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# Supplementary Materials for

## Same rule, different genes: *Blimp1* is a pair-rule gene in the milkweed bug *Oncopeltus fasciatus*

Katie Reding *et al.*

Corresponding author: Leslie Pick, lpick@umd.edu

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**The PDF file includes:**



**Other Supplementary Material for this manuscript includes the following:**

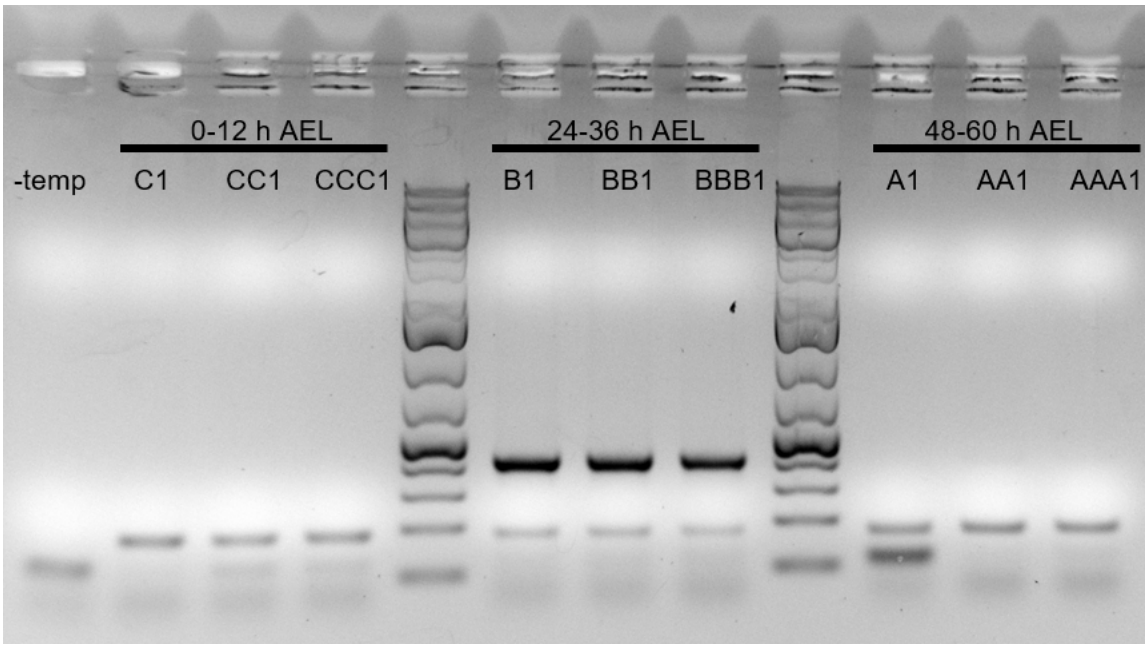


**Fig. S1. RT-PCR of *E75A* across three embryonic time points**

An 366 bp amplicon of *E75A* was amplified at 0-12, 24-36, and 48-60 hours after egg laying (h AEL) to validate staging for RNA-seq. A 173 bp amplicon of *actin* was simultaneous amplified as a positive control. The first lane is a no template control. Three replicates were performed per time point.

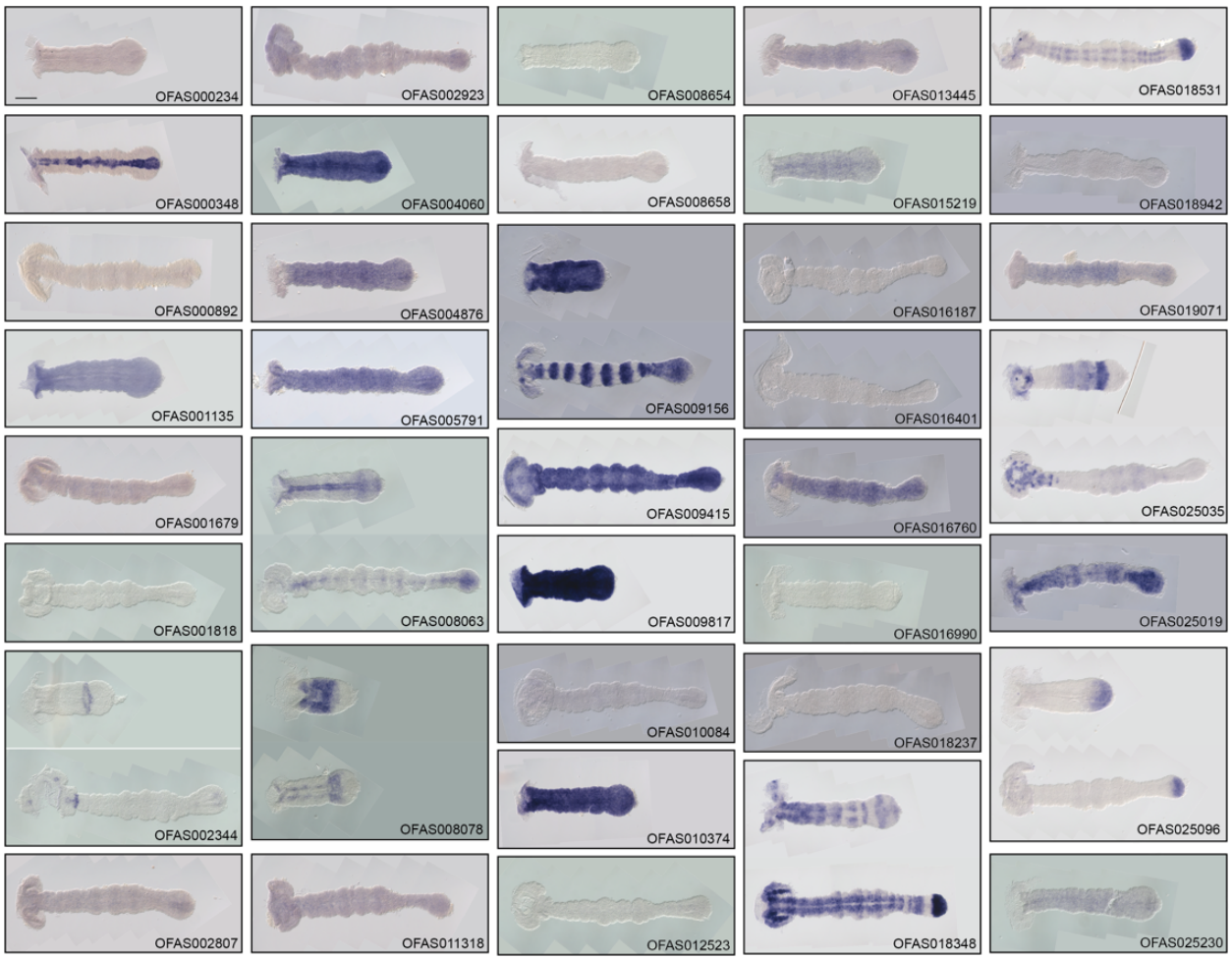


**Fig. S2. In situ hybridization screen for *E75A*-coexpressed genes.**

Expression patterns identified in the initial screen *Of-E75A*-coexpressed genes are shown. These 38 genes are in addition to the 8 presented in Fig. 1 for a total of 46 transcription factor-encoding genes screened by in situ hybridization to date. Expression patterns are shown at 24-48 h AEL for the following genes: OFAS000234, OFAS000348, OFAS000892, OFAS001135, OFAS001679, OFAS001818, OFAS002344 (annotated as *Knirps2*), OFAS002807, OFAS002923, OFAS004060, OFAS004876, OFAS005791, OFAS008063 (annotated as *retained*), OFAS008078 (annotated as *SoxN*), OFAS008654, OFAS008658, OFAS009156 (annotated as *cubitus interruptus-partial*), OFAS009415, OFAS009817, OFAS010084, OFAS010374, OFAS011318, OFAS012523, OFAS013445, OFAS015219, OFAS016187, OFAS016401, OFAS016760, OFAS016990, OFAS018237, OFAS018348 (annotated as *hunchback*), OFAS018531 (annotated as *Dichaete*), OFAS018942, OFAS019071, OFAS025035 (annotated as *giant*), OFAS025019 (annotated as *extramacrochaetae*), OFAS025096 (annotated as *caudal*), and OFAS025230. Scale bar corresponds to 200 µm. Genes are named based on published annotations (Panfilio et al. 2019).

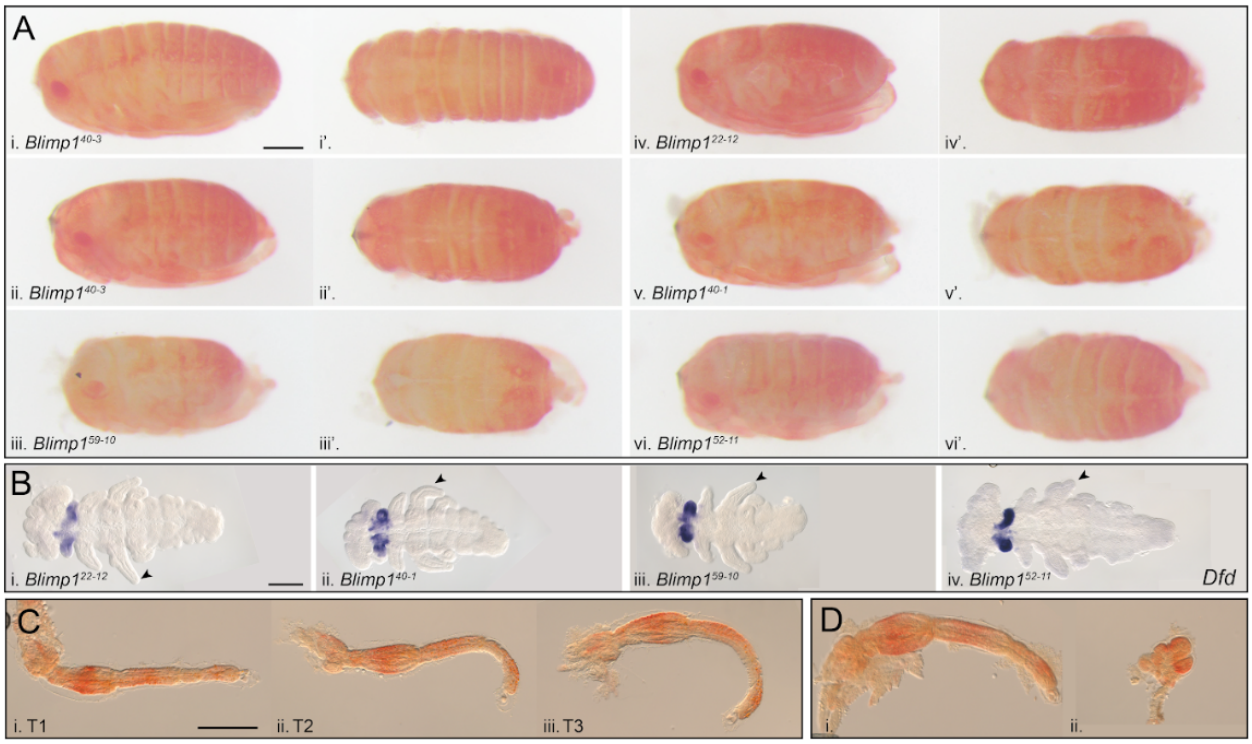


**Fig. S3. Pair-rule phenotypes in all *Of-Blimp1* mutant lines**

All embryos are offspring of self-crosses for the indicated line. **A)** Pre-hatchling phenotypes. i) Wildtype-like *Of-Blimp1*$^{40\text{-}3}$ offspring, Pair-rule defects for: (ii) *Of-Blimp1*$^{40\text{-}3}$, (iii) *Of-Blimp1*$^{59\text{-}10}$, (iv) *Of-Blimp1*$^{22\text{-}12}$, (v) *Of-Blimp1*$^{40\text{-}1}$, and (vi) *Of-Blimp1*$^{52\text{-}11}$ offspring. i-vi) lateral view; i'-vi') dorsal view. Scale bar corresponds to 200 µm. **B)** *Dfd* expression in embryos from line (i) *Of-Blimp1*$^{22\text{-}12}$, (ii) *Of-Blimp1*$^{40\text{-}1}$, (iii) *Of-Blimp1*$^{59\text{-}10}$, (iv) *Of-Blimp1*$^{52\text{-}11}$. Arrowhead indicates fused appendage. C) T1 (i), T2 (ii), and T3 (iii) leg dissected from a wild type-like *Of-Blimp1*$^{22\text{-}12}$ individual. D) Leg (i) and nub-like appendage (ii) dissected from a presumptive *Of-Blimp1*$^{22\text{-}12}$ homozygote. Scale bars represent 200 µm.

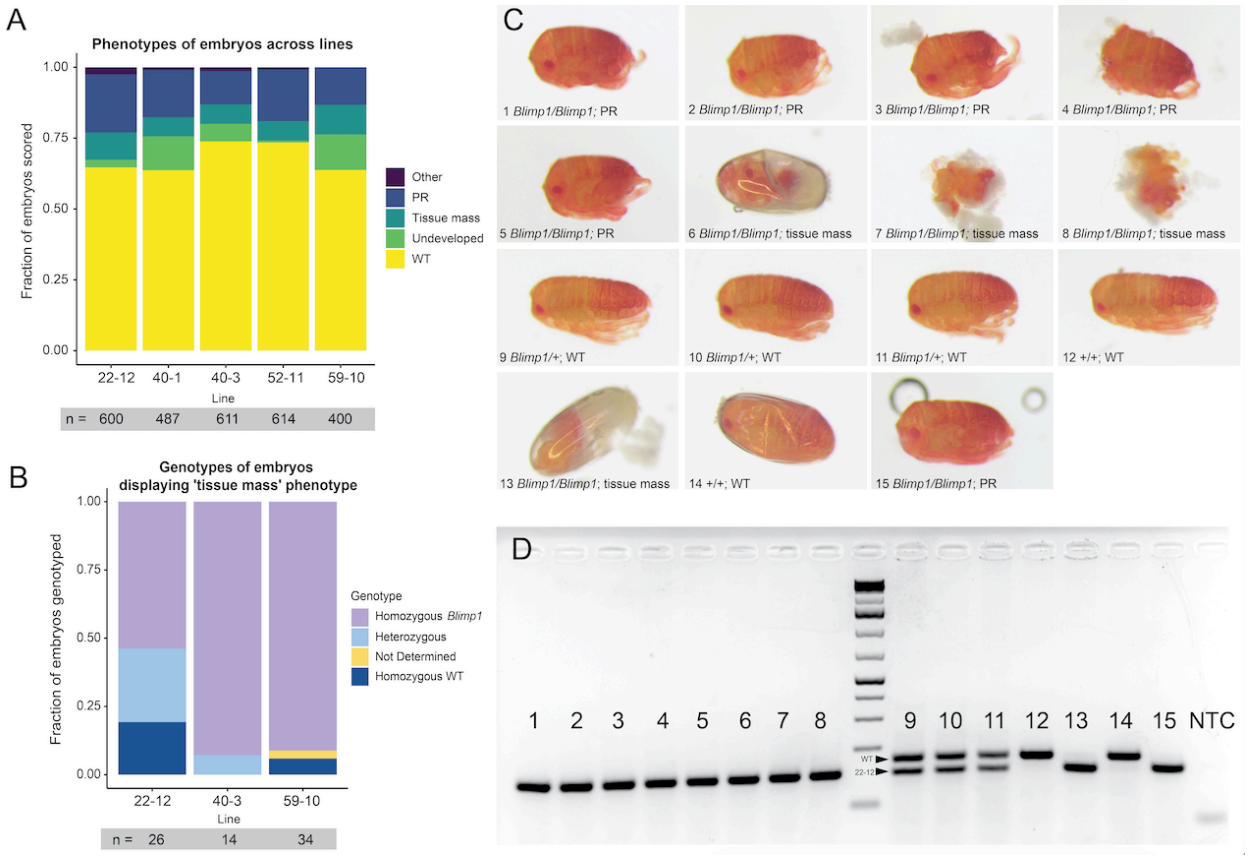


**Fig. S4. Phenotyping and genotyping 'tissue mass' phenotype across lines.**

**A)** Graph showing overall phenotypes of embryos derived from self-crosses of indicated *Blimp 1* mutant lines. A large portion of embryos from all five lines displayed an unexpected 'tissue mass' phenotype. Number of embryos examined from each line are presented in a gray box beneath the plot. **B)** Genotypes of embryos displaying 'tissue mass' phenotype from lines *Blimp1*$^{22\text{-}12}$, *Blimp1*$^{40\text{-}3}$, and *Blimp1*$^{59\text{-}10}$. While most 'tissue mass' embryos are *Blimp1* homozygotes, many–particularly from line *Blimp1*$^{22\text{-}12}$–are heterozygous or homozygous WT, suggesting that this phenotype is not caused by *Blimp1* mutation, but may be due to a linked off-target mutation. The number of embryos genotyped from each line are displayed in a gray box beneath the plot. **C)** 15 *Blimp1*$^{22\text{-}12}$ embryos that were phenotyped prior to being sacrificed for genotyping. Embryos 6-8 and 13 display the 'tissue mass' phenotype. Embryos are labeled: Genotype; Phenotype. **D)** Genotyping gel from embryos in C. A single lower band is present for *Blimp1*$^{22\text{-}12}$ homozygotes (1-8, 13, and 15), a single upper band is present for samples homozygous for the WT allele (12 and 14), and both bands are present in heterozygotes (9-11). NTC = no template control.

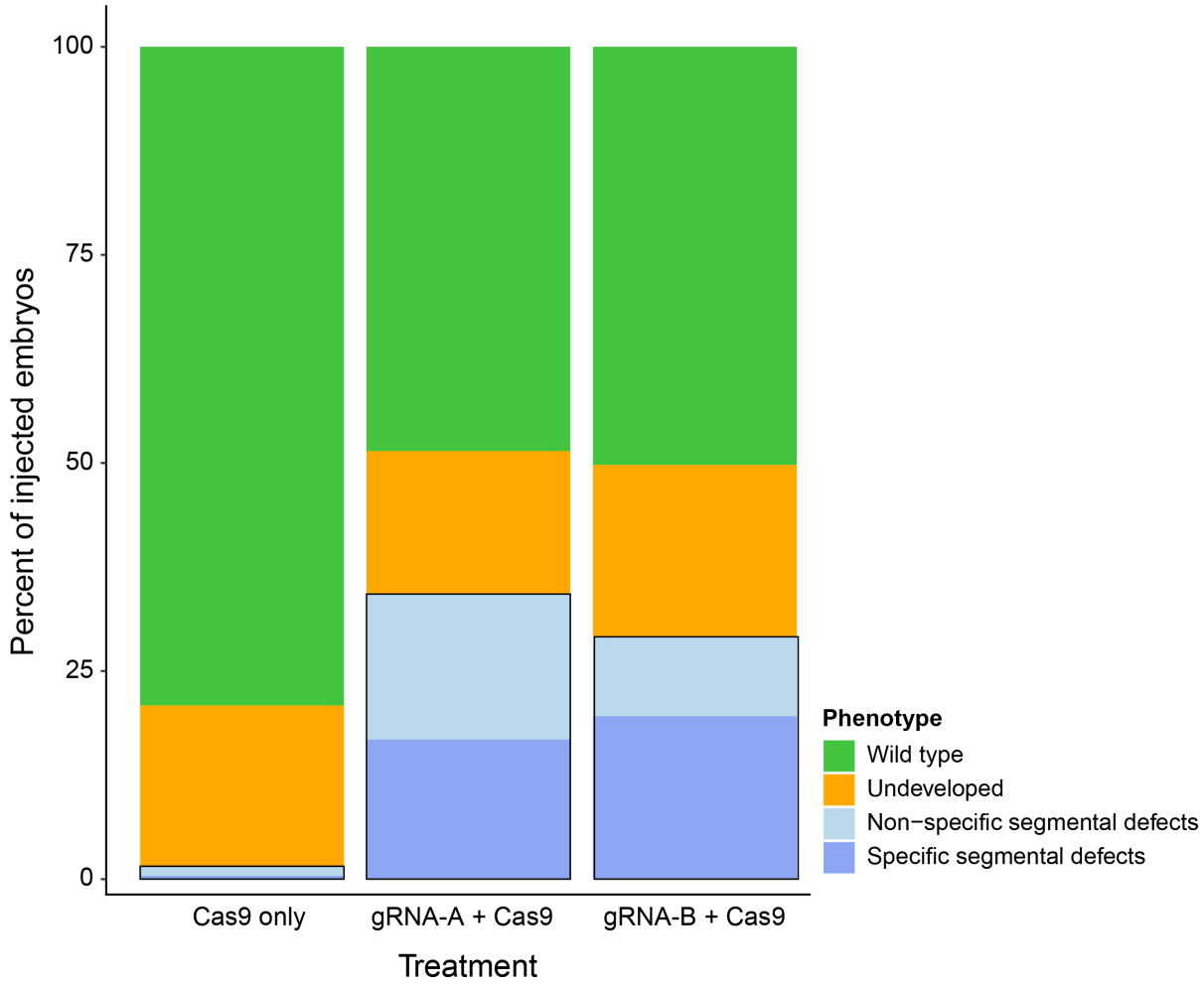


**Fig. S5. Frequencies of phenotypes observed after somatic *Of-Blimp1* CRISPR/Cas9 somatic mutagenesis**

Embryos injected with Cas9 mRNA only (n=259), Cas9 mRNA and *Of-Blimp1* gRNA-A (n=698), or Cas9 mRNA and *Of-Blimp1* gRNA-B (n=639) were broadly categorized as wild type, undeveloped (no clear structures, likely killed by injection or unfertilized), having non-specific segmental defects (defects were too severe or segment loss too complete to accurately identify affected segments), or as having specific partial segmental defects. Only embryos in this last category could be accurately identified and used for analysis presented in main text Fig. 6. The percentage of embryos displaying any segmental defects (both specific and non-specific) is outlined in the plot.

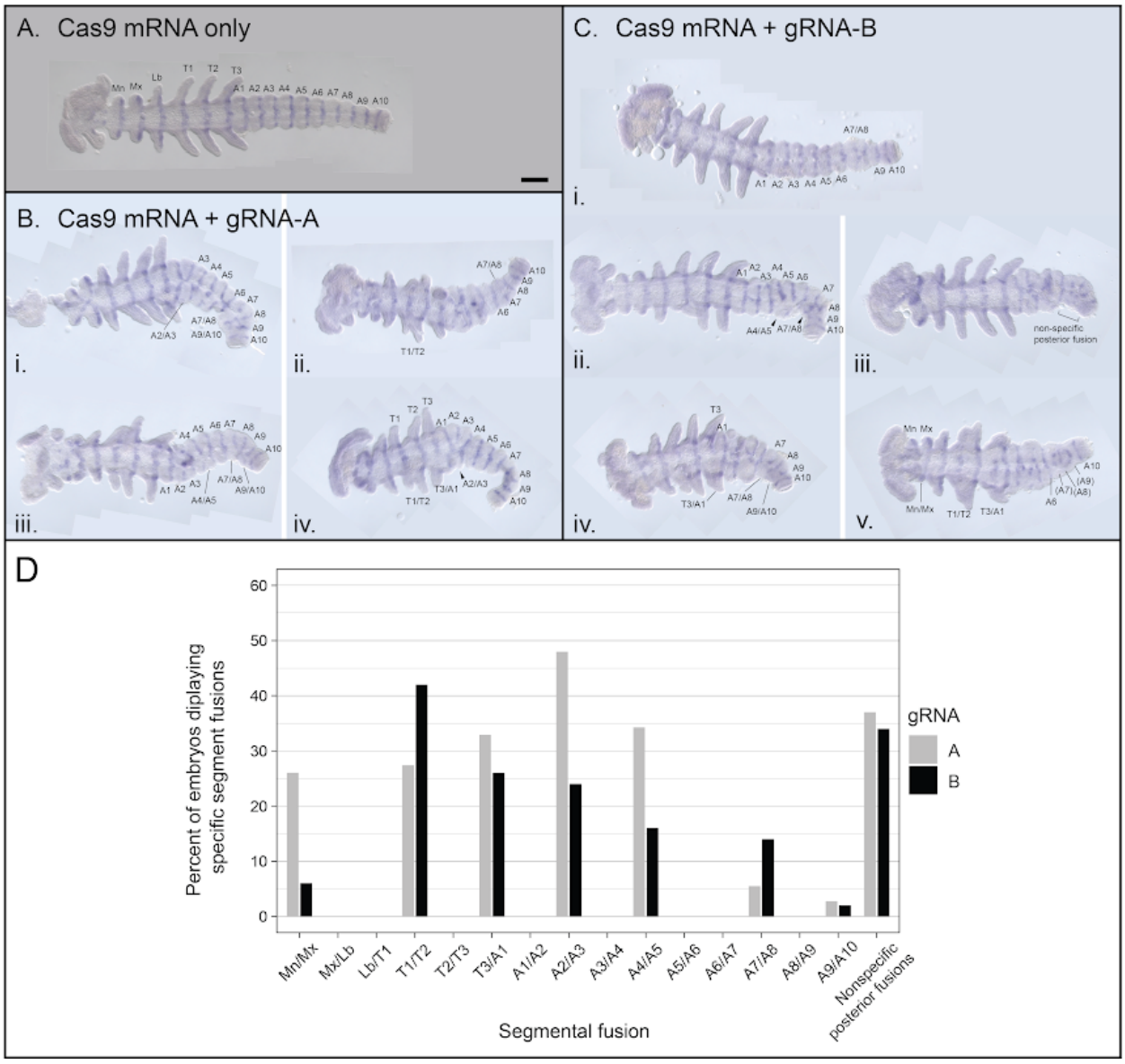


**Fig. S6. Expression of *invected (inv)* in *Of-Blimp1* somatic mutants**

**A)** An embryo injected with Cas9 mRNA only, displaying wildtype segment morphology and a wildtype *inv* expression pattern. **B)** Embryos injected with Cas9 mRNA and *Of-Blimp1* gRNA-A. (i) Embryo displaying disrupted segment boundaries, including fusion of segments A2/A3, A7/A8, and A9/A10 on one side. (ii) Embryo displaying fusion of segments T1 and T2, A7 and A8. (iii) Embryo displaying A4/A5, A7/A8, and A9/A10 segmental fusions. (iv) An embryo displaying T1/T2, T3/A1, and A2/A3 fusions on one side. **C)** Embryos injected with Cas9 mRNA and *Of-Blimp1* gRNA-B. (i) An embryo displaying fusion of segments A7 and A8. (ii) An embryo displaying A4/A5 and A7/A8 segmental fusions. (iii) An embryo displaying reduced *inv* expression and posterior curling in the posteriormost segments (bracket), which was scored as having nonspecific posterior defects. (iv) An embryo displaying T3/A1, A7/A8, and A9/A10 segmental fusions. (v) An embryo with Mn/Mx, T1/T2 and T3/A1 segmental fusions, and nonspecific posterior fusions. In the region of A7 through A9, *inv* expression did not form complete stripes. All embryos were fixed at 67-71 h AEL. **D)** Plot displaying the percent of each segmental fusion observed in embryos injected with Cas9 mRNA and gRNA-A (gray) or -B

(black). Nonspecific posterior fusions refer to segment fusions in the region of A7 through A10 which could not be specifically identified, which were marked by reduced or disrupted *inv* expression and often by curling of the posterior abdomen. Scale bar represents 200 μm.

**Table S1.**

Primer sequences used to amplify probe template for in situ hybridization screen of genes co-expressed with *Of-E75A*.

| Primer name | Primer Sequence | Amplicon (bp) |
|---|---|---|
| ofas008150-F | GCAGTGTATCTAGTACTGGACC | 1,164 |
| ofas008150-RT7 | taatacgactcactatagggagaGTAGTCGGAGTATGCAGGAG | |
| ofas013944-F | GCACCAATGAAGAATCAGGACTTCCAAAGATG | 605 |
| ofas013944-RT7 | taatacgactcactatagggagaCTTCTGATGACCAAGCACTGTTTCCATCTTG | |
| ofas000035-F | GCTTGATTAACCTGTTGCAGAAGCAGGGTC | 686 |
| ofas000035-RT7 | taatacgactcactatagggagaCGTGATGCCCTTCGTGGTAGAGGATCG | |
| ofas012059-F | CTAGAAGCCCTCTTCGCCAAGAC | 518 |
| ofas012059-RT7 | taatacgactcactatagggagaGTACTGGTATTCAGGATACGATCCCGAGG | |
| ofas025181-F | GGGACTAAGTGTGAGAAGTGCAGTTTAAGC | 711 |
| ofas025181-RT7 | taatacgactcactatagggagaGAGTGCGAAGTCTGTGAGAGCCTTCC | |
| ofas025116-F | GAAGGAGAAGTCGAAGAATGC | 685 |
| ofas025116-RT7 | taatacgactcactatagggagaGTGCAACTTGATCTCGGTG | |
| ofas025203-F | GAGTTGGAAGAACGTGATCTCTGGAGAAG | 601 |
| ofas025203-RT7 | taatacgactcactatagggagaCCAACCAGCCACTTTCATATTGCTGTTG | |
| ofas003956-F | GATGAAGAAGGAATCAGACGGAACCTCC | 642 |
| ofas003956-RT7 | taatacgactcactatagggagaGAGGTGCTTCAGAACCACTATAGGACTTATTGAC | |
| ofas000234-F | CTAGTTCTGATAGTAGTGCAGGTTCTGTGTCATC | 677 |
| ofas000234-RT7 | taatacgactcactatagggagaCTGGAGACCAGCAGGAAGTCCC | |
| ofas000348-F | GATTAGCCATGACGAAAGTGCCGTCCTTC | 727 |
| ofas000348-RT7 | taatacgactcactatagggagaGCCCTTCACTCCTCTCCTCGTTGC | |

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|---|---|---|
| ofas000892-F | GTTATAGAGCCATCCAAGGAACCTAC | 717 |
| ofas000892-RT7 | taatacgactcactatagggagaCAGATGCTGTTCCAAGGCATTG | |
| ofas001135-F | GAGTGGTTTTAGGACATTTGAATC | 339 |
| ofas001135-RT7 | taatacgactcactatagggagaCCATTTTCAAACTTGCAACAAC | |
| ofas001679-F | GCCATCGATAATAAAATTGAGCAGGC | 598 |
| ofas001679-RT7 | taatacgactcactatagggagaCAA GTG GAA GGG TAT GCT ACG C | |
| ofas001818-F | CTGGTCCTAAATGTGTTGTGTTGG | 833 |
| ofas001818-RT7 | taatacgactcactatagggagaCTGTCCACAATTAGTGGCAAGAATTAC | |
| ofas002344-F-2 | GAGCCAGCGGCAGGATTTCAC | 516 |
| ofas002344-RT7 | taatacgactcactatagggagaCCTGTGGTTGAAGTTGTCGTCGTAGCAG | |
| ofas002807-F | GTGACCATAGAAGAAAATGGGATACAGAAG | 447 |
| ofas002807-RT7 | taatacgactcactatagggagaCTTTCATCCTCTGTTCCAAGTCATAGTCTC | |
| ofas002923-F | GTCCGTAATTCTAGTGTGCCTAAGATGGAGC | 733 |
| ofas002923-RT7 | taatacgactcactatagggagaCCACTTTTGGCATGTTCCATTACTTGCTG | |
| ofas004060-F | GCAGATGACTGGGGTGGATG | 960 |
| ofas004060-RT7 | taatacgactcactatagggagaCAGAGTCACAACTCTCACGTCG | |
| ofas004876-F | GACTCCTGTTCGACGTAAACGAAGGGAAAC | 599 |
| ofas004876-RT7 | taatacgactcactatagggagaGGTAACCATTGTCTTCTTCAGAGGACCACACAG | |
| ofas005791-F | GGATTTCCAACCAGAAGAGGTGACGGTAAC | 568 |
| ofas005791-RT7 | taatacgactcactatagggagaGACAACCATAGCTGCTTCCACGAGG | |
| ofas008063-F | GAAATACTTGTACCCCTACGAATGTG | 575 |
| ofas008063-RT7 | taatacgactcactatagggagaCATTATCTGGGTAATGTCTCTTAGGTG | |

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|---|---|---|
| ofas008078-F | GTACCATCGCCGGCAGCCAAG | 916 |
| ofas008078-RT7 | taatacgactcactatagggagaGCGTGTGGATGCCCTGCG | |
| ofas008654-F | CAGCACTACTGTTTACGTTGG | 880 |
| ofas008654-RT7 | taatacgactcactatagggagaGATGATCCATGTCCATCGC | |
| ofas008658-F | GAGATTGGAGCCAAGGTGTTATAAGACG | 334 |
| ofas008658-RT7 | taatacgactcactatagggagaCCTTTGAGCTAAGTCTAACGGGGC | |
| ofas009156-F | GACTAGCAAGCCCTAGACCTC | 996 |
| ofas009156-RT7 | taatacgactcactatagggagaCTCCTCCATCTTCTGAGCCAC | |
| ofas009415-F | GACAGCAGAGGCACCTAAATCTG | 771 |
| ofas009415-RT7 | taatacgactcactatagggagaCCATCCACAATAACTGAATGAGCC | |
| ofas009817-F | GAATTCTGGAGCAAATTCGAGGAG | 866 |
| ofas009817-RT7 | taatacgactcactatagggagaCTGAGTTCTTCAAATGCGTTCC | |
| ofas010084-F | GCATATGAGGATAAGACACAGTGATG | 842 |
| ofas010084-RT7 | taatacgactcactatagggagaCGAACTAATCTGTGCTAACTTATCTGC | |
| ofas010374-F | CAGACCAATCATCTTGTACTGTAGGAATATGGGCTG | 801 |
| ofas010374-RT7 | taatacgactcactatagggagaCAGGTGGTTCATTTCCATACCAAAATTGTCTACTCC | |
| ofas011318-F | GTGCAAGTGCCTAAAGACTGCCCAATATC | 851 |
| ofas011318-RT7 | taatacgactcactatagggagaGCGACACTCCAAGAGAACACTCGACC | |
| ofas012523-F | CGAATTATAGCGGCAGAGTTGC | 857 |
| ofas012523-RT7 | taatacgactcactatagggagaGAGAAGCTCAGTTGGATTGTTCAC | |
| ofas013445-F | CTCTTCTGTGATTACAGTGCTCCTTATGCAAGTAAC | 641 |
| ofas013445-RT7 | taatacgactcactatagggagaGCAAGACTCAACTGTACTGTATTCGCAATGTGG | |

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|---|---|---|
| ofas015219-F | GACCTGCATCAAGATAGAGGAGC | 957 |
| ofas015219-RT7 | taatacgactcactatagggagaGTCTGAATTATGGGCTGCTGAAC | |
| ofas016187-F | GTAAATAACACTGTAGATCCCCTGGCTTCAG | 757 |
| ofas016187-RT7 | taatacgactcactatagggagaCTATAAGAGTTTCACTCAATGGGCGAATGAGTTC | |
| ofas016401-F | GACTCAGTCAGGTGCTCGTCCTAATATTTGG | 501 |
| ofas016401-RT7 | taatacgactcactatagggagaCCAGAGAGGAGAGATTCGGTAACTGGC | |
| ofas016760-F | GGAAATTTAGAGACATTGATGTGGACACCTAACCAC | 604 |
| ofas016760-RT7 | taatacgactcactatagggagaGCCCACCATACTTCTTCCAATAACACCAAC | |
| ofas016990-F | CGAAACAGTGACTTCGGCG | 847 |
| ofas016990-RT7 | taatacgactcactatagggagaGAGGTCCGATCCGCTCTG | |
| ofas018237-F | CAGAGGACGAGTTTGGTGACAGTG | 879 |
| ofas018237-RT7 | taatacgactcactatagggagaCGGTATGCTTAGACATAATGTGTCTCTTCATGC | |
| ofas018348-F | CCAGCATGACGGGAGGGGTG | 1,025 |
| ofas018348-RT7 | taatacgactcactatagggagaCGCCGCTGTCTTCAACCATAGG | |
| ofas018531-F | GTCAGGTTACAGTTTCCCCATG | 722 |
| ofas018531-RT7 | taatacgactcactatagggagaGTCTTCTTAGGCTGTCCATGTC | |
| ofas018942-F | GAAGCGAAAGCACAGGAGTAGATC | 405 |
| ofas018942-RT7 | taatacgactcactatagggagaGGAGGCTTGATAGTTCCATCGTC | |
| ofas019071-F | CCACCCTTCAAGGCAGAAATGC | 436 |
| ofas019071-RT7 | taatacgactcactatagggagaGAAGTCCTTGGCAAATAAATAGGTTCAGTAGG | |
| ofas025035-F | GCAGGAGTACTGGACCTAC | 553 |
| ofas025035-RT7 | taatacgactcactatagggagaCGTAGAACATTCGCCTCATGTG | |

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|---|---|---|
| ofas025019-F | GAAAGTGTCAAATGTTTGTGGTTCCTC | 303 |
| ofas025019-RT7 | taatacgactcactatagggagaCAAGAGACGGGTGGTACGATG | |
| ofas025096-F | CCTCTGAGAGTATGTACTATCCTCAG | 462 |
| ofas025096-RT7 | taatacgactcactatagggagaCTTGTCGTTCTGACAATCCAAG | |
| ofas025230-F | CCTGTGAAAGAAGAGACACCTTTG | 905 |
| ofas025230-RT7 | taatacgactcactatagggagaGTATGTTTGGCGAGTATGTGTCTATTC | |

**Table S2. Primer sequences not related to expression pattern screen**

Primer sequences used to amplify probe template for in situ hybridization screen of genes co-expressed with *Of-E75A*.

| Primer name | Primer Sequence | Notes |
|---|---|---|
| Of-Blimp-gDNAseq-F1 | GCAATACAATGCAATAGGCTTG | Used for sequencing |
| Of-Blimp-gDNAseq-R2 | GCAAATCTCACACTGATGAGG | |
| Of-Blimp-exon10-R | GTTGGCTGATACTCATAGTAGTATTGAG | |
| Of-Blimp-exon8-F | GTTCCAGTAAGCCCTGATTCAAC | Used for sequencing and screening |
| Cas9-mRNA-F-T7 | taatacgactcactatagggagaGGTGGGAGGTCTATATAAGCAG | To amplify template for *Cas9* mRNA; amplicon: 4,300 bp |
| Cas9-mRNA-R | GCTGATCAGCGGGTTTAAACTC | |
| Of-Blimp-gRNA-A | ttaatacgactcactataggTCAAGGATGGATGGTTGCTGgttttagagctagaaatag | Used with gRNArev to amplify template for *Of-Blimp1* gRNA-A |
| Of-Blimp-gRNA-B | ttaatacgactcactataggTAAGGGTAGTCGGAGTATGCgttttagagctagaaatag | Used with gRNArev to amplify template for *Of-Blimp1* gRNA-B |
| gRNArev | AAAAGCACCGACTCGGTGCC | Used to amplify templates for gRNA synthesis |
| Of-Blimp-screening-F1 | CAGTTCTCGTAAGCCCAGAGCC | Used for screening |
| Of-Blimp-screening-R1 | ATCTCAGTAGGACTACTCGGCGG | |
| Of-Blimp-screening-R2 | CCGAGATCCTGTAGGTGAGAGTG | |
| Dmel-Blimp-gRNA-F | TATATAGGAAAGATATCCGGGTGAACTTCgtggttgtgatgtccatgcaGTTTTAGAGCTAGAAATAGCAAG | Used to construct gRNA expression plasmid |
| Dmel-Blimp-gRNA-R | ATTTTAACTTGCTATTTCTAGCTCTAAAACtctacgtgcattcgatgatcGACGTTAAATTG | |

| | | |
|---|---|---|
| | AAAATAGGTC | |
| Dmel-Blimp-5-HDR-F | cgacggccagtgaattcgagctcggtacccGTGGGTCAGTAGTAGGAACTCTC | To amplify 5' homology arm for *Dmel-Blimp1* HDR template; amplicon: 1,097 bp |
| Dmel-Blimp-5-HDR-R | gctcgaattaaccatGGCGACTATAGCTAGATTGTTGCTCAG | |
| Dmel-Blimp-3-HDR-F | catcaatgtatcttaAAGGTACATGGTACATGGTATATGGAG | To amplify 3' homology arm for *Dmel-Blimp1* HDR template; amplicon: 942 bp |
| Dmel-Blimp-3-HDR-R | atgcctgcaggtcgactctagaggatccccGCTAGGCGATACTAGTTCAACAGATG | |
| Dmel-Blimp-HDR-insert-F | ctagctatagtcgccATGGTTAATTCGAGCTCGCC | To amplify *3XP3>EGFP>SV40* transgene from plasmid; amplicon: 1,245 bp |
| Dmel-Blimp-HDR-insert-R | tgtaccatgtaccttTAAGATACATTGATGAGTTTGGACAAAC | |
| ofas008150-F | GCAGTGTATCTAGTACTGGACC | To amplify *Of-Blimp1 (OFAS008150)* probe template; amplicon: 1,164 bp |
| ofas008150-RT7 | taatacgactcactatagggagaGTAGTCGGAGTATGCAGGAG | |
| ofas008150-FT7 | taatacgactcactatagggagaGCAGTGTATCTAGTACTGGACC | Used with ofas008150-RT7 to amplify *Of-Blimp1* dsRNA #1 template; amplicon: 1,164 bp |
| OfE75AF_New | tcaagagggactccatacac | To amplify *Of-E75A* probe template; amplicon: 413 bp |
| OfE75ART7_New | taatacgactcactatagggagaTGTACCAGGACTCTTGCTCT | |
| Of-slp F2 | CTTACAGCTACAACGCCCTC | To amplify *Of-slp* probe template; amplicon: 1,476 bp |
| Of-slp3UTR-R1T7 | taatacgactcactatagggagaTGAGGAATGTGACGACTTTAGG | |
| Of-enRT7 | taatacgactcactatagggagaTGTCTTCCTTGCTCTTGTCT | To amplify *Of-inv* probe template |

| | | |
|---|---|---|
| Of-enF | aatcggatgtagtgaggatg | (primers were named before gene identified as *inv*; forward primer is found closest to *inv* in genome, reverse primer is not specific to either gene); amplicon: |
| Of-Ubx-F | GGATTCTACGGTTCACATCACC | To amplify *Of-Ubx* probe template; amplicon: 544 bp |
| Of-Ubx-RT7 | taatacgactcactatagggagaGATTGGCAGGCTGTTGATGG | |
| Of-lab-F | GTGAGCAGGAGAACTACTGCCAG | To amplify *Of-lab* probe template; amplicon: 712 bp |
| Of-lab-RT7 | taatacgactcactatagggagaGAGGTGCTGGAGTGTGAGAG | |
| Of-AbdB-F | GACCGAGCGGCAGGTGAAG | To amplify Of-AbdB probe template; amplicon: 602 bp |
| Of-AbdB-RT7 | taatacgactcactatagggagaGTTGAATGAGGCGCTGCGG | |
| Dmel-Blimp1-F | GAGCTAATGGTTTGGTACTGCAAG | To amplify Dmel-Blimp1 probe template; amplicon: 798 bp |
| Dmel-Blimp1-RT7 | taatacgactcactatagggagaCTCATTCTTGTCCGTTTCGCAAATGG | |
| Of-Blimp1-dsRNA2-FT7 | taatacgactcactatagggagaGCAGCAGCATGTGGCTAGGAAATG | To amplify Of-Blimp1 dsRNA #2 template; amplicon: 626 bp |
| Of-Blimp1-dsRNA2-RT7 | taatacgactcactatagggagaACCAAGAGGAACTTCAGTTGGCTG | |
| turbo GFP 1R_T7_2 | ttaatacgactcactataggCTCGGTGTTGCTGTGATCCTC | To amplify *tGFP* control dsRNA; amplicon: 636 bp |
| T7-tGFP-F | taatacgactcactatagggagaATGGAGAGCGACGAGAGC | |
| Of-Blimp1-upstream-2 | GTCATGGTAATTTAGAGTGAAGATCATAGAAG | To amplify more complete Blimp1 cDNA; amplicon: 3,539 bp |
| Of-Blimp1-downstream-2 | CAGTCGATTTGATTGGTAGCAG | |

| | | |
|---|---|---|
| Of-E75a-3-RT7 | taatacgactcactatagggagaGCAAGCGCGGCAGAGTACTG | Used to amplify *Of-E75A* - RT-PCR to determine correct staging for RNA-seq; Amplicon: 366 bp, but T7 promoters add an additional 46 bp (band size = 412 bp) |
| Of-E75a-F-T7-2 | taatacgactcactatagggagaTCGGCACTCGCCCGGTTATCA | |
| F_Of_actin | ATGGTCGGTATGGGACAGAA | Used to amplify *Of-actin* as positive control for RT-PCR; Amplicon: 173 bp |
| R_Of_actin | TGTTCTTCAGGGGCAACTCT | |

**Data S1. (separate file)**
Sanger-sequenced DNA fragments

**Data S2. (separate file)**
Data set including all *Oncopeltus* gene models found to be differentially expressed and passed to WGCNA for clustering into co-expression modules, as well as their module membership.