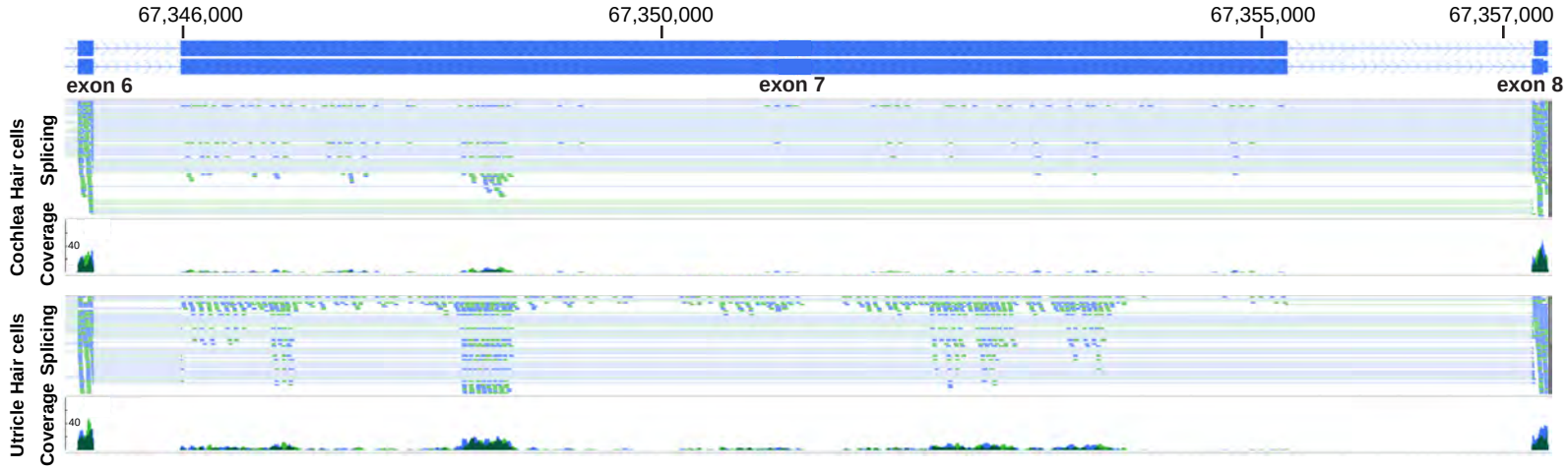


Figure S1 related to Figure 2. RNA-Seq read alignments for the *Xirp2* gene in cochlear and utricular hair cells, for exons 6-8.

The five panels are (from top to bottom) reference sequences, cochlea splicing, cochlea coverage, utricle splicing, and utricle coverage. The Y axis is the raw depth of the coverage of the alignments. Blue represents regions covered by reads in the forward direction, light green the reverse direction, and dark green in both directions. Reads covering exon 6-8 junctions as well as reads spanning exons 6-7 and 7-8 were also found. The coverage difference between exons 9, 8, 6 and exon 7 illustrates the presence of both XIRP2 short isoforms and long isoforms in hair cells, with XIRP2 short isoform being predominant.

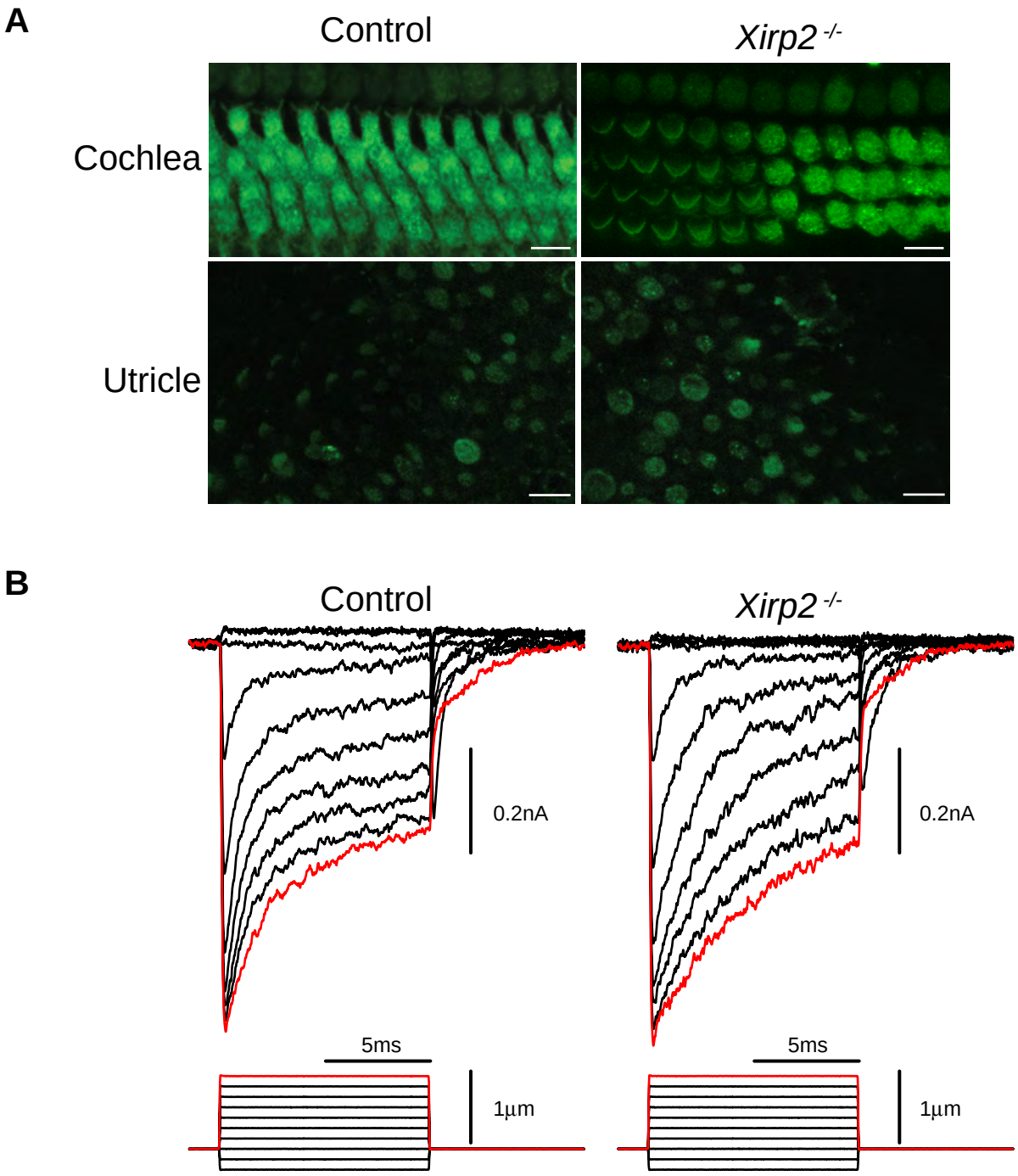
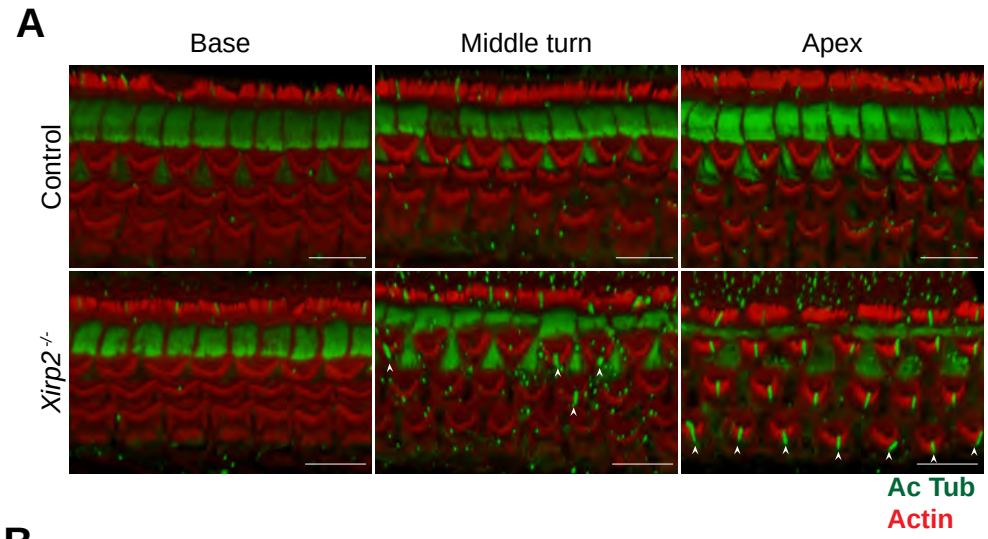


Figure S2 - related to Figure 3. Normal Transduction in *Xirp2* $^{-/-}$ Mice.
(A) FM1-43 uptake in control and *Xirp2* $^{-/-}$ cochleas (top panel) and utricles (lower panel) at P5. Transduction assessed by dye loading was not affected in *Xirp2* $^{-/-}$ animals. Scale bars = 10 µm.
(B) Mechanotransduction recordings from control and *Xirp2* $^{-/-}$ outer hair cells at P5+2 days in vitro. Stiff probe deflection of hair bundles with 10-ms steps of up to 1 µm elicited inward currents exceeding 700 pA, and there was no significant difference in peak amplitude between control and knockout at this age.



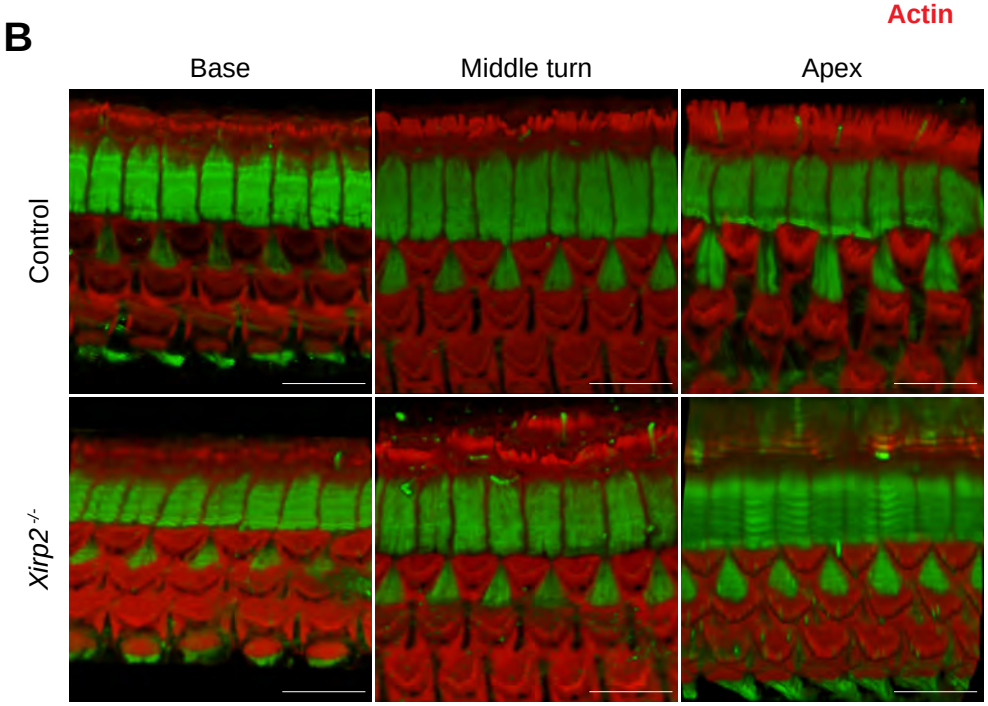


Figure S3 - related to Figure 3. Abnormal Persistence of Kinocilia in *Xirp2* $^{-/-}$ mice
Whole mount immunostaining of cochleas from control and *Xirp2* $^{-/-}$ mice showing acetylated tubulin (green) and phalloidin counterstaining for actin (red).
(A) At P9, kinocilia were absent from all control OHCs from base to apex. In *Xirp2* $^{-/-}$ mice, kinocilia (arrowheads) remained in some first-turn and all apical OHCs.
(B) At P11, OHCs did not exhibit kinocilia in either control or *Xirp2* $^{-/-}$ mice.
Scale bars = 10µm.

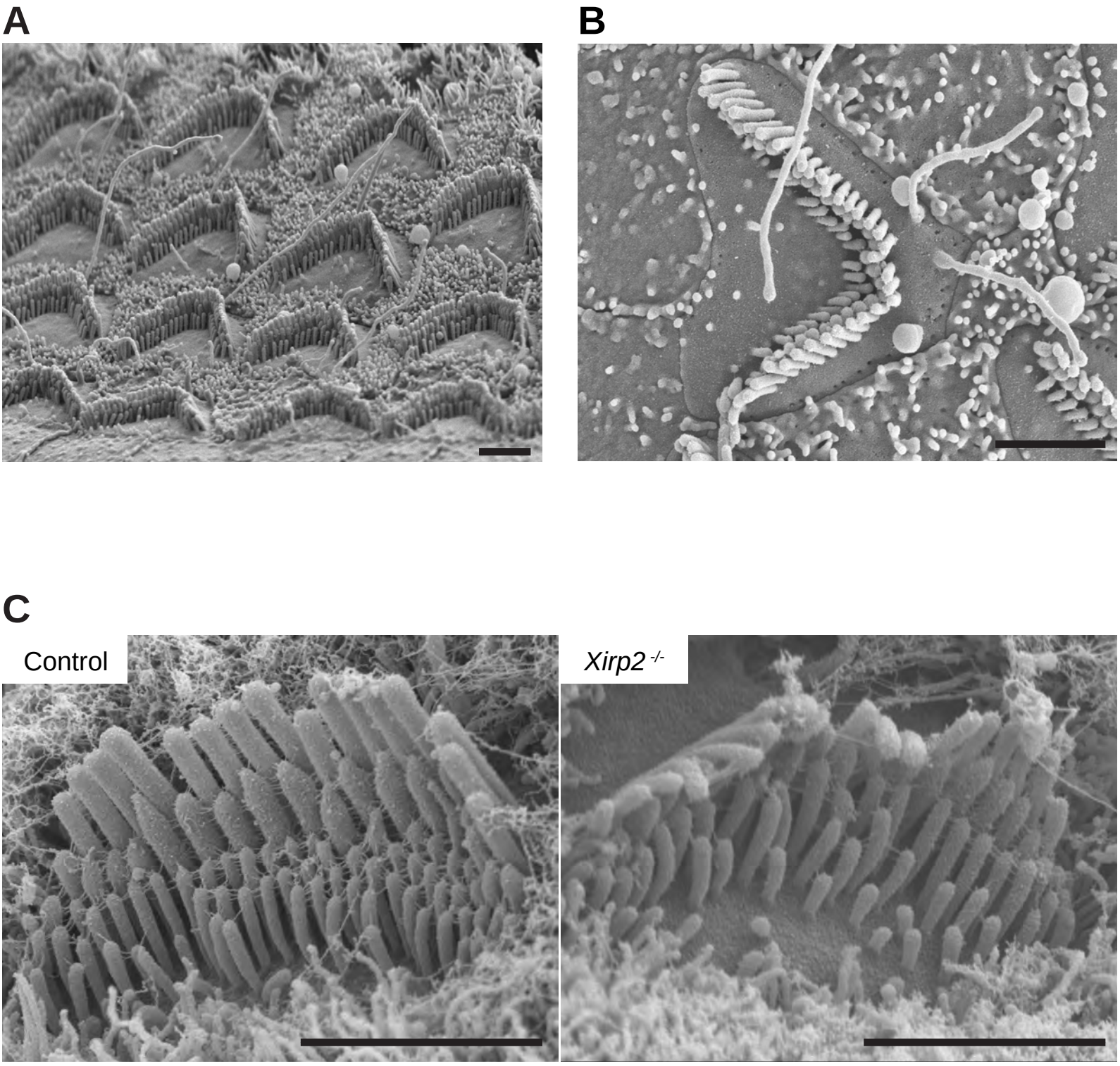


Figure S4 - related to Figure 3. Membrane Protrusions at the Hair Cell Surface in *Xirp2* $^{-/-}$ Mice
(A) SEM image of the OHC region of a *Xirp2* $^{-/-}$ cochlea at P11. Note the length of the protrusions.
(B) Top view of a single OHC showing the presence of two protrusions at the fonticulus region, and a third coming from an adjacent cell.
(C) Control and *Xirp2* $^{-/-}$ P5 IHCs without osmium treatment. Note the curling of the *Xirp2* $^{-/-}$ stereocilia induced by the electron beam. Scale bars = 2 µm.

Supplemental Experimental Procedures

PCR Primers

mXirp2 exon9 (primer c in Fig. 1B and 2)		10165R GGCTTGCAGTATATTCGTCCG
mXirp2 exon7 (primer b in Fig. 1B and 2)	9921F CAGCGCGTGGGATATGAAACC	
mXirp2 exons2/3 (primer a in Fig. 2)	286F GCAGCTTCTCGGCTAATGTCA	
mGAPDH	CTCATGACCACAGTCCATGC	AGGTCCACCACCCTGTTGC

Transmission electron microscopy analysis

The images were analyzed by using two sets of custom computation functions created in MATLAB (MathWorks) in addition to a software suite created for automated file sorting and regions of interest cropping. First, we selected a region of interest within a homogeneous region of the actin core, and performed an autocorrelation on each stereocilium image in two dimensions. Aligning the Y direction with the long axis of each stereocilium, we plotted the autocorrelation in the X direction. We then calculated the power spectral density (PSD) using a Fourier transform in the X direction. Each stereocilium image was subdivided along the Y axis into segments of 100 pixels (56.1 nm). The image intensities were then averaged along the Y

direction for each segment, giving a single average profile in the X direction. The PSD of each segment's X profile was then calculated using Welch's method (MATLAB function PWELCH) with a moving window size of 129 pixels (72.4 nm). All segmental PSDs were averaged to create a single PSD for each stereocilium. This allowed a calculation of local periodicity without loss of signal from long-range inhomogeneity.

Immunohistochemistry

Antibodies: Primary antibodies were rabbit polyclonal anti-XIRP2 raised against Xin domains (Huang et al., 2006), against C-terminal amino acids 3255-3278 of XIRP2 isoform 2 and 3256-3300 of XIRP2 isoform 1 (used at 1/200) (Wang et al., 2010), mouse monoclonal anti-MYO7A (1/2000) (Developmental Studies Hybridoma Bank, University of Iowa #138-1), mouse monoclonal anti-acetylated tubulin (1/2000) (Sigma-Aldrich #T6793), rabbit polyclonal anti-PMCA2 (1/200). Secondary antibodies were Alexa Fluor 488 or 594 goat anti-rabbit or anti-mouse antibodies (Invitrogen). Hair bundles were counterstained with Alexa Fluor 594 phalloidin (Invitrogen).