Supplementary Data

In vitro optimization of 2'-OMe-4-thioribonucleoside modified anti-microRNA oligonucleotides (AMOs) and its targeting delivery to mouse liver using a liposomal nanoparticle

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Analytical data of synthesized oligonucleotides (Table S1).

Table S1. *MALDI-TOFF mass analytical data of synthesized AMOs.*

AMO	Molecular formulae	Calculated mass	Observed mass
AM21SM	$C_{231}H_{302}N_{82}O_{128}P_{21}S_{22}$	7627.80 (M–H)	7626.50
AM21SMF1	$C_{220}H_{269}F_{11}N_{82}O_{117}P_{21}S_{22}$	7498.83 (M–H)	7492.32
AM21SMF2	$C_{213}H_{248}F_{18}N_{82}O_{120}P_{21}S_{12}$	7251.67 (M–H)	7249.67
AM21M	$C_{231}H_{302}N_{82}O_{150}P_{21}$	7276.3 (M–H)	7273.80
AM21MF1	$C_{220}H_{269}F_{11}N_{82}O_{139}P_{21}$	7145.38 (M–H)	7141.34
AM21MF2	$C_{213}H_{248}F_{18}N_{82}O_{132}P_{21}$	7059.95 (M–H)	7056.82
AM21SM-L	$C_{333}H_{437}N_{111}O_{191}P_{31}S_{32}$	11029.08 (M–H)	11028.41
AM21M-L	$C_{333}H_{437}N_{111}O_{223}P_{31}$	10520.82 (M–H)	10525.64
AM122SM	$C_{240}H_{318}N_{85}O_{131}P_{22}S_{23}$	7906.87 (M–H)	7906.72
AM122SM-PS	$C_{240}H_{318}N_{85}O_{109}P_{22}S_{45}$	8261.36 (M–H)	8261.66
AM122SM-PS 20nt	$C_{208}H_{276}N_{72}O_{96}P_{19}S_{39}$	7159.88 (M–H)	7159.56
AM122SM-PS 15nt	$C_{154}H_{206}N_{49}O_{75}P_{14}S_{29}$	5307.08 (M–H)	5305.49
AM122SM-PS 8nt	$C_{82}H_{111}N_{27}O_{38}P_{7}S_{15}$	2780.71 (M–H)	2777.38
AM122M	$C_{240}H_{318}N_{85}O_{154}P_{22}$	7539.40 (M–H)	7538.52
AM122M-PS	$C_{240}H_{318}N_{85}O_{132}P_{22}S_{22}$	7891.89 (M–H)	7886.64

Stability of AMOs in 50% human plasma. Nuclease degradation analyses of AMOs in 50% human plasma was shown in Figure S1.

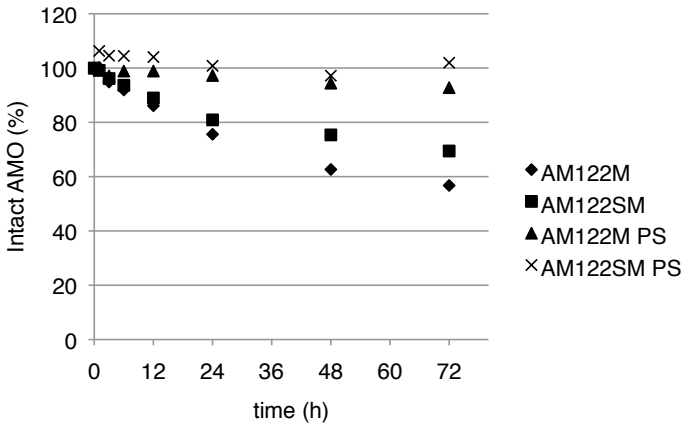

Figure S1. *Stability of AMOs against human plasma nuclease degradation.* Each AMO, labeled with ^{32}P at the 5'-end (5 pmol), was mixed with the corresponding unlabeled AMO (95 pmol). The AMO was incubated in PBS (20 μL) containing 50% human plasma at 37 ˚C. At appropriate time intervals, 3 μL aliquots of the reaction mixture were added to 12 μL of loading buffer (1 x TBE, 90% formamide, 0.05% bromophenol blue, 0.05% xylene cyanol), and then the mixtures were frozen immediately in liquid nitrogen. After the final sample was taken, all samples were then dissolved on ice. The mixtures were analyzed by electrophoresis on a denaturing 20% PAGE. Radioactive densities of remaining full-length AMOs were visualized and measured by a Bio-imaging analyzer (Bas 2500, Fuji Co., Ltd).

Table S2. *Physical properties of YSK05-MEND formulated AMOs.*

	AM122SM PS	AM122M PS
Diameter (nm)	72 ± 3	71 ± 2
PdI	0.20 ± 0.05	0.20 ± 0.00
Zeta potential (mv)	4.4 ± 0.6	3.1 ± 0.5
Encapsulation efficiency (%)	98.3 ± 3.3	98.9 ± 1.2

Particle diameter, polydispersity and zeta-potential were measured using a Malvern Zetasizer. Percentage of encapsulation efficiency was determined by RiboGreen fluorescence assay to measure the amount of AMO relative to total AMO present. Data points are expressed as the mean±SD (n=3).

Serum alanine aminotransferase (ALT) levels in mice. In order to assess hepatotoxicity, serum ALT level was measured after injection of YSK05-MEND formulated AMOs. No significant increase of ALT level was observed at 48 hours after the last injection. See Materials and Methods.

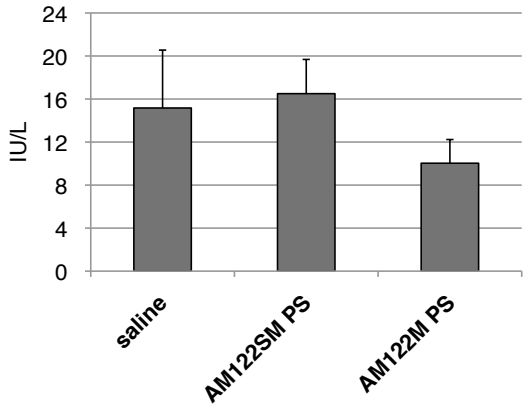

Figure S2. *Serum alanine aminotransferase (ALT) levels in mice.*