Recombinant tandem of pore-domains in a Weakly Inward rectifying K^+ channel 2 (TWIK2) forms active lysosomal channels

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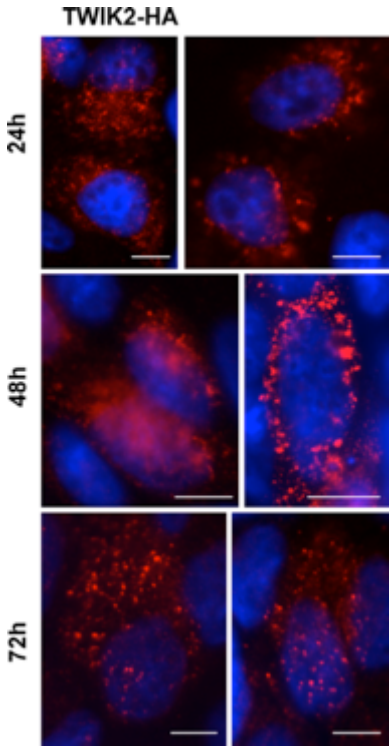
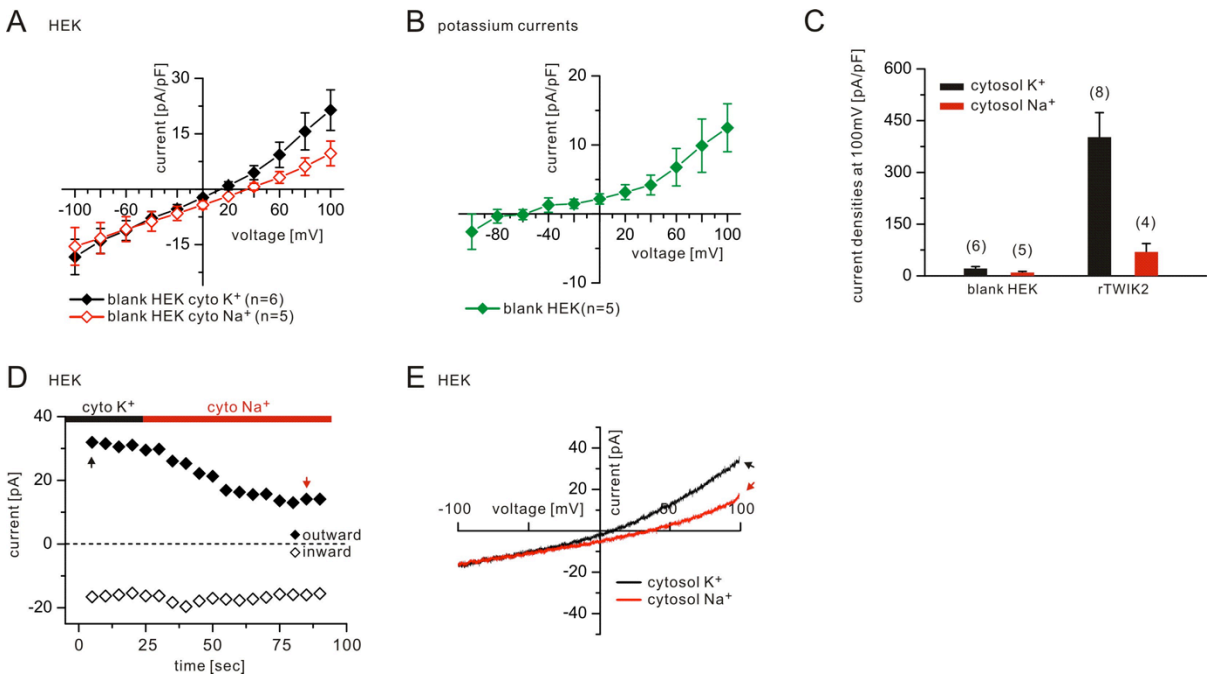
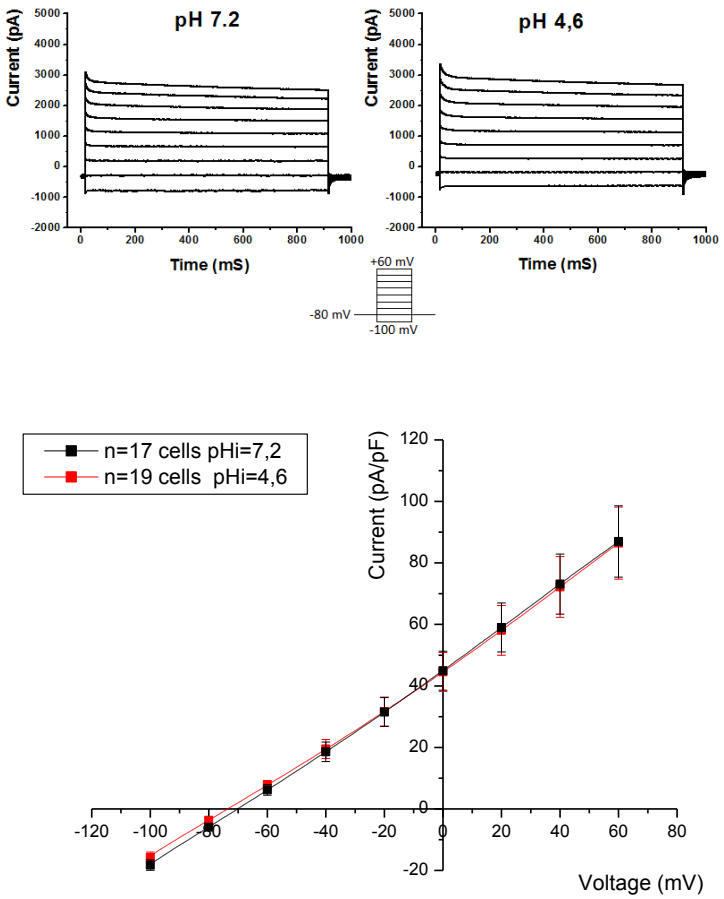


SUPPLEMENTAL FIGURE 1. ***TWIK2 expression stability during 72h in MDCK cells.*** TWIK2-HA was transfected into MDCK cells and stained with HA-antibody 24h, 48h and 72h post transfection. Scale bar 10µm.



SUPPLEMENTAL FIGURE 2. ***Endolysosomal K^+ currents from non-transfected HEK293 cells.*** Cation currents were recorded in endolysosomes released from HEK293T cells. Representative currents were elicited by voltage step protocols from -100 to +100 mV (20 mV step) **(A and B)** or by voltage ramps from -100 to +100 mV (voltage holding at 0 mV) **(D and E)** with 145 mM K^+ and perfused with 145 mM Na^+ in the cytosol (bath) as indicated. Shown in **B** are potassium I-V curves ($I_{\text{cyto high K}^+}$ - $I_{\text{cyto high Na}^+}$) from blank HEK293T cells. **(C)** Column figures summarizing the current densities obtained at +100 mV. **(D)** Current amplitudes measured at -100 mV and +100 mV were used to plot the time course of activation. Data are represented as mean ± s.e.m.

The profile of these small endogenous K^+ currents is in agreement with previous data that suggest that other K^+ permeable ion channels are present on endolysosomal membranes such as TMEM175, TRPMLs and BK channels (Cang et al, 2015; Cao et al, 2015; Chen et al, 2014a).



SUPPLEMENTAL FIGURE 3. ***Recordings of rat TWIK2 $Y_{308}A$ $IL_{289/290}AA$ at the plasma membrane of transfected HEK293T cells.***

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H. Sapiens      -  DLHGLTELIL LPPPCPASFN ADEDDRVDIL GPQPESHQQL SASSHTDYAS IPR
M. musculus     -  DLHGLTELIL LPDPDPASLS QDEDDQVAVL DARTDLHQHL SAASHADYAS IPR
R. norvegicus   -  DLHGLTELIL LPDPDPARLH QDEDDQVDIL DARTDLHQHL SAASHANYAS IPR
X. tropicalis   -       TDIFY LPRLQDQDDQ EPILETTDYS TRDLEPKRPL ASESQPDYSS INR
D. rerio        -       HLPSC EEDEEDKEPI IEAGPEDDSP EAEKASIKPL DPSSQVSYNS INR
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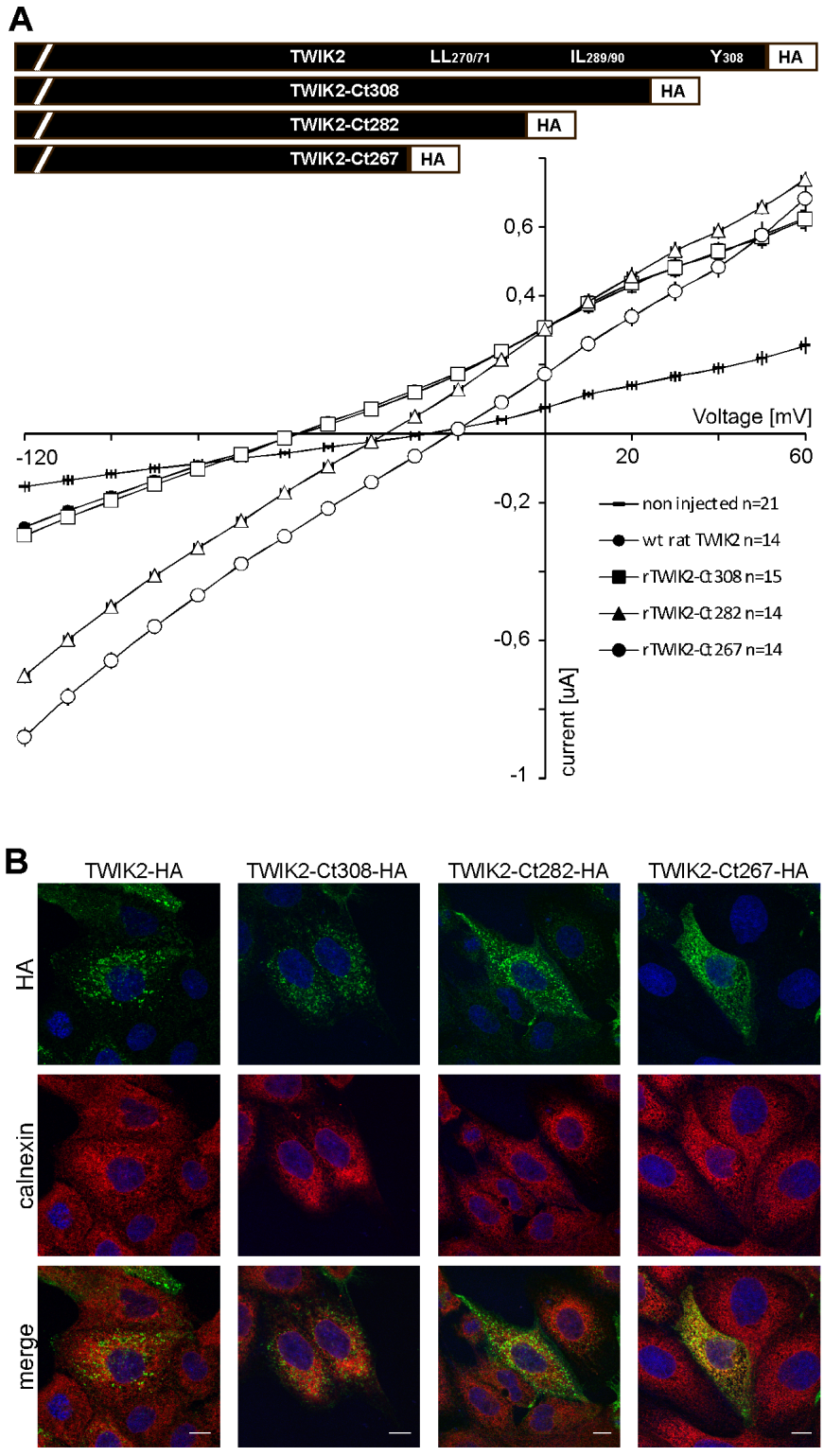
SUPPLEMENTAL FIGURE 4. ***Conserved trafficking motifs in the C-terminus of TWIK2.*** TWIK2-C-terminal-sequences of *Homo sapiens*, *Mus musculus*, *Rattus norvegicus*, *Xenopus tropicalis* and *Danio rerio*.



SUPPLEMENTAL FIGURE 5. ***Effect of the truncation of the TWIK2 C-ter on cell trafficking.*** (**A**) Truncated channels did not show significant changes in current amplitude when expressed and recorded in *Xenopus* oocytes and (**B**) showed intracellular localization when expressed and stained in MDCK cells. Scale bar: 10 µM.

SUPPLEMENTAL VIDEO

LEGEND OF THE VIDEO: Live HEK293 cells expressing TWIK2-GFP fusion protein (in green). Lysosmes are labelled with Lysotracker dye (in red).