RNA toxicity in a zebrafish model for C9orf72 ALS is abrogated by Pur-alpha and p62

Bart Swinnen, MD
Leuven, Belgium

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Background

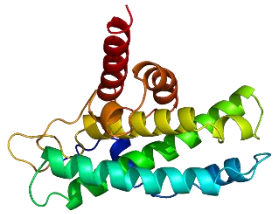
1) Research Question

5’ - CTAGGGGCCGGGGCCGGGG … GGGGCCGGGGCCGGGGCG – 3’
3’ - GATCCCCGGCCCCGGCCCC … CCCCGGCCCCGGCCCCGC – 5’

DPR toxicity

RNA toxicity

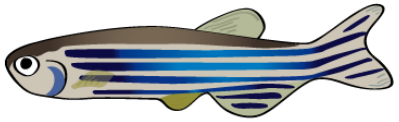
C9orf72 LOF

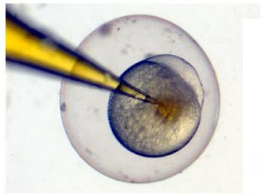


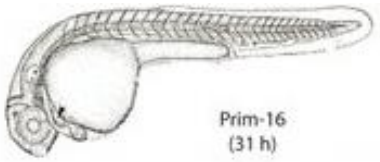
Evidence for direct RNA toxicity in an *in vivo* model?

2) Research model

Vertebrate model

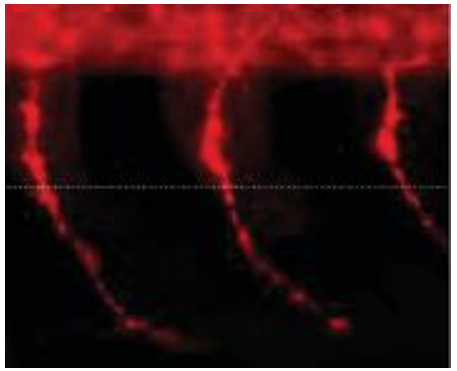


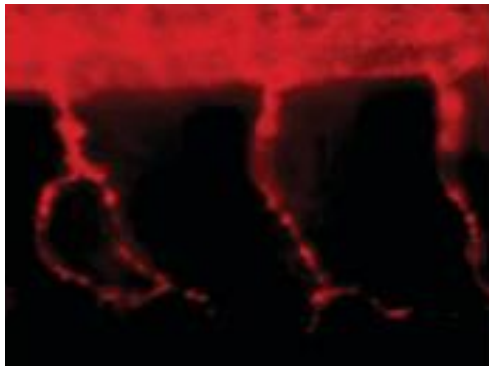




Motor Neurons

Axons

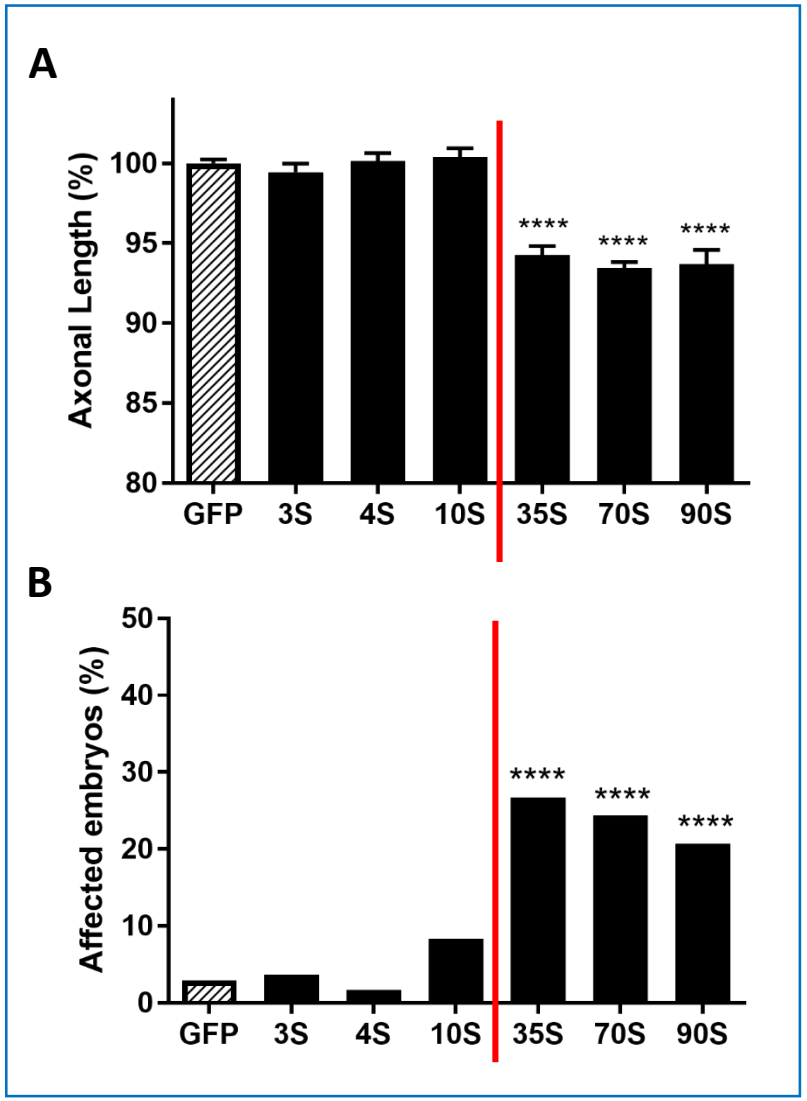




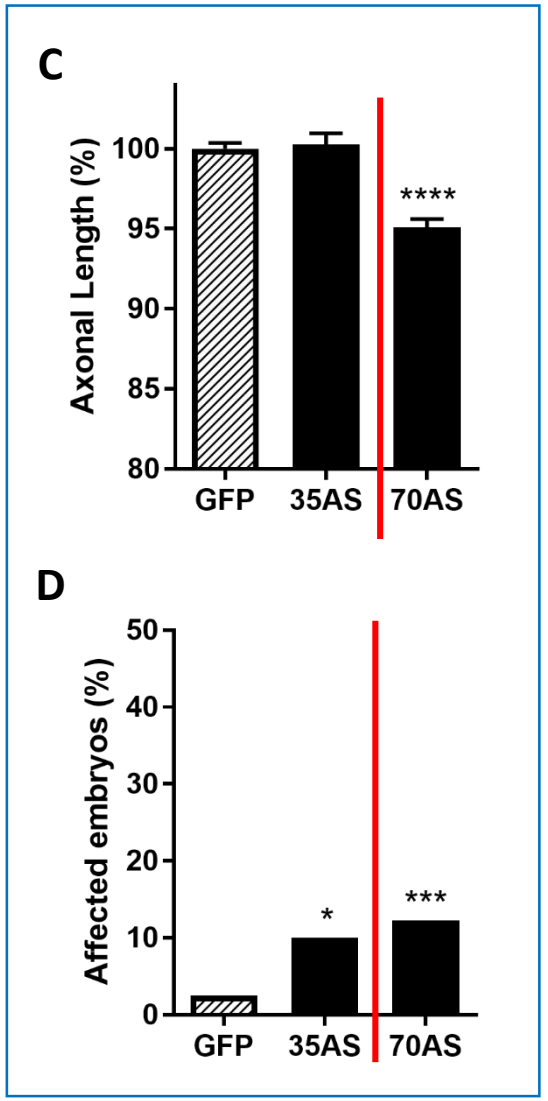
Lemmens et al, HMG 2007

1) Is repeat RNA toxic in zebrafish?

***Sense* RNA is toxic**



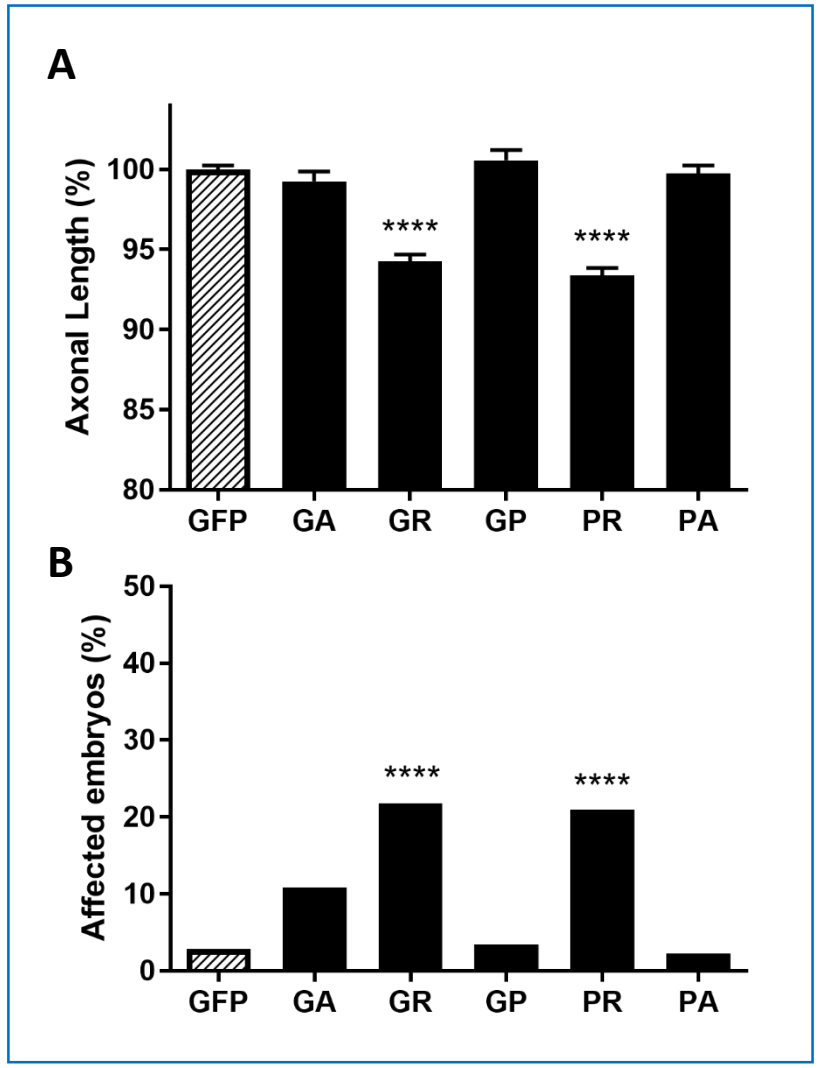
***Antisense* RNA is toxic**



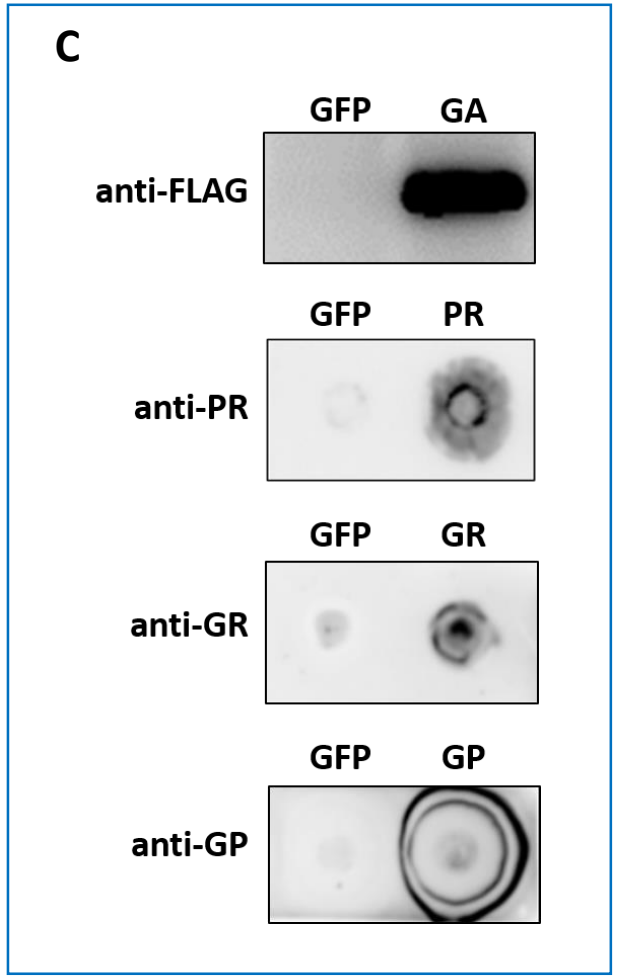
2) Are DPRs toxic in zebrafish?

Codon optimized DPR constructs

PR and GR are toxic

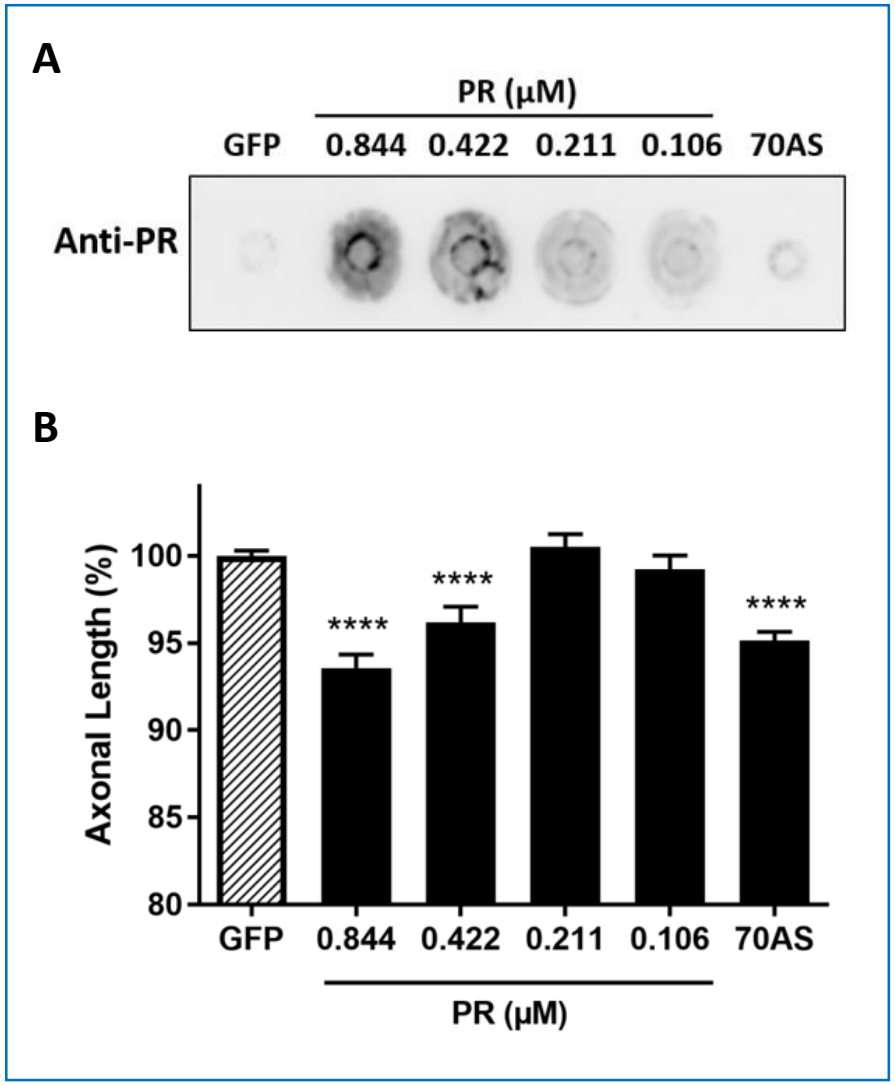


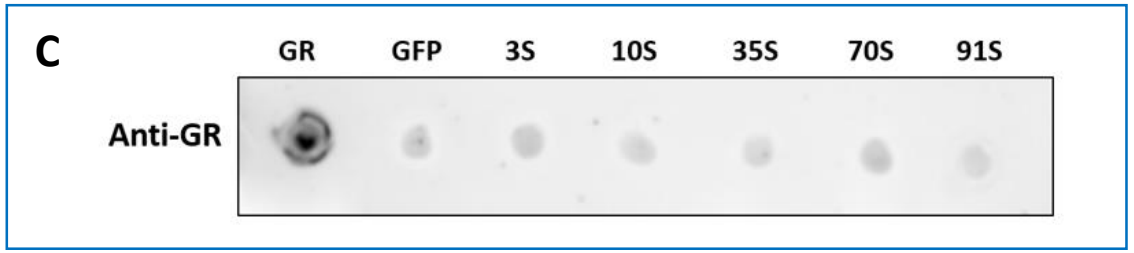
All (except PA) DPRs are detected

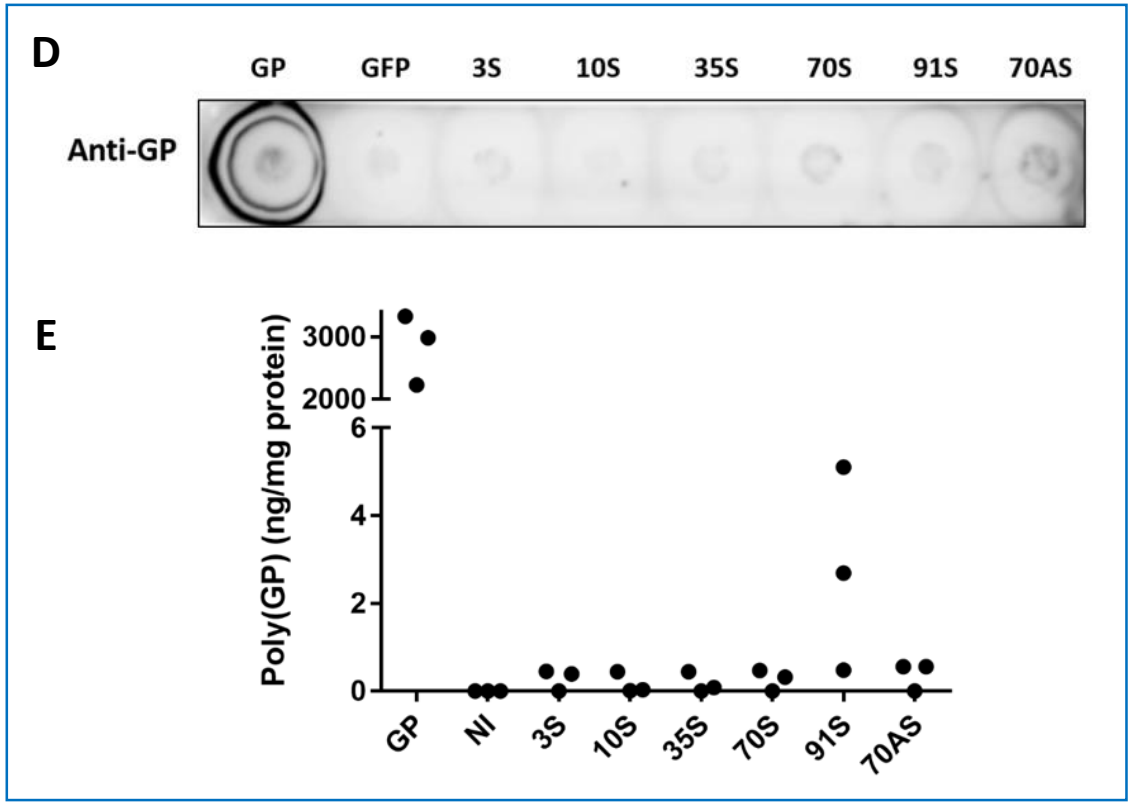


3) Can repeat RNA toxicity be caused by DPR toxicity?

GR, PR and GP are not detected in repeat RNA zebrafish





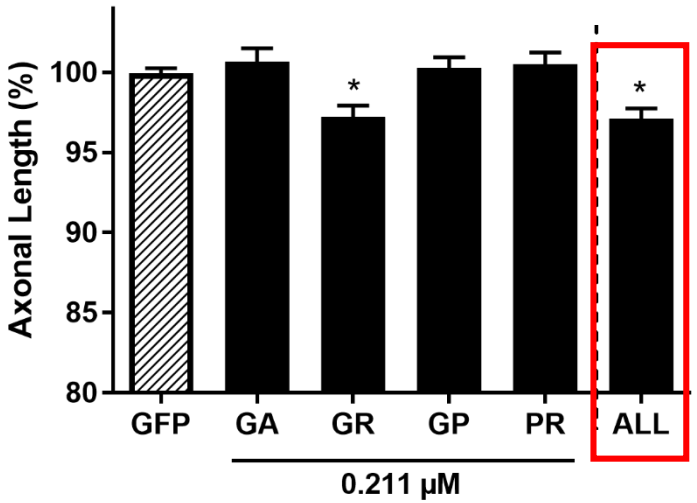


T. Gendron & L. Petrucelli

3) Can repeat RNA toxicity be caused by DPR toxicity?

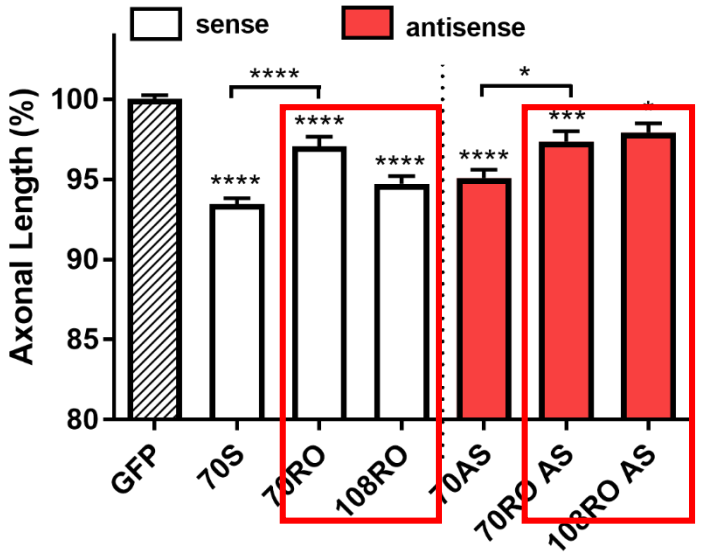
No synergistic toxicity between low doses of DPRs

A



'RNA only' constructs are toxic in zebrafish

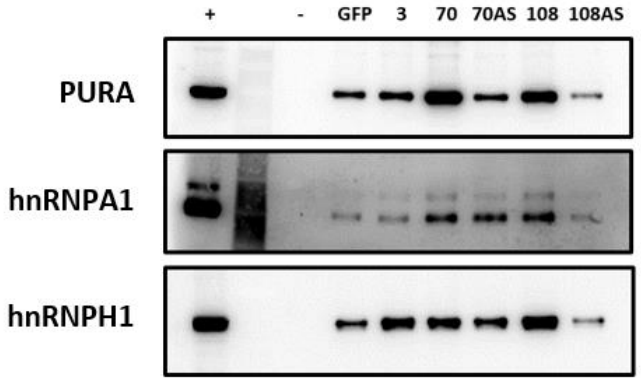
B



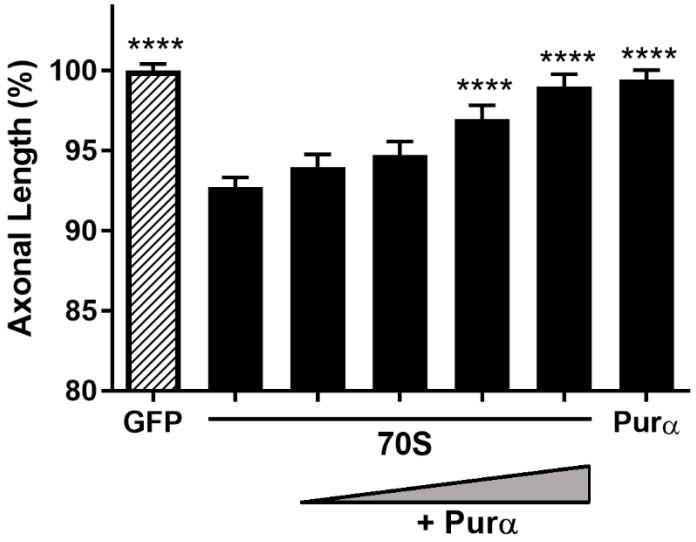
Mizielinska et al, Science 2014

4) Can repeat RNA toxicity be modified?

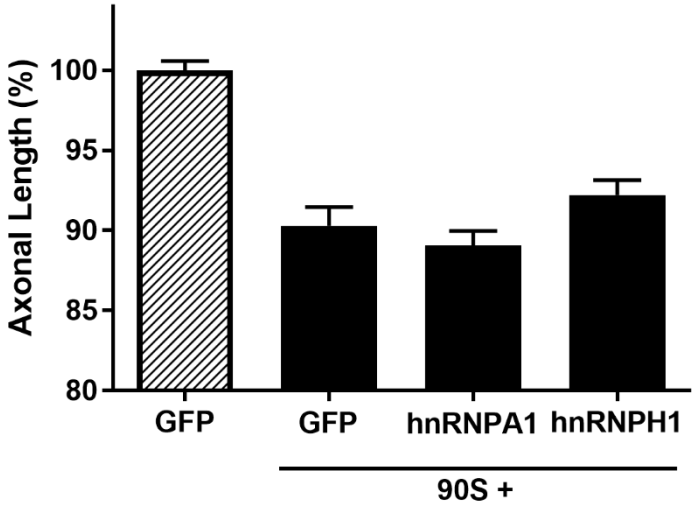
Purα, hnRNPA1 and hnRNPH1 bind repeat RNA



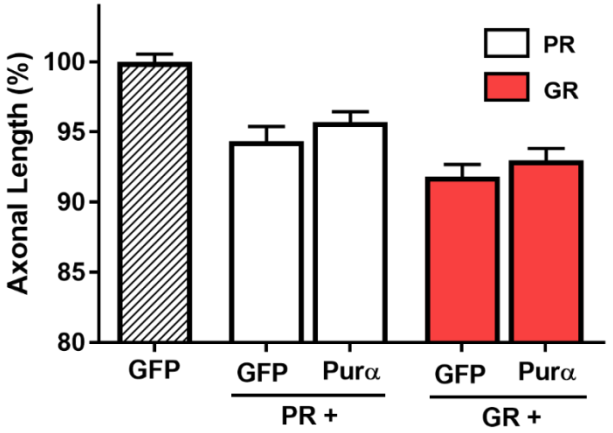
Purα prevents RNA toxicity



No effect of hnRNPA1 and hnRNPH1



Purα does not rescue DPR toxicity



5) p62 in RNA toxicity zebrafish model?

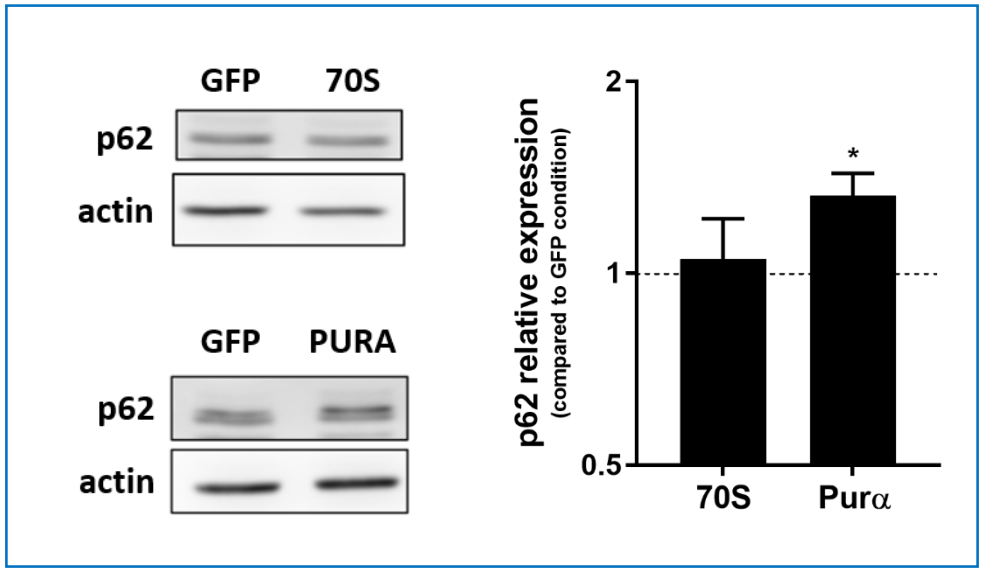
Almeida et al, Acta Neuropathol 2013

Dafinca et al, Stem Cells 2016

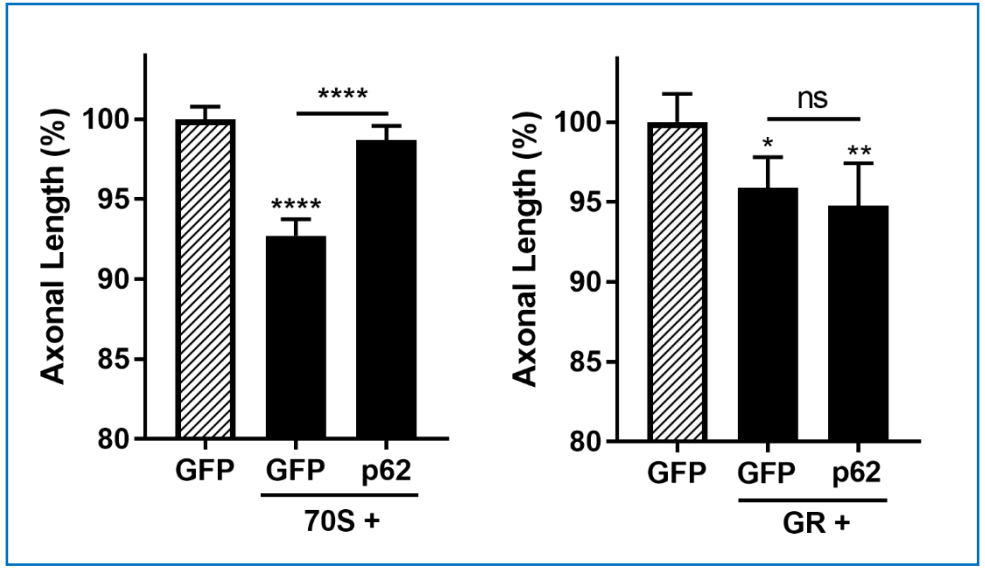
p62 is increased in C9orf72 iPSNs

p62 levels not altered in zebrafish model

Pur-alpha increases p62 levels



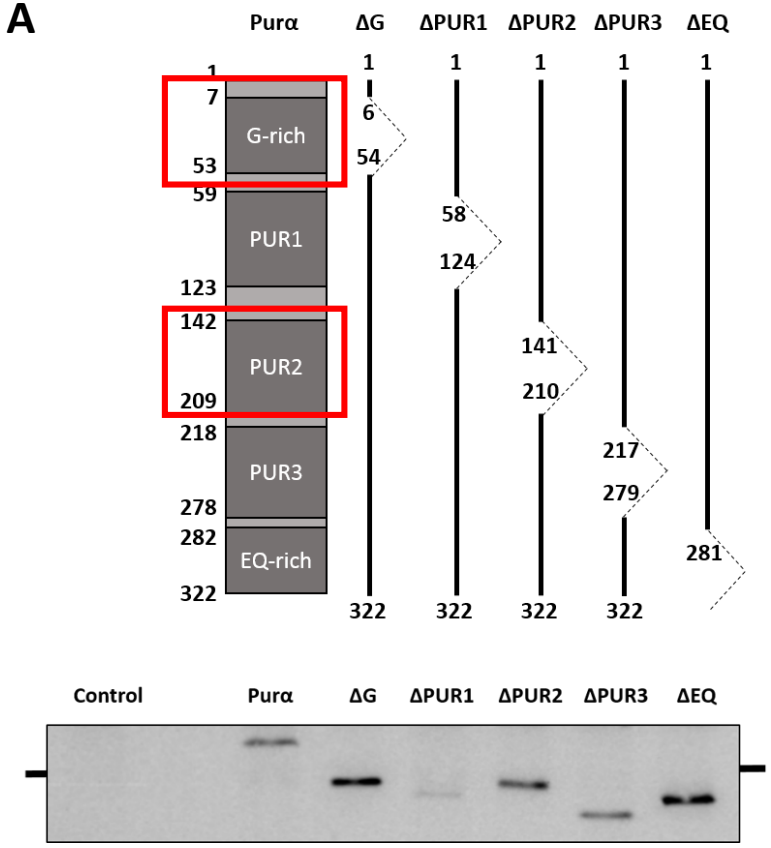
p62 prevents RNA toxicity



6) What is the mechanism of the rescue by Purα?

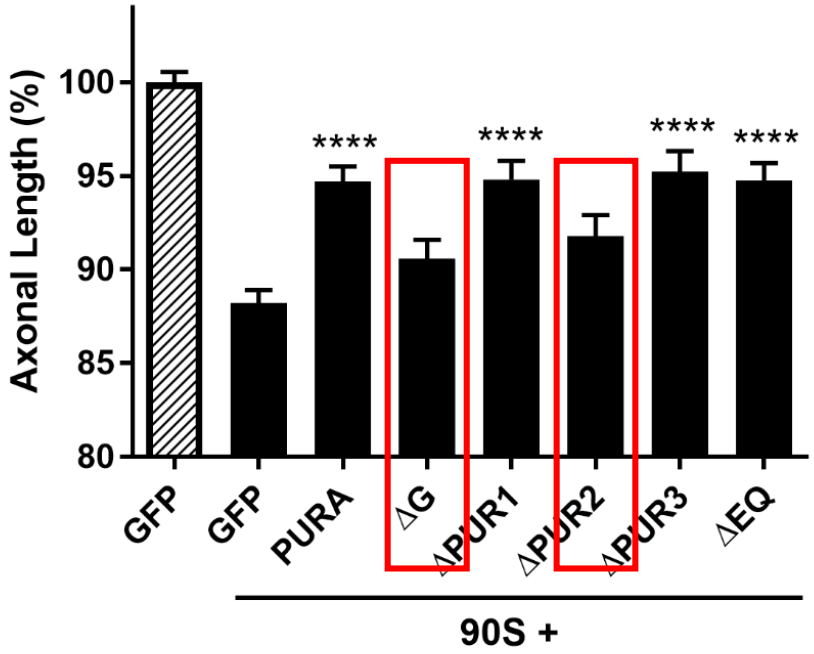
Purα deletion constructs

A

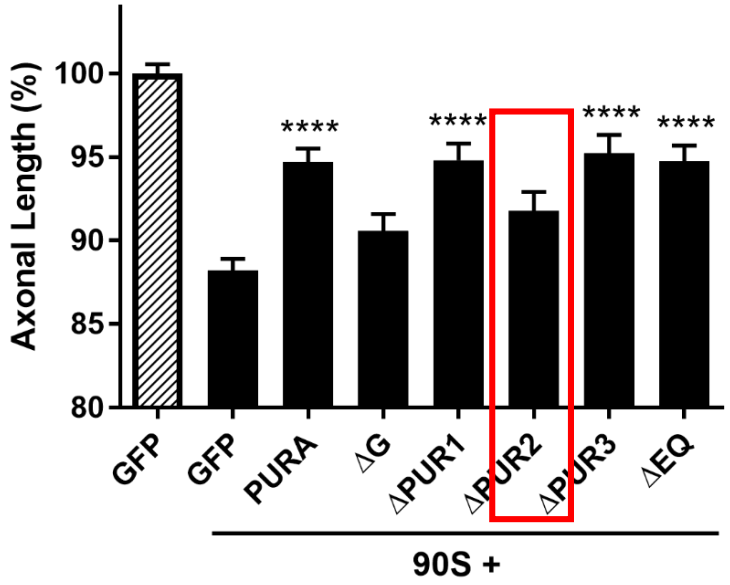


Deletion of <u>G-rich</u> or <u>PUR2</u> domain abrogates rescue of Purα

B

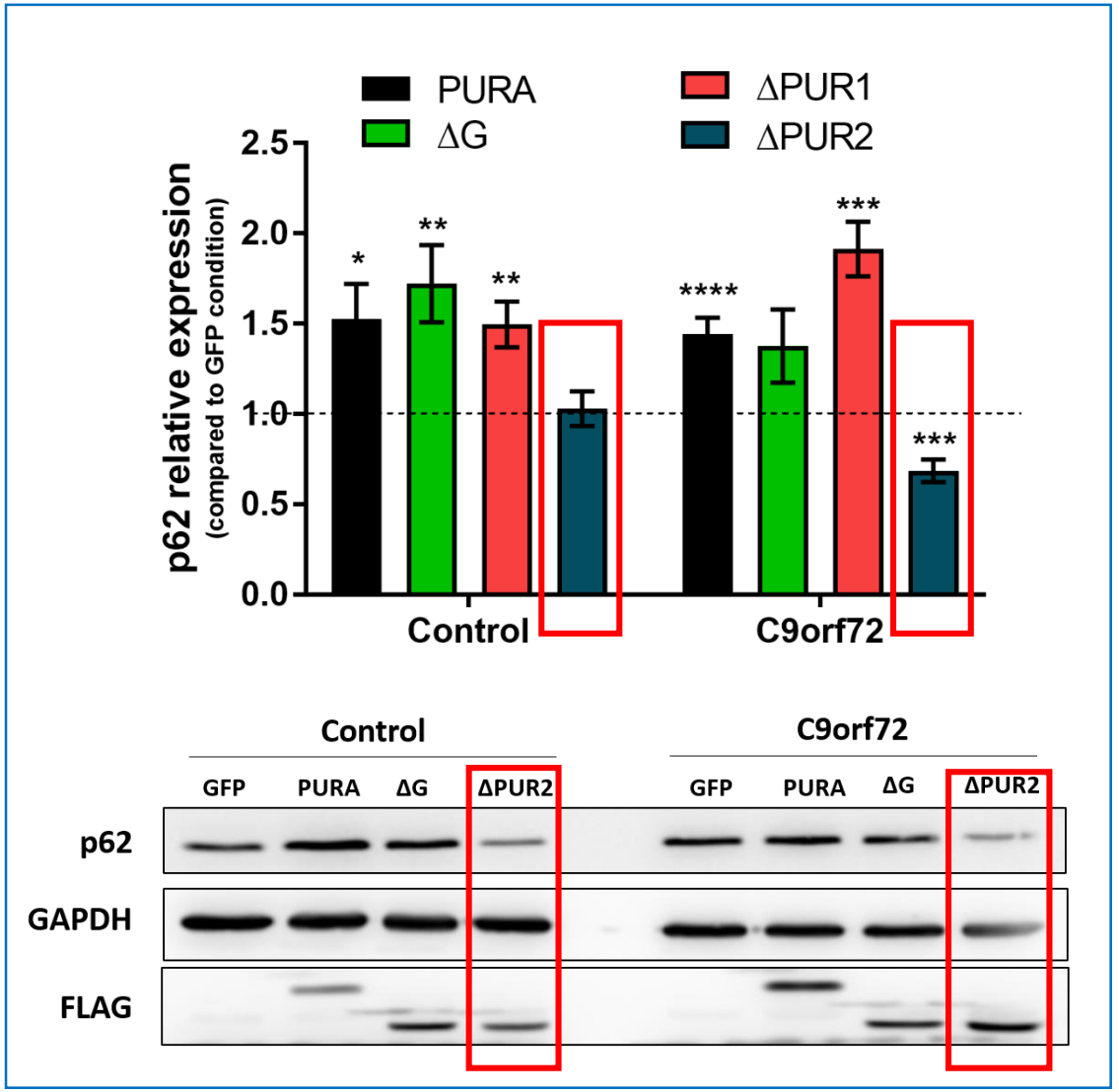


6) What is the mechanism of the rescue by Purα? – PUR2 domain

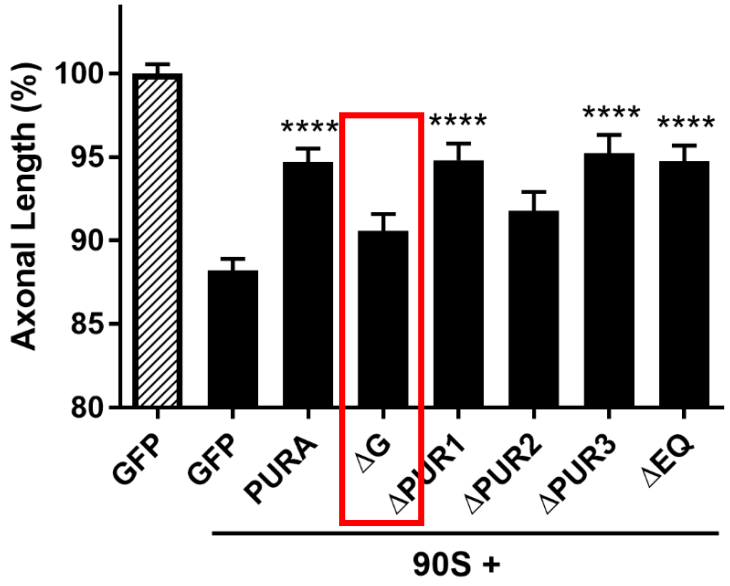


Patient derived fibroblasts

The PUR2 domain mediates p62 induction



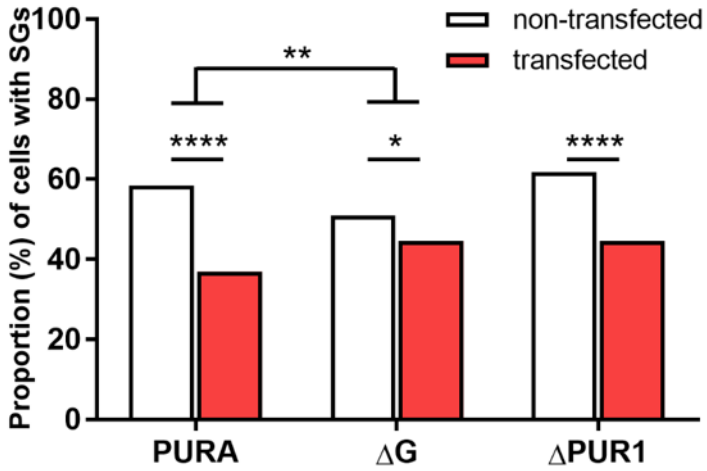
6) What is the mechanism of the rescue by Purα? – G-rich domain



Daigle et al, Acta Neuropathol 2016:

- Pur-alpha regulates SG dynamics
- Pur-alpha localizes to SGs
- G-rich domain is low complexity domain

The <u>G-rich</u> domain is involved in SGs (?)



Summary

1) Arginine rich DPRs (PR & GR) are toxic in zebrafish

2) Sense and antisense repeat RNA cause direct RNA toxicity in the zebrafish independent of DPR toxicity

3) Overexpression of Purα prevents direct RNA toxicity
   - PUR2 domain: p62
   - G-rich domain: stress granules

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  - Adrian Isaacs
  - Sarah Mizielinska
- **Department of Neuroscience, Mayoclinic, Jacksonville, USA**
  - Leonard Petrucelli
  - Tania Gendron
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