

1 **Title**

2 Intrinsic apoptosis is evolutionarily divergent among metazoans

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22 **Supplementary Figure 1:**

23 Topology of metazoan CARD-caspase phylogeny obtained by Bayesian inference. Selected out
24 group is the unique CARD-caspase of Ctenophora *Pleurobrachia bachei*. The four strongly
25 supported monophyletic groups identified, caspase-9, caspase-2, inflammatory caspases, and
26 caspase-Y, are the same as when the unique CARD-caspase of the Porifera *Amphimedon*
27 *queenslandica* was used as out group. Also, sequences from bilaterian animals remain
28 monophyletic.

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30 **Supplementary Figure 2:**

31 Phylogeny of CARD-caspases at the deuterostomian scale. Maximum likelihood and Bayesian
32 inference methods produce similar topologies. Despite the unstable position of
33 cephalochordate *Branchiostoma belcheri* caspase-9A (green arrow), it unequivocally appears
34 to belong to the caspase-9 group. Numbers given correspond to posterior probabilities. We
35 used respectively as out groups *Capitella teleta* caspase-Y (ELT97848.1) (**A1**), *Aplysia*
36 *californica* caspase-2 (XP_005113266) (**A2**), *Hydra vulgaris* caspase-X2 (NP_001274285.1) (**A3**),
37 and *Caenorhabditis elegans* Ced3 (AAG42045.1) (**A4**).

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39 **Supplementary Figure 3:**

40 Topology of metazoan CARD-caspase phylogeny obtained by Bayesian inference. Dataset used
41 is similar to Figure 3 but with the absence of *Branchiostoma belcheri* caspase-9A. The four
42 strongly supported monophyletic groups are identified, caspase-9, caspase-2, inflammatory
43 caspases, and caspase-Y. Robustness of Caspase-2 clade increased by removing the long and
44 divergent branch of *Branchiostoma belcheri* caspase-9A.

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46 **Supplementary Figure 4:**

47 Topology of metazoan CARD-caspase phylogeny obtained by Bayesian inference. Dataset used
 48 is similar to Figure 3 but with the absence of nematodes Ced-3. The four monophyletic groups
 49 are identified, caspase-9, caspase-2, inflammatory caspases, and caspase-Y. Robustness of
 50 Caspase-2 clade increased by removing the long and divergent branches of nematodes Ced-3.
 51 Importantly, the robustness of the branching of insects Dronc with Caspase-2 is even
 52 reinforced.

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54 **Supplemental Table 1: List of CARD-caspases used for phylogenetic analysis.**

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	Species	Accession number	Name
Porifera	<i>Amphimedon queenslandica</i>	XP_003383519.1	Casp. Card a
		XP_019854115.1	Casp. Card b
	<i>Geodia cydonium</i>	CAC83013.1	Casp. Card
Cnidaria	<i>Acropora digitifera</i>	XP_015766400.1	Casp. X2
		XP_015768208.1	Casp. X1
	<i>Exaiptasia pallida</i>	KXJ12672.1	Casp. X1
		KXJ20965.1	Casp. X2
	<i>Hydra vulgaris</i>	KXJ12683.1	Casp. X3
		ACY95435.1	Casp. X1
	ACY95436.1	Casp. X2	
Ctenophora	<i>Pleurobrachia bachei</i>	Sb 2658116	Casp. X
Placozoa	<i>Trichoplax adherens</i>	RDD44781.1	Casp. X
Lophotrocozoa	<i>Aplysia californica</i>	XP_005113266.2	Casp. 2
		XP_012945422.1	Casp. Y
	<i>Biomphalaria glabrata</i>	XP_013082356.1	Casp. 2
		<i>Bulinus truncatus</i>	KAH9499437.1
	KAH9513478.1		Casp. Y2
	<i>Capitella teleta</i>	ELU00616.1	Casp. 2
		ELT97848.1	Casp. Y
	<i>Crassostrea angulata</i>	AGN75137.1	Casp. 2
		<i>Crassostrea gigas</i>	XP_011419292.2
	XP_011414267.3		Casp. Y1
	XP_011449817.2		Casp. Y2
		XP_011432762.2	Casp. Y3
	<i>Octopus bimaculoides</i>	XP_014783442.1	Casp. 2
		XP_014790087.1	Casp. Y1
		XP_014784582.1	Casp. Y2
<i>Owenia fusiformis</i>	CAH1774650.1	Casp. Y1	
	CAH1774000.1	Casp. Y2	

Chelicerata	<i>Limulus polyphemus</i>	XP_013788138.1	Casp. 2A
		XP_013776997.1	Casp. 2B
Insecta	<i>Agrilus planipennis</i>	XP_018324425.1	Casp. 2
	<i>Apis mellifera</i>	XP_016771440.1	Casp. 2
	<i>Drosophila melanogaster</i>	CAB53565.1	Casp. 2
	<i>Habropoda laboriosa</i>	XP_017788772.1	Casp. 2
	<i>Helicoverpa armigera</i>	AEK20835.1	Casp. 2
	<i>Papilio machaon</i>	XP_014362236.1	Casp. 2
	<i>Tribolium castaneum</i>	XP_001813274.1	Casp. 2
Nematoda	<i>Ascaris suum</i>	ERG86894.1	Casp. 2
	<i>Caenorhabditis elegans</i>	AAG42045.1	Casp. 2
Vertebrata	<i>Salmo salar</i>	XP_014009539.1	Casp. 2
		XP_013991583.1	Casp. 9
	<i>Mus musculus</i>	NP_033937.2	Casp. 1
		EDL13489.1	Casp. 2
		NP_031635.2	Casp. 4
		AAH56447.1	Casp. 9
		AAT91067.1	Casp. 12
	<i>Xenopus laevis</i>	NP_001081223.1	Casp. 1
		NP_001081404.1	Casp. 2
		NP_001079035.1	Casp. 9
	<i>Gallus gallus</i>	XP_040543522.1	Casp. 1
		NP_001161173.2	Casp. 2
		XP_046759217.1	Casp. 9
	<i>Homo sapiens</i>	NP_001214.1	Casp. 1
		NP_116764.2	Casp. 2
		KAI4078767.1	Casp. 9
Echinodermata	<i>Holothuria leucospilota</i>	UTQ93743.1	Casp. 9
	<i>Lytechinus variegatus</i>	XP_041480663.1	Casp. 9
	<i>Strongylocentrotus purpuratus</i>	XP_789183.3	Casp. 9A
		XP_011661242.1 1	Casp. 9B
		XP_011661359.1	Casp. 9C
Hemichordata	<i>Saccoglossus kowalevskii</i>	XP_006811879.1	Casp. 9
Cephalochordata	<i>Branchiostoma belcheri</i>	XP_019646757.1	Casp. 2A
		XP_019642903.1	Casp. 2B
		XP_019623612.1	Casp. 9A
		XP_019644208.1	Casp. 9B
	<i>Branchiostoma floridae</i>	EEN68002.1	Casp. 2
Urochordata	<i>Botryllus schlosseri</i>	Boschl.CG.Botznik2013.chrUn.g02816.01.p	Casp. 2A1
		Boschl.CG.Botznik2013.chrUn.g08767.01.p	Casp. 2A2
		Boschl.CG.Botznik2013.chrUn.g09831.01.p	Casp. 2B
	<i>Ciona intestinalis</i>	KH2012:KH.C4.463.v1.A.ND1-1	Casp. 2A
	<i>Ciona savignyi</i>	CISAVI-CG-ENS81-R15-461426-463207-09349-P	Casp. 2A
		CISAVI-CG-ENS81-R54-461275-467018-10343-P	Casp. 2B
	<i>Molgula oculata</i>	Moocul-CG-ELv1_2-S113854-g13164-01-p	Casp. 2A
		Moocul-CG-ELv1_2-S103067-g09526-01-p	Casp. 2B
	<i>Phallusia mammillata</i>	PHMAMM-CG-MTP2014-S310-G07060-01-P	Casp. 2A

		PHMAMM-CG-MTP2014-S92-G02989-01-P	Casp. 2B
	<i>Halocynthia roretzi</i>	HARORE-CG-MTP2014-S35-G03390-01-P	Casp. 2A
		HARORE-CG-MTP2014-S130-G07320-01-P	Casp. 2B

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Supplemental Table 2: List of Apaf-1 among metazoans. N/A: No Apaf-1 has been identified.

	Species	Accession number	Name
Porifera	<i>Amphimedon queenslandica</i>	XP_019855714.1	Apaf-1
Ctenophora	<i>Pleurobrachia bachei</i>	N/A	
Placozoa	<i>Trichoplax sp.</i>	RDD40813.1	Apaf-1
Cnidaria	<i>Acropora digitifera</i>	XP_015776356.1	Apaf-1
	<i>Acropora millepora</i>	AJG37574.1	Apaf-1-1
		XP_029185121.1	Apaf-1-2
	<i>Actinia tenebrosa</i>	XP_031572908.1	Apaf-1
	<i>Dendronephthya gigantea</i>	XP_028393942.1	Apaf-1
	<i>Exaiptasia pallida</i>	KXJ17575.1	Apaf-1
	<i>Fimbriaphyllia ancora</i>	QMS47765.1	Apaf-1
	<i>Hydra vulgaris</i>	CDG72123.1	Apaf-1
	<i>Nematostella vectensis</i>	XP_032220711.2	Apaf-1
		XP_032223290.2	Apaf-1 like1
	XP_001626725.1	Apaf-1 like2	
	XP_032225984.2	Apaf-1 like3	
	<i>Orbicella faveolata</i>	XP_020620792.1	Apaf-1
	<i>Paramuricea clavata</i>	CAB4025087.1	Apaf-1
	<i>Pocillopora damicornis</i>	XP_027042245.1	Apaf-1
	<i>Stylophora pistillata</i>	XP_022790799.1	Apaf-1
Nematoda	<i>Caenorhabditis elegans</i>	CAA48781.1	Ced-4
	<i>Caenorhabditis briggsae</i>	XP_002642027.2	Ced-4
Insecta	<i>Aedes aegypti</i>	EAT48066.2	Dark
	<i>Agrilus planipennis</i>	XP_025834330.1	Dark
	<i>Apis mellifera</i>	XP_006565907.1	Dark
	<i>Drosophila melanogaster</i>	NP_725637.1	Dark
	<i>Drosophila pseudoobscura</i>	XP_001360832.3	Dark
	<i>Habropoda laboriosa</i>	XP_017794472.1	Dark
	<i>Helicoverpa armigera</i>	XP_021181657.1	Dark
	<i>Papilio machaon</i>	XP_014359375.1	Dark
	<i>Tribolium castaneum</i>	XP_015840766.1	Dark
Crustacea	<i>Daphnia pulex</i>	XP_046440096.1	Apaf-1
Chelicerata	<i>Centruroides sculpturatus</i>	XP_023242710.1	Apaf-1
	<i>Dermacentor andersoni</i>	XP_050040075.1	Apaf-1
	<i>Dermacentor silvarum</i>	XP_037568890.1	Apaf-1
	<i>Limulus polyphemus</i>	XP_022249946.1	Apaf-1-1

		XP_022244405.1	Apaf-1-2
	<i>Rhipicephalus microplus</i>	XP_037276898.1	Apaf-1
	<i>Rhipicephalus sanguineus</i>	XP_037506725.1	Apaf-1
Platyhelminthes	<i>Dibothriocephalus latus</i>	VDN16554.1	Apaf-1
	<i>Echinococcus granulosus</i>	XP_024354545.1	Apaf-1
	<i>Echinococcus_multilocularis</i>	CDI98465.1	Apaf-1
	<i>Hydatigera taeniaeformis</i>	VDM18691.1	Apaf-1
	<i>Macrostomum lignano</i>	PAA53960.1	Apaf-1-1
		PAA76335.1	Apaf-1-2
	<i>Mesocestoides corti</i>	VDD80977.1	Apaf-1
	<i>Schistosoma haematobium</i>	XP_035588882.1	Apaf-1
	<i>Schistosoma japonicum</i>	KAH8853391.1	Apaf-1
	<i>Spirometra erinacei</i>	VZI41625.1	Apaf-1
	<i>Sparganum proliferum</i>	VZI22297.1	Apaf-1
Lophotrocozoa	<i>Aplysia californica</i>	N/A	
	<i>Biomphalaria glabrata</i>	N/A	
	<i>Crassostrea angulata</i>	N/A	
	<i>Crassostrea gigas</i>	N/A	
	<i>Octopus bimaculoides</i>	N/A	
	<i>Capitella teleta</i>	N/A	
Vertebrata	<i>Homo sapiens</i>	XP_047284715.1	Apaf-1
	<i>Mus musculus</i>	NP_033814.2	Apaf-1
	<i>Canis lupus familiaris</i>	XP_038544278.1	Apaf-1
	<i>Salmo salar</i>	XP_014063984.1	Apaf-1
	<i>Gallus gallus</i>	XP_040518239.1	Apaf-1
	<i>Xenopus laevis</i>	NP_001085834.1	Apaf-1
	<i>Takifugu rubripes</i>	XP_011611631.2	Apaf-1
	<i>Tetraodon nigroviridis</i>	CAG01878.1	Apaf-1
Echinodermata	<i>Acanthaster planci</i>	XP_022083681.1	Apaf-1
	<i>Apostichopus japonicus</i>	PIK57251.1	Apaf-1
	<i>Asterias rubens</i>	XP_033645922.1	Apaf-1
	<i>Lytechinus variegatus</i>	XP_041472197.1	Apaf-1
		XP_041469796.1	Apaf-1 like1
		XP_041459069.1	Apaf-1 like2
	<i>Patiria miniata</i>	XP_038054179.1	Apaf-1
	<i>Patiria pectinifera</i>	AUQ44343.1	Apaf-1
	<i>Strongylocentrotus purpuratus</i>	XP_030844937.1	Apaf-1
		XP_030854919.1	Apaf-1 like1
		XP_030828596.1	Apaf-1 like2
Hemichordata	<i>Saccoglossus kowalevskii</i>	XP_006818297.1	Apaf-1
Cephalochordata	<i>Branchiostoma belcheri</i>	XP_019621685.1	Apaf-1
	<i>Branchiostoma floridae</i>	XP_035681983.1	Apaf-1
		XP_035678916.1	Apaf-1 like1
		XP_035672288.1	Apaf-1 like2
		XP_035690210.1	Apaf-1 like3
		XP_035667061.1	Apaf-1 like4
		XP_035696241.1	Apaf-1 like5

		XP_035693308.1	Apaf-1 like6
		XP_035673101.1	Apaf-1 like7
		XP_035692718.1	Apaf-1 like8
Urochordata	<i>Botryllus schlosseri</i>	N/A	
	<i>Ciona intestinalis</i>	N/A	
	<i>Ciona savignyi</i>	N/A	
	<i>Molgula oculata</i>	N/A	
	<i>Phallusia mammillata</i>	N/A	
	<i>Halocynthia roretzi</i>	N/A	

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Supplemental Table 3: List of Bcl-2 used for phylogenetic analysis.

Species		Accession number	Names
Porifera	<i>Amphimedon queenslandica</i>	XP_003383425.1	Bcl-2 like 1
		XP_003387574.1	Bcl-2 like 2
Cnidaria	<i>Hydra vulgaris</i>	EF104645.1	Bak 1
		EU035760.1	Bak 2
		XP_012562061.1	Bax
		EU035764.1	Bcl-2 like 1
		EF104646.1	Bcl-2 like 2
		EU035765.1	Bcl-2 like 3
		EU035763.1	Bcl-2 like 4
		EU035761.1	Bok
		EF104647.1	Mcl-1 1
		EU035762	Mcl-1 2
Nematoda	<i>Caenorhabditis elegans</i>	AAA20080.1	Ced-9
Insecta	<i>Drosophila melanogaster</i>	AAF44120.1	Buffy (Bok-1)
		AAF26289.1	Debcl (Bok-2)
Lophotrocozoa	<i>Biomphalaria glabrata</i>	XP_013085524.1	Bax 1
		XP_013096338_1	Bax 2
		XP_013086802.1	Bax 3
		XP_013070177.1	Bak
		XP_013081872.1	Bcl-2 like 1
		XP_013093137.1	Bcl-2 like 2
		XP_013068612.1	Bcl-2 like 3
		XP_013083436.1	Bcl-2 like 4
		XP_013069706.1	Bok
		XP_013065969.1	Mcl-1
	<i>Crassostrea gigas</i>	XP_011424481.1	Bax
		XP_011439700.1	Bak
		XP_011449013.1	Bcl-2 like 1
		ACH42081.1	Bcl-2 like 2
		EKC18663.1	Bcl-2 like 3
		XP_011436990.1	Mcl-1 1
		EKC40007.1	Mcl-1 2

Vertebrata	<i>Homo sapiens</i>	AAA74466.1	Bak
		NP_001278357.1	Bax
		API71171.1	Bcl-2
		AAK48715.1	Bcl-B
		AAB09055.1	Bcl-w
		CAA80661.1	Bcl-xL
		NP_004040.1	Bfl-1
		NP_115904.1	Bok
		AAF64255.1	Mcl-1
	<i>Mus musculus</i>	NP_031549.2	Bak
		NP_031553.1	Bax
		AAH95964.1	Bcl-2
		Q9Z0F3.1	Bcl-B
		AAA51039.1	Bcl-xL
		AAB09056.1	Bcl-w
		Q07440.1	Bfl-1
		NP_058058.1	Bok
		NP_032588.1	Mcl-1
	<i>Xenopus laevis</i>	NP_001089587.1	Bak
		AAR84081.1	Bax
		BAH28834.1	Bcl-2
		AAI10791.1	Bcl-xl
		XP_018089640.1	Bcl-w
		NP_001139563.1	Bok
		ACI47310.1	Mcl-1
Urochordata	<i>Ciona intestinalis</i>	KH2012:KH.C4.794.v1.A.SL1-1	Bax
		KH2012:KH.S653.2.v2.A.SL1-1	Bcl-xl
		KH2012:KH.L87.39.v1.A.ND1-1	Bok
	<i>Molgula oculata</i>	Moocul.CG.ELv1_2.S96550.g08147.01.p	Bax
		Moocul.CG.ELv1_2.S112899.g12639.01.p	Bok

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70 **Supplementary Table 4:** summary of species sampling across phylogenetic analyses. Species
71 used for an analysis are denoted by an “x”. As the basis of our study, the CARD-caspase
72 analysis determined the sampling strategy for other phylogenetic analyses. Species sampled
73 in the Bcl-2 analysis and the broad analysis containing all types of caspases are also present in
74 the sampling for the CARD-caspase only analysis. The sequences sampled for the Apaf-1
75 analysis based on those used in previous works are highlighted by a “*”. Other Apaf-1
76 sequences were identified in this study. Apaf-1 sampling comprise all species used to conduct
77 the CARD-caspases analysis. Among them, the ones not represented are species for which we
78 did not detected an Apaf-1 candidate in the genome (N/A). Concerning the Apaf-1 analysis,
79 we added several species including echinoderms and cnidarians to test the paraphyly of these
80 two groups. We were not able to detect Apaf-1 in any of the currently available NCBI genome
81 assemblies of molluscs, annelids, and urochordates. In order to prevent bias due to absence

82 of lophotrochozoan in the topology, we incorporated all Apaf-1 sequences that we detected
 83 in platyhelminths by searching all currently available flatworm genome assemblies.
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Species	All caspases	CARD-caspases	Bcl-2	Apaf-1
<i>Acanthaster planci</i>				x
<i>Acropora digitifera</i>		x		x
<i>Acropora millepora</i>				x
<i>Actinia tenebrosa</i>				x
<i>Aedes aegypti</i>				x*
<i>Agrilus planipennis</i>		x		x
<i>Amphimedon queenslandica</i>	x	x	x	x
<i>Apis mellifera</i>		x		x
<i>Aplysia californica</i>		x		N/A
<i>Apostichopus japonicus</i>				x
<i>Ascaris suum</i>	x	x		N/A
<i>Asterias rubens</i>				x
<i>Biomphalaria glabrata</i>		x	x	N/A
<i>Botryllus schlosseri</i>		x		N/A
<i>Branchiostoma belcheri</i>		x		x
<i>Branchiostoma floridae</i>		x		x*
<i>Bulinus truncatus</i>		x		N/A
<i>Caenorhabditis briggsae</i>				x*
<i>Caenorhabditis elegans</i>	x	x	x	x*
<i>Canis lupus familiaris</i>				x*
<i>Capitella teleta</i>		x		N/A
<i>Centruroides sculpturatus</i>				x
<i>Ciona intestinalis</i>		x	x	N/A
<i>Ciona savignyi</i>		x		N/A
<i>Crassostrea angulata</i>		x		N/A
<i>Crassostrea gigas</i>		x	x	N/A
<i>Daphnia pulex</i>				x
<i>Dendronephthya gigantea</i>				x
<i>Dermacentor andersoni</i>				x
<i>Dermacentor silvarum</i>				x
<i>Dibothriocephalus latus</i>				x
<i>Drosophila melanogaster</i>	x	x	x	x*
<i>Drosophila pseudoobscura</i>				x*
<i>Echinococcus granulosus</i>				x
<i>Echinococcus multilocularis</i>				x
<i>Exaiptasia pallida</i>		x		x
<i>Fimbriaphyllia ancora</i>				x
<i>Gallus gallus</i>	x	x		x*
<i>Geodia cydonium</i>		x		N/A
<i>Habropoda laboriosa</i>		x		x

<i>Halocynthia roretzi</i>		x		N/A
<i>Helicoverpa armigera</i>	x	x		x
<i>Holothuria leucospilota</i>		x		N/A
<i>Homo sapiens</i>	x	x	x	x
<i>Hydatigera taeniaeformis</i>				x
<i>Hydra vulgaris</i>	x	x	x	x
<i>Limulus polyphemus</i>	x	x		x
<i>Lytechinus variegatus</i>		x		x
<i>Macrostomum lignano</i>				x
<i>Mesocestoides corti</i>				x
<i>Molgula oculata</i>		x	x	N/A
<i>Mus musculus</i>		x	x	x
<i>Nematostella vectensis</i>				x*
<i>Octopus bimaculoides</i>	x	x		N/A
<i>Orbicella faveolata</i>				x
<i>Owenia fusiformis</i>		x		N/A
<i>Papilio machaon</i>		x		x
<i>Paramuricea clavata</i>				x
<i>Patiria miniata</i>				x
<i>Patiria pectinifera</i>				x
<i>Phallusia mammillata</i>		x		N/A
<i>Pleurobrachia bachei</i>		x		N/A
<i>Pocillopora damicornis</i>				x
<i>Rhipicephalus microplus</i>				x
<i>Rhipicephalus sanguineus</i>				x
<i>Saccoglossus kowalevskii</i>	x	x		x
<i>Salmo salar</i>		x		x
<i>Schistosoma haematobium</i>				x
<i>Schistosoma japonicum</i>				x
<i>Sparganum proliferum</i>				x
<i>Spirometra erinaceieuropaei</i>				x
<i>Strongylocentrotus purpuratus</i>		x		x*
<i>Stylophora pistillata</i>				x
<i>Takifugu rubripes</i>				x*
<i>Tetraodon nigroviridis</i>				x*
<i>Tribolium castaneum</i>		x		x*
<i>Trichoplax sp.</i>		x		x
<i>Xenopus laevis</i>		x	x	x*