Supplemental Materials

Molecular Biology of the Cell

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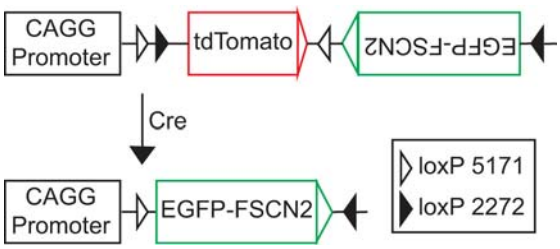
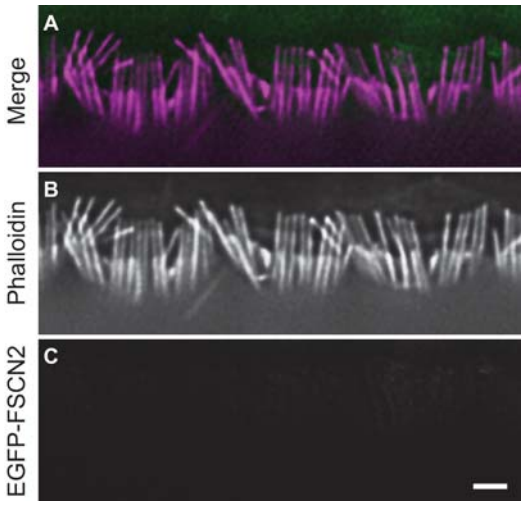
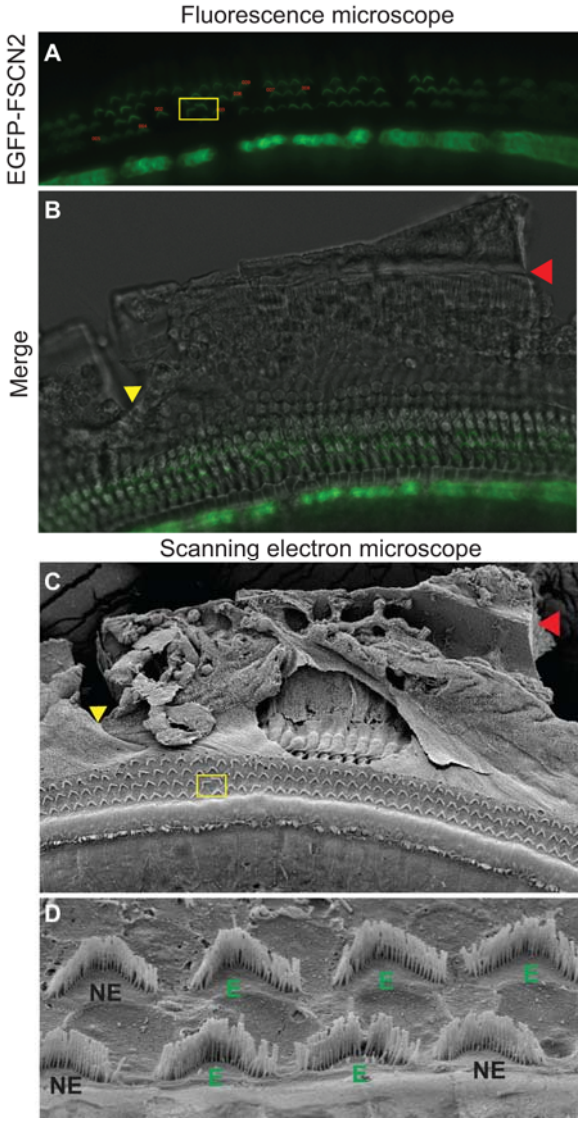


Supplemental figure 1:Schematic diagram representing Cre-mediated induction of EGFP-FSCN2. Cre-mediated recombination inverts EGFP-FSCN2 from its antisense to sense orientation, while deleting tdtomato. This results in irreversibleexpression of EGFP-FSCN2.



Supplemental figure 2: Tamoxifen is required for CreER to induce EGFP-FSCN2. A) Merged image of EGFP-FSCN2 and phalloidin stained F-actin. B) Phalloidin staining only. C) EGFP-FSCN2, which was undetectable. In the merged image, phalloidin is in magenta. Scale bar=1 µm.



Supplemental figure 3: CorrelativeSEM**.** A) EGFP-FSCN2, marking several pairs of non-expressing and expressing EGFP-FSCN2 OHCs. B) Fluorescence merged with brightfield. C) SEM. Landmarks used to identify specific regions of the cochlear turn are indicated by yellow and red arrow heads while the yellow rectangle highlights distinctive stereocilia bundles.D) Magnified view of representative bundles not-expressing and expressing EGFP-fascin2 (marked NE and E respectively).

Supplemental table 1: Percentage of outer hair cells, inner hair cells and utricular hair cells expressing EGFP-FSCN2.

FLEx-EGFP-FSCN2 transgenic mouse line	% of expressing cells in inner hair cells	% of expressing cells in outer hair cells	% of expressing cells in utricular hair cells
Line C	68%	64%	62%
Line E	93%	88%	80%
Line F	98%	96%	95%