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Consistent Liver Function Support from Gene-Edited Pigs to Non-Human Primate Acute Liver Failure Models via Extracorporeal Liver Cross-Circulation

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Article

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Consistent Liver Function Support from Gene-Edited Pigs to Non-Human Primate Acute Liver Failure Models via Extracorporeal Liver Cross-Circulation

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Abstract

Acute liver failure (ALF) and acute-on-chronic liver failure (ACLF) are severe, rapidly progressing conditions. Existing non-biological artificial liver support has limited efficacy, and liver transplantation is constrained by donor shortage and the time window. Extracorporeal liver cross-circulation (ELC) using gene-edited (GE) pigs offers a promising bridging therapy. Here, six Tibetan macaque ALF models were connected via ELC to 8GE pig livers under normothermic machine perfusion for 74–144 h to assess feasibility and functional support. Basic vital signs of the recipients, including heart rate, body temperature, and mean arterial pressure, remained relatively stable. Their AST/ALT rose then declined, total bilirubin trended upward, albumin and total protein remained stable, and bile secretion was continuous, indicating effective replacement of impaired liver function. INR and fibrinogen remained relatively stable, while platelets dropped sharply and stayed low, suggesting generally maintainable coagulation but thrombocytopenia. Complement, immunoglobulins, and B/T/NK cells remained low, indicating good immunosuppression. Donor liver injury was mainly ischemia/reperfusion- and inflammation-mediated, without typical hyperacute or acute rejection. In summary, 8-GE pig livers under room-temperature ex vivo cross-perfusion maintained stable vital signs in ALF recipients, supported liver function for >72 h in all six recipients (up to 144 h), and effectively avoided hyperacute and acute rejection, providing a feasible strategy and key data for the safety and efficacy of clinical ELC.

Key words: Xenogeneic liver perfusion; Normothermic machine perfusion; Acute liver failure; Coagulation dysfunction; Inflammatory response; Non-human primate model

1. Introduction

Acute liver failure (ALF) and acute-on-chronic liver failure (ACLF) are characterized by acute decompensation of hepatocyte function, hyperactive systemic inflammatory response, and multiple organ dysfunction. They can rapidly induce accumulation of toxic metabolites, coagulopathy, and metabolic disturbance, and the condition often progresses swiftly to hepatic encephalopathy, circulatory failure, and even death[1,2]. Bernal et al. observations of more than 50 patients with severe ACLF showed that those who did not receive timely transplantation all died within 7 days of registration, whereas transplanted patients achieved an 81% survival rate at 212 days of follow-up[3], highlighting the decisive significance of the time window in treatment. However, the donor liver procurement cycle, preoperative evaluation procedures, and surgical tolerance in critically ill states all severely limit the clinical accessibility of this window. Therefore, the therapeutic goal for ALF/ACLF should not rest on short-term correction of individual parameters, but should be committed to securing a genuinely effective bridging opportunity for liver function recovery or liver transplantation before irreversible multiple organ failure occurs.

Liver transplantation can rebuild long-term liver function through whole-organ replacement, but its widespread use is limited by donor shortage, waiting time, surgical risk, and patient tolerance, making it difficult to cover rapidly progressing patient populations. Non-biological artificial liver systems, represented by plasma exchange, albumin dialysis, and the molecular adsorbent recirculating system, can only remove some water-soluble toxins and cannot assume integrated liver functions such as synthesis, metabolism, detoxification, and immune regulation, with limited clinical survival benefit[4-6]. Although hepatocyte-based bioartificial livers have metabolic potential, they remain constrained by cell sources, effective cell numbers, functional stability, and uncertain efficacy[7,8]. Therefore, there is an urgent need for a liver replacement approach that can provide genuine biosynthetic and metabolic support. In contrast, artificial liver support and extracorporeal liver cross-circulation (ELC) utilize

an intact extracorporeal donor liver to provide comprehensive support for metabolism, detoxification, synthesis, and exocrine secretion, and allow real-time monitoring of blood flow, bile secretion, and immune and coagulation responses during operation, with timely termination when risks rise. Combining functional integration with operational controllability, this approach is expected to overcome the integrated-function shortcomings of existing modalities and provide a bridging therapy pathway with greater clinical translation prospects for ALF/ACLF patients. In recent years, gene-edited pig livers have been shown, in a 10-day continuous observation of pig-to-brain-dead patient[9] and in 38 days of normal functioning in auxiliary liver transplantation from pig to living patient[10], to effectively perform liver functions including synthesis, secretion, metabolism, and detoxification, and to achieve controllable immune rejection under conventional immunosuppressive regimens, fully demonstrating the unlimited availability and effectiveness of gene-edited pigs as a donor liver source for ELC.

Recently, major breakthroughs have been made in preclinical research on xenogeneic extracorporeal liver support (ELC). In an ELC study using 11 gene-edited pig livers in four brain-dead human decedents, the xenogeneic pig livers maintained parenchymal structural integrity, produced bile, supplemented hepatocyte function, and maintained hemodynamic stability and stability of key parameters such as pH, lactate, ammonia, and the international normalized ratio in the anhepatic state[11]. Subsequently, multi-omics studies of this case further revealed the mechanisms related to immune cell infiltration, complement dynamics, coagulation responses, and thrombocytopenia in human–pig ex vivo cross-circulation, providing a systematic biological basis for optimizing the xenogeneic liver support platform[12]. Meanwhile, the U.S. Food and Drug Administration (FDA) has cleared a Phase 1 clinical trial of the donor pig developed by eGenesis in combination with the OrganOx ELC system, planning to enroll 20 patients with acute-on-chronic liver failure (ACLF) complicated by hepatic encephalopathy (https://egenesisbio.com/news-media/press-releases/egenesis-and-

organox-announce-u-s-fda-clearance-of-ind-application-for-the-treatment-of-patients-with-acute-on-chronic-liver-failure). In China, in April 2026, the National Health Commission issued the Guidelines for the *Filing of Clinical Research on New Xenotransplantation Technologies (Version 1)*, which explicitly stipulates that, in the evaluation of liver graft function, for extracorporeal liver perfusion support therapy in non-human primate acute liver failure models, the normothermic perfusion treatment time of pig livers should be no less than 72 hours, and no fewer than 6 cases should be conducted, setting clear feasibility standards for the ELC strategy to move toward clinical application.

Our team previously successfully constructed 8-GE donor pigs, with knockout of 3 xenogeneic antigens and overexpression of 5 human genes (hCD46, hCD55, hCD59, hTBM, and hCD39), and validated the effectiveness of 8-GE pigs in a non-human primate kidney xenotransplantation model[13]. In this study, 8-GE donor pigs were used to perform liver ELC in 6 monkey models of liver failure, confirming the effectiveness of gene-edited pigs for rescuing patients with acute liver failure and laying the foundation for xenogeneic liver ELC to enter clinical practice.

2. Materials and Methods

2.1 Animal Ethics

The 8-gene-edited pigs were bred by the Yunnan Province Key Laboratory for Porcine Gene Editing and Xenotransplantation, female, weighing 20-40 kg. Tibetan macaques were purchased from the Institute of Laboratory Animals of Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital. All animal experiments were approved by the Life Science Ethics Committee of Yunnan Agricultural University (approval number: APYNAU202511004).

2.2 Construction of the Non-Human Primate Model of Liver Failure

Six male Tibetan macaques weighing 13-17 kg were screened by cross-matching to establish non-human primate models of acute liver failure (hereinafter referred to as recipients). The acute liver failure monkey model was constructed by ligating and

transecting the hepatic artery and common bile duct and restricting portal vein flow (narrowing the main portal vein to 40%-50% of its original circumference, reducing the portal vein cross-sectional area by approximately 3/4)[14]. In this study, under the premise of confirmed irreversible anatomical blockade for no less than 18 hours, the recipient acute liver failure model was considered successfully established and extracorporeal pig liver perfusion was initiated when any one of the following conditions was met: serum ALT/AST >1000 U/L, indicating hepatocyte injury; total bilirubin at 2 times the baseline value, designated as metabolic dysfunction; or INR >1.5 or PT at 1.5 times the baseline value, designated as coagulopathy. Before starting ELC, the donor livers were confirmed to meet functional criteria (perfusate lactate <2.8 mmol/L; pH >7.2; alanine aminotransferase <6000 IU/L; evidence of glucose consumption; portal vein flow >500 mL/min).

In the first cross-perfusion, to test the reliability and stability of the perfusion equipment, we first established an extracorporeal circulation circuit between the pig liver and the Tibetan macaque and observed for 26 hours, during which the recipient monkey's vital signs remained stable. Subsequently, we constructed the model by ligating and transecting the hepatic artery and common bile duct and restricting portal vein flow, and continued to maintain extracorporeal normothermic machine cross-perfusion.

In the second cross-perfusion, we first performed acute liver failure modeling. To reserve a certain therapeutic window for acute liver failure, cross-perfusion was started when relevant indicators were found to be elevated but had not yet reached the set thresholds. The recipient's vital signs subsequently remained stable, preliminarily confirming the feasibility of this strategy. Based on the feasibility established in the first two cases, from the third case onward, we strictly followed the above indicators for acute liver failure modeling and carried out extracorporeal normothermic machine cross-perfusion.

2.3 Normothermic Pig Liver Perfusion and Extracorporeal Liver Cross-Circulation

After anesthesia, the donor pigs underwent a cruciate incision for abdominal access. The portal vein and abdominal aorta were perfused with HTK solution and normal saline at low temperature until the liver turned clay-yellow and the effluent became clear. After procurement, the donor liver was trimmed to preserve the main portal vein, the hepatic artery to the abdominal aorta segment, and the common bile duct and gallbladder were cannulated with drainage tubes. The pig liver was then placed on an automated normothermic liver perfusion device (TH-LS LIV100, True Health Medical Technology Development Co., China) and primed with monkey washed red blood cells, artificial blood, and perfusate for closed-circuit continuous perfusion, with cold ischemia time controlled within 100 minutes.

Before connecting the perfusion circuit to the recipient monkey's circulation, extracorporeal hemofiltration of the circuit was performed to adjust the electrolyte concentrations in the perfusate (especially potassium) to the normal range. The device maintained continuous inflow and outflow by placing a double-lumen venous catheter in the recipient monkey's femoral vein (both inflow and outflow of perfusion passed through this femoral vein double-lumen catheter). The flow rate was gradually increased to 400 mL/min within the first 80 minutes after the start of ELC and maintained at this exchange rate during the subsequent connection period. The system allowed continuous observation of the extracorporeal liver, observation of bile characteristics and accurate measurement of bile production, and collection of blood samples before, during, and after perfusion. To prevent circuit coagulation, the heparin infusion rate was adjusted to maintain the activated clotting time (ACT) target at 160-200 s.

The study endpoint was considered reached when any of the following occurred: (i) mean arterial pressure (MAP) could not be maintained >40 mmHg despite norepinephrine at 2 μg/kg/min, or (ii) uncorrectable malignant arrhythmia or cardiac arrest occurred, or (iii) extracorporeal liver perfusion failed, or (iv) the study objective had been achieved. At the end of the study or upon reaching other endpoints, the

recipients were euthanized by intravenous injection of 40 mg/kg pentobarbital.

2.4 Immunosuppression Regimen

Preoperative induction therapy consisted of rituximab (10 mg/kg) combined with ATG (5 mg/kg). Postoperative maintenance therapy consisted of FK506 (0.025 mg/kg), MMF (10 mg/kg), and methylprednisolone (starting at 160 mg, halved daily). During ICU management, targeted anti-rejection therapy was administered as clinically indicated.

2.5 Monitoring of Hematological Parameters

Physiological and hematological parameters were all measured at the Yunnan Province Key Laboratory for Porcine Gene Editing and Xenotransplantation, Yunnan Agricultural University. Complete blood count, blood biochemistry (including liver and kidney function, blood glucose, blood lipids, electrolytes, etc.), blood gas, activated clotting time (ACT), six coagulation parameters (FIB, D-dimer, TT, PT, APTT, INR), and basic vital signs were monitored using the following devices, respectively: Mindray BC-5000 Vet automated hematology analyzer, Mindray BS-240Vet automated biochemistry analyzer, epoc Blood Analysis System blood gas analyzer, Abbott i-STAT 1 handheld blood analyzer, PUSHKANG MC100 portable coagulation analyzer, Comen C80-V multi-parameter veterinary monitor, and Mindray WATO EX-20 Vet anesthesia machine. In addition, detection of complement (C3, C4), immunoglobulins (IgM, IgG, IgA), and cytokines (IL-6, IL-8, IL-17, IFN-α) in whole blood was entrusted to the Kunming First People's Hospital.

2.6 Flow Cytometry

Peripheral blood was collected from the recipients, subjected to red blood cell lysis and washing, and then incubated with the corresponding antibodies in the dark. Detection was performed using a Beckman Coulter CytoFLEX flow cytometer, and the absolute cell counts of $CD3^+$ T lymphocytes (Cat# 556612, BD Biosciences, 1:12.5), $CD8^+$cytotoxic T cells (Tc) (Cat# A23346, abclonal, 1:10), $CD4^+$helper T cells (Th) (Cat# A22773, abclonal, 1:10), double-positive T cells ($CD4^+CD8^+$), B lymphocytes

(Cat# A23008, abclonal, 1:10), and $CD56^+$ and/or $CD16^+$ natural killer (NK) (Cat# 17-0566-42, ThermoFisher, 1:12.5 and/or Cat# A23400, abclonal, 1:12.5) cells were recorded.

2.7 Liver Histopathological Analysis

Liver tissue specimens were collected at the experimental endpoint, with liver from donor pigs of the same genotype serving as controls. After formalin fixation, H&E staining and immunohistochemical (IHC) staining were performed. IHC staining markers included IgM (Cat# A18842, ThermoFisher, 1:200), IgG (Cat# AA406878, ThermoFisher, 1:200), C4d (Cat# RMA-0857, MXB Biotechnologies, 1:200), C3d (Cat# NBP3-07741, novusbio, 1:100), CD68 (macrophages) (Cat# MA5-13324, ThermoFisher, 1:500), CD56 (natural killer cells) (Cat# 14255-1-AP, Proteintech, 1:8000), and vWF (von Willebrand factor) (Cat# A008229-5, Dako Agilent, 1:600).

2.8 Data Analysis

This study was descriptive and no statistical testing was performed. Graphs were generated using Prism v.8 (GraphPad Software).

3 Results

3.1 Basic Physiological Status of the Recipient Monkeys

ALF recipient Tibetan macaque models were constructed by ligating and transecting the hepatic artery and common bile duct and restricting portal vein flow (Fig. 1A). After confirming that the acute liver failure criteria set in this study were met (Table 1), the perfused 8-GE pig livers were connected to a normothermic machine perfusion device to establish extracorporeal liver cross-circulation with the recipient monkeys for continuous perfusion.

To evaluate the overall physiological status of the recipient monkeys during cross-circulation, we continuously monitored vital signs, biochemical parameters, and changes in multiple organ functions. Throughout the perfusion period, the vital signs of the six recipient monkeys remained relatively stable: body temperature was maintained at 34-38 °C (Fig. 1B); heart rate and mean arterial pressure (MAP) were maintained at

70-150 bpm (Fig. 1C) and 60-150 mmHg (Fig. 1D), respectively, both showing considerable fluctuation; the abnormal elevation of MAP was caused by bolus administration of vasopressors; pH remained generally stable at 7.2-7.6 (Fig. 1E). In terms of biochemical monitoring, serum creatinine in all recipients showed a continuous upward trend after 24 h of cross-circulation (Fig. 1F); lactate levels remained relatively stable in all individuals except No. 5, which increased markedly in the late perfusion period (Fig. 1G). Overall, extracorporeal cross-perfusion of pig livers in the acute liver failure monkey model effectively maintained the recipients' basic vital signs, although the recipients exhibited marked creatinine elevation.

3.2 Monitoring of Recipient Liver Function

To dynamically evaluate the functional integrity of the transplanted livers during extracorporeal liver perfusion and cross-circulation, we monitored AST and ALT (reflecting hepatocyte injury), ALB and TP (reflecting synthetic function), and TBIL (reflecting excretory function). Recipient AST and ALT generally showed a pattern of initial increase followed by decrease after cross-perfusion (Fig. 2A, B). ALB and TP decreased after cross-perfusion and remained relatively stable, with a slow decline in the later period (Fig. 2C, D). TBIL, DBIL, and IBIL generally showed a rapid upward trend in the early phase (Fig. 2E-G), and the upward trend slowed after cross-perfusion. Blood ammonia showed a downward trend after cross-perfusion (Fig. 2H). Except for the donor livers of No. 3 and No. 5, which secreted very little bile in the late perfusion period, the remaining donor livers secreted bile continuously throughout the entire process (Fig. 2I). These results indicate that pig liver cross-perfusion can replace the impaired liver function in the acute liver failure monkey model.

To determine whether blood test parameters were consistent between the perfusion device side and the recipient side, we collected blood samples from both sides and performed complete blood count, liver function, renal function, blood gas, and electrolyte tests. The results showed (taking one case as an example) that all parameters on both sides were highly consistent after cross-perfusion, including blood cell counts

and platelet counts in the complete blood count, ALT, AST, and TBIL in liver function, serum creatinine in renal function, pH and lactate in blood gas, and Na, K, and Ca in electrolytes (Fig. S1), indicating that the cross-perfusion flow rate and efficiency were sufficient and that values were highly consistent after cross-perfusion.

3.3 Coagulation Assessment of Extracorporeal Liver Cross-Circulation

Coagulation dysfunction is one of the major obstacles in xenogeneic liver transplantation, and its severity directly affects recipient safety and graft survival. To dynamically evaluate the coagulation status of the recipients during perfusion, we systematically monitored ACT, FIB, INR, D-dimer, and platelet count. First, ACT mainly reflects the management of circulating anticoagulation. After initiation of cross-perfusion, ACT rose from a baseline of approximately 120-150 s to a peak of approximately 350 s, then decreased and was maintained between 150 and 250 s (Fig. 3A), suggesting that anticoagulation regulation was generally stable during perfusion. INR and PT generally showed a prolonging trend after modeling and fell after cross-perfusion (Fig. 3B, C). FIB generally showed an increasing trend after modeling, decreased overall after cross-perfusion, and then was maintained at 0.6-1.5 g/L (Fig. 3D). D-dimer gradually increased after modeling and decreased overall after cross-perfusion (Fig. 3E). Platelet count dropped sharply after initiation of perfusion and remained persistently low, a response that was highly consistent across all five recipients (Fig. 3F). These results indicate that pig liver cross-perfusion can correct some coagulation abnormalities, with marked platelet reduction, consistent with the situation in previous pig-to-human orthotopic auxiliary liver transplantation.

3.4 Immune Response to Liver Cross-Perfusion

To dynamically evaluate changes in the immune status of the recipients during cross-perfusion, we monitored complement, immunoglobulins, lymphocyte subsets, and inflammatory cytokines. After immune induction, recipient complement (C3, C4) and immunoglobulin (IgA, IgG, IgM) levels were markedly reduced and remained at low levels throughout the perfusion period (Fig. 4A-E). T lymphocytes, cytotoxic T

cells (Tc), helper T cells (Th), double-positive T cells (Tdp), B lymphocytes, and natural killer (NK) cells in all recipients also showed trends consistent with those of humoral immunity (Fig. 4F-K). Regarding inflammation, IL-6 in all recipients gradually increased after initiation of cross-perfusion (Fig. 4L); IL-8 and IL-17 showed marked elevation only in the late perfusion period of recipient No. 1, while the remaining recipients maintained low levels (Fig. 4M, N); IFN-α remained relatively stable across all monitoring time points (Fig. 4O). The above results suggest that the 8-gene editing and immunosuppression regimen in this study effectively suppressed humoral and cellular immune rejection, with no hyperacute or acute rejection observed, but that liver extracorporeal cross-perfusion was associated with an IL-6-driven inflammatory response.

3.5 Recipient Survival Analysis

During the six cross-perfusion procedures, based on clinical ICU management experience, recipients received necessary blood transfusion, anti-infection, coagulation correction, and anti-rejection therapy. The six recipients had stable vital signs after cross-perfusion and were actively terminated upon reaching the study endpoint, achieving 74 h, 98 h, 84 h, 110 h, 144 h, and 101 h, respectively. In addition, after donor liver procurement, we used a normothermic machine perfusion device to repair the pig livers, with repair times of 26 h, 22 h, 22 h, 34 h, 38 h, 13 h, and 23 h, respectively, and total extracorporeal perfusion times of 100 h, 120 h, 106 h, 148 h, 157 h, and 124 h, reflecting the long-term stability of extracorporeal liver perfusion. Interestingly, we found that in the fifth case of 144 h liver cross-perfusion, compared with before cross-perfusion, the donor liver margin changed markedly from sharp to blunt, and the donor liver weight increased by 168 g within 144 h, suggesting that marked liver regeneration occurred during cross-perfusion.

3.6 Liver Histopathology

We performed synchronous histopathological analysis of recipient livers and 8-GE donor livers (Fig. 5A) at the experimental endpoint. The recipient's native liver was a

liver failure model established by ligation and division of the hepatic artery and common bile duct combined with portal vein flow restriction; its pathological changes represent background lesions secondary to model construction. At the experimental endpoint, all recipient livers showed moderate-to-severe injury, mainly manifested as hepatocellular edema, steatosis, and patchy necrosis predominantly around the central veins, accompanied by chronic inflammatory cell infiltration in the portal areas and central venous dilatation and congestion (Fig. 5B). These pathological features were consistent with the expected manifestations of ischemic, hypoxic, and cholestatic liver injury after model construction, confirming that they were background changes following induction of acute liver failure.

Regarding the donor livers, all donor livers appeared grossly normal or showed only mild nonspecific changes before cross-perfusion (mild sinusoidal dilatation, no or only sparse chronic inflammatory cell infiltration in portal areas, and no fibrous tissue proliferation), indicating good donor liver quality at procurement with no obvious underlying lesions. After cross-perfusion, no hyperacute rejection occurred in the gene-edited pig livers, but the degree of donor liver injury at the endpoint varied among individuals: livers No. 1, 2, and 4 showed moderate-to-severe injury, characterized mainly by hepatocellular necrosis (focal to massive infarction/necrosis), sinusoidal dilatation and congestion, acute inflammatory cell (neutrophil and eosinophil) infiltration, and bile duct cholestasis, accompanied by varying degrees of fibrous tissue proliferation; livers No. 3, 5, and 6 showed only mild injury, with mild hepatocellular edema or grossly normal appearance, no definite necrosis, sparse chronic inflammatory cell infiltration in portal areas with mild fibrous tissue proliferation and a small amount of bile duct cholestasis (Fig. 5C). The above injury patterns were consistent with ischemia/reperfusion injury and inflammation-mediated injury associated with ex vivo perfusion, with no evidence of hyperacute rejection, nor any manifestations of acute cellular rejection such as venous endothelialitis, bile duct injury, or severe lymphocytic infiltration. Immunofluorescence and immunohistochemical staining showed a small

amount of IgM and IgG antibody deposition in the donor livers, which was more pronounced in livers No. 5 and 6; however, no C3d or C4d antibody deposition was found in any donor liver, suggesting mild antibody attachment without triggering complement cascade activation. Sparse CD56-positive NK cell infiltration was observed in donor livers No. 1-4, and no CD68-positive macrophage infiltration was seen in any donor liver, indicating that NK cell- and macrophage-mediated innate immune attack was not active. pvWF was detected in all donor livers, suggesting a certain degree of activation of porcine vascular endothelial cells (Fig. 5D).

4. Discussion

In China, the overall mortality rate of patients with acute liver failure (ALF) who do not receive liver transplantation is close to 50%, while fewer than 10% of patients can obtain emergency liver transplantation[15]. The combination of xenotransplantation and modern machine perfusion technology provides a promising bridging strategy for patients with liver failure, with the potential to extend transplant viability to the current limits. In recent years, orthotopic auxiliary liver transplantation of gene-edited donor pig livers into brain-dead human recipients[9] and into living patients[10], have both confirmed the efficacy and safety of gene-edited pig livers as bridging therapy. However, liver function is complex, and after transplantation it may be difficult to maintain function in vivo over the long term due to physiological incompatibility. In contrast, xenogeneic extracorporeal liver cross-perfusion, as a bridging approach while awaiting native liver recovery or allogeneic liver transplantation, is significant not only because it fills the gap between the limited functionality of existing artificial liver systems and the severe shortage of human donor livers, but also because it advances liver support from toxin clearance to full functional replacement[11]. Moreover, by using an extracorporeal circuit, it circumvents long-term in vivo compatibility issues, making the support process adjustable and removable, enabling minimally invasive treatment with minimal trauma and no need for lifelong medication, thereby providing ALF patients with temporary liver support approaching full function. This strategy is suitable

for patients without underlying liver disease whose liver function may recover without transplantation, and can also extend waiting time and serve as bridging therapy before allogeneic liver transplantation, ultimately reducing mortality in ALF patients.

In this study, by constructing acute liver failure monkey models and establishing extracorporeal cross-circulation with 8-GE gene-edited pig livers, we achieved xenogeneic extracorporeal liver cross-perfusion for up to 144 h, breaking through the 72-hour bottleneck of extracorporeal machine liver perfusion and systematically confirming that ELC is an effective, feasible, and usable ALF support modality that can effectively perform liver function. During cross-perfusion, the core vital signs (body temperature, heart rate, mean arterial pressure, pH) of the six ALF models remained within the preset safety range, suggesting that extracorporeal liver cross-perfusion has acceptable tolerability at the hemodynamic level; ALB and TP remained relatively stable, ALT and AST gradually declined 24-48 h after cross-perfusion following an initial increase, and most donor livers maintained continuous bile production throughout, suggesting that donor livers can maintain synthetic, metabolic, and excretory functions under normothermic perfusion conditions; the donor liver's improvement of coagulation function was generally stable, further supporting that the extracorporeal donor liver has partial compensatory capacity. In previous use of hCD55 transgenic pig livers, perfusion time was extended to approximately 21 hours, but recipients still faced hemolysis risk (https://kaken.nii.ac.jp/report/KAKENHI-PROJECT-10357010/103570102001kenkyu_seika_hokoku_gaiyo/). In this study, cross-perfusion with 8-GE pig livers supported up to 144 hours, expanding the time window nearly 7-fold. The median waiting time from onset to obtaining liver transplantation for acute liver failure patients is approximately 7 days[16] , and the 144-hour (6-day) support duration is already close to the core window of this clinical bridging need. This suggests that under extracorporeal support with gene-edited pig livers, recipients can maintain basic vital signs and key liver function parameters over a relatively long period, thereby providing preliminary affirmative evidence for the core

question of whether pig livers can sustain patients until a donor liver becomes available. At the endpoint, the donor liver injury pattern was dominated by ischemia/reperfusion-related hepatocyte necrosis, acute inflammatory infiltration, and cholestasis, without typical features of hyperacute rejection or acute cellular rejection, confirming that gene editing and immunosuppression effectively alleviated immune rejection. Notably, we found that the fifth pig liver exhibited marked regenerative capacity after cross-perfusion. In summary, extracorporeal cross-perfusion with 8-GE pig livers can achieve effective liver function support in ALF models with controllable immune rejection, and is feasible for clinical translation, with the potential to provide liver failure patients with a sufficient clinical treatment window, increasing the likelihood of obtaining liver transplantation or of native liver recovery.

Total hepatectomy modeling causes severe bleeding in recipients, and even with liver cross-perfusion, only short-term maintenance can be achieved[11]. In this study, an ALF monkey model was constructed by ligating and transecting the hepatic artery and common bile duct combined with portal vein flow restriction[14], and after narrowing the recipient portal vein by 75%-85%, extracorporeal cross-perfusion was performed, with maintenance durations all exceeding 72 h; whereas previous studies with 50% narrowing maintained only 48 h after cross-perfusion in recipients[17], further demonstrating that this modeling approach is relatively stable. Compared with other models, this modeling method is simple to operate, time-saving, minimally invasive, stable, and highly reproducible, and can both maintain basic intestinal return and avoid portal hypertensive shock, while also avoiding the risk of continuous oozing from the hepatectomy surface or portocaval anastomosis. Previous studies started cross-perfusion 1 h after portal vein ligation[18], whereas we performed cross-perfusion only after no less than 18 h following ligation and transection of the hepatic artery and common bile duct combined with portal vein flow restriction, suggesting that the ALF model construction in this study is reliable. In addition, the histopathological changes in the recipients' own livers at the endpoint were dominated by massive hepatocyte

necrosis, steatosis, and perivenular injury, highly consistent with the characteristics of ischemic liver injury after portal vein ligation, further confirming the validity of the model and consistent with the core pathophysiological features of clinical fulminant liver failure[19].

Xenogeneic cross-perfusion still faces some important obstacles, among which coagulation dysfunction is a common complication of xenogeneic liver transplantation[20]. In this study, all five recipients showed a highly consistent sharp drop in platelets. Mechanistically, the sinusoidal endothelial layer of pig livers is rich in a large number of resting macrophages (Kupffer cells), which can rapidly recognize and phagocytose recipient platelets after initiation of xenogeneic perfusion, leading to platelet retention and consumption within the graft[21], consistent with our previous results of pig-to-living-patient auxiliary liver transplantation[10]. Immunological monitoring showed that the immune induction regimen successfully maintained complement and immunoglobulins at low levels, indicating that this regimen provides sufficient immunosuppressive intensity at the humoral immunity level. However, IL-6 increased consistently after initiation of perfusion, suggesting that perfusion-induced inflammatory response is an issue that requires careful consideration in future research[22]. However, the elevation of IL-6 may originate from multiple sources: ischemia/reperfusion injury of the donor liver, circuit-related inflammatory responses, and interactions between porcine-derived antigens and the recipient immune system[23]. Histopathological analysis of the donor livers at the endpoint showed that the injury pattern was dominated by ischemia/reperfusion-related hepatocyte necrosis, acute inflammatory infiltration, and cholestasis, with no hyperacute or acute cellular rejection observed, suggesting that the main injury caused by cross-perfusion is ischemia/inflammation-mediated.

In summary, this study, through normothermic extracorporeal cross-perfusion of gene-edited pig livers for the treatment of non-human primate models of acute liver failure, confirmed that xenogeneic extracorporeal liver cross-perfusion can effectively

provide liver function support including synthesis, metabolism, detoxification, and coagulation, maintain stable recipient vital signs, support ALF recipients' survival for more than 72 h (up to 144 h), and effectively avoid hyperacute rejection and acute rejection. This study advances ELC from short-term proof of concept to long-term functional support in large-animal acute liver failure models, providing important data support for the safety and efficacy of ELC in clinical application, offering a feasible strategy for bridging therapy in patients with acute liver failure, and laying an important foundation for xenogeneic liver ELC to move toward clinical practice.

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Author Contributions

Conceptualization: H.J.W., J.R., B.S., and Y.S. Methodology: J.R., Y.S., Y.M., J.M., C.Y., Y.W., R.W., and W.Z. Validation: H.J.W., J.R., B.S., and Y.S. Formal analysis: H.J.W., K.X., X.L., and Y.M. Investigation: T.W., H.Z., Z.L., X.L., X.Z., Y.L., Y.Q., G.S., X.S., S.L., Q.X., R.X., X.Q., L.Y., Y.D., S.W., Y.Z., L.Z., J.G., and J.S. Resources: H.J.W., H.R.Z, and X.C. Data curation: H.J.W., K.X., X.L., F.H., and Y.F. Visualization: K.X., H.J.W., and X.L. Supervision: H.J.W., J.R., B.S., and Y.S. Project administration: H.J.W., and H.Y.Z. Writing—original draft: K.X. and J.M. Writing—review and editing: all authors.

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Conflicts of Interest

All authors declare no conflicts of interest.

Figure Legends

Fig. 1 Basic physiological status of the recipient monkeys during extracorporeal liver cross-circulation. (A) Construction of the acute liver failure (ALF) recipient Tibetan macaque model by ligation and transection of the hepatic artery and common bile duct combined with restriction of portal vein flow. (B–E) Vital signs monitored continuously throughout the perfusion period: body temperature (B), heart rate (C), mean arterial pressure (MAP) (D), and pH (E). (F, G) Biochemical monitoring: serum creatinine (F) and lactate (G) levels during cross-circulation.

Fig. 2 Monitoring of recipient liver function during extracorporeal liver cross-circulation. (A–H) Dynamic changes in AST (A) and ALT (B), ALB (C) and TP (D), TBIL (E), DBIL (F), and IBIL (G), and blood ammonia (H) after cross-perfusion. (I) Bile secretion from donor livers throughout perfusion. AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALB, albumin; TP, total protein; TBIL, total bilirubin; DBIL, direct bilirubin; IBIL, indirect bilirubin.

Fig. 3 Coagulation assessment of extracorporeal liver cross-circulation. (A–F) Dynamic monitoring of ACT (A), INR (B), PT (C), FIB (D), D-dimer (E), and platelet count (PLT) (F) in recipients during perfusion. ACT, activated clotting time; INR, international normalized ratio; PT, prothrombin time; FIB, fibrinogen; PLT, platelet.

Fig. 4 Immune response to extracorporeal liver cross-circulation. (A–E) Complement (C3, C4) and immunoglobulin (IgA, IgG, IgM) levels. (F–K) Lymphocyte subsets, including T lymphocytes, cytotoxic T cells (Tc), helper T cells (Th), double-positive T cells (Tdp), B lymphocytes, and natural killer (NK) cells. (L–O) Inflammatory cytokines: IL-6 (L), IL-8 (M), IL-17 (N), and IFN-α (O).

Fig. 5 Liver histopathology at the experimental endpoint. (A) Photographs of recipient livers and 8-gene-edited pig livers at the experimental endpoint. (B) HE staining of recipient livers at the experimental endpoint. (C) HE staining of 8-gene-edited pig livers at the experimental endpoint. (D) Immunofluorescence and immunohistochemical staining of donor livers, including IgM, IgG, C3d, C4d, CD56, CD68, and pvWF.

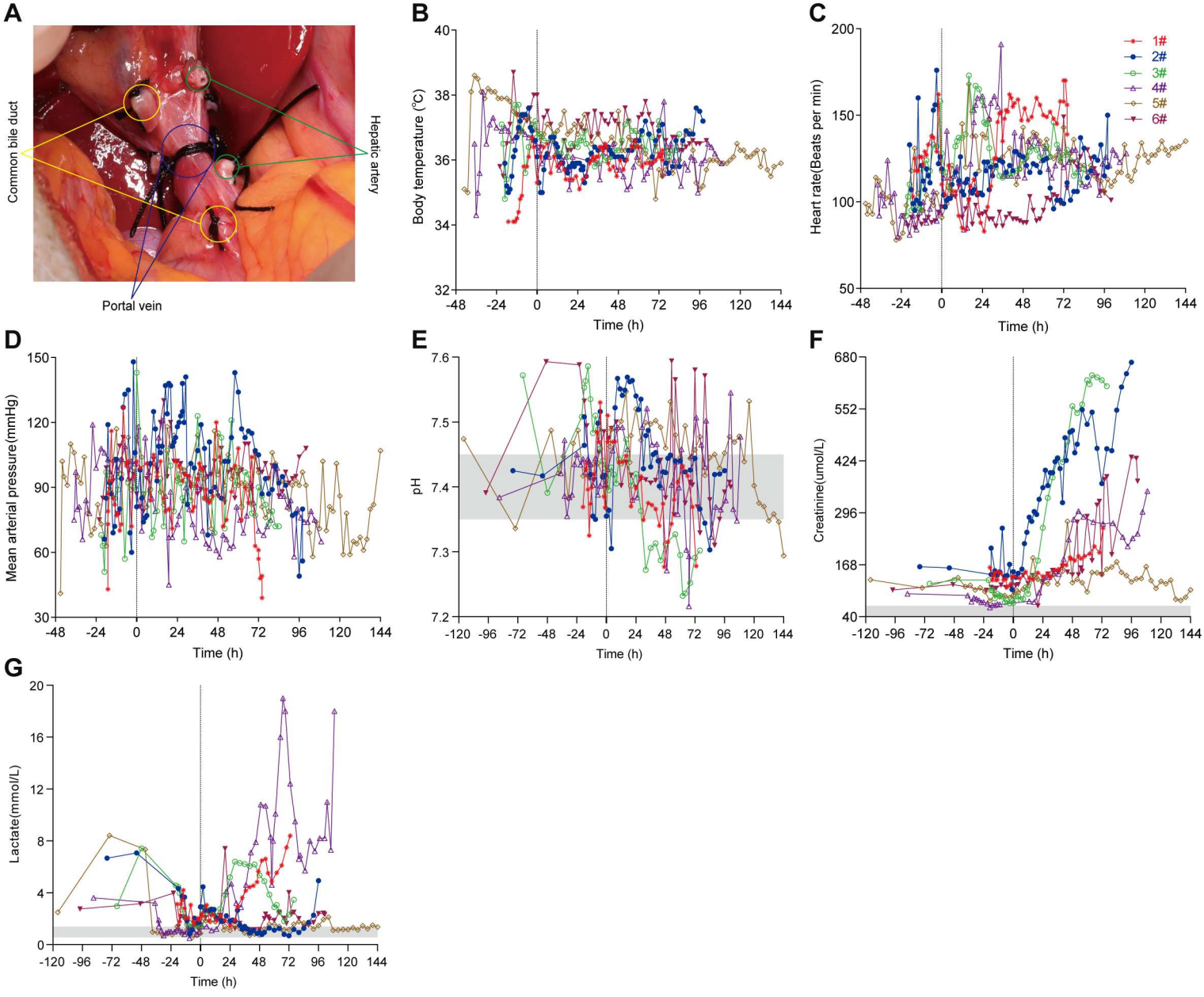
Fig. S1 Consistency of blood test parameters between the perfusion device side and the recipient side after cross-perfusion.

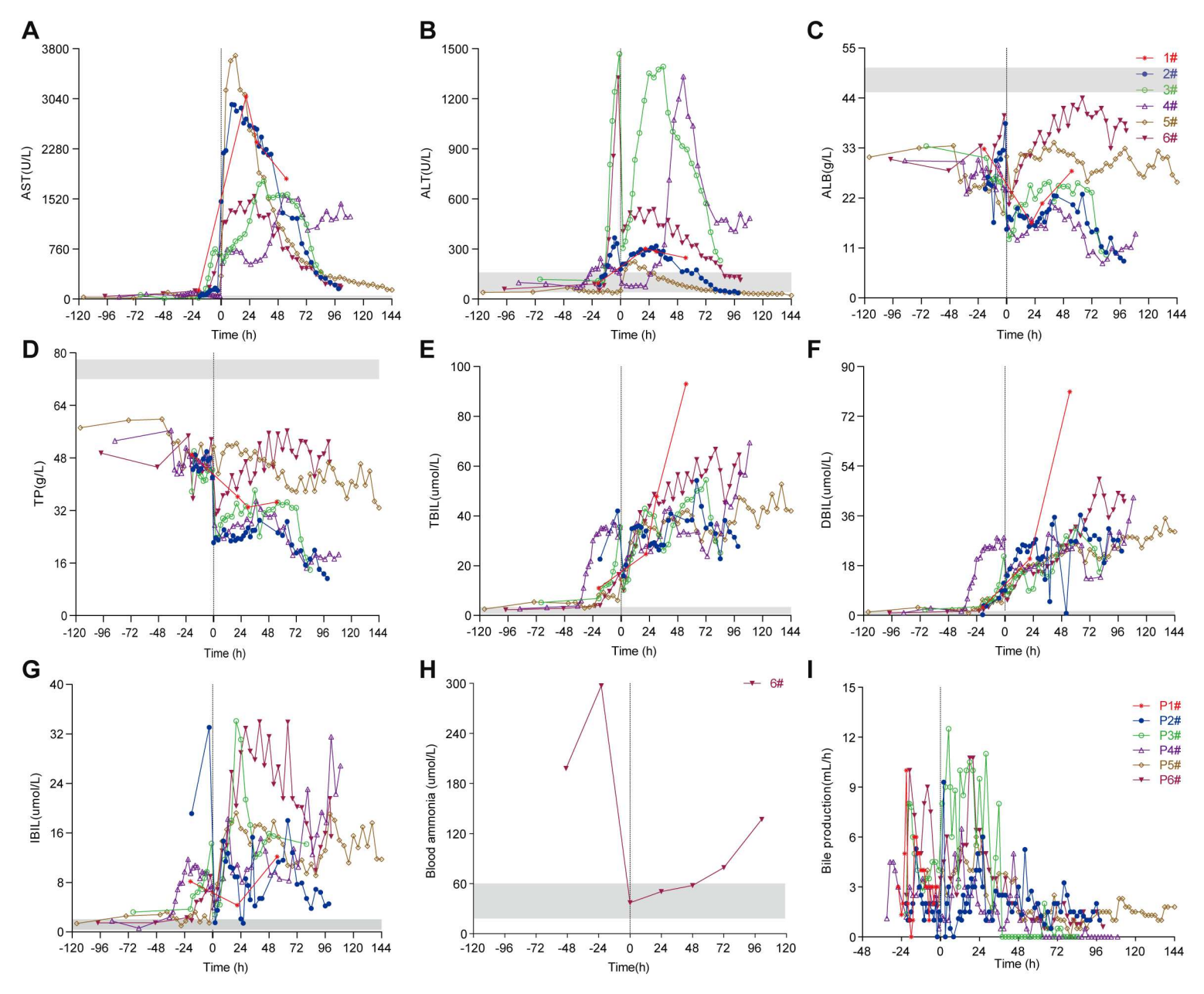
Table 1 Criteria for establishing the acute liver failure models

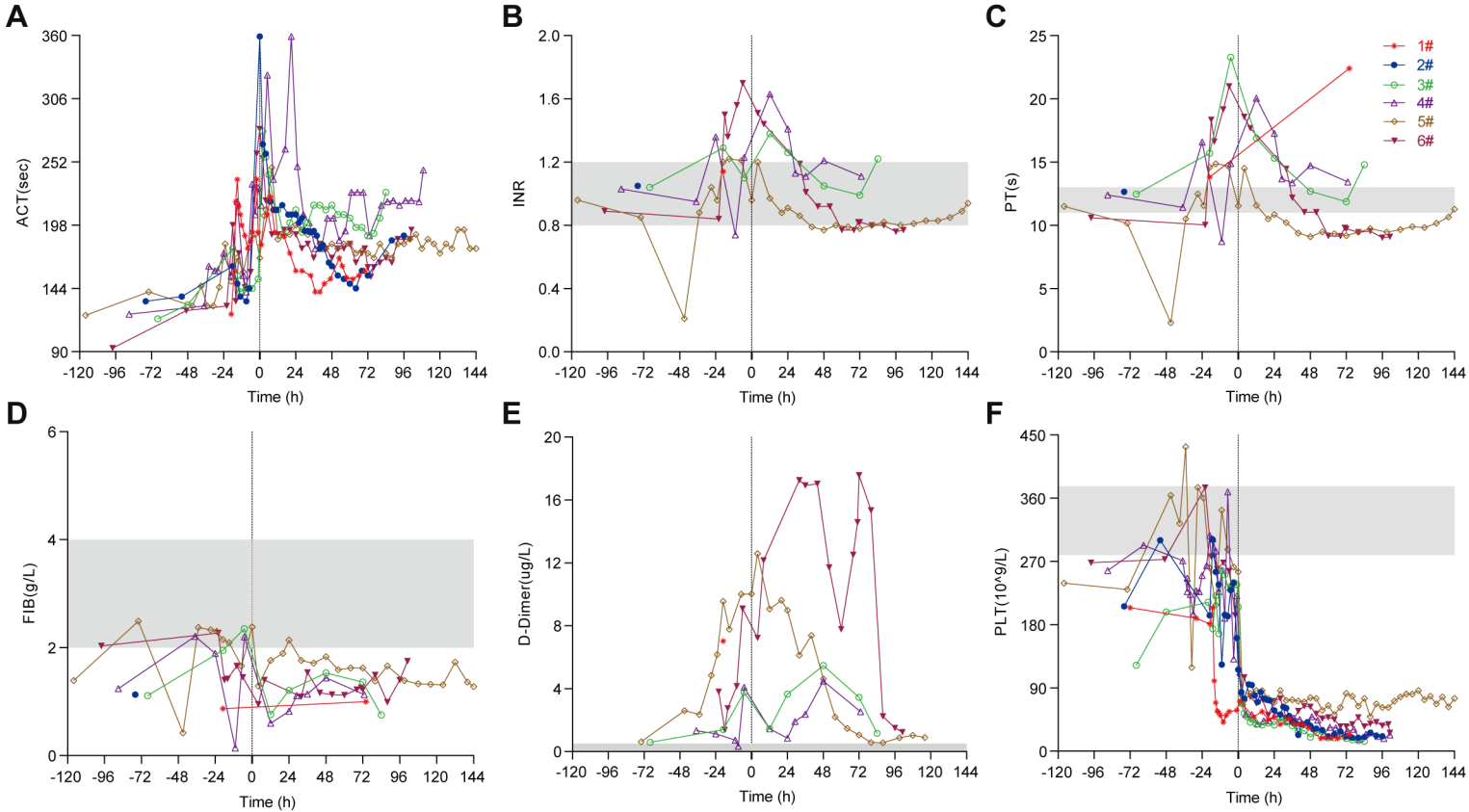
Table 1. Criteria for establishing the acute liver failure models

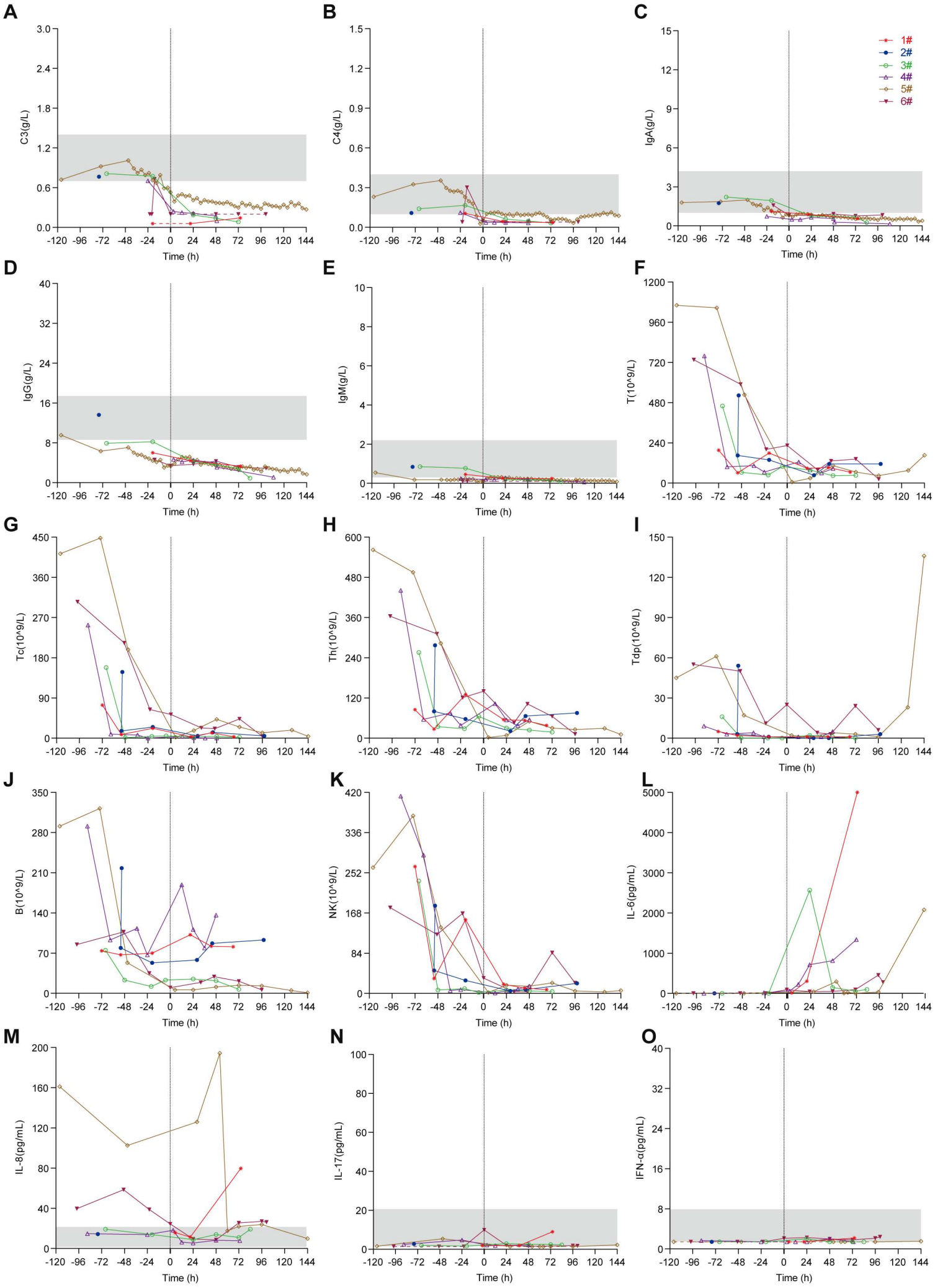
Case No.	Irreversible Blockade Duration (h)	ALT (U/L) Baseline → Pre-Cross-Perfusion	AST (U/L) Baseline → Pre-Cross-Perfusion	Total Bilirubin (μmol/L) Baseline → Pre-Cross-Perfusion (Fold Change)	PT (s) Baseline → Pre-Cross-Perfusion (Fold Change)	INR Baseline → Pre-Cross-Perfusion	Outcome
1#	0	94→—	131→—	11→—	13.81→—	1.14→—	—
2#	18	84.6→332.5	43.2→129.8	22.63→42.02 (1.9)	12.64→—	1.05→—	Not met
3#	19	117.8→1468.4*	61.8→679	5.23→35.66 (6.8)*	12.46→23.28 (1.9)*	1.04→1.1	3 criteria met
4#	36	98.1→157.1	30.5→47.3	2.69→35.49 (13.2)*	12.39→14.88 (1.2)	1.03→1.23	1 criterion met
5#	43	39.3→49.4	26.8→348.4	2.59→14.43 (5.6)*	11.49→14.71 (1.3)	0.96→1.21	1 criterion met
6#	20	59→1325.3*	40.7→648.2	2.28→16.44 (7.2)*	10.59→20.98 (2.0)*	0.89→1.7*	4 criteria met

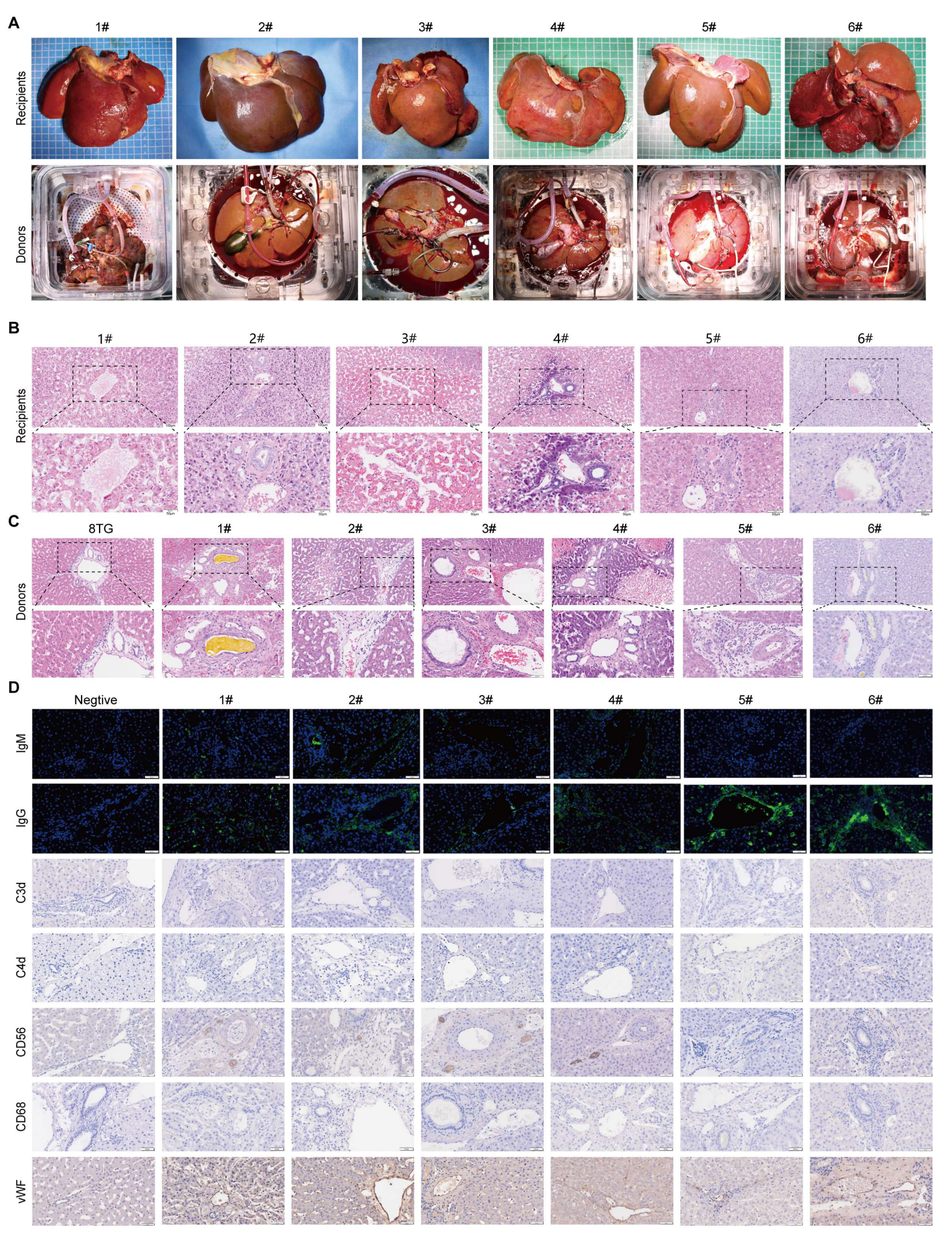
Note: Assessment required confirmed anatomical irreversible blockade in the recipient monkey lasting at least 18 h. Criteria for fulfilment — Hepatocellular injury: ALT or AST > 1000 U/L; Metabolic dysfunction: total bilirubin ≥ 2× baseline; Coagulation dysfunction: INR > 1.5 or PT ≥ 1.5× baseline. "Baseline → Pre-Cross-Perfusion" indicates the values from each recipient's pre-operative baseline to the value before cross-perfusion; figures in parentheses are the pre-cross-perfusion/baseline fold increase. Case 1# had baseline data only; in Case 2#, coagulation parameters (PT, INR) were not measured before cross-perfusion, and the corresponding cells are shown as "—". * denotes a criterion that was met.


A
Common bile duct
Hepatic artery
Portal vein
B
Body temperature (℃)
Time (h)
C
Heart rate(Beats per min)
1#
2#
3#
4#
5#
6#
Time (h)
D
Mean arterial pressure(mmHg)
Time (h)
E
pH
Time (h)
F
Creatinine(umol/L)
Time (h)
G
Lactate(mmol/L)
Time (h)


A
AST(U/L)
3800
3040
2280
1520
760
0
-120 -96 -72 -48 -24 0 24 48 72 96 120 144
Time (h)
B
ALT(U/L)
1500
1200
900
600
300
0
-120 -96 -72 -48 -24 0 24 48 72 96 120 144
Time (h)
C
ALB(g/L)
55
44
33
22
11
0
-120 -96 -72 -48 -24 0 24 48 72 96 120 144
Time (h)
1#
2#
3#
4#
5#
6#
D
TP(g/L)
80
64
48
32
16
0
-120 -96 -72 -48 -24 0 24 48 72 96 120 144
Time (h)
E
TBIL(umol/L)
100
80
60
40
20
0
-120 -96 -72 -48 -24 0 24 48 72 96 120 144
Time (h)
F
DBIL(umol/L)
90
72
54
36
18
0
-120 -96 -72 -48 -24 0 24 48 72 96 120 144
Time (h)
G
IBIL(umol/L)
40
32
24
16
8
0
-120 -96 -72 -48 -24 0 24 48 72 96 120 144
Time (h)
H
Blood ammonia (umol/L)
300
240
180
120
60
0
-120 -96 -72 -48 -24 0 24 48 72 96 120
Time(h)
6#
I
Bile production(mL/h)
15
12
9
6
3
0
-48 -24 0 24 48 72 96 120 144
Time (h)
P1#
P2#
P3#
P4#
P5#
P6#


A
ACT(sec)
360
306
252
198
144
90
-120 -96 -72 -48 -24 0 24 48 72 96 120 144
Time (h)
B
INR
2.0
1.6
1.2
0.8
0.4
0.0
-120 -96 -72 -48 -24 0 24 48 72 96 120 144
Time (h)
C
PT(s)
25
20
15
10
5
0
-120 -96 -72 -48 -24 0 24 48 72 96 120 144
Time (h)
1#
2#
3#
4#
5#
6#
D
FIB(g/L)
6
4
2
0
-120 -96 -72 -48 -24 0 24 48 72 96 120 144
Time (h)
E
D-Dimer(ug/L)
20
16
12
8
4
0
-120 -96 -72 -48 -24 0 24 48 72 96 120 144
Time (h)
F
PLT(10^9/L)
450
360
270
180
90
0
-120 -96 -72 -48 -24 0 24 48 72 96 120 144
Time (h)


A
C3(g/L)
B
C4(g/L)
C
IgA(g/L)
1#
2#
3#
4#
5#
6#
D
IgG(g/L)
E
IgM(g/L)
F
T(10^9/L)
G
Tc(10^9/L)
H
Th(10^9/L)
I
Tdp(10^9/L)
J
B(10^9/L)
K
NK(10^9/L)
L
IL-6(pg/mL)
M
IL-8(pg/mL)
N
IL-17(pg/mL)
O
IFN-α(pg/mL)
Time (h)
-120 -96 -72 -48 -24 0 24 48 72 96 120 144


A
1#
2#
3#
4#
5#
6#
Recipients
Donors
B
1#
2#
3#
4#
5#
6#
Recipients
C
8TG
1#
2#
3#
4#
5#
6#
Donors
D
Negtive
1#
2#
3#
4#
5#
6#
IgM
IgG
C3d
C4d
CD56
CD68
vWF

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- FigureS1.tif