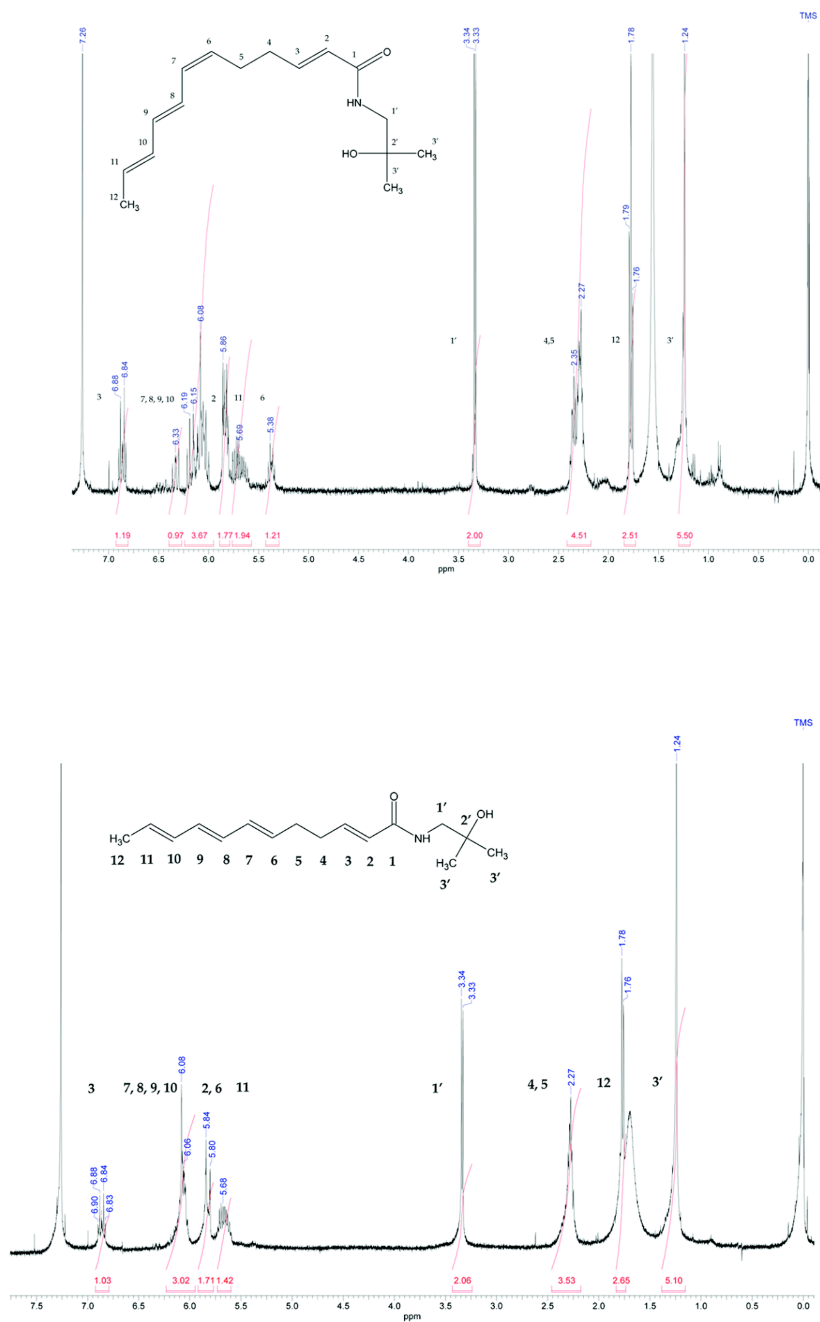
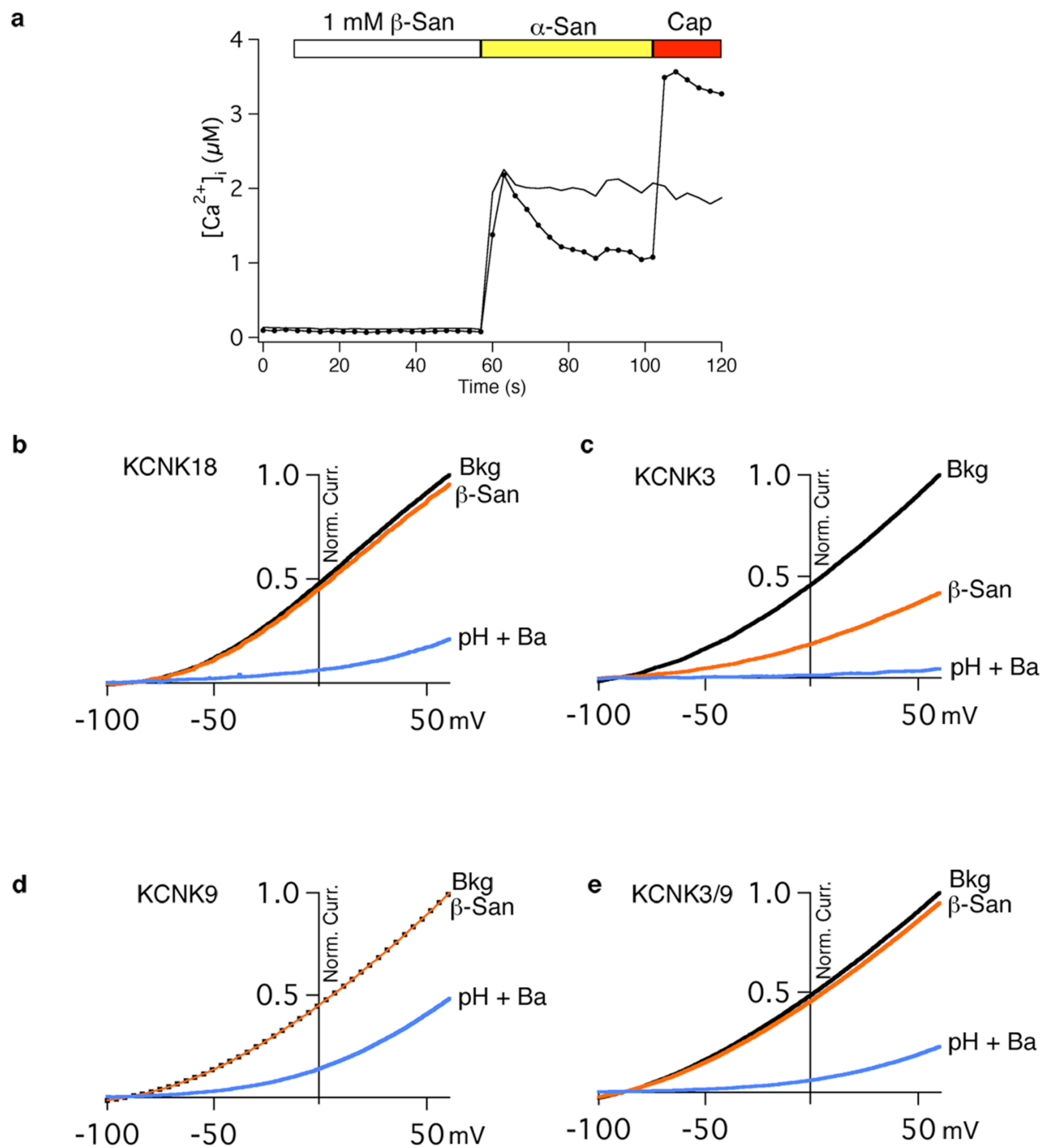
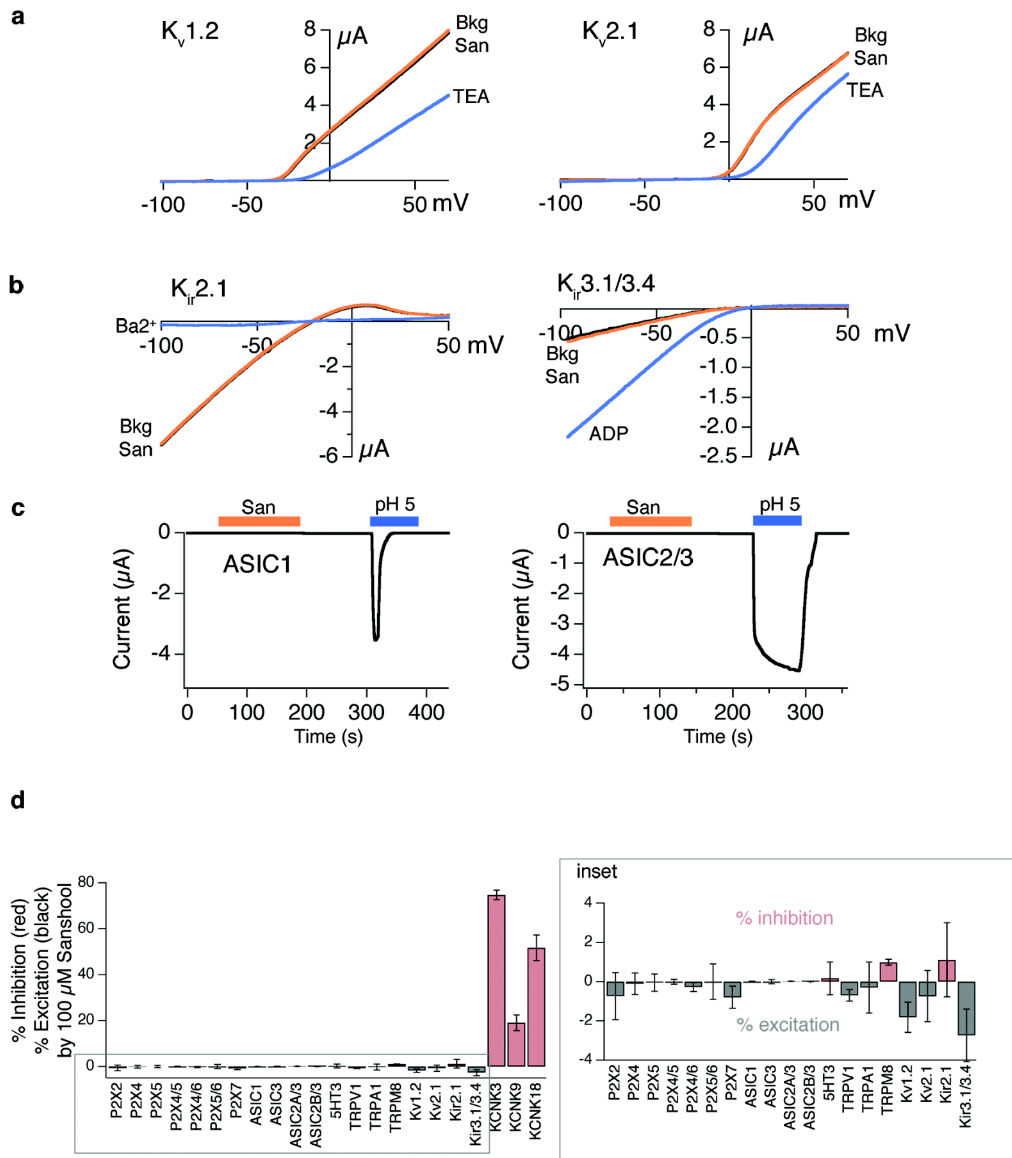


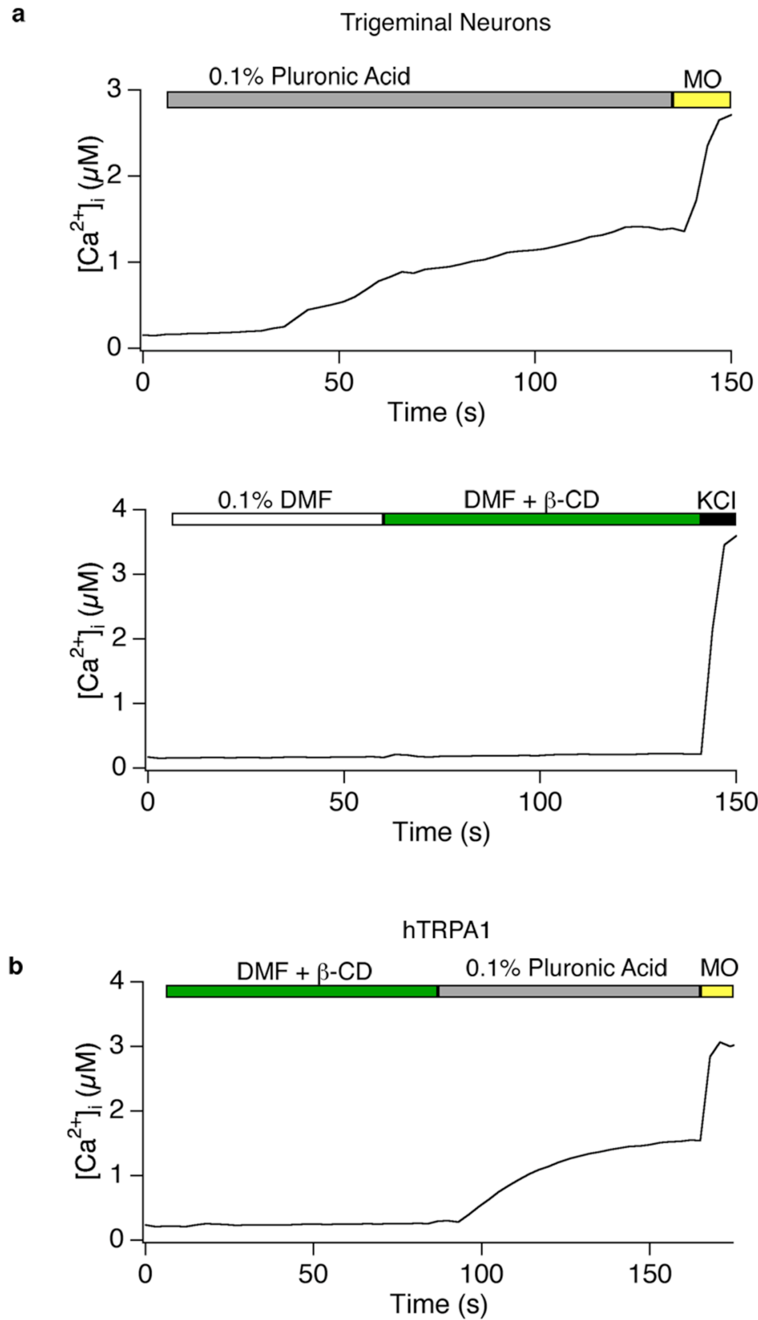
Supplementary Figure 1 NMR spectra of hydroxy α– and β-sanshool isomers. **(Top)** Hydroxy-α-sanshool (2E,6Z,8E,10E)-2'-hydroxyl-*N*-isobutyl-2,6,8,10-dodeca-tetraenamide) and (**bottom**) hydroxy-β-sanshool (2E,6E,8E,10E)-2'-hydroxyl-*N*-isobutyl-2,6,8,10-dodeca-tetraenamide) were purified and ^1H spectra were recorded on a Varian 400 spectrometer at 400 MHz.



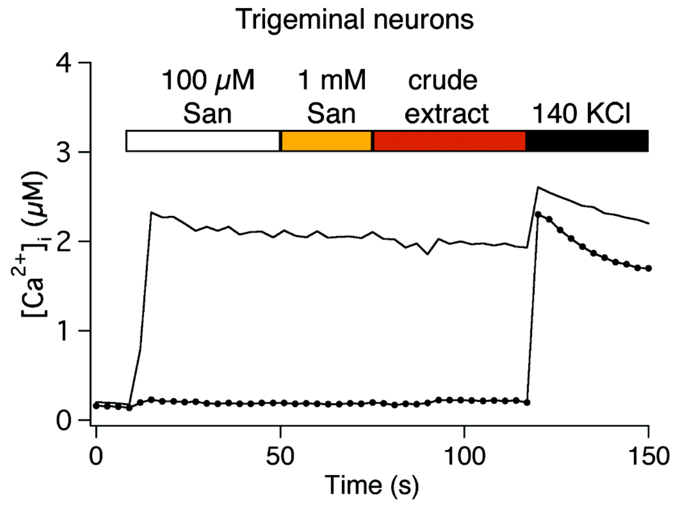
Supplementary Figure 2 Hydroxy-β-sanshool does not activate sensory neurons, but is a specific antagonist for KCNK3. **(a)** Representative calcium response of cultured sensory neurons to hydroxy-β-sanshool (1 mM), hydroxy-α-sanshool (100 μM), and capsaicin (1 uM). **(b-e)** Representative traces of hydroxy-β-sanshool-evoked (100 μM; orange) inhibition of KCNK18, KCNK3, KCNK9, and KCNK3/9 heteromers (b–e, respectively) expressed in oocytes (holding potential = –80 mV, n=3–5). Inhibition by 2 mM $BaCl_2$ (pH = 6.5; blue).



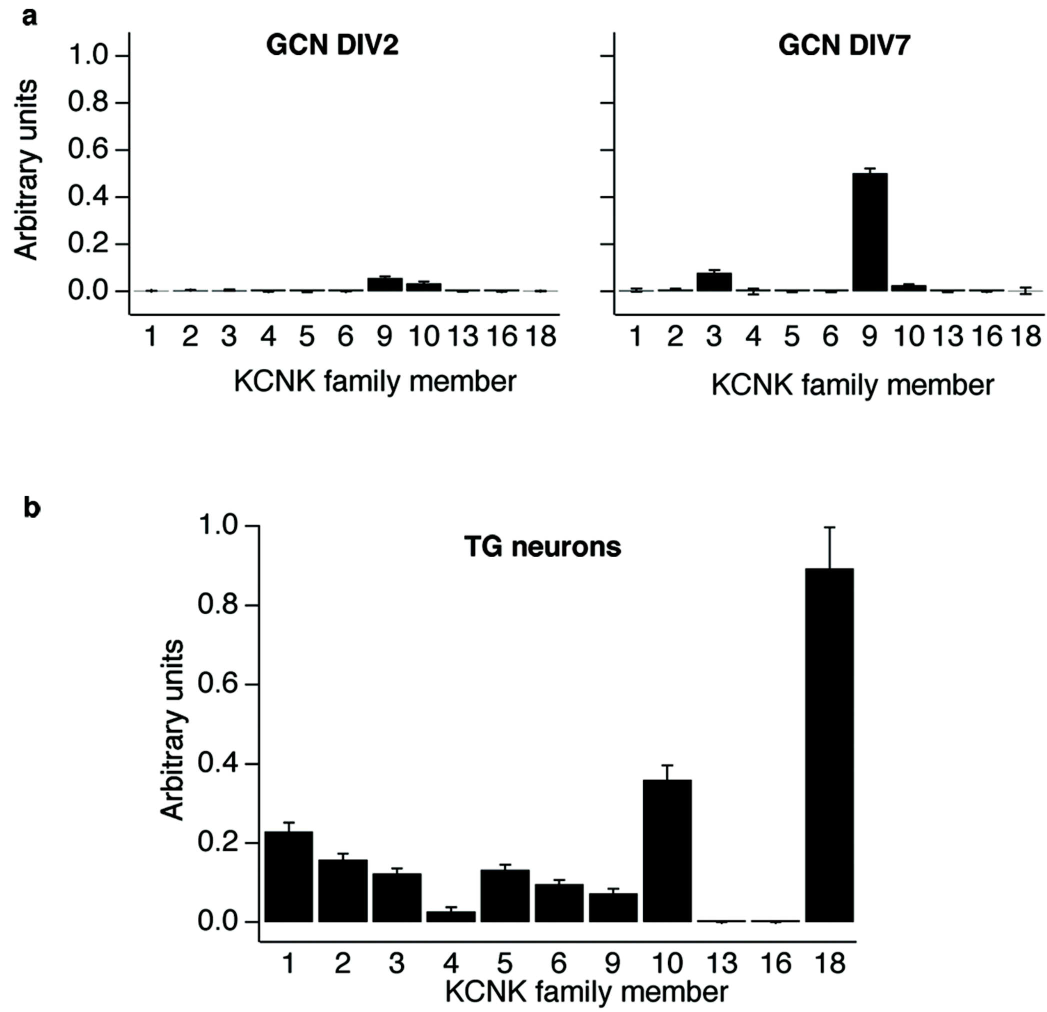
Supplementary Figure 3 Sanshool does not effect a variety of ion channels expressed by sensory neurons. (a) Representative traces showing lack of sanshool sensitivity of K_v1.2 (left) and K_v2.1 (right; orange) (holding potential = –80 mV, n=5). Inhibition by TEA is shown for comparison (10 mM; blue). (b) Representative traces showing lack of sanshool sensitivity of K_{ir}2.1 (left) and K_{ir}3.1/3.4 co-expressed with P2Y12 (right) (orange; holding potential = –80 mV, n=5). Inhibition of K_{ir}2.1 by 1 mM $BaCl_2$ and activation of K_{ir}3.1/3.4 co-expressed with $P2Y_{12}$ by 10 μM ADP are shown for comparison (blue). (c) Representative traces showing lack of sanshool-evoked (100 μM; orange) activation of ASIC1 (left) and ASIC2b/3 heteromer (right) (holding potential = –80 mV, n=5). Activation by ND96, pH 5.0 is shown for comparison (blue). (d) Summary showing effect of sanshool on a variety of ion channels expressed in *Xenopus* oocytes. Membrane currents were recorded in two-electrode voltage-clamp mode (holding potential = –60 mV for all channels except K_v1.2 and K_v2.1 where holding potential = +30 mV) and response to bath applied sanshool (100 μM) compared to activation (black) or inhibition (red) by the relevant agonist/antagonist (10 mM TEA for K_v1.2 and K_v2.1, 1 mM $BaCl_2$ for K_{ir}2.1, 10 μM ADP for K_{ir}3.1/3.4 co-expressed with $P2Y_{12}$R, 100 μM ATP for P2X channels, pH 5.0 for ASIC channels, 100 μM serotonin for 5-HT3R-A channel, and 1 μM capsaicin, 100 μM mustard oil, or 100 μM menthol for TRPV1, TRPA1, or TRPM8, respectively). 100 μM sanshool was used for all experiments. Panel at left shows comparison with sanshool effects on KCNK channels; panel at right shows expanded scale view of data in box.



Supplementary Figure 4 Pluronic activates endogenous and heterologous TRPA1. **(a)** Cultured trigeminal neurons show an increase in calcium in response to 0.1% pluronic acid (top; n=178), but not 0.1% dimethylformamide (DMF) or 0.1% DMF and 0.1 % β-cyclodextrin (β-CD)(bottom; n=167). **(b)** Human TRPA1 was transiently transfected into HEK293 cells. Calcium influx was observed in response 0.1% pluronic acid and mustard oil (100 uM), but not 0.1% DMF and 0.1 % β-CD (n=131).



Supplementary Figure 5 Crude Szechuan pepper extract and purified hydroxy-α-sanshool activate the same population of cultured sensory neurons. A representative calcium response of cultured sensory neurons to application of 100 μM, then 1mM hydroxy-α-sanshool, followed by crude Szechuan pepper extract (3%).



Supplementary Figure 6 Quantitative PCR analysis of KCNK transcripts from cerebellar granule and trigeminal sensory neurons **(a)** Comparison of KCNK channel subtype expression in cerebellar granule neurons (CGN) on day 2 (left) versus day 7 (right) in culture (n=3 per condition). **(b)** KCNK channel subtype expression in trigeminal (TG) neurons (n=3).

Primers sequences used to clone mouse KCNK gene coding regions		
KCNK1	5′	ATGCTGCAGTCCCTGGCCGGCAGC
	3′	TCAGTGGTCTGCAGAGCCATCCTCATAGGG
KCNK2	5′	ATGCTTGCCAGCGCCTCGCGG
	3′	CTACTTGCCAGCGCCTCGCGG
KCNK3	5′	ATGAAGCGGCAGAATGTGCGCACGTTGG
	3′	TCACACCGAGCTCCTGCGCTTCATGAG
KCNK4	5′	ATGCGCAGCACCACACTCCTGGCTCTG
	3′	CTACACCGGCACGGCCTTGTCTCGGAG
KCNK5	5′	ATGGTGGACCGGGGTCCTTTACTCACC
	3′	TCACGTGCCCCTGGGGTTATCTGCCTT
KCNK9	5′	ATGAAGCGGCAGAACGTGCGTACCC
	3′	TTAGATGGACTTGCGACGGAGGTGCAGCCTATG
KCNK10	5′	ATGAAATTTCCAATCGAGACGCCAAG
	3′	TTAGTTTCTGTCTTCAAGTAAAGAATTGTTCTCC
KCNK13	5′	ATGGCTGGCCGCGGTTGCGG
	3′	CTACCTATCTCCACTGGTCTCTGCCAACCTG
KCNK18	5′	ATGGAGGCTGAGGAGCCACCTGAGGCC
	3′	TTACCAAGGTAGCGAAACTTCCCTTTGGC

Supplementary Table 1 - Specific primers designed to clone all functional KCNK family members. Primer sequences were designed to specific sections of the 5' and 3' ends of the coding regions of all known functional KCNK channels. PCR amplification was performed as described in **Methods.**

Primer sequences used for qPCR experiments		
KCNK1	5′	GTTCCTGTATACATTTCTACCCTCTTG
	3′	CATAATCTGTTCAGGGGAGAGG
KCNK2	5′	CACTGTGAGTTTTGCACATGG
	3′	GGGACTGGACTTTTTCTGAATC
KCNK3	5′	TGCTCGTGCCTCTGGTACA
	3′	CGTGGACACCGAGCTGAT
KCNK4	5′	CATCCAAAAAGCCTTCCAGA
	3′	ATTTGGCAACCACTGGACTC
KCNK5	5′	TTGCTGATTCAGGCACGTAG
	3′	GAGAACCACATGCCAAACCT
KCNK6	5′	TTGATGCTCTGCATGGCTAC
	3′	TCCCCCGTGTGACTTTCTAC
KCNK9	5′	CTACTGGAGGGAGAAGTTGCGGAGA
	3′	ACATCATCATCATCATCATCGTCATC
KCNK10	5′	GCAGCTTTCCCTTAGACCAG
	3′	CCAGGGACATTCATTTTGGA
KCNK13	5′	CTGTTTTGTGGCTTTCAGCA
	3′	CCGCTATCCAGTTTCCTCAG
KCNK16	5′	GTCTTCTGTGTCTTCTATGCTCTGAT
	3′	AGAGATGGGGATTTTCTGTGATCTA
KCNK18	5′	CTCTCTTCTCCGCTGTCGAG
	3′	AAGAGAGCGCTCAGGAAGG
RPL19	5′	AGCCTGTGACTGTCCATTCC
	3′	GGCAGTACCCTTCCTCTTCC

Supplementary Table 2- Specific primers used in qPCR experiments. Primers were designed to amplify a specific 100-250 base pair fragment of each known functional KCNK gene for use in qPCR experiments. qPCR experiments were performed as described in **Methods.**