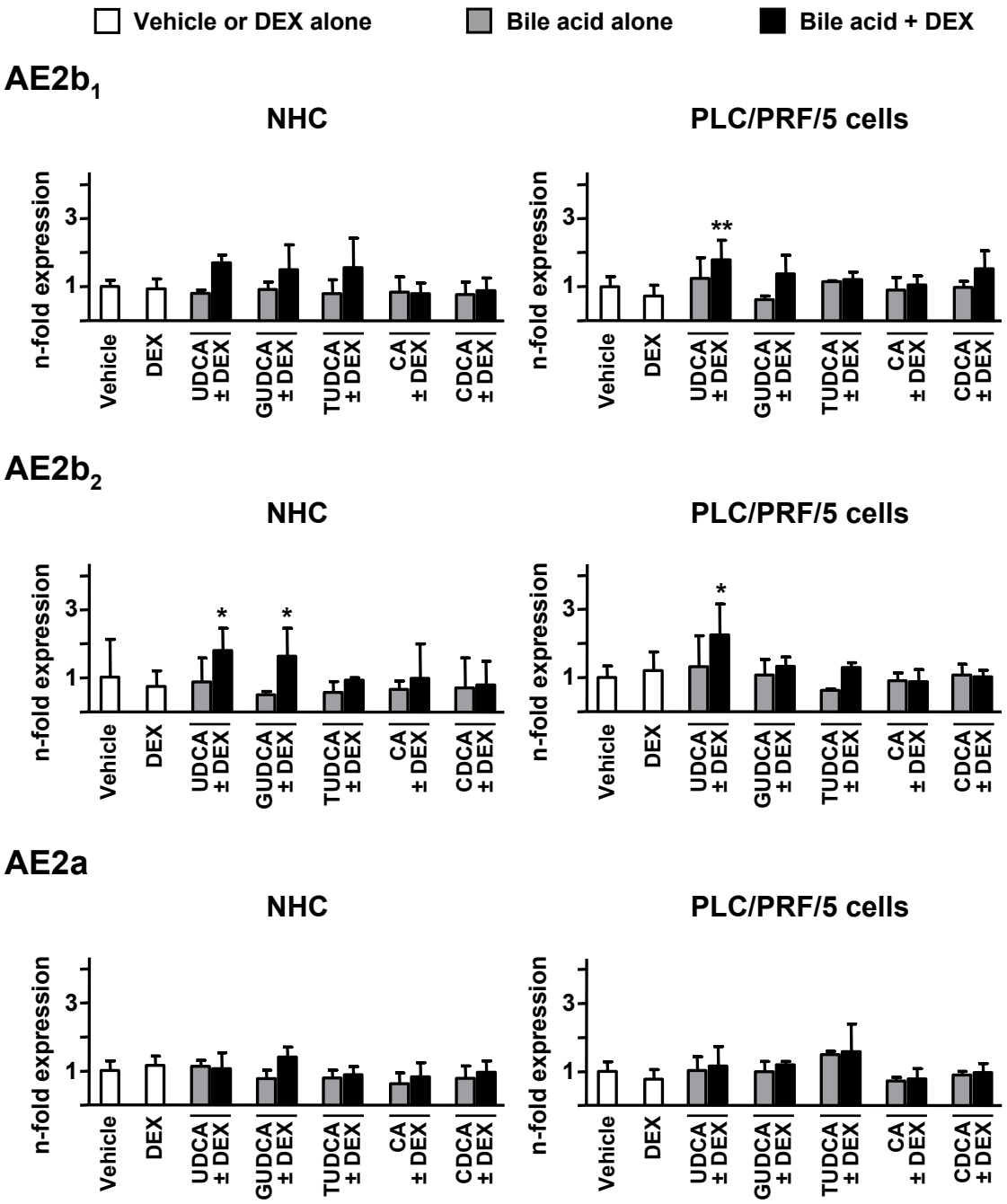
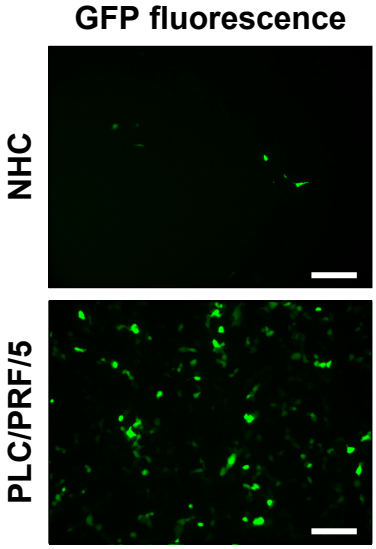


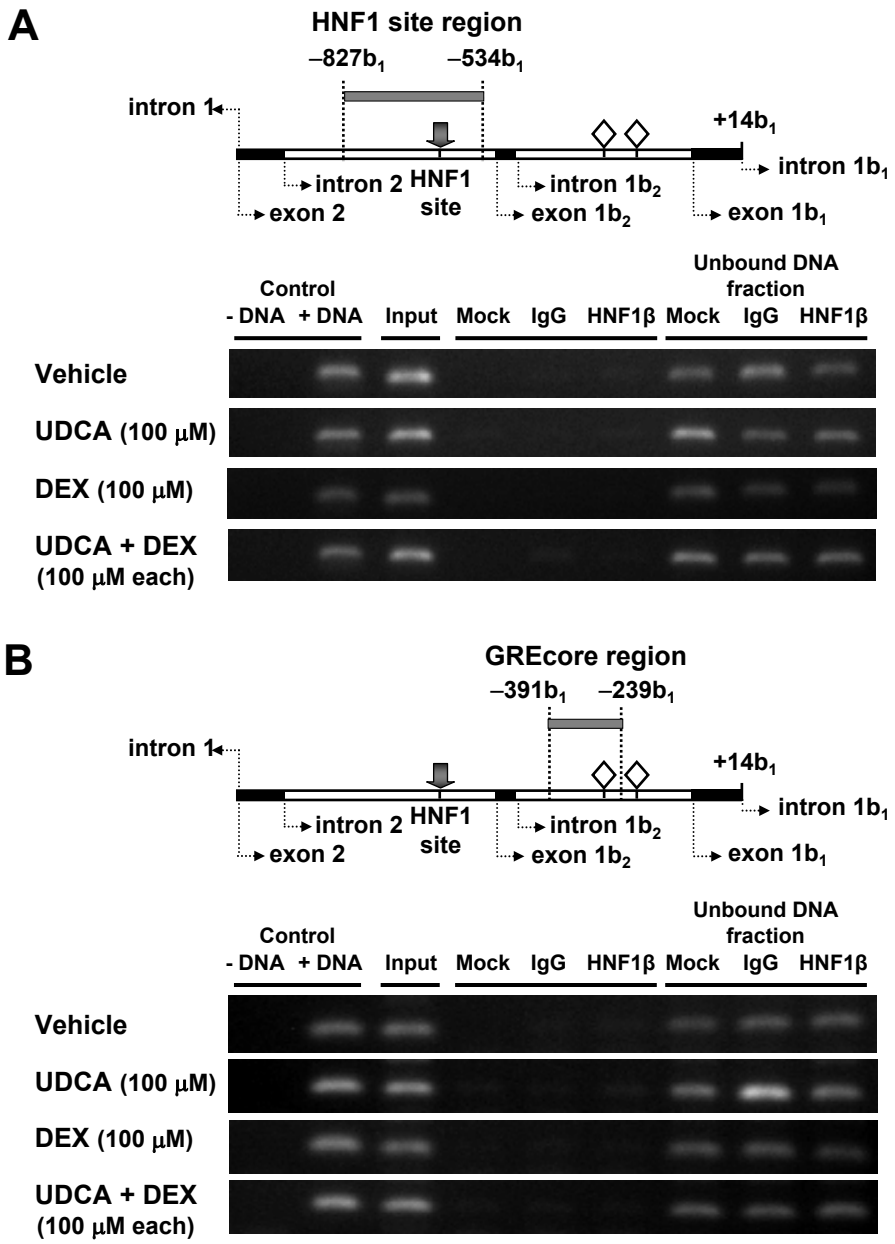
Supplemental Figure 1

Early effects of the combination of UDCA and dexamethasone on *AE2* alternative expression in human liver cells. Real-time PCR analysis was performed to determine the mRNA levels for AE2b1, AE2b2 and AE2a isoforms in cultured normal human cholangiocytes (NHCs) or hepatocyte lineage PLC/PRF/5 cells, treated for 6 hours either with dexamethasone (DEX) and/or UDCA, GUDCA, TUDCA, CA or CDCA (100 μM each) or with just vehicle. Values (normalized with GAPDH mRNA levels) are given as fold expression relative to values in the respective controls with just vehicle. Data are mean ± SD; *n*=6 (except for GUDCA, TUDCA, CA and CDCA – with and without DEX –, for which *n* = 3 each). **P* < 0.05, ***P* < 0.01 versus vehicle control.



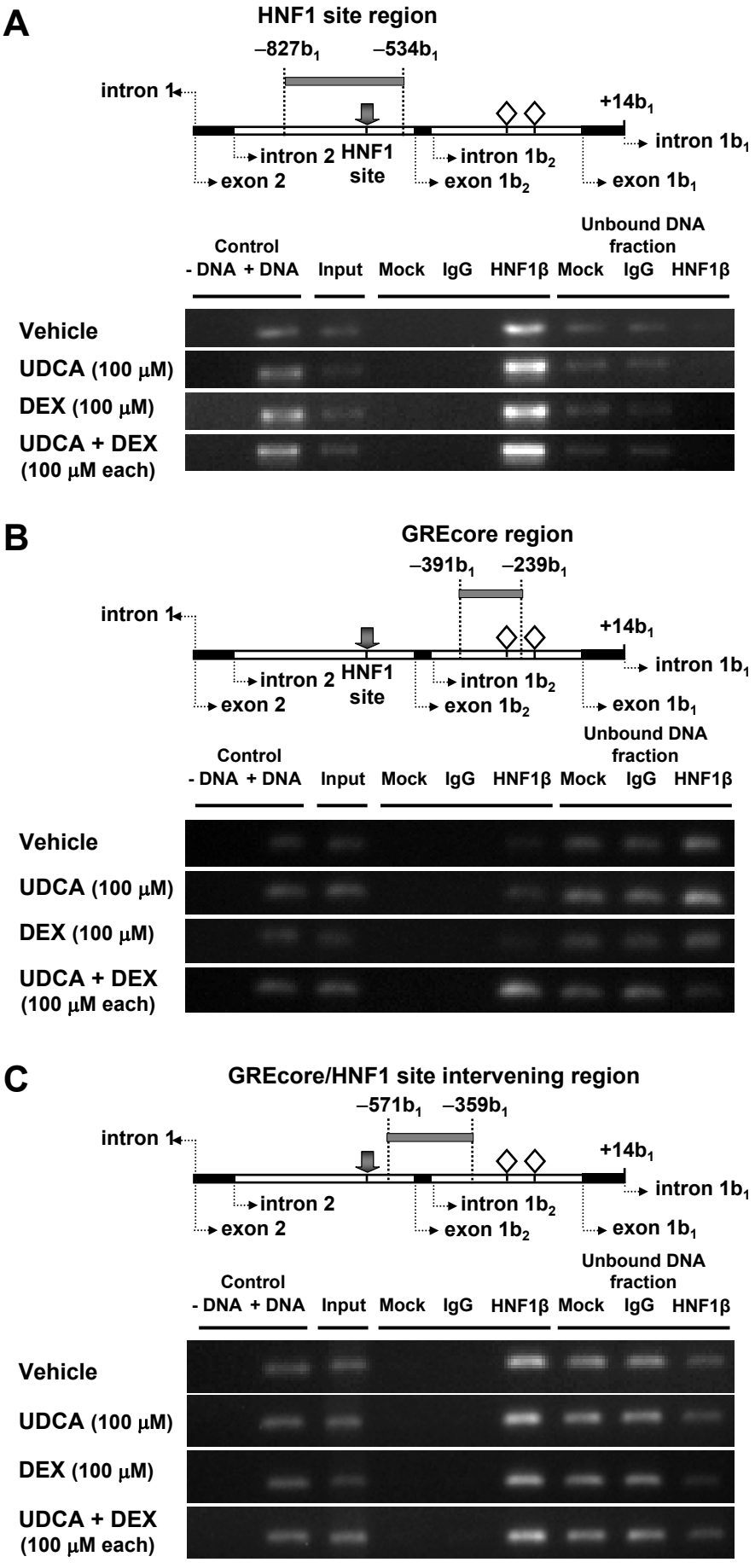
Supplemental Figure 2

Fluorescence microscopy of confluent-cell monolayers to compare transfection efficiency and determine functional silencing in cultured NHCs and hepatocyte lineage PLC/PRF/5 cells. Cells were seeded on 15 mm glass slips for their transient cotransfection with 2 μg of shRNA-1 construct (employed for *AE2* silencing) and 1 ng of GFP-expression vector using Lipofectamine 2000. After 72 hours of transfection, GFP-transfected cells in confluent monolayers (as visualized by phase-contrast microscopy) could be easily discriminated by fluorescence microscopy. Following BCECF-AM loading, microfluorimetric determination of the AE activity could be differentially determined in transfected cells and compared with that in nontransfected cells. White bars: 200 μm.



Supplemental Figure 3

HNF1β seems to play no major role in the *AE2* alternative transcription in nontransfected hepatocyte lineage PLC/PRF/5 cells. ChIP assays against HNF1β in nuclear extracts from non-transfected PLC/PRF/5 cells treated for 24 hours either with UDCA, dexamethasone (DEX), UDCA plus DEX, or just vehicle were carried out using the following templates: DNA negative (just water as negative control), DNA positive (human genomic DNA as positive PCR control), input (start material), mock (no antibody), IgG (goat total IgG) – the latter two as negative immunoprecipitation controls – and HNF1β immunoprecipitates. Protein A/G agarose unbound DNA fractions were also used as control templates. Band amplifications were negligible when using HNF1β immunoprecipitates as templates, both (**A**) for the amplicon –827b$_1$/–534b$_1$ (which corresponds to the HNF1 site region; see the gray bar in the upper diagram), and (**B**) for the amplicon –391b$_1$/–239b$_1$ (which corresponds to the GREcore –327b$_1$ region; see the upper diagram in panel **B**). Gray arrows in the upper diagrams indicate to the HNF1 site and open rhombuses represent the GREcore motifs.



Supplemental Figure 4

Transfection of hepatocyte-lineage PLC/PRF/5 cells with an HNF1β expression vector may result in interaction of HNF1β with its HNF1 element on the *AE2* alternate promoter. PLC/PRF/5 cells were cultured in 150 mm-diameter plates and transiently transfected with 14 μg of pBJ5-HNF1β expression vector supplemented with 56 μg of carrier DNA (pGL3-basic) using 140 μl of Lipofectamine 2000. One day after transfection, cells were treated for 24 hours either with UDCA, dexamethasone (DEX), UDCA plus DEX, or just vehicle. ChIP assays against HNF1β in nuclear extracts from these transfected PLC/PRF/5 cells were carried out using the templates equivalent to those described in Supplemental Figure 3. These templates were used in PCR with specific primers to detect (**A**) the HNF1-site region (amplicon $-827b_1$/$-534b_1$), (**B**) the GREcore $-327b_1$ region (amplicon $-391b_1$/$-239b_1$), or (**C**) the intervening region between HNF1 and GREcore $-327b_1$ elements (amplicon $-571b_1$/$-359b_1$). The gray bars in the upper diagrams refer to the corresponding expected amplicons; gray arrows indicate the HNF1 site and open rhombuses represent the GREcore motifs