Supplementary Methods

Animal models. FVB-Luc^{+} (FVB-Tg(CAG-luc,-GFP)L2G85Chco/J) mice were obtained from our in-house breeding colony. Mice were maintained at the 12-h light/12-h dark cycle at 22℃ and had free access to food and water. To generate a mouse strain with liver-specific luciferase expression (L-Luc mice), mice bearing the Gt(ROSA)26Sor$^{tm1(Luc)Kael}$ allele and Tg(Alb-cre)21Mgn mice were purchased from Jackson Laboratories (stocks 005125 and 003574, respectively) and crossed. Gray-fur mice were bred several times to generate white-fur litters. To examine the effect of fenofibrate, mice were fed a standard laboratory chow diet with or without fenofibrate (0.2% w/w) for 10 days, while having free access to drinking water. All animal studies were approved by and performed according to the guidelines of the Animal Care and Use Committee of the University of California, Berkeley.

General animal imaging methods and data analysis. A Xenogen IVIS Spectrum instrument (Caliper Life Sciences) was used to obtain luminescence images in all animal experiments. Image analysis was performed using the IVIS Living Image software. Total photon flux in each mouse was determined by drawing a region of interest in the animal and integrating photon flux over the total imaging period. Mice were anesthetized prior to injection and during imaging by isoflurane inhalation.

Intraperitoneal injection of FFA-Luc and luciferin. Anesthetized mice were intraperitoneally (IP) injected with 100μL of 200μM FFA-Luc in 0.1% (w/v) BSA-containing PBS or with 100μL of 2 mM luciferin in PBS immediately before imaging. Luminescence images were acquired by auto exposure back to back for 50 min in case of FFA-Luc and 25 min in case of luciferin.

Quantification of free luciferin in serum. Sera were collected before and 10 and 20 min after IP injection of FFA-Luc and luciferin, and 50μl of serum samples was plated on 96-well plates containing reaction solution; firefly luciferase (L9420; Sigma-Aldrich, USA) and ATP (A2383; Sigma-Aldrich, USA) were added immediately before recording the results using a SpectraMax i3 (Molecular device, USA). Luminescence signals were measured for 10seconds, and the amount of luciferin was calculated based on a standard curve constructed using a series of luciferin concentrations.

In vivo monitoring of FFA hepatic uptake using fluorescence-labeled FA (BODIPY). The assay was performed as described previously[1] with minor modifications. In brief, mice fasted overnight were anesthetized with isoflurane and injected IP with DCA (6.4 mg/kg body weight) and then BODIPY (100μl of 2μM solution). After 30 min, mice were euthanized, and livers were harvested and homogenized in RIPA buffer. Liver lysates were prepared with three volumes of Dole's reagent (heptane:2-propanol:2N sulfuric acid = 10:40:1 v/v/v), centrifuged at 18,000 × *g* for 10 min, and clear organic-phase supernatant (top layer) was collected and added to a 96-well plate for fluorescence measurement.

Diurnal rhythm of FFA hepatic uptake. Two cohorts of male mice were maintained at a 12-h light/12-h dark cycle; zeitgeber time zero (ZT0) referred to lights on and ZT12 to lights off. The first injection of FFA-Luc was conducted at ZT06 (1 PM) in one group and ZT18 (1AM) in the other, followed by subsequent injections every 30 hours. For fasting and refeeding study, additional two groups of male mice were measured hepatic FFA

uptake assay under different feeding states at ZT6 and ZT12 periods. The signal was measured for 20 min during each imaging and the total photon flux was determined. To avoid light application during dark period assessments, we used night vision goggles and dim red lighting. Serum was collected at the same time points and FFA levels were measured using the FFA kit (Sigma-Aldrich).

Measurement of quenching of luciferase-luciferin bioluminescence. Incubation of different concentration of DCA or fenofibrate with luciferin was performed in 2% DMSO/PBS for 10minutes. At the end of the incubation, an equal volume (100 µl) of a reaction solution containing luciferase and ATP was added and mixed well. Bioluminescence signals were measured for 10seconds using a SpectraMax i3 (Molecular device, USA).

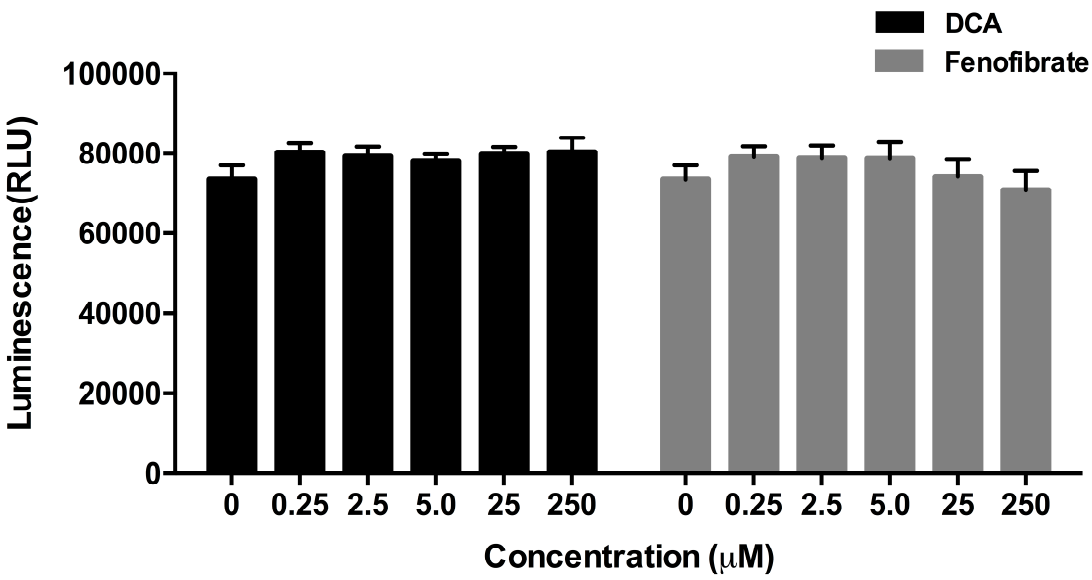
Supplementary References

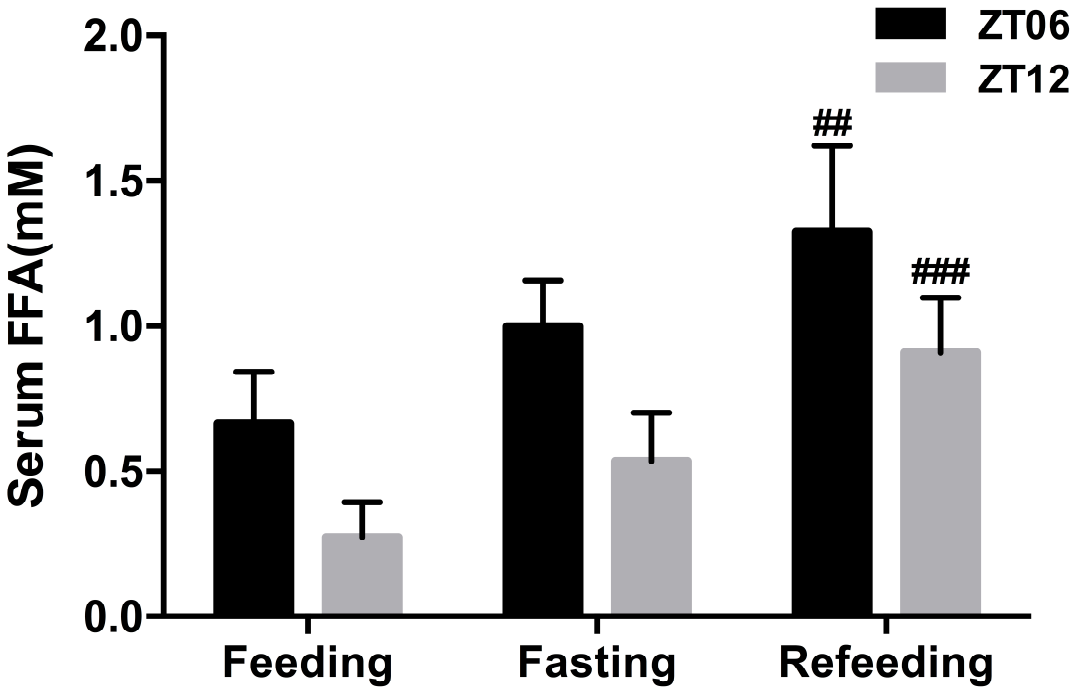
1. Nie B, et al. Hepatology 2012;56:1300-1310.

Supplementary figure legend

Supplementary Figure 1
Determination of DCA or fenofibrate induced quenching of luciferase-luciferin bioluminescence. Luciferin was pre-incubated with varying concentrations of DCA or fenofibrate for 10 minutes, followed by addition of reaction solution containing luciferase and ATP. Relative bioluminescence signals were measured for 10seconds. Statistical analyses were performed using a Student's t-test. Error bars are ± SEM (n=4)

Supplementary Figure 2
Serum FFA level after regular feeding, 24hr of fasting and refeeding during the light (ZT6, ZT12) periods. Statistical analyses were performed using a paired Student's t-test. $^{\#\#}P<0.01$ between feeding and refeeding at ZT06 and $^{\#\#\#}P<0.005$ at ZT12. Error bars are ± SEM.


DCA
Fenofibrate
Luminescence(RLU)
100000
80000
60000
40000
20000
0
0
0.25
2.5
5.0
25
250
0
0.25
2.5
5.0
25
250
Concentration (μM)


ZT06
ZT12
Serum FFA(mM)
2.0
1.5
1.0
0.5
0.0
##
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Feeding
Fasting
Refeeding