Supplementary Materials
Generating Cisgenic Sexing Strains in Insect Pests

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Fig. S1 Oxford Nanopore genome sequencing of the *white pupae* genomic site.
Diagrams depicting the nanopore sequencing reads (top grey) aligning to the *white pupae* genomic location (blue) with the *tra* intron inserted in the IMPERIAL strain.

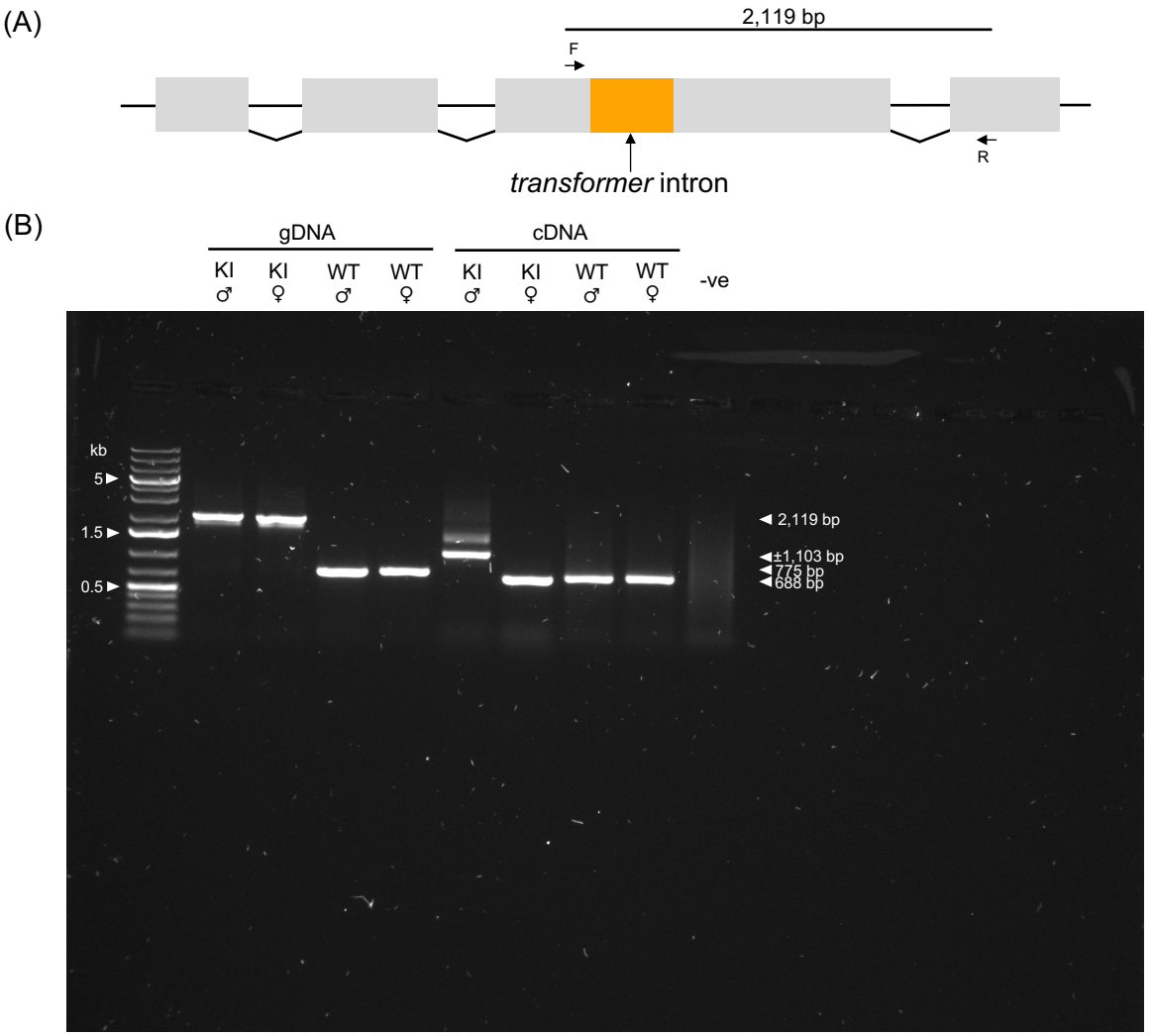

Fig. S2 *white pupae* is spliced sex-specifically in the IMPERIAL strain.
(A) The diagram showing the *white pupae* gene in the genome of the *transformer* (*tra*) intron IMPERIAL knock-in (KI) strain with forward and reverse primers labelled as F and R, accordingly (table S4). (B) The annotated electrophoresis gel of the PCR products using primers from (A). The PCR was performed on genomic and complementary DNA templates from wild-type and IMPERIAL adults. The DNA ladder and the negative control were run in the first and tenth wells, respectively.

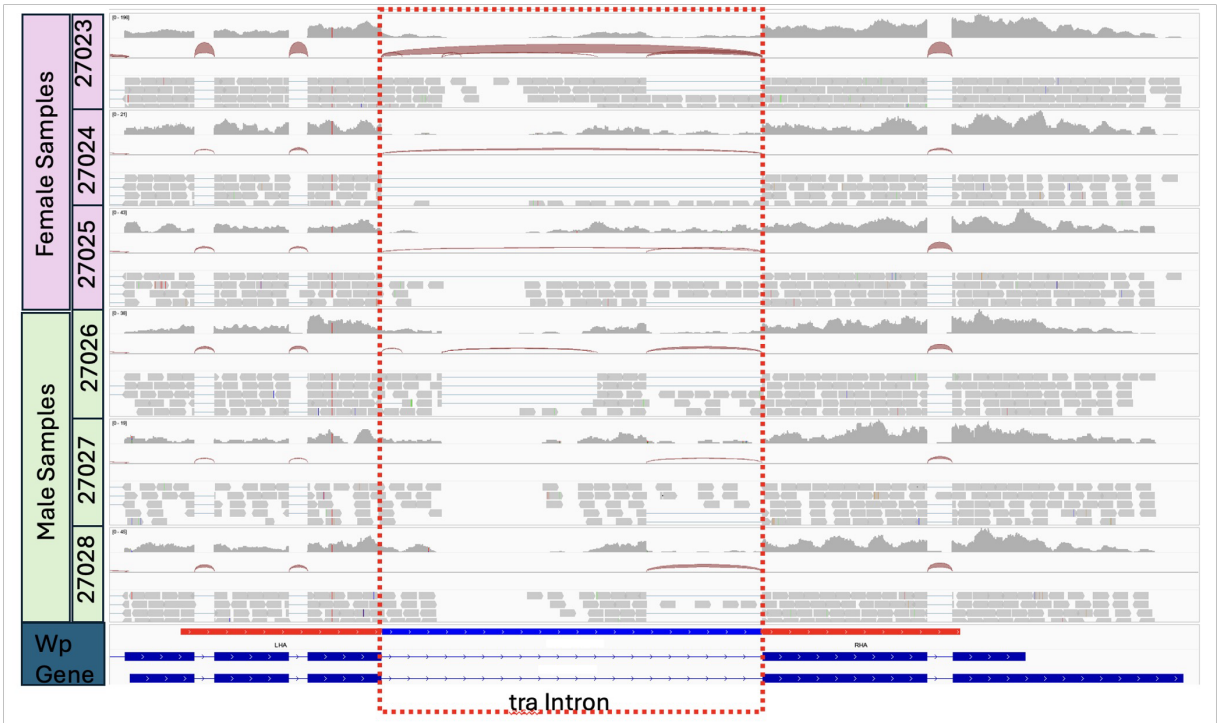

Fig. S3 RNA sequencing alignments to the *wp* genomic locus.
A zoomed-out genome browser view of the *white pupae* genomic locus and gene structure (blue bars on bottom). RNAseq reads were aligned (light grey bars) for the female samples (27023-27025) and the male samples (27026-27028). The inserted *tra* intron is indicated in dotted red box.

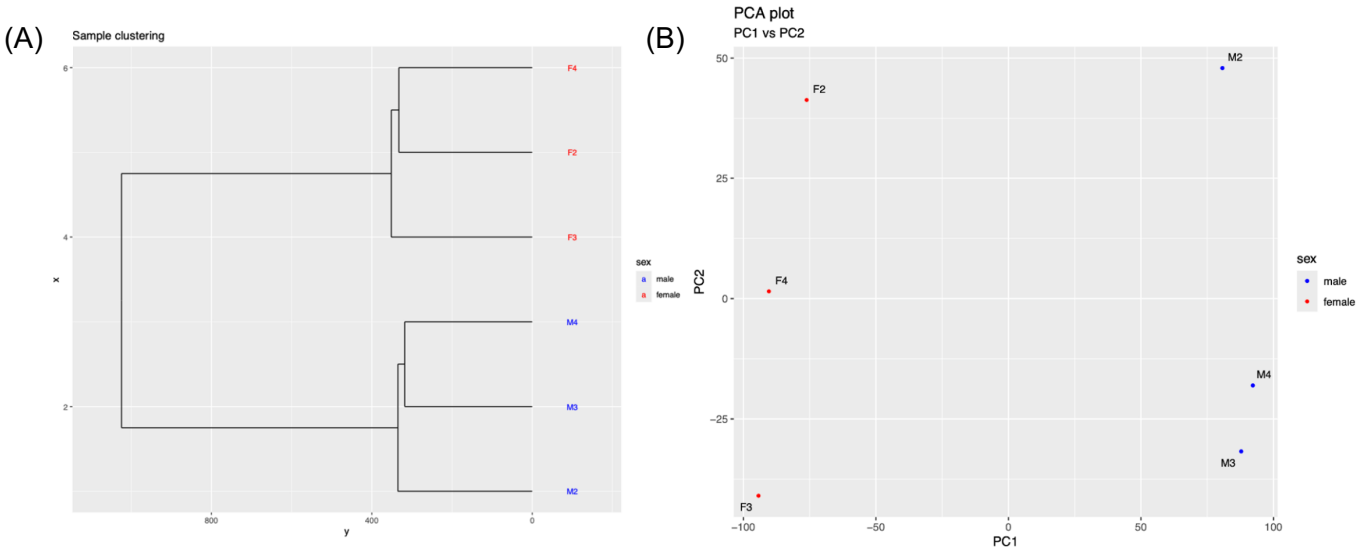

Fig. S4 Medfly RNAseq Clustering and Principal Component Analysis.

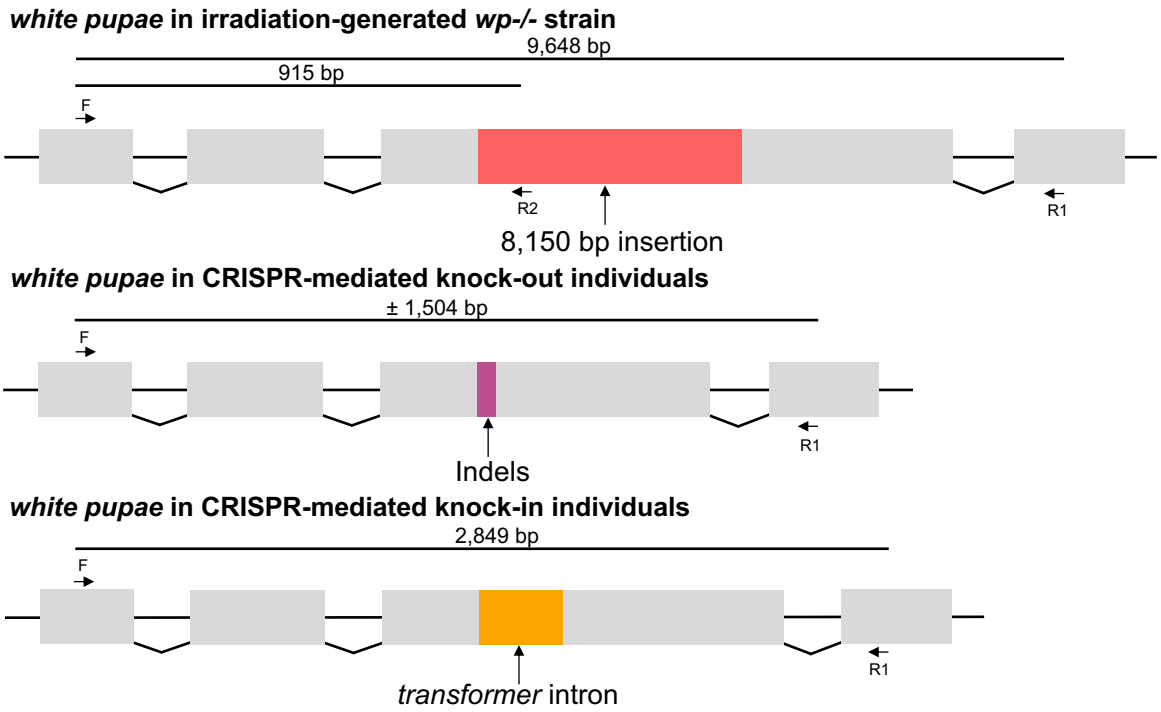

Fig. S5 *white pupae* genotyping for homozygous IMPERIAL strain generation.
Diagrams depicting the genotyping strategy to distinguish the three possible alleles, used at every generation from G2 till G7.

Table S1 Statistical analysis of adult longevity by strain.
P-values for strain-by-strain pairwise log-rank test comparisons for adult longevity with Bonferroni corrections.

	VIENNA 8	Wild-type	IMPERIAL
VIENNA 8			
Wild-type	0.0022**		
IMPERIAL	0.0048**	0.3920	

Table S2 Statistical analysis of adult longevity by strain and sex.
P-values for pairwise log-rank test comparisons by sex and strain for adult longevity with Bonferroni corrections.

		♀			♂		
	Strain	**VIENNA 8**	**Wild-type**	**IMPERIAL**	**VIENNA 8**	**Wild-type**	**IMPERIAL**
♀	**VIENNA 8**						
	Wild-type	<0.0001****					
	IMPERIAL	<0.0001****	0.14512				
♂	**VIENNA 8**	<0.0001****	0.00236**	0.05774			
	Wild-type	0.08615	<0.0001****	0.00065***	0.09250		
	IMPERIAL	0.00668**	<0.0001****	0.00236**	0.19649	0.40373	

Table S3 Statistical analysis of pupal eclosion times by strain and sex.
P-values for pairwise comparisons of *post hoc* Tukey HSD test after nested ANOVA by strain and sex.

		♀			♂		
	Strain	**VIENNA 8**	**Wild-type**	**IMPERIAL**	**VIENNA 8**	**Wild-type**	**IMPERIAL**
♀	**VIENNA 8**						
	Wild-type	<0.0001****					
	IMPERIAL	<0.0001****	0.91126				
♂	**VIENNA 8**	<0.0001****	<0.0001****	<0.0001****			
	Wild-type	<0.0001****	0.94854	0.99999	<0.0001****		
	IMPERIAL	<0.0001****	0.71737	0.99781	<0.0001****	0.99232	

Table S4 Primer summary.
Sequences of primers used for construct cloning and cisgenic strain characterisation.

Purpose	Primer Name	Primer Sequence (5'-3')
gDNA sequencing pre-cloning	1167.s1F	CTGCATACTGTGAGTGGAATTACAATGGTCTGG
	1167.s2R	GCAGGTGATAAGGAAGAGCACGGAGCC
Cloning	1167A.c1F	CTTAAAACAAACTTATAATATAACCCATATGCACGACACGAATTGCCAAAGCAATGTTCG
	1167A.c2R	AAGAAAAAAAATATGCTTTTAAAATTACCAATCGCTTTACGTTCTGGTTCCTTGAGTGTGG
	1167A.c3F	AACGTAAAGCGATTGGTAATTTTAAAAGCATATTTTTTTCTTTGAAATTCATAAGTTATC
	1167A.c4R	TGGTACGATCGCCCTCACCTATAGATACCATAGATGTATGGATTAGTATCATATACATAC
	1167A.c5F	TCCATACATCTATGGTATCTATAGGTGAGGGCGATCGTACCAAAGATGGCAAAAAGATTG
	1167A.c6R	AATTCAACGCACACTTATTACGTGAGGTACCGAAGCAGGTGATAAGGAAGAGCACGGAGC
Genotyping PCR	1167A_F	CGAGTTGGATTATGGTGATAAGGCT
	1167A_R	CACCGCTGTTGTAGAATCGTTATC
	8kb_B_R	CATAGATGCTTATGTTGTTGTAAAGGCA
Splicing PCR	1167A_F_V1_splicing	CACTCAAGGAACCAGAACGTAAAG