

Supporting information

Index of supplementary tables and figure:

Supplementary Table S1. Primer pairs used for qPCR analysis.

Supplementary Table S2. Primary antibodies used for western blot analysis.

Supplementary Table S3. Biochemical analysis of serum and liver.

Supplementary Figure S1. PEM does not affect FA/TG metabolism in eWAT.

Supplementary Figure S2. PEM does not affect FA/TG metabolism in BAT.

Supplementary Figure S3. Photomicrographs of hematoxylin and eosin-stained heart and kidney tissue.

Supplementary Figure S4. Clinically relevant dose of PEM activates hepatic PPAR α without enhancing PPAR β or PPAR γ .

Supplementary Figure S5. Full-length blots

Supplementary Table S1. Primer pairs used for qPCR analysis.

Gene	Accession #	Primer sequence (5'-3')
<i>18S rRNA</i>	NR_003278	F 5'-CACGGACAGGATTGACAGATTG-3' R 5'-CAGACAAATCGCTCCACCAA-3'
<i>Acaa1</i>	NM_130864	F 5'-TCTACGGTCAACAGACAGTGTTC-3' R 5'-GGCCATGCCAATGTCATAAGA-3'
<i>Acaca</i>	NM_133360	F 5'-GGGCACAGACCGTGGTAGTT-3' R 5'-CAGGATCAGCTGGGATACTGAGT-3'
<i>Acadl</i>	NM_007381	F 5'-AGAAACATGGCGGCATTG-3' R 5'-CAATATCTGAGTGGAGGCTGAAG-3'
<i>Acadm</i>	NM_007382	F 5'-TGCTTTTGATAGAACCAGACCTACAGT-3' R 5'-CTTGGTGCTCCACTAGCAGCTT-3'
<i>Acads</i>	NM_007383	F 5'-CTCCACAGCTAACCTCATCTTTG-3' R 5'-GGGTTTGCATGGCTATTTTG-3'
<i>Acadvl</i>	NM_017366	F 5'-GCGTGTGCTCCGAGATATTC-3' R 5'-CCAGTGAGTTCCTTTCCTTTG-3'
<i>Acox1</i>	NM_015729	F 5'-TGGTATGGTGTGCTACTTGAATGAC-3' R 5'-AATTTCTACCAATCTGGCTGCAC-3'
<i>Acs11</i>	NM_007981	F 5'-TCCTACGGCAGTGATCTGGTG-3' R 5'-GGTTGCCTGTAGTTCCACTTGTG-3'
<i>Apob</i>	NM_009693	F 5'-TCACCCCCGGGATCAAG-3' R 5'-TCCAAGGACACAGAGGGCTTT-3'
<i>Cd36</i>	NM_007643	F 5'-CCAAATGAAGATGAGCATAGGACAT-3' R 5'-GTTGACCTGCAGTCGTTTTGC-3'
<i>Ces1d</i>	NM_053200	F 5'-TGGTATTTGGTGTCCCATCA-3' R 5'-GCTTGGGCGATACTCAAAC-3'
<i>Cpt1</i>	NM_013495	F 5'-TGGCATCATCACTGGTGTGTT-3' R 5'-GGTCCGATTGATCTTTGCAATC-3'
<i>Cpt2</i>	NM_009949	F 5'-ATCGTACCCACCATGCACTAC-3' R 5'-CTGTCATTCAAGAGAGGCTTCTG-3'
<i>Dgat1</i>	NM_010046	F 5'-CTGCTACGACGAGTTCTTGAGA-3' R 5'-GATAGTAGGGACCATCCACTGTTG-3'
<i>Dgat2</i>	NM_026384	F 5'-GCTTCGCGAGTACCTGATGT-3' R 5'-CACCACGATGATGATAGCATTG-3'
<i>Ehhadh</i>	NM_023737	F 5'-CGATACTCTTCCCCCACTACCA-3' R 5'-CAGTTACCAACAACGACTCCAATC-3'
<i>Fabp1</i>	NM_017399	F 5'-GCAGAGCCAGGAGAACTTTGAG-3' R 5'-TTTGATTTTCTTCCCTTCATGCA-3'
<i>Fasn</i>	NM_007988	F 5'-ATCCTGGAACGAGAACACGATCT-3' R 5'-AGAGACGTGTCACTCCTGGACTT-3'

<i>Hsl</i>	NM_010719	F 5'-GAGCGCTGGAGGAGTGTTTT-3' R 5'-TGATGCAGAGATTCCCACCTG-3'
<i>Lipc</i>	NM_008280	F 5'-ACGGGAAGAACAAGATTGGAAG-3' R 5'-CGTTCCCTCAAACATAGGGC-3'
<i>Lpl</i>	NM_008509	F 5'-CGCTCCATTCATCTCTTCATT-3' R 5'-GGCAGAGCCCTTTCTCAAAGG-3'
<i>Mttp</i>	NM_008642	F 5'-GAGCGGTCTGGATTTACAACG-3' R 5'-GTAGGTAGTGACAGATGTGGCTTTTG-3'
<i>Ppargc1a</i>	NM_008904	F 5'-GACTCAGTGTCACCACCGAAATC-3' R 5'-GACCTGTGTGCGAGAAAAGGATCTT-3'
<i>Pnpla2</i>	NM_025802	F 5'-CGTGTTTCAGACGGAGAGAAC-3' R 5'-TTGGAGGGTAGGAGGAATGAG-3'
<i>Slc25a20</i>	NM_020520	F 5'-GAGCCGAAACCCATCAGTCC-3' R 5'-CAGTCGGACCTTGACCGTG-3'
<i>Slc27a1</i>	NM_011977	F 5'-ACCACCGGGCTTCCTAAGG-3' R 5'-CTGTAGGAATGGTGGCCAAAG-3'
<i>Ucp1</i>	NM_009463	F 5'-AGGATGGTGAACCCGACAAC-3' R 5'-GGCCTTCACCTTGGATCTGA-3'

F, forward sequence; R, reverse sequence.

Acaa1, acetyl-CoA acyltransferase 1

Acaca, acetyl-CoA carboxylase α

Acadl, long-chain acyl-CoA dehydrogenase

Acadm, medium-chain acyl-CoA dehydrogenase

Acads, short-chain acyl-CoA dehydrogenase

Acadvl, very long-chain acyl-CoA dehydrogenase

Acox1, acyl-CoA oxidase 1

Acs1l, long chain acyl-CoA synthase

Apob, apolipoprotein B

Cd36, fatty acid translocase

Ces1d, carboxylesterase 1d

Cpt1, carnitine palmitoyl transferase 1

Cpt2, carnitine palmitoyl transferase 2

Dgat1, diacylglycerol acyltransferase 1

Dgat2, diacylglycerol acyltransferase 2

Ehhadh, enoyl-Coenzyme A, hydratase/3-hydroxyacyl Coenzyme A dehydrogenase

Fabp1, fatty acid binding protein 1

Fasn, fatty acid synthase

Hsl, hormone-sensitive lipase

Lipc, hepatic lipase

Lpl, lipoprotein lipase

Mttp, microsomal triglyceride transfer protein

Ppargc1a, peroxisome proliferator-activated receptor gamma coactivator 1
alpha

Pnpla2, patatin-like phospholipase domain containing 2

Slc25a20, mitochondrial carnitine/acylcarnitine translocase

Slc27a1, fatty acid transport protein 1

Ucp1, uncoupling protein 1

Supplementary Table S2. Primary antibodies used for western blot analysis.

Protein name	Abbreviation	Manufacturer	Catalog #	Dilution
4-hydroxy-nonenal modified protein	4HNE	JalCA (Shizuoka, Japan)	#MHN-020P	1:100
Acetyl-CoA carboxylase alpha	ACC α	Santa Cruz Biotechnology (Dallas, TX)	#sc-30212	1:200
Actin, cytoplasmic 1	ACTB	Abcam (Cambridge, MA)	#ab8227	1:1000
Cluster of differentiation 36	CD36	Biotechnology (Dallas, TX)	#sc-9154	1:200
Fatty acid synthase	FAS	Biotechnology (Dallas, TX)	#sc-16147	1:200
Histone H1	Histone H1	Santa Cruz Biotechnology (Dallas, TX)	#sc-10806	1:200
Microsomal TG transfer protein	MTP	Santa Cruz Biotechnology (Dallas, TX)	#sc-33116	1:200
Peroxisome proliferator-activated receptor α	PPAR α	Santa Cruz Biotechnology (Dallas, TX)	#sc-9000	1:200

Other antibodies used were described previously (Aoyama et al., J Biol Chem 1998).

Supplementary Table S3. Biochemical analysis of serum and liver.

Serum biochemical characteristics

Item	Control group (n=8)	PEM-0.1 group (n=10)	PEM-0.3 group (n=9)
Glucose (mg/dL)	205.8 ± 55.90	212.93 ± 75.06	174.96 ± 35.61
Insulin (ng/dL)	0.37 ± 0.13	0.30 ± 0.12	0.19 ± 0.14
HMW adiponectin (mg/dL)	129.86 ± 93.27	107.36 ± 67.91	104.53 ± 58.63

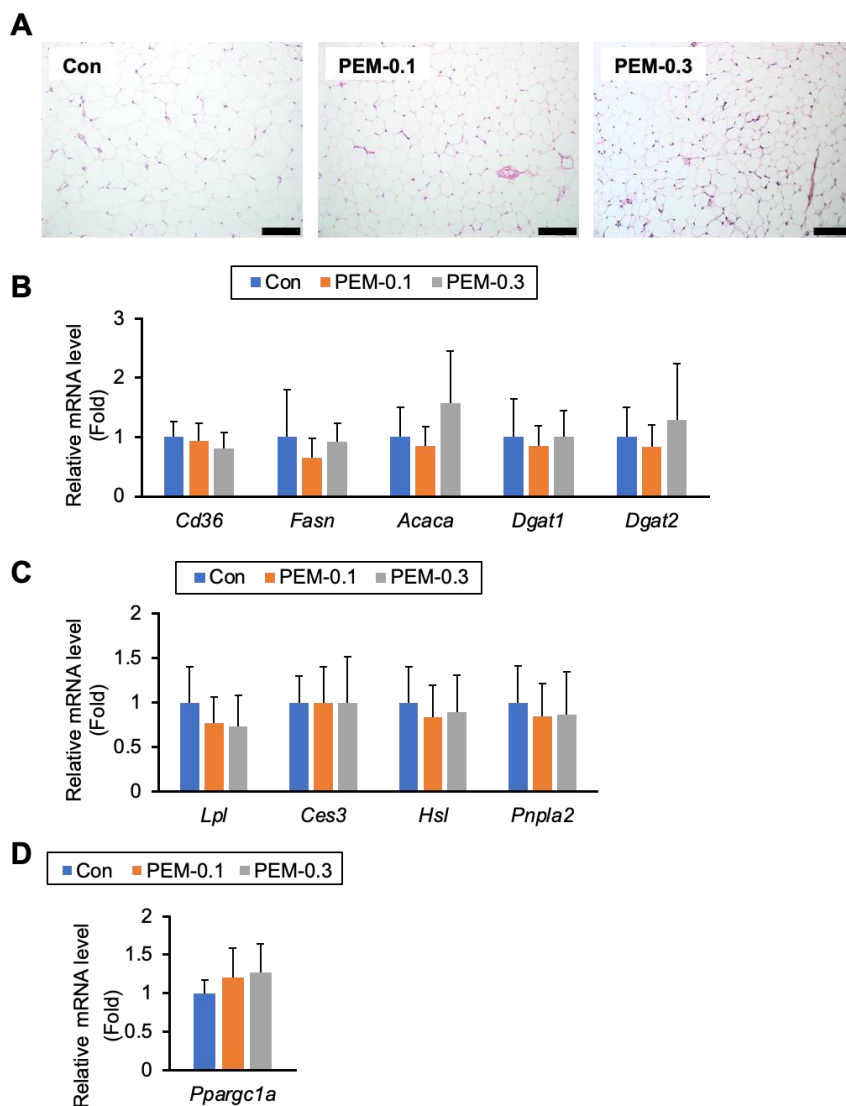
Liver biochemical characteristics

Item	Control group (n=8)	PEM-0.1 group (n=10)	PEM-0.3 group (n=9)
PL (mg/g liver)	48.03 ± 10.85	43.19 ± 9.48	47.23 ± 14.19

Values are expressed as the mean ± SD.

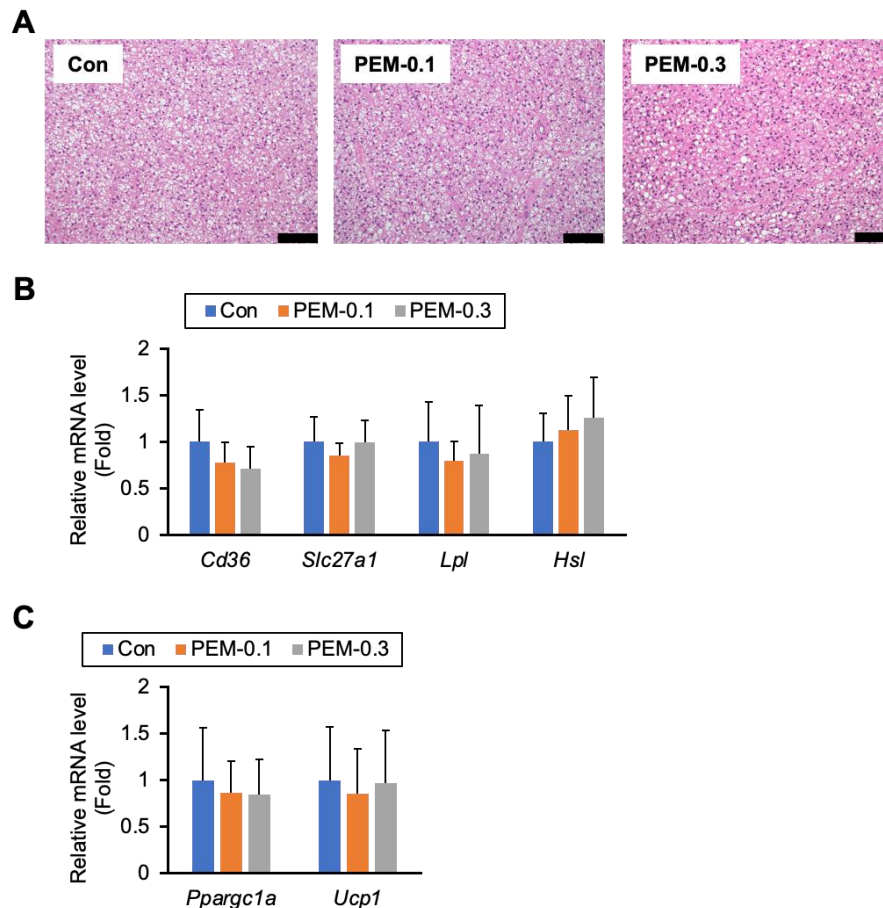
Supplementary Figure S1. PEM does not affect FA/TG metabolism in eWAT.

(A) Representative photomicrographs of hematoxylin and eosin-stained eWAT sections. Scale bar= 50 μ m. (B-D) Hepatic mRNA levels of genes related to FA uptake (*Cd36*), FA synthesis (*Fasn* and *Acaca*), TG synthesis (*Dgat1* and *Dgat2*), TG decomposition (*Lpl*, *Ces1b*, *Hsl* and *Pnpla2*), and fat burning (*Ppargc1a*) were quantified by qPCR, normalized to that of 18s ribosomal RNA levels, and expressed as values relative to those of control mice. Data are expressed as the mean \pm SD. Con, vehicle-treated mice; PEM-0.1, pemafibrate-treated mice at a clinically relevant dose (0.1 mg/kg/day); PEM-0.3, pemafibrate-treated mice at a relatively high dose (0.3 mg/kg/day).



Supplementary Figure S2. PEM does not affect FA/TG metabolism in BAT.

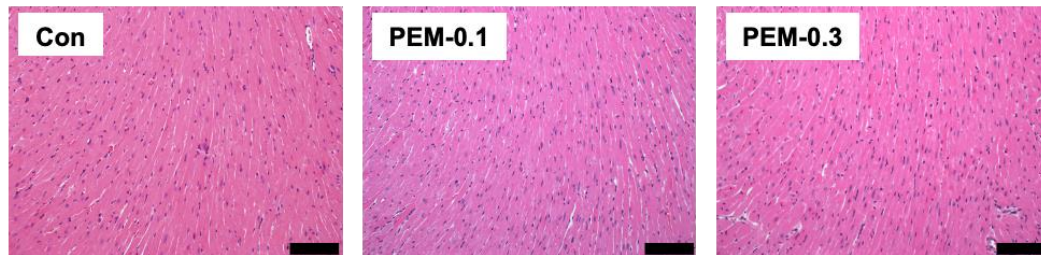
(A) Representative photomicrographs of hematoxylin and eosin-stained BAT sections. Scale bar= 50 μ m. (B-D) Hepatic mRNA levels of genes related to FA uptake (*Cd36*), intracellular FA transport (*Slc27a1*), TG decomposition (*Lpl* and *Hsl*), and fat burning (*Ppargc1a* and *Ucp1*) were quantified by qPCR, normalized to that of 18s ribosomal RNA levels, and expressed as values relative to those of control mice. Data are expressed as the mean \pm SD. Con, vehicle-treated mice; PEM-0.1, pemaifibrate-treated mice at a clinically relevant dose (0.1 mg/kg/day); PEM-0.3, pemaifibrate-treated mice at a relatively high dose (0.3 mg/kg/day).



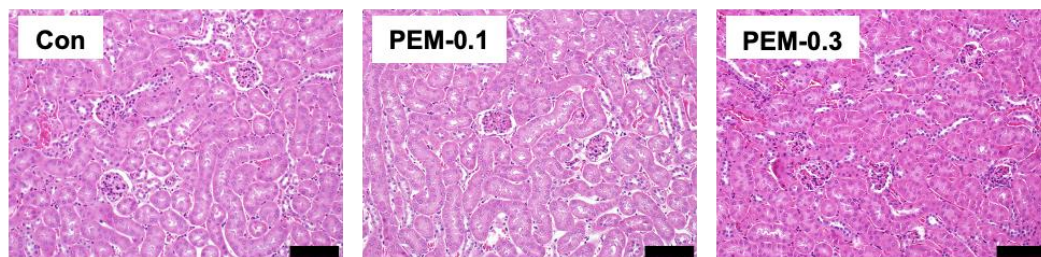
Supplementary Figure S3. Representative photomicrographs of hematoxylin and eosin-stained heart (A) and kidney (B) tissue.

Scale bar= 50 μ m. Con, vehicle-treated mice; PEM-0.1, pemaibrate-treated mice at a clinically relevant dose (0.1 mg/kg/day); PEM-0.3, pemaibrate-treated mice at a relatively high dose (0.3 mg/kg/day).

A

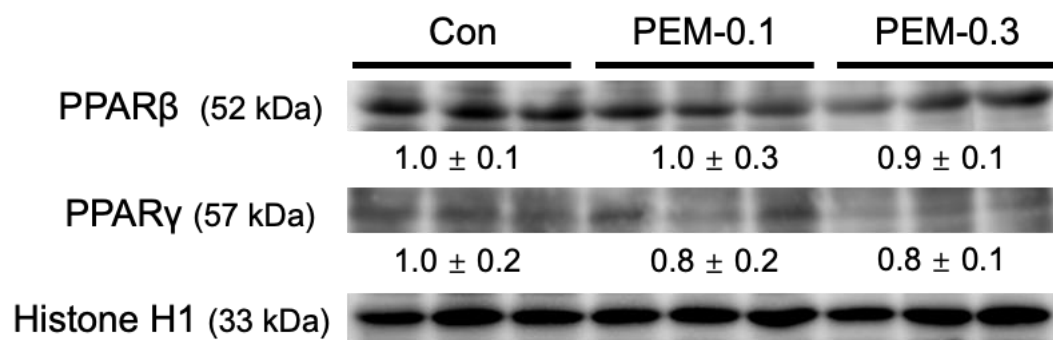


B



Supplementary Figure S4. Clinically relevant dose of PEM activates hepatic PPAR α without enhancing PPAR β or PPAR γ .

Immunoblot analysis of PPAR β and PPAR γ . The band of histone H1 was used as a loading control. Data are expressed as the mean \pm SD. Con, vehicle-treated mice; PEM-0.1, pemafibrate-treated mice at a clinically relevant dose (0.1 mg/kg/day); PEM-0.3, pemafibrate-treated mice at a relatively high dose (0.3 mg/kg/day).



Supplementary Figure S5. Full-length blots.

The protein bands inside the black dotted lines are used in this paper.

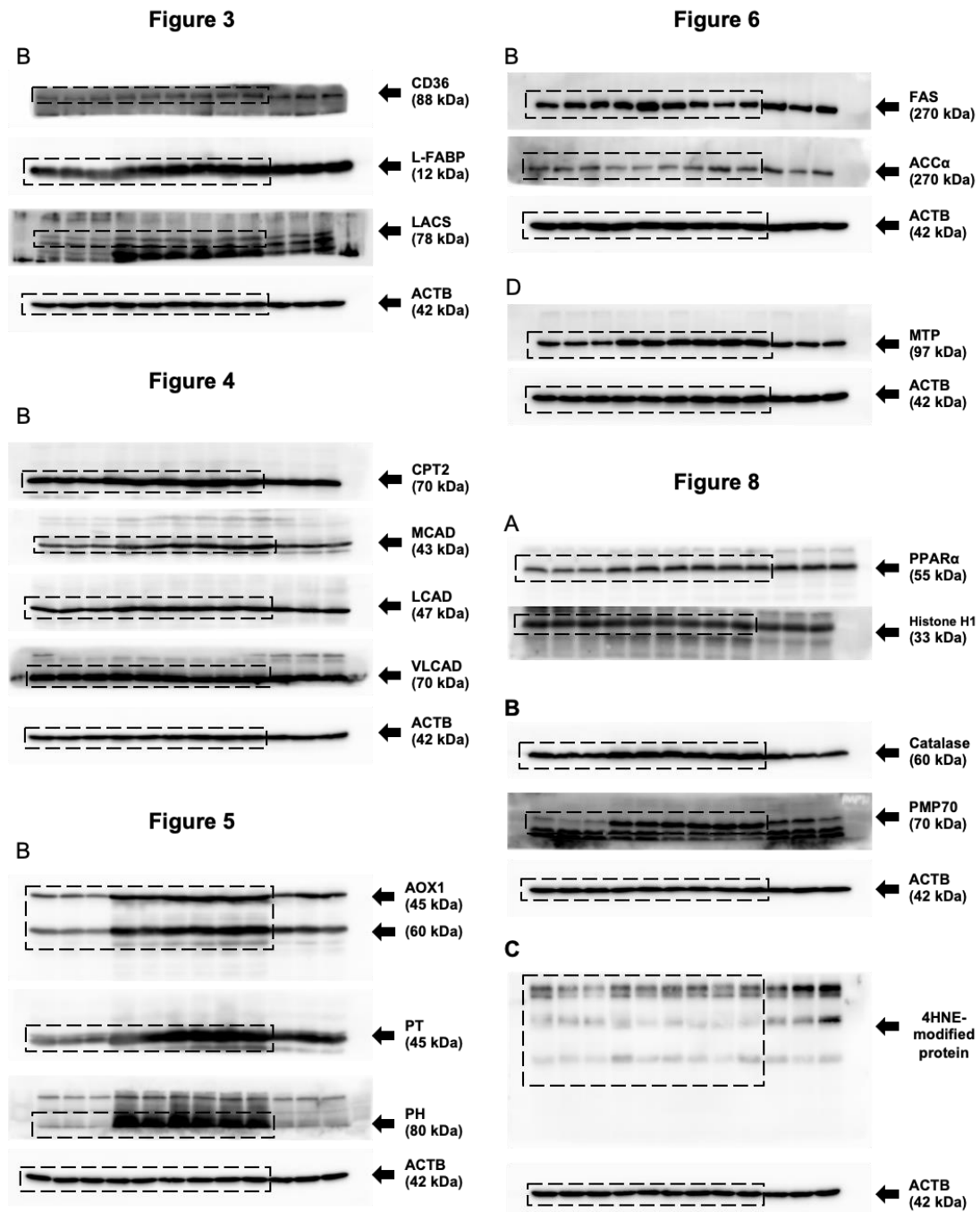


Figure S4

