Figure S1 *Drosophila* miR-3 family miRNAs. Sequence alignment of *Drosophila* miR-3 family miRNAs. The nucleotides in the seed region are shown in bold.

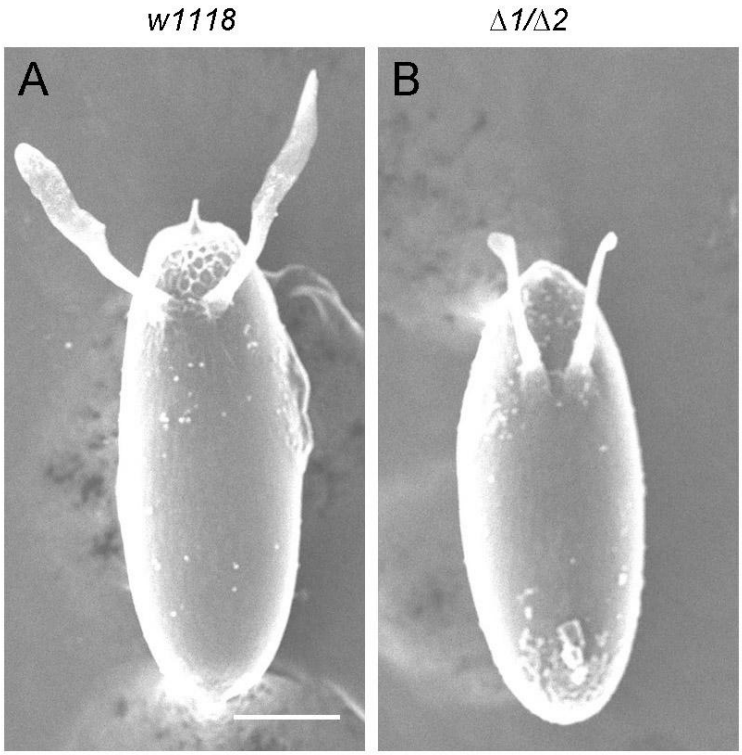

Figure S2 Characterization of miR-318 mutants. (A, B) miR-318 maternal loss-of-function mutants lay eggs with abnormal morphology. Eggs laid by control and miR-318 mutant females were visualized by scanning electron microscopy. Eggs are presented in a dorsal view, with anterior to the upper side. Scale bar: 100 um.

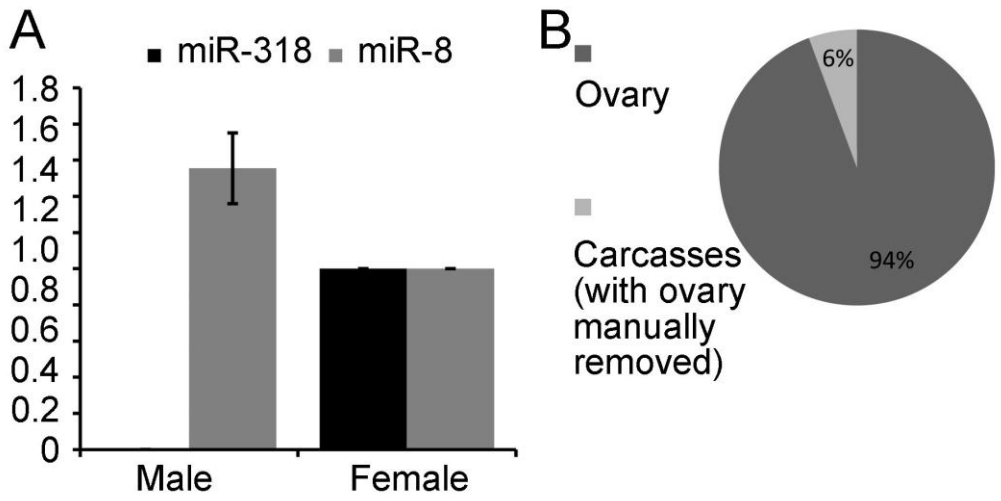

Figure S3 miR-318 is expressed in *Drosophila* ovaries. (A) miR-318 is only expressed in female adults. Mature miR-318 levels were measured by miRNA quantitative RT-PCR in RNAs isolated from adult males and females. miR-8 served as a control miRNA that is expressed in both adult males and females. miRNA expression levels were normalized to miR-184. Error bars represent standard deviation from three independent experiments. (B) miR-318 is enriched in ovaries. Mature miR-318 levels were measured by miRNA quantitative RT-PCR in RNAs extracted from ovaries and the rest part of the female body. miRNA levels were normalized to miR-184. Note the small fraction of miR-318 in carcasses is likely due to incomplete dissection of ovaries.

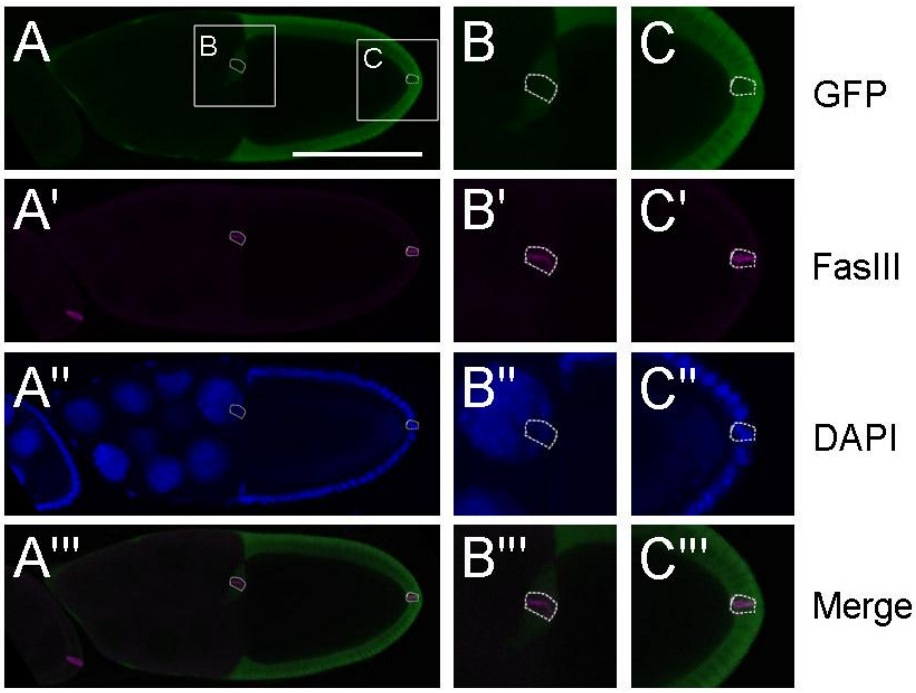

Figure S4 miR-318 is not expressed in the polar cells. (A-A''') miR-318 is not detected in two central polar cells within the border cell cluster (B-B''', Higher magnification) and the posterior polar cells (C-C''', Higher magnification). Shown are confocal images of egg chambers from *miR-318*$^{\Delta 1}$*/+* heterozygous females, stained with anti-GFP (Green), anti-FasIII (Purple) and DAPI (Blue). Scale bar: 100um.

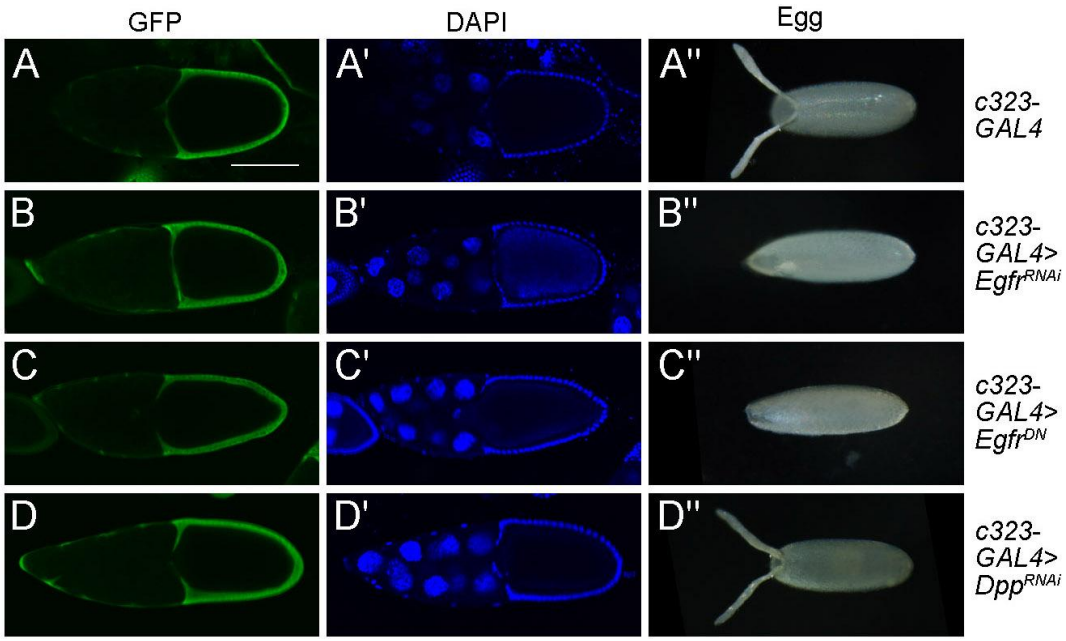

Figure S5 EGFR and Dpp pathways are not required for miR-318 expression. (A-D'') Depletion of EGFR or Dpp does not affect miR-318 expression. Shown are confocal images of stage 10B egg chambers from *miR-318$^{\Delta 1}$/+* female flies expressing UAS-Egfr^{RNAi}, UAS-Egfr^{DN} or Dpp^{RNAi} transgenes under the control of c323-GAL4, stained with anti-GFP (Green) and DAPI (Blue). Phase contrast images of dorsal views of eggs from female flies of indicated genotypes are shown in the right panels (A''-D''). Note the abnormal dorsal appendages caused by depletion of EGFR or Dpp. Scale bar: 100 um.

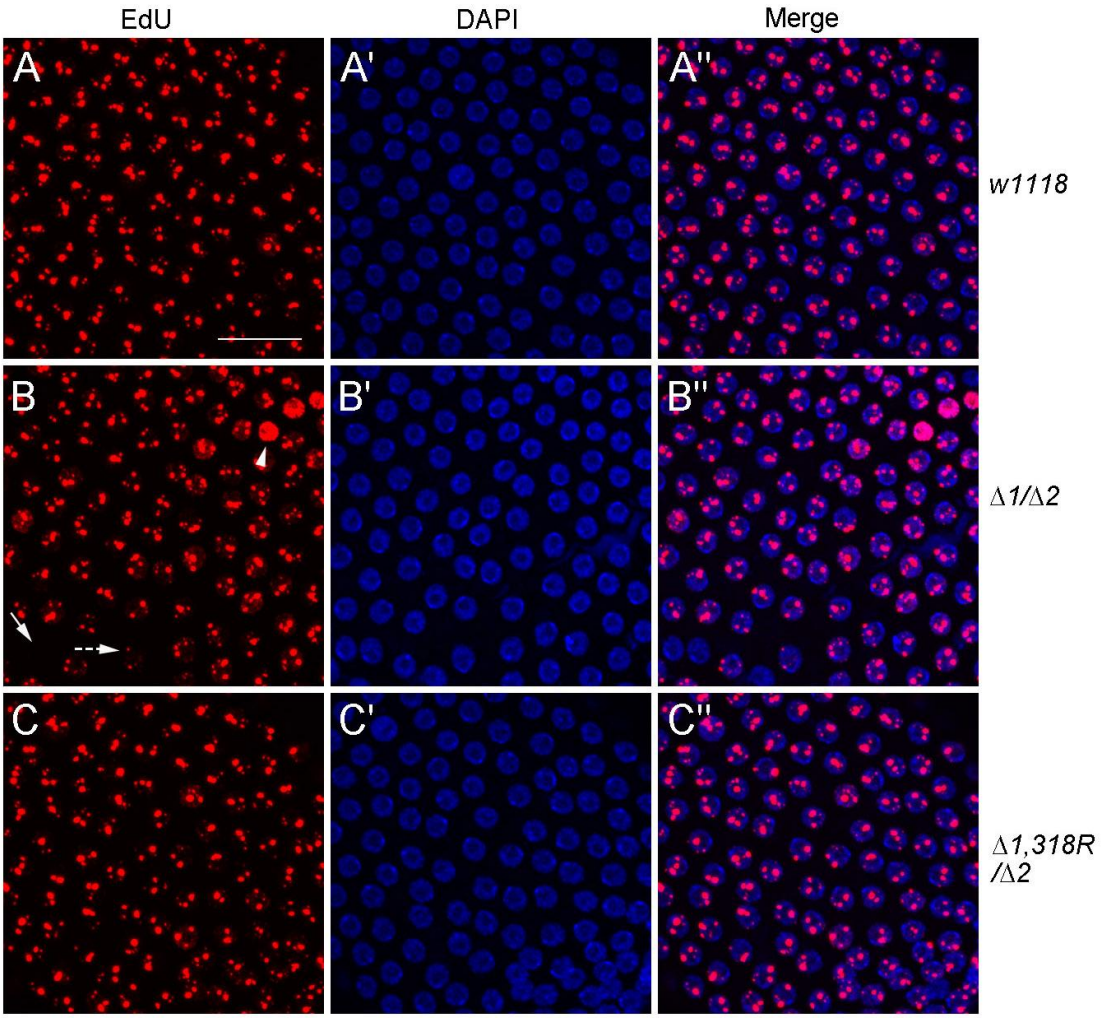

Figure S6 miR-318 mutation causes gene amplification defects. (A-C'') The EdU incorporation pattern is abnormal in miR-318 mutants. Shown are maximum intensity projections of Z-stacks of confocal images with higher magnification of stage 10B egg chambers from females of indicated genotypes. Samples were labeled with EdU (Red) and DAPI (Blue). EdU incorporation throughout the nucleus (Arrowhead in B), reduced EdU incorporation (Dotted arrow in B) and absent EdU incorporation (Arrow in B). Scale bar: 10 um.