

# Essential nucleotide- and protein-dependent functions of *Actb*/β-actin

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**The highly similar cytoplasmic β- and γ-actins differ by only four functionally similar amino acids, yet previous in vitro and in vivo data suggest that they support unique functions due to striking phenotypic differences between *Actb* and *Actg1* null mouse and cell models. To determine whether the four amino acid variances were responsible for the functional differences between cytoplasmic actins, we gene edited the endogenous mouse *Actb* locus to translate γ-actin protein. The resulting mice and primary embryonic fibroblasts completely lacked β-actin protein, but were viable and did not present with the most overt and severe cell and organismal phenotypes observed with gene knockout. Nonetheless, the edited mice exhibited progressive high-frequency hearing loss and degeneration of actin-based stereocilia as previously reported for hair cell-specific *Actb* knockout mice. Thus, β-actin protein is not required for general cellular functions, but is necessary to maintain auditory stereocilia.**

β-actin | γ-actin | gene edited | auditory hair cell | stereocilia

The actin cytoskeleton contributes to a wide range of cellular functions, such as muscle contraction, cytokinesis, migration, chromatin remodeling, transcriptional regulation, and regulating cell shape (1). Although often thought of as a single protein, there are actually six actin isoforms encoded by separate genes. Four actins are considered muscle specific while the cytoplasmic β- and γ-actin isoforms are ubiquitously expressed. The cytoplasmic actins are highly similar at the level of both gene and protein: the coding sequences of *Actb* and *Actg1* are 89% identical and β- and γ-actin differ at just 4 out of 375 aa residues. Furthermore, human and mouse *Actb* coding sequences are 90% identical and β- and γ-actin primary sequences are identical across birds and mammals (2). The fact that selective pressure has maintained these seemingly slight differences for hundreds of millions of years has confounded cell biologists since the cytoplasmic actins were initially cloned four decades ago: Why do cells apparently require two such highly similar proteins?

Corresponding to the evolutionary conservation of β- and γ-actin, several lines of evidence demonstrate that both isoforms are required for normal cellular function. In humans, point mutations in *ACTB* and *ACTG1* cause a spectrum of developmental defects as well as progressive hearing loss, demonstrating lack of compensation between the isoforms (3–5). In mice, genetic ablation of each isoform results in different phenotypes. Loss of *Actb* results in embryonic lethality and severe cellular proliferation and migration defects in null fibroblasts, while *Actg1* knockouts survive, although with decreased fitness (6–8). Finally, ablation of each isoform in auditory sensory hair cells causes different patterns of progressive hearing loss and degeneration of actin-based protrusions (9).

The phenotypes arising from conventional gene knockout may result from loss of isoform specific transcript or protein. Most notably, the *Actb* transcript has a 3'-UTR “zipcode” sequence not found in the *Actg1* transcript that targets the transcript to subcellular regions and regulates translation (10, 11). In addition, the transcripts differ in

ribosome density and translation rate (12). On the protein level, purified β- and γ-actin have slightly different polymerization rates and differentially interact with a subset of actin binding proteins (13, 14). Considering that nucleotide and amino acid sequences are evolutionarily maintained, both may contribute to critical cellular functions.

To separate the effects of noncoding regulation from differences in β- and γ-actin protein, we edited the *Actb* gene encoding β-actin to instead encode γ-actin while retaining all of the endogenous *Actb* regulatory elements. We found the resulting mice, despite lacking β-actin protein, were born in Mendelian ratios and that the mice and derived fibroblasts appeared phenotypically normal. These results, together with those of a recent study using a similar approach (12) demonstrate that the nucleotide sequence is a critical determinant of actin isoform-specific function. However, we also found that β-actin protein is required to maintain the shape and function of auditory hair cells, demonstrating that other functions of cytoplasmic actins are defined by their amino acid sequences.

## Results

***Actb*<sup>cγ</sup> Mice Coding γ-Actin Instead of β-Actin Protein Are Grossly Normal.** We designed transcription activator-like effector nucleases (TALENs) and a single-stranded oligo (ssOligo) donor

### Significance

Actin is required for many general and specialized cellular functions. Two isoforms, β-actin and γ-actin, are ubiquitously expressed and 99% identical in amino acid sequence, yet previous studies indicated only β-actin is indispensable for life. The nucleotide sequence of each gene also varies, providing additional regulation that may make β-actin indispensable. To separate the effects of protein and nucleotide sequences, the β-actin gene was edited to encode γ-actin protein, while retaining any regulation contained in the nucleotide sequence. The mice, which lack β-actin protein, are viable and appear to be normal. However, they develop progressive hearing loss as auditory sensory cells degenerate. Together, these results show that β-actin function depends on both its nucleotide and protein sequence.

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Conflict of interest statement: D.F.V. is a named inventor on several patents involving the use of transcription activator-like effector nucleases (TALENs) to create targeted genome modifications.

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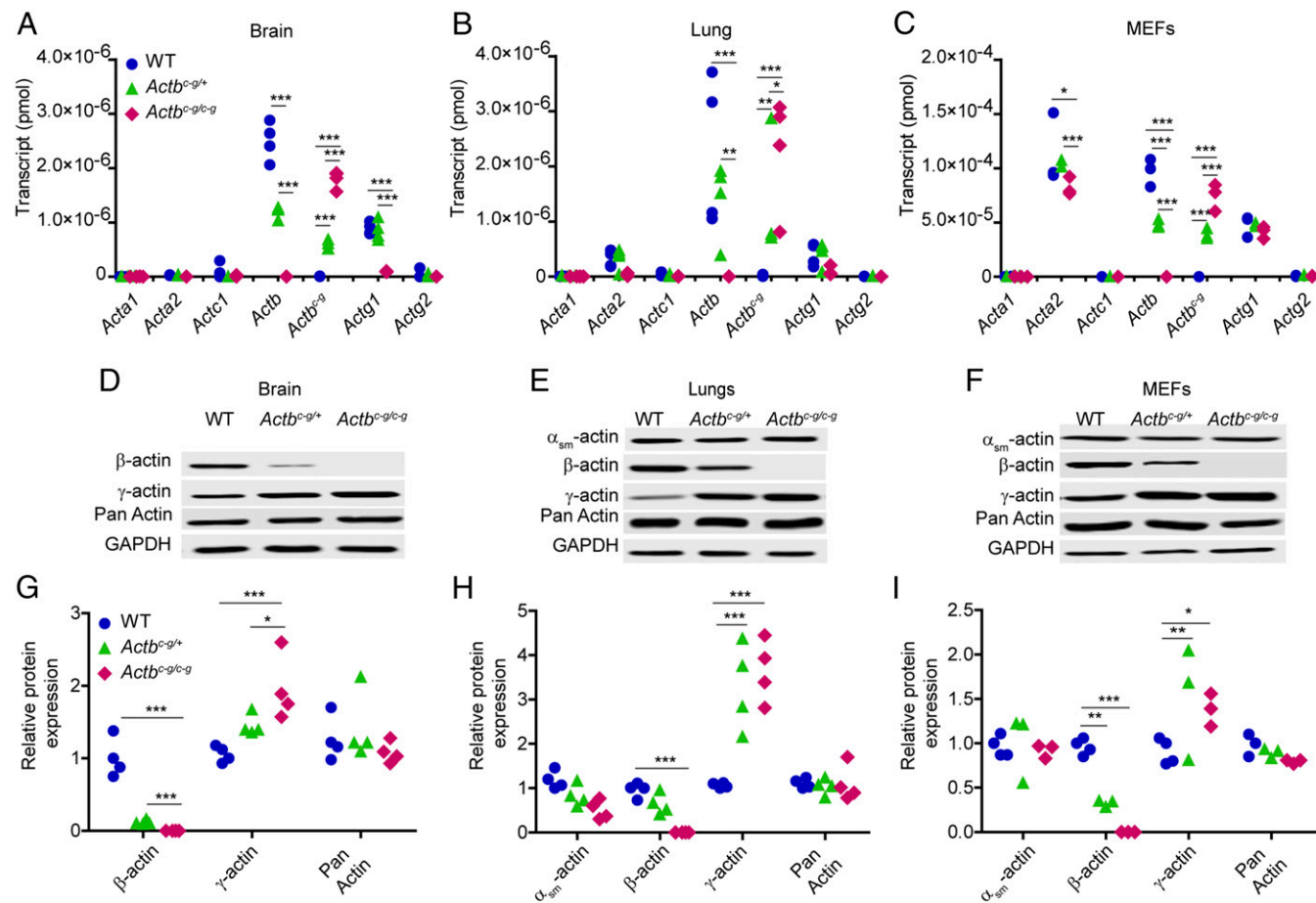
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template to edit the endogenous *Actb* locus within the mouse genome (SI Appendix, Fig. S1A). A single founder was edited so the *Actb* exon 2 encoded E2-4 and I10 instead of D2-4 and V10, thus producing  $\gamma$ -actin protein instead of  $\beta$ -actin (SI Appendix, Fig. S1B and C). We named the edited allele as *Actb*<sup>c-g</sup> for *Actb* coding gamma.

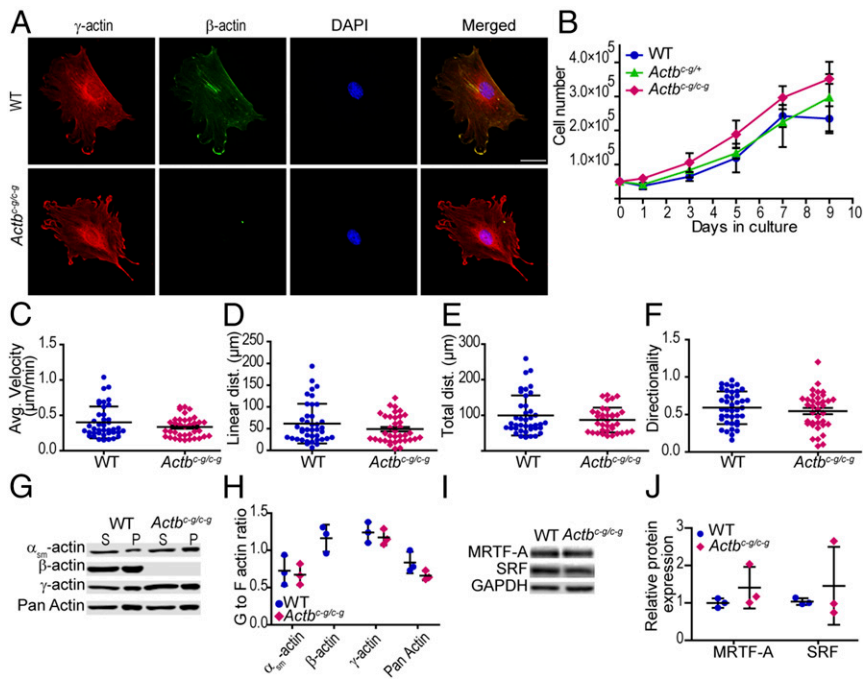
Whereas conventional cre-mediated knockout of *Actb* results in embryonic lethality (7), homozygous *Actb*<sup>c-g</sup> mice were born in Mendelian ratios (27% *Actb*<sup>+/+</sup>, 50% *Actb*<sup>c-g/+</sup>, 23% *Actb*<sup>c-g/c-g</sup>;  $\chi^2 = 0.80$ ) and in normal litter sizes (average of 7 pups per litter; 17 litters) (15). In addition, homozygous *Actb*<sup>c-g</sup> mice were visibly indistinguishable from *Actb*<sup>+/+</sup> littermates, grew at the same rate as their littermates, and exhibited no perinatal or early onset death out to 6 mo of age ( $n = 30$ ).

Cre-mediated knockout of *Actb* in skeletal muscle or the central nervous system resulted in a mild myopathy or hyperactivity, respectively (16, 17). These phenotypes were not detected in homozygous *Actb*<sup>c-g</sup> mice. *Actb*<sup>c-g</sup> homozygous mice displayed no evidence of histopathology in quadriceps muscles at 6 mo of age (SI Appendix, Fig. S2A–C), had no defects in muscle contractile function (SI Appendix, Fig. S2D and Table S1), and behaved normally in an open field assay (SI Appendix, Fig. S2E and F). Overall, these results suggest that  $\beta$ -actin protein is dispensable for mouse viability if  $\gamma$ -actin protein is expressed from the endogenous *Actb* locus.

***Actb*<sup>c-g</sup> MEFs Proliferate and Migrate Normally.** We previously demonstrated that MEFs with cre-mediated knockout of *Actb* failed to proliferate and migrated less efficiently than control cells (7). *Actb*<sup>c-g/c-g</sup> MEFs, as gauged by immunostaining with actin isoform-specific antibodies, localize  $\gamma$ -actin to stress fibers and the periphery of lamellipodial protrusions, and do not express  $\beta$ -actin



**Fig. 1.** *Actb*<sup>c-g</sup> transcript is synthesized from the edited *Actb* locus and correlates with a twofold increase in  $\gamma$ -actin protein expression. (A–C) Calculated quantity of isoactin transcript via qRT-PCRs in brain ( $n = 4$  mice), lung tissues ( $n = 4$  mice), and MEFs ( $n = 3$  embryos). The x axis denotes actin isoform; the y axis denotes transcript in pmol. (D–F) Representative Western blots in brain, lung, and MEFs. (G–I) Calculated relative isoactin protein expression in WT, heterozygous, and homozygous *Actb*<sup>c-g</sup> mice brain ( $n = 4$  mice), lung ( $n = 4$  mice), and MEFs ( $n \geq 3$  embryos). The x axis denotes actin isoform; the y axis denotes relative protein expression which was normalized to GAPDH and relative to a WT sample. Two-way ANOVA with Bonferroni posttest was performed. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .



**Fig. 2.** *Actb<sup>G</sup>* MEFs display cell proliferation and random migration rates not different from WT. (A) Representative immunofluorescence images of WT MEFs and *Actb<sup>G</sup>* MEFs.  $\gamma$ -Actin is labeled in red,  $\beta$ -actin is labeled in green, nucleus is labeled in blue, and the merged image is on the Right. (Scale bar: 50  $\mu$ m.) (B) MEF growth curve analysis, cultured from 0 to 9 d.  $n = 3$  embryos. (C–F) Calculated average velocity, linear distance, total distance, and directionality from random migration assay.  $n \geq 4$  embryos,  $n \geq 40$  cells per genotype. (G and H) G-to-F-actin ratio in WT and *Actb<sup>G</sup>* MEFs. The x axis denotes actin isoform; the y axis denotes G- to-F-actin ratio.  $n = 3$  embryos. (I and J) Representative and calculated relative isoactin protein expression in WT, homozygous *Actb<sup>G</sup>* MEFs for SRF and MRTF-A.  $n = 3$  embryos. The x axis denotes actin isoform; the y axis denotes relative protein expression which was normalized to GAPDH and relative to a WT sample. Error bars are SD.

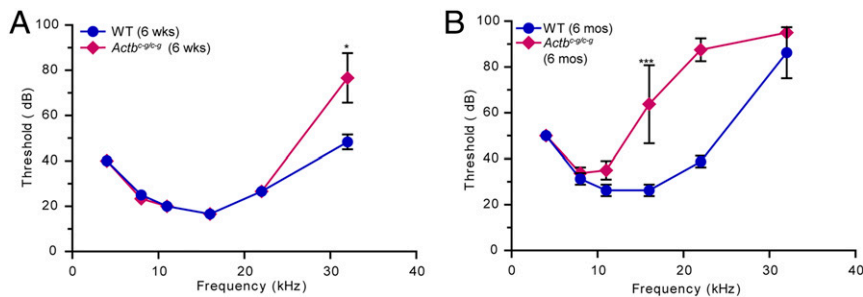
(Fig. 2A). Despite lacking  $\beta$ -actin, heterozygous and homozygous *Actb<sup>G</sup>* MEFs proliferated similarly to control cells in culture (Fig. 2B). Similarly, *Actb<sup>G</sup>* homozygous MEFs migrated normally, displaying average velocity, linear distance, total distance, and directionality persistence comparable to WT MEFs (Fig. 2C–F). Thus,  $\beta$ -actin protein is not required for cell proliferation or migration.

Finally, actin exists both as a monomer (G-actin) and as filaments (F-actin); and the G-to F-actin dynamic plays a key role in the serum response factor (SRF)/myocardin-related transcription factor (MRTF) signaling pathway (19). Cre-mediated  $\beta$ -actin knockout in primary MEFs caused a significant decrease in G-actin which correlated with altered gene expression (8). In contrast, we found  $\alpha_{sm}$ - $\gamma$ , and total actin G- to F-actin ratios were not altered in *Actb<sup>G</sup>* homozygous MEFs (Fig. 2G and H). Expression of SRF and MRTF-A proteins were also not altered compared with WT MEFs (Fig. 2I and J). Overall, these results suggest that  $\gamma$ -actin and  $\beta$ -actin are functionally redundant for G-actin-mediated regulation of gene expression.

**Progressive Hearing Loss and Stereocilia Degeneration in *Actb<sup>G</sup>* Mice.** We previously demonstrated that cre-mediated knockout of *Actb* resulted in progressive, high-frequency hearing loss corresponding to inappropriate shortening of stereocilia, which are

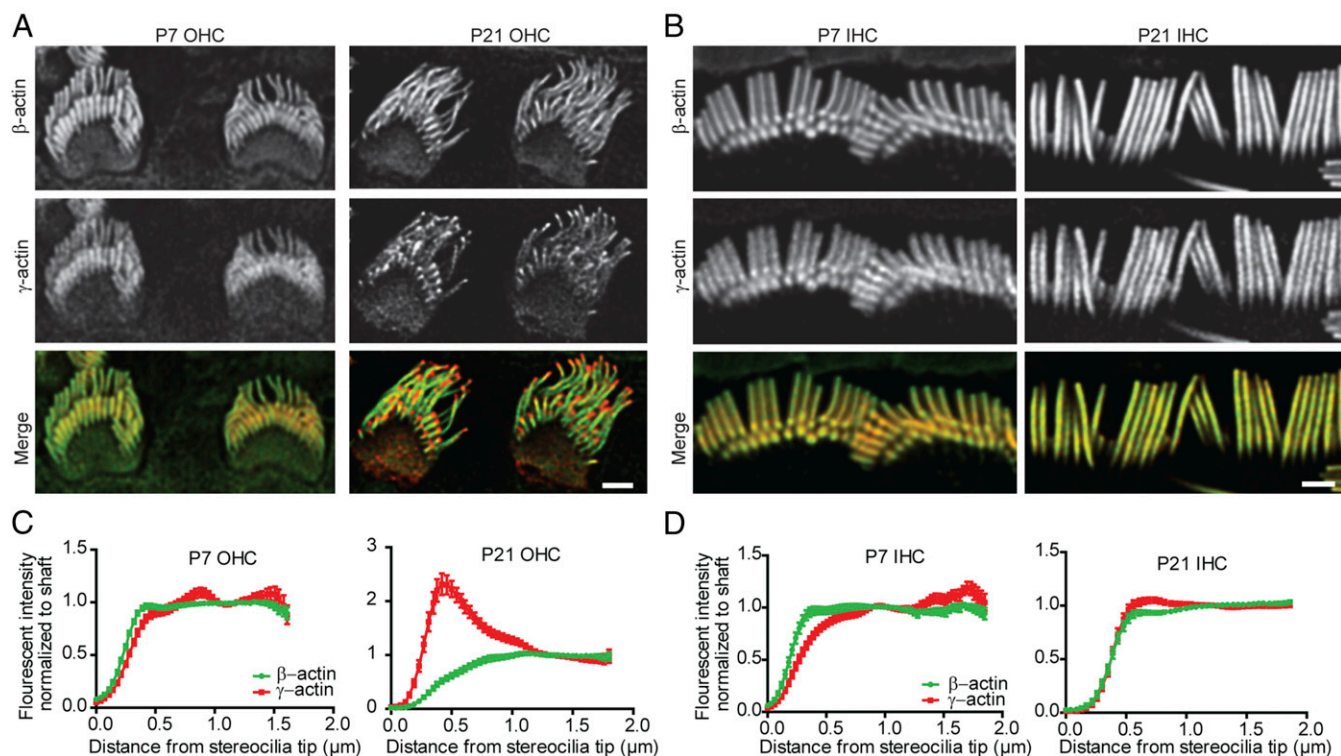
actin-based protrusions on auditory hair cells that detect sound (9, 20). We therefore assessed hearing in *Actb<sup>G</sup>* mice by measuring auditory brainstem responses (ABRs) to a range of pure frequency tones. At 6 wk of age, control and *Actb<sup>G</sup>* mice had comparable thresholds, except at 32 kHz, the highest frequency tested, where *Actb<sup>G</sup>* mice had some hearing loss as demonstrated by their higher ABR threshold (Fig. 3A). This high-frequency hearing loss was more advanced by 6 mo of age, with significant elevation of ABR thresholds at and above 16 kHz in *Actb<sup>G</sup>* mice compared with control mice (Fig. 3B).

We immunostained cochlear tissue to assess the distribution of  $\beta$ - and  $\gamma$ -actin in stereocilia on inner hair cells (IHCs) and outer hair cells (OHCs), which both detect sound but have different stereocilia morphologies. As expected,  $\beta$ -actin was not detected in *Actb<sup>G</sup>* stereocilia (SI Appendix, Fig. S3). In wild-type hair cells at P7, when bundles are developing,  $\beta$ -actin and  $\gamma$ -actin are colocalized to the OHC stereocilia. However,  $\beta$ -actin was reduced and  $\gamma$ -actin markedly enriched at the tips of OHC stereocilia in mature P21–P40 mice, where stereocilia lengths are unchanging (Fig. 4A and C). IHC stereocilia showed a similar trend, with a higher proportion of  $\beta$ -actin at the tips of developing stereocilia (Fig. 4B and D). Thus, increased levels of  $\beta$ -actin at stereocilia



**Fig. 3.** *Actb<sup>G</sup>* mice suffer from progressive hearing loss. (A) ABR of 6-wk-old WT and homozygous *Actb<sup>G/G</sup>* mice.  $n = 3$  mice. (B) ABR of 6-mo-old WT and homozygous *Actb<sup>G/G</sup>* mice.  $n = 4$  mice. The x axis denotes defined frequency in kilohertz; the y axis denotes threshold (in decibels) sound that elicits an ABR. Student *t* test. \* $P < 0.05$ , \*\*\* $P < 0.001$ . Error bars are SD.





**Fig. 4.**  $\beta$ -Actin and  $\gamma$ -actin colocalized in developing OHC stereocilia. (A) Representative immunofluorescent images of the OHC in P7 and P21 C57BL/6 mice.  $\beta$ -Actin is labeled in green,  $\gamma$ -actin is labeled in red. (B) Representative immunofluorescent images of the IHC in P7 and P21 C57BL/6 mice.  $\beta$ -Actin is labeled in green,  $\gamma$ -actin is labeled in red. (C) Calculated fluorescent intensity of  $\beta$ - and  $\gamma$ -actin normalized to the shaft in the OHC.  $n = 3$  mice,  $n \geq 40$  stereocilia. (D) Calculated fluorescent intensity of  $\beta$ - and  $\gamma$ -actin normalized to the shaft in the IHC. (Scale bar:  $1 \mu\text{m}$ .)  $n = 3$  mice,  $n \geq 40$  stereocilia. Error bars are SEM.

tips correlates with growth, suggesting that  $\beta$ -actin protein drives stereocilia growth.

The morphology of stereocilia bundles is closely associated with their mechanotransductive function (Fig. 5C). We used scanning electron microscopy (SEM) to examine OHC stereocilia from the middle turn of the cochlea, which corresponds to the frequency range where ABR thresholds in *Actb*<sup>c-g</sup> mice are normal at 6 wk of age, but elevated at 6 mo of age. Six-week-old control and *Actb*<sup>c-g</sup> mice had OHC bundles that appeared normal, featuring similar numbers of stereocilia that were of similar height (Fig. 5A and D). In contrast, at 6 mo of age, the mechanotransducing stereocilia in *Actb*<sup>c-g</sup> mice had started to degenerate and were shorter and fewer in number than in control OHCs. In addition, some OHC stereocilia bundles were missing in *Actb*<sup>c-g</sup> mice (Fig. 5B and D). Toward the base of the cochlea where higher pitch sounds are detected, a similar phenotype was observed in *Actb*<sup>c-g</sup> OHC stereocilia at 6 wk of age (SI Appendix, Fig. S4). Together, these data suggest that *Actb*<sup>c-g</sup> mechanotransducing OHC stereocilia selectively and progressively degenerate, leading to hearing loss. Notably, this pattern of hearing loss and stereocilia degeneration is similar to that previously observed in hair cell-specific *Actb* knockout mice (9, 20), demonstrating that this protein isoform is required for stereocilia length maintenance.

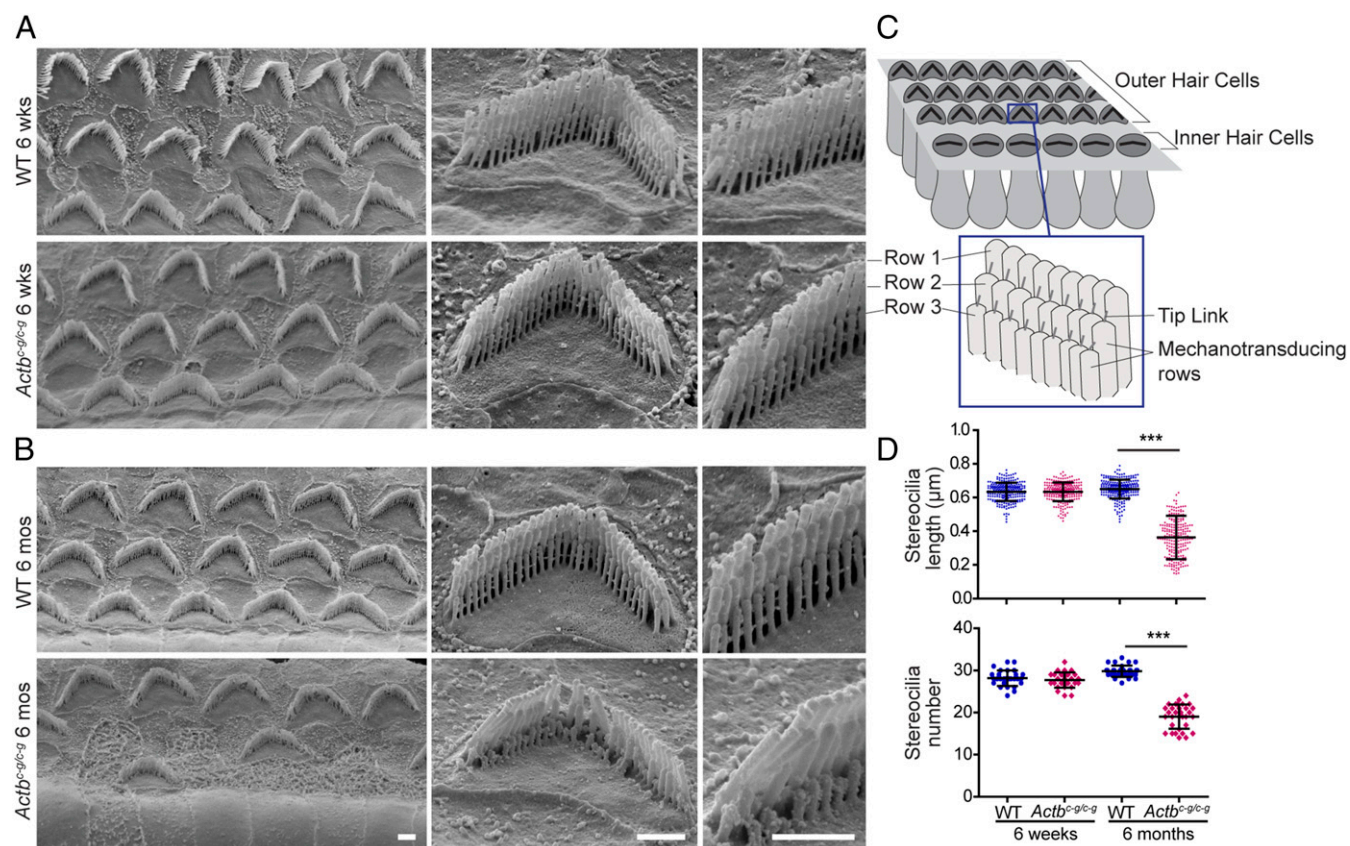
## Discussion

To distinguish the relative contributions of nucleotide and amino acid sequences to the unique functions of closely related cytoplasmic actins, we used TALENs to engineer the endogenous *Actb* gene to express  $\gamma$ -actin instead of  $\beta$ -actin. This approach edited only the nucleotides required to substitute the  $\beta$ -actin N-terminal amino acids D2–4 and V10 for  $\gamma$ -actin residues E2–4 and I10. The resulting mice and cells, which produce no  $\beta$ -actin

protein, lacked the most severe and overt phenotypes of previously characterized *Actb* knockouts. These findings demonstrate that many functions of the highly conserved and expressed *Actb* gene reside in the nucleotide sequence and do not depend on the protein sequence. However, homozygous *Actb*<sup>c-g</sup> mice did develop progressive hearing loss due to degeneration of actin-rich protrusions on sensory cells. Therefore,  $\beta$ -actin protein has functions in some specialized cell types that cannot be compensated for by  $\gamma$ -actin.

A recent study using clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 methodology also edited the endogenous *Actb* locus to express  $\gamma$ -actin protein (12), and the resulting mouse line is nearly identical to the one we independently generated and characterized here. Several of the same phenotypes were assessed, and very similar results were obtained. In particular, *Actb*<sup>c-g</sup> mice are viable and appear to develop normally without evidence of premature mortality (12). In addition, *Actb*<sup>c-g</sup> MEFs also have proliferation and migration rates similar to WT (Fig. 2 and ref. 12). We further show that *Actb*<sup>c-g</sup> muscle viability and physiology, as well as the activity level of the mice, was comparable to wild type. Overall, these two independent studies are highly consistent and support the major finding that  $\beta$ -actin protein is dispensable for most cellular functions.

The *Actb* gene-edited mice (herein, ref. 12) contrast with conventional cre-mediated knockout of a floxed *Actb* allele, where exons 2 and 3 were deleted, and which caused embryonic lethality in mice (8). Tissue-specific knockout of the same allele also resulted in a variety of phenotypes with muscle showing progressive myofiber degeneration/regeneration, muscle weakness, impaired mitochondrial fission (17, 21), and the CNS showing tissue morphology defects that correlated with hyperactivity and cognitive behavior impairments (16). Additionally, isolated MEFs failed to divide or migrate, suggesting  $\beta$ -actin was



**Fig. 5.** *Actb<sup>c-g</sup>* mice show evidence of stereocilia degeneration. Representative scanning electron microscopy images of (A) 6-wk-old and (B) 6-mo-old OHC stereocilia from the middle turn of the cochlea. (Scale bar: 1 μm.) (C) Cartoon image of the stereocilia. (D) Length (in micrometers) of stereocilia in row 3 of OHC bundles in 6-wk-old and 6-mo-old WT and homozygous *Actb<sup>c-g</sup>* mice.  $n = 4$  mice,  $n \geq 200$  stereocilia. Number of stereocilia in row 3 of OHC bundles in 6-wk-old and 6-mo-old WT and homozygous *Actb<sup>c-g</sup>* mice.  $n = 4$  mice,  $n \geq 28$  cells. One-way ANOVA with Tukey's posttest was performed.  $***P < 0.001$ . Error bars are SD.

required for fundamental cellular processes (6, 8). None of these phenotypes were observed in *Actb<sup>c-g</sup>* mice. It is possible that some of the phenotypes observed following cre-mediated deletion of *Actb* exons 2 and 3 are independent of normal *Actb* function. For example, there may be an essential element in the intervening intron. It is also possible that recombination produces toxic transcript and/or truncated protein. However, differences between cre-mediated knockouts and *Actb<sup>c-g</sup>* mice most likely suggest that regulatory information embedded in the nucleotide sequence is critical, as this would be altered in the *Actb* floxed knockout but not in the *Actb<sup>c-g</sup>* mice.

Singer and coworkers (22) first discovered and established the *Actb* zipcode as a key difference between *Actb* and *Actg1* gene and transcript, which is conserved from birds to mammals (23). ZBP1 binds to this sequence to regulate both transcript localization and translation of β-actin (11). Consistent with well-regulated temporal and spatial β-actin protein translation, *Actb* transcript is more abundant than *Actg1* transcript in primary MEFs even when β- and γ-actin protein levels are similarly expressed (6). Based on ref. 12 and our findings that the four amino acid variances between β- and γ-actin protein are not critical to most cellular functions, it seems likely that transcript targeting and regulated translation via the zipcode is the essential feature of *Actb*. A similar gene-editing approach can potentially address the importance of the *Actb* zipcode in vivo.

While β-actin protein is not essential to the function of many cell types, some tissues nonetheless seem to specifically detect and regulate the levels of β- and γ-actin. As *Actb<sup>c-g</sup>* transcript increased in heterozygous and homozygous brain tissue, the level

of γ-actin protein increased as it was produced both from the engineered *Actb* locus and the endogenous *Actg1* locus (Fig. 1A–C). Interestingly, as γ-actin protein concentration increased, the amount of transcript from the endogenous *Actg1* alleles decreased. In *Actb<sup>c-g</sup>* cells, as well as in most cells and tissues where *Actb* or *Actg1* were knocked out, total actin protein levels remained constant across all genotypes even as transcript levels varied (7, 8). Thus, most cell and tissue types appear to maintain actin homeostasis via a protein-based feedback mechanism.

Clinically, spontaneous missense mutations in both *ACTB* and *ACTG1* have been associated with Baraitser–Winter syndrome, a rare disease with complex phenotypes, including facial dysmorphism, learning disabilities, and deafness (24). Some of the mutations affect the polymerization properties of actin or interaction with actin binding proteins to cause a gain-of-function or a dominant-negative effect (25–27). Interestingly, the more severe forms of Baraitser–Winter syndrome are associated with mutations in *ACTB* over *ACTG1* (4). Loss-of-function mutations in human *ACTB* also cause developmental delays, intellectual disability, internal organ malformations, hearing loss, and facial malformations (3). Together, these studies suggest that loss or mutation of cytoplasmic actins, although not necessarily lethal, perturbs a variety of specialized cells or cellular processes and decreases organismal fitness.

Stereocilia maintenance is one such specialized process that requires β-actin protein, which likely relates to deafness observed in humans with mutations in *ACTB* and *ACTG1* (27). *Actb<sup>c-g</sup>* hair cells, like cre-mediated *Actb* knockout hair cells characterized previously, have a particular pattern of degeneration where the shorter rows of the bundle shorten irregularly as mice age, but



the tallest row of the bundle is largely unaffected (9). In contrast, *Actg1* knockout hair cells have a different phenotype where individual stereocilia are lost from bundles (9, 28). Together, these data point to a particular requirement for  $\beta$ -actin in maintaining stereocilia length. As with *Actb* knockout, blocking mechanotransduction also results in degeneration of the shorter rows of stereocilia, which can subsequently regrow when mechanotransduction is restored (29). Considering that the mechanotransductive apparatus is under constant mechanical stress, one possibility is that damage and repair during aging results in cycles of stereocilia shortening and regrowth (30). Efficient regrowth likely requires  $\beta$ -actin. Indeed, preferential localization of  $\beta$ -actin to the growing tips of developing stereocilia further supports a role for  $\beta$ -actin over  $\gamma$ -actin elongating stereocilia.

The ability of  $\beta$ -actin to lengthen stereocilia may be because it has a faster polymerization rate than  $\gamma$ -actin (13) under calcium bound conditions, which may reasonably occur in mechanotransducing stereocilia. Besides polymerization kinetics, multiple actin binding proteins may also differentially interact with  $\beta$ - and  $\gamma$ -actin. For example, nonmuscle myosinIIa and myosinVIIa both exhibit higher ATPase activity with  $\gamma$ -actin filaments (31). MyosinVIIa is a particularly interesting candidate for mediating actin isoform-specific effects since it is also localized to stereocilia. Finally, the actin filament severing protein cofilin regulates  $\beta$ - and  $\gamma$ -actin differently (14), which is intriguing since mice lacking the severing protein actin depolymerizing factor (ADF) phenocopy the pattern of stereocilia degeneration seen in *Actb<sup>c-g</sup>* mice (30).

Whether through polymerization kinetics or differential binding partners, differences in  $\beta$ - and  $\gamma$ -actin function in stereocilia depend on the four amino acid differences in the N terminus. One substitution is a valine for an isoleucine, while the other three substitutions are in an acidic block (DDD in  $\beta$ - and EEE in  $\gamma$ -actin). Auditory

function in mice expressing  $\beta$ -actin from the *Actg1* locus seem likely to phenocopy *Actg1* nulls, while swapping the EEE acidic block or the isoleucine from *Actg1* into the *Actb* locus would help resolve the relative contributions of each feature to  $\beta$ -actin protein function.

Regardless of the mechanism by which  $\beta$ -actin protein contributes to stereocilia maintenance, this isoform has a cellular function that is not met by  $\gamma$ -actin protein. Therefore, it is likely that other cell types also depend on  $\beta$ -actin protein to form or maintain specialized features. However, this scenario is considerably less common than previously appreciated and, in agreement with Vedula et al. (12), we conclude that the nucleotide sequence rather than amino acid sequence determines the majority of the divergent phenotypes previously associated with *Actb* gene disruption.

## Methods

**Animal Care.** Animals were housed and treated in accordance with the standards set by the University of Minnesota Institutional Animal Care and Use Committee.

**Statistics.** All statistical analysis was analyzed using the GraphPad Prism software.

Detailed descriptions of materials and methods, including TALEN construction, cell culture, the open field activity assay, muscle histology and physiology, immunofluorescence microscopy of MEFs and cochlea, scanning electron microscopy, auditory brainstem response, live cell imaging, qRT-PCR, Western blotting, and the G-to F-actin ratio assay are given in *SI Appendix, SI Materials and Methods*.

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