Supporting Information

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SI Materials and Methods

Generation of the *mre11*^{58S} Allele by Ends-in Targeting. A 6-kb genomic fragment of *mre11* was PCR amplified with primers mre11(+)-A2 and mre11(+)-Xba-B2, and cloned into pCR2.1 by TOPO TA cloning. An I-SceI cut site was added by ligating the annealed product of I-siteblunt+ and I-siteblunt- to a unique PmeI site. The H230Y change was made by site directed mutagenesis using primers mre11-His230Tyr-A and mre11-His230Tyr-B so that a CAT codon for His was changed to a TAT codon for Tyr. The *mre11*-coding region was sequenced to verify the CAT to TAT change as the only mutation. The final 6-kb fragment was cloned as a NotI fragment into the targeting vector pTV2 (1).

Targeting crosses and the subsequent reduction crosses were performed by published protocols (1, 2). We discovered that an *mre11*^{58S} transgene rescued viability to homozygotes bearing the *mre11*$^{\Delta 35K1}$ deletion mutation, but the surviving females were sterile; *mre11*$^{\Delta 35K1}$ was then introduced to all reduction lines for testing female fertility. All female sterile reduction lines along with a few female fertile lines were subjected to molecular characterization. First, the overall integrity of the *mre11* genomic region was assayed by Southern blot analysis using genomic DNA from homozygous flies digested with either ClaI or NdeI individually. The membranes were analyzed with probes from 2 regions of *mre11* using the following primers: mre11-UP-seq2 and mre11-4916B (corresponds to probe 1 in Fig. S1); mre11-DO-seq2 and mre11(+)-B (corresponds to probe 2 in Fig. S1). Second, reduction lines with an intact *mre11* region were further characterized by PCR and sequencing to verified the presence of the H230Y mutation. Third, cDNAs from *mre11*^{58S} mutants were made and sequenced to verify H230Y as the only mutation. Total RNA was isolated using the Qiagen RNA easy kit. The first strand was reverse transcribed using oligo-dT20 primer from Invitrogen. The *mre11* coding region was amplified with mre11-KPN-1A and mre11-KPN-0B, and cloned by TOPO TA cloning. Multiple *mre11*^{58S} lines were recovered, and the line S44 was used for all subsequent studies.

Generation of the *nbs*^{2K} Allele. We recovered a female sterile *nbs* mutant during our work on the site-specific integrase mediated repeated targeting (SIRT) method (3). We checked the integrity of the *nbs* region by PCR using primers nbs1991d and nbs6968u to amplify a 5-kb fragment. The primers lies outside of the homology used for targeting *nbs* (3). The *nbs* region was sequenced, and we identified a 180-bp deletion at the 5′ end of *nbs*. This deletion eliminates the predicted "ATG" start codon. However, several in-frame ATG codons are present downstream of the deletion, which might be used. This is consistent with the truncated Nbs protein that we detected on Western blot analyses (Fig. 4*B*).

Live Imaging. Embryos (0–2 h) were collected from females with an *h2AvD-gfp* transgene that had been mated to wild-type males. They were placed under Halocarbon 27 oil in a depression slide covered with a coverslip, and observed with a 40× oil lense. Photographs were taken every 30 s for 2 h on a ZEISS Axioplan 2 motorized microscope using the AxioVision software. The pictures were assembled into a movie file using AxioVision.

Time Course of Loss of Maternal Mre11-Rad50-Nbs (MRN) Contribution During Larval Development. To estimate the time for the loss of Mre11 function during development, we took advantage of the fact that telomere fusions in the *mre11*$^{\Delta 35K1}$ mutant result in chromosome bridges during mitosis (4). These bridges can be detected by staining mitotic chromatin for phosphorylated histone H3 (H3P). We staged homozygous mutants as second and third instar larvae. With *mre11*$^{\Delta 35K1}$, 3.5% of mitotic nuclei (n = 972) taken from 11-s instar larvae displayed bridges, and 12.9% of mitotic nuclei (n = 1622 nuclei) taken from 6 third instar larvae did so. This suggests that the loss of the telomere protection function of maternal Mre11 happens mainly during the third instar stage.

Fly Stocks and Other Reagents. All balancers were obtained from the Bloomington Stock Center. An *h2AvD-gfp* line was a gift from Kami Ahmad (Harvard Medical School, Boston) (5). The *mre11*$^{\Delta 35K1}$ and *nbs*$^{\Delta}$ have been described previously (3, 6).

Anti-MRN Antibodies. The entire Mre11 coding region was amplified with primers MRE11-KPN-1A and MRE11-ECOR-620B using DNA from the cDNA clone LD08638 obtained from DGRC (https://dgrc.cgb.indiana.edu) as a template. The PCR product was cloned into the KpnI and EcoRI sites of pRSETb from Invitrogen. Purified His-tagged Mre11 fusion proteins were used to immunize rabbits and guinea pigs (GP). Rabbit sera were purified using the Melon Gel IgG Purification Kit from Pierce.

A rabbit anti-Rad50 antibody and a plasmid used for antigen production were kindly provided by Maurizio Gatti (University of Rome, Rome) (7). We raised rabbit and GP anti-Rad50 antibodies using the same antigen. Anti-Rad50 antibodies were affinity purified by the Antibody Production and Purification Unit of National Cancer Institute.

An *nbs* fragment encoding amino acids 384–733 were amplified using the LD44438 cDNA clone from DGRC with the primer pair: Fnbs and Rnbs. The PCR product was cloned into BamHI and HinDIII sites in pQE-30a (Qiagen). His-tagged fusion proteins were purified and used as antigens in GP. The anti-sera were purified by using the Melon Gel IgG Purification Kit (Pierce).

Immunoblotting and IP. Embryos were collected at 0- to 2-h intervals, washed with washing buffer (0.7% NaCl/0.05% Triton X-100), dechorionated with 50% bleach for 60 s, rinsed with distilled water, frozen in liquid nitrogen (N_2), and stored at −80 °C before use. White pupae were collected, washed with PBS then with dH_2O, frozen and stored at −80 °C. Normally 200 pupae were used for 1 IP experiment. Frozen embryos or pupae were pulverized in N_2 using a mortar and pestle as N_2 evaporates. All subsequent steps were carried out on ice.

For whole-cell extract preparation, homogenate (in powder form) was suspended in a buffer of 50 mM Tris·HCl, pH 7.5/150 mM NaCl/1% SDS (all concentrations are final), and sonicated for 15 min with a program of 10-s "ON" and 30-s "OFF". Cellular debris was removed by centrifugation at 15,000 × g for 1 h. Protein concentration was determined with the Bradford assay. Equal amounts of protein was loaded per lane and fractionated in 8% SDS gels for standard Western blot analyses.

To obtain good quality extracts for IP experiments using pupae, we adapted protocols for purifying crude nuclear extracts. We monitored materials after homogenization by DAPI staining, and discovered that our protocols resulted in a mixture of intact cells and intact nuclei. Therefore, extracts used for IP contained both cytoplasmic and nuclear proteins. Similarly,

extracts from embryos contain proteins from both compartments.

Homogenate (in powder form) was suspended in Buffer I (10 mM Hepes, pH 7.6/10 mM KCl/5 mM MgCl/0.5 mM EDTA/5% Glycerol/0.35 M sucrose/1 mM DTT/1 mM PMSF/1 mM Na_3VO_4/1 mM NaF, supplemented with protein inhibitor from Roche), homogenized with a pestle (Kontes) for several strokes. The homogenate was filtered through two layers of Miracloth (Calbiochem), and centrifuged at 5,000 $\times$ g for 20 min. The pellets were suspended in Buffer II (10 mM Hepes, pH 7.6/1% NP/200 mM KCl/2 mM MgCl/0.5% sodium deoxycholate/0.5 mg/mL BSA/0.5 mM EDTA/5% Glycerol/1 mM DTT/1 mM PMSF/1 mM Na_3VO_4/1 mM NaF, supplemented with protein inhibitor from Roche), and briefly sonicated to solublize chromatin. Final extracts were obtained after centrifugation at 15,000 times] g for 1 h. For IP experiments, extracts with equal amount of total protein were incubated with appropriate antibodies for 4 h at 4 °C, followed by addition of 20 μL protein A/G agarose beads (Santa Cruz) and overnight storage at 4 °C. The immunocomplexes were washed 5 times in Buffer II, boiled in Laemmli sample buffer (BioRad), and fractionated in 8% SDS gels for standard Western blot analyses.

1. Rong YS, et al. (2002) Targeted mutagenesis by homologous recombination in D. melanogaster. *Genes Dev* 16:1568–1581.
2. Rong YS, Golic KG (2001) A targeted gene knockout in Drosophila. *Genetics* 157:1307–1312.
3. Gao G, McMahon C, Chen J, Rong YS (2008) A powerful method combining homologous recombination and site-specific recombination for targeted mutagenesis in Drosophila. *Proc Natl Acad Sci USA* 105:13999–14004.
4. Bi X, Wei SC, Rong YS (2004) Telomere protection without a telomerase; the role of ATM and Mre11 in Drosophila telomere maintenance. *Curr Biol* 14:1348–1353.
5. Clarkson M, Saint R (1999) A His2AvDGFP fusion gene complements a lethal His2AvD mutant allele and provides an in vivo marker for Drosophila chromosome behavior. *DNA Cell Biol* 18:457–462.
6. Gong M, Bi X, Rong Y (2005) Targeted mutagenesis of Drosophila atm and mre11 genes. *Drosophila Information Service* 88:79–83.
7. Ciapponi L, et al. (2004) The Drosophila Mre11/Rad50 complex is required to prevent both telomeric fusion and chromosome breakage. *Curr Biol* 14:1360–1366.

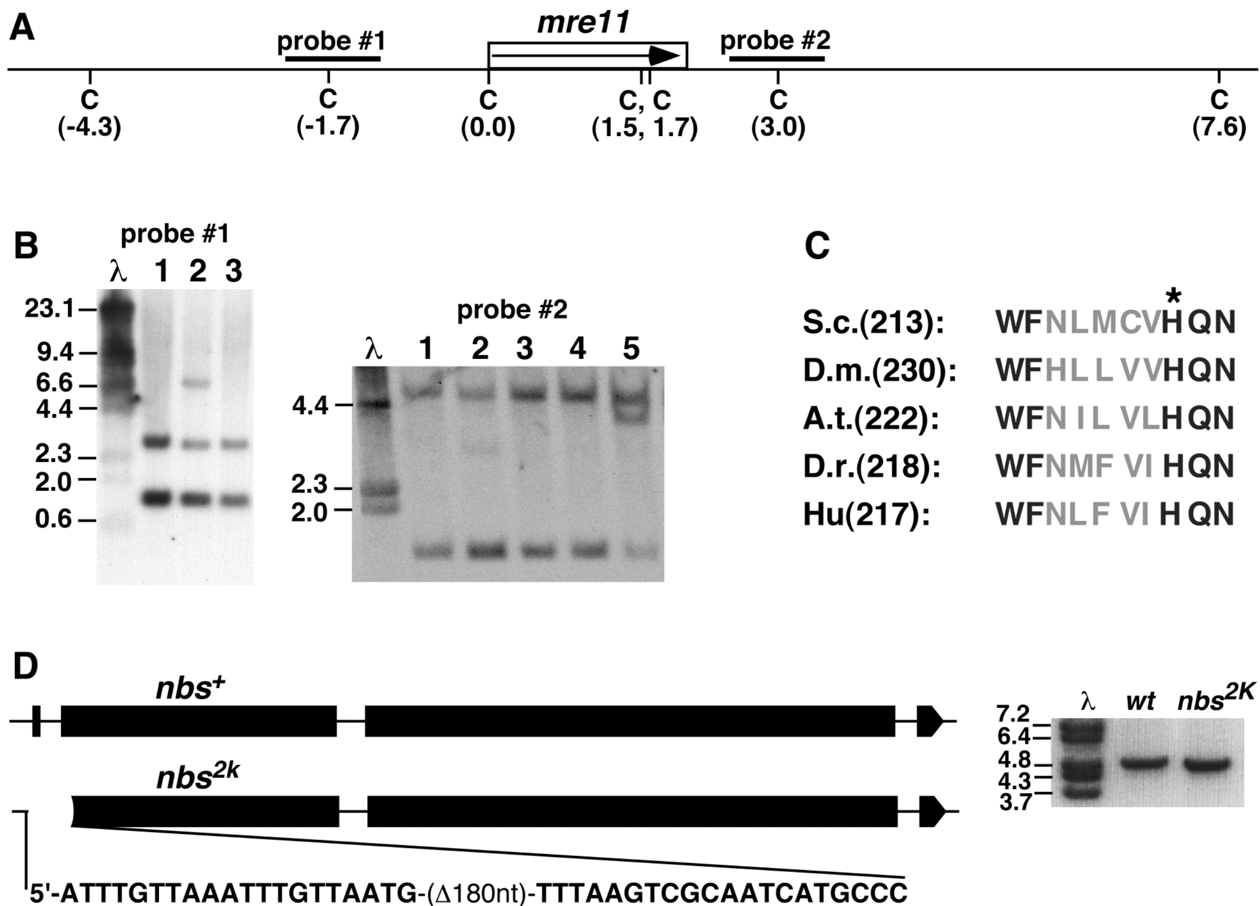


Fig. S1. Molecular characterization of *mre11*^{58S} and *nbs*^{2K}. (*A*) Genomic organization of the *mre11* locus. The positions for ClaI (C) sites are indicated with the coordinates in kilobase shown in parentheses. The 0.0 position corresponds to the start codon for Mre11. The *mre11* coding region is shown with the direction of transcription indicated with an arrow. The regions for probe 1 and 2 are indicated. (*B*) Southern blot analyses of *mre11* targeting events. The left membrane shows genomic DNA cut with ClaI and probed with probe 1. Lane λ, markers with sizes in kilobase; lane 1, homozygous wild-type DNA; lane 2, wild type with the *mre11* targeting donor P element (from pTV2) showing an extra band around 8 kb; lane 3, *mre11*^{58S} reduction line S44 homozygotes. The wild-type and *mre11*^{58S} samples show identical patterns. The right membrane shows genomic DNA cut with ClaI and probed with probe 2. Lane 1, wild type; lanes 2, 3, and 4, homozygotes from 3 independent reduction lines; lane 5, homozygotes from an *mre11* targeting event (before reduction) showing an extra band at 4 kb. (*C*) Mre11 alignment around H230, with conserved residues in black and others in gray. The invariant Histidine is marked with *. S.c., *Saccharomyces cerevisiae*; D.m., *Drosophila melanogaster*; A.t., *Arabidopsis thaliana*; D.r., *Danio rerio*; Hu, human. The coordinates for the conserved his in different organisms are shown in parentheses. (*D*) The organization of the *nbs*^{2K} allele. The diagram shows exon (boxes) and intron (line) structures of *nbs* modified from a similar diagram at http://flybase.org. The nature of the deletion is shown with sequences surrounding the180-nt deletion in bold (*Left*). PCR result (*Right*). The band in *nbs*^{2K} sample is slightly smaller due to the 180-bp deletion.

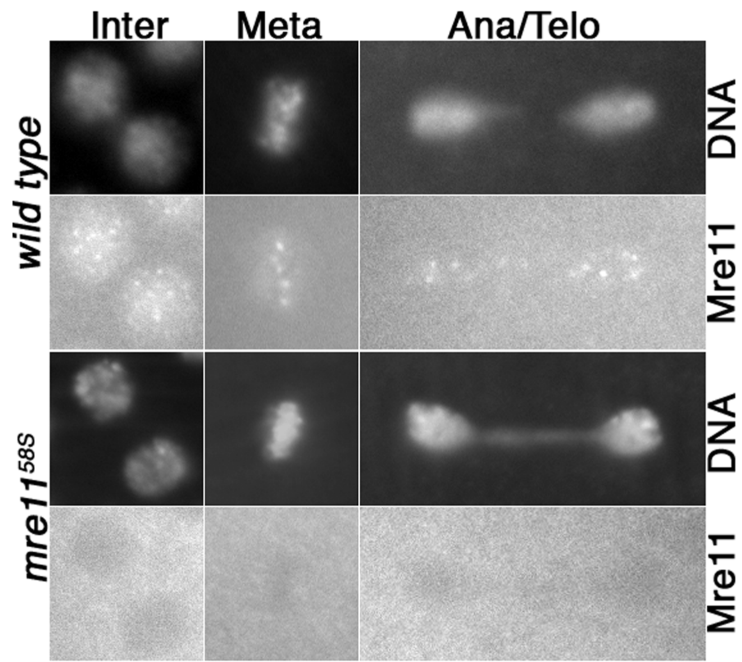
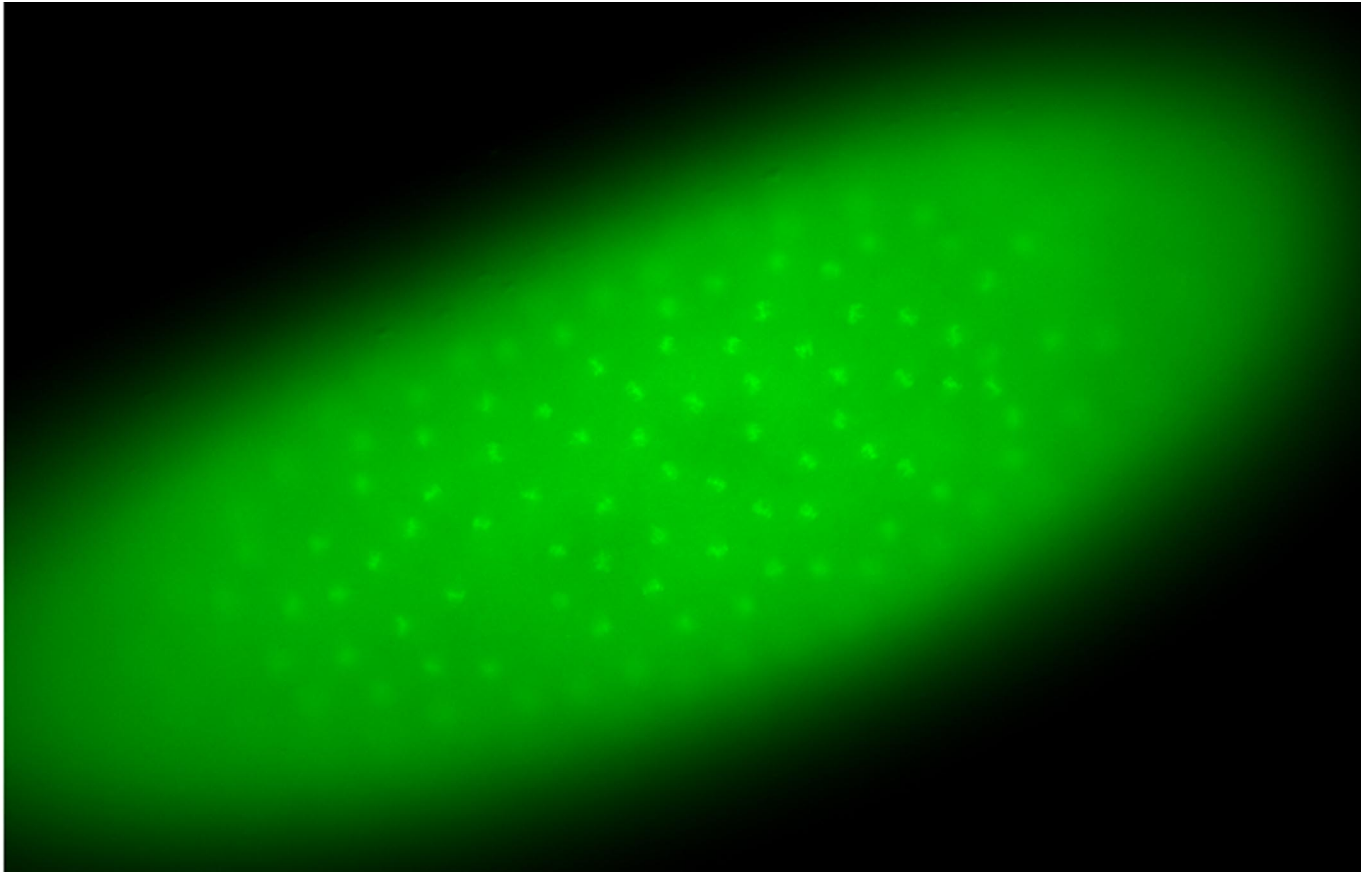
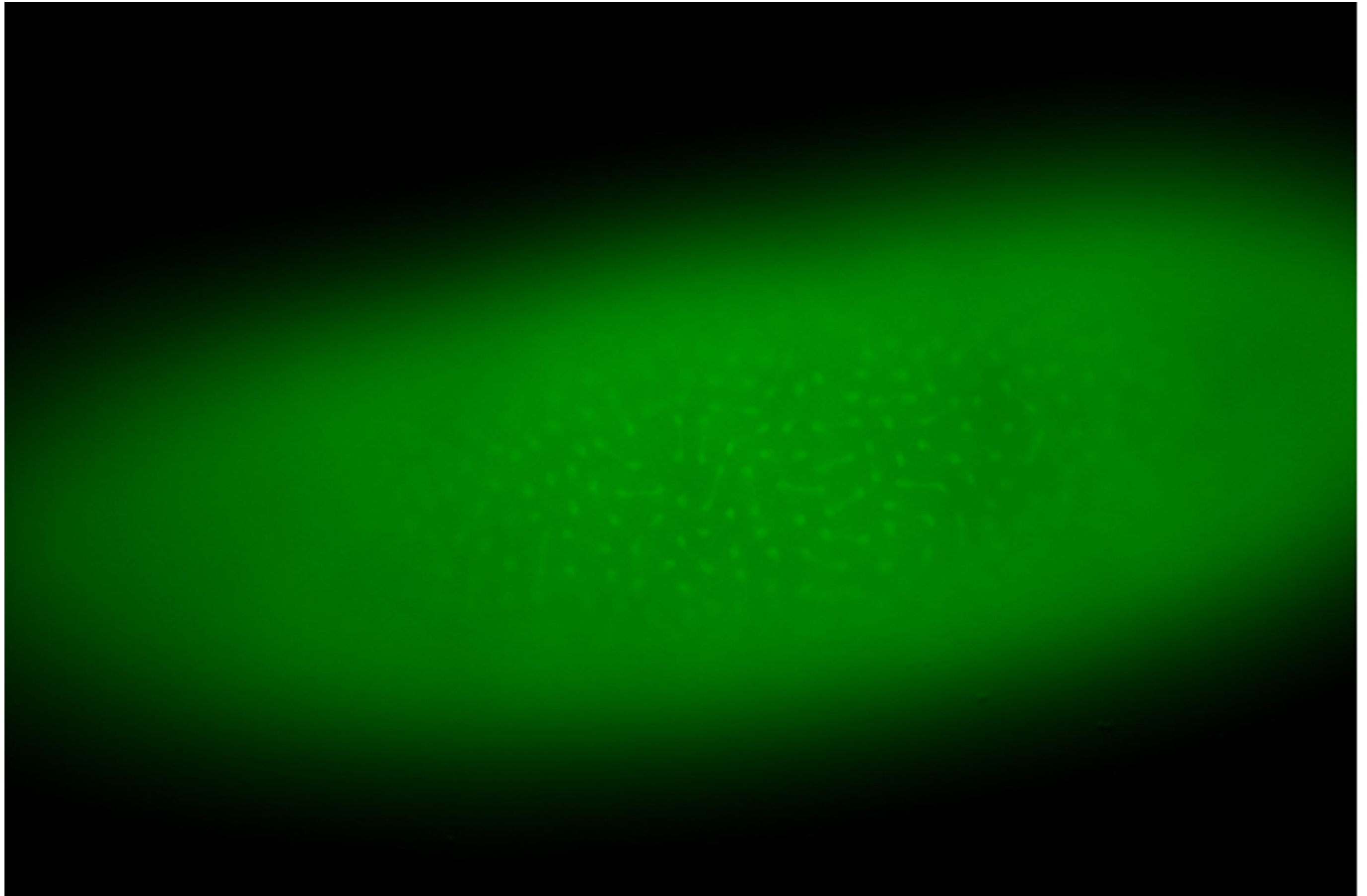


Fig. S2. Mre11 forms large nuclear foci in wild-type, but not mutant embryos. DNA (DAPI) and anti-Mre11 (Mre11) signals are shown as grayscale pictures for wild type (*Top*) and *mre11*^{58S} (*Bottom*) embryos. Cell-cycle phases: Inter, interphase; Meta, metaphase; Ana, anaphase; and Telo, telophase.



Movie S1. Movie of a syncytial wild-type embryo expressing GFP-tagged histones so that the chromosomes are labeled green.

Movie S1 (MOV)



Movie S2. Movie of a syncytial mre1158S embryo expressing GFP-tagged histones so that the chromosomes are labeled green.

Movie S2 (MOV)

Table S1. Primers

Name	Sequence
mre11(+)-A2	5′-cggagaacatcattatccagcagc
mre11(+)-Xba-B2	5′-gctctagatgacggatgcccgtcaatttggcaac
I-siteblunt+	5′-tagggataacagggtaat
I-siteblut-	5′-tattaccctgttatcccta
mre11-His230Tyr-A	5′-ccatctgttagtggtatatcagaaccgcgccgatc
mre11-His230Tyr-B	5′-gatcggcgcggttctgatataccactaacagatgg
mre11-UP-seq2	5′-gcggctaataaagatacgg
mre11–4916B	5′-ctcacttactgccgtattg
mre11-DO-seq2	5′-gcttacctgttatgttcacc
mre11(+)-B	5′-gcctgaaacagaaagcccttcatgc
mre11-ECOR-620B	5′-gcgaattctcaatcggaatcatccgatataac
mre11-KPN-1A	5′-taggtaccatgaatggcaccacgac
mre11-KPN-0B	5′-taggtaccatcggaatcatccgatataac
Fnbs	5′-caaggatccacagttgactcctcggacga
Rnbs	5′-ctgaagcttatccgccttaatagaggtttcc
HeT-A453rev	5′-acttcccattgcatcgctcgtt
HeT-A1196rev	5′-ggcggaaaaatgctgggagttac
HeT-A1751rev	5′-cgcatggggccacctgtag
HeT-A1997rev	5′-gtggcgggggtggttcttg
HeT-A2615rev	5′-atttttggcgatggcgtggta
nbs1991d	5′-tgttcgtggtcatcaacagg
nbs6968u	5′-aggaagaggaactgctcctt