Real time fluorescence quantitative RT-PCR

The whole RNA of U251MG cells was extracted by the Trizol method. Real time fluorescence quantitative RT-PCR was carried out using a protocol from the Applied Biosystems. SYBR Premix Ex TapTMII (including TaKaRa Ex TapTM HS, dNTP compound, Mg2+, SYBR Green I; TaKaRa DRR041) and the primers (GAPDHTGACTTCAACAGCGACACCCA CACCCTGTTGCTGTAGCCAAA; KCNK2, Trek-1, GATTATACCGTTAGGAAACACC TCCCAGTAAGGCATAGATGA) were added in the reaction system. The melting curves and electrophoresis on agarose gel were applied to detect the specificity of the PCR product of every primer reaction. The comparative Trek-1 mRNA expression was calculated by dividing the GAPDH mRNA expression. The threshold cycle (Ct) was determined by the SDS program of ABI Prism. ΔCt represented the comparative mRNA expression, ΔCt = Ct (Trek-1 mRNA) - Ct (GAPDH).
Result: Average ΔCt (U251MG) was 15.36±0.064, showing the moderate abundance of gene expression in U251MG cells.

Immunofluorescence staining

Cultured U251MG cell was separated using trypsin and planted onto glass coverslip as used in patch-clamp recordings. Then U251MG cells were fixed with 4% paraformaldehyde for 1h. After washing with PBS, Triton X-100 (0.1%) was used to permeabilize the cells and goat serum (5%) was used to block nonspecific reactions. The cell was treated by the primary rabbit anti- -KCNK2 (TREK-1) antibody (1:100, Alomone Labs) overnight in 4°C condition. When washing cells by PBS for three times at 5 min interval, cells were treated by the fluorescent secondary antibody (FITC-conjugated goat anti-rabbit IgG, 1:1000, Sigma) at room temperature. One hour later, the cell nuclei were re-stained by 4',6-diamidino-2-phenylindole (DAPI). The fluorescence image was captured using a Zeiss 710 confocal laser scanning microscope (Zeiss, Germany).
Result: TREK-1 was expressed in the cell membrane.

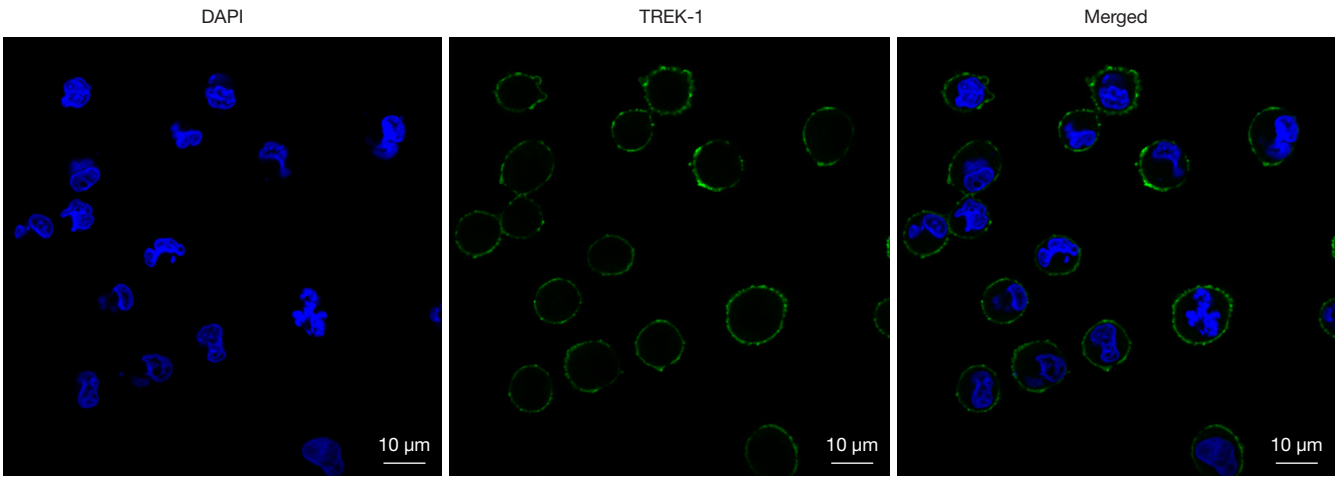

Figure S1 Immunofluorescence staining of TREK-1 in U251MG cells. After being 4% Paraformaldehyde-fixed and 0.1% Triton X-100 permeabilized the U251MG cells were labeled with primary rabbit anti-KCNK2 (TREK-1) antibody (1:100, Alomone Labs) followed by the fluorescent secondary antibody (FITC-conjugated goat anti-rabbit IgG, 1:1000, Sigma). The cell nuclei were re-stained by 4',6-diamidino-2-phenylindole (DAPI). The fluorescence image was captured using a Zeiss 710 confocal laser scanning microscope (Zeiss, Germany). TREK-1 was expressed in the cell membrane.

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