Supplemental Figure Legends for

Automated and customizable quantitative image analysis of whole *C. elegans* germlines
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Supplemental Figure 1. Position of individual nuclei within whole germlines identified for computational analysis. Bar plots representing the proportion of nuclei in ten equal bins across the lengths of the two gonads analyzed in Figure 2. P values in all panels were calculated by Chi Square Test of Goodness of Fit (expected frequency 0.1 in each bin). (A-B) Number of nuclei within bins across the lengths of analyzed individual germlines. The distribution of nuclei computationally identify by the Whole Gonad Pipeline along the length of each gonad was nonuniform. (C) Number of nuclei within bins of combined gonads analyzed in panels A-B. The distribution of nuclei within bins is indistinguishable from a uniform distribution by this same test when the nuclei from the two germlines are taken together.

Supplemental Figure 2. Validation of Gonad Linearization algorithm. (A) Plots of simulated dataset of 100 'germlines' each with 100 'nuclei' points dispersed along their lengths. Points were realigned to the central lines using the Gonad Linearization algorithm, and points that were aligned to the correct line segment are marked in grey while points marked in red were aligned to the incorrect line segment. (B) Density plot demonstrating the distribution of accuracy of point alignment to line segments among the 100 individual simulated 'gonads'. (C) Comparison of the known rank order of correctly aligned spots to the rank order of spots as determined by the Gonad Linearization algorithm. R^2 and p values were calculated by linear regression analysis. (D) Calculation of the deviation of assigned positions as determined by the Gonad Linearization algorithm from 'actual' known positions from the original simulation.

Supplemental Figure 3. DSB-2 normalized intensity per nucleus correlates with RAD-51 foci per nucleus. Assessment of nonparametric correlation by Spearman correlation tests between RAD-51 foci per nucleus and normalized DSB-2 staining intensity among nuclei within the premeiotic through early pachytene stages, and in mid- through late pachytene stages. Specific meiotic stages determined based on DNA morphology.

Supplemental Figure 4. RAD-51 foci per nucleus quantification in meiotic mutants. Immunofluorescence images of (A) a *spo-11(me44)* null mutant hermaphrodite germline (no endogenous DSBs made during meiotic prophase I progression), and (B) a *rad-54(ok615)* null mutant hermaphrodite germline (RAD-51 cannot be unloaded during meiotic prophase I progression) stained with DAPI (DNA; blue) and RAD-51 (green). Scale bar represents 20μm. Inset images display representative late pachytene nuclei. The position these nuclei are located within the germline are indicated by white boxes on the whole gonad images. Specific meiotic stages determined based on DNA morphology. Image in (B) is an immunofluorescence image from a supplemental figure in Rosu *et al., Science* 2011 that has been requantified utilizing the method described in this manuscript. (C) Visualization of numbers of RAD-51 foci associated with individual nuclei across the germlines displayed in A-B. Mutants deficient in *spo-11* are unable to induce meiotic DNA breaks, resulting in very few DSBs observed within the germline (Colaiácovo *et al.* 2003). Mutants for *rad-54* are unable to complete DSB repair, and so exhibit elevated and persistent RAD-51 marked DSBs (Mets and Meyer 2009; Rosu *et al.* 2011; Nottke *et al.* 2011).

Supplemental Figure 5. Quantification of 'bright' MSH-5 foci associated with the chromosome axes of individual meiotic nuclei. Visualization of numbers of MSH-5 foci associated with SYP-1 within individual nuclei across the germline displayed in Figure 3A. As

MSH-5 foci are known to become brighter in late prophase I, we restricted our analysis to account for only the brightest foci in the germline. This dataset, composed of identical nuclei to those quantified in the top panel of Figure 3C, demonstrates the flexible capacity of Imaris software in conjunction with our Whole Gonad Pipeline to specifically identify and quantify specific subpopulations of meiotic biological features.

Supplemental Figure 6. Colocalization analysis of meiotic features which infrequently colocalize. To determine the accuracy of our colocalization analysis (Figure 4), we assessed the association of MSH-5 and RAD-51, which mark distinct recombination intermediates and rarely colocalize (Schvarzstein *et al.* 2014). (A) Immunofluorescence image of a *C. elegans* hermaphrodite germline stained with DAPI (DNA; blue), RAD-51 (green), and MSH-5 (red). Our analysis focused on early-mid pachytene where MSH-5 and RAD-51 foci are most frequently observed in the same nuclei. Scale bar represents 10μm. Inset images display a representative early pachytene nucleus. The position at which this nucleus is located within the germline is indicated by white boxes on the gonad images. Numbered arrowheads respectively indicate examples of: (1) a RAD-51 focus not colocalized with MSH-5, (2) a MSH-5 focus not colocalized with RAD-51, and (3) colocalized RAD-51 and MSH-5 foci. (B) Proportion of MSH-5 (7.1%; 28/392) and RAD-51 (5.8%; 28/481 foci) foci determined to be colocalized with the respective other protein foci. (C) Density plot displaying the distributions of distances between MSH-5 foci and their respective colocalized RAD-51 focus, or between non-colocalized MSH-5 foci and the nearest RAD-51 focus.