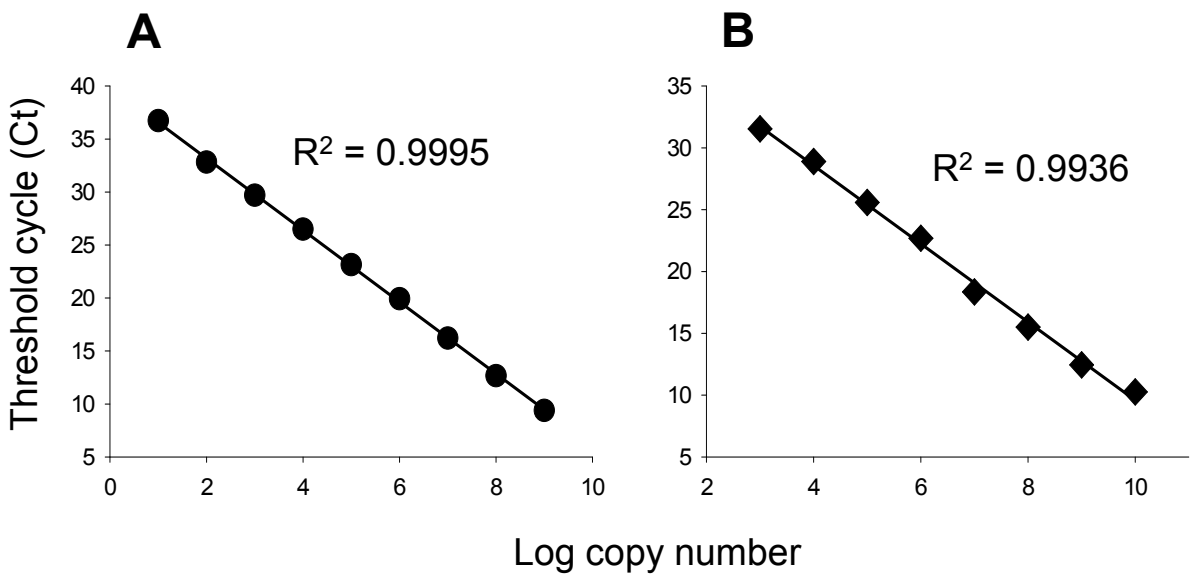
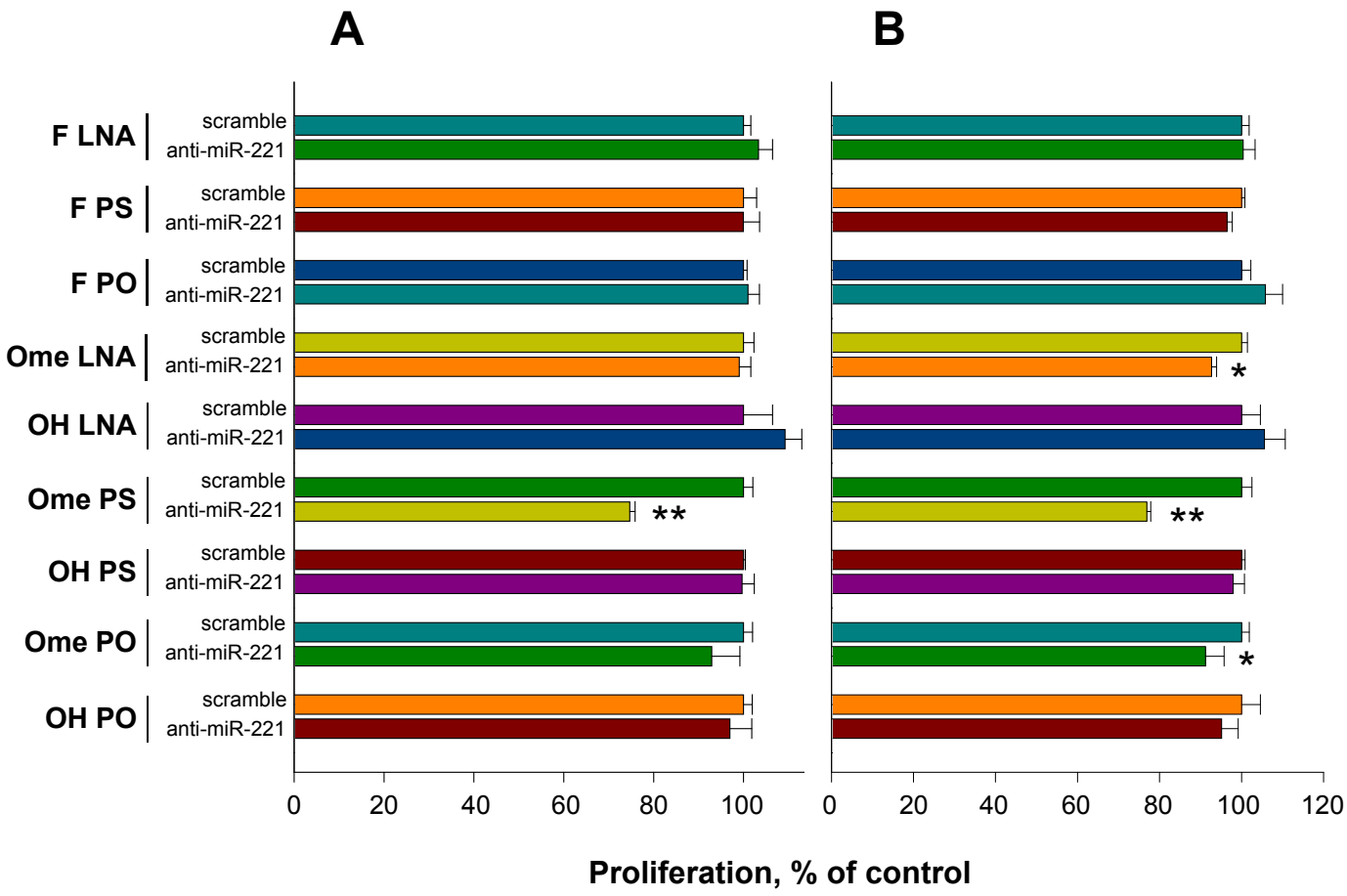
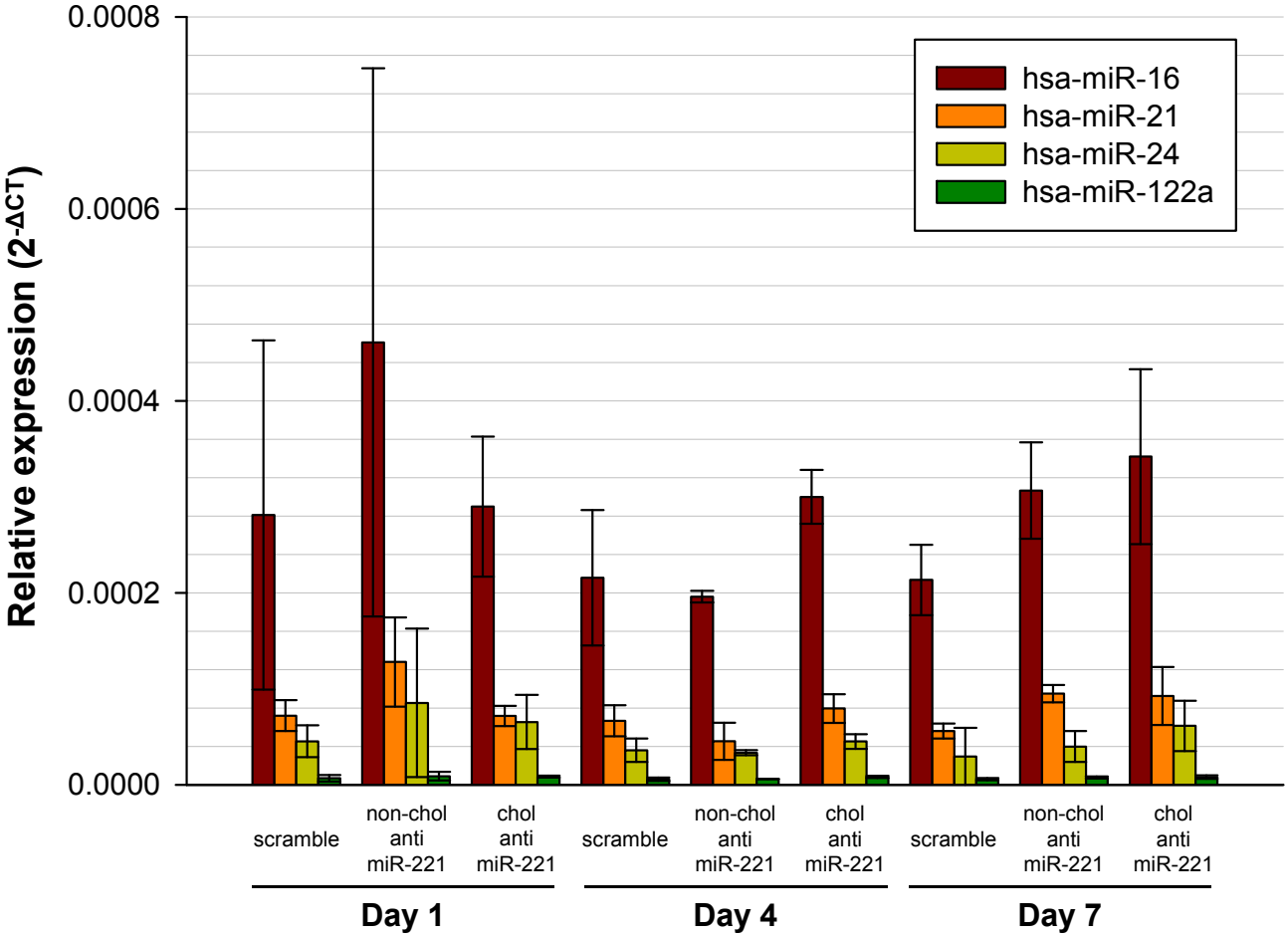


Supplemental Figure S1. The anti-miR-221 was converted to cDNA by priming with an anti-miR-221 specific RT (reverse transcription) adapter primer containing universal sequences (5' CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAGCTACAT 3'). qPCR was then performed using forward primer (5' ACACTCCAGCTGGGGAAACCCAGCAGACAA 3'), reverse primer (5' CTCAACTGGTGTCGTGGAGT 3') and TaqMan® MGB Probe (6FAMTTCAGTTGAGAGCTACATMGBNFQ).

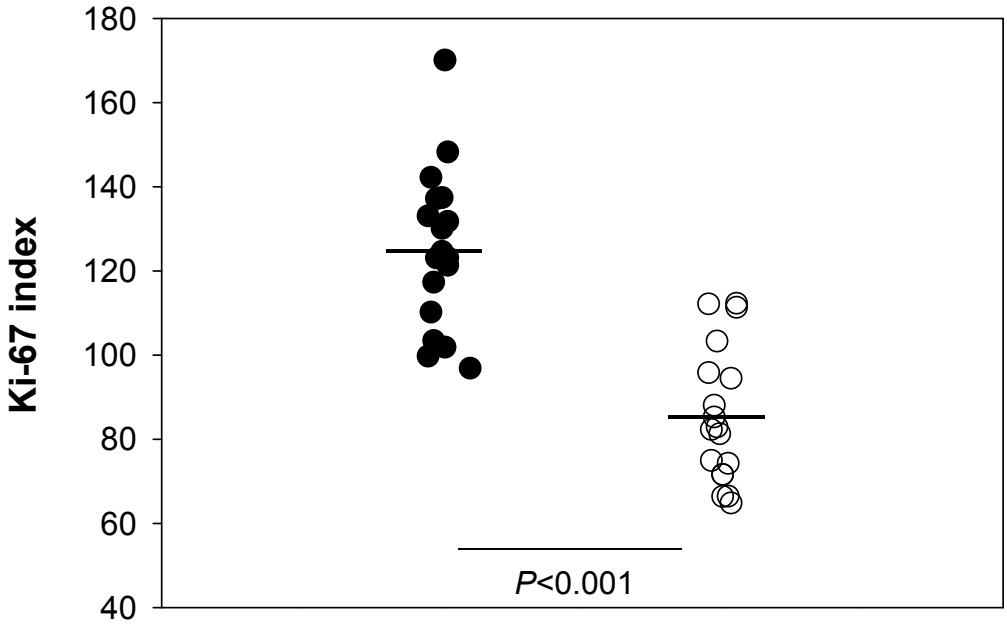


Supplemental Figure S2. *In vitro* activity of chemically modified anti-miR-221 oligos. The anti-proliferative activity of antisense oligos containing nine different chemical modifications was determined in PLC/PRF/5 cells. Cells were lipofectamine transfected with 25 nM of the anti-miR-221 or scrambled control oligo of the identical chemical composition for 48 h (A) and 72 h (B). Cell viability was assessed using a WST-1 assay and the survival was compared to the scrambled control oligo treated cells. The chemical composition of each antisense oligo is shown in Supplemental Table 1. Data represent the mean of duplicate experiments. * $P < 0.05$, ** $P < 0.01$.

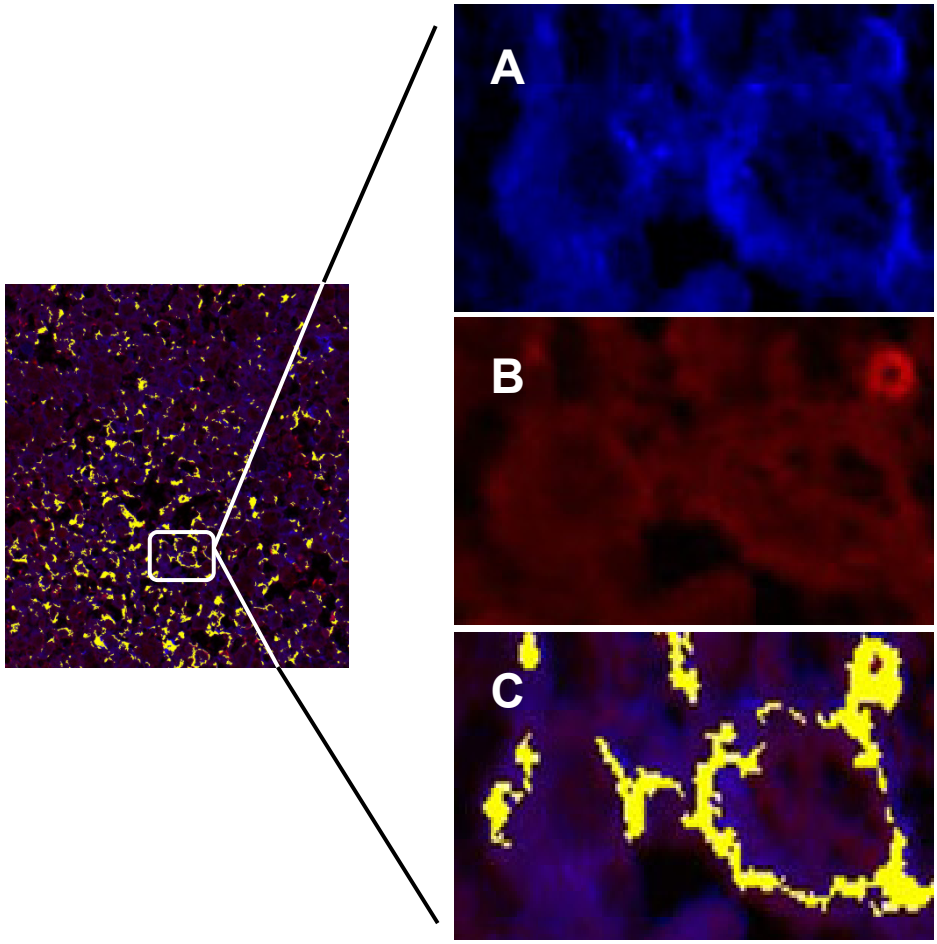
Note.
LNA, locked nucleic acids
PS, phosphorothioate
PO, phosphodiester
F, fluorine
Ome, 2′-O-methyl
OH, 2′-hydroxyl.



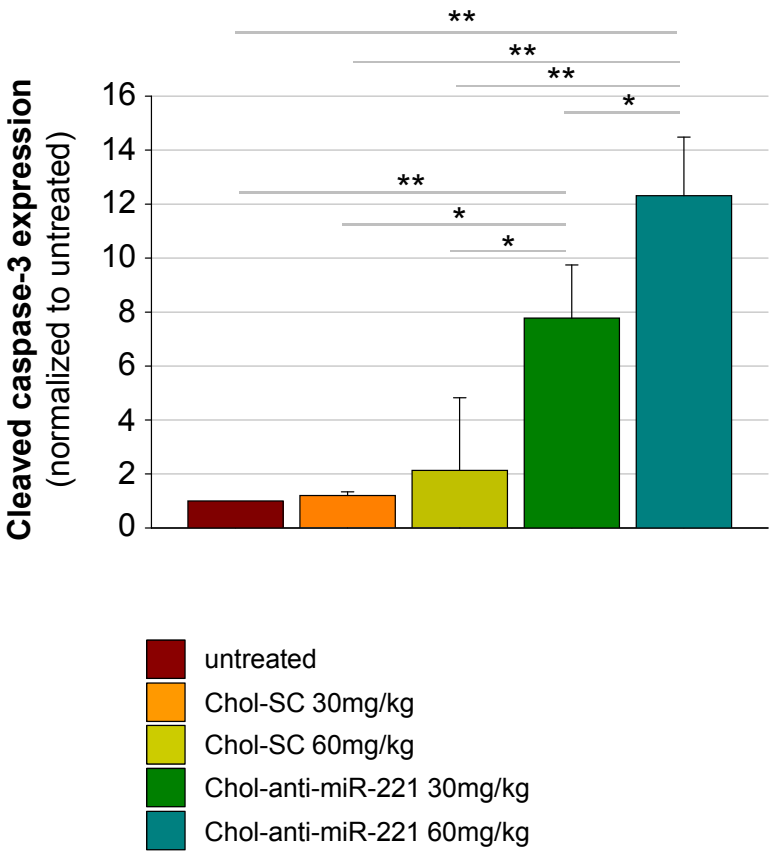
Supplemental Figure S3. Expression of randomly selected microRNAs in the chol-anti-miR-221 treated mice. C57Bl/6 mice injected with 3 daily doses of 30 mg/kg via the tail vein were sacrificed on days 1, 4, and 7. The amount of the mature miRNAs in the liver was quantified by qPCR using 18S rRNA as the reference gene.



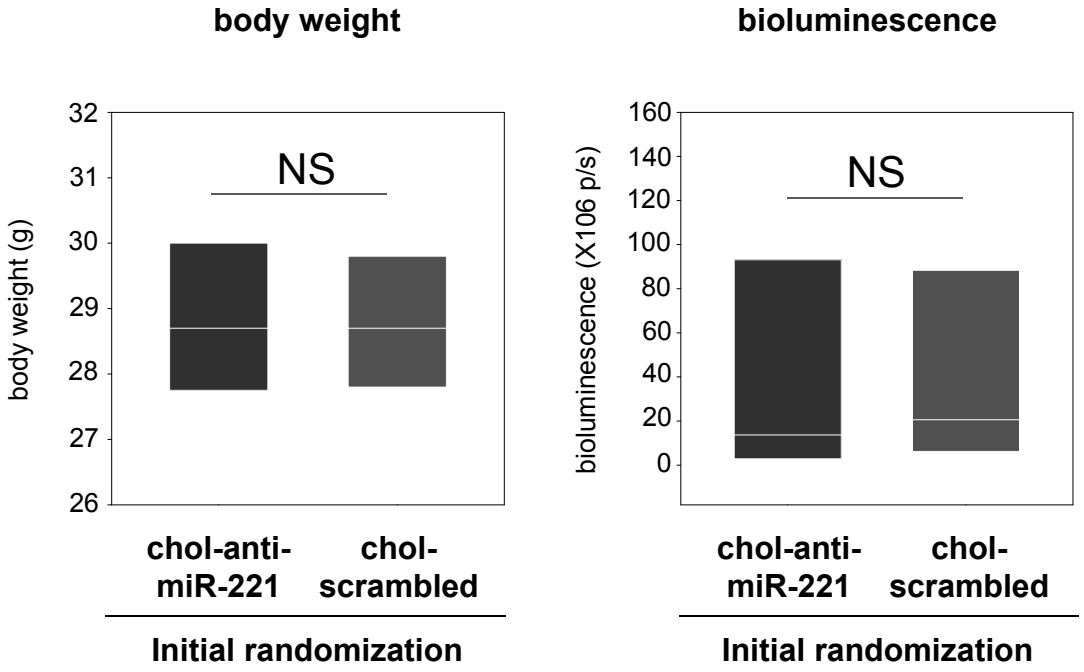
Supplemental Figure S4. Mice were injected via the tail vein with 3 daily doses of 60 mg/kg chol-anti-miR-221 and were sacrificed 7 days after receiving the initial dose. Ki-67 expression was determined by immunohistochemistry and was quantified by ImageJ software. Scatter plot (with median bars) shows Ki-67 labeling indices of cholesterol-labeled control oligo (●) and chol-anti-miR-221 treated tumor (○) samples. Each circle represents the staining intensity of Ki-67 staining positive cells in randomly selected areas.



Supplemental Figure S5. Co-localization of chol-anti-miR-221 and p27^{Kip1} in the tumors of treated mice. Tumor bearing orthotopic mice were sacrificed 7 days after receiving 3 daily doses of 60 mg/kg chol-anti-miR-221 or cholesterol-labeled control oligo. In situ hybridization was used to detect anti-miR-221 using fluorescent blue as a marker (A). Immunohisto-cytochemistry was applied to the identical section to determine the cellular location of p27^{Kip1} using a fluorescent red dye (B). Co-expression mixing analysis of A and B shows that many of the tumor cells co- expressed both the chol-anti-miR-221 and p27^{Kip1} protein (seen as fluorescent yellow, C). Many of the tumor cells co-expressed both the chol-anti-miR-221 and p27^{Kip1} protein that co-localized to the cytoplasm.



Supplemental Figure S6. Quantitation of apoptotic cells in the tumors of treated mice. Cells staining for activated (cleaved) caspase-3 were determined by immunohistochemistry and were quantified by ImageJ software. Positive cells were more numerous in the tumors of mice treated with chol-anti-miR-221 compared to the tumors of mice receiving cholesterol labeled scrambled control (chol-SC). * $P < 0.05$, ** $P < 0.01$.



Supplemental Figure S7. Baseline parameters of mice at the start of the treatment protocol of chol-anti-miR-221 and cholesterol-labeled control oligo. There were no significant differences in bioluminescence or body weight between the groups at randomization. NS, not significant (*P > 0.05).*

Supplemental Table S1

	Sequence
Control F PO	5'- CA/i2FU/ GA/ i2FC/ A/ i2FU/G AG/i2FC/ /i2FU/AA /i2FC//i2FC/A GA/i2FC/ AG -3'
Control F PS	5'- C*A*/i2FU/ GA/ i2FC/ A/ i2FU/G AG/i2FC/ /i2FU/AA /i2FC//i2FC/A GA/i2FC/* A*G -3'
Control F LNA	5'- +C+A/i2FU/ GA/ i2FC/ A/ i2FU/G AG/i2FC/ /i2FU/AA /i2FC//i2FC/A GA/i2FC/ +A+G -3'
Control OH PO	5'- CAT GAC ATG AGC TAA CCA GAC AG -3'
Control OH PS	5'- C*A*T GAC ATG AGC TAA CCA GAC* A*G -3'
Control OH LNA	5'- +C+AT GAC ATG AGC TAA CCA GAC +A+G -3'
Control Ome PO	5'- mCmAmU mGmAmC mAmUmG mAmGmC mUmAmA mCmCmA mGmAmC mAmG -3'
Control Ome PS	5'- mC*mA*mU mGmAmC mAmUmG mAmGmC mUmAmA mCmCmA mGmAmC* mA*mG -3'
Control Ome LNA	5'- +C+AmU mGmAmC mAmUmG mAmGmC mUmAmA mCmCmA mGmAmC +A+G -3'
Control Ome PS Chol	5'-mC*mA*mU mGmAmC mAmUmG mAmGmC mUmAmA mCmCmA mGmAmC* mA*mG/3CholTEG/ -3'

	Sequence
Anti-miR-221 F PO	5'- GAA A/i2FC//i2FC/ /i2FC/AG /i2FC/AG A/i2FC/A A/i2FU/G /i2FU/AG /i2FC/T -3'
Anti-miR-221 F PS	5'- G*A*A A/i2FC//i2FC/ /i2FC/AG /i2FC/AG A/i2FC/A A/i2FU/G /i2FU/AG* C*T -3'
Anti-miR-221 F LNA	5'- +G+AA A/i2FC//i2FC/ /i2FC/AG /i2FC/AG A/i2FC/A A/i2FU/G /i2FU/AG +C+T -3'
Anti-miR-221 OH PO	5'- GAA ACC CAG CAG ACA ATG TAG CT -3'
Anti-miR-221 OH PS	5'- G*A*A ACC CAG CAG ACA ATG TAG* C*T -3'
Anti-miR-221 OH LNA	5'- +G+AA ACC CAG CAG ACA ATG TAG +C+T -3'
Anti-miR-221 Ome PO	5'- mGmAmA mAmCmC mCmAmG mCmAmG mAmCmA mAmUmG mUmAmG mCmU -3'
Anti-miR-221 Ome PS	5'- mG*mA*mA mAmCmC mCmAmG mCmAmG mAmCmA mAmUmG mUmAmG* mC*mU -3'
Anti-miR-221 Ome LNA	5'- +G+AmA mAmCmC mCmAmG mCmAmG mAmCmA mAmUmG mUmAmG +C+T -3'
Anti-miR-221 Ome PS Chol	5'-mG*mA*mA mAmCmC mCmAmG mCmAmG mAmCmA mAmUmG mUmAmG*mC*mU/3CholTEG/ -3'

Note.

LNA, locked nucleic acids
PS, phosphorothioate
PO, phosphodiester
F, fluorine
Ome, 2′-O-methyl
OH, 2′-hydroxyl

/i2FN/: internal 2‘ fluoro modification
*: phosphorothioate linkages
+N: LNA base
mN: 2'-O-Methyl modification
CholTEG: cholesteryl triethyleneglycol

Supplemental Table S2

Sample (blood)		CV (%)			% relative errors	
		Within-day	Between-day	Total	Within-day	Between-day
Chol-anti-miR-221	10 pM	0.24 – 1.28	0.41 – 0.97	0.29 – 1.2	-0.58±0.4	-0.27±1.13
	1 nM	0.69 – 1.05	0.37 – 0.69	0.59 – 1.07	-0.43±0.26	0.09±0.24
	100 nM	0.27 – 0.56	0.23 – 0.37	0.29 – 0.56	-0.37±0.15	-0.15±0.58
Non-chol-anti-miR-221	10 pM	0.23 – 2.23	0.12 – 2.81	0.43 – 3.51	-0.7±0.91	0.19±3.78
	1 nM	0.02 – 0.67	0.35 – 0.68	0.42 – 0.86	-0.38±0.1	-0.09±0.92
	100 nM	0.001 – 0.38	0.35 – 0.4	0.22 – 0.45	-0.18±34	-0.06±0.38

Sample (liver)		CV (%)			% relative errors	
		Within-day	Between-day	Total	Within-day	Between-day
Chol-anti-miR-221	10 pM	0.04 - 0.46	0.3 – 0.64	0.67 – 1.58	-0.46±1.9	-0.2±1.44
	1 nM	0.01 - 0.69	0.07 – 0.71	0.77 – 0.84	-0.37±0.9	0.04±0.73
	100 nM	0.23 - 0.45	0.27 – 0.47	0.29 – 1.18	-0.56±0.04	0.17±1.4
Non-chol-anti-miR-221	10 pM	0.41 - 0.9	0.44 – 0.91	0.4 – 2	-0.45±2.12	-0.29±2.32
	1 nM	0.07 - 0.32	0.07 – 0.01	0.79 – 1.79	-0.5±2.16	0.01±0.87
	100 nM	0.32 - 0.42	0.04 – 0.11	1.04 – 1.79	-0.47±2.15	-0.08±1.1