

2 **Enzymatic decontamination of paraoxon-ethyl**
3 **limits long-term effects in planarians**

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11 **Supplementary table 1.** Multiple sequence alignment of planarian cholinesterases (Smed_4101, Smed_4800, Smed_10334,
 12 Smed_14790 and Smed_15930) with acetylcholinesterase isoform E4-E5 precursor (NP_056646.1) and butyrylcholinesterase
 13 precursor (NP_000046.1) using Clustal 2.1. Catalytic triad is indicated in red.

Identity (%)	NP_000046.1	NP_056646.1	Smed_4101	Smed_4800	Smed_10334	Smed_14790	Smed_15930
NP_000046.1	-	46.0133	27.3743	25.7475	22.4252	23.588	23.588
NP_056646.1		-	23.2775	23.825	23.1767	23.6629	24.9595
Smed_4101			-	25.5121	22.5326	24.0223	21.9739
Smed_4800				-	29.5238	37.8086	29.7297
Smed_10334					-	24.6032	26.6667
Smed_14790						-	28.3951
Smed_15930							-

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15 **CLUSTAL 2.1 multiple sequence alignment**

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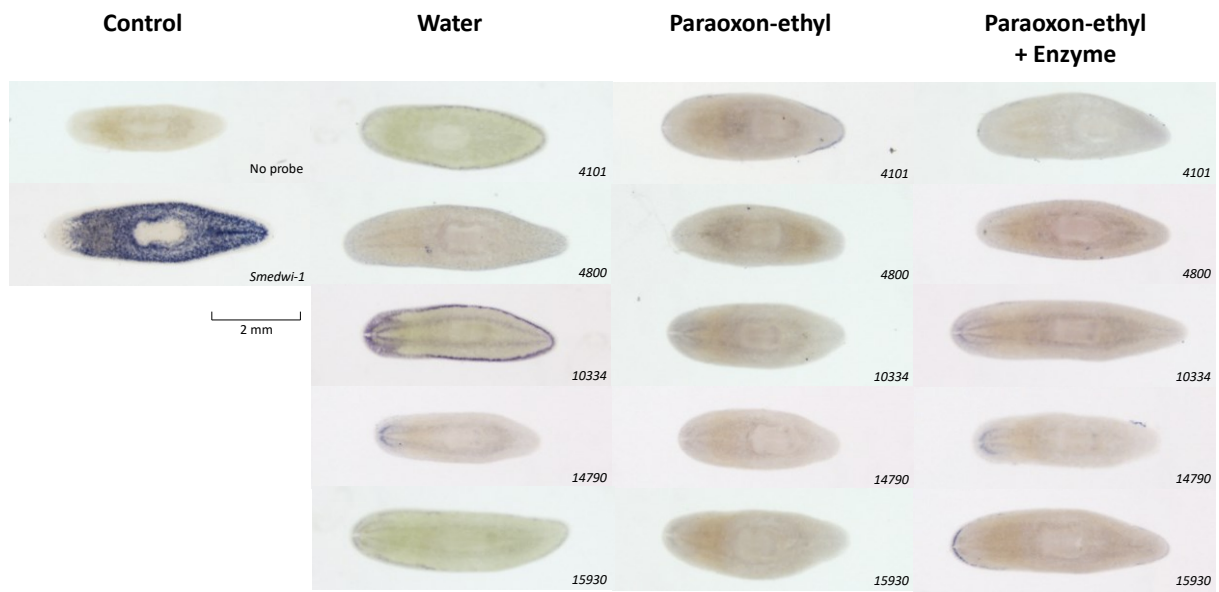
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18 NP_000046.1   -----MHSKVTIICIRFLWFLLLCMLIGKSHTEDDII I A T K N G K V R G M N L T V F - G G
19 NP_056646.1   -MRPPQCLLHTPSLASPLLLLLLWLLGGVGAEGREDAELLVTVRGGRLRGIRLKTP-GG
20 Smed_15930    -----MEFWSYSLWIFVWVTRGLTFNRNGEYVTVSTTNGNIMGIKKDVL-GK
21 Smed_10334    -----MEFVSFIVLLTAYSAVSYPI T P N Y T P S V R T K I G T I N G F I S K V E W N D R S A T
22 Smed_4101     -----MLNFSVSNGLVQGGQTRKVLHR
23
24
25 Smed_4800      T I D H F A R I P Y A L P P I N N L R F K Y P Q E I V G N Q W K G V Y N A T T S P N T C W Q V E - G G E F D K L N P A A
26 Smed_14790    K I F H F S K I P Y A K P P I G N R R F R Y P E K L N N P P W T G V Y D S T V Q P L T C W Q G A P S I E F E L K N P I G
27 NP_000046.1   T V T A F L G I P Y A Q P P L G R L R F K K P Q S L -- T K W S D I W N A T K Y A N S C C Q N I D Q S F P G F H G -- S
28 NP_056646.1   P V S A F L G I P F A E P P M G P R R F L P P E P K -- Q P W S G V V D A T T F Q S V C Y Q Y V D T L Y P G F E G -- T
29 Smed_15930    P V Y V F L G I P Y A K P P V G R L R F R N P E L V N -- K W E G T Y S A T K L P K T C Y Q N L P E H Y E L N N P - A W
30 Smed_10334    T V H I Y Y G I P Y A I P P V K N L R F K K P V P F P - E I S E K V V N A Q T H K P S C H Q Y N D T S Y L N T A G -- A
31 Smed_4101     D I A E F L S I P Y C K P P I G E L R F Q K P P F S -- S F S K S I I N R G K F D T C P P Q L P M R I F N G E N L D E
32
33
34 Smed_4800      R L W L S K T P M S E D C L Y L T I W S P R T ----- V K T K L P V M V W I Y G G S F F G S S T L E V Y
35 Smed_14790    K M W F S K T V M S E D C L Y L T I W T P Q I N V ----- A N E L L P V M I W I Y G G S Y M S G S S T L E V Y
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37 NP_056646.1   E M W N P N R E L S E D C L Y L N V W T P Y P R ----- P T S P T P V L V W I Y G G G F Y S G A S S L D V Y
38 Smed_15930    R I W T N N T E M S E D C L Y L S I W T P K I N K N Q N R K M - F N E K F I I S T L V W I H G G G F T G G S S T L D I Y
39 Smed_10334    R M W V S P I P F D E D C L Y L N I W V P Q N P A P D V H K M N Q K T T D K L A V M V W I Y G G S F A S G A A G L E V Y
40 Smed_4101     E T W I P K E R Q D E N S L Y V N V W C P L E I L K ----- E K E N Q P V L V W I Y G G G Y V S G S S S L E I Y
41
42
43 Smed_4800      D G S I L A A K H E V V V S L Q Y R L G P L G F L Y L D D T L - A P G N Q G L M D Q R M A L K W V K D N I Y N F N G D
44 Smed_14790    D G S I L A S L H N V V I V S F Q F R S G P L G F L Y L D D T E - S P G N Q A I M D Q H M L L K W V N N H I V S F G G D
45 NP_000046.1   D G K F L A R V E R V I V S M N Y R V G A L G F L A L P G N P E A P G N M G L F D Q Q L A L Q W V Q N I A A F G G N
46 NP_056646.1   D G R F L V Q A E R T V L V S M N Y R V G A F G F L A L P G S R E A P G N V G L L D Q R L A L Q W V Q E N V A A F G G D
47 Smed_15930    N G E I L A S K M N V I V V N I Q Y R L G P F G F L S Y - N V K E I P G N Q G L M D Q V L A L Q W I R D N I G F F G G D
48 Smed_10334    E G R Y L A A R Q N V I V S M N Y R L G P F G F I Y L K N G L - I P G N M G L W D Q R L A L K W V K E N I E F F G G D
49 Smed_4101     D G S V L A S K E N I V V S F N Y R I A M L G F I S L D D E I - L N G N L G I W D Q V C A L Q W I H E N I S H F K G N
50
51
52 Smed_4800      S E S I T I F G E S A G S V G V S I H V L S P L S N N L F K R A I M E S A T A S A A W A I E T K K E S K E K G L A I S K
53 Smed_14790    P K K V T L F G E S S G A T S I S V H L F S K I S E K Y F Q R A I L Q S G T I F V P W A I E S S S A S Y I K A K Y L A E
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58 Smed_4101     P K N V T I V G N S A G G S V S L H L L I E K S W E Y F Q N A I M L S G V C L S D W G F L T K N E V R D R S K K A V N
59
60
61 Smed_4800      F V N C N Y D S ---- W Q V N V L L K C L Q K V K P D L L V S K Q L D L K N I V N K Q R S D L L R R R G V K N L F T D
62 Smed_14790    K A N C T S R N ---- N Q E -- I V K C L R S T K P E V L V N L N F L I R D Q I A K T R K E K L M K E G Y K D F Y S D
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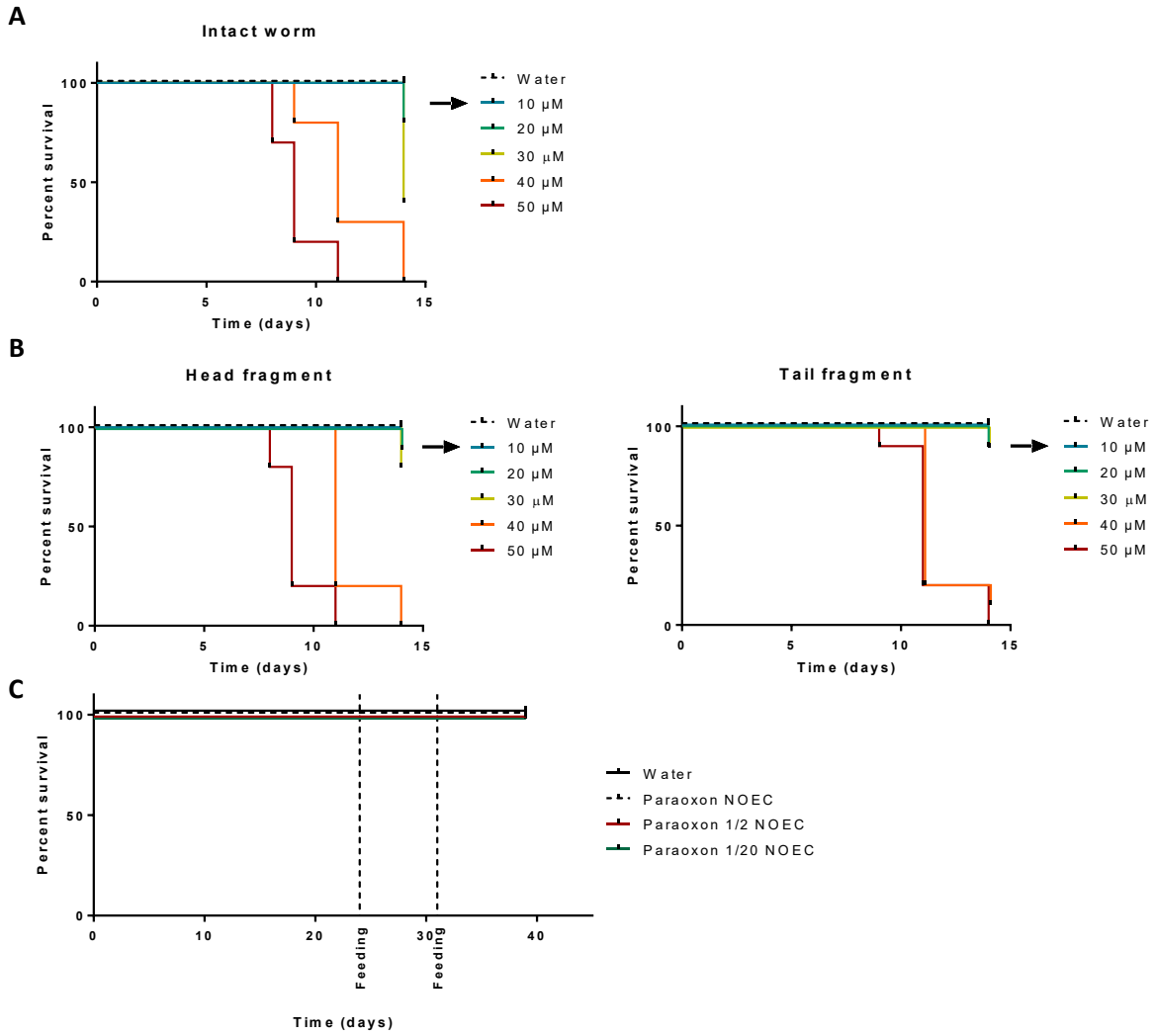
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66 Smed_10334 RLKCRGSLH-----AKVECLRQLPAKQLTDAHSYLYDANS-----
67 Smed_4101 KILEKFD FPMKWNEEITRENKIAIKFLQNI NFKEFLLDTELVTG-----
68
69
70 Smed_4800 ASFFFDIIFRPTYDANFINQPVNLLLNDSTFKPN-IELLMGYNANEGMFFLLYGLNKWLN
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72 NP_000046.1 ---PLSVNFGPTVDGDFLTDMPDILLELGQFKK--TQILVGVNKDEGTAFLVYGAPGFSK
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74 Smed_15930 ASFYFDI PFKPTIGGLYLPKPPSSYFAGKEPKILRKRVM LGTTKNEGLIHLFKGFRHYFM
75 Smed_10334 ---YFSVFPFPVIDNHFLYPNSQAFKNLVHVKPTGALMLGMNKNEGSYFMLSFI PNSD
76 Smed_4101 ---ILQFAWAPCVDGELIKDDPRRLFNEKKFKICPTIFLN--VANEGTYFII SRFPNIDV
77
78
79 Smed_4800 FFNKGEVEDTSLSSNNTNYIKASKFLLLENLTPNDIAYTNLLPLLHEYKIPSTIFNWKK
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84 Smed_10334 WLNNKTSVQIAN-----NKDYQEKL RKVLDLEAKIPSQILSLVDFEYTDYNLP-
85 Smed_4101 DNHK-----MIDNKYFTQTFFQYYPHFPIICSQSTRKKISVEYVL
86
87
88 Smed_4800 WNSTDVLSALDQLTGDQNFVCPVIDYAELLAD-----KNVKVYLYSFQHRTSR
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90 NP_000046.1 QRPENYREALGDVVG DYNFICPALEFTKKFSE-----WGNNAFFYFEHRSSK
91 NP_056646.1 EDPARLREALSDVVG DHNVCVPAQLAGRLAA-----QGARVYAYVFEHRAST
92 Smed_15930 WKSQDIINVDNIAGDFSFKCPVVD FADKYLK-----IAQVYLYSFEQQTQF
93 Smed_10334 DTPQNRVNRLEEISSDRSFKCPTIEMAKMVNNDNRFEKGLGKR SVTLPTYFYEFYRTRTKS
94 Smed_4101 NEPSLSIDILDRIIADQYFISGSIELANLISKS-----NSKVYLYWFETFTQF
95
96
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103 Smed_4101 EHWPNWCGVMHSEEIYYLFGHYLTQQSE-----TRQSATS IKIMKEFGNFVKHGNLSE
104
105
106 Smed_4800 -----QKIEYFDSTLSWPLYNTTSKLSVYFTT PHESQLNAARLNDKAMKCKFWNK
107 Smed_14790 -----TDFTEKG---FWPAYTNKNKRYLKI SG-TNSGGFKVENHFKQK HCRFWTK
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113
114
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123
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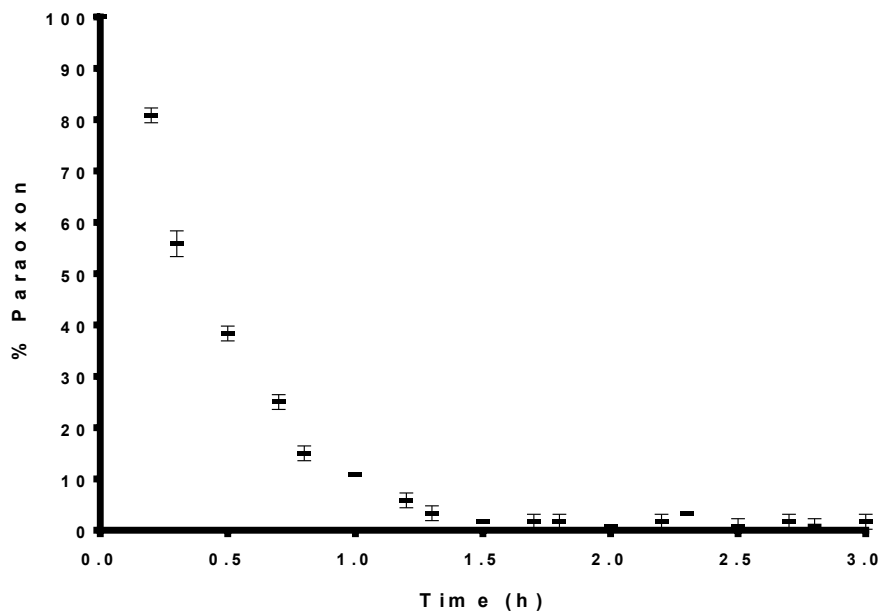
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133 **Supplementary figure 1.** Localization of planarian cholinesterases by WISH (Whole-mount in situ hybridization). *Smedwi-1*
 134 is a marker of proliferation cells. All cholinesterase numbers were retrieved from PlanMine database and specified in the
 135 lower right corner in italics for each picture. Distinct nervous pattern systems were identified and impact of paraoxon-ethyl (5
 136 μM , 1/2 of NOEC) was determined. Scale bar represents 2 mm.
 137



138

139 **Supplementary figure 2.** Survival rates of planarians. The toxicity of paraoxon-ethyl was evaluated for concentrations
 140 ranging from 10 µM up to 50 µM for intact planarians (A) and after amputation on head and tail fragments (B). The curves
 141 represent the survival rates for 10 planarians exposed to paraoxon-ethyl. Arrows indicate NOEC values. (C) As control
 142 NOEC value of 10 µM was verified over 39 days on intact worms.
 143



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145 **Supplementary figure 3.** 5 μ M Paraoxon-ethyl degradation curve. The degradation of 5 μ M paraoxon-ethyl by the enzyme
146 *SsoPox* α sD6 at 0.25 μ M was followed over time. Full degradation was reached after 1.5h incubation.

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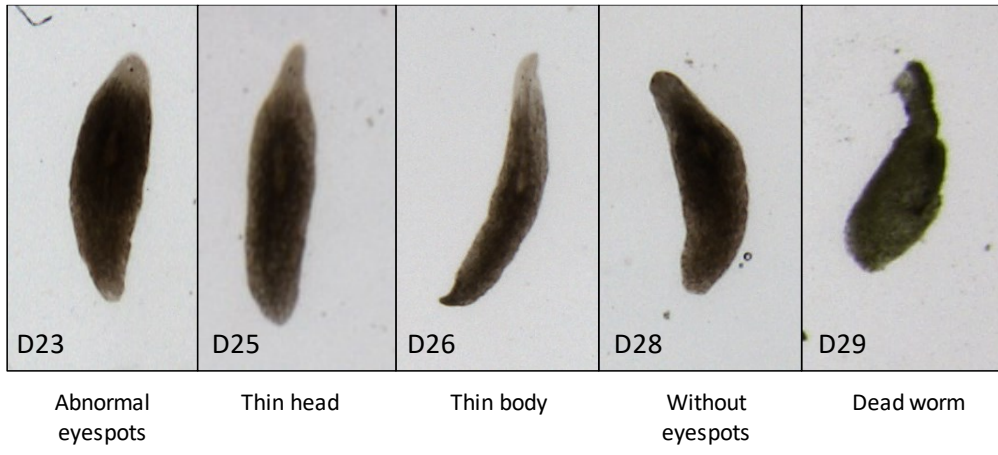
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Head fragment (Posterior regeneration)			
	No regeneration	Amputated part	
	Blastema	Start of regeneration	
	Regeneration in progress	Emergence of the pharynx	
	Regenerated	Complete regeneration	

157

158 **Supplementary figure 4.** Planarian posterior regeneration is presented using a color code from grey to green describing
 159 initial to final regeneration stages.

160



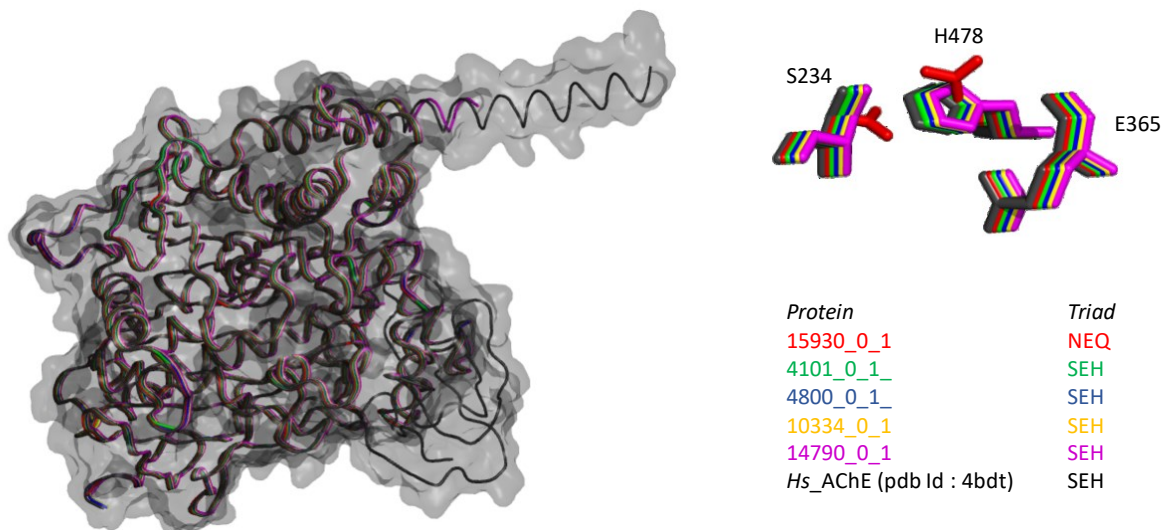
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Supplementary figure 5. Temporal evolution of an abnormal worm exposed to paraoxon-ethyl 5 μ M. The worm arose from a G1 tail fragment.

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168 **Supplementary figure 6.** Structural predictions of planarian cholinesterases compared to the best template c4bdA_
169 corresponding to human acetylcholinesterase in complex with huprine w and fasciculin 2 (pdb : 4bdt). Conservation of the
170 catalytic triad in all sequences, except dd_Smed_v6_15930_0_1.
171