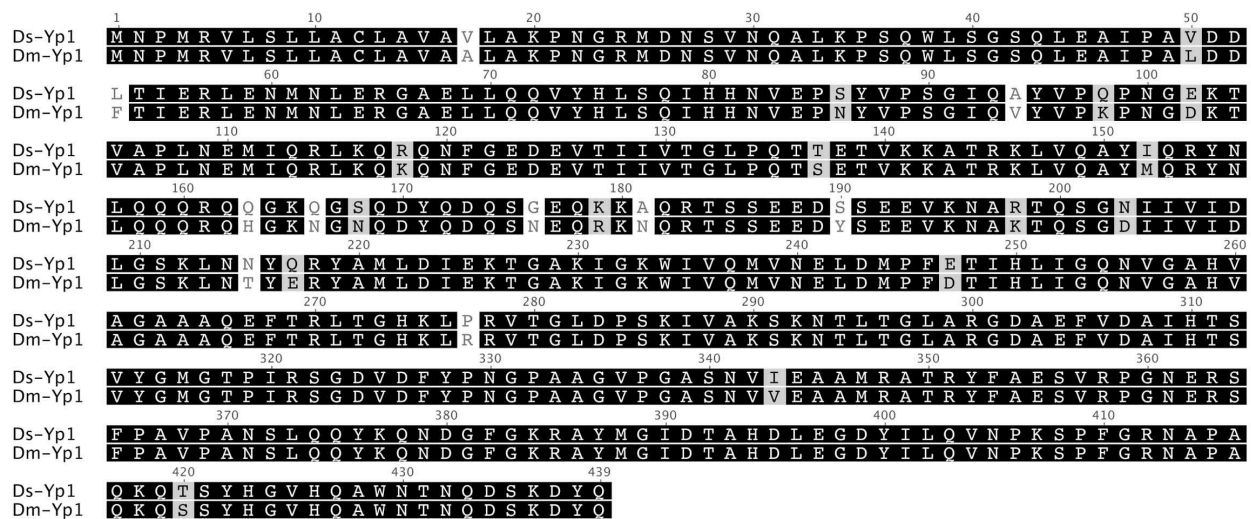


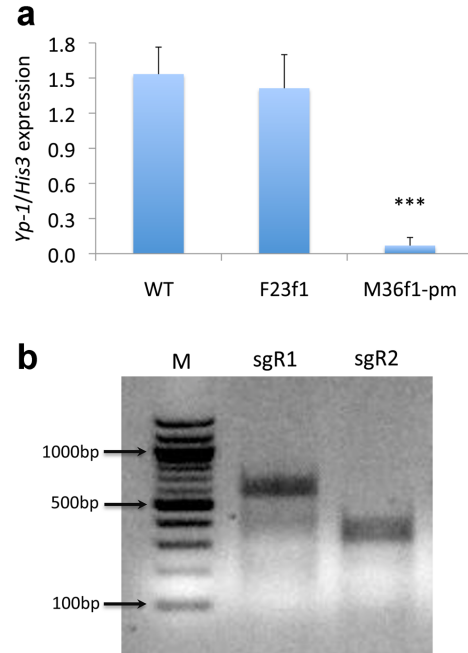
**spotted wing drosophila, *Drosophila suzukii***

Jianwei Li\* and Alfred M. Handler

## Supplementary Information

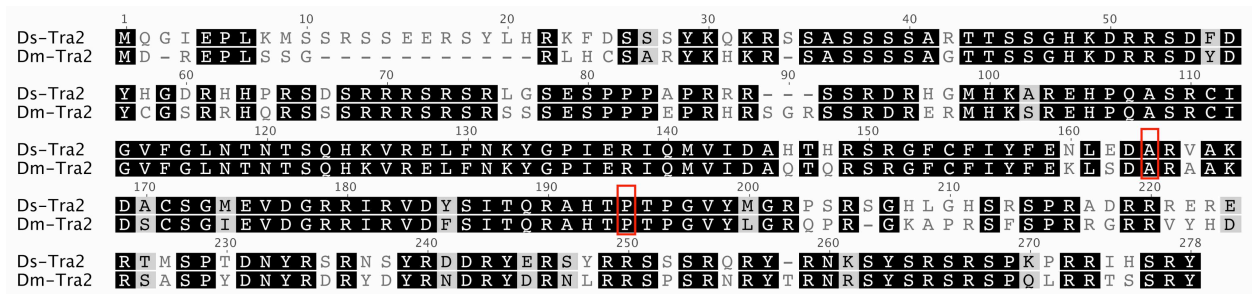


**Figure S1. ClustalW multiple sequence alignment of the *D. suzukii* Ds-Yp1 and *D. melanogaster* Dm-Yp1 proteins.**

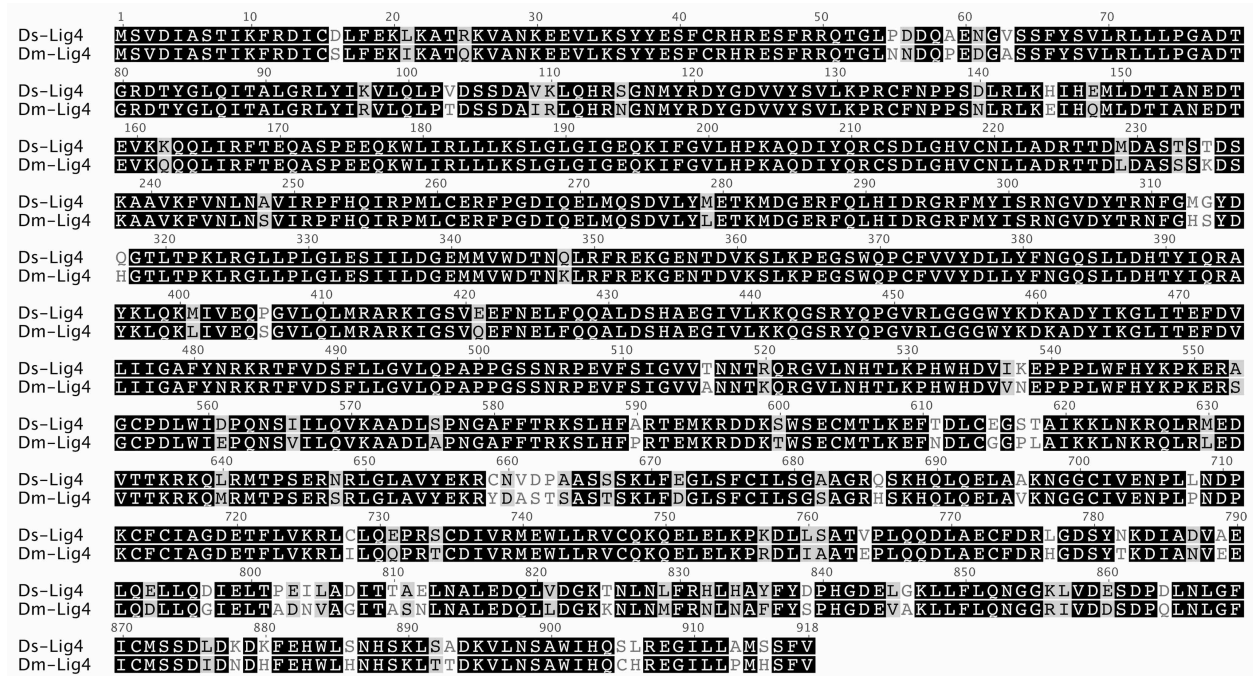


**Figure S2. Comparison of *Ds-Yp1* expression and confirmation of Cas9/sgrRNAs cutting specificity.**

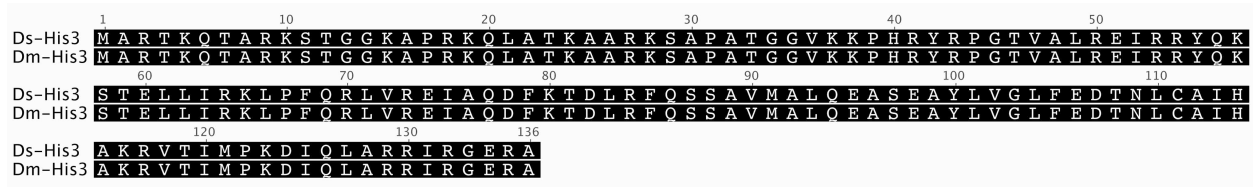
a) Comparison of *Ds-Yp1* expression in 7 day old WT and control females, and M36f1 pseudo-males at 26°C. Abbreviations: WT, wild type females; F23f1, control line females; M36f1-pm, XX; *Ds-tra-2<sup>ts2</sup>* pseudo-males. F23f1 control females are *Ds-tra-2<sup>+</sup>*, but express the *IE1hr5-DsRed* marker gene from an independent pB{*IE1hr5-DsRed*/SV40} transformation. The asterisks (\*) above M36f1 mark the student *t*-test results compared to WT: \*\*\*:  $P < 0.001$  (0.0002). F23f1 is not significantly different from wild type ( $P = 0.36$ ). b) Confirmation of Cas9/sgrRNAs cutting specificity. For sgRNA1 (sgR1), the amplified fragment was 597 bp using primers AH1082/1083, while the Surveyor test generated fragments of ~333 bp and ~264 bp due to a relatively low number of mutations generated by Cas9/sgrRNA1. For sgRNA2 (sgR2), primers AH1084/1052 amplified a fragment of 332 bp, while the Surveyor test generated fragments of ~243 bp and ~96 bp due to a relatively low number of mutations generated by Cas9/sgrRNA2.



**Figure S3. ClustalW multiple sequence alignment of the Ds-TRA-2 and Dm-TRA-2 proteins. Red boxes mark the position of the Ds-TRA-2<sup>ts1</sup> and Ds-TRA-2<sup>ts2</sup> mutations.**



**Figure S4.** ClustalW multiple sequence alignment of the Ds-Lig4 and Dm-Lig4 proteins.



**Figure S5.** ClustalW multiple sequence alignment of the Ds-His3 and Dm-His3 proteins.

**Table S1. Phenotype and fertility of the *Ds-tra-2<sup>ts2</sup>* line under temperature shifts to improve viability**

| Group | Temperature shifts <sup>a</sup> | XY Males |               |                             | XX Females |                       |               |
|-------|---------------------------------|----------|---------------|-----------------------------|------------|-----------------------|---------------|
|       |                                 | No.      | Fertility (%) | Immotile sperm <sup>b</sup> | No.        | Intersex <sup>c</sup> | Fertility (%) |
| 1)    | 22°_AE > 26°_3d > 26°           | 20       | 10            | 3/5                         | 31         | +                     | 0             |
| 2)    | 22°_11d > 26°_AE_3d > 26°       | 33       | 5             | 3/5                         | 34         | +                     | 0             |
| 3)    | 22°_9d > 26°_AE > 29°_3d > 26°  | 95       | 0             | 4/5                         | 35         | ++                    | 0             |
| 4)    | 22°_9d > 29°_AE_3d > 26°        | 89       | 0             | 4/5                         | 32         | ++                    | 0             |

<sup>a</sup>Eggs were collected at 16°C or 20°C for a 24-48h period and reared for indicated number of days (d) or to adult eclosion (AE) at 22°C; subsequent shifts in temperature and time periods indicated by (>); the time to AE for each group: 1) 12d, 2) 1d, 3) 2d, and 4) 2d; fertility was tested at the last temperature. The viability in all groups was between 80% and 90%, which was estimated based on the progeny from same number of WT adults. Wild type flies were phenotypically normal and fertile under all four temperature shifts ( $n \geq 20$ ).

<sup>b</sup>males with only immotile sperm/ males dissected (see Video S1 and S2 for comparison to wild type sperm number and motility).

<sup>c</sup>(+) denotes sex combs and abnormal genitalia; and (++) denotes sex combs, abnormal genitalia and wing spots.

**Table S2. Relative *Ds-Yp1* transcript expression to the internal control *Ds-His3* under different temperature conditions**

| Temperature conditions <sup>a</sup> | Time points | Relative <i>Ds-Yp1</i> expression (mean $\pm$ SD) |                   |      | <i>t</i> -test of M36f1 to WT-f<br>( <i>P</i> value) |
|-------------------------------------|-------------|---------------------------------------------------|-------------------|------|------------------------------------------------------|
|                                     |             | WT-f                                              | M36f1             | WT-m |                                                      |
| 16°>16°>16°C                        | 3d-16°C     | 2.7 $\pm$ 3.6                                     | 4.3 $\pm$ 7.2     | -    | 0.8015                                               |
|                                     | 6d-16°C     | 37.8 $\pm$ 26.6                                   | 32.8 $\pm$ 12.6   | -    | 0.7814                                               |
|                                     | 9d-16°C     | 29.7 $\pm$ 14.7                                   | 15.6 $\pm$ 6.7    | -    | 0.3079                                               |
| 29°>29°>29°C                        | 3d-29°C     | 26.9 $\pm$ 2.8                                    | 1.0 $\pm$ 0.99    | 0    | 0.0003                                               |
|                                     | 6d-29°C     | 6.2 $\pm$ 0.9                                     | 0.44 $\pm$ 0.22   | 0    | 0.0002                                               |
|                                     | 9d-29°C     | 3.8 $\pm$ 0.3                                     | 0.017 $\pm$ 0.006 | 0    | 0.0001                                               |
| 29°>16°>16°C                        | 3d-29°C     | 26.9 $\pm$ 2.8                                    | 1.0 $\pm$ 0.99    | -    | 0.0003                                               |
|                                     | 6d-16°C     | 16.9 $\pm$ 1.9                                    | 9.4 $\pm$ 4.9     | -    | 0.1413                                               |
|                                     | 9d-16°C     | 14.2 $\pm$ 7.5                                    | 10.8 $\pm$ 1.9    | -    | 0.4957                                               |
| 29°>16°>29°C                        | 3d-29°C     | 26.9 $\pm$ 2.8                                    | 1.0 $\pm$ 0.99    | -    | 0.0003                                               |
|                                     | 6d-16°C     | 16.9 $\pm$ 1.9                                    | 9.4 $\pm$ 4.9     | -    | 0.1413                                               |
|                                     | 9d-29°C     | 8.5 $\pm$ 5.2                                     | 0.18 $\pm$ 0.11   | -    | 0.0246                                               |

<sup>a</sup> Fresh XX; *Ds-tra2*<sup>ts2</sup> and wild type flies reared at 16°C were constantly kept at 16°C or 29°C for 9 d; shifted to 29°C for 3 d, then to 16°C for another 3 d (indicated by (>)), then kept at 16°C or shifted to 29°C for additional 3 d.

**Table S3. Oligonucleotide primer sequences (5' to 3' orientation) and their annealing temperature**

| Primer | Ta <sup>a</sup><br>(°C) | Sequence                                                              |
|--------|-------------------------|-----------------------------------------------------------------------|
| AH1001 | 72                      | GAACACCAACACCTCGCAGCAC                                                |
| AH1002 | 72                      | CGTGAGCGGCTGTAGCTTTTGT                                                |
| AH992  | 60                      | AAAAGCACCGACTCGGTGCCACTTTTCAAGTTGATAACGGACTAGCCTTATTTAACTTGCTATTCTAGC |
| AH1053 | 60                      | GAAATTAATACGACTCACTATAGGCGCGAACCTTGTGCTGCGGTTTAGAGCTAGAAATAGC         |
| AH1054 | 60                      | GAAATTAATACGACTCACTATAGGTAGGCCAGTTCCAGGCTCGTTTAGAGCTAGAAATAGC         |
| AH1020 | 70                      | GGCCGAGTAAAGGTTAAGGTGTCT                                              |
| AH1021 | 70                      | CCGCTGATGGAGGTGTATAGGTTAC                                             |
| AH1026 | 80                      | ATTCGAGCTCGGTACGGCCGAGTAAAGGTTAAGGTGTCT                               |
| AH1027 | 80                      | CCTTGTGCTGCGACGTGTTGGTGTTCAGACC                                       |
| AH1028 | 82                      | ACACCAACACGTGCGCAGCACAAGGTTCCGC                                       |
| AH1029 | 82                      | CCGGAGTAGAAGTGTGCGCGGTTGGGTATGGAGTA                                   |
| AH1030 | 77                      | CAACGCGCGCACACTTCTACTCCGGGTGTCTATATG                                  |
| AH1031 | 77                      | CCAAGCTTGCATGCCAGACTCAACTTTCTGTACAATTCA                               |
| AH1032 | 70                      | ATTCGAGCTCGGTACGTTGAGTCTTTTGTCTTTATTAT                                |
| AH1033 | 70                      | GCATGTGATAAATCAGAGCCTGGAAC                                            |
| AH1034 | 71                      | TCCAGGCTCTGATTATCACATGCATATCC                                         |
| AH1035 | 71                      | CCAAGCTTGCATGCCGCTGATGGAGGTGTATAGGTTA                                 |
| AH1044 | 72                      | TGAGTCTGCTTTAAGATACATTGATGAGTTTGACA                                   |
| AH1045 | 72                      | AAAGACTCAACCGAATTCCTGCAGCCCG                                          |
| AH1043 | 69                      | TCTTAAAGCAGACTCAACTTTCTGTACAATTCAATT                                  |
| AH1046 | 69                      | AATTCGGTTGAGTCTTTTGTCTTTATTATTGAATTGGT                                |
| AH1003 | 73                      | TCTGGCCGAGTGCTTTGACCGTC                                               |
| AH1004 | 73                      | GCCCTCCCGAAGCGATTGATGA                                                |
| AH1093 | 71                      | TGCATCACCTCGAACAGCAGAG                                                |
| AH986  | 71                      | CCTTCGCCTGGGACATCCT                                                   |
| AH987  | 71                      | GATGGTGTAGTCCTCGTTGTGGG                                               |
| AH1094 | 71                      | CTTGGTGGAGTTGCTGGAGTTCTT                                              |
| AH1133 | 69                      | ACAACCTCCGTCAACCAGGC                                                  |
| AH1134 | 69                      | GCACCGACATTCTGGCCAATC                                                 |
| AH1135 | 69                      | CCACGCAAACTGGCTACT                                                    |
| AH1136 | 69                      | CAGAGCCATAACCGCCGAG                                                   |
| AH1139 | 56                      | CTGCAGCAAGTCTACACCTG                                                  |
| AH1140 | 56                      | GCGCTGGATCATCTCGTTGAG                                                 |
| AH1137 | 56                      | GAACGGTTGCCCTGCGTG                                                    |
| AH1138 | 56                      | AGCTCTGGAATCGCAGGTCAG                                                 |
| AH1082 | 68                      | GGACTTCGATTACCATGGCGAT                                                |
| AH1083 | 68                      | GAACGATGGGTCTGTATGGCA                                                 |
| AH1084 | 69                      | CTTGCCGACGATGAAGTGGAG                                                 |
| AH1052 | 69                      | CTCCAACGAACCTACCCAATCCCT                                              |

<sup>a</sup> Ta = annealing temperature.

**Video S1. Immobile sperm from XY; *Ds-tra-2<sup>ts2</sup>* chromosomal males at 3 d after adult eclosion.** This is a time-lapse of a 21 second period compressed into one second.

**Video S2. Motile sperm from XY WT males at 3 d after adult eclosion.** This is a time-lapse of a 21 second period compressed into one second.