

SUPPLEMENTARY FIGURES 1-4.

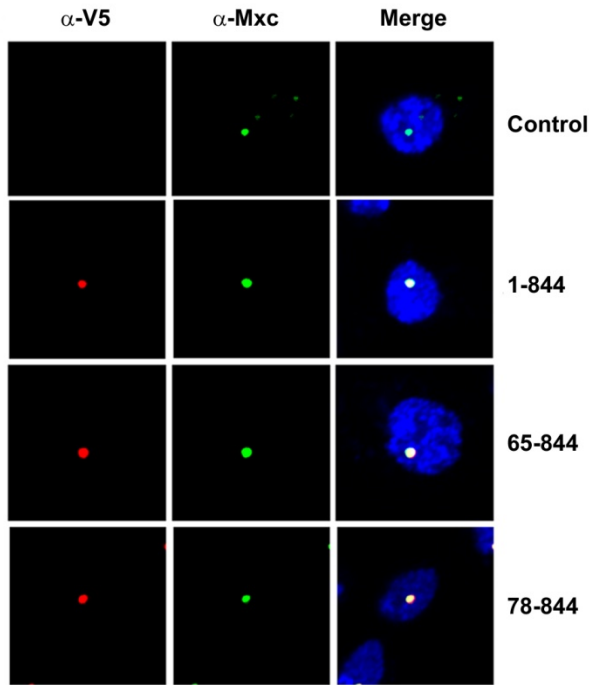


Figure S1. The first 77 amino acids of dFLASH are not required for localization to the HLB. *Drosophila* D.Mel-2 cells were transfected with the indicated constructs and stained with antibodies against the V5 epitope, recognizing recombinant dFLASH, and Mxc, which localizes to the HLB in *Drosophila*. DAPI was used to stain the nucleus. The cells were transfected with the indicated clones and then stained and imaged at the same time, with the same exposure time. Representative confocal microscopy images are shown.

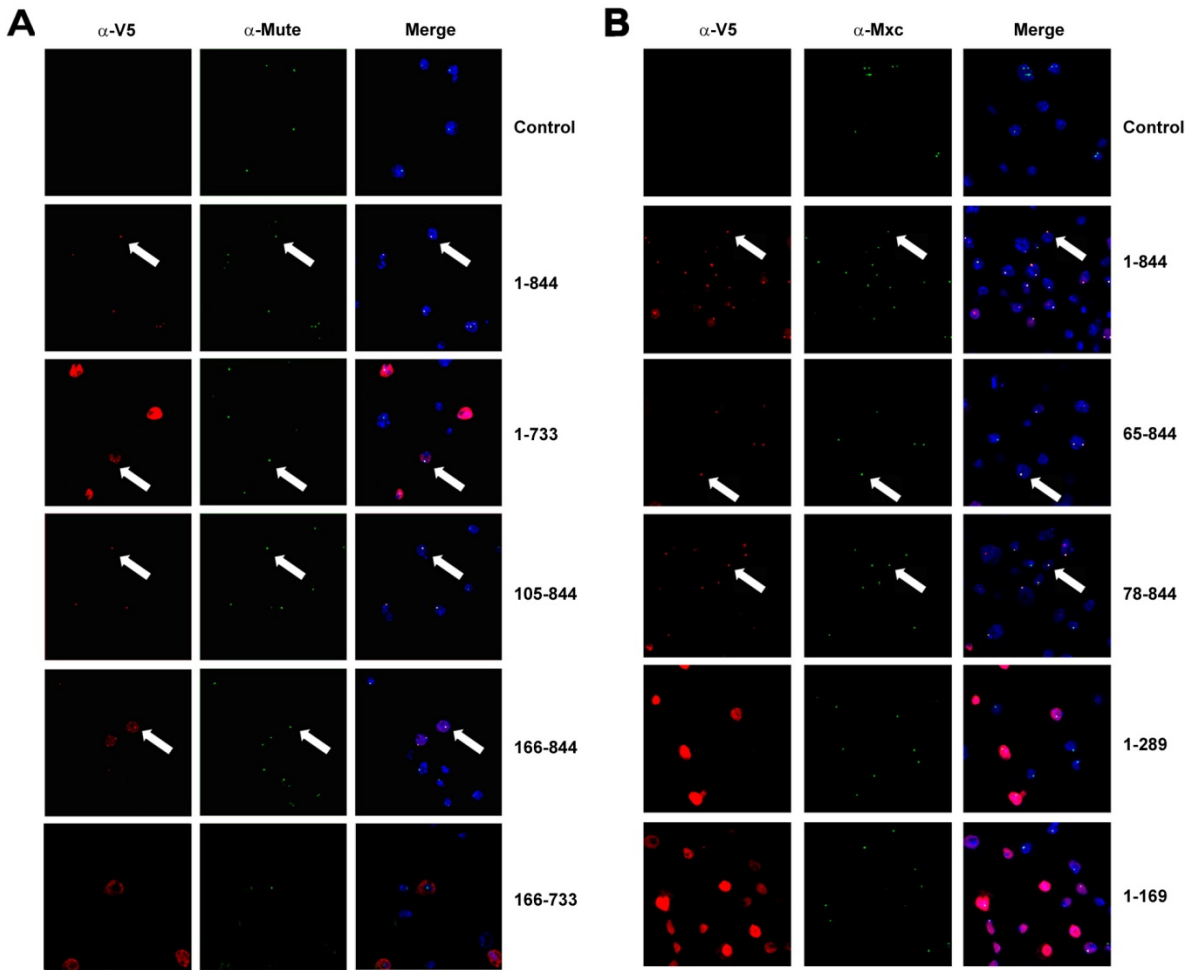


Figure S2. Requirements for nuclear localization of dFLASH.

Drosophila D.Mel-2 cells were transfected with the indicated constructs and stained with antibodies against the V5 epitope, recognizing recombinant dFLASH, and either Mute (A) or Mxc (B), each of which localize to the HLB in *Drosophila*. DAPI was used to stain the nucleus. Wider image fields are shown to include multiple cells. For each panel, cells were transfected with the indicated clones and then stained and imaged at the same time, with the same exposure time. White arrows indicate representative histone locus bodies.

In panel A the 1-733 protein localizes to the nucleus but not the HLB, and the 166-733 protein is cytoplasmic, indicating there is a nuclear localization sequence in the C-terminal region between 733 and 844. In panel A, the 1-289 and 1-169 proteins (bottom) localize to the nucleus but not the HLB, indicating there is also a nuclear localization signal in the N-terminus between amino acids 1-169.

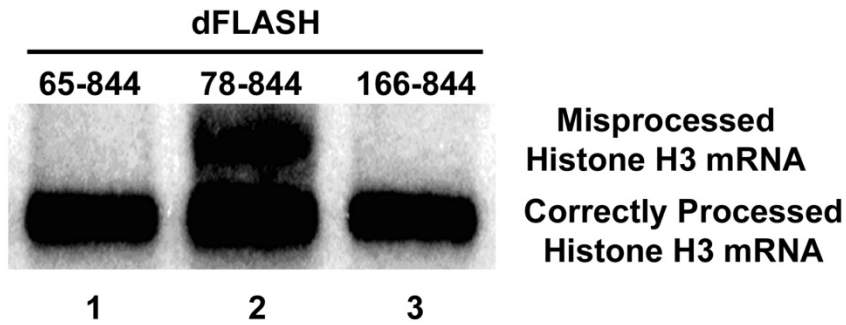


Figure S3. Misprocessing of histone mRNA as a result of expressing a FLASH mutant which can bind Lsm11. *Drosophila* D.Mel-2 cells were transfected with the indicated constructs and total RNA was analyzed by Northern blotting using a probe against histone H3 mRNA. The 65-844 protein is fully active in processing, and the 166-844 protein lacks the Lsm11 binding site. The 78-844 lacks the putative binding site for a factor required to activate processing.

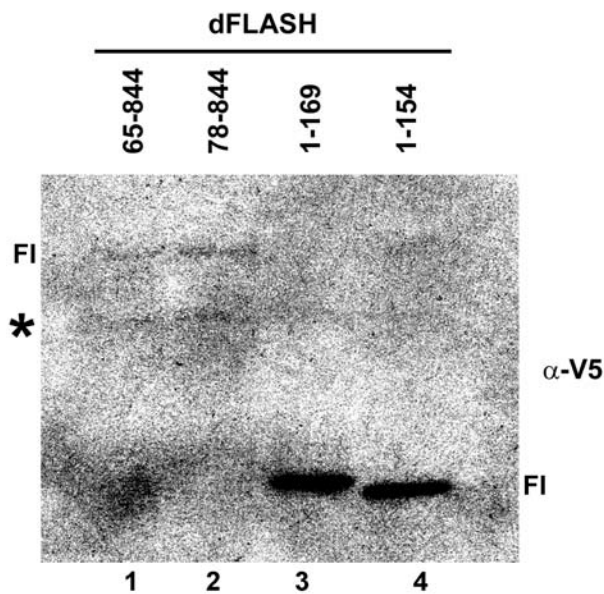


Figure S4. Expression of v5-tagged FLASH mutants in S2 cells.

Cell lysates from the cells expressing different FLASH mutants in Fig. 7 were analyzed by Western blotting using V5 antibody to detect the epitope tag. Lanes 1 and 2 are the large FLASH proteins that localized to the HLB and lanes 3 and 4 are the smaller FLASH proteins which are largely in the nucleoplasm (specific proteins indicated above each lane). FI= tagged FLASH proteins; * indicates a proteolytic product of the large tagged FLASH proteins.