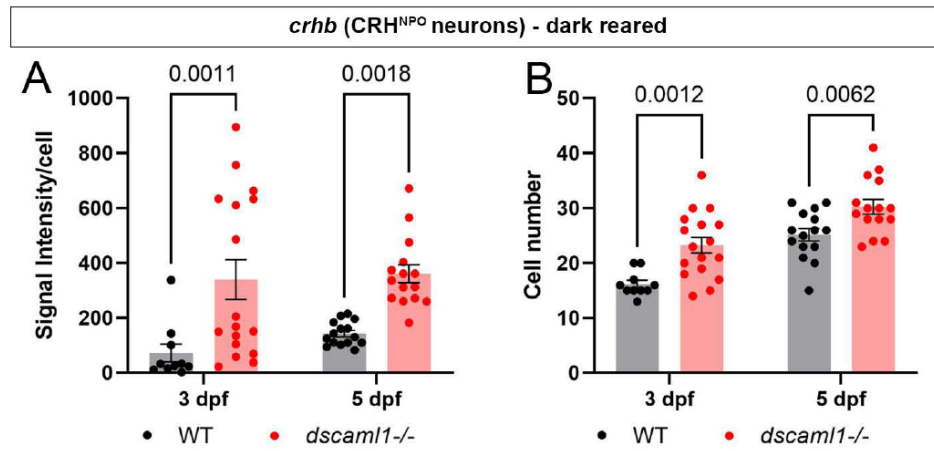
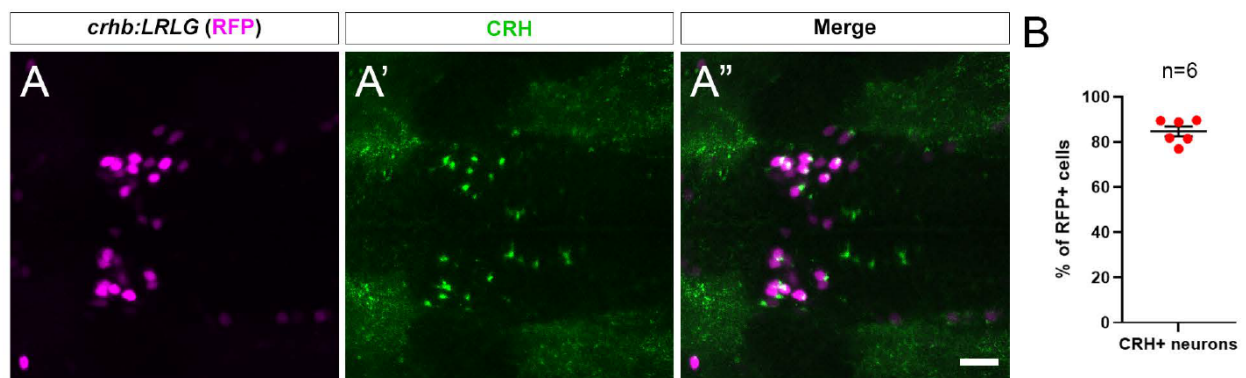


Ma et al. 2023.

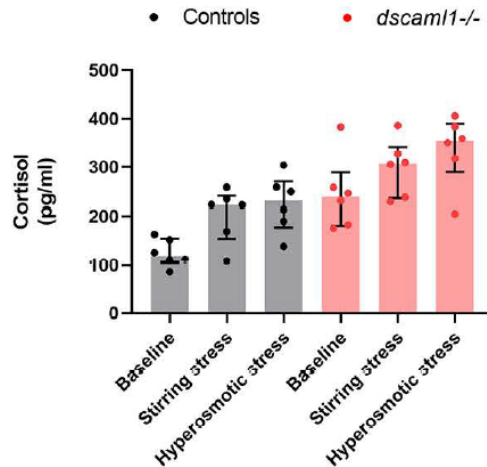
## Supplementary Figures and Table



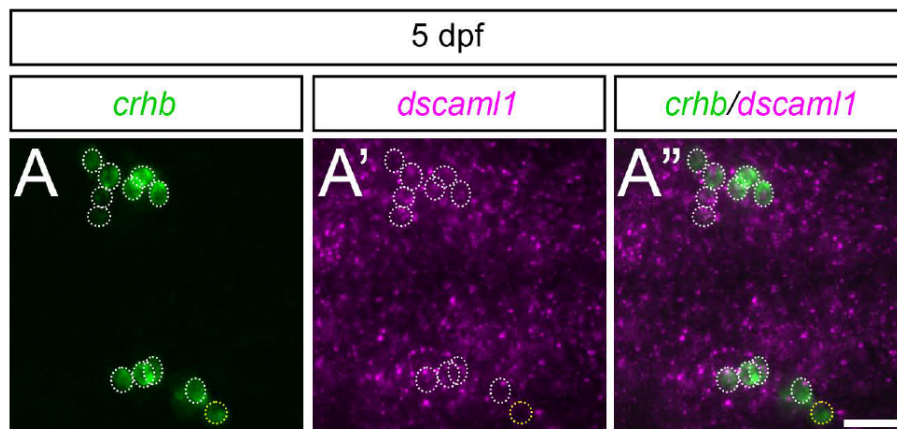
**Supplementary Fig. S1. CRH<sup>NPO</sup> neuron development is impaired in the absence of ambient light (dark reared).** Quantification of CRH<sup>NPO</sup> neurons, labeled by *crhb* FISH. Graphs show the signal intensity per cell (A) and cell number (B). Multiple-comparison corrected *p* values are as shown. WT: n=10 (3 dpf), 15 (5 dpf). *dscaml1*<sup>-/-</sup>: n=17 (3 dpf), 15 (5 dpf)



**Supplementary Fig. S2. The *crhb*:LRLG transgenic line labels CRH-expressing neurons.** (A-A'') Most RFP+ neurons (magenta, anti-RFP) express the CRH protein (green, anti-CRH). (B) Percentage of RFP+ neurons that express CRH. Mean, standard error, and sample size are shown. The scale bar is 20  $\mu$ m.



**Supplementary Fig. S3. Non-normalized baseline and stress-induced cortisol profile for 5 dpf larvae at ZT6-8.** Same measurements as Fig. 5B, but without normalization. For each sample (dot), cortisol was extracted from a pool of 30 animals. n=6 for all groups. Controls in black and *dscaml1*<sup>-/-</sup> animals in red. Median and interquartile range are shown.



**Supplementary Fig. S4. *dscaml1* is expressed in CRH<sup>NPO</sup> neurons during larval development.** Double FISH with *crhb* (green, A) and *dscaml1* (magenta, A') RNA probes at 5 dpf. Merged image is shown in A''. Dotted circles mark the outline of *crhb*<sup>+</sup> cells that express *dscaml1* (white) or do not express *dscaml1* (yellow). The scale bar is 20  $\mu$ m.

<b>CRH<sup>NPO</sup> neurons (normal day/night cycle) (Fig. 2E-F)</b>			
<b><i>crhb</i> signal intensity per cell</b>	<b>F (DFn, DFd)</b>	<b>P value</b>	<b>Significant?</b>
Interaction	F (2, 71) = 7.649	P=0.0010	Yes
Developmental stage	F (2, 71) = 10.48	P=0.0001	Yes
Genotype	F (1, 71) = 43.31	P<0.0001	Yes
<b>Cell number</b>	<b>F (DFn, DFd)</b>	<b>P value</b>	<b>Significant?</b>
Interaction	F (2, 71) = 9.102	P=0.0003	Yes
Developmental stage	F (2, 71) = 75.67	P<0.0001	Yes
Genotype	F (1, 71) = 4.865	P=0.0306	Yes

<b>CRH<sup>NPO</sup> neurons (dark reared) (Supp. Fig. S1)</b>			
<b><i>crhb</i> signal intensity per cell</b>	<b>F (DFn, DFd)</b>	<b>P value</b>	<b>Significant?</b>
Interaction	F (1, 53) = 0.2373	P=0.6282	No
Developmental stage	F (1, 53) = 0.8534	P=0.3598	No
Genotype	F (1, 53) = 24.36	P<0.0001	Yes
<b>Cell number</b>	<b>F (DFn, DFd)</b>	<b>P value</b>	<b>Significant?</b>
Interaction	F (1, 53) = 0.5935	P=0.4445	No
Developmental stage	F (1, 53) = 36.83	P<0.0001	Yes
Genotype	F (1, 53) = 21.35	P<0.0001	Yes

<b><i>crhb</i>:LRLG fluorescent positive (Fig. 5G)</b>			
<b>Cell number/hemisphere</b>	<b>F (DFn, DFd)</b>	<b>P value</b>	<b>Significant?</b>
Interaction	F (1, 45) = 0.5128	P=0.4776	No
Developmental stage	F (1, 45) = 50.97	P<0.0001	Yes
Genotype	F (1, 45) = 7.544	P=0.0086	Yes

<b>Dex treatment (Fig. 7C)</b>			
<b><i>crhb</i> signal intensity per cell</b>	<b>F (DFn, DFd)</b>	<b>P value</b>	<b>Significant?</b>
Interaction	F (1, 35) = 8.210	P=0.0070	Yes
Treatment	F (1, 35) = 59.48	P<0.0001	Yes
Genotype	F (1, 35) = 11.91	P=0.0015	Yes

**Supplementary Table I**