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Supplemental Material to:

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Brahma regulates a specific trans-splicing event at the *mod(mdg4)* locus of *Drosophila melanogaster*

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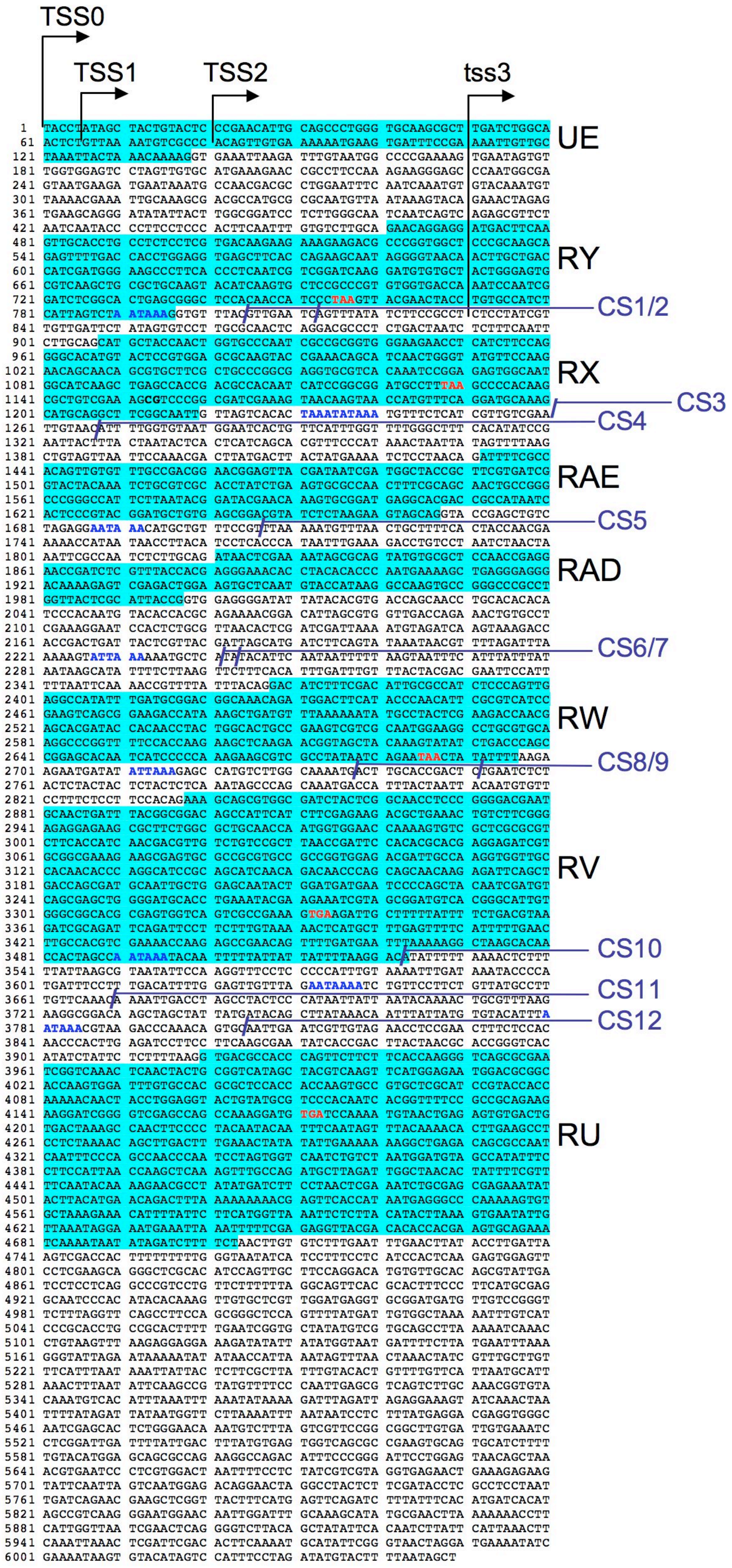

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 241 GTAATGAAGA TGAATAAATG CCAACGACGC CTGGAATTTC AATCAAATGT GTACAAATGT
 301 TAAAACGAAA TTGCAAAGCG ACGCCATGCG CGCAATGTTA ATAAAGTACA GAAACTAGAG
 361 TGAAGCAGGG ATATATTACT TGGCGGATCC TCTTGGGCAA TCAATCAGTC AGAGCGTTCT
 421 AATCAATACC CCTTCCTCCC ACTTCAATTT GTGTCTTGCA GAACAGGAGG ATGACTTCAA
 481 GTTGCACCTG CCTCTCCTCG TGACAAGAAG AAAGAAGACG CCCGGTGGCT CCCGCAAGCA
 541 GAGTTTTGAC CACCTGGAGG TGAGCTTCAC CAGAAGCAAT AGGGGTAACA ACTTGCTGAC
 601 CATCGATGGG AAGCCCTTCA CCCTCAATCG TCGGATCAAG GATGTGTGCT ACTGGGAGTG
 661 CGTCAAGCTG CGCTGCAAGT ACATCAAGTG CTCCGCCCGT GTGGTGACCA AATCCAATCG
 721 GATCTCGGCA CTGAGCGGGC TCCACAACCA TCCCTAAGTT ACGAACTACC TGTGCCATCT
 781 CATTAGTCTA ATAAAGGTGT TTACGTTGAA TCAGTTTATA TCTTCCGCCT CTCCTATCGT
 841 TGTTGATTCT ATAGTGTCCT TGCGCAACTC AGGACGCCCT CTGACTAATC TCTTTCAATT
 901 CTTGCAGCAT GCTACCAACT GGTGCCCAAT CGCCGCGGTG GGAAGAACCT CATCTTCCAG
 961 GGGCACATGT ACTCCGTGGA GCGCAAGTAC CGAAACAGCA TCAACTGGGT ATGTTCCAAG
1021 AACAGCAACA GCGTGCTTCG CTGCCCGGCG AGGTGCGTCA CAAATCCGGA GAGTGGCAAT
1081 GGCATCAAGC TGAGCCACCG ACGCCACAAT CATCCGGCGG ATGCCTTTAA GCCCCACAAG
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1981 GGTTACTCGC ATTACCGGTG GAGGGGATAT TATACACGTG ACCAGCAACC TGCACACACA
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2101 CGAAAGGAAT CCACTCTGCG TTAACACTCG ATCGATTAAA ATGTAGATCA AGTAAAGACC
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2701 AGAATGATAT ATTAAAGAGC CATGTCTTGG CAAAATGACT TGCACCGACT CTGAATCTCT
2761 ACTCTACTAC TCTACTCTCA AATAGCCCAG CAAATGACCA TTTACTAATT ACAATGTGTT
2821 CCTTTCTCCT TCCACAGAAA GCAGCGTGGC GATCTACTCG GCAACCTCCC GGGGACGAAT
2881 GCAACTGATT TACGGCGGAC AGCCATTCAT CTTCGAGAAG ACGCTGAAAC TGTCTTCGGG
2941 AGAGGAGAAG CGCTTCTGGC GCTGCAACCA ATGGTGGAAC CAAAAGTGTC GCTCGCGCGT
3001 CTTCACCATC AACGACGTTG TCTGTCCGCT TAACCGATTC CACACGCACG AGGAGATCGT
3061 GCGGCGAAAG AAGCGAGTGC GCCGCGTGCC GCCGGTGGAG ACGATTGCCA AGGTGGTTGC
3121 CACAACACCC AGGCATCCGC AGCATCAACA GACAACCCAG CAGCAACAAG AGATTCAGCT
3181 GACCAGCGAT GCAATTGCTG GAGCAATACT GGATGATGAA TCCCCAGCTA CAATCGATGT
3241 CAGCGAGCTG GGGATGCACC TGAAATACGA AGAAATCGTA GCGGATGTCA CGGGCATTGT
3301 GGGCGGCACG CGAGTGGTCA GTCGCCGAAA GTGAAGATTG CTTTTTATTT TCTGACGTAA
3361 GATCGCAGAT TCAGATTCCT TCTTTGTAAA AACTCATGCT TTGAGTTTTC ATTTTTGAAC
3421 TTGCCACGTC GAAAACCAAG AGCCGAACAG TTTTGATGAA TTTAAAAAGG CTAAGCACAA
3481 CCACTAGCCA ATAAATACAA TTTTTATTAT TATTTTAAGG ACATATTTTT AAAACTCTTT
3541 TTATTAAGCG TAATATTCCA AGGTTTCCTC CCCCATTTGT AAAATTTGAT AAATACCCCA
3601 TGATTTCCTT TGACATTTTG GAGTTGTTTA GAATAAAATC TGTTCCTTCT GTTATGCCTT
3661 TGTTCAAACA AAATTGACCT AGCCTACTCC CATAATTATT AATACAAAAC TGCGTTTAAG
3721 AAGGCGGACA AGCTAGCTAT TATGATACAG CTTATAAACA ATTTATTATG TGTACATTTA
3781 ATAAACGTAA GACCCAAACA GTGCAATTGA ATCGTTGTAG AACCTCCGAA CTTTCTCCAC
3841 AACCCACTTG AGATCCTTCC TTCAAGCGAA TATCACCGAC TTACTAACGC ACCGGGTCAC
3901 ATATCTATTC TCTTTTAAGG TGACGCCACC CAGTTCTTCT TCACCAAGGG TCAGCGCGAA
3961 TCGGTCAAAC TCAACTACTG CGGTCATAGC TACGTCAAGT TCATGGAGAA TGGACGCGGC
4021 ACCAAGTGGA TTTGTGCCAC GCGCTCCACC ACCAAGTGCC GTGCTCGCAT CCGTACCACC
4081 AAAAACAACT ACCTGGAGGT ACTGTATGCG TCCCACAATC ACGGTTTTCC GCCGCAGAAG
4141 AAGGATCGGG GTCGAGCCAG CCAAAGGATG TGATCCAAAA TGTAACTGAG AGTGTGACTG
4201 TGACTAAAGC CAACTTCCCC TACAATACAA TTTCAATAGT TTACAAAACA CTTGAAGCCT
4261 CCTCTAAAAC AGCTTGACTT TGAAACTATA TATTGAAAAA AAGGCTGAGA CAGCGCCAAT
4321 CAATTTCCCA GCCAACCCAA TCCTAGTGGT CAATCTGTCT AATGGATGTA GCCATATTTC
4381 CTTCCATTAA CCAAGCTCAA AGTTTGCCAG ATGCTTAGAT TGGCTAACAC TATTTTCGTT
4441 TTCAATACAA AAGAACGCCT ATATGATCTT CCTAACTCGA AATCTGCGAG CGAGAAATAT
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4621 TTAAATAGGA AATGAAATTA AATTTTTCGA GAGGTTACGA CACACCACGA AGTGCAGAAA
4681 TCAAAATAAT ATAGATCTTT TCTAACTTGT GTCTTTGAAT TTGAACTTAT ACCTTGATTA
4741 AGTCGACCAC TTTTTTTTTG GGTAATATCA TCCTTTCCTC ATCCACTCAA GAGTGGAGTT
4801 CCTCGAAGCA GGGCTCGCAC ATCCAGTTGC TTCCAGGACA TGTGTTGCAC AGCGTATTGA
4861 TCCTCCTCAG GCCCGTCCTG TTCTTTTTTA GGCAGTTCAC GCACTTTCCC TTCATGCGAG
4921 GCAATCCCAC ATACACAAAG TTGTGCTCGT TGGATGAGGT GCGGATGATG TTGTCCGGGT
4981 TCTTTAGGTT CAGCCTTCCA GCGGGCTCCA GTTTTATGAT TGTGGCTAAA AATTTGTCAT
5041 CCCGCACCTG CCGCACTTTT TGAATCGGTG CTATATGTCG TGCAGCCTTA AAAATCAAAC
5101 CTGTAAGTTT AAGAGGAGGA AAGATATATT ATATGGTAAT GATTTTCTTA TGAATTTAAA
5161 GGGTATTAGA ATAAAAATAT ATAACCATTA AATAGTTTAA CTAAACTATC GTTTGCTTGT
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5281 AAACTTTAAT ATTCAAGCCG TATGTTTTCC CAATTGAGCG TCAGTCTTGC AAACGGTGTA
5341 CAAATGTCAC ATTTAAATTT AAATATAAAA GATTTAGATT AGAGGAAAGT ATCAAACTAA
5401 TTTTATAGAT TATAATGGTT CTTAAAATTT AATAATCCTC TTTATGAGGA CGAGGTGGGC
5461 AATCGAGCAC TCTGGGAACA AATGTCTTTA GTCGTTCCGG CGGCTTGTGA TTGTGAAATC
5521 CTCGGATTGA TTTTATTGAC TTTATGTGAG TGGTCAGCGC CGAAGTGCAG TGCATCTTTT
5581 TGTACATGGA GCAGCGCCAG AAGGCCAGAC ATTTCCCGGG ATTCCTGGAG TAACAGCTAA
5641 ACGTGAATCC CTCGTGGACT AATTTTCCTC TATCGTCGTA GGTGAGAACT GAAAGAGAAG
5701 TATTCAATTA GTCAATGGAG ACAGGAACTA GGCCTACTCT TCGATACCTC GCCTCCTAAT
5761 TGATCAGAAC GAAGCTCGGT TACTTTCATG AGTTCAGATC TTTATTTCAC ATGATCACAT
5821 AGCCGTCAAG GGAATGGAAC AATTGGATTT GCAAAGCATA TGCGAACTTA AAAAAACCTT
5881 CATTGGTTAA TCGAACTCAG GGGTCTTACA GCTATATTCA CAATCTTATT CATTAAACTT
5941 CAAATTAAAC TCGATTCGAC ACTTCAAAAT GCATATTCGG GTAACTAGGA TGAAAATATC
6001 GAAAATAAGT GTACATAGTC CATTTCCTAG ATATGTACTT TTAATAGCT
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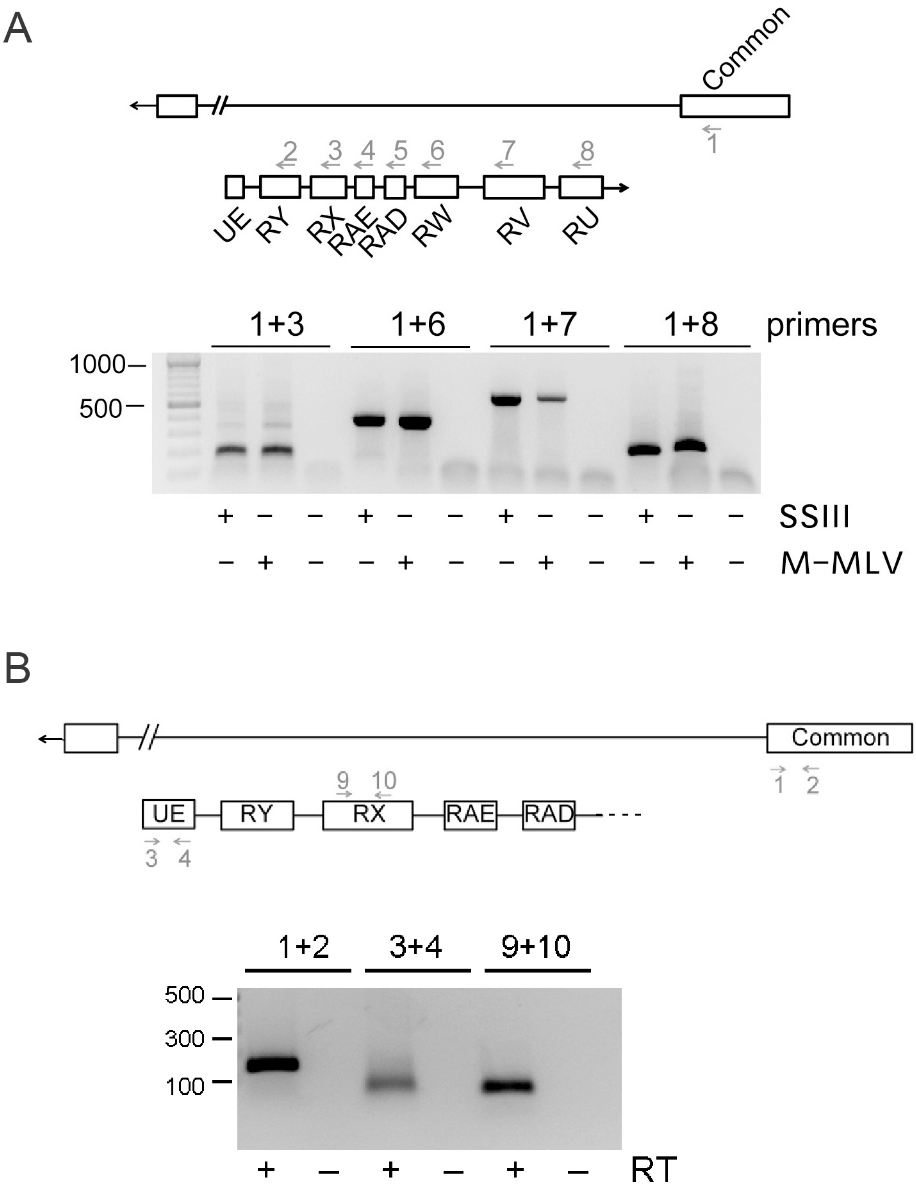
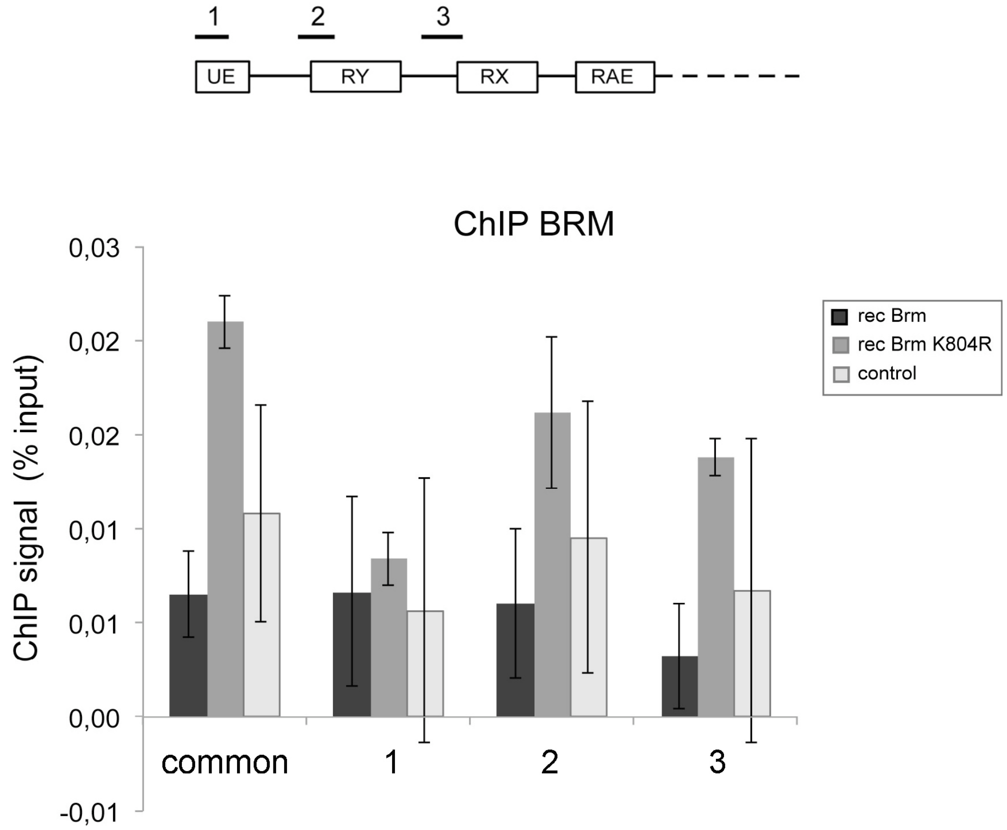
Supplementary Figure S1: The sequence features of the anti-sense 1 transcript
The figure shows the sequence of the anti-sense region 1 based on the DGM2-chr 3R gene prediction and on our own data. The trans-spliced exons are shown as blue boxes. The TSS and CS sites are indicated in the figure. Polyadenylation signals are highlighted in blue, and stop codons in frame are highlighted in red.



Supplementary Figure S2. RT control reactions

A. *In vivo* analysis of the *mod(mdg4)* trans-splicing. The figure shows RT-PCR reactions in which the same trans-spliced products were amplified from *D. melanogaster* fly cDNA using two different reverse transcriptases, SuperScript III (*SSIII*) and Moloney Murine Leukemia Virus reverse transcriptase (*M-MLV*). The experiment also included control reactions without reverse transcriptase, as indicated. The primer-pairs used in each reaction and their positions are indicated in the figure.

B. RT(–) control reactions using total RNA purified from S2 cells. The purified RNA was treated with DNase (Turbo DNA-free kit) and reverse transcribed with Superscript III (Invitrogen) using random primers. Control reactions without reverse transcriptase (RT–) were carried out in parallel. The resulting cDNAs were used as templates in PCR reactions with the primer-pairs indicated in the figure.



Supplementary Figure S3. The association of BRM with the *mod(mdg4)* gene
The association of BRM with the *mod(mdg4)* gene was analyzed by ChIP. Chromatin was extracted from either S2 control cells or from S2 cells that over-expressed either recombinant recBRM or recBRM-K804R proteins, as in Figure 7. The cells were fixed with formaldehyde and immunoprecipitation reactions were carried out with an antibody against the human BRG1 protein that cross-reacts with the BRM of *D. melanogaster*. A negative control immunoprecipitation was carried out in parallel without primary antibody. The immunoprecipitated DNA was analyzed by qPCR using a standard curve for each primer pair. Primer pairs we designed to quantify the sense promoter region (*common*) and three genomic regions within the anti-sense region 1. The values obtained in no-antibody controls were substracted, and the resulting signals were expressed relative to input. The histograms show average folds and the error bars represent standard deviations calculated from three independent experiments (n=3).

Supplementary Table S1.
The primer-pair combinations that were used in the different RACE reactions.

RACE	1st PCR primers	2nd PCR primers	
TSS1	5RACE outer+RY1R	5RACE inner+RY1R	Clone nested PCR product
TSS2	5RACE outer+RY1R	5RACE inner+RY1R	Clone nested PCR product
TSS3	5RACE outer+RXR	5RACE inner+RXR	Clone nested PCR product
CS1	COMMON F+3RACE outer	RY F+3RACE inner	Purify gel band from nested PCR
CS2	COMMON F+3RACE outer	RY exon junc F+ 3RACE inner	Clone nested PCR product
CS3	COMMON F+3RACE outer	RX exon junc F+ 3RACE inner	Clone nested PCR product
CS4	COMMON F+3RACE outer	RX exon junc F+ 3RACE inner	Clone nested PCR product
CS5	COMMON F+3RACE outer	RAE F+3RACE inner	Clone nested PCR product
CS6	COMMON F+3RACE outer	RAE F+3RACE inner	Clone nested PCR product
CS7	COMMON F+3RACE outer	RAD or RAE F+3RACE inner	Purify gel band from nested PCR
CS8	COMMON F+3RACE outer	RW F+3RACE inner	Purify gel band from nested PCR
CS9	COMMON F+3RACE outer		Clone PCR product
CS10	COMMON F+3RACE outer	RV F+3RACE inner	Purify gel band from nested PCR
CS11	COMMON F+3RACE outer	RV F+3RACE inner	Purify gel band from nested PCR
CS12	COMMON F+3RACE outer	RV F+3RACE inner	Purify gel band from nested PCR

Supplementary Table S2.
The nucleotide sequences of the bands in Figure 4.

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>Figure4B_a
CAGGCGCACATGTACTCCGTGGAACGCAAGTACCGAAACAGCATCAACTGGGTATGTTCC
AAGAACAGCAACAGTGTGTTACGCTGCCCGGCGAGGTGCGTCACAAATCCGGAGAGTGGC
AATGGCATCAAGCTGAGCCACCGACGCCACAATCATCCGGCGGATGCCTTTAAGCCCCAC
AAGCGCTGTTGAAAGCGTCCCGGCGATCGAAAGTAACAAGTAACCATGTTTCAGGATGCA
AAGCATGCAGGCTTCGGCAATTATTTTCGCCACGATT

>Figure4B_b
AGGGCACATGTACTCCGTGGAACGCAAGTACCGAAACAGCATCAACTGGGTATGTTCCAA
GAACAGCAACAGTGTGTTACGCTGCCCGGCGAGGTGCGTCACAAATCCGGAGAGTGGCAA
TGGCATCAAGCTGAGCCACCGACGCCACAATCATCCGGCGGATGCCTTTAAGCCCCACAA
GCGCTGTTGAAAGCGTCCCGGCGATCGAAAGTAACAAGTAACCATGTTTCAGGATGCAAA
GCATGCAGGCTTCGGCAATTATTTTCGCCACAGTTGTGTTTGCCGACGGAACGGAGTTAC
GATAATCGATGGCTACCGCTTCGTGATCGGTACTACAAATCTGCGTCGCACCTATCTGAA
GTGCGCCAACTTTCGCAGCAACTGCCGGGCCCGGGCCATTCTTAATACGGACACGAACAA
AGTGCGGATGAGGCACGACCGCCATAATCACTCTCGTACGGATGCTGTGAGCGGACGTAT
CTCTAAGAAGTAGCAGATAACTCGAAAATAGCGCAGTATGTGCGCTCCAACCGAGGAACC
GATCTCGTTTACCACGAGGGAAACACCTACACACCCAATGAAAACTTGG

>Figure4B_c
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AGCGCTGTTGAAAGCGTCCCGGCGATCGAAAGTAACAAGTAACCATGTTTCAGGATGCAA
AGCATGCAGGCTTCGGCAATTATTTTCGCCACAGTTGTGTTTGCCGACGGAACGGAGTTA
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TCTCTAAGAAGTAGCAGATAACTCGAAAATAGCGCAGTATGTGCGCTCCAAACCGAGGAA
CCGATCTCGTTTACCACGAGGGAAACACCTACACACCCAATGAAAAGCTGAGGGAGGGAC
AAAAGAGTCGAGACTGGAAGTGCTCAATGTACCATAAGGCCAAGTGCCGGGCCCGCCTGG
TTACTCGCATTACCGGTGGAGGGGATATTATACACGTGACCAGCAACCTGCACACACATC
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ACATTGCGCCATCTCCCAGTTGAGGCCATATTTGATGCGGACGGCAAACAGATGGACTTC
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>Figure4B_d
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ATGGCATCAAGCTGAGCCACCGACGCCACAATCATCCGGCGGATGCCTTTAAGCCCCACA
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TATTTGATGCGGACGGCAAACAGATGGACTTCATACCCAACATTCGCGTCATCCGAAGTC
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CTTCTGC

>Figure4B_e
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AGTGGCAATGGCATCAAGCTGAGCCACCGACGCCACAATCATCCGGCGGATGCCTTTAAG
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AGGGACAAAAGAGTCGAGACTGGAAGTGCTCAATGTACCATAAGGCCAAGTGCCGGGCCC
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CACATCCCACAATGTACACCACGCAGAAAACGGACATTAGCGTGGTTGACCAGAAACTGT
GCCTCGAAAGGAATCCACTCTGCGTTAACACTCGATCGATTAAAATGTAGATCAAGTGAC
GCCACCCAGTTCTTCTTCACCAAGGGTCAGCGCGAATCGGTCAAACTTAACTACTGCGGT
CATAGCTACGTCAGTCTG

>Figure4C_d
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GCCATTCATCTTCGAGAAGACGCTGAAACTGTCTTCGGGAGAGGAGAAGCGCTTCTGGCGCT
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TCGGTCAAACTAACTACTGCGTCATGCAC

>Figure4C_f
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GGATGCATCTGAAATACGAAGAAATCGTAGCGGATGTCACGGGCATTGTGGGCGGCACGCGA
GTGGTCAGTCGCCGAAAGTGAATATTGCTTTTTATTTTCTGACGTGACGCCACCCAGTTCTT
CTTCACCAAGGGTCAGCGCGAATCGGTCAAACTAACTAC
```

Supplementary Table S3
Nucleotide sequences of PCR primers.

Primers used for RT-PCR and RT-qPCR
Actin 2 F GCACACCCACAAGCTTACACA
Actin 2 R TTGCGCTTTGGGAAATATCTTC
Mod(mdg4) Common F CACCCACGCTATCGTATTCC
Mod(mdg4) Common R CGTTAGCCCCTTGATTTGC
Mod(mdg4) UE F outer TGTACTCCCGAACATTGCAG
Mod(mdg4) UE R TGTGGGCGACATTTTAACAG
Mod(mdg4) RY intron F TTACTTGGCGGATCCTCTTG
Mod(mdg4) RY F CCCGGTGGCTCCCGCAAGCAGAG
Mod(mdg4) RY middle R ATCCTTGATCCGACGATTGA
Mod(mdg4) RYRX junc F CTAATAAAGCATGCTACCAACTG
Mod(mdg4) RX intron F GGACGCCCTCTGACTAATCTC
Mod(mdg4) RX F CTACCAACTGGTGCCCAATC
Mod(mdg4) RX R AGTACATGTGCCCCTGGAAG
Mod(mdg4) RAE F AATCTGCGTCGCACCTATCT
Mod(mdg4) RAE R CAAACACAACTGTGGCGAAA
Mod(mdg4) RAD F GGAACCGATCTCGTTTACCA
Mod(mdg4) RAD R CTCCCTCAGCTTTTCATTGG
Mod(mdg4) RW F AGACCAACGAGCACGATAC
Mod(mdg4) RW R TCCGGCTGGGTCAGATATAC
Mod(mdg4) RV F GAAACTGTCTTCGGGAGAGG
Mod(mdg4) RV R ACAATGCCCGTGACATCC
Mod(mdg4) RU R GCGTCCATTCTCCATGAACT
Mod(mdg4)-prom F AACACTTCTAATTTTCACGTC
Mod(mdg4)-prom R ACTAACTAAAGCCAGAGTTTTT
Mod(mdg4) RY-RU nt 57 F GGCAACTCTGTTAAAATGTC
Mod(mdg4) RY-RU nt 150 R TCTTAATTTCACCTTTTGTTT
Mod(mdg4) RY-RU nt 829 F CTCTCCTATCGTTGTTGATT
Mod(mdg4) RY-RU nt 907 R CTGCAAGAATTGAAAGAGAT

Primers used for synthesis of dsRNA
Brm BKNT7 F taatacgactcactatagggagaAAGCCCAATCGCATTACAAC
Brm BKNT7 R taatacgactcactatagggagaTGAACTGTATCAGCCGCTTG
Brm HFAT7 F TTAATACGACTCACTATAGGGAGAgtttcgctgtacaataacaatc
Brm HFAT7 R TTAATACGACTCACTATAGGGAGAatgtggagcaggacttaaag
GFP T7 F taatacgactcactatagggagaATGGTGAGCAAGGGCGAGGAGCTG
GFP T7 R taatacgactcactatagggagaGCGGTCACGAACTCCAGCAG

Primers used for Northern blot probe
Mod(mdg4) const probe 2 CTTGAGCCTCTGTGTTGCCCTGATC
Mod(mdg4) RX probe 2 CGATTGGGCACCAGTTGGTAGCATG

Mod(mdg4) const+RX junction probe
CGATTGGGCACCAGTTGGTAGCATGCTTGAGCCTCTGTGTTGCCCTGATC

Primers for RACE
5’RACE adapter
GCUGAUGGCGAUGAAUGAACACUGCGUUUGCUGGCUUUGAUGAAA
5RACE outer GCTGATGGCGATGAATGAACACTG
5RACE inner CGCGGATCCGAACACTGCGTTTGCTGGCTTTGATG
3’RACE adapter GCGAGCACAGAATTAATACGACTCACTATAGGT12VN
3RACE outer GCGAGCACAGAATTAATACGACT
3RACE inner CGCGGATCCGAATTAATACGACTCACTATAGG
Mod(mdg4) RY F CCCGGTGGCTCCCGCAAGCAGAG
Mod(mdg4) RY exon junc F CAGAGGCTCAAGGAACAGGA
Mod(mdg4) RY 1R GGGATGGTTGTGGAGCCCGC
Mod(mdg4) RX F CTACCAACTGGTGCCCAATC
Mod(mdg4) RX exon junc F CAGAGGCTCAAGCATGCTACC
Mod(mdg4) RX R AGTACATGTGCCCCTGGAAG
Mod(mdg4) RAE F AATCTGCGTCGCACCTATCT
Mod(mdg4) RAD F GGAACCGATCTCGTTTACCA
Mod(mdg4) RW F AGACCAACGAGCACGATAC
Mod(mdg4) RV F GAAACTGTCTTCGGGAGAGG

Primers used for cloning the Brm wide type and K804 mutant
Brm-K804R CCGATGAAATGGGTTTGGGTCGAACCATTCAAACCATTTCGC
Brm-K804R antisence
GCGAAATGGTTTGAATGGTTCGACCCAAACCCATTTCATCGG
Brm-SpeI F GATAACTAGTAAGTCAAGAAAGGAGCTGCAA
Brm-SacII R GAATCCGCGGGTCCATGTCATCGTCGTCAT

Primers used for cloning the mod(mdg4) anti-sense strand
Mod(mdg4) AS 5UTR F SpeI GCACTAGTTGTACTCCCGAACATTGCAG
Mod(mdg4) RW intron R EcoRV ATCCGGATATCTTCCTTTCGAGGCACAGTTT

Supplementary Table S4.
The nucleotide sequences of the PCR products obtained in the RACE reactions.

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>TSS1_RY1R_RY1R
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