Live imaging of RNA dynamics for genetic forms of ALS in zebrafish

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« Treatments of ALS: from genetics to zebrafish »
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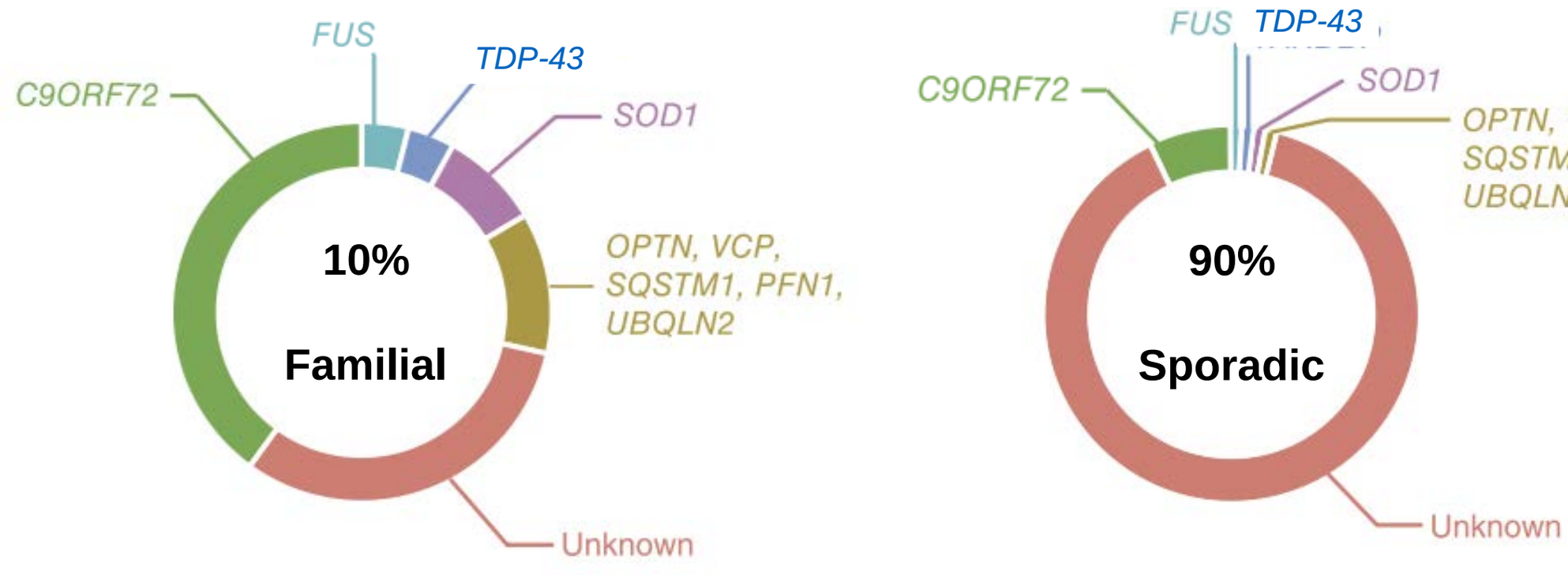
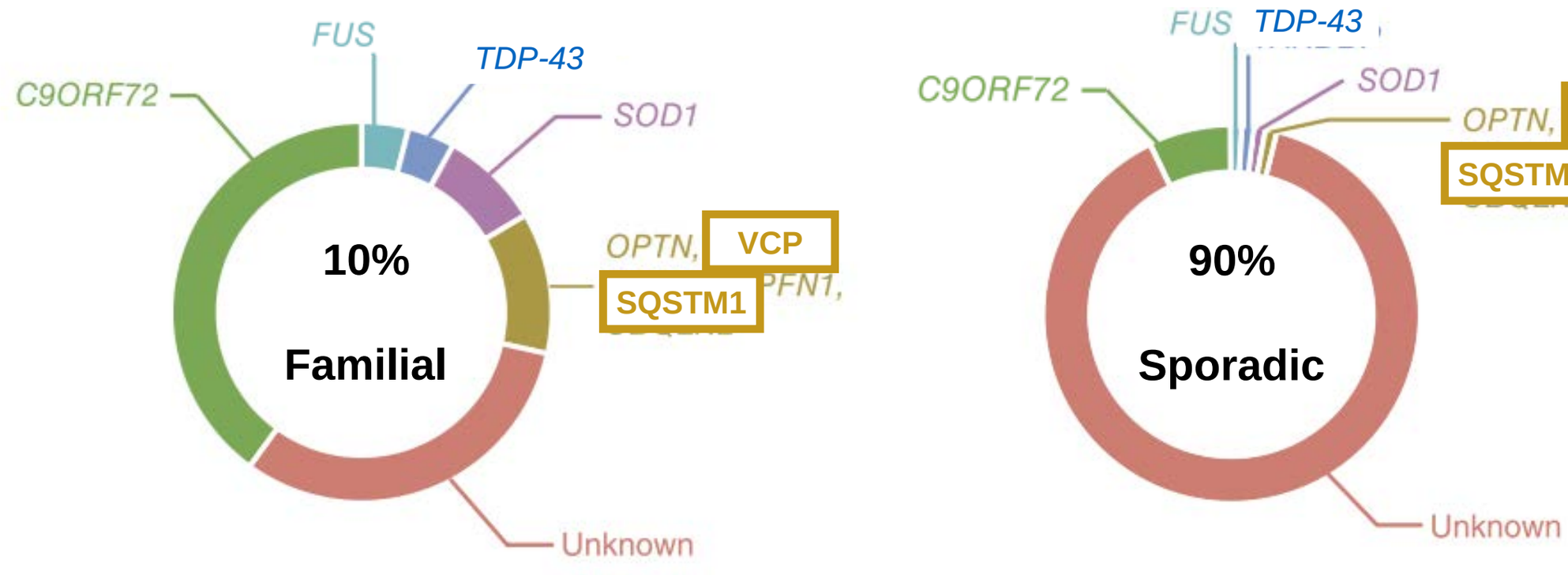
The Genetics of ALS



Modified from **Renton** et al. 2014

The Genetics of ALS



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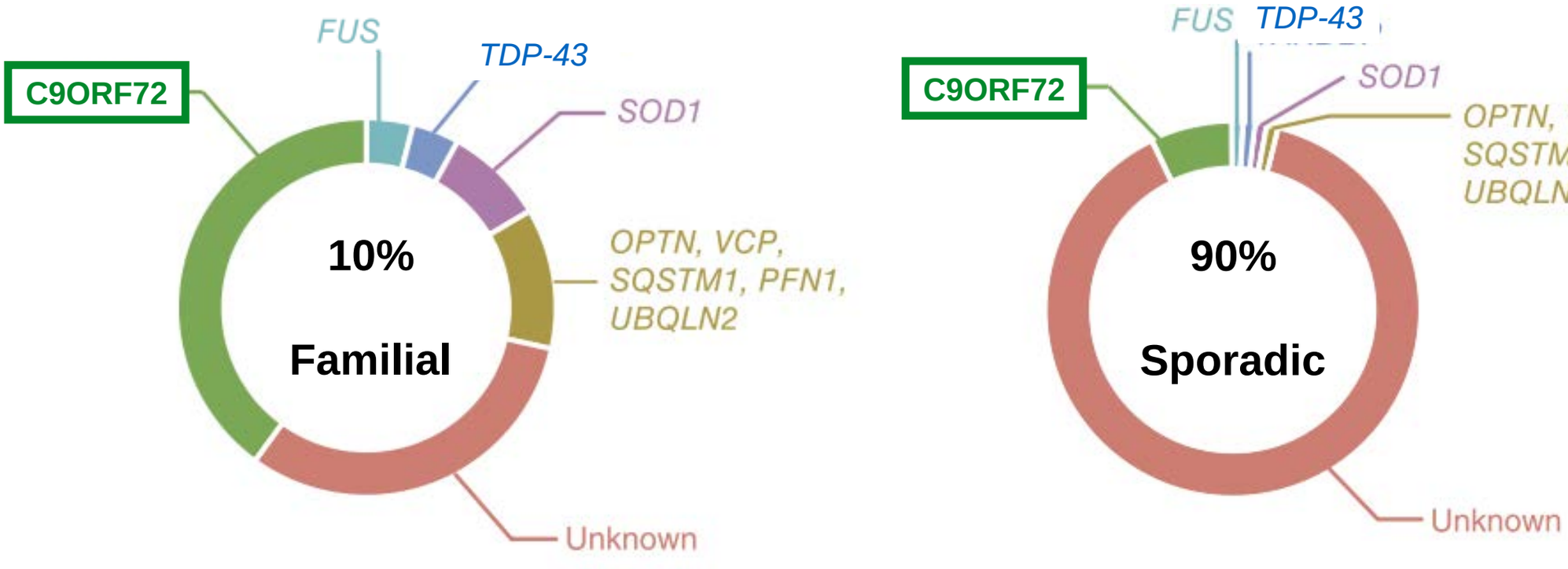
The Genetics of ALS



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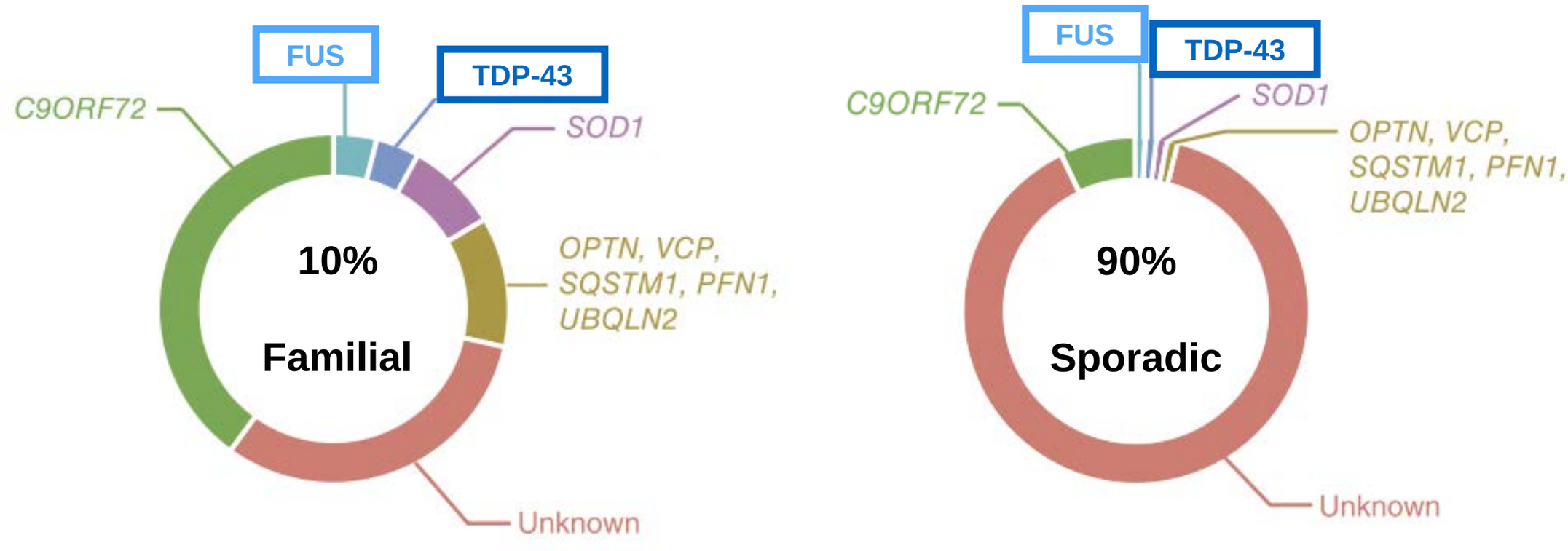
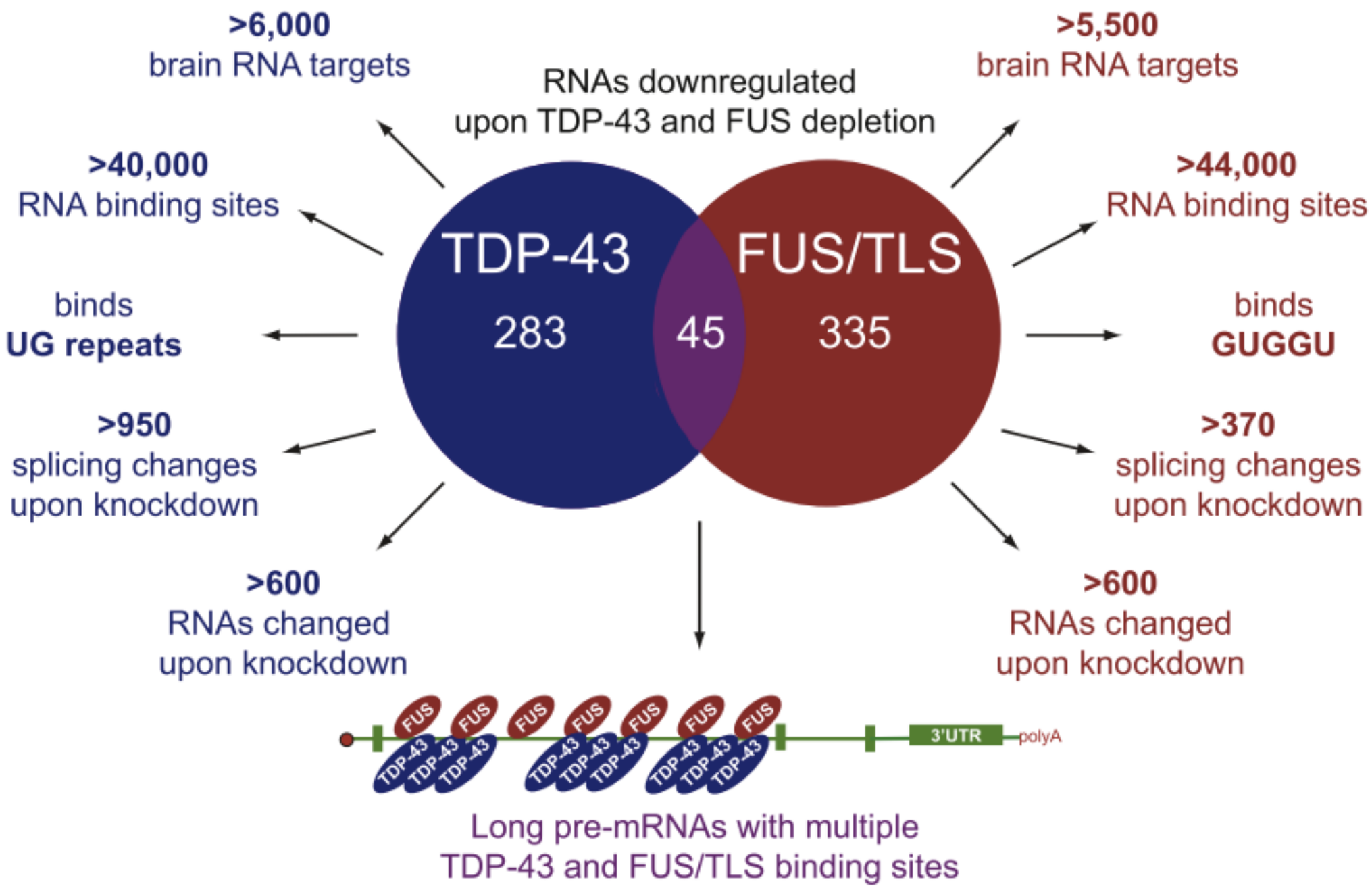
The Genetics of ALS



Modified from **Renton** et al. 2014

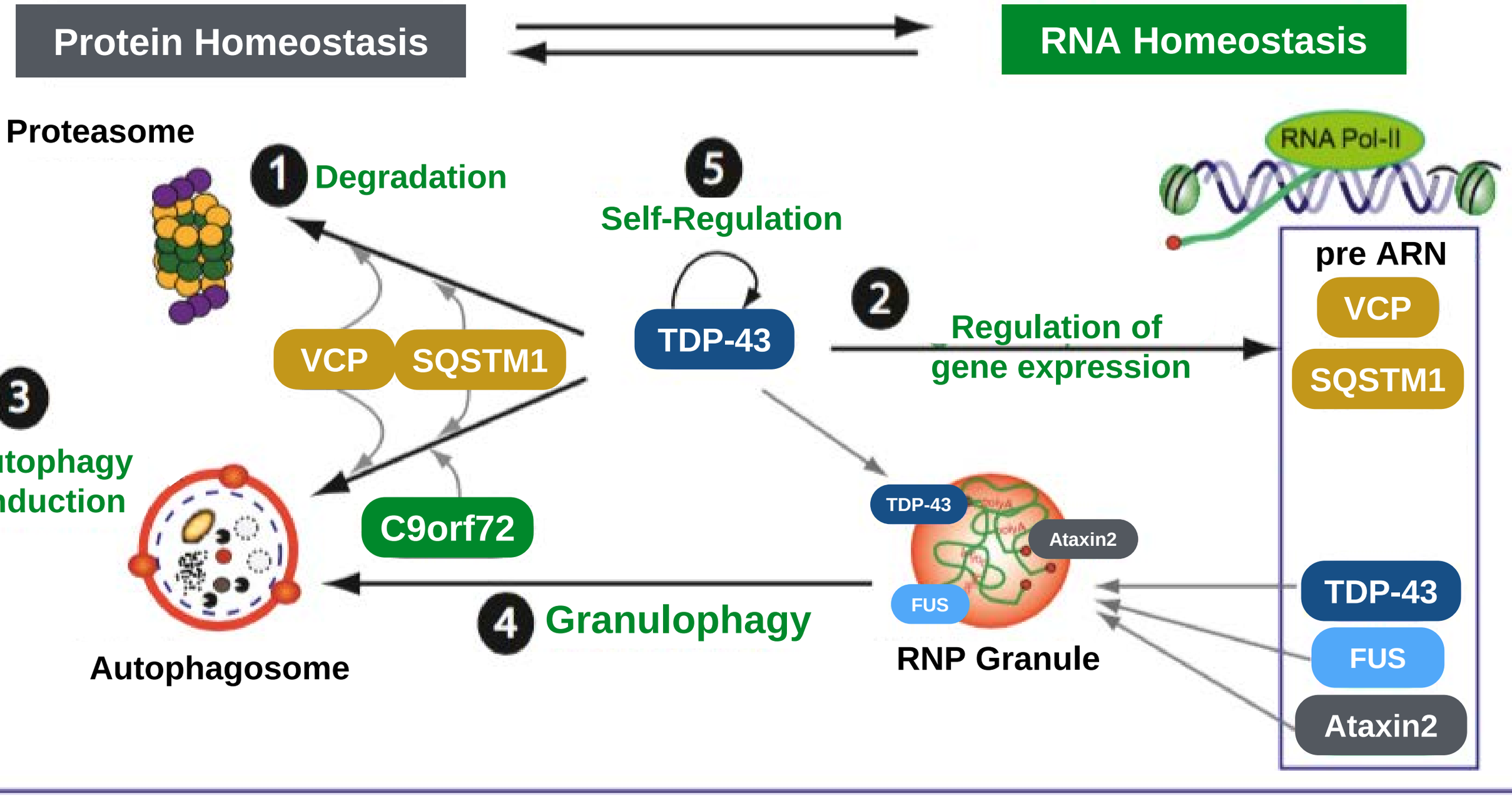
TDP-43 and FUS: RNA-binding proteins



(from **Ling** et al. 2013)

Protein and RNA homeostasis

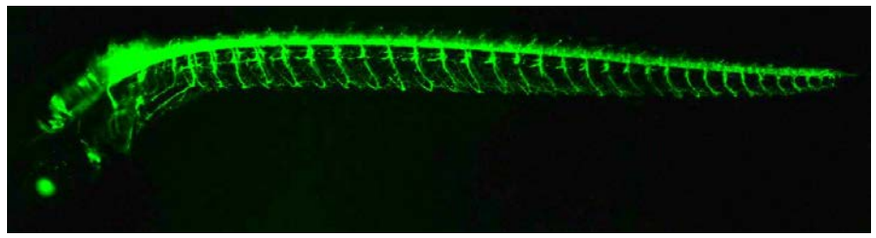
TDP-43 self-regulatory mechanism



(modified from **Ling** et al. 2013)

In vivo study of RNA dynamics: the zebrafish model

-Large progeny generating model
-Vertebrate
-Transparency at embryonic stages
-Great range of genetic tools

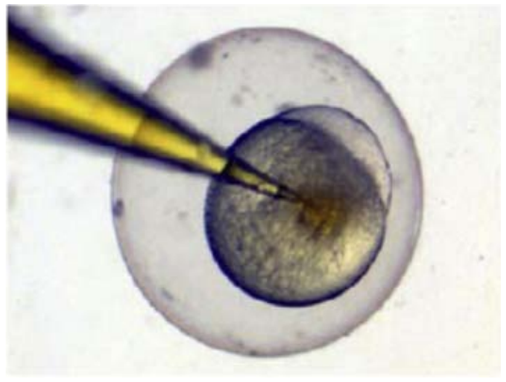


Hb9-GFP transgenic line

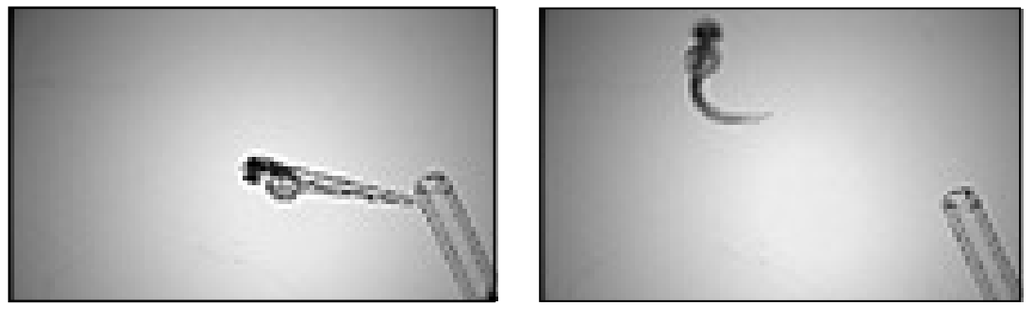
=> Generate pathological models

=> Unveil genetic interactions

Injections



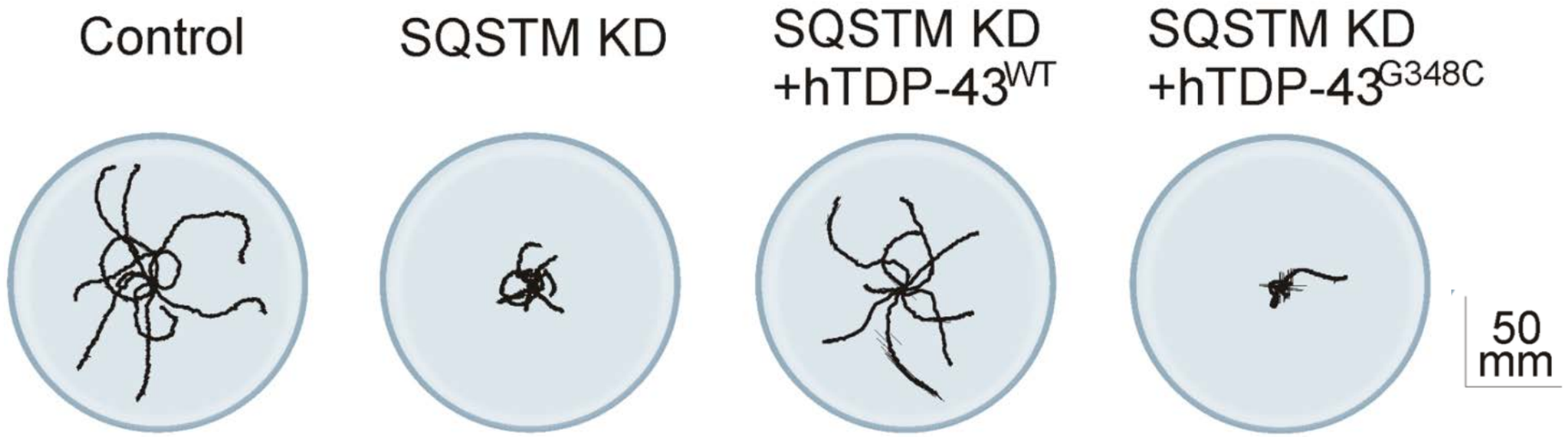
Touch evoked escape response (TEER)



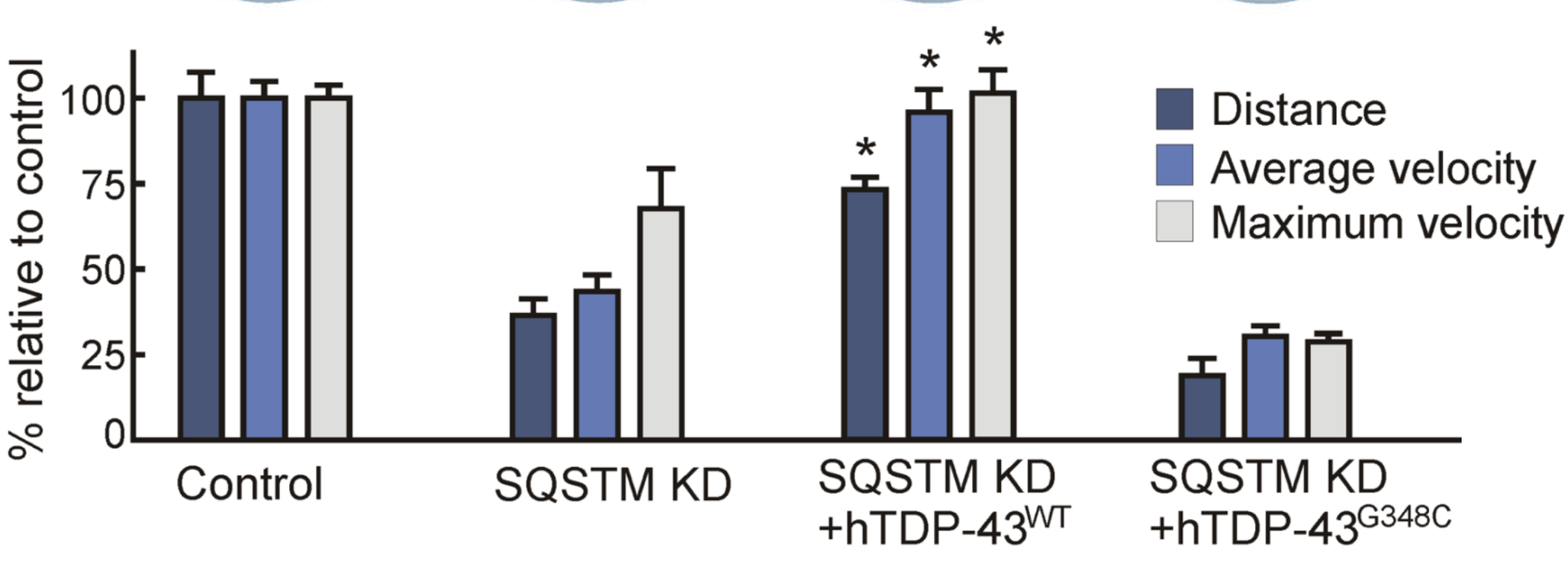
2-3 dpf

Genetic interaction between TDP-43 and SQSTM1

Escape response



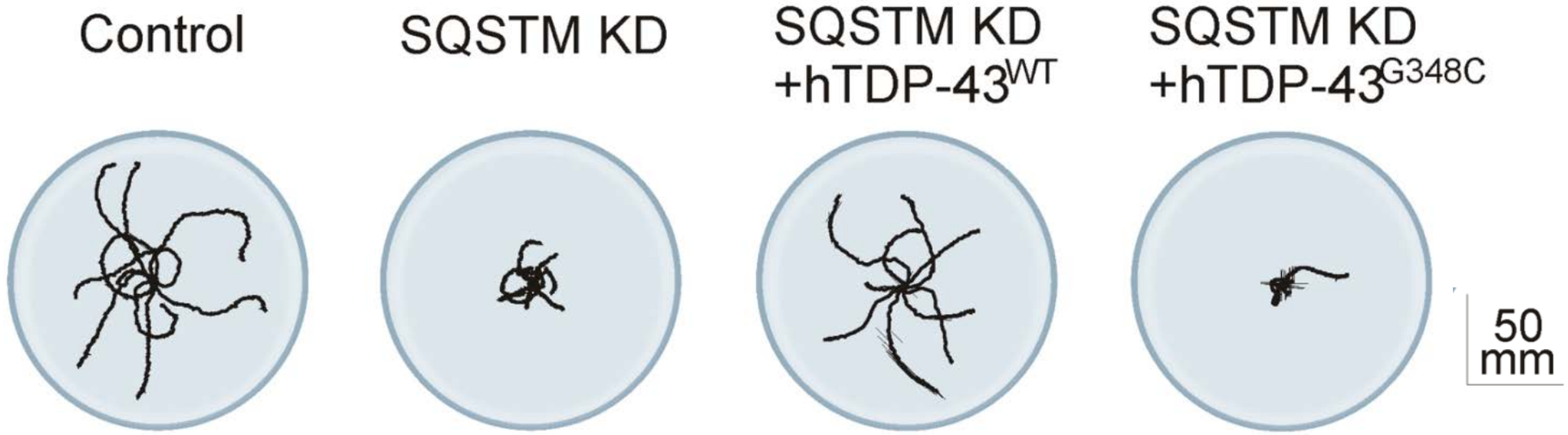
Motor phenotype



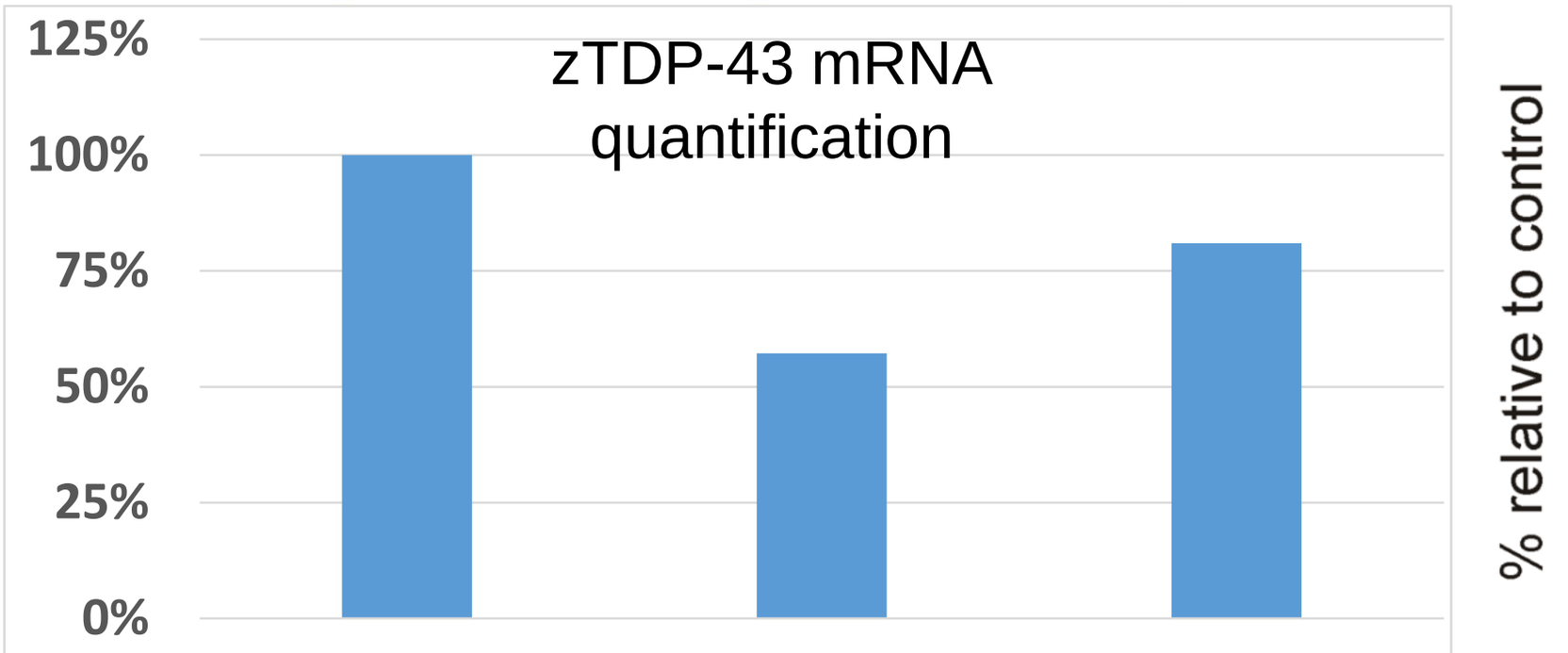
KD of SQSTM1 leads to **reduced motricity**
=> significantly **ameliorated** by human **WT TDP-43 RNA**

Genetic interaction between TDP-43 and SQSTM1

Escape response



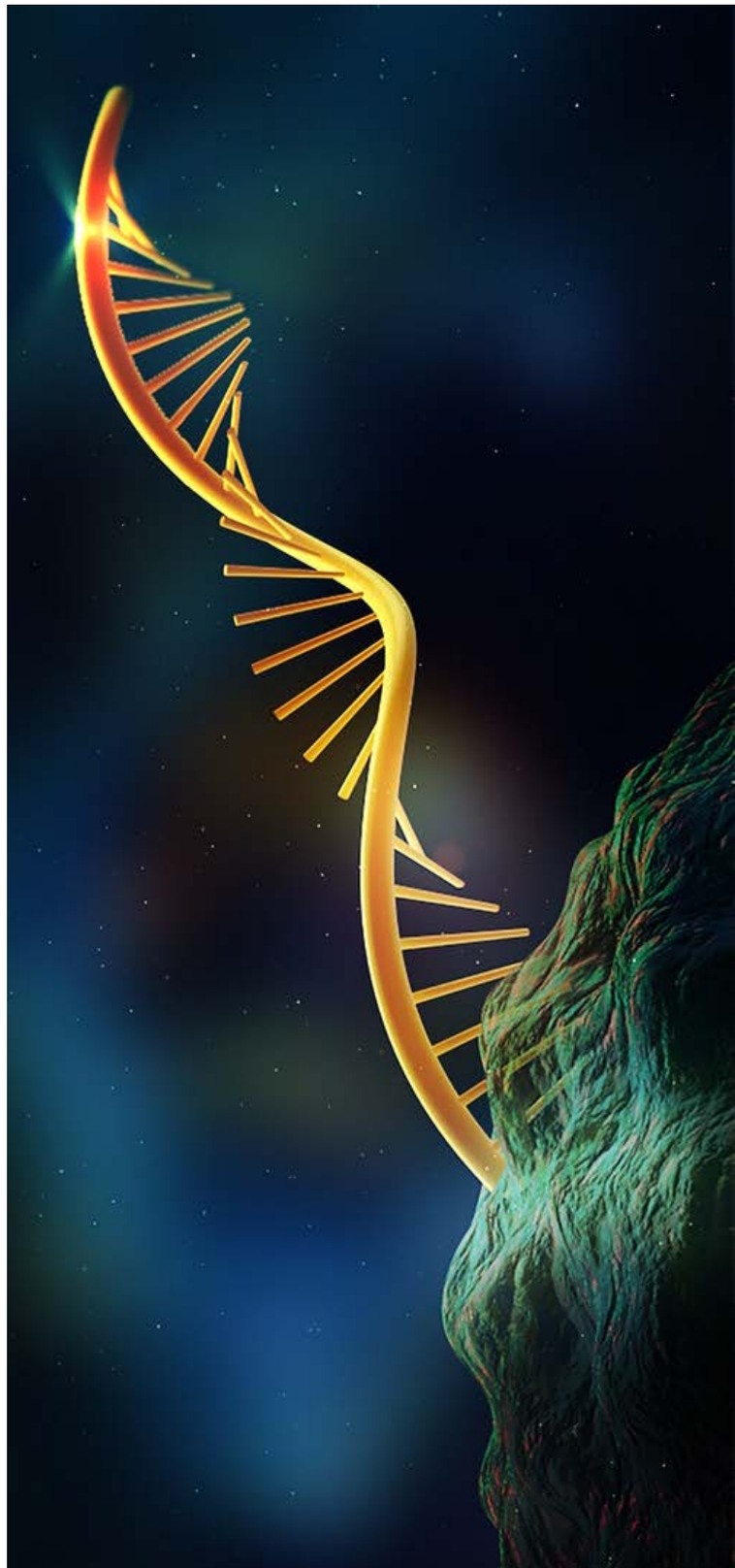
qPCR targeting zTDP-43 RNA



KD of SQSTM1 leads to **reduced zTDP-43 mRNA levels**
=> significantly **ameliorated** by human **WT TDP-43 RNA**

Studying RNA metabolism in ALS

Understand RNA-binding proteins central role and their interactions with key ALS transcripts (SQSTM1, C9orf72, VCP) in motor neurons



- Precise **localization** and colocalization
- **Interaction partners**
- **Quantification**
- **Dynamics** through live-imaging time-lapse
- **Axonal transport**
- **Healthy VS Pathological condition**

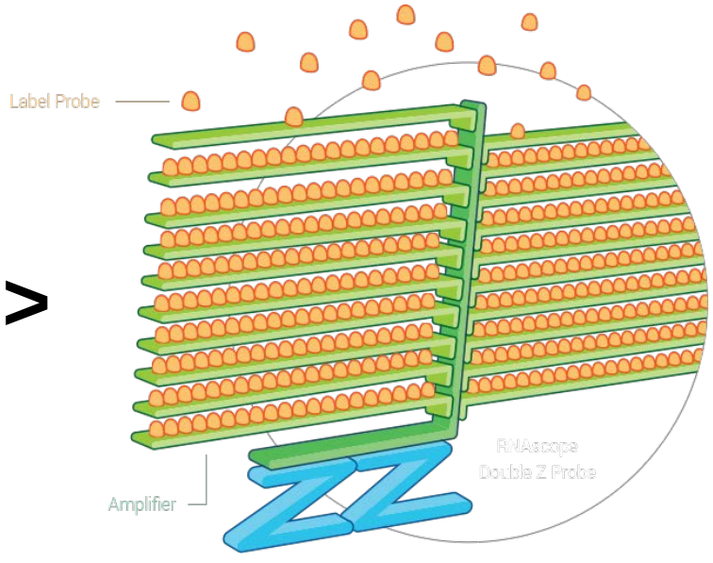
Investigating RNA metabolism: Precise *in situ* hybridization

Control FISH with ACD's RNAscope:

- Single-molecule precision

- IHC colocalization

=>



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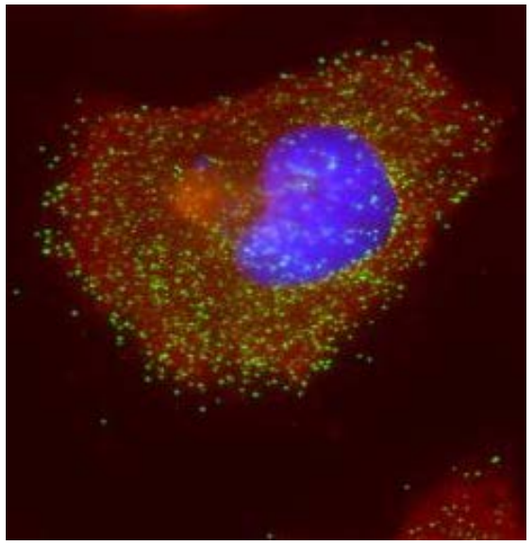


Double Z probe fixation

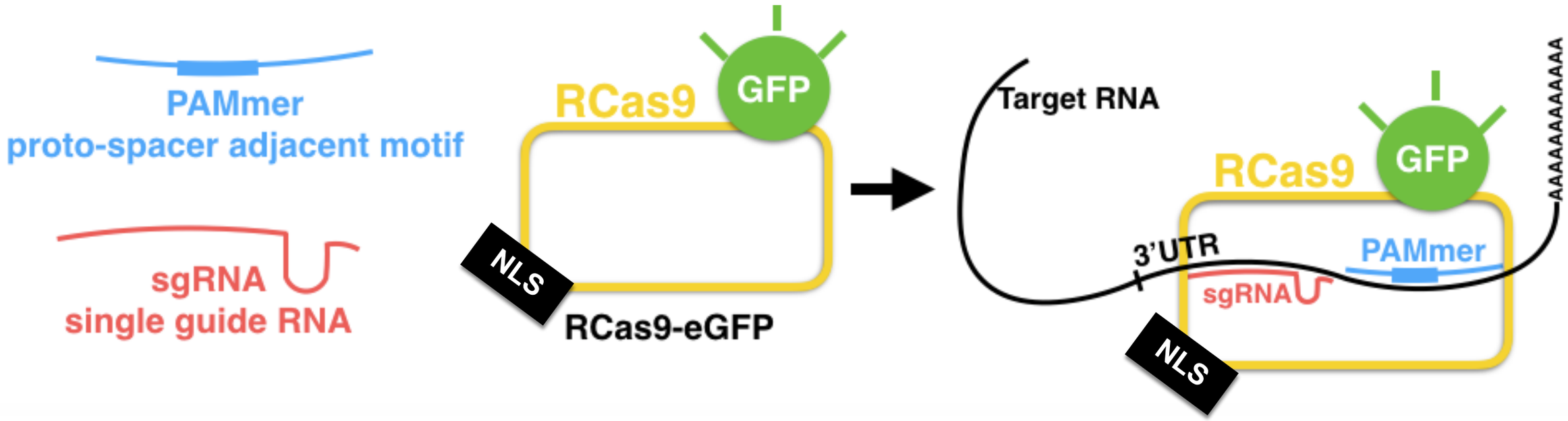
Signal amplification and fluorescent labeling

Imaging

In vivo study of RNA dynamics: a modified CRISPR/Cas9 system

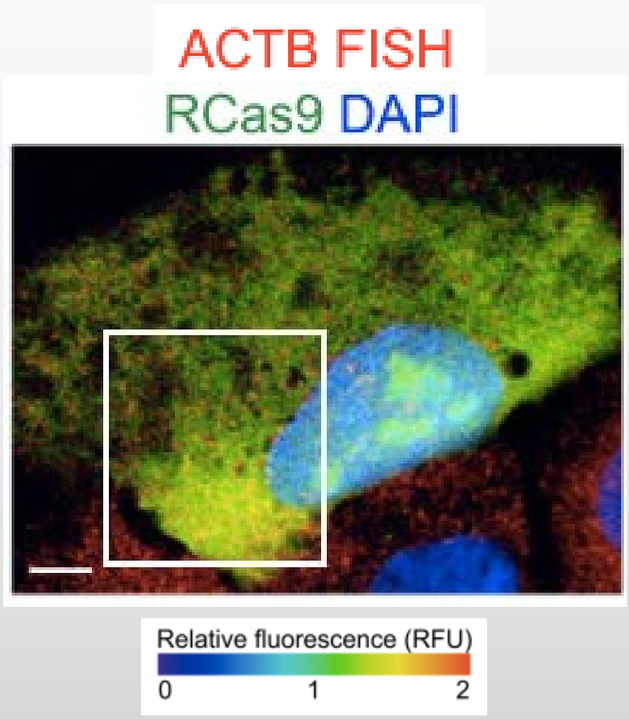
Classic Crispr/Cas9 System:

Modified RNA targeting Crispr/Cas9 System:



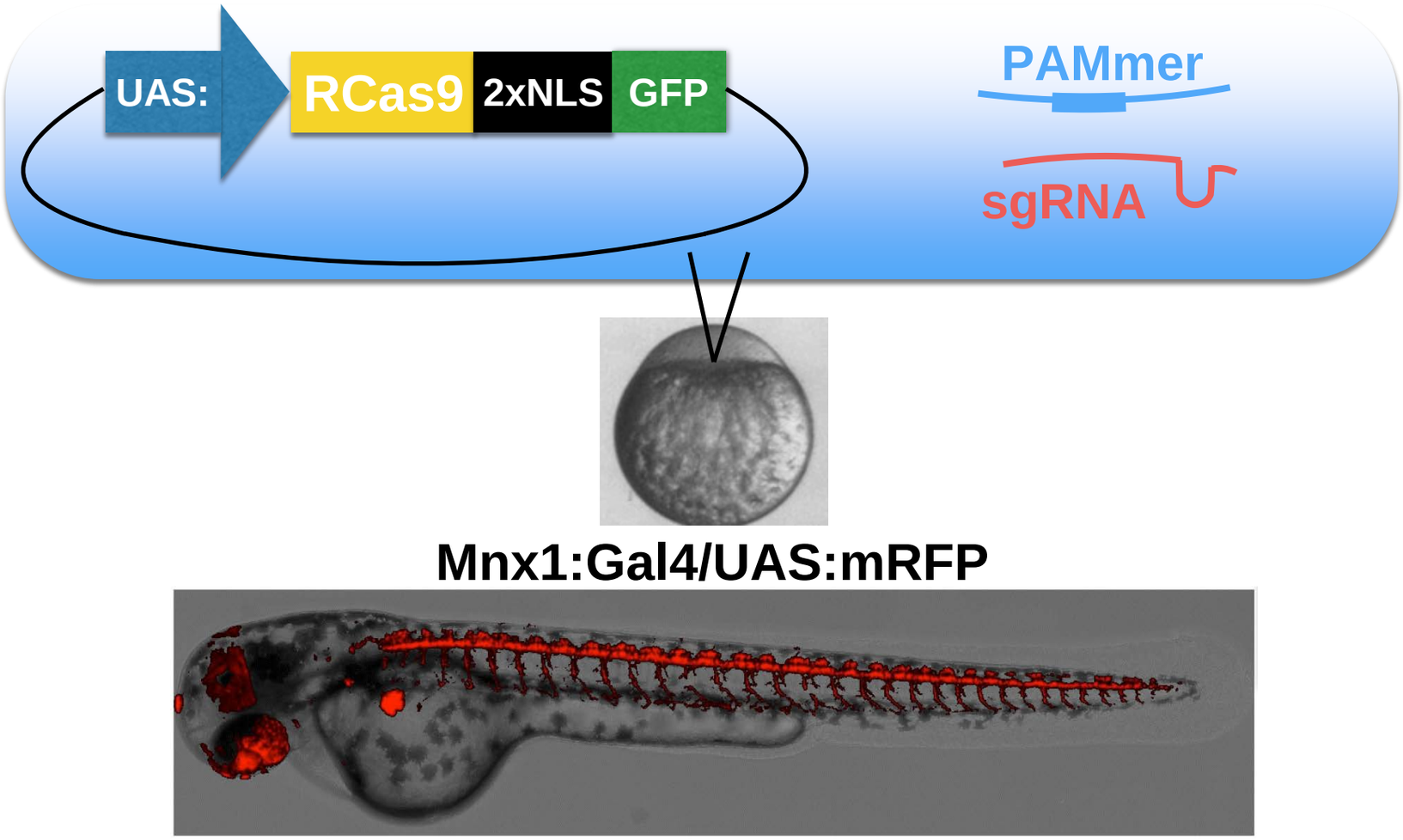
The RNA targeting CRISPR/Cas9

***In vitro* use of the technique:**



- Targets endogenous RNAs
- No secondary effects
- Localization is highly correlated with control FISH

(from **Nelles** et al. 2016)



NLS **Nuclear Localisation Signal**

FISH: Fluorescent *in situ* Hybridization

Future directions

Conclusions:

-Zebrafish models combines genetic and imaging tools

-TDP-43 and SQSTM1 functionally interact

-First *in vivo* of a modified CRISPR/Cas9 to target RNA

Perspectives:

-Central role of RNA-binding proteins in ALS

-RNA homeostasis defects in pathogenicity

-Precise the level of interactions between key factors

Acknowledgements

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Anca Marian
Corinne Besnard-Guerin

Edor Kabashi's Team

and thank you to: Hervé Tostivint, François Giudicelli

… also featuring Maria-Letizia Campanari !

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