ONLINE APPENDIX

Cannabinoid receptor stimulation impairs mitochondrial biogenesis in mouse white adipose tissue, muscle, and liver: the role of eNOS, p38 MAPK, and AMPK pathways

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SUPPLEMENTAL DATA

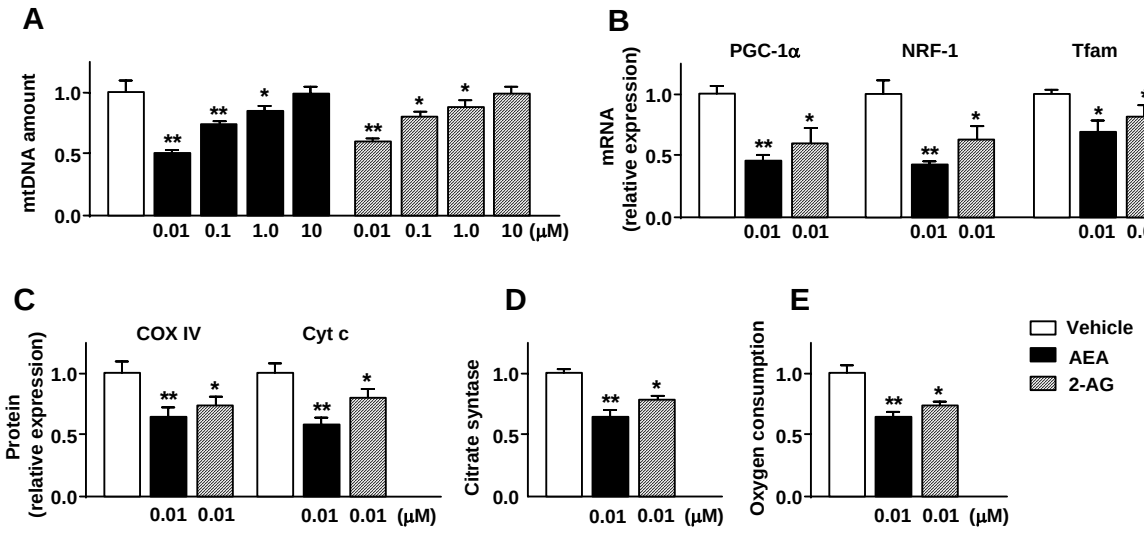

Figure S1.

The endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG) decrease mitochondrial biogenesis in cultured mouse white adipocytes. *A*: mtDNA amount, analyzed by means of quantitative PCR in white adipocytes treated either with vehicle (0.002% DMSO) or different doses of endocannabinoids for 2 days. Values of the vehicle-treated cells were taken as 1.0 (n = 3 experiments). *B*: PGC-1α, NRF-1, and Tfam mRNA levels were analyzed by means of quantitative RT-PCR The cycle number at which the various transcripts were detectable was compared to that of β-actin, and expressed as relative expression versus values in vehicle-treated cells taken as 1.0 (n = 3 experiments). *C*: COX IV and Cyt c protein levels detected by immunoblot with densitometric analysis referred to β-actin, with values from vehicle-treated cells taken as 1.0 (n = 3 experiments). *D*: Citrate synthase activity (nmol citrate/min/mg protein) and *E*: oxygen consumption (nmol O_2/min/mg protein) were expressed as fold-change *vs*.vehicle-treated cells taken as 1.0 (n = 3 experiments). *A-E:* $^{*}P < 0.05$ and $^{**}P < 0.01$ *vs*. vehicle-treated cells. All data represent mean ± SEM.

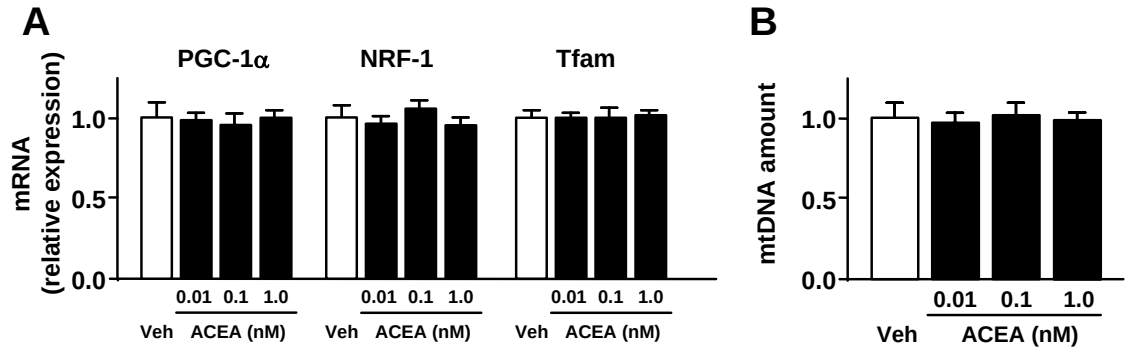

Figure S2.

Low concentrations of ACEA do not affect mitochondrial biogenesis in mouse white adipocytes. *A*: PGC-1α, NRF-1, and Tfam mRNA levels were analyzed by means of quantitative RT-PCR white adipocytes treated either with vehicle (0.002% DMSO, Veh) or ACEA for 2 days. The cycle number at which the various transcripts were detectable was compared to that of β-actin, and expressed as relative expression versus values in vehicle-treated cells taken as 1.0 (n = 5 experiments). *B*: mtDNA amount, analyzed by means of quantitative PCR and expressed as mtDNA copy number per nuclear DNA copy number. All data represent mean ± SEM.

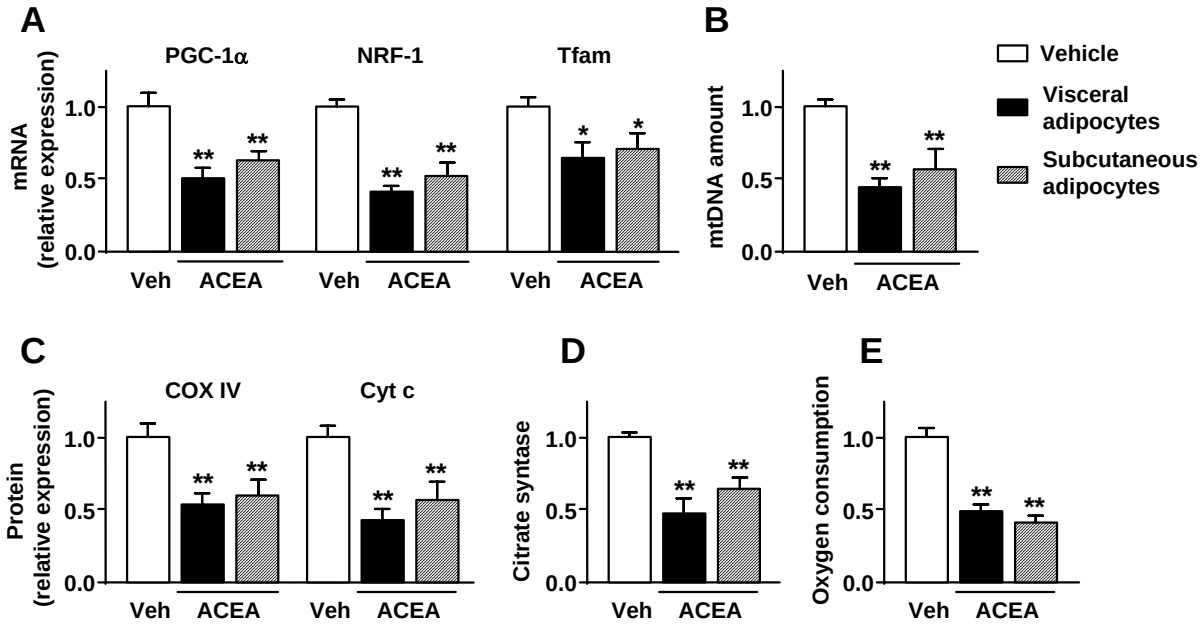

Figure S3.

The CB1 receptor agonist ACEA decreases mitochondrial biogenesis in human white adipocytes. *A*: PGC-1α, NRF-1, and Tfam mRNA levels were analyzed by means of quantitative RT-PCR in visceral and subcutaneous white adipocytes treated either with vehicle (0.002% DMSO, Veh) or 0.01 μM ACEA for 2 days. The cycle number at which the various transcripts were detectable was compared to that of β-actin, and expressed as relative expression versus values in vehicle-treated cells taken as 1.0 (n = 5 experiments). $^{*}P < 0.05$ and $^{**}P < 0.01$ *vs*. vehicle-treated cells. *B*: mtDNA amount, analyzed by means of quantitative PCR and expressed as mtDNA copy number per nuclear DNA copy number. $^{**}P < 0.01$ *vs*. vehicle-treated cells. *C*: COX IV and Cyt c proteins were detected by immunoblot analysis. The relative values from the densitometric analysis are referred to β-actin levels; vehicle-treated cell measurement is given a value of 1.0. $^{**}P < 0.01$ *vs*. vehicle-treated cells. *D*: Citrate synthase activity (nmol citrate/min/mg protein) and *E*: oxygen consumption (nmol O_2/min/mg protein) were expressed as fold-change *vs*.vehicle-treated cells taken as 1.0 (n = 3 experiments). $^{**}P < 0.01$ *vs*. vehicle-treated cells. All data represent mean ± SEM.

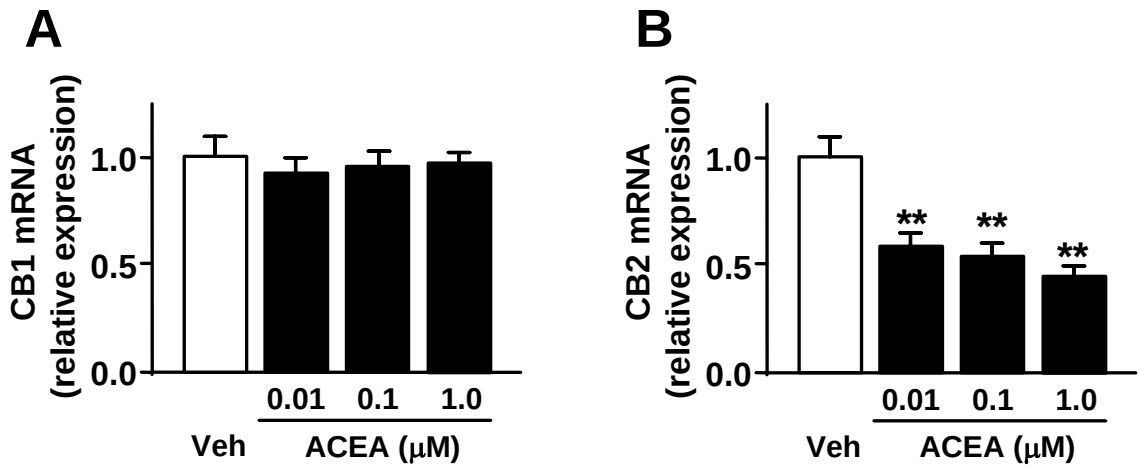

Figure S4.
CB1 and CB2 receptor expression in mouse white adipocytes. CB1 and CB2 receptor mRNA levels were analyzed by means of quantitative RT-PCR in white adipocytes treated either with vehicle (0.002% DMSO, Veh) or different doses of ACEA for 2 days. The cycle number at which the various transcripts were detectable was compared to that of β-actin, and expressed as relative expression versus values in vehicle-treated cells taken as 1.0 (n = 5 experiments). $^{**}P <$ 0.01 *vs*. vehicle-treated cells. All data represent mean ± SEM.

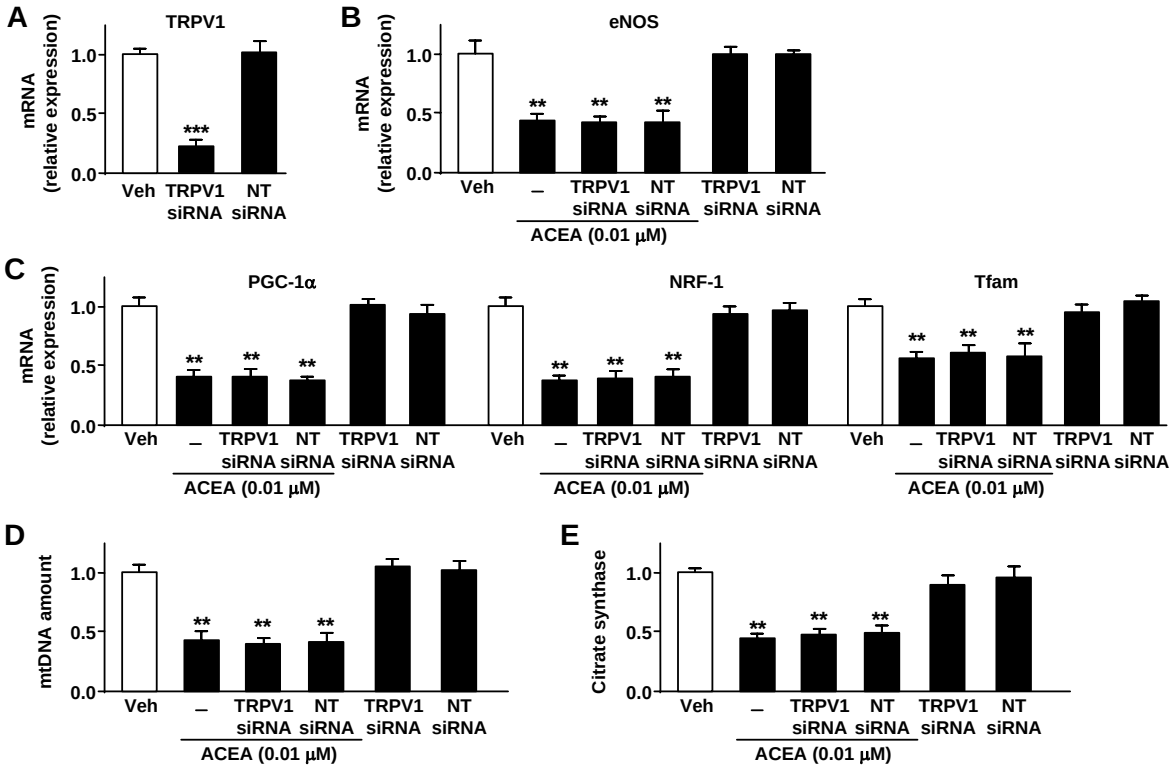

Figure S5.

TRPV1 activation is not involved in ACEA-mediated decrease of mitochondrial biogenesis in cultured mouse white adipocytes. *A*: TRPV1 expression in white adipocytes transfected with either TRPV1 siRNA or non-targeting (NT) siRNA. $^{***}P < 0.001$ *vs*. vehicle-treated cells. *B*-*E*: mitochondrial biogenesis parameters in white adipocytes transfected with either TRPV1 siRNA or NT-siRNA and treated with vehicle (0.002 % DMSO, Veh) or with ACEA for 2 days. *B*: eNOS expression and *C*: PGC-1α, NRF-1, and Tfam expression. Relative expression values of the vehicle-treated cells were taken as 1.0 ($n = 5$ experiments; $^{**}P < 0.01$ *vs*. vehicle-treated cells). *D*: mtDNA amount and *E*: citrate synthase activity. $^{**}P < 0.01$ *vs*. vehicle-treated cells. All data represent mean ± SEM.

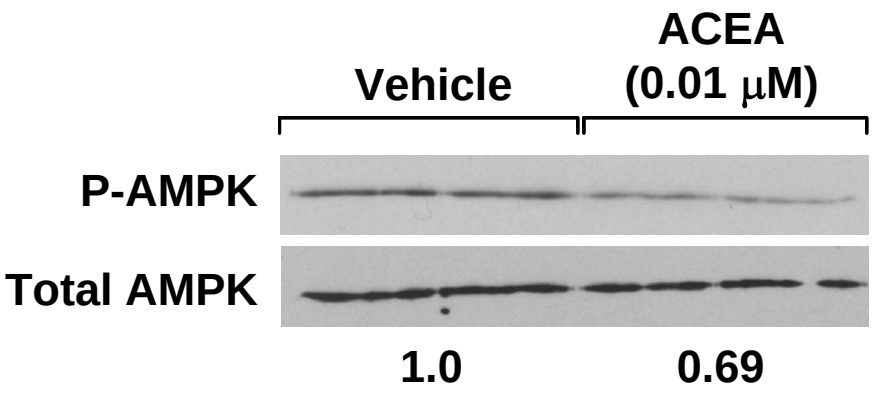

Figure S6.

ACEA reduces AMPK phosphorylation in cultured mouse white adipocytes. AMPK phosphorylation in primary white adipocytes exposed to vehicle (0.002% DMSO) or ACEA for 48 h (one experiment representative of three reproducible ones. Relative values from the densitometric analysis (phospho-AMPK/total AMPK) are shown below the blots, with vehicle values taken as 1.0.

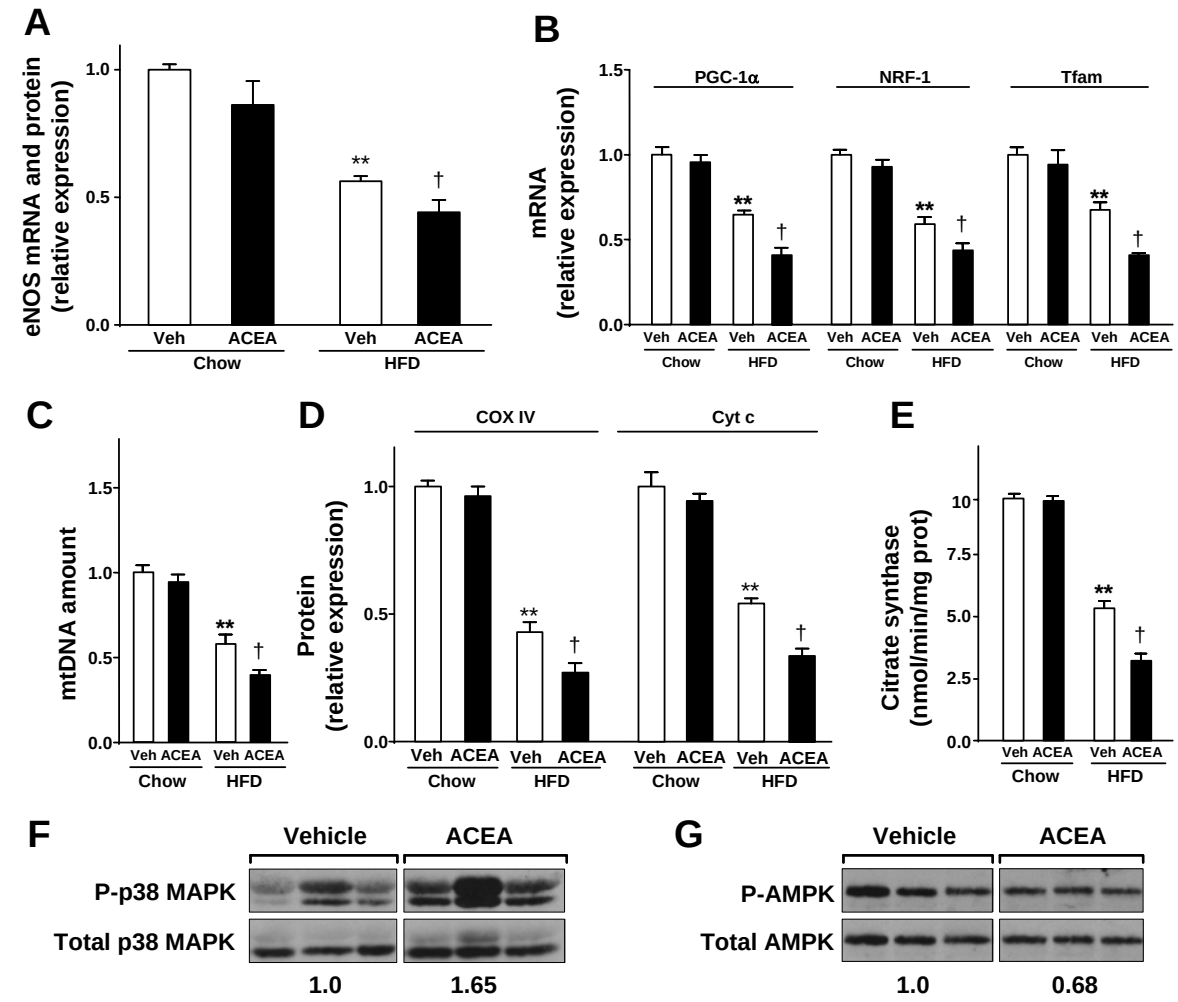

Figure S7.

CB1 receptor stimulation down-regulates mitochondrial biogenesis in the tibialis muscle of obese mice. *A*: eNOS mRNA, analyzed by means of quantitative RT-PCR, in tibialis muscle of both ACEA- and vehicle-treated mice on chow regular diet or HFD ($n = 8$ animals; $^{**}P < 0.01$ *vs.* vehicle-treated mice on chow regular diet, and $^{\dagger}P < 0.05$ *vs.* vehicle-treated mice on HFD). *B-C*: PGC-1α, NRF-1, Tfam mRNA levels and mtDNA amount, in muscle of both ACEA- and vehicle-treated mice on chow regular diet or HFD ($n = 8$ animals; $^{**}P < 0.01$ *vs.* vehicle-treated mice on chow regular diet, and $^{\dagger}P < 0.05$ *vs.* vehicle-treated mice on HFD). *D*: COX IV and Cyt c protein levels detected by immunoblot with densitometric analysis ($n = 3$ experiments; $^{**}P < 0.01$ *vs.* vehicle-treated mice on chow regular diet and $^{\dagger}P < 0.05$ *vs.* vehicle-treated mice on HFD). *E*: Citrate synthase activity ($n = 3$ experiments; $^{**}P < 0.01$ *vs.* vehicle-treated mice on chow regular diet and $^{\dagger}P < 0.05$ *vs.* vehicle-treated mice on HFD). *F*: p38 MAPK phosphorylation in muscle of vehicle- and ACEA-treated mice on HFD. *G*: AMPK phosphorylation in muscle of vehicle- and ACEA-treated mice on HFD. All data represent mean ± SEM.

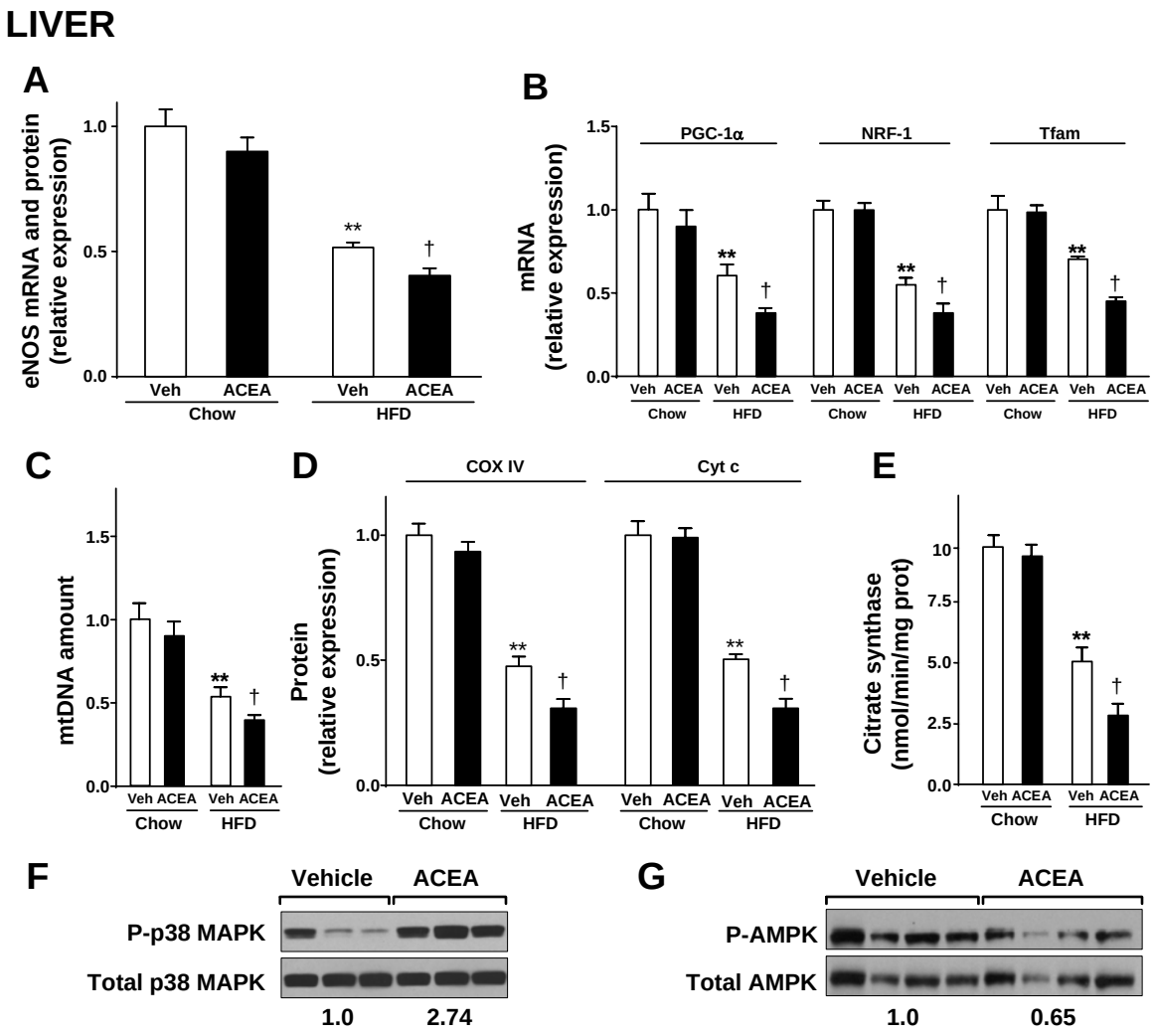

Figure S8.

CB1 receptor stimulation down-regulates mitochondrial biogenesis in the liver of obese mice. *A*: eNOS mRNA, analyzed by means of quantitative RT-PCR, in liver of both ACEA- and vehicle-treated mice on chow regular diet or HFD ($n = 8$ animals; $^{**}P < 0.01$ *vs*. vehicle-treated mice on chow regular diet, and $^{\dagger}P < 0.05$ *vs*. vehicle-treated mice on HFD). *B-C*: PGC-1α, NRF-1, Tfam mRNA levels and mtDNA amount, in liver of both ACEA- and vehicle-treated mice on chow regular diet or HFD ($n = 8$ animals; $^{**}P < 0.01$ *vs*. vehicle-treated mice on chow regular diet, and $^{\dagger}P < 0.05$ *vs*. vehicle-treated mice on HFD). *D*: COX IV and Cyt c protein levels detected by immunoblot with densitometric analysis ($n = 3$ experiments; $^{**}P < 0.01$ *vs*. vehicle-treated mice on chow regular diet and $^{\dagger}P < 0.05$ *vs*. vehicle-treated mice on HFD). *E*: Citrate synthase activity ($n = 3$ experiments; $^{**}P < 0.01$ *vs*. vehicle-treated mice on chow regular diet and $^{\dagger}P < 0.05$ *vs*. vehicle-treated mice on HFD). *F*: p38 MAPK phosphorylation in liver of vehicle- and ACEA-treated mice on HFD. *G*: AMPK phosphorylation in liver of vehicle- and ACEA-treated mice on HFD. All data represent mean ± SEM.