

Supplementary Material

M. Ray & S. C. Lakhota: Altered *hsrw* lncRNA levels in activated Ras background further enhance Ras activity in *Drosophila* eye and induces more R7 photoreceptors

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**Supplementary Table S1. Altered levels of *hsrw* transcripts in *sev-GAL4>Ras<sup>V12</sup>* background specifically enhance R7 photoreceptors**

| Genotypes   | Mean ( $\pm$ S.E.) Number of different cells           |   |  |   |
|---|--|---|--|---|
|   | Elav+ve<br>GFP-ve<br>(Other<br>photoreceptor<br>cells) | Elav-ve<br>GFP+ve<br>(Future Cone<br>cells) | Elav+ve<br>GFP+ve<br>(photoreceptor<br>cells expressing<br><i>sev-GAL4</i> ) | Runt +ve<br>GFP+ve<br>(R7<br>photoreceptor) |
| <i>sev-GAL4&gt;UAS-GFP</i><br>(N=25)                                    | 3.84 $\pm$ 0.2   | 1.2 $\pm$ 0.1                               | 3.5 $\pm$ 0.1  | 1 $\pm$ 0<br>(N=57)                         |
| <i>sev-GAL4&gt;UAS-RasI<sup>V12</sup></i><br>(N=12)                     | 3.7 $\pm$ 0.3  | 1 $\pm$ 0. 0.13                             | 4.2 $\pm$ 0. 0.4   | 1.5 $\pm$ 0.1<br>(N=75)                     |
| <i>sev-GAL4&gt;UAS-RasI<sup>V12</sup></i><br><i>hsrw-RNAi</i><br>(N=17) | 4 $\pm$ 0  | 1.4 $\pm$ 0. 0.1                            | 9.6 $\pm$ 0.8  | 3.7 $\pm$ 0.3<br>(N=61)                     |
| <i>sev-GAL4&gt;UAS-RasI<sup>V12</sup></i><br><i>EP3037</i><br>(N=11)    | 3.8 $\pm$ 0.12   | 0.8 $\pm$ 0. 0. 2                           | 6.6 $\pm$ 0.5  | 3.0 $\pm$ 0.2<br>(N=47)                     |

N values in column 1 indicate the number of ommatidia examined in different genotypes and apply to columns 2-4, while those in column 5 apply to that column

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**Supplementary Table S2.** Forward and reverse primers used for qRT-PCR/semi quantitative RT-PCR for quantification of different transcripts

| Transcripts and Primers |                         | SEQUENCE (5'-3')            | AMPLICON SIZE |
|-------------------------|-------------------------|-----------------------------|---------------|
| <b>g3pdh</b>            | Reverse                 | GGGTGCTTAATCCCACAGAA        | <b>129bp</b>  |
|                         | Forward1                | AGGCGTTTTGTGACTTCTGGA       |               |
| <b>g3pdh</b>            | Reverse1                | TGTCCTCCAGACCCTTGTTTC       | <b>144bp</b>  |
|                         | Forward2                | CCACTGCCGAGGAGGTCAACTA      |               |
| <b>sev</b>              | Forward (LP)            | GGCAGACATACGTACACGTGGCAGCAT | <b>238 bp</b> |
|                         | hsrw-n specific Reverse | TTGCGCTCACAGGAGATCAA        |               |
| <b>sos</b>              | Forward                 | AAGATGACCACCACCCACAT        | <b>161 bp</b> |
|                         | Reverse                 | CGCAGCAGATCGAGAGTAGA        |               |
| <b>Dsor</b>             | Forward                 | GAGCAACGGCCTACGACTAC        | <b>155 bp</b> |
|                         | Reverse                 | GTGCTTGTGCTGTGCATTCT        |               |
| <b>gap</b>              | Forward                 | CCACCCTGGAGTCGATATTC        | <b>123bp</b>  |
|                         | Reverse                 | GTCCTTGAACCTCGGTGGAGA       |               |
| <b>rin</b>              | Forward                 | ATGCGTGTA AACCCGCAAAG       | <b>174bp</b>  |
|                         | Reverse                 | TTTTGCACGCACTAGCAGAC        |               |
| <b>lz</b>               | Forward                 | TTTGCACTATTTTCGAGACG        | <b>163 bp</b> |
|                         | Reverse                 | GACCATAGCTGGCGTTTGAT        |               |
| <b>kibra</b>            | Forward                 | CAGCCCATAGTGGGCATAGT        | <b>157 bp</b> |
|                         | Reverse                 | TGTTCTGTGTCGCTTCTG          |               |
| <b>dome</b>             | Forward                 | TACCTTGCCGGAAGCTAATC        | <b>173 bp</b> |
|                         | Reverse                 | GGTGGCTCTATGAGGGTGCT        |               |
| <b>E(Pc)</b>            | Forward                 | CTCACGTCTCGACTGGGAAC        | <b>193 bp</b> |
|                         | Reverse                 | CTAGCTGGAAGGGATTGTGG        |               |
| <b>hers</b>             | Forward                 | AGCCTCATCGACAACCACTC        | <b>185 bp</b> |
|                         | Reverse                 | ATCGTCCTCAGCTTCATCGT        |               |
| <b>Ras</b>              | Forward Normal          | GGTCGTCGTTGGAGCCGG          | <b>242bp</b>  |
|                         | Forward Mutant          | GGTCGTCGTTGGAGCCGT          |               |
|                         | Reverse                 | CACTGTTGACGGCAAAGACC        |               |

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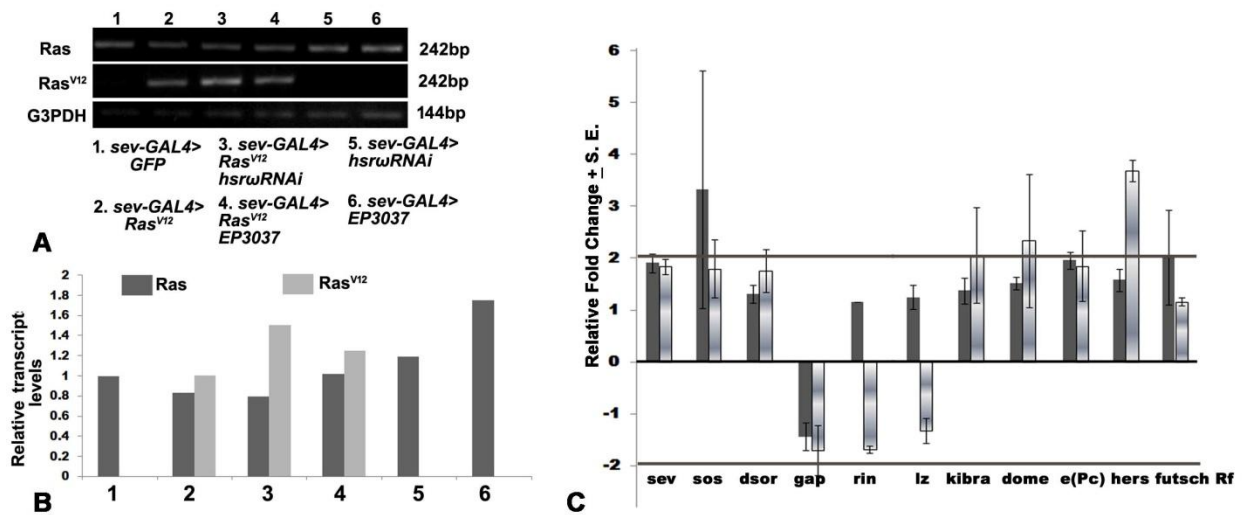
**Supplementary Table S3.** The PCR conditions used for semi-quantitative RT- PCR

| Gene                      | Initial Denaturation | Denaturation    | Annealing       | Extension       | No. of cycles | Final Extension |
|---------------------------|----------------------|-----------------|-----------------|-----------------|---------------|-----------------|
| <i>g3pdh</i>              | 3min at 94 °C        | 30 sec at 94 °C | 30 sec at 60 °C | 30 sec at 72 °C | 30            | 7 min at 72°C   |
| <i>Ras</i>                | 3 min at 94 °C       | 30 sec at 94 °C | 30 sec at 63 °C | 45 sec at 72 °C | 30            | 7 min at 72°C   |
| <i>Ras</i> <sup>VI2</sup> | 3min at 94 °C        | 30 sec at 94 °C | 30 sec at 63 °C | 45 sec at 72 °C | 30            | 7 min at 72°C   |

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Supplementary Figure S1.



**Fig. S1. Levels of transcripts of Ras signaling pathway genes remained unaltered in activated Ras background with either down- or up-regulation of *hsrw* transcripts.** **A** Agarose gel images of RT-PCR amplicons to show levels of transcripts of endogenous *Ras*, activated *Ras<sup>V12</sup>* and *G3pdh* (internal control) in different genotypes (numbers at top of each lane and explained in the key provided at bottom). **B** Histogram showing relative levels (Y-axis) of endogenous *Ras* (dark grey) and transgenic activated *Ras<sup>V12</sup>* (light grey) transcripts compared to a reference gene (*G3pdh*) transcripts in third instar larval eye discs in different genotypes based on semi-quantitative RT-PCR (X-axis, key to the numbers is provided at the bottom of panel **A**). **C** Histogram showing real-time qRT-PCR based relative fold changes (Y-axis) in levels of transcripts of different genes (named on the X-axis) involved in Ras signaling cascade in *sev-GAL4>UAS-Ras<sup>V12</sup>UAS-hsrwRNAi* (dark grey) and *sev-GAL4>UAS-Ras<sup>V12</sup>EP3037* (light grey) eye discs compared to *sev-GAL4>UAS-Ras<sup>V12</sup>* eye discs.