Supplementary Information for

Multisensory interactions regulate feeding behavior in *Drosophila*

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Fig. S1

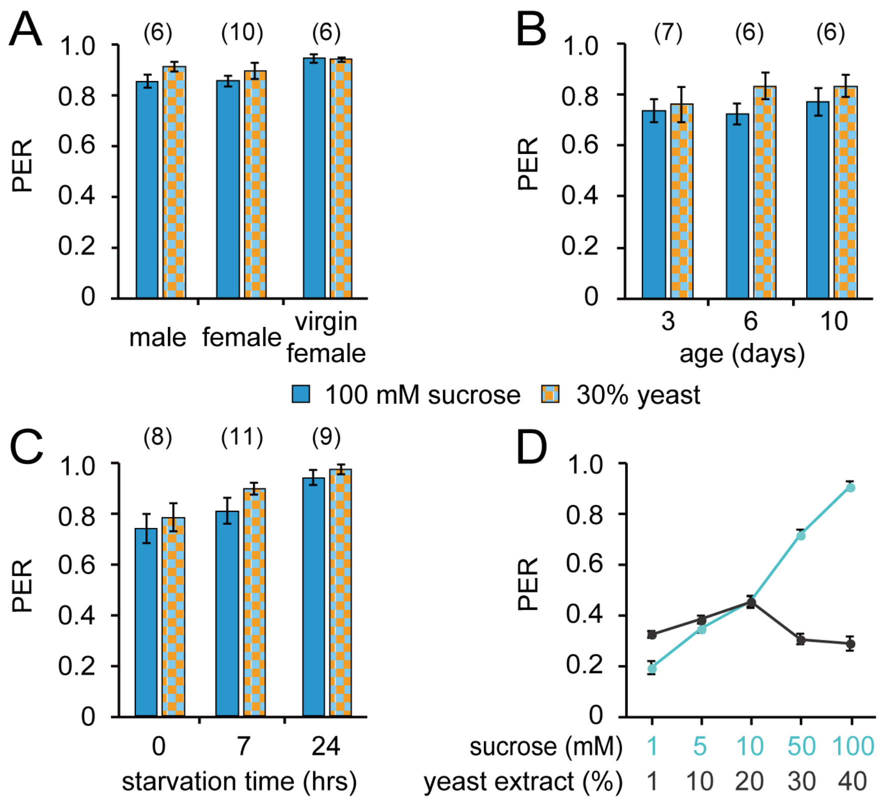

Fig. S1. Effects of various conditions on yeast odor-evoked PER.

(*A*-*C*) PER evoked by yeast in flies under different conditions. (*A*) Sex and mating. (*B*) Aging. (*C*) Starvation time. n is indicated above the bar. Unpaired Student's *t*-tests or Mann-Whitney *U*-tests for A-C. (*D*) PER evoked by various concentrations of yeast extract. The data for PER induced by sucrose are the same as those in Fig. 1*B*. n=6–10. All data are presented as means ± SEM.

Fig. S2

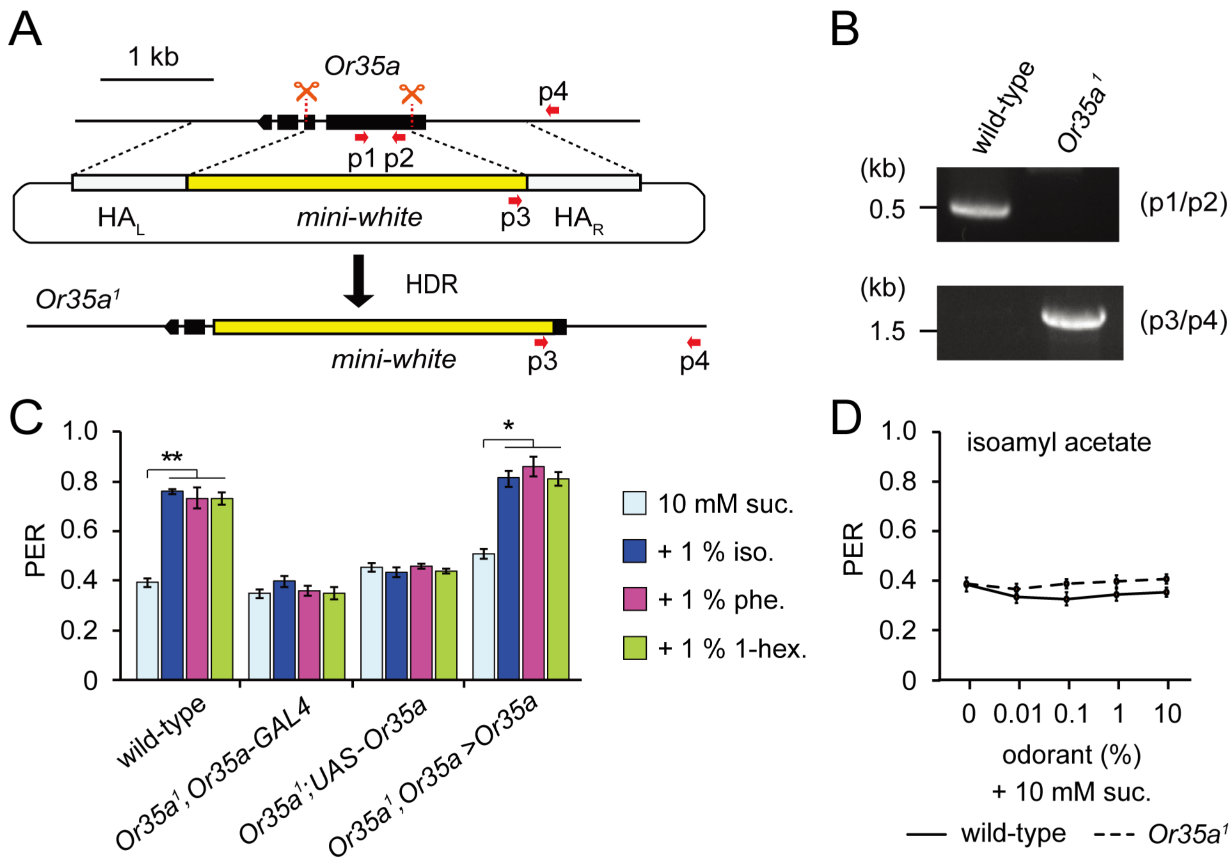

Fig. S2. *Or35a^1* flies and the modified PER evoked by sucrose and volatile compounds. (*A*) Schematic showing the *Or35a* locus and the strategy for making *Or35a^1* flies using the CRISPR/Cas9 system combined with homology-directed repair (HDR). The black boxes indicate exons. The orange scissors indicate the guide RNA targeting sites cut by the Cas9 nuclease. (*B*) The deletion of the *Or35a* coding sequence was confirmed by genomic PCR using the indicated primers (p1, p2, p3, p4; red arrows in (*A*)). (*C*) Modified PER using sucrose and the indicated volatile compounds. iso; isoamyl alcohol, phe; phenylethyl alcohol, 1-hex; 1-hexanol. Genotypes of tested flies are indicated. n=6. One-way ANOVAs with Tukey *post-hoc* tests or Kruskal-Wallis with Mann-Whitney *U*-tests. The asterisks indicate statistically significant differences from PER evoked by sucrose alone. *$p < 0.01$, **$p < 0.001$. (*D*) Modified PER using sucrose and isoamyl acetate. n=6. Unpaired Student's *t*-tests or Mann-Whitney *U*-tests. All data are presented as means ± SEM.

Fig. S3

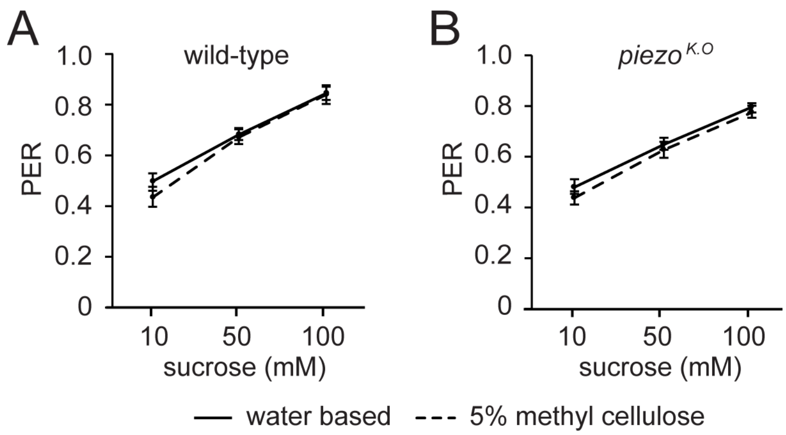

Fig. S3. Effect of food viscosity on sucrose-evoked PER. (*A*-*B*) PER evoked by water based- and 5% methyl cellulose based-sucrose in wild-type (*A*) and *piezo*$^{K.O}$ flies (*B*) by touching the legs. n=6–12. All data are presented as means ± SEM. Unpaired Student's *t*-tests.

Fig. S4

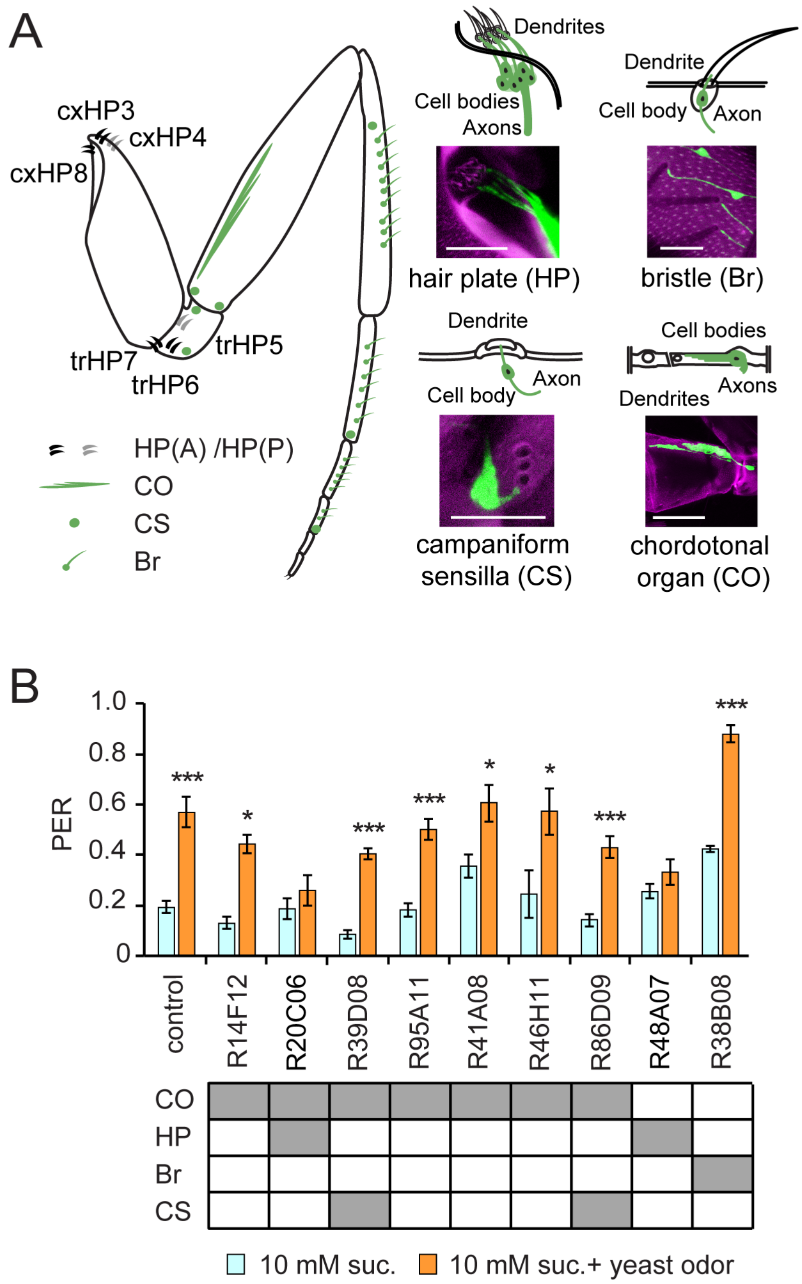

Fig. S4. Mechanosensory organs are involved in yeast odor-enhanced PER. (*A*) Schematics of the mechanosensory organs of the leg. Hair plate (HP), bristle (Br), campaniform sensilla (CS), and chordotonal organ (CO). There are three hair plates on the coxa (cxHP) and three hair plates on the trochanter (trHP) in specific locations of the foreleg. Each distinct number was assigned based on the number of neurons innervating the cluster. Anterior and posterior HPs were marked with black and gray, respectively. The schematics of each type of mechanosensory organ were modified from Tuthill and Wilson, 2016 (1). Scale bar, 25 µm. (*B*) Screening of mechanosensory organs for their roles in yeast odor-enhanced PER. Mechanosensory organs expressing *GAL4* lines were crossed to *UAS-Kir2.1* to silence mechanosensory neurons. n=4–8. All data are presented as means ± SEM. Unpaired Student's *t*-tests and Mann-Whitney *U*-tests. The asterisks indicate statistically significant differences from PER evoked by sucrose alone. $^*p < 0.05$, $^{***}p < 0.001$.

Fig. S5

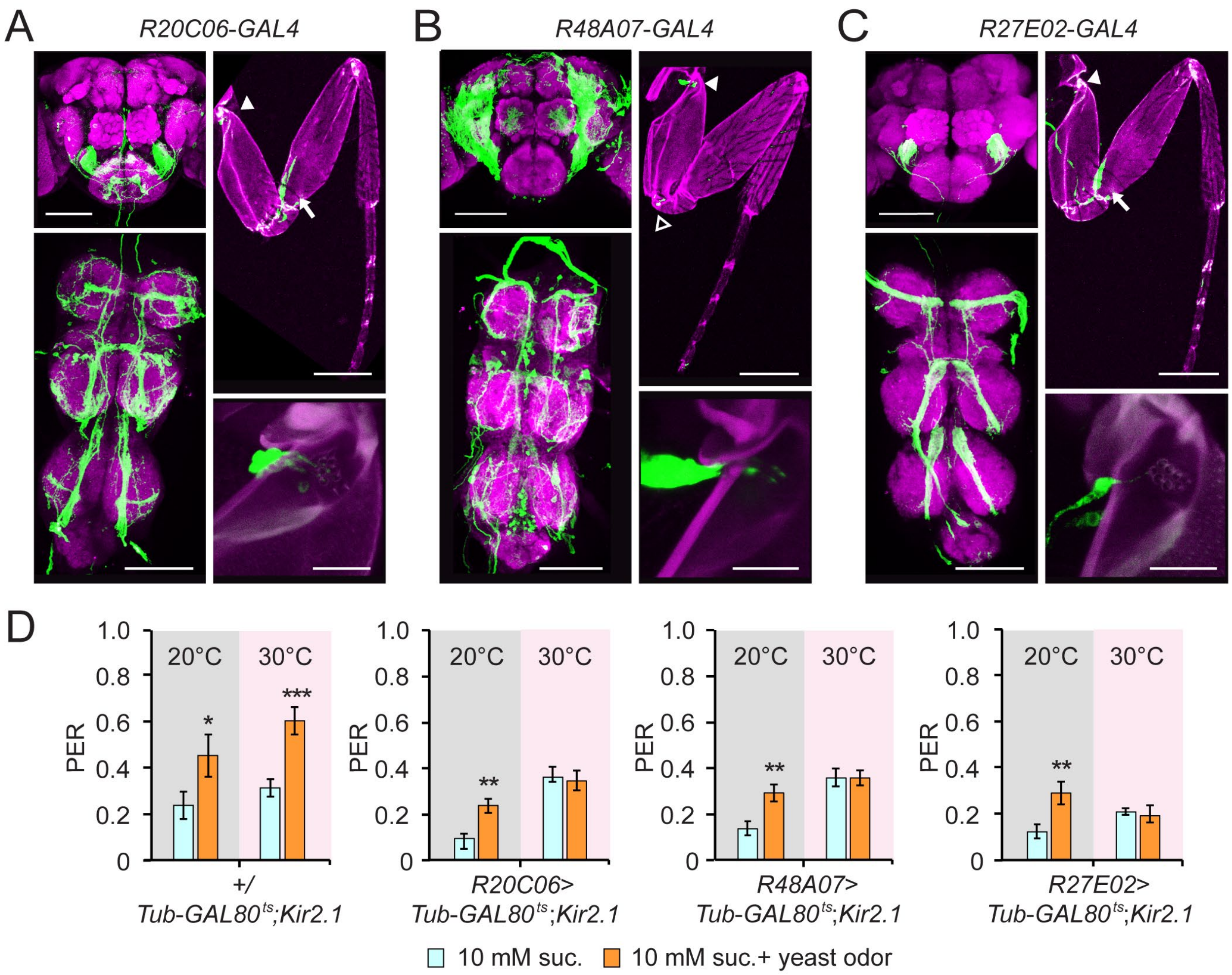

Fig. S5. Leg hair plates are involved in yeast odor-enhanced PER. (*A-C*) Expression of hair plate-expressing *GAL4* drivers in the brain, ventral nerve cord (VNC), and legs. VNCs and brains were stained with rabbit anti-GFP (green) and nc82 (magenta). Magenta in the leg images shows cuticle auto-fluorescence. White arrowheads indicate the hair plate shown at a higher magnification below. Arrows and the unfilled arrowhead indicate the chordotonal organs and an additional hair plate, respectively. Scale bars: 100 µm in the brain and VNC, 200 µm in the leg, 25 µm in the magnified hair plate view. (*D*) Effect of silencing hair plate mechanosensory neurons on yeast odor-enhanced PER. n=6–11. All data are presented as means ± SEM. Unpaired Student's *t*-tests or Mann-Whitney *U*-tests. The asterisks indicate statistically significant differences from PER evoked by sucrose alone. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Fig. S6

Fig. S6. *split*^{HP}-expressing coxa hair plate MNs in the foreleg. (*A*) High resolution image of leg segments of *split*^{HP}>mCD8::GFP flies. Only eight MNs in cxHP8 are labeled in *split*^{HP}>mCD8::GFP flies. Scale bars: leg, 200 µm; coxa, femur, tibia, and tarsus, 100 µm; hair plate, 25 µm. (*B*) Scanning electron micrographs of the coxa hair plate, cxHP8. Scale bars: left, 200 µm; middle, 100 µm; right, 20 µm. Black boxes indicate cxHP8.

Fig. S7

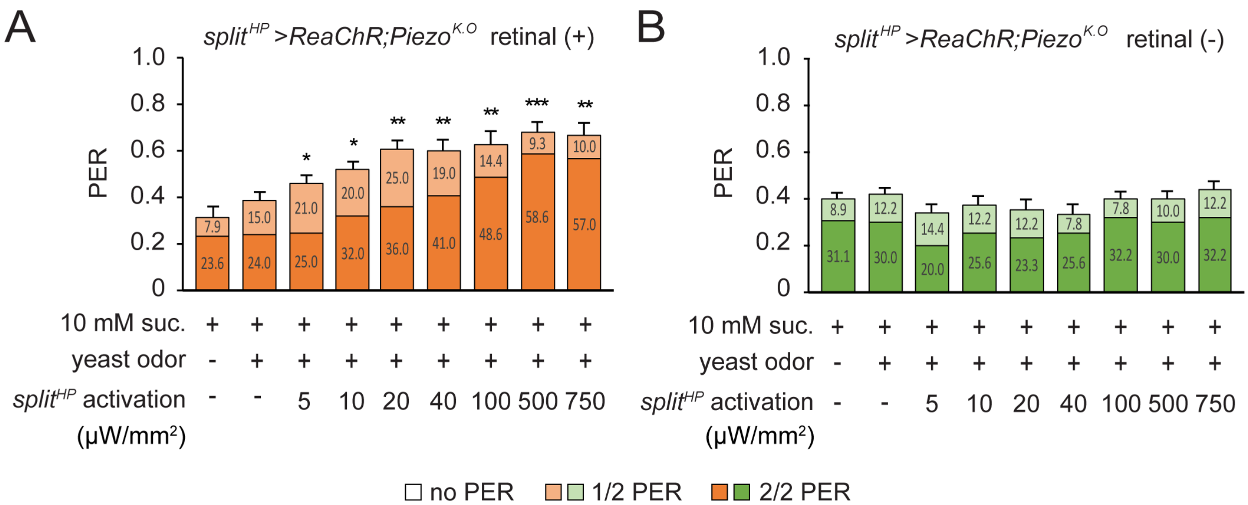

Fig. S7. Relationship between MN activation intensity and the percentage of PER evoked by taste paired with yeast odor. Modified PER combined with optogenetic activation of *split*^{HP} MNs was performed in the indicated genotypes with (A) or without (B) retinal. A wide range of intensities of red light (617 nm) were applied to activate *split*^{HP} MNs. Proportions of the number of PER recorded are indicated by the different orange and green shades (1/2 PER: one PER upon two optogenetic stimulations; 2/2 PER: two PER upon two optogenetic stimulations). n=9–14. All data are presented as means ± SEM. Unpaired Student's *t*-tests or Mann-Whitney *U*-tests. The asterisks indicate statistically significant differences between PER of retinal (+) and retinal (-) fed flies. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

Fig. S8

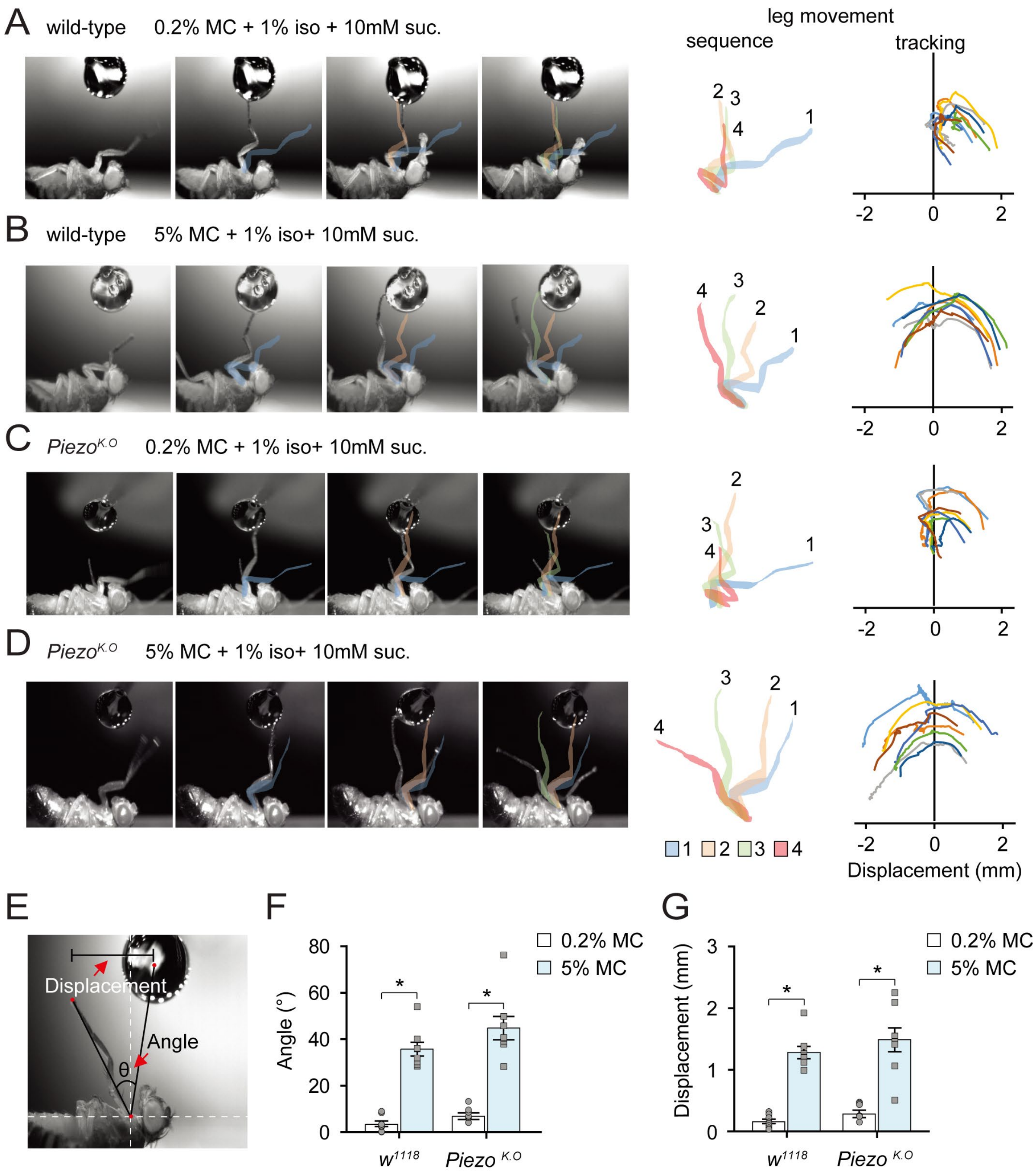

Fig. S8. Distinct leg motions in response to different food viscosities. Leg movements during PER evoked by the indicated foods in the indicated genotypes. Wild-type (*A and B*) and $Piezo^{K.O}$

(*C* and *D*). The left colored “sequence” diagrams are superimposed outlines of the left leg, representing the initial (1) to final (4) positions of leg movement. The right colored "tracking" diagrams show the superimposed displacement patterns from distinct flies. PER was observed only in the wild type presented droplets containing 0.2% methyl cellulose with 1% isoamyl alcohol in 10 mM sucrose. (*E*-*G*) Quantification of leg movement measuring maximum displacement (i.e., the distance between the center of a droplet and the tip of the tarsus as it reached maximum displacement) and degree of maximum leg rotation (i.e., the largest angle formed by the leg from the center of a droplet with the vertex located at the thoraco-coxal joint). n=8. All data are presented as means ± SEM. Unpaired Student’s *t*-tests and Mann-Whitney *U*-tests. The asterisks indicate statistically significant differences from viscosity, 0.2% MC or 5% MC. *$p < 0.001$.

Movie S1. Leg movement during PER assay with 0.2% methyl cellulose with 1% isoamyl alcohol in 10 mM sucrose in wild-type. PER was evoked.

Movie S2. Leg movement during PER assay with 5% methyl cellulose with 1% isoamyl alcohol in 10 mM sucrose in wild-type. PER was not observed.

Supplementary Table S1. Statistic test results.

Figure		Group	Method	Value
Figure 1 *$p < 0.01$ **$p < 0.001$	C	10 mM suc.	ANOVA	0.2600
		100 mM suc.	Kruskal-Wallis	0.8700
		yeast	Kru-Mann-Bon ($\alpha < 0.017$)	0.0030
	D	10 mM suc.	Kruskal-Wallis	0.2000
		100 mM suc.	Kruskal-Wallis	0.2200
		yeast	ANOVA with Turkey	<0.0001
	E	w^{1118}	ANOVA with Turkey	<0.0001
Figure 2 *$p < 0.05$ **$p < 0.01$ ***$p < 0.001$	B	+/*Kir2.1*	Unpaired Student's *t*-test	<0.0001
		Or35a-GAL4	Unpaired Student's *t*-test	0.4521
		Or67a-GAL4	Unpaired Student's *t*-test	<0.0001
		Or67b-GAL4	Unpaired Student's *t*-test	0.0003
		Or69a-GAL4	Unpaired Student's *t*-test	<0.0001
		Or9a-GAL4	Unpaired Student's *t*-test	<0.0001
		Or43b-GAL4	Unpaired Student's *t*-test	<0.0001
		Or7a-GAL4	Unpaired Student's *t*-test	<0.0001
		Or22a-GAL4	Unpaired Student's *t*-test	0.0001
	C	w^{1118}	Mann-Whitney *U*-test	0.0020
		$Or35a^{1}$,*Or35a-GAL4*	Unpaired Student's *t*-test	0.0872
		$Or35a^{1}$;*UAS-Or35a*	Mann-Whitney *U*-test	0.2790
		$Or35a^{1}$,*Or35a>Or35a*	Unpaired Student's *t*-test	<0.0001
	D	w^{1118}	Unpaired Student's *t*-test	<0.0001
		Or35a-GAL4;$Orco^{1}$	Unpaired Student's *t*-test	0.2580
		UAS-Orco;$Orco^{1}$	Mann-Whitney *U*-test	0.3520
		Or35a>Orco;$Orco^{1}$	Unpaired Student's *t*-test	0.0001
	E Isoamyl Alcohol (IA)	10 mM suc.	Unpaired Student's *t*-test	0.0959
		10 mM suc. + 0.01% IA	Mann-Whitney *U*-test	0.3940
		10 mM suc. + 0.1% IA	Unpaired Student's *t*-test	0.0140
		10 mM suc. + 1% IA	Unpaired Student's *t*-test	0.0005
		10 mM suc. + 10% IA	Unpaired Student's *t*-test	0.0060
	E Phenylethly	10 mM suc.	Mann-Whitney *U*-test	0.5890
		10 mM suc. + 0.01% PA	Mann-Whitney *U*-test	0.0020
		10 mM suc. + 0.1% PA	Mann-Whitney *U*-test	0.0020

	Alcohol (PA)	10 mM suc. + 1% PA	Unpaired Student's *t*-test	<0.0001
		10 mM suc. + 10% PA	Unpaired Student's *t*-test	0.0001
	E	10 mM suc.	Unpaired Student's *t*-test	0.1125
		10 mM suc. + 0.01% HA	Unpaired Student's *t*-test	0.1158
	1-hexanol (HA)	10 mM suc. + 0.1% HA	Unpaired Student's *t*-test	0.0020
		10 mM suc. + 1% HA	Unpaired Student's *t*-test	<0.0001
		10 mM suc. + 10% HA	Unpaired Student's *t*-test	0.0102
	F	retinal (-)	Kru-Mann-Bon ($\alpha < 0.017$)	0.0010
		retinal (+)	ANOVA with Turkey	<0.0001
Figure 3 *$p < 0.01$ **$p < 0.001$	A	10 mM suc. ± odor	Unpaired Student's *t*-test	0.0005
		50 mM suc. ± odor	Mann-Whitney *U*-test	0.0060
		100 mM suc. ± odor	Mann-Whitney *U*-test	0.1640
	B	10 mM suc. ± odor	Mann-Whitney *U*-test	0.3330
		50 mM suc. ± odor	Unpaired Student's *t*-test	0.0898
		100 mM suc. ± odor	Unpaired Student's *t*-test	0.0716
	C	10 mM suc. with 0.2% MC ± odor	Mann-Whitney *U*-test	0.0020
		10 mM suc. with 1% MC ± odor	Mann-Whitney *U*-test	<0.0001
		10 mM suc. with 2% MC ± odor	Mann-Whitney *U*-test	<0.0001
		10 mM suc. with 3% MC ± odor	Mann-Whitney *U*-test	0.0020
		10 mM suc. with 4% MC ± odor	Mann-Whitney *U*-test	0.0020
		10 mM suc. with 5% MC ± odor	Mann-Whitney *U*-test	0.8630
	D	w^{1118}	Unpaired Student's *t*-test	<0.0001
		$pain^{1}$	Unpaired Student's *t*-test	<0.0001
		$NompC^{f00642}$	Mann-Whitney *U*-test	<0.0001
		nan^{36a}	Unpaired Student's *t*-test	0.1179
		iav^{3621}	Mann-Whitney *U*-test	0.5790
		$piezo^{K.O}$	Unpaired Student's *t*-test	0.2566
Figure 4 *$p < 0.01$ **$p < 0.001$	B	+/*Kir2.1*	Unpaired Student's *t*-test	<0.0001
		$split^{HP}$>*Kir2.1*	Unpaired Student's *t*-test	0.2536
	C	+/shi^{ts} (20℃)	Unpaired Student's *t*-test	0.0002
		$split^{HP}$/+ (20℃)	Mann-Whitney *U*-test	0.0020
		$split^{HP}$>shi^{ts} (20℃)	Unpaired Student's *t*-test	<0.0001
		+/shi^{ts} (30℃)	Unpaired Student's *t*-test	<0.0001
		$split^{HP}$/+ (30℃)	Unpaired Student's *t*-test	0.0001
		$split^{HP}$>shi^{ts}	Unpaired Student's *t*-test	0.3996
	D	*iav RNAi*/+	Unpaired Student's *t*-test	0.0002

		split^{HP}>iav RNAi	Unpaired Student's *t*-test	0.4552
		nan RNAi/+	Unpaired Student's *t*-test	<0.0001
		split^{HP}>nan RNAi	Unpaired Student's *t*-test	0.2158
		piezo RNAi/+	Unpaired Student's *t*-test	<0.0001
		split^{HP}>piezo RNAi	Unpaired Student's *t*-test	0.4010
	E	*w^{1118}*	Unpaired Student's *t*-test	0.0001
		piezo$^{K.O}$;split^{HP}	Unpaired Student's *t*-test	0.3151
		piezo$^{K.O}$;UAS-piezo	Unpaired Student's *t*-test	0.2405
		piezo$^{K.O}$;split^{HP}>piezo	Unpaired Student's *t*-test	0.0002
	F	body	ANOVA with Turkey	<0.0001
		head	ANOVA with Turkey	<0.0001
	G	+/*Kir2.1* 0.2% MC	Kru-Mann-Bon (α < 0.0167)	<0.0001
		+/*Kir2.1* 5% MC	ANOVA with Turkey	<0.0001
		split^{HP}>Kir2.1 0.2% MC	Kruskal-Wallis	0.142
		split^{HP}>Kir2.1 5% MC	Kruskal-Wallis	0.08
Figure 5 *$p < 0.001$	A	retinal (-)	Kru-Mann-Bon (α < 0.0023)	<0.0001
		retinal (+)	Kru-Mann-Bon (α < 0.0023)	<0.0001
	B	retinal (-)	Kru-Mann-Bon (α < 0.0083)	<0.0001
		retinal (+)	Kru-Mann-Bon (α < 0.0083)	<0.0001

Supplemental Figure		Group	Method	Value
Figure S1	A	male	Mann-Whitney *U*-test	0.1320
		female	Unpaired Student's *t*-test	0.1497
		virgin	Mann-Whitney *U*-test	0.8180
	B	3 days	Unpaired Student's *t*-test	0.3862
		6 days	Unpaired Student's *t*-test	0.0670
		10 days	Unpaired Student's *t*-test	0.1949
	C	0 hr	Unpaired Student's *t*-test	0.2987
		7 hrs	Mann-Whitney *U*-test	0.2700
		24 hrs	Mann-Whitney *U*-test	0.2970
Figure S2 *$p < 0.01$ **$p < 0.001$	C	*w^{1118}*	ANOVA with Turkey	<0.0001
		Or35a^{1},Or35a-GAL4	ANOVA	0.3057
		Or35a^{1};UAS-Or35a	Kruskal-Wallis	0.6910
		Or35a^{1},Or35a>Or35a	Kru-Mann-Bon (α < 0.0083)	0.0030
	D	10 mM suc.	Mann-Whitney *U*-test	0.8180

	Isoamyl Acetate (IAC)	10 mM suc. + 0.01% IAC	Unpaired Student's *t*-test	0.1640
		10 mM suc. + 0.1% IAC	Mann-Whitney *U*-test	0.3940
		10 mM suc. + 1% IAC	Unpaired Student's *t*-test	0.0787
		10 mM suc. + 10% IAC	Mann-Whitney *U*-test	0.1800
Figure S3	A	10 mM suc. ± 5% MC	Unpaired Student's *t*-test	0.1226
		50 mM suc. ± 5% MC	Unpaired Student's *t*-test	0.3919
		100 mM suc. ± 5% MC	Unpaired Student's *t*-test	0.4390
	B	10 mM suc. ± 5% MC	Unpaired Student's *t*-test	0.1483
		50 mM suc. ± 5% MC	Unpaired Student's *t*-test	0.2648
		100 mM suc. ± 5% MC	Unpaired Student's *t*-test	0.2958
Figure S4 *$p < 0.05$ **$p < 0.01$ ***$p < 0.001$	B	+/*Kir2.1*	Unpaired Student's *t*-test	0.0005
		R14F12-GAL4	Mann-Whitney *U*-test	0.0290
		R20C06-GAL4	Unpaired Student's *t*-test	0.1692
		R39D08-GAL4	Mann-Whitney *U*-test	<0.0001
		R95A11-GAL4	Unpaired Student's *t*-test	<0.0001
		R41A08-GAL4	Unpaired Student's *t*-test	0.0125
		R46H11-GAL4	Unpaired Student's *t*-test	0.0229
		R86D09-GAL4	Unpaired Student's *t*-test	0.0006
		R48A07-GAL4	Unpaired Student's *t*-test	0.1024
		R38B08-GAL4	Unpaired Student's *t*-test	<0.0001
Figure S5 *$p < 0.05$ **$p < 0.01$ ***$p < 0.001$	D	+/*Tub-GAL80^{ts}*;*Kir2.1* (20℃)	Unpaired Student's *t*-test	0.0411
		+/*Tub-GAL80^{ts}*;*Kir2.1* (30℃)	Mann-Whitney *U*-test	<0.0001
		R20>Tub-GAL80^{ts};*Kir2.1* (20℃)	Unpaired Student's *t*-test	0.0017
		R20>Tub-GAL80^{ts};*Kir2.1* (30℃)	Unpaired Student's *t*-test	0.3931
		R48>Tub-GAL80^{ts};*Kir2.1* (20℃)	Unpaired Student's *t*-test	0.0047
		R48>Tub-GAL80^{ts};*Kir2.1* (30℃)	Unpaired Student's *t*-test	0.4916
		R27>Tub-GAL80^{ts};*Kir2.1* (20℃)	Unpaired Student's *t*-test	0.0080
		R27>Tub-GAL80^{ts};*Kir2.1* (30℃)	Mann-Whitney *U*-test	0.5620
Figure S7 *$p < 0.05$ **$p < 0.01$ ***$p < 0.001$	A, B	10 mM suc.	Mann-Whitney *U*-test	0.305
		10 mM suc. + yeast odor	Mann-Whitney *U*-test	0.661
		10 mM suc. + yeast odor + 5	Unpaired Student's *t*-test	0.036
		10 mM suc. + yeast odor + 10	Mann-Whitney *U*-test	0.043
		10 mM suc. + yeast odor + 20	Unpaired Student's *t*-test	0.001
		10 mM suc. + yeast odor + 40	Unpaired Student's *t*-test	0.001
		10 mM suc. + yeast odor + 100	Mann-Whitney *U*-test	0.003
		10 mM suc. + yeast odor + 500	Unpaired Student's *t*-test	<0.0001

		10 mM suc. + yeast odor + 750	Unpaired Student's *t*-test	0.001
Figure S8 *$p < 0.001$	F	w^{1118}	Unpaired Student's *t*-test	<0.0001
		Piezo$^{K.O}$	Unpaired Student's *t*-test	<0.0001
	G	w^{1118}	Mann-Whitney *U*-test	<0.0001
		Piezo$^{K.O}$	Unpaired Student's *t*-test	<0.0001

Kru-Mann-Bon: Kruskal-Wallis with Mann-Whitney *U*-tests combined with Bonferroni-Corrections

MC: methyl cellulose

SI Reference

1. J. C. Tuthill, R. I. Wilson, Mechanosensation and Adaptive Motor Control in Insects. *Curr Biol* **26**, R1022-r1038 (2016).