Supplementary Figure legends

Supplementary Figure S1. Structures of the luciferase expression vectors used in this study. (**A**) Structure of the *firefly* luciferase expression plasmid vector, pTK4.12. (**B**) Structure of the *Renilla* luciferase reporter without insertion; pGL4.74. (**C**) Structure of the *Renilla* luciferase reporter pGL4.74-miR21T harbouring a 22bp insert which is fully complementary to the mature miR-21 just downstream of the *Renilla* luciferase gene. (**D**) Structure of the *Renilla* luciferase reporter pGL4.74-miR200cT containing a 23bp insert, which is fully complementary to mature miR-200c, just downstream of the *Renilla* luciferase gene. (**E**) Structure of the *Renilla* luciferase reporter pGL4.74-miR16T with a 22bp insert just downstream of the *Renilla* luciferase gene which is fully complementary to mature miR-16. (**F**) Structure of the *Renilla* luciferase reporter pGL4.74-miR106bT containing a 21bp insert just downstream of the *Renilla* luciferase gene which is fully complementary to the mature miR-106b.

Supplementary Figure S2. Inhibitory effects of 2'-O-methylated RNA and PNA AMOs. 2'-O-methylated RNA oligonucleotide-based antisense or PNA oligonucleotide-based antisense molecules were transfected into HCT-116 cells together with the *Renilla* luciferase miR-21 reporter (miR-21-RL) or the untargeted control

Renilla luciferase reporter (UT-RL), as well as the *Firefly* luciferase reporter (FL) as a transfection control. After performing a dual luciferase assay, the ratios of miR-21-RL/FL to UT-RL/FL expression were determined and are represented by the mean ± s.d (n = 3). These expression levels were normalized to the ratio of miR-21-RL/FL to UT-RL/FL in 2'-OMe-NC transfected HCT-116 cells and are represented by the mean ± s.d (n = 3).

Supplementary Figure S3. Sequence and structure of S-TuD for miR-21, miR-200c, miR-16 and miR-106b. MBS is the miRNA binding site, which is fully or partially complementary to the target miRNA (left). G-U pairs are indicated by a dot. A 4 nt insertion and 1 nt mismatch in the MBS regions of S-TuD molecules are represented by red and blue characters, respectively.

Supplementary Figure S4. Effects of the Stem I length on miRNA inhibiting activity of S-TuD molecules. (**A**) Structures and sequences of S-TuD-miR21, S-TuD-miR21-14bp and S-TuD-miR21-10bp. A 1 nt mismatch in the MBS regions within S-TuD is represented by blue characters. (**B**) The miR-21 inhibiting activity of the three types of S-TuD-miR21s was assessed by luciferase activity. HCT-116 cells

were co-transfected with the *Renilla* luciferase miR-21 reporter (miR-21-RL; open bars) or the untargeted control *Renilla* luciferase reporter (UT-RL; black bars) as well as the *Firefly* luciferase reporter (FL) as a transfection control. After performing a dual luciferase assay, the expression levels were normalized to the ratio of the activity of miR-21-RL to that of FL in 1 nM S-TuD-NC transfected HCT-116 cells and are represented by the mean ± s.d (n = 3). (**C**) Normalized UV melting curves for 1.5 μM of S-TuDs, S-TuD-miR21-10mut, S-TuD-miR21-10mut-stemI-14bp and S-TuD-miR21-10mut-stemI-10bp, in buffers of 10 mM sodium phosphate (pH 7.0) containing 10 mM NaCl. Melting was assessed by UV absorbance at 260 nm and a melting rate of 0.5 °C/min. Vertical black bars indicate T_m points.

Supplementary Figure S5. The levels of free miR-106b detected in RNA from HCT-116 cells transfected with miRNA inhibitors at concentrations of 0.05 nM by quantitative real time RT-PCR. The miR-106b expression levels were normalized to those of HCT-116 cells transfected with S-TuD-NC and are represented by the mean ± s.d (n = 3). U6 snRNA was served as an endogenous control.

Supplementary Figure S6. (**A**) The expression levels of miR-200b/200c/429 family in HCT-116 cells. The expression levels of miRNAs were determined by miRNA-microarray (Agilent). (**B**) Sequence and structure (predicted by CentroidFold) of S-TuD-miR200c-pf and S-TuD-miR429-pf. (**C**) The expression levels of miR-15a/15b/16/195/424/497 family in HCT-116 cells. The expression levels of miRNAs were determined by miRNA-microarray (Agilent). (**D**) Sequence and structure (predicted by CentroidFold) of S-TuD-miR16-pf, S-TuD-miR195-pf and S-TuD-miR497-pf.

Supplementary Figure S7. The miRNA inhibitory effects and 5-FAM levels of 5-FAM labeled S-TuD and sorting of 5-FAM positive cells. (**A**) Comparison of the miRNA inhibitory effects of non-labeled and 5-FAM-labeled S-TuD. S-TuD-miR200c-pf, S-TuD-NC, 5-FAM-S-TuD-miR200c-pf or 5-FAM-S-TuD-NC were transiently transfected into HCT-116 cells together with the *Renilla* luciferase miR-200c reporter (miR-200c-RL; open bars) or the untargeted control *Renilla* luciferase reporter (UT-RL; black bars). In all cases, the *Firefly* luciferase reporter (FL) was co-transfected as a transfection control. After performing a dual luciferase assay, the expression levels were normalized to the ratio of the activity of miR-200c-RL to that of FL in 0.03 nM

S-TuD-NC transfected HCT-116 cells and are represented by the mean ± s.d (n = 3). Two days after the transfection FACS analysis of the 5-FAM levels in MOCK-treated HCT-116 cells (**B**), 10 nM 5-FAM-S-TuD-NC transfected HCT-116 cells (**C**) or 10 nM 5-FAM-S-TuD-miR200c-pf transfected HCT-116 cells (**D**) was performed. M1 markers represent the gates used for sorting.

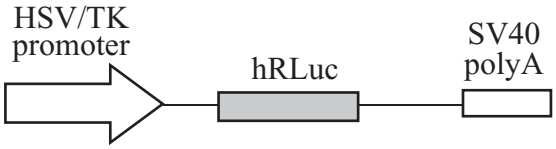
Supplementary Figure S8. TuD-miR200c-expression lentivirus vector. (**A**) Structure of a HIV-based self-inactivating TuD RNA expression vector, pLSP. The provirus structure of pLSP is shown. ΔU3, U3 sequence in which the major enhancer sequences were deleted; R, lentiviral R sequence; U5, lentiviral U5 sequence; ψ, lentiviral packaging signal. (**B**) A TuD RNA expression lentivirus vector was transduced into HCT-116 cells with an MOI of three followed by selection with puromycin. These cells were transfected with the *Renilla* luciferase miR-200c reporter (miR-200c-RL; open bars) or the untargeted control *Renilla* luciferase reporter (UT-RL; black bars) as well as the *Firefly* luciferase reporter (FL) as a transfection control at 13 days after transduction. A dual luciferase assay was performed at 15 days after transduction, after which the expression levels were normalized to the ratio

of the activity of miR-200c-RL to that of FL in TuD-NC vector-transduced HCT-116 cells. These values are represented by the mean ± s.d (n = 3).

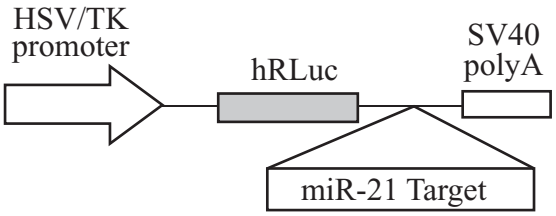
Supplementary Figure S1

A pTK4.12

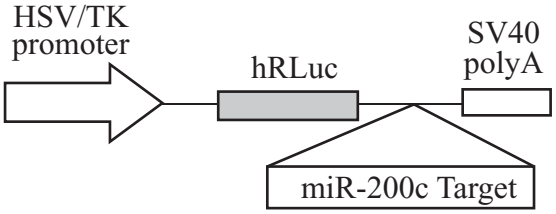
B pGL4.74



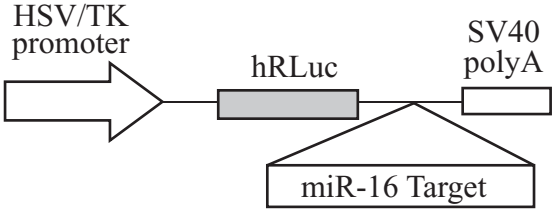
C pGL4.74-miR21T



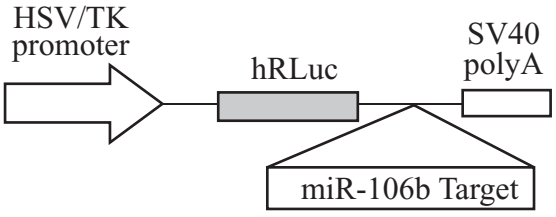
D pGL4.74-miR200cT



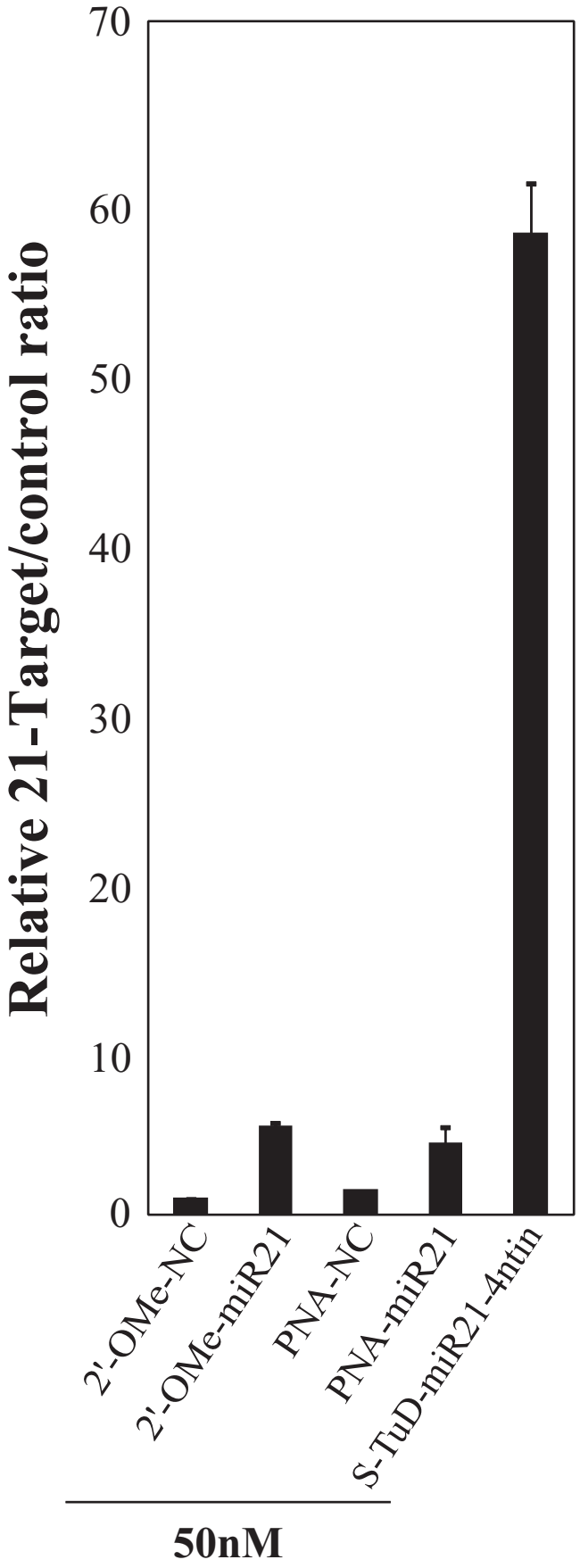
E pGL4.74-miR16T



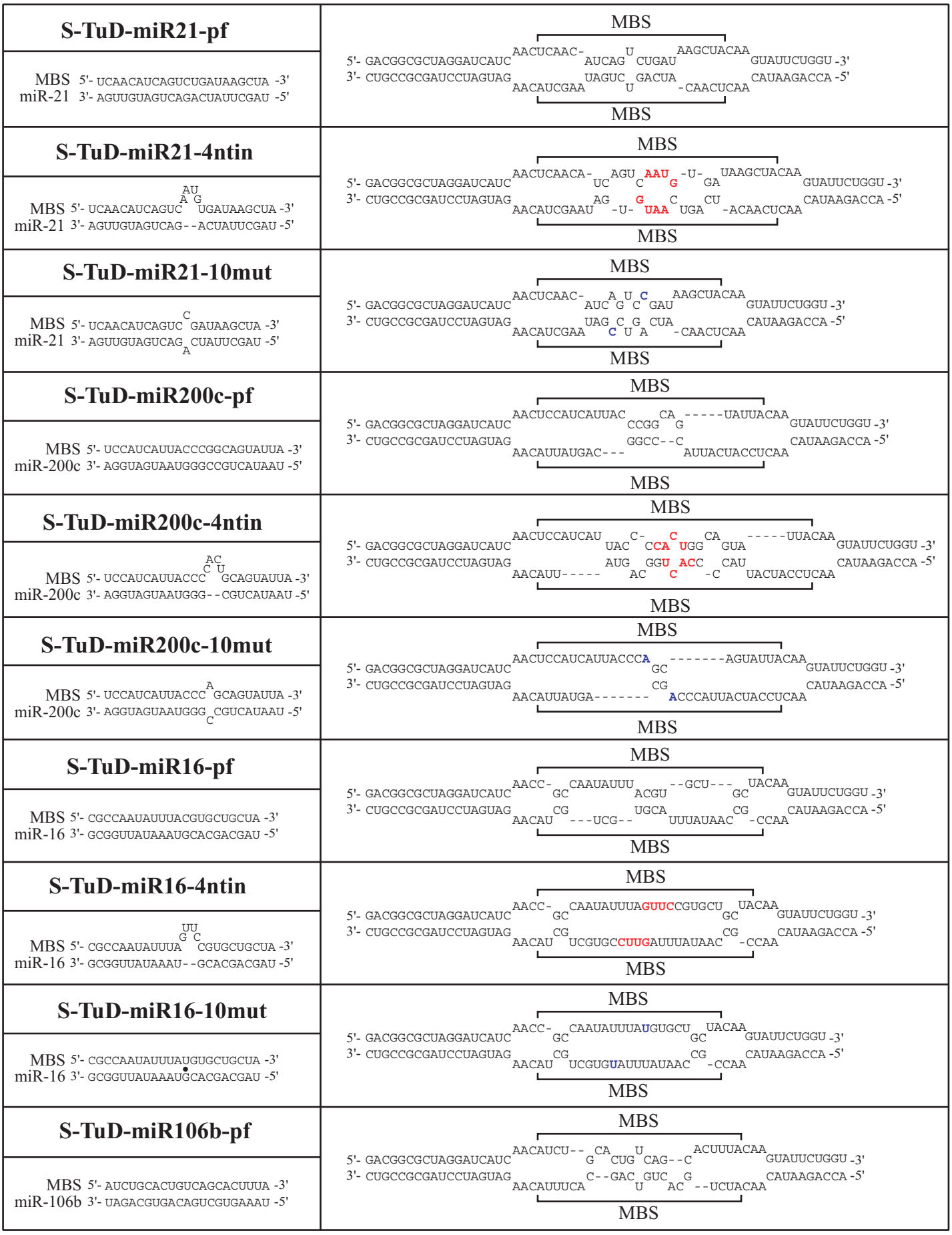
F pGL4.74-miR106bT



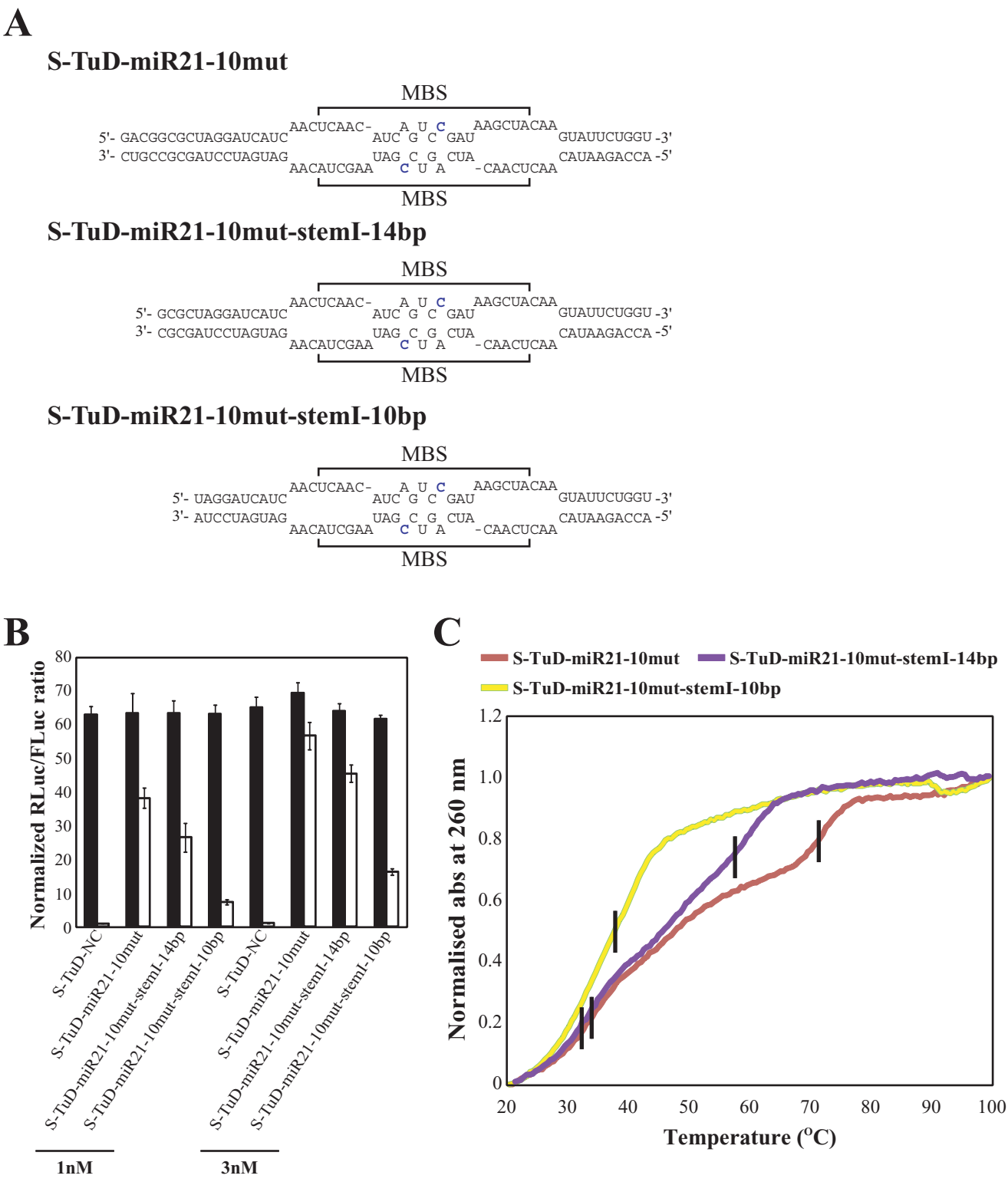
Supplementary Figure S2



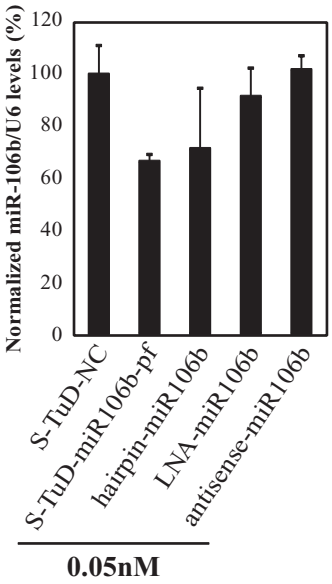
Supplementary Figure S3



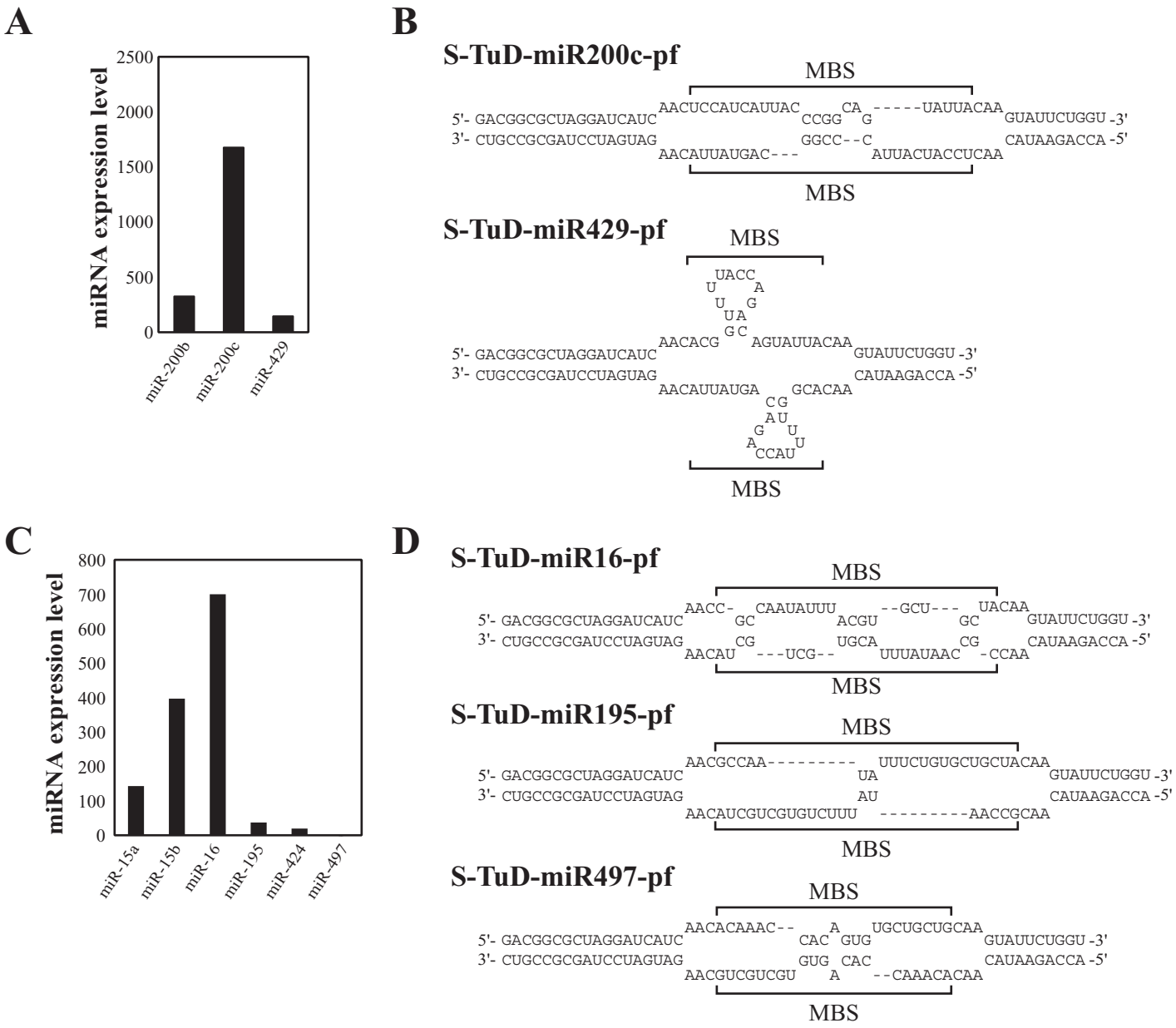
Supplementary Figure S4



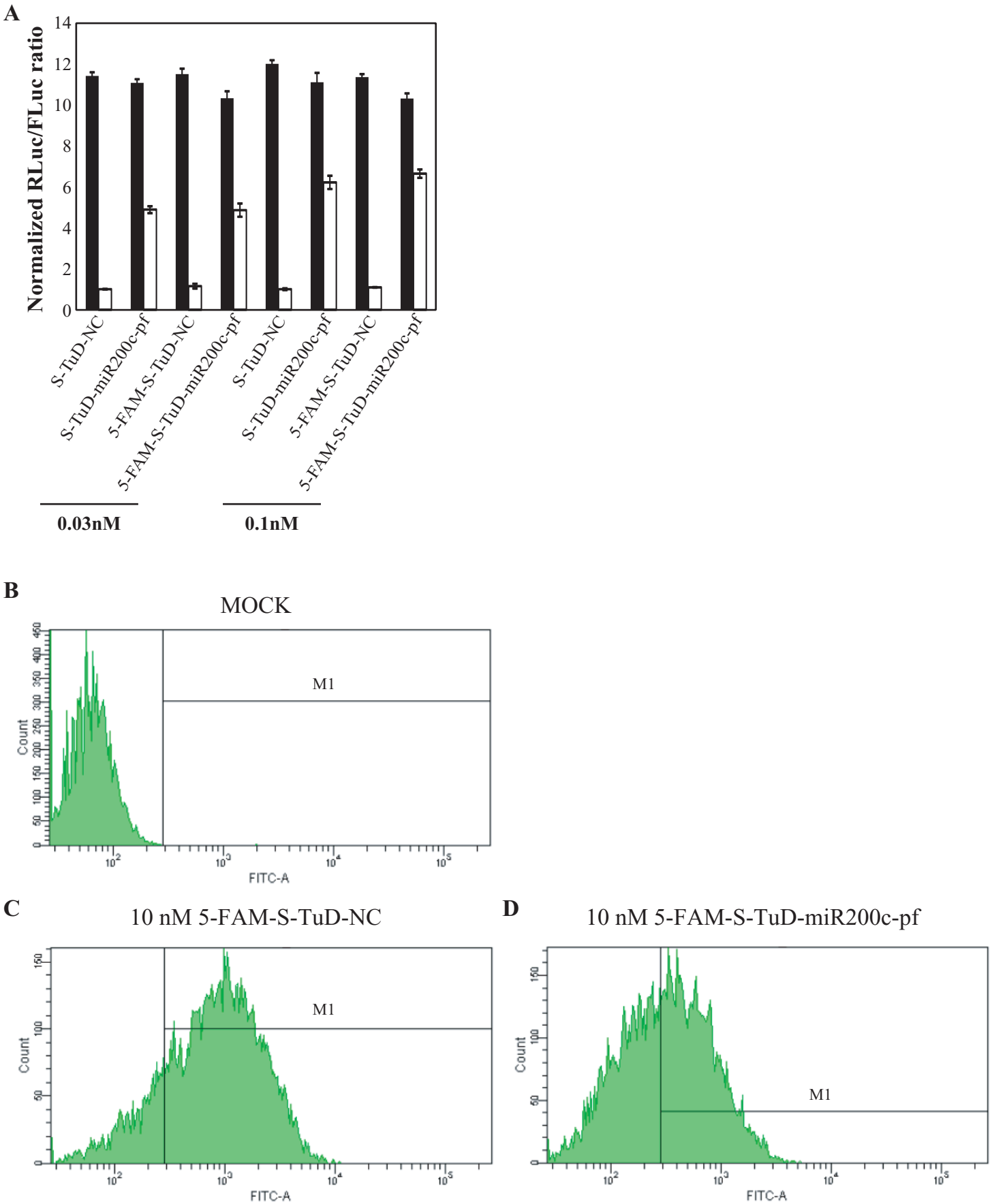
Supplementary Figure S5



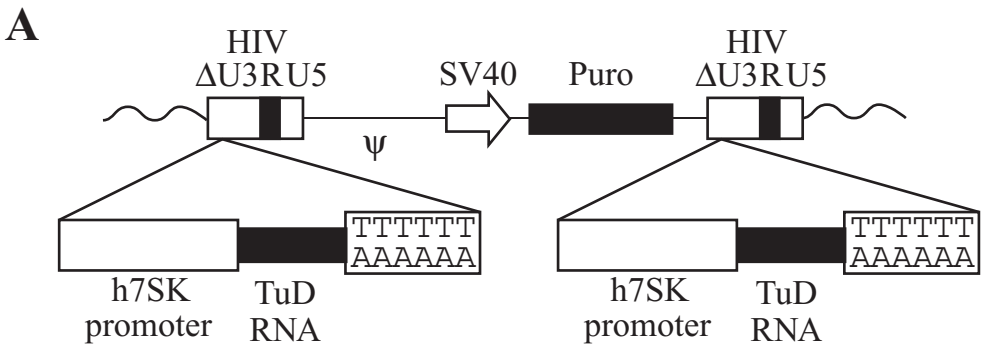
Supplementary Figure S6

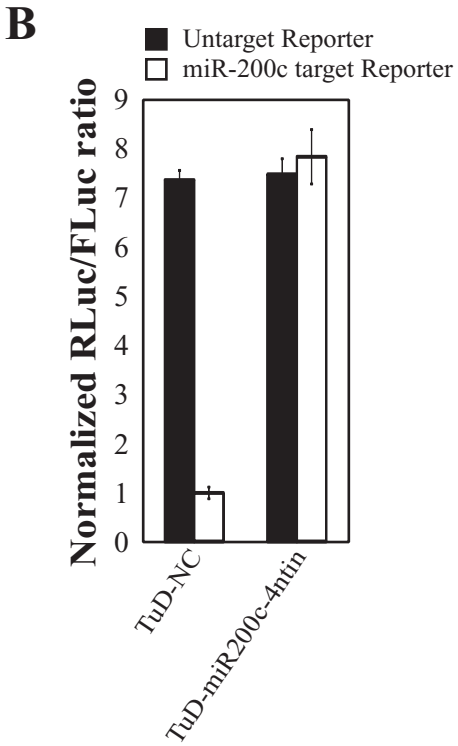


Supplementary Figure S7



Supplementary Figure S8





15 days after transduction

Supplementary Table S1. The 2'-O-methylated RNA oligo pairs for S-TuD preparation.

Primers		Sequence
S-TuD-miR21-pf	s^+	5'- GACGGCGCUAGGAUCAUCAACUCAACAUCAGUCUGAUAAGCUACAAGUAUUCUGGU -3'
S-TuD-miR21-pf	a^+	5'- ACCAGAAUACAACUCAACAUCAGUCUGAUAAGCUACAAGAUGAUCCUAGCGCCGUC -3'
S-TuD-miR21-4ntin	s	5'- GACGGCGCUAGGAUCAUCAACUCAACAUCAGUCAAUGUGAUAAGCUACAAGUAUUCUGGU -3'
S-TuD-miR21-4ntin	a	5'- ACCAGAAUACAACUCAACAUCAGUCAAUGUGAUAAGCUACAAGAUGAUCCUAGCGCCGUC -3'
S-TuD-miR21-10mut	s	5'- GACGGCGCUAGGAUCAUCAACUCAACAUCAGUCCGAUAAGCUACAAGUAUUCUGGU -3'
S-TuD-miR21-10mut	a	5'- ACCAGAAUACAACUCAACAUCAGUCCGAUAAGCUACAAGAUGAUCCUAGCGCCGUC -3'
S-TuD-miR21-10mut-stemI-10bp	s	5'- UAGGAUCAUCAACUCAACAUCAGUCCGAUAAGCUACAAGUAUUCUGGU -3'
S-TuD-miR21-10mut-stemI-10bp	a	5'- ACCAGAAUACAACUCAACAUCAGUCCGAUAAGCUACAAGAUGAUCCUA -3'
S-TuD-miR21-10mut-stemI-14bp	s	5'- GCGCUAGGAUCAUCAACUCAACAUCAGUCCGAUAAGCUACAAGUAUUCUGGU -3'
S-TuD-miR21-10mut-stemI-14bp	a	5'- ACCAGAAUACAACUCAACAUCAGUCCGAUAAGCUACAAGAUGAUCCUAGCGC -3'
S-TuD-miR200c-pf	s	5'- GACGGCGCUAGGAUCAUCAACUCCAUCAUUACCCGGCAGUAUUACAAGUAUUCUGGU -3'
S-TuD-miR200c-pf	a	5'- ACCAGAAUACAACUCCAUCAUUACCCGGCAGUAUUACAAGAUGAUCCUAGCGCCGUC -3'
S-TuD-miR200c-4ntin	s	5'- GACGGCGCUAGGAUCAUCAACUCCAUCAUUACCCCACUGGCAGUAUUACAAGUAUUCUGGU -3'
S-TuD-miR200c-4ntin	a	5'- ACCAGAAUACAACUCCAUCAUUACCCCACUGGCAGUAUUACAAGAUGAUCCUAGCGCCGUC -3'
S-TuD-miR200c-10mut	s	5'- GACGGCGCUAGGAUCAUCAACUCCAUCAUUACCCAGCAGUAUUACAAGUAUUCUGGU -3'
S-TuD-miR200c-10mut	a	5'- ACCAGAAUACAACUCCAUCAUUACCCAGCAGUAUUACAAGAUGAUCCUAGCGCCGUC -3'
S-TuD-miR16-pf	s	5'- GACGGCGCUAGGAUCAUCAACCGCCAAUAUUUACGUGCUGCUACAAGUAUUCUGGU -3'
S-TuD-miR16-pf	a	5'- ACCAGAAUACAACCGCCAAUAUUUACGUGCUGCUACAAGAUGAUCCUAGCGCCGUC -3'
S-TuD-miR16-4ntin	s	5'- GACGGCGCUAGGAUCAUCAACCGCCAAUAUUUAGUUCCGUGCUGCUACAAGUAUUCUGGU -3'
S-TuD-miR16-4ntin	a	5'- ACCAGAAUACAACCGCCAAUAUUUAGUUCCGUGCUGCUACAAGAUGAUCCUAGCGCCGUC -3'
S-TuD-miR16-10mut	s	5'- GACGGCGCUAGGAUCAUCAACCGCCAAUAUUUAUGUGCUGCUACAAGUAUUCUGGU -3'
S-TuD-miR16-10mut	a	5'- ACCAGAAUACAACCGCCAAUAUUUAUGUGCUGCUACAAGAUGAUCCUAGCGCCGUC -3'
S-TuD-miR106b-pf	s	5'- GACGGCGCUAGGAUCAUCAACAUCUGCACUGUCAGCACUUUACAAGUAUUCUGGU -3'
S-TuD-miR106b-pf	a	5'- ACCAGAAUACAACAUCUGCACUGUCAGCACUUUACAAGAUGAUCCUAGCGCCGUC -3'
S-TuD-miR195-pf	s	5'- GACGGCGCUAGGAUCAUCAACGCCAAUAUUUCUGUGCUGCUACAAGUAUUCUGGU -3'
S-TuD-miR195-pf	a	5'- ACCAGAAUACAACGCCAAUAUUUCUGUGCUGCUACAAGAUGAUCCUAGCGCCGUC -3'
S-TuD-miR497-pf	s	5'- GACGGCGCUAGGAUCAUCAACACAAACCACAGUGUGCUGCUGCAAGUAUUCUGGU -3'
S-TuD-miR497-pf	a	5'- ACCAGAAUACAACACAAACCACAGUGUGCUGCUGCAAGAUGAUCCUAGCGCCGUC -3'
S-TuD-miR429-pf	s	5'- GACGGCGCUAGGAUCAUCAACACGGUUUUACCAGACAGUAUUACAAGUAUUCUGGU -3'
S-TuD-miR429-pf	a	5'- ACCAGAAUACAACACGGUUUUACCAGACAGUAUUACAAGAUGAUCCUAGCGCCGUC -3'
S-TuD-NC	s	5'- GACGGCGCUAGGAUCAUCAACUAUCGCGAGUAUCGACGUCGAGGCCCAAGUAUUCUGGU -3'
S-TuD-NC	a	5'- ACCAGAAUACAACUAUCGCGAGUAUCGACGUCGAGGCCCAAGAUGAUCCUAGCGCCGUC -3'

s^+; sense strand

a^+; antisense strand

Supplementary Table S2. Information of purchased miRNA inhibitors.

miRNA inhibitors	Product name	Product code	Supplier	Characteristics
hairpin-miR21 hairpin-miR106b	miRIDIAN microRNA Hairpin Inhibitors	IH-300492-05 IH-300649-07	Thermo Scientific	The entire oligoribonucleotides are 2'-O-methylated and miRIDIAN microRNA Hairpin Inhibitors might contain some other modifications. In miRIDIAN microRNA Hairpin Inhibitor, a single antisense strand of the miRNA are flanked by oligonucleotides at the 5' and 3' ends, both of which are designed to form hairpin structure with a 8 base-pair stem.
LNA-miR200c LNA-miR106b	miRCURY LNA™ microRNA Inhibitor	410126-00 426648-00	Exiqon	The sequence of miRCURY LNA™ microRNA inhibitors are completely complementary to the corresponding miRNA and spiked by LNA.
antisense-miR106b	Anti-miR™ miRNA Inhibitor	AM10067	Life Technologies	Anti-miR™ miRNA Inhibitor is 2'-0-methylated oligoribonucleotides that is fully complementary to the corresponding miRNA.

Supplementary Table S3. Sequences of 2'-O-methyl antisense oligos and LNA/DNA antisense oligos.

Primers	Sequence
2'OMe-miR21*	5’- GUCAACAUCAGUCUGAUAAGCUA -3’
2'OMe-NC*	5’- AAGGCAAGCUGACCCUGAAGU -3’
LNA-NC**	5’- CATTAATGTCGGACAACTCAAT -3’

* : All of bases are 2'-O-methylated RNA.

** : LNAs are indicated by underline and other bases are DNA.

Supplementary Table S4. Primer pairs used for luciferase reporter vectors.

Primers		Sequence
pGL4.74-T21	s^+	5’- CTAGACCGGAATTCTCAACATCAGTCTGATAAGCTACTCGAGCGGAGGCCGG-3’
pGL4.74-T21	a^+	5’- CCTCCGCTCGAGTAGCTTATCAGACTGATGTTGAGAATTCCGGT-3’
pGL4.74-T200c	s	5’- CTAGACCGGAATTCTCCATCATTACCCGGCAGTATTACTCGAGCGGAGGCCGG-3’
pGL4.74-T200c	a	5’- CCTCCGCTCGAGTAATACTGCCGGGTAATGATGGAGAATTCCGGT-3’
pGL4.74-T16	s	5’- CTAGACCGGAATTCCGCCAATATTTACGTGCTGCTACTCGAGCGGAGGCCGG-3’
pGL4.74-T16	a	5’- CCTCCGCTCGAGTAGCAGCACGTAAATATTGGCGGAATTCCGGT-3’
pGL4.74-T106b	s	5’- CTAGACCGGAATTCATCTGCACTGTCAGCACTTTACTCGAGCGGAGGCCGG-3’
pGL4.74-T106b	a	5’- CCTCCGCTCGAGTAAAGTGCTGACAGTGCAGATGAATTCCGGT-3’

s^+; sense strand

a^+; antisense strand

Supplementary Table S5. Primer pairs used for the construction of h7SK promoter TuD RNA shuttle vectors.

Primers		Sequence
h7SK promoter PCR	F^+	5'- GGATCCTGCAGTATTTAGCATGCCCCA -3'
h7SK promoter PCR	R^+	5'- GAATTCAAAAAAGGATGTGAGGGCGTCATCGAGACGGTACCGTCTCCGATGACGCCCTCACATCCGAGGTACCCAGGCGGCGCACAAGC -3'
h7SK TuD RNA shuttle	s^+	5'- CTCGGATGTGAGGGCGTCATCGGAGACGACACCATCCACAGCCAGCGTCTCGATGACGCCCTCACATCCTTTTTTGAATTCA -3'
h7SK TuD RNA shuttle	a^+	5'- AGCTTGAATTCAAAAAAGGATGTGAGGGCGTCATCGAGACGCTGGCTGTGGATGGTGTCGTCTCCGATGACGCCCTCACATCCGAGGTAC -3'

F^+; Forward primer
R^+; Reverse primer
s^+; sense strand
a^+; antisense strand

Supplementary Table S6. Primer pairs used for TuD RNA expression vectors.

Primers		Sequence
TuD RNA-miR200c	s^+	5'- CATCAACTCCATCATTACCCATTAGGCAGTATTACAAGTATTCTGGTCACAGAATACAACTCCATCATTACCCATTAGGCAGTATTACAAG -3'
TuD RNA-miR200c	a^+	5'- TCATCTTGTAATACTGCCTAATGGGTAATGATGGAGTTGTATTCTGTGACCAGAATACTTGTAATACTGCCTAATGGGTAATGATGGAGTT -3'
TuD RNA-NC	s	5'- CATCAACTATCGCGAGTATCGACGTCGAGGCCCAAGTATTCTGGTCACAGAATACAACTATCGCGAGTATCGACGTCGAGGCCCAAG -3'
TuD RNA-NC	a	5'- TCATCTTGGGCCTCGACGTCGATACTCGCGATAGTTGTATTCTGTGACCAGAATACTTGGGCCTCGACGTCGATACTCGCGATAGTT -3'

s^+; sense strand

a^+; antisense strand

Supplementary Table S7. Primer pairs used for the detection of interferon response.

Primers		Sequence
OAS1	F^+	5’- AGGTGGTAAAGGGTGGCTCC -3’
OAS1	R^+	5’- ACAACCAGGTCAGCGTCAGAT -3’
OAS2	F	5’- ATCTGGATTTTGTGCCTAGTTC -3’
OAS2	R	5’- AACCACATGCTTGGTCTTTC -3’
MX1	F	5’- TCAGATGCACATGAGCTGG -3’
MX1	R	5’- GCACTCATGCTCCTAAAACAC -3’
IRF9	F	5’- GACTTGGTCAGGTACTTTCAGG -3’
IRF9	R	5’- TCTACACCAGGGACAGAATG -3’
IFITM1	F	5’- CCAAAGCCAGAAGATGCAC -3’
IFITM1	R	5’- GCTATGAAGCCCAGACAGC -3’
GAPDH	F	5’- ACTTTGTCAAGCTCATTTCCTG -3’
GAPDH	R	5’- CTCTCTTCCTCTTGTGCTCTTG -3’

F^+; Forward primer

R^+; Reverse primer