Supplementary Material

Table S1. Primers used in this study.

qRT-PCR		
Name	**Sense (5'-3')**	**Antisense(5'-3')**
BmAgo2RT	AGGTCAATTTCCTGGTCGTG	CGATTCTCCAATGCCTGATT
Bmw-2RT	TGTACTCGAACGAAGCGATG	GGTGATGTAGAGCAGCAGCA
BmBlos2RT	GATGTATGCCCAGCAGATCC	AACGAGCCTTCAATTGCTTC
DsRedRT	GCCACTACCTGGTGGAGTTC	TGGTGTAGTCCTCGTTGTGG
Bmrp49RT	CCTGTTTACAGGCCGACAAT	GACGGGTCTTCTTGTTGGAA
Open Reading Frame cloning		
BmAgo2	TAGAGCTCGCCACCATGGCTAGAGGAAAAAAC AA	TTAGCGGCCGCTTAGACGAAGAACATACGGCT
shRNA and dsRNA synthesis		
U6 promoter	AGGTTATGTAGTACACATTG	ACTTGTAGAGCACGATATTT
RL shRNA	TAGCTAGCAGGTTATGTAGTACACATTGTTGTA	TGCATATGAAAATTTCACTACTCCTACGAGCACGGACAGC ACACGTGCTCGTAGGAGTAGTGAAAACTTGTAGAGCACG ATATT
BmBlos2 shRNA	TAAGATCTAGGTTATGTAGTACACATTGTTGTA	TGAGATCTAAAAGGAACACTACATGCTGCTTGAGGACAG CACACTCAAGCAGCATGTAGTGTTCCACTTGTAGAGCACG ATATT
BmAgo2T7	AATACGACTCACTATAGGGACTCGCAGCAGGA AACCTAAATTTAC	AATACGACTCACTATAGGGATGACACTTCAAACGGCATGT CTTTG
RLT7	TAATACGACTCACTATAGGGGTAACGCTGCCTC CAGCTAC	TAATACGACTCACTATAGGGGTAGGCAGCGAACTCCTCAG
DsRedT7	TAATACGACTCACTATAGGGGCTCCTCCAAGAA CGTCATC	TAATACGACTCACTATAGGGTGGTCTTCTTCTGCATCACG
EGFPT7	TAATACGACTCACTATAGGGAGGACGACGGCA ACTACAAG	TAATACGACTCACTATAGGGGAACTCCAGCAGGACCATGT
Junction PCR		
DsRedFirst round	ACGGATTCGCGCTATTTAGA	GTGCTTGTCAATGCGGTAAG
DsRedSecond round	TCAAGAATGCATGCGTCAAT	GGGCCGATACATTGATGAGT
EGFPFirst round	TGCGGTTTACCGGTACTTTC	TCAAACTAAAGGCGGAGTGG
EGFPSecond round	GTAAGGGGTCCGTCAAAACA	GAAAGGCAAATGCATCGTGC

Table S2. Detailed information of the matched peptides in MALDI-TOF/TOF analysis.

Gene name	ID	Matched peptides
Heat shock protein 70	**gi|320526705**	LSKEEIER
		MVNEAEKYR
		FELTGIPPAPR
		VEIIANDQGNR
		MKETAEAYLGK
		AQIHDIVLVGGSTR
		TTPSYVAFTDTER
		STAGDTHLGGEDFDNR
Heat shock protein 70B	**gi|336454474**	TTPSYVAFTDTER
		STAGDTHLGGEDFDNR
Cellular Retinoic Acid Binding Protein	**gi|108793850**	APDGLEVTYVR
		SVCTFEGNTLK
Enolase	**gi|119381542**	YNQILR
		TGAPCRSER
		ANLEVTQQR
		KNGWGTMVSHR
BmDicer2	**gi|302318907**	FNLGGRMK

		EYPWDQR
		ALYDFIKR
		AATLKAFTDK
		GDPYSNTKTAK
		ARPDEFEFLK
		ELKPGEMTDLR
		KPLCGIIFTKQR
		TDVEKILNYTFK
		NISTRMNCLLPR
		QSFLIKYDAFQK
BmAgo2	**gi|166706853**	MACFNIR
		VVIKDMNGK
		DMPFEVSFK
		QLNDRQLSTMVR
		AAEAFNEFIRGLK
BmTudor-sn	**gi|302190081**	FPSDPDDR
		TANNDTETK
		TAEENAIKK
		QGFAKCVMK
		VQDTSGDPTKAK
		KVNVTVDYIQPAK
		DGLVLVEQVRDSR
		SSQYDKLLEAELK

Figure S1-S7: **The MALDI-TOF/TOF mass spectrum.** The X axis is the relative molecular weight (m/z) and Y axis is the peptide intensity. Green arrows indicate the major peptides identified from the digested protein complex.

Figure S8: **Schematic overviews of the experiments performed in this study. A.** Double RNAi in the BmN cell line. Luciferase was used as a target gene to measure RNAi efficiency. If the candidate gene does not function in the silkworm RNAi response, knocking down the candidate gene has no effect on luciferase dsRNA- or shRNA-triggered RNAi. However, luciferase RNAi will be repressed if the candidate gene is involved in the silkworm RNAi response. **B.** Double RNAi in silkworm embryos. *Bmw-2* was used as the reporter, and its down-regulation disrupted serosa pigmentation.

Figure S9: **Efficient knocking down of *Renilla Luciferase* (*RL*) by dsRNA or shRNA.** Either dsRNA targeting *RL* or the *pBac[3xp3-EGFP-U6-Blos2 shRNA]* (*U6-RL shRNA*) plasmid was co-transfected with the two luciferase expression plasmids. Three independent replicates were performed to quantify the relative luciferase activity. The asterisks indicate statistical significance ($p<0.05$), and error bars are means ± S.E.M.

Figure S10：Genome insertion of three transgenic silkworm lines revealed by inverse

PCR and sequencing. A. *OpIE2-BmAgo2*, **B.** *IE1-DsRed*, **C.** *U6-Blos2 shRNA*. Chromosome localization was shown. At least two individual lines for each transgene were used for detection. The TTAA insertion sites have been mapped for all of the lines.

Figure S1.

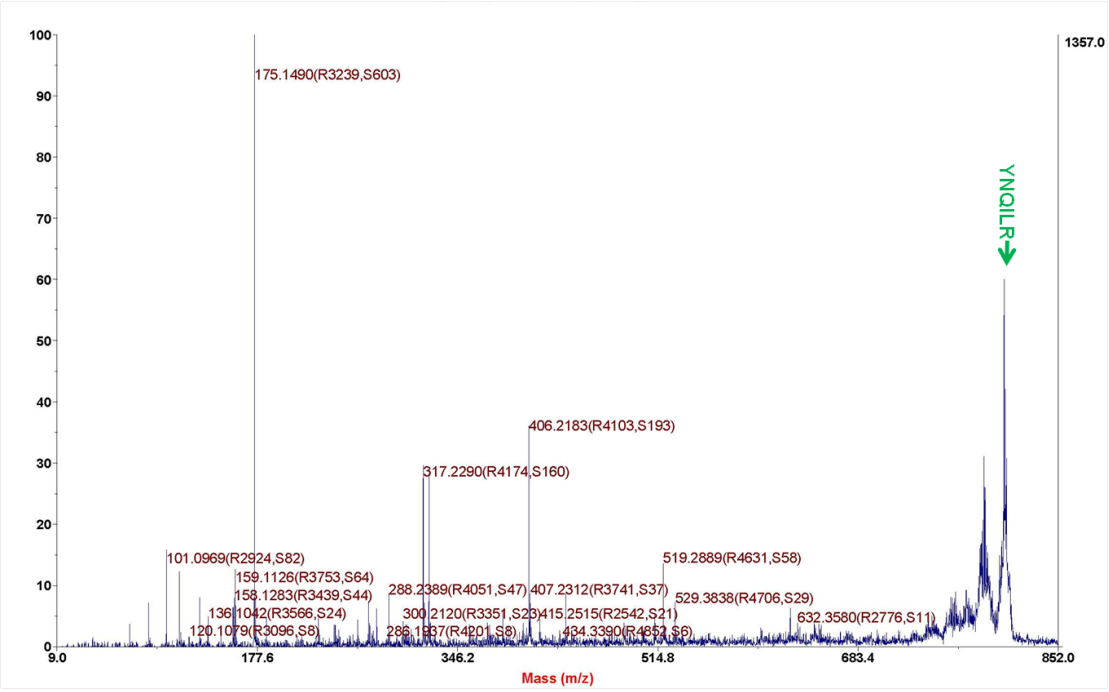


Figure S2.

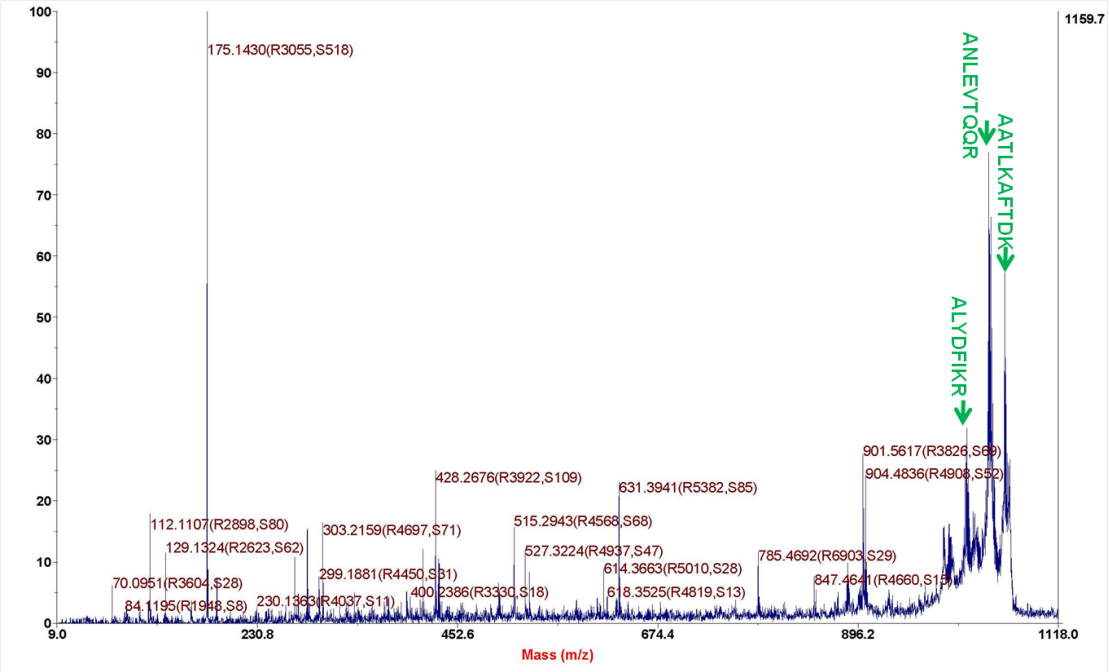


Figure S3.

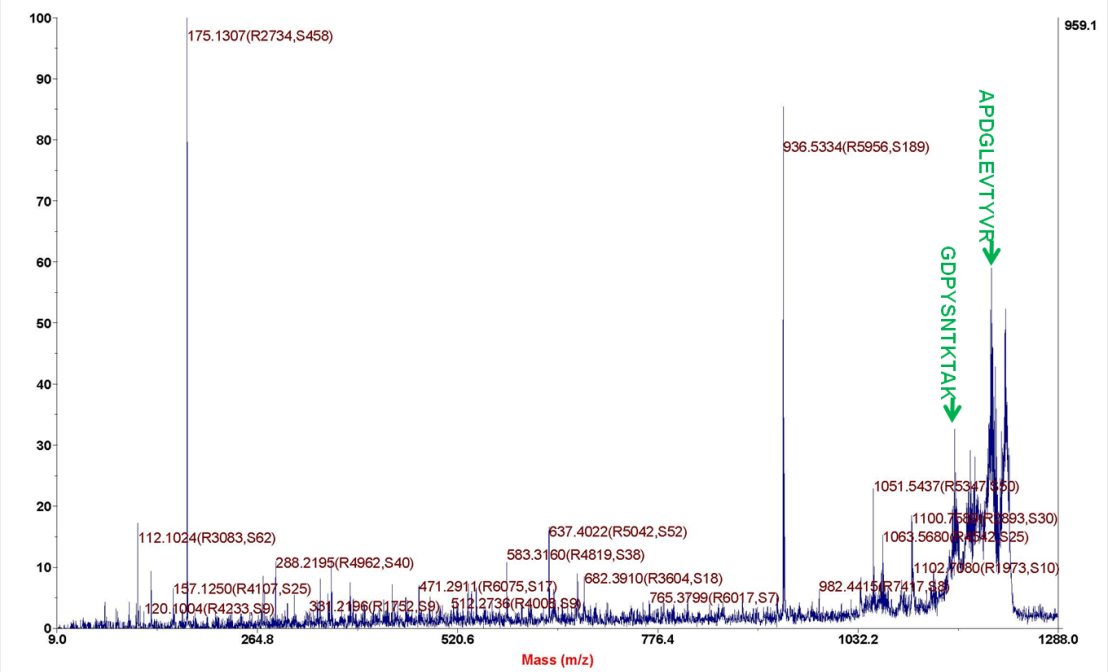


Figure S4.

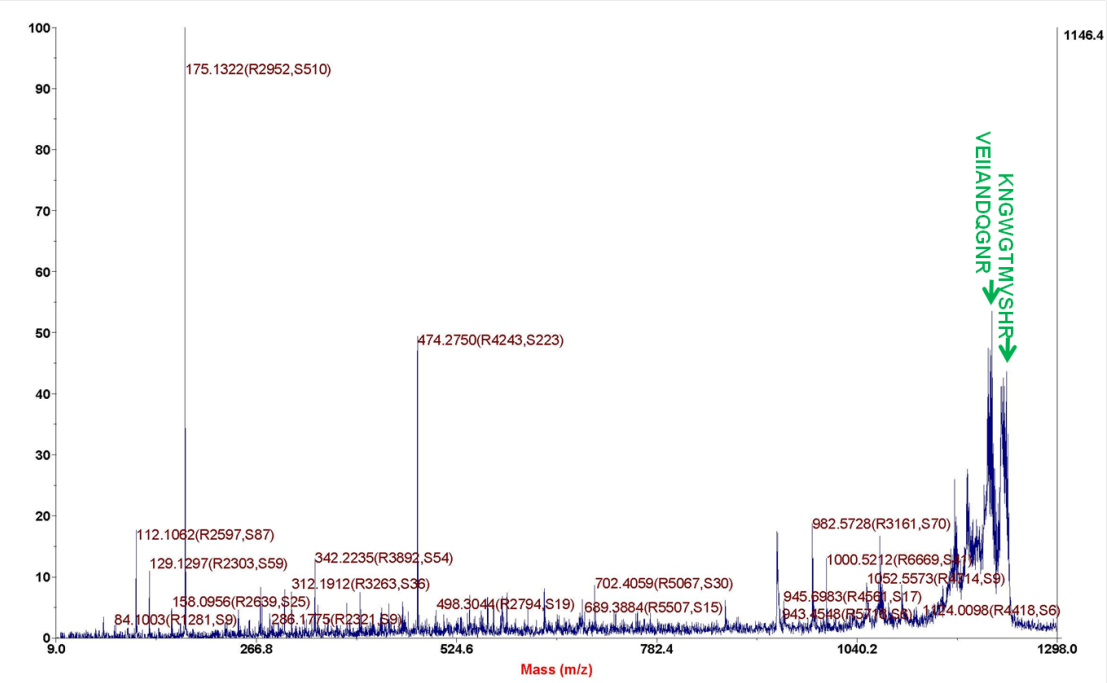


Figure S5.

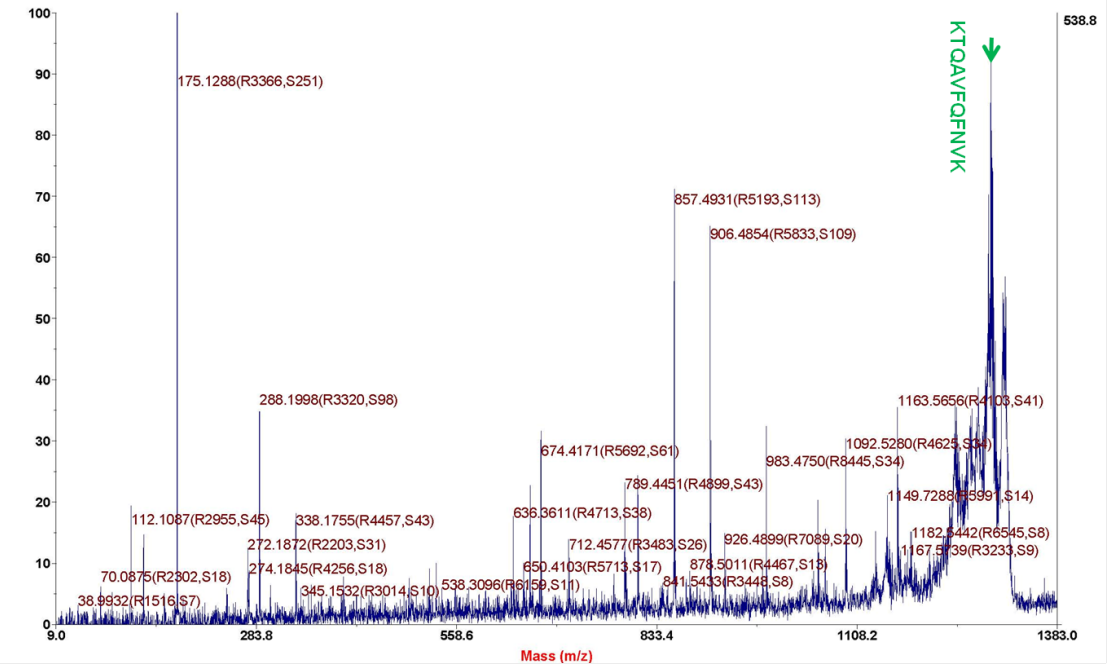


Figure S6.

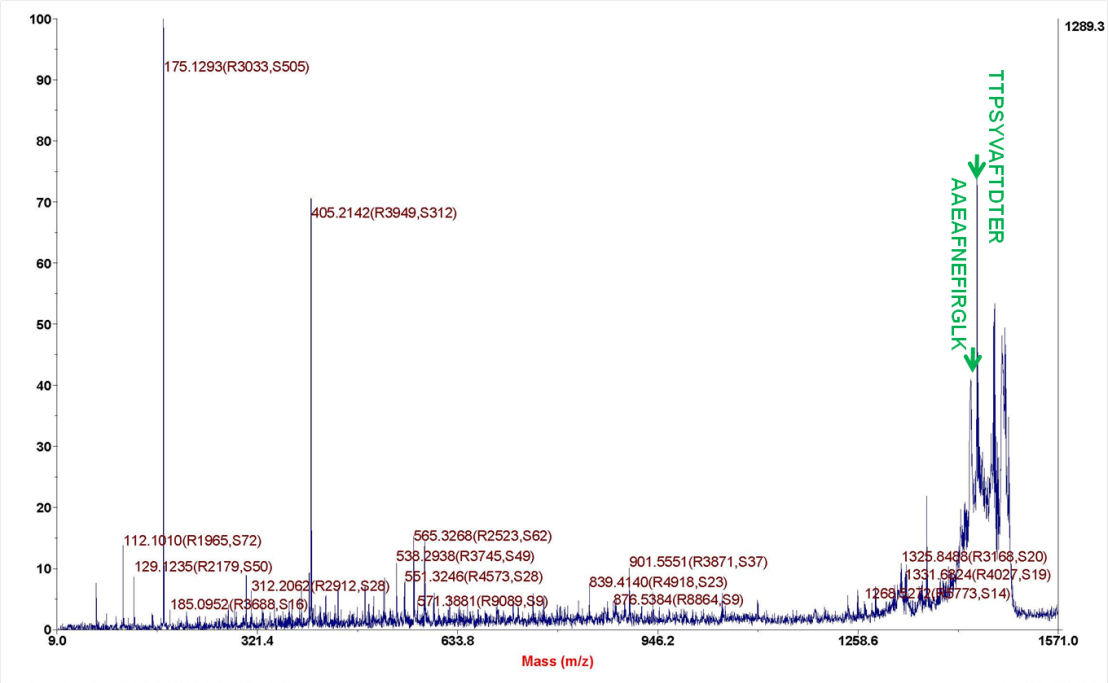


Figure S7.

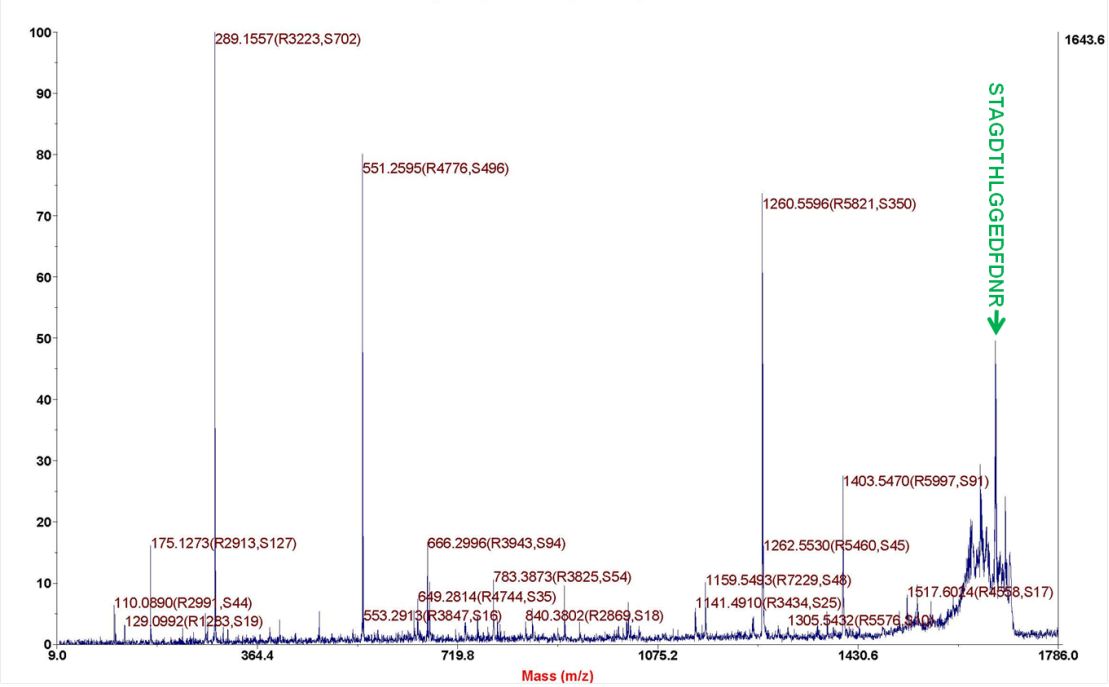


Figure S8.

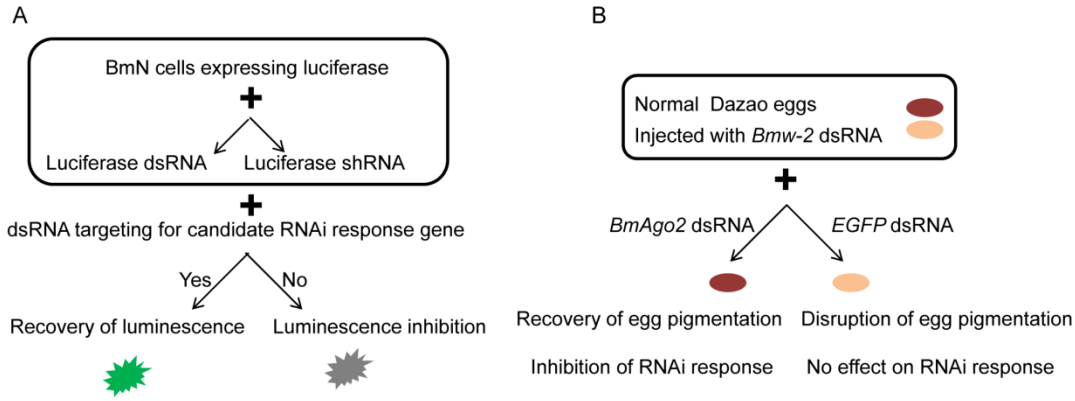


Figure S9.

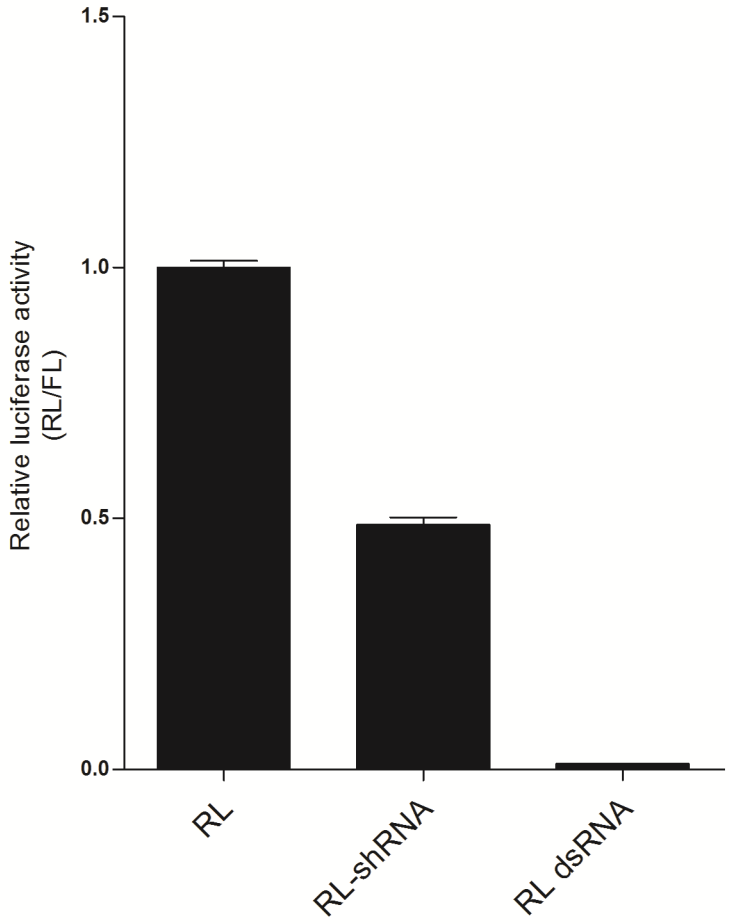
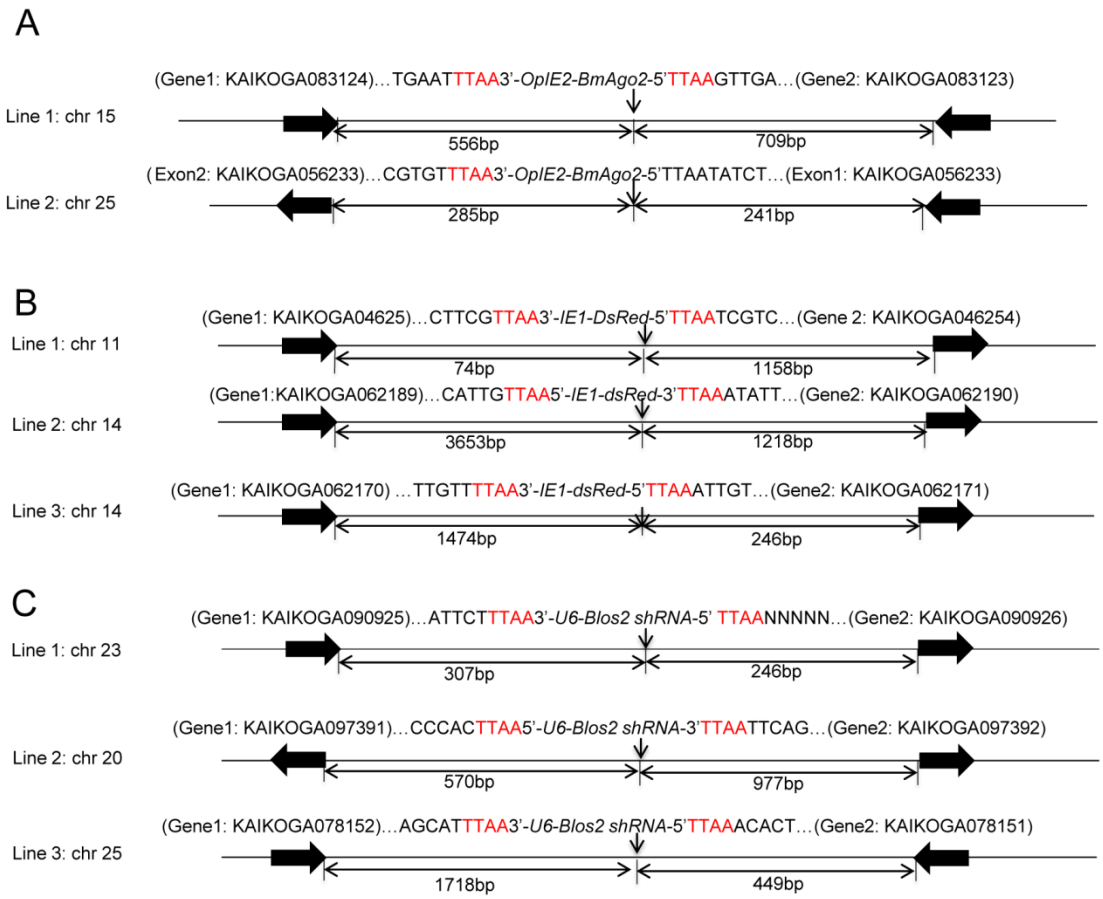


Figure S10.


A
(Gene1: KAIKOGA083124)…TGAATTTAA3'-OpIE2-BmAgo2-5'TTAAGTTGA…(Gene2: KAIKOGA083123)
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556bp
709bp
(Exon2: KAIKOGA056233)…CGTGTTTAA3'-OpIE2-BmAgo2-5'TTAATATCT…(Exon1: KAIKOGA056233)
Line 2: chr 25
285bp
241bp
B
(Gene1: KAIKOGA04625)…CTTCGTTAA3'-IE1-DsRed-5'TTAATCGTC…(Gene 2: KAIKOGA046254)
Line 1: chr 11
74bp
1158bp
(Gene1:KAIKOGA062189)…CATTGTTAA5'-IE1-dsRed-3'TTAAATATT…(Gene2: KAIKOGA062190)
Line 2: chr 14
3653bp
1218bp
(Gene1: KAIKOGA062170) …TTGTTTTAA3'-IE1-dsRed-5'TTAAATTGT…(Gene2: KAIKOGA062171)
Line 3: chr 14
1474bp
246bp
C
(Gene1: KAIKOGA090925)…ATTCTTTAA3'-U6-Blos2 shRNA-5' TTAANNNNN…(Gene2: KAIKOGA090926)
Line 1: chr 23
307bp
246bp
(Gene1: KAIKOGA097391)…CCCACTTAA5'-U6-Blos2 shRNA-3'TTAATTCAG…(Gene2: KAIKOGA097392)
Line 2: chr 20
570bp
977bp
(Gene1: KAIKOGA078152)…AGCATTTAA3'-U6-Blos2 shRNA-5'TTAAACACT…(Gene2: KAIKOGA078151)
Line 3: chr 25
1718bp
449bp