Figure S1. Related to Figure 1. Additional data on phonotaxis behavior and substrate-borne vibration-activated behavior.

(A) Additional data on phonotaxis. In Figure 1C, we tested phonotaxis behavior by slowly and gently moving the NGM plate by hand while the speaker was held fixed in place. Here, we performed the converse experiment by moving the speaker with the manipulator while holding the NGM plate in place. This yielded a similar result. Error bars: SEM. ****p<0.0001(t-test). n≥10.

(B) mec-4 mutant shows a defect in tap avoidance, while des-2 and deg-3 mutant do not. Error bars: SEM. ****p<0.0001 (ANOVA with Bonferroni test). n≥10.
Figure S2. Related to Figure 2. Additional data on sound-evoked cuticle vibrations in WT and cuticle mutants.

(A-B) Worm cuticle vibrates at 1 kHz in response to 1 kHz sound stimulus (80 dB SPL). The small peak near 0 Hz arose from background building vibrations and was present in all recordings, including no sound control.

(C-D) Sound evokes a higher value of cuticle displacement in the head when the stimulus is applied to the head than to the tail, and vice versa. Sound stimulus: 1 kHz, 80 dB SPL. Error bars: SEM. ***p<0.0001 (ANOVA with Bonferroni test). n≥10.

(D) bli mutants show avoidance response to osmotic stimulus. Worms were tested with glycerol (2 M), which triggered reversals. osm-9 mutant served as a positive control. Error bars: SEM. **p<0.0001 (ANOVA with Bonferroni test). n≥10.
Figure S3. Related to Figure 3. Additional data on phonotaxis behavior. The sound-delivery protocol used for calcium imaging of FLP/PVD neurons is slightly different from that for behavioral measurements, due to the setup of the calcium imaging system (see methods). We thus repeated the behavioral test using the condition adopted for the calcium imaging experiments. The scoring protocol remained the same. We obtained similar results using this protocol in WT worms, and des-2 deg-3 mutant worms also showed a severe defect under this condition. Error bars: SEM. ****p<0.0001 (t-test). n≥10.
Figure S4

A  Osmotic avoidance

B  Nose touch

C  Sound avoidance

D  FLP  Sound

E  Peak GCaMP ∆R/R (fold)

F  des-2::mNG knockin

G  Sound avoidance (% response)

H  PVD  Sound

I  Peak GCaMP ∆R/R (fold)
Figure S4. Related to Figure 5. Additional data on des-2 and deg-3

(A-B) des-2 deg-3(xu482) mutant worms showed normal osmotic avoidance and nosh touch avoidance behavioral responses. osm-9 mutant worms, which were defective in both behaviors, were used as a control. Error bars: SEM. ***p<0.0001 (ANOVA with Bonferroni test). n≥10.

(C) des-2::mNG and deg-3::mNG knockin worms show no defect in phonotaxis behavior. Head-avoidance response was tested. Error bars: SEM. p>0.05 (ANOVA with Bonferroni test). n≥10.

(D-E) des-2::mNG and deg-3::mNG knockin worms show no defect in sound-evoked calcium responses in FLP neurons. (D) Average traces. Shades along the traces indicate error bars (SEM). (E) Bar graph. Error bars: SEM. P>0.05 (ANOVA with Bonferroni test). n≥10.

(F) des-2 and deg-3 are expressed in additional neurons in the tail area. des-2 and deg-3 were previously reported to be expressed in IL2 and PVC neurons besides FLP and PVD (Treinin et al., 1998). We did not detect reliable expression of des-2 and deg-3 in IL2 neurons in des-2::mNG and deg-3::mNG knockin worms. However, knockin worms expressed des-2 and deg-3 in additional neurons in the tail region (consistent expression in PVC and PVN and inconsistent expression in a few other tail neurons). Scale bars, 10 μm.

(G) des-2 deg-3(xu482) mutant worms are defective in sound-stimulated forward movement. Error bars: SEM. ***p<0.0001 (t test). n≥10.

(H-I) des-2 deg-3(xu482) mutant worms lack sound-evoked calcium responses in PVD neurons. (H) Average traces. Shades along the traces indicate error bars (SEM). (I) Bar graph. Error bars: SEM. ***p<0.0001 (t test). n≥10.
**Figure S5**

**A**

Amino acid sequences for DES-2 and DEG-3:

**DES-2**

274 - V S L G I T T L L S M S I L M L M V S D -

**DEG-3**

302 - I N L G I T T L L A M S I L M L M V S D -

α7 nAChR

240 - I S L G I T V L L S L T V F M L L V A E -

**B**

Graphs showing current density for Mock, DES-2, DEG-3, DES-2 + DEG-3, DES-2(L282S) + DEG-3(L310S), DES-2(S292R) + DEG-3(S320R), DES-2(G277K) + DEG-3, and DES-2(G277K) + DEG-3(G305K).}

**C**

Graph showing the decay constant τ and current density at 200 pA and 50 ms.

**D**

Bar graphs comparing current density (pA/pF) for different treatments.

**E**

Graph showing the decay constant τ for different treatments.
Figure S5. Related to Figure 7. Electrophysiological characterization of wild-type and mutant forms of DES-2/DEG-3 in HEK293T cells.

(A) Sequence alignment of C. elegans DES-2 and DEG-3 and human nAChR7 in the pore-lining M2 segment. The residues in DES-2 and DEG-3 that were mutated in this study are marked in red.

(B) Representative current traces from HEK293T cells expressing wild-type and mutant forms of DES-2/DEG-3. Horizontal bars above each trace indicate the application of choline (10 mM), which is known as a potent agonist for DES-2/DEG-3. Agonist was applied to mutant forms of DES-2/DEG-3 for a longer duration to ensure that no current was evoked (for channel-dead mutants) or the current was able to deactivate for a sufficient amount of time needed for data quantification (for L-S mutant). Holding potential: -70 mV. RIC-3, a chaperon for nAChRs, was co-transfected with DES-2 and DEG-3 into HEK293T cells.

(C) No notable mechanically-activated currents were detected in DES-2/DEG-3 expressed in HEK293T cells. Cells were stimulated with a glass probe driven by a piezo actuator as described previously (Li et al., 2011). The stimulus steps, ranging from 0 to 8 µm, were shown to the top.

(D) Bar graph summarizing the data in (B). Mock, DEG-3, DES-2(G277K)+DEG-3, DES-2(S292R)+DEG-3, DES-2(S292R)+DEG-3(S320R), and DES-2(G277K)+DEG-3(G305K) all showed no current (n≥5). DES-2: 8.0±3.4 (pA/pF) (n=10). DES-2+DEG-3: 267.4±145.3 (pA/pF) (n=17). DES-2(L282S)+DEG-3(L310S): 179.5±135.6 (pA/pF) (n=10).

(E) Bar graph showing that DES-2(L282S)/DEG-3(L310S) desensitizes/inactivates more slowly than wild-type DES-2/DEG-3 channel. The decay phase of the currents were fitted with exponential function to calculate the decay constant τ. For DES-2+DEG-3: 0.97±0.19 (s) (n=11); DES-2(L282S)+DEG-3(L310S): 21.76±5.51 (s) (n=9). Data are presented as mean±s.d. **p<0.001 (t test).
Figure S6. Related to Figure 7. Knockin worms carrying channel-dead mutations show normal expression of DES-2. The channel-dead mutations G277K and S292R were introduced into the endogenous locus of des-2 by CRISPR-based genome editing. Prior to introducing G277K and S292 point mutations, we first introduced the mNG (mNeonGreen) tag into the endogenous des-2 locus to produce des-2::mNG knockin line by CRISPR-based genome editing. This mNG tag did not affect DES-2 function (Figure S4). We then performed genome editing in this des-2::mNG background to introduce the G277K and S292 channel-dead mutations. des-2(G277K)::mNG (B) and des-2(S292R)::mNG (C) knockin lines show normal expression of DES-2 in FLP and PVD neurons compared to the parental control (A). Scale bars, 10 μm.
Figure S7. Related to Figures 1-7. Schematic and parts list for miniature microphone power supply. The miniature microphone requires its own power supply. (A) A schematic for the design of the custom power supply used to operate the Knowles miniature microphone. (B) The parts list for the items required for assembling the microphone power supply.

| Description                              | Part #        | Source                        |
|------------------------------------------|---------------|-------------------------------|
| SPDT Toggle switch                       | M2013SS1W01   | Digi-Key Electronics          |
| Panel mount TRS jack                     | 4832.23       | Digi-Key Electronics          |
| Metalized film capacitor                 | 335MWR050K    | Digi-Key Electronics          |
| AA Battery holder with leads             | BCAAW         | Digi-Key Electronics          |
| Analog electret condenser microphone     | FG-23329-P07  | Digi-Key Electronics          |
| TRRS phono cable                         | 10-02153      | Digi-Key Electronics          |
| Aluminum enclosure                       | PRT-13839     | Digi-Key Electronics          |
| Panel mount BNC connector                | 031-10-RFXG1  | Digi-Key Electronics          |
| TRS plug (3.5mm)                         | 4832.13       | Digi-Key Electronics          |
| BNC to 1/4" audio adapter               | 1297          | Digi-Key Electronics          |
| Focusrite Scarlett solo                  | SCARLETT-SOLO-3G | B&H Photo Video               |