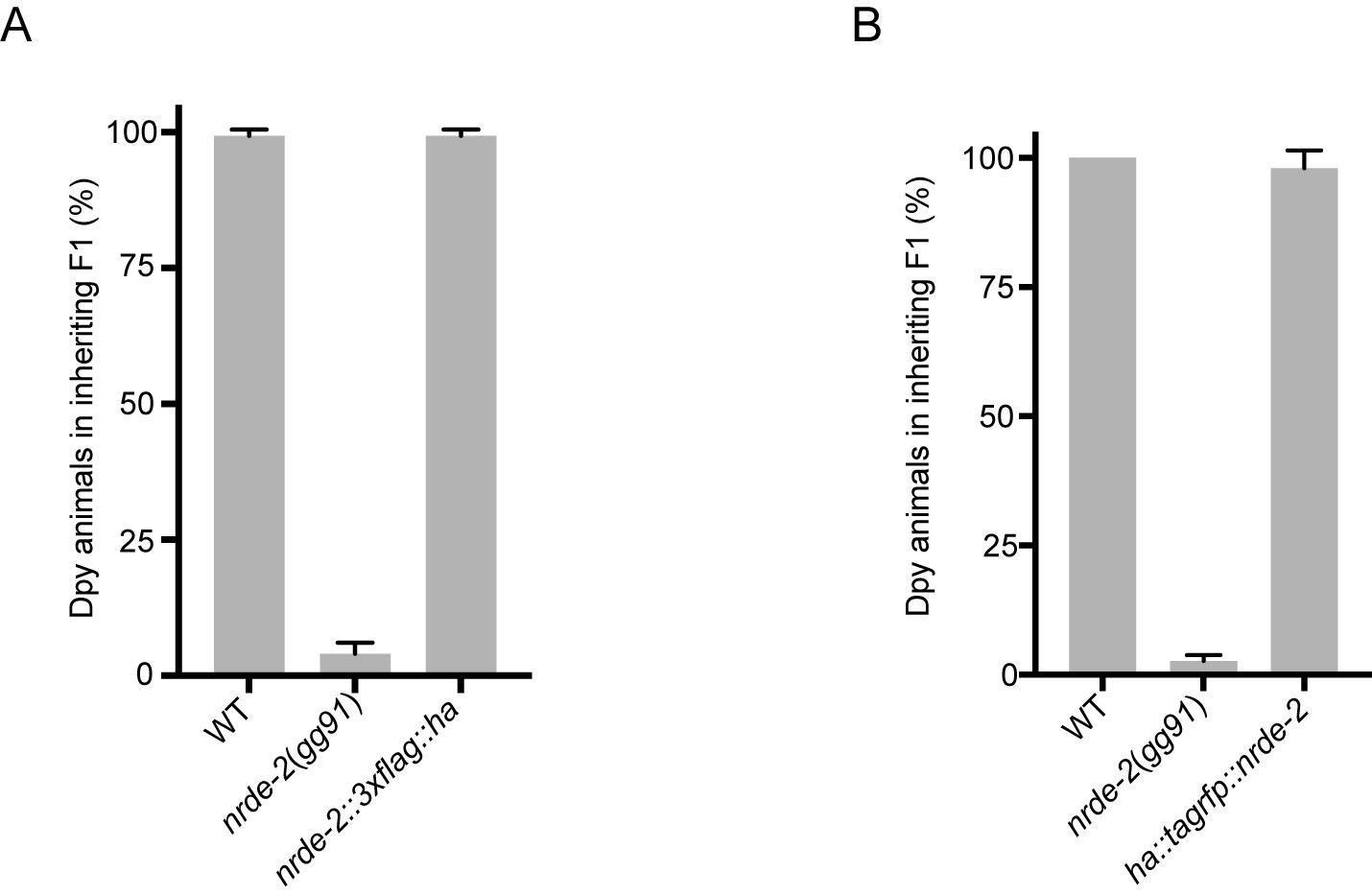
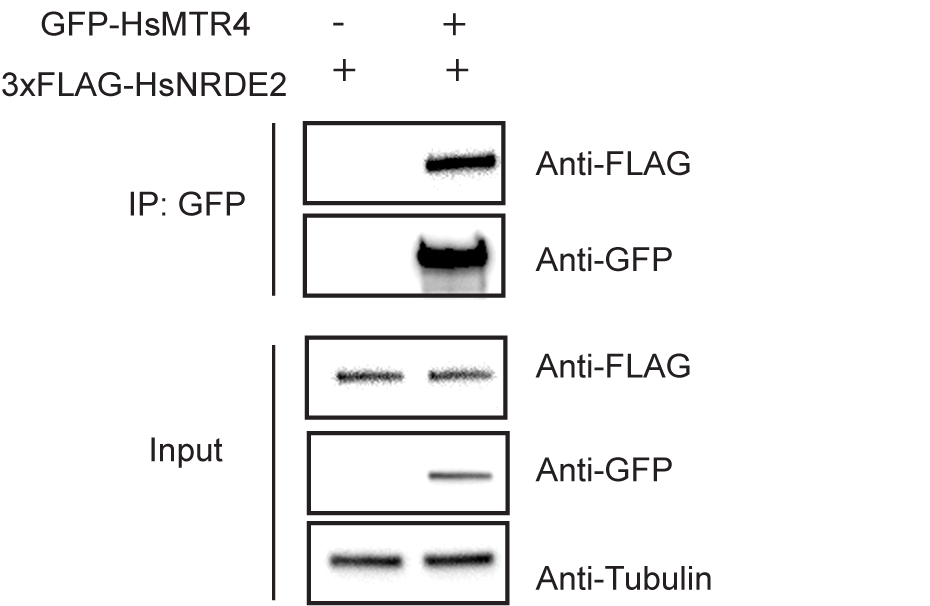
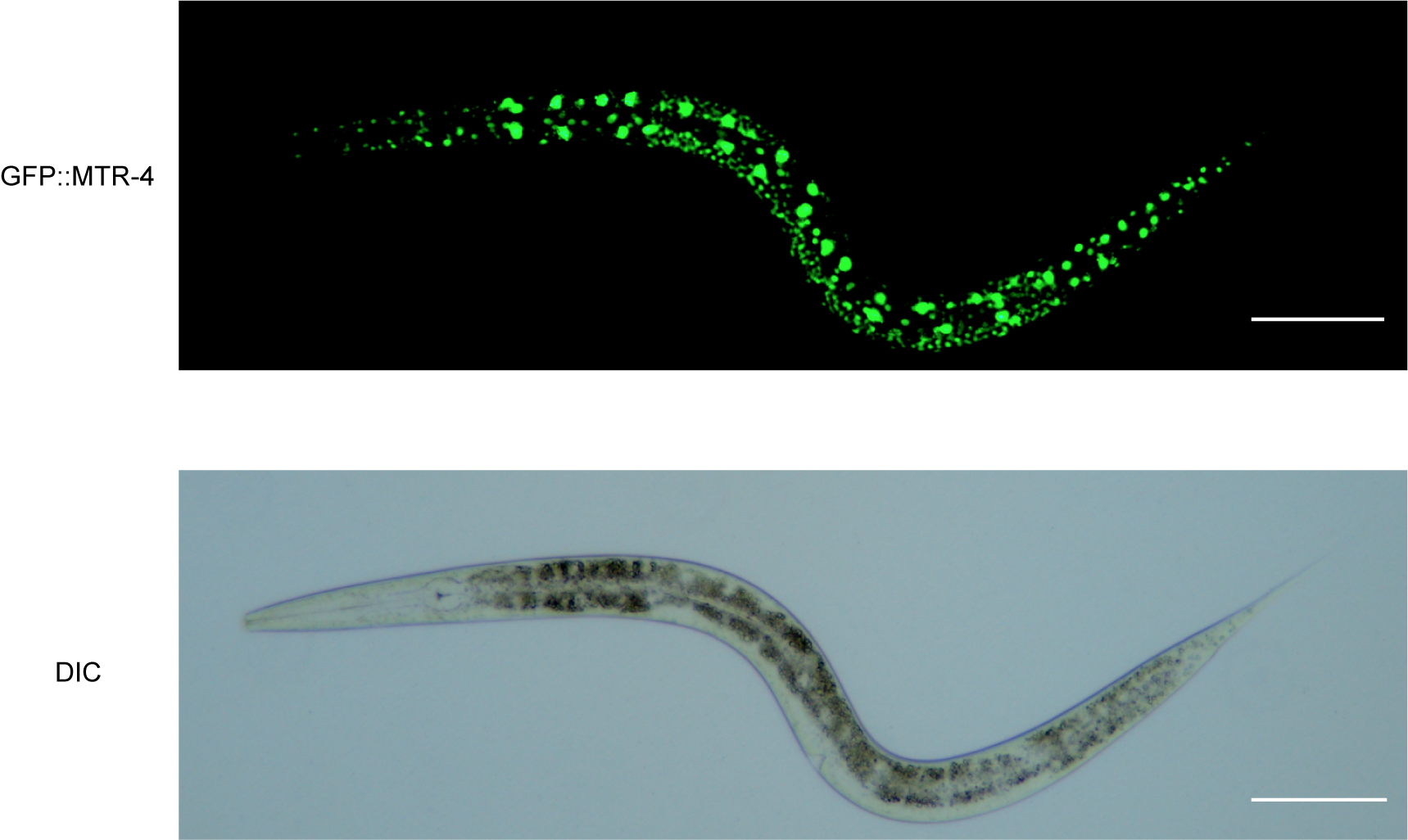
**Supplemental figure legends**:



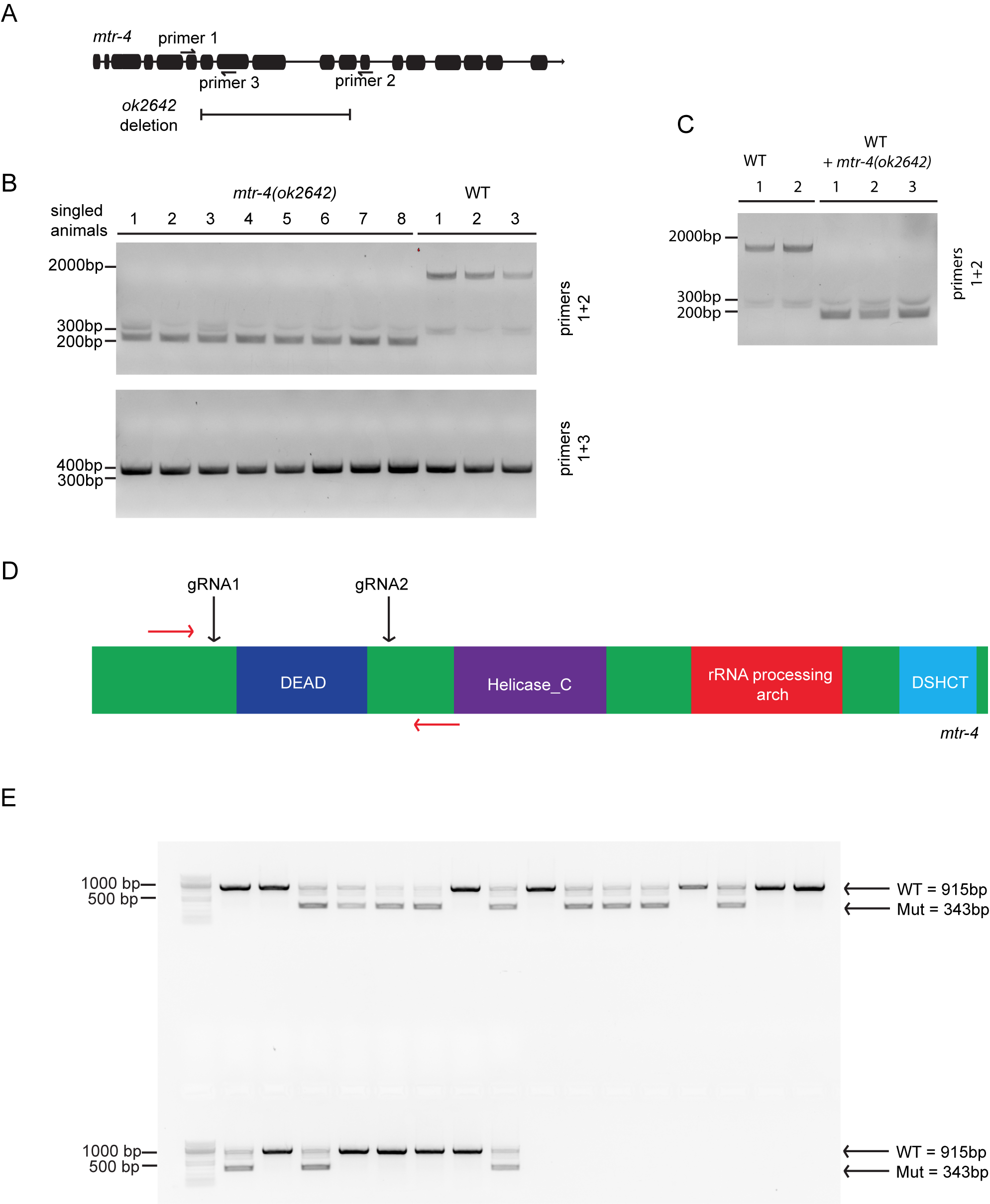
**Supplemental figure 1. CRISPR/Cas9 tagged *nrde-2* produces functional proteins.** (A-B) Animals of the indicated genotypes were subjected to *dpy-11* RNAi. The % of progeny exhibiting a Dpy phenotype is indicted. Note, epitope tagged alleles of *mtr-4* described in this study also generated functional MTR-4 proteins as indicated by the fact that these animals appeared normal, while animals with the function of MTR-4 disrupted are lethal (see Figure 3 and S4).



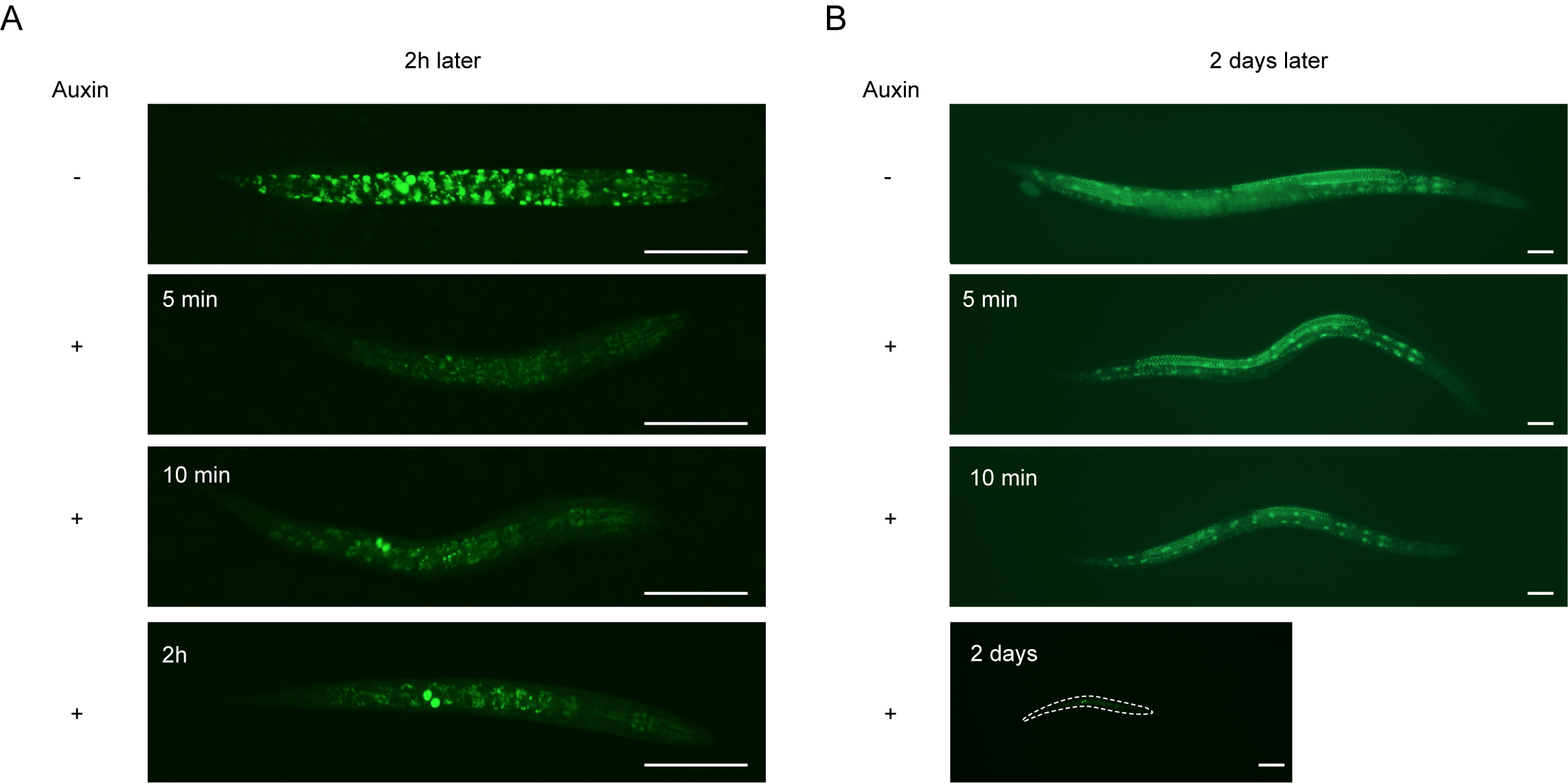
**Supplemental figure 2. Human NRDE2 and MTR4 interact.** Co-IP analysis of GFP-HsMTR4 and 3xFLAG-HsNRDE2 from human HEK293T cells were IP’ed with anti-GFP antibodies and detected by Western blot with indicated antibodies.



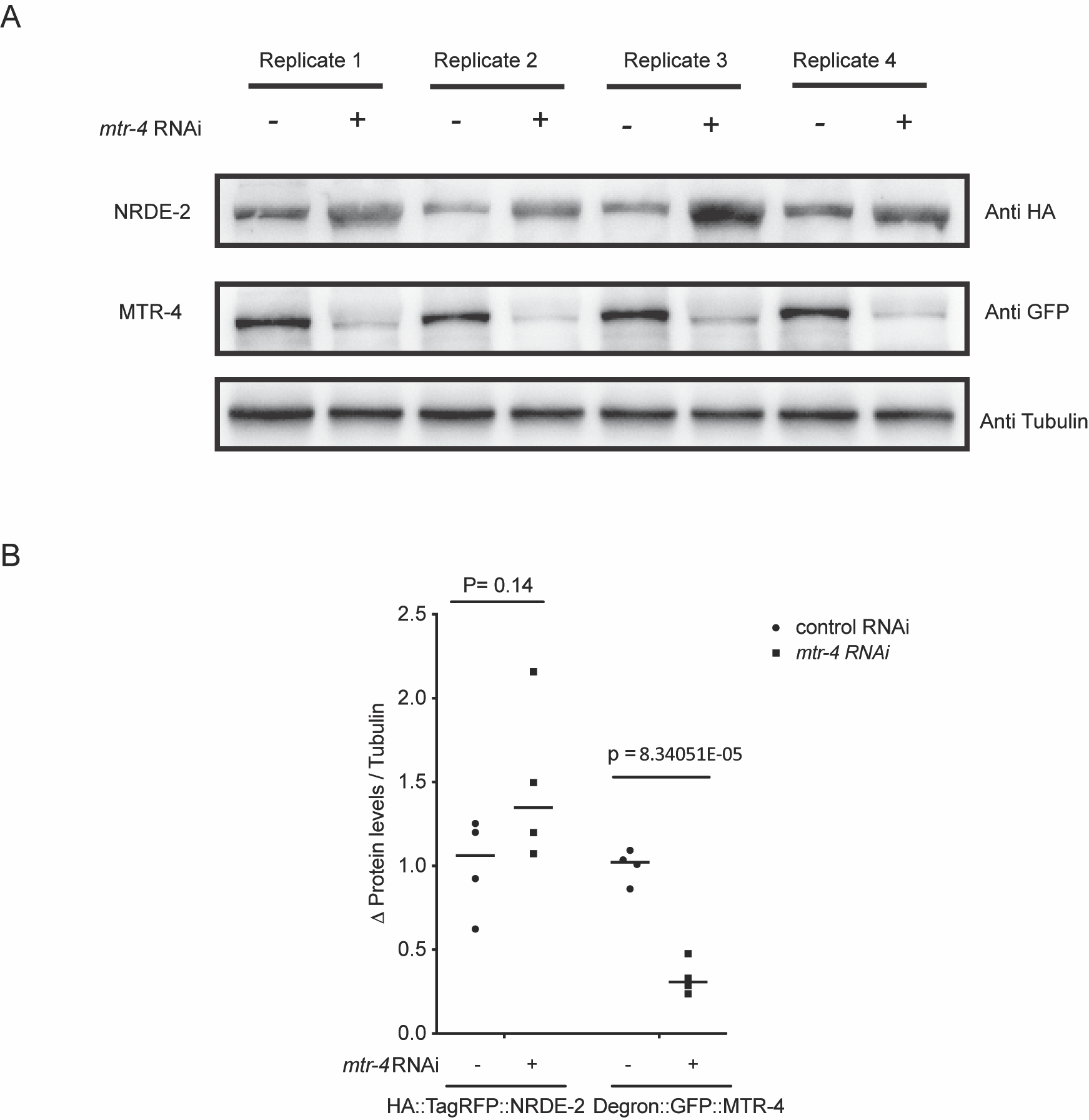
**Supplemental figure 3.** Fluorescent and light micrographs of a larva animal expressing GFP::MTR-4. DIC, differential interference contrast. Scale bar: 20μm.

****

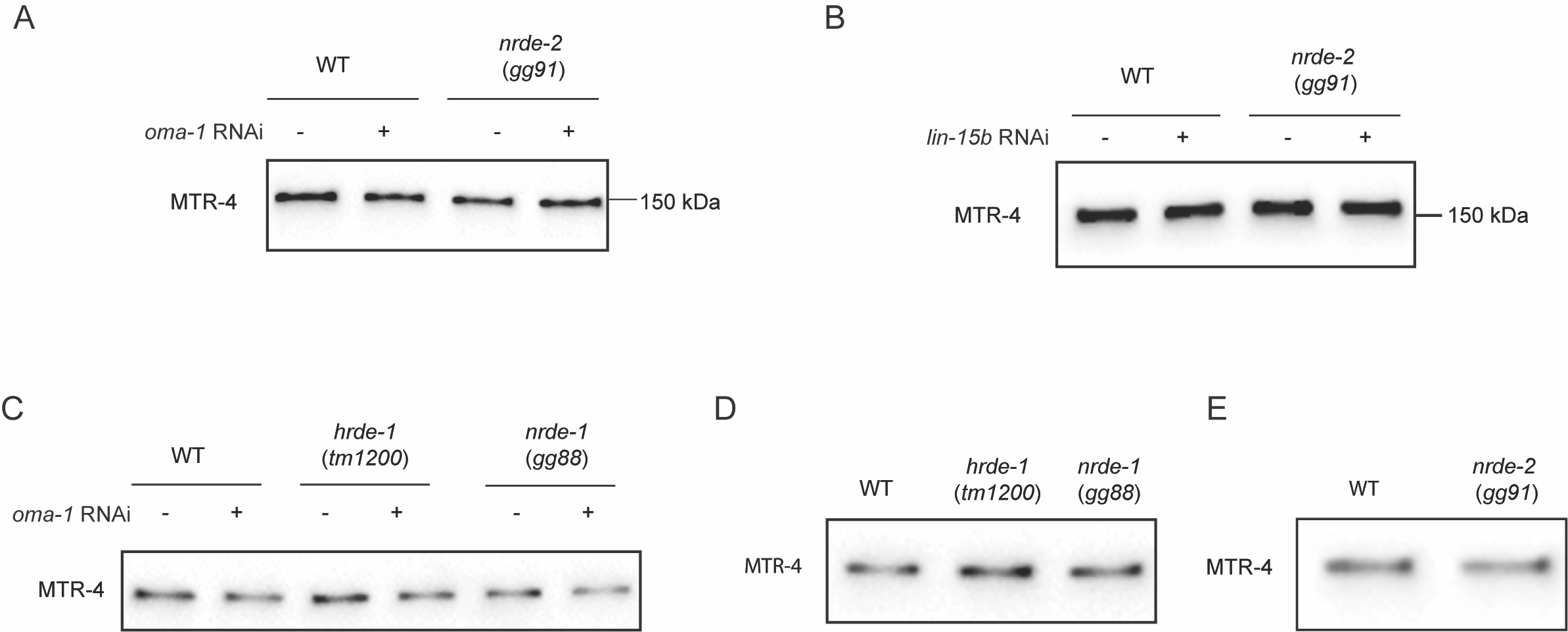
**Supplemental Figure 4. Characterization of *mtr-4 (ok2642)* and attempts to identify a *mtr-4* null allele.** (A) Cartoon representation of *mtr-4* gene. Solid blocks represent exons. Primers used in genotyping assay in (B-C) are indicated with arrows. Location of presumed *ok2642* deletion is indicated. (B, upper panel) PCR-based detection of predicted wild-type (1797bp) and *ok2642* (243bp) sized PCR products using primers 1 and 2. (B, lower panel) PCR-based detection of a predicted wild-type (378bp) sized PCR product in both wild-type and *ok2642* animals using primers 1 and 3. (C) Mixing wild-type and *ok2642* DNA at a 1:1 ratio causes only the shorter *ok2642* deletion PCR product to be detected, possibly explaining why the longer WT PCR product is not observed in *ok2642* animals shown in (B). (B-C) Together, these data suggest that *ok2642* may harbor the predicted *ok2642* deletion but also a wild-type copy of the *mtr-4* gene. It seems reasonable to speculate that the PCR-based screening used to identify *ok2642* selected for this type of complex *mtr-4* locus rearrangement, as evidence presented below (and elsewhere in this work) indicate that a clean deletion would be lethal. (D) Cartoon representation of the MTR-4 protein as well as locations of guide RNAs used to delete the MTR-4 DEAD box helicase domain. Red arrows indicate genotyping primers used in (E). (E) PCR-based detection of the anticipated MTR-4 DEAD box helicase deletion in 24 progeny of an animal heterozygous for the DEAD box deletion. No progeny are homozygous for the deletion. Genotyping of an additional 36 animals failed to detect a homozygote. The data argue that the MTR-4 DEAD box deletion allele is likely lethal.



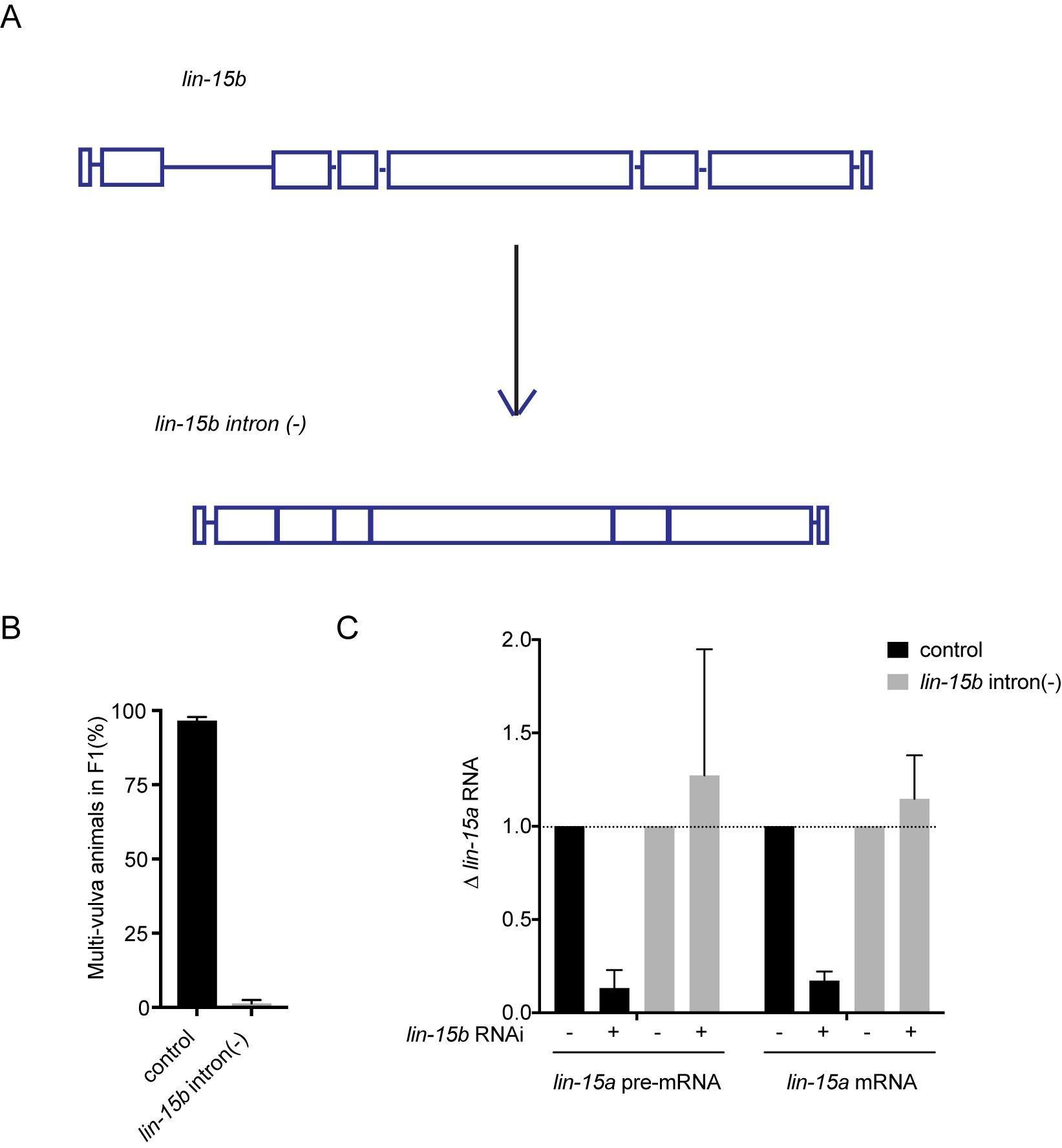
**Supplemental Figure 5.Short-term 5-10 minute auxin exposure temporarily depletes degron::GFP::MTR-4 but is not sufficient to cause larval arrest.** Animals were treated with -/+ 1mM auxin for indicated time, fluorescent images were taken 2h **(A)** or 2 days **(B)** post-treatment. Scale bar, 50μm. Dashed line indicates the shape of the arrested larval animal. Larva images were taken at 20x magnification, while adult images were taken at 10x.



**Supplemental figure 6. Loss of MTR-4 does not trigger a loss of NRDE-2.** (A) Western blotting was used to monitor NRDE-2::3xFLAG::HA and 3xFLAG::GFP::MTR-4 levels in animals treated with +/- *mtr-4* RNAi. Replicates 1 and 2 are technical replicates of Figure 5A. Replicates 1-4 are biological replicates. The data show that RNAi-mediated depletion of MTR-4 does not alter levels of NRDE-2. (B) Proteins level in (A) were quantified with imageJ. Results are expressed as a ratio relative to a Tubulin loading control. Error bars are +/- s.d. N=4. P value, student’s-t test, two-tailed.



**Supplemental figure 7. Controls showing that similar levels of MTR-4 were IP’ed for RIP experiments shown in the main text.** (A) figure 5B, (B) figure 5C, (C) figure 6A, (D) figure 6C, (E) figure 6D.



**Supplemental figure 8. Removal of introns from the *lin-15b* gene causes *lin-15b* to become recalcitrant to cTGS.** (A) Schematic of the *lin-15b* locus or the *lin-15b* intron(-) locus after introns 2-6 were removed by CRISPR/Cas9. (B) *lin-15b* RNAi-induced multi-vulva (Muv) formation is, like *lir-1* RNAi, an “operon assay” that lets nuclear RNAi or cTGS be scored [(Guang et al. 2008)](https://paperpile.com/c/jZWS2b/BNUHY). *lin-15b* RNAi triggers a NRDE-dependent silencing of the downstream *lin-15a* gene, which induces Muv [(Guang et al. 2008)](https://paperpile.com/c/jZWS2b/BNUHY). An *eri-1(mg366)* genetic background was used for this analysis because *eri-1(mg366)* enhances RNAi, which allows Muv to be easily scored after *lin-15b* RNAi [(Guang et al. 2008)](https://paperpile.com/c/jZWS2b/BNUHY). *eri-1(mg366)* (control) or *eri-1(mg366);lin-15b intron(-)* (*lin-15b intron(-)*) animals were treated with *lin-15b* RNAi for two generations and % multi-vulva animals in F1 were scored. (C) WT (control) or *lin-15b* intron(-) animals were treated with control RNAi or *lin-15b* RNAi, total RNA was isolated and converted to cDNA. *lin-15a* is co-transcribed with *lin-15b* as part of an operon. *lin-15a* RNAs levels were quantified with qRT-PCR. Data from control RNAi was set at 1 and data from *lin-15b* RNAi was expressed as fold change compared with respective control RNAi. Error bars are +/- s.d. N=3.

**Table S1.** List of proteins identified by NRDE-2 IP-MS. Sheet 1 provides a list of proteins identified by 0 peptides from control extracts and more than 20 peptides from *nrde-2::3xflag::ha* extracts. Sheet 2 is a list of all proteins identified from each sample. For WT control, protein FDR is 5.68%, peptide FDR is 0.71%, and for *nrde-2::3xflag::ha*, protein FDR is 3.57%, peptide FDR is 0.73%.

**Primers used in this study:**

Primers for H3K9me3 ChIP:

|  |  |
| --- | --- |
| Primer name | Primer sequence |
| *oma-1* 1F | TTGTTAAGCATTCCCTGCAC |
| *oma-1* 1R | TCGATCTTCTCGTTGTTTTCA |
| *oma-1* 2F | GTGAGTTTAACAAAATTCAAATGAAAA |
| *oma-1* 2R | AGTGGTGAAACGGGCAAAGT |
| C09G9.5 F | CCAACGAAGCTTTGTGGATA |
| C09G9.5 R | GCGTGTGGGAACCTAGAAAA |
| *spr-2* F | AAACTTTCGCAATGTAACTCTTCC |
| *spr-2* R | CTGGTAGTGCTGCGTACGTG |
| *rad-26* F | CGATGAAAGAAGAGCAAGAAGAA |
| *rad-26* R | TTTTCATGATAAAACACTGGGAAA |
| *eft-3* | CCTGCAAGTTCAACGAGCTTA |
| *eft-3* | TGAAAAACAAATTGGTACATAAACG |

Primers for detecting *lin-15b* pre-mRNA after RIP:

|  |  |
| --- | --- |
| Primer name | Primer sequence |
| *lin-15b* 1F | ACAGCACGACTTACATCAAACCC |
| *lin-15b* 1R | TGGTTGGAATGCATTTGGAATAG |
| *lin-15b* 2F | ATCTGGCGTTTTCGATTCATTAC |
| *lin-15b* 2R | CCCAAATTTCACAACTACATTTG |
| *lin-15b* 3F | CCTAACAATCATAAACTCTC |
| *lin-15b* 3R | TGTCTAATTTTCCACAGAAC |
| *lin-15b* 4F | GGCTCAACGGCTTATGTACAGTG |
| *lin-15b* 4R | GGAAATGAAACTGAGGTTCAGAA |
| *lin-15b* 5F | GCCCTCCGTCCACACATTATTGC |
| *lin-15b* 5R | CAAAATGTGCAATTTTAAAAACG |
| *lin-15b* 6F | GAGGAGGAAGAGTATGAAGAATC |
| *lin-15b* 6R | CAAAGCATATATTTTTCAAAAAC |
| *lin-15a* 1F | GCATTGGAACGGATGCTCTGCAG |
| *lin-15a* 1R | GGGAGAAAATCCTTAAAAAGCCA |
| *lin-15a* 2F | TCCGCTTTACTCATTCAAGAATT |
| *lin-15a* 2R | AATTTTCACAAGTGTCACAAGAC |

Primers for detecting *oma-1* pre-mRNA or mRNA after RNAi or RIP:

|  |  |
| --- | --- |
| Primer name | Primer sequence |
| *oma-1* mRNA F | GCTTGAAGATATTGCATTCAACC |
| *oma-1* mRNA R | AACTGTTGAAATGGAGGTGC |
| *oma-1* pre-mRNA 1F | GTGCGTTGGCTAATTTCCTG |
| *oma-1* pre-mRNA 1R | CTGAATCGCGCGAACTTG |
| *oma-1* pre-mRNA 2F | TGTCTCAAACTAATCCGAATGC |
| *oma-1* pre-mRNA 2R | CAGGAAATTAGCCAACGCAC |
| *eft-3* mRNA | CGAGCTTAAGGAGAAGGTTG |
| *eft-3* mRNA | CCAGCATCTCCAGACTTGAG |