Supplementary Information for

The voltage-gated sodium channel TPC1 confers endolysosomal excitability

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Supplementary Results

Supplementary Figure 1: Whole-endolysosomal ionic conductances. K⁺ (a), H⁺ (b) and Cl⁻ (c) currents were recorded in endolysosomes from macrophages. The bath solution contained (in mM) 1 HCl, 10 HEPES (for pH 7.2 in a and c, and pH 7.0 in b) or MES (for pH 4.0, 5.0, and 6.0 in b), and 150 mM ion as indicated. pHs were adjusted with methanesulfonic acid or NMDG. Pipette solution contained (in mM) 1 HCl, 150 mM K⁺ (in a) or NMDG (in b and c), 10 MES, pH 4.6 (or 10 HEPES, pH 7.0 in b) adjusted with methanesulfonic acid. H⁺ currents were minimal when low pH (4.6) pipette solution was used (n = 6, not shown). A ramp protocol (-100 mV to +100 mV in 1 s, V_h = 0 mV) was used in a1, b1, c1, c2. Step protocols (-100 mV to +100 mV; V_h = 0 mV) in step of 20 mV were used in a2, a3, b2, b3 and c3. Bar graphs are averaged current amplitudes (at +100 mV). Numbers of endolysosomes are in parentheses. Data are represented as means ± SEMs.
**Supplementary Figure 2:** LysoNaV recorded from mIMCD3 mouse kidney collecting duct cells.  

- **a, b:** Representative currents elicited by voltage step pulses (illustrated in panel a) before (a) and after (b) ATP (5 mM) bath application. The averaged current amplitudes measured at the end of the voltage step pulses at +150 mV are summarized in (d).  
- **c:** Similar to (a), processed with an on-line leak subtraction. Bath solutions contained (in mM) 150 NaCl, 0.001 PI(3,5)P2, 10 HEPES, pH 7.2. Pipette solution contained (in mM): 145 Na-methanesulfonate, 5 NaCl, 10 glucose, 10 HEPES, 10 MES, pH 4.6. Asterisks indicate statistical significance (*, p<0.05).
Supplementary Figure 3: Lack of inactivation in lysoNav and TPC1. Currents were recorded with a 10-s pulse of +100 mV from a cardiac myocyte (a, representative of 3) and a TPC1-transfected HEK293T cell endolysosome (b, representative of 3).
Supplementary Figure 4: LysoNa\textsubscript{V} is abolished in \textit{tpc} knockout and is restored by TPC1 transfection. Currents were recorded from endolysosomes from WT (a-d), \textit{tpc} KO (e, h) mice, or mTPC1-transfected \textit{tpc} KO (f-h) myocytes. Recording conditions were the same as the ones used in Supplementary Figure 2. Asterisks indicate statistical significance (**, p<0.01).
Supplementary Figure 5: TPC1 is functionally conserved between mouse and human.
Endolysosomal recordings were done from HEK293T cells transfected with mouse TPC1.  a, Representative currents.  b, Averaged I-Ψ relationship.
Supplementary Figure 6: LysoNaV recorded from cardiac myocytes and TPC1 expressed in HEK293T cells have similar sensitivities to phosphatidylinositol bisphosphates.  

**a-c**, current amplitudes normalized to peak values (a) or averaged current amplitudes (b, c) at +100 mV recorded with concentrations of PI(3,5)P2, PI(3,4)P2 or PI(4,5)P2 as indicated.  

**d-f**, currents recorded with voltage steps (-100 mV to +120 mV) from a TPC1-transfected HEK293T cell with 0.1 μM (d) or 10 μM (e) PI(3,5)P2.  
Current sizes normalized to that at +120 mV are in (f).  
Asterisks indicate statistical significance (*, p<0.05; **, p<0.01).
Supplementary Figure 7: ATP inhibits TPC1 but does not change the channel’s voltage dependence.  

**a, b,** Representative $I_{\text{TPC1}}$ recorded before (a) and after (b) application of 0.5 mM ATP in the bath.  

**c,** Averaged currents ($n = 5$).  

**d,** Current amplitudes normalized to the peak value obtained at +150 mV.  

Recordings were done with hTPC1-expressing HEK293 cells.
**Supplementary Figure 8:** Averaged I-Ψ curves showing that NAADP does not change I_{TPC1}'s voltage-dependence or amplitude (n = 6). Similar results were obtained with 100 nM NAADP (n = 3). Recordings were done with hTPC1-expressing HEK293 cells.
Supplementary Figure 9: Effects of cytosolic Ca\textsuperscript{2+} on I\textsubscript{TPC1}.  

\textbf{a-c}, Representative currents recorded from the same hTPC1-expressing endolysosome in bath solutions containing 100 nM (a), 10 \(\mu\)M (b) or 1 mM (c) free Ca\textsuperscript{2+}.  

\textbf{d, e}, I-\(\Psi\) relationships under various [Ca\textsuperscript{2+}]\textsubscript{cyt}.  

\textbf{e}, Current amplitudes were normalized to the peak value at +100 mV.  
Representative of 4.
Supplementary Figure 10: TPC1 pharmacology.  a, c, Representative I_{TPC1} recordings showing inhibition by verapamil (a) and Cd^{2+} (c).  b, d, Current amplitudes normalized to those recorded without drug and fitted with the Hill equation.  e, I_{TPC1} amplitudes (at 100 mV) in the presence of channel blockers in the bath (cytosol) normalized to the those before drug application.  f, I_{TPC1} (left panel) and cardiac myocyte lysoNaV (right panel) amplitudes (at +100 mV) recorded in pipette (lumen) solutions containing no or 10 μM TTX. All recordings were done with hTPC1-expressing HEK293 cells except the right panel in (f). In (f), pipette solution with increased pH (pH 7.4) was used to mitigate the effect of conformational changes of TTX under low pH.
Supplementary Figure 11: TPC1 expression confers endolysosomal excitability to HEK293 cells. Current clamp recordings were performed using endolysosomes from hTPC1-expressing (a, representative of 5) or non-transfected (b, representative of 6) HEK293T cells as done in Figure 6.