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SUPPLEMENTARY INFORMATION

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An integrated perfusion machine preserves injured human livers for 1 week

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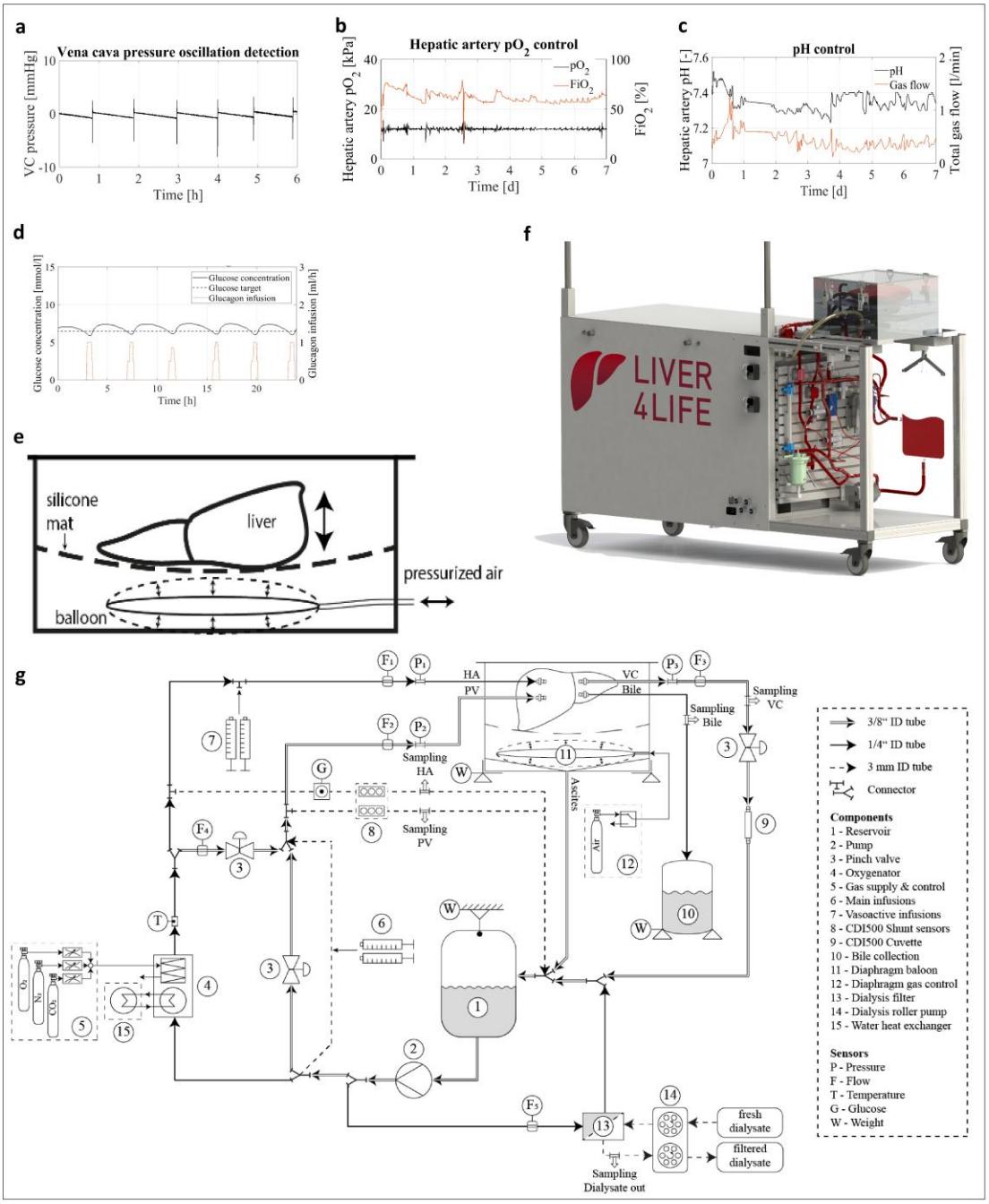
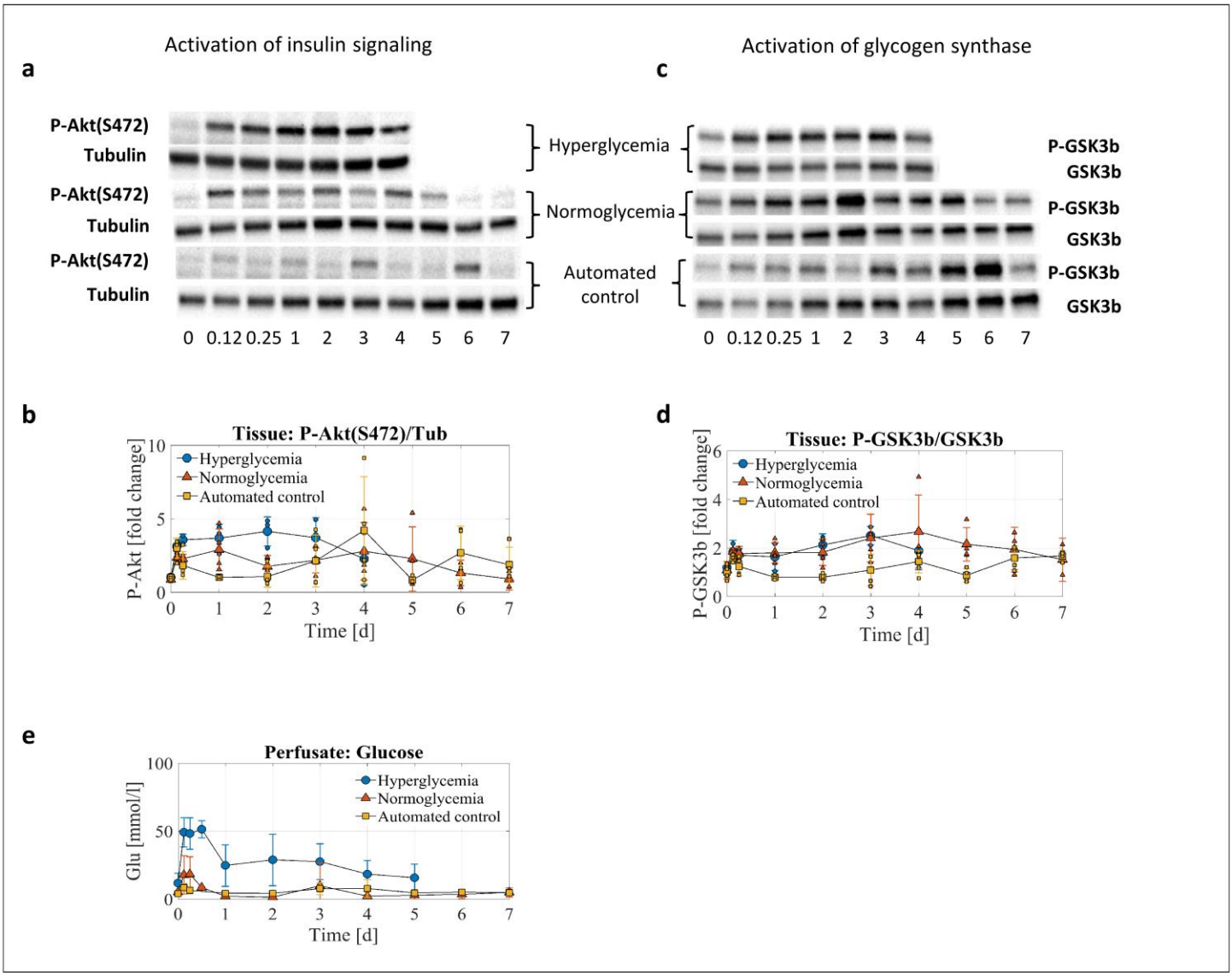


Supplementary Figure 1

Machine specifications.

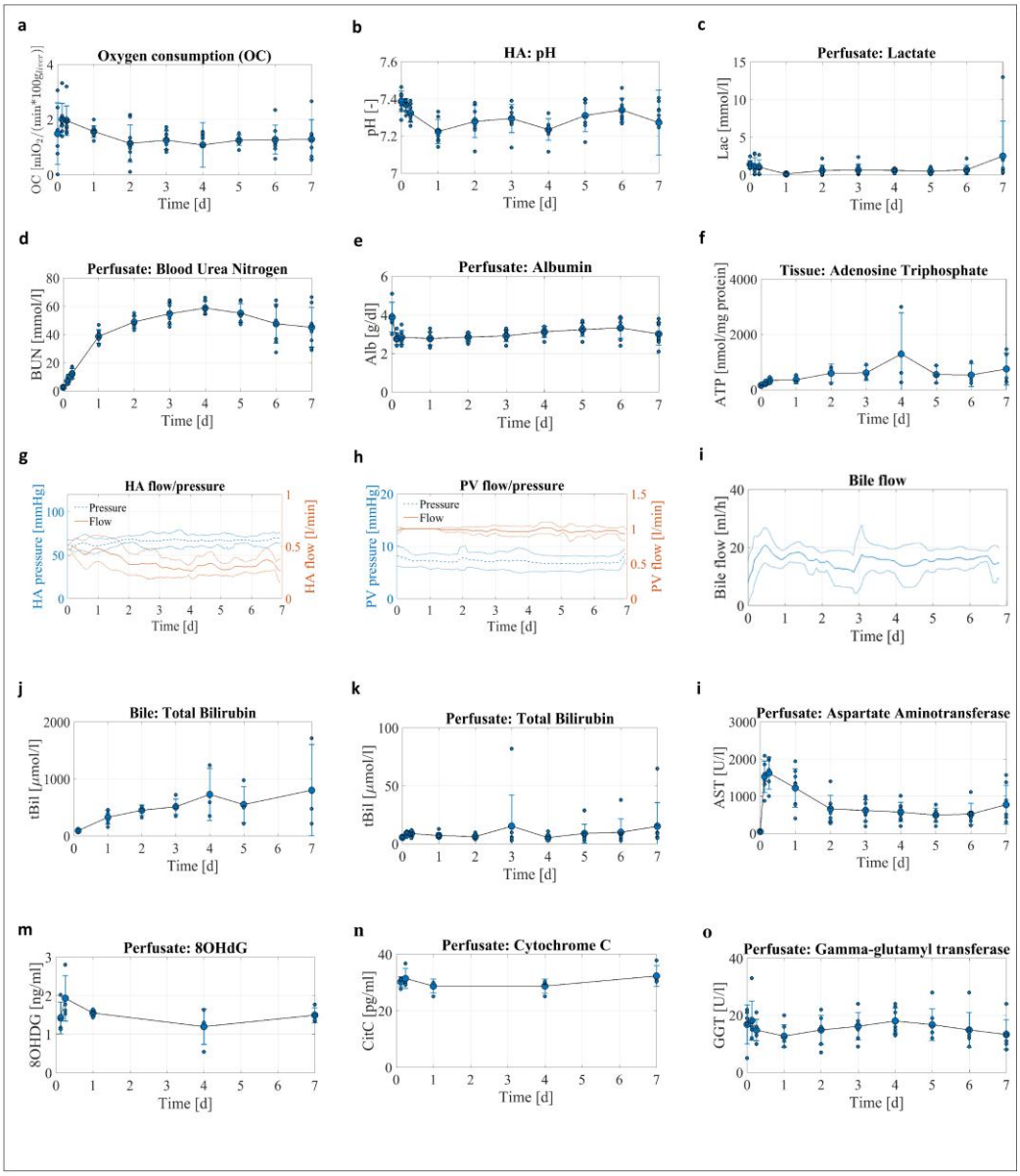
a, A representative graphic showing the continuous reduction of the caval pressure until the fluctuation point (pressure <0 mmHg) is reached. After detection of the fluctuation point, the vena cava (VC) target pressure is raised by 1 mmHg. For further details please refer to the Methods. **b**, Representative illustration of the pO_2 control, where the system automatically adapts FiO_2 of the gas supply to keep a targeted pO_2 in the hepatic artery (HA). **c**, Representative illustration of the control system adjusting the wash-out rate of CO_2 from the perfusate and thereby, controlling the pH. **d,** Representative illustration of the automated glucagon infusion. The glucagon supply is integrated as a safety feature to prevent severe hypoglycemia. Glucagon is injected, if the desired glucose level is undershot after spontaneous glucose level recovery was not sufficient. **e**, Illustration of periodic liver movement with pressurized air. **f,** Illustration of the current mobile laboratory prototype with internal gas and power supply. Liver4Life refers to the name of our research group. **g**, Detailed schematics of the perfusion loop. The functions of the integrated components were described in the Methods of the manuscript.



Supplementary Figure 2

Glucose metabolism in pig livers

The activation of insulin signaling pathway during perfusion for the “hyperglycemic” (n=4 pig livers), “normoglycemic” (n=4 pig livers) and “automated control” (n=4 pig livers) groups. **a-b**, Insulin induces phosphorylation of Akt and an activation of its signaling pathway. **a,** P-Akt Western blot analysis and **b,** quantification. **c-d**, Glycogen synthase activation depending on insulin application in each study group. P-Akt induces phosphorylation and inactivation of GSK3b, leading to the activation of glycogen synthase. **c**, P-GSK3b Western blot analysis and **d**, quantification. **e**, Glucose level during perfusion for every study group. (hyperglycemic, normoglycemic, automated control). Data reported as mean ± s.d.

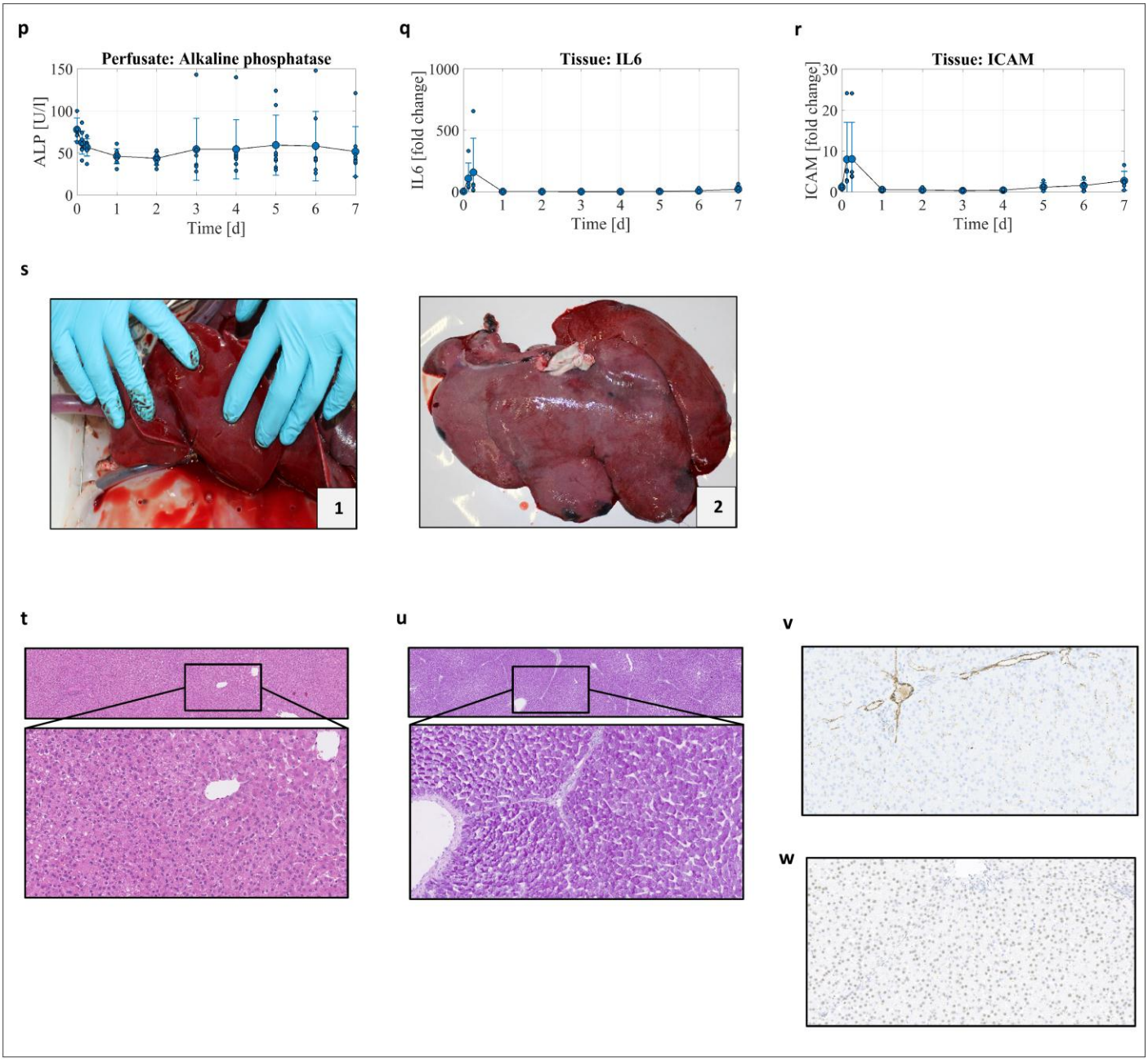


Supplementary Figure 3

Pig liver performance during 7 days *ex vivo* perfusion (n=8 pig livers).

n=8 pig livers for measurements in perfusate and n=5 pig livers for measurements in tissue. Livers with the intention to transplant (n=3 pig livers) were not biopsied on a daily basis during perfusion to prevent bleeding after transplantation.

a, b, Oxygen consumption and pH: Perfused pig livers consumed a substantial amounts of oxygen (**a**) and maintained mean pH >7.2 (**b**). **c**, Lactate clearance: Compared to the perfusion of human livers with a high lactate at start caused by the packed blood products, the pig blood was collected freshly with a minimal storage time. Thus, lactate was less than 2 mmol/l at perfusion start. **d, e, f,** Synthetic functions: Perfused livers produced blood urea nitrogen (BUN) (**d**) and maintained albumin within physiologic levels (**e**). ATP synthesis in tissue shown as a parameter of maintenance of cell energy (**f**). **g**, Flow and pressure in the hepatic artery (HA). **h**, Flow and pressure in the portal vein (PV). **i**, Continuous bile flow was present in all of the eight pig livers. **j, k** Total bilirubin level in bile (**j**) and blood (**k**). **l, m, n, o,** Injury markers: The initially increased injury marker AST declined during perfusion (**l**). 8-Hydroxydesoxyguanosin (8OHdG)(n=5) presented as an injury marker for DNA (**m**) and Cytochrome C representing an injury marker for mitochondria (**n**), (n=5). **o**, One week course of Gamma-glutamyl transferase. Data reported as mean ± s.d.

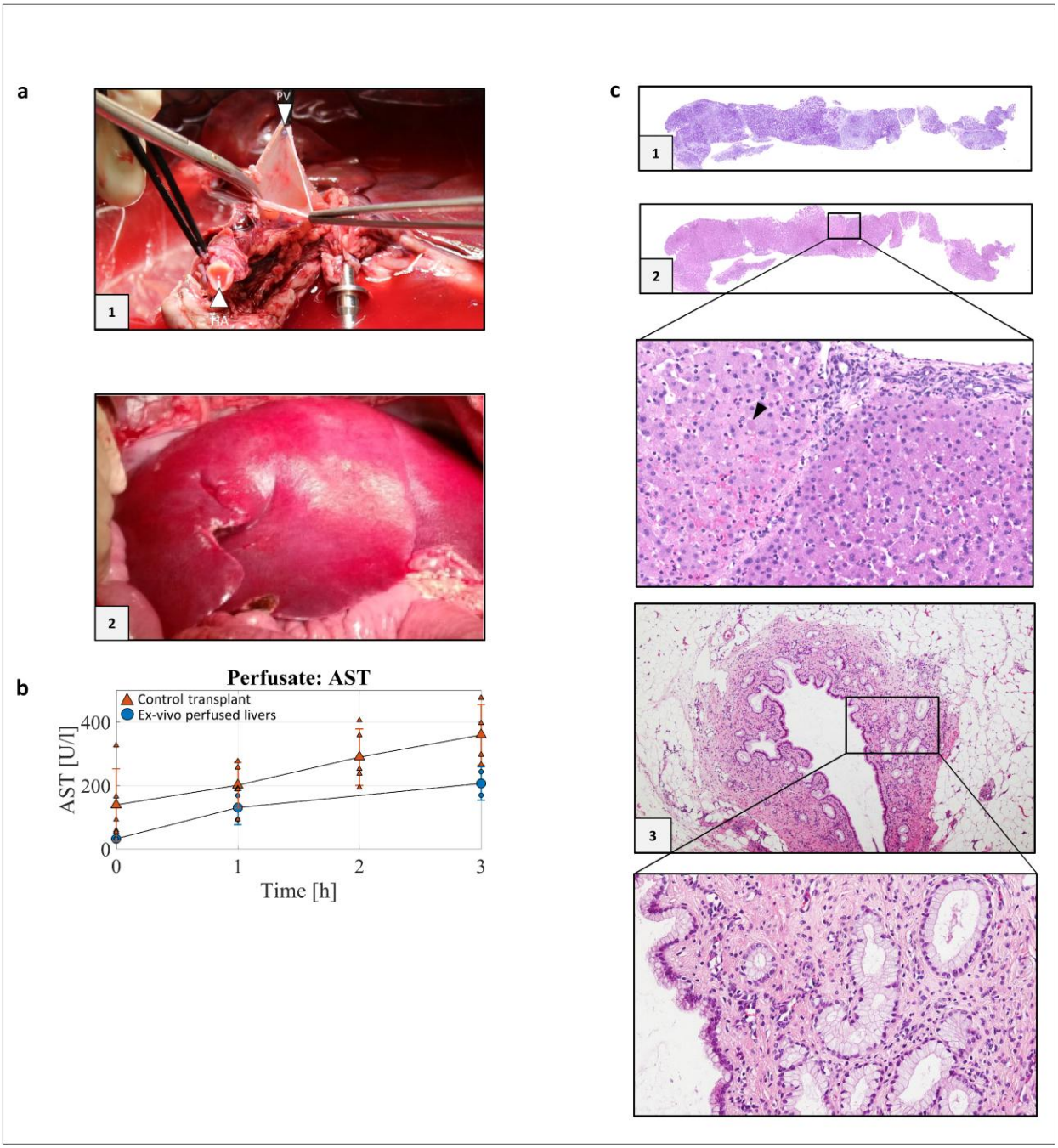


Supplementary Figure 4

Pig liver performance during 7 days ex vivo perfusion (n=8 livers).

n=8 pig livers for measurements in perfusate and n=5 pig livers for measurements in tissue. Livers with the intention to transplant (n=3 pig livers) were not biopsied on a daily basis during perfusion to prevent bleeding after transplantation.

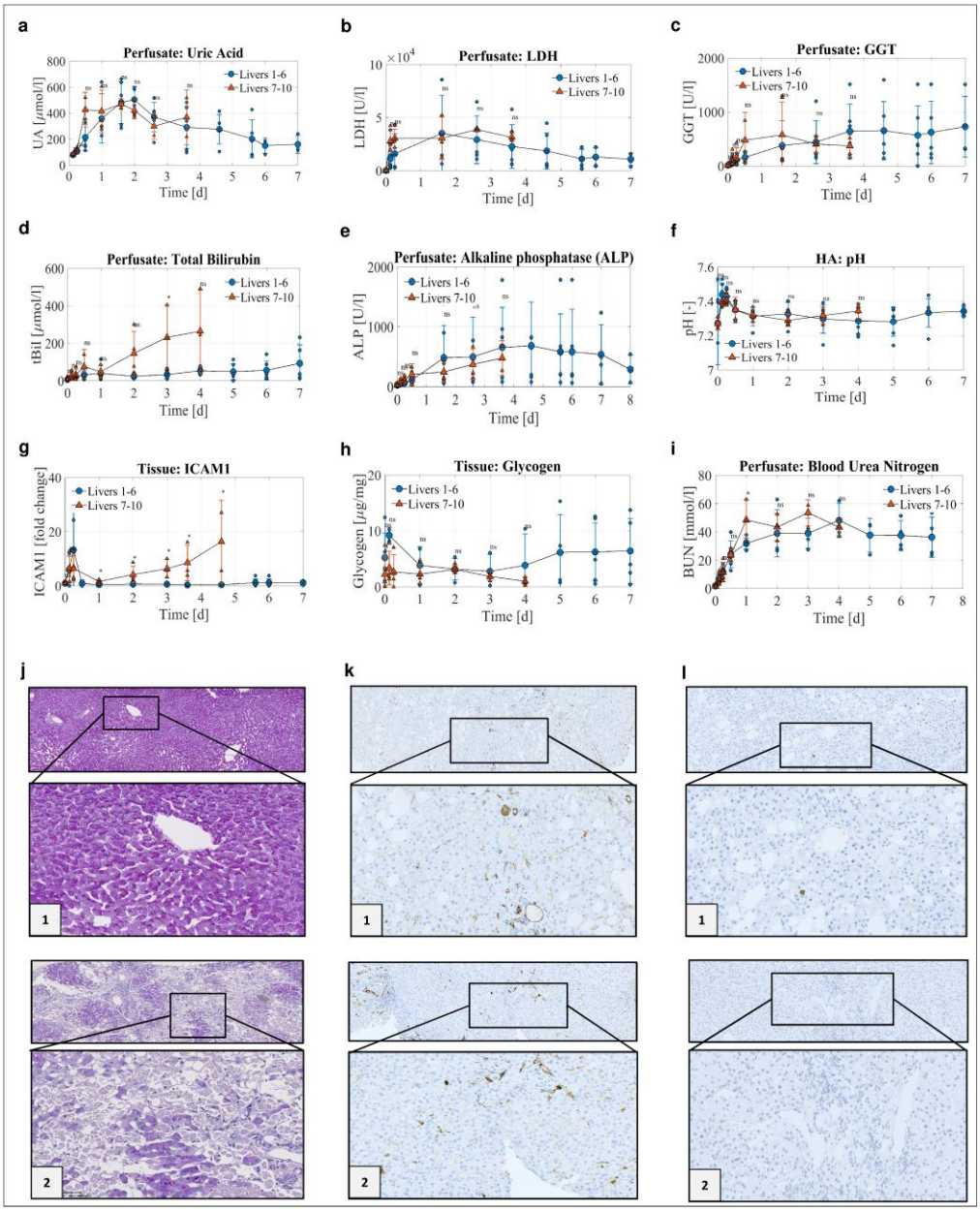
p, Cholestasis marker alkaline phosphatase (ALP) remained low in the perfusate during 7 days. **q,** Inflammation marker IL-6 in tissue illustrated as fold change at mRNA level. **r**, Intercellular adhesion molecule 1 (ICAM-1) as a marker of endothelial cell activation shown as fold change at mRNA level in tissue. **s**, Representative macroscopic view on day 7 of perfusion with the contact areas presented (**1**) and shortly after termination of perfusion (**2**). Dark areas correspond to biopsy spots during perfusion. **t, u, v, w,** Representative histology slides on day 7: Preserved liver integrity shown on H&E staining (**t**) with preserved glycogen seen on PAS staining (**u**) (slides shown in 5x and 20x magnification). **v**, Endothelial cells were not activated as shown with von Willebrand immunohistochemistry staining (20x magnification). Caspase 3 staining showing the absence of relevant cell apoptosis on day 7. Data reported as mean ± s.d.



Supplementary Figure 5

Pig liver transplantation after 7 days of ex-vivo perfusion (n=3 pig livers after ex vivo perfusion, n=5 pigs as control after standard cold storage). Representative images and histology were shown only for ex vivo perfused livers.

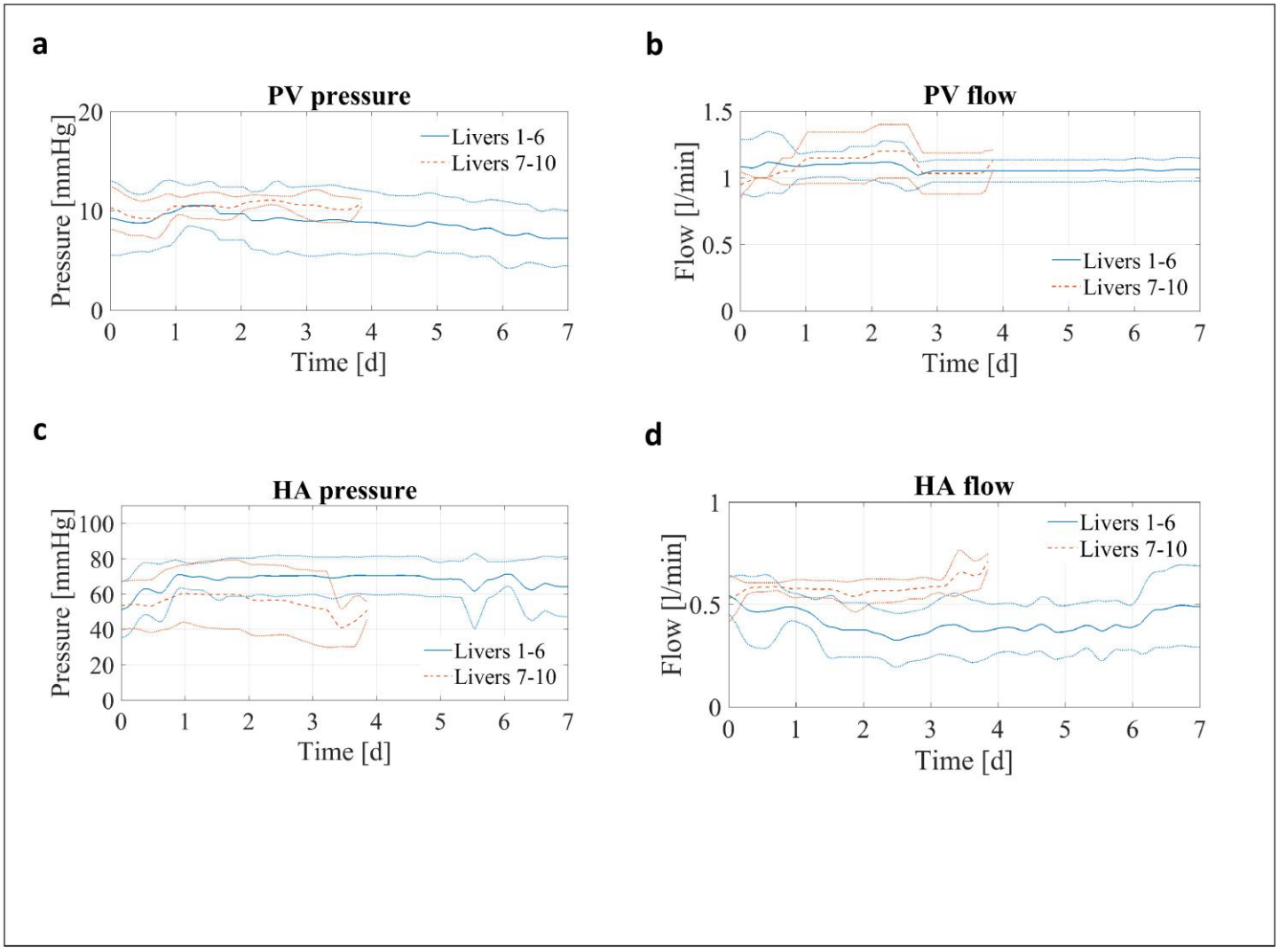
a, Representative macroscopic view: The portal vein (PV) and hepatic artery (HA) during back-table preparation after ex vivo perfusion (1). Liver after reperfusion (2). **b**, AST release during the first 3 post-transplant hours (n=3) compared to control transplants without ex vivo perfusion (n=5). **c**, Representative core needle biopsies at 3 hours of reperfusion showing retained glycogen storages on PAS staining (1) and preserved liver architecture on H&E staining (2). Higher magnification (20x) shows vital hepatocytes but with few apoptotic cells (arrow)). Representative extrahepatic bile duct after reperfusion on H&E staining (5x and 20x magnification) showed a preserved epithelial lining and subepithelial glands on H&E staining (3). Data reported as mean ± s.d.



Supplementary Figure 6

Performance of human livers during 7 days *ex vivo* perfusion (n=10 livers).

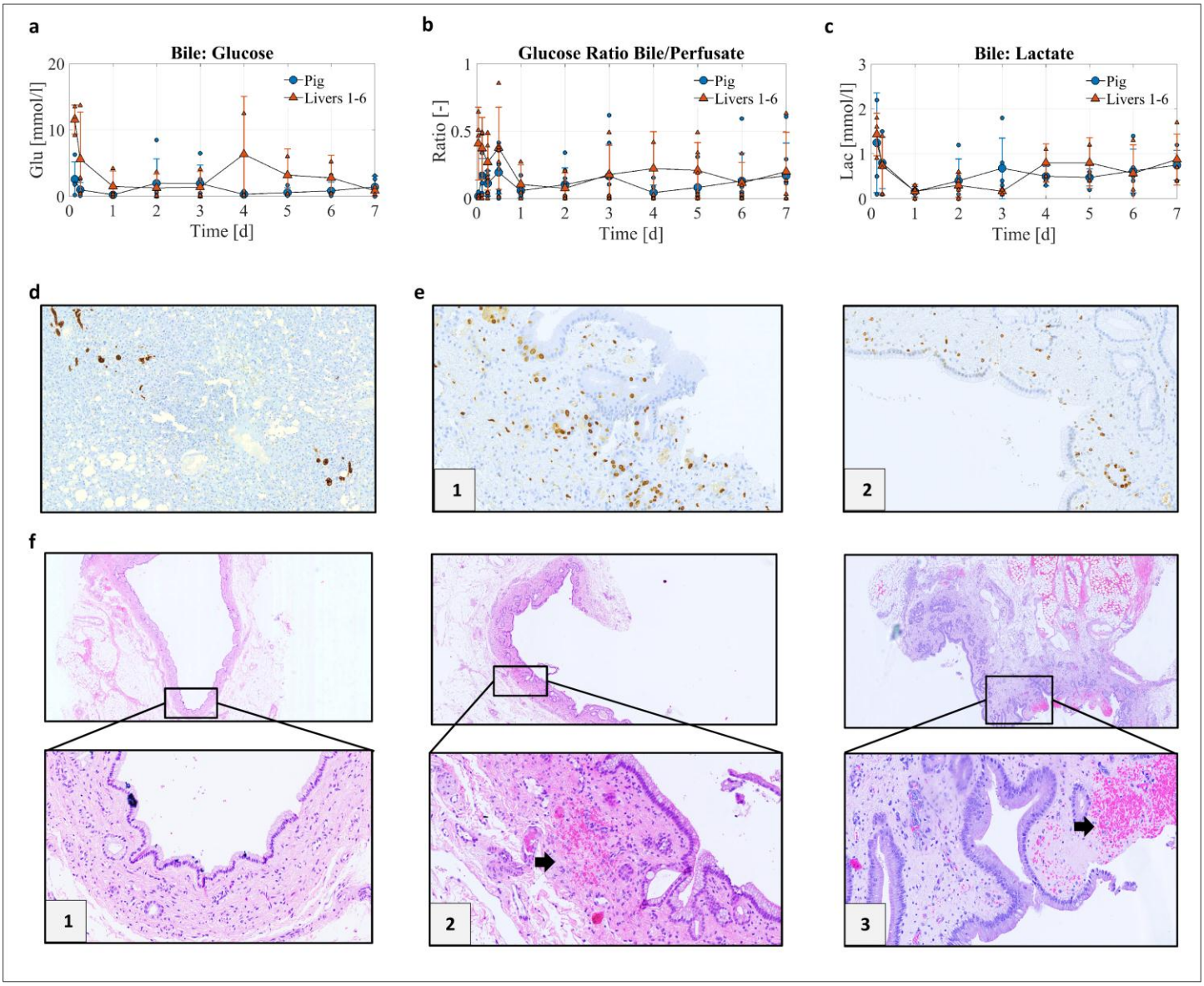
Human livers 1-6 (blue line, n=6 livers), human livers 7-10 (red line, n=4 livers)). **a, b, c, d,** Injury marker release into perfusate shown for uric acid (UA) (**a**), lactate dehydrogenase LDH (**b**), gamma-glutamyl transferase (GGT) (**c**) and total bilirubin (**d**). Increase of GGT and total bilirubin was observed with some delay, similar to the clinical setting. **e**, Course of Alkaline phosphatase level in perfusate. **f**, pH maintenance in both groups without significant difference. **g**, ICAM-1 course shown as fold change at mRNA level in tissue. **h**, Glycogen amount in tissue measured chemically. **i**, Blood urea nitrogen (BUN) level in perfusate. **j, k, l,** Representative histology slides at the end of the experiment. (Slides shown in 5x and 20x magnification): preserved glycogen stores on PAS staining in livers 1-6 (**j1**)**.** Scattered loss of tissue glycogen in necrotic areas of livers 7-10. (**j2**). Endothelial cells were not activated as shown with von Willebrand immunohistochemistry staining (livers 1-6 **k1,** livers 7-10 **k2**). Caspase 3 staining showing the absence of relevant cell -apoptosis on day 7 (livers 1-6 **l1,** livers 7-10 **l2**). Data reported as mean ± s.d.. P-value *<0.05, **<0.01, *** <0.001. ns, not significant. For comparison of two groups two-tailed Student's t-test was used. Exact P values were provided in the Supplementary Table 3 for p values.



Supplementary Figure 7

Flow and pressure during one week perfusion of human livers (n=10 livers).

Human livers 1-6 (blue line, n=6 livers), human livers 7-10 (red line, n=4 livers). **a, b**, Pressure and flow in the portal vein (PV). **c, d,** Pressure and flow in the hepatic artery (HA). Data reported as mean ± s.d.. Livers 1-6: solid blue lines for mean value, dotted blue lines for s.d.. Livers 7-10: dashed red lines for mean value, dotted red lines for s.d.



Supplementary Figure 8

Bile duct viability in injured human livers (n=10) and healthy pig livers (n=8) during one week of perfusion.

Human livers 1-6 (red line, n=6 livers), pig livers blue line (n=5 livers). **a,** Glucose level in bile. **b,** Bile/perfusate glucose ratio ≤0.7 has been recommended as a reliable viability sign. During perfusion the mean ratio was <0.5. pH of the bile was not reported due to contact of bile with ambient air during collection. **c**, Lactate content of bile during perfusion. **d**, Intrahepatic bile ductuli shown with a staining for CK7 without signs of cholestasis in a human liver (10x magnification) in livers 1-6 (n=6 livers). Of note, human livers with cirrhosis or fibrosis showed bile duct metaplasia at perfusion start related to the primary liver disease**. e, f** Extrahepatic bile ducts from three representative experiments on day 7. The biopsy samples were taken close to the point, where the cannulas had been attached. **e 1 and 2,** Ki-67 immunohistochemistry staining demonstrated mitotic activity in extrahepatic bile ducts. **f 1, 2, 3**, Extrahepatic bile ducts disclosed some hemorrhagic changes of the surrounding soft tissue (black arrows) with less than 50% epithelial denudation and preserved submucosal glands on H&E staining. (Slides presented in 5x magnification and 20x magnification). Data reported as mean ± s.d.

	Seven days perfusion						Four days perfusion			
Livers	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Donor age	48	81	73	54	31	78	43	70	53	69
BMI (kg/m^2)	23	28	26	52	37	36	26	26	26	27
Cause of death	CVA	Trauma	CVA	CVA	Anoxia	Anoxia	Anoxia	Trauma	CVA	CVA
DBD/DCD	DBD	DCD	DBD	DBD	DCD	DCD	DCD	DCD	DCD	DCD
ICU stay (day)	17	6	18	2	5	9	21	6	11	1
Peak ALT/AST	82/121	80/89	133/196	26/35	385/481	61/31	3579/8882	236/490	70/69	12/20
Peak sodium >165 mmol/l	No	No	No	No	No	No	No	No	No	No
Peak bilirubin >3 mg/dl	No	No	No	No	No	No	Yes	No	No	No
Histology at start										
- Macro steatosis	<5%	<5%	<5%	25%	50%	<5%	<5%	<5%	<5%	10%
- Fibrosis grade	3	0	4	2	0	0	0	0	0	0
- Inflammatory infiltrates	yes	no	no	yes	no	no	yes	no	no	no
Functional WI (min)	n.a.	26	n.a.	n.a.	30	19	36	25	20	29
Asystolic WI (min)	n.a.	19	n.a.	n.a.	20	14	23	16	15	15
Total cold storage (min)	360	572	360	330	597	510	496	250	280	643
HOPE	No	Yes	No	No	Yes	Yes	Yes	No	Yes	Yes
Assessment of mitochondrial injury (complex I fragments during HOPE)(1-3)	-	high risk	-	-	high risk		high risk	-	high risk	high risk
Donor risk index	1.4	2.53	2.12	1.52	1.73	2.69	2.12	2.5	2.3	2.92
Reason for declining	fibrosis	mitochondrial injury	cirrhosis	fibrosis	mitochondrial injury	Amyloidosis	mitochondrial injury	poor flushout	mitochondrial injury	mitochondrial injury
Liver weight start/end (kg)	2.1 / 1.5	1.8 / 1.1	2.3 / 1.6	2.5 / 1.8	3.2 / 2.1	1.8 / 1.3	1.8 / 1.5	1.5 / 1.3	1.6 / -	2.6 / -

Supplementary Table 1. Human liver details

Targeted perfusion duration was 7 days. Livers 1-6 were perfused for targeted one week. Ongoing cell death with signs of liver failure were the reason for abortion of the perfusion within 4 days in livers 7-10. Hypothermic oxygenated perfusion (HOPE), cerebrovascular accident (CVA), donation after circulatory death (DCD), donation after brain death (DBD), Aspartate- and Alanine-Aminotransferase (AST, ALT).

1. Muller X, Schlegel A, Kron P, Eshmuminov D, Wurdinger M, Meierhofer D, et al. Novel Real-time Prediction of Liver Graft Function During Hypothermic Oxygenated Machine Perfusion Before Liver Transplantation. Ann Surg. 2019;270(5):783-90.
2. Chouchani ET, Pell VR, James AM, Work LM, Saeb-Parsy K, Frezza C, et al. A Unifying Mechanism for Mitochondrial Superoxide Production during Ischemia-Reperfusion Injury. Cell Metab. 2016;23(2):254-63.
3. Murphy MP. How mitochondria produce reactive oxygen species. Biochem J. 2009;417(1):1-13.

Perfusate preparation		
	Amount	**Comment**
Conserved red blood cells	~ 1.4 liter	conserved erythrocytes have high lactate at delivery
Fresh frozen plasma	~ 0.8 liter	Add 5000 unit heparin
Thrombocyte concentrate	1 unit	
Albumin 20 % solvent (Human Albumin)	Usually 100 to 300 ml required	Add to reach target albumin in perfusate >20g/l prior to perfusion start.
Dialysis		
	Rate	**Comment**
Prior liver connection	1000-2000 ml/h	Correct pH (~7.2), normalise sodium, adjust hematocrit (target 27-30%)
During perfusion	200 ml/h	Dialysate solution: multiBIC, Fresenius Medical Care
Additives		
Bolus infusions at perfusion start	**Dose**	**Comment**
Piperacillin-Tazobactam (Sandoz)	2.2 g	
Solu-Medrol (Pfizer, Methylprednisolonum)	500 mg	
Bicarbonates	**-**	Add bicarbonates if pH< 7.0 with start of dialysis to correct pH.
Constant infusions during perfusion		
Piperacillin-Tazobactam (Sandoz)	2.2g/24 h	Concentration: 2.2 g in 24 ml solvent.
Solu-Medrol (Pfizer, Methylprednisolonum)	500 mg/24h	Concentration: 500 mg in 24 ml solvent
Heparin-NA (B. Braun Medical AG, Heparin)	Adjustable	Concentration: 1000 U/ml. Target active clotting time (ACT >300 s.)
Nutriflex special 70/240 (B. Braun Medical AG, Parenteral Nutrition)	15 ml/h	Only amino acid bag (upper bag). No glucose (do not open lower bag containing glucose).
Ursodeoxycholic acid (PCA, bile acid)	3.4 g/24h	Concentration: 3.4 g in 24 ml solvent.
Controlled infusions		
Actrapid (Novo Nordisk Pharma, Human insulin)	Automated to control perfusate glucose	Concentration: 0.45 U/ml
GlucaGen, (Novo Nordisk Pharma, Glucagon)	Automated to control perfusate glucose	Concentration: 0.111 U/ml
Neo-Synephrine HCL (Ospedalia AG, Phenylephrin)	Automated to control HA resistance	Concentration: 0.4 mg/ml
Flolan (GlaxoSmithKline, Epoprostinolium)	Automated to control HA resistance	Concentration: 4 ug/ml

Supplementary Table 2. Perfusate constitute and additives for human liver perfusion