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Supplemental information

The neuropeptide Pth2 modulates social behavior and anxiety in zebrafish

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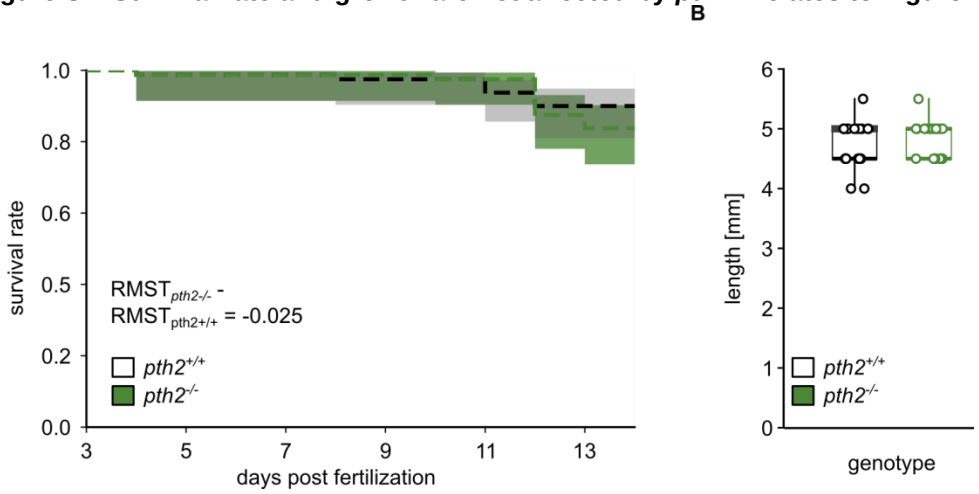
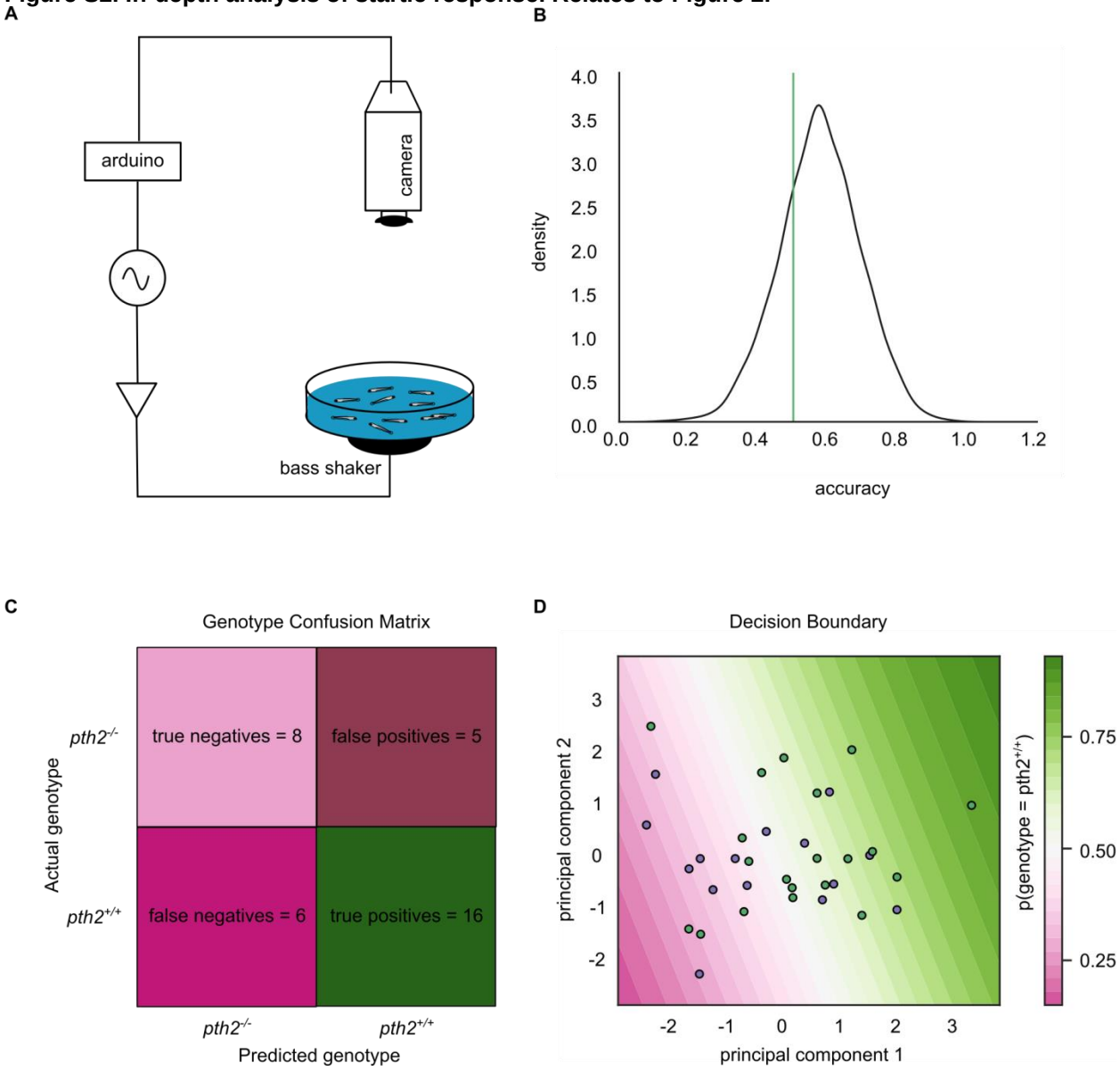
Figure S1. Survival rate and growth are not affected by *pth2.* Relates to Figure 1.



(A) Kaplan-Meier curve shows the survival rate of *pth2*$^{-/-}$ and wildtype fish over the first two weeks of development. Shaded areas indicate the .95 confidence interval. For both groups, n = 80. We analyzed the curves with a log-rank test and found no significant difference ($p_{X2=1.15}$= 0.28), supported by a difference in mean restricted survival times between groups of 0.025 days.

(B) Box plots highlight the average body length at 2 weeks post fertilization. For both groups, n = 20. Distributions were compared using a two-sided, unpaired t-test ($p_{t=0.7,\ df=38}$= 0.48, effect size: 0.22, mean of wildtype: 4.78 cm, mean of mutant: 4.85 cm).

Figure S2. In-depth analysis of startle response. Relates to Figure 2.



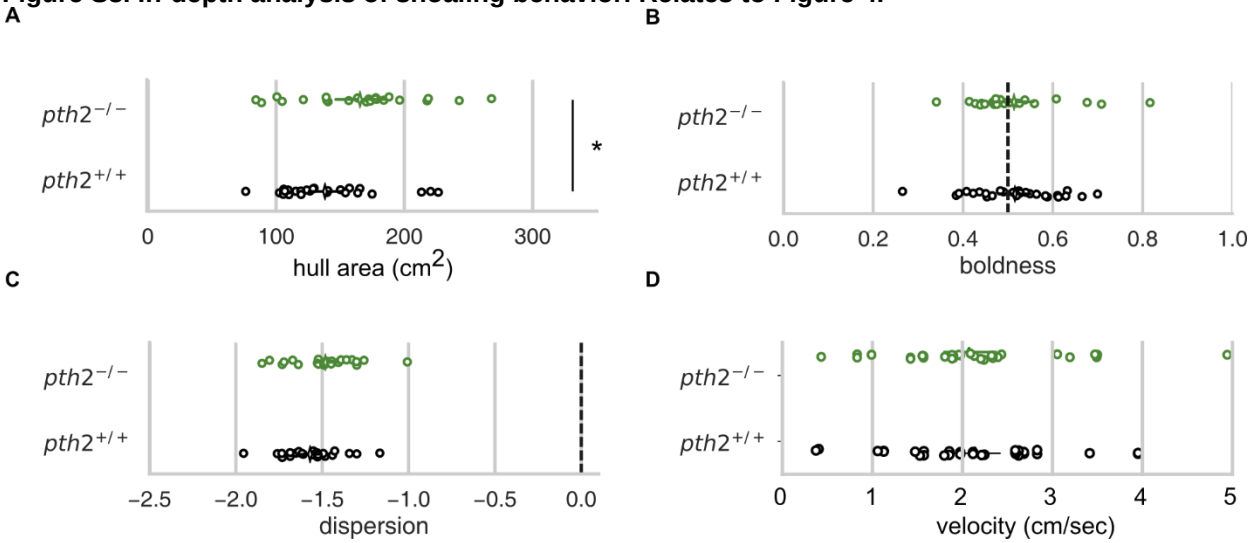
(A) Experimental scheme for the startle experiment. Both the bass shaker for startle cue delivery and the high-speed camera were triggered by an Arduino board at the same time to ensure alignment of videos and cue.

(B) Graph shows the bootstrapped accuracy of the logistic regression model fit to the data. We trained the model 1,000 times sampling 70 % of the full dataset as training set and testing the model on the remaining 30 % as test set. The vertical green line indicates chance level of correct assignment of genotype.

(C) Confusion matrix of the full model, highlighting the number of true and false predictions.

(D) The fraction of SLCs, LLCs, and failures to respond were projected onto the space of the first two principal components to facilitate visualization. Wildtype are plotted as green dots, *pth2*$^{-/-}$ in magenta. The background color indicates the decision boundary computed by the logistic regression model.

Figure S3. In-depth analysis of shoaling behavior. Relates to Figure 4.



(A) Dot plot shows the median area occupied by a convex hull covering all 20 fish over 30 minutes as a measure of shoal cohesion. The mean and its .95 confidence interval are overlaid. For all graphs in this figure, n = 26 for *pth2*$^{-/-}$ and n = 29 for *pth2*$^{+/+}$. Groups were compared with a two-sided, unpaired t-test ($p_{t=2.24,\ df=50}$= 0.03, effect size: 0.64, mean of mutants: 165.4 cm^2, mean of wildtype: 138.1 cm^2).

(B) The boldness parameter indicates the fraction of time the animals spend in the center of the arena versus the periphery. Chance level is indicated by the dashed vertical line. No difference was found between groups (two-sided, unpaired t-test, $p_{t=0.07,\ df=50}$=0.94, effect size: 0.02, mean of mutants: 0.51, mean of wildtype: 0.51).

(C) Dispersion relates the median nearest-neighbor distance to a random distribution (random distance indicated by dashed vertical line)(Harpaz *et al.*, 2021). No difference was detected between groups (two-sided, unpaired t-test, $p_{t=1.74,\ df=50}$= 0.08, effect size: 0.49, mean of mutants: -1.48, mean of wildtype: -1.57).

(D) Velocity of 56 dpf animals during the habituation phase in the tripartite chamber from figure 3E. In the absence of conspecifics, no differences in movement speed are observed (two-sided, unpaired t-test, $p_{t=0.29,\ df = 63}$ = 0.76, effect size: 0.07, mean of mutants: 2.07 cm/sec, mean of wildtype: 2.14 cm/sec).