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Supplemental Information

Nucleotide Modification Alters MicroRNA-Dependent Silencing of MicroRNA Switches

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Supplementary Material

Nucleotide modification alters microRNA-dependent silencing of microRNA switches

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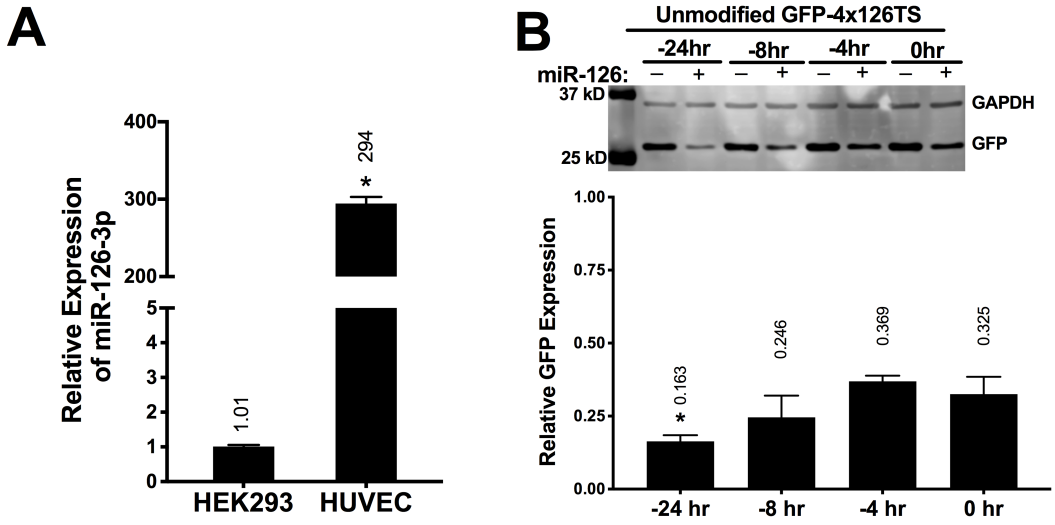
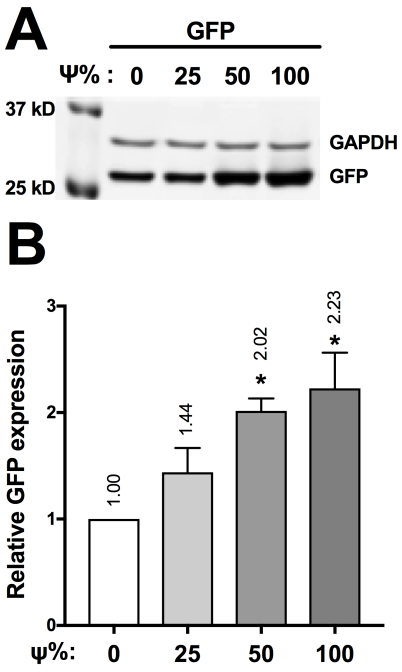
Supplementary Figure S1



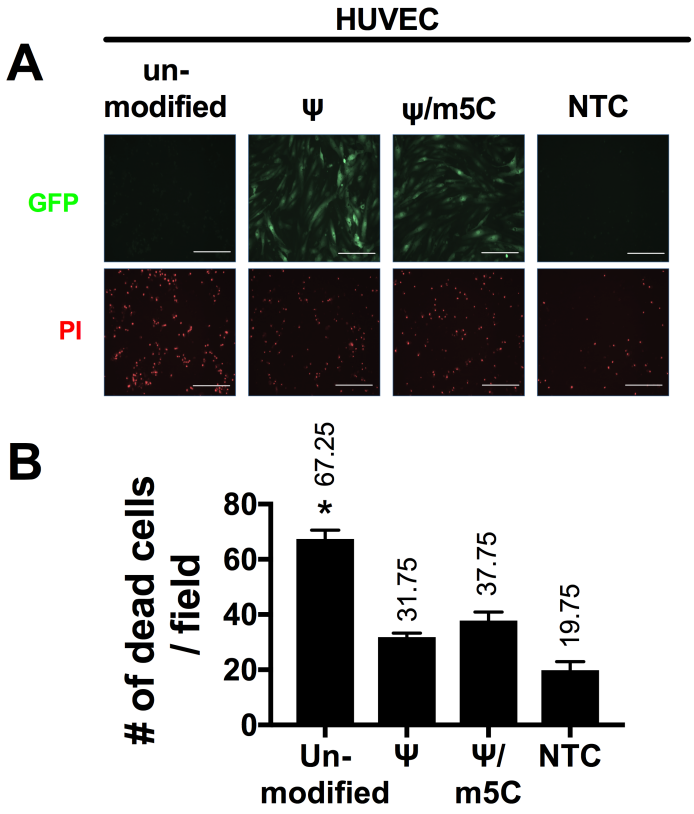
Supplementary Figure S1: Expression of miR-126-3p and optimization of miRNA mimic delivery in HEK293 cells. (A) *Real-time PCR analysis of the indicated miRNA expression in HEK293 and HUVEC.* ***(B)*** *Representative immunoblot and densitometric quantification of HEK293 cells transfected with miR-126-3p mimics or vehicle control at the indicated time point before transfection with unmodified GFP-4x126TS miRNA switches. GFP and GAPDH expression were measured after 24 hours. Data represent the mean ± SEM of three independent experiments, normalized against U18 (A) or GAPDH (B) and relative to HEK293 cells (A) or vehicle control (B). *p < 0.05 versus HEK293 (A) or 0 hour time point (B). Numbers above bars indicate the mean values.*

Supplementary Figure S2



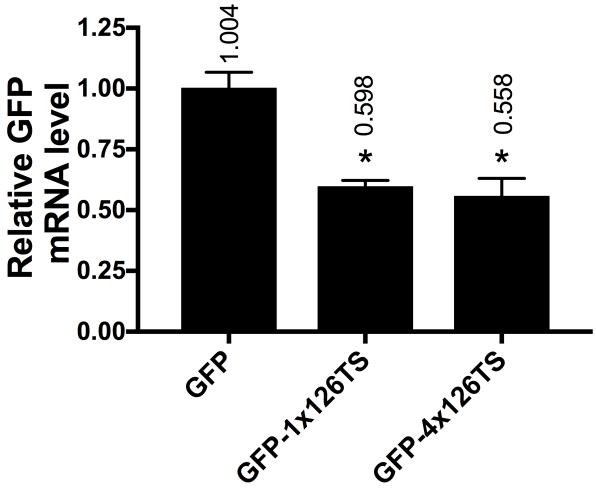
Supplementary Figure S2: Ψ-substitution increases GFP expression from IVT mRNA. *Representative immunoblot and densitometric quantification of HEK293 cells transfected with with GFP-encoding IVT mRNA with the indicated percentage of Ψ-substitution. GFP and GAPDH expression were measured after 24 hours. Data represent the mean ± SEM of three independent experiments, normalized against GAPDH and relative to unmodified GFP-encoding IVT mRNA. *p < 0.05 versus unmodified GFP-encoding IVT mRNA. Numbers above bars indicate the mean values.*

Supplementary Figure S3



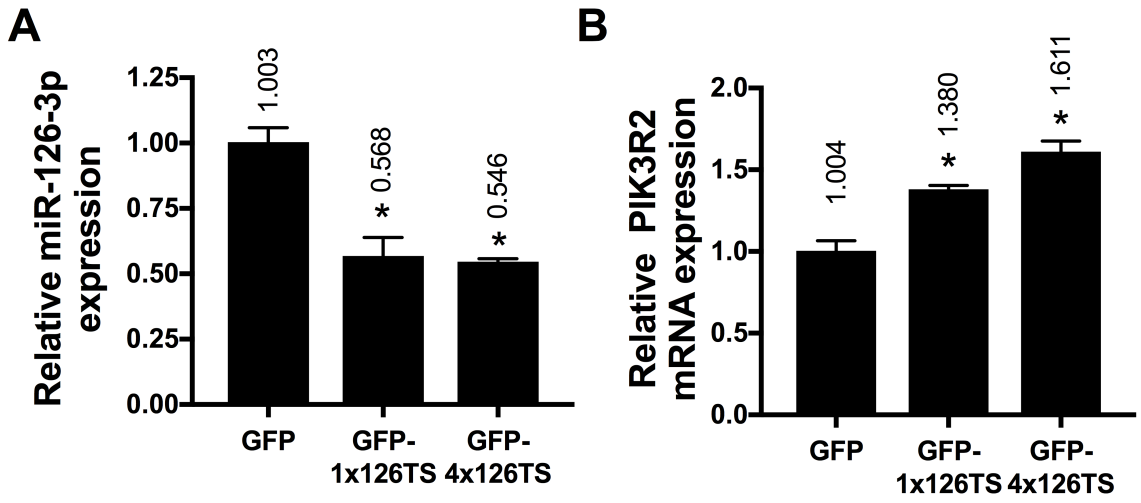
Supplementary Figure S3: Nucleotide modification decreases cytotoxicity and increases GFP expression in HUVEC. (A-B) *Representative fluorescence microscopy images (A) and number of dead cells per field (B) of HUVEC 24 hours after transfection with GFP-encoding IVT mRNA with the indicated modified nucleotides (Scale bars: 200 µm; 20x magnification). Media was changed four hours after mRNA transfection. Fluorescence Imaging and quantification of dead cells was performed by adding propidium iodide after 24 hours without removal of the media. Data represent the mean ± SEM from four non-overlapping fields. Numbers above bars indicate the mean values.*

Supplementary Figure S4

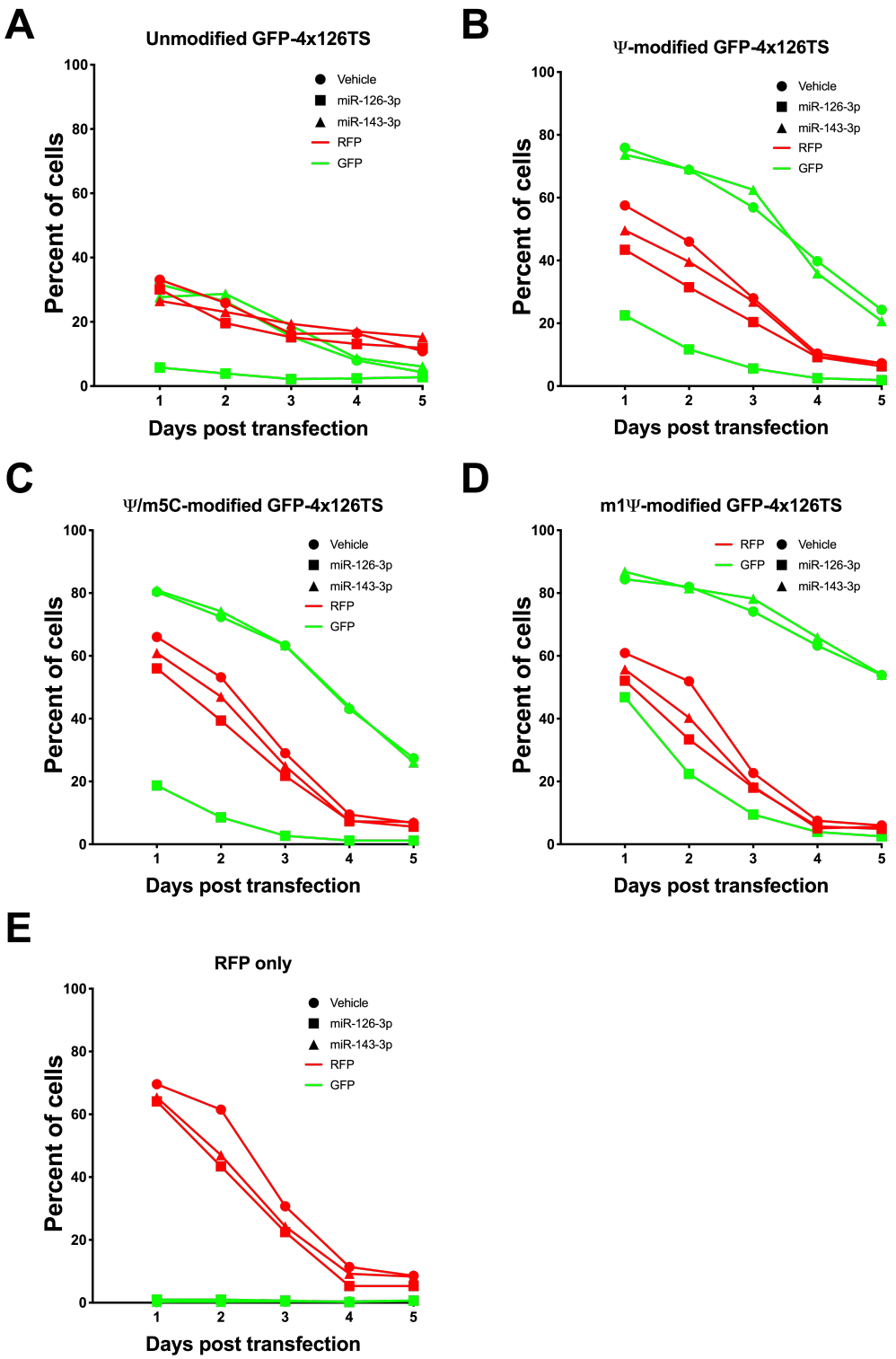


Supplementary Figure S4: Additional miRNA target sites do not increase cleavage of miRNA switches. *Real Time-PCR analysis of GFP normalized to GAPDH mRNA from HUVEC transfected with the indicated 100% Ψ-modified GFP miRNA switch. Data represent the mean ± SEM of one representative experiment performed in triplicate. *p < 0.05 versus GFP mRNA transfected cells. Numbers above bars indicate the mean values.*

Supplementary Figure S5



Supplementary Figure S5: miRNA sponging by miRNA switches in HUVEC. (A-B) *Real Time-PCR analysis of miR-126-3p normalized to U18 snRNA (A) and PIK3R2 normalized to GAPDH mRNA (B) from HUVEC transfected with the indicated 100% Ψ-modified GFP miRNA switch. Data represent the mean ± SEM of one representative experiment performed in triplicate. *p < 0.05 versus GFP mRNA transfected cells. Numbers above bars indicate the mean values.*



Supplementary Figure 6: Long-term kinetics of miRNA switch silencing. (A-E) HEK293 cells were transfected with the indicated miRNA mimic or vehicle control 24 hours prior to transfection with GFP-4x126TS miRNA switches with the indicated nucleotide modifications and control Ψ/m5C-modified RFP mRNA. GFP and RFP expression was analyzed by flow cytometry of 5000 cells every 24 hours after transfection.

Supplementary Table S1. **List of oligonucleotides used to generate plasmids and templates for *in vitro* transcription**

Construction of Plasmid Templates			
1	EcoRI-BamHI-PacI-SphI-*βglobin 3'UTR*	Forward	5'-ATTA-gaattc-ggatcc-ttaattaa-gcatgc-*GCTCGC TTTCTTGCTGTCCAATTTCTA*
2	EcoRI-*βglobin 3'UTR*	Reverse	5'-ATTA-gaattc-*GCAATGAAAATAAATGTTTTTT ATTAGGCAGAATCCAGAT*
3	BamHI-1x126TS-BamHI	Forward	5'-gatcc-CGCATTATTACTCACGGTACGA-g
4	BamHI-1x126TS-BamHI	Reverse	5'-gatcc-TCGTACCGTGAGTAATAATGCG-g
5	BamHI-2x126TS-BamHI	Forward	5'-gatcc-CGCATTATTACTCACGGTACGAGA TCGATCCGCATTATTACTCACGGTACGA-g
6	BamHI-2x126TS-BamHI	Reverse	5'-gatcc-TCGTACCGTGAGTAATAATGCGGA TCGATCTCGTACCGTGAGTAATAATGCG-g
7	PacI-2x126TS-PacI	Forward	5'-taa-CGCATTATTACTCACGGTACGAGATC GATCCGCATTATTACTCACGGTACGA-ttaat
8	PacI-2x126TS-PacI	Reverse	5'-taa-TCGTACCGTGAGTAATAATGCGGATC GATCTCGTACCGTGAGTAATAATGCG-ttaat
9	BamHI-2x126seed-BamHI	Forward	5'-gatcc-TTTAACTAAATGTAAGGTACGAatt taTTTAACTAAATGTAAGGTACGAA-g
10	BamHI-2x126seed-BamHI	Reverse	5'-gatcc-TTCGTACCTTACATTTAGTTAAAta aatTCGTACCTTACATTTAGTTAAA-g
11	BamHI-2x21TS-BamHI	Forward	5'-gatcc-TCAACATCAGTCTGATAAGCTAgatc gatcTCAACATCAGTCTGATAAGCTA-g
12	BamHI-2x21TS-BamHI	Reverse	5'-gatcc-TAGCTTATCAGACTGATGTTGAgatc gatcTAGCTTATCAGACTGATGTTGA-g
13	PacI-2x21TS-PacI	Forward	5'-taa-TCAACATCAGTCTGATAAGCTAgatcgat cTCAACATCAGTCTGATAAGCTA-ttaat
14	PacI-2x21TS-PacI	Reverse	5'-taa-TAGCTTATCAGACTGATGTTGAgatcgat cTAGCTTATCAGACTGATGTTGA-ttaat
15	BamHI-2x145TS-BamHI	Forward	5-gatcc-AGGGATTCCTGGGAAAACTGGACgat cgatcAGGGATTCCTGGGAAAACTGGAC-g
16	BamHI-2x145TS-BamHI	Reverse	5'-gatcc-GTCCAGTTTTCCCAGGAATCCCTgat cgatcGTCCAGTTTTCCCAGGAATCCCT-g
17	PacI-2x145TS-PacI	Forward	5'-taa-AGGGATTCCTGGGAAAACTGGACgatc gatcAGGGATTCCTGGGAAAACTGGAC-ttaat
18	PacI-2x145TS-PacI	Reverse	5'-taa-GTCCAGTTTTCCCAGGAATCCCTgatcg atcGTCCAGTTTTCCCAGGAATCCCT-ttaat
19	BamHI-2x122TS-BamHI	Forward	5-gatcc-CAAACACCATTGTCACACTCCAgatcg atcCAAACACCATTGTCACACTCCA-g
20	BamHI-2x122TS-BamHI	Reverse	5'-gatcc-TGGAGTGTGACAATGGTGTTTGgatc gatcTGGAGTGTGACAATGGTGTTTG-g
21	PacI-2x122TS-PacI	Forward	5'-taa-CAAACACCATTGTCACACTCCAgatcgat cCAAACACCATTGTCACACTCCA-ttaat
22	PacI-2x122TS-PacI	Reverse	5'-taa-TGGAGTGTGACAATGGTGTTTGgatcgat cTGGAGTGTGACAATGGTGTTTG-ttaat
Templates for in vitro transcription			
23	**T7**-*βglobin 5' UTR*-GFP	Forward	5'-CTTATGTCAA**TAATACGACTCACTATA GGG**-*ACATTTGCTTCTGACACAACTGTGTTCA CTAGCAACCTCAAACAGACCACC*-ATGGTGA GCAAGGGCGAGGAGCTGTT
24	*βglobin 3'UTR*	Reverse	5'-*GCAATGAAAATAAATGTTTTTTATTAGGCAG AATCCAGAT*

25	**T7**-*βglobin 5'UTR*-1x126TS-GFP	Forward	5'-CTTATGTCAA**TAATACGACTCACTATA GGG-***ACATTTGCTTCTGACACAACTGTGTTCA CTAGCAACCTCAAACAGACCACC*-CGCATTAT TACTCACGGTACGA-ctagcgctaccggtcgccacc ATGGTGAGCAAGGGCGAGGAGCTGTT

Supplementary Table S2. Summary of miRNA switch silencing in HEK293 cells.

	Unmodified	Ψ	Ψ/m5C	m1Ψ
GFP-1x126TS	0.235	0.364	0.184	N/A
GFP-2x126TS	0.264	0.35	0.18	N/A
GFP-3x126TS	0.077	0.236	0.135	N/A
GFP-4x126TS	0.068	0.123	0.145	0.318
GFP-4x21TS	0.105	0.415	0.256	0.194
GFP-4x145TS	0.11	0.438	0.326	0.329
GFP-4x122TS	0.095	0.116	0.202	0.21
GFP-2x126seed	0.206	0.449	0.416	N/A
5'UTR-1x126TS-GFP	0.011	0.09	0.109	0.057

Supplementary Table S2: Summary of miRNA switch silencing in HEK293 cells. The mean relative GFP expression from the indicated miRNA switches in the presence of their cognate miRNA mimics.