Supplementary Material

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

Table S1. 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
g3pdh	Reverse	GGGTGCTTAATCCCACAGAA	**129bp**
	Forward**1**	AGGCGTTTGTGACTTCTGGA	
g3pdh	Reverse**1**	TGTCCTCCAGACCCTTGTTC	**144bp**
	Forward**2**	CCACTGCCGAGGAGGTCAACTA	
sev	Forward (LP)	GGCAGACATACGTACACGTGGCAGCAT	**238 bp**
	hsrω-n specific Reverse	TTGCGCTCACAGGAGATCAA	
sos	Forward	AAGATGACCACCACCCACAT	**161 bp**
	Reverse	CGCAGCAGATCGAGAGTAGA	
Dsor	Forward	GAGCAACGGCCTACGACTAC	**155 bp**
	Reverse	GTGCTTGTGCTGTGCATTCT	
gap	Forward	CCACCCTGGAGTCGATATTC	**123bp**
	Reverse	GTCCTTGAACTCGGTGGAGA	
rin	Forward	ATGCGTGTAAACCCGCAAAG	**174bp**
	Reverse	TTTTGCACGCACTAGCAGAC	
lz	Forward	TTTGCACTATTTCGCAGACG	**163 bp**
	Reverse	GACCATAGCTGGCGTTTGAT	
kibra	Forward	CAGCCCATAGTGGGCATAGT	**157 bp**
	Reverse	TGTTCCTGTTGTCGCTTCTG	
dome	Forward	TACCTTGCCGGAAGCTAATC	**173 bp**
	Reverse	GGTGGCTCTATGAGGGTGCT	
E(Pc)	Forward	CTCACGTCTCGACTGGGAAC	**193 bp**
	Reverse	CTAGCTGGAAGGGATTGTGG	
hers	Forward	AGCCTCATCGACAACCACTC	**185 bp**
	Reverse	ATCGTCCTCAGCTTCATCGT	
Ras	Forward Normal	GGTCGTCGTTGGAGCCGG	**242bp**
	Forward Mutant	GGTCGTCGTTGGAGCCGT	
	Reverse	CACTGTTGACGGCAAAGACC	

Table S2. The PCR conditions used for semi-quantitative RT- PCR

Gene	Initial Denaturation	Denaturation	Annealing	Extension	No. of cycles	Final Extension
g3pdh	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
Ras	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
Ras ^{V12}	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.

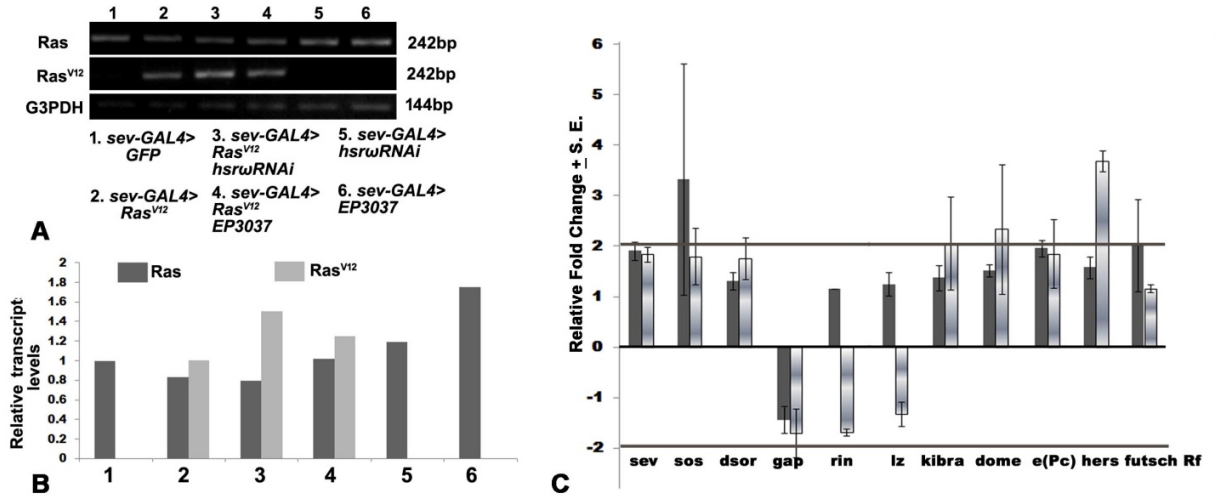

Fig. S1. Levels of transcripts of Ras signaling pathway genes remained unaltered in activated Ras background with either down- or up-regulation of hsrω transcrtipts. A Agarose gel images of RT-PCR amplicons to show levels of transcripts of endogenous *Ras*, activated *Ras^{V12}* and *G3pdh* (internal 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^{V12}* (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^{V12}UAS-hsrωRNAi* (dark gray) and *sev-GAL4>UAS-Ras^{V12}EP3037* (light grey) eye discs compared to *sev-GAL4>UAS-Ras^{V12}* eye discs..