Supporting Information

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SI Materials and Methods

Plant Materials and Growth Conditions. *Arabidopsis thaliana* (*At*) *suppressor of gene silencing (sgs)3-11* and *rna-dependent rna polymerase (rdr)6-11* mutants were in a Columbia genetic background and have been described previously (1). Seeds were sown on soil mixed with Supermix (Sakata) and vermiculite (Nittai) (1:1), and placed at 4 °C for 3–5 d before transfer to growth chambers under 16-h day/8-h night illumination cycles with a temperature of 22 °C.

Oligonucleotides. The oligonucleotides used in this study are listed in Table S1 (primers) and Table S2 (oligoRNAs).

Transgenic Plants. cDNAs for N-terminally myc-tagged or N-terminally myc- and C-terminally FLAG-tagged SGS3 were generated by PCR using the primers myc-AtSGS3 forward primer (FP), AtSGS3 stop reverse primer (RP), or AtSGS3-3×FLAG RP. The PCR fragments were cloned into pENTR/D-TOPO (Invitrogen) and transferred into pEarleyGate 100 (2). The constructs were transformed into the *sgs3-11 rdr6-11* double mutant.

RNA Immunoprecipitation with SGS3 from Plant Tissue Extracts. Extracts were prepared from flower buds of transgenic *sgs3-11 rdr6-11* plants expressing myc-tagged SGS3 or myc- and FLAG-double-tagged SGS3, loaded onto discontinuous gradients composed of 9 mL of 20% (wt/vol) glycerol and 5 mL of 50% (wt/vol) glycerol in 20 mM Hepes (pH 7.5), 100 mM KCl, and 10 mM $MgCl_2$, and centrifuged at 39,000 rpm for 90 min at 4 °C using an SW40Ti rotor (Beckman Coulter). The gradients were fractionated to 12 fractions (1 mL each) using a density gradient fractionation system (ISCO). Dilution buffer (4 mL) was added to the eighth fraction (1 mL each) to give a final concentration of 20 mM Hepes (pH 7.5), 120 mM KCl, 10 mM $MgCl_2$, 0.2% Nonidet P-40, 1% plant protease inhibitor mixture (Sigma), and 100 U/mL RNasin Plus Ribonuclease Inhibitor (Promega). Fifty microliters of EZview Red Anti-FLAG M2 affinity gel (Sigma) was added, and the mixture was incubated for 3 h at 4 °C. Next, gels were transferred to a SigmaPrep spin column (Sigma), washed five times with 500 μL of wash buffer A (20 mM Hepes, pH 7.5, 120 mM KCl, 10 mM $MgCl_2$, 0.2% Nonidet P-40, and 1% plant protease inhibitor mixture), and eluted with 240 μL of wash buffer A containing 170 ng/μL 3×FLAG peptide. RNA was purified from a 200-μL aliquot using an RNeasy Mini Kit (QIAGEN) by mixing with 700 μL of RLT buffer (QIAGEN) containing 1% β-mercaptoethanol or from 170 μL of the input samples before addition of anti-FLAG M2 agarose gel by mixing with 595 μL of RLT buffer containing 1% β-mercaptoethanol. Following elution with 100 μL of elution buffer, an 85-μL aliquot was incubated with 4 U of RQ1 RNase-Free DNase (Promega) and 40 U RNasin Plus Ribonuclease Inhibitor for 20 min at 37 °C. After extraction with phenol/chloroform/isoamylalcohol, ethanol precipitation was carried out and recovered RNA was dissolved in 25 μL of water. A 5-μL aliquot was used to synthesize cDNA using gene-specific primers and SuperScript III (Invitrogen). cDNAs were analyzed by quantitative PCR [SYBR Premix ExTaq II (Takara); Prism 7000 (Applied Biosystems)] and the relative amount of RNA in each precipitate was estimated as described previously (3). Statistical significance was determined by Kruskal–Wallis test followed by Tukey–Kramer test.

Coimmunoprecipitation of ARGONAUTE 1 with SGS3 from Plant Tissue Extracts. Extracts were prepared from flower buds of transgenic plants expressing myc-tagged or myc- and FLAG-double-tagged SGS3 as described above. Packed tissue powder (400 μl) was combined with 1.6 mL of extraction buffer (30 mM Hepes, pH 7.6, 80 mM KOAc, 1.8 mM $MgCl_2$, 2 mM DTT, and 1% plant protease inhibitor mixture). After clarification by centrifugation at 12,000 × g for 5 min at 4 °C, a 1.5-mL aliquot of the supernatant was taken for second-round clarification by centrifugation at 12,000 × g for 2 min at 4 °C, and 1.4 mL of the supernatant was finally recovered. After addition of 24 μL of 10% Nonidet P-40, 1.2 mL of the supernatant was mixed with 60 μL of EZview Red Anti-FLAG M2 affinity gel and incubated for 60 min on ice. The gels and supernatants were transferred to a SigmaPrep spin column (Sigma), and gels were washed five times with 200 μL of wash buffer B (30 mM Hepes, pH 7.6, 80 mM KOAc, 1.8 mM $MgCl_2$, 2 mM DTT, 0.2% Nonidet P-40, and 1% plant protease inhibitor mixture). Finally, FLAG-tagged proteins were eluted from the gels with 200 μL of wash buffer B containing 170 ng/μL 3×FLAG peptide for 30 min on ice. Myc-tagged SGS3 and endogenous Argonaute (AGO)1 were detected with anti-myc (Applied Biological Materials) and anti-AGO1 (Agrisera), respectively. The signals were detected with horseradish peroxidase-conjugated anti-rabbit IgG secondary antibody (GE Healthcare) and an ECL Plus Kit (GE Healthcare) using an LAS-3000 image analyzer (Fujifilm).

Determination of the Nucleotide Sequences of *NtSGS3a* and *NtSGS3b* mRNAs. Based on the nucleotide sequence of *Solanum lycopersicum SGS3* (*SlSGS3*) (4), we designed primers, amplified the cDNA of a part of *Nicotiana tabacum* (*Nt*) *SGS3* mRNA, and determined the nucleotide sequences. The nucleotide sequences of full-length *N. tabacum SGS3* mRNAs were determined using 5′ and 3′ RACE. We obtained two *NtSGS3* cDNAs, which are designated *NtSGS3a* and *NtSGS3b*. The GenBank accession numbers for the *NtSGS3a* and *NtSGS3b* cDNA sequences are AB690269 and AB690270, respectively.

Preparation of Template RNA for in Vitro Translation. *FLAG-NtAGO1* mRNA was synthesized as described previously (5). To obtain the plasmid for synthesis of *FLAG-LUC* (luciferase) mRNA, we used the oligonucleotides FLAG-Luc FP and Luc-stop RP, and cloned the PCR products between the SalI and BamHI sites of pSP64. To obtain the plasmid for synthesis of *AtSGS3-myc* mRNA, we used the oligonucleotides AtSGS3 SalI FP, AtSGS3-3xmyc RP1st, and AtSGS3-3xmyc RP2nd, and cloned the PCR product between the SalI and XbaI sites of pSP64. To obtain the plasmid for synthesis of *LUC-myc* and *NtSGS3a-myc*, we used [Luc-SalI FP and Luc-KpnI RP] and [NtSGS3a-SalI FP and NtSGS3a-KpnI RP]. The PCR products were cloned between the SalI and KpnI sites of pSP64-AtSGS3-myc. To obtain a plasmid carrying the full-length *TAS2* sequence, we used the oligonucleotides TAS2 FP and TAS2 RP. The PCR product was cloned into pCR4Blunt-TOPO (Invitrogen). To generate a plasmid carrying the *TAS2-171* sequence, we used the oligonucleotides TAS2 171 FP and TAS2 171 RP. The PCR product was cloned into pCR4Blunt-TOPO. To amplify the template DNA for in vitro transcription of *TAS2* target RNAs by PCR, we used the oligonucleotides SP6-TAS2 FP and TAS2-polyA70.

In vitro transcription using SP6 RNA polymerase followed by a capping reaction was performed as described previously (5).

Analysis of RNAs Copurified with AGO1 and SGS3. To analyze RNAs that were copurified with FLAG-NtAGO1 and NtSGS3a-myc, 5 nM ^{32}P-labeled target RNAs and 25 ng/μL unlabeled luciferase mRNA were added to the RNA-induced silencing complex (RISC) solutions, and the mixtures were incubated for 20 min at 25 °C. The reaction mixtures (30 μL) were mixed with 0.6 μL of 2–5 μg/μL of anti-FLAG antibody and 9.4 μL of the translation reaction (TR) plus NP-40 (TRN) buffer (30 mM HEPES [pH 7.4], 80 mM KOAc, 1.8 mM MgCl2, 2 mM DTT, 0.2% NP-40, 1 tablet/50 ml complete protease inhibitor [Roche]) and incubated for 40 min on ice, followed by further incubation with 6 μL of protein G-conjugated Dynabeads and 30 μL of TRN buffer for 20 min on ice. The magnetic beads were washed four times with 200 μL TRN buffer and eluted with 25 μL TRN buffer containing 170 ng/μL 3×FLAG peptide for 30 min on ice. The elution samples (15 μL each) were mixed with 1 μL of 0.5 μg/μL luciferase mRNA, 0.3 μL of 1 μg/μL anti-myc antibodies, and 3.7 μL of TRN buffer and incubated for 40 min on ice, followed by further incubation with 3 μL of protein G-conjugated Dynabeads (Invitrogen) and 15 μL of TRN buffer for 20 min on ice. The magnetic beads were then washed twice with 100 μL TRN buffer. Extraction and analysis of copurified RNAs were performed as in *Materials and Methods*.

Depletion of Endogenous NtSGS3 in Evacuolated Tobacco Protoplasts. Anti-NtSGS3 antibody was raised in rabbits using a synthetic peptide (NH_2-CQWVPQNPSPKTWGNQNT-COOH; underlined residues correspond to the 63rd to 79th amino acids of NtSGS3a and NtSGS3b) as an antigen (Medical & Biological Laboratories). The affinity-purified anti-NtSGS3 antibody was prepared from the immune serum using the above peptide. To deplete endogenous NtSGS3, 60 μL of RISC solution, AtSGS3-myc–translated evacuolated tobacco protoplasts (BYL), or mock-translated BYL was separately mixed with 1.5 μg of anti-NtSGS3 antibody or anti-b-glucuronidase (GUS) antibody (Sigma-Aldrich; product G5545), incubated for 60 min on ice, and followed by further incubation with 12 μL protein A Sepharose (GE Healthcare) equilibrated with TR buffer (30 mM HEPES [pH 7.4], 80 mM KOAc, 1.8 mM MgCl2, 2 mM DTT, 1 tablet/50 ml complete protease inhibitor [Roche]) for 60 min on a rotating stage at 4 °C. The supernatant was recovered after centrifugation at 1,000 × *g* for 1 min at 4 °C and used for RNA analyses as described above.

1. Peragine A, Yoshikawa M, Wu G, Albrecht HL, Poethig RS (2004) SGS3 and SGS2/SDE1/RDR6 are required for juvenile development and the production of *trans*-acting siRNAs in *Arabidopsis*. *Genes Dev* 18(19):2368–2379.
2. Earley KW, et al. (2006) Gateway-compatible vectors for plant functional genomics and proteomics. *Plant J* 45(4):616–629.
3. Schmittgen TD, Livak KJ (2008) Analyzing real-time PCR data by the comparative C(T) method. *Nat Protoc* 3(6):1101–1108.
4. Glick E, et al. (2008) Interaction with host SGS3 is required for suppression of RNA silencing by tomato yellow leaf curl virus V2 protein. *Proc Natl Acad Sci USA* 105(1): 157–161.
5. Iki T, et al. (2010) In vitro assembly of plant RNA-induced silencing complexes facilitated by molecular chaperone HSP90. *Mol Cell* 39(2):282–291.

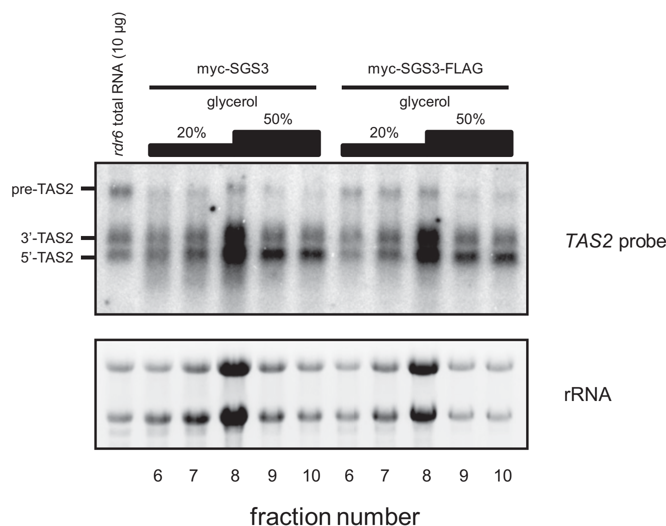

Fig. S1. Distributions of *TAS2*-derived RNAs after fractionation by 20–50% stepwise glycerol gradient centrifugation. Floral tissue extracts from transgenic plants expressing *myc-SGS3* or *myc-SGS3-FLAG* in *sgs3 rdr6* double mutants were used. The eighth fractions were used for the experiments in Fig. 1*B*.

Fig. S2. Alignment of SGS3 proteins from *A. thaliana* (GenBank accession no. NP_197747) and *N. tabacum* (GenBank accession nos. AB690269 and AB690270 for NtSGS3a and NtSGS3b, respectively). Alignment was performed by using the ClustalW2 algorithm (http://www.ebi.ac.uk/tools/clustalw2) with the default settings. The red underline indicates the peptide that is used as an antigen for anti-NtSGS3 antibody.

A

full-length *TAS2* RNA
(accession No. DQ000170)

1 1055

431 452

cleavage site

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TAS2 RNA        5'—UGGUUUUGUGAUUUUUCUCUCCAAGCGAAUGAUGAU—3'
                             |||||||:||||| ||||||||
miR173                    3'-CACUAAAGAGAGACGUUCGCUU-5'
(ΔG = -32.2 kcal/mol)

TAS2-m1m2 RNA   5'—UGGUUUUCAGAUUUUUCUCUCCAAGCGAAUGAUGAU—3'
                               |||||:||||| ||||||||
miR173                    3'-CACUAAAGAGAGACGUUCGCUU-5'
(ΔG = -29.8 kcal/mol)

TAS2-m1 RNA     5'-UGGUUUUCUGAUUUUUCUCUCCAAGCGAAAUGAUGAU—3'
                              |||||:||||| |||||||||
miR173                    3'-CACUAAAGAGAGACGUUCGCUU-5'
(ΔG = -30.3 kcal/mol)

TAS2-m2 RNA     5'—UGGUUUUGAGAUUUUUCUCUCCAAGCGAAUGAUGAU—3'
                             | |||||:||||| ||||||||
miR173                    3'-CACUAAAGAGAGACGUUCGCUU-5'
(ΔG = -29.8 kcal/mol)

TAS2-m3 RNA     5'—UGGUUUUGUAAUUUUUCUCUCCAAGCGAAUGAUGAU—3'
                             || ||||:||||| ||||||||
miR173                    3'-CACUAAAGAGAGACGUUCGCUU-5'
(ΔG = -26.5 kcal/mol)

TAS2-171 RNA    5'—UGGUUUUGAUAUUGGCGCGGCUCAAUCA-UGAUGAU—3'
                             |  |||       |: | : |
miR173                    3'-CACUAAAGAGAGACGUUCGCUU-5'

TAS2-171 RNA    5'—UGGUUUUGAUAUUGGCGCGGCUCAAUCA-UGAUGAU—3'
                             ||||||||||||||||||||
miR171                    3'-CUAUAACCGCGCCGAGUUAGU-5'
(ΔG = -41.9 kcal/mol)
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B

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                     miR173(22-nt)
                          G       A
miR173          5'—UUCGCUUGCA AGAGAA UCAC—3'
                   |||||||:|| |||||| ||
                3'—ACAAGCGAAUGU UCUCUU-AG—5'
                          G
                    miR173* (21-nt)

                     amiR173(22-nt)
                          G
amiR173         5'—UUCGCUUGCA AGAGAAAUCAC—3'
                   |||||||:|| |||||||||
                3'—ACAAGCGAAUGU UCUCUUUAG—5'
                          G
                    amiR173sy* (22-nt)

                     amiR173(22-nt)
                          G       A
amiR173bul      5'—UUCGCUUGCA AGAGAA UCAC—3'
                   |||||||:|| |||||| ||
                3'—ACAAGCGAAUGU UCUCUU-AG—5'
                          G
                    amiR173sy* (21-nt)

                     amiR173(21-nt)
                          G
amiR173-21      5'—UUCGCUUGCA AGAGAAAUCA—3'
                   |||||||:|| ||||||||
                3'—ACAAGCGAAUGU UCUCUUUA—5'
                          G
                    amiR173sy* (21-nt)

                     amiR173(21-nt)
                          G       A
amiR173-21bul   5'—UUCGCUUGCA AGAGAA UCA—3'
                   |||||||:|| |||||| |
                3'—ACAAGCGAAUGU UCUCUU-A—5'
                          G
                    amiR173sy* (21-nt)
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Fig. S3. Structures of *TAS2*-related target RNAs and miR173/miR173*–related duplexes used in in vitro analyses. (*A*) Structures of *TAS2*-related target RNAs. The mutated bases are shown as red characters. The miR171 target site was obtained from *SCL6-III* mRNA (At3g60630). (*B*) Structures of miR173/miR173*–related duplexes. These forms of amiR173s were used in Fig. 3.

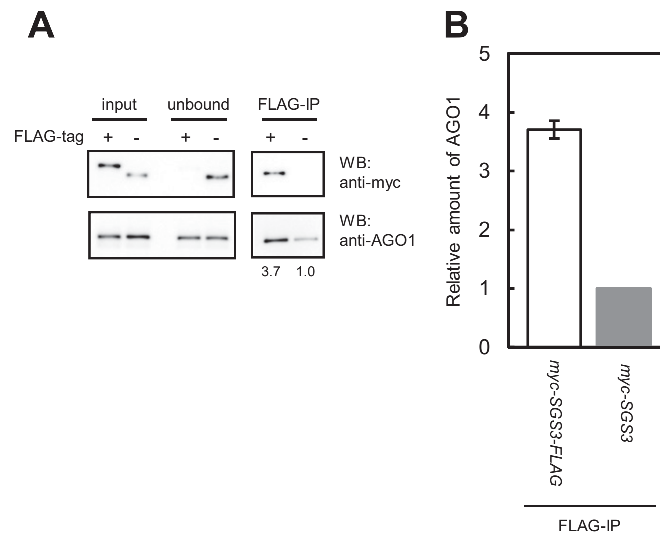

Fig. S4. SGS3 associates with AGO1 in vivo. (*A*) Copurification of endogenous AGO1 with FLAG-tagged SGS3. FLAG-tagged SGS3 was immunoprecipitated (IP) with anti-FLAG antibody from floral tissue extracts of the transgenic plants used in Fig. 1. SGS3 and AGO1 were detected with anti-myc and anti-AGO1 antibodies, respectively. (*B*) Relative amount of AGO1 copurified with FLAG-tagged SGS3. The strength of signals relative to those for FLAG tag minus controls is shown. The error bar shows the SD calculated from three biological replicates. Similar results were obtained from another experiment using an independent transgenic line for each tag.

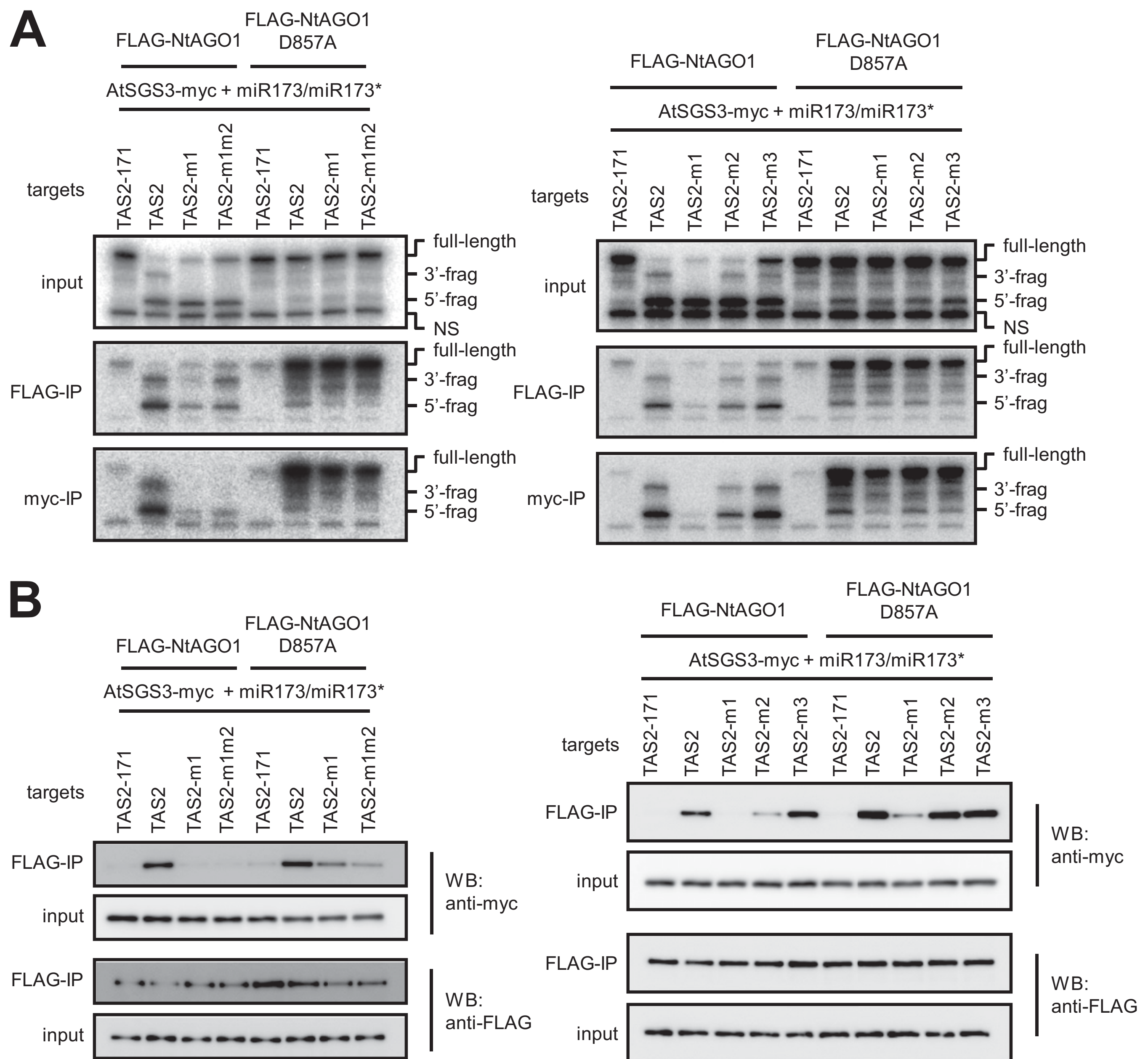

Fig. S5. Association of AtSGS3-myc with miR173-bound FLAG-NtAGO1 in the presence of *TAS2*-related target RNAs in vitro. (*A*) Copurification of *TAS2*-related RNAs with FLAG-NtAGO1 and FLAG-NtAGO1^{D857A} (*Middle*) or AtSGS3-myc (*Lower*). (*B*) Copurification of AtSGS3-myc with FLAG-NtAGO1 and FLAG-NtAGO1^{D857A} (*Upper* two) in the presence of *TAS2*-related RNAs. Experiments were performed as described in Fig. 4, except that myc-tagged *SGS3* mRNA from *A. thaliana* was used in place of *NtSGS3a-myc* mRNA. WB, Western blotting.

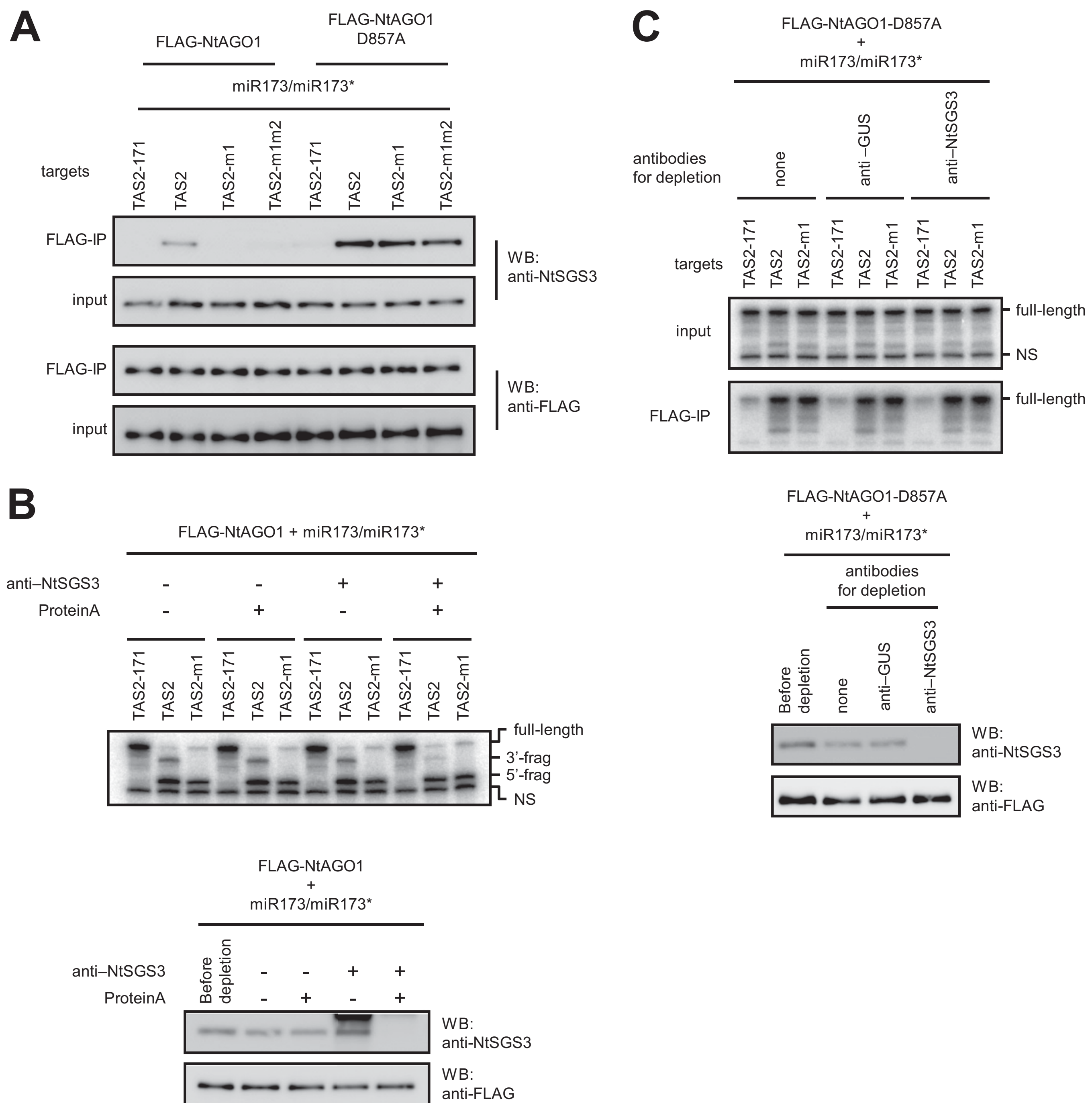

Fig. S6. Effects of depletion of endogenous NtSGS3 in BYL on the stability of miR173–RISC–cleaved 3′ fragments and complexes containing target RNA and slicer-defective FLAG-NtAGO1. (*A*) Copurification of endogenous NtSGS3 with miR173-bound FLAG-NtAGO1 and FLAG-NtAGO1^{D857A} in the presence of *TAS2*-related RNAs . Experiments were performed as described in the legend for Fig. 3*B*, except that NtSGS3a-myc was not added. (*B*) Analysis of *TAS2* target RNAs cleaved by endogenous NtSGS3-depleted miR173–RISC and the amounts of endogenous NtSGS3 and FLAG-NtAGO1 in the mixtures. Experiments were performed as described in the legend for Fig. 5*A*, except that immunoprecipitation using anti-FLAG antibody was not performed. (*C*) Effects of depletion of endogenous NtSGS3 on the formation of target RNA–RISC complexes containing slicer-defective FLAG-NtAGO1^{D857A} and the amounts of endogenous NtSGS3 and FLAG-NtAGO1^{D857A} in the mixtures. The band indicated by 'NS' shows an in vitro transcription byproduct that does not have the miR173 target site. Experiments were performed as described in the legend for Fig. 5*A*, except that FLAG-NtAGO1^{D857A} was used.

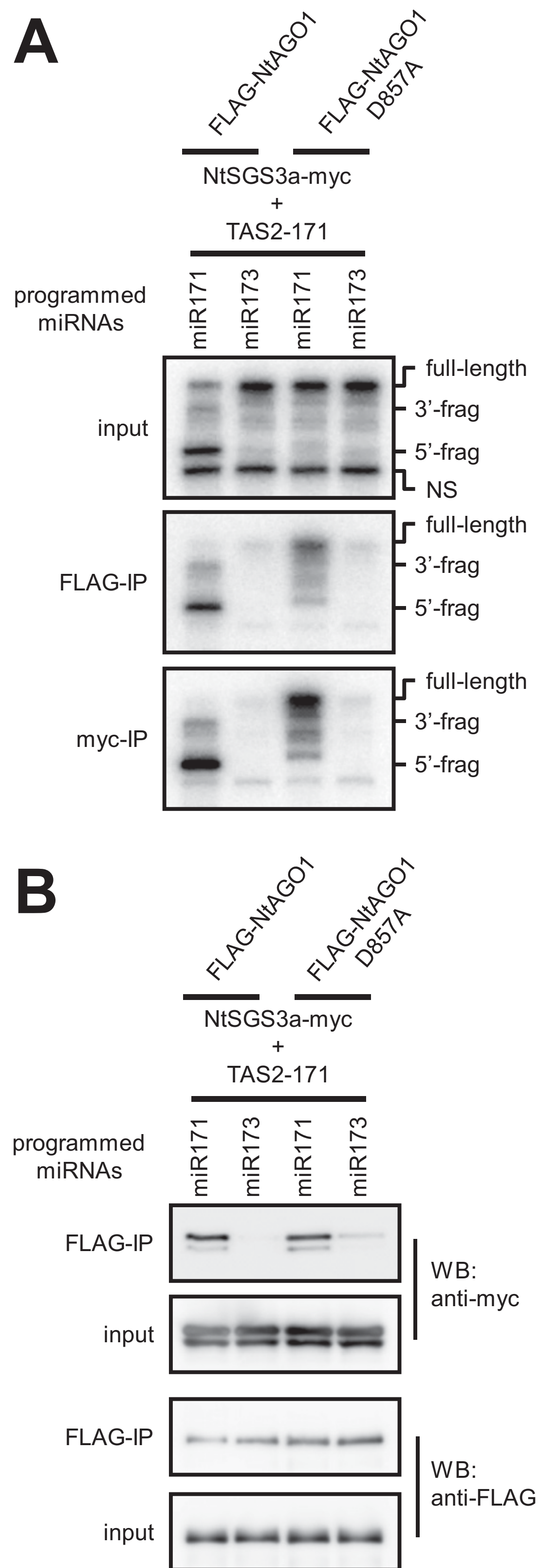

Fig. S7. Association of target RNAs with miR171-programmed FLAG-NtAGO1 or NtSGS3a-myc, and association of NtSGS3a-myc with miR171-programmed FLAG-NtAGO1. (*A*) Copurification of *TAS2-171* RNA with FLAG-NtAGO1 and FLAG-NtAGO1^{D857A} that bound miR171 or miR173 (*Middle*) or NtSGS3a-myc (*Lower*). Experiments were performed as described in the legend for Fig. 2*B*. (*B*) Copurification of NtSGS3a-myc with FLAG-NtAGO1 bound to miR171 or miR173 (*Upper* two panels) in the presence of *TAS2-171* RNA. Experiments were performed as described in the legend for Fig. 2*A*.

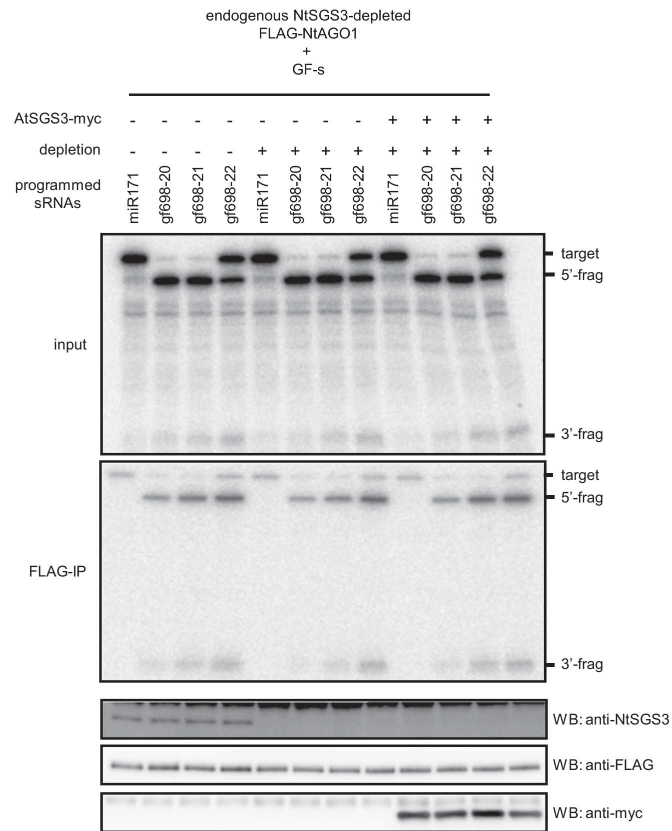

Fig. S8. Depletion of endogenous NtSGS3 does not affect the stability of GF-s cleavage fragments and the complexes containing GF-s target RNAs and the RISC that bound gf698 siRNAs. The target GF-s RNA has a partial sequence of GFP mRNA. The experiment was performed as in Fig. 5*B*.

Table S1. DNA primers used in this study

Name	Sequence (5′-3′)	Purpose	Vector
TAS2-F1	CTCCTGTCACACTGATGGGTTTCGAAGA	*TAS2* probe	
TAS2-R1	TGATCCGAGAAAATTACAAGTGCAAAAGTG	*TAS2* probe	
myc-AtSGS3 FP	CACCATGGAACAAAAGTTGATTTCTGAAGAAG ATTTGAGTTCTAGGGCTGGTCCAATG	*myc-SGS3* expression	pENTR/D-TOPO
AtSGS3 stop RP	TCAATCATCTTCATTGTGAAGGCCATGC	*myc-SGS3* expression	pENTR/D-TOPO
AtSGS3-3×FLAG RP	CTACTTATCATCATCATCCTTATAATCAATATCA TGATCCTTATAATCTCCATCATGATCCTTATAATCGGT ACCATCATCTTCATTGTGAAGGCCATGC	*myc-SGS3-FLAG* expression	pENTR/D-TOPO
AtSGS3-SalI FP	TTTGTCGACATGAGTTCTAGGGCTGGTCCA	*AtSGS3-myc* mRNA	pSP64
AtSGS3-3×myc RP1st	GGAGATTAGCTTTTGTTCACCGTTCAAATCTTCT TCAGAAATCAACTTTTGTTCGG TACCATCATCTTCATTGTGAAGGCCATGC	*AtSGS3-myc* mRNA	pSP64
AtSGS3-3×myc RP2nd	TTTTCTAGATCACAAGTCTTCTTCTGAGATTA ATTTTTGTTCACCGTTCAAGTCT TCCTCGGAGATTAGCTTTTGTTCAC	*AtSGS3-myc* mRNA	pSP64
FLAG-Luc FP	ACTGACGTCGACATGGACTACAAGGATGACGATGACA AGGGAGGTGAAGACGCCAAAAACATA	*FLAG-Luc* mRNA	pSP64
Luc-stop RP	ACTGACGGATCCTTACACGGCGATCTTTCC	*FLAG-Luc* mRNA	pSP64
Luc-SalI FP	ACTGACGTCGACATGGAAGACGCCAAAAAC	Replacement of AtSGS3-myc with LUC-myc	pSP64
Luc-KpnI RP	TTTTGGTACCCACGGCGATCTTTCCGCCCT	Replacement of AtSGS3-myc with LUC-myc	pSP64
NtSGS3a-SalI FP	ACTGACGTCGACATGAGTTCCAGCAAAGG	Replacement of AtSGS3-myc with NtSGS3a-myc	pSP64
NtSGS3a-KpnI RP	TTTGGTACCTTCAGATTGCTTTGGGGAGTA	Replacement of AtSGS3-myc with NtSGS3a-myc	pSP64
TAS2 FP	AAACCTAGTCGTGACGTCAAAAACAAAGAG	Full-length *TAS2*	pCR4Blunt-TOPO
TAS2 RP	AGCAACGAAACTGATTATTTGATTAGACAG	Full-length *TAS2*	pCR4Blunt-TOPO
TAS2-miR171 FP	GATATTGGCGCGGCTCAATCATGATGATACTTAAA CTATTCACTTGATTA	Replacement of miR173 target site with miR171 target site	pCR4Blunt-TOPO
TAS2-miR171 RP	TCATCATGATTGAGCCGCGCCAATATCAAAACCAT GTCGTTTCAACTCGT	Replacement of miR173 target site with miR171 target site	pCR4Blunt-TOPO
SP6-TAS2 FP	TTTATTTAGGTGACACTATAGAAAAACCTAGT CGTGACGTCAAAAACAA	*TAS2* target RNA	
TAS2-70polyA	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTAGCAACGAAACTGATTATTT	*TAS2* target RNA	
TAS2qRT-PCRa1	TTGGGTTTGGGAGTGAGTTTACGAGTTACA	Quantitative RT-PCR	
TAS2qRT-PCRa2	GTGACTGACACATGCTTGGTAACAAATCCA	cDNA synthesis and quantitative RT-PCR	
TAS2qRT-PCRb1	GTTTCACTAGAATAAATACGGCGGTTTACGA	Quantitative RT-PCR	
TAS2qRT-PCRb2	GTGAATAGTTTAAGTATCATCATTCGCTTGGA	cDNA synthesis and quantitative RT-PCR	
TAS2qRT-PCRc1	TCCAGATGGTAGAAATGGGATATACATATATG	Quantitative RT-PCR	
TAS2qRT-PCRc2	CCTATGTTTCTACCATCCGATCAACAAGAG	cDNA synthesis and quantitative RT-PCR	
TAS1aqRT-PCRa1	CTTCTCTTGATTAGCGTCTATAGCTAGATTG	Quantitative RT-PCR	
TAS1aqRT-PCRa2	GAAACTCCTACATGGCTGGTGACAAATAGA	cDNA synthesis and quantitative RT-PCR	
TAS1aqRT-PCRb1	GGAATACGGAGATATATTTTCAAGAGGAGAAA	Quantitative RT-PCR	
TAS1aqRT-PCRb2	GGACTTAGGATGAATGACTCATTCGCTTGTA	cDNA synthesis and quantitative RT-PCR	
TAS1aqRT-PCRc1	GGATGTTGGTATTCTTATTTTGCAAGGCTTG	Quantitative RT-PCR	
TAS1aqRT-PCRc2	ATTAGCTGACTTGCTTCATGTAGACTTCACA	cDNA synthesis and quantitative RT-PCR	
SCL6qRT-PCRa1	AAACGACCAAGACCAGTCAGCGGTAATCA	Quantitative RT-PCR	
SCL6qRT-PCRa2	TCCCTTGCGCGAGAACGGGATTGTTATC	cDNA synthesis and quantitative RT-PCR	
SCL6qRT-PCRb1	GCTCACCACAAACGGCGGAGATAACAATC	Quantitative RT-PCR	
SCL6qRT-PCRb2	TCTGTTATATACGAAGCTGCTCTGTGGAAC	cDNA synthesis and quantitative RT-PCR	
SCL6qRT-PCRc1	AACAACAATCCTAAACCTCCGTTCCACAGA	Quantitative RT-PCR	
SCL6qRT-PCRc2	GAGAGACGGTGGTGATAATGATGAGTCTTG	cDNA synthesis and quantitative RT-PCR	
EIF4AqRT FP1	GACATATTCCAGCTTCTTCCACCAAAGATC	Quantitative RT-PCR	
EIF4AqRT RP1	TGCTCATGAACTTCCTTGTGATCTCAAGAG	cDNA synthesis and quantitative RT-PCR	
ACT2qRT FP1	ATCAGGAAGGATCTGTACGGTAACATTGTG	Quantitative RT-PCR	
ACT2qRT RP1	GTGATTTCTTTGCTCATACGGTCAGCGATA	cDNA synthesis and quantitative RT-PCR	

Table S2. RNAs used in this study

Name	Sequence (5′-3′)
miR173	UUCGCUUGCAGAGAGAAAUCAC
miR173*	GAUUCUCUGUGUAAGCGAAAG
amiR173-22asy*	GAUUCUCUGUGUAAGCGAACA
amiR173-22sy*	GAUUUCUCUGUGUAAGCGAACA
amiR173-21	UUCGCUUGCAGAGAGAAAUCA
amiR173-21sy*	AUUUCUCUGUGUAAGCGAACA
amiR173-21asy*	AUUCUCUGUGUAAGCGAACA
miR171	UGAUUGAGCCGCGCCAAUAUC
miR171*	UAUUGGCCUGGUUCACUCAGA
gf698-20 guide strand	UAGUUCAUCCAUGCCAUGUG
gf698-20 passenger strand	CAUGGCAUGGAUGAACUAUA
gf698-21 guide strand	UAGUUCAUCCAUGCCAUGUGU
gf698-21 passenger strand	ACAUGGCAUGGAUGAACUAUA
gf698-22 guide strand	UAGUUCAUCCAUGCCAUGUGUA
gf698-22 passenger strand	CACAUGGCAUGGAUGAACUAUA

All RNAs have 2′ hydroxymethyl groups in the 3′-terminal nucleotide.