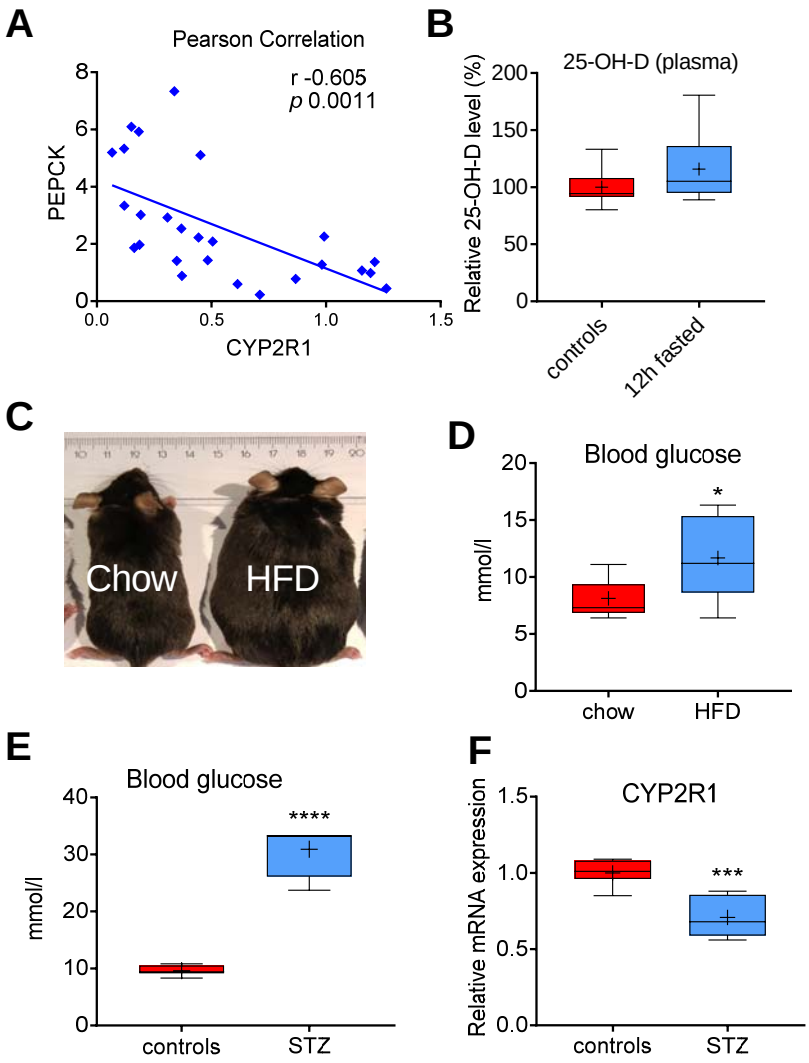
SUPPLEMENTARY DATA

Reagents and antibodies

Dexamethasone, mifepristone, streptozotocin, DMSO, calcitriol, TRI reagent, RNAzol reagent, William's E medium, cholecalciferol and XCT790 were purchased from Sigma-Aldrich (St. Louis, MO, USA). High-fat diet (Envigo TD.06414, 60% of calories from fat) and regular chow (Envigo TD.2018) from Envigo Teklad Diets, USA. Rabbit polyclonal CYP2R1 antibody (#T2849, Figure 1c) was purchased from Epitomics (Burlingame, CA, USA), Rabbit polyclonal CYP2R1 antibody (Center, SAB1300955, Figure 1i, 2j), and mouse monoclonal β-actin antibody (A1978) were purchased from Sigma-Aldrich. Rabbit polyclonal CYP24A1 antibody (H-87, sc-66851) was from Santa-Cruz Biotechnology (Santa Cruz, CA, USA), Goat anti-rabbit IgG-HRP and anti-mouse IgG-HRP used as secondary antibodies were purchased from Santa Cruz Biotechnology and GE healthcare (Little Chalfont, UK), respectively. Amersham ECL Prime Blocking Reagent (RPN418) was purchased from GE Healthcare Bio-Sciences (USA). FuGENE® HD Transfection Reagent (E2311) and Dual-Glo(R) Luciferase Assay System (E2940) were purchased from Promega (Madison, WI, USA). Quickchange II site-directed mutagenesis kit (#200523) was purchased from Agilent, USA.

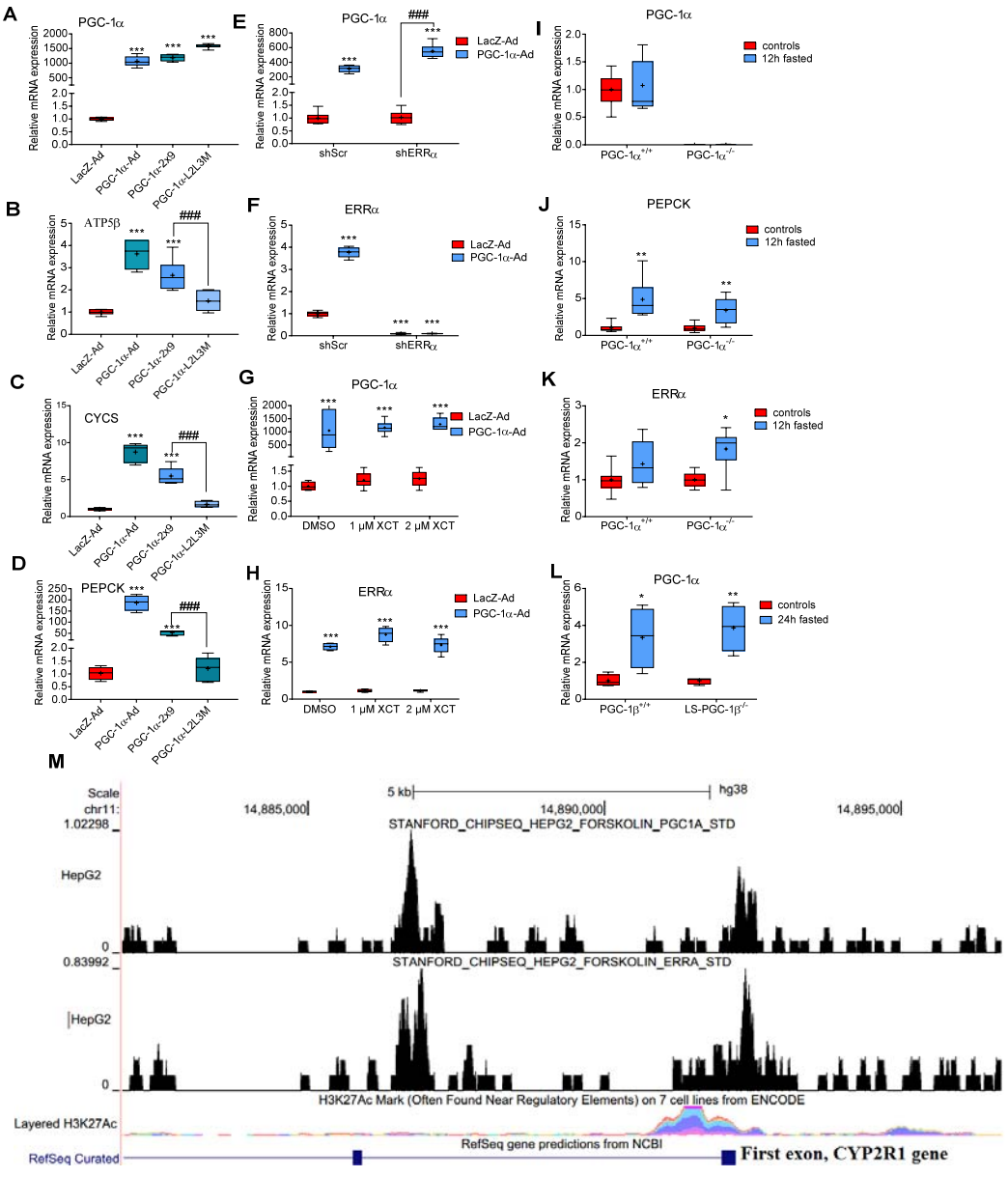
SUPPLEMENTARY DATA

Supplementary Figure S1. CYP2R1 expression and 25-OH-D levels were studied in fasting and diabetes models. *A*: Fasting represses CYP2R1 in mouse liver in a negative correlation with the fasting induced gluconeogenic gene PEPCK. The line represents best-fit by the linear regression. Furthermore, the Pearson correlation was calculated. *B*: The plasma levels of the 25-OH-D in the fed and the 12h fasted mice (n=10). *C*: The HFD-treatment induced obesity and diabetes in mice. *D*: The fasted blood glucose was significantly higher in the HFD-treated mice compared to lean controls (n= controls 10, HFD 9). *E*: The nonfasted blood glucose was higher in the STZ-treated mice compared to controls (n= controls 8, STZ 4). *F*: Analysis of published microarray data (GSE39752) indicate that CYP2R1 was repressed in the livers of STZ-treated mice compared to vehicle-treated controls (n= controls 7, STZ 6). The box-and-whisker plots indicate the minimum, the 25th percentile, the median, the 75th percentile, and the maximum. In addition, the mean is indicated with +. The data was analyzed by two-tailed *t*-test.



SUPPLEMENTARY DATA

Supplementary Figure S2. Role of the PGC-1α-ERRα axis in the CYP2R1 suppression. *A*: WT PGC-1α and the PGC-1α-2x9 and PGC-1α-L2L3M mutants were similarly expressed in mouse primary hepatocytes (n=6). *B-D*: PGC-1α/ERRα-target genes ATP5β, CYCS, and PEPCK were induced with WT PGC-1α and the PGC-1α-2x9 mutant, but not with the PGC-1α-L2L3M mutant (n=6). *E*: PGC-1α is induced efficiently by adenovirus in both Scr-Ad and shEERα-Ad infected cells (n= 6). *F*: ERRα was knocked down by shERRα-Ad. *G and H*: XCT790 (XCT) does not affect PGC-1α and ERRα expression induced by PGC-1α-Ad (n= DMSO 4, 1 μM XCT 6, 2 μM XCT 5). *I*: PGC-1α mRNA was not detected in the livers of the PGC-1α knockout mice (n= PGC-1α$^{+/+}$ 7, PGC-1α$^{-/-}$ 6). *J and K*: PEPCK and ERRα were induced in the livers of fasted wild type and PGC-1α$^{-/-}$ mice livers. *L*: PGC-1α was induced similarly by fasting in the livers of liver-specific PGC-1β knockout mice and the WT mice (n=4). *M*: Analysis of the published ChIP-seq data (GSE31477) indicate PGC-1α and ERRα bind to two overlapping regions, in the proximal promoter and in the first intron of the human *CYP2R1* gene in the HepG2 cells treated with forskolin. The box-and-whisker plots indicate the minimum, the 25th percentile, the median, the 75th percentile, and the maximum. In addition, the mean is indicated with +. The data was analyzed by One-way ANOVA (Tukey's *post hoc* test, 95 % confidence interval).



SUPPLEMENTARY DATA

Supplementary Figure S3. Role of the GR in the CYP2R1 suppression *in vivo*. *A*: Analysis of the published microarray data (GSE24256) indicates that dexamethasone (DEXA) significantly represses the CYP2R1 in mouse liver compared to vehicle-treated mice (n=3). *B*: Analysis of the published ChIP-seq data (GSE72084) indicate that GR binds (both monomer (GRDIM) and homodimer (WT) to the proximal promoter, close to TSS, of the mouse *Cyp2r1* gene in the mouse liver. The box-and-whisker plots indicate the minimum, the 25th percentile, the median, the 75th percentile, and the maximum. In addition, the mean is indicated with +. The data was analyzed by A, two-tailed *t*-test.