Conditional inactivation of Mrg15 gene function limits survival during larval and adult stages of *Drosophila melanogaster*

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Supplementary Materials

Supplementary Materials and Methods

Generation of DNA constructs

pDmMRG15. Drosophila *DmMRG15* full-length cDNA was obtained from DGRC and was amplified by PCR using primer SET1, which yields flanking *PstI* and *SpeI* restriction sites. A Gate-Way vector containing a 3xHA tag was also obtained from the Carnegie Institution of Washington and DGRC. The DNA fragment from the Gate-Way vector that includes the 3xHA tag and the 3' UTR/polyA signal was amplified using primer SET2, which yields flanking restriction sites *SpeI* and *EcoRI*. The two PCR fragments described above were then subcloned into the *PstI* to *EcoRI* sites of pUSC1.0 vector (Allikian et al., 2002) by three-fragment ligation.

phMRG15 and phMORF4. These constructs were generated using a strategy analogous to that above. Human MRG15 and MORF4 were amplified from plasmids pcDNA3.1(+)hMRG15 and pcDNA3.1(+)hMORF4 (Tominaga et al., 2005) using primer SET3 and SET4, respectively. These fragments were then subcloned along with the 3xHA tag and 3'UTR/polyA signal fragment into pUSC.1 vector by three-fragment ligation. Multiple independent transgenic strains were generated for both human constructs, however no expression was observed for any of the resultant transgenic lines using Western blot analysis (data not shown).

pDmMT1. According to the DNA sequence of human MORF4, a Drosophila MRG15 mutant lacking the chromo domain was generated. PCR reactions were performed using primer SET5 which yields flanking *PstI* and *SpeI* sites. The fragment was then subcloned along with the 3xHA tag and 3'UTR/polyA signal fragment into pUSC1.0 vector by three-fragment ligation.

pRNAi-DmMRG15. The RNAi vector called pWIZ (Lee and Carthew, 2003) was obtained from DGRC. A fragment of pWIZ including two MCSs, the *white* gene intron 2 and the SV40 polyA signal sequence was amplified using primer SET6, which yields flanking *PstI* and *EcoRI* sites. This fragment was subcloned into pUSC1.0 vector to generate an intermediate construct called pUSC-RNAi-intermediate. Drosophila *DmMRG15* exon 4 was amplified using primer SET7 and SET8, and sequentially subcloned into pUSC-RNAi-intermediate to generate p*RNAi-DmMRG15*.

Primer sequences.

SET1: 5' primer, AGCTCTGCAG(*PstI*)TGAAAATAAAATGGGAGAAGTAA; 3' primer, AGCTACTAGT(*SpeI*)CTGTGCATTTCGCACGTAC

SET2: 5' primer, AGCTACTAGT(*SpeI*)ACC ATG GAT CTC CAC CGC; 3' primer, AGCTGAATTC (*EcoRI*)GATCCAGACATGATAAGATAC

SET3: 5' primer, AGCTCTGCAG(*PstI*)CCACCATGGCGCCG AAG ; 3' primer, AGCTACTAGT(*SpeI*)CACAGCTTTCCGATGGTAC

SET4: 5' primer, AGCTCTGCAG(*PstI*)CCACCATGAGATGGGCTG; 3' primer, AGCTACTAGT(*SpeI*)CACAGCTTTCCGATGGTAC

SET5: 5' primer, AGCTCTGCAG(*PstI*)TGAAAATAAAATGAAAGGCAGCGCCA-AGGC; 3' primer, AGCTACTAGT(*SpeI*)CTGTGCATTTCGCACGTAC

SET6: 5’ primer, AGCTCTGCAG(*PstI*)GTTAACAGATCTGCGGCC; 3’ primer, AGCTGAATTC (*EcoRI*)GATCCAGACATGATAAGATAC

SET7: 5’ primer, AGCTACTAGT(*SpeI*)GCAGCGCCAAGGCCAAGA; 3’ primer, AGCTACGCGT(*MluI*)CTGTGCATTTCGCACGTAC

SET8: 5’ primer, AGCTCCTAGG(*AvrII*)GCAGCGCCAAGGCCAAGA; 3’ primer, AGCTCTGCAG(*PstI*)CTGTGCATTTCGCACGTAC

SET9: 5’ primer, CGT CAC GCA CGG TCA CTC; 3’ primer CTT TCT TAT TAT CCT TCT TGG A

Supplementary Tables

Supplemental Table S1. Transgenic strains

Construct	Independent line	Chromosome
DmMRG15	7	2nd
	8	3rd
	21	2nd
	26	3rd
	33	3rd
	47	X
DmMT1	3	2nd
	11	3rd
	53	2nd
	106	3rd
RNAi-DmMRG15	4-1	X

	4-3	3rd
	53	2nd
hMORF4	1-1	2nd
	15	X
	23-1	3rd
	24-2	3rd
	76	3rd
hMRG15	61	3rd
	63-1	2nd
	63-2	3rd
	66	3rd
	67	3rd

Supplemental Table S2. Life span statistics summary

Exp 1

Genotype	Drug	Sex	Median	SD	Δ Median	*p*
Control (Or-R)	+DOX	M	89	13.6	-2.20%	0.195
Control (Or-R)	-DOX	M	91	13.3	N/A	N/A
Control (Or-R)	+DOX	F	90	8.2	2.27%	0.0147
Control (Or-R)	-DOX	F	88	8.6	N/A	N/A
DmMRG15-7	+DOX	M	96	12.5	14.29%	<0.001
DmMRG15-7	-DOX	M	84	12.0	N/A	N/A
DmMRG15-7	+DOX	F	92	17.4	4.55%	0.00114
DmMRG15-7	-DOX	F	88	17.9	N/A	N/A
DmMRG15-8	+DOX	M	108	18.2	2.86%	<0.001
DmMRG15-8	-DOX	M	105	14.6	N/A	N/A
DmMRG15-8	+DOX	F	105	8.4	0.96%	<0.001
DmMRG15-8	-DOX	F	104	14.1	N/A	N/A
RNAi-DmMRG15-53	+DOX	M	88	15.9	-10.20%	0.0463
RNAi-DmMRG15-53	-DOX	M	98	12.2	N/A	N/A
RNAi-DmMRG15-53	+DOX	F	99	10.1	-3.88%	<0.001
RNAi-DmMRG15-53	-DOX	F	103	11.9	N/A	N/A
RNAi-DmMRG15-4-1	+DOX	F	92	12.8	-8.00%	0.0191
RNAi-DmMRG15-4-1	-DOX	F	100	14.8	N/A	N/A
hMORF4-76	+DOX	M	99	11.0	-2.94%	0.151
hMORF4-76	-DOX	M	102	12.2	N/A	N/A

Exp 2

Genotype	Drug	Sex	Median	SD	Δ Median	*p*
Control (Or-R)	+DOX	M	92	24.7	6.98%	0.0096
Control (Or-R)	-DOX	M	86	26.5	N/A	N/A
Control (Or-R)	+DOX	F	88	11.7	0.00%	0.806
Control (Or-R)	-DOX	F	88	13.0	N/A	N/A
DmMRG15-7	+DOX	M	98	14.2	2.08%	0.458
DmMRG15-7	-DOX	M	96	18.1	N/A	N/A
DmMRG15-7	+DOX	F	94	20.1	2.17%	0.0253
DmMRG15-7	-DOX	F	92	15.6	N/A	N/A
DmMRG15-8	+DOX	M	90	19.7	4.65%	0.00181
DmMRG15-8	-DOX	M	86	18.6	N/A	N/A
DmMRG15-8	+DOX	F	104	15.2	1.96%	0.0153
DmMRG15-8	-DOX	F	102	10.9	N/A	N/A
DmMRG15-21	+DOX	M	88	24.2	0.00%	0.862
DmMRG15-21	-DOX	M	88	21.1	N/A	N/A
DmMRG15-21	+DOX	F	100	23.0	0.00%	0.255
DmMRG15-21	-DOX	F	100	28.6	N/A	N/A
DmMRG15-33	+DOX	M	108	16.4	0.00%	0.797
DmMRG15-33	-DOX	M	108	18.7	N/A	N/A
DmMRG15-33	+DOX	F	100	28.5	-3.85%	0.0049
DmMRG15-33	-DOX	F	104	36.3	N/A	N/A
DmMRG15-47	+DOX	M	82	13.0	-2.38%	0.159
DmMRG15-47	-DOX	M	84	14.5	N/A	N/A
DmMRG15-47	+DOX	F	96	24.2	9.09%	0.258
DmMRG15-47	-DOX	F	88	28.3	N/A	N/A
RNAi-DmMRG15-4-1	+DOX	M	76	13.4	-7.32%	0.00143
RNAi-DmMRG15-4-1	-DOX	M	82	10.2	N/A	N/A
RNAi-DmMRG15-4-1	+DOX	F	98	18.8	-2.00%	0.754
RNAi-DmMRG15-4-1	-DOX	F	100	19.9	N/A	N/A
RNAi-DmMRG15-4-3	+DOX	M	76	20.1	-7.32%	0.0177
RNAi-DmMRG15-4-3	-DOX	M	82	24.4	N/A	N/A
RNAi-DmMRG15-4-3	+DOX	F	80	20.3	-16.67%	0.0029
RNAi-DmMRG15-4-3	-DOX	F	96	23.9	N/A	N/A
RNAi-DmMRG15-53	+DOX	M	74	17.3	-15.91%	<0.001
RNAi-DmMRG15-53	-DOX	M	88	23.8	N/A	N/A

Exp 3

Genotype	Drug	Sex	Median	SD	Δ Median	*p*
Control (Or-R)	+Dox	M	68	12.7	0 %	0.8321
Control (Or-R)	-Dox	M	68	10.5	N/A	N/A
Control (Or-R)	+Dox	F	70	6.6	-5.41%	0.0198
Control (Or-R)	-Dox	F	74	8.9	N/A	N/A
Control (w1118)	+Dox	M	89	16.7	4.71%	0.2458
Control (w1118	-Dox	M	85	13.8	N/A	N/A
Control (w1118	+Dox	F	83	12.7	-2.35%	0.1153
Control (w1118)	-Dox	F	85	12.8	N/A	N/A
MT1-3	+Dox	M	88	15.2	4.76 %	0.0569
MT1-3	-Dox	M	84	14.4	N/A	N/A
MT1-3	+Dox	F	86	11.9	2.38 %	0.0237
MT1-3	-Dox	F	84	10.8	N/A	N/A
MT1-11	+Dox	M	78	14.3	2.63 %	0.0312
MT1-11	-Dox	M	76	13.6	N/A	N/A
MT1-11	+Dox	F	82	16.5	5.13%	<0.001
MT1-11	-Dox	F	78	15.8	N/A	N/A
MT1-53	+Dox	M	84	12.5	-2.33 %	0.6742
MT1-53	-Dox	M	86	17.8	N/A	N/A
MT1-53	+Dox	F	88	9.8	2.33 %	0.1037
MT1-53	-Dox	F	86	15.3	N/A	N/A
MT1-106	+Dox	M	80	13.7	0%	0.0473
MT1-106	-Dox	M	80	12.0	N/A	N/A
MT1-106	+Dox	F	82	20.6	0%	0.3266
MT1-106	-Dox	F	82	19.2	N/A	N/A
PGM3-23	+Dox	M	75	14.8	-1.32 %	0.7079
PGM3-23	-Dox	M	76	15.4	N/A	N/A
PGM3-23	+Dox	F	86	14.1	4.88 %	0.00112
PGM3-23	-Dox	F	82	11.2	N/A	N/A

PGM2-12	+Dox	M	91	17.9	7.06 %	<0.001
PGM2-12	-Dox	M	85	12.4	N/A	N/A
PGM2-12	+Dox	F	85	11.4	4.94%	0.2458
PGM2-12	-Dox	F	81	14.9	N/A	N/A

Exp 4

Genotype	**Drug**	**Sex**	**Median**	**SD**	**Δ Median**	***p***
Control (Or-R)	+RU486	M	61	10.8	-3.17%	0.5778
Control (Or-R)	-RU486	M	63	10.1	N/A	N/A
Control (Or-R)	+RU486	F	70	10.8	-5.41%	0.6877
Control (Or-R)	-Ru486	F	74	7.4	N/A	N/A
45A2	+RU486	M	54	15.9	-3.57%	0.8656
45A2	-RU486	M	56	14.4	N/A	N/A
45A2	+RU486	F	60	16.0	-1.64%	0.1325
45A2	-RU486	F	61	16.0	N/A	N/A
189E	+RU486	M	40	10.5	-23.07%	<0.001
189E	-RU486	M	52	12.0	N/A	N/A
189E	+RU486	F	52	10.0	0%	<0.001
189E	-RU486	F	52	15.2	N/A	N/A

Supplemental Table S3. Female fertility assay

Exp 1 (40 day old flies)

Genotype	Drug	Mean(eggs)	SD	Δ Mean	*p*
Control (Or-R)	+Dox	5.40	2.0	-10.60%	0.198
Control (Or-R)	-Dox	6.04	1.6	N/A	N/A
MT1-3	+Dox	1.17	1.1	-59.49%	<0.001
MT1-3	-Dox	2.88	1.1	N/A	N/A
MT1-11	+Dox	2.59	1.5	-48.84%	<0.001
MT1-11	-Dox	5.07	1.4	N/A	N/A
MT1-53	+Dox	2.49	1.7	-52.08%	<0.001
MT1-53	-Dox	5.19	1.9	N/A	N/A
MT1-106	+Dox	5.29	2.5	-30.02%	0.0166
MT1-106	-Dox	7.56	2.4	N/A	N/A
Control (Or-R)	+RU486	3.41	1.3	-65.75%	<0.001
Control (Or-R)	-RU486	9.95	1.5	N/A	N/A
45A2	+RU486	1.93	0.9	-61.43%	<0.001
45A2	-RU486	5.01	1.0	N/A	N/A
189E	+RU486	3.33	2.7	-64.44%	<0.001
189E	-RU486	9.38	3.0	N/A	N/A

Exp 2 (40 day old flies)

Genotype	Drug	Mean (eggs)	SD	Δ Mean	*p*
Control (Or-R)	+DOX	4.51	2.8	-26.17%	0.0034
Control (Or-R)	-DOX	6.10	3.2	N/A	N/A
DmMRG15-8	+DOX	1.09	1.6	-62.35%	<0.001
DmMRG15-8	-DOX	2.91	2.8	N/A	N/A
DmMRG15-26	+DOX	3.30	2.9	-66.15%	<0.001

DmMRG15-26	-DOX	9.74	3.0	N/A	N/A
RNAi-DmMRG15-4-1	+DOX	3.95	3.7	-49.72%	<0.001
RNAi-DmMRG15-4-1	-DOX	7.85	3.3	N/A	N/A
RNAi-DmMRG15-4-3	+DOX	5.10	4.5	-44.73%	<0.001
RNAi-DmMRG15-4-3	-DOX	9.22	4.7	N/A	N/A
RNAi-DmMRG15-53	+DOX	2.26	2.9	-71.72%	<0.001
RNAi-DmMRG15-53	-DOX	7.99	3.4	N/A	N/A

Exp 3 (60 day old flies)

Genotype	**Drug**	**Mean (eggs)**	**SD**	**Δ Mean**	***p***
Control (Or-R)	+DOX	2.42	2.4	43.77%	0.372
Control (Or-R)	-DOX	1.68	1.7	N/A	N/A
DmMRG15-7	+DOX	4.12	1.4	23.01%	0.165
DmMRG15-7	-DOX	3.35	1.3	N/A	N/A
DmMRG15-8	+DOX	2.39	0.8	-25.03%	0.0710
DmMRG15-8	-DOX	3.18	1.3	N/A	N/A
DmMRG15-21	+DOX	1.85	0.6	-48.56%	<0.001
DmMRG15-21	-DOX	3.59	1.3	N/A	N/A
DmMRG15-33	+DOX	2.72	1.3	-23.95%	0.147
DmMRG15-33	-DOX	3.58	1.6	N/A	N/A
DmMRG15-47	+DOX	3.32	1. 0	19.68%	0.324
DmMRG15-47	-DOX	2.78	1.7	N/A	N/A
RNAi-DmMRG15-4-1	+DOX	2.89	1.3	-37.95%	0.0101
RNAi-DmMRG15-4-1	-DOX	4.66	1.8	N/A	N/A
RNAi-DmMRG15-4-3	+DOX	4.60	2.1	-23.11%	0.0464
RNAi-DmMRG15-4-3	-DOX	5.98	1.1	N/A	N/A
RNAi-DmMRG15-53	+DOX	0.96	0.8	-52.66%	0.00434
RNAi-DmMRG15-53	-DOX	2.02	0.9	N/A	N/A

Supplemental Table S4. Male fertile period

Exp 1

Genotype	**Drug**	**Number**	**Median**	**Δ Median**	***p***
Control (Or-R)	+Dox	20	58	0.00%	0.988
Control (Or-R)	-Dox	20	58	N/A	N/A
DmMrg15-8	+Dox	20	58	7.41%	0.856
DmMrg15-8	-Dox	20	54	N/A	N/A
DmMrg15-26	+Dox	16	54	8.00%	0.112
DmMrg15-26	-Dox	11	50	N/A	N/A
RNAi-4-3	+Dox	20	46	0.00%	0.525
RNAi-4-3	-Dox	20	46	N/A	N/A
RNAi-53	+Dox	20	42	-16.0%	<0.001
RNAi-53	-Dox	20	50	N/A	N/A
MT1-3	+Dox	20	62	6.90%	0.077
MT1-3	-Dox	20	58	N/A	N/A
MT1-106	+Dox	20	54	17.4%	0.049
MT1-106	-Dox	20	46	N/A	N/A

Exp 2

Genotype	**Drug**	**Number**	**Median**	**Δ Median**	***p***
Control (Or-R)	+Dox	20	48	5.49%	0.6213
Control (Or-R)	-Dox	20	45.5	N/A	N/A
DmMrg15-7	+Dox	20	43	0%	0.5148
DmMrg15-7	-Dox	20	43	N/A	N/A
RNAi 4-1	+Dox	16	25	0%	0.2228
RNAi 4-1	-Dox	17	25	N/A	N/A
MT1-3	+Dox	20	47	9.3%	0.9870
MT1-3	-Dox	20	43	N/A	N/A
MT1-11	+Dox	20	48	11.63%	0.1773
MT1-11	-Dox	19	43	N/A	N/A
MT1-53	+Dox	20	48	0%	0.7822
MT1-53	-Dox	20	48	N/A	N/A
Control (Or-R)	+RU486	20	50	0%	0.9934
Control (Or-R)	-RU486	20	50	N/A	N/A
45A2	+RU486	20	31	-6.06%	0.9518
45A2	-RU486	17	33	N/A	N/A
189E	+RU486	18	31	6.90%	0.9134
189E	-RU486	18	29	N/A	N/A

Supplemental Table S5. Larval survival assay

Genotype	Drug	Sex	Ratio*	SD	Δ Mean	*p*
Control (Or-R)	+Dox	M	51.45%	0.0178	-4.19%	0.2728
Control (Or-R)	-Dox	M	53.70%	0.0563	N/A	N/A
Control (Or-R)	+Dox	F	52.13%	0.0383	-4.29%	0.2558
Control (Or-R)	-Dox	F	54.47%	0.0410	N/A	N/A
RNAi 4-1	+Dox	M	39.22%	0.0113	-28.79%	<0.001
RNAi 4-1	-Dox	M	55.08%	0.0327	N/A	N/A
RNAi 4-1	+Dox	F	48.31%	0.0392	-13.51%	0.0293
RNAi 4-1	-Dox	F	55.86%	0.0308	N/A	N/A
RNAi 4-3	+Dox	M	52.99%	0.0064	-4.37%	0.0185
RNAi 4-3	-Dox	M	55.41%	0.0120	N/A	N/A
RNAi 4-3	+Dox	F	50.84%	0.0346	-10.22%	0.0326
RNAi 4-3	-Dox	F	56.63%	0.0195	N/A	N/A
RNAi 53	+Dox	M	39.86%	0.0067	-24.50%	<0.001
RNAi 53	-Dox	M	52.80%	0.0236	N/A	N/A
RNAi 53	+Dox	F	43.60%	0.0336	-6.05%	0.1332
RNAi 53	-Dox	F	46.40%	0.0171	N/A	N/A
DmMrg15-8	+Dox	M	52.82%	0.0356	-0.73%	0.4346
DmMrg15-8	-Dox	M	53.21%	0.0143	N/A	N/A
DmMrg15-8	+Dox	F	47.99%	0.0207	-7.38%	0.1008
DmMrg15-8	-Dox	F	51.82%	0.0381	N/A	N/A
MT1-53	+Dox	M	52.05%	0.0903	1.42%	0.4601
MT1-53	-Dox	M	51.33%	0.0763	N/A	N/A
MT1-53	+Dox	F	51.01%	0.0860	-5.47%	0.3034
MT1-53	-Dox	F	53.96%	0.0319	N/A	N/A

* The number of male or female flies containing both the driver and the transgene divided by the total number of F1 male or female progeny.