

Supplemental Material

Supplemental Methods

Male B6 mice treated with control ASO or FMO3 ASO#2

Six month old male C57BL/6J mice on a chow diet were treated with a control ASO (ISIS 141923, 100 mg/kg body weight per week) or an ASO specific for FMO3 (FMO3 ASO#2, ISIS 555926, 5'-TTTTCCCTTCCATATTCCTG-3', 100 mg/kg body weight per week) through weekly i.p. injection for a total of 6 injections. Five days after the last ASO injection, mice were fasted for 4 hr before mice were euthanized for blood and tissue collection.

Supplemental Figure Legends

Supplemental Figure I. Liver FMO activity and plasma TMAO level of FMO3 Tg and wild-type mice. (A) Liver FMO activity and (B) plasma TMAO levels of FMO3 Tg and wild-type (WT) mice maintained on 1.3% choline chloride water for 6 weeks were determined. n = 6 to 9 for each group. *: p < 0.05 as compared to the WT group.

Supplemental Figure II. Altered plasma lipoprotein profile in FMO3 Tg/E3L Tg mice fed a HF/HC diet. FPLC was used to fractionate pooled plasma samples (n = 5 per pool) from FMO3 Tg/E3L Tg and E3L Tg mice fed the HF/HC diet for 16 weeks. (A) Triglyceride, (B) total cholesterol, and (C) phosphatidylcholine concentrations of each FPLC fraction were measured.

Supplemental Figure III. Liver and small intestine gene expression pattern of FMO3 Tg/E3L Tg and E3L Tg mice fed a HF/HC diet. (A) Hepatic (n = 8 per group) and (B) small intestine (n = 4 per group) mRNA levels of various genes were determined by RT-qPCR.

Supplemental Table I. Primers used for quantitative RT-PCR

Species	Primer Name	Sequence
mouse	Alt-F	CTCTAAGGGGCTACATGGGCG
mouse	Alt-R	ACCTGCTCCGTGAGTTTAGC
mouse	Abcg5-F	GGTGCCTGCATGTGTCCTA
mouse	Abcg5-R	ATTTGCCTGTCCCCTTCTG
mouse	Abcg8-F	AACCCTGCGGACTTCTACG
mouse	Abcg8-R	CTGCAAGAGACTGTGCCTTCT
mouse	Asbt-F	GACTCGGGAACGATTGTGAT
mouse	Asbt-R	GGTTCAATGATCCAGGCACT
mouse	Bsep-F	ACAGAAGCAAAGGGTAGCCATC
mouse	Bsep-R	GGTAGCCATGTCCAGAAGCAG
mouse	Cd68-F	GACCTACATCAGAGCCCAGT
mouse	Cd68-R	CGCCATGAATGTCCACTG
mouse	Cyp7a1-F	CAACGGGTTGATTCCATAACC
mouse	Cyp7a1-R	ATTTCCCATCAGTTTGCAG
mouse	Cyp8b1-F	GCAGCACTGAATACCCATCC
mouse	Cyp8b1-R	TCTGAGAGCTGGGGAGAGG
mouse	Cyp27a1-F	CCTCACCTATGGGATCTTCATC
mouse	Cyp27a1-R	TTTAAGGCATCCGTGTAGAGC
mouse	Fasn-F	GCTGCTGTTGGAAGTCAGC
mouse	Fasn-R	AGTGTTTCGTTCCCTCGGAGTG
mouse	Fgf15-F	GAGGACCAAAAACGAACGAAATT
mouse	Fgf15-R	CAGTCCATTTCCCTCCCTGAA
mouse	Fmo3-F	CCTTTAAACAGAACACTC
mouse	Fmo3-R	TCCTCAAACACAGCGGAC
mouse	Foxo1-F	AAGGATAAGGGCGACAGCAA
mouse	Foxo-1R	TCTTGCCAGACTGGAGAGAT
mouse	G6pc-F	CCGGATCTACCTTGCTGCTC
mouse	G6pc-R	TTGTAGATGCCCGGATGTG
mouse	Hmgcr-F	GAATGCCTTGTGATTGGAGTTG
mouse	Hmgcr-R	ACACAGGCCCGGAAGAATG
mouse	Ibabp-F	GGCAAAGAATGTGAAATGCAG
mouse	Ibabp-R	CCGAAGTCTGGTGATAGTTGG

Species	Primer Name	Sequence
mouse	Me-F	GCAGCTCTTCGAATAACCAAG
mouse	Me-R	AAGTGAGCAATCCCCAAGG
mouse	Mrp2-F	CTGAGTGCTTGGACCAGTGA
mouse	Mrp2-R	GTTAACAGCTGCCTGTGCAA
mouse	Npc111-F	GGTGCTGGCTGTGGGAGCTG
mouse	Npc111-R	GGTGCGGCCAATGTGAGCCT
mouse	Ntcp-F	TCTGTCATCAATGTGGGCAAC
mouse	Ntcp-R	TTGGAATCCTGTTTCCATGCTG
mouse	Ostb-F	TGACAAGCATGTTCTCCTCTG
mouse	Ostb-R	TGCAGGTCTTCTGGTGTCTTCT
mouse	Pepck-F	CAGTGATGGTGGCGTGTACT
mouse	Pepck-R	ATGATGGCGTCAATGGGGAC
mouse	Pparg1-F	GTGAACCACTGATATTCAGGACATTT
mouse	Pparg1-R	CCACAGAGCTGATTCCGAAGT
mouse	Rpl13a-F	CCCTCCACCCTATGACAAGA
mouse	Rpl13a-R	TTCTCCTCCAGAGTGGCTGT
mouse	Scd-1-F	TTCCCTCCTGCAAGCTCTAC
mouse	Scd-1-R	CAGAGCGCTGGTCATGTAGT
mouse	Shp-F	TCTGGAGCCTTGAGCTGGGT
mouse	Shp-R	GCCTTGGCTGGCTGGGTAC
mouse	Srebp-1c-F	GGAGCCATGGATTGCACATT
mouse	Srebp-1c-R	GGCCCGGGAAGTCACTGT
mouse	Tnf- α -F	CTGTAGCCCACGTCGTAGC
mouse	Tnf- α -R	TTGAGATCCATGCCGTTG
human	ALT-F	CAGCCCTGGCCCACTAAG
human	ALT-R	TGACCTCGGTGAAAGGCTTC
human	G6PC-F	CACTTCCGTGCCCTGATAA
human	G6PC-R	TAGTATACACCTGCTGTGCC
human	OCT-F	TTTTGGTGTGGGAAGCCAGT
human	OCT-R	CAGCAGAGCAAAGCCTGTTT
human	PEPCK	CAGTGATGGTGGCGTGTACT
human	PEPCK	ATGATGGCGTCAATGGGGAC

Supplemental Table II. Differentially expressed PPAR α target genes in the livers of LDLRKO mice treated with FMO3 ASO vs. control ASO

Gene	FMO3ASO/Control ASO	P Value
Elov16	0.29	0.0045
Mod1	0.33	0.0074
Srebf1	0.34	0.0001
Pck1	0.40	0.0004
Decr1	0.42	0.0001
Mrpl15	0.47	0.0015
Cyp8b1	0.48	0.0253
Rdh5	0.49	0.0001
Apoa5	0.49	0.0020
Ppara	0.50	0.0001
Sfxn1	0.51	0.0002
Pank1	0.52	0.0029
Gstt2	0.53	0.0108
Accn5	0.53	0.0007
Angptl4	0.53	0.0123
Pcx	0.54	0.0106
Es22	0.55	0.0057
Cpox	0.55	0.0054
Fgf1	0.55	0.0008
Mttp	0.56	0.0089
G0s2	0.57	0.0121
Suox	0.57	0.0015
Mgl1	0.58	0.0009
Gstm4	0.58	0.0333
Ccb12	0.58	0.0167
Slc2a2	0.59	0.0019
Met	0.59	0.0242
Fkbp4	0.59	0.0007
Tgoln1	0.60	0.0020
Ptplb	0.61	0.0037
Abhd1	0.61	0.0008
Gpam	0.61	0.0350
Dnajb10	0.61	0.0017
Acad10	0.61	0.0024
Pdk1	0.61	0.0140
Ehhadh	0.62	0.0357
Pex19	0.62	0.0061
Coq3	0.62	0.0026
Slc22a5	0.62	0.0095
Uck1	0.63	0.0012
Slc27a1	0.63	0.0216
Slc25a30	0.63	0.0390
Bpnt1	0.63	0.0152
Mmd	0.64	0.0009
Tmem98	0.64	0.0009
Asb13	0.65	0.0075
Ephx2	0.65	0.0020
Elov12	0.65	0.0125
Akap1	0.65	0.0094
abcb6	0.66	0.0040

Gene	FMO3ASO/Control ASO	P Value
Nudt1	0.66	0.0152
Acad8	0.67	0.0039
Creb3l3	0.67	0.0024
Pex5	0.67	0.0021
Slc25a10	0.67	0.0105
Coq4	0.68	0.0368
Sorbs1	0.68	0.0298
Abcg8	0.68	0.0396
Pctp	0.68	0.0125
Bace1	0.69	0.0095
Abcd3	0.69	0.0114
Rnf14	0.69	0.0049
Bet1	0.69	0.0111
Dhrs4	0.69	0.0140
Wipi2	0.69	0.0195
Chn2	0.69	0.0018
Abhd6	0.70	0.0383
Adipor2	0.70	0.0071
Fmo1	0.70	0.0039
Rusc2	0.70	0.0117
Pvrl2	0.70	0.0199
Stat5a	0.70	0.0338
Chdh	0.71	0.0107
Anapc5	0.71	0.0127
Pkp2	0.71	0.0096
Pxmp4	0.71	0.0072
Decr2	0.71	0.0224
Hadhsc	0.71	0.0058
Mfn2	0.71	0.0026
Crat	0.72	0.0471
Pdzrn3	0.72	0.0381
Apba3	0.72	0.0073
Copz2	0.72	0.0077
Acs11	0.72	0.0296
Chrna2	0.73	0.0118
Fbp1	0.73	0.0040
Slc25a20	0.73	0.0372
Acot7	0.73	0.0444
Oplah	0.73	0.0455
Pex13	0.73	0.0229
Arpp19	0.74	0.0192
Carhsp1	0.74	0.0067
Synj2bp	0.74	0.0156
Nfe2l1	0.74	0.0146
Usp10	0.75	0.0346
Rbpms	0.75	0.0140
Pex16	0.75	0.0120
Khdrbs3	0.75	0.0463
Galk1	0.75	0.0132
Peci	0.75	0.0174

Supplemental Table II. continued

Gene	FMO3ASO/Control ASO	P Value
Mlycd	0.75	0.0152
Agpat3	0.75	0.0334
Acot8	0.76	0.0186
Slc25a11	0.76	0.0366
Gstk1	0.77	0.0312
Coq5	0.77	0.0134
Cpt2	0.77	0.0313
Acox1	0.77	0.0294
Gtf2h4	0.77	0.0130
Nln	0.77	0.0086
Qpct	0.77	0.0304
Zfp574	0.78	0.0077
Acads	0.78	0.0135
Mtap	0.78	0.0197
Gpr172b	0.78	0.0203
Rab9	0.79	0.0161
Acadl	0.79	0.0427
Ccs	0.79	0.0121
Hmgcs2	0.80	0.0217
Chkb	0.80	0.0431
Hyal2	0.80	0.0285
Coasy	0.80	0.0325
Pex3	0.81	0.0182
Zadh1	0.81	0.0312
Usp38	0.81	0.0370
Ctnnb1	0.81	0.0478
Ciapin1	0.81	0.0277
Abcf3	0.81	0.0384

Gene	FMO3ASO/Control ASO	P Value
Bag4	0.81	0.0408
Nit2	0.82	0.0417
Aldh3a2	0.82	0.0309
Dnajb9	0.82	0.0523
Ppm1b	0.82	0.0385
Prkrir	0.83	0.0407
Chordc1	0.84	0.0500
Etfdh	0.84	0.0457
Adh1	0.84	0.0495
Spop	1.20	0.0481
Ppp2r5c	1.20	0.0461
Scarb2	1.24	0.0457
Tmem77	1.25	0.0436
Psap	1.28	0.0123
Snapap	1.32	0.0146
Arl6ip1	1.33	0.0066
Ccng1	1.33	0.0120
Mpp1	1.36	0.0038
Slc40a1	1.38	0.0065
Ccnd3	1.40	0.0408
Atp11c	1.40	0.0363
Slk	1.50	0.0013
Galnt1	1.50	0.0084
Ostf1	1.55	0.0014
Elk3	1.56	0.0027
Mcm7	1.75	0.0160
Cugbp2	1.79	0.0004
Car2	2.54	0.0312
Ctse	4.68	0.0068

Supplemental Table III. Differentially expressed Klf15 and its target genes in the livers of LDLRKO mice treated with FMO3 ASO vs. control ASO

Gene	FMO3 ASO/Control ASO	P value
G6pc	0.31	0.0067
Pck1	0.40	0.0004
Otc	0.54	0.0005
Prodh	0.57	0.0024
Klf15	0.58	0.0179
Hpd	0.70	0.0096

Supplemental Table IV. Plasma lipid, lipoprotein, glucose, and insulin levels of male B6 mice injected with control ASO or FMO3

ASO#2 and maintained on a chow diet

	n	Triglyceride	Total Cholesterol	HDL Cholesterol	VLDL/IDL/LDL Cholesterol	Unesterified Cholesterol	Free Fatty Acid	Glucose	Insulin
Control ASO	5	38 (4)	120 (8)	94 (6)	26 (4)	23 (1)	30 (2)	217 (2)	3485 (1114)
FMO3 ASO#2	6	25 (2)*	92 (5)*	63 (4)*	29 (2)	18 (1)*	28 (1)	195 (7)*	976 (199)*

Mice were fasted for 4 hr. Values shown are means (standard errors) of the groups. Units are mg/dL except insulin. For insulin, unit is pg/ml. *: $p < 0.05$ vs. control ASO

Supplemental Table V. Chromosome 1 region near *Fmo3** is associated with variations in plasma lipid traits in a HMDP study.

Trait Name	P-value	SNP name	SNP position
VLDL/IDL/LDL Cholesterol	2.50E-08	rs3689151	163768134
Total Cholesterol	3.93E-08	rs3689151	163768134
HDL Cholesterol	1.32E-07	rs31353696	163879627
Unesterified Cholesterol	3.73E-07	rs3689151	163768134
Triglyceride	1.02E-05	rs3689151	163768134

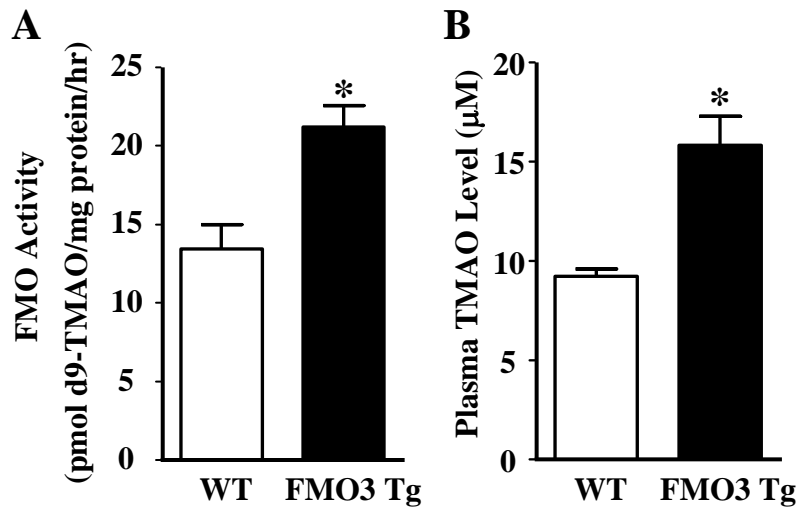
*: *Fmo3* is located at 164883933-164914659 of mouse chromosome 1.

Supplemental Table VIA. Biweight midcorrelation analyses between plasma TMAO, HDL cholesterol, VLDL/IDL/LDL cholesterol levels, and lesion size of female apoB Tg/HMDP mice

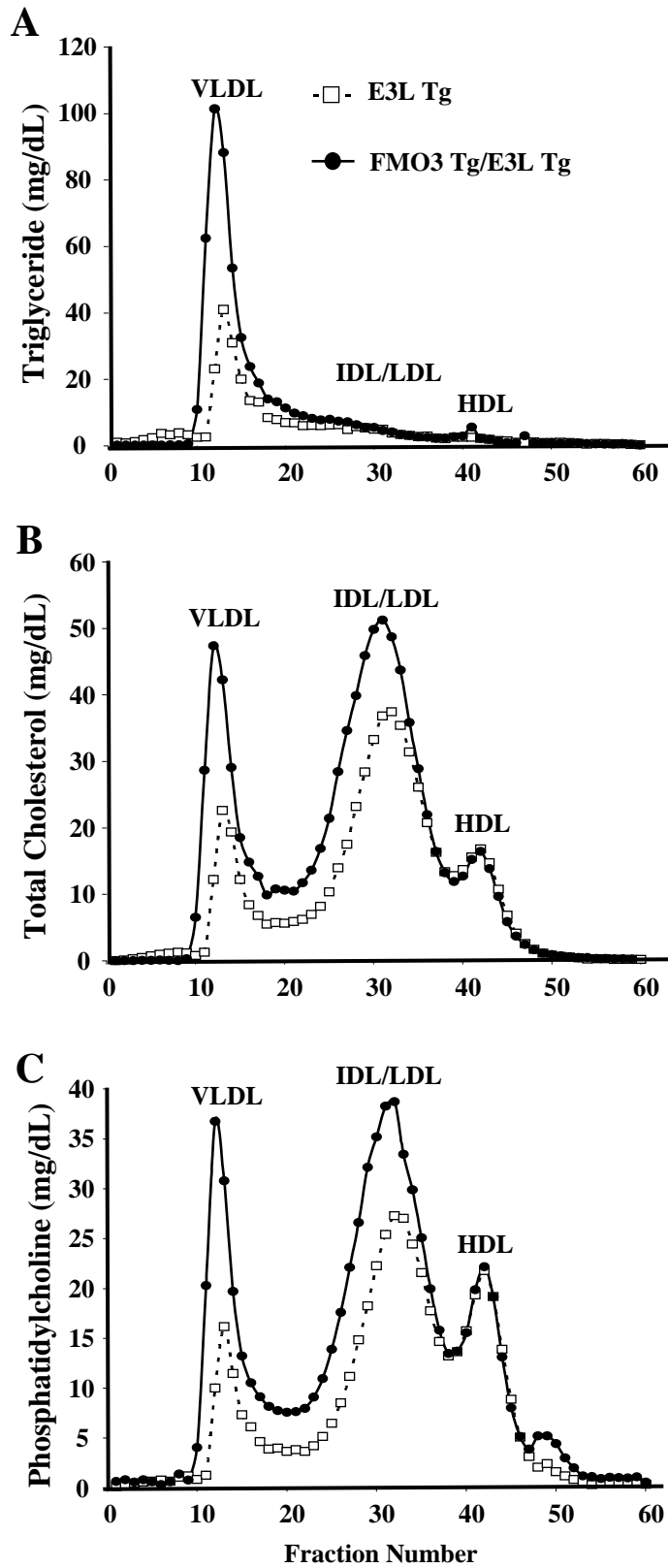
	Correlation coefficient (r)	P value
TMAO:HDL	-0.22	0.01
TMAO:VLDL/IDL/LDL	0.23	0.01
HDL:VLDL/IDL/LDL	-0.56	<0.0001
TMAO:lesion size	0.26	0.002
HDL:lesion size	-0.33	0.0001
VLDL/IDL/LDL:lesion size	0.53	<0.0001

Supplemental Table VIB. Relative importance of plasma HDL, VLDL/IDL/LDL, and TMAO levels in influencing atherosclerotic lesion size in a population of female apoB Tg/HMDP mice

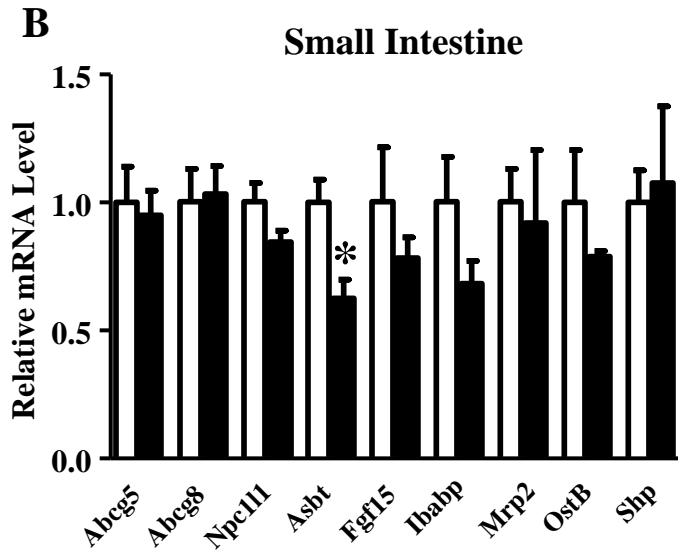
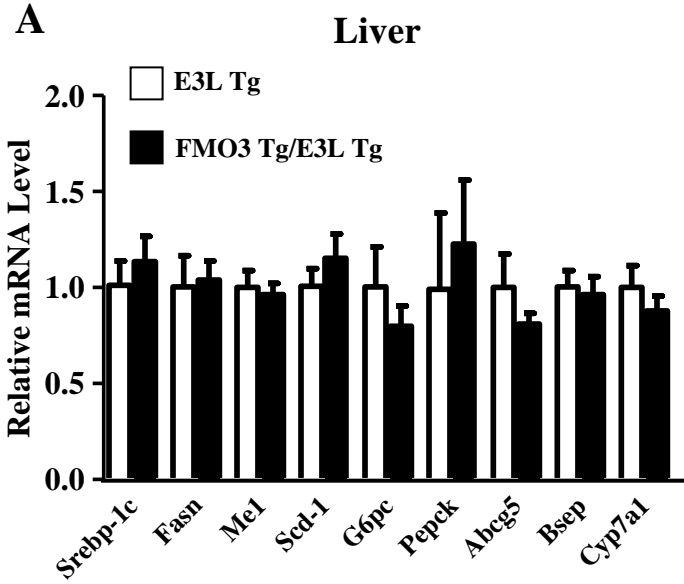
Predictor	Variance explained
VLDL/IDL/LDL	24.4%
HDL	5.4%
TMAO	2.8%
Residuals	67.5%



Supplemental Figure I



Supplemental Figure II



Supplemental Figure III