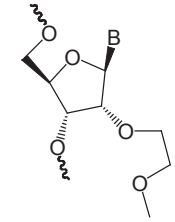
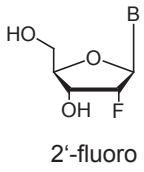
Supplementary Figure 1

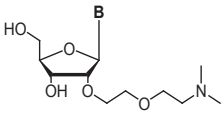
Chemistry	Modification property	Modification pattern	T_m
2'-MOE	High affinity, 2' sugar	5'-$A_s{}^mC_sA_sA_sA_s{}^mC_sA_s{}^mC_s{}^mC_sA_sT_sT_sG_sT_s{}^mC_sA_s{}^mC_sA_s{}^mC_sT_s{}^mC_s{}^mC_sA$-3'	78.9
F-MOE	High affinity, 2' sugar	5'-$A_sC_sA_sA_sA_sC_sA_sC_sC_sA_sU_sU_sG_sU_sC_sA_sC_sA_sC_sU_sC_sC_sA$-3'	80.7
DMAEOE/ 2'-MOE	Charged group, 2 sugar	5'-$A_s{}^mC_sA_sA_sA_s{}^mC_sA_s{}^mC_s{}^mC_sA_sT_sT_sG_sT_s{}^mC_sA_s{}^mC_sA_s{}^mC_sT_s{}^mC_s{}^mC_sA$-3'	71.6
Tri-MOE/ 2'-MOE	Bulk, 2' sugar	5'-$A_s{}^mC_sA_sA_sA_s{}^mC_sA_s{}^mC_s{}^mC_sA_sT_sT_sG_sT_s{}^mC_sA_s{}^mC_sA_s{}^mC_sT_s{}^mC_s{}^mC_sA$-3'	78.4
Phenoxazine/ 2'MOE	High affinity, nucleobase	5'-$A_sC_sA_sA_sA_sC_sA_sC_sC_sA_sT_sT_sG_sT_sC_sA_sC_sA_sC_sT_sC_sC_sA$-3'	>95
MMI-2'OMe/2'-MOE	Neutral backbone	5'-$A_s{}^mCA_sA_sA_s{}^mCA_s{}^mC_s{}^mC_sAT_sT_sG_sT{}^mC_sA_s{}^mC_sA{}^mC_sT_s{}^mC_s{}^mCA$-3'	n/a



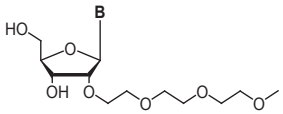
2'-*O*-methoxyethyl



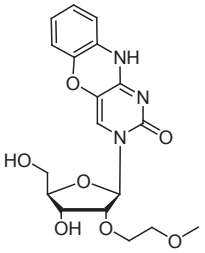
2'-fluoro



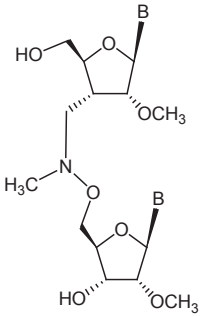
2'-DMAEOE



2'-TriMOE

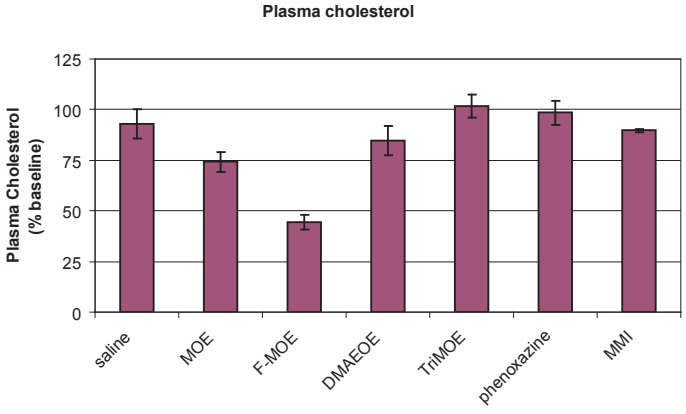


2'-*O*-methoxyethyl phenoxazine

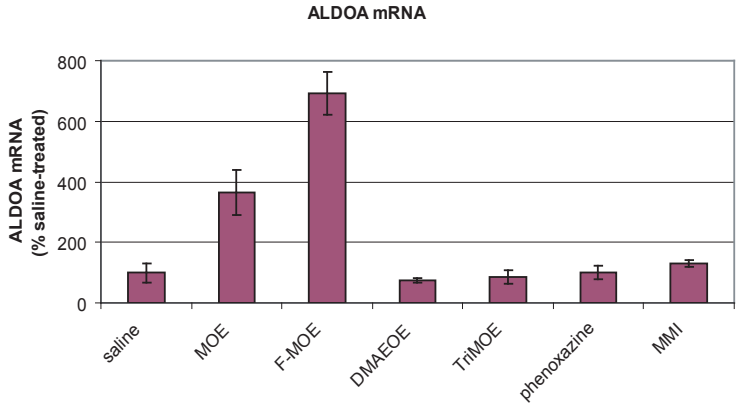


2'-O-methyl MMI dimer

A



B



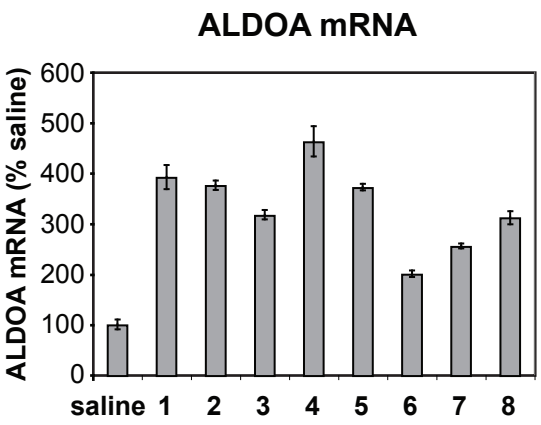
Supplementary Figure 2

A

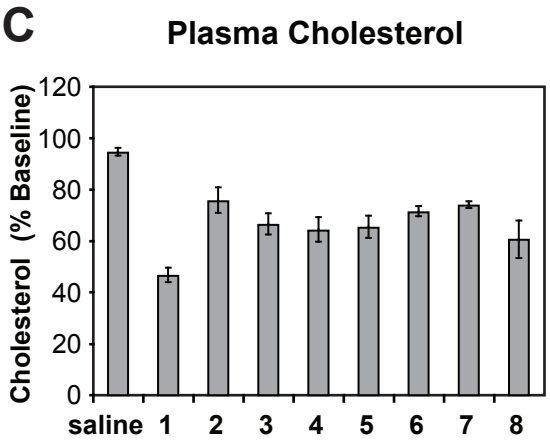
Compound #	Sequence
1	ACAAACACCAUUGUCACACUCCA
2	ACAAACACCAUUGUCACACUCCA
3	ACAAACACCAUUGUCACACUCCA
4	ACAAACACCAUUGUCACACUCCA
5	ACAAACACCAUUGUCACACUCCA
6	ACAAACACCAUUGUCACACUCCA
7	ACAAACACCAUUGUCACACUCCA
8	ACAAACACCAUUGUCACACUCCA

2'-MOE 2'-F

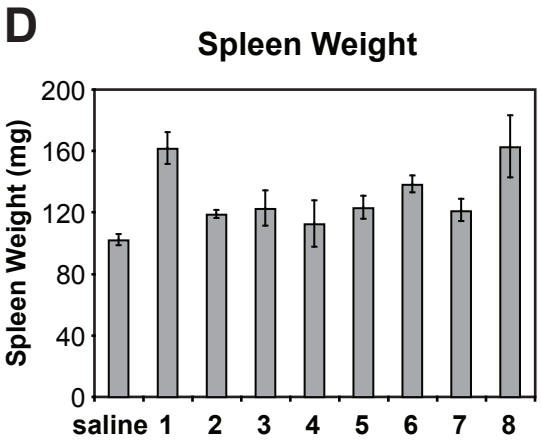
B



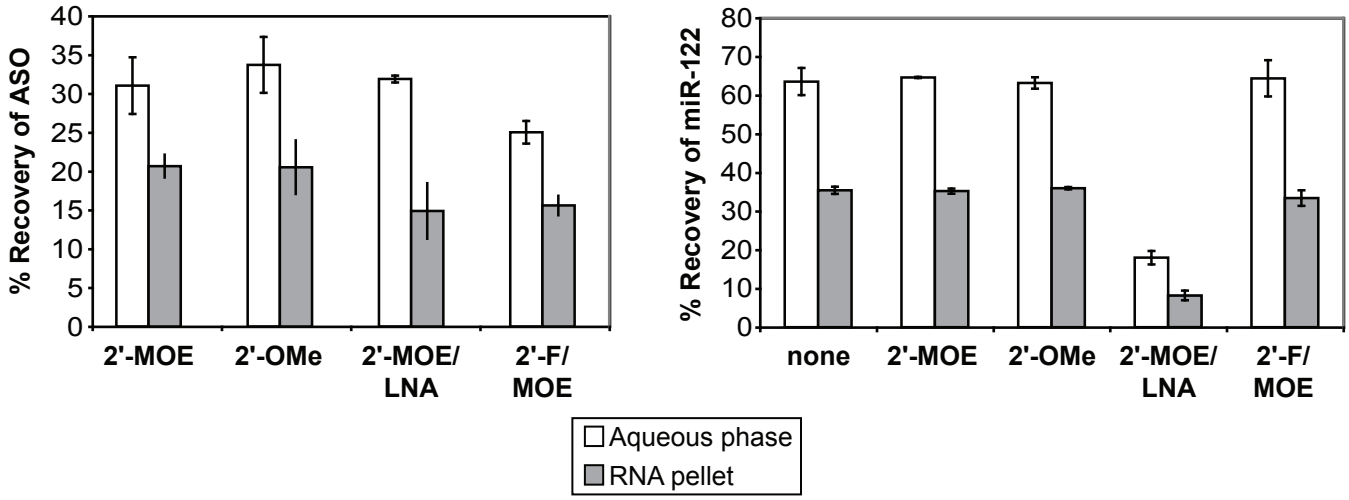
C



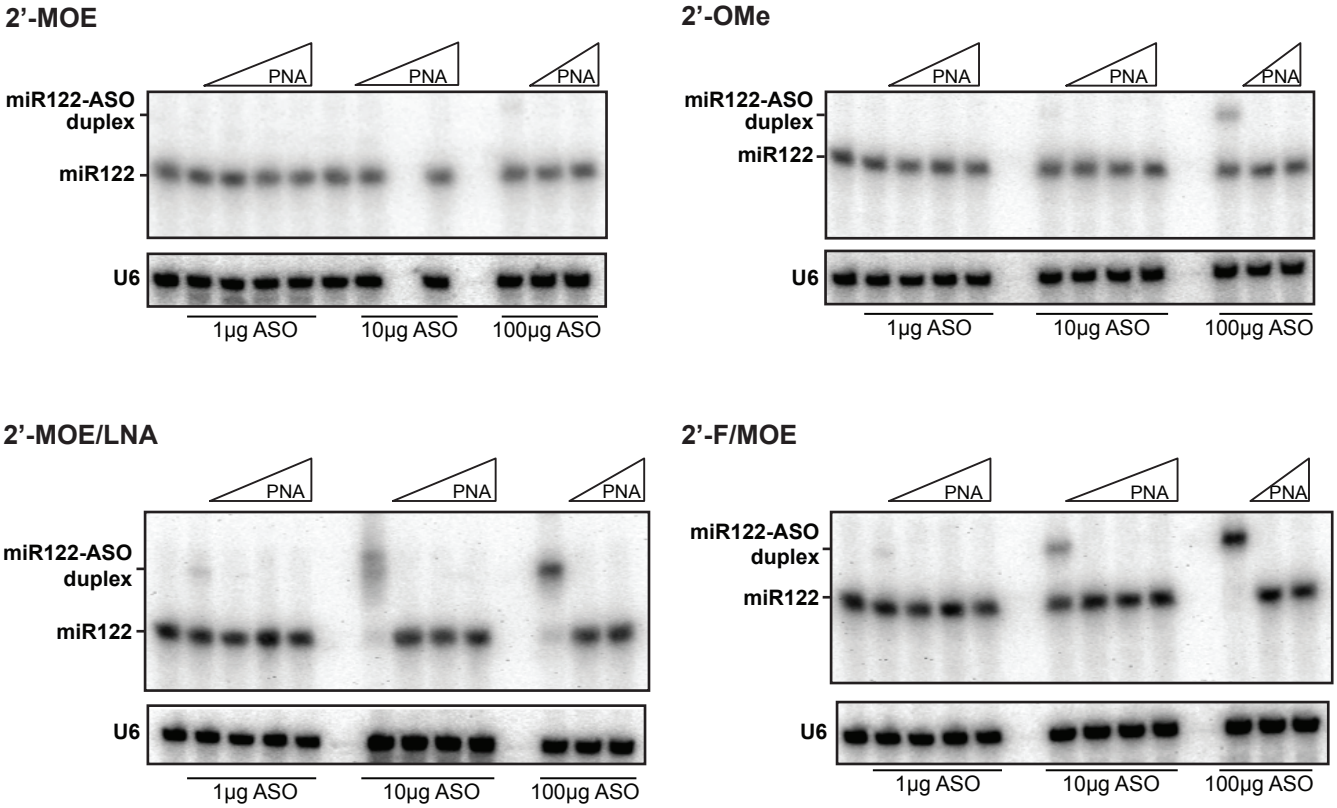
D



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure Legends

Supplementary Figure 1 Evaluation of a panel of chemically-modified miR-122 ASOs. Normal mice were treated i.p. with 25 mg/kg of miR-122 ASOs twice weekly for 3 weeks. n=4. Error=SEM (A) Plasma cholesterol levels. (B) Aldolase A mRNA levels in liver by RTPCR.

Supplementary Figure 2 Insertion of additional 2'-MOE modifications into the 2'-F/MOE cap ASO ameliorates splenomegaly while retaining ASO activity. Normal mice were treated i.p. with 25 mg/kg of miR-122 ASOs twice weekly for 3 weeks. n=5. Error=SEM (a) Chemical composition of anti-miR ASOs incorporating additional 2'-MOE modifications into 2'-F/MOE ASO. (b) Real-time RT-PCR measuring levels of miR-122 target gene ALDOA in liver RNA. (c) Change in total plasma cholesterol levels. (d) Spleen weight at sacrifice.

Supplementary Figure 3 Recovery of ASOs and miR-122 in presence of ASOs through Trizol purification process. (a) Recovery of radiolabeled ASOs after Trizol purification of liver RNA. (b) Recovery of radiolabeled miR-122 in presence of ASOs after Trizol purification of liver RNA.

Supplementary Figure 4 Competitor PNA has ability to free miR-122 from miR-122-ASO duplex in mouse total liver RNA. The indicated ASOs were added to liver lysates and RNA was Trizol-purified. Radiolabeled miR-122 RNA was added to total RNA sample, RNA was separated by denaturing PAGE in presence of competitor PNA and transferred to membrane, which was exposed on a phosphoimager. Northern blotting for U6 was also performed for normalization.

Supplementary Table 1 miR-122 inhibition by 2'-MOE and 2'-F/MOE ASOs *in vivo* has little effect on global expression of microRNAs. In one experiment, mice were treated i.p. with 75mg/kg of the 2'-MOE ASO twice weekly for 4 weeks and real time RT-PCR was used to measure the levels of 162 miRNAs in the liver compared to saline- or ASO-treated mice. In the second experiment, mice were treated with 5mg/kg of the 2'-F/MOE ASO twice weekly for six weeks and miRNA microarray profiling was performed.

Supplementary Methods

ASO labeling and gel shift assay

ASOs and miR-122 RNA were 5'-^{32}P-labelled with T4 polynucleotide kinase and purified on Sephadex columns (Roche). Labeled ASOs were added into 1ml Trizol preparations containing 50mg mouse liver. Labeled miR-122 RNA was added into 1ml Trizol preparations containing 50mg liver and 10μg ASO. After Trizol purification, the percent recovery was determined by scintillation counting. For gel shift assays, ^{32}P-labeled miR-122 RNA was added into total RNA prepared from 50mg mouse liver containing 1, 10, or 100μg ASO. 10 μg total RNA in 1x TBE-Urea loading buffer (Invitrogen) was separated on a 15% TBE-Urea polyacrylamide gel (Invitrogen) in presence of PNA competitor and transferred to membrane, which was exposed on a phosphoimager. Northern blotting for U6 was also performed for normalization.

miRNA Profiling

Total RNA extracted from whole liver tissue using miRNeasy kit (Qiagen). Array of 2'-MOE ASO treated samples was performed using Taqman microRNA Early Access kit

(Applied Biosystems). Array of 2'-F/MOE ASO treated samples performed by LCSciences (array version: miRRodentia_9.1).