METHODS

LV production

pSIN-CSGWdlNotl-based LV were produced from the parent vector pSIN-CSGWdlNotl-SFFV-EGFP that contained the *EGFP* transgene under the control of the spleen focus forming virus (SFFV) promoter. Initially, the SFFV promoter was replaced with a construct that contained two copies of a human α1-microglobulin/bikunin enhancer and the promoter from the human thyroid hormone-binding globulin (TBG) gene (*23*). Subsequently, the pig *Fah* cDNA was cloned into the TBG vector in place of EGFP to produce the LV-TBG-*Fah* expression construct. Similarly, the pig *Nis* cDNA was cloned into the TBG vector in place of EGFP to produce the LV-TBG-*Nis* expression construct. In order to generate viral vectors, the LV-TBG-*Fah* expression construct, along with the packaging plasmid pCMVR8.91 and the VSV-G–expressing plasmid, pMD.G, were transiently transfected into HEK293T cells using PEI. Transfected cells were washed after 16 hours, and grown for 48-60 hours, after which supernatants were harvested and passed through a 0.45-μm filter. Vector supernatants were concentrated by ultracentrifugation (25,000 RPM, 1.5 hours at 4°C), and resuspended in serum-free media (OptiMEM, Life Technologies), aliquoted, and stored at −80°C. Viral titers were calculated using the p24 lentivirus titration kit (Clontech Laboratories, Inc). Virus titers and MOI are reported as physical LPs (lentiviral particles) based on p24 concentration.

In vitro LV transduction assays

For determining the transduction efficiency of single and double LV-transduced cells, either one or both of LV-GFP (*40*) and LV-H2B-RFP (*41*) were added to primary pig hepatocytes at the stated MOI. Seventy-two hours later, cells were fixed in 1% PFA for 10 minutes and

fluorescence analyzed with a FACScalibur flow cytometer (BD Biosciences). Data were analyzed using FlowJo (Treestar). Analysis of ^{125}I uptake by LV-TBG-NIS transduced pig hepatocytes was performed in 6-well culture plates as previously described (*9*).

Western blot assays

Liver samples were homogenized in cell lysis buffer (Cell Signaling) and isolated total protein separated by SDS-PAGE, followed by immunoblotting onto a polyvinylidene fluoride membrane (TransBlot Turbo, BioRad). The primary antibodies against FAH (*44*) and β-actin (13E5; Cell Signaling) were detected with a secondary HRP conjugated anti-rabbit antibody (Cell Signaling), and imaged using a chemiluminescent substrate for detection of HRP (Thermo Scientific).

Blood biochemical analysis

For pigs, blood was obtained via the right femoral vein using an ultrasound guided percutaneous technique under sedation with intramuscular injection of 5 mg/kg telazol and 2 mg/kg xylazine. Serum and plasma were separated for analysis using standard protocols. For amino acid analysis, blood samples were collected and dried on 903 Protein Saver Cards (GE Healthcare). Amino acids and SUAC were measured in dried blood spots by tandem mass spectrometry as described previously (*46*). For mice, biochemical analyses on plasma were analyzed using the VetScan VS2 benchtop analyzer (Mammalian Liver Profile; Abaxis) according to the manufacturer's instructions.

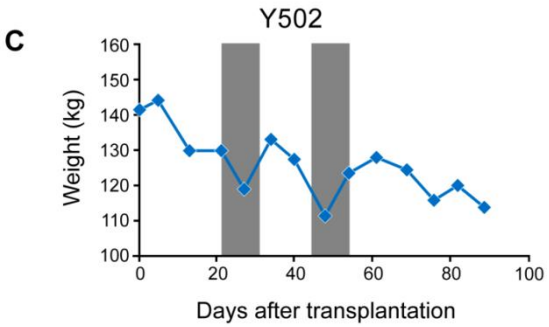
Supplementary Figures

A

# Births	# Crossovers	Frequency of Crossover Event (%)	Estimated Distance (cM)	Estimated Distance ($x10^6$ bp)
56	15	26.8	26.8	27

B

Pig ID	Sex	Hap-1	Hap-2	Sire ID	Hap-1	Hap-2	Dam ID	Hap-1	Hap-2
Y501	F	22.15b	55.13	7821	35.13	55.13	4927	22.15b	35.23
Y502	M	22.15b	55.13	10612	22.15b	35.13	1977	29.24	55.13



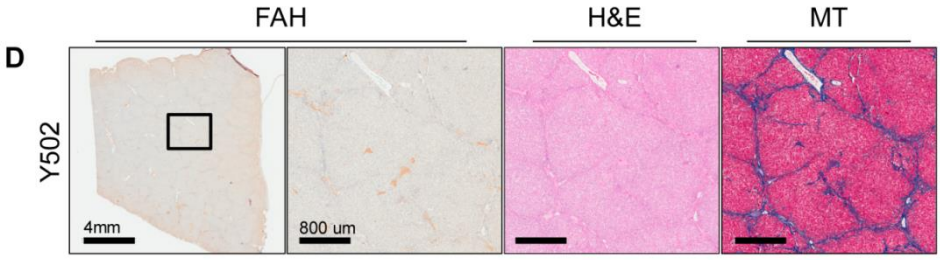


Fig. S1. **Allogeneic transplanted hepatocytes were not detected in recipient pig Y502.** (**A**) Distance between *Fah* and swine leukocyte antigen (*Sla*) locus on porcine chromosome 7 was estimated based on the frequency of crossover events in 56 births. (**B**) SLA-typing was performed to select an allogeneic $Fah^{+/-}$ cell donor for $Fah^{-/-}$ pig Y502. Based on SLA-typing, donor pig Y501 was a perfect match. Haplotypes of the sire and dam of each animal are included for completeness. (**C**) Weight chart of pig Y502 in the proceeding days after transplantation. The animal was cycled on/off NTBC to provide a selective proliferative advantage for transplanted cells. Time on NTBC is depicted in the grey shading (days 21-30 and 44-53). (**D**) Pig Y502 was euthanized 3 months after transplantation. Representative FAH immunohistochemistry in liver tissue is shown at low and high magnification (the square indicates the area of enlargement). Serial sections of H&E and Masson's Trichrome (MT) stained liver are also shown. Scale bars for H&E and MT, 800 µm.

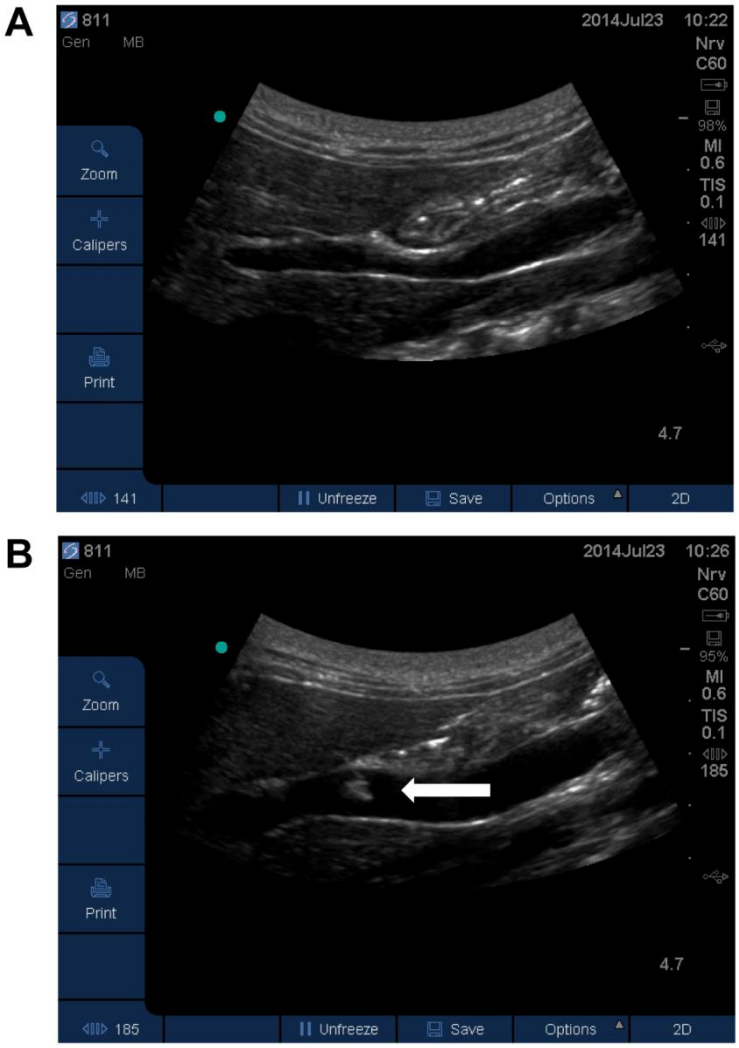


Fig. S2. **Ultrasound guidance allows a less invasive method to inject cells in the portal vein.** (**A**) Baseline screen-shot of ultrasound of portal vein at baseline in a recipient *Fah*$^{-/-}$ pig. (**B**) Ultrasound of portal vein at time of cannulation in the same *Fah*$^{-/-}$ pig. A white arrow depicts location of the needle in the portal vein.

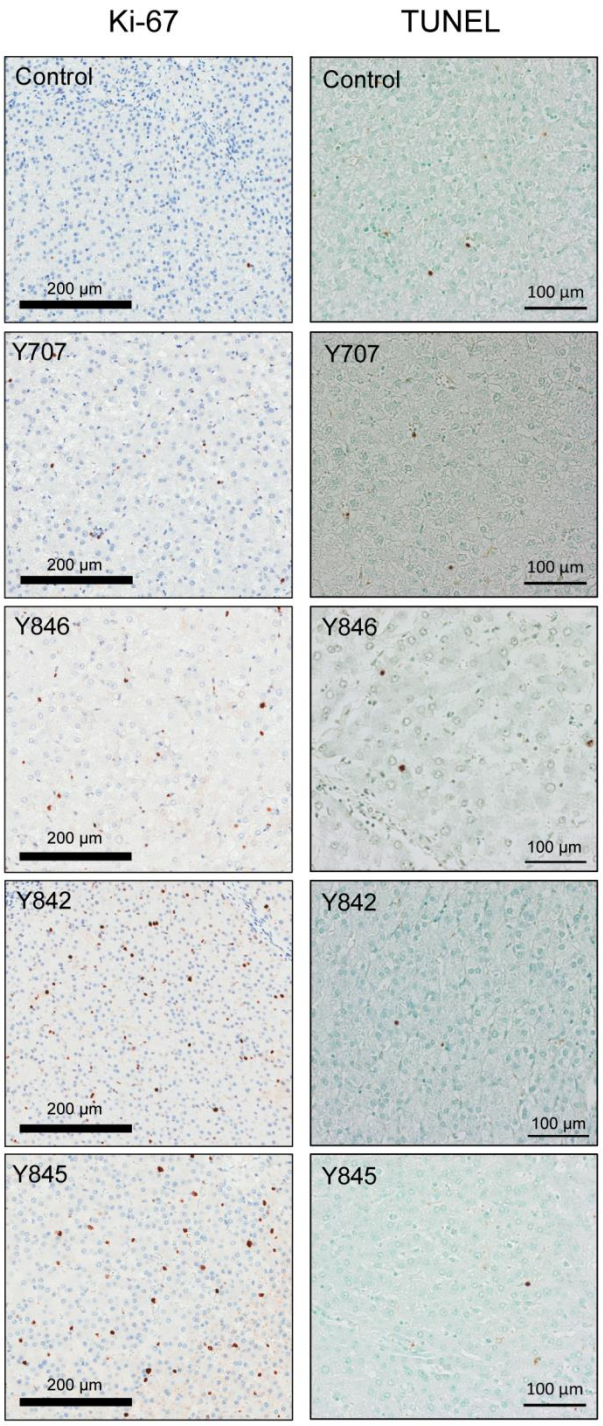


Fig. S3. Assessment of cell proliferation and apoptosis in transplanted *Fah*$^{-/-}$ pigs. Cell proliferation was quantified in random paraffin-embedded tissue sections of pigs Y707, Y846, Y842, Y845, and a control WT pig. Ki67-positive nuclei were stained brown (left panels) and TUNEL-positive cells were stained brown (right panels). Representative images are shown for each animal.

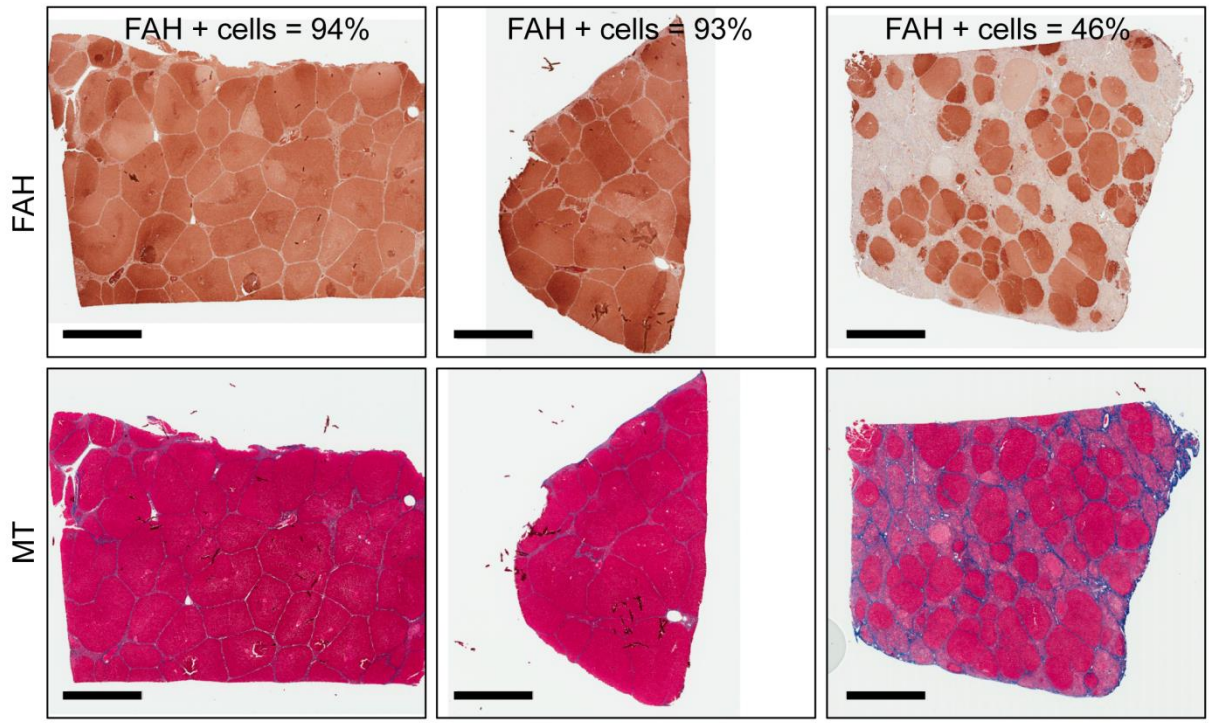


Fig. S4. Variation in fibrosis is associated with differences in cell repopulation in pig Y845. FAH and Masson's Trichrome (MT) staining of liver specimens from three different lobes of one animal are shown at time of euthanasia at 12 months. Lobes in which FAH+ hepatocytes have repopulated >90% (left and middle) are associated with normal fibrous connective tissue staining, whereas lobes with less FAH+ cell repopulation (right) are associated with areas in which distortion of the normal lobular architecture occurs primarily because of extensive interlobular fibrosis. Scale bars, 3 mm.

SUPPLEMENTARY TABLE

Table S1. Summary of cell transplantations. *Fah*$^{-/-}$ pigs received transplants of either allogeneic (allo) or *ex vivo* gene corrected autologous (auto) hepatocytes that were transduced with one or both of LV-*Fah* and LV-*Nis* at the doses described. L768 did not receive any hepatocyte transplantation. Animals were euthanized at 2, 3, 4, or 12 months after transplantation. Y842 was not euthanized, but liver biopsies and biochemical data were collected at 12 months. PV, portal vein infusion; PC, percutaneous ultrasound-guided portal vein injection; Tx, treatment.

Pig ID	**Weight at transplant (kg)**	**Cells harvested (g)**	**Auto or allo**	**# Cells Tx (× 10^6)**	**Cell viability (%)**	**LV-*Fah* (LPs)**	**LV-*Nis* (LPs)**	**Route of injection**	**Months in study**
Y502	141.0	6	Allo	895	90	-	-	PV	3
Y707	7.6	8	Auto	350	91	1.8×10^{11}	-	PV	2
Y846	11.5	10	Auto	400	92	2.6×10^{11}	-	PC	4
Y842	11.0	10	Auto	400	88	2.3×10^{11}	2.6×10^{11}	PC	12
Y845	19.5	11	Auto	864	90	7.5×10^{11}	-	PC	12
L768	4.0	0	-	0	-	0	0	-	3

SUPPLEMENTARY MOVIES

Movie S1. Ultrasound-guided percutaneous portal vein injection.. Under ultrasound guidance, the portal vein of the pig is identified and the hepatocytes are infused into the portal circulation by direct injection using an 18-gauge needle.