**Supplemental Information:**

**Coordinated action of CRH and cortisol shapes acute stress-induced behavioural response in zebrafish**

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**Fig S1: Representative image of the light-dark paradigm.**

Zebrafish are subject to alternating periods (7.5 minutes each) of light and dark. Wildtype zebrafish larvae (4 dpf) will show limited locomotor activity during the light periods, and increased activity during the dark. Each dot represents the sum of 30 seconds of activity ± SEM n = 12 larvae).



**Fig S2: Cortisol levels in WT larvae 15 min post hCRH exposure.**

Cortisol levels in WT larvae at 15 minutes post-hCRH exposure. Bars represent mean ± SEM. There were no significant differences between treatments (one-way ANOVA; n= 4-8 [each n=pools of 10 larvae]); p<0.05).



**Fig S3: Stress does not affect locomotor activity during the dark phase of the behavioural trial.**

Stressed larvae (black bars) have a similar activity level during the intermittent dark periods to WT -Sham larvae (white bars). The total distance moved was calculated as distance each zebrafish larvae moved in the dark during the 30-minute behavioural assay (total time in the dark – 15 minutes). CRHR1-KO larvae have lower activity, regardless of stress at both the 15 minutes and 60 minutes time points (grey bars). Bars represent mean ± SEM (n= 12-50 individual larvae). Data points with different letters/asterisks denote significant differences (two-way ANOVA for each time point, p<0.05).



**Fig S4: Effects of different doses of human corticotropin releasing hormone (hCRH) on larval behaviour**

Larval zebrafish were treated with different doses (1 ng/ml – 5000 ng/ml) of hCRH

15 minutes prior to the behavioural assessment. Doses below 5000 ng/ml did not cause a significant change in locomotor activity from the control. The total distance moved was calculated as distance each zebrafish larvae moved in the light during the 30-minute behavioural assay (total time in the light – 15 minutes). Bars represent mean ± SEM (n= 12-24 individual larvae). Data points with different letters denote significant differences (one-way ANOVA, p<0.05).

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**Fig S5: Epinephrine and not Phenylephrine cause hyperactivity**

Larval zebrafish were treated with either epinephrine (30 µM) or phenylephrine (30 µM)

15 minutes prior to the behavioural assessment. Epinephrine caused a 4-fold increase in locomotion levels, whereas phenylephrine was no different from the control larvae. The total distance moved was calculated as distance each zebrafish larvae moved in the light during the 30-minute behavioural assay (total time in the light – 15 min). Bars represent mean ± SEM (n= 12-24 individual larvae). Data points with different letters denote significant differences (one-way ANOVA, p<0.05).