Supplemental material

Supplemental Methods

Animals. Transgenic mice (PiZ) were originally generated by Sifers et al (1) by introducing a 14.4 kb DNA fragment containing the entire AAT-Z gene plus 2 kb of 5' and 3' flanking genomic DNA sequences into the germ line. PiZ and C57Bl/6-Rosa26 (beta-galactosidase transgenic) mice (Jackson Laboratory) were bred and maintained at the Animal Institutes of Albert Einstein College of Medicine and the University of Nebraska College of Medicine. All animal experiments were performed with approval of the Animal Care and Use Committees of Albert Einstein College of Medicine and the University of Nebraska College of Medicine, and were within the guidelines for humane care of laboratory animals.

Serological testing for liver function and injury. Serum albumin was determined by Western blot and densitometric analysis of immunoreactive bands. Serum bilirubin was determined according to Jendrassik and Grof (2). Serum alanine aminotransferase was measured using a kit (Catachem) according to the manufacturer's protocol.

Diastase/periodic acid Schiff (D/PAS) staining (3): Liver cryosections (5μm) were incubated with 0.5% amylase to digest glycogen, treated with 1.0% periodic acid and stained with Schiff's reagent (Electron Microscopy Sciences) to visualize AAT-Z globules.

LASER capture microscopy (LCM): D/PAS-stained PiZ mouse liver cryosections showed clusters of AAT-Z globule containing cells, separated by clusters of globule-devoid cells. Using a Leica AS LMD microscope (Leica Microsystems), areas liver sections containing AAT-Z globule-containing hepatocytes and those containing globule-devoid cells were collected in separate tubes.

DNA PCR for *AAT-Z* transgene content. DNA (12.5 ng in 5µl) samples extracted from the AAT-Z globule-containing and globule-devoid regions of the liver sections were amplified using the forward primer: 5'-TGA GTT CGC CTT CAG CCT TAT-3' and the reverse primer: 5'-GTG TCC CCG AAG TTG ACA GT-3', employing the following PCR program: 94^{o}C (1 min), 55^{o}C (45 sec), 72^{o}C (30 sec), 72^{o}C (5 min), 35 cycles.

Quantitative RT PCR for AAT-Z mRNA content. RNA was extracted from the AAT-Z globule-containing and globule-devoid regions using the RNAqueous-Micro kit (Ambion). Quantitative RT-PCR was performed using PowerSYBR Green RNA-to-C_T 1-Step Kit (Applied Biosystems) was performed in a single step using the forward primer (5'-ACT GTC AAC TTC GGG GAC AC-3') and the reverse primer (5'-CAA GCT CCT TGA CCA AAT CC-3'). The PCR program was: 48°C for 30 min, 95°C for 10 min; 95°C for 15 sec, 60°C for 1 min, 40 cycles followed by dissociation. Mouse GAPDH was used as internal control.

Quantitative DNA PCR for the *lacZ* gene: To estimate the extent of liver repopulation by ROSA26 hepatocytes, LacZ gene content in donor hepatocytes and liver specimens after transplantation, DNA was extracted using QIAamp

DNA Micro Kit (Qiagen,) and amplified using SYBR green PCR master Mix (Applied Biosystems) and the following primers: forward: 5'-GAC CGC TGG GAT CTG CCA TTG TCA GAC ATG-3', reverse: CCA TGT GCC TTC TTC TTC CGC GTG CAG CAG ATG-3'. The PCR program was: 95°C for 10mins; 95°C for 15secs, 60°C for 1min; 40 cycles followed by dissociation. Mouse GAPDH was used as internal control.

Hepatocyte isolation. Hepatocytes were isolated by in situ portal vein collagenase (Wako Chemicals) perfusion (4). Viability, assessed by Trypan blue exclusion, was 90% or greater.

Hepatocyte transplantation and immunosuppression. Initial experiments showed that transplanted ROSA26 hepatocytes were rejected in 4-6 weeks, suggesting that the PiZ mice are not fully congeneic with C57Bl/6-ROSA 26 mice. To prevent allograft rejection, the recipients were injected with tacrolimus (1 mg/kg subcutaneously daily). ROSA26 mouse hepatocytes ($1x10^6$) were delivered to the liver by injection into the splenic pulp through a left subcostal incision (5).

Bioluminescence imaging of hepatic repopulation. A recombinant lentiviral vector (lenti-Alb-luc), expressing firefly luciferase (*luc*) from an albumin promoter was generated as described (6), using a transduction plasmid, Alb-luc (gift of Dr. Antonella Follenzi, Albert Einstein College of Medicine, New York). Gene transfer titers were determined by infecting HEPG2 cells and immunostaining. For gene transfer into hepatocytes, the lentiviral vectors were mixed for 4 hours at 37^oC with Rosa26 mouse hepatocytes at MOI 10 in DMEM containing 10% FBS and

8ug/ml Polybrene (7). After washing, the cells were transplanted by intrasplenic injection (5). For in vivo imaging, luciferin 1 (50 mg/kg) was injected intraperitoneally into the transplant recipients and photons were captured using a CCD camera (IVIS 200, Xenogen Corp).

Human hepatocyte growth factor (HGF) gene transfer. Some recipient PiZ mice were injected with 10^{11} particles of a recombinant adenoviral vector expressing human HGF intravenously (8) on the day of hepatocyte transplantation. One control group of mice received an adenoviral vector expressing an irrelevant gene, human UGT1A1 (9)

Tissue staining for β-galactosidase. Liver cryosections were stained using the 5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside (X-gal) (Invitrogen) substrate (8).

Immunofluorescence staining of paraffin sections of formalin-fixed liver tissue was performed using an anti-E. coli β-galactosidase rabbit polyclonal antibody (MP Biomedicals) and a rhodamine-labeled secondary antibody.

Immunofluorescence staining for Ki67. Paraffin sections of formalin-fixed liver tissue were stained using a goat polyclonal antibody (Santa Cruz) and an Alexa fluor 488-labeled anti-goat donkey secondary antibody (BD Biosciences).

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL). TUNEL staining was performed on liver cryosections using the TACS™ 2 TdT-DAB In Situ Apoptosis Detection Kit (Trevigen) according to the manufacturer's protocol.

Statistical analysis. Sets of data in the various groups were compared using Student's paired two-tailed T test. P values <0.01 were considered statistically significant.

References for Supplemental Methods

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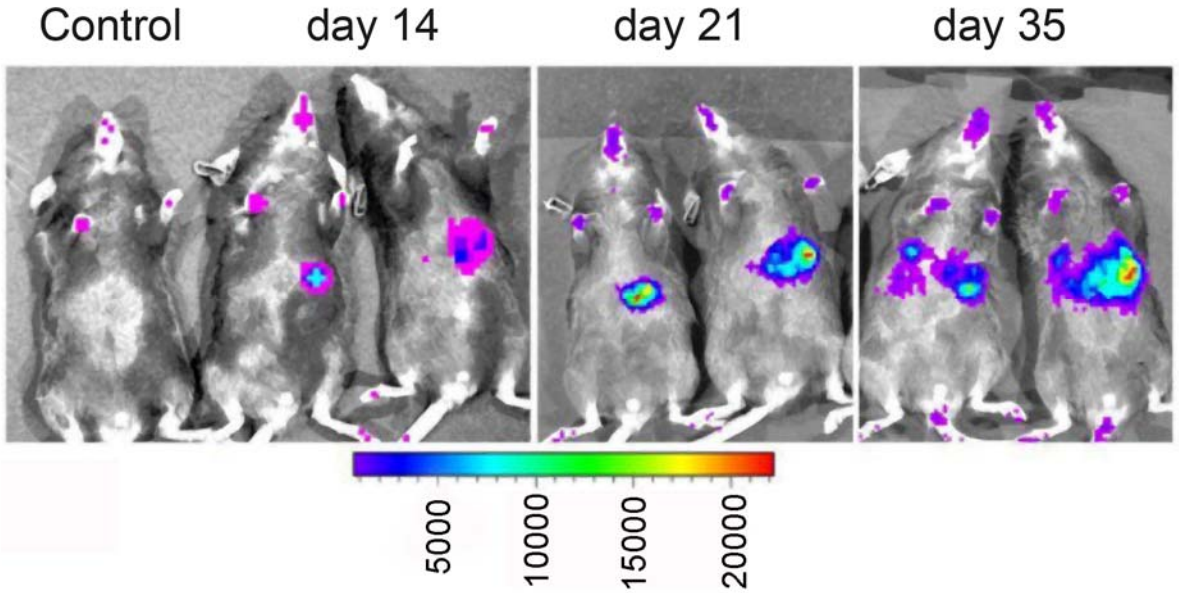
Legend to supplemental Figure 1.

Supplemental Figure 1. Non-invasive bioluminescence analysis of hepatic repopulation. Hepatocytes from C57Bl/6 mice were transduced with a lentivector expressing firefly luciferase from an albumin promoter at an MOI of 30, as described in the Methods section. The transduction efficiency was ~ 30%. The hepatocytes ($1x10^6$) were then transplanted into PiZ mice, which received tacrolimus (1 mg/kg daily subcutaneously), beginning 7 days before transplantation. At various time points after transplantation, luciferin was administered intraperitoneally to mice at 150 mg/kg, and a cooled charge coupled device (CCD) camera (IVIS 200, Xenogen Corp) was used to capture photons for 5 minutes from anesthetized transplant and control recipients. Images at various time points after hepatocyte transplantation are shown.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