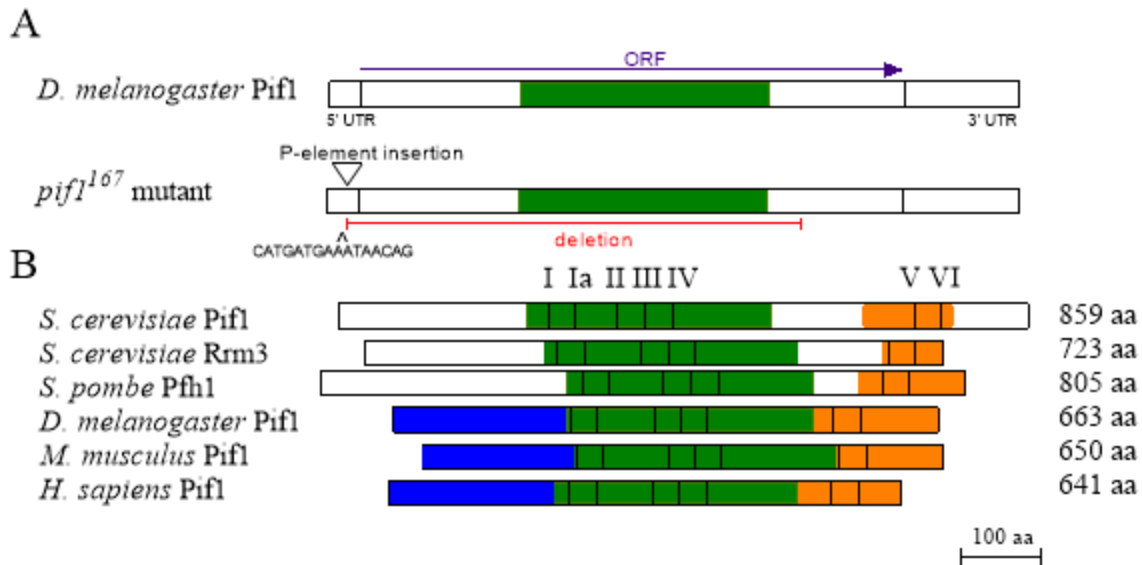


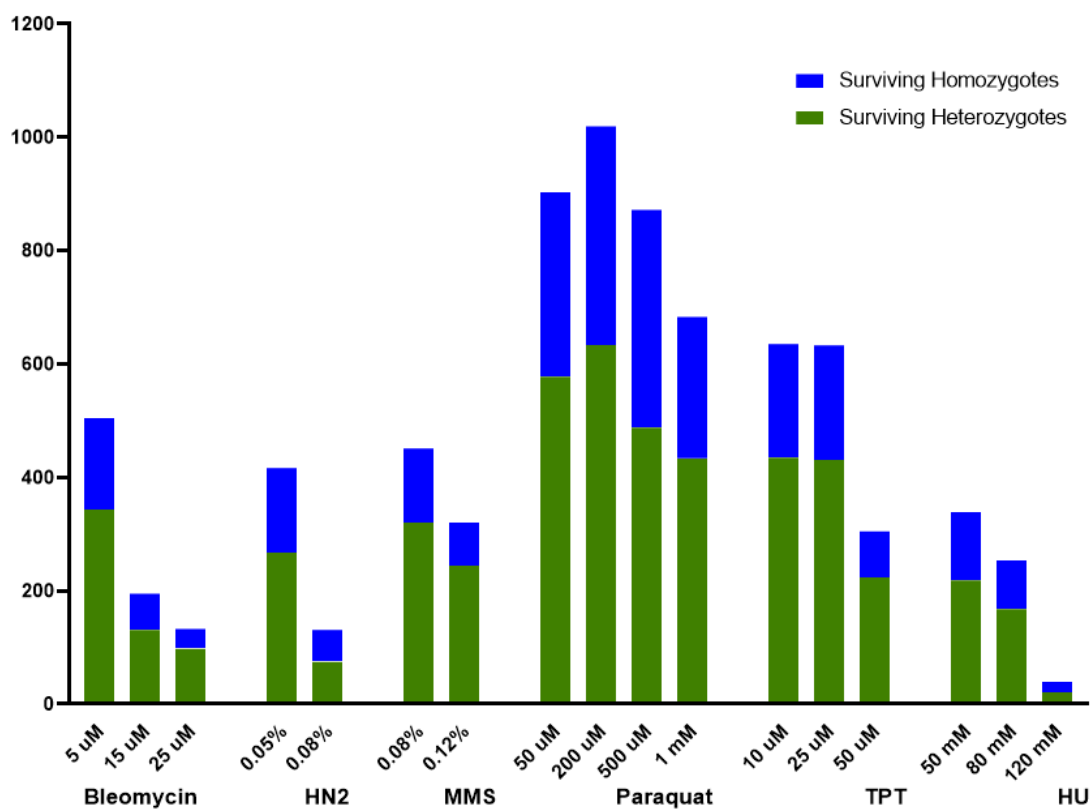
**Kocak *et al.***

**The *Drosophila melanogaster* PIF1 helicase promotes survival during replication stress  
and processive DNA synthesis during double-strand gap repair**

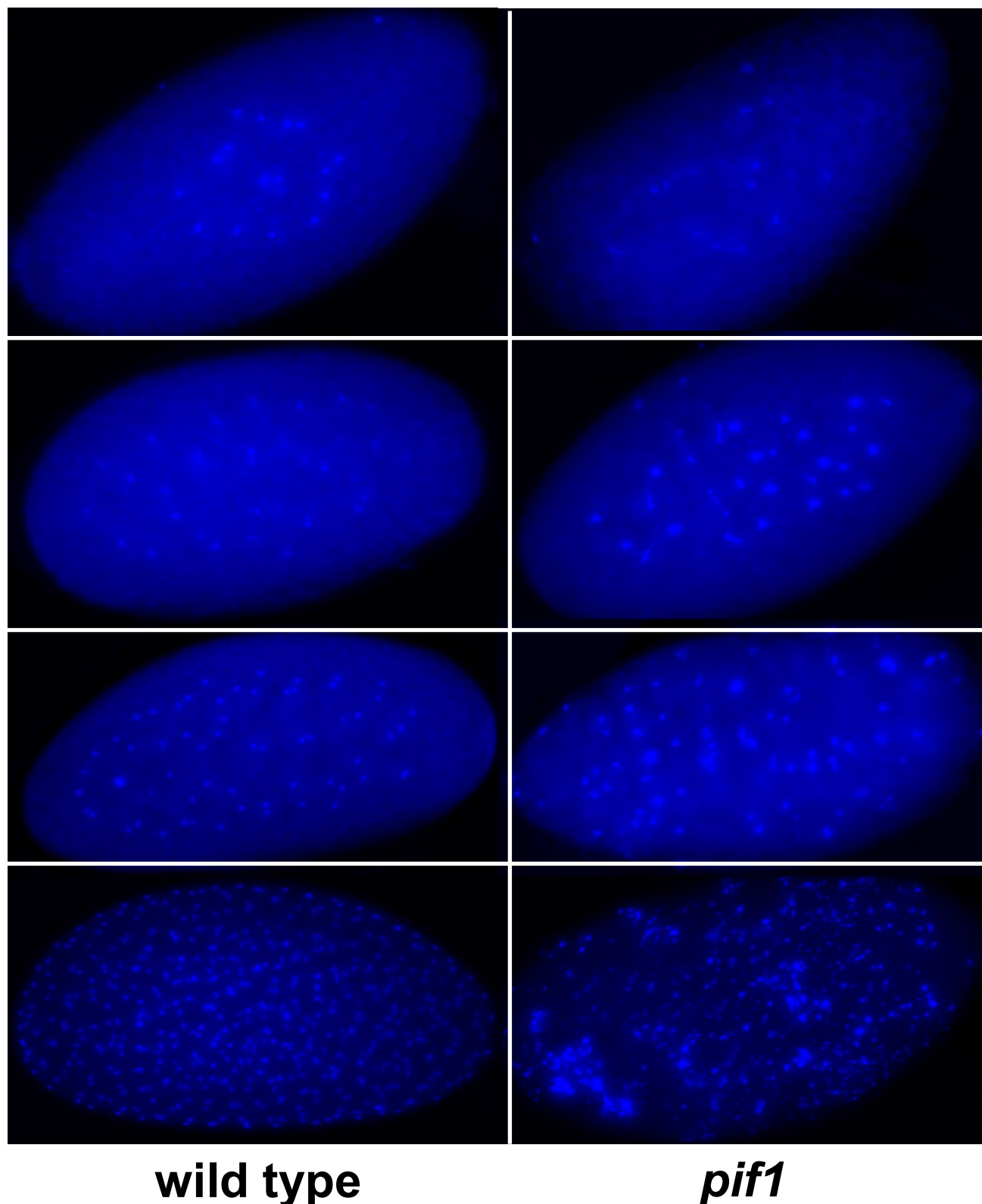
**Supplemental Figures 1-5**



**Figure S1** – Creation of a *Drosophila pifl* mutant. (A) A large deletion that removes most of the *PIF1* coding sequence was created through imprecise excision of a *P* element inserted in the 5' UTR. The extent of the deletion is indicated by a red bar. A carat indicates an accompanying insertion at the deletion site. (B) Alignment of PIF1 orthologs from multiple species. The conserved helicase domain is shown in green, the conserved N-terminus in shown in blue, and the conserved C-terminal is shown in orange. The seven conserved helicase motifs are indicated in roman numerals and represented by black bars.



**Figure S2** – Numbers of surviving adults (both *pif1* heterozygotes and homozygotes) for increasing doses of bleomycin, nitrogen mustard (HN2), methyl methane sulfonate (MMS), paraquat, topotecan (TPT) and hydroxyurea (HU).



**Figure S3:** DAPI-stained embryos, showing defects in *pif1* mutants occur very early in embryogenesis.

$\text{♀♀ } y,w; pif1 \quad \times \quad \text{♂♂ } \frac{y,w^a, Ste^1}{B^s Y y^+}$			
↓			
Normal progeny (X chromosome genotype)		Nondisjunction progeny (X chromosome genotype)	
♀♀	♂♂	♀♀	♂♂
$\frac{y,w}{y,w^a, Ste1}$	$\frac{y,w}{B^s Y y^+}$	$\frac{y,w/y,w}{B^s Y y^+}$	$\frac{y,w^a, Ste^1}{\emptyset}$
WT 305	144	0	1
<i>pif1</i> 32	13	0	1
NDJ for WT = $(1/450) \times 2 = 0.44\%$ NDJ for <i>pif1</i> = $(1/46) \times 2 = 4.4\%$ P = 0.0588 (Chi-Square test)			

**Figure S4:** *pif1* mutants have a non-significant increase in X chromosome nondisjunction. Wild type (WT) or *pif1* mutant females were mated to males of the indicated genotype. Gametes from females in which X chromosome nondisjunction occurred will be *yw/yw* or nullo-X. Fertilization of the *yw/yw* egg by a Y-bearing sperm will result in females with Bar-shaped eyes and wild-type body color. Fertilization of the nullo-X egg by an X-bearing sperm will result in males with normal-shaped eyes and yellow bodies. The frequency of non-disjunction is calculated by dividing the number of nondisjunction progeny by the total number of progeny and multiplying by 2 to account for the lethal combinations.

♀♀	<u><i>pif1</i><sup>167</sup></u> ; <u><i>rad51</i><sup>1057</sup></u>	x	♂♂	<u><i>pif1</i><sup>167</sup></u> ; <u><i>rad51</i><sup>1057</sup></u>
	<u>CyO</u> ; <u>TM3,Sb</u>			<u>CyO</u> ; <u>TM3,Sb</u>
	↓			
	<i>pif1</i> ; <i>rad51</i>	<i>pif1</i> ; <u><i>rad51</i></u>	<u><i>pif1</i></u> ; <i>rad51</i>	<u><i>pif1</i></u> ; <u><i>rad51</i></u>
		<u>TM3</u>	<u>CyO</u>	<u>CyO</u> <u>TM3</u>
Observed	19	63	35	52
Expected	19	38	38	75

♀♀	<u><i>pol32</i><sup>L2</sup></u> ; <u><i>rad51</i><sup>1057</sup></u>	x	♂♂	<u><i>pol32</i><sup>L2</sup></u> ; <u><i>rad51</i><sup>1057</sup></u>
	<u>CyO</u> ; <u>TM3,Sb</u>			<u>CyO</u> ; <u>TM3,Sb</u>
	↓			
	<i>pol32</i> ; <i>rad51</i>	<i>pol32</i> ; <u><i>rad51</i></u>	<u><i>pol32</i></u> ; <i>rad51</i>	<u><i>pol32</i></u> ; <u><i>rad51</i></u>
		<u>TM3</u>	<u>CyO</u>	<u>CyO</u> <u>TM3</u>
Observed	8	14	47	31
Expected	11	22	22	44

**Figure S5:** Mutation of RAD51 is not lethal in a *pif1* or *pol32* mutant background. Females and males heterozygous for the indicated mutations were mated and the progeny that survived to adulthood were recorded.