SUPPLEMENTARY INFORMATION

Restoration of Bile Duct Injury of Donor Livers During Ex Situ Normothermic Machine Perfusion

Iris E.M. de Jong, Silke B. Bodewes, Otto B. van Leeuwen, Dorenda Oosterhuis, Veerle A. Lantinga, Adam M. Thorne, Bianca Lascaris, Marius C. van den Heuvel, Rebecca G. Wells, Peter Olinga, Vincent E. de Meijer, and Robert J. Porte

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SUPPLEMENTARY TABLES

Table S1. List of antibodies used for immunostainings.

Antigen	Marker for	Host	Supplier	Catalog no.	Dilution
AE2	Mature cholangiocytes – bicarbonate transporter	Mouse	Santa Cruz	Sc-376632	1:50
CD31	Endothelial cells	Mouse	Roche-VENTANA	760-4378	Ready-to-use
K19	Cholangiocytes	Mouse	Dako Cytomation	M0888	1:100
Glut-1	Mature cholangiocytes – glucose transporter	Rabbit	Millipore	07-1401	1:750
Ki-67	Proliferation	Mouse	Dako Cytomation	M7240	1:300
Sox9	Biliary progenitor cells	Rabbit	Millipore	AB5535	1:300
vWF	Endothelial cells	Mouse	Dako Cytomation	M0616	1:100

Abbreviations: AE2, anion exchanger 2; CD31, cluster of differentiation 31; K19, keratin 19; Glut-1, glucose transporter 1; vWF, von Willebrand factor.

Table S2. Perfusion parameters after 2.5 hrs of normothermic machine perfusion.

		Transplanted[a]	Not transplanted[a]
Lactate (mmol/l)		0.4 [0.3 - 1.1]	0.9 [0.7 - 1.5]
Glucose (mmol/l)		25 [17-28]	24 [22-28]
Bicarbonate (mmol/l)		15 [13-18]	14 [12-17]
pO2 (kPa)		23 [21-39]	22 [22-35]
pCO2 (kPa)		3.3 [2.8-3.7]	3.2 [2.7-3.7]
Portal	flow (ml/min)	1790 [1630-1900]	1740 [1600-1860]
	pressure (mmHg)	10 [8-11]	9 [7-9]
Arterial	flow (ml/min)	494 [430-653]	505 [425-560]
	pressure (mmHg)	55 [45-65]	56 [48-70]

[a]Values shown are median and interquartile ranges.

Table S3. Donor characteristics.

	Transplanted (n=25)	Not transplanted (n=17)	P - value
Age (years)	63 (52-69)	68 (54-73)	0.156
BMI (kg/m^2)	25.0 (23.0-26.8)	27 (24.5-30.0)	0.089
Gender			**0.019**
Male	18 (75%)	10 (59.8%)	
Female	6 (25%)	7 (41.2%)	
Cause of death			0.191
Trauma	5 (20.8%)	3 (17.6%)	
Cerebrovascular accident	7 (29.2%)	8 (47.1%)	
Anoxia	10 (41.7%)	2 (11.8%)	
Other	2 (8.4%)	4 (17.6%)	
Warm ischemia time (min)	16 (14.25-18.00)	16.0 (14.5-18.5)	0.407
Hepatectomy time (min)	37.5 (29.5-49.5)	43.5 (39.0-55.0)	0.134
Static cold ischemia time (min)	257 (238-289)	292.5 (271.0-254.2)	**0.004**
Last GGT (U/L)	53.0 (26.3-90.0)	32.0 (11.5-137.5)	0.327
Last bilirubin (mmol/L)	8.4 (5.7-16.0)	8 (5-10.5)	0.432
Last ALP (U/L)	70 (58-90)	73.5 (57.5-73.5)	0.601
ET DRI	2.87 (2.64-3.08)	3.07 (2.62-3.31)	0.239

Abbreviations: ALP, alkaline phosphatase; BMI, body mass index; ET DRI, Eurotransplant donor risk index; GGT, gamma-glutamyl transferase.

Table S4. Posttransplant outcomes of the patients that received a liver from the transplanted group.

Variable	All livers (n=25)
Posttransplant outcomes	
Actuarial graft survival	
3-months	98%
6-months	98%
12-months	95%
Retransplantation for	
Chronic rejection	1 (2%)
Venous outflow tract obstruction	1 (2%)
Actuarial patient survival	
12-months	100%
Peak ALT (U/L)	691 (379-941)
Peak AST (U/L)	988 (519-1684)
Bilirubin day 7 (µmol/L)	18.0 (9.5-42)
INR day 7	1.0 (1.0-1.1)
GGT (IU/L)	
day 30	103 (46-251)
day 90	60 (16-217)
day 180	92 (23-183)
Alkaline phosphatase (U/L)	
day 30	123 (79-295)
day 90	142 (83-314)
day 180	139 (92-248)
Biliary complications	
Nonanastomotic strictures	1 (2%)
Anastomotic stricture	8 (19%)
Bile leakage	3 (7%)
Primary nonfunction	0
Hepatic artery thrombosis	0
Acute rejection	3 (7%)
Chronic rejection	2 (6%)
Relaparotomy for:	
bleeding	2 (5%)
bile leakage	3 (7%)
other	4 (10%)
ICU stay (days)	2 (1-4)
Hospital stay (days)	18 (14-25)

Abbreviations: ALT, alanine transaminase; AST, aspartate aminotransferase; INR, international normalized ratio; GGT, gamma-glutamyl transferase; ICU; intensive care unit.

Table S5. Histological features of the donor bile duct before and after machine perfusion of the patient that underwent liver retransplantation for bile duct complications.

One patient that received a perfused liver graft presented with bile duct complications that was classified as NAS and/or recurrence PSC for which he/she required liver retransplantation. Indications for first and second transplantation for this patient: Primary indication for transplantation: PSC Reason for retransplantation: recurrence PSC/NAS									
Histology scoring[a]	**Mucosal loss**	**Bleeding**	**Thrombi**	**Vascular lesions**	**Arteriolo necrosis**	**Stroma necrosis**	**Inflamm ation**	**Luminal PBGs**	**Deep PBGs**
Before MP	4	0	0	2	0	3	0	4	3
After reperfusion	4	0	0	2	1	4	0	2	1
PBG mass									
Before MP	0.3 %								
After reperfusion	10.3 %								
PBG proliferation rate									
Before MP	1.7 %								
After reperfusion	3.0 %								
Glut-1 expression									
Before MP	0.7 %								
After reperfusion	53.3 %								

[a]Refer to table S6 for details of the scoring system. Abbreviations: MP, machine perfusion; NAS, nonanastomotic strictures; PBG, peribiliary gland; PSC, primary sclerosing cholangitis.

Table S6. Comparison between samples taken directly after NMP and samples taken after reperfusion in the transplanted group.

	Transplanted livers Median – IQR		
	Directly after NMP	After reperfusion	P – value
MVD-PBGs	0.88 [0.71-1.40]	0.57 [0.49-1.54]	0.35
Outer layer	3.60 [2.95-4.45]	4.60 [3.00-5.10]	0.26
Middle layer	5.80 [4.35-6.25]	4.80 [3.80-7.00]	0.99
Inner layer	15.60 [8.55-20-70]	16.00 [12.20-19.80]	0.72
PBG mass	2.34 [1.39-4.69]	2.16 [1.71-4.63]	0.75
PAS	2.86 [1.06-3.33]	3.04 [1.47-5.54]	0.55
Alc-B	1.50 [1.17-3.78]	0.40 [0.21-2.74]	0.22
Sox9	745.5 [305.3-883.5]	783.0 [398.0-1586]	0.75
Glut-1	34.99 [22.99-53.52]	23.97 [20.81-39.97]	0.39
Ki-67	2.13 [1.22-3.61]	2.49 [1.29-4.56]	0.65

Abbreviations: Alc-B, alcian blue; Glut-1, glucose transporter 1; IQR, interquartile range; MVD, microvascular density; NMP, normothermic machine perfusion; PAS, periodic acid Schiff; PBG, peribiliary gland.

Table S7. Histological grading of samples taken before and after machine perfusion of the transplanted and the not transplanted group.

Bile duct component	Before MP Injury score (%)			After MP Injury score (%)		
	Transplanted (n=24)	Not transplanted (n=14)	P -value	Transplanted (n=25)	Not transplanted (n=14)	P - value
Mucosal loss			0.05			0.10
Grade 0	0	0		0	0	
Grade 1	0	0		4	0	
Grade 2	4.2	0		4	0	
Grade 3	20.8	0		8	0	
Grade 4	75	100		84	100	
Bleeding			>0.99			**0.02**
Grade 0	100	100		60	85.7	
Grade 1	0	0		36	14.3	
Grade 2	0	0		4	0	
Grade 3	0	0		0	0	
Grade 4	0	0		0	0	
Thrombi			0.69			0.46
Absent	95.8	92.9		68	78.6	
Present	4.2	7.1		32	21.4	
Vascular lesions			**0.02**			0.16
Grade 0	4.2	0		0	0	
Grade 1	70.8	35.7		24	7.1	
Grade 2	25	64.3		76	92.9	
Arteriolonecrosis			0.21			0.26
Grade 0	58.3	28.6		16	0	
Grade 1	37.5	64.3		48	50	
Grade 2	4.2	7.1		36	50	
Stroma necrosis			0.71			0.17
Grade 0	0	0		0	0	
Grade 1	16.7	7.1		8	0	
Grade 2	41.7	50		8	0	
Grade 3	37.5	42.9		20	28.6	
Grade 4	4.2	0		64	71.4	
Inflammation			0.46			**0.01**
Grade 0	75	85.7		40	85.7	
Grade 1	12.5	7.1		24	7.1	
Grade 2	12.5	7.1		36	7.1	
Luminal PBG injury			0.31			**0.02**
Grade 0						
Grade 1	0	0		4	0	
Grade 2	29.2	7.1		16	0	
Grade 3	16.7	28.6		24	7.1	
Grade 4	20.8	35.7		24	35.7	
No PBGs	29.2	21.4		28	57.1	
	4.2	7.1		4	0	
Deep PBG injury			0.21			0.60
Grade 0	16.7	0		12	0	
Grade 1	50	42.9		48	42.9	
Grade 2	4.2	28.6		12	35.7	
Grade 3	20.8	7.1		12	14.3	
Grade 4	0	7.1		8	7.1	
No PBGs	4.2	14.3		8	0	

Abbreviations: PBG, peribiliary gland; MP, machine perfusion.

SUPPLEMENTARY FIGURES

A

	Before MP						
	ANOVA		Bonferroni's Post-hoc (p)				
	F	P	Donor CNT-Healthy CNT	Donor CNT – T before	Donor CNT – NT before	Healthy CNT – T before	Healthy CNT – NT before
PBG mass	10.67	**0.00**	**0.00**	NS	NS	**0.00**	**0.00**
Ki-67	2.64	0.06	NS	NS	NS	NS	NS
Glut-1	1.30	0.29	NS	NS	NS	NS	NS
PAS	2.04	0.13	NS	NS	NS	NS	NS
Outer PVP layer	0.72	0.55	NS	NS	NS	NS	NS
Middle PVP layer	2.81	**0.05**	NS	NS	NS	NS	NS
Inner PVP layer	6.86	**0.00**	NS	**0.00**	**0.00**	NS	NS
PBG MVD	0.65	0.59	NS	NS	NS	NS	NS

B

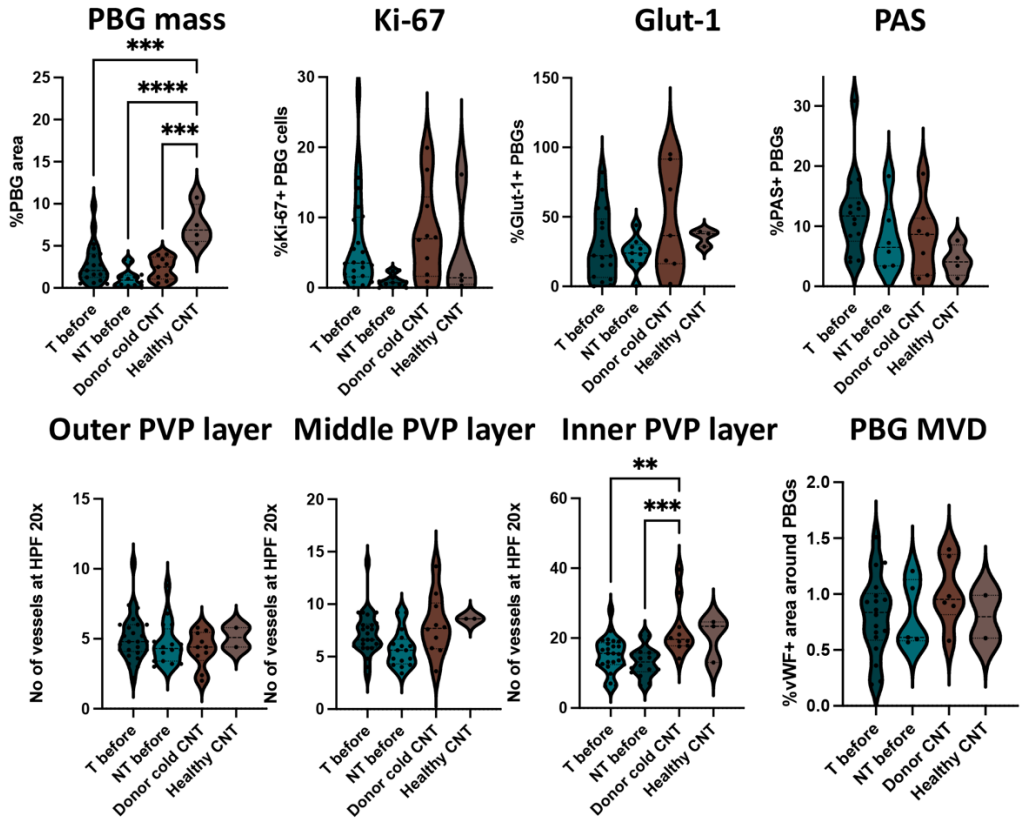

Figure S1. Biliary histological parameters before machine perfusion. (A) One-way ANOVA and Bonferroni's post hoc test results for the main histological parameters. Bile duct biopsies taken directly after static cold storage of donor livers that met the regular criteria for transplantation (Donor CNT) and biopsies from healthy donor livers (Healthy CNT) were

compared to bile duct biopsies taken directly after static cold storage from extended criteria donor livers that were included in this study. These livers were either transplanted (T before) or not transplanted (NT before) after machine perfusion. **(B)** Histograms of the main histological parameters. Abbreviations: MVD, microvascular density; PBG, peribiliary gland; NS, not significant; PVP, peribiliary vascular plexus. **$p<0.01$;*** $p<0.001$;****$p<0.0001$.

A

	After MP						
	ANOVA		Bonferroni's Post-hoc (p)				
	F	P	Donor CNT-Healthy CNT	Donor CNT – T after	Donor CNT – NT after	Healthy CNT – T after	Healthy CNT – NT after
PBG mass	8.92	**0.00**	**0.00**	NS	NS	**0.00**	**0.00**
Ki-67	8.21	**0.00**	NS	**0.00**	**0.00**	NS	NS
Glut-1	3.52	**0.02**	NS	NS	NS	NS	NS
PAS	0.78	0.51	NS	NS	NS	NS	NS
Outer PVP layer	0.68	0.57	NS	NS	NS	NS	NS
Middle PVP layer	1.82	0.16	NS	NS	NS	NS	NS
Inner PVP layer	2.95	0.04	NS	NS	NS	NS	NS
PBG MVD	2.07	0.13	NS	NS	NS	NS	NS

B

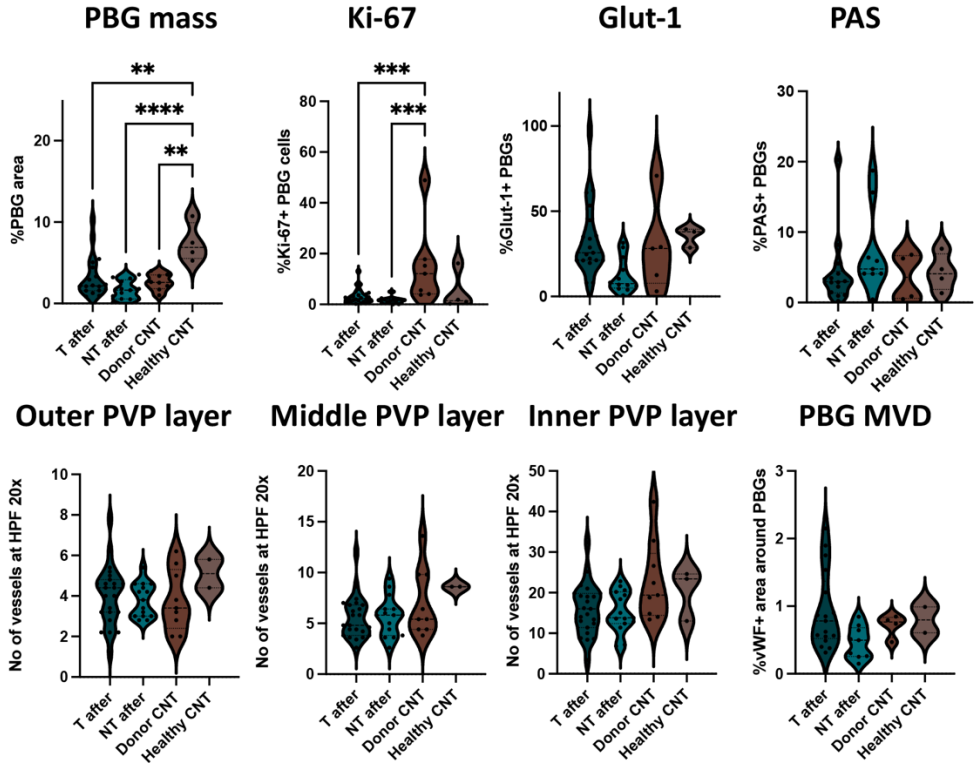

Figure S2. Biliary histological parameters after machine perfusion. (A) One-way ANOVA and Bonferroni's post hoc test results for the main histological parameters. Bile duct biopsies taken after reperfusion of donor livers that met the regular criteria for transplantation (Donor CNT) and biopsies from healthy donor livers (Healthy CNT) were compared to bile duct biopsies taken after machine perfusion from extended criteria donor livers that were

included in this study. These livers were either transplanted (T after) or not transplanted (NT after) after machine perfusion. **(B)** Histograms of the main histological parameters. Abbreviations: MVD, microvascular density; PBG, peribiliary gland; NS, not significant; PVP, peribiliary vascular plexus. **$p<0.01$;***$p<0.001$;****$p<0.0001$.

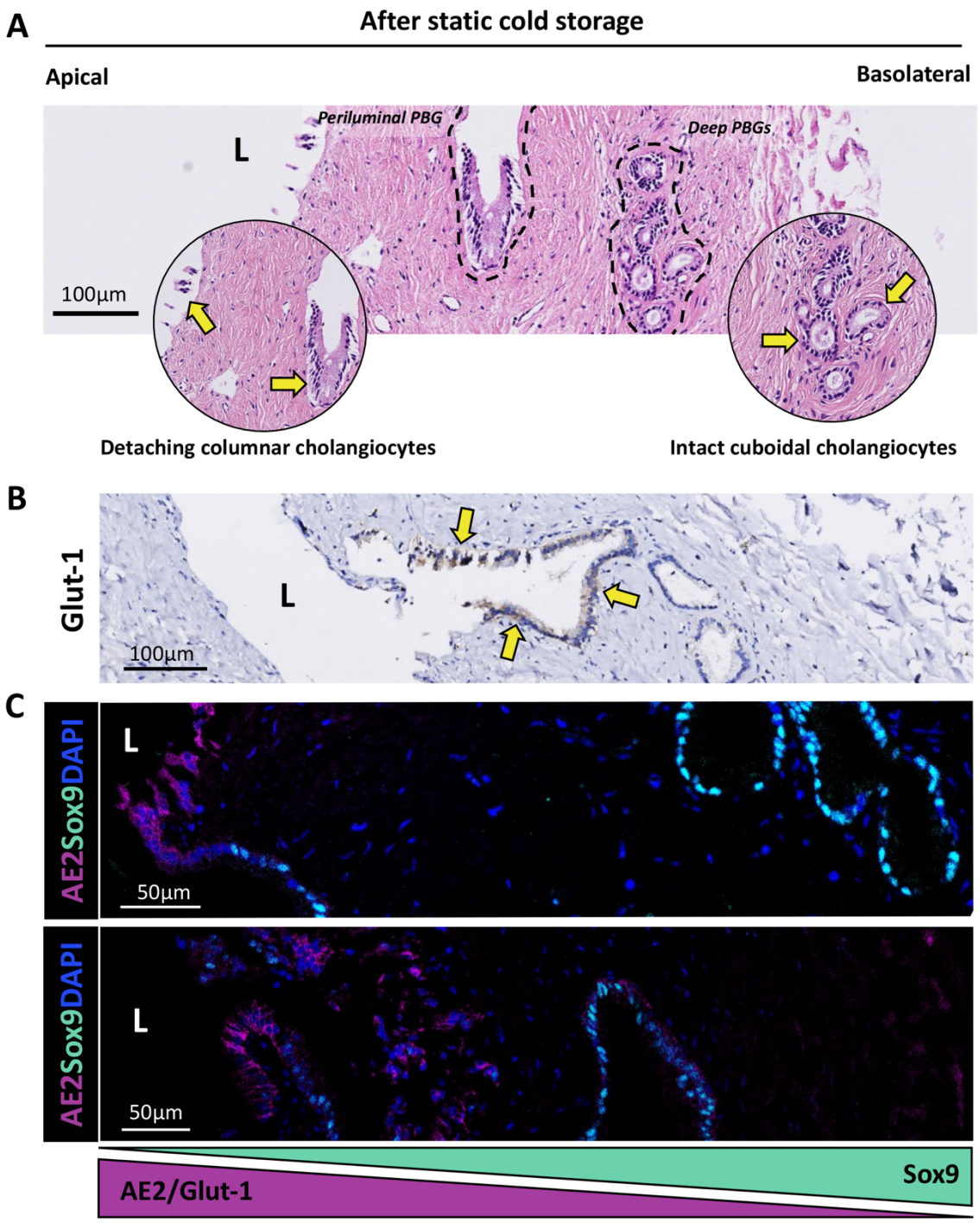

Figure S3. Biliary epithelial cell maturation along the radial axis. (A) Hematoxylin and eosin (H&E) staining of a bile duct specimen before machine perfusion. **(B)** Immunohistochemistry for Glut-1. **(C)** Immunofluorescence of AE2 and Sox9 and a schematic overview of their location in the bile duct wall.