Expanded View Figures

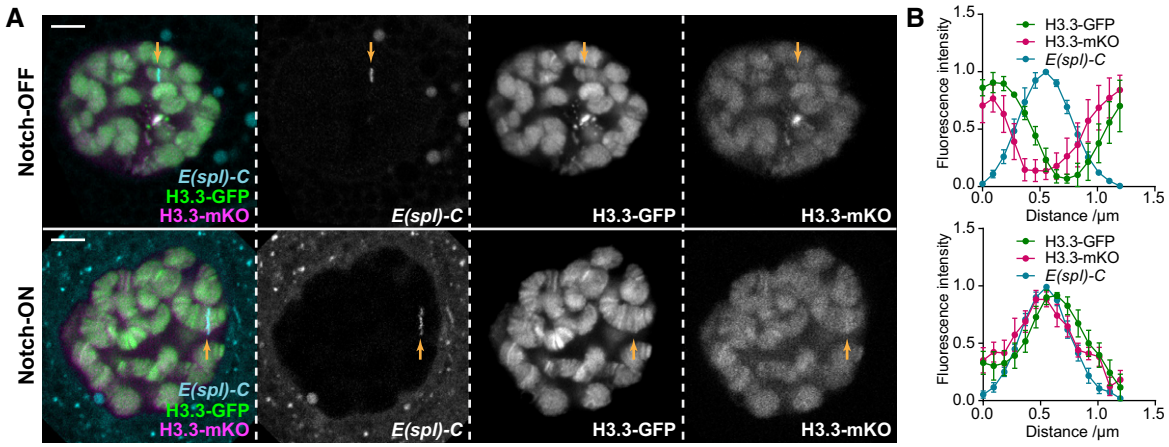

Figure EV1. Histone H3.3 is incorporated dynamically at the *E(spl)-C* in Notch-ON nuclei.

A Live imaging of H3.3-GFP (green), H3.3-mKO (magenta), and ParB-mCherry (cyan) expressed in larval salivary gland nuclei using 1151-Gal4 with the *UAS-FRT-H3.3-GFP-PolyA-FRT-H3.3-mKO-PolyA* transgene [34]. H3.3-mKO expression was initiated by heat-shock-inducible flippase expression approximately 24 h before imaging (see Materials and Methods for details), and ParB-mCherry indicates the *E(spl)-C* as in Fig 1. H3.3-mKO incorporation shows a similar pattern to H3.3-GFP in Notch-OFF (LacZ expression) and Notch-ON ($N^{\Delta ECD}$ expression) nuclei, as higher levels are present relative to surrounding regions in the Notch-ON condition. Yellow arrow indicates position of *E(spl)-C* on chromosome. Scale bars (white) = 5 µm.

B Quantifications of relative fluorescence intensity of H3.3-GFP, H3.3-mKO, and ParB-mCherry across the *E(spl)-C* in Notch-OFF (upper) and Notch-ON (lower) conditions. Mean ± SEM; n_{nuclei} = 3 and 5 and n_{glands} = 3 and 3 where each gland represents a biological replicate (from top to bottom).

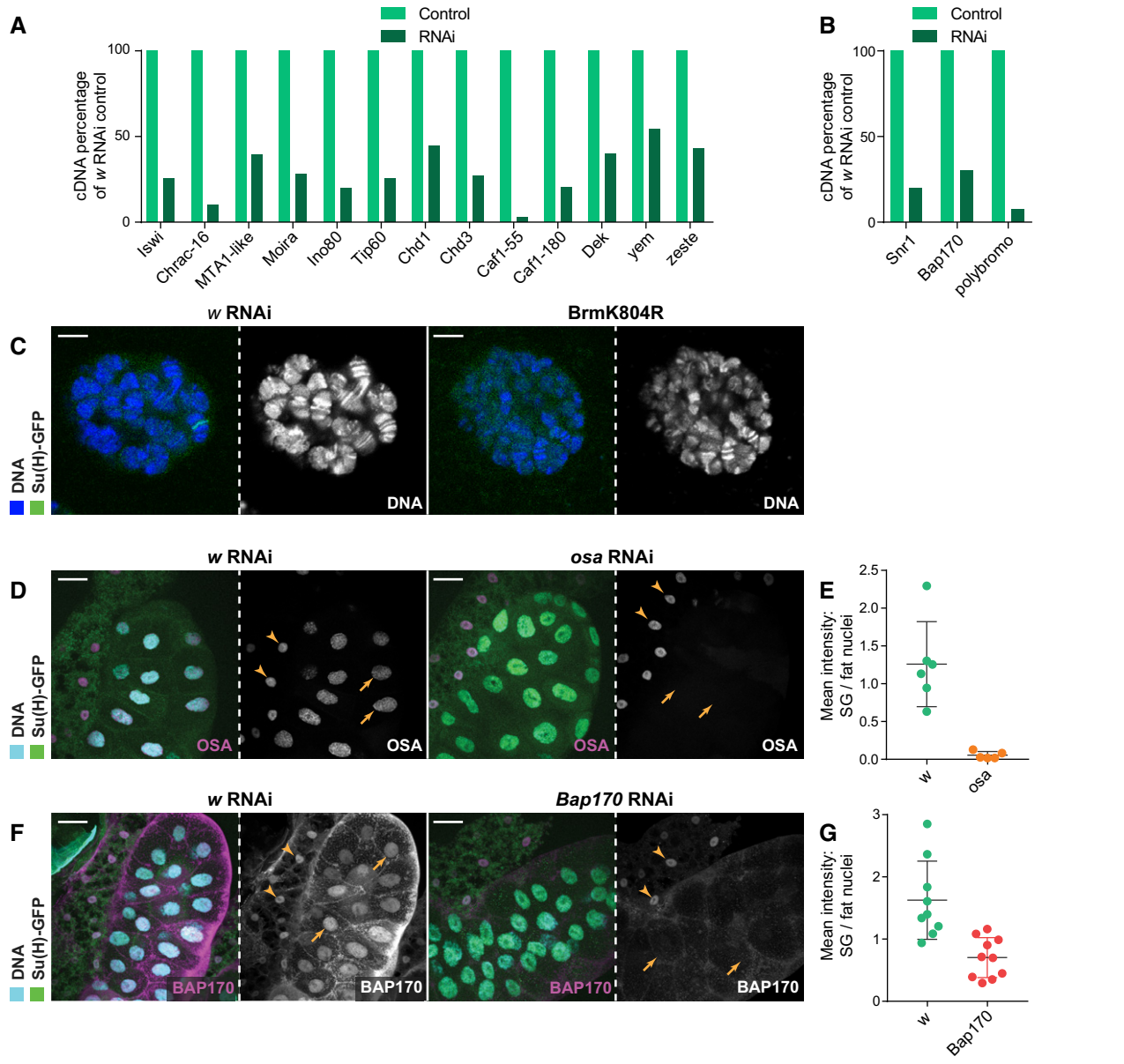

Figure EV2. The RNAi lines used successfully reduce RNA and protein levels.

A, B Effects of the indicated RNAi expression in salivary glands on cDNA levels, measured by reverse transcription–qPCR; percentage cDNA compared to *w* RNAi control after normalizing to an internal control for each sample (*RpL32* for most but *RpII215* for *Moira* as *Moira* RNAi appeared to affect *RpL32* levels). All reduce their respective cDNA levels, with *polybromo* RNAi causing a greater reduction than *Snr1*, despite not having an effect on Su(H) recruitment (B). Note that samples included RNA extracted from adjoining fat cells as well as salivary glands, and thus, some variability in the knockdowns is attributed to residual expression of chromatin remodelers and chaperones in the fat tissue where the RNAi was not expressed. Mean, $n = 2$.

C Live Hoechst 33342 staining (blue) of salivary glands expressing either *w* RNAi or BrmK804R. Chromosomes are observed with distinctive banding patterns under both conditions despite chromosomes being slightly smaller with BrmK804R expression. Scale bars (white) = 5 μm.

D–G (D, F) Immunofluorescence staining of OSA (C; magenta) and BAP170 (E; magenta) in salivary glands expressing *osa* (stock (2) in Fig 2) and *Bap170* RNAi, respectively, compared to *w* RNAi control glands. *osa* RNAi depletes all detectable OSA protein and *Bap170* RNAi removes most BAP170 protein. Yellow arrows indicate salivary gland nuclei and yellow arrowheads indicate fat cell nuclei for comparison where RNAi is not expressed. Scale bars (white) = 50 μm. (E, G) Quantifications of OSA ($n = 5$) (E) and BAP170 ($n = 10$) (G) nuclear levels from maximum projection images with salivary gland nuclei normalized to fat cell nuclei. Mean ± SEM.

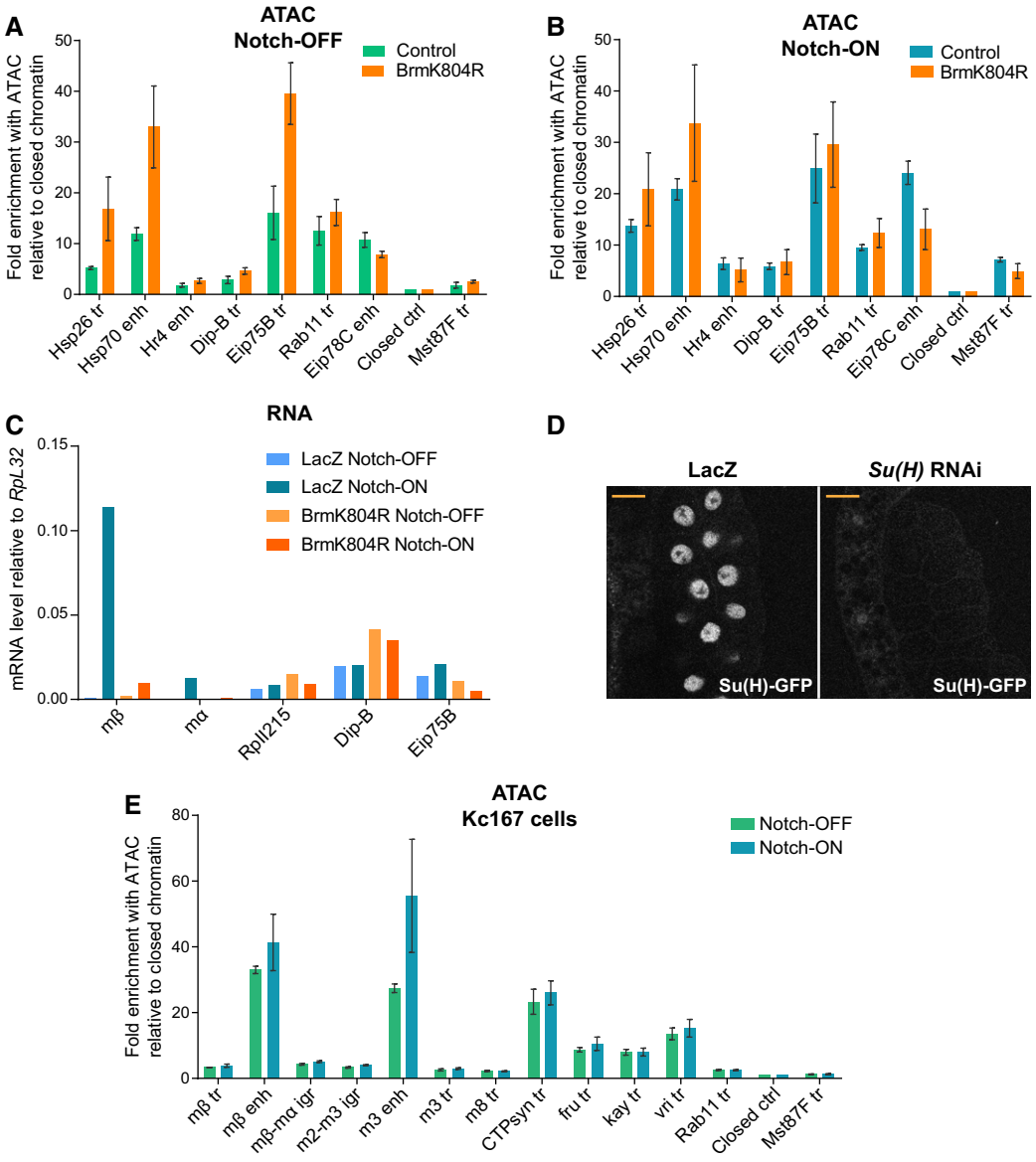

Figure EV3. The effects of BrmK804R on the accessibility and expression of Notch-inducible genes are not widespread.

A, B Chromatin accessibility in Notch-OFF (A) and Notch-ON ($N^{\Delta ECD}$ expression, B) salivary gland nuclei measured by ATAC-qPCR; fold enrichment at the indicated regions compared to a "closed ctrl" region. Expression of dominant-negative Brm, BrmK804R, had little effect or increased the accessibility of some regions. "Hsp26 tr" and "Hsp70 enh" are heat-shock-responsive regions, while "Hr4 enh", "Dip-B tr", and "Eip75B" are ecdysone-responsive. "Rab11 tr", "Eip78C", "Closed ctrl", and "Mst87F tr" are the same as shown in Fig 3B and C. Mean ± SEM; $n = 3$.

C Effect of Notch activation ($N^{\Delta ECD}$ versus LacZ expression) and BrmK804R expression on gene expression in salivary glands measured by reverse transcription–qPCR. Genes shown are Notch-responsive *E(spl)mβ-HLH* (mβ) and *E(spl)mα-HLH* (mα), housekeeping gene *RpII215*, and ecdysone-responsive *Dip-B* and *Eip75B*. Out of those shown, BrmK804R expression only reduces the Notch-responsive expression of *E(spl)mβ-HLH* and *E(spl)mα-HLH*. Mean, $n = 2$.

D Effect of *Su(H)* RNAi expression on Su(H)-GFP levels detected with live imaging compared to LacZ expression control. No Su(H)-GFP was left detectable, and this genotype was used for the ATAC experiment in Fig 3D. Scale bars (yellow) = 50 μm.

E An acute Notch response in Kc167 cells involves increased enhancer accessibility. Chromatin accessibility across the *E(spl)-C* in Notch-ON (EGTA-treated) and Notch-OFF (PBS control) Kc167 cells detected by ATAC-qPCR. Fold enrichment of the indicated regions compared to a "closed ctrl" region; positions of *E(spl)-C* primers in the genome are shown in Fig 3A. "CTPsyn tr", "fru tr", "kay tr", and "vri tr" are highly accessible control regions which do not respond to Notch. Mean ± SEM; $n = 3$.

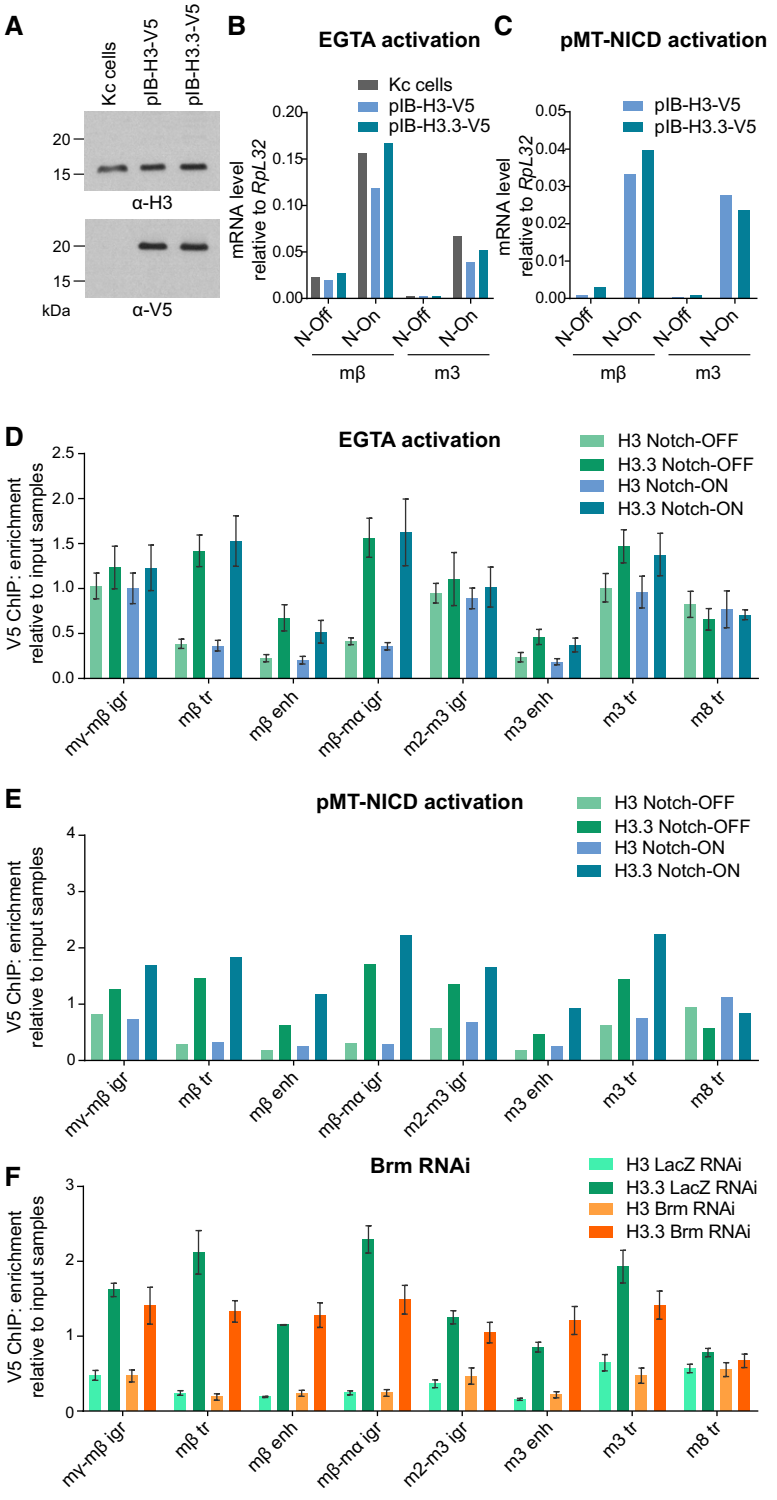

Figure EV4. Notch activation does not affect the distribution of histones H3 and H3.3.

A H3-V5 and H3.3-V5 expression in stable cell lines compared to un-transfected "Kc cells", demonstrated by Western blots probed with H3 and V5 antibodies. V5-tagged histones have a larger molecular weight and are not detectable in the H3 blot due to low levels of expression in comparison with endogenous H3.

B, C Effect of Notch activation by EGTA (B) or copper-inducible NICD expression (C) on expression of *E(spl)mβ-HLH* and *E(spl)m3-HLH* in stable cell lines expressing H3-V5 and H3.3-V5, measured by reverse transcription–qPCR. Both methods of activation strongly induce both genes. "N-On" denotes EGTA or copper treatment, and "N-Off" denotes PBS alone or no copper.

D, E Notch activation does not affect H3 and H3.3 levels across the *E(spl)*-C. V5 ChIP–qPCR in Kc cells expressing H3-V5 or H3.3-V5 from a ubiquitous promoter with Notch signaling activated by EGTA (D) or 6 h of copper-inducible NICD expression (E), shown as fold enrichment over input samples. H3-V5 and H3.3-V5 show a differential pattern across the *E(spl)-C*, but Notch activation causes no detectable change in levels compared to controls treated with PBS (D) or no copper (E). Mean ± SEM, n = 3 (D); mean, n = 2 (E).

F V5 ChIP–qPCR in Kc cells expressing H3-V5 or H3.3-V5 from a ubiquitous promoter after *lacZ* or *brm* RNAi treatment, shown as fold enrichment over input samples. The changes caused by *brm* RNAi are minimal and do not occur at enhancer regions. Mean ± SEM; n = 3.

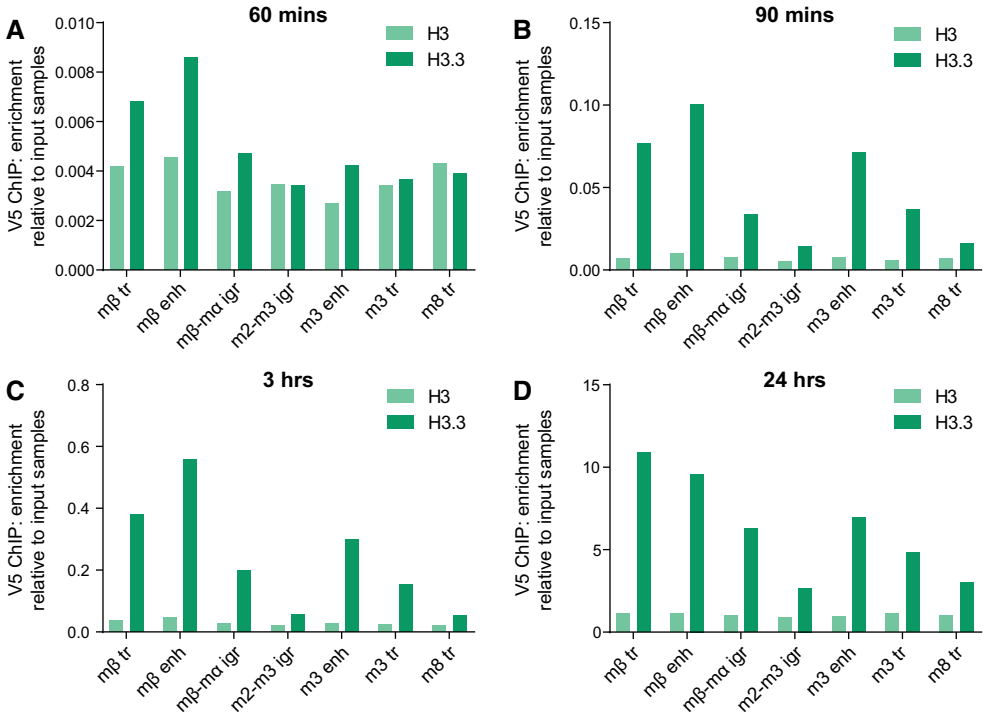

Figure EV5. Time-course of copper-inducible histone-V5 expression.

A–D V5 ChIP–qPCR in Kc cells with H3-V5 and H3.3-V5 expression induced by copper from the pMT promoter for 60 min (A), 90 min (B), 3 h (C), and 24 h (D), shown as fold enrichment over input samples. Differential incorporation of H3.3 across the *E(spl)-C* is clear after 90 min. $n = 1$ (A and B); mean, $n = 2$ (C and D).