PNAS
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Supplementary Information for

Essential Nucleotide- and Protein-Dependent Functions of *Actbl*β-Actin

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www.pnas.org/cgi/doi/10.1073/pnas.1807895115

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Supplemental Materials and Methods

TALEN construction and purification: TALEN constructs were generated using the Golden Gate TALEN assembly method (1) into RCIscript-GoldyTALEN backbone (Addgene #38142, Carlson *et al.*, 2012). TALEN DNA constructs were transformed into DH5α cells, miniprepped with Wizard ® *Plus* SV Minipreps DNA Purification System (Promega, Cat. #A1460), linearized with the BamHI restriction enzyme (New England Biolabs Inc. Cat. #R3136S), and then phenol/chloroform purified. TALEN RNAs were *in-vitro* transcribed from purified linearized DNA using the mMESSAGE mMACHINE® T3 Transcription Kit. (Thermo Fisher Scientific, Cat. #AM1348) and then purified using the MEGAclear™ Kit. (Thermo Fisher Scientific, Cat. #AM1908M) following manufacture protocols. Purified RNAs were stored at -80°C until use.

TALEN activity validation. TALEN RNA pairs (15µg each arm) were nucleofected (Amaxa® Cell Line Nucleofector® Kit R, Amaxa® Nucleofector® II) into $1x10^6$ NIH3T3 fibroblasts. 3-days post nucleofection, gDNA was harvested from the cells with the PureLink® Genomic DNA Mini Kit, Invitrogen according to manufacture protocols. TALEN activity was assayed via the Surveyor Mutation Detection Kit (Transgenomic, Cat. #706020.) (Figure S5). Primers: *Actb* Exon2 F 5'-GGTAATAATGCGGCCGGTCT-3', *Actb* Exon2 R 5'-TACCCGGGATACTGACCTGG-3'. Additionally, *in-silico* off-target analysis using https://tale-nt.cac.cornell.edu with the most stringent cutoff showed that the most similar potential off-target sequences had a minimum of 7 nucleotides that differed between the on- and off-targets.

Generating genome engineered mice and validation:
TALEN RNA pairs (25ng/µL) and the corresponding Exon 2 PAGE Ultramer ssOligo donor (15ng/µL) template (Integrated DNA Technologies) were sent to the Murine Genetics Core at The Scripps Research Institute for pronuclear microinjection into fertilized C57BL/6 blastocysts, which were then implanted into pseudo-pregnant female mice. The donor template contained 8 nucleotide alterations to exchange the translation of β-actin D2-4, V10 to the γ-actin E2-4, I10, to disrupt TALEN re-binding to the edited locus, and to insert a BamHI site for restriction fragment length polymorphism (RFLP) analysis. Genomic DNA from all F0 mouse pup tail samples was purified (PureLink® Genomic DNA Mini Kit) and assessed by RFLP assay with the BamHI restriction enzyme (New England Biolabs Inc. Cat. #R3136S) and Sanger sequenced for donor template integration. Primers used for RFLP: *Actb* Exon2 F 5'-GGTAATAATGCGGCCGGTCT-3', *Actb* Exon2 R 5'-TACCCGGGATACTGACCTGG-3'.

TALEN sequences:

Target Site	RVD Sequence (N-terminus to C-terminus)
Actb Exon 2, left arm	**RVD:** HD NN HD HD NI NG NN NN NI NG NN NI HD NN NI NG HD NN HD HD NI NG NN NN NI **DNA sequence (5' to 3'):** CTGACCCCGGACCAAGTGGTGGCTATCGCCAGCAACGGTGGCG GCAAGCAAGCGCTCGAAACGGTGCAGCGGCTGTTGCCGGTGCT

	GTGCCAGGACCATGGCCTGACTCCGGACCAAGTGGTGGCTATCG CCAGCCACGATGGCGGCAAGCAAGCGCTCGAAACGGTGCAGCG GCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGAC CAAGTGGTGGCTATCGCCAGCAACAATGGCGGCAAGCAAGCGCT CGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCAT GGCCTGACTCCGGACCAAGTGGTGGCTATCGCCAGCCACGATG GCGGCAAGCAAGCGCTCGAAACGGTGCAGCGGCTGTTGCCGGT GCTGTGCCAGGACCATGGCCTGACTCCGGACCAAGTGGTGGCTA TCGCCAGCCACGATGGCGGCAAGCAAGCGCTCGAAACGGTGCA GCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCG GACCAAGTGGTGGCTATCGCCAGCAACATTGGCGGCAAGCAAGC GCTCGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGAC CATGGCCTGACCCCGGACCAAGTGGTGGCTATCGCCAGCAACG GTGGCGGCAAGCAAGCGCTCGAAACGGTGCAGCGGCTGTTGCC GGTGCTGTGCCAGGACCATGGCCTGACCCCGGACCAAGTGGTG GCTATCGCCAGCAACAATGGCGGCAAGCAAGCGCTCGAAACGGT GCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACC CCGGACCAAGTGGTGGCTATCGCCAGCAACAATGGCGGCAAGCA AGCGCTCGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAG GACCATGGCCTGACCCCGGACCAAGTGGTGGCTATCGCCAGCAA CATTGGCGGCAAGCAAGCGCTCGAAACGGTGCAGCGGCTGTTG CCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGACCAAGTGG TGGCTATCGCCAGCAACGGTGGCGGCAAGCAAGCGCTCGAAAC GGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTG ACCCCGGACCAAGTGGTGGCTATCGCCAGCAACAATGGCGGCAA GCAAGCGCTCGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGC CAGGACCATGGCCTGACCCCGGACCAAGTGGTGGCTATCGCCA GCAACATTGGCGGCAAGCAAGCGCTCGAAACGGTGCAGCGGCT GTTGCCGGTGCTGTGCCAGGACCATGGCCTGACTCCGGACCAAG TGGTGGCTATCGCCAGCCACGATGGCGGCAAGCAAGCGCTCGA AACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGC
Actb Exon 2, right arm	**RVD:** NN HD HD NN NN NI NN HD HD NN NG NG NN NG HD NN NI HD NN NI NN HD HD NN NN NI NN HD HD NN **DNA sequence (5’ to 3’):** CTGACCCCGGACCAAGTGGTGGCTATCGCCAGCAACAATGGCGG CAAGCAAGCGCTCGAAACGGTGCAGCGGCTGTTGCCGGTGCTGT GCCAGGACCATGGCCTGACTCCGGACCAAGTGGTGGCTATCGCC AGCCACGATGGCGGCAAGCAAGCGCTCGAAACGGTGCAGCGGC TGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACTCCGGACCAA GTGGTGGCTATCGCCAGCCACGATGGCGGCAAGCAAGCGCTCG AAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGG CCTGACCCCGGACCAAGTGGTGGCTATCGCCAGCAACAATGGCG GCAAGCAAGCGCTCGAAACGGTGCAGCGGCTGTTGCCGGTGCT GTGCCAGGACCATGGCCTGACCCCGGACCAAGTGGTGGCTATC GCCAGCAACAATGGCGGCAAGCAAGCGCTCGAAACGGTGCAGC

	GGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGA CCAAGTGGTGGCTATCGCCAGCAACATTGGCGGCAAGCAAGCGC TCGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCAT GGCCTGACCCCGGACCAAGTGGTGGCTATCGCCAGCAACAATGG CGGCAAGCAAGCGCTCGAAACGGTGCAGCGGCTGTTGCCGGTG CTGTGCCAGGACCATGGCCTGACTCCGGACCAAGTGGTGGCTAT CGCCAGCCACGATGGCGGCAAGCAAGCGCTCGAAACGGTGCAG CGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACTCCGG ACCAAGTGGTGGCTATCGCCAGCCACGATGGCGGCAAGCAAGC GCTCGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGAC CATGGCCTGACCCCGGACCAAGTGGTGGCTATCGCCAGCAACAA TGGCGGCAAGCAAGCGCTCGAAACGGTGCAGCGGCTGTTGCCG GTGCTGTGCCAGGACCATGGCCTGACCCCGGACCAAGTGGTGG CTATCGCCAGCAACGGTGGCGGCAAGCAAGCGCTCGAAACGGT GCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACC CCGGACCAAGTGGTGGCTATCGCCAGCAACGGTGGCGGCAAGC AAGCGCTCGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCA GGACCATGGCCTGACCCCGGACCAAGTGGTGGCTATCGCCAGC AACAATGGCGGCAAGCAAGCGCTCGAAACGGTGCAGCGGCTGTT GCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGACCAAGTG GTGGCTATCGCCAGCAACGGTGGCGGCAAGCAAGCGCTCGAAA CGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCT GACTCCGGACCAAGTGGTGGCTATCGCCAGCCACGATGGCGGC AAGCAAGCGCTCGAAACGGTGCAGCGGCTGTTGCCGGTGCTGT GCCAGGACCATGGCCTGACCCCGGACCAAGTGGTGGCTATCGC CAGCAACAATGGCGGCAAGCAAGCGCTCGAAACGGTGCAGCGG CTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGACCA AGTGGTGGCTATCGCCAGCAACATTGGCGGCAAGCAAGCGCTCG AAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGG CCTGACTCCGGACCAAGTGGTGGCTATCGCCAGCCACGATGGCG GCAAGCAAGCGCTCGAAACGGTGCAGCGGCTGTTGCCGGTGCT GTGCCAGGACCATGGCCTGACCCCGGACCAAGTGGTGGCTATC GCCAGCAACAATGGCGGCAAGCAAGCGCTCGAAACGGTGCAGC GGCTGTTGCCGGTGCTGTGCCAGGACCATGGC-3’

ssOligo Donor Sequences:

Target Site	**Sequence (5’→3’)**
Actb Exon 2	AGTGGGCAGGGCGGCAGCGGCTGCTCTTGGCGGCCCCGAGGTGACT ATAGCCTTCTTTTGTGTCTTGATAGTTCGCAATGGAAGAAGAAATCGCT GCGCGGTCATAGACAACGGATCCGGAATGTGCAAAGCCGGCTTCGCG GGCGACGATGCTCCCCGGGCTGTATTCCCCTCCATCGTGGGCCGCCC TAGGCACCAG

Cell Culture: Primary MEFs were cultured from E13.5 WT, *Actb*$^{c\text{-}g}$ heterozygous and homozygous mouse embryos as described previously (3). Cells were grown to 80% confluency on 10-cm plates and frozen down at passage 1 at 1x10^6 cells/mL in MEF

freezing media (95% FBS and 5% dimethyl sulfoxide). Primary MEFs from individual embryos were thawed and cultured in MEF media (DMEM supplemented with 10% FBS, 1% Pen/Strep, 0.5ug/mL Fungizone), grown to 80% confluency. NIH3T3 fibroblasts were cultured in 3T3 media (DMEM supplemented with 10% BS, 1% Pen/Strep, 0.5ug/mL Fungizone).

Open Field Activity Assay:
Activity was measured using an AccuScan system by Columbus Instruments, Inc. Total horizontal distance (meters) was determined by measuring infrared beam breaks. Mice were placed in the open-field apparatus and horizontal distance was measured for 15 minutes.

Muscle histology and physiology
Quadriceps muscles from each mouse line were cryopreserved in melting isopentane for 30 seconds and 10 µm transverse cryosections were obtained (Leica CM3050 S). For histology, sections were stained with hematoxylin and eosin and imaged on a Leica DM5500 B microscope equipped with a Leica HC PLAN APO 20x objective. Centrally nucleated fibers (CNFs) were counted using the Cell Counter plugin on ImageJ software (NIH) and expressed as a percentage of the total number of myofibers (%CNFs). For *ex-vivo* force analysis, mice were anesthetized with sodium pentobarbital and extensor digitorum longus (EDL) muscles dissected. Silk suture was used to attach the distal tendon to a static structure and the proximal tendon to a force transducer (Model 300B-LR, Aurora Scientific). The EDL was incubated in Krebs-Ringer bicarbonate buffer [120.5 mM NaCl, 4.8 mM KCl, 1.2 mM $MgSO_4$ 1.2 mM Na_2HPO_4, 20.4 mM $NaHCO_3$, 10 mM glucose, 10 mM pyruvate, 1.5 mM $CaCl_2$], oxygenated with 95% O_2/5% CO_2. Muscles were set to their anatomic length (L_o) which was measured from myotendonous junction to myotendonous junction using digital calipers. Muscles remained quiescent for 5 min before passive stiffness was determined by passively stretching the muscles sinusoidally from 97.5% L_o to 102.5% L_o at 0.5 Hz while measuring the resulting force. Isometric tetanic contractions separated by 2 min followed 30s later until a plateau was attained (within 5 mN) and –dP/dt and +dP/dt were measured. EDL muscles were stimulated for 200 ms at 175 Hz. Peak twitch force was then measured 2 min later using a 0.5 ms pulse at 150 V (Grass S48 stimulator delivered through a SIU5D stimulus isolation unit; Grass Telefactor, Warwick, RI) before active stiffness was calculated using a sinusoidal length oscillation of 0.01% at 500 Hz during a tetanic isometric contraction(4, 5). Two minutes later, the muscle completed 10 eccentric contractions in which muscles were passively shortened to 90 % L_o and then stimulated for 200ms while being simultaneously lengthened to 110 % L_o at 1.0 L_o/s. Each eccentric contraction was separated by 3 min of rest to prevent fatigue. Immediately following the 10^{th} contraction, muscles were readjusted to L_o before a post P_o was measured and compared to pre P_o as a percentage.

Primary MEFs fixation, staining and immunofluorescent imaging
MEFs were plated on 5 µg/ml fibronectin coated coverslips at a density of 1×10^4 cells/coverslip and incubated in MEF culturing media. The next day, cells were fixed with fresh 4% paraformaldehyde in PBS for 15 min at room temperature, washed in

phosphate-buffered saline (1xPBS) 3x5min, and blocked in 5% Bovine serum albumin (BSA) for 30min. Coverslips were incubated with 1° antibodies for β-actin (AC15; Sigma-Aldrich) and γ-actin (affinity purified γ-cyto actin rabbit 7577) in 5% BSA at 4°C overnight, washed in 1xPBS 3x5min and incubated with Alexa-488- or 568-conjugated 2° antibodies (Life Technologies). Coverslips were mounted in Prolong (P36931; Life Technologies) mounting medium prior to imaging. All images were obtained were acquired using a 20x/NA0.75 objective on a Delta Vision personalDV microscope using softWoRx 3.7.1 software (GE Technologies).

Cochlea Immunofluorescent microscopy

Dissected cochlea were fixed in 4% paraformaldehyde (PFA) in PBS for 4 hours at room temperature before decalcification in 170 mM EDTA in PBS for 16 hrs at 4°C. Organ of Corti was dissected, incubated in methanol for 10 mins at -20°C, permeabilized with 0.2% triton X-100 for 10 minutes at room temperature and blocked with 5% goat serum in PBS before incubation with Alexa-546 conjugated monoclonal anti γ-actin antibody clone 1-37 and FITC conjugated anti-β-actin antibody clone AC-15 (AbCam, 1:400) overnight at 4°C. Samples were mounted either in Prolong Gold or Prolong Diamond anti-fade reagent (P36930 or P36965, Life Technologies) imaged with a 63X NA 1.4 objective on a Leica SP8 confocal microscope operating in resonant mode. Images were deconvolved using Huygens X11 Essentials software. Fluorescence intensities of actin immunostaining along the stereocilia length were measured using the line function in Leica LasX software.

Scanning electron microscopy

Dissected cochlea were fixed in 2.5% glutaraldehyde, 0.1 M sodium cacodylate, 2 mM $CaCl_2$ overnight at 4°C and then decalcified in 170 mM EDTA in PBS for 16 hours at 4°C. Dissected organ of Corti was incubated in 2% each of arginine, glutamine, glycine and sucrose in water overnight at room temperature, followed by incubation in 2% tannic acid and guanidine hydrochloride for 2 hours at room temperature and 1% $Os0_4$ in water for 1 hour at room temperature, with extensive water washes between steps. The samples were transitioned to 100% ethanol, critical point dried from CO_2 and sputter coating with gold. Samples were imaged using a JEOL JSM-7800F field emission scanning electron microscope. The stereocilia length and number measurements were analyzed using Fiji software (National Institutes of health, Bethesda, MD).

Auditory Brainstem Response (ABR)

ABR waveforms were collected for mice using a Tucker Davis Technologies System 3 at frequencies of 4 kHz, 11 kHz, 16 kHz, 22kHz and 32 kHz as described previously (6). Mice were anesthetized with Avertin following which scalp potentials were recorded using subdermal electrodes. Waveforms for each frequency were collected starting at 90 dB, decreasing in 5 dB to a sub-threshold level. The collected waveforms were stacked and the lowest level of stimulation that resulted in a definite waveform was considered as the threshold.

Live cell imaging

$10x10^4$ MEFs were plated on a Nunc™ Glass Base Dish (Thermo Scientific) and cultured overnight in regular MEF media. Second day, the dish was sealed with vacuum grease and a glass coverslip. MEF culture media containing 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) was used to stabilize the pH, and the cells were maintained at 37°C by an environmental chamber enclosing the microscope. For random migration assay, images were captured on a Delta Vision personalDV (GE Technologies) with a 10x/NA0.25 objective with phase contrast illumination. Images were captured at 10-minute intervals for 4 hours, and cells were tracked using the Manual Tracking plugin for ImageJ software (National Institutes of health, Bethesda, MD). Cells that divided or contacted other cells during the experiment were excluded for data analysis. Velocity was calculated as the total track distance divided by the total time (240 min), and directionality (D/T) was calculated as the linear distance (D) divided by the total track distance (T).

qRT-PCR

Generation of WT mouse isoactin control constructs were previously described in Patrinostro *et al.*, 2017. *Actb*$^{c\text{-}g}$ control construct was generated by site-directed mutagenesis using the QuikChange II XL kit (Agilent Technologies, Cat# 200521) according to manufacture protocols from the WT pENTR/D-TOPO-*Actb* vector generated in Patrinostro *et al.*, 2017. Each actin isoform qRT-PCR primer set was tested for amplification of all control constructs to assess primer specificity (7) and Figure S6. Total RNA was extracted from WT, *Actb*$^{c\text{-}g}$ heterozygous and homozygous MEF samples using the Bio-Rad-Aurum Total RNA Mini Kit following the manufacturer's instructions. RNA concentration and purity (260/280 ratio) were determined using a NanoDrop spectrophotometer (Wilmington, DE). First-strand cDNA was synthesized with a Bio-Rad iScript Advanced cDNA Synthesis Kit for qRT-PCR using the same initial RNA amount for all samples. Individual control constructs were used in a 10-fold dilution to generate a standard curve, and MEF samples were amplified in parallel with each specific qRT-PCR primer set using Bio-Rad SsoAdvanced Universal SYBR polymerase on the Bio-Rad CFX96 Real Time System C1000 Touch Thermal Cycler to profile each actin isoform transcript amount (picomoles).

G- to F- Actin ratio

WT and *Actb*$^{c\text{-}g}$ homozygous primary MEFs were counted and equal number of cells from each genotype were pelleted prior to the experiment. G-to F-actin ratio as assed by the commercially available G-Actin/F-Actin In Vivo Assay Biochem Kit (Cytoskeleton, Cat# BK037) according to manufacture protocols and then Westered blotted. Please see Western blotting methods for list of antibodies. LiCor fluorescence intensity for each Western blot fraction band is used to determine the ratio of G-to F-actin in each embryo.

Western blotting

MEF and tissue protein was extracted with 1% SDS buffer in 1x PBS and a cocktail of protease inhibitors (100 µM aprotinin, 0.79 mg/ml benzamide, 10 nM E-64, 10 µM leupeptin, 0.1 mg/ml pepstatin, 1 mM phenylmethylsulfonyl fluoride), sonicated (Model 150V/T Ultrasonic homogenizer; BioLogics), boiled, and centrifuged to remove the

insoluble fraction. Equal amounts of cleared total lysate protein (25 μg) were blotted with antibodies β-actin (AC-15; Sigma-Aldrich), γ-actin (mAb 1-17), α_{sm}-actin (A14; Sigma-Aldrich), Pan-actin (C4), SRF (G-20; Santa Cruz Biotechnology), MRTF-A (H-140; Santa Cruz Biotechnology), with glyceraldehyde 3-phosphate dehydrogenase (GAPDH; G9545 or G8795; Sigma-Aldrich) as loading control.

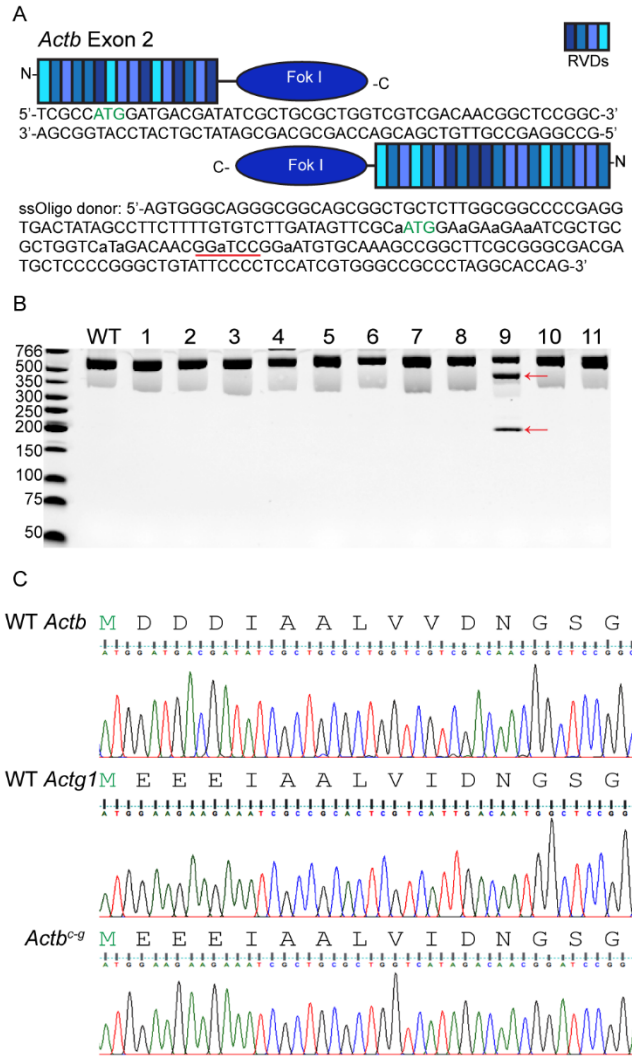

Fig. S1. Genetically engineered *Actb*$^{c\text{-}g}$ mice via TALENs and a single-strand oligo donor. (A) Schematic of TALEN target sequence within WT mouse *Actb* exon 2 and the ssOligo homologous repair donor sequence. The start codon is labeled in green, BamHI restriction enzyme site is underlined in red, edited nucleotides are denoted in lowercase letters. (B) RFLP results of WT and live born F0 pups using the BamHI restriction enzyme digestion. Red arrows indicate the correct digested fragments. (C) Sanger sequence results for WT mouse pup number 9. Aspartic acids 2-4 and Valine 10 in WT β-actin have been changed to glutamic acids 2-4 and isoleucine 10 in the corresponding location in γ-actin.

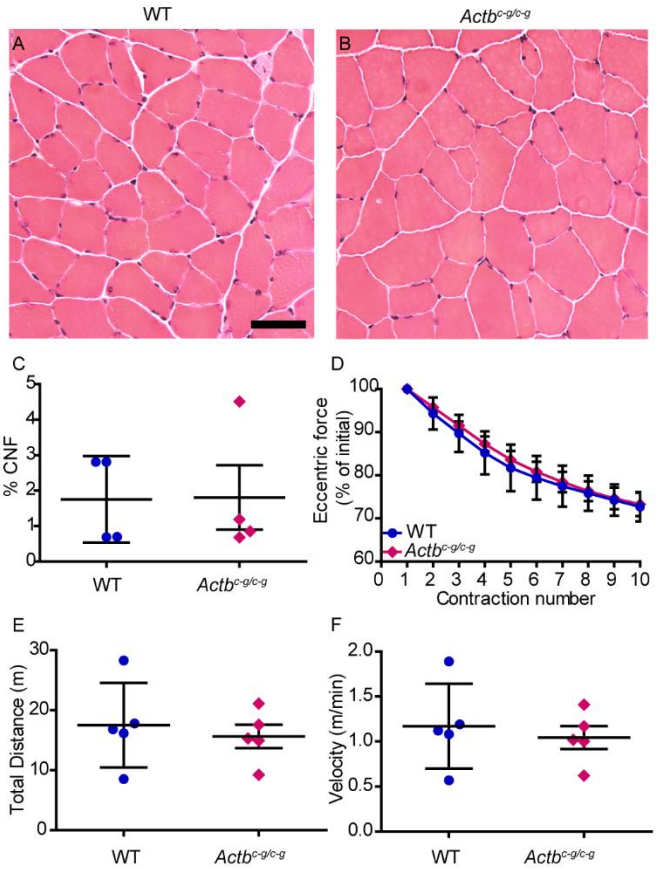

Fig. S2. *Actb*$^{c\text{-}g}$ mice display CNF percentage and open field activity not different than WT. (A-B) Representative H&E images of WT and homozygous *Actb*$^{c\text{-}g}$ mice quadricep muscle. (C) Calculated CNF % of WT and homozygous *Actb*$^{c\text{-}g}$ mice quadricep. N=4 mice, n≥400 fibers per mouse. (D) Graph of eccentric force drop as a percentage of initial force N=5. (E) Total distance traveled during an open field activity assay. (F) Average velocity of each mouse during an open field activity assay. N=5. Error bars are S.D.

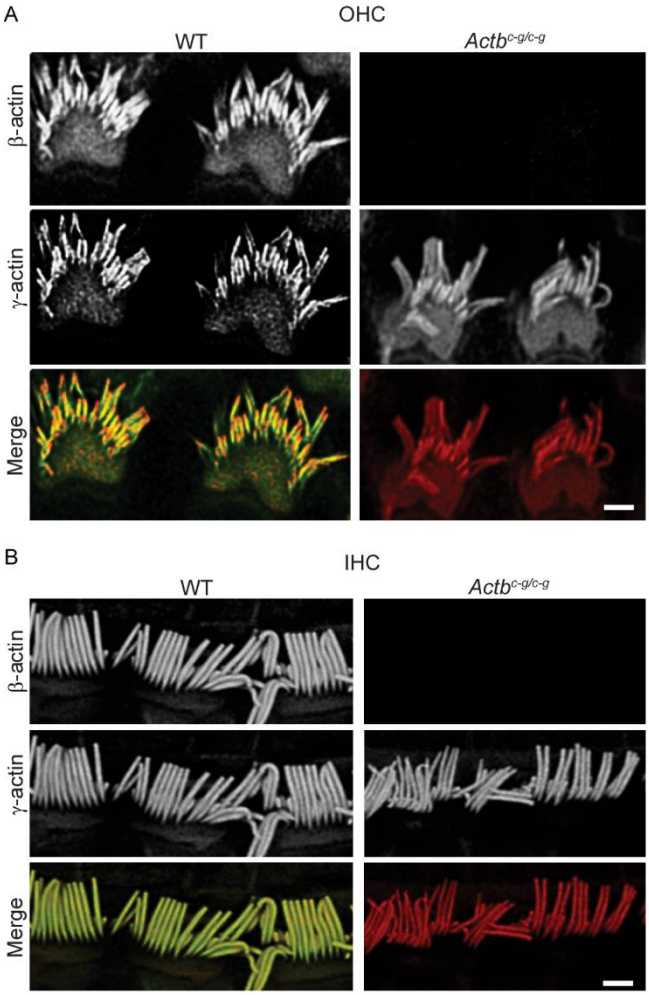

Fig. S3. β-actin protein is not localized to stereocilia in *Actb*$^{c\text{-}g}$ mice. (A) Representative immunofluorescent images of β-actin (green), γ-actin (red) and merged image in the OHC between WT and homozygous *Actb*$^{c\text{-}g}$ mice. (B) Representative immunofluorescent images of β -actin (green), γ -actin (red) and merged image in the IHC between WT and homozygous *Actb*$^{c\text{-}g}$ mice. Scare bar = 1μm. N=3 mice.

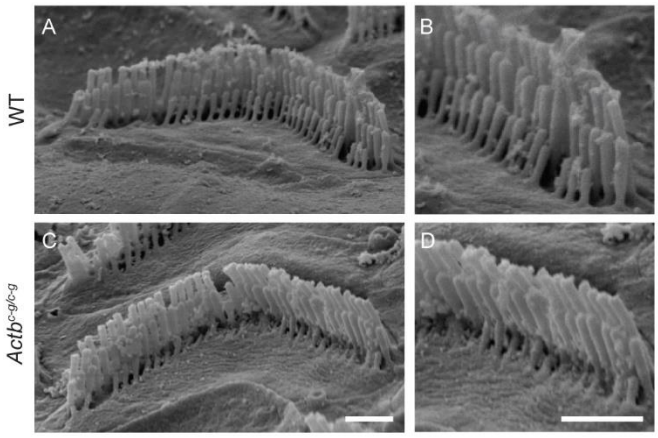

Fig. S4. 6 week old *Actb*$^{c\text{-}g}$ mice display stereocilia degeneration. (A-D) Representative scanning electron microscopy images of 6 week old OHC stereocilia from the base of the cochlea. Scare bars = 1µm. N=4 mice.

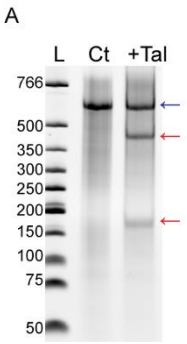

Fig. S5. TALEN activity validation in NIH3T3 fibroblasts. (A) Control and TALEN nucleofected fibroblast gDNA post the Surveyor Assay. Blue arrow indicates the WT band, red arrows indicate the Surveyor nuclease digested bands.

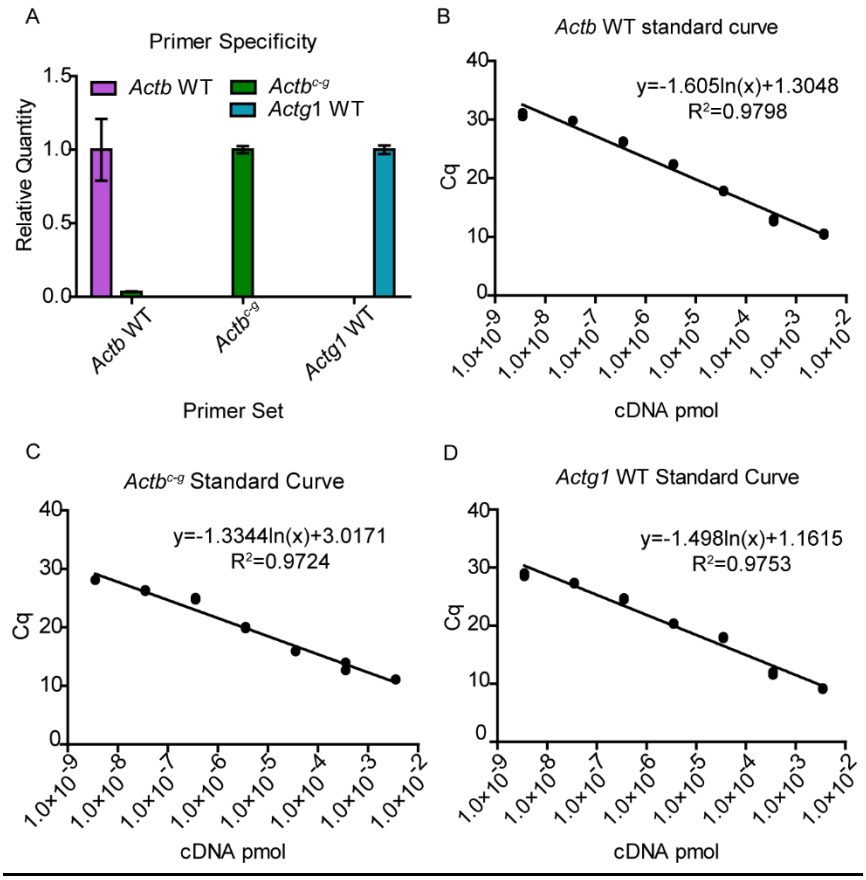

Fig. S6. Mouse actin isoform standard curves and primer specificity analysis. (A) Representative graph of qRTPCR primer specificity. Two new primer sets were designed to specifically amplify WT *Actb* and *Actb*$^{c\text{-}g}$ edited transcript. Each primer set was used to amplify WT *Actb*, edited *Actb*$^{c\text{-}g}$, and WT *Actg1* control constructs to calculate relative quantity. Color bars represent individual actin isoform, y-axis denotes relative quantity, x-axis denotes primer set. (B-D) Representative standard curves generated using specific actin isoform primers with the corresponding control actin construct in a ten-fold dilution.

Table S1.

Physiological parameters of isolated EDL muscles used in *ex vivo* force measurements

Parameter	WT	*Actb*$^{c\text{-}g/c\text{-}g}$	P value
EDL mass (mg)	13.4 ± 1.1	13.8 ± 1.1	0.587
L_o (mm)	13.7 ± 0.3	13.5 ± 0.4	0.397
CSA (mm^2)	2.1 ± 0.2	2.2 ± 0.2	0.500
Passive stiffness (N/m)	13.3 ± 1.0	14.6 ± 2.9	0.367
P_o (mN)	451 ± 67	452 ± 45	0.979
Specific P_o (N/cm^2)	21.6 ± 3.1	20.7 ± 1.7	0.592
ΔP_0 (%)	16.6 ± 5.9	18.4 ± 12.1	0.772
Peak eccentric force (mN)	857 ± 138	834 ± 74	0.752
Specific eccentric force (N/cm^2)	40.9 ± 5.7	38.3 ± 2.3	0.378
Eccentric force loss (%)	27.3 ± 3.4	26.7 ± 2.7	0.763
Active stiffness (N/m)	866 ± 106	844 ± 59	0.696
Peak twitch (mN)	133 ± 20	130 ± 11	0.777
Twitch Force development time (ms)	16.9 ± 2.5	16.4 ± 1.2	0.690
Twitch ½ relaxation time (ms)	17.1 ± 3.1	16.7 ± 3.2	0.845
Tetanic Maximal rate of contraction (mN/s)	14.4 ± 1.6	14.3 ± 1.0	0.910
Tetanic Maximal rate of relaxation (mN/s)	21.8 ± 4.4	21.9 ± 2.7	0.966

Values are mean ± SD.

L_o = optimal muscle length, CSA = physiological cross-sectional area, P_o = maximal isometric tetanic force. ΔP_o = change in P_o after eccentric contractions. n=5 for each line. Statistics were performed using unpaired *t*-test analyses with $p < 0.05$ considered significant.

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