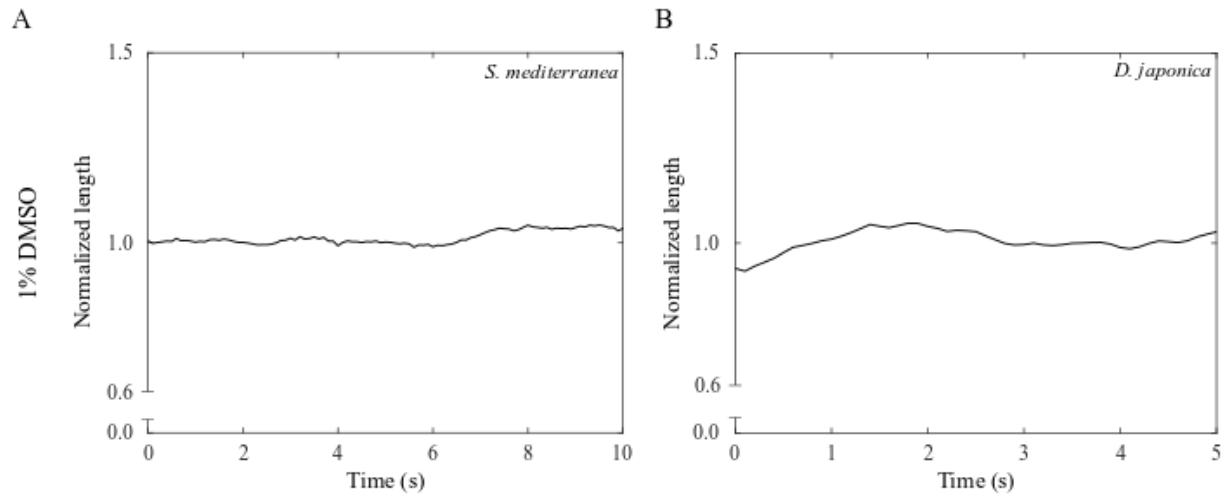


Supporting Information

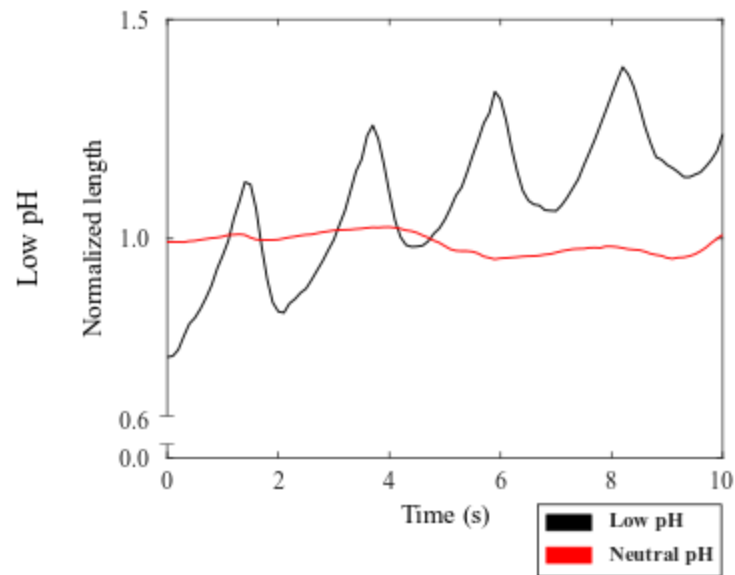
S1 Table. Maximum elongation and speed of scrunching are dependent on AITC

concentration. Scrunching parameters for *D. japonica* and *S. mediterranea* exposed to 50, 75, or 100 μM AITC, denoted as mean $\pm$ standard deviation. For each concentration, planarians were observed to scrunch with the parameters listed within the first minute in the bath. * denotes $p < 0.05$ and ** denotes $p < 0.01$ significance level compared to 50 μM AITC given by a two-tailed t-test. ^ denotes $p < 0.05$ and ^^ denotes $p < 0.01$ significance level compared to amputation given by a two-tailed t-test. ^aAmputation data are previously published values (4) provided for reference.

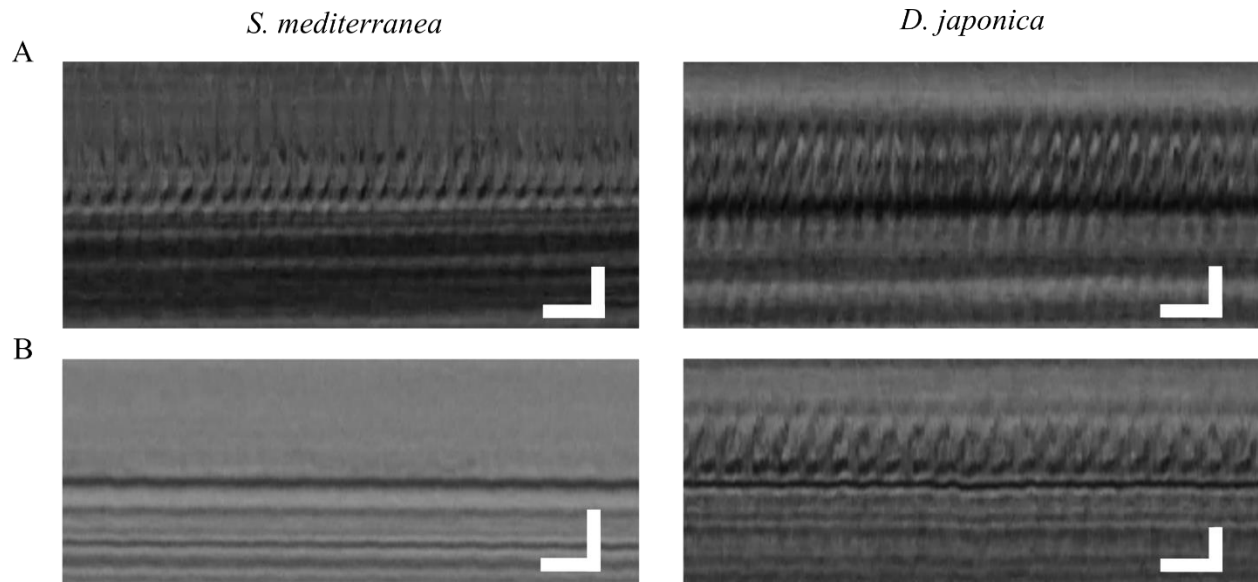
Species	Induction	Frequency (cycles s ⁻¹)	Maximum elongation	Speed (body length s ⁻¹)	N=
<i>D. japonica</i>	50 μM AITC	0.72 $\pm$ 0.08	0.52 $\pm$ 0.04	0.37 $\pm$ 0.04	9
<i>D. japonica</i>	75 μM AITC	0.78 $\pm$ 0.13	0.56 $\pm$ 0.06	0.44 $\pm$ 0.09*	9
<i>D. japonica</i>	100 μM AITC	0.92 $\pm$ 0.06**, ^	0.60 $\pm$ 0.03**, ^^	0.55 $\pm$ 0.05**, ^^	8
<i>D. japonica</i>	Amputation ^a	0.70 $\pm$ 0.27	0.50 $\pm$ 0.08	0.34 $\pm$ 0.12	15
<i>S. mediterranea</i>	50 μM AITC	0.33 $\pm$ 0.02	0.43 $\pm$ 0.05	0.14 $\pm$ 0.01	5
<i>S. mediterranea</i>	75 μM AITC	0.42 $\pm$ 0.14	0.49 $\pm$ 0.02*	0.21 $\pm$ 0.07*	5
<i>S. mediterranea</i>	100 μM AITC	0.41 $\pm$ 0.09	0.51 $\pm$ 0.12*	0.22 $\pm$ 0.02**	5
<i>S. mediterranea</i>	Amputation ^a	0.40 $\pm$ 0.09	0.44 $\pm$ 0.09	0.17 $\pm$ 0.09	77



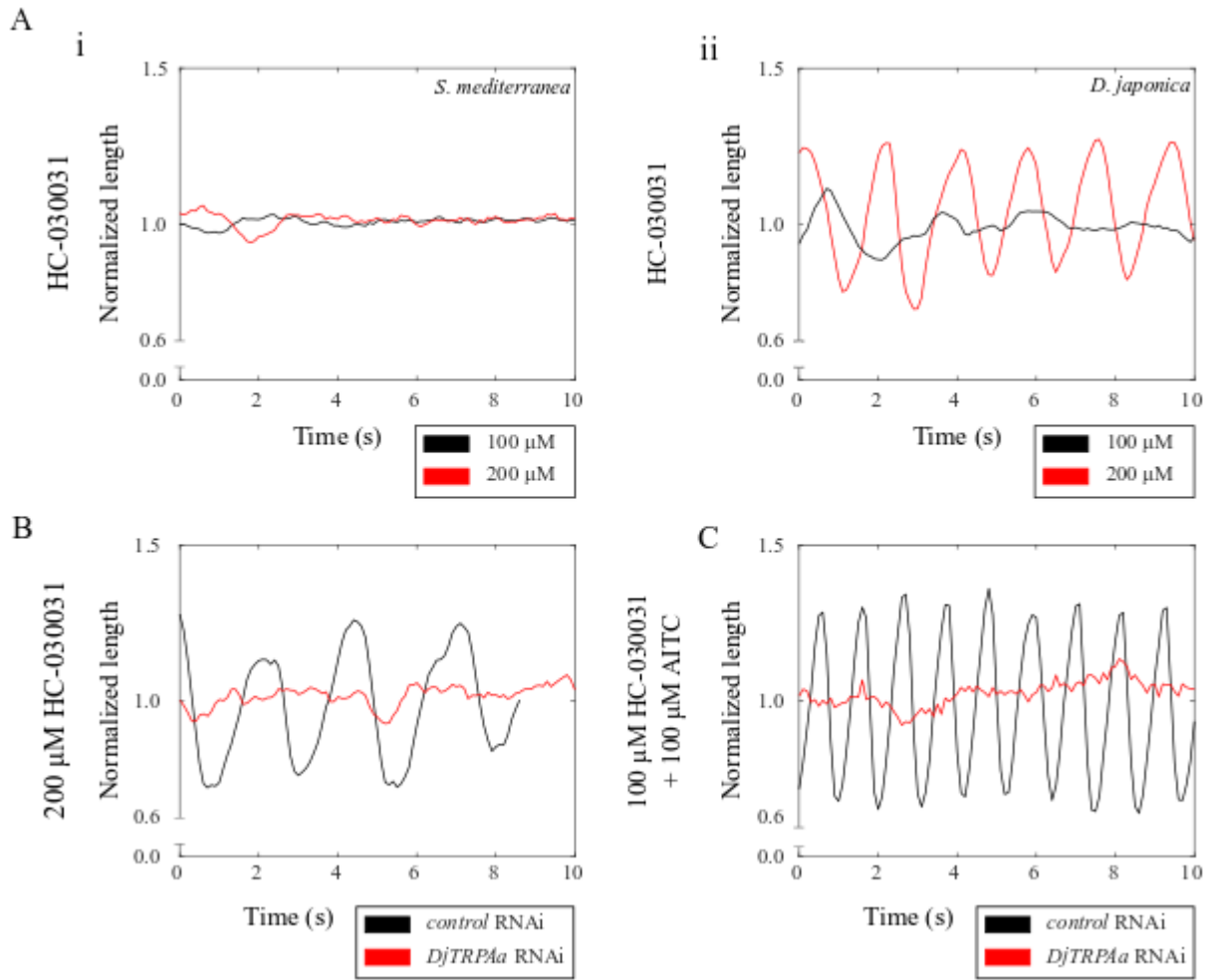
S1 Fig. 1% DMSO does not induce scrunching in either *S. mediterranea* or *D. japonica*. Representative length versus time plot for wildtype (A) *S. mediterranea* or (B) *D. japonica* planarians in 1% dimethyl sulfoxide (DMSO) (N=10). Planarians were exposed to 1% DMSO by directly pipetting 100 μ L.



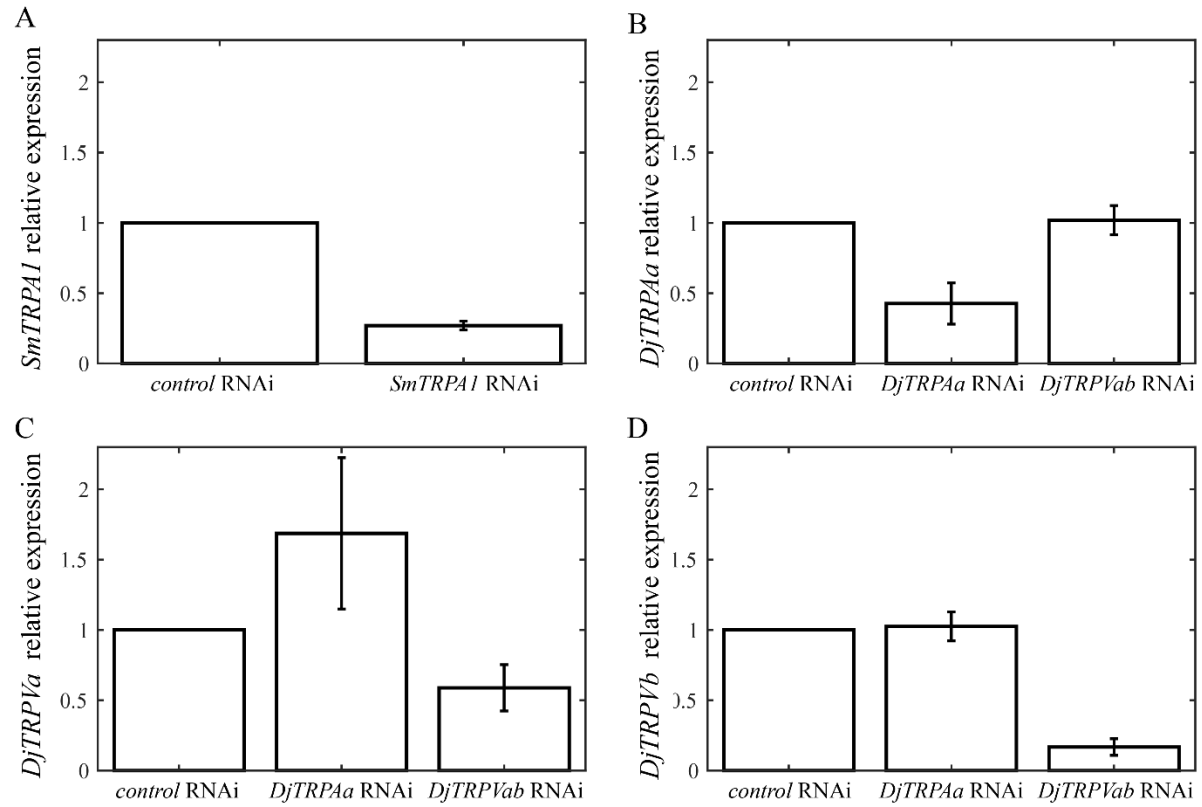
S2 Fig. *S. mediterranea* scrunch in response to low pH exposure. Representative length versus time plot for wildtype *S. mediterranea* planarians in Instant Ocean water at neutral pH (red, N=5) and pH 2.7 (black, N=10). Scrunching was induced by directly pipetting 100 μ L onto planarians



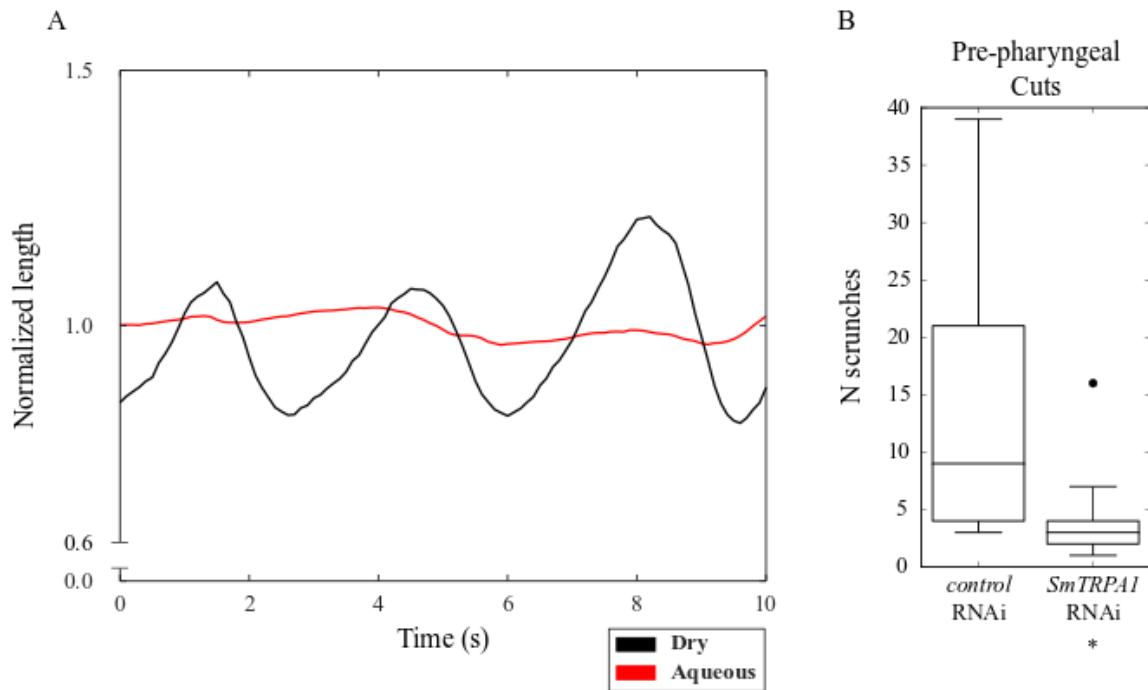
S3 Fig. Anandamide impairs cilia beating in *S. mediterranea* but not *D. japonica*. Representative (N=3/3) 1 s kymograph of cilia beating for *S. mediterranea* (left) and *D. japonica* (right). (A) Controls in planarian water and (B) when exposed to 100 μ M anandamide for 5 minutes. Notice that cilia beating is almost completely lost in *S. mediterranea* while cilia beat normally in *D. japonica*. Scale bar shows 0.1 s horizontally and 1 μ m vertically.



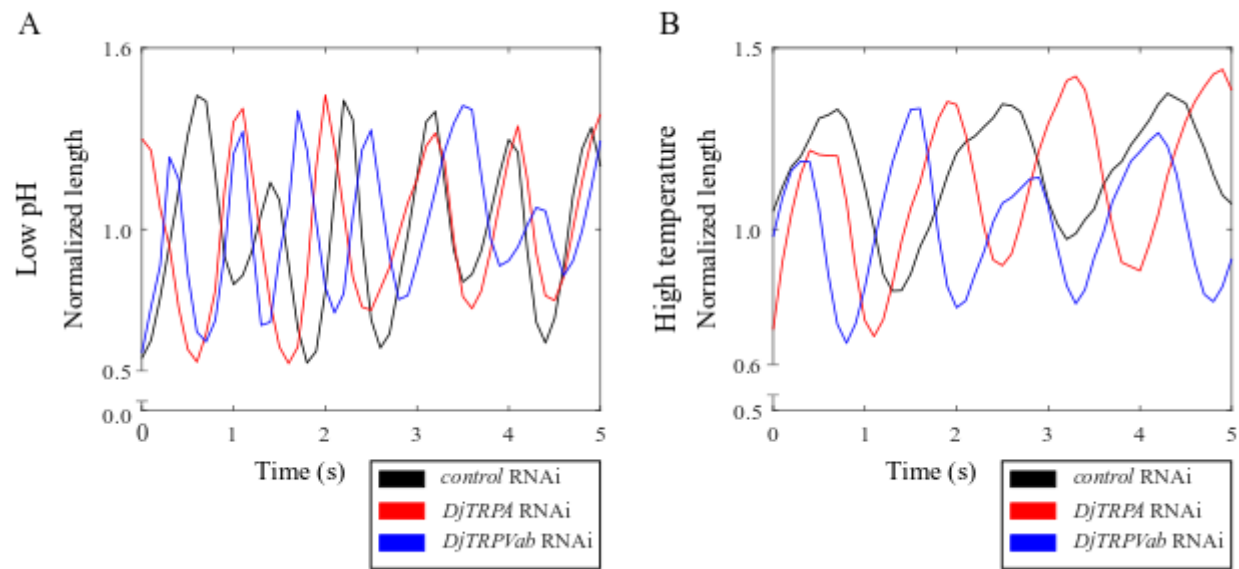
S4 Fig. 200 μ M HC-030031 induction of scrunching in *D. japonica* is dependent on TRPA1. (A) Representative length versus time plot for wildtype (i) *S. mediterranea* and (ii) *D. japonica* planarians in 100 μ M (black) and 200 μ M (red) HC-030031. Plots are representative of N=10. (B) Representative length versus time plot for *D. japonica* control RNAi (N=13) and *DjTRPAa* RNAi (N=11) planarians in 200 μ M HC-030031. (C) Representative length versus time plot for *D. japonica* control RNAi (N=8) and *DjTRPAa* RNAi (N=9) planarians in 100 μ M HC-030031 + 100 μ M AITC.



S5 Fig. Confirmation of RNAi knockdown by qRT-PCR. (A-D) Relative expression of (A) *SmTRPA1*, (B) *DjTRPAa*, (C) *DjTRPVa* and (D) *DjTRPVb* in the respective RNAi populations compared to the *control* RNAi population in that species. Data are shown as the mean of two biological replicates (each including 3 technical replicates). Error bars represent the standard error.



S6 Fig. *SmTRPA1* mediates scrunching in response to amputation. (A) Representative length versus time plot for wildtype *S. mediterranea* planarians in an aqueous (red, N=5) and dry (black, N=10) environment, created by placing the planarians on wet filter paper as in (10). (B) Distribution showing median and quartiles of the number of scrunches directly following amputation in *control* RNAi (N=21) and *SmTRPA1* RNAi (N=24) planarians. * denotes $p < 0.01$ significance from *control* RNAi given by a two-tailed t-test.



S7 Fig. Heat and low pH sensing are not significantly impaired in *DjTRPAa* or *DjTRPVab* RNAi planarians. (A-B) Representative oscillation plots for *control*, *DjTRPAa*, and *DjTRPVab* RNAi planarians exposed to (A) pH 2.7 and (B) 65°C IO water via pipette. No significant differences in scrunching induction are seen in any of the conditions. N=10 for all conditions.

Supplementary Movies

S1 Movie. *D. japonica* and *S. mediterranea* planarians in 165 μ M capsaicin. Comparison of *D. japonica* and *S. mediterranea* behaviors in 165 μ M capsaicin. Planarians exhibit vigorous head shaking and turning as well as scrunching. Movie is recorded and played at 10 fps.

S2 Movie. *D. japonica* exposed to 50 μ M AITC alone and with 100 μ M HC-030031. First 50 s of exposure of *D. japonica* to 50 μ M AITC alone or co-administered with 100 μ M HC-030031. Movie is recorded and played at 10 fps. Scale bar: 1 cm.

S3 Movie. *S. mediterranea* exposed to 50 μ M AITC alone and with 100 μ M HC-030031. First 50 s of exposure of *S. mediterranea* planarians to 50 μ M AITC alone or co-administered with 100 μ M HC-030031. Movie is recorded and played at 10 fps. Scale bar: 1 cm.

S4 Movie. *D. japonica* control and *DjTRPAa* RNAi planarians in 100 μ M AITC. Behavior of *D. japonica* control RNAi and *DjTRPAa* RNAi in 100 μ M AITC during the first 30 seconds of exposure. *Control* RNAi planarians scrunch whereas *DjTRPAa* RNAi planarians exhibit rapid head turning but lack a scrunching response. Movie is recorded and played at 10 fps. Scale bar: 1 cm.

S5 Movie. *S. mediterranea* control and *SmTRPA1* RNAi *S. mediterranea* planarians in 100 μ M AITC. Behavior of *S. mediterranea* control RNAi and *SmTRPA1* RNAi planarians in 100 μ M AITC during the first 30 s of exposure. *Control* RNAi planarians scrunch whereas *SmTRPA1* RNAi planarians glide. Movie is recorded and played at 10 fps. Scale bar: 1 cm.

S6 Movie. *D. japonica* control, *DjTRPAa*, and *DjTRPVab* RNAi planarians in 165 μ M capsaicin. First 60 seconds of *D. japonica* RNAi populations when exposed to 165 μ M capsaicin. Movie is recorded and played at 10 fps. Scale bar: 1 cm.

S7 Movie. *D. japonica* behavior in 1 μ M and 10 μ M SB-366791. Movie showing *D. japonica* behavior in SB-366791. *D. japonica* planarians exhibit no abnormal behaviors in 1 μ M SB-366791 but display vigorous head turning at 10 μ M SB-366791. Movie is recorded and played at 10 fps. Scale bar: 1 cm.

S8 Movie. *D. japonica* control, *DjTRPAa*, and *DjTRPVab* RNAi planarians in 125 μ M anandamide. First 60 seconds of *D. japonica* RNAi populations when exposed to 125 μ M anandamide. *Control* RNAi *D. japonica* planarians exhibit either scrunching or a non-scrunching behavior. Conversely, both *DjTRPAa* and *DjTRPVab* RNAi planarians show a significant decrease in both reactions. Movie is recorded and played at 10 fps. Scale bar: 1 cm.