Supplemental Information

Drosophila orthologue of WWOX, the chromosomal fragile site *FRA16D* tumour suppressor gene, functions in aerobic metabolism and regulates reactive oxygen species

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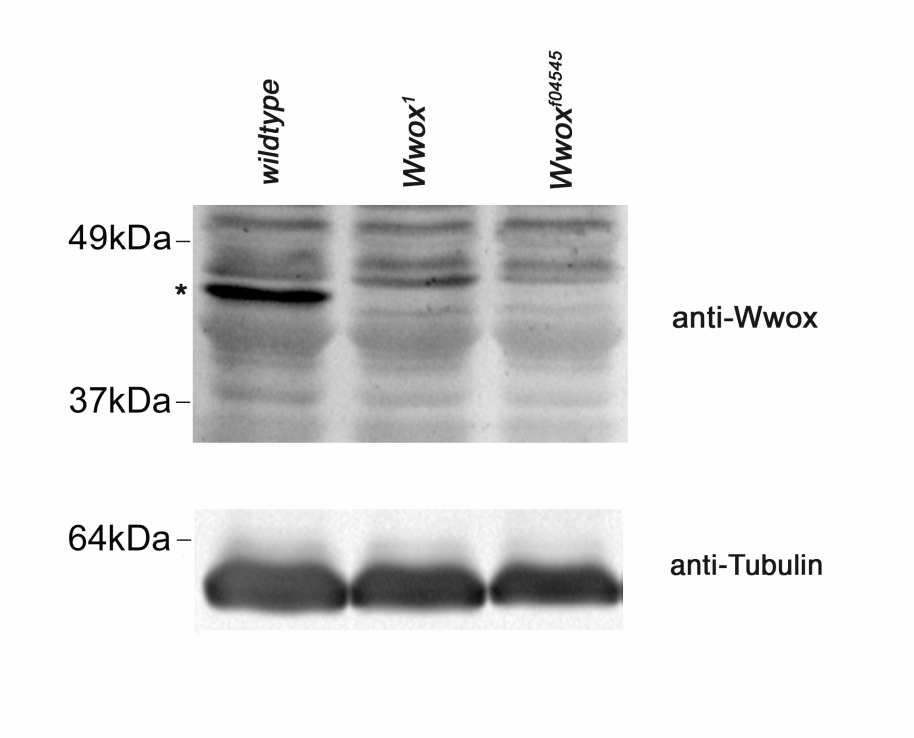

Figure S1. Related to Figure 1, Western analysis of Wwox protein levels in *Wwox*^{1} and *Wwox*^{f04545} homozygous adult flies.
(A) Anti-Wwox antibody detects a band corresponding to Wwox (*), as previously determined (28), that is absent in *Wwox*^{1} as well as in *Wwox*^{f04545} adult mutant flies.
(B) Alpha Tubulin loading control.
Westerns were performed using anti-N-terminal Wwox antibody as previously described (28) with the following modifications: anti-Rabbit Cy5 secondary antibody was used at 1:2,500 (Rockland) and amplification of the signal was achieved using SignalBoost™ Immunoreaction Enhancer Kit (Calbiochem). Alpha Tubulin was detected using anti-alpha tubulin at 1:2000 (Sigma) and anti-mouse Alexa 488 at 1:2000 (Molecular Probes). Protein size markers are shown in kDaltons (kDa).

Table S1. MS Identification of proteins in spots that exhibited significant changes in abundance as identified by DIGE. Related to Figure 1.

Spot	Ave Ratio	T-test	Accession	Name	Combined# MOWSE score	Queries* Matched	Sequence coverage	Theoretical mol. mass (Da)/pI	Observed mol. mass (Da)/pI
***w*^{1118} vs *Wwox*^{1} adults**									
15	2.70	0.011	gi|24664081	Fat body protein 1	630 (35)	18 (9)	20%	119,666 / 6.39	~47,000 / 5.9
19	2.15	0.001	gi|8647	Cu-Zn superoxide dismutase	94 (35)	2 (2)	20%	15,974 / 5.67	~15,000 / 5.5
9	1.53	0.004	gi|8282	Alcohol dehydrogenase	114 (35)	4 (1)	19%	27,858 / 7.74	~28,000 / 6.2
10	1.25	0.034	gi|20129399	Aldehyde dehydrogenase	212 (35)	8 (2)	16%	57,325 / 6.37	~54,000 / 5.4
14^	1.16	0.006	gi|157561	Glycerol-3-phosphate dehydrogenase	80(60)	7(0)	6%	35,351 / 8.42	~37,000 / 6.1
5	1.14	0.006	gi|21358499	CG7430	72(31)	4(1)	7%	53,565 / 6.41	~54,000 / 5.65
12	1.14	0.006	gi|24650465	CG31075	315(35)	8(4)	18%	52,607 / 6.64	~54,000 / 5.55
25	1.13	0.017	gi|24649832	CG11089	380 (35)	9 (7)	16%	63,797 / 7.97	~65,000 / 7.65
13	1.10	0.003	gi|24650465	CG31075	311(35)	9(5)	22%	52,607 / 6.64	~54,000 / 5.85
23	-1.08	0.003	gi|45550132	Hsp60C	88 (35)	3 (1)	6%	61,890 / 6.75	~60,000 / 5.1
2	-1.12	0.003	gi|24648872	CG6439 / Isocitrate dehydrogenase	83(31)	4(1)	10%	40,382 / 8.74	~40,000 / 6.65
3	-1.30	0.006	gi|24647881	CG7998	138(35)	6(1)	26%	35,524 / 9.2	~35,000 / 7.5
20	-2.23	0.001	gi|8647	Cu-Zn superoxide dismutase	63 (24)	1 (1)	9%	15,974 / 5.67	~15,000 / 5.9
***w*^{1118}*; da>GAL4* vs *w*^{1118}*; da>Wwox* adults**									
16^	3.04	0.002	gi|24664081	Fat body protein 1	483 (60)	40 (6)	33%	119,350 / 5.82	~46,000 / 6.45
17	1.60	0.001	gi|7961	Fat body protein 1	273 (34)	6(4)	6%	119,350 / 5.82	~47,000 / 5.65
1	1.49	0.001	gi|20129347	Wwox	101 (35)	2 (2)	8%	47052 / 7.19	~35,000 / 6.10
6^	1.48	0.006	gi|7248650	Malic enzyme	128 (36)	4(1)	9%	84,368 / 7.23	~60,000 / 5.8
7	1.43	0.019	gi|7248650	Malic enzyme	192 (35)	9(4)	19%	84,368 / 7.23	~60,000 / 5.65
24	1.31	0.021	gi|20130249	CG2852	45 (32)	1 (1)	9%	22,185 / 8.69	~20,000 / 6.75
4	1.23	0.042	gi|17136394	Phosphoglycerate kinase	144 (32)	4 (1)	9%	44,119 / 7.01	~45,000 / 6.45
28	1.22	0.006	gi|19921864	CG8193	79 (35)	3 (1)	4%	79,520 / 6.49	~80,000 / 6.35
8^	1.22	0.004	gi|157476	Glyceraldehyde-3-phosphate dehydrogenase	72 (60)	7 (0)	19%	35,351 / 8.42	~35,000 / 6.4
27	-1.23	0.047	gi|20130403	Tudor-SN	593 (35)	14 (8)	18%	103,436 / 8.14	~100,000 / 6.5
19	-1.23	0.035	gi|8647	Cu-Zn Superoxide dismutase	94 (35)	2(2)	20%	15,988 / 5.85	~15,000 / 5.45
26	-1.26	0.013	gi|21358001	CG14526	60 (35)	3 (1)	5%	79,172 / 5.69	~80,000 / 5.7
21	-1.37	0.046	gi|21357673	CG5590	57 (32)	2 (1)	5%	44,611 / 8.11	~46,000 / 7.15
22	-1.43	0.001	gi|21357643	CG7470	308 (34)	7 (4)	13%	84,093 / 6.71	~85,000 / 5.7
11	-1.57	0.031	gi|1079042	Acetyl Coenzyme A synthase	60 (33)	2 (1)	4%	75,946 / 5.44	~75,000 / 5.45
18	-2.20	0.004	gi|24664081	Fat body protein 1	575 (43)	14 (8)	16%	119,350 / 5.82	~47,000 / 5.95

Numbers in parentheses represents the peptide 'identity' threshold score

* Numbers in parentheses represents number of queries over the peptide 'identity' threshold score

^ Analysis performed using MALDI-TOF/TOF-MS

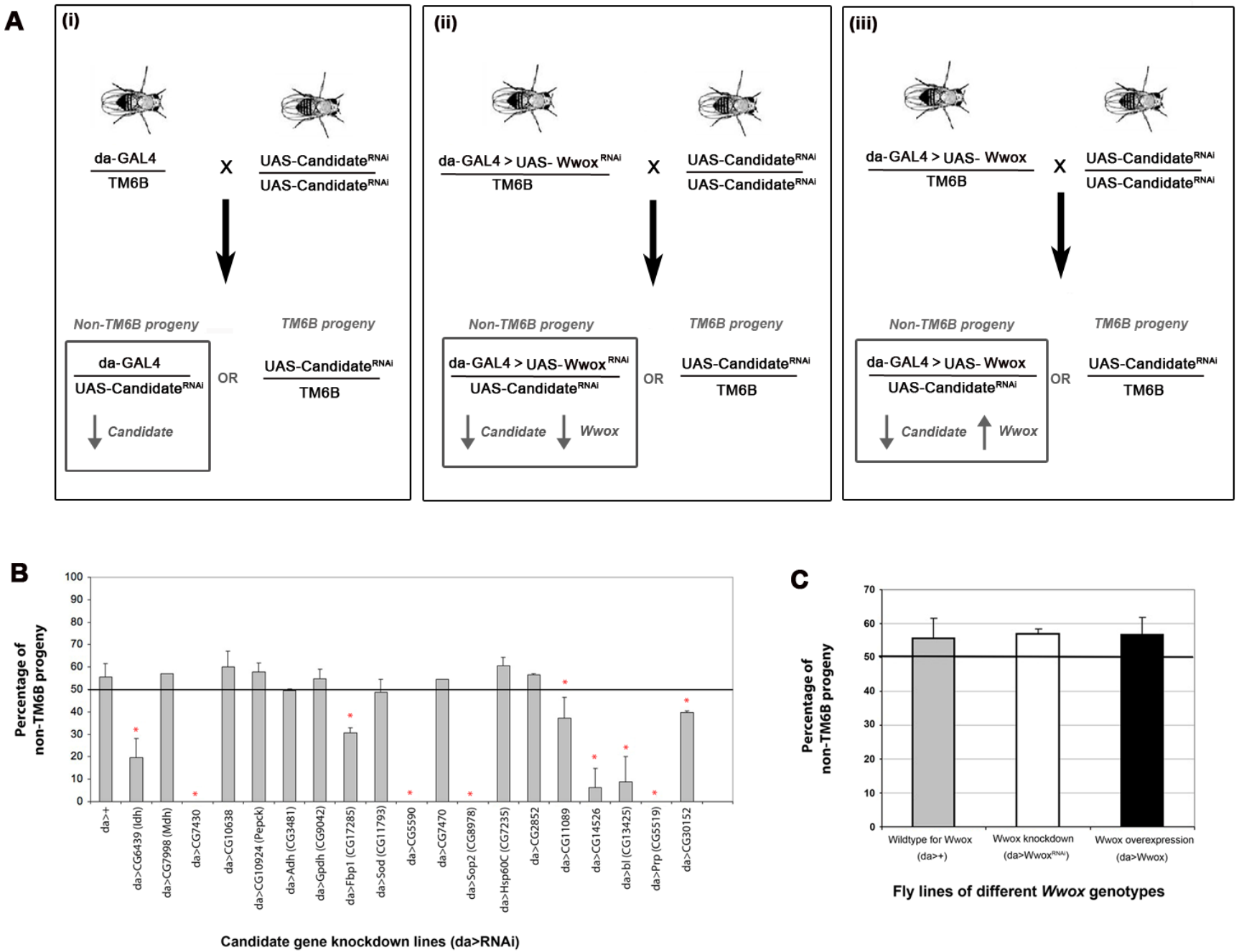
A
(i)
da-GAL4
TM6B
X
UAS-Candidate^RNAi
UAS-Candidate^RNAi
Non-TM6B progeny
TM6B progeny
da-GAL4
UAS-Candidate^RNAi
Candidate
OR
UAS-Candidate^RNAi
TM6B
(ii)
da-GAL4 > UAS- Wwox^RNAi
TM6B
X
UAS-Candidate^RNAi
UAS-Candidate^RNAi
Non-TM6B progeny
TM6B progeny
da-GAL4 > UAS-Wwox^RNAi
UAS-Candidate^RNAi
Candidate
Wwox
OR
UAS-Candidate^RNAi
TM6B
(iii)
da-GAL4 > UAS- Wwox
TM6B
X
UAS-Candidate^RNAi
UAS-Candidate^RNAi
Non-TM6B progeny
TM6B progeny
da-GAL4 > UAS- Wwox
UAS-Candidate^RNAi
Candidate
Wwox
OR
UAS-Candidate^RNAi
TM6B
B
Percentage of non-TM6B progeny
100
90
80
70
60
50
40
30
20
10
0
da>+
da>CG6439 (ldh)
da>CG7998 (Mdh)
da>CG7430
da>CG10638
da>CG10924 (Pepck)
da>Adh (CG3481)
da>Gpdh (CG9042)
da>Fbp1 (CG17285)
da>Sod (CG11793)
da>CG5590
da>CG7470
da>Sop2 (CG8978)
da>Hsp60C (CG7235)
da>CG2852
da>CG11089
da>CG14526
da>bl (CG13425)
da>Prp (CG5519)
da>CG30152
Candidate gene knockdown lines (da>RNAi)
C
Percentage of non-TM6B progeny
70
60
50
40
30
20
10
0
Wildtype for Wwox (da>+)
Wwox knockdown (da>Wwox^RNAi)
Wwox overexpression (da>Wwox)
Fly lines of different Wwox genotypes

Figure S2. Related to Figure 3, effect of knocking down expression of Wwox candidate interactors and altering Wwox expression on viability in *Drosophila.*

(A) Crosses were set up for ubiquitous RNAi knockdown of the candidate gene either with normal levels of WWOX (i) or with altered (ii – decreased, iii – increased) levels of WWOX. The da-GAL4 driver was used to drive ubiquitous expression of the UAS constructs using the binary UAS-GAL4 system. From each cross, progeny of two different genotypes could be obtained at a 1:1 ratio, in accordance with Mendel's law of segregation. The two types of progeny obtained were those that carried a copy of the UAS-candidate RNAi construct with either the da-GAL4 driver (non-TM6B progeny, in which the candidate gene is ubiquitously knocked down) or the TM6B balancer (TM6B progeny, in which there is no knockdown of the candidate gene). Crosses with a minimum of 30 TM6B progeny were analysed and the ratio of non-TM6B: TM6B progeny were compared from (i), (ii) and (iii).

(B) Viability of *Drosophila* expressing candidate RNAi lines ubiquitously with the da-GAL4 driver. The percentage of non-TM6B progeny was scored for each RNAi line. The mean percentage for each RNAi line was compared to the expected percentage (50%, as indicated with the bold line) and error bars represent standard deviation of experimental replicates when the experiment has been replicated. Any negative deviation from the expected percentage was taken as an indication of reduced viability and "*" denotes statistical significance ($p<0.05$) determined by t-test analysis.

(C) No significant difference in viability ($0.90 \geqslant p > 0.10$) was observed when Wwox levels were increased (da>*Wwox*) or decreased (da>$Wwox^{RNAi}$) compared to the wildtype control (da>+).

Table S2: Summary of results from the viability screen testing the effect of altering Wwox levels in *Drosophila* expressing candidate RNAi constructs.

Gene name/symbol	VDRC line(s)	Effect on viability with da>*Wwox*^{RNAi}			Effect on viability with da>*Wwox*		
Reduced viability with da-GAL4							
CG6439	14443^	#1	↓	P=0.256	#1	↑	P=0.757
		#2	↓	P=0.768	#2	↑	P=0.072
		#3	↓	**P<0.001 ****	#3	↑	**P=0.016 ****
		#4	↓	**P=0.002 ****	#4	↑	**P<0.001 ****
		#5	↓	P=0.405	#5	↑	**P<0.001 ****
CG11089	31421	#1	↓	P=0.185	#1	↓	P=0.238
		#2	↓	**P=0.007 ****	#2	↑	P=0.471
Bancal/CG13425‡	2912	#1	↓	P=0.052	#1	↓	P=0.413
		#2	↓	**P<0.001 ****	#2	↓	**P=0.003 ****
		#3	No change		#3	↑	P=0.197
		#4	No change		#4	↑	P=0.158
CG14526	47138	#1	↑	P=0.198	#1	↓	P=0.102
		#2	↑	**P=0.036 ****	#2	↓	**P=0.005****
	47139	#1	↑	P=0.565	#1	↑	P=0.096
		#2	↓	P=0.529	#2	↑	P=0.933
	39838^	#1	↓	P=0.897	#1	↑	P=0.090
		#2	↑	P=0.207	#2	↑	P=0.483
Fat body protein 1(Fbp1)/CG17285	37881	#1	↓	P=0.425	#1	↓	P=0.685
		#2	↑	P=0.613	#2	↑	P=0.215
CG30152	38848	#1	↑	P=0.520	#1	↑	P=0.132
		#2	↓	**P=0.033 ****	#2	↑	P=0.663
Lethal with da-GAL4							
Prp19/CG5519	22147	#1	No rescue of lethality		#1	No rescue of lethality	
		#2	No rescue of lethality		#2	No rescue of lethality	
CG5590	42040	#1	No rescue of lethality		#1	No rescue of lethality	
		#2	No rescue of lethality		#2	No rescue of lethality	
CG7430	28011	#1	No rescue of lethality		#1	No rescue of lethality	
Sop2/CG8978	42171	#1	No rescue of lethality		#1	No rescue of lethality	
		#2	No rescue of lethality		#2	No rescue of lethality	

Related to Figure 3. 10 of the candidate genes identified by proteomic or microarray analysis were tested in this screen. For *CG14526*, line 47138 was the original line tested and included in Figure S1; lines 47139 and 39838 were later tested as two other independent lines for that gene. Numbers #1-5 refer to the number of experimental replicates tested for that particular RNAi line. The arrows indicate the direction in which altering WWOX levels affects the viability of the genotype. The p-values highlighted in grey indicate statistical significance ($p<0.05$) determined by chi-square test analysis.

^These RNAi lines have 1 reported off target (http://stockcenter.vdrc.at).

‡For candidate gene *Bancal/CG13425,* ubiquitously expressing the RNAi construct in *Drosophila* resulted in reduced viability in two experiments (#1 and #2) but complete lethality in two other replicates (#3 and #4). In experiments #3 and #4, decreasing WWOX levels in *Drosophila* expressing the *Bancal* RNAi construct also resulted in lethality, hence the results are reported as “no change”.

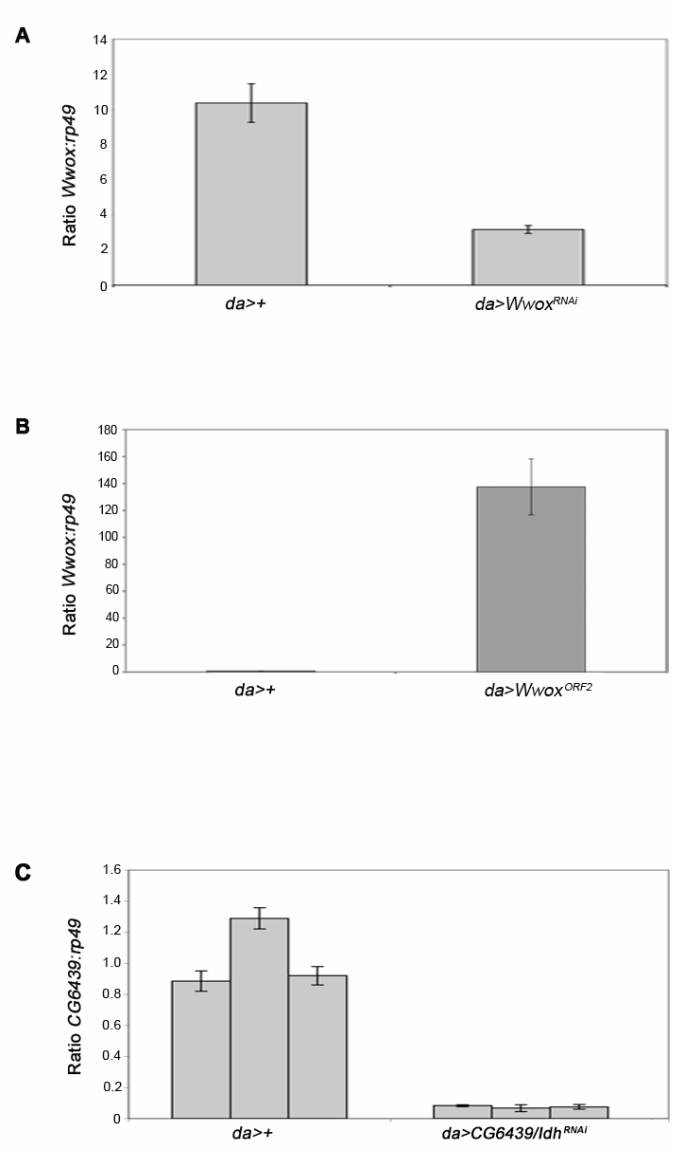

Figure S3. Related to Figure 3, qPCR analysis of *Wwox* and *CG6439* transcript levels in 3rd instar larvae.
(A) Ubiquitous expression of RNAi directed against *Wwox* (*da>Wwox*^{RNAi}) resulted in knockdown of endogenous *Wwox* transcript levels compared to the control with the ubiquitous driver alone (*da>+*).
(B) Ubiquitous ectopic expression of Wwox open reading frame (ORF) under the daughterless promoter *(da>Wwox*^{ORF}*)* results in increased levels of *Wwox* transcript compared to the control *(da>+).*
(C) Knockdown of endogenous *CG6439/Idh* transcripts was observed following ubiquitous expression of *CG6439/Idh*^{RNAi} (*da>CG6436/IdhRNAi*) compared to the control (*da>+*).

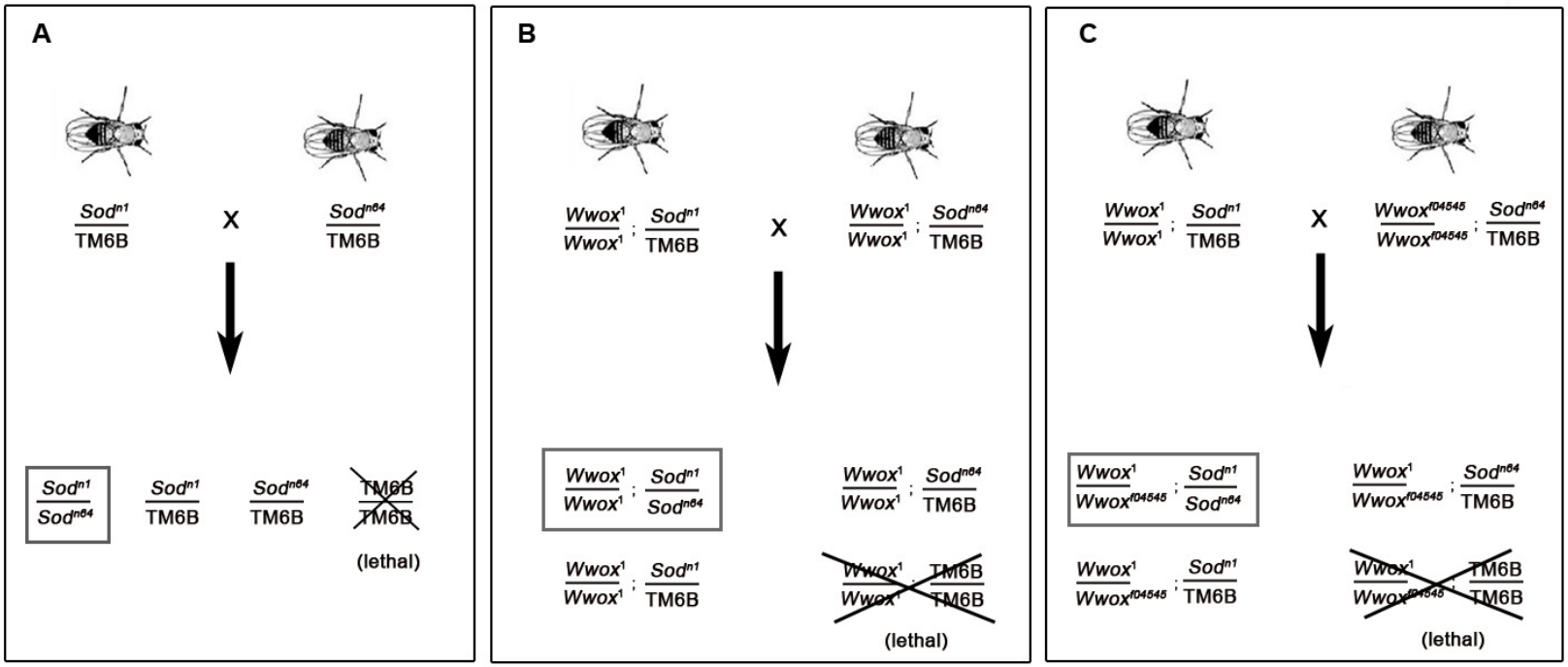

Figure S4. Related to Figure 4, crosses set up to test the effect of the *Wwox* mutations on the viability of *Drosophila* trans-heterozygous for the Sod^{n1}/Sod^{n64} mutations.
(A) In order to generate *Drosophila* that are trans-heterozygous for the Sod^{n1}/Sod^{n64} mutations, Sod^{n1} flies that are balanced over a TM6B balancer were crossed to Sod^{n64} flies balanced over a TM6B balancer. In accordance to Mendel's law of segregation, progeny of 4 different genotypes could be obtained at a 1:1:1:1 ratio. However, as having two copies of TM6B balancer results in lethality, only progeny of the 3 other genotypes were obtained and it was expected that 33.3% of the total progeny would be of the desired Sod^{n1}/Sod^{n64} genotype.
(B) & (C) Crosses set up to test the effect of the *Wwox* mutations on the viability of *Drosophila* trans-heterozygous for the Sod^{n1}/Sod^{n64} mutations. The percentage of progeny being *Wwox;Sod* double mutants out of the total progeny was calculated to determine if there was a decrease from the expected 33.3%.