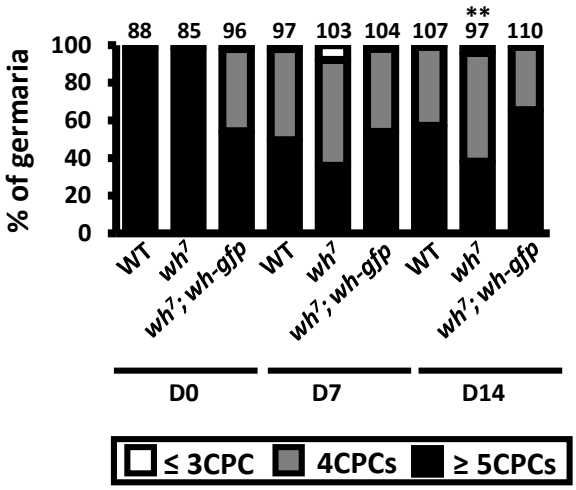

Fig. S1. Wh depletion has small effects on cap cell number.

The percentage of germaria carrying indicated cap cell (CpC) number per germarium in 1-, 7-, and 14-day (D)-old flies of indicated genotypes. Numbers of analyzed germaria are shown above each bar. Genotype of wild-type is w^{1118}. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

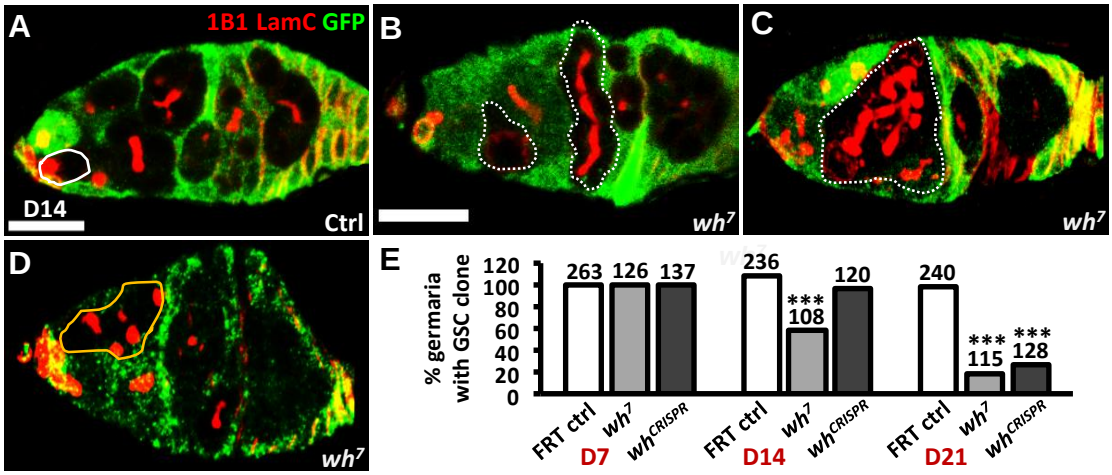

Fig. S2. Wh controls germ cell homeostasis in a cell-autonomous manner.

(A-D) Conventional clonal analysis revealed that Wh is required in germ cells for germline homeostasis. *FRT19A* control (A) and wh^{7} mosaic mutant germaria (B) with 1B1 (red, fusomes), LamC (red, TF and cap cell nuclear envelopes) and GFP (green) labeling. Clones of GSCs and their progeny and stem-cyst (identified by the absence of GFP) are outlined by solid, white dashed and yellow dashed lines, respectively. **(E)** Wh cell-autonomously controls GSC maintenance. The percentage (%) of germaria of flies with indicated genotypes carrying GSC clones at 7, 14, 21 days after the clone induction. Data are normalized with respect to D7 *FRT* control. Representative 14-day (D)-old germaria are shown in 3D-reconstructed images. Scale bar, 10 µm

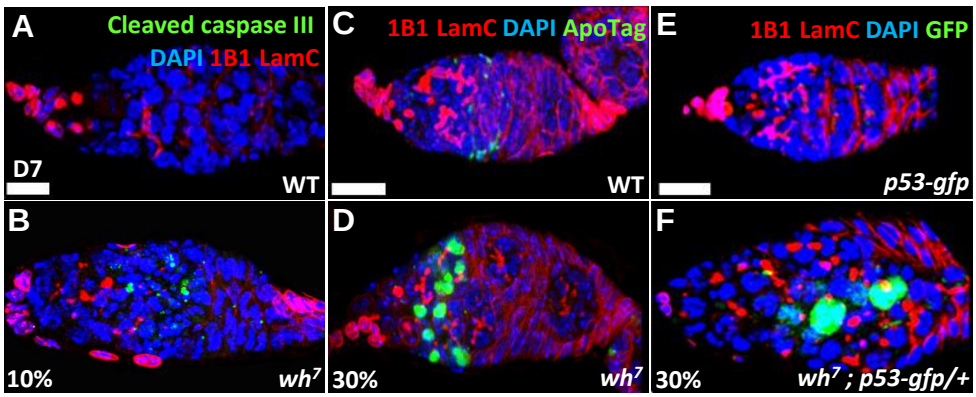

Fig. S3. Wh depletion causes germ cell apoptosis.

(A-F) 10-30% of *wh*^{7} mutant germaria show germ cells positive for apoptosis markers and p53 signals. (A and B) Wild-type (WT) (A and C) and *wh7* mutant germaria (B) with Cleaved caspase III (green, early apoptosis marker), 1B1 (red, fusomes), LamC (red, TF and CpC nuclear envelopes), and DAPI (blue, DNA) labeling. (C and D) Wild-type (C) and *wh7* mutant germaria (D) with ApoTag (green, late apoptosis marker), 1B1 (red, fusomes), LamC (red, TF and cap cell nuclear envelopes) , and DAPI (blue) labeling. (E and F) *p53-gfp* (a P53 signaling reporter) (E) and *wh7*; *p53-gfp/+* germaria (F) with GFP (green), 1B1(red, fusomes), LamC .(red, TF and cap cell nuclear envelopes) , and DAPI (blue) labeling. Scale bar, 10 µm. Representative 7-day (D)-old germaria are shown in 3D-reconstructed images. Genotype of wild-type is *w*^{1118}.

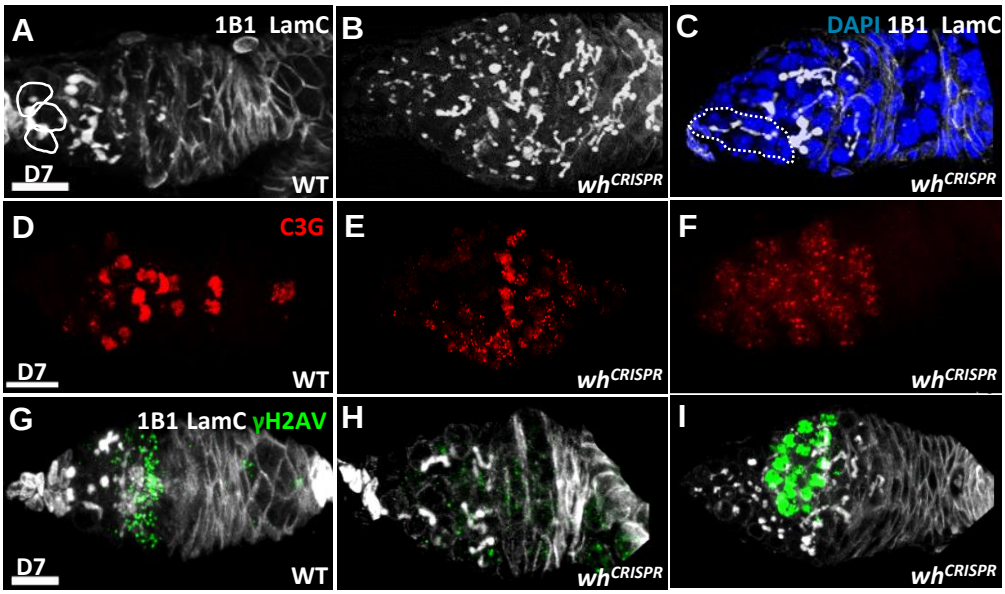

Fig. S4. *wh*^{CRISPR} mutant germ cells show branched fusome, stem-cyst phenotype and impaired meiosis.

(A-C) *wh*^{CRISPR} mutant germaria carrying massive branched fusome and stem-cysts phenotype. (A-B) wild-type (WT) (A) and *wh*^{CRISPR} mutant germaria (B and C) with 1B1 (gray, fusomes), LamC (gray, TF and CpC nuclear envelopes) and DAPI (blue, DNA) labeling. Ovals outline GSCs, dashed line outlines stem-cysts **(D-F)** *wh*^{CRISPR} mutant germaria carrying germ cells with impaired synaptonymal complex formation and distribution. Wild-type (WT) (D) and *wh*^{CRISPR} mutant germaria (E-F) with C3G (red, synaptonymal complex) labeling. In wild-type germaria, C3G proteins assemble into the long ribbons in several cells in a cyst; however, as oogenesis proceeds, the SC is restricted to the two pro-oocytes and finally to the single oocyte in the egg chamber. In the *wh*^{CRISPR} mutant, this pattern is disrupted. **(G-I)** *wh*^{CRISPR} mutant germaria carrying germ cells without meiosis-induced double-strand breaks, as revealed in the wild-type by punctate γH2AV signals in most cells; as meiosis proceeds, the DSBs are repaired and γ-H2Av–positive foci are gradually reduced. Wild-type (WT) (G) and *wh*^{CRISPR} mutant germaria (G-I) with 1B1 (red, fusomes), LamC (red, TF and CpC nuclear envelopes), γH2AV (green) labeling. Scale bar, 10 μm. Representative 7-day (D)-old germaria are shown in 3D reconstructive images. Genotype of wild-type is w^{1118}.

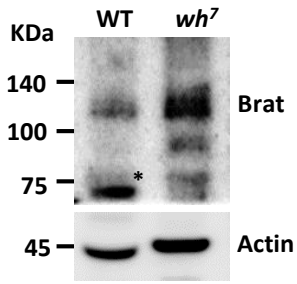

Fig. S5. Brat expression is upregulated in *wh*^{7} mutant germ cells.

Representative western blot for Brat levels (110 kDa) in wild-type and *wh*^{7} mutant ovaries. Actin (42 kDa) was used as an internal control. Molecular weight markers are indicated to the left of the blots. (E)

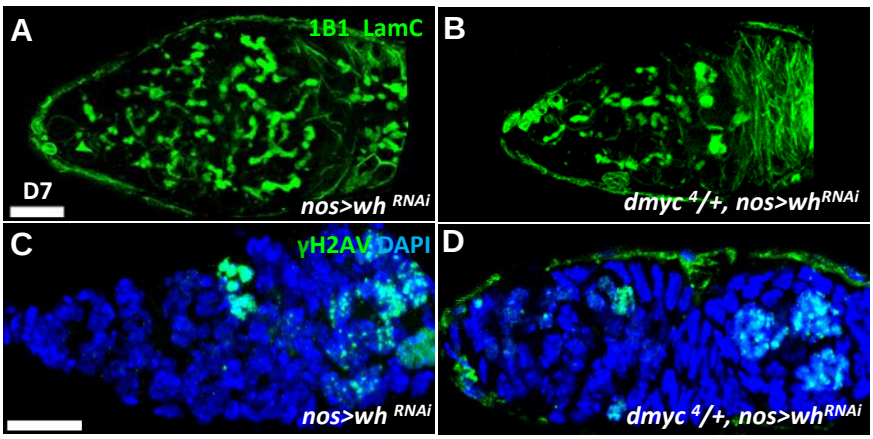

Fig. S6. Removal a copy of dmyc does not rescue abnormal fusome morphology and defective meiosis wh-knockdown germ cells.

(A-B) Removal of one *dmyc* copy in the wh-knockdown germline l does not rescue abnormal fusome morphology. *nos>wh^{RNAi}* (A) and and *dmyc^{4}/+, nos>wh^{RNAi}* germaria (B) with 1B1 (green, fusomes), LamC (green, TF and CpC nuclear envelopes). **(C-D)** Removal of one *dmyc* copy from *nos>wh^{RNAi}* germaria does not rescue meiosis. Ctrl (*nos>wh^{RNAi}*) (D) and *dmyc^{4}/+ nos>wh^{RNAi}* germaria (D) with γH2AV (green) and DAPI (blue, DNA) labeling. Scale bar, 10 μm. Representative 7-day (D)-old germaria are shown in 3D reconstructive images. Genotype of wild-type is *w^{1118}*.

Table S1. Primer sequences and probe numbers used in this study

	Primers and Taq Man probes used for qRT-PCR	Probe
mei-p26	**F-**5′-TCGGGCAAGATATACGGATG-3′ **R-**5′-TTGCTGTTGCAGATGGTGTT-3′	#74
RpL32	**F-**5′-CGGATCGATATGCTAAGCTGT-3′ **R-**5′- CGACGCACTCTGTTGTCG-3′.	#117
	Primers used for genomic PCR	
CRISPR cassette (upstream)	**F** 5′-CGAGGGTTCGAAATCGATAA-3′ **R** 5′-CAGAAGCTCTCCGGCTTAAA-3′	
CRISPR cassette (downstream)	**F** 5′-GACAAGTCCTCGGGAATGAA-3′ **R** 5′-AACGCAAGCAAATGTGTCAG-3′	
gapdh	**F** 5′-TTTCTCAGCCATCACAGTCG-3′ **R** 5′-CGACCTCCTCATCGGTGTAT-3′	
	Primers used for WDR4 fragment amplification	
WDR4	**F** 5'- CCTGCACTAGTTTCTTATAGCTGCAATA-3' **R** 5'- GATTAGGATCCTGAATTCATGGCGGGCTC-3'	
	Primers used for *in situ* hybridization	
bam	**F** 5′-GCACATCGGGCGTTTTATCC-3′ **R** 5′-CGATCAGAGCGGAGAGGAAC -3′	
	Primers used for immunoprecipitation RT-PCR	
nanos 3′UTR	**F** 5′- CTGACGCGTAGAGGGCGAATCCAGCTC-3′ **R** 5′-CAGGCGGCCGCGTATTACGATATTGTAAG-3′	

F, forward primer; R, reverse primer.

Table S2. TaqMan™ microRNA assay miRBase ID and assay ID used in this study

Target	miRBase ID	Assay ID
mir-1	dme-miR-1-3p	000260
mir-289	dme-miR-289-5p	000311
mir-316	dme-miR-316-5p	000326
snoRNA227	dmesnoRNA-227	CTXGPYT