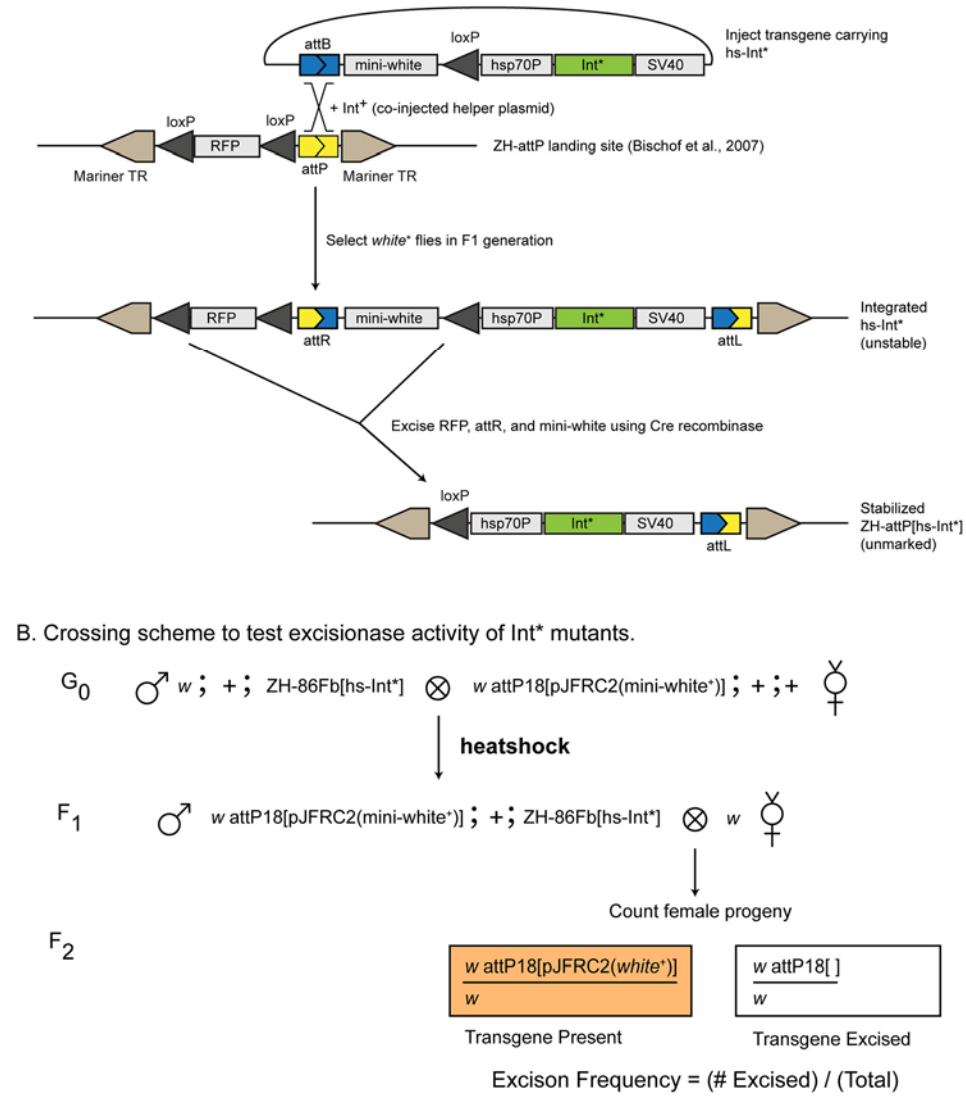

Figure S1. Testing Int* variants for excisionase activity *in vivo*.

Figure S1 Testing Int* variants for excisionase activity *in vivo.* (A) Strategy to stably and site-specifically introduce Int* into the genome: Int* transgene constructs bearing a single loxP site (5′ to hs-Int*) were integrated into the landing sites *ZH-51C* or *ZH-86Fb* (Bischof *et al.* 2007), and integrants were identified by mini-white expression. To prevent Int* from excising itself, sequences between the upstream-most loxP in the landing site and the loxP in the integrated transgene were eliminated by Cre recombinase. The removal of RFP and mini-white leaves the *hs-Int** transgene unmarked, to avoid interference with downstream applications. (B) Crossing scheme to test Int* variants for excisionase activity: Virgins bearing *attP18[JFRC2]* were crossed to males bearing *hs-Int**, and progeny were heat shocked for one hour during the third larval instar. Following eclosion, individual males were crossed to *white* virgins, and the numbers of female progeny that were *mini-white*$^{+}$ (transgene present) and *white* (transgene excised) were counted. The frequency of transgene excision was determined by comparing the number of *white* female progeny to the total number of female progeny. Also see Figure 1D and Table S1.