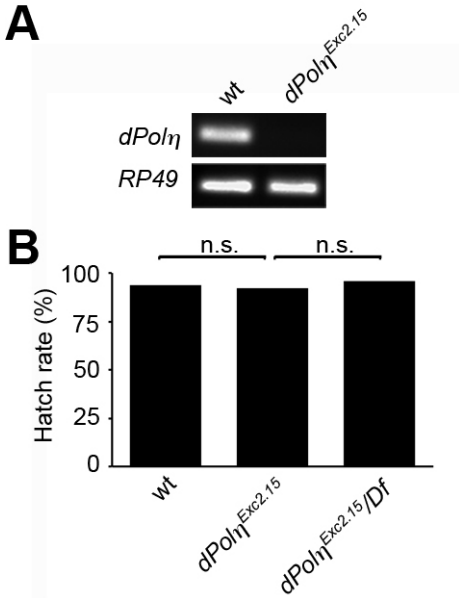

Figure S1. RT-PCR and hatch rates of *dPolη*-derived embryos. (A) RT-PCR confirming loss of *dPolη* expression in *dPolη*-derived embryos. *RP49* served as a loading control. (B) Bar graph showing hatch rates of *dPolη*-derived embryos are similar to that of wild-type embryos. *Df=Df(3L)BSC284*.

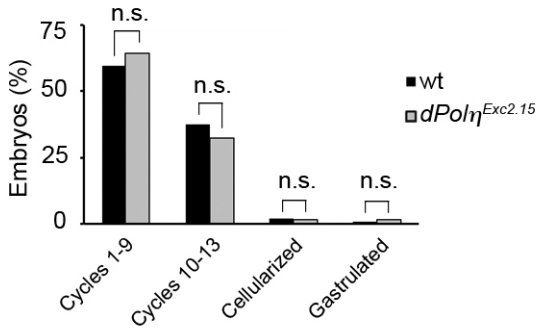

Figure S2. Stage distribution of *dPolη*$^{Exc2.15}$ embryo collections. Embryos (0-2 hours) collected from females of indicated genotypes were fixed and stained for α-tubulin and DNA to assess developmental stage. *dPolη*$^{Exc2.15}$-derived embryos progressed through syncytial development at a rate similar to that of wild-type embryos. n.s. indicates non-significance.

Sensitivity to hydroxyurea			
	% homozygotes/hemizygotes		
Genotype	-HU	+HU	*p*-value
mei-41^{RT1}	52.6 (435)	33.0 (299)	<0.0001
dPolη$^{Exc2.15}$	29.5 (298)	19.6 (138)	<0.05
dPolη$^{Exc2.15}$*/Df*	34.8 (322)	12.5 (178)	<0.0001

Figure S3. Sensitivity of *dPolη* mutants to hydroxyurea (HU). The following mating crosses were performed (10 males and 10 virgin females per vial): *mei-41*/Y males x *mei-41/FM7a* females, *dPolη*$^{Exc2.15}$*/TM3* males x *dPolη*$^{Exc2.15}$*/TM3* females, and *Df/TM3* males x *dPolη*$^{Exc2.15}$*/TM3* females. After 48 hours of egg laying, adults were removed from vials. Larvae were grown on food ± 20 μM HU and allowed to develop for 2 weeks. Numbers of adult progeny were scored. Data are represented as the ratio of homozygous or hemizygous adult progeny to total adult progeny expressed as a percentage. Expected Mendelian ratios were 50% and 33% for *mei-41* (X chromosome) and *dPolη* (3rd chromosome) homozygotes/hemizygotes, respectively. The total number of adult flies scored is shown in parentheses. *p*-values were calculated using a two-tailed Fisher's exact test. *Df=Df(3L)BSC284*.

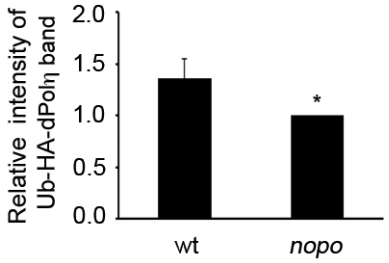

Figure S4. Quantification of ubiquitylated HA-dPolη in embryos. Representative immunoblot from His-ubiquitin assays in *Drosophila* embryos is shown in Fig. 7B. Quantification of the intensity of the major ubiquitylated HA-dPolη band for each genotype was performed using ImageJ with normalization to the intensity of the corresponding total HA-dPolη band on immunoblots of embryonic lysates. A small but significant reduction in the intensity of the major ubiquitylated HA-dPolη band was observed in *nopo*-derived embryos compared to wild-type embryos. Asterisk, $p<0.05$, paired Student's t-test, n=5 experiments. Data are shown as mean ± s.e.m.

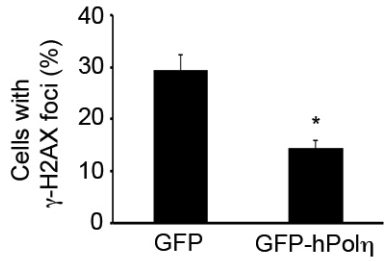

Figure S5. Overexpression of GFP-hPOLη does not increase double-stranded break formation. HeLa cells were transfected as indicated, fixed, and stained for gamma-H2AX. Quantification of gamma-H2AX foci revealed that overexpressing GFP-hPOLη decreases the percentage of gamma-H2AX-positive cells. Asterisk, $p<0.01$.

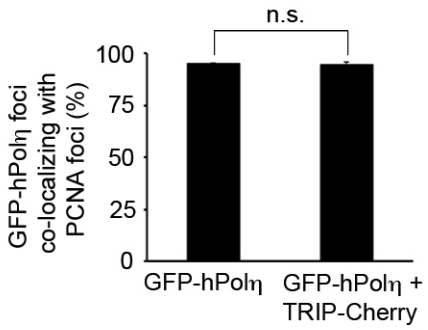

Figure S6. Co-localization frequency of GFP-hPOLη foci with PCNA foci. HeLa cells were transfected as indicated, fixed, and stained for PCNA (endogenous). Quantification of GFP-hPOLη foci revealed that co-expression of TRIP-mCherry does not alter the frequency of co-localization of GFP-hPOLη foci with PCNA foci. n.s. indicates non-significance.

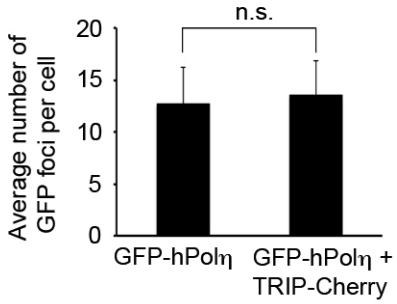

Figure S7. TRIP does not alter the average number of hPOLη nuclear foci per cell. HeLa cells were transfected as indicated and fixed. Quantification of GFP-hPOLη foci revealed that co-expression of TRIP-mCherry has no effect on the average number of GFP-hPOLη foci per cell.

Table S1. Primers used for RT-PCR.

Gene	Primer sequences
RP49	5′-TCCTTCCAGCTTCAAGATGACC-3′ 5′-CTTGGGCTTGCGCCATTTGTG-3′
nopo	5′-CATCAGCAGCTATGTCGAGCA-3′ 5′-GAAGATTGAATACTTTTCACTGAGATC-3′
hTRIP	5′-GCTCTTCTTTGATCTTGCCCA-3′ 5′-CCACAGGAAGGTGTCCAGCTTGGCCT-3′
dPolη	5′-AGCCAGGTTCCATTCTTCTCGTCA-3′ 5′-AATTCGGTGAGGTGGTGTGTGAGA-3′
dPolι	5′-CCAATATCCGTGCGTACCGATGTGGA-3′ 5′-CGAGTCGAACTTGCGCAACAC-3′
dRev1	5′-CGATCTGGCACACGAACTCAATGT-3′ 5′-TGCTCGAATCCAACAAGAACTGGC-3′

Table S2. Primers used for generating DNA constructs.

Insert*	Primer sequence
nopo genomic region	5′-TTTTGGCACAAAT-3′ 5′-GTCCACAGCCATG-3′
NOPO	5′-ATGTTGAACTTAAACT-3′ 5′-CTTATTTGCCCAGGTTA-3′
TRIP	5′-ATGCCTATCCGTGCTC-3′ 5′-CTCAACGACCACAGGA-3′
hPolη	5′-ATGGCTACTGGACAGGATCG-3′ 5′-CTAATGTGTTAATGGCTTAAAAAATGATTCCAATG-3′
N-hPolη	5′-ATGGCTACTGGACAGGATCG-3′ 5′-TTACTAATTGCAACAGCCACCATTGTAC-3′
C-hPolη	5′-ATGGTACAATGGTGGCTGTTGCAATTAG-3′ 5′-CTAATGTGTTAATGGCTTAAAAAATGATTCCAATG-3′
hPolκ	5′-ATGGATAGCACAAAGGAGAAGTG-3′ 5′-CTTTTTGTTCTTGTTACAGCCTTCTG-3′
N-hPolκ	5′-ATGGATAGCACAAAGGAGAAGTG-3′ 5′-TTACACATTCTTCAACTTAATGGTAACAGTTCTACC-3′
C-hPolκ	5′-GATCGGCCGGCCAATGGGTAGAACTGTTACCATTAAGTTGAA GAATGT-3′ 5′-GACTGGCGCGCCCTTTTTGTTCTTGTTACAGCCTTCTG-3′
dPolη	5′-ATGTCCAGCGCACGCA-3′ 5′-CTAATTGCTTTGGCTGAAAAACTGGG-3′
N-dPolη	5′-ATGTCCAGCGCACGCA-3′ 5′-TTACACTAATGCCAAGGAACTTGATAGC-3′
C-dPolη	5′-ATGGCTATCAAGTTCCTTGGCATTAGTG-3′ 5′-CTAATTGCTTTGGCTGAAAAACTGGG-3′
dPolι	5′-ATGGACTTCGCTAGCGTACTC-3′ 5′-TCACTTATTCCGGAGAAAGTAGCG-3′
N-dPolι	5′-ATGGACTTCGCTAGCGTACTC-3′ 5′-TTAGTTGTACCTTGCTCACACCAGTTTC-3′
C-dPolι	5′-ATGGAAACTGGTGTGAGCAAGGTACAA-3′ 5′-TCACTTATTCCGGAGAAAGTAGCG-3′

*Protein (full-length or fragment) encoded by cDNA insert is listed unless otherwise indicated.