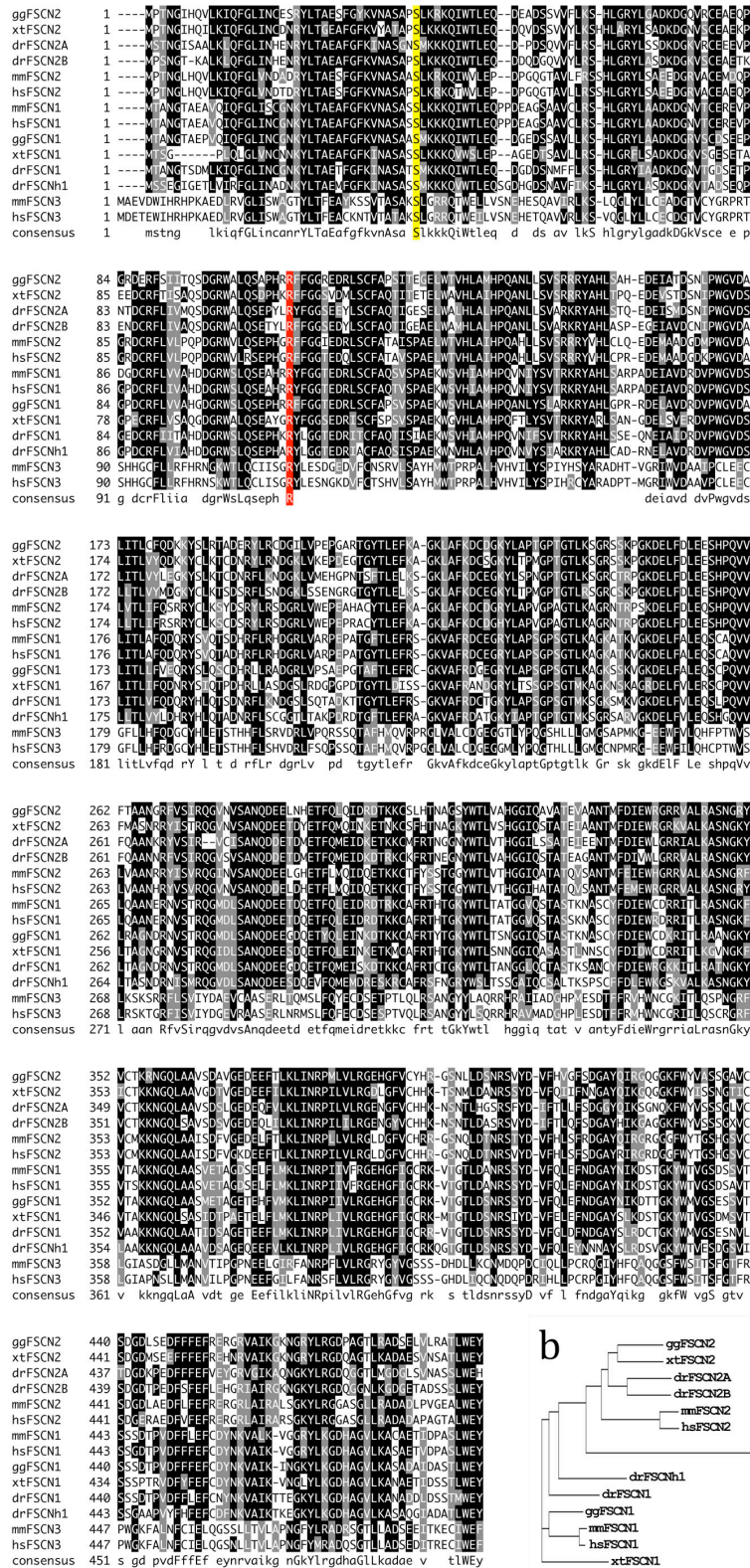


Supplemental Material

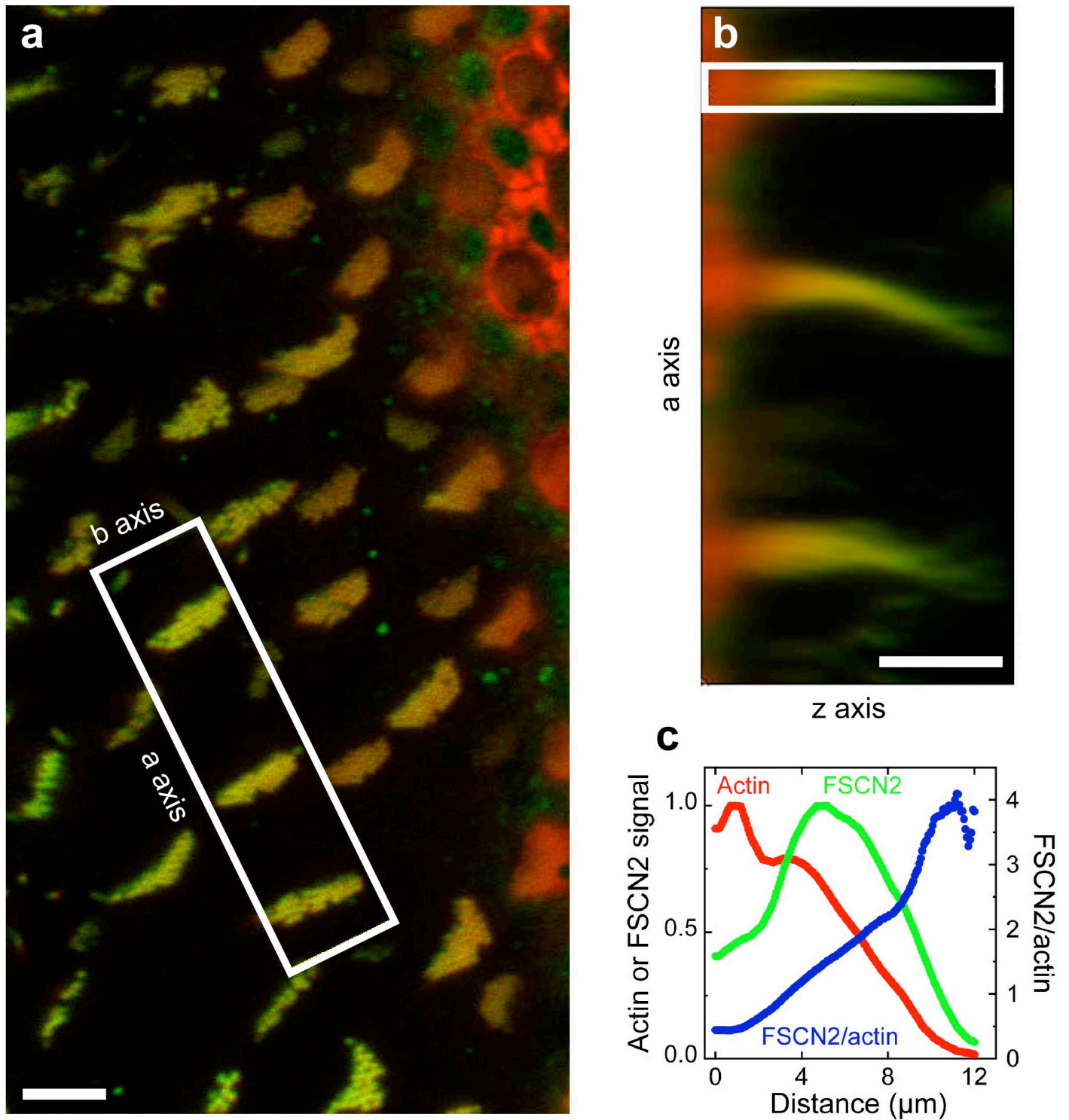
Supplementary Table 1. Hearing loss variability among DBA-related inbred strain mice. Average ABR thresholds, standard errors, and sample sizes are given for each strain-age combination tested.

| Strain | Age tested weeks | number | Average ABR thresholds and standard errors | | | | | | | |
|-------------|------------------|--------|--|-----|-------|------|--------|------|--------|-----|
| | | | click | | 8 kHz | | 16 kHz | | 32 kHz | |
| | | | AVG | SE | AVG | SE | AVG | SE | AVG | SE |
| DBA/1J | 12 | 3 | 48 | 1.7 | 43 | 1.7 | 28 | 1.7 | 60 | 0.0 |
| DBA/1J | 48 | 5 | 57 | 2.0 | 40 | 2.8 | 42 | 7.6 | 59 | 7.4 |
| DBA/2HaSmnJ | 6.5 | 4 | 39 | 1.3 | 30 | 2.1 | 19 | 1.3 | 51 | 2.4 |
| DBA/2HaSmnJ | 14 | 5 | 36 | 1.0 | 26 | 2.5 | 22 | 1.2 | 45 | 1.6 |
| DBA/2HaSmnJ | 16 | 2 | 35 | 0.0 | 30 | 0.0 | 25 | 0.0 | 43 | 2.8 |
| DBA/2DeJ | 7.5 | 1 | 50 | — | 50 | — | 20 | — | 85 | — |
| DBA/2DeJ | 15 | 2 | 50 | 5.0 | 50 | 0.0 | 33 | 2.5 | 88 | 2.5 |
| DBA/2DeJ | 17 | 1 | 50 | — | 50 | — | 35 | — | 95 | — |
| DBA/2DeJ | 28 | 5 | 55 | 4.0 | 49 | 6.3 | 53 | 12.1 | 98 | 1.3 |
| DBA/2NCrI | 4 | 3 | 40 | 0.0 | 42 | 6.0 | 17 | 4.4 | 78 | 1.7 |
| DBA/2NCrI | 7.5 | 3 | 47 | 4.4 | 43 | 1.7 | 18 | 3.3 | 82 | 4.4 |
| DBA/2NCrI | 14 | 4 | 48 | 4.8 | 41 | 4.3 | 36 | 5.1 | 94 | 2.4 |
| DBA/2NCrI | 17 | 4 | 44 | 2.4 | 43 | 3.3 | 39 | 7.4 | 84 | 2.4 |
| DBA/2NCrI | 21 | 4 | 51 | 1.3 | 43 | 1.5 | 45 | 6.5 | 95 | 2.1 |
| DBA/2NCrI | 26 | 4 | 54 | 4.3 | 44 | 4.3 | 73 | 9.3 | 95 | 2.9 |
| DBA/2J | 3.5 | 6 | 43 | 1.7 | 28 | 1.7 | 28 | 7.1 | 53 | 4.8 |
| DBA/2J | 5.0 | 7 | 60 | 4.3 | 44 | 3.5 | 53 | 5.7 | 74 | 5.9 |
| DBA/2J | 8.0 | 5 | 55 | 3.5 | 46 | 4.0 | 62 | 4.3 | 67 | 5.1 |
| DBA/2J | 12.0 | 10 | 67 | 3.3 | 50 | 4.1 | 65 | 2.3 | 72 | 3.8 |
| DBA/2J | 17.0 | 2 | 73 | 7.5 | 80 | 10.0 | 70 | 5.0 | 90 | 0.0 |
| DBA/2J | 23.0 | 4 | 90 | 5.4 | 89 | 9.7 | 91 | 5.6 | 100 | 0.0 |
| DBA/2J | 28.0 | 7 | 97 | 2.9 | 95 | 2.7 | 92 | 3.9 | 100 | 0.7 |

a



Suppl. Fig. 1. Fascin sequences are highly homologous across species. **a.** Multiple protein sequence alignments done in ClustalW2. Serine 39, which is a regulatory phosphorylation site, is highlighted in yellow. Arginine 109, which is mutated to a histidine in DBA/2J mice, is highlighted in red. **b.** Phylogenetic tree of fascin genes. Branch lengths are proportional to the amount of evolutionary distance between related genes.



Suppl. Fig. 2. FSCN2 concentration towards tips in P10 mouse utricle hair bundles. Using ImageJ, an a-b-z stack was generated from a confocal stack of bundles labeled with anti-FSCN2 (a single slice of that stack is shown in **a**, with the area used for the a-b-z stack indicated with a box). That stack was flattened along the b-axis using average z-projection in ImageJ, generating the image in **b**. Using the Plot Profile function in ImageJ, profiles of red (actin) and green (FSCN2) fluorescence were generated. These profiles and the FSCN2/actin ratio were plotted in **c**. Note that the FSCN2/actin ratio is highest in the last $\sim 3 \mu\text{m}$ of the hair bundle. Similar profiles were observed in other hair bundles.