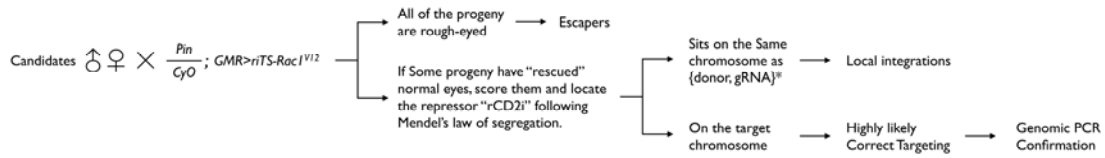
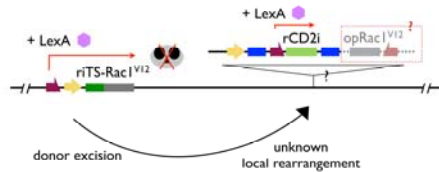


## A Chromosome Mapping and Genomic PCR



## B Local integrations



## Escapers



**Figure S3** Candidate characterization and false positives

(A) A flowchart to characterize GT candidates. Crossing candidates to *GMR>riTS-Rac1<sup>V12</sup>* allows deduction of the presence and location of the rCD2i repressor. Candidates with the repressor relocating to the target chromosome likely carry correct GTs. Those with the repressor remaining on the original chromosome are categorized as local integrations. Escapers, by contrast, carry a defective *5XLexAop2-FRT-riTS-Rac1<sup>V12</sup>* and have therefore, escaped the lethality selection. The escapers without the rCD2i repressor show no suppression of the rough-eyed phenotype in their progeny. (B) Local integrations refer to those retaining the repressor yet losing the non-repressible toxic module and hence surviving the lethality selection, possibly due to local hopping given their presence on the chromosome where {donor} originates from. By contrast, escapers have excised without the repressor-marked GT DNA due to failure in the reconstitution of a functional repressible toxic module at the {donor} residual site, apparently because of imprecise flip-out or premature I-SceI cutting.