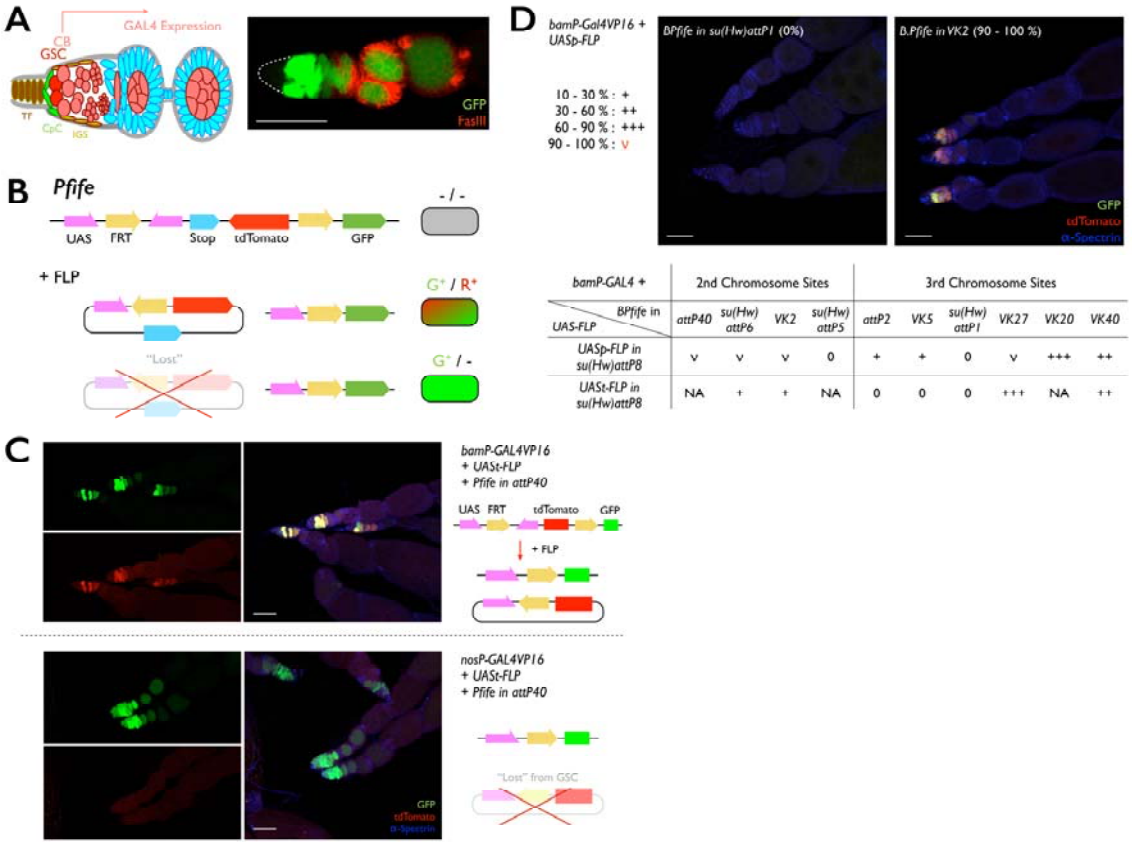

bamP-GAL4 + UAS-FLP / BPfife in	2nd Chromosome Sites				3rd Chromosome Sites					
	attP40	su(Hw) attP6	VK2	su(Hw) attP5	attP2	VK5	su(Hw) attP1	VK27	VK20	VK40
UASp-FLP in su(Hw)attP8	v	v	v	0	+	+	0	v	+++	++
UASt-FLP in su(Hw)attP8	NA	+	+	NA	0	0	0	+++	NA	++

Figure S1 Optimizing cystoblast-specific excision of FRT cassettes
(A) *bamP-GAL4::VP16* drives GFP expression in newborn CBs, but not in the preceding GSCs located at the tip of the germarium outlined with anti-FasIII immuostaining (red). TF: terminal filament; GSC: germline stem cell; CB: cystoblast; CpC: cap cells; IGC: inner germarium sheath cell (B) Pfife carries two interrupted UAS-reporters that are reconstituted based on FLP based recombination at the FRT sites. Upon flip-out, UAS-GFP is expressed by the residual Pfife and UAS-tdTomato is expressed by the circularized FRT cassette. Co-expression of GFP and tdTomato indicates persistence of the excised cassette following flip-out, while GFP alone reports older flip-out events where the circularized FRT cassette has been lost through cell division. (C) Induction of flip-out in ovarioles using *bamP-GAL4::VP16* versus *nosP-GAL4::VP16*. *bamP* elicited flip-out occurs specifically in cystoblasts that were persistently labeled with GFP plus tdTomato, while the *nosP*-mediated flip-out occurred in GSCs prior to adult oogenesis as indicated by expression of GFP alone throughout the female germline. (D) The larger BPfife placed at various attP sites on second and third chromosomes was assayed for flip-out mediated by *bamP-GAL4::VP16*-driven *UASp*- versus *UASt-FLP*. As summarized in the table below, flip-out efficiency varied drastically with the insertion site, and *UASp-FLP* outperformed *UASt-FLP*. Note: 0% flip-out in *su(Hw)attP1* versus almost 100% in *VK2* on a per ovariole basis . Scale bars: 50 μm.

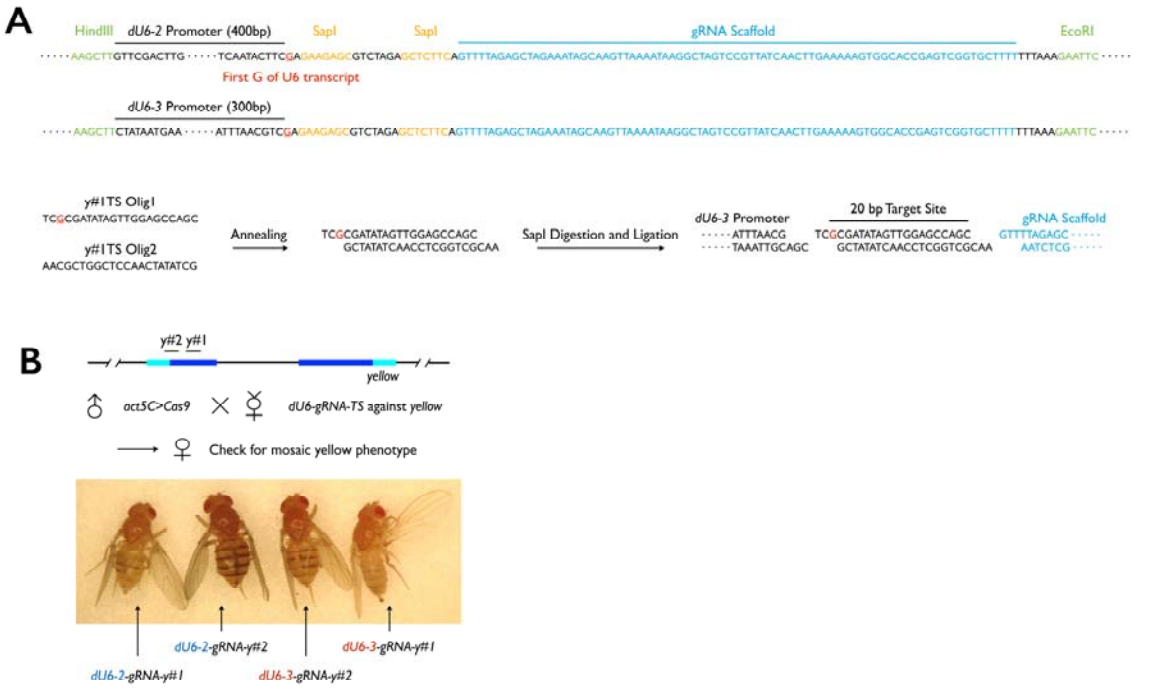

Figure S2 Generation and validation of dU6-gRNA transgenes

Sequence arrangement of the dU6-gRNA scaffolds are shown on top. dU6-gRNA backbone was cloned into pJFRC28 using *Hin*dIII and *Eco*RI sites. Two *Sap*I sites were put in-between *dU6* promoter and the gRNA scaffold for easy target site cloning. gRNA scaffold is the same as the published one (Cong *et al*. 2013; Mali *et al*. 2013). After annealing two corresponding target site primers, the target site can be directly ligated with the SapI-digested empty dU6-gRNA with its TCG and AAG 5' overhangs to constitute a functional dU6-gRNA. (B) Females carrying various *U6-gRNAs* against *yellow* were crossed to males with *act5C>Cas9*. Their female progeny showed allele-dependent yellow body color mosaicism, with *dU6-3-gRNA-y#1* causing a yellow phenotype throughout almost the whole body whereas *dU6-2-gRNA-y#2* affected few, if any, cells.

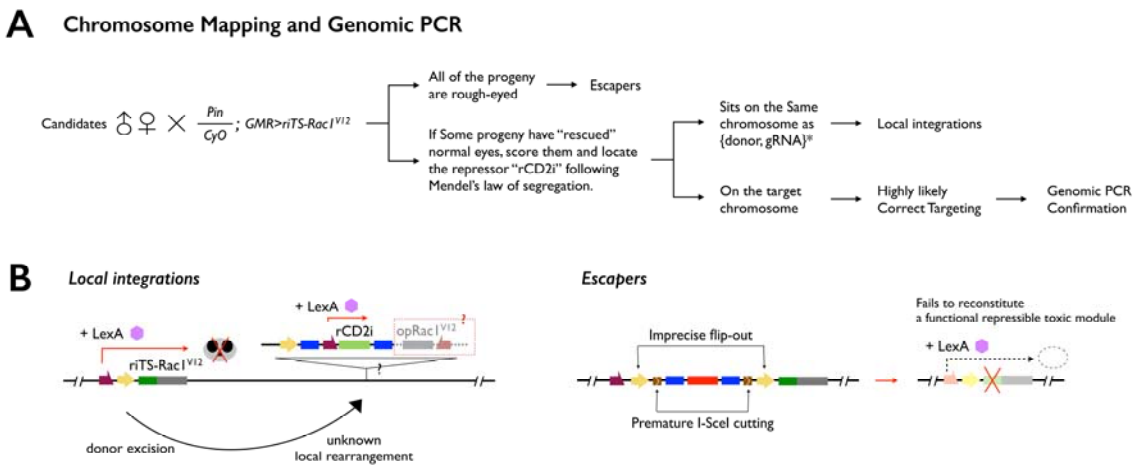

Figure S3 Candidate characterization and false positives

(A) A flowchart to characterize GT candidates. Crossing candidates to *GMR>riTS-Rac1^{V12}* 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^{V12}* 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 eclosed 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.

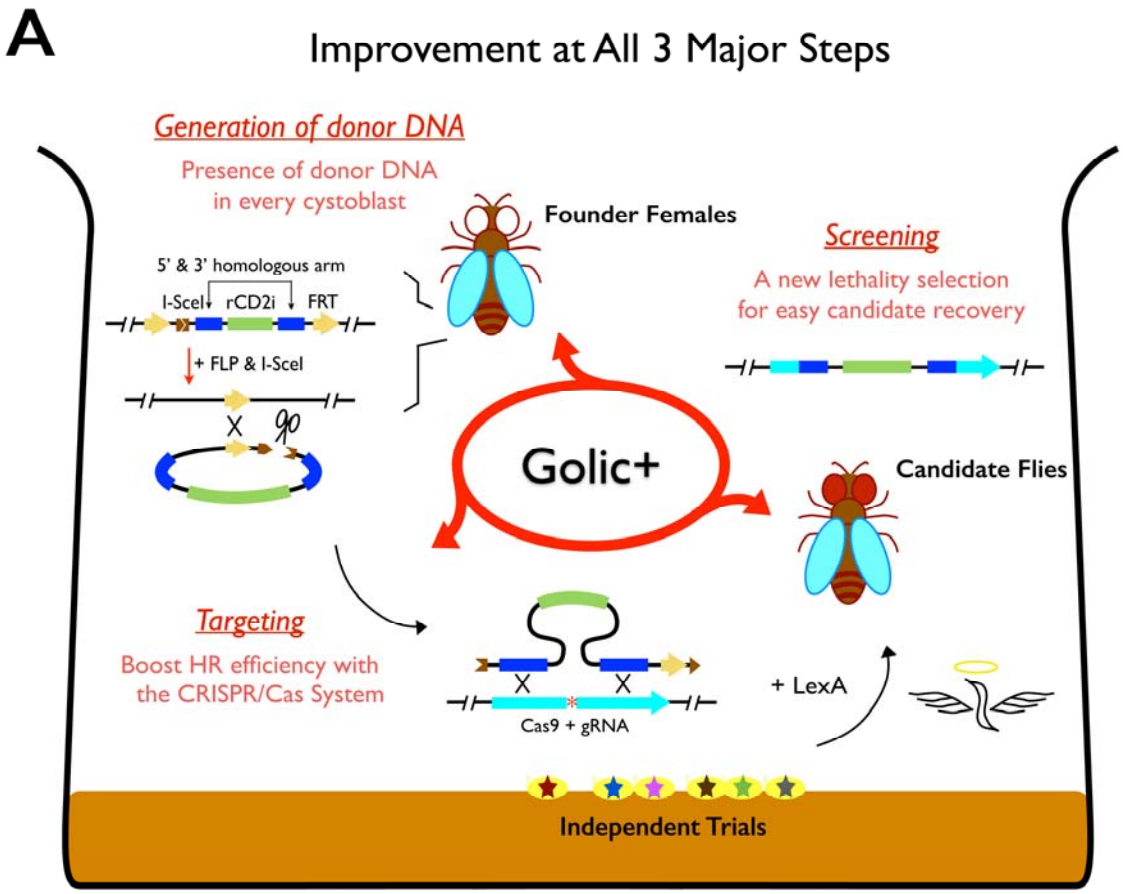

Figure S4 Golic+
Golic+ founder females can be raised en masse in bottles because the linear donor DNA is released in each cystoblast for independent GT trials. Ends-out GT is greatly boosted by CRISPR/Cas and candidates are selectively recovered via suppression of *nSyb-LexA::p65*-mediated pupal lethality.