

hsCNGB1	MLGWVQRVLQPPTGPRKTKMQEEEEVEPEPEMEAEVEPEPNPEEAETESESMPPPESEFKEEVAVADPSPQETKEAALTSTISLRAQGA	90
hsCNGB1	EISEMNSPSSRRVLTWLMKGVKVPVQVHSITEDPAQILGHGSDTGTCTDEPNEALEAQDTRPGLRLLWLEQNLERVLPQPPKSSSEVW	180
hsCNGB1	RDEPAVATGAASDPAPPGRPQEMGPKLQARETPSLPTPIPLQKKEEPKEAPEPQPQGSQAQTSSLPPTRDPAFLVAVLWLRLEMLALPQP	270
hsCNGB1	VLHGKIGEQEPDPSGICDVQITISILPGGQVEPDLVLEVEVPPWDAHQDVSTSPQGTVEVVPAYEENKAVEKMPRELSRIEEKEDEEEEE	360
hsCNGB3	-----FKSLTKVVK-----	10
hsCNGA1	-----M	1
hsCNGA2	-----	0
hsCNGA3	-----	0
hsCNGA4	-----	0
ceTAX-4	-----	0
hsCNGB1	EEEEEEEEEEVTVLLDSCVVSQVGVGQ-----SEEDGTRPQSTSDQKLWEEVGEAEKKEEAEKAEAEVVEVEEAEKPEQDWAET	443
hsCNGB3	-----VKPIENNNEQSSRRNEGSHSPNSQQTTA-----DE	45
hsCNGA1	KLMSKNNININQOQSFV-TMPN--VIVPDIEKEI--RRMENGACSSFSDEDD-----DSA---STSSE-	54
hsCNGA2	---MT--EKTNGVKSSP-ANNHNNHAPPAIKANG---KDDHRTSSRPHSAAD-----DDTS---SELQRL	53
hsCNGA3	-----MAKINTQYSHP-SRTH--LKVKTSDRDL--NRANGLSRAHSSE-----ETS---SVLQPG	49
hsCNGA4	-----LAPTTNGIGSPPTA-----S-----MSTAEPPD-----PT---NPSTSG	17
ceTAX-4	-----KEEPEEAEEAASGVPAKQHPVEVQVEDTADSCPLMAEENPSTVLPSPSPA--KSDTLVPSVSAAGTHRKKLPSEDDAEELKALSPA	531
hsCNGB1	---NKGEKSLKTSTPTVTEEPHTNIQDKL-----SKNSSDILTNPPDQNAEPTGTVPE-----QKEMDPE	107
hsCNGA1	---SENENPHARGSFYSYSLRKG-----PSQREQLPGAIALFVNNSSNKDQEPBEKKKKKK-----EKKSKD--DKNENKND-	125
hsCNGA2	AD---VDAPOQGR-----S-----GFRRIVRLVGIIRWANKNFREERPRDPSFLERFRGPPELQVTTTQEGDGGK-	116
hsCNGA3	IAETRGLADSGQGSFTGGIARL-----SRLIFL---LRRWAARVHHQDQDPSFDRFRGAELEKVESQESNAQANVGSQD-	125
hsCNGA4	-----LAPTTNGIGSPPTA-----S-----MSTAEPPD-----PT---NPSTSG	75
ceTAX-4	-----ESPVVAVSDPTPKDITDGDRAASTASTNSAIINRLQELVKKLFK-----ERTEKVKKLLDP-----DVTSDDESPPK	600
hsCNGB1	K-----EGNSPQN-----KPPAAPVINEYDAQLHNLVKRKE-----QRTALYKKKIVVEG-----DLSSEASPT	164
hsCNGA1	-----PEKKKK---KDK---EKKKKEEK-----SKDKKEEKKVEVVIDPSGN-TYINWLFCTITLPMVYNNWMTVIARACFDLQD-	194
hsCNGA2	-----PADRGRSAMFLAK---CNTNTSNN-----GEDKTKKFFVLDVDPAGD-WYICWLFVIAMVLYNWCILVARACFDLQD-	169
hsCNGA3	-----MSQD---TKVKTTES-----SPPASRARKLLVLDPSGD-YYWNLNTMVPVPMYNIILVCRACFPDLQH-	63
hsCNGA4	-----PAATGGQPASSDGGSAIEVPPKPE-----SYAVRIRKLYANLQDPSD-NFYIYTCVVTVAYIYNLLVFIARQVNDLIGP-	151
ceTAX-4	SPAKKAPAPADTKPAEAEVPEEEHYCDMLCCKFKHRPWK-----KYQFPOSIDLPTNLMYVLWLFVVMAMWNNCWLLPVRWAFPYQTP	685
hsCNGB1	-----AKTAVVPVKE---SDDKPTHEHYRLLWFRVKKMPLTLYLKRILKLPNSIDSYTDRLYLWLLVLYTDAYNWNCCPIPLRIFPYQTA	247
hsCNGA1	-----DYLEWYLIDYVSDIYVLDIM-FVTRRTGYLEOGLLVKEELK-	236
hsCNGA2	-----GYLWVWLVDYVSDIYVLDI-FIRLRTPLEOGLLVKDTKLL	211
hsCNGA3	-----BYLMLWLVLDYSDVLYVLDV-LVRAHGTGFLBQGLMYSINRL	239
hsCNGA4	-----GYLWVWLVDYSDIYVLDIM-VVRFHTGFLEOGLLVVDKGR	105
ceTAX-4	SSQSLCRFYNGTLNSTTQVECTYNMNTMKEMPTYSQYDPLGWSKYWVHFRMLWVFDLMDCVYLDIT-FLNRYRGMYDQGLVREAEKV	240
hsCNGB1	-----DNIHWWLLMDYCDLIYFLDITVFQTRIQFVGGDIIIDDKDM	728
hsCNGB3	-----DNIHWYLIADICDIYLYDMLFIQFRLQVGGDIIYVDSNEL	290
hsCNGA1	INIKYSNLQPKLDVLSLIPDILLYFKLGW-----NYPIRLNRLRFSRMFEFFQRTETRTNYPNIFRISNLVMYIVIIHWNACVF	318
hsCNGA2	RDNYIHTLQFKLDVASIIIPDILYFAVDI-----HSPVFRNRLHFAFMFEFFDRTETRTNYPNIFRISNLVYLIVIHWNACIY	293
hsCNGA3	WQHYYKTTFQPKLDVLSLVPDILAYLVGT-----NYPEVRNRLKLFSLFEFFDRTETRTNYPNMFRIINLVLYLIIIVIHWNACIY	321
hsCNGA4	SSRYVTRVSPFDLDASLMPDIDVYVRLGP-----HTPTLRNRLFRAPRLEAFDRTETRTAYPNAPRIAKMLYIFVVIHWNACIY	187
ceTAX-4	TRAYWQSKQYRIDGSLIPLDYI---LWGPVPIYINWRGLPILRLNRLIRYKRVNRCLERTETRSMPNAPFRVWVWVYIVIIHWNACIY	327
hsCNGB1	RNYLKSRREKMDLISLIPDILYLVKGV-----NPLRLPRCLYMAFFENSRLESILSKAYVVRVIRTTAYLISLHNSCLY	809
hsCNGB3	RKHYYTQKPKLDVLSLIPDILYLVKGV-----NPLRANRMLYTFEFENHLESIMDKAYVVRVIRTTAYLISLHNSCLY	371
hsCNGA1	YISIKAIKGFNDITWVYDINDPE----FGRLARKYVYSLYWSLTLITIGETPPVVRDSYEVVVDVFLIGVLIIFATIFGNIGSMISNM	403
hsCNGA2	YAIKSIKGFVDTWVYDINDPE----YGLAREYIYCLYWSLTLITIGETPPVVKDEYLFVDFLIGVLIIFATIVGNVGSMSNM	378
hsCNGA3	FALISKFIKGFDTSDWVYDINDPE----HGRLSKYYIYSLYWSLTLITIGETPPVVKDEYLFVDFLIGVLIIFATIVGNVGSMSNM	406
hsCNGA4	FALSRYLGFGRDAWYDPPAQPC---FERLRQVLYSVEFSLITITIGETPPPARBEYLFVDFLIGVLIIFATIVGNVGSMSNM	272
ceTAX-4	FWSSEWIGLGTDAWYGHKQSLPDDITDILRRVYVSPWSTLITIGETVSPVRIEYAVTLDLMDCVLIFATIVGNVGSMSNM	417
hsCNGB1	YWASAYQGLGSHHWYDGV-----GNSYIRCYFYAVKLLITIGLPPDKLFEIIVFQLLNFTGVFAFVSMIGQMRDVGAA	886
hsCNGB3	YWASNYEGIGTTRWYDGE-----GNEYLRXYWAVLITITIGLPEPQTLFELIVFQLLNFTGVFAFVSMIGQMRDVGAA	448
hsCNGA1	NAARAEFQARIDAIAIKQYMHFRNVSKDMEKRVIKWFDYLVWTKKTVDEKEVLKYLPLDKLRAEIAINVHLDLTKKVRIFADCEAGLLVEVLV	493
hsCNGA2	NATRAEFQAKIDAVKHVMQFRKVSQKMEAKVIRWFDYLVWTKKTVDEKEVLKYLPLDKLRAEIAINVHLDLTKKVRIFADCEAGLLVEVLV	468
hsCNGA3	NATRAEFQAKIDAVKHVMQFRKVSQKMEAKVIRWFDYLVWTKKTVDEKEVLKYLPLDKLRAEIAINVHLDLTKKVRIFADCEAGLLVEVLV	468
hsCNGA4	N7ADAAEFQPDHLLVKKYMKLOHNRKLERRVIDWYQHLQINKMTNEVALIQLHPLERLAEVAVSVLSTLRSVQIFQNCBALELVEVL	362
ceTAX-4	SAARTEFQKMDIKQYMLRKKVSKOLEIRVWFDYLVWTKKTVDEKEVLKYLPLDKLRAEIAINVHLDLTKKVRIFADCEAGLLVEVLV	507
hsCNGB1	TAGQTYRSCMDSTVKYMNYPKPKSVQNRVKTWYETVWDSQGLMDESELMVQLPDKMRLDLADIVNINIVSKVALPQGGCDRQMIFDMLK	976
hsCNGB3	TANQNYFACMDDTIAVNNNSIPKLVQKRVKTVWYETVWDSQGLMDESELMVQLPDKMRLDLADIVNINISKVDLPQGGCDTQMIDMLL	538
hsCNGA1	KLQPQVSPGDYICKKGGDIREMYIIKEGKLAVVA-DDGVTFVFLSDGSGYFGEISILNIRKSGKAGNRRTANIKSIGYSDLFCLSKDDLM	582
hsCNGA2	KLRPQVSPGDYICKKGGDIREMYIIKEGKLAVVA-DDGVTFVFLSDGSGYFGEISILNIRKSGKAGNRRTANIKSIGYSDLFCLSKDDLM	557
hsCNGA3	KLRPTVSPGDYICKKGGDIREMYIINEGKLAVVA-DDGVTFVFLSDGSGYFGEISILNIRKSGKAGNRRTANIKSIGYSDLFCLSKDDLM	585
hsCNGA4	KLQPQVSPGDYICKKGGDIREMYIIKEGKLAVVA-DDGVTFVFLSDGSGYFGEISILNIRKSGKAGNRRTANIKSIGYSDLFCLSKDDLM	451
ceTAX-4	KLQGLVSPGDYICKKGGDIREMYIVKRGRLQVVD-DDGVTQYAVLGDQVFLQEGSVFGEISILNIRKSGKAGNRRTANIKSIGYSDLFCLSKDDLM	596
hsCNGB1	RLRSVYVLPNDYVCKKGEIKREMYIIKAGVQVLLGDKKSVLVTILKAGSVFGEISILLAVGG---GNRRTANVVAHGFNTLFDLQKDKLN	1063
hsCNGB3	RLKSVLVPNDYVCKKGEIKREMYIIKAGVQVLLGDPGDKVLTILKAGSVFGEISILLAVGG---GNRRTANVVAHGFNTLFDLQKDKLN	625
hsCNGA1	EALTEYPAKTMLEEKQKILMKDGLLDLNIANAGS-D-----PKDLEEKVT-----RMEGSDVLLQTRFARILAEYESMOQ	653
hsCNGA2	EAVTEYPAKTVLEERGERILMKEGLLDENEVAT-S-M-----EVDVQKELG-----QLETNMMLTQTRFARILAEYTAGAQ	627
hsCNGA3	EALTEYPAKTMLEEKQKILMKDGLLDLNIANAGS-D-----PKDLEEKVT-----RMEGSDVLLQTRFARILAEYESMOQ	656
hsCNGA4	EVLSEYPAQGTIMBEKGRILLKMNKLDVNAEAEAL-----QEATESRLR-----GLDQQLDLDLQTRFARILAEYTAGAQ	523
ceTAX-4	NALREYPAKRLLLKAGREILKDKLDLLENAPTE-----QKTEAEAE-----HLNNAVKVLLQTRFARILAEYTAGAQ	694
hsCNGB1	EILVHYPEKQILRKKARRMLRSNNK-----PKEEKSVLILPRAAGTPKLFNAALAMTKMGGKAGKGLAHLRKLKLAALAEAA-	1145
hsCNGB3	EILVHYPEKSERILMKARVLLKQAKTAEATPPRKDLALLFPKKEETPKLFTLLGGTGKASLA-----R---LLKLEQAA--	700
hsCNGA1	KLKQRLTKVEKFLKPLIDTE-F---SSIEGPAESGPDIST-----	690
hsCNGA2	KLKQRTVLETKMKQNNEDD-Y---LSDGMNSPELAAADPE-----	664
hsCNGA3	KLRQRLTKVEKFLKPLIDTE-F---SSIEGPAESGPDIST-----	694
hsCNGA4	KIARIIRLELWQTRWMPEDLAEADDEGPEEETGSDDEGRASQEG-----	575
ceTAX-4	KLMKRIEMLEKHLRSYKALARRQ-KTMHGVSDIGDGIITDGVDRVRRPPLRQ-----	729
hsCNGB1	---AKQDEL---VEQAKSSQDVKGEI---GSAAPDQHTHPEAAADPPAPRTPEEPPGS-PPSSPPPASLQ-RPE---GEEGPADEPEEH	1221
hsCNGB3	---QKKNSEGGEEEGKENEKQKEN---EDRKQENEDKGG-ENEDKDKGRPEEKELDRPECTASPIAVEEPEPHVRRVLPGRGTSQ	782
ceTAX-4	SLLK-----	733
hsCNGB1	SVRIKMSPPGPEGQILSVKMPPEEREKAE	1251
hsCNGB3	SIIISMSPASREGGEVLTIEVKKAKQ---	809

Supplementary Fig. 1. Amino acid sequence alignment of human CNG channel subunits and *C. elegans* CNGA subunit TAX-4. Secondary structures are marked for A3. The SF of CNGA 1-3 is boxed in blue. The cavity gate is boxed in black. Cavity gate-forming residues in A3 are shown in magenta. Cavity gate- and inner gate-forming residues and SF-projecting R403 in B3 are shown in cyan. Residues involved in gating ring/TMD interactions between B3 and A3I and A3III (**Fig. 6c, d**) are shown in red. Residues involved in A'B'/C'D' interactions (**Extended Data Fig. 5**) are shown in green. Missense DAMs in A3 and B3 are highlighted with a yellow and brown background, respectively.