Pek et al., http://www.jcb.org/cgi/content/full/jcb.201507065/DC1

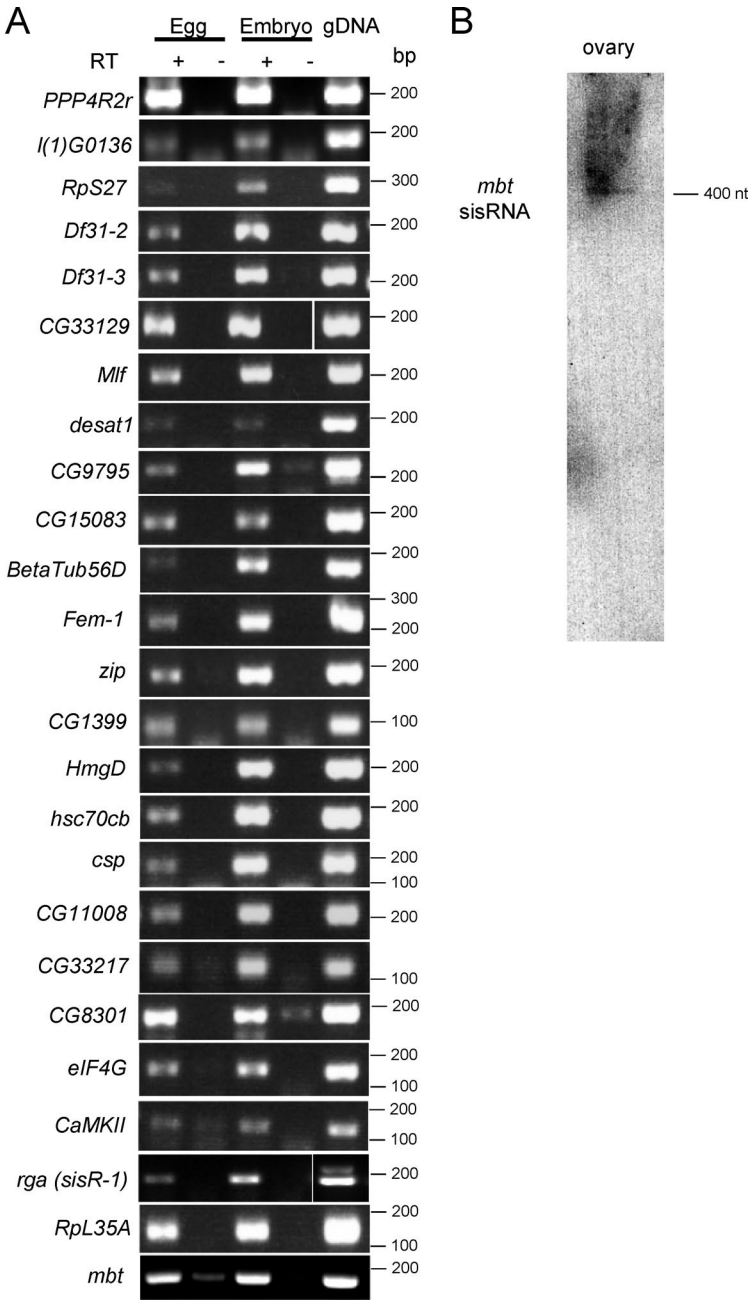

Figure S1. **Identification of sisRNAs.** (A) RT-PCR showing expression of sisRNAs in unfertilized eggs. (B) Northern blot showing the expression of *mbt* sisRNA in ovaries.

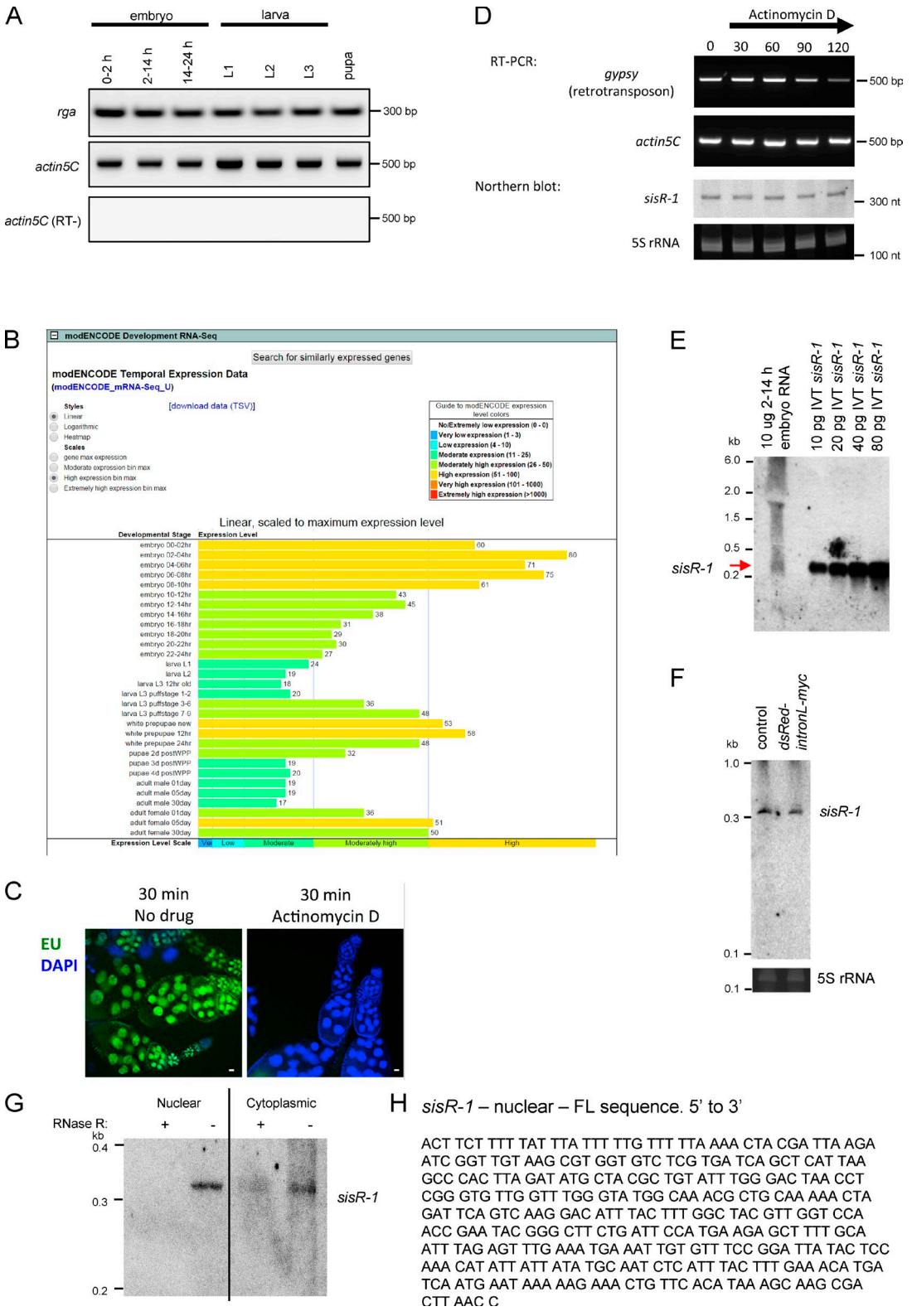

Figure S2. **Characterization of *sisR-1*.** (A) RT-PCR showing the expression of *rga* mRNA during development. *Actin5C* was used as a loading control. (B) Screen shot of the *rga* expression profile by modENCODE shown on FlyBase. (C) Actinomycin D effectively inhibits transcription in *Drosophila* ovarioles. (left) Control ovarioles incubated in ethynyluridine for 30 min show newly transcribed RNA (green). (right) Ovarioles incubated in the presence of actinomycin D fail to incorporate the RNA precursor. Ovarioles were stained with DAPI (blue) to visualize DNA. (D) RT-PCR and Northern blots showing the steady-state abundance of *gypsy* mRNA, *actin5C* mRNA, *sisR-1*, and 5S rRNA before and after actinomycin D treatment. (E) Northern blot showing the relative expression of *sisR-1* versus lariats versus pre-mRNA in 2–14-h embryos. IVT *sisR-1* was used as standards. (F) Northern blot showing the expression of *sisR-1* in controls (*y w*) versus *UAS-dsRed-intron-L-myc* females. (G) Northern blots showing the presence of *sisR-1* before and after RNase R treatment. Both nuclear and cytoplasmic *sisR-1* were degraded by RNase R, indicating that they are not circular molecules. (H) Full-length sequence of nuclear *sisR-1*.

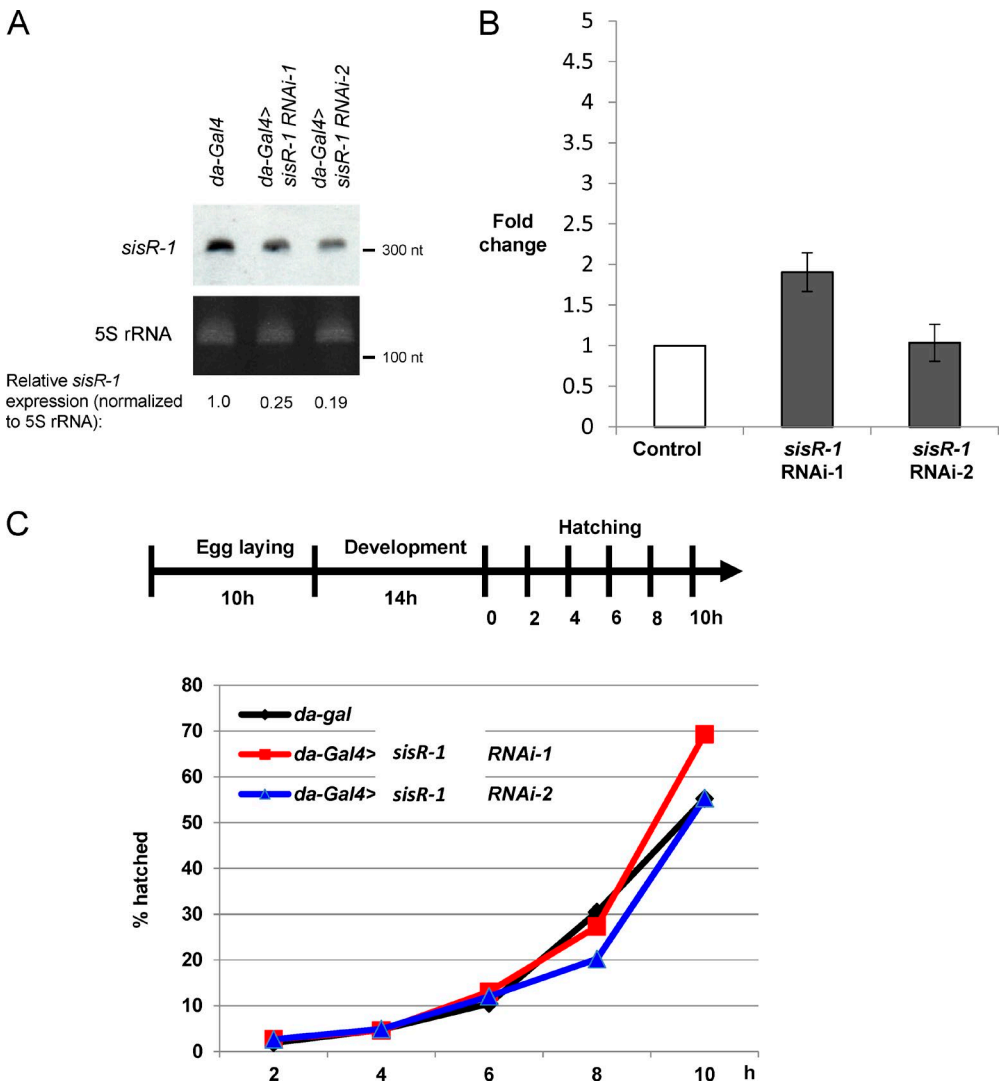

Figure S3. **shRNA-mediated knockdown of *sisR-1*.** (A) Northern blots showing the expression of *sisR-1* in controls versus ovaries expressing two independent *sisR-1* shRNAs. (B) qPCR data showing relative expression of *rga* pre-mRNA normalized to *actin5C* in controls versus ovaries expressing two independent *sisR-1* shRNAs. (C) Chart showing hatching rates of controls and shRNA-expressing embryos. Crosses were setup and allowed to lay eggs for 10 h and then develop for another 14 h. The number of hatched embryos were counted ever 2 h for a total of 10 h.

Table S1. **List of candidate sisRNAs in 0–2-h embryos**

No.	Chromosome	Host gene	CG number	Coordinates of the approximate center of sisRNAs	Location	RT-PCR in eggs
1	X	Act5C	CG4027	chrX:5,796,367	5′UTR	N
2	X	PPP4R2r	CG2890	chrX:10,373,299	5′UTR	Y
s3	X	l(1)G0136	CG8198	chrX:15,628,779	CDS	Y
4	X	mbt	CG18582	chrX:16,506,899	CDS	Y
5	2L	toc	CG9660	chr2L:3,071,980	5′UTR	N
6	2L	cl	CG11024	chr2L:5,520,836	CDS	Y
7	2L	Df31	CG2207	chr2L:21,628,530	CDS	Y
8	2L	Df31	CG2207	chr2L:21,628,988	CDS	Y
9	2L	Df31	CG2207	chr2L:21,629,453	CDS	Y
10	2L	RpS13	CG13389	chr2L:8,364,446	CDS	Y
11	2L		CG33129	chr2L:10,967,648	5′UTR	Y
12	2R	Mlf	CG8295	chr2R:11,825,014	CDS	Y
13	2R	Khc	CG7765	chr2R:12,158,613	CDS	Y
14	2R		CG15083	chr2R:14,723,144	CDS	Y
15	2R	HmgD	CG17950	chr2R:17,602,032	5′UTR	Y
16	2R	betaTub56D	CG9277	chr2R:15,338,652	5′UTR	Y
17	2R		CG1399	chr2R:3,568,133	CDS	Y
18	2R	Fem-1	CG9025	chr2R:16,553,821	CDS	Y
19	2R	zip	CG15792	chr2R:20,898,466	5′UTR	Y
20	3L	hsc70cb	CG6603	chr3L:14,031,059	CDS	Y
21	3L	csp	CG6395	chr3L:22,263,179	CDS	Y
22	3L	capr	CG18811	chr3L:18,665,583	CDS	Y
23	3L	Mkrn1	CG7184	chr3L:21,526,468	5′UTR	N
24	3L		CG11008	chr3L:12,808,197	CDS	Y
25	3L		CG33217	chr3L:23,123,899	CDS	Y
26	3R	rga (sisR-1)	CG2161	chr3R:1,435,140	CDS	Y
27	3R		CG8301	chr3R:5,410,682	CDS	Y
28	3R	desat1	CG5887	chr3R:8,268,685	5′UTR	Y
29	3R		CG9795	chr3R:145,807	5′UTR	Y
30	3R	RpL35A	CG2099	chr3R:1,292,496	5′UTR	Y
31	3R	RpS27	CG10423	chr3R:21,072,909	5′UTR	Y
32	3R		CG12054	chr3R:27,047,539	CDS	Y
33	4	eIF4G	CG10811	chr4:945,544	CDS	Y
34	4	CaMKII	CG18069	chr4:1,061,498	CDS	Y

sisRNAs verified by Northern blots *mbt*, *csp*, *rga*, and *RpS27*. N, no; Y, yes.

Table S2. **List of oligonucleotides used in the study**

Oligos	Sequence (5′–3′)
rga Northern probe Fw (intron)	TGAACAGTTTCTTTTTATTCATTGAT
rga Northern probe Rv (intron)	GAATCGGTTGTAAGCGTGGT
rga exon Fw	ACTGCCCACCAGTCCTACAT
rga exon Rv	GCCGATGAATTCGATAGTGG
rga 5′race nested	AAAAGCTCTTCATGGAATCA
rga 3′Race nested	TCTCGTGATCAGCTCATTAAG
rga intron A AscI Fw	CGATGCGGCGCGCCAGGTGAGTTCAAATCA AAATCC
rga intron B AscI Fw	CGATGCGGCGCGCCAGGTAATTTCTTTACCTCT TGT
rga intron NotI Rv	TAGCATGCGGCCGCATCTGAAATCAGAAGAGGA GCG
rga intron second half probe Fw (also for ASTR, and rga pre-mRNA Fw)	CAGAAGAGGAGCGAGATGGT
rga intron second half probe Rv (also for ASTR)	AAAGCAAGCGACTTAACCTTTT
Sis-rga-shRNA-1-top-oligo	CGATGCGCTAGCAGTAGCCCACTTAGATATGCT ACGTAGTTATATTCAAGCATACGTAGCATA TCTAAGTGGGCTGCGAATTCATGCTA
Sis-rga-shRNA-1-bottom-oligo	TAGCATGAATTCGCAGCCCACTTAGATATGCTA CGTATGCTTGAATATAACTACGTAGCATAT CTAAGTGGGCTACTGCTAGCGCATCG
Sis-rga-shRNA-2-top-oligo	CGATGCGCTAGCAGTACGTTGGTCCAACCGAAT ACGTAGTTATATTCAAGCATACGTATTCGG TTGGACCAACGTGCGAATTCATGCTA
Sis-rga-shRNA-2-bottom-oligo	TAGCATGAATTCGCACGTTGGTCCAACCGAATA CGTATGCTTGAATATAACTACGTATTCGGT TGGACCAACGTACTGCTAGCGCATCG
rga sisRNA nuclear 3′ extreme end	GGTTAAGTCGCTTGCTTTATGTG
rga sisRNA cyto predicted 3′ extreme end	TTCTTTTTATTCATTGATCATGTTTCA
actin5C-Fw	TGCCCATCTACGAGGGTTAT
actin5C-Rv	AGTACTTGCGCTCTGGCGG
gypsy Fw DE-1	GCCTTAAAGGTTGTGGCGGG
gypsy Rv DE-2	GGGTAGACGGCGACTTTCTTGC
dsRed Fw	AGTTCATGCGCTTCAAGGTG
dsRed Rv	TTCACGCCGATGAACTTCAC
rga mRNA Fw	CCGTCCAAGTGGTTCTCTGT
rga mRNA Rv (also for rga pre-mRNA Rv)	TGGCGGTCCTTTGAATAACT
rga intron A 5′ss mut AscI Fw	CGATGCGGCGCGCCAGCTGAGTTCAAATCAAAA TCC
Act5C sisRNA Fw	TTTGCGGCTTTCTTTTGCAC
Act5C sisRNA Rv	TACAAAATAGAAGGCCCCGC
PPP4R2r sisRNA Fw	AGAAAATGTGTGCTTGTGTGC
PPP4R2r sisRNA Rv	CAATTGTGTGCAGGTAGGTATG
l(1)G0136 sisRNA Fw	GCATTCTATCGCCTTCCACG
l(1)G0136 sisRNA Rv	GCCCTCTTGCGAACTTCCTA
mbt sisRNA Fw	TTGTGCCTTTGTACCTTGCC
mbt sisRNA Rv	ATTTCACCTGGCACTTTGGG
toc sisRNA Fw	TTGGATCTCGAGAGTTTGCG
toc sisRNA Rv	GGATTTTCCCGGCAACCTTT
cl sisRNA Fw	GAGACTGCGAACATCTGCTG
cl sisRNA Rv	ACAGTTGGGGTTGAGTGACT
Df31-1 sisRNA Fw	TGCATTGTTACATCTGCCGG
Df31-1 sisRNA Rv	ACCCTGTTCTCATCTGTTGGT
Df31-2 sisRNA Fw	AGTCGTTTCTCTCGCACTCA
Df31-2 sisRNA Rv	AAGACGCAGGTTGTATGACG
Df31-3 sisRNA Fw	GTCGTCCGCATACACTCAAC
Df31-3 sisRNA Rv	TGTAGTTGTCTCGTTCCCGC

Table S2. **List of oligonucleotides used in the study** *(Continued)*

Oligos	Sequence (5′–3′)
RpS13 sisRNA Fw	GCGCCTGTCAAATGCGAATA
RpS13 sisRNA Rv	CCTGTGTGCCAAGCTGAATG
CG33129 *sisRNA Fw*	AATCGCAGCTGGAACACATC
CG33129 *sisRNA Rv*	GGTTCCAGTTGCCGCTAAAT
Mlf sisRNA Fw	CGTTGCTACCCATGAAGTCG
Mlf sisRNA Rv	TGTCAGCTTCGAACCTCATT
Khc sisRNA Fw	GTCATCGCGCTTCTTTCGAT
Khc sisRNA Rv	CTTCCCCAAAATTGCCGACC
CG15083 *sisRNA Fw*	ATTGCCCAGACTGACGCTAT
CG15083 *sisRNA Rv*	TGAATTGTCAGCTCGAATTTGTC
HmgD sisRNA Fw	AGGCAGTCATCGCATACAGA
HmgD sisRNA Rv	ACGCGCGCCACTTAATAATT
betaTub56D sisRNA Fw	TATGCGAATGTCAATGGCCC
betaTub56D sisRNA Rv	TCCCGGCATCTGTGTTTGTA
CG1399 *sisRNA Fw*	TAGTATGTTGGGACGTTTGTGA
CG1399 *sisRNA Rv*	ACTGAACACTTATTCCATACCCA
Fem-1 sisRNA Fw	TAAAACGCAACAGCAGAGGT
Fem-1 sisRNA Rv	CACCAAAAGTCACTGTCGCA
zip sisRNA Fw	CTTTATCTCGGAAGTGCGGC
zip sisRNA Rv	CACCACCCCTTCTTCTACGT
hsc70cb sisRNA Fw	CGGTCTGCATAATCGTTCGA
hsc70cb sisRNA Rv	GCACATATCTGGGGTACACG
csp sisRNA Fw	GTCCAAATATAATACCCGCCCC
csp sisRNA Rv	AGTACAGTGTGTGGGTGGAT
capr sisRNA Fw	ACTGGTTCTAATTGACATGCCG
capr sisRNA Rv	TCCTCCGTGTTGCTTTGTTT
Mkrn1 sisRNA Fw	CCCCAAACGAACATACCTACAG
Mkrn1 sisRNA Rv	TGCAAAAGGTGTCACGAGTG
CG11008 *sisRNA Fw*	AAACCGTTACAAACCCAGGG
CG11008 *sisRNA Rv*	CGAAAAGAAAGTGAAGGCGC
CG33217 *sisRNA Fw*	TCGGGGTATAAAGTGATGTCAGT
CG33217 *sisRNA Rv*	TCACTCGAGCCAGGATTGTT
rga sisRNA Fw	TCATGGAATCAGAAGCCCGT
rga sisRNA Rv	GGTTGTAAGCGTGGTGTCTC
CG8301 *sisRNA Fw*	AAGAATCCCTGGAGAGCAGC
CG8301 *sisRNA Rv*	AACGGATGGCTAGTCGTAGA
desat1 sisRNA Fw	GTGATAACGGGCCACAACAA
desat1 sisRNA Rv	ATCAGAGGCACGCATTGAAC
CG9795 *sisRNA Fw*	GGAGCTAGCAGGAGGAAGAA
CG9795 *sisRNA Rv*	AAGTTTTGACACGCGCTCAA
RpL35A sisRNA Fw	CATGGAACTTTTGGACGGCA
RpL35A sisRNA Rv	ATAACCTGCAAACGCCAACC
RpS27 sisRNA Fw	TGGCACATTTTCTCTCGGTG
RpS27 sisRNA Rv	CCCGCTTCCATTGAATGTGT
CG12054 *sisRNA Fw*	CTGGAAATGGCTACCTGTGC
CG12054 *sisRNA Rv*	CCACTACACCGCAAACAACA
eIF4G sisRNA Fw	ACCCGAAATTTGAAGTCGAGAC
eIF4G sisRNA Rv	GGCTCTGATTCTGCGCAAAT
CaMKII sisRNA Fw	TGTCGTCAAGGTACCGTAACA
CaMKII sisRNA Rv	CGTAAGGGGAGAGTGACACA