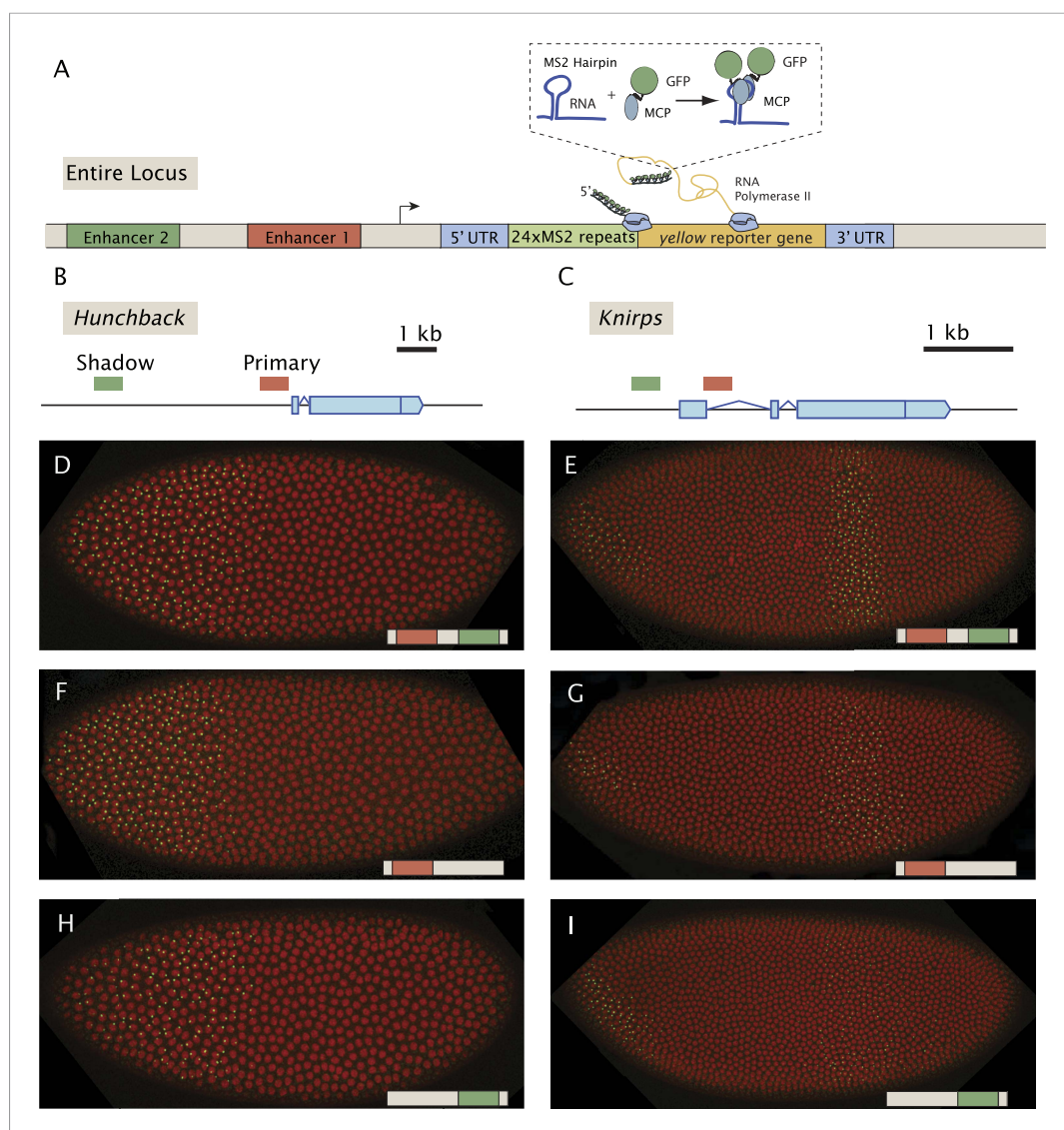


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## Figures and figure supplements

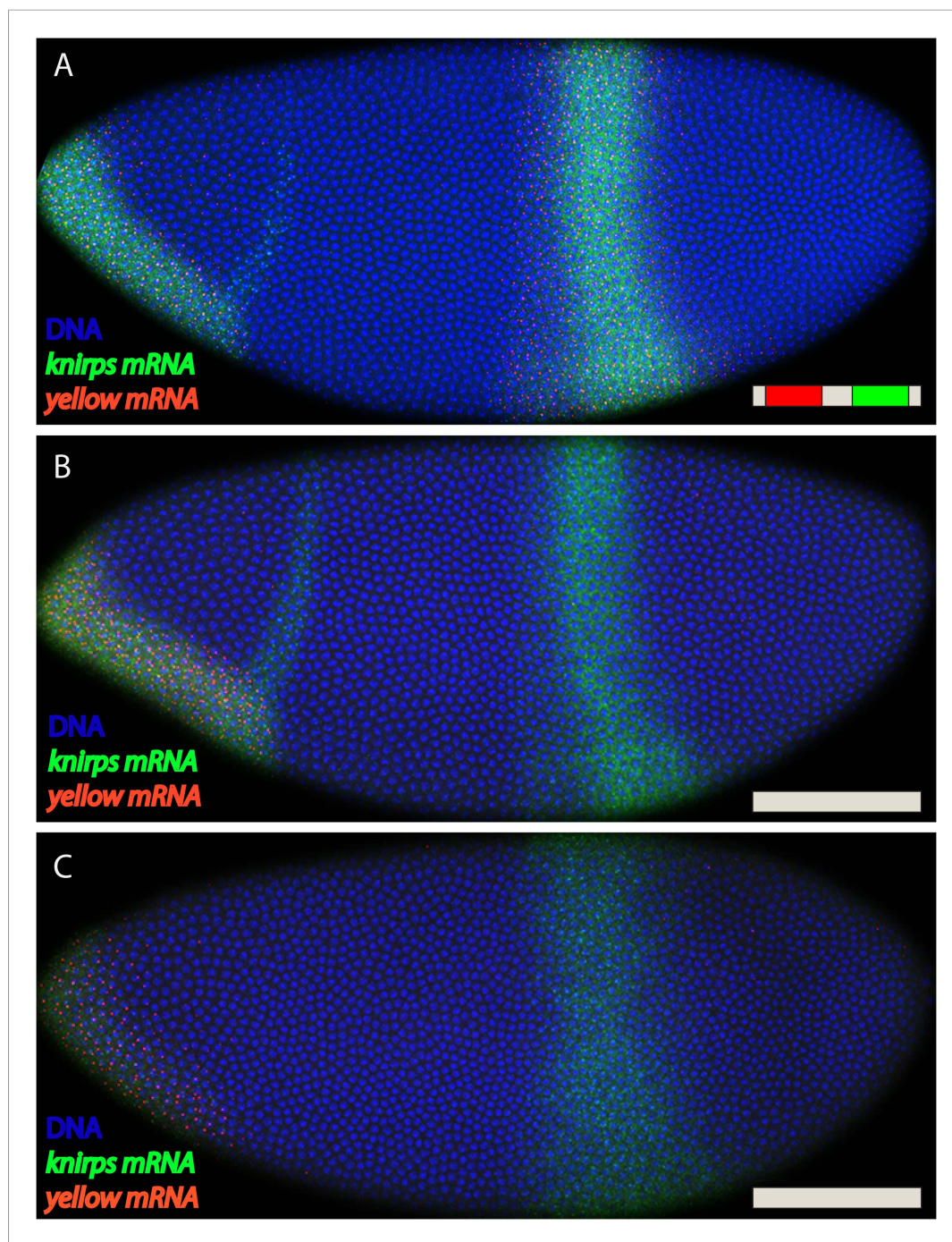
Enhancer additivity and non-additivity are determined by enhancer strength in the *Drosophila* embryo

**Jacques P Bothma, et al.**



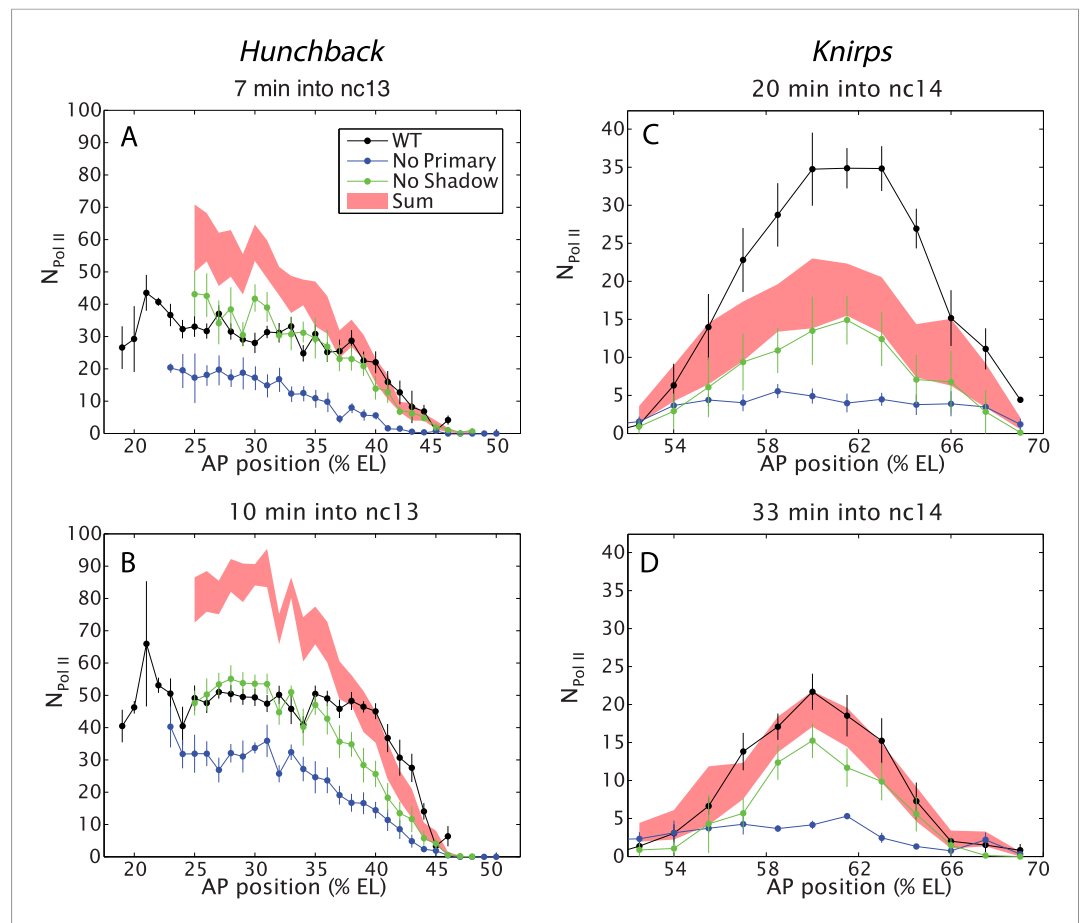
**Figure 1.** Live-imaging of transcriptional activity of *hb* and *kni* loci lacking different enhancers. **(A)** General structure of the reporter constructs. A reporter construct with 24 repeats of the MS2 stem loops and the *yellow* gene was recombined into BACs spanning the *hb* and *kni* loci. The 5' UTR and 3' UTR of the endogenous genes were left intact. The MCP::GFP protein that binds to the MS2 stem loops is present in the unfertilized egg and in the early embryo. Gene models of **(B)** the *hb* and **(C)** *kni* loci showing the location of the primary and shadow enhancers (Perry et al., 2011). **(D, F, H)** Snapshots of *Drosophila* embryos expressing different versions of the *hb* BAC>MS2 reporter containing different combinations of the two enhancers 10 min into nuclear cleavage cycle 13 (nc13). The colored bar on the bottom right indicates which enhancer was removed. **(E, G, I)** Snapshots of *Drosophila* embryos expressing different versions of the *kni* BAC>MS2 reporter containing different combinations of the two enhancers in nc14.

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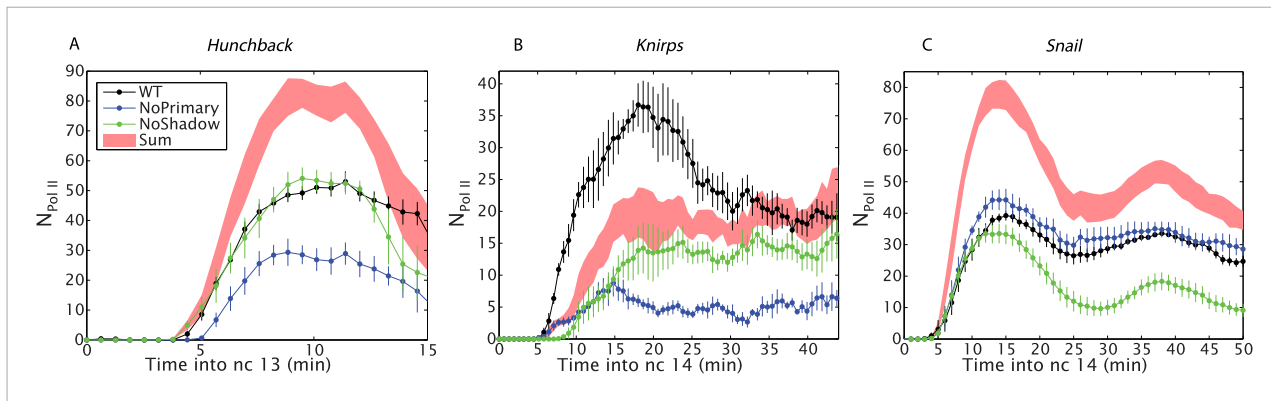
**Figure 1—figure supplement 1.** *kni* BAC expression lacking both shadow and primary enhancers. Fluorescent in situ hybridization of endogenous *kni* and *kni* BAC>*yellow* transgenes. (A) Shows an embryo with the fully intact *kni* BAC>*yellow* transgene in late nc 14. (B, C) Show embryos with the *kni* BAC>*yellow* transgene lacking both primary and shadow enhancers, removing both enhancers abolishes all activity in the stripe domain. In (A) an embryo is in late nc14 and (B) shows an embryo in early nc 14.

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**Figure 2.** Combined effect of multiple enhancers as a function of AP position. (**A, B**) Mean number of Pol II molecules transcribing per nucleus ( $N_{\text{Pol II}}$ ) in the *hb* BAC reporters containing different combinations of enhancers as a function of AP position for two time points in nc13.  $N_{\text{Pol II}}$  is calculated by averaging data from at least three embryos at each AP position. The predicted sum of the individual enhancers is also shown. Note the additivity at the boundary vs the sub-additivity at the core, anterior domain of the pattern. (**C, D**) Mean number of Pol II molecules transcribing per nucleus ( $N_{\text{Pol II}}$ ) in the *kni* BAC reporters in nc14 as a function of AP position. For *kni*, we see super-additive behavior in the beginning of nc14 which then becomes additive later in nc14. The absolute number of transcribing Pol II molecules was estimated following a previous calibration (Garcia et al., 2013). Error bars are the standard error of the mean over multiple embryos.

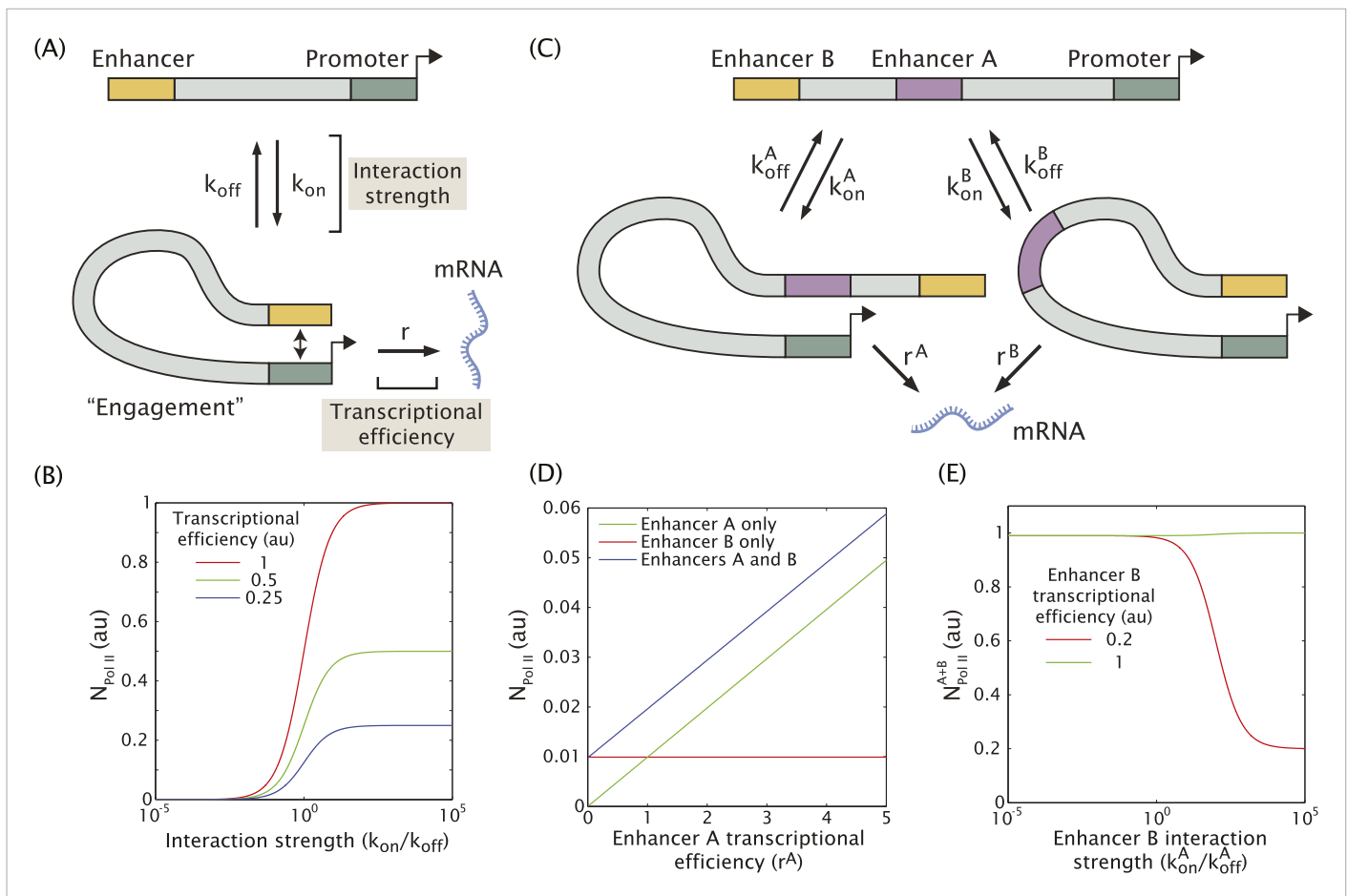
DOI: [10.7554/eLife.07956.008](https://doi.org/10.7554/eLife.07956.008)



**Figure 3.** Combined effect of multiple enhancers as a function of time. **(A)** Time course of the mean number of Pol II molecules transcribing per nucleus ( $N_{\text{Pol II}}$ ) for the different *hb* BAC transgenes and sum of individual enhancers at 27% EL for the duration of nc13. **(B)** *kni* BAC transgenes activities and the sum of individual enhancer activity at 60% EL for the first 50 min of nc14. **(C)** *sna* BAC transgenes and the sum of individual enhancer activities averaged over the central mesoderm for the initial 50 min of nc14. Error bars are the standard error of the mean over multiple embryos.

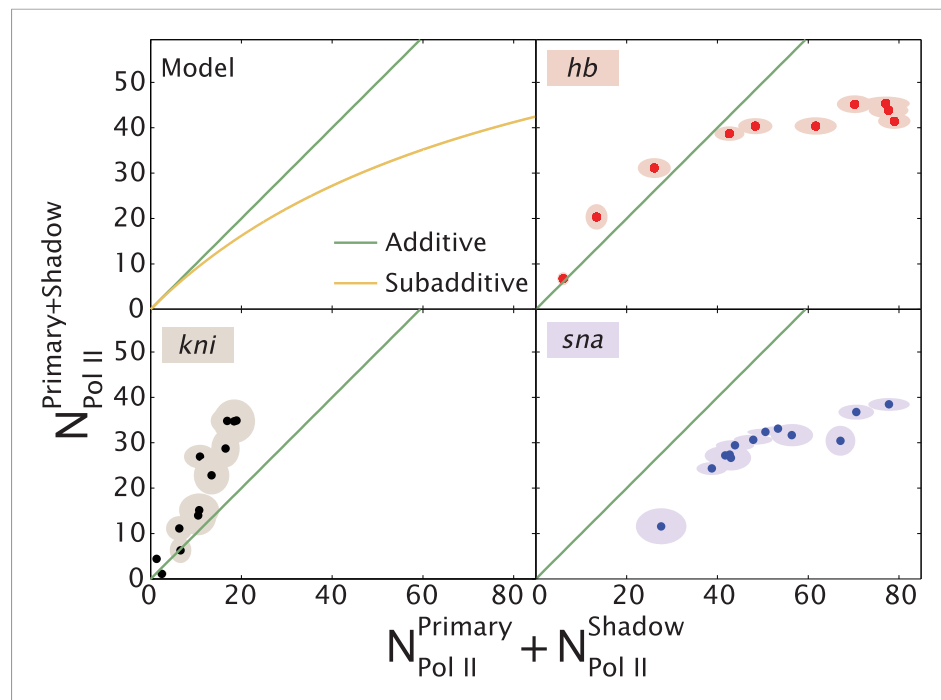
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**Figure 4.** Model of enhancer–promoter interactions and its predictions for mRNA production. **(A)** Minimal model of one enhancer engaging a promoter.  $k_{on}$  and  $k_{off}$  are the rates of promoter engagement and disengagement, respectively, and determine the interaction strength.  $r$  is the rate of mRNA production when the promoter is engaged and is a measure of the transcriptional efficiency. The mean number of Pol II molecules transcribing per nucleus ( $N_{Pol II}$ ) is proportional to the rate of mRNA production. **(B)** As the interaction strength of a single enhancer is increased, the amount of mRNA produced increases up to a maximum value dictated by the transcriptional efficiency. **(C)** The model in **(A)** can be generalized to allow for multiple enhancers interacting with the same promoter. **(D)** In the regime where the interaction strength of both promoters is weak ( $k_{on}/k_{off} = 0.01$ ), the amount of mRNA produced by having both **A** and **B** is simply the sum of the individual contributions of **A** and **B**, ( $r = 1$ ). **(E)** In the regime where the interaction strength is large, the combined activity of both enhancers can be significantly less than the sum the individual enhancers. A less efficient enhancer A ( $r^A = 0.2$  au) can interfere with the more efficient enhancer B ( $r^B = 1$  au) such that their combined activity is significantly less than the sum of the activities of individual enhancers.

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**Figure 5.** Theoretical expectation and experimental results showing different regimes of combined enhancer action. (Upper left) Theoretical predictions (yellow) illustrating how the rate of mRNA production from both enhancers,  $N_{\text{Pol II}}^{\text{Primary+Shadow}}$ , varies with the sum of the activity of the individual enhancers,  $N_{\text{Pol II}}^{\text{Primary}} + N_{\text{Pol II}}^{\text{Shadow}}$  (yellow). Mean number of Pol II molecules transcribing per nucleus ( $N_{\text{Pol II}}$ ) is proportional to the rate of mRNA production. The green line shows perfect additivity for comparison. The model predicts additive behavior ( $N_{\text{Pol II}}^{\text{Primary+Shadow}} \approx N_{\text{Pol II}}^{\text{Primary}} + N_{\text{Pol II}}^{\text{Shadow}}$ ) when the rate of production is low and sub-additive behavior ( $N_{\text{Pol II}}^{\text{Primary+Shadow}} < N_{\text{Pol II}}^{\text{Primary}} + N_{\text{Pol II}}^{\text{Shadow}}$ ) as the production rate increases. As the interaction strength of individual enhancers increases so does the rate of mRNA production, but the combined activity of both enhancers becomes sub-additive. (Upper right, lower left, lower right) Transcriptional activity of intact loci vs the sum of activities of individual enhancers for *hb*, *kni*, and *sna* at different times. A green line has been drawn in to indicate where  $N_{\text{Pol II}}^{\text{Primary+Shadow}}$  is equal to  $N_{\text{Pol II}}^{\text{Primary}} + N_{\text{Pol II}}^{\text{Shadow}}$ . For *hb* and *kni*, the plots show data taken at different AP positions at 10 min into nc 13 and 20 min into nc 14, respectively, while for *sna* the datapoints were at different times. Ellipses indicate standard error of the mean.

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