

Supplementary Materials

Choline dehydrogenase interacts with SQSTM1 to activate mitophagy and promote coelomocyte survival in *Apostichopus japonicus* following *Vibrio splendidus* infection

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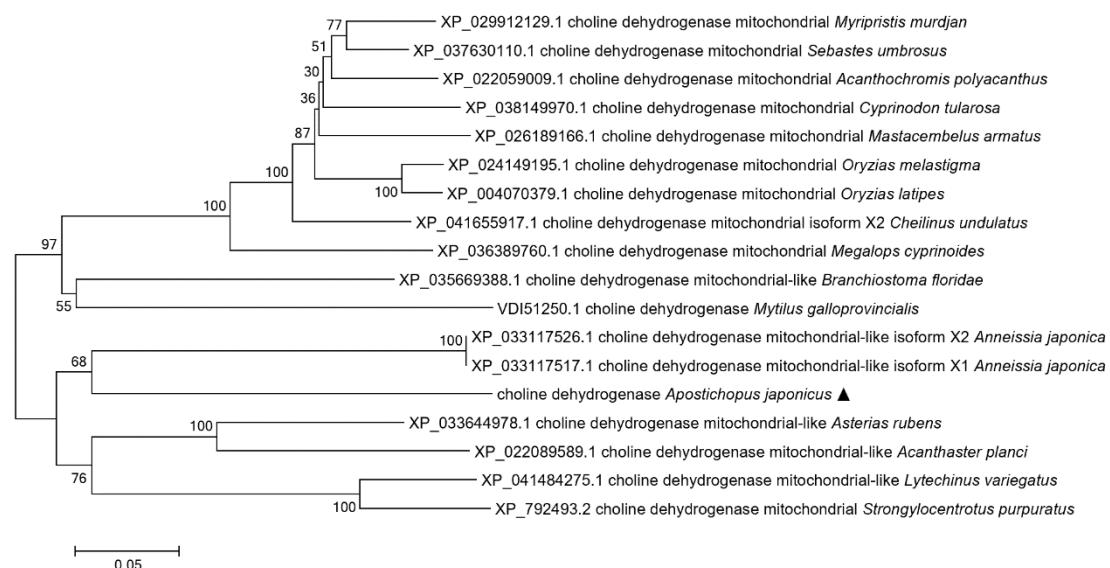
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<i>Apostichopus japonicus</i> ▲	MESFLJFVFLACSVLHDLTKMAFQVTNTVLKSLNMQALRKG.	LMRNASSLSETHIIVG GS	61
<i>Asterias rubens</i>	. MTCRSGLMNRWGLSLWQNQLRLPVDGCCRHRAFIL.	VSTTVRKDISETHIIVG GS	62
<i>Lytechinus variegatus</i>	.. MESMKARQGLQAVKQLAIPNSCRSQ., RLFSVIIR.	GEDEETHIIVG GS	47
<i>Strongylocentrotus purpuratus</i>	... MNLSLARARQGLHAKVHLISFPASSSSQQSRPFVCQSN.	GSJDEETHIIVG GS	53
<i>Acanthaster planci</i> MWSRITALVRSLOWRWTMPWQINILRTHIIHSICR.	VQADQSEYETHIIVG GS	52
<i>Cheilinus undulatus</i> MFLATRGPTGARNQRIGOFFFSITLNHCROLSAKATHRN.	ASPPAAFNQKTPSYSYIIVG GS	63
<i>Mastacembelus armatus</i> MFLSLATVQAGRAOR . FSGTGTGRTLKNGGFFTCSPKHILP1PNIRQQ.	SSPPAANGKTPSYSYIIVG GS	55
<i>Oryzias melastigma</i> TFGSPSLKHRYCFSASPDQNGKTPSFSYVVVG GS	83	
<i>Myripristis murdjan</i> MSLSLVGLGRAGARL . GVSRTWGR1EEEGRCPLACAYAHTVPRTR.	NWHTPSFSPARCVSTAACRSTSSPVANGKTPSISYVVG GS	89
<i>Oryzias latipes</i> MFLSLATVAPAGARCR . FSGJALGRTRLSGRWFLTCSPKHILP1RHEE.	RFGSPSPFHRYCFSASAPDRNGRTPSFSYVVVG GS	83
<i>Megalops cyprinoides</i> MFLT1LANRQVRNL . RVIWAQK RCFSASAARCS.	ASPPAANGKTPSYSYIIVG GS	56
<i>Acanthochromis polyacanthus</i> MSLSLATLQGTGAWR . VVTGTRGRMRMNSCFITRLPDCHSVPETR.	KTRSFPANICRCFTTASPASQKTPSISYIIVG GS	80
<i>Cyprinodon tularosa</i> MSLSLAMGRAVLPR . LVTRGRWKTEFLKDGRFTTASHWN.	TSSPSAIIQKTPSYSYVVVG GS	61
<i>Sebastes umbrosus</i> MSLSLATSGQTGARR . VVTATRGR1MRDGRLLSCLSQSPVKDRNMGSFSRSSNQYRCFSATAAHWSASPSTANGKTPSYSYIIVG GS	90	
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Supplementary Figure S1 Multiple sequence alignment of AjCHDH

Multiple sequence alignment of deduced amino acid sequences of CHDH proteins from 14 vertebrate and invertebrate organisms. Consensus residues are shaded with a threshold of >50% identity using DNAMAN software. Identical residues are indicated in black, similar residues (>75% identity) are indicated in dark gray, and similar residues (>50% identity) are indicated in light gray. AjCHDH shared the highest similarity with CHDH from *Asterias rubens* (71.14%) and lowest similarity with CHDH from *Oryzias melastigma* (63.06%), with the remaining somewhere in between. Numbers on right indicate amino acid positions. Identical and similar residues are indicated below alignments with letters.



Supplementary Figure S2 Phylogenetic tree based on 18 amino acid sequences of the CHDH family from distinct organisms

Tree was obtained by bootstrap analysis using the neighbor-joining method. Numbers on branches represent bootstrap values for 1 000 replications. CHDH proteins clustered into two major groups, namely, vertebrate CHDH and invertebrate CHDH.

Supplementary Table S1 Primers and siRNA sequences used in this study

Primer name	Primer sequence (5'-3')	Used for
AjCHDH-qF	CAATGCGATGTGCTATGTC	real-time PCR
AjCHDH-qR	ACCCAAATCCTCCTGTT	
SQSTM1-qF	CAGAAGACCAGTAATGGAAGCGG	real-time PCR
SQSTM1-qR	AACCTCCTCGTCTGTGAAACCC	
LC3-qF	AGACACAGTTGCCTTGTTGGAC	real-time PCR
LC3-qR	CCATCCTCATCTTCTCTTCCCG	
Ajβ-actin-qF	CCATTCAACCCTAAAGCCAACA	real-time PCR
Ajβ-actin-qR	ACACACCGTCTCCTGAGTCCAT	
si-AjCHDH (sense)	GGCGGUAGUUAAUAAAATT	siRNA interference
si-AjCHDH (antisense)	UUUAUUAUUAACUACCGCCTT	
si-NC (sense)	UUCUCCGAACGUGUCACGUUTT	siRNA interference
si-NC (antisense)	ACGUGACACGUUCUGGAGAATT	
AjCHDH-F	ATGGAATCTTTCTATTGTGT	ORF
AjCHDH-R	TTATCTATCCGTTCTAATG	
pET-28a-AjCHDH-F	GGATCCATGGAATCTTTCTATTGTGT	Recombinant expression
pET-28a-AjCHDH-R	GCGGCCGTTATCTATCCGTTCTAATG	
pCMV-N-mCherry-CHDH-F	AAGCTTATGGAATCTTTCTATTGTGT	Recombinant expression
pCMV-N-mCherry-CHDH-R	CTCGAGTCTATCCGTTCTAATG	
pEGFP-N3-SQSTM1-F	CTCGAGATGTCTGTGACAGTTAAAGCATAT	Recombinant expression
	C	
pEGFP-N3-SQSTM1-R	GTCGACGCGTTCTGAGGGAGGTTAGG	
pCMV-Flag-2C-SQSTM1-F	GGATCCATGTCTGTGACAGTTAAAGCATAT	Recombinant expression
	C	
pCMV-Flag-2C-SQSTM1-R	CTCGAGTTAGTTCTGAGGGAGGTTAGG	
pIRES2-EGFP-LC3-F	CTCGAGATGGGATCCGATTCTCAAAGCA	Recombinant expression
pIRES2-EGFP-LC3-R	GAATTGATTACCGAACATTCTTGCG	