

1 **Supporting Information**

2 **For**

3
4 **Rapid recapitulation of non-alcoholic steatohepatitis upon**
5 **loss of HCF-1 function in mice**
6

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Supplementary experimental procedures

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Primers and PCR conditions for genotyping:

For HCF-1: p1 (5'-GGAGGAACATGAGCTTTAGG-3'), p2 (5'-CAATAGGCGAGTACCATCACAC-3'), and p3 (5'-GGGAAAGTAGACCCACTCTG-3').

The annealing was done at 62°C for 15 seconds with an extension at 72°C for 10 seconds (22).

For AlbCre: p1 (5'-ATCATTCTTTGTTTTTCAGG-3'), p2 (5'-GGAACCCAAACTGATGACCA-3'), and p3 (5'-TTAAACAAGCAAAACCAAAT-3'). The annealing was done at 53°C for 1 minute with an extension at 72°C for 1 minute. Combination of p1 and p2 was used to detect the *wildtype* allele (229bp). Combination of p2 and p3 was used to detect the *Cre* allele (444bp).

Tissue immunohistochemistry and histology:

For (a) fluorescence and diaminobenzidine (DAB) immunostaining, and (b) colorations, the liver tissues were either paraffin-embedded and sectioned into 4 µm thick sections using a MICROM HM325 microtome or cut with the help of a cryostat MICROM HM550 microtome to generate 8 µm thick sections.

(a) *Fluorescence and DAB immunostaining:* The paraffin-embedded sections were first (i) deparaffinized in xylene, (ii) rehydrated through graded alcohol washes, and (iii) rinsed twice with PBS. For DAB immunostaining, endogenous peroxidase activity was quenched at this stage with 6% hydrogen peroxide in methanol for 10 min and rapidly washed once with H₂O. Subsequently antigens for both fluorescence and DAB immunostaining were revealed by heating in a 750 W microwave oven until boiling for approximately 10 min in citrate buffer (10mM, pH 6.0), allowed to slowly cool to 4°C, washed twice with PBS, and then blocked for 30 min with 2% normal goat serum (NGS)

57 (Sigma-Aldrich, cat. # G9023) in PBS at room temperature (RT). After blocking,
58 primary immunostaining was performed by incubation of the slices with specific
59 primary antibody (see below) diluted in 2% NGS overnight at 4°C followed by three
60 washes with PBS.

61 For secondary fluorescence immunostaining, incubation with the appropriate
62 secondary antibody (see below) was for 30 min in the dark at RT, followed by (i) three
63 PBS washes, (ii) counterstaining with 4',6-diamidino-2-phenylindole (DAPI) (Sigma-
64 Aldrich, CAS # 28718-90-3), (iii) two PBS washes, and (iv) embedding with Mowiol
65 mounting medium (Sigma-Aldrich, CAS # 9002-89-5). The sections were subsequently
66 analyzed using an AxioImager M1 microscope with AxioCam MRm monochrome and
67 AxioCam MRc color cameras (Carl Zeiss AG, Oberkochen, Germany), or a Zeiss
68 CLSM 710 spectral confocal laser scanning microscope. Images were processed using
69 AxioVision 4.8.2 (Carl Zeiss AG, Oberkochen, Germany) or Imaris 8.2 (Bitplane Inc.)
70 software.

71 For secondary DAB immunostaining, the primary antibodies were detected by
72 incubating the sections for 30 min with anti-mouse (Dako cat. # K4000) or anti-rabbit
73 (Dako cat. # K4002) horseradish peroxidase (HRP) secondary antibody. Visualization
74 was performed with DAB substrate (Dako cat. # K3468) before being counterstained
75 with Mayer's hematoxylin. The sections were analyzed using the AxioImager M1
76 microscope with AxioCam MRm monochrome and AxioCam MRc color cameras (Carl
77 Zeiss AG, Oberkochen, Germany). Tilescan imaging of entire liver lobes was acquired
78 at high resolution using a Leica DMI8 inverted microscope with Andor Zyla black and
79 white and DFC7000T color cameras.

80 The primary antibodies used were: rabbit anti-HCF-1 (1:1000, H12, (56)), rat
81 anti-Ki67 (1:60, eBioscience cat. # 41-5698), anti-HNF4 α (1:100, R&D Systems cat. #

82 PP-H1415-00), anti- β -catenin (1:75, BD Biosciences, cat. # 610153), anti-SMA (1:400,
83 Abcam cat. # ab5694), anti-Oxphos (1:100, Abcam cat. # ab110413), anti-PGC1 α
84 (1:100, Millipore cat. # ST1202), anti-ATP5a (1:400, Abcam cat. # ab14748), anti-
85 UQCRC2 (1:400, Abcam cat. # ab14745), anti-MTCO1 (1:200, Abcam cat. # ab14705),
86 and anti-PCNA (1:50, BD Transduction Laboratories cat. # 610665) mouse monoclonal
87 antibodies, rabbit anti-Histone H3 phospho Ser10 (1:100, Abcam cat. ab5176), rabbit
88 anti-cytokeratin (1:100, Dako cat. # Z0622), rat anti-F4/80 (1:800, Abcam cat. #
89 ab6640), and rat anti-CD31 (1:20, Dianova GmbH cat. # DIA-310-M).

90 The secondary antibodies used were: goat anti-rabbit Alexa 488 (1:400, Molecular
91 Probes cat. # A11034), goat anti-mouse Alexa 568 (1:500, Molecular Probes cat. #
92 A11019), goat anti-rabbit Alexa 568 (1:1000, Molecular Probes cat. # A21069), goat
93 anti-mouse Alexa 488 (1:400, Molecular Probes cat. # A11029), donkey anti-mouse
94 Alexa 594 (1:500, Molecular Probes cat. # A11005), and goat anti-mouse Alexa 635
95 (1:300, Molecular Probes cat. # A31575).

96

97 *b) Colorations:*

98 *Hematoxylin (H) and Eosin (E) staining*

99 Standard hematoxylin and eosin staining was performed on deparaffinized and
100 rehydrated liver sections (57).

101

102 *Periodic acid-Schiff (PAS) staining*

103 For glycogen detection, PAS staining was performed on deparaffinized and rehydrated
104 liver sections by incubation in 1% periodic acid for 10 mins followed by staining with
105 Schiff's reagent for 15 mins, followed by Mayer's hematoxylin staining for 5 mins.

106

107 *Sirius red staining*

108 Sirius red staining was done to assess collagen deposition in injured liver paraffin
109 sections. After hydrating, sections were rinsed in 0.2% phosphomolybdic acid for 5
110 minutes followed by staining with 0.1% sirius red stain for 90 minutes. Thereafter, the
111 sections were stained with Mayer's hematoxylin for 1 minute.

112

113 *Oil Red O assay*

114 Liver samples were cut with the help of a cryostat microtome (MICROM HM550) to
115 generate 8 µm thick cryo-sections for this assay. After drying the cryo-sections were (i)
116 fixed with 4% paraformaldehyde (PFA) for 10 minutes at RT, (ii) quickly washed with
117 distilled water, (iii) rinsed with 60% isopropanol for 1 minute, (iv) stained with Oil Red
118 O solution (Sigma cat. # 0625) for 10 minutes, (v) quickly rinsed with 60% isopropanol,
119 (vi) quickly rinsed with distilled water, (vii) co-stained with hematoxylin for 45 seconds,
120 (viii) washed very well with water, and (ix) mounted. With this staining protocol, lipids
121 and nuclei can be seen in red and blue color, respectively.

122

123 *Succinate dehydrogenase assay*

124 Liver samples were cut with the help of a cryostat microtome (MICROM HM550) to
125 generate 8 µm thick cryo-sections for this assay. After drying, the cryo-sections were
126 incubated in succinate dehydrogenase solution containing succinic acid (Sigma cat. #
127 S2378) and nitro blue tetrazolium chloride, NBT (Roche cat. # 11 383 213 001) for 30
128 minutes at 37°C. Thereafter, the sections were washed thrice with 1X PBS. This
129 staining causes formation of a purple precipitate over top of mitochondria.

130

131 ***Immunoblotting:***

132 For immunoblotting, approximately 100 mg of liver tissue from each time point was
133 homogenized in RIPA buffer (50 mM Tris-HCl pH7.4, 150 mM NaCl, 1 mM EDTA, 0.2%
134 sodium deoxycholate, 1 mM DTT, 1mM PMSF, and 1% Triton X) containing protease inhibitor
135 (Roche). Samples (10-20 µg) were boiled for 5 mins before PAGE and transfer to nitrocellulose
136 membrane. Membranes were blocked for 60 mins with 5 ml of LI-COR blocking buffer,
137 incubated with primary antibody in 50% LI-COR blocking buffer and 50% PBST (PBS
138 containing 0.1% Tween 20) overnight at 4°C, washed three times and incubated with secondary
139 antibody (dilution 1:10,000) for 30 mins at RT. The membranes were washed three times and
140 scanned with an Odyssey infrared imager (LI-COR).

141

142 ***TUNEL assay:***

143 Terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling (TUNEL) was
144 performed on paraffin-embedded liver sections with the *in situ* cell death detection kit (Roche
145 Applied Science, cat. # 11684795910), according to the manufacturer's directions.

146

147 ***Metabolic Assessments:***

148 *Glucose and pyruvate tolerance tests:* After a 15-hour overnight fast, age- and sex-
149 matched 12- to 14-week old mice received glucose or pyruvate by intraperitoneal injections (2
150 g/kg, as a 20% solution). Blood samples were obtained from tail-tip bleedings, and blood
151 glucose levels were measured with a Glucometer (GlucoTrend Premium; Boehringer Mannheim
152 GmbH, Mannheim, Germany). Glycemic areas were measured from time 0 to 120 minutes.

153

154 *Insulin tolerance tests:* After a 5-hour fast, age- and sex-matched 10- to 16-week old
155 mice received insulin by intraperitoneal injections (0.3U/kg). Blood samples were collected
156 like glucose/pyruvate tolerance tests. Plasma insulin levels were determined by ELISA

157 (Merckodia AB, Uppsala, Sweden).

158

159 *Diagnostics:* Aspartate transaminase (AST) levels were measured by using the Cobas
160 C111 robot (Roche Diagnostics). Alanine transaminase (ALT), HDL/LDL/total cholesterol
161 and triglyceride levels were measured by using the Xpand clinical chemistry system (Siemens
162 Healthcare GmbH).

163

164 *Estimation of metabolites, energy metabolism intermediates, and bile acids:* All these
165 measurements were done at a metabolomics facility (Biocrates Life Sciences AG, Austria). To
166 extract metabolites, age- and sex-matched 10- to 14-week old mice liver samples were
167 homogenized using Precellys® with ethanol phosphate buffer. An aliquot of each sample was
168 centrifuged and the supernatant was used for analysis. AbsoluteIDQ® p180 kit assay was used
169 for the quantification of amino acids, acylcarnitines, sphingomyelins, phosphatidylcholines,
170 hexoses, and biogenic amines. The fully automated assay was based on PITC
171 (phenylisothiocyanate)-derivatization in the presence of internal standards followed by FIA-
172 MS/MS (acylcarnitines, lipids, and hexose) and LC/MS (amino acids, biogenic amines) using
173 an AB SCIEX 4000 QTrap® mass spectrometer (AB SCIEX, Darmstadt, Germany) with
174 electrospray ionization.

175 For the quantitative analysis of energy metabolism intermediates (glycolysis, citrate
176 cycle, pentose phosphate pathway, urea cycle) hydrophilic interaction liquid chromatography
177 (HILIC)-ESI-MS/MS method in highly selective negative MRM detection mode was used. The
178 MRM detection was performed using an ABSCIEX 4000 QTrap® tandem mass spectrometry
179 instrument (AB SCIEX, Darmstadt, Germany). The sample was protein precipitated and
180 extracted simultaneously with aqueous methanol in a 96 well plate format. Internal standards
181 (ratio external to internal standard) and external calibration were used for highly accurate

182 quantitation.

183 A highly selective reversed phase LC-MS/MS analysis method in negative MRM
184 detection mode was applied to determine the concentration of bile acids. Samples were
185 extracted via dried filter spot technique in 96 well plate format, which is well suitable for high
186 throughput analysis. For highly accurate quantification internal standards and external
187 calibration were applied. In brief, internal standards and 10 μ L sample volume placed onto
188 filter spots were extracted and simultaneously protein precipitated with aqueous methanol.
189 These sample extracts were measured by LC-ESI-MS/MS with a tandem mass spectrometry
190 instrument (AB SCIEX, Thermo Scientific, Waters). Data of bile acids were quantified with a
191 corresponding MS software (AB SCIEX – Analyst, Thermo Scientific – x-calibur, Waters -
192 Masslynx, Darmstadt, Germany) and finally exported into MetIDQ software for comprehensive
193 statistical analysis.

194

195 ***RNA-sequencing (RNA-seq):***

196 Poly(A)-containing RNA from individual livers was used for RNA-seq. RNA was extracted
197 with RNeasy kit (Qiagen cat. # 74104). The disruption and homogenization was done with
198 TissueLyser II. Samples were quantified with MySpec machine and 200 μ g (in 100 μ l) of RNA
199 sample was used for further analysis. Strand-specific libraries were prepared with the TruSeq
200 Stranded mRNA Library Prep kit (Illumina catalog # RS-122-9004DOC). The fragment ends
201 (50 nucleotides) were sequenced with single-end sequencing technology from HiSeq 2100
202 (Illumina).

203 RNA-seq reads were aligned to the Mouse mm9 genome assembly and transcriptome
204 using TopHat version 2.0.13 with Bowtie version 1.1.2. Counts per gene were calculated using
205 the Rsubreads R package and transformed in RPKM. Normalization was done via the edgeR
206 R package using TMM. The cutoff for genes considered as non-transcribed was calculated

207 according to the method described in <https://bmcgenomics.biomedcentral.com/articles/10.1186/1471-2164-14-778>. Genes showing a change during the time-course
208 between zero and ten days were selected using MaSigPro with a significance level of 0.05. The
209 4549 selected genes were then submitted to a PAM analysis and split into three categories,
210 namely: 1) genes going down during time-course, 2) genes going up during the first half of the
211 time-course, and 3) genes going up during the second half of the time-course. The mean value,
212 and the fold-change (KO minus WT) were calculated for each time point. The selected genes
213 were ranked by the fold-change, and a GSEA was performed using this ranked gene list against
214 the HALLMARKS set
215 (http://software.broadinstitute.org/gsea/msigdb/collection_details.jsp#H).

217

218 **ChIP-seq analysis:**

219 *Chromatin isolation, immunoprecipitation, and preparation of high throughput*
220 *sequencing:* Chromatin was isolated from the liver of individual male *Alb-Cre-ERT2^{tg}* ;
221 *Hcfc1^{lox/Y}* mice at 0 days and at 4 days after *Hcfc1^{hepKO}*-allele induction by tamoxifen
222 administration as described in Methods. Chromatin was immunoprecipitated and prepared for
223 high-throughput sequencing as described (58) using the anti-HCF-1 H12 antibody (56).

224

225 *Mapping:* Both sequenced fragment ends R1 and R2 were first mapped individually on
226 the Mouse genome Mm10 release, allowing up to 50 multiple matches. For both ends, the best
227 matches were extracted for each read. All multiple matches with the same mapping scores were
228 kept at this stage. The two mapped reads R1 and R2 were then merged. If whole fragments
229 had multiple matches with the same score, only the matches giving fragments smaller than 1
230 kb were kept. The multiple matches were weighted according to the ratio (times

231 sequenced)/(number of multiple matches). Regions from the ENCODE blacklisted list as well
232 as telomeric regions were discarded for further analysis.

233

234 *Peak detection:* Peaks were first detected with MACS2, with an FDR of 0.01. Those
235 peaks were then compared to the enriched regions determined by another method using
236 genomic bins, as described in Renaud et al (Genome Res. 2014 Jan;24(1):37-51. doi:
237 10.1101/gr.161570.113. Epub 2013 Oct 9, PMID: 24107381). Only the MACS peaks
238 overlapping with the enriched genomic bins were kept. Peaks positions were then compared
239 sequentially to the following annotated features:

240 - Pol2 TSS from the Gencode genomic annotation, Mouse Mm10

241 - Repeats (Alus, MIR, LTR, satellite regions)

242 - RefSeq genes, coding and non-coding

243 - Ensembl genes

244

245 *Scoring:* Fragments within a window of minus 250 to plus 250 nucleotides around the
246 Pol2 TSSs (Gencode annotation, Mouse Mm10) were summed, in the IP and in the
247 corresponding Input. One pseudocount was added for log2 calculation. The final score was
248 then calculated as follows: $\text{score} = \log_2(\text{IP}) - \log_2(\text{Input})$. All scores were scaled to a total of 15
249 mio fragments per experiment.

250

251 ***Electron microscopy:***

252 Small pieces of liver (2 mm³) were fixed in 2.5% glutaraldehyde solution (EMS, Hatfield, PA,
253 USA) in phosphate buffer (PB 0.1 M, pH 7.4) (Sigma, St Louis, MO, USA) for 2 hours at room
254 temperature (RT). Then, the samples were post-fixed in 1% osmium tetroxide (EMS, Hatfield,
255 PA, USA) in phosphate buffer (PB 0.1 M, pH 7.4) (Sigma, St Louis, MO, USA) for 2 hours at

256 RT. The samples were then washed two times in distilled water and dehydrated in acetone
257 solution (Sigma, St Louis, MO, USA) at graded concentrations (30%-30 min; 70%-30 min;
258 100%-2x1 h). This procedure was followed by infiltration in epoxy resin (Sigma, St Louis,
259 MO, USA) at graded concentrations (Epon 1/3 acetone-1 h; Epon 3/1 acetone-1 h, Epon 1/1-2
260 h, Epon 1/1-12h). Pieces of liver were then placed in molds filled with resin and then
261 polymerized for 48 h at 60°C in an oven. Ultrathin sections (50 nm) were cut on Leica Ultracut
262 (Leica Mikrosysteme GmbH, Vienna, Austria) and picked up on a copper slot grid 2x1 mm
263 (EMS, Hatfield, PA, USA) coated with a polystyrene film (Sigma, St Louis, MO, USA).
264 Micrographs were taken with a Philips CM100 TEM (FEI, Eindhoven, The Netherlands) at an
265 acceleration voltage of 80 kV with a TVIPS TemCam-F416 digital camera (TVIPS GmbH,
266 Gauting, Germany).

267

268 **Supplementary Figure Legends**

269

270 **Supplementary Figure 1.** Knockout *Alb-Cre-ERT2^{tg}* ; *Hcfc1^{hepKO/Y}* males display rapid loss
271 of *Hcfc1* expression whereas heterozygous *Alb-Cre-ERT2^{tg}* ; *Hcfc1^{hepKO/Y}* females continue to
272 maintain *Hcfc1* expression. View of the reads mapped on the 26 exons of the *Hcfc1* gene,
273 shown in blue at the top, in control *Hcfc1^{lox/Y}* male (A), heterozygous *Alb-Cre-ERT2^{tg}* ;
274 *Hcfc1^{hepKO/+}* female (B), and knockout *Alb-Cre-ERT2^{tg}* ; *Hcfc1^{hepKO/Y}* male (C) livers beginning
275 from 0d to 14d post-tamoxifen treatment with RNA-seq analysis. Arrows point to the reads
276 observed at exon 2 and 3 of the *Hcfc1* gene. The total *Hcfc1* transcript expression level in
277 control *Hcfc1^{lox/Y}* male (blue), heterozygous *Alb-Cre-ERT2^{tg}* ; *Hcfc1^{hepKO/+}* female (green), and
278 knockout *Alb-Cre-ERT2^{tg}* ; *Hcfc1^{hepKO/Y}* male (red) livers is depicted as log₂(Reads Per
279 Kilobase of transcript per Million mapped reads or RPKM) values.

280

281 **Supplementary Figure 2.** *Hcfc1*^{hepKO/+} heterozygous female livers do not show any abnormal
282 features. (A) Macroscopic comparison of livers from control liver (0d), and 7d, 14d, and 18d
283 post-tamoxifen treated heterozygous *Alb-Cre-ERT2*^{tg}; *Hcfc1*^{hepKO/+} female livers. (B) Boxplot
284 showing liver-to-body weight ratio of control liver (0d; n=9), and 7d (n=4) and 18d (n=4) post-
285 tamoxifen treated knockout *Alb-Cre-ERT2*^{tg}; *Hcfc1*^{hepKO/Y} male livers. The difference between
286 liver-to-body weight ratio of 0d control liver and 7d post-tamoxifen treated knockout *Alb-Cre-*
287 *ERT2*^{tg}; *Hcfc1*^{hepKO/Y} male liver was significant (p-value 0.01). The difference between liver-
288 to-body weight ratio of 7d and 18d post-tamoxifen treated knockout *Alb-Cre-ERT2*^{tg};
289 *Hcfc1*^{hepKO/Y} male livers was marginally significant (p-value 0.05). The difference between
290 liver-to-body weight ratio of 0d control liver and 18d post-tamoxifen treated knockout *Alb-Cre-*
291 *ERT2*^{tg}; *Hcfc1*^{hepKO/Y} male liver was significant (p-value 0.03). (C) Boxplot showing liver-to-
292 body weight ratio of control liver (0d; n=4), and 7d (n=4) and 18d (n=2) post-tamoxifen treated
293 heterozygous *Alb-Cre-ERT2*^{tg}; *Hcfc1*^{hepKO/+} female livers. Scale bar: 1 cm.

294

295 **Supplementary Figure 3.** *Hcfc1*^{hepKO/Y} knockout males display metabolic abnormalities. (A)
296 Graphs showing the body weights of control *Hcfc1*^{lox/Y} (n=7/time point) and knockout *Alb-Cre-*
297 *ERT2*^{tg}; *Hcfc1*^{hepKO/Y} (n=11/time point) males after tamoxifen treatment. (B) Glucose levels
298 (or glycemia) during glucose tolerance test in control *Hcfc1*^{lox/Y} (n=6) and knockout *Alb-Cre-*
299 *ERT2*^{tg}; *Hcfc1*^{hepKO/Y} (n=6) males 7d post-tamoxifen treatment. (C) Glycemia during glucose
300 tolerance test in control *Hcfc1*^{lox/+} (n=5) and heterozygous *Alb-Cre-ERT2*^{tg}; *Hcfc1*^{hepKO/+} (n=6)
301 females 7d post-tamoxifen treatment. (D) Glycemia during pyruvate tolerance test in control
302 *Hcfc1*^{lox/Y} (n=6) and knockout *Alb-Cre-ERT2*^{tg}; *Hcfc1*^{hepKO/Y} (n=8) males 4d post-tamoxifen
303 treatment. (E) Glycemia during pyruvate tolerance test in control *Hcfc1*^{lox/+} (n=5) and
304 heterozygous *Alb-Cre-ERT2*^{tg}; *Hcfc1*^{hepKO/+} (n=6) females 4d post-tamoxifen treatment. (E)
305 Basal and post-15 minutes plasma insulin levels during glucose-stimulated insulin secretion

306 test in control *Hcfc1*^{lox/Y} (n=10) and knockout *Alb-Cre-ERT2*^{tg} ; *Hcfc1*^{hepKO/Y} (n=10) males 4d
307 post-tamoxifen treatment. (F) Glycemia during insulin tolerance test in control *Hcfc1*^{lox/Y} (n=9)
308 and knockout *Alb-Cre-ERT2*^{tg} ; *Hcfc1*^{hepKO/Y} (n=10) males 4d post-tamoxifen treatment.

309

310 **Supplementary Figure 4.** *Hcfc1*^{hepKO/Y} knockout males present an altered hepatic metabolism
311 by 7d post-tamoxifen treatment. (A) Column graphs showing levels of intermediates of energy
312 metabolism in 0d control liver (suffix WT; n=4) and 7d post-tamoxifen treated knockout *Alb-*
313 *Cre-ERT2*^{tg} ; *Hcfc1*^{hepKO/Y} male (suffix KO; n=2) livers. The intermediates assayed were
314 aspartic acid (Asp); glutamate (Glu); dihydroxyacetone phosphate (DHAP) and 3-
315 phosphoglycerate (3-PGA); hexose (e.g. glucose; H1); glucose-1-phosphate, glucose-6-
316 phosphate, and fructose-6-phosphate (abbreviated as Hex-P here); fumaric acid (Fum); and
317 succinic acid (Suc). The p-values are indicated in the figure. (B) Column graphs showing
318 levels of acylcarnitines in 0d control liver (suffix WT; n=4) and 7d post-tamoxifen treated
319 knockout *Alb-Cre-ERT2*^{tg} ; *Hcfc1*^{hepKO/Y} male (suffix KO; n=2) livers. The acylcarnitines
320 assayed were free carnitine (C0); acetylcarnitine (C2); propionylcarnitine (C3);
321 butyrylcarnitine (C4); isovalerylcarnitine/ 2-methylbutyrylcarnitine (C5); glutarylacarnitine/
322 hydroxyhexanoylcarnitine (C5-DC); methylglutarylacarnitine (C5-M-DC);
323 hydroxyisovalerylcarnitine/ hydroxy-2-methylbutyryl/ methylmalonylcarnitine (C5-OH);
324 caproylcarnitine/ fumarylacarnitine (C6); tetradecanoylcarnitine/ myristylcarnitine (C14);
325 hexadecanoylcarnitine/ palmitoylcarnitine (C16); octadecanoylcarnitine/ stearylacarnitine
326 (C18); and octadecenoylcarnitine/ oleylcarnitine (C18:1). The p-values are indicated in the
327 figure. (C) Column graphs showing levels of bile acids in 0d control liver (suffix WT; n=4)
328 and 7d post-tamoxifen treated knockout *Alb-Cre-ERT2*^{tg} ; *Hcfc1*^{hepKO/Y} male (suffix KO; n=6)
329 livers. The bile acids assayed were cholic acid (CA); glycocholic acid (GCA); α -muricholic
330 acid (MCA(a)); β -muricholic acid (MCA(b)); ω - muricholic acid (MCA(o)), taurocholic acid

331 (TCA), taurodeoxycholic acid (TDCA) and tauroursodeoxycholic Acid (TUDCA). The p-
332 values are indicated in the figure. (D) Column graphs showing levels of amino acids in 0d
333 control liver (suffix WT; n=4) and 7d post-tamoxifen treated knockout *Alb-Cre-ERT2^{tg}* ;
334 *Hcfc1^{hepKO/Y}* male (suffix KO; n=2) livers. The amino acids assayed were aspartate (Asp);
335 glutamine (Gln); histidine (His); and serine (Ser). The p-values are indicated in the figure.

336

337 **Supplementary Figure 5.** *Hcfc1^{hepKO/Y}* knockout male livers display significant tissue
338 disorganization. Liver histology was assessed by haematoxylin (blue) and eosin (shades of
339 pink) staining of paraffin-embedded sections from control liver (0d), and 4d, 7d, 9d, 11d, 14d,
340 and 18d post-tamoxifen treated knockout *Alb-Cre-ERT2^{tg}* ; *Hcfc1^{hepKO/Y}* male livers. Yellow
341 arrows point to some hepatocytes showing variable nuclear sizes. Dotted white boundaries
342 point to distinct tightly packed hepatocyte clusters. Scale bar: 100 μ m.

343

344 **Supplementary Figure 6.** Intracellular glycogen levels are progressively depleted in
345 *Hcfc1^{hepKO/Y}* knockout male livers. Hepatic glycogen content was detected by PAS staining
346 (purple) in paraffin-embedded sections from control liver (0d), and 4d, 7d, 14d, and 18d (two
347 images) post-tamoxifen treated knockout *Alb-Cre-ERT2^{tg}* ; *Hcfc1^{hepKO/Y}* male livers. The
348 sections were also stained with nuclear Mayer's hematoxylin (blue). Scale bar: 100 μ m for all.

349

350 **Supplementary Figure 7.** *Hcfc1^{hepKO/Y}* knockout male livers display presence of significant
351 inflammatory infiltrate from 7d post-tamoxifen treatment onwards. Hepatic inflammation was
352 visualized by DAB immunostaining for macrophage marker, F4/80 (brown) in paraffin-
353 embedded sections from control liver (0d), and 7d, 9d, 11d, 14d, and 18d post-tamoxifen treated
354 knockout *Alb-Cre-ERT2^{tg}* ; *Hcfc1^{hepKO/Y}* male livers. Scale bar: 100 μ m for all.

355

356 **Supplementary Figure 8.** *Hcfc1*^{hepKO/Y} knockout male livers display progressive build-up of
357 collagen fibers from 9d post-tamoxifen treatment onwards. Hepatic fibrosis was identified by
358 Sirius Red staining (red) of paraffin-embedded sections from control liver (0d), and 7d, 9d, 11d,
359 14d, and 18d post-tamoxifen treated knockout *Alb-Cre-ERT2*^{tg} ; *Hcfc1*^{hepKO/Y} male livers.
360 Black arrows point to some collagen fibers. Scale bar: 100 μm for all.

361

362 **Supplementary Figure 9.** *Hcfc1*^{hepKO/Y} knockout livers display hepatic steatosis, altered
363 mitochondria morphology and fibrosis. Electron microscopy was performed to examine the
364 ultrastructural details of control liver (0d), and 4d, 7d, and 14d post-tamoxifen treated knockout
365 *Alb-Cre-ERT2*^{tg} ; *Hcfc1*^{hepKO/Y} male liver samples. The regions highlighted by dotted squares
366 in A1, B1, C1, and D1 are shown at higher magnification in A2, B2, C2, and D2, respectively.
367 Mitochondria pointed by black arrows in A2, B2, C2, and D2, respectively can be seen at even
368 higher magnification in A3, B3, C3, and D3, respectively. Black arrowheads in D1 point to
369 perisinusoidal collagen fibers. Yellow arrows in D1-D2 point to vacuoles. Red arrow in D1
370 points to a lysosome. d, days post-tamoxifen treatment; Gly, glycogen; KO, knockout; L, lipid
371 droplets, Mito, mitochondria; N, nuclei, S, sinusoid. Scale bar: 100 μm.

372

373 **Supplementary Figure 10.** *Hcfc1*^{hepKO/Y} knockout male livers display significantly alterations
374 in levels of mitochondrial-gene-specific RNAs. Heat map of down-regulated (A) and up-
375 regulated (B) mitochondrial-gene-specific RNA levels from 0d to 9d post-tamoxifen treatment
376 in knockout *Alb-Cre-ERT2*^{tg} ; *Hcfc1*^{hepKO/Y} males. The color key, shown beside the heat map,
377 indicates the associated Z-score, with white signifying the lowest score.

378

379 **Supplementary Figure 11.** *Hcfc1*^{hepKO/Y} knockout male livers display up-regulation of genes
380 related to chronic injury. (A) Summary of the results of the functional enrichment analysis on

381 the transcripts that are up-regulated in *Hcfc1*^{hepKO/Y} knockout males. Only the GO terms
382 displaying an enrichment p-value lower than 10⁻¹⁰ were kept for analysis with the REVIGO
383 tool. REVIGO aggregates synonymous GO terms and displays the aggregated terms as circles
384 where the distance among circles indicates their similarity within the GO structure and their
385 color indicates the associated p-value, with blue signifying the lowest p-values. Selected GO
386 terms with highest p-values are shown with the circle aggregates. The scale for p-values is
387 shown beside the REVIGO-representation. (B) The genes up-regulated in knockout male livers
388 were ranked by the fold-change (knockout value minus wildtype value for each time point), and
389 a gene-enrichment analysis was performed using this ranked gene list against the
390 HALLMARKS set (http://software.broadinstitute.org/gsea/msigdb/collection_details.jsp#H).
391 The most statistically significant terms in the up-regulated category were related to
392 inflammatory response, epithelial-to-mesenchymal transition, TNF α signaling via NF κ B,
393 IL6/JAK/STAT3 signaling, apoptosis, mitotic spindle, angiogenesis, cholesterol homeostasis,
394 complement, KRAS signalling, estrogen response (early and late), p53 pathway, MTORC1
395 signaling, hypoxia, IL2/STAT5 signaling, allograft rejection, UV response, myogenesis and
396 apical junction.

397

398 **Supplementary Figure 12.** Plot showing fold-change in expression of HCF-1 bound genes
399 that still retain a significant HCF-1 peak upon loss of HCF-1 at 4d post-tamoxifen treatment in
400 knockout *Alb-Cre-ERT2*^{tg}; *Hcfc1*^{hepKO/Y} male livers.

401

402 **Supplementary Figure 13.** Mitochondria-specific protein levels are significantly reduced in
403 *Hcfc1*^{hepKO/Y} knockout males by 5d post-tamoxifen treatment. (A) Immunofluorescence
404 analysis of paraffin-embedded sections from control (0d) liver and 7d post-tamoxifen treated
405 knockout *Alb-Cre-ERT2*^{tg}; *Hcfc1*^{hepKO/Y} male livers stained with DAPI (blue) and antibody

406 against ATP5 α (red) or UQCRC2 (red) or MTCO1 (red). (B) Immunofluorescence analysis of
407 paraffin-embedded sections from control (0d) liver, and 7d and 18d post-tamoxifen treated
408 knockout *Alb-Cre-ERT2*^{tg}; *Hcfc1*^{hepKO/Y} male livers stained with antibody against OXPHOS.
409 (C) Succinate dehydrogenase (SDH) activity assay on paraffin-embedded sections from control
410 (0d) liver, and 7d and 18d post-tamoxifen treated knockout *Alb-Cre-ERT2*^{tg}; *Hcfc1*^{hepKO/Y} male
411 livers. Dotted circles point to hepatocyte clusters with higher SDH activity. Scale bar: 50 μ m.
412

413 **Supplementary Figure 14.** *Hcfc1*^{hepKO/+} heterozygous females display characteristics typical
414 of mild liver injury by 18d post-tamoxifen treatment. (A) Haematoxylin (blue) and eosin
415 (shades of pink) staining of paraffin-embedded sections from 0d control and 18d post-
416 tamoxifen treated heterozygous *Alb-Cre-ERT2*^{tg}; *Hcfc1*^{hepKO/+} female livers. (B and C)
417 Immunofluorescence analysis of paraffin-embedded sections from 0d control and 18d post-
418 tamoxifen treated heterozygous *Alb-Cre-ERT2*^{tg}; *Hcfc1*^{hepKO/+} female livers stained with DAPI
419 (blue) together with antibodies against β -catenin (red) and HCF-1 (green). (D) Hepatic
420 glycogen was visualized by PAS staining (purple) of paraffin-embedded sections from 0d
421 control and 18d post-tamoxifen treated heterozygous *Alb-Cre-ERT2*^{tg}; *Hcfc1*^{hepKO/+} female
422 livers. The sections were also stained with nuclear Mayer's hematoxylin (blue). (E) DAB
423 immunostaining for macrophage marker, F4/80 (brown) on paraffin-embedded sections from
424 0d control and 18d post-tamoxifen treated heterozygous *Alb-Cre-ERT2*^{tg}; *Hcfc1*^{hepKO/+} female
425 livers. The sections were also stained with nuclear Mayer's hematoxylin (blue). (F)
426 Immunofluorescence analysis of paraffin-embedded sections from 0d control and 18d post-
427 tamoxifen treated heterozygous *Alb-Cre-ERT2*^{tg}; *Hcfc1*^{hepKO/+} female livers stained with DAPI
428 (blue) together with antibody against smooth muscle α -actin (SMA; green). (G) Sirius red
429 staining of paraffin-embedded sections from 0d control and 18d post-tamoxifen treated
430 heterozygous *Alb-Cre-ERT2*^{tg}; *Hcfc1*^{hepKO/+} female livers. (H) TUNEL assay was performed

431 on paraffin-embedded sections from 0d control and 18d post-tamoxifen treated heterozygous
432 *Alb-Cre-ERT2^{tg}* ; *Hcfc1^{hepKO/+}* female livers co-stained with HCF-1 (green). TUNEL-positive
433 apoptotic cells are shown in red. Scale bar: 100 μ m.

434

435 **Supplementary Figure 15.** *Hcfc1^{hepKO/+}* heterozygous female livers display mild steatosis and
436 transient loss of glycogen. (A) The presence of steatosis was estimated by Oil Red O staining
437 of cryo-sections from control liver (0d), and 7d, 11d, and 18d post-tamoxifen treated
438 heterozygous *Alb-Cre-ERT2^{tg}* ; *Hcfc1^{hepKO/+}* female livers. (B) Hepatic glycogen content was
439 detected by PAS staining (purple) in paraffin-embedded sections from control liver (0d), and
440 7d, 11d, and 18d post-tamoxifen treated heterozygous *Alb-Cre-ERT2^{tg}* ; *Hcfc1^{hepKO/+}* female
441 livers. The sections were also stained with nuclear Mayer's hematoxylin (blue). Scale bar: 100
442 μ m.

443

444 **Supplementary Figure 16.** *Hcfc1^{hepKO/+}* heterozygous female livers display presence of
445 significant inflammatory infiltrate from 9d post-tamoxifen treatment onwards. Hepatic
446 inflammation was visualized by DAB immunostaining for macrophage marker, F4/80 (brown)
447 in paraffin-embedded sections from control liver (0d), and 7d, 9d, 11d, 14d, and 18d post-
448 tamoxifen treated heterozygous *Alb-Cre-ERT2^{tg}* ; *Hcfc1^{hepKO/+}* female livers. Scale bar: 100
449 μ m for all.

450

451 **Supplementary Figure 17.** *Hcfc1^{hepKO/+}* heterozygous female livers display mild presence of
452 collagen fibers from 9d post-tamoxifen treatment onwards. Hepatic fibrosis was identified by
453 Sirius Red staining (red) of paraffin-embedded sections from control liver (0d), and 7d, 9d, 11d,
454 14d, and 18d post-tamoxifen treated heterozygous *Alb-Cre-ERT2^{tg}* ; *Hcfc1^{hepKO/+}* female livers.
455 Black arrows point to some collagen fibers. Scale bar: 100 μ m for all.

456

457 **Supplementary Figure 18.** HCF-1-positive hepatocytes proliferate and replace HCF-1-
458 negative hepatocytes in *Hcfc1*^{hepKO/+} heterozygous females. (A-B) Immunofluorescence
459 analysis of paraffin-embedded sections from 7d (A) and 18d (B) post-tamoxifen treated
460 heterozygous *Alb-Cre-ERT2*^{tg} ; *Hcfc1*^{hepKO/+} female livers stained with DAPI (blue) and
461 antibodies against HCF-1 (green) and Ki67 (red). White arrows point to HCF-1⁺- and Ki67⁺-
462 hepatocytes. d, days post-tamoxifen treatment.

Supplementary Tables

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Supplementary Table 1. Raw tag counts on selected *Hcfc1* exons along with the expected-splicing events. The size of the *Hcfc1* exon 1 is 193; exon 2 is 149; exon 3 is 161; and exon 4 is 209 bp.

Supplementary Table 2. List of identified 654 down-regulated genes in *Alb-Cre-ERT2^{tg}*; *Hcfc1^{hepKO/Y}* knockout male livers.

Supplementary Table 3. List of identified 521 initially up-regulated genes in *Alb-Cre-ERT2^{tg}*; *Hcfc1^{hepKO/Y}* knockout male livers.

Supplementary Table 4. List of identified 2871 later up-regulated genes in *Alb-Cre-ERT2^{tg}*; *Hcfc1^{hepKO/Y}* knockout male livers.

Supplementary Table 5. Enriched GO terms in identified 654 down-regulated genes in *Alb-Cre-ERT2^{tg}*; *Hcfc1^{hepKO/Y}* knockout male livers.

Supplementary Table 6. Enriched GO terms in identified 521 initially up-regulated genes in *Alb-Cre-ERT2^{tg}*; *Hcfc1^{hepKO/Y}* knockout male livers.

Supplementary Table 7. List of total HCF-1 peaks near transcription start site (TSS) or not associated with a TSS (i.e., more than +/- 250 bp) identified by ChIP-seq analysis.

487 **Supplementary Table 8.** List of transcription units associated with HCF-1 Peaks within +/-
488 250 bp of only one gene/transcription unit (unidirectional) and with two or sometimes (but
489 rarely so) more than two transcription units.

490

491 **Supplementary Table 9.** Enriched GO terms in identified 280 HCF-1 bound down-regulated
492 genes in *Alb-Cre-ERT2^{tg}* ; *Hcfc1^{hepKO/Y}* knockout male livers at 4d post-tamoxifen treatment.

493

494 **Supplementary Table 10.** Enriched GO terms in identified 163 HCF-1 bound up-regulated
495 genes in *Alb-Cre-ERT2^{tg}* ; *Hcfc1^{hepKO/Y}* knockout male livers at 4d post-tamoxifen treatment.

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