Supporting information

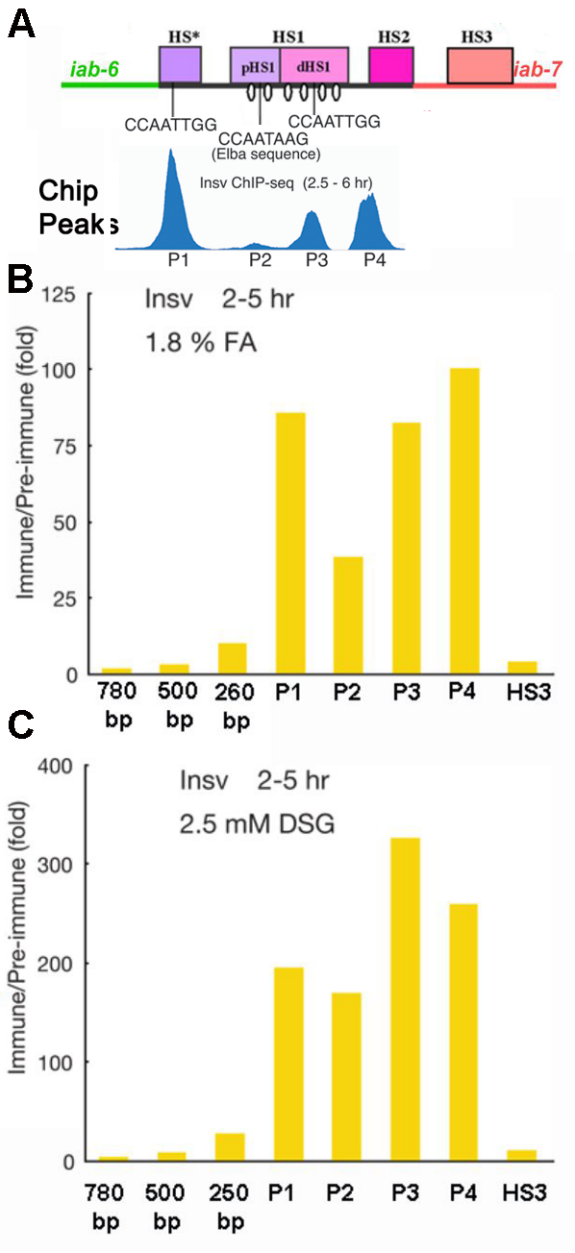

Fig. S1. Insv chromatin immunoprecipitation across the *Fab-7* hypersensitive sites HS*, HS1, and HS2. Recovery of fragments (measured by real time PCR) spanning the indicated Insv binding sites, P1, P2, P3, and P4, plus sequences located to either side of the *Fab-7* boundary, as indicated. Two different procedures were used for the ChIP

experiments (Aoki *et al.* 2014). The first follows the standard formaldehyde cross-linking procedure. The second uses a procedure developed for ChIP experiments on the Elba factor. Plotted is the ratio of DNA recovered after immunoprecipitation with Insv antibody (immune) to pre-immune serum.

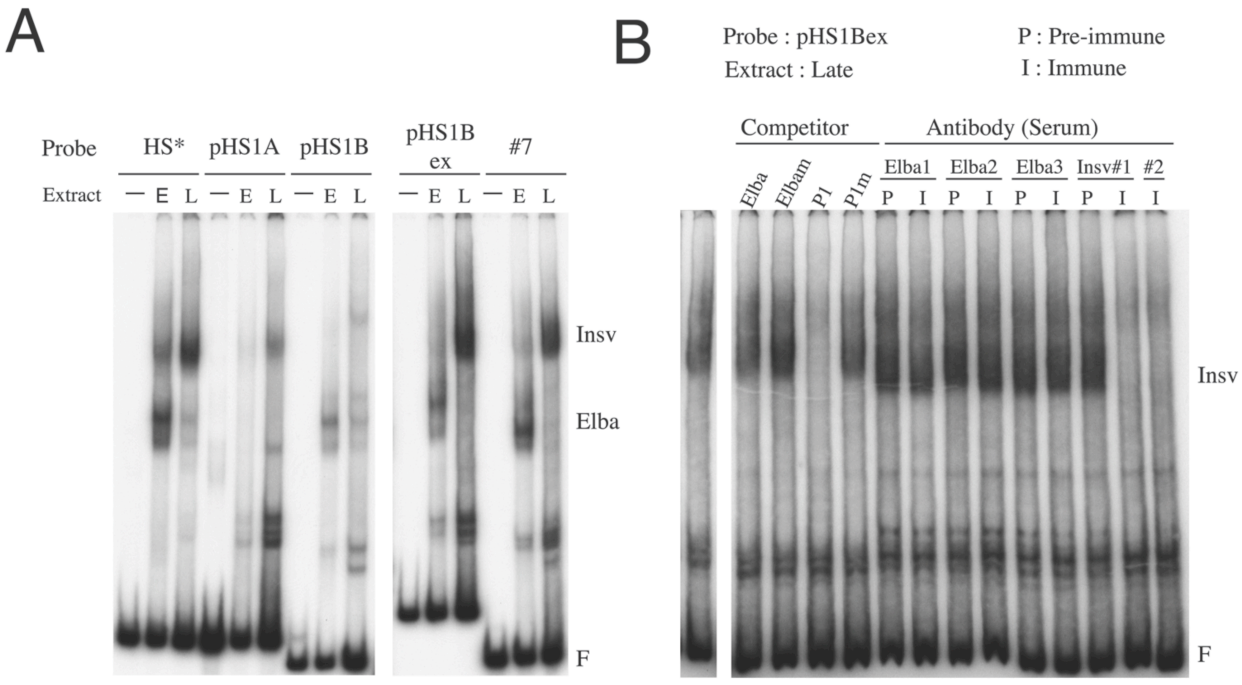

Fig. S2. Insv binds to P2. Panel A: EMSA of different P2 probes as indicated in Fig 5. (-) no extract; E: 0-6 hr "early" embryo nuclear extract, L: 6-18 hr "late" embryo extract. The probe HS* corresponds to the entire sequence of DNase-hypersensitive region HS* (See the schematic diagram of Fab-7 in Fig 1A), which include the palindromic P1 (CCAATTGG) sequence. The positions of other probes are indicated in Fig 5A. Panel B: Competition and supershift analysis of Insv binding to the P2 recognition sequence. EMSA of pHS1Bex incubated with the late embryo nuclear extracts. Lanes as follows: C: control, late nuclear extract only. Competitors: Elba, Elbamut, P1 P1mut. Incubation

contained cold competitors as indicated. Antibody supershifts: Elba1, Elba2, Elba3, Insv1#, and #2 (Insv2#2). Incubation contained either pre-immune (P) or immune (I) serum, as indicated.

Probe	Length (bp)	Sequence (top strand, proximal5'-3'distal)	Genomic position
P1	32	AAGGACGCATTTCCAATTGGGAAAGAAACCCA	16898566 - 16898597
P1mut (P1m)	32	AAGGACGCATTTCCCCGTGGGAAAGAAACCCA	N/A
P3	32	CCACCGCAAAATCCAATTGGAAGAGAGCGACT	16899239 - 16899270
P3mut (P3m)	32	CCACCGCAAAATCCCCGTGGAAGAGAGCGACT	N/A
Elba	27	TGCAGCGCCCAATAAGCAAATGGCAGC	16898981 - 16899007
Elbamut (Elbam)	27	TGCAGCGCCCCCGAAGCAAATGGCAGC	N/A
HS*	147	ATAATTTCACCTATAATTCAATGAGATCGAAATATACTCTG ATTAAGATGATCTTAAATTAAATCCAACTGCAGTGAAGACA CGAACCCCAAGGACGCATTTCCAATTGGGAAAGAAACCCAT TGGTGCAGACTTTGTTCAACATTG	16898476 - 16898622
pHS1A	133	GTGGCAAAAGCTGGCAAAGCAGCAAAAATCGTAAAAAAGAA AATTGCATTTCCCCAAAGCAGCGAAACTTGCGCAGGACTTT TGAGATTCTATTAAATTCTAACAAGATTTCAAGCTGTGTGG CGGGGGAAAG	16898831 - 16898963
pHS1B	121	CTGTGTGGCGGGGGAAAGAGGAAGAGAGCGGAAAGTGCAGC GCCCAATAAGCAAATGGCAGCTGTCACGGGGAAGCACAGAG AGTGCAGAAAGGGGAAAAAACATTGGGGCATATCAACGC	16898946 - 16899066
pHS1Bex	169	CTTGCGCAGGACTTTTGAGATTCTATTAAATTCTAACAAGA TTTCAAGCTGTGTGGCGGGGGAAAGAGGAAGAGAGCGGAAA GTGCAGCGCCCAATAAGCAAATGGCAGCTGTCACGGGGAAG CACAGAGAGTGCAGAAAGGGGAAAAAACATTGGGGCATATC AACGC	16898898 - 16899066
pHS1BexME	169	CTTGCGCAGGACTTTTGAGATTCTATTAAATTCTAACAAGA TTTCAAGCTGTGTGGCGGGGGAAAGAGGAAGAGAGCGGAAA GTGCAGCGCCCCCGAAGCAAATGGCAGCTGTCACGGGGAAG CACAGAGAGTGCAGAAAGGGGAAAAAACATTGGGGCATATC AACGC	N/A
pHS1BexMG	169	CTTGCGCAGGACTTTTGAGATTCTATTAAATTCTAACAAGA TTTCAAGCTGTGTGGCGGGGGAAAGAGGAATTTGTCGGAAA GTGCAGCGCCCAATAAGCAAATGGCAGCTGTCACGGGGAAG CACAACTAGTGCAGAAAGGGGAAAAAACATTGGGGCATATC AACGC	N/A
#7	117	CTTGCGCAGGACTTTTGAGATTCTATTAAATTCTAACAAGA TTTCAAGCTGTGTGGCGGGGGAAAGAGGAAGAGAGCGGAAA GTGCAGCGCCCAATAAGCAAATGGCAGCTGTCACG	16898898 - 16899014
#8	98	ATTCTATTAAATTCTAACAAGATTTCAAGCTGTGTGGCGGG	16898917 - 16899014

		GGAAAGAGGAAGAGAGCGGAAAGTGCAGCGCCCAATAAGCAAATGGCAGCTGTCACG	
#9	110	CTTGCGCAGGACTTTTGAGATTCTATTAAATTCTAACAAGATTTCAAGCTGTGTGGCGGGGGAAAGAGGAAGAGAGCGGAAAGTGCAGCGCCCAATAAGCAAATGGCAGC	16898898 - 16899007
#10	102	CTTGCGCAGGACTTTTGAGATTCTATTAAATTCTAACAAGATTTCAAGCTGTGTGGCGGGGGAAAGAGGAAGAGAGCGGAAAGTGCAGCGCCCAATAAGCAA	16898898 - 16898999

Table S1. Probe sequences

The sequences of probes used in this work. The genomic positions correspond to base numbers of Drosophila melanogaster genome version 6. The binding sites for each factors are highlighted in the colors below; palindromic Insv/Elba site : blue, original Elba site : light blue, GAF : orange. The mutations introduced in the probes are highlighted in red.