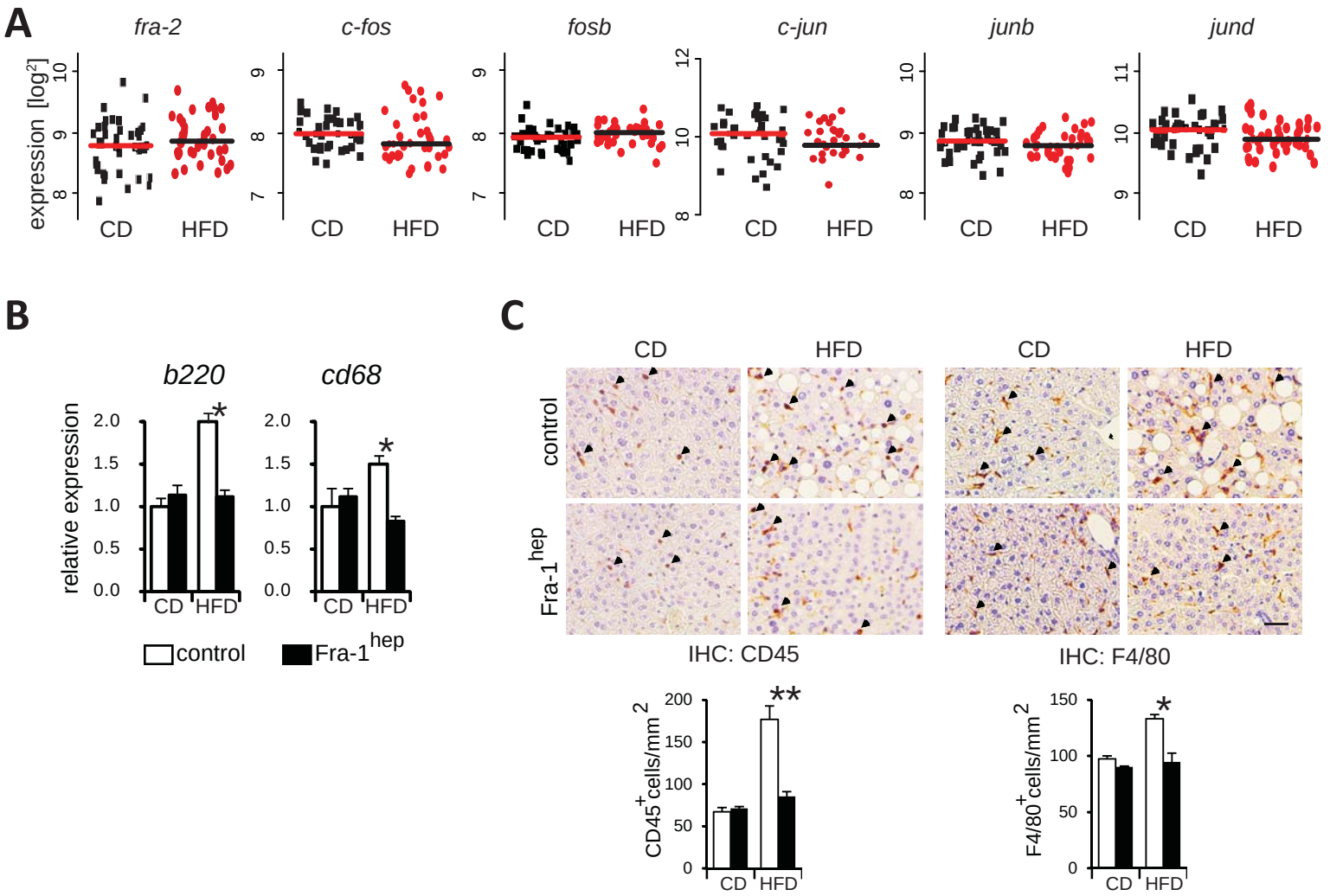
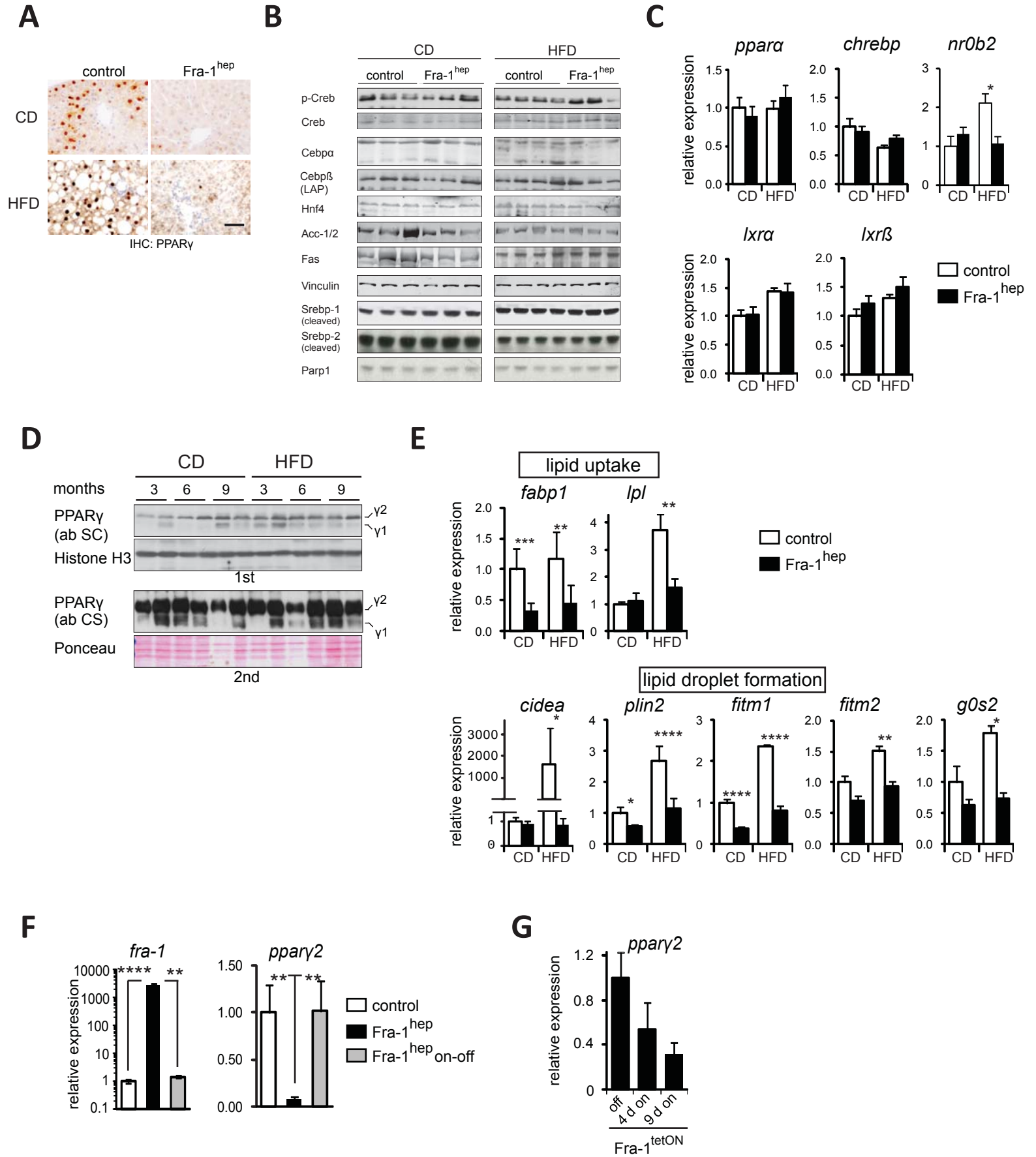
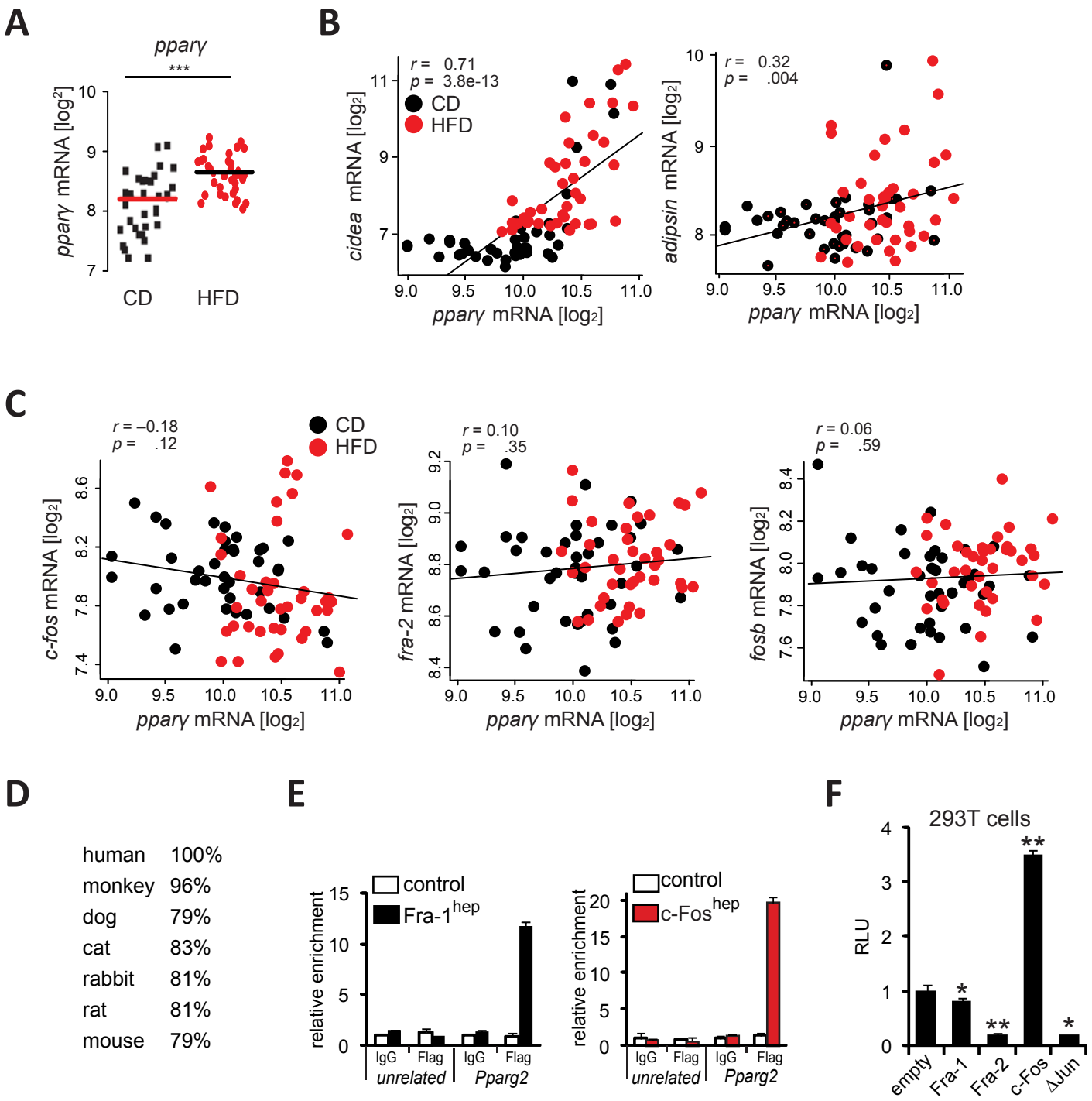


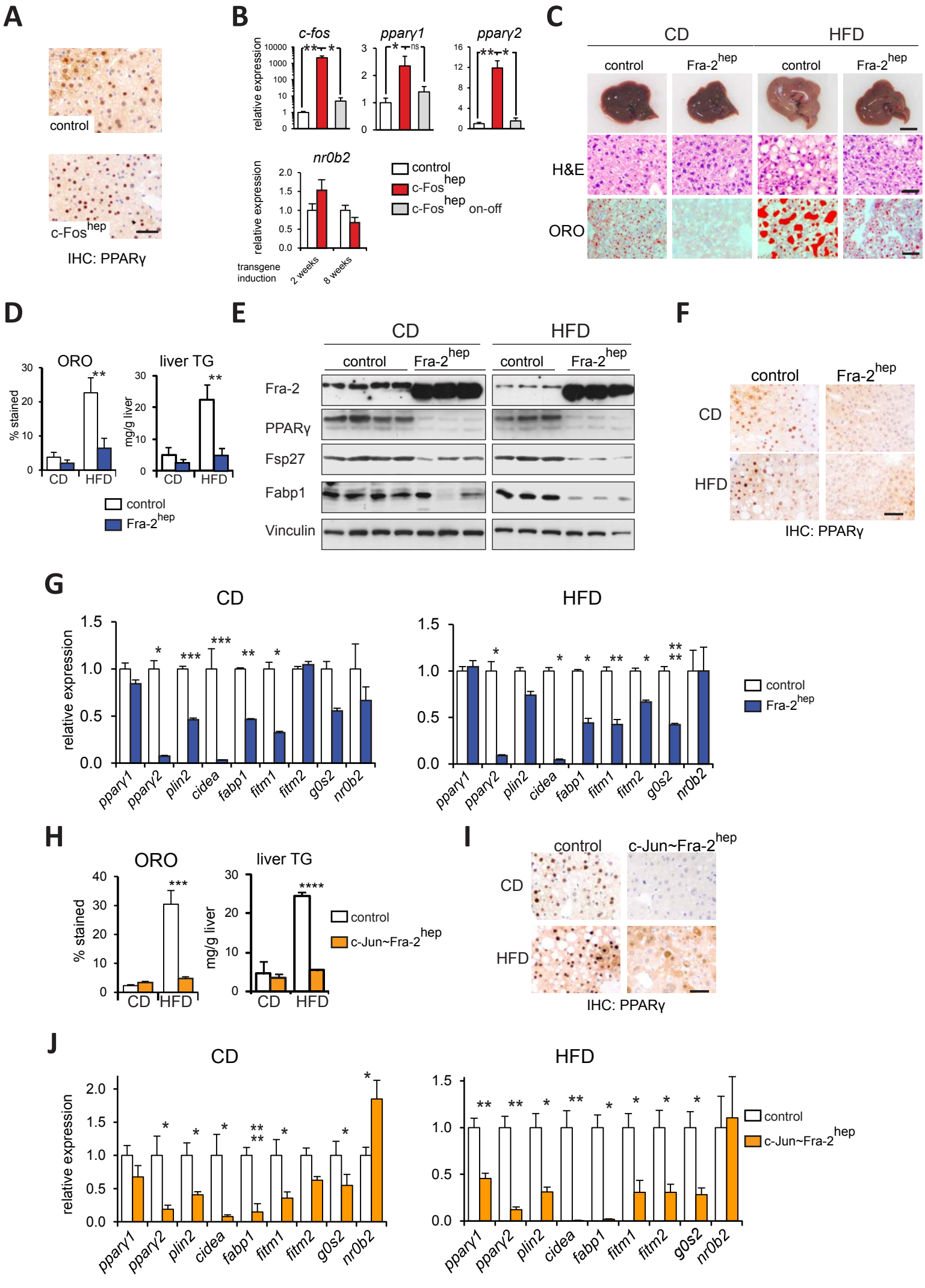

Hasenfuss et al., Figure S1



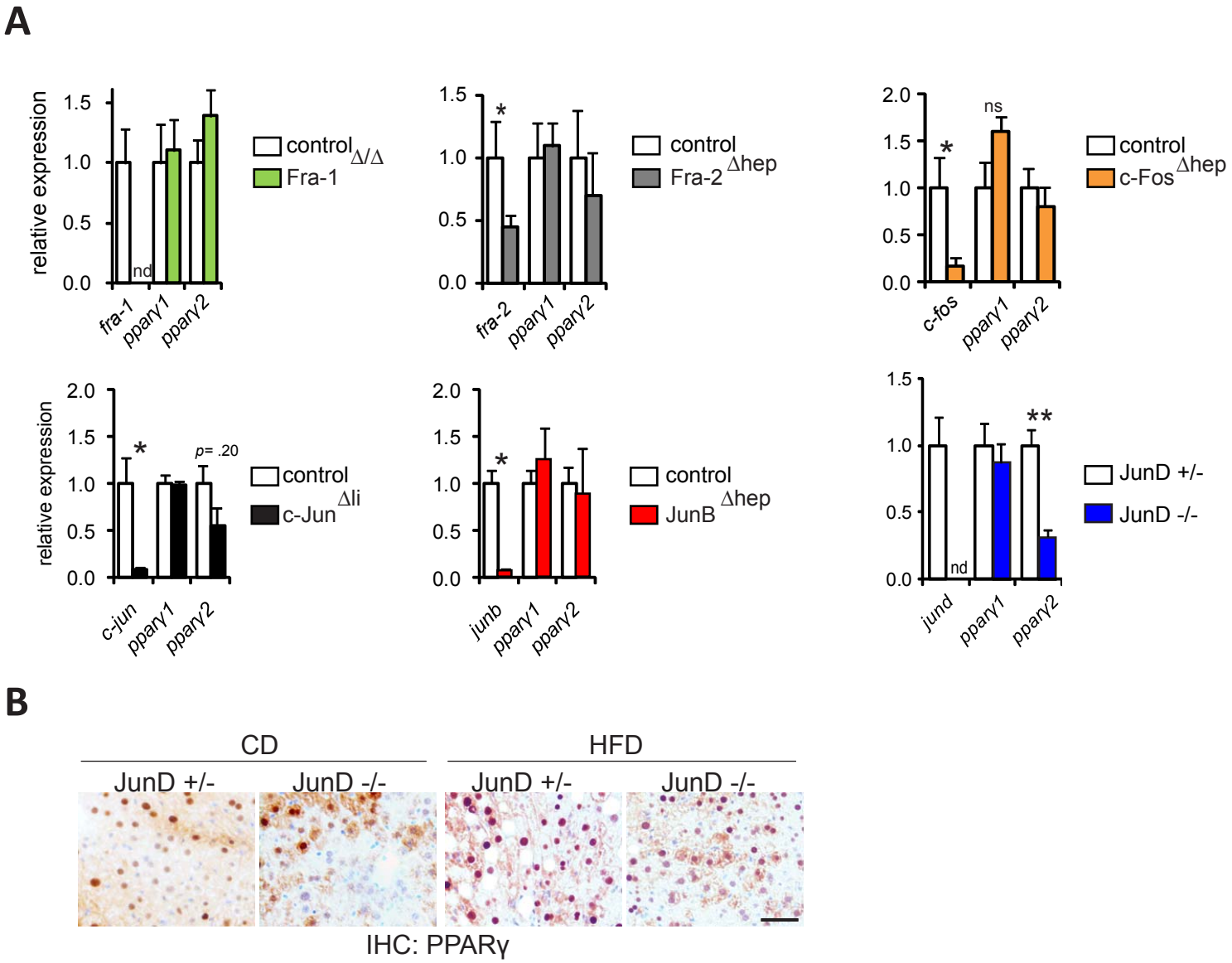

Hasenfuss et al., Figure S2




Hasenfuss et al., Figure S3




Hasenfuss et al., Figure S4




Hasenfuss et al., Figure S5

## Legends for Supplementary Figures

**Figure S1: Fra-1 regulates NAFLD development - Related to Figure 1.**

**(A)** Hepatic AP-1 mRNA expression in BXD inbred strains, which were fed CD or HFD (for 5 months; 60% kCal/fat) prior to sacrifice. Each data point represents the mean expression of 5 mice. Inflammation marker analysis by qRT-PCR **(B)** and IHC **(C)** in CD-/HFD (for 5-9months; 45% kCal/fat)-fed Fra-$1^{hep}$ and control mice. Representative liver sections and quantitation of $CDF45^{+}$ and $F4/80^{+}$ cells are shown; arrowheads mark positively stained cells, bars are 50µm, n≥4/cohort. Bar graphs are presented as mean±SEM. **(D)** Intraperitoneal glucose tolerance test (GTT) and insulin tolerance test (ITT) in CD-/HFD (for 3,5 months; 45% kCal/fat)-fed Fra-$1^{hep}$ and control mice. Data bars are mean+SEM, n≥5/cohort. *p*-values indicate statistical difference between HFD-fed Fra-$1^{hep}$ and control mice, as determined by 2-way ANOVA analysis.

**Figure S2: Fra-1 regulates the PPARγ pathway - Related to Figure 2.**

**(A-C, E)** Fra-$1^{hep}$ mice and control littermates were maintained on CD or HFD (for 5-9 months; 45% kCal/fat); n≥5/condition. Representative PPARγ IHC of liver sections **(A)** (bars: 50µm) and immunoblot analysis **(B)** in CD-/HFD-fed Fra-$1^{hep}$ and control mice. Vinculin and PARP1 were used to control loading in whole liver and nuclear extracts respectively. **(C)** Metabolic gene expression by qRT-PCR in CD-/HFD-fed Fra-$1^{hep}$ and control mice. **(D)** Male wild-type C57BL/6J mice were fed HFD (45% kCal/fat) starting at 5 weeks of age or maintained on CD. PPARγ immunoblot analyses of liver nuclear extracts from mice at 3, 6 or 9 months of age in 2 mouse cohorts using 2 different commercial

antibodies (sc: antibody sc-7273; CS: antibody CS2443). Histone H3 or Ponceau staining were used to control for loading. **(E)** qRT-PCR analyses of PPARγ target genes involved in lipid uptake and lipid droplet formation. **(F)** *fra-1* and *pparγ2* mRNA expression in control, Fra-1$^{hep}$ mice (Fra-1 on) and Fra-1$^{hep}$ mice (on-off; Fra-1 expression was switched off 1 month prior to analysis); n≥5/cohort; control is set to 1. **(G)** Fra-1$^{tetON}$ and control mice were analyzed for *pparγ2* mRNA expression in the "Fra-1 off" state (off) or upon 4 or 9 days of Fra-1 induction; n ≥ 2/time point; off state is set to 1. Bar graphs are presented as mean±SEM.

**Figure S3: Regulation of the PPARγ pathway by AP-1 - Related to Figure 5.**

**(A)** Hepatic *pparγ* mRNA expression in 42 BXD inbred strains, which were fed CD or HFD (for 5 months; 60% kCal/fat) prior to sacrifice. **(B,C)** Correlation plots for *pparγ* with *cidea* and *adipsin* **(B)** and *c-fos, fra-2* and *fosb* with *pparγ* **(C)** in the BXD inbred strains. Each data point represents the mean expression of 5 mice in **(A,B)**; Pearson's r was used to analyze correlations. **(D)** Conservation of the *Pparg2* proximal promoter in different species. **(E)** ChIP assays using hepatic chromatin from Fra-1$^{hep}$ (Fra-1 on), c-Fos$^{hep}$ (c-Fos on) and control mice and α-Flag or IgG. Bars are mean±s.d of technical duplicates; the results are representative of at least 3 independent experiments. **(F)** Human *PPARG2* reporter assays in 293T cells. RLU: relative luminescence units. ΔJun: truncated c-Jun; Bar graphs are presented as mean±SEM of 5 independent experiments. Control (empty vector) is set to 1.

**Figure S4: Antagonistic regulation of the PPARγ pathway by AP-1 *in vivo* - Related to Figure 6.**

**(A)** PPARγ IHC in c-Fos$^{hep}$ (1 week on) and control mice; n=2, bars= 50µm. **(B)** Top: qRT-PCR analyses of *c-fos* (endogenous+ectopic) and *pparγ1/2* expression in c-Fos$^{hep}$ and control mice. c-Fos expression was induced for 8 weeks (c-Fos on) or induced for 8 weeks and switched off for 8 weeks (c-Fos on-off). n≥4 for controls and c-Fos$^{hep}$ mice (on), n=3 for c-Fos$^{hep}$ mice (on-off). Bottom: *nrob2* expression in c-Fos$^{hep}$ mice after 2 weeks (n=6/cohort) and 8 weeks (n=8/cohort) of transgene induction. **(C-G)** Analyses of Fra-2$^{hep}$ mice (Fra-2 on) and control littermates on CD or HFD (for 5 months, 45% kCal/fat); n≥5/condition. **(C)** Representative liver macroscopy and histology; bars=1cm and 50µm. **(D)** Quantitation of ORO-positive areas and liver TG content. **(E)** Fra-2, PPARγ, Fsp27, Fabp1 and Vinculin immunoblots. **(F)** PPARγ IHC, bars= 50µm. **(G)** PPARγ target gene expression. **(H-J)** Analyses of c-Jun~Fra-2$^{hep}$ mice (c-Jun~Fra-2 was switched on at 1 month of age) and control littermates on CD or HFD (for 4 months; 60% kCal/fat); n≥5/condition. (**H)** Liver TG content. **(I)** PPARγ IHC (bars=50µm). **(J)** PPARγ target gene mRNA expression. Bar graphs are presented as mean±SEM.

**Figure S5: JunD is essential for PPARγ expression and NAFLD- Related to Figure 7.**

**(A)** qRT-PCR analyses of *pparγ* isoforms expression in c-Jun$^{\Delta li}$, JunB$^{\Delta hep}$, JunD-/-, Fra-1$^{\Delta/\Delta}$, Fra-2$^{\Delta hep}$, c-Fos$^{\Delta hep}$ and respective control mice. n≥5/cohort. **(B-D)** Analyses of JunD$^{-/-}$ and JunD$^{+/-}$ control littermates (males and females) on CD or HFD (for 9 months; 45% kCal/fat); n≥7/condition. **(B)** PPARγ IHC of liver sections (bar=50µm). **(C)** Hepatic PPARγ

target gene mRNA expression. **(D)** *pparγ1* and *pparγ2* mRNA expression in different organs. Bar graphs are presented as mean±SEM.

| LOF strain | genotype | control genotype | deletion | background | Reference |
|---|---|---|---|---|---|
| Fra-1$^{\Delta/\Delta}$ | Fosl1$^{f/\Delta}$; Mox2$^{+/Cre}$ | Fosl1$^{f/\Delta}$; Mox2$^{+/+}$ | broad | C57BL/6J | Eferl et al., EMBO J. 2004 |
| Fra-2$^{\Delta hep}$ | Fosl2$^{f/f}$; Tg. Alfp Cre | Fosl2$^{f/f}$ or Fosl2$^{+/f}$ Tg., Alfp Cre | liver parenchyme | C57BL/6Jx129sv | this study |
| c-Fos$^{\Delta hep}$ | c-Fos$^{f/f}$; Tg. Alfp Cre | c-Fos$^{f/f}$ or c-Fos$^{+/f}$; Tg. Alfp Cre | liver parenchyme | C57BL/6Jx129sv | this study |
| c-Fos$^{\Delta li}$ | c-Fos$^{f/f}$; Tg. Mx Cre | c-Fos$^{f/f}$ or c-Fos$^{+/f}$; Tg. Mx Cre | liver + BM | C57BL/6Jx129sv | Min et al., Nature Cell biology 2012 |
| JunD KO | JunD$^{-/-}$ | JunD$^{+/-}$ | broad | C57BL/6J | Thepot et al., Development 2000 |
| JunB$^{\Delta hep}$ | JunB$^{f/f}$; Tg. Alfp Cre | JunB$^{+/f}$; Tg. Alfp Cre | liver parenchyme | C57BL/6J | Hasselblatt et al., PNAS 2007 |
| JunB$^{\Delta li}$ | JunB$^{f f/f}$; Tg. Mx Cre | JunB$^{f/f}$ | liver + BM | C57BL/6Jx129sv | Passegue et al., Cell 2004 |
| c-Jun$^{\Delta li}$ | c-Jun$^{f/f}$; Tg. Mx1 Cre | c-Jun$^{f/f}$ | liver + BM | C57BL/6Jx129sv | Behrens et al., EMBO J. 2002 |

| GOF strain | Genotype | control genotype | transgene expression | background | Reference |
|---|---|---|---|---|---|
| Fra-1$^{tet}$ | Col1a1$^{+/TetOP\text{-}Fosl1}$; Rosa 26$^{+/rtTA}$ | Col1a1$^{+/+}$; Rosa 26$^{+/rtTA}$ | broad (on Dox) | C57BL/6J | Hasenfuss et al., Hepatology 2013 |
| Fra-1$^{hep}$ | Col1a1$^{+/TetOP\text{-}Fosl1}$; LAP$^{+/tTA}$ | Col1a1$^{+/+}$; LAP$^{+/tTA}$ or Col1a1$^{+/ TetOP\text{-}Fosl1}$; LAP$^{+/+}$ | hepatocytes (no Dox) | C57BL/6J or C57BL/6Jx129sv | Hasenfuss et al., Hepatology 2013 |
| Fra-2$^{hep}$ | Col1a1$^{+/TetOP\text{-}Fosl2}$; LAP$^{+/tTA}$ | Col1a1$^{+/+}$; LAP$^{+/tTA}$ or Col1a1$^{+/ TetOP\text{-}Fosl2}$; LAP$^{+/+}$ | hepatocytes (no Dox) | C57BL/6Jx129sv | Hasenfuss et al., Hepatology 2013 |
| c-Jun~Fra-2$^{hep}$ | Col1a1$^{+/TetOP\text{-}c\text{-}Jun\sim Fosl2}$; LAP$^{+/tTA}$ | Col1a1$^{+/TetOP\text{-}c\text{-}Jun\sim Fosl2}$;LAP$^{+/+}$ or Col1a1$^{+/+}$;LAP$^{+/tTA}$ | hepatocytes (no Dox) | C57BL/6Jx129sv | this study |
| c-Fos$^{hep}$ | Col1a1$^{+/TetOP\text{-}c\text{-}Fos}$; Rosa 26$^{+/lsl\ rtTA;}$ Tg.Alfp Cre | Col1a1$^{+/TetOP\text{-}c\text{-}Fos}$; Rosa 26$^{+/lsl\ rtTA}$ | liver parenchyme (on Dox) | C57BL/6Jx129sv | this study |
| c-Jun~c-Fos$^{hep}$ | Col1a1$^{+/TetOP\text{-}c\text{-}Jun\sim c\text{-}Fos}$; Rosa 26$^{+/lsl\ rtTA}$; Tg.Alfp Cre | Col1a1$^{+/TetOP\text{-}c\text{-}Jun\sim c\text{-}Fos}$; Rosa 26$^{+/lsl\ rtTA}$ | liver parenchyme (on Dox) | C57BL/6Jx129sv | this study |
| JunB~c-Fos$^{hep}$ | Col1a1$^{+/TetOP\text{-}Junb\sim c\text{-}Fos}$; Rosa 26$^{+/lsl\ rtTA}$; Tg.Alfp Cre | Col1a1$^{+/TetOP\text{-}Junb\sim c\text{-}Fos}$; Rosa 26$^{+/lsl\ rtTA}$ | liver parenchyme (on Dox) | C57BL/6Jx129sv | this study |
| JunD~c-Fos$^{hep}$ | Col1a1$^{+/TetOP\text{-}JunD\sim c\text{-}Fos}$; Rosa 26$^{+/lsl\ rtTA}$; Tg.Alfp Cre | Col1a1$^{+/TetOP\text{-}JunD\sim c\text{-}Fos}$; Rosa 26$^{+/lsl\ rtTA}$ | liver parenchyme (on Dox) | C57BL/6Jx129sv | this study |

**Table S1. Overview of mouse strain – related to Experimental Procedures.** Upper panel describes all loss-of-function (LOF) and the lower panel all gain-of-function (GOF) mouse strains employed in this study. Control genotypes, genetic background, sites of transgene expression/gene inactivation and references are indicated. Mice of a C57BL/6Jx129sv background are referred to as "mixed background". In the column "transgene expression", it is also indicated, whether the transgene is expressed in the presence or absence of Dox. To account for Dox-effects, control and mutant mice always received the same treatment. BM: bone marrow; Tg.: transgenic.

| A | CD | | HFD | |
|---|---|---|---|---|
| **Parameter** | **control** | **Fra-1$^{hep}$** | **control** | **Fra-1$^{hep}$** |
| Body weight (grams) | 38.6 ± 1.5 | 37.4 ± 1.0 | 49.9 ± 1.6 | 48.7 ± 3.1 |
| Glucose (mg/dl) | 169 ± 17 | 168 ± 15 | 185 ± 6 | 196 ± 5 |
| Cholesterol (mg/dl) | 159 ± 9 | 157 ± 13 | 211 ± 22 | 272 ± 21 (*p*= .06) |
| FFA (µM) | 53 ± 3 | 59 ± 4 | 74 ± 7 | 72 ± 6 |
| TG (mg/dl) | 158 ± 11 | 195 ± 7 (*p*= .07) | 299 ± 9 | 369 ± 10*** |
| ß-HB (µM) | 471 ± 53 | 416 ± 33 | 344 ± 23 | 314 ± 19 |
| Insulin (ng/ml) | 1.31 ± 0.36 | 0.76 ± 0.18 | 4.52 ± 1.22 | 5.43 ± 1.45 |
| Adiponectin (mg/ml) | 12.0 ± 0.3 | 10.0± 0.5 | 10.0± 0.4 | 9.3± 0.5 |
| Leptin (ng/ml) | 469 ± 47 | 354 ± 50 | 575 ± 44 | 701 ± 129 |
| Resistin (ng/ml) | 19.2 ± 1.6 | 21.7 ± 1.9 | 21.2 ± 2.3 | 29.4 ± 3.2 |
| IL-6 (pg/ml) | nd | nd | 36.0 ± 12.9 | 13.6 ± 4.3 (*p*= .06) |
| | | | | |
| **B** | **CD** | | **HFD** | |
| **Parameter** | **control** | **Fra-1$^{hep}$ (C57Bl6/J)** | **control** | **Fra-1$^{hep}$ (C57Bl6/J)** |
| Body weight (grams) | 32.3 ± 0.7 | 31.3 ± 0.4 | 44.7 ± 1.2 | 44.5 ± 0.5 |
| Liver/body (%) | 4.2 ± 0.2 | 4.6 ± 0.3 | 6.0 ± 0.0 | 3.9 ± 0.0*** |
| Fat pad/body (%) | 2.1 ± 0.2 | 2.1 ± 0.1 | 3.1 ± 0.2 | 3.5 ± 0.1 |
| ALT (U/l) | 55.0 ± 5.5 | 42.2 ± 4.7 | 119.7 ± 71.7 | 44.1 ± 11.7** |
| TG (mg/dl) | 116 ± 5 | 119 ± 1 | 137 ± 12 | 172 ± 4 (*p*= .06) |
| TG (fasted) (mg/dl) | 154 ± 3 | 184 ± 16 (*p*= .07) | 160 ± 8 | 203 ± 14* |
| Cholesterol (mg/dl) | 123 ± 6 | 122 ± 1 | 172 ± 12 | 149 ± 5 |
| Cholesterol (fasted) (mg/dl) | 124 ± 4 | 125 ± 2 | 218 ± 22 | 277 ± 40 (*p*= .28) |

**Table S2. Serum and metabolic parameters in Fra-1$^{hep}$ mice – related to Figure 1.**

**(A)** Serum parameters of male Fra-1$^{hep}$ mice and control littermates on a mixed genetic background, which were fed CD/HFD (for 5-9 months; 45 % kCal/fat). n≥6/cohort. nd: not determined. ß-HB: ß-Hydroxybutyrate. **(B)** C57BL/6J Fra-1$^{hep}$ mice and control littermates were fed HFD starting at 4 weeks (for 5 months; 45 % kCal/fat) or received CD during the entire experiment; n≥5/cohort. Bar graphs are presented as mean±SEM. All parameters were determined in the fed state, if not indicated differently. *p*-values and statistical significance refer to control vs. mutant for the same diet condition.

| | AP-1 | | | PPARγ | | |
|---|---|---|---|---|---|---|
| **TFBS** | **%** | ***p*-value** | **Rank** | **%** | ***p*-value** | **Rank** |
| All | 58.1 | 7.2E-32 | 1 | 76.1 | 2.4E-23 | 9 |
| Up | 61.3 | 1.7E-24 | 1 | 77.2 | 1.5E-13 | 45 |
| Down | 56.8 | 9.0E-13 | 7 | 75.6 | 3.3E-11 | 11 |

**Table S3. AP-1 and PPARγ binding sites in genes dysregulated in Fra-1$^{hep}$ livers – related to Figure 2.**

Up- and down-regulated genes were analyzed for putative transcription factor binding sites. The ranking of AP-1 and PPARγ binding sites and modified Fisher exact *p*-values are indicated, as calculated by the DAVID web resource.

| | CD | | HFD | |
|---|---|---|---|---|
| **Parameter** | **JunD +/-** | **JunD -/-** | **JunD +/-** | **JunD -/-** |
| Glucose (mg/dl) | 163 ± 7 | 134 ± 4** | 221 ± 8 | 185 ± 5* |
| Cholesterol (mg/dl) | 123 ± 6 | 121.7 ± 1 | 132 ± 7 | 131 ± 5 |
| Cholesterol (fasted) (mg/dl) | 103 ± 1 | 111 ± 1* | 174 ± 12 | 149 ± 5 |
| TG (mg/dl) | 116 ± 5 | 118.7 ± 1 | 137 ± 12. | 172 ± 4 (*p*= .06) |
| TG (fasted) (mg/dl) | 146± 9 | 163 ± 7 | 165 ± 12 | 171 ± 7 |
| ALT (U/l) | 23.9 ± 2.4 | 26.6 ± 1.1 | 80 ± 17.1 | 75 ± 15.2 |
| Fat pad/body (%) | 4.7 ± 0.6 | 3.4 ± 0.2 | 4.6 ± 0.5 | 2.3 ± 0.4* |

**Table S4. Serum and metabolic parameters in JunD$^{-/-}$ mice – related to Figure 7.**

JunD-/- and JunD+/- control littermates were fed HFD (for 8,5 months; 45% fat) or maintained on CD. All parameters were determined in the fed state, if not indicated differently; *p*-values and statistical significance refer to control vs. mutant for the same diet condition; n≥7/cohort. All data are mean±SEM.

## Extended experimental procedures

**Chromatin immunoprecipitation (ChIP).** Whole liver (between 0.2 and 1g) was homogenized in 10ml of DMEM/1%PFA using a needle homogenizer and fixed for 15 minutes at 37°C. Cells were washed twice in PBS and once in PIPES (5 mM PIPES, 5mM KCl, 1% NP40, adjusted to pH7.5) containing protease inhibitor cocktail (Sigma). Nuclear lysis and chromatin fragmentation was performed in lysis buffer (10 mM EDTA, 1%SDS, 50mM Tris pH 8) using a Diagenode Bioruptor sonicater (settings: maximum power, 15 sec on, 15 sec off for 15 minutes). The extracts were diluted 10 times in dilution buffer (0.01% SDS, 1.1% Triton, 1.2 mM EDTA, 16.7 mM Tris-HCl, pH 8.1, 167 mM NaCl) and incubated with 100µl of Protein A/G Agarose (Santa Cruz) for 2hours at 4°C on a tube roller. After centrifugation, the extracts were centrifuged and the supernatant was transferred to a new tube. 2% of the supernatant was kept as Input control and the rest was incubated with 2-5µg of the indicated antibody and 60µl of Protein A/G Agarose for 12-16h hours at 4°C on a tube roller. After centrifugation and removal of the supernatant, the beads were washed three times for 15min at 4°C using 15ml of following buffers in a consecutive manner: Wash buffer 1 (0,1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.1, 150 mM NaCl), Wash buffer 2 (Wash buffer 1 supplemented with NaCl to a final concentration of 500mM NaCl) and Wash buffer 3 (0.25 M LiCl, 1% NP-40, 1% Sodium Deoxycholate, 1 mM EDTA, 10 mM Tris-HCl, pH 8.1). After the last wash, ChIP samples were eluted from the beads in 500µl elution buffer (10%SDS, 0.1M $NaHCO_3$) and, including the input samples, de-crosslinked with 1µl Proteinase K (20 µg/ml) at 55°C for 6 hours. The samples were purified using DNA-Mini

columns (Qiagen), eluted in 120µl and 5µl of each sample were used for PCR. For ChIP analysis of primary hepatocytes, cells were harvested in 1%PFA/DMEM and processed as described above. Following antibodies were used: Flag (F3165, Sigma), Fra-1 (SC-183, Santa Cruz), Fra-2 (rat, CNIO polyclonal), c-Fos (PC-05, Calbiochem), c-Jun (BD), JunB (SC-73, Santa Cruz), JunD (CS5000, Cell Signaling). Pan Jun ChIP with HuH7 cells has been performed with a mixture of 2 antibodies raised against an epitope present in all Jun proteins.

**Supplemental References**

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