Figures and figure supplements

Diverse functions of homologous actin isoforms are defined by their nucleotide, rather than their amino acid sequence

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Figure 1. β-actin (Actbc-g) mice exhibit no phenotypic changes compared to control. (A) CRISPR/Cas9 editing strategy used to generate Actbc-g mouse. (B) Photos of Actbc-g E12.5 mouse embryos, with genotypes indicated. (C) Photos of Actbc-g mice after gene editing, alone (top left) and next to age-matched (top right) and littermate wild type (WT) (bottom). Three mice from two different litters are shown. (D) H&E-stained sagittal sections of the heads (top) and bodies (bottom) of littermate P0 wild type (WT) and Actbc-g mice. Scale bar, 1 mm.

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Figure 1—figure supplement 1. Generation of Actbc-g mouse. Top left, genotyping strategy: editing of the N-terminal codons of the β-actin gene abolishes an EcoRV restriction site, enabling the screening of the edited gene variants by EcoRV digestion of the PCR-generated DNA fragments corresponding to the 5' of the actin sequence. Top right, PCR products before (top) and after (bottom) EcoRV digestion. Bottom, western blots of wild type, heterozygous, and Actbc-g mouse tail lysates with antibodies to β- and γ- actin.

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**Figure 1—figure supplement 2.** Sequencing result for wild type Actb and the edited Actbc-g alleles. Screen shots from the Chromas sequence viewing software.

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Figure 2. Actb gene editing abolishes β-actin protein from multiple organs and is accompanied by up-regulation of γ-actin without changing the total actin levels. Western blot analysis showing images (left) and quantifications (right) of whole tissue lysates from wild type (Actb+/+) and Actbcg mice. Fluorescence images obtained from the Odyssey gel imager are shown. For quantification, total fluorescence from the 43 kDa actin band was normalized to the loading control and to the actin level in the first lane for each blot. Error bars represent SEM, n = 3.

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Figure 2—figure supplement 1. Actin levels in Actbc-g mice are similar to control. Western blots of wild type, heterozygous, and Actbc-g mouse brain lysates probed with antibodies to β- and γ-actin and total actin (pan-actin). Mouse genotypes are indicated on top of each lane, and the antibodies used are listed below each blot. DOI: https://doi.org/10.7554/eLife.31661.007
Figure 2—figure supplement 2. 2D gel distribution of actin isoforms in wild type (top) and Actbc-g (bottom) mouse tissue lysates. DOI: https://doi.org/10.7554/eLife.31661.008
Figure 2—figure supplement 3. Generation of Actg1c-b mouse. Top, genotyping strategy: editing of the N-terminal codons of the actin gene generates an EcoRV restriction site, enabling the screening of the edited gene variants by EcoRV digestion of the PCR-generated DNA fragments corresponding to the beginning of the actin sequence. Bottom, PCR products before (top) and after (bottom) EcoRV digestion.
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**Figure 2—figure supplement 4.** γ-coded β-actin (Actg1c-b) mice exhibit no phenotypic changes compared to control. Top, CRISPR/Cas9 editing strategy used to generate Actg1c-b mouse. Bottom left, photos of Actg1c-b mouse after gene editing, to age-matched wild type. Bottom right, H and E-stained sagittal sections of the heads (top) and bodies (bottom) of littermate wild type (WT) and Actg1c-b mice. Scale bar, 1 mm.

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Figure 2—figure supplement 5. Partial editing of the γ-actin gene to encode β-actin-like protein abolishes γ-actin protein from multiple organs. Western blots with the actin antibodies indicated on the left using tissue homogenates from wild type control and Actgc-b mouse.

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Figure 3. Mouse embryonic fibroblasts derived from Actbc-g mice have normal actin cytoskeleton, despite complete lack of β-actin. Top, quantification of total F-actin detected by Phalloidin-AlexaFluor488 staining of wild type (Actb+/+) and Actbc-g primary mouse embryonic fibroblasts. Numbers were averaged from 69 cells in WT and 76 cells in Actbc-g, obtained from two different primary cultures independently derived from two different littermate embryos for each set. Bottom, representative images of both cell types stained with Phalloidin-AlexaFluor488 or antibodies to both actin isoforms as indicated.

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Figure 4. Mouse embryonic fibroblasts show no major changes in morphology and actin distribution. Representative images of wild type (WT) and Actbc-g primary mouse embryonic fibroblasts stained with antibodies to both actin isoforms as indicated. DOI: https://doi.org/10.7554/eLife.31661.013
Figure 5. Mouse embryonic fibroblasts derived from Actbc-g mice migrate at normal rates. Left, phase contrast images of the first (0') and last (600') frame taken from a representative time lapse videos of the WT and Actbc-g cells at the edge of a monolayer migrating into an infinite scratch wound. Overlay of the two frames is shown in the bottom row. Scale bar, 100 μm. Right top, quantification of the cell migration rate as μm²/min, WT: n = 28, Actbc-g: n = 29 averaged from two independently derived primary cultures for each set. See Supplemental Videos 1 and 2. Right bottom, quantification of cell directionality in single cell migration assays (calculated as persistence over time, WT: n = 49, Actbc-g: n = 50) suggests that single cell migration was not affected in Actbc-g cells. DOI: https://doi.org/10.7554/eLife.31661.014