**Table S1 Excisionase activity of all Int* variants, and accessibility of several genomic locations.**

A.

| Int* | Excision Frequency | N (vials) | Int* location | Vector | Landing Site | Resident Transgene |
|---|---|---|---|---|---|---|
| **E449K** | 64% ± 8% | 10 | ZH-86Fb | Gen1 | attP18 | pJFRC2-UAS-mCD8gfp |
| **E449K** | 51% ± 11% | 10 | ZH-86Fb | Gen2 | attP18 | pJFRC2-UAS-mCD8gfp |
| **E449K, E463K** | 61% ± 10% | 10 | ZH-86Fb | Gen1 | attP18 | pJFRC2-UAS-mCD8gfp |
| **E456K** | 0.1% ± 0.2% | 10 | ZH-86Fb | Gen2 | attP18 | pJFRC2-UAS-mCD8gfp |
| **E449H** | 40% ± 8% | 10 | ZH-86Fb | Gen2 | attP18 | pJFRC2-UAS-mCD8gfp |
| **E449H, E463H** | 1% ± 2% | 10 | ZH-86Fb | Gen2 | attP18 | pJFRC2-UAS-mCD8gfp |
| **E449H, E463G** | 23% ± 6% | 10 | ZH-86Fb | Gen2 | attP18 | pJFRC2-UAS-mCD8gfp |
| **E449G, E463H** | 3% ± 2% | 10 | ZH-86Fb | Gen2 | attP18 | pJFRC2-UAS-mCD8gfp |
| **E449K, E456K, E463K** | 16% ± 7% | 8 | ZH-86Fb | Gen1 | attP40 | pJFRC2-UAS-mCD8gfp |

B.

| Landing Site | Location | Landing Site Reported | Int* | Int* location | N (vials) | Excision Frequency |
|---|---|---|---|---|---|---|
| **attP18** | **X - 6C** | **Markstein et al., 2008** | **E449K, E463K** | **ZH-51C** | **10** | **36% ± 8%** |
| **attP18** | **X - 6C** | **Markstein et al., 2008** | **E449K, E463K** | **ZH-86Fb** | **10** | **62% ± 16%** |
| attP40 | 2L - 25C | Markstein et al., 2008 | E449K | ZH-86Fb | 6 | 34% ± 10% |
| su(Hw)attP5 | 2R - 51E | Ni et al., 2009 | E449K | ZH-86Fb | 7 | 60% ± 16% |
| attP2 | 3L - 68A | Groth et al., 2004 | E449K | ZH-51C | 5 | 28% ± 10% |
| VK00005 | 3L - 75B | Venken et al., 2006 | E449K | ZH-51C | 7 | 26% ± 10% |
| su(Hw)attP1 | 3R - 87B | Ni et al., 2009 | E449K | ZH-51C | 7 | 63% ± 10% |
| su(Hw)attP2 | 3R - 92D | Ni et al., 2009 | E449K | ZH-51C | 7 | 59% ± 14% |

Table S1. The excisionase activity of Int* variants, and accessibility of several genomic locations.

(A) The activity of each Int* variant (column 1) was assayed using the genetic scheme detailed in Figure S1B. The observed activity (column 2) of variants ranges from virtually undetectable to greater than 60%. The fifth column, "Vector," indicates the plasmid in which Int* was cloned. These two plasmids are identical in the core transgene (hsp70P-Int*-SV40), though sequence differences outside this region may influence Int* expression (compare first and second rows). See also Figure 1D. (B) Int* activity assayed at multiple genomic locations: Int* at *ZH-51C* or *ZH-86Fb* were used to excise pJFRC2 from various landing sites on the *X* and third or second chromosomes, respectively. The first two rows (bold) permit a comparison between levels of Int* activity when expressed from *ZH-51C* or *ZH-86Fb*. Int* excised pJFRC2 from landing sites on every major chromosome arm, suggesting that the most (and perhaps all) sites in the genome are accessible to Int*.