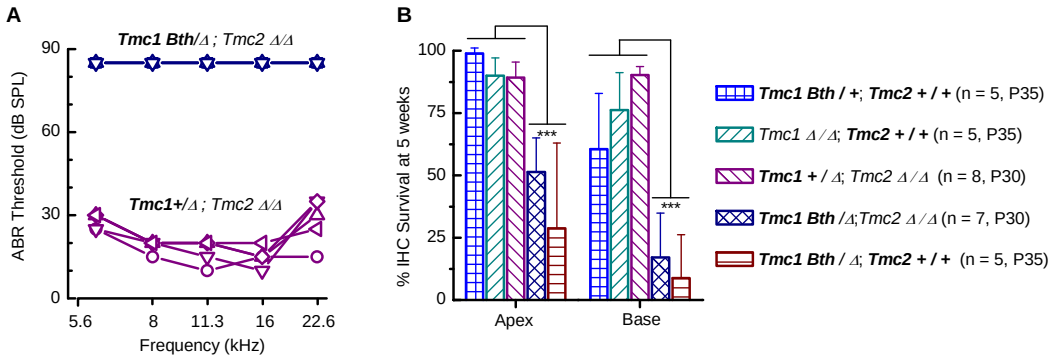
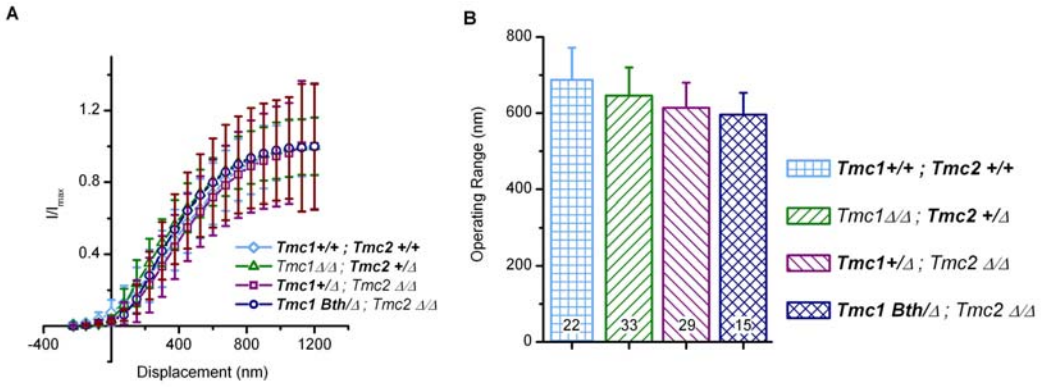
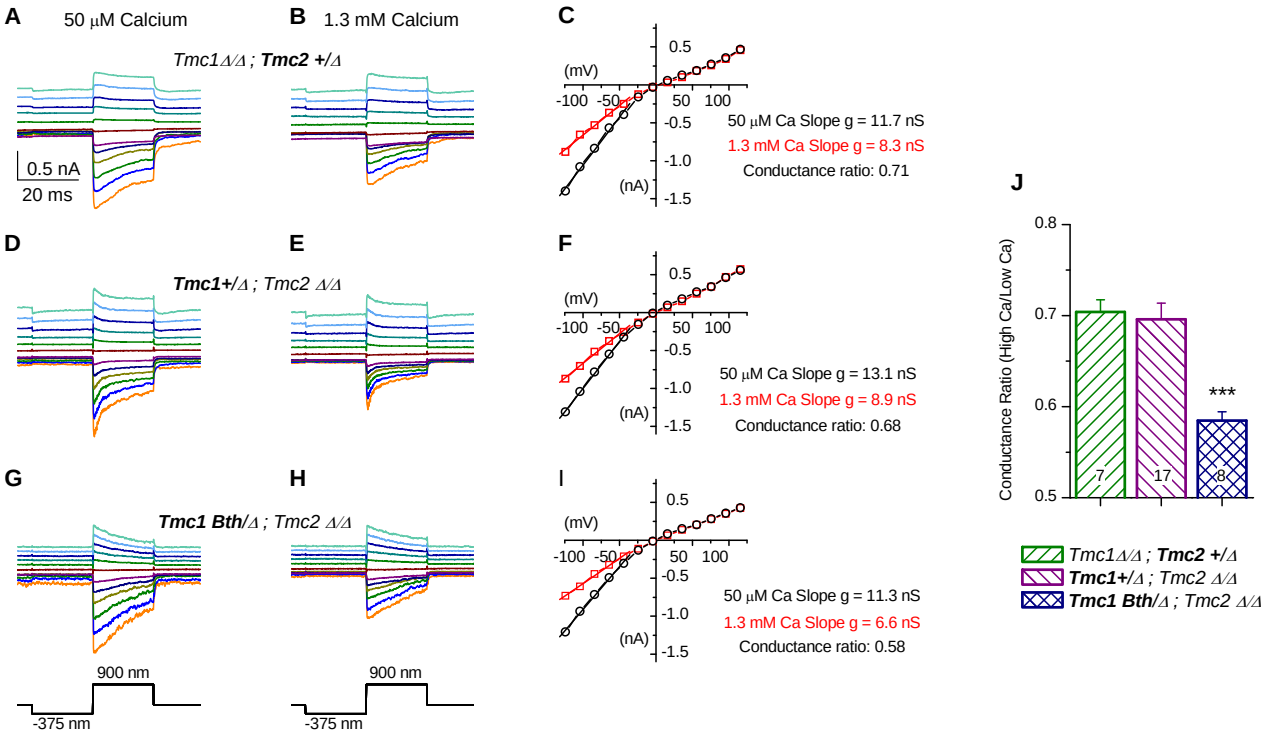
Supplementary Figures



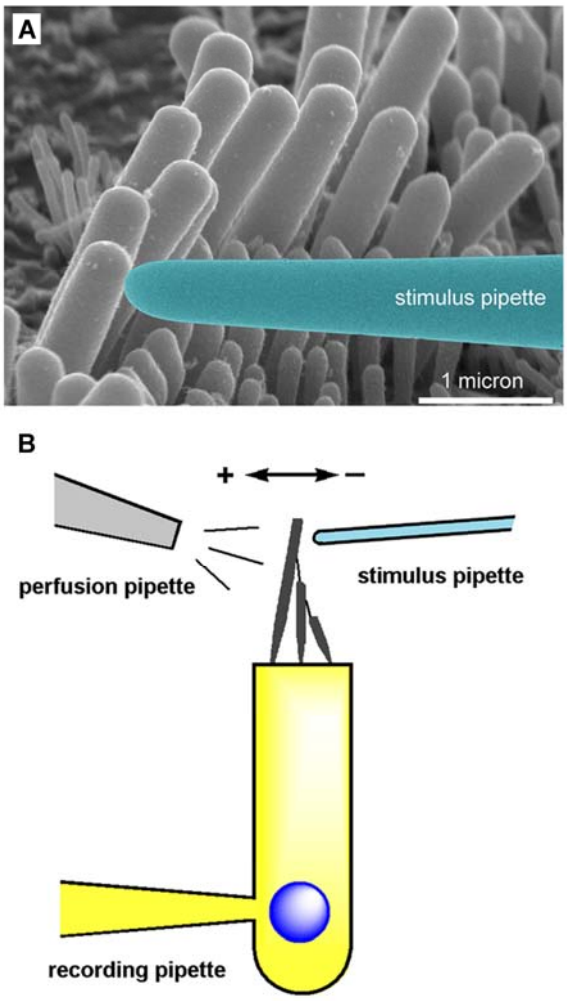
Supplementary Figure 1 Auditory brainstem responses and inner hair cell counts in *Tmc1* mutant mice. (A) ABR thresholds plotted as function of stimulus frequency for five $Tmc1^{+/\Delta}$ mice and four $Tmc1^{Bth/\Delta}$ mice at postnatal day 30. $\boldsymbol{Tmc1^{+/\Delta}}$;$Tmc2^{\Delta/\Delta}$ mice (purple) had normal ABRs. $\boldsymbol{Tmc1^{Bth/\Delta}}$;$Tmc2^{\Delta/\Delta}$ mice (blue) had no detectable ABRs at any frequency or sound pressure level (dB SPL). Wild-type control ABR data are shown in Figure 3C of Kawashima et al. (2011). (B) Inner hair cell counts from cochleas excised at 4-5 weeks of age from mice of the genotypes indicated. The tissue was fixed and stained with rhodamine-conjugated phalloidin to illuminate hair bundles. Number of mice and age tested is indicated in the legend. Cells with hair bundles present were scored positive for survival. The data revealed a significant loss (***) of inner hair cells in $\boldsymbol{Tmc1^{Bth/\Delta}}$ mice regardless of the *Tmc2* genotype, consistent with a gain-of-function mutation. $\boldsymbol{Tmc1^{Bth/+}}$, $Tmc1^{\Delta/\Delta}$ and $\boldsymbol{Tmc1^{+/\Delta}}$ hair cell counts were not significantly different from each other.



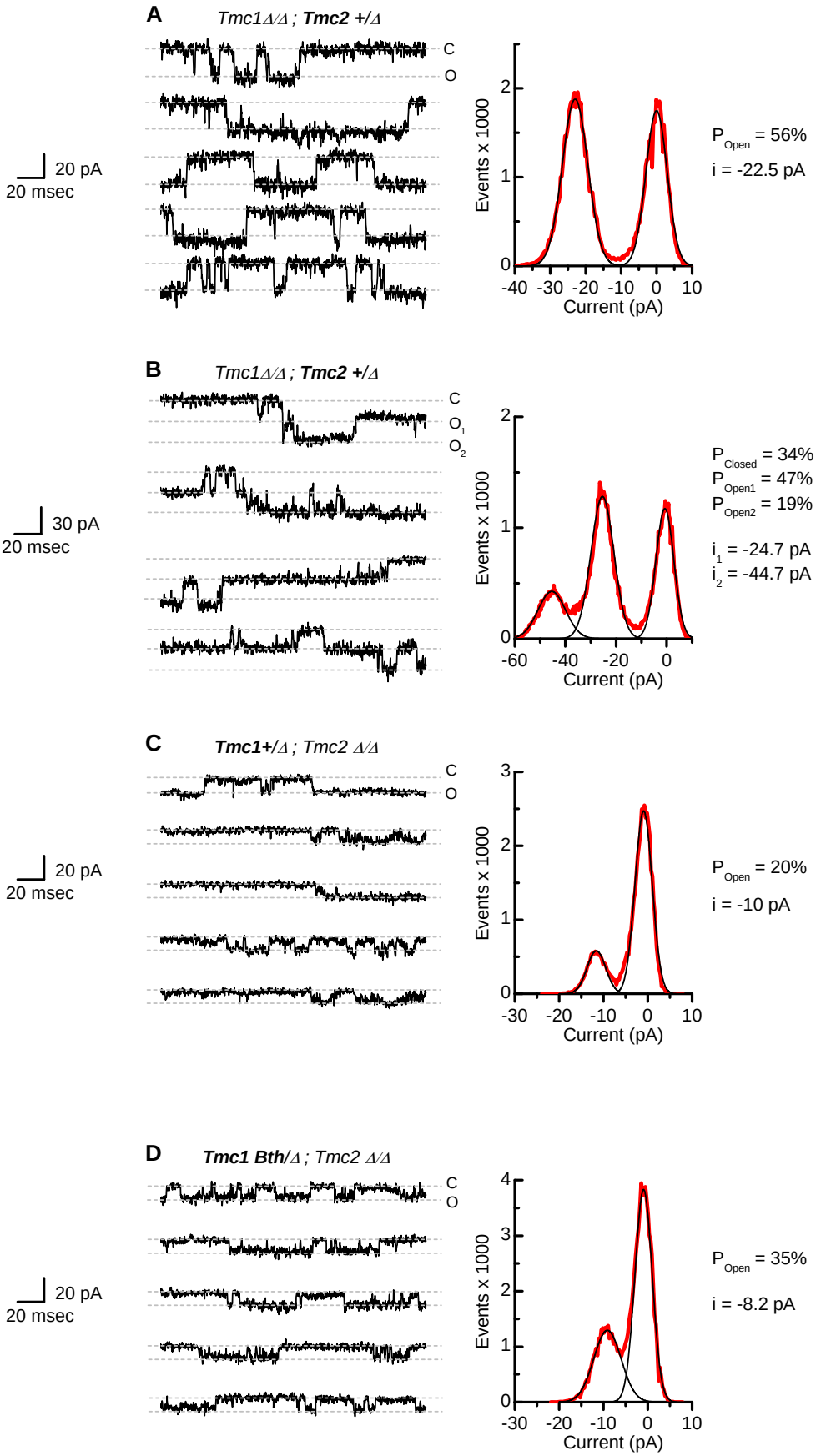
Supplementary Figure 2 Stimulus-response relationships for mechanosensitive currents recorded from *Tmc* mutant hair cells. (A) Peak values from families of transduction currents, as shown in Fig. 1, were plotted as a function of stimulus amplitude. Data from 15 to 33 P0-P7 inner hair cells were averaged for each genotype. Mean values ± s.d. were fitted with a 2nd order Boltzmann equation. (B) The 10-90% operating range was measured from the Boltzmann curves of 99 inner hair cells. Mean operating ranges (+1 s.d.) are plotted on the bar graph for each genotype. Number of samples is indicated at the bottom.



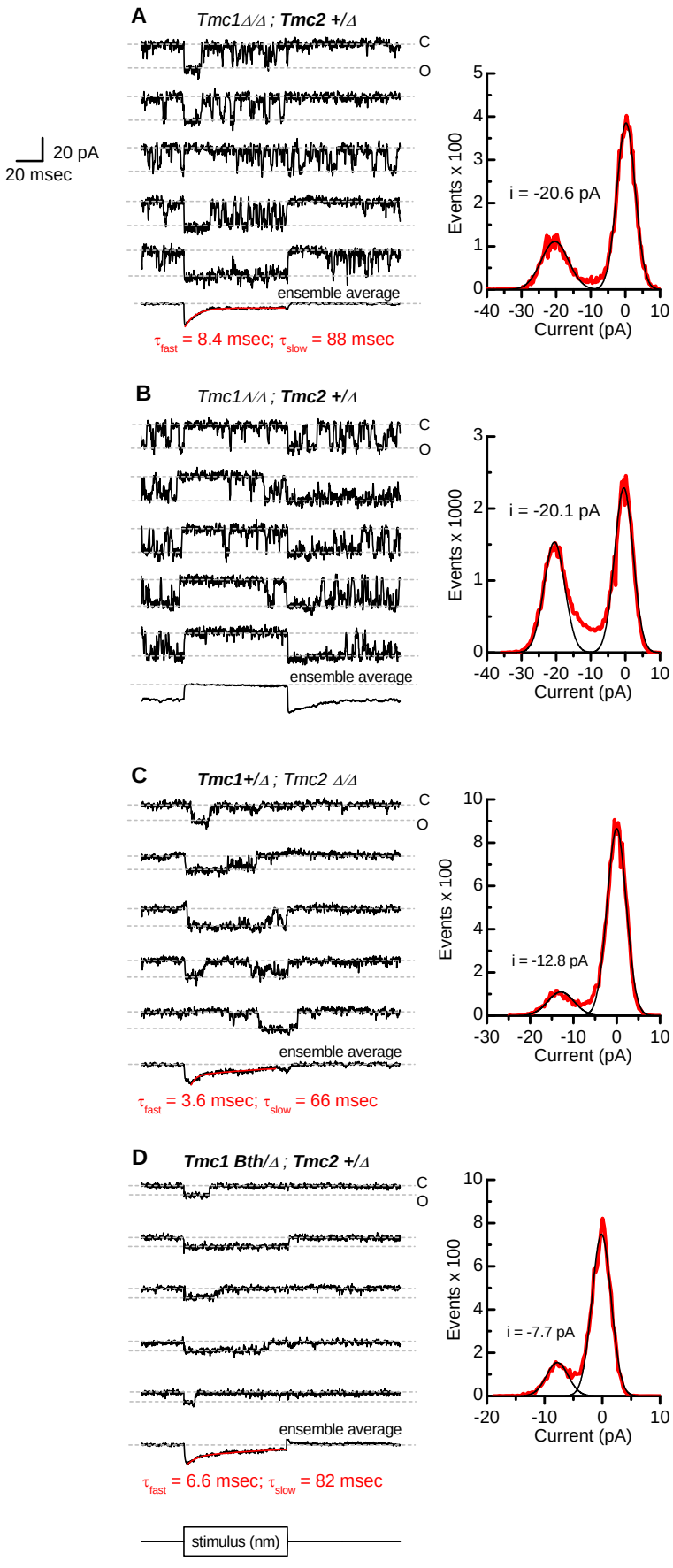
Supplementary Figure 3 Whole-cell mechanotransduction current-voltage relationships in endolymph and perilymph calcium concentrations. (A-B), Families of transduction currents recorded from a representative $Tmc1^{\Delta/\Delta}$;$\boldsymbol{Tmc2^{+/\Delta}}$ apical inner hair cell at P7. Membrane potential was stepped between −124 and 116 mV in 20-mV increments. Cells were bathed in 50 μM (A) or 1.3 mM (B) Ca^{2+} as indicated. The scale bar at the left and the displacement protocols at the bottom apply to all current families. (C) Peak transduction currents plotted as function of membrane potential for both Ca^{2+} concentrations. The I-V curves were fitted with linear regressions between −124 and −44 mV and slope was taken as the transduction conductance. Conductance ratio was calculated as the slope conductance in 1.3 mM Ca^{2+} divided by the slope conductance in 50 μM Ca^{2+}. (D-E), Current families from a representative $\boldsymbol{Tmc1^{+/\Delta}}$;$Tmc2^{\Delta/\Delta}$ apical inner hair cell at P7. (F) Transduction I-V curves taken from the data shown in panels D and E. (G-H) Current families from a representative $\boldsymbol{Tmc1^{Bth/\Delta}}$;$Tmc2^{\Delta/\Delta}$ apical inner hair cell at P6. (I) Transduction I-V curves taken from the data shown in panels G and H**.** (J) Mean conductance ratio (+1 s.e.) for the genotypes indicated. Number of samples is indicated below. Conductance ratios from $\boldsymbol{Tmc1^{Bth/\Delta}}$;$Tmc2^{\Delta/\Delta}$ cells were significantly (***P<0.001*) less than those measured from $\boldsymbol{Tmc1^{+/\Delta}}$;$Tmc2^{\Delta/\Delta}$ cells, indicating Ca^{2+} block was more effective in $\boldsymbol{Tmc1^{Bth/\Delta}}$;$Tmc2^{\Delta/\Delta}$ cells.



Supplementary Figure 4 Montage illustrating the stimulation protocol for deflecting single stereocilia and measuring single transduction channel currents. (A) Scanning electron micrograph of a P14 inner hair bundle with an image of a stiff glass stimulus pipette (blue) superimposed to illustrate the method of stimulation. The tissue was excised, fixed and prepared for SEM imaging using the OTOTO method (Hunter-Duvar, 1978) with some minor modifications (Lelli et al., 2010). Scale bar = 1 µm. (B) Schematic diagram illustrating the recording configuration. Stimulus, perfusion and recording pipettes are shown.


A
Tmc1Δ/Δ ; Tmc2 +/Δ
C
O
20 pA
20 msec
Events x 1000
Current (pA)
P Open = 56%
i = -22.5 pA
B
Tmc1Δ/Δ ; Tmc2 +/Δ
C
O1
O2
30 pA
20 msec
Events x 1000
Current (pA)
P Closed = 34%
P Open1 = 47%
P Open2 = 19%
i1 = -24.7 pA
i2 = -44.7 pA
C
Tmc1+/Δ ; Tmc2 Δ/Δ
C
O
20 pA
20 msec
Events x 1000
Current (pA)
P Open = 20%
i = -10 pA
D
Tmc1 Bth/Δ ; Tmc2 Δ/Δ
C
O
20 pA
20 msec
Events x 1000
Current (pA)
P Open = 35%
i = -8.2 pA

Supplementary Figure 5 Single-channel currents recorded from inner hair cells that displayed spontaneous activity. (A) Five representative traces recorded in 50 µM Ca^{2+} at −84 mV from a P3 apical inner hair cell of a *Tmc1*$^{\Delta/\Delta}$;***Tmc2***$^{+/\Delta}$ mouse in the absence of mechanical stimulation. Closed (C) and open (O) states are indicated by the dashed lines. An event histogram was generated (right, red trace) and fitted with two Gaussians (black traces) that had peaks at of 0 and −22.5 pA and widths of 6 and 7.2 pA. For spontaneously active channels we estimated open probability by integrating the area under the Gaussian curves for the closed and open states. For this channel P_{Open} = 0.56. (B) Four representative traces recorded from the same cell shown in panel A. Later in the recording we observed two channels that were spontaneously active. The closed state (C), one channel open (O_1) and two channels open (O_2) are indicated by the dashed lines. The event histogram (right, red trace) indicated three prominent peaks. The data were fitted with three Gaussian functions that had peaks at 0, −24.7, and −44.7 pA and widths of 7.1, 9.1 and 10.9 pA. P_{Open} for the three states is indicated on the graph. (C) Representative traces recorded at −84 mV from a P3 basal inner hair cell of a ***Tmc1***$^{+/\Delta}$;*Tmc2*$^{\Delta/\Delta}$ mouse in the absence of mechanical stimulation. Closed (C) and open (O) states are indicated by the dashed lines. An event histogram was generated (right, red trace) and fitted with two Gaussians (black traces) that had peaks at 0 and −10 pA and widths of 3.9 and 4.2 pA. P_{Open} = 0.2. (D) Representative traces recorded at −84 mV from a P7 apical inner hair cell of a ***Tmc1***$^{Bth/\Delta}$;*Tmc2*$^{\Delta/\Delta}$ mouse in the absence of mechanical stimulation. Closed (C) and open (O) states are indicated by the dashed lines. The event histogram was generated (right, red trace) and fitted with two Gaussians (black traces) that had peaks at 0 and −8.2 pA and widths of 3.9 and 6.2 pA. P_{Open} = 0.35. The corresponding scale bar is shown to the left of each dataset.


A
Tmc1Δ/Δ ; Tmc2 +/Δ
C
O
20 pA
20 msec
ensemble average
τfast = 8.4 msec; τslow = 88 msec
Events x 100
i = -20.6 pA
Current (pA)
B
Tmc1Δ/Δ ; Tmc2 +/Δ
C
O
ensemble average
Events x 1000
i = -20.1 pA
Current (pA)
C
Tmc1+/Δ ; Tmc2 Δ/Δ
C
O
ensemble average
τfast = 3.6 msec; τslow = 66 msec
Events x 100
i = -12.8 pA
Current (pA)
D
Tmc1 Bth/Δ ; Tmc2 +/Δ
C
O
ensemble average
τfast = 6.6 msec; τslow = 82 msec
Events x 100
i = -7.7 pA
Current (pA)
stimulus (nm)

Supplementary Figure 6 Single-channel currents recorded from inner hair cells that displayed prominent adaptation. Inner hair cells with adaptation were encountered occasionally, but less frequently than those that did not have prominent adaptation (**Figure 2**). (A) Five representative traces recorded at −84 mV from a P3 apical inner hair cell of a *Tmc1*$^{\Delta/\Delta}$;***Tmc2***$^{+/\Delta}$ mouse. Channel opening was evoked by a +200 nm step deflection. The stimulus protocol is shown at the bottom of panel D. Closed (C) and open (O) states are indicated by the dashed lines. An ensemble average of 100 traces is shown at the bottom (black trace). The data were fitted with a double exponential equation (red trace) with fast and slow time constants as indicated. An event histogram was generated (right, red trace) and fitted with two Gaussians (black traces) that had peaks at of 0.2 and −20.4 pA and widths of 5.3 and 7.9 pA. (B) Five representative traces recorded from the same cell shown in panel A. In this case, a small pipette offset in the positive direction was imposed and channel closure was evoked by a −200 nm step deflection. The stimulus protocol is shown at the bottom of panel D. Closed (C) and open (O) states are indicated by the dashed lines. An ensemble average of 100 traces is shown at the bottom (black trace). An event histogram was generated (right, red trace) and fitted with two Gaussians (black traces) that had peaks at of −0.4 and −20.5 pA and widths of 5.3 and 6 pA. (C) Five representative traces recorded at −84 mV from a P8 apical inner hair cell of a ***Tmc1***$^{+/\Delta}$;*Tmc2*$^{\Delta/\Delta}$ mouse. Closed (C) and open (O) states are indicated by the dashed lines. An ensemble average of 84 traces is shown at the bottom (black trace). The data were fitted with a double exponential equation (red trace) with fast and slow time constants as indicated. An event histogram was generated (right, red trace) and fitted with two Gaussians (black traces) that had peaks of 0 and −12.8 pA and widths of 4.4 and 6 pA. (D) Five representative traces recorded at −84 mV from a P5 basal inner hair cell of a ***Tmc1***$^{Bth/\Delta}$;*Tmc2*$^{\Delta/\Delta}$ mouse. Closed (C) and open (O) states are indicated by dashed lines. An ensemble average of 23 traces is shown at the bottom (black trace). The data were fitted with a double exponential equation (red trace) with fast and slow time constants as indicated. An event histogram was generated (right, red trace) and fitted with two Gaussians (black traces) that had peaks of −0.1 and −7.8 pA and widths of 3.3 and 3.8 pA.