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The Nucleosome Remodeling Factor *ISWI* Functionally Interacts With an Evolutionarily Conserved Network of Cellular Factors

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ABSTRACT

ISWI is an evolutionarily conserved ATP-dependent chromatin remodeling factor playing central roles in DNA replication, RNA transcription, and chromosome organization. The variety of biological functions dependent on ISWI suggests that its activity could be highly regulated. Our group has previously isolated and characterized new cellular activities that positively regulate ISWI in *Drosophila melanogaster*. To identify factors that antagonize ISWI activity we developed a novel *in vivo* eye-based assay to screen for genetic suppressors of *ISWI*. Our screen revealed that *ISWI* interacts with an evolutionarily conserved network of cellular and nuclear factors that escaped previous genetic and biochemical analyses.

THE eukaryotic cell has evolved regulatory mechanisms to induce structural changes to chromatin in response to environmental and cellular stimuli. Chromatin covalent modifiers catalyze specific post-translational modifications of the histones' amino- and carboxy-terminal tails (Kouzarides 2007), while chromatin remodeling complexes use the energy of ATP hydrolysis to change nucleosome positions or to incorporate histone variants into chromatin (Eberharter and Becker 2004; Saha *et al.* 2006). These chromatin modifications, occurring without a change in DNA sequence, set different chromatin functional states and constitute the epigenetic marks of our genome (Imhof 2006; Martin and Zhang 2007; Sala and Corona 2009). Although it is expected that a cross talk should exist between ATP-dependent remodelers and covalent modifiers of chromatin, little is known about how these activities are coordinated and integrated with each other in complex chromatin signaling pathways (Strahl and Allis 2000; Jenuwein and Allis 2001).

ISWI is the catalytic subunit of several ATP-dependent chromatin remodeling complexes, highly conserved during evolution and essential for cell viability (Corona and Tamkun 2004). ISWI-containing complexes play central roles in DNA replication, gene expression, and chromosome organization (Dirscherl and Krebs 2004). ISWI uses the energy of ATP hydrolysis to catalyze nucleosome spacing and sliding reactions (Corona and Tamkun 2004). In Drosophila, loss of *ISWI* function causes global transcription defects and leads to dramatic alterations in higher-order chromatin structure, including the apparent decondensation of both mitotic and interphase chromosomes (Deuring *et al.* 2000; Corona *et al.* 2007). Recent findings indicate that ISWI controls chromosome compaction *in vivo*, in part through its ability to promote the association of the linker histone H1 with chromatin (Corona *et al.* 2007; Siriaco *et al.* 2009).

In vitro and *in vivo* studies carried out in several model organisms have also shown the involvement of ISWI-containing complexes in a variety of nuclear functions including telomere silencing, stem cell self-renewal, neural morphogenesis, and epigenetic reprogramming occurring during nuclear transfer in animal cloning (Dirscherl and Krebs 2004; Xi and Xie 2005; Parrish *et al.* 2006). Inactivation of *ISWI* also interferes with the *ras* pathway (Andersen *et al.* 2006), and loss of *ISWI* function seems to be associated with a subset of melanotic tumors and the human multisystemic disease Williams–Beuren syndrome (Mellor 2006).

The variety of functions associated with ISWI is probably connected to the ability of other cellular and nuclear factors to regulate its ATP-dependent chromatin remodeling activity (Corona *et al.* 2002; Ferreira *et al.* 2007; Hogan and Varga-Weisz 2007). Due to the broad spectrum of functions played by ISWI *in vivo*, it is likely that chromatin factors, nuclear enzymatic activities, and a variety of histone modifications may influence its activity *in vivo*. To identify new regulators of *ISWI* function, we developed an eye-based assay to identify

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ISWI genetic interactors in the higher eukaryote *Drosophila melanogaster* (Corona *et al.* 2004). Loss of *ISWI* function, by eye-specific misexpression of the dominant negative allele $ISWI^{K159R}$, produces catalytically inactive ISWI that is incorporated into native complexes, giving rise to rough and reduced eye phenotypes in otherwise healthy flies (Deuring *et al.* 2000; Burgio *et al.* 2008). In a previous study, we used this *in vivo* eye assay to conduct an unbiased genetic screen for mutations in genes that dominantly modify phenotypes resulting from the misexpression of $ISWI^{K159R}$ in the eye (Burgio *et al.* 2008).

The screen produced the first genetic interaction map for the ATP-dependent chromatin remodeler ISWI in a higher eukaryote (Burgio *et al.* 2008). The characterization of the network of factors we isolated revealed unanticipated roles for ISWI in the cell as well as novel mechanisms by which its activity could be regulated (Burgio *et al.* 2008). Interestingly, one class of mutants isolated in the $ISWI^{K159R}$ screen included chromatin components and nuclear enzymatic activities that could regulate ISWI function by covalently modifying chromatin factors or ISWI itself (Burgio *et al.* 2008; Sala *et al.* 2008). The biochemical characterization of the genetic interactions recovered between $ISWI^{K159R}$ and genes encoding for chromatin covalent modifiers, established that eye-based genetic screens in flies could be a powerful tool for the *in vivo* dissection of chromatin-remodeling signaling pathways occurring in the nucleus (Corona *et al.* 2004; Armstrong *et al.* 2005; Burgio *et al.* 2008; Sala and Corona 2009). Moreover, the $ISWI^{K159R}$ screen established that *ISWI* function could be modulated *in vivo* by a variety of cellular factors that have escaped previous biochemical analyses (Burgio *et al.* 2008).

The nonsaturating $ISWI^{K159R}$ F_1 screen was designed to specifically isolate enhancers of *ISWI* (Burgio *et al.* 2008). To identify novel factors working in antagonism with ISWI, we developed a new *in vivo* assay that allowed us to screen for genetic suppressors of eye phenotypes caused by true loss-of-function *ISWI* alleles. We took advantage of the ***E**y-**G**al4, **U**AS-**F**lip (EGUF*) approach to produce flies with eyes composed exclusively of mitotic clones that have lost *ISWI* function (Stowers and Schwarz 1999). Loss of *ISWI* in the eye caused reduced rough eyes, eye color variegation, and loss of cell identity. We employed the *ISWI-EGUF* eye phenotypes to set up a dominant modifier screen to isolate factors antagonizing ISWI activity *in vivo*. Employing classic gene network bioinformatics analysis, we combined the results of our screen with those obtained in two others screens conducted in Drosophila and in *Caenorhabditis elegans*, where an *ISWI* allele and its worm ortholog were isolated (Andersen *et al.* 2006; Parrish *et al.* 2006). The combination of genetic and bioinformatics approaches employed resulted in the identification of an evolutionarily conserved network of modifiers of *ISWI* eye phenotypes, which included several potential antagonists of *ISWI* function. Our analysis revealed new roles for ISWI in cell cycle progression as well as unanticipated mechanisms by which its activity could be regulated, shedding new light into the evolutionarily conserved physiological function of ISWI family members in cell cycle regulation.

MATERIALS AND METHODS

Drosophila stocks and genetic crosses: Flies were raised at 25° on K12 medium (Genovese and Corona 2007). Unless otherwise stated, strains were obtained from Bloomington, Szeged, and Drosophila Genetic Resource Center Stock Centers and are described in FlyBase (http://www.flybase.org).

***ISWI-EGUF* eye characterization:** The *EGUF* approach (Stowers and Schwarz 1999) was employed to obtain, by mitotic recombination, flies with eyes entirely composed of homozygous wild-type, $ISWI^2$, brm^2, and kis^1 clones (*ISWI-EGUF*). The control wild-type *EGUF* adults *FRT42D, +/FRT42D, GMR-hid; EGUF/+* were obtained by crossing *yw; P{neoFRT}42D* males with *yw; P{neoFRT}42D,GMR-hid2R,CL2R,y^+/CyO; EGUF* virgins. *ISWI-EGUF* adults *FRT42D, $ISWI^2$/FRT42D, GMR-hid; EGUF/+* were obtained by crossing yw; P{neoFRT}42D,$ISWI^2$,sp/SM5,Cy,sp males with *yw; P{neoFRT}42D,GMR-hid2R,CL2R,y^+/CyO; EGUF* virgins. *ISWI-EGUF* adults bearing a copy of wild-type *ISWI* (Deuring *et al.* 2000), *FRT42D, $ISWI^2$/FRT42D, GMR-hid; $ISWI^+$/EGUF* were obtained by crossing *yw; P{neoFRT}42D,$ISWI^2$,sp/+;P{w^+,$ISWI^+$}/+* males with *yw; P{neoFRT}42D,GMR-hid2R,CL2R,y^+/CyO;EGUF* virgins. *ISWI-EGUF* adults bearing a copy of the enzymatic inactive $ISWI^{K159R}$ (Deuring *et al.* 2000), *FRT42D, $ISWI^2$/FRT42D, GMR-hid; $ISWI^{K159R}$/EGUF* were obtained by crossing *yw;P{neoFRT}42D,$ISWI^2$,sp/+;P{w^+,$ISWI^{K159R}$}/+* males with *yw; P{neoFRT}42D, GMR-hid2R,CL2R,y^+/CyO;EGUF* virgins. *brm-EGUF* adults *EGUF/+; FRT79B, brm^2/FRT79B, GMR-hid* were obtained by crossing *yw; P{neoFRT}79B,brm^2/TM6,Hu,Tb* males with *yw; EGUF/CyO; P{neoFRT}79B,GMR-hid3R,CL3R,y^+/TM3,Sb* virgins. *kis-EGUF adults* FRT40A, kis^1/FRT40A, GMR-hid; +/EGUF were obtained by crossing *yw; P{neoFRT}40A,kis^1/CyO* males with *yw; P{neoFRT}40A,GMR-hid2L,CL2L,y^+/CyO; EGUF* virgins. *ISWI-EGUF* adults bearing a copy of the *acf1[1]* allele (Fyodorov *et al.* 2004), *FRT42D, $ISWI^2$/FRT42D, GMR-hid; acf1[1]/EGUF* were obtained by crossing *yw; P{neoFRT}42D,$ISWI^2$,sp/+; acf1[1]/+* males with *yw; P{neoFRT}42D,GMR-hid2R,CL2R,y^+/CyO;EGUF* virgins. *ISWI-EGUF* adults bearing a copy of the $E(bx)^{Nurf301-2}$ allele (Badenhorst *et al.* 2002), *FRT42D, $ISWI^2$/FRT42D, GMR-hid; $E(bx)^{Nurf301-2}$/EGUF* were obtained by crossing *yw; P{neoFRT}42D,$ISWI^2$,sp/+; $E(bx)^{Nurf301-2}$/+* males with *yw; P{neoFRT}42D,GMR-hid2R,CL2R,y^+/CyO; EGUF* virgins.

***ISWI-EGUF PiggyBac* genetic screen:** New *PiggyBac* insertions were generatad by crossing small batches of ~20 *pHer{3xP3-ECFP,αtub-piggyBacK10} jumpstarter* males with ~20 *yw,pBac{3xP3-EYFP,p-tTA-K10}; P{neoFRT}42D,$ISWI^2$,sp/CyO mutator* bearing virgins. In the F_1, males *yw,pBac{3xP3-EYFP,p-tTA-K10}; P{neoFRT}42D,$ISWI^2$,sp/pHer{3xP3-ECFP,a-tub-piggyBacK10}* carrying the $ISWI^2$ allele and both *mutator* and *jumpstarter* elements, recognizable by the double CFP/YFP eye fluorescence and the absence of the Cy marker, were crossed with isogenic w^{1118} virgins. In the F_2, single males w^{1118}; *P{neoFRT}42D,$ISWI^2$,sp/pBac{3xP3-EYFP,p-tTA-K10}* if the *mutator* hopped on the second chromosome or w^{1118}; *P{neoFRT}42D,$ISWI^2$,sp/+; pBac{3xP3-EYFP,p-tTA-K10}/+ }* if the *mutator* hopped on the third chromosome, recognizable by the presence of the YFP and absence of the CFP eye fluorescence, were crossed with *yw; P{neoFRT}42D,GMR-hid2R,CL2R,y^+/CyO;*

EGUF virgins. In the F_3, males and females *P{neoFRT}42D,GMR-hid2R,CL2R,y⁺/P{neoFRT}42D,ISWI²,pBac{3xP3-EYFP,p-tTA-K10},sp; EGUF/+* for second chromosome *mutator* insertions and *P{neoFRT}42D,GMR-hid2R,CL2R,y⁺/P{neoFRT}42D,ISWI²,sp; EGUF/pBac{3xP3-EYFP,p-tTA-K10}* for third chromosome *mutator* insertions were scored for their ability to dominantly modify (enhance or suppress) *ISWI-EGUF* eye phenotypes. Flies carrying only one *FRT42D* site linked with the *GMR-hid* transgene could be easily recognized because they cannot undergo eye-specific mitotic recombination, thus generating slightly pigmented adult eyes without ommatidia (Figure 2A) (Stowers and Schwarz 1999). Interacting *mutator* insertions were retested and balanced on both chromosomes. The PiggyBac *mutator* line *pBac{3xP3-EYFP, p-tTA-K10}* (Horn *et al.* 2003), containing the tetracycline transactivator gene (tTA) instead of the yeast GAL4 transactivator, was employed to avoid interference with the *EGUF* system.

***ISWI-EGUF EP* genetic screen:** The *EP* collection on the third chromosome (Rorth *et al.* 1998) was tested by crossing males carrying a balanced or unbalanced *EP* insertion with *yw; P{neoFRT}42D,ISWI²,sp/CyO* virgins. In the F_1, *yw; P{neoFRT}42D,ISWI²,sp/+;EP/+* males, recognizable by the loss of the Cy and *EP* balancer markers, were crossed with *yw; P{neoFRT}42D,GMR-hid2R,CL2R,y⁺/CyO; EGUF* virgins. In the F_2, male and female *yw; P{neoFRT}42D,ISWI²,sp/P{neoFRT}42D,GMR-hid2R,CL2R,y⁺; EGUF/EP* were scored for their ability to dominantly modify (enhance or suppress) *ISWI-EGUF* eye phenotypes.

***ISWI-EGUF* genetic screen scoring system:** The *ISWI-EGUF* eye phenotypes are highly penetrant (100%; $n = 1000$) and show a very low expressivity ($< \sim 15\%$ of progeny show phenotypes less or more severe than the standard *ISWI-EGUF* eye phenotypes; $n = 1000$). Nevertheless, both the PiggyBac and the EP *ISWI-EGUF* screens were conducted in F_3 and F_2, respectively (see Figure 2), to increase the chance to identify true *ISWI-EGUF* modifiers. A *PiggyBac* or an *EP* insertion was considered an *Enhancer of ISWI* [*En(ISWI)*] if >50% of the experimental class showed an eye with a more severe phenotype (reduced eye size and increased roughness), when compared to the control *ISWI-EGUF* eye. A *PiggyBac* or an *EP* insertion was considered a *Suppressor of ISWI* [*Su(ISWI)*] if >50% of the experimental class showed an eye with a less severe phenotype (increased eye size and reduced roughness), when compared to the control *ISWI-EGUF* eye. *PiggyBac* and *EP* insertions giving in the experimental class both *En(ISWI)* and *Su(ISWI)* were termed bimodal, *Bi(ISWI)*.

***ISWI-EGUF* secondary screens:** To eliminate false *ISWI-EGUF* interactors, distinct secondary screens were conducted on the basis of the type of insertions (*PiggyBac vs. EP* transposons), the chromosome arm hit (2L, 2R, 3L, or 3R), and the type of interaction class scored (enhancer *vs.* suppressor). *PiggyBac En(ISWI)*'s mapping the 2L, 3L, and 3R chromosomes were tested for their inability to cause eye defects in the absence of an *FRT42D, ISWI²* chromosome (supporting information, File S1 and Figure S4). *PiggyBac En(ISWI)* and *Su(ISWI)* mapping the 2R chromosome, because of the presence of the FRT42D sites, produce eyes with the 2R chromosome in homozygosis. Therefore, false *En(ISWI)*'s were eliminated because they failed to enhance *ISWI-EGUF* eyes in the presence of an ectopic copy of the wild-type $ISWI^+$ transgene (Figure S4). On the other hand, false *Su(ISWI)*'s were identified by their inability to suppress *ISWI-EGUF* eyes in the presence of an extra copy of the *GMR-hid* transgene not linked to the *FRT42D* recombination site (Figure S4). Finally, En(ISWI) and Su(ISWI) isolated with the EP screen were eliminated for their failure to enhance or suppress *ISWI-EGUF* eye phenotypes in the absence of the *FRT42D, ISWI²* chromosome (Figure S4).

iPCR and candidate allele analysis: We mapped *ISWI-EGUF* modifiers to 99 potential protein-coding loci (Table S1), combining iPCR data available in FlyBase (www.flybase.org) for the EP interacting insertions with the iPCR sequencing data we generated for the PiggyBac interactors, using standard protocols (www.fruitfly.org/about/methods/inverse.pcr.html). Some *ISWI-EGUF* modifiers (*i.e.*, *mbf1*, *ttk*, *eff*, *stg*, and *cpo*) were validated by testing other alleles of these genes, available from public stock centers, using the genetic screen scheme used for testing EP insertions. Mutations in genes corresponding to "neuronal morphogenesis," "multiple cell fate," and "connecting" nodes were obtained from public stock centers and tested for their ability to interact in the *ISWI-EGUF* eye assay, using the genetic screen scheme used for testing EP insertions, and in the $ISWI^{K159R}$ eye assay according to Burgio *et al.* (2008).

Bioinformatic and cell cycle analyses: For the BioGrid analysis (Breitkreutz *et al.* 2008), each *En(ISWI)*, *Su(ISWI)*, and *Bi(ISWI) ISWI-EGUF* interaction was associated with a single gene on the basis of the insertion DNA sequence data available in FlyBase (www.flybase.org) and the iPCR analysis we conducted on the PiggyBac insertions. The gene ontology data and all the genetic and physical interactions existing between the *ISWI-EGUF* modifiers were obtained from the BioGrid website (www.thebiogrid.org) and were represented in a graphical format using the Osprey software (Breitkreutz *et al.* 2003) (http://biodata.mshri.on.ca/osprey/servlet/Index). For Gene Ontology (GO) analysis the FatiGO data mining tool (Al-Shahrour *et al.* 2007) and the latest gene annotation and Gene Ontology provided by FlyBase were used. The *C. elegans* genes isolated in the multiple cell fate screening (Andersen *et al.* 2006) were converted to fly orthologs using the WormBase database (www.wormbase.org).

For the GO analysis the gene annotation and the Gene Ontology provided by FlyBase were used with Ontologizer (www.ohloh.net/p/ontologizer) to determine overrepresented GO terms. The parent–child method of Ontologizer, which takes into account the parent–child relationships of the GO hierarchy, was applied and the *P*-values were adjusted using Westfall–Young single-step multiple testing correction. A corrected *P*-value threshold of 0.1 was used as a cutoff for reporting significant matches. To compute statistical significance of the frequencies of GO-component terms hypergeometric distributions were calculated on the basis of the occurrence of the indicated terms and corrected for multiple testing by the Bonferroni correction method.

Cell cycle profiles from imaginal discs and brain cells were obtained according to Collesano and Corona (2007), using a BD FACSCanto flow cytometer (BD Biosciences), with a laser wavelength set at 488 nm (~6000 events were recorded for each cell cycle profile). Cell cycle profiles were quantitatively analyzed using the ModFit software. Homozygous *ISWI²*, $wun^{PBacF48}$ and *ISWI²*, $casp^{PBacG75\alpha}$ brains and total imaginal discs used for the cell cycle analysis were dissected from third instar larvae, obtained from stocks balanced with the *T(2:3),CyO, Tb* balancer and selected for the absence of the Tb marker. Imaginal eye discs subjected to cell cycle analysis were obtained by crossing *ISWI²; eyGAL4/T(2:3), CyO, Tb* virgins with *ISWI²; eyGAL4/T(2:3), CyO, Tb* or *ISWI²;* eff^{EP3627}*/T(2:3), CyO, Tb* or *ISWI²;* ttk^{EP3314}*/T(2:3), CyO, Tb* or *ISWI²;* $mbf1^{EP3684}$*/T(2:3), CyO, Tb* males.

RESULTS AND DISCUSSION

An *in vivo* assay to identify factors antagonizing ISWI activity: Suppressors of *ISWI* function are a very important class of genes to identify, as they may give us a complete picture of factors regulating chromatin remodeling reactions *in vivo*. We previously showed that

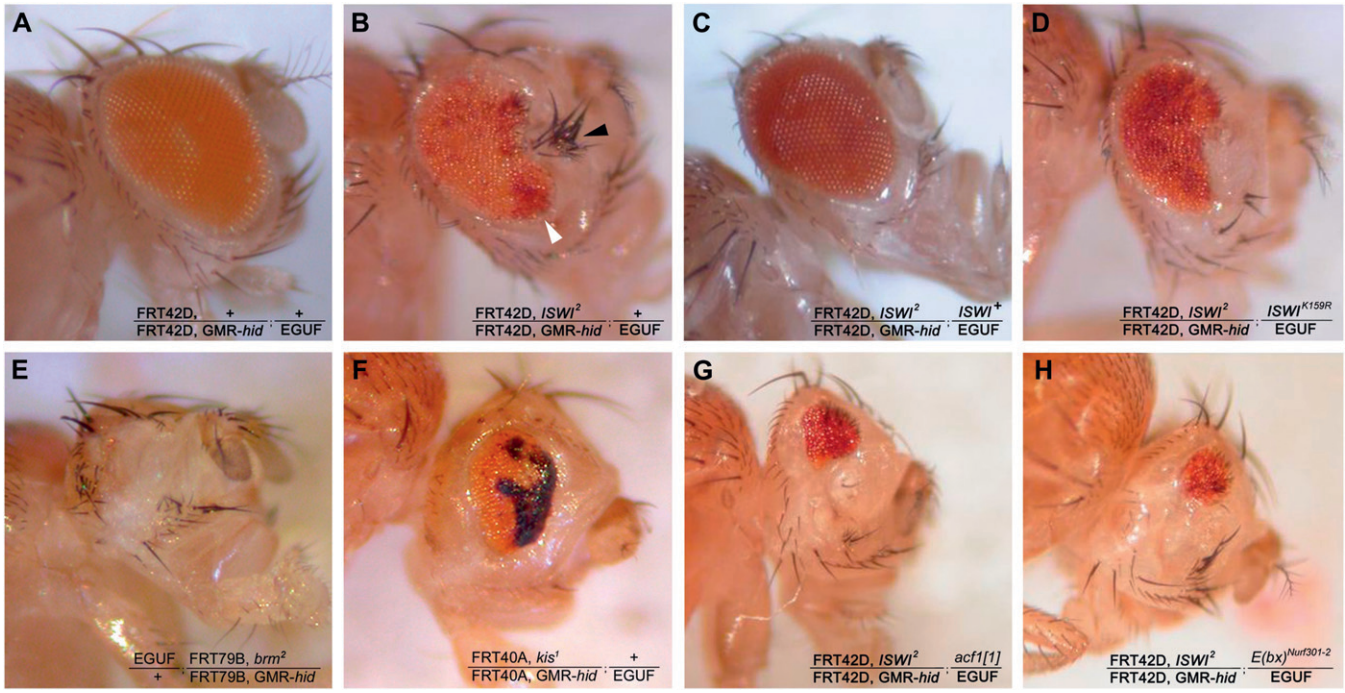

Figure 1.—Loss of *ISWI* function by the *EGUF* approach causes specific eye phenotypes. Drosophila adult eyes obtained with the *EGUF* mitotic clonal approach (Stowers and Schwarz 1999) bear in homozygosis a wild-type 2R chromosome (A), an $ISWI^2$ allele (B), an $ISWI^2$ allele in the presence of one extra copy of the wild-type $ISWI^+$ gene (C), or a copy of $ISWI^{K159R}$ defective in its ATPase activity (D), a brm^2 (E) or kis^1 allele (F), an $ISWI^2$ allele in the presence of one copy of *acf1[1]* (G), or $E(bx)^{Nurf301-2}$ (H) alleles. The white arrowhead indicates the eye color variegation, while the black arrowhead indicates loss of cell identity defects in which bristles grow in eye territories normally occupied by photoreceptors. The *ISWI-EGUF* eye phenotype is caused by the progressive depletion, during eye development, of the ISWI mRNA/protein pool present before mitotic recombination in $ISWI^2$ heterozygous mother cells. As a consequence, ISWI progressive loss of activity during eye development, occurring after mitotic recombination, could be accelerated by loss of factors positively regulating its activity, like *acf1* and *E(bx)*, thus explaining the enhancement of *ISWI-EGUF* eye defects we observe. On the other hand, the presence of an extra copy of the wild-type $ISWI^+$ gene can complement eye defects caused by the recombination-dependent *ISWI* genetic loss.

the misexpression of $ISWI^{K159R}$ in the eye-antennal discs caused defects that could be enhanced by null alleles of *ISWI*, indicating that $ISWI^{K159R}$ eye phenotypes resulted from the specific reduction of *ISWI* function in the eye. However, due to the conditions used (cross temperature and choice of *Gal4* driver), we generated mild eye phenotypes that probably prevented us from identifying suppressors of $ISWI^{K159R}$ (Burgio *et al.* 2008). On the other hand, we could not formally exclude that the misexpression of $ISWI^{K159R}$ might also have had some unspecific dominant effects that compromised eye development, preventing us from recovering suppressors of $ISWI^{K159R}$ (Burgio *et al.* 2008).

Because of the limitations derived from the previous eye assay and the potential interfering effect of the dominant negative allele $ISWI^{K159R}$, we decided to develop a new *in vivo* eye assay. To isolate an antagonist of *ISWI* function we generated flies with eyes composed exclusively of clones that had lost *ISWI* activity using the *EGUF* approach (Stowers and Schwarz 1999). The *EGUF* method uses the eye-specific *eyGAL4* driver in combination with the *UAS-FLP* transgene to express the site-specific recombinase FLP in mitotically active eye precursor cells. When homologous chromosomes containing FRT's recombination sites are present in these cells, FLP-mediated site-specific mitotic recombination occurs. Since one of the two FRT recombination sites is distally linked with the dominant photoreceptor cell lethal transgene *GMR-hid*, after mitotic recombination all photoreceptor cells bearing one or two copies of *GMR-hid* will die because of the eye-specific expression of the cell death gene *hid* during metamorphosis. Thus, this technique allows the generation of adult eyes that are homozygous for a specific mutation in an otherwise heterozygous adult fly (Stowers and Schwarz 1999).

Using the *EGUF* approach we generated flies with eyes homozygous for the $ISWI^2$ null allele (compare Figure 1A with 1B) (Deuring *et al.* 2000). Loss of *ISWI* in the eye by the *EGUF* approach caused reduced rough eyes, eye color variegation, and loss of cell identity (*ISWI-EGUF* eye phenotype) (compare Figure 1B). The *ISWI-EGUF* eye phenotypes are specifically caused by the mitotic recombination of the $ISWI^2$ allele occurring in the developing eye-antennal discs (Figure S1) and are characterized by defects in the retina structure consisting of the loss of ommatidia boundaries and orientation and a reduced number of photoreceptors (Figure 1B and Figure S2). Indeed, some photoreceptors appear to

undergo a process of degeneration probably contributing to the observed ommatidia loss in the *ISWI-EGUF* adult eye (Figure S2).

The *ISWI-EGUF* eye phenotypes are very specific for the loss of *ISWI* activity since they can be fully suppressed by an ectopic copy of wild-type $ISWI^{+}$ (Deuring *et al.* 2000), under the control of its natural promoter (Figure 1C), but not by a copy of $ISWI^{K159R}$ (Deuring *et al.* 2000) defective in its ATPase activity (Figure 1D). These data strongly suggest that the *ISWI-EGUF* eye defects are specifically caused by the loss of ISWI enzymatic activity and that *ISWI-EGUF* eye phenotypes could be in principle suppressed. Interestingly, we observed that loss of the *brm* chromatin remodeling factor, the Drosophila ortholog of the yeast SWI2/SNF2 protein, and *kis*, another ATP-dependent chromatin remodeling factor (Becker and Horz 2002), results in eye phenotypes distinct from *ISWI-EGUF* (compare Figure 1B with 1E and 1F), highlighting the specificity of the *ISWI-EGUF* phenotype. Remarkably, mutations in *acf1* and *E(bx)*, two genes encoding for known physical interactors of ISWI (Badenhorst *et al.* 2002; Corona and Tamkun 2004; Fyodorov *et al.* 2004), enhance *ISWI-EGUF* eye defects (compare Figure 1B with 1G and 1H), further indicating that the *ISWI-EGUF* phenotypes are specific for loss of *ISWI* activity and that they could also be used to recover dominant enhancers.

Previous work has shown that individuals that are homozygous for the $ISWI^{2}$ allele survive until late larval development, due to the high maternal contribution of ISWI (Corona *et al.* 2007; Burgio *et al.* 2008). Interestingly, loss of *ISWI* function in very late developing larvae (21 days) also caused global polytene chromosome condensation defects (Figure S3), highly reminiscent of chromosome condensation defects observed when misexpressing $ISWI^{K159R}$ (Corona *et al.* 2007; Burgio *et al.* 2008). Therefore, we reasoned that similar chromatin organization defects in $ISWI^{2}$ null cells in the eye discs could also directly or indirectly contribute to the observed *ISWI-EGUF* eye defects, thus facilitating the genetic isolation of factors antagonizing ISWI chromatin remodeling activity.

A genetic screen for suppressors of *ISWI*: We decided to exploit the *ISWI-EGUF* eye phenotypes as an *in vivo* assay to conduct a dominant modifier genetic screen to isolate factors suppressing *ISWI* activity. The rationale of this screen was that mutations in genes that dominantly modify *ISWI-EGUF* eye phenotypes are likely to encode for regulators or effectors of *ISWI* function *in vivo*. To circumvent the known limitations of *P*-element-based mutagenesis (*i.e.*, preference to integrate into hotspots) we decided to use a PiggyBac-based insertional mutagenesis approach (Hacker *et al.* 2003). To identify and stably establish novel insertion lines without the need of balancers, we used two independent and distinguishable fluorescent markers to track a PiggyBac *mutator* line (marked with YFP) and a *jumpstarter* (marked with CFP), carrying a source of PiggyBac transposase (Figure 2A) (Hacker *et al.* 2003). Since each strain can be followed independently by fluorescence of adult fly eyes (Figure 2A), we did not use balancer chromosomes to track new insertions during the screening process. In particular, the dominant YFP fluorescence was used as a visible marker to identify novel insertions in both larval and adult stages, thus facilitating stock keeping. Furthermore, the PiggyBac transposon insertions also marked the mutated gene, thus helping us in mapping and cloning the mutations of interest.

To identify potential loss-of-function interactions suppressing *ISWI-EGUF* eye phenotypes, we screened ~2000 newly generated insertions of the transposable mutator element PiggyBac (Figure 2, A and B) (Hacker *et al.* 2003). After crossing the *jumpstarter* and the *mutator* lines, flies carrying both elements as well as the FRT42D, $ISWI^{2}$ chromosome were identified on the basis of the eye-specific CFP and YFP fluorescence and the absence of the CyO balancer (Figure 2, A and B). In the next generation, the CFP-marked *jumpstarter* was crossed out to allow the stable inheritance of the YFP-marked autosomal insertion (Figure 2, A and B). Finally, males carrying second and third chromosome stable PiggyBac insertions were crossed with the *EGUF* line to score for *ISWI* interactions (Figure 2, A and B). In a second complementary approach, we screened potential gain-of-function interactions using the EP collection on the third chromosome that may lead to the GAL4-dependent overexpression of the gene downstream the insertion site (Rorth *et al.* 1998) (Figure 2C). All PiggyBac and EP interacting lines were retested and rescreened in specific secondary screens to eliminate false positives (Figure S4).

Combining iPCR data for the EP interacting insertions available on FlyBase with iPCR sequencing data we generated for the PiggyBac interactors, we mapped *ISWI-EGUF* modifiers to 99 potential protein-coding loci (Table S1, A and B). We found 21 PiggyBac (~1% of total insertions screened) and 78 EP (~8% of total insertions screened) interactors (Table S1, A and B). Our scoring strategy defined three different classes of modifiers of *ISWI-EGUF* eye phenotypes: enhancers, *En(ISWI)*, suppressors, *Su(ISWI)*, and few mutations giving a bimodal population comprising both enhancers and suppressors in the same progeny that we called *Bi(ISWI)* (Figure 3A and Table S1, A and B). One easy way to explain the bimodal type of interaction is that we scored two independent mutations, one enhancing and the other suppressing, coming from the same EP line tested. Indeed, for the bimodal interactions we isolated we confirmed that the presence of two independent mutations (probably arising from a second nonannotated segregating independent insertion or lesion) were likely responsible for the bimodal genetic interaction initially scored (Table S1 C).

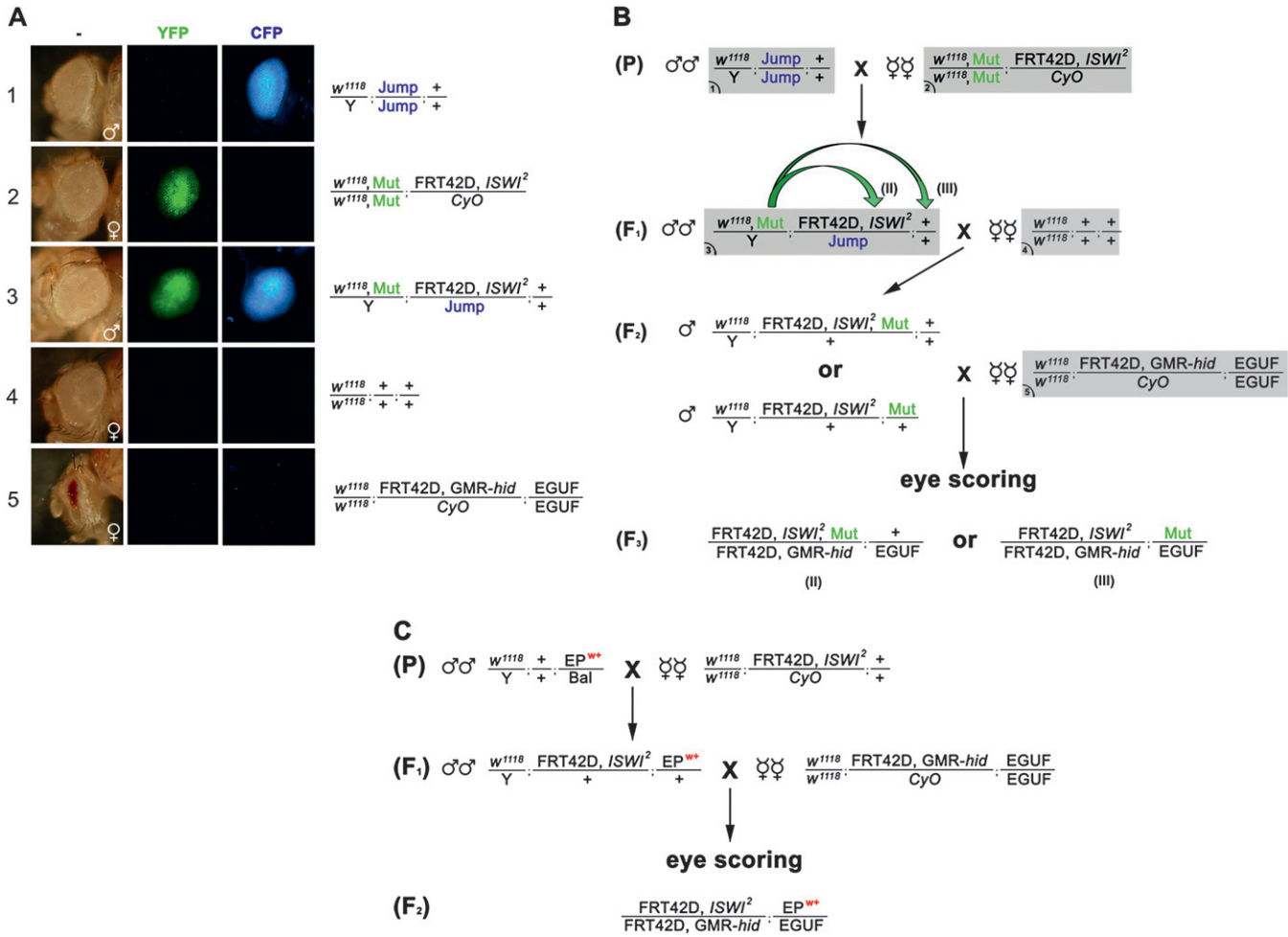

FIGURE 2.—*ISWI-EGUF* genetic screen strategy. (A) The use of distinguishable fluorescent transformation markers in the adult eyes allows us to follow the mutator (YFP) and the jumpstarter (CFP) elements independently. (B and C) Crossing scheme for the identification of new *PiggyBac mutator* (B) and third chromosome *EP* insertions (C) dominantly modifying *ISWI-EGUF* eye phenotypes. For both the *PiggyBac mutator* and the *EP* screen, in the absence of mitotic recombination, the presence of the *GMR-hid* transgene generates adult eyes without ommatidia but slightly pigmented (STOWERS and SCHWARZ 1999), allowing their unambiguous distinction from the mitotic recombinant experimental class. The presence of w^+-marked EP insertions allowed the scoring of the mitotic recombinant experimental class in the EP screen. Mut, *mutator*; Jump, *jumpstarter*.

To understand the biological processes regulated by the *ISWI-EGUF* modifiers we isolated, we conducted a GO analysis employing the latest protein annotations available on FlyBase. Our GO analysis revealed that *ISWI* interacts with a network of cellular and nuclear factors involved in a variety of biological functions not previously linked with known ISWI activities (Figure 3B).

Furthermore, to gain an integrated view of the interaction network potentially existing between the genes isolated in our screen, we annotated the known genetic and physical interactions existing among the *ISWI-EGUF* modifiers with the help of the BioGrid database, containing the information of all known physical and genetic interactions reported for a variety of model organisms, including *D. melanogaster* (http://www.thebiogrid.org). Our analysis revealed that some of the *ISWI-EGUF* modifiers are known to interact with each other, forming small interaction networks, emphasizing the functional correlation existing between some of the factors isolated in our screen (Figure 3).

Finally we compared the list of *ISWI-EGUF* modifiers we recovered with the one we obtained with our previous $ISWI^{K159R}$ eye assay (BURGIO *et al.* 2008). This analysis revealed that the two screens resulted in the isolation of a number of common modifiers (~25%; Figure 3C, Table S1 B, and Figure S5). However, the *ISWI-EGUF* screen allowed us also to isolate cellular and nuclear factors that have escaped from our previous $ISWI^{K159R}$ genetic approach, including several *Su(ISWI)*'s (Figure 3C and Table S1 B).

***ISWI* interacts with factors antagonizing its activity *in vivo*:** One of the main goals of the *ISWI-EGUF* screen was to isolate factors encoding activities that could antagonize ISWI function *in vivo*. Our genetic screen resulted in the isolation of several modifiers of *ISWI-EGUF* eye phenotypes behaving as suppressors (Figure

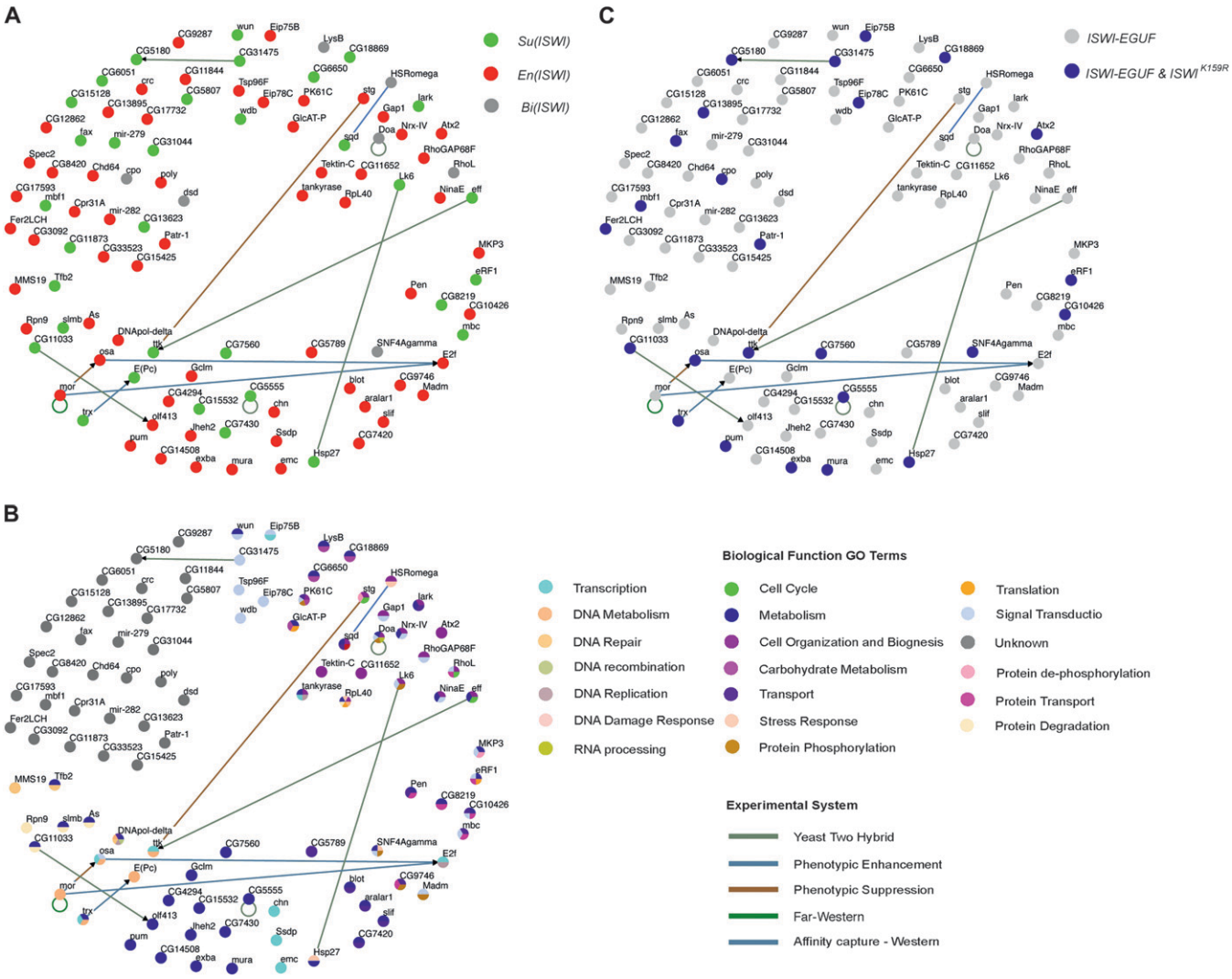

FIGURE 3.—*ISWI* genetically interacts with a wide range of cellular components. The 99 potential protein-coding loci corresponding to *ISWI-EGUF* dominant modifiers are clustered in concentric circles as nodes, colored according to their interaction class (A), current gene ontology (GO) categories, as indicated in the key (B), and their intersection with *ISWI* interactors isolated in the *ISWI*^{K159R} screen (BURGIO *et al.* 2008) (C). The edges represent known physical and genetic interactions identified with the experimental system indicated in the key.

3A and Table S1). As expected, some *ISWI* suppressors are associated with enzymatic activities regulating chromatin function like *trx*, *E(Pc)*, the poly-ADP-ribose polymerase *tankyrase*, and the class I ubiquitin-conjugating (E2) enzyme *effete* (*eff*) (Figures 3A and 4A). Indeed, *eff* is thought to play essential roles in telomere function (CENCI *et al.* 2005). Eff biochemically interacts with the zinc-finger transcriptional repressor encoded by the *tramtrack* gene (*ttk*) (BADENHORST *et al.* 1996; TANG *et al.* 1997), whose mutations also suppress *ISWI-EGUF* eye phenotypes (Figures 3A and 4A). Another gene that suppresses the *ISWI-EGUF* eye is *multiprotein bridging factor 1* (*mbf1*) (Figures 3A and 4A). Mbf1 is a highly conserved protein in archaea and eukaryotes (DE KONING *et al.* 2009) that allows cells to maintain adequate activity of the cell proliferation transcription factor AP-1 under oxidative stress (JINDRA *et al.* 2004). Interestingly, Mbf1 also enhances transcription by forming a bridge between distinct regulatory DNA-binding proteins and the TATA-box-binding protein (TBP) (DE KONING *et al.* 2009). Moreover, Mbf1 is predicted to have a methyl-CpG binding domain (inferred from sequence similarity with UniProtKB: Q9Z2E1) that would in principle allow the selective binding of methylated cytosine/guanine DNA, potentially linking ISWI activity with DNA methylation in flies. To validate the specificity of some of the genetic interactions identified, we tested whether other alleles of *ttk*, *eff*, and *mbf1* also behaved as *Su(ISWI)*'s. Indeed, some of the alleles tested for *ttk*, *eff*, and *mbf1* suppress *ISWI-EGUF* eye phenotypes (Figure 4B).

***ISWI* interacts with an evolutionarily conserved network of cellular factors:** In at least two other screens independently conducted in flies and worms *ISWI* was

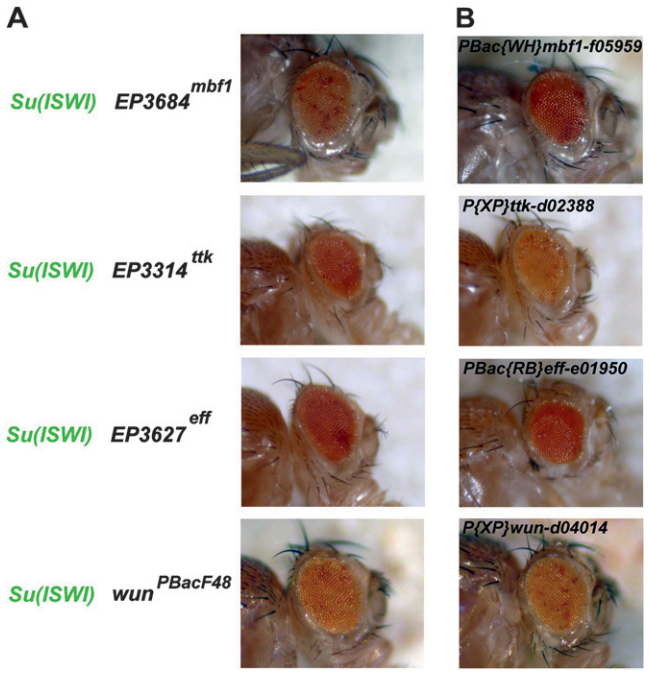

Figure 4.—Loss of *ISWI* function in the eye can be dominantly suppressed by mutations in *mbf1*, *ttk*, *eff*, and *wun*. (A) Eye phenotypes resulting from an eye homozygous for $ISWI^2$ (*ISWI-EGUF* eye) carrying an *EP* insertion mapping the *mbf1*, *ttk*, and *eff* genes or a *PiggyBac* insertion in the *wun* gene. (B) To validate the genetic interactions we scored, other alleles mapping *mbf1*, *ttk*, *eff*, and *wun* were tested in the *ISWI-EGUF* eye assay.

picked up as a genetic interactor. An RNA interference screen identified *ISWI* as one of the factors involved in the proper morphogenesis of Drosophila sensory neuron dendrites (Parrish *et al.* 2006). In another genetic screen for factors regulating the expression of vulval cell fates in *C. elegans,* mutations in *ISW-1*, the worm ortholog of fly *ISWI*, suppressed the multivulva phenotype caused by the hyperactivation of the Ras pathway (Andersen *et al.* 2006).

To gain some insights into the evolutionarily conserved network of regulation of *ISWI* we decided to identify known functional connections existing between genes scored in the four different *ISWI*-based genetic screens ($ISWI^{K159R}$, *ISWI-EGUF*, the fly neuronal morphogenesis, and the worm multiple cell fate screens). We used the BioGrid to search for known genetic or physical interactions existing between the genes identified in the fly neuronal morphogenesis and the worm multiple cell fate screens and the modifier genes we picked in both the *ISWI-EGUF* and the $ISWI^{K159R}$ eye screens (Andersen *et al.* 2006; Parrish *et al.* 2006; Burgio *et al.* 2008). Our analysis identified 1 big (93 nodes) and 12 small networks comprising *ISWI* genetic interactors isolated in the four different genetic screens analyzed (Figure 5A, Figure S6, and Table S2, A–D). Next, to verify whether the 12 small networks (comprising a total of 33 nodes with a network size ranging from 2 to 5 nodes) were functionally related to each other, we again queried the BioGrid to search for factors bridging any of the genes belonging to the 12 networks. Interestingly, this analysis identified 41 connecting nodes linking the 33 genes included in the 12 small networks, defining a second big network (Figure 5B, Figure S6, and Table S2 E). The bioinformatic analysis allowed us to predict that *ISWI* genetically and physically interacted with a series of factors, in part shared among the four different genetic screens analyzed (purple, yellow, green, and blue nodes in Figure 5) and in part novel (red nodes in Figure 5), that may have escaped *ISWI*-based screens.

To verify whether the predicted interacting nodes were true *ISWI* genetic interactors, we tested multiple alleles for each of the neuronal morphogenesis and the worm multiple cell fate screens and the connecting nodes, for their ability to interact in the *ISWI-EGUF* or the $ISWI^{K159R}$ eye assays. Remarkably, alleles of genes corresponding to 63% of the neuronal morphogenesis, 90% of the multiple cell fate, and 50% of the connecting nodes genetically interacted with at least one of the *ISWI-EGUF* or $ISWI^{K159R}$ eye assays (Figure 5C and Table S3). Our data strongly indicate that the nucleosome remodeling factor *ISWI* functionally interacts with an evolutionarily conserved network of cellular factors, predicted by the gene network analysis we conducted.

Loss of *ISWI* causes cell cycle defects that can be suppressed by *Su(ISWI)*'s: A GO analysis on the evolutionarily conserved network of *ISWI* interactors suggested a significant enrichment of regulators of cell cycle and signal transduction (*i.e.*, *Stg*, *Abl*, *Rbf1*, *E2F*, and *Ras85D*) (Figure 5 and Table S2). In particular, an analysis conducted with *Ontologizer* (to quantify GO terms representation) showed that *cell cycle regulation* is an overrepresented category within the combined interacting nodes shown in Figure 5 (*P*-value = 0.03; Table S2). Loss of *ISWI* activity has been linked to different aspect of cell cycle regulation connected, for example, to the development of melanotic tumors (Mellor 2006), the regulation of the germline stem cell self-renewal, and the Rb pathway (Dirscherl and Krebs 2004; Xi and Xie 2005; Parrish *et al.* 2006). Moreover, our data show that loss of *ISWI* in the eye-antennal discs causes an eye phenotype characterized by patches of ommatidia that are missing, disorganized, dedifferentiated, or variegated (Figure 1B and Figure S2; Burgio *et al.* 2008), suggesting a possible role for ISWI in cell viability and the control of differentiation.

To directly test whether loss of *ISWI* causes cell cycle defects, we analyzed isolated cell populations from imaginal discs and larval brains, obtained *ex vivo* from wild-type (w^{1118}) and *ISWI* mutant larvae using a new method we recently developed (Collesano and Corona 2007). We decided to analyze imaginal discs because they contain highly proliferating cells and also because we conducted the two *ISWI* screens in

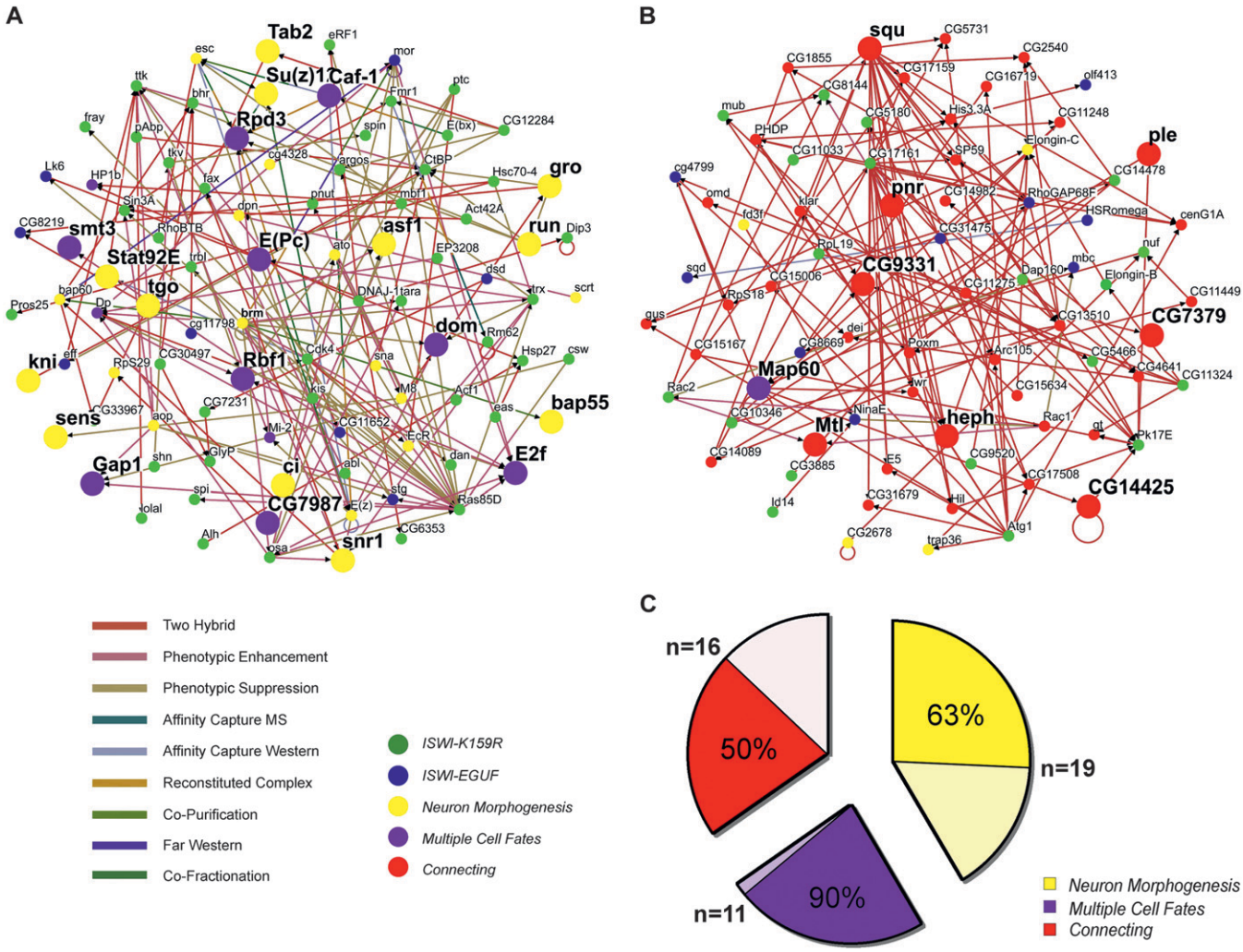

FIGURE 5.—Evolutionarily conserved network of regulation of *ISWI*. BioGrid analysis is shown of known genetic and physical interactions existing between the genes identified in the fly "neuronal morphogenesis" and the worm "multiple cell fate" screens and the modifier genes we picked in both the *ISWI-EGUF* and the *ISWI*^{K159R} eye screens (ANDERSEN *et al.* 2006; PARRISH *et al.* 2006; BURGIO *et al.* 2008). Our analysis predicted (A) 1 big and (B) 12 small interaction networks comprising *ISWI* genetic interactors isolated in the four different genetic screens analyzed, linked by "connecting" nodes corresponding to interactors not isolated in previous *ISWI* related screens. The big network comprises 93 nodes while the 12 small networks are constituted of 33 original nodes and 41 connecting new nodes. (C) Alleles of genes corresponding to 63% of the neuronal morphogenesis, 90% of the multiple cell fate, and 50% of the connecting nodes genetically interacted with the *ISWI-EGUF* or the *ISWI*^{K159R} eye assays. These interaction frequencies are much greater than the frequencies of *ISWI* interaction we normally get with eye-based screens (usually in the ~1–11% range), suggesting that the gene network analysis we conducted increased our ability to predict *ISWI* interactors. The edges represent known physical and genetic interactions identified with the experimental system indicated in the key. *ISWI-EGUF* or *ISWI*^{K159R} interacting genes are highlighted in boldface type and have a bigger node size.

eye-antennal imaginal discs. However, we also extended our analysis to brain tissues to test potential *ISWI* cell cycle defects also in highly differentiating cells (COLLESANO and CORONA 2007). Isolated cell populations were directly analyzed by flow cytometry and cell cycle profiles were subjected to quantification (Figure S7) (COLLESANO and CORONA 2007).

Our analysis revealed significant differences in cell cycle profiles between wild-type and *ISWI* mutant larvae. Loss of *ISWI* in total imaginal or in eye imaginal discs caused a marked decrease of both G_1 and G_2/M peaks as well as a dramatic increase of the pre-G_1 peak (red and purple arrows in Figure 6, B and C; Figure S7, E and G). We reasoned that the increase in the pre-G_1 peak reflects DNA fragmentation due to cell death likely caused by a G_1 or G_2/M block caused by *ISWI* mutant disc defects (*i.e.*, alterations in chromosome condensation, replication, or gene expression). Interestingly, the pre-G_1 defect is specific for proliferating *ISWI* mutant discs and it is not detected in *ISWI* mutant differentiating brain cells that are a mixture of differentiated and cycling cells, with a significant contribution of cells in G_1 (Figure 6A and Figure S7 C). On the other hand, *ISWI* mutant brain cells show a reproducible approximately

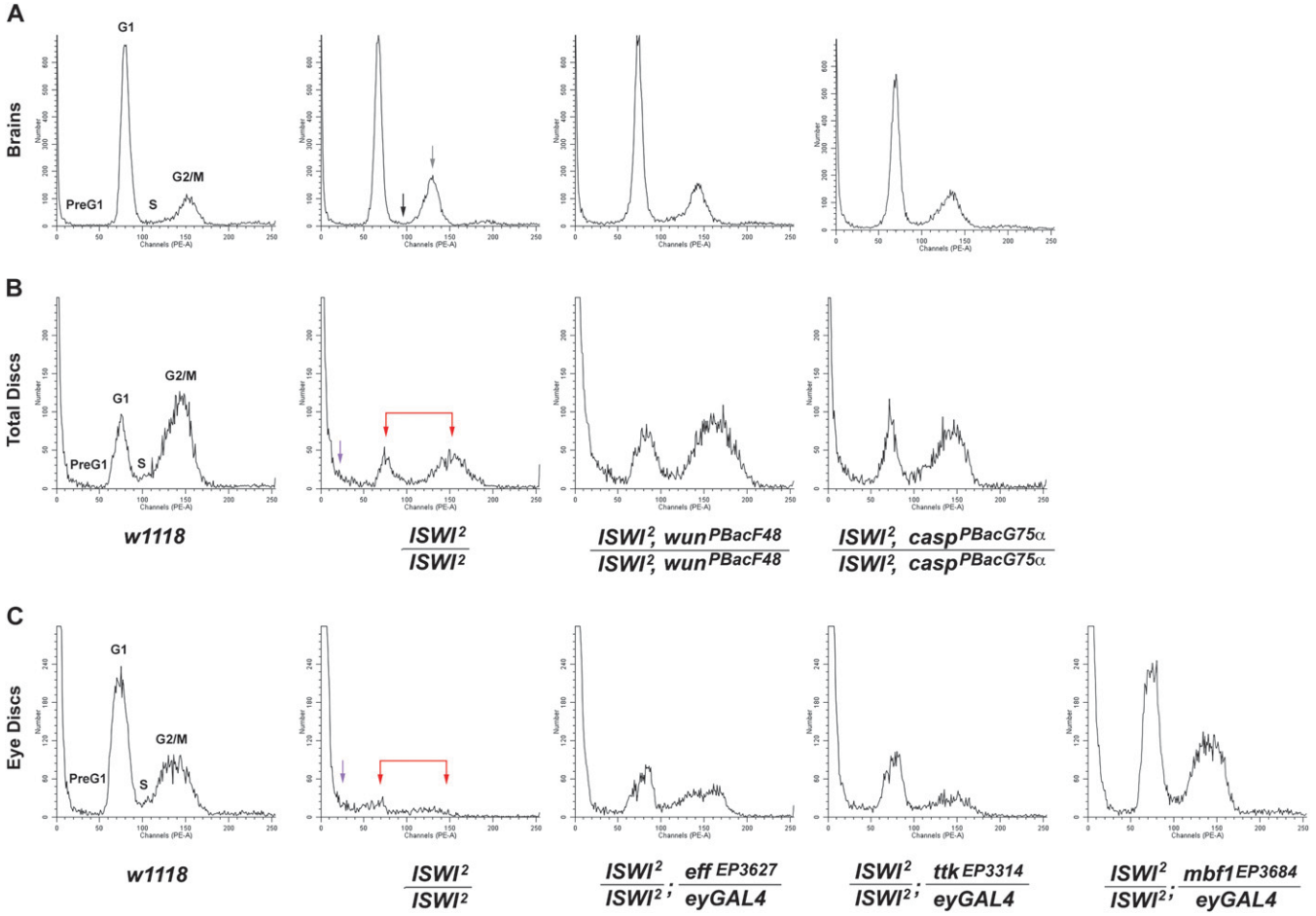

FIGURE 6.—Cytofluorimetric analysis of *ISWI* mutant cells. Cell populations derived *ex vivo* from wild-type and ISWI mutant neuroblasts (A) and total imaginal (B) and eye-antennal imaginal (C) discs were analyzed by flow cytometry. *ISWI* imaginal disc cells showed a significant decrease of G_1 and G_2/M peaks (red arrows) and an increase in the pre-G_1 peak (purple arrow). On the other hand, when compared to their wild-type counterpart, *ISWI* mutant neuroblasts showed a small but reproducible increase in the G_2/M peak (green arrow) and a faster S phase (blue arrow). These cell cycle defects can be in part or completely suppressed by some of the *Su(ISWI)*'s we isolated.

twofold increase in the G_2/M cell population and a shorter S phase when compared to wild type (green and blue arrows in Figure 6A and Figure S7 B), probably reflecting a G_2/M block or a faster S phase [as previously shown in SL2 cells silenced for the ISWI regulator Acf1 (FYODOROV *et al.* 2004)].

Our cell cycle analysis suggests that highly proliferating cells tend to be more sensitive to loss of *ISWI* activity while differentiating cells resist *ISWI* loss by shortening the S phase and accumulating in G_2/M. To check if *ISWI* cell cycle defects could be suppressed by some of the *Su(ISWI)*'s we identified in the *ISWI-EGUF* screen, we conducted the same analysis on double *ISWI, Su(ISWI)* mutants that survived to third instar larval stage, where brains and imaginal discs could be dissected. For these reasons, we limited our cell cycle analysis to the EP lines: eff^{EP3627}, ttk^{EP3314}, $mbf1^{EP3684}$, and the newly generated PiggyBac insertion $wun^{PBacF48}$, a gene encoding for a lipid phosphate phosphatase guiding germ cell migration (Table S1 A and Figure 4). As an internal positive control to evaluate the level of cell cycle suppression we could get in the double *ISWI, Su(ISWI)* mutant discs and brains, we also included in our analysis an allele of *casp* ($casp^{PBacG75\alpha}$), a false positive *Su(ISWI)* we isolated in our *ISWI-EGUF* screen. The *casp* gene encodes for a homolog of the mammalian Fas-associating factor 1, an apoptotic signaling factor that acts downstream of the Fas signal transduction pathway. Due to its role in promoting apoptosis, loss of *casp* is expected to suppress cell death and cell cycle defects linked to apoptotic signaling present in *ISWI* mutant cells.

Indeed, the false positive *Su(ISWI)* $casp^{PBacG75a}$ mutant suppressed the shortening of the S phase observed in *ISWI* mutant brain cells (Figure 6A and Figure S7 B). Interestingly, $wun^{PBacF48}$ *Su(ISWI)* also suppressed the *ISWI* S-phase defect (Figure 6A and Figure S7 B); however, both $casp^{PBacG75a}$ and $wun^{PBacF48}$ failed to suppress the *ISWI* G_2/M increase observed in differentiating brain cells (Figure 6A and Figure S7 B). These results indicate that the specific S-phase shortening and the G_2/M increase observed in brain cells are very likely independent noncoupled *ISWI* defects.

Interestingly, *wun*^{PBacF48} and *casp*^{PBacG75a} can also suppress *ISWI* pre-G_1 defects observed in imaginal disc cells (Figure S7, D and E) and restore a cell cycle profile similar to wild type (Figure 6B). Remarkably, while the *Su(ISWI)* EP lines *eff*^{EP3627} and *ttk*^{EP3314} in the presence of the eyGAL4 driver weakly suppress eye disc-specific *ISWI* pre-G_1 and G_1–G_2/M defects, the *mbf1*^{EP3684} *Su(ISWI)* strongly suppresses these defects (Figure S7, F and G; Figure 6C).

Concluding remarks: The *ISWI-EGUF* screen allowed us to isolate new *ISWI* genetic interactors that increased our understanding of the complex network of factors regulating ISWI nuclear signaling pathways. The *ISWI-EGUF* screen revealed that *ISWI* interacts with an evolutionarily conserved network of cellular and nuclear factors that escaped previous genetic and biochemical analyses, indicating the participation of ISWI in a variety of biological processes not linked to date with known ISWI functions. Moreover, the combination of gene network bioinformatics tools with classic fly genetics approaches has been instrumental to isolate some of the factors that might have been missed in the not saturating *ISWI*-based screens. The unexpected functional interactions we found between *ISWI* and factors playing central roles in cell cycle regulation have a high potential to shed light on the mechanistic aspects of cell cycle progression directly or indirectly regulated by ISWI and more generally by chromatin remodelers. Although the molecular basis of the genetic antagonism existing between *ISWI* and the variety of modifiers we identified will need further characterization and many of the isolated modifiers may act indirectly (*i.e.*, by regulating the perdurance, the expression, or regulators of ISWI), we believe that the network of *ISWI* enhancers and suppressors we isolated represents an invaluable selected cohort of genes that could be potentially assayed in any biological process where an ISWI-dependent functional assay is available.

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LITERATURE CITED

Al-Shahrour, F., P. Minguez, J. Tárraga, I. Medina, E. Alloza *et al.*, 2007 FatiGO +: a functional profiling tool for genomic data. Integration of functional annotation, regulatory motifs and interaction data with microarray experiments. Nucleic Acids Res. **35:** W91–W96.

Andersen, E. C., X. Lu and H. R. Horvitz, 2006 C. elegans ISWI and NURF301 antagonize an Rb-like pathway in the determination of multiple cell fates. Development **133:** 2695–2704.

Armstrong, J. A., A. S. Sperling, R. Deuring, L. Manning, S. L. Moseley *et al.*, 2005 Genetic screens for enhancers of brahma reveal functional interactions between the BRM chromatin-remodeling complex and the delta-notch signal transduction pathway in Drosophila. Genetics **170:** 1761–1774.

Badenhorst, P., S. Harrison and A. Travers, 1996 End of the line? Tramtrack and cell fate determination in Drosophila. Genes Cells **1:** 707–716.

Badenhorst, P., M. Voas, I. Rebay and C. Wu, 2002 Biological functions of the ISWI chromatin remodeling complex NURF. Genes Dev. **16:** 3186–3198.

Becker, P. B., and W. Horz, 2002 ATP-dependent nucleosome remodeling. Annu. Rev. Biochem. **71:** 247–273.

Breitkreutz, B. J., C. Stark and M. Tyers, 2003 Osprey: a network visualization system. Genome Biol. **4:** R22.

Breitkreutz, B. J., C. Stark, T. Reguly, L. Boucher, A. Breitkreutz *et al.*, 2008 The BioGRID Interaction Database: 2008 update. Nucleic Acids Res. **36:** D637–D640.

Burgio, G., G. La Rocca, A. Sala, W. Arancio, D. Di Gesu *et al.*, 2008 Genetic identification of a network of factors that functionally interact with the nucleosome remodeling ATPase ISWI. PLoS Genet. **4:** e1000089.

Cenci, G., L. Ciapponi and M. Gatti, 2005 The mechanism of telomere protection: a comparison between Drosophila and humans. Chromosoma **114:** 135–145.

Collesano, M., and D. F. Corona, 2007 Flow cytometry and karyotype analysis of D. melanogaster eye disc cells. Fly (Austin) **1:** 242–244.

Corona, D. F., and J. W. Tamkun, 2004 Multiple roles for ISWI in transcription, chromosome organization and DNA replication. Biochim. Biophys. Acta **1677:** 113–119.

Corona, D. F., C. R. Clapier, P. B. Becker and J. W. Tamkun, 2002 Modulation of ISWI function by site-specific histone acetylation. EMBO Rep. **3:** 242–247.

Corona, D. F., J. A. Armstrong and J. W. Tamkun, 2004 Genetic and cytological analysis of Drosophila chromatin-remodeling factors. Methods Enzymol. **377:** 70–85.

Corona, D. F., G. Siriaco, J. A. Armstrong, N. Snarskaya, S. A. McClymont *et al.*, 2007 ISWI regulates higher-order chromatin structure and histone H1 assembly in vivo. PLoS Biol. **5:** e232.

de Koning, B., F. Blombach, H. Wu, S. J. Brouns and J. van der Oost, 2009 Role of multiprotein bridging factor 1 in archaea: Bridging the domains? Biochem. Soc. Trans. **37:** 52–57.

Deuring, R., L. Fanti, J. A. Armstrong, M. Sarte, O. Papoulas *et al.*, 2000 The ISWI chromatin-remodeling protein is required for gene expression and the maintenance of higher order chromatin structure in vivo. Mol. Cell **5:** 355–365.

Dirscherl, S. S., and J. E. Krebs, 2004 Functional diversity of ISWI complexes. Biochem. Cell. Biol. **82:** 482–489.

Eberharter, A., and P. B. Becker, 2004 ATP-dependent nucleosome remodelling: factors and functions. J. Cell Sci. **117:** 3707–3711.

Ferreira, R., A. Eberharter, T. Bonaldi, M. Chioda, A. Imhof *et al.*, 2007 Site-specific acetylation of ISWI by GCN5. BMC Mol. Biol. **8:** 73.

Fyodorov, D. V., M. D. Blower, G. H. Karpen and J. T. Kadonaga, 2004 Acf1 confers unique activities to ACF/CHRAC and promotes the formation rather than disruption of chromatin in vivo. Genes Dev. **18:** 170–183.

Genovese, S., and D. F. V. Corona, 2007 A New Medium to Grow Live Insects. European Patent: MI2007A001420/8145 PTIT.

Hacker, U., S. Nystedt, M. P. Barmchi, C. Horn and E. A. Wimmer, 2003 PiggyBac-based insertional mutagenesis in the presence of stably integrated P elements in Drosophila. Proc. Natl. Acad. Sci. USA **100:** 7720–7725.

Hogan, C., and P. Varga-Weisz, 2007 The regulation of ATP-dependent nucleosome remodelling factors. Mutat. Res. **618:** 41–51.

Horn, C., N. Offen, S. Nystedt, U. Hacker and E. A. Wimmer, 2003 PiggyBac-based insertional mutagenesis and enhancer detection as a tool for functional insect genomics. Genetics **163:** 647–661.

Imhof, A., 2006 Epigenetic regulators and histone modification. Brief. Funct. Genomic Proteomic **5:** 222–227.

Jenuwein, T., and C. D. Allis, 2001 Translating the histone code. Science **293:** 1074–1080.

Jindra, M., I. Gaziova, M. Uhlirova, M. Okabe, Y. Hiromi *et al.*, 2004 Coactivator MBF1 preserves the redox-dependent AP-1 activity during oxidative stress in Drosophila. EMBO J. **23:** 3538–3547.

Kouzarides, T., 2007 Chromatin modifications and their function. Cell **128:** 693–705.

Martin, C., and Y. Zhang, 2007 Mechanisms of epigenetic inheritance. Curr. Opin. Cell Biol. **19:** 266–272.

Mellor, J., 2006 Imitation switch complexes. Ernst Schering Res. Found. Workshop **57:** 61–87.

Parrish, J. Z., M. D. Kim, L. Y. Jan and Y. N. Jan, 2006 Genome-wide analyses identify transcription factors required for proper morphogenesis of Drosophila sensory neuron dendrites. Genes Dev. **20:** 820–835.

Rorth, P., K. Szabo, A. Bailey, T. Laverty, J. Rehm *et al.*, 1998 Systematic gain-of-function genetics in Drosophila. Development **125:** 1049–1057.

Saha, A., J. Wittmeyer and B. R. Cairns, 2006 Chromatin remodelling: the industrial revolution of DNA around histones. Nat. Rev. Mol. Cell. Biol. **7:** 437–447.

Sala, A., and D. F. Corona, 2009 Poly-ADP-ribose (PAR) as an epigenetic flag. Epigenetics **4:** 287–290.

Sala, A., G. La Rocca, G. Burgio, E. Kotova, D. Di Gesu *et al.*, 2008 The nucleosome-remodeling ATPase ISWI is regulated by poly-ADP-ribosylation. PLoS Biol. **6:** e252.

Siriaco, G., R. Deuring, M. Chioda, P. B. Becker and J. W. Tamkun, 2009 Drosophila ISWI regulates the association of histone H1 with interphase chromosomes in vivo. Genetics **182:** 661–669.

Stowers, R. S., and T. L. Schwarz, 1999 A genetic method for generating Drosophila eyes composed exclusively of mitotic clones of a single genotype. Genetics **152:** 1631–1639.

Strahl, B. D., and C. D. Allis, 2000 The language of covalent histone modifications. Nature **403:** 41–45.

Tang, A. H., T. P. Neufeld, E. Kwan and G. M. Rubin, 1997 PHYL acts to down-regulate TTK88, a transcriptional repressor of neuronal cell fates, by a SINA-dependent mechanism. Cell **90:** 459–467.

Xi, R., and T. Xie, 2005 Stem cell self-renewal controlled by chromatin remodeling factors. Science **310:** 1487–1489.

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The Nucleosome Remodeling Factor *ISWI* Functionally Interacts With an Evolutionarily Conserved Network of Cellular Factors

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FILE S1

SUPPORTING MATERIALS AND METHODS

Salivary gland chromosomes cytology. Polytene chromosome structure analysis, on *ISWI^1/ISWI^2* 21 days old male salivary gland nuclei, was conducted by crossing *w/Y; ISWI^1, Bc/ SM5, Cy* males to *y w; ISWI^2/T(2:3) CyO;TM6B, Tb* virgin females at 18°C. 21 days old *ISWI^1/ISWI^2* trans-heterozygous male larvae were recognized by their yellow mouth hooks, the presence of the dominant Bc marker and the absence of the Tb marker. Polytene chromosome preparations and DAPI stainings were conducted according to previously published protocols (CORONA *et al.* 2004).

Eye imaginal discs Analysis. Immunofluorescence on GFP^{nls}-marked *ISWI^2/ISWI^2* homozygous eye disc nuclei were conducted in larvae obtained by crossing males from the FIG line (FRT42D, ISWI, GFP), generated by classic recombination (XU and RUBIN 1993): w/Y; *P{neoFRT}42D,ISWI^2*, P{Ubi-GFP(S65T)nls}/CyO with *yw; P{neoFRT}42D,GMR-hid2R,CL2R,y$^+$/CyO; EGUF* virgins. Heterozygous and homozygous GFP^{nls}-marked wild type eye disc nuclei were obtained from *yw; P{neoFRT}42D,* P{Ubi-GFP(S65T)nls}/CyO larvae or from larvae obtained by crossing *yw; P{neoFRT}42D,* P{Ubi-GFP(S65T)nls}/CyO males with *yw; P{neoFRT}42D,GMR-hid2R,CL2R,y$^+$/CyO; EGUF* virgins, respectively. Double immunofluorescence on eye-antennal imaginal discs with anti-ISWI (DEURING *et al.* 2000) and anti-GFP (Abcam #5450) antibodies were conducted according to standard protocols (SULLIVAN *et al.* 2000).

Adult eyes histology: Retina from *ISWI-EGUF* adults *FRT42D, ISWI^2 / FRT42D, GMR-hid; EGUF/+* were sectioned, processed and analyzed according to (MONTRASIO *et al.* 2007).

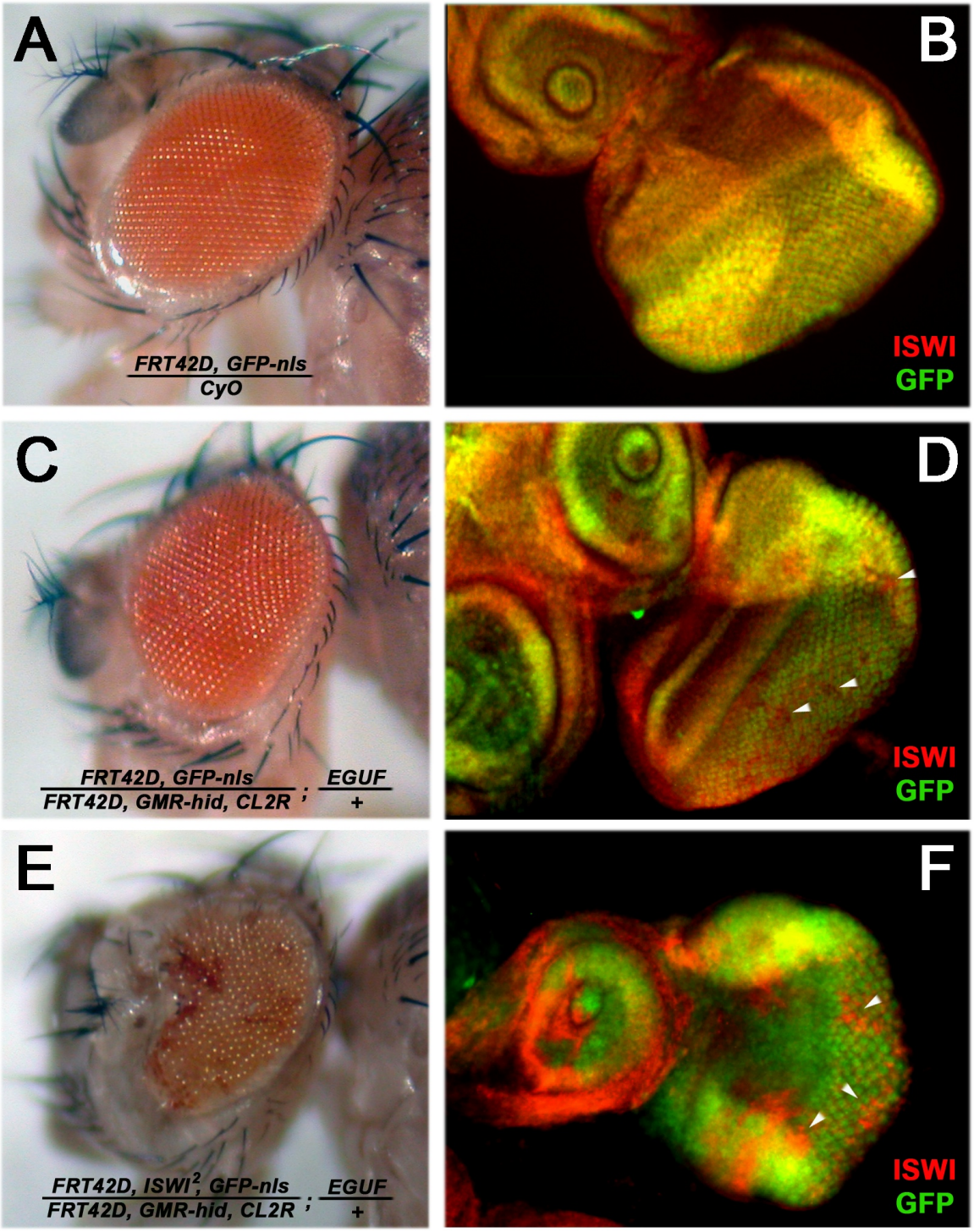

FIGURE S1.–Adult eye structures and double immunostaining, with anti-ISWI (red) and anti-GFP (green), of eye-antennal imaginal discs derived from *FRT42D, GFP^{nls} / CyO* (A,B), *FRT42D, GFP^{nls}/FRT42D, GMR-hid, CL2R; EGUF/+* (C,D) and *FRT42D, ISWI^2, GFP^{nls}/FRT42D, GMR-hid, CL2R; EGUF/+* larvae (E,F). The *GFP^{nls}* allele produces an ubiquitously expressed nuclear localized GFP. Arrowhead indicates small dying clones with only one copy of *GFP^{nls}*. Eye discs where *ey-GAL4*, *UAS-Flp* (*EGUF*) induced mitotic recombination and *GMR-hid* expression has occurred showed a near complete loss of ISWI signal and a homogenous GFP staining (E & F), suggesting that *ISWI-EGUF* eyes are very likely composed exclusively of homozygous *ISWI^2* cells.

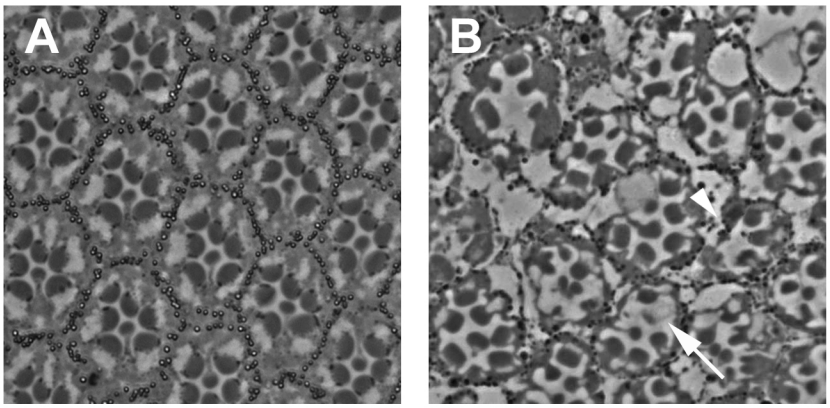

FIGURE S2.–Ommatidia organization is visualized by tangential sections through wild type (A) or *ISWI-EGUF* eyes (B). Loss of *ISWI* function in the adult eyes causes defects in the retina structure associated with loss of ommatidia boundaries and orientation. Several ommatidia are composed by a reduced number of photoreceptors (arrowhead) or photoreceptors that are undergoing degeneration (arrow).

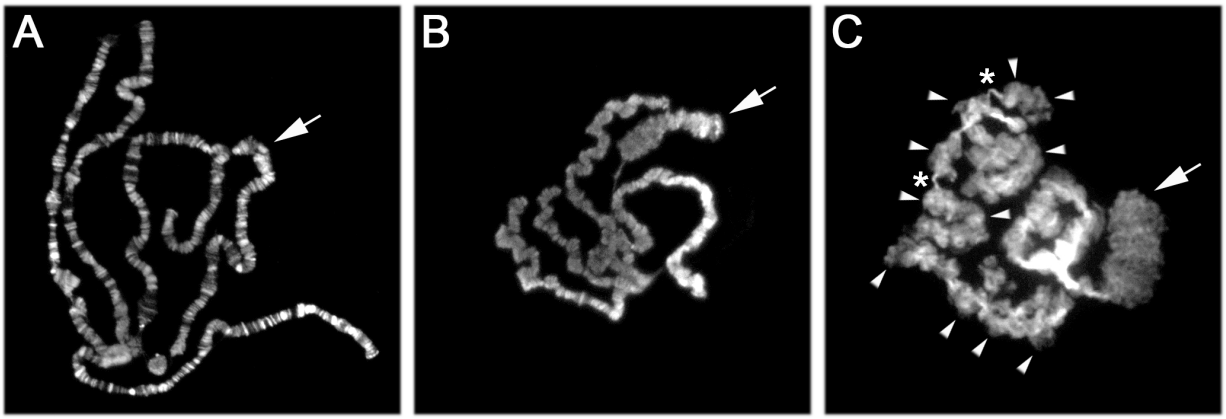

FIGURE S3.–Wild type male polytene chromosomes (A). Loss of ISWI activity not only causes defects on the male X chromosome organization in 14 days old 3rd instar larvae (B) but also extensive polytene chromosome condensation defects in the autosomes, when *ISWI*^{2} homozygous larvae are allowed to age for 21 days (C). The arrows point to the male X chromosome, showing the most extensive condensation defects (DEURING *et al.* 2000). The arrowheads point to autosome condensation defects, while the asterisks indicate chromatin hinges/breakpoints or chromatin domains that appear to be resistant to de-condensation.

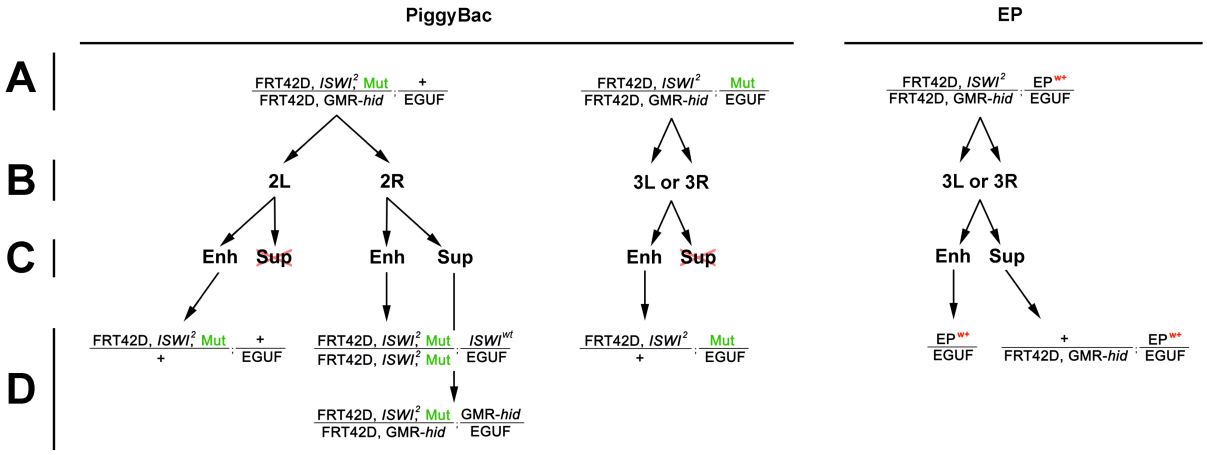

FIGURE S4.–False positives in which the *PiggyBac* insertion alone or the *ey-GAL4*, *EP* combination alone behaved as modifier of *ISWI-EGUF* eye phenotypes were sorted out by secondary screens. All the *ISWI-EGUF* interacting genetic loci shown in Figure 3 and listed in Table S1 passed the following secondary screens: PiggyBac and EP insertion genotypes where an *ISWI-EGUF* interaction was scored (A) were sorted according to the chromosome arm in which the interacting insertion was mapped (B). All the insertions, sorted by the interacting class isolated (C), were tested for their inability to interact alone in the *ISWI-EGUF* eye assay (D). We distinguished between interactions mapping chromosome 2L and 2R because *ISWI* lies on the 2R chromosome. Therefore, in the *ISWI-EGUF* assay the presence of the FRT42D sites produces eye with the 2R chromosome in homozygosis. Thus, all the interacting insertions on 2L will be dominant, while the one lying on 2R will be scored as recessive. The interaction classes that were not isolated, in the *ISWI-EGUF* screen for a specific chromosome arm, were crossed out in red. Bimodal interactions were screened for both the Enhancer and Suppressor secondary screens described. Ehn=Enhancer; Sup=Suppressor. For other details check MATERIALS AND METHODS.

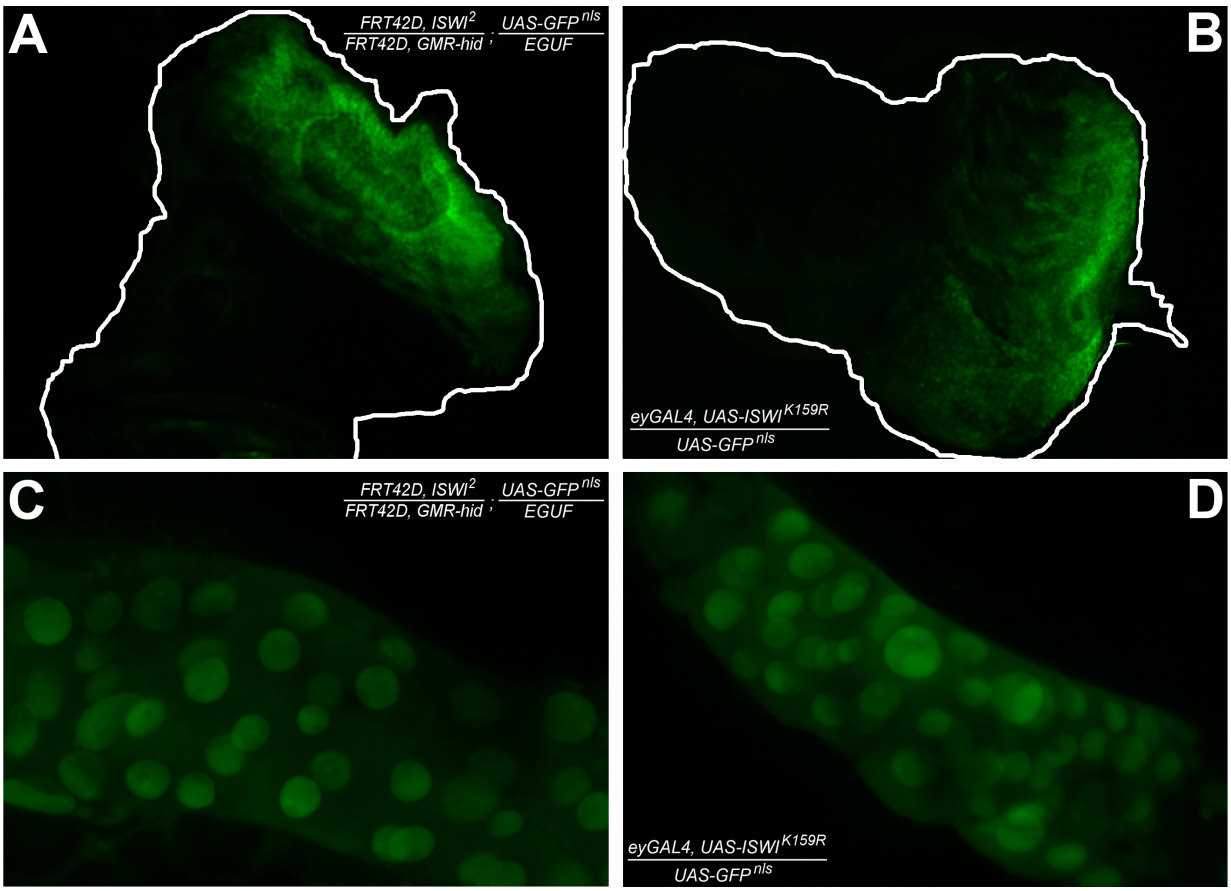

FIGURE S5.–The GFP expression in eye-antennal discs (A, B) and salivary glands nuclei (C, D) in *ISWI-EGUF* and *ISWI^{K159R}* genetic backgrounds, respectively, does not appear to significantly change.

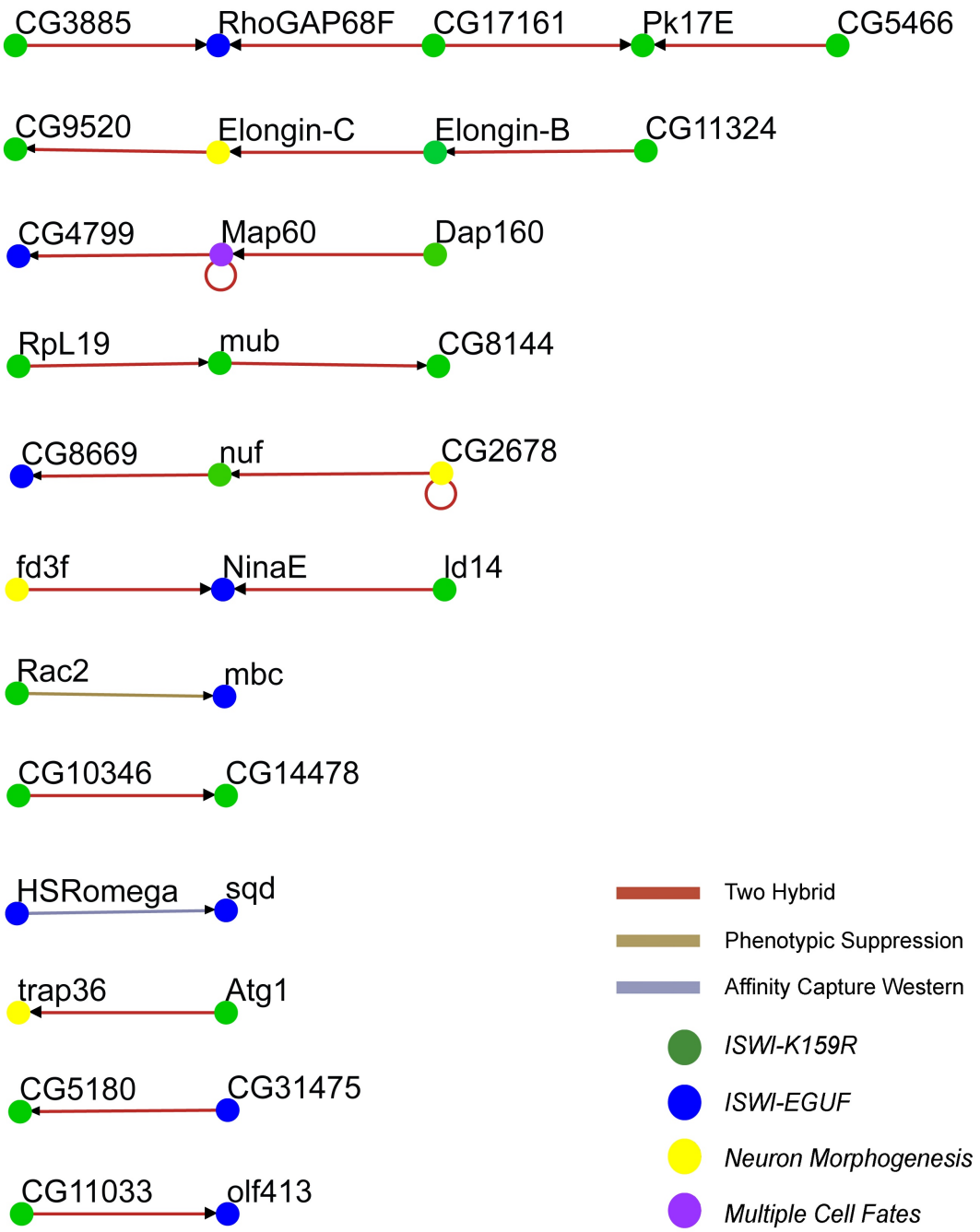

FIGURE S6.–Listed are the 12 small networks comprising *ISWI* genetic interactors isolated in the four different genetic screens analyzed: "Neuronal Morphogenesis", the worm "Multiple Cell Fate", the *ISWI-EGUF* and *ISWI*^{K159R} eye screens (ANDERSEN *et al.* 2006; BURGIO *et al.* 2008; PARRISH *et al.* 2006).

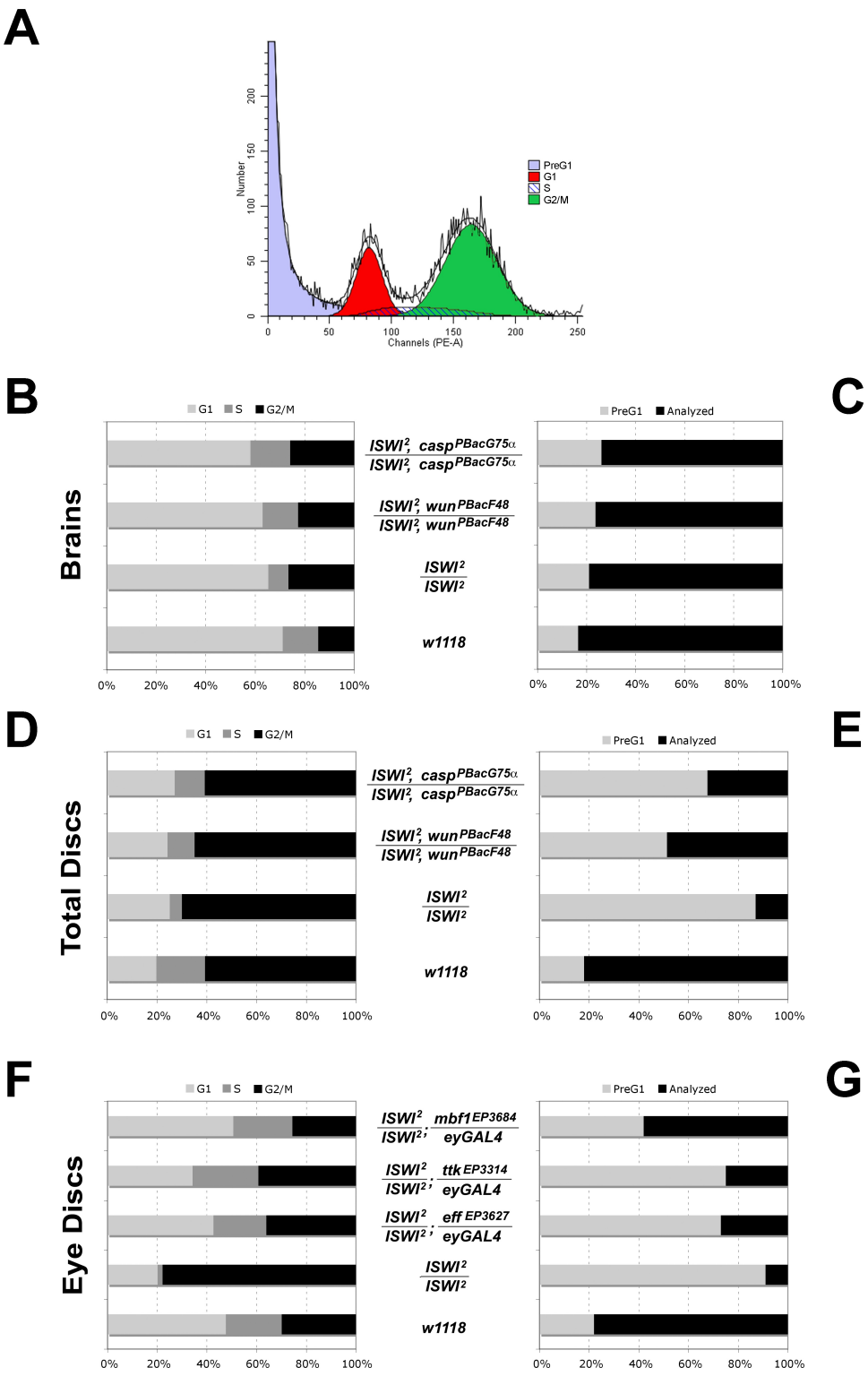

FIGURE S7.–Representative example of the quantitative analysis done with the ModFit software on different cell cycle phases (A). Quantification of the cell cycle profiles shown in Figure 6 (B, D and F). Relative contribution of the PreG1 and G1, S, G2/M (Analyzed) data shown in Figure 6 (C, E and G).

TABLE S1

***ISWI-EGUF* screening data**

(**A**) ISWI dominant modifiers have been mapped to specific gene loci combining iPCR sequencing data we generated for the *PiggyBac* interactors with mapping data available on Flybase (www.flybase.org) for the *EP* interacting insertions. (**B**) *ISWI-EGUF* genetic interaction classes [*Su(ISWI), En(ISWI), Bi(ISWI)*] and the intersection with the $ISWI^{K159R}$ screen are shown in separate columns for every gene locus listed. If the same gene was picked in the *ISWI-EGUF* and $ISWI^{K159R}$ screens, the details of the EP insertion and strength of interaction (+, ++, +++) identified in the $ISWI^{K159R}$ is reported. When the EP identified in the *ISWI-EGUF* and $ISWI^{K159R}$ is identical it is highlighted in light blue. The data from this table have been used to generate the graphs shown in Figure 3. Interestingly, few genes that in the $ISWI^{K159R}$ screen were isolated as enhancers were instead scored as *Su(ISWI)* in the *ISWI-EGUF* screen. In particular, 4 of the mutations that enhanced $ISWI^{K159R}$ and suppressed *ISWI-EGUF* eye phenotypes corresponded to different EP's insertions (Table S1). On the other hand, we also found 7 EP's enhancing $ISWI^{K159R}$ while suppressing *ISWI-EGUF* eye phenotypes. These genetic interaction differences are unlikely to be explained by differences in the expressivity of the *eyGAL4* drivers used in the $ISWI^{K159R}$ and *ISWI-EGUF* screens (Figure S5), and are probably due to the differences in the nature of the two *ISWI* alleles employed to generate the eye-defects used for the two different genetic screens (interfering misexpressing dominant negative $ISWI^{K159R}$ allele versus recessive lethal $ISWI^{2}$ allele in homozygosis). (**C**) The *ISWI-EGUF* screen resulted in the isolation of few mutations that produced both enhancement and suppression of *ISWI-EGUF* eye phenotypes in the same progeny, that we called *Bi(ISWI)* or bimodal. In particular, we recovered 7 bimodal interactions only in the EP but not in the PiggyBac screen. We tested other misexpression allele available on Flybase for the 7 *Bi(ISWI)* gene loci we isolated. None of new alleles corresponding the 7 Bi(ISWI) gene loci tested gave a bimodal interaction (see 'Other Alleles' column). Males *FRT42D,* $ISWI^{2}$ */ FRT42D, GMR-hid; EGUF/Bi(ISWI)* giving a bimodal interactions and behaving as *En(ISWI)* when re-crossed with *FRT42D, GMR-hid / CyO; EGUF/EGUF* females kept giving *En(ISWI),* while *Su(ISWI)* kept giving *Su(ISWI)* (see 'Re-Cross' column). The EP lines corresponding to the 7 *Bi(ISWI)* where out-crossed with the wild type isogenic w^{1118} line and independent males carrying the EP (marked with the *w+)* and control males with white eyes were re-tested in the *ISWI-EGUF* assay (see 'Out-Cross' Column). Combining the 'Other Allele' and 'Out-Cross' data we can clearly conclude that the *EP3115* maps a *Su(ISWI)*, the *EP3176* an *En(ISWI)*, the *EP3400* a *Su(ISWI)*, the *EP3608* an *En(ISWI)*, while we cannot conclusively say much about *EP3011*, *EP3239* and *EP3651*, probably because the two lesions were too close and did not segregate (*n* = independent males tested).

A

MUTATION	GENE	CHR	*ISWI-EGUF*	*ISWIK159R*	iPCR DATA
PBacE10	*PK61C*	3	***En(ISWI)***		NNNNNNNNNNNNNNCNNTTNANANNNGAGAGCAATATTTCNA GAATGCATGCGTCAATTTTACGCAGANTNNNNNNNNGNGTTAA TAAATAAATAATTAATGGGCGGNGCATTCNAAATGNAAAAGAAA ACGCAAATAGCTTGCCCACTCGCGGCAATAACGGAACCGTGAC TTGCAAATCGGTGAAATGAATAGGATATGCATAACTGTTTTTTC GAAAGACCTGCAAATAATTCAAGAAAGTTCCAGAAATACCCAAA TTTAATAGCTATCCAACATTAGGGTGACAATAACGNGTTTCAAC AGATGCAATATATACGCAAAATCAAAGAAAAACAANCNAAACCC GTNNNANNCTNATNATNTAAANAANAATNCNNNANCNTNNNNN NNNNGNTAA
PBacE24	*CG4294*	2R	***En(ISWI)***		NNNNNNTNTNNCGCTNTTNNGNNNAGAGAGNNNATTTCAAGAA TGCATGCGTCAATTTTACGCAGACTATCTTTCTAGGGTTAAATT CGCAGTTTTGACTTGTGGTTTCTGAACTGCTGAGGTAGCCTTA TCGTTGGTCATAGGGGAACTTGTTGTACTGCCTAGGTCTTTCT TGGCTGGGTTGTTTACAATATTCGCCGTGCTTAACGATGCATT GTTTTTCGGCGTTGGCTTATCAACAGTAGGCGCCTTGCTTTTG GCAACAGGAGCAGCAGTCTTGGCCCTATATATGGATCCAATTG CAATGATCATCATGACAGATCTGACAATGTTCAGTGCAGAGACT CGGCTACGCCTCGTGGACTTTGAAGTTGACCAACAATGTTTAT TCTTACCTCTAATAGTCCTCTGTGGCAAGGTCANN
PBacF48	*wun*	2R	***Su(ISWI)***		NNNNNNNNNNNTTTCNNGGGTTATGGCAGCGAATTTTGAATT AGACAGGGCGAAGAAGTCGCTGACAGATTTCCCTGGGCGGAAT TACTTTATGATCCACAATTGAGACCCCGCACACGTTCGCCTGTC ACTGGGAAATCTTATCGACGGCTTAGTCGTATCATAAGTCAATA GGGAGGGTCTCTATGACGTTTTTACCCCCAAGAAGATAGTGCC GCAGCACTTAGGGCCTATGGCATTATTGTACGGAATGATAAACA TTGCCTGCATAAATTCTTTTATTATATACAGCCATAATGTCAGT AGCAAGGGAGAAAAGGTTCAAAGTCGCAAAAAATTTATGAGAAA CCTTTACATGAGCCTGACGTCATCGTTTATGCGTAAGCGTTTA GAAGCTCCTACTTTGAAGAGATATTTGCGCGATAATATCTCTAA TATTTTGCCAAATGAAGTGCCTGGTACNTCAGATGACAGTACT GAANAGCCAGTAATGAAAAAACGTACTTACTGTACTTACTGCCC CTCTAAAATAAGGNGAAAGGCNNNNGNATNNNGNNANNNATGN NNNNNNTNNNTCNGNNNNNANCNNCCNANNNNN
PBacF57	*CG11652*	3	***En(ISWI)***		NNNNNNNNNNNNNTTCNNGGGTTAACNTAGTGGTAACATTTG GTGTTTAAATAGTTTTCGTGCTANNNNNNNATNNNTCTGTGCA TTGTTCGAAATTAATTTCAATATTTTAAACAATATTACTTTCACC ATTAACAACAACAAAATATGTTTACATGCTACNCTGTTTACCAT AAANNACAACGCCAGTTTGTTTTGCCATTTTATTGCCAATTGTT AATTCANACCTTGATGGCNCNNNNNTATCTCTAATATTTTGCC NAATGAANTGCCTGGNACATCACATGANNNTNCTGANNANCCA

				CNNNNNNNNTNNNNNNTTATTNANTTACTGNCCCTNNNNNNN NNTATNNNN
PBacF60	*stg*	3	***En(ISWI)***	NNNNNNNTTNACGTACGTCNNAATATGATTATCTTTCTAGGGT TAAAGACACACGGTCCATCGTGTAGAGTGCTACCTGTGAATGG ATCTCTGTGGCTGGAAGCTTTGCTCATTTAACGACATTGGCGT CTACGTCTGCAGAACACAAAGGAGCCACGCCCCCTCCACGACC GCCCATCCCGCCGGTGCGGCGATCATATGACTGCGGCCTATGG CATTATTGTACGGAATGATAAACATTGCCTGCATAAATTCTTTT ATTATATACAGCCATAATGTCAGTAGCAAGGGAGAAAAGGTTCA AAGTCGCAAAAAATTTATGAGAAACCTTTACATGAGCCTGACGT CATCGTTTATGCGTAAGCGTTTAGAAGCTCCTACTTTGAAGAGA TATTTGCGCGATAATATCTCTAATATTTTGCCAAATGAAGTGCC TGGTACATCAGATGACAGTACTGAAGAGCCAGTAATGAAAAAAC GTACTTACTGTACTTACTGCCCCTCTAAAATAAGGCGAAAGGCA AATGCATCGTGCAAAAAATGCAAAAAAGTTATTTGTCGAGAGCA TAATATTGATATGTGCCAAAGTTGTTTCTGACTGACTNNN
PBacG00	*slif*	3	***En(ISWI)***	NNNNNNNTNACGTACGTCNNAATATGATTATCTTTCTAGGGTT AAGGTATACAATTTCTATTTCTTTGTTACTATTGGTTCTAAAGT CTGTCATAGAATTCGTTAACTAAGAACTTGACCTTCAGAGACCA TCTGGGCAACAGCAGTCAGAATAATATCCGTCAATTAAAGTTGT TTTAGACGCAAATAGCACGACTAGTCTCTCTCGTTAATTACTGA CTAGCCCAGCGACTAACCCATGCCAGCTTTCATTAATTTACCCG TATGCATTTGAGCAATATAAAGACGCTTCCGCATCGCACCCCGC ACAAAAGCGCGATAATATCTCTAATATTTTGCCAAATGAAGTGC CTGGTACATCAGATGACAGTACTGAAGAGCCAGTAATGAAAAAA CGTACTTACTGTACTTACTGCCCCTCTAAAATAAGGCGAAAGGC AAATGCATCGTGCAAAAAATGCAAAAAAGTTATTTGTCGAGAGC ATAATATTGATATGTGCCAAAGTTGTTTCTGACTNACTANNNN
PBacG36alfa	*CG14508*	3	***En(ISWI)***	NNNNNNNNNNNNNNTCNAGGNNNNNANACATATCGATCGAAT GNACTTAAAAGCTCTGCTAAACTATNTGGAAAANATCTAAGCCC CTCTCCCTTGTGTAAGNAGTATTAANNAAGNNNGNTNNNGGNN NNNNNNNNNANGCNGGANGNTTNNNNANNNNNNNNNCNNNNN NCNTNNNNNNCNGNTNCNCTCNCCNNNNNNCTTANNNNNGAT TCNNNNNANANNGNNTNNGNNNNNNNNCNNTNCCNNNNNNNN NNNNNNNNNNNNANAAAANNNNNNNNACNNCNNCNNTNNNNA ANNNNCNNNNNNNCCNTTATNNNNNCNNNNNNNNNNTNNNNN ANNNNNNNNNNCATACCCNTCNNNNNNNTNNNANNNNNNNNN NNNNCTANCNNNNANCANAANCNNNANACTNNNANNNNNCTNN NTNCTN
PBacG80	*CG15128*	2R	***Su(ISWI)***	NNNNNNTNACGTACGTCNNAATATGATTATCTTTCTAGGGTT AAACAACACGTTTTACGGCTCATTATAAATGAACTAGGAAAAAC AAAGAGCAAACAAAATAGTTTATGGAAAACCTAATAAATCATAG GAAACGTTGAAATTTACTTTCTTTCAAAATACTGATGTTAAGCA ACACATATCATCGAAACATATCGGACTCCTTTCAACCCACACTT GTTTTAATCTTTATTAAATTTATAGTAAAATTAACAGCCAAGTT

				TTAATAACACTTTAACACTTACTTTATCAAGTTGTACACAATACT CATTTTAAGAGCGTGAAAACGATACCCATTAGAATTTTGGCAAG GTCTACATCGCAAAACTAGTGAATACAAGAAGCCAAAATGACGT CGATTGTGGAGATGCCAAGGGCAAATTTAGAGGAGGCCTATGG CATTATTGTACGGAATGATAAACATTGCCTGCATAAATTCTTTT ATTATATACAGCCATAATGTCAGTAGCAAGGGAGAAAAGGTTCA AAGTCGCAAAAAATTTATGAGAAACCTTTACNTGAGCCTGACGT CATCGTTTATGCGTAAGCGTTTAGAAGCTCCTACTTTNNANANA TATTTGCGCGATNATATCTCNAANATTNNGCCAAATGANNTGN NCTGGNNNNTCANATNAN
PBacG92	*Cpr31A / Pen*	2L	***En(ISWI)***	NNNNNNNNNACGTACGTCNNAATATGATTATCTTTCTAGGGT TAATAATTAATTTACCACATTAATTNAGCGGATTACTTCTCAAA GAATCGAAAAACAAACAATTGAAAAAGGGACTAAATCGATTGGC AGCAATCATCTCTAAAAAAAATTTTGTTTATTTCTGACAACCGC ATATAAACAAAATGAAAAGAGCATTAACCGGAAATATCTGCGCA TTTATTGCATTATTACTGATAATCGGGAGATTAAATGGTATTAG TGCAATGATAACATATTTGCAAGCAAAAAAAAANANACAATCAA ATTTTATATTTATAGATAGACAGATAAAATCNNTNCCTTTTATT ANCCNNTTAANTNTTGATTTANGNGGNTATTTTTTGTCTTANN NNATATGGATANAGCTTTACATTTNANNNCATATTNGNTGANT NANNNNNAANNNANNNNNNNNNNNNNNNANNNNNNNGANN NNG
PBacG97	*CG7420*	2L	***En(ISWI)***	NNNNNNTTNACGTACGTCNNAATATGATTATCTTTCTAGGGTT AATTTACTTTTCCAGTTGCACCCCAACTCTTTTACAACTGCCAC CAGTGAAATTGTGCGGCGCGATAATATCTCTAATATTTTGCCAA ATGAAGTGCCTGGTACATCAGATGACAGTACTGAAGAGCCAGT AATGAAAAAACGTACTTACTGTACTTACTGCCCCTCTAAAATAA GGCGAAAGGCAAATGCATCGTGCAAAAAATGCAAAAAAGTTATT TGTCGAGAGCATAATATTGATATGTGCCAAAGTTGTTTCTGACT GNNNNNNNNNNNNNNAAAANNNNNNNNNNNNNANCNNANAN NAAAAAANANNNNNNNNGNNNNNNNANNNN
PBacK17	*CG11844*	3	***En(ISWI)***	NNNNNNNNNNNNNNNNNNNNNNNNANNANTATCTTTCTAGGG TTAATAAAATTGCAACACCTTCAAAATAGTTTAAATTTGTTATAC ATTTTTAAGGTATAACTTTTCCTAAATTATTGTAAATATACATAT ATGGTTTATTTAAGGCTGTAAATTATCAAATCCCTTTTCAAAAA CCAACCATTGTTCAGAAAATATAAATTATATATTTTAATTTTTAC AGATATATACTGATATTGAACCGGNAACTATCNATTGTNNNANT GNANTTANNNNANAAACN
PBacK18	*CG3092*	2R	***En(ISWI)***	NNNNNNNNNNANNGNACGTNNNNNNATGATTATCTTTCTAGG GTTAAAGGGCAATCGAACTTCTGGCCCAGTTCTTTAAACTTTAA AGCATGTGTTCNTTTAAATCANTGNCNTGGGCACCCAAATCNA TTGNCAT
PBacL45	*CG9287*	2L	***En(ISWI)***	NNNNNNNNNCTTTCTAGGGTTNNNTTTATGCTTCTAAACCTG TTTATGCAAACTCAGTAATCNTATAATTATGCTATTCATAGGCG CGATAATATCTCTAATATTTTGCCAAATGAAGTGCCTGGTACAT

				CAGATGACAGTACTGAAGAGCCAGTAATGAAAAAACGTACTTAC TGTACTTACTGCCCCTCTAAAATAAGGCGAAAGGCAAATGCATC GTGCNAAAAATGNAAAAANNTNNNNNNNCNNNNNNNNNNNNNN NNNNNNNNNCNNNNNNNNNTGCNATANNNNATGCNANTTGNN NNCTCNNTGANNCNNNNNNNNNNNNNNNNNNNNTTGCNCCCT CTGGNATTCCTCAGGNCNTGCCNGTTTGANCNTCNCTGAAAAC CCNNNCNNNGAATCACNTGGNGCTGATGNAGNNGNCTTGNNC CGCNNCGNCGGTGGCCGGGTCCACCCTGTNNAATTTCNTTGN NANNANNACNGACCCCCGNCTGCTGANTNNNNNNANNNNN
PBacL68	*Gap1*	3	***En(ISWI)***	NNNNNNNNNNCTTTCNNGGGTTACGGNNTTTTGCAGGTAAA GAGGGTAAACATGAACCACAAAGNCNNNCNAATGAAATGAAGG TAANAAGGTGACAGCTAATTTNNNNNNACNACCCGATAATCNA NGGAAGTGCNTNACTTTCGTTCTTCNNTCNNCGCGATAATATC NCTNNTATTTNGNNNNNTGANNNGNNGNNNACNTCNNATGAC NNNNNNGNNNNNNNNTANNNNNNNNNNNNNNNNNNTNNNNNN TCNNNNCNNNNNNNNANNNNGANNNNNNNCNGNTNNNNNCNN NNNNNNNCNAANNNNCNNCNNNGNNNGNANNGNNTNCGCNNN NCNNGNNNNNNCNNNNGNNNNNNNNNNNNNCNNCNTNNCNN NNNNNNNNNNAAANNNNNNNNNNNNNNNNNNNNTTTNNCNCN CNNNNNNNNNGNCNNCNNCNNCNNNNNNNNNNNNNNNNNNN NNNNCNNNCNNNNNNTNNNANNTTCNTNNNNNNNNNNNN
PBacL78	*CG17593*	2L	***En(ISWI)***	NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNATGATTATCT TTCTAGGGTTAAATGTCCTAAAACTTTTCACACTAATTAAGAAA AAAAAACTAGGTTAAATGGTTAGTGTGCCAGTCGCGTGCAGAT TGAAGAATAAAACGCGTTAGTGCCTTTTAGTTTGAATGCNATTC GGTATTCCNANNNANCACACNCTTGTTAAATGNTTTNNNNNNN NTTNTTTTANTTTNTANNNATTNNNTTCN
PBacM04alfa	*E2f*	3	***En(ISWI)***	NNNNNNNNTTACGTACGTNNCAATANGATTATCTTTCTAGGGT TAAATATTTATAGAATTAGTATAANNNNTTAAAAAAAACAAAACA ATAAACTTCAAAGAAGTGTCNNNNTCGACTTCCATTAAACACCC ATACATTTTTCCTTAAAAGGCGCGATAATATCTCTAATATTTTG CCAAATGAAGTGCCTGGTACATCAGATGACAGTACTGAAGAGC CAGTAATGAAAAAACGTACTTACTGTACTTACTGCCCCTCTAAA ATAAGGCGAAAGGCAAATGCATCGTGCAAAAAATGCAAAAAAGT TATTTGTCGAGAGCATAATATTGATATGTGCCAAAGTTGTTTCT GACTGACTANNNNNNNNANNNNNNNNANNNNNNNNNNCNNNN NNNNNNNCNNCNNNNNNNNNNNNNNAAAATANNNNNNAAAAA NN
PBacN13	*RpL40 / CG15425*	2L	***En(ISWI)***	NNNNNNNNNNNNNNNNNNNCNNAATATGATTATCTTTCTAGG NTTAAAGGTATATCATATTTCGTGCTAGGTAATTAAAATAAAAA CGAATAAAAATTTTTCTTTTCAAAAATGCTGAAAATAATATTAGA TTCCAAAAGCTGTAATATTTTTTCAATATCCAGCTGTTTGTACT TCTGTCTGCACTGAGCGAAAAACAGTCGACCGCAGTTGGAGCT

				CAAGTTTGGGGATGTCTGCTAACCGCCGCGCGATAATATCTCT AATATTTTGCCAAATGAAGTGCCTGGTACATCAGATGACAGTAC TGAAGAGCCAGTAATGAAAAAACGTACTTACTGNACTTACTGCC CCTCTAAAATAAGGCGAAAGGNAANTNNNNNNTGCAAANNNNG NNNNNNNNNNANNNGTCCAGANCNTAANTTTGNNNNGNGCCA AAAANTNN
PBacN16	*CG17732*	3	***En(ISWI)***	NNNNNNNTTNACGTACGTCNNAATATNATTATCTTTCTAGGGT TAATGTCCGTAGTTTCGGGATCATGGNNGNCTTCNTTGGATTT TGGATAACATGCCTCCTTCATTTTCTTANCCACGTATCGAGGAC GACCCGAACCGATCGGAAATTTCAGCATTCGGAGATGGTACTC ACGATTCTTGGCGTATCTAGTCAGTGTCCTTGTGCGCGATAAT ATCTCTAATATTTTGCCAAATGAAGTGCCTGGTACATCAGATGA CAGTACTGAAGAGCCAGTAATGAAAAAACGTACTTACTGNNCTT ACTGNCNCTNNNNNNTAANGN
PBacN43	*CG12862*	2R	***En(ISWI)***	NNNNNNNNNNNNNNNNNNNNNNNNNNNNTATGATTATCTT TCTAGGGTTAATAAACTCTCGCCGACGCTTCGCACAGGGCTTA TGTCCTGAAAGGCGTCCTTTAATTCATTTTCCATGCACACACAC TAGCACACACATAGTCGGACTAAAAAAACAGCGGAANANGATTA AAAACTACTTTAATATATCNTCNTTGTCNCTCCTGCGAGCCGCG NANGAGANCNNGANGNGNATGGNN
PBacO02	*E(Pc)*	2R	***Su(ISWI)***	NNNNNNNNNNNNNNNCNTNNNNNNNNNNNANCTTTCNNNNNN NNNNNTTTTTNGTGCAGTNNGCANNNNNNNNNNNNNNCTGTCA GAGCTGNNNNNGCCATTTAAGANNNNNNNNNNNNNNNNNNNN ATGCNGACAAGGTAGNNNNNGNNACNNNNNNNTNNNNNTNNN GCNTGNGTTTTNNNNNNNNCAAANANNN
PBacO68	*chn*	2R	***En(ISWI)***	_NNNNNNNNNNNNNTACGTNNNANTANNNTTATCTTTCTAGG NTTAAAACCGAATCGAAGGGTTCGNNNNNNNNNNNNNGNNTTC AATTAGTAAAATGACGGGTTANNNNNNNNNNNNNGNGGNTTCN TCATGTGTCTACCTGGACCATGTATNGNANGCCCTCCTTCTGG NCATATACCNCNCNCCAAACCCAAAAAAGCNNNNGNNNNNNNT AANGANNNNCNNNGTCNNANNNNNCTCCTNNTCNTTCTTT

B

MUTATION	GENE	CHR	*ISWI-EGUF*	*ISWI*	*K159R*
				Interaction	*EP (strength)*
EP0572	*Tfb2*	3	***Su(ISWI)***		
EP0681	*slmb*	3	***Su(ISWI)***		
EP0711	*Tektin-C*	3	***En(ISWI)***		
EP0809	*Nrx-IV*	3	***En(ISWI)***		
EP1038	*Fer2LCH*	3	***En(ISWI)***	✔	**EP1059 (++)**
EP1064	*heterochromatin*	3	***En(ISWI)***		
EP1070	*heterochromatin*	3	***En(ISWI)***		
EP1082	*mura*	3	***En(ISWI)***	✔	**EP1202 (++)**
EP1196	*pum*	3	***En(ISWI)***	✔	**EP1196 (+++)**
EP1219	*NinaE*	3	***En(ISWI)***		
EP1247	*Gclm*	3	***En(ISWI)***		
EP3002	*Chd64*	3	***En(ISWI)***		
EP3006	*Crc*	3	***En(ISWI)***		
EP3011	*Doa*	3	***Bi(ISWI)***		
EP3041	*mir-282*	3	***En(ISWI)***		
EP3059	*CG13623*	3	***Su(ISWI)***		
EP3063	*CG7560*	3	***Su(ISWI)***	✔	**EP3063 (++)**
EP3065	*CG5789*	3	***En(ISWI)***		
EP3068	*CG9746 / CG8420*	3	***En(ISWI)***		
EP3072	*CG8219*	3	***Su(ISWI)***		
EP3073	*CG5180*	3	***Su(ISWI)***	✔	**EP3073 (++)**
EP3086	*mor*	3	***En(ISWI)***		
EP3089	*CG6051*	3	***Su(ISWI)***		
EP3097	*Ssdp*	3	***En(ISWI)***		
EP3098	*Eip78C*	3	***En(ISWI)***	✔	**EP3098 (++)**
EP3115	*HSRomega*	3	***Bi(ISWI)***		

EP3121	*eRF1*	3	***Su(ISWI)***	✔	**EP3195** (+)
EP3135	*mbc*	3	***Su(ISWI)***		
EP3137	*Madm*	3	***En(ISWI)***		
EP3139	*mir-282*	3	***En(ISWI)***		
EP3142	*MKP3*	3	***En(ISWI)***		
EP3145	*Atx2*	3	***En(ISWI)***	✔	**EP3022** (+)
EP3146	*CG5807*	3	***Su(ISWI)***		
EP3152	*RhoGAP68F*	3	***En(ISWI)***		
EP3166	*emc*	3	***En(ISWI)***		
EP3176	*SNF4Agamma*	3	***Bi(ISWI)***	✔	**EP3332** (++)
EP3179	*CG11873*	3	***Su(ISWI)***		
EP3186	*CG5555 / CG31475*	3	***Su(ISWI)***	✔	**EP3186 (+)**
EP3190	*osa*	3	***En(ISWI)***	✔	**EP3190 (++)**
EP3202	*CG15532*	3	***Su(ISWI)***		
EP3207	*MMS19*	3	***En(ISWI)***		
EP3210	*Jheh2*	3	***En(ISWI)***		
EP3214	*As*	3	***En(ISWI)***		
EP3216	*Lk6*	3	***Su(ISWI)***		
EP3230	*olf413*	3	***En(ISWI)***		
EP3231	*CG7430*	3	***Su(ISWI)***		
EP3234	*Patr-1*	3	***En(ISWI)***	✔	**EP562** (+)
EP3239	*RhoL*	3	***Bi(ISWI)***		
EP3242	*blot*	3	***En(ISWI)***		
EP3259	*lark*	3	***Su(ISWI)***		
EP3292	*DNApol-delta*	3	***En(ISWI)***		
EP3314	*ttk*	3	***Su(ISWI)***	✔	**EP3062** (++)
EP3385	*wdb*	3	***Su(ISWI)***		
EP3400	*dsd*	3	***Bi(ISWI)***		
EP3439	*Rpn9*	3	***En(ISWI)***		
EP3476	*tankyrase*	3	***En(ISWI)***		

EP3483	*Tsp96F*	3	***En(ISWI)***		
EP3500	*Eip75B*	3	***En(ISWI)***	✔	**EP3380** (++)
EP3541	*trx*	3	***Su(ISWI)***	✔	**EP3541** (++)
EP3548	*GlcAT-P*	3	***En(ISWI)***		
EP3583	*Hsp27*	3	***Su(ISWI)***	✔	**EP3583** (++)
EP3591	*exba*	3	***En(ISWI)***	✔	**EP3591** (+)
EP3608	*cpo*	3	***Bi(ISWI)***	✔	**EP3608** (+++)
EP3626	*mir-279 / CG31044*	3	***Su(ISWI)***		
EP3627	*eff*	3	***Su(ISWI)***		
EP3631	*sqd*	3	***Su(ISWI)***		
EP3636	*CG10426*	3	***En(ISWI)***	✔	**EP3636** (+)
EP3648	*CG33523*	3	***En(ISWI)***		
EP3651	*LysB*	3	***Bi(ISWI)***		
EP3653	*CG6650*	3	***Su(ISWI)***		
EP3666	*CG13895*	3	***En(ISWI)***	✔	**EP3666** (+)
EP3675	*aralar1*	3	***En(ISWI)***		
EP3676	*CG18869*	3	***Su(ISWI)***	✔	**EP3676** (+)
EP3684	*mbf1*	3	***Su(ISWI)***	✔	**EP3684** (++)
EP3699	*CG11033*	3	***Su(ISWI)***	✔	**EP3471** (++)
EP3703	*poly*	3	***En(ISWI)***		
EP3736	*fax*	3	***Su(ISWI)***	✔	**EP3355** (++)
EP3740	*Spec2*	3	***En(ISWI)***		

C

MUT	GENE	*ISWI-EGUF*	*Other Alleles*	*Re-Cross*	*Out-Cross*
			P{XP}Doa-d02760 -> En(ISWI)	*En(ISWI) -> En(ISWI) [n=12]*	*Bi(ISWI)-w+ [n=10]*
EP3011	*Doa*	*Bi(ISWI)*	*P{EPgy2}Doa-EY11301 -> No Interaction*	*Su(ISWI) -> Su(ISWI) [n=8]*	*Bi(ISWI) [n=8]*
				En(ISWI) -> En(ISWI) [n=7]	*Su(ISWI)-w+ [n=4]*
EP3115	*HSRw*	*Bi(ISWI)*	*P{XP}Hsrω-d02763 -> Su(ISWI)*	*Su(ISWI) -> Su(ISWI) [n=5]*	*Bi(ISWI) [n=17]*
				En(ISWI) -> En(ISWI) [n=4]	*En(ISWI)-w+ [n=2]*
EP3176	*SNF4Ag*	*Bi(ISWI)*	*PBac{WH}SNF4A-f01641-> En(ISWI)*	*Su(ISWI) -> Su(ISWI) [n=9]*	*Bi(ISWI) [n=13]*
			PBac{WH}f02848 -> *Su(ISWI)*	*En(ISWI) -> En(ISWI) [n=13]*	*Bi(ISWI)-w+ [n=5]*
EP3239	*RhoL*	*Bi(ISWI)*	*P{XP}d00094 -> En(ISWI)*	*Su(ISWI) -> Su(ISWI) [n=11]*	*Bi(ISWI) [n=10]*
				En(ISWI) -> En(ISWI) [n=9]	*Su(ISWI)-w+ [n=9]*
EP3400	*dsd*	*Bi(ISWI)*	*P{EPgy2}EY05482 -> Su(ISWI)*	*Su(ISWI) -> Su(ISWI) [n=8]*	*Bi(ISWI) [n=12]*
				En(ISWI) -> En(ISWI) [n=3]	*En(ISWI)-w+ [n=7]*
EP3608	*cpo*	*Bi(ISWI)*	*P{XP}cpo-d09064 -> En(ISWI)*	*Su(ISWI) -> Su(ISWI) [n=11]*	*Bi(ISWI) [n=14]*
			P{XP}d11180 -> *En(ISWI)*	*En(ISWI) -> En(ISWI) [n=5]*	*Bi(ISWI)-w+ [n=10]*
EP3651	*LysB*	*Bi(ISWI)*	**P{XP}d01620 -> *En(ISWI)***	*Su(ISWI) -> Su(ISWI[n=6]*	*Bi(ISWI) [n=11]*

TABLE S2

Predicted ISWI functional connections

Known genetic or physical interactors existing among the genes identified in the fly "Neuronal Morphogenesis" (**A**) and the worm "Multiple Cell Fate" (**B**) screens, the modifier genes we picked in both the *ISWI-EGUF* (**C**) and *ISWI^{K159R}* (**D**) eye screens (ANDERSEN *et al.* 2006; BURGIO *et al.* 2008; PARRISH *et al.* 2006) and the "Connecting" nodes (**E**). This table contains the raw data used to compose Figure 5.

A

Fly Gene	Description	GO Special
aop	anterior open	;transport;transcription;signal transduction;
asf1	anti-silencing factor 1	;DNA metabolic process;
ato	atonal	;transcription;signal transduction;cell cycle;
bap55	Brahma associated protein 55kD	;protein catabolic process;cell organization and biogenesis;
bap60	Brahma associated protein 60kD	;DNA metabolic process;
brm	brahma	;DNA metabolic process;transcription;
CG4328	CG4328	NONE
Tab2	Tab2	NONE
ci	cubitus interruptus	;signal transduction;
dpn	deadpan	;transcription;
E(z)	Enhancer of zeste	;metabolic process;transcription;DNA metabolic process;
esc	extra sexcombs	;metabolic process;DNA metabolic process;signal transduction;transcription;
EcR	Ecdysone receptor	;autophagy;signal transduction;transcription;metabolic process;
gro	groucho	;transcription;cell organization and biogenesis;signal transduction;
kni	knirps	;transcription;signal transduction;
M8	Enhancer of split	;transcription;signal transduction;
RpS29	Ribosomal protein S29	;translation;

run	CG1849-PA	;transcription;
scrt	scratch	NONE
sens	senseless	;transcription;signal transduction;
sna	snail	NONE
snr1	Snf5-related 1	;DNA metabolic process;
Stat92E	Signal-transducer and activator of transcription protein at 92E	;signal transduction;transcription;
Su(z)12	Su(z)12	;metabolic process;transcription;DNA metabolic process;
tgo	tango	;transcription;metabolic process;signal transduction;
trap36	Mediator complex subunit 4	NONE
fd3f	forkhead domain 3F	NONE
cg2678	CG2678	;transcription;
Elongin-C	Elongin C	;transcription;

B

Worm Gene	Fly Gene	Description	GO Special
smo-1	smt3	smt3	;protein transport;metabolic process;
lin-53	Caf-1	Chromatin assembly factor 1 subunit	;DNA metabolic process;metabolic process;transcription;DNA repair;
dpl-1	Dp	DP transcription factor	;cell cycle;transcription;
efl-1	E2f	E2F transcription factor	;DNA replication;transcription;
lin-35	Rbf1	Retinoblastoma-family protein	;DNA replication;cell cycle;transcription;DNA metabolic process;
lin-13	CG7987	CG7987	;metabolic process;transcription;
ssl-1	dom	domino	;DNA replication;DNA metabolic process;response to DNA damage stimulus;DNA repair;metabolic process;DNA recombination;
epc-1	E(Pc)	Enhancer of Polycomb	;DNA metabolic process;
gap-1	Gap1	GTPase-activating protein 1	;signal transduction;
hpl-2	HP1b	HP1b	;DNA metabolic process;
let-418	Mi-2	Mi-2	;DNA metabolic process;transcription;metabolic process;
lin-8	Map60	Microtubule-associated protein 60	;cell organization and biogenesis;
hda-1	Rpd3	Rpd3	;metabolic process;transcription;DNA metabolic process;

C

Fly Gene	Description	GO Special
mor	moira	;DNA metabolic process;
dsd	distracted	NONE
stg	string	;cell cycle;protein amino acid dephosphorylation;
CG11652	CG11652	NONE
CG11798	charlatan	;transcription;
eff	effete	;metabolic process;cell organization and biogenesis;cell cycle;
Lk6	Lk6	;signal transduction;cell organization and biogenesis;protein amino acid phosphorylation;
CG8219	CG8219	;protein transport;
fray	frayed	;metabolic process;signal transduction;protein amino acid phosphorylation;
HSRomega	Heat shock RNA omega	goProcess
olf413	olf413	nuclear speck organization and biogenesis;response to heat;oogenesis (sensu Insecta);protein localization;
RhoGAP68F	RhoGAP68F	histidine catabolic process;catecholamine metabolic process;synaptic transmission;
mbc	myoblast city	cytoskeleton organization and biogenesis;cell motility;intracellular signaling cascade;
CG31475	CG31475	phagocytosis;intracellular signaling cascade;intracellular protein transport;cell morphogenesis;larval visceral muscle development;striated muscle development;myoblast fusion;mesoderm development;dorsal closure;
CG8669	CG8669-PA, isoform A	calcium-mediated signaling;
NinaE	neither inactivation nor afterpotential E	molting cycle (sensu Insecta);regulation of transcription, DNA-dependent;regulation of transcription;pupation;pupariation;metamorphosis (sensu Insecta);
sqd	squid	rhabdomere development;adult locomotory behavior;G-protein coupled receptor protein signaling pathway;phototransduction;visual perception;sensory perception;protein-chromophore linkage;signal transduction;photoreceptor cell morphogenesis (sensu Endopterygota);
CG4799	Pendulin	RNA export from nucleus;oocyte localization during oogenesis;mRNA catabolic process, nonsense-mediated decay;dorsal/ventral axis determination, follicular epithelium (sensu Insecta);follicle cell migration (sensu Insecta);oocyte anterior/posterior axis determination (sensu Insecta);egg chamber formation (sensu Insecta);intracellular mRNA localization;regulation of alternative nuclear mRNA splicing, via spliceosome;oogenesis (sensu Insecta);pole plasm mRNA localization;negative regulation of translation;pole plasm oskar mRNA localization;oocyte microtubule cytoskeleton organization (sensu Insecta);dorsal/ventral pattern formation;mRNA export from nucleus;

D

Fly Gene	Description	GO Special
trbl	tribbles	;signal transduction;protein amino acid phosphorylation;
pnut	peanut	;cell organization and biogenesis;cell cycle;
tara	taranis	;DNA metabolic process;
kis	kismet	;DNA metabolic process;
Dip3	Dorsal interacting protein 3	NONE
CG12284	thread	;metabolic process;
CG7231	CG7231	NONE
csw	corkscrew	;signal transduction;protein amino acid dephosphorylation;
ptc	patched	;metabolic process;protein catabolic process;signal transduction;
CG30497	CG30497	NONE
RhoBTB	RhoBTB	;signal transduction;protein transport;cell cycle;cell organization and biogenesis;
fax	failed axon connections	NONE
argos	argos	;cell organization and biogenesis;
mbf1	multiprotein bridging factor 1	NONE
EP3208	bantam	NONE
Sin3A	Sin3A	;metabolic process;
tkv	thickveins	;signal transduction;protein amino acid phosphorylation;
Ras85D	Ras oncogene at 85D	;signal transduction;transport;cell organization and biogenesis;protein transport;
spin	spinster	;transport;
CtBP	C-terminal Binding Protein	;metabolic process;
Act42A	Actin 42A	;cell organization and biogenesis;
Rm62	Rm62	;metabolic process;
Cdk4	Cyclin-dependent kinase 4	;cell cycle;signal transduction;cell organization and biogenesis;protein amino acid phosphorylation;
Acf1	ATP-dependent chromatin assembly factor large subunit	;DNA metabolic process;
abl	Abl tyrosine kinase	;cell organization and biogenesis;signal transduction;protein amino acid phosphorylation;
GlyP	Glycogen phosphorylase	;carbohydrate metabolic process;
Hsp27	Heat shock protein 27	;response to stress;metabolic process;
DNAJ-1	DnaJ-like-1	;response to stress;metabolic process;

ttk	tramtrack	;transcription;DNA metabolic process;
eas	easily shocked	;metabolic process;
CG6353	CG6353	NONE
osa	osa	;transcription;signal transduction;DNA metabolic process;
Alh	CG1070-PC, isoform C	NONE
lolal	lola like	;DNA metabolic process;
trx	trithorax	;DNA metabolic process;transcription;metabolic process;
dan	distal antenna	NONE
spi	spitz	;cell cycle;signal transduction;
shn	schnurri	;transport;signal transduction;
pAbp	polyA-binding protein	;metabolic process;RNA processing;
bhr	bhringi	NONE
Fmr1	Fmr1	;metabolic process;transport;
E(bx)	Enhancer of bithorax	;DNA metabolic process;transcription;signal transduction;
Hsc70-4	Heat shock protein cognate 4	;transport;metabolic process;response to stress;
eRF1	eukaryotic release factor 1	;protein transport;signal transduction;translation;
Pros25	Proteasome 25kD subunit	;protein catabolic process;
CG33967	CG33967	NONE
CG33967	CG33967	NONE
CG11324	homer	;signal transduction;
nuf	CG33991-PC, isoform C	;cell organization and biogenesis;
Elongin-B	Elongin B	;metabolic process;
CG5466	CG5466	NONE
Pk17E	Protein kinase-like 17E	;cell cycle;signal transduction;protein amino acid phosphorylation;
CG9520	CG9520	;metabolic process;
Atg1	Autophagy-specific gene 1	;protein amino acid phosphorylation;signal transduction;autophagy;
CG3885	CG3885	;transport;protein transport;
ld14	ld14	NONE
CG10346	Grip71	NONE
Rac2	Rac2	;signal transduction;cell organization and biogenesis;RNA localization;protein transport;
RpL19	Ribosomal protein L19	;translation;

CG11033	CG11033	;metabolic process;protein catabolic process;
mub	mushroom-body expressed	;signal transduction;RNA processing;protein transport;metabolic process;transcription;
CG8144	pasilla	;RNA processing;
CG5180	CG5180	NONE
CG17161	grapes	;response to DNA damage stimulus;cell cycle;signal transduction;cell organization and biogenesis;DNA metabolic process;protein amino acid phosphorylation;
Dap160	Dynamin associated protein 160	;transport;protein transport;
CG14478	CG14478	;DNA metabolic process;

E

Fly Gene	Description	GO Special
Arc105	Mediator complex subunit 15	NONE
cenG1A	centaurin gamma 1A	;signal transduction;
CG1855	CG11138	NONE
CG2540	CG2540	NONE
gus	gustavus	;signal transduction;
CG4641	CG4641	NONE
CG5731	CG5731	;carbohydrate metabolic process;
CG7379	CG7379	;cell cycle;
CG9331	CG9331	;metabolic process;carbohydrate metabolic process;
omd	oocyte maintenance defects	NONE
CG11248	CG11248	;protein transport;cell cycle;
CG11449	CG11449	NONE
CG13510	CG13510	NONE
CG14089	CG14089	;transport;
CG14425	CG14425	NONE
CG14982	CG14982	NONE
CG15006	CG15006	NONE
CG15167	CG15167	NONE
CG15634	CG15634	NONE
CG16719	CG16719	NONE
CG17159	CG40460-PC.3	NONE
CG17508	CG17508	NONE
CG31679	CG31679	NONE
dei	delilah	NONE

E5	E5	NONE
gt	giant	;transcription;signal transduction;
heph	hephaestus	;RNA processing;signal transduction;
Hil	Hillarin	NONE
His3.3A	Histone H3.3A	;DNA metabolic process;
CG11275	CG11275	NONE
klar	klarsicht	;cell organization and biogenesis;
lwr	lesswright	;metabolic process;protein transport;
Mtl	Mig-2-like	;signal transduction;cell cycle;cell organization and biogenesis;
PHDP	Putative homeodomain protein	NONE
ple	pale	;metabolic process;signal transduction;
pnr	pannier	;transcription;signal transduction;metabolic process;
Poxm	Pox meso	;signal transduction;transcription;
RpS18	Ribosomal protein S18	;translation;
SP59	CG11824	;protein catabolic process;
squ	squash	NONE
Rac1	Rac1	;signal transduction;cell organization and biogenesis;

TABLE S3

Some of the predicted interacting nodes genetically interacts with *ISWI*

Alleles corresponding to genes picked in the fly "Neuronal Morphogenesis" (**A**) and the worm "Multiple Cell Fate" (**B**) screens and the "Connecting" nodes (**C**), genetically interacted with the *ISWI-EGUF* or the *ISWI^{K159R}* eye assays. *ISWI-EGUF* and *ISWI^{K159R}* genetic interaction classes are shown in separate columns for every gene locus listed.

A

Gene	Cyto Map	Allele	Stock	*ISWI-EGUF*	*ISWI^{K159R}*
asf1	76B9-76B9	Asf1^{1}	9547 (Bloom)	En(ISWI)	-
brm	72C1-72C1	brm^{2}	3619 (Bloom)	-	-
		brm^{2}	3622 (Bloom)	-	-
E(z)	67E5-67E5	E(z)^{EY21318}	22471 (Bloom)	-	-
gro	96F10-96F10	gro^{1}	511 (Bloom)	En(ISWI)	++
		gro^{DG12307}	20592 (Bloom)	Su(ISWI)	-
kni	77E3-77E3	kni^{10}	3307 (Bloom)	-	-
		kni^{ri-1}	566 (Bloom)	-	-
		kni^{d10922}	19340 (Bloom)	En(ISWI)	-
sens	70A8-70A8	sens^{E2}	5311 (Bloom)	En(ISWI)	++
		sens^{Ly-1}	8136 (Bloom)	-	++
		sens^{f06181}	18946 (Bloom)	-	-
snr1	83A4-83A4	Snr1^{01319}	11529 (Bloom)	En(ISWI)	-
Stat92E	92F1-92F1	Stat92E^{06346}	11681 (Bloom)	En(ISWI)	-
		Stat92E^{DG17607}	21574 (Bloom)	En(ISWI)	-
		Stat92E^{HJ}	24510 (Bloom)	En(ISWI)	-
Su(z)12	76D4-76D4	Su(z)^{123}	5068 (Bloom)	En(ISWI)	++
tgo	85C2-85C2	tgo^{1}	9588 (Bloom)	En(ISWI)	++

		tgo^{EY03802}	15914 (Bloom)	-	-
EcR	42A9-42A12	EcR^{06410}	11770 (Bloom)		-
aop	22D1-22D1	aop^{03953a}	11359 (Bloom)		-
		aop^{1}	3101 (Bloom)		-
		aop$^{yan-XE18}$	8777 (Bloom)		-
Tab2	56C8-56C9	Tab2^{201Y}	4440 (Bloom)		++
esc	33A2-33A2	esc^{1}	813 (Bloom)		
		esc^{21}	3623 (Bloom)		
Elongin-C	56D7-56D7	Elongin-C^{e01107}	17924 (Bloom)		
Bap55	54B7-54B7	Bap55^{EY15967}	21174 (Bloom)		+++
sna	35D2-35D2	sna^{18}	2311 (Bloom)		
		sna^{19}	101332 (DGRC)		
run	19E2-19E2	run^{2}	8128 (Bloom)		+
		run^{3}	4496 (Bloom)		
		run^{29}	4497 (Bloom)		+
ci	102A1-102A3	ci^{1}	642 (Bloom)		+
		ci^{36}	643 (Bloom)		+++
		ci^{57}	644 (Bloom)		+
		ci^{W}	646 (Bloom)		

B

Gene	Cyto Map	Allele	Stock	*ISWI-EGUF*	*ISWI^{K159R}*
Caf-1	88E3-88E3	Caf1^{DG25308}	21275 (Bloom)	En(ISWI)	+
E2f	93E9-93F1	E2f^{07172}	11717 (Bloom)	En(ISWI)	++
		E2f^{f2}	7274 (Bloom)	En(ISWI)	++
CG7987	88C6-88C6	CG7987^{EY22125}	22538 (Bloom)	En(ISWI)	-
Gap1	67C10-67C11	Gap1^{EY00011}	14996 (Bloom)	-	+
Mi-2	76D3-76D4	Mi-2^{S000606}	l(3)S000606 (Szeg)	-	-
Rpd3	64B12-64B12	Rpd3^{04556}	11633 (Bloom)	En(ISWI)	+++
smt3	27C7-27C7	smt3^{04493}	11378 (Bloom)		+
Map60	45F5-45F6	Map60^{KG00506}	14880 (Bloom)		++
dom	57D11-57D12	dom^{9}	9261 (Bloom)		-
		dom^{EY01281}	15337 (Bloom)		+
E(Pc)	47F13-47F14	E(Pc)^{EP608}	17286 (Bloom)		+
Rbf	1C5-1C5	Rbf^{14}	7435 (Bloom)		-
		Rbf^{EY07209}	16803 (Bloom)		+

C

Gene	Cyto Map	Allele	Stock	*ISWI-EGUF*	*ISWI^{K159R}*
CG7379	90C5-90C5	CG7379	14430 (Bloom)	En(ISWI)	+++
dei	97B2-97B2	dei^{e01478}	17955 (Bloom)	-	-
heph	100D3-100E1	heph^{2}	635 (Bloom)	En(ISWI)	-
		heph^{PL00536}	19538 (Bloom)	-	-
klar	61C1-61C3	klar^{1}	3330 (Bloom)	-	-
Mtl	98A13-98A13	Mtl^{EP855}	17297 (Bloom)	En(ISWI)	++
		Mtl^{f07113}	19043 (Bloom)	-	+
		Mtl$^{\Delta}$	6679 (Bloom)	-	-
ple	65C3-65C3	ple^{4}	3279 (Bloom)	-	-
		ple^{f01945}	18492 (Bloom)	-	+
pnr	89A13-89B1	pnr^{1}	3106 (Bloom)	-	-
		pnr^{MD237}	3039 (Bloom)	En(ISWI)	-
Rac1	61F5-61F5	Rac1^{J10}	6679 (Bloom)	-	-
CG5731	31B1-31B1	CG5731^{KG05192}	13881 (Bloom)		-
CG9331	38F1-38F1	CG9331^{EY06494}	16397 (Bloom)		+
cenG1AE	34D6-34E2	cenG1A^{EY10943}	20232 (Bloom)		-
squ	36A10-36A10	squ^{PP32}	5114 (Bloom)		+
gt	3A3-3A3	gt^{X11}	1530 (Bloom)		-

CG14425-Sxl	**6F3-6F5**	**CG14425-Sxl**	**17354 (Bloom)**	+