**Supplemental Table 1**: List of primers used in this study

**Plasmid Construction** – Cloning primers

| Gene name | Forward | Reverse |
|---|---|---|
| CYP2B6_+131HindIII | | TCCAAGCTTCTGGTCTGACTGC |
| CYP2B6-2654 | TGACAATGCTTGCCCGAAACTT | |
| CYP2B6-800 | CCTCCCAGGTTCAAGTGATT | |
| CYP2B6-481 | AAGGATACACACATAAGCACC | |
| CYP2B6_-347 | CATGCAAGCACAGACAAACA | |
| CYP2B6-10918 | AAGAGAAATTGGCCAAAATGAA | |
| CYP2B6-7428 | | GAGTCATAAACCACTTAGCAA |
| CYP2B6-1433 | | GGACTCCCATATGTCTCTATCCTC |

**Chromatin immunoprecipitation** - Quantitative PCR primers

| Gene name | Forward | Reverse | Amplicon size |
|---|---|---|---|
| PCR1-12026 | AGGTTGCAGTGAGCCAAGAT | AAGAGCCCTGTATCCCTAGACA | 213 bp |
| PCR2-10918 | AAGAGAAATTGGCCAAAATGAA | CGTCCAGACCCCAGAATAGTAG | 286 bp |
| PCR3-9336 | GGAAACTTTAGGCTCCAAGC | CCATCACAGCACTCGTCACT | 181 bp |
| PCR4-8634 | TGAGGACCCAGAGTACCCGTAT | AAACCACTTGCCGCCACT | 314 bp |
| PCR5-4956 | GCAGCAAGCCTTTTGTTCTC | CCAGCTTGAGCTGAGCTTTT | 347bp |
| PCR6-2507 | TCCCTCCCCTTTTGAAAATC | AGTGGCATTATCAGGGGAAA | 348bp |
| PCR7-1788 | ATGAGCACCCAATCTTAGTGTCA | GGACTCCCATATGTCTCTATCCTC | 356bp |
| PCR8-347 | CATGCAAGCACAGACAAACA | CCCAGTGACCTGATGCCTAT | 176bp |
| RPLPO | ACCCAGCTCTGGAGAAGTCA | GAGGTCCTCCTTGGTGAACA | 175bp |

**mRNA expression** – Quantitative RT PCR primers

| Gene name | Accession nº | Forward | Forw. 5'nt | Reverse | Amplicon size |
|---|---|---|---|---|---|
| PBGD | NM_000190 | CGGAAGAAAACAGCCCAAAGA | 189 | TGAAGCCAGGAGGAAGCACAGT | 294bp |
| GAPDH | NM_002046 | ATGCTGGCGCTGAGTACGTC | 368 | GGGCAGAGATGATGACCCTT | 104bp |
| CYP2B6 | NM_000767 | GACGCCTTCAATCCTGACCACT | 1446 | ATTTTGCCCACACCACACTCCT | 225bp |
| C/EBPα | NM_004364 | GTGGAGACGCAGCAGAAG | 922 | TTCCAAGGCACAAGGTTATC | 450bp |
| CAR | NM_005122 | TGCTGCCTCTGGTCACACACTT | 664 | TCAATCTCATCTCTCTGGGTAAC | 385bp |
| HNF4α | NM_000457 | GCCTACCTCAAAGCCATCAT | 948 | GACCCTCCCAGCAGCATCTC | 275bp |
| CYP2C9 | NM_000771 | CCTCTGGGGCATTATCCATC | 1549 | ATATTTGCACAGTGAAACATAGGA | 141bp |
| CYP3A4 | NM_ 017460 | CCTTACATATACACACCCTTTGGAAGT | 1389 | AGCTCAATGCATGTACAGAATCCCCGGTTA | 352bp |
| UGT1A1 | NM_000463 | AGACGTACCCTGTGCCATTC | 245 | CCGTCAGCATGACATCAAAG | 222bp |
| UGT2B4 | NM_021139 | CACAGAATTCAGCCACTGGA | 137 | CTGCCCATCTCTTAACCAGC | 206bp |
| OSTbeta | NM_178859 | ATCCAGGCAAGCAGAAAAGA | 205 | CTGGTACATCCGGAAGGAAA | 199bp |
| GST1A1 | NM_145740 | CCTGAGGAAAAAGATGCCAA | 452 | GACTGGAGTCAAGCTCCTCG | 181bp |

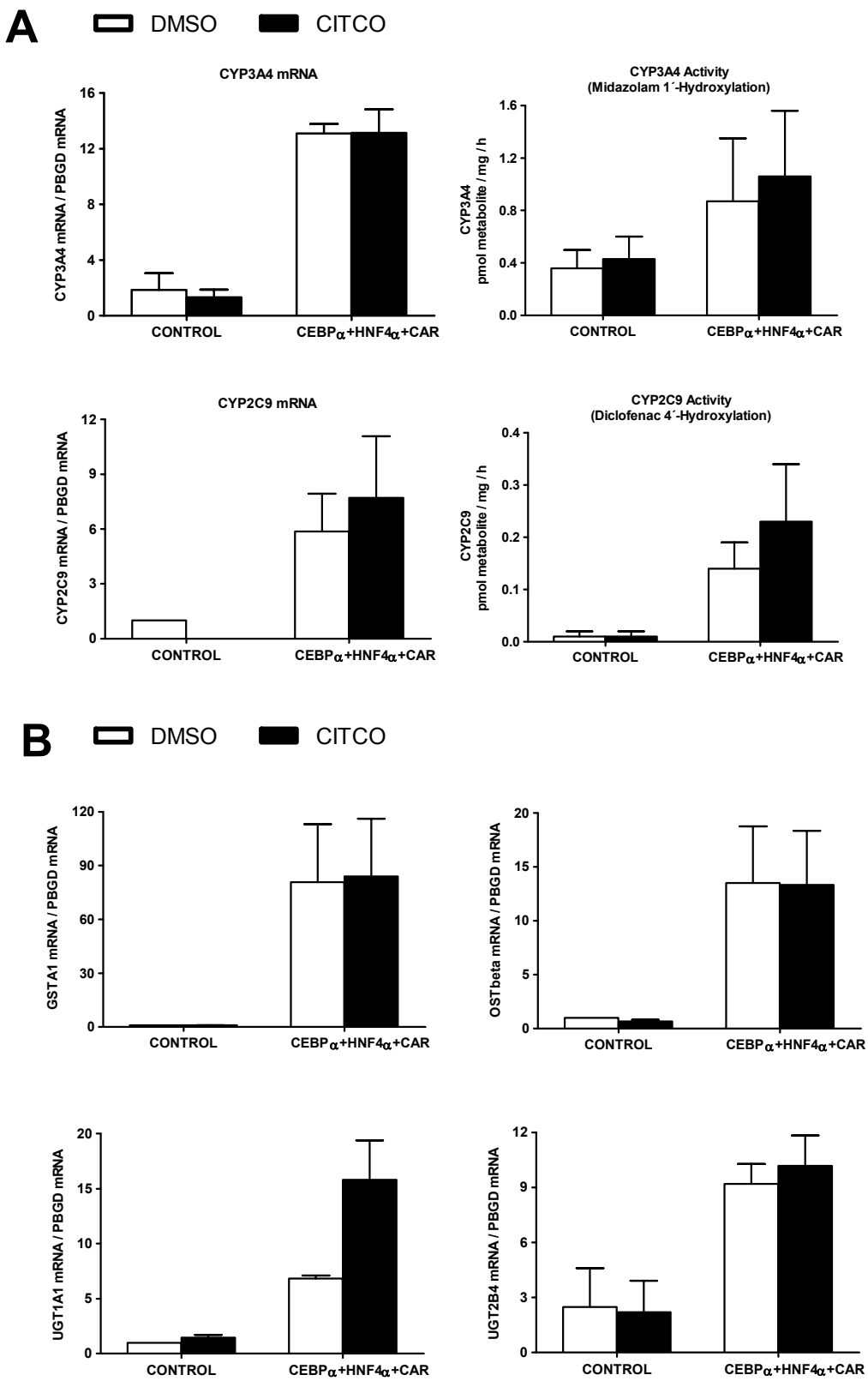
**Supplemental Table 2**: Statistically overrepresented pathways from the list of induced genes in the upgraded HepG2 cells by ConsensusPathDB analysis:

| p-value | q-value | Pathway | Source | Members input overlap | Related genes | Effective input size | Overlap with input list |
|---|---|---|---|---|---|---|---|
| 0.000 | 0.002 | Glycogen synthesis | Reactome | GBE1; GYG1; GYS1 | | 7 | 3 |
| 0.000 | 0.002 | Glycogen branching enzyme transfer…. | Reactome | GBE1; GYG1 | | 3 | 2 |
| 0.000 | 0.002 | Biological oxidations | Reactome | ACSM2B; ADH4; CYP19A1; CYP24A1; CYP26A1; CYP2B6; CYP3A5; CYP3A7; CYP4F2; GSTA1; TPMT; UGT1A1; UGT2B4 | | 126 | 13 |
| 0.000 | 0.003 | Cytochrome P450 - arranged by substrate type | Reactome | CYP19A1; CYP24A1; CYP26A1; CYP2B6; CYP3A5; CYP3A7; CYP4F2 | | 49 | 7 |
| 0.000 | 0.005 | Phase 1 - Functionalization of compounds | Reactome | ADH4; CYP19A1; CYP24A1; CYP26A1; CYP2B6; CYP3A5; CYP3A7; CYP4F2 | | 67 | 8 |
| 0.001 | 0.011 | Xenobiotics | Reactome | CYP2B6; CYP3A5; CYP3A7 | | 15 | 3 |
| 0.001 | 0.008 | Vitamins & Steroid hormones | Reactome | CYP24A1; CYP26A1 | CYP19A1 | 6 | 2 |
| 0.003 | 0.026 | Synthesis of bile acids and bile salts | Reactome | AKR1D1; SLC27A2 | OSTbeta, BAAT | 10 | 2 |
| 0.006 | 0.040 | PPAR signaling pathway | KEGG | ACSL1; ACSL5; AQP7; CD36; PCK1; SLC27A2 | RXRB | 69 | 6 |
| 0.001 | 0.008 | Urea cycle | HumanCyc | ARG1; ASL | | 6 | 2 |
| 0.002 | 0.015 | Urea synthesis | Reactome | ARG1; ASL | CA2; CA12; CA9 | 8 | 2 |
| 0.005 | 0.033 | Superpathway of methionine degradation | HumanCyc | BHMT; CDO1; CTH | | 22 | 3 |
| 0.000 | 0.002 | Dissolution of Fibrin Clot | Reactome | PLAUR; SERPINB2; SERPINE1 | | 8 | 3 |
| 0.009 | 0.048 | Fibrinolysis pathway | BioCarta | SERPINB2; SERPINE1 | | 14 | 2 |
| 0.006 | 0.037 | Response to elevated platelet cytosolic Ca++ | Reactome | CD36; KNG1; PRKCA; SERPINE1; TGFB3; TMSB4X | | 67 | 6 |
| 0.004 | 0.032 | Activation of PKC through G-protein coupled receptors | BioCarta | PLCB1; PRKCA | | 11 | 2 |
| 0.000 | 0.002 | HIF-1-alpha transcription factor network | PID | ADM; CA9; CITED2; EGLN3; FOS; HNF4A; MCL1; PFKFB3; RORA; SERPINE1 | | 66 | 10 |
| 0.004 | 0.029 | Trk receptor signaling mediated by the MAPK pathway | PID | EGR1; FOS; MEF2C; RPS6KA1 | | 33 | 4 |

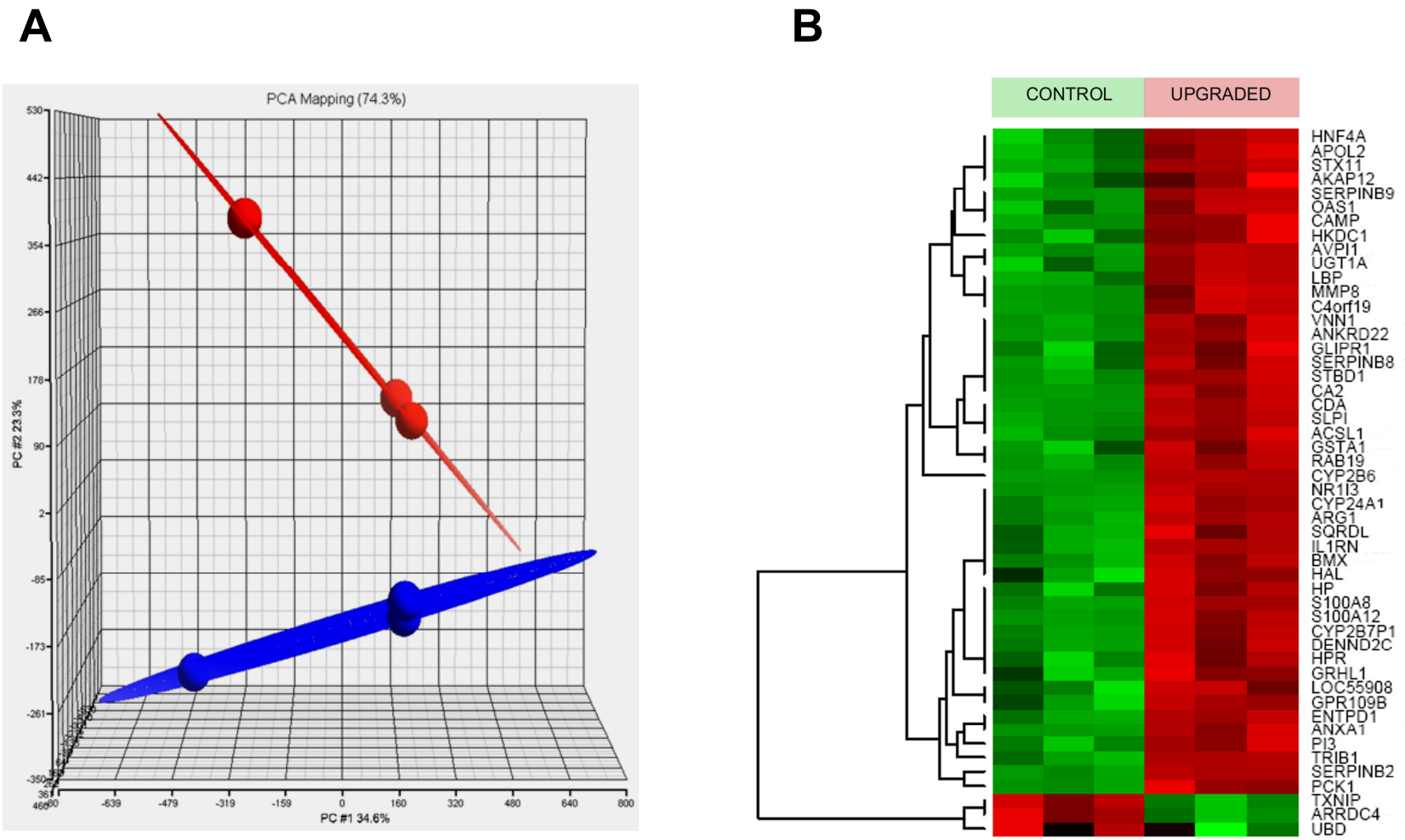
**Supplemental Table 3**: Overrepresented pathways in the upgraded HepG2 cells according to the *Pharmacogenomics Knowledge Base*:

| p-value | q-value | Pathway | Source | Members input overlap | Related genes | Effective input size |
|---|---|---|---|---|---|---|
| 0.000 | 0.002 | Irinotecan Pathway | PharmGKB | CES1; CES2; CYP3A5; UGT1A1 | 13 | 4 |
| 0.000 | 0.003 | Antiplatelet Drug Clopidogrel Pathway (PK) | PharmGKB | CES1; CYP2B6; CYP3A5 | 9 | 3 |
| 0.000 | 0.003 | Phenytoin PK Pathway | PharmGKB | CYP3A5; CYP3A7; UGT1A1 | 10 | 3 |
| 0.001 | 0.008 | Anti-estrogen Pathway (Tamoxifen PK) | PharmGKB | CYP2B6; CYP3A5 | 6 | 2 |
| 0.001 | 0.008 | Ifosfamide Pathway (PK) | PharmGKB | CYP2B6; CYP3A5 | 6 | 2 |
| 0.001 | 0.011 | Cyclophosphamide Pathway (PK) | PharmGKB | CYP2B6; CYP3A5 | 7 | 2 |
| 0.002 | 0.015 | Erlotinib Pathway (PK) | PharmGKB | CYP3A5; UGT1A1 | 8 | 2 |
| 0.002 | 0.020 | Irinotecan Pathway (Cancer) | PharmGKB | CES1; CES2; UGT1A1 | 18 | 3 |
| 0.003 | 0.024 | Thiopurine Pathway | PharmGKB | GSTA1; MTHFS; MTRR; TPMT | 31 | 4 |
| 0.003 | 0.026 | Statin Pathway (Atorvastatin, Lovastatin...PK) | PharmGKB | CYP3A5; UGT1A1 | 10 | 2 |
| 0.006 | 0.038 | Etoposide Pathway | PharmGKB | CYP3A5; UGT1A1 | 12 | 2 |
| 0.007 | 0.040 | Fluoropyrimidine PK | PharmGKB | CDA; CES1; CES2 | 24 | 3 |
| 0.007 | 0.041 | Statin Pathway (Fluvastatin PK) | PharmGKB | CYP3A5; UGT1A1 | 13 | 2 |

PK: Pharmacokinetic pathways




**Supplemental Figure 1** - Adenoviral-mediated transfection of C/EBPa, HNF4a and CAR reactivates multiple drug metabolism and disposition genes, and improves metabolic competence in human hepatoma HepG2 cells. HepG2 cells were infected with the combined adenoviral vectors for 24 h. Next, they were exposed to 500 nM CITCO or solvent for an additional 24-h period. A, CYP mRNA levels were determined by Q-RT-PCR and normalized with the housekeeping PBGD. CYP3A4 and CYP2C9 activities were assayed using a cocktail mixture containing midazolam and diclofenac as specific substrates. Enzymatic activity was expressed as pmol of metabolite formed/h/mg total protein. B,The mRNA concentration of phase II conjugating enzymes and transporters was determined by quantitative RT-PCR. Data represent the mean ± S.D. from 3-4 independent experiments.




**Supplemental Figure 2 - Transcriptome analysis of the upgraded HepG2 cells**. Total RNA was purified from matched-pair HepG2 cells infected with Ad-C/EBPα/Ad-HNF4α/Ad-CAR, or with a control (insertless) adenovirus, and their expression profiles were determined by microarray analysis. **A,** Unsupervised Principal Component Analysis (PCA) shows the non forced grouping of the control *versus* the upgraded HepG2 cells. The figure depicts a three-dimensional PCA representation where blue and red represent the controls and the upgraded HepG2 cells, respectively. **B,**Heatmap of the top 50 differentially expressed genes, showing preferential gene induction over gene repression in the upgraded HepG2 cells. *Red*, *green*, and *black* represent up-, down-, and non-regulation, respectively