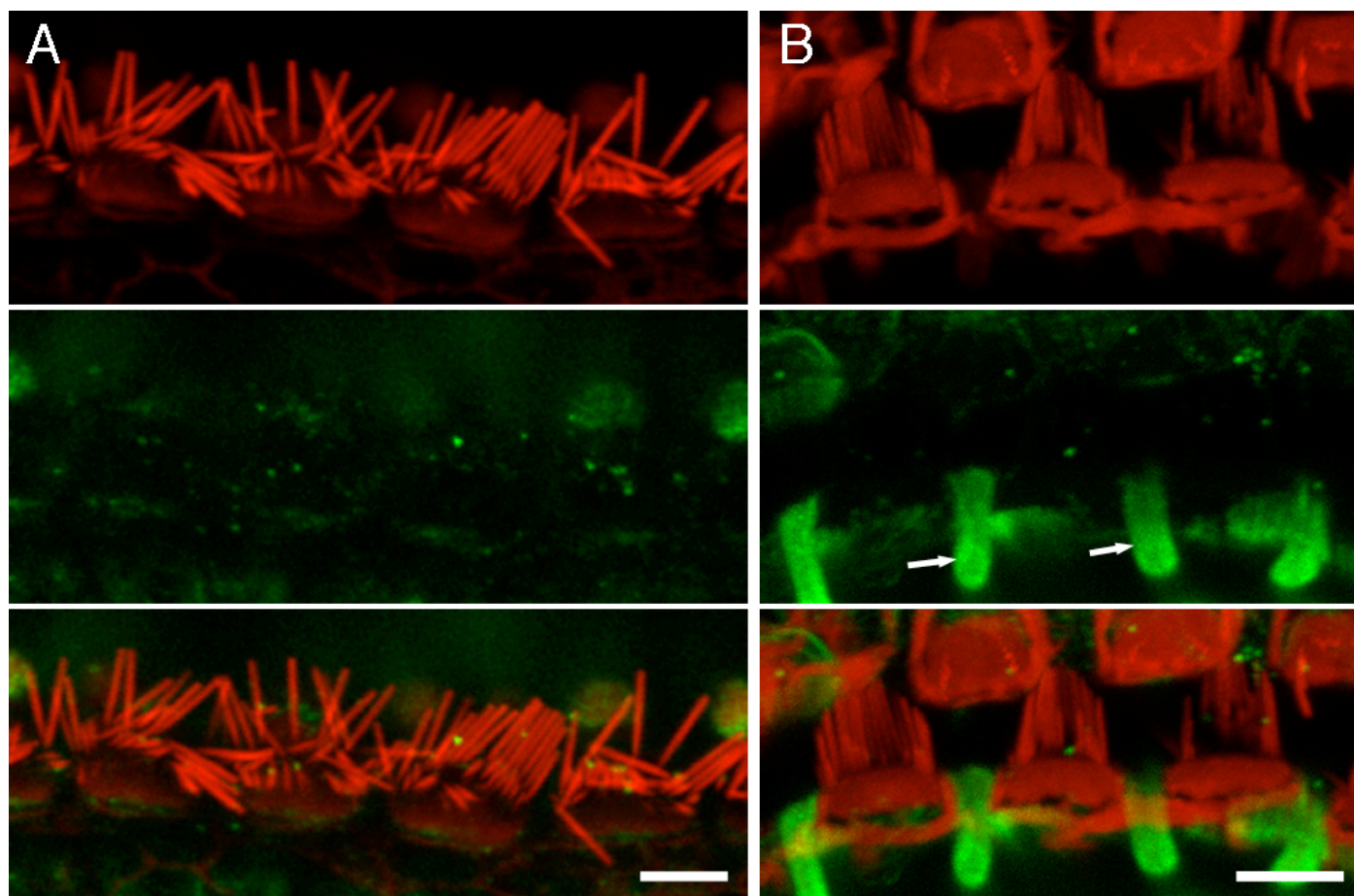


## Supplementary Information



**Supplementary Figure S1.** Twinfilin 1 labelling is absent in cochlear stereocilia. Twinfilin 1 staining (green) of cochlear stereocilia is absent in both (A) P28 inner hair cells and (B) P50 outer hair cells. Staining is strongly present in Deiters' cell phalangeal processes (arrows). Stereocilia F-actin is labeled with TritC-phalloidin (red). Scale bar = 5 $\mu$ m.

CLUSTAL 2.0.10 multiple sequence alignment

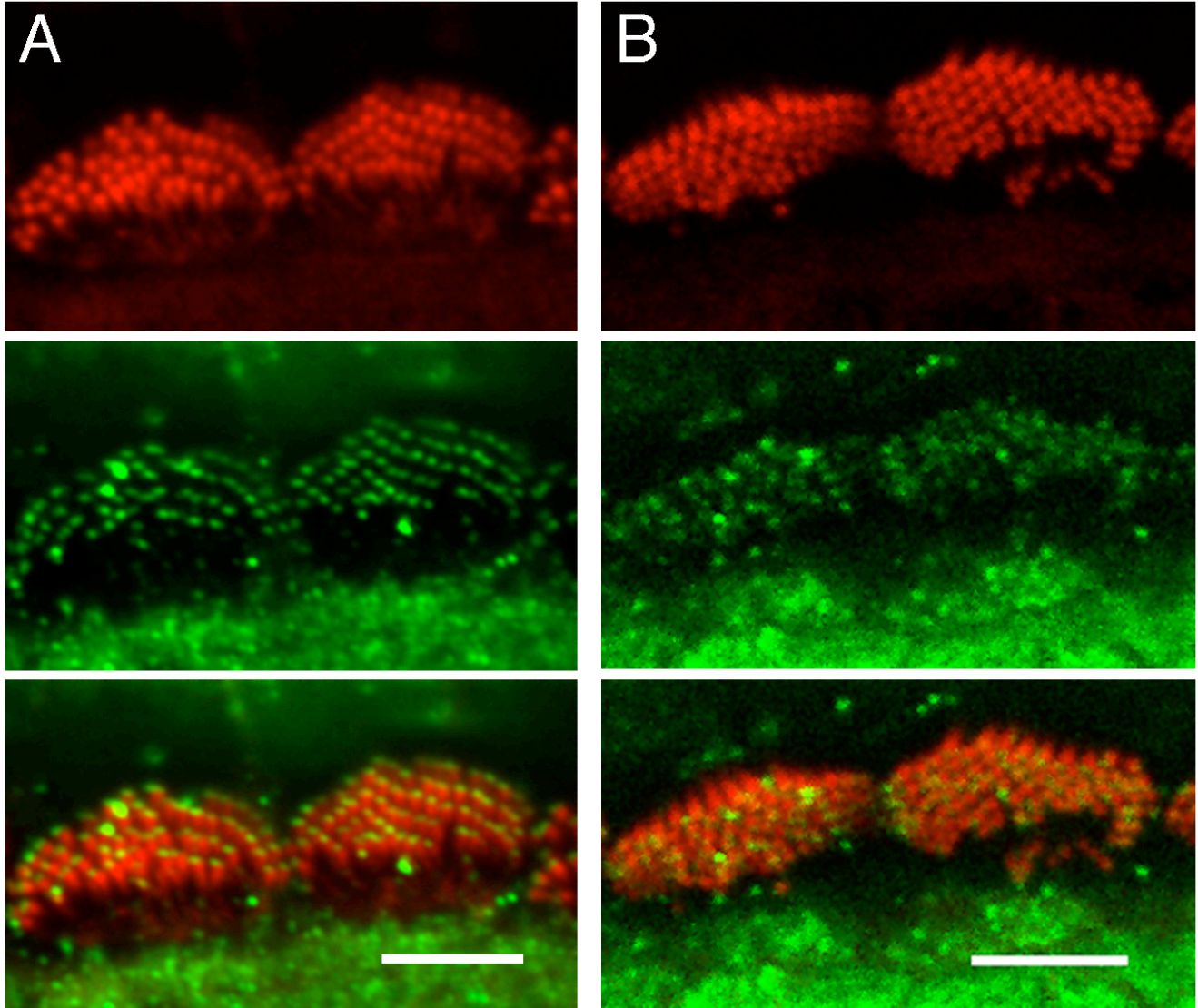
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sp|Q9Z0P5|TWF2_MOUSE     MAHQTGIHATEELKEFFAKARAGSIRLIKVIEDEQLVLGASQEPVGRWDQDYDRAVLPL 60
*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*
                                     _____
sp|Q5ZM35|TWF2_CHICK      LDEQQPCYVLYRLDSQNAQGYEWLFISWSPDNSPVRLKMLYAATRATVKKEFGGGHIKDE 120
sp|Q9Z0P5|TWF2_MOUSE     LDAQEPCYLLFRLDSQNAQGFEWFLAWSPDNSPVRLKMLYAATRATVKKEFGGGHIKDE 120
** *:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*
                                     _____
sp|Q5ZM35|TWF2_CHICK      MFGTVKEDVSLSGYQKHVSSCSAPAPLTAAEQELQQIRINEVKTEISVESKHQTLQGLAF 180
sp|Q9Z0P5|TWF2_MOUSE     LFGTVKDDLSLAGYQKHLSSCAAPLTSAERELQQIRINEVKTEISVESKHQTLQGLAF 180
*:*****:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*
                                     _____
sp|Q5ZM35|TWF2_CHICK      PLQLDAQQAIQTLKQKKNINYIQLKLDLERETIDLVHTSPTDISDLPKRIPQDSARYHFFL 240
sp|Q9Z0P5|TWF2_MOUSE     PLQPEAQRALQQLKQKTVNYIQLKLDLERETIELVHTEPTNVAQLPSRIPRDAARYHFFL 240
*** *:*:*:*:* * * * * * . : * * * * * * * * * * * * * * * * * * * * * *
                                     _____
sp|Q5ZM35|TWF2_CHICK      YKHSHEGDYLESVVFIIYSMPGYKCSIKERMLYSSCKSRLLDTVEQFCLEIAKKIEIDDG 300
sp|Q9Z0P5|TWF2_MOUSE     YKHTHEGDALESVVFIIYSMPGYKCSIKERMLYSSCKSRLLDSVEQDFOLEIAKKIEIGDG 300
***:*:*:* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
                                     _____
sp|Q5ZM35|TWF2_CHICK      AELTAEFLYDEVHPKQHAFKQAFAPKGPVGKRGQKRLIKGPGENGEDS 349
sp|Q9Z0P5|TWF2_MOUSE     AELTAEFLYDEVHPKQHAFKQAFAPKGPGGKRGHKRLIRGPGENGEDS 349
*****:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*

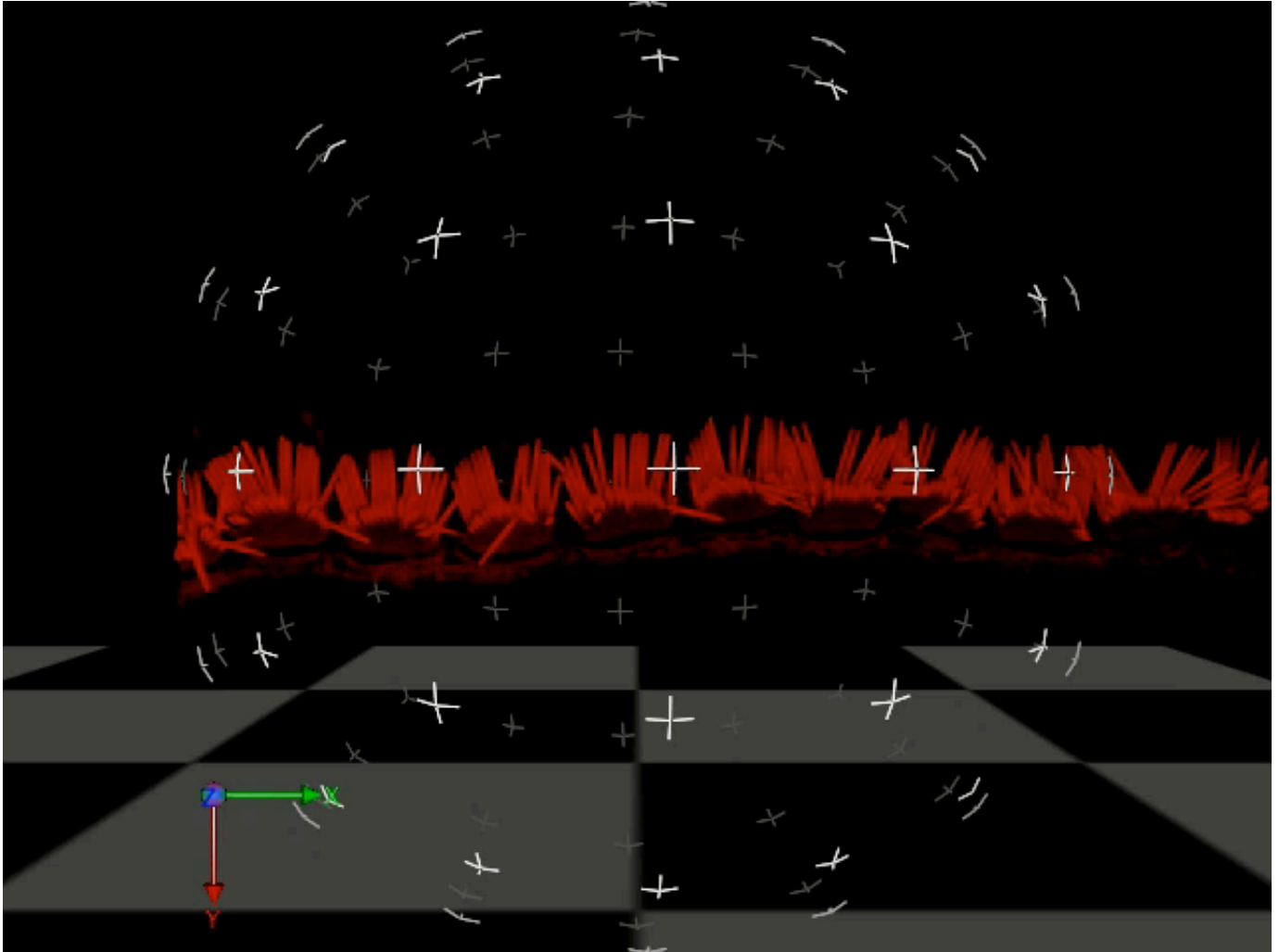
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**Supplementary Figure S2.** Clustal alignment of chicken and mouse twinfilin 2 sequences. Red bar above sequence indicates predicted actin depolymerizing factor-homology domain (according to the NCBI conserved domain database (Marchler-Bauer et al., 2009)). Blue bar indicates the carboxyl-terminal tail region.

Marchler-Bauer A et al. (2009) CDD: specific functional annotation with the Conserved Domain Database. *Nucleic Acids Res* 37:D205-210.



**Supplementary Figure S3.** Twinfilin 1&2 is localized to the tips of all stereocilia in *shaker 2* and *whirler* mice. (A) P13 *shaker 2* mouse and (B) P12 *whirler* mouse inner hair cells showing twinfilin 1&2 labelling (green) and rhodamine phalloidin to visualize stereocilia (red). Scale bars = 5 $\mu$ m



**Supplementary Movie.** Movie illustrating how length measurements were done using Velocity (Improvisation Inc., Waltham, MA). 3D rendering is used to visualize the structure, and lines are drawn where stereocilia are present to determine the lengths of stereocilia.