MCD Diet-induced steatohepatitis generates a diurnal rhythm of associated biomarkers and worsens liver injury of *Klf10* deficient mice

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Supplementary Methods

Real-time quantitative PCR analysis: TaqMan gene expression assays were purchased from Thermo Fisher Scientific Inc.: *B2m (*Mm00437762_m1); *Klf10 (*Mm00449812_m1*)*; *Cidec (*Mm00617672_m1*); Pnpla2 (*Mm00503040_m1*; Tnfa (*Mm00443258_m1*); Ccl2 (*Mm00441242_m1*) ; Tgfβ1* (Mm03024053_m1) ; *Col1a1* (Mm00801666_g1) ; *Timp1* (Mm00441818_m1) ; *Klf4 (*Mm00516104_m1*); Klf6 (*Mm00516184_m1*) Klf11*(Mm00462958*). ; Il-6 (Mm00446190_m1) ; Ikb* (Mm00456849_m1).

Primers for SYBR green approach: *B2m* F: TGCTATCCAGAAAACCCCTCAA, R: GGGGTGAATTCAGTGTGAGCC; *Klf10* F: AGTGACTTTGAAGCGGTGGA, R: AAGGTGCGTTAAACAAAATGC; *Bmal1* F: CTCATTGATGCCAAGACTGG, R: GGTGGCCAGCTTTTCAAATA; *Rev-erbα*: F: AACCTCCAGTTTGTGTCAAGGT, R: GATGACGATGATGCAGAAGAAG; *Rev-erbβ:* F: TTCCAGTAGGTGGATGTTCTCA, R: CTGCTGGGGTAAAACTCATTG; *Rorc*: F: TTTTGAGGAAACCAGGCATC, R: CCACATCTCCCACATTGACTTC; *Dbp*: F: CTGAGGAACAGAAGGATGAGAAG, R: GTAGCGTGAAAGCACAGCAC.

Mouse primary hepatocytes preparation. Mouse hepatocytes were isolated with a two-step collagenase procedure. Briefly, mouse livers were perfused with HEPES buffer containing 8 g/l NaCl, 33mg/l Na2HPO4, 200 mg/l KCl and 2.38 g/l HEPES, pH 7.5, supplemented with 0.5mM EGTA for 3min at 3ml/min, then with HEPES buffer for 3 min at 3ml/min and finally with HEPES buffer supplemented with 1.5 g/l CaCl2 and 0.026% collagenase type IV (#C5138) (Sigma-Aldrich;, Saint-Quentin-Fallavier, FR) for 7 min at 3 ml/min. Livers were then carefully removed and minced in Williams' E medium (Life Technologies, St Aubin, FR) supplemented with 10% fetal bovine serum (PAA Laboratories, Velizy-Villacoublay, FR), 100 units/ml penicillin, 100 mg/ml streptomycin, 2mM L-glutamine and 0.02 UI/ml insulin (Umulin, Lilly France, Neuilly-sur-Seine, FR). The cell suspension was then filtered (100 µm) and hepatocytes were collected by centrifugation at 50 x *g* for 5 min. Viability was evaluated by trypan blue exclusion (Sigma-Aldrich, Saint-Quentin-Fallavier, FR).

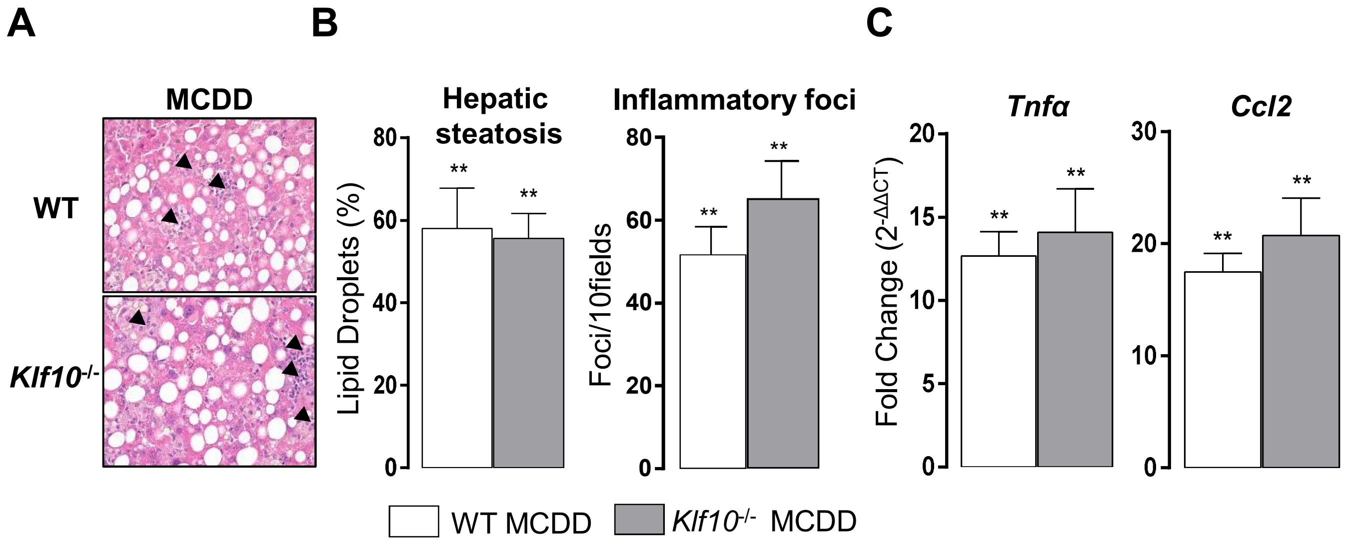

Fig. S1 *Klf10* deficiency does not impact the development of hepatic steatosis and inflammation during steatohepatitis in mice sampled at ZT9. WT and *Klf10*$^{-/-}$ male mice were fed a methionine and choline deficient diet (MCDD) for 4 weeks and sacrificed at ZT9 (n=9 mice/ group). (**A**) Representative images, showing the presence of lipid droplets and inflammatory foci (black arrows), from H&E stained liver sections of WT and *Klf10*$^{-/-}$ upon MCDD. (**B**) Quantification of hepatic steatosis and inflammatory foci from H&E stained liver sections. (**C**) Hepatic expression of *Tnfα* and *Ccl2*. All the data are expressed as mean ± SEM. The mRNA levels are normalized to *B2m* and expressed relative the CD level ZT9 (n=3). Statistical significance was tested using the Mann-Whitney test, ** $p<0.01$.

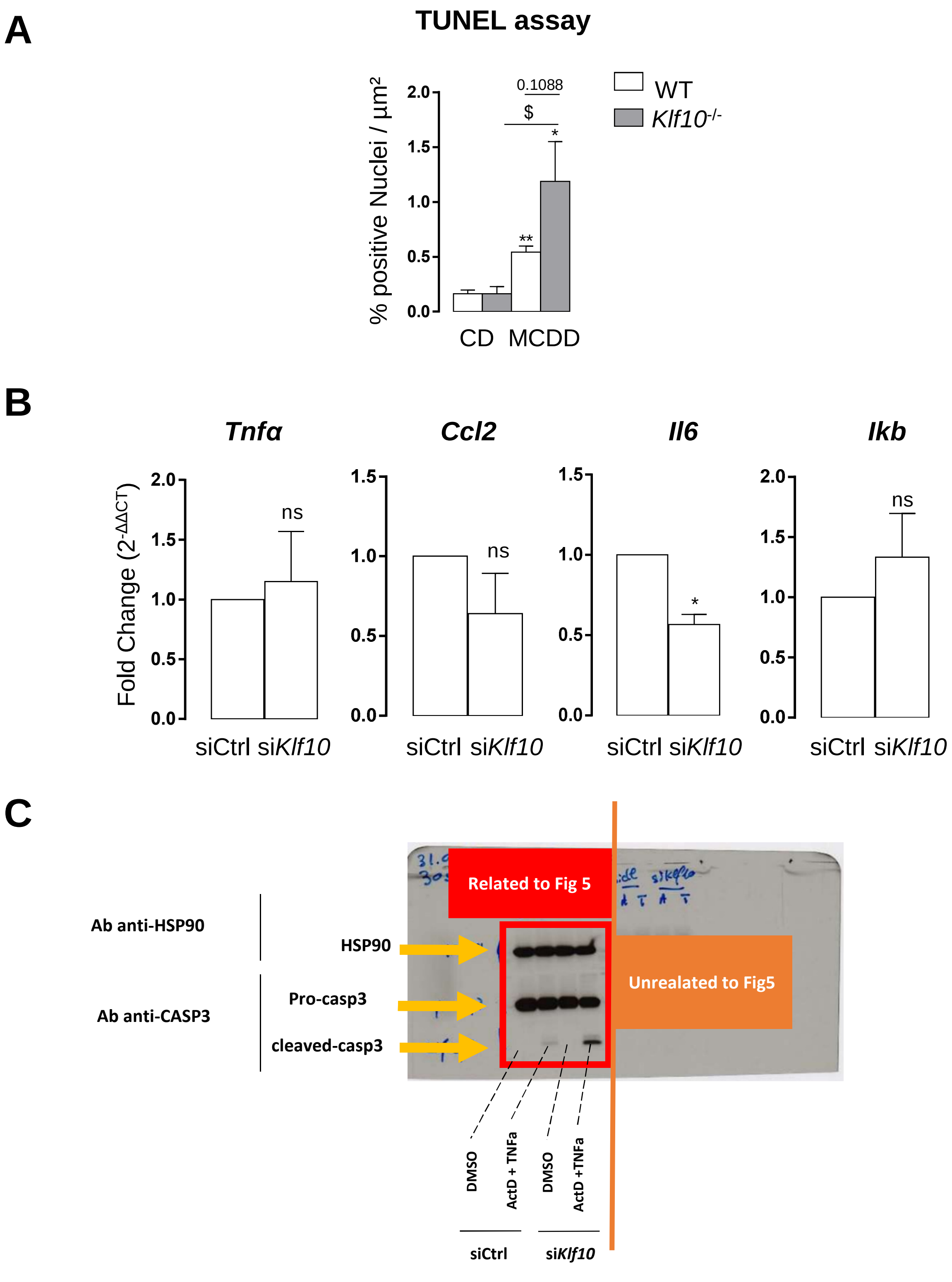

Fig. S2 *Klf10* deficient mice may display increased hepatic apoptotic cells upon MCDD challenge. **(A)** quantification of the TUNEL assay performed on liver sections of WT and *Klf10*$^{-/-}$ mice fed a CD or MCDD and sacrificed at ZT3 (n=3-10 mice /group). **(B)** Mouse primary hepatocytes were transfected with control (siCtrl) or *Klf10* (si*Klf10*) siRNA. Gene expression of *Tnfα*, *Ccl2*, *Il6* and *Ikb* were evaluated. Gene expression was normalized to *B2m* and expressed as relative expression of the untreated siCtrl condition, 72h after transfection (n=4). **(C)** Corresponding uncropped image of the western blot shown in Fig. 5d. All the data are expressed as mean ± SEM. Statistical significance was tested using the Mann-Whiney test vs the control group (stars) or the indicated groups (dollar symbols), ** $p<0.01$; * and $ $p<0.05$.

Items	Diet	Mean level	Amplitude	Acrophase (ZT : min)
Steatosis (%)	CD	ND	ND	ND
	MCDD	12.10 (7.83 ; 16.42)*	13.19 (7.32 ; 19.25)	08:07 (06:19 ; 10:06)
Inflammatory foci (foci / 10 fields)	CD	ND	ND	ND
	MCDD	12.49 (7.08 ; 19.10)*	12.27 (3.72 ; 21.08)	07:38 (04:24 ; 11:08)
Hepatic TG (mg/g of liver)	CD	2.98 (2.47 ; 3.58)	1.59 (0.87 ; 2.35)	14.27 (12.19 ; 16.36)
	MCDD	7.35 (5.59 9.49)*	NSR	NSR
ALT (IU/L)	CD	20.02 (16.36 ; 25.13)	NSR	NSR
	MCDD	84.20 (74.35 ; 94.92)*	NSR	NSR
AST (IU/L)	CD	202 (179 ; 227)	NSR	NSR
	MCDD	620 (553 ; 689)	307 (211 ; 400)	00:57 (00:15 ; 02:00)
Pnpla2	CD	1.08 (0.93 ; 1.24)	0.43 (0.20 ; 0.64)	08:35 (06:29 ; 10:46)
	MCDD	2.02 (1.79 ; 2.30)*	0.66 (0.30 ; 1.01)	08:44 (06:22 ; 10:59)
Fsp27/Cidec	CD	1.28 (0.85 ; 1.75)	NSR	NSR
	MCDD	17.96 (14.90 ; 20.92)	7.73 (3.73 ; 11.93)	06:25 (04:04 ; 08:31)
Tnfα	CD	1.20 (1.00 ; 1.43)	NSR	NSR
	MCDD	8.73 (7.01 ; 10.78)*	5.77 (3.21 ; 8.39)	4:26 (02:19 ; 06:13)
Ccl2	CD	0.88 (0.49 ; 1.48)	NSR	NSR
	MCDD	6.73 (5.40 ; 8.29)*	4.55 (2.61 ; 6.53)	02:20 (00:32 ; 04:11)
Tgfβ1	CD	0.82 (0.72 ; 0.92)	NSR	NSR
	MCDD	2.02 (1.79 ; 2.24)*	NSR	NSR
Col1α1	CD	1.00 (0.78 ; 1.20)	NSR	NSR
	MCDD	3.63 (2.81 ; 4.49)*	1.89 (0.72 ; 3.15)	06.42 (03.44 ; 09.15)
Timp1	CD	1.11 (0.69 ; 1.57)	NSR	NSR
	MCDD	13.41 (10.05 ; 16.85)*	8.00 (3.36 ; 12.57)	06.02 (03.16 ; 08.27)

Table S1. NAFLD features cosinor analysis

Bootstrap analysis was performed to generate the 95% confidence intervals. * indicates statistical difference between CD and MCDD fed mice ($p<0.05$). NSR, not significantly rhythmic, ND, not detectable.

Gene	Diet	Mean level	Amplitude	Acrophase (ZT : min)
Bmal1	CD	0.83 (0.67 ; 1.00)	0.75 (0.52 ; 0.99)	22:20 (21:10 ; 23:35)
	MCDD	1.10 (0.96 ; 1.28)	1.21 (0.96 ; 1.46)	19:12 (18:29 ; 19:54)*
Rev-erbα	CD	0.43 (0.28 ; 0.61)	0.57 (0.33 ; 0.81)	04: 26 (02:44 ; 06:07)
	MCDD	0.36 (0.22 ; 0.54)	0.56 (0.33 ; 0.80)	03:17 (01:38 ; 04:53)
Per2	CD	7.13 (5.58 ; 8.88)	7.63 (5.31 ; 9.97)	11:36 (10:22 ; 12:49)
	MCDD	7.68 (6.91 ; 8.47)	5.85 (4.74 ; 6.95)	10:34 (09:50 ; 11:16)
Cry1	CD	2.04 (1.65 ; 2.38)	1.64 (1.18 ; 2.12)	19:23 (18:10 ; 20:39)
	MCDD	2.22 (2.09 ; 2.37)	1.88 (1.69 ; 2.07)	16:26 (16:01 ; 16:50)*
Rev-erbβ	CD	0.78 (0.64 ; 0.95)	0.55 (0.34 ; 0.78)	08:07 (06:31 ; 09:49)
	MCDD	0.47 (0.41 ; 0.54)*	0.35 (0.26 ; 0.44)	08:19 (07:11 ; 09:23)
Rorc	CD	2.66 (2.27 ; 3.15)	1.16 (0.56 ; 1.77)	15:53 (13:34 ; 18:02)
	MCDD	2.87 (2.63 ; 3.13)	2.44 (2.12 ; 2.78)*	14:35 (13:58 ; 15:10)
Dbp	CD	1.69 (1.22 ; 2.19)	2.32 (1.69 ; 3.00)	07:44 (07:41 ; 09:56)
	MCDD	0.63 (0.47 ; 0.80)*	0.88 (0.62 ; 1.14)*	8:42 (07:33 ; 09:46)
Klf10	CD	1.99 (1.40 ; 2.67)	1.50 (0.59 ; 2.41)	9:23 (6:46 ; 12:00)
	MCDD	2.50 (2.06 ; 3.00)	NSR	NSR
Klf11	CD	0.70 (0.49 ; 0.97)	0.51 (0.18 ; 0.87)	02:57 (23:54 ; 05:58)
	MCDD	0.95 (0.83 ; 1.07)	0.50 (0.34 ; 0.68)	01:31 (00:09 ; 2:52)
Klf4	CD	0.98 (0.78 ; 1.24)	NSR	NSR
	MCDD	2.64 (2.34 ; 2.94)*	NSR	NSR
Klf6	CD	0.88 (0.75 ; 1.03)	NSR	NSR
	MCDD	4.19 (3.68 ; 4.71)*	1.66 (0.97 ; 2.41)	01:17 (23:30 ; 03:06)

Table S2. Liver clock genes cosinor analysis

Bootstrap analysis was performed to generate the 95% confidence intervals. * indicates statistical difference between CD and MCDD fed mice ($p<0.05$). NSR, not significantly rhythmic.

Gene	Diet	Mean level	Amplitude	Acrophase (ZT : min)
Bmal1	CD	0.88 (0.66 ; 1.11)	0.64 (0.32 ; 0.97)	21:15 (19:09 ; 23:17)
	MCDD	1.06 (0.96 ; 1.18)	1.03 (0.87 ; 1.18)	18:19 (17:44 ; 18:53)*
Nr1d1	CD	0.68 (0.53 ; 0.85)	0.46 (0.24 ; 0.70)	7:03 (05:07 ; 08:52)
	MCDD	0.63 (0.46 ; 0.90)	0.94 (0.60 ; 1.29)	03:29 (02:04 ; 04:54)*
Per2	CD	1.41 (1.15 ; 1.66)	0.93 (0.57 ; 1.29)	13:18 (11:29 ; 14:39)
	MCDD	1.44 (0.97 ; 2.04)	1.24 (0.48 ; 2.02)	08:45 (06:02 ; 11:18)*
Cry1	CD	4.11 (3.24 ; 4.97)	3.77 (2.58 ; 4.93)	16:19 (14:59 ; 17:37)
	MCDD	8.68 (5.67 ; 3.22)*	8.28 (3.65 ; 4.06)	10:38 (07:44 ; 13:29)*
Nr1d2	CD	0.88 (0.77 ; 1.00)	0.46 (0.29 ; 0.62)	08:59 (07:38 ; 10:16)
	MCDD	1.09 (0.67 ; 1.68)	1.23 (0.51 ; 1.93)	05:52 (03:00 ; 08:15)
Rorc	CD	2.39 (2.46 ; 3.51)	2.54 (1.85 ; 3.24)	16:30 (15:15 ; 17:36)
	MCDD	3.38 (3.17 ; 4.76)	2.2 (1.11 ; 3.38)	13:07 (11:03 ; 15:28)
Dbp	CD	1.77 (51.38 ; 2.15)	2.01 (1.44 ; 2.60)	09:41 (08:40 ; 10:43)
	MCDD	2.22 (1.88 ; 2.57)	2.68 (2.20; 3.19)	05:49 (05:08 ; 06:29)*
Klf10	CD	2.02 (1.71 ; 2.35)	1.18 (0.77 ; 1.59)	14:41 (13: 07 ; 16:22)
	MCDD	2.15 (1.71 ; 2.67)	NSR	NSR
Klf11	CD	0.68 (0.56 ; 0.83)	0.33 (0.16 ; 0.51)	03:15 (00:36 ; 05:42)
	MCDD	0.85 (0.69; 1.02)	0.60 (0.37; 0.83)	03:34 (01: 55; 05:03)

Table S3. Kidney clock genes cosinor analysis

Bootstrap analysis was performed to generate the 95% confidence intervals. * indicates statistical difference between CD and MCDD fed mice ($p<0.05$). NSR, not significantly rhythmic.

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