

Supplementary Materials

Knockdown of superoxide dismutase 6 inhibits neuronal remodelling during metamorphosis in *Tribolium castaneum*

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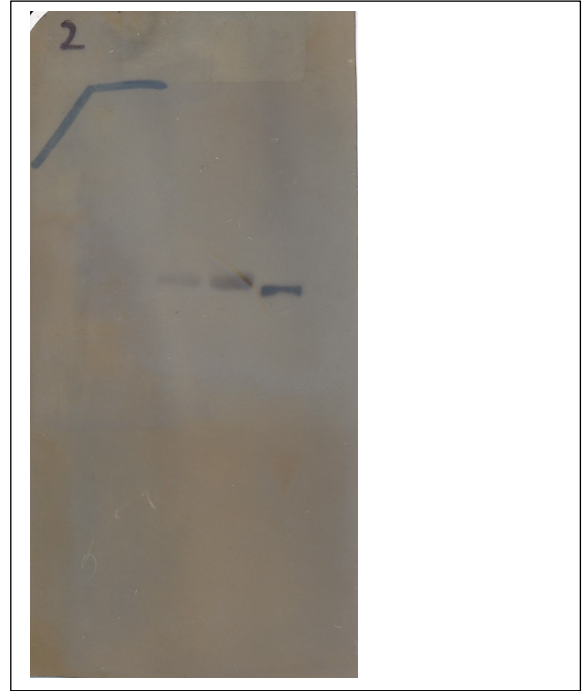
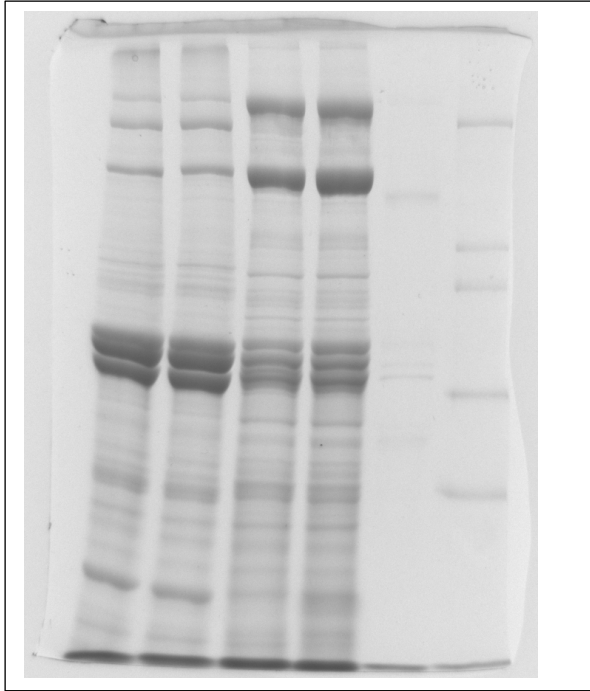
Supplementary Table 5. Primers used for dsRNA synthesis.

TcSOD6_clorning	1	MGQII FVWFILLKLATSLELKSYSVSDHGLHGTITFRKIGPEIKIITDLNATLEYPNQVSWAVVEFFVDY	100
TC011770-RA	1	S.....	100
TcSOD6_clorning	101	NGTSEFSTQELQINGDVGIYGKSLLFKDPETNTRICATITMVDKAQEKIARFNSPIAGS	200
TC011770-RA	101	HFWFSTKDNHNDMVVITDLYHVSNNKHSQSLDFTEH	200
TcSOD6_clorning	201	LWKIYATDILSDSKERPDCNQLVDFPDNTGDQALGDLDSRLGKIKISTDYKRKKFKTLFTDQRLILLPSDLQGPQ	300
TC011770-RA	201	SLYLVIFERKHPDSFLACAKIR	300
TcSOD6_clorning	301	YERPINAKVIIQSGGIGKEVRMTQFSKFEPFVNFDLITARGDLETRLVYSSSVAGYKIHELFPVKPS	400
TC011770-RA	301	TLGQENNSCLITKRVNPNKIDPNVAPPSSGLG	400
TcSOD6_clorning	401	TQNQYLVGDLGKLGGRNQT	500
TC011770-RA	401	LVPTGHELSGLYDWTLLPLQGKFSIIHRTLVIYKNTQVPKGEILSEPWICGGIALYEPNFRYQKPIFTA	500
TcSOD6_clorning	501	GRVLMRQVRDEPWSDTISILVEYLVDHGAALNNSKDRWAIHEEPPGKDFYSWKERCLSSGQIYNPYKIDALDACTG	600
TC011770-RA	501	GLCRVGDLTARLGT	600
TcSOD6_clorning	601	SDRVSRITMTDPFVPLTGQFSVLGKSLVYDDFGPKARGERLACSIIGGVYR	700
TC011770-RA	601	KAVVKDWFNPNGVPISVKGKIEFYQTEYDITNVEVNLQGLSDNSGYH	700
TcSOD6_clorning	701	IHITPVEENLEFPCEASSLYDHWNPINVDPASSPRTYHGTDPQYEMGDLGKFGTLDNQTYKYTDYNDTMLPLFGPK	799
TC011770-RA	701	SIVGRSIIHK	800
TcSOD6_clorning	800	ERGYSPSEARELRATASFHHPDGAYGYLKMQLIYNDGSKSDTTIEVKLRHPGKNDNRNVRDHDWAIYVNFVGVDAAVKTQ	899
TC011770-RA	801	NTRCVAGGYIWNPFYFTQL	900
TcSOD6_clorning	900	ADPLNGDLYRQECGPENPLRCHVGDLSARLGTIDIGLQKQVFTDANFPLEGDVTAGRSIVIMSPNRRPERYACANIEPDY	999
TC011770-RA	901	DIKYANIEKPPRFVLSQF	1000
TcSOD6_clorning	1000	IEEVREILGIPDWFLTVDSRKTKSLHSDACVQLLHFKGPLANLEQDFSRLLASGRLDFTLYIPGFINTKRKVKIAYKQC	1099
TC011770-RA	1001	GQVQDPNEKSKKRGFFFA	1090
TcSOD6_clorning	1100	RSGSNPAKFAHSLIVVIVLSYLL	1122
TC011770-RA	1091		1111

Supplementary Figure 1. Comparison of *T. castaneum* SOD6 amino acid sequences obtained from BeetleBase and translated via cDNA cloning. *TcSOD6* amino acid sequences obtained from the BeetleBase and cDNA cloning were aligned and compared. Different amino acid sequences are shown the black-colored.



Supplementary Figure 3. Verification of decreased TcSOD6 mRNA expression in TcSOD6-knockdown pupae. Verification of *TcSOD6* mRNA expression by RNA-seq analysis. Histograms show the frequency of *TcSOD6* between *TcVer* and *TcSOD6* knockdown groups (n=3, respectively).



Supplementary Figure 4. Full image for Figure 4. a, SDS-PAGE, b, Immunoblot

Tables

Table S1. SOD sequence IDs obtained from BeetleBase.

SOD name	Position	Length	Beetle base ID
<i>TcSOD1</i>	ChLG4:13586289..13586750	462	TC007011
<i>TcSOD2</i>	ChLG8:9478941..9479642	702	TC005780
<i>TcSOD3</i>	ChLG9:20121715..20122308	594	TC011676
<i>TcSOD5</i>	ChLG9:20124698..20125624	927	TC011675
<i>TcSOD6</i>	ChLG9:18441998..18445842	3845	TC011770
<i>TcCCS</i>	ChLG7:15791708..15792438	731	TC010027

Table S2. Gene ID was used the phylogenic tree in this study.

Species	Product	UniProt ID
<i>A.mellifera</i>	SOD1	A0A088A933
<i>A.mellifera</i>	SOD2	A0A088A1K0
<i>A.mellifera</i>	SOD2	A0A088A3Z0
<i>A.mellifera</i>	SOD6	A0A087ZNA5
<i>A.mellifera</i>	CCS	A0A088A6F0
<i>B.mori</i>	SOD1	P82205
<i>B.mori</i>	SOD2	H9JD56
<i>B.mori</i>	SOD3	H9J7J7
<i>B.mori</i>	SOD4	H9J072
<i>B.mori</i>	SOD5	H9IZW3
<i>B.mori</i>	SOD6	H9IYH7
<i>B.mori</i>	CCS	H9IWR6
<i>D.melanogaster</i>	SOD1	P61851
<i>D.melanogaster</i>	SOD1	M9PF91
<i>D.melanogaster</i>	SOD2	A0A0B4LGQ1
<i>D.melanogaster</i>	SOD3	Q0E9C3
<i>D.melanogaster</i>	SOD3	Q7JR71
<i>D.melanogaster</i>	SOD3	A1Z8K9
<i>D.melanogaster</i>	SOD5	Q9VBK8
<i>D.melanogaster</i>	SOD6	Q9VA99
<i>D.melanogaster</i>	CCS	A1Z850
<i>D.rerio</i>	SOD1	O73872
<i>D.rerio</i>	SOD1	B2GRH9
<i>D.rerio</i>	SOD2	Q6P980
<i>D.rerio</i>	SOD3	F1R0Y7
<i>D.rerio</i>	SOD3	E9QIM2
<i>D.rerio</i>	CCS	F1QCS3
<i>H.sapience</i>	SOD1	H7BYH4
<i>H.sapience</i>	SOD2	A0A0C4DFU2
<i>H.sapience</i>	SOD3	P08294
<i>H.sapience</i>	CCS	J3KNF4
<i>M.musculus</i>	SOD1	P08228

<i>M.musculus</i>	SOD2	P09671
<i>M.musculus</i>	SOD3	O09164
<i>M.musculus</i>	CCS	Q9WU84
<i>R.norvegicus</i>	SOD1	P07632
<i>R.norvegicus</i>	SOD2	P07895
<i>R.norvegicus</i>	SOD3	Q08420
<i>R.norvegicus</i>	CCS	Q9JK72
<i>T.castaneum</i>	SOD1	D2A2T2
<i>T.castaneum</i>	SOD2	D6WWA9
<i>T.castaneum</i>	SOD3	D6X0S2
<i>T.castaneum</i>	SOD5	D6X0S3
<i>T.castaneum</i>	SOD6	D6WZU6
<i>T.castaneum</i>	CCS	D6WRL1
<i>X.tropicalis</i>	SOD1	F6VW32
<i>X.tropicalis</i>	SOD1	Q0IIW3
<i>X.tropicalis</i>	SOD2	Q6DJC5
<i>X.tropicalis</i>	SOD3	A9JTW5
<i>X.tropicalis</i>	CCS	F6W0J3
<i>X.tropicalis</i>	CCS	F6Y0R2

UniProt IDs obtained from the UniProt database (<http://www.uniprot.org/>).

Table S3. TcSOD6 primers used in cDNA cloning

Gene name	Forward	Reverse
<i>TcSOD6</i>	5'-ATGGGACAAATTATCTTCGT-3'	5'-TTACAATAAATAGCTTAAAA-3'

Table S4. TcSODs primers used in quantitative reverse transcription PCR

Gene name	Forward	Reverse
<i>TcRpS6</i>	5'-GGACCCAAAAGAGCATCAAA-3'	5'-CCTCAAGCAACGCTTCTTCT-3'
<i>TcSOD1</i>	5'-GGTGATGGAGTTGCCAAAGT-3'	5'-GATTCCAACAACACCACACG-3'
<i>TcSOD2</i>	5'-CAAGTCCGGGAAATTGAAAA-3'	5'-CTCGAAAATGGCCTTGACAT-3'
<i>TcSOD3</i>	5'-CTTCCACATCCACGAGAAGG-3'	5'-CACCACAGCCCTCCCTATAA-3'
<i>TcSOD5</i>	5'-ACCAAAGCGACCTATACGAA-3'	5'-GCTGCACCCTTGTCTCAAAT-3'
<i>TcSOD6</i>	5'-GGCAAGATTGCAGACAGTGA-3'	5'-AACCAATCCTTGACCACAGC-3'
<i>TcCCS</i>	5'-CCTTATGGGGACTTGGGAGT-3'	5'-CTCTGGGTGGGATTTTCTCA-3'
<i>TcVer</i>	5'-CGTAATGAGTTGCCCACTGAGAC-3'	5'-CGACTCTTCTAAAACGTCGCTGA-3'

Table S5. Primers used for dsRNA synthesis

Target gene	Forward	Reverse
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<i>TcSOD6</i>	5'- CACAGCCAAAGTCTGGACTT- 3'	5'- GGACTGTATTATAACCTTGG- 3'
<i>TcSOD6</i> with T7	5'- TAATACGACTCACTATAGGC ACAGCCAAAGTCTGGACTT- 3'	5'- TAATACGACTCACTATAGGG GACTGTATTATAACCTTGG-3'
<i>TcVer</i>	5'- TGATCCTCAAGGTATTGGTG- 3'	5'- TGATCAGCGAGTCTATGTCC- 3'
<i>TcVer</i> with T7	5'- TAATACGACTCACTATAGGT GATCCTCAAGGTCTTGGTG- 3'	5'- TAATACGACTCACTATAGGT GATCAGCGAGTCTATGTCC- 3'

Supplementary Video 1. TcSOD6-knockdown insect movement after dsRNA injection in the prepupa.

Supplementary Video 2. TcVer-knockdown insect movement after dsRNA injection in the prepupa.

Supplementary Video 3. TcSOD6-knockdown insect movement following dsRNA injection immediately after adult eclosion.

Supplementary Video 4 Video. TcVer-knockdown insect movement following dsRNA injection immediately after adult eclosion.