

# GENETICS

## Supporting Information

<http://www.genetics.org/cgi/content/full/genetics.110.118166/DC1>

### **Behavioral Responses to Hypoxia in *Drosophila* Larvae Are Mediated by Atypical Soluble Guanylyl Cyclases**

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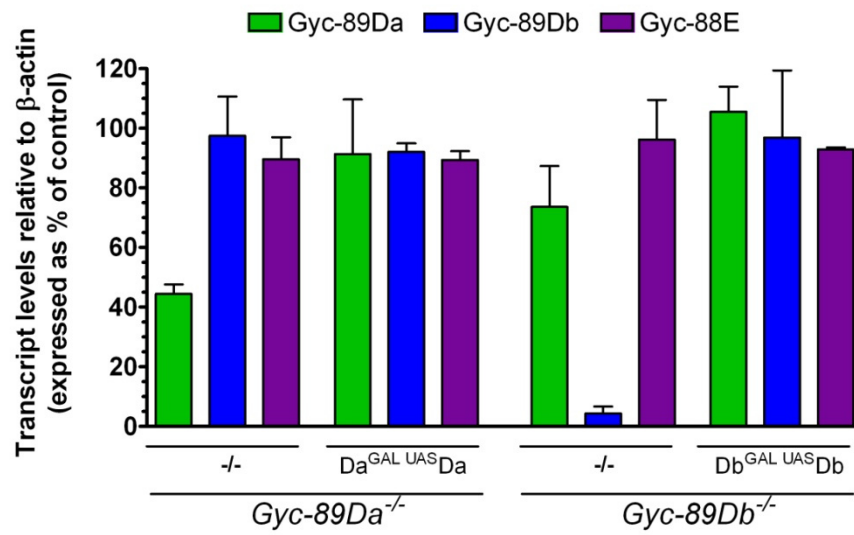


FIGURE S1.—Expression of *Gyc-89Da*, *Gyc-89Db* and *Gyc-88E* relative to actin and expressed as % of wild type controls. Total RNA was extracted from adult heads and 1  $\mu$ g was used to prepare cDNA. After diluting the cDNA 1:4, 2  $\mu$ l was used for the real-time PCR reaction. Results are the average of three separate RNA preparations.

0 copies	1 copy	2 copies	3 copies	4 copies
<i>Gyc-89Da<sup>-/-</sup>Db<sup>-/-</sup></i>	<i>Gyc-89Da<sup>-/-</sup>Db<sup>-</sup></i>	<i>Gyc-89Da<sup>-/-</sup></i>	<i>Gyc-89Da<sup>-/+</sup></i>	<i>w<sup>1118</sup></i>
<i>Gyc-89DaDb Df / Gyc-89Da<sup>-</sup>Db<sup>-</sup></i>	<i>Gyc-89Da<sup>-</sup>Db<sup>-/-</sup></i>	<i>Gyc-89Db<sup>-/-</sup></i>	<i>yc-89Db<sup>-/+</sup></i>	
	<i>Gyc-89DaDb Df / Gyc-89Da<sup>-</sup></i>	<i>Gyc-89Da<sup>-</sup> / Gyc-89Db<sup>-</sup></i>		
	<i>Gyc-89DaDb Df / Gyc-89Db<sup>-</sup></i>	<i>Gyc-89Da<sup>-/+</sup>Db<sup>-/+</sup></i>		
		<i>Gyc-89DaDb Df / +</i>		

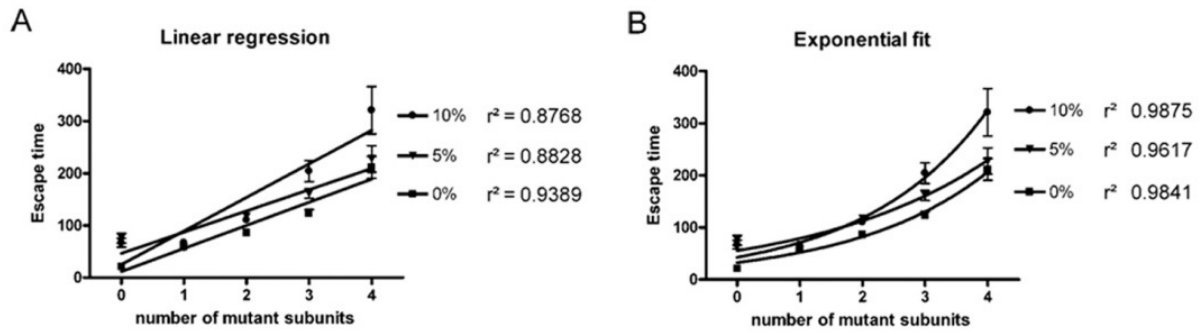


FIGURE S2.—Non-linear relationship between *Gyc-89Da/Db* copy number and hypoxia escape response. Larvae with the genotypes shown in the following table were assayed for their hypoxia escape response as described in the methods section. Data from larvae with different genotypes, but the same number of wild type copies of *Gyc-89Da* or *Gyc-89Db* were pooled. When the results were plotted the data fitted exponential curves (B) better than linear regression (A) for all O<sub>2</sub> concentrations tested. All values are mean ± SEM. N=20-60.

**FILE S1****Supporting Movie**

Three 3<sup>rd</sup> instar larvae were placed in a small pile of yeast on a layer of agar in a petri dish, the dish inverted and filmed with a video camera fitted with a red filter to reduce glare. The first portion of the movie shows wild type larvae (*w<sup>1118</sup>*) and the second portion shows larvae with ChR2-H134R expressed in the Gyc-89Da neurons. In both cases, when the caption “Light on” shows, the larvae were illuminated for 50s with a blue LED flashing at 17Hz.

**TABLE S1****List of primers used RT-PCR**

Primer	Sequence	Fragment size
Gyc-89Da, 1 <sup>st</sup> round	5'-TTCAGTGAATCCCATCCGGTGG-3'	882 bp
	5'-ATCGCCGGTGTACTTCGATA-3'	
Gyc-89Da, 2 <sup>nd</sup> round	5'-TGAATCTGCGAGAGAGGATGAG-3'	708 bp
	5'-CCAACTCGAATAGCCACGTC-3'	
Gyc-89Db, 1 <sup>st</sup> round	5'-AGTGCGGAGAACGCCAAGGAAG-3'	889 bp
	5'-CCTTCAGCGCAGTATAGTTGG-3'	
Gyc-89Db, 2 <sup>nd</sup> round	5'-GGCAGCCACCTCCAAGGACGAA-3'	714 bp
	5'-TTAATGCCACCCGTATAGC-3'	
$\beta$ -actin, 1 <sup>st</sup> - 2 <sup>nd</sup> rounds	5'-TCGCAGTTCTACAGCGAAAG-3'	1132 bp gDNA
	5'-AGGGCGTAACCCTCGTAGAT-3'	592 bp cDNA

**TABLE S2****List of primers used real time PCR**

Primer	Sequence	Fragment size
Gyc-89Da	5'-CGATGCTGTCTTAAACATGGACT-3'	171 bp
	5'-GAAGATCGACGTTCCCCAGT-3'	
Gyc-89Db	5'-TGGCCTTAAGGGTGATGAAG-3'	237 bp
	5'-CAACCTTATAGCCCACCTTTTG-3'	
Gyc-88E	5'-GTGCACATATCCGAGTCTACGA-3'	238 bp
	5'-GAGTCAACGGCTTGGATACAG-3'	
$\beta$ -actin	5'-GGTGTGGTGCCAGATCTTCT-3'	150 bp
	5'-CAGAGCAAGCGTGGTATCCT-3'	

TABLE S3

**Escape response times for Gyc-89Da and Gyc-89Db single and double mutants compared to parental fly lines used for Gyc-89Da and Gyc-89Db rescue assays**

	Mutant backgrounds			
	Gyc-89Da	Gyc-89Db	Gyc-89Da/Db	
-/-	91.93 ± 10.09	86.98 ± 7.02	178.5 ± 17.81	0%
	121.8 ± 9.53	128.3 ± 12.28	243.8 ± 23.14	5%
	144.1 ± 13.13	128.9 ± 13.08	233.5 ± 26.11	10%
Da <sup>GAL4</sup> ;-/-	96.70 ± 9.403	93.60 ± 14.93	167.3 ± 23.92	0%
	136.40 ± 19.77	139.7 ± 20.47	209.30 ± 31.76	5%
	141.0 ± 15.84	120.8 ± 20.18	235.2 ± 30.80	10%
Db <sup>GAL4</sup> ;-/-	ND	91.95 ± 11.24	180.7 ± 27.45	0%
	ND	142.60 ± 19.84	216.40 ± 33.16	5%
	ND	118.7 ± 16.18	224.1 ± 36.67	10%
UASDa;-/-	95.20 ± 19.29	84.65 ± 11.50	182.10 ± 24.71	0%
	129.40 ± 13.83	133.70 ± 19.97	221.50 ± 33.11	5%
	134.6 ± 45.42	115.8 ± 14.57	225.1 ± 30.39	10%
UASDb;-/-	ND	93.60 ± 11.11	177.40 ± 28.44	0%
	ND	147.3 ± 22.45	217.20 ± 28.00	5%
	ND	113.2 ± 19.43	206.2 ± 33.49	10%

Values represent mean ± SEM at three O<sub>2</sub> concentrations (0%, 5% and 10%). (-/-) represents mutant backgrounds (N=40); (Da<sup>GAL4</sup>;-/- and Db<sup>GAL4</sup>;-/-) represents the GAL4 construct in the different mutant backgrounds (N=20), and (UASDa;-/- and UASDb;-/-) represents the UAS construct in the mutant background (N=20). ND = not determined (90% of animals were Db<sup>GAL4</sup>;-/-Sb or UASDb;-/-Sb)