Supplementary Figure Legends

Fig. S1 AT-rich satellite binding protein D1 serves as a marker for the *D. simulans* Y and 4th chromosomes. **(A)** Mitotic preparations of larval neuroblast cells from wildtype *D. melanogaster w1118* and **(B)** *D. simulans w501* were stained with a D1 antibody (green). The pericentric localization of D1 to the X, Y, 2nd, and 4th chromosomes in *D. melanogaster* is consistent with previous studies (*1*) but note the lack of D1 staining on the *D. simulans* X chromosome, which is due to the absence of the pericentric SATIII (1.688 g/cm^3) (*2*) D1 target in *D. simulans*. **(C)** Summary of D1 staining on *D. simulans* chromosomes. We are unable to unambiguously determine whether the 2nd or 3rd chromosomes show D1 staining in the pericentric regions.

Fig. S2 Scheme for mapping OdsHsim and OdsHmau localization in *D. simulans* sister species. *D. simulans* males or females homozygous for a transgene (shown as a red triangle) inducibly expressing either a FLAG-OdsH^{sim} or Venus-OdsH^{mau} fusion protein were mated to either *D. mauritiana* or *D. sechellia* males and females to generate interspecies hybrids. All interspecies progeny inherit one parental chromosome with an OdsH transgene and there are 3 possible combinations of a hybrid karyotype depending on the sex of the parents (represented by classes I-III).

Fig. S3 OdsH varies in its localization to chromosomes in closely related sister species. **(A)** Full spreads of mitotic preparations (as summarized in Fig. 2) from male and female larval neuroblast cells from either *D. simulans/D. mauritiana* or **(B)** *D. simulans/D. sechellia*. Hybrid flies are either expressing 1XFLAG-OdsHsim or Venus-OdsHmau fusion proteins (red) counterstained with D1 (green) and DNA (blue).

Fig. S4 Additional examples of how OdsH protein decondenses chromatin at sites of localization. **(A)** Venus-OdsHmel and **(B)** Venus-OdsHmau (both shown in red) fusion proteins on mitotic chromosomes from *D. melanogaster* male larval neuroblast cells decondense chromatin at their respective sites of localization (arrows). DNA staining shown in blue.

Fig. S5 Antibody raised to OdsH C-terminal region (*D. simulans* amino acids 210-370) in mouse specifically recognizes the OdsH protein. **(A)** Protein lysates from non-heatshocked (-) or heatshocked (+) transgenic Venus-OdsHmau or 1XFLAG-OdsHsim larval lines were separated by electrophoresis on a denaturing polyacrylamide gel and subsequently Western blotted using anti-OdsH (left panel) or antibodies to the FLAG or Venus tags, respectively (right two panels). Note that the OdsH protein runs approximately 10 kD higher than predicted molecular weights of ~43 kD for 1xFLAG-OdsHsim and ~66 kD for Venus-OdsHmau and recognizes both proteins with high specificity. **(B)** Expression of a Venus-OdsHsim fusion protein in *D. melanogaster* S2 cell culture and subsequent immunofluorescence using anti-OdsH (red) recognizes the fusion protein (green).

Fig. S6 Fertile and sterile introgression lines differ in that the last two exons of *OdsH* are derived from *D. mauritiana*. A region of the *D. mauritiana* X chromosome (red) had been introgressed into a *D. simulans* background (blue) to produce two types of introgression lines: one is fertile and encodes a chimeric *D. mauritiana/D. simulans* protein and the other is sterile and encodes the OdsH protein completely derived from *D. mauritiana* coding sequence. The homeodomain is encoded by exons 2 and 3.

Fig. S7 OdsH protein is absent in spermatogonia and early G2 nuclei. **(A)** Squashed testes from *D. simulans* wildtype and **(B)** *D. simulans* sterile introgression lines were stained for OdsH (green) and counterstained with DAPI (blue). The small and round morphology of the nuclei allowed for the identification of a domain consisting of the spermatogonia and early G2 spermatocytes and is demarcated with the hashed line. Note the exclusive localization of OdsH in those nuclei that are larger and fainter, which represent spermatocytes in mid- to late-G2. Only the approximate one-third of each testis is shown. Final images are a composite of several smaller images. Scale bar represents 20 μm.

Fig. S8 OdsH protein expression does not extend into meiosis. **(A)** Whole-mount testes from *D. simulans* wildtype and **(B)** *D. simulans* sterile introgression lines were stained for OdsH (red) and phospho-Histone H3 serine 10 (green), which is a chromatin mark specific to condensed mitotic and meiotic chromosomes. Meiotic nuclei staining for phospho-H3ser10 are outlined with a hashed box and a close-up is shown in the right panels **(A', B')**. No OdsH protein is observed in these nuclei. Final images are a composite of several smaller images. Exposure times were constant for the individual pictures but some variation in color was still observed. Scale bar represents 20 μm.

1. N. Aulner *et al.*, *Mol Cell Biol* **22**, 1218 (Feb 1, 2002).
2. A. R. Lohe, A. J. Hilliker, P. A. Roberts, *Genetics* **134**, 1149 (Aug, 1993).