## **Supplemental Figures**



Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 4

## **Supplemental Figure Legends**

Supplement Figure 1. *Strategy used to create the Fab-7<sup>attp50</sup> platform* (see material and methods section). Panel A. The hypersensitive sites "\*", HS1 and HS2 are shown in red following the same scheme shown in Figure 2. HS3, which corresponds to the *iab-*7PRE, is shown in blue. Relevant restrictions sites for the cloning strategy are drawn. The top line in Panel B depicts the structure of the *KSattBFLFab7ry* plasmid that is injected to integrate modified *Fab-7* elements within *Fab-7<sup>attp50</sup>*. Note that the Nco I and Xba I sites are unique in the *KSattBFLFab7ry* vector, allowing rapid replacement by *Fab-7* or other heterologous DNAs. The middle line in panel B shows *KSattBFLFab7ry* once integrated within *Fab-7<sup>attp50</sup>*. Finally the bottom line shows the reconstituted *Fab-7* region after floxing the *rosy* and plasmid cassette. The scale is indicated at the bottom of the grey panel. Note that despite the fact the *rosy* and plasmid cassette displace *Fab-7* from *iab-7* by about 10.2 kb, the resulting flies look perfectly wild type upon integration of the WT *Fab-7* control construct.

Supplement Figure 2. Fab-7 dHS1 probes are bound by the same late stage specific factor.

A) GAGA3 and GAGA4 are more effective competitors than GAGA5. Labeled probe:
GAGA4; Cold competitors added at 50- and 100- fold excess): hsp70 GAGAG, GAGA4,
GAGA3, and GAGA5 (lanes 3-10); LBC: black arrowhead; F: free probe.
B) GAGA6 inefficiently competes LBC binding to GAGA5. Labeled probe: GAGA5; cold
competitors added at 50-, 100-, and 200- fold excess): *Fab-7* MultiR (A multimerized

28bp region located between GAGA5 and GAGA6), GAGA4, GAGA5, and GAGA6; LBC: black arrowhead; F: free probe.

Supplement Figure 3. *LBC binding to GAGA3-65 and GAGA3-65 are competed by each other and by the larger LBC probes.* 

(A) EMSAs with labeled GAGA3-65 and unlabeled cold competitors as indicated above the lanes. Cold competitors were added at 50- and 100- fold excess. The cold competitors were: *hsp70* GAGAG, GAGA3-65, GAGA3, GAGA4, and GAGA5, LBC: black arrowhead; F: free probe.

(B) EMSA with labeled GAGA4-65 and unlabeled cold competitors as indicated above the lanes. Cold probes were added at 100-fold excess. The cold competitors were: *hsp70* GAGA, GAGA3-65, GAGA4-65, and GAGA4. LBC: black arrowhead; F: free probe.

Supplement Figure 4. *The LBC is a large protein complex.* Gel filtration profile. Nuclear extract derived from 6-18 hr nuclear extracts was fractionated by size exclusion chromatography. Probe G3+4 was incubated with nuclear extracts from late embryos, (lanes 2 and 3) and 10  $\mu$ l of the column fractions (lanes 4-31) along with rat serum. A black arrowhead and the letter F indicate the identity of the shifted LBC band and the free probe. In this experiment the "peak" fractions (14-17) were estimated to between ~600-750 kD.