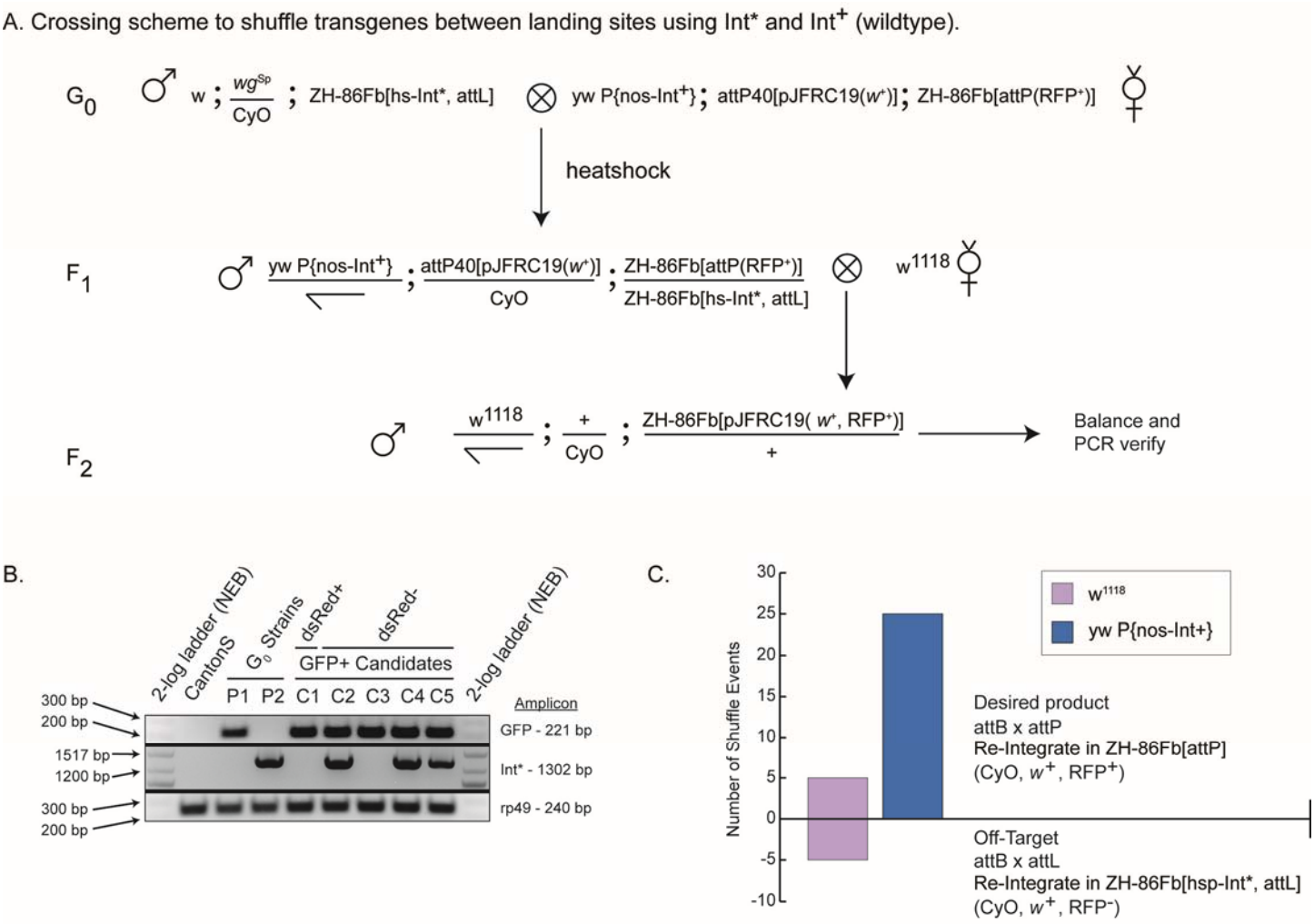

Figure S6. Genetics and integration characteristics of Int*/Int transgene shuffling.

Figure S6 Genetics and integration characteristics of Int*/Int transgene shuffling. (A) Crossing scheme to shuffle pJFRC2 between two autosomal landing sites. In this example, wild-type integrase is supplied by an *X*-linked transgene (Bischof *et al.* 2007). The movement of pJFRC19 from *attP40* (donor on *II*) to *ZH-86Fb* (receiver on *III*) was followed using the transgene's mini-white marker. Flies in the F_2 generation that carried mini-white and *CyO* carried candidate shuffle events; genetic mapping and PCR were used to confirm the presence of pJFRC19 at the receiver site. (B) Molecular characterization of selected shuffle candidates. Top – Genomic PCR confirmed the presence of GFP in five shuffle candidates (C1-C5). P1 corresponds to the maternal G_0 genotype, *w; attP40[pJFRC19(w+)]; ZH86Fb[attP(RFP+)].* P2 corresponds to the paternal G0 genotype, *w; wg^{Sp}/CyO; ZH86Fb[hs-Int*, attL].* Middle – Genomic PCR detected Int* in three shuffle candidates. Int* was not detected in candidate C1, since pJFRC19 shuffled into the receiver landing site (marked by *3xP3-DsRed*). Int* was detected in C2, C4, and C5. This indicates that pJFRC19 re-integrated on the hs-Int* chromosome, which is corroborated by the absence of DsRed expression in these flies. The attL sequence downstream of hs-Int* (see Figure S1A) presented a potential re-integration target due to the relaxed integration site specificity of Int*. The re-integration site of candidate C3 was not determined, though the lack of DsRed strongly suggests this was off-target. Bottom – PCR control with rp49. (C) Wild-type Int enforces canonical attP x attB recombination during transgene shuffling: Flies were scored for the presence of the *ZH-86Fb* landing site marker 3xP3-DsRed to distinguish re-integration at the receiver site from off-target integration (see panel B). When Int* provided both excisionase and integrase activities, half of the recovered candidates lacked DsRed, indicating re-integration at a site other than the receiver site. In contrast, in the presence of wild-type integrase, all candidates re-integrated at receiver landing site.