

**Figure S1. Fourteen *C. elegans* wild isolates display temperature sensitive Mrt Phenotype at 25°C. Related to Figure 1.**

**(A)** The Mrt phenotype of 14 wild *C. elegans* isolates was followed over 25 generations at 25°C in 6 replicates. The proportion of sterile replicates accumulating along generations is plotted. Bars represent standard errors (SE). **(B)** Details on the wild isolates' origin (see also CeDNR and Wormbase websites).

**Figure S2. Quantifications of germline integrity defects and chromosomal aberrations in the final generations before or at full sterility. Related to Figure 2.**

**(A)** Quantification of germline phenotypes in JU1395 G2 and MY10 G2 adult animals (24 hrs after the L4 stage). The numbers of animals scored per condition (n) are represented for the different brood size levels observed: full (F), medium (M), low-to-sterile (L/S). The degree of the gonad disruption defects is positively associated with the brood size reduction in MY10 G2 (L4+24hrs) animals. **(D)** Quantification of gamete differentiation defects in MY10 animals grown at 20°C or 25°C. The numbers of animals scored per condition (n) are represented for the different brood size levels observed: full (F), medium (M), low (L). **(B)** Proportions of animals with more than six chromatin-DAPI stained bodies. The numbers of animals scored per condition (n) are represented for the different brood size levels observed: full (F), medium (M).

**Figure S3. Composition of the small RNA populations in JU1395 and MY10 across generations when grown at 23°C. Related to Figure 2.**

Plots represent the proportions of siRNAs grouped according to their length and their first 5' nucleotide. Small RNA data were obtained from the sequencing of 5'-independent small RNA libraries obtained from adult hermaphrodites. Small RNA composition in JU1395 and in MY10 were followed across 3 generations for three independent replicates, and 1 replicate only for MY10 generation 3. Data were normalized to the total number of reads with a length between 16 to 33 nucleotides.

**Figure S4. *set-24(mfP23)* in MY10, transformation rescue and *set-24* edition alleles. Related to Figure 4.**

(A) Wormbase (WS262) browser view of *set-24* and *set-24(mfP23)* location in the transcript prediction. (B) Sequence of the *set-24(mfP23)* flanking regions. Sequences were obtained by Sanger sequencing of the PCR product amplified in MY10 using the primers MY10*set24*delF & MY10*set24*delR. (C) Transgenic rescue of *set-24* in MY10 by introduction of extrachromosomal arrays. JU3250 and JU3249 carry the YAC with the *set-24(N2)* allele. JU3301 and JU3310 carry control arrays without the YAC. Their Mrt phenotype was assayed in five replicates at 25°C (Mrt-value) by transferring ten GFP-positive L4-larvae. Controls were performed in five replicates at 15°C. The experiment was stopped at generation 15, considering fertility at G15 as non-Mrt. \*\* Wilcoxon rank sum test with continuity correction between YAC (JU3250 and JU3249) no YAC (JU3301 and JU3310),  $p = 0.0025$ . (D) Sequence of CRISPR/Cas9 alleles obtained within the *set-24* coding regions in the JU3253, JU3254, JU3292, JU3293 strains. (E) JU3292 [*set-24(mf130)* in JU1395] and JU3293 [*set-24(mf131)* in JU1395] were phenotyped at 25°C over 20 generations.

## Supplemental References

- S1. Ashe, A., BÉlicard, T., Le Pen, J., Sarkies, P., Frezal, L., Lehrbach, N.J., Félix, M.A., and Miska, E.A. (2013). A deletion polymorphism in the *Caenorhabditis elegans* RIG-I homolog disables viral RNA dicing and antiviral immunity. *eLife* 2, e00994.
- S2. Haber, M., Schüngel, M., Putz, A., Müller, S., Hasert, B., and Schulenburg, H. (2005). Evolutionary history of *Caenorhabditis elegans* inferred from microsatellites: evidence for spatial and temporal genetic differentiation and the occurrence of outbreeding. *Mol. Biol. Evol.* 22, 160-173.
- S3. Petersen, C., Dirksen, P., Prahl, S., Strathmann, E.A., and Schulenburg, H. (2014). The prevalence of *Caenorhabditis elegans* across 1.5 years in selected North German locations: the importance of substrate type, abiotic parameters, and *Caenorhabditis* competitors. *BMC ecology* 14, 4.