Chitinase 3-like-1 Promotes Intrahepatic Activation of Coagulation Through Induction of Tissue Factor in Mice

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Abstract

Coagulation is a critical component in the progression of liver disease. Identification of key molecules involved in the intrahepatic activation of coagulation (IAOC) will be instrumental in the development of effective therapies against liver disease. Using a mouse model of concanavalin A (Con A)-induced hepatitis, in which IAOC plays an essential role in causing liver injury, we uncovered a previously unknown procoagulant function of chitinase 3 like 1 (Chi3l1). Chi3l1 expression is dramatically elevated after Con A challenge, which is dependent on Con A-induced T cell activation, and the resulting IFN-γ and TNF-α productions. Compared to wild type mice, Chi3l1$^{-/-}$ mice show less IAOC, reduced tissue factor (TF) expression and attenuated liver injury. Reconstituting Chi3l1$^{-/-}$ mice with recombinant TF triggers IAOC and augments liver injury. Conclusion: Our data demonstrate that Chi3l1, through induction of TF via MAPK activation, promotes IAOC and tissue injury.

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Keywords

Chi3l1; intrahepatic activation of coagulation; liver disease; tissue factor; concanavalin A

Introduction

The activation of blood coagulation cascade is evident in a variety of acute liver injury[1–3]. Growing evidence indicates that coagulation is not merely a process reactive to toxicity, but rather a critical determinant of acute liver disease progression[4–10]. A reduction in liver damage by administration of anticoagulant has been observed in a variety of animal models, including APAP-induced hepatotoxicity, hepatic ischemia reperfusion injury, as well as liver injury caused by lipopolysaccharide/D-galactosamine, anti-Fas antibody and concanavalin A (Con A)[4–10]. The alleviation of liver injury in hepatocyte TF deficient mice further demonstrates the significance of intrahepatic activation of coagulation (IAOC) in acute liver injury[11,12]. However, the mechanisms by which coagulation cascade exacerbates liver disease are not completely understood.

To gain mechanistic insights and identify key players involved in intrahepatic coagulation, we used a mouse model of Con A-induced liver injury (CILI), in which excessive IAOC drives tissue damage[9,10,13,14]. Con A is a lectin with agglutination activities resulting in prominent intra-sinusoidal stasis. This phenomenon is manifested by erythrocyte agglutination, adherence of lymphocytes and neutrophils to the endothelium, as well as platelet aggregation/degranulation. Together, these events result in a marked decrease of intrahepatic blood flow and elevated portal pressure, culminating in severe liver injury[9,15]. It has been demonstrated that anticoagulant treatment significantly attenuates CILI, supporting that the sinusoidal hypercoagulation with increased thrombin generation plays a paramount role in the injury process[9,14]. To identify novel mediators of IAOC, we screened a panel of soluble factors produced by innate and adaptive immune cells that are known to be activated by Con A. We found that both mRNA and protein levels of chitinase 3 like 1 (Chi3l1) were significantly elevated after Con A challenge.

Chi3l1 is a chitinase like soluble protein without chitinase activities[16]. It is produced by multiple cell types, including neutrophils, macrophages, chondrocytes, synovial cells, smooth muscle cells, endothelial cells, and tumor cells[16,17]. Increased levels of Chi3l1 has been correlated with poor prognosis in liver diseases, such as hepatic fibrosis, non-alcoholic fatty liver, alcoholic liver disease, and hepatocellular carcinoma[18,19]. However, the biological functions of Chi3l1 in liver remain mysterious. Our study indicates that Chi3l1 plays a critical role in promoting IAOC and liver injury through inducing tissue factor (TF) expression via MAPK activation. This discloses a previously unappreciated pro-coagulant function of Chi3l1. The findings suggest that Chi3l1 may serve as a therapeutic target in the treatment of liver disease associated with intrahepatic coagulation.

Results

Chi3l1 contributes to CILI

Con A challenge causes soluble mediators released by a variety of immune cells in the liver, including natural killer (NK) cell, NKT cell, neutrophils, macrophages, and T cells[20–23]. We measured the hepatic mRNA expression of a panel of these mediators in wild-type (WT) C57Bl/6J mice at 3h after Con A challenge (Fig. S1). Among these mediators, both mRNA and protein levels of Chi3l1 were dramatically increased post-Con A (Fig. 1, A–C). Interestingly, we found that mice deficient in Chi3l1 (Chi3l1$^{-/-}$) were less susceptible to CILI than WT mice. The ALT levels in Chi3l1$^{-/-}$ mice were 1,200 and 600 IU/l at 8 and 24 h, respectively; whereas the levels in WT mice were as high as 5600 IU/l (Fig. 1D). In accordance with the reduced ALT activities, the histopathology of the liver showed less hepatocyte injury in Chi3l1$^{-/-}$ (Fig. 1, E&F). These results suggest that Chi3l1 is produced and released after Con A challenge and it contributes to CILI.

Con A-induced immune responses are not affected by Chi3l1

The activation of immune cells (T, NKT, neutrophils, macrophages) and their cytokine products, especially IFN-γ and TNF-α, have been reported to contribute to CILI[20,21,24,25]. We first investigated whether Chi3l1 could potentially affect these immune cells and their cytokine production. Our data showed no significant differences in the number of CD3$^+$ T cells in the livers of WT and Chi3l1$^{-/-}$ mice (Fig. 2, A&B). The amounts of IFN-γ and TNF-α production by hepatic T cells were also similar between WT and Chi3l1$^{-/-}$ mice (Fig. 2, C–F). The serum levels of TNF-α were even higher in Chi3l1$^{-/-}$ than WT mice at 3h post-Con A (Fig. 2F). NKT cells and their IL-4 production are important in CILI[20]. However, our data (Fig. S3, A&B) showed that the numbers of hepatic NKT cells and their intracellular expression of IL-4 were comparable between WT and Chi3l1$^{-/-}$ mice. It has been reported that neutrophils and macrophages are important in promoting T cell recruitment and subsequent development of hepatitis[21,24,26]. However, we did not find any differences in the numbers of neutrophils or macrophages in the liver between WT and Chi3l1$^{-/-}$ mice (Fig. S4, A–D). Taken together, these data suggest that Chi3l1 does not affect Con A-induced activation of the innate and adaptive immune systems.

Chi3l1-deficiency attenuates CILI

Aside from the pro-inflammatory immune responses, IAOC represents another critical underlying mechanism for CILI[9,10]. To explore whether Chi3l1 contributes to CILI through promoting coagulation, we compared fibrin(ogen) deposition, thrombin anti-thrombin complex (TAT) levels and platelets influx between WT and Chi3l1$^{-/-}$ mice. WT mice had higher levels of fibrin(ogen) deposition (Fig. 3, A&B) in liver tissues than Chi3l1$^{-/-}$ mice at both 6h and 24h post-Con A. TAT complexes are used as an indicator for thrombin formation in the circulation[27]. Our data showed that the plasma TAT levels were significantly elevated after Con A challenge in WT mice, but not in Chi3l1$^{-/-}$ mice (Fig. 3E). Moreover, platelets influx significantly increased at 3h post-Con A, but there was no difference between WT and Chi3l1$^{-/-}$ mice (Fig. S5, A&B). Taken together, these results demonstrate that Chi3l1 is critically involved in IAOC.

The effect of Chi3l1 on CILI depends on T cell-derived IFN-γ and TNF-α

It has been reported that T cell activation and their production of IFN-γ and TNF-α contribute to CILI[24]. To determine if the Chi3l1 is a downstream mediator of this pathway, we treated WT mice with a T cell suppressor, FK506, 1 h before Con A challenge. As expected, FK506 treatment inhibited Con A-induced up-regulation of *IFN-γ* and *TNF-α* mRNA levels in the liver (Fig. 4A). Interestingly, the mRNA expression levels of *Chi3l1* were also markedly reduced by FK506 (Fig. 4A). Consistently, the Chi3l1 protein expression in the liver and serum were also dramatically reduced by the FK506 treatment (Fig. 4, B&C). These results indicate that T cell activation is necessary to induce Chi3l1 production. Systematic time course studies also showed that the elevation of IFN-γ and TNF-α preceded that of Chi3l1 (Fig. 5A). Thus, to determine if IFN-γ and TNF-α induce Chi3l1 expression, we treated mice with anti-IFN-γ and TNF-α antibodies prior to Con A challenge. Blocking IFN-γ and TNF-α prevented Con A-induced up-regulation of both mRNA and protein expressions of Chi3l1 (Fig. 5, B–D).

In agreement with the notion that IFN-γ and TNF-α are key players causing CILI, our data showed that blocking IFN-γ and TNF-α significantly reduced fibrin(ogen) deposition, the degree of hepatic necrosis, and ALT activities (Fig. S6, A–C). To further demonstrate that Chi3l1 can be induced by IFN-γ and TNF-α, we isolated liver sinusoid endothelial cells (LSEC) and hepatic macrophages (MΦ) from naïve WT mice and treated the cells with either IFN-γ or TNF-α. We found that both IFN-γ and TNF-α could significantly up-regulate Chi3l1 mRNA expression in LSEC and hepatic MΦ (Fig 5, E&F). These data suggest that IFN-γ and TNF-α are potent regulators in inducing of Chi3l1 both *in vivo* and *in vitro*.

Chi3l1 plays a crucial role in the induction of TF

To understand the mechanism by which Chi3l1 contributes to CILI, we screened a number of coagulation factors after Con A treatment (Fig. S7). The baseline levels of the coagulation factors in WT and Chi3l1$^{-/-}$ mice were similar (Fig. S7A). At 3h after Con A treatment, the hepatic mRNA levels of *TF*, *PAI-1*, *anti-thrombin*, *protein C* and *fibrinogen* were significantly lower in Chi3l1$^{-/-}$ compared to WT mice among the factors we screened (Fig. S7B). It has been reported that TF-mediated IAOC contributes to CILI[10]. Through systematic time course studies, we also noticed that TF induction proceeds coagulation and liver injury caused by Con A (Fig. S8 A&B), indicating that Chi3l1 may be important in inducing TF production, thereby promoting coagulation. We found that compared with WT mice, Chi3l1$^{-/-}$ mice expressed significantly lower mRNA and protein levels of TF (Fig. 6, A–C; Fig. S9). To further demonstrate that Chi3l1 can induce TF, we reconstituted Chi3l1$^{-/-}$ mice with recombinant Chi3l1 (rChi3l1). As shown in Fig 6E, administration of rChi3l1 significantly increased the mRNA expression of *TF* in the liver. The combination of rChi3l1 and Con A dramatically up-regulated the mRNA and protein levels of TF (Fig. 6, D&E; Fig. S10A). Consistent with the induction of TF, reconstitution of Chi3l1$^{-/-}$ mice with rChi3l1 significantly increased the levels of fibrin(ogen) deposition in the liver and augmented CILI, evident by the increased ALT activities and worsened histology (Fig. 6, D&F; Fig. S10B). These data indicate that Chi3l1 plays a critical role in IAOC through the induction of TF. Interestingly, in the absence of Con A treatment, rChi3l1 alone did not cause coagulation in

Chi3l1$^{-/-}$ mice, suggesting that Chi3l1 regulates the expression of TF, but the coagulant activity of TF is induced by Con A in a Chi3l1-independent manner.

TF-induced by Chi3l1 drives activation of coagulation

To confirm that TF drives IAOC, we blocked endogenous TF protein using an anti-TF (1H1) antibody and found that Con A-induced IAOC and liver injury were significantly decreased (Fig. S11, A&B). To further examine our hypothesis that in the Chi3l1$^{-/-}$ mice, it is the attenuated TF production that results in reduced liver injury, we administered exogenous rTF to Chi3l1$^{-/-}$ mice. Because the rTF we injected was not functionally activated by relipidation and lacked procoagulant activity, it alone did not cause systemic coagulation (Fig. S11C). However, in the presence of Con A treatment, likely caused activation of rTF, we observed significant increases of Con A-induced IAOC and liver injury in Chi3l1$^{-/-}$ mice (Fig. 7A and B). These data implicate that Chi3l1 induces TF expression but does not affect its activation. To further substantiate the role of Chi3l1 in TF induction, we reconstituted Chi3l1$^{-/-}$ mice with rChi3l1 and meanwhile neutralized TF. The data showed that rChi3l1-induced worsening of CILI and IAOC was abrogated by TF neutralization (Fig. 7).

Chi3l1 induces TF expression through MAPK signaling pathway

It is reported that TF is expressed by hepatic MΦ, LSEC, and hepatocytes during CILI[24]. We examined *TF* mRNA expression in hepatic MΦ, LSEC, and hepatocytes from both naïve WT and Chi3l1$^{-/-}$ mice and mice treated with Con A for 3h. The baseline levels of *TF* mRNA in hepatocytes are about 8 fold higher than that in hepatic MΦ and LSEC in both WT and Chi3l1$^{-/-}$ mice (Fig. 8A). After Con A, *TF* mRNA increased 18-fold in hepatic MΦ of both WT and Chi3l1$^{-/-}$ mice. However, *TF* mRNA increased nearly 40-fold in LSEC and 45-fold in hepatocytes of WT mice, but only increased around 9-fold in LSEC and 19-fold in hepatocytes of Chi3l1$^{-/-}$ mice (Fig. 8A). To directly address whether Chi3l1 induces TF in these cells, we stimulated hepatic MΦ, LSEC, and hepatocytes isolated from naïve WT mice with rChi3l1. *TF* mRNA expression were induced in LSEC and hepatocytes but not in hepatic MΦ *in vitro* (Fig. 8B). Interestingly, hepatocytes are more responsive to rChi3l1. The elevation of *TF* mRNA levels is much more dramatic in hepatocytes (80 fold) than LSEC (3 fold) (Fig. 8B). The measurement of endotoxin levels and experiment using polymyxin B ruled out the possibility of TF induction due to endotoxin contamination of rChi3l1 (Fig. S12 A&B). To compare the potencies of IFN-γ, TNF-α and Chi3l1 in the induction of TF, we treated LSEC with the concentrations of these mediators detected in serum at 3h after Con A treatment. Among the three potential mediators, only rChi3l1 induced TF up-regulation (Fig. 8C). These data further suggest that Chi3l1 is an important downstream mediator of IFN-γ/TNF-α in inducing TF expression. To shed light on the molecular mechanisms by which Chi3l1 regulates TF expression, we screened several potential signaling pathways. Our data showed that Chi3l1 activated AKT and the mitogen-activated protein kinase (MAPK) family, including ERK1/2, c-Jun amino terminal kinases (JNKs), and p38 in transformed sinusoid endothelial cell line (TSEC) (Fig. 8D). However, rChi3l1 did not activate NF-κB or STAT1 (Fig. 8D). We then treated LSEC with rChi3l1 in combination with various MAPK inhibitors (JNK: SP600125, p38: SB203580, ERK: U0126), all of which significantly reduced TF expression levels (Fig. 8E). These results suggest Chi3l1 induces TF expression through activating MAPK signaling pathway.

Discussion

In this study, we demonstrate that after Con A administration, activated T cells produce IFN-γ and TNF-α, which induce Chi3l1 production. The released Chi3l1 triggers MAPK activation and promotes TF production by LSEC and hepatocytes, thereby initiating IAOC and leading to liver injury (Fig. 8F).

Our observations that i) IFN-γ and TNF-α production precedes Chi3l1 release (Fig. 5A), ii) T cell suppression abrogates the elevation of Chi3l1 (Fig. 4, A–C), and iii) neutralization of IFN-γ and TNF-α dramatically reduces the levels of Chi3l1 (Fig. 5, B–D), suggest that Con A-triggered T cell activation and the production of IFN-γ and TNF-α play crucial roles in the up-regulation of Chi3l1. It has been reported that IFN-γ and TNF-α are important in inducing TF production and promoting hepatic thrombosis within the liver[24]. However, after Con A treatment, WT and Chi3l1$^{-/-}$ mice produce similar amount of IFN-γ and TNF-α (Fig. 2, C–F), but TF expression is much lower in Chi3l1$^{-/-}$ mice (Fig. 6, A–C, Fig. S9). Moreover, at the levels that can be detected *in vivo*, IFN-γ and TNF-α could not directly induce TF expression *in vitro* (Fig. 8C). These findings suggest that Chi3l1 is one of the downstream mediators of IFN-γ and TNF-α that plays an important role in inducing TF expression. Nonetheless, blocking IFN-γ/TNF-α did not completely abrogate Chi3l1 expression (Fig. 5). Thus, we cannot exclude the possibility that Con A may directly induce Chi3l1 expression, as Con A can activate LSEC and MΦs [9,26,28]. Aside from IFN-γ and TNF-α, other cytokines produced by Con A-activated T cells may also induce Chi3l1 expression. For example, it is reported that the expression of Chi3l1 can be regulated by IL-6, IL-13, IL-18, IL-1β[29–32]. Our data show that IFN-γ and TNF-α induce Chi3l1 expression in both LSEC and hepatic MΦ (Fig. 5, E&F). However, it is possible that other cells in the liver may also produce Chi3l1. Therefore, questions such as the cellular source of Chi3l1 and the receptor(s) through which it signals, warrant further research.

T cell activation plays a critical role in the mechanism of CILI[25]. Moreover, IFN-γ and TNF-α, produced by Con A-activated T cells, are critical in mediating tissue damage. In addition to triggering T cell activation, Con A has agglutination properties that cause stasis of blood components in liver sinusoids, preceding liver damage[9]. Ample evidence suggests that Con A-induced microcirculatory disturbance is of paramount importance contributing to CILI[9,10,13,14]. Anti-coagulant (heparin, cyproheptadine and antithrombin III) pretreatment decrease hepatic injury through reducing hemostasis, but it does not affect inflammatory responses[9,14]. In agreement with these findings, our data demonstrate that Chi3l1-deficiency confers a profound resistance to CILI without impairing T cell activation or inhibiting IFN-γ and TNF-α production (Fig. 2). In contrast, Con A-induced IAOC is markedly attenuated in Chi3l1$^{-/-}$ mice (Fig. 3). Taken together, our study and published reports demonstrate that Con A-induced IAOC and T cell activation are both critical events contributing to liver injury.

TF initiates coagulation and plays an integral role in blood coagulation and thrombin generation[11]. Our data suggest that in Chi3l1$^{-/-}$ mice, it is the reduced TF expression that results in attenuated CILI (Fig. 6&7). Both mRNA and protein levels of TF are markedly lower in the livers of Chi3l1$^{-/-}$ compared to WT mice (Fig. 6, A–C; Fig. S9). Reconstituting

Chi3l1^{−/−} mice with rChi3l1 dramatically increased the TF mRNA and protein expressions, and also augmented intrahepatic coagulation and CILI (Fig. 6, D–F; Fig. S7). Our data showed that administering exogenous TF alone did not cause coagulation, as the TAT level was not increased (Fig. S11C). Although TF is important in initiating coagulation, endothelial activation/injury resulting in platelet aggregation and activation is also required[33]. In Chi3l1^{−/−} mice treated with Con A, the platelets were recruited similarly as in WT mice (Fig. S5), but coagulation was suppressed (Fig. 3) due to the lack of TF (Fig. 7A). Thus, administering exogenous TF could promote coagulation in Con A-treated Chi3l1^{−/−} mice, recapitulating the IAOC and liver injury observed in WT mice. Moreover, neutralizing TF abrogated the effect of rChi3l1 treatment in Chi3l1^{−/−} mice (Fig. 7C–D), strongly suggesting that TF contributes to CILI and that the absence of Chi3l1 results in reduced TF expression and attenuated CILI in Chi3l1^{−/−} mice. It is reported that hepatocyte-derived TF contributes to IAOC caused by APAP treatment [11,12]. Our data show that Chi3l1 induces TF expression in both hepatocytes and LSEC (Fig. 8B; Fig. S9), suggesting that both cell types may be important source of TF production in CILI.

Liver diseases are consistently associated with coagulation activation mediated by TF[24,34–36]. TF-dependent activation of the coagulation cascade occurs and contributes to the progression of acute cholestatic hepatitis caused by α-naphthylisothiocyanate[35]. Myeloid cell-derived TF plays an important role in modulating hepatic necrosis during cholestasis[36]. In mice deficient of TF, overdose of acetaminophen-induced liver injury and activation of coagulation are significantly reduced[12]. Hematopoietic cell-derived TF is required for liver inflammation and steatosis in nonalcoholic fatty liver disease[37]. TF pathway contributes to liver fibrosis induced by chronic cholestasis by increasing expression of the αVβ6 integrin[38]. Administration of TF-antisense oligodeoxynucleotides successfully prevents monocrotaline/lipopolysaccharide-induced liver injury[34]. Therefore, the identification of Chi3L1 as a regulator of TF indicates an important role of Chi3l1 in liver diseases.

In summary, the present study demonstrates a novel pro-coagulant function of Chi3l1 and provides evidence that Chi3l1 induces TF production via MAPK activation. These findings identify Chi3l1 as a potential therapeutic target in treating coagulopathy in liver diseases and potentially other coagulation disorders.

Methods

Animal experiments and procedures

All animal experiments were performed according to guidelines from UC Denver Institutional Animal Care and Use Committee. 8–12 weeks old mice were injected with 15mg/kg Con A (Sigma, St. Louis, MO) intravenously (i.v.). Male and female mice respond to Con A treatment similarly, although their dose responses are different (Fig. 1D and Fig. S2)[39]. Because most of the published studies used male mice[25], we used male mice in our studies. In some experiments, mice received Con A followed immediately injection with anti-murine IFN-γ antibody (500ng, Peprotech 500 p119), anti-murine TNF-α antibody (500ng, Peprotech 500-P64), normal rabbit IgG (Peprotech, 500-p00), recombinant Chi3l1 (rChi3l1 500ng, Sino Biological 50929-M08H) by intraperitoneal (i.p.) or TF protein (500ng, R&D 3178-PA-010), anti-TF antibody (1H1, 500μg, Genentech Inc.) by intravenous

injection (i.v.). FK506 (32mg/kg body weight, Cayman 104987-11-3) was performed as previously described[9]. The levels of alanine aminotransferase (ALT) in serum and IFN-γ, TNF-α and Chi3l1 in serum and liver tissues were determined using commercially available kits (ALT, Teco Dignostics; ELISA, R&D).

Preparation of Liver Cells

Hepatic nonparenchymal cells and hepatocytes were isolated as previously described[40,41].

Histology

Hematoxylin and eosin (H&E) were performed by the Histology core at CUAMC on paraffin sections. Fibrin(ogen) was performed as previously described[24]. TF were stained with anti-mouse TF antibody (abcam151748). CD41 were stained by anti-mouse CD41 antibody (BD Bioscience, Clone MWReg 30) followed by Alexa 488-conjugated donkey anti-rat secondary antibody(Invitrogen Life Technologies).

Thrombin Antithrombin III Complex (TAT) measurement

Plasma were collected by retro-orbital puncture into a 3.2% solution of sodium citrate. TAT was measured by a commercially available kit (AssayMax, cat#EMT1020-1).

Cell culture and treatment with cytokines or inhibitors

Isolated primary LSEC and MΦ were cultured in DMEM with 10% fetal bovine serum and treated with indicated amount of murine recombinant rChi3l1, IFN-γ (Peprotech 315-05), or TNF-α (Peprotech 500-P64) for various times. In some experiments, the cells were pre-treated with inhibitors for one hour before rChi3l1 treatment. The concentrations of the inhibitors used were as follows: c-JNK inhibitor (20μM SP600125, sigma S5567); P38 inhibitor (20μM SB203580, sigma S8307); ERK inhibitor (20μM U0126, sigma U120). The transformed sinusoids endothelial cell (TSEC) is a generous gift from Dr. Vijay Shah (Mayo Clinic, Minnesota). LSEC or TSEC cells were cultured in DMEM supplemented with 10% ECGS(SclenCell, cat#1052).

Flow cytometric analysis

Intracellular staining for IFN-γ, TNF-α, IL-4 was performed as previously described[42].

Statistics

Data are presented as mean ± SEM. Statistical analyses were performed using GraphPad Prism (GraphPad Software). Comparisons between two groups were analyzed using unpaired Student t test. Comparisons among multiple groups (n>=3) were analyzed using one-way ANOVA. P values are as labeled and less than 0.05 was considered significant. Positive areas of protein expression were analyzed by Image J software.

Supplementary Material

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Acknowledgments

Abbreviations

Con A	concanavalin A
Chi3l1	Chitinase 3 like 1
IFN-γ	interferon gamma
TNF-α	tumor necrosis factor alpha
TF	tissue factor
MAPK	mitogen-activated protein kinase
CILI	concanavalin A (Con A)-induced liver injury
NK cell	natural killer cell
WT	wild-type
TAT	thrombin anti-thrombin complex
LSEC	liver sinusoid endothelial cells
MΦ	macrophages
rChi3l1	recombinant Chi3l1
IAOC	Intrahepatic activation of coagulation

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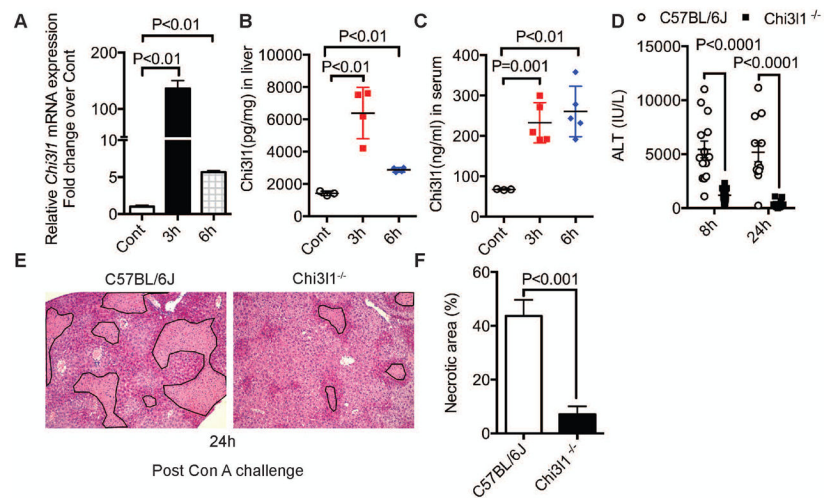
Fig. 1. Chi3l1 contributes to Con A-induced liver injury in mice

Hepatic mRNA expression of Chi3l1 (A), and protein levels of Chi3l1 in liver (B) and serum (C) were measured in male WT (C57BL/6J) mice treated with 15 mg/kg Con A by intravenous injection (n=3–5 mice per group). (D) Serum ALT levels were determined at 8 and 24 h post-Con A treatment (n=15–22 mice per group). (E) Shown are representative H&E-stained liver sections from at least 4 mice per group at 24h after Con A treatment (magnification, 100×). Necrotic areas are outlined. (F) Necrotic areas are quantified by Image J. P values are as indicated. Two-tailed, unpaired t test in D and F; one-way ANOVA in A, B, C.
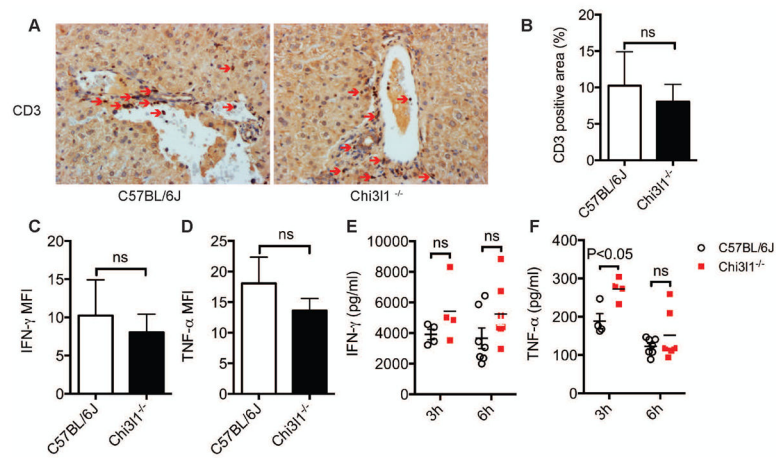
Fig. 2. Con A-induced T cell responses are not affected by Chi3l1

Male WT and Chi3l1$^{-/-}$ mice were treated with Con A as described in Fig. 1. (A) At 6h after Con A administration, hepatic T cells were identified by immunohistochemical staining using anti-CD3 antibody (magnification: 200×). Red arrows indicate CD3$^+$ T cells. Shown are representative images from at least 4 mice per group. (B) The number of hepatic CD3$^+$ T cells are quantified by Image J. (C–D) At 3h after Con A administration, CD3$^+$ T cell-derived IFN-γ (C) and TNF-α (D) were measured by intracellular staining and flow cytometry (n=5 mice per group). (E–F) At 3h and 6h after Con A administration, Serum levels of IFN-γ (E) and TNF-α (F) were measured by ELISA (n=4–7 mice per group). P values are as indicated. Two-tailed, unpaired t test were performed in B–F.
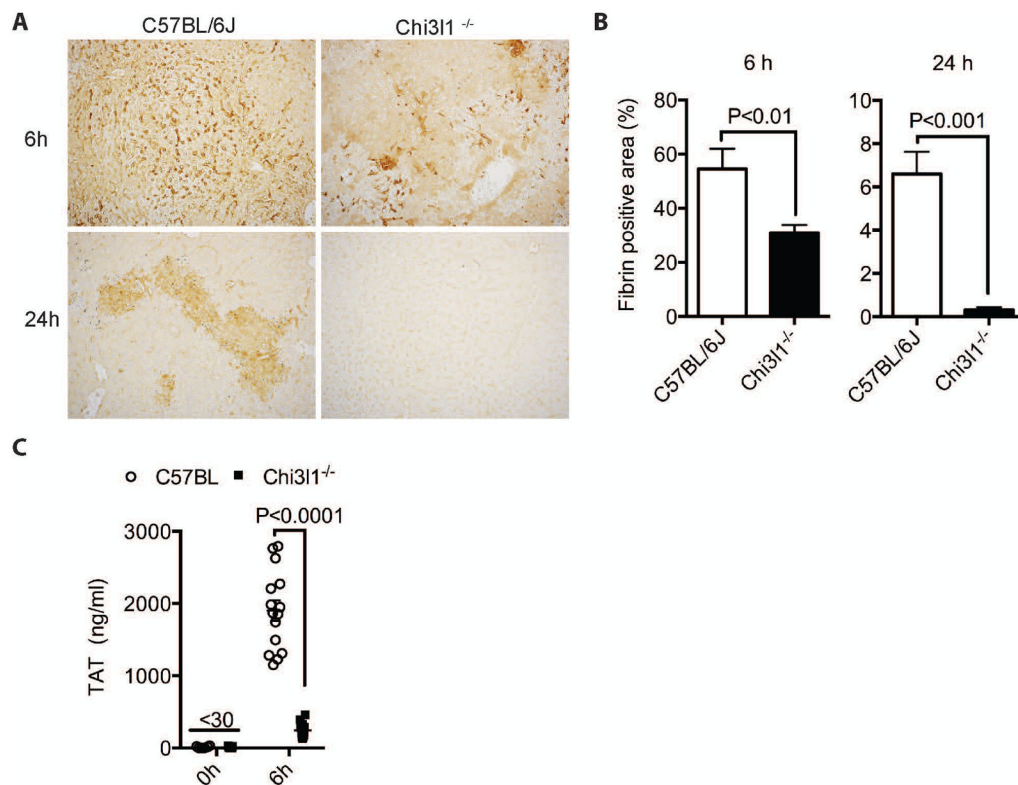
**Fig. 3. Chi3l1⁻/⁻ mice exhibit attenuated intrahepatic coagulation upon Con A challenge**
(A–B) Male WT and Chi3l1⁻/⁻ were treated with Con A as described in Fig. 1 for 6h and 24h. Immunohistochemical staining for fibrin(ogen) (magnification: 200x) were performed and quantified. Images shown are representative of at least 4 mice per group. (C) The levels of TAT complex were measured in plasma of WT and Chi3l1⁻/⁻ mice at 6h post-Con A treatment (n=14–15 per group). P values are as indicated. Two- tailed, unpaired t test were performed in B, D–F.
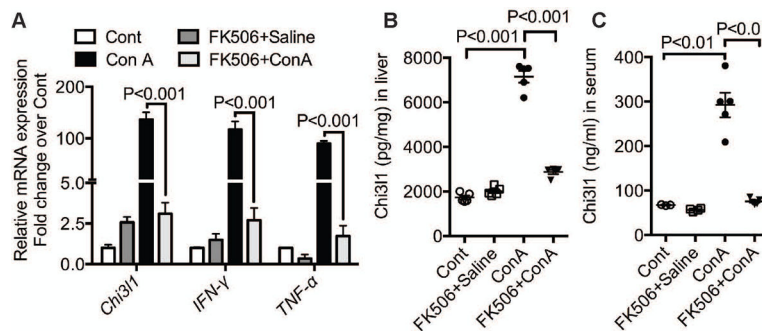
Fig. 4. Chi3l1 production is dependent on T cell activity
Male WT mice were orally treated with a T cell inhibitor FK506 at 1h prior to Con A administration. (A) Hepatic mRNA expression levels of *Chi3l1*, *IFN-γ* and *TNF-α* were measured at 3h post-Con A by qRT-PCR. (B–C) Protein levels of Chi3l1 in the liver (B) and serum (C) were measured by ELISA (n=3–5 mice per group). P values are as indicated. One-way ANOVA was performed in A–C.
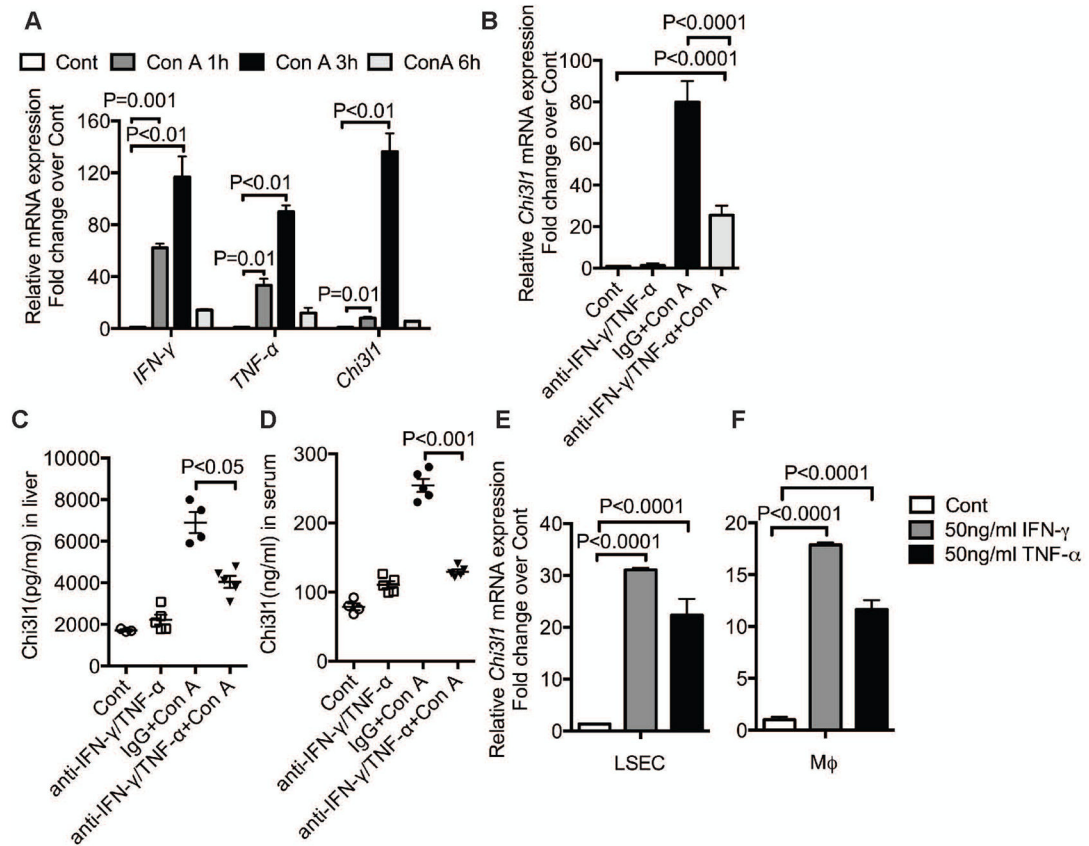
Fig. 5. Chi3l1 production is dependent on IFN-γ and TNF-α

(A) Con A-induced up-regulation of *IFN-γ*, *TNF-α*, and *Chi3l1* mRNA levels in the liver were measured in WT mice at various time points (n=4 mice per group). (B–F) WT mice were treated with anti-IFN-γ and TNF-α antibodies by i.p. injection at the same time of Con A administration and sacrificed at 3h post-Con A treatment. The hepatic mRNA levels of *Chi3l1* (B), and protein levels of Chi3l1 in the liver (C) and serum (D) were measured (n=3–5 mice per group). (E–F) LSEC and MΦ were isolated from naïve WT mice and treated with IFN-γ (50ng/ml) or TNF-α (50ng/ml) for 3h *in vitro. Chi3l1* mRNA expression levels were measured by qRT-PCR. P values are as indicated. One-way ANOVA was performed in A–F.
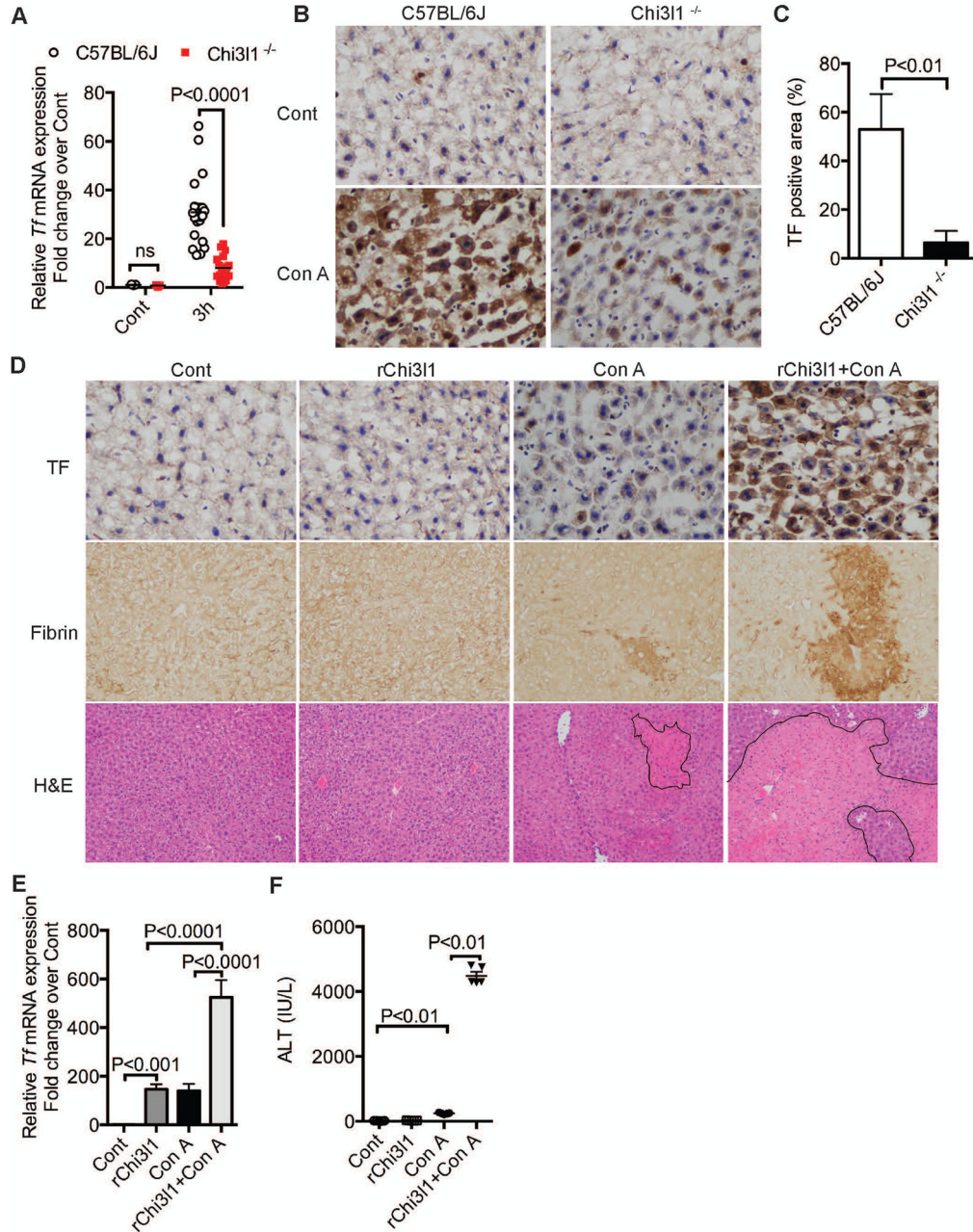
Fig. 6. Chi3l1 plays a critical role in the induction of TF

(A–C) Male WT and Chi3l1$^{-/-}$ mice were treated with Con A for 3h as described in Fig. 1.
(A) Hepatic *TF* mRNA expression was measured by qRT-PCR (n=5–20 mice per group). (B)
TF protein expression was measured by immunohistochemical staining (magnification:
200×). Shown are representative images from at with mouse recombinant Chi3l1 (rChi3l1)
by i.p. injection at the same time of Con A administration. (D) The levels of TF and
fibrin(ogen) were measured by immunohistochemical staining (magnification: 200×).
Photomicrophages of H&E stained liver sections are shown with outlined necrotic areas
(magnification: 100×). Representative images were from at least 4 mice per group. (E)
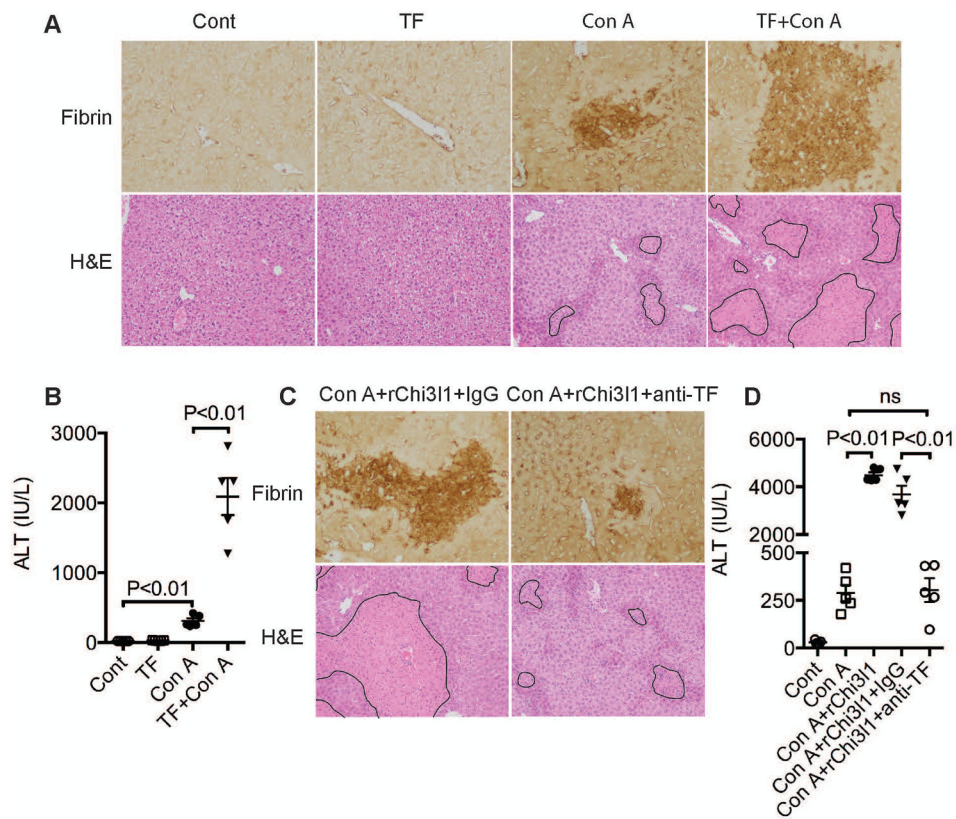
Hepatic *TF* mRNA expression levels were measured at 3h post-Con A. (F) Serum ALT activities were determined at 24 h post Con A treatment (n=4–6 mice per group). P values are as indicated. Two-tailed, unpaired t test was performed in A, C and one-way ANOVA in E–F.

Fig. 7. TF induced by Chi3l1 drives activation of coagulation

(A–D) Male Chi3l1$^{-/-}$ mice were treated with Con A as described in Fig. 1 or recombinant TF(rTF), anti-TF (1H1) antibody or rChi3l1 as indicated. (A, C) Immunohistochemical staining for fibrin(ogen) (magnification: 200x), and H&E-stained liver sections (magnification, 100×) were performed at 24h after Con A treatment. Necrotic areas are outlined. (B, D) Serum ALT levels were determined at 24 h post-Con A treatment (n=5 mice per group). Images shown are representative of at least 4 mice per group. P values are as indicated. One-way ANOVA in A, C.
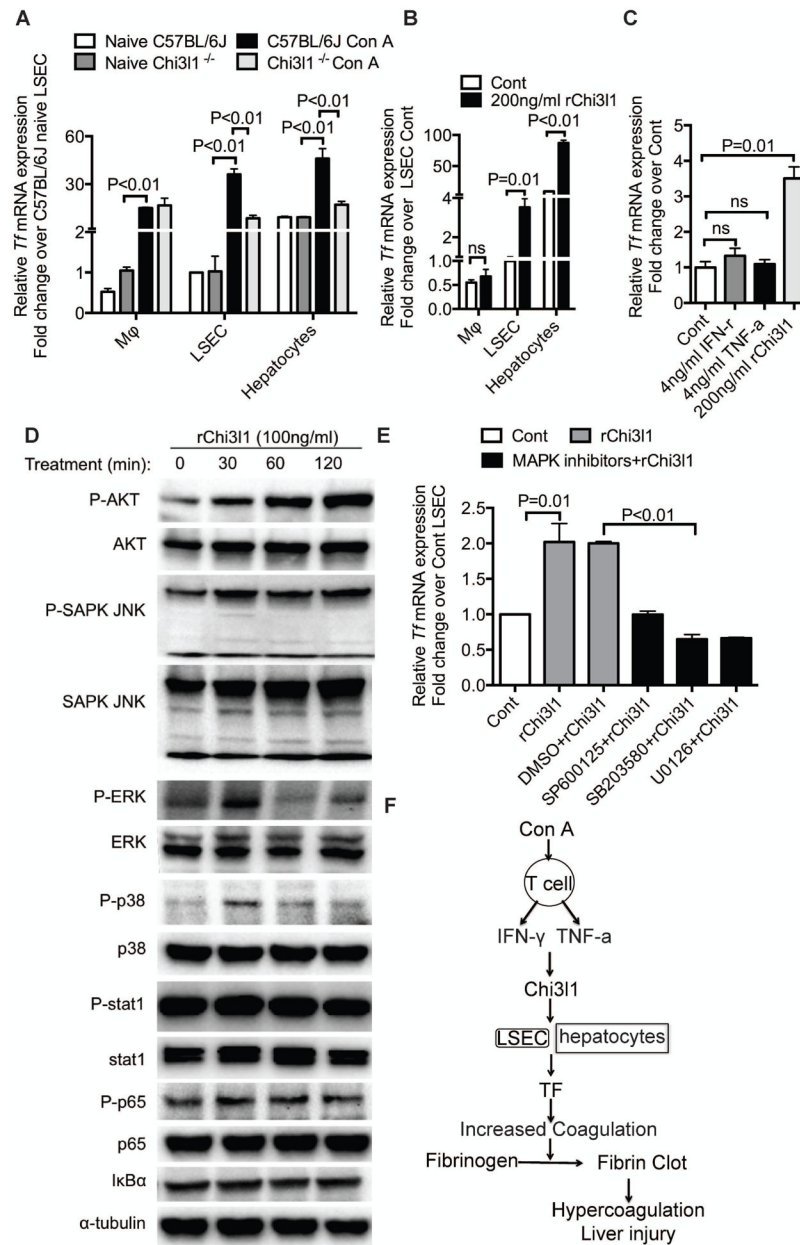
Fig. 8. Chi3l1 induces TF expression through the MAPK signaling pathway

(A) LSEC, MΦ and hepatocytes were isolated from naïve (Cont) and Con A-treated WT mice (n=6 per group). The *TF* mRNA expression levels in these cells were measured. (B) LSEC, MΦΦ and hepatocytes were isolated from naïve WT mice. The cells were treated with rChi3l1 for 5h, and *TF* mRNA expression levels were measured. (C) LSECs isolated from naïve WT mice were treated with indicated doses of IFN-γ, TNF-α, and rChi3l1 that match the levels of these factors detected *in vivo* (Fig. 2, E&F; Fig. 1C). After 5h, *TF* mRNA levels were measured. (D) TSEC cells (a mouse LSEC cell line) were treated with 100ng/ml of rChi3l1 for various time periods as indicated. Activation of various signaling pathways was measured by western blotting. (E) LSECs isolated from naïve WT mice were

pretreated with MAPK inhibitors for 1h, followed by rChi3l1 treatment for 5h. *TF* mRNA expression was measured by qRT-PCR. (F) Schematic for the involvement of Chi3l1 in CILI and Con A-induced IAOC. Results shown are from three independent experiments. P values are as indicated. Two-tailed, unpaired t test was performed in B. One-way ANOVA was performed in A, C and E.