Supplementary material: figures and tables

A common theme in extracellular fluids of beetles: extracellular superoxide dismutases crucial for balancing ROS in response to microbial challenge

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SUPPLEMENTARY FIGURES

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<i>Pc</i> SOD3.1	MFKFAVLAALIT	
PcSOD3.2	MFKFVVAVVLLSGTY	
PcSOD1		
HsSOD1		
HsSOD3	MLALLCSCLLLAAGASDAWTGEDSAEPNSDSAEWIRDMYAKVTEIWQEVM	

100

	<u> .</u> .
PcSOD3.1	ITYAQRSAVVYLFDPSGASGVHGNLTFEQRDSQIQISGEVH
PcSOD3.2	QFCNCDVHSATVRLYDPTGNTEVTGELNFTQVENGVRITGEIH
PcSOD1	MPTKAVCVLNGEVKGTVFFNQANSTSPVEVTGEIT
<i>Hs</i> SOD1	MATKAVCVLKGDGPVQGIINFEQKESNGPVKVWGSIK
HsSOD3	QRRDDDGALHAACQVQPSATLDAAQPRVTGVVLFRQLAPRAKLDAFFALE

		*	150
		* *	
PcSOD3.1	GLTPGKHGF <mark>HVH</mark> QLGNIGL <mark>G</mark>	LGTGG <mark>H</mark> FNPHNKH <mark>H</mark> G	APTDKER <mark>H</mark> VG
PcSOD3.2	GIHEGKHGG <mark>HVH</mark> VDADISKG	CIGAGG <mark>P</mark> FNPHNVR <mark>H</mark> A	GPDDEIR <mark>N</mark> TG
PcSOD1	GLQKGLHGF <mark>HVH</mark> EFGDNTNG	CISAGP <mark>H</mark> FNPRNVE <mark>H</mark> G	GPDHDIR <mark>H</mark> IG
HsSOD1	GLTEGLHGF <mark>HVH</mark> EFGDNTAG	CTSAGP <mark>HFNPLSRK</mark> HG	GPKDEER <mark>H</mark> VG
HsSOD3	GFPTEPNSSSRAI <mark>HVH</mark> QFGDLSQG	ESTGP <mark>H</mark> YNPLAVP <mark>H</mark> P	°−−−−−Q <mark>H</mark> PG
			200

		 *	

PcSOD3.1	DLGNIVADATGVAHVHIEDDVIAL-QGNHNIIGRAMVVHAGEDDLGRGGQ
PcSOD3.2	DLGNVVGDRFGVAHIDVVDKRIKLNKGIDGIIGRGFVI <mark>H</mark> EKEDDLGRGNN
PcSOD1	DLGNIQATDNGVAKVNIKDKFISL-EGENSIIGRTVVVHADPDDLGQGGH
HsSOD1	DLGNVTADKDGVADVSIEDSVISL-SGDHCIIGRTLVVHEKADDLGKGGN
HsSOD3	DFGNFAVRDGSLWRYRAGLAASLAGPHSIVGRAVVVHAGEDDLGRGGN

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PcSOD3.1	SDSLTTGHAGGRLACGVIGILTE
PcSOD3.2	AESGKTGNSGGRIA GVIEVLEE
PcSOD1	ELSKTTGNAGGRLA GVIGLAKI
<i>Hs</i> SOD1	EESTKTGNAGSRLA GVIGIAQ
<i>Hs</i> SOD3	QASVENGNAGRRLACCVVGVCGPGLWERQAREHSERKKRRRESECKAA
	*

Figure S1. Multiple sequence alignment of Cu,Zn-SODs from *P. cochleariae* and *H. sapiens* (SOD1, **P00441; SOD3, P08294).** Red amino acid residues, metal cation binding and catalysis; yellow residues, cysteine involved in disulfide bridges; green residues, N-glycosylation sites; pink box, signal peptide; torques box, heparin binding region; blue stars, Cu²⁺-binding; orange stars, Zn²⁺-binding; black star, position of the *Hs*SOD3 Cys residue involved in the intersubunit disulfide bridge.



Figure S2. Comparison of the predicted tertiary structure of *Pc***SOD3.1 with the solved HsSOD1 (Uniprot P00441) structure 1HL5 chain A by using Phyre2** (Kelley and Sternberg, 2009). Beta-Strands are displayed as flat arrows, and helices are displayed as ribbons. Amino acid residues ligating metal ions in the active center are shown as red sticks. Key structural elements are color-coded (Perry et al., 2010): dark blue, electrostatic loop (A138-R160) contains charged amino acid residues guiding negatively charged substrates to the active site entrance); light blue, GK1 (G54-G58) and GK2 (D119-I129), Greek key loops 1 and 2; yellow, S-S, disulfide bridge (C74 and C163); purple, V-loop (R42-Q45), variable loop ; light red, Zn²⁺ binding region (H80-D100); green, asparagine residue (N 36) for N-glycosylation.



Figure S3. *In vitro* activities of purified recombinant *Pc*SODs. A, purified r*PcS*ODs after heterologous expression in *E. coli* cells, left: elution fraction, stained with Coomassie blue. **B**, 4 μ g of purified protein each was used for the SOD activity assays. SOD activity was determined colorimetrically at 440 nm as the inhibition of the reduction of the tetrazolium salt, WST-1. (n=3)



Figure S4A. Phylogenetic tree of SOD1 and SOD3 of selected insect species. Red, *P. cochleariae* (Pc); green, *C. populi* (Cp); blue, *C. lapponica* (ClW, willow-feeder; ClB, birch-feeder); orange, *H. sapiens* (Hsp) and *M. musculus* (Mmus). Numbers at nodes are bootstrap values. Corresponding accession numbers are listed in table S2.

	10 20 30 40 50
PcSOD3.1	MFKFAVLAALITITYAQRSAVVYLFDPSGASGVHGNLTFEQRDSQ
PcSOD3.2	MFKFVVAVVLLSGTYQFCNCDVHSATVRLYDPTGNTEVTGELNFTQVENG
CpSOD03	MFKILVAAAILSVSCHQCNCAIKSAHVKLRDPSGSTEVTGELNFTQTANG
Clsodw03	MFKVLLAAAILSFSCHLCNCAVKTAYVHLRDPSGSTEVTGELNFTQTANG
C1SODB03	MFKVLLAAAILSFSCHLCNCAVKTAYVHLRDPSGSTEVTGELNFTQTANG
	60 70 80 * 90 100
PcSOD3.1	IQISGEVHGLTPGKHGF <mark>HVH</mark> QLGNIGLGCLGTGGHFNPHNKH <mark>H</mark> GAPTDKE
PcSOD3.2	VRITGEIHGIHEGKHGGHVHVDADISKGCIGAGGPFNPHNVRHAGPDDEI
CpSOD03	VRIHGIIHGIHEGLHGGHVHENAKIEASCAGAGGVFNPHKVPHGSPRDEI
C1SODW03	VRIHGKILGIHEGLHGGHVHESANIGRSCAGAGGVFNPHNVPHGSPKDKI
C1SODB03	VRIHGKILGIHEGLHGGHVHESANIGRSCAGAGGVFNPHNVPHGSPKDKI
	110 120 130 140 150
	.**
PcSOD3.1	RHVGDLGNIVADATGVAHVHIEDDVIAL-QGNHNIIGRAMVVHAGEDDLG
PcSOD3.2	RNTGDLGNVVGDRFGVAHIDVVDKRIKLNKGIDGIIGRGFVIHEKEDDLG
CpSOD03	RTVGDLGNVIADGSGVAVVDFIDRMVOLNTGVEGVIGRSYVIHEKEDDLG
C1SODW03	RTVGDLGNVVADEYGVAVVDFFDDLVRLNTDVDGVIGRSYVIHEKEDDLG
C1SODB03	RTVGDLGNVVADEYGVAVVDFFDDLVRLNTDVDGVIGRSYVIHEKEDDLG
	160 170
PcSOD3.1	RGGQSDSLTTGHAGGRLACGVIGILTE-
PcSOD3.2	RGNNAESGKTGNSGGRIACGVIEVLEE-
CpSOD03	RGANEESKTTGNCGGLIACGVIEVLEED
C1SODW03	RGNNKESKTNGNCGGLIACGVIEVLEED
	RGNNKESKTNGNCGGLTACGVTEVLEED

Figure S4B. Multiple sequence alignment of extracellular Cu,Zn-SODs from Chrysomelina species. Red amino acid residues, metal cation binding and catalysis; blue stars, Cu²⁺-binding; orange stars, Zn²⁺-binding.



Figure S5. Relative normalized expression levels of *PcSODs* **in different tissues of third-instar** *P. cochleariae* **larvae.** Tissues of 3 larvae were pooled for one sample. 4-12 biological replicates were examined. Reference genes were *Pcrps3* and *Pcrpl6*.



Figure S6. SOD activity after protein isolation. Tissues from six third-instar larvae of *P. cochleariae* were pooled in one sample and proteins were separated into cytosolic and membrane enriched fractions according to the protocol of the Calbiochem ProteoExtract Native Membrane Protein Extraction Kit (Millipore). Protein concentration was measured with bicinchoninic acid protein assay and 6 μ g of total protein was used for the SOD activity assay. SOD activity was determined colorimetrically at 440 nm as the inhibition of the reduction of the tetrazolium salt, WST-1. (n=3)



Figure S7. Silencing effects on *PcSOD3.1* expression in different tissues of the larval F1-generation after RNAi induction in the parent generation. A, *P. cochleariae* third-instar larvae were injected with 500 ng of *Pc*SOD3.1-dsRNA or *gfp*-dsRNA and mRNA levels were assayed in larvae of the next generation. B, *P. cochleariae* pupae were injected with 4 µg of *Pc*SOD3.1-dsRNA or *gfp*-dsRNA and mRNA levels were assayed in larvae of the next generation. Reference genes were *PcRPS3* and *PcRPL6*. The Δ Cq values of *SOD*-silenced samples have been normalized to the Δ Cq values of the *gfp*-injected samples ($\Delta\Delta$ Cq). (n=3)



Figure S8. Effect of Cu/Zn SOD silencing on the chrysomelidial synthesis in *P. cochleariae* larvae. GC/MS analysis of secretions was carried out six days after triggering RNAi. The amount of chrysomelidial was normalized to the internal standard methylbenzoate. (n=5)

		pcSOD3.1	PC50D3.2	PCSODI
PcSOD3.1(-)	Gut	1,62	189,30	97,29
	Fat body	0,07	108,55	85,82
	MPT	3,30	49,69	98,54
	Glands	0,65	177,77	87,59
PcSOD3.2(-)	Gut	124,12	33,98	56,73
	Fat body	24,75	1,90	81,29
	MPT	96,74	2,38	49,94
	Glands	99,04	19,61	43,98
PcSOD1(-)	Gut	55,31	261,07	6,01
	Fat body	236,28	373,94	6,57
	MPT	43,77	20,81	8,48
	Glands	110,40	65,53	0,44

Figure S9. Expression of Cu/Zn SODs genes after their individual silencing by RNAi in juvenile *P. cochleariae*. 200 ng dsRNA of each Cu/Zn SOD or *gfp* has been used to induce RNAi in early second-instar larvae. RNA from different tissues of *P. cochleariae* larvae has been used as template ten days after ds RNA injection. Reference genes were *PcRPS3* and *PcRPL6*. The Δ Cq values of *SOD*-silenced samples have been normalized to the Δ Cq values of the *gfp*-injected samples ($\Delta\Delta$ Cq). Green: decreased expression level, red: increased expression level. (%means of n=3/treatment)



Figure S10. Graphical representation of life stage development of *P. cochleariae*. First-instar larvae were injected with 200 ng of *Pc*SOD3.1-, *Pc*SOD3.2-, *Pc*SOD1-dsRNA or *gfp*-dsRNA. Markers indicate 50% of population in this state, lines represent first and last turnover. L1-L3, larval instars.



Figure S11. Longevity of *P. cochleariae* after silencing of Cu/Zn SODs by RNAi. The probability of survival is depicted as Kaplan-Meier curves by applying Gehan-Breslow statistic test. Second-instar larvae were injected with 200 ng of *PcSOD3.1-*, *PcSOD3.2-*, *PcSOD1-*dsRNA or *gfp-*dsRNA (n=60/treatment).



Figure S12. Relative normalized expression levels of *sod*'s in whole third-instar *P. cochleariae* **larvae 1 to 6 days after infection with** *M. anisopliae.* Non-infected and infected larvae in comparison, 1 to 6 biological replicates of whole larvae were examined. Reference genes were *PcRPS3* and *PcRPL6*. * = significantly different to their non-infected group (p<0.05, two-tailed t-test).



Figure S13. Effect of defensive secretions from juvenile *P. cochleariae* on the growth of *Metarhizium anisopliae* in agar diffusion tests after three days of incubation. A, Effect of secretions collected from *PcSOD3.1* silenced larvae (1:10 (w/v) in 50 mM NaPO₄ pH 5.8, 10 μ l per spot); B, Effect of secretions collected from *yellow* silenced larvae. (1:30 (w/v) in 50 mM NaPO₄ pH 5.8, 5 μ l per spot).

Kelley, L.A., Sternberg, M.J.E., 2009. Protein structure prediction on the Web: a case study using the Phyre server. Nat. Protoc. 4, 363-371.

Perry, J.J.P., Shin, D.S., Getzoff, E.D., Tainer, J.A., 2010. The structural biochemistry of the superoxide dismutases. Biochim. Biophys. Acta 1804, 245-262.



Figure S14. Full-length gel corresponds to figure 1A in the main text. It shows the detection of SOD activity in defensive exudates and hemolymph collected from larvae of different leaf beetle species. From each species 1 μ l hemolymph and 0.3 μ l secretion was separated on native PAGE and functionally stained for SOD activity by using a qualitative zymography method based on the conversion of the tetrazolium salt, MTT. SOD activity inhibits the formation of a dark blue formazane dye and corresponds to white bands on a dark background.

Lane 1/2, *P. cochleariae*, hemolymph/secretion; lane 3/4, bovine erythrocyte SOD; lane 5/6, *Gastrophysa viridula*, hemolymph/ secretion; lane 7/8, *Chrysomela populi*, hemolymph/ secretion.



Figure S15. Full-length gel corresponds to figure 1A in the main text. It shows the detection of SOD activity in defensive exudates and hemolymph collected from larvae of *Chrysomela lapponica* (C. lapp.) One μ l hemolymph and 0.3 μ l secretion was separated on native PAGE and functionally stained for SOD activity by using a qualitative zymography method based on the conversion of the tetrazolium salt, MTT. SOD activity inhibits the formation of a dark blue formazane dye and corresponds to white bands on a dark background.

Lane 1, C. lapp. affiliated to *Salix* ssp., hemolymph; lane 2, C. lapp. affiliated to *Betula* ssp., hemolymph; lane 3, C. lapp. affiliated to *Salix* ssp., secretion; lane 4, C. lapp. affiliated to *Betula* ssp., secretion.



Figure S16. Full-length gel corresponds to figure 2C in the main text. Coomassie stained SDS-PAGE with 0.3 µl of larval defence secretions from *P. cochleariae* nine days after RNAi induction. Injection was carried out with 200 ng *PcSOD3.1*-dsRNA or 200 ng *gfp* as a control. M, marker (Bio-Rad Laboratories, Munich, Germany); NIC, non-injection control.

SUPPLEMENTARY TABLES

Table S1. Peptides identified from superoxide dismutases from juvenile P. cochleariae.

Peptides identified from *Pc*SOD 3.1 in hemolymoph.

peptide sequence ^a	theor. [M+H] ^{+ b}	exp. [M+H] ^{+ c}	mass error (ppm)	peptide score	retention time (min)
DSQIQISGEVHGLTPGK	1765.9177	1765.9156	4.4	8.0	33.07
HVGDLGNIVADATGVAHVHIEDDVIALQGNHNIIGR	3739.9205	3739.9205	-1.6	7.8	43.86
AMVVHAGEDDLGR	1369.6487	1369.6507	-1.6	7.7	26.37
GGQSDSLTTGHAGGR	1400.6741	1400.6707	13.9	6.3	25.34
AM _{ox} VVHAGEDDLGR	1385.6495	1385.6515	2.7	7.9	23.16

 \rightarrow exemplary table of identified peptides from *Pc*SOD3.1 in hemolymph; results for defensive secretions are similar

Peptides identified from *Pc*SOD 3.2 in hemolymoph.

peptide sequence ^a	theor. [M+H] ^{+ b}	exp. [M+H] ^{+ c}	mass error (ppm)	peptide score	retention time (min)
NTGDLGNVVGDR	1216.6014	1216.5986	5.7	7.9	28.06
FGVAHIDVVDKR	1355.7344	1355.7367	-4.7	7.7	30.36
ITGEIHGIHEGK	1290.6637	1290.6663	-10.7	7.6	21.63
GIDGIIGR	800.4588	800.46	-3.1	7.5	31.62
GC*IGAGGPFNPHNVR	1552.7408	1552.7418	-1.3	7.3	29.52
GNNAESGKTGNSGGR	1405.6543	1405.6495	5.7	6.1	29.74

Peptides identified from *Pc*SOD1 in Malpighian tubules and fat body.

peptide sequence ^a	theor. [M+H] ^{+ ♭}	exp. [M+H] ^{+ c}	mass error (ppm)	peptide score	retention time (min)
TVVVHADPDDLGQGGHELSK	2074.0231	2074.02	1.5	6.4	28.96
FISLEGENSIIGR	1434.7636	1434.7587	3.4	6.2	40.59
LAC*GVIGLAK	1001.5863	1001.5813	4.9	6.2	33.78
GTVFFNQANSTSPVEVTGEITGLQK	2624.3185	2624.3202	-0.7	5.6	42.78
HIGDLGNIQATDNGVAK	1722.893	1722.8769	9.3	5.4	43.38
IYC*IVELGTESFIIGNYNK	2233.118	2233.121	-1.3	5.7	40.36

 \rightarrow exemplary table of identified peptides from *Pc*SOD1 in Malpighian tubules; results for fat body are similar

^a C*, C-carbamidomethylation; Mox, M-oxidation

^b theoretical monoisotopic mass of the peptide

^c calculated monoisotopic mass of the lock-mass corrected peptide ion cluster

name	similar to protein	fmol protein					
		NIC	E. coli	M. luteus	M. anisopliae		
PcAMP01	acaloleptin A (coleoptericin)	252.93	3354.64	884.15	1750.57		
PcAMP02	attacin-like immune protein	27.89	1536.42	1419.46	634.22		
	(attacin_C)						
PcAMP03	defensin_2	0	659.06	309.20	123.43		

Table S2. Low molecular proteins identified in hemolymph from juvenile *P. cochleariae*.

Peptides identified from PcAMP01

peptide sequence ^a	theor.	exp.	mass	peptide	retention
	[M+H] ^{+ b}	[M+H] ^{+ c}	error	score	time
			(ppm)		(min)
QQPWEVSPNINR	1467.7278	1467.7339	-4.2	8.9	35.13
SLQPGAPDINNGK	1310.6626	1310.6699	-5.6	7.7	25.51
AKPTWHAGGTIR	1294.7073	1294.7015	4.5	8.6	21.07
SLQPGAPDINNDKK	1496.7773	1496.7703	4.6	8.0	23.55
QQPWEVSPNINRDGAGNVR	2137.0648	2137.0533	5.3	8.1	33.49
DHDFNAGWGK	1146.4921	1146.4963	-3.7	7.5	27.08
SLQPGAPDINNDK	1368.6796	1368.6754	3.1	7.7	26.40
TDIEVQNHGKDHDFNAGWGK	2268.0656	2268.0428	10.0	6.7	33.71
AKPTWHAGGTIR	1294.701	1294.7015	-0.4	7.9	81.18
TDIEVQNHGK	1140.5675	1140.5644	2.7	7.5	17.31
SLQPGAPDINNGKK	1438.7498	1438.7649	-10.5	8.1	23.33
TDIEVQNHGKDHDFNAGWGK	2268.1397	2268.0428	42.7	7.2	41.40
DHDFNAGWGK	1146.5051	1146.4963	7.7	7.4	80.92
VSPNINRDGAGNVR	1468.7331	1468.7615	-19.3	6.6	45.14
KQQPWEVSPNINR	1595.8306	1595.8289	1.1	8.5	31.31

Peptides identified from PcAMP02

peptide sequence ^a	theor.	exp.	mass	peptide	retention
	[M+H] ^{+ b}	[M+H] ^{+ c}	error	score	time
			(ppm)		(min)
LDYTHTPSGSGAFIQADR	1935.9195	1935.9259	3.3	9.0	32.93
YNIYTSPK	985.4989	985.4993	0.4	8.4	25.63
KDFGVDATAQYQR	1498.7285	1498.7335	3.4	8.6	26.76
NYGTDVAAGGK	1052.5007	1052.5005	-0.2	8.2	20.54
DFGVDATAQYQR	1370.6335	1370.6495	11.7	8.5	32.01
GGRLDYTHTPSGSGAFIQADR	2206.0636	2206.0589	-2.1	7.3	30.70
TRNYGTDVAAGGK	1309.6495	1309.6444	-3.9	7.4	80.87

Peptides identified from PcAMP03

peptide sequence ^a	theor. [M+H] ^{+ b}	exp. [M+H] ^{+ c}	mass error (ppm)	peptide score	retention time (min)
VTCDVLNFEIK	1337.677	1337.679	1.5	6.4	42.09
LNDAACAAHCLTLGK	1614.7727	1614.7946	13.5	5.2	37.38

^a C*, C-carbamidomethylation; Mox, M-oxidation

^b theoretical monoisotopic mass of the peptide

^c calculated monoisotopic mass of the lock-mass corrected peptide ion cluster

Table S3. Proteome analysis via shotgun from hemolymph of *P. chochleariae* larvae

protein			fmol protein		
	gfp	gfp	PcSOD3.1(-)	PcSOD3.1(-)	PcSOD3.2 (-)
	not infected	infected with M.	not infected	infected with M.	not infected
		anisopliae		anisopliae	
PcSOD3.1	5.48 ± 3.67	3.76 ± 4.43	0	0	1.38 ± 2.75
PcSOD3.2	14.49 ± 1.11	9.07 ± 3.33	16.25 ± 8.18	8.77 ± 3.8	0

Table S4. Peptides identified from PcYellow-like in secretions of juvenile P. cochleariae.

peptide sequence ^a	theor. [M+H] ^{+ b}	exp. [M+H] ^{+ c}	mass	peptide	retention
			error	score	time
			(ppm)		(min)
NNPFAAPQIFHTYR	1675.834	1675.841	4.4	8.6	40.78
IHSGTPVTLGTVTDR	1553.828	1553.831	1.8	8.7	33.12
QANIDGGYFR	1140.543	1140.546	2.1	8.4	33.59
TNHLLLR	866.5207	866.5207	0.0	8.6	30.37
LLAFDLR	847.5036	847.5021	-1.8	8.0	42.40
QELWILTSR	1145.631	1145.633	1.8	8.3	43.78
NTFVYLGDVR	1183.611	1183.618	5.9	8.0	39.71
ITIDECER	1035.478	1035.483	4.8	6.7	28.24
STQSSAEAIDR	1164.549	1164.552	2.1	8.0	23.61
SWAVR	618.3358	618.3402	7.1	7.2	28.49
DLSIVHEWSQIEFDYHSEVER	2618.216	2618.219	1.0	7.8	47.43
LWVIDIGPLNDVITCPAR	2052.095	2052.1	2.4	8.0	53.14
STQSSAEAIDRDGIMYYGLLGDIK	2603.266	2603.338	27.9	6.0	41.85
RDLSIVHEWSQIEFDYHSEVER	2774.317	2774.334	6.3	6.0	45.35
GRQELWILTSR	1358.754	1358.764	7.7	8.2	40.53
RLFYHAMSSPTENWVYTSYLR	2621.261	2621.228	-12.3	6.3	30.43
LFYHAMSSPTENWVYTSYLR	2481.154	2481.164	3.7	8.1	44.43

AQYVASGTMFDVNETNFR	2065.928	2065.933	2.1	8.1	37.70

^aC*, C-carbamidomethylation; Mox, M-oxidation

^b theoretical monoisotopic mass of the peptide

^c calculated monoisotopic mass of the lock-mass corrected peptide ion cluster

Table S5. Primer used for cloning, quantitative real time PCR, dsRNA production.

Primer type	Target	\leftrightarrow	Sequence	Amplicon length
Coding	PcSOD1	\rightarrow	ATGCCTACAAAAGCAGTTTGCGTTCTC	462
sequence		\leftarrow	CTATATTTTTGCCAATCCAATGACTCCA	-
	PcSOD3.1	\rightarrow	GAGAAAAAATTTGATACTTCG	670
		\leftarrow	TTTTCACTTATCTAAAATCGTTTAT	
	PcSOD3.2	\rightarrow	ATGTTCAAGTTTGTAGTAGCCGTGGTTT	553
		\leftarrow	CTCCCTAATCGTAGGTTATTCATTCTTCC	
dsRNA template	PcSOD1	\rightarrow	TAATACGACTCACTATAGGGAGACTCCAATGTGGCGA ATA	243
		~	TAATACGACTCACTATAGGGAGATGCCTACAAAAGCA GTT	
	PcSOD3.1	\rightarrow	TAATACGACTCACTATAGGGAGAACATCGTAGCCGAC GCA	193
		~	TAATACGACTCACTATAGGGAGACCGATGACCCCGCA A	
	PcSOD3.2	\rightarrow	TAATACGACTCACTATAGGGAGAGACTCCGCAAGCTA TAC	508
		~	TAATACGACTCACTATAGGGAGACAAGTTTGTAGTAG CCG	
	gfp	\rightarrow		523
		4	TAATACGACTCACTATAGGGAGATAATCCCAGCAGCA GTT	
	PcYellow- like	\rightarrow	GTAATACGACTCACTATAGGGAGCTTTCTTGGCGTCA CTGGTGGTAG	
		<i>←</i>	GTAATACGACTCACTATAGGGAGCCAGCAAACCATAA TACATTATTCCATCC	
qPCR	PcSOD1	\rightarrow	ATCATGATCTGGGCCACCATGC	77
		\leftarrow	ACAACACCAACGGGTGCATTTCC	
	PcSOD3.1	\rightarrow	AAACATCGTAGCCGACGCAACAG	89
		\leftarrow	TGCCGATGATGTTGTGGTTGC	
	PcSOD3.2	\rightarrow	TCAATGTGAGCGACGCCGAAAC	111
		\leftarrow	TGGACCTTTCAATCCCCACAACG	
	PcRP-L6	\rightarrow	CAAGACAGGATCAGACCTAGAC	126
		\leftarrow	CACTCTCTTTCCTTTGTGGGAAC	
	PcRP-S3	\rightarrow	AAGGCTGTGAAGTTGTGGTATCTGG	
		\leftarrow	ATGGCGGGTGGCAGTGTCTAC	
pET100	PcSOD1	\rightarrow	CACCATGCCTACAAAAGCAGTTTGC	466
Insert		\leftarrow	CTATATTTTTGCCAATCCAATGACT	

PcSOD3.1	\rightarrow	CACCATGCGTAGCGCAGTCGTCTATCT	487
	\leftarrow	AGCCAGAACGCGTATTTACTC	
PcSOD3.2	\rightarrow	CACCGATGTGCATTCAGCAACAGTT	478
	\leftarrow	TCATTCTTCCAATACTTCGA	

Table S6. Accession numbers of sequences used for all the studies of this publication.

Species	Designation in figure	Accession number
Anoplophora glabripennis	AglaSOD01	gi 550258789 gb JAB66410.1
	AglaSOD02	gi 550254905 gb JAB65445.1
Acyrthosiphon pisum	ApisSOD01	gi 242247393 ref NP_001156153.1
	ApisSOD02	gi 239789313 dbj BAH71287.1
	ApisSOD03	gi 242247211 ref NP_001156243.1
Bombyx mori	BmorSOD01	gi 112982998 ref NP_001037084.1
	BmorSOD02	gi 512930962 ref XP_004932190.1
	BmorSOD03	gi 512885538 ref XP_004921611.1
	BmorSOD04	gi 68144076 gb AAY86076.1
Chrysomela lapponica	CIBSOD01	gb KT002136
	CIBSOD02	gb KT002134
	CIBSOD03	gb KT002135
	CIBSOD04	gb KT002137
	CIBSOD05	gb KT002138
	CIWSOD01	gb KT002139
	CIWSOD02	gb KT002140
	CIWSOD03	gb KT002141
	CIWSOD04	gb KT002142
	CIWSOD05	gb KT002143
Chrysomela populi	CpSOD01	gb KT270576
	CpSOD02	gb KT270577
	CpSOD03	gb KT270578
	CpSOD04	gb KT270579
	CpSOD05	gb KT270580
Phaedon cochleariae	PcSOD1	gb KT002146
	PcSOD3.1	gb JQ424878
	PcSOD3.2	gb KT002145
	PcSOD3.3	gb KT002144
	PcSOD04	gb KT270585
	PcAMP01	gb KT270583
	PcAMP02	gb KT270582
	PcAMP03	gb KT270581
	PcYellow-like	gb KT270584
	PcRPL6	gb KT270586

	PcRPS3	gb KC109783
Dastarcus helophoroides	DhelSOD01	gi 667755291 gb AlG92782.1
	DhelSOD02	gi 667755289 gb AIG92781.1
	DhelSOD03	gi 667755293 gb AlG92783.1
Drosophila melanogaster	DmelSOD1	Q7JR71 Q7JR71_DROME
	DmelSOD3	gi 17136496 ref NP_476735.1
Dendroctonus ponderosae	DponSOD01	gi 546674530 gb ERL85890.1
	DponSOD02	gi 478251452 gb ENN71916.1
	DponSOD03	gi 332380651 gb AEE65524.1
	DponSOD04	gi 332376316 gb AEE63298.1
	DponSOD05	gi 332376945 gb AEE63612.1
	DponSOD06	gi 478253114 gb ENN73487.1
	· ·	
Homo sapiens	HspSOD1	P00441 SODC HUMAN
	HspSOD3	P08294 SODE HUMAN
	· ·	·
Leptopilina boulardi	LbouSOD01	gi 357535425 gb AET83767.1
· · ·	LbouSOD02	gi 357535429 gb AET83769.1
	LbouSOD03	gi 357535423 gb AET83766.1
	LbouSOD04	gi 357535431 gb AET83770.1
	LbouSOD05	gi 357535433 gb AET83771.1
	LbouSOD06	gi 506969309 gb AGM32998.1
Lygus hesperus	LhesSOD01	gi 732607915 gb JAG19381.1
	LhesSOD02	gi 732607911 gb JAG19379.1
	LhesSOD03	gi 732613469 gb JAG22158.1
	LhesSOD04	gi 732610069 gb JAG20458.1
	LhesSOD05	gi 732576017 gb JAG03432.1
	LhesSOD06	gi 732622201 gb JAG26524.1
Mus musculus	MmusSOD1	sp P08228 SODC_MOUSE
	MmusSOD3	sp O09164 SODE_MOUSE
Nasonia vitripennis	NvitSOD01	gi 156548615 ref XP_001608103.1
	NvitSOD02	gi 345481696 ref XP_001602916.2
	NvitSOD03	gi 645035824 ref XP_008216838.1
	NvitSOD04	gi 645035835 ref XP_008216841.1
	NvitSOD05	gi 645035832 ref XP_008216840.1
Panstrongylus megistus	PmegSOD01	gi 656761599 gb JAC85673.1
•	PmegSOD02	gi 656761385 gb JAC85566.1
Solenopsis invicta	SinvSOD01	gi 322782495 gb EFZ10444.1
	SinvSOD02	gi 322782511 gb EFZ10460.1

Tribolium castaneum	TcasSOD01	D6X0S2D6X0S2_TRICA
	TcasSOD02	D2A2T2 D2A2T2_TRICA
	TcasSOD03	gi 91091194 ref XP_972244.1
Tenebrio molitor	TmolSOD01	gi 669688118 gb All26027.1
	TmolSOD02	gi 669688116 gb All26026.1
Streptomyces coelicolor	ScoeSOD	O51917 SODF_STRCO