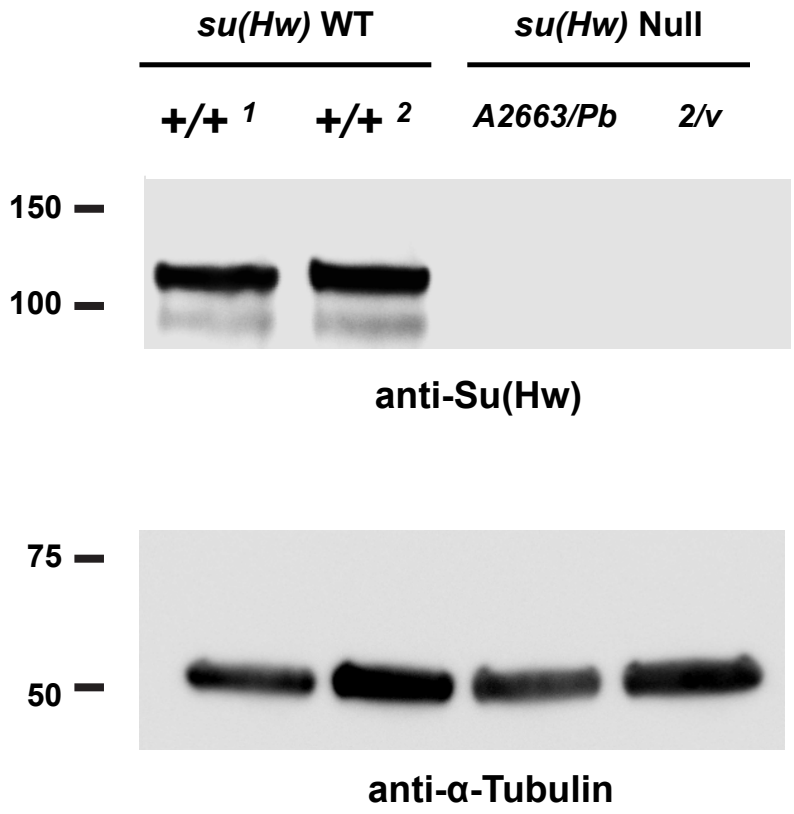
Supplemental Table 1. Primers used in qPCR analyses

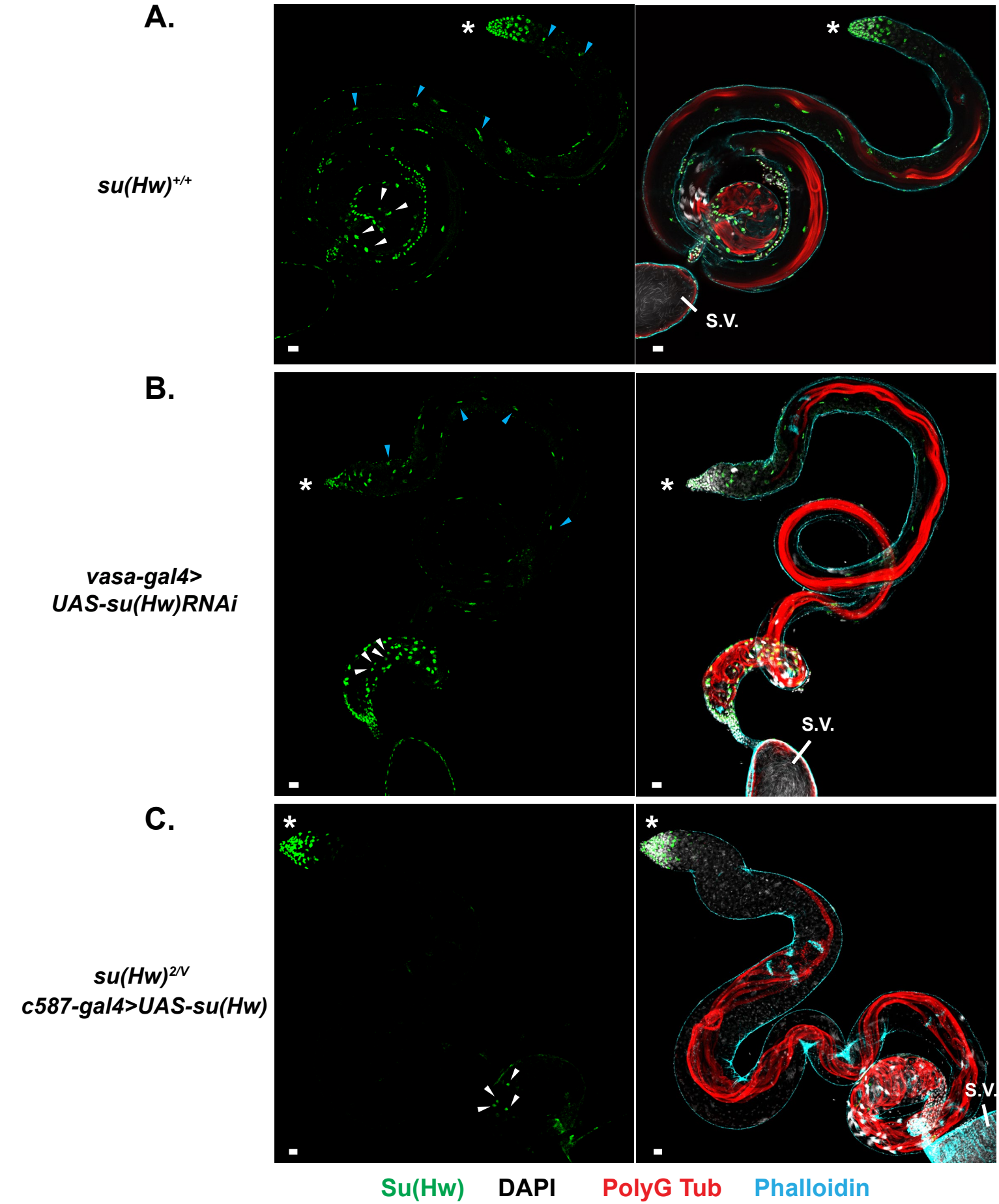
Category	Gene name	Forward primer	Reverse primer
controls	*cycA*	AAGAGGATGAGCACCAGCAG	CAACGGGAGCTTGTCAATCG
	Gapdh2	GGAGGTCGTTTCTACCGATTTC	TACCGCGCCCTAATCTTTAAC
	Ras64B	ATTACCAATCGTGGCTCGCA	TGCCGTGGGTGTAATACAGA
	rp49	AAGATGACCATCCGCCCAGCATAC	ACGCACTCTGTTGTCGATACCCTTG
	vasa	TGGCACCATTGTGTTTGTGG	TCTTGCTGGGCATGTCATAGT
Chr. Y genes	*ARY*	TTGCCGCTACAACGGAATTA	GTAACCGAGTATCCGCTTCATC
	CCY	GAGAGCTCACCTACATGCATAA	GACTGAACCTACATCTCGGAATAG
	fdy	CCCTGATGTTTCCTGCCATC	CGGCAATCTGTGGCTGTTC
	kl-2	ATTGCGATGTTCTCACTGGT	CACATTGTCTCCCCATTCCA
	kl-3	GATGGTGCTGGTTGGGATAA	CCAGTGGTCAGGATGCTTATT
	KL-5	GTGATCCTGCTGCTCATATCTT	CTTCCCAAGGTACTCTGGTATTG
	ORY	TAGCAGCAGAGCGTTCATTAG	ACTTCGCACACATCGTTGA
	Pp1-Y1	GGCTCGCCAGGTTCTTATGT	GAGGGTCTCCACCGAGTATTT
	Pp1-Y2	CGGTCTCAGTCCCGACTTATTA	CTCGTAACCATCCTCAACAACC
	Ppr-Y	ATGACCGTGACGCCATTT	CATCGTGTGGTAAGGATGTCATA
	PRY	TCAACCAAACACGGGACATAA	CGTAGCTTACCGCAGTGAAA
	WDY	GTTGAAGATCGGGAGGAAGAAA	TCAGTTTGGACACGGCTAAC
Repressed target genes	*CanB*	GATTCGCCTTCCGCATCTAT	CACACACACACACACTCATTAC
	CG3104	CCGTAATCACCGTGCTGCTCAAAT	TCAGACCATCGTGGTTCTTCAGCA
	CG6282	GCATCCGAACTACTTTGGCG	CTCCACGTAGACAGCAGGAG
	CG9813	GCAGGTATCACCAAACCAAATC	CATCTCAACCTCGGAATGTATCT
	CG15760	GCAACTCGTCCACGATTCACAAAG	GCAACTCGTCCACGATTCACAAAG
	dimm	CAATCGCTTCGAGAGGTCATAC	CTCCACTTTCGGAGGAAAGATT
	dpr19	TGAATCCCTCGGTCAGTGTG	AGAGTTACGCCGCTAGTTCC

	fz2	AGAGCTTGGTCGTAGCGAAA	GAGGAAAGTGTTTGTGGAAGGG
	Jhe	GCCAGCATGAAGTCAACGAG	CGGATGTATAGGCAGAGGCA
	Mob2	AAATCCGAAGCATAAATCTGGTTATCTCC	CCAACACAACCACAAATCGCACC
	Nlg2	ACATCTGAGTCCAAGTTTTAGCC	TGATGCCAGAGAACAGAAACA
	lap	ACAAGTCCAAGGTGTCGTCG	CGAAAGGATTGCCCAACTGC
	nrv3	GCGACTACTATCCCCGAATG	TGACATCTTCCCAACGACTCT
	rn	GCCATCAGCTTCCCGAACA	GCTATCCCTTGTCCTTCCCA
	Rph	GATGCTTCTCTCGCTCTGCT	CGCTGGTCTTGTGCTTCTTG
	Sse	TGAAACCGTGCCCAATGA	GACACAGAGCTCCATGGTAATAA
	Syn2	AATGCCAAGTCCTGCAAAGTCTGCTG	TATATCATTTTGAGTGTGAGTCCATGCG
	CG14459	ATTGAGAGCTGTCTGGTTGG	TTGCGTGCAAATTATGGCAA
	Nlg3	ATATGATGGACGGCGATCAC	GGGTCTTGTCCCTTTGGTAG
	Or35a	CTCCGAAAGAATGTGGCTCT	AGCTCAACTGTCTTAGCACC
	Sh	GAGAAAAGTCTGGCTGCTCT	CACCTCGTCTTCCTCGATTT
Activated target genes	*CG13285*	ATCTCTTTGGAACTGGCTCTG	GTCGGATAACTGCTAGTGGAATAA
	Cyp6a20	CAGTTGTTCCGAAATGGCTG	ATGGTCCGTCAGTCTTGTTC
	Drsl5	ACCGACAACATGCAGATCAA	GCACTTCAGACTGGGACTG
	Flo2	TACGGCGATGCAGCAATTA	GCTTCCTGTCGTCTCTTTCTT
	imd	GTAGCACCAGTACCGCAGA	TTCCGACATGCCAAGATCCC
	NetA	ATCCCAATCCCAATCCCAATC	GTCCCGACCCAAGATCAAATAG
	Prx2540-2	GCAACAAGTGTGCGAGCTAA	TGGTGCAGACGGGAGTAAAG
	twi	GCTGCTGGACATAAGCTACAA	TGTGTCCTCCAGTTCGGTAATA

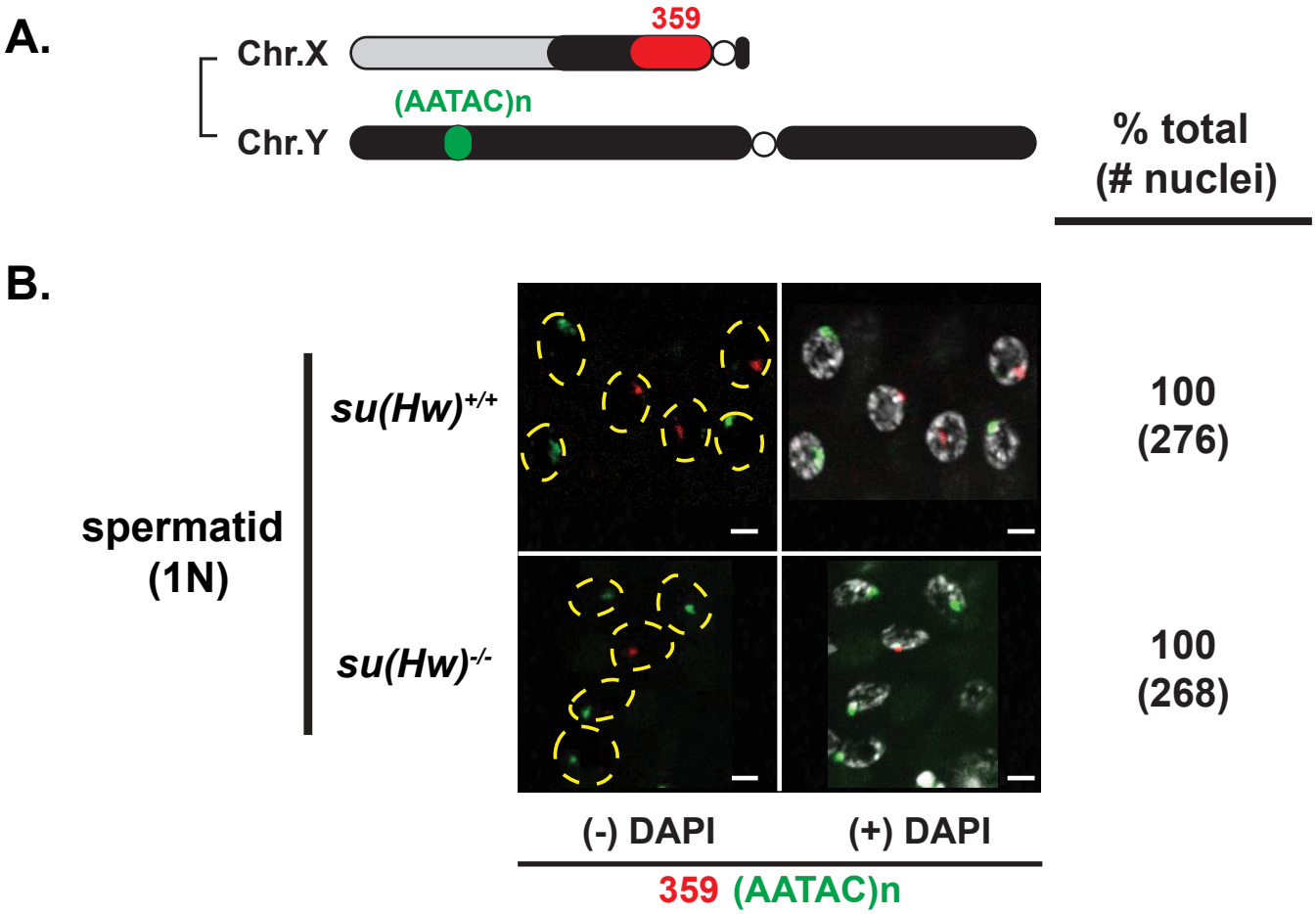
Supplemental Figure 1



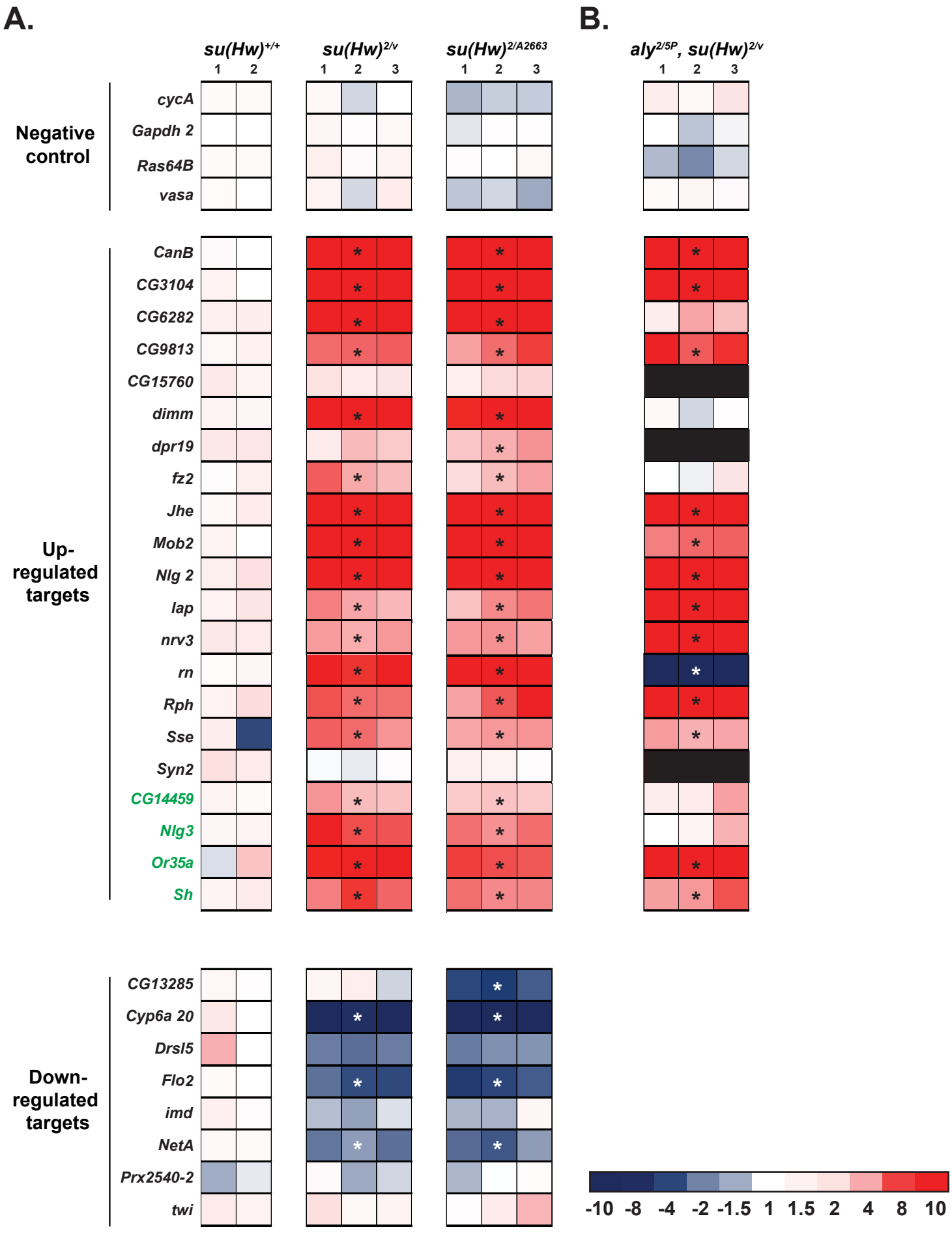
Supplemental Figure 2



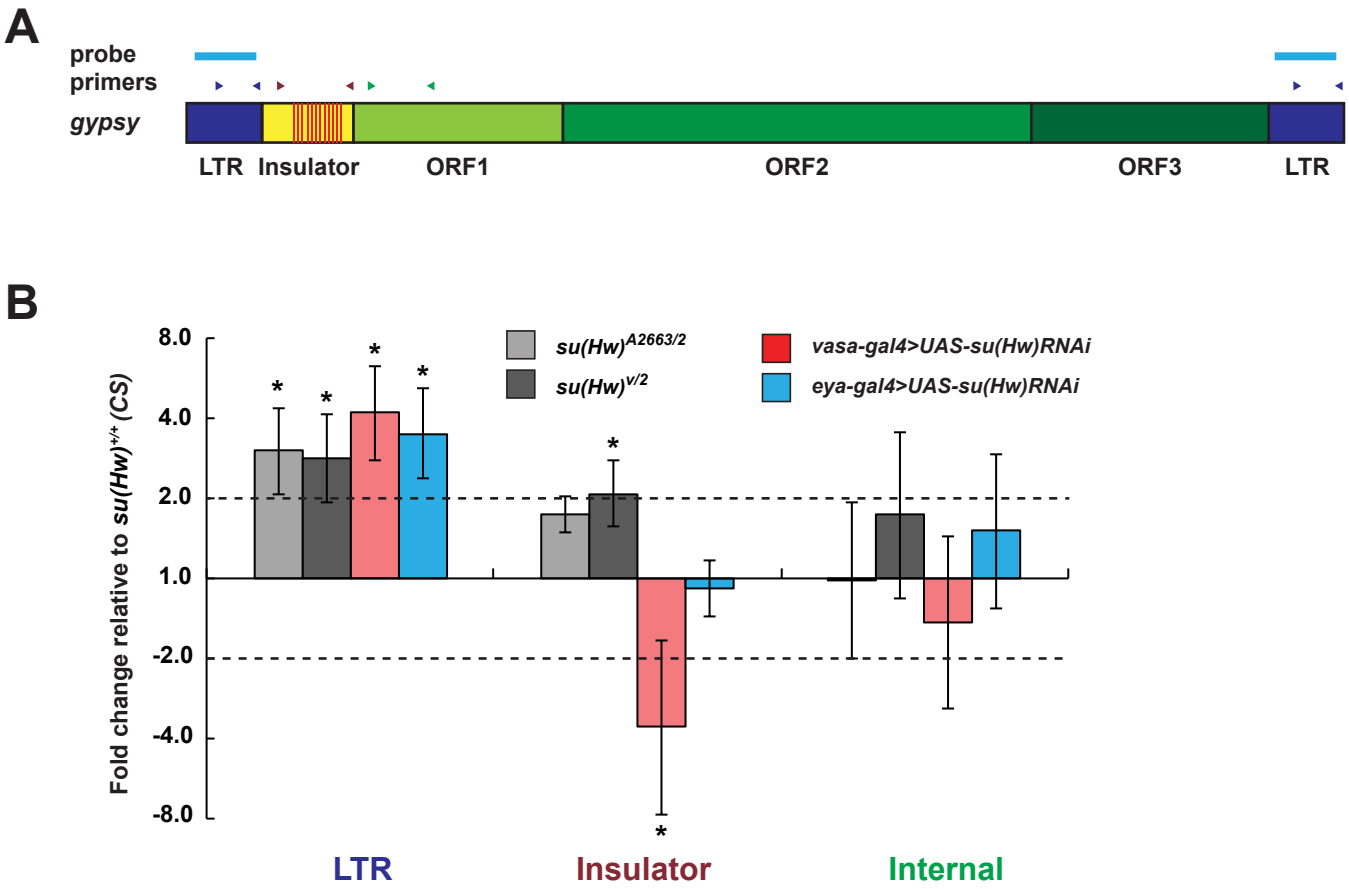
Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5



Supplemental Figure 1. The *su(Hw)* alleles used in this study produce no Su(Hw) protein. Shown is a western blot of protein lysates isolated from males of two *su(Hw)*$^{+/+}$ reference strains (1: *Canton S*, 2: *y*^{1} *w*^{1118}) and two *su(Hw)*$^{-/-}$ mutants. The blot was probed with antibodies against (Top) Su(Hw) or (Bottom) α-Tubulin.

Supplemental Figure 2: Su(Hw) distribution and testis phenotypes associated with cell type-specific Su(Hw) knockdown and expression. (A) Confocal images of a 7-day-old *su(Hw)* $^{+/+}$ testis stained with antibodies against Su(Hw) (green) and spermiogenesis markers, PolyG Tub (red) and Phalloidin (blue). DNA was stained for DAPI (white). The image on the right shows Su(Hw) only and on the left shows the merge of all staining. Su(Hw) is found in somatic and germ cells in early stages of spermatogenesis at the anterior end of the testis (asterisk). In later stages, Su(Hw) is lost in germ cells, but retained in all cyst cells (blue arrowheads). Su(Hw) is also found in somatic cells at the posterior end of the testis (white arrowheads). (B). Confocal image of a 7-day-old *vasa-gal4>UAS-su(Hw)RNAi* testis stained as described in (A). Su(Hw) is found only in somatic cells throughout the testis. The merged image shows that germ cell knockdown does not disrupt spermatogenesis. (C) Confocal image of a 7-day-old a *su(Hw)*$^{2/v}$*, c587-gal4>UAS-su(Hw)* testis stained as described in (A). Su(Hw) is restored in only in early cyst cells, with weak staining of posterior somatic cells at the base of the testis. The merged image shows that restoration of Su(Hw) in early cyst cells restores spermatogenesis. Scale bars: 20 µm.

Supplemental Figure 3. Segregation of the *X* and *Y* chromosomes occurs normally in *su(Hw)* mutants. (A) Chromosome schematic showing the pericentric locations of the 356 (*X*-chromosome, red) and AATAC (*Y*-chromosome, green) satellite repeats used in FISH analysis. (B) Confocal images of a representative field of spermatids in 3-day-old *su(Hw)*$^{+/+}$ and *su(Hw)*$^{-/-}$ testes hybridized with the 359 and AATAC satellite probes, shown either without (-) or with (+)

DAPI staining. The table at the right shows the percentage of total nuclei that have either a 359 or AATAC signal, with the total number of nuclei scored indicated in parentheses. The yellow dotted lines outline the nucleus. Scale bars: 2 μm.

Supplemental Figure 4. **Transcriptional analysis of Su(Hw) target genes**. (A) Heat map of fold changes of gene expression defined by RT-qPCR for 21 randomly selected Su(Hw) repressed and 8 randomly selected activated target genes in RNA isolated from 3-day-old *su(Hw)$^{+/+}$* (*CS*), *su(Hw)$^{2/A2663}$* or *su(Hw)$^{2/v}$* testes. Fold change in expression was determined by normalizing levels to the housekeeping gene *RpL32* and is relative to one RNA sample from *su(Hw)$^{+/+}$* (*Canton S*, *CS*). Asterisks indicate genes that change expression at least 2-fold with $p<0.05$ (Student's t-test). Negative controls are genes that lack SBSs. (B) Heat map of fold changes of gene expression for Su(Hw) repressed target genes in RNA isolated from 3-day-old *aly$^{2/5P}$; su(Hw)$^{2/v}$* testes. Mis-regulated genes identified in *su(Hw)$^{-/-}$* and *kmg$^{-/-}$* testes are shown in green. The color key for corresponding fold change is shown below the heat map.

Supplemental Figure 5. Su(Hw) loss does not have a general effect on *gypsy* transcription. (A) Schematic of the structure of the *gypsy* retrotransposon. The location of the probe sets (blue bars) present on the microarray is shown above the schematic. For RT-qPCR, three primer sets (arrowheads) were used, including a set encompassing the LTR (blue), a set encompassing the 12 binding sites of Su(Hw) insulator region (red), and a set encompassing ORF1(green). (B) Quantitative RT-qPCR of RNA isolated from 3-day-old testes dissected from two *su(Hw)$^{-/-}$* (grey or black), *vasa-gal4; UAS-su(Hw)RNAi* (red) and *eya-gal4; UAS-su(Hw)RNAi* (blue) males. Fold change in expression was determined by normalizing levels to the housekeeping gene *RpL32* and is relative to RNA levels in *su(Hw)$^{+/+}$* (*Canton S*, *CS*). Asterisks indicate greater than 2-fold changes with $p<0.05$ (Student's t-test). Only LTR sequences showed a significant increase in mRNA levels in the *su(Hw)* mutant backgrounds.