Fig. S1. Cdh23 genotyping and sequencing of Fat-1 mice.
Table S1. Summary table for the two-way ANOVA.

### Fig. 1B

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degrees of Freedom</th>
<th>Sum of Squares</th>
<th>F Ratio</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain</td>
<td>1</td>
<td>426.603</td>
<td>11.2518</td>
<td>0.0011</td>
</tr>
<tr>
<td>Stimulus Frequency</td>
<td>4</td>
<td>38240.047</td>
<td>252.149</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Strain * Stimulus Frequency</td>
<td>4</td>
<td>928.508</td>
<td>6.1224</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

### Fig. 1C

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degrees of Freedom</th>
<th>Sum of Squares</th>
<th>F Ratio</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain</td>
<td>1</td>
<td>551.25</td>
<td>4.7533</td>
<td>0.0306</td>
</tr>
<tr>
<td>Stimulus Frequency</td>
<td>4</td>
<td>38536.944</td>
<td>83.0737</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Strain * Stimulus Frequency</td>
<td>4</td>
<td>1570.278</td>
<td>3.385</td>
<td>0.0108</td>
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</tbody>
</table>

### Fig. 1D

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degrees of Freedom</th>
<th>Sum of Squares</th>
<th>F Ratio</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain</td>
<td>1</td>
<td>17.09</td>
<td>0.1332</td>
<td>0.7165</td>
</tr>
<tr>
<td>Stimulus Frequency</td>
<td>4</td>
<td>13262.69</td>
<td>25.8408</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Strain * Stimulus Frequency</td>
<td>4</td>
<td>773.459</td>
<td>1.507</td>
<td>0.2128</td>
</tr>
</tbody>
</table>

### Fig. 1E

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degrees of Freedom</th>
<th>Sum of Squares</th>
<th>F Ratio</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain</td>
<td>1</td>
<td>78.6594</td>
<td>6.0265</td>
<td>0.0166</td>
</tr>
<tr>
<td>Age</td>
<td>2</td>
<td>1856.9686</td>
<td>71.1355</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Strain * Age</td>
<td>2</td>
<td>171.9292</td>
<td>6.5862</td>
<td>0.0024</td>
</tr>
</tbody>
</table>

### Fig. 2A

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degrees of Freedom</th>
<th>Sum of Squares</th>
<th>F Ratio</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain</td>
<td>1</td>
<td>4532.99</td>
<td>24.7196</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Stimulus Frequency</td>
<td>4</td>
<td>4948.2461</td>
<td>6.746</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Strain * Stimulus Frequency</td>
<td>4</td>
<td>951.9461</td>
<td>1.2978</td>
<td>0.2769</td>
</tr>
</tbody>
</table>
Table S1. Summary table for the two-way ANOVA.

**Fig. 3E**

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degrees of Freedom</th>
<th>Sum of Squares</th>
<th>F Ratio</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain</td>
<td>1</td>
<td>84.62137</td>
<td>0.0481</td>
<td>0.8267</td>
</tr>
<tr>
<td>Cochlear Frequency</td>
<td>7</td>
<td>739.12335</td>
<td>0.0601</td>
<td>0.9997</td>
</tr>
<tr>
<td>Strain * Cochlear Frequency</td>
<td>7</td>
<td>89.19892</td>
<td>0.0072</td>
<td>1</td>
</tr>
</tbody>
</table>

**Fig. 3F**

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degrees of Freedom</th>
<th>Sum of Squares</th>
<th>F Ratio</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain</td>
<td>1</td>
<td>2877.697</td>
<td>3.179</td>
<td>0.0769</td>
</tr>
<tr>
<td>Cochlear Frequency</td>
<td>7</td>
<td>55730.092</td>
<td>8.795</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Strain * Cochlear Frequency</td>
<td>7</td>
<td>2025.967</td>
<td>0.3197</td>
<td>0.944</td>
</tr>
</tbody>
</table>

**Fig. 3K**

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degrees of Freedom</th>
<th>Sum of Squares</th>
<th>F Ratio</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain</td>
<td>1</td>
<td>116.046</td>
<td>4.1698</td>
<td>0.0514</td>
</tr>
<tr>
<td>Area</td>
<td>2</td>
<td>1718.6094</td>
<td>30.8766</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Strain * Area</td>
<td>2</td>
<td>134.3344</td>
<td>2.4135</td>
<td>0.1093</td>
</tr>
</tbody>
</table>

**Fig. 3L**

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degrees of Freedom</th>
<th>Sum of Squares</th>
<th>F Ratio</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain</td>
<td>1</td>
<td>4.764406</td>
<td>1.0556</td>
<td>0.3137</td>
</tr>
<tr>
<td>Area</td>
<td>2</td>
<td>10.222521</td>
<td>1.1325</td>
<td>0.3376</td>
</tr>
<tr>
<td>Strain * Area</td>
<td>2</td>
<td>8.354084</td>
<td>0.9255</td>
<td>0.409</td>
</tr>
</tbody>
</table>
### Table S2. Fatty Acid Composition of CE-2 Diet.

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>CE-2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C12:0</td>
<td>n.d.</td>
</tr>
<tr>
<td>C14:0</td>
<td>0.82</td>
</tr>
<tr>
<td>C14:1</td>
<td>n.d.</td>
</tr>
<tr>
<td>C15:0</td>
<td>n.d.</td>
</tr>
<tr>
<td>C16:0</td>
<td>16.25</td>
</tr>
<tr>
<td>C16:1n-7</td>
<td>1.34</td>
</tr>
<tr>
<td>C17:0</td>
<td>0.52</td>
</tr>
<tr>
<td>C17:1</td>
<td>n.d.</td>
</tr>
<tr>
<td>C18:0</td>
<td>2.13</td>
</tr>
<tr>
<td>C18:1n-9 cis</td>
<td>20.95</td>
</tr>
<tr>
<td>C18:1n-9 trans</td>
<td>n.d.</td>
</tr>
<tr>
<td>C18:1n-7</td>
<td>2.11</td>
</tr>
<tr>
<td>C18:2n-6 cis</td>
<td>44.44</td>
</tr>
<tr>
<td>C18:2n-6 trans</td>
<td>n.d.</td>
</tr>
<tr>
<td>C18:3n-6</td>
<td>n.d.</td>
</tr>
<tr>
<td>C18:3n-3</td>
<td>3.26</td>
</tr>
<tr>
<td>C20:0</td>
<td>0.49</td>
</tr>
<tr>
<td>C20:1n-9</td>
<td>0.72</td>
</tr>
<tr>
<td>C20:4n-6</td>
<td>n.d.</td>
</tr>
<tr>
<td>C20:5n-3</td>
<td>2.27</td>
</tr>
<tr>
<td>C22:0</td>
<td>0.25</td>
</tr>
<tr>
<td>C22:5n-3</td>
<td>n.d.</td>
</tr>
<tr>
<td>C22:6n-3</td>
<td>1.29</td>
</tr>
<tr>
<td>C24:0</td>
<td>0.18</td>
</tr>
<tr>
<td>C24:1</td>
<td>0.2</td>
</tr>
<tr>
<td>Unidentified</td>
<td>2.78</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100</td>
</tr>
</tbody>
</table>

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total n-6</td>
<td>44.44</td>
</tr>
<tr>
<td>Total n-3</td>
<td>6.82</td>
</tr>
</tbody>
</table>

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>n-6/n-3 ratio</td>
<td>6.52</td>
</tr>
</tbody>
</table>

n.d.=not detected.

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Additional methods

Cdh23 genotyping

To confirm that the Fat-1 transgenic mice had the Cdh23$^{753A/753A}$ genotype for Cdh23, which is common in the C57BL6 strain and accelerates age-related hearing loss, we isolated genomic DNA from three Fat-1 transgenic mice, amplified the DNA fragments by PCR, and then sequenced the region of DNA containing the 753rd nucleotide in the Cdh23 gene. The primer sequences were as follows:

Forward: 5’- GATCAAGACAAGACCAGACCTCTGTC-3’
Reverse: 5’-GAGCTACCAGGAACAGCTTGGGCCTG-3’

The cycling conditions for PCR were as follows: 95°C for 2 min; 35 cycles of 95°C for 10 sec, 63.7°C for 10 sec, 72°C for 30 sec; and 72°C for 5 min.

The expected band size of the PCR product was 360 bp. The Cdh23 gene was sequenced in three Fat-1 transgenic mice for both strands.