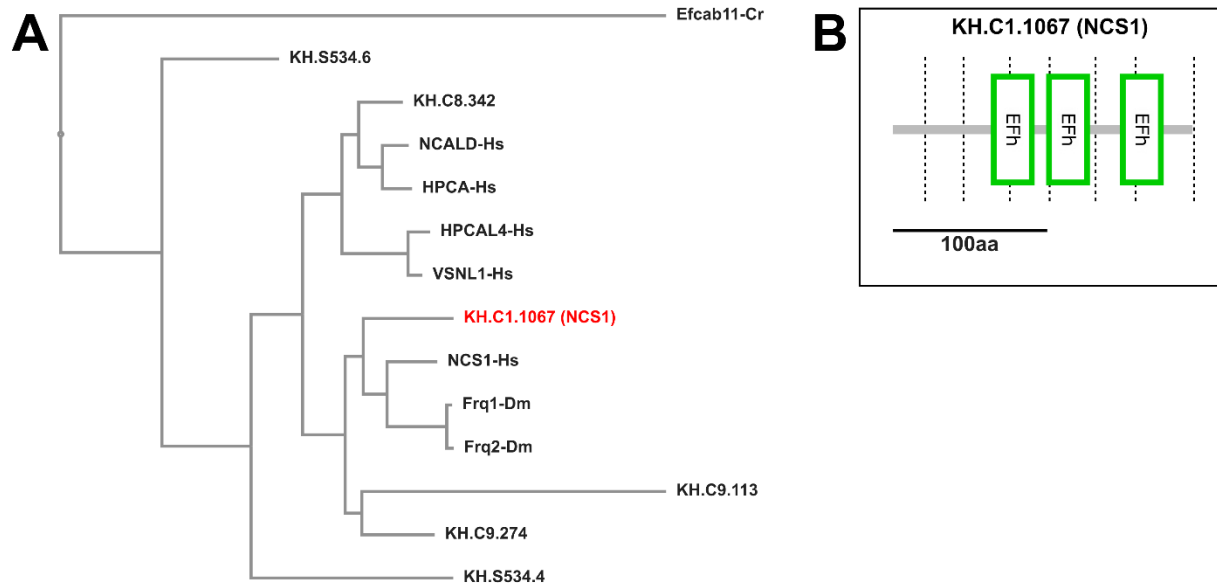


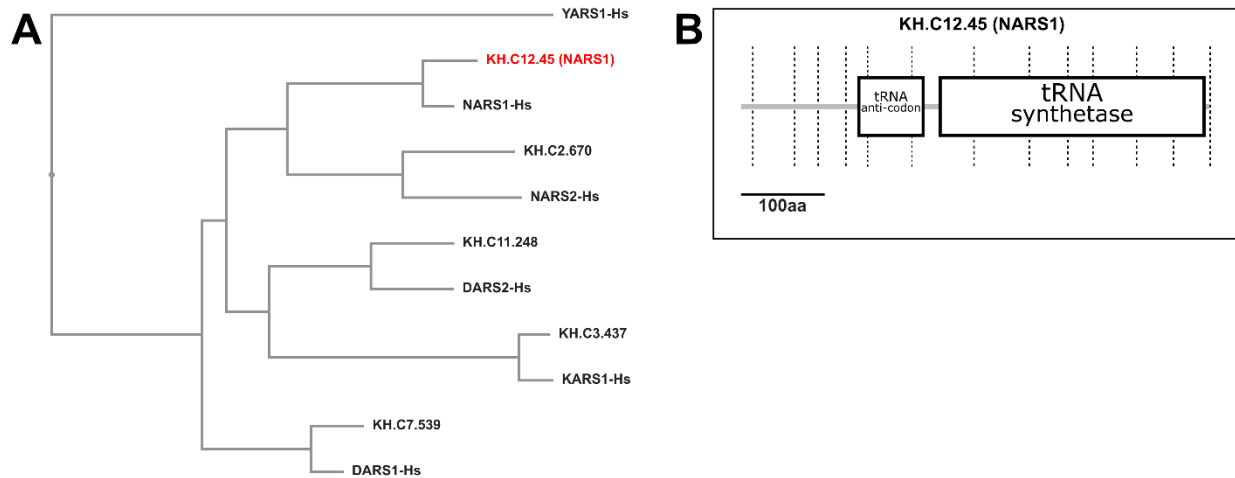
**Fig. S1. Phylogenetic tree of Vamp proteins.**

Tree showing phylogenetic analysis of predicted proteins encoded by *VAMP* family genes from human (Hs) and *Ciona robusta* (KH gene models). See methods for details and supplemental sequences file for protein sequences used.



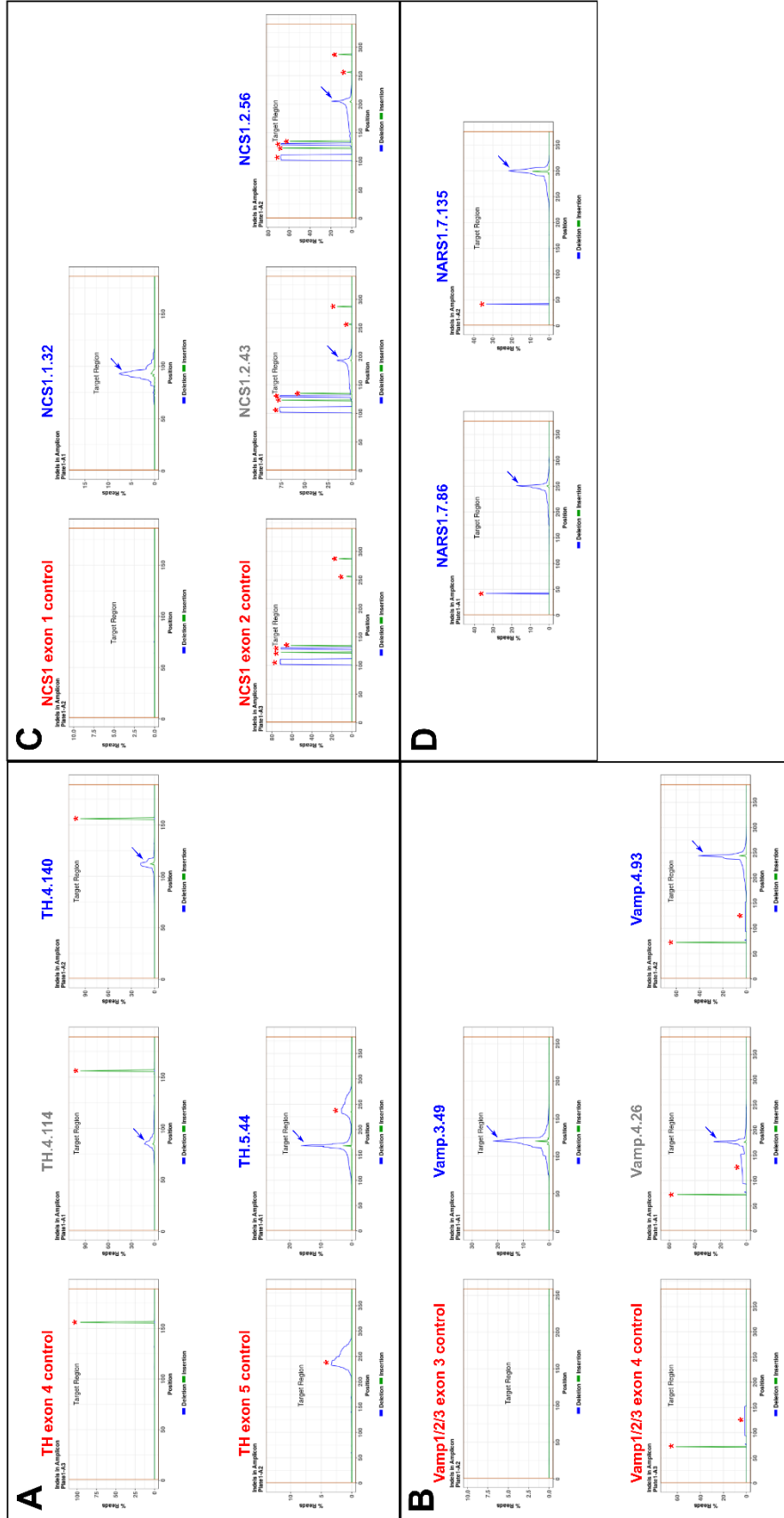
**Fig. S2. Phylogenetic tree of NCS proteins and diagram of *Ciona robusta* NCS1 domains.**

A) Tree showing phylogenetic analysis of proteins encoded by *Neuronal Calcium Sensor* (NCS) family genes from human (Hs), *Drosophila melanogaster* (Dm) and *Ciona robusta* (KH gene models). Efcab11 from *C. robusta* (Cr) was used to root the tree. See methods for details and supplemental sequences file for protein sequences used. B) Protein domain analysis diagram of *Ciona robusta* NCS1 from SMART (Letunic et al. 2021) showing its predicted three EF-hand (Efh) domains. Dashed lines indicate exon-exon junctions.



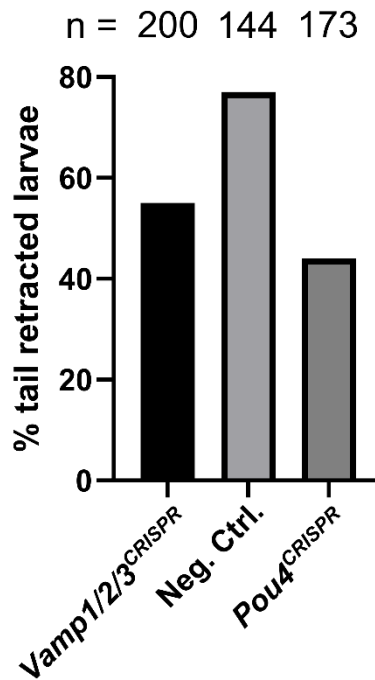
**Fig. S3. Phylogenetic tree of select aminoacyl-tRNA synthetases and *Ciona* NARS1 domains.**

A) Tree showing phylogenetic analysis of proteins encoded by a sampling of aminoacyl-tRNA synthetase genes from human (Hs) and *Ciona robusta* (KH gene models). See methods for details and supplemental sequences file for protein sequences used. B) Protein domain analysis diagram of *Ciona robusta* NARS1 from SMART (Letunic et al. 2021) showing its predicted domains. Dashed lines indicate exon-exon junctions.



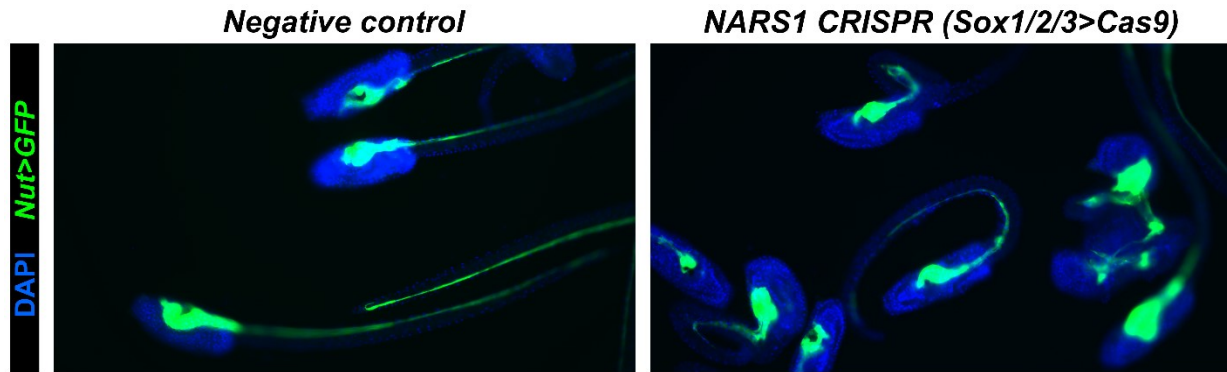
**Fig. S4. Indel plots for all sgRNAs tested.**

A-D) NGS indel validation plots (including negative controls) for all the sgRNAs tested in this study. No amplicon was obtained for the third *NARS1* sgRNA nor the *NARS1* negative control. Blue arrows indicate CRISPR-generated indels, red asterisks indicate naturally occurring indels.



**Fig. S5. Replicate of *Vamp1/2/3* CRISPR.**

Independent replicate of papilla-specific *Vamp1/2/3* CRISPR in *Ciona* larvae. Embryos were electroporated with 40  $\mu\text{g}/700 \mu\text{l}$  *Foxc*>*Cas9* and gene-specific pairs of sgRNA vectors (40  $\mu\text{g}/700 \mu\text{l}$  each sgRNA vector). Negative control embryos were electroporated with 40  $\mu\text{g}/700 \mu\text{l}$  *Foxc*>*Cas9* alone. Tail retraction was scored at 48 hours post-fertilization without screening for mCherry+ individuals.



**Fig. S6. Neurectoderm-specific knockout of *NARS1* impairs neurulation.**

Negative control larvae showing normal neural tube and tail morphogenesis, compared to *NARS1* CRISPR larvae. *Nut>Unc-76::GFP* (green) labels the central nervous system. Nuclei counterstained by DAPI (blue). See text for quantification, and for detailed amounts of plasmids used. For approximate sense of scale, larvae can be compared to that in Figure 4C.

**Table S1.**

[Click here to download Table S1](#)

**Table S2.** Approximate costs of CRISPR course activities (based on USA pricing). Price for custom sgRNA plasmid (\*) can be much lower if using cloning methods described in Stolfi et al. 2014 or Gandhi et al. 2018. Costs of basic reagents for embryo dechorionation, electroporation, fixing, etc. not included.

<b>Reagent or service</b>	<b>Supplier</b>	<b>Approx. price per sgRNA</b>
Custom sgRNA plasmid	Twist Bioscience	\$120*
Maxiprep	Macherey-Nagel (Takara)	\$9
Sanger plasmid sequencing	Eurofins	\$6
Whole-plasmid sequencing	Plasmidsaurus	\$15
Primers for NGS assay	Eurofins	\$6
Amplicon sequencing	Azenta	\$84



## Supplemental sequences file – Johnson et al. 2023 (Using CRISPR/Cas9 to...)

>Nut -1155/-1 (Nut promoter based on Shimai et al. 2010)  
(Sequence of the Nut driver based on genome assembly, not verified)  
atctgttctaggaatcttttaactcggcgagcctgtgttttcttttttagactataataaccctccaataac  
agggttaagatgcatgcgaaaaaacgattgattttgtgcatataaaaatatttataatagtctggggttat  
gtgagtgaatagctggttataatctaactgcagccgaaatacgtgctctttgcagaaaggaaaatccat  
ttctcgtgtattgcaacatacgggtatagtaggtttagatgggtcacctttaccacaaataacatccaat  
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ttaaataatgctttgtttactacaaaatgggacgggaaaataaaaataaaaagggtgtcctatcttcccccat  
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tggtgtatcatcttaccatcctgctacaaatataaacaatttcatgataagaaaatgcacatgtttgt  
tatgccgcgtgttggtgtacagttctggttaggatttcagaacacacacagaaaatcggctcgtgcctgc  
tgctgcaactagtgcgacacctcaataattatgagactgcctcagtgcatcctatcaacgtgttttgcg  
ctcgtatcacccaaaagcgtgcctcgtgctgcctacacgggtgtggctacataaaaacgtcgcgtgtttt  
actgttccagttcgtttttgtattcgtggaaataggttcgcattttttattaagaaagagtttggttttc  
gaggtcactgaattgcaattagaaggcatttaatatagtagcactaggacactctattcactgcgttgctc  
aatccatgcgaaacaacaaaataagtcgcaagacatgctgtgcgtgtattgtttttttaaaccctcgc  
ctttgtgaaaatctggttgattatcttctgaccagttttacagtttaataacgactgtgcttcagttt  
ttgttagtattgagttgtacactatatcaacaac

### sgRNAs used in this study:

TH.4.114

**GCTGGAATCAATTGGTTGCA (G+N19)**

TH.4.140

**GAATTGTTAACGGGACTGAA (G+N19)**

TH.5.44

**GCTGAGCTTGAATTGTGCAG (G+N19)**

Vamp.3.49

**GGTCCAGCTGCACCAACTGG (G+N19)**

Vamp.4.26

**GGACATCATGAGGACAAATG (G+N19)**

Vamp.4.93

**GGGCAGATGCATTACAGCA (G+N19)**

NCS1.1.32

**GGCAGCAAACCTGAGCGCAG (G+N19)**

NCS1.2.43

**GTGGTATCGAGACTTTATGA (G+N19)**

NCS1.2.56

**GTTATGAAGGATTGTCCCAA (G+N19)**

NARS1.4.25

**GAGAACAAGAAGATGCGGAG (G+N19)**

NARS1.7.86

**GATAGATACAGTCCTCAACC (G+N19)**

NARS1.7.135

**GACAGACATCTAGTTATCCG (G+N19)**

Pou4.3.21 (Johnson et al. 2023)

**GCTGAGTGGTGGAAAGCGGG (G+N19)**

Pou4.4.106 (Johnson et al. 2023)

**GAGGATGGAAATGATTCGGG (G+N19)**

*Primers for amplicon sequencing to measure mutagenesis efficacy*

Gene + exon	Forward primer	Reverse primer
TH exon 4	ACACTAATTA AAAACGTTGCCG	CTATATGTGCCAATAGTGCC
TH exon 5	TGGGCACTATTGGCACATA	ACATAAATGACGGTAGAATACCC
Vamp1/2/3 exon 3	GCACTTAAACCTTATATCCCTG	CGATTTTTGACCCGAAGAACA
Vamp1/3/4 exon 4	AGAAACCATGGTGTGCCTC	ATAAGCGTCCACCATTTC
NCS1 exon 1	GTGATCTTTGTAGGTCTTCTTT	AGTTAGAAACTAAACGACCGC
NCS1 exon 2	CTGGTTGCTCGCTATAAGTAA	CGTATTCTTGCGACTCTTATAGG
NARS1 exon 4	GGCTACCCTGAATAATCAAACTT	TTAGCCGTTGTACTGAAGTCC
NARS1 exon 7	GTTGGTATGCCCTAATTACC	CAAATCACCTCATAATATCCTCG

**Sequences for Vamp1/2/3 phylogenetic tree:**

>KH.C1.165.v3.A.SL2-1 (Vamp1/2/3)

MSGYDAVNPNSYGGTSNYGGPAAPTGGSSQASKKLQQTQAQVDDVVDIMRTNVDKVLERDQKLSELDDR  
ADALQQGASQFETQAAKLRKYWWKNCKMWAIIIVILVIIIIIVVSVVTQTQKKT

>KH.C8.1.v3.A.SL2-2

MVKLFSLSLFYKGESCVIPLIAEYDTSFSFFQRKSVQEFLSFTSKIITERTHPGMRQSVKEQEHLCHVY  
VREDRLAAVLISDEDYPRRVAFTVLTQVCEDFAKKFPPELNNPAPMNIQFEGVREMLCKYQNPKEADSM  
SRVQAEDELTKIVLHGTMESLLQRGEKLDLVAKSDQLSSQSKMFYKQIKIHKHDKHLWKKCTIAYRAN  
FHLV

>KH.C14.274.v1.B.ND1-1

MTILFSIIARGTTVLARYASCAGNFQEVSEQILSKITADDAKLTYSHGSYLFHYICDDRIVYMAITEDDF  
ERSKAFRYLSDIRKKFQSTYGNVHTALPYAMDTDFARVLMVQMKRFSNEEPENKVEEVQDQLNDLKI  
MVKNIDSIANRGENLNLVLDKTEDLSESAVTFKKQSTTLRRLWKNVKITVILVIVAIIVLYFIICAAC  
GGMNWRPCVHQSNPNKTLTLT

>KH.L57.23.v2.A.ND1-1

MTDNLVYLCAADKEFRRIPYLFLEELKKQFCSSGSLQRTTGASAFEFNRDFRNILSGIMDDFNKGKG  
DQLSTMQNQVGEVTGIMRQNIKVIKIERGDKLDDLDKTEDLQAGAATFKVTAKRIQRKYFWQNKMLII  
IVIVLIIITLIVLFATGTI

>KH.C8.46.v1.A.ND1-1

MTRYNKGKNTERVALDDYESDDDFFLKGPSSASDKVKRVQSQVNEVVDVMQTNIGKVLERGDKLEDLQD  
KSESLADSATHFNTHATRLRKKMWWKDMRTKIIIGVLLLMILIIIIIVSIAVKNKGS

>VAMP1-Hs

MSAPAQPPAEGTEGTAPGGGPPGPPNMTSNRRLQQTQAQVVEEVVDIIRVNVDKVLERDQKLSELDDRAD  
ALQAGASQFESSAAKLRKYWWKNCKMMIMLGAICAIIVVVIVYFF'T

>VAMP2-Hs

MSATAATAPPAAPAGEGGPPAPPNLTSNRRLQQTQAQVDEVVDIMRVNVDKVLERDQKLSELDDRADAL  
QAGASQFETSAAKLRKYWWKNLKMIIILGVICAIILIIIIIVYFST

>VAMP3-Hs

MSTGPTAATGSNRRLQQTQNVDEVVDIMRVNVDKVLERDQKLSELDDRADALQAGASQFETSAAKLRK  
YWWKNCKMWAIGITVLVIFIIIIIVVVSS

>VAMP4-Hs

MPPKFKRHLNDDVDTGSKSERRNLEDDSDDEEDFFLRGSPGPRFGPRNDKIKHVQNQVDEVIDVMQEN  
ITKVIERGERLDELQDKSESLSDNATAFNSRSKQLRRQMWWRGCKIKAIMALVAAIILLVIIILIVMKYR  
T

>VAMP5-Hs

MAGIELERCQQQANEVTEIMRNNFGKVLERGVLKLAELQQRSDQLDMSSTFNKTTQNLAQKKWENIRYR  
ICVGLVVVGVLLIILIVLLVVFLPQSSDSSAPRTQDAGIASGPGN

>YKT6-Hs

MKLYSLSVLYKGEAKVLLKAAYDVSSFSFFQRSSVQEFMTFTSQLIVERSSSKGTRASVKEQDYLCYVYV  
RNDSLAGVVIADNEYPSRVAFTLLEKVLDEFKQVDRIDWPVGSPATIHYPALDGHLSTRYQNPREADPMT  
KVQAELETKIILHNTMESLLERGEKLDLVSSEVLGTQSKAFYKTARKQNSCCAIM

>VAMP7-Hs

MAILFAVARGTTILAKHAWCGGNFLEVTEQILAKIPSENNKLTYSHGNYLFHYICQDRIVYLCITDDDF  
ERSRAFNFLNEIKKRFQTTYGSRAQTALPYAMNSEFSSVLAQKHHSENKGLDKVMETQAQVDELKGMIM  
VRNIDLVAQRGERLELLIDKTENLVDSSVTFKTTSRNLARAMCMKNLKLTIIIIIIVSIVFIYIIIVSPLCG  
GFTWPSCVKK

>VAMP8-Hs

MEEASEGGNDRVRNLQSEVEGVKNIMTQNVVERILARGENLEHLRNKTEDLEATSEHFKTTSQKVARKFW  
WKNVKMIVLICVIVFIIILFIVLFATGAFS

>SNAP25-Hs

MAEDADMRNELEEMQRRADQLADESLESTRMLQLVEESKDAGIRTLVMLDEQGEQLERIEEGMDQINKD  
MKEAEKNLTDLGKFCGLCVCPCNKLKSSDAYKAWGNNQDGVVASQPARVVDEREQMAISGGFIRRVTND  
ARENEMDENLEQVSGIIGNLRHMALDMGNEIDTQNRQIDRIMEKADSNKTRIDEANQRATKMLGSG

### Sequences for NCS1 phylogenetic tree:

>KH.C1.1067.v1.A.SL1-1 (NCS1)

MGKSGSKLSAEDAQTLQTTTHFDKKEIQKWYRDFMKDCPNGYLKKEEFQKVYQQFFPKGNPSKFANFVFN  
VFSDSKDGFITFKEFISALSVTSRGNLDEKLDWAFNLYDLHDGDFITREMLNIVDAIYSMVGNAMDLPE  
DENTPEKRVNKIFCQMDQNKDGKLTKDEFREGSKCDPYIVKALSAGLGAESCPS

>KH.C9.113.v1.A.ND1-1

MGNQESTQSLSEHEIARLSAETNFTPAEVAQWYKGFKRDCPSGKFTMARFQAI AEGFFPAGDAENFTKFI  
FNGMDLEEDASLNFESEFIKVTSLLAGRTKEEKLKWAFHVVRDIDHTGFITKEKMQLVEDSVFSMIANHVPL  
PDDENTAERTEKLYNLI EKDQDGKVTVDQFKVGLNQDRDIVNALS LHDTVQ

>KH.S534.4.v2.A.SL3-1

MGCAKSKTRDEDCKQLES LNFTPEELKKCYDDFQQESTSGKMDR TKFDEFYKFFNRDPRFVDHLFRTF  
DFNNDGFINFREFVCGLSITTRGTPEEKLTWTFNVYDVNNDGTITRDEMLQIMRAIYAMNGISEPEQLKS  
GSDAFEGLD SNGDGLISVAEFVKGVKRDERLLEFLQRTIDVQQK

>KH.S534.6.v1.A.nonSL2-1

MGQQHPSLKPRMLDDLMRMSDFSADELKIWYNEFSKDSVSGFLSKNDFIKIYKGLFPHGDASGFADHVFR  
TFDANKDGV LNFRFVIGLSLTMKGPLDDKLYWAFKLYDVGNGFVTKDEMNEIIAVSNAIFTVCCDTDT  
DDD VIMSGREAFKTM DTEDGKVS WGEFRKGIHSNRVFM L IVEAKMKNAGVTSR

>KH.C8.342.v1.A.SL2-1

MGKQNSKLPEDIRDLRAATEFNEHELQEWYKGF IKDCPSGNLTMDEFQKIYANFFPQGDASKFAAHVFR  
TFDSNGDSTIDFREFIVALSVTSRGNLEEKLKWAFSMYDCDNGIISRDEM LEIVRAIYKMGVAVMKMPE  
DESTPEKR TDKIFKQMDKNLDGSI SLEEFVEGAKKDPSIVRLLQCTPGSGGLGM

>KH.C9.274.v1.A.SL1-1

MGNRKSCLKPEVLEKLTQTKFTEAELHQWHKGF LHCPTGKLSYEEFQGIYRQFFPQGN SAKFAKL VFT  
TFDDNKDGTVEFEFFIIALS VTSRGTLD EKLHWAFQLYDLNDNGFITKDEMLNIVEAIFAMVGD AVNLP A  
EENTPQKRVEKIFKVM DKNKDGKLT KDEF L V GAKSDPSIVQALS IYDGLV

>NCS1-Hs

MGKSNSCLKPEVVEELTRKTYFTEKEVQQWYKGF IKDCPSGQLDAAGFQKIYKQFFPFGDPTKFATFVFN  
VF DENKDGRIEFSEFIQALS VTSRGTLD EKLHWAFKLYDLNDNGYITRNEMLDIVDAIYQMVGN TVELPE  
EENTPEKRVDRI FAMDKNADGKLT LQEFQEGSKADPSIVQALS IYDGLV

>NCALD-Hs

MGKQNSKLRPEVMQDLLESTDFTEHEIQEWYKGF LRDCPSGHL SMEEFKKIYGNFFPYGDASKFAEHVFR  
TFDANGDGTIDFREFIIALS VTSRGRLEQKLMWAFSMYDL DNGYISKAEMLEIVQAIYKMVSSVMKMPE  
DESTPEKRTEKIFRQMDTNRDGKLSLEEFIRGAKSDPSIVRLLQCDPSSAGQF

>HPCA-Hs

MGKQNSKLRPEMLQDLRENTFSELELQEWYKGF LKDCPTGILNVDEFKKIYANFFPYGDASKFAEHVFR  
TFD TNSDGTIDFREFIIALS VTSRGRLEQKLMWAFSMYDL DNGYISREEMLEIVQAIYKMVSSVMKMPE  
DESTPEKRTEKIFRQMDTNN DGKLSLEEFIRGAKSDPSIVRLLQCDPSSASQF

>HPCAL4-Hs

MGKTNSKLAPEVLEDLVQNTFSEQELKQWYKGF LKDCPSGILNLEEFQQLYIKFFPYGDASKFAQHAFR  
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NQDGLTPQQRVDKIFKMDQDKDDQITLEEFKEAAKSDPSIVLLQLQCDMQK

>VSNL1-Hs

MGKQNSKLAPVEMEDLVKSTEFNEHELKQWYKGFLLKDCPSGRNLNLEEFQQLYVKFFPYGDASKFAQHAFR  
TFDKNGDGTIDREFICALSITSRGSFEQKLNWAFNMYDLDDGDKITRVEMLEIEIAIYKMGVTVIMMKM  
NEDGLTPEQRVDKIFSKMDKNKDDQITLDEFKEAAKSDPSIVLLLQCDIQK

>Frq1-Dm

MGKKSSKLKQDITDRLTTDTYFTEKEIRQWHKGFLLKDCPNGLLTEQGFIKIYKQFFPQGDPSKFASLVFR  
VFDENNDSIEFEEFIRALSITSRGNLDEKLQWAFRLYDVDNDGYITREEMYNIVDAIYQMVGGQPQSED  
ENTPQKRVDKIFDQMDKNHDKLTLLEEFREGSKADPRIVQALS LGG

>Frq2-Dm

MGKKNSKLKQDITDRLTTDTYFTEKEIRQWHKGFLLKDCPNGLLTEQGFIKIYKQFFPDGDPSKFASLVFR  
VFDENNDGAIEFEEFIRALSITSRGNLDEKLHWAFLYDVDNDGYITREEMYNIVDAIYQMVGGQPQTED  
ENTPQKRVDKIFDQMDKNHDDRLTLLEEFREGSKADPRIVQALS LGG

>Efcab11-Cr (KH.C10.577.v1.A.ND1-1)

MAVSLRRLQTI FNHCDEDKKGFLNREDLKVAMLI LFGYKPSKYEIQQLLDDGEGNKKLMNFETFKSIMAA  
SKHDPDHEIRDI FRMFDT HCRGFLIFDDVKKAFHAVAPHISDATIRACCEEMCRESDGRI SYREFAEAMR  
HGHVHMEHHENYDHFNSNVCFS

### Sequences for NARS1 phylogenetic tree:

>KH.C12.45.v1.A.SL1-1 (NARS1)

MGDSVEQQVNKLSIGELYTSEKTGCDESGDGTEAKPFKTI LRAMMFHQSEPFPTLYVDSKEPNKKFDPIA  
KAQLKKQTKI FTAEKRKDVARKVREQEDAERREKNLEDAKKII I SEDKSLPAAKSIKIGAATAHRGQRVK  
VNGWSHRIRRQKTLMFIVLRDGSYQLQAVLNDQLCQTYNAVMLSTESTVCLYGVITPVMEGKSAPGGHE  
LICDYWELIGLAPPGIDTVLNQEADVDMVLDNRHLVIRGEQTSKILRVRSYLMQAFRDHYFERGYEVT  
PPCLVQTQCEGGSTLFLKLDFFGEQAYLTQSSQLYLETCLPSLGDVFCLAESYRAEQSRTRRHAEYTHVE  
AECAFITFNDLLDRLEDLICDVVDKLLKSPAGELIKDLNPGFVAPKRPFRRMDYAEAI VYLKEHDIKED  
GSYYEFGEDIPEMPERKMTDQINEPILLCRFPYKIKSFYMQKCKDNLELTESVDLLMPNVGEIVGGS MR  
DNHEELLEGYKSEGVDP SKYYWYTDQRKFGTCEHGGYGLGLERLLTWLTD RYHIRDVCLYPRFLERCCP

>KH.C7.539.v1.A.SL1-1

MADVENVPLGDDGKPM SKKALKKQKDAEKAAKKAQRQQEQADAQQKADADDVSKDLYGDMVMIQSSEKP  
DRCLQPIGDIGPNLAEKNIWVRGRRLHTSRGTGKQCFTVLRHQOSTIQALIFVGEKISKQMVKFCANISKE  
SIVDVYGVVKKVEQKVESCTQDDVEIHIGKIFVVSKAAPQLPLLI EDASRPELEDGKNEEGGRPRVNLDT  
RLDNRI LDLR TTTKQAI FRLEAGVCKLFRD TLTAKGFC EIHTPKIISAASEGGANVFTVSYFKTNAFLAQ  
SPQLYQMAIAADFEKVFTVGAVFRAEDSNTHRHLTEFVGLDLEMAFYHYHEVLQVIGDMFISIFKGLR  
ESYQKEITTVARQYPAEPFKFLEPTLILQYPEAVKMLNDAGVEMQDNEDLSTPSEKLLGRLVRQKYD TDY  
YILDKYPLEVRPFYTMPDPDNPKWSNSYDMFMRGEEILSGAQR IHDPEFLSERAKAHGIDLKTI EAYIDS  
FKYGCPPHAGGGIGMERVVMLYLGLHNIRSSSLFPRDPKRLTP

>KH.C2.670.v1.A.ND1-1

MFRLCKLSYSTVTTALKRFKIKDVVNGEAVSQNGWIQGWVQSIRKHKNFMFVDIVDGTSLNPLQVVLPSH  
MFNSQLQNGAAV SANGTFVETDLNVNKIEMVCEEIQVVGPCDPETY PFLKHTPHDMQHLRSYPHLRMRRT  
SMINAFKLRNQLLEMLFHEFFQNEGFTHVHTPIITLSDCEGAGELFSIKSDTSTNTDMENSENHFFNKPAY  
LTVSGQMHL EACAMSLG SVYTLSPVFRAEHGISRKHLS EFRMLEVEVAFTQDLHEILDIEDSIKFCIER  
VQSVPLKFFDDGYKDIPPQHHKAVKNATCSNYVRIPYSEALEILQKNSHKLTTAAPVWGDDFNTDQESFL  
VKHFGLTPVFIINFPKVAKPFYMYANNNDNTVA AVDLIFPFCGEICGGS LREHRLELLQDSLRTSGLNEK  
DYEWYDLRRFGSAPHGGYGLGFDRLVHFLLGTRNIKDAVFPFRTPHSCPL

>KH.C3.437.v2.A.SL1-1

MADNGDCEEKIKSKNEMKRQLKAQQKAKEKAEKAAKALENAPKEVKPEKTASKEEETLDPNQYLQIRKNT  
ITTLRQNNIEPYPHKFHVSISLSDYVEKYNNIEVGSHLNDQQVSIAGRIHAKREAGPKLIFYDVRGDGVK  
LQVMANSKMYSSSEEAYQEINERTRRGDIIGVIGHPAKTKKGELSIVPNTIEILSPCLHMLPHLHFGLKDK  
ETRYRQRYLDLIMNDQTRQKFITRAKIIISYIRSFQMGFLEVETPMNMVAGGATAKPFITHHNDLDM  
LYMRVAPELYLKLMLVVGGLDRVYEIGRLFRNEGIDMTHNPEFTSCEFYMAYADYEDLMKISSETLISGMVK  
QICGSYKLTYPDGPDSEGYEVDYTPPFRRLRMLPDLERLTGMEFPKPTTELHTAGAARLDEICVKLGVE  
CPPPRTTARLLDKLVGDYLEVNCINPTFITHEPEIMSPLAKWHRSIKGLTERFELFVNKEICNAYTELN  
DPMIQRQRFEQQALDKAAGDDEAQMVDENFCTALEYGLPPTGGWGMGIDRLTMFLTDSNNIKEVLLFPAM  
KPDDQVPKKAEDSPST

>KH.C11.248.v1.A.SL2-1

MLKLFVRVVRPILFKFRPKFNPTVICIVTKQFTSNSAIPQVVQSIKNEKLPNSKFSYRSHTCGELRIKNAG  
EAVSLCGWLEYKRKDFLVRDSFGSVQILITGYEDKNQTFQNVKPEVVKVTGKVTRPEGQANPNMITG  
EIEIIPDNIEILNKCKFLPFRVQKFAGKGERIRQKYRYLDIRGDIQQLRRLRSKVLKMRKSLEENCFV  
EVETPTLFHPTPGGAREFIVPSSSNPGKFYSLPQSPQQLKQLLMIGGVDRYYQFARCYRDEPFKRDQVE  
FTQLDIEMSFVDVNDVIKLSSEVIKSSWPRKFSDESFKLITYDDAMSKYGSDDKPDTRFGMLLHDITTFIP  
DTLVELFQGDHSDPVNIQAVVVENAQKHFSSKFRRYIGSRMTSAIDDPSPYALLGLVNGQWSGYHKNTNI  
GSMVTKQLVDHLQLGGSDIVVVSWEKRSQKTLGALRRIISRIFDEISVKHRNVLDLQFLWVVDVDFLFE  
ADPYTGSCLTVHHPFTSPHPDDVHLLVVDPLKVRSAQAYDLVLNGNEVGGGSIRIHDSKLQRQIFTLNLD  
FTELSYLLEALNSGAPPHGGIAFGIDRIMSILCGTETIRDVIAFPKNSRGEDLMTNSPISINSNILKEFH  
IKTNVSDK

>YARS1-Hs

MGDAPSPEEKHLITRNLQEVLGEEKLKEILKERELKIYWGATTTGKPHVAYFVPMISKIADFLKAGCEVT  
ILFADLHAYLDNMKAPWELLELRVSYENVIKAMLESIGVPLEKLFKIKGTDYQLSKEYTLDVYRLSSV  
TQHDSSKAGAEVVKQVEHPLLSGLLYPGLQALDEEYLVDAQFQGGIDQRKIFTFAEKYLPALGYSKRVHL  
MNPMPGLTGSKMSSSEESKIDLLDRKEDVKKLKKAFCEPGNVENNGVLSFIKHVLFPLKSEFVILRD  
EKWGGNKTYTAYVDLEKDFAAEVVHPGDLKNSVEVALNKLLDPIREKFNTPALKKLASAAYPDPKQKPM  
AKGPAKNSEPEEVI PSRLDIRVGKIIITVEKHPDADSLYVEKIDVGEAEPRTVVSGLVQFVPKEELQDRLV  
VVLGNLKPQKMRGVESQGMLLCASIEGINRQVEPLDPPAGSAPGEHVVFVKGYEKGQPDEELKPKKKVFEK  
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>DARS1-Hs

MPSASASRSQEKPREIMDAEDYAKERYGISSMIQSQEKPDRLVLRVRLDTIQKADEVVWVRARVHTSR  
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INKGFVEIQTPKIIISAASEGGANVFTVSYFKNNAYLAQSPQLYKQMCICADFEKVFVSIQVVFRAEDSNTH  
RHLTEFVGLDIEMAFNYHYHEVMEEIADTMVQIFKGLQERFQTEIQTVNKQFPCEPFKLEPTLRLEYCE  
ALAMLREAGVEMGDEDDLSTPNEKLLGHLVKEKYDITDFYILDKYPLAVRPFYTMPDPRNPKQSNSYDMFM  
RGEEILSGAQRIHDPQLLTERALHHGIDLEKIKAYIDSFRTGAPPHAGGGIGLERVTMLFLGLHNVRQTS  
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>DARS2-Hs

MYFPSWLSQLYRGLSRPIRRTTQPIWGSLYRSLQSSQRRIPFSSFVVRTNTCGELRSSHLGQEVTLCG  
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IKVKTAELLNACKKLPFEIKNFVKKTEALRLQYRYLDLRSFQMQLNRLRSQMVMKMREYLCNLHGFDI  
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IDIEMSFVDQGTGQSLIEGLLQYSWPNDKDPVVVFPPTMTFAEVLATYGTDKPDTRFGMKIIDISDVFRN  
TEIGFLQDALSHPGTVKAICIEGAKYLKRDIESIRNFAADHFNQEIILPVFLNANRNWNSPVANFIME  
SQRLLELIRLMETQEEDVLLTAGEHNKACSLGKLRLECADLLETGRVLRDPTLFSFLWVVDVDFLFLPK  
EENPRELESAAHPFTAPHPSDIHLLYTEPKKARSQHYDLVLNGNEIGGGSIRIHNAELQRYILATLLKED

VKMLSHLLQALDYGAPPHGGIALGLDRLICLVGTGSPSIRDVIAFPKSFGRGHDLMSTPDSVPPPEELKPYH  
IRVSKPTDSKAERAH

>NARS1-Hs

MVLAELYVSDREGSDATGDGTKEKPFKTGLKALMTVGKEPFPTIYVDSQKENERWNVISKSQKNIKKMW  
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LNRLLEDLVCDVVDRI LKSPAGSIVHELNPNFQPPKRPFKRMYSDAIVWLKEHDVKEDGTFYEFGEDIP  
EAPERLMTDTINEPILLCRFPVEIKSFYMQRCPEDSRLTESVDVLMPNVGEIVGGSMRIFDSEELLAGYK  
REGIDPTPYWYTDQQRKYGTCPHGGYGLGLERFLTWILNRYHIRDVCLYP  
RFVQRCTP

>NARS2-Hs

MLGVRCLLRSVRFCSSAPFPKHKPSAKLSVRDALGAQNASGERIKIQGWIRSVRSQKEVLFVHNDGSSL  
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QYPHFRCRTNVLGSILRIRSEATAAIHSFFKDSGFVHIHTPIITSNDSEGAGELFQLEPSGKLVPEENF  
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KATTMMVLSKCPEDVELCHKFIAPGQKDRLEHMLKNNFLIISYTEAVEILKQASQNFFTTPEWGADLRTE  
HEKYLKHCIGNIPVVFVINYPLTLKPFYMRDNEDGPQHTVAAVDLLVPGVGELFGGGLREERYHFLEERLA  
RSGLTEVYQWYLDLRRFGSVPHGGFGMGFERYLQCILGVDNIKDVIPFRFPHSCLL

>KARS1-Hs

MAAVQAAEVKVDGSEPKLSKNEIKRRLKAEKKVAEKEAKQKELSEKQLSQATAAATNHTTDNGVGPEEES  
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KLIFYDLRGEVGLQVMANSRNYKSEEEFIHINNKLRGDIIGVQGNPGKTKKGELSIIPEYITLLSPCL  
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KPFITYHNELDMNLYMRIAPELYHKMLVGGIDRVYEIGRQFRNEGIDLTHNPEFTTCEFYMAYADYHDL  
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KILDDICVAKAVECPPRRTTARLLDKLVGEFLEVTCINPTFICDHPQIMSPLAKWHRSEGLTERFELFV  
MKKEICNAYTELNDPMRQRQLFEEQAKAKAAGDDEAMFIDENFCTALEYGLPPTAGWGMGIDRVAMFLTD  
SNNIKEVLLFPAMKPEDKENVATDTLESTTVGTSV

Cr: *Ciona robusta*

Hs: *Homo sapiens*

Dm: *Drosophila melanogaster*