

Fig. S1. Differential expression of self and non-self siRNAs. **(A)** siRNA track plots for *oma-1* and the LTR retrotransposon *Cer3* in WT adult animals from the same sequencing run. Each sequenced small RNA read is indicated as a black block above (sense sRNA) or under (antisense sRNA) the gene track. **(B)** Box plot of average siRNA levels of native germline nuclear RNAi targets (non-self siRNAs) and germline genes (self siRNAs) in WT animals. The native germline nuclear RNAi target genes were obtained from (Ni, Chen et al. 2014). The germline genes are the oogenic genes identified in (Ortiz, Noble et al. 2014).

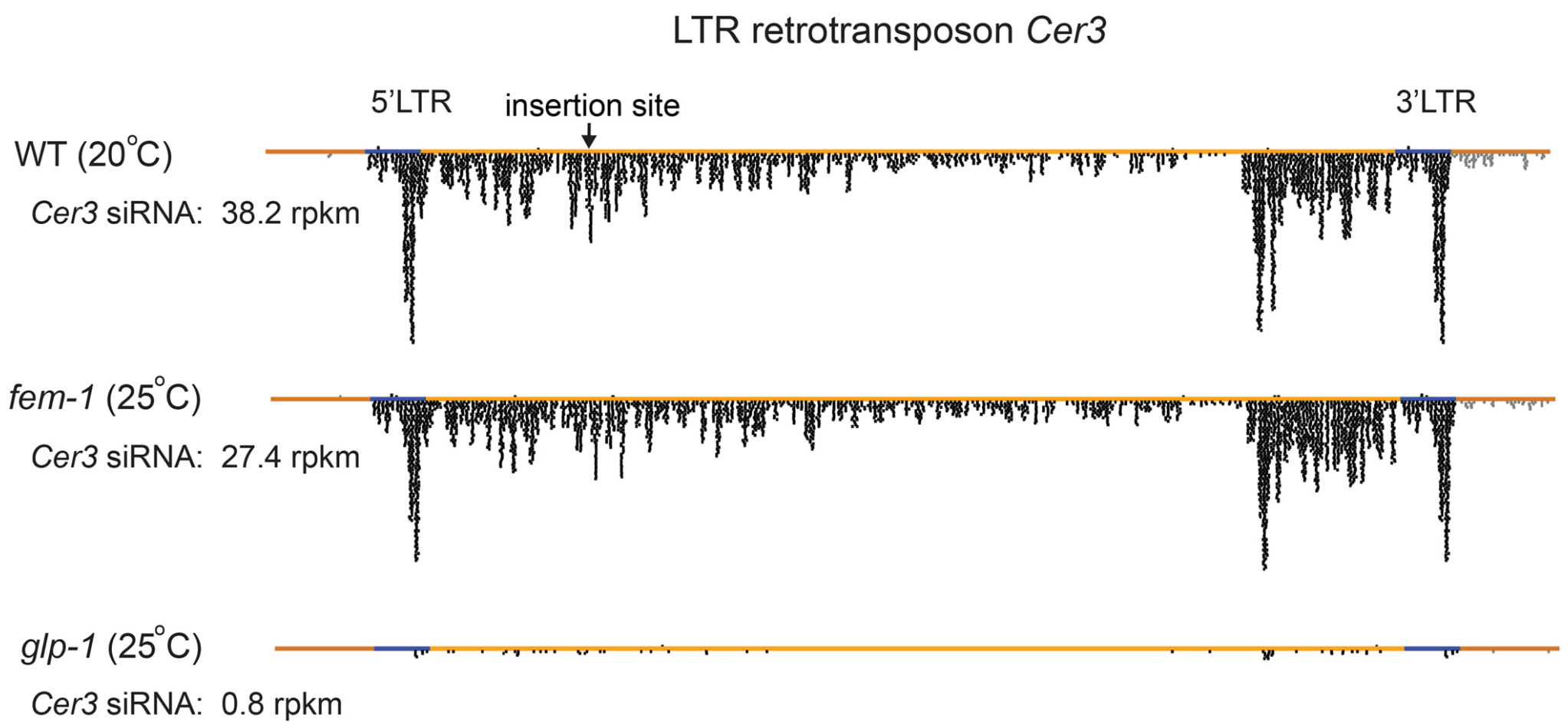


Fig. S2. Germline-enriched expression of *Cer3* siRNAs. *Cer3* siRNA track plots for adult WT (20°C), *fem-1*(*hc17*) (25° C, producing functional female germline, but lack of embryo in the uterus due to spermatogenesis defect) (Nelson, Lew et al. 1978), and *glp-1*(*e2141*) (25°C, germline depleted) (Kodoyianni, Maine et al. 1992) animals. As a quality control for the sRNA-seq, 30%, 12.7%, and 37% of sequenced small RNAs were mapped to microRNAs for WT, *fem-1*, and *glp-1*, respectively. The rpkms values of *Cer3* siRNAs are indicated in the figure. The insertion site in *Cer3* used in this study to express ectopic siRNAs is indicated.

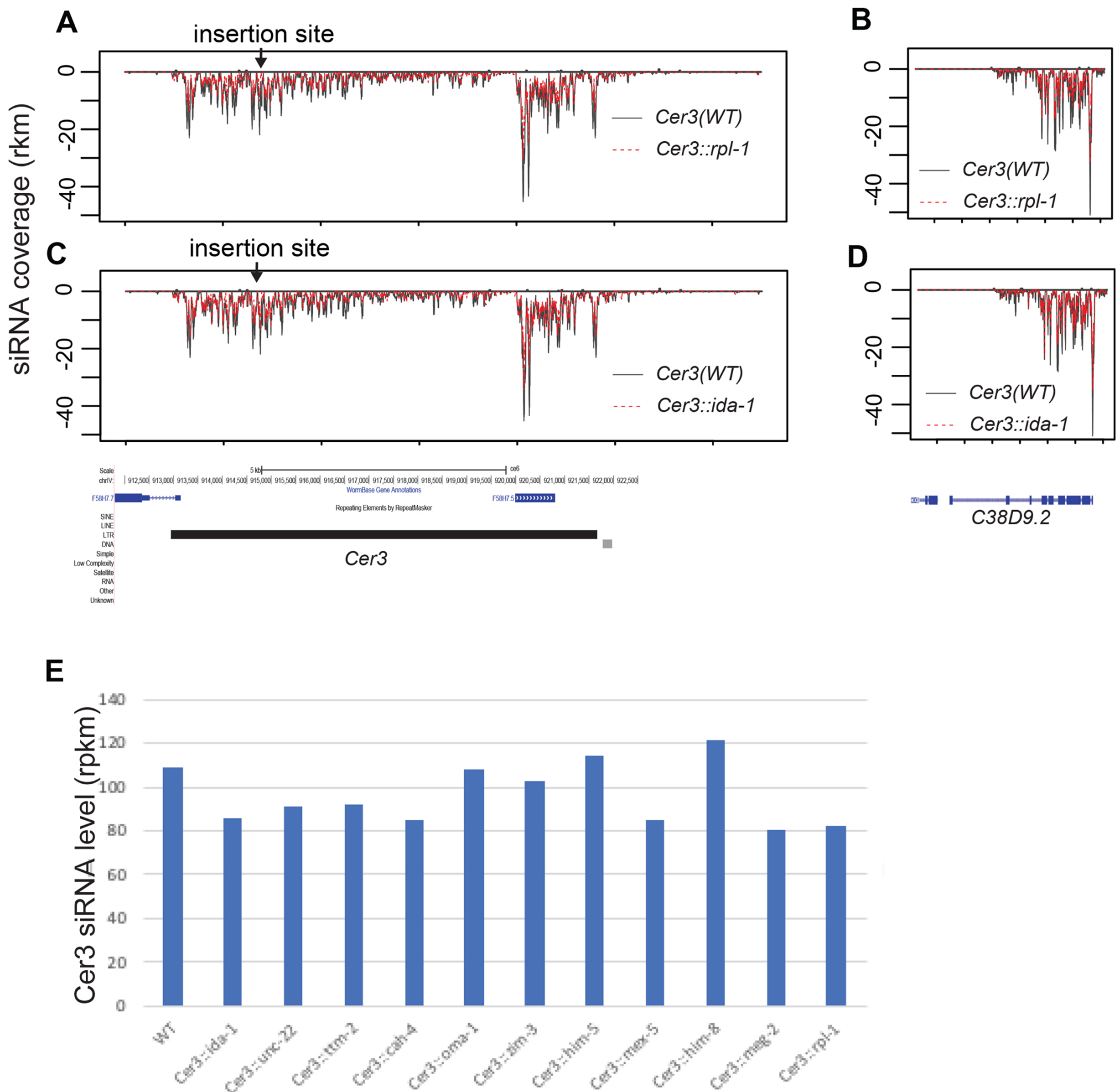


Fig. S3. *Cer3* siRNA expression is not affected by insertions. siRNA coverage plots at *Cer3* (A and C) and *c38d9.2* (B and D) are shown for strains carrying WT *Cer3*, *Cer3::rpl-1*, and *Cer3::ida-1* as indicated. Sense and antisense siRNA coverages are separately plotted as positive and negative values. siRNAs derived from the insertion were excluded from this analysis. The WT animals had slightly higher siRNA expressions than the two *Cer3* mutants for *Cer3* and other native nuclear RNAi targets, such as *c38d9.2* (B and D). This is likely due to a slight age difference between the samples (data not shown). (E) Bar graph showing *Cer3* siRNA levels (rpkm) for WT and various *Cer3::insertion* mutant strains.

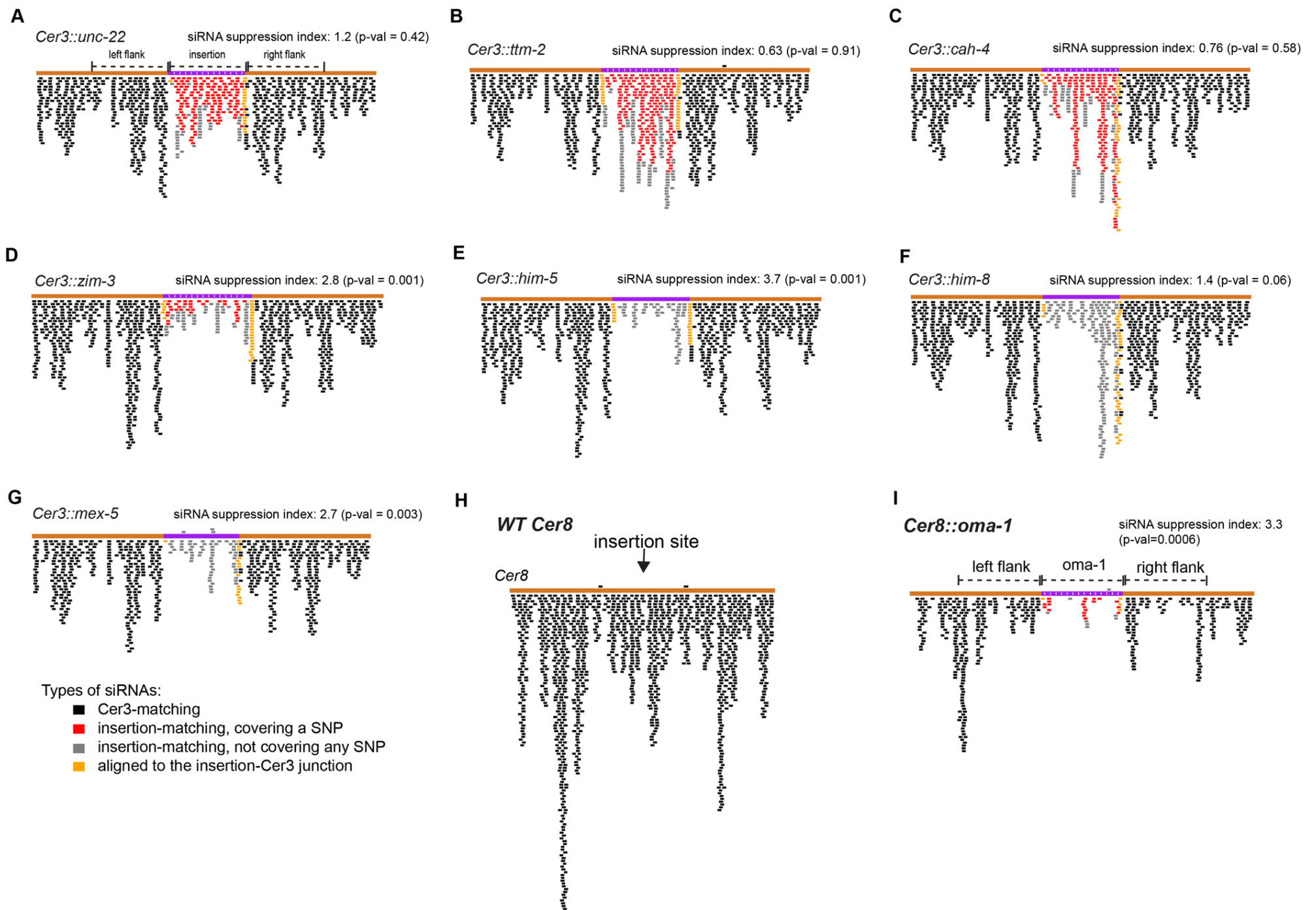


Fig. S4. siRNA track plots of additional *Cer3*::insertions (**A-G**), WT LTR retrotransposon *Cer8* (**H**), and *Cer8*::*oma-1* (**I**). Only the 700 nt *Cer8* sequence that flank each side of the insertion site is used for the plots. For *Cer3*::*him-5* (**E**), *Cer3*::*him-8* (**F**), and *Cer3*::*mex-5* (**G**), no SNPs were included in the insertion and therefore the insertion-matching siRNA reads were all colored in gray.

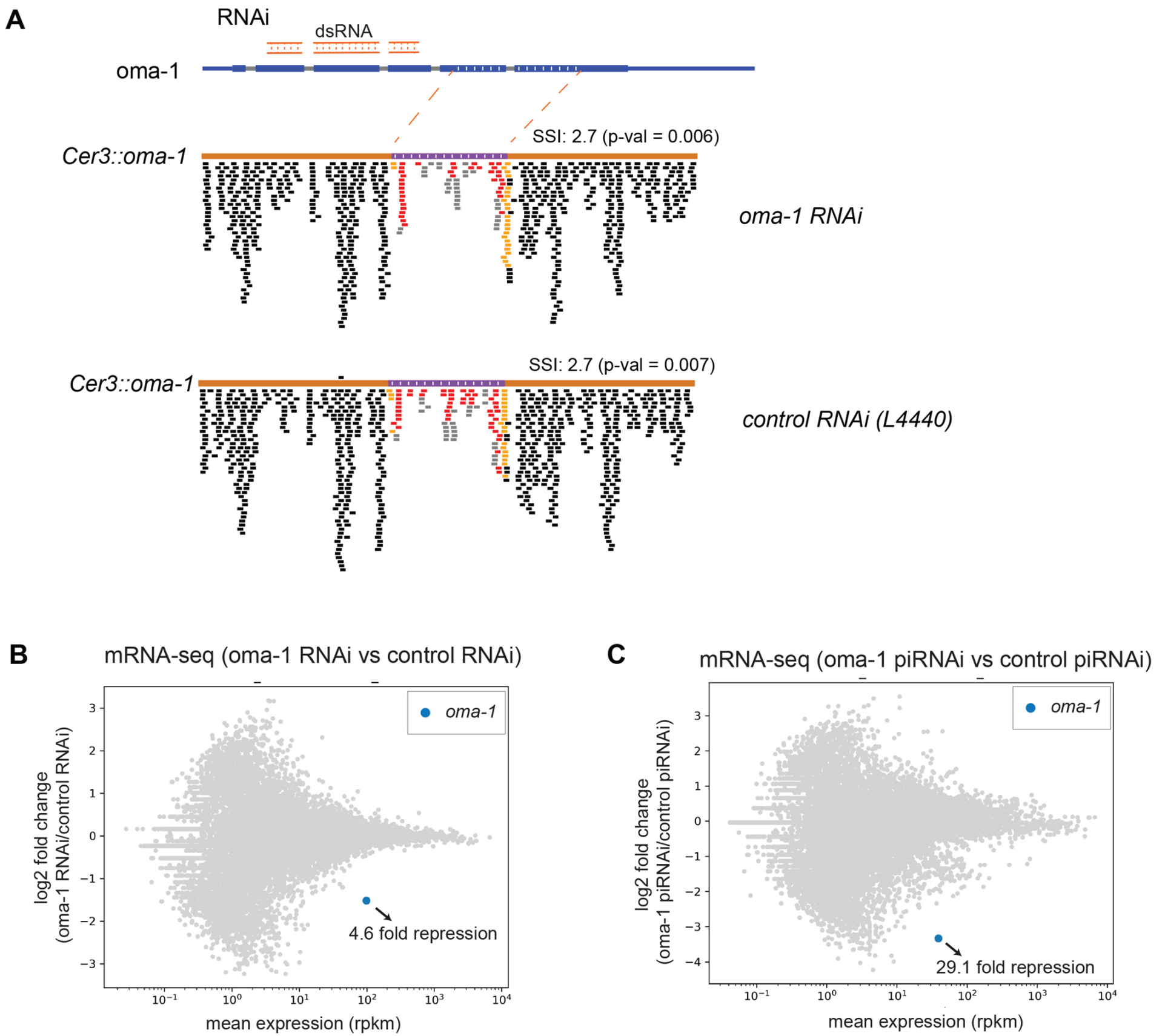


Fig. S5. *oma-1* RNAi did not affect siRNA suppression of *Cer3::oma-1*. **(A):** *Cer3::oma-1* siRNA track plot for *oma-1* RNAi or control RNAi (L4440 empty vector) animals. dsRNA targeted region in *oma-1* is indicated. **(B-C):** RNA-seq MA-plots of *oma-1* RNAi vs control (L4440) RNAi and *oma-1* piRNAi vs control (*unc-119*) piRNAi, showing that both *oma-1* dsRNA and piRNA led to *oma-1* mRNA repression, but the dsRNA-triggered repression was weaker than the piRNA-triggered repression.

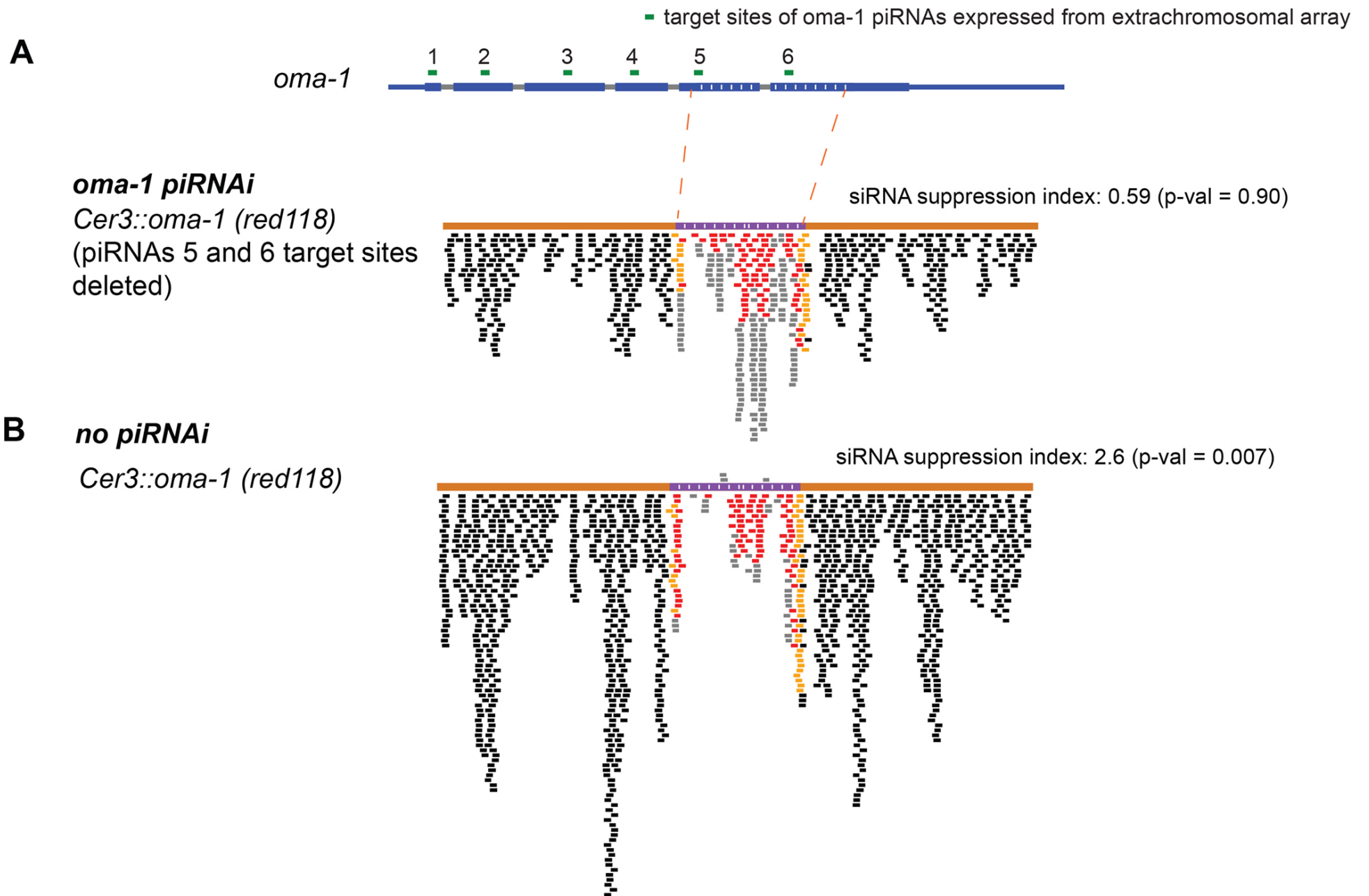


Fig. S6. Additional *oma-1* piRNAi experiments. The *oma-1* piRNAi transgene encodes six piRNAs. Their target sites in *oma-1* are indicated in (A). Two of the six piRNAs also target the *oma-1* fragment in *Cer3::oma-1* used in this study. To determine whether the effect of *oma-1* piRNAi on siRNA suppression was mediated by the piRNA target sites in the *Cer3::oma-1*, we generated a *Cer3::oma-1* allele (red118) that lacks these two target sites. The siRNA track plots with and without *oma-1* piRNAi are shown in A and B.

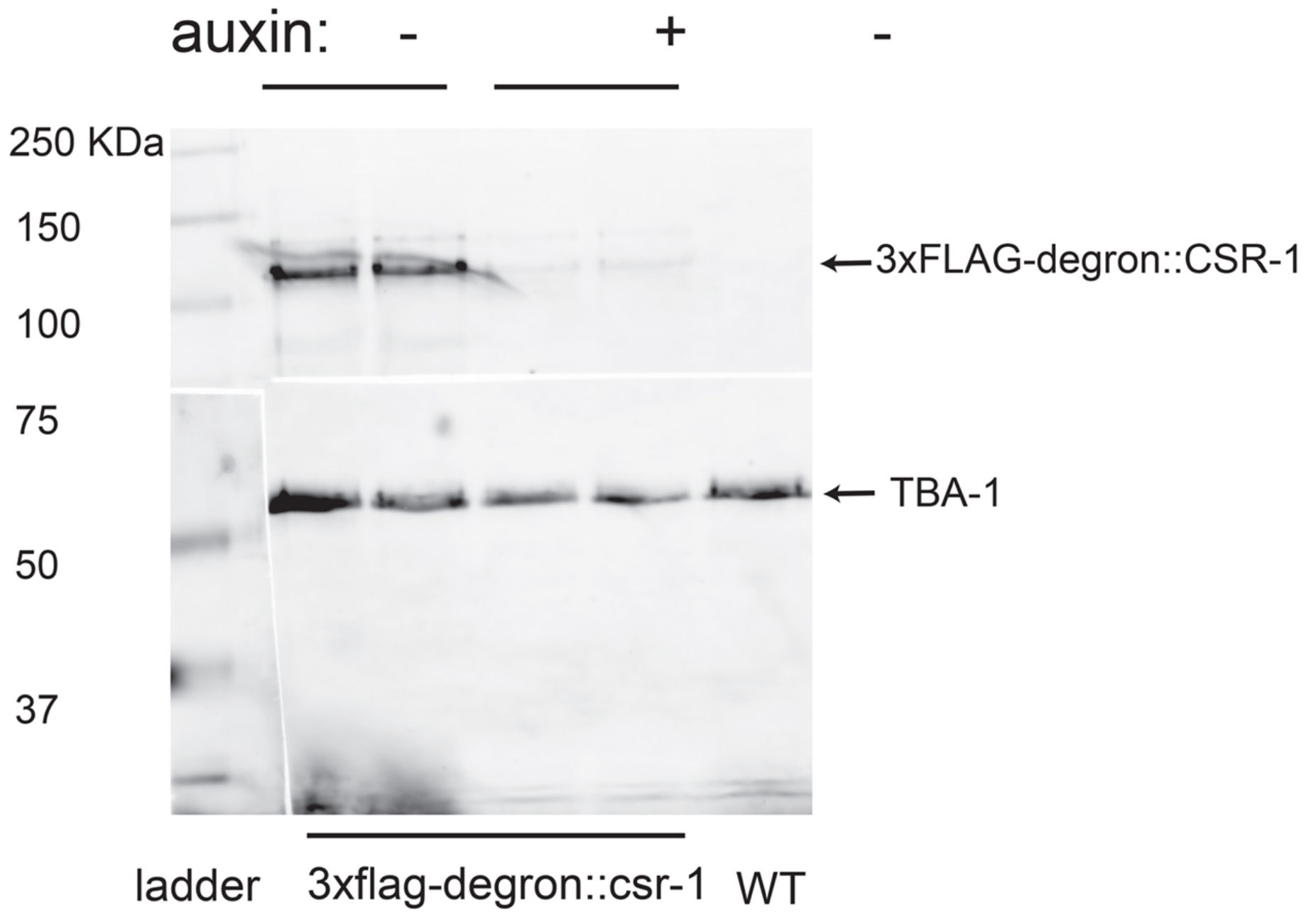


Fig. S7. Anti-FLAG western blot of 3xFLAG::degtron::CSR-1 confirming auxin-induced degradation (AID) of CSR-1 (87% reduction). We note that, although the CSR-1 depletion was not complete, the animals exhibited a fully penetrant embryonic lethality, a phenotype expected for the loss-of-function *csr-1* mutation (data not shown).

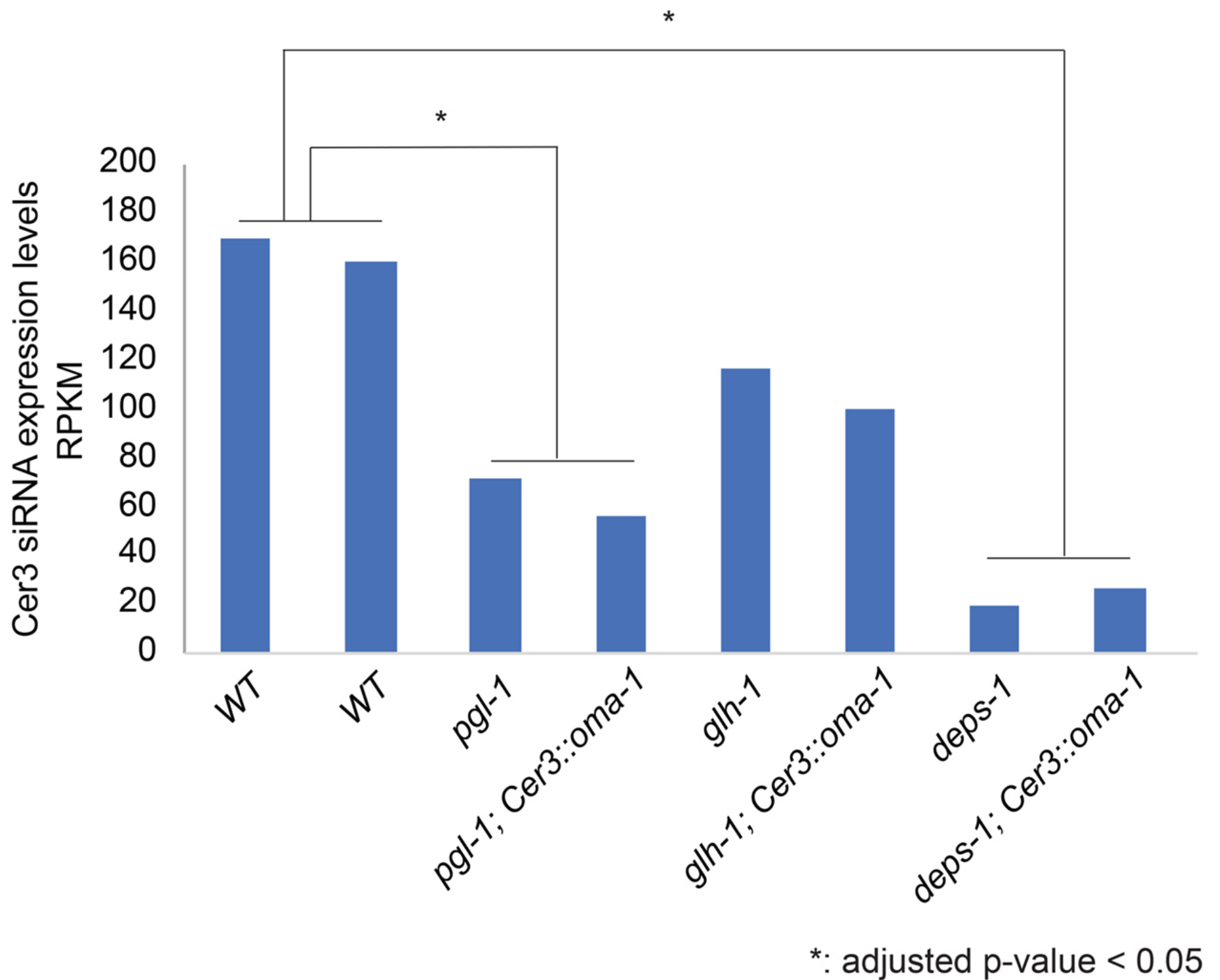


Fig. S8. *Cer3* siRNA expression levels in WT and P-granule mutant strains that carry either WT *Cer3* or *Cer3::oma-1*. The full-length WT *Cer3* sequence was used for the alignment to calculate the *Cer3* siRNA levels. DESeq2 (Love, Huber et al. 2014) was used to calculate the adjusted p-values for the comparison between WT and a mutant background. Both *pgl-1* and *deps-1* mutations were associated with significant reductions in *Cer3* siRNA production (3.7 and 9.2-fold reductions, respectively, adjusted p-values < 1.0×10^{-18}). A modest *Cer3* siRNA reduction was observed in the *glh-1* mutant animals (1.7-fold, adjusted p-values = 0.2).

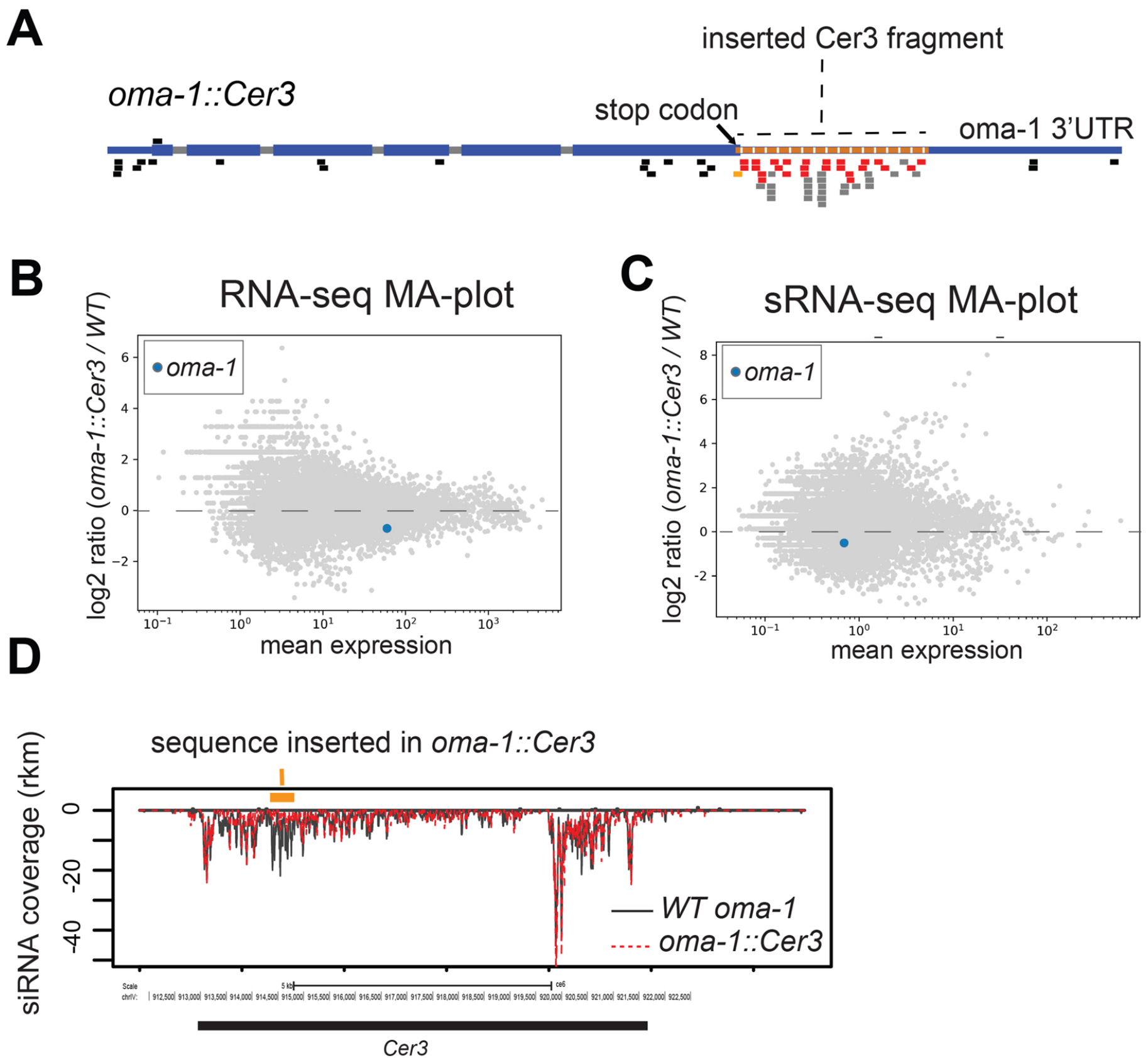


Fig. S9. Additional analysis for *oma-1::Cer3* mutant animals. **(A)** A track plot of *Cer3::oma-1* siRNA profile, with SNPs-containing siRNAs colored in red and siRNAs that do not cover any SNP position colored in gray. **(B-C)** MA-plots comparing *oma-1::Cer3* and WT mRNA (B) and siRNA (C) expressions for all genes, with *oma-1* highlighted in blue. The *Cer3* insertion was excluded from the analyses. **(D)** siRNA coverage plot at *Cer3*, normalized to the sequencing depth, for WT and *oma-1::Cer3* animals using the same data as Fig. 5A and 5B.

Table S1. NGS libraries used in this study. All Fastq files have been deposited in NCBI with the GEO accession number GSE196847.

[Click here to download Table S1](#)

Supplementary Materials and Methods

DNA sequence of mutations created in this study:

1. Insertion site in Cer3 used in this study:

TCGGTTTCAAGACAGCCGCC [insertion]ACTTTGCCTCCGTTCAATGG

2. red20 (Cer3::oma-1), upper case: SNPs:

catgactaaCggtcgcattgcagctccccgctttctgcGattcagcatcctttagaaatgtttgccagA
ccatcaactccagatgagccagcggctaaGttgccactaggaccaactcctgtagtacCcggtggtccaa
gatatgagctaccaacgaaAgaattgcatgacgcggaaggtgcatgacTtatccaccgtctcgtggcc
attggatccGtcgatgtttgctctagacgcttggaaatggcCcatcggccagctagtccactcgattcA
atggttttgggttccgctccaaatgctggTtcgctcggaaatgctcggaaagcaaaatacCctggaggag
ttctggatattcatctgcCggatccacgccttctcaggatctcagttcCtcgctcactcaatgcagcat

3. red52 (Cer3::gfp), upper case: SNPs:

tccaatgcttctcccgtAaccagaccacatgaagcgtcacgacttcAtcaagtccgcatgccagaggg
atcgtcGaagagcgtaccatcttctcaaggacgacCgaaactacaagaccggtgccgaggtcaagAtc
gaggagacaccctcgtcaaccgtatcCagctcaagggaaatcgacttcaaggaggacCgtaacatcctgg
gacacaagctggagtacTactacaactcccacaacgtctacatcatgCccgacaagcaaaagaacggaat
caaggtcTacttcaagatccgtcacaacatcgaggacCgatcgggtccaactcggccaccactacaaGaa
aacacccaatcgggtgacggaccagtcGtcctcccagacaaccactacctctccaccGaatccgacctc
ccaaggacccaacgagTagcgtgaccacatggtcctcctcaggttcCt

4. red123(Cer3::rpl-1), upper case: SNPs:

agcaccatcttgacgaggctgcccgggaCacattccatcgatgagcgcgatgacttgTagaagcttaa
caagcagaagaagctcatcTagaagctcggcaagagctacgatgctttcTtcgcttccgaatccttgatc
aagcagatcGcacgtatcctcgggtccaggactcaacaagCccggaaaattcccttccgctcgttaccacC
gagaatctctccaaagcaagagtgcagagTtccgcgccaccgtcaagttccagatgaagTaggttctctg
cctctccgctcgcgctcgggtGacgttggactcaccagaggagcttgtcAccaacatctccctctccatc
aacttctcCtctcgtcttgaagaagaactggcagaacCtgagatcggt

5. red124(Cer3::unc-22), upper case: SNPs:

gaggaagagcatgatgaatgggtcagatgGaatcagaaccatctccaccaataactaGaatgttccaa
atctcatcgacggaagaaaCtatagataccgagtatttgctgtcaatgaAgcaggactttccgatctagc
cgagcttgaAcaactttgttccaagcatccggttctggTgaaggaccaagattgtcagtcattgagG
gatttgaacgaagaagttggaagatgtgtGacatttgagtgtgaaatcagtggtatctccTagacctgat
acagatgggtcaaggatgGaaggaacttgttgacaccagcaagtacacActtattaataaggagacaa
gcaagtcctAattatcaatgacttaacgtctgatgatgcAgatgaataga

6. red126(Cer3::ttm-2), upper case: SNPs:

tggaagtatcaaacagtttagcgaatgctAggattggatgtattctatgatgaaaatcaTaatcggcatt
tttcaagcgaatctgcatcAgcatcatcttctctttattcacaacagcAgcaacatttctattcaaat
tgaactaccAcattttgcacctccgagtttttattgcccTggatctccggtatccataaggttcacggtC
gaaatccaactttacaaccaaggatttaaTattgcatctcacgaagaaaatctcgtcgtTctaaattatg
aatctatcaaacggcaggtGactccgaagccggtcaattttcagaaaacAtttaactttccaaggagcg
atcaatttcAttgaaatgctcctacacagatgttttGacaactact

7. red127(Cer3::cah-4), upper case: SNPs:

ctggaacaccagctatgagtcggttaatgAggcactcagcaagcccgatggattggcggAtgttggagtc
ttcttgaaggaaggaaaatTcaatgacaattaccatggcctgatcgacaGagtgcgcaaagccaccgaa
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attcgttacatacctcggatcccttaccGcccaccatacaacgagtggttatctggaGcttgttcaca
gagcctgtggaggtctcctAcggacagctcaacgtgctccgtaatatcaAccccgcaatcatcgcgct
gccaagacaCatgcgaccgtgaaatccgatcttccctcaTcttttaatt

8. red47(Cer3::zim-3), upper case: SNPs:

cttaacaagcgaacgagcaaatgaataaGaatcatattattaaatgtaatttcgaaggCtgtaagcaaa
ttctctcgtggaaacttctGtacggcaacaacgacttctggatcatgcGatgactcatgaaatgaaa
gtgtctcgtCgtcgcacatgtgaatactcttgagtagcGatccccaaatgaagtatcactatcaaaaG
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gagataaaGaaatgtaaaaactcgtgaaaaactccGgaagtcgacaatgagaatgtagataaagaT
tctccaggaccatcacacgatcttttctaCtttcgcagaattcgc

9. red145(Cer3::him-5):

cggtcggttcacagaacagaaatacatcgaaaaaattgccgatggccatattttttgccgagatacaga
attcgagacaatgcagagagatcagtaggtgcacgtttcaagagcttccacaaaaagaacaagacgaag
tagtgaatgaagctttttcgaatcttcgagaatatttgaaaaagagggaaacctttctatgcaaagctccg
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gatagacgcaataagggaaataagaaaactcaaaaaatgcgagtaagaactgccaattgagaaatcgt
cgaataattctggaattctcaaaaaaagtggatcaggaatcagcgtggcttcta

10. red146(Cer3::him-8):

cgggtgtagacaacgatggaaatattgatgattatgaagagtatcagtcattaccaccgaatgatgatgtt
attatgaatgaaacagaattgatggatgttgatcgaaccacagtgatgactccattacgaagtcctacat
ttttcgattatcacaacgaatccggtgatgaagatcagttgaatgagaatgaaatgaaatctccagacag
caaaaacgacgagattaataaagatgaaatccacaatattcaatgtcatttcccgaactgcaatcgagca
attgctggaagaggaaatattgaaaactctgtcttatcgcattcattggtccattgcgataaaaact
ttctgaaatgcaaaaaatgcaagcacacttgtcatacaatccgtcaaatgcg

11. red147a(Cer3::mex-5):

cacccttgacaatcacaacgatgacacgatgagcgtgaaaaggaaaatcatttccatgaacatcgtggc
gagaagttcggtcgctcgtggattcccaattccagaaactgacagtcacaaccaccaactacaagactc
gtctttgatgatgcacgcactctggaatcaaacatgtgatatgggtgctcgcgatgcaagtttgcctatgg
gctcaaagagctcagagctactgatgctccggtcgcctatccgaacaacaagtacaagacaagctgtgc
aagaactttgcgctggaggaactggattctgcccgtatggacttcggttgcgagtttgttcatccaacgg
acaaggaattccagaatattcctccatatacagcgcgatgtctc

12. red40 (Cer3::oma-1), upper case: SNPs:

atgctgcattgagtgacgaGgaactgagatcctgagaaggcgtggatccGgcagatgaatatccagaaac
tcctccaggGgtattttgctttccgagcattccgaacgaAccagcatttggagcggaaacccaaaaccatT
gaatcgagtggactagctggccgatgGgccatattccaagcgtctagagcaaacatcgaCggatccaatg
gccagcgagacgggtggataAgtcatcgcaccttccgctcatgcaattcTttcgttggtagctcatact
tgaccacgGgtactaacaggagttggctcctagtggcaaCttagccgctggctcatctggagttgatggT
ctggcaaacatttctaaggatgctgaaatCgcagaaagcgggggagctgcaatgcgaccGttagtcatg

13. prg-1(red115), upper case: WT sequence, lower case: mutation :
TTATTCATCAGCCCGCTCAgctagctGTGAATTCGTGAAGGATGGG
14. prde-1(red139), upper case: WT sequence, lower case: mutation :
GCATCGATTACCGATGAGGgctagcaACTTttCAGGTCTGAAGGAGCAAAAAG
15. pgl-1(red116), upper case: WT sequence, lower case: mutation :
AAGTTGTGAACCATATCGCTtAAgCTagcTTTCGAGGAAGCGAGTAAAA
16. glh-1(red117), upper case: WT sequence, lower case: mutation :
TGAATGTCCGGAGCCACCCCGcTaGctGATGTTTCAATTGTGGCGAG
17. oma-1(red57), oma-1::Cer3 , upper case: oma-1 sequence with stop codon underlined, lower case: Cer3 insertion (with SNPs indicated in the upper case):
TGACCAAGCATCTCAATTGAaccccccaagaAacgtggtgtctggaagcagtgacgtagtGacgctactga
ctcttctggaagtggatccTcgatgccatcgagctcaccagaatcgtgacAtcgtcgaagaacaatcggaa
gatcagcaaTtcaatagtggtgagcttgccggagatcaaAatgatctagaagaagcaacaattcaattT
gtggaatttcgcttcaattggagtgatcAgtttcaagacagccgccactttgcctccgCtcaatggaaa
cttgactgaagatttcacaGccttcgtgcaaaagttcaaggatcggctaAcagcgtctacagagatggac
aacgatcaaGagaggtctacattccttttatttttggacGatcgcgcgaggagtagcggccgatggaataG
ttggagcacagcctgcgattacactcagAatcttatcacaagatgtcagcagtggtcAagaatgcTTG
TGGTGAACAACCTTCTCT
18. Cer8(red35), Cer3::oma-1, upper case: Cer8 sequence, lower case: oma-1 insertion (with SNPs indicated in the upper case):
CCATTTGAAGAAATCCAAGTcatcatgactaaCggtcgcattgcagctcccccgctttctgcGattcagc
atcctttagaaatgtttgccagAccatcaactccagatgagccagcggctaaGttgccaactaggaccaac
tcctgttagtacCggtggtccaagatatgagctaccaacgaaAgaattgcatgacgcggaagggtgcatg
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ggccagctagtccactcgattcAatggttttgggttccgctccaaatgctggTtcggttcggaatgctcgg
aaagcaaaatacCcttgaggagtttctggatattcatctgcCggatccacgccttctcaggatctcagt
tcCtctcactcaatgcagcatCATCGCCTGAGTAGTGCAGC
19. red118 (Cer3::oma-1), upper case: SNPs:
attgcagctccccgctttctgcGattcagcatcctttagaaatgtttgccagAccatcaactccagatg
agccagcggctaaGttgccaactaggaccaactcctgttagtacCggtggtccaagatatgagctaccaac
gaaAgaattgcatgacgcggaagggtgcatgacTtatccaccgtctcgtggtccattggatccGtcgatg
tttgccCcatcggccagctagtccactcgattcAatggttttgggttccgctccaaatgctggTtcggttc
ggaatgctcggaaagcaaaatacCcttgaggagtttctggatattcatctgcCggatccacgccttctc
aggatctcagttcCtctcactcaatgcagcat

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