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ORIGINAL ARTICLE

Coagulation factor XIII is a critical driver of liver regeneration after partial hepatectomy

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Abstract

Background: Activation of coagulation and fibrin deposition in the regenerating liver appears to promote adequate liver regeneration in mice. In humans, perioperative hepatic fibrin deposition is reduced in patients who develop liver dysfunction after partial hepatectomy (PHx), but the mechanism underlying reduced fibrin deposition in these patients is unclear.

Methods and Results: Hepatic deposition of cross-linked (ie, stabilized) fibrin was evident in livers of mice after two-thirds PHx. Interestingly, hepatic fibrin cross-linking was dramatically reduced in mice after 90% PHx, an experimental setting of failed liver regeneration, despite similar activation of coagulation after two-thirds or 90% PHx. Likewise, intraoperative activation of coagulation was not reduced in patients who developed liver dysfunction after PHx. Preoperative fibrinogen plasma concentration was not connected to liver dysfunction after PHx in patients. Rather, preoperative and postoperative plasma activity of the transglutaminase coagulation factor (F)XIII, which cross-links fibrin, was lower in patients who developed liver dysfunction than in those who did not. PHx-induced hepatic fibrin cross-linking and hepatic platelet accumulation were significantly reduced in mice lacking the catalytic subunit of FXIII ($FXIII^{-/-}$ mice) after two-thirds PHx. This was coupled with a reduction in both hepatocyte proliferation and liver–to–body weight ratio as well as an apparent reduction in survival after two-thirds PHx in $FXIII^{-/-}$ mice.

Conclusion: The results indicate that FXIII is a critical driver of liver regeneration after PHx and suggest that perioperative plasma FXIII activity may predict posthepatectomy liver dysfunction. The results may inform strategies to stabilize proregenerative fibrin during liver resection.

KEYWORDS
coagulation, fibrin, fibrinogen, hepatocytes, platelets

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1 | INTRODUCTION

Partial liver resection (eg, partial hepatectomy [PHx]) is a common surgical procedure used to remove diseased liver tissue (eg, tumors). The regenerative capability and functional reserve of the remnant liver ensure that hepatic function quickly recovers, even after removal of a substantial part of the liver [1]. Notably, in approximately 10% to 15% of patients undergoing partial liver resection, liver regeneration is insufficient, and as a consequence, patients can develop post-hepatectomy liver failure (PHLF), a condition where the liver remnant cannot sustain critical hepatic functions [2]. Detecting patients at risk of PHLF and developing targeted therapies to improve liver regeneration are continued needs, even though the molecular mechanisms of liver regeneration are increasingly understood [3].

Cellular and molecular mechanisms driving liver regeneration after PHx have largely been discovered using the well-characterized experimental setting of two-thirds PHx in rodents [4]. PHx is rapidly followed by a sequence of cell signaling events leading to coordinated cell proliferation and restoration of liver mass and function in 7 to 10 days in mice [4–6]. Standard two-thirds PHx induces activation of the blood coagulation cascade in mice, indicated by biomarkers of thrombin generation and deposition of the thrombin substrate fibrin(ogen) in the regenerating liver (ie, remnant) within 30 minutes of surgery [7]. Reducing thrombin generation using genetic or pharmacologic approaches reduced hepatic fibrin(ogen) deposition and hepatocyte proliferation after PHx in mice [7,8]. Likewise, depletion of plasma fibrinogen with ancrod, prior to PHx, significantly reduced hepatic fibrin(ogen) deposition and hepatocyte proliferation after PHx [7]. Prior studies suggest that fibrin(ogen) plays a pivotal role in promoting rapid accumulation of platelets in the regenerating liver [7]. Indeed, like fibrin(ogen), platelets rapidly accumulate in the liver remnant after PHx in mice [9] and humans [10]. Moreover, depleting or inhibiting platelets significantly reduced hepatocyte proliferation and delayed liver regeneration after PHx in mice [9,11]. Overall, experimental and clinical evidence suggests that components of the hemostatic system are among the earliest triggers of liver regeneration after PHx.

Rapid hepatic activation of coagulation was also evident in patients undergoing liver resection [7,12]. Consistent with observations in mice, a rapid intraoperative increase in hepatic fibrin(ogen) deposition in the regenerating liver of patients undergoing partial liver resection was demonstrated in biopsy samples obtained during surgery [7]. Remarkably, hepatic fibrin(ogen) accumulation was reduced in patients who ultimately developed posthepatectomy liver dysfunction, suggesting a functional connection between rapid hepatic fibrin(ogen) deposition and successful regeneration [7]. Preoperative plasma concentrations of clottable fibrinogen were not connected to postoperative liver dysfunction, and the mechanistic basis for this failure of rapid intrahepatic fibrin(ogen) deposition is unknown. We tested the hypothesis that intrahepatic fibrin(ogen) stability and liver regeneration after PHx is driven by coagulation factor (F)XIII. When activated (ie, FXIIIa), this multifunctional transglutaminase cross-links multiple proteins, including fibrin polymers [13,14]. The role of FXIII was determined using experimental settings of PHx-induced liver regeneration (two-thirds PHx) and liver failure (90% PHx) as well as analysis of perioperative samples from patients undergoing partial liver resection.

2 | MATERIALS AND METHODS

2.1 | PHx in mice

Wild-type C57Bl/6J mice were purchased from the Jackson Laboratory. Mice lacking the FXIII catalytic A subunit (FXIII-A$^{-/-}$ mice) [15,16] and a matched line of wild-type mice originally generated from heterozygous mice were bred at Michigan State University (MSU). Surgeries were performed on male and female mice aged between 8 and 14 weeks (see figure legends). Mice were housed under a 12-hour light/dark cycle, fed a standard diet (Teklad 8940, Envigo), and provided drinking water *ad libitum*. All procedures on mice were performed at MSU and approved by the Institutional Animal Care and Use Committee of MSU, East Lansing, USA. Age-matched cohorts of mice underwent a standard two-thirds or extended 90% PHx according to published protocols with some modifications [4,17]. Two-thirds PHx was performed in unfasted mice during the light cycle (0800-1300) by resection of the left lateral lobe, the right portion of the median lobe, and the left portion of the median lobe using 3 separate ligatures to preserve the gall bladder. For extended 90% PHx, the lower right lateral lobe was also resected [18]. Sham surgery was identical but included gentle manipulation of the liver lobes without removal of liver tissue. Surgical procedures were performed under deep surgical anesthesia induced by isoflurane (Abbott), and carprofen (5 mg/kg, subcutaneously) was administered as an analgesic (Pfizer). Blood and liver samples were collected 30 minutes or 48 hours after surgery. Blood was collected under deep surgical anesthesia induced by isoflurane by exsanguination from the inferior vena cava immediately after injection of 3.8% sodium citrate (Merck) in the spleen (8 μL/g). Blood samples were centrifuged to obtain plasma and were stored at −80 °C. Livers were rinsed in phosphate-buffered

saline and either fixed in 10% neutral-buffered formalin for 96 hours prior to routine processing or snap-frozen in liquid nitrogen.

2.2 | Patient sample collection and cohorts

In total, 98 patients were recruited at 3 different hospitals in Vienna, Austria (General Hospital, Clinic Landstraße, and Clinic Favoriten), and followed up prospectively over a period of 90 days after surgery. Within this group of 98 patients, blood samples were assessed 1 day prior to surgery as well as on postoperative day 1 (POD1) and postoperative day 5 (POD5) after liver resection in 88 patients. In 25 of these 88 patients, additional intraoperative blood samples were obtained from the portal and hepatic vein (draining the regenerating liver lobe) 2 hours after induction of liver regeneration. Patient-related data were collected, including baseline characteristics, surgical procedure, perioperative routine laboratory parameters, and baseline liver pathology, and were distributed as illustrated in Supplementary Table S1 (intraoperative blood sample cohort, $N = 25$), Supplementary Table S2 (perioperative blood samples cohort, $N = 88$), and Supplementary Table S3 (patient demographics). This study was conducted in adherence to the Declaration of Helsinki and was approved by the institutional ethics committee (Medical University of Vienna) and the ethics committee of the city of Vienna (EK#1186/2018 and EK 16-253-0117), and informed consent was obtained from all participants.

2.3 | Definition of PHLF

To evaluate PHLF in our patient cohort, the definition published by the International Study Group of Liver Surgery was used [19]. Here, PHLF is characterized by an increase in the international normalized ratio and concomitant hyperbilirubinemia on or after POD5. Further, this definition classifies the severity of PHLF in grades A to C. Patients suffering from grade A PHLF only show a deviation in laboratory parameters but do not require a change in clinical management. Grade B, on the other hand, does require a change in management but without the need for invasive treatment. Lastly, grade C is defined as patients needing invasive treatment and at great risk for postoperative mortality [19].

2.4 | Plasma biomarkers of liver injury and coagulation

Plasma alanine aminotransferase activity was determined using commercial reagents (Thermo Fisher and Pointe Scientific) according to the manufacturers' instructions and adapted for measurement in a microplate reader (ie, final reaction volume, 110 μL). Data were collected using an Infinite M200 plate reader (Tecan). Plasma fibrinogen concentration in mouse plasma was measured by enzyme-linked immunosorbent assay (ELISA) using 2 distinct polyclonal antibodies directed against fibrinogen (Capture, A0080 [Agilent]; Detection, ASMFBGN-GF-horseradish peroxidase [HRP] [Innovative Research]) as described previously [20]. Plasma thrombin-antithrombin (TAT) and prothrombin fragment 1 + 2 concentrations were determined using commercial ELISAs (Enzygnost TAT micro; Siemens Healthcare Diagnostics). Plasma D-dimer was determined using a commercial ELISA (Asserachrom D-Di, Diagnostica Stago). FXIII-A concentration in mouse plasma was detected using capillary Western blotting (Wes) using a sheep anti-FXIII-A antibody (1:2000 [SAF13A-IG], Affinity Biologicals) and HRP-conjugated rabbit anti-sheep antibody (1:1000, Jackson ImmunoResearch), and other reagents accompanying the Wes Master Kit were used according to the manufacturer's protocol (ProteinSimple). Prior studies have used this antibody to detect plasma FXIII-A by Wes [21]. Plasma FXIIIa activity in human plasma samples was determined as described previously [22], and plasma fibrinogen concentration in human plasma was determined using an ELISA [22].

2.5 | Measurement of cell proliferation in liver

Formalin-fixed paraffin-embedded liver sections were stained for Ki-67 (SP6 clone, Cell Marque) by the Investigative Histopathology laboratory at MSU, as previously described [7]. Slides were scanned using a Virtual Slide System VS110 (Olympus), and ~500 high-power fields randomly sampled images were used for quantification of Ki67-positive hepatocyte nuclei. Ki67-positive nuclei were identified using ImageJ (Fiji) (version 1.5w, National Institutes of Health, Bethesda) and expressed as percentage of total hepatocyte nuclei.

2.6 | Hepatic fibrin(ogen) and platelet accumulation

The urea-insoluble protein fraction was enriched from snap-frozen mouse liver as described previously, and fibrin(ogen) was detected using automated capillary Wes (Protein Simple), as described previously [23]. Insoluble protein fractions were resolved using Wes 12 to 230 kDa (fibrin(ogen)-β [Fib-β]) or 66 to 440 kDa 25-capillary gels (Fibα) (ProteinSimple). Fibrinogen polypeptides were detected using antibodies selective for each individual fibrinogen chain [24] (Proteintech) (1:100 dilution for α chain and 1:1000 dilution for β chain), as described previously [23]. A goat anti-rabbit HRP-conjugated secondary antibody (1:400 dilution for detection of Fibα antibody and 1:700 dilution for Fib-β antibody; Jackson ImmunoResearch) and other reagents accompanying the Wes Master Kit were used according to the manufacturer's protocol. Quantification of fibrin(ogen) peak area was performed using Compass for Simple Western software (version 6.0.0, ProteinSimple). Hepatic platelet accumulation was evaluated by Wes for the platelet-specific integrin α_{IIb} in detergent-soluble liver extracts. Protein samples were diluted in Laemmli sample buffer containing β-mercaptoethanol and heat-denatured for 10 minutes at 95 °C. Ten micrograms of protein were loaded and separated using sodium dodecyl-sulfate polyacrylamide gel electrophoresis on a 4% to 12% Bis-Tris gel (Bio-Rad) in Tris/Glycine/SDS buffer (Bio-Rad). Proteins were transferred to a polyvinylidene fluoride membrane (Millipore Sigma) using the Criterion Blotter System (Bio-Rad),

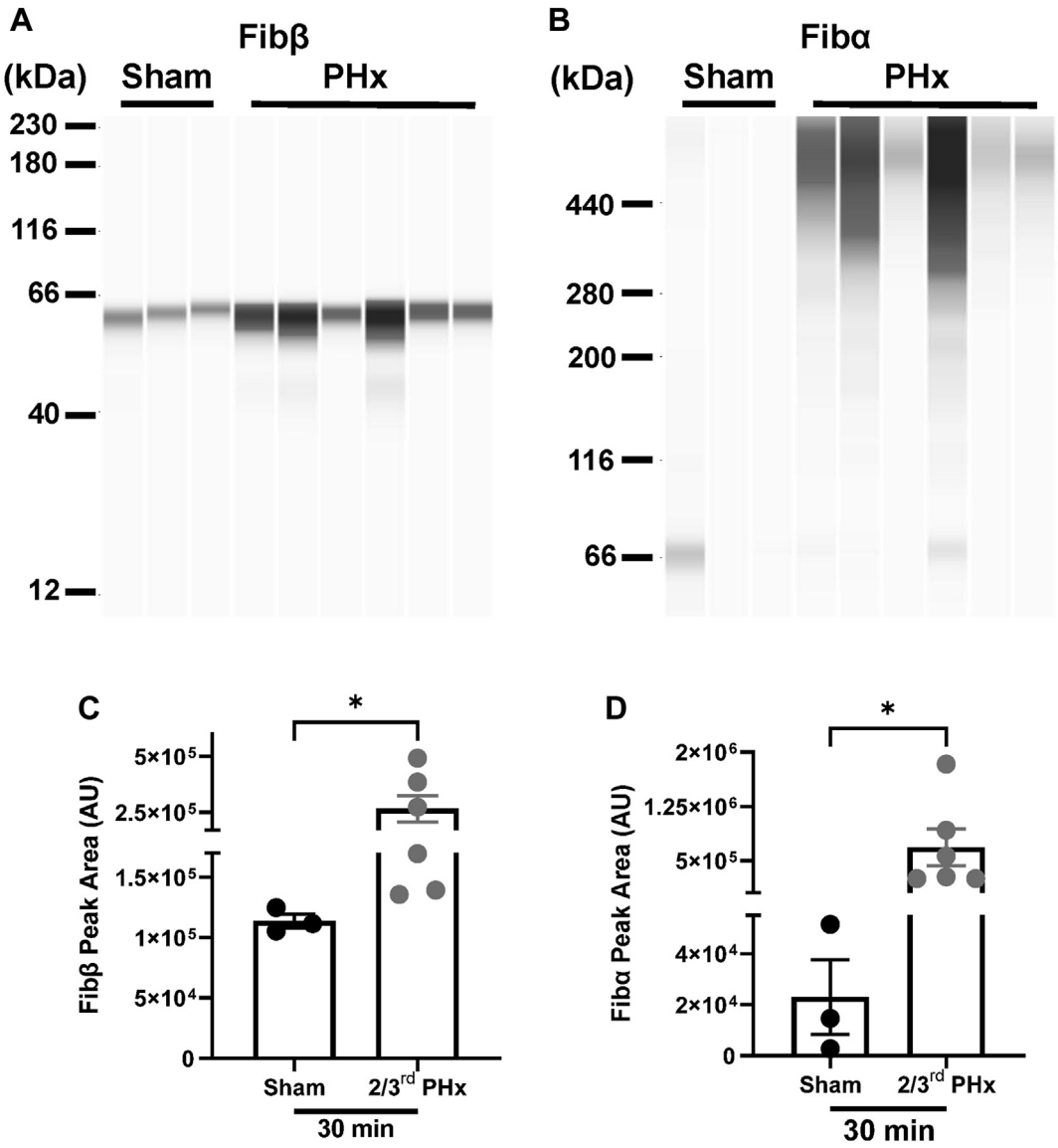

FIGURE 1 Two-thirds partial hepatectomy (PHx) induces rapid accumulation of insoluble cross-linked fibrin in the liver. Male wild-type mice underwent two-thirds PHx or sham surgery (see Methods), and livers were collected 30 minutes after the last lobe was resected. Fibrin(ogen) levels were measured in enriched insoluble liver extracts using automated capillary Western blotting. Representative digital capillary images show fibrin(ogen) detected by rabbit polyclonal antibodies selective for fibrin(ogen) (A) Bβ chain and (B) Aα chain. Quantification of peaks is shown in panels C and D. Results from individual mice are plotted, and bars represent mean ± SEM. N = 3 to 6 mice per group. $^*P < .05$.

and the membrane was blocked for 1 hour at room temperature in 5% bovine serum albumin in Tris-buffered saline with 0.1% Tween-20. Platelets were detected by a recombinant rabbit monoclonal anti-CD41 antibody (EPR17876) (Abcam 181582, 1:1000 dilution in blocking buffer). Peroxidase AffiniPure Goat Anti-Rabbit IgG (H + L) was used as the secondary antibody (1:10.000 dilution in 1% bovine serum albumin/Tris-buffered saline with 0.1% Tween-20, Jackson ImmunoResearch). For chemiluminescent detection, membranes were incubated with EcoBright Pico HRP substrate (Innovative Solutions) and exposed to blue autoradiography film (DOT Scientific). Total protein was evaluated using Revert 700 reagent and imaged using the Odyssey CLx Infrared Imaging System (Licor). Quantification of bands was performed using the gel analysis tool in Image Studio (Licor).

2.7 | Transmission electron microscopy

Liver biopsy samples (collected at Medical University of Vienna) were fixed in 4% paraformaldehyde in phosphate-buffered saline. After washing and fixing in 1% osmium tetroxide (resolved in 3% potassium hexacyanoferrate), the material was dehydrated and embedded in EPON Resin 812 (Serva). For ultrastructural assessment, thin sections were cut using an Ultracut UCT Ultramicrotome (Leica Microsystems), mounted on copper grids, and further counterstained with uranyl acetate and lead citrate. The sections were then examined at 60 kV in a JEOL JEM-1400 Plus transmission electron microscope, whereas images were obtained by using an Olympus Quemesa bottom-mounted TEM CCD camera and RADIUS—EM Imaging Software (Emsis GmbH).

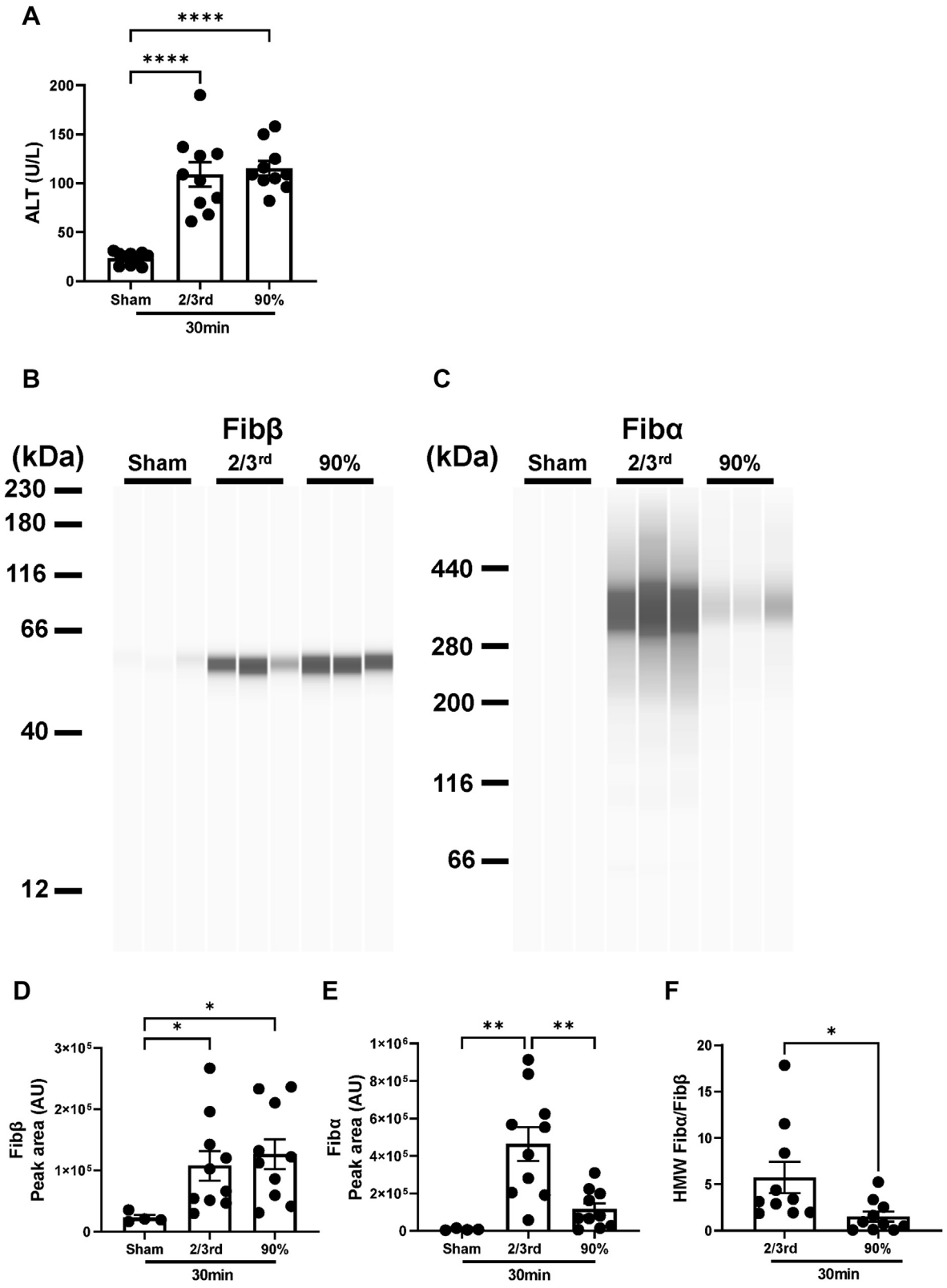

FIGURE 2 Hepatic fibrin cross-linking is reduced in mice after extended 90% partial hepatectomy (PHx). Male wild-type mice underwent two-thirds PHx, 90% extended PHx, or sham surgery (see Methods), and livers and plasma were collected 30 minutes after the last lobe was resected. (A) Plasma alanine aminotransferase (ALT) activity. Fibrin(ogen) levels were measured in enriched insoluble liver extracts using automated capillary Western blotting. Representative digital capillary images show fibrin(ogen) detected by rabbit polyclonal antibodies selective for fibrin(ogen) (B) Bβ chain and (C) Aα chain. Quantification of peaks is shown in panels D and E. Cross-linked high-molecular-weight (HMW) Aα chain was expressed relative to total insoluble fibrin levels (ie, Bβ chain). Results from individual mice are plotted, and bars represent mean ± SEM. N = 4 to 10 mice per group. $^{*}P < .05$; $^{**}P < .01$; $^{****}P < .0001$.

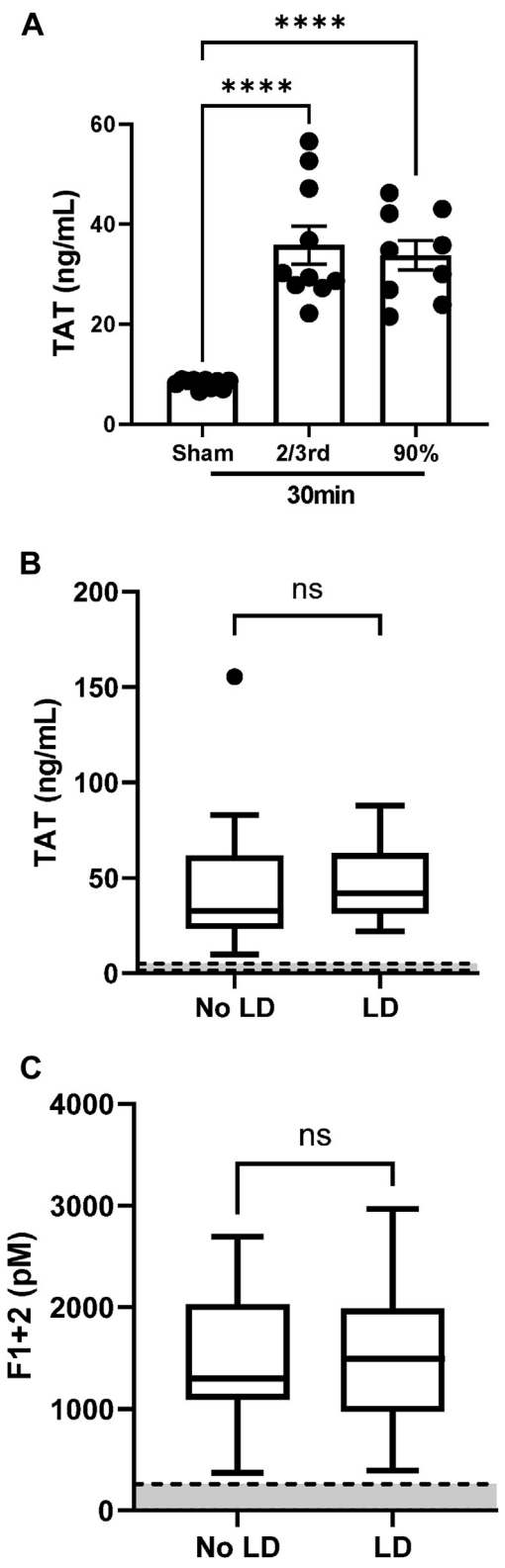

FIGURE 3 Biomarkers of coagulation cascade activation in mice and humans after partial hepatectomy (PHx). Male wild-type mice underwent two-thirds PHx, 90% extended PHx, or sham surgery (see Methods), and plasma was collected 30 minutes after the last lobe was resected. (A) Plasma thrombin-antithrombin (TAT) complexes were determined using a commercial enzyme-linked immunosorbent assay (see Methods). For B and C, the concentrations of (B) TAT complexes and(C) prothrombin fragment 1 + 2 were measured in hepatic vein plasma samples collected intraoperatively from 25 patients who underwent hemihepatectomy using commercial enzyme-linked immunosorbent assays (see Methods). For A, results from individual mice are plotted, and bars represent mean ± SEM. $N = 9$ or 10 mice per group. For B and C, results from 25 patients are expressed as Tukey box and whisker plots with the normal range indicated by gray space demarcated by dashed lines. ****$P < .0001$. LD, liver dysfunction; ns, not significant.

2.8 | Statistics

Statistical analyses were performed using GraphPad Prism v.9 software package. Continuous variables are presented as mean ± SEM. Comparison of 2 groups was performed using Student's *t*-test. Comparison of 3 or more groups was performed using 1-way analysis of variance with Tukey post hoc test. Results from patient samples were analyzed using a mixed-effects analysis with Ŝídák's post hoc test because of missing results for certain samples. A *P* value of less than .05 was considered statistically significant.

2.9 | CTAT methods

The CTAT methods are provided in the Supplementary Material.

3 | RESULTS

3.1 | Hepatic fibrin(ogen) cross-linking after two-thirds PHx in mice

Plasma TAT, a biomarker of coagulation cascade activation, increased within 30 minutes after two-thirds PHx, in agreement with prior studies [7], and returned to levels observed in sham mice by 6 hours after PHx in wild-type mice (Supplementary Figure S1). Prior studies used immunohistochemistry to observe rapid sinusoidal fibrin(ogen) accumulation after two-thirds PHx in mice and humans [7]. In agreement with this observation, hepatic levels of Fib-β increased in the insoluble protein fraction 30 minutes after two-thirds PHx (Figure 1A, C). Thrombin-mediated fibrin polymerization precedes fibrin cross-linking [25]. Notably, a robust increase in high-molecular-weight fibrin(ogen) complexes in the liver remnant, likely cross-linked α-polymer, was evident after two-thirds PHx (Figure 1B, D). Importantly, sinusoidal accumulation of fibrin was discernable in intraoperative liver biopsy samples collected from the liver remnant 2 hours after ligation of the portal vein in patients undergoing partial liver resection (red arrows, Supplementary Figure S2). The results support prior observations to suggest that insoluble cross-linked fibrin polymer (ie, traditional fibrin clot) formation occurs rapidly after PHx in the liver remnant.

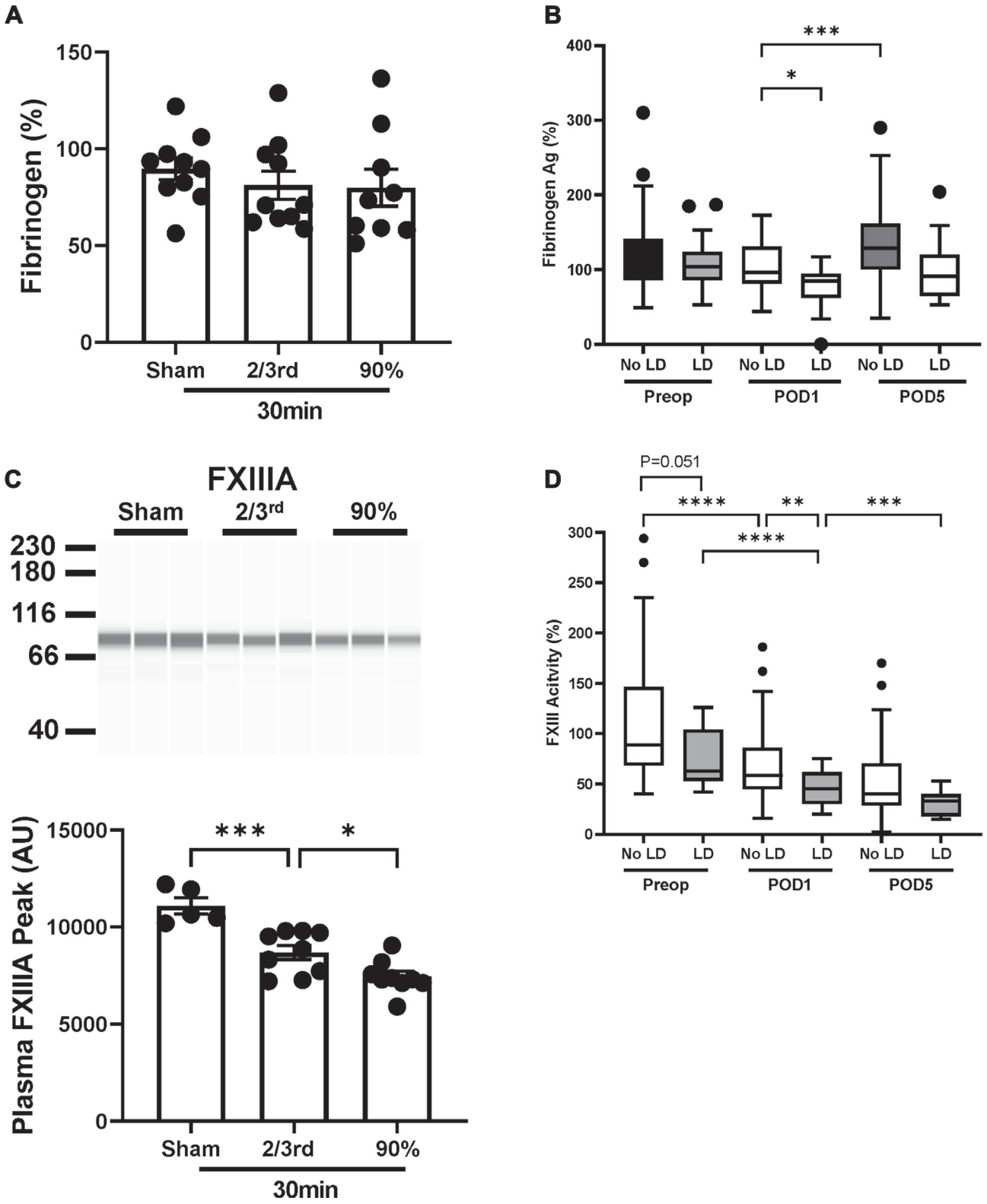

FIGURE 4 Plasma fibrinogen and factor (F)XIII in mice and humans after partial hepatectomy (PHx). Male wild-type mice underwent two-thirds PHx, 90% extended PHx, or sham surgery (see Methods), and plasma was collected 30 minutes after the last lobe was resected. (A) Plasma fibrinogen concentration was determined by enzyme-linked immunosorbent assay (see Methods). (B) Plasma fibrinogen concentration was determined by enzyme-linked immunosorbent assay (see Methods) in plasma samples collected from 88 patients 1 day prior to surgery as well as on postoperative day 1 (POD1) and postoperative day 5 (POD5) after liver resection. Liver dysfunction (LD) at 90 days postoperative was determined using the International Study Group of Liver Surgery criteria (see Methods). (C) Plasma FXIII-A antigen levels in mice after PHx were determined using capillary-based Western blotting. Representative samples are shown in a digital capillary rendering. (D) Plasma FXIII-A activity was determined in 88 patients as above for fibrinogen (see Methods). For A and C, results from individual mice are plotted, and bars represent mean ± SEM. $N = 5$ to 10 mice per group. For B and D, results from 88 patients are expressed as Tukey box and whisker plots. $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$; $^{****}P < .0001$.

3.2 | Impact of 90% PHx on hepatic fibrin(ogen) deposition

Notably, hepatic fibrin(ogen) accumulation in the liver remnant is evident in patients undergoing partial liver resection, but this response was attenuated in patients who developed postoperative liver dysfunction [7]. Seeking the basis for this observation, we compared the rapid fibrin(ogen) deposition in the standard setting two-thirds PHx in mice with that of 90% PHx (extended hepatectomy), a model of failed liver regeneration, hepatic dysfunction, and liver failure [18,26]. Both two-thirds PHx and 90% PHx caused a slight but significant increase in plasma alanine aminotransferase activity (Figure 2A). Standard two-thirds PHx significantly increased hepatic fibrin(ogen) accumulation 30 minutes after PHx, indicated by an increase in insoluble Fib-β (Figure 2B, D). A similar increase in Fib-β chain was observed in mice after 90% PHx (Figure 2B, D). Interestingly, levels of cross-linked α-polymer (apparent molecular weight, ~360 kD) in urea-extracted insoluble protein were markedly reduced in livers of mice after 90% PHx compared to mice that underwent two-thirds PHx (Figure 2C, E). The results suggest that cross-linking of hepatic fibrin(ogen) deposits is decreased in the liver after 90% PHx compared to two-thirds PHx.

3.3 | Coagulation activation, fibrinogen, and FXIII plasma levels in mice and humans after PHx

Our studies in mice and in humans point to altered fibrin(ogen) deposition/cross-linking as a common mechanism leading to impaired liver regeneration. Prior studies indicate that hepatic fibrin(ogen) deposition requires activation of the coagulation cascade [7]. Indeed, the extent of coagulation activation and thrombin levels play a key role in penultimate fibrin structure and stability [27]. Thus, we evaluated biomarkers of coagulation activation in both mice and patients after PHx. Plasma TAT complexes were significantly increased after two-thirds PHx and similarly increased after 90% PHx compared to sham surgery (Figure 3A). Likewise, the concentration of markers of coagulation activation (ie, TAT complexes [normal, 1.6-5.1 ng/mL] and prothrombin fragment 1 + 2 [normal, 34.4-260.3 pM]) measured in plasma collected from the hepatic vein (ie, blood draining from the regenerating lobe) 2 hours after induction of regeneration were similar in patients who did or did not develop liver dysfunction (Figure 3B, C). Plasma fibrinogen concentration decreased only slightly after either two-thirds or 90% PHx (Figure 4A). Preoperative plasma fibrinogen antigen concentration was also similar in patients who did or did not develop hepatic dysfunction after PHx (Figure 4B). Notably, plasma fibrinogen concentration in patients developing liver dysfunction reduced on POD1 compared to baseline values and remained low on POD5 (Figure 4B), whereas fibrinogen levels in patients who did not develop liver dysfunction did not change over time. Collectively, the results suggest that changes in intrahepatic fibrin(ogen) deposition are not attributed to insufficient thrombin generation or insufficient fibrinogen. Plasma FXIII circulates in a complex with fibrinogen. Upon activation by thrombin, FXIIIa imposes covalent cross-links at specific residues on the fibrin α and γ polypeptides, which increases clot stability and improves resistance to clot lysis [28]. Plasma FXIII-A antigen concentration was significantly reduced after two-thirds PHx and 90% PHx in mice, with the greatest reduction evident after 90% PHx (Figure 4C). Mirroring changes in mice after PHx, plasma FXIIIa activity was reduced on POD1 in patients after partial liver resection, and FXIIIa activity was lowest in patients who developed liver dysfunction (Figure 4D). Interestingly, preoperative plasma FXIIIa activity tended to be lower (P = .051) in patients who ultimately developed liver dysfunction after surgery (42%-101%; median, 63%) compared to those who did not (40%-294%; median, 89%) (Figure 4D).

3.4 | FXIII-mediated fibrin(ogen) cross-linking is a key driver of hepatic platelet accumulation after PHx

We next determined the impact of FXIII deficiency on hepatic fibrin(ogen) cross-linking in mice after two-thirds PHx, using mice deficient in the catalytic subunit FXIII-A [29]. Insoluble cross-linked fibrin was evident in livers of wild-type mice 30 minutes after two-thirds PHx (Figure 5A–D). Deposition of cross-linked fibrin was dramatically reduced in livers of FXIII-$A^{-/-}$ mice after two-thirds PHx (Figure 5B, D). Notably, overall levels of insoluble fibrin(ogen) were reduced in livers of FXIII-$A^{-/-}$ mice. Prior studies have shown that plasma fibrinogen depletion reduced hepatic accumulation of platelets after PHx, a key proregenerative signal [30,31]. Notably, hepatic integrin α_{IIb} levels were significantly reduced in livers of FXIII-$A^{-/-}$ mice compared to wild-type mice after two-thirds PHx (Figure 5E, F). Collectively, the results suggest that stabilization of hepatic fibrin deposits and initial hepatic platelet accumulation after PHx is driven in part by FXIII-dependent fibrin cross-linking.

3.5 | Impact of FXIII deficiency on hepatocyte proliferation after two-thirds PHx

As our study thus far suggested that FXIII-dependent fibrin cross-linking was altered in experimental 90% PHx-induced liver dysfunction and plasma FXIIIa activity was reduced in patients who developed liver dysfunction, we sought to determine the impact of FXIII-A deficiency on liver regeneration after two-thirds PHx. Proliferating hepatocytes ($Ki67^{+}$) were abundant in livers of male wild-type mice 48 hours after two-thirds PHx (Figure 6A, C), and FXIII-A deficiency significantly reduced the number of $Ki67^{+}$ hepatocytes (Figure 6B, C). This corresponded to a reduction in liver–to–body weight ratio 48 hours after two-thirds PHx in both male and female mice (Figure 6D). Remarkably, although no obvious morbidity was evident in wild-type mice, several FXIII-deficient mice died between 24 and 48 hours after two-thirds PHx (Figure 6E). The results indicate that FXIII

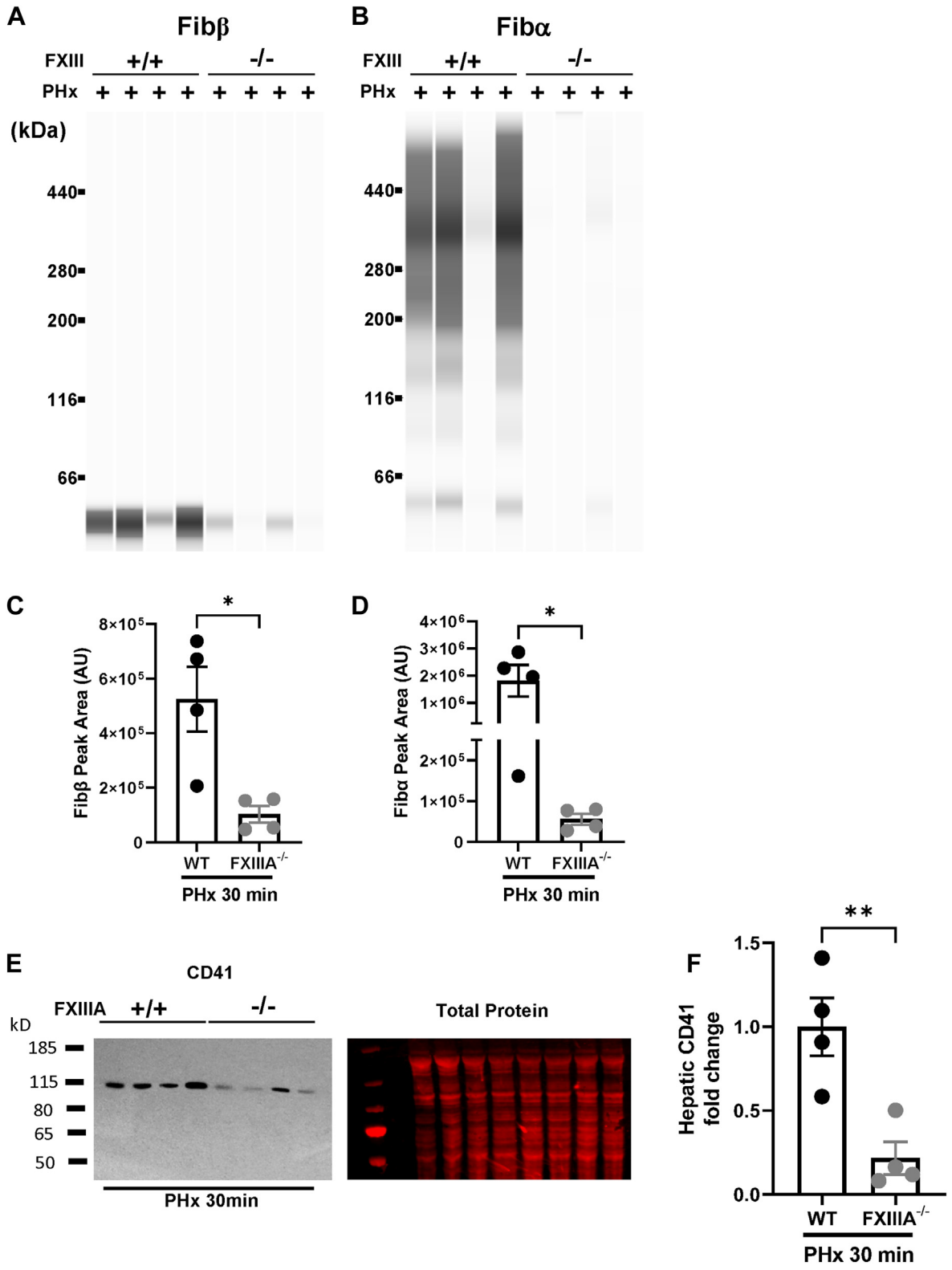

FIGURE 5 Factor (F)XIII drives hepatic fibrin cross-linking and platelet accumulation after partial hepatectomy (PHx). Male wild-type (WT) mice and FXIII-A$^{-/-}$ mice underwent two-thirds PHx (see Methods), and livers and plasma were collected 30 minutes after the last lobe was resected. Fibrin(ogen) levels were measured in enriched insoluble liver extracts using automated capillary Western blotting. Representative digital capillary images show fibrin(ogen) detected by rabbit polyclonal antibodies selective for fibrin(ogen) (A) Bβ chain and (B) Aα chain. Quantification of peaks is shown in panels C and D. (E, F) Hepatic levels of the platelet integrin CD41 were determined by Western blotting. Results from individual mice are plotted, and bars represent mean ± SEM. N = 4 mice per group. *$P < .05$; **$P < .01$.

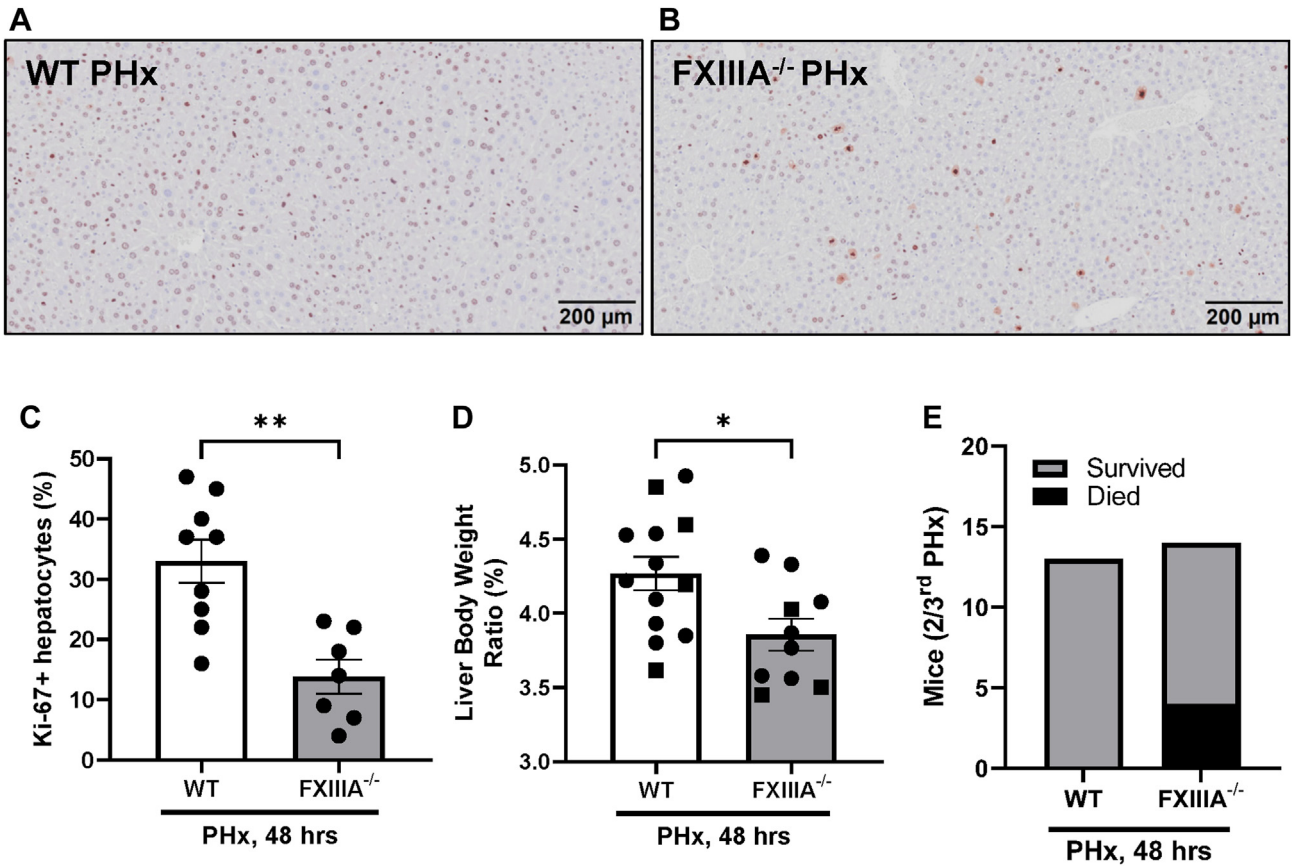

FIGURE 6 Factor (F)XIII promotes hepatocyte proliferation after partial hepatectomy (PHx). Male and female wild-type (WT) mice and FXIII-A$^{-/-}$ mice underwent two-thirds of PHx (see Methods), and livers were collected 48 hours later. Representative photomicrographs showing immunohistochemical labeling of Ki67-positive (brown, DAB) hepatocytes in (A) WT and (B) FXIII-A$^{-/-}$ mice. (C) Percentage Ki67+ hepatocytes was quantified (see Methods), and (D) liver–to–body weight ratio. (E) Observed mortality. Results from individual mice are plotted, and bars represent mean ± SEM. Male mice are shown as circles, whereas female mice are shown as squares. $^{*}P < .05$; $^{**}P < .01$.

deficiency and a lack of hepatic fibrin cross-linking are coupled to reduced hepatocyte proliferation.

4 | DISCUSSION

PHx induces rapid intrahepatic activation of the coagulation cascade in the regenerating liver remnant [7], and hepatic accumulation of fibrin(ogen) has been linked to hepatocyte proliferation and liver regeneration in both humans and mice [7,8]. Compared to liver regeneration induced by standard two-thirds PHx, we found that cross-linking of insoluble hepatic fibrin was altered in mice after 90% PHx, an experimental setting of hepatic dysfunction/failed regeneration. Plasma levels of the transglutaminase FXIIIa, which can cross-link fibrin, were reduced before and after surgery in patients who developed posthepatectomy liver dysfunction compared to patients who did not develop liver dysfunction. Notably, we found that complete FXIII-A deficiency in mice dramatically reduced hepatic levels of cross-linked fibrin, attenuated hepatic platelet accumulation, and reduced hepatocyte proliferation in mice after standard two-thirds PHx. These results suggest that reduced FXIIIa activity or altered FXIII-directed fibrin(ogen) cross-linking are associated with reduced liver regeneration and liver dysfunction after PHx.

Ten percent to 15% of patients have insufficient regeneration and develop liver dysfunction after hepatectomy. Intraoperative hepatic fibrin(ogen) deposition, evident in the liver remnant in the first few hours after portal vein ligation, is diminished in patients who develop liver dysfunction [7]. We sought to uncover which aspect of intrahepatic hemostasis was "failing" in patients who ultimately developed liver dysfunction. One potential explanation for altered fibrin(ogen) accumulation in the liver remnants of patients who ultimately developed liver dysfunction is relatively low preoperative plasma fibrinogen levels. However, preoperative fibrinogen concentration was not reduced in patients who developed liver dysfunction compared to those who did not, as measured by ELISA (Figure 4B) or by Clauss assay, as described previously [7]. Postoperative changes in plasma fibrinogen may also portend liver dysfunction [7], likely reflecting a reduction in de novo expression of fibrinogen by the liver and perhaps a reduction in continued hepatic fibrinogen deposition. The collective consequences of these and other hemostatic changes could ultimately also connect to the requirement for postoperative transfusion. We also considered the possibility that coagulation activity (eg, thrombin

generation) could be reduced after PHx. However, biomarkers of coagulation activation were roughly equivalent after standard (two-thirds) and 90% PHx in mice. Moreover, there was no association of TAT complexes or prothrombin fragment 1 + 2 with the propensity to develop liver dysfunction in patients. Thus, a reduction in fibrin(ogen) accumulation in the liver remnant cannot be ascribed to a relative lack of fibrinogen or a failure to generate sufficient amounts of thrombin.

Despite equivalent coagulation activation after standard PHx (two-thirds) and 90% PHx in mice, altered hepatic fibrin cross-linking was evident in the liver remnant 30 minutes after 90% PHx. The mechanistic basis for this observation is unclear, but we hypothesize that this is connected to FXIII as we found that FXIII was largely responsible for fibrin cross-linking in the liver after PHx in mice. Moreover, we observed reduced FXIIIa activity in patients who developed liver dysfunction compared to patients who did not develop liver dysfunction after PHx. The mechanistic basis for this reduction and how it may potentially relate to patient demographics (Supplementary Table S3), such as surgical differences or tumor type, is not yet known. FXIII is a heterotetramer composed of regulatory (B) and catalytic (A) subunits synthesized by different cell types (ie, FXIII-A [hematopoietic cells] [32,33]; FXIII-B [hepatocytes] [32,34]). Deficiency in each FXIII subunit impacts the plasma concentration of the other [16,35–37]. Thus, altered expression of either FXIII-A or FXIII-B could explain reduced FXIIIa activity in patients who developed liver dysfunction after PHx. Prior transcriptomic analysis did not uncover changes in *F13B* or *F13A1* in livers of patients who did or did not develop liver dysfunction after PHx [38]. Additional possibilities worth exploring include extrahepatic alterations in FXIII-A production, as they may occur in inflammatory disease [13], or the presence of a FXIII inhibitor.

FXIII-deficient mice displayed a substantial reduction in hepatocyte proliferation after PHx. The precise mechanisms linking FXIII to liver regeneration are not known, and additional studies are required to pinpoint downstream substrates driving liver regeneration after PHx. Reduced FXIII-dependent fibrin cross-linking may favor premature fibrinolysis. Notably, the Hemorrhage During Liver Resection: Tranexamic Acid trial (NCT02261415) seeks to determine the beneficial effect of tranexamic acid on bleeding during partial liver resection but may very well hint at related pro-regenerative effects. Notably, FXIIIa cross-links multiple proteins and contributes not only to hemostasis but also to wound healing [14], leaving fibrinogen-independent effects of FXIII fully plausible. Notably, acute depletion of plasma fibrinogen with ancrod also reduced hepatocyte proliferation after PHx [7]. Moreover, FXIII deficiency reduced fibrin cross-linking and insoluble fibrin accumulation after PHx. The reduction in fibrin cross-linking in FXIII-deficient mice after PHx was associated with a marked reduction in hepatic platelet accumulation. This is important because multiple studies support the concept that rapid (but transient) platelet accumulation drives liver regeneration in mice and humans [30,31]. Although multiple mechanisms alter platelet function in the context of liver regeneration [39], FXIII-induced cross-linking stabilizes fibrin [40], and this may be critical to drive platelet accumulation in the early moments after PHx. Moreover, multiple studies have shown that FXIII directly contributes to platelet adhesion to fibrin and drives platelet responses secondary to fibrin(ogen)-integrin $\alpha_{IIb}\beta_3$ engagement [41–43]. In addition to platelets, fibrin engagement of leukocyte β2 integrins [44] could contribute to liver regeneration by modulating effector functions of either neutrophils or macrophages, both cell types linked to liver regeneration in mice and humans [45,46]. Collectively, multiple mechanisms support our results and the concept that FXIII-mediated fibrin cross-linking supports early hepatic platelet accumulation critical for liver regeneration.

In summary, we uncovered a failure of fibrin cross-linking in experimental PHx-induced liver dysfunction and found that complete FXIII deficiency substantially reduced hepatocyte proliferation after standard two-thirds PHx in mice. Interestingly, plasma FXIII activity was reduced in patients who developed liver dysfunction after PHx compared to those who did not, even before surgery. The precise basis for this reduction and the impact of underlying disease states (eg, steatotic liver disease) on plasma FXIIIa activity could help identify patients at risk of PHLF. Importantly, correction of plasma FXIII plasma levels could be accomplished using US Food and Drug Administration–approved plasma-purified or recombinant FXIII, each of which could be repurposed and administered during surgery.

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AUTHOR CONTRIBUTIONS

Z.W., D.J.G., J.A., L.G.P., H.C., A.E.K., B.L., L.B., A.A., P.S., T.L., and J.P.L. contributed to the design of these studies. Experiments were performed and data were collected and interpreted by all authors. Previously collected samples from patients who underwent partial liver resection were from the tissue repository developed and maintained by P.S. and A.A.. Analysis of repository samples was performed by J.A. and T.L.. Experimental partial hepatectomy and related results acquisition was performed by Z.W., D.J.G., L.G.P., H.C., and J.P.L.. The manuscript was drafted by J.P.L.. All authors reviewed and approved the submitted version of the manuscript.

DECLARATION OF COMPETING INTERESTS

There are no competing interests to disclose.

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SUPPLEMENTARY MATERIAL

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