

Title:

Splice form variant and amino acid changes in MDR49 confers DDT resistance in transgenic
Drosophila

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Supplementary table S1. Cloning primers used in this study.

Gene	Sequence	Remarks
Dmel_MDR49A	F: GCAGTATCGCCTCATAGACACAATGG R: TCACTCCAGTTCGCTGTGTTAG	For the cloning of full Dmel_MDR49 ORF
Dmel_MDR49B	F: CCTCATAGACACAATGACCTTTGTG R: TCACTCCAGTTCGCTGTGTTAG	

Supplementary table S2. Summary of RNA-seq data from 91-R and 91-C.

Strains	Replicates	Total reads	Genes	Coverages	Mapped reads
91-R	R1	152,800,522	<i>MDR49A</i>		3,270
			<i>MDR49B</i>	137.3	2,733
			<i>MDR49N</i>		3,142
			<i>MDR50</i>	92	2,385
			<i>MDR65</i>	80.3	1,499
	R2	152,219,476	<i>MDR49A</i>		3,492
			<i>MDR49B</i>	159.1	3,263
			<i>MDR49N</i>		ND
			<i>MDR50</i>	162.2	3,953
			<i>MDR65</i>	84.3	1,696
	R3	167,856,374	<i>MDR49A</i>		4,766
			<i>MDR49B</i>	189.7	3,802
			<i>MDR49N</i>		4,607
			<i>MDR50</i>	175.4	4,303
			<i>MDR65</i>	91.3	1,697
91-C	R1	154,166,490	<i>MDR49A</i>	265.9	5,414
			<i>MDR49B</i>		5,440
			<i>MDR50</i>	133.7	3,265
			<i>MDR65</i>	112.6	2,523
	R2	142,269,050	<i>MDR49A</i>	203.9	4,563
			<i>MDR49B</i>		4,031
			<i>MDR50</i>	176	4,097
			<i>MDR65</i>	98.9	2,234
	R3	155,930,278	<i>MDR49A</i>	224.9	5,652
			<i>MDR49B</i>		4,564
			<i>MDR50</i>	208	5,006
			<i>MDR65</i>	117.5	2,178

ND describes 'not determined'

Supplementary table S3. Comparison of expression level of *MDR49A* and *MDR49B* in *91-R* and *91-C*, based on RNA-seq data.

Gene	Expression value*		ratio**	p-value
	<i>91-R</i>	<i>91-C</i>		
<i>MDR49A</i>	6.72 ± 0.13	8.01 ± 0.21	0.86	<i>p</i> >0.05
<i>MDR49B</i>	6.67 ± 0.58	6.75 ± 0.32	0.99	

*Expression value was log₂ transformed from raw read coverages.

**Ratio was calculated as *91-R/91-C*.

Supplementary figure legend

Supplementary figure S1. Sequence alignment of putative amino acids deduced of the MDR50 proteins from *91-C* and *91-R* strain. Three mutations, T393A, S881F, and V1012L were observed between two strains. The NBDs are homologous for *91-C* and *91-R* strain and each NBD has seven highly conserved motifs. The conserved aromatic, Walker A, Q-loop, ABC signature, Walker B, D-loop, and H-loop are boxed.

Supplementary figure S2. Sequence alignments of the putative amino acids deduced from the MDR65 proteins from the *91-C* and *91-R* strains. Three mutations that resulted in amino acid replacements, K277R, I646M, and L992P, were observed when the *91-R* strain was compared with the *91-C* strain. The NBDs were homologous for both strains and each NBD had seven highly conserved motifs (aromatic, Walker A, Q-loop, ABC signature, Walker B, D-loop, and H-loop), which appear in boxes

Supplementary figure S3. Prediction of the alternative splice variants in *MDR50*. (A) One alternative splice variant was predicted from *91-C-MDR50*. (B) One alternative splice variant was predicted from *91-R-MDR50*.

Supplementary figure S4. Prediction of the alternative splice variants in *MDR65*. (A) One alternative splice variant was predicted from *91-C-MDR65*. (B) One alternative splice variant was predicted from *91-R-MDR65*.

Supplementary figure S1.

91-C-MDR50	MTVKNGDVVKDDVNSRSQYKTNIVLGAKLESDRRDRKSFEPNKKKKSKHDESASDEED	60
91-R-MDR50	MTVKNGDVVKDDVNSRSQYKTNIVLGAKLESDRRDRKSFEPNKKKKSKHDESASDEED	60

91-C-MDR50	GSQYHEDVKQVSYFQLFRYATKKDRALYVIGLLSAVATGLTTPANSLIFGNLANDMIDLS	120
91-R-MDR50	GSQYHEDVKQVSYFQLFRYATKKDRALYVIGLLSAVATGLTTPANSLIFGNLANDMIDLS	120

91-C-MDR50	GLLESQSYRADDIAISTLLLDKVRQFSLQNTYIGIIMLVCSYLSITCFNYAAHSQILTIR	180
91-R-MDR50	GLLESQSYRADDIAISTLLLDKVRQFSLQNTYIGIIMLVCSYLSITCFNYAAHSQILTIR	180

91-C-MDR50	SKFFRSILHQDMKWYDFNQSGEVASRMNEDLSKMEDGLAEKVVMFVHYLVAFVGSVLVAF	240
91-R-MDR50	SKFFRSILHQDMKWYDFNQSGEVASRMNEDLSKMEDGLAEKVVMFVHYLVAFVGSVLVAF	240

91-C-MDR50	VKGWQLSLVCLTSLPLTFIAMGLVAVATSRSLAKKEVTMYAGA AVVAEGALSGIRTVKAFE	300
91-R-MDR50	VKGWQLSLVCLTSLPLTFIAMGLVAVATSRSLAKKEVTMYAGA AVVAEGALSGIRTVKAFE	300

91-C-MDR50	GEAKEVAAYKERVVAAKILNIKRNMFSGIGFLLWFFIYASYALAFWYGVGLVIESAYEN	360
91-R-MDR50	GEAKEVAAYKERVVAAKILNIKRNMFSGIGFLLWFFIYASYALAFWYGVGLVIESAYEN	360

91-C-MDR50	YDAGTMITVFFSVMMGSMNIGMAAPYIEAFGI T KGACAKVFHIEQIPEINPIDGEGKKL	420
91-R-MDR50	YDAGTMITVFFSVMMGSMNIGMAAPYIEAFGI A KGACAKVFHIEQIPEINPIDGEGKKL	420

	Aromatic	Walker A
91-C-MDR50	NEPLTTIEFKEVEFEQYPTREPEVSILNKLNLKIHRGQTVALV G PSGCGKSTCIQLVQRFYD	480
91-R-MDR50	NEPLTTIEFKEVEFEQYPTREPEVSILNKLNLKIHRGQTVALV V PSGCGKSTCIQLVQRFYD	480

	Q-loop	
91-C-MDR50	PQAGNLLFNGTNLKDLDINWLRSRIGVV G QEPILFATSIYENIRYGRE DATREEIEAAAA	540
91-R-MDR50	PQAGNLLFNGTNLKDLDINWLRSRIGVV G QEPILFATSIYENIRYGRE DATREEIEAAAA	540

	ABC Signature	Walker B D-loop
91-C-MDR50	AANAAIFIKKLPKGYDTLVGERGAL S GGGQKRIAIARALIRDPE I LLDEATSALD T PAS	600
91-R-MDR50	AANAAIFIKKLPKGYDTLVGERGAL L SGGQKRIAIARALIRDPE I LLDEATSALD T PAS	600

	H-loop	
91-C-MDR50	EAKVQAALAEKVSAGRTTIIV A HR LSTVRRADRIVVINKGEVVE SGTHQELMELKDHYNL	660
91-R-MDR50	EAKVQAALAEKVSAGRTTIIV A HR LSTVRRADRIVVINKGEVVE SGTHQELMELKDHYNL	660

91-C-MDR50	VTTQLGEDDGSVLSPTGDIYKNFDIKDEDEEEIKVLSSEDEDEDVMVTDEKNKKKKMKVK	720
91-R-MDR50	VTTQLGEDDGSVLSPTGDIYKNFDIKDEDEEEIKVLSSEDEDEDVMVTDEKNKKKKMKVK	720

91-C-MDR50	DPNEVKPMLEVMMKNKPEWLQI AVGCISSVIMGCAMP IFAVLFGSILQILSVKDNDQYVR	780
91-R-MDR50	DPNEVKPMLEVMMKNKPEWLQI AVGCISSVIMGCAMP IFAVLFGSILQILSVKDNDQYVR	780

91-C-MDR50	ENSNQYSLYFLIAGIVVGIATFLQIYFFGIAGERLTERLRGLMFEAMLRQEVAFDDKAN	840
91-R-MDR50	ENSNQYSLYFLIAGIVVGIATFLQIYFFGIAGERLTERLRGLMFEAMLRQEVAFDDKAN	840

91-C-MDR50	GTGSLCARLSGDAAVQGATGQRIGTIIQISISTLALGIAL S MYEWSLGLVALAFTPFIL	900
91-R-MDR50	GTGSLCARLSGDAAVQGATGQRIGTIIQISISTLALGIAL F MYEWSLGLVALAFTPFIL	900

91-C-MDR50	IAFYMQRTLMAKENMGSAKTMENCTKLAVEVVSNI RTVASL GREEMFHQNYIGMLIPAVE	960
91-R-MDR50	IAFYMQRTLMAKENMGSAKTMENCTKLAVEVVSNI RTVASL GREEMFHQNYIGMLIPAVE	960

91-C-MDR50	ISKRNTHFRGLVYGLARSLMFFAYAACMYGTWCVIHRGILFGDVFKV S QALIMGTASIA	1020
91-R-MDR50	ISKRNTHFRGLVYGLARSLMFFAYAACMYGTWCVIHRGILFGDVFKV Q ALIMGTASIA	1020

91-C-MDR50	NALAFAPNMQKGVSAAKTIFTFLRRQPSIVDRPGVSRDPWHSEGYVRFDKVKFSYPTRSE	1080
91-R-MDR50	NALAFAPNMQKGVSAAKTIFTFLRRQPSIVDRPGVSRDPWHSEGYVRFDKVKFSYPTRSE	1080

91-C-MDR50	IQVLKGLELAVSKGQKIALVGPSCGKSTCIQLIQRFYDVDEGATLIDEC DV RNVSMTNL	1140
91-R-MDR50	IQVLKGLELAVSKGQKIALVGPSCGKSTCIQLIQRFYDVDEGATLIDEC DV RNVSMTNL	1140

91-C-MDR50	RNQLGIVSQEPILFDRTIRENISYGDNARNVTDQEIISACKKSNIHEFIANLPLGYDTRM	1200
91-R-MDR50	RNQLGIVSQEPILFDRTIRENISYGDNARNVTDQEIISACKKSNIHEFIANLPLGYDTRM	1200

91-C-MDR50	GEKGAQLSGGQKRIAIARALIRNPKIMLLDEATSALDAESEKVVQDALDAASEGRTTIS	1260
91-R-MDR50	GEKGAQLSGGQKRIAIARALIRNPKIMLLDEATSALDAESEKVVQDALDAASEGRTTIS	1260

91-C-MDR50	IAHRLSTVVHSDVIFVFENGLVCEAGDHQQLLANRGLYTYLYKLQSGAM	1309
91-R-MDR50	IAHRLSTVVHSDVIFVFENGLVCEAGDHQQLLANRGLYTYLYKLQSGAM	1309

Supplementary figure S2.

91-C-MDR65	MERDEVSTSSSEGKSQEEAPMAEGLEPTEPIAFLKLFSTYGEIGWLFQFGFIMCCIKAL	60
91-R-MDR65	MERDEVSTSSSEGKSQEEAPMAEGLEPTEPIAFLKLFSTYGEIGWLFQFGFIMCCIKAL	60

91-C-MDR65	TLPAVVIYSEFTSMLVDRAMQFGTSSNVHALPLFGGKTLTNASREENNEALYDDISY	120
91-R-MDR65	TLPAVVIYSEFTSMLVDRAMQFGTSSNVHALPLFGGKTLTNASREENNEALYDDISY	120

91-C-MDR65	GILLTIASVVMFISGIFSVDFNMVALRQVTRMRIKLFSSVIRQDIGWHDLASKQNFQTS	180
91-R-MDR65	GILLTIASVVMFISGIFSVDFNMVALRQVTRMRIKLFSSVIRQDIGWHDLASKQNFQTS	180

91-C-MDR65	MVDDVEKIRDGISEKVGHFVYLVVGFIIITVAISFSYGWKLTAVSSYIPLVILLNYYVAK	240
91-R-MDR65	MVDDVEKIRDGISEKVGHFVYLVVGFIIITVAISFSYGWKLTAVSSYIPLVILLNYYVAK	240

91-C-MDR65	FQGKLTAREQESYAGAGNLAEEILSSIRTVVSVFGGKSEVQRYENFLVPARKASQWKGA	300
91-R-MDR65	FQGKLTAREQESYAGAGNLAEEILSSIRTVVSVFGGERSEVQRYENFLVPARKASQWKGA	300

91-C-MDR65	SGLSDAVLKSMYLSLSCAGAFWYGVNLIIDDRNVENKEYTPAILMIAFFGIIVGADNIART	360
91-R-MDR65	SGLSDAVLKSMYLSLSCAGAFWYGVNLIIDDRNVENKEYTPAILMIAFFGIIVGADNIART	360

Aromatic		
91-C-MDR65	APFLESFATARGCATNLFKVIDLTSKIDPLSTDGKLLNYGLRGDVEFQDVFFHYPSRPEV	420
91-R-MDR65	APFLESFATARGCATNLFKVIDLTSKIDPLSTDGKLLNYGLRGDVEFQDVFFHYPSRPEV	420

Walker A		
91-C-MDR65	IVHRGLNIRIRAGQTVLVGSSGGCKSTCVQLLQRFYDPVFGSVLLDDLDIRKYNIQWLR	480
91-R-MDR65	IVHRGLNIRIRAGQTVLVGSSGGCKSTCVQLLQRFYDPVFGSVLLDDLDIRKYNIQWLR	480

Q-loop		
91-C-MDR65	SNIAVVGQEPVLFGLGTIAQNISYKPGATQKEIEAAATQAGAHEFITNLPESYRSMIGER	540
91-R-MDR65	SNIAVVGQEPVLFGLGTIAQNISYKPGATQKEIEAAATQAGAHEFITNLPESYRSMIGER	540

ABC Signature Walker B D-loop H-loop		
91-C-MDR65	GSQLSGGQKQRIAIARALIQNPKILLLDEATSALDYQSEKQVQALDLASKGRTTIVVSH	600
91-R-MDR65	GSQLSGGQKQRIAIARALIQNPKILLLDEATSALDYQSEKQVQALDLASKGRTTIVVSH	600

91-C-MDR65	RLSAIRGADKIVFIHDGKVLVEEGSHDDLMALEGAYNMVRAGDINIPDEVEKEDSIEDTK	660
91-R-MDR65	RLSAIRGADKIVFIHDGKVLVEEGSHDDLMALEGAYNMVRAGDINIPDEVEKEDSIEDTK	660

91-C-MDR65	QKSLALFEKSFETSPLNFEKGQKNSVQFEETIKALIKDTNAQSAEAPPEKPNFFRTFSR	720
91-R-MDR65	QKSLALFEKSFETSPLNFEKGQKNSVQFEETIKALIKDTNAQSAEAPPEKPNFFRTFSR	720

91-C-MDR65	ILQLAKPEWCYLILGTISAVAVGLYPAFAVIFGEFYAALAEKDPEDALRRTAVLSWACL	780
91-R-MDR65	ILQLAKPEWCYLILGTISAVAVGLYPAFAVIFGEFYAALAEKDPEDALRRTAVLSWACL	780

91-C-MDR65	GLAFLTGLVCFLQTYLFNYAGIWLTTTRMRAMTFNAMVNQEVGFDDENNSVGLSARLSG	840
91-R-MDR65	GLAFLTGLVCFLQTYLFNYAGIWLTTTRMRAMTFNAMVNQEVGFDDENNSVGLSARLSG	840

91-C-MDR65	EAVDIQGAIGYPLSGMIQALSNISSVSVAMYNNWKLALLCLANCPPIVGSVILEAKMMS	900
91-R-MDR65	EAVDIQGAIGYPLSGMIQALSNISSVSVAMYNNWKLALLCLANCPPIVGSVILEAKMMS	900

91-C-MDR65	NAVVRKQVIEEACRIATESITNIRTVAGLRREADVIREYTEEIQRVEVLIRQKLRWRGV	960
91-R-MDR65	NAVVRKQVIEEACRIATESITNIRTVAGLRREADVIREYTEEIQRVEVLIRQKLRWRGV	960

91-C-MDR65	LNSTMQASAFFAYAVALCYGGVLVSEGLLFQDIKVSSETLLYGSMMLAQSLAFTPAFSA	1020
91-R-MDR65	LNSTMQASAFFAYAVALCYGGVLVSEGLLFQDIKVSSETLLYGSMMLAQSLAFTPAFSA	1020

91-C-MDR65	ALIAGHRLFQILDRKPKIQSPMGTIKNTLAKQLNLFEGVRYRGIQFRYPTRPDAKILNGL	1080
91-R-MDR65	ALIAGHRLFQILDRKPKIQSPMGTIKNTLAKQLNLFEGVRYRGIQFRYPTRPDAKILNGL	1080

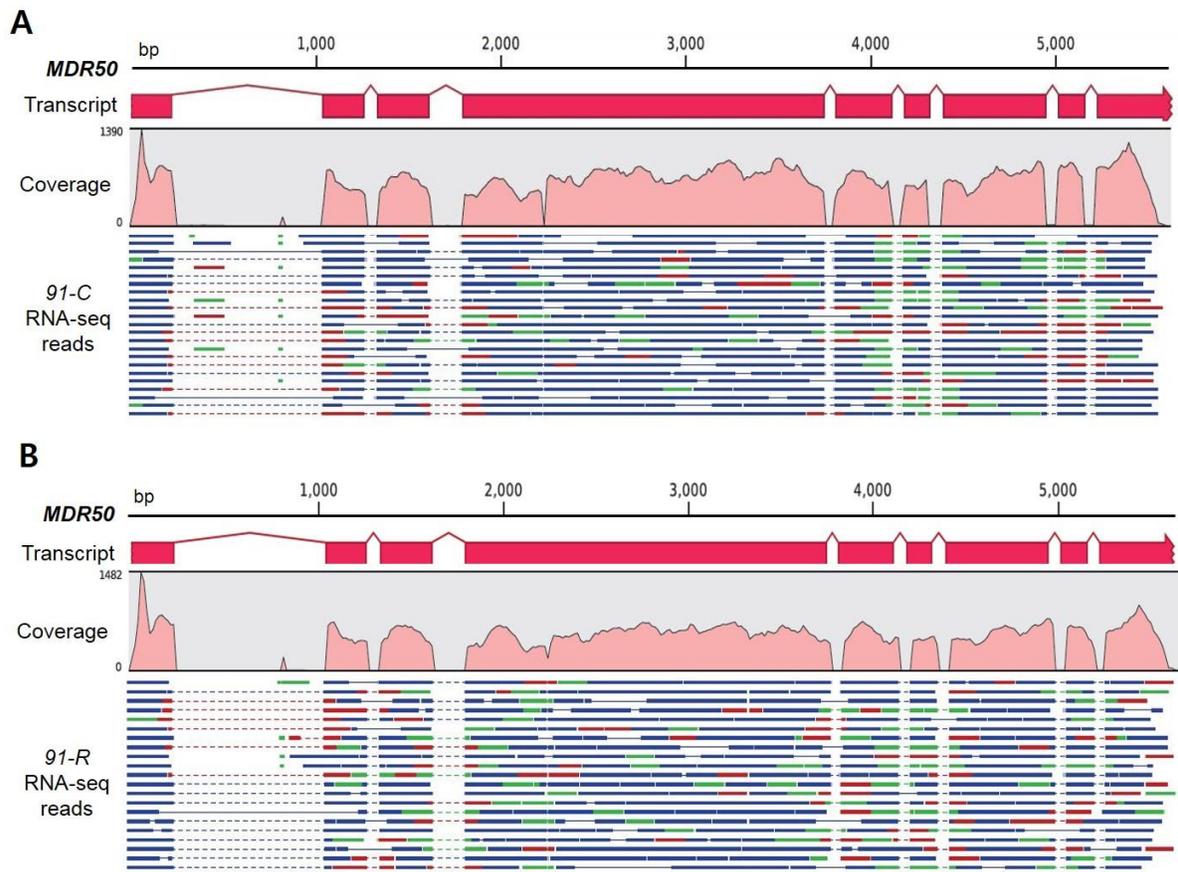
91-C-MDR65	DLEVLKGQTVLVGHSGCGKSTCVQLLQRYDPEGTIHDHDDIQHDLTLDGVRTKLG	1140
91-R-MDR65	DLEVLKGQTVLVGHSGCGKSTCVQLLQRYDPEGTIHDHDDIQHDLTLDGVRTKLG	1140

91-C-MDR65	VSQEPFLFERSIAENIAYGDNRRSVSMVEIIAAAKSANAHSFIIISLPNGYDTRMGARGTQ	1200
91-R-MDR65	VSQEPFLFERSIAENIAYGDNRRSVSMVEIIAAAKSANAHSFIIISLPNGYDTRMGARGTQ	1200

91-C-MDR65	LSGGQKQRIAIARALVRNPKILLLDEATSALDLQSEQLVQALDTACSGRTCVIAHRLS	1260
91-R-MDR65	LSGGQKQRIAIARALVRNPKILLLDEATSALDLQSEQLVQALDTACSGRTCVIAHRLS	1260

91-C-MDR65	TVQNADVICVIQNGQVVEQGNHMLISQGGIYAKLHKTQKDH	1302
91-R-MDR65	TVQNADVICVIQNGQVVEQGNHMLISQGGIYAKLHKTQKDH	1302

Supplementary figure S3.



Supplementary figure S4.

