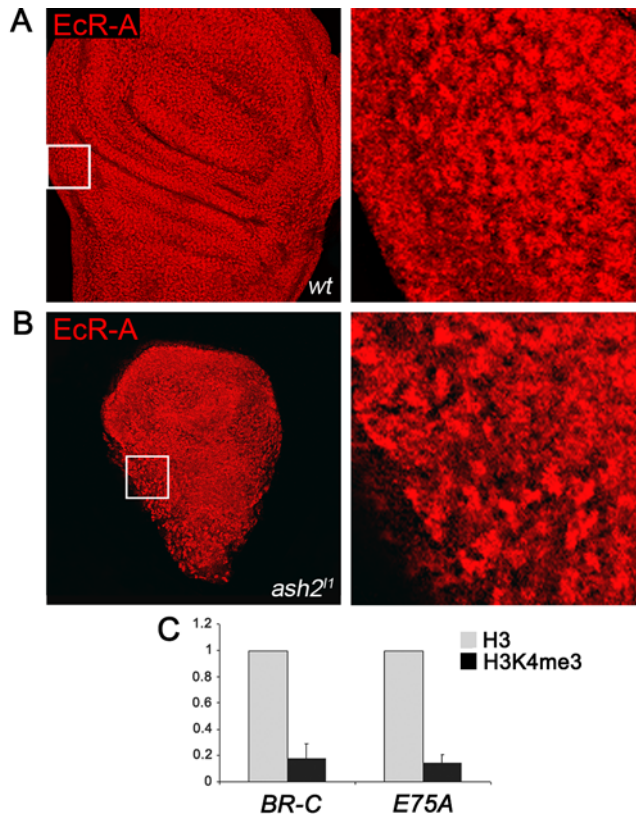
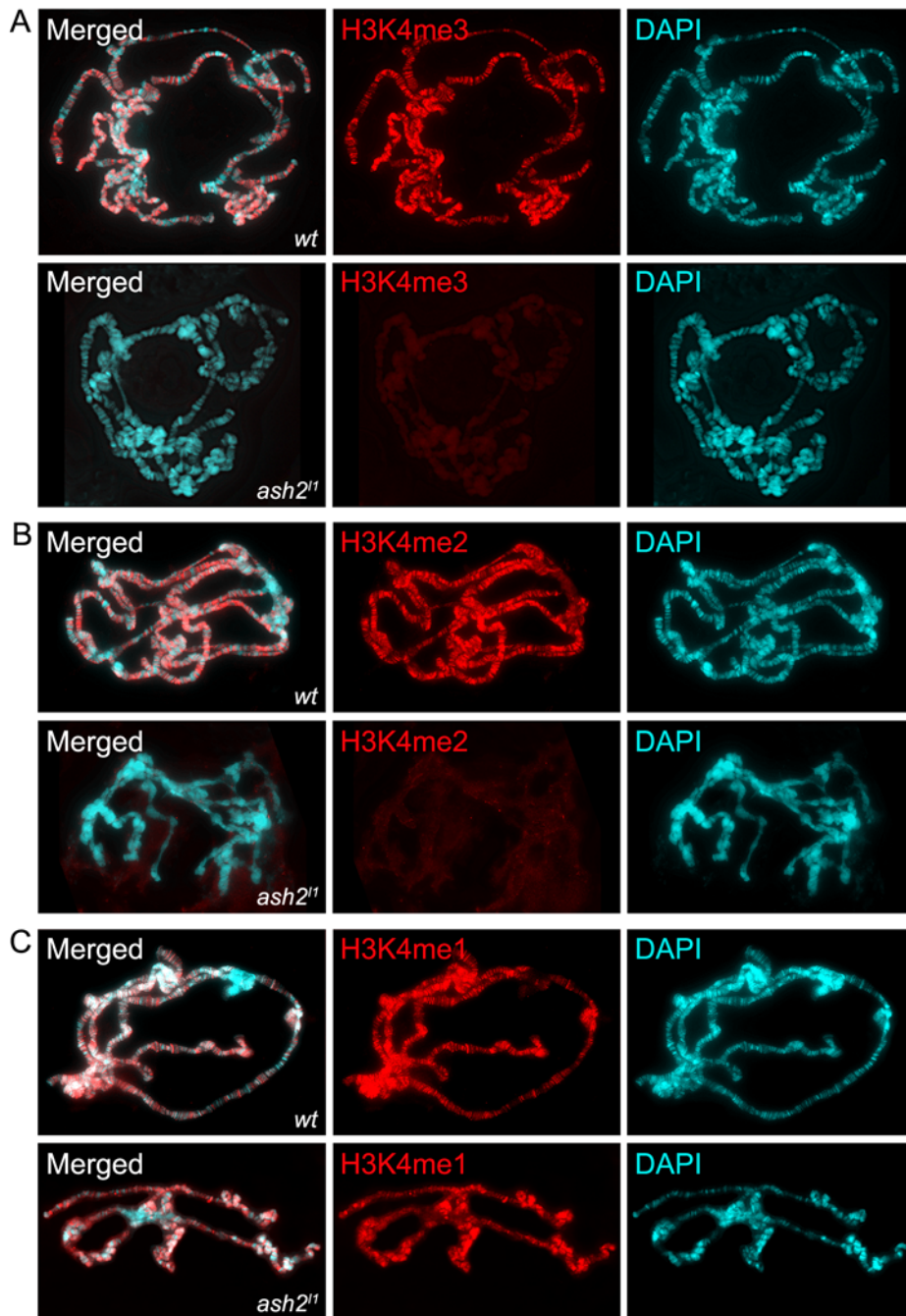


Supplemental Figure 1. Characterization of Ash2 antibodies. (A,B) Western blots with anti-Ash2^{Nt} (A) and anti-Ash2^{Ct} (B) were performed using S2 cells transfected with *ash2-RC-V5*. Anti-V5 was used as a control. (C) Overexpression of Ash2 with the *daughterless* driver did not produce additional Ash2 bands. The distribution of endogenous Ash2 (top panel) and overexpressed Ash2-HA (bottom panel) on a representative region of wild-type polytene chromosome corresponding to the 2L arm is shown. Immunostaining was performed using anti-Ash2^{Nt} and anti-HA.



Supplemental Figure 2. The loss of H3K4me3 in *ash2* mutants is not associated with a decrease of EcR or with a change in nucleosome occupancy. (A,B) Detection of EcR-A in wild-type (A) and *ash2^{1/1}* homozygous (B) wing imaginal disc. Both wing imaginal discs are presented at the same scale. Right panels show a magnification of the squared regions in left panels. (C) A ChIP analysis of wild-type and *ash2^{1/1}* late wandering larva using H3 (grey) and H3K4me3 (black) antibodies. H3 was used as a mark of nucleosome positioning, and ChIP results are depicted as a ratio of fold enrichment from wild-type compared to *ash2^{1/1}*. H3K4me3 results were normalized against H3 results. Error bars represent SEM.



Supplemental Figure 3. The loss of ASH2 affects the overall deposition of H3K4me3 and H3K4me2 but not that of H3K4me1. Distribution of H3K4me3 (A), H3K4me2 (B), and H3K4me1 (C) on polytene chromosomes from wild-type (top panels) and *ash2¹* (bottom panels) third instar larvae.

**Early responsive genes (<3h)
downregulated (>0,5 FC) in *ash2¹* wing
imaginal discs**

bip1
BR-C
CG11163
CG13784
CG13813
CG14629
CG18212
CG4586
CG6124
CG6234
CG6630
CG8249
crol
Cyp4e2
E23
Eip55E
Eip71CD
Eip74EF
E75A
Eip78C
Hr39
Hsp27
InR
Pdi

Supplemental Table 1. Early responsive genes are downregulated in *ash2*¹ mutants. A subset of EcR targets genes that were found to be up-regulated in response to 20HE at an early time (≤ 3 h) (Gauhar *et al.*, 2009) and to be downregulated in wing imaginal discs from *ash2*¹ mutants (Beltran *et al.*, 2007) are listed.