Supporting Information

Cichoric Acid Prevents Free Fatty Acids Induced Lipid Metabolism Disorders via Regulating Bmal1 in HepG2 Cells

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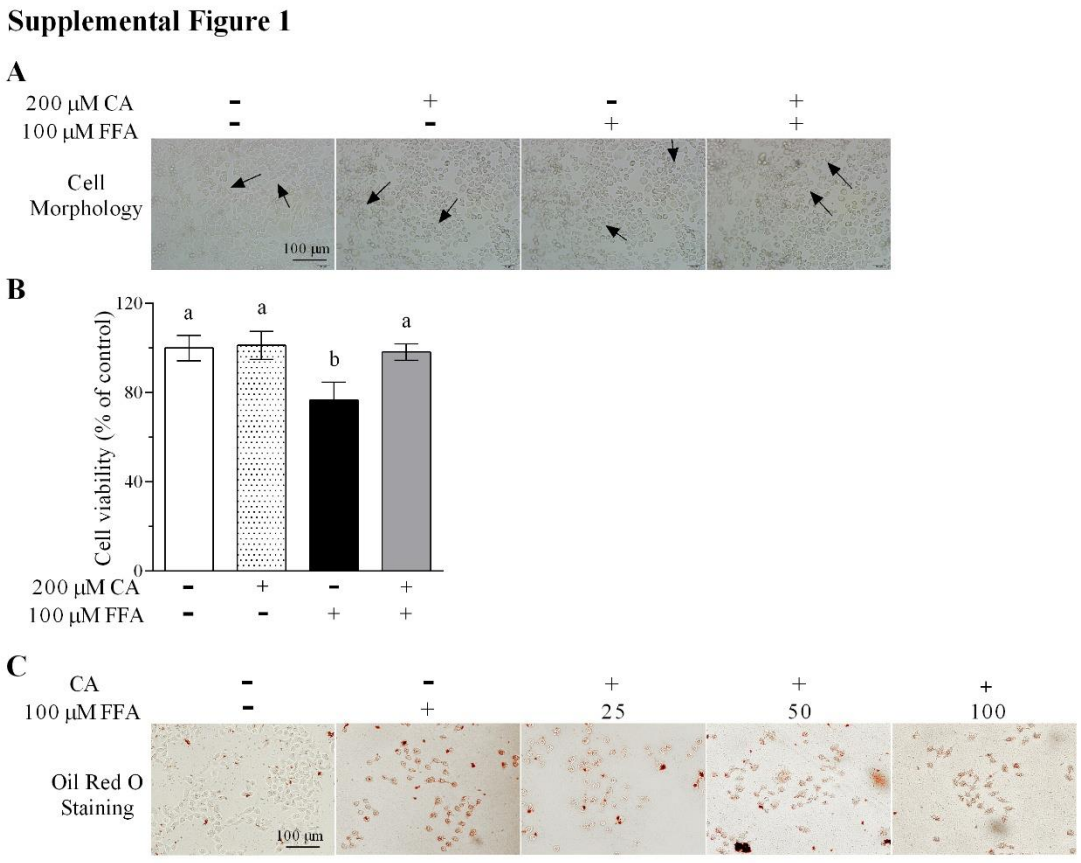
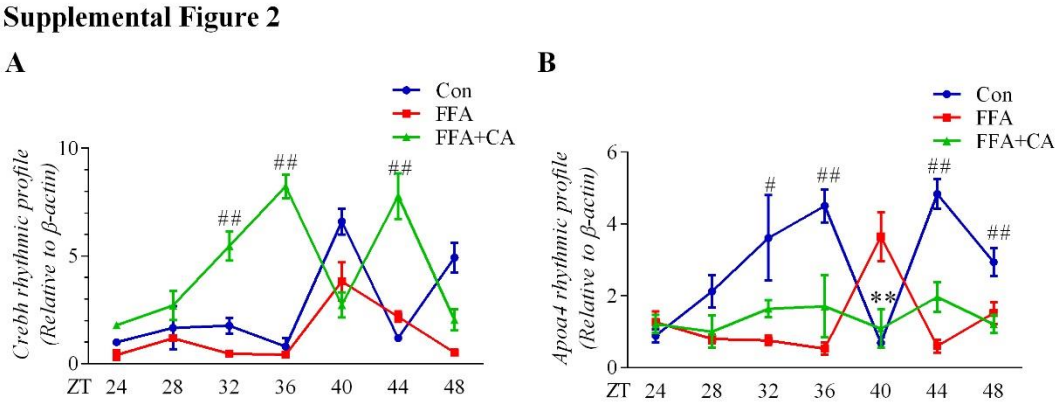
Supplemental Fig. 1 Effects of CA on morphology changes and cell viability.

Cells were treated with or without 100 μM FFA and 200 μM CA for 24 hours. (A) Cells morphology were observed by an inverted microscope. (B) Effect of CA on FFA induced HepG2 cell viability measured by MTT assay. (C) Cells were treated with or without 100 μM FFA and disparate concentrations of CA (0, 25, 50 and 100 μM) for 24 hours, Oil Red O staining were observed by an inverted microscope. Values are expressed as the fold change compared with a vehicle control that was arbitrarily set to 100. The results are expressed as the means ± SD, n≥3. Values having different superscripts are significantly different, $p < 0.05$.



Supplemental Fig. 2 CA had no effect on rhythmic expression of *Crebh* and *Apoa4* under high fatty acid conditions.

HepG2 cells were serum-shocked for 2 hours and treated with FFA (100 μM) and CA (200 μM) for 24 hours. Cells then were collected for both mRNA and protein analysis at 4 hours intervals between 24 hours and 52 hours time points. (A) The mRNA level of *Crebh* in HepG2 cells. (B) The mRNA level of Apoa4 in HepG2 cells. Transcript levels were measured by qRT-PCR and normalized to β-actin. The results are expressed as the means ± SD, n≥3. $p < 0.05$, $^{**}p < 0.01$, versus control group, $\#p < 0.05$, $\#\#p < 0.01$ versus FFA group.

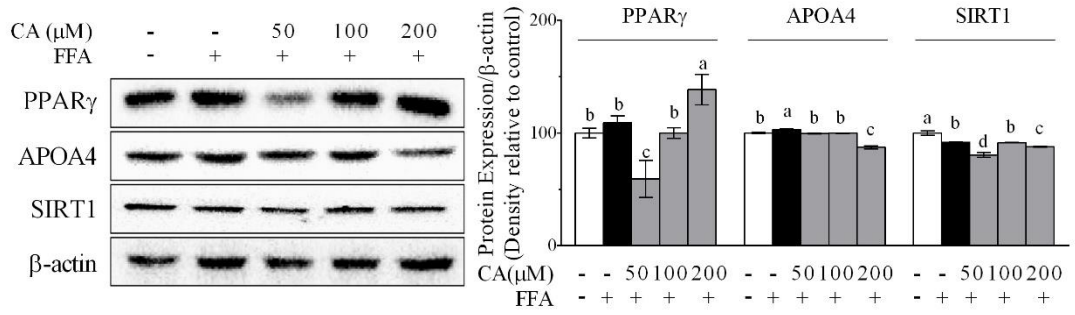


Supplemental Fig. 3 CA had no effect on FFA induced lipid metabolism imbalance of PPARγ, APOA4 and SIRT1 in protein levels.

HepG2 cells were treated with or without 100 μM FFA and disparate concentrations of CA (0, 50, 100 and 200 μM) for 24 hours. The effects of CA on FFA induced PPARγ, APOA4 and SIRT1 changes, determined by western blots,

and β-actin was used as a loading control. The densitometric analysis of the blots are expressed as the means ± SD, n≥3. Values having different superscripts are significantly different, $p<0.05$.

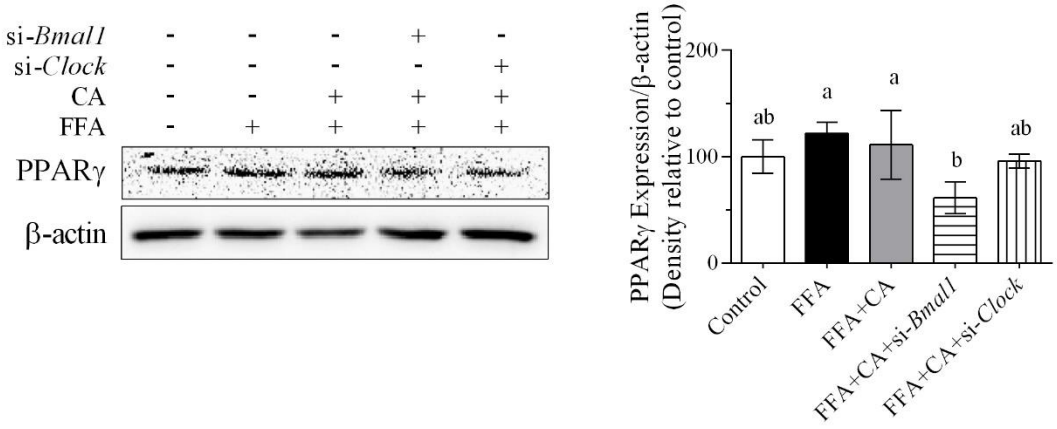
Supplemental Figure 3



Supplemental Fig. 4 CA had no effect on FFA induced protein expression disorders of PPARγ via modulating of the circadian clock.

HepG2 cells were transfected with the si-*Control* or si-*Bmal1*/si-*Clock* using lipofectamine 2000 transfection reagent. After transfection with siRNAs for 48 hours, the cultured cells treated with FFA (100 μM) and CA (200 μM) for 24 hours. The protein levels of PPARγ, and β-actin was used as a loading control. The results are expressed as the means ± SD, n≥3. Values having different superscripts are significantly different, $p < 0.05$.

Supplemental Figure 4



Supplemental Fig. 5 CA had no effect on FFA induced mRNA expression disorders via modulating of the circadian clock.

HepG2 cells were transfected with the si-*Control* or si-*Bmal1*/si-*Clock* using lipofectamine 2000 transfection reagent. After transfection with siRNAs for 48 hours, the cultured cells treated with FFA (100 μM) and CA (200 μM) for 24 hours. The mRNA levels of *Fgf21*, *Srebp-1*, *Apoa4*, *Crebh* and *Cpt-1*, and *β-actin* was used as a loading control. The results are expressed as the means ± SD, n≥3. Values having different superscripts are significantly different, $p < 0.05$.

Supplemental Figure 5