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Supplementary information

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In vivo magnetogenetics for cell-type-specific targeting and modulation of brain circuits

In the format provided by the authors and unedited

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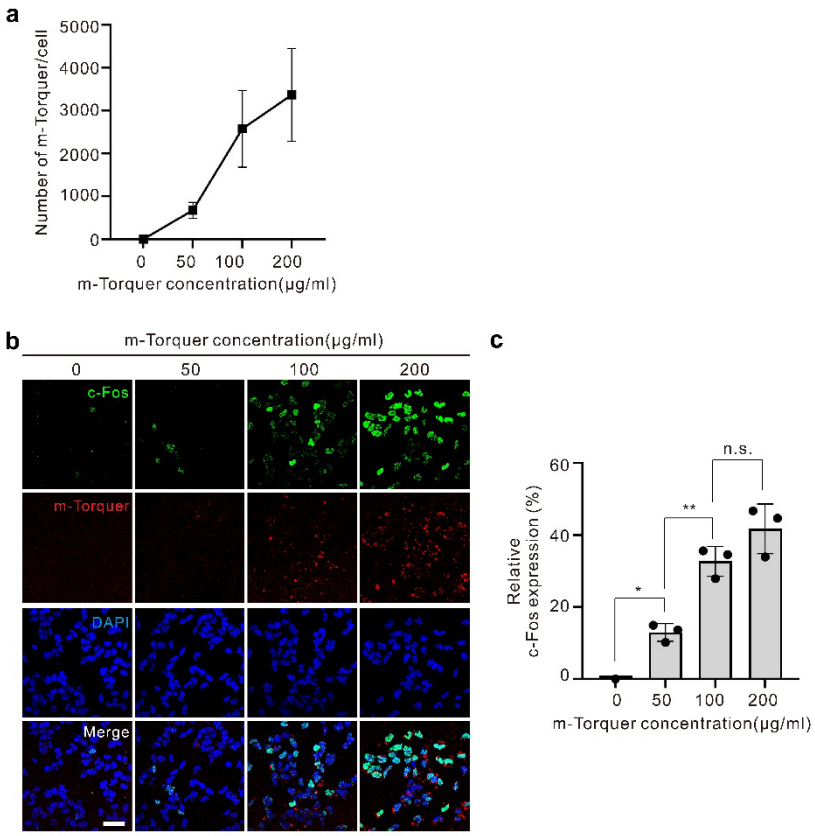
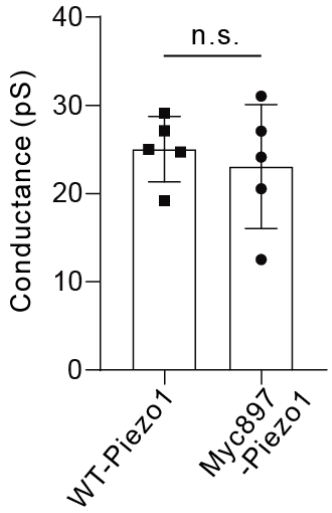
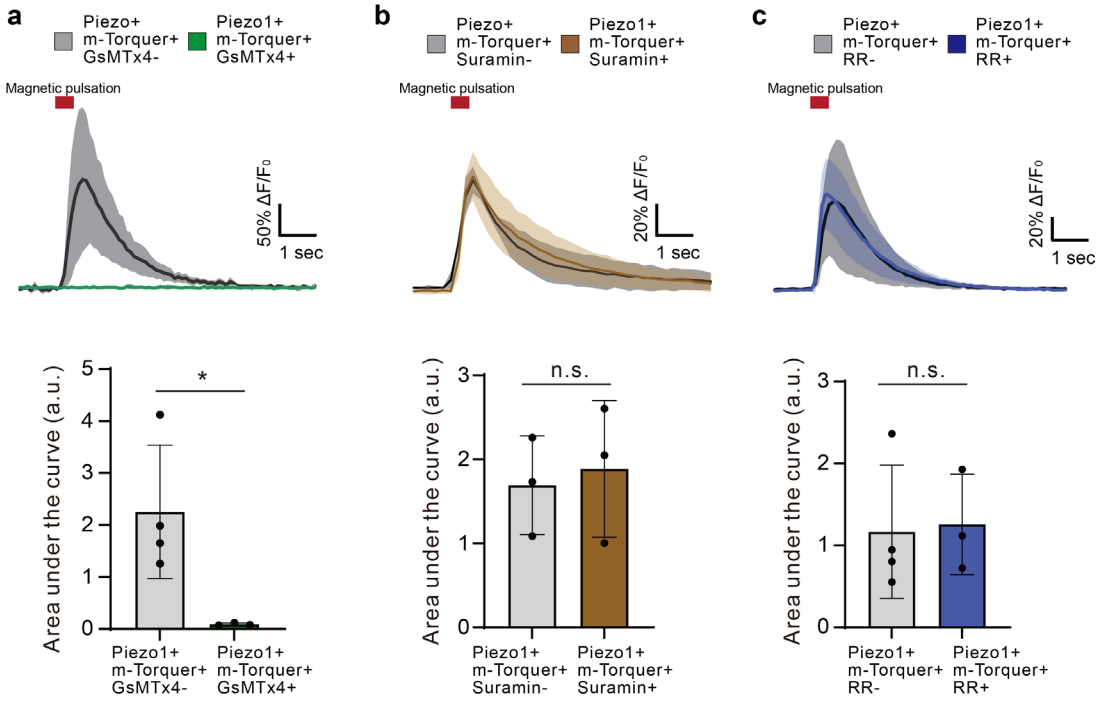


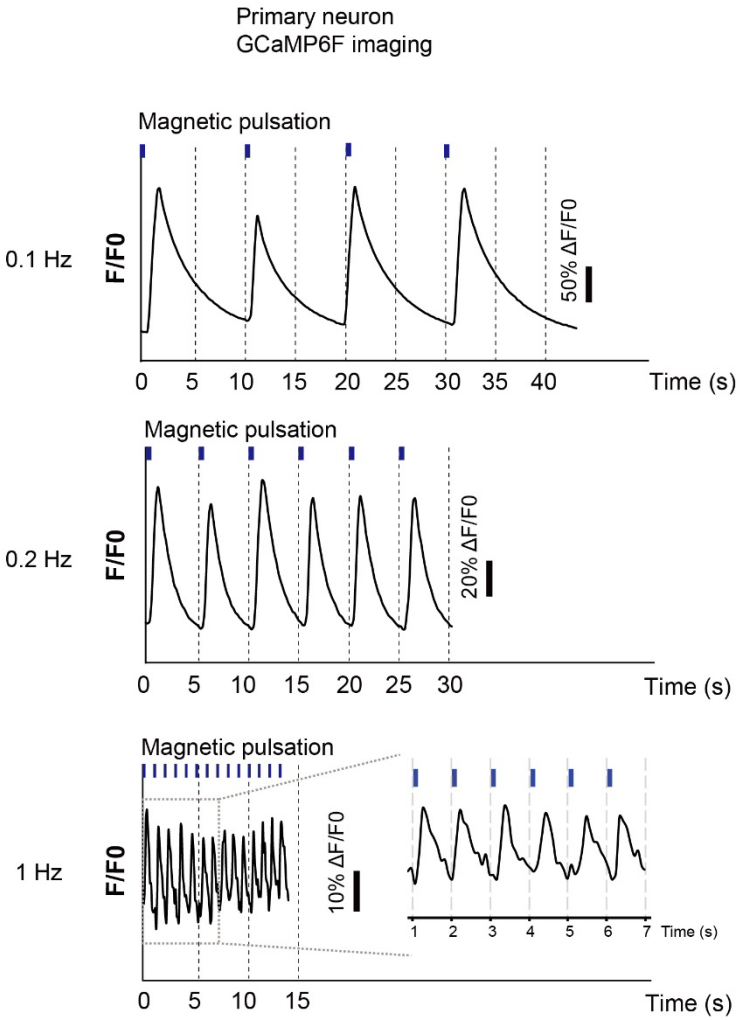
Supplementary Figure 1. The m-Torquer binding on a single cell and its effect on *in vitro* cell activation. (a) The number of labeled m-Torquer per Piezo1+HEK293 cell when applied with different m-Torquer concentrations, measured by ICP spectroscopy. (b) Immunocytochemistry images of Piezo1+ HEK293 cells against c-Fos to evaluate Ca^{2+} influx induced by MMG stimulation with different m-Torquer concentrations. Green, c-Fos; red, m-Torquer; blue, DAPI (nucleus). Scale bar, 20 μm. (c) Quantification of c-Fos-positive cells among Piezo1+ HEK293 cells after MMG stimulation. One-way ANOVA with multiple comparison test; $F(2, 6)=20.70$, $p=0.0020$. Data are mean ± s.d. *$p=0.0451$, **$p=0.0021$; n.s., non-significant; two-tailed unpaired Student's *t* test; n=3 samples for each m-Torquer concentration, respectively.



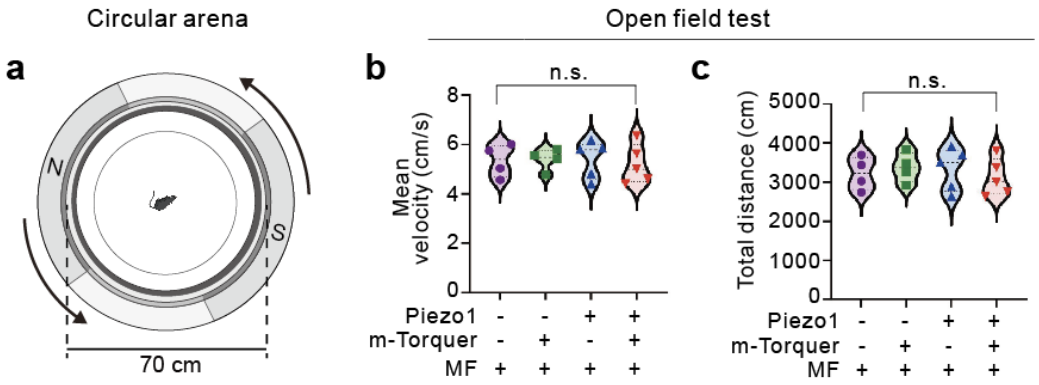
Supplementary Figure 2. **Effects of genetic modification of Piezo1 on channel function.** The values of single ion channel conductance of WT-Piezo1[1–4] and Myc897-Piezo1 from our measurements. All data are presented as mean ± s.d. of n=5; n.s., non-significant; two-tailed unpaired Student's *t* test.



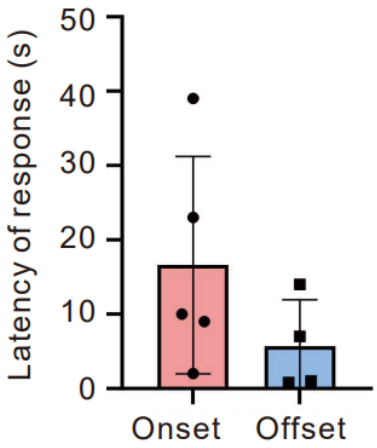
Supplementary Figure 3. Effects of pharmacological inhibition of Piezo1 and other mechanosensitive ion channels. (a) Calcium responses of Piezo1+ neurons to a magnetic pulsation of 0.5 s in the presence of GsMTx4 (10 μM). Data are mean ± s.d.; **p*=0.0283; two-tailed unpaired Student's *t* test; n=3 for Piezo1+m-Torquer without GsMTx4, n=3 for Piezo1+m-Torquer with GsMTx4. **(b)** Calcium responses of Piezo1+m-Torquer+ neurons to MMG stimulation with or without Suramin treatment (60 μM). Data are mean ± s.d.; n.s., non-significant; two-tailed unpaired Student's *t* test; n=3 for Piezo1+m-Torquer without Suramin, n=3 for Piezo1+m-Torquer with Suramin. **(c)** Calcium responses of Piezo1+m-Torquer+ neurons to MMG stimulation with or without ruthenium red (RR, 500 nM) treatment. Data are mean ± s.d.; n.s., non-significant; two-tailed unpaired Student's *t* test; n=3 for Piezo1+m-Torquer without RR, n=3 for Piezo1+m-Torquer with RR.



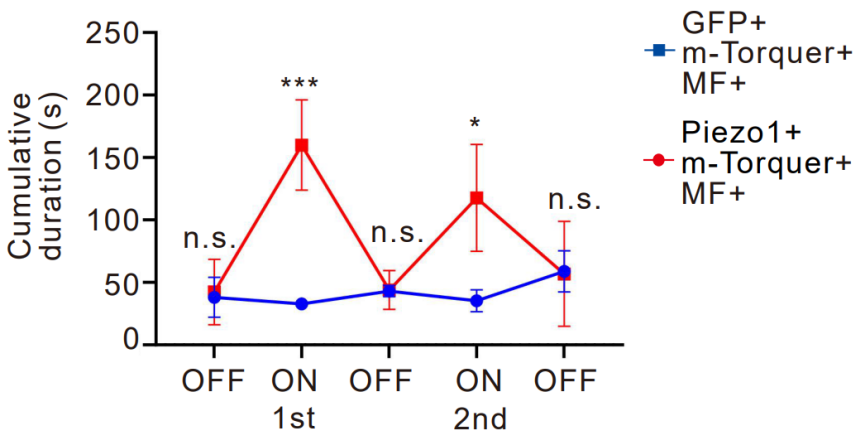
Supplementary Figure 4. Optical recording of neuronal responses upon MMG stimulation of varying frequencies. Representative calcium influx traces recorded from Piezo1-expressing neurons in response to a train of repetitive magnetic pulses of different frequencies (0.1, 0.2, and 1.0 Hz).



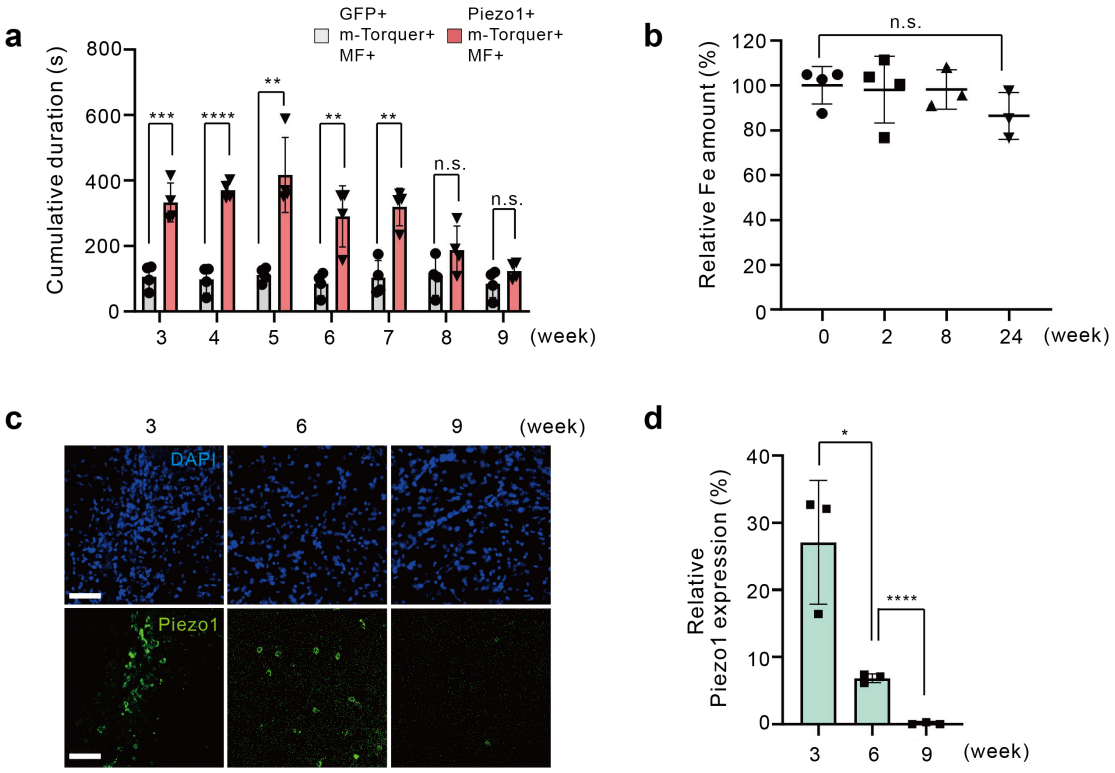
Supplementary Figure 5. Open field test to demonstrate the effect of MMG stimulation on animal locomotion. (a) Animals were placed in an open-field arena (70 cm circular arena) and their movements were recorded using a camera placed above. The change of **(b)** mean velocity traveled, and **(c)** total distance traveled, were negligible in LHA Vgat-MMG mice. (b, c) One-way ANOVA with multiple comparison test, $F(3, 14)=0.08787$ $p=0.9655$ for (b). One-way ANOVA with multiple comparison test, $F(3, 14)=0.2677$, $p=0.8476$ for (c). Data are mean ± s.d. from n=4 (Piezo1-m-Torquer-), 4 (Piezo1-m-Torquer+), 5 (Piezo1+m-Torquer-) and 5 (Piezo1+m-Torquer+) animals, respectively.



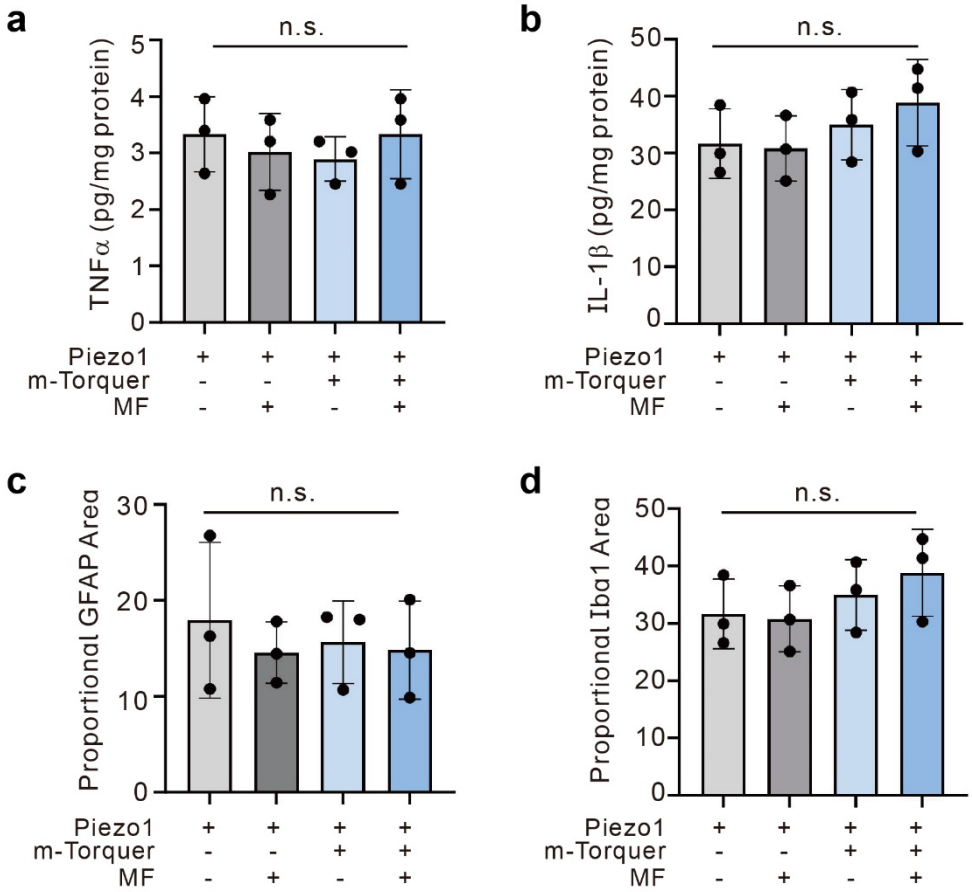
Supplementary Figure 6. *In vivo* latency time analysis for real-time feeding tests upon MMG application. Onset and offset latency times were measured as 16.6 s (n=5) and 5.65 s (n=4). Data were obtained for n=4 or 5 independent animals, mean ± s.d.



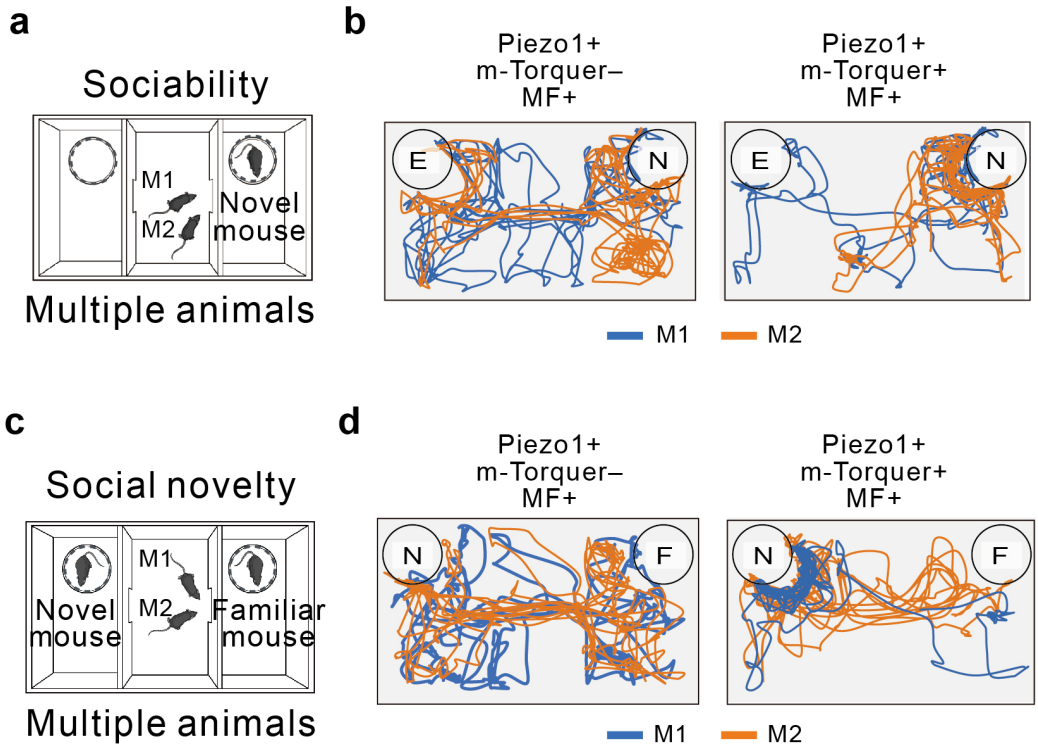
Supplementary Figure 7. Duration of feeding behavior of Vgat-Piezo1 or Vgat-GFP mice during 2x repeated MMG stimulations. Data are mean ± s.d.; **p*=0.0119; ****p*=0.0008; n.s., non-significant; two-tailed unpaired Student's *t* test; n=4 for Vgat-GFP with m-Torquer, n=3 for Vgat-Piezo1 with m-Torquer animals per group.



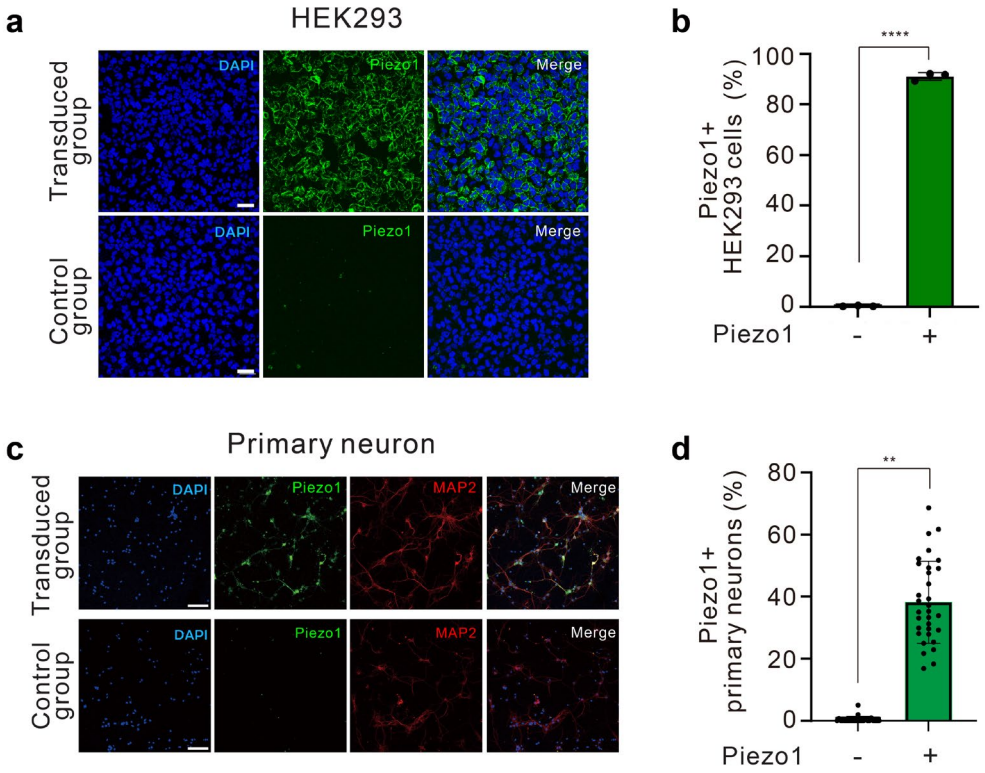
Supplementary Figure 8. Assessment of longevity of behavioral effects by MMG stimulation. **(a)** Cumulative duration of feeding behavior of control (GFP+m-Torquer+; grey) and MMG (Piezo1+m-Torquer+; red) in response to MMG stimulation (180°/s rotation speed, 10 minutes) over 9 weeks. Two-way ANOVA with multiple comparison test, $F(6, 42)=5.524$, $p=0.0003$. Data are mean ± s.d.; $^{**}p=0.002$ (5 week), $^{**}p=0.0063$ (6 week), $^{**}p=0.0015$ (7 week), $^{***}p=0.0006$; $^{****}p<0.001$; n.s., non-significant; two-tailed unpaired Student's *t* test; n=4 for Vgat-GFP with m-Torquer, n=4 for Vgat-Piezo1 with m-Torquer animals per group. **(b)** Analysis of the amount of Fe in the LHA over time after m-Torquer injection. One-way ANOVA with multiple comparison test, $F(3, 10)=0.9869$, $p=0.4377$. Data are mean ± s.d.; n.s., non-significant; two-tailed unpaired Student's *t* test; n=3 or 4 animals for each weeks per group. **(c)** Immunohistochemistry analysis of the LHA region showing Piezo1 expression at 3, 6, and 9 weeks after Piezo1 adenovirus transduction. Scale bar, 50 μm. **(d)** Quantification of Piezo1-expressing cells in the LHA over time. One-way ANOVA with multiple comparison test, $F(2, 6)=20.70$, $p=0.0020$. Data are mean ± s.d.; $^{*}p=0.0194$; $^{****}p<0.001$; two-tailed unpaired Student's *t* test; n=3 animals for each weeks per group.



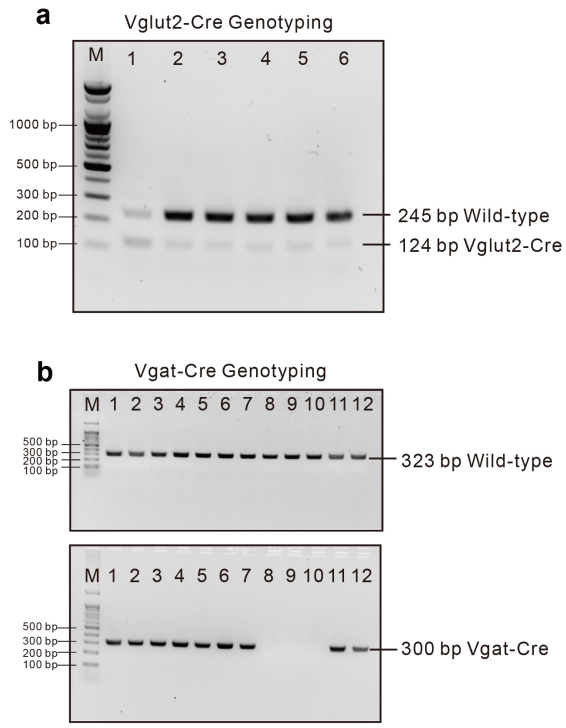
Supplementary Figure 9. Assessment of chronic immune response and biocompatibility of m-Torquer injection and MMG stimulation. **(a, b)** Quantification of the expression of proinflammatory cytokines, (a) TNF-α and (b) IL-1β, in brain homogenates. One-way ANOVA with multiple comparison test; $F(3, 8)=0.3593$, $p=0.7841$ for (a) One-way ANOVA with multiple comparison test; $F(3, 8)=0.9659$, $p=0.4547$ for (b). **(c, d)** Quantification of the (c) GFAP+ astrocytes, and (d) IbaI+ microglia at the lateral hypothalamus of Piezo1-expressing Cre mice in the absence and in the presence of m-Torquer with and without MMG stimulation. One-way ANOVA with multiple comparison test; $F(3, 8)=0.2348$, $p=0.8697$ for (c). One-way ANOVA with multiple comparison test; $F(3, 8)=0.3227$, $p=0.9916$ for (d). All data are presented as mean ± s.d. of n=3 animals; n.s., non-significant; two-tailed unpaired Student's *t* test.



Supplementary Figure 10. (a, c) Schematics of (a) three-chamber sociability and (c) social novelty tests with multiple mice. **(b, d)** Spatial map tracking the movement of two Vgat-Piezo1 mice without (left) and with (right) m-Torquer in (b) sociability and (d) social novelty assays.



Supplementary Figure 11. Transduction efficiency of Myc897-Piezo1 in HEK293 and neurons *in vitro*. (a) Immunofluorescence confocal images for Myc897-Piezo1 expression in HEK293 cells. Robust Piezo1 signals are detected only in the transduced group. Blue, DAPI (nucleus); Green, Myc-tag (Piezo1). Scale bars, 50 μm. **(b)** Quantification of Piezo1-expressing cells in control vs. transduced group. All data are presented as mean ± s.d. of n=3; ****p<0.0001; two-tailed unpaired Student's t test. **(c)** Immunofluorescence confocal images of primary neurons transduced with recombinant adenovirus (10^9 virus particles (VPs)·ml^{-1}) for expressing Myc897-Piezo1. Blue, DAPI (nucleus); green, Piezo1-IRES-GFP; red, MAP2 (neurons). Scale bars, 100 μm. **(d)** Quantification of Piezo1-expressing cells in control vs. transduced primary neurons. All data are presented as mean ± s.d. of n=32; **p=0.0078; two-tailed unpaired Student's t test.



Vglut2 Genotype	PCR Band size	Mouse number
WT/WT	245 bp	
WT/Cre	245 bp/124 bp	#1~6

Vgat Genotype	PCR Band size	Mouse number
WT/WT	323 bp	#8~9
WT/Cre	323 bp/300 bp	#1~7, #11,12

Supplementary Figure 12. PCR results from genomic DNA (gDNA) purified from tail biopsies. Gel electrophoresis results for genotyping of **(a)** Vglut2-Cre mice and **(b)** Vgat-Cre mice.PCR results from genomic DNA (gDNA) purified from tail biopsies.

Captions for Supplementary Video 1-7

Supplementary Video 1. Rotational magnetic force generators (MFGs) and their real-time magnetic field strength profiles with various size 16, 35, and 70 cm.

Supplementary Video 2. Wireless MMG stimulation of a Vgat-Cre mouse with Piezo1 and m-Torquer in an open chamber, free-access feeding task. Vgat-specific MMG stimulation of the lateral hypothalamus induced fast and robust feeding behaviors in this freely behaving mouse.

Supplementary Video 3. Reversible control of feeding behaviors in a Vgat-Cre mouse with Piezo1 and m-Torquer. Each movie clip shows the behaviors of the same mouse for 3 minutes during pre-stimulation (left), stimulation (middle), and post-stimulation (right). During the stimulation, the mouse exhibited markedly increased feeding behavior while the same animal did not during pre- and post-stimulation.

Supplementary Video 4. Reversible control of feeding behaviors in a Vglut2-Cre mouse with Piezo1 and m-Torquer. Each movie clip shows the behaviors of the same mouse for 3 minutes during pre-stimulation (left), stimulation (middle), and post-stimulation (right). During the stimulation, the mouse exhibited markedly reduced feeding behavior while the same animal tended to show more interest in food.

Supplementary Video 5. A Vgat-Cre mouse with Piezo1 expression and m-Torquer in the LH during the 3-chambered sociality test upon MMG stimulation. The mouse showed an increased preference for a Novel mouse (right chamber) than Empty chamber (left chamber) in response to magnetic fields.

Supplementary Video 6. A Vgat-Cre mouse with Piezo1 expression and m-Torquer in the LH during the 3-chambered social novelty test upon MMG stimulation. The mouse showed an increased preference for a Novel mouse (left chamber) than a Familiar mouse (right chamber) in response to magnetic fields.

Supplementary Video 7. Sociality test of two freely moving mice upon wireless MMG stimulation. This video highlights a pair of Vgat-Piezo1 mice with m-Torquer showed synchronized behaviors with increased interactions with a Novel mouse than the empty chamber, while a pair of control mice without m-Torquer showed random activity without such preference for social interaction.

Supplementary Video 8. Social novelty test of two freely moving mice upon wireless MMG stimulation. This video highlights that a pair of Vgat-Piezo1 mice showed increased interactions with a Novel mouse than a Familiar mouse in a synchronized fashion upon MMG stimulation, while a pair of control mice without m-Torquer showed random activity without such preference for social novelty.

Supplementary Video 9. Parental behavior test of two freely moving mice upon wireless MMG stimulation in a semi-natural setup. This video highlights increased parental behavior, such as pup retrieval, of Vgat-Piezo1 mice in response to MMG, whereas the control mice (GFP+ m-Torquer+) did not show any noticeable increase in such behavior.

Supplementary Note 1.

To determine which ion channels are involved in MMG neuromodulation, we used chemical blockers to inhibit mechanosensitive ion channels and conducted *in situ* optical recording of calcium response to MMG stimulation in primary neurons. Upon treating GsMTx4 to inhibit Piezo1 channel [5], Piezo1 transduced (Piezo1+) neurons showed no calcium response to magnetic stimulation, whereas Piezo1+ neurons without GsMTx4 showed strong calcium influxes (**Supplementary Fig. 3a**). For neurons not transduced with Piezo1, magnetic stimulation did not elicit any calcium influx as shown in Fig. 1d, suggesting that the mechanical ion gating of transduced Piezo1 is necessary for MMG. We also tested suramin and ruthenium red (RR), which block GPCR signaling and TRPV family channels, respectively, to assess the effects of other ion channels [6,7]. Treatment of these inhibitors did not have any noticeable effect on neuronal responses to magnetic stimulation, suggesting that other mechanosensitive channels are not involved in the MMG-mediated neuronal activation (**Supplementary Fig. 3b, c**). Together, these results confirm the calcium influx in response to magnetic stimulation is induced by mechanical gating of transduced Piezo1 channels, not by unintended side effects on the cells.

Supplementary Note 2.

Additionally, similar to optogenetics, the neuromodulation with MMG can be lasted for weeks (Supplementary Fig. 8). When the real-time feeding assay was repeated weekly for 9 weeks, Vgat-Piezo1 mice with m-Torquer demonstrated an increased feeding in response to magnetic stimulation, as evidenced by a significantly increased time spent near the food zone, up to 7 weeks after Piezo1 transduction (**Supplementary Fig. 8a**). Since m-Torquer materials remain stable in the injected region without chemical decomposition, as evidenced by unaltered Fe amount in the LHA for over 6 months, the sustained behavioral responses of MMG stimulation persist as long as Piezo1 expression endures in the LHA (**Supplementary Fig. 8b-d**).

Supplementary Methods

Synthesis of m-Torquer conjugated with Myc antibodies

For Myc antibody surface functionalization, 100 μg protein A (Sigma Aldrich) was first conjugated onto 1 mg carboxylated m-Torquer via EDC/NHS coupling. After purification by centrifugation, 40 μg Myc antibody (Sigma Aldrich) was added to protein A conjugated m-Torquer. Myc antibody conjugated m-Torquer was purified by centrifugation and redispersed in 10 mM phosphate buffer (pH 7.2) at a final concentration of 50 $mg \cdot ml^{-1}$.

Magnetic setup for *in vivo* MMG stimulation

For *in vivo* MMG stimulation of freely moving mice in a minimally restrained setup, the 70 cm (diameter) in-house-built magnetic arena was used. To build the 70 cm magnetic setup, ten 1T NdFeB magnets (12.5 cm × 15 cm × 12.5 cm) were assembled in a stainless stress cage. The 70 cm magnetic setup was mounted on an in-house-built motorized rotation stage and controlled by Arduino. A specialized arena designed for various animal behavioral assays, including feeding, social interactions, and parental behaviors, was assembled and placed into the center of the magnetic arena. The magnetic field strength of the various configurations of magnetic setup was simulated by finite element analysis using COMSOL Multiphysics.

Plasmid and viral vector construction

For *in vivo* and *in vitro* magneto-mechanical-genetics (MMG) experiments, Ad-hSyn-FLEX-Myc897-Piezo1. Mouse Piezo1 with myc tag inserted at residue 897 (Myc897-Piezo1) was cloned in an antisense direction to create Ad-pEnt-hSyn-FLEX-Myc897-Piezo1. Myc897-Piezo1 was flanked by a pair of canonical loxP sites and a pair of adenoviral constructs were then sent to KOMABIOTECH Inc., for adenovirus packaging with serotype 5 (1×10^{12} viral $particles \cdot mL^{-1}$). Lentiviral particles were produced in-home using HEK293T cells and 3rd generation lentiviral packaging system. For transduction, in LHA with Ad-hSyn-FLEX-Myc897-Piezo1 virus, it has been reported that the synapsin (Syn) promotor is neuron-specific with little glial expression.

Pharmacological inhibition of Piezo1 and other mechanosensitive ion channels

Primary neurons were co-transduced with Ad-Piezo1 virus and AAV-GCaMP6f, followed by labbeing with m-Torquer as described above. Subsequently, the neurons were treated with chemical blockers to inhibit various mechanosensitive ion channels. To inhibit Piezo1, the neurons were treated with GsMTx4 (10 μM) for 1hr. To examine other endogenous mechanosensitive ion channels, the neurons were treated with either suramin (60 μM) or

ruthenium red (500 nM) to inhibit GPCRs and TRPV channels, respectively. Calcium response to MMG stimulation was then assessed via *in situ* optical imaging using EMCCD camera.

Genotyping

DNA isolated from the tail biopsies was used for mouse genotyping of the Vgat::IRES-Cre or Vglut2::IRES-Cre gene (**Supplementary Fig. 12**). The DNA extraction process from tissue followed the MyTaq Extract-PCR Kit (Meridian Bioscience). The presence of the Vgat::IRES-Cre allele was verified by PCR amplification using the WT-Vgat and KI-Vgat primer sets. PCR conditions were as follows: denaturation steps at 95 °C for 3 min, followed by 35 cycles at 95 °C for 15 s, 60.5 °C for 15 s, and an elongation step at 72 °C for 20 s. The genotyping primers used for the RT-PCR assays are as follows: WT-Vgat primers, Forward 5'-CTTCGTCATCGGCGGCATCTG-3'; Reverse 5'-CAGGGCGATGTGGAATAGAAA-3'. KI-Vgat primers, Forward 5'- CCTTGAGGCTGTCCAAGTGATTCAGGCCATCG -3'; Reverse 5'- GAGTCATCCTTAGCGCCGTA -3'. The presence of the Vglut2::IRES-Cre allele was verified by PCR amplification using the WT-Vglut2 and KI-Vglut2 primer sets. Conditions of PCR were as follows: denaturation step at 95 °C for 3 min, followed by 35 cycles at 95 °C for 15 s, 60 °C for 15 s, and an elongation step at 72 °C for 20 s. The genotyping primers used for the RT-PCR assays are as follows: WT-Vglut2 primers, Forward 5'-AAGAAGGTGCGCAAGACG-3'; Reverse 5'- CTG CCACAG ATTGCA CTTGA-3'; KI-Vglut2 primers, Forward 5'- AAGAAGGTGCGCAAGACG-3', Reverse 5'- ACACCG GCCTTATTCCAAG-3'.

***In vivo* open field test (OFT)**

Animals were placed in a custom circular, single-chamber (50 cm × 30 cm) arena. Mice were allowed to freely explore for 1 min and then received 10 min of continuous magnetic stimulation during which they were allowed to freely move and explore around the arena. Their spatial locations, total distance, and velocity were recorded via a CCD camera at 15 fps interfaced with EthoVision XT 10 (Noldus, Leesburg, VA) for individual mice in a semi-dark condition. All behavioral tests were performed under low light conditions, and animals were allowed to acclimate to the behavior room for a least 1 hr before the beginning of behavioral testing. Mice were habituated at least 3 days before with magnetic field sounds without stimulation for 30 min. After each experiment, the open field arena was cleaned with 70% ethanol and then with dry wipe.

ELISA

Mice were perfused with phosphate-buffered saline (PBS, pH 7.4) prior to brain collection. The collected brain samples were sectioned into 400 μm sections of LHA using a vibratome (Leica

Microsystems GmbH, Germany). Two 400 μm sections were used for ELISA protein analysis. The brains were homogenized in 2x cell lysis buffer with cold PBS. The samples were centrifuged at 12,000 × g for 15 min. The supernatant was collected for a protein assay. Serum and brain levels of IL-1β (R&D Systems, Minneapolis, MN) and TNF-α (R&D Systems, Minneapolis, MN) were measured using ELISA kits, following the manufacturer's instructions. Ready-to-used kits with 96-well antibody pre-coated plates were employed for both IL-1β and TNF-α. A linear regression was used to fit the optical densities for the standard curve against their concentration, with the standard curve range for IL-1β being 12.5 to 800 $pg \cdot ml^{-1}$, and for corticosterone, 10.9 to 7000 $pg \cdot ml^{-1}$. Concentrations were calculated from the optical density at 450 nm of each sample. Appropriate sample dilutions were carried out to maintain detection in the linear part of the standard curve. Data acquired from the performed ELISAs are presented as absolute values. Differences between groups were identified by Student's *t* test or ANOVA.

In vitro 3D human brain phantom experiment

Tissue culture*:* Human embryonic kidneys cell line, HEK293, passage number 3-5, were purchased from ATCC (ATCC® CRL-1573) and grown in Dulbecco's modified Eagle's medium with 4.5 g·l-1 glucose (DMEM; Gibco) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco) and 1% penicillin-streptomycin (Gibco). The cells were maintained at 37°C in a humidified incubator with 5% CO_2 and passaged every 2–3 days, depending on confluency, using 0.05% Trypsin-EDTA (Yonsei University cell culture facility). The cells were authenticated based on their STR/DNA profiling, morphology, growth condition, and immunostaining with specific markers by ATCC.

Sample preparation: For MMG stimulation in a 3D human brain-like phantom, HEK293 cells were co-transfected with pcDNA3.1-pCMV-FLEX-Myc897-Piezo1 and pCMV-Cre (Addgene #123133) plasmids using 10 μl lipofectamine 3000 with 5000 ng of total DNA in Opti-MEM medium (Gibco). After 2 days, the cells were treated with 8 μl of m-Torquer (50 $mg \cdot ml^{-1}$) diluted to 1 ml in culture medium for m-Torquer labeling. After a 3 hr incubation at 37 ℃, cells were washed with complete medium to remove unbound m-Torquers. A total of 5×10^5 cells were embedded in the Matrigel matrix (size = 1.8 cm × 1.8 cm × 1.8 cm, volume = 120 μl). MMG stimulation was provided for 30 min with 180°/s frequency using the MMG-70 apparatus. The samples were analyzed using RT-PCR for *c-Fos*, *Piezo1*, *Cr*e, and *β-actin* mRNA expression.

RT-PCR*:* Total RNA was extracted from cells using the RNAeasy Mini Kit (QIAGEN, Hilden, Germany). cDNAs were synthesized using 3 μg of total RNA, oligo dT (10 pmol), and SuperScript reverse transcriptase III (200 $units \cdot \mu l^{-1}$) in a total reaction volume of 20 μl. The primer sequences used for *c-Fos, Piezo1, Cre, and β-actin.* The primers used for the RT-PCR assays are as follows: *c-Fos* primers, Forward 5'-CAAGCGGAGACAGACCAACT-3'; Reverse 5'-AGTCAG ATCAAGGGAAGCCA-3'; *Piezo1* primers, Forward 5'-TTCTTCGGGTTGGAGAGGTA-3', Reverse 5'-TGTCACCATGT GGTTAAGGATG-3; *Cre*

primers, Forward 5'-GCCTGCATTACCGGTCGA TGCAAC-3', Reverse 5'-CGTATATCCTGGCAGCGATCGC-3; *β-actin* primers, Forward 5'-AGAGCTACGAGCTGCCTGAC -3', Reverse 5'-AGCACTGTGTTGGCGTAC AG-3. Target gene expression were analyzed by Image Lab software (Bio-Rad chemiDoc MP imaging system 6.1.0).

IVIS optical imaging*:* HEK293 cells were co-transfected with pCMV-Luc and pcDNA3.1-pCMV-Myc897-Piezo1 expression plasmids using 10 µl Lipofectamine 3000 with 5000 ng of total DNA in Opti-MEM medium (Gibco). After 2 days of recovery and Piezo1 expression, the cells were treated with 8 µl of m-Torquer (50 $mg \cdot ml^{-1}$) diluted to 1 ml with culture medium for m-Torquer labeling. After a 3 hr incubation at 37 °C, cells were washed with complete medium to remove unbound m-Torquer. A total of 5×10^5 cells were transferred to and embedded in the Matrigel matrix (size = 1.8 cm × 1.8 cm × 1.8 cm, volume = 120 µl). MMG stimulation was provided for 30 min with 180°/s using the MMG-70 apparatus. Cells embedded in the Matrigel were treated with 150 $\mu g \cdot ml^{-1}$ D-luciferin diluted in media for 30 min. Chemi-luminescence signals from luciferase expression were captured using the IVIS Lumina III In Vivo Imaging System (PerkinElmer). Total radiant efficiency of the samples was measured using Living Image software 4.7.4 (PerkinElmer).

Luciferase reporter assay: For luciferase assay, Ca^{2+} dependent luciferase reporter-expressing HEK293 cell line was generated using lentiviral transduction. Lentivirus for CSN-mCMV-eGFP-2A-FLuc were applied to 5×10^4 of HEK293 cells cultured in a 6-well plate. Luciferase reporter-expressing HEK293 cells were co-transfected with pcDNA3.1-pCMV-FLEX-Myc897-Piezo1 (2000 ng) and pCMV-Cre (Addgene #123133, 4000 ng) expression plasmids using 10 µl Lipofectamine 3000 in Opti-MEM medium (Gibco). After 2 days of recovery and Piezo1 expression, cells were treated with 8 µl of m-Torquer (50 $mg \cdot ml^{-1}$) diluted to 1 ml with culture medium for m-Torquer labeling. *In vitro* MMG stimulation was performed using a 35-cm MMG apparatus with 180°/s rotating magnetic fields for 24 hr. Then, cells were washed with complete medium to remove unbound m-Torquer and analyzed for luciferase activity using an EnSight Multimode Microplate Reader (PerkinElmer). All reactions were performed in triplicate. Reporter activity was normalized to the total protein amount to determine transfection efficiency.

Statistics and Reproducibility

The number of independent experiments for representative results in all figures: The number of independent experiments repeated for each representative result shown in Figures, Extended Data figures and Supplementary figures is provided here: for Fig. 1h, g, n = 4 independent experiments; Fig. 1h, n = 3 independent experiments; for Fig. 1b, g, n = 4 independent experiments; for Fig. 5b, n = 3 independent experiments; for Fig. 5f-h, n = 6 independent animals, (GFP group), n=4-5 independent animals (Piezo1 group); Extended Data Fig. 1e, n = 10 independent experiments; Extended Data Fig. 3a, n = 5 biologically independent

samples over two independent experiments; Extended Data Fig. 4a, $n = 10$ independent experiments; Extended Data Fig. 4b, $n \geq 20$ independent experiments; Extended Data Fig. 5c, d, $n = 3$ independent experiments; Extended Data Fig. 7a-c, $n = 3$ independent experiments; Supplementary Fig. 12a, b, $n \geq 30$ biologically independent animals, All mice were tested independently before being used in experiments.

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Source Data_Supplementary Information Fig 12_RT-PCR gel

Related to Supplementary Information Fig 12