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Supplemental Information

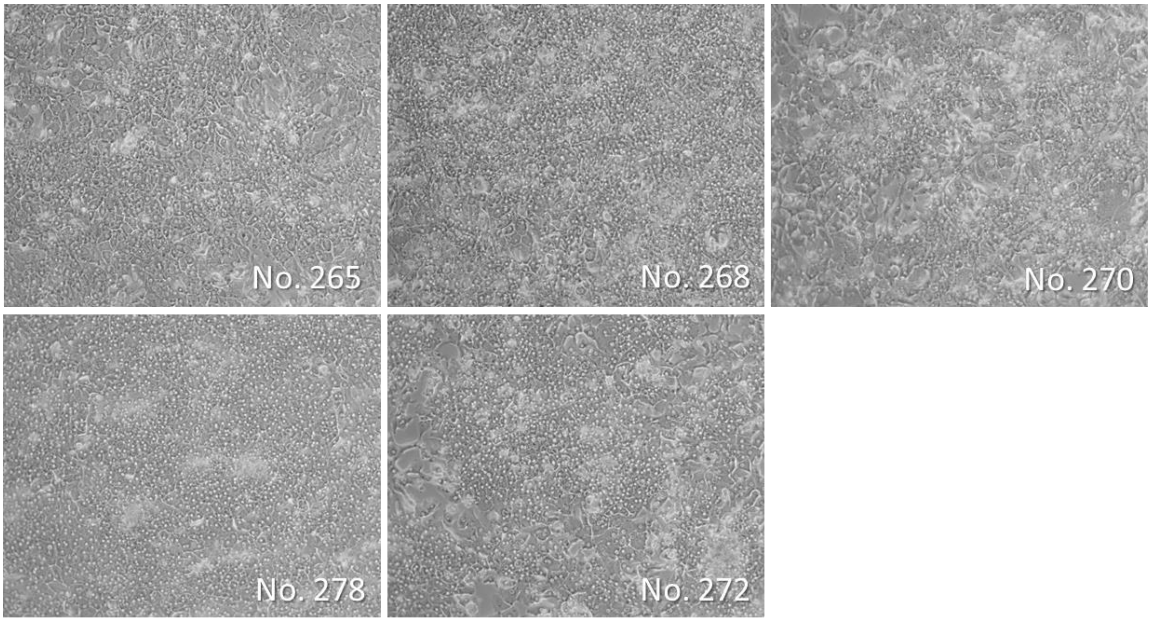
Ex Vivo Cell Therapy by Ectopic Hepatocyte Transplantation Treats the Porcine Tyrosinemia Model of Acute Liver Failure

Clara T. Nicolas, Robert A. Kaiser, Raymond D. Hickey, Kari L. Allen, Zeji Du, Caitlin J. VanLith, Rebekah M. Guthman, Bruce Amiot, Lukkana Suksanpaisan, Bing Han, Maria Giovanna Francipane, Amin Cheikhi, Huailei Jiang, Aditya Bansal, Mukesh K. Pandey, Ishan Garg, Val Lowe, Aditya Bhagwate, Daniel O'Brien, Jean-Pierre A. Kocher, Timothy R. DeGrado, Scott L. Nyberg, Eric Lagasse, and Joseph B. Lillegard

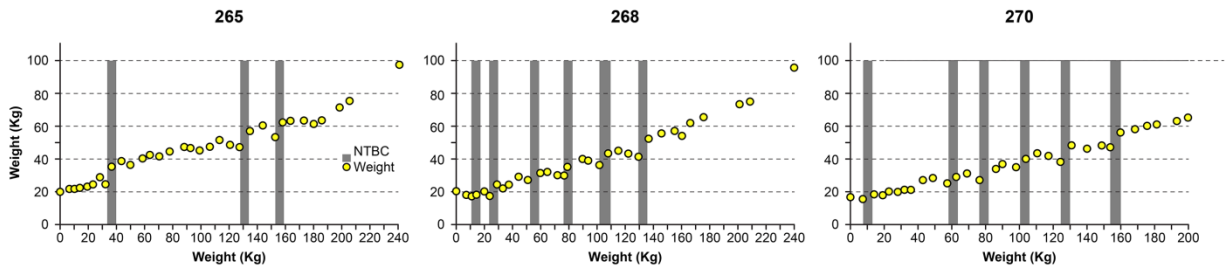
Supplementary Materials

Supplementary Table 1. Summary of cell transplantations.

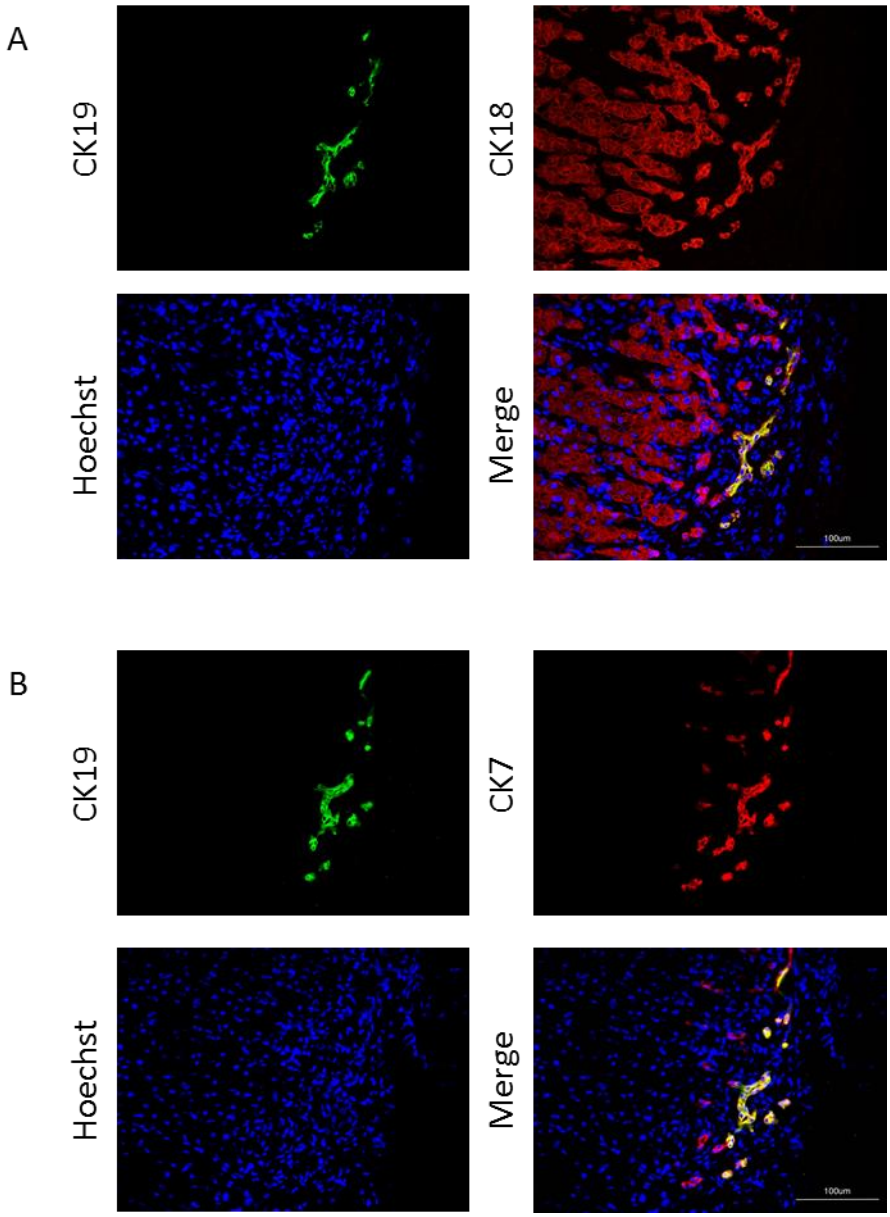
Pig ID	Sex	Weight at Transplant (kg)	Live Cells Harvested (x 10^6)	Viability (%)	Injection Volume of Saline (mL)	Number of Lymph Nodes Injected	Cells Transplanted (x 10^6)	Cells Transplanted (x 10^6/kg)	Days of Follow-up
265	Female	20.8	1160	84	10	10-20	600	28.8	239
268	Male	19.8	770	77	20	10-20	600	30.3	235
270	Male	16.4	995	82	20	10-20	600	36.6	241
272	Male	19.0	975	74	20	10-20	600	31.6	212
278	Female	19.6	671	85	20	10-20	600	30.6	239



Supplementary Figure 1. Isolated hepatocytes for autotransplantaton were platable in culture. Representative hepatocytes from all pigs (Nos. 265, 268, 270, 278, and 272) were cultured for a full 48 hours after isolation to evaluative platability, which was qualitatively evaluated as a surrogate indicator for predicting engraftment *in vivo*.

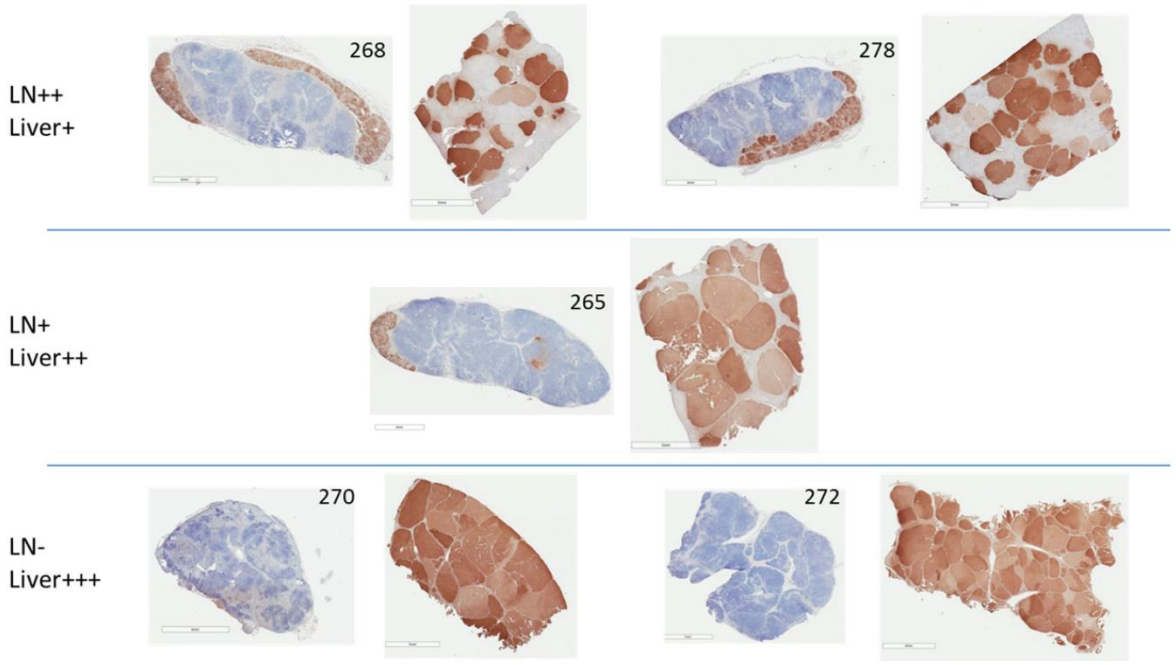


Supplementary Figure 2. Weight stabilization of pigs 265, 268, and 270. NTBC-independent weight gain was achieved at days 158, 133, and 163, respectively, after three to six cycles on the drug (represented by gray vertical bars).

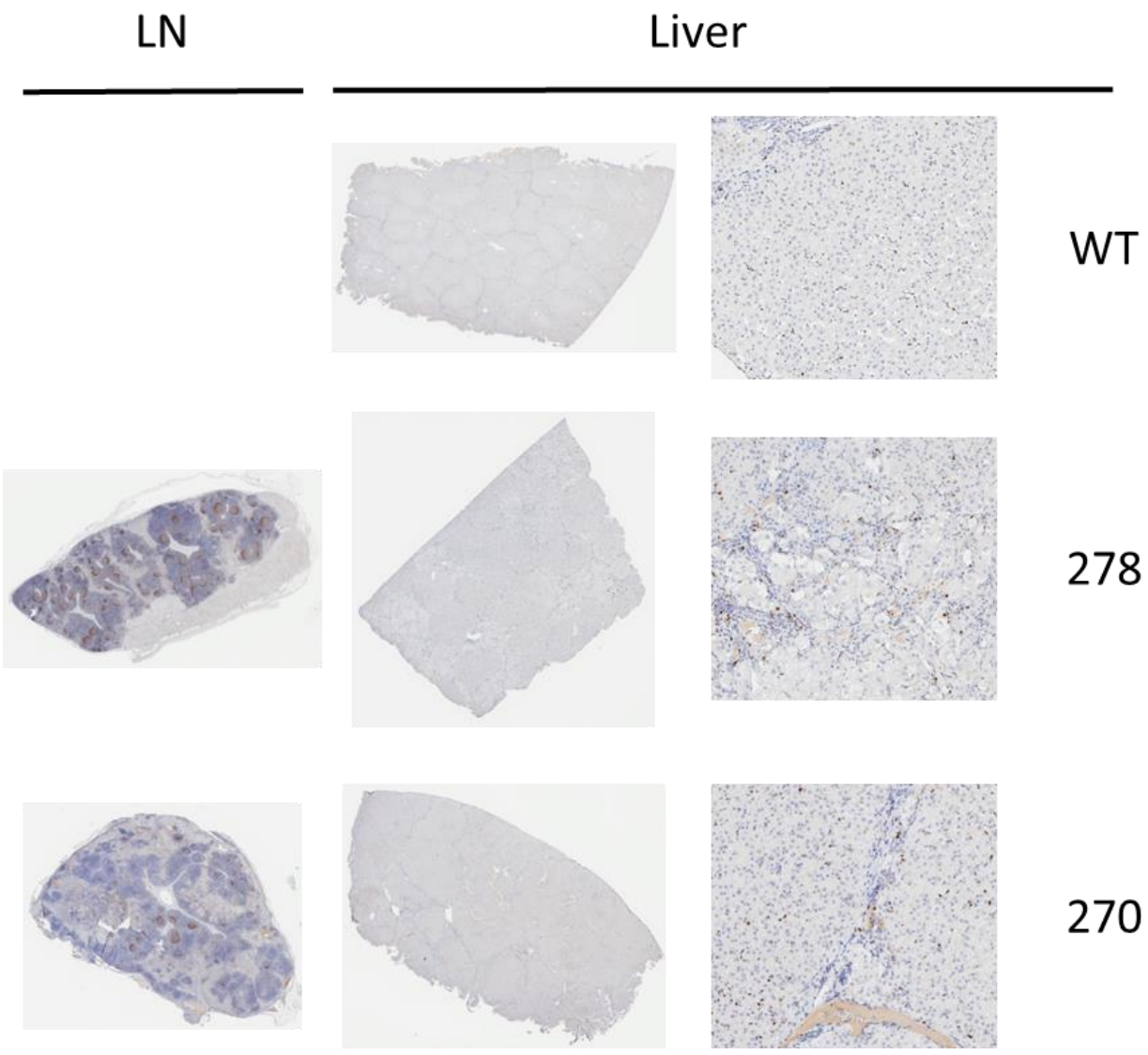


Supplementary Figure 3. Regions of hepatocyte engraftment in lymph nodes develop bile duct cells. Bile duct cell presence was further explored in Pig No. 265. A) At the periphery of nodules of hepatocyte engraftment and expansion, rare sections also contain cells that express CK19 (green), a common marker for bile duct cells. CK18 (red) and Hoechst staining demonstrate the presence of the hepatocytes in this section, verifying that it is a site of engraftment. B) All CK19-positive cells (green) were also positive for CK7 (red), although the

merged panel indicates that only the majority of CK7 positive cells were also positive for CK19 (co-localization in yellow).



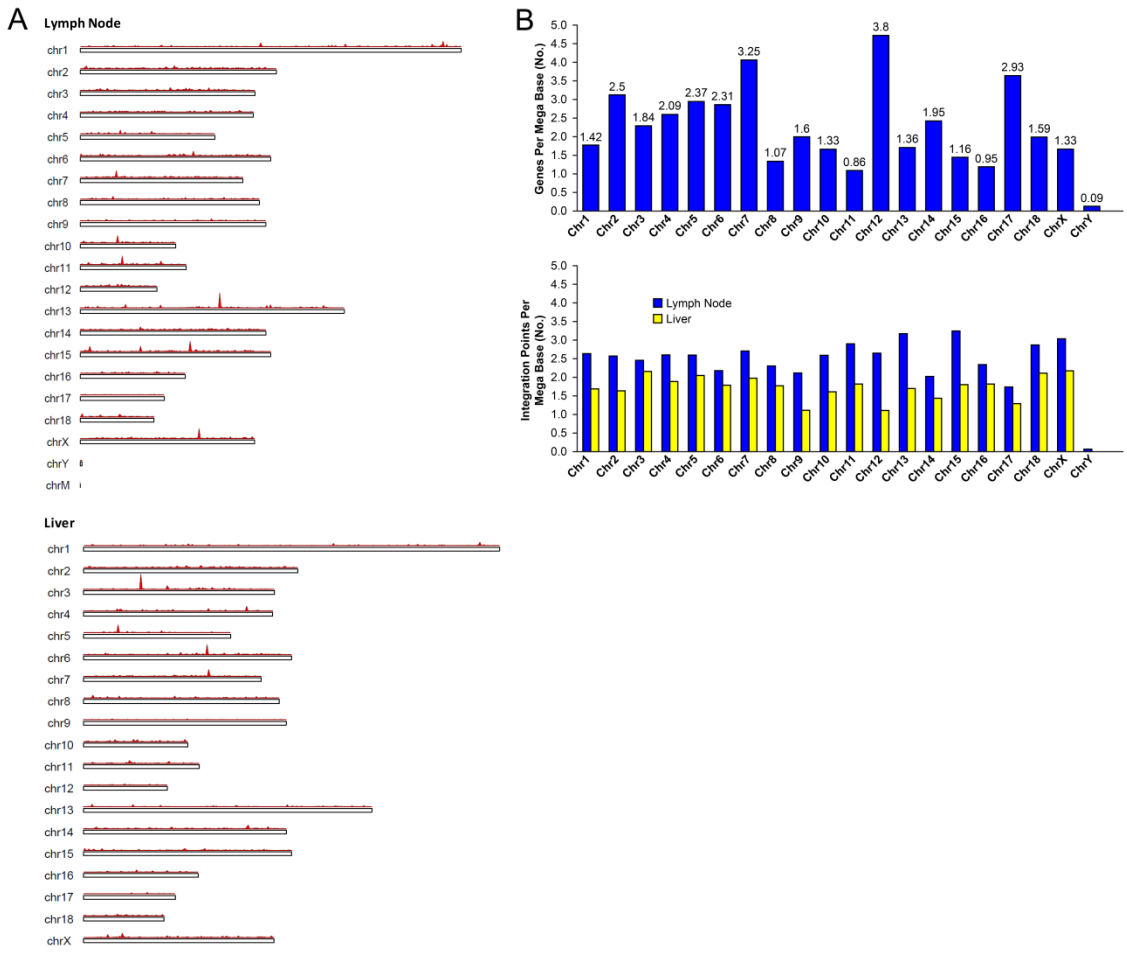
Supplementary Figure 4. Correlation between presence of FAH-positive hepatocytes in lymph node and liver. Animals with partial liver repopulation with FAH-positive hepatocytes (pigs 268 and 278) still had robust ectopic liver tissue presence in lymph nodes at the time of euthanization. Animals with complete liver repopulation with FAH-positive hepatocytes (pigs 270 and 272) had little to no presence of ectopic liver tissue in lymph nodes at the time of euthanization.



Supplementary Figure 5. Eight months after transplantation, hepatocytes in lymph nodes and livers are not actively proliferating. At termination (approximately 8 months post-transplantation) animals with partial liver repopulation with FAH-positive hepatocytes (such as pig 268) and those with complete liver repopulation with FAH-positive hepatocytes (such as pig 270) showed unremarkable Ki-67 positivity relative to wild type pig liver (top row), indicating that proliferation in both tissues was largely complete at the time of termination.

Supplementary Table 2. Next generation sequencing mapping statistics

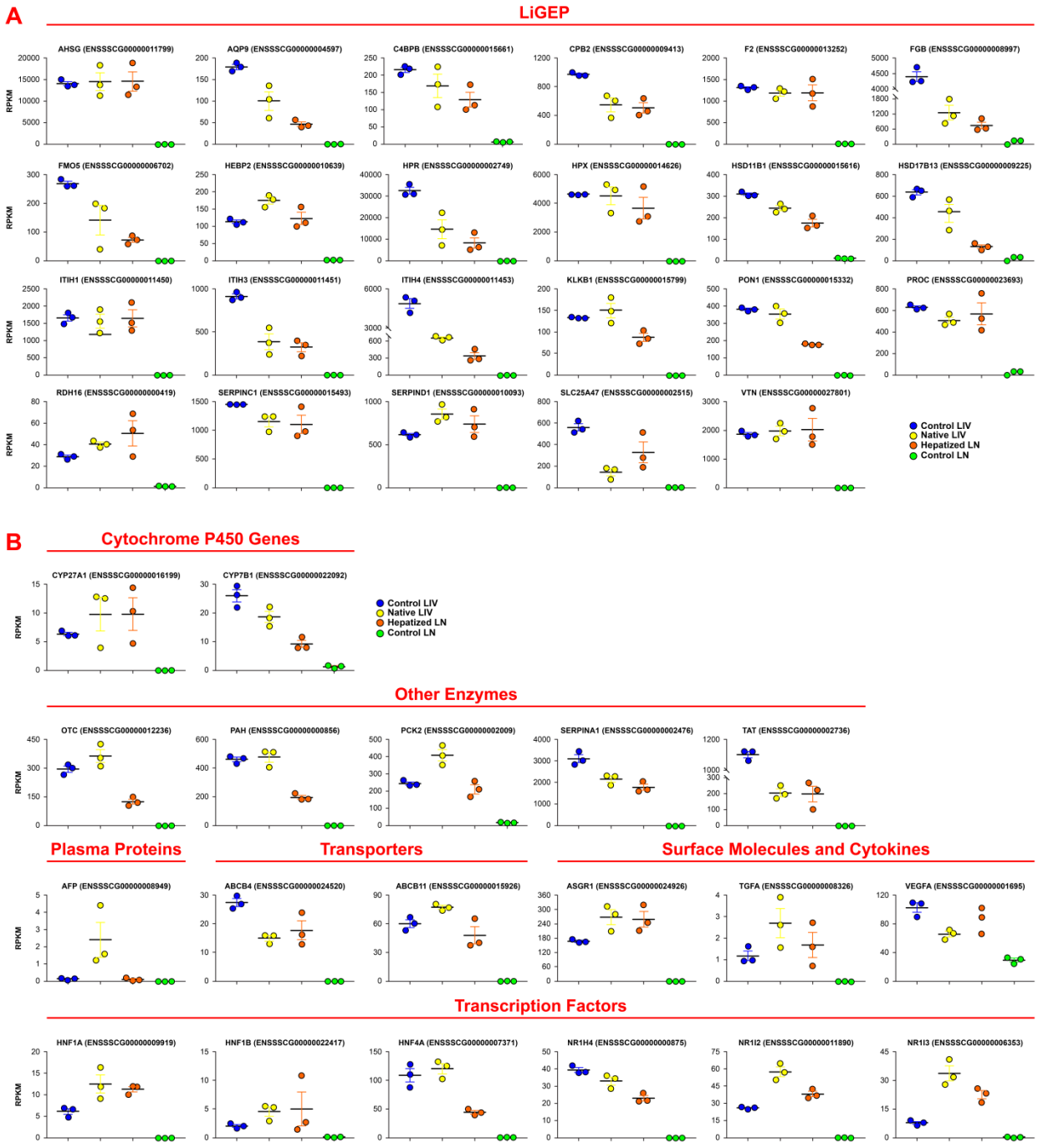
Sample	Total Reads	Mapped Reads (Total)	Integration Points at 1X	Integration Points at 5X
Lymph node	115.748.080	5,465,009 (7.34 %)	40.239	6.335
Liver	116.490.845	21,126,444 (22.29 %)	27.987	4.270



Supplementary Figure 6. Lentiviral integration profile in lymph node vs. liver hepatocytes. (A) Chromosomal integration map for transplanted hepatocytes that remained in lymph nodes (top) and transplanted hepatocytes that migrated to the liver (bottom). Red vertical bars represent integration points. (B) Relative gene density per chromosome (top) compared to relative integration density per chromosome (bottom).

Supplementary Table 3. Liver-specific genes included in RNA sequencing analysis

LiGEP		
ENSSSCG00000011799	AHSG	Alpha 2-HS glycoprotein
ENSSSCG00000008948	ALB	Albumin
ENSSSCG00000004597	AQP9	Aquaporin 9
ENSSSCG00000015661	C4BPB	C4b-binding protein beta chain
ENSSSCG00000009413	CPB2	Carboxypeptidase B2
ENSSSCG00000013252	F2	Coagulation factor II (thrombin)
ENSSSCG00000008997	FGB	Fibrinogen beta chain
ENSSSCG00000006702	FMO5	Flavin containing monooxygenase 5
ENSSSCG00000010639	HABP2	Hyaluronan binding protein 2
ENSSSCG00000002749	HPR	Haptoglobin
ENSSSCG00000014626	HPX	Hemopexin
ENSSSCG00000015616	HSD11B1	Hydroxysteroid (11-beta) dehydrogenase 1
ENSSSCG00000009225	HDD17B13	Hydroxysteroid (17-beta) dehydrogenase 13
ENSSSCG00000011450	ITIH1	Inter-alpha-trypsin inhibitor heavy chain H1
ENSSSCG00000011451	ITIH3	Inter-alpha-trypsin inhibitor heavy chain H3
ENSSSCG00000011453	ITIH4	Inter-alpha-trypsin inhibitor heavy chain H4
ENSSSCG00000015799	KLKB1	Plasma kallikrein
ENSSSCG00000015332	PON1	Paraoxonase 1
ENSSSCG00000023693	PROC	Vitamin K-dependent protein C
ENSSSCG00000000419	RDH16	Retinol dehydrogenase 16
ENSSSCG00000015493	SERPINC1	Antithrombin-III
ENSSSCG00000010093	SERPIND1	Heparin cofactor 2
ENSSSCG00000002515	SLC25A47	Solute carrier family 25 member 47
ENSSSCG00000027801	VTN	Vitronectin
Additional Liver-Specific Genes		
ENSSSCG00000016199	CYP27A1	Cytochrome P450 family 27 subfamily A member 1
ENSSSCG00000022092	CYP7B1	Cytochrome P450 family 7 subfamily B member 1
ENSSSCG00000001780	FAH	Fumarylacetoacetate hydrolase
ENSSSCG00000012236	OTC	Ornithine transcarbamylase
ENSSSCG00000000856	PAH	Phenylalanine hydroxylase
ENSSSCG00000002009	PCK2	Phosphoenolpyruvate carboxykinase 2
ENSSSCG00000002476	SERPINA1	Alpha-1-antitrypsin
ENSSSCG00000002736	TAT	Tyrosine aminotransferase
ENSSSCG00000008949	AFP	Alpha-fetoprotein
ENSSSCG00000024520	ABCB4	Adenosine triphosphate-binding cassette, subfamily B, member 4
ENSSSCG00000015926	ABCB11	Adenosine triphosphate–binding cassette B11
ENSSSCG00000009919	HNF1A	Hepatocyte nuclear factor 1 homeobox A
ENSSSCG00000022417	HNF1B	Hepatocyte nuclear factor-1-beta
ENSSSCG00000007371	HNF4A	Hepatocyte nuclear factor 4 alpha
ENSSSCG00000000875	NR1H4	Nuclear receptor subfamily 1 group H member 4
ENSSSCG00000011890	NR1I2	Nuclear receptor subfamily 1 group I member 2
ENSSSCG00000006353	NR1I3	Nuclear receptor subfamily 1 group I member 3
ENSSSCG00000024926	ASGR1	Asialoglycoprotein receptor 1
ENSSSCG00000008326	TGFA	Transforming growth factor alpha
ENSSSCG00000001695	VEGFA	Vascular endothelial growth factor A



Supplementary Figure 7. Further RNA sequencing analysis. Scatter dot plot graphs showing RPKM values for LiGEP (liver-specific gene expression panel) (A) or additional liver-specific genes (B) in control liver, native liver, hepatized lymph node, and control lymph node. Data are mean ± SEM.

Supplementary Video 1. 3D render movie of PET-CT images of ^{89}Zr-labeled hepatocytes at 6 h post-transplantation into mesenteric lymph nodes in pig.

Supplementary Video 2. 3D render movie of PET-CT images of NIS-labeled hepatocytes at 3 months post-transplantation into mesenteric lymph nodes in pig 265.

Supplementary Video 3. 3D render movie of PET-CT images of NIS-labeled hepatocytes at 6 months post-transplantation into mesenteric lymph nodes in pig 265.

Supplementary Video 4. 3D render movie of PET-CT images of NIS-labeled hepatocytes at 5 months post-transplantation into mesenteric lymph nodes in pig 268.

Supplementary Video 5. 3D render movie of PET-CT images of NIS-labeled hepatocytes at 6 months post-transplantation into mesenteric lymph nodes in pig 268.