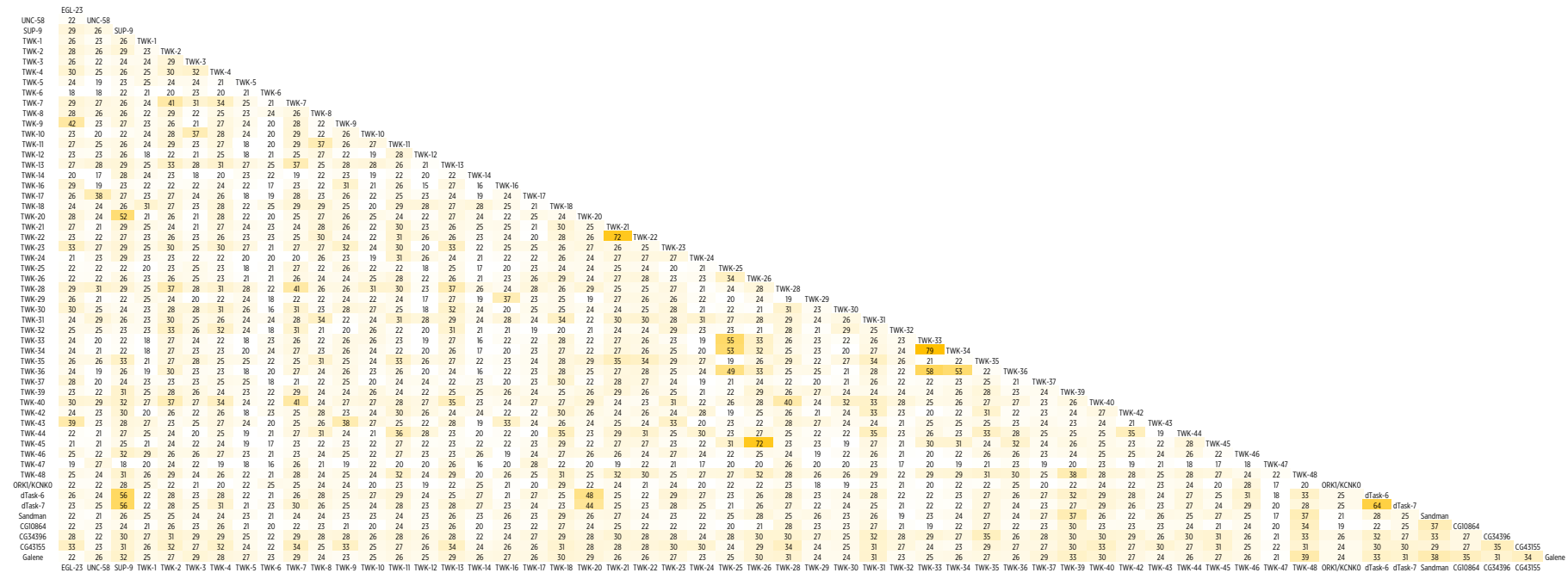Supplementary Information

Mutation of a single residue promotes gating of vertebrate and invertebrate two-pore domain potassium channels

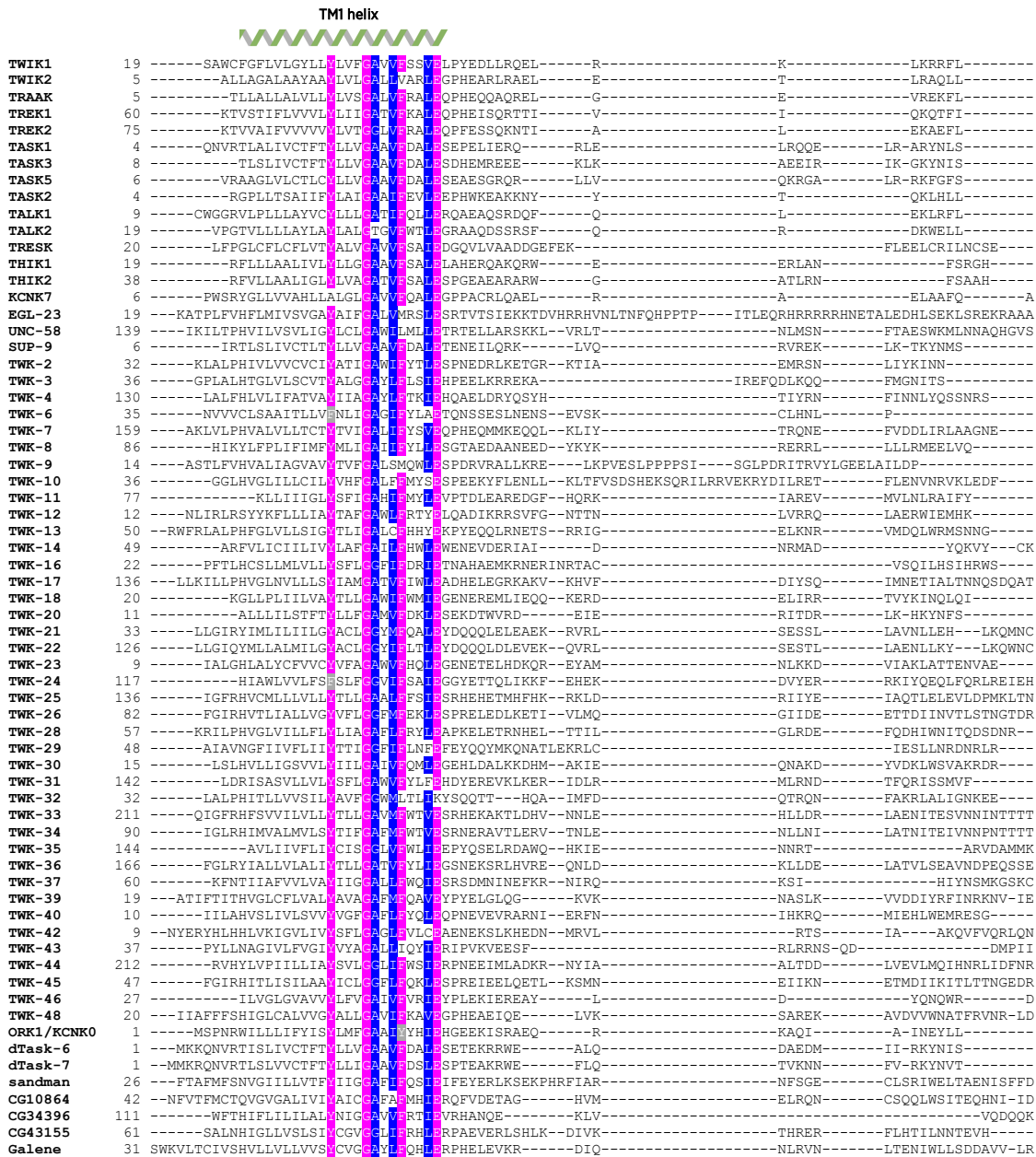Ben Soussia*, El Mouridi*, *et al.*

Supplementary Figure 1 – One-to-one sequence identity matrix based on clustal omega alignement. Genbank identifiers for aligned sequences are listed below. N- and C-terminal cytoplasmic portions were truncated before alignment.

	TWIK1	TWIK2	TRAAK	TREK1	TREK2	TASK1	TASK3	TASK5	TASK2	TALK1	TALK2	TRESK	THIK1	THIK2	KCNK7
TWIK2	47														
TRAAK	33	35													
TREK1	31	31	52												
TREK2	33	29	56	70											
TASK1	32	29	31	33	35										
TASK3	32	29	31	34	32	74									
TASK5	31	29	31	35	33	67	68								
TASK2	29	30	33	32	33	33	32	34							
TALK1	34	33	39	38	38	37	35	38	39						
TALK2	27	31	38	34	32	30	30	33	37	44					
TRESK	26	25	25	28	30	31	32	29	21	29	23				
THIK1	32	25	28	29	28	32	31	29	26	30	24	26			
THIK2	32	31	27	30	27	30	28	30	26	29	23	25	70		
KCNK7	42	36	29	27	28	26	26	29	24	26	24	21	21	25	
EGL-23	23	24	28	26	26	29	29	28	26	25	24	25	20	23	21
UNC-58	23	25	25	22	23	24	23	24	24	27	23	27	20	20	19
SUP-9	27	25	31	33	32	58	58	52	31	33	30	30	31	31	25
TWK-1	29	27	24	25	27	26	24	23	24	21	26	21	19	26	
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dTask-7	28	25	30	29	31	54	55	49	29	34	29	27	32	30	22
Sandman	27	25	25	28	27	24	26	25	25	25	27	25	23	23	22
CG10864	22	24	26	27	26	22	24	26	23	23	23	27	25	24	22
CG34396	28	24	27	27	24	30	30	30	27	26	27	25	25	24	25
CG43155	31	29	28	28	29	30	30	31	31	31	31	28	25	25	24
Galene	28	28	31	31	30	34	34	34	25	28	30	27	27	27	22

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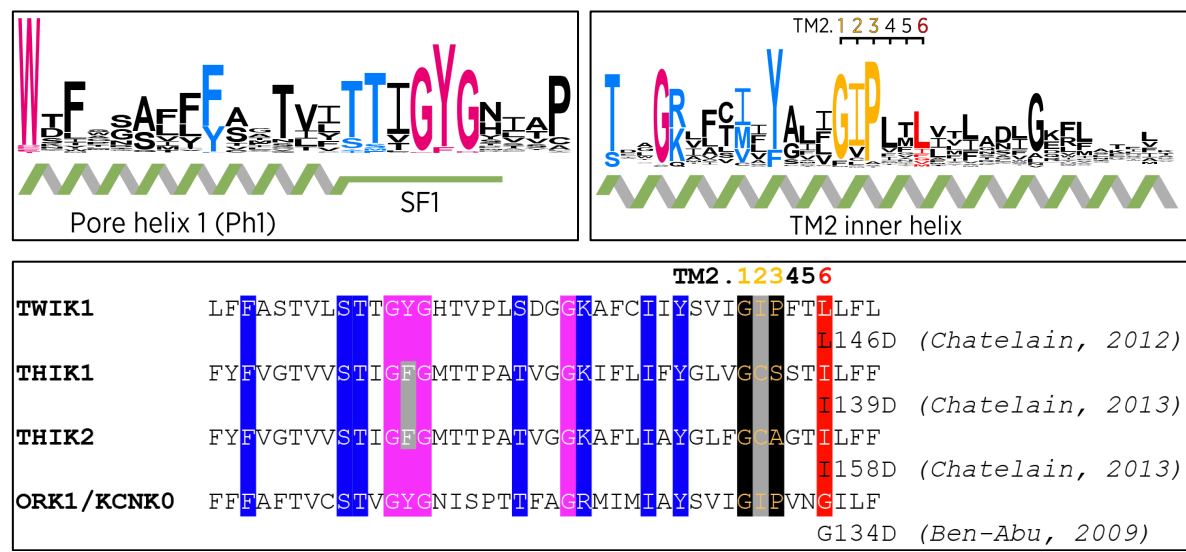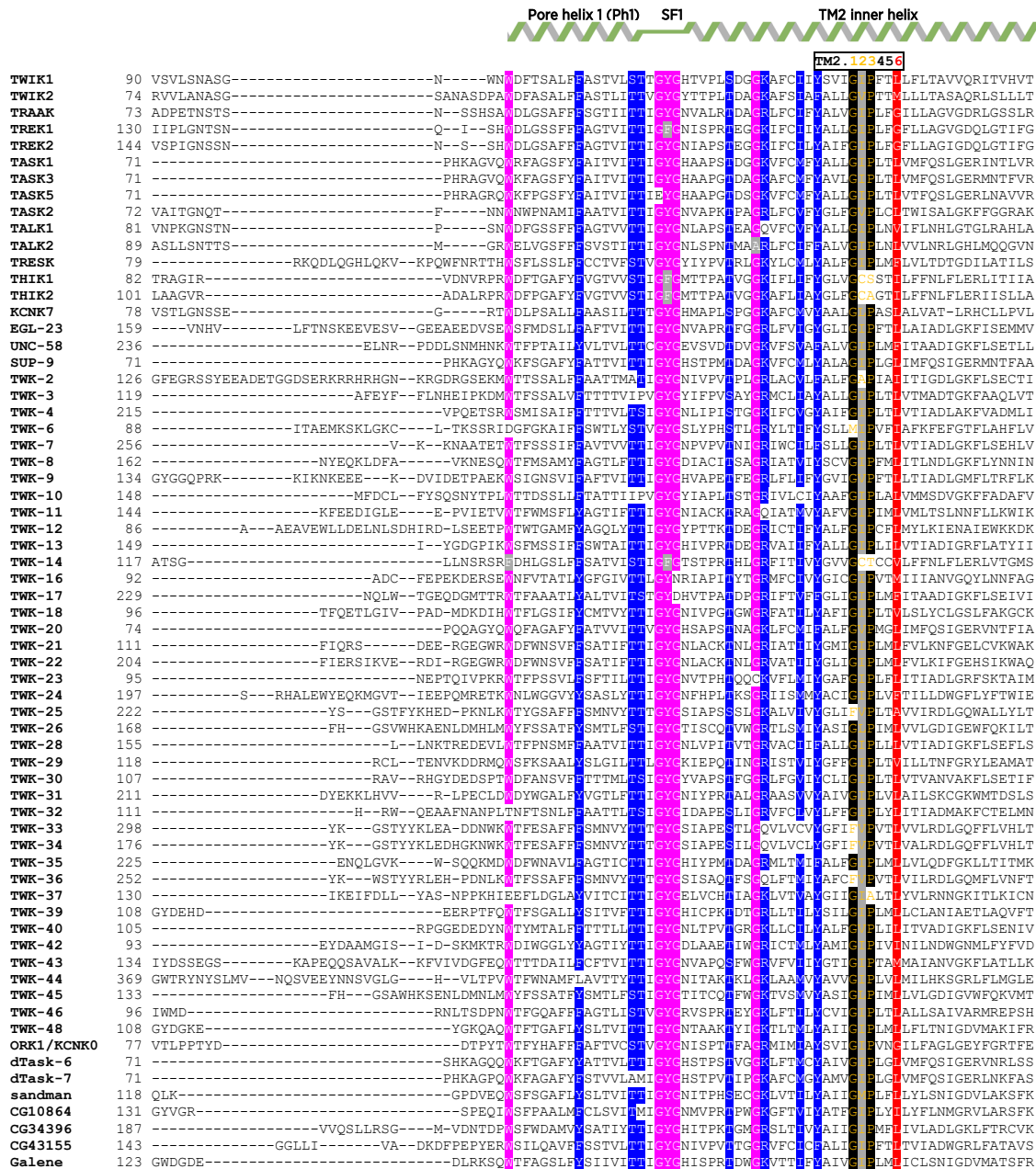
Supplementary Figure 2

TM1 helix

```
TWIK1       19 ------SAWCFGFLVLGYLLLVFGAVVLSSVVLPYEDLLRQEL------R-------------------K-------------LKRRFL--------
TWIK2        5 ---------ALLAGALAAYAALVLVLGALLVARLGPHEARLRAEL------E-------------------T-------------LRAQLL--------
TRAAK        5 ---------TLLALLALVLLVLVSGALLVRALGPHEQQAQREL------G-------------------E-------------VREKFL--------
TREK1       60 --------KTVSTIFLVVVVLIIGATVVKALPQHEISQRTTI------V-------------------I-------------QKQTFI--------
TREK2       75 --------KTVVAIFVVVVVLVTGGLVVRALPQFESSQKNTI------A-------------------L-------------EKAEFL--------
TASK1        4 -----QNVRTLALIVCFTYLLVGAVVLDALSEPELIERQ------RLE-------------------LRQQE-------LR-ARYNLS-------
TASK3        8 --------TLSLIVCFTYLLVGAVVLDALSDHEMREEE------KLK-------------------AEEIR-------IK-GKYNIS-------
TASK5        6 --------VRAAGLVLCTLCYLLVGAVVLDALSEAESGRQR------LLV-------------------QKRGA-------LR-RKFGFS-------
TASK2        4 --------RGPLLTSAIIFYLAIGAVVLEVLEPHWKEAKKNY------Y-------------------T-------------QKLHLL--------
TALK1        9 -----CWGGRVLPLLLAYVCYLLLGATIFQLLRQAEAQSRDQF------Q-------------------L-------------EKLRFL--------
TALK2       19 --------VPGTVLLLAYLAYLALGTGVVWTLEGRAAQDSSRSF------Q-------------------R-------------DKWELL--------
TRESK       20 ----LFPGLCFLCFLVTYALVGAVVLSATEDGQVLVAADDGEFEK-----------------------------------FLEELCRILNCSE----
THIK1       19 --------RFLLLAALIVLYLLGGAVVLSALDLAHERQAKQRW------E-------------------ERLAN-------------FSRGH-----
THIK2       38 --------RFVLLAALIGLYLVAGATVVSALSPGEAEARARW------G-------------------ATLRN-------------FSAAH-----
KCNK7        6 -------PWSRYGLLVVAHLLALGLGAVVLQALEGPPACRLQAEL------R-------------------A-------------ELAAFQ-------A
EGL-23      19 ---KATPLFVHFLMIVSVGAYAIFGALVVMRSLSRTVTSIEKKTDVHRRHVNLTNFQHPPTP----ITLEQRHRRRRRHNETALEDHLSEKLSREKRAAA
UNC-58     139 ----IKILTPHVILVSVLIGYLCLGAVILMLLTRTELLARSKKL--VRLT-------------------NLMSN-------FTAESWKMLNNAQHGVS
SUP-9        6 --------IRTLSLIVCTLTYLLVGAVVLDALETENEILQRK------LVQ-------------------RVREK-------LK-TKYNMS-------
TWK-2       32 -----KLALPHIVLVVCVCIYATIGAWLIYTLSPNEDRLKETGR--KTIA-------------------EMRSN-------LIYKINN---------
TWK-3       36 -----GPLALHTGLVLSCVTYALGGAYLLSTLSHPEELKRREKA-----------------------IREFQDLKQQ------FMGNITS-------
TWK-4      130 -----LALFHLVLIFATVAYIIAGAYLTKLSHQAELDRYQSYH----------------------TIYRN-------FINNLYQSSNRS-----
TWK-6       35 -------NVVVCLSAAITLLVGNLIGAGLIYLAPTQNSSESLNENS--EVSK-------------------CLHNL-------P------------
TWK-7      159 ----AKLVLPHVALVLLTCTYTVIGAILIYSVLQPHEQMMKEQQL--KLIY-------------------TRQNE-------FVDDLIRLAAGNE----
TWK-8       86 -------HIKYLFPLIFIMFYMLIGAILIYLLSGTAEDAANEED--YKYK-------------------RERRL-------LLLRMEELVQ-------
TWK-9       14 ----ASTLFVHVALIAGVAVYTVFGALSMQWLSPDRVRALLKRE----LKPVESLPPPPSI----SGLPDRITRVYLGEELAILDP----------
TWK-10      36 -------GGLHVGLILCILYVHFGALFMYSLSPEEKYFLENLL--KLTFVSDSHEKSQRILRRVEKRYDILRET-------FLENVNRVKLEDF----
TWK-11      77 -----------KLLIIIGLYSFIGAHIFMYLSVPTDLEAREDGF--HQRK-------------------IAREV-------MVLNLRAIFY-------
TWK-12      12 ---NLIRLRSYYKFLLLIAMTAFGAYLLRTYSLQADIKRRSVFG--NTTN-------------------LVRRQ-------LAERWIEMHK-------
TWK-13      50 --RWFRLALPHFGLVLLSIGYTLIGALCFHHYSKPYEQQLRNETS--RRIG-------------------ELKNR-------VMDQLWRMSNNG-----
TWK-14      49 --------ARFVLICIILIVYLAFGAILFHWLSWENEVDERIAI------D-------------------NRMAD-------------YQKVY---CK
TWK-16      22 ------PFTLHCSLLMLVLLYSFLGAFILDRITTNAHAEMKRNERINRTAC------------------------------VSQILHSIHRWS----
TWK-17     136 ----LLKILLPHVGLNVLLLSYIAMGATVYIWLSADHELEGRKAKV--KHVF-------------------DIYSQ-------IMNETIALTNNQSDQAT
TWK-18      20 ---KGLLPLIILVAYTLLGAWVLSWMISGENEREMLIEQQ--KERD-------------------ELIRR-------TVVYKINQLQI-------
TWK-20      11 ----ALLILSTFTYLLFGAWVVDKLSEKDTWVRD------EIE-------------------RITDR-------LK-HKYNFS-------
TWK-21      33 ------LLGIRYIMLILIILGYACLGGYMVQALYDQQQLELEAEK--RVRL-------------------SESSL-------LAVNLLEH---LKQMNC
TWK-22     126 -----LLGIQYMLLALMILGYACLGGYILTLYDQQQLDLEVEK--QVRL-------------------SESTL-------LAENLLKY---LKQWNC
TWK-23       9 ------IALGHLALYCFVVCYVFAGAWIVHQLSGENETELHDKQR--EYAM-------------------NLKKD-------VIAKLATTENVAE----
TWK-24     117 ----------HIAWLVVLFSESLFGCVLISALSGGYETTQLIKKF--EHEK-------------------DVYER-------RKIYQEQLFQRLREIEH
TWK-25     136 ------IGFRHVCMLLLVLLYTLLGAALIFSILSRHEHETMHFHK--RKLD-------------------RIIYE-------IAQTLELEVLDPMKLTN
TWK-26      82 -----FGIRHVTLIALLVGYVFLGGFMLEKLSPRELEDLKETI--VLMQ-------------------GIIDE-------ETTDIINVTLSTNGTDR
TWK-28      57 -----KRILPHVGLVILLFLYLIAGAFLRYLSAPKELETRNHEL--TTIL-------------------GLRDE-------FQDHIWNITQDSDNR--
TWK-29      48 ------AIAVNGFIIVFLIIYTTIGCFILDNFFEYQQYMKQNATLEKRLC--------------------IESLLNRDNRLR----
TWK-30      15 -----LSLHVLLIGSVVLYIILGAVIYQMFGEHLDALKKDHM--AKIE-------------------QNAKD-------YVDKLWSVAKRDR----
TWK-31     142 -------LDRISASVLLVLYSFLGAWVGYLFHDYEREVKLKER--IDLR-------------------MLRND-------TFQRISSMVF-------
TWK-32      32 ------LALPHITLLVVSILMAVFGCWMLTLLKYSQQT---HQA--IMFD-------------------QTRQN-------FAKRLALIGNKEE----
TWK-33     211 ----QIGFRHFSVVILVLLYTLLGAVMWTVFSRHEKAKTLDHV--NNLE-------------------HLLDR-------LAENITESVNNINTTTT
TWK-34      90 ------IGLRHIMVALMVLSYTIFGAFMWTVFSRNERAVTLERV--TNLE-------------------NLLNI-------LATNITEIVNNPNTTTT
TWK-35     144 ----------AVLIIVFLIYCISGCLVLWLIEPYQSELRDAWQ--HKIE-------------------NNRT---------------ARVDAMMK
TWK-36     166 -----FGLRYIALLVLALIYTLLGATVYLIEGSNEKSRLHVRE--QNLD-------------------KLLDE-------LATVLSEAVNDPEQSSE
TWK-37      60 ------KFNTIIAFVVLVAYIIGGALLWQISRSDMNINEFKR--NIRQ-------------------KSI---------------HIYNSMKGSKC
TWK-39      19 ---ATIFTITHVGLCFLVALYAVAGAFMQAVDYPYELGLQG------KVK-------------------NASLK-------VVDDIYRFINRKNV-IE
TWK-40      10 ----IILAHVSLIVLSVVYVGFGAFLTYQLSQPNEVEVRARNI--ERFN-------------------IHKRQ-------MIEHLWEMRESG----
TWK-42       9 --NYERYHLHHLVKIGVLIYSFLGAGLYVLCGAENEKSLKHEDN--MRVL-------------------RTS-------IA----AKQVFVQRLQN
TWK-43      37 ------PYLLNAGIVLFVGIYVYAGALLIQYTFRIPVKVEESF-----------------------RLRRNS-QD--------------DMPII
TWK-44     212 -------RVHYLVPIILLIAMSVLGCLIWSTFRPNEEIMLADKR--NYIA-------------------ALTDD-------LVEVLMQIHNRLIDFNR
TWK-45      47 -------FGIRHITLISILAAYICLGGFLLQKLSPREIEELQETL--KSMN-------------------EIIKN-------ETMDIIKITLTTNGEDR
TWK-46      27 ------ILVGLGVAVVYLFVGAIVVRISYPLEKIEREAY------L-------------------D-------------YQNQWR-------D
TWK-48      20 ---IIAFFFSHIGLCALVVGYALLGAVILKAVEGPHEAEIQE------LVK-------------------SAREK-------AVDVVWNATFRVNR-LD
ORK1/KCNK0   1 -----MSPNRWILLLIFYISYLMFGAFLTYHIHGEEKISRAEQ------R-------------------KAQI-------A-INEYLL-------
dTask-6      1 -----MKKQNVRTISLIVCFTYLLVGAVVLDALSETEKRRWE------ALQ-------------------DAEDM-------II-RKYNIS-------
dTask-7      1 --MMKRQNVRTLSLVVCFTYLLLIGAAVLDSLSPTEAKRWE------FLQ-------------------TVKNN-------FV-RKYNVT-------
sandman     26 ---FTAFMFSNVGIILLVTFYIIGGAFILQSIDIFEYERLKSEKPHRFIAR------------------NFSGE------CLSRIWELTAENISFFD
CG10864     42 --NFVTFMCTQVGVGALIVYAICGAFAFMHITRQFVDETAG------HVM-------------------ELRQN-------CSQQLWSITEQHNI-ID
CG34396    111 ------WFTHIFLILILALYNIGGAVVYRTIDVRHANQE-------KLV--------------------------------------VQDQQK
CG43155     61 ------SALNHIGLLVSLSIYCGVGCLLIRHLDRPAEVERLSHLK--DIVK-------------------THRER-------FLHTILNNTEVH----
Galene      31 SWKVLTCIVSHVLLVLLVVSYCVGGAYLIQHLDRPHELEVKR------DIQ-------------------NLRVN-------LTENIWLLSDDAVV-LR
```

TM1 helix

Supplementary Figure 2 - Clustal Omega alignment of human, *C. elegans*, and *D. melanogaster* two-pore domain potassium channel sequences.

Genbank identifiers for aligned sequences are listed below. N- and C-terminal cytoplasmic portions were truncated before alignment. Cartoon representations on top mark the four transmembrane helix regions (TM1, TM2, TM3, and TM4), the two pore helix domains (Ph1 and Ph2), and two selectivity filters (SF1 and SF2). Sequence logos were generated using WebLogo 3 (Crooks et al., 2004). Highly conserved residues are indicated in *hotpink* and well conserved residues in *marine*, as in Figure 1.
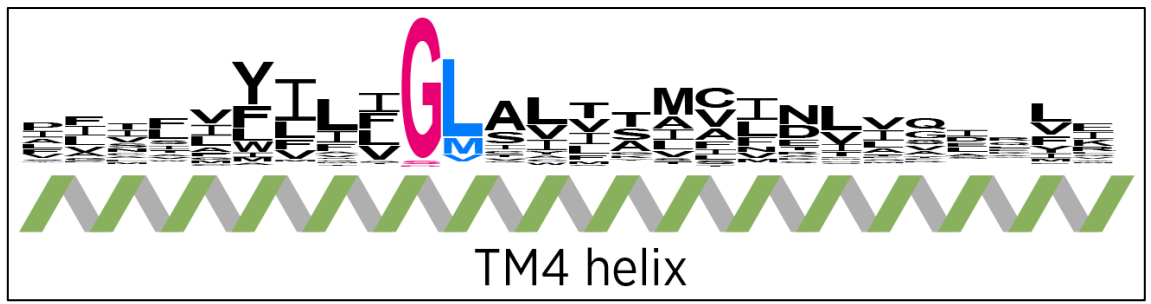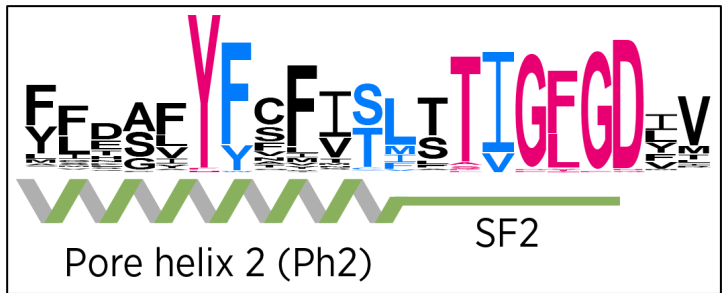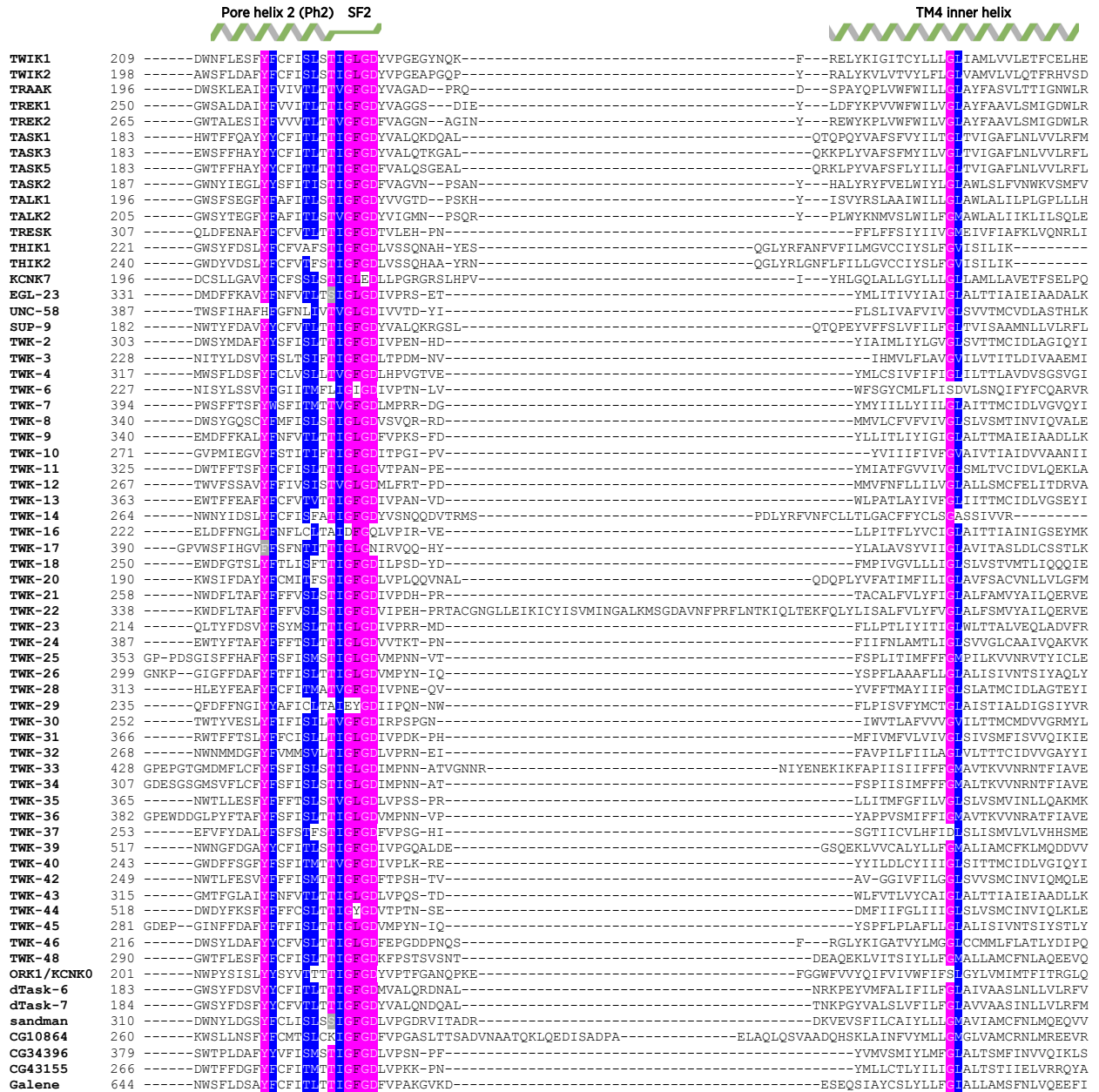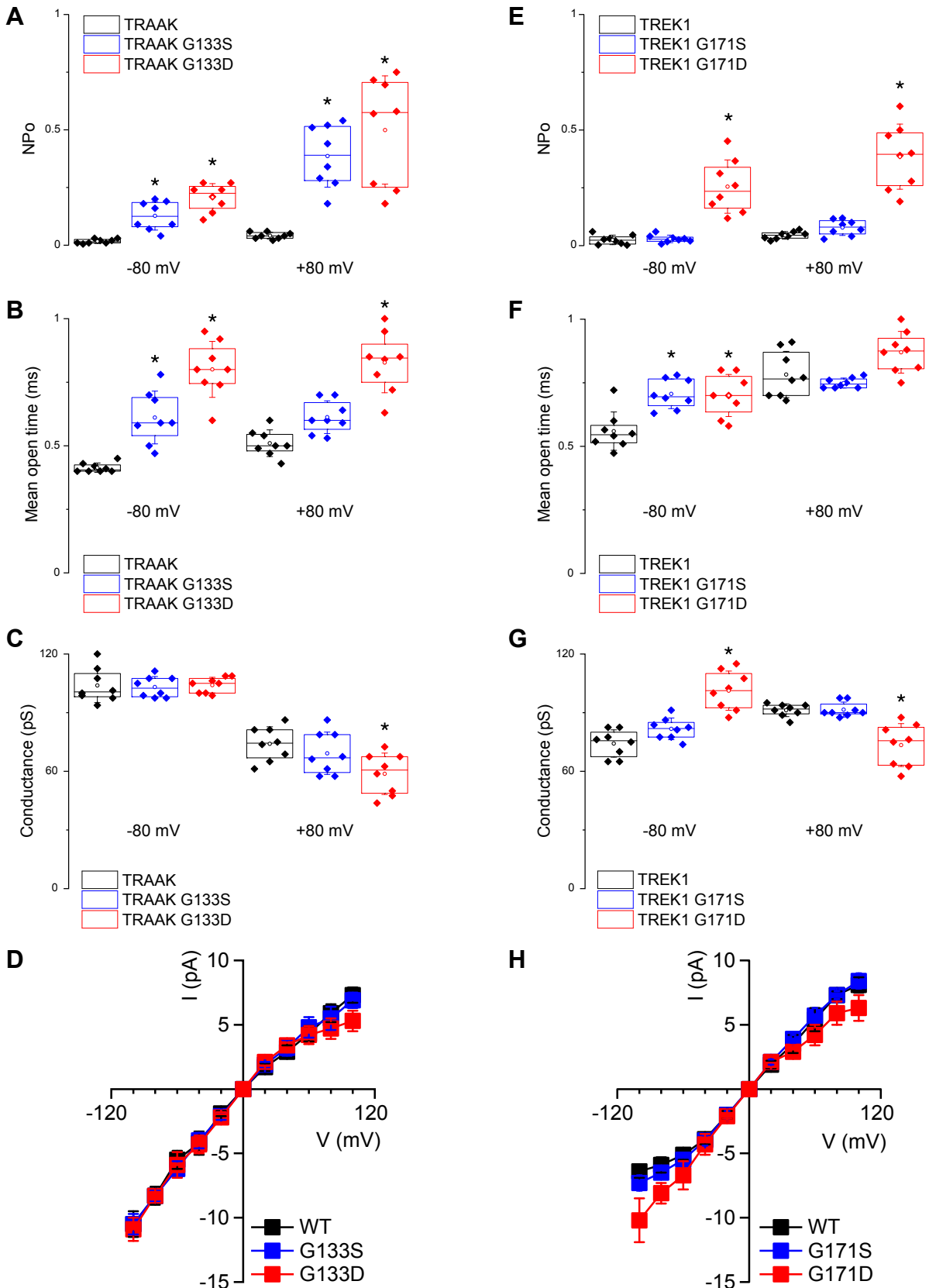
Supplementary Figure 2

Previously published gain-of-function mutants of TM2.6 (see manuscript for references).

Supplementary Figure 2

TM3 helix

```
TWIK1      179 -----------------------------------------------------------------VAIVHAVLLGFVTVSCFFFIPAAVFSVLED--
TWIK2      168 -----------------------------------------------------------------AACWHLVALLGVVVTVCFLVPAVIFAHLEE--
TRAAK      167 -----------------------------------------------------------------VRVLSAMLFLLIGCLLFVLTPTFVFCYME---
TREK1      221 -----------------------------------------------------------------IRIISTIIFILFGCVLFVALPAIIFKHIE---
TREK2      236 -----------------------------------------------------------------IRVISTILFILAGCIVFVTIPAVIFKYIE---
TASK1      155 -------------------------------------------------------------------SMANMVLIGFFSCISTLCIGAAAFSHYE--
TASK3      155 -------------------------------------------------------------------SMENMVTVGFFSCMGTLCIGAAAFSQCE--
TASK5      155 -------------------------------------------------------------------STENLVVAGLLACAATLALGAVAFSHFE--
TASK2      158 -------------------------------------------------------------------AQITCTVIFIVWGVLVHLVIPPFVFMVTE--
TALK1      167 -------------------------------------------------------------------LQVLGLALFLTLGTLVILIFPPMVFSHVE--
TALK2      179 --------------------------------------------------------------------LA---GSGALLSGLLLFLLLPPLLFSHME--
TRESK      268 -----------------------------------------------------------------N-LDEVGQQVERLDIPLPIIALIVFAYISCAAAILPFWET--
THIK1      182 ------------------------------------------------------CEVDSLA--------GWKPSVYYVMLILCTA-SILISCCASAMYTPIE--
THIK2      201 ------------------------------------------------------SEADSLA--------GWKPSVYHVLLILGLF-AVLLSCCASAMYTSVE--
KCNK7      166 -----------------------------------------------------------------AALLQAVALGLLVASSFVLLPALVLWGLQG--
EGL-23     294 ------------------------------------------------------E-VSEEEDDLTETEATSLFILFLVYIAFGGFMLAAYEP--
UNC-58     348 ------------------------------------------------------D-ILGVDGTEEKLWFPIGAYVSCICIYCSIGSAMFITWER--
SUP-9      155 -----------------------------------------------------------------SSDLIIFCTGWG-GLLIFGGAFMFSSYE--
TWK-2      265 ------------------------------------------------------I-LDMDMDEIDKSEVPVLMVFTIILLYI-AFGGILFSILE--
TWK-3      205 -----------------------------------------------------------------AAIFVCLLFAYPLVVGFILCST-S--
TWK-4      285 ------------------------------------------------------TEDPKTGRQLLVLVFLLGYMTISACVYTILEP--
TWK-6      191 ------------------------------------------------------E-TPSNSL-QHDYLIFLSSLLLCSISLLSSSALFSSIE--
TWK-7      356 ------------------------------------------------------H-GMGHDMNIEEKRIPAFLVLAILIVYT-AFGGVLMSKLE--
TWK-8      302 ------------------------------------------------------E-DIEDEEERSMPRMSVKVALGITVGWIFFCSALFKLWE--
TWK-9      302 ------------------------------------------------------E-E-EPENNEPRKTEESIALGITFTCYLVAGAKILSVYEP--
TWK-10     240 -----------------------------------------------------------------KIQNITAFMVVLLILLVAYSVIGGIAYSKI-V--
TWK-11     288 ------------------------------------------------------E-DDE-EEEEIHQDPPVLSTLIATVAWIILSAAVFCLFE--
TWK-12     228 ------------------------------------------------------E-DPEAAEERKKKPFFPIPIAIIMLIIWICFSASMFCIWED--
TWK-13     325 ------------------------------------------------------E-QIQFDPSNHEKRVSVLFILLIMLGYV-AGGAYIVRWWE--
TWK-14     225 ------------------------------------------------SCGGHMD-------NWRPSVYKVFFILFSM-CLVLITASAGIYSVVE--
TWK-16     188 ------------------------------------------------------------IYKESSIQVTSLALLCVFLIYVAVGALLLPLLNG--
TWK-17     351 ------------------------------------------------------D-DEECEDEEDRLQLPIASYFTLIIGYCCVGSLLFNTFEK--
TWK-18     213 ------------------------------------------------------E-KTE-NNDDDLLSFPISGLLLITVIWVIFCAVLFTFLE--
TWK-20     162 --------------------------------------------------------------TPTHLLMVSLTIGFMVIVSGTYMFHTIE--
TWK-21     220 ------------------------------------------------------F-FEVPEDDKEDTTFQLRWGLLVIVLFVVLCSFVVSFWE--
TWK-22     300 ------------------------------------------------------E-SGISEDEEQITTFPVKWALFIVFFFMVVCSLIVSFWE--
TWK-23     176 -----------------------------------------------------------KQSDEHLLREIAEVLLVAGLFVVFIAIGSAVIPLWEN--
TWK-24     348 ------------------------------------------------------L-DVDVPMGEQVQTVPIKSAAIFFFLWIMISAFIVRLWEY--
TWK-25     312 ------------------------------------------------------G-FVDKLDEDEIITLPIKFSVSVMILYLLSATMFIFEYDELS
TWK-26     259 ------------------------------------------------------K-QPVNRKKN-EILLPMWLALFLVLAYILICTLTIKMFDHNE--
TWK-28     275 ------------------------------------------------------D-SDSEDSAGDELRIPVFMVLLVLLAYT-AIGGFLFQSWE--
TWK-29     205 ------------------------------------------------------------EDENVSGSTLFFIVLVYILGATMIPLMSG--
TWK-30     215 ------------------------------------------------------EEEILDRVRLVRFPPLTVFFFVFVYG-CIAAWVVRYWE--
TWK-31     327 ------------------------------------------------------A-DPEKKPEVESRTIPIWLALLICVVYVAGCSSLFLLWET--
TWK-32     230 ------------------------------------------------------F-LWTHLEHAQFVEVPFLLVIGILLLYI-GLSSWIISWVE--
TWK-33     388 ------------------------------------------------------G-N-KHVDEDEIISLPIKACLLLLASYLGACTIFIYFYDELS
TWK-34     267 ------------------------------------------------------G-D-TSIDVNEIIKIPIKACLLLLALYLAFCTIFIHVFDELS
TWK-35     327 ------------------------------------------------------ERQERHDLDIFDLPLPVGIALIVTWIFICSFVLSVWDH--
TWK-36     342 ------------------------------------------------------G-K-REVDEDEIIQLPIKFCMTILIAYLLLCTTFVYLYDAVM
TWK-37     226 ------------------------------------------------------YKMTVLKAFILLVTFWGFGALAIAVYE--
TWK-39     427 SA-RELR----EKEMMMMNSLNHKQPSMDSSTSRRLRDIDGRSYRSDRSERSDEMSLHSLRRNGHRNQEKMPV--SVGICIVFAFISGGAWLFSWWE---
TWK-40     207 ------------------------------------------------------LNLDHLENYISIPIFLIVAILLSYI-TFGAVVLSMWE--
TWK-41     213 ------------------------------------------------------P-SP--QNPNGTRPIPLLLVLIVLFFWMIQCVAYFAYFE--
TWK-43     277 ------------------------------------------------------E-E-AEEEVEESDVTETIVLFVAFLVYIIAGSLLMSYYEE--
TWK-44     482 ------------------------------------------------------RNTGEDRISEMPLILAIGVAFGWMFLCAAIFLRFEK--
TWK-45     241 ------------------------------------------------------K-QSIEIKRK-ETLLPMWLAMLVVFTYIIICTLTILLFDDNE
TWK-46     186 ------------------------------------------------------------IQLIHVGVVFASLLLFVFAIPAWVFSSIET--
TWK-48     247 M------------EQL----------------------------------------EVRETAAAQLESMTVPISLVVFTMLGYLGVGTTIFKVWE--
ORK1/KCNK0 172 -----------------------------------------------------------------GLITTVVIALIPGIALFLLLPSWVFTYFE--
dTask-6    155 -----------------------------------------------------------------SEVDLICVVTTLSSLTIAGGAAAFSKFE--
dTask-7    156 -----------------------------------------------------------------TEMNLMLATGMLSSIIITTGAAVFSRYE--
sandman    264 -------------------------------------------QYTESDS---------DIEREIRGSTDEITVPVTVCVFVMVGYILWGALLFGRWE---
CG10864    221 -----------------------------------------------------------------ALEGGVGMTRKKVIVPSTACLWVIFFYVLTGTVMFANWE---
CG34396    340 ------------------------------------------------------P-YPETFEVDDEFNLPVSVASLLLITYILLGSFGFLMMEP--
CG43155    234 ------------------------------------------------------FIGKTWFYAILAVGFGLGVYLAAGAGLLLLWED--
Galene     552 SRSREPKRNRRRERAERLPPSPRIMSPMGFPVQRQIRRRPSYDYDDDD-----SMYGDEYGDYGDLLPKDRPVPIWLCVFLVVSYILGGAVLFAYWE---
```


TM3 helix

Supplementary Figure 2

Pore helix 2 (Ph2)   SF2

TM4 inner helix

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TWIK1      209 ------DWNFLESFYFCFISLSTIGFGDYVPGEGYNQK----------------------------------------F---RELYKIGITCYLLLGTIAMLVVLETFCELHE
TWIK2      198 ------AWSFLDAFYFCFISLSTIGFGDYVPGEAPGQP----------------------------------------Y---RALYKVLVTVYLFLGTVAMVLVLQTFRHVSD
TRAAK      196 ------DWSKLEAIYFVIVTLTTVGFGDYVAGAD--PRQ----------------------------------------D---SPAYQPLVWFWILLGTAYFASVLTTIGNWLR
TREK1      250 ------GWSALDAIYFVVVTLTTIGFGDYVAGGS---DIE----------------------------------------Y---LDFYKPVVWFWILVGTAYFAAVLSMIGDWLR
TREK2      265 ------GWTALESIYFVVVTLTTVGFGDFVAGGN--AGIN----------------------------------------Y---REWYKPLVWFWILVGTAYFAAVLSMIGDWLR
TASK1      183 ------HWTFFQAYYFCFITLTTIGFGDYVALQKDQAL----------------------------------------QTPQPQYVAFSFVYILTGTTVIGAFLNLVVLRFM
TASK3      183 ------HWSFFHAYYYCFITLTTIGFGDYVALQTKGAL----------------------------------------QKKPLYVAFSFMYILVGTTVIGAFLNLVVLRFL
TASK5      183 ------GWTFFHAYYYCFITLTTIGFGDFVALQSGEAL----------------------------------------QRKLPYVAFSFLYILLGTTVIGAFLNLVVLRFL
TASK2      187 ------GWNYIEGLYYSFITLTTIGFGDYVAGVN--PSAN----------------------------------------Y---HALYRYFVELWIYLGTAWLSLFVNWKVSMFV
TALK1      196 ------GWSFSEGFYFAFITLSTVGFGDYVVGTD--PSKH----------------------------------------Y---ISVYRSLAAIWILLGTAWLALILPLGPLLLH
TALK2      205 ------GWSYTEGFYFAFITLSTVGFGDYVIGMN--PSQR----------------------------------------Y---PLWYKNMVSLWILFGVAWLALIIKLILSQLE
TRESK      307 ------QLDFENAFYFCFITLTTIGFGDYVLEH-PN----------------------------------------FFLFFSIYIIVGVEIVFIAFKLVQNRLI
THIK1      221 ------GWSYFDSLYFCFVAFSTIGFGDLVSSQNAH-YES----------------------------------------QGLYRFANFVFILMGVCCIYSLFGVISILIK--------
THIK2      240 ------GWDYVDSLYFCFVFFSTIGFGDLVSSQHAA-YRN----------------------------------------QGLYRLGNFLFILLGVCCIYSLFGVISILIK--------
KCNK7      196 ------DCSLLGAVYFCFSSLSTIGFGDLPGRGRSLHPV----------------------------------------I---YHLGQLALLGYLLGTILAMLLAVETFSELPQ
EGL-23     331 ------DMDFFKAVYFNFVTLTTIGLGDIVPRS-ET----------------------------------------YMLITIVYIAIGTALTTIAIEIAADALK
UNC-58     387 ------TWSFIHAFHYGFNLIVTVGFGDIVVTD-YI----------------------------------------FLSLIVAFVIVGLSVVTMCVDLASTHLK
SUP-9      182 ------NWTYFDAVYFCFVTLTTIGFGDYVALQKRGSL----------------------------------------QTQPEYVFFSLVFILFGTTVISAAMNLLVLRFL
TWK-2      303 ------DWSYMDAFYYSFISLTTIGFGDIVPEN-HD----------------------------------------YIAIMLIYLGVGTSVTTMCIDLAGIQYI
TWK-3      228 ------NITYLDSVYFSLTSIFTIGFGDLTPDM-NV----------------------------------------IHMVLFLAVGVILVTITLDIVAAEMI
TWK-4      317 ------MWSFLDSFYFCLVSLLTVGFGDLHPVGTVE----------------------------------------YMLCSIVFIFIGLILTTLAVDVSGSVGI
TWK-6      227 ------NISYLSSVYFGIITMFLICIGDIVPTN-LV----------------------------------------WFSGYCMLFLISDVLSNQIFYFCQARVR
TWK-7      394 ------PWSFFTSFYWSFITMTTVGFGDLMPRR-DG----------------------------------------YMYIILLYIILGTAITTMCIDLVGVQYI
TWK-8      340 ------DWSYGQSCYFEMFISLSTIGFGDYVSVQR-RD----------------------------------------MMVLCFVFVIVGLSLVSMTINVIQVALE
TWK-9      340 ------EMDFFKALYFNFVTLTTIGFGDFVPKS-FD----------------------------------------YLLITLIYIGITALTTMAIEIAADLLK
TWK-10     271 ------GVPMIEGVFESTITLTTICFGDITPGI-PV----------------------------------------YVIIIFIVFGVAIVTIAIDVVAANII
TWK-11     325 ------DWTFFTSFYFCFISLTTIGFGDYVTPAN-PE----------------------------------------YMIATFGVVIVGLSMLTVCIDVLQEKLA
TWK-12     267 ------TWVFSSAVYFFIVSISTVGLGDMLFRT-PD----------------------------------------MMVFNFLLILVGLALLSMCFELITDRVA
TWK-13     363 ------EWTFFEAFYFCFVTLTTICFGDYVTPN-VD----------------------------------------WLPATLAYIVFGLIITTMCIDLVGSEYI
TWK-14     264 ------NWNYIDSLYFCFISFATIGFGDYVSNQQDVTRMS----------------------------------------PDLYRFVNFCLLTLGACFFYCLSGASSIVVR-------
TWK-16     222 ------ELDFFNGLYFNFLGLTADFCFQLVPIR-VE----------------------------------------LLPITFLYVCIGTAITTIAINIGSEYMK
TWK-17     390 ----GPVWSFIHGVFESFNTITTIGCNIRVQQ-HY----------------------------------------YLALAVSYVIITLVITASLDLCSSTLK
TWK-18     250 ------EWDFGTSLFFTLISFTTIGFGDILPSD-YD----------------------------------------FMPIVGVLLLIGLSLVSTVMTLIQQQIE
TWK-20     190 ------KWSIFDAYFECMTIFSTIGFGDLVPLQQVNAL----------------------------------------QDQPLYVFATIMFILIGTAVFSACVNLLVLGFM
TWK-21     258 ------GWDFLTAFYFFFVSLSTIGFGDIVPDH-PE----------------------------------------TACALFVLYFITLALFAMVYAILQERVE
TWK-22     338 ------KWDFLTAFYFFFVSLSTIGFGDVIPEH-PRTACGNGLLEIKICYISVMINGALKMSGDAVNFPRFLNTKIQLTEKFQLYLISALFVLYFVGLALFSMVYAILQERVE
TWK-23     214 ------QLTYFDSVYFSYMSLTTIGFGDIVPRR-MD----------------------------------------FLLPTLIYITIGTWLTTALVEQLADVFR
TWK-24     387 ------EWTYFTAFYFFFTSLTTIGFGDVVTKT-PN----------------------------------------FIIFNLAMTLIGLSVVGLCAAIVQAKVK
TWK-25     353 GP-PDSGISFFHAFYFSFIMSTIGLGDVMPNN-VT----------------------------------------FSPLITIMFFFGMPILKVVNRVTYICLE
TWK-26     299 GNKP--GIGFFDAFFTFISLTTIGLGDVMPYN-IQ----------------------------------------YSPFLAAAFLLGLALISIVNTSIYAQLY
TWK-28     313 ------HLEYFEAFYFCFITMATVGFGDIVPNE-QV----------------------------------------YVFFTMAYIIFGLSLATMCIDLAGTEYI
TWK-29     235 ------QFDFFNGIYYAFIGLTALEYQIIPQN-NW----------------------------------------FLPISVFYMCTGTAISTIALDIGSIYVR
TWK-30     252 ------TWTYVESLFFIFISTLTVCFGDIRPSPGN----------------------------------------IWVTLAFVVVGVILTTMCMDVVGRMYL
TWK-31     366 ------RWTFFTSLYFCFISLLTIGLGDYVPDK-PH----------------------------------------MFIVMFVLVIVGLSIVSMFISVVQIKIE
TWK-32     268 ------NWNMMDGFYFVMMSVLTICFGDLVPRN-EI----------------------------------------FAVPILFIILACLVLTTTCIDVVGAYYI
TWK-33     428 GPEPGTGMDMFLCFYFSFISLSTIGFGDIMPNN-ATVGNNR----------------------------------------NIYENEKIKFAPIISIIFFFGVAVTKVVNRNTFIAVE
TWK-34     307 GDESGSGMSVFLCFYFSFISTIGLGDIMPNN-AT----------------------------------------FSPIISIMFFFGVALTKVVNRNTFIAVE
TWK-35     365 ------NWTLLESFYFFFTSLSTVGLGDLVPSS-PR----------------------------------------LLITMFGFILVGLSLVSMVINLLQAKMK
TWK-36     382 GPEWDDGLPYFTAFYFSFISLSTIGLGDVMPNN-VP----------------------------------------YAPPVSMIFFIGMAVTKVVNRATFIAVE
TWK-37     253 ------EFVFYDALYFSFSTFSTIGFGAFVPSG-HI----------------------------------------SGTIICVLHFIDLSLISMVLVLVHHSME
TWK-39     517 ------NWNGFDGAYYCFITLSTIGFGDYVPGQALDE----------------------------------------GSQEKLVVCALYLLGVALIAMCFKLMQDDVV
TWK-40     243 ------DWDFFLSFYYCFITMTTVGFGDYVPLK-RE----------------------------------------YYILDLCYIIILTSITTMCIDLVGIQYI
TWK-42     249 ------NWTLFESVFEFFISMTTIGFGDFTPSH-TV----------------------------------------AV-GGIVFILGLSVVSMCINVIQMQLE
TWK-43     315 ------GMTFGLAIYFNFVTLTTIGFGDLVPQS-TD----------------------------------------WLFVTLVYCAIGLALTTIAIEIAADLLK
TWK-44     518 ------DWDYFKSFYFFFCSLLTICYGDVTPTN-SE----------------------------------------DMFIIFGLIIIGLSLVSMCINVIQLKLE
TWK-45     281 GDEP--GINFFDAFYFTFISLTTIGLGDVMPYN-IQ----------------------------------------YSPFLPLAFLLGLALISIVNTSIYSTLY
TWK-46     216 ------DWSYLDAFYYCFVSLTTIGLGDFEPGDDPNQS----------------------------------------F---RGLYKIGATVYLMGGTCCMMLFLATLYDIPQ
TWK-48     290 ------GWTFLESFYFCFISLTTIGLGDKFPSTSVSNT----------------------------------------DEAQEKLVITSIYLLFGVALLAMCFNLAQEEVQ
ORK1/KCNK0 201 ------NWPYSISLYMSYVFITTICFGCIYVPTFGANQPKE----------------------------------------FGGWFVVYQIFVIVWFIFSGYLVMIMTFITRGLQ
dTask-6    183 ------GWSYFDSVYIYCFIFITTICFGDIMVALQRDNAL----------------------------------------NRKPEYVMFALIFILFGTLAVVAASLNLLVLRFV
dTask-7    184 ------GWSYFDSVYIYCFIFITTIGFGDYVALQNDQAL----------------------------------------TNKPGYVALSLVFILFGTLAVVAASINLLVLRFM
sandman    310 ------DWNYLDGSYFCLISLSTIGFGDLVPGDRVITADR----------------------------------------DKVEVSFILCAIYLLLGVAVIAMCFNLMQEQVV
CG10864    260 ------KWSLLNSFYFCFISLCKTIGFGDFVPGASLTTSADVNAATQKLQEDISADPA----------ELAQLQSVAADQHSKLAINFVYMLLGVGLVAMCRNLMREEVR
CG34396    379 ------SWTPLDAFYFVFISMSTIGFGDLVPSN-PF----------------------------------------YVMVSMIYLMFGLALTSMFINVVQIKLS
CG43155    266 ------DWTFFDGFYFCFITMTTIGFGDLVPKK-PN----------------------------------------YMLLCTLYILIGTALTSTIIELVRRQYA
Galene     644 ------NWSFLDSAYFCFITLSTIGFGDFVPAKGVKD----------------------------------------ESEQSIAYCSLYLLFGTALLAMSFNLVQEEFI
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Pore helix 2 (Ph2)   SF2

TM4 helix

4

Supplementary Figure 3

Single channel properties of TRAAK and TREK1 TM2.6 mutants
(A) TRAAK, TRAAK G133S, TRAAK G133D, (E) TREK1, TREK1 G171S, and TREK1 G171D channel activity determined by NPo analysis at -80 and +80 mV.
(B), (F) Mean open times were obtained from channel openings recorded at -80 mV and +80 mV.
(C), (G) Unitary conductances obtained from channel openings recorded at -80 mV and +80 mV.
(D), (H) Current-voltage relationships of single channels.
Center lines, medians; open circles, means; box limits, 25th and 75th percentiles; whiskers, standard deviation.
Kruskal-Wallis, Dunn's multiple comparison test, * p < 0.05.

Supplementary Table 1: Two-electrode voltage-clamp experiments (Figure 1B)

Channel	TM2.6	cRNA	Day	E$_{rev}$ (mV)	I (µA) at 0 mV
hTWIK1	WT	10 ng	1	-38.1 ± 2.4	0.2
	L146N			-88.4 ± 1	2.7 ± 0.2
rTWIK2	WT	10 ng	1	-33.1 ± 1.1	0.1
	M135D			-66.4 ± 2.2	0.4
hTASK1	WT	0.8 ng	3	-63.4 ± 2.5	0.7 ± 0.1
	L122N			-71.0 ± 1.1	13.4 ± 2
hTASK3	WT	50 pg	1	-73.4 ± 4.3	1.8 ± 0.3
	L122N			-77.6 ± 2.5	5 ± 0.8
mTREK1	WT	1 ng	1	-66.1 ± 1.5	0.7 ± 0.1
	G171D			-72.0 ± 0.6	8.8 ± 0.8
mTREK2	WT	1 ng	2	-52.5 ± 2	0.5 ± 0.1
	G196D			-72.1 ± 0.9	1.7 ± 0.2
mTRAAK	WT	1 ng	2	-55.6 ± 1.5	0.3
	G133D			-65.8 ± 0.8	32.9 ± 7.2
mTRESK	WT	1 ng	1	-70.8 ± 1.1	0.9 ± 0.1
	F156N			-68.9 ± 2	24.3 ± 3.6
mTASK2	WT	5 ng	1	-87.5 ± 2.8	3.8 ± 0.6
	L127N			-90.3 ± 6.6	14.2 ± 1.6
hTALK1	WT	20 ng	1	-80.5 ± 3.9	1 ± 0.1
	V137N			-93.6 ± 2.2	2.9 ± 0.2
hTALK2	WT	20 ng	1	-42.4 ± 2.1	0.3
	L145N			-51.4 ± 3.5	1.4 ± 0.1
hTHIK2	WT	50 ng	2	-31.1 ± 5.1	0.1
	I158D			-83.3 ± 0.9	1
hTASK2	WT	10 ng	1	-77.4 ± 2.7	0.4
	L127N			-93.4 ± 0.6	6.8 ± 0.2
rTWIK2 LY	WT	10 ng	1	-84.3 ± 0.8	2.2 ± 0.1
	M135D			-79.9 ± 0.8	8.5 ± 0.2
hTWIK1 AA	WT	10 ng	1	-71.7 ± 4	0.4
	L146N			-85.1 ± 0.9	9.3 ± 0.4
hTHIK2 5RA	WT	15 ng	1	-31.2 ± 1.4	0.2
	I158N			-76.5 ± 1	1.7 ± 0.2

Supplementary Table 2: Strain list

Strain	Genotype	Description
JIP1658	*egl-23(bln309) V*	EGL-23-wrmScarlet
JIP1506	*egl-23(bln309bln334) V*	EGL-23(L229N)-wrmScarlet
JIP1559	*egl-23(bln309bln359) V*	EGL-23(L229S)-wrmScarlet
JIP1620	*unc-58(bln448) X*	UNC-58(F294S)
JIP1621	*unc-58(bln449) X*	UNC-58(F294T)
JIP1622	*unc-58(bln450) X*	UNC-58(F294N)
JIP1623	*twk-18(bln451) X*	TWK-18(V158N)
JIP1636	*twk-18(bln458) X*	TWK-18(V158S)
JIP1706	*twk-18(bln491) X*	TWK-18(V158T)
JIP1677	*sup-9(bln476) II*	SUP-9(L122S)

Supplementary Table 3: List of single strand oligonucleotides (DNA and RNA) for CRISPR/Cas9 genome engineering

	Sequence (5' to 3')	Description
crRNA *unc-58*	gcggcagugauagacauuag	Use to generate UNC-58(F294x)
oPT271	cggaaaggttttctcagtagcattcgcgcttgttggta taccactAatgtCTatCacTgcCgccgatattggtaaa tttttatctgaaacattactccagtttgtgagct	Repair template (Ultramer) for UNC-58(F294S)
oPT273	tcggaaaggttttctcagtagcattcgcgcttgttggt ataccactAatgACAatTacTgcCgccgatattggtaa attttatctgaaacattactccagtttgtgagctt	Repair template (Ultramer) for UNC-58(F294T)
oPT275	tcggaaaggttttctcagtagcattcgcgcttgttggt ataccactAatgAATatTacGgcCgccgatattggtaa attttatctgaaacattactccagtttgtgagctt	Repair template (Ultramer) for UNC-58(F294N)
crRNA *egl-23*	ugguauuccguuuacacugc	Use to generate EGL-23(L229x)
oSEM402	ttcgcctccaccatcatttctgatatgaattttccgag atctgcaattgcTagACTtgtaaacggaataccaatta gaccataaccaatgacaaatagacggccac	Repair template (Ultramer) for EGL-23(L229S)
oSEM356	ttcgcctccaccatcatttctgatatgaattttccgag atctgcaattgcTagGTTtgtaaacggaataccaatta gaccataaccaatgacaaatagacggccac	Repair template (Ultramer) for EGL-23(L229N)
crRNA *twk-18*	uaaagacugagcacugugag	Use to generate TWK-18(V158x)
oSEM435	ctggatggggtcgttttgctactatcctttacgcattc attggaattccaTtGacaAACTtAagtTtGtactgtct tggtagtcttttttgccaagggatg	Repair template (Ultramer) for TWK-18(V158N)
oSEM436	ctggatggggtcgttttgctactatcctttacgcattc attggaattccaTtGacaAGTTtAagtTtGtactgtct tggtagtcttttttgccaagggatg	Repair template (Ultramer) for TWK-18(V158S)
oSEM437	ctggatggggtcgttttgctactatcctttacgcattc attggaattccaTtGacaACTTtAagtTtGtactgtct tggtagtcttttttgccaagggatg	Repair template (Ultramer) for TWK-18(V158T)
crRNA *sup-9*	cgcacuggcgggaauuccau	Use to generate SUP-9(L122x)
oNZ161	tggaactcctggaaaattttcagcaactcaccaatact ctgaaacatgataTTtccTaGtggaattcccgccagtg cgtagagcatgcagaaaacttttccgg	Repair template (Ultramer) for SUP-9(L122N)
oMG798	tggaactcctggaaaattttcagcaactcaccaatact ctgaaacatgatGCTGccTaGtggaattcccgccagtg cgtagagcatgcagaaaacttttccgg	Repair template (Ultramer) for SUP-9(L122S)

Supplementary Table 4: Genotyping primer combinations for *C. elegans* mutants

Allele	Primer	Sequence (5' to 3')	Description
unc-58 F294X	oTB522	cggggttagtaatatggatgaagg	338bp PCR product; verify by sequencing.
	oTB508	ggaccacccgttgattgtaa	
egl-23 L229X	oSEM357	cccaccattaaccgagtttg	487bp PCR product digested by FspBI; WT: 134/353bp Mutant: 104/134/249bp
	oSEM358	tcccagattataagcaggcaat	
twk-18 V158X	oSEM430	tgcggcgcttaagtatttg	673bp PCR product digested by RsaI; WT: 673bp Mutant: 384/289bp
	oSEM431	ggagatggggaaagacaaca	
sup-9 L122X	oMG850	caactgtgataacgacgatcgg	PCR oMG850-852-856: WT:986/324bp L122N&L122S:986bp PCR oMG850-852-855 WT:986bp L122N:986/324bp PCR oM850-852-853 WT:986bp L122S:986/324bp
	oMG852	caccgcgtcgaaatatgtcc	
	oMG853	cgggaattccaCtAggCAGC	
	oMG855	gcgggaattccaCtAggaAA	
	oMG856	gcgggaattccaTtGggACT	

Supplementary Table 5: List of plasmids

Plasmid/gene	Primer	Sequence (5' to 3')	Isothermal Ligation with EcoRV digested pTB207 and	
pTB301/ *TWK-18* *WT*	oTB553	ccactagtaacggccgccagtgtgctggaattctgcagatatggcgattgttgcgcaagg	oTB553-554	from pOX_twk-18[1]
	oTB554	cgggccccccctcgagcggccgccagtgtgatggatctagatgtcatgctctagat		
pNZ19/ *TWK-18* *(V158D)*	oNZ52	tgtgagtggaattccaatga	oTB553-oNZ52 oTB554-oNZ53	
	oNZ53	tgctactatcctttacgcattcattggaattccactcacaGACctcagtctttactgtcttgg		
pTB314/ *TWK-18* *(M280I)*	oTB571	actccaacaattggGATgaaatcgtaatcc	oTB553-oTB571 oTB554-oTB572	
	oTB572	catattgataggactcgctgtattctccacttgtgtcaat		
pOA23/ *hTASK1* *WT*	oOA21	ccactagtaacggccgccagtgtgctggaattctgcagatatgaagcggcagaacgtgcg	oOA21-22	from hTASK-1-pSGEM[2]
	oOA22	agctcgggccccccctcgagcggccgccagtgtgatggattcacacggagctcctgcgct		
pOA24/ *hTASK1* *(L122N)*	oOA23	ctggaacatgacATTcgtgagcgggatgcc	oOA21-23 oOA24-22	
	oOA24	ggcatcccgctcacgAATgtcatgttccag		
pOA21/ *hTASK3* *WT*	oOA18	ccactagtaacggccgccagtgtgctggaattctgcagatatgaagaggcagaacgtgcg	oOA18-oIS17	from hTASK-3-pSGEM[2]
	oIS17	agctcgggccccccctcgagcggccgccagtgtgatggatctaaacggacttccggcgtt		
pOA22/ *hTASK3* *(L122N)*	oOA26	gctctggaacatgacATTtgtcagcgggat	oOA18-26 oIS17-oOA25	
	oOA25	atcccgctgacaAATgtcatgttccagagc		
pOA25/ *mTASK2* *WT*	oNZ56	ccactagtaacggccgccagtgtgctggaattctgcagatatggtggaccggggtcctttt	oNZ56-57	from pTLN mTASK-2[3]
	oNZ57	agctcgggccccccctcgagcggccgccagtgtgatggattcacgtgcccctggggtta		
pNZ20/ *mTASK2* *(L127N)*	oNZ55	gcacagcggcaccccgaaga	oNZ56-55 oNZ54-57	
	oNZ54	ctgtgtcttctacggcctcttcggggtgccgctgtgcAACacatggatcagtgccctggg		
pOA26/ *mTREK2* *WT*	oOA30	ccactagtaacggccgccagtgtgctggaattctgcagatatgaaatttccaatcgagac	oOA30-31	from mTREK2c-pEXO
	oOA31	agctcgggccccccctcgagcggccgccagtgtgatggatttagtttctgtcttcaagta		
pOA27/ *mTREK2* *(G196D)*	oOA43	tccagccaataagaaATCaaaaagcgggat	oOA30-43 oOA42-31	
	oOA42	atcccgcttttttGATttcttattggctgga		
pOA28/ *mTRAAK* *WT*	oOA34	ccactagtaacggccgccagtgtgctggaattctgcagatatgcgcagcaccacactcct	oOA34-35	from mTRAAK-pEXO
	oOA35	agctcgggccccccctcgagcggccgccagtgtgatggatctacaccggcacggccttgt		
pOA29/ *mTRAAK* *(G133D)*	oOA45	tcccgccagcagcatATCgaacagtgggat	oOA34-45 oOA44-35	
	oOA44	atcccactgttcGATatgctgctggcggga		

pOA30/ *mTRESK* *WT*	oOA38	ccactagtaacggccgccagtgtgctggaa ttctgcagatatggaggctgaggagccacc	oOA38-39	from mTRESK- pEXO
	oOA39	agctcgggccccccctcgagcggccgccag tgtgatggatttaccaaggtagcgaaactt		
pOA31/ *mTRESK* *(F156N)*	oOA41	gtctgtgaggaccagATTcattagagggat	oOA38-41 oOA40-39	
	oOA40	atccctctaatgAATctggtcctcacagac		
	oTB			