



**Figure S1 Immunolocalization of RAD-51 and MSH-5 during wild-type meiosis.** Images show a region of the gonad extending from meiotic prophase entry (left) until the end of the pachytene stage (right); Scale bar = 10  $\mu$ m. Images show that RAD-51 foci are abundant during early pachytene, decline during mid-pachytene, and are nearly absent by late pachytene. Images also show that faint MSH-5 foci appear during early pachytene, become brighter and more abundant during mid-pachytene, then reduce in number at late pachytene, where they persist at CO-designated sites.

Although both RAD-51 foci and MSH-5 foci are frequently present in the same nuclei during early and mid pachytene, the two types of foci rarely overlap in either wild-type or *him-6* mutant germ lines. We evaluated the rarity of colocalization as follows: Maximum intensity projection images of 10 full gonads of each genotype (wild type and *him-6*) were examined for incidences of apparent colocalization where green and red immunofluorescence signals appeared coincident (yielding a yellow signal in projected images). The incidence of such cases of apparent colocalization was extremely low ( $1.1 \pm 1.0$  per gonad for wild type;  $1.5 \pm 1.4$  per gonad for *him-6*), and no significant difference was observed between the genotypes ( $p = 0.62$ ). Further, for each case of possible colocalization, the relevant nucleus was examined in 3D rotation using Volocity (Perkin Elmer) three-dimensional rendering software. For 5/11 cases in wild type and 5/15 cases in the *him-6* mutant, the RAD-51 and MSH-5 foci were well separated, indicating that apparent colocalization was an artifact of image projection. In most of the remaining cases, the RAD-51 and MSH-5 foci were either immediately adjacent to each other (5/11 for wild-type, 7/15 for *him-6*) or exhibited a very small degree of overlap (1/11 for wild type, 2/15 for *him-6*). There was only one case (in a *him-6* late pachytene nucleus) where substantial overlap between a RAD-51 and a MSH-5 immunofluorescence signal was verified.