Supplementary Information

Characterization of TK-NOG mice. The structure of the HSVtk transgenic construct, which contains the mouse albumin enhancer/promoter (mAlb En/Pro), the chimeric intron, *HSVtk* cDNA, and the 3'-UTR of the human growth hormone gene with a polyadenylation signal (hGH pA) is shown in Fig. S1A. There was a single site of transgene integration, and HSVtk mRNA was selectively expressed in the liver of TK-NOG mice (Fig. S1B-C). Administration of ganciclovir (GCV; 0.5 to 12.5 mg/kg I.P) on days -7 and -5 prior to transplantation induced significant liver damage in TK-NOG mice, while administration of 100-fold higher GCV doses to control NOG mice did not cause any liver damage (Fig. S1D-E). No lesions were observed after GCV treatment in any tissues other than liver obtained from TK-NOG mice; except fewer elongating spermatids and abnormally shaped sperm heads were observed in the testis (data not shown).

After GCV conditioning and transplantation of 10^6 human liver cells via intra-splenic injection, human albumin (hAlb) was detected in the plasma of all transplant recipients at 4 weeks, and the amount increased steadily thereafter to a maximum of 5.9 mg/mL (Fig. S2A). After correcting for methodological differences, the hAlb-concentration in humanized TK-NOG mice was ~70% of that in human serum, which indicates a very robust human hepatocyte reconstitution.

We also carefully assessed the significance of the hAlb measurements. We examined whether the measured plasma hAlb level was linearly correlated with the extent of human liver replacement. The replacement index (RI) is the ratio of the area occupied by h-CK8/18-positive liver cells relative to the total area in a stained liver section. We found that the RI was highly correlated with the plasma albumin concentration (Fig. S2B). The RI for three liver lobules obtained from mouse TK009-18 averaged 91.4%. The plasma hAlb level (7.5 mg/mL) for the mouse is indicated as a blue asterisk located on the regression line.

Transgenic mice harboring the HSVtk gene are also known to have a lower breeding efficiency that is caused by male sterility [1]. Consistent with this, we found that male TK-NOG mice were also sterile. Nevertheless, breeding of these mice is easily performed; female TK-NOG mice are mated with male NOG mice, and the transgenic offspring are selected by genotyping. With this method, we have established a breeding colony with >60 breeding pairs with an average litter size of ~5.5 (1,636 mice/299 deliveries), and ~50% transmit the transgene to the next generation: 797 out of 1,636 mice were transgenic.

Optimization of the transplantation protocol. There are several methods by which the extent of humanization can be increased in TK-NOG liver. After our pilot human liver transplantation studies were completed, we identified two variables that improved the extent of human liver reconstitution in TK-NOG mice: 1) the age of the mice at the time of transplantation, and 2) the dose and timing of GCV administration. The level of transgene expression and serum ALT activity in TK-NOG mice at 4 weeks of age was much lower than at 8 weeks (Fig. S3A). Therefore, TK-NOG mice were transplanted at 8-10 weeks of age in the optimized protocol. We also found the optimal level of hepatic injury, which facilitated human liver cell engraftment, was when 200 to 400 IU/L of serum ALT activity was detected at 1 week after the 1st GCV dose (Fig. S3B).

Supplementary Figures

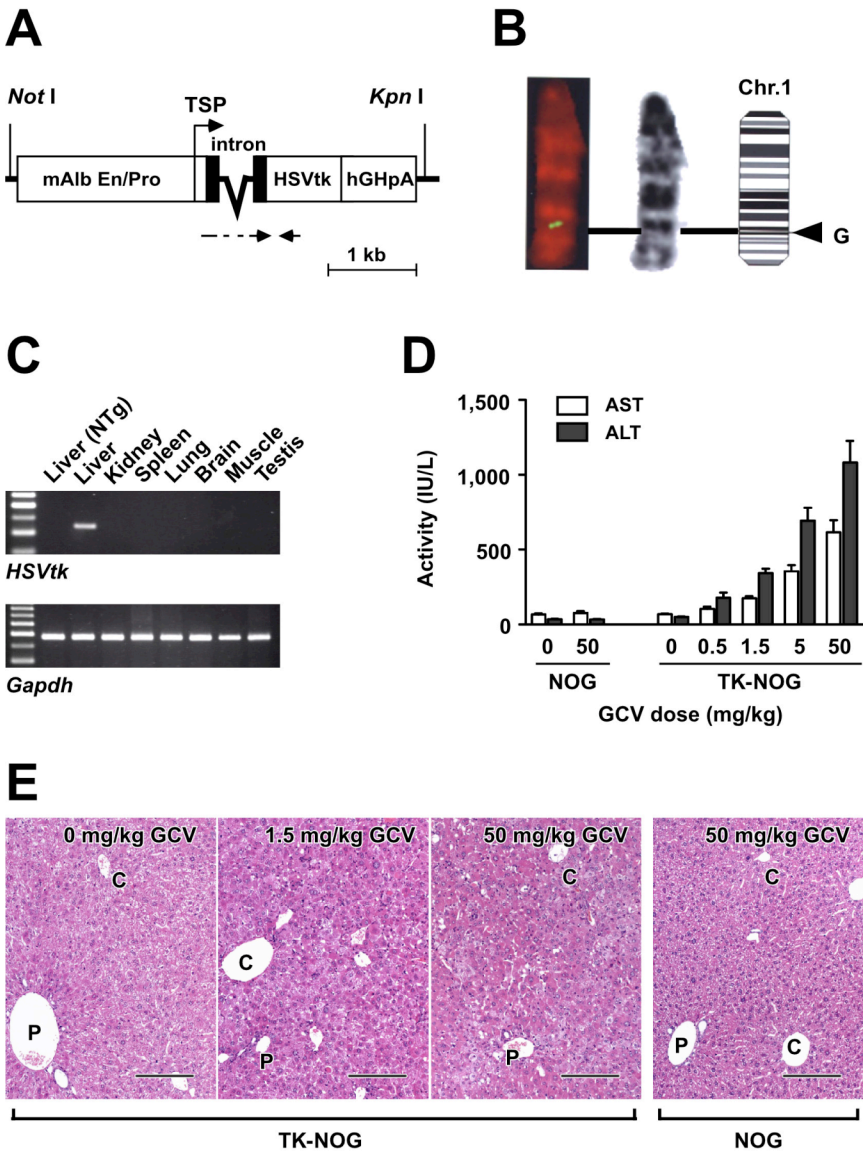

Figure S1

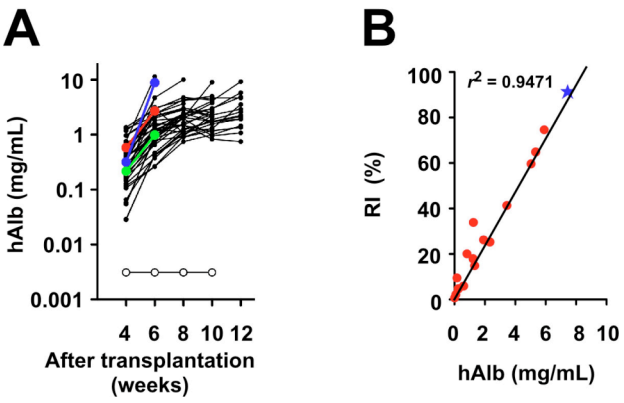

Figure S2

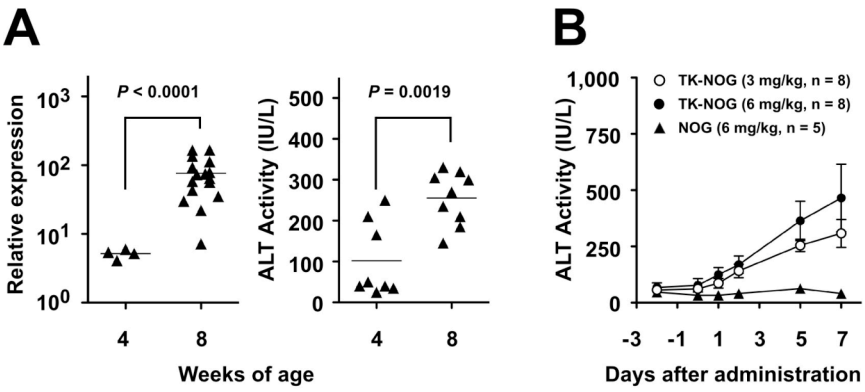

Figure S3

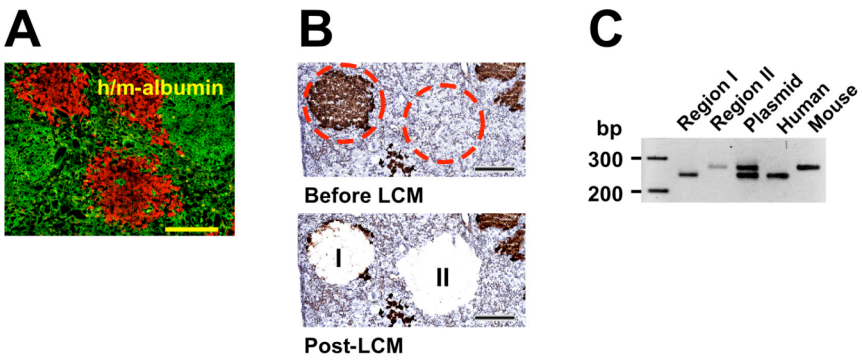

Figure S4

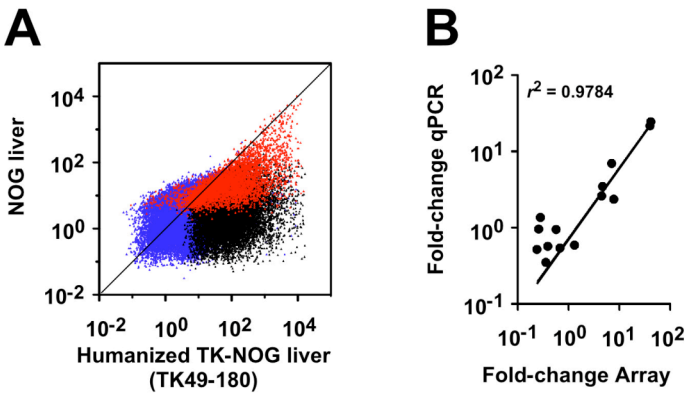

Figure S5

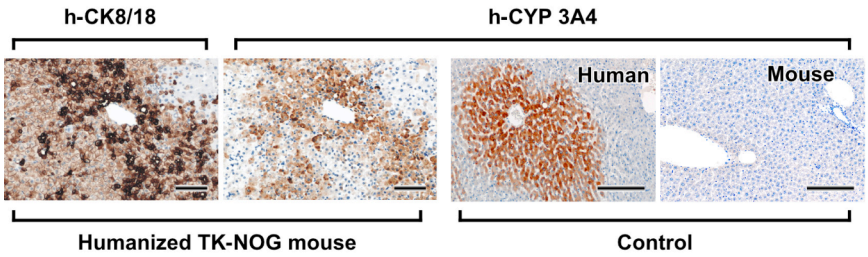

Figure S6

Supplementary Figure Legends

Figure S1. Characterization of TK-NOG mice. (**A**) The HSVtk transgenic construct contains the mouse albumin enhancer/promoter (mAlb En/Pro), the chimeric intron, *HSVtk* cDNA, and the 3'-UTR of the human growth hormone gene with a polyadenylation signal (hGH pA). The arrowheads depict the positions and directions of the primers used for detecting the transgene-specific transcript, with the tip of each arrow representing the 3' end of the oligonucleotide. (**B**) The site of integration of the HSVtk expression unit was determined by fluorescence in situ hybridization analysis, which indicated that there was a single site of transgene insertion located at the chromosome 1G loci. (**C**) RT-PCR analyses of HSVtk transgene expression were performed using a primer that recognizes the sequence of the spliced chimeric intron. The following tissues were analyzed: non-transgenic NOG mouse liver (NTg); and liver, kidney, spleen, lung, brain, skeletal muscle, and testis obtained from transgenic TK-NOG mice. Glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) amplicons were used as an internal control. (**D**) The aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were measured in sera obtained from NOG mice (Control) and TK-NOG mice (TK-NOG) 7 days after they were treated with the indicated dose of GCV. GCV administration to TK-NOG mice caused hepatocellular injury that was dose dependent. (**E**) The microscopic appearance of livers in ganciclovir-administered TK-NOG mice. TK-NOG mouse were treated twice on alternate days with phosphate-buffered saline (0 mg), 1.5, or 50 mg/kg GCV; and control NOG mice were treated with 50 mg/kg GCV. Seven days after the initial treatment, livers obtained from the GCV-treated TK-NOG mice showed hepatocyte megalocytosis, moderate-to-severe cytoplasmic vacuolization, and focal cell death. In addition, there were no lesions in non-transgenic NOG mice treated with 50 mg/kg GCV. Scale bars, 200 μm; P, portal tract; C, central vein.

Figure S2. (**A**) The amount of hAlb in the sera of humanized TK-NOG mice was measured using an enzyme-linked immunosorbent assay at the indicated times after human liver cell transplantation. The green, red, and blue lines indicate the assays performed on mice #12-14, #6-6-10, and #2-4-7, respectively. (**B**) The correlation between the measured hAlb concentration in the plasma of humanized TK-NOG mice and the replacement index, which was assessed by morphometric analysis of liver sections stained with h-CK8/18 antibody. Fifteen humanized TK-NOG mice were analyzed for these measurements. The blue asterisk indicates the data obtained from humanized mouse TK009-18.

Figure S3. Optimization of the transplantation protocol. (**A**) Hepatic HSVtk transgene expression (left) and serum ALT activity (right) after I.P. administration of 6.25 to 12.5 mg/kg GCV was measured in 4 week and 8 week old male TK-NOG mice. Transgene expression and serum ALT activity after GCV treatment (which is a an indicator of hepatic injury) were significantly higher in mice that were 8 weeks old. The *p*-values represent an unpaired *t* test with Welch's correction. (**B**) The time course and dose dependency of GCV induced hepatic injury in TK-NOG mice. The indicated dose of GCV was administered to 8-week-od TK-NOG mice (n=5-8 mice per group), and the serum ALT activity was measured on alternate days.

Figure S4. No evidence for mouse-human cell fusion in the humanized TK-NOG liver. (**A**) Immunofluorescent double staining of a liver section obtained from a humanized TK-NOG mouse for mouse albumin (green) and human albumin (red). Scale bar, 200 μm. (**B**) Microdissection of clonally expanding human hepatocyte colonies from a h-CK8/18 stained frozen section before (top) and after microdissection (bottom) of the regions indicated by the broken lines. The human (region I) and nonhuman (region II) target regions are indicated. Scale bars, 200 μm. (**C**) The captured tissue fragments ware analyzed by PCR for discrimination between human and mouse hepatocytes. The region I tissue fragment containing h-CK8/18 positive liver cells creates a 245-bp amplicon that corresponds with the human β-actin gene, which was never produced from the region II fragment.

Figure S5. (A) Only 8% (4,484) of the 54,613 probes analyzed on the array, cross-hybridize with mRNAs in liver obtained from control NOG mice. In this image, the red closed triangles are the probes with 'present' detection calls for the control NOG mouse liver sample, which indicates the probe sets that cross-react with mouse genes. The blue and black closed triangles indicate 'absent or marginal' and 'present' detection calls in the fully 'humanized liver' of TK-NOG mice, respectively. (**B**) The gene expression levels measured by

microarray and qPCR analysis were highly correlated with each other (r^2 = 0.9784).

Figure S6. A section of humanized liver obtained from a TK-NOG mouse was stained with anti-human CK8/18 (h-CK8/18) or anti-human CYP3A4 (h-CYP3A4) antibodies. Scale bars, 100 μm. As a specificity control, sections of mouse and human liver tissues were stained with the anti-human CYP3A4 antibody. This antibody exhibited specificity for binding to the pericentral region of human liver tissue.

Supplementary Table 1

TaqMan probe information

Gene name	Gene description	TaqMan Assay No.	Specificity
18S rRNA	Eukaryotic 18S rRNA	Hs99999901_s1	Mouse/Human
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	Hs99999905_m1	Human
ALB	Albumin	Hs99999922_s1	Human
CYP1A1	Cytochrome P450, family 1, subfamily A, polypeptide 1	Hs00153120_m1	Human
CYP1A2	Cytochrome P450, family 1, subfamily A, polypeptide 2	Hs00167927_m1	Human
CYP2A6	Cytochrome P450, family 2, subfamily A, polypeptide 6	Hs00868409_s1	Human
CYP2B6	Cytochrome P450, family 2, subfamily B, polypeptide 6	Hs03044634_m1	Human
CYP2C8	Cytochrome P450, family 2, subfamily C, polypeptide 8	Hs00258314_m1	Human
CYP2C9	Cytochrome P450, family 2, subfamily C, polypeptide 9	Hs00426397_m1	Human
CYP2C18	Cytochrome P450, family 2, subfamily C, polypeptide 18	Hs00426400_m1	Human
CYP2C19	Cytochrome P450, family 2, subfamily C, polypeptide 19	Hs00426380_m1	Human
CYP2D6	Cytochrome P450, family 2, subfamily D, polypeptide 6	Hs00164385_m1	Human
CYP2E1	Cytochrome P450, family 2, subfamily E, polypeptide 1	Hs00559368_m1	Human
CYP3A4	Cytochrome P450, family 3, subfamily A, polypeptide 4	Hs00430021_m1	Human
CYP3A5	Cytochrome P450, family 3, subfamily A, polypeptide 5	Hs00241417_m1	Human
UGT1A1	UDP glucuronosyltransferase 1 family, polypeptide A1	Hs02511055_s1	Human
UGT2B15	UDP glucuronosyltransferase 2 family, polypeptide B15	Hs00870076_s1	Human
SLC22A1	Solute carrier family 22 (organic cation transporter), member 1	Hs00427552_m1	Human
SLC22A6	Solute carrier family 22 (organic anion transporter), member 6	Hs00537914_m1	Human
SLC22A7	Solute carrier family 22 (organic anion transporter), member 7	Hs00198527_m1	Human
SLC22A8	Solute carrier family 22 (organic anion transporter), member 8	Hs01056647_m1	Human
SLC22A9	Solute carrier family 22 (organic anion transporter), member 9	Hs00971064_m1	Human
ABCB1	ATP-binding cassette, subfamily B (MDR/TAP), member 1	Hs00184500_m1	Human
ABCB11	ATP-binding cassette, subfamily B (MDR/TAP), member 11	Hs00184824_m1	Human
ABCC2	ATP-binding cassette, subfamily C (CFTR/MRP), member 2	Hs00166123_m1	Human
ABCG2	ATP-binding cassette, subfamily G (WHITE), member 2	Hs01053790_m1	Human
NR1H4	Nuclear receptor subfamily 1, group H, member 4	Hs00231968_m1	Human
NR1I2	Nuclear receptor subfamily 1, group I, member 2	Hs00243666_m1	Human
NR1I3	Nuclear receptor subfamily 1, group I, member 3	Hs00901571_m1	Human

Supplementary Materials and Methods

Transgenic mice. The herpes simplex virus type 1 thymidine kinase (*UL23* or HSVtk) gene expression unit is diagramed in Fig. S1A. The 42 nucleotides of the polylinker (GATCCAAGCTTATGCAGTCGACCCGGGCATGCGAATTCTCGA) were introduced into pBlueScript II (pBSII; Promega, Madison, WI, USA) at the *Bam* HI-*Xho* I site (pBSII/linker). The HSVtk gene was PCR amplified with the following primers (annealing temperature 62˚C): HTKF, 5'-GCTAGCATGGCTTCGTACCCCTGC-3'; HTKR, 5'-GTCGACTCAGTTAGCCTCCCCCATCTC-3'. It was then cloned into a pCI plasmid (Promega) at the *Nhe* I-*Sal* I site (pCI-TK). The human growth hormone (hGH) 3' flanking region was PCR amplified with following PCR primers (annealing temperature 60˚C): hGHF, 5'-GCTCTACTGCTTCAGGAAGGACAT-3'; hGHR, 5'-GAATTCAACAGGCATCTACTGA-3'. It was cloned into the pBSII/linker plasmid at the *Sma* I- *Eco*R I site (pBSII/linker/hGH). A 2,345-bp *Not* I- *Bam*H I fragment of the mouse albumin enhancer/promoter from plasmid p2335A-1 [2] and a 1,456-bp *Hin*d III-*Sal* I fragment of the chimeric intron/HSVtk gene from pCI-TK were cloned into corresponding cloning sites of the pBSII/linker/hGH plasmid (pmAlbEPintUL23GH; GenBank accession no. AB453181). A vector-free 4.4-kb HSVtk expression fragment was prepared by cleavage of the pmAlbEPintUL23GH plasmid DNA at unique *Not* I and *Kpn* I sites. The fusion construct was microinjected into fertilized NOD/Shi strain mouse eggs using standard methods. Transgenic offspring were identified by PCR (annealing temperature 63˚C) using the HTKF1 forward primer, 5'-CACGTCTTTATCCTGGATTACG-3' and hGHR1 reverse primer, 5'-CACTGGAGTGGCAACTTCCA-3'. Genomic DNA extracted from tail tissue was amplified in a 20-μl reaction mixture using the following conditions: 2 min at 94˚C, then 30 cycles of 30 s at 94˚C, 30 s at 63˚C, and 30 s at 72˚C, and finally 3 min at 72˚C. Transgene DNA showed an amplified product band of 236 bp on an agarose gel. Transgenic females were mated with NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Sug}/ShiJic (NOG) males to give the *scid* and IL2Rg^{null} mutations. The *scid* and IL2Rg^{null} mutations were genotyped by a PCR method described previously [3,4]. The line of mice used here have been assigned the following genetic designation: NOG-*Tg*(*Alb-UL23*)*7-2*/ShiJic (formally, NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Sug} *Tg*(*Alb-UL23*)*7-2*/ShiJic, abridged name: **TK-NOG**). This study was performed in accordance with institutional guidelines and was approved by the Animal Experimentation Committee of the Central Institute for Experimental Animals.

Fluorescence in situ hybridization analysis. Chromosomal location of the transgene was determined by fluorescence *in situ* hybridization (FISH) [5]. Nine metaphases derived from mitogen-activated splenocytes obtained from TK-NOG mice were analyzed with the biotin-16-dUTP-labeled pCI-TK plasmid. The biotin-labeled DNA was visualized using avidin-FITC (Vector Laboratories, Burlingame, CA, USA) and then counterstained with propidium iodide (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). Observations were carried out with a Leica Q550 Cytogenetic Workstation (Leica Microsystems Imaging Solutions, Cambridge, UK) using Leica filter sets A4, L5, and N3, and chromosomes with fluorescent signals were identified according to the G banding standards.

Detection of HSVtk transcripts by RT-PCR. Total cellular RNA of liver, kidney, spleen, lung, brain, skeletal muscle, and testis was obtained from a 65-day-old TK-NOG mouse using the RNeasy Mini kit (Qiagen K.K., Tokyo, Japan). RT-PCR was performed using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA). The spTKF forward primer 5'-GAGGCACTGGGCAGGTGTCC-3' and HTKR1 reverse primer 5'-GTAAGTCATCGGCTCGGGTAC-3' were used to detect the spliced form of the HSVtk transcript as a 343-bp band (annealing temperature 68˚C). The 479-bp glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) fragment amplified using the G3PDHF forward primer 5'-TCACCATCTTCCAGGAGCGAGA-3' and the G3PDHR reverse primer 5'-GAAGGCCATGCCAGTGAGCTT-3' was used as an internal control (annealing temperature 65˚C).

Induction of liver damage. Mice received intraperitoneal injections of ganciclovir (GCV) sodium (Denosine-IV; Mitsubishi Tanabe Pharma, Osaka, Japan) or vehicle on days -7 and -5 prior to transplantation. GCV was dissolved in physiological saline and filter-sterilized before being administered. All mice were monitored daily. The degree of liver damage was examined by determining biochemical serum values and pathological analysis. One week after GCV treatment, the mice were killed under isoflurane anesthesia following peripheral blood collection from the inferior vena cava for analysis of serum aspartate aminotransferase (AST)

and alanine aminotransferase (ALT). Clinical chemical analysis of serum samples was carried out using a FUJI DRI-CHEM 7000 (Fujifilm, Tokyo, Japan). Data were analyzed using the Prism 5 for Macintosh (GraphPad Software, San Diego, CA, USA). The tissues were fixed with 4% (v/v) phosphate-buffered formalin, and paraffin-embedded sections were stained using hematoxylin and eosin (H&E).

Transplantation of human liver cells. An un-optimized protocol for transplantation of human liver cells into TK-NOG mice was initially used. In this protocol, adult 6–12-week-old TK-NOG recipients were injected intraperitoneally with ganciclovir (GCV; 0.5–5 mg/kg) twice, 5 and 3 days prior to transplantation. An optimized protocol was developed in which adult 8-week-old TK-NOG recipients were injected intraperitoneally with GCV (3 mg/kg) on days -7 and -5 prior to transplantation. For transplantation, 1 x 10^6 cryopreserved human liver cells (Lonza Walkersville, MD, USA) were transplanted by intra-splenic injection as described [6].

Human albumin measurement. Small amounts of blood were collected biweekly from the retro-orbital venous plexus with a plastic capillary. After a 500–135,000 fold-dilution with Tris-buffered saline containing 1% bovine serum albumin and 0.05% Tween 20, human albumin (hAlb) concentration was measured with the Human Albumin ELISA Quantitation Kit (Bethyl Laboratories, Montgomery, TX, USA) according to the manufacturer's protocol. Human albumin was not detected in sera obtained from control un-transplanted non-transgenic mice (n = 6)(<6.25 ng/mL) even with a 500 fold reduced dilution. The threshold concentration in this system was ~3 x 10^3 ng/mL.

Histology and immunohistochemistry. Formalin-fixed livers and kidneys were embedded in paraffin and prepared in 5-μm sections. Some sections were autoclaved for 10 min in target retrieval solution (0.1 M citrate buffer, pH 6.0; 1 mM EDTA, pH 9.0) and then placed at room temperature for 20 min. Monoclonal mouse antihuman Cytokeratin (8/18) (h-CK8/18, clone 5D3; Novocastra Laboratories, Newcastle, UK), monoclonal mouse anti-HLA class I-A, B, C (clone EMR8-5; Hokudo, Sapporo, Japan), polyclonal rabbit antihuman asialoglycoprotein receptor 1 (ASGR1, Sigma-Aldrich), polyclonal goat antihuman albumin (Bethyl Laboratories), polyclonal rabbit anti-Cytochrome P450 3A4 (Abcam Inc., Cambridge, MA), and polyclonal rabbit antihuman glutamine synthase (GS; glutamate-ammonia ligase, Sigma-Aldrich) antibodies were used as primary antibodies. For bright-field immunohistochemistry, the antibodies for mouse, rabbit, and goat Ig were visualized using amino acid polymer/peroxidase complex-labeled antibodies [Histofine Simple Stain Mouse MAX PO (M, R, and G); Nichirei Bioscience, Tokyo, Japan], and diaminobenzidine (DAB; Dojindo Laboratories, Kumamoto, Japan) substrate (0.2 mg/mL 3,3'-diaminobenzidine tetrahydrochloride, 0.05 M Tris–HCl, pH 7.6, and 0.005% H_2O_2). Sections were counterstained with hematoxylin. A periodic acid-Schiff (PAS) staining kit (Muto Pure Chemicals, Tokyo, Japan) was used for visualizing glycogen. The images were captured under an upright microscope Axio Imager (Carl Zeiss, Thornwood, NY, USA) equipped with AxioCam HRm and AxioCam MRc5 CCD cameras (Carl Zeiss). To estimate the replacement index (RI), which is the percentage of donor human liver cells in recipient livers, the ratio of the area occupied by h-CK8/18-positive cells to the entire area examined in immunohistochemical sections of three to five lobes was measured. The livers used to generate frozen sections were embedded in OCT compound (Sakura Finechemicals, Tokyo, Japan), frozen in liquid nitrogen, and sectioned 5–10 μm thick. A biotinylated polyclonal goat antihuman albumin antibody, which was labeled using the FluoReporter Mini-Biotin-XX Protein Labeling Kit (Invitrogen, Carlsbad, CA, USA), Alexa Fluor 594-labeled streptavidin (Invitrogen), and a FITC-labeled polyclonal goat anti-mouse albumin antibody (A90-234F; Bethyl Laboratories) was used for immunofluorescence studies.

Immunoblotting. Diluted serum samples (dilution factor: 50–6,000) were solubilized in SDS sample buffer with 5% β-mercapthoethanol. Proteins were subjected to SDS-PAGE and transferred to Hybond-ECL membranes (GE Healthcare Bio-Sciences, Piscataway, NJ, USA). Membranes were incubated for 90 min with a polyclonal goat antihuman albumin antibody (A80-229A; Bethyl Laboratories), a polyclonal goat antihuman complement C3 antibody (D-19; Santa Cruz Biotechnology), a polyclonal rabbit antihuman transferrin antibody (H-65; Santa Cruz Biotechnology), or a polyclonal goat anti-ceruloplasmin antibody (H-60; Santa Cruz Biotechnology), washed, and incubated with horseradish peroxidase-labeled secondary antibodies against goat IgG (Bethyl Laboratories), or rabbit IgG (GE Healthcare Bio-Sciences) for 60 min. Immunoblots were developed using the ECL Western Detection System (GE Healthcare Bio-Sciences) and Hyperfilm ECL (GE Healthcare Bio-Sciences).

Laser-capture microdissection (LCM). Serial frozen tissue sections (10 μm) were fixed and rehydrated sequentially in decreasing concentrations of ethanol and DNase and RNase-free water followed by immunohistochemical staining with the h-CK8/18 antibody. By microscopic visualization, positive and negative staining areas for h-CK8/18 antibody in the sectioned tissue were mapped for LCM. Microdissection of each area was performed using an AutoPix LCM system (Molecular Devices, San Diego, CA, USA) according to the manufacturer's instructions. Genomic DNA was extracted with TPMK buffer (10 mM Tris–HCl, pH 8.3, 50 mM KCl, 2 mM $MgCl_2$, and 40 μg/mL proteinase K). After overnight incubation at 55°C, proteinase K was inactivated by heating the samples at 95°C for 5 min. Part of the buffer was used as template DNA for genomic PCR to discriminate between human and mouse cells. The human and mouse β-actin genes were amplified by PCR with the following primers: forward primer, 5'-TAGGTACTAACACTGGCTCGTGTGACAA-3'; and reverse primer, 5'-GGTGTTGAAGGTCTCAAACATGATCTGTA-3' (annealing temperature 60˚C). This set of primers amplifies not only the human β-actin gene, but also the mouse β-actin gene (245 bp and 271 bp, respectively). The control plasmid (pBSII-hm/β-*ACT*) was prepared by inserting corresponding sequences obtained from human and mouse DNA into *Sma* I and *Hind*III sites of a pBSII vector, respectively.

Oligonucleotide array hybridization. Global gene expression was analyzed using the HG-U133A Plus 2 GeneChip array (Affymetrix Inc., Santa Clara, CA). Signal intensity for each transcript (background subtracted and adjusted for noise) and detection call (present, absent, or marginal) were determined using Affymetrix Expression Console Software (Affymetrix Inc.). The reconstituted humanized liver in TK-NOG mouse contains not only human liver cells but also mouse liver cells, therefore the signal intensities in the humanized liver had to be normalized by house keeping gene, of which probe set can detect human and mouse gene expression equally. We choose the human 18S rRNA gene, 10098_M_at probe that is consisted of 20 set of 25 oligonucleotide as internal standard, because of following reasons; 1) oligonucleotide sequence of 19 out of 20 set are completely identical with mouse counterpart, 2) and the other set of 25 oligomer has only one nucleotide mismatch at second position of 5' prime end. The MIAME compliant microarray data was deposited in the Center for Information Biology gene EXpression database (CIBEX) at DDBJ (Japan)(CIBEX Accession: CBX102).

Real-time Quantitative Reverse Transcription-PCR (qPCR) for drug metabolizing related gene expression. Total cellular RNA was isolated from the liver using the RNeasy mini kit (Qiagen K.K.). Complementary DNA was synthesized using a High Capacity cDNA Reverse Transcription kit (Applied Biosystems) with a random primer. TaqMan Gene Expression Master Mix and TaqMan Gene Expression Assays (Applied Biosystems) were used for Real-time quantitative PCR reaction, then amplification was carried out using an ABI Prism 7000 Sequence Detection System (Applied Biosystems). The comparative CT method was used to determine the relative ratio of gene expression for each gene corrected using 18S rRNA and referenced to RNA extracted from normal human liver cells (nHeps; Lonza Walkersville). The TaqMan Assay number is listed in Supplementary Table 1.

in vivo biotransformation studies. Debrisoquine (DEB) sulfate (Sigma-Aldrich Chemie GmbH) was dissolved in sterile water and suspended in 0.5% methycellulose (Wako Pure Chemicals), and administered orally by gavage at 2.0 mg/kg. Urine samples were collected between 0-7 hrs after DEB administration. Small volume (40 μL) blood samples were collected from the retro-orbital venous plexus with a plastic capillary at 0.5, 1, 2, 4 and 7 hrs after the oral drug administrations from 6-9 mice per time point. Serum was separated by centrifugation at 12,000*g*, 4°C, for 15 min. The serum concentrations of DEB and its 4-hydroxydebrisoquine (4-OH DEB) metabolite were measured according to the methods by Katoh et al [7] with the LC-10AD HALC System (Shimadzu Corp., Kyoto, Japan) and PE Sciex API4000 tandem mass spectrometer (Applied Biosystems). The detection and quantification of compounds were performed using MS/MS in the multiple reaction monitoring mode. Two mass/charge (*m*/*z*) ion transitions were recorded: *m*/*z* 176 and 134 for DEB, *m*/*z* 192 and 157 for 4-OH DEB, *m*/*z* 281 and 193 for internal standard for DEB. Pharmacokinetics parameters for DEB and 4-OH DEB were estimated from the serum concentration-time data using a non-compartmental approach with the software package WinNonlin Professional version 5.2 (Pharsight Corporation, Mountain View, CA). The area under the serum concentration versus time curves from 0 to 7 h (AUC_{0-7h}) was determined with a combination of linear and logarithmic trapezoidal methods.

***Statistical Analyses*.** Statistical analyses were performed with the Prism 5 software (GraphPad Software, CA, USA) and SAS preclinical package software ver. 5.0 (SAS Institute, Tokyo, Japan).

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