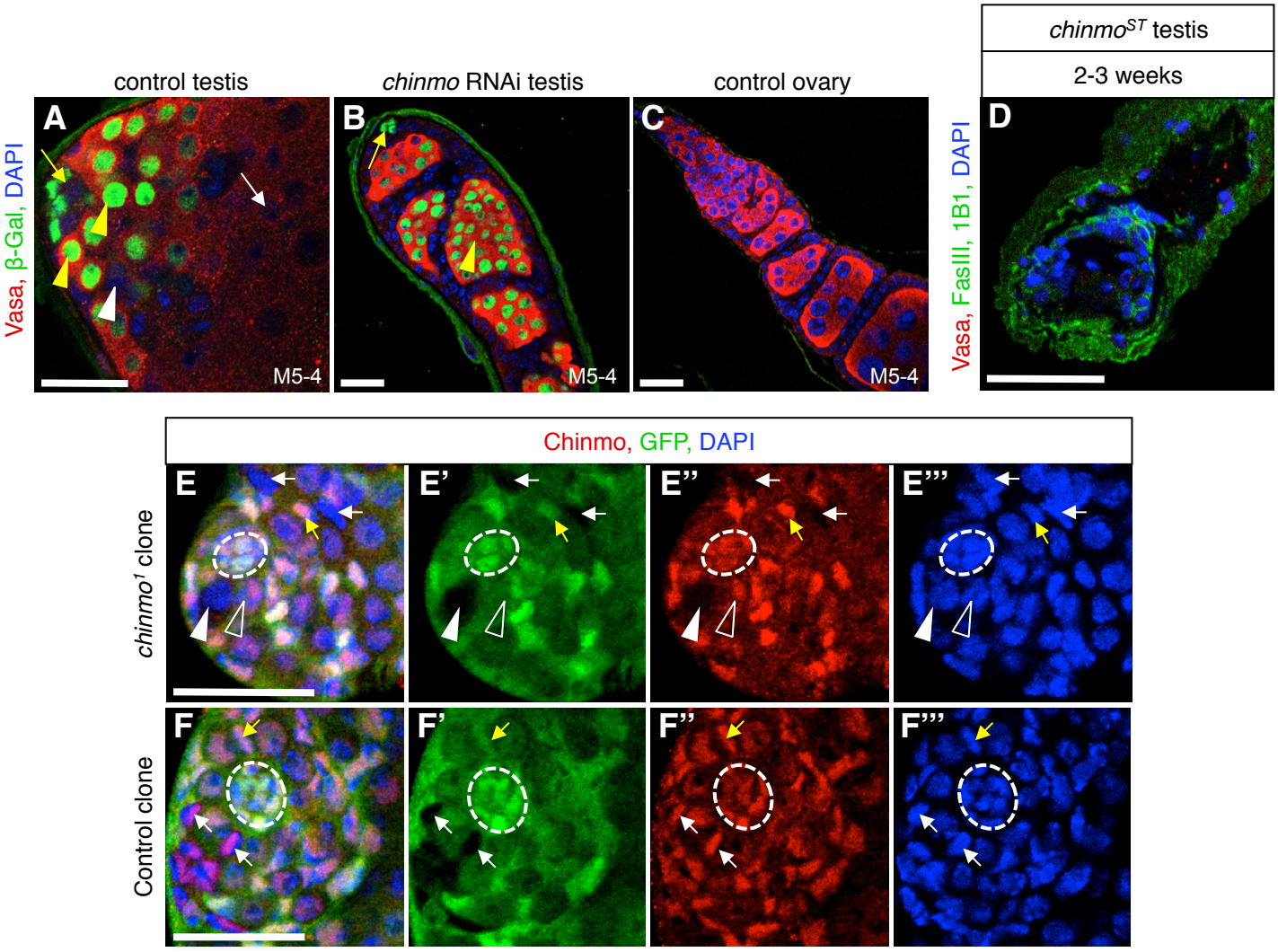
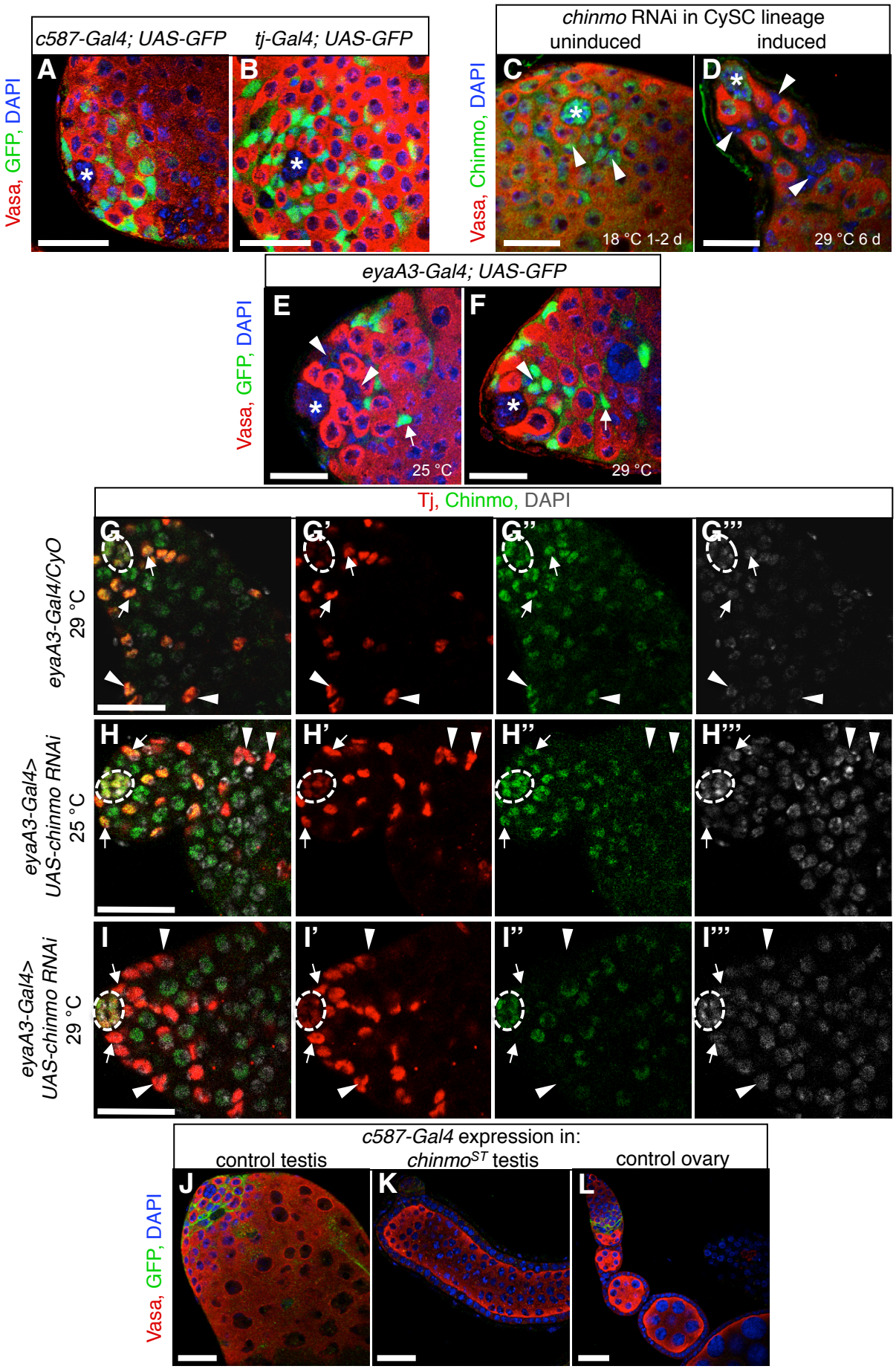
**Supplemental Inventory:**

# Figure S1 (related to Figure 1 and 2)



# Figure S2 (related to Figure 5)



# Figure S3 (related to Figure 7)

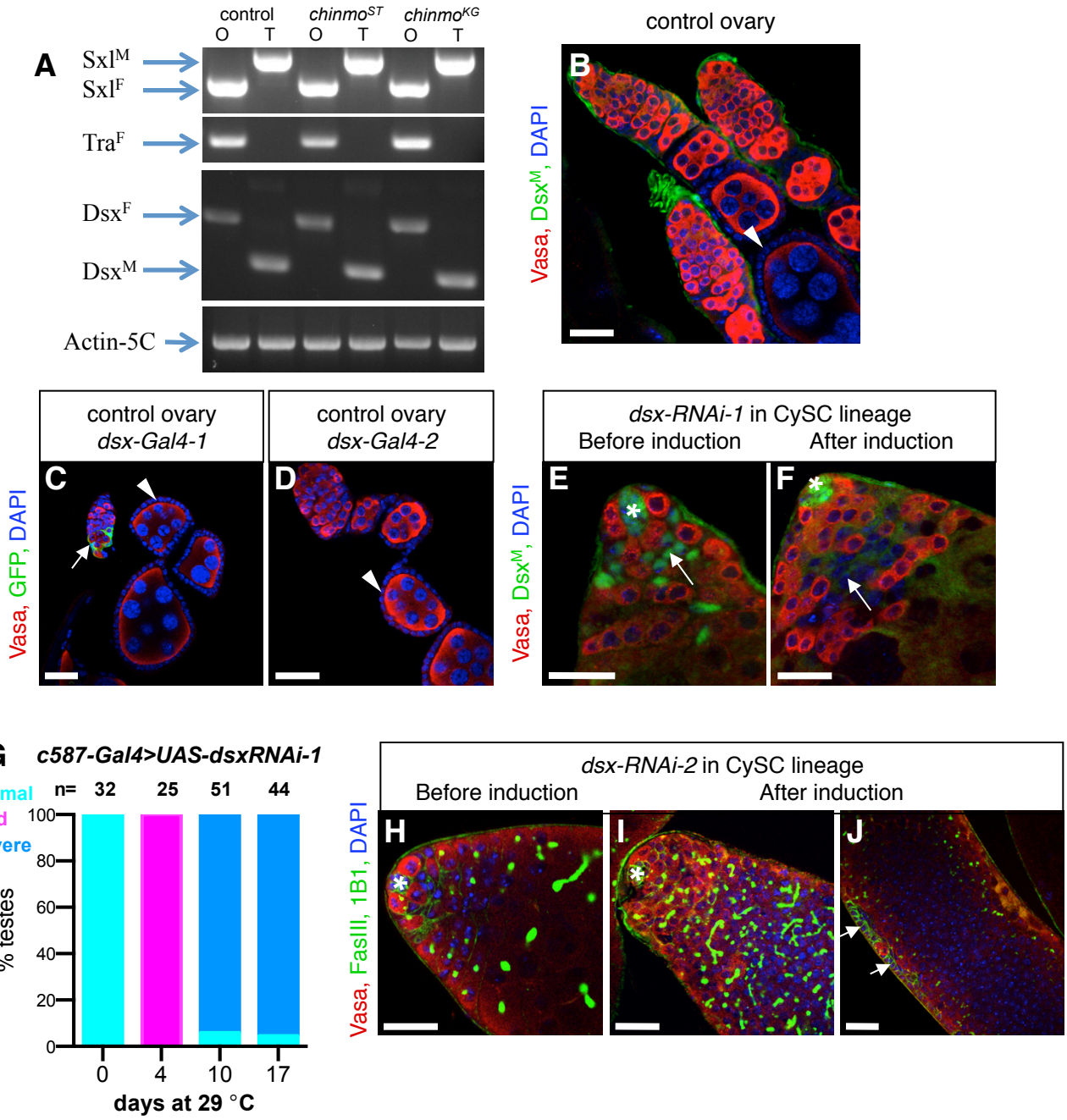


**Figure S1. (Related to Figure 1 and 2) Reduction of Chinmo causes somatic cells in adult testes to be gradually replaced by cells resembling ovarian follicle cells.**

**(A-C)** Immunofluorescence detection of β-galactosidase (green) to visualize M5-4, a male-specific *escargot* enhancer trap, and Vasa (red) to visualize germ cells. In control testes (A), M5-4 is expressed in hub cells (yellow arrow), GSCs and early differentiated germ cells (yellow arrowheads), but not in CySC lineage cells (white arrowhead) or later differentiated germ cells (white arrow). After RNAi-mediated depletion of *chinmo* from the CySC lineage (*c587-Gal4; UAS-chinmo-RNAi*) (B), hub cells and germ cells continue to express M5-4, suggesting that they maintain characteristics of their male identity. Germ cells away from the testis apex fail to downregulate M5-4 (yellow arrowhead), indicating that they do not differentiate properly. M5-4 is absent from ovaries (C). (**D**) Immunofluorescence detection of FasIII (green at cell periphery) to visualize follicle-like cells in testes, and Vasa (red) to visualize germ cells. At 2-3 weeks of age, germ cells in $chinmo^{ST}$ testes degenerate; only somatic cells remain at the testis apex. (**E-F**) Immunofluorescence detection of Chinmo (red) in adult testes containing $chinmo^{1}$ or control clones verifies the specificity of anti-Chinmo antisera in adult testes. Hubs marked by dashed line. Chinmo levels are decreased in a $chinmo^{1}$ germ cell clone (white arrowhead) and somatic clones (white arrows), identified by the absence of GFP (green), compared to neighboring GFP-positive heterozygous germ cells (one indicated, open arrowhead) and somatic cells (yellow arrow) (E). Wild-type control somatic clones and neighboring cells (white and yellow arrows, respectively) (F) have similar levels of Chinmo. DAPI marks nuclei (blue). Scale bars = 20 μm.

**Figure S2. (Related to Figure 5) Chinmo is required autonomously in adult CySC lineage cells to prevent their transformation into female soma.**

(**A-B**) Immunofluorescence detection of nuclear-localized UAS-GFP (green) in adult testes reveals that *c587-Gal4* (A) and *tj-Gal4* (B) are expressed in early CySC lineage cells but not in hub cells (asterisk) or germ cells (red). (**C-D**) Immunofluorescence detection of Chinmo (green) in adult testes verifies the efficacy of *chinmo RNAi* in the CySC lineage (genotype: *c587-Gal4; UAS-chinmo RNAi/tub-Gal80$^{ts}$*). Before expression of *chinmo* RNAi (C), Chinmo is detected in hub cells (asterisk), CySC lineage cells (arrowheads), and germ cells (red). After 6 days of *chinmo*-RNAi expression in CySC lineage cells (D), Chinmo is no longer detected in CySC lineage cells (arrowhead). (**E-F**) Immunofluorescence detection of GFP (green), revealing the *eyaA3-Gal4* expression pattern. At 25 ˚C (E), *eyaA3-Gal4* drives expression of *UAS-GFP* at high levels in cyst cells (arrow) but not in CySCs (arrowheads). At 29 ˚C (F), *eyaA3-Gal4* drives expression in both CySCs and cyst cells. (**G-I**) Immunofluorescence detection of Chinmo (green) and Tj (red) in adult testes. Hubs marked by dashed line. In control testes (G), Chinmo is detected in CySCs and their immediate daughters (arrows), which are identified as Tj$^{+}$ nuclei near the hub, as well as in cyst cells (arrowheads). After *chinmo*-RNAi is expressed only in cyst cells (*eyaA3-Gal4* at 25 ˚C) (H), Chinmo remains detectable in CySCs and their immediate daughters (arrows) but not in older cyst cells (arrowheads). After *chinmo*-RNAi is expressed in both CySCs and cyst cells (*eyaA3-Gal4* at 29 ˚C) (I), Chinmo is no longer detected in CySCs (arrows) or cyst cells (arrowheads). (**J-L**) Immunofluorescence detection of cytoplasmic GFP (green), revealing the *c587-Gal4* expression pattern. *c587-Gal4* drives expression of *UAS-GFP* in the cytoplasm of early CySC lineage cells in

control testes (J) and in somatic cells in the germarium in control ovaries (L). Older follicle cells in control ovaries (L) and follicle-like cells in *chinmo*$^{ST}$ testes (K) lack *c587-Gal4* activity. DAPI marks nuclei (blue in A-E, J-L and white in G-I). Scale bars = 20 μm.

**Figure S3. (Related to Figure 7) *chinmo*$^{ST}$ regulates *dsx* transcription rather than female-specific splicing of mRNAs in the canonical sex determination pathway, and *dsx*-RNAi partially phenocopies the *chinmo* mutant phenotype.**

(**A**) RT-PCR detection of male and female spliced forms of *sxl*, *tra* or *dsx* mRNA shows that the female spliced forms are not ectopically expressed in control, *chinmo*$^{ST}$ or *chinmo*$^{KG}$ testes. Actin-5C is used as a control. (**B**) Immunofluorescence detection of Dsx$^{M}$ (green) in control ovaries. Dsx$^{M}$ is not detectable in follicle cells (arrowhead) or any other cells in ovarioles. The green staining outside the ovarioles reflects non-specific staining of the ovarian sheath. (**C-D**) Immunofluorescence detection of GFP (green) in control ovaries to reflect the transcription of *dsx-Gal4* (two different lines). *dsx-Gal4-1* is expressed in escort cells (arrow) but not in follicle cells (arrowhead). *dsx-Gal4-2* is absent from control ovaries, including follicle cells (arrowhead). (**E-F**) Immunofluorescence detection of Dsx$^{M}$ (green) in adult testes before and after induction of *dsx-RNAi* (line 1) in the CySC lineage. Before RNAi induction (E), Dsx$^{M}$ is expressed in the nuclei of hub cells (asterisk) and CySCs lineage cells (arrow) (n=18 testes). After RNAi induction (F), Dsx$^{M}$ levels are low or absent in most CySCs and cyst cells (arrow) but remain high in hub cells (n=25 testes). (**G**) Composite bar graph showing the percentage of testes with normal, mild, or severe phenotypes after expression of *dsx-RNAi* (line 1) in the CySC lineage in adult testes for the number of days indicated (genotype: *c587-Gal4; UAS-dsx-*

*RNAi-1; tub-Gal80$^{ts}$*). In this experiment, morphologically wild type testes were scored as normal; testes with somatic cell aggregates and/or over-proliferating early germ cells at the apex, but not away from the apex, were scored as having a mild phenotype; and testes with over-proliferating early germ cells throughout the entire testis were scored as having a severe phenotype (see Fig. 6J). Before RNAi induction (0 d at 29 ˚C), all testes look normal. After RNAi induction in CySC lineage cells, testes display a range of mild to severe phenotypes. (**H-J**) Immunofluorescence detection of FasIII and 1B1 (green) and DAPI (blue) in adult testes before and after induction of *dsx-RNAi* (line 2) in the CySC lineage (genotype: *UAS-dsx-RNAi-2; eyaA3-Gal4; tub-Gal80$^{ts}$*). Before RNAi induction (H), testes look normal (100% of testes, n =35). After RNAi induction (29 ˚C for 2 weeks) (I, J), germ cells overproliferate and arrest at early spermatogonial stages (72% of testes, n = 39). In addition, these testes contain some aggregates of FasIII$^{+}$ cells at the periphery, which resemble the follicle-like cells in *chinmo-RNAi* testes (J, arrows). Vasa marks germ cells (red) and DAPI marks nuclei (blue). Scale bars = 20 μm.

**Table S1 (Related to Figure 2): Phenotype characterization for combinations of *chinmo* mutant alleles**

| | *chinmo*$^{ST}$ [1] | *chinmo*$^{KG}$ [2] | *chinmo*$^{M33}$ [3] | *chinmo*$^{1}$ [4] | *Df(chinmo)* [5] | *Other Df* [6] |
|---|---|---|---|---|---|---|
| *chinmo*$^{ST}$ | FC [7] | FC | FC | FC | FC | No FC |
| *chinmo*$^{KG}$ | FC | lethal | FC | FC | FC | not tested |

[1], [2] *chinmo*$^{ST}$ is a partial loss-of-function allele. It has no mutation in the coding region but has a 10 bp deletion in the 5'UTR region. *chinmo*$^{KG}$ denotes *chinmo*$^{KG05386}$, which has an 11.5 kb transposable element inserted in the 2$^{nd}$ intron (Flybase). *chinmo*$^{ST}$ and *chinmo*$^{KG}$ are fully viable when crossed to each other or to deficiencies for *chinmo.*

[3], [4] *chinmo*$^{M33}$ encodes a missense mutation (F88I) in the BTB domain (Flybase). *chinmo*$^{1}$ is a protein null allele of *chinmo*. *chinmo*$^{M33}$ and *chinmo*$^{1}$ are lethal when crossed to each other or to deficiencies for *chinmo*, which indicates that they are stronger alleles than *chinmo*$^{ST}$ or *chinmo*$^{KG}$. *chinmo*$^{M33}$ or *chinmo*$^{1}$ mutant CySC clones are lost from the adult testis stem cell niche (Flaherty et al., 2010) and no sex transformation is observed. It is possible that CySC clones are lost before the male-to-female transformation phenotype can develop.

[5] Df(*chinmo*) includes the following deficiencies for *chinmo: Df(2L)ast2, Df(2L)Exel6005, Df(2L)dp-79b, Df(2L)S2, Df(2L)ED7762, Df(2L)BSC480,* and *Df(2L)BSC521.*

[6] Other Df includes 58 deficiencies from the Bloomington Stock Center's deficiency kit for the second chromosome that do not remove *chinmo*.

[7] FC = follicle cell-like phenotype

**Table S2 (Related to Figure 2): The *chinmo*$^{ST}$ phenotype can be rescued by Chinmo overexpression in the CySC lineage**

| Genotype[1] | % testes with follicle-like cell phenotype |
|---|---|
| *chinmo*$^{ST}$ (*c587-Gal4; chinmo*$^{ST}$*; TM6B/tub-Gal80*$^{ts}$) | 85.7 (42/49) |
| *chinmo*$^{ST}$ + Chinmo OE[2, 3] (*c587-Gal4; chinmo*$^{ST}$*; UAS-FL-chinmo/tub-Gal80*$^{ts}$) | 0 (0/47) |

[1] Flies were raised and aged for 14-16 days at 18 ˚C.

[2] *UAS-FL-chinmo* (full length *chinmo*) alone, without a Gal4 driver, does not rescue *chinmo*$^{ST}$.

[3] With a Gal4 driver (*c587-Gal4*), *UAS-FL-chinmo* expresses enough Chinmo at 18 ˚C to rescue *chinmo*$^{ST}$. Therefore, we cannot use temperature to control the timing of Chinmo overexpression, and we are not able to determine if *chinmo*$^{ST}$ can be rescued by Chinmo overpression only in adult flies, after formation of the follicle-like cell phenotype.

**Table S3a (Related to Figure 5): Chinmo knockdown in CySCs and cyst cells (CC), but not in late cyst cells alone, induces the follicle cell-like phenotype**[1]

| Express *UAS-chinmo RNAi* with[2] | | | | % testes | | |
|---|---|---|---|---|---|---|
| Gal4 Driver | Pattern | RNAi allele[2] | No. of testes | No phenotype | Mild[3] phenotype | Severe[4] phenotype |
| - | - | 1 | 40 | 95 | 5 | 0 |
| - | - | 2 | 16 | 100 | 0 | 0 |
| c587 | CySC, CC | 1 | 59 | 0 | 59 | 41 |
| tj | CySC, CC | 1 | 19 | 5 | 42 | 53 |
| tj | CySC, CC | 2 | 26 | 31 | 58 | 11 |
| Simj[BG00403][5] | Late CC | 1 | 20 | 100 | 0 | 0 |
| CG3964[BG00933][6] | Late CC | 1 | 29 | 100 | 0 | 0 |
| CG42669[BG02427][7] | Late CC | 1 | 12 | 100 | 0 | 0 |
| tai[BG01746][8] | Late CC | 1 | 29 | 100 | 0 | 0 |

[1] All flies were raised at 18 ˚C, at which temperature RNAi is not induced. After eclosion, adult flies were shifted to 29 ˚C or 31 ˚C for 1-2 weeks to induce RNAi.

[2] *UAS-chinmo RNAi-1 = chinmo$^{HM04048}$; UAS-chinmo RNAi-2 = chinmo$^{HMS00036}$*

[3], [4] See Methods for quantitation of phenotype severity.

[5]-[8] Gal4 enhancer traps that are expressed in testes: *simj[BG00403]* (late cyst cells, pigment cells, muscle sheath), *CG3964[BG00933]* (pigment cells, muscle sheath), *CG42669[BG02427]* (late cyst cells), *tai[BG01746]* (late cyst cells, pigment cells) (Bunt et al., 2012).

**Table S3b (Related to Figure 5): Chinmo knock down in CySCs and cyst cells induces the follicle cell-like phenotype with time**

| Express *UAS-chinmoRNAi-1* with[1] | | | | % testes | | |
|---|---|---|---|---|---|---|
| Driver | Pattern | Days of RNAi induction[2] | No. of testes | No phenotype | Mild[3] phenotype | Severe[4] phenotype |
| c587 | CySC, CC | 0 | 30 | 100 | 0 | 0 |
| | | 7-9 | 37 | 3 | 97 | 0 |
| | | 14[5] | 59 | 0 | 59 | 41 |
| | | 21-28 | 90 | 1 | 23 | 76 |

[1] *UAS-chinmoRNAi-1 = chinmo*$^{HM04048}$

[2] All flies were raised at 18 ˚C, at which temperature RNAi is not induced. After eclosion, adult flies were shifted to 29 ˚C for the indicated amount of time to induce RNAi.

[3], [4] See Methods for quantitation of phenotype severity.

[5] These are the same data as in Table S3a (*c587-Gal4>UAS-chinmoRNAi-1*).

**Table S4 (Related to Figure 6): Follicle-like cells arise from the CySC lineage**

| | % normal testes that have marked CySC lineage cells | % testes with mild phenotype[1] that have marked somatic aggregates | % testes with severe phenotype[2] that have marked follicle-like cells |
|---|---|---|---|
| CySC lineage tracing[3] | 100.0 (9/9) | 100.0 (72/72) | 94.0 (47/50) |
| Hub lineage tracing[4] | 4.5 (4/88) | 4.3 (1/23) [5] | 6.3 (1/16) [6] |

[1], [2] See Methods for quantitation of phenotype severity.

[3] 100% testes have at least some marked CySC lineage cells, but not all CySC lineage cells within each testis are marked. Full Genotype: (*c587-Gal4; chinmo$^{ST}$/chinmo$^{KG}$; UAS-FLP/Actin>Stop>lacZ*)

[4] 100% testes have at least some marked hub cells, but not all hub cells within each testis are marked. Full genotype: (*E132-Gal4; chinmo$^{ST}$/chinmo$^{KG}$; UAS-FLP/Actin>Stop>lacZ*)

[5] One testis has one marked cyst cell.

[6] One testis has three marked follicle-like cells.

## Supplementary Experimental Procedures

*Immunostaining*

Testes and ovaries were dissected, fixed, and stained as described previously (Matunis et al., 1997). Tyramide signal amplification (Invitrogen) was used to increase sensitivity of rat anti-$Dsx^M$ (from B. Oliver, 1:500 dilution). The following antibodies were also used: rabbit anti-Vasa (d-260) and goat anti-Vasa (dN-13) (Santa Cruz Biotechnology, 1:400); rabbit anti-GFP (Torrey Pines Biolabs, 1:10,000); chicken anti-GFP (Abcam, 1:10,000); mouse anti-β-Galactosidase (Promega, 1:1000); mouse 1B1 (1:25), mouse anti-Fasciclin III (1:50), mouse anti-Armadillo (1:50), mouse anti-Eya 10H6 (1:50), and mouse anti-Cut (1:20) (all from Developmental Studies Hybridoma Bank, University of Iowa); rat-anti-Chinmo (from N. Sokol, 1:500); rabbit anti-ZFH1 (from R. Lehmann, 1:5000); guinea pig anti-Tj (from D. Godt, 1:4000); mouse anti-phospho-Histone H3 (Cell Signaling, 1:200); and rat anti-BrdU MCA2060 (Serotec, 1:40). Alexa fluor-conjugated secondary IgG (H+L) antibodies were diluted at 1:200 for 568 and 633 conjugates and 1:400 for 488 conjugates. Secondary antisera were: goat anti-rat 488, goat anti-rabbit 488 and 568, goat anti-mouse 488, 568 and 633, goat anti-chick 488, and goat anti guinea-pig 568 (Molecular Probes/Invitrogen). DNA was stained with 4,6-diamidino-2-phenylindole (DAPI; Sigma) at 1 mg/ml.

*mRNA extraction and PCR*

mRNA extraction and reverse transcription-PCR were performed as previously described (Issigonis and Matunis, 2012) with the following primers (Amrein and Maniatls, 1994; Deshpande and Schedl, 1999; Graham and Schedl, 2011; Tarone et al., 2005):

| **Gene** | **Forward primer 5' to 3'** | **Reverse primer 5' to 3'** |
|---|---|---|
| Sxl | CGCTGCGAGTCCATTTCC | GTGGTTATCCCCCATATGGC |
| Tra | GGTCACACTGAGGAAAGTGC | CTTCTCACCCGATCCTGTTCTC |
| $dsx^F$ | TTCCGCTATCCTTGGGAGC | ACAGAGGTTTTGCTCCATAA |
| $dsx^M$ | TTCCGCTATCCTTGGGAGC | AAGTGCGCCCCATAGCGACC |
| Yp | TGAGCGTCTGGAGAACATGAA | GCGACAGGTGGTAGACTTGCT |
| GAPDH | CAACAATAACAAAATATGGCGGATA | CTATGGCCGAACCCCAGTT |
| Act5C | GTATCCTCACCCTGAAGTAC | CATGATGGAGTTGTAGGTGG |

GAPDH and Act-5C were used as controls.

**Supplemental references**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