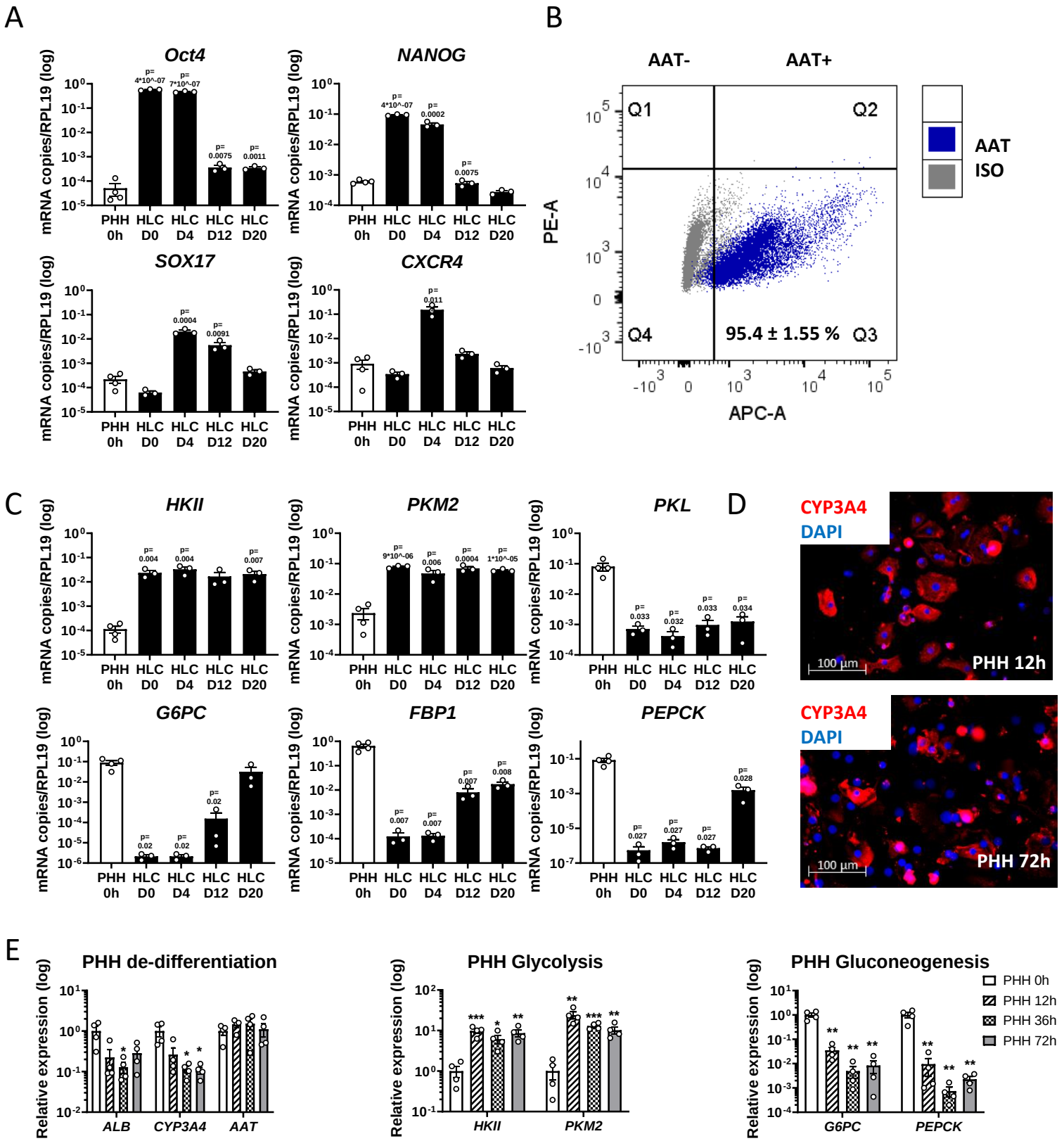
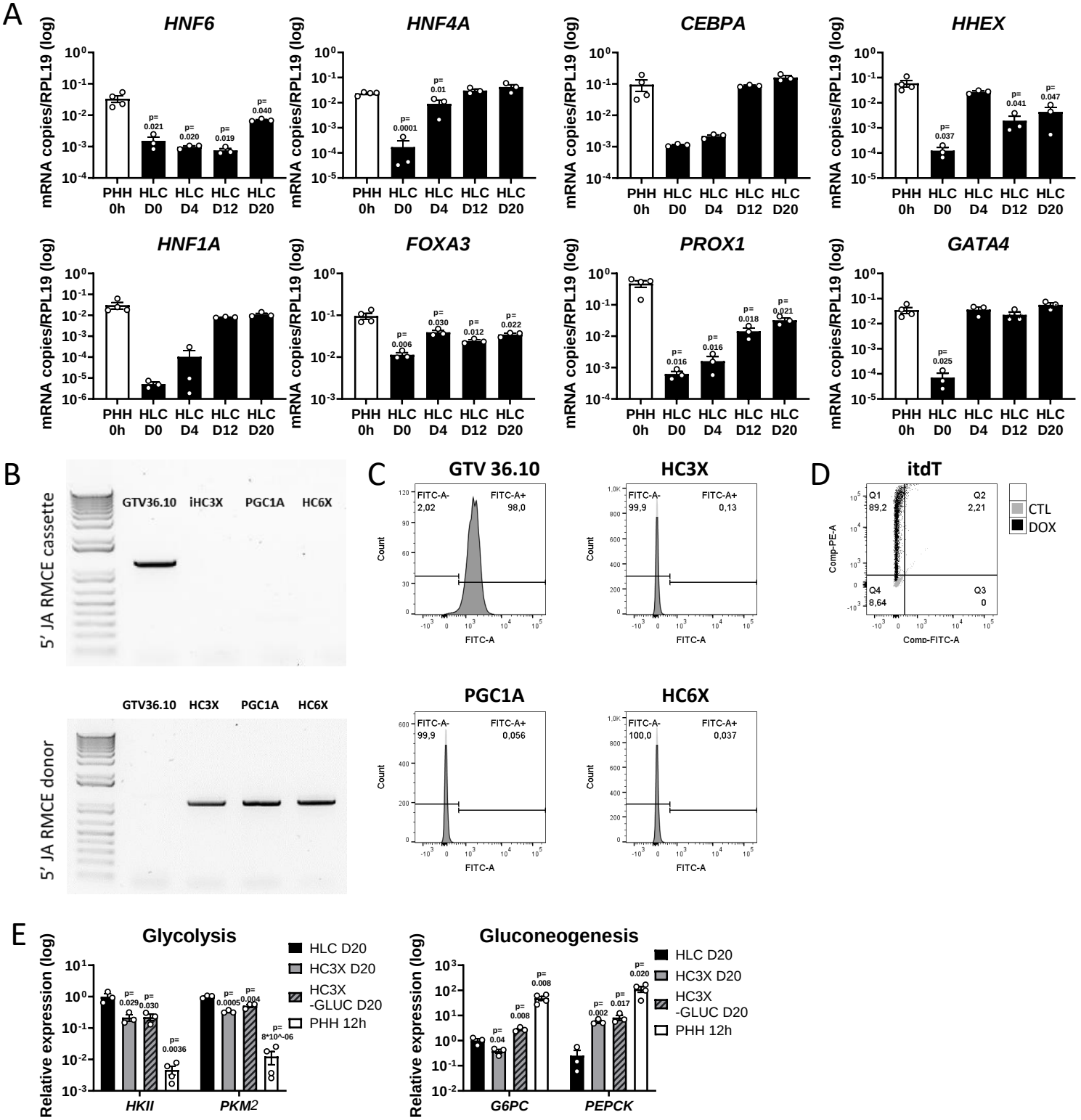
**Supplementary Information**

# Amino acid levels determine metabolism and CYP450 function of hepatocytes and hepatoma cell lines

**Boon *et al.***



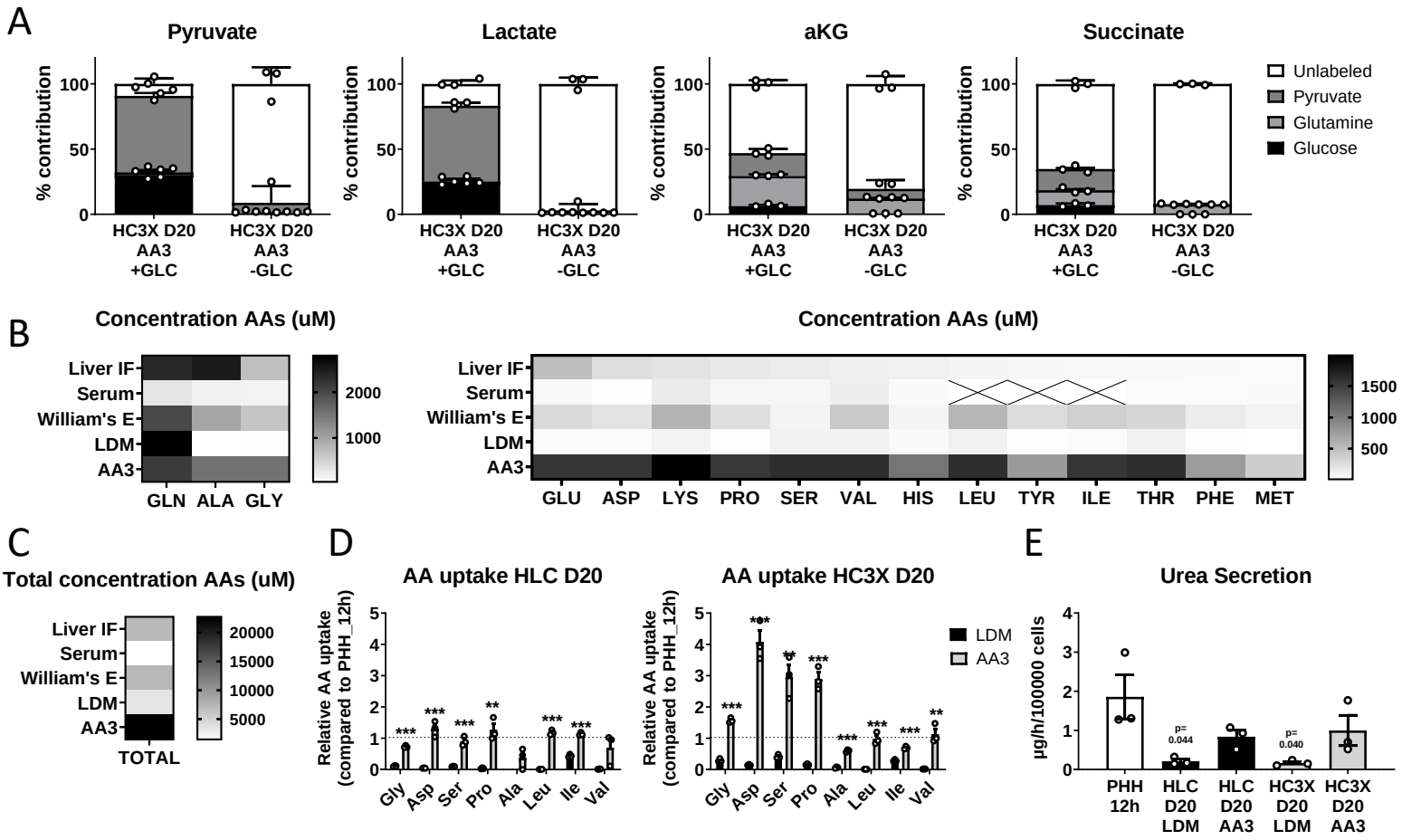

**Supplementary figure 1. HLC D20 display a phenotype similar to that of 2D cultured PHHs.** **(A)** Expression of *OCT4, NANOG, SOX17 and CXCR4 in* PHHs and HLCs at different time points (D0, D4, D12, D20). Data was normalized for expression of ribosomal protein lysine 19 (*RPL19*). N=3 independent differentiations. N=4 donors for PHHs. Significance was calculated as compared to PHH 0h by unpaired two-tailed Student's t-test. **(B)** Representative FACS plots for AAT, overlying isotype (gray) and AAT staining (blue) Quantification. N=4 independent differentiations. **(c)** Expression of *HKII, PKM2, PKL, G6PC, FBP1* and *PEPCK* in PHHs and differentiating HLCs at different time points (D0, D4, D12, D20). N=3 independent differentiations. N=4 donors for PHHs. Significance is calculated as compared to PHH 0h by unpaired two-tailed Student's t-test. **(D)** Immunostaining for CYP3A4 in PHH 12h and PHH 72h. representative image of plating of 2 donors of PHHs. (**E)** Relative expression of *ALB, CYP3A4*, AAT, HKII, PKM2, G6PC and PEPCK in PHH 12h, -36h, and -72h compared to PHH 0h. N=3 independent differentiations. N=4 donors for PHHs. Significance is calculated as compared to PHH 0h by unpaired two-tailed Student's t-test. ∗p < 0.05, ∗∗p < 0.01, ∗∗∗p < 0.001. Data in all panels represents mean ± standard error of mean (SEM) with P-values indicated when significant. Source data are provided as a Source Data file.



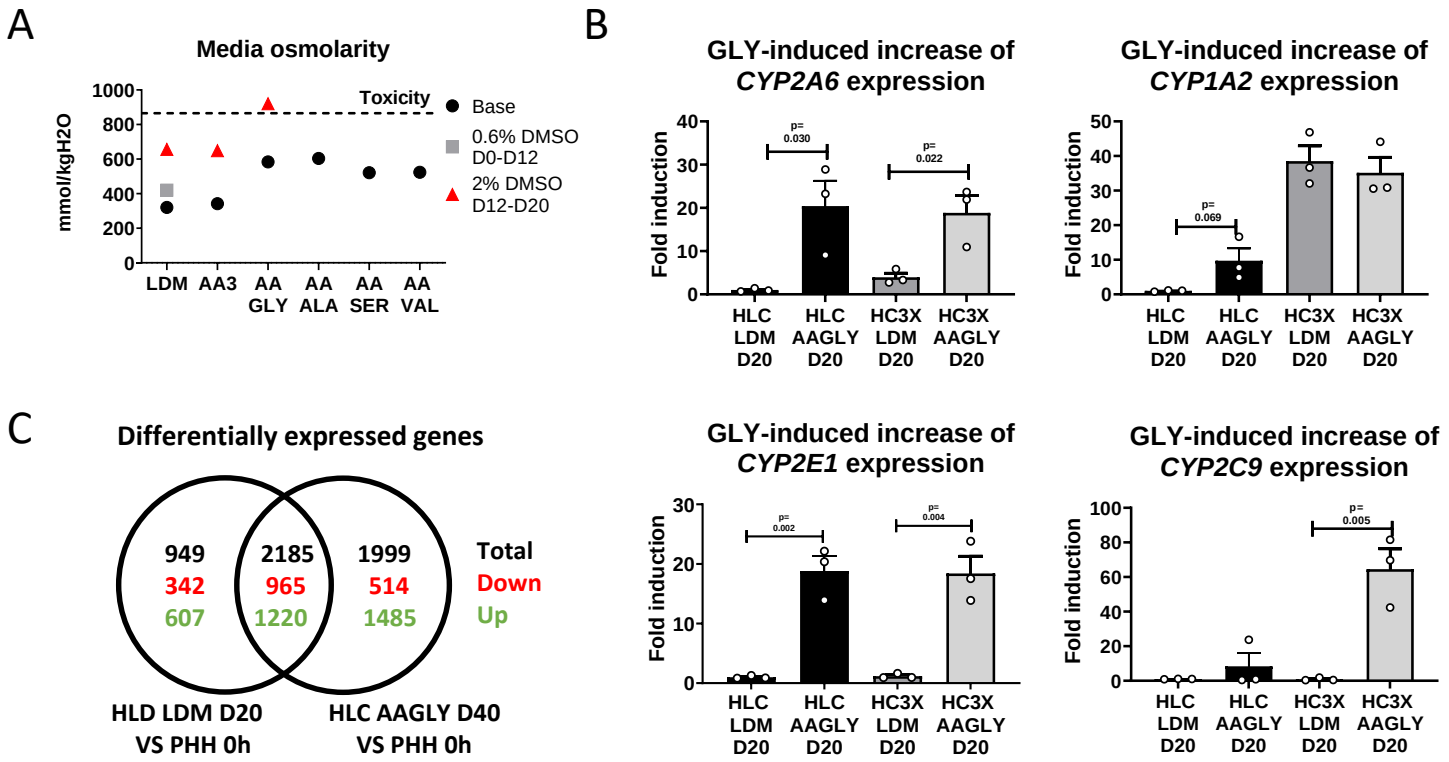

**Supplementary figure 2. Overexpression of hepatic transcription factors induces metabolic reprogramming.** **(A)** Expression of the hepatic transcription factors *HNF6, HNF4A, CEBPa, HHEX, HNF1A, FOXA3, PROX1* and *GATA4* in PHH 0h and HLCs at different time points (D0, D4, D12, D20). Data was normalized for expression of ribosomal protein lysine 19 (*RPL19*). N=3 independent differentiations. N=4 donors for PHHs. Significance is calculated as compared to PHH 0h by unpaired two-tailed Student's t-test. **(B)** PCR genotyping for presence of the exchangeable RMCE cassette (5'JA RMCE cassette) and of the integrated overexpression cassettes (5'JA RMCE donor) for the basic RMCE H9 cell line (GTV36,10) and the generated RMCE lines (HC3X, PGC1A and HC6X). One repeat. **(C)** FACS analysis demonstrating that following RMCE, the GFP cassette in the initial FRT-GFP.HYG/TK-FRT line in the *AAVS1* locus was eliminated: GFP signal for GTV36.10, HC3X, PGC1A and HC6X. One repeat. **(D)** FACS analysis for tdT signal in HLC D20 following RMCE with a tdT cassette and addition of 5µg/ml doxycycline from D4 to D20 (DOX) compared with HLC D20-tdT without doxycycline administration. Representative plot for 3 repeats. **(E)** Relative expression of genes involved in glycolysis and gluconeogenesis In HLC D20 and HC3X D20 when compared to PHH 0h. Data was normalized for expression of ribosomal protein lysine 19 (*RPL19*). N=3 independent differentiations. N=3 donors for PHHs. Significance is calculated as compared to HLC D20 by unpaired two-tailed Student's t-test. Data in all panels represents mean ± standard error of mean (SEM) with P-values indicated when significant. Source data are provided as a Source Data file.

| Amino Acid | LDM (mg/l) | AA1 (mg/l) | AA2 (mg/l) | AA3 (mg/l) | AA3+GLY mg/l) | William's E (mg/l) |
|---|---|---|---|---|---|---|
| Glycine | 3.01 | 17.96 | 62.77656 | 122.53512 | 20122.53 | 50 |
| L-Alanine | 3.56 | 21.29 | 74.47888 | 145.39376 | 145.39 | 90 |
| L-Asparagine | 60 | 85.2 | 160.8 | 261.6 | 261.6 | 20 |
| L-Aspartic acid | 5.2 | 31.69 | 111.184 | 217.168 | 217.16 | 30 |
| L-Glutamic Acid | 5.6 | 34.88 | 122.752 | 239.904 | 239.90 | 45 |
| L-Proline | 2.28 | 25.23 | 94.09 | 185.91 | 185.91 | 30 |
| L-Serine | 12.42 | 33.17 | 95.43 | 178.43 | 178.43 | 10 |
| L-Arginine hydrochloride | 25.25 | 25.25 | 88.19 | 277.04 | 277.04 | 50 |
| L-Cystine | 14 | 25.86 | 61.44 | 108.88 | 108.88 | 40 |
| L-Histidine hydrochloride-H2O | 8.02 | 28.94 | 91.70 | 175.38 | 175.38 | 15 |
| L-Isoleucine | 5.26 | 31.41 | 109.85 | 214.44 | 214.44 | 50 |
| L-Leucine | 15.66 | 41.70 | 119.83 | 224 | 224 | 75 |
| L-Lysine hydrochloride | 15.27 | 51.37 | 159.66 | 304.05 | 304.05 | 87 |
| L-Methionine | 1.76 | 9.29 | 31.88 | 62.01 | 62.01 | 15 |
| L-Phenylalanine | 1.96 | 18.44 | 67.88 | 133.80 | 133.80 | 25 |
| L-Threonine | 14.05 | 37.71 | 108.69 | 203.33 | 203.33 | 40 |
| L-Tryptophan | 2.40 | 7.48 | 22.71 | 43.01 | 43.01 | 10 |
| L-Tyrosine | 4.47 | 22.42 | 76.29 | 148.11 | 148.11 | 50 |
| L-Valine | 14.05 | 37.31 | 107.09 | 200.131888 | 200.13 | 50 |
| L-Glutamine | 408.86 | 404.7714 | 392.5056 | 376.1512 | 376.1512 | 292 |
| Total Amount | 623.08 | 991.3714 | 2159.227 | 3821.274 | 23821.25 | 1074 |

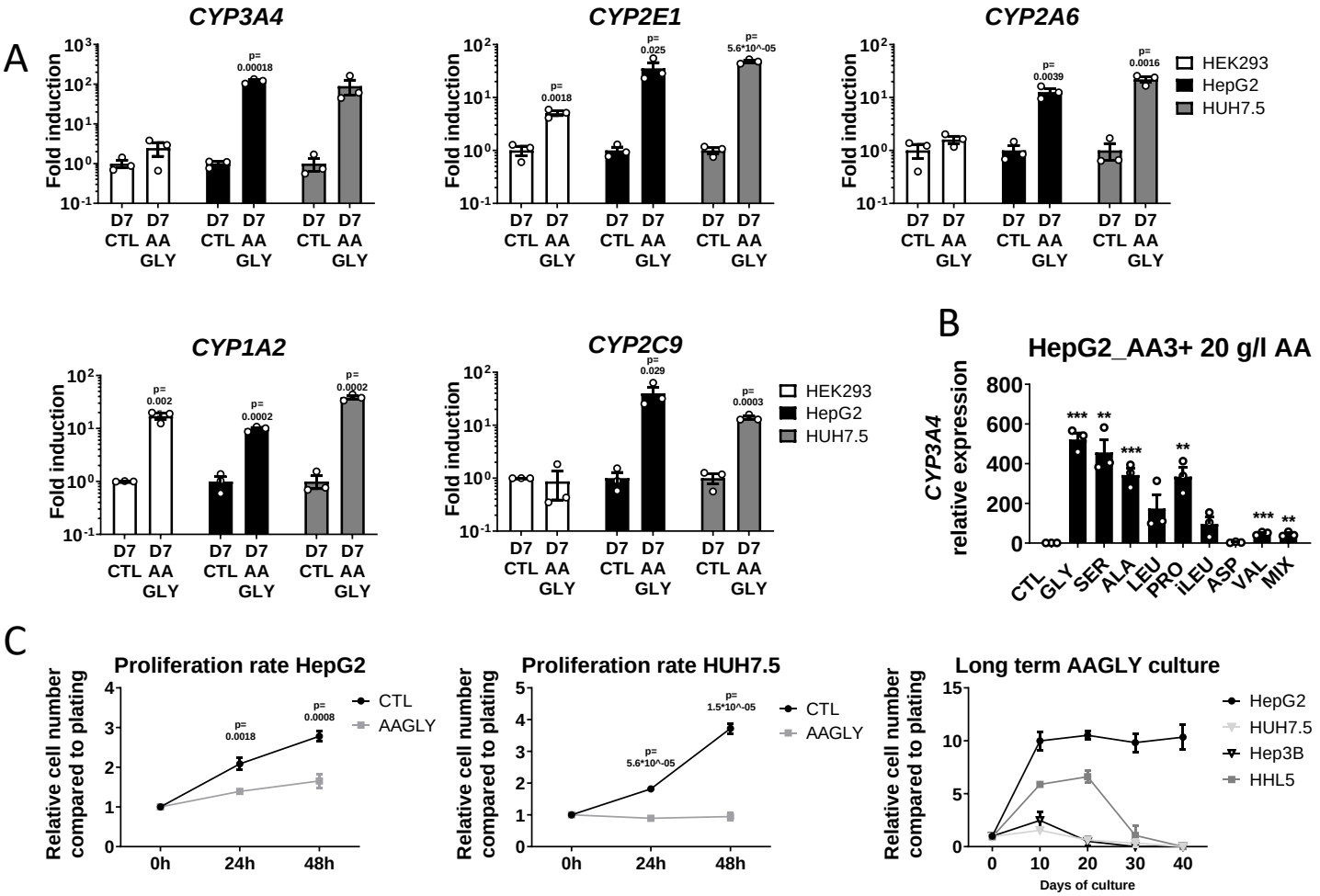
**Supplementary table 1. Concentrations of AAs in utilized media and in William's E medium.** Table showing the amino acid composition of utilized media.



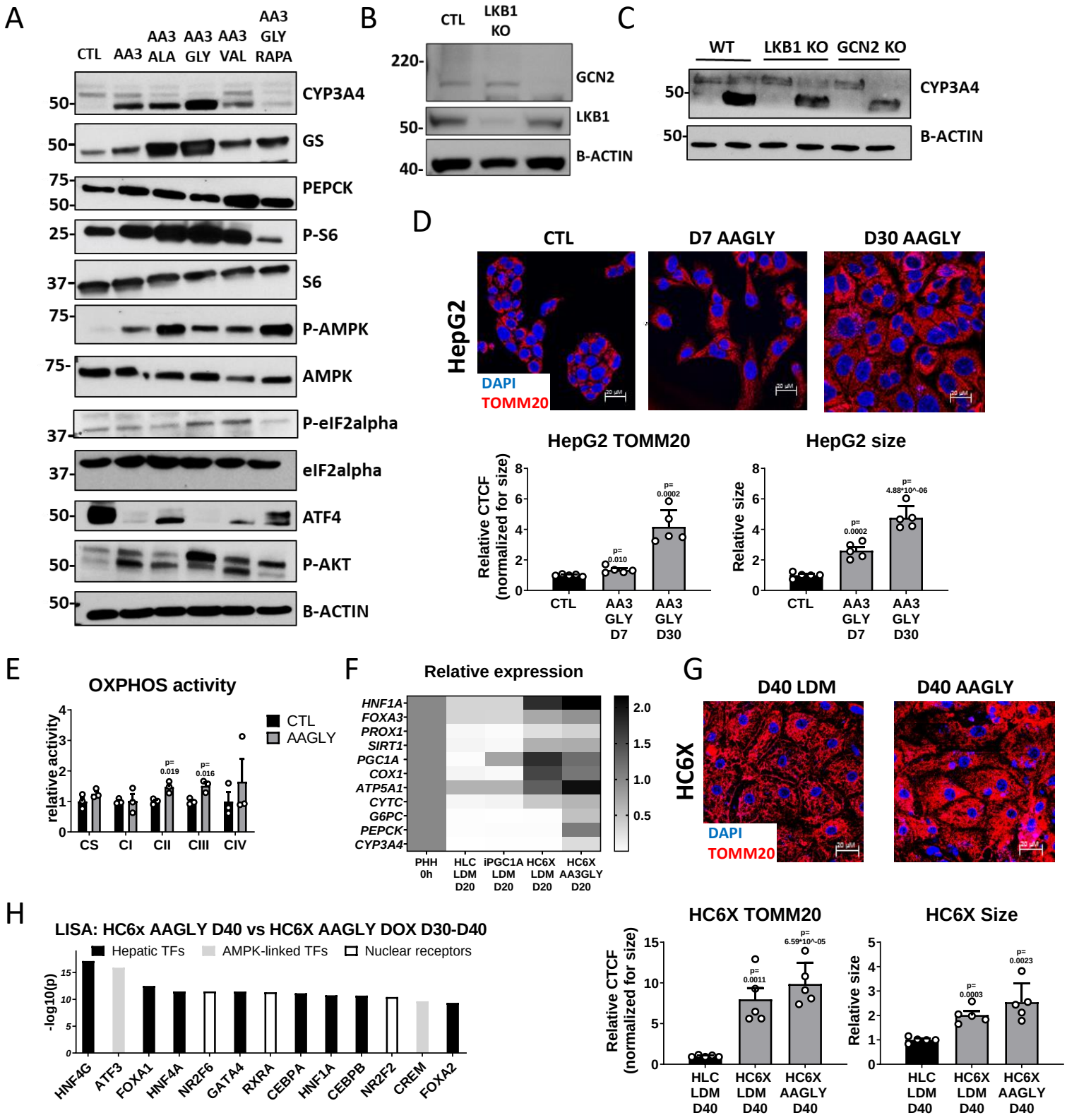

**Supplementary figure 3. AA supplementation induces metabolic reprogramming.** **(A)** Percentage of $^{13}C$ labeled glucose, glutamine or pyruvate contribution to pyruvate, lactate, alpha-ketoglutarate (AKG) and succinate in HC3X D20 cultured in medium supplemented with AA3 supplement with glucose (AA3+GLLC) and AA3 supplement without glucose (AA3-GLC). The non-labeled fraction was designated as unlabeled. N=3 independent differentiations. **(B)** and **(C)** Concentration of AAs found in mouse liver interstitial fluid, mouse serum, and in the different media used. Intensity of color correlates with concentrations. X signifies undetectable values. N= 5 mice for IF and N= 13 mice for serum measurements. **(D)** Amino acid uptake rates for glycine (GLY), aspartate (ASP), serine (SER), proline (PRO), alanine (ALA), leucine (LEU), isoleucine (iLEU) and valine (VAL) in HLC D20 and HC3X D20 cultured in LDM medium and LDM medium with AA3 supplementation (AA3), compared to PHH 12h cultured in WE medium (stippled line). N=3 independent differentiations, N=3 donors for PHHs. Significance is calculated by unpaired two-tailed Student's t-test by comparing against PHH 12h.$^{**}p < 0.01$, $^{***}p < 0.001$. **(E)** Urea secretion rates for HLC and HC3X D20 progeny cultured in LDM medium and LDM medium with AA3 supplementation (AA3) compared to PHH 12h cultured in William's E medium. N=3 independent differentiations, N=3 donors for PHHs. Significance is calculated by unpaired two-tailed Student's t-test compared with PHH 12h. Data in all panels represents mean ± standard error of mean (SEM) with P-values indicated when significant. Source data are provided as a Source Data file.



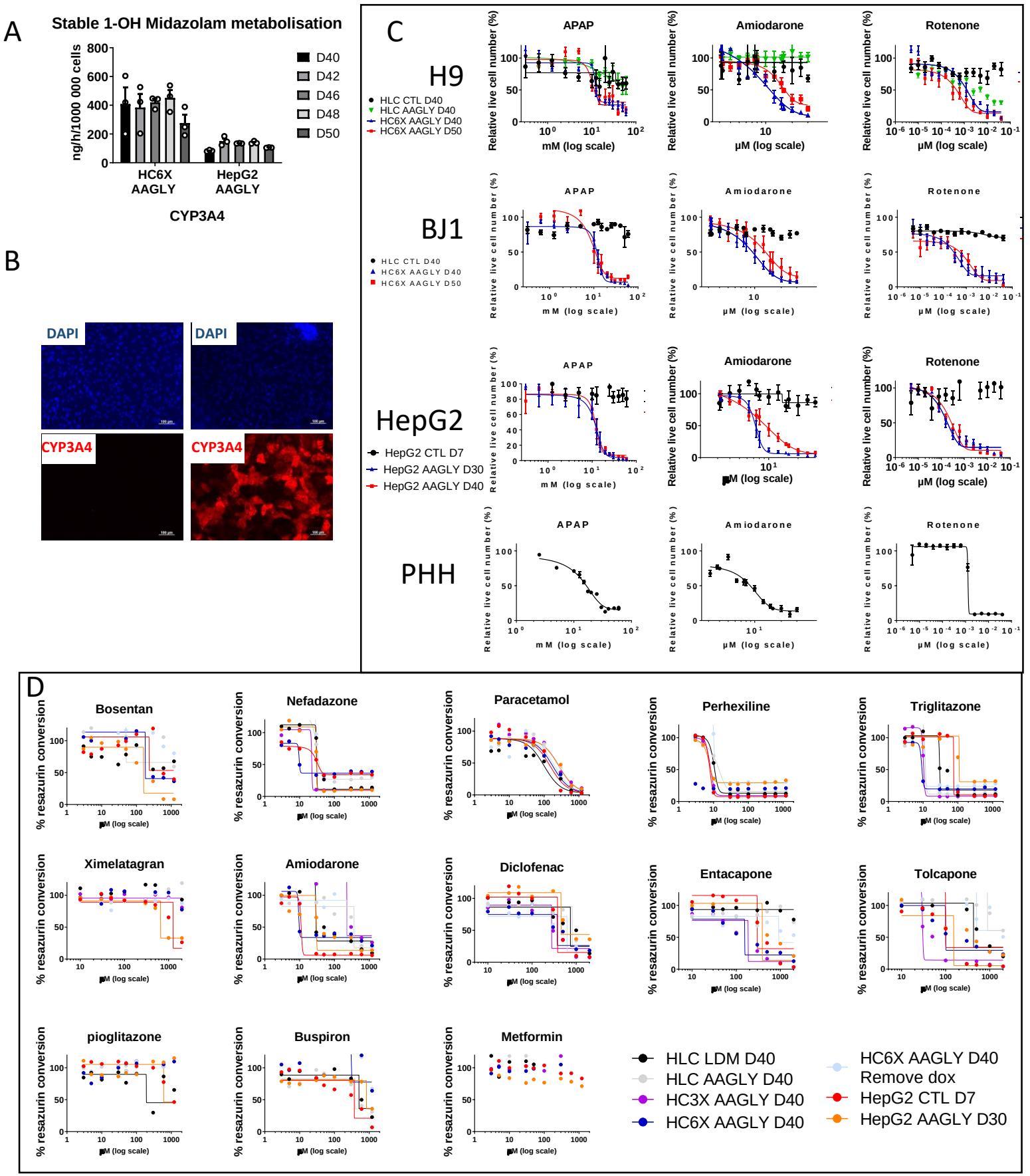

**Supplementary figure 4. Elevating AA levels induces CYP450 function and global maturation of HLC and HC3X.** **(A)** Osmolarity of different media compositions. **(B)** Relative expression of *CYP450* isoforms in HLCs and HC3X-progeny cultured with or without AAGLY-supplementation from D14 until D20 of differentiation. N=3 independent differentiations. Significance is compared to LDM conditions and calculated by two-tailed unpaired Student;s t-test. **(C)** Venn diagram displaying the number of up- and downregulated genes when comparing PHHs to either HLC LDM D20 or HLC AAGLY D40. N=3 independent differentiations Data in all panels represents mean ± standard error of mean (SEM) with P-values indicated when significant. Source data are provided as a Source Data file.




**Supplementary figure 5. AA supplementation drives global differentiation of HepG2.** **(A)** Relative expression of *CYP450* isoforms in HepG2 cultured with or without AAGLY-supplementation for 7 days. N=3 independent maturations. Significance is compared to CTL media by unpaired two-tailed Student's t-test. **(B)** Relative gene expression analysis for *CYP3A4* in HepG2 cultured in medium supplemented with AA3 with or without 2% of either glycine (GLY), serine (SER), alanine (ALA), leucine (LEU), proline (PRO), isoleucine (iLEU), aspartate (ASP), valine (VAL), or a mix of all of the above. N=3 independent maturations. Significance is calculated compared to CTL medium by unpaired two-tailed Student's t-test. $**p < 0.01$, $***p < 0.001$. **(C)** Relative proliferation rates for HepG2, HUH7.5, HHL5 and Hep3B cells grown with or without AAGLY-Supplementation. N=3 independent treatments. Significance is compared to CTL conditions by upaired two-tailed Student's t-test. Data in all panels represents mean ± standard error of mean (SEM) with P-values indicated when significant. Source data are provided as a Source Data file.




**Supplementary figure 6. AAs allow activation of the hepatic transcriptional network** **(A)** Western blot showing expression of hepatic markers and the activity of metabolic signaling pathways in HepG2 D30 differentiated in standard media (CTL), in AA3+ALA, AA3+GLY or AA3+VAL medium, or in AA3+GLY media supplemented with rapamycin (RAPA). N=2. **(B)** Western blot analysis for GCN2 or LKB1 in WT or KO HepG2. N=1**(C)** Western blot showing induction of CYP3A4 in control or KO HepG2 after AAGLY-mediated differentiation at day 30. N=1 **(D)** Staining for the mitochondrial subunit TOMM20 and analysis of cell size and normalized TOMM20 intensity of HepG2 grown with or without AAGLY. Representative pictures (N=mean of 10 random images of 5 wells). Significance is calculated by comparing to CTL by unpaired two tailed Student's t-test. **(E)** Activity measurements of the different mitochondrial complexes (CI, CII, CIII, and CIV) and for the mitochondrial enzyme citrate synthase (CS) in HepG2 grown with or without AAGLY supplementation for 7 days. N=3 independent differentiations. Significance is calculated by comparing to CTL by paired two tailed Student's t-test. $*p < 0.05$ **(F)** Heatmap representing mean expression levels for transgenes, and genes linked to the PGC-1α pathway in PHHs, HLCs and iPGC1A or HC6X cells. N=3 independent differentiations. **(G)** Staining for the mitochondrial subunit TOMM20 and analysis of cell size and normalized TOMM20 intensity for HC6X D20 grown with or without AAGLY supplementation. Representative pictures. N=mean of 10 random images from 5 differentiations. Significance is calculated by comparing to CTL by unpaired two tailed Student's t-test. **(H)** LISA analysis identifying top TFs responsible for the difference between HC6X AAGLY D40 and HC6X AAGLY D40 with removal of doxycycline between D30 and D40. Data in all panels represents mean ± standard error of mean (SEM) with P-values indicated when significant. Source data are provided as a Source Data file.



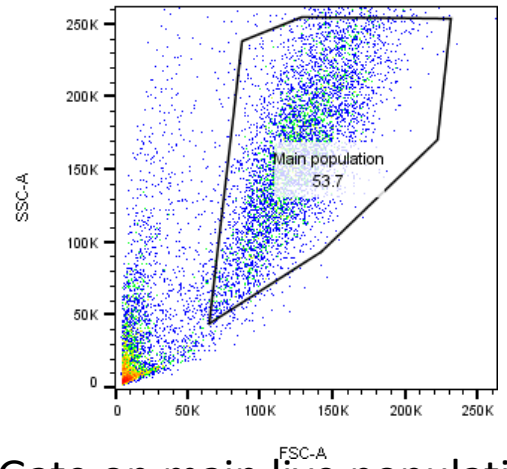

**Supplementary figure 7 AA supplementation allows for identifying DILI compounds.** **(A)** CYP3A4-dependent metabolization of 1-OH midazolam over a 10-day period in HC6X AAGLY D40 and HepG2 AAGLY D30 (N=1 treatment of 3 simultaneous differentiation). **(B)** Representative image of CYP3A4 staining in HC6X LDM D40 and HC6X AAGLY D40 cells for two independent differentiations **(C)** Relative number of living cells measured by Hoechst and Draq7 staining after 24h exposure to different concentrations of acetaminophen (APAP), rotenone and amiodarone. As a positive control PHH 12h (2 donors) grown in WE medium were used. Sigmoidal kill curves were plotted using Graphpad Prism software. N=6 wells per concentration for treated cells. N= 18 wells for control cells. 6 replicate samples of 1 donor of PHHs were analyzed. **(D)** Sigmoidal kill curves representing resazurin conversion upon 3 day exposure to 9 hepatotoxic and 4 non-hepatotoxic compounds. N=6 wells for treated conditions, N= 14 wells for control conditions. Data in all panels represents mean ± standard error of mean (SEM). Source data are provided as a Source Data file.

| Drug | Reference | Cell model | Exposure time | Measurement | IC50 (µM) |
|---|---|---|---|---|---|
| APAP (Boon et al: 10 000 µM) | [10] | Spheroid of immortalized PHHs | 24 h | ATP | 40 000 |
| | [3] | Spheroid of PHHs | 48h | ATP | 10 000 |
| | [11] | 3D Bioreactor with primary rat hepatocytes | 24h | Live Death | 14 290 |
| | [12] | Co-culture of endothelial cells and mouse hepatocytes | 36h | Cell count | 7000 |
| | [13] | Primary rat hepatocytes | 24h | WST-1 assay | 14 000 |
| | [14] | Rat liverbeads (Alginate beads of rat hepatocytes) | 24h | LDH | 10 240 |
| | [15] | PHH | 24h | ALT release | ± 10 000 |
| | [16] | Cryopreserved PHH | 48h | ATP | > 25 000 |
| | [17] | Rat hepatocytes | 24h | MTT | 7 600 |
| | | Human cryopreserved hepatocytes | 24h | | 28 200 |
| | [4] | Co-culture of PHHs and fibroblasts | 24h | ATP | 5 500 |
| Amiodarone (Boon et al. 5-10 µM) | [10] | Spheroid of immortalized PHHs | 24 h | ATP | 260 |
| | [3] | Spheroid of PHHs | 48h | ATP | >100 |
| | [4] | Co-culture of PHHs and fibroblasts | 24h | ATP | 33 |

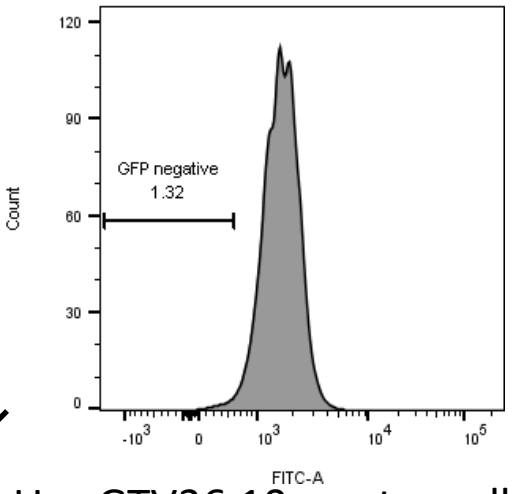
**Supplementary table 2. IC50 values for APAP and amiodarone exposure to PHH systems in published studies.**

| Reference | Cell model | Basal media | Total AA concentration |
|---|---|---|---|
| [1] | Primary hepatocytes | Williams' Medium E | 0.782 g/l |
| [2] | Primary hepatocytes | William's E medium | 0.782 g/l |
| [3] | Primary hepatocytes | William's E medium with 2 mM of glutamine | 1.075 g/l |
| [4] | Primary hepatocytes | William's E medium with 2 mM of glutamine | 1.075 g/l |
| [5] | PSC differentiation | CMRL/Hepatozyme (Invitrogen) | 0.944 g/l |
| [6] | PSC differentiation | Hepatocyte Basal Medium (Lonza CC-3199) | 0.782 g/l |
| [7] | PSC differentiation | Williams' E medium | 0.782 g/l |
| [8] | PSC differentiation | DMEM/F12 with 1 mmol/L nonessential amino acids | 1.189 g/l |
| [9] | PSC differentiation | RPMI B27 | 0.86808 g/l |
| Boon et al | PSC differentiation Cell line maturation | AA3-medium | 3.696 g/l |
| Boon et al | PSC differentiation Cell line maturation | AA3-glycine medium | 23.696 g/l |

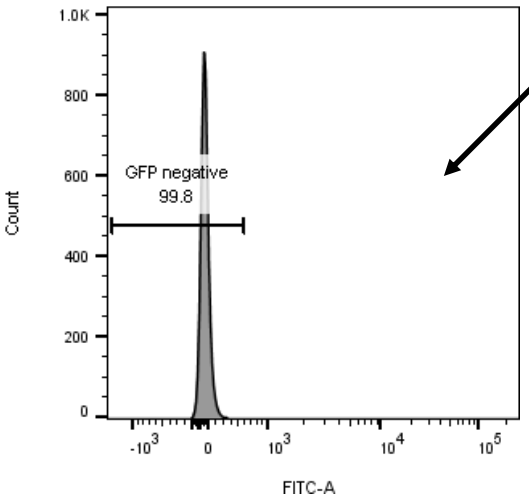
**Supplementary table 3 Comparison of AA concentrations in PHH culture and HLC differentiation.** Comparison of the total amount of amino acids present in hepatic differentiation and maintenance systems.




Gate on main live population




Use GTV36.10 master cell line to define GFP positive and negative population




Analyze cell lines after RMCE recombination

**Supplementary figure 8. Flow cytometry gating strategy for GFP signal utilized in supplementary Figure 2C.** This representative schema describes the gating for evaluating RMCE exchange efficiency for the generation of new RMCE PSC lines. Exchange of the master RMCE cassette was evaluated by loss of GFP signal. In order to evaluate % of GFP positivity, we first gated on the main population in a FSC-A/ SSC-A plot as shown in A. During this step we excluded the FSC-A low/ SSC-low population. The same gating was used for all samples. Next, we separated negative and positive cells based on signal from the GFP positive RMCE master cell line GTV36.10 as as shown in B. We then analyzed the loss of GFP in exchanged cell lines as shown in C.

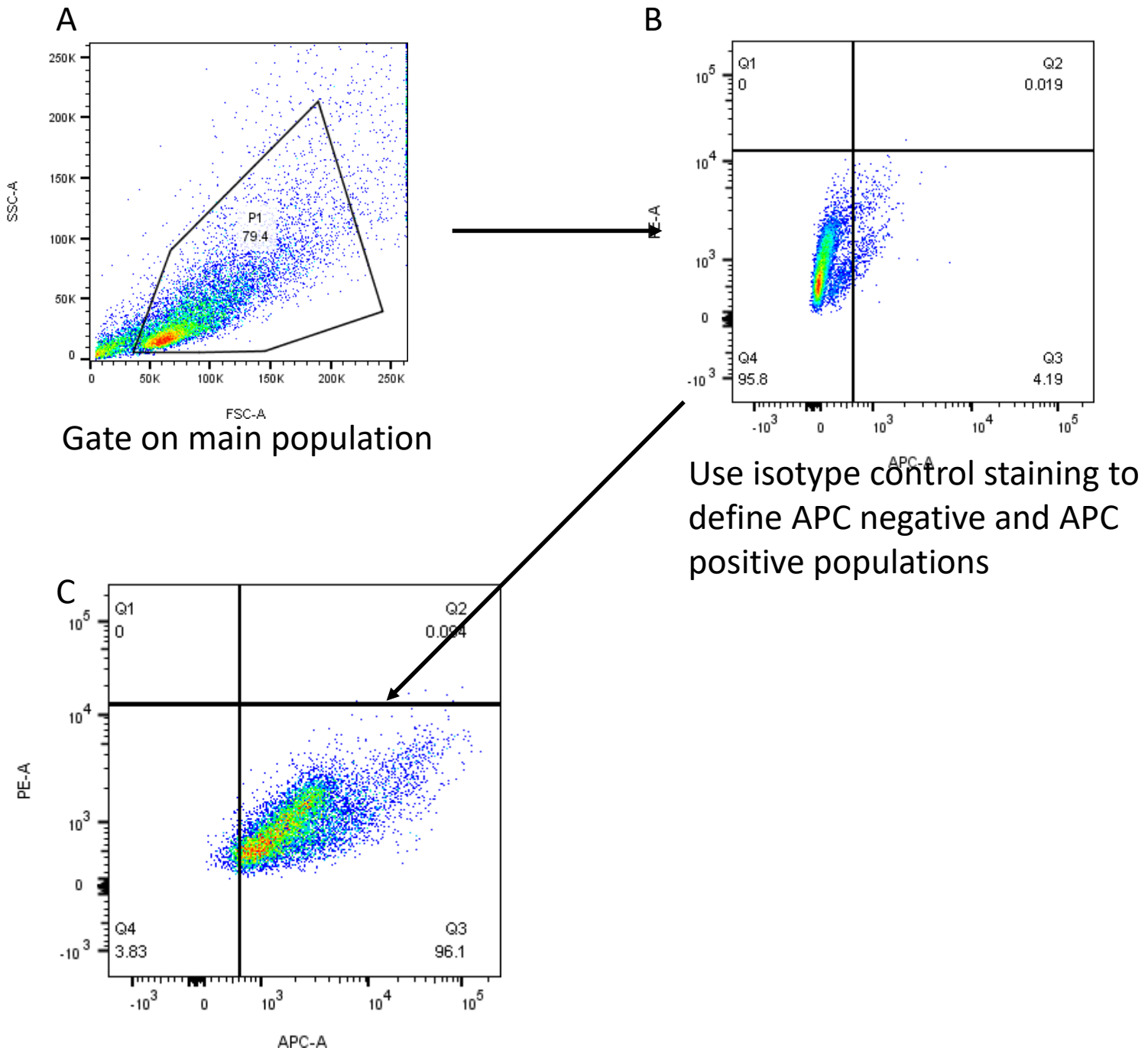
| Liver Differentiation Media (LDM) | Catalog number | Supplier | Volume for 500 ml (ml) |
|---|---|---|---|
| DMEM LG | 31885 | Invitrogen | 285 |
| MCDB pH=7.2 | M6770 | Sigma | 200 |
| Pen/Strep | 15140 | Invitrogen | 5 |
| L-Ascorbic Acid | A8960 | Sigma | 5 |
| ITS | 4140-045 | Invitrogen | 1.25 |
| LA-BSA | L9530 | Sigma | 1.25 |
| b-Mercapto | 31350 | Invitrogen | 0.5 |
| Dexamethasone | D-2915 | Sigma | 2 |

| HepG2,Hep3B and HHL5 | Catalog number | Supplier | Volume for 500 ml (ml) |
|---|---|---|---|
| DMEM LG | 31885 | Invitrogen | 445 |
| FBS | F7524 | Sigma | 50 |
| Pen/Strep | 15140 | Invitrogen | 5 |

| Huh7.5 | Catalog number | Supplier | Volume for 500 ml (ml) |
|---|---|---|---|
| DMEM HG | 41965 | Invitrogen | 440 |
| FBS | F7524 | Sigma | 50 |
| Pen/Strep | 15140 | Invitrogen | 5 |
| NEAA | 11140 | Invitrogen | 5 |

| HEK293T | Catalog number | Supplier | Volume for 500 ml (ml) |
|---|---|---|---|
| DMEM HG | 41965 | Invitrogen | 429 |
| FBS | F7524 | Sigma | 50 |
| Pen/Strep | 15140 | Invitrogen | 5 |
| L-Glutamine | 25030 | Invitrogen | 10 |
| Sodium Pyruvate | 11360 | Invitrogen | 6 |

**Supplementary table 4. Media composition.** Media compositions of all basal media used.




**Supplementary figure 9. Flow cytometry gating strategy for AAT staining utilized in supplementary Figure 1B.** This representative schema describes the gating for PSC-derived HLC cells stained with an antibody for AAT. Cells were fixed, permeabilized and stained according to the description in the material and methods. In order to evaluate % of AAT positivity, we first gated on the main population in a FSC-A/ SSC-A plot as shown in A. During this step we excluded the FSC-A low/ SSC-low population. The same gating was used for all samples. Next, we separated negative and positive cells based on an isotype staining as shown in B. We then analyzed the AAT-stained samples as shown in C.

| Name | Company | CAT-NR (LOT-NR) | Dilution |
|---|---|---|---|
| Rabbit anti-Oct4 (H-134) | Santa Cruz | sc-9081 | 1:100 |
| Mouse anti-HNF4α (K9218) | Abcam | ab41898 (72) | 1/200 |
| Rabbit anti-SLC10A1=NTCP | Sigma | HPA042727 (C106087) | 1/500 |
| Polyclonal Rabbit Anti-Human Alpha-1-Antitrypsin (Multipurpose) | DAKO | A0012 (00092029) | |
| Anti-Human CYP3A4 | Tebu-Bio (Cypex limited (Dundee, UK)) | PAP 011 (150616) | 1/100 |
| Rabbit anti-PEPCK (H-300) | Santa Cruz | sc-32879 (F2008) | 1/1000 |
| Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody. Alexa Fluor 488 | Molecular probes | A-11029 | 1/500 |
| Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody. Alexa Fluor 555 | Molecular probes | A21429 | 1/500 |
| Rabbit IgG | BD Pharmingen | 550875 (6175659) | Used as control in same concentration as primary antibody |
| Mouse BALB/c IgG1. κ | BD Pharmingen | 550878 | Used as control in same concentration as primary antibody |
| p70 S6 Kinase Antibody (49D7) | Cell signaling technology. | 2708 | 1/1000 |
| Phospho-p70 S6 Kinase (Thr389) Antibody (108D2) | Cell signaling technology. | 9234 (12) | 1/1000 |
| S6 Ribosomal Protein (5G10) Rabbit mAb | Cell signaling technology. | 2217 (7) | 1/1000 |
| Phospho-S6 Ribosomal Protein (Ser235/236) Antibody (D57.2.2E) | Cell signaling technology. | 4858 (16) | 1/1000 |
| Anti-rabbit IgG. HRP-linked Antibody | Cell signaling technology. | 7074 | 1/10000 |
| Anti-TOMM20 antibody | Abcam | ab56783 | 1/500 |
| Anti-B actin (D6A8) | Cell Signaling | 8475 | 1/1000 |
| Anti GCN2 (E9H60) | Cell Signaling | 40457S | 1/1000 |
| Anti LKb1 (27D10) | Cell Signaling | 3050S | 1/1000 |
| ANTI P- eIF2alpha (ser51) | Cell Signaling | 9721 (21) | 1/500 |
| ANTI- eIF2alpha (D7D3) | Cell Signaling | 5324 (6) | 1/1000 |
| Anti ATF4 (D4B8) | Cell Signaling | 11815S | 1/1000 |
| Anti P-AMPK (T172)(40H9) | Cell Signaling | 2535 (21) | 1/1000 |
| Anti-AMPK (D5A2) | Cell Signaling | 5831 (6) | 1/1000 |
| Anti-Hamartin (TSC1)(5C8A12) | Thermo Fisher | 37-0400 (QG21065) | 1/2000 |

**Supplementary table 5. Antibodies.** Table showing all relevant antibodies used in this study

| Primer name | Sequence | Primer name | Sequence |
|---|---|---|---|
| RPL19 | ATT GGT CTC ATT GGG GTC TAA C AGT ATG CTC AGG CTT CAG AAG A | HNF4A | ACTACGGTGCCTCGAGCTGT GGCACTGGTTCCTCTTGTCT |
| AAT | AGGGCCTGAAGCTAGTGGAT TCCTCGGTGTCCTTGACTTC | CEBPa | AAAGGGGTGGAAACATAGGG GGAGAGGCGTGGAACTAGAG |
| NTCP | ATCGTCCTCAAATCCAAACG CCACATTGATGGCAGAGAGA | HHEX | AGGAGAACCCTCAAAGCA TCTGAACATGCCAATGCC |
| ALB | ATGCTGAGGCAAAGGATGTC AGCAGCAGCACGACAGAGTA | GATA4 | TCCAAACCAGAAAACGGAAG CTGTGCCCGTAGTGAGATGA |
| OCT4 | GATGGCGTACTGTGGGCCC TGGGACTCCTCCGGGTTTTG | CYP2A6 | GTTGTACATCTGCTCTGTGTTC GTGGCCTTGCTGGTCTG |
| NANOG | CCTGTGATTTGTGGGCCTG GACAGTCTCCGTGTGAGGCAT | CYP1A2 | CAGCTCTGGGTCATGGTTG CCTCCTTCTTGCCCTTCAC |
| SOX17 | CGCTTTCATGGTGTGGGCTAAGGACG TAGTTGGGGTGGTCCTGCATGTGCTG | CYP2E1 | GCACACAACAAAAGAAACAACTC AGCCAGAACACTTCCTGAATG |
| CXCR4 | CACCGCATCTGGAGAACCA GCCCATTTCCTCGGTGTAGTT | PGC1A | CCTTGCAGCACAAGAAAACA TGCTTCGTCGTCAAAAACAG |
| HKII | CTTCTTCACGGAGCTCAACC AGCCCTTTCTCCATCTCCTT | SIRT1 | CATAGACACGCTGGAACAGG CAAATCAGGCAAGATGCTGT |
| PKM2 | GAGGCCTCCTTCAAGTGC CCAGACTTGGTGAGGACGAT | KAT2A | CTGGAAAAGTTCCGAGTGGA GCCCCATAGATCTCCTCCTC |
| PKL | ACTAAGCCGTGATCCCACTG CGGTACCGAGACAGAAGCTG | NRF1 | TGTGGGACAGCAAGCTATTG GCAGACTCCAGGTCTTCCAG |
| G6PC | GTGTCCGTGATCGCAGACC GACGAGGTTGAGCCAGTCTC | NRF2 | GACGGGATATTCTCTTCTGTGC ACTCTTTCCGTCGCTGA |
| FBP1 | ACGTCCAGCTTCTTAACTTGA AGCAGTCAAAGCCATCTCTTC | TFAM | GGGAAGGTCTGGAGCAGAG TGGACAACTTGCCAAGACAG |
| PEPCK | AAGAAGTGCTTTGCTCTCAG CCTTAAATGACCTTGTGCGT | COX1 | CATCAATGTCTCCATACAATTCCT CTGCAGCCCTTCAATGAGT |
| HNF1A | ACACCTCAACAAGGGCACTC TGGTAGCTCATCACCTGTGG | CYTC | TGTGCCAGCGACTAAAAAGA AGATTTGGCCCAGTCTTGTG |
| FOXA3 | ATTCTCTCTGGCATGGGTTG AAATTCCCCACACCCTAACC | ATP5A1 | TCCAGAAATGCTTTGGGTTC GCTCCAAGAATACGCTCTTCA |
| FOXA_exogenous | TCAGAACAGACACCAGCACC GGCCCTGGTAGTAGACTCCA | 5'JA RMCE cassette | CACTTTGAGCTCTACTGGCTTC CATGTTAGAAGACTTCCTCTGC |
| PROX1 | TCACCTTATTCGGGAAGTGC GGAGCTGGGATAACGGGTA | 5'JA RMCE donor | TTCACTGCATTCTAGTTGTGG AAGGCAGCCTGGTAGACA |
| HNF6 | AAATCACCATTTCCCAGCAG ACTCCTCCTTCTTGCGTTCA | | |
| CYP3A4 | TTCCTCCCTGAAAGATTCAGC GTTGAAGAAGTCCTCCTAAGCT | | |

**Supplementary table 6. Primer sequences.** Table showing primer sequences used for qRT-PCR analysis

# Supplementary references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