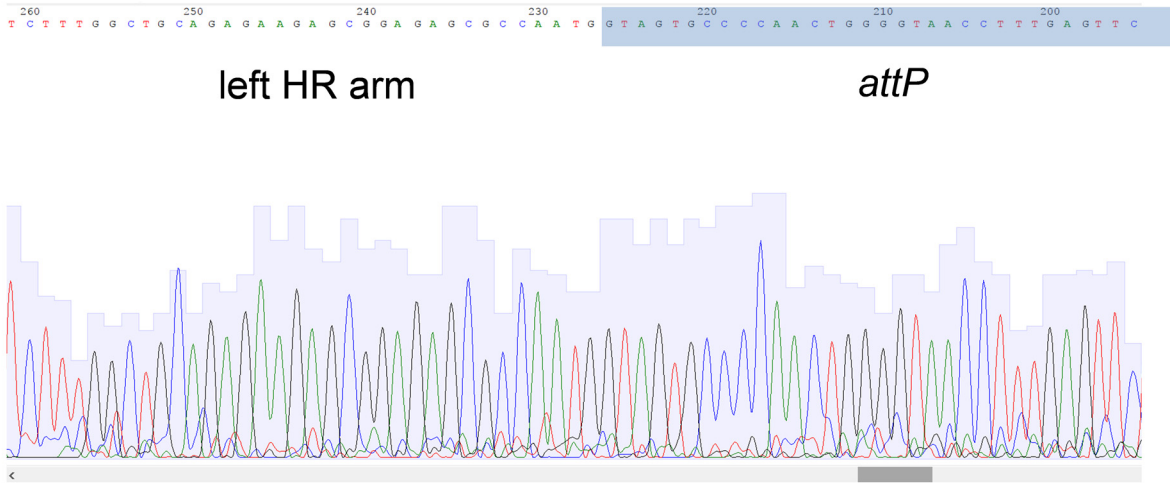
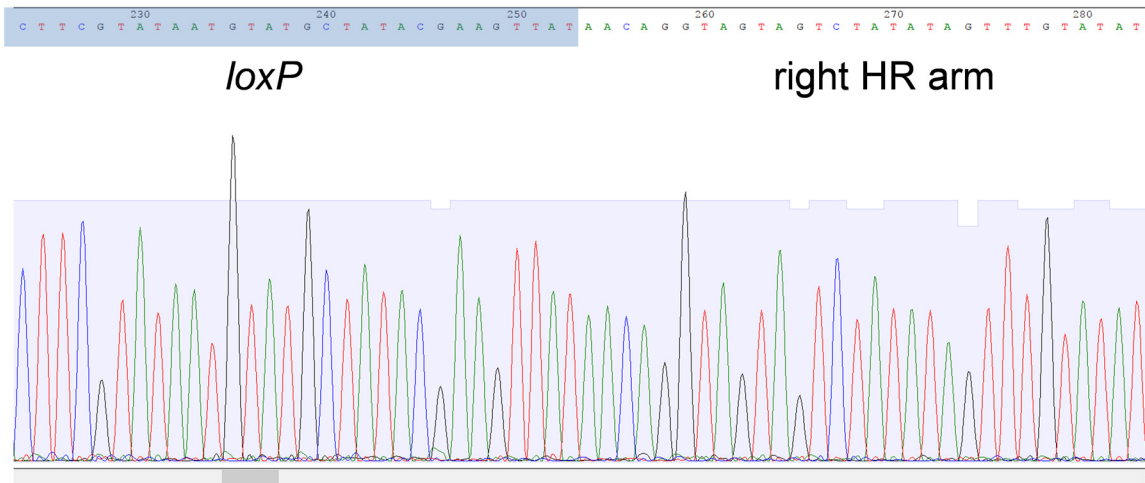


S1. Generation of *wit* null allele. (A) Schematic of null allele generation. Two chimeric guideRNAs are used to remove the *wit* locus. Florescent red eyes are used as positive selection and this positive selection is floxed for removal (cyan). The adjacent *attP* site serves as docking site for future modifications (blue). The location of *witZ* and *wit6* enhancers is denoted. (B) Rescue allele donors are targeted to the *wit* locus by ϕ C31, in this case the WT allele. (C) Homozygous null flies fail to eclose from the pupal casing. Number of pupae scored is denoted by “n”. Scale bars are 1Kb in size.

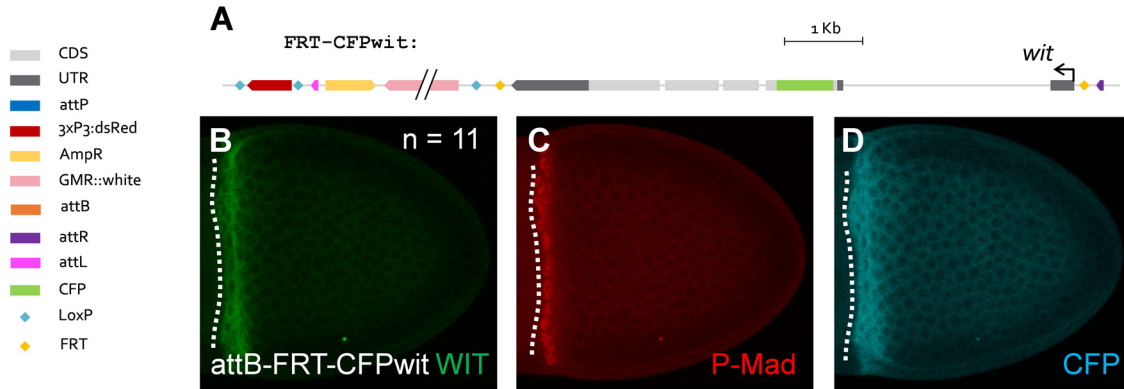
A



B



S2. Sequences of attP *wit* null allele. (A) Sequence of the left arm of the null allele includes the *attP* site. **(B)** Sequencing of the right arm of the null allele includes the *loxP* site.



S3. The *wit* null CRISPR/Cas9 fly is rescued by insertion of engineered *D. melanogaster cfp-wit* allele. (A) Diagram of N-terminal fusion of CFP-WIT inserted into the *wit* locus. (B) CFP-WIT is detected by the WIT antibody in the WT anterior pattern. (C) P-Mad is expressed in the WT anterior domain in two-cell rows wide. (D) CFP is detected by and anti-GFP antibody in the anterior. Dotted line denoted the anterior border of the oocyte-associate follicle cells. Number of egg chambers scored with a similar pattern are denoted by “n”. Scale bars are 1Kb in size.



S4. Diagrams of all *wit* rescue constructs. Depicted are diagrams of all of the rescue alleles utilized in this report. Scale bars are 1Kb in size.

Supplemental Table 1 – List of primers for enhancers

Name	Sequence	Description
witZ1_forward	AGGCTCACATTCTCCTTCG	wit1
witZ1_reverse_new	TACACCGACTGTCGCAGTTT	
witZ2_forward	GTCCCCATCCTCAGACACAT	wit2
witZ2_reverse	CCCATTGTTTACTGTGTGGAA	
witZ3_forward	GCGAAGGGAATTTGAATGG	wit3
witZ3_reverse	TGTCTGCTCACAGTACACATGC	
witZ4_forward	AATGGCCCCTTGATCCTACT	wit4
witZ4_reverse	TCCACTCTGCGCAAGTACAC	
witZ5_forward	CCCTTCGCTAGTCAGCGTAT	wit5
witZ5_reverse	CGAGTGTGCAACTTCTTTTCG	
witZ6_forward	TGCGAGCAGCACTTACACTT	wit6
witZ6_reverse	GCCTATCGGGCAAACCTTGT	
witZ7_forward	GTCTGCTTTGCGCTGCAC	wit7
witZ7_reverse	CCAATGGGAGCATAGCTGAG	
witZ8_forward	CCGACTTTTCGAGGAATGAAT	wit8
witZ8_reverse	TTTCGCTCTCTCGCTACGAT	
witZ9_forward	TTTACGAAGCTGCGTGGAAT	wit9
witZ9_reverse	GGCCATCCATAGTAGCGTATACG	
vir_wit_big_forward	GGTCATCATTGCGGGACAC	
vir_wit_small_reverse	ACTCGTCTCGTTTCAAGTGC	
vir_wit_small_split1_reverse	TCGGGATACGATGTCAAAAACA	
vir_wit_small_split2_forward	TTTGACATCGTATCCCGACTC	
wit6_mut_Forward	TTATTGGGGCACCGatCCAGTCGCGTCTGCT	wont bind Brk or Mad
wit6_mut_Reverse	AGCAGACGCGACTGGatCCGGTGCCCCAATAA	
wit6_mut2_Forward	TTATTGGGGCACCGaCGtCAGTCGCGTCTGCT	wont bind Brk but will mad
wit6_mut2_Reverse	AGCAGACGCGACTGaCGtCGGTGCCCCAATAA	
wit6_mut3_Forward	GGAAAATTTATTATTGGtGaACCGGCGCCAGTCGCG	wont bind Med+3
wit6_mut3_Reverse	CGCGACTGGCGCCGGtCaCCAATAATAAATTTTCC	
wit6_mut4_Forward	GGCACCGGCGCCAtTaGCGTCTGCTTTGC	wont bind Med+1
wit6_mut4_Reverse	GCAAAGCAGACGCAaTGCGCCGGTGCC	
wit6_mut5_Forward	CACCGGCGCCAGTCGCAaTGCTTTGCGCTGCAC	wont bind Med+6
wit6_mut5_Reverse	GTGCAGCGCAAAGCAaGCGACTGGCGCCGGTG	

Supplemental Table 2 – List of primers for CRISPR

Name	Sequence	Description
wit_sense_guide1	cttcGTCTTGGACAAGAGCGAAAC	wit CRIPSR
wit_anti_guide1	aaacGTTTCGCTCTTGTCCAAGAC	
wit_sense_guide2	cttcGCGCTCAGCTATGCTCCCAT	
wit_anti_guide2	aaacATGGGAGCATAGCTGAGCGC	
wit_break1_hom_arm_for	CAAGcacctgcCAAGtgcGTGCCAGGGATATTCAGAAGTGG	wit DONOR
wit_break1_hom_arm_rev	CAAGcacctgcCAAGctacCATTGGCGCTCTCCGCTC	
wit_break2_hom_arm_for	CAAGgctcttcCtatAACAGGTAGTAGTCTATATAGTTTGTATATGTGC	
wit_break2_hom_arm_rev	CAAGgctcttcCgacGTATTATCCCTTGCCAGACCACC	
wit_crispr_conf_left	ATCCCCTTCATTCCCTACTCCCT	confirm CRISPR wit locus
wit_crispr_conf_right	GGGCCGCGACTCTAGATCATAAT	
wit_crispr_WT_right	AAGACACTGAGCTTGTACGACGA	
wit_crispr_conf2_for	GTGCTCAACTCTTTCGCTCTCTCG	
wit_crispr_conf2_rev	TAGCGACGTGTTCACTTTGCTTGT	
wit_inside_for	GGGGAAACGAGTGTGAGGTGGAG	
wit_inside_rev	ATCAATAGACGGGCCACACTCGC	
wit_outside_1_for	AGTGCCAAGGACGAGCTATCCA	
wit_outside_2_rev	CACCAATGTCGGCATTATGTTCC	

Supplemental Table 3 – List of primers for rescue vectors

Name	Sequence	Description
wit_locus_left	TGGGTATAAGTAAGTCGCCAGAGC	
wit_locus_right	GAAAAGAACGGACTGCGAATCGG	
wit_locus_L_FRT	GAAGTTCCTATTcctagaaaGtATAGGAACTTcTGGGTATAAGTAAGTCGCCAGAGC	
wit_locus_R_FRT	GAAGTTCCTATaCtttctagaGAATAGGAACTTcGAAAAGAACGGACTGCGAATCGG	
wit_locus_left_R	AGTGAGTACCTCAAAGTCTTCTTATCTATG	
wit_locus_right_F	TGCTCTGCCAGCGAGATC	
wit_CFP_F	tcgctgggcagagcaATGGTGAGCAAGGGCGAG	
wit_CFP_R	ttaggtactcactCTTGTACAGCTCGTCCATGC	
pSC_R	ACAGTGGATATCAAGCTTATCG	vir CRM in mel locus
pSC2vir_wit_L_F	agcttgatccactgtTGGGTATAAGTAAGTCGCC	
vir_wit_L2R_R	gctcagacggcgccaacctcaCCAATAATAAATTTCCGACGAGTGTGCAAC	
vir_wit_R2L_F	tgaggtggcgccgtctgagcTGCTTTGCGCTGCACGGC	
vir_wit_R2pSC_R	ctctagaactagtgatGAAAAGAACGGACTGCGAATCG	
pSC_F	ATCCACTAGTTCTAGAGCGG	
assembly_delSmad_for	CGGAAAATTTATTATTGGGGCACCAGTCGCGTCTGCTTTGCG	BRK site removed
assem_dSmad_rev_sho	CCCCAATAATAAATTTCCG	
vir_locus_F	CCGGTGGAGCAATAAAAATG	
vir_locus_R	AACGTTCCCTTGACACAG	