

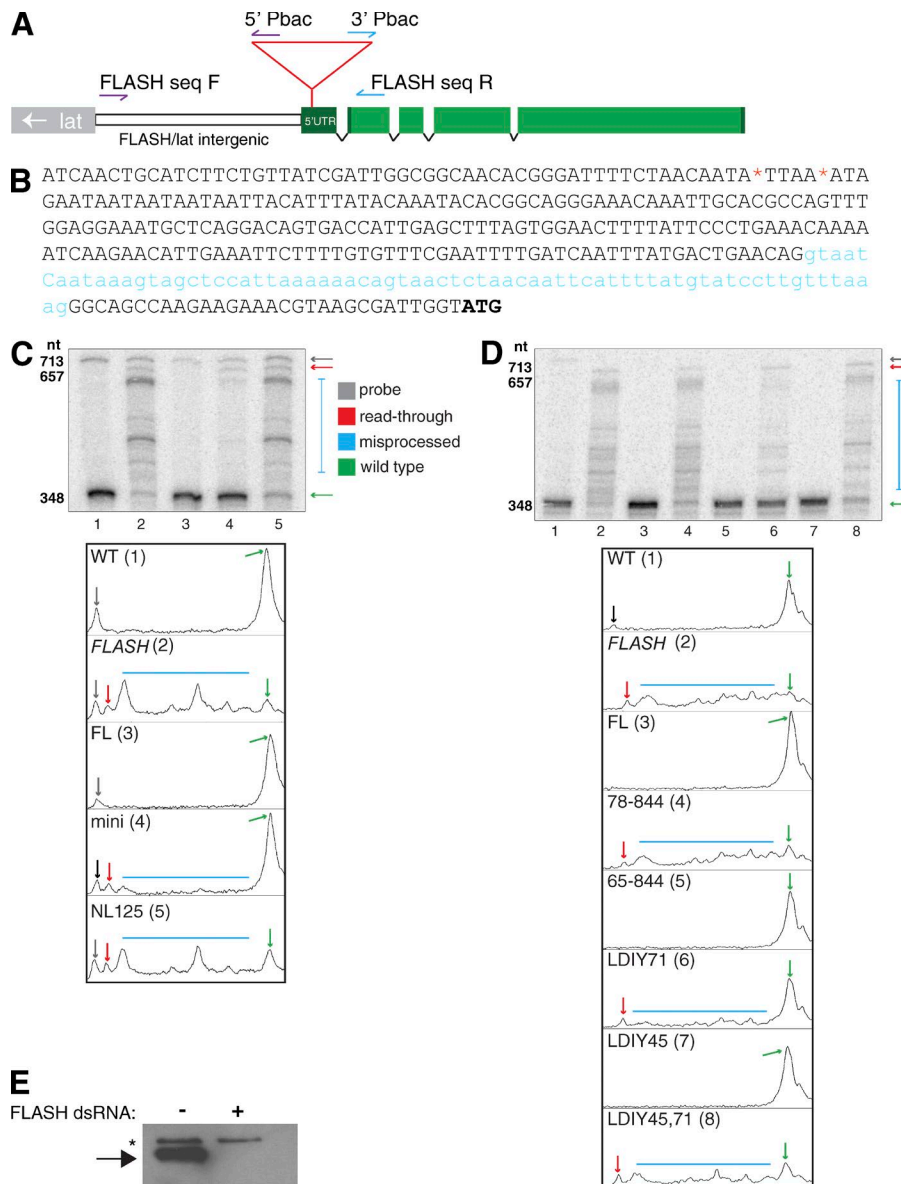
Tatomer et al., <http://www.jcb.org/cgi/content/full/jcb.201504043/DC1>

Figure S1. **Characterization of *FLASH*<sup>LL01602</sup> PBac.** (A) Diagram of the FLASH locus (open reading frame, green; 5' UTR, dark green) indicating the location of PCR primers used to identify the exact location of PBac LL01602. (B) Sequence of the FLASH 5' UTR indicating the confirmed insertion site (red stars). An intron present in the 5' UTR is indicated in lowercase blue letters, and the start of translation is the ATG sequence in bold. (C) Phosphorimager scan of the same S1 protection experiment shown in Fig. 1 E. The signal intensities in each lane of the gel were plotted as a line scan using the ImageJ gel-analysis function, with the top of the gel on the left of the scan and the properly processed histone mRNA on the right (green arrows). The gray arrow indicates undigested probe and the red arrow the read-through product, as indicated next to the image of the gel. Nucleotide markers are shown to the left for the undigested probe, read-through fragment, and properly processed H2a mRNA. (D) Phosphorimager scan of the same S1 protection experiment shown in Fig. 2 D. ImageJ-derived line scan, arrows, and nucleotide markers are the same as in C. (E) Equal amounts of nuclear extract prepared from WT or FLASH-depleted S2 cells were resolved by SDS-gel electrophoresis and analyzed by Western blotting using an anti-FLASH antibody. The nuclear extracts were used in the complementation processing assay in Fig. 2. Asterisk, cross-reacting protein that serves as a loading control. dsRNA, double-stranded RNA.

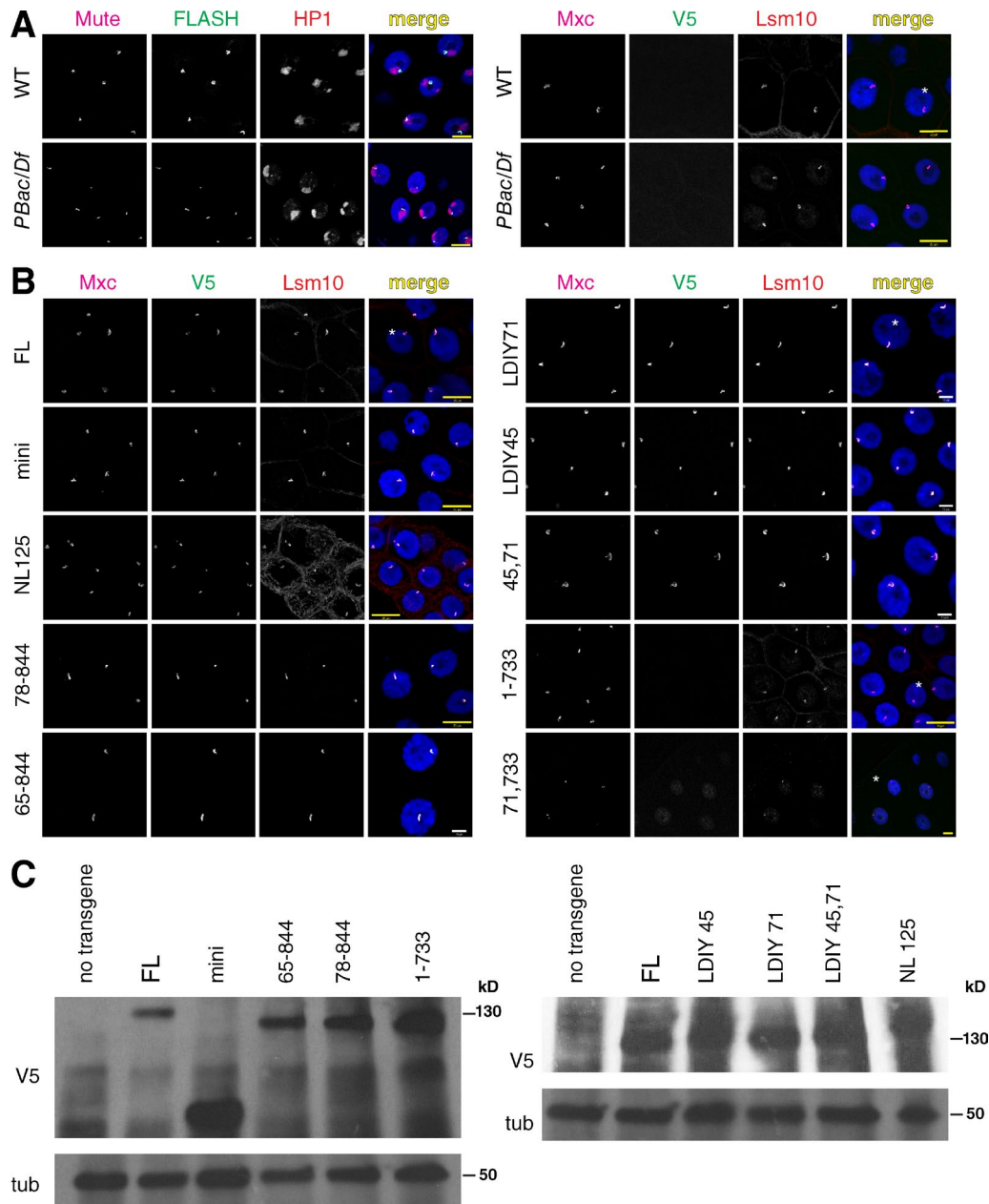


Figure S2. **Expression and localization of transgenic FLASH proteins.** (A) WT and *FLASH<sup>PBac/Df</sup>* wandering third-instar larval salivary glands stained with a panel of HLB markers. In contrast to *FLASH<sup>PBac/Df</sup>* mutant ovaries analyzed in Fig. 3, endogenous FLASH is detectable in the HLB in salivary glands from the same mutant, likely because of the presence of a stable, maternal pool of FLASH in this tissue. As a consequence, Lsm10 is also detected in these FLASH-positive HLBs. (B) Localization of exogenous FLASH protein tagged with V5 epitope (V5) in *FLASH<sup>PBac/Df</sup>* larval salivary glands expressing the indicated FLASH transgenes. Only proteins lacking the C-terminal 111 aa of FLASH fail to localize to the HLB. Asterisks here and in A indicate images that were cropped and presented in Fig. 3 C. Bars: (yellow): 20  $\mu$ m; (white) 10  $\mu$ m. (C) Equal amounts of protein from wandering third-instar WT larvae expressing the indicated FLASH transgenes were resolved by SDS-gel electrophoresis and analyzed by Western blotting with anti-V5 antibody followed by anti-tubulin (tub) antibody.

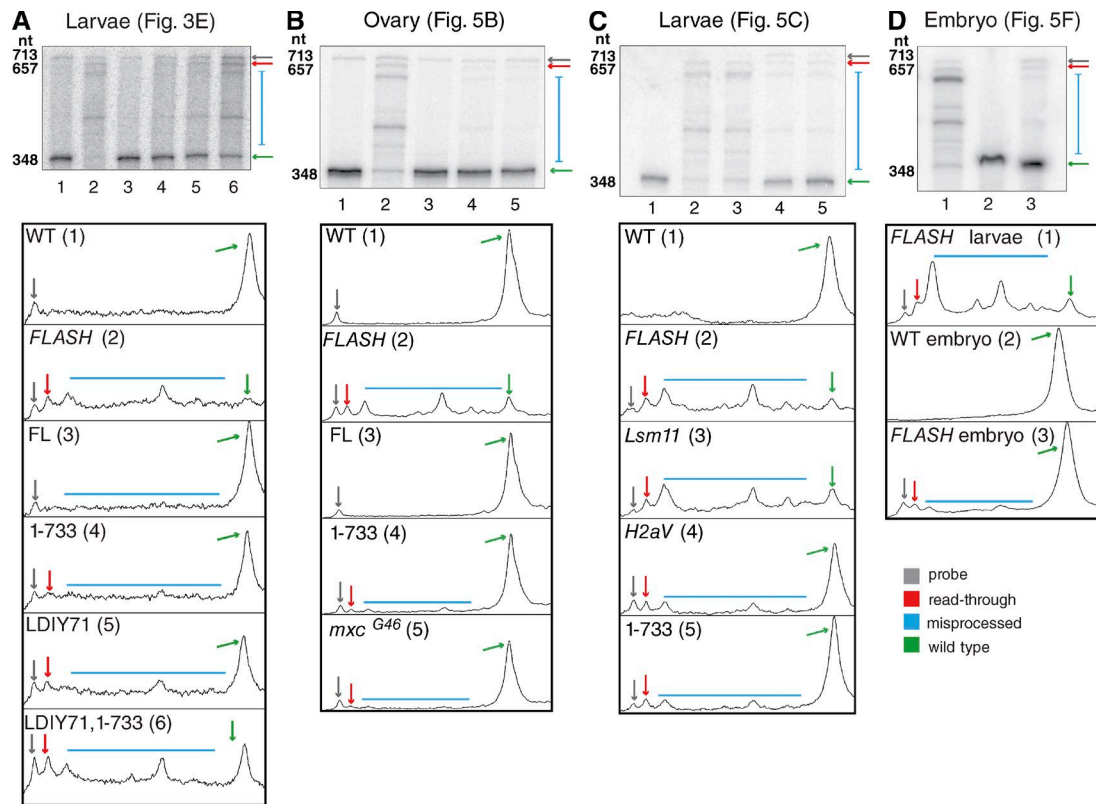


Figure S3. **RNA phenotypes in animals where FLASH concentration and/or biochemical activity of FLASH in the HLB is altered.** (A–D) Phosphorimages of the gels shown in Fig. 3 E (A), Fig. 5 B (B), Fig. 5 C (C), and Fig. 5 F (D) are reproduced as in Fig. S1. ImageJ-derived line scans of each lane of the S1 protection assays are shown for larval RNA (A and C), ovary RNA (B), and embryo RNA (D). The gels and scans are labeled as in Fig. S1.

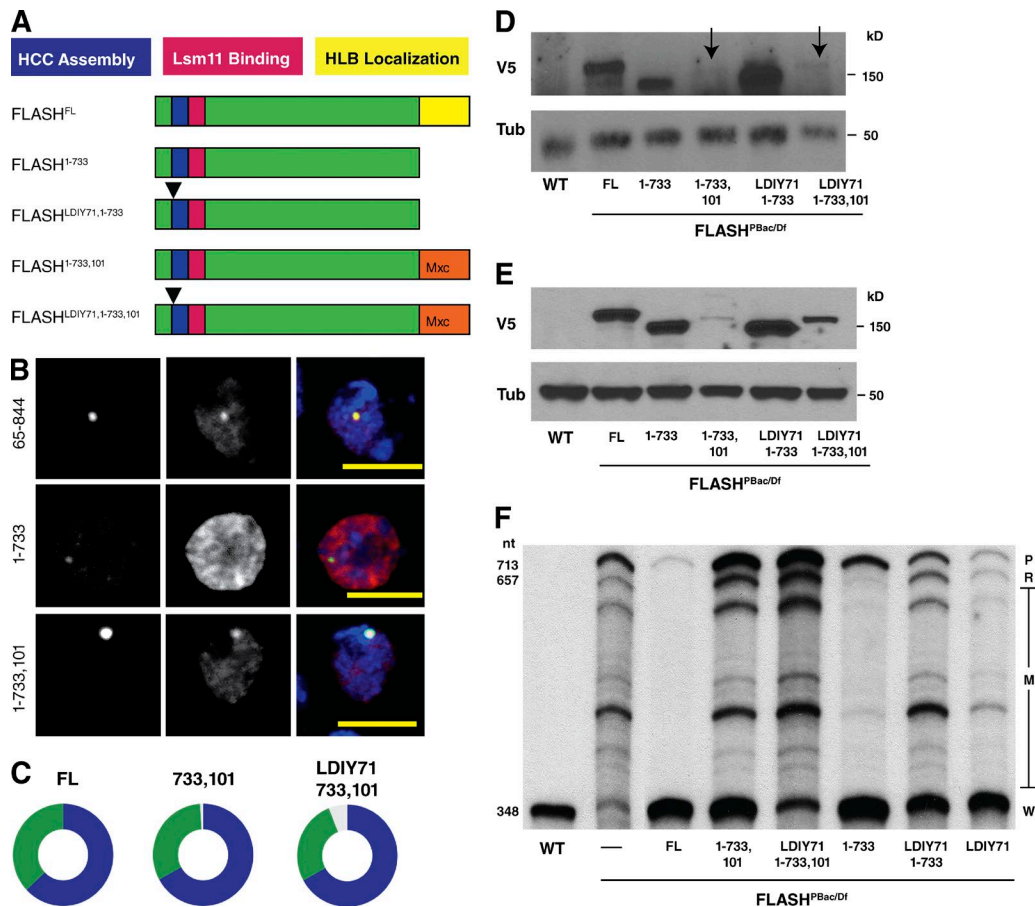


Figure S4. **Attempts to target FLASH<sup>1-733</sup> to the HLB using the HLB localization site from Mxc.** (A) To further demonstrate that localization of FLASH to the HLB is critical for efficient histone pre-mRNA processing, we attempted to localize the C-terminal truncated FLASH to the HLB using a heterologous signal. Taking advantage of our recent findings that the N-terminal 101 aa of Mxc will localize heterologous proteins to the HLB and are also essential for localizing Mxc to the HLB (Terzo et al., 2015), we fused the N-terminal region of Mxc (aa 1–101) followed by the V5 epitope to the C terminus of FLASH<sup>1-733</sup> and FLASH<sup>LDIY71,733</sup> to express the fusion proteins FLASH<sup>1-733,101</sup> and FLASH<sup>LDIY71,1-733,101</sup>. (B) Retargeted FLASH constructs tagged with V5 at the C terminus were expressed in S2 cells and stained with anti-Mxc and V5. The FLASH<sup>1-733,101</sup> protein colocalized with Mxc, as did the control FLASH<sup>65-844</sup> construct that contains the C terminal of FLASH. FLASH<sup>1-733</sup> was dispersed throughout the nucleoplasm as previously reported (Burch et al., 2011) and, like other mislocalized FLASH proteins, is overexpressed in S2 cells. Bar, 5  $\mu$ m. The FLASH<sup>LDIY71,1-733,101</sup> protein was also concentrated in the HLB when it was expressed in S2 cells (not shown). (C) Visual representation of transgenic rescue of FLASH<sup>PBac/Df</sup>. We created transgenic animals expressing FLASH<sup>1-733,101</sup> and FLASH<sup>LDIY71,733,101</sup> and determined their ability to rescue the reduced viability of the FLASH mutant. Expression of these genes rescued the reduced viability of the FLASH mutant, although both the FLASH<sup>733,101</sup> and FLASH<sup>LDIY71,733,101</sup> females were sterile (Table 1). Circles indicate the proportion of FLASH<sup>PBac/Df</sup> mutant with the indicated transgene (green) and control heterozygous sibling (blue) adult flies obtained. The expected fraction of control siblings is two thirds. Thus, one third green indicates full viability, and the absence of green indicates lethality. (D and E) Protein from an equal number of wandering third-instar larvae (D) and ovaries (E) expressing the indicated FLASH transgenes in FLASH<sup>PBac/Df</sup> mutants was resolved by SDS-gel electrophoresis and probed with anti-V5 antibody and antitubulin (Tub) antibody. This Western blotting data demonstrated that there was very low accumulation of FLASH<sup>1-733,101</sup>, and FLASH<sup>LDIY71,733,101</sup> in both larvae (D) and ovaries (E) compared with the accumulation of FL FLASH and the corresponding V5-tagged FLASH constructs lacking the Mxc101 domain, all of which were expressed at similar levels. Because the fusion proteins were expressed from the same FLASH promoter construct and inserted into the same site in the *Drosophila* genome, these proteins are likely less stable in animals than the parent FLASH constructs. (F) A total of 5  $\mu$ g of total mRNA from third-instar larvae expressing FLASH transgenes in FLASH<sup>PBac/Df</sup> was analyzed in the S1 nuclease protection as in Figs. S1 and S3. The bands are labeled to the right and left of the gel as in Figs. S1 and S3. Qualitatively similar results were obtained with RNA from ovaries (not shown). The presence of the FLASH<sup>1-733,101</sup> and FLASH<sup>LDIY71,733,101</sup> transgenes increased the percentage of properly processed H2a mRNA compared with the FLASH<sup>PBac/Df</sup> mutant. However, the amount of increase was much less than with the corresponding mislocalized V5-tagged FLASH proteins lacking the Mxc101 domain (i.e., FLASH<sup>1-733</sup> and FLASH<sup>LDIY71,733</sup>), and there was a much larger proportion of misprocessed RNA. The failure of these transgenes to promote high levels of properly processed histone mRNA likely accounts for their failure to rescue fertility, because the Slbp and U7 snRNA mutants, which express large amounts of misprocessed histone mRNA, are also infertile (Sullivan et al., 2001; Godfrey et al., 2006).

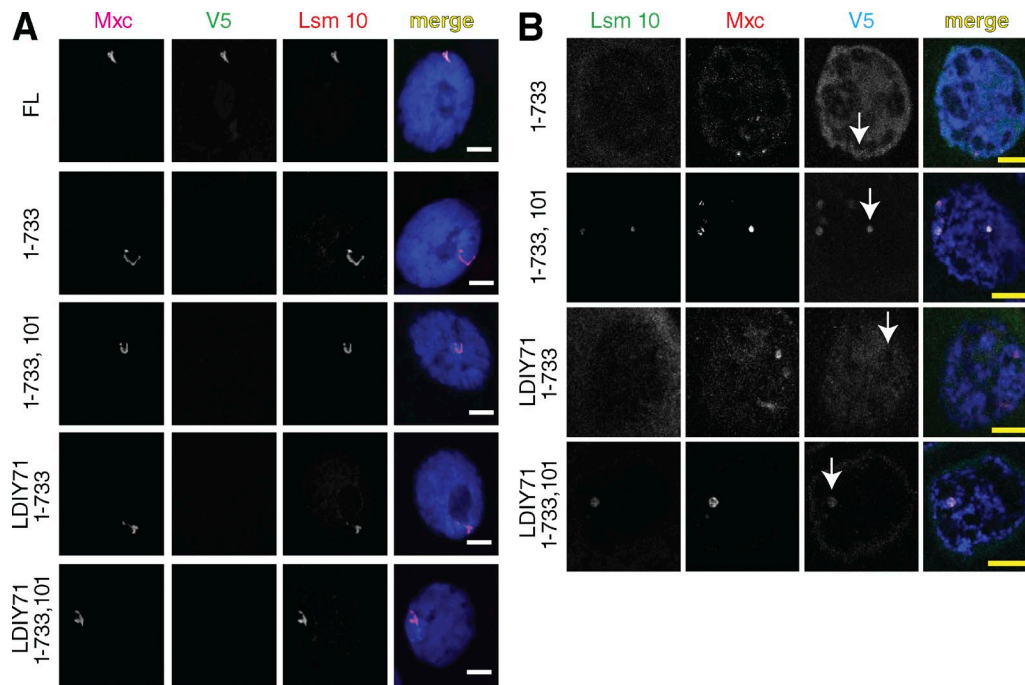


Figure S5. **HLB localization analysis of transgenic FLASH-mxc fusion constructs.** (A) Third-instar larval salivary glands expressing transgene-derived, V5-tagged FLASH mutant proteins in *FLASH<sup>PBac/Df</sup>* were stained with anti-V5, anti-Lsm10, and anti-Mxc antibodies. Only the FL FLASH protein was detected in the HLB in this tissue. As noted in Fig. S2, because of the likely persistence of maternally loaded FLASH in salivary glands of third-instar larvae, Lsm10 is observed in the HLBs in the *FLASH<sup>PBac/Df</sup>* mutant. (B) Similarly stained stage 11 ovaries from the indicated genotypes. Weak anti-V5 staining is present in the nurse cell nuclei of *FLASH<sup>1-733,101</sup>* and *FLASH<sup>LDIY71,1-733,101</sup>* transgenic animals, but not in the *FLASH<sup>1-733</sup>* transgenic animals (arrows). Bars, 10  $\mu$ m.

## References

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