

Additional file 1:

Table S1. Accession number of the different genes used in this study. Asterisks denotes the sequences used as templates in *ensembl* and NCBI databases.

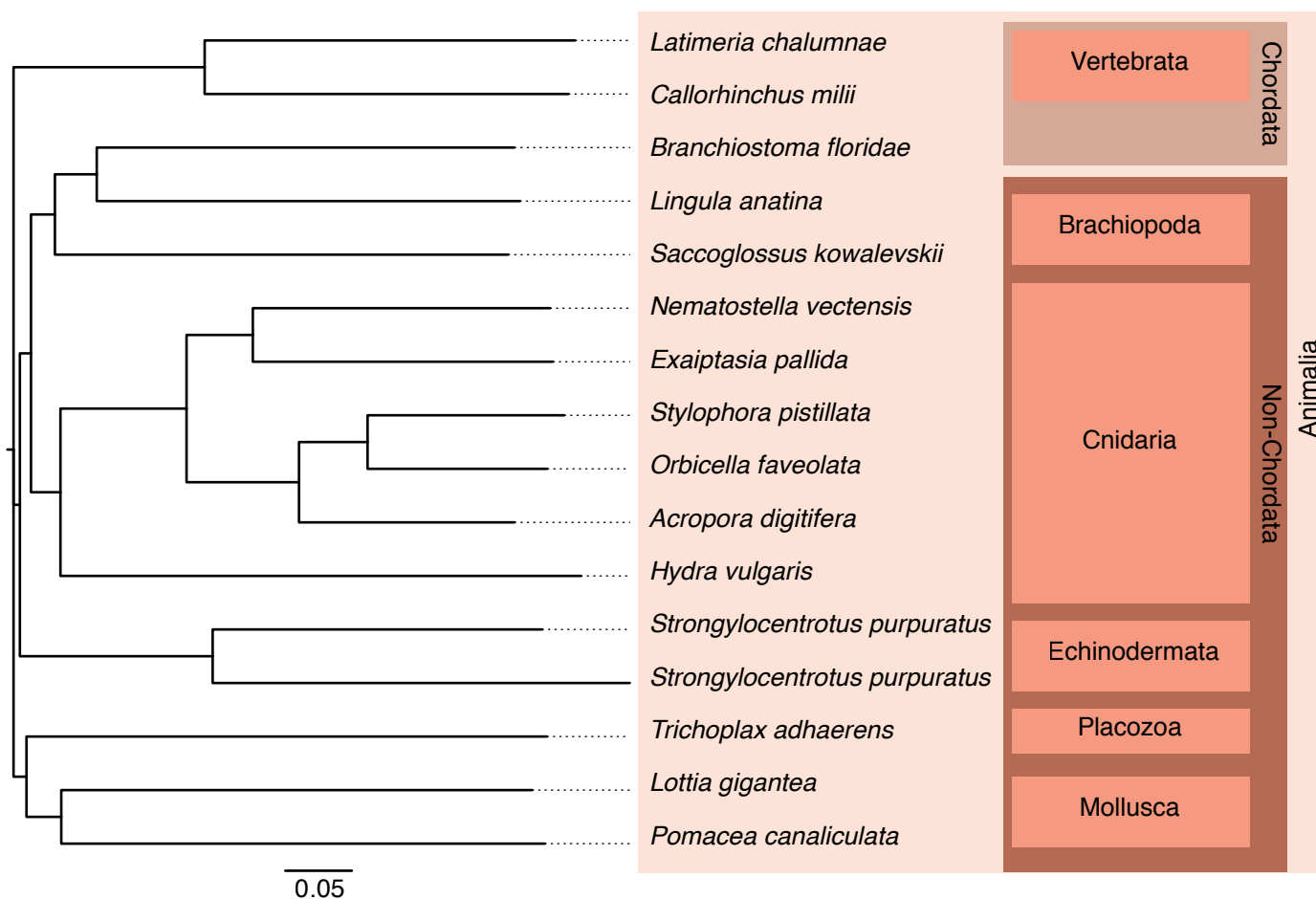
Filo/Class	Specie	Common name	Gene	Accession number
Chordata/ Mammalia	<i>Homo sapiens</i>	Human	<i>gsdma</i> * <i>gsdmb</i> * <i>gsdmc</i> * <i>gsdmd</i> * <i>gsdme</i> * <i>pjvk</i> *	Q96QA5 Q8TAX9 Q9BYG8 P57764 O60443 Q0ZLH3
	<i>Mus musculus</i>	Mouse	<i>gsdma1</i> <i>gsdma2</i> <i>gsdma3</i> <i>gsdmc1</i> <i>gsdmc2</i> <i>gsdmc3</i> <i>gsdmc4</i> <i>gsdmd</i> <i>gsdme</i> <i>pjvk</i>	Q9EST1 Q32M21 Q5Y4Y6 Q99NB5 Q2KHK6 Q8CB12 Q3TR54 Q9D8T2 Q9Z2D3 Q0ZLH2
	<i>Bos taurus</i>	Cow	<i>gsdma</i> <i>gsdmb</i> <i>gsdmc</i> <i>gsdmd</i> <i>gsdme</i> <i>pjvk</i>	F1MUE4 F1MCQ4 Q2KJ26 Q29S10 E1BFC3 E1BK64
	<i>Sus scrofa</i>	Pig	<i>gsdma</i> <i>gsdmb</i> <i>gsdmc</i> <i>gsdmd</i> <i>gsdme</i> <i>pjvk</i>	F1RXA6 I3LUA2 F1RRS0 F1SEV7 F1ST94 F1RZD1
	<i>Gorilla gorilla gorilla</i>	Gorilla	<i>gsdma</i> <i>gsdmb</i> <i>gsdmc</i> <i>gsdmd</i> <i>gsdme</i> <i>pjvk</i>	G3QQR5 G3QZ02 G3QNY2 A0A2I2ZBS6 G3QR26 G3R652
	<i>Neomonachus schauinslandi</i>	Seal	<i>gsdmax1</i> <i>gsdmax2</i> <i>gsdmb</i> <i>gsdmd</i> <i>gsdme</i> <i>pjvk</i>	XP_021560075 XP_021560077 XP_021560045 XP_021544405 XP_021558921 XP_021545343
	<i>Orcinus orca</i>	Whale	<i>gsdmb</i> <i>gsdmc</i> <i>gsdme</i> <i>pjvk</i>	XP_033275830 XP_033266659 XP_004265687 XP_033279542

Chordata/ Aves	<i>Ficedula albicollis</i>	Flycatcher	<i>gsdma</i> <i>gsdme</i> <i>pjvk</i>	U3JCH6 U3K4L7 U3JL98
	<i>Meleagris gallopavo</i>	Turkey	<i>gsdma</i> <i>gsdme</i> <i>pjvk</i>	H9H0H2 G3UR30 G1NB24
	<i>Gallus gallus</i>	Chicken	<i>gsdma</i> <i>gsdme</i> <i>pjvk</i>	H9L060 F1NP12 E1BYV7
Chordata/ Reptilia	<i>Anolis carolinensis</i>	Lizard	<i>gsdma</i> <i>gsdme</i> <i>pjvk</i>	G1KWU3 G1KNI3 H9GP49
	<i>Pelodiscus sinensis</i>	Turtle	<i>gsdma</i> <i>gsdme</i> <i>pjvk</i>	K7FU99 K7GG46 K7F4E5
Chordata/ Amphibian	<i>Xenopus Tropicalis</i>	African frog	<i>gsdme</i> <i>pjvk</i>	A0A7D9NJQ2 A0A6I8SVR2
Chordata/ Sarcopterygii	<i>Latimeria chalumnae</i>	Coelacant	<i>gsdme</i> <i>pjvk</i>	H3AEY8 H3A539
Chordata/ Osteichthyes	<i>Oryzias latipes</i>	Medaka	<i>gsdmea</i> <i>gsdmeb</i> <i>pjvk</i>	H2LKI2 H2LUH2 H2L2X9
	<i>Tetraodon nigroviridis</i>	Tetraodon	<i>gsdmea</i> <i>gsdmeb</i> <i>pjvk</i>	H3C1H7 H3C9T5 H3CZ40
	<i>Danio rerio</i>	Zebra fish	<i>gsdmea</i> <i>gsdmeb</i> <i>pjvk</i> *	E7F9U2 Q6J2R6 F8W4T7
	<i>Takifugu rubripes</i>	Japanese puffer	<i>gsdmea</i> <i>gsdmeb</i> <i>pjvk</i>	A0A3B5K424 H2UMH9 H2U9V8
	<i>Oreochromis niloticus</i>	Tilapia	<i>gsdmeb</i> <i>pjvk</i>	I3J4F1 I3JJG0
Chordata/ Chondrichthyes	<i>Callorhynchus milii</i>	Elephant shark	<i>gsdme</i> <i>pjvk</i>	K4FYP8 A0A4W3IS58
Chordata/ Hyperoartia	<i>Petromyzon Marinus</i>	Lamprey	<i>gsdm</i>	S4RLH7
Chordata/ Leptocardii	<i>Branchiostoma floridae</i>	Florida lancelet	<i>gsdm</i>	C3ZVN8
Non-Chordata/ Cnidaria	<i>Nematostella vectensis</i>	Anemone	<i>gsdm</i> *	A7T146
	<i>Exaiptasi pallida</i>	Anemone	<i>gsdm</i>	XP_020910515.1
	<i>Orbicella faveolata</i>	Coral	<i>gsdm</i>	XP_020607257.1
	<i>Stylophora pistillata</i>	Coral	<i>gsdm</i>	XP_022788280.1
	<i>Acropora digitifera</i>	Coral	<i>gsdm</i>	XP_015769608.1
	<i>Hydra vulgaris</i>	Fresh water polyp	<i>gsdm</i>	XP_012557585.1
Non-Chordata/ Brachiopoda	<i>Lingula anatina</i>	Marine worm	<i>gsdm</i>	A0A1S3HNP5
	<i>Saccoglossus kowalevskii</i>	Marine worm	<i>gsdm</i>	XP_006824139.1
	<i>Lottia gigantea</i>	Sea snail	<i>gsdm</i>	V4CLM6

Non-Chordata/ Mollusca	<i>Pomacea canaliculata</i>	Sea snail	<i>gsdm</i>	XP_025094681
	<i>Octopus bimaculoides</i>	Octopus	<i>gsdm</i>	A0A0L8I419
Non-chordata/ Echinodermata	<i>Strongylocentrotus purpuratus</i>	Purple sea urchin	<i>gsdm1</i> <i>gsdm2</i>	W4ZF48 XP_030830372.1
Non-chordata/ Placozoa	<i>Trichoplax adhaerens</i>		<i>gsdm</i>	B3RMM6

Figure S1

A



B

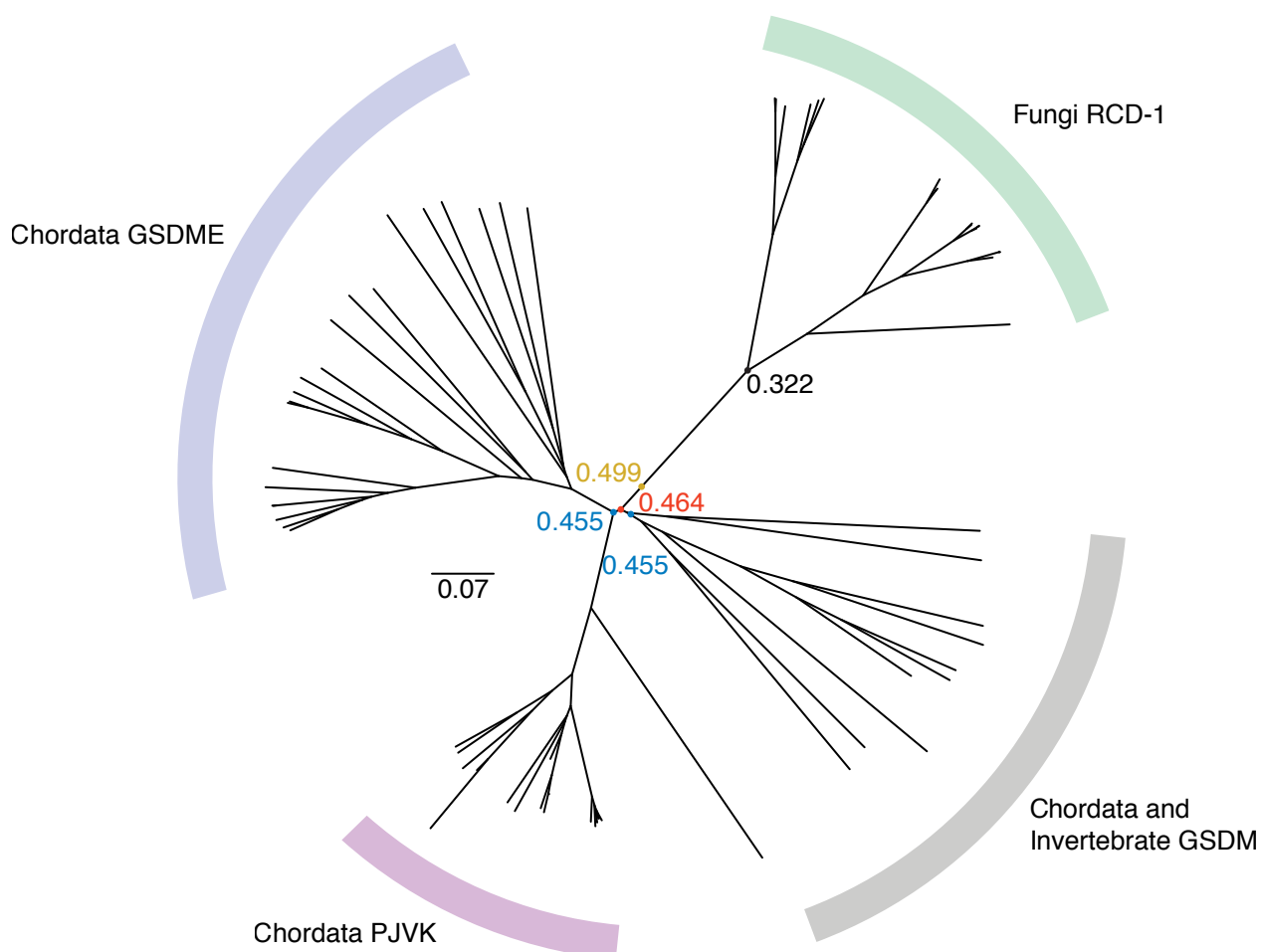


Figure S1. Phylogeny of the gasdermin gene family in invertebrates. (A)

Phylogenetic tree inferred from amino acid sequence data using the neighbor-joining method for the different gasdermin sequences found in invertebrates. Chordata *gsdme* sequences of coelacanth (*Latimeria chalumnae*) and shark (*Callorhinchus milii*) were also included in the analysis. **(B)** Phylogenetic tree inferred from amino acid sequence data using the neighbor-joining method for the different gasdermin sequences and fungal RCD-1 sequences as annotated.

Figure S2

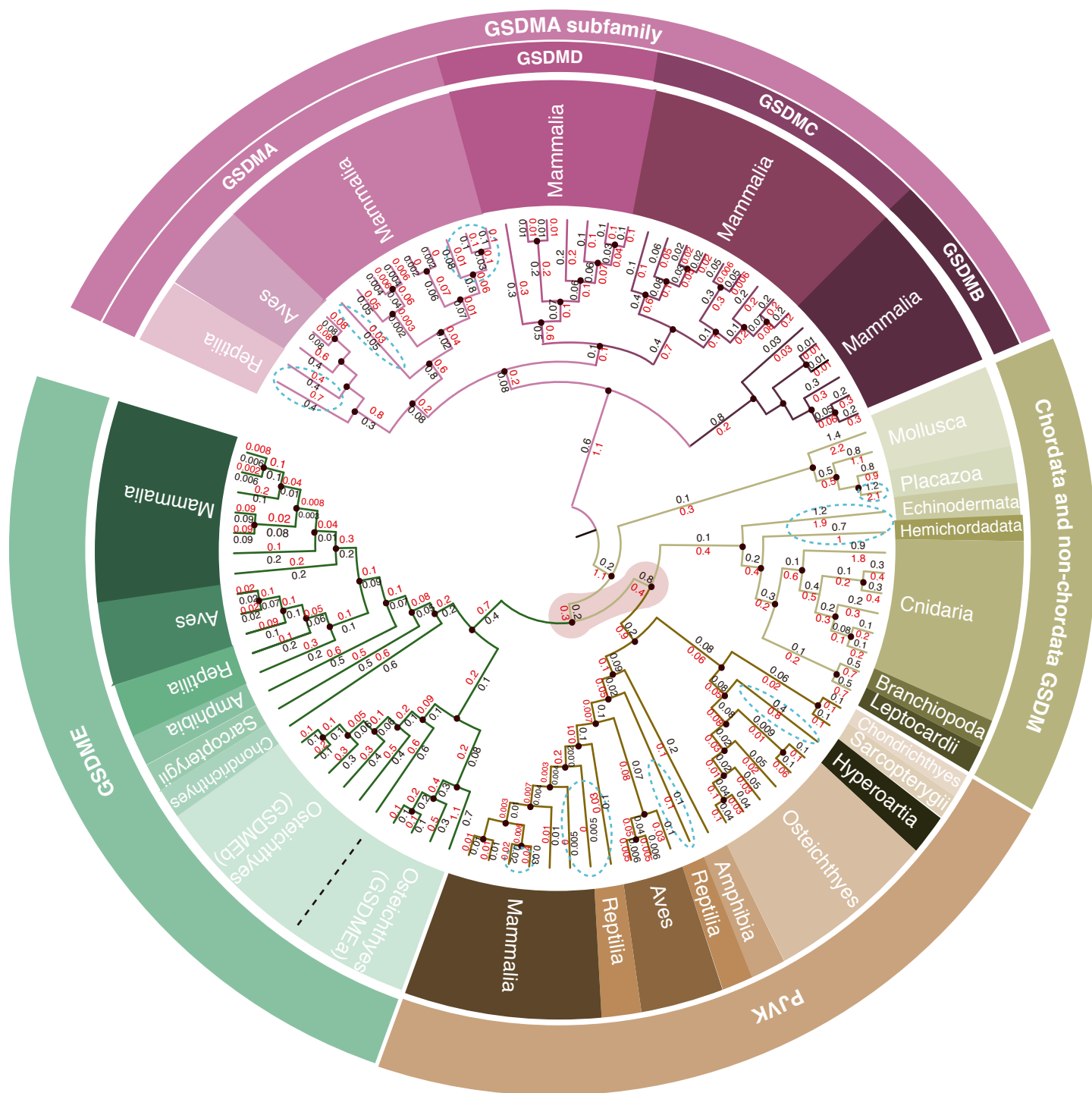


Figure S2. Phylogeny of the gasdermin family. Phylogenetic tree inferred from amino acid sequence data using the maximum likelihood method showing divergence of full-length gasdermins in mammals, birds, reptiles, amphibians, fish, jawless Chordata and non-Chordata. Black numbers represent relative branch length of Bayesian analysis and red numbers relative branch length of maximum likelihood. Light blue dashed circles show small changes between the Bayesian and maximum likelihood methods. Pink circle show how maximum likelihood analysis place PJVK sequences in the same node as the gasdermins from invertebrates and gasdermin E in a different node, which is the main difference when compared to the Bayesian phylogenetic tree.

Figure S3

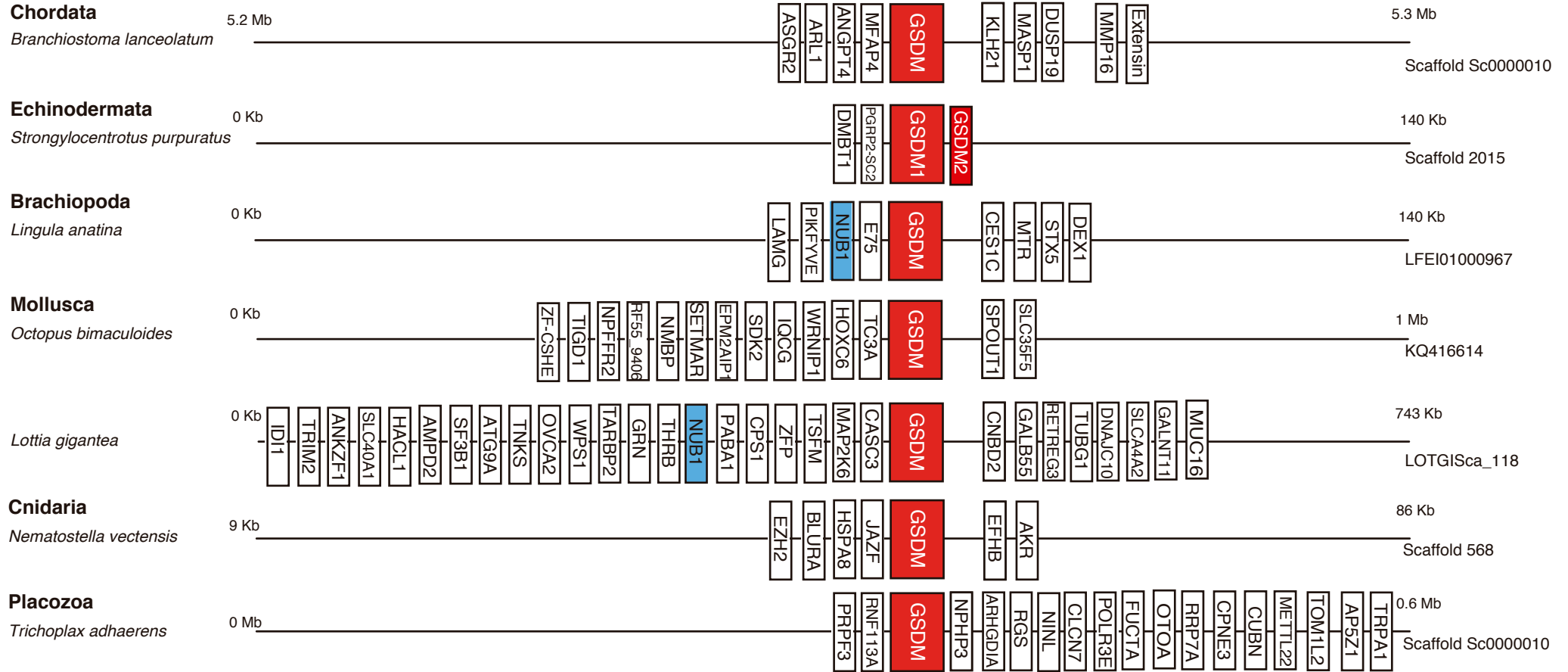


Figure S3. Synteny analysis of the gasdermin loci in early- and non-Chordata species. Schematic diagram showing the conservation of synteny in the *gsdm* loci of different early Chordata (*Branchiostoma lanceolatum*) and non-Chordata species (*Stongylocentrotus purpuratus*, *Lingula anatina*, *Octopus bimaculoides*, *Lottia gigantea*, *Nematostella vectensis* and *Trichoplax adhaerens*).

Figure S4

A

Sarcopterygii

Latimeria chalumnae



Osteichthyes

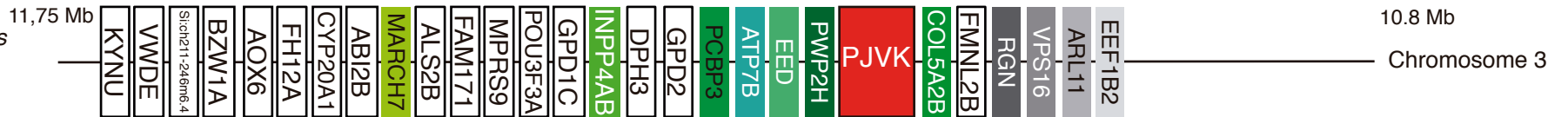
Danio rerio



Oreochromis niloticus



Tetraodon nigroviridis



Takifugu rubripes



B

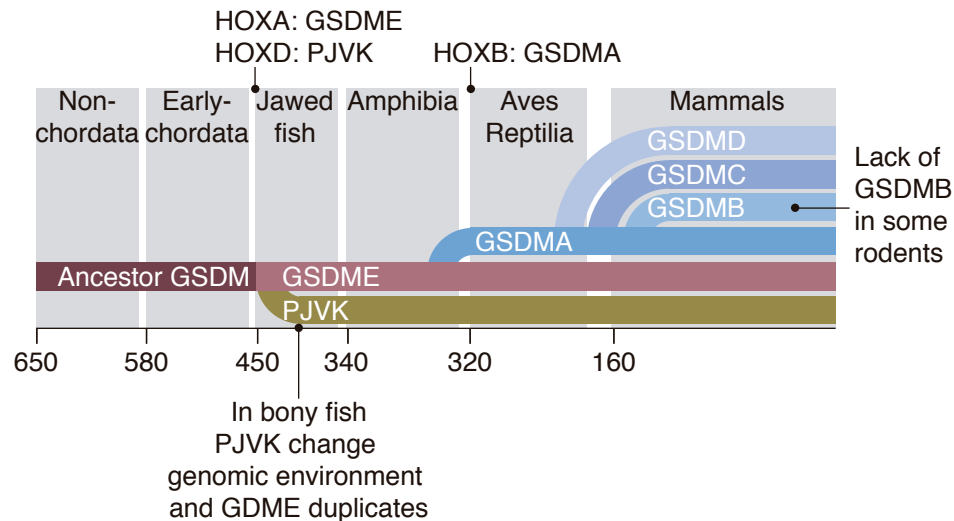


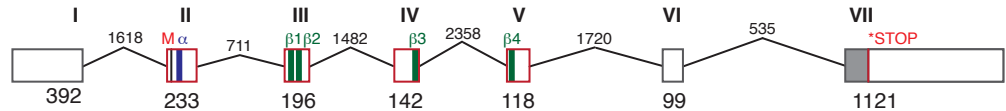
Figure S4. Synteny analysis of PJVK loci in fish. (A) Schematic diagram showing the conservation of synteny in the *pjvk* loci of different fish, including coelacanth (*Latimeria chalumnae*), zebra fish (*Danio rerio*), tilapia (*Oreochromis niloticus*), tetraodon (*Tetraodon nigroviridis*) and fugu (*Takifugu rubripes*). (B) Schematic representation of the evolution of gasdermins.

Figure S5

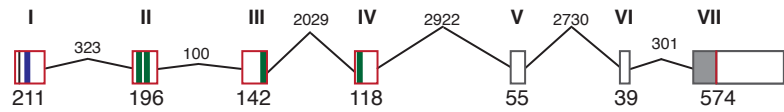
A

PJVK

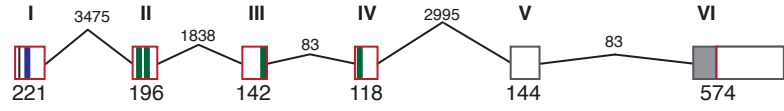
Mammalia

Homo sapiens

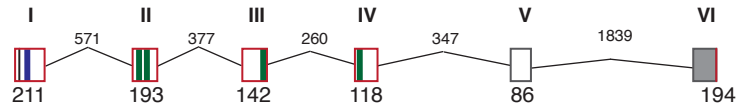
Sarcopterygii

Latimeria chalumnae

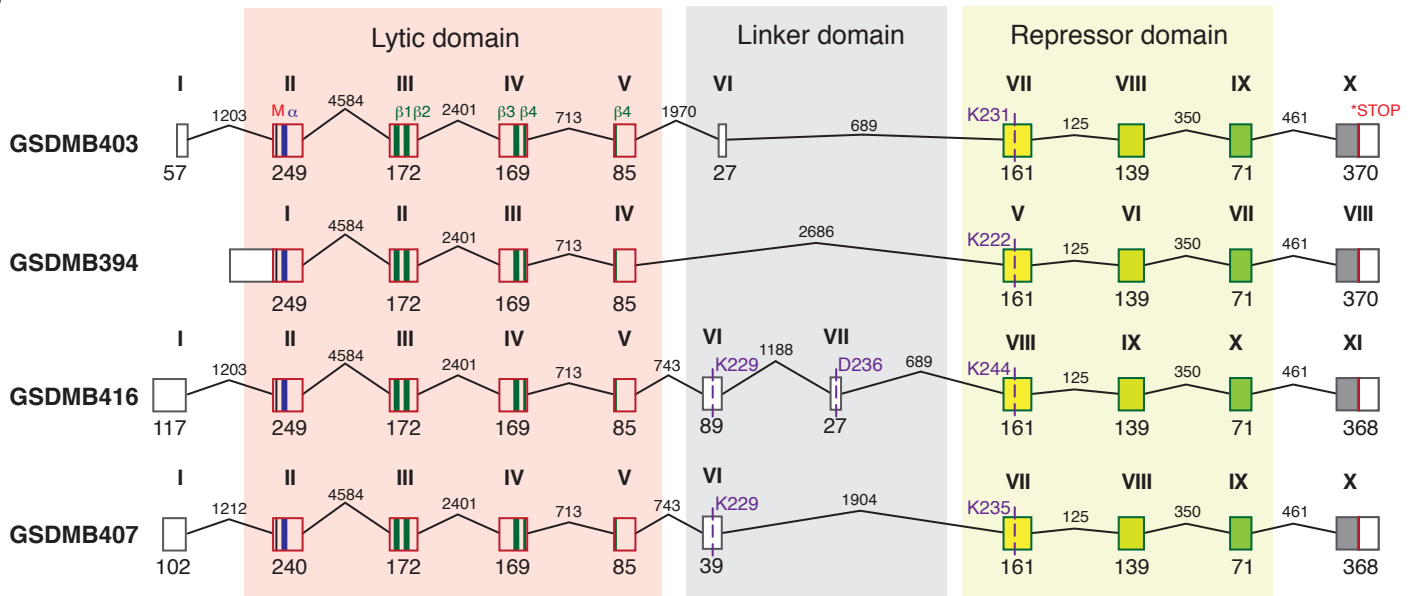
Osteichthyes

Danio rerio

Chondrichthyes

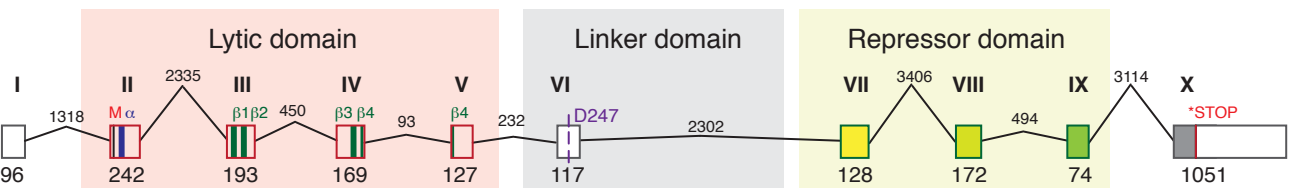
Callorhynchus milii

B

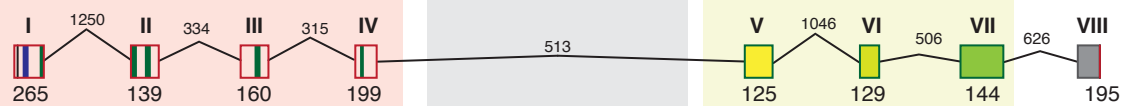


C

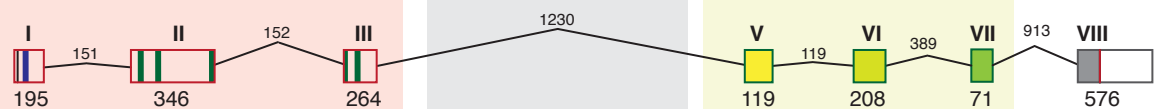
Chordata

Danio rerio
(GSDMEb)

Brachiopoda

Lingula anatina

Cnidaria

Nematostella vectensis

Mollusca

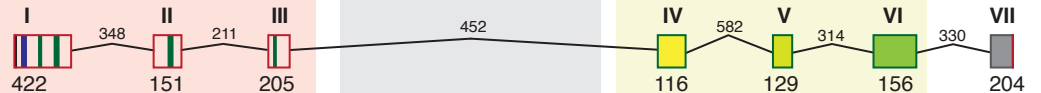
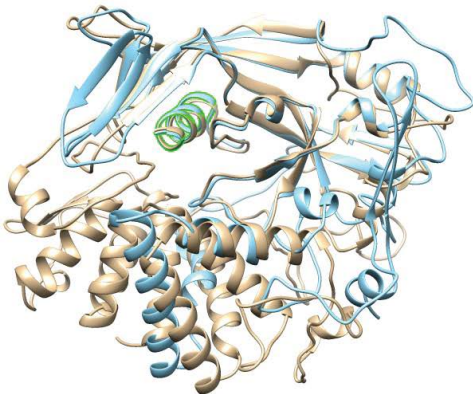
Octopus bimaculoides*Lottia gigantea*

Figure S5. Analysis of the exons and introns of different gasdermin genes. (A)

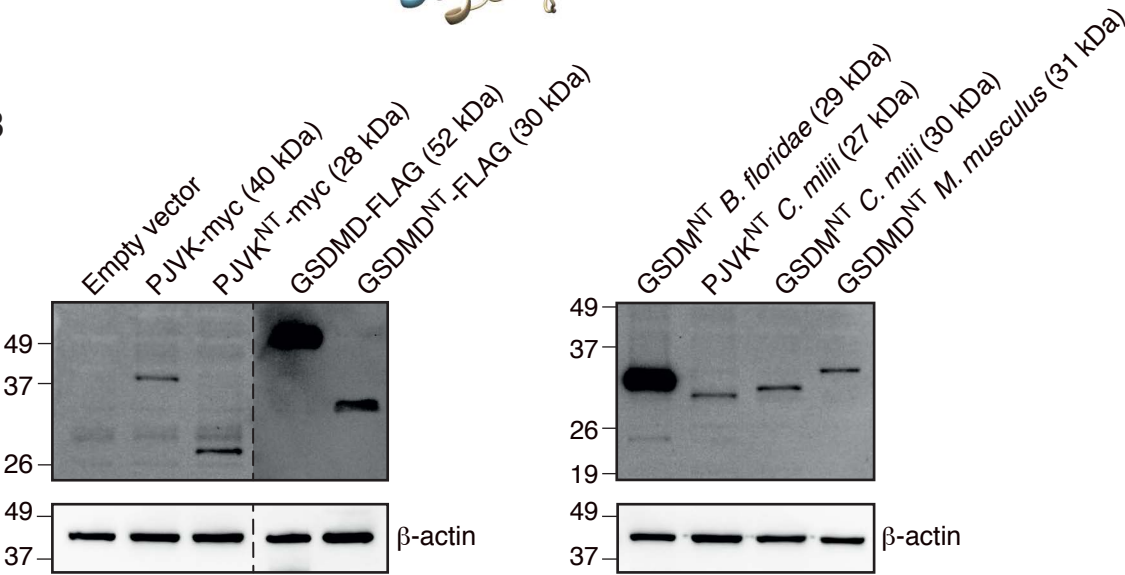
Schematic representation of the different exons and introns present in the genomic DNA of *pjvk* found in human (*Homo sapiens*), coelacanth (*Latimeria chalumnae*), zebra fish (*Danio rerio*) and shark (*Callorhinchus milii*). **(B)** Schematic representation of the different exons and introns of the different human GSDMB splice variants; the granzyme A cleavage site at K²²⁹, K²³¹, K²²² and K²³⁵ and the caspase-1 cleavage site (D²³⁶) are shown. **(C)** Schematic representation of the different exons and introns found in zebra fish (*Danio rerio*) *gsdmeb* and in non-Chordata gasdermin from Brachiopoda (*Lingula anatina*), Cnidaria (*Nematostella vectensis*) and Mollusca (*Octopus bimaculoides* and *Lottia gigantea*). In **A**, **B** and **C** the first methionine is indicated with a black line, the stop codon with a red line, the first α -helix of the N-terminal domain is indicated with a blue bar and the four β -sheets are indicated with green bars. The exons of the repressor C-terminal domain are represented with different colors to show the conserved similarity between them.

A

H. sapiens PJKV vs
H. sapiens GSDME



B



C

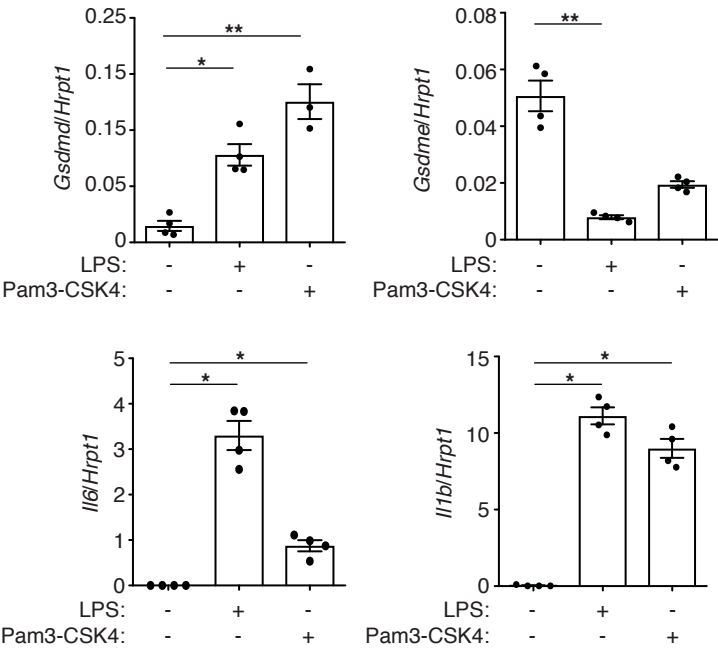


Figure S6. Molecular models of the full-length human PJVK and GSDME proteins. (A) Structural alignment of human GSDME (brown) and human PJVK (Cyan) showing 27.1% similarity. Green represents the first α -helix of the N-terminal domain. Both gasdermins have been generated using an intensive modelling mode with Phyre² software. (B) Immunoblot for anti-myc (to detect myc tagged *H. sapiens* PJVK and PJVK^{NT}, *B. floridae* GSDM^{NT}, *C. milii* PJVK^{NT} and GSDM^{NT}, and *M. musculus* GSDMD^{NT}), anti-FLAG (to detect FLAG tagged *H. sapiens* GSDMD and GSDMD^{NT}) and β -actin in cell lysates from transfected HEK293; numbers on the left correspond to the molecular weight marker expressed in kDa. (C) Expression of *Gsdmd*, *Gsdme*, *Il6* and *Il1b* from mouse bone marrow derived macrophages by quantitative PCR after incubation for 4h with LPS or Pam3-CSK4.