Supplemental Material:

MATERIALS AND METHODS

RNA interference

Mouse CHOP siRNA (ON-TARGETplus SMARTpool Cat# L-062068-00) and control siRNA (ON-TARGETplus Control) were purchased from Dharmacon. Transfection reagent designed for 3T3-L1 cells was also purchased from Dharmacon (Dharmafect 3). All transfection procedures were as per manufacturer's protocol. Cells were grown at a density of 2.5 x 10^5 cells/ml in 35 mm dishes and treated with 100 nM CHOP siRNA with DharmaFect transfection reagent in penicillin/streptomycin-free growth media 2 days prior to differentiation (day -2). Differentiation media was added on day 0 (48 hrs post-initial siRNA transfection). Every two days cells were re-transfected with CHOP siRNA. Cells were treated with 100 nM thapsigargin on day -2, day 0, and day 2 for 48 hrs to induce ER stress and CHOP expression. Total protein lysates were collected and separated on a 10% SDS polyacrylamide gel. On day 0 and 2, 10 mM 4-PBA was added to the media.

All other procedures were as described in the manuscript text.

FIGURE LEGENDS

Figure S1. Treatment of 3T3-L1 cells with tauro-ursodeoxycholic acid (TUDCA) blocks 3T3-L1 differentiation in a dose-dependent manner. ***A)*** Confluent day 0 3T3-L1 cells were cultured in differentiation media with increasing concentrations of TUDCA (0.1, 1, or 2 mg/ml). On day 4, cells were fixed and stained with Oil red O. Representative images of Oil red O stained cells are shown for each condition, with arrows indicating differentiated adipocytes. ***B)*** *Quantification of Oil red O indicated a significant decrease in lipid droplets with TUDCA treatment.* Following extraction and collection of Oil red O in isopropanol, absorbance was measured at 510 nm. Data are presented as the mean values +/- standard deviation (n=3). (*$*p<0.01$*)

Figure S2. 4-PBA treatment inhibits 3T3-L1 differentiation through a mechanism independent of CHOP. ***A)*** *CHOP protein expression is repressed in CHOP siRNA transfected 3T3-L1 cells when treated with 100 nM thapsigargin or 10 mM 4-PBA.* Cells were treated with 100 nM CHOP siRNA in penicillin/streptomycin-free growth media (day -2). Differentiation media was added on day 0 (48 hrs post-initial siRNA transfection) and post-stimulation media was added on day 2. Cells were transfected with CHOP siRNA on day -2 and 0 and treated with 100 nM of the ER stress inducer thapsigargin (Tg) on day -2, day 0, and day 2 for 48 hrs to induce CHOP expression. On day 0 and 2, 10 mM 4-PBA was added to the media. Total protein lysates were collected at various time points during the experiment and run on a 10% SDS polyacrylamide gel. Western blotting was performed and the membrane was probed with an anti-CHOP antibody as well as anti-β-actin as a loading control. **A-** Day 0 Control, **B-** Day 0

after 48 hr 100 nM Tg, **C-** Negative Control siRNA + 100 nM Tg, **D-** Day 0 CHOP siRNA (48 hrs), **E-** Day 0 CHOP siRNA + 100 nM Tg (48 hrs), **F-** Day 2 CHOP siRNA (96 hrs), **G-** Day 2 CHOP siRNA (96 hrs) + 100 nM Tg (48 hrs), **H-** Day 2 48 hrs 4-PBA treated, **I-** Day 2 CHOP siRNA (96 hrs) + 48 hrs 4-PBA treated, **J-** Day 4 Control, **K-** Day 4 after 48 hrs 100 nM Tg, **L-** Day 4 CHOP siRNA treated day -2 to day 2 + 48 hrs 100 nM Tg, **M-** Day 4 96 hrs 4-PBA treated, **N-** Day 4 CHOP siRNA treated day -2 to day 2 + 96 hrs 4-PBA treated. ***B)*** *siRNA knock-down of CHOP did not rescue the cells from the anti-adipogenic effects of 4-PBA.* 3T3-L1 cells were seeded at 70% confluence and transfected with CHOP siRNA. Two days later on day 0, cells were transfected again and differentiation media was added in the presence or absence of 10 mM 4-PBA. On day 2, cells were washed and post-stimulation media with or without 10 mM 4-PBA was added. Cells were fixed and stained with Oil red O on day 4. Images were taken at 20X magnification. ***C)*** Oil red O quantification indicated a significant decrease in lipid content in 4-PBA and CHOP siRNA+4-PBA treated cells as compared to untreated control cells. However, there was no difference in adipogenic differentiation with 4-PBA when CHOP was knocked down versus non-transfected cells *(*$p<0.001$*).*

Figure S3. Salubrinal, a selective inhibitor of eIF2α dephosphorylation, blocks adipogenesis in a dose-dependend manner. ***A)*** 3T3-L1 cells at day 0 were stimulated to differentiate in the presence or absence of 10, 50 or 100 μM salubrinal. On day 4, the cells were fixed and stained with Oil red O. Representative images are shown. ***B)*** *Salubrinal dose-dependently inhibited lipid accumulation.* Oil red O staining was quantified and a significant decrease in staining intensity was observed with increasing dose of salubrinal (* $p<0.05$,

p<0.01).* ***C) Western blotting confirmed that 100 μM salubrinal decreases splicing of XBP1 in differentiating day 4 adipocytes.

Figure S4. Rosiglitazone can partially rescue the differentiation of 3T3-L1 cells in the presence of 4-PBA by enhancing GRP78/GRP94 expression levels. ***A)*** Day 0 cells were treated with 4 μM rosiglitazone in the absence or presence of 10 mM 4-PBA in differentiation media. Cells were re-supplemented with these drugs on day 2. On day 5, cells were fixed and stained with Oil red O. ***B)*** 4-PBA alone and the rosiglitazone/4-PBA co-treatment resulted in a significant decrease in Oil red O staining *(*p<0.001)* though co-treatment allowed for some adipogenic conversion to occur. ***C)*** *Rosiglitazone and 4-PBA co-treatment allows for some adiponectin secretion by 3T3-L1 cells that was completely blocked by 4-PBA treatment alone.* Media was collected on day 5 of differentiation and an ELISA was performed to detect secreted adiponectin protein. Media from day 0 untreated and undifferentiated 3T3-L1 cells was used as a negative control. Data represent the mean values +/- standard deviation. *(*p=0.0003, ** p≤0.0001)* ***D)*** *Rosiglitazone exerts its pro-adipogenic effect in the presence of 4-PBA by enhancing GRP78 expression in differentiating adipocytes.* Western blotting using lysates collected on day 5 of differentiation with various treatments. Rosiglitazone treatment during 5 days of differentiation (day 0-5) increased GRP78 expression. 4-PBA blocked GRP78 expression, while rosiglitazone and 4-PBA co-treatment enhanced GRP78 expression.

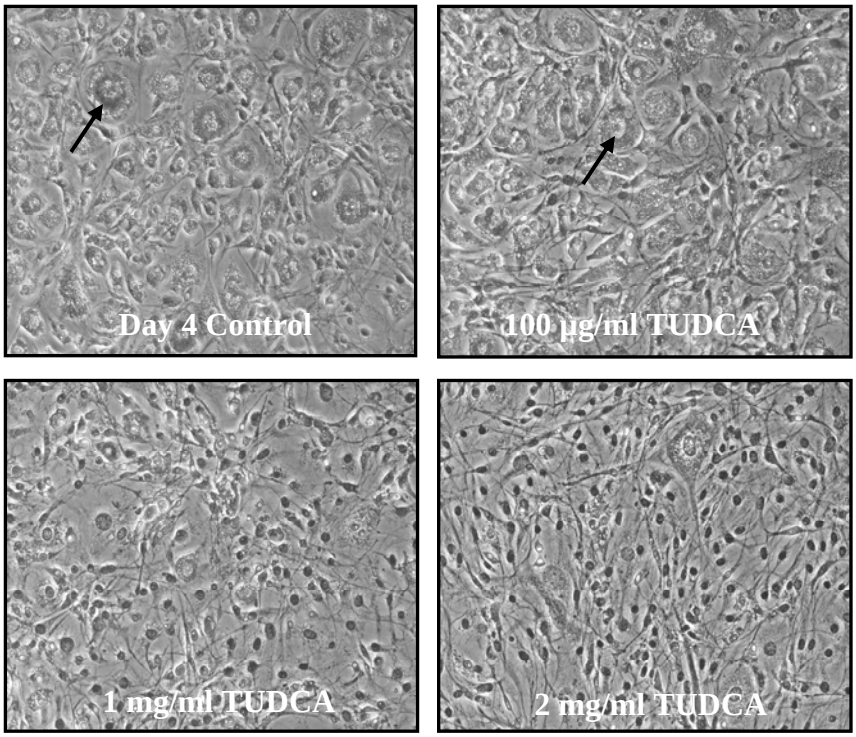
Figure S5. Markers of inflammation or oxidative stress were not altered with 4-PBA treatment. ***A)*** 3T3-L1 cells treated with varying doses of 4-PBA over 48 hours did not change the expression of NFκB or peroxiredoxin-1 on Western blots. Expression of β-catenin, a known

inhibitor of adipogenesis was also not affected by 4-PBA. ***B)*** Western blot analysis of the WAT and liver lysates from the 4-PBA supplemented mice showed no change in NFκB or peroxiredoxin1 expression as compared to the non-supplemented group. β-actin was used as a loading control.

Figure S6. XBP-1 deficient MEFs show reduced expression of adipogenic markers. Protein was collected on days 0, 2, or 4 of differentiation following stimulation of XBP-1 deficient or wildtype MEFs with adipogenic media. Protein from 3T3-L1 cells was collected as an additional control. Western blotting was performed to detect PPARγ, C/EBPα isoforms, as well as β-actin as a loading control. MEFs deficient in XBP-1 express reduced adipogenic markers as compared to wildtype control MEFs.

Supplementary Figure S1.

A.



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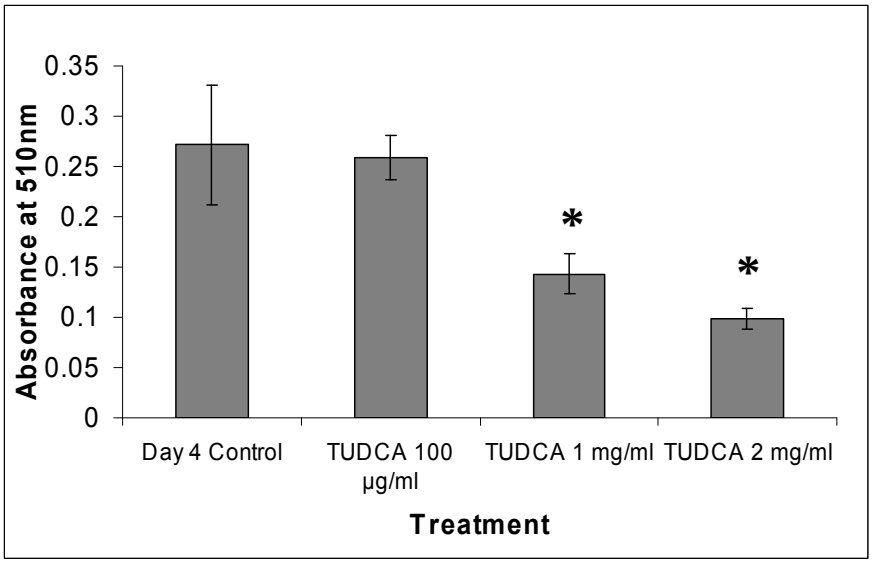
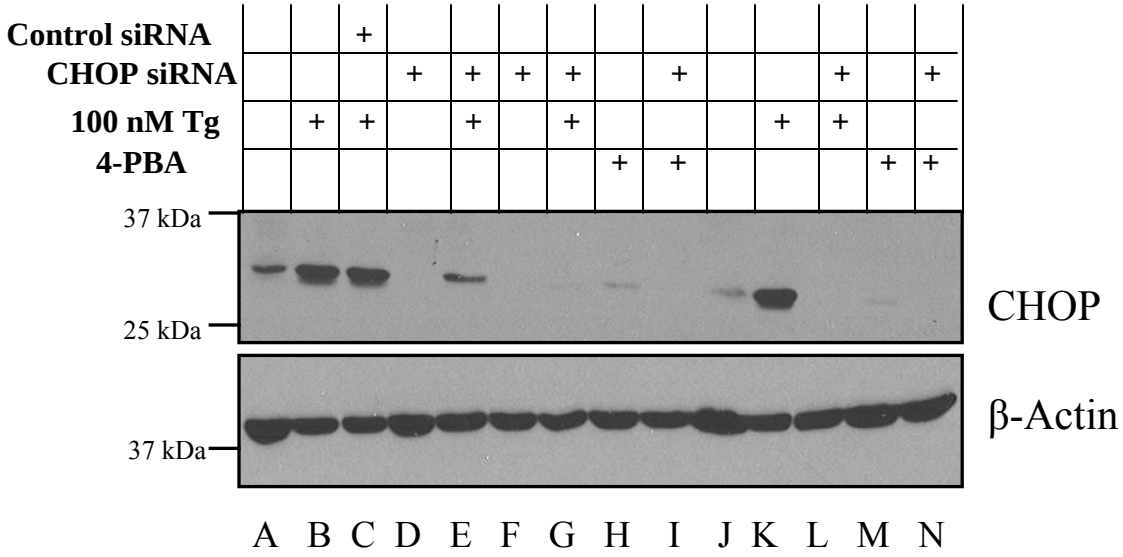


Basseri S et al. The chemical chaperone 4-phenylbutyrate inhibits adipogenesis by modulating the unfolded protein response.

Supplementary Figure S2.

A.



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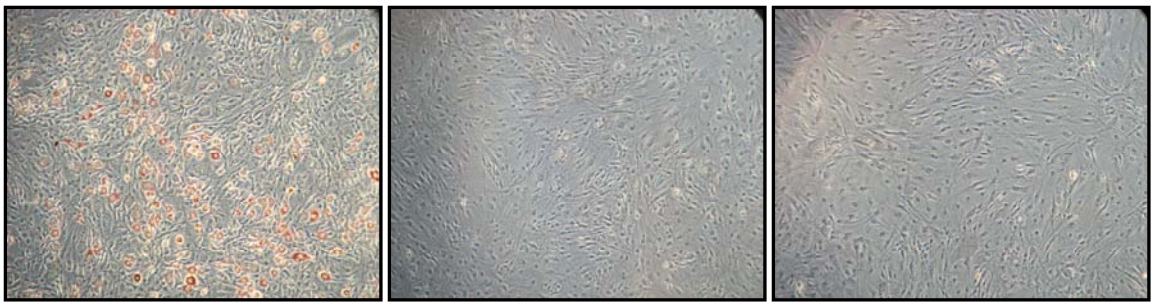

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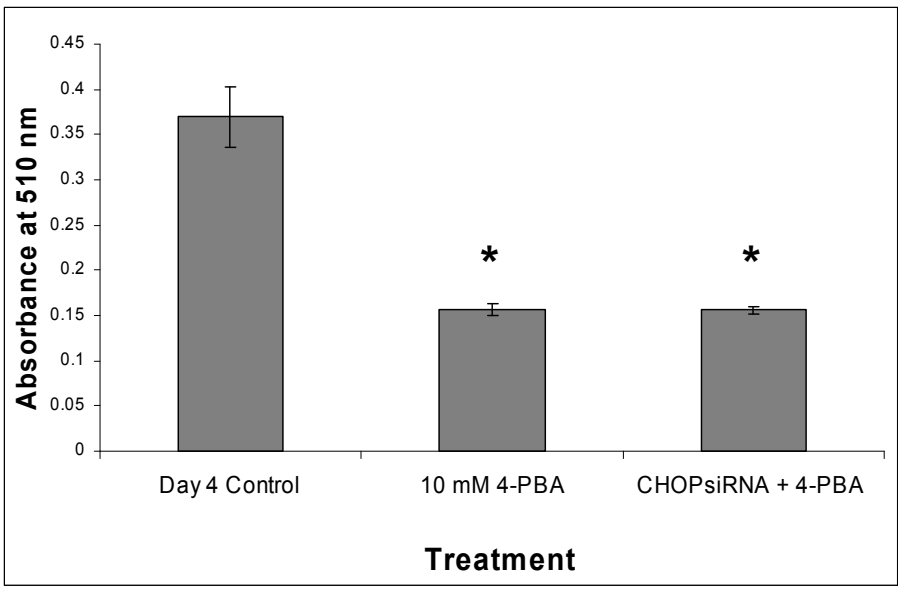

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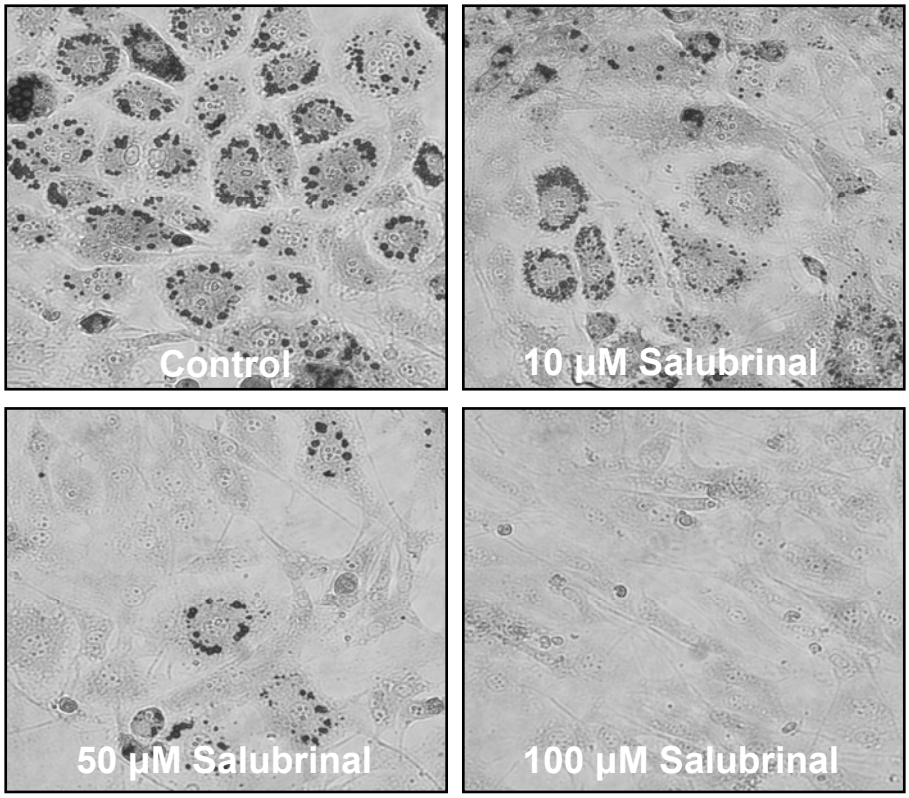
Supplementary Figure S3.

A.



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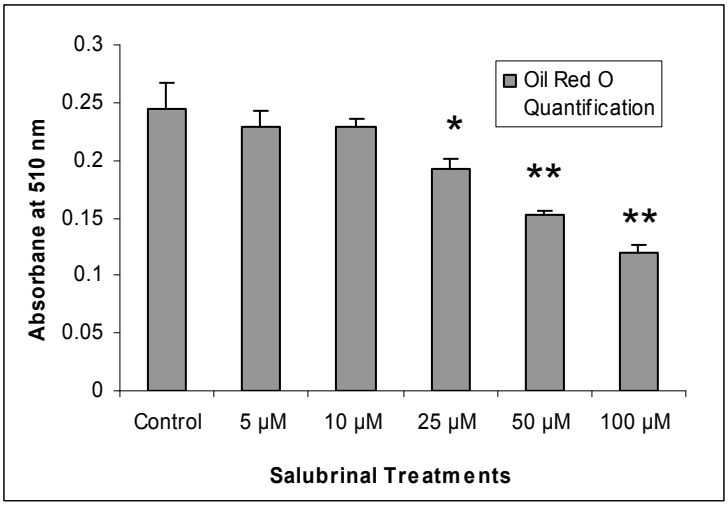

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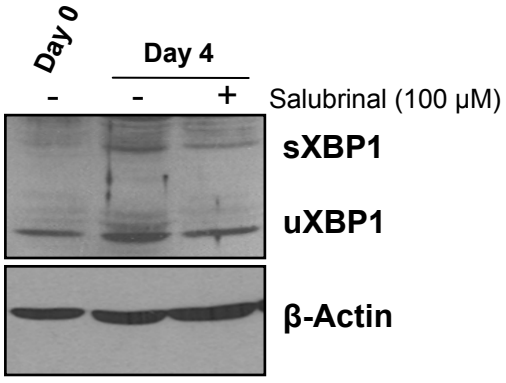

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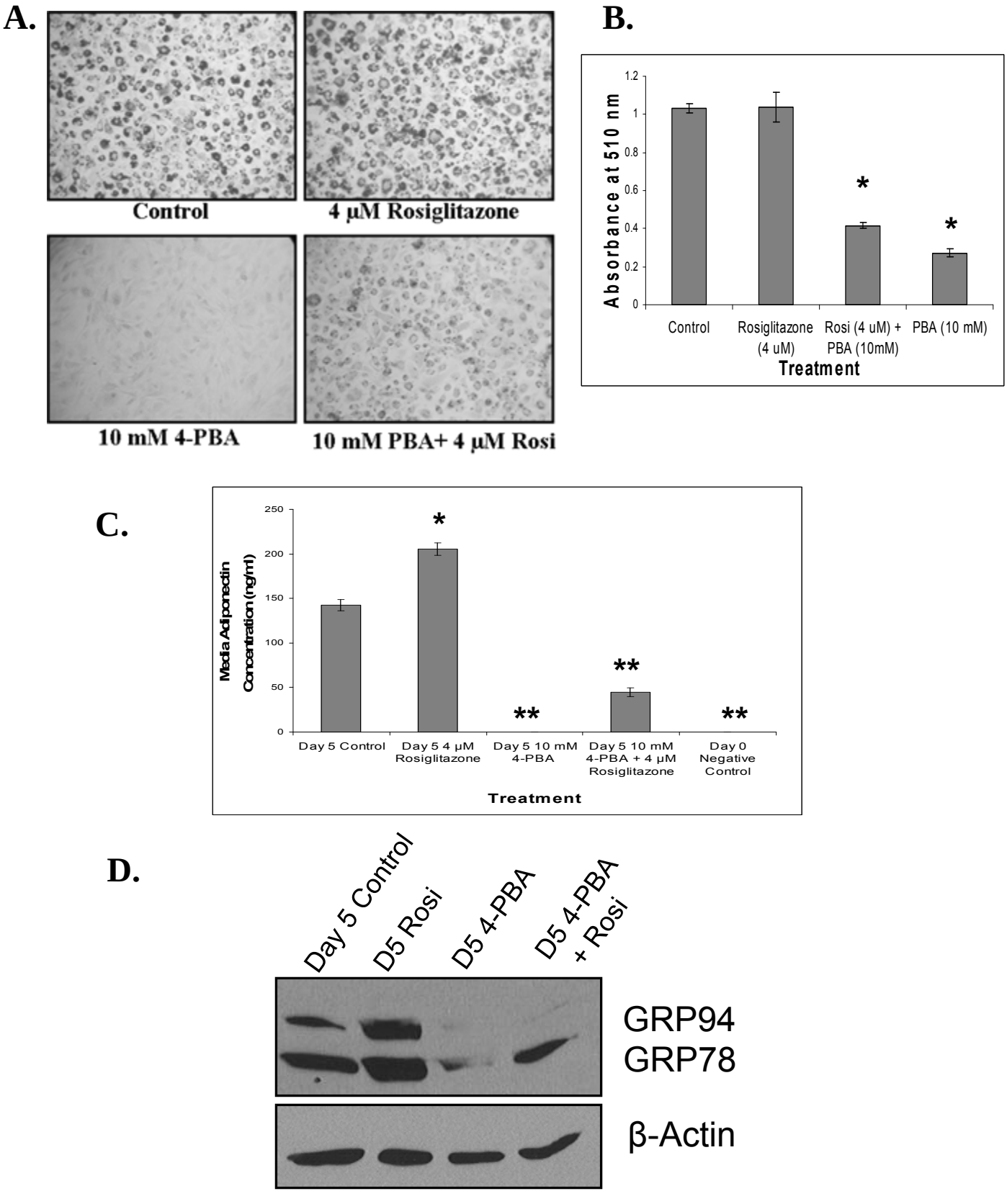
Supplementary Figure S4.



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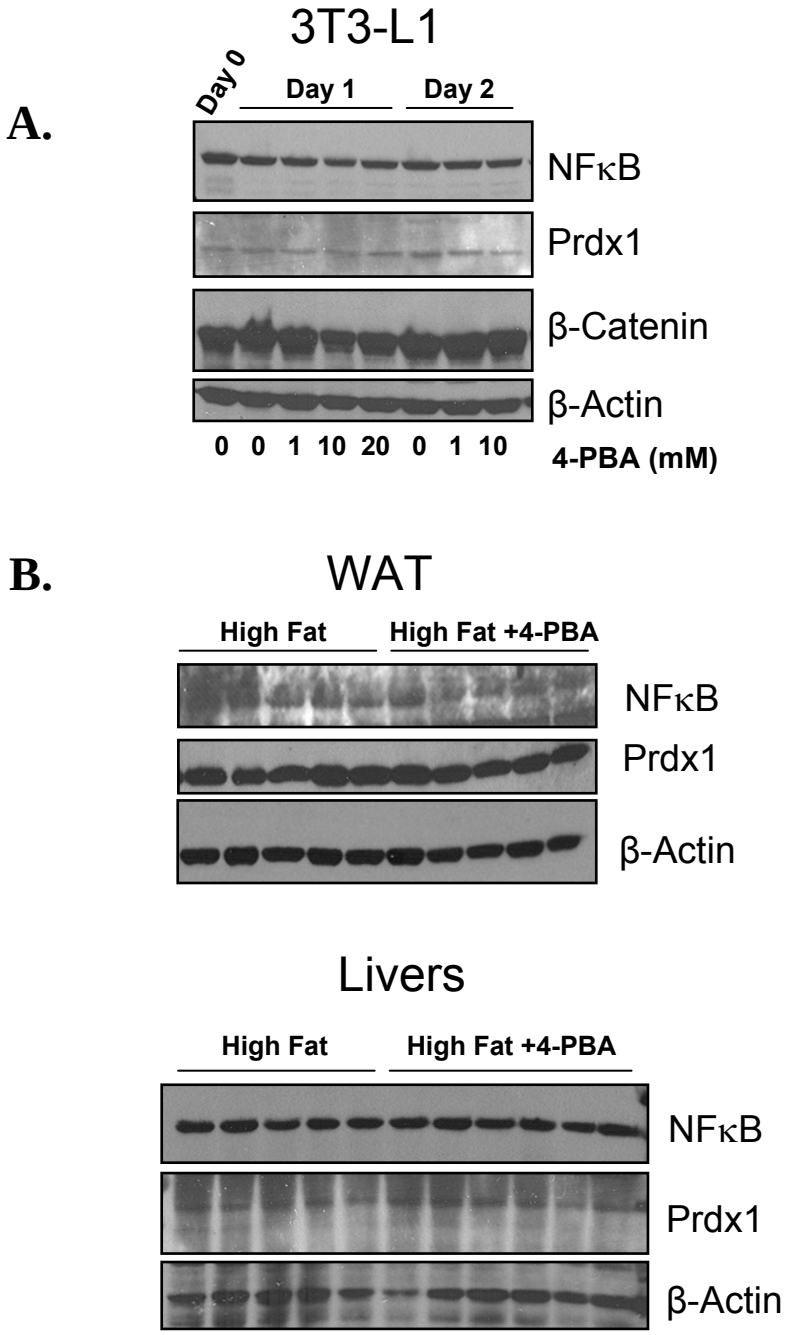
Supplementary Figure S5.



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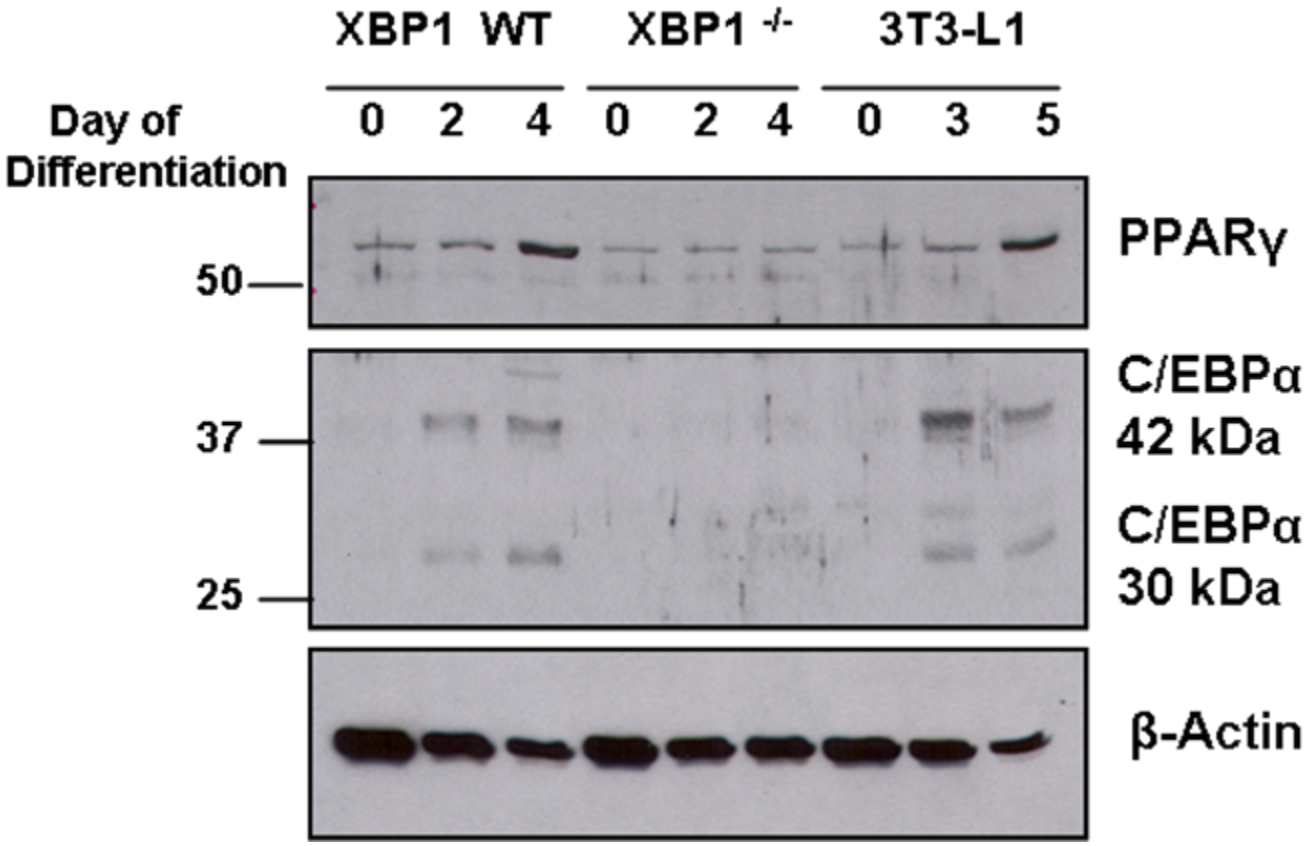
Supplementary Figure S6.



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