

# Supplementary Results

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## *Yippee*<sup>s1</sup> cDNA sequences

*Yippee*<sup>s1</sup> cDNAs were amplified using RT-PCR, then, products were cloned and sequenced as described in Materials and Methods in the main text. Alignment of cDNAs to *s*<sup>1</sup> genomic DNA are shown in Figure 6 (cDNA from pupae) and in Figure S6 (cDNA from adults) and annotated in the genomic sequence of the *s*<sup>1</sup> insertion deposited at Genbank (accession OM135585). We chose to separate the data into two figures for clarity of presentation because different primer sets were used for the two experiments (pupa and adults). The obtained *s*<sup>1</sup> *Yippee* cDNA sequences are:

### >Pupa-type1

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### >Pupa-type2

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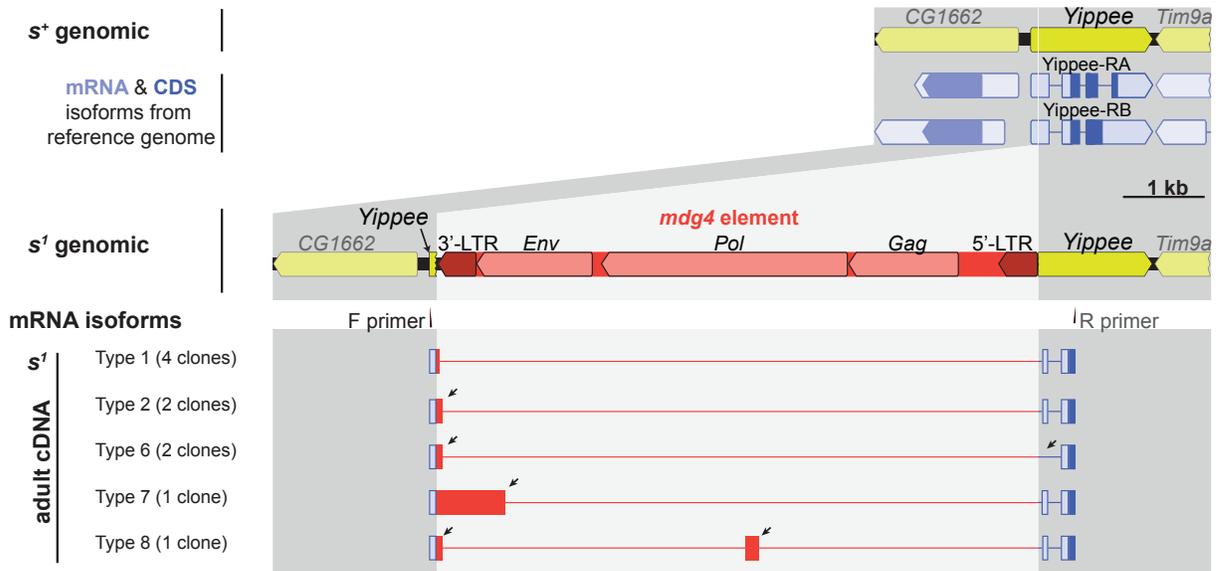
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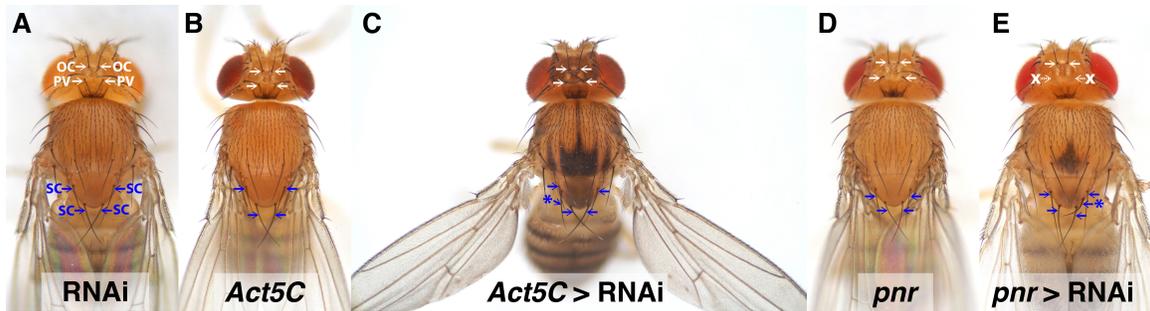
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TCTGCGC



**Figure S6.** Identified isoforms of *Yippee* from adult *s<sup>1</sup>* cDNA. This is a similar experiment to the pupal data shown in Figure 6. Arrowheads point to segments of *s<sup>1</sup>* cDNAs that differed from the typical splice pattern. Adult male *w<sup>1118</sup> s<sup>1</sup>* flies were ground in Trizol (Thermo Fisher) using microtube pestles and RNA isolated with the Direct-Zol RNA Miniprep kit (Zymo Research). RNAs were then DNase treated, oligo-dT annealed, and cDNA synthesized using the SuperScript IV First-Strand Synthesis System with EZ DNase (Thermo Fisher). cDNA was PCR amplified using the primers Yippee-9F and Yippee-seq-R3, products cloned into pGem-T-Easy, and sequenced. The primers used in this assay did not contain the entire *Yippee* coding sequence, so inference about the coding capacity was not possible. mRNA isoforms Type 1 and Type 2 were also observed in pupae (Figure 6).



**Figure S7.** Loss of *Yippee* function affects bristle numbers. High-resolution version of Figure 3A-E to emphasize examples of *Yippee* bristle phenotypes. (A) RNAi-only control ( $w^{1118}; +/UAS-YippeeRNAi$ ). (B) *Act5C*-GAL4-only control, ( $w^{1118}; Act5C-GAL4/+$ ). Both (A) and (B) have the wild-type complement of 4 scutellar bristles on the posterior thorax (SC, blue arrows), two ocellar bristles just anterior to the ocelli (OC, white arrows), and two postvertical bristles just posterior to the ocelli (PV, white arrows). (C) *Act5C > RNAi* fly ( $w^{1118}; Act5C-GAL4/UAS-YippeeRNAi$ ), with dark body color, outheld/curved wings, and also an ectopic scutellar bristle (\*). (D) A *pnr*-GAL4-only control ( $w^{1118}; pnr-GAL4/+$ ) has the wild-type number of scutellar, ocellar, and postvertical bristles. (E) In addition to having dark body color in patches along the dorsal midline, this *pnr > RNAi* fly ( $w^{1118}; pnr-GAL4/UAS-YippeeRNAi$ ) has an ectopic scutellar bristle (\*) and is missing both postvertical bristles (X marks with dashed white arrows). In other cases not shown in this figure, *pnr > UAS-YippeeRNAi* and *s<sup>1</sup>/Df(1)Exel6245* flies were missing ocellar bristles instead of or in addition to postvertical bristles. See Table 1 for quantitative bristle data.