

Supplementary Figures and Legends

Acoustic modulation of mechanosensitive genes and adipocyte differentiation

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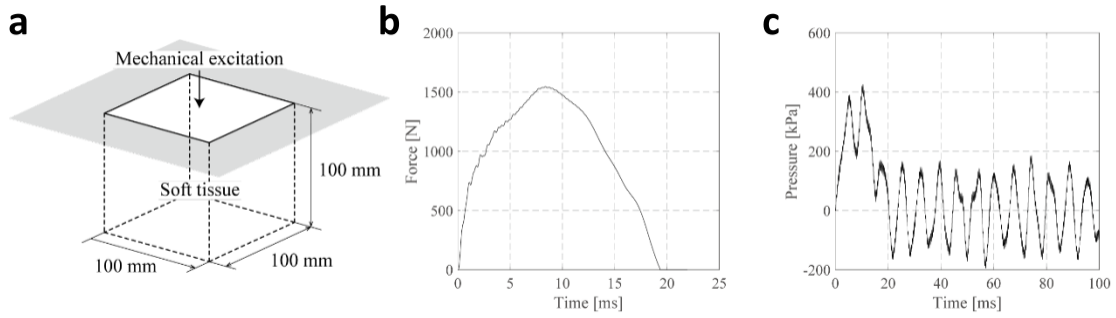
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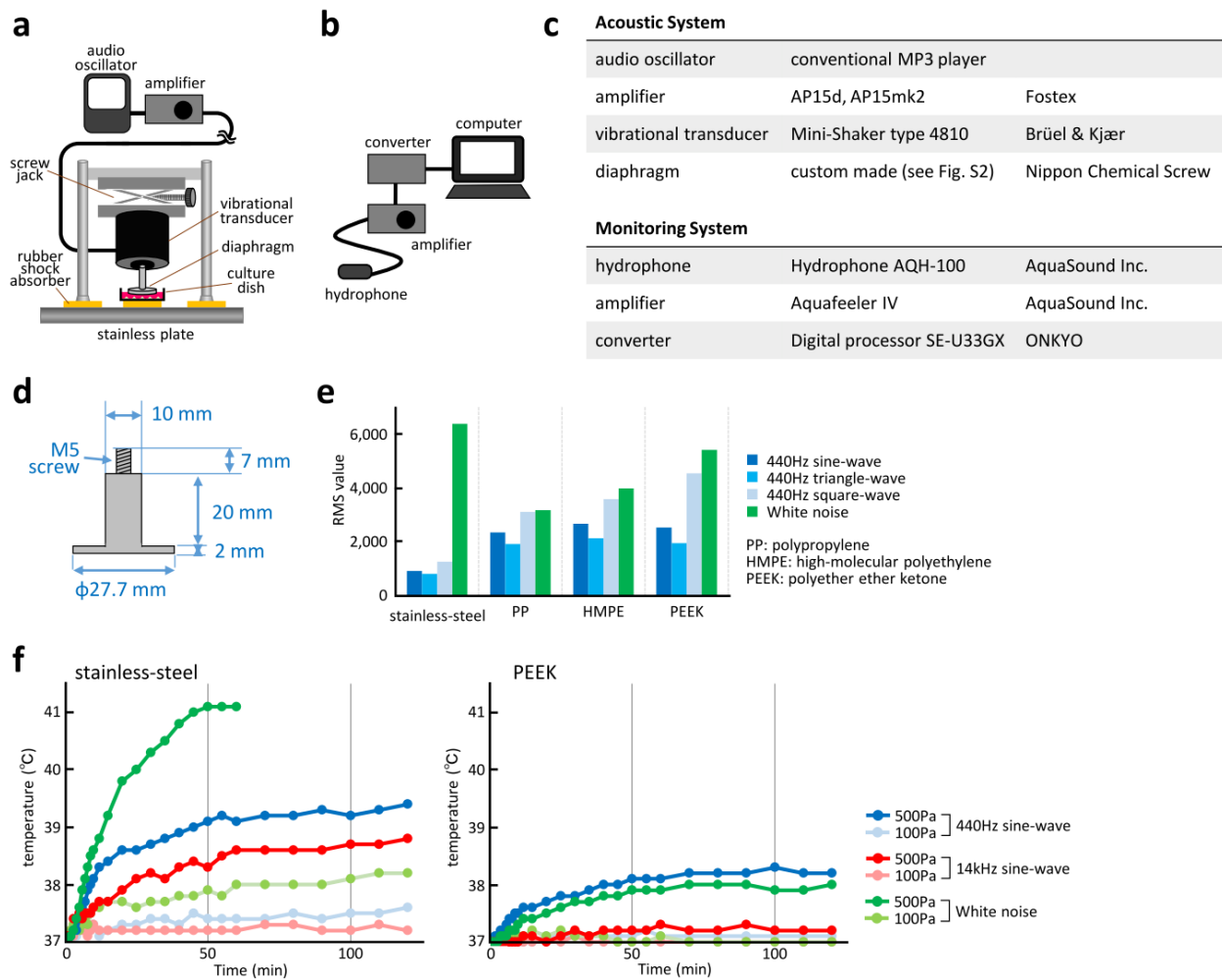
13 **Supplementary Fig. 1**



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15 **Supplementary Fig. 1 | Simulation analysis of sound transmission in an elastic object via physical**
 16 **contact.**

17 **a)** An elastic object of 0.001 m^3 volume with a $1 \times 10^5 \text{ N/m}^2$ shear elastic constant was subjected to
 18 physical contact with an impact ball (YI-01, Rion). Analysis was performed based on the vibroacoustic
 19 finite-difference time-domain method. **b)** Properties of the input force applied to the top of the object
 20 via physical contact. **c)** Properties of the vibroacoustic pressure detected at the centre of the object. As
 21 the simulation condition did not consider energy dissipation, circulation of pressure was observed. The
 22 first pressure peak indicates that the maximum pressure owing to this physical contact was $\sim 400 \text{ kPa}$.

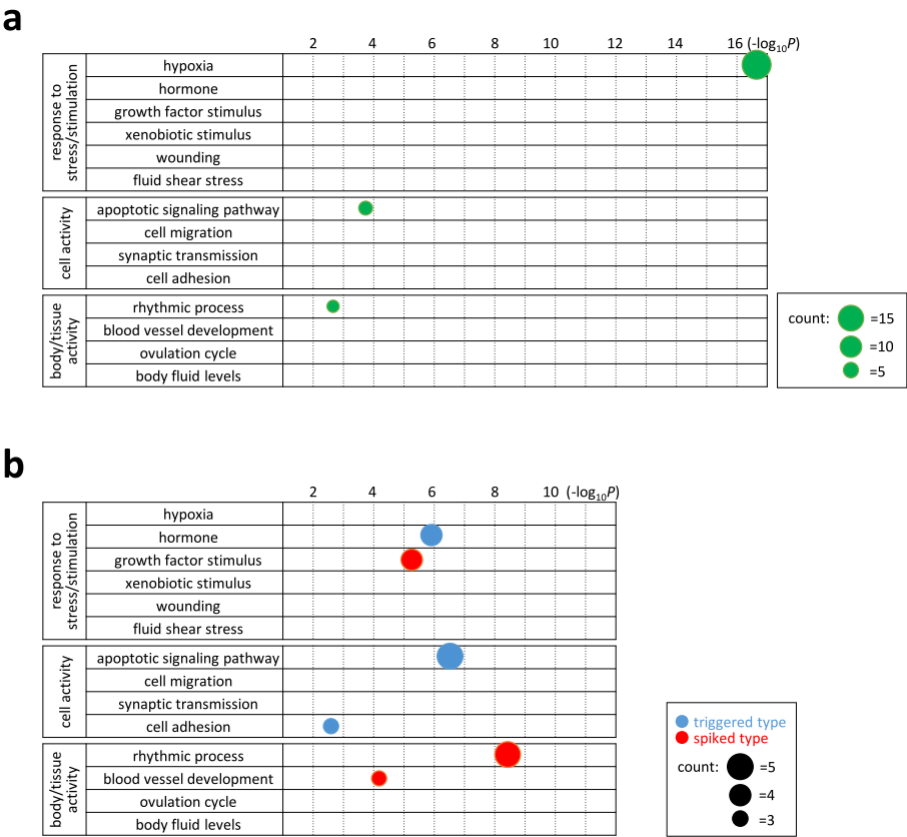


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26 **Supplementary Fig. 2 | Setup of the acoustic irradiation and monitoring systems.**
27 **a)** Schematic illustration of the direct sound emission system used in this study. The vibrational
28 transducer was attached to a small screw jack and fixed to a metal shelf. A custom-made diaphragm
29 was attached to the transducer. The system and dish were put on a 5-mm-thick 450 × 450 mm stainless
30 plate through rubber shock absorbers to cancel vibrations and avoid slipping during acoustic emission.
31 The tip of the diaphragm was attached to the culture medium by adjusting the screw jack. The sound
32 signal was output using a conventional MP3 player through an amplifier. **b)** Schematic illustration of
33 the sound monitoring system. A hydrophone was placed in a water tank to monitor the intensity of
34 sound transmitted into the culture medium. The details of the sound intensity measurement procedure
35 are described in the [Methods](#). **c)** Equipment used in the acoustic and monitoring systems. **d)** Design of
36 custom-made diaphragm. Head was made of a 2-mm-thick plate with diameter of 27.7 mm, designed
37 to cover 80% of the 30 mm dish (actual diameter: 31 mm). The tail contained an M5 screw attached
38 to a vibrational transducer. **e)** The diaphragm was made of four different materials: stainless-steel,
39 polypropylene (PP), high-molecular polyethylene (HMPE), and polyether ether ketone (PEEK). The
40 440 Hz sine-wave, triangle-wave, square-wave, and white noise sound was emitted at the same output

level, and the sound intensity was measured in water. RMS values show that the stainless-steel diaphragm exhibits the highest intensity when emitting white noise, whereas the PEEK diaphragm shows a high and constant output. **f)** Changes in temperature owing to acoustic stimulation. Stainless-steel and PEEK diaphragms were used to emit acoustic signals in a 30 mm dish containing 3 ml of water; 440 Hz sine-wave, 14 kHz sine-wave, and white noise sound was output at 500 Pa and 100 Pa for 2 h. Time-lapse monitoring of the temperature clearly shows the high thermal conductivity of the stainless-steel diaphragm, which transferred heat from the vibrational transducer to the sample. The temperature changes using the PEEK diaphragm were smaller; in particular, increases in temperature with 100 Pa output were limited within 0.2 °C. Based on these findings, the PEEK diaphragm was selected as the standard.

51 **Supplementary Fig. 3**

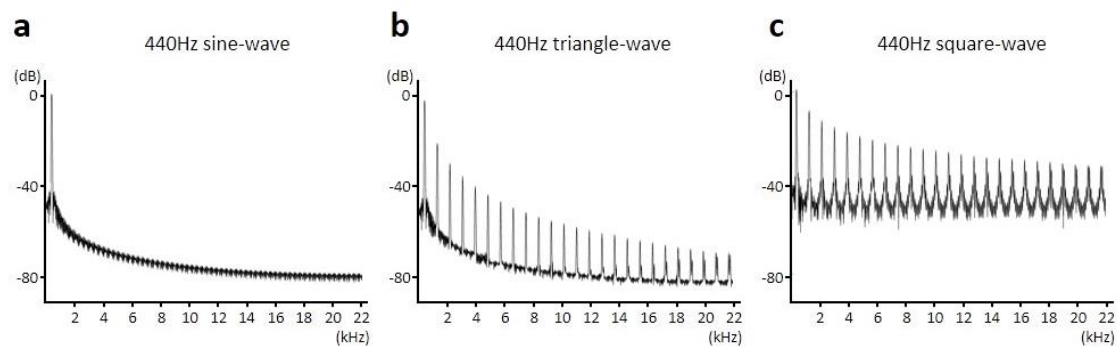


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53 **Supplementary Fig. 3 | Gene annotation analyses of specific groups of sound-sensitive genes.**

54 **a)** Annotation analysis of genes upregulated by 440 Hz stimulation and downregulated by 14 kHz
55 sound, indicated in red in Fig. 1f. **b)** Annotation analysis of trigger-type (blue) and spike-type (red)
56 upregulated genes presented in Fig. 2a.

57 **Supplementary Fig. 4**



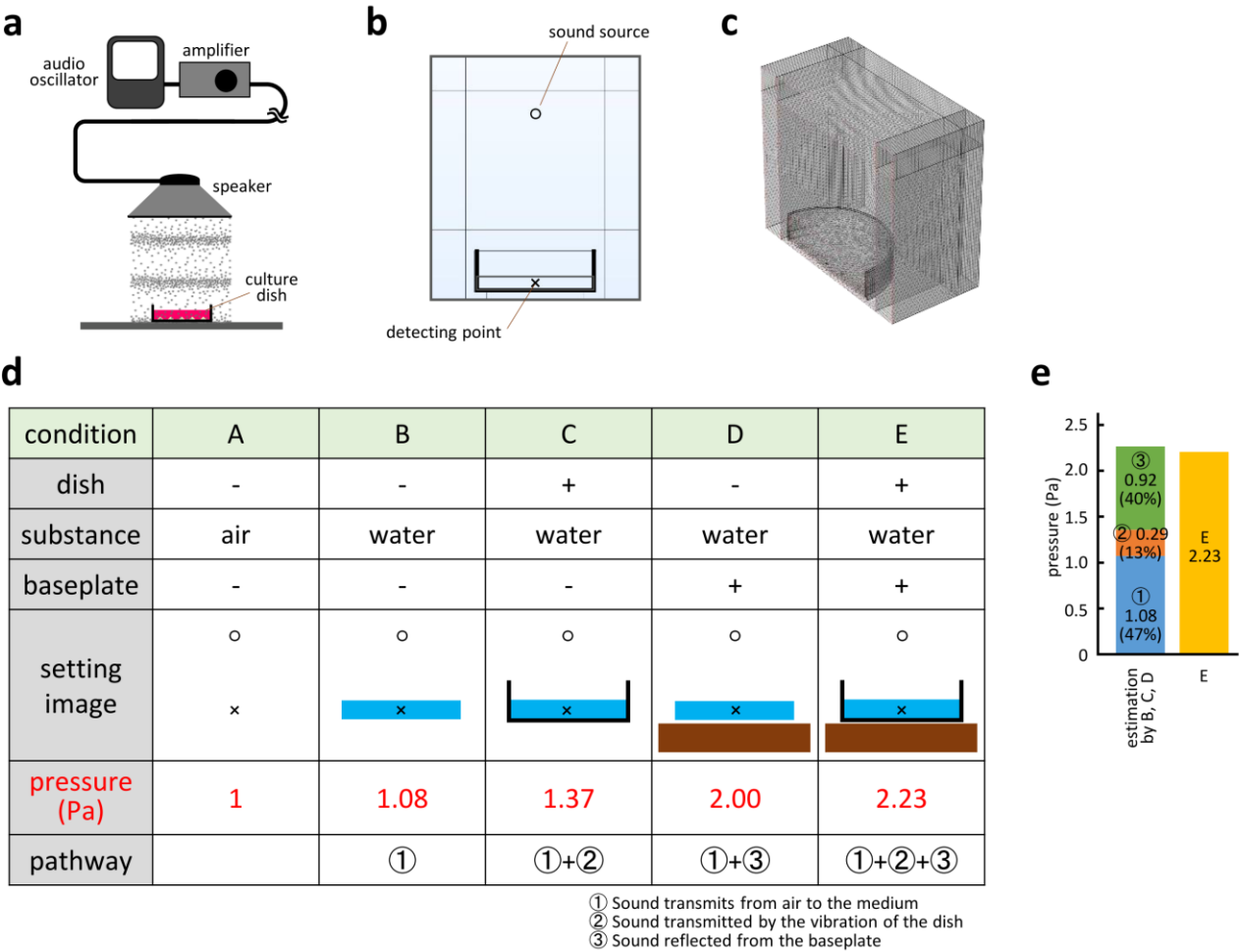
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59 **Supplementary Fig. 4 | Frequency characteristics of sine-, triangle-, and square-waves.**

60 The averaged spectrum of 440 Hz for different waveforms was obtained using a Fourier transform.

61 Results of **a)** sine-wave, **b)** triangle-wave, and **c)** square-wave signals are shown.

62 **Supplementary Fig. 5**

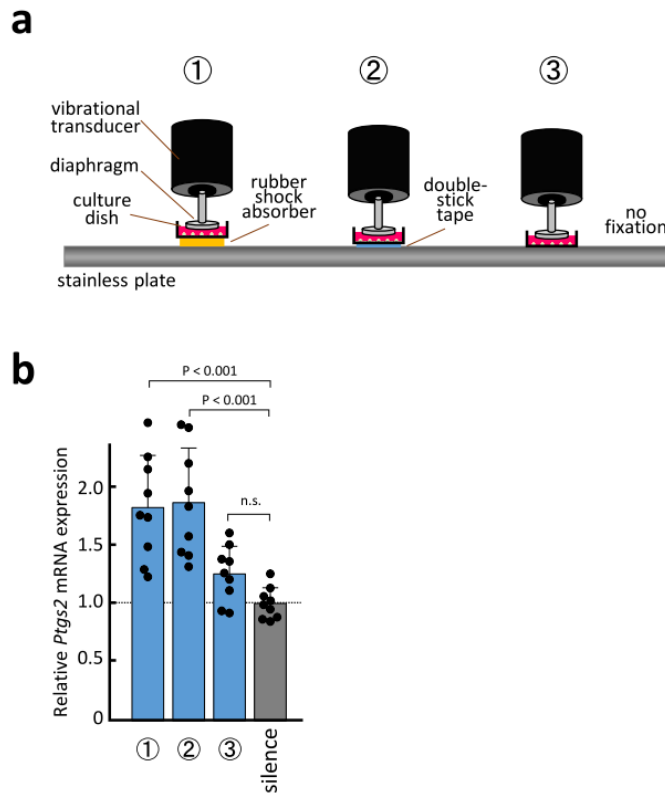


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64 **Supplementary Fig. 5 | Simulation analyses of acoustic propagation using the indirect sound**
65 **emission system.**

66 **a)** Schematic illustration of indirect sound emission system used in a previous study¹². A full-range
67 loudspeaker was set above the culture dish to emit sound, as described in the [Methods](#). **b)** Simulation
68 field indicating the sound source, detection point, and substances such as water and dishes. **c)** 3D image
69 of the simulated acoustic field. The dissection image is presented, showing the sound source, detection
70 point, and dish in the field. **d)** Pressure levels detected using different settings. The pressure level
71 simulated in air (A) was set as the standard, and the same sound source was used. Pressures propagated
72 by the sound transmitted from air to the medium (①), transmitted by the vibration of the dish (②),
73 and reflected from the baseplate (③) were estimated by comparing conditions B, C, and D. **e)** The sum
74 of the pressures ①, ②, and ③ was relatively equal to the pressure detected under condition E,
75 demonstrating that cells received only 40% of the direct transmitted sound and approximately 60% of
76 the reflected/re-irradiated sound that might be altered.

77 **Supplementary Fig. 6**

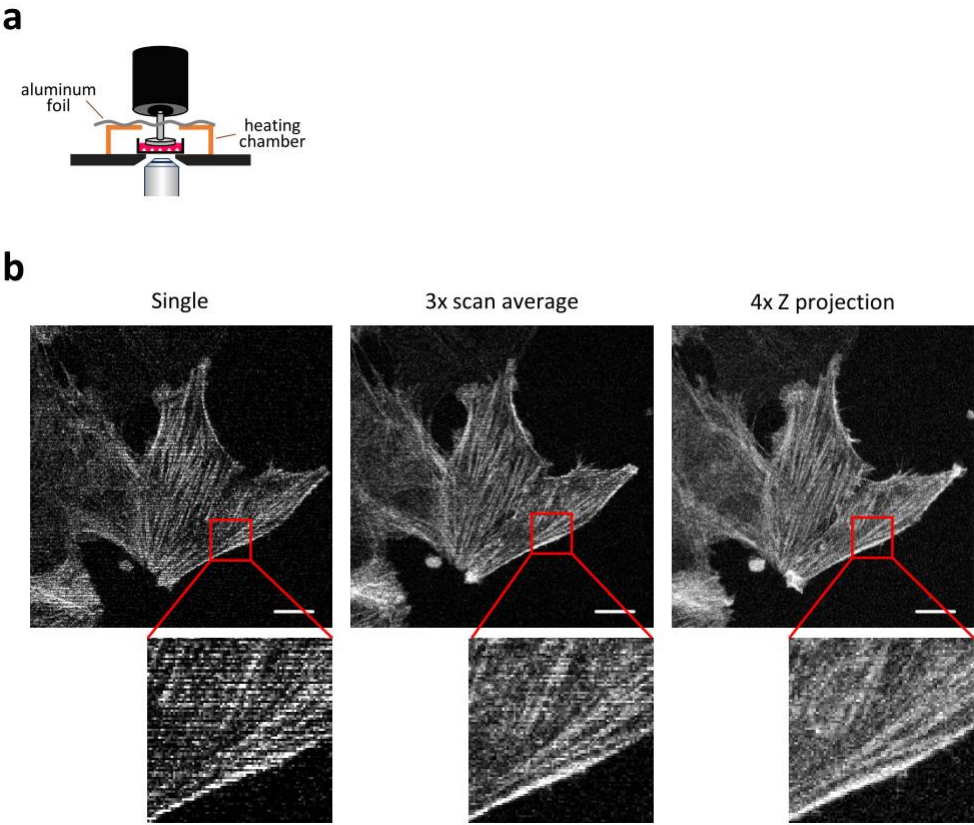


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79 **Supplementary Fig. 6 | Effect of dish vibration on the acoustic response of *Ptgs2*.**

80 **a)** Schematic illustration of the direct sound emission system used to observe the effect of dish
81 vibrations on gene regulation. Standard dish setting (①), dish firmly attached to the plate by a double-
82 stick tape (②), and dish without fixation (③) were prepared. After 2 h of emission of 440 Hz sine-
83 wave sound at 100 Pa, total RNA was extracted, and *Ptgs2* expression was measured using qPCR
84 analysis. **b)** qPCR results clearly demonstrate that the vibration of the dish abolished the effect of
85 directly emitted sound, suggesting an opposing effect of indirectly transmitted sound on sound-
86 sensitive gene responses. Bars represent +SD from three biological replicates of three independent
87 experiments, and statistical significance was evaluated using one-way ANOVA followed by Tukey's
88 HSD test.

89 **Supplementary Fig. 7**

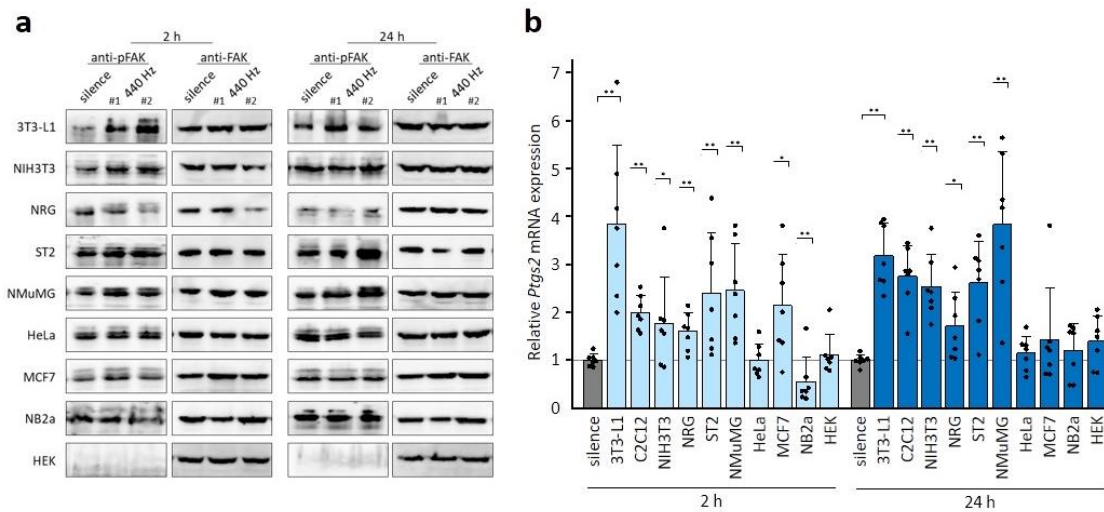


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91 **Supplementary Fig. 7 | Image-processing procedure for live cell observation under acoustic**
92 **stimulation.**

93 **a)** Schematic illustration of the direct sound emission system at the microscope stage. Cells transfected
94 with EGFP-Lifeact were grown in a glass-bottomed dish and observed under a confocal laser-scanning
95 microscope. The diaphragm was inserted through a hole in the lid of the heating chamber and covered
96 with aluminum foil to stabilise the temperature. Images were captured every minute for 1 h. **b)** Image-
97 processing procedure. Sound at 440 Hz emitted at 100 Pa disturbed the laser scanning image
98 acquisition and generated scratching noise in the single-plane image. To cancel this noise, data from
99 three scans were averaged on a single plane, and four differently focused images with 1 μm Z-scale
100 intervals were projected.

101 **Supplementary Fig. 8**



102

103 **Supplementary Fig. 8 | Responses of FAK phosphorylation and *Ptgs2* gene expression to acoustic**

104 **stimulation in different cell lines.**

105 **a)** 3T3-L1, HEK, HeLa, MCF7, NB2a, NIH3T3, NMuMG, NRG, ST2 cells were subjected to 440 Hz

106 acoustic stimulation at 100 Pa intensity for 2 and 24 h. Phosphorylation states of FAK Y397 was

107 analysed by quantifying pFAK/FAK signal intensities of Western blotting using anti-FAK and anti-

108 pFAK antibodies and the effect of acoustic stimulation was evaluated over silent condition. Results of

109 two biological replicates (#1 and #2) are shown. **b)** qPCR analysis of *Ptgs2* expression in different cell

110 lines. Cells were subjected to acoustic stimulation by 440 Hz sine-wave sound at 100 Pa for 2 h and

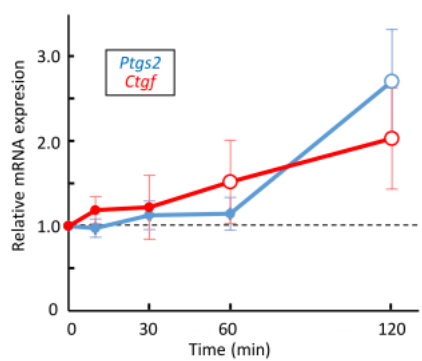
111 24 h. The results clearly demonstrate a significant upregulation of *Ptgs2* expression following acoustic

112 stimulation. Bars represent +SD from three biological replicates of two independent experiments, and

113 statistical significance was evaluated using Tukey's multiple comparison test against silent condition.

114 *: P<0.5, **: P<0.1.

115 **Supplementary Fig. 9**

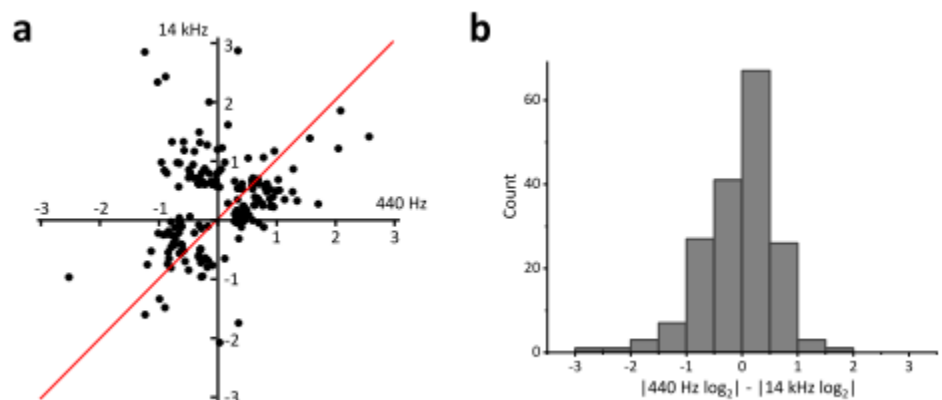


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117 **Supplementary Fig. 9 | Sound-sensitive gene responses in shorter time points.**

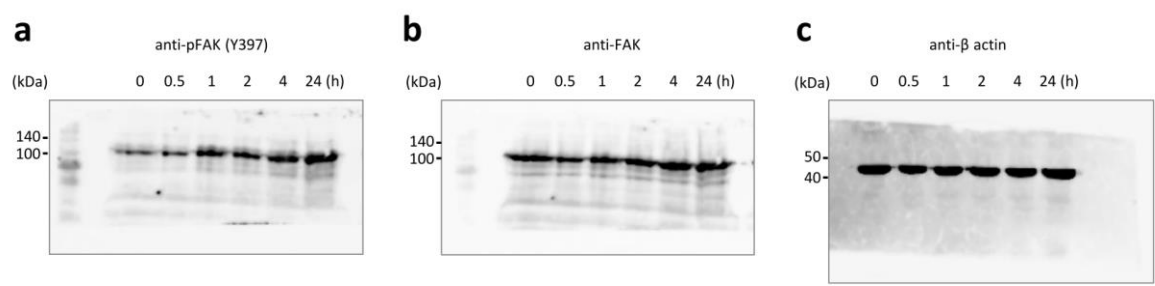
118 Expression levels of *Ptgs2* (blue) and *Ctgf* (red) in C2C12 cells treated with 440 Hz sound at 100 Pa
119 for 10, 30, 60 and 120 minutes. Bars represent $\pm$ SD from 3 biological replicates of 2 independent
120 experiments, and statistical significance was evaluated using Welch's t-test. Open circles indicate
121 statistical significance of $P < 0.05$ compared with the silent sample.

122 **Supplementary Fig. 10**



123
124 **Supplementary Fig. 10 | Correlation of gene responses against 440 Hz and 14 kHz acoustic**
125 **stimulation.**
126 **a)** Correlation of log₂ fold differences of sound-sensitive gene responses after 2 and 24 of 440 Hz (X-
127 axis) and 14 kHz (Y-axis) acoustic stimulation. Red line indicates X = Y. 54.5% of the genes were X
128 > Y, while 45.5% were X < Y. **b)** Histogram of the differences between 440 Hz and 14 kHz gene
129 responses.

130 **Supplementary Fig. 11**



131
132 **Supplementary Fig. 11 | Unedited images of western blots using anti-pFAK, FAK, and β-actin.**
133 Unedited images of data shown in Fig. 3d. Western blot analysis of C2C12 whole-cell lysate taken at
134 the indicated time point after starting 440 Hz continuous acoustic stimulation at 100 Pa. After blotting
135 with an anti-phospho Y397 FAK antibody **(a)**, the membrane was stripped and reblotted with an anti-
136 FAK antibody **(b)**. Anti-β actin blotting was performed as a loading control **(c)**.

137 **Supplementary Movie 1:** Live cell observation of C2C12 cells under acoustic stimulation. C2C12
138 cells expressing EGFP-Lifeact were observed under acoustic stimulation with 440 Hz sine-wave sound
139 at 100 Pa. Images were acquired every minutes for 60 min. Image processing procedures are described
140 in Supplementary Fig. 7.

141
142 **Supplementary Movie 2:** Live cell images of expanded and retracted areas of C2C12 cells under
143 acoustic stimulation. Based on the data presented in Supplementary Movie 1, expanded and retracted
144 areas in 1 min were shown in green and red, respectively.

145
146 **Supplementary Movie 3:** Live cell observation of C2C12 cells in silent condition. C2C12 cells
147 expressing EGFP-Lifeact were observed under silent condition.

148
149 **Supplementary Movie 4:** Live cell images of expanded and retracted areas of C2C12 cells in silent
150 condition. Based on the data presented in Supplementary Movie 3, expanded and retracted areas in 1
151 min were shown in green and red, respectively.

152
153 **Supplementary Audio 1: 440 Hz Sine Wave (60s)**

154 **Supplementary Audio 2: 14k Hz Sine Wave (60s)**

155 **Supplementary Audio 3: White Noise (60s)**

156 **Supplementary Audio 4: 440 Hz Triangle Wave (60s)**

157 **Supplementary Audio 5: 440 Hz Square Wave (60s)**