

*PCR primer design and qRT-PCR.* Genomic DNA and mRNA sequences were downloaded from NCBI LocusLink at <http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene> for all known potassium channels in the Human Genome (Table M1). For each gene of interest PCR primer pairs were designed to span the 3'-most intron and to generate an amplicon that was shared by all known splice variants. Primer characteristics, such as melting temperature ( $T_m$ ), GC content, secondary structure formation and primer-end self complementarity, were evaluated and secondary structure minimized for each amplicon. Selected primers were validated initially *in silico* using NCBI BLAST and subsequently validated empirically on a control whole body cDNA library. Before running qRT-PCR experiments, the efficiency of amplification was determined for each primer set by using 2-fold serial dilutions of input cDNA. All primer sets exhibited an efficiency > 90. All PCR products exhibited a single peak as determined by melt-curve analysis and produced correct products when sequenced. Data was collected using the 7500 Real-Time PCR System (Applied Biosystems) and SensiMix Plus SYBR (Quantace) as fluorescent reporter. Relative quantification was performed using the comparative threshold (CT) method after determining the CT values for reference (18SrRNA) and target genes in each sample sets according to the  $2^{-\Delta\Delta C_t}$  method (Pfaffl, 2006) as described by the manufacturer (Applied Biosystems; User Bulletin 2). Changes in mRNA expression level were calculated after normalization to 18SrRNA.

$$\Delta C_{t \text{ Total}} = C_{t \text{ Mean Total}} - C_{t \text{ Mean 18SrRNA}}$$

$$\Delta C_{t \text{ LCM}} = C_{t \text{ Mean LCM}} - C_{t \text{ Mean 18SrRNA}}$$

$$\Delta\Delta C_t = \Delta C_{t \text{ Total}} - \Delta C_{t \text{ LCM}}$$

$$\text{Gene expression level} = (1 + \text{Efficiency})^{-\Delta\Delta C_t}$$

Table M1.

PCR primers designed in the current study

| Gene                            | Also known as                                                                                                    | Working Primers<br>(5' - 3')                                | Size<br>(bp) | T <sub>m</sub> |
|---------------------------------|------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------|--------------|----------------|
| <b>Human Potassium Channels</b> |                                                                                                                  |                                                             |              |                |
| KCNK1                           | TWIK-1, DPK; HOHO; TWIK1; K2p1.1;                                                                                | GTCCTGGAGGATGACTGGAA<br>CACCTGATCCTCGTCCTTGT                | 261          | 82.0           |
| KCNK2                           | TREK; TPKC1; TREK1; K2p2.1; TREK-1; hTREK-1c; hTREK-1e; MGC126742; MGC126744                                     | CCGTTAGGAAACACCTCCAA<br>CTTTGGCAATTCTTTTCCA                 | 229          | 80.4           |
| KCNK3                           | TASK-1; OAT1; TASK; TBAK1; K2p3.1                                                                                | CCTTCTACTTCGCCATCACC<br>GAACATGACGAGCGTGAGC                 | 128          | 87.0           |
| KCNK4                           | TRAAK; K2p4.1; TRAAK1                                                                                            | GTGCCACCGAGCTAGTAAG<br>AGGATCCAGAACCACACCAG                 | 236          | 87.0           |
| KCNK5                           | TASK2; K2p5.1; TASK-2; FLJ11035                                                                                  | TTCATCACCATCTCCACCAT<br>CAGCCCCAAGTAGATCCAGA                | 114          | 84.4           |
| KCNK6                           | TOSS; KCNK8; TWIK2; K2p6.1; TWIK-2; FLJ12282                                                                     | CCCTCTACAAGGTGCTGGTC<br>GTCTCATCCGCAATTGAAAC                | 158          | 87.3           |
| KCNK7                           | TWIK3; K2p7.1; MGC118782; MGC118784                                                                              | CCTCACCACCACAGGTTATG<br>ACCAGCAGTCCAGTGCAA                  | 228          | 89.3           |
| KCNK9                           | KT3.2; TASK3; K2p9.1; TASK-3; MGC138268; MGC138270                                                               | CTACTTTGCGATCACGGTCA<br>GGAGAAGAAGCCACAGTCA                 | 247          | 88.0           |
| KCNK12                          | THIK2; THIK-2                                                                                                    | CACCGTGGTGTC AACCATAG<br>GCTCCAGGAAGAGTTGAAG                | 128          | 85.9           |
| KCNK13                          | THIK1; THIK-1; K2p13.1                                                                                           | CGTTTCCACCATAGGTTTG<br>CAGGCTGTCCACCTCACACT                 | 241          | 85.9           |
| KCNK16                          | TALK1; TALK-1; K2p16.1; MGC133123                                                                                | CAAGTCACACCCAGGACTT<br>GCTTGTCACCTCTTGCTTC                  | 143          | 83.5           |
| KCNK17                          | TASK4, TALK2; TALK-2; TASK-4; K2p17.1                                                                            | GGGATGTGTCCTCAAGCATAC<br>AAGGGCAAAGAAGATGCAGA               | 179          | 86.0           |
| KCNK18                          | TRIK; TRESK; TRESK2; K2p18.1; TRESK-2                                                                            | ATGGAGGTCTCGGGGCAC<br>GTTTTCTGTCTTCCACCCTGTTTCACTGCAGTTCAAG | 242          | 88.0           |
| KCNT1                           | KCa4.1; bA100C15.2                                                                                               | CGTCTTCAGCATCAGCATGT<br>GAAGACGTGGCTCTCTGTCC                | 241          | 87.0           |
| KCNT2                           | SLICK; KCa4.2; SLO2.1; MGC119610; MGC119611; MGC119612; MGC119613; RP11-58O13.1                                  | CAAAACGACGACAGGGTACA<br>GAAACCTGTAAGCCCCACAA                | 248          | 77.0           |
| KCNU1                           | Slo3; KCNMC1; KCa5.1; Kenma3                                                                                     | CTGGGGCTTCTGTCTTACA<br>CGGGTGATCAGAAACCTTTT                 | 173          | 79.1           |
| KCNV1                           | HNKA; KCNB3; KV2.3; KV8.1                                                                                        | TTGCCATCTTGCCCTTCTAC<br>AGGCCGACTTCTTCGTAACA                | 202          | 81.6           |
| KCNV2                           | Kv8.2; MGC120515                                                                                                 | AATGTCTGAGCCCTAGCTG<br>TCGTCTGTCTGCTCCTCGTA                 | 590          | 88.6           |
| <b>voltage-gated channel</b>    |                                                                                                                  |                                                             |              |                |
| KCNA1                           | EA1; MK1; AEMK; HBK1; HUK1; MBK1; RBK1; KV1.1; MGC126782; MGC138385                                              | CCATCATTCCTTATTTATCAC<br>CTCTTCCCCCTCAGTTTCTC               | 488          | 85.6           |
| KCNA2                           | HK4; MK2; HBK5; NGK1; RBK2; HUKIV; KV1.2; MGC50217                                                               | AGAGAATTGGGCCTCCTGAT<br>CCCCTATCTTTCCCCCAAT                 | 197          | 83.5           |
| KCNA3                           | MK3; HGK5; HLK3; PCN3; HPCN3; KV1.3; HUKIII                                                                      | CATCCTGGGAGGAATGAAA<br>CAGGGTAGGCAACCGAAATA                 | 169          | 77.9           |
| KCNA4                           | HK1; HBK4; PCN2; HPCN2; HUKII; KCNA8; KV1.4; KCNA4L                                                              | GCTTGAGGGGGAACAAAG<br>TACCCAGGTACAATGCCAAA                  | 233          | 82.2           |
| KCNA5                           | HK2; HCK1; PCN1; HPCN1; KV1.5; MGC117058; MGC117059                                                              | CGAGGATGAGGGCTTCATTA<br>AGGGTCTCCAAGCAGAAGGT                | 174          | 85.6           |
| KCNA6                           | HBK2; KV1.6                                                                                                      | CAATGTGTGTGTGAGTCGAG<br>GCGTCTCCACCAGAAAGAAG                | 185          | 83.5           |
| KCNA7                           | HAK6; KV1.7                                                                                                      | GTCCAAGCAAGGCTATCTTC<br>AGAGGACCACACCGATGAAG                | 284          | 88.1           |
| KCNA10                          | Kcn1; Kv1.8                                                                                                      | GCAGAACATCCCAGGAGAAA<br>GAGACAGGATGGACCCAA                  | 151          | 80.9           |
| KCNAB1                          | Shaker-related subfamily, beta member 1 (hKvb3; AKR6A3; KCNA1B; Kvb1.3; hKvBeta3; KV-BETA-1)                     | TTGCCGTGTGAATCATCTCA<br>CCAGGAGCACAGAACTCACA                | 231          | 81.8           |
| KCNAB2                          | shaker-related subfamily, beta member 2 (AKR6A5; KCNA2B; HKvbeta2; KV-BETA-2; MGC117289; HKvbeta2.1; HKvbeta2.2) | TGTTCCAGCGTGAGAAAGTG<br>GGGATGCCACTGTCTACTT                 | 124          | 85.0           |
| KCNAB3                          | Shaker-related subfamily, beta member 3 (AKR6A9; KCNA3B; KCNA3.1B; KV-BETA-3; MGC116886)                         | CCTCCATCAAGGGCTACCA<br>GCTGGCTCAGCACCTGTAG                  | 234          | 87.7           |
| KCNB1                           | Shab-related subfamily, member 1 (Kv2.1; DRK1; KV2.1; h-DRK1)                                                    | CCGCTACCACCAGAAGAAAG<br>ATGGTGGAGAGACGATGAA                 | 207          | 85.0           |
| KCNB2                           | Shab-related subfamily, member 2 (Kv2.2)                                                                         | CTGGAAGTGTGCGACGACTA<br>GGCAGCAGGACTCCAAGTAG                | 193          | 80.7           |
| KCNC1                           | Shaw-related subfamily, member 1 (Kv3.1; KV4; NGK2; FLJ41162; FLJ42249; FLJ43491; MGC129855)                     | CCGTACTCGTCCGCTAC<br>ACGCCCTCGATGTAGGTAAG                   | 206          | 86.0           |
| KCNC3                           | Shaw-related subfamily, member 3 (Kv3.3; SCA13; KSHIID)                                                          | GCTCTTCGAGGACCCCTACT<br>TATGGATGAAGCCCTCATGG                | 119          | 84.0           |

|                                    |                                                                                                                      |                                                       |     |      |
|------------------------------------|----------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------|-----|------|
| KCNC4                              | Shaw-related subfamily, member 4 (Kv3.4; KSHIIC; HKSHIIC; MGC126818)                                                 | CGCTCTTCGAGGATCCCTAC<br>TCACGTTGCGGTGCATATTA          | 132 | 83.1 |
| KCND1                              | Shal-related subfamily, member 1 (Kv4.1)                                                                             | GAGAAGACAACGTGCCATGA<br>TGACTGAGGCAGTGGAGTTG          | 199 | 87.7 |
| KCND2                              | Shal-related subfamily, member 2 (Kv4.2; RK5; KIAA1044; MGC119702; MGC119703)                                        | CTTCGGCTAGCAAGTTCACC<br>TGCTCTGCTCGTTGATTCCTGG        | 216 | 81.8 |
| KCND3                              | Shal-related subfamily, member 3 (Kv4.3; KCND3L; KCND3S; KSHIVB; MGC142035; MGC142037)                               | CATGGCCATCATCATCTTTTG<br>CTGGTGGTAAATCCGGCTAA         | 250 | 84.8 |
| KCNE1                              | Isk-related family, member 1 (ISK; JLNS; LQT5; MinK; JLNS2; MGC33114)                                                | CGTGCTCAGGAGGAAGAGAC<br>AACTGGCCAGAGAAAGCAGA          | 167 | 76.0 |
| KCNE1L                             | KCNE1-like (KCNE5)                                                                                                   | ATTTTCTTGCCCTCCCAGTT<br>AAGTTCCACGGATGAAGCAG          | 169 | 80.3 |
| KCNE2                              | Isk-related family, member 2 (LQT5; LQT6; MiRP1; MGC138292)                                                          | TTGTGTGCAACCCAGAAGAG<br>CTTCCAGCGTCTGTGTGAAA          | 147 | 82.0 |
| KCNE3                              | Isk-related family, member 3 (HOKPP; MiRP2; MGC102685; MGC129924; DKFZp781H21101)                                    | GTCTGAGCTTCTACCGAGTCTTCC<br>CTCGTGTTAGATCATAGACACACGG | 363 | 84.4 |
| KCNE4                              | Isk-related family, member 4 (MiRP3; MGC20353)                                                                       | ACCTCTTGGAAGTGGACGAT<br>GTGCTGTTACAGAGGTCAT           | 109 | 81.7 |
| KCNF1                              | subfamily F, IK8; kH1; KCNF; Kv5.1; MGC33316                                                                         | CGACACCATCTTCTCCCTGT<br>ATGCCCTTCTTCATGTGGAC          | 132 | 82.1 |
| KCNG1                              | subfamily G, member 1 (K13; kH2; KCNG; Kv6.1; MGC12878)                                                              | GTCAACCTCTCCGTGACGAC<br>AGGAGGAATCCAGGGAGAA           | 131 | 85.5 |
| KCNG2                              | subfamily G, member 2 (KCNF2; Kv6.2)                                                                                 | CGTGTCGGTGTCCTTCGT<br>GGAAGTCTGAGGAGAACGAG            | 158 | 88.7 |
| KCNG3                              | subfamily G, member 3 (Interacts Kv2.1); also known as Kv6.3; Kv10.1                                                 | GTGCATCGTGAGGTTCAATTG<br>GGCAAGCTTAATCACCCAAA         | 205 | 81.0 |
| KCNG4                              | subfamily G, member 2 (Kv6.3; Kv6.4; MGC4558; MGC129609)                                                             | GTGAGCCTGTGTGTCAGCA<br>CGACACGTAGTATGGGAGA            | 222 | 84.9 |
| KCNH1                              | subfamily H (eag-related), member 1 (EAG, EAG1, Kv10.1, h-eag )                                                      | GGAGTTCAGACGGTGCAC<br>CCTCATCATCTTGGATCACC            | 116 | 82.9 |
| KCNH2                              | subfamily H (eag-related), member 2 (ERG1, HERG, HERG1, Kv11.1, LQT2, SQT1 )                                         | GCCTTCTCAGGAGTGTCCAA<br>CGTCATCTGCCTCTGTAGCA          | 249 | 89.3 |
| KCNH2B                             |                                                                                                                      | ACACCTTCCTGGACACCATC<br>AAGCCGTGTTGTCAGTAGAT          | 109 | 84.0 |
| KCNH3                              | subfamily H (eag-related), member 3 (Bec1, Elk2 )                                                                    | TTCTTTTATGGGCCAGACAC<br>TTCGCTGATGTCTTGTGAG           | 201 | 85.3 |
| KCNH4                              | subfamily H (eag-related), member 4 (BEC2; ELK1; Kv12.3)                                                             | ATTCCCTTTTCCAAGCCTTC<br>GGTCCTGGGAGAAAGTAGG           | 137 | 84.3 |
| KCNH5                              | subfamily H (eag-related), member 5 (EAG2; H-EAG2; Kv10.2)                                                           | GCCTGGCTGGTACTGGATAG<br>CAGTCGTAAGAGACGCCCA           | 258 | 80.7 |
| KCNH7                              | subfamily H (eag-related), member 7 (ERG3; HERG3; Kv11.3; MGC45986)                                                  | ATTGTCTGGGTCAGAAAGAGA<br>GGTGGCAGCGTTTTCATTAT         | 166 | 80.0 |
| KCNH8                              | subfamily H (eag-related), member 8 (ELK, ELK1, Kv12.1)                                                              | CAAAGGAATCCCCATTTTT<br>TTGACATGAGTTGGCAGAGC           | 184 | 79.1 |
| KCNQ1                              | KQT-like subfamily, member 1 (LQT; RWS; WRS; LQT1; SQT2; ATFB1; JLNS1; KCNA8; KCNA9; Kv1.9; Kv7.1; KVLQT1; FLJ26167) | TTTGCCATCTCCTTCTTTGC<br>CTGGGTGACAGCAGAGTGTG          | 224 | 86.6 |
| KCNQ2                              | KQT-like subfamily, member 2 (EBN; BFNC; EBN1; ENB1; HNSPC; KV7.2; KCNA11; KVEBN1)                                   | CGGCAGAACTCAGAAGAAGC<br>ATGACGTCCATCAGTTCGTA          | 194 | 88.9 |
| KCNQ3                              | KQT-like subfamily, member 3 (BFNC2, EBN2, KV7.3)                                                                    | ATCGGGTTCGCCTTTCTAAT<br>CATGTCTTCGATGGGGAAGT          | 251 | 84.4 |
| KCNQ4                              | KQT-like subfamily, member 4 (DFNA2; KV7.4)                                                                          | CAAACCTCGGGTGACCAAAT<br>GGTAGTCGGAGGTGATGTCG          | 268 | 89.2 |
| KCNQ5                              | KQT-like subfamily, member 5 (Kv7.5 ) M current forms heteromer with Q3                                              | CGCTTTCGTTTTTCTCCTTG<br>CGAGCAAACCTCAGTCTTCC          | 207 | 81.9 |
| KCNS1                              | delayed-rectifier, subfamily S, member 1 (Kv 9.1)                                                                    | GGAGGGAAGGAGATGAGTC<br>GCAGGGATTGGACCACTTTA           | 206 | 82.2 |
| KCNS2                              | delayed-rectifier, subfamily S, member 2 (Kv 9.2)                                                                    | GGACGTGTCGGAGGCTAAC<br>TTGCCGGTGTGATAGAAATG           | 245 | 89.0 |
| KCNS3                              | delayed-rectifier, subfamily S, member 3 (Kv 9.3)                                                                    | CAAAATGCCTATGTGCCACCAC<br>TGCTTTGGTCAACAGACTGC        | 271 | 84.8 |
| <b>Inwardly-rectifying channel</b> |                                                                                                                      |                                                       |     |      |
| KCNJ1                              | Subfamily J, member 1 (ROMK; ROMK1; KIR1.1)                                                                          | TGGACATCTGGACAACGGTA<br>CACACAGGAGTGTGATTGG           | 170 | 81.0 |
| KCNJ2                              | Subfamily J, member 2 (IRK1; LQT7; SQT3; HHIRK1; KIR2.1; HHBIRK1)                                                    | TGTTGGGTTTGACAGTGGAA<br>CCACAGGATTTTCATTGCT           | 215 | 81.6 |
| KCNJ3                              | Subfamily J, member 3 (KGA; GIRK1; KIR3.1)                                                                           | GGACGGAAACTCACGCTTA<br>TCAAGTTGGTCAAGGGAAG            | 135 | 83.9 |
| KCNJ4                              | Subfamily J, member 4 (HIR; HRK1; IRK3; HIRK2; Kir2.3; MGC142066; MGC142068)                                         | ATCGCGCTTGGGATGTA<br>GTCCGTGCATGTCTGAAG               | 115 | 88.3 |
| KCNJ5                              | Subfamily J, member 5 (CIR; GIRK4; KATP1; KIR3.4)                                                                    | GCTTTTCGAGTGTCTTTTGG<br>GGCCAGATGCTGATTTTAA           | 250 | 82.8 |
| KCNJ6                              | Subfamily J, member 6 (BIR1; GIRK2; KATP2; KCNJ7; KIR3.2; hiGIRK2; MGC126596)                                        | CGCTGATCATTAGCCATGAA<br>CCAGCTCTTTGGCACTAAGG          | 285 | 84.0 |

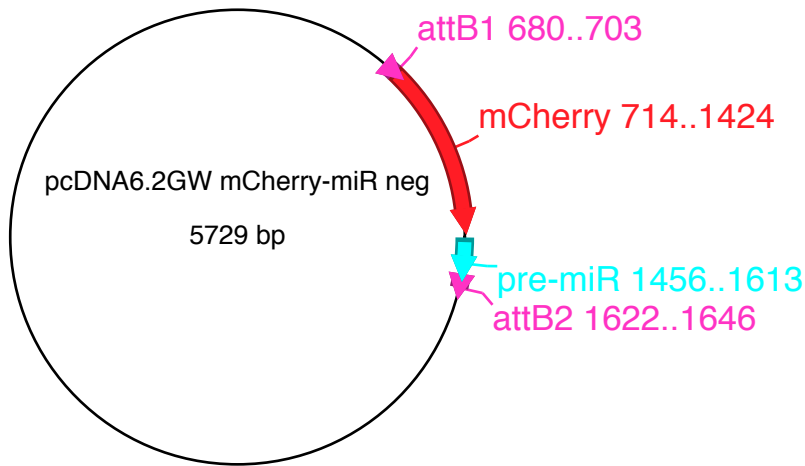
|                                                                 |                                                                                  |                                                  |     |      |
|-----------------------------------------------------------------|----------------------------------------------------------------------------------|--------------------------------------------------|-----|------|
| KCNJ8                                                           | Subfamily J, member 8 (KIR6.1; uKATP-1)                                          | CCATGGGGACATCTATGCTT                             | 155 | 79.3 |
| KCNJ9                                                           | Subfamily J, member 9 (GIRK3; KIR3.3)                                            | TCATCATCCTCCCTCCAAAC<br>TGGAGGGCTTGGTAAACAC      | 183 | 82.8 |
| KCNJ10                                                          | Subfamily J, member 10 (KIR1.2; KIR4.1; BIRK-10; KCNJ13-PEN )                    | CCCATGTCTCTGAAACGACTT<br>AGCTCCGGGTTTAAGAGTCC    | 262 | 88.3 |
| KCNJ11                                                          | Subfamily J, member 11 (BIR; HHF2; PHHI; IKATP; TNDM3; KIR6.2; MGC133230)        | CAATGTGCTCCATTCTCACG<br>ATCATCGTCATCCTGGAAGG     | 162 | 85.8 |
| KCNJ12                                                          | Subfamily J, member 12 (IRK2, KCNJN1, Kir2.2, Kir2.2v, hIRK, hkir2.2x, kcnj12x)  | GGTGTGCGCAAACTTGGAGT<br>TCGATGTGGGCTTCGACAA      | 310 | 87.4 |
| KCNJ13                                                          | Subfamily J, member 13 (KIR1.4, KIR7.1, MGC33328)                                | GTAGAGGGCACCTCATAGG<br>TTGGGGAATCCTAATGGACA      | 211 | 81.6 |
| KCNJ14                                                          | Subfamily J, member 14 (IRK4; KIR2.4; MGC46111)                                  | CAGGAGAGAAGAGAATGCAG<br>CTGGTCTTCAGCGAGAACG      | 220 | 89.6 |
| KCNJ15                                                          | subfamily J, member 15 (KIR1.3, KIR4.2, MGC13584)                                | GGGACACGAGGAAGATACGA<br>CCTGCCTTGAAGAAGACACC     | 274 | 85.6 |
| KCNJ16                                                          | subfamily J, member 16 (BIR9; KIR5.1; MGC33717)                                  | AGCGAACAGGGTGAGTTTGT<br>TTGAGCTGGGAAATCCTTTG     | 187 | 79.5 |
| <b>Large conductance calcium-activated channel</b>              |                                                                                  | TTGCGTCCGCATTGATAATA                             |     |      |
| KCNMA1                                                          | subfamily M, alpha member 1 (MaxiK, SAKCA, SLO, SLO-ALPHA )                      | GGAATGGGAGACGCTTCATA                             | 155 | 80.2 |
| KCNMB1                                                          | subfamily M, beta member 1(K(VCA) beta, SLO-BETA, hslo-beta)                     | CCTGCAGCGAAGTATCATCA<br>CTTTGCCCTGGGTGTAACCAT    | 141 | 82.9 |
| KCNMB2                                                          | subfamily M, beta member 2 (MaxiK channel beta 2 subunit)                        | CTGGTCCCTGATGTTGGTCT<br>AATCACACTCCTGCGCTCATACAT | 202 | 83.0 |
| KCNMB3                                                          | subfamily M, beta member 3 (Maxi K beta 3 subunit)                               | TTCTGTGTGGTAGAGGAGAGC<br>TGCCTCAGGGAAGAAGAGAG    | 269 | 84.0 |
| KCNMB4                                                          | subfamily M, beta member 4 (calcium activated potassium channel beta 4 subunit ) | AGTGACACACCACAGGTGAAG<br>GATTGGTTCCCGAGCCATTTA   | 232 | 85.2 |
| <b>Intermediate/small conductance calcium-activated channel</b> |                                                                                  | TACAAGCCTCCTTCCCCTTT                             |     |      |
| KCNN1                                                           | subfamily N, member 1 (SK1; hSK1; SKCA1; KCa2.1)                                 | AAGCTGAACGACCAGGCTAA                             | 81  | 81.7 |
| KCNN2                                                           | subfamily N, member 2 (SK2; hSK2; SKCA2; KCa2.2)                                 | GTGCAGCTCCGATACAAGGT<br>AGCTGTAGTGGCAAGGAAGC     | 279 | 78.5 |
| KCNN3                                                           | subfamily N, member 3 (SK3; hSK3; SKCA3; KCa2.3)                                 | GCCAAGTCCACCAAAGTGTT<br>TCCTCCAAGCTATCCACCAG     | 135 | 80.9 |
| KCNN4                                                           | subfamily N, member 4 (IK1, IKCA1, KCA4, KCa3.1, SK4, hIKCa1, hKCa4, hSK4)       | TCCGGTCATTGAGTTCTGTG<br>CATCACATTCTGACCATCG      | 159 | 88.0 |
|                                                                 |                                                                                  | ACGTGCTTCTCTGCCTTGTT                             |     |      |

## Molecular cloning and lentiviral miRNA construction:

### miR RNAi cloning

#### Creation of pcDNA6.2-GW\_mCherry-miR neg

Emerald GFP (emGFP: Invitrogen) was replaced in pcDNA6.2-GW\_EmGFP-miR negative control vector (miR neg sequence 5'-gtctccacgcgcagtacattt-3') with mCherry (Clontech) by excising with restriction endonuclease *DraI* and inserting PCR amplified *DraI* digested mCherry, (Table 2: PCR primers; forward *DraI* mCherry, reverse *DraI* mCherry) to create pcDNA6.2-GW\_mCherry-miR neg (Figure M1).

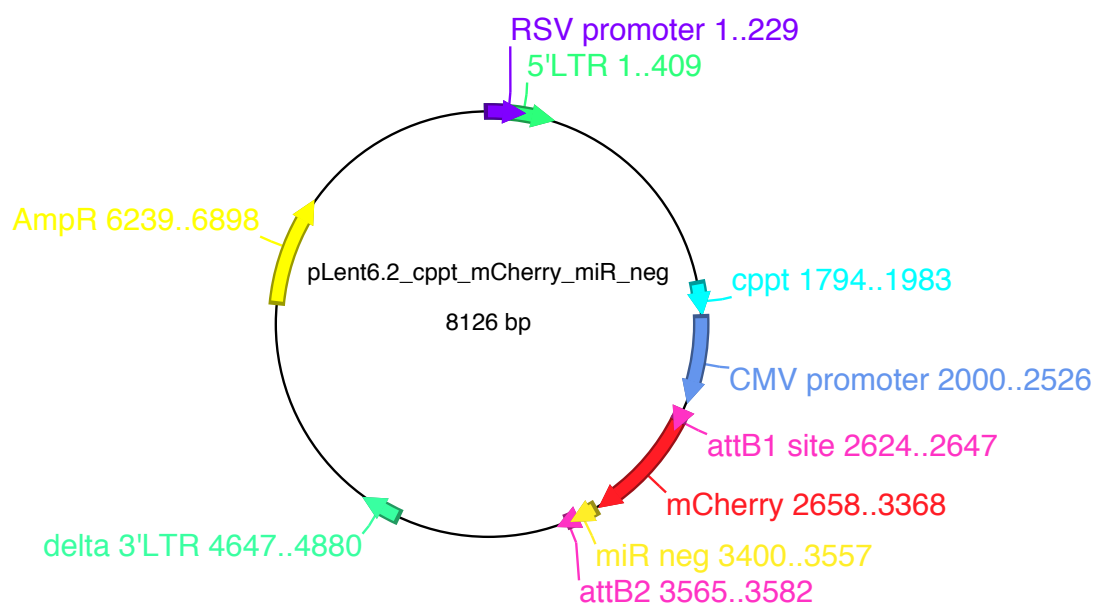


**Figure M1.** Plasmid map of negative control pcDNA6.2-GW\_mCherry-miR neg. A GATEWAY (Invitrogen) enabled cassette flanked by recombination sites attB1 and attB2 consisted of mCherry (Clontech) and a pre-miR containing a sequence 5'-gtctccacgcgcagtacattt-3' with no similarity to the human or mouse transcriptome.

### Design of pLenti6.2-GW-mCherry-KCNJ13 miR RNAi

We used the Invitrogen Block-iT RNAi designer web programme to identify DNA sequences within KCNJ13 (NM\_002242) that could be incorporated into the pre-miR then aligned human (NM\_002242) and mouse (NM\_001110227.1) DNA sequences using Lasergene (DNASTar Inc.) and chose sequences that were also conserved between species as putative sequences to silence target genes. The miR RNAi were numbered according to their distance from the start codon (ATG) of KCNJ13; 404 5'-tcgccttactgccatacaaa-3', h543G 5'-ggatggcaaacctaattcttat-3', m543A 5'-agatggcaaacctaattcttat-3' and 1028 5'-tcaatggacaaagcattgaca-3'. DNA sequence 543 was species specific, due to a one base-pair mismatch, necessitating construction of human (h543G) and mouse (m543A) specific sequences. These were incorporated into oligonucleotides encoding the full pre-miR expression cassette with *Bam*HI and *Xho*I compatible overhangs (Table M2), annealed and inserted into *Bam*HI and *Xho*I digested pcDNA6.2-GW\_mCherry-miR neg replacing miR neg

to create pcDNA6.2-GW\_mCherry-miR 404, -h543G, -m543A and -1028. Inserts were verified by DNA sequencing before recombining into pLenti6.2 (Figure M2).

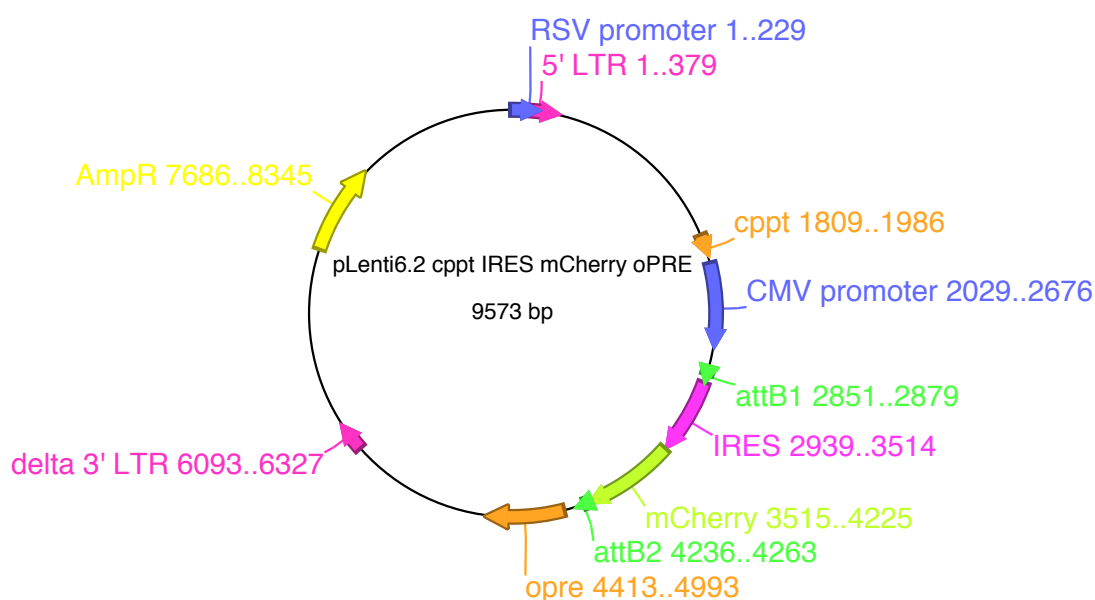


**Figure M2.** Plasmid map of pLenti6.2-cppt-mcherry-miR-neg. RSV, rous sarcoma virus promoter; 5'LTR, 5' long terminal repeat; cppt, central polypurine track; CMV, cytomegalovirus promoter; attB1 and attB2, recombination sites; delta 3'LTR, modified 3' long terminal repeat; AmpR, ampicillin resistance.

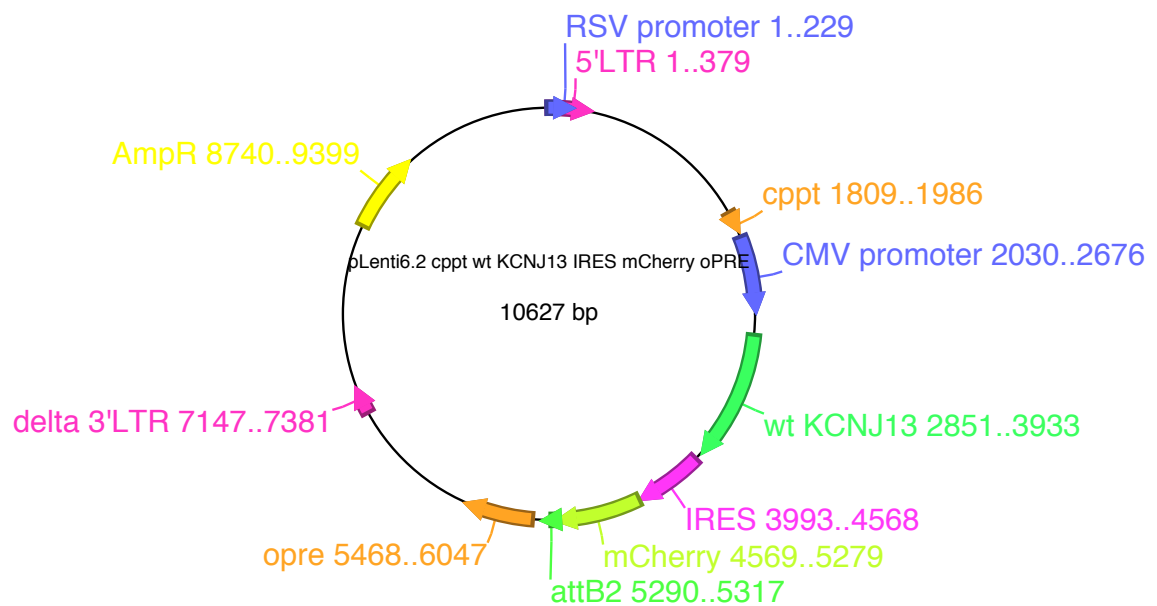
### Cloning KCNJ13

pDONR221-KCNJ13 (Invitrogen) was converted using conventional PCR and laboratory techniques to match the protein coding region of human KCNJ13 (NM\_002242). Three nucleotide mismatches were contained within a 688 bp fragment bounded by *Acc65I* and *BglII* restriction enzyme digestion sites. This fragment was amplified from the Invitrogen

clone using forward *Acc65I* and reverse *BglII* PCR primers containing bases that matched the NM\_002242 sequence. The PCR product was gel purified then further amplified with GATEWAY enabled PCR primers attB1\_*Acc65I* and attB5R\_*BglII* gel purified and recombined into pDONR221-P1-P5r to generate pDONR221-B1-*Acc65I*\_KCNJ13\_*BglII*-B5r and verified by DNA sequencing. The KCNJ13 (Invitrogen) clone was digested with *Acc65I* and *BglII* and the 688 bp insert replaced with the corrected PCR amplified fragment, which was obtained by digestion and gel purification of *Acc65I*\_KCNJ13\_*BglII* to create pDONR221-B1-wtKCNJ13-B5r (wt; wild-type). Two IRES-mCherry fragments were amplified by PCR from plasmid pLVX-IRES-mCherry (Clontech) using two primer pairs: attB1-IRES-mCherry and attB2-mcherry and attB5-IRES-mCherry and attB2-mcherry (Table M2) and recombined into pDONR221 P1-P2 and pDONR221 P5-P2 respectively to create pDONR221 B1-IRES-mCherry-B2 and pDONR221 B5-IRES-mCherry-B2. Clones were recombined with plenti6.2-cppt-DEST-opre to create negative control; pLenti6.2-cppt-IRES-mCherry-opre (Figure M3) and pDONR221-B1-wt KCNJ13-B5r to create pLenti6.2-cppt-wtKCNJ13-IRES-mCherry-opre (Figure M4).



**Figure M3.** Plasmid map of pLenti6.2-cppt-IRES-mcherry-opre. RSV, rous sarcoma virus promoter; 5'LTR, 5' long terminal repeat; cppt, central polypurine track; CMV, cytomegalovirus promoter; attB1 and attB2, recombination sites; IRES, internal ribosome entry site; opre, optimized post-translational response element; delta 3'LTR, modified 3' long terminal repeat; AmpR, ampicillin resistance.



**Figure M4.** Plasmid map of pLenti6.2-cppt-wt KCNJ13-IRES-mcherry-opre. RSV, rous sarcoma virus promoter; 5'LTR, 5' long terminal repeat; cppt, central polypurine track; CMV, cytomegalovirus promoter; attB1 and attB2, recombination sites; wt, wild-type; KCNJ13, coding region of human potassium inwardly-rectifying channel, subfamily J, member 13; IRES, internal ribosome entry site; opre, optimized post-translational response element; delta 3'LTR, modified 3' long terminal repeat; AmpR, ampicillin resistance.

## Generation of Lentivirus

Lentiviral particles Lv-mCherry-miR-neg; Lv-mCherry-KCNJ13-404; Lv-mCherry-hKCNJ13-543G; Lv-mCherry-mKcnj13-543A, Lv-mCherry-KCNJ13-1028 and Lv-KCNJ13-IRES-mCherry were generated for *in vitro* use as described in Evans et al. *Faseb J* 23, 2165-75, 2009. We generated lentivirus for *in vivo* experiments as follows:  $1 \times 10^7$  HEK293T cells were seeded into 150 mm plates coated with a dried Matrigel basement membrane (Becton Dickinson), previously diluted 1:30 in PBS, and transfected as described (Sena-Esteves et al. *J. Virol Meth.* 122, 131-139, 2004) with a ratio of 28.35 mg pLenti6.2 shuttle vector: 20.25 mg psPAX2 (Addgene): 8.1 mg pVSV-G and left 16 h before washing 2 x with opti-MEM (Invitrogen) and cultured in 18 ml opti-MEM for a further 48 h with a change of media at 24 h. The harvests were centrifuged at 500 g filtered through a 0.45 mm filter (Millipore), pooled and concentrated using a centrifugal filter with a 100,000 molecular weight cut off (Millipore). Volumes were scaled up to generate a final titre of  $1 \times 10^8$  transduction units/ml (TU/ml). Lentiviruses were aliquoted and stored at  $-80^\circ\text{C}$  before titting on HT1080 cells. Briefly,  $2 \times 10^5$  HT1080 cells were plated per well of a 6 well plate and serially diluted virus added with 6 mg/ml polybrene (Sigma) and left for a minimum of 6 h before washing off and culturing cells for a further 72 h. Fluorescent cells were counted to calculate a titre, which was confirmed by a multiplicity of infection (MOI) test by adding 1, 2 and 5 viruses per HT1080 cell plated and imaged after 72 h ensuring 1:1 cellular transduction. Only titres in excess of  $1 \times 10^8$  were used in subsequent experiments.

**Table M2.** Synthetic Oligonucleotides used for PCR and miR RNAi experiments

| PCR | Name | DNA Sequence (5'-3') |
|-----|------|----------------------|
|-----|------|----------------------|

|             |                                  |                                                                                                                                                                                                                       |
|-------------|----------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|             | forward <i>Dra</i> I<br>mCherry* | GGGGGTTTAAACACCATGGTGAGCAAGGGCGAGGAG                                                                                                                                                                                  |
|             | reverse <i>Dra</i> I<br>mCherry  | GGGGTTTAAACGTTCACTTGTACAGCTCGTCCATG                                                                                                                                                                                   |
|             | forward<br><i>Acc65</i> I^       | GGTTATGGTACCATGTTCCCCAGTGGTGACTGTCCAAGTGCAAT<br>CGCCTTACTTGCCATACAAATGCTCCTAGGCCTCATGCTAGAG<br>GCTTTTATCACAGGTGCTTTTGTGGCGAAGATTGCCCGGCCAA<br>AAAATCGAGCTTTTTCAATTCGCTTTACTGACACAGCAGTAGT<br>AG                       |
|             | reverse <i>Bgl</i> II            | CAGAGATCTGAAAATTGTCAATGCTTTGTCCATTGATGTGGATA<br>TCCAGGTCAGTCCTGTTTGGGCTTTTAGAAACCAGAGGAGTTG<br>GAAATTCAGGGACAGTCTTGTCAAATTCTCCATCTTGATTG<br>ATATTCACCTTTGGAACCTCGGGTCAACAGAGATGCAAAACAG<br>TGATGTAACATGATTCAGACGGTAGG |
|             | attB1_ <i>Acc65</i> I            | GGGGACAAGTTTGTACAAAAAAGCAGGCTGGTTATGGTACCAT<br>GTTCCCCAG                                                                                                                                                              |
|             | attB5R_ <i>Bgl</i> II            | GGGGACAACCTTTGTATACAAAGTTGTCAGAGATCTGAAAATT<br>GTCAATGC                                                                                                                                                               |
|             | attB1-IRES-<br>mCherry           | GGGGACAAGTTTGTACAAAAAAGCAGGCTACTAGAGGATCTA<br>TTTCCGGTG                                                                                                                                                               |
|             | attB2-mcherry                    | GGGGACCACTTTGTACAAGAAAGCTGGGTCAGACGCGTTCCT<br>TGTACAG                                                                                                                                                                 |
|             | attB5-IRES-<br>mCherry           | GGGGACAACCTTTGTATACAAAGTTGACTAGAGGATCTATTTC<br>CGGTG                                                                                                                                                                  |
| miR<br>RNAi | KCNJ13 404<br>top                | GATCCTGGAGGCTTGCTGAAGGCTGTATGCTGTTTGTATGGCA<br>AGTAAGGCGAGTTTTGGCCACTGACTGACTCGCCTTATGCCAT<br>ACAAACAGGACACAAGGCCTGTTACTAGCACTCACATGGAAC                                                                              |

|  |                        |                                                                                                                                                                      |
|--|------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|  |                        | AAATGGCCCAGATCTGGCCGCAC                                                                                                                                              |
|  | KCNJ13 404<br>bottom   | TCGAGTGCGGCCAGATCTGGGCCATTTGTTCCATGTGAGTGCTA<br>GTAACAGGCCTTGTGTCCTGTTTGTATGGCATAAGGCGAGTCA<br>GTCAGTGGCCAAAACCTCGCCTTACTTGCCATACAAACAGCATA<br>CAGCCTTCAGCAAGCCTCCAG |
|  | KCNJ13<br>h543G top    | GATCCTGGAGGCTTGCTGAAGGCTGTATGCTGATAAGATTAGG<br>TTTGCCATCCGTTTTGGCCACTGACTGACGGATGGCACCTAATC<br>TTATCAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAA<br>ATGGCCCAGATCTGGCCGCAC  |
|  | KCNJ13<br>h543G bottom | TCGAGTGCGGCCAGATCTGGGCCATTTGTTCCATGTGAGTGCTA<br>GTAACAGGCCTTGTGTCCTGATAAGATTAGGTGCCATCCGTCA<br>GTCAGTGGCCAAAACGGATGGCAAACCTAATCTTATCAGCATA<br>CAGCCTTCAGCAAGCCTCCAG  |
|  | KCNJ13<br>m543A top    | GATCCTGGAGGCTTGCTGAAGGCTGTATGCTGATAAGATTAGG<br>TTTGCCATCTGTTTTGGCCACTGACTGACAGATGGCACCTAATC<br>TTATCAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAA<br>ATGGCCCAGATCTGGCCGCAC  |
|  | KCNJ13<br>m543A bottom | TCGAGTGCGGCCAGATCTGGGCCATTTGTTCCATGTGAGTGCTA<br>GTAACAGGCCTTGTGTCCTGATAAGATTAGGTGCCATCTGTCA<br>GTCAGTGGCCAAAACAGATGGCAAACCTAATCTTATCAGCATA<br>CAGCCTTCAGCAAGCCTCCAG  |
|  | KCNJ13 1028<br>top     | GATCCTGGAGGCTTGCTGAAGGCTGTATGCTGTGTCAATGCTTT<br>GTCCATTGAGTTTTGGCCACTGACTGACTCAATGGAAAGCATT<br>GACACAGGACACAAGGCCTGTTACTAGCACTCACATGGAACA<br>AATGGCCCAGATCTGGCCGCAC  |
|  | KCNJ13 1028<br>bottom  | TCGAGTGCGGCCAGATCTGGGCCATTTGTTCCATGTGAGTGCTA<br>GTAACAGGCCTTGTGTCCTGTGTCAATGCTTTCCATTGAGTCAG                                                                         |

|  |  |                                                                    |
|--|--|--------------------------------------------------------------------|
|  |  | TCAGTGGCCAAACTCAATGGACAAAGCATTGACACAGCATA<br>CAGCCTTCAGCAAGCCTCCAG |
|--|--|--------------------------------------------------------------------|

*Dra*I, *Acc*65I, *Bgl*II, *Bam*HI and *Xho*I restriction enzyme sites are italicized (\*) and DNA nucleotides (^) altered to match NM\_002242 are underlined.

### **Compound screening**

#### **Cell line generation;**

hKir7.1 (NM\_002242.4) expressing Chinese hamster ovary (CHO) cells were generated using the Flp-In system (Invitrogen)<sup>1</sup>. Flp-in host CHO cells were transfected with pOG44 (3.6μg) and hKir7.1-pcDNA5-FRT (0.4μg) plasmids using Lipofectamine 2000 according to the manufacturer's guidelines. After 48 hours cells were harvested and cultured in media (see cell culture) containing Hygromycin-B (400μg/ml). After approximately 14 days under selection resultant cell colonies were expanded and cells were tested for hKir7.1 functional responses using the automated electrophysiology assay.

#### **Cell culture;**

Cells were routinely cultured at 37°C in 5% CO<sub>2</sub> in Hams F12 Nutrient Mix Glutamax (Invitrogen), supplemented with 10% FBS (Invitrogen), 1X Penicillin-Streptomycin (Invitrogen) and 200 ug/ml Hygromycin-B (Invitrogen). Cells were not permitted to exceed 85% confluence and were passaged every 2-3 days following dissociation with 0.25% trypsin-EDTA solution (Invitrogen). Prior to assay, cells were transferred to 30°C in 5% CO<sub>2</sub> and incubated for 90 - 120h.

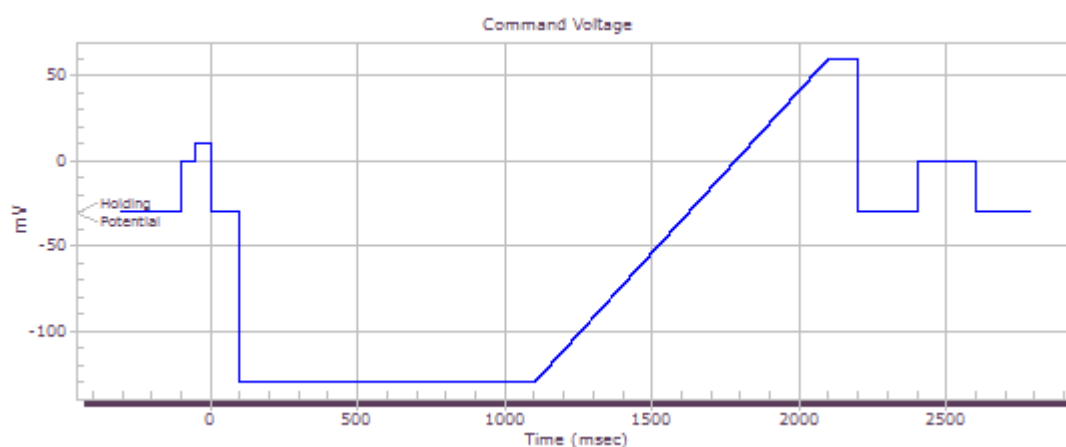
#### **Kir7.1 Automated Electrophysiology Assay;**

Standard methodologies of the IonWorks®(Molecular Devices™) automated patch clamp electrophysiology system were followed as described<sup>2</sup>. Using the population patch clamp

(PPC) configuration<sup>3</sup>, an ensemble average of the current from up to 64 cells per well was recorded in a 384-well microtitre plate.

External (4mM NaCl, 50 mM RbCl, 90mM KCl, 10mM HEPES, 1mM MgCl<sub>2</sub>, 1.8mM CaCl<sub>2</sub>, pH7.3) and internal (90mM K-gluconate, 40mM KCl, 10mM NaCl, 3.2mM EGTA, 5mM HEPES, 3.2mM MgCl<sub>2</sub>, pH 7.3) recording solutions were prepared (Sigma-Aldrich) and electrical access achieved using amphotericin (200mg/ml) in the internal solution.

Cells were initially held for a period of 1s at -130mV before a ramp to +60mV was applied over a 1s period. This was followed by a 200ms step from -30 to 0mV. An identical voltage protocol was applied before and after (5-7min) application of test compounds.



#### Data analysis;

Peak current amplitude was determined by subtracting the baseline current at 0 mV (2500 to 2590 ms) from the current evoked at -130 mV (800 to 1090 ms). Functional responses were expressed as a ratio of current amplitudes in the presence and absence of test compound and subsequently normalised (% inhibition) to control well responses within a plate (DMSO and 3.3mM Ba<sup>2+</sup>).

$$Response = \left( \frac{Presence}{Absence} \right) \times 100$$

$$\% Inhibition = \left( \frac{DMSO \text{ Control Response} - Test \text{ Compound Response}}{DMSO \text{ Control Response} - Barium \text{ Control Response}} \right) \times 100$$

Non-linear regression of concentration-effect curves was performed using the 4 parameter logistic model to yield estimates of pIC<sub>50</sub> and slope value (XLfit Version 5.1.0.0).

**References;**

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