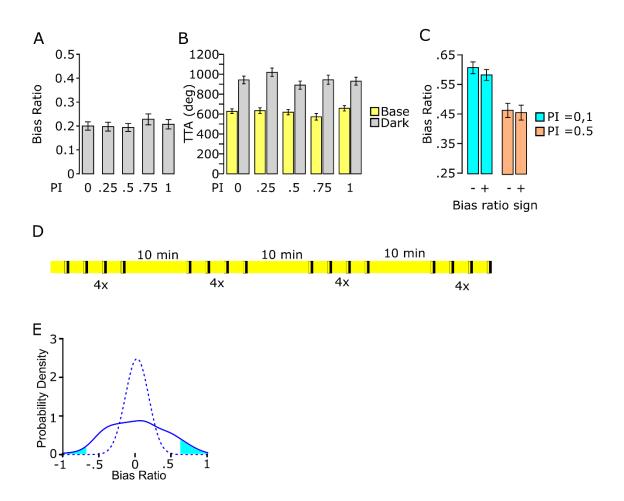
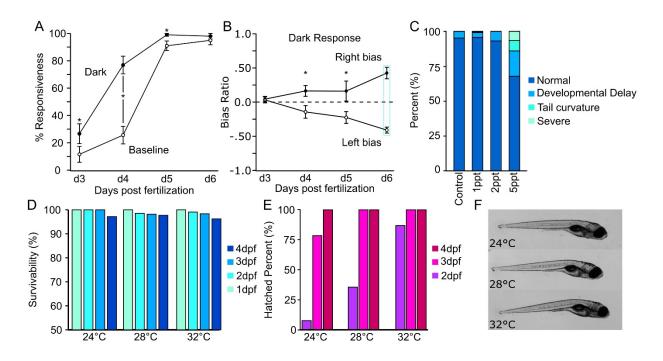


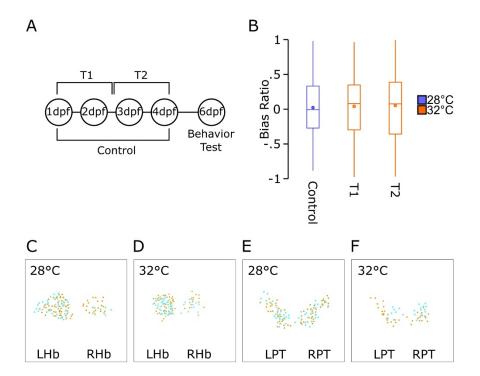
**Supplemental Figure 1. (A)** 4X match index for paired baseline (yellow) and dark response (grey). Red dotted line indicates random choice. Asterisk, p <0.05 Wilcoxon signed-rank test (N = 374). (B) Representation and calculation used for generating bias ratio. NTA is the sum of left (-) and right (+) angular displacement, while TTA is the absolute sum of all angular displacement. Bias ratio is the dividend of NTA to TTA. (C) Population average of 4X recordings (n = 374) (D-F) Same analysis as in Figure 1C-E except for 8X recordings. Asterisk p <0.05. Asterisk in circle, p <0.05 to MAD permutation value. (G-H) 4x (N=374) and 8X (N=189) TTA for baseline (yellow) and dark response (grey).



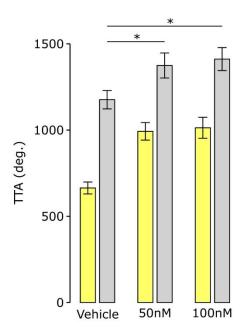
**Supplemental Figure 2. (A)** Absolute bias ratio for 4x baseline illumination responses per PI (PI 0, N=66; PI 0.25, N =74; PI 0.5, N=75; PI 0.75, N=75; PI 1, N= 67). **(B)** TTA for data in A, showing baseline (yellow) and dark (grey) responses. **(C)** Single event absolute bias ratio for matched (PI 0,1 cyan) and unbiased (PI 0.5, orange) individuals sorted by direction (+, rightward; -, leftward). **(D)** Diagram of q4x recording. Yellow and black indicating lights on and off, respectively. Black outline areas indicate 30 second recording windows. **(E)** Average q4X probability density distribution for dark responses (solid line, N= 114) and permutation populations generated from the average of 1000 resampled populations (dotted line). Cyan fill displays percent of population with strong left (<-0.3) and right (>0.3) average bias ratios.



Supplemental Figure 3. (A-B) Developmental onset of turn bias. Individual larvae were tested daily in a 4X assay from 3 to 6 dpf. (A) Proportion of individuals that show motor responsiveness (N=25) (B) Presence of turn bias during development. At 6dpf (dotted blue box), larvae were categorized as right (N=9) or left (N=16) bias based on average bias ratio (+, right bias; -, left bias) to group responses over the testing interval. Asterisk p <0.05, t-test between points at same developmental stage in A and B. No significance shown for 6dpf in B as this timepoint was used to group larvae by performance. (C) Phenotypic scoring at 4dpf following variable salinity exposures during early development. As 5ppt generated some developmental abnormalities this treatment was excluded from behavior testing. (D) Survival of larvae raised from 1-4dpf at different temperatures (24°C, N=103; 28°C, N=180; 32°C, N=209). (E) Proportion of hatched (dechorinated) embryos due to different temperatures (24°C, N=103; 28°C, N=103; 28°C, N=180; 32°C, N=209). (F) Representative images of 6dpf larvae after varying temperature exposure from 1-4 dpf.



**Supplemental Figure 4. (A)** Timeline of elevated temperature testing showing control (28°C) and T1 (31-55 hpf, 32°C) and T2 (55-79 hpf, 32°C) testing conditions which are not statistically different from random (1 sample t-test against 0: control-  $0.023 \pm 0.043 t(100) = 0.53$ , p=0.60; T1-  $0.040 \pm 0.042 t(100) = 0.97$ , p=0.33; T2-  $0.052 \pm 0.045 t(106) = 1.151$ , p=0.25). **(B)** Average population bias ratio for 28°C control (purple, N=101) and T1 (orange, N=111) and T2 (orange, N=107) elevated temperature groups. Circles show means. **(C-F)** Representative neuron counts showing two overlaid examples (represented by orange and cyan, respectively) with circles representing the location of individual neurons for the habenula (28°C **(C)**; 32°C **(D)**) and the PT (28°C **(E)**; 32°C **(F)**).



**Supplemental Figure 5**. TTA following Notch inhibitor exposure during early development (vehicle, N=69;  $50\mu M$ , N=69;  $100\mu M$ , N=48).