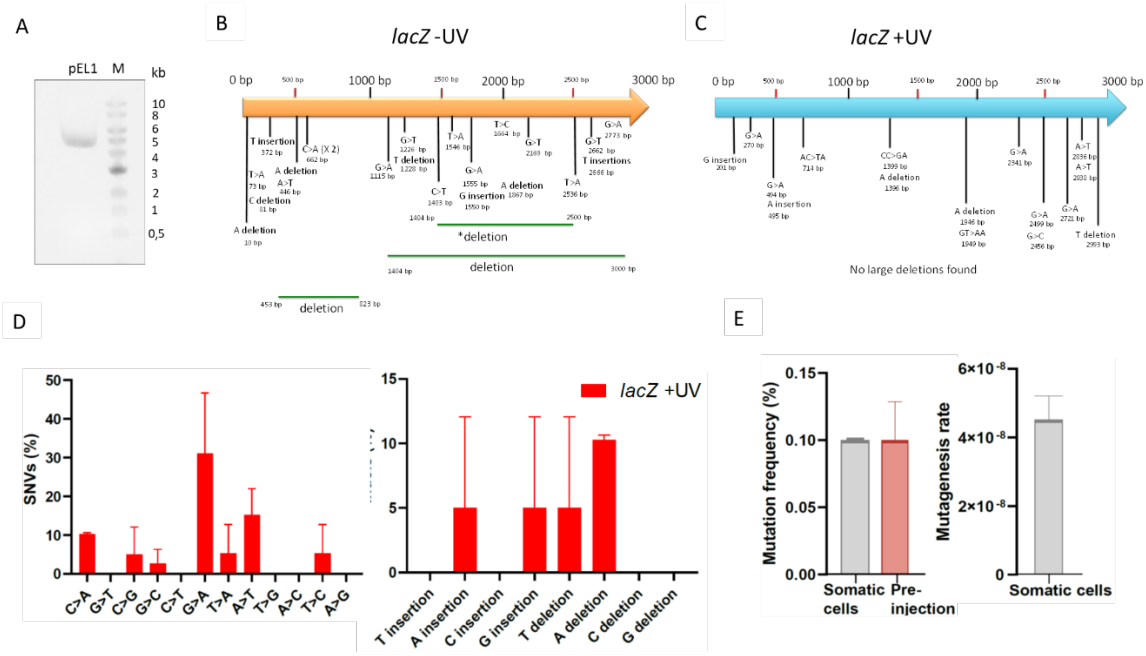
Translesion DNA synthesis-driven mutagenesis in very early embryogenesis of fast cleaving embryos

Elena Lo Furno[1], Isabelle Busseau[2], Antoine Aze[1], Claudio Lorenzi[3], Cima Saghira[4], Matt C Danzi[4], Stephan Zuchner[4] and Domenico Maiorano[1*]

Supplementary Material

Supplementary material includes 8 supplementary Figures S1-8.



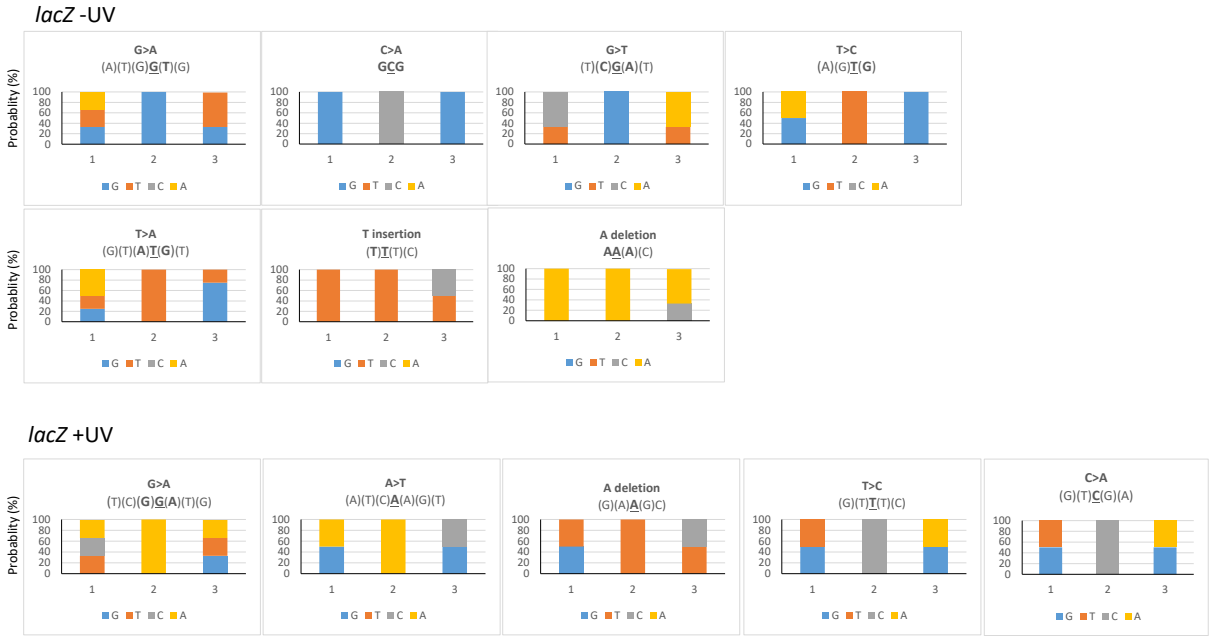
Supplementary Figure S1. Mutation maps and SNVs in early *Xenopus* embryos

(A) Agarose gel electrophoresis of *lacZ*-containing plasmid (pEL1). M indicates DNA ladder molecular weight marker.

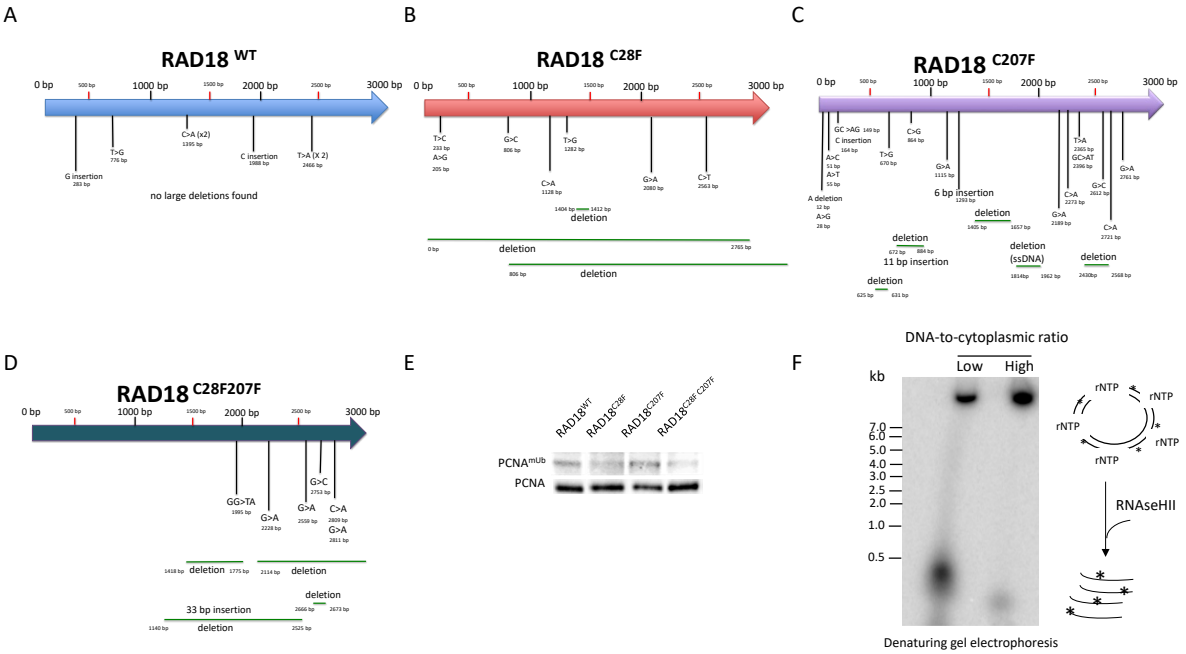
(B-C) Mutations map (SNVs, insertions, deletions) on the *lacZ* gene recovered from pre-MBT embryos irradiated (+UV) or not (*lacZ*) with UV-C (n=3).

(D) Mutation spectra of UV-irradiated *lacZ* gene recovered from *Xenopus* pre-MBT embryos after Sanger sequencing. Data are presented as means ± SD (n=2).

(E) Mutation frequency (left panel) and rate (right panel) of cultured somatic A6 *Xenopus* cells. Mutagenesis rate is expressed as mutations per base pair/locus per generation (see Materials and Methods), normalized to the pre-injection background values. Data are presented as means ± SD (n=2).



Supplementary Figure S2. Analysis of SNVs dinucleotide environment generated on a plasmid bearing the *lacZ* reporter gene not irradiated (-UV) or irradiated (+ UV) with UV-C recovered from *Xenopus* early embryos. The histogrammes represent the probability to find the indicated base at the positions immediately 5' (position 1) end 3' (position 3) of the SNV (position 2). A consensus is indicated above each histogram. Slashs separate alternate bases at each position while hyphens separate bases. DNA bases in bold highlight most abundant base at that position. Underlined and bold bases indicate the position where the change has occurred.



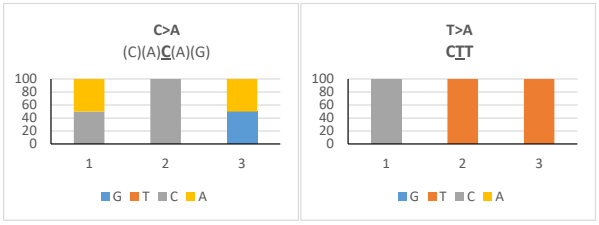
Supplementary Figure S3. Mutations map of *lac*Z co-injected with RAD18 mRNAs

(A-D) Mutations map of the *lacZ* gene recovered from pre-MBT embryos co-injected with the indicated Rad18 forms (n=3).

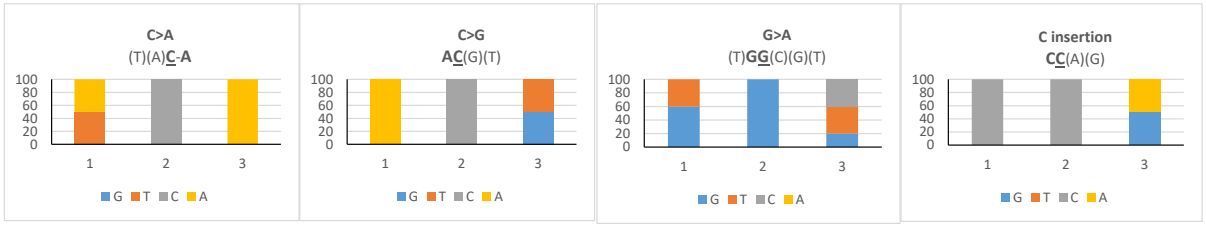
(E) Western blot of 16-cell *Xenopus* embryos protein extracts with either a $PCNA^{mUb}$- or a PCNA-specific antibody expressing the indicated Rad18 variants.

(F) High level of ribonucleotides incorporation during DNA synthesis at low DNA-to-cytoplasmic ratio. Autoradiograph of radioactive-labeled M13 ssDNA replicated in egg extracts at different doses: low (0.66 ng/μL^{-1}) for pre-MBT-like or high DNA-to-cytoplasmic ratio (6.6 ng/ μL^{-1}) for post-MBT-like dose, treated with NaOH and separated by denaturing urea-gel electrophoresis. Products were compared with DNA ladder of know size (n=2).

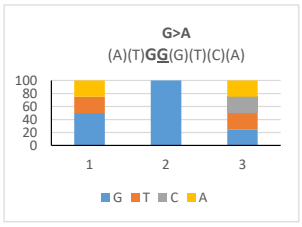
Rad18^{WT}



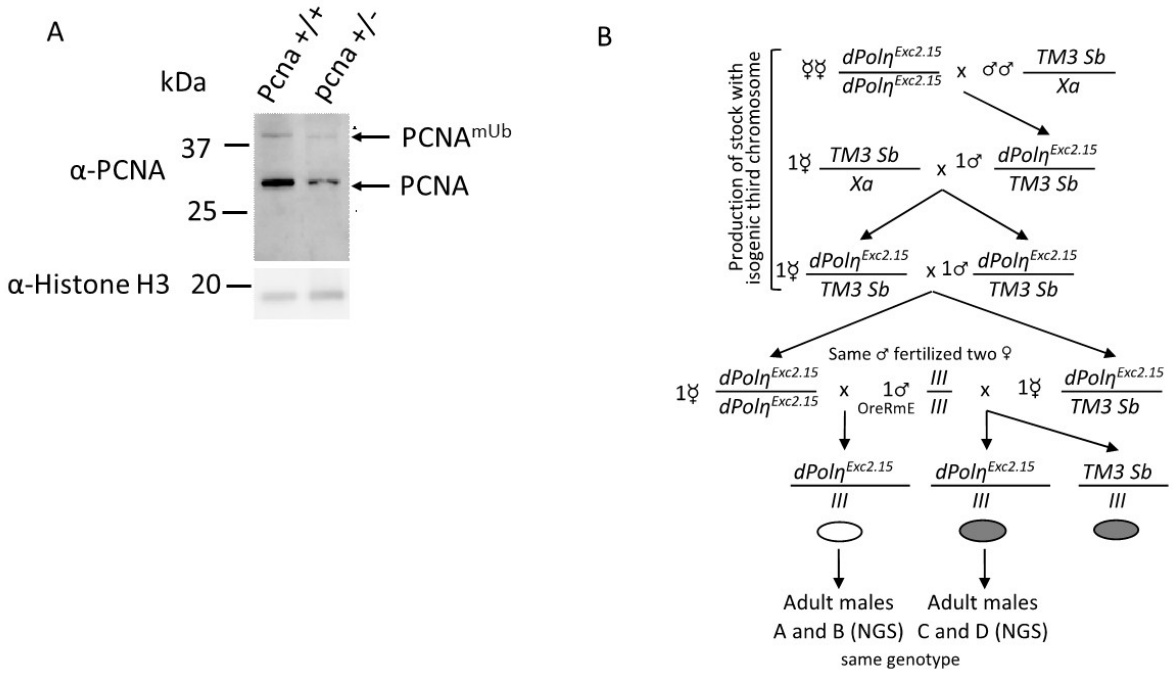
Rad18^{C207F}



Rad18^{C28FC207F}



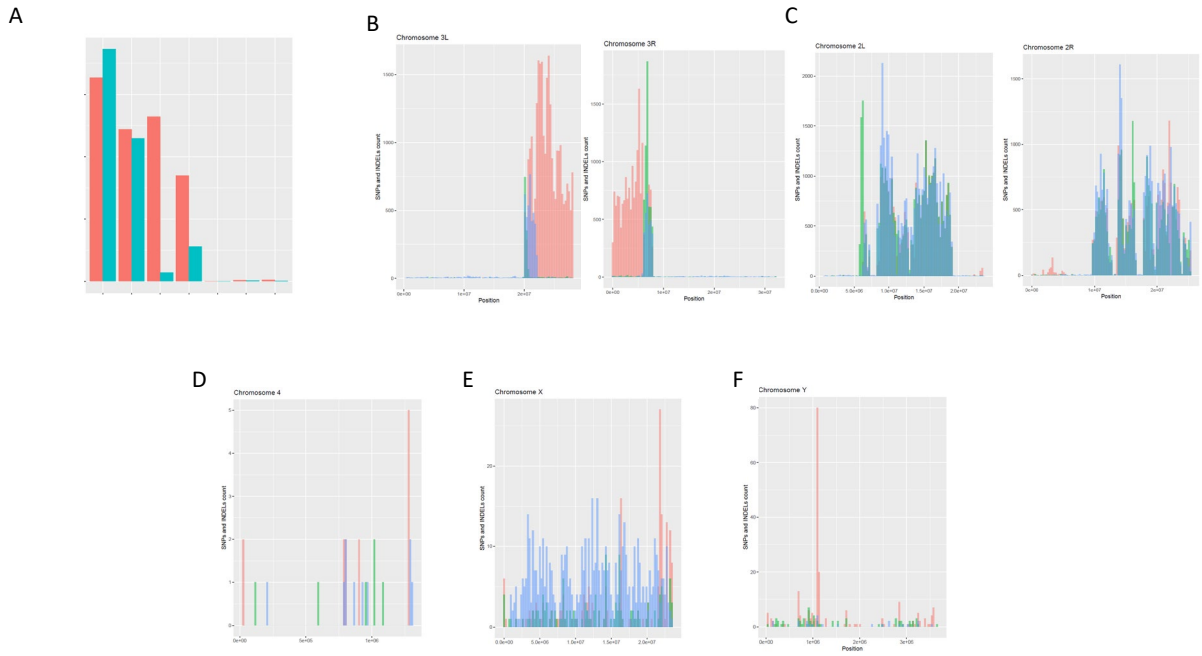
Supplementary Figure S4. Analysis of SNVs dinucleotide environment generated on a plasmid bearing the *lacZ* reporter gene recovered from *Xenopus* early embryos expressing the indicated mRNAs. The histogrammes represent the probability to find the indicated base at the positions immediately 5' (position 1) end 3' (position 3) of the SNV (position 2). A temptative consensus is indicated above each histogram. Slashs separate alternate bases at each position while hyphen separate bases. DNA bases in bold highlight most abundant base at that position.



Supplementary Figure S5. PCNA detection in *Drosophila* and genetic crosses used to generate maternally-depleted *dpolη* flies

(A) Western blot of total protein extracts obtained from flies of the indicated genotype with the PC10 antibody (n=2).

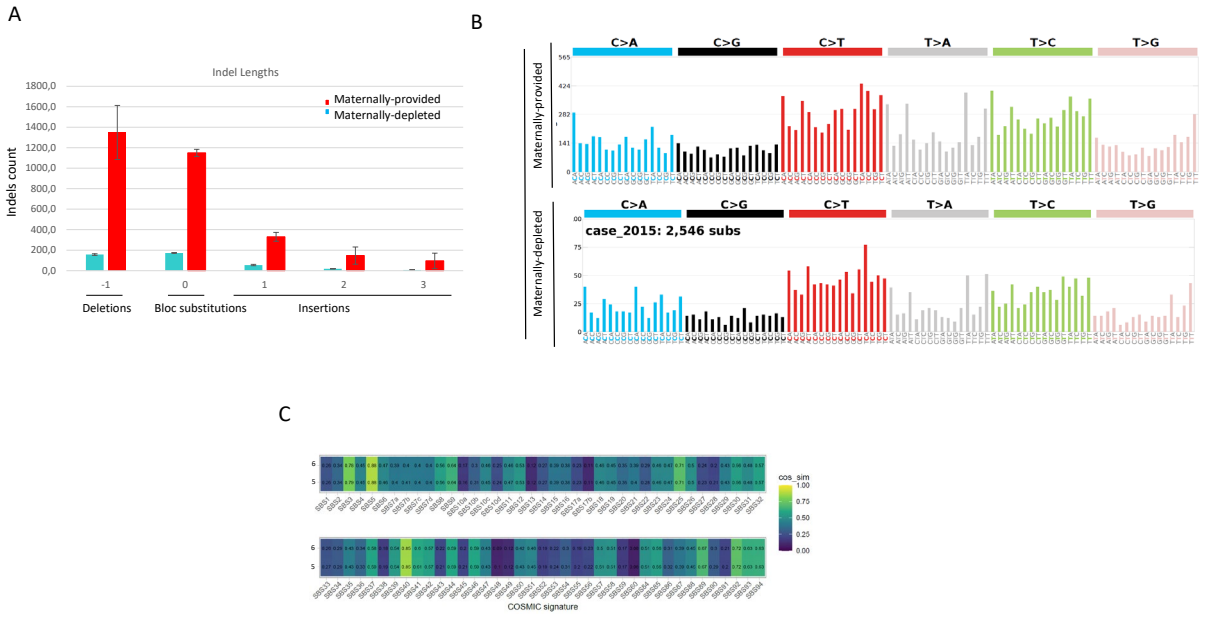
(B) Genetic crosses set up to generate either maternally-depleted or maternally-provided dPolη adult flies. The first two crosses generated a balanced stock over TM3 marked with Sb with a isogenic 3rd chromosome carrying the *dpolη*$^{Exc2.15}$ mutation. From this stock, one homozygous (left) and one hetreozygous (right) virgin females were collected and mated with the same wild type male, to produce embryos which were respectively depleted or not of maternal deposited dPolη. Then, genotypically identical adult males, devoid of the Sb marker, and containing the isogenic 3rd chromosome carrying the *dpolη*$^{Exc2.15}$ mutation, were collected for WGS processing and analysis.



Supplementary Figure S6. Distribution of mutations in the absence of maternal *dpol η* across chromosomes

(A) SNVs and Indels count on the indicated *Drosophila melanogaster* chromosomes.

(B-F) Mean of SNVs and Indels distribution on the indicated *Drosophila* chromosomes (n=2). The x-axis describes the position of counted variants on the chromosome and y-axis displays how many variants were counted on that position. Means were compared with an unpaired-two tailed Student's t test.

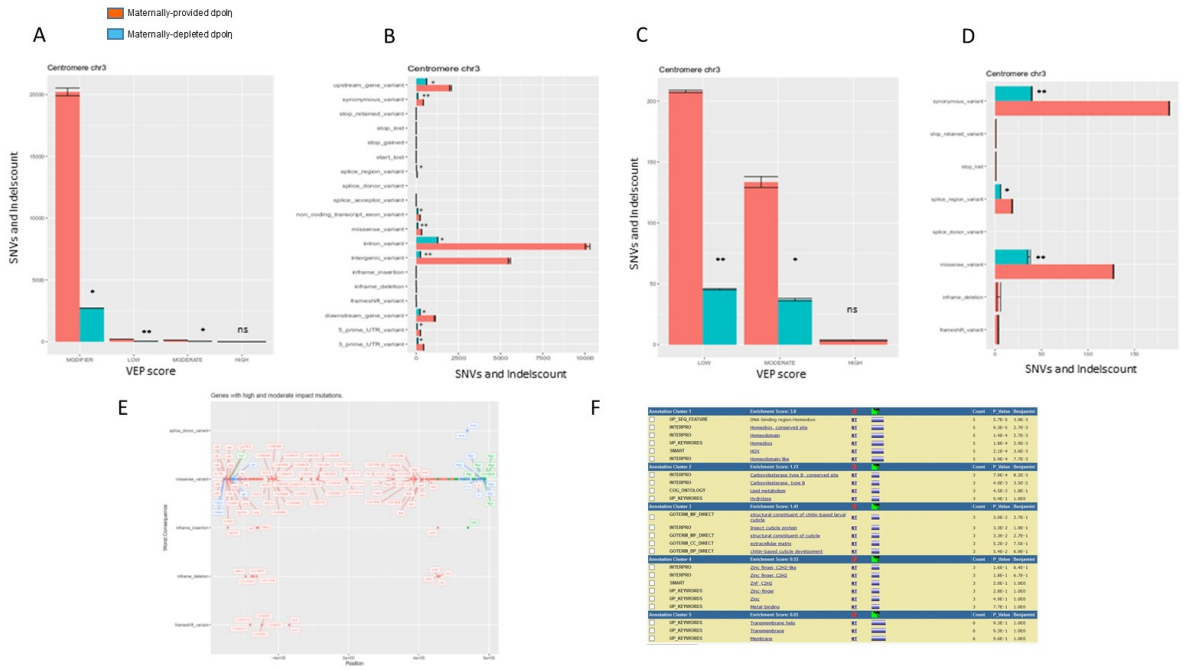


Supplementary Figure S7. Analysis of dPolη mutation signatures on the pericentromeric region of *Drosophila* chromosome 3.

(A) Statistical analysis of Indels length on the pericentromeric region of chromosome 3. The sizes refer to whether the variant is an insertion (positive numbers), deletion (negative numbers), or a block substitution (0).

(B) Average of number of single base substitutions in all possible trinucleotide contexts on the pericentromeric region of chromosome 3 in either *dpolη* maternally-provided or maternally-depleted flies.

(C) Heatmap showing the normalized relative contribution of dPolη mutational signatures from human tumors.



Supplementary Figure S8. Impact of dPolη maternal depletion on gene function

(A) Diagram classifying the impact of mutations found on chromosome 3L (left panel) and chromosome 3R (right panel). Mutations are classified according to their effect by attribution of a VEP score. These panels describe how many SNVs (y-axis) have high, low, moderate or modifier effect (x-axis) in each sequenced fly. Most mutations generated in maternally-provided dPolη flies do not particularly affect genes, transcripts, protein sequence, or regulatory regions (n=2).

(B) In this plot, types of variants with modifier VEP score are listed on the y-axis and the counts for the type of polymorphisms are shown on the x-axis. Most of mutations are slightly altering introns or intergenic regions (n=2).

(C) In this panel, modifier VEP scores have been discarded to focus on the consequences of other scored variants. Most polymorphisms have a moderate effect on altered gene (n=2).

(D) Evaluation of mutation impact upon discarding variants with modifier score. In this graph, types of variants with other scored are illustrated on the y-axis and the counts for each type of polymorphisms are shown on the x-axis. Most of SNVs give raise to missense variants (n=2).

Data are presented as means ± SD. Means were compared with an unpaired Student's t test.
(E) Graphic representation of SNVs consequences on genes found on the pericentromeric region of chromosome 3 in either dPolη maternally-provided (red) or maternally-depleted (green) flies. Common genes are indicated in blue.

(F) Gene ontology analysis of genes found on the pericentromeric region of chromosome 3.