Supplemental Data for Chen et al.: *Cutoff* and *Aubergine* mutations result in upregulation of retrotransposons and activation of a checkpoint in the *Drosophila* germline

Experimental Procedures

Fly Stocks

Cuff^{WM25}, *Cuff* ^{QQ37}, *Cuff*^{RI67}, *Cuff* ^{WL25}, *Cuff*^{RN48}, *aub*^{QC} and *aub*^{HN} were isolated in female sterile screens on the second chromosome and were further mapped to a small region on chromosome 2R [6, 7]. The original *cuff* mutations from the female sterile screen failed to complement *KG05951,* which was isolated by the BDGP Gene Disruption Project (Bloomington stock #14462), and corresponds to a P-element insertion in the N terminal coding region of the CG13190 gene. *Chk2* has been previously described [4, 39] ; *Mei41*^{D1} , *Mei41*^{D3} and *c(3)g* are gifts from Dr. Hawley [16]. *OreR, cn bw* or *cuff* heterozygous flies were used as wild type control. Marker mutations and balancers are described in flybase (http://flybase.org).

Whole Mount immunohistology staining, and in situ RNA hybridization

Antibody staining, RNA *in situ* hybridization and karyosome staining were performed as previously described [4, 24, 29, 40, 41]. Mouse anti Grk antibody 1D12 was used at 1:10, mouse anti alpha Spectrin antibody 3A9 at 1:100 (Developmental Studies Hybridoma Bank), mouse anti Fasciclin III antibody was used at 1:5 (Developmental Studies Hybridoma Bank), guinea pig anti C(3)G antibody [16] was used at 1:500, goat anti Vasa antibody (Santa Cruz Biotechnology) was

used at 1:100. Mouse anti HA antibody (Santa Cruz) was used at 1:500. Secondary antibodies and Hoechst (Invitrogen) were used at 1:1000.

Western Blot and Molecular techniques

Western Blots were performed as previously described [4, 29]. Goat anti Vasa antibody (Santa Cruz Biotechnology) was used at 1:100.

For RNA analysis, total RNA was isolated using Trizol (Invitrogen). Reverse Transcription (RT) was performed with the SuperScript™ III Reverse Transcriptase (Invitrogen). Subsequent PCR was performed with the following primers, 5'-ATGAATTCTAATTACACAATATT-3' and 5'-ATCCGCGCGGCCGCTTAA-3', which amplify the *cuff* coding region.

For the *cuff* genomic rescue construct, we obtained Bacterial Artificial Chromosome (BAC) clone BACR14L19 (Accession # AC007474) from BACPAC Resource Center. We digested the BAC construct with EagI, and cloned the fragments into the pBlueScript SK+ vector. The genomic fragment containing the *cuff* gene was identified and subsequently cloned into the pCasper4 vector (detailed cloning procedure is available upon request). The genomic construct rescues both the eggshell defect and sterility of *cuff* mutant females.

For the *cuff* cDNA rescue construct, we amplified *cuff* coding sequence from an ovarian cDNA library using primers 5'- ATCCGCTCTAGAATGAATTCTAATTA CACAATATT-3' and 5'-ATCCGCGCGGCCGCTTAAACTATAGAAGACATGGTT-3'. The PCR fragment was digested with Xba I and Not I, and subsequently cloned into a pUASp vector with either a triple HA tag at the C-terminus or a FLAG tag at the N-terminus.

For tagged dRAT1 (CG10354) construct, we obtained Expressed Sequence Tag (EST) clone RE15821 from the Drosophila Genomic Resource Center (DGRC), and subsequently cloned the

dRat1 coding sequence into the pUASp vector with a triple HA tag at the N-terminus. Transgenic flies were generated by Genetic Services, Inc.

Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR)

Quantitative RT-PCR was performed with the ABI Prism® 7900 system (AME Bioscience). Each reaction consisted of 50ng first strand cDNA template. For each mutant genotype, three separate RNA samples were prepared. Statistical analysis was performed with Microsoft Office Excel software. For *Het-A* transcript, we used primer pair 5'-ATCCTTCACCGTCATCACCTTCCT-3', 5'-GGTGCGTTTAGGTGAGTGTGTGTT-3'; for *Tart* transcript, we used primer pair 5'-AGAGAGGGAAAGAAGGGAAAGGGA-3', ATTTCCTGCCTGGTTAGATCGCCA-3'; we used *rpr49* as internal control, with primer pair 5'-ATGACCATCCGCCCAGCATAC-3', 5'-CTGCATGAGCAGGACCTC CAG-3'. $SpnB^{BU}/+$ females were used to construct the standard curve.

Northern Blot Analysis for small interfering RNA

Ovaries were manually dissected with forceps into Drosophila Ringer's solution (182 mM KCl, 46 mM NaCl, 3 mM CaCl 2, 10 mM Tris-HCl, pH 7.5). RNA was isolated from ovaries using Trizol (Invitrogen). Total RNA was quantified by absorbance at 260 nm, and 30 μg of total RNA was resolved by 15% denaturing polyacrylamide/urea gel electrophoresis (Invitrogen). After electrophoresis, the polyacrylamide gel was transferred to Hybond N+ (Amersham-Pharmacia) in 0.5x TBE by semi-dry transfer (X-Cell Surelock, Invitrogen) at 20 V for 1–2 h. The RNA was crosslinked to the membrane by UV irradiation (1200 μjoules/cm; Stratalinker, Stratagene) and pre-hybridized as previously described [42] for 1 h at 42°C. 20 pmol of single stranded DNA

probe was 5′-32P-radiolabeled with polynucleotide kinase (New England Biolabs) and 330 μCi γ-32P-ATP (7,000 μCi/mmol; New England Nuclear) and purified using a Sephadex G-25 spin column (Roche). The 32P-radiolabeled probes were hybridized for 4–12 h at 42°C. After hybridization, membranes were washed twice with 2x SSC/0.1% (w/v) sodium dodecyl sulfate (SDS) and once with 1x SSC/0.1% (w/v) SDS for 30 min.