

Fig. S1 (supports Fig. 1): *chinmo* is not required in the ovary.

(A-D) Immunofluorescence detection of nuclear-localized GFP (green) in adult ovaries reveals that *c587-Gal4* (A) is expressed in escort cells, follicle stem cells, and some follicle precursor cells; *eyaA3-Gal4* (B) is expressed in some escort cells, follicle cells, and stalk cells; *tj-Gal4* (C) is expressed in escort cells, follicle cells, and stalk cells; and *nanos-gal4* (D) is expressed in germ cells. (E-F) Immunofluorescence detection in adult ovaries of FasIII (green at cell periphery) to visualize somatic cell membranes and 1B1 (green in germ cells) to mark fusomes. Ovaries are indistinguishable from control ovaries when *chinmo* is knocked down (via RNAi induction) in somatic cells. (G-K) Immunofluorescence detection of Chinmo (green) in adult testes and ovaries. In control testes (G), Chinmo is expressed in the nuclei of hub cells (asterisk), CySCs and early cyst cells (arrows), and germ cells (red) (Flaherty et al., 2010). In control ovaries (H), Chinmo is expressed weakly in a few germ cells (arrowheads, white in inset) but is absent from somatic cells (arrows). Chinmo is slightly increased in germ cells when *chinmo* is overexpressed in germ cells (I) in adult ovaries and ovaries are indistinguishable from control ovaries. Chinmo is detected in the *chinmo*-expressing somatic cells with ectopic expression of *chinmo-3'UTR* (without the 5'UTR) (J) and *chinmo-5'UTR* (without the 3'UTR) (K). The expression pattern is similar to ectopic expression of *chinmo-FL* (full length construct of *chinmo*) described in Figure 1C. They also give same phenotype as expression of full length *chinmo* for about 4 days. (L-N) Immunofluorescence detection in adult ovaries of FasIII (green at cell periphery) to visualize somatic cell membranes and 1B1 (green in germ cells) to mark fusomes. Ectopic expression of *chinmo-3'UTR* (L) or *chinmo-5'UTR* (M, N) gives the same phenotype as expression of full length *chinmo* as described in figure 1. Vasa (red) marks germ cells; DAPI (blue) marks nuclei; and anterior is to the left in all panels. Scale bars = 20 μ m.

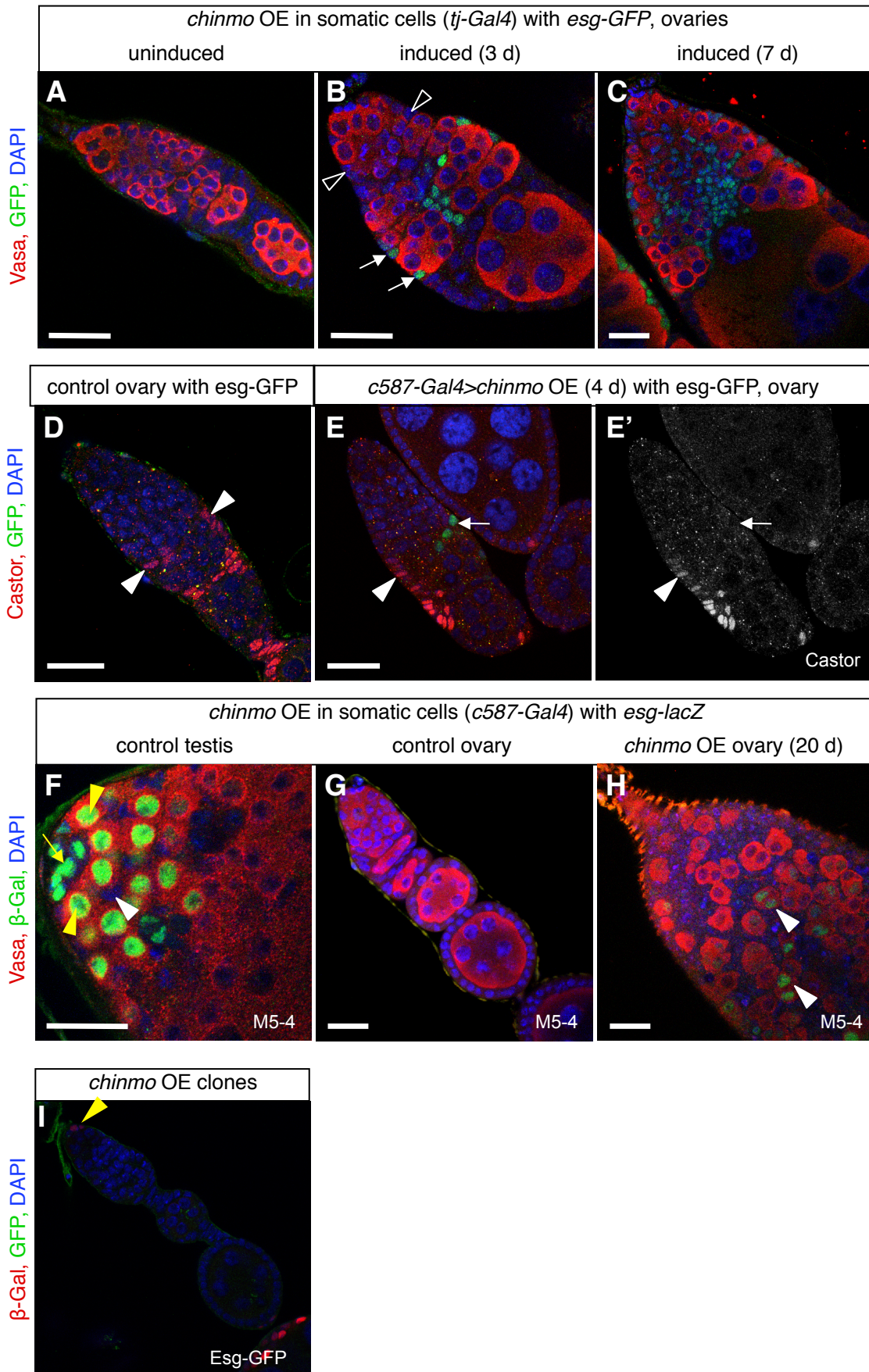


Fig. S2 (supports Fig. 2): Somatic cells and germ cells in the ovary express a male specific marker when *chinmo* is expressed ectopically in somatic cells.

(A-C) Immunofluorescence detection in adult ovaries of GFP (green) to visualize expression of a male-specific enhancer trap *esg-GFP* and Vasa (red) to visualize germ cells. Before induction of ectopic *chinmo* expression (A), the adult ovary looks normal and does not express *esg-GFP*. After *chinmo* is expressed in adult somatic cells for 3 days (B), somatic cells (arrows) in the posterior of the germarium, where follicle stem cells and their early progeny reside, start to express *esg-GFP*. At this time point, *esg-GFP* is not expressed in escort cells (arrowheads) or cap cells. After *chinmo* is expressed for longer times (C), the ovaries develop a more severe phenotype as described in Figure 1, and *esg-GFP* is expressed in more somatic cells. Compare these results (with *tj-Gal4*) to Fig. 2 C-F (with *c587-Gal4*). **(D-E)**

Immunofluorescence detection of GFP (green), to visualize expression of the male-specific marker *esg-GFP*, and Castor (red), a female-specific marker for follicle stem cells and early follicle cells. In control ovaries carrying *esg-GFP* (D), Castor marks follicle stem cells and early follicle cells (arrowheads) as expected, but GFP is not expressed. In *c587-Gal4>UAS-chinmo* ovaries carrying *esg-GFP* (E), after 4 days of ectopic *chinmo* expression, Castor is not expressed in the somatic cells that express GFP. The red channel (Castor only) is shown in panel E'.

(F-H) Immunofluorescence detection of β -galactosidase (green) to visualize expression of *M5-4*, a male-specific *escargot* enhancer trap, and Vasa (red) to visualize germ cells. In control testes (F), *M5-4* is expressed in hub cells (yellow arrow), GSCs (yellow arrowheads) and early differentiating germ cells, but not in cyst stem cells (white arrowhead) or cyst cells. *M5-4* is not expressed in control ovaries (G), but after ectopic expression of *chinmo* in the somatic cells of the germarium for 20 days (H), *M5-4* expression can be detected in some germ cells (arrowheads).

(I) Immunofluorescence detection in the adult ovaries of GFP (green) to visualize expression of male-specific *esg-GFP* and β -Gal (red) to visualize clones with *chinmo* overexpressed. The germarium looks wild-type and have no *esg-GFP* expressing cells with *chinmo* overexpression clones (arrowhead) in the anterior half of germarium. DAPI marks nuclei (blue) in all panels. Scale bars = 20 μ m.

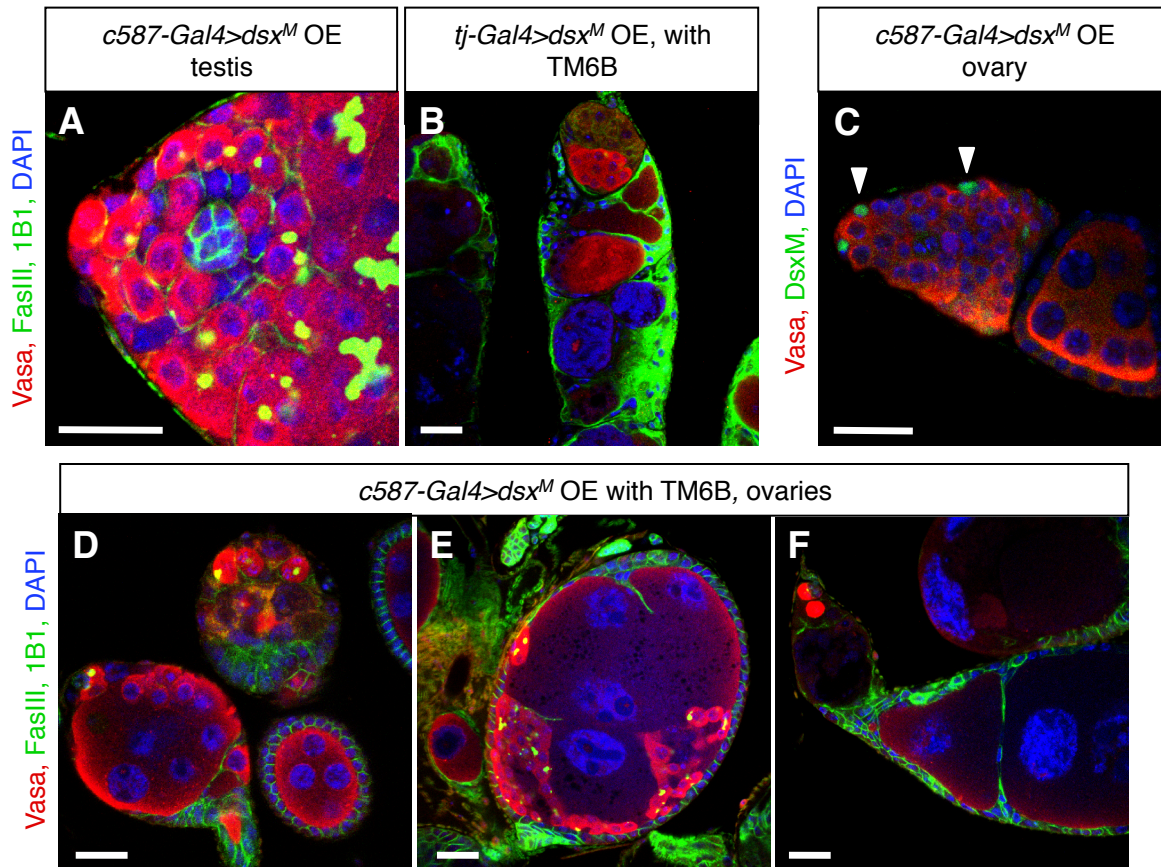


Fig. S3 (supports Fig. 3): Ectopic somatic *Dsx^M* is sufficient to disrupt the morphology of adult ovaries but not testes.

(A-B) Immunofluorescence detection of FasIII (green at cell periphery) to visualize somatic cell membranes and 1B1 (green in germ cells) to mark fusomes. (A) After 1-2 weeks of *Dsx^M* overexpression in the cyst stem cell lineage in adult testes (with *c587-Gal4*), testes look normal. (B) After 1-2 weeks of *Dsx^M* overexpression in the somatic cells of adult ovaries (with *tj-Gal4*, in TM6B background), ovaries display a range of phenotypes including degenerating or abnormal egg chambers. (C) Immunofluorescence detection of *Dsx^M* (green). After 7 days of ectopic *Dsx^M* expression in somatic cells in adult germaria, *Dsx^M* can be detected in some of the *Dsx^M*-expressing cells (arrowheads). (D-F) Immunofluorescence detection of FasIII (green at cell periphery) to visualize somatic cell membranes and 1B1 (green in germ cells) to mark fusomes. After 1-2 weeks of *Dsx^M* overexpression in the somatic cells of adult ovaries (with *c587-Gal4*, in TM6B background), ovaries display a range of phenotypes including degenerating egg chambers or abnormal egg chambers containing both early and late differentiating germ cells. However, in contrast to ectopic expression of *chinmo*, egg chambers with layers of columnar epithelial somatic cells continue to form and the phenotypes are different with *chinmo* overexpressing phenotypes. Vasa (red) marks germ cells; DAPI (blue) marks nuclei. Scale bars = 20 μ m.

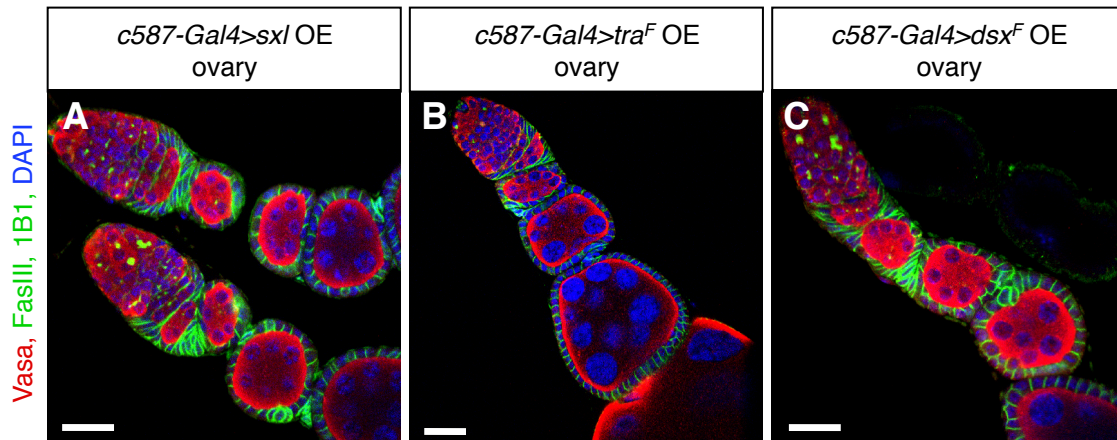


Fig. S4 (supports Fig. 4): Ectopic expression of female sex determinants in ovaries does not disrupt morphology.

(A-C) Immunofluorescence detection of FasIII (green at cell periphery), 1B1 (green in germ cell fusomes), and Vasa (red, germ cells) to visualize the morphology of adult testes and ovaries after overexpression of different female determinants. After overexpression of *sxI* (A), *tra^F* (B), or *dsx^F* (C) in the somatic cells of adult germaria, ovaries look normal. DAPI (blue) marks nuclei in all panels; yellow Scale bars = 20 μ m.

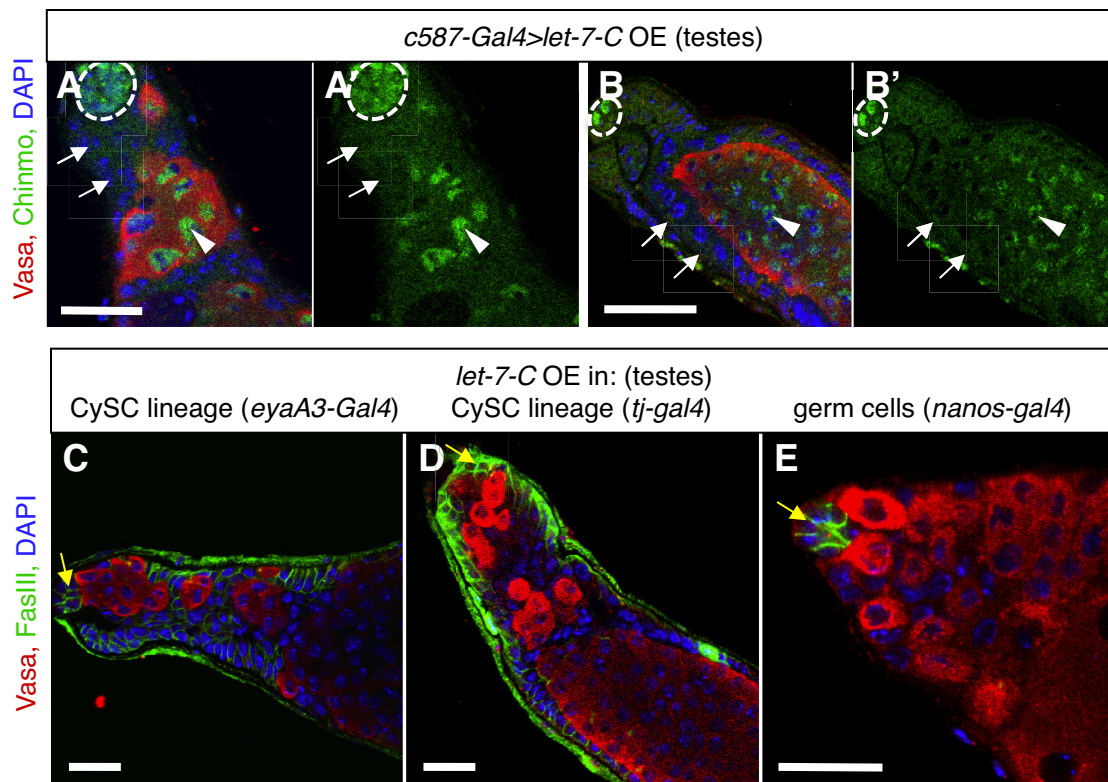


Fig. S5 (supports Fig. 5): Overexpression of miRNA *let-7* in the adult testis phenocopies loss of *chinmo*.

(A-B) Immunofluorescence detection of Chinmo (green) in adult testes after overexpression of *let-7-C* in the cyst stem cell lineage. Chinmo is depleted from somatic aggregates (A, arrows) and follicle-like cells (B, arrows) but remains expressed in germ cells (arrowheads) and in the hub (dashed circles). A' and B' show the green channel (Chinmo) only. (C-E) Immunofluorescence detection of FasIII (green at cell periphery) to visualize testis morphology after *let-7-C* overexpression in different types of cells in adult testes. After *let-7-C* overexpression in the cyst stem cell lineage with the drivers *eyaA3-gal4* (C) or *tj-gal4* (D), 59% (n = 41) or 83% (n = 18) of testes, respectively, contain aggregates of somatic cells or monolayers of follicle-like cells similar to *chinmo* mutant testes. After *let-7-C* overexpression in germ cells (E), all testes look normal (n = 31). Hubs are marked by yellow arrows. Vasa (red) marks germ cells and DAPI (blue) marks nuclei in all panels. Scale bars = 20 μ m.