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me98 is a new allele of *rad-54*

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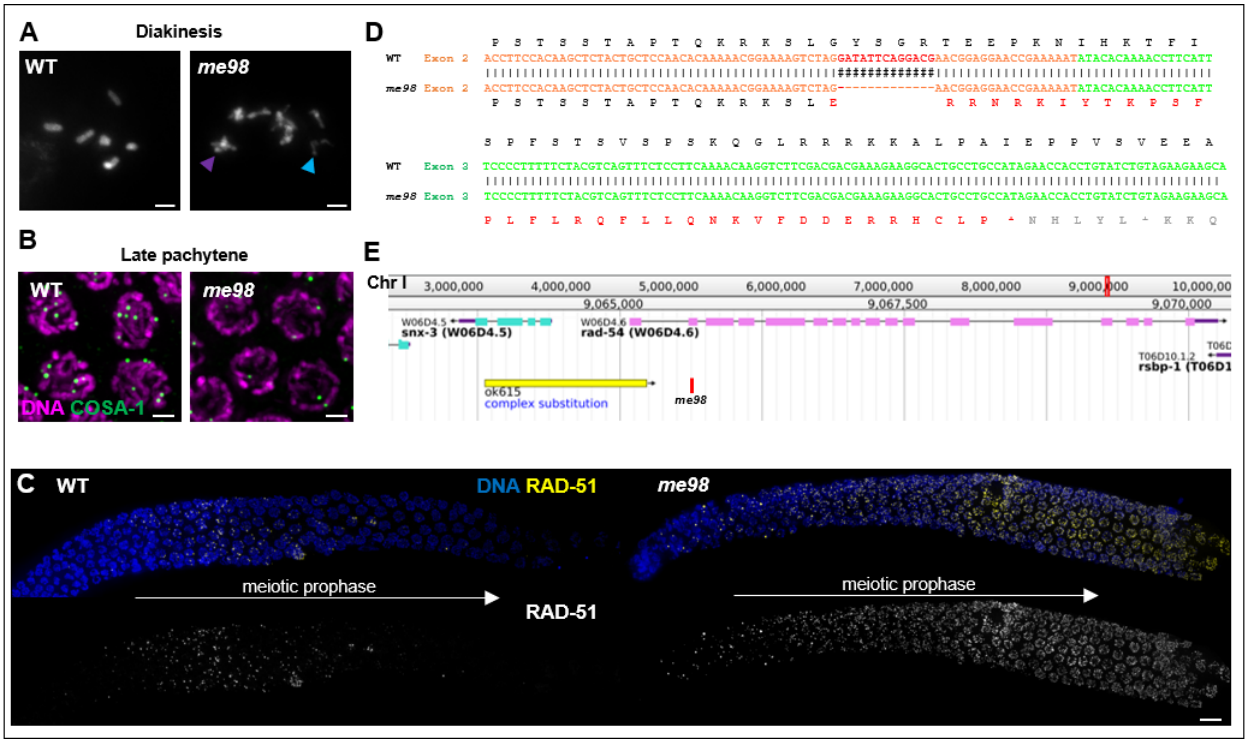

Figure 1 A. DAPI stained chromosomes in oocytes of the indicated genotype at diakinesis, the last stage of meiotic prophase; while six pairs of attached homologs are detected in wild-type animals, chromosome structural defects such as fragments (blue arrowhead) or partially decondensed chromosomes (purple arrowhead) are observed in the *me98* mutant. **B.** Detection of the crossover site marker GFP::COSA-1 in WT (left) and *me98* mutant germ-cells (right). Scale bars on panels A and B represent 2μm. **C.** Detection of the recombinase RAD-51 in the region of the gonad corresponding to early to middle stages of meiotic prophase in WT (left) and *me98* mutant (right) worms. RAD-51 foci are transiently detected in wild-type animals in early prophase as DNA breaks are formed and repair progresses. In contrast, in *me98* mutants, RAD-51 foci accumulate and remain at high levels. Scale bar represents 10μm. **D.** Position and nature of the *me98* deletion, located in the second exon of the *rad-54* coding sequence, and its consequences on the encoded protein product. **E.** Screenshot of genome browser centered on the *rad-54* locus. Positions of the previously described *rad-54* allele, *ok615*, and the newly identified *me98* mutation are represented respectively as a yellow and a red bar.

Description

We isolated the *me98* mutant in a genetic screen for *C. elegans* mutants with an altered number of GFP::COSA-1 foci, which mark the sites of crossovers in wild-type *C. elegans* germ cells (Rosu *et al.* 2013). After multiple rounds of outcrossing, we confirmed that the *me98* mutant is defective in meiotic prophase as *i*) chromosomes in diakinesis oocytes appear partially decondensed and structurally compromised (Fig. 1A), *ii*) *me98* fails to form the six GFP::COSA-1 foci observed in wild-type late pachytene meiocytes (Fig. 1B) and *iii*) *me98* accumulates RAD-51 foci during the course of meiotic prophase (Fig. 1C). Further, 100% of eggs laid by *me98* mutant hermaphrodites are inviable. These defects are reminiscent of those caused by the previously-described *rad-54(ok615)* mutation (Mets and Meyer 2009), and sequencing of the *rad-54* locus in *me98* mutants revealed the presence of a 13bp deletion in the second exon of the annotated transcript (I:9065652 to I:9065664 of WS269). This lesion creates a frameshift that would result in premature termination of translation in the third exon (of seventeen) of the predicted transcript (Fig. 1D), suggesting that it is likely a null allele. Of note, the previously described *rad-54* loss-of-function allele, *ok615*, is an insertion/deletion that also affects the neighboring gene *snx-3* (Fig 1E). As gonads of *rad-54(me98)* mutants appear overall healthier than those in the *ok615* mutant, *me98* could be a valuable tool to analyze the specific function of *rad-54*.

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Materials

Cytology:
Immunofluorescent detection of GFP::COSA-1 and RAD-51 was performed as described in (MARTINEZ-PEREZ AND VILLENEUVE 2005) using a mouse anti-GFP antibody (Sigma-Aldrich #11814460001) and a rabbit anti-RAD-51 antibody (COLAIACOVO *et al.* 2003).

Reagents

Strains:
AV727: *meIs8[pie-1p::gfp::cosa-1 + unc-119(+)] II;ltIs37[pie-1p::mCherry::his-58 + unc-119(+)] IV;ltIs38[pie-1p::gfp::ph(PLC1delta1) + unc-119(+)]*
AV762: *rad-54(me98)/hT2[qIs48] (I;III);meIs8[pie-1p::gfp::cosa-1 + unc-119(+)] II;ltIs37[pie-1p::mCherry::his-58 + unc-119(+)] IV;ltIs38[pie-1p::gfp::ph(PLC1delta1) + unc-119(+)]*

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Author Contributions The genetic screen was initially designed by KAZ and AMV, performed by KAZ. The *me98* mutant was characterized by BR. The manuscript was written and edited by BR and AMV.

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