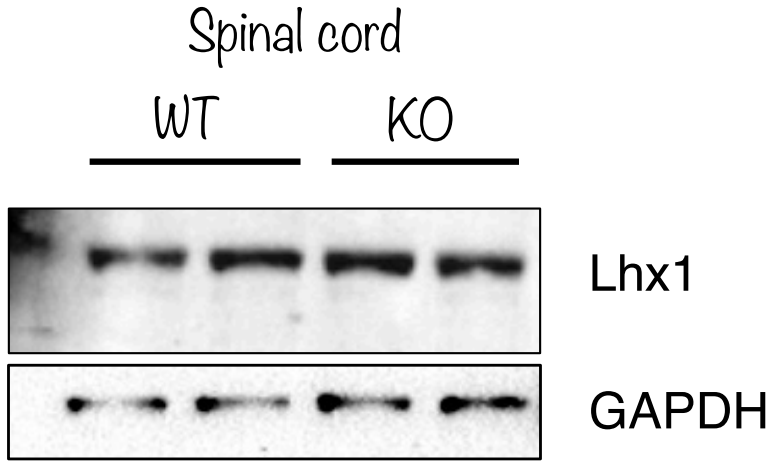
Lhx1os $^{-/-}$





Lhx1 expression in Lhxlos $^{-/-}$ mouse





Looking for Lhx1os $^{-/-}$ *in vivo* phenotype

Tests:

- Open field
- Hanging wire
- Hanging steel

Elvira De Leonibus
IGB-cnr. EMBL

- Treadmilll

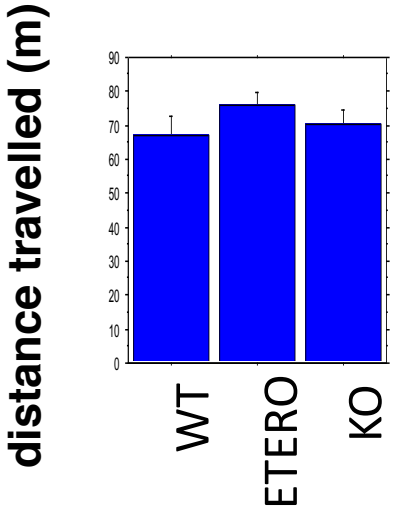
CARMINE

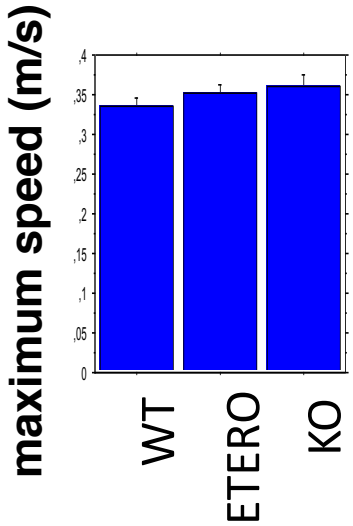
Open field

task to evaluate exploratory activity

3 Months

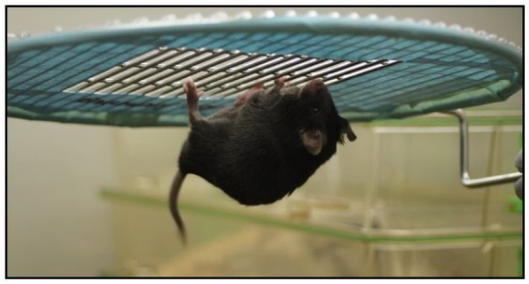
n=10





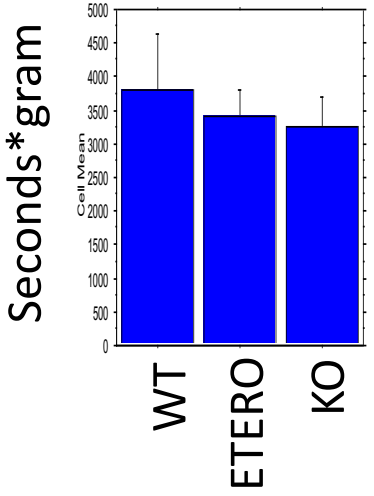
Hanging wire test

to evaluate their grip strength



3 Months

n=10

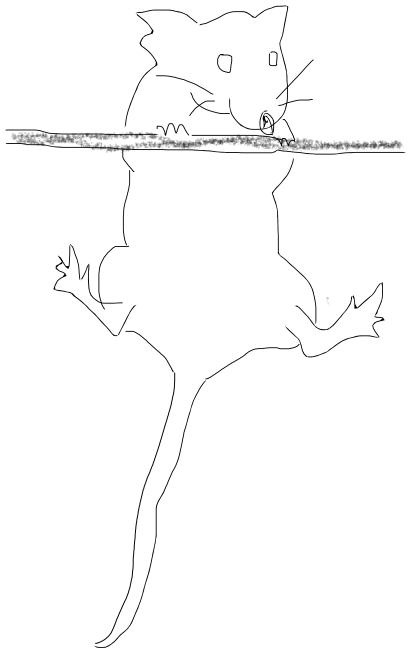


H.W.*weight

There are no differences between genotypes

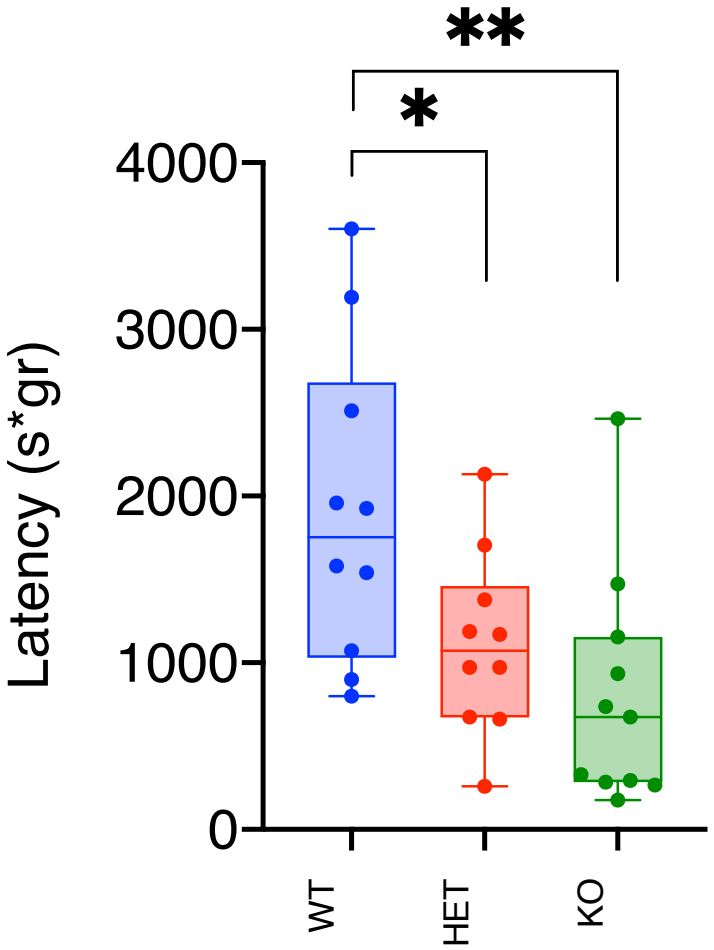
Elvira De Leonibus
IGB-cnr

Hanging steel test



3 Months

n=10

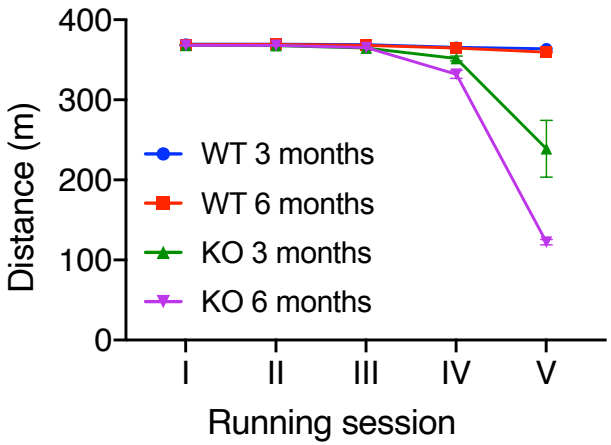


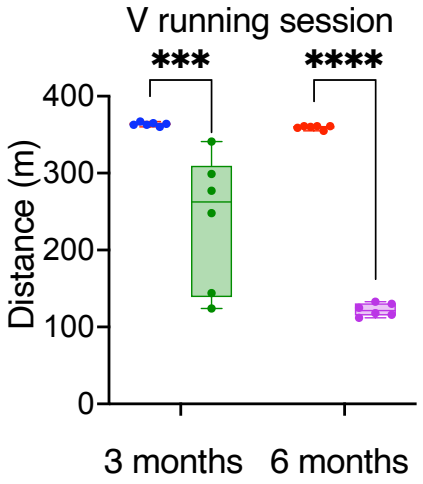
Elvira De Leonibus (IGB-CNR)

Treadmilll

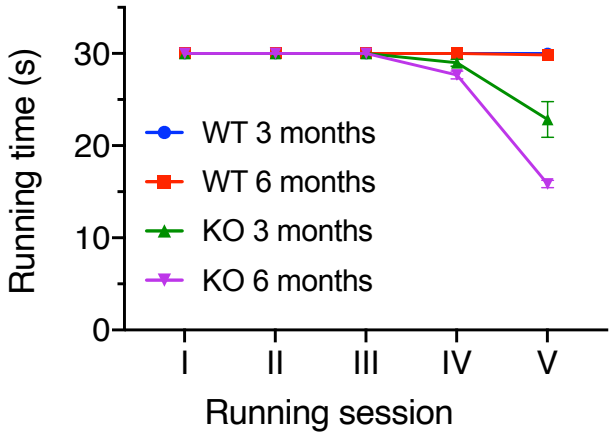
3 months WT n=8, HET n=5, KO n=8
6 months WT n=6, HET n=6, KO n=6

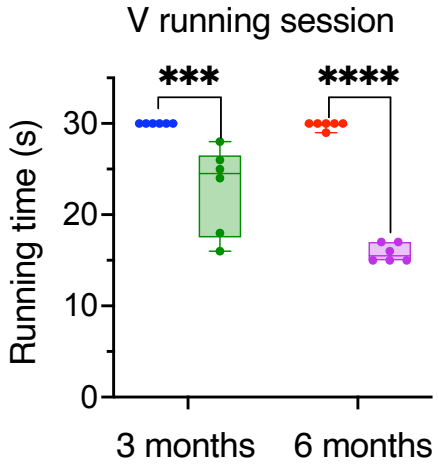
1- METRES





2- RUN TIME



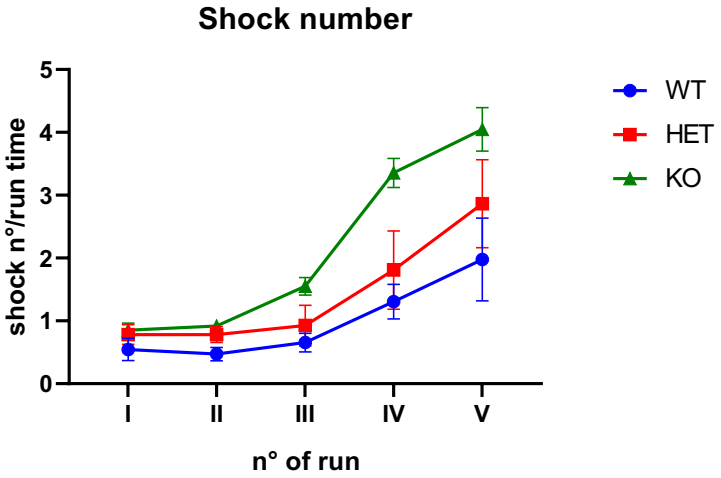


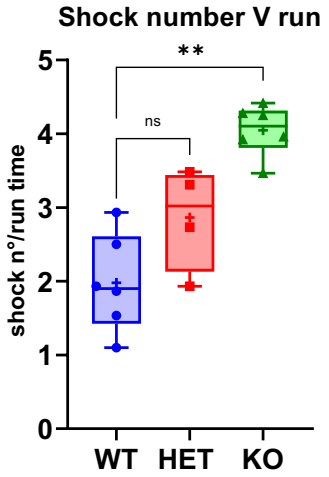
Treadmilll

3- SHOCK NUMBER/RUN TIME

3 Months

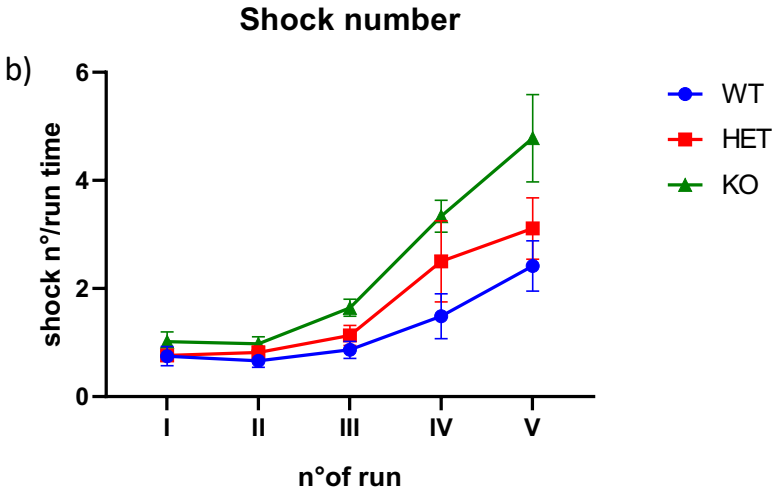
WT n=6, HET n=4, KO n=6

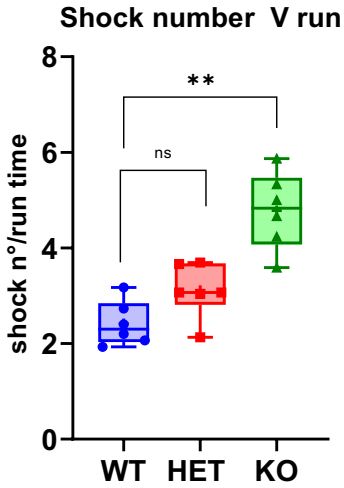




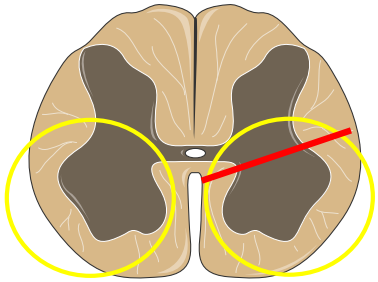
6 Months

WT n=6, HET n=6, KO n=6

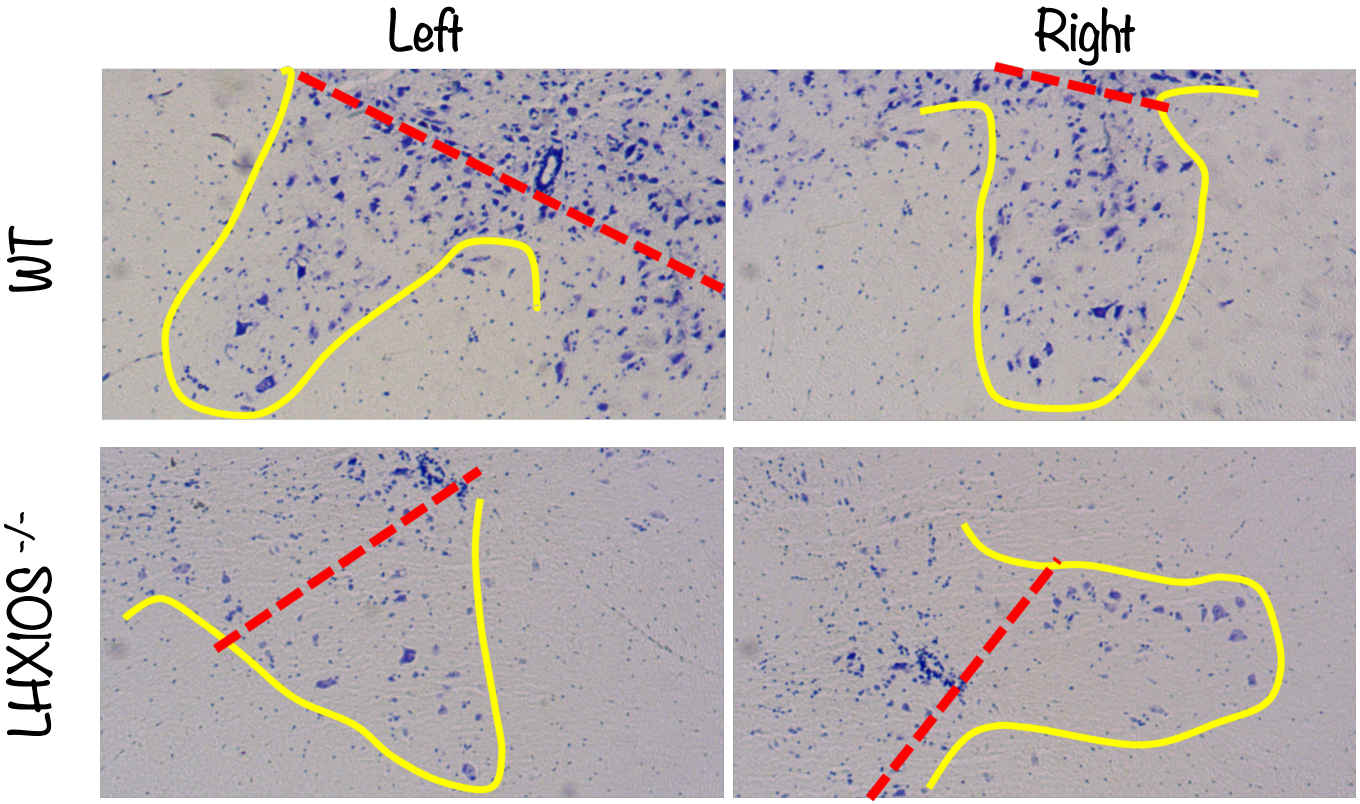


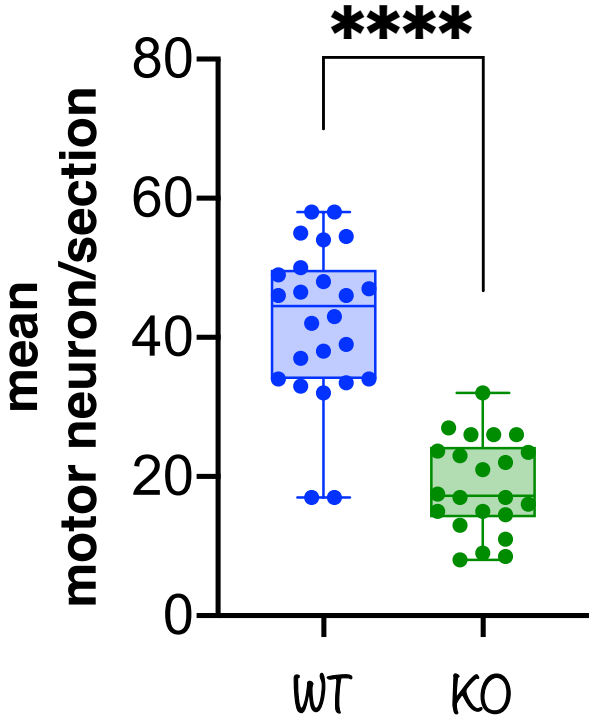


Nissl-stained motor neuron count in the lumbar spinal cord



Ventral horn of SC

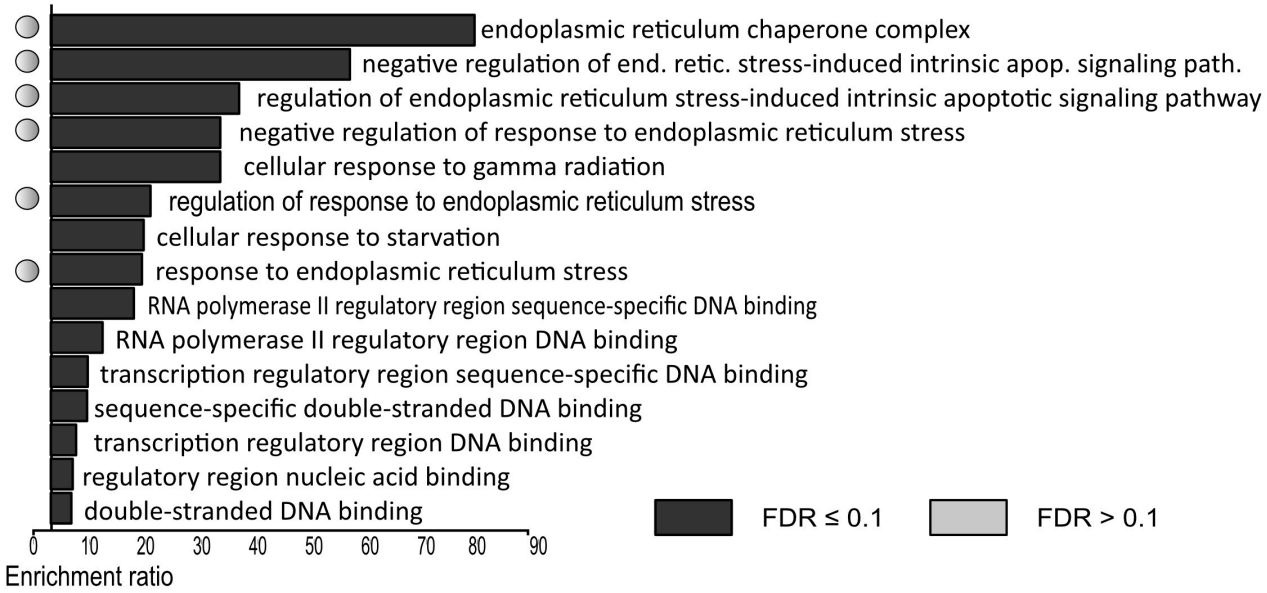




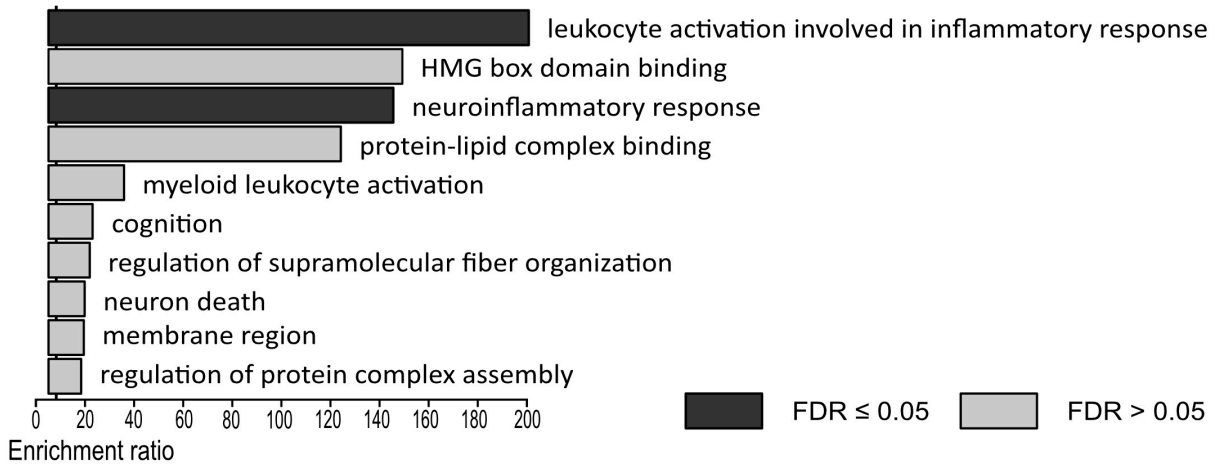
mRNAs differential expression

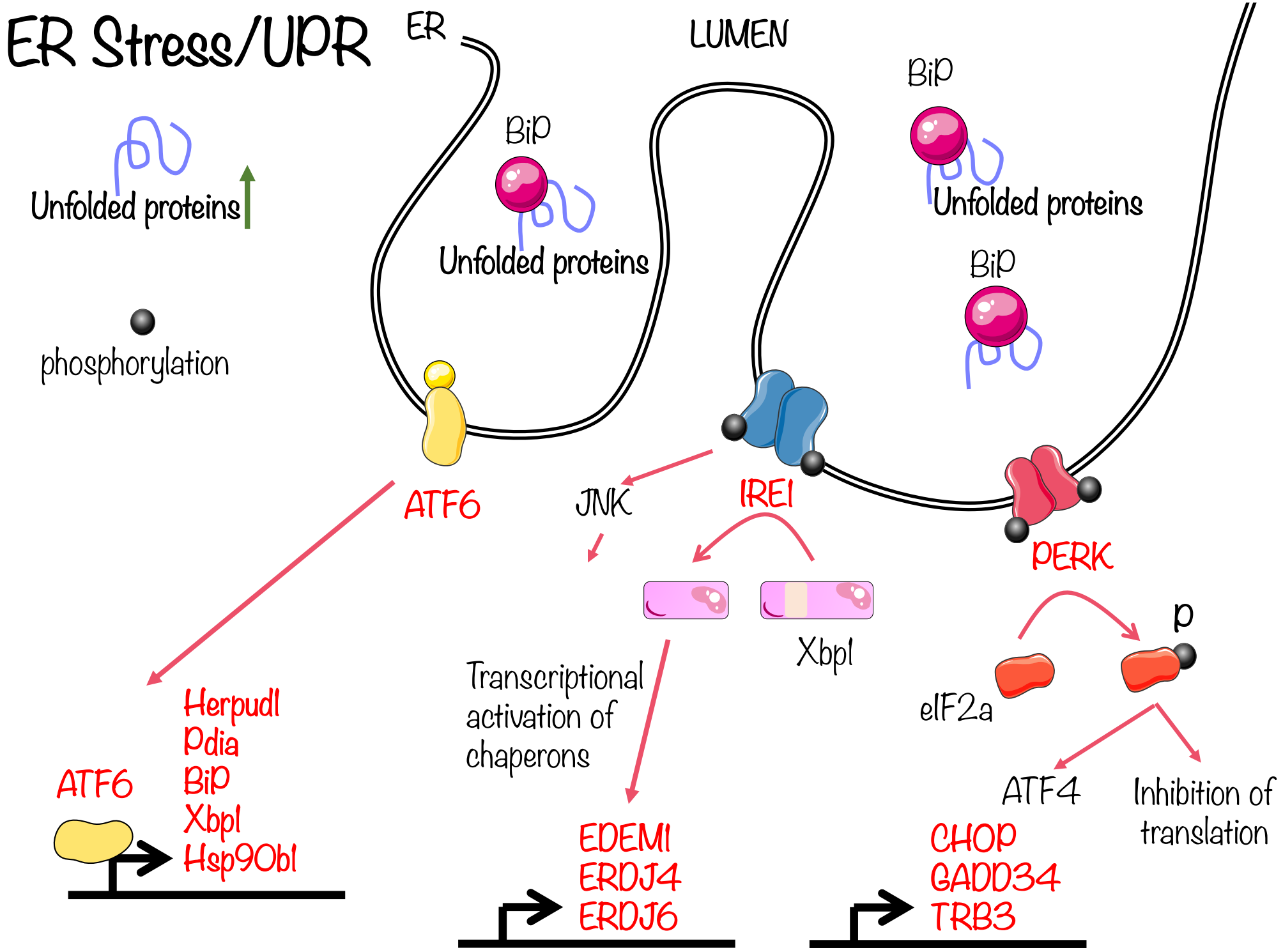
SPINAL CORD 3 months RESTING WT vs KO

Down-regulated (52 mRNAs)

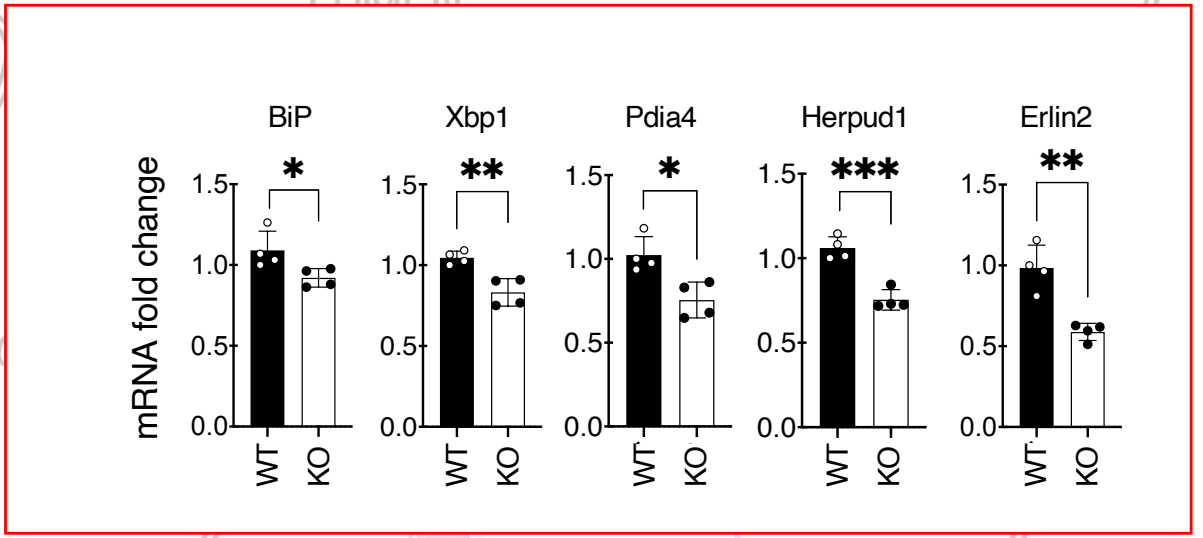


Up-regulated (5 mRNAs)




ER Stress/UPR
ER
LUMEN
Unfolded proteins
phosphorylation
BiP
Unfolded proteins
BiP
Unfolded proteins
BiP
ATF6
JNK
IRE1
PERK
Xbpl
p
eIF2a
ATF4
Inhibition of translation
Transcriptional activation of chaperons
ATF6
Herpudl
Pdia
BiP
Xbpl
Hsp90bl
EDEM1
ERDJ4
ERDJ6
CHOP
GADD34
TRB3

ER Stress



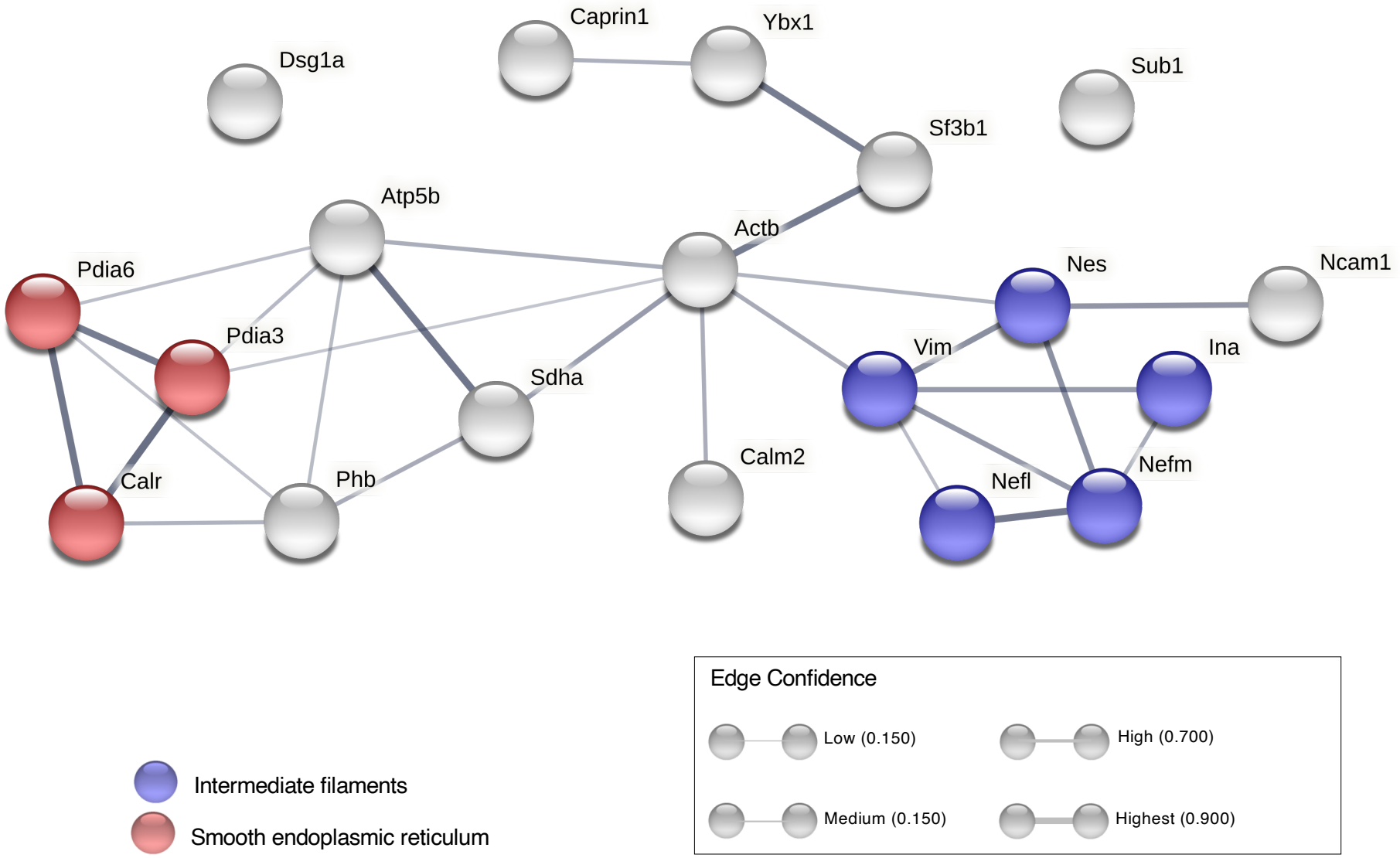
ATF6

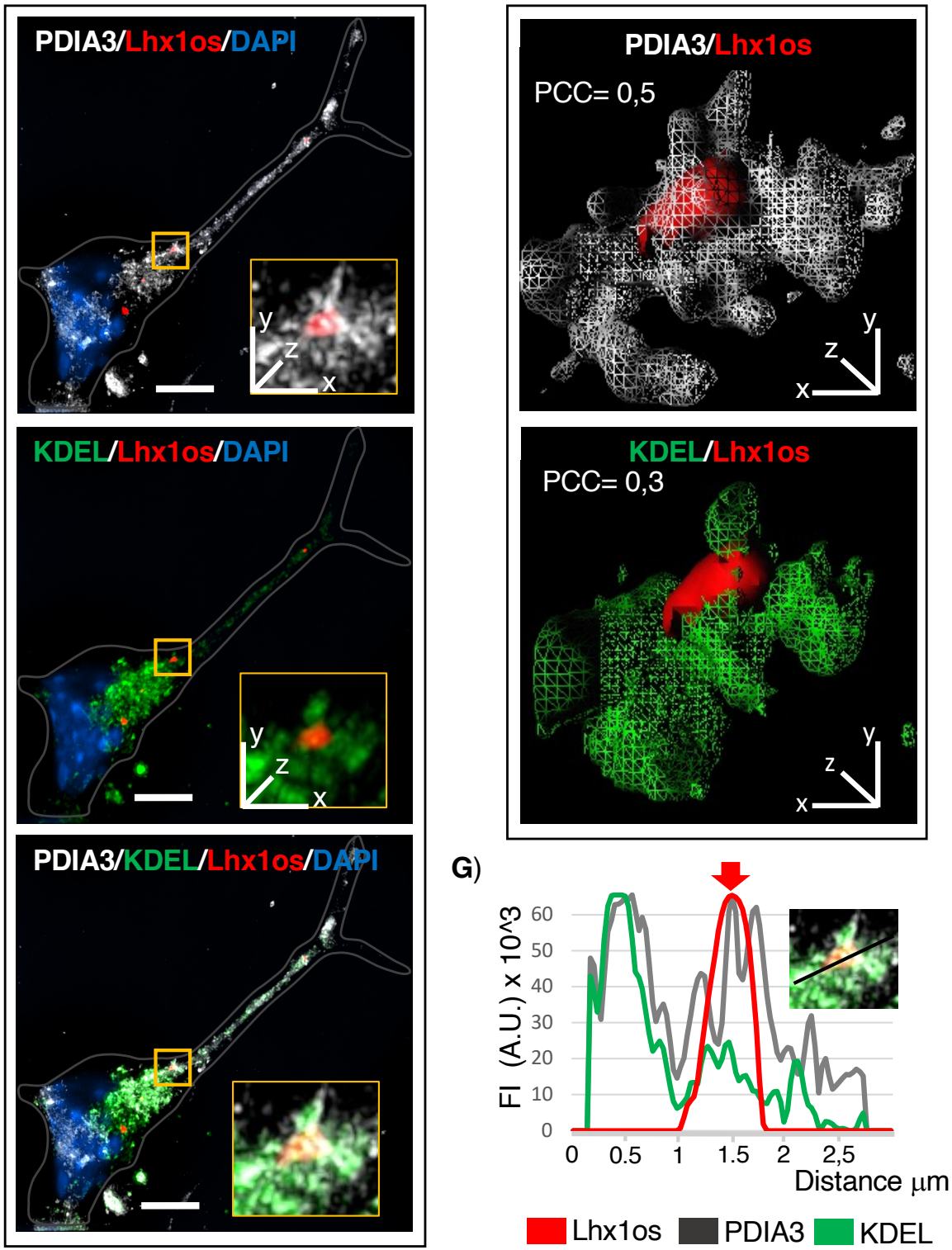
Those targets are down-regulated in the RNA-seq from Lhxlos-/- mice

ATF6

Herpud1
Pdia
BiP
Xbp1
Hsp90b1

Lhx1os protein interactors




PDIA3/Lhx1os/DAPI
KDEL/Lhx1os/DAPI
PDIA3/KDEL/Lhx1os/DAPI
PDIA3/Lhx1os
PCC= 0,5
KDEL/Lhx1os
PCC= 0,3
G)
FI (A.U.) x 10^3
60
50
40
30
20
10
0
0
0.5
1
1.5
2
2,5
Distance µm
Lhx1os
PDIA3
KDEL

ER-stress mediated induction of Lhx1os