#### Legends for Supplementary Figures:

### Supplementary Figure 1. Hb does not directly activate *hb* transcription in neuroblasts. Related to Figure 2

Ventral view of wild type stage 11 embryo (left) hybridized with mRNA probe against 3'UTR of the endogenous *hb* transcript, which is not present in the *UAS-hb* construct and thus specifically detects endogenous *hb* RNA. Overexpression of ectopic Hb protein in neuroblasts (right, image taken from E13 embryo which does not express any Hb protein in neuroblasts) does not activate endogenous *hb* transcription (middle, *hb* mRNA expression is similar to that of the same stage wild type embryo). Midline, dotted line.

## Supplementary Figure 2. *lamin<sup>sz18</sup>* mutants show decreasing lamin protein levels at E11-E13. Related to Figure 5

(A-C) Lamin protein levels decrease by E13 in *lamin<sup>sz18</sup>* mutants.

## Supplementary Figure 3. Hb, Svp, Dan expression are unchanged in *lamin<sup>sz18</sup>* mutants. Related to Figure 5

Hb, Svp and Dan expression all display the same temporal dynamics in *lamin<sup>sz18</sup>* mutants as they do in wild type embryos.

## Supplementary Figure 4. Downregulation of Dan neuroblast expression is correlated with the *hb* gene movement to the nuclear periphery.

### **Related to Figure 6**

(A-D) Dan (green) nuclear protein is detectable in all neuroblasts (Miranda, magenta) until it is rapidly downregulated at stage 12 (C), the stage when *HA:hb* and *hb* loci move to the nuclear periphery.

## Supplementary Figure 5. *hb* gene positioning and NB7-1 competence window is unchanged in *dan danr<sup>ex56</sup>* mutants.

### Related to Figure 6

(A) HA:Hb+ (green) U motoneurons (marked by Eve, red) were detected in embryos with prolonged ectopic Hb protein expression in neuroblasts in either wild type or *dan* mutants.

(B) quantified results of (A). Numbers scored are as follows: wild type N=6 embryos (99 hemisegments), dan danr<sup>ex56</sup> mutants N=4 embryos (78 hemisegments).

(C,D) *dan danr<sup>ex56</sup>* mutants lack detectable Dan protein (C) and have no significant change in the percentage of hb loci at the nuclear periphery at stage 11 (D). Error bars = SD. n.s. = not significant



Figure S1 Kohwi/Doe



# Figure S2 Kohwi/Doe





Figure S3



Figure S4 Kohwi/Doe

