METHODS

Isolation of primary human hepatocytes and T lymphocytes

Human livers were obtained from the University of Pittsburgh via the NIH-sponsored Liver Tissue and Cell Distribution System. Hepatocytes were isolated as described [1]. Freshly-isolated cells were shipped to Oregon Health & Science University via overnight courier in University of Wisconsin solution. Patient details are described in Supplementary Table 1. Mobilized peripheral blood was obtained from patients after informed consent in accordance with Oregon Health & Science University institutional review board policies. Cells were processed within 24h and cryopreserved in DMSO. After thawing, cells were stained with a PE-conjugated CD3 antibody (Beckton Dickinson) per manufacturer's instructions. Cells were sorted with a FACS Vantage SE/Diva flow cytometer (Beckton Dickinson) using a 70 μm nozzle. Dead cells were excluded on the basis of 5 μg/ml propidium iodide (Invitrogen) incorporation.

Mouse surgery

Triple knockout mice, *Fah*$^{-/-}$ *Rag2*$^{-/-}$ *Il2rg*$^{-/-}$ (FRG), were transplanted intrasplenically with 500,000 primary human hepatocytes and allowed to repopulate the host liver [2]. Following extensive repopulation (>75%) expanded hepatocytes were isolated by collagenase perfusion [3]. Transplantation experiments were approved by the Institutional Animal Care and Use Committee of Oregon Health & Science University.

Fluorescence *in situ* hybridization (FISH)

Hepatocytes or T lymphocytes were incubated for 10 min in 56 mM KCl and fixed with methanol:acetic acid (3:1 ratio). After dropping onto glass slides, nuclei were hybridized with up to 4 different sets of probes per manufacturer's guidelines (Abbott Molecular).

Chromosome probes 1, 9, 16
Chr 1: CEP 1-SpectrumOrange
Chr 9: CEP 9-SpectrumAqua
Chr 16: CEP 16-SpectrumGreen

Chromosome probes 13, 18, 21, X, Y
MultiVysion PGT Multi-color Probe Kit
Chr 13: LSI 13-SpectrumRed
Chr 18: CEP 18-Spectrum Aqua
Chr 21: LSI 21-Spectrum Green
Chr X: CEP X-SpectrumBlue
Chr Y: CEP Y-Spectrum Gold

Chromosome probes 1, 13
Chr 1: CEP 1-SpectrumOrange
Chr 13: LSI 13-SpectrumGreen

Chromosome probes 9, 18
Chr 9: CEP 9-SpectrumGreen
Chr 18: CEP 18-SpectrumOrange

Samples were analyzed and scored under a Nikon Eclipse E800 photoscope. For analysis of interphase nuclei, signals were counted for ≥200 nuclei/sample. Signals were scored as separate if they were further than a signal's diameter apart in distance, according to established clinical practices of FISH signal interpretation and as described [4]. Photographs were captured using CytoVision software from Applied Imaging.

Karyotypes
First, freshly-isolated or cryopreserved human hepatocytes were seeded at 10,000 cells/cm^2 on collagen-coated Primaria tissue culture plastic (Beckton Dickinson) in DMEM/F12 (Invitrogen, 0.5% FBS (Hyclone), ITS Supplement (Invitrogen; contains 5 μg/ml insulin, 5 μg/ml transferrin and 5 ng/ml sodium selenite), 15 mM HEPES and antibiotic-antimycotic (Cellgro). After 16-20h, cells were washed once with DMEM/F12 to remove non-adherent cells and provided fresh culture medium supplemented with 50 ng/ml human epidermal growth factor (Invitrogen) and 1.0 μM 3,3',5-Triiodo-L-thyronine sodium salt (T_3) (Sigma). Secondly, after 48-72h in culture, hepatocytes were treated with 30 μg/ml KaryoMAX colcemid (Invitrogen) for 16 hr and harvested by trypsinization. Third, after extensive washing, cells were incubated for 20 min in 56 mM KCl and fixed with methanol:acetic acid (3:1 ratio). Finally, chromosomes from 20 metaphase-arrested hepatocytes per sample were G-banded with a standard trypsin/Wright's stain protocol. Photographs were taken using CytoVision software from Applied Imaging.

High resolution imaging of dividing hepatocytes
Hepatocytes were seeded at a density of 5,000-10,000 cells/cm^2 on collagen-coated ibi-Treat 8-well μ-slides (Ibidi) using the culture media described earlier. After expansion for 3d, cells were immunostained as described previously [5, 6]. Briefly, cells were fixed with methanol, incubated with primary antibodies for alpha-Tubulin (clone DM1A, Sigma) and Centrin-2 (N-17, Santa Cruz) and detected with species-specific secondaries conjugated to Alexafluor 488 or Alexafluor 555 (Invitrogen). Hoechst 33342 (Invitrogen) was used to visualize nuclei. Images were acquired on a high resolution wide field Core DV system (Applied Precision) equipped with a Nikon Coolsnap ES2 HQ camera. Images were acquired as Z-stacks (every 0.2 μm from the bottom to top of each mitotic spindle) and deconvolved with the appropriate optical transfer function using an algorithm of 10 iterations using SoftWoRx Image Restoration Software (Applied Precision).

Statistical significance
Statistical significance was determined using 2-sided Student's t-test. *P* values less than 0.05 were considered significant.

REFERENCES

1. Strom SC, et al. Methods Enzymol 1996;272:388-401.
2. Azuma H, et al. Nat Biotechnol 2007;25:903-10.
3. Overturf K, et al. Nat Genet 1996;12:266-73.
4. Bayani J, et al. Curr Protoc Cell Biol 2004;Chapter 22:Unit 22 4.
5. Duncan AW, et al. Nature 2010;467:707-10.
6. Ganem NJ, et al. Nature 2009;460:278-82.