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Supplemental information

CD36 regulates diurnal glucose metabolism and hepatic clock to maintain glucose homeostasis in mice

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Supplemental Text and Figures

Figure S1

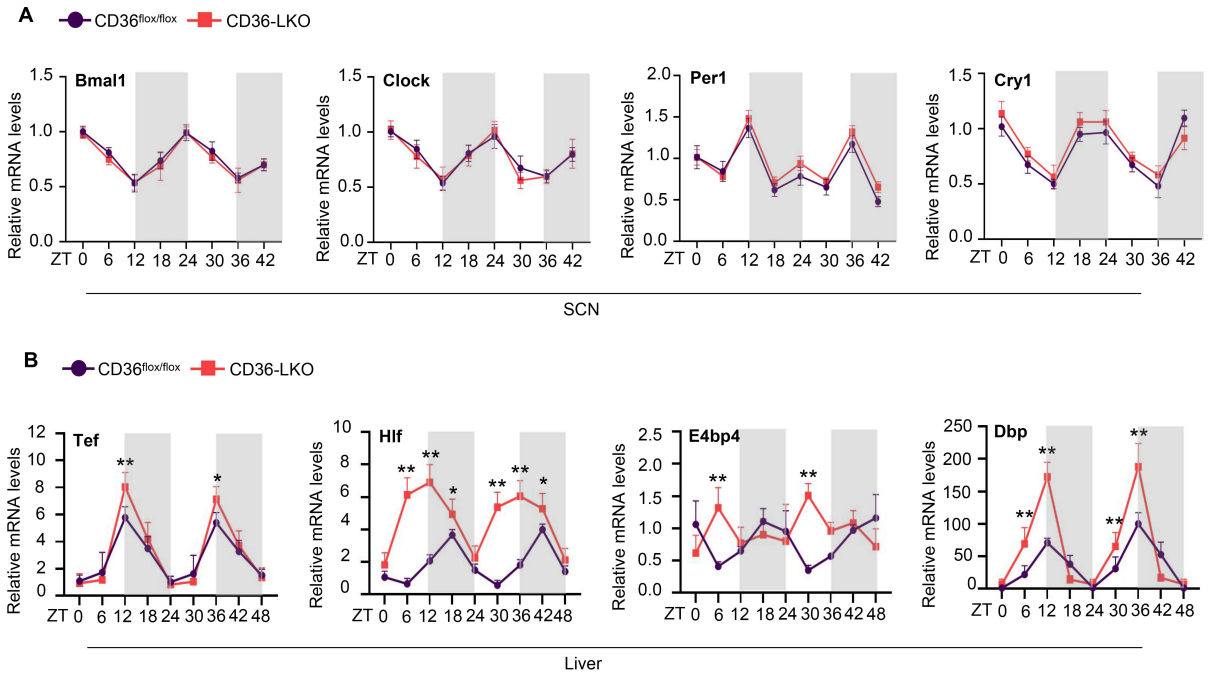

Figure S1 (related to Figure 1). Effect of CD36-LKO on the gene expression of circadian components in mice SCN and liver.

(A) The relative mRNA levels of core clock genes in the SCN of CD36$^{flox/flox}$ and CD36-LKO mice. (B) The relative mRNA levels of genes downstream of circadian components in the liver of CD36$^{flox/flox}$ and CD36-LKO mice. n=4-5 in each group. Data are shown as the mean ± SEM. Comparison of different groups was carried out using two-way ANOVA. *P<0.05 and **P<0.01 indicate a significant difference between the two groups at the corresponding time point. ZT0, the beginning of a subjective circadian period (6:00 AM). Gray shading represents the lights-off period from 6:00 PM to 6:00 AM.

Figure S2

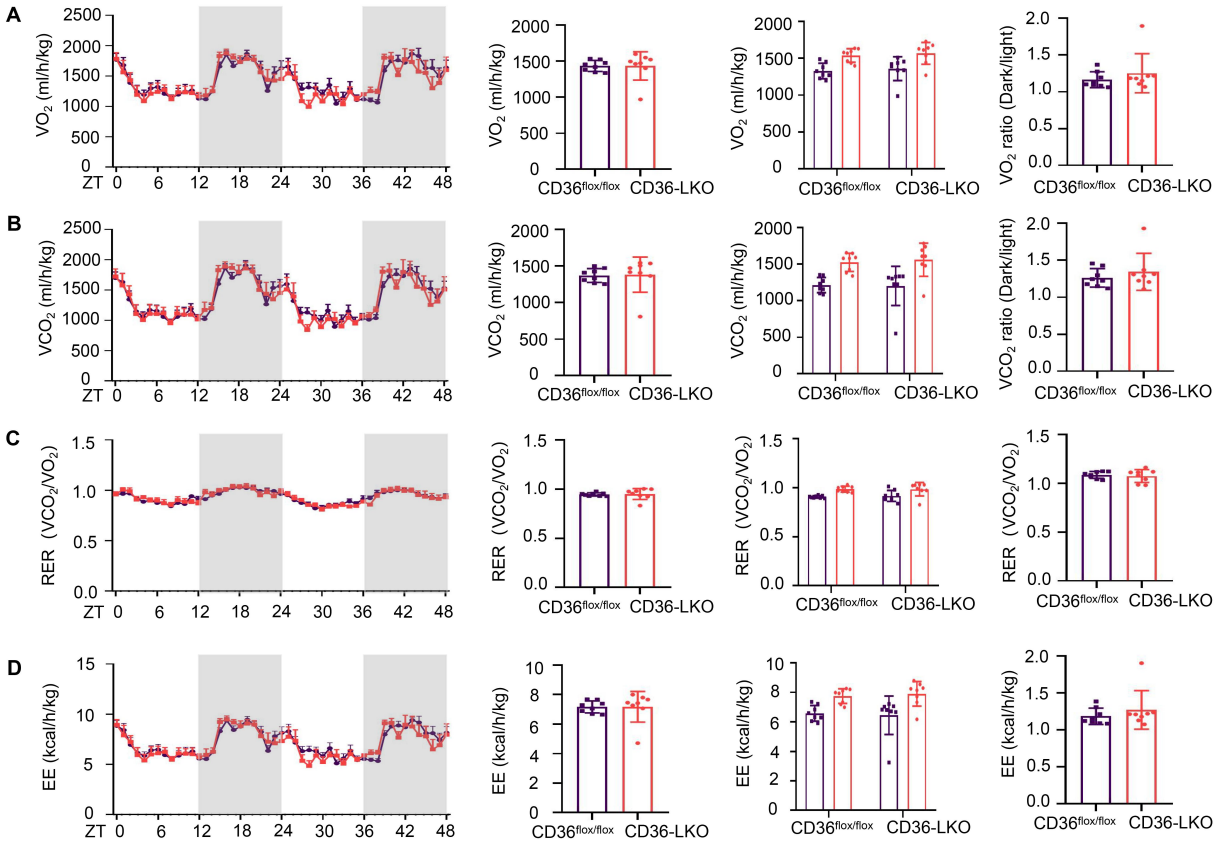

Figure S2 (related to Figure 2). Effects of CD36-LKO on whole body metabolic status.

Mouse oxygen consumption (VO_2), carbon dioxide output (VCO_2), respiratory exchange ratio (RER) and energy expenditure (EE) were monitored for 48 h in 8 to 12-week-old $CD36^{flox/flox}$ and CD36-LKO mice. (A) VO_2 per hour in the light/dark cycle, average VO_2 per hour, VO_2 during light and dark phases and dark/light VO_2 ratio in $CD36^{flox/flox}$ and CD36-LKO mice. (B) VCO_2 per hour during the light/dark cycle, average VCO_2 per hour, VCO_2 during light and dark phases, and dark/light VCO_2 ratio in $CD36^{flox/flox}$ and CD36-LKO mice. (C) RER per hour during the light/dark cycle, average RER per hour, RER during light and dark phases, and dark/light RER ratio in $CD36^{flox/flox}$ and CD36-LKO mice. (D) EE per hour during the light/dark cycle, average EE per hour, EE during light and dark periods, and dark/light EE ratio in $CD36^{flox/flox}$ and CD36-LKO mice. n=8 in each group. Data are shown as the mean ± SEM. Comparison of different groups was carried out using two-tailed unpaired Student's t-test a or two-way ANOVA. *P<0.05, **P<0.01 compared with the $CD36^{flox/flox}$ group; $^{\#}$P<0.05, $^{\#\#}$P<0.01 compared with the light phase in the identical genotype mice. ZT0, the beginning of a subjective circadian period (6:00 AM). Gray shading represents the lights-off period from 6:00 PM to 6:00 AM.

Figure S3

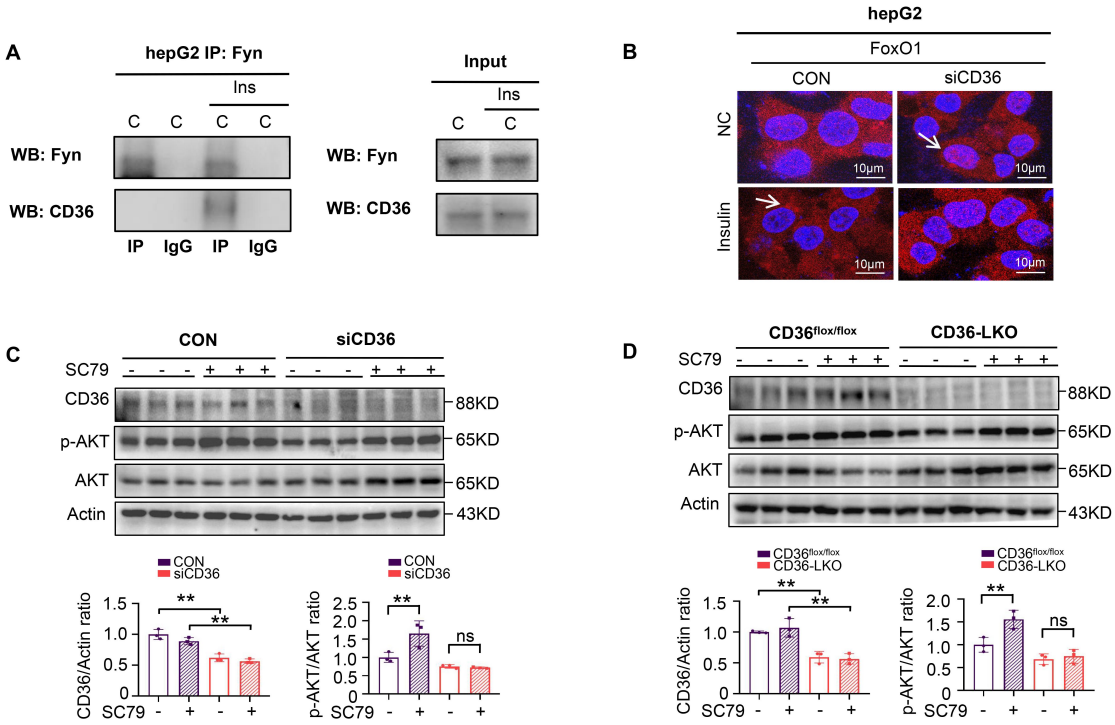

Figure S3 (related to Figure 5). The binding of CD36 to Fyn promotes the activation of insulin signaling pathway.

(A) Insulin promotes Fyn association with CD36 in HepG2 cells. Cells were incubated with or without insulin (Ins; 100 nmol/L, 5 min), IP was performed with anti-Fyn antibody, and immunoblotting was used to detect CD36 and Fyn. (B) Effect of CD36 knockdown and the insulin treatment on the cellular localization of FoxO1 in HepG2 cells. Scale bars= 10μm. (C) Western blot analyses and densitometric quantification of the expression levels of AKT and p-AKT (Ser473) in CD36-knockout HepG2 cells treated with SC79. (D) Western blot analyses and densitometric quantification of the expression levels of AKT and p-AKT (Ser473) in the livers of $CD36^{flox/flox}$ and CD36-LKO mice treated with SC79. n=3 in each group. All data are shown as the mean ± SEM. Comparison of different groups was carried out using two-way ANOVA (C and D). *P<0.05, **P<0.01 versus control groups.

Figure S4

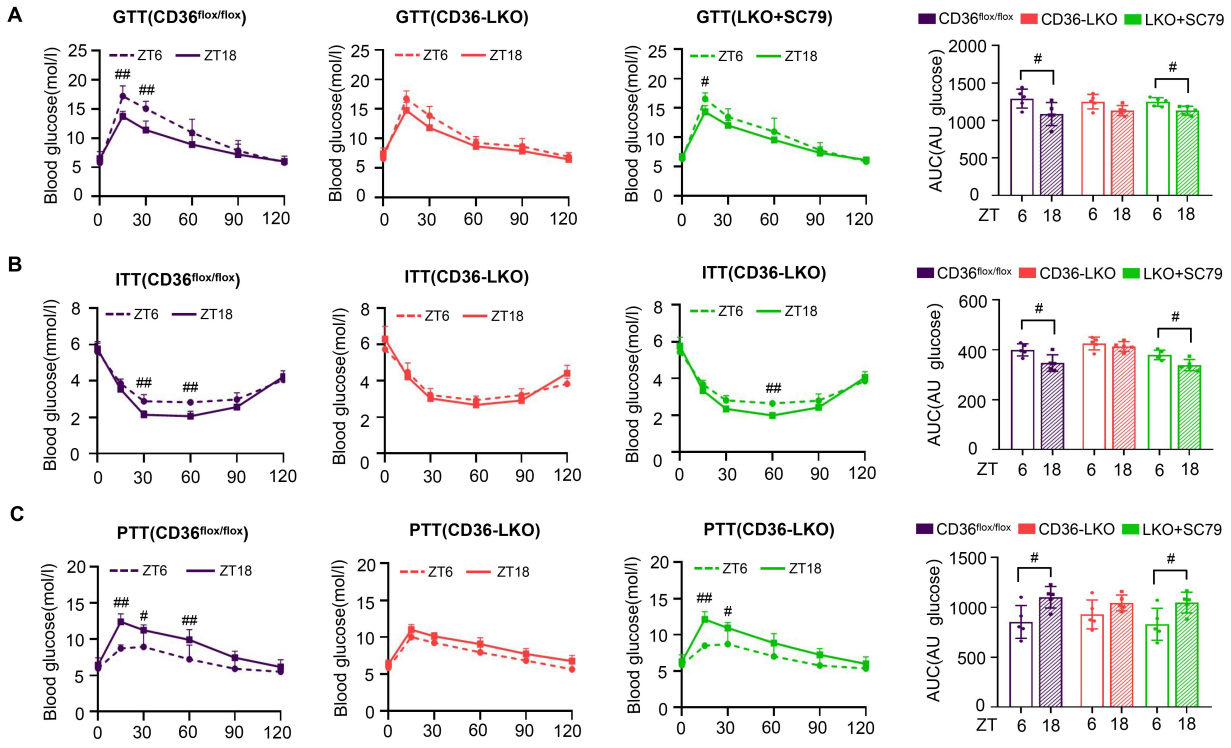

Figure S4 (related to Figure 7). Injection of SC79 restored the diurnal glucose metabolic rhythm in CD36-LKO mice.

(A) ITTs were performed at ZT6 and ZT18 in CD36-LKO mice after 12 h of SC79 injection. The area under the curve (AUC) was used to quantify the ITT results. (B) GTTs were performed at ZT6 and ZT18 in CD36-LKO mice after 12 h of SC79 injection. The area under the curve (AUC) was used to quantify the GTT results. (C) PTTs were performed at ZT6 and ZT18 in CD36-LKO mice after 12 h of SC79 injection. The area under the curve (AUC) was used to quantify the PTT results. n=5 in each group. All data are shown as the mean ± SEM. Comparison of different groups was carried out using two-way ANOVA. $^{\#}P<0.05$, $^{\#\#}P<0.01$ compared with ZT6 in the identical genotype mice. ZT, zeitgeber time.

Figure S5

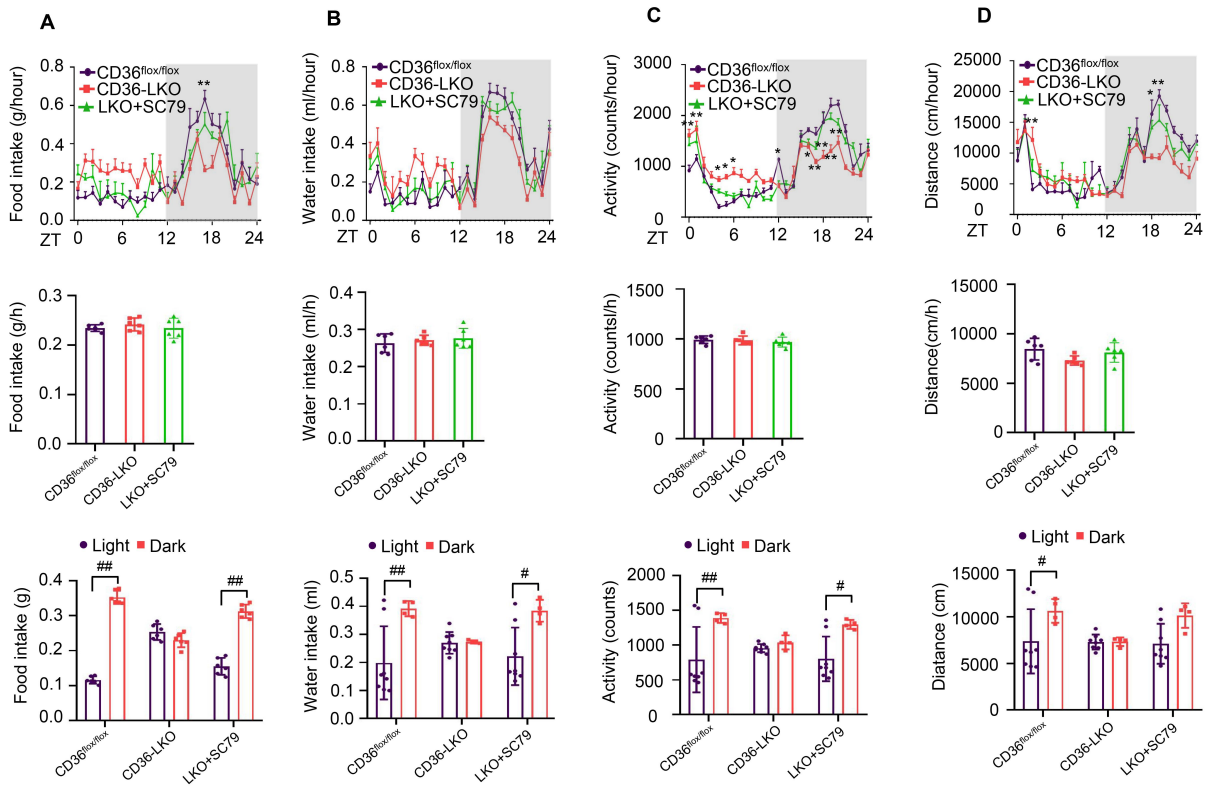

Figure S5 (related to Figure 7). Effect of SC79 injection on biological behavior rhythms in CD36-LKO mice.

Food intake, water intake, locomotor activity and movement distance were monitored for 24 h in 8 to 12-week-old $CD36^{flox/flox}$ and CD36-LKO mice after SC79 injection. (A) Food intake per hour during the light/dark cycle, average food intake per hour and food intake during light and dark phases in $CD36^{flox/flox}$ and CD36-LKO mice injected with SC79. (B) Water intake per hour during the light/dark cycle, average water intake per hour and water intake during light and dark phases in $CD36^{flox/flox}$ and CD36-LKO mice injected with SC79. (C) Locomotor activity per hour during the light/dark cycle, average locomotor activity per hour and locomotor activity during light and dark phases in $CD36^{flox/flox}$ and CD36-LKO mice injected with SC79. (D) Movement distance per hour during the light/dark cycle, average movement distance per hour and movement distance during light and dark phases in $CD36^{flox/flox}$ and CD36-LKO mice injected with SC79. n=6 in each group. Data are shown as the mean ± SEM. Comparison of different groups was carried out using two-tailed unpaired Student's t-test a or two-way ANOVA. *P<0.05, **P<0.01 CD36-LKO group compared with the $CD36^{flox/flox}$ group; $^{\#}$P<0.05, $^{\#\#}$P<0.01 compared with the light phase in the same group. ZT0, the beginning of a subjective circadian period (6:00 AM). Gray shading represents the lights-off period from 6:00 PM to 6:00 AM.

Supplemental Tables

Table S1. Information on Primers (mouse) Used in RT-qPCR. Related to STAR Methods.

Gene	Upstream sequences（5'-3'）	Downstream sequences（5'-3'）
Pklr	CCGAGATACGCACTGGAGTC	GTGGTAGTCCACCCACACTG
PEPCK	ATGAAAGGCCGCACCATGTA	GCACAGATATGCCCATCCGA
Gck	GAGGTCGGCATGATTGTGGG	ACCATCCGGTCGTACTCCAG
FoxO1	AGTGGATGGTGAAGAGCGTG	GAAGGGACAGATTGTGGCGA
Gys2	CACCTAGAGCCCACATCACC	ATTTAGCCGATCCCTCTCAGC
PGC-1a	AAGCACTTCGGTCATCCCTG	CAATGAATAGGGCTGCGTGC
Glut2	GTGTCTGCTACTGCTCTTCTGTC	GACATCCTCAGTTCCTCTTAGTCTC
G6pase	GCTGGAGTCTTGTCAGGCAT	ATCCAAGCGCGAAACCAAAC
Gsk3β	AGTGGTGTGGATCAGTTGGTG	TGCTCCTGGTGAGTCCTTTGT
Bmal1	TGACCCTCATGGAAGGTTAGAA	GGACATTGCATTGCATGTTGG
Clock	ATGGTGTTTACCGTAAGCTGTAG	CTCGCGTTACCAGGAAGCAT
Per1	GAATTGGAGCATATCACATCCGA	CCCGAAACACATCCCGTTTG
Per2	CAGGTTGAGGGCATTACCTCC	AGGCGTCCTTCTTACAGTGAA
Per3	AAAAGCACCACGGATACTGGC	GGGAGGCTGTAGCTTGTCA
Cry1	CACTGGTTCCGAAAGGGACTC	CTGAAGCAAAAATCGCCACCT
Cry2	CACTGGTTCCGCAAAGGACTA	CCACGGGTCGAGGATGTAGA
Rev-erba	AACAGTCTACGGCAAGGCAA	GTGCTGAGAAAGGTCACGGA
Rora	ATTAGGATGTGCCGTGCCTT	GCGATTTCGTCTTCGGTCAG
CD36	ATGGGCTGTGATCGGAACTG	GTCTTCCCAATAAGCATGTCTCC
β-actin	TGCCCTGAGGCTCTTTTCC	TCGTGGATGCCACAGGATT
Dbp	GGATGGGTGGGTAGAATCATTGT	CGGGATCAGGTTCAAAGGTCATT
E4bp4	TGCTTATGTTTGGACTGGCTTTC	GACTTCAGCCTCTCATCCATCAA
Hlf	GCCACAGCCCATGATTAAGAAAG	TTTGCCCAGCTCCTTCCTTAAAT
Tef	CCGACCTGGTCCTCTCTAGT	GAGCGTTTAGCTGCCACATT

Table S2. Information on Primers (human) Used in RT-qPCR. Related to STAR Methods.

Gene	Upstream sequences（5'-3'）	Downstream sequences（5'-3'）
Per1	AACTCATGACAGCACTTCGAGAG	CACTGCTGGTAGTATTCCTGGTT
FoxO1	GGCTGAGGGTTAGTGAGCAG	TTGCTGCCAAGTCTGACGAA
β-actin	GTTGTCGACGACGAGCG	GCACAGAGCCTCGCCTT

Table S3. Information on Primers Used in ChIP-qPCR. Related to STAR Methods.

Gene	Upstream sequences（5'-3'）	Downstream sequences（5'-3'）
Per1-P1	CCACATTCCGAGGCAGCTT	TGCATAGACAAAAGGCTCCAG
Per1-P2	AGTGAATGAGACAGATGACGTTCC	GAAGCCAGGACTACAGAGGGA
Per1-P3	CTCAAGGACCACCCATCTCAT	TGGAAGGGAGGAGAGAGGGAGATA