Hm-MyD88 and *Hm*-SARM: Two key regulators of the neuroimmune system and neural repair in the medicinal leech.

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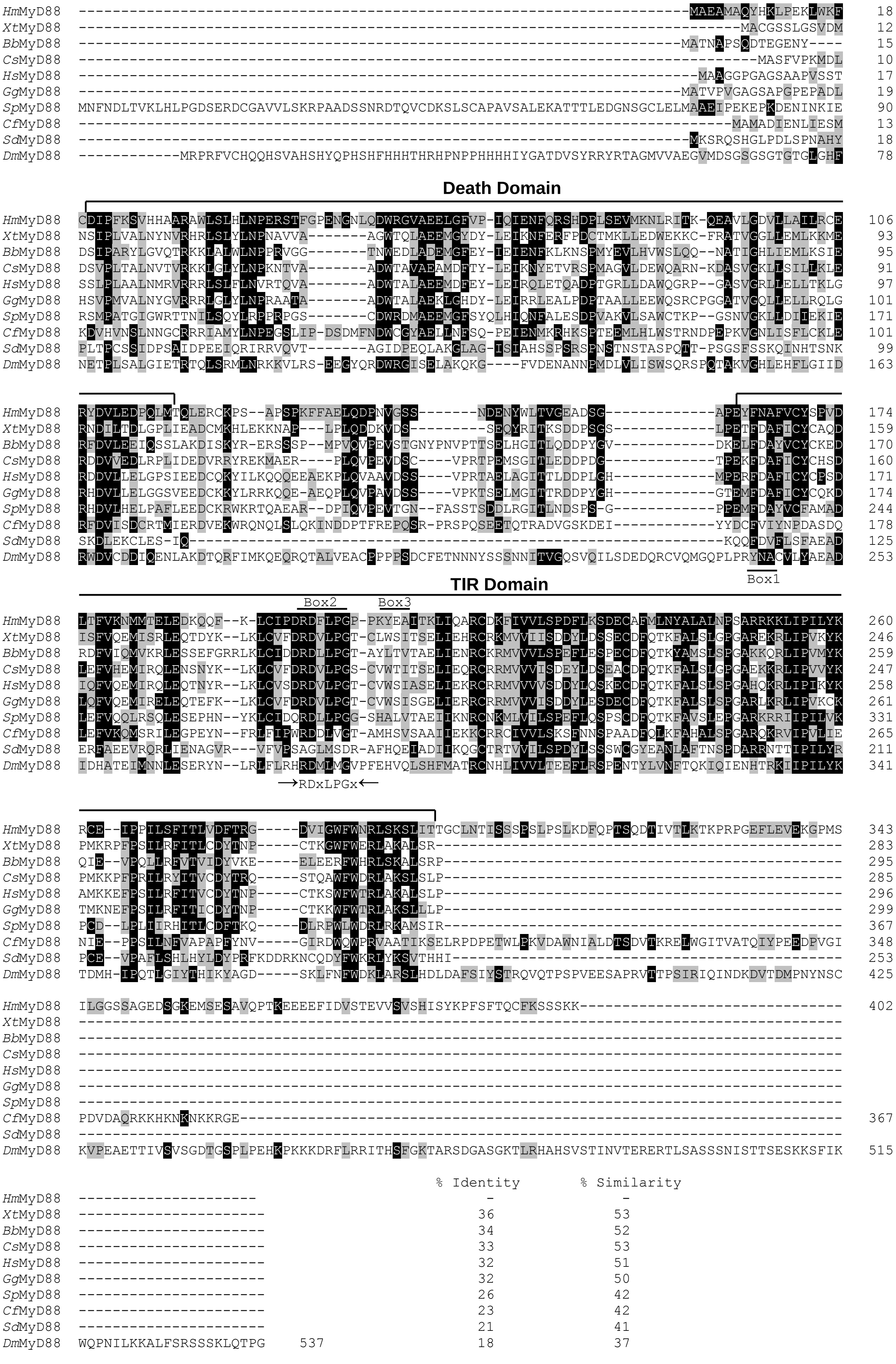
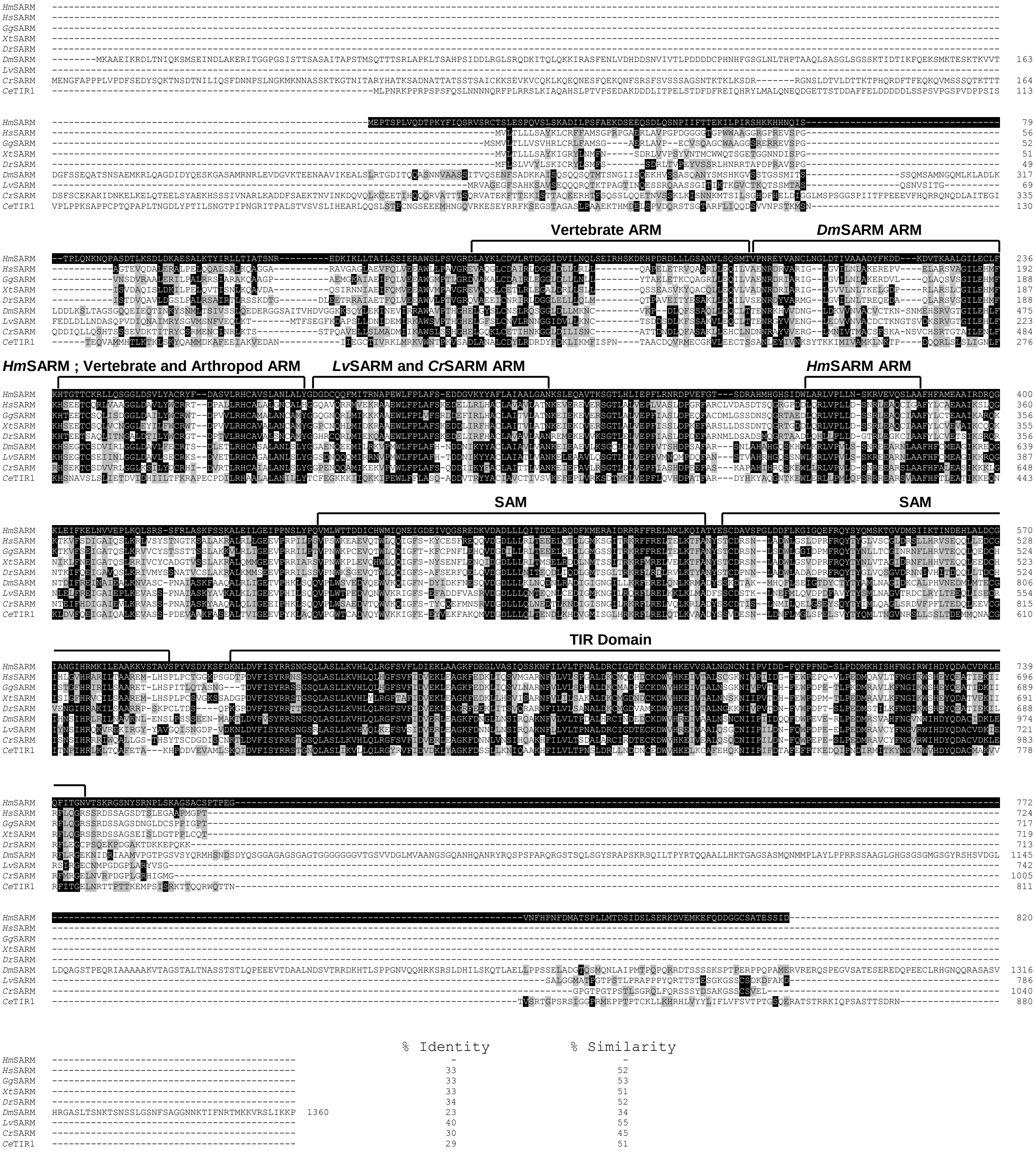

HmMyD88
XtMyD88
BbMyD88
CsMyD88
HsMyD88
GgMyD88
SpMyD88
CfMyD88
SdMyD88
DmMyD88
Death Domain
TIR Domain
Box1
Box2
Box3
→RDxLPGx←
% Identity
% Similarity
HmMyD88 - -
XtMyD88 36 53
BbMyD88 34 52
CsMyD88 33 53
HsMyD88 32 51
GgMyD88 32 50
SpMyD88 26 42
CfMyD88 23 42
SdMyD88 21 41
DmMyD88 18 37

Supplementary Material 1: Comparison of the amino acid sequence of *Hirudo medicinalis* MyD88 (*Hm*MyD88) with vertebrate and invertebrate MyD88 orthologs.

Alignment was generated by using the program CLUSTAL W. Identical residues with *Hm*-MyD88 are shaded in black, homologous residues in grey. Gaps (-) were introduced to optimize sequence alignment. →RDxLPGx← indicates the key motif for MyD88 dimerization and its interaction with TLRs. Accession numbers in EMBL/Genbank: *Xt*MyD88: *Xenopus tropicalis* (NP_001016837); *Bb*MyD88: *Branchiostoma belcheri* (ABQ32299); *Cs*MyD88: *Cynoglossus semilaevis* (ACU31062.1); *Hs*MyD88: *Homo sapiens* (Q99836.1); *Gg*MyD88: *Gallus gallus* (A5HNF6.1); *Sp*MyD88: *Strongylocentrotus purpuratus* (XP_780590); *Cf*MyD88: *Chlamys farreri* (ABB76627); *Sd*MyD88: *Suberites domuncula* (Q4W1E7); *Dm*MyD88: *Drosophila melanogaster* (NP_610479).



	% Identity	% Similarity
*Hm*SARM	-	-
*Hs*SARM	33	52
*Gg*SARM	33	53
*Xt*SARM	33	51
*Dr*SARM	34	52
*Dm*SARM	23	34
*Lv*SARM	40	55
*Cr*SARM	30	45
*Ce*TIR1	29	51

Supplementary Material 2: Comparison of the amino acid sequence of *Hirudo medicinalis* SARM (*Hm*-SARM) with vertebrate and invertebrate SARM orthologs.

Alignment was generated by using the program CLUSTAL W. Identical residues with *Hm*SARM are shaded in black, homologous residues in grey. Gaps (-) were introduced to optimize sequence alignment. Accession numbers in EMBL/Genbank: *Hs*SARM: *Homo sapiens* (Q6SZW1); *Gg*SARM: *Gallus gallus* (A5HNF6.1); *Xt*SARM: *Xenopus tropicalis* (XM_002937143.2); *Dr*SARM: *Danio rerio* (B3DK97); *Dm*SARM: *Drosophila melanogaster* (Q6IDD9); *Lv*SARM: *Litopenaeus vannamei* (**G8GV23);** *Cr*SARM: *Carcinoscorpius rotundicauda* (A9X3T4); *Ce*TIR-1: *Caenorhabditis elegans* (Q86DA5).

H. medicinalis myd88

Intron	5'-Donor	3'-Donor	Intron phase
1	G gtatgtg Glu	ttttcag AA Glu	1
2	G gtttaac Val	aattaag TT Val	1
3	G gtgcgta Ala	tttttag CT Ala	1
4	AG gtaggtc Arg	ttatcag G Arg	2
5	A gtaagtc Ser	tatttag GT Ser	1

Deuterostoma
Vertebrates

H. sapiens myd88

Intron	5'-Donor	3'-Donor	Intron phase	*H. medicinalis* I/E junctions
1	G gtgaggga Glu	cccacag AG Glu	1	
2	G gtaaggg Gly	cacccag GG Gly	1*	i3
3	AG gttggct Arg	cccctag G Arg	2*	i4
4	G gtaagct Gly	cttgcag GT Gly	1*	i5

D. rerio myd88

Intron	5'-Donor	3'-Donor	Intron phase	*H. medicinalis* I/E junctions
1	G gtacgtc Asp	tgcacag AT Asp	1*	i1
2	G gtcagtg Gly	tcaacag GA Gly	1*	i3
3	AG gcatgtt Arg	ttcttag G Arg	2*	i4
4	G gtacaaa Gly	tcttcag GA Gly	1*	i5

G. gallus myd88

Intron	5'-Donor	3'-Donor	Intron phase	*H. medicinalis* I/E junctions
1	G gtgagcc Glu	tttgcag AG Glu	1	
2	G gtgagtg Gly	cttacag GG Gly	1*	i3
3	AG gtgagtg Arg	tctgaag G Arg	2*	i4
4	G gtaagat Gly	ctttcag GG Gly	1*	i5

X. tropicalis myd88

Intron	5'-Donor	3'-Donor	Intron phase	*H. medicinalis* I/E junctions
1	G gtaagta Glu	gttacag AG Glu	1*	i1
2	G gtacgcc Gly	cttacag GG Gly	1*	i3
3	CG gtaaggg Arg	gttatag G Arg	2*	i4
4	G gtaagat Gly	cttacag GG Gly	1*	i5

Protostoma
Ecdyzozoa

D. melanogaster myd88

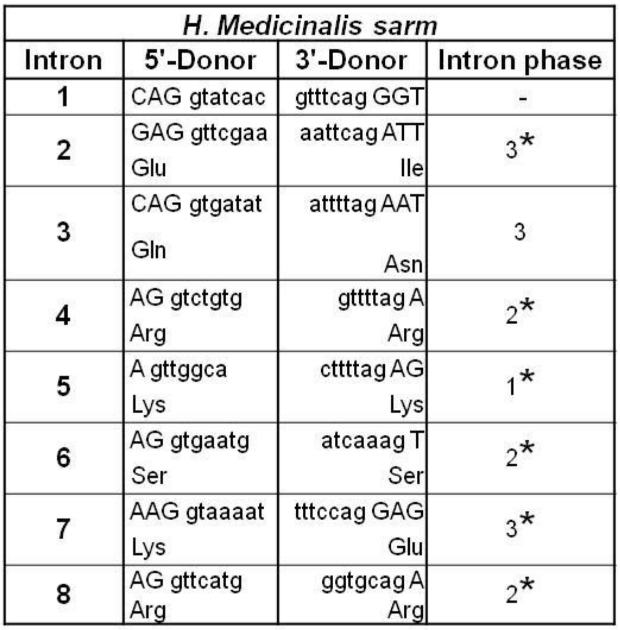
Intron	5'-Donor	3'-Donor	Intron phase	*H. medicinalis* I/E junctions
1	G gtaggca Ala	tcaaaag CC Ala	1	
2	AGG gtgagtg Arg	cttacag CTA Leu	3	
3	G gtgagta Glu	tccccag AG Glu	1*	i4
4	AC gtaagtg Thr	tatgcag T Thr	1	

L. vannamei myd88

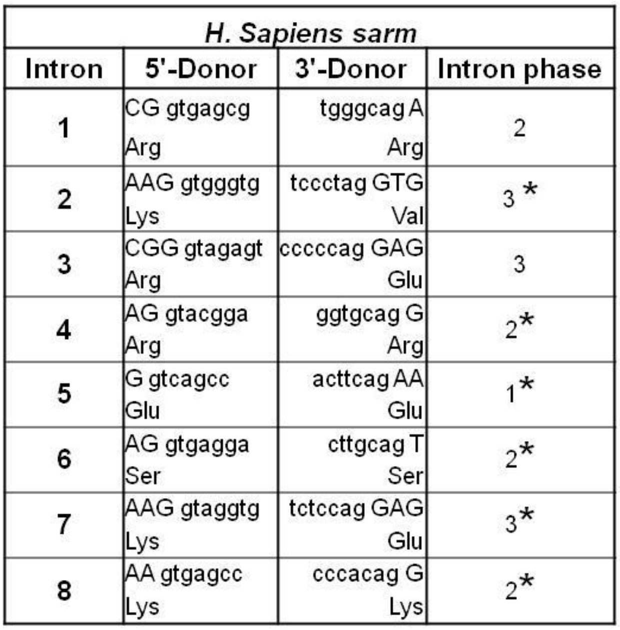
Intron	5'-Donor	3'-Donor	Intron phase	*H. medicinalis* I/E junctions
1	AG gtgaata Arg	tttacag A Arg	2	
2	T gtaatat Tyr	tttacag AT Tyr	1 *	i1
3	G gtaagga Asp	attgcag AT Asp	1*	i3
4	AAG gtaagt Lys	cttgcag CTG Leu	3	
5	AG gtaacgt Ser	ctttcag C Ser	2	
6	GAT gtaagt Asp	tcagcag TCT Ser	3	

<u>Supplementary Material 3:</u> Intron-exon boundaries of the leech, vertebrates and ecdyzozoa *myd88* genes.

The sequence of each of the intron-exon boundaries is shown, as well as the codons for the amino acid residues. Uppercase and lowercase letters represent nucleotides in the exons and introns, respectively. Asterisks (*) indicate that the position of an intron-exon junction is the same in leech and a vertebrate or an ecdyzozoa gene. The column entitled “*H. medicinalis* I/E junctions” point out which leech intron-exon junction is conserved in vertebrate or ecdyzozoa genes.

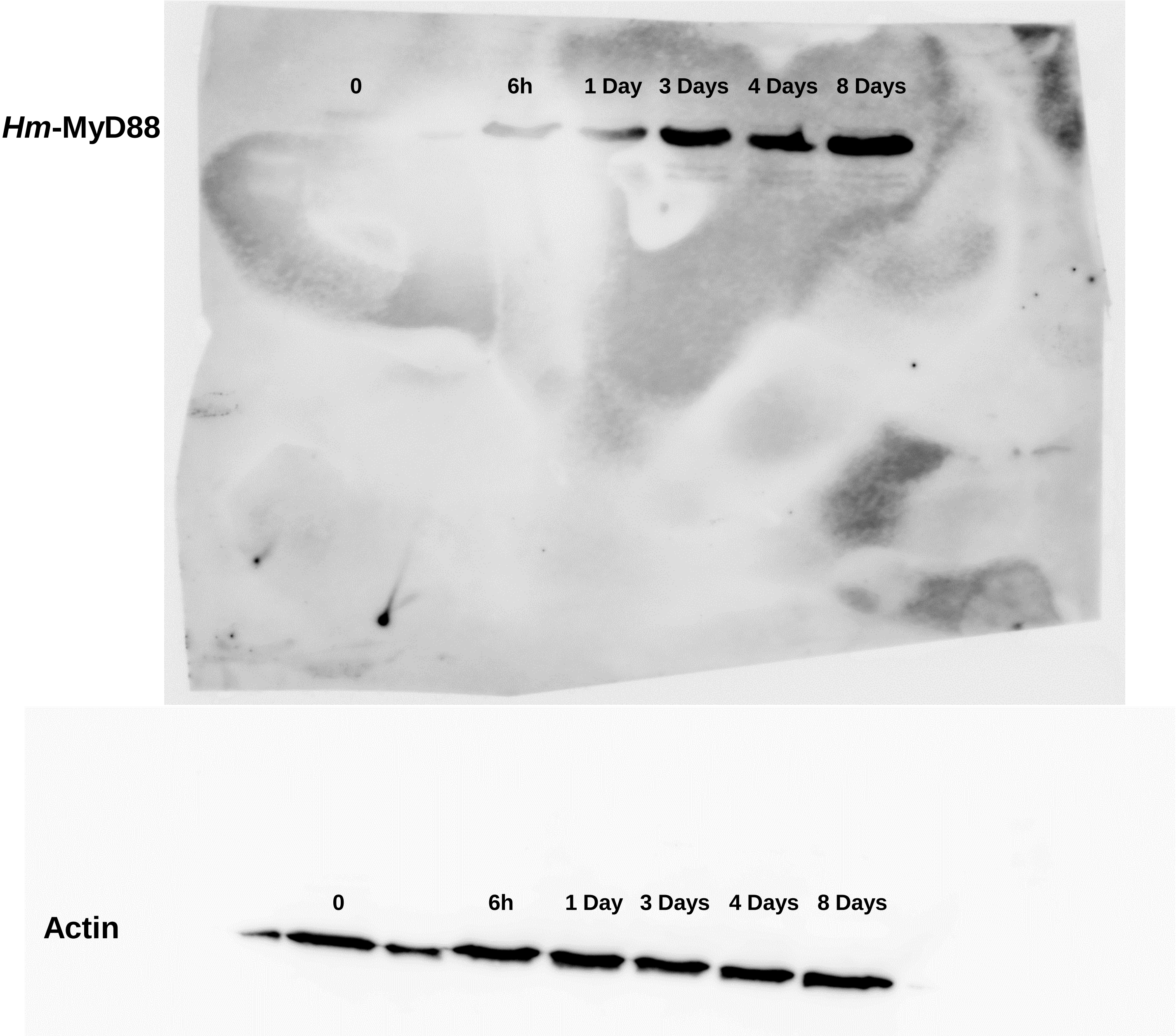


H. Medicinalis sarm			
Intron	**5'-Donor**	**3'-Donor**	**Intron phase**
1	CAG gtatcac	gtttcag GGT	-
2	GAG gttcgaa Glu	aattcag ATT Ile	3*
3	CAG gtgatat Gln	attttag AAT Asn	3
4	AG gtctgtg Arg	gttttag A Arg	2*
5	A gttggca Lys	cttttag AG Lys	1*
6	AG gtgaatg Ser	atcaaag T Ser	2*
7	AAG gtaaaat Lys	tttccag GAG Glu	3*
8	AG gttcatg Arg	ggtgcag A Arg	2*



H. Sapiens sarm			
Intron	**5'-Donor**	**3'-Donor**	**Intron phase**
1	CG gtgagcg Arg	tgggcag A Arg	2
2	AAG gtgggtg Lys	tccctag GTG Val	3 *
3	CGG gtagagt Arg	cccccag GAG Glu	3
4	AG gtacgga Arg	ggtgcag G Arg	2*
5	G gtcagcc Glu	acttcag AA Glu	1*
6	AG gtgagga Ser	cttgcag T Ser	2*
7	AAG gtaggtg Lys	tctccag GAG Glu	3*
8	AA gtgagcc Lys	cccacag G Lys	2*

Supplementary Material 4: Intron-exon boundaries of the leech and human *sarm* genes. The sequence of each of the intron-exon boundaries is shown, as well as the codons for the amino acid residues. Uppercase and lowercase letters represent nucleotides in the exons and introns, respectively. Asterisks (*) indicate that the position of an intron-exon junction is the same in leech and human genes and has the same intron phasing.



<u>Supplementary Material 5:</u> Unprocessed original scans for the blots in figure 10 (A) Kinetics of expression in the course of neural regeneration.

Hm-MyD88 protein expression was assessed by western blot in isolated nerve cords cultured *ex vivo* for up to 8 days under sterile conditions. Samples were collected at 0 h (T0), 6 h, 1 day, 3 days and 8 days. Membrane was incubated with anti-*Hm*-MyD88. To assess that an equal amount of proteins was loaded on gels, membrane was stripped and reprobed with anti-Actin.

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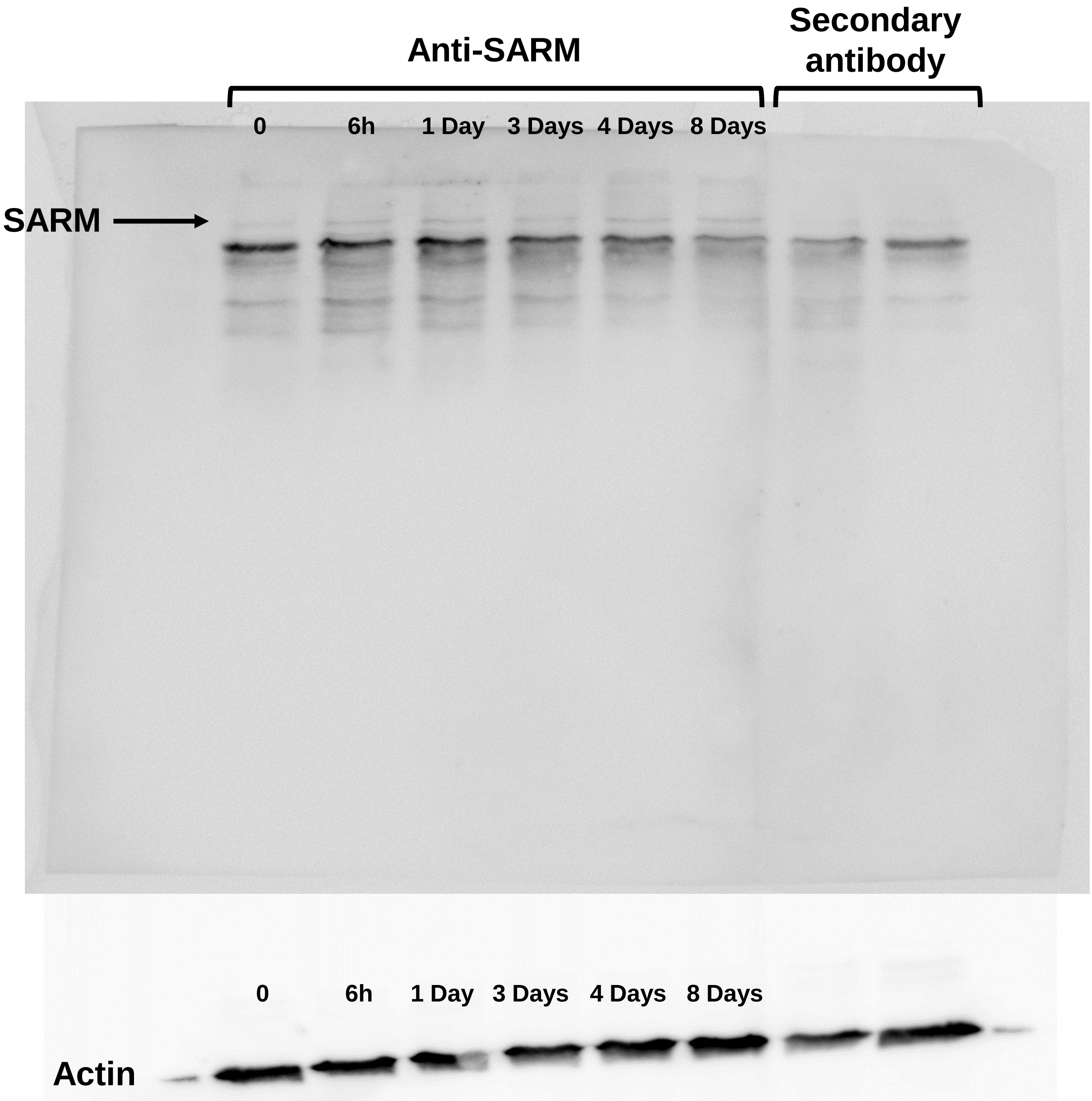

Supplementary Material 6: Unprocessed original scans for the blots in figure 10 (B) Kinetics of expression in the course of neural regeneration.

Hm-SARM protein expression was assessed by western blot in isolated nerve cords cultured *ex vivo* for up to 8 days under sterile conditions. Samples were collected at 0 h (T0), 6 h, 1 day, 3 days and 8 days. Membrane was incubated with anti-SARM. As a control, a membrane was incubated with the secondary antibody alone. To assess that an equal amount of proteins was loaded on gels, membrane was stripped and reprobed with anti-Actin.