Reducing endoplasmic reticulum stress through a macrophage lipid chaperone alleviates atherosclerosis

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Supplemental Materials and Methods:

Reagents and plasmids: Western diet #TD88137 was obtained from Harlan Teklad, Madison, Wisconsin. Antibodies against total eIF2–α, phospho-(Thr980) PERK, activated caspase 3, cleaved PARP, and PDI were from Cell Signaling, phospho-(serine 52) eIF2–α and CD3 from Invitrogen, CHOP, tubulin, ATF-3 and LXR–α from Santa Cruz, MOMA-2 from Accurate Chemical & Scientific Corp., Westbury, NY, CD11c from BD Biosciences, α-SMA from Sigma and LXR–β antibody from Thermo Scientific. The anti-FABP antibody was generated in our laboratory. Horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG and goat anti-mouse IgG were from Ambion. Biotinylated secondary antibodies to rat or rabbit IgG were from BD Pharmingen. Tissue culture materials were purchased from VWR and media were obtained from Invitrogen. Fetal bovine serum (FBS) was obtained from Hyclone Laboratories and was heat inactivated for 30 minutes at 65^0C. The ACAT inhibitor was a generous gift of Alan Edgar (Fournier, France). AcLDL was obtained from Biomedical Technologies. Thapsigargin, tunicamycin, T0901317 and PBA were from Calbiochem, 25 hydroxy cholesterol and cycloheximide from Sigma and the aP2 inhibitor (BMS309403) was obtained from Bristol-Myers Squibb Pharmaceutical Research Institute. The caspase-Glo 3/7 and dual luciferase assays were obtained from Promega. The TK-LXRE-X3luc reporter was a generous gift of David

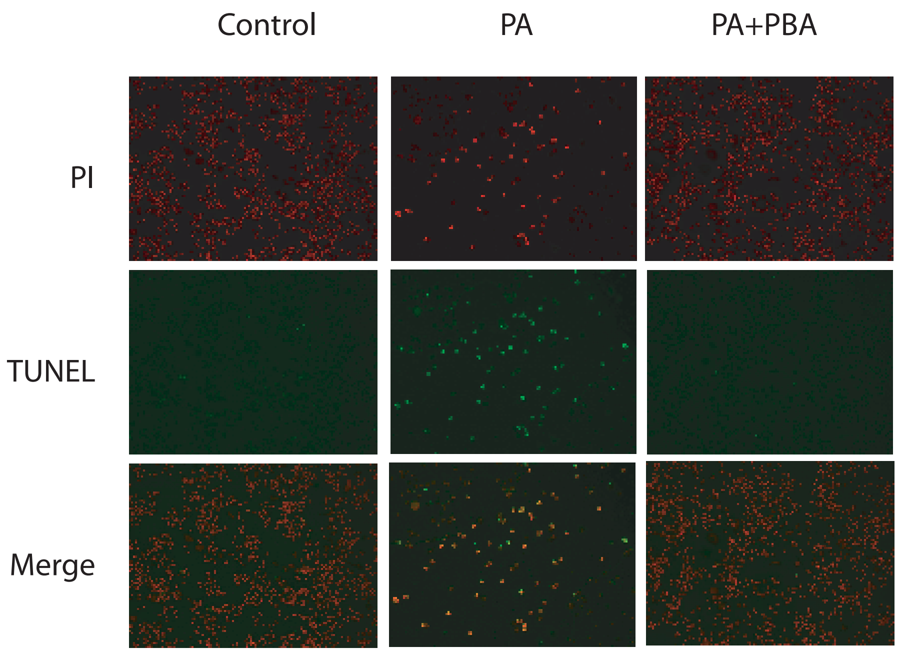
Mangelsdorf (UT Southwestern). Glucose oxidase kit was from Sigma. Mouse insulin ELISA kit from Alpco Diagnostics.

Primers and siRNA: Primer sequences for *aP2*, *Ddit3*, *Fasn*, *SCD-1*, s*XBP-1*, *Abca1*, *Abcg1*, and *CD51* were previously published[29,37,39]. All primers used for qRT-PCR were synthesized at Qiagen and for siRNA at Ambion. The pGEX-aP2-LM (R126L, Y128F) plasmid was a generous gift of David Bernlohr, was recloned into a pCDNA3 vector[30]. The siRNA sequence for *SCD-1* was sense: 5'GCCUUU AAUCAACCCAAG-ATT–3' and antisense: 5'–UCUUGGGUUGAUUAAAGGCTT–3' and for *Nr1h3* was sense: 5'–GGACUUCAGUUACAACCGGTT–3' and anti-sense: 5'–CCGGUUGUAACUGAAGUCCTT–3'.

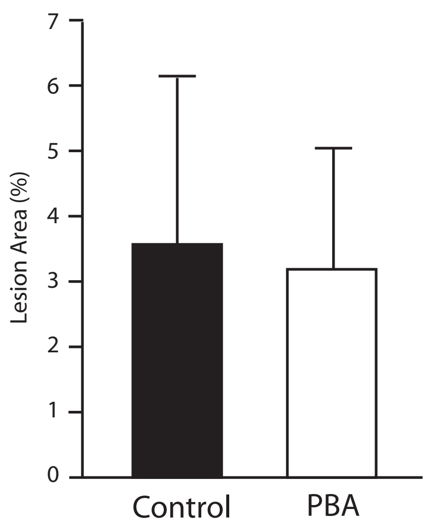
Fatty acid uptake assay: Macrophages were incubated with ^{3}H labeled palmitic acid at a final concentration of 100μM palmitic acid at 37^{0}C for 1 hour. The uptake was terminated by the addition of ice-cold stop solution containing 0.1% BSA and 200μM phloretin in Krebs-Ringer phosphate solution. The cells were than washed three times with Krebs-Ringer phosphate buffer to remove unincorporated fatty acids. Cells were lysed in Krebs-Ringer buffer with 0.3% Triton-X and, lysate was transferred into liquid scintillation tubes and counted with a liquid scintillation counter. From the lysates, total protein concentrations were measured using Biorad assay and fatty acid uptake data was represented as the ratio of total cpm counts to total protein concentration.

SCD activity assay: SCD activity was determined from the production of ^3H_2O using [9, 10-^{3}H]-stearoyl-CoA as substrate[50]. Macrophages were lysed and microsomal fractions (105,000xg) were isolated by sequential centrifugations. Reactions were performed at 37^{0}C for 30 min with 100 μg/mL protein homogenate, 10 μM (2 μCi/mL) of [9, 10-^{3}H]-stearoyl-CoA and 30 μM NADH in 100 μL of 10 mM potassium phosphate buffer (pH 7.4). After the reaction, 100 μL of 10 mg mL^{-1} fatty acid-free BSA and 200 μL of 10% trichloroacetic acid were added. After centrifugation (5000 g for 10 min) radioactive counts in the supernatant were measured by a scintillation counter. The enzyme activity was expressed as nmol min^{-1} mg^{-1} protein.

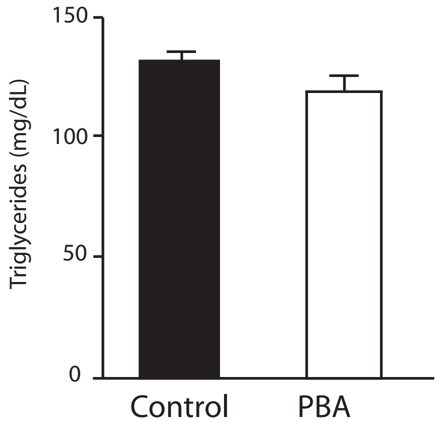
a.


Control
PA
PA+PBA
PI
TUNEL
Merge

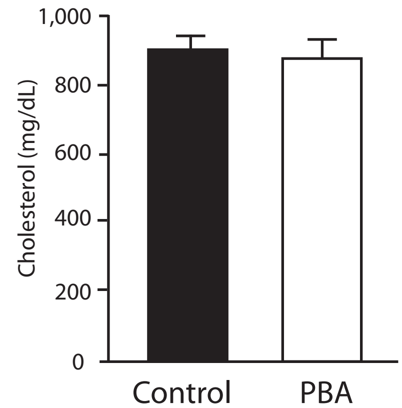
b.


7
6
5
4
3
2
1
0
Lesion Area (%)
Control
PBA

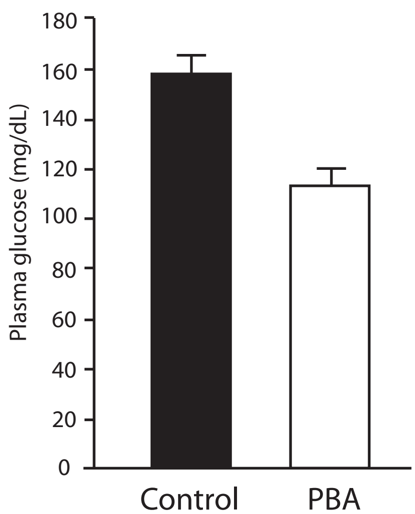
c.


Triglycerides (mg/dL)
150
100
50
0
Control
PBA

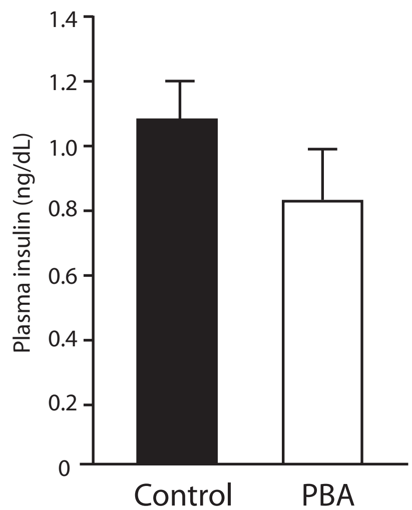
d.


Cholesterol (mg/dL)
1,000
800
600
400
200
0
Control
PBA

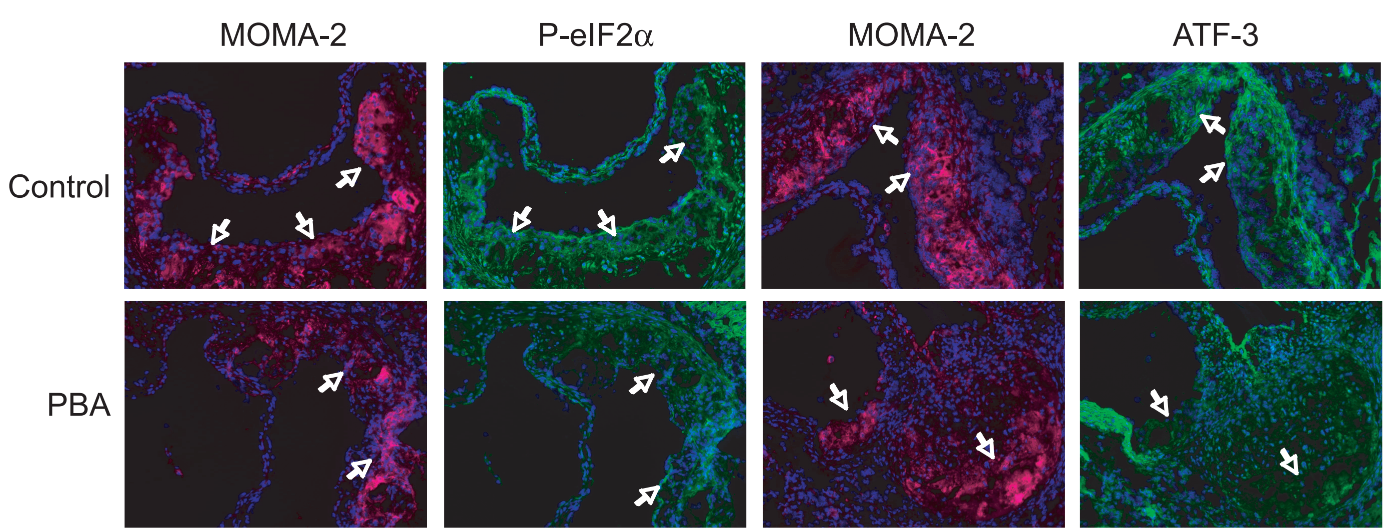
e.


Plasma glucose (mg/dL)
180
160
140
120
100
80
60
40
20
0
Control
PBA

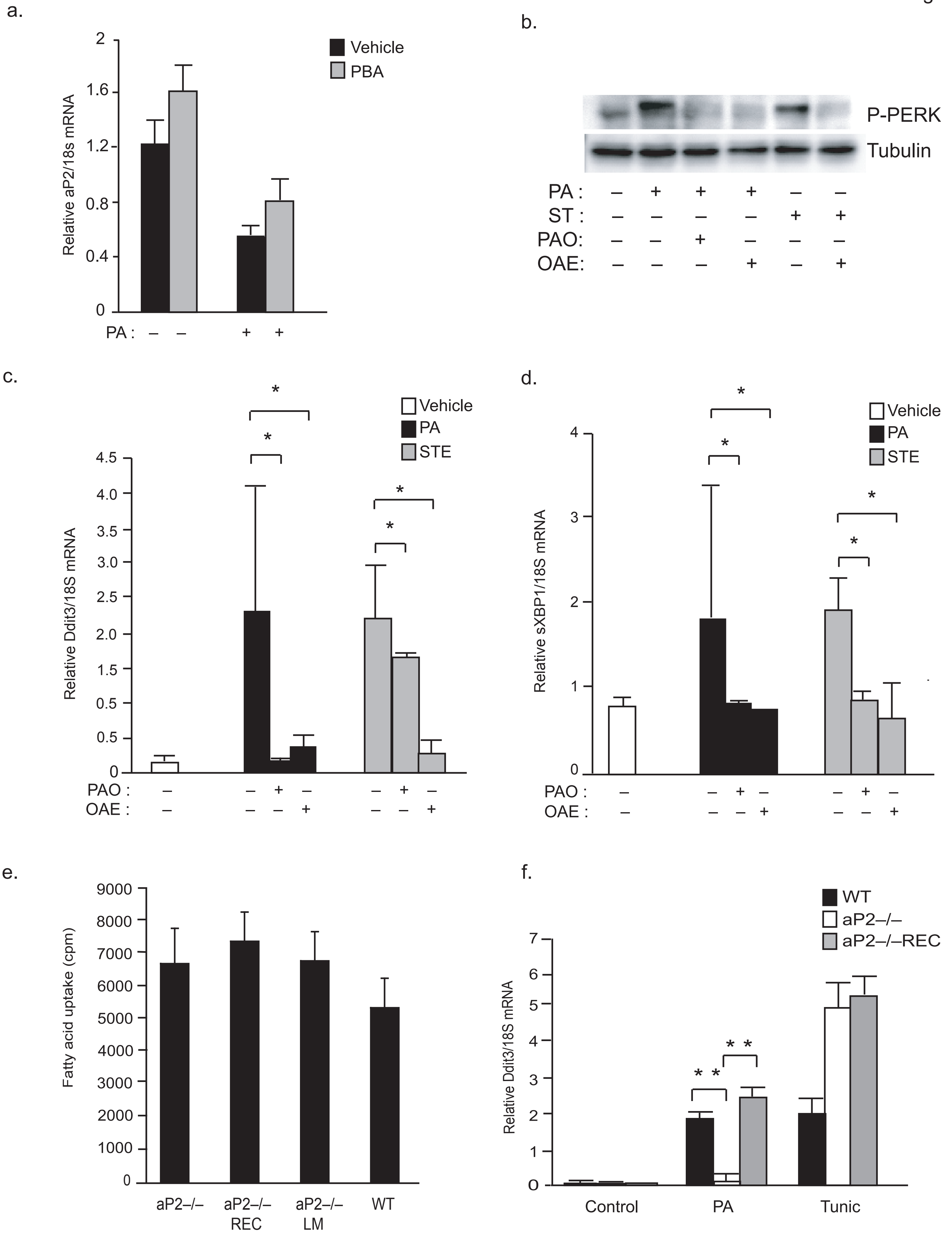
f.


Plasma insulin (ng/dL)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
Control
PBA

g.

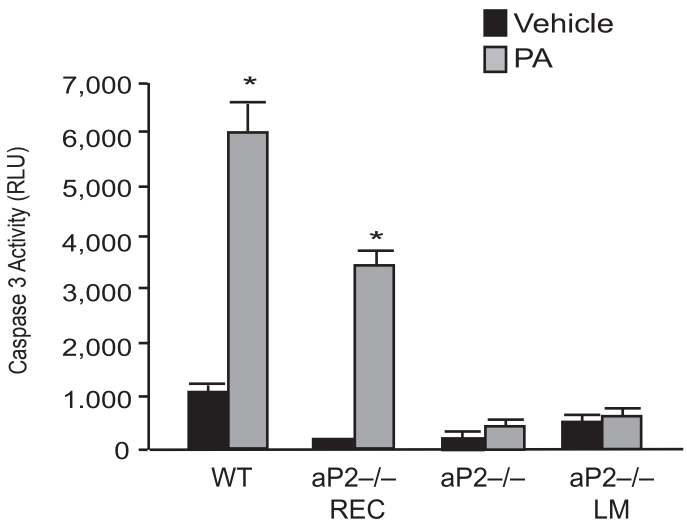

MOMA-2
P-eIF2α
MOMA-2
ATF-3
Control
PBA

S.Fig.2

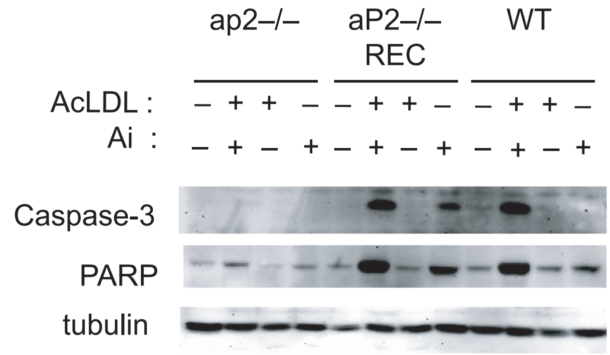


a.
Vehicle
PBA
Relative aP2/18s mRNA
2
1.6
1.2
0.8
0.4
0
PA : – – + +
b.
P-PERK
Tubulin
PA : – + + + – –
ST : – – – – + +
PAO: – – + – – –
OAE: – – – + – +
c.
Vehicle
PA
STE
Relative Ddit3/18S mRNA
4.5
4.0
3.5
3.0
2.5
2.0
1.5
0.5
0.5
0
PAO : – – + – – + –
OAE : – – – + – – +
d.
Vehicle
PA
STE
Relative sXBP1/18S mRNA
4
3
2
1
0
PAO : – – + – – + –
OAE : – – – + – – +
e.
Fatty acid uptake (cpm)
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
aP2–/–
aP2–/– REC
aP2–/– LM
WT
f.
WT
aP2–/–
aP2–/–REC
Relative Ddit3/18S mRNA
7
6
5
4
3
2
1
0
Control
PA
Tunic

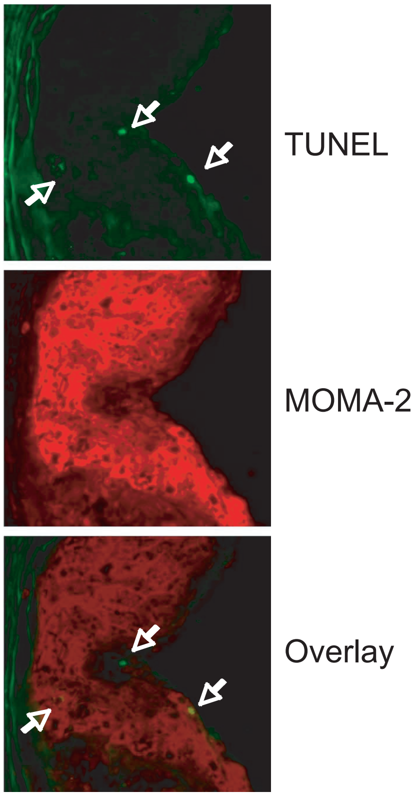
g.


Vehicle
PA
Caspase 3 Activity (RLU)
7,000
6,000
5,000
4,000
3,000
2,000
1,000
0
*
*
WT
aP2–/–
REC
aP2–/–
aP2–/–
LM

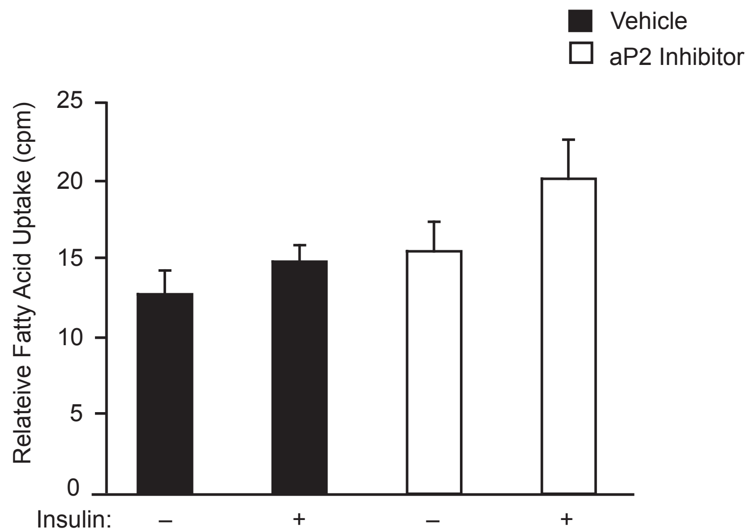
h.


ap2–/–
aP2–/–
REC
WT
AcLDL :
Ai :
Caspase-3
PARP
tubulin

a.

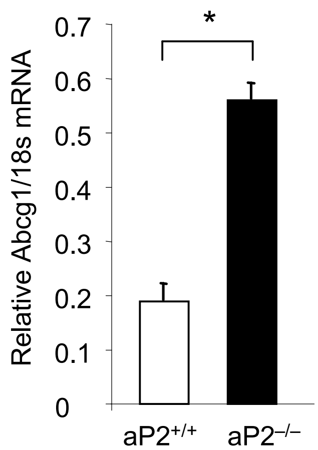

TUNEL
MOMA-2
Overlay

b.

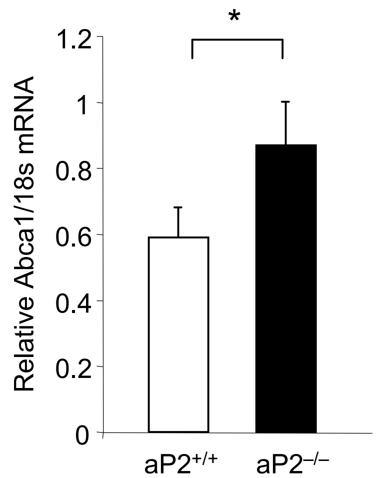

Vehicle
aP2 Inhibitor
Relateive Fatty Acid Uptake (cpm)
0
5
10
15
20
25
Insulin:
–
+
–
+

S.Fig.4

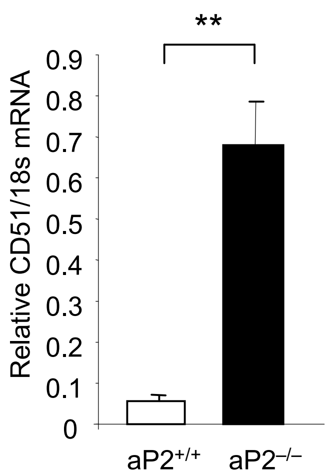
a.


Relative Abcg1/18s mRNA
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
*
aP2+/+
aP2–/–

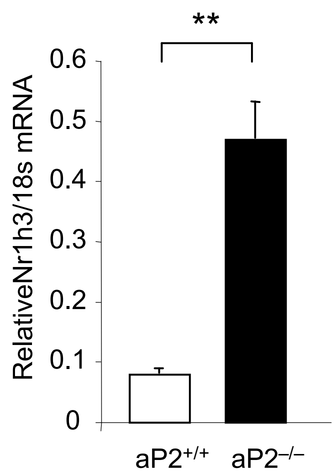
b.


Relative Abca1/18s mRNA
1.2
1
0.8
0.6
0.4
0.2
0
*
aP2+/+
aP2–/–

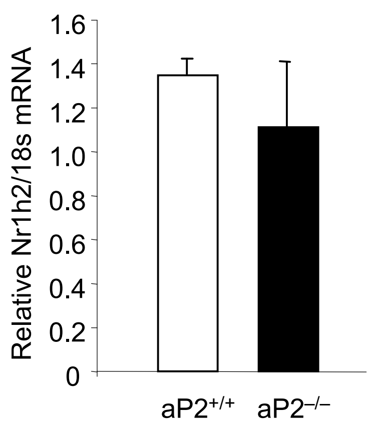
c.


Relative CD51/18s mRNA
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
**
aP2+/+
aP2–/–

d.

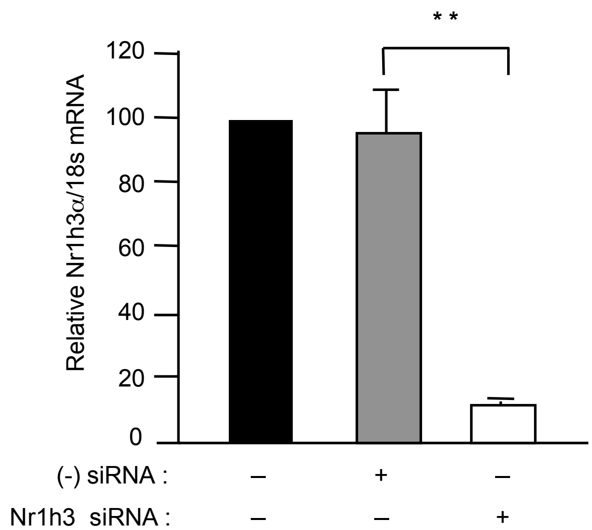

RelativeNr1h3/18s mRNA
0.6
0.5
0.4
0.3
0.2
0.1
0
**
aP2+/+
aP2–/–

e.


Relative Nr1h2/18s mRNA
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
aP2+/+
aP2–/–

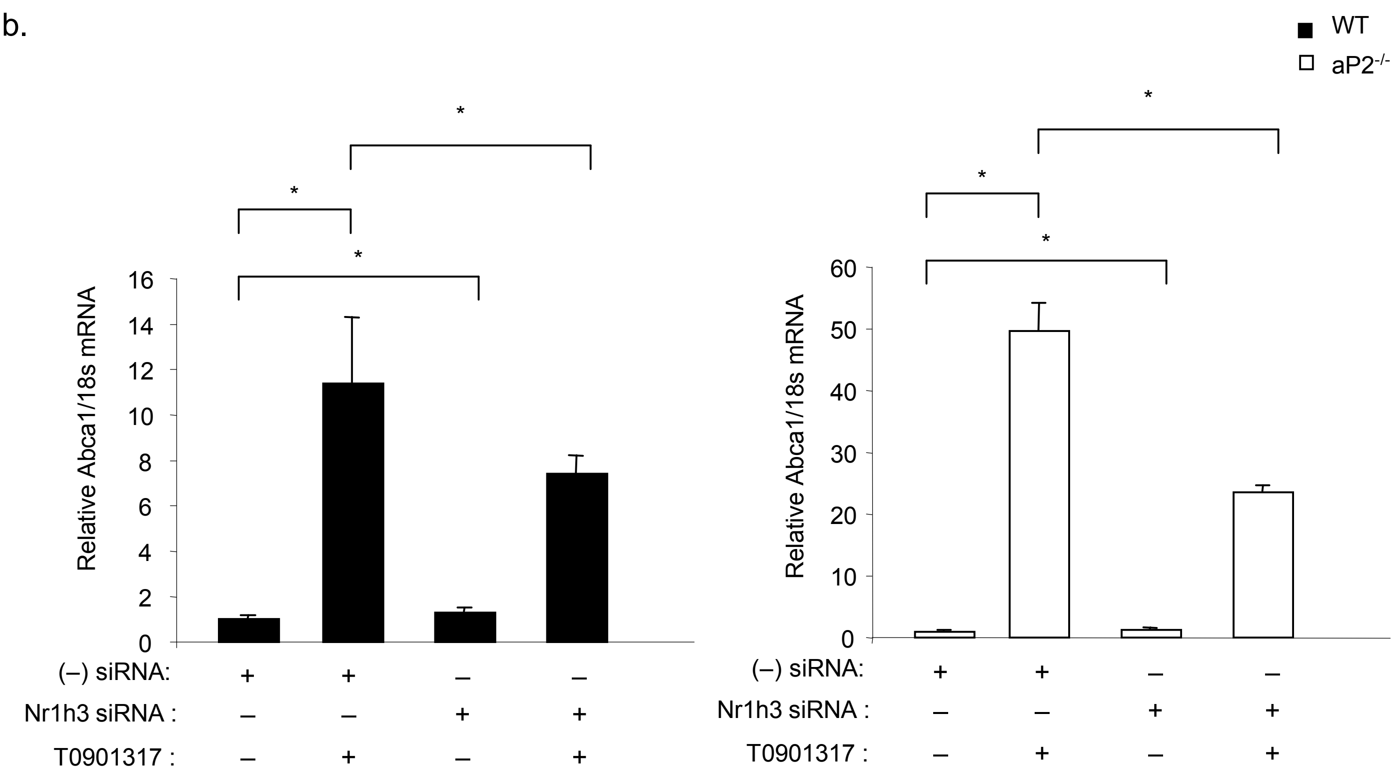
S.Fig 5

a.



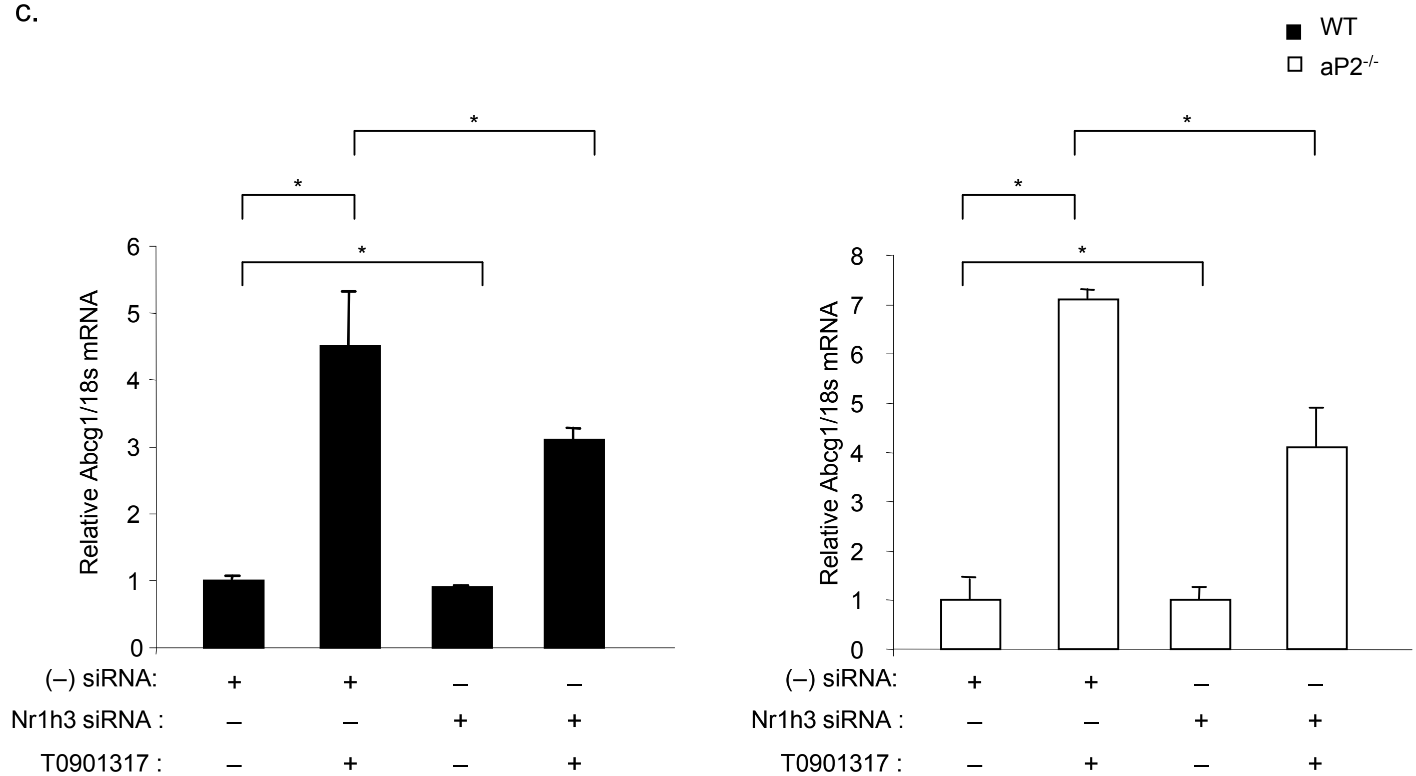
**
120
100
80
60
40
20
0
Relative Nr1h3α/18s mRNA
(-) siRNA : – + –
Nr1h3 siRNA : – – +

b.

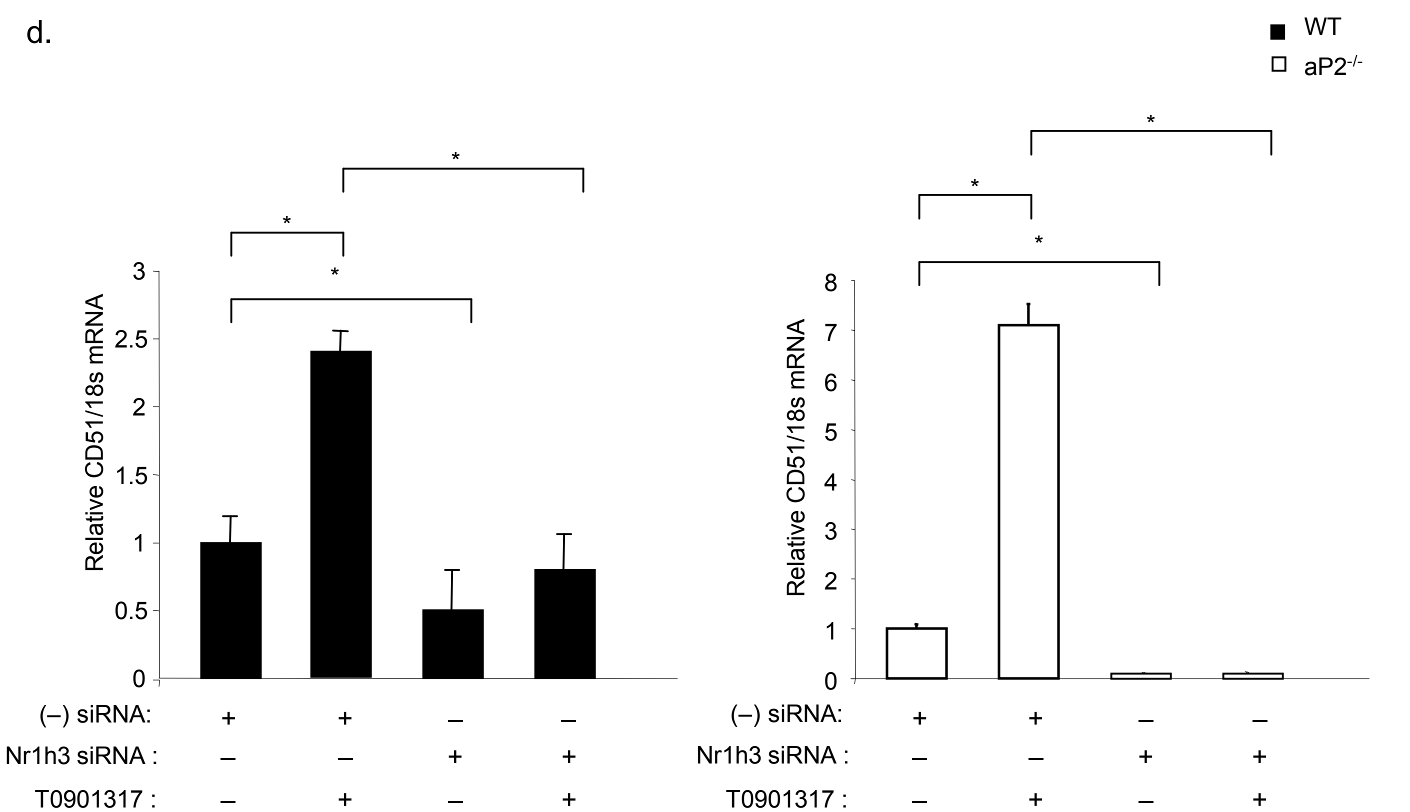


WT
aP2-/-
*
*
*
16
14
12
10
8
6
4
2
0
Relative Abca1/18s mRNA
(–) siRNA: + + – –
Nr1h3 siRNA : – – + +
T0901317 : – + – +
*
*
*
60
50
40
30
20
10
0
Relative Abca1/18s mRNA
(–) siRNA: + + – –
Nr1h3 siRNA : – – + +
T0901317 : – + – +

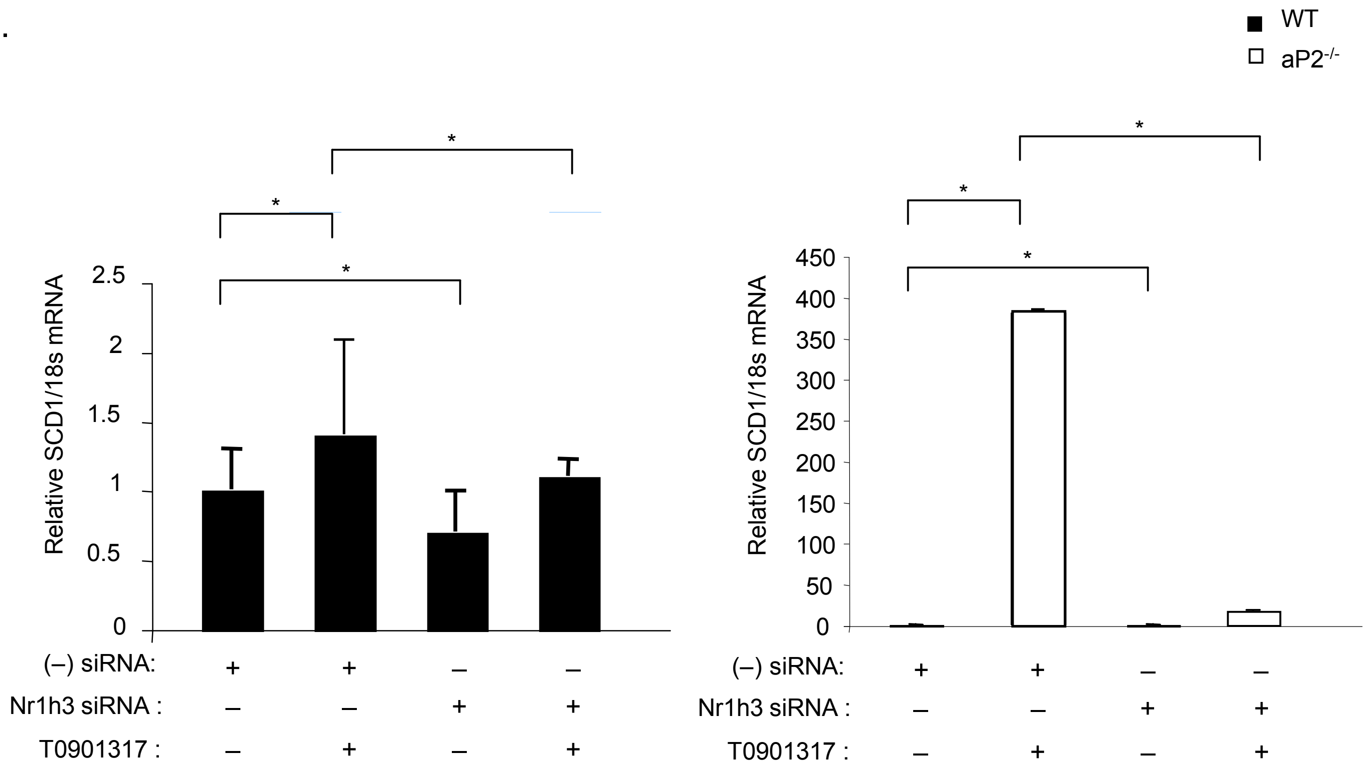
c.


WT
aP2-/-
Relative Abcg1/18s mRNA
0
1
2
3
4
5
6
7
8
*
(–) siRNA: + + – –
Nr1h3 siRNA : – – + +
T0901317 : – + – +

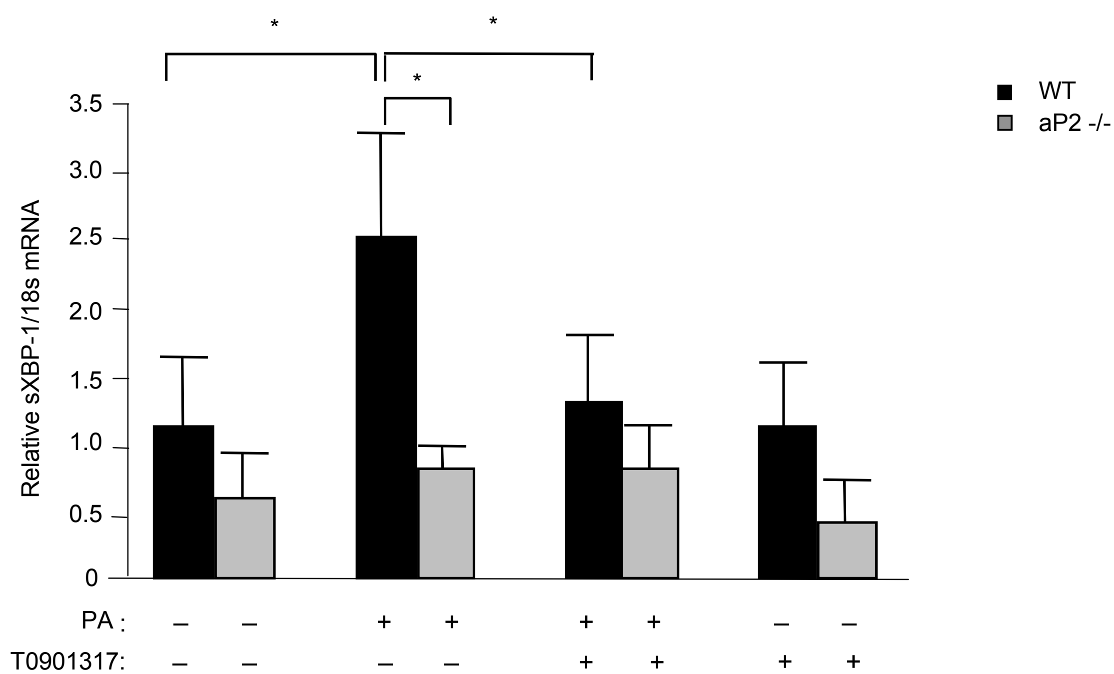
d.


WT
aP2-/-
Relative CD51/18s mRNA
0
0.5
1
1.5
2
2.5
3
4
5
6
7
8
*
(–) siRNA: + + – –
Nr1h3 siRNA : – – + +
T0901317 : – + – +

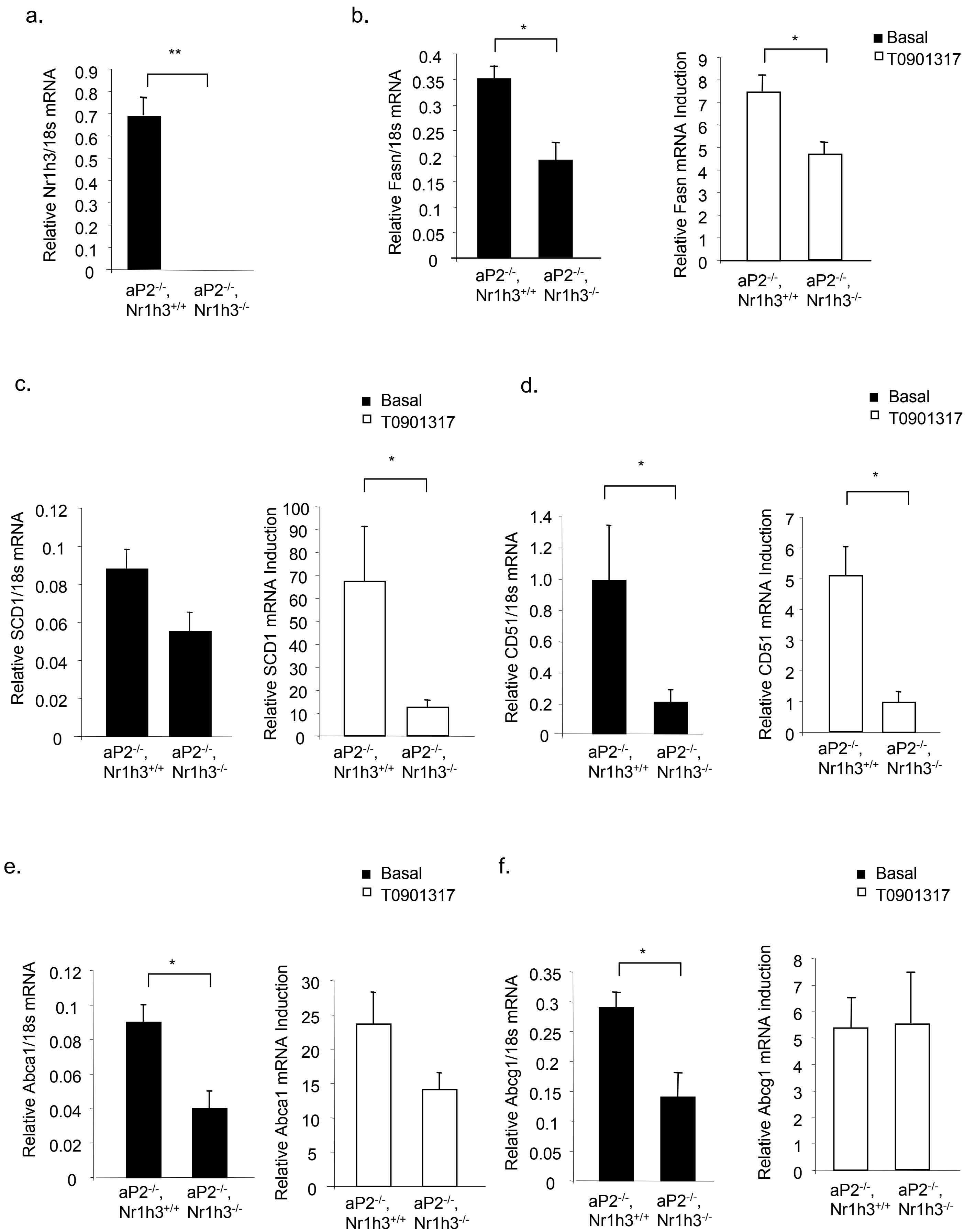
e.


WT
aP2-/-
Relative SCD1/18s mRNA
2.5
2
1.5
1
0.5
0
*
*
*
(–) siRNA: + + – –
Nr1h3 siRNA : – – + +
T0901317 : – + – +
Relative SCD1/18s mRNA
450
400
350
300
250
200
150
100
50
0
*
*
*
(–) siRNA: + + – –
Nr1h3 siRNA : – – + +
T0901317 : – + – +

f.


WT
aP2 -/-
Relative sXBP-1/18s mRNA
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
*
*
*
PA : – – + + + + – –
T0901317: – – – – + + + +

S.Fig.6


a.
**
Relative Nr1h3/18s mRNA
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9
aP2-/-, Nr1h3+/+
aP2-/-, Nr1h3-/-
b.
*
Relative Fasn/18s mRNA
0 0.05 0.1 0.15 0.2 0.25 0.3 0.35 0.4
Relative Fasn mRNA Induction
0 1 2 3 4 5 6 7 8 9
Basal
T0901317
c.
Relative SCD1/18s mRNA
0 0.02 0.04 0.06 0.08 0.1 0.12
Relative SCD1 mRNA Induction
0 10 20 30 40 50 60 70 80 90 100
d.
Relative CD51/18s mRNA
0 0.2 0.4 0.6 0.8 1.0 1.2 1.4
Relative CD51 mRNA Induction
0 1 2 3 4 5 6 7
e.
Relative Abca1/18s mRNA
0 0.02 0.04 0.06 0.08 0.1 0.12
Relative Abca1 mRNA Induction
0 5 10 15 20 25 30
f.
Relative Abcg1/18s mRNA
0 0.05 0.1 0.15 0.2 0.25 0.3 0.35
Relative Abcg1 mRNA induction
0 1 2 3 4 5 6 7 8

= Significant in Contrast of "aP2-/- vs aP2-/-REC" Supp.Table 1
Critical Value = .05

	Mean (SEM)*		
	WT	aP2-/-	aP2-/- REC
Metabolites			
CE14:0	326.95 (13.71)	207.12 (34.52)	383.72 (46.02)#
CE16:0	305.13 (28.25)	210.48 (26.66)	431.15 (32.92)#
CE18:0	227.36 (25.92)	81.77 (11.37)	240.85 (20.24)#
CE20:0	18.72 (2.8)	5.71 (0.83)	19.4 (1.03)#
CE22:0	5.89 (0.55)	1.97 (0.24)	7.89 (0.65)#
CE24:0	24.85 (5.93)	5.34 (1.96)	15.31 (2.28)
CE16:1n7	42.92 (6.65)	67.53 (5.84)	87.94 (9.76)
CE18:1n7	236.47 (29.08)	443.73 (25.5)	629.56 (70.58)#
CE18:1n9	512.23 (75.09)	392.41 (46.49)	931.1 (122.69)#
CE20:3n9	1.47 (0.26)	1.21 (0.33)	0.4 (0.1)
CE24:1n9	26.36 (3.84)	18.3 (1.67)	33.41 (2.92)#
CE18:2n6	20.84 (15.27)	11.24 (5.74)	21.09 (7.19)
CE18:3n6	0.92 (0.3)	0.77 (0.43)	0.49 (0.23)
CE20:4n6	30.57 (6.26)	36.73 (8.79)	70.13 (8.76)#
CE22:2n6	0.9 (0.13)	0.72 (0.12)	1.22 (0.22)#
CE18:3n3	0.54 (0.26)	0.63 (0.12)	2.87 (0.27)#
CE20:5n3	0.91 (0.23)	0.89 (0.42)	0.98 (0.24)
CE22:5n3	8.37 (2)	7.38 (2.36)	13.23 (1.68)
CE22:6n3	1.81 (0.6)	2.33 (1.11)	5.23 (1.22)#
CETL	1921.88 (161.95)	1536.81 (49.85)	3030.81 (320.91)#
CESFA	998.42 (131.62)	512.4 (67.65)	1098.32 (94.38)#
CEMUFA	845.15 (115.38)	954.17 (36.18)	1796.64 (219.94)#
CEPUFA	78.31 (23.28)	70.24 (18.29)	135.85 (10.46)#
CEn3	11.63 (2.59)	11.23 (3.45)	22.31 (1.85)#
CEn6	65.21 (20.84)	57.8 (15.08)	113.14 (9.4)#
CEn7	279.39 (35.73)	511.26 (29.87)	717.5 (80.31)#
CEn9	565.16 (79.29)	439.65 (54.22)	1072.88 (139.48)#
DG14:0	24.56 (3.7)	54.66 (4.66)	87.8 (9.91)#
DG15:0	2.72 (0.44)	4.24 (0.46)	5.01 (0.4)
DG16:0	148.51 (17.34)	298.62 (28.98)	306.4 (11.19)
DG18:0	133.62 (9.88)	108.35 (10.01)	161.97 (9.4)#
DG20:0	2.66 (0.35)	2.3 (0.1)	5.14 (0.5)#
DG22:0	1.75 (0.32)	2 (0.35)	2.63 (0.29)
DG24:0	2.71 (0.5)	2.08 (0.3)	3.15 (0.85)
DG14:1n5	0.97 (0.28)	1.52 (0.25)	1.96 (0.34)
DG16:1n7	25.69 (4.04)	72.87 (10.04)	52.01 (3.73)
DG18:1n7	76.21 (6.89)	158.66 (21.53)	139.28 (13.56)
DG18:1n9	237.51 (10.78)	303.38 (36.54)	477.82 (61.64)#
DG20:1n9	7.09 (0.57)	6.7 (0.93)	17.81 (3.19)#
DG20:3n9	5.39 (1.34)	1.69 (0.35)	1.57 (0.21)
DG22:1n9	3.3 (0.53)	3.9 (0.57)	5.11 (1.27)
DG24:1n9	12.97 (2.48)	14.87 (6.72)	6.1 (1.87)

DG18:2n6	19.64 (2.85)	17.53 (1.81)	23.23 (3.43)
DG20:2n6	2.72 (1.24)	1.83 (0.38)	3.96 (1.15)#
DG20:3n6	5.45 (0.68)	3.41 (0.69)	6.37 (0.78)#
DG20:4n6	30.45 (4.16)	27.99 (2.29)	34.91 (3.47)
DG22:4n6	1.58 (0.42)	1.58 (0.7)	11.13 (3.31)#
DG22:5n6	1.45 (0.23)	1.57 (0.24)	2 (0.63)
DG18:3n3	0.27 (0.23)	1.03 (0.37)	1.77 (0.63)
DG18:4n3	0.96 (0.58)	1.33 (0.44)	1.88 (0.99)
DG20:4n3	0.13 (0.06)	0.53 (0.22)	0.43 (0.1)
DG20:5n3	2.03 (0.9)	2.72 (1.58)	2.86 (0.68)
DG22:5n3	3.73 (0.3)	4.37 (0.78)	6.34 (1.66)
DG22:6n3	3.85 (0.4)	6.64 (1.33)	8.51 (1.44)
DGTL	383.53 (18.15)	551.99 (54.41)	697.3 (50.97)
DGSFA	317.76 (27.43)	472.24 (37.5)	572.1 (17.4)
DGMUFA	363.74 (20.6)	561.9 (67)	718.28 (87.33)#
DGPUFA	85.56 (10.89)	69.83 (8.09)	104.23 (7.76)#
DGn3	14.86 (5.56)	14.57 (2.7)	22.52 (2.79)
DGn6	66.66 (7.55)	53.57 (5.97)	80.45 (8.58)#
DGn7	101.89 (10.92)	231.53 (31.54)	191.28 (17.18)
DGn9	264.92 (12.65)	330.54 (36.7)	471.92 (61.17)#
FA14:0	37.75 (5.89)	65.37 (11.55)	69.3 (11.46)
FA15:0	4.96 (0.79)	6.94 (1.04)	9.05 (2.06)
FA16:0	161.37 (33.2)	328.59 (75.34)	366 (51.52)
FA18:0	137.39 (25.5)	191.8 (54.71)	244.35 (43.52)
FA20:0	4.11 (0.61)	3 (0.54)	5.77 (0.68)
FA22:0	4.3 (0.47)	3.79 (0.82)	4.65 (0.53)
FA24:0	7.04 (1.48)	6.64 (1.45)	7.17 (0.93)
FA14:1n5	2.58 (0.34)	3.97 (0.46)	3.96 (0.34)
FA16:1n7	13.34 (2.16)	77.24 (26.57)	53.9 (12.62)
FA18:1n7	48.65 (9)	211.17 (57.9)	250.12 (64.73)
FA18:1n9	133.71 (24.61)	347.31 (100.15)	370.39 (75.22)
FA20:1n9	3.02 (0.42)	7.69 (1.62)	16.91 (5.08)#
FA20:3n9	1.4 (0.34)	2.27 (0.93)	1.18 (0.26)
FA22:1n9	3.13 (0.75)	3.3 (0.76)	5.95 (1.08)#
FA24:1n9	12.37 (0.95)	14.92 (2.26)	14.53 (1.91)
FA18:2n6	8.77 (1.48)	16.02 (6.25)	15.62 (2.52)
FA20:2n6	0.73 (0.2)	1.66 (0.46)	4.12 (1.4)
FA20:3n6	0.81 (0.13)	4.28 (2.63)	2.81 (0.73)
FA20:4n6	19.22 (4.37)	43.78 (20.18)	31.57 (4.75)
FA22:2n6	0.55 (0.17)	0.43 (0.21)	0.64 (0.07)
FA22:4n6	2.02 (0.14)	2.89 (0.72)	3.59 (0.69)
FA22:5n6	0.35 (0.07)	1.61 (0.9)	0.97 (0.27)
FA18:3n3	0.6 (0.21)	1.08 (0.31)	1.07 (0.21)
FA18:4n3	0.19 (0.02)	0.8 (0.33)	0.94 (0.37)
FA20:5n3	0.87 (0.29)	1.8 (0.72)	1.74 (0.25)
FA22:5n3	2.07 (0.44)	7.39 (3.43)	4.43 (1.28)
FA22:6n3	2.17 (0.44)	10.2 (4.62)	6.84 (1.64)
FATL	614.83 (111.01)	1376.83 (375.04)	1634.6 (328.22)

FASFA	356.93 (66.3)	606.13 (143.45)	712.26 (110.85)
FAMUFA	216.79 (37.13)	665.59 (188.21)	839.47 (218.04)
FAPUFA	41.11 (7.99)	105.11 (45.38)	82.87 (16.08)
FAn3	5.85 (1.37)	21.4 (8.68)	14.75 (3.41)
FAn6	33.86 (7.08)	81.44 (35.98)	67.17 (13.72)
FAn7	61.99 (10.88)	288.41 (84.33)	304.02 (77.23)
FAn9	153.62 (26.33)	375.49 (104.54)	532.43 (140.94)

PL14:0	1032.18 (33.09)	1766.39 (46.91)	1066.87 (55.73)#
PL15:0	83.41 (2.37)	131.16 (4.1)	95.79 (3.62)#
PL16:0	5528.26 (184.12)	11913.91 (1045.58)	7721.08 (245.4)#
PL18:0	8792.84 (197.39)	8209.33 (693.81)	7680.21 (547.42)
PL20:0	66.27 (4.07)	40.94 (4.27)	88.93 (8.23)#
PL22:0	227.88 (26.85)	200.6 (40.46)	217.08 (30.32)
PL24:0	347.27 (47.24)	294.2 (55.67)	357.54 (66.04)
PL14:1n5	23.56 (0.85)	31.81 (5.35)	34.94 (1.16)
PL16:1n7	1672.49 (44.46)	4525.46 (556.48)	2119.86 (99.82)#
PL18:1n7	3842.25 (132.16)	9323.17 (947.16)	5110.12 (288.6)#
PL18:1n9	14179.03 (370.24)	19249.84 (1424)	15889.77 (1422.48)#
PL20:1n9	392.66 (9.64)	314.09 (36.49)	367.59 (35.58)
PL20:3n9	371.58 (9.22)	208.56 (62.54)	112.84 (8.31)
PL22:1n9	32.44 (1.42)	25.57 (2.9)	27.35 (1.75)
PL24:1n9	489.4 (66.57)	592.25 (77.06)	594.97 (71.92)
PL18:2n6	1441.06 (25.77)	1647.94 (116.13)	1437.71 (89.99)
PL18:3n6	14.6 (1.38)	17 (1.55)	13.04 (1.55)
PL20:2n6	95.82 (8.28)	119.01 (14.11)	102.7 (4.38)
PL20:3n6	499.81 (10.6)	463.16 (28.69)	490.29 (29.01)
PL20:4n6	2740.78 (89.39)	3276.45 (270.61)	2608.55 (128.52)#
PL22:4n6	337.18 (12.21)	355.91 (22.22)	340 (17.78)
PL22:5n6	128.78 (4.01)	152.11 (8.13)	128.8 (6.38)#
PL18:3n3	6.09 (0.3)	14.09 (1.35)	13.14 (1)
PL20:4n3	4.84 (0.71)	4.11 (1.19)	4.35 (1.03)
PL20:5n3	121.96 (5.18)	130.52 (25.48)	88.13 (6.06)
PL22:5n3	445.83 (16.57)	630.11 (82.97)	431.43 (22.35)#
PL22:6n3	600.76 (21.66)	811.39 (103.93)	579.14 (27.99)#
PLdm16:0	1369.76 (115.4)	1787.11 (284.67)	1392.81 (230.34)
PLdm18:0	1024.32 (20.19)	449.81 (68.74)	627.1 (97.86)
PLdm18:1n7	764.39 (31.5)	976.09 (115.53)	556.6 (72.79)#
PLdm18:1n9	446.96 (22.86)	317.06 (33.68)	255.11 (30.8)
PLTL	23565.02 (525.38)	32729.5 (3001.27)	25336.96 (1564.48)#
PLSFA	16078.11 (393.35)	22572.33 (1745.03)	17227.5 (775.81)#
PLMUFA	20636.4 (498.37)	31520.47 (3422.48)	24144.61 (1792.56)#
PLPUFA	6810.09 (192.27)	7836.15 (635.65)	6470.21 (309.71)#
PLn3	1180.48 (42.96)	1595.52 (214.75)	1120.68 (52.07)#
PLn6	5258.03 (140.68)	6032.06 (452.82)	5236.68 (254.69)#
PLn7	5514.74 (153.65)	15156.87 (1769.62)	7229.98 (366.97)#
PLn9	15469.69 (422.1)	20378.08 (1428.26)	16992.53 (1429.84)#
PLdm	3605.43 (153.98)	3530.07 (455.58)	2831.61 (405.12)

TG14:0	586.09 (32.53)	1057.83 (266.62)	827.9 (148.87)
TG15:0	30.44 (0.85)	40.62 (7.04)	39.96 (6.51)
TG16:0	2152.47 (67.98)	3972.27 (906.51)	3449.14 (529.19)
TG18:0	1594.01 (39.94)	1172.71 (238.29)	1769.31 (283.31)
TG20:0	49.63 (3.35)	41.65 (4.47)	77.25 (7.18)#
TG22:0	8.39 (0.42)	6.73 (0.88)	17.71 (0.9)#
TG24:0	11.64 (0.39)	9.95 (1.3)	22.11 (0.8)#
TG14:1n5	7.94 (0.63)	28.01 (6.44)	16.55 (1.85)#
TG16:1n7	228.3 (13.45)	690.93 (166.14)	337.02 (41.15)#
TG18:1n7	1196.11 (44.89)	2465.73 (560.49)	1523.57 (234.97)#
TG18:1n9	3271.27 (131.33)	3652.67 (701.61)	3676.08 (633.79)
TG20:1n9	151.64 (3.84)	114.44 (15.26)	191.14 (32.37)#
TG20:3n9	16.47 (1.5)	7.11 (1.14)	6.01 (0.81)
TG22:1n9	18.28 (0.96)	18.27 (1.75)	34.29 (4.7)#
TG24:1n9	10.23 (0.61)	10.53 (1.22)	27.51 (2.33)#
TG18:2n6	102.41 (3.61)	85.03 (14.89)	88.16 (11.28)
TG18:3n6	1.15 (0.27)	1.39 (0.37)	2.71 (0.87)
TG20:2n6	11.05 (0.76)	13.58 (3.28)	24.62 (8.97)
TG20:3n6	28.55 (1.64)	16.23 (2.01)	23.75 (3.54)#
TG20:4n6	148.42 (13.85)	143.1 (17.62)	145.1 (27.96)
TG22:2n6	0.99 (0.23)	1.31 (0.22)	3.02 (0.79)#
TG22:4n6	20.33 (1.75)	18.64 (3.29)	19.73 (3.8)
TG22:5n6	3.86 (0.26)	4.45 (1.14)	4.4 (0.46)
TG18:3n3	2.43 (0.37)	4.77 (0.48)	4.73 (0.88)
TG18:4n3	5.63 (1.34)	8.37 (2.59)	8.86 (1.62)
TG20:4n3	0.41 (0.12)	0.72 (0.2)	0.42 (0.16)
TG20:5n3	3.34 (0.48)	4.11 (1.42)	2.83 (0.41)
TG22:5n3	28.23 (1.85)	32.46 (4.4)	25.67 (5.08)
TG22:6n3	17.08 (1.19)	31.16 (4.6)	19.33 (3.06)#
TGTL	3235.59 (81.19)	4550.95 (970.62)	4129.44 (656.95)
TGSFA	4432.67 (131.62)	6301.76 (1414.95)	6203.38 (973.85)
TGMUFA	4883.76 (194.16)	6980.58 (1451.49)	5806.16 (950.15)
TGPUFA	390.34 (24.77)	370.52 (49.35)	378.79 (53.6)
TGn3	57.11 (3.51)	80.48 (11.87)	61.84 (10.4)
TGn6	316.76 (20.38)	282.93 (38.83)	310.95 (42.66)
TGn7	1424.41 (58.06)	3156.66 (726.12)	1860.59 (275.71)#
TGn9	3467.88 (137.33)	3803.02 (718.91)	3935.03 (673.41)

Supplemental Figure Legends:

S.Fig.1: ***Macrophage apoptosis and systemic response to PBA treatment in lipotoxic conditions*** **(a)** ER stress was induced in wild-type, bone marrow derived, mouse macrophage lines by 500 μM PA with or without the presence of 3 μM PBA where indicated. Apoptosis was measured by performing TUNEL assays. **(b)** Total lesion area was determined from enface aorta preparations of control mice treated with PBS or 100 mg kg^{-1} PBA (n≥13). **(c-f)** Serum triglyceride **(c)**, cholesterol **(d)**, plasma glucose **(e)** and insulin **(f)** were determined from control and 100 mg kg^{-1} PBA treated mice at the time of sacrifice (n≥13). **(g)** Double immunofluorescent staining was performed in the same section from lesions of *ApoE*$^{-/-}$ mice treatd with PBA (100 mg kg^{-1}) or vehicle using antibodies against MOMA-2 and ATF3 or MOMA-2 and P-eIF2–α (Arrows indicate ER stress marker expression, ATF3 or P-eIF2–α, in macrophage-rich areas of the lesions.).

S.Fig.2: ***Fatty acid induced stress response*** **(a)** Relative *aP2* mRNA was measured by qRT-PCR in macrophage lines treated with PA (500 μM) with or without the presence of PBA (3 mM). **(b-d)** ER stress was assessed in bone marrow derived macrophages upon 500 μM PA or ST treatment. PERK phosphorylation **(a)**, *Ddit3* **(b)** and s*XBP-1* induction **(c)** in bone marrow-derived macrophages can be prevented by simultaneous addition of palmitoleate (PA) or oleate (OAE) (500 μM). **(e)** C^{14} labeled PA uptake was determined in various bone marrow-derived macrophage lines. **(f)** Relative *Ddit3* mRNA was determined by qRT-PCR from bone marrow-derived macrophage lines treated with PA (500 μM) or tunicamycin (2 mg mL^{-1}) for 12 hours. **(g)** The apoptotic indicator, caspase 3/7 activity, was examined in the various bone marrow derived macrophage lines after treatment with vehicle or PA (500 μM) and reported as relative luciferase units (RLU). **(h)** Apoptosis was assessed in bone marrow derived macrophage lines in the absence or presence of AcLDL and ACAT inhibitor by detecting active caspase 3 and cleaved PARP by Western blotting.

S.Fig.3: ***Macrophage apoptosis in lesions and aP2i effect on fatty acid uptake*** **(a)** TUNEL assay and immunofluorescent staining was performed in the same section from atherosclerotic lesion of *ApoE*$^{-/-}$ mice. Arrows point to TUNEL positive cells (green) in MOMA-2 staining macrophages (red). **(b)** C^{14} labeled palmitic acid uptake was determined in insulin and vehicle or aP2-i treated bone marrow-derived macrophage lines.

S.Fig.4: ***Endogenous gene expression in aP2***$^{-/-}$ ***and WT macrophages*** **(a)** *Abcg1* **(b)**, *Abca1* **(c)**, *CD51*, **(d)** *Nr1h3* and **(e)** *Nr1h2*, mRNA levels were determined from *aP2*$^{-/-}$ and WT macrophages by qRT-PCR. Student's t-test was performed to statistically analyze the quantitative data and all data shown represent mean±; SEM; * indicates $p<0.05$ and ** indicates $p<0.01$.

S.Fig.5: ***LXRα activity protects against PA-induced macrophage ER stress:*** **(a)** 100nM of *Nr1h3* siRNA suppressed LXR–α expression more than 80% in bone marrow derived WT macrophage line as determined by qRT-PCR. The LXR-regulated genes, **(b)** *Abca1*, **(c)** *Abcg1*, **(d)** *CD51* and **(e)** *SCD-1*, were reduced to varying degrees in the *Nr1h3* siRNA treated *aP2*$^{-/-}$ or WT macrophages in the presence or absence of an LXR agonist T0901317 (10 μM) treatment for 24 hours. **(f)** Pretreatment of macrophages with T0901317 (10 μM) prevents PA-induced *XBP-1* mRNA splicing in WT macrophage line (data represent mean±; SEM; * indicates $p<0.05$ and ** indicates $p<0.01$).

S.Fig.6: ***Endogenous gene expression in aP2***$^{-/-}$ ***and aP2***$^{-/-}$***Nr1h3***$^{-/-}$ ***primary peritoneal macrophages*** **(a)** *Nr1h3*, **(b)** *Fasn*, **(c)** *SCD-1* **(d)** *CD51*, **(e)** *Abca1* and **(f)** *Abcg1* mRNA levels were determined by qRT-PCR from *aP2*$^{-/-}$ and *aP2*$^{-/-}$

Nr1h3$^{-/-}$ macrophages treated with vehicle (basal) or T0901317 (10 μM) for 24 hours (data represent mean±; SEM; * indicates $p<0.05$ and ** indicates $p<0.01$).

S.Table-1: Distribution of fatty acids in various lipid species. Quantitative measurement of various fatty acids was determined for WT, *aP2*$^{-/-}$, *aP2*$^{-/-}$REC macrophages. (CE: cholesterol ester, DG: diacylglcyerol, FA: fatty acid, PL: phospholipid, TL: total lipids FAS: Fattyacid synthase, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids). Statistical analysis: In order to evaluate the effects of genotype, a one-way ANOVA was used. All data represent mean±; SEM; * indicates $p<0.05$, n=5.