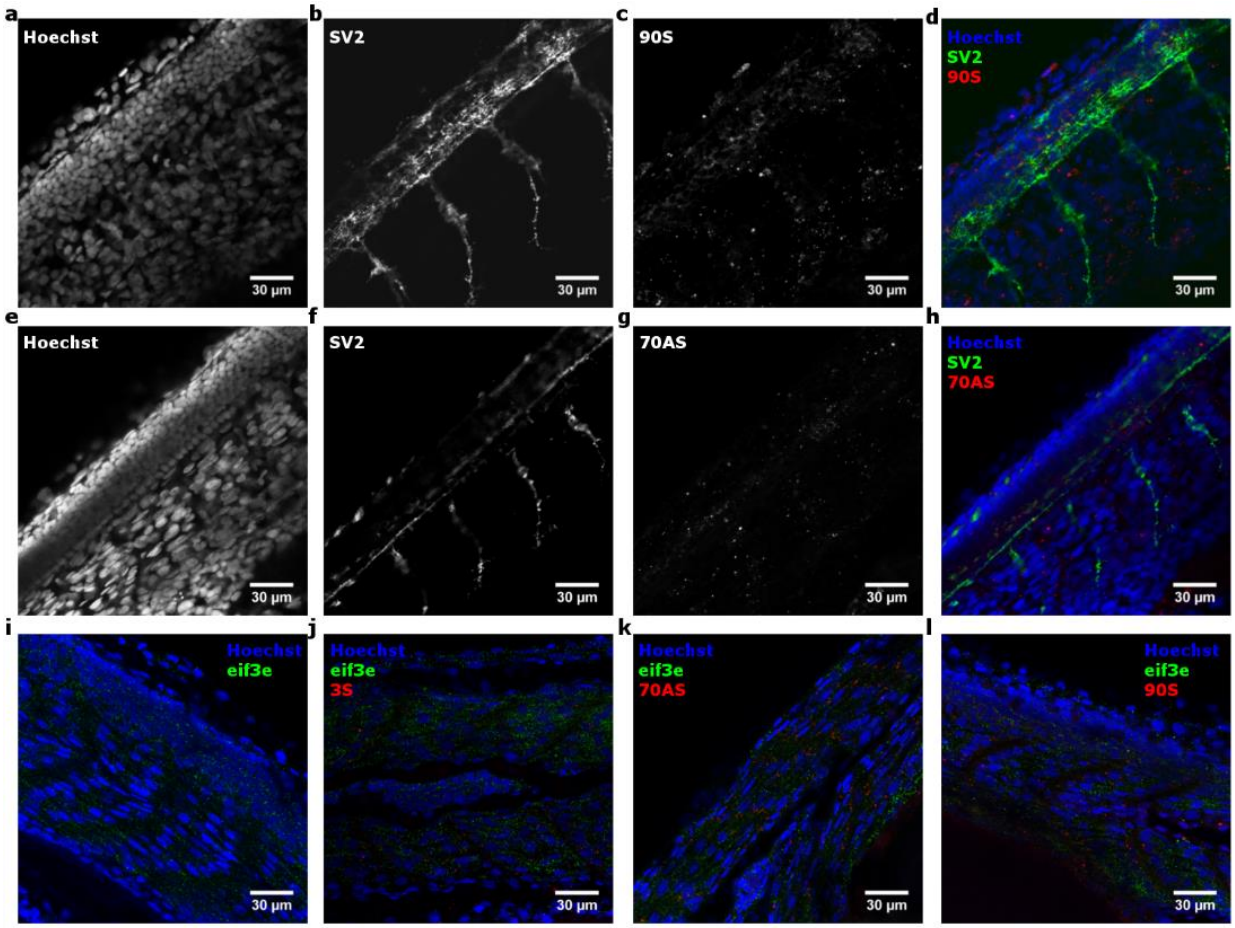
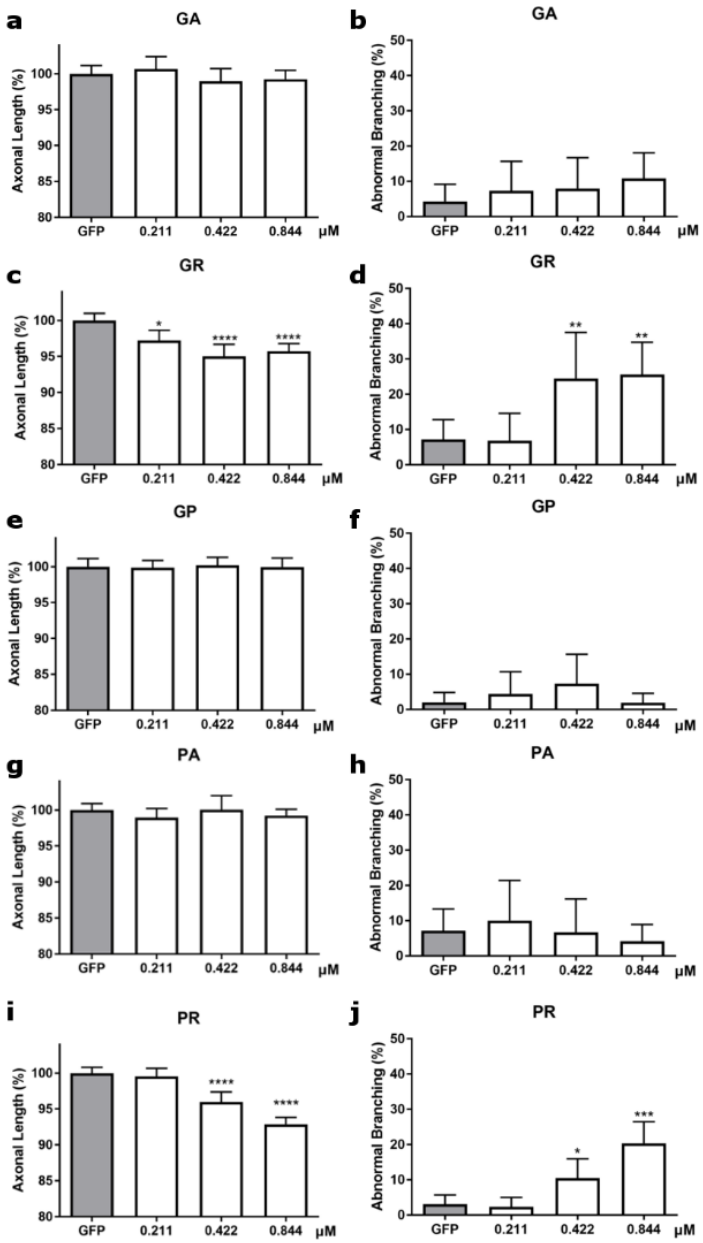
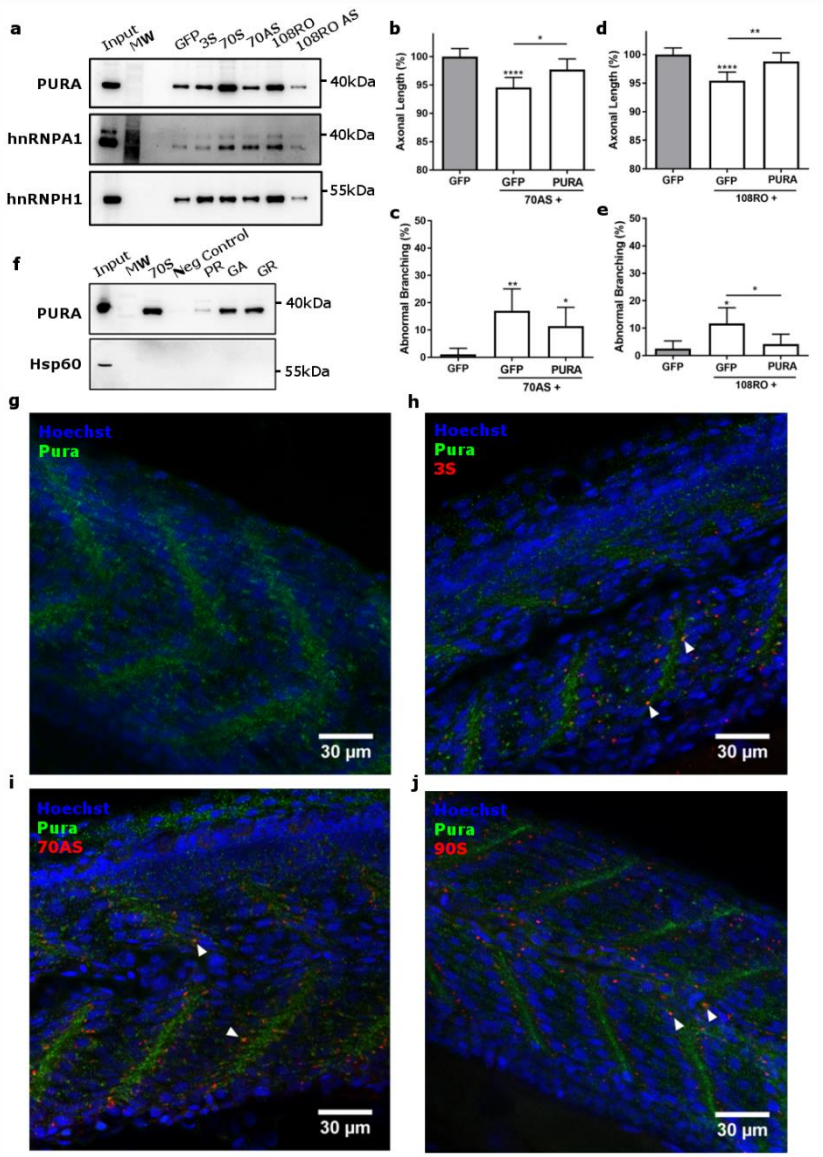


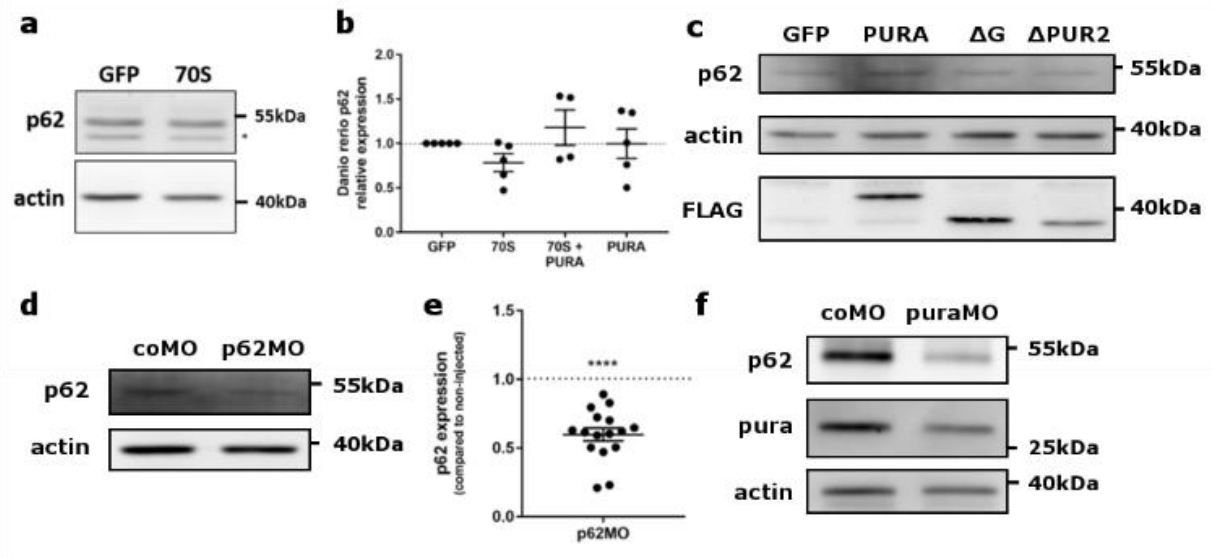
Online Resource 1. Localization of sense and antisense repeat RNA. (**a,b,c,d**) Whole mount immunohistochemistry (SV2 antibody, Hoechst) of 30 hpf zebrafish embryos injected with fluorescently labeled ~90S repeat RNA. (**e,f,g,h**) Whole mount immunohistochemistry (SV2 antibody, Hoechst) of 30 hpf zebrafish embryos injected with fluorescently labeled ~70AS repeat RNA. (**i,j,k,l**) Whole mount immunohistochemistry (eif3e antibody, Hoechst) of 30 hpf zebrafish embryos injected with 3S (**j**), ~70AS (**k**) or ~90S (**l**) and non-injected embryos (**i**).



Online Resource 2. Dose dependent toxicity of DPRs in zebrafish. (**a,b**) Dose response of (GA)50 mRNA, compared to GFP condition (0.844 μM) (n = 3 experiments). (**a**) Data represent mean +/- 95% CI, one-way ANOVA, F(3, 220) = 0.9918. (**b**) Data represent mean +/- 95% CI, logistic regression (z-values: 0.674, 1.184, 1.425). (**c,d**) Dose response of (GR)50 mRNA, compared to GFP condition (0.844 μM) (n = 3 experiments). (**c**) Data represent mean +/- 95% CI, one-way ANOVA, F(3, 258) = 14.83, *p<0.05, ****p<0.0001. (**d**) Data represent mean +/- 95% CI, logistic regression (z-values: -0.068, 2.623, 3.067), **p<0.01. (**e,f**) Dose response of (GP)50 mRNA, compared to GFP condition (0.844 μM) (n = 6 experiments). (**e**) Data represent mean +/- 95% CI, one-way ANOVA, F(3, 287) = 0.02847. (**f**) Data represent mean +/- 95% CI, logistic regression (z-values: 0.810, 1.451, -0.049). (**g,h**) Dose response of (PA)50 mRNA, compared to GFP condition (0.844 μM) (n = 3 experiments). (**g**) Data represent mean +/- 95% CI, one-way ANOVA, F(3, 198) = 0.8611. (**h**) Data represent mean +/- 95% CI, logistic regression (z-values: 0.480, -0.086, -0.760). (**i,j**) Dose response of (PR)50 mRNA, compared to GFP condition (0.844 μM) (n = 9 experiments). (**i**) Data represent mean +/- 95% CI, one-way ANOVA, F(3, 585) = 43.84, ****p<0.0001. (**j**) Data represent mean +/- 95% CI, logistic regression (z-values: -0.376, 2.432, 4.257), *p<0.05, ***p<0.001.



Online Resource 3. Pura in zebrafish embryos. (**a**) RNA pull-down from mouse brain lysate. Western blot analysis of Pur-alpha, hnRNPA1 and hnRNPH1, showing the binding affinity to different sense and antisense repeat RNAs, n = 2 biological replicates (Input = brain lysate, MW = molecular weight marker). (**b,c**) Effect of Pur-alpha (0.573µM) on ~70AS (0.844 µM) axonal toxicity (n = 6 experiments). (**b**) Data represent mean +/- 95% CI, one-way ANOVA, $F(2, 262) = 10.52$, *p<0.05, ****p<0.0001. (**c**) Data represent mean +/- 95% CI, logistic regression (z-values compared to GFP: 2.782, 2.308; 70AS + GFP vs 70AS + Pur-alpha: z-value = 1.043, p = 0.297), *p<0.05, **p<0.01. (**d,e**) Effect of Pur-alpha (0.573µM) on 108RO (0.844 µM) axonal toxicity (n = 8 experiments). (**d**) Data represent mean +/- 95% CI, one-way ANOVA, $F(2, 357) = 10.89$, **p<0.01, ****p<0.0001. (**e**) Data represent mean +/- 95% CI, logistic regression (z-values compared to GFP: 2.521, 0.712; 108RO + GFP vs 108RO + Pur-alpha: z-value = -2.065, p = 0.0389), *p<0.05. (**f**) RNA pull-down from mouse brain lysate. Western blot analysis of Pur-alpha, showing the binding affinity to different DPR coding codon-optimized mRNAs, n = 2 biological replicates. (Input = brain lysate, MW = molecular weight marker, Negative Control = non-biotinylated ~70S repeat RNA). Hsp60 was used as negative control for the RNA pull-down. (**g, h, i, j**) Whole mount immunohistochemistry (pura antibody, Hoechst) of 30 hpf zebrafish embryos injected with 3S (**h**), ~70AS (**i**) or ~90S (**j**) and non-injected embryos (**g**). Arrowheads indicate sites of colocalization of pura with repeat RNA.



Online Resource 4. p62 in zebrafish embryos. (**a**) *Danio rerio* p62 protein levels in 30 hpf zebrafish embryos injected with equimolar amounts (0.844 µM) of GFP or ~70S RNA as assessed by Western blot (n = 7 experiments) (***** aspecific band). (**b**) *Danio rerio* p62 mRNA levels in 30 hpf zebrafish embryos as assessed by qPCR. Data represent mean +/- SEM, statistics are compared to GFP condition, n = 5 experiments, one-way ANOVA, $F(3, 15) = 1.437$. (**c**) *Danio rerio* p62 protein levels in 30hpf zebrafish embryos upon overexpression of PURA, ΔG or ΔPUR2 as assessed by Western blot. Transgene expression was confirmed with FLAG antibody. (**d**) *Danio rerio* p62 protein levels in 30 hpf zebrafish embryos upon injection of p62 morpholino as assessed by Western blot (n = 16 experiments), quantification in (**e**). Data represent mean +/- SEM, statistics are compared to non-injected condition, ratio-paired t-test ($t_{15} = 5.695$), ****p<0.0001. (**f**) *Danio rerio* p62 and pura protein levels in 30 hpf zebrafish embryos upon injection of pura morpholino as assessed by Western blot.