Supplementary Material:

An internally potentiated, thermostable, pH sensitive Cys loop receptor from the hydrothermal vent worm *Alvinella pompejana*

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Supplementary Figure 1A

Multiple sequence alignment for Alv-a1-pHCl, its mutants and Alv-a9 with other members of the Cysteine loop receptor family. GI accession numbers of sequences are as follows, *C. elegans* GluCl α, 559559 (CE_GluC_alpha); Human glycine α1, 119372310 (HS_GlyR_alpha1), Human GABA ρ1, 194097386 (HS_GABArho1) Human GABAA α1, 38327554 (HS_GABA_A_alpha1) Human α7 nAChR, 496607 (HS_Nic_alpha7) subunits from *T. marmorata* : α, 213216 (HS_Nic_alpha), β, 39653645 (HS_Nic_beta), δ ,39653649 (HS_Nic_delta), γ , 39653647 (HS_Nic_gamma), *L. stagnalis* AChBP, 14285341 (LS_AChBP) *A. californica* AChBP, 325296909 (AC_AChBP), Human 5-HT3A, 37514834 (HS_5HT3A) and the bacterial GLIC and ELIC receptors (37523766 and 169791754). Transmembrane helices M1-M4 are indicated as green boxes according to PDB structure 3rhw (*C.elegans* GluCl). The signal peptide for Alv-a1-pHCl and its mutants is indicated by a grey bar. Conserved residues are marked in yellow. N-terminal residues of Alv-a1-pHCl, predicted to be disordered are highlighted in red and were absent in the construct tAlv-a1-pHCl. In thAlv-a1-pHCl, in addition to these N-terminal residues those of the predicted N-terminal helix, highlighted in grey, were deleted. M3-M4 loop residues are highlighted in cyan. In the tAlv-a1-pHCl-AGT and thAlv-a1-pHCl-AGT constructs the predicted M3-M4 loop residues were replaced by the tripeptide AGT.
For an overview of the constructs see Fig. 1 of the main text.

Supplementary Figure 1B

Sequence alignment for M2 transmembrane helices
Alignments of several anion specific CLRs (A) and cation specific CLRs (B).
The conservation pattern clearly groups Alv-a1-pHCl as an anionic channel and Alv-a9 as a cationic channel, in accord with other authors and our results.

Supplementary Figure 2

Alignment of EST sequences of Alv-a1-pHCl, EST sequences GO149973.1, GO164508.1, GO215979.1, GO215980.1 were taken from NCBI database and TERA02171 was taken from *Alvinella pomejana* EST sequences database available at www.alvinella.igbmc.fr.

Supplementary Figure 3

Western Blot with Anti His-tag Antibody of membranes preparations from Sf9 cells for different Alv-a1-pHCL constructs: Lane 1- thAlv-a1-pHCl, Lane 2- thAlv-a1-pHCl-AGT, Lane 3- tAlv-a1-pHCl, and Lane 4- tAlv-a1-pHCl-AGT.
Lanes C: Molecular weight markers from top to bottom (kDa): 170, 130, 100, 70(red), 55, 40, 35, 25 and 15.

Supplementary Figure 4

Statistics of channel activities of tAlv-a1-pHCl in 8 different oocytes and of Alv-a1-pHCl in 7 different oocytes.

Supplementary Figure 1

Signal Peptide

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Alv-a1-pHCl          -------------IASVKMLLYA-ICLILVLQLVPTSGT---------EVTDATSPDQDSGTYSVTTTTYVGQRADTTATPNTSVN------V
tAlv-a1-pHCl         ------------------MWGFAGGRLFGIFSAPVLVAVVCC--------------------------------------------------A
tAlv-a1-pHCl-AGT     ------------------MWGFAGGRLFGIFSAPVLVAVVCC--------------------------------------------------A
Alv-a9               ------------------MK---MAELAVVAACILLILAGD---YSA---------------------------------------------G
CE_GluC_alpha        ------------------MA------TWIVGKLIIASLILGIQAQQARTKSQDIFEDDNDN-----------------GTTTLESLARLTSPI
HS_GlyR_alpha1       ------------------MYSFNTLRLYLWETIVFFSLAASKEAEA----------------------------------------------A
HS_GABArho1          ------------------MLAVPNMRFGIFLLWWGWVLATESRMHWPGREVHEMSKKGRPQR--------------QRREVHEDAHKQVSPIL
HS_GABA_A_alpha1     ------------------MRKSPGLSDCLWAWILLLSTLTGRSYGQP---------------------------------------------S
HS_Nic_alpha7        ------------------MR---CSPGGVWLGLAASL--------------------------------------------------------
TM_Nic_alpha         ------------------MI---LCSYWHVGLVLLLFSC------------------------------------------------------
TM_Nic_beta          ------------------MEDVRRMALGLVVMMALALS-------------------------------------------------------
TM_Nic_delta         ------------------MG------NIHFVYLLISCLY------------------------------------------------------
TM_Nic_gamma         ------------------MV------LTLLLIICLALE-------------------------------------------------------
LS_AChBP             ------------------MR------RNIFCLACLWIV-------------------------------------------------------
AC_AChBP             ------------------ML------VSVYLALLVACV-------------------------------------------------------
HS_5HT3A             ------------------ML---LWVQQALLALLLPTLLAQG--------------------------------------------------E
HS_NIC_alpha9        ------------------MN---WSHSCISFCWIYFAASRLR---------------------------------------------------
GLIC                 MFPTGWRPKLSESIAASRML---WQPMAAVAVVQIGLL-------------------------------------------------------
ELIC                 ---------------------------------------------------------------------------------------------
3rhw_chainA_p001     ---------------------------------------------------------------------------------------------

Alv-a1-pHCl          SNQEAYSTDFDQRSFLERLL---MDMD--PTVRPVS--GQNDGPVEVKVDFHVLSISAMSEANMEYQLDIYFRQTWTDRRLAYNLS---DLGP
tAlv-a1-pHCl         SNQEAYSTDFDQRSFLERLL---MDMD--PTVRPVS--GQNDGPVEVKVDFHVLSISAMSEANMEYQLDIYFRQTWTDRRLAYNLS---DLGP
tAlv-a1-pHCl-AGT     SNQEAYSTDFDQRSFLERLL---MDMD--PTVRPVS--GQNDGPVEVKVDFHVLSISAMSEANMEYQLDIYFRQTWTDRRLAYNLS---DLGP
Alv-a9               VLGFQDRSMSRELGLYEHIV---GNDTRLRFIRPAR---NANESVHVEISIALIGLEDLDEKRQVMTSHIWLRQRWKNPYVSWSPD---DFGG
CE_GluC_alpha        HIPIEQPQTSDSKILAHLFT---SGYD--FRVRPPT---DNGGPVVVSVNMLLRTISKIDVVNMEYSAQLTLRESWIDKRLSYGVK---GDGQ
HS_GlyR_alpha1       RSAPKP---MSPSDFLDKLMGRTSGYD--ARIRPNF----KGPPVNVSCNIFINSFGSIAETTMDYRVNIFLRQQWNDPRLAYNEY---PDDS
HS_GABArho1          RRSPDITKSPLTKSEQLLRI---DDHD--FSMRPGF----GGPAIPVGVDVQVESLDSISEVDMDFTMTLYLRHYWKDERLSFPST---NNLS
HS_GABA_A_alpha1     LQDELKDNTTVFTRILDRLL---DGYD--NRLRPGL----GERVTEVKTDIFVTSFGPVSDHDMEYTIDVFFRQSWKDERLKFKGP----MTV
HS_Nic_alpha7        --LHVSLQGEFQRKLYKELV---KNYN--PLERPVA---NDSQPLTVYFSLSLLQIMDVDEKNQVLTTNIWLQMSWTDHYLQWNVS---EYPG
TM_Nic_alpha         --CGLVLGSEHETRLVANLL---ENYN--KVIRPVE---HHTHFVDITVGLQLIQLINVDEVNQIVETNVRLRQQWIDVRLRWNPA---DYGG
TM_Nic_beta          ----GVGASVMEDTLLSVLF---ENYN--PKVRPSQ---TVGDKVTVRVGLTLTSLLILNEKNEEMTTSVFLNLAWTDYRLQWDPA---AYEG
TM_Nic_delta         --YSGCSGVNEEERLINDLLI-VNKYN--KHVRPVK---HNNEVVNIALSLTLSNLISLKETDETLTTNVWMDHAWYDHRLTWNAS---EYSD
TM_Nic_gamma         ------VRSNEEGRLIEKLL---GDYD--KRIKPAK---TLDHVIDVTLKLTLTNLISLNEKEEALTTNVWIEIQWNDYRLSWNTS---EYEG
LS_AChBP             --------QACLSLDRADIL---YNIR--QTSRPDVIPTQRDRPVAVSVSLKFINILEVNEITNEVDVVFWQQTTWSDRTLAWNSS---HSPD
AC_AChBP             ---GQAHSQANLMRLKSDLF---NRSP----MYPGP---TKDDPLTVTLGFTLQDIVKADSSTNEVDLVYYEQQRWKLNSLMWDPN---EYGN
HS_5HT3A             ARRSRNTTRPALLRLSDYLL---TNYR--KGVRPVR---DWRKPTTVSIDVIVYAILNVDEKNQVLTTYIWYRQYWTDEFLQWNPE---DFDN
HS_NIC_alpha9        --AAETADGKYAQKLFNDLF---EDYS--NALRPVE---DTDKVLNVTLQITLSQIKDMDERNQILTAYLWIRQIWHDAYLTWDRD---QYDG
GLIC                 --------------WFSPPV---WGQD--MVSPPPP---IADEPLTVNTGIYLIECYSLDDKAETFKVNAFLSLSWKDRRLAFDPV----RSG
ELIC                 -----------------------------APADNAA----DARPVDVSVSIFINKIYGVNTLEQTYKVDGYIVAQWTGKPRKTPGDKPLIVEN
3rhw_chainA_p001     ---------SDSKILAHLFT---SGYD--FRVRPPT---DNGGPVVVSVNMLLRTISKIDVVNMEYSAQLTLRESWIDKRLSYGVK---GDGQ
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Cys-Loop

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Alv-a1-pHCl          GSRMGYFKLGKDPRNLIWVPDLFFPFEKQASFHVITVPNIMVQIYPSGEVMYSTRLTLVIACKMQLSSFPMDSQTCPFDIESYSYQTSEMILL
tAlv-a1-pHCl         GSRMGYFKLGKDPRNLIWVPDLFFPFEKQASFHVITVPNIMVQIYPSGEVMYSTRLTLVIACKMQLSSFPMDSQTCPFDIESYSYQTSEMILL
tAlv-a1-pHCl-AGT     GSRMGYFKLGKDPRNLIWVPDLFFPFEKQASFHVITVPNIMVQIYPSGEVMYSTRLTLVIACKMQLSSFPMDSQTCPFDIESYSYQTSEMILL
Alv-a9               L------RDVMIPADEVWTPDITAHNSLSTSYHED--HTSQLVVHSDGLIVWAVQSRLETFCFLDLTYFPLDTHNCPIVYGSWSYDSSKLNLS
CE_GluC_alpha        P-----DFVILTVGHQIWMPDTFFPNEKQAYKHTIDKPNVLIRIHNDGTVLYSVRISLVLSCPMYLQYYPMDVQQCSIDLASYAYTTKDIEYL
HS_GlyR_alpha1       L------DLDPSMLDSIWKPDLFFANEKGAHFHEITTDNKLLRISRNGNVLYSIRITLTLACPMDLKNFPMDVQTCIMQLESFGYTMNDLIFE
HS_GABArho1          M------TFDGRLVKKIWVPDMFFVHSKRSFIHDTTTDNVMLRVQPDGKVLYSLRVTVTAMCNMDFSRFPLDTQTCSLEIESYAYTEDDLMLY
HS_GABA_A_alpha1     L------RLNNLMASKIWTPDTFFHNGKKSVAHNMTMPNKLLRITEDGTLLYTMRLTVRAECPMHLEDFPMDAHACPLKFGSYAYTRAEVVYE
HS_Nic_alpha7        V------KTVRFPDGQIWKPDILLYNSADERFDAT--FHTNVLVNSSGHCQYLPPGIFKSSCYIDVRWFPFDVQHCKLKFGSWSYGGWSLDLQ
TM_Nic_alpha         I------KKIRLPSDDVWLPDLVLYNNADGDFAIV--HMTKLLLDYTGKIMWTPPAIFKSYCEIIVTHFPFDQQNCTMKLGIWTYDGTKVSIS
TM_Nic_beta          I------KDLSIPSDDVWQPDIVLMNNNDGSFEIT--LHVNVLVQHTGAVSWHPSAIYRSSCTIKVMYFPFDWQNCTMVFKSYTYDTSEVILQ
TM_Nic_delta         I------SILRLRPELIWIPDIVLQNNNDGQYNVA--YFCNVLVRPNGYVTWLPPAIFRSSCPINVLYFPFDWQNCSLKFTALNYNANEISMD
TM_Nic_gamma         I------DLVRIPSELLWLPDVVLENNVDGQFEVA--YYANVLVYNDGSMYWLPPAIYRSTCPIAVTYFPFDWQNCSLVFRSQTYNAHEVNLQ
LS_AChBP             Q--------VSVPISSLWVPDLAAYNAISKPEVL---TPQLARVVSDGEVLYMPSIRQRFSCDVSGVDTE-SGATCRIKIGSWTHHSREISVD
AC_AChBP             I------TDFRTSAADIWTPDITAYSSTRPVQVL---SPQIAVVTHDGSVMFIPAQRLSFMCDPT-GVDSEEGATCAVKFGSWVYSGFEIDLK
HS_5HT3A             I------TKLSIPTDSIWVPDILINEFVDVGKSP---NIPYVYIRHQGEVQNYKPLQVVTACSLDIYNFPFDVQNCSLTFTSWLHTIQDINIS
HS_NIC_alpha9        L------DSIRIPSDLVWRPDIVLYNKADDESSEP--VNTNVVLRYDGLITWDAPAITKSSCVVDVTYFPFDNQQCNLTFGSWTYNGNQVDIF
GLIC                 V------RVKTYEPEAIWIPEIRFVNVENARDA----DVVDISVSPDGTVQYLERFSARVLSPLDFRRYPFDSQTLHIYLIVRSVDTRNIVLA
ELIC                 T------QIERWINNGLWVPALEFINVVGSPDT----GNKRLMLFPDGRVIYNARFLGSFSNDMDFRLFPFDRQQFVLELEPFSYNNQQLRFS
3rhw_chainA_p001     P-----DFVILTVGHQIWMPDTFFPNEKQAYKHTIDKPNVLIRIHNDGTVLYSVRISLVLSCPMYLQYYPMDVQQCSIDLASYAYTTKDIEYL
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M1

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Alv-a1-pHCl          WKDNP------------VTLEDFFELPRFSLKLPIKTTVCTKEYKT-------GSFPCLQAKFEMSRFFGDYLLQTYIPSGLIVILSWVAFWI
tAlv-a1-pHCl         WKDNP------------VTLEDFFELPRFSLKLPIKTTVCTKEYKT-------GSFPCLQAKFEMSRFFGDYLLQTYIPSGLIVILSWVAFWI
tAlv-a1-pHCl-AGT     WKDNP------------VTLEDFFELPRFSLKLPIKTTVCTKEYKT-------GSFPCLQAKFEMSRFFGDYLLQTYIPSGLIVILSWVAFWI
Alv-a9               HWGDPELD---------LRHGSALDNSEWYIGNSSVVLHSKMRGVGT------QPYQDITVYVTLSRRHAYVIQLYVAPVVTFVLLTPAVFIL
CE_GluC_alpha        WKEHSPLQ---------LKVGLSSSLPSFQLTNTSTTYCTSVTNT--------GIYSCLRTTIQLKREFSFYLLQLYIPSCMLVIVSWVSFWF
HS_GlyR_alpha1       WQEQGA-----------VQVADGLTLPQFILKEEKDLRYCTKHYNT-------GKFTCIEARFHLERQMGYYLIQMYIPSLLIVILSWISFWI
HS_GABArho1          WKKGNDS----------LKTDERISLSQFLIQEFHTTTKLAFYSST-------GWYNRLYINFTLRRHIFFFLLQTYFPATLMVMLSWVSFWI
HS_GABA_A_alpha1     WTREPARS--------VVVAEDGSRLNQYDLLGQTVDSGIVQSST--------GEYVVMTTHFHLKRKIGYFVIQTYLPCIMTVILSQVSFWL
HS_Nic_alpha7        MQE--------------ADISGYIPNGEWDLVGIPGKRSERFYECCK------EPYPDVTFTVTMRRRTLYYGLNLLIPCVLISALALLVFLL
TM_Nic_alpha         PESDR------------PDLSTFMESGEWVMKDYRGWKHWVYYTCCPD-----TPYLDITYHFIMQRIPLYFVVNVIIPCLLFSFLTVLVFYL
TM_Nic_beta          HALDAKGERE--VKEIMINQDAFTENGQWSIEHKPSRKNWRSDD---------PSYEDVTFYLIIQRKPLFYIVYTIVPCILISILAILVFYL
TM_Nic_delta         LMTDTIDGKDYPIEWIIIDPEAFTENGEWEIIHKPAKKNIYGDKFPNG-----TNYQDVTFYLIIRRKPLFYVINFITPCVLISFLAALAFYL
TM_Nic_gamma         LSAEEGEVV----EWIHIDPEDFTENGEWTIRHRPAKKNYNWQLTKDD-----IDFQEIIFFLIIQRKPLFYIINIIAPCVLISSLVVLVYFL
LS_AChBP             PTTENS-----------DDSEYFSQYSRFEILDVTQKKNSVTYSCCP------EAYEDVEVSLNFRKKGRSEIL-------------------
AC_AChBP             TDTDQ------------VDLSSYYASSKYEILSATQTRQVQHYSCCP------EPYIDVNLVVKFRERRAGNGFFRNLFD-------------
HS_5HT3A             LWRLPEK--------VKSDRSVFMNQGEWELLGVLPYFREFSMESS-------NYYAEMKFYVVIRRRPLFYVVSLLLPSIFLMVMDIVGFYL
HS_NIC_alpha9        NALDS------------GDLSDFIEDVEWEVHGMPAVKNVISYGCCS------EPYPDVTFTLLLKRRSSFYIVNLLIPCVLISFLAPLSFYL
GLIC                 VDLEK------------VGKNDDVFLTGWDIESFTAVVKPANFALED------RLESKLDYQLRISRQYFSYIPNIILPMLFILFISWTAFWS
ELIC                 DIQVY------------TENIDNEEIDEWWIRKASTHISDIRYDHLSSVQPNQNEFSRITVRIDAVRNPSYYLWSFILPLGLIIAASWSVFWL
3rhw_chainA_p001     WKEHSPLQ---------LKVGLSSSLPSFQLTNTSTTYCTSVTNT--------GIYSCLRTTIQLKREFSFYLLQLYIPSCMLVIVSWVSFWF
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                                      M2                               M3

Alv-a1-pHCl           NKEAIPARVSLSIVTVMTLVSHIAGSKTSIPSVTY-LKAVDVWMMMAMVFVFAAVIEYAFVNSLDRKAMKAKK--------------------
tAlv-a1-pHCl          NKEAIPARVSLSIVTVMTLVSHIAGSKTSIPSVTY-LKAVDVWMMMAMVFVFAAVIEYAFVNSLDRKAMKAKK--------------------
tAlv-a1-pHCl-AGT      NKEAIPARVSLSIVTVMTLVSHIAGSKTSIPSVTY-LKAVDVWMMMAMVFVFAAVIEYAFVNSLDRAGT------------------------
Alv-a9                PAES-AHKFVLGSGLLISEVLLMISLNDIIPAAHHTPPRLGLYYLCSLLMICCSLGIAALVANIWNRSKRRSFGV-----PSCVRVVLLKGLG
CE_GluC_alpha         DRTAIPARVTLGVTTLLTMTAQSAGINSQLPPVSY-IKAIDVWIGACMTFIFCALLEFALVNHIANKQGVERKAR------TEREKAEIPLLQ
HS_GlyR_alpha1        NMDAAPARVGLGITTVLTMTTQSSGSRASLPKVSY-VKAIDIWMAVCLLFVFSALLEYAAVNFVSRQHKELLRFR------RKRRHHKEDEAG
HS_GABArho1           DRRAVPARVPLGITTVLTMSTIITGVNASMPRVSY-IKAVDIYLWVSFVFVFLSVLEYAAVNYLTTVQERKEQKL------REKLPCTSGLPP
HS_GABA_A_alpha1      NRESVPARTVFGVTTVLTMTTLSISARNSLPKVAY-ATAMDWFIAVCYAFVFSALIEFATVNYFTKRGYAWDGKSVVPEKPKKVKDPLIKKN-
HS_Nic_alpha7         PADS-GEKISLGITVLLSLTVFMLLVAEIMPATSDSVPLIAQYFASTMIIVGLSVVVTVIVLQYHHHDPDGGKMP------KWTRVILLNWCA
TM_Nic_alpha          PTDS-GEKMTLSISVLLSLTVFLLVIVELIPSTSSAVPLIGKYMLFTMIFVISSIIVTVVVINTHHRSPSTHTMP------QWVRKIFINTIP
TM_Nic_beta           PPDA-GEKMSLSISALLALTVFLLLLADKVPETSLSVPIIISYLMFIMILVAFSVILSVVVLNLHHRSPNTHTMP------NWIRQIFIETLP
TM_Nic_delta          PAES-GEKMSTAICVLLAQAVFLLLTSQRLPETALAVPLIGKYLMFIMSLVTGVVVNCGIVLNFHFRTPSTHVLS------TRVKQIFLEKLP
TM_Nic_gamma          PAQAGGQKCTLSISVLLAQTIFLFLIAQKVPETSLNVPLIGKYLIFVMFVSLVIVTNCVIVLNVSLRTPNTHSLS------EKIKHLFLEFLP
LS_AChBP              ---------------------------------------------------------------------------------------------
AC_AChBP              ---------------------------------------------------------------------------------------------
HS_5HT3A              PPNS-GERVSFKITLLLGYSVFLIIVSDTLPATAIGTPLIGVYFVVCMALLVISLAETIFIVRLVHKQDLQQPVP------AWLRHLVLERIA
HS_NIC_alpha9         PAAS-GEKVSLGVTILLAMTVFQLMVAEIMPASEN-VPLIGKYYIATMALITASTALTIMVMNIHFCGAEARPVP------HWARVVILKYMS
GLIC                  TSY--EANVTLVVSTLIAHIAFNILVETNLPKTPY-MTYTGAIIFMIYLFYFVAVIEVTVQHYLKVESQ--PARA------------------
ELIC                  ESF--SERLQTSFTLMLTVVAYAFYTSNILPRLPY-TTVIDQMIIAGYGSIFAAILLIIFAHHRQANGV---EDD------------------
3rhw_chainA_p001      DRTAIPARVTLGVTTLLTMTAQSAGINSQLPPVSY-IKAIDVWIGACMTFIFCALLEFALVNHIAN---------------------------

Alv-a1-pHCl           -------AGQPASKVAGAE--------------------------------------------------------------------------
tAlv-a1-pHCl          -------AGQPASKVAGAE--------------------------------------------------------------------------
tAlv-a1-pHCl-AGT      ---------------------------------------------------------------------------------------------
Alv-a9                RVLCLFYEDFLCVDSSPDHIFDQYTTSLD---------------------------------------TISQGSS------------NHP---
CE_GluC_alpha         -------NLHNDVPTKVFNQEEKV--RTV---------------------------------------PLNRRQM------------NSF---
HS_GlyR_alpha1        -------EGRFNFSAYGMGPACLQ--AKD---------------------------------------GISVKGA--NNSNTTNPPPAPS---
HS_GABArho1           -------PRTAMLDGNYSDGEVND--LDN---------------------------------------YMPENGE--KPDRMMVQLTLAS---
HS_GABA_A_alpha1      ---------NTYAPTATSYTPNLA--RGD----------------------------------------PGLATI--AKSATIEPKEVKP---
HS_Nic_alpha7         WFLRMKRPGEDKVRPACQHKQRRCSLASVEMSAVG---PPPASNGNLLYIGFRGLDGVHCVPTPDSGVVCGRMACSPTHDEHLLHGGQPPEGD
TM_Nic_alpha          NLMFFSTMKRASKEKQENKIFADDIDISDI---------------------------------------------SGKQVTGEVIFQTPLIKN
TM_Nic_beta           PFLWIQRPVTTPSPDSKPTIISRANDEYFIRKPAG-----------DFVCP-----------VDNARVAVQPERLFSEMKWHLNGLTQPVTLP
TM_Nic_delta          RILHMSRVDEIEQPDWQNDLKLRRSSSVGYISKAQEYFNIKSRS-ELMFEK-----------QSERHGLVPRVTPRIGFGNNNENIAASDQLH
TM_Nic_gamma          KYLGMHLEPSEETPEKPQPRRRSSFGIMIKAEEYI---LKKPRS-ELMFEEQ---------KDRHGLKRVNKMTSDIDIGTTVDLYKDLANFA
LS_AChBP              ---------------------------------------------------------------------------------------------
AC_AChBP              ---------------------------------------------------------------------------------------------
HS_5HT3A              WLLCLREQSTSQRPPATSQATKTDDCSAMGNHCS---------------------------------HMGGPQDFEKSPRDRCSPPPPPREAS
HS_NIC_alpha9         RVLFVYDVGESCLSPHHSRERDHLTKVYSKLPESN---LKAARN------------------KDLSRKKDMNKRLKNDLGCQGKNPQEAESYC
GLIC                  ---------------------------------------------------------------------------------------------
ELIC                  ---------------------------------------------------------------------------------------------
3rhw_chainA_p001      ---------------------------------------------------------------------------------------------

                                                                M4

Alv-a1-pHCl           -------------------DGQDEP---KYADHAKRVDKVSRIVFPLAFVLFNAVYWPYYTRS----------IPS----------
tAlv-a1-pHCl          -------------------DGQDEP---KYADHAKRVDKVSRIVFPLAFVLFNAVYWPYYTRGH---HHHHHHHHH----------
tAlv-a1-pHCl-AGT      ------------------------P---KYADHAKRVDKVSRIVFPLAFVLFNAVYWPYYTRGH---HHHHHHHHH----------
Alv-a9                ----------LDDAKMIREYNSHVVHRAEWRQLAAVVDRT----FCLIFWVMSIVFIVAMAIKLH--HHHHHHH------------
CE_GluC_alpha         -----------------LNLLETKT---EWNDISKRVDLISRALFPVLFFVFNILYWSRFGQQN---VLF----------------
HS_GlyR_alpha1        -----------------KSPEEMRK---LFIQRAKKIDKISRIGFPMAFLIFNMFYWIIYKIVR---REDVHNQ------------
HS_GABArho1           ----------ERSSPQRKSQRSSYV---SMRIDTHAIDKYSRIIFPAAYILFNLIYWSIFS-------------------------
HS_GABA_A_alpha1      ----------ETKPPEPK----------KTFNSVSKIDRLSRIAFPLLFGIFNLVYWATYLNRE---PQL--KAPTPHQ-------
HS_Nic_alpha7         PDLAKILEEVRYIANRFRCQDESEAVCSEWKFAACVVDRLCLMAFSVFTIICTIGILMSAPNFVEAVSKDFA--------------
TM_Nic_alpha          PDVKSAIEGVKYIAEHMKSDEESSNAAEEWKYVAMVIDHILLCVFMLICIIGTVCVFAGRLIE-------LSQEG-----------
TM_Nic_beta           QDLKEAVEAIKYIAEQLESASEFDDLKKDWQYVAMVADRLFLYIFITMCSIGTFSIFLDASHNVP-PDNPFA--------------
TM_Nic_delta          DEIKSGIDSTNYIVKQIKEKNAYDEEVGNWNLVGQTIDRLSMFIITPVMVLGTIFIFVMGNFNRP-PAKPFEGDPFDYSSDHPRCA
TM_Nic_gamma          PEIKSCVEACNFIAKSTKEQNDSGSENENWVLIGKVIDKACFWIALLLFSLGTLAIFLTGHLNQV-PEFPFPGDPRKYVP------
LS_AChBP              --------------------------------------------------------------------------------------
AC_AChBP              --------------------------------------------------------------------------------------
HS_5HT3A              LAVCGLLQELSSIRQFLEKRDEIREVARDWLRVGSVLDKLLFHIYLLAVLAYSITLVMLWSIWQYA-------------------
HS_NIC_alpha9         AQYKVLTRNIEYIAKCLKDHKATNSKGSEWKKVAKVIDRFFMWIFFIMVFVMTILIIARAD-------------------------
GLIC                  ----------------------------------ASITRASRIAFPVVFLLANIILAFLFFGF-----------------------
ELIC                  ----------------------------------LLIQ-RCRLAFPLGFLAIGCVLVIRGITL-----------------------
3rhw_chainA_p001      --------------------AGTT---EWNDISKRVDLISRALFPVLFFVFNILYWSRFGH------------------------
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Supplementary Figure 1B

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A)
                           -1' 2'     9'
    Alv-a1-pHCl           NKEAIPARVSLSIVTVMTLVSHIAGSKTSIPSVTY
    CE_GluC_alpha         DRTAIPARVTLGVTTLLTMTAQSAGINSQLPPVSY
    HS_GlyR_alpha1        NMDAAPARVGLGITTVLTMTTQSSGSRASLPKVSY
    HS_GABArho1           DRRAVPARVPLGITTVLTMSTIIITGVNASMPRVSY
    HS_GABA_A_alpha1      NRESVPARTVFGVTTVLTMTTLSISARNSLPKVAY
B)
    Alv-a9                PAES-AHKFVLGSGLLISEVLLMISLNDIIPAAHH
    HS_Nic_alpha7         PADS-GEKISLGITVLLSLTVFMLLVAEIMPATSD
    HS_5HT3A              PPNS-GERVSFKITLLLGYSVFLIIVSDTLPATAI
    HS_NIC_alpha9         PAAS-GEKVSLGVTILLAMTVFQLMVAEIMPASEN
```

Supplementary Figure 2

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TERA02171     ATGTGTCACCCTCAGTGGATGGCGGCCGCCGGTCTATTTTAAGACACCGACCTTCATCTC
GO149973      ------------------------------------------------------------
GO164508      ------------------------------------------------------------
GO215979      ------------------------------------------------------------
GO215980      ------------------------------------------------------------


TERA02171     AAAGGAACGCCTTGTTTTAACCCGGTTCATTTTAAAGGTAACAACTTTTTCCGTCGGCTC
GO149973      ------------------------------------------------------------
GO164508      ------------------------------------------------------------
GO215979      ------------------------------------------------------------
GO215980      ------------------------------------------------------------


TERA02171     GCGGCCAGGTGAGACAATTCTTTGTGGGCTACAGCAGCTAGAAGTTCAGGTAAACATCGT
GO149973      ------------------------------------------------------------
GO164508      ------------------------------------------------------------
GO215979      ------------------------------------------------------------
GO215980      ------------------------------------------------------------


TERA02171     CTTCGACTGAGATCGTCGTGAAGTCTAGGCAGGTCGCTTATCATCAAACGTCGATAGATC
GO149973      ------------------------------------------------------------
GO164508      ------------------------------------------------------------
GO215979      ------------------------------------------------------------
GO215980      ------------------------------------------------------------


TERA02171     GACATTCTGACAATAAATCGCGTCAGTCAAGATGCTTCTGTACGCCATTTGCCTTATCCT
GO149973      ------------------------------------------------------------
GO164508      ------------------------------------------------------------
GO215979      ------------------------------------------------------------
GO215980      ------------------------------------------------------------


TERA02171     CGTGCTGCAGTTGGTGCCGACCAGCGGCACAGAGGTCACGGACGCCACGTCTCCGGATCA
GO149973      ------------------------------------------------------------
GO164508      ------------------------------------------------------------
GO215979      ------------------------------------------------------------
GO215980      ------------------------------------------------------------


TERA02171     AGATTCTGGAACATACTCGGTCACCACGACAACATACGTAGGTCAGAGAGCAGACACCAC
GO149973      ------------------------------------------------------------
GO164508      ------------------------------------------------------------
GO215979      ------------------------------------------------------------
GO215980      ------------------------------------------------------------


TERA02171     GGCCACACCGAATACAAGCGTAAACGTGTCAAACCAGGAGGCGTATAGCACCGACTTTGA
GO149973      ------------------------------------------------------------
GO164508      ------------------------------------------------------------
GO215979      ------------------------------------------------------------
GO215980      ------------------------------------------------------------


TERA02171     CCAACGATCGTTTCTTGAGCGTCTGCTTATGGATATGGATCCGACGGTGCGACCGGTCAG
GO149973      ------------------------------------------------------------
GO164508      ------------------------------------------------------------
GO215979      ------------------------------------------------------------
GO215980      ------------------------------------------------------------


TERA02171     TGGTCAGAACGACGGACCGGTTGAGGTTAAAGTAGATTTCCATGTTCTCAGCATAAGCGC
GO149973      --GTCAGAACGACGGACCGGTTGAGGTTAAAGTAGATTTCCATGTTCTCAGCATAAGCGC
GO164508      ------------------------------------------------------------
GO215979      ------------------------------------------------------------
GO215980      ------------------------------------------------------------


TERA02171     AATGAGCGAAGCTAATATGGAATATCAGCTGGACATCTACTTTCGACAGACATGGACAGA
GO149973      AATGAGCGAAGCTAATATGGAATATCAGCTGGACATCTACTTTCGCCAGACATGGACAGA
GO164508      ------------------------------------------------------------
GO215979      ------------------------------------------------------------
GO215980      ------------------------------------------------------------


TERA02171     TCGCCGGCTGGCATACAACCTCTCCGATCTGGGTCCCGGTTCCAGGATGGGTTACTTTAA
GO149973      TCGCCGGCTGGCATACAACCTCTCCGATCTGGGTCCCGGTTCCAGGATGGGTTACTTTAA
GO164508      ------------------------------------------------------------
GO215979      ------------------------------------------------------------
GO215980      ------------------------------------------------------------
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TERA02171     ACTGGGCAAGGATCCGAGAAATCTAATCTGGGTACCAGATCTTTTCTTTCCGTTCGAGAA
GO149973      ACTGGGCAAGGATCCGAGAAATCTAATCTGGGTACCAGATCTTTTCTTTCCGTTCGAGAA
GO164508      ------------------------------------------------------------
GO215979      ------------------------------------------------------------
GO215980      ------------------------------------------------------------


TERA02171     GCAGGCCTCTTTCCATGTCATTACCGTGCCAAACATCATGGTCCAGATTTATCCGAGCGG
GO149973      GCAGGCCTCTTTCCATGTCATTACCGTGCCAAACATCATGGTCCAGATTTATCCGAGCGG
GO164508      -CAGGCCTCTTTCCATGTCATTACCGTGCCAAACATCATGGTCCAGATTTATCCGAGCGG
GO215979      -CAGGCCTCTTTCCATGTCATTACCGTGCCAAACATCATGGTCCAGATTTATCCGAGCGG
GO215980      ------------------------------------------------------------


TERA02171     AGAGGTCATGTACAGCACCAGGTTAACCCTTGTCATAGCGTGCAAGATGCAGCTTTCGTC
GO149973      AGAGGTCATGTACAGCACCAGGTTAACCCTTGTCATAGCGTGCAAGATGCAGCTTTCGTC
GO164508      AGAGGTCATGTACAGCACCAGGTTAACCCTTGTCATAGCGTGCAAGATGCAGCTTTCGTC
GO215979      AGAGGTCATGTACAGCACCAGGTTAACCCTTGTCATAGCGTGCAAGATGCAGCTTTCGTC
GO215980      ------------------------------------------------------------


TERA02171     CTTTCCCATGGACAGCCAGACGTGTCCTTTTGACATCGAAAGTTACTCCTACCAAACGAG
GO149973      CTTTCCCATGGACAGCCAGACGTGTCCTTTTGACATCGAAAGTTACTCCTACCAAACGAG
GO164508      CTTTCCCATGGACAGCCAGACGTGTCCTTTTGACATCGAAAGTTACTCCTACCAAACGAG
GO215979      CTTTCCCATGGACAGCCAGACGTGTCCTTTTGACATCGAAAGTTACTCCTACCAAACGAG
GO215980      -----------------------------------------------------------G


TERA02171     TGAGATGATCCTGTTGTGGAAGGACAACCCCGTGACACTTGAGGATTTTTTTGAACTGCC
GO149973      TGAGATGATCCTGTTGTGGAAGGACAACCCCGTGACACTTGAGGATTTTTTTGAACTGCC
GO164508      TGAGATGATCCTGTTGTGGAAGGACAACCCCGTGACACTTGAGGATTTTTTTGAACTGCC
GO215979      TGAGATGATCCTGTTGTGGAAGGACAACCCCGTGACACTTGAGGATTTTTTTGAACTGCC
GO215980      TGAGATGATCCTG-TGTGGAAGGACAACCCCGTGACACTTGAGGATTTTTTTGAACTGCC


TERA02171     CCGGTTTTCTC-------------------------------------------------
GO149973      CCGGTTTTCTCTGAAGCTCCCCATTAAAACGACCGTCTGTACAAAGGAATACAAAACAGG
GO164508      CCGGTTTTCTCTGAAGCTCCCCATTAAAACGACCGTCTGTACAAAGGAATACAAAACAGG
GO215979      CCGGTTTTCTCTGAAGCTCCCCATTAAAACGACCGTCTGTACAAAGGAATACAAAACAGG
GO215980      CCGGTTTTCTCTGAAGCTCCCCATTAAAACGACCGTCTGTACAAAGGAATACAAAACAGG


TERA02171     ------------------------------------------------------------
GO149973      AAGCTTTCCTTGTCTACAGGCGAAGTTTGAAATGAGCCGTTTCTTTGGTGATTACCTGCT
GO164508      AAGCTTTCCTTGTCTACAGGCGAAGTTTGAAATGAGCCGTTTCTTTGGTGATTACCTGCT
GO215979      AAGCTTTCCTTGTCTACAGGCGAAGTTTGAAATGAGCCGTTTCTTTGGTGATTACCTGCT
GO215980      AAGCTTTCCTTGTCTACAGGCGAAGTTTGAAATGAGCCGTTTCTTTGGTGATTACCTGCT


TERA02171     ------------------------------------------------------------
GO149973      CCAGACGTATATCCCATCCGGATTGATCGTCATCCTCTCCTGGGTGGCATTCTGGATCAA
GO164508      CCAGACGTATATCCCATCCGGATTGATCGTCATCCTCTCCTGGGTGGCATTCTGGATCAA
GO215979      CCAGACGTATATCCCATCCGGATTGATCGTCATCCTCTCCTGGGTGGCATTCTGGATCAA
GO215980      CCAGACGTATATCCCATCCGGATTGATCGTCATCCTCTCCTGGGTGGCATTCTGGATCAA


TERA02171     ------------------------------------------------------------
GO149973      TAAAGAAGCCATTCCGGCTAGGGTTTCGCTTAGCATTGTCACGGTCATGACACTGGTGTC
GO164508      TAAAGAAGCCATTCCGGCTAGGGTTTCGCTTAGCATTGTCACGGTCATGACACTGGTGTC
GO215979      TAAAGAAGCCATTCCGGCTAGGGTTTCGCTTAACATTGTCACGGTCATGACACTGGTGTC
GO215980      TAAAGAAGCCATTCCGGCTAGGGTTTCGCTTAGCATTGTCACGGTCATGACACTGGTGTC


TERA02171     ------------------------------------------------------------
GO149973      CCACATTGCCGG------------------------------------------------
GO164508      CCACATTGCCGGTTCTAAGACTTCCATTCCCAGTGTCACTTACCTGAAGGCCGTCGATGT
GO215979      CCACATTGCCGGTTCTAAGACTTCCATTCCCAGTGTCACTTACCTGAAGGCCGTCGATGT
GO215980      CCACATTGCCGGTTCTAAGACTTCCATTCCCAGTGTCACTTACCTGAAGGCCGTCGATGT



TERA02171     ------------------------------------------------------------
GO149973      ------------------------------------------------------------
GO164508      GTGGATGATGATGGCCATGGTATTTGTGTTCGCGGCGGTCATCGAGTACGCCTTCGTCAA
GO215979      GTGGATGATGATGGCCATGGTATTTGTGTTCGCGGCGGTCATCGAGTACGCCTTCATCAA
GO215980      GTGGATGATGATGGCCATGGTATTTGTGTTCGCGGCGGTCATCGAGTACGCCTTCGTCAA


TERA02171     ------------------------------------------------------------
GO149973      ------------------------------------------------------------
GO164508      CTCTCTCGATCGTAAGGCGATGAAAGCAAAAAAGGCCGGACAACCAGCCAGCAAGGTGGC
GO215979      CTCTCTCGATCGTAAGGCGATGAAAGCAAAAAAGGCCGGACAACCAGCCAGCAAGGTGGC
GO215980      CTCTCTCGATCGTAAGGCGATGAAAGCAAAAAAGGCCGGACAACCAGCCAGCAAGGTGGC
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TERA02171     ------------------------------------------------------------
GO149973      ------------------------------------------------------------
GO164508      CGGCGCGGAAGACGGCCAGGATGAGCCCAAGTACGCAGACCACGCCTAGAGAGTTGACAA
GO215979      CGGCGCGGAAGACGGCCAGGATGAGCCCAAGTACGCAGACCAC-----------------
GO215980      CGGCGCGGAAGACGGCCAGGATGAGCCCAAGTACGCAGACCACGCCAAGAGAGTTGACAA

TERA02171     ------------------------------------------------------------
GO149973      ------------------------------------------------------------
GO164508      AGTCAGCAGAATCGTCTTTCCTTTGGCCTTTGTACTCTTNCACGCCGTCTACTGGCC---
GO215979      ------------------------------------------------------------
GO215980      AGTCAGCAGAATCGTCTTTCCTTTGGCCTTTGTACTCTTCAACGCCGTCTACTGGCCATA

TERA02171     ------------------------------------------------------------
GO149973      ------------------------------------------------------------
GO164508      ------------------------------------------------------------
GO215979      ------------------------------------------------------------
GO215980      CTACACACGATCTATTCCGTCCGAGTAAGAAGAACGCACATCACCTGACAAGGGTTCGCT

TERA02171     ------------------------------------------------------------
GO149973      ------------------------------------------------------------
GO164508      ------------------------------------------------------------
GO215979      ------------------------------------------------------------
GO215980      GGCTGTTTTATATTTATTCACCTTCTTTGTTAGCACGCAGTTTATTAAATGATTACCATA

TERA02171     ------------------------------------------------------------
GO149973      ------------------------------------------------------------
GO164508      ------------------------------------------------------------
GO215979      ------------------------------------------------------------
GO215980      TCTAATGTCACAGGCCATATTTTTCGATTCTTTCCAATAAATATTATAGGAGTTTACCCT

TERA02171     --------
GO149973      --------
GO164508      --------
GO215979      --------
GO215980      CAAAAAAA
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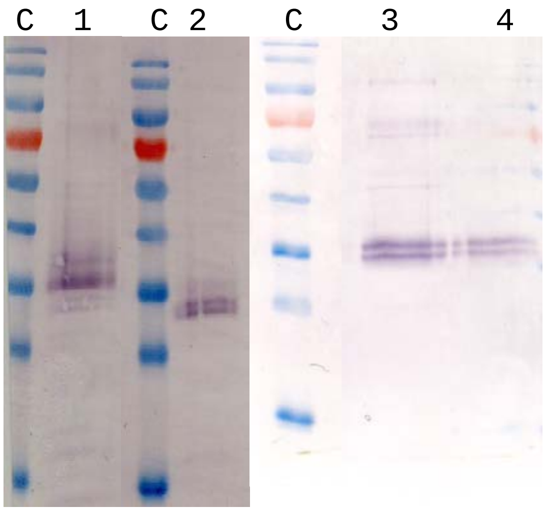
Supplementary Figure 3



Supplementary Figure 4