Supplementary Information

Heterochromatin components in germline stem cell maintenance

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Table S1. Requirement for HP1 in GSC maintenance

Days after clone induction	Clone genotype	Testes with GSC clones/testes scored
1	WT control	15/18 (83%)
	Su(var)205^{5}	2/20 (10%)
2	WT control	36/37 (83%)
	Su(var)205^{5}	3/16 (19%)
5	WT control	8/11 (73%)
	Su(var)205^{5}	1/14 (7.1%)
7	WT control	27/31 (87%)
	Su(var)205^{5}	0/38 (0%)

"Twin-spot" clones homozygous for *Su(var)205*^{05} (loss-of-function allele) or wild-type (control) were identified as GFP^{-} cells. Testes with identifiable clones were scored. Those with at least one clone at GSC position were counted as "testes with GCS clone".

Figure S1

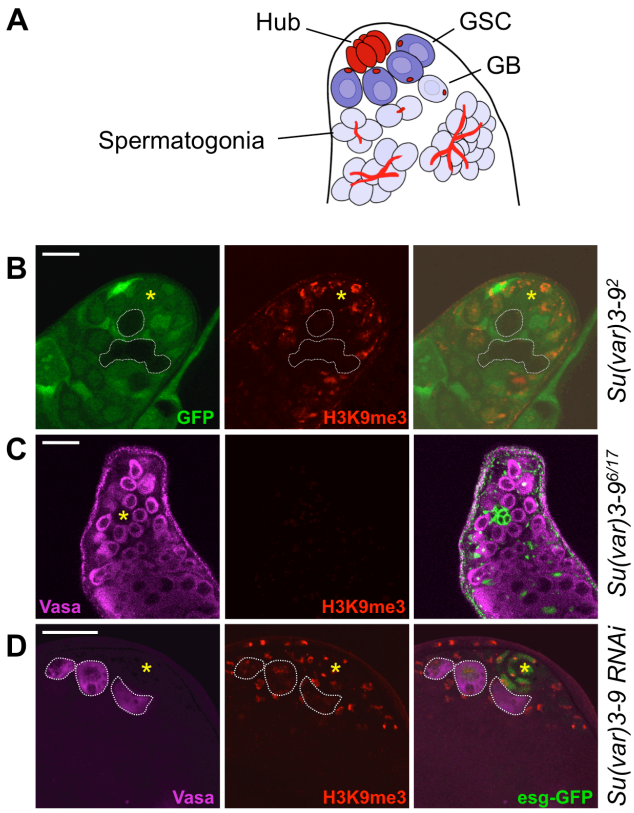

Fig. S1. Su(var)3-9 mutation or RNAi knock-down reduces heterochromatin

Scale bar = 20 μm

(A) Schematic representation of stem cell differentiation in the *Drosophila* testis. Germline stem cells (GSCs; blue) are adjacent to hub cells (red). GSCs differentiate to give rise to gonialblasts (GBs; light blue), which divide to give rise to spermatogonia. See text for more detail.

(B) Testes from *hsp70-flp; FRT*^{82B} *ubi-GFP/FRT*^{82B} *Su(var)3-9*^{2} were dissected 2 days after heat-shock induction of somatic recombination and were stained for H3mK9 (red). Note that the *Su(var)3-9*^{2} homozygous cells (GFP^{-}; circled by dotted line) have no detectable H3mK9 signal.

(C) Testes from 2-day old *Su(var)3-9*$^{6/17}$ males were stained for Vasa (magenta) and H3mK9 (red), and the hub and fusome (green). Note that the testis lacks H3mK9 signal.

(D) Testes from 2-day old wild-type males expressing esg-GFP, nos-Gal4, and UAS-Su(var)3-9 RNAi were stained for Vasa (magenta) and H3mK9 (red). Note that the remaining Vasa+ cells (circled by dotted lines) exhibit much reduced H3mK9 signal compared with the rest of testis area.

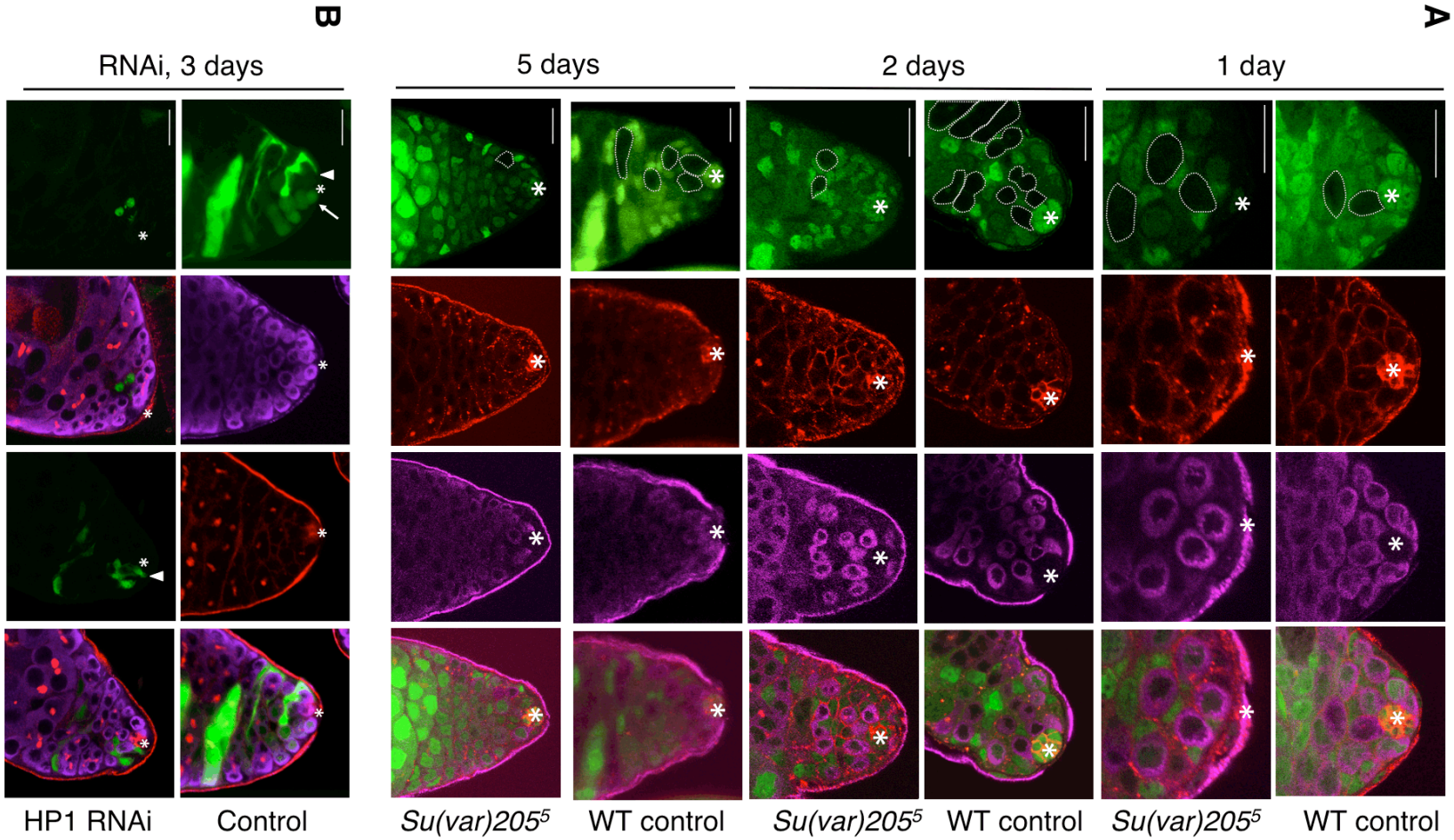
B
RNAi, 3 days
HP1 RNAi
Control
A
5 days
2 days
1 day
Su(var)205^5
WT control
Su(var)205^5
WT control
Su(var)205^5
WT control

Figure S2. Effects of loss of HP1 on GSC maintenance

Testes from male flies that had been subjected to induction of marked clones for loss-of-function of HP1 (GFP^-) (**a**) or over-expression of HP1 RNAi (GFP+) (**b**) were immunostained for Vasa (magenta cytoplasmic staining) and for fusomes and hub cells (both red). Asterisk indicates the position of the hub. Scale bar=20 μm.

(A) Testes were dissected after 1, 2, or 5 days after clone induction. Wild-type control and *Su(var)205*^{5} homozygous mutant clonal cells were marked by the absence of GFP (lack of green). Note that wild-type control clones were found at GSC position (next to the hub), and that *Su(var)205*^{5} homozygous mutant clones were found at he GSC position only 1 day after clone induction, but not after 2 or more days.

(B) In control testes (which did not express HP1 RNAi) 3 days after clone induction, clonal GFP+ cells were found in both the germline (round Vasa+ cells) and somatic (elongated Vasa-minus cells) lineages. Arrow marks a GSC, and arrowhead a CPC. Three days after HP1 RNAi was expressed, no HP1 RNAi-expressing (GFP+) GSCs were observed next to the hub. GFP+ germline cells (round green cells in left panels) were found away from the hub, and they now cease to express Vasa. GFP+ somatic cells (long $Vasa^-$ cells in right panels), however, were still observed.

Figure S3

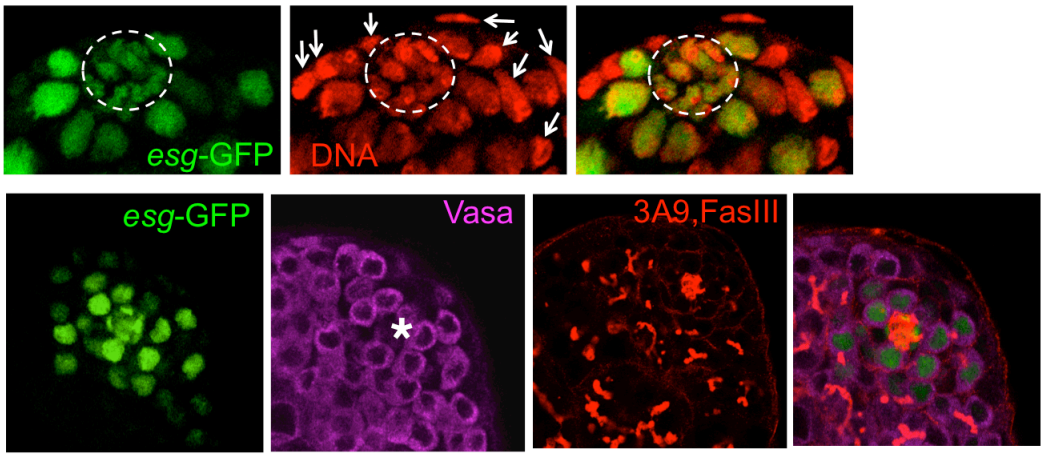

Fig. S3. Expression and localization of esg-GFP

Testes from 2-day old *Drosophila* wild-type males expressing esg-GFP were stained for DNA (red; upper), or Vasa (magenta; lower), or fusome and hub cells (red; lower). Hub cells are circled with dashed line (upper) or marked by asterisk (lower). Arrows point to CySCs. Note that esg-GFP+ cells colocalize with Vasa+ cells, but not with CySCs ($Vasa^{-}$ cells).