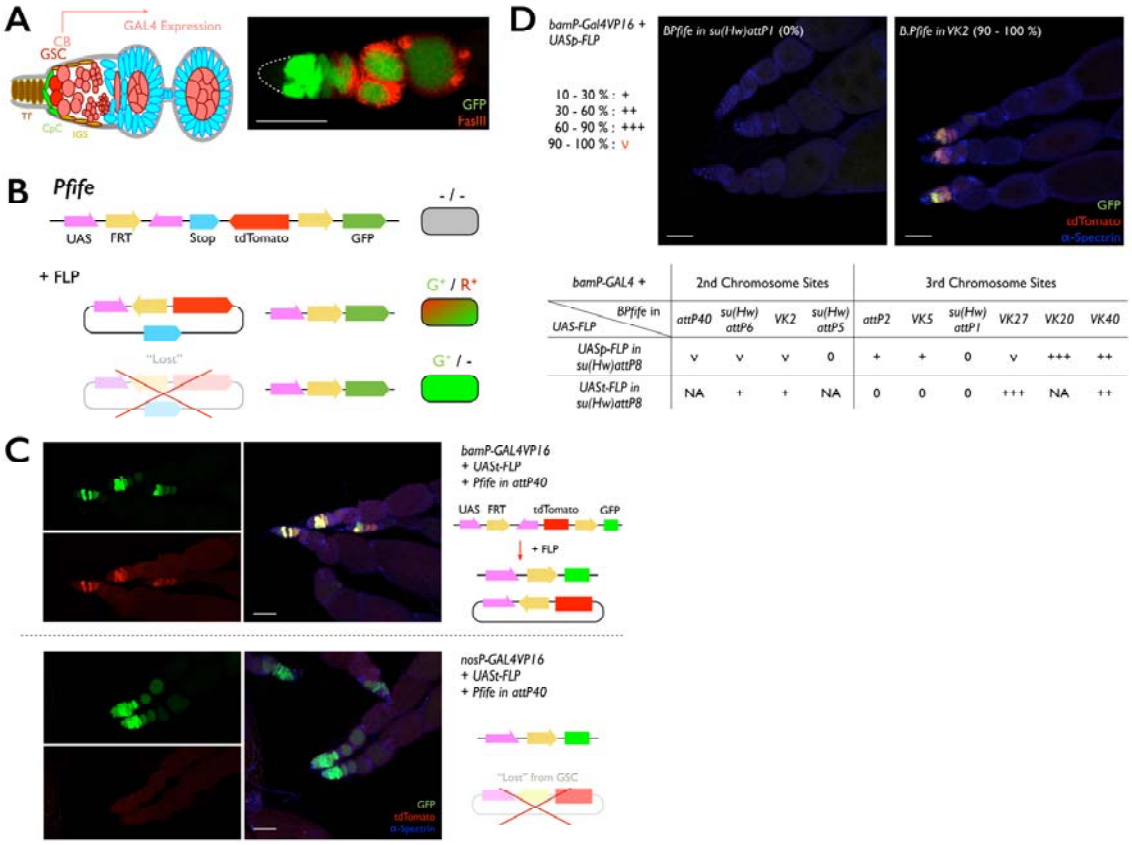

bamP-GAL4 + UAS-FLP / BPfife in	2nd Chromosome Sites				3rd Chromosome Sites					
	attP40	su(Hw) attP6	VK2	su(Hw) attP5	attP2	VK5	su(Hw) attP1	VK27	VK20	VK40
UASp-FLP in su(Hw)attP8	v	v	v	0	+	+	0	v	+++	++
UASt-FLP in su(Hw)attP8	NA	+	+	NA	0	0	0	+++	NA	++

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.