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Supplementary Information for

A small number of cholinergic neurons mediate hyper-aggression in female *Drosophila*

Caroline B. Palavicino-Maggio[1*], Yick-Bun Chan[1], Claire McKellar[2] and Edward A. Kravitz[1*]

Corresponding authors[*]
caroline_palavicino-maggio@hms.harvard.edu
edward_kravitz@hms.harvard.edu

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List of detailed genotypes and sample sizes

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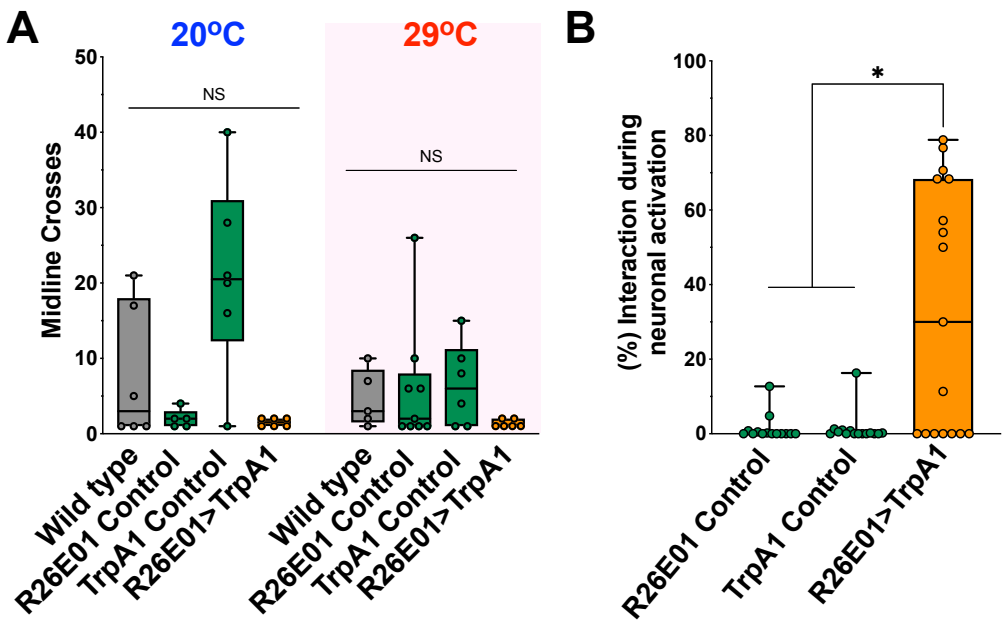

Fig. S1. Behavioral measures of *R26E01>TrpA1.*

(A) TrpA1-mediated activation of *R26E01* neurons does not alter locomotor activity. **(*B*)** During activation *R26E01>TrpA1* female flies interact with headless wild type females (Kruskal-Wallis, H=6.304, $P < 0.05$, n=14-17 flies) longer than control genotypes *R26E01/+* (*P = 0.0272) and *TrpA1/+* (*P = 0.0487). Dunn's multiple comparisons *post hoc* tests determined these values. Data **(*A* and *B*)** center line, median; boxes, first and third quartiles; whiskers, range; circles represent individual values. NS, not significant $P > 0.05$.

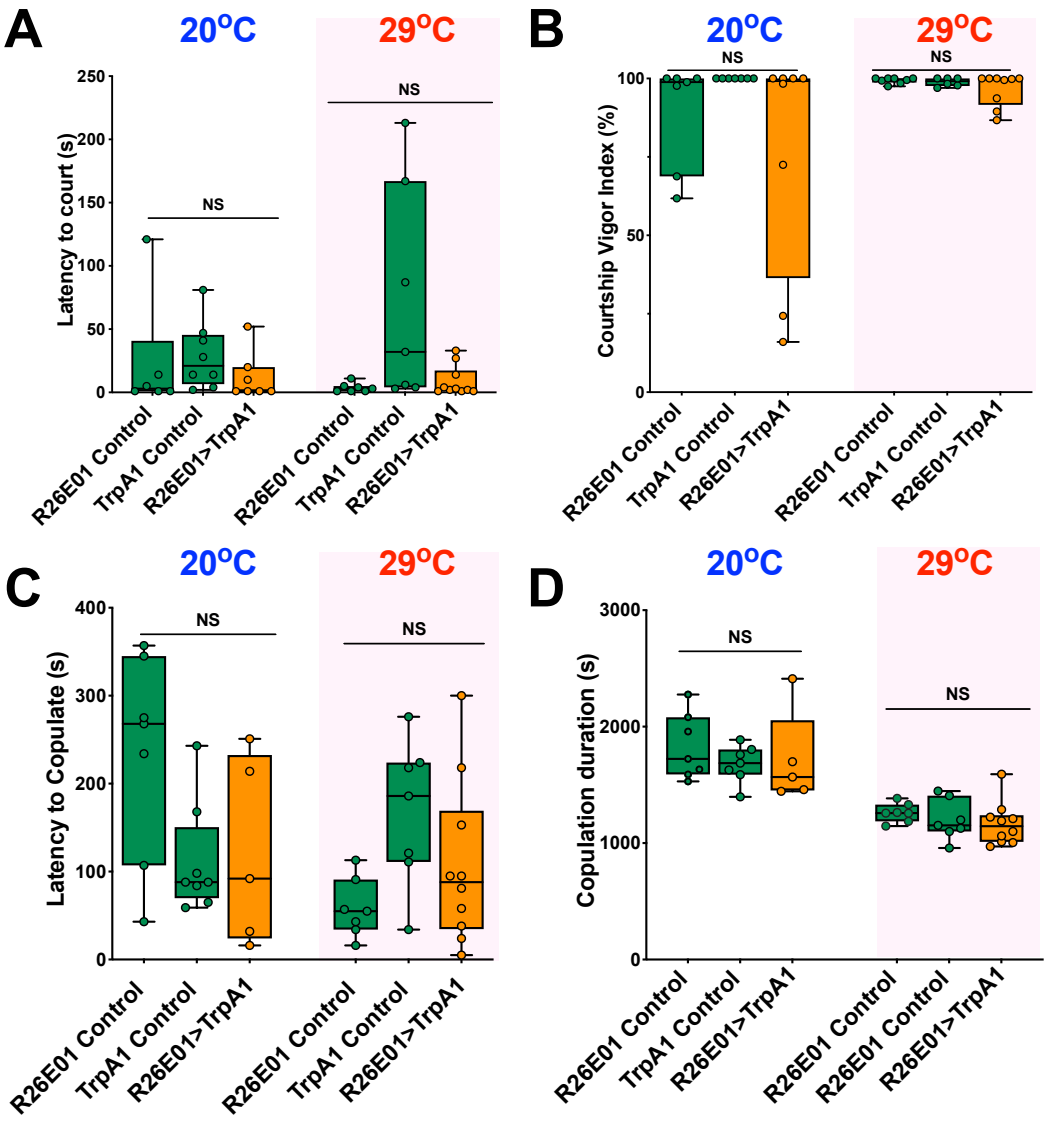

Fig. S2. Thermogenetic activation of *R26E01-Gal4* neurons does not modulate courtship behavior.

No effect on their latencies to (***A***) court or (***B***) copulate, (***C***) CVI or in the (***D***) duration of copulation when control genotypes *R26E01/+, TrpA1/+* or *R26E01>TrpA1* females were paired with wild type *Canton-S* males. NS, not significant $P > 0.05$. Courtship behaviors were measured over 30-minute period.

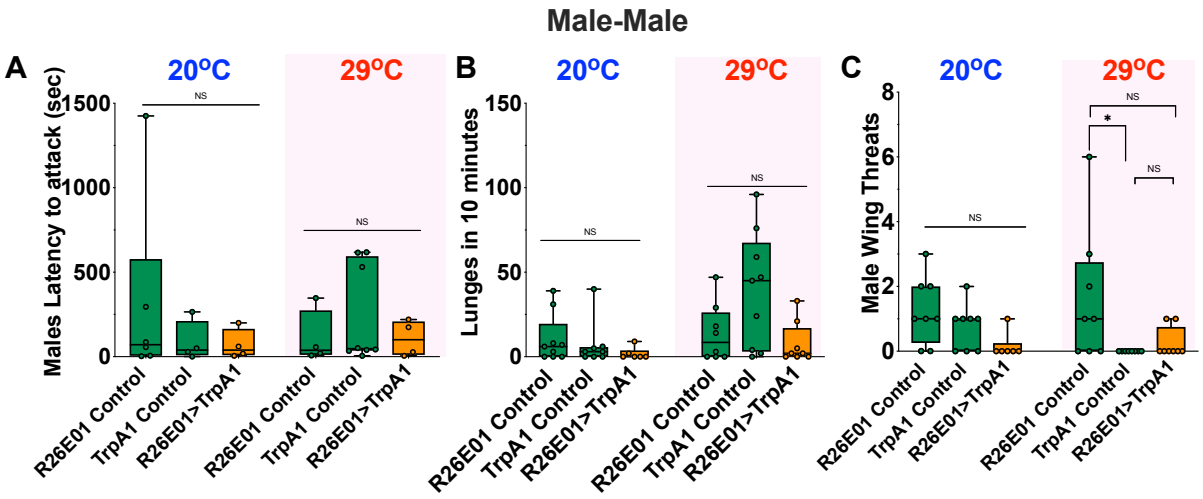

Fig. S3. Thermogenetic activation of *R26E01-Gal4* neurons does not enhance male aggression.

No behavioral differences found in **(*A*)** latencies or in the **(*B*)** number of lunges in *R26E01>TrpA1* male fight pairings compared to control genotypes *R26E01/+* or *TrpA1/+* male fight pairings. An increase in the **(*C*)** number of wing threats (Kruskal-Wallis, H=8.028, P = 0.0181, n=8-9 pairs) was observed in *R26E01/+ (*P< 0.05)* control pairings compared to *TrpA1/+* control pairings at 29°C. However, no differences were seen between *R26E01>TrpA1* and control pairings. Dunn's multiple comparisons *post hoc* tests determined. Data **(*A-C*)** center line, median; boxes, first and third quartiles; whiskers, range; circles represent individual values. NS, not significant $P > 0.05$. Aggressive behaviors were measured over 40-minute period.

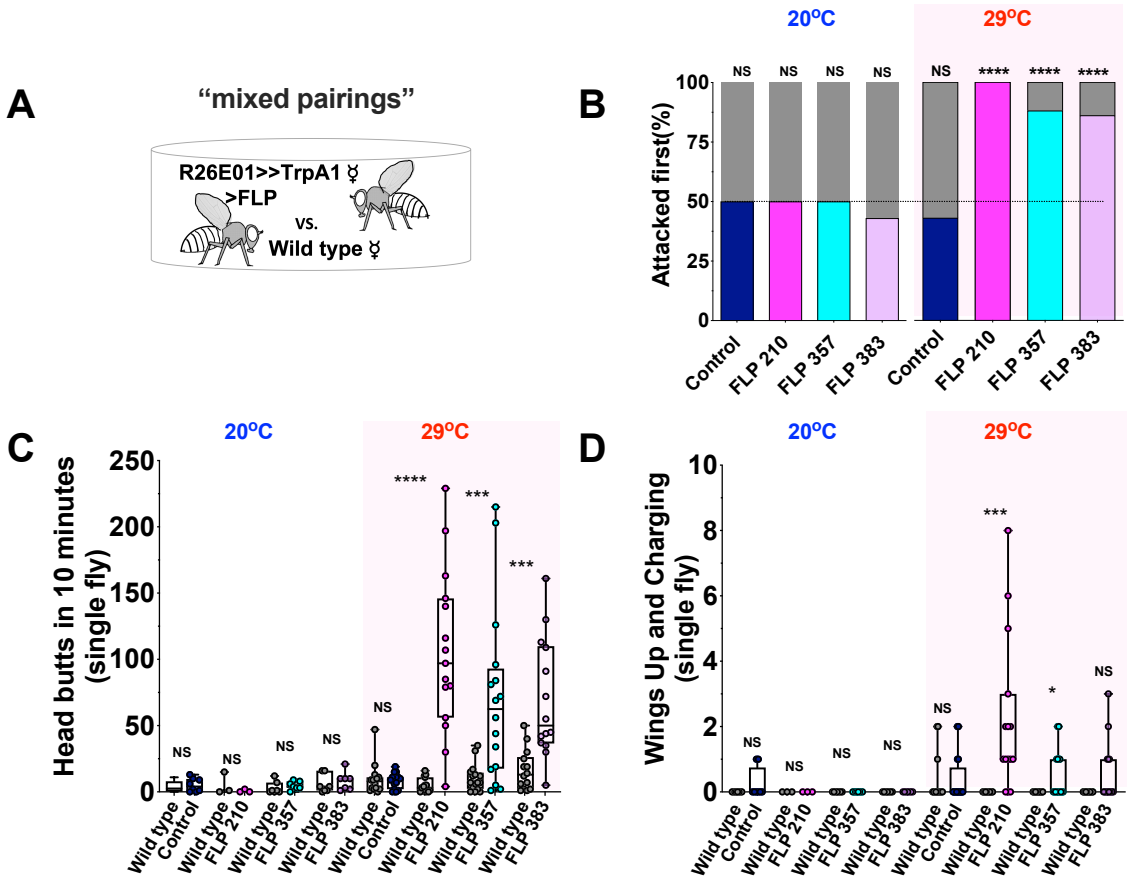

Fig. S4. *et-^{FLP}∩R26E01* versus *Canton-S* wildtype flies.

(*A*) Schematic of assay in which a *FLP#∩R26E01* female is paired with a wild type fly in a fighting chamber at 20°C or 29°C. **(*B*)** Percentage of flies that attacked first during fights against *Canton-S* (gray bars) opponents (Chi-square test, $\chi 2=113.5$). **(*C*)** Number of head butts and times flies had their **(*D*)** wings up and charging versus their wild type opponent. Wilcoxon matched-pairs signed rank test determined the differences between measures. ****$P < 0.0001$; ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; NS, not significant $P > 0.05$; Data **(*C* and *D*)** center line, median; boxes, first and third quartiles; whiskers, range; circles represent individual values. Aggressive behaviors were measured over 40-minute period.

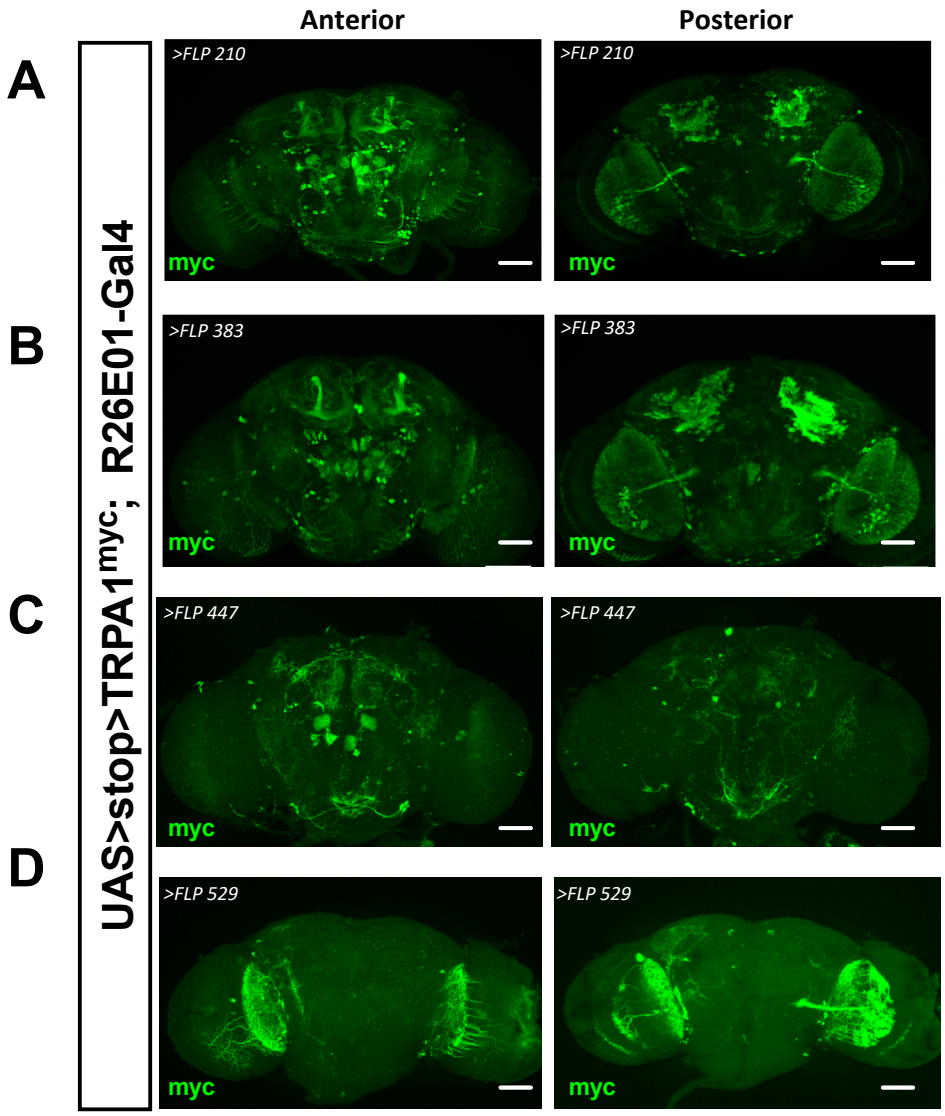

Fig. S5. FLP library TrpA1^{myc} expression patterns.

Confocal Z-stack images of brains expressing TrpA1^{myc} with anti-myc antibody (green) in **(*A*)** *210^{FLP}∩R26E01*, **(*B*)** *383^{FLP}∩R26E01*, **(*C*)** *447^{FLP}∩R26E01* and **(*D*)** *529^{FLP}∩R26E01.*

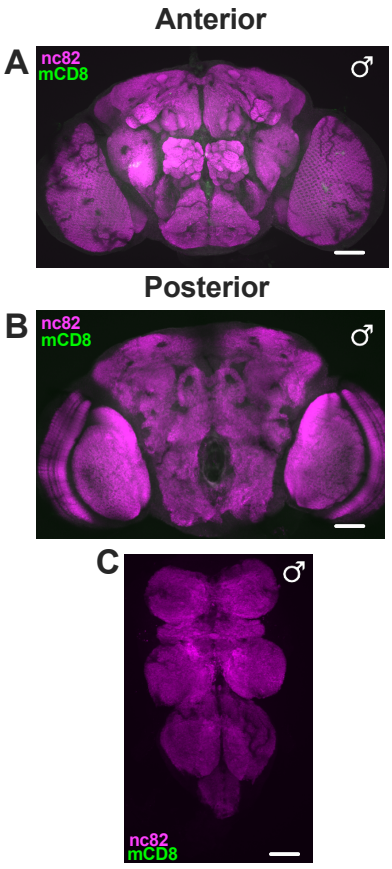

Fig. S6. Expression in *UAS>stop>mCD8::GFP; R26E01-Gal4/dsx^{FLP}* males.
No GFP (green) expression of pC1 *dsx* neurons in *UAS>stop>mCD8::GFP; R26E01-Gal4/dsx^{FLP}* male adult brains **(*A* and *B*)** or abdominal ganglion ***(C)*** counterstained with nc82. Scale bar represents 50*μ*m.

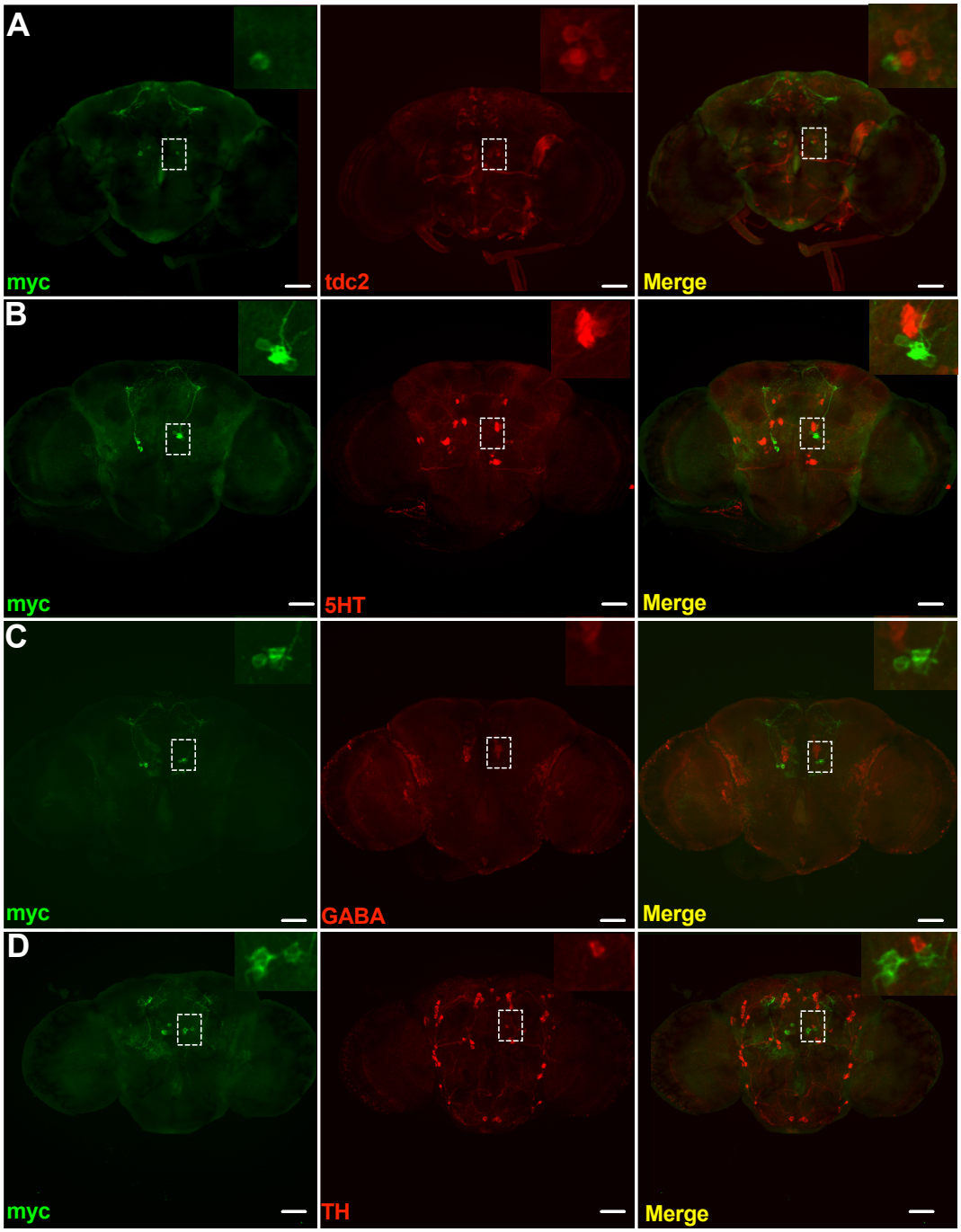

Fig. S7. Identification of the pC1 *dsx* neurons in *UAS>stop> TrpA1^{myc};R26E01-Gal4/dsx^{FLP}*. Myc expression pattern in genotype *UAS>stop> TrpA1^{myc};R26E01-Gal4/dsx^{FLP}* female brain counterstained with ***(A)*** anti-tdc2 ***(B)*** anti-5HT ***(C)*** anti-GABA **(*E*)** anti-TH. Zoomed in view of cell bodies on upper right corner of image. Scale bars represent 50μm.

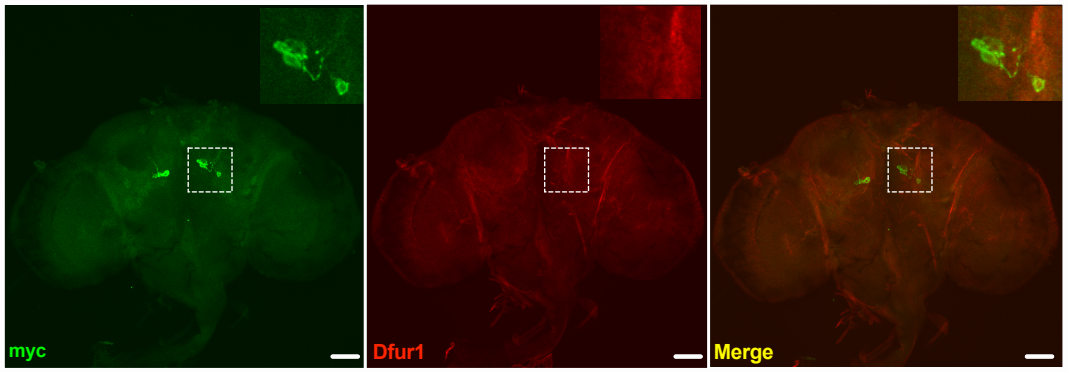

Fig. S8. No colocalization of female pC1 *dsx* neurons and furin. Immunostaining of myc (green) in the adult female brain of genotype *UAS>stop>TrpA1^{myc};R26E01-Gal4/dsx^{FLP}* counterstained anti-dfur1 (red). Zoomed view of cell bodies on upper right corner of image. Scale bar represents 50μm.

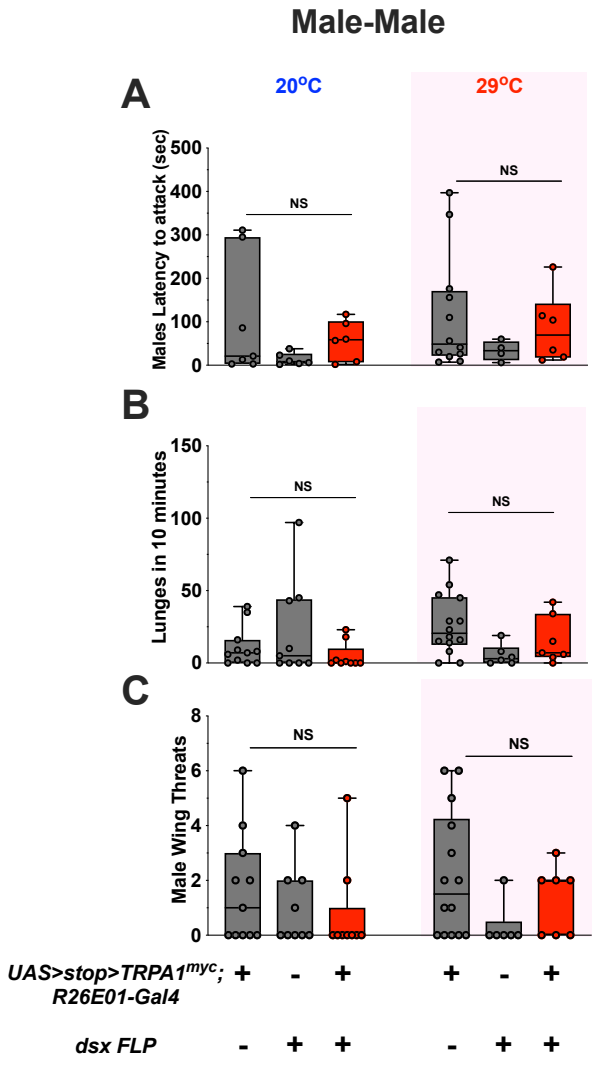

Fig. S9. *dsx^{FLP}∩R26E01* males show no aggressive phenotype.

Activation of *dsx^{FLP}∩R26E01* neurons does not affect **(*A*)** latencies, **(*B*)** number of lunges or in the **(*C*)** number of wing threats in male fight pairings. Data **(*A-C*)** center line, median; boxes, first and third quartiles; whiskers, range; circles represent individual values. NS, not significant $P > 0.05$. Aggressive behaviors were measured over 40-minute period.

Captions for Supplementary Movies 1-7

Movie. S1. Wildtype *Drosophila* females fight at low intensity. *Canton-S* vs. *Canton-S*.

Females fights contain low posture fencing, extending the middle or rear legs and contacting the opponent with the adjacent legs, head butts, and pushing. They commonly result in the sharing of resources. Movie courtesy of ref. 17. Copyright (2004) National Academy of Sciences, U.S.A

Movie. S2*. R26E01>TrpA1* vs. *R26E01>TrpA1* females.

Two females with *TrpA1* activation displaying female patterns of hyper-aggression behavioral phenotype at 29ºC**.**

Movie. S3*. R26E01>TrpA1* females display female fight behavior patterns.

Fight between two *R26E01>TrpA1* females at 29ºC. This behavior included females ‘‘charging’’ their opponent often with wings up to a 45°– 90° angle and high posture fencing. During high posture fencing both flies stand tall on their middle and rear legs while attacking their opponent with their forelegs.

Movie S4*. R26E01>TrpA1* vs. *Canton-S* females at 29ºC.

Female flies showed ‘‘territorial behavior.’’ *R26E01>TrpA1* would charge and head butt its wild type opponent until the opponent retreats. Once the fly retreats *R26E01>TrpA1* remains on the food source.

Movie. S5. Close up high-speed video (400 fps) of *R26E01>TrpA1* vs. *R26E01>TrpA1* female fights.

A fight between two *R26E01>TrpA1* females at 29ºC (slow rate of 10fps). One female quickly charges the other female with their wings up and then head butts her opponent. This behavior is repeated until the fly retreats and one female remains on the food cup.

Movie. S6. *R26E01>TrpA1 vs. R26E01>TrpA1* male fights.

At 29ºC, *TrpA1* activated males do not display aggressive behavior.

Movie. S7. *dsx^{FLP}∩R26E01 vs. dsx^{FLP}∩R26E01* female fights.

At 20ºC, *dsx^{FLP}∩R26E01* females do not display any aggressive phenotype until *TrpA1* is activated at 29ºC. Females attack the other females and display similar fighting behavior as seen in *R26E01>TrpA1* thermogenetically activated female pairings.

Detailed genotypes and sample size for each condition

Figure 1*B* Females - Females

	Control Groups	Experimental Groups
At 20ºC	Wild type *Canton-S* (n=4 flies)	*w;UAS-TrpA1/+; R26E01-Gal4/+* (n=9 flies)
	w;+/+; R26E01-Gal4/+ (n=14 flies)	
	w;UAS-TrpA1/+;+/+ (n=9 flies)	
At 29ºC	Wild type *Canton-S* (n=13 flies)	*w;UAS-TrpA1/+; R26E01-Gal4/+* (n=15 flies)
	w;+/+; R26E01-Gal4/+ (n=10 flies)	
	w;UAS-TrpA1/+; +/+ (n= 14 flies)	

Figure 1*C* Females - Females

	Control Groups	Experimental Groups
At 20ºC	Wild type *Canton-S* (n=9 pairs)	*w;UAS-TrpA1/+; R26E01-Gal4/+* (n=12 pairs)
	w; +/+; R26E01-Gal4/+ (n=14 pairs)	
	w;UAS-TrpA1/+; +/+ (n=13 pairs)	
At 29ºC	Wild type *Canton-S* (n=13 pairs)	*w;UAS-TrpA1/+; R26E01-Gal4/+* (n=15 pairs)
	w;+/+; R26E01-Gal4/+ (n=13 pairs)	
	w;UAS-TrpA1/+; +/+ (n= 17 pairs)	

Figure 1*D* Females - Females

	Control Groups	Experimental Groups
At 20ºC	Wild type *Canton-S* (n=9 pairs)	*w;UAS-TrpA1/+; R26E01-Gal4/+* (n=12 pairs)
	w; +/+; R26E01-Gal4/+ (n=14 pairs)	

	w;UAS-TrpA1/+; +/+ (n=13 pairs)	
At 29°C	Wild type *Canton-S* (n=14 pairs)	*w;UAS-TrpA1/+; R26E01-Gal4/+* (n=15 pairs)
	w; +/+; R26E01-Gal4/+ (n=17 pairs)	
	w;UAS-TrpA1/+; +/+ (n= 19 pairs)	

Figure 1*F* Females - Females

	Control Groups	Experimental Groups
At 20°C	*w;+/+;R26E01-Gal4/+* (n=6) versus Wild type *Canton-S* (n=6)	*w;UAS-TrpA1/+;R26E01-Gal4/+* (n=5) versus Wild type *Canton-S* (n=5)
	w;UAS-TrpA1/+; +/+ (n=8) versus Wild type *Canton-S* (n=8)	
At 29°C	*w;+/+;R26E01-Gal4/+* (n=11) versus Wild type *Canton-S*(n=11)	*w;UAS-TrpA1/+; R26E01-Gal4/+* (n=11) versus Wild type *Canton-S* (n=11)
	w;UAS-TrpA1/+; +/+ (n=7 flies) versus Wild type *Canton-S* (n=7 flies)	

Figure 1*G* Females - Females

	Control Groups	Experimental Groups
At 20°C	*w; +/+;R26E01-Gal4/+* (n=6) versus Wild type *Canton-S* (n=6)	*w;UAS-TrpA1/+;R26E01-Gal4/+*(n=5) versus Wild type *Canton-S* (n=5)
	w;UAS-TrpA1/+;+/+ (n=8) versus Wild type *Canton-S* (n=8)	
At 29°C	*w;+/+;R26E01-Gal4/+*(n=11) versus Wild type *Canton-S* (n=11)	*w;UAS-TrpA1/+;R26E01-Gal4/+* (n=10) versus Wild type *Canton-S* (n=10)
	w;UAS-TrpA1/+; +/+ (n=7) versus Wild type *Canton-S* (n=7)	

Figure 1*H* Females - Females

	Control Groups	Experimental Groups
At 20°C	*w; +/+; R26E01-Gal4/+*(n=6) versus Wild type *Canton-S* (n=6)	*w;UAS-TrpA1/+;R26E01-Gal4/+* (n=5) versus Wild type *Canton-S* (n=5)
	w;UAS-TrpA1/+; +/+ (n=8) versus Wild type *Canton-S* (n=8)	
At 29°C	*w; +/+;R26E01-Gal4/+* (n=11) versus Wild type *Canton-S* (n=11)	*w;UAS-TrpA1/+;R26E01-Gal4/+* (n=10) versus Wild type *Canton-S* (n=10)
	w;UAS-TrpA1/+;+/+ (n=7) versus Wild type *Canton-S* (n=7)	

Figure 1*I* Females - Females

Control Groups	Experimental Groups

At 20°C	*w;+/+; R26E01-Gal4/+* (n=6) versus Wild type *Canton-S* (n=6 flies)	*w;UAS-TrpA1/+;R26E01-Gal4/+* (n=5) versus Wild type *Canton-S* (n=5)
	w;UAS-TrpA1/+; +/+ (n=8) versus Wild type *Canton-S* (n=8)	
At 29°C	*w;+/+;R26E01-Gal4/+* (n=11) versus Wild type *Canton-S* (n=11)	*w;UAS-TrpA1/+;R26E01-Gal4/+* (n=11) versus Wild type *Canton-S* (n=11)
	w; UAS-TrpA1/+;+/+ (n=10)versus Wild type *Canton-S* (n=10)	

Figure 1*J* Female: *w; UAS-mCD8::GFP/+; R26E01-Gal4/+*

Figure 2*C* Females - Females

	Control Groups	Experimental Groups
At 20°C	*w; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/+* (n=13 flies)	*w; UAS>STOP>TrpA1^{myc}/210^{FLP}; R26E01-Gal4/+* (n=8 flies)
		w; UAS>STOP>TrpA1^{myc}/357^{FLP}; R26E01-Gal4/+ (n=9 flies)
		w; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/383^{FLP} (n=7 flies)
		w; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/447^{FLP} (n=6 flies)
		w; UAS>STOP>TrpA1^{myc}/529^{FLP}; R26E01-Gal4/+ (n=5 flies)
At 29°C	*w; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/+* (n=20 flies)	*w; UAS>STOP>TrpA1^{myc}/210^{FLP};R26E01-Gal4/+* (n=15 flies)
		w; UAS>STOP>TrpA1^{myc}/357^{FLP};R26E01-Gal4/+ (n=15 flies)
		w; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/383^{FLP} (n=15 flies)
		w; UAS>STOP>TrpA1^{myc}/+;R26E01-Gal4/447^{FLP} (n=15 flies)
		w; UAS>STOP>TrpA1^{myc}/529^{FLP}; R26E01-Gal4/+ (n=15 flies)

Figure 2*D* Females - Females

	Control Groups	Experimental Groups
At 20°C	*w;UAS>STOP>TrpA1^{myc}/+;R26E01-Gal4/+*(n=9 pairs)	*w; UAS>STOP>TrpA1^{myc}/210^{FLP};R26E01-Gal4/+* (n=7 pairs)
		w; UAS>STOP>TrpA1^{myc}/357^{FLP};R26E01-Gal4/+ (n=10pairs)
		w; UAS>STOP>TrpA1^{myc}/+;R26E01-Gal4/383^{FLP} (n=7 pairs)
		w; UAS>STOP>TrpA1^{myc}/+;R26E01-Gal4/447^{FLP} (n=9 pairs)
		w; UAS>STOP>TrpA1^{myc}/529^{FLP};R26E01-Gal4/+ (n=7 pairs)
At 29°C	*w; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/+* (n=17 pairs)	*w;UAS>STOP>TrpA1^{myc}/210^{FLP};R26E01-Gal4/+* (n=15 pairs)
		w;UAS>STOP>TrpA1^{myc}/357^{FLP};R26E01-Gal4/+ (n=15 pairs)
		w;UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/383^{FLP} (n=15 pairs)
At 29°C		*w;UAS>STOP>TrpA1^{myc}/+;R26E01-Gal4/447^{FLP}* (n=12 pairs)
		w;UAS>STOP>TrpA1^{myc}/529^{FLP}; R26E01-Gal4/+ (n=14 pairs)

Figure 2*E* Females - Females

	Control Groups	Experimental Groups
At 20°C	*w; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/+* (n=9 pairs)	*w; UAS>STOP>TrpA1^{myc}/210^{FLP}; R26E01-Gal4/+* (n=7 pairs)
		w; UAS>STOP>TrpA1^{myc}/357^{FLP}; R26E01-Gal4/+ (n=10 pairs)
		w; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/383^{FLP} (n=7 pairs)
		w; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/447^{FLP} (n=9 pairs)
		w; UAS>STOP>TrpA1^{myc}/529^{FLP}; R26E01-Gal4/+ (n=7 pairs)
At 29°C	*w; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/+* (n=17 pairs)	*w;UAS>STOP>TrpA1^{myc}/210^{FLP};R26E01-Gal4/+* (n=15 pairs)
		w;UAS>STOP>TrpA1^{myc}/357^{FLP};R26E01-Gal4/+ (n=15 pairs)
		w;UAS>STOP>TrpA1^{myc}/+;R26E01-Gal4/383^{FLP} (n=15 pairs)
		w;UAS>STOP>TrpA1^{myc}/+;R26E01-Gal4/447^{FLP} (n=11 pairs)
		w;UAS>STOP>TrpA1^{myc}/529^{FLP};R26E01-Gal4/+ (n=14 pairs)

Figure 2*F* Female: *w; UAS>STOP>TrpA1^{myc}/357^{FLP}; R26E01-Gal4/+*

Figure 2*F'* Female: *w;UAS>STOP>TrpA1^{myc}/357^{FLP};R26E01 Gal4/+* Male: *w/Y; UAS>STOP>TrpA1^{myc}/357^{FLP}; R26E01-Gal4/+*

Figure 3*A-C* Female: *w; UAS>STOP>mC8::GFP/+; R26E01-Gal4/dsx^{FLP}*

Figure 3*D-E* Female: *w; UAS>STOP>nysb/+; R26E01-Gal4/dsx^{FL}*

Figure 4 Females: *w; UAS>STOP>mC8::GFP/+; R26E01-Gal4/dsx^{FLP}*

Figure 5*A* Females - Females

	Control Groups	Experimental Groups
At 20°C	Wild type *Canton-S* (n=7 flies)	*w; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/dsx^{FLP}* (n=5 flies)
	w; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/+ (n=7 flies)	
	w; +/+; dsx^{FLP}/+ (n=7 flies)	
At 29°C	Wild type *Canton-S* (n=4 flies)	*w; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/dsx^{FLP}* (n=11 flies)
	w; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/+ (n=11 flies)	
	w; +/+; dsx^{FLP}/+ (n=4 flies)	

Figure 5*B* Females - Females

	Control Groups	Experimental Groups
At 20°C	Wild type *Canton-S* (n=5 pairs)	*w; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/dsx^{FLP}* (n=9 pairs)

	w; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/+ (n=9 pairs)	
	w; +/+; dsx^{FLP}/+ (n=9 pairs)	
At 29°C	Wild type *Canton-S* (n=9 pairs)	*w; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/dsx^{FLP}* (n=11 pairs)
	w; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/+ (n=9 pairs)	
	w; +/+; dsx^{FLP}/+ (n=7 pairs)	

Figure 5*C* Females - Females

	Control Groups	Experimental Groups
At 20°C	Wild type *Canton-S* (n=6 pairs)	*w; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/dsx^{FLP}* (n=9 pairs)
	w; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/+ (n=9 pairs)	
	w; +/+; dsx^{FLP}/+ (n=7 pairs)	
At 29°C	Wild type *Canton-S* (n=9 pairs)	*w; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/dsx^{FLP}* (n=11pairs)
	w; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/+ (n=8 pairs)	
	w; +/+; dsx^{FLP}/+ (n=7 pairs)	

Figure 5*D* Females - Females

Control Groups	Experimental Groups
w; UAS>STOP>Kir2.1/+; R26E01-Gal4/+ (n=7 flies)	*w;UAS>STOP> Kir2.1/+; R26E01-Gal4/dsx^{FLP}* (n=7 flies)
w; +/+; dsx^{FLP}/+ (n=7 flies)	

Figure 5*E* Females - Females

Control Groups	Experimental Groups
w;UAS>STOP>Kir2.1/+; R26E01-Gal4/+ (n=8 pairs)	*w; UAS>STOP> Kir2.1/+; R26E01-Gal4/dsx^{FLP}* (n=7 pairs)
w; +/+; dsx^{FLP}/+ (n=7 pairs)	

Figure 5*F* Females - Females

Control Groups	Experimental Groups
w;UAS>STOP>Kir2.1/+; R26E01-Gal4/+ (n=8 pairs)	*w; UAS>STOP> Kir2.1/+; R26E01-Gal4/dsx^{FLP}* (n=7 pairs)
w; +/+; dsx^{FLP}/+ (n=7 pairs)	

Figure S1*A* Females - Females

	Control Groups	Experimental Groups
At 20°C	Wild type *Canton-S* (n=6 flies)	*w;UAS-TrpA1/+; R26E01-Gal4/+* (n=6 flies)

	w; +/+; R26E01-Gal4/+ (n=5 flies)	
	w;UAS-TrpA1/+; +/+ (n=6 flies)	
At 29°C	Wild type *Canton-S* (n=5 flies)	*w;UAS-TrpA1/+; R26E01-Gal4/+* (n=6 flies)
	w; +/+; R26E01-Gal4/+ (n=9 flies)	
	w;UAS-TrpA1/+; +/+ (n= 6 flies)	

Figure S1*B* Females - Headless females (Wild type *Canton-S*)

Control Groups	Experimental Groups
w; +/+; R26E01-Gal4/+ (n=14 flies)	*w;UAS-TrpA1/+; R26E01-Gal4/+* (n=17 flies)
w;UAS-TrpA1/+; +/+ (n= 15 flies)	

Figure S2*A* Females - Males (Wild type *Canton-S*)

	Control Groups	Experimental Groups
At 20°C	*w;+/+;R26E01-Gal4/+* (n=6 flies)	*w;UAS-TrpA1/+; R26E01-Gal4/+* (n=7 flies)
	*w;UAS-TrpA1/+; +/+ (*n= 8 flies)	
At 29°C	*w; +/+; R26E01-Gal4/+ (*n=7 flies)	*w;UAS-TrpA1/+; R26E01-Gal4/+* (n=10 flies)
	*w;UAS-TrpA1/+; +/+ (*n=7 flies)	

Figure S2*B* Females - Males (Wild type *Canton-S*)

	Control Groups	Experimental Groups
At 20°C	*w;+/+;R26E01-Gal4/+ (*n=7 flies)	*w;UAS-TrpA1/+;R26E01-Gal4/+* (n=8 flies)
	w;UAS-TrpA1/+;+/+ (n= 8 flies)	
At 29°C	*w;+/+;R26E01-Gal4/+* (n=8 flies)	*w;UAS-TrpA1/+; R26E01-Gal4/+* (n=9 flies)
	*w;UAS-TrpA1/+; +/+ (*n=6 flies)	

Figure S2*C* Females - Males (Wild type *Canton-S*)

	Control Groups	Experimental Groups
At 20°C	*w;+/+;R26E01-Gal4/+* (n=7 flies)	*w;UAS-TrpA1/+;R26E01-Gal4/+* (n=5 flies)
	w;UAS-TrpA1/+; +/+ (n= 8 flies)	
At 29°C	*w;+/+;R26E01-Gal4/+* (n=7 flies)	*w;UAS-TrpA1/+;R26E01-Gal4/+* (n=10 flies)
	*w;UAS-TrpA1/+; +/+ (*n=7 flies)	

Figure S2*D* Females - Males (Wild type *Canton-S*)

Control Groups	Experimental Groups

At 20°C	*w;+/+;R26E01-Gal4/+ (*n=7 flies)	*w;UAS-TrpA1/+;R26E01-Gal4/+* (n=5 flies)
	*w;UAS-TrpA1/+; +/+ (*n= 7 flies)	
At 29°C	*w;+/+;R26E01-Gal4/+ (*n=7 flies)	*w; UAS-TrpA1/+;R26E01-Gal4/+*(n=10 flies)
	*w;UAS-TrpA1/+; +/+ (*n=7 flies)	

Figure S3*A* Males - Males

	Control Groups	Experimental Groups
At 20°C	*w/Y; +/+; R26E01-Gal4/+ (*n=6 flies)	*w/Y;UAS-TrpA1/+; R26E01-Gal4/+* (n=4 flies)
	w/Y;UAS-TrpA1/+; +/+ (n=4 flies)	
At 29°C	*w/Y; +/+; R26E01-Gal4/+* (n=4 flies)	*w/Y;UAS-TrpA1/+; R26E01-Gal4/+* (n=4 flies)
	w/Y;UAS-TrpA1/+; +/+ (n=8 flies)	

Figure S3*B* Males - Males

	Control Groups	Experimental Groups
At 20°C	*w/Y; +/+; R26E01-Gal4/+ (*n=9 pairs)	*w/Y;UAS-TrpA1/+; R26E01-Gal4/+* (n=6 pairs)
	*w/Y;UAS-TrpA1/+; +/+ (*n=8 pairs)	
At 29°C	*w/Y; +/+; R26E01-Gal4/+ (*n=8 pairs)	*w/Y;UAS-TrpA1/+; R26E01-Gal4/+* (n=8 pairs)
	*w/Y;UAS-TrpA1/+; +/+ (*n=9 pairs)	

Figure S3*C* Males - Males

	Control Groups	Experimental Groups
At 20°C	*w/Y; +/+; R26E01-Gal4/+* (n=8 pairs)	*w/Y;UAS-TrpA1/+; R26E01-Gal4/+* (n=6 pairs)
	w/Y;UAS-TrpA1/+; +/+ (n=8 pairs)	
At 29°C	*w/Y; +/+; R26E01-Gal4/+* (n=8 pairs)	*w/Y;UAS-TrpA1/+; R26E01-Gal4/+* (n=8 pairs)
	w/Y;UAS-TrpA1/+; +/+ (n=9 pairs)	

Figure S4*B* Females - Females

	Control Groups	Experimental Groups
At 20°C	*w; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/+* (n=8) versus Wild*type Canton-S* (n=8)	*w; UAS>STOP>TrpA1^{myc}/210^{FLP}; R26E01-Gal4/+* (n=4) versus Wild type *Canton-S* (n=4)
		w; UAS>STOP>TrpA1^{myc}/357^{FLP}; R26E01-Gal4/+ (n=8) versus *Wild type Canton-S* (n=8)
		w; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/383^{FLP} (n=8) versus Wild type *Canton-S* (n=8)
At 29°C	*w; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/+* (n=13) versus *Wild type Canton-S* (n=13)	*w; UAS>STOP>TrpA1^{myc}/210^{FLP}; R26E01-Gal4/+* (n=8) versus Wild type *Canton-S* (n=8)
At 29°C		*w; UAS>STOP>TrpA1^{myc}/357^{FLP}; R26E01-Gal4/+* (n=9) versus Wild type *Canton-S* (n=9)
		w; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/383^{FLP} (n=10) versus Wild type *Canton-S* (n=10)

Figure S4*C*

Females - Females

	Control Groups	Experimental Groups
At 20°C	*w; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/+* (n=8) versus Wild type *Canton-S* (n=8)	*w; UAS>STOP>TrpA1^{myc}/210^{FLP}; R26E01-Gal4/+* (n=3) versus Wild type *Canton-S* (n=3)
		w; UAS>STOP>TrpA1^{myc}/357^{FLP}; R26E01-Gal4/+ (n=7) versus *Wild type Canton-S* (n=7)
		w; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/383^{FLP} (n=7) versus Wild type *Canton-S* (n=7)
At 29°C	*w; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/+* (n=16) versus Wild type *Canton-S* (n=16)	*w; UAS>STOP>TrpA1^{myc}/210^{FLP}; R26E01-Gal4/+* (n=15) versus *Wild type Canton-S* (n=15)
		w; UAS>STOP>TrpA1^{myc}/357^{FLP}; R26E01-Gal4/+ (n=16) versus *Wild type Canton-S* (n=16)
		w; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/383^{FLP} (n=14) versus Wild type *Canton-S* (n=14)

Figure S4*D*

Females - Females

	Control Groups	Experimental Groups
At 20°C	*w; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/+* (n=8) versus Wild type *Canton-S* (n=8)	*w; UAS>STOP>TrpA1^{myc}/210^{FLP}; R26E01-Gal4/+* (n=3) versus Wild type *Canton-S* (n=3)
		w; UAS>STOP>TrpA1^{myc}/357^{FLP}; R26E01-Gal4/+ (n=7) versus Wild type *Canton-S* (n=7)
		w; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/383^{FLP} (n=7) versus Wild type *Canton-S* (n=7)
At 29°C	*w; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/+* (n=16) versus Wild type *Canton-S* (n=16)	*w; UAS>STOP>TrpA1^{myc}/210^{FLP}; R26E01-Gal4/+* (n=15) versus *Wild type Canton-S* (n=15)
		w; UAS>STOP>TrpA1^{myc}/357^{FLP}; R26E01-Gal4/+ (n=16) versus *Wild type Canton-S* (n=16)
		w; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/383^{FLP} (n=14) versus Wild type *Canton-S* (n=14)

Figure S5*A* Female: *w; UAS>STOP>TrpA1^{myc}/210^{FLP}; R26E01-Gal4/+*

Figure S5*B* Female: *w; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/383^{FLP}*

Figure S5*C* Female: *w; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/447^{FLP}*

Figure S5*D* Female: *w; UAS>STOP>TrpA1^{myc}/529^{FLP}; R26E01-Gal4/+*

Figure S6 Male*: w/Y; UAS>STOP>mC8::GFP/+; R26E01-Gal4/dsx^{FLP}*

Figure S7-S8 Female: *w; UAS>STOP> TrpA1^{myc} /+; R26E01-Gal4/dsx^{FLP}*

Figure S9*A*

Males - Males

	Control Groups	Experimental Groups
At 20°C	*w/Y; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/+* (n=7 flies)	*w/Y; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/dsx^{FLP}* (n=6 flies)

	w/Y; +/+; dsx^{FLP}/+ (n=6 flies)	
At 29°C	*w/Y; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/+* (n=12 flies)	*w/Y; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/dsx^{FLP}* (n=6 flies)
	w/Y; +/+; dsx^{FLP}/+ (n=4 flies)	

Figure S9*B* Males - Males

	Control Groups	Experimental Groups
At 20°C	*w/Y; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/+* (n=11 pairs)	*w/Y; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/dsx^{FLP}* (n=9 pairs)
	w/Y; +/+; dsx^{FLP}/+ (n=9 flies)	
At 29°C	*w/Y; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/+* (n=14 pairs)	*w/Y; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/dsx^{FLP}* (n=7 pairs)
	w/Y; +/+; dsx^{FLP}/+ (n=6 pairs)	

Figure S9*C* Males - Males

At 20°C	*w/Y; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/+* (n=11 pairs)	*w/Y; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/dsx^{FLP}* (n=9 pairs)
	w/Y; +/+; dsx^{FLP}/+ (n=9 pairs)	
At 29°C	*w/Y; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/+* (n=14 pairs)	*w/Y; UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/dsx^{FLP}* (n=7 pairs)
	w/Y; +/+; dsx^{FLP}/+ (n=6 pairs)	

Movie S1 Females: Wild type *Canton-S*

Movies S2-S5 Females: *w;UAS-TrpA1/+; R26E01-Gal4/+*

Movie S6 Males: *w/Y;UAS-TrpA1/+; R26E01-Gal4/+*

Movie S7 Females: *w;UAS>STOP>TrpA1^{myc}/+; R26E01-Gal4/dsx^{FLP}*