SUPPLEMENTAL MATERIAL

RESULTS

cTRPA-1::GFP colocalizes with pdel-2::RFP in the OLQ and IL1 neurons

In our reporter line *ljEx117[pdel-2::RFP]*, expression of RFP was detected in neurons which by position could be detected in the OLQ and IL1 neurons (Supplemental Fig. 2). In contrast to the results of Harbinder et al ¹, who also observed expression of a *pdel-2::lacZ* transgene in neurons provisionally identified as ASH, we did not detect expression of *pdel-2::RFP* in ASH or any other dyefilling amphid neuron. It should be noted that our promoter fragment differed in both its 5' and 3' ends from the promoter used in the earlier study, which could account for the differences in observed expression pattern. As with any study of this type, it is impossible to rule out the possibility that our *del-2* promoter transgene was expressed at very low levels that were below the detection limit of our experiment.

Effects of cTRPA-1 on other behaviors

Since cTRPA-1 appears to play a mechanosensory role in *C. elegans*, we tested for defects in other well-characterized mechanosensory behaviors in *trpa-1* mutants. The two *trpa-1* mutant strains showed normal escape responses to gentle body touch, which requires the ALM, AVM and PLM, PVM touch neurons (Supplementary Fig. 3a)². cTRPA-1::GFP expression was also observed in the PVD neurons which is required for response to harsh touch ³. Harsh touch can only

be assayed in animals that lack functional light touch response ⁴. Therefore, harsh touch was assayed in a *mec-4* null mutant background by poking the animal on the anterior or the posterior side with a platinum wire and scoring for escape responses. We observed that the *trpa-1(ok999); mec-4(u253)* double mutants showed a normal response to harsh touch (Supplementary Fig. 4b). We did not observe evidence for a role of cTRPA-1 in response to either gentle body touch or harsh body touch.

Mammalian TRPA1 (mTRPA1) and *Drosophila* TRPA1 (dTRPA1) respond to temperature, and are required for cold hyperalgesia and thermotaxis in adult rats and fly larvae, respectively ⁵⁻⁸. Thermosensory behaviors have also been characterized in *C. elegans*, though the mechanisms underlying thermosensory transduction are not well understood ⁹. Thermosensory signal transduction requires the function of a cyclic nucleotide-gated channel encoded by the *tax-2* and *tax-4* genes in the AFD neuron ¹⁰. However it has not been determined whether *tax-2* and *tax-4* act as direct thermosensors. We, therefore, explored if *trpa-1* mutants showed defects in temperature sensation. In a linear thermotaxis assay paradigm ¹¹, we did not find evidence for a role of cTRPA-1 in *C. elegans* thermotaxis (Supplemenatary Fig. 4c)

cTRPA-1::GFP is also expressed in several non-neuronal cells such as the pharyngeal muscle, vulva epithelium, and the rectal gland (Fig 2a, b). We found that *trpa-1* mutants have a moderate effect on these behaviors. *trpa-1* mutant worms have a slightly faster rate of pharyngeal pumping (Supplementary Fig. 4e).

It is unclear if cTRPA-1 is playing a direct role in pumping or if the higher rate of pumping is a secondary effect of defects in foraging behavior. In addition, their defecation cycle is also slightly faster than wild-type animals (Supplementary Fig. 4d). *trpa-1* mutant worms also show slight alterations in the timing of egg laying behavior 12 . Here, the inter-cluster time constant (indicating the average time between bursts of egg-laying events) was slightly longer in *trpa-1* mutants than wild-type (1477 s, compared to 1201 s in wild-type). In addition the intra-cluster time constant (indicating the rate of egg-laying within a burst) was longer in the mutants, 32.2 s compared to 21.2 s in wild-type.

SUPPLEMENTARY METHODS

Behavioral assays: For all behavioral assays, 4 day old young adults were used for the assays, unless otherwise mentioned.

For light touch, 10 worms per strain were given 10 stimulations with an eyelash, 5 anterior and 5 posterior, with one minute between stimulations. No difference was seen between anterior and posterior response. A null allele, touch insensitive allele of *mec-4* was used as a positive control. The percent responding represents both types of stimuli. A *trpa-1(ok999); mec-4(u253)* double mutant was generated to assay for harsh touch. The same assay as light touch was used for harsh touch, although a platinum wire was used in place of an eyelash to stimulate animals.

Population thermotaxis assays using a linear thermal gradient were performed as follows¹¹. A stable linear thermal gradient was established on a 60cm long aluminum platform with one end of the platform placed in an ice water bath and the other end in a water bath at 45 °C. A rectangular plate (14 cm x 10 cm, Nalge Nunc) containing TTX media (2% agar, 3% sodium chloride and 25 mM potassium phosphate) was placed for 15 min on the linear thermal gradient such that the center of the plate was at 20 °C. A gradient in the range of 17 °C to 23 °C was established on the TTX plate. About 200-300 animals grown at 17 °C, 20 °C or 23°C under well-fed non-crowded conditions were placed in the 20°C range on TTX plate and allowed to thermotax for 1 hr. The TTX plate was divided into eight equal segments. At the end of 1 hr animals were killed by chloroform gas and the number of animals at the eight different temperature regions was counted.

To perform the pumping assay, lawns were seeded the day before with one drop OP50 spread onto plate and grown overnight at 37 °C. Young adults were placed on these lawns for at least 1 hr before videos were recorded. At least 18 videos were recorded per strains at 20 Hz for 20 seconds on the axioscope described in the still image method section. Videos were slowed down 10X and pumping was scored by eye.

For analysis of egg-laying and defecation, up to 6 hr videos were recorded using the automated worm tracking system previously described ¹². Egg-laying analysis was performed in the same manner as previously reported ¹². Here, the inter-cluster time constant (indicating the average time between bursts of egglaying events) was slightly longer in *trpa-1* mutants than wild-type (1477 s, compared to 1201 s in wild-type). In addition the intra-cluster time constant (indicating the rate of egg-laying within a burst) was longer in the mutants, 32.2 s compared to 21.2 s in wild-type. For defecation analysis, the time of 5 defecation cycles averaged per worm, n=18 in wild-type and 16 for *trpa-1*.

Supplementary Figure 1. Phylogenetic tree of TRPA subfamily. *H. sapiens, M. musculus, D. melanogaster and C. elegans* members of these families are color coded, and the proposed names of the two *C. elegans* members is in parenthesis. ClustalW was used to align the transmembrane domains and the alignment is presented as a phylogram. The number of predicted ankyrin repeats is listed. **Supplementary Figure 2.** cTRPA-1 is a member of the TRPA subfamily of TRP channels.

Comparison of *C.elegans* TRPA-1 (C29E6.2) and TRPA-2 (M05B5.6) protein sequences to mouse TRPA1(Genbank accession number AY23117), human TRPA1 (NM_007332.1) and *Drosophila* TRPA1 (AY302598). The alignment was generated using Megalign and Boxshade. Identical or conserved residues are shown in blue and similar residues are shown in yellow. **Supplementary Figure 3** The full length cTRPA-1 GFP fusion, *ljEx114*

colocalizes with *pdel-2::RFP* in the Il 1 and OLQ neurons.

Left panels indicate *pdel-2::RFP* expression in the OLQ (top panels) or IL1 (bottom panels) neurons, center panels are *ljEx114* expression, and right panels are the merged images.

Supplementary Figure 4 cTRPA-1 is not required for body touch responses and normal thermotaxis.

The error bars indicate Standard Error of Mean (SEM), p values were calculated according to a student's t test, * denotes p<0.05, *** denotes p<0.001. (a) *trpa-1* mutants respond normally to gentle body touch with an eyelash (n=100 trials). (b) In a *mec-4* (light touch-insensitive) background, harsh touch response is not affected in *trpa-1* mutants (n=100 trials). (c) cTRPA-1 is not required for normal response to thermotaxis. The distribution of wild-type animals (filled circle, n=6 trials) and *trpa-1* mutants (*ok999* and *tm1402*, filled triangle and filled square respectively, n=4 trials) cultivated at 20°C on TTX plate with thermal gradient. 20°C on the gradient is at 0. (d) Defecation in *trpa-1* is slightly faster than wild-type animals (n=18 animals for wild-type, 16 for *trpa-1*, 5 cycles averaged per animal. (e) Quantification of pharyngeal pumping in wild-type animals (n=18 animals for each strain).

Supplementary Figure 5.

(a) Prolonged suction at -50 mmHg does not desensitize cTRPA1 currents. (b) Response of an uninduced cTRPA1 stable cell to application of 500 μ M Gd3+ (indicated by black bar). (c) Changes in cell size due to whole cell suction. Pictures of a CHO cell in whole-cell configuration with 0, -50, and -100 mmHg applied pressure. Area of the cell is calculated at 0-100 mmHg suction and compared to the cell's original size as a percentage.

Supplementary Figure 6 Baseline cameleon levels in wildtype and *trpa-1* mutants, and raw imaging data.

(a) Shown are the raw cyan baseline intensity measurements of wildtype and *trpal* mutants expressing cameleon in the OLQ neurons in array *ljEx130[pocr-4::YCD3]*. No significant difference was observed according to the Mann-Whitney rank test. (b) Similar to cyan baseline intensities, no difference was seen in the baseline ratio, where the baseline ratio is equal to the raw fluorescence of YPF/CFP intensites. (c) Raw data of a ratiometric FRET response to response to nose touch in a wildtype OLQ neuron. The YPF and CFP fluorescence intensity measurements are shown as green and blue respectively in the bottom panel. The YPF/CFP ratio of these traces is shown in the panel above. The ratio is equal to YFP/CFP intensities –0.6. (d) A similar trace as shown in panel c, except a nose touch response in a *trpa-1* OLQ neuron is indicated.

Supplementary Movie 1. Reversal response to nose touch.

As animal makes a nose-on collision with an eyelash, ~80% of the time a reversal or backward movement is initiated.

Supplementary Movie 2. Head withdrawal response to nose touch.

In addition to a reversal response, ~15% of the time animals display a head

withdrawal upon encounter with the eyelash.

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Supplementary Figure 1. Phylogenetic tree of TRPA subfamily. *H. sapiens, M. musculus, D. melanogaster and C. elegans* members of these families are color coded, and the proposed names of the two *C. elegans* members is in parenthesis. ClustalW was used to align the transmembrane domains and the alignment is presented as a phylogram. The number of predicted ankyrin repeats is listed.

hTRPA1 mTRPA-1 dTRPA1 cTRPA-1 cTRPA-2	1 1 1 1 1 1	- MKC5LEKMWRPGEKKEPQOVŸYEDŸDDTEDFKES- - MKRGLRFLLPEEREVQOVŸYGV2BØDCSESS- - MKRGLRFLLPEEREVQOVŸYGV2BØDCSESS- - MKG5LGLVRLELEGEISHDDTERSEKDORQASIFRVAELDARTEADHLR-
hTRPA1 mTRPA-1 dTRPA1 cTRPA-1 cTRPA-2	36 36 45 52 1	LEVVFEGSATGLONPNK-QK-KLKTCDDHDTPPLHTAAABGQ FKVDIEGDKCKLEDFINNR-KLGKTDDHDCFLHTAAABGQ RILKNSACSTPFIITUVTLAABGONDFKTPKINA SIIEGSARKGNYNALGEALLKAPLAYNAGDGDFHTPLHTAARTON
hTRPA1 mTRPA-1 dTRPA1 cTRPA-1 cTRPA-2	76 77 105 97	IRLNERVITROSSLEVILHENDDYGNTPLNCAVE
hTRPA1 mTRPA-1 dTRPA1 cTRPA-1 cTRPA-2	112 113 139 155 1	ESYKELLERGÄNPNECHPINKAPLHIAVQGNINEVKKULLERG-TIDYNLEGENGNTAVI ESYKELLEQGÄPPNEGNRANNOPLHIAVIGHINEVKULVENK-ATNINLEGENGNTAVI DALOTLEITVOTVULKEKAPVKAPILNINVSILVEVTENK-ATNINLEGENGNTALI KIINALVENKEIDPVKKTGETPLHYAAKKENFALUALILLEADVOADDIKKMPLL
hTRPA1 mTRPA-1 dTRPA1 cTRPA-1 cTRPA-2	171 172 199 213 1	IACTINSEALOILENG - AFCCENTRG (PIGAFCGESECTEILENGERG'SEC STCARDNE ACTILECT. ALCORENTGO VOICAFCGESCARCUEILCOURS DALIDERCALL TIPOACTECUNGY DIEAANNACSKYNEYPONGECGE LACYNGSQEITQELIKAN - SNYTERDORLNYPFIYALRGPEYLENNHDID VEAI
hTRPA1 mTRPA-1 dTRPA1 cTRPA-1 cTRPA-2	230 231 259 269 1	LHEN FYNNIGEATELHLAN COMDLENTEKKELDNEA CUD PYRCH-CTALHRAAT COATEIY RHEN YN HARAFLLAN COMDLENTEKKELDNEA CUD ANLENN RA'C CALHRAAT COATEIY RHEN YN DERONVELHAN NOGDEXAVEKCE GOARANLDHNEN RA'C CALHRAAT COATEIY RALN PYNNEKETELRAN COMDLENTEKKELDNEA CUD ANLENN RA'C CALHRAAT COATEIY RALN PYNNEKETELRAN COMD FETEKKELONGA CUD YRCH CTALHRAAT COATEIY RALN PYNNEKETELRAN COMD FETEKKELONGA CUD YRCH CTALHRAAT COATEIY
hTRPA1 mTRPA-1 dTRPA1 cTRPA-1 cTRPA-2	289 290 318 329 1	KLMISSYSGSVDIVN-TTDGCHETMLHEAGLFDHHELADYLISVG-ADIKKLDSGGBFDL KLMISSYGGSDIVN-AVDONSTLLHEAGLFDHHDLADYLISVG-ADIKKLDSGGBFDL KLMFBNQPNERARCISCTDGTDHVANDHDDDIVSXLGAGEADINALDKENERPL KALVBAGGNKNERNBURAVDLHVAAQNNQLEVVSXLIBBEKDNIDVVDBQGLTPL
hTRPA1 mTRPA-1 dTRPA1 cTRPA-1 cTRPA-2	347 348 377 384 1	ILATA SAMWAI YWLLLGAGA OVD I KUN MORNFELHTYOO ILATA SAMWAI YWLLLGAGA YD YRU HAGNFELHTYOO LLAAS RSOWRTYWLLIA GAC I SYKD AAKNYLHYYYN N MMAYTHUSKKCYEYL <mark>I</mark> AKKAMLTI TEKDERTPYYT GAKPNALSSYEYI LUHLAKKNKETE
hTRPA1 mTRPA-1 dTRPA1 cTRPA-1 cTRPA-2	386 387 416 444 1	PYGLENLEPEPKOKO OLKELYMABENDGCTPLENKACROGOPOSYMNLLOPINYSIISK PYGLENLEPEPKOKO OLKELYMBENDGCTPLENKACROJPYSYMLLOPINYSIISK GGLEDPARGYANCOTAQORILLENKENSKGCEPLENKASRDGHIESEPHIIKASACINL RSALKSPTENTERIYSEDVRETHYNMYDRDQNTPHHIVASNGYLEMHQLLORNSIISY
hTRPA1 mTRPA-1 dTRPA1 cTRPA-1 cTRPA-2	443 444 476 504	SKEKKEPLMFAASYGRINTCORELOOIIGDIGTELLNGGDLHOMTPLILAARMGHDKVVOLLL NNNNEPLMFAASYGRINTCORELOOIIGTELLNGGDLHOMTPLILAARMGHDKVVOLLL NNNNEPLMFAASYGRINTVROLLOEKG PTINS DOAGMTPLILAARGHDKVVOLLL NEBERTALHFAAIGVRQLLENDIKLLINKEBMGALHLAARSGHDATTKUL NEBERTALHFAAIGVRQLLENDIKLLINKEBMGALHLAARSGHDATTKUL MEAERVPLQELGHRNEKINDEISIDPC
hTRPA1 mTRPA-1 dTRPA1 cTRPA-1 cTRPA-2	503 504 536 557 28	KKGALPLSDHN-GWTALHHÄSNOGYTOTNKVILDTNLKCTDRLDEDGNTALHPAAREGHA KKGALFLSDHN-GWTALHHÄSNOGYTOTNYVILDTNLKCTDRLDEEGNTALHPAAREGHA NGALHRADHT-GRWFQLGAANGGTETIELUSVIBALDGVDRJONTALHAFNAREGHA DNGADKEAKNYOYGFFLGVAVDGGLETCORLYAGAQIESSD-TTKVLHTAAPTGNE NERSTIRANKAESTTPIFVIGGGGUTLWNKELKKES
hTRPA1 mTRPA-1 dTRPA1 cTRPA-1 cTRPA-2	562 563 595 615 67	KAVALLLSHNADIVLNKQQ-ASFUHALHNKREMVULIISSKMDSCLKIFCH KAVALLSYNADILJKKQ-ASFUHALHNKREMVLIISSKMDSCQVFTH HASVLNGKCVYNVLNGKSIVASIY SIYXYFIASQVIJDROBECKTAFDIACHDHKQVARAFLSTQVKNLHIPCDVIPLDLK SIYXYFIASQVIJDROBECKTAFDIACHDHKQVARAFLSTQVKNLHIPCDVIPLDLK
hTRPA1 mTRPA-1 dTRPA1 cTRPA-1 cTRPA-2	616 617 647 675 81	
hTRPA1 mTRPA-1 dTRPA1 cTRPA-1 cTRPA-2	672 673 703 734 95	KT-P-ROPUTYEB LTALMANUONNE ELLANPYC KYAD-TODVÜYEB LTLANVONNE ELLANPYC DER.TGSZGCKSAVDEDFKLEKDAQSYASNYDRVYTYELKAADARKHALANPLS TMI VEDDOGRQEICCKSAVDEDFKLEKDAQSYASNYDRVYTYELKAADARKHALANPLS MILAFL
hTRPA1 mTRPA-1 dTRPA1 cTRPA-1 cTRPA-2	704 706 740 794 102	EFFLUERRILANGPRANNINGSTCHEIPHTLVVNIRPGMAPNTG EFFLUERRICAGPRANNINGSTCHEIPHTLVVNIRPGMAPNTG CXXLORRINGYGRYHLANGLIYSJFUPPTTYSSLMNNISELAGONRTNSGTCHMGME EALEXYENRLGPMYTPAFIPHLIPTSJFUPTTYSSLMNNISE FALEXYENRLGPMYTPAFIPHLIPTSJFUPTTYSSLMNNISE VPFLUEANNVRSRIFFCDFITTTGIPMFLVLRGTIKÄRITKUS TM2
hTRPA1 mTRPA-1 dTRPA1 cTRPA-1 cTRPA-2	751 753 800 841 149	TIN-ESSDERILDTING
hTRPA1 mTRPA-1 dTRPA1 cTRPA-1 cTRPA-2	800 803 860 893 177	PHDISHVLENIIITGGIPVLELPVLED
hTRPA1 mTRPA-1 dTRPA1 cTRPA-1 cTRPA-2	855 858 917 953 213	NCGIPYUMLEVILKTLÜKTSTUPPIPLLAFGLEPYILLA NGGIPYUMLEVILKTLÜKTSTUPPIPLLAFGLEPYILLA VGGIPYUMLEVIPKLÜKTGUPFIPLLAFGLEPYILLA VGGIPYUMLEVIPKLÜKTLÄKUMPILLAFGLEPYILLA RØGIPVUMLEVILKTLÄKUMPILLAFGLEPYILLA RØGIPVUMLEVILKTLÄKUMPILLAFGLEPYILLA RØGIPVUMLEVILKTLÄKUMPILLAFGLEPYILLA NGGIPVUMLEVILKTLÄKUMPILLAFGLEPYILLA NGGIPVUMLEVILKTLÄKUMPILLAFGLEPYILLA NGGIPVUMLEVILKTLÄKUMPILLAFGLEPYILLA NGGIPVUMLEVILKTLÄKUMPILLAFGLEPYILLAFGLEPYILLA NGGIPVUMLEVILKTLÄKUMPILLAFGLEPYILLAFG
hTRPA1 mTRPA-1 dTRPA1 cTRPA-1 cTRPA-2	908 911 977 1006 272	TFSHMLGDINGRESFL FYLENELAHFVLSFACUVSFTFYFYLVLNHLIGLANG TFSHMLGDINGRADAFLEFLFHRELAHFVLSFACUVSFTFYFYLVLNHLIGLANG TFSHMLGDINGRADAFLEFLFHRELAHFNTSFILAFTHYLVLNHLIGLANG TSVMLTSFFFGTGTUNTTALSFNGGANFNTSFILAFTHYLVSLLNHLVGLAND TSVMLTSFFGTGTUNTALSFNGGANFGTANTANACALFYFYCINTILHNUGAND
hTRPA1 mTRPA-1 dTRPA1 cTRPA-1 cTRPA-2	963 966 1032 1066 330	DIA BUOKUMA CLARIANQUELHI SOLEKKUP UNFLAYUDOK SIYUPANIPA SOG MLP DIA BUOKUMA CLARIANQUELHI SOLEKKUP UNFLAYUDOK SIYUPANIPA SOG MLP DIA BUOKUMA CLARIANQUELHI SOLEKKUP UNFLAYUDOK SIYUPANIPA SOG MLP DIA BUOKUMA CLARIANU BUTU BUKUPANIPANIPANIPANIPANIPANIPANIPANIPANIPANI
hTRPA1 mTRPA-1 dTRPA1 cTRPA-1 cTRPA-2	1018 1021 1092 1119 389	HIE COLORCIGE ROZIPH - ADEC LEMEIL KOCY RLKDLP LLER QHELKLIIQKHEII - PPHTPLNNQER QEVN - IDTCLEMEIL KOCY RLKDLT BLER QHELKLIIQKHEII KWSHPPTESSHOVI SPHTDTINAEL KOCY RLKDLT BLER QHELKLIIQKHEI TOFANH MYRAK VLVI - TPHIDDINAEL KOCY RLKDLT BHAG QHELKLIIQKHEI DYPHAA Y II DAAFIISHMET MARAKITKI KOVYTTI BHOT GLOQQAAL KHIQAN DY DYPHAA Y II DAAFIISHMET OKRAKITKI KOVYTTI BHOT GLOQDAAL KHIQAN DY
hTRPA1 mTRPA-1 dTRPA1 cTRPA-1 cTRPA-2	1076 1078 1152 1178 448	SETEDDDSHCSPQDAFKKEQMEQRN-SFWNTVLRAVKAKTHHLEP SETEDEDHCSPQDAFKKELEQML-SKWHFVLNVKTKTHCSINHDP TRADDVDBCIPMELRSVVCKSAGGURHMISPVLNVKLAALSPNKAM- VKLANIIAKLATGUNINIELBEKON- OVELENIIAKLATGUNINIELBEKON- SSTYEDSSCMVOYAK

Supplementary Figure 2. cTRPA-1 is a member of the TRPA subfamily of TRP channels.

Comparison of *C.elegans* TRPA-1 (C29E6.2) and TRPA-2 (M05B5.6) protein sequences to mouse TRPA1(Genbank accession number AY23117), human TRPA1 (NM_007332.1) and *Drosophila* TRPA1 (AY302598). The alignment was generated using Megalign and Boxshade. Identical or conserved residues are shown in blue and similar residues are shown in yellow.



Supplementary Figure 3. The full length cTRPA-1 GFP fusion, *ljEx114*

colocalizes with *pdel-2::RFP* in the II 1 and OLQ neurons.

Left panels indicate *pdel-2::RFP* expression in the OLQ (top panels) or IL1 (bottom panels) neurons, center panels are ljEx114 expression, and right panels are the merged images.



Supplementary Figure 4. cTRPA-1 is not required for body touch responses and normal thermotaxis.

The error bars indicate Standard Error of Mean (SEM), p values were calculated according to a student's t test, * denotes p<0.05, *** denotes p<0.001. (a) *trpa-1* mutants respond normally to gentle body touch with an eyelash (n=100 trials). (b) In a *mec-4* (light touch-insensitive) background, harsh touch response is not affected in *trpa-1* mutants (n=100 trials). (c) cTRPA-1 is not required for normal response to thermotaxis. The distribution of wild-type animals (filled circle, n=6 trials) and *trpa-1* mutants (*ok999* and *tm1402*, filled triangle and filled square respectively, n=4 trials) cultivated at 20°C on TTX plate with thermal gradient. 20°C on the gradient is at 0. (d) Defecation in *trpa-1* is slightly faster than wild-type animals (n=18 animals for wild-type, 16 for *trpa-1*, 5 cycles averaged per animal. (e) Quantification of pharyngeal pumping in wild-type and *trpa-1* mutant worms shows that *trpa-1* worms pump faster than wild type animals (n=18 animals for each strain).



Supplementary Figure 5. Baseline cameleon levels in wildtype and *trpa-1* mutants, and raw imaging data.

(a) Shown are the raw cyan baseline intensity measurements of wildtype and *trpa-1* mutants expressing cameleon in the OLQ neurons in array *ljEx130[pocr-4::YCD3]*. No significant difference was observed according to the Mann-Whitney rank test. (b) Similar to cyan baseline intensities, no difference was seen in the baseline ratio, where the baseline ratio is equal to the raw fluorescence of YPF/CFP intensites. (c) Raw data of a ratiometric FRET response to response to nose touch in a wildtype OLQ neuron. The YPF and CFP fluorescence intensity



Supplementary Figure 6. Mechanical stimuli activate TRPA-1–expressing CHO cells. (a) Prolonged suction at -50 mmHg does not desensitize cTRPA1 currents. (b) Response of an uninduced cTRPA1 stable cell to application of $500 \mu \text{M Gd3+}$ (indicated by black bar). (c) Changes in cell size due to whole cell suction. Pictures of a CHO cell in whole-cell configuration with 0, -50, and -100 mmHgapplied pressure. Area of the cell is calculated at 0-100 mmHg suction and compared to the cell's original size as a percentage.