Supplementary Information

Supplementary File Captions.

Supplementary File S1. Electropharyngeogram recordings of *pezo-1* mutants induced to perform pharyngeal pumping by immersion in a 10mM serotonin bath displayed no pharyngeal pumping deficits in pumping frequency (**A**), pump duration (**B**), and inter-pump intervals (**C**) N>20. Values reported are mean+/- SEMs.

Supplementary File S2. Increasing food density caused the brightness of the pharyngeal gland cells to decrease. NGM agar plates were seeded with a 30 μ l lawn of OP50 *E.coli* bacteria at four different densities. Wild type animals (AVG09, N=5-12) were then placed into each plate and allowed to feed for 30 minutes. After this, pharyngeal gland cells were filmed, and their brightness measured and normalized against their background. Values reported are mean+/- SEMs. Coefficient of determination r²=0.32.

Supplementary Tables Supplementary Table S1. Animal strains used in this study.

Strain	Allele	Position	Mutation/genotype	Description		
N2	n/a	n/a	WT	Reference strain		
TU3311	n/a	unknown	uls60 [unc-119p::YFP + unc-119p::sid-1]	Hypersensitive neuronal RNAi		
COP1367	knu384	IV:9366441	<i>pezo-1</i> (knu384 - 198bp deletion and 23bp insertion): gaatcggtggtcgtaacacagcaacaacaga//ATG ACGGTCCCGCC - deletion of 198bp and insertion of ACAGCAACAACAGAATGACGGTC - TCGAAAGTCTTTAGCACTTCCCAgtaaga aattaaagtccgatgt	leads to premature stop - MTVPPQQQQNDGLESL*		
COP1524	knu490	IV:9366455	oezo-1 GOF: R2373K (based on isoform G amino acid residues sequence)	Gain of function mutation affecting all isoforms.		
COP1553	knu508	unknown	<i>pezo-1</i> 6,616bp deletion	6.6kb deletion at 5' end of gene affects 8 longest isoforms (a-h)		
AG405	av143	unknown	<i>pezo-1</i> C-terminus deletion of Exon 27-33 + introns, generated by CRISPR/Cas9, Inserted STOP CODON AFTER deletion	Deletion affecting 8 longest isoforms (a- h)		
AG406	av144	unknown	<i>pezo-1</i> N-terminus deletion of Exon 1-13 + introns, generated by CRISPR/Cas9, Inserted STOP CODON AFTER deletion	Deletion affecting all isoforms of <i>pezo-1</i>		
AG467	av182	n/a	oezo-1(av182 [pezo-1::mScarlet]) IV; CRISPR/Cas9 edit	mScarlet in C terminus generated through CRISPR/Cas9		
AVG09	ury09	n/a	uryEx09[Ppezo- 1::GCaMP6s::unc54_3'UTR, Pmyo- 3::mCherry::unc54_3'UTR]	GCaMP6s in pharyngeal glands, gonad and anal cells, red bodywall		
AVG10	ury10	n/a	uryEx10[Ppezo-1ah::GFP::unc54_3'UTR, Pmyo-3::mCherry::unc54_3'UTR]	GFP <i>pezo-1</i> a-h isoforms, red bodywall		
AVG11	ury11	n/a	uryEx11[Ppezo-1ij::GFP::unc54_3'UTR, Pcoel::GFP::unc54_3'UTR]	GFP <i>pezo-1</i> i and j isoforms, GFP in coelomocyte		
AVG23	ury12	n/a	uryEx12[Ppezo-1k::GFP::unc54_3'UTR, Pmyo-3::mCherry::unc54_3'UTR]	GFP <i>pezo-1</i> isoform k, mCherry in body wall muscles		
AVG22	ury13	n/a	uryEx13[Ppezo-1l::GFP::unc54_3'UTR, Pcoel::GFP::unc54_3'UTR]	GFP <i>pezo-1</i> isoform I, GFP in coelomocyte		

Supplementary Table S2. Primers used for the construction of strains used in this study.

Primer	Sequence (5'-3')	location	Product
<i>Ppezo-1</i> a-h FWD	CATTCAAAAGGAAGAGGCAGA	IV: 9371411	
Ppezo-1 a-h REV	CTGAACAATCGCCGAGAAATCAAG	IV: 9366539	4,872bp
Ppezo-1 i-j FWD	TTGCAAAGTTCCCATGGTTT	IV: 9360555	
Ppezo-1 i-j REV	GTCGATGGAGCTGATGTTGA	IV: 9355693	4,862bp
Ppezo-1GCAMP6s FWD	GGCGCGTCGAGACCT	IV: 9368065	
Ppezo-1GCAMP6s REV	TCTGTTGTTGCTGTGTTACGA	IV: 9366455	1,610bp
Ppezo-1 k FWD	TTGCTCGCACAGTAGAATAGAAC	IV: 9364926	
Ppezo-1 k REV	TGCTCTTCTTGGTGACGTTG	IV: 9359527	5,399bp
Ppezo-1 FWD	GCGATACCCTTGTCAAGAAGC	IV: 9353590	
Ppezo-1 REV	GACCCCATGAACACCAAATC	IV: 9348570	5,020bp

Supplementary Table S3. Sequencing results from Source Bioscience for their new *pezo-1* RNAi clone (top), and alignments of the sequence to *C. elegans* genome (bottom) obtained through WormBase through ENSEMBL and BLASTX (Yates et al., 2020; Harris et al., 2020).

NNNNNNNNNNNNNNNNACTCCNNNAGGGNGNNNNCAGATCTGATTGAGTGGGAACGATCGGAATTTGA TATGTGTAGAGAACCAAGAAATGAAGCGCCGAGTAGAATATGAGAAACTGAAAAATATCGAAAAGGTTTTACAG CACGTGGATATGAACAGAAAAGCGAGAAAGTAAATTGAGTTACTCAGCGATGGTTTCACGATTCCAACATATG CCGTGAACAGGAAAAGCACGAAATTAGTCAATCTTCGTAGAGAATTTCCGATTGCACTCCACATTATAGCGGC TAACTTTCTTTGTCGTCGTCGCTCGCCGCCCACTGAAATAGTTAATATTATTTTGAGTTCATAAGTATATTCTAA CGCACGATTCACTCCTGACTGTTACAACATCACCAACGACGTCCAATTGCTCGTCTCTATGCGACAAAAACAT GACGATTATGATAGTGAGCAGAGCTGCAGACGTGGCAATAATCTCCGGAAGAAATGCTCGAGTTGATTCAAAT GTTCCTGTTGGATGGAATCGGACAAGGCCAATGCTCCGGAACAGAGTAGTGTCGGATCGATTGCAAATGTAT GTCCGGTCTTTTTTATGAACAACTTGTTCTGAGATCTGTAANATAATATTTAATCAACTGTTTCATAANCTATTTT TACCTGGNAGGAACCAACTCCTAGTGCGACTGCTAGGCAGAAGAGAAAAGTGATAATANCGAATGTNCCAACT AGTTNTNNNAAAGAATATTGATTTCAACGACNTCNNACTTTAATTTCTTACNGGGAAGTGCTAAANACTTTCGA ATTGGTGGNAAANCGGCNCTGACAAGTGCAAGTAGNACNTANCCAATTGNTAAAAAGCTCGCCGGTTTATGG GCAGCTGAAAAANTTGAGCNTTANNNTNTACTANNTTNNGGAAACACCTGTTNAAATGAAANAAAANNNAGCT ANCCTGCNANNNGTGCTGCCNGATAGCAGCANTTTGANNANNGCANNATTNNAAAAGNCGGCNNGNCCNNCT ATCNGTNNNNNTGGNNNNNCNNCCCCCGNATNCNNNNNNANCNNANNATNNNANNNTNNTCNNNNCCNN CNNCNNNNNNNGGGGGGNNGACCNNNTNNCCCNN



Supplementary Table S4. Statistical tests used for the different comparisons made in this study. Tests used and sample sizes (for each treatment) for the figures presented in this paper. For Figure 5 and 7D capitalized letters indicate P<0.001 while lower case letters indicate p<0.05. For Figure 6B food density was combined into *low density* (A600=0.32-0.34), *medium density* (A600=2.41-3.49), and *high density* (A600=14.82-15.14).

Figure	Statistic test	Ν	Significance	
Figure 5A	One-Way ANOVA with Holm-Sidak test (all pairwise), F=16.9, DF=5	10	*p<0.05	
Figure 5B	One-Way ANOVA with Holm-Sidak test (all pairwise), F=13.5, DF=5	10-12	*p<0.05	
Figure 6A	Mann-Whitney Rank Sum Test, U statistic =194	29	**p<0.001	
Figure 6B	Two-Way ANOVA (with Holm-Sidak method at significance level =0.05). Strain F=8.5 DF=5 p<0.001; Food density F=37.2 DF=2 p<0.001; Strain x Food density F=3.9 DF=10 p<0.001. Power=1.0 for three conditions at alpha=0.05 Food density	>10	*p<0.05, **p<0.001	
	N2: High vs Low ^{**} , High vs Med [*] , Medium vs Low ^{**} Cop1553: High vs Low ^{ns} , High vs Med [*] , Medium vs Low [*] Cop1367: High vs Low ^{ns} , High vs Med ^{ns} , Medium vs Low ^{ns} AG405: High vs Low ^{**} , High vs Med ^{ns} , Medium vs Low ^{**} Cop1524: High vs Low ^{**} , High vs Med ^{ns} , Medium vs Low [*]			
	AG406: High vs Low**, High vs Medium ^{ns} , Medium vs Low ^{ns}			
	N2 vs: Cop1553**, Cop1367**, Cop1524*, AG406 ^{ns} , AG405 ^{ns} Cop1553 vs: Cop1367 ^{ns} , Cop1524 ^{ns} , AG406**, AG405** Cop1367 vs: Cop1524 ^{ns} , AG406*, AG405** Cop1524 vs: AG406 ^{ns} , AG405* AG406 vs AG405 ^{ns}			
	Strain in medium food density N2 vs Cop1524* (all other comparisons n.s.)			
	<u>Strain in low food density</u> Cop1524 vs Cop1367** (all other comparisons n.s.)			
Figure 6C	One-Way ANOVA on Ranks (Dunn's on all Pairwise)	10-26	*p<0.05	
Figure 6D	One-Way ANOVA on Ranks (Dunn's on all Pairwise)	10-26	*p<0.05	
Figure 6E	One-Way ANOVA on Ranks (Dunn's on all Pairwise)	10-26	*p<0.05	
Figure 7B	2nd order regression	>10	r²=0.44	
Figure 7C	2nd order regression	>10	r²=0.23	
Figure 7D	Two-Way ANOVA (with Holm-Sidak method at significance level =0.05). RNAi treatment F=65.5 DF=1 p<0.001; Food exposure F=15.5 DF=2 p<0.001; RNAi treatment x Food exposure F=20.5 DF=2 p<0.001. Power=0.99-1.0 for three conditions at alpha=0.05	10	*p<0.05, **P<0.001	
	Food exposure RNAi_Ctrl on food 1 vs: off food**, on food 2** Off food vs: on food 2*			
	RNAi treatment On food 1: RNAi_Ctrl vs RNAi <i>_pezo-1**</i> Off food: RNAi_Ctrl vs RNAi <i>_pezo-1*</i> On food 2: RNAi_Ctrl vs RNAi <i>_pezo-1*</i>			
SuppFig 1A	One-Way ANOVA, F=1.9, DF=2	>20	p=0.16	
SuppFig 1B	One-Way ANOVA on Ranks	>20	p=0.24	
SuppFig 1C	One-Way ANOVA on Ranks	>20	p=0.41	
SuppFig 2	1st order regression	5-12	r²=0.32	

Supplementary Table S5. Expression profile for *pezo-1* in L1-L4 *C. elegans* larvae obtained through The *C. elegans* Neuronal Gene Expression Map & Network (CeNGEN, Hammarlund et al., 2018). A total of 135 Cells/tissues were found to express *pezo-1* during development. Expression levels were calculated using *n*etwork Differential Gene Expression (nDGE) analysis. Isoforms identified to be expressed in relevant tissues (through PCR-fusion) are indicated.

Cell	Exp. level	Isoform	Cell	Exp. level	Isoform	Cell	Exp. level	Isoform
NSN	338.659	a-h	RIH	30.358		RMF	6.588	
VA12	321.08		PVW	29.583		SMD stress	6.424	
PVD	273.595	i-j	AVB	28.631		RMD DV	6.091	
PVT	233.318		PHA	28.086	k	PLM	5.817	
CAN	200.129		AS	27.483		ASER	5.685	
VB	162.988	k	AWC OFF	27.445		FLP	5.601	i-j
Marginal cell	112.52	i-j	AVF	25.945		Vulva muscle	5.34	a-h
BDU	105.389		Glia 2	25.194		SIB	5.301	
NSM	105.13		DVC	25.178		Anal muscle	5.222	a-h
PVP	100.069		PVC	23.789		AIM	5.13	
DB	97.148		Epidermis	22.811		VB01	5.114	
PHsh	90.926		ADF	22.45		CEPsh	4.859	
RIR	86.999		IL2 LR	21.27		AIB	4.563	
PHso	84.345		CEP	20.118		AVL	4.45	
URA	77.389		RIC	19.994		Body wall muscle ant	3.943	a-h
AMso	74.719	a-h	AWC ON	19.589		VC 4&5	3.963	
VA	70.921		ASG	19.085		SMD	3.803	
DA9	70.243		IL2 DV	18.652		Unknown cell 2	3.63	
14	69.573		ASK	18.651		RIS	3.592	
VB02	68.162		SIA	18.118		RIB	3.574	
Spermatheca	65.54		Unknown	17.921		Pharyngeal muscle	3.412	a-h
Glia 4	63.117		ASJ	17.154		МС	3.386	
Glia 1	62.07		ADA	16.9		AFD	3.376	
Gon. sheath	59.384		Germline	16.375		AWB	3.355	
AVD	57.962		Body wall	15.647	a-h, k	ADL	3.294	
Glia 3	56.962		PDB	14.718		URX	3.113	
DA	54.272		VD DD	14.571		AIN	3.05	
Arcade cell	53.602	k	Coelom	14.14		RMD LR	2.907	
DB01	52.126		Rectal gland	13.392	a-h	BAG	2.791	
RIP	45.997		AVE	12.256		SAB	2.334	
Sperm	45.68		AVJ	12		VC	2.178	
DVB	45.997		ASEL	11.957		AUA	2.03	
M2	43.768		Spermatheca	11.805		AMsh	1.987	a-h
Unkn. cell 1	43.121		ASH	11.654		AVK	1.787	
Dist. tip cell	41.971		PLN	11.441		AIY	1.7	
Unannotated	39.01		RMH	11.336		RIA	1.51	
AIA	37.666		Excretory	10.749	i-j	Vulval cells	1.364	
Uterine	36.89	a-h	Seam cell	10.43		RIM	1.333	
Intestine	35.362	i-j	RIV stress	10.392		AVM	1.253	
ASI	34.146		Pharyngeal gland	10.006	a-h	PVQ	1.227	
AVG	33.602		AVA	9.402		RIV	1.156	
13	33.109	a-h, i-j	AVH	8.986		RID	1.056	
LUA	31.109		SMB	8.698		M5	0.749	
PHB	30.693		ALM	8.441		RIG	0.549	
AWA	30.638		11	7.236		AIZ	0.539	