

# Starfish Apaf-1 activates effector caspase-3/9 upon apoptosis of aged eggs

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## Supplemental Materials and Methods

**Cytochrome *c* assay.** Samples were incubated with 10  $\mu$ M horse heart cytochrome *c* (Sigma) and 1 mM dATP (Invitrogen) final concentration for 60 min at 20°C. DEVDase activity was measured at indicated time by using Ac-DEVD-MCA.

**Immunoprecipitation.** Immunoprecipitation was performed according to the method described previously (Hiraoka *et al.*, 2004). Protein A Sepharose 4B (Sigma) was pre-adsorbed to the anti-caspase-3/9 antibody or rabbit IgG (Amersham). 20  $\mu$ L of cell-free preparation was added to 2  $\mu$ L of Sepharose beads, and incubated at 4°C for 120 min. The 2  $\mu$ L pellet of Sepharose beads was used for western blotting.

**a**

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caspace-3/9_A.pectinifera 1 GDSNQEPMDSLPSKSYIASPTDELKAPWPGTTPADLTTOEMP-----MAPLPLADQ
caspace-3_B.taurus 1 -----NTENSVDSSKSIKT-----SEKILHGSKSMDSGCI-----
caspace-3_M.musculus 1 -----NNKTSVDSSKSIINN-----FEVHTIHGSKSVDSGCI-----
caspace-3_R.norvegicus 1 -----NNETSVDSKSIINN-----FETHTIHGSKSMDSGCI-----
caspace-3_H.sapiens 1 -----NTENSVDSSKSIKKN-----LEPKILHGSKSMDSGCI-----

caspace-3/9_A.pectinifera 54 DASDVEDVYQMKSKPRFIAVTINNKHFRT--MNRKCTDIDGRNINHVFERKGFPTLYR
caspace-3_B.taurus 30 ---SLEESYKMDYFEMGICILINNNKFNENTGMACRSCTDDVDAANLRETFMNRKVEVRIRK
caspace-3_M.musculus 30 ---YLDSSYKMDYFEMGICILINNNKFNKSTGMSSRSCTDDVDAANLRETFMGLKRYEVRNRK
caspace-3_R.norvegicus 30 ---YLDSSYKMDYFEMGICILINNNKFNKSTGMSSRSCTDDVDAANLRETFMGLKRYEVRNRK
caspace-3_H.sapiens 30 ---SLDNSYKMDYFEMGICILINNNKFNKSTGMSRSCTDDVDAANLRETFRNLRKVEVRNRK

caspace-3/9_A.pectinifera 111 TDOTGEEEMOQIILRDAASHNHOTFDCLFILAILSHGVEGATYGVDERIVKIEHITTYFEGGR
caspace-3_B.taurus 87 NDILTCKEMLELMSNVSRKEDHSKRSSFICVILLSHGEEGTFIFGTNG-PVNTLKKLASFFRGGDY
caspace-3_M.musculus 87 NDILTREDILELMSVSRKEDHSKRSSFVFCVILLSHGDEGVIFGTNG-PVLEKLLTSFFRGGDY
caspace-3_R.norvegicus 87 NDILTREDILELMSVSRKEDHSKRSSFVFCVILLSHGDEGVIFGTNG-PVLDLKKLTSFFRGGDY
caspace-3_H.sapiens 87 NDILTREDIIVLELMSVSRKEDHSKRSSFVFCVILLSHGEEGTFIFGTNG-PVDLKKLITFFRGGDY

caspace-3/9_A.pectinifera 171 CPTLACKPKLFLFOACRGERFDGGHEATDSKAVAPSDANAEOQDSIEPLSDEELAGRMLER
caspace-3_B.taurus 146 CRSLTCKPKLFLFOACRGETELDCGIE-ADSGA-----EDDM-----
caspace-3_M.musculus 146 CRSLTCKPKLFLFOACRGETELDCGIE-ADSGT-----DEEM-----
caspace-3_R.norvegicus 146 CRSLTCKPKLFLFOACRGETELDCGIE-ADSGT-----DDM-----
caspace-3_H.sapiens 146 CRSLTCKPKLFLFOACRGETELDCGIE-ADSGV-----DDDM-----

caspace-3/9_A.pectinifera 231 ELEDTDASNAIRSKLFSOSDMLLAFATVPGFVSWRNSERGSWFVQALSEVFLHANKED
caspace-3_B.taurus 181 -----AC-OKIIVVEADFLVAVSTAPGVYSWRNSRDCGSWFIQSLCAMLKLAHAKLE
caspace-3_M.musculus 181 -----AC-OKIIVVEADFLVAVSTAPGVYSWRNSRDCGSWFIQSLCAMLKLAHAKLE
caspace-3_R.norvegicus 181 -----AC-OKIIVVEADFLVAVSTAPGVYSWRNSRDCGSWFIQSLCAMLKLAHAKLE
caspace-3_H.sapiens 181 -----AC-HKIVVEADFLVAVSTAPGVYSWRNSRDCGSWFIQSLCAMLKQVADKLE

caspace-3/9_A.pectinifera 291 LLSMMTRVNNKVARAFESSS--GRNKOIPAPVTMLRKRKYFNPNGR
caspace-3_B.taurus 230 FMHILTRVNNRRIKVAIEYFSFSDSAFHAKKOIIPCTIVSMLTKKELVYVH--
caspace-3_M.musculus 230 FMHILTRVNNRRIKVAIEYFSFSDSAFHAKKOIIPCTIVSMLTKKELVYVH--
caspace-3_R.norvegicus 230 FMHILTRVNNRRIKVAIEYFSFSDSAFHAKKOIIPCTIVSMLTKKELVYVH--
caspace-3_H.sapiens 230 FMHILTRVNNRRIKVAIEYFSFSDSAFHAKKOIIPCTIVSMLTKKELVYVH--

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**b**

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caspace-3/9_A.pectinifera 1 GDSNQE---PMDTSLPSKSYIASPTDELKAPWPGTTPADLTTOEMP-----MAP
caspace-9_B.taurus 1 -----DPSKPSQGGKA--PVVILGEEELWPAKLRPEVLRPEVPRAVDAGSGGCTD
caspace-9_M.musculus 1 GLTAKEQRVVKLDPSQAVGNLTA--PVVILGEEELWPAKLRPEVLRPEVPRAVDAGSGCAHD
caspace-9_R.norvegicus 1 GLKSKEQKVVKLDPSQAVGNLTA--PVVILGEEELWPTRLRPEVLRPEVPRAVDAGSGCAHD
caspace-9_H.sapiens 1 -----SKPTLENLTA--PVVILRE-----IRKPEVLRPEVPRAVDAGSGGCTD

caspace-3/9_A.pectinifera 48 LPLADQDASDVEDVYQMKSKPRFIAVTINNKHFRT--MNRKCTDIDGRNINHVFERKGLG
caspace-9_B.taurus 48 VCPDRAKGNADLAVVYNADPCEGCLILINNVNFCRESGLRARTGSDIDCGRMKRRRFLLO
caspace-9_M.musculus 60 VCVPGKIRGHADMAVTLDSDFCEGCLILINNVNFCPSGGLCTRAGSNLDRDRKRRRRLWR
caspace-9_R.norvegicus 60 VCTPGKIERHADMAVTLDSDFCEGCLILINNVNFCPSGGLSTRIGSHVDCEKLRORRQWLR
caspace-9_H.sapiens 61 WGALESIRGNADLAVIILSMPCGEGCLILINNVNFCRESGLRRTRTGSDNIDCERLRRRSSLH

caspace-3/9_A.pectinifera 105 FTTLTKTDOTGEEEMOQIILRDAASHNHOTFDCLFILAILSHGVE-----GAIYGVDERIV
caspace-9_B.taurus 108 FMVEVKNDLTAKRMVLTALMOLARQDHSALDCCVVVILSHGCOASHLOFPFGAVYGTDCGCV
caspace-9_M.musculus 120 FMVEVKNDLTAKRMVLTALMEMAHRNRHALDCCVVVILSHGCOASHLOFPFGAVYGTDCGCV
caspace-9_R.norvegicus 120 FMVEVKNDLTAKRMVLTALMEMAHRNRHALDCCVVVILSHGCOASHLOFPFGAVYGTDCGCV
caspace-9_H.sapiens 101 FMVEVKNDLTAKRMVLTALLELAQDDHGRALDCCVVVILSHGCOASHLOFPFGAVYGTDCGCV

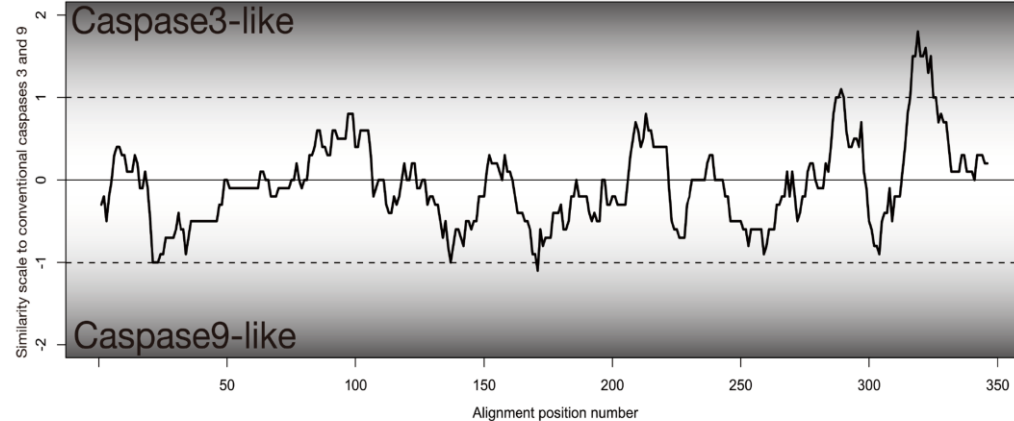
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caspace-9_B.taurus 168 SVERIVNIFNFGSCPSLGGKPKLFFFOACGGEGKDHGFE-VESTS--PEDKTESSDS---
caspace-9_M.musculus 180 SIEKIVNIFNFGSCPSLGGKPKLFFFOACGGEGKDHGFE-VACTS--SOGRTLSDS---
caspace-9_R.norvegicus 180 SIEKIVNIFNFGSCPSLGGKPKLFFFOACGGEGKDHGFE-VACTS--SODKAFSDS---
caspace-9_H.sapiens 161 SIEKIVNIFNFGSCPSLGGKPKLFFFOACGGEGKDHGFE-VACTS--PEDSESGSNB---

caspace-3/9_A.pectinifera 218 LSDEELAORMLERELEDTDASNIAIRSKLFSOSDMLLAFATVPGFVSWRNSERGSWFVQAL
caspace-9_B.taurus 222 --EADATPF--CEGPRSIDEPDAV--SLEFPTSDILVSYSTFFPGFVSWRDRKSGSNVIEA
caspace-9_M.musculus 234 --EADAVPY--CEGPRLLDQDAV--SLEFPTSDILVSYSTFFPGFVSWRDRKSGSNVIEA
caspace-9_R.norvegicus 234 --EADAVPY--CEGPRLLDQDAV--SLEFPTSDILVSYSTFFPGFVSWRDRKSGSNVIEA
caspace-9_H.sapiens 215 --EADATPF--CEGLRTRFDQDAV--SLEFPTSDILVSYSTFFPGFVSWRDRKSGSNVIEA

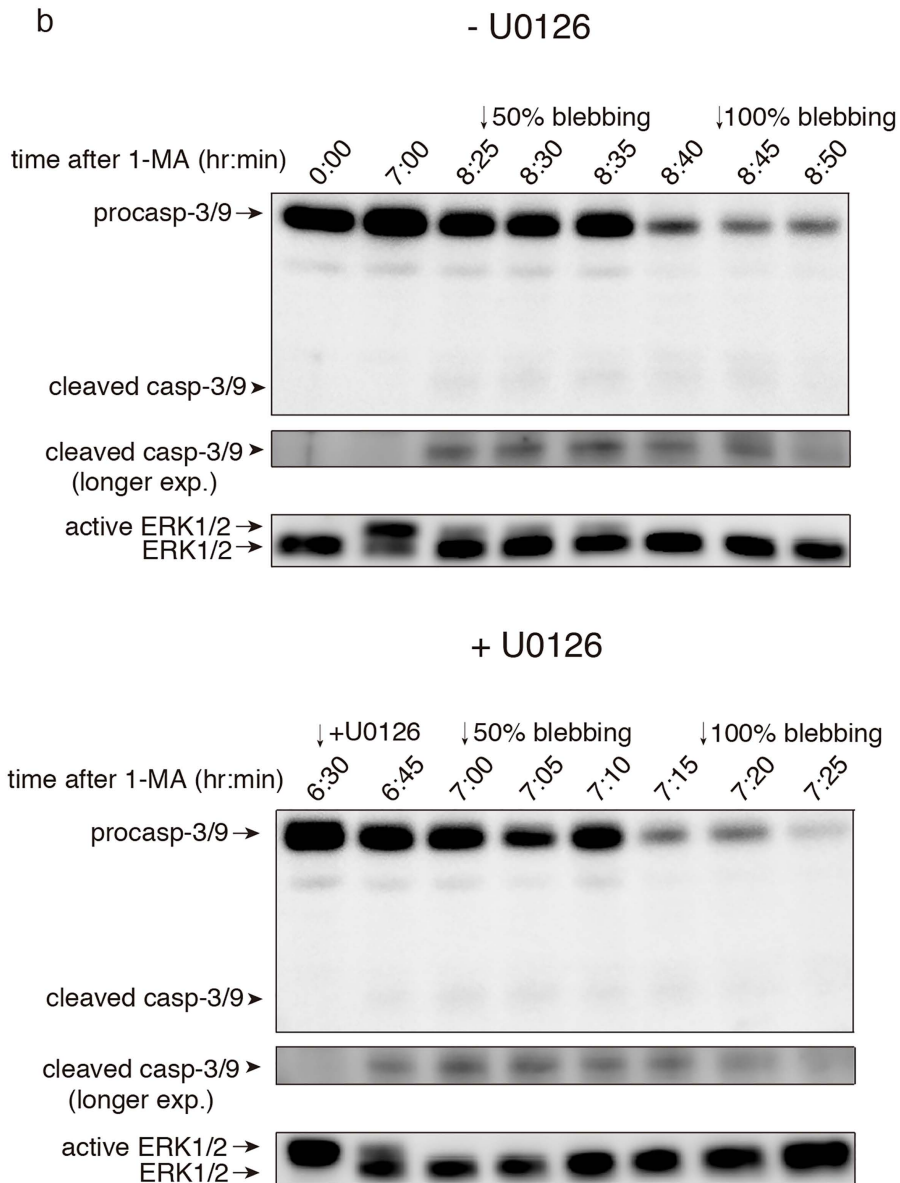
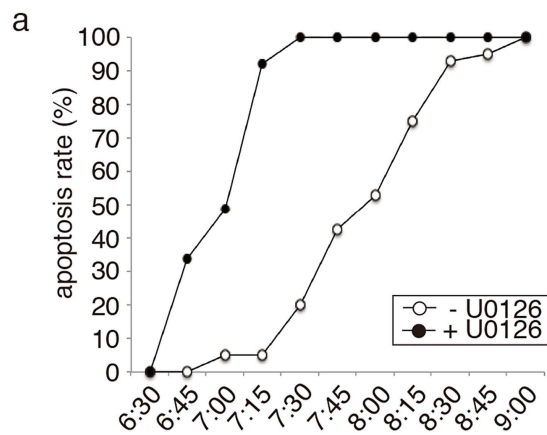
caspace-3/9_A.pectinifera 278 TSEVFLHANKEDLLSMMTRVNNKVARAFESSS--GRNKOIPAPVTMLRKRKYFNPNGR
caspace-9_B.taurus 276 LDISIFEQWAHSEDLLOTLLLRVANAVSVK-----CTYKQIPGCFNFIKRLKFLFKTS-
caspace-9_M.musculus 288 LIDGILEQWAHSEDLLOSLLLRVANAVSAK-----CTYKQIPGCFNFIKRLKFLFKTS-
caspace-9_R.norvegicus 288 LIDGILEQWAHSEDLLOSLLLRVANAVSEK-----CTYKQIPGCFNFIKRLKFLFKTS-
caspace-9_H.sapiens 269 LDDIFEQWAHSEDLLOSLLLRVANAVSVK-----CTYKQIPGCFNFIKRLKFLFKTS-

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**c**



**Supplemental Figure S1.** (a, b) Multiple sequence alignment of caspase-3 (a) and caspase-9 (b) from *Homo sapiens*, *Rattus norvegicus*, *Mus musculus*, and *Bos taurus* against starfish, *Asterina pectinifera*, caspase-3/9. Positions with conserved amino acid residues are in a black background. The alignment was performed by MAFFT. (c) Conservation tendency graph of starfish caspase-3/9. The total number of black amino acid residues in a sliding window (every 15 amino acids) of caspase-3 (Supplemental Figure S1 a) was subtracted by the total number of black amino acid residues in the same sliding window of caspase-9 (Supplemental Figure S1 b). The subtracted number of each window was divided by 15, and obtained each value was plotted against the alignment position. A positive value means that the region in starfish caspase-3/9 has a caspase-3-like sequence and a negative value means that the region has a caspase-9-like sequence. The graph shows that starfish caspase-3/9 has a caspase-9-like sequence in the N-terminal side, and a caspase-3-like sequence in the C-terminal side.



**Supplemental Figure S2.** Inhibition of ERK1/2 activates caspase-3/9. (a) The number of apoptotic eggs was counted at the indicated time after 1-MA treatment without U0126 (white circle) or with U0126 (black circle). Eggs were treated with 1  $\mu$ M of U0126 at 6.5 h after 1-MA treatment. (b) Time course of endogenous caspase-3/9 activation with or without U0126. Samples were analyzed by SDS-PAGE and western blotting with anti-caspase-3/9 and anti-ERK1/2 antibodies. Cleaved caspase-3/9 was visible after longer exposures.



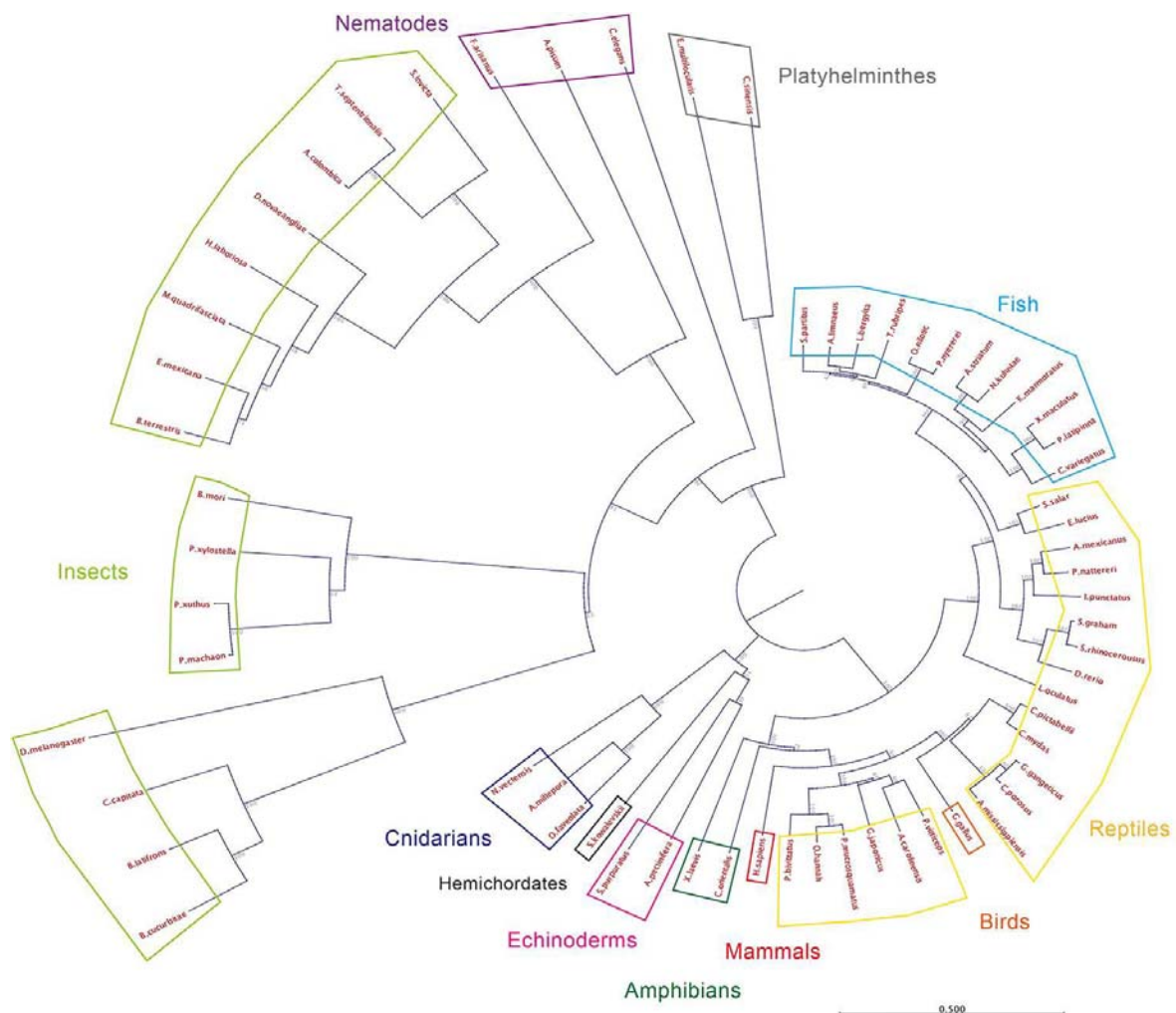


H.sapiens	766	TKL	-MDAT	-NENW-SHWQFLNLEP	-QDEWTKVCS	-WSD-GAR	-TVAANN-KLFL	-DHTSGLQETHG	-HST-ITQD-CF	849
G.gallus	767	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
C.mys	768	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
C.pitella	769	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
G.japonica	770	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
P.vitticeps	771	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
A.cariolinensis	772	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
P.bivittatus	773	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
O.hannah	774	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
P.macrognathus	775	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
A.mississippiensis	776	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
C.rosalia	777	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
G.gonostictus	778	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
D.rosio	779	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
S.rhinocerosus	780	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
S.graban	781	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
P.nattereri	782	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
A.mexicana	783	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
I.punctatus	784	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
E.lucius	785	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
S.salar	786	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
P.latiipinna	787	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
A.maculatus	788	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
C.variatus	789	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
P.nyereii	790	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
O.notic	791	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
L.bergi	792	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
A.limeus	793	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
S.partitus	794	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
T.rubripes	795	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
N.kuhate	796	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
A.atriatum	797	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
K.nemoratus	798	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
A.orientalis	799	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
X.laevis	800	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
A.milegor	801	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
G.favosita	802	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
N.vectensis	803	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
S.kowalevskii	804	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
A.pectinifera	805	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
S.purpuratus	806	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
E.mexicana	807	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
A.orientalis	808	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
M.quadrifasciata	809	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
H.laboriosa	810	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
D.noveboracensis	811	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
A.colombiana	812	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
S.sivicta	813	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
F.ariensis	814	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
A.pisum	815	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
B.mori	816	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
P.yostellia	817	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
L.ocalata	818	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
P.athous	819	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
C.olegans	820	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
C.siniensis	821	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
E.mollis	822	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
B.latifrons	823	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
B.cucurbitae	824	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849
C.capitata	825	TKL	-MNS	-NENW-TIENQWQDQ	-QDEWTKVCS	-WSD-SOT	-TLVANN-KLL	-DHTSGLQETHG	-HST-ITQD-CF	849

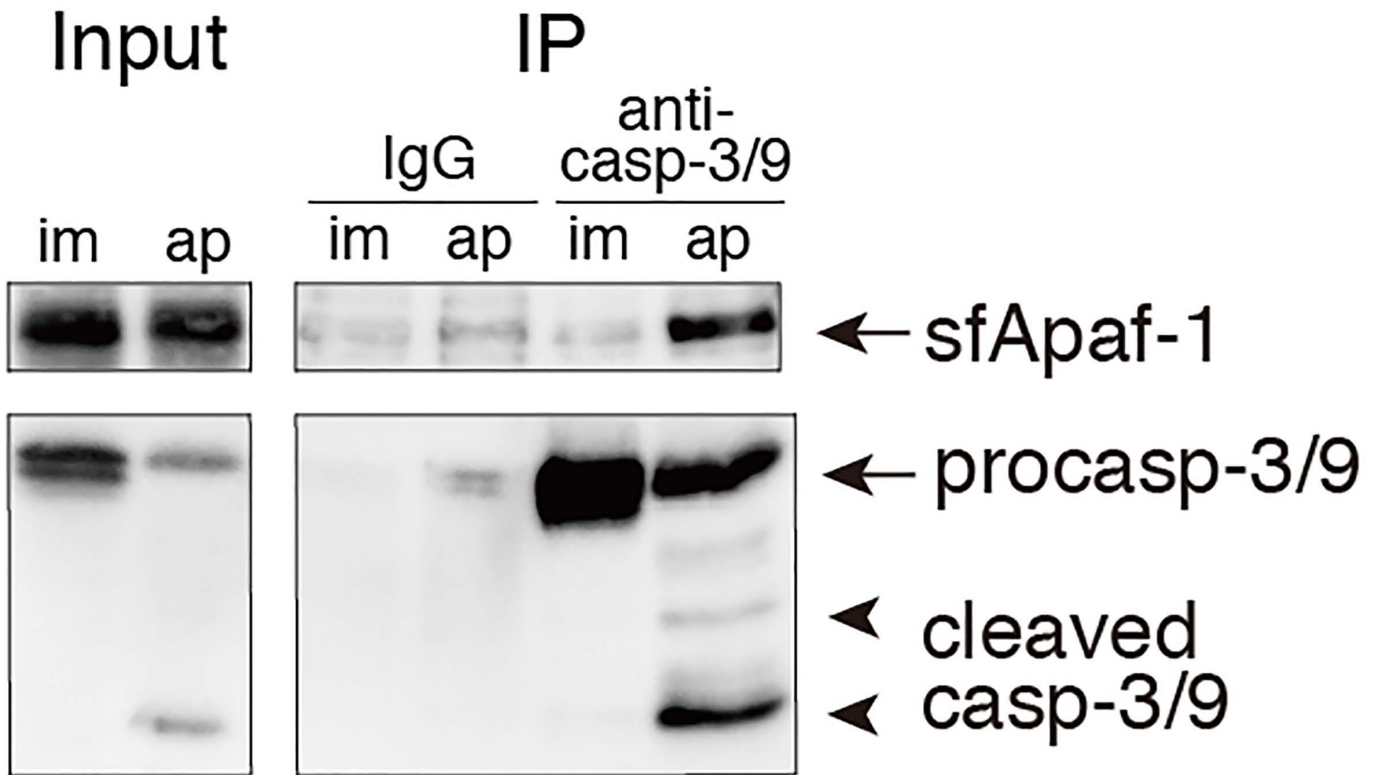
H.sapiens	837	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
G.gallus	838	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
C.mys	839	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
C.pitella	840	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
G.japonica	841	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
P.vitticeps	842	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
A.cariolinensis	843	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
P.bivittatus	844	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
O.hannah	845	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
P.macrognathus	846	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
A.mississippiensis	847	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
C.rosalia	848	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
G.gonostictus	849	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
D.rosio	850	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
S.rhinocerosus	851	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
S.graban	852	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
P.nattereri	853	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
A.mexicana	854	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
I.punctatus	855	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
E.lucius	856	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
S.salar	857	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
P.latiipinna	858	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
A.maculatus	859	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
C.variatus	860	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
P.nyereii	861	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
O.notic	862	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
L.bergi	863	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
A.limeus	864	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
S.partitus	865	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
T.rubripes	866	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
N.kuhate	867	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
A.atriatum	868	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
K.nemoratus	869	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
A.orientalis	870	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
X.laevis	871	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
A.milegor	872	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
G.favosita	873	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
N.vectensis	874	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
S.kowalevskii	875	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
A.pectinifera	876	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
S.purpuratus	877	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
E.mexicana	878	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
A.orientalis	879	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
M.quadrifasciata	880	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
H.laboriosa	881	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG	-TEELE	-NMFVDFGPH	-KXWTH	-QFT	1012
D.noveboracensis	882	-VAVDNRRLQ	-DHWRT	-DIDTL	-EAO	-VSSCCSP	-RQVTAFOGNG</					

H.sapiens	1007	ATKFSSTADKT-AK	---TSPFLDPLL	---MELRHGHCVRCTFSFD	---STLALT	---GDGKEIRLMDVSR	---GELLMLCAP	---LS	EEGATHTGGWTLDFSPD	1188	H.sapiens	1246	TLE	1248
G.gallus	1008	GSFSPSTADKT-AK	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKOTDIRMLT	---GELLFCSF	---VT	VEEPTHTGGWTLDFSPD	1189	G.gallus	1248	KL	1249
C.mydas	1026	ATKFSSTADKT-AK(104)	TSPFESSVL	---MELRHGHCVRCTFSFD	---DKFLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1192	C.mydas	1264	LUN(1)	1267
C.picta bellii	1104	ATKFSSTADKT-AK	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1202	C.picta bellii	1250	NLE	1252
G.japonicas	1104	ATKFSSTADKT-AK	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1194	G.japonicas	1254	TMD(3)	1259
P.vitticeps	1101	GRFSTADKT-AK	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1186	P.vitticeps	1244	IME(3)	1249
A.carolinensis	1101	ATKFSSTADKT-AK	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1193	A.carolinensis	1246	IME	1248
P.hisvittatus	1007	ATKFSSTADKT-AK	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1188	P.hisvittatus	1246	IME	1248
O.hannah	1099	ATKFSSTADKT-AK	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1188	O.hannah	1156	IME	1158
P.mucrosquamatus	1091	ATKFSSTADKT-AK	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1188	P.mucrosquamatus	1246	IME	1248
A.mississippiensis	1095	ATKFSSTADKT-AK	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1187	A.mississippiensis	1225	---	1225
C.porosus	1095	ATKFSSTADKT-AK	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1188	C.porosus	1246	IME	1247
G.gangeticus	1096	ATKFSSTADKT-AK	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1187	G.gangeticus	1246	IME	1248
B.terrieri	1105	GRFSTADKT-AK	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1197	B.terrieri	1256	RLE(4)	1261
S.rhinocerosus	1105	GRFSTADKT-AK	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1197	S.rhinocerosus	1256	---	1258
S.graham	1105	GRFSTADKT-AK	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1197	S.graham	1255	KVE(4)	1261
P.nattereri	1106	GVFSTADKT-AK	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1198	P.nattereri	1256	OVE(4)	1262
A.americanus	1118	GSFSTADKT-AK	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1202	A.americanus	1259	OVE(4)	1264
I.punctatus	1106	GMFSTADKT-AK	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1198	I.punctatus	1256	OVE(4)	1262
E.lucius	1122	GLFSTADKT-AK	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1214	E.lucius	1272	KVE	1274
S.salar	1113	GLFSTADKT-AK	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1197	S.salar	1263	KVE	1265
P.latiplana	1107	GLFSTADKT-AK	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1200	P.latiplana	1258	RVA	1260
X.naculatus	1107	GLFSTADKT-AK	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1200	X.naculatus	1256	RVA	1258
C.variegatus	1105	GLFSTADKT-AK	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1197	C.variegatus	1255	RVA	1257
P.yererei	1106	GFSTADKT-AK	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1198	P.yererei	1256	RVA	1258
O.niotic	1106	GFSTADKT-AK	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1198	O.niotic	1256	RVA	1258
L.beryllia	1111	GLFSTADKT-AK	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1203	L.beryllia	1263	RVA	1263
A.limous	1145	GFSTADKT-AK	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1227	A.limous	1292	RVA(1)	1294
S.partitus	1106	GLFSTADKT-AK	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1198	S.partitus	1255	RVA	1257
T.ubripes	1105	GLFSTADKT-AK	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1198	T.ubripes	1255	RVA	1257
N.kuhntae	1106	GFSTADKT-AK	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1198	N.kuhntae	1255	RVA	1257
A.striatus	1105	GLFSTADKT-AK	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1197	A.striatus	1254	RVA	1256
K.namoratus	1105	GLFSTADKT-AK	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1197	K.namoratus	1254	RVA	1256
L.oculata	1181	GFSTADKT-AK	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1193	L.oculata	1258	IME	1262
C.orientalis	1106	GFSTADKT-AK	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1198	C.orientalis	1246	IME	1248
X.laevis	1096	SKFSTADKT-AK	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1188	X.laevis	1246	LIE	1248
A.milolepora	1197	DKRLASADKT-AK	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1200	A.milolepora	1337	KIS(1)	1340
O.faveolata	1084	DKRLASADKT-AK	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1188	O.faveolata	1644	RIN(3)	1648
N.vectensis	1076	SKRLASADKT-AK	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1157	N.vectensis	1215	QI	1216
S.kowalevskii	1189	ESLLSTADKT-AK	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1172	S.kowalevskii	1229	KVE(9)	1239
A.pectinifera	1084	EFKFSSTADKT-AK	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1167	A.pectinifera	1224	LIX(9)	1265
S.purpuratus	1106	SKFSTADKT-AK	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1198	S.purpuratus	1242	RVA(15)	1294
E.mexicana	1234	RSLVLAENGD-VV	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1226	E.mexicana	1367	LOG(2)	1368
B.terrestris	1131	KSLVLAENGD-VV	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1223	B.terrestris	1324	LOG(2)	1327
H.quadrifasciata	1128	KCLVLAENGD-VV	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1220	H.quadrifasciata	1322	LYG(2)	1324
H.laboriosa	1103	KRLVLAENGD-VV	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1218	H.laboriosa	1241	CE(2)	1245
D.novaeangliae	1132	KMLVLAENGD-VV	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1224	D.novaeangliae	1364	VG(2)	1368
A.columbica	1125	KYLALLENGD-VV	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1220	A.columbica	1358	LE(7)	1359
S.imvicta	1104	EDMLALLENGD-VV	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1194	S.imvicta	1377	LIG(9)	1388
F.arsianus	1118	EDMLALLENGD-VV	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1200	F.arsianus	1415	LFG(2)	1419
A.pisum	1211	PSNLVLAENGD-VV	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1209	A.pisum	1355	LPS(2)	1359
B.mori	1235	NMLVLAENGD-VV	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1232	B.mori	1427	WR(1)	1430
P.xylostella	1281	KMLVLAENGD-VV	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1369	P.xylostella	1538	WR(15)	1537
P.machon	1274	KMLVLAENGD-VV	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1362	P.machon	1523	WR(3)	1528
P.vuthus	1408	KYLALLENGD-VV	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1401	P.vuthus	1448	---	1448
C.elegans	543	---	---	---	---	---	---	---	---	544	C.elegans	543	---	549
C.simensis	1815	GRVLAENGD-VV	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1810	C.simensis	2384	PIG(9)	2385
E.multilocularis	1176	GMLAATADKT-AK	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1170	E.multilocularis	1963	PIE(47)	2012
O.melanogaster	1158	NLLVLAENGD-VV	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1150	O.melanogaster	1369	LIE(57)	1429
B.latifrons	1154	NQLVLAENGD-VV	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1146	B.latifrons	1418	LMD(25)	1445
B.cucurbitae	1211	NMLVLAENGD-VV	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1203	B.cucurbitae	1467	LMD(25)	1494
C.capitata	1244	NMLVLAENGD-VV	---TSPFESSVL	---MELKHGHCVRCTFSFD	---MILLAT	---GDGKEIRLMDVSR	---GELLMLCAP	---VT	VEEPTHTGGWTLDFSPD	1237	C.capitata	1508	LMD(28)	1522
H.sapiens	1189	GKH	---	---	---	---	---	---	---	1245	H.sapiens	1245	---	1245
G.gallus	1191	SMH	---	---	---	---	---	---	---	1247	G.gallus	1247	---	1247
C.mydas	1223	SMH	---	---	---	---	---	---	---	1249	C.mydas	1249	---	1249
C.picta bellii	1193	SMH	---	---	---	---	---	---	---	1249	C.picta bellii	1249	---	1249
G.japonicas	1197	SMH	---	---	---	---	---	---	---	1253	G.japonicas	1253	---	1253
P.vitticeps	1185	NEL	---	---	---	---	---	---	---	1243	P.vitticeps	1243	---	1243
A.carolinensis	1186	NEL	---	---	---	---	---	---	---	1243	A.carolinensis	1243	---	1243
P.hisvittatus	1189	SMH	---	---	---	---	---	---	---	1245	P.hisvittatus	1245	---	1245
O.hannah	1099	GKY	---	---	---	---	---	---	---	1155	O.hannah	1155	---	1155
P.mucrosquamatus	1089	GKY	---	---	---	---	---	---	---	1155	P.mucrosquamatus	1155	---	1155
A.mississippiensis	1088	SMH	---	---	---	---	---	---	---	1225	A.mississippiensis	1225	---	1225
C.porosus	1088	SMH	---	---	---	---	---	---	---	1245	C.porosus	1245	---	1245
G.gangeticus	1089	SMH	---	---	---	---	---	---	---	1244	G.gangeticus	1244	---	1244
B.terrieri	1105	NRY	---	---	---	---	---	---	---	1258	B.terrieri	1258	---	1258
S.rhinocerosus	1106	NRY	---	---	---	---	---	---	---	1258	S.rhinocerosus	1258	---	1258
S.graham	1198	NRE	---	---	---	---	---	---	---	1258	S.graham	1258	---	1258
P.nattereri	1199	NRY	---	---	---	---	---	---	---	1258	P.nattereri	1258	---	1258
A.americanus	1203	NRY	---	---	---	---	---	---	---	1258	A.americanus	1258	---	1258
I.punctatus	1199	NRY	---	---	---	---	---	---	---	1258	I.punctatus	1258	---	1258
E.lucius	1122	NRY	---	---	---	---	---	---	---	1258	E.lucius	1258	---	1258
S.salar	1107	NRY	---	---	---	---	---	---	---	1258	S.salar	1258	---	1258
P.latiplana	1201	NRY	---	---	---	---	---	---	---	1258	P.latiplana	1258	---	1258
X.naculatus	1200	NSV	---	---	---	---	---	---	---	1256	X.naculatus	1256	---	1256
C.variegatus	1199	NSV	---	---	---	---	---	---	---	1256	C.variegatus	1256	---	1256
P.yererei	1199	NSL	---	---	---	---	---	---	---	1254	P.yererei</			

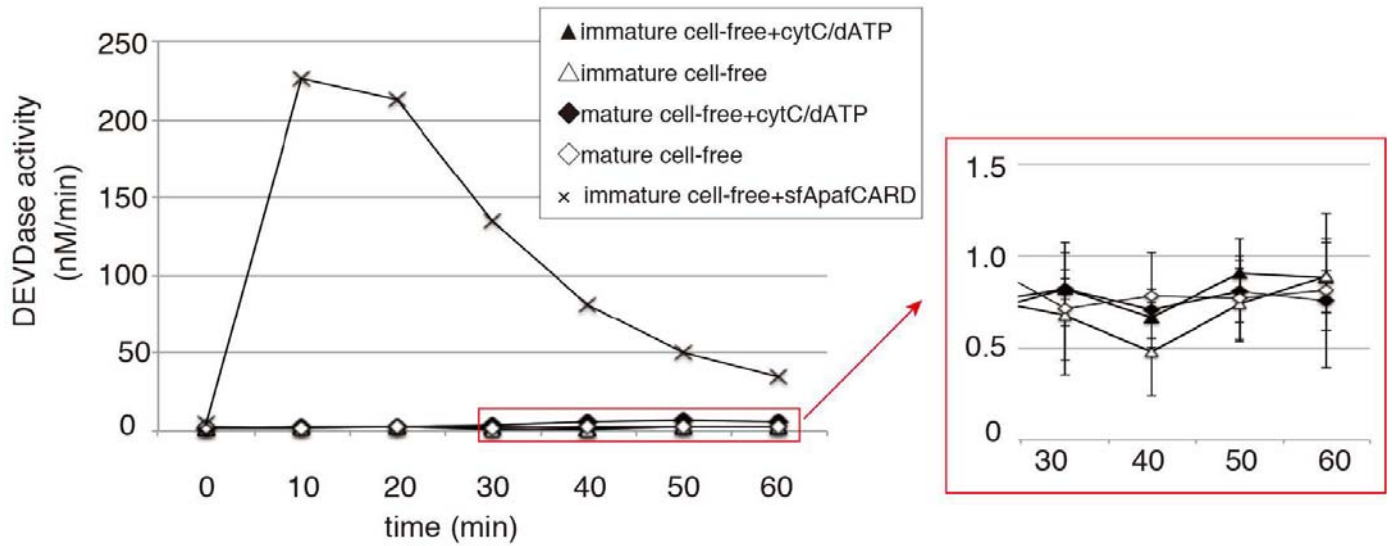




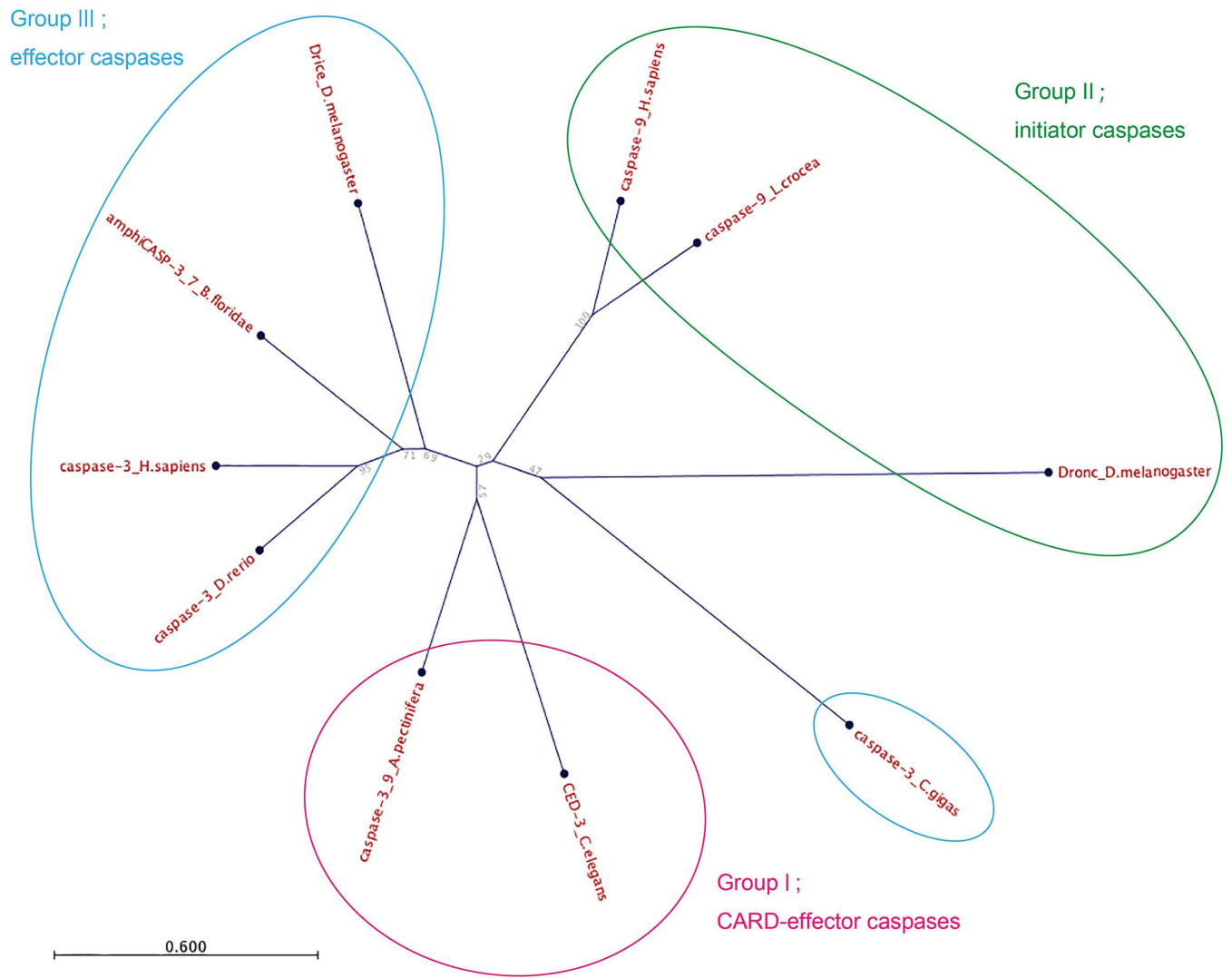
**Supplemental Figure S4.** Phylogenetic tree of Apaf-1 family proteins. The phylogenetic tree was calculated using CLC Sequence Viewer ver.7.6 (CLC Bio A/S, Aarhus, Denmark) based on the alignment in Fig. S1. The species used for the alignment were mammals (red), birds (orange), reptiles (yellow), fishes (light blue), amphibians (green), cnidarians (blue), hemichordates (black), echinoderms (pink), insects (light green), nematode (purple), platyhelminthes (gray). A bootstrap value, a measure of the confidence in the branch, is attached to each node.



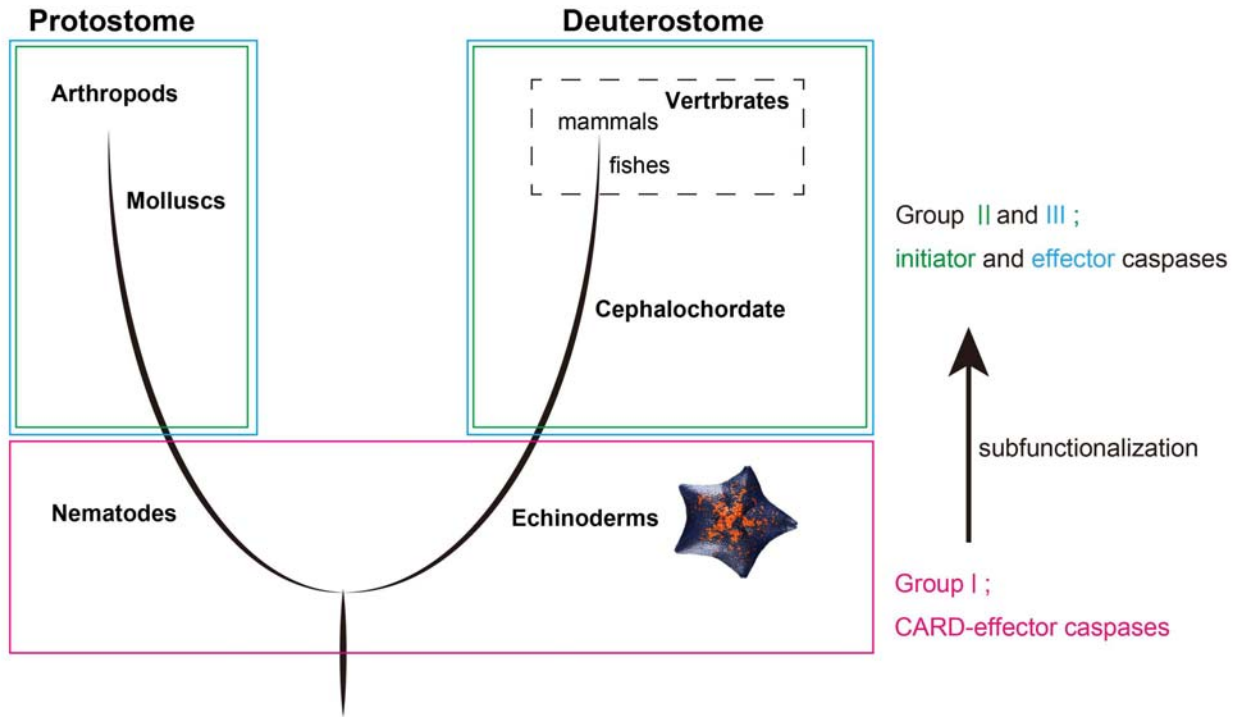
**Supplemental Figure S5.** Co-immunoprecipitation of endogenous sfApaf-1 and caspase-3/9. Cell-free preparations from immature oocytes (im) or apoptotic eggs (ap) were incubated with anti-caspase-3/9 antibody or control IgG-coupled Protein A Sepharose beads. Immunoprecipitates were analyzed by western blotting using anti-sfApaf-1 antibody (upper panel) or anti-caspase-3/9 antibody (lower panel).



**Supplemental Figure S6.** Cytochrome *c* does not activate caspase-3/9 in starfish cell-free preparations. Cell-free preparations using immature oocytes and mature eggs were made according to the method of Chiba *et al.* (1999). Time course of DEVDase activity was measured in the presence (immature▲, mature◆) or absence (immature△, mature◇) of 10  $\mu$ M cytochrome *c* and 1 mM dATP. Positive control experiments were performed in the presence of 2 mM GST-A-CARD (×).



**Supplemental Fig. S7.** Phylogenetic tree of the caspases whose proteolytic activities were examined using recombinants. The phylogenetic tree was calculated using CLC Sequence Viewer ver.7.6 (CLC Bio A/S, Aarhus, Denmark) based on the alignment which the long insertions were deleted. The species used for phylogenetic tree were *H. sapiens* caspase-3 (CAC88866) and caspase-9 (BAA87905), *D. rerio* caspase-3 (BAB32409), *Pseudosciaena crocea* caspase-9 (ACJ65026), *C.gigas* caspase-3 (XP\_011449627), *B. floridae* AmphiCASP-3/7 (AAN45849), *C. elegans* CED-3 (AAG42045), *D. melanogaster* Drice (CAA72937) and Dronc (AAD26625), and *A. pectinifera* caspase-3/9. A bootstrap value, a measure of the confidence in the branch, is attached to each node.



**Supplementa Figure S8.** Evolutionary trends of caspases. Conservation of caspases throughout animal evolution. Group I (CARD-effector caspases), Group II (initiator caspases), and Group III (effector caspases) are boxed in pink, green, and blue, respectively.

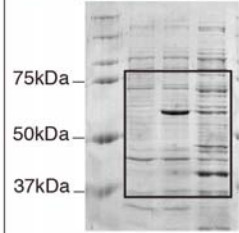
```

caspase-3/9   YIASPTDELPKAPWPGTTPADLTTQEMPMAPLPLADQDASDVD 178
caspase-9     VVLRPEIRKPEVLRP-ETPRPVDIGSGGFGDVGALESIRGNAD 150
               : * . *:. * ** : . :. : :. :..*

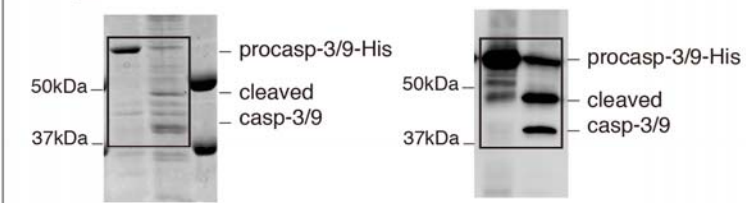
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**Supplemental Figure S9.** Conservation of the MAPK consensus motif. MAPK consensus motif is boxed in starfish caspase-3/9 (136–178 aa) and human caspase-9 (109–150 aa). Sequence alignment was generated using MAFFT alignment program. Symbols at the bottom of protein sequences are indicated conserved sequences (\*), conservative mutations (:), semi-conservative mutations (.), and non-conservative mutations ( ), respectively.

For Figure 2a



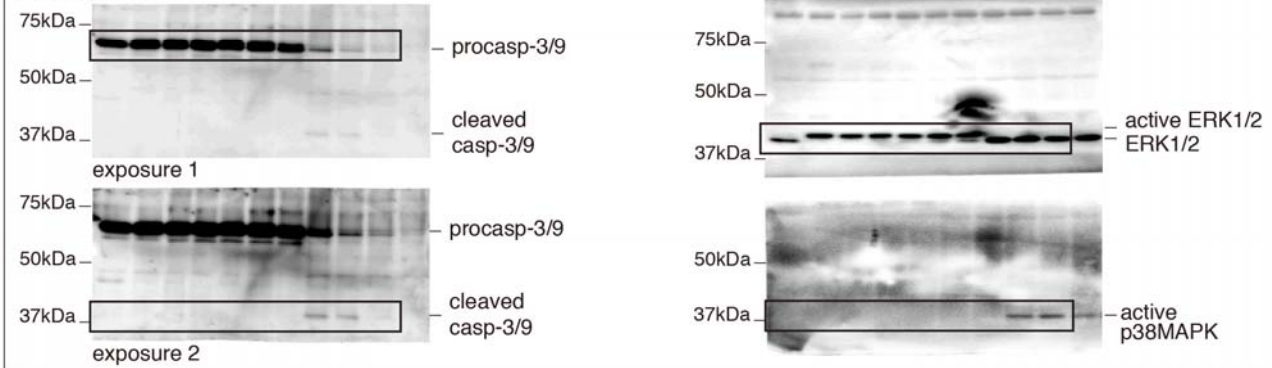
For Figure 3a



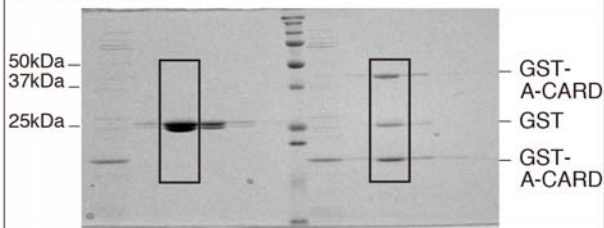
For Figure 3b



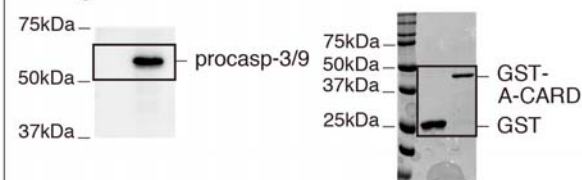
For Figure 3c



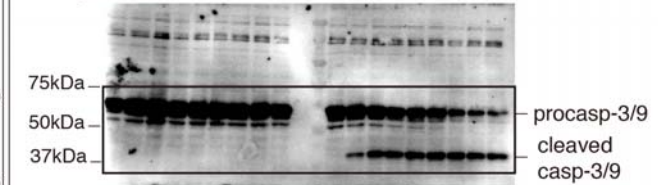
For Figure 5a



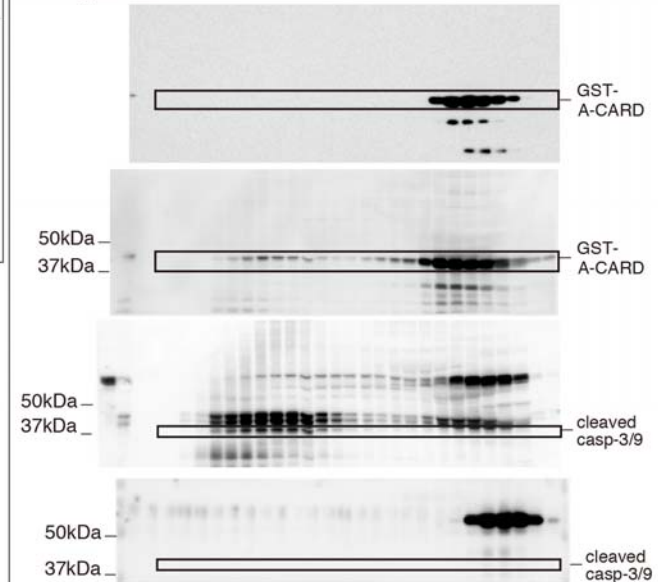
For Figure 5b

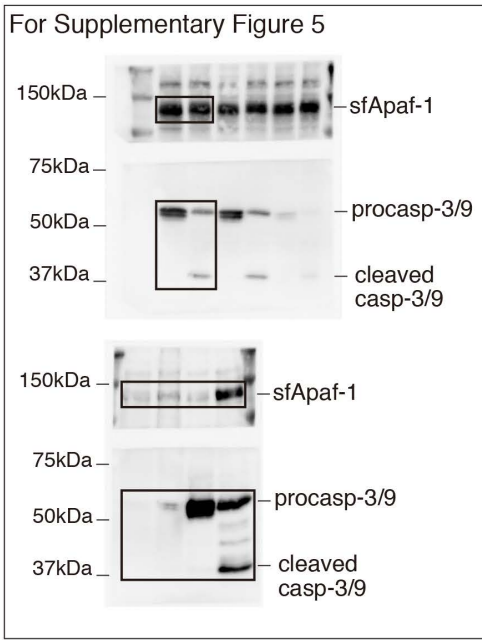
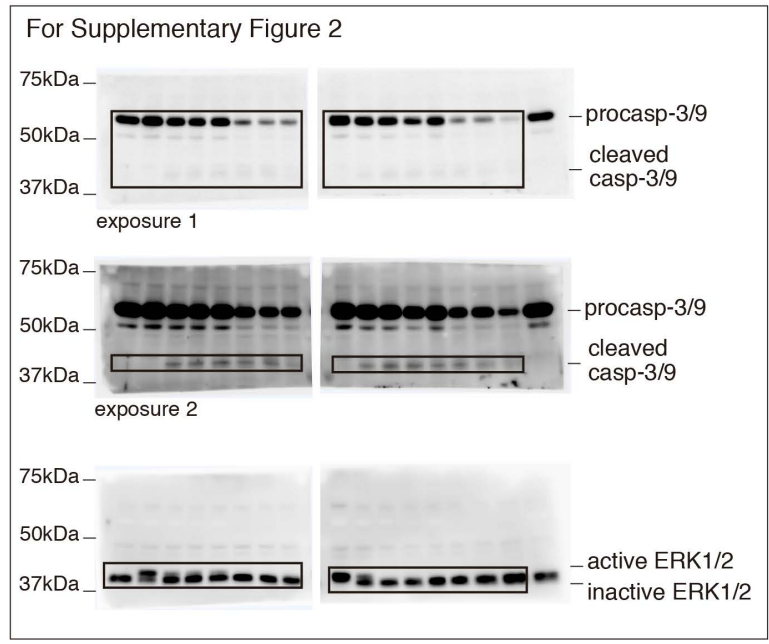
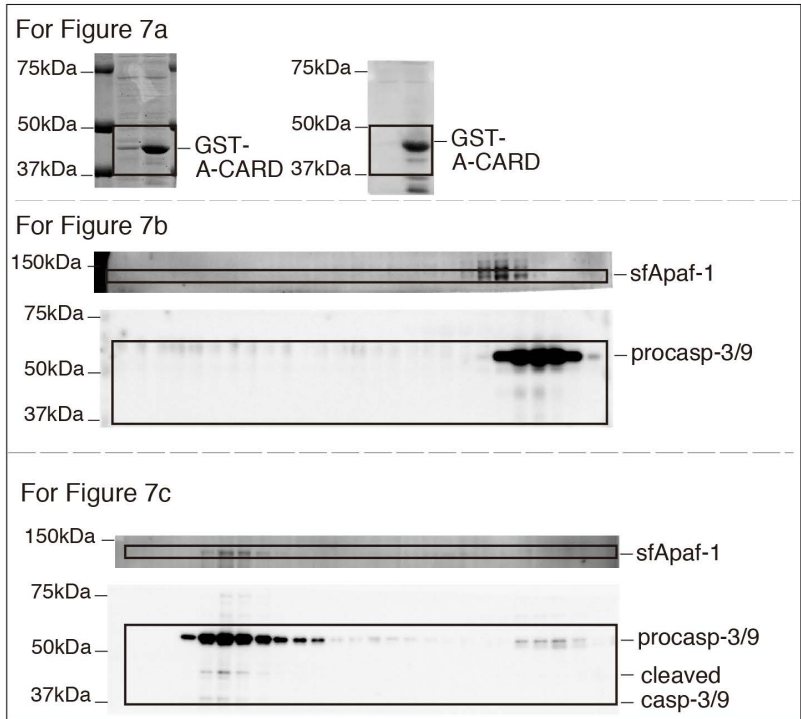


For Figure 6b



For Figure 6c





**Supplemental Figure S10.** Full-length gels and blots used for Figure 1-7 and Supplementary Figures.



**Supplemental Table S1. Primers for cDNA cloning.**

primer	sequences (5'-3')	
degenerate primers for caspase-3/9	Fw	ATCATHAAYAAYAARAAYTTYSA
	Re	GCCTGRATRAARAANAGTTTRGGYTT
specific primers for caspase-3/9	Fw	TGGCAAGAACCTGAAGCACGTG
	Re	TCTGCTGCATTTGCTTCCCGGTC
degenerate primers for a partial sfApaf-1	Fw	GGNGGNGTNCCNATNCCICC
	Re	ARRAARTCNARYGNARRTYRTG
degenerate primers for a partial sfApaf-1 for nested PCR	Fw	CATGGNATGGGNGGNATNGGIAAR
	Re	ARRTARTAYTCCANCKIKTIGG
specific primers for 5' RACE for cDNA transcription		[Phos]CTCAATCTATCCTT
specific primers for 5' RACE		CACAGGTCTGTAGCTTGGT
		AGACTAGACCGTGAGTCC
specific primers for 5' RACE for nested PCR		CTGATGGCGACCCAGAACAC
		CCCACTCAACCTGGAGGAG
specific primers for 3' RACE		ATAAGCTGCGCTGCATGA
specific primers for 3' RACE for nested PCR		CAGGAATGGGATGATGATAAGCT