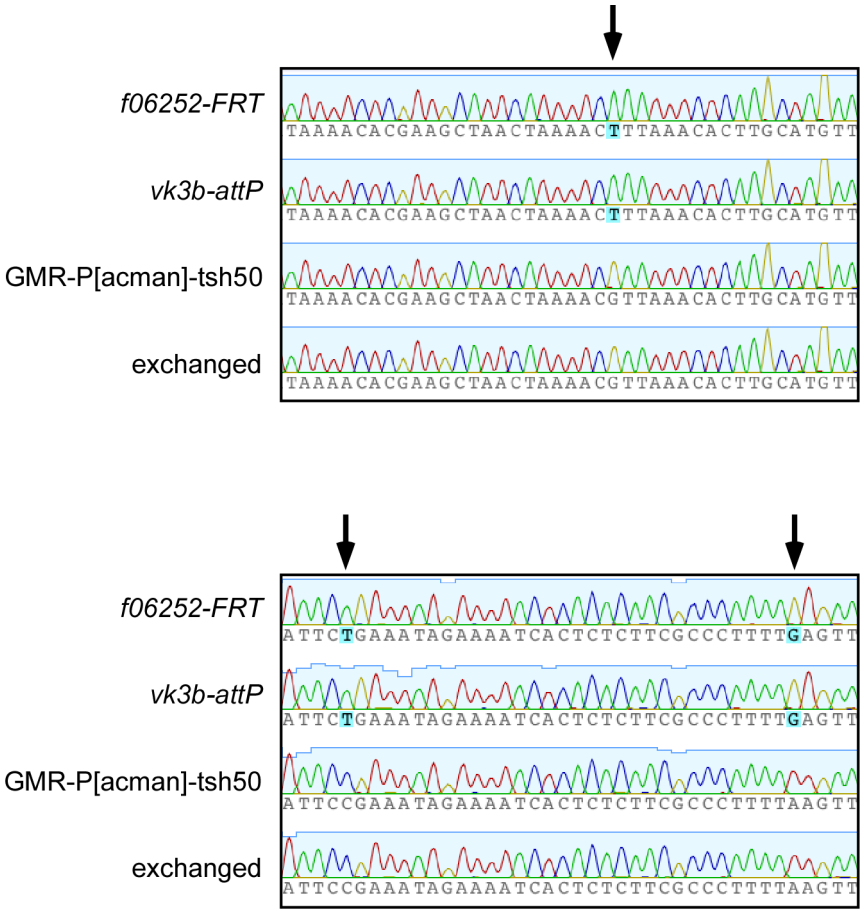

Figure S1 SNP analysis of candidate exchange flies. Shown are traces identifying three SNPs in the 706 bp genomic region amplified by the primer pair SNP2F/SNP2R within the captured segment. For each box, the templates for amplification were: Row 1, *f06252-FRT* genomic DNA (parent chromosome); Row 2, *vk3b-attP* genomic DNA (parent chromosome); Row 3, purified BAC GMR-P[acman]-tsh50 (donor plasmid); Row 4, genomic DNA from candidate $y^- w^-$ flies following exchange. For each SNP, only the BAC allele is observed in genomic DNA of candidate flies. We identified a total of 5 SNPs in this region that differentiate BAC DNA from both parent chromosomes, and in all cases, the allele observed in the BAC was the only allele observed in the amplified region from the genome of candidate flies. In addition, a total of 3 SNPs from a second independently amplified 797 bp region defined by primers SNP5F/SNP5R gave identical results.

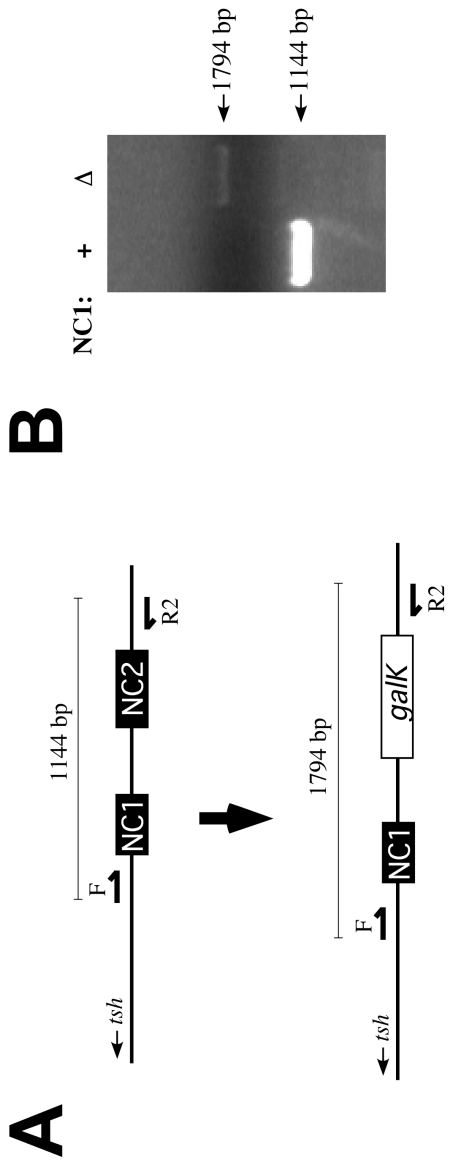

Figure S2 Confirmation of *tshNC2* deletion following captured segment exchange. A, schematic showing relative positions of primers NC1check1for (F) and NC2check1rev (R2). Diagrams are not to scale. B, Ethidium-stained gel showing PCR products from templates where tshNC2 is unaltered (+) or deleted (Δ) and replaced with *galK* using purified BAC DNA or *Drosophila* genomic DNA as templates. Candidate flies homozygous for the recombinant chromosome carrying the deletion (right-most lane) show the predicted PCR product.

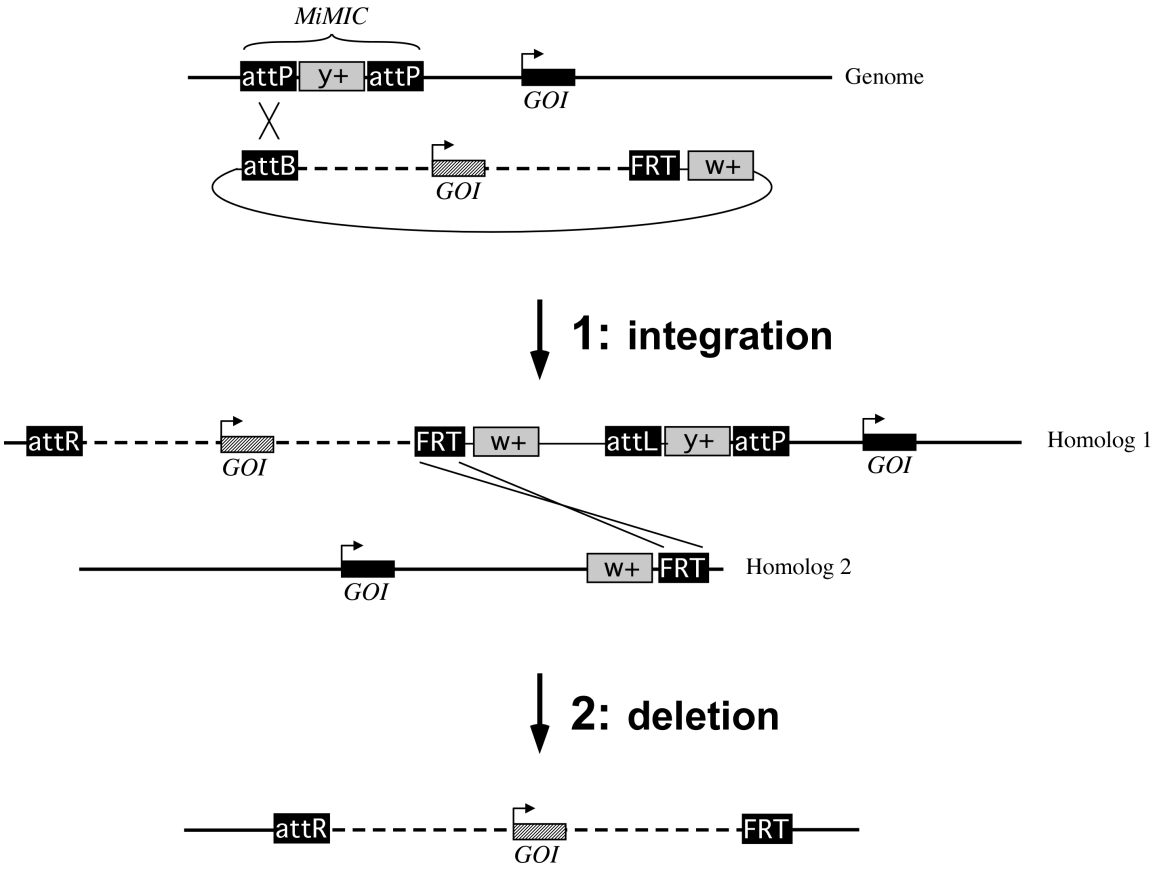

Figure S3 Schematic for two-step dual RMCE using a MiMIC element. MiMIC differs from the *VK3b-attP* element used in the present study in that it carries two attP sites in opposite orientations flanking a *yellow* marker gene. Captured segment exchange can be performed following the same steps as those demonstrated in the main text; in the initial step of inserting the donor BAC, half of all insertions will be in the correct orientation to proceed as outlined in the figure, while, by chance, half of the insertions will use the attP in the opposite orientation (not shown).

Table S1 Primers used in this study.

Primer name	Sequence (5'-3')	Additional information
VK3b_5_3	GGCGCGCCGTACGCGCCCGGGGAGCCCAAGGGCACGCCCTGGCACCCGTAAACGAGACAATTTATAAATGC	Forward primer for LA: includes AscI (orange) and 40 bp attB (blue)
VK3b_3_1	GGATCCTCTGTTTGCTATGGGCTGAA	Reverse primer for LA: includes BamHI (orange)
f06252_5_2	TTCAGCCCATAGCAAACAGAGGATCCAGAGGCTATACAAGTTAGAAAGTTGA	Forward primer for RA: includes complementary sequence to VK3B_3_1 for SOEing (orange)
f06252_3_1	TTAATTAATGAAGTTCCTATACTTTCTAGAGAATAGGAACTTCGCATTAAATAGTCGCAATTATATTTCA	Reverse primer for RA: includes PacI (orange) and 35 bp FRT (blue)
PacmanMCS-F	TTTAAACCTCGAGCGGTCCGTTATC	
tsh-5-vk3b-check-R	GGTTCAGCGGGACTAAGTGA	
tsh-3-06252-check-F	TGAACACACCCATAGGACGA	
PacmanMCS-R	CTAAAGGGAACAAAAGCTGGGTAC	
tshNC1_GalK_F	GCATCTTCTGCTTCTTCTTCATCTTCTTTTTGCTCTTCTTGATGGACAGAACCTGTTGACCAATTAATCATCGGCA	Forward primer for tshNC1 deletion: includes 50 bp homology adjacent to tshNC1 (blue)
tshNC1_GalK_R	CCACCCTCTTCCCAACCAACATCCCCTTCACTCAGTGTTGAAGAAGAATCCTCAGCACTGTCCTGCTCCTT	Reverse primer for tshNC1 deletion: includes 50 bp homology adjacent to tshNC1 (blue)
tshNC2_GalK_F	TATGTACACACTCCGGTACCAGTATTTTTGCCTGCCTTGACGTAAATGCCGCCTGTTGACAATTAATCATCGGCA	Forward primer for tshNC2 deletion: includes 50 bp homology adjacent to tshNC2 (blue)
tshNC1_GalK_R	CCACCCTCTTCCCAACCAACATCCCCTTCACTCAGTGTTGAAGAAGAATCCTCAGCACTGTCCTGCTCCTT	Reverse primer for tshNC2 deletion: includes 50 bp homology adjacent to tshNC2 (blue)
NC1check1for	TGCATCGGAGACAGAGGGAGCA	
NC1check1rev	ACCACACCCATGCCCTATGCC	
NC2check1rev	TGGCCGAAAGGAGGCAGCAAC	
tsh_RRF3	TTGGCACGCCAACTCAACGC	
tsh_RRR3	TGGCAATGGTGCGGCCATCT	

tsh_RLF	TCTTCGTTGCGTGCGGTGGG
tsh_RLR	TGCTGCAGTAGAGAGACACGGGG
SNP2F	TGCGCAGCAGTGCCTCTTGAAA
SNP2R	AAAAGCCGCAAACAGCAGGCA
SNP5F	AGCAAGCGCGGCGTGAAAAT
SNP5R	AGGATTTGGTGCGGCTTGGTGA
SOE_eF	TCGCGGTGCGAGCTGTGATT
SOE_eR	TCGCACGTTCCTGCTCGCTT
PacBK_5	GATCGGCGGCGCCGGGTACCGGGC
PacBK_3	GATCGCCCGGTACCCGGCGCCGCC

Table S2 Integration efficiency of GMR-P[acman] constructs.

Insert Name	Insert Size (kb)	Total Vials Scored	Vials With Integrants	Efficiency of Integration
tsh1	1	45	4	8.8%
tsh50	50	90	5*	5.5%*
tsh50ΔNC1	50	120	5*	4.2%*
tsh50ΔNC2	50	65	5*	7.7*

All constructs were injected by BestGene Inc. using *PBac(y^{+}-attP-3B)VK00003b* as a target site and *M[vas-int.Dm]ZH2A* as a source of the integrase. Integrants were identified based on *mini-white* eye pigmentation. Efficiency of integration is calculated as the number of vials with integrants divided by the total vials scored, expressed as a percentage.

*In cases where 5 positive vials were identified, no further screening was carried out. Thus, the efficiency in these cases represents a lower limit on the true value.

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