Figure S1. Onset and length of PaSNA quantification. Related to Figure 1.

(A) Developmental time metric used to quantify PaSNA onset and progression. **(B)** Onset and hatching measurements of PaSNA (n = 33). **(C)** Number of total episodes from PaSNA onset to hatching (n = 33). Bar plots represent mean with 95% confidence interval. **(D)** Interval stereotypy analysis in which the distribution of interpeak intervals across all embryos are compared to Poisson processes with the same mean (see Methods). ** $p < 0.01$, **** $p < 0.0001$; two-sample t-test, Holm-Bonferroni correction.
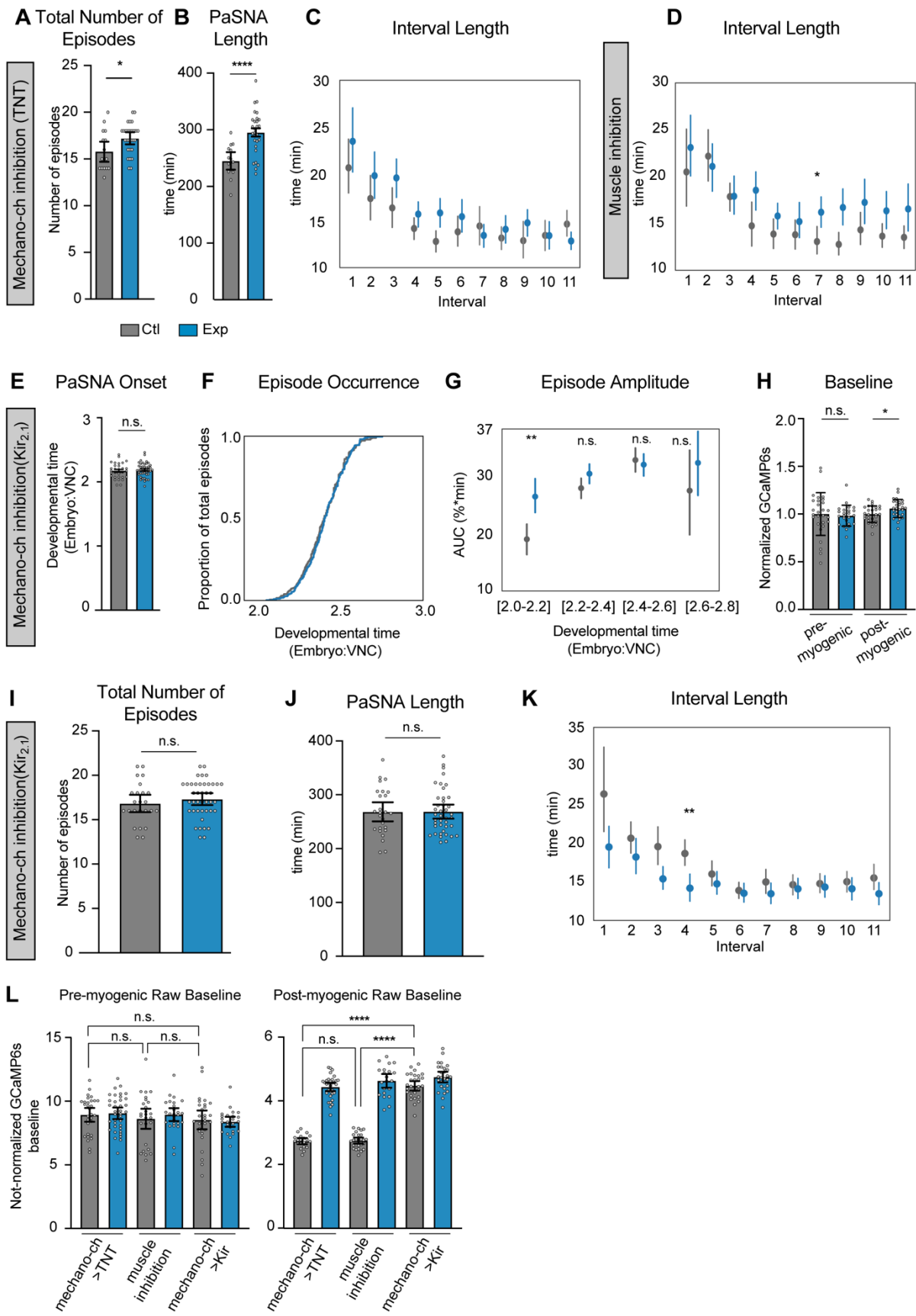
A Total Number of Episodes

B PaSNA Length

C Interval Length

D Interval Length

Mechano-ch inhibition (TNT)

Muscle inhibition

Ctl   Exp

E PaSNA Onset

F Episode Occurrence

G Episode Amplitude

H Baseline

Mechano-ch inhibition(Kir$_{2.1}$)

I Total Number of Episodes

J PaSNA Length

K Interval Length

Mechano-ch inhibition(Kir$_{2.1}$)

L Pre-myogenic Raw Baseline   Post-myogenic Raw Baseline

Figure S2. Mechanosensory neurons modulate the amplitude of PaSNA episodes. Related to Figure 3.

(A-C) Quantification of PaSNA phenotypes in control embryos in gray and experimental embryos expressing TNT in mechano-ch in blue. **(A)** Total number of episodes from PaSNA onset to hatching (n = 16 control; n = 28 experimental). **(B)** Time span from PaSNA onset to hatching (n = 16 control; 28 experimental). **(C)** Quantification of the first eleven interbout interval length (n = 17 control; n = 32 experimental). **(D)** Quantification of the first eleven interbout interval lengths (n = 28 control; n = 28 experimental) for control embryos in gray and experimental embryos expressing $Kir_{2.1}$ in muscles in blue. **(E-K)** Measurements of the timing and intensity of PaSNA for control embryos (gray) and experimental embryos expressing $Kir_{2.1}$ in mechano-ch neurons (blue). **(E)** Quantification of PaSNA onset (n = 30 control; n = 41 experimental). **(F)** Cumulative occurrence of the first twelve episodes plotted as the proportion of total episodes across developmental time (n = 27 control; n = 29 experimental). **(G)** AUC quantification for the first twelve episodes plotted against binned developmental time (n = 26, control; n = 41 experimental). **(H)** Quantification of GCaMP6s baseline levels normalized against control mean before (n = 28 control; n = 24 experimental) and after the myogenic phase (n = 28 control; n = 28 experimental). **(I)** Total number of episodes from PaSNA onset to hatching (n = 24 control; n = 41 experimental). **(J)** Time span from PaSNA onset to hatching (n = 26 control; n = 41 experimental). **(K)** Quantification of the first eleven interbout interval lengths (n = 27 control; n = 29 experimental). **(L)** Raw GCaMP6s baselines for control (gray) and experimental (blue) groups. Different experiments labeled on the X axis. As GCaMP6s and TdTomato are expressed at low levels during the pre-myogenic stage, we increased excitation power at this stage, making direct comparisons between pre- and post-myogenic stages impossible. For all point plots, points represent mean and lines depict the 95% confidence interval. For all bar graphs the mean and 95% CI are displayed. ****p<0.0001, ***p<0.005, **p<0.005, *p<0.05. For **(A)**, **(B)**, **(E)**, **(I)** and **(J)** we used a two-sample t-test. For **(C)**, **(D)**, **(G)** and **(K)** we used two-sample t-tests with Holm-Bonferroni correction. For **(H)** we used two-sample Welch's t-tests to account

for difference in variance. For (**L**) we used a Brown-Forsythe and Welch ANOVA followed by a Dunnett's T3 multiple comparison test to account for differences in variance. For genotypes information see Table S1**.**
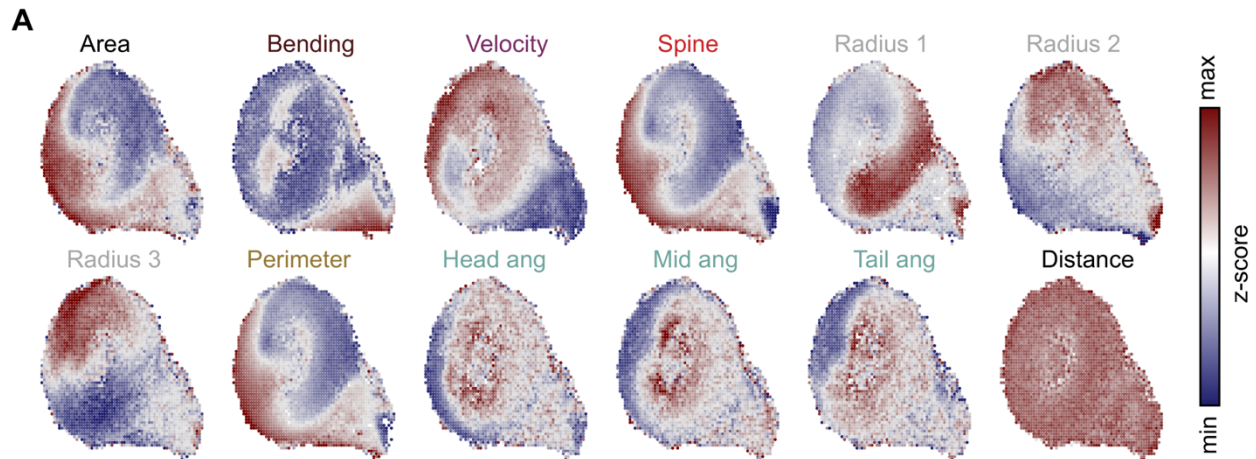

Figure S3. Primary behavioral metrics as a function of larval behavioral space. Related to Figure 4.

The distribution of area, bending, velocity, spine length, radius 1, radius 2, radius 3, perimeter, head angle, middle body angle, tail angle, and distance as a function of larval behavior space (z-scores). Z-score scale to the right.