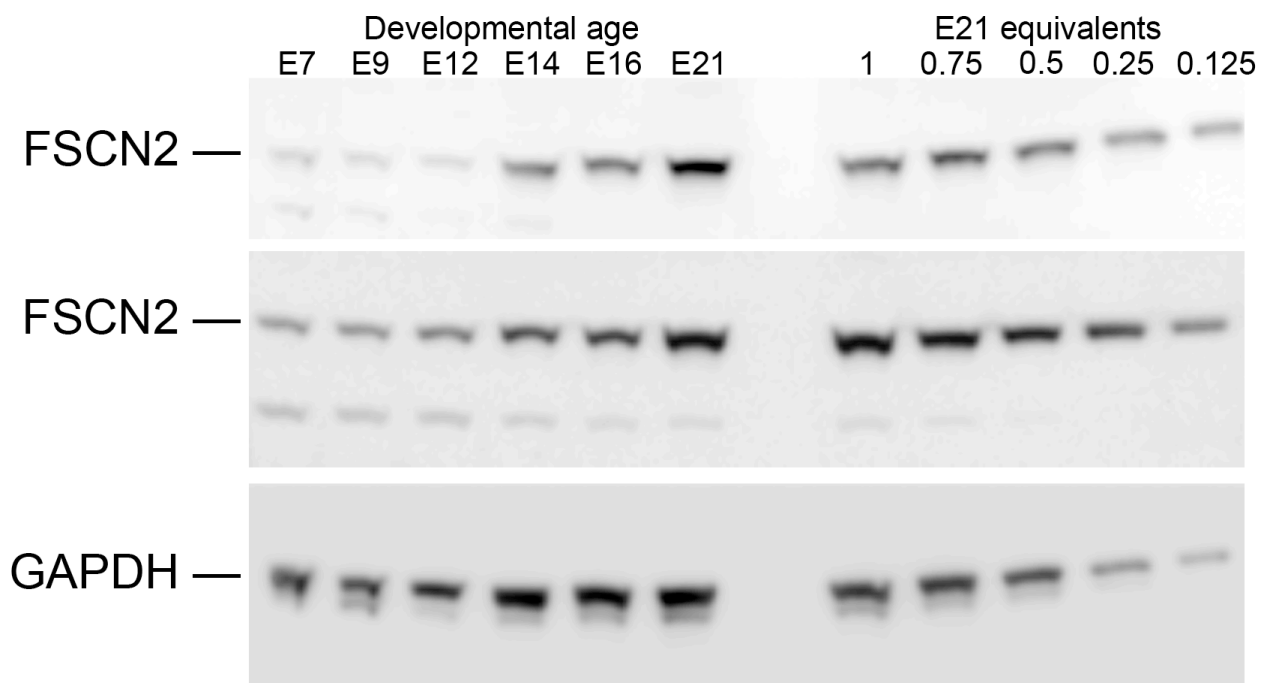


## Supplemental Information

In support of:

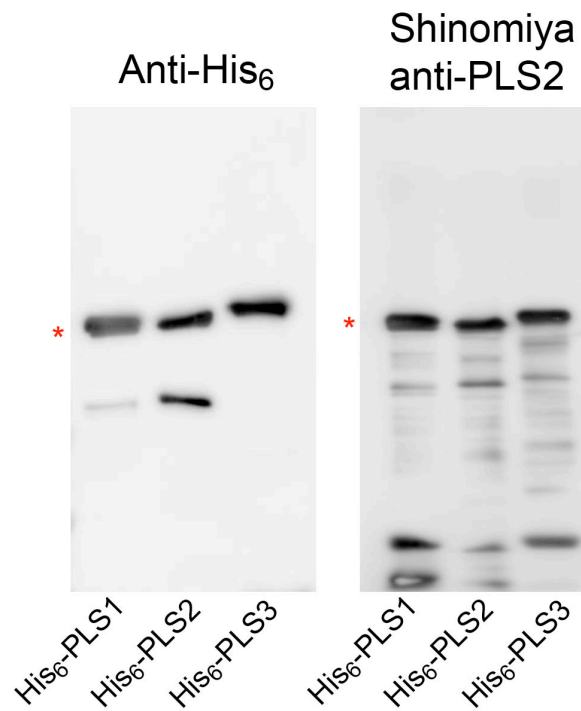
### Correlation of Actin Crosslinker and Capper Expression Levels with Stereocilia Growth Phases

Matthew R. Avenarius, Katherine W. Saylor, Megan R. Lundeberg, Phillip A. Wilmarth, Jung-Bum Shin, Kateri J. Spinelli, James M. Pagana, Leonardo Andrade, Bechara Kachar, Dongseok Choi, Larry L. David, and Peter G. Barr-Gillespie



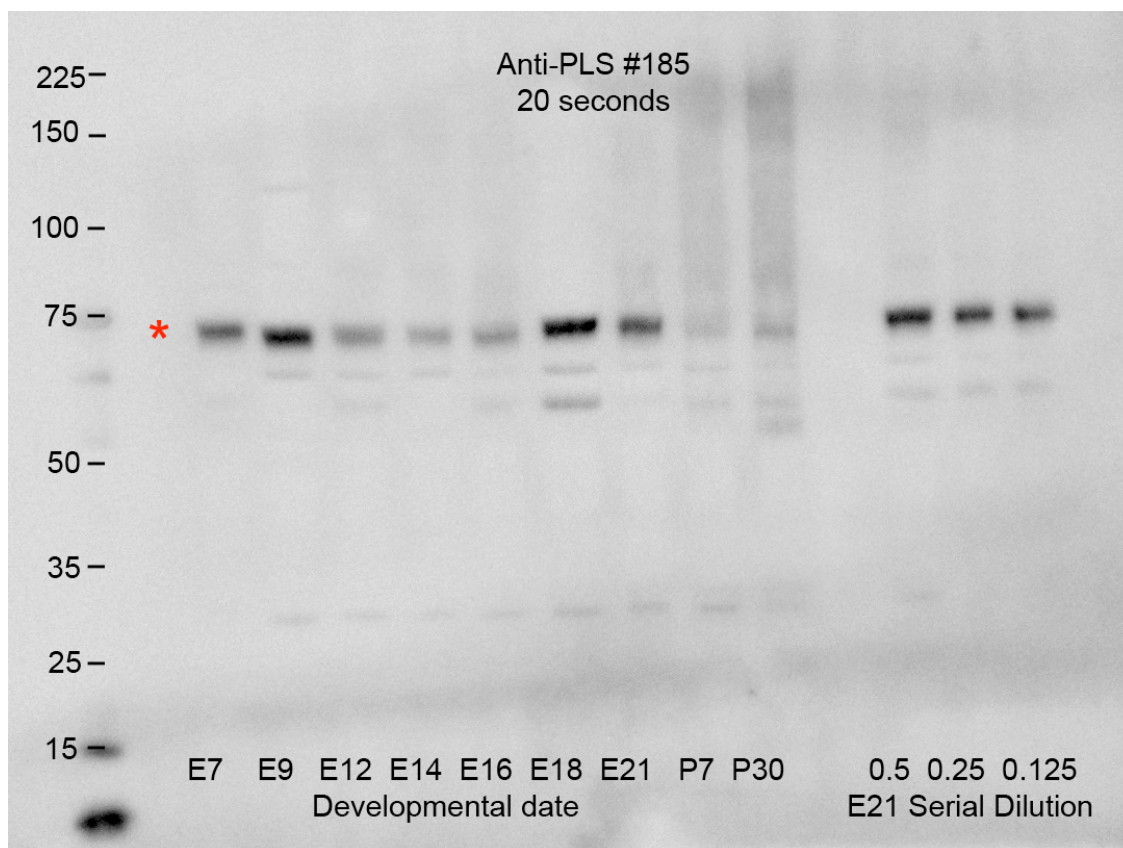
**Figure S1. Examples of developmental immunoblot profile of FSCN2 and GAPDH expression in developing chick cochlea.**

Example of immunoblot analysis of chick cochlea developmental time points using pan-PLS antibody #185. Developmental age is indicated for each lane on the top. Callouts indicate the antibody used and the position of the correct-sized band, quantified as described elsewhere. Identical amounts of total protein were loaded for each developmental age.



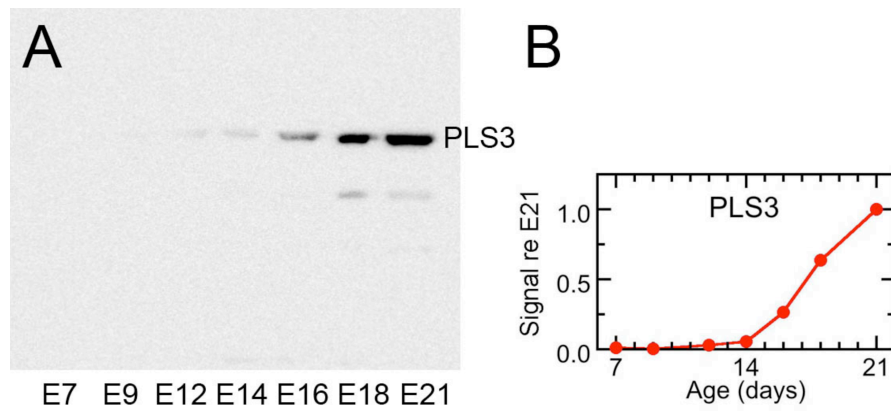
**Figure S2. Characterization of pan-PLS antibody.**

Left, immunoblot detection of bacterially expressed, His<sub>6</sub>-tagged chicken plastin cDNA clones. Red asterisk indicates approximate molecular mass for full-length plastins. Right, immunoblot signal from the same samples using the Shinomiya antibody against mouse PLS2. Note that all three chicken plastins are detected approximately equally by the antibody.



**Figure S3. Example of developmental immunoblot profile of PLS expression in developing chick cochlea.**

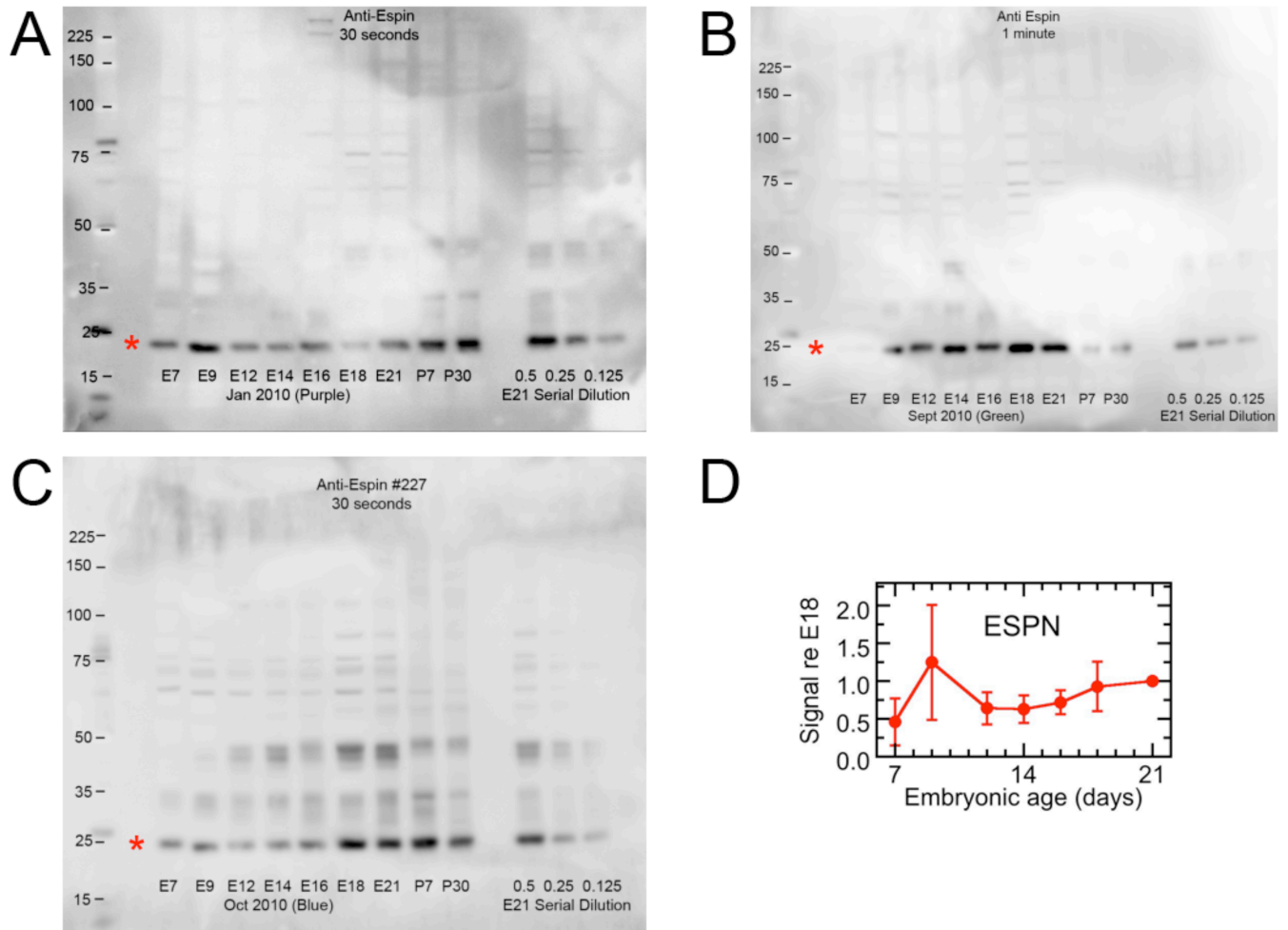
Example of immunoblot analysis of chick cochlea developmental time points using pan-PLS antibody #185. Molecular mass markers are indicated on the left, and developmental age is indicated for each lane on the bottom. The red asterisk indicates the band that was quantified. Identical amounts of total protein were loaded for each developmental age.



**Figure S4. Developmental immunoblot profile of PLS3 expression in developing chick cochlea.**

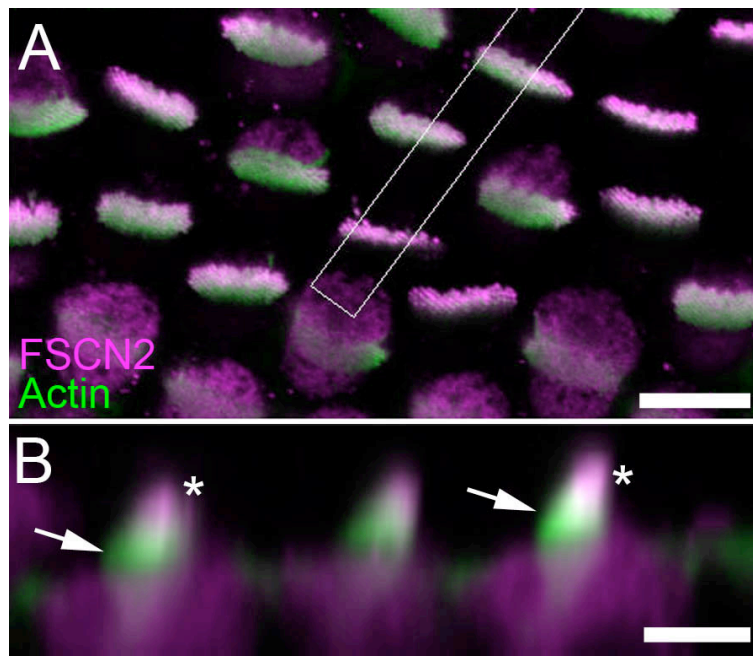
A, Immunoblot detection of PLS3. Developmental age is indicated for each lane on the bottom. D, Quantitation of PLS3 in the blot in panel B. The signal at each developmental age was plotted relative to the signal at E21. Identical amounts of total protein were loaded for each developmental age.





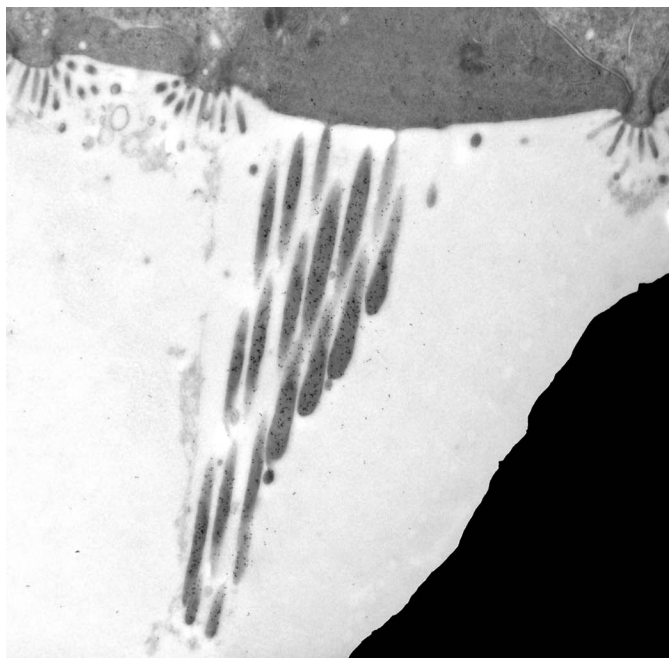
**Figure S5. Developmental immunoblot profiles of espin expression in developing chick cochlea.**

A-C, Examples of espin detection. Molecular mass markers are indicated on the left, and developmental age is indicated for each lane on the bottom. The red asterisk indicates the band that was quantified. D, Quantitation of espin in three experiments. The signal at each developmental age was plotted relative to the signal at E18. Identical amounts of total protein were loaded for each developmental age.



**Figure S6. Example of FSCN2 bundle profile staining.**

A, Single slice of stack through E16 cochlea base region. FSCN2 and actin channels are indicated; scale bar is 10  $\mu\text{m}$ . Box indicates region chosen for XZ reslice. B, XZ reslice; average z-projection. Asterisks indicate strong FSCN2 staining in upper half of the hair bundle; arrows indicate weak FSCN2 staining at bundle bases. Images are indistinct because of averaging and the limited z-axis resolution of the confocal microscope.



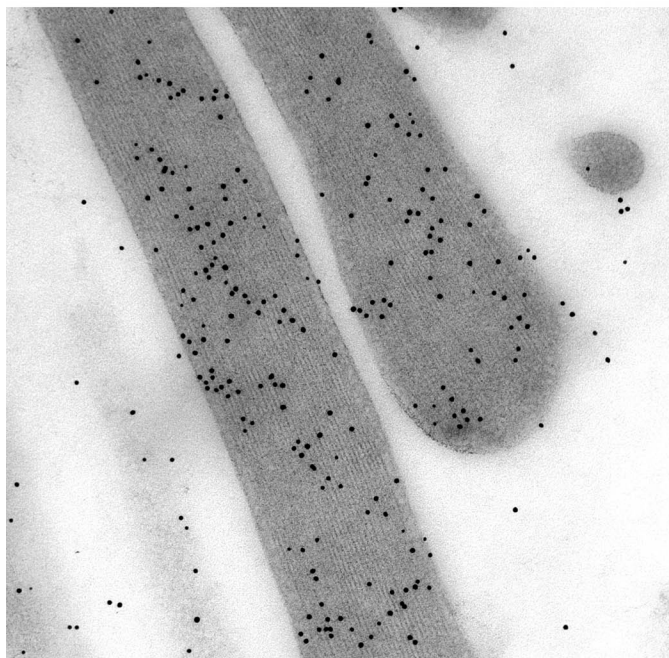
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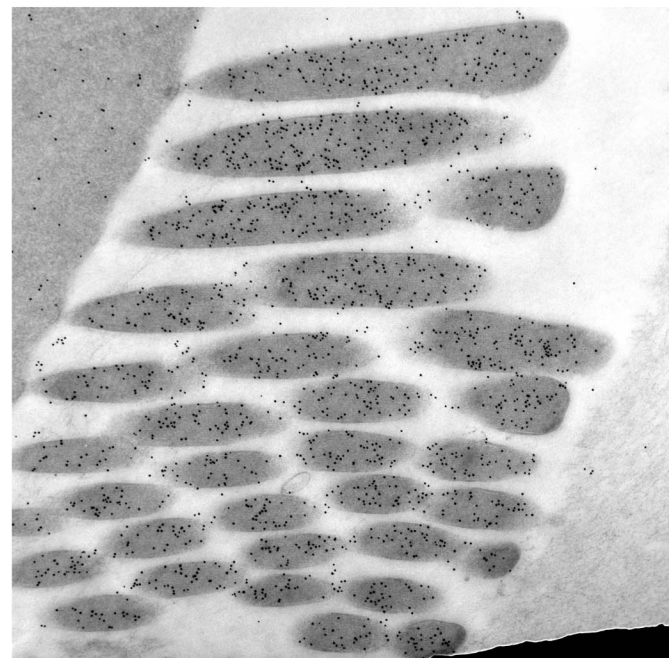
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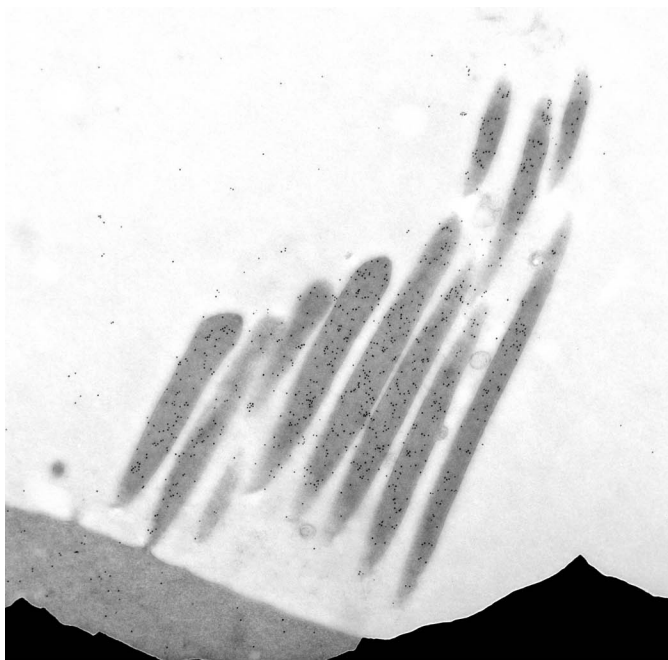


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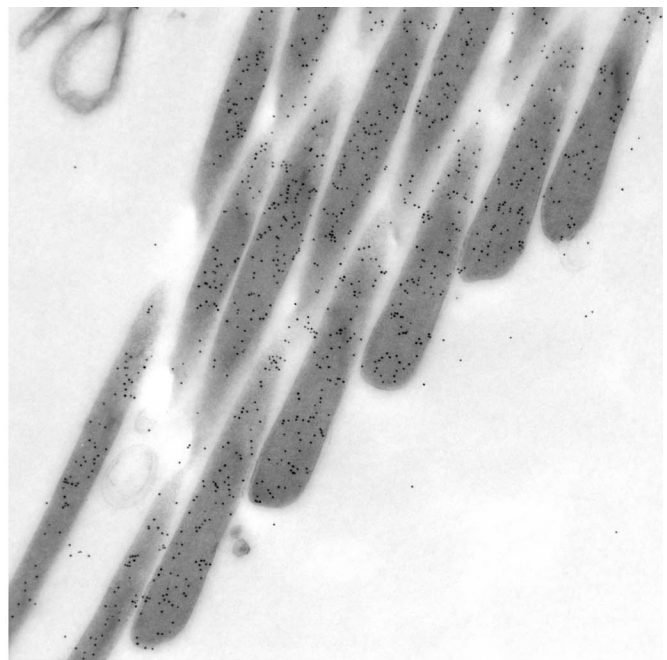
**Figure S7. Additional immunogold TEM images showing FSCN2 distribution in cochlea.**

Immunogold electron microscopy with anti-FSCN2 antibodies. Note that the density of labeling appears uniform within stereocilia. Scale bars are indicated for each panel.



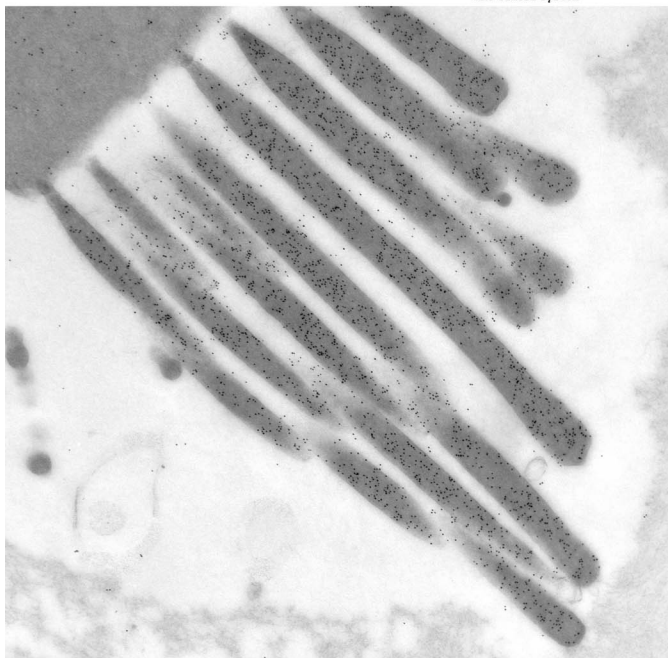
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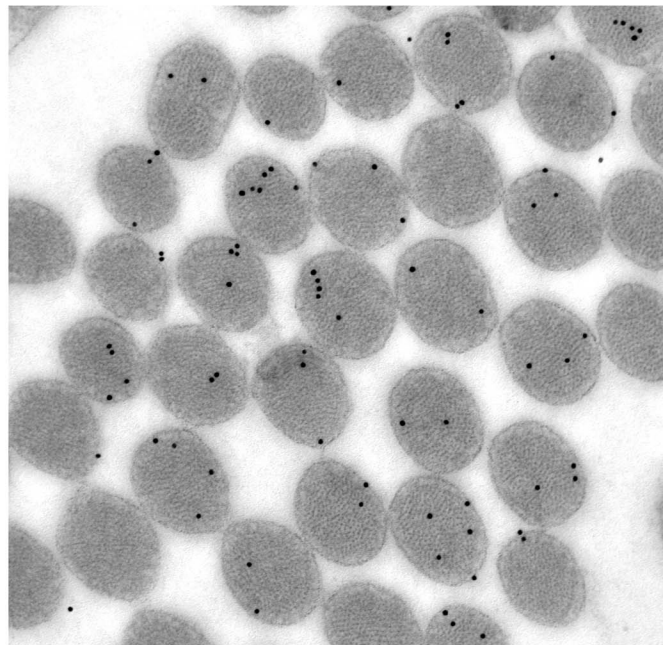
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**Figure S8. Additional immunogold TEM images showing FSCN2 distribution in cochlea.**

More immunogold electron microscopy with anti-FSCN2 antibodies. Note that the density of labeling appears uniform within stereocilia. Scale bars are indicated for each panel.

**Table S7. Primers used for quantitative PCR analysis**

<b>Gene</b>	<b>Forward</b>	<b>Reverse</b>
FSCN1-a	GACTCCAACCGCTCCTCCTACGAC	AAAAAGAAGTCCACGGGCGTATCG
FSCN1-b	GTCCACCGCCTCCACAAAGAAC	TCCGTCTCACCTGCTGTCTCCAT
FSCN2-a	GTGGGGGAGGACGAGGAGTTCAC	GTAGACGGAGCGGTTGGAGTCG
FSCN2-b	CAGCCAACGGCAGATTCGTCTC	CTGGAAGGTCTCGTGGTTCAGC
PLS1-a	GTTGCTCGAAAAATTGGTGCTCGT	CCTCTTCCCATCAAACATGCAAACA
PLS1-b	TGAAATGACTCGTGTGCCAGTTGAC	CCAGCAATACCGACCAGGGAGAAT
PLS2-a	TTGGTGGTGGAGAGAAGGTGAACG	ATGCTGGTGTGATTTTGCCATC
PLS2-b	TCGTACAGCCACTGATGTTGTCC	TCCTCTTCCCTTGTCTCACCTTC
PLS3-a	ATTCTCCCTGGTTGGCATTGGA	ATGTCGTCGTTGGCTTTCTGACC
PLS3-b	CAGTGAGGGAACGCAGCACTCATA	CAGGGTCATTTTCCAGGGCTTTGT
GAPDH-a	GCTAAGGCTGTGGGGAAAGT	CTTGGCTGGTTTCTCCAGAC
CAPZA-a	CATCACACCACCAACAGCTC	GCTGTCTGAACATCGCTTGA
CAPZB1-a	ACTCTTCTCCGCACATAGCC	TTGTCGGGGATAGCATCAAT
CAPZB2-a	ACTCTTCTCCGCACATAGCC	CTCTTCAAAGCCTCCACCAG