SUPPLEMENTAL INFORMATION

A novel orally available delta-5 desaturase inhibitor prevents atherosclerotic lesions accompanied with changes of fatty acid composition and eicosanoid production in *ApoE* knockout mice

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Supplemental table S1. Selectivity profiling of compound-326.

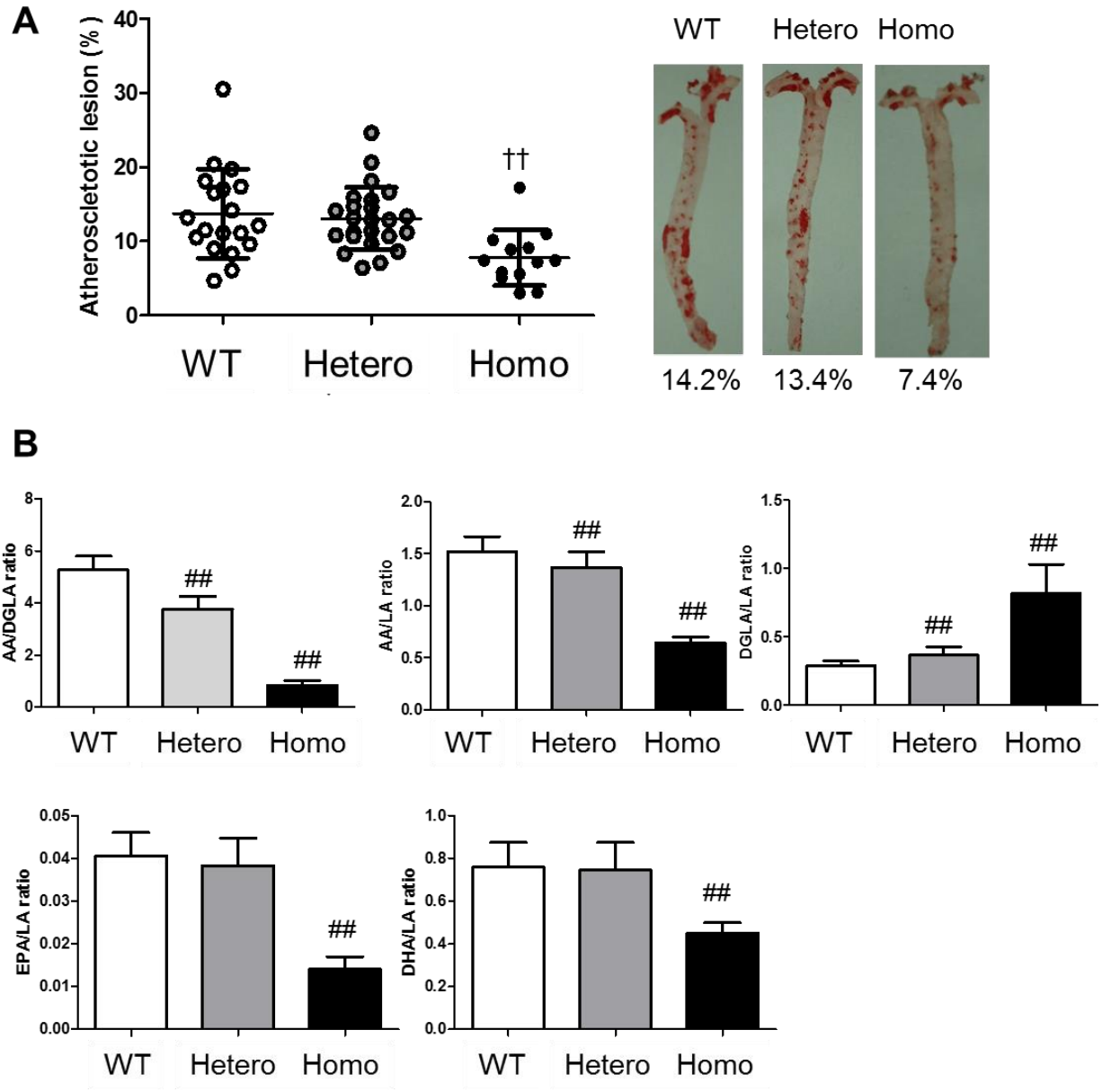
Target	% of inhibition
ATPase, Ca2+, skeletal muscle, pig	-4
ATPase, Na+/K+, heart, pig	4
Carbonic anhydrase	19
Chelinesterase, acetyl	11
Cyclooxigenase COX-1	22
Cyclooxigenase COX-2	8
HMG-CoA reductase	7
Lipoxygenase 5-LO	28
Monoamine oxidase MAO-A	63
Monoamine oxidase MAO-B	1
Nitric oxidase synthase, inducible (iNOS)	8
Nitric oxidase synthase, neuronal (nNOS)	3
Matrix metalloproteinase-1 (MMP-1)	-8
Matrix metalloproteinase-7 (MMP-7)	1
Matrix metalloproteinase-13 (MMP-13)	-25
PDE10A1	-2
PDE3	-2
PDE4	16
PDE5	7
PDE6	8
PKA	-15
PKC	-8
EGF receptor	2
Steroid 5a-reductase	2
Xanthine oxidase	3
Adenosine A1	-2
Adenosine A2A	-5
Adenosine A2B	7
Adrenergic a1 receptor	-3
Adrenergic a2 receptor	6
Adrenergic b1 receptor	-11
Adrenergic b2 receptor	-5

Adrenergic b3 receptor	-7
Androgen (AR)	9
Angiotensin AT1	-9
Angiotensin AT2	-17
Bradykinin B1	-14
Bradykinin B2	2
Calcium channel L-type, benzothiazepine	3
Calcium channel L-type, dihydropyridine	6
Calcium channel L-type, phenylalkylamine	14
Calcium channel N-type	-5
Cannabinoid CB1	8
Cholecystokinin CCK1	-3
Cholecystokinin CCK2	-3
Dopamine D1	-5
Dopamine D2L	-3
Dopamine D3	6
Dopamine D4.2	-5
Estrogen ERa	0
GABAA, chloride channel	6
GABAA, flunitrazepam	8
GABAA, muscimol	10
GABAB	-2
GABAB1A	11
GABAB1B	-2
Glucocorticoid	11
Glutamate, AMPA	0
Glutamate, kainate	-10
Glutamate, NMDA, agonism	-1
Glutamate, NMDA, glycine	-3
Glutamate, NMDA, phencyclidine	-5
Glycine, strychnine-sensitive	4
Growth hormone secretagogue (Ghrelin)	2
Hisatamine H1	6
Hisatamine H2	-8

Imidazoline I2	16
Muscarinic M1	1
Muscarinic M2	-20
Muscarinic M3	-12
Nicotinic acetylcholine	-19
Opiate d (OP1)	15
Opiate k (OP2)	4
Opiate m (OP3)	4
Potassium channel [KATP]	4
Potassium channel [SKCA]	0
Progesterone PR-B	1
Thromboxane A2 (TP)	18
5-HT1	-5
5-HT2	-2
5-HT2B	-2
5-HT3	-10
5-HT4	20
Sigma	24
Sodium channel, site 2	-24
Tachykinin NK1	1
Tachykinin NK2	1
Tachykinin NK3	14
Transporter, Dopamine (DAT)	7
Transporter, GABA	22
Transporter, Norepinephrine	4
Transporter, Serotonin (SERT)	-9
Vasopressin V1A	3
Vasopressin V2	-3

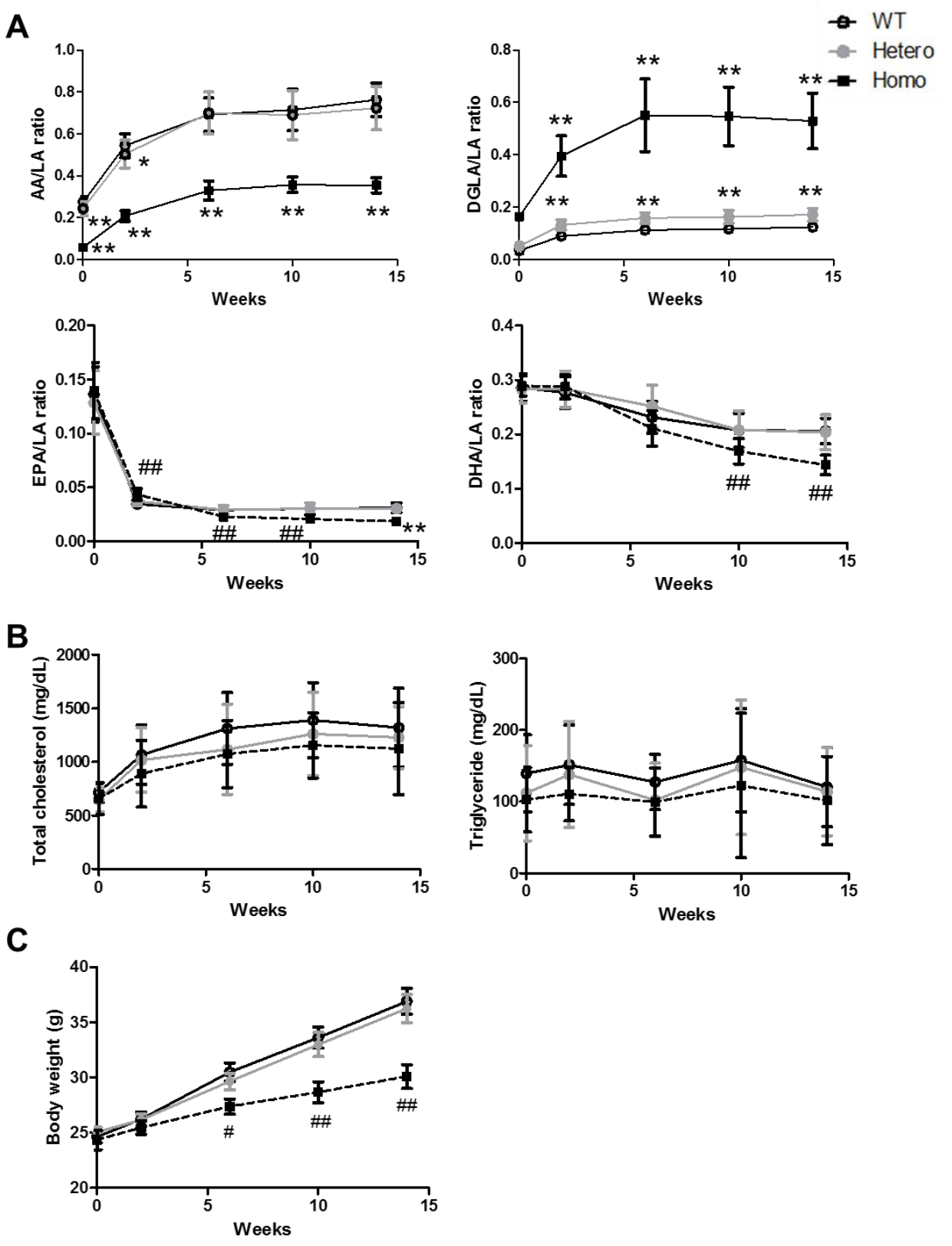
Supplemental table S2. Fatty acid composition in diets utilized in the study.

	Normal cow (CE2)	Western diet (D12079B)	Control diet (98121701)
Fatty acid	(%)	(%)	(%)
C2	0	0	0
C4	0	3.3	0
C6	0	1.9	0
C8	0	1.1	0
C10	0	2.5	0
C12	0	2.8	0
C14	0.4	10.2	0
C14:1	0	1.5	0
C16	15.4	27.2	5.7
C16:1	1.3	2.3	0
C18	1.8	12.4	1
C18:1	22.7	26.8	13.1
C18:2	49.4	5.4	31.6
C18:3	3.4	1.5	0.7
C18:4	0	0	0
C20	0	1	0
C20:1	1	0	0
C20:4	0.2	0	0
C20:5	1.8	0	0
C22	0.2	0	0
C22:1	0.4	0	0
C22:4	0	0	0
C22:5	0	0	0
C22:6	1.7	0	0
C24	0	0	0

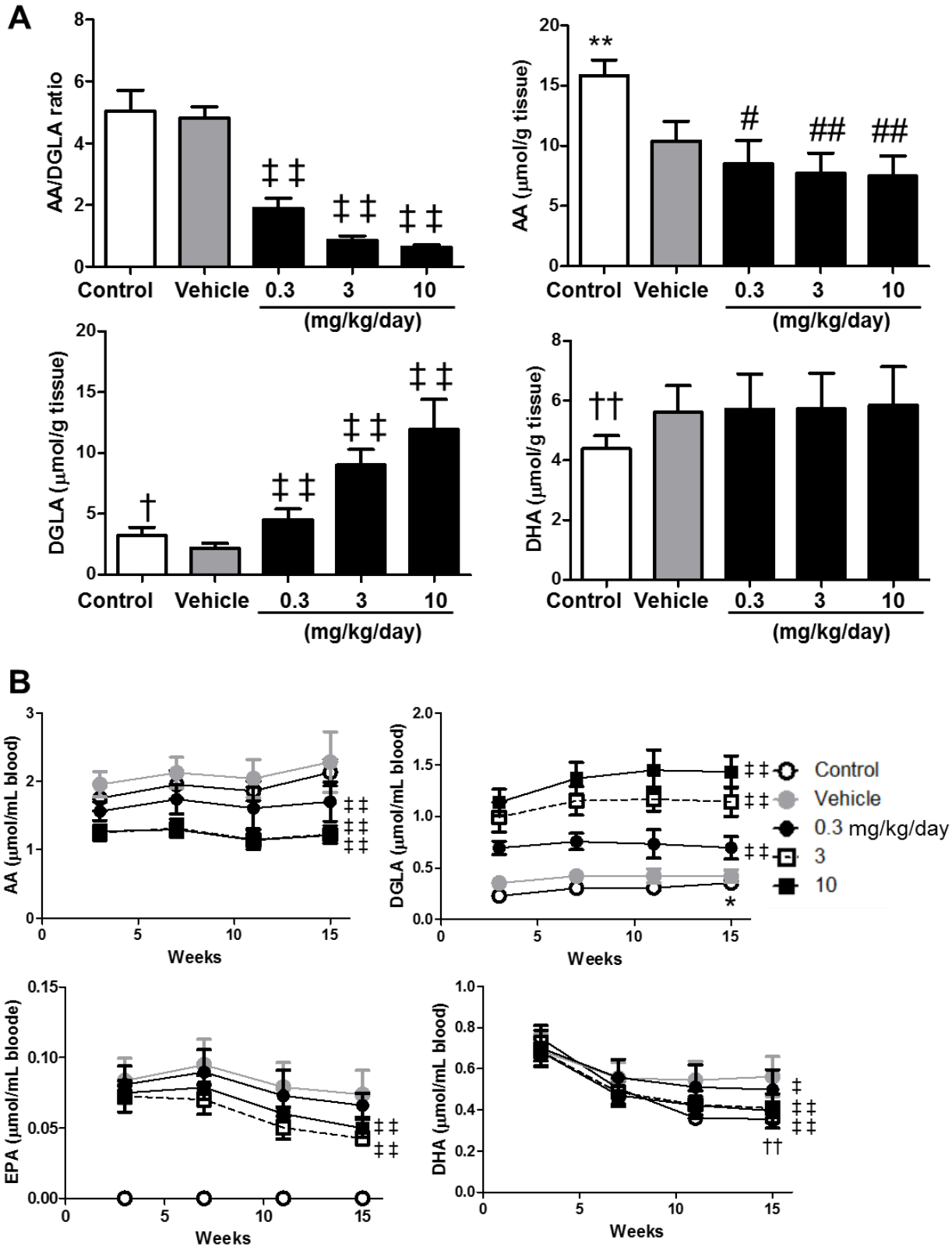


Supplemental Fig S1. Western diet-fed *Fads1* x *ApoE* double knockout (KO) mice were less susceptible to the development of atherosclerotic lesions, and showed alterations in ω-6 and ω-3 fatty acid levels in the liver. (A) The percentage of stained atherosclerotic lesion area in the aorta. Representative images of each group were presented. The values of the percentage of the lesion were

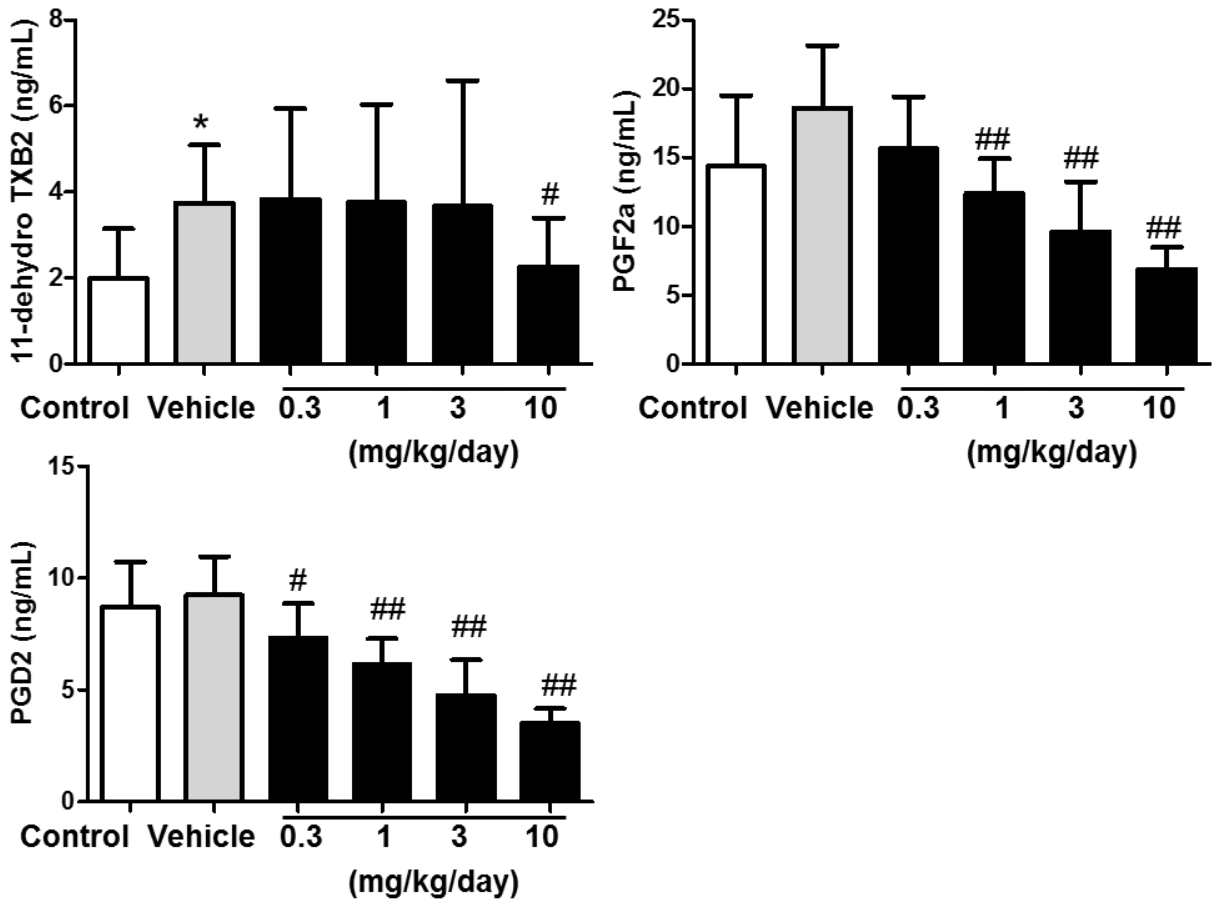
described below the images. (B) Fatty acid analysis in the liver. The number of mice in each group was as follows: WT, wild mice (n=19); Hetero, hetero KO mice (n=24); Homo, homo KO mice (n=13). Data are presented mean ± standard deviation (S.D.). ††: $P < 0.01$ vs. WT by *Dunnett*-test, ##: $P < 0.01$ vs. WT by *Steel*-test. Abbreviations: LA, linoleic acid; AA, arachidonic acid; DGLA, dihomo-γ-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.


A
WT
Hetero
Homo
AA/LA ratio
DGLA/LA ratio
EPA/LA ratio
DHA/LA ratio
Weeks
B
Total cholesterol (mg/dL)
Triglyceride (mg/dL)
C
Body weight (g)

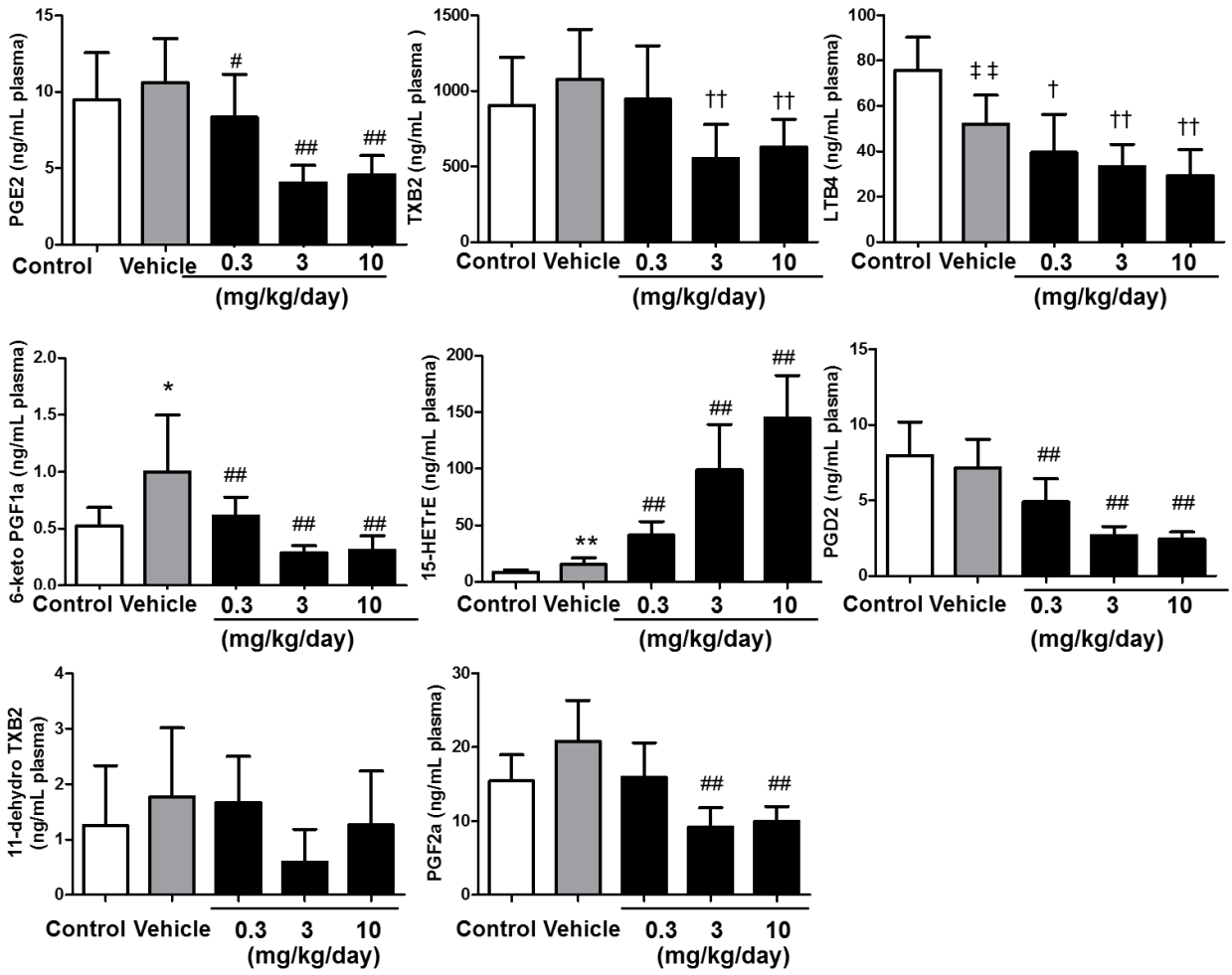
Supplemental Fig S2. ω-6 and ω-3 fatty acid levels in the blood, plasma total cholesterol and triglyceride levels, and body weight of Western diet-fed *Fads1* x *ApoE* double KO mice. Blood and plasma were obtained at 0, 2, 6, 10, and 14 weeks of the study period. Time course of (A) blood fatty acids and (B) plasma total cholesterol and triglyceride. Data are presented as mean ± standard deviation (S.D.). Time course of (C) body weight. Data are presented as mean ±standard error of the mean (S.E.M.). The number of mice in each group was as follows: WT, wild mice (n=19); Hetero, hetero KO mice (n=24); Homo, homo KO mice (n=13). *: $p<0.05$, **: $p<0.01$ vs. WT by *Steel*-test. #: $p<0.05$, ##: $p<0.01$ vs. vehicle by *Dunnett*-test. Abbreviations: LA, linoleic acid; AA, arachidonic acid; DGLA, dihomo-γ-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.


A
AA/DGLA ratio
AA (μmol/g tissue)
DGLA (μmol/g tissue)
DHA (μmol/g tissue)
Control
Vehicle
0.3
3
10
(mg/kg/day)
B
AA (μmol/mL blood)
DGLA (μmol/mL blood)
EPA (μmol/mL bloode)
DHA (μmol/mL blood)
Weeks
Control
Vehicle
0.3 mg/kg/day
3
10

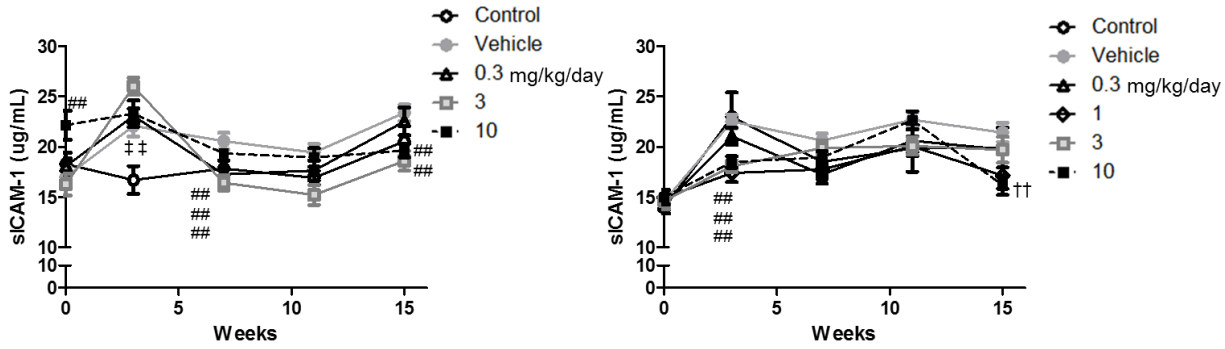
Supplemental Fig S3. ω-6 and ω-3 fatty acid levels from *ApoE* KO mouse liver and the blood administered with compound-326 (Study 1). Fatty acid analysis of (A) mouse liver and (B) mouse blood. After 15 weeks of compound-326 administration, livers were harvested. The number of mice in each group is described in the legend of Figure 1. The EPA levels in the blood from the control were undetectable. Data are presented mean ± standard deviation (S.D.). *: $p<0.05$, **: $p<0.01$ vs. Vehicle by *Student's t*-test, †: $p<0.05$, ††: $p<0.01$ vs. Vehicle by *Aspin-Welch* test, #: $P < 0.025$, ##: $P < 0.005$ vs. Vehicle by *Williams*-test, ‡: $P<0.025$, ‡‡: $P<0.005$ vs. Vehicle by *Shirley-Williams* test. Abbreviations: AA, arachidonic acid; DGLA, dihomo-γ-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.



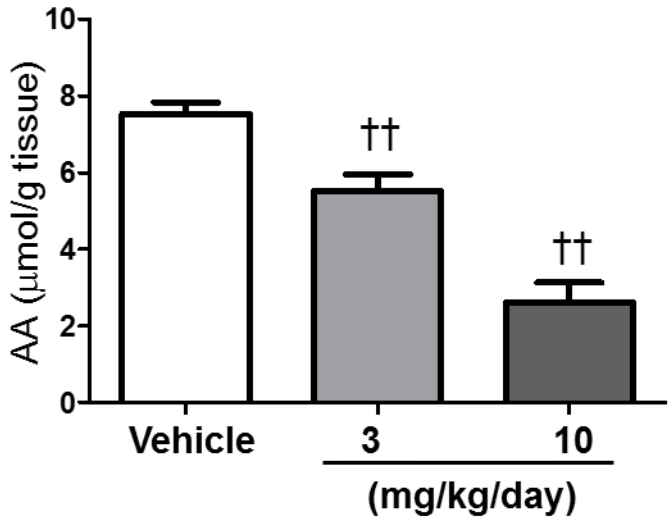
Supplemental Fig S4. Eicosanoid production from *ApoE* KO mouse blood stimulated with A23187 and fMLP (Study 2). *ApoE* KO mouse blood was stimulated with A23187 (30 μM) and fMLP (10 μM). Both reagents were added to blood samples at the same time. The number of mice in each group is described in the legend of Figure 2. Data are presented as mean ± standard deviation (S.D.). *: $p<0.05$ vs. Control by *Student's t*-test, #: $P < 0.025$, $P < 0.005$ vs. vehicle by *Shirley-Williams* test. Abbreviations: PG, prostaglandin; TXB2, thromboxane B2.



Supplemental Fig S5. Eicosanoid production from ApoE KO mouse blood stimulated with A23187 and fMLP (Study 1). ApoE KO mouse blood was stimulated with A23187 (30 μM) and fMLP (10 μM). Both reagents were added to blood samples at the same time. Data are presented as mean ± standard deviation (S.D.). *: p<0.05, **: p<0.01 vs. Control by *Aspin-Welch* test, ‡‡: P<0.01 vs. Vehicle by *Student's t*-test, #: P < 0.025, ##: P < 0.005 vs. Vehicle by *Shirley-Williams* test, †: P < 0.025, ††: P < 0.005 vs. Vehicle by *Williams* test. Abbreviations: PG, prostaglandin; TXB2, thromboxane B2; LTB4, leukotriene B4; 15-HETrE, 15-hydroxyeicosatrienoic acid.



Supplemental figure S6. Plasma sICAM-1 levels from ApoE KO mice administered with compound-326 (Studies 1 and 2). Plasma was obtained at 0, 3, 7, 11, and 15 weeks of the study period. The number of mice in each group is described in the legend of Figure 1. Data are presented as mean ± standard error of the mean (S.E.M.). ‡ ‡: $p<0.01$ vs. Control by *Student's t*-test, ##: $p<0.005$ vs. Vehicle by *Williams* test, ††: $p<0.005$ vs. Vehicle by *Shirley-Williams* test.



Supplemental figure S7. Effects of compound-326 on the AA levels in the mouse brain. Male C57BL/6 mice fed standard rodent chow (CE2, CLEA) were administered with vehicle or Compound-326 (3–10 mg/kg/day, p.o., suspended in 0.5% [w/v] methylcellulose) for 18 weeks. The AA levels in the brain were determined. The number of mice is as follows: Vehicle (n=10), 3mg/kg/day (n=8), 10mg/kg/day (n=9). Data are presented mean ± standard deviation (S.D.). ††: $p<0.005$ vs. Vehicle by *Williams* test.