Figures and figure supplements

TMC1 is an essential component of a leak channel that modulates tonotopy and excitability of auditory hair cells in mice

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Figure 1. TMC1 mediates a background current in outer hair cells. (A) Diagram of the recording configuration. The P6 outer hair cells (OHCs, mostly P6 apical-middle OHCs if not specified otherwise) in acutely dissociated cochlea were whole-cell voltage-clamped with Cs⁺ in the recording electrode and
perfused with either 144 Na or 144 NMDG external solutions. 144 Na, regular recording solution; 144 NMDG, Na⁺ substituted with NMDG⁺. (B) Representative traces of membrane current (Iₘ) in OHCs from wild-type and Tmc1-knockout (Tmc1 KO) mice. The light gray and pink traces were recorded traces that were low-pass filtered to less noisy traces shown in black and red (similar filtering applied in the following figures). Iᵦ (background current) was calculated by subtraction of Iₘ in 144 Na (I Na) and Iₘ in 144 NMDG (I NMDG) to exclude technical leak. (C–E) Quantification of the I Na (C), I NMDG (D), and I BG (E) measured from recordings similar to (B). Wild-type I Na, –73 ± 6 pA, Tmc1-knockout I Na, –21 ± 3 pA; wild-type I NMDG, –7 ± 1 pA, Tmc1-knockout I NMDG, –4 ± 1 pA; wild-type I BG, –71 ± 5 pA, Tmc1-knockout I BG, –18 ± 2 pA (F). Example of Iₘ in wild-type (black and gray) and Tmc1-knockout (red and pink) OHCs undergoing a series of membrane depolarization, with tissues bathed in 144 Na followed by 144 NMDG. (G–I) Composite data showing I-V curve (G), reversal potential (H), and I BG (I) measured and calculated from recordings similar to (F). (G) I-V curve from recordings in 144 Na. (H) Mean reversal potentials calculated from I-V curve recorded in OHCs in 144 Na. (I) I BG-V curve after subtracting I NMDG. Only inward current was measured because NMDG was only applied extracellularly. The external solution contained 1.3 mM Ca²⁺. The holding potential was –70 mV. Data are presented as mean ± SEM. N values are shown in each panel. *p<0.05, **p<0.01, ***p<0.001, Student’s t-test. DOI: https://doi.org/10.7554/eLife.47441.002
Figure 2. TMC1 but not TMC2 conducts the background current. (A) Exogenous expression of TMC1 in wild-type OHCs from organotypic P3 cochlear tissue cultured for 1 day in vitro (P3 + 1DIV). EGFP was co-expressed as an indicator. The OHCs were stained to show spatial distribution of TMC1 (recognized by HA antibody, red), EGFP (by GFP antibody, green), and actin-enriched stereocilia (by Phalloidin, magenta), with two OHCs from the white dashed frame shown in detail. (B) Diagram of the recording configuration. The OHCs expressed engineered TMC1 with EGFP and whole-cell voltage clamped with Cs\(^+\) in the recording electrode and Na\(^+\) extracellularly. (C) Examples of \(I_m\) of wild-type OHCs at P3 + 1DIV, expressing control (EGFP), deafness TMC1 (TMC1_dn), or wild-type TMC1 (TMC1_WT). (D) Quantification of \(I_{BG}\) from wild-type OHCs expressing EGFP, TMC1_dn, and TMC1_WT under conditions similar to those in (C). \(I_{BG}\) values: EGFP, –17 ± 3 pA; TMC1_dn, –16 ± 3 pA; TMC1_WT, –43 ± 7 pA. (E) Representative traces of \(I_{BG}\) in P6 Tmc2\(^{-}\) and Lhfpl5-knockout OHCs from acutely dissociated cochleae. (F) Quantification of \(I_{BG}\) measured from recordings similar to (E) from Tmc2\(^{-}\) and Lhfpl5-knockout mice at assigned ages. \(I_{BG}\) values: P1 wild-type, –1 ± 0 pA; P1 Tmc2-knockout, –0 ± 0 pA; P3 wild-type, –19 ± 4 pA; P3 Tmc2-knockout, –26 ± 4 pA; P6 wild-type, –66 ± 5 pA; P6 Tmc2-knockout, –73 ± 7 pA; P6 Lhfpl5-knockout, –14 ± 2 pA. The external solution contained 1.3 mM Ca\(^{2+}\). The holding potential was –70 mV. Data are presented as mean ± SEM. N values are shown in each panel. *p<0.05, **p<0.01, ***p<0.001, one-way ANOVA.

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Figure 2—figure supplement 1. Localization of ectopically expressed TMC2 in OHCs. (A) Exogenous expression of TMC2 in P3 + 1DIV OHCs. The OHCs were stained to show spatial pattern of TMC2 (by HA antibody, red) and EGFP (by GFP antibody, green). The hair bundle was stained by Phalloidin (magenta). Two transfected OHCs are shown enlarged from the white dashed frame.

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Figure 3. TMC1-mediated leak current is not carried by the resting open MET channel. (A) Representative $I_m$ trace showing fluid jet (FJ)-induced open and closed status of MET current and DHS-induced alteration of baseline current. The OHCs were bathed in external solution with 0.3 mM Ca$^{2+}$ instead of 1.3 mM Ca$^{2+}$. Insets: left, a diagram of fluid jet stimulation on a hair bundle; right, a 40 Hz sinusoidal stimulation protocol was used to induce forward and reverse deflection of the hair bundle. (B) Dashed frames #1, #2, and #3 in (A) are shown as enlarged traces. The baseline current was similar when the MET channels were closed by either FJ (#1) or DHS ($I_{DHS}$, #2), as highlighted with a red dashed line. As shown in #3, the DHS-sensitive resting MET current ($I_{Resting-MET}$) was calculated by subtraction of $I_{Na}$ and $I_{DHS}$. The baseline current was further closed by NMDG ($I_{NMDG}$). $I_{Leak}$ was defined as the subtraction of $I_{DHS}$ and $I_{NMDG}$. (C) Quantification of subtracted currents under different conditions: background, $-113 \pm 7$ pA; FJ-NMDG ($I_{Leak}$ subtracted from current baseline closed at negative FJ), $-72 \pm 6$ pA; DHS-NMDG ($I_{Leak}$ subtracted from that closed by 100 μM DHS), $-65 \pm 6$ pA. (D) Quantification of ratio of $I_{Leak}$ to $I_{BG}$ ($I_{Leak}/I_{BG}$) under different [Ca$^{2+}$]$_o$ conditions: 0.1 mM, $0.29 \pm 0.04$; 0.3 mM, $0.45 \pm 0.05$; 0.5 mM, $0.47 \pm 0.07$; 1.3 mM, $1.00 \pm 0.03$; 3.0 mM, $0.96 \pm 0.03$. (E) Quantification of the $I_{Leak}$ of OHCs measured in 1.3 [Ca$^{2+}$]$_o$. Wild-type $I_{Leak}$, $-51 \pm 3$ pA; Tmc1-knockout $I_{Leak}$, $-17 \pm 2$ pA. The external solution contained variable Ca$^{2+}$ concentration as indicated. The holding potential was $-70$ mV. Data are represented as mean $\pm$ SEM. N values are shown in each panel. *p<0.05, **p<0.01, ***p<0.001, (C) ANOVA; (E) Student’s t-test.

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Figure 3—figure supplement 1. Removal of hair bundles disrupts leak current of OHCs. (A) A photo showing the OHC in recording with hair bundle removed. The circles with white dashed line indicate hair-bundle removed OHCs. (B) Quantification of $I_{\text{Leak}}$ recorded in wild-type OHCs with or without hair-bundle removal. The holding potential was $-70 \text{ mV}$. Data are represented as mean ± SEM. N values are shown in each panel. *p<0.05, **p<0.01, ***p<0.001, Student’s t-test.

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**Figure 4.** Amino-acid substitution in TMC1 alters the leak current. (A) TMC1 with 10 putative transmembrane domains. The six substituted amino acids are highlighted as colored balls in the predicted positions, and the deafness (dn) truncation is at the third extracellular loop between TMS and TM6. (B) Figure 4 continued on next page.
Figure 4 continued

Diagram of the analysis of leak current in cultured Tmc1-knockout OHCs (P3 + 1 DIV) expressing modified TMC1 (TMC1*). (C) Representative traces showing the rescue of leak conductance in OHCs by control full-length TMC1 (TMC1_WT), deafness TMC1 (TMC1_dn), TMC1-G411C (G411C), TMC1-M412C (M412C), TMC1-N447C (N447C), TMC1-D528C (D528C), TMC1-T532C (T532C), and TMC1-D569C (D569C). Perfusion contents are indicated below. An 800 nm step deflection was applied to the hair bundle every 10 s by a glass probe. The glass probe induced MET currents are marked ‘+’, accompanying unwanted MET currents and electrical artefacts induced by switching the perfusion system (#). Note that the MET current was truncated to better show the leak current. (D) Quantification of rescue by mTMC1 constructs. $I_{\text{Leak}}$ values: TMC1_WT, $-49 \pm 5$ pA, G411C, $-23 \pm 3$ pA; M412C, $-40 \pm 6$ pA, N447C, $-15 \pm 1$ pA, D528C, $-22 \pm 4$ pA, T532C, $-32 \pm 4$ pA, D569C, $-23 \pm 3$ pA, TMC1_dn, $-18 \pm 3$ pA. The rescue indexes of FL and dn were used to evaluate significant difference. Cell numbers are shown on each bar. The external solution contained 1.3 mM Ca$^{2+}$. The holding potential was $-70$ mV. Data are presented as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001, ANOVA. DOI: https://doi.org/10.7554/eLife.47441.011
Figure 4—figure supplement 1. Cysteine substitution in TMC1 affects the MET current and the leak current. (A) Plots of amplitude of the background current recorded from Tmc1-knockout OHCs expressing engineered TMCs as indicated, before and after MTSET treatment. (B) Representative trace of $I_m$ recording in a P1 + 3 DIV Tmc1; Tmc2 double-knockout OHC expressing TMC1-M412C. A 10 Hz train of 800 nm step deflection was applied to the hair bundle by a glass probe. (C) Summary of absolute values and normalized ratios of $I_{\text{Leak}}$ and $I_{\text{MET}}$. The $I_{\text{Leak}}$ values were measured from data in Figure 4—figure supplement 1 continued on next page
Figure 4. The restored MET values of all TMC1 constructs were measured from Pan et al. (2018), excepting that of dn, which was collected in vestibular hair cells from Kawashima et al. (2011).

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Figure 5. TMC1-mediated leak conductance is antagonized by MET channel blockers. (A and B) Representative trace (A) and statistical curve (B) of I_m inhibition by DHS. A 10 Hz train of 800 nm step deflection was applied to the hair bundle by a glass probe to induce MET currents. I_{MET} and I_{BG} were calculated and plotted against the DHS concentration. As fitted, the IC_{50} of DHS was 15 μM for the MET channels and 487 μM for the leak conductance. Cell numbers, 7–11. Hill slope: I_{MET}, /C_0^{1.10}; I_{BG}, /C_0^{0.65}. (C and D) Statistical dose curve of I_m with graded concentrations of d-tubocurarine (dTC) (C) and amiloride (D). dTC IC_{50}, I_{MET}, 6 μM, I_{BG}, 82 μM. dTC Hill slope: I_{MET}, −0.47; I_{BG}, −2.80. dTC cell numbers, 5–15. Amiloride IC_{50}, I_{MET}, 46 μM, I_{BG}, 365 μM. Amiloride Hill slope: I_{MET}, −1.36; I_{BG}, −1.67. Amiloride cell numbers, 7–16. (E and F)Dosage effect of Gd^{3+}. Example trace (E) and Figure 5 continued on next page.
Figure 5 continued

statistical curve (F) of $I_{\text{m}}$ in OHCs during perfusion with solutions containing graded concentrations of Gd$^{3+}$. A train of 800 nm step deflection was applied to the hair bundle by a glass probe to induce MET currents. The MET and leak current amplitudes changed because of the channel sensitivity of Gd$^{3+}$ and NMDG. IC$_{50}$: $I_{\text{MET}}$, 66 µM; $I_{\text{BG}}$, 524 µM. Hill slope: $I_{\text{MET}}$, −0.48; $I_{\text{BG}}$, −2.49. Cell numbers, 7–16. (G and H) Dose effect of La$^{3+}$. Example trace (G) and dosage curve (H) of $I_{\text{m}}$ with La$^{3+}$ treatment. A train of 800 nm step deflection was applied to the hair bundle by a glass probe to induce MET currents. IC$_{50}$: $I_{\text{MET}}$, 259 µM; $I_{\text{BG}}$, 531 µM. Hill slope: $I_{\text{MET}}$, −1.06; $I_{\text{BG}}$, −5.67. Cell numbers, 7–8. For space reasons, 144 NMDG is shown as 0 Na. The external solution contained 1.3 mM Ca$^{2+}$. The holding potential was −70 mV. Data are presented as mean ± SEM.

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Figure 6. High-concentration Ca\(^{2+}\) blocks the leak current but not MET current. (A) Monovalent cations Li\(^{+}\) and Cs\(^{+}\) conducted the leak current. In this experiment, 150 mM NaCl was substituted with 150 mM LiCl or 150 mM CsCl in the external solution. (B) Divalent cations 10 mM Ba\(^{2+}\), 75 mM Zn\(^{2+}\), 75 mM Co\(^{2+}\), 150 mM Mg\(^{2+}\), and 75 mM Ca\(^{2+}\), conducted the leak current. The 150 mM NaCl was partially or completely replaced with cations as described in the Materials and methods. (C) Representative I\(_{\text{m}}\) traces by ramp stimulation for calculation of ionic permeability of the leak channel. The extracellular ion was switched from 150 mM Na\(^{+}\) to 75 mM Ca\(^{2+}\), and to 150 NMDG\(^{+}\), all containing 100 µM DHS. In the intracellular solution, 150 mM CsCl was used. (D) Quantification of ionic permeability calculated from similar recordings in (C). (E) Example trace of I\(_{\text{m}}\) of OHCs during perfusion with solutions containing graded concentrations of Ca\(^{2+}\) and Na\(^{+}\). An 800 nm step deflection was applied to the hair bundle by a glass probe. The glass probe induced MET currents are marked ‘+’, accompanying unwanted MET currents and artefacts induced by switching the perfusion system (#). (F) Dose curves of I\(_{\text{BG}}\) and I\(_{\text{MET}}\) in wild-type OHCs in different Ca\(^{2+}\) and Na\(^{+}\) concentrations (cell numbers, 9–20). (G) Quantification of dose-dependent background leak current in OHCs from wild-type (black) and Tmc1-knockout (red) mice when bathed in mixed Ca\(^{2+}\) and Na\(^{+}\). The ions and concentrations used in test external solutions were variable, as described in this figure legend and the Materials and methods. The holding potential was –70 mV. Data are presented as mean ± SEM. N values are shown in each panel. *p<0.05, **p<0.01, ***p<0.001, (B,D) ANOVA.

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Figure 7. IHC excitability is down-regulated in Tmc1-knockout mice. (A) Representative current-clamp recording in IHCs bathed in external solution with 100 μM DHS from wild-type (black) and Tmc1-knockout (red) mice. For the most part, the IHCs were held at 0 pA. To define excitability, a ramp
current was injected into the Tmc1-knockout IHCs to induce a burst of spikes. (B) Quantification of \( V_m \) recorded in IHCs similar to (A). Values of \( V_m \) in wild-type IHCs were defined as two states, bursting and non-bursting, which did not apply to Tmc1-knockout IHCs. \( V_m \) of wild-type in bursting state, 49 ± 2 mV; wild-type in non-bursting state, 60 ± 2 mV; Tmc1-knockout, 73 ± 2 mV. (C) Quantification of firing rate (spikes/s) in IHCs similar to (A). Values of firing rate: wild-type, 3.2 ± 0.7 Hz; Tmc1-knockout, 0 ± 0 Hz. (D) Quantification of \( I_{\text{leak}} \) from voltage-clamp recording in IHCs. Values of \( I_{\text{leak}} \): wild-type, 24 ± 4 pA; Tmc1-knockout, 14 ± 2 pA. (E) Representative current-clamp traces of \( V_m \) in IHCs with ramp-current injection from –100 pA to +100 pA for 3 s. (F) Representative current-clamp recording in IHCs stimulated by a family of depolarization currents from –50 pA to +125 pA at 25 pA steps. (G) Quantification of firing threshold from data as in (E). Values of threshold were –47 ± 1 mV in wild-type OHCs and –47 ± 1 mV in Tmc1-knockout OHCs. (H) Quantification of minimum current injected (Injected \( I_{\text{min}} \)) to evoke an action potential from data as in (E). In wild-type OHCs: –0 ± 7 pA; in Tmc1-knockout OHCs: –21 ± 4 pA. (I) Quantification of numbers of spikes per second from data as in (F). Wild-type: 0 pA, 1.3 ± 0.7; 25 pA, 9.1 ± 0.8; 50 pA, 12.3 ± 0.7; 75 pA, 13.1 ± 0.7; 100 pA, 13.3 ± 0.6; 125 pA, 13.6 ± 0.7. Tmc1-knockout: 0 pA, 0 ± 0; 25 pA, 5.1 ± 1.3; 50 pA, 10.6 ± 1.1; 75 pA, 12.7 ± 0.7; 100 pA, 13.0 ± 0.9; 125 pA, 13.2 ± 1.1. In this figure, the external solution contained 1.3 mM Ca\(^{2+}\) and 100 μM DHS. K\(^+\) was used in the intracellular solution for current-clamp recordings in this figure except that Cs\(^+\) was used for voltage-clamp recording in (D). Data are presented as mean ± SEM. N values are shown in each panel. *p<0.05, **p<0.01, ***p<0.001, (B) ANOVA; (C,D,G,H,I) Student’s t-test. DOI: https://doi.org/10.7554/eLife.47441.019
Figure 8. TMC1-mediated leak and MET currents in OHCs. (A) Diagram showing the tonotopic map in mouse hair cells (adapted from Figure 1B in Kim and Fettiplace, 2013), labeled with response frequencies (kHz, gray) and location (D% to apex, black). The apex and base are defined as 0 and 1, with reference to which D05, D20, D40, D60, and D80 represent distances of 0.05, 0.2, 0.4, 0.6, and 0.8. (B) Representative traces of $I_m$ recorded in OHCs at different locations along the cochlear coil, from wild-type (black) and Tmc1-knockout (red) mice. The external solution contained 1.3 mM Ca$^{2+}$. (C) Wild-type and Tmc1 KO expression. (D) Representative traces of $I_m$ recorded in OHCs at different locations along the cochlear coil, from wild-type + 1.3 Ca, TMC1 KO + 1.3 Ca, and wild-type + 35 Ca. (E) Wild-type and Tmc1 KO expression. Figure 8 continued on next page.
The apex and base are defined as 0 and 1, with reference to which D05, D20, D40, and D60 represent distances of 0.05, 0.2, 0.4, and 0.6. (C) Quantification of location-specific $I_{\text{leak}}$ from similar recordings to those in (B). Values of $I_{\text{leak}}$ in wild-type OHCs (pA): D05, $-23 \pm 4$; D20, $-63 \pm 4$; D40, $-67 \pm 7$; D60, $-84 \pm 7$. $I_{\text{leak}}$ values in Tmc1-knockout OHCs (pA): D05, $-18 \pm 3$; D20, $-18 \pm 2$; D40, $-10 \pm 2$; D60, $-5 \pm 1$. (D) Representative traces of location-specific MET current in wild-type OHCs when bathed in 1.3 mM or 35 mM Ca$^{2+}$ and Tmc1-knockout OHCs when bathed in 1.3 mM Ca$^{2+}$. A sinusoidal deflection was applied to the hair bundle by a fluid jet. (E) Quantification of location-specific macroscopic MET current. Values of $I_{\text{MET}}$ in wild-type OHCs in 1.3 mM Ca$^{2+}$ (pA): D05, $-505 \pm 37$ pA; D20, $-780 \pm 24$ pA; D40, $-872 \pm 21$ pA; D80, $-939 \pm 22$ pA. Values of $I_{\text{MET}}$ in wild-type OHCs in 35 mM Ca$^{2+}$ (pA): D05, $-369 \pm 13$ pA; D20, $-369 \pm 13$ pA; D40, $-384 \pm 30$ pA; D60, $-461 \pm 31$ pA. Values of $I_{\text{MET}}$ in Tmc1-knockout OHCs in 1.3 mM Ca$^{2+}$ (pA): D20, $-371 \pm 35$ pA; D40, $-177 \pm 19$ pA; D60, $-117 \pm 15$ pA; D80, $-102 \pm 9$ pA. The holding potential was $-70 \text{mV}$. In (C) and (E), data are presented as mean $\pm$ SEM with N values. *p<0.05, **p<0.01, ***p<0.001, ANOVA. DOI: https://doi.org/10.7554/eLife.47441.021
Figure 8—figure supplement 1. TMC1-dependent background leak current in ageing hair cells. (A) Representative $I_m$ traces in whole-cell voltage-clamp recorded OHCs from P14 wild-type and Tmc1-knockout mice. (B) Quantification of $I_{\text{leak}}$ recorded from P3, P6, and P14 OHCs under conditions similar to those in (A). $I_{\text{leak}}$ values (pA) for wild-type: P3, $-26 \pm 8$; P6, $-66 \pm 5$; P14, $-150 \pm 21$. For Tmc1-knockout: P3, $-10 \pm 2$; P6, $-18 \pm 2$; P14, $-23 \pm 7$. The holding potential was $-70$ mV. Data are presented as mean ± SEM and N values are shown in panel B. *p<0.05, **p<0.01, ***p<0.001, ANOVA. DOI: https://doi.org/10.7554/eLife.47441.022
Figure 9. High Ca²⁺ removes the MET conductance gradient as revealed by unitary channel analysis. (A) Location-specific single MET channel recording from wild-type OHCs in solution with 3 mM or 35 mM Ca²⁺ at D05 or D60. The traces were chosen to show nice dual-peak fitting but did not represent normal flickers. A 100 nm step deflection was applied to the hair bundle by a glass probe. (B) Statistical analysis of location-specific unitary MET channel current. Values of unitary $I_{\text{MET}}$ in 3 mM Ca²⁺: D05, –7.0 ± 0.2 pA; D20, –7.9 ± 0.2 pA; D60, –10.6 ± 0.2 pA. Values of $I_{\text{MET}}$ in 35 mM Ca²⁺: D05, –4.7 ± 0.1 pA; D20, –4.8 ± 0.1 pA; D60, –4.9 ± 0.1 pA. The holding potential was –70 mV. N values are shown as events/cells. Data are presented as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001, ANOVA.