Supplementary Materials and Methods

Maintenance of mice

All mice were maintained and bred at the Cleveland Clinic's Biological Resources Unit in accordance with institutional guidelines and National Institutes of Health standards. This study was approved by the Institutional Animal Care and Use Committee. Mice were regularly monitored and had free access to standard mouse chow and water. Mice received humane care in accordance with institutional guidelines and National Institutes of Health recommendations outlined in the "Guide for the Care and Use of Laboratory Animals."

Generation of fibronectin-floxed and 'liver fibronectin-null' mice

The fibronectin targeting vector was constructed as described previously[1] with a modification (Supplementary Fig. 1A). A 11.3-kb *Xba*I 129 strain genomic fragment spanning from the promoter to the fifth intron was isolated from a PAC library and subcloned into a pBS vector (Stratagene). A *loxP* site and a *neo-tk* cassette flanked by *loxP* sites were inserted into the *Msc*I site (5' untranslated region) and *Nhe*I site (within the first intron), respectively. Thus the fibronectin construct consisted of a 4.2-kb left arm, a single *loxP* site, a 1.6-kb genomic fragment, a *loxP-neo-tk-loxP* cassette, and a 3.7-kb right arm. After homologous recombination in embryonic stem (ES) cells, positive clones were transfected with a *Cre*-expressing plasmid, counterselected in 1-(2-deoxy-2-fluoro-beta-D-arabinofuranosyl)-5-iodouracil (FIAU), and clones that had retained two *loxP* sites flanking the first coding exon were identified by Southern blotting (Supplementary Fig. 1B, C; 'floxed'). Then germline-transmitting chimeras were generated (Supplementary Fig. 1D). It was confirmed that the fibronectin-floxed allele did not affect fibronectin mRNA and fibronectin protein expression (data not shown).

To produce mice carrying the null allele, fibronectin-floxed mice carrying the interferon- and polyinosinic-polycytidic acid (pI-pC)-inducible *Mx-Cre* transgene (Fn(fl/fl)/*Mx-Cre*+) were generated (*Mx-Cre* mice were provided by Dr. Klaus Rajinsky [Harvard Medical School])[2]. "Liver fibronectin-null" mice were established in the Fn(fl/fl)/*Mx-Cre*+ strain by intraperitoneal injections of pI-pC. It was confirmed that *fibronectin* gene was deleted from all cell types from the liver, including parenchymal as well as non-parenchymal cells, by Southern blotting (Supplementary Fig. 1E). It was also confirmed that, although plasma fibronectin became undetectable after pI-pC injections, the levels of other plasma components such as fibrinogen and TSP-1 were not affected in fibronectin mutant mice (Supplementary Fig. 2A). The fibronectin protein levels in fibronectin mutant livers decreased to ~8% of wild-type liver levels by Western analysis at 8 weeks after pI-pC injections (Supplementary Fig. 2B). The deposition of fibronectin in the liver matrix (mainly sinusoid) was also considerably diminished (Supplementary Fig. 2C). The mutant livers showed normal liver morphology, and no obvious inflammation or fibrosis was noted after pI-pC injections (Supplementary Fig. 2D). Thus, the overall

depletion of fibronectin in the liver in fibronectin mutant mice was ~92%, and these mice were used for experiments at least 2 months after deletion.

Antibodies, cytokines and reagents

The following antibodies were used for the analyses: rabbit polyclonal antibody (pAb) against mouse fibronectin (Chemicon); goat pAb against fibrinogen (Nordic Immuno. Lab.); mouse monoclonal antibody (mAb) against TSP-1 (clone D4.6, Thermo Fisher Sci.); mouse mAb (clone 1A4, Sigma) and rabbit mAb (E184, Epitomics) against αSMA; rabbit pAb against phosphor-Smad2 (pSmad2), which specifically recognizes the phosphorylated C-terminal serine 465/467 of Smad2, was a kind gift from Dr. Koichi Matsuzaki (Kansai Medical University); another pSmad2 antibody (#3108) which recognizes the phosphorylated C-terminal serine 465/467 was from Cell Signaling; mouse mAb against total Smad2/3 (BD Biosciences); rabbit mAb against glyceraldehyde 3-phosphate dehydrogenase (GAPDH: clone EPR1977Y, Epitomics); mouse mAb against β-actin (clone AC15, Sigma); mouse mAb against heat shock cognate protein 70 (HSC70, Santa Cruz). The preparation and characterization of rabbit pAbs against bovine type I collagen and type III procollagen are described elsewhere[3]. Affinity-purified pAbs against mouse LAP of TGF-β1, mouse LTBP-3, human LTBP-4, and human type V collagen were generated by immunization of rabbits to purified or full-length recombinant proteins. We confirmed that those antibodies had no cross-reactivities with other isoforms. Rabbit pAb against LTBP-1 was a kind gift from Dr. Lynn Sakai (Research Center, Shriners Hospitals for Children). FITC- and Cy3-conjugated donkey anti-rabbit IgG, and peroxidase-conjugated donkey anti-mouse, anti-rabbit, and anti- goat IgG were from Jackson ImmunoRes Lab. Alexa Fluor488- and 568-conjugated donkey anti-goat IgG, and 4'6-diamidino-2-phenylindole (DAPI) were from Molecular Probes. Envision^{TM}+ System-HRP labeled polymer was from DAKO. Recombinant human TGF-β1, rat PDGF-AA and mouse amphiregulin were from R&D Systems. Bovine plasma fibronectin was from Sigma. Human placental type V collagen was solubilized by pepsin, separated from other collagens by fractional NaCl precipitation, and purified by chromatography on a Mono-Q column[4]. Purified type V collagen was solubilized by 0.1 M acetic acid, then neutralized by sodium hydroxide and used for the analysis. The MMP inhibitor GM6001 was from Chemicon.

Induction of acute and chronic liver injury by carbon tetrachloride (CCl_4)

Acute liver injury was induced intraperitoneally by a single dose administration of CCl_4 solution in olive oil (Fluka) (1.0 ml/kg body weight as 50% [vol/vol]) in sex-matched, 12- to 15-week-old mice. The chronic liver injury was induced also by CCl_4 (0.5 ml/kg weight, twice a week for 8 weeks) and mice were sacrificed at 72 hrs after the final injection[5]. Control and liver fibronectin-null mice were derived from the same litters.

Isolation of primary HSCs and generation of adult HSC lines

HSCs were isolated principally according to the method described by Schafer et al.[6], with a modification. The liver was perfused with Gey's balanced salt solution containing 0.02% collagenase (type IV, Sigma; 130-150 units/mg solid) and 0.05% protease (Sigma) from the portal vein for 10 min. After perfusion, the liver was excised, cut into small pieces, and added to agitation buffer (Gey's buffer containing 0.1% collagenase, 0.1% protease, and 0.005% DNase [Sigma]). The solution was stirred at 70 rpm for 20 min at 37˚C. Then the cell suspension was mixed with Optiprep (Axis-Shield), and density-gradient centrifugation was performed at 2,500 x *g* for 17 min. HSCs were separated by gradient fractions; being present at the lowest density (1.025-1.035 g/ml; most upper fraction).

Adult control and fibronectin-null HSC lines were generated from HSCs of mice on p53- and p21-null genetic background[7] (p53- and p21-knockout mice were provided by Dr. Dusko Ilic [University of California, San Francisco])[8]. To establish these HSC lines, primary HSCs from adult livers of 15-week-old Fn(fl/fl)/p53(-/-) and Fn(fl/fl)/p21(-/-) mice were isolated, cloned, and several immortalized clones were generated. Subsequently, several clones were treated with a *Cre*-transducing adenovirus to delete the *fibronectin-floxed* genes. The deletion of Fn alleles was confirmed by PCR and the lack of fibronectin protein secretion by radioimmunoprecipitation using metabolically labeled conditioned medium as described previously[9] (Supplementary Fig. 3A, B). Both parental fibronectin-floxed and fibronectin-null HSC lines showed similar morphology and highly express the HSC marker αSMA (Supplementary Fig. 3C). For characterization of collagen mRNA expression and protein secretion and assembly, those cells were cultured for indicated time periods with Dulbecco's modified Eagle's medium (DMEM) containing 8% fibronectin-depleted FBS, 0.2 mM ascorbic acid, and with indicated supplements. It was confirmed that both parental fibronectin-floxed and fibronectin-null HSC lines expressed similar levels of *Col3a1* or *Col1a1* mRNAs by real-time PCR and the type I collagen secretion by pulse chase analysis (Supplementary Fig. 3D, E). The upregulation of *Col3a1* or *Col1a1* mRNA by TGFβ1 (2 pM) was similar between fibronectin-null and its parental HSCs (for *Col3a1:* 1.33 ± 0.24 and 1.20 ± 0.21 fold [*n* = 3], respectively; for *Col1a1:* 2.37 ± 0.28 and 2.32 ± 0.48 fold [*n* = 3], respectively, compared to untreated controls).

Histological analysis, immunohistochemistry and immunofluorescence, and electron microscopy

For histological analyses, liver samples were either directly frozen in OCT compound (Tissue-Tek, Sakura Finetek) or fixed overnight in 4% paraformaldehyde in PBS, pH 7.2, and dehydrated in a graded alcohol series and embedded in paraffin. Hematoxylin/eosin, Periodic Acid-Schiff, Mallory-Azan, and Sirius-Red stainings were performed according to the standard protocols. Immunohistochemistry and immunofluorescence studies were performed as described previously[1,10]. Double-immunohistochemical analysis with paraffin-embedded tissue sections was performed as described[11]. Briefly, after the first

antigen-antibody reaction, the slides were incubated with dissociation buffer containing 0.1 M Glycine-HCl (pH 2.2) to dissociate immunoglobulins from antigenic sites. Then the second antigen-antibody reaction was subsequently performed. We carried out the second reaction without antibodies and confirmed the complete dissociation of the first immunoglobulins. For quantification of signal intensity, images were captured with the same gain, offset, magnitude and exposure time. Then a minimum of 5 different images were randomly selected and the intensities were quantified using Image-Pro Plus software (version 6.1, Media Cybernetics). The background fluorescence intensity measured in control or vehicle only was subtracted from each value.

For electron microscopy, liver samples were fixed immediately after sacrifice with 1.5% glutaraldehyde, 1.5% paraformaldehyde, 0.05% $CaCl_2$ and 0.05% tannic acid in 0.1M cacodylate buffer (pH 7.35) for 12 hrs at 4°C, rinsed overnight in cacodylate buffer, then immersed in 1% osmium tetroxide. Samples were dehydrated in a graded ethanol series to 100%, rinsed in propylene oxide, infiltrated and embedded in Spurrs epoxy, and ultrathin sections of 80-nm thickness were prepared. The sections were mounted on 1 x 2 mm formvar-coated grids and examined using a FEI G2 transmission electron microscope (TEM) operated at 120 kV. Digital micrographs were collected using a 2K x 2K AMT side entry camera with magnifications calibrated using a carbon grating replica having 2,160 lines/mm. For morphometric analysis, micrographs in transverse sections from non-overlapping regions were randomly selected and fibril diameters (1,500 fibrils in each) were measured using Image-Pro Plus software.

Real-time PCR

Real-time PCR was performed as described previously[12]. The following primers were used (please see also supplementary Table 1): *Colla1* forward, 5'-GGGCGAGTGCTGTGCTTT-3'; *Colla1* reverse, 5'-GGTCCCTCGACTCCTACATCTTC-3'; *Col3a1* forward, 5'-CTGTAACATGGAAACTGGGGAAA-3'; *Col3a1* reverse, 5'-CCATAGCTGAACTGAAAACCACC-3'; *Col5a1* forward, 5'-GAGGACCACACAGGGAAGC-3'; *Col5a1* reverse, 5'-CTTGTAGACACTGAGAGCAATTCG-3'; *18S rRNA* forward, 5'-GGCGACGACCCATTCG-3'; *18S rRNA* reverse, 5'-ACCCGTGGTCACCATGGTA-3'. All samples were analyzed in triplicate as a minimum. After the reactions, the specificity of amplifications in each sample was confirmed by dissociation analysis, showing that each sample gave a single melting peak. The relative mRNA levels were normalized to the level of *18S rRNA*.

siRNA analysis

Col5a1 siRNA (5'-AAGGAGAGGGUGAGACCUAUUA-3') and its scrambled control dsRNA (5'-CAGAGGGAGUGGGAGCCAAUAAUUA-3') were designed using the BLOCK-iT™ RNAi Designer algorithm from Invitrogen. Both siRNA and scrambled dsRNA were transfected into fibronectin-null HSCs using Lipofectamine™ 2000 (Invitrogen) according to the manufacturer's instruction. At 27 hrs

after transfection, TGF-β1 (2 pM) or plasma fibronectin (10 μg/ml) was added and cells were incubated for an additional 18 hrs at 37°C before running the analysis. Block-iT™ Fluorescent Oligo (Invitrogen) was used initially for optimization and documentation of transfection efficacy.

Southern, Northern, and Western blot analyses, and radioimmunoprecipitation

Southern, Northern, and Western blot analyses were performed as described elsewhere[10]. In some immunoblotting analyses, samples were transferred onto Immobilon-FL polyvinylidene fluoride (PVDF) membrane (Millipore Corp.) and probed with primary and IRDye 800CW- or IRDye 680-conjugated secondary antibodies (LI-COR Biosci.). Immunoreactive bands were detected using the Odyssey Infrared Imaging System (LI-COR Biosci.). Radioimmunoprecipitation analysis was described elsewhere[13]. For pulse chase analysis, 3 hrs after plating on culture dish in methionine- and cycteine-free DMEM supplemented with Fn-depleted FBS and ascorbic acid, the cells received L-[^{35}S]methionine/cysteine at 100 μCi/ml medium (TRAN^{35}S-Label™, MP Biochemicals) and were incubated for 30 min (pulse labeling). The cells were then washed with normal medium and either lysed immediately or incubated for a further 120 min (chase periods). After incubation, the conditioned media were collected and the same volumes were used for the radioimmunoprecipitation analysis. Northern blots were hybridized with a mouse fibronectin cDNA probe (clone M20/2) and a 1.1-kb cDNA fragment of GAPDH (Clontech) as described previously[1].

Hydroxyproline assay

Hydroxyproline content was measured by amino acid analysis. Briefly, mouse liver tissue samples were weighed and hydrolyzed for 24 hrs with 6 N HCl/0.05% mercaptoethanol/0.02% phenol at 115°C. Hydrolysate was evaporated using a Speed Vac and the residue was dissolved in a citrate buffer (pH 2.2). Samples and standards containing common amino acids were loaded into System 6300 Amino Acid Analyzer (Beckman), and ninhydrin positive amino acid content was calculated by measuring the optical densities at 440, 570, and 690 nm.

Hepatic biochemical markers

Serum alanine aminotransferase (ALT) level was determined using standard kit (Genzyme Diagnostics P.E.I. Inc.). Serum total protein (by Biuret method), albumin (by BCG method), and bilirubin (by Azobilirubin method) levels were measured using Hitachi 7180 Auto Analyzer.

Supplementary References

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Supplementary Figure Legends

sFig. 1. Generation of mutant [Fn(fl/fl)/*Mx-Cre*+] mice.

(**A**) Partial maps of the wild-type, recombinant, fibronectin (Fn)-floxed, and fibronectin-null alleles. Exons and *loxP* sequences are indicated as rectangles and triangles, respectively. Lines with arrows under the restriction maps show the size of the restriction fragments produced after Southern blotting and external (probe 1) and internal (probe 2) probes are indicated. Restriction sites are written on the map. PGKpro, *phosphoglycerate kinase promoter*; Neo, *neomycin* gene; TK, *thymidine kinase* gene; RI, *Eco*RI.

(**B**) Southern blot analysis of ES cell clone DNA, following digestion of *Xba*I and hybridization with probe 1. (+/+), wild type; (+/targeted), heterozygous for the fibronectin-recombinant allele. The sizes of the wild type (11.3 kb) and targeted (7.6 kb) bands are indicated.

(**C**) Southern blot analysis of ES cell clone DNA before and after transient expression of Cre, following digestion of *Eco*RI and hybridization with probe 2. (+/targeted), heterozygous for the fibronectin-recombinant allele; (+/fl), heterozygous for the fibronectin-floxed gene; (+/-), heterozygous for the fibronectin-null gene. The sizes of the targeted (5.3 kb), wild type (5.0 kb), fibronectin-floxed (2.3 kb), and fibronectin-null (1.4 kb) bands are indicated.

(**D**) Southern blot analysis of mouse tail DNA, following digestion of *Eco*RI and hybridization with probe 2. (+/+), wild type; (+/fl), heterozygous for the fibronectin-floxed gene; (fl/fl) homozygous for the fibronectin-floxed gene. The sizes of the wild type (5.0 kb) and Fn-floxed (2.3 kb) bands are indicated.

(**E**) Southern blot analysis of livers from fibronectin-floxed (Fn[fl/fl]) and Fn(fl/fl)/*Mx-Cre*+ mice at 4 weeks after the final injection of pI-pC. DNA was digested with *Eco*RI and hybridized with probe 2. The sizes of the fibronectin-floxed (2.3 kb) and fibronectin-null (1.4 kb) bands are indicated.

sFig. 2. Generation of liver fibronectin-null mice.

(**A**) Western blot analysis of fibronectin, fibrinogen, and TSP-1 protein levels in plasma from control (Fn[fl/fl]) and mutant (Fn[fl/fl]/*Mx-Cre*+) mice at 8 weeks after the final injection of pI-pC. The same samples were stained with Coomassie brilliant blue (CBB) to confirm that comparable amounts of protein were loaded.

(**B**) Upper panel: Western blot analysis of fibronectin (plasma and cellular types) in liver tissue lysates from control and mutant mice at 8 weeks after the final injection of pI-pC. Lower panel: Analysis of fibronectin intensities. The fibronectin expression levels are shown relative to the control value of 100 (percent of control) (n = 5 for each group). Note that the fibronectin protein levels in mutant livers decreases to ~8% of wild type liver levels. **, $P < .01$.

(**C**) Double immunofluorescent staining for fibronectin (in red) and DAPI (in blue) in control and mutant (Fn[fl/fl]/*Mx-Cre*+) livers at 3 months after pI-pC injections. Note that the expression level of fibronectin is remarkably diminished in mutant livers, whereas intense fibronectin expression is evident in the sinusoidal ECM of control livers. CV, central vein. Bar = 25 μm.

(**D**) Normal histology of control and Fn(fl/fl)/*Mx-Cre*+ mouse livers at 3 months after the final injection of pI-pC. Hematoxylin/eosin (HE; upper panels), Periodic Acid-Schiff (PAS; middle panels), and Mallory-Azan (Azan; lower panels). Bar = 100 μm.

sFig. 3. Generation of fibronectin-null HSC lines.

(**A**) Upper panel: Partial maps of fibronectin-floxed and Fn-null alleles. *LoxP* sequences (triangle), exon1 (rectangle) and the position of primers (a, b and c) for PCR analysis to monitor *Cre*-mediated recombination are indicated. Lower panel: PCR analysis of HSC DNA in clones cell #1 and #2 before and after treatment with a *cre*-transducing adenovirus. Primary HSCs were isolated from adult Fn(fl/fl)/p53(-/-) and Fn(fl/fl)/p21(-/-) mouse livers, cloned, and infected with adenovirus-*cre* to delete the fibronectin-floxed genes. PCR products for fibronectin-floxed (283 bp) using primers 'a' and 'b', and for fibronectin-floxed (1,310 bp) and fibronectin-null (365 bp) using primers 'a' and 'c', are indicated.

(**B**) Fibronectin protein is not produced after the deletion of *fibronectin-floxed* genes by adenovirus-*cre*. Fluorograph of immunoprecipitates from the conditioned medium of metabolically labeled HSC lines by SDS-PAGE. The cells received L-[^{35}S]methionine/cysteine (100 μCi/ml medium), and the same volumes of the conditioned medium were immunoprecipitated with antibodies against fibronectin before addition of Protein A. Samples were then subjected to SDS-PAGE. The positions of molecular weight markers are indicated. Fn(fl/fl), parental fibronectin-floxed HSCs in each clone; Fn(-/-), adenovirus-*cre*-treated fibronectin-null HSCs in each clone.

(**C**) Double immunofluorescent staining for αSMA (in green) and DAPI (in blue). Note that the fibronectin-floxed (Fn[fl/fl]/p53[-/-] and Fn[fl/fl]/p21[-/-]) and fibronectin-null (Fn[-/-]/p53[-/-] and Fn[-/-]/p21[-/-]) HSC lines show similar morphology and highly express the activated HSC marker αSMA. Bar = 50 μm.

(**D**) Real-time PCR analysis of *Col3a1* and *Col1a1* mRNAs in fibronectin-floxed (Fn[fl/fl]) and fibronectin-null (Fn[-/-]) HSC lines. Relative mRNA expression levels are shown relative to the control value of 1.0 (Fn(fl/fl) liver) (n = 3 for each group). Note that fibronectin-floxed and fibronectin-null HSC cells express similar levels of collagen mRNAs.

(**E**) *De novo* type I collagen secretion in fibronectin-floxed (Fn[fl/fl]) and fibronectin-null (Fn[-/-]) HSC lines by pulse chase analysis (0 and 120 min after labeling): The fluorograph of SDS-PAGE gel of immunoprecipitates from the conditioned media of metabolically labeled cells. The intensity of the bands at 120 min after labeling was measured by densitometry, and the intensity of fibronectin-

floxed sample was set to 1.0. Note that fibronectin-floxed and fibronectin-null cells secret a similar amount of type I collagen protein.

sFig. 4. Deposition and assembly of type I collagen fibrils (type I collagen in red; DAPI in blue) in control and fibronectin-null HSCs by immunofluorescent staining. Cells were incubated for 18 hrs with no addition, or either TGF-β1 (2 pM), PDGF-AA (800 pM) or amphiregulin (3,500 pM). Note that TGF-β1 induces type I collagen fibril network formation, whereas PDGF-AA and amphiregulin have no effect on collagen assembly in fibronectin-null HSCs. Bar = 50 μm.

sFig. 5. Deposition and assembly of type I collagen fibrils (type I collagen in red; DAPI in blue) in control and fibronectin-null HSCs by immunofluorescent staining. Cells were incubated for 18 hrs with no addition, or either plasma fibronectin (pFn, 10 μg/ml), TGF-β1 (2 pM) or both plasma fibronectin and TGF-β1. Note that there is a synergistic effect on type I collagen organization when both TGF-β1 and plasma fibronectin are added simultaneously to fibronectin-null HSCs. Bar = 50 μm.

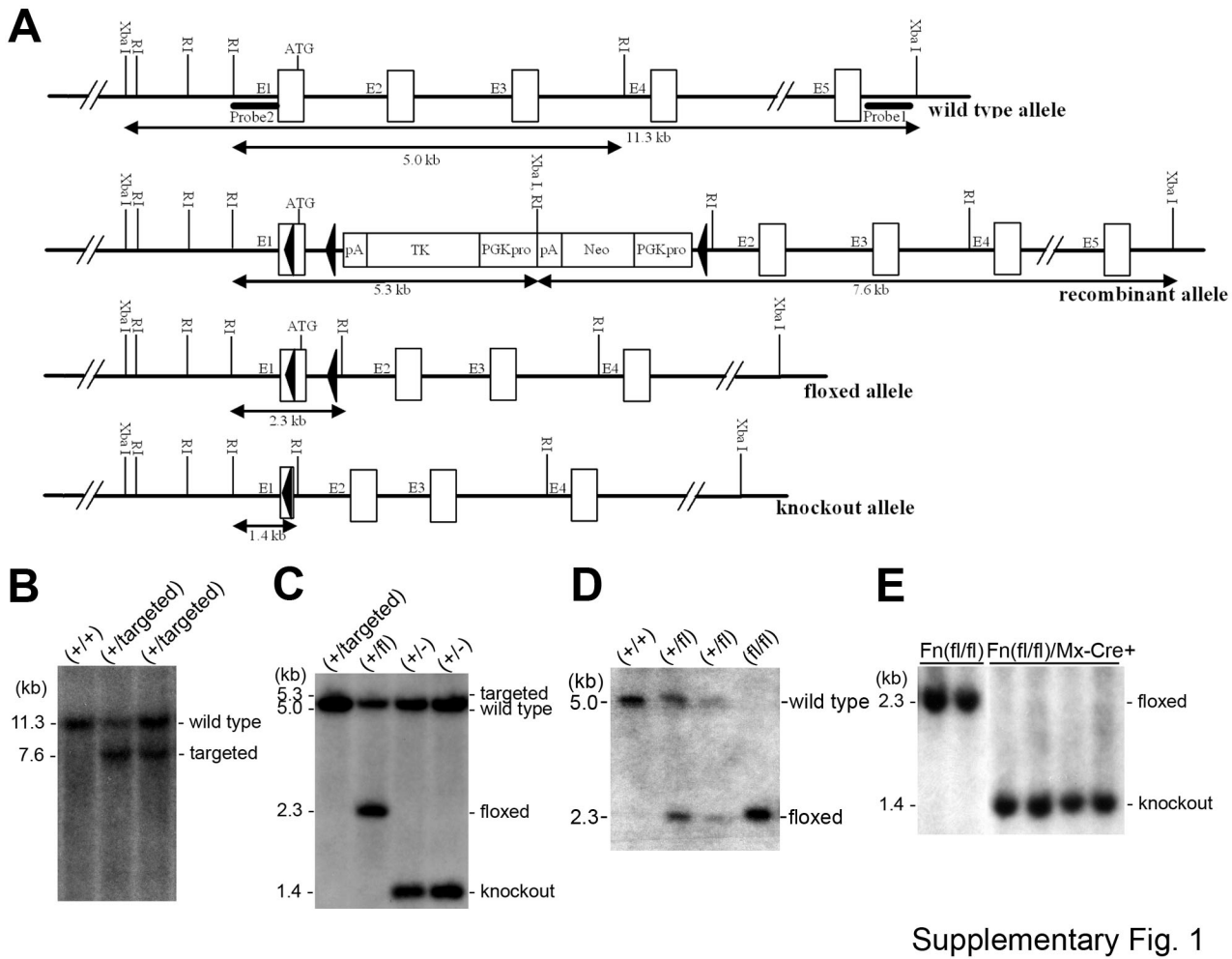
sFig. 6. Effect of exogenous type V collagen on the deposition and assembly of type I collagen fibrils (type I collagen in red; DAPI in blue) in fibronectin-null HSCs *in vitro* by immunofluorescent staining. Cells were incubated for 18 hrs in the presence or absence of 10 μg/ml type V collagen. For control, the same amounts of 0.1 M acetic acid and 1.0 M sodium hydroxide used for neutralization were added. Upper panels: merged images showing type I collagen (in red) and DAPI (in blue). Lower panels: The same images for collagen type I single staining (black and white). Bar = 50 μm. Note that type V collagen induces short and thin type I collagen fibril networks in fibronectin-null HSCs.

sFig. 7. Left panels: Deposition and assembly of type III, type I, and type V collagen fibrils in fibronectin-null embryonic fibroblastic cells by immunofluorescent staining (type III and type I collagen in red; type V collagen in green; DAPI in blue). Cells were incubated for 18 hrs in DMEM containing 8% Fn-depleted FBS, 0.2 mM ascorbic acid, and with plasma fibronectin (pFn, 10 μg/ml) or TGF-β1 (500 pM). Bar = 50 μm. Right panel: Analysis of intensity in assembled collagen fibrils. Relative fluorescence intensities are shown relative to each control value of 100 (no addition). Note that in contrast to the fibronectin-null HSCs (Fig. 4B), TGF-β1 (up to 500 pM) does not induce type III, type I, or type V collagen assembly at all in fibronectin-null embryonic fibroblasts. **, $P < .01$.

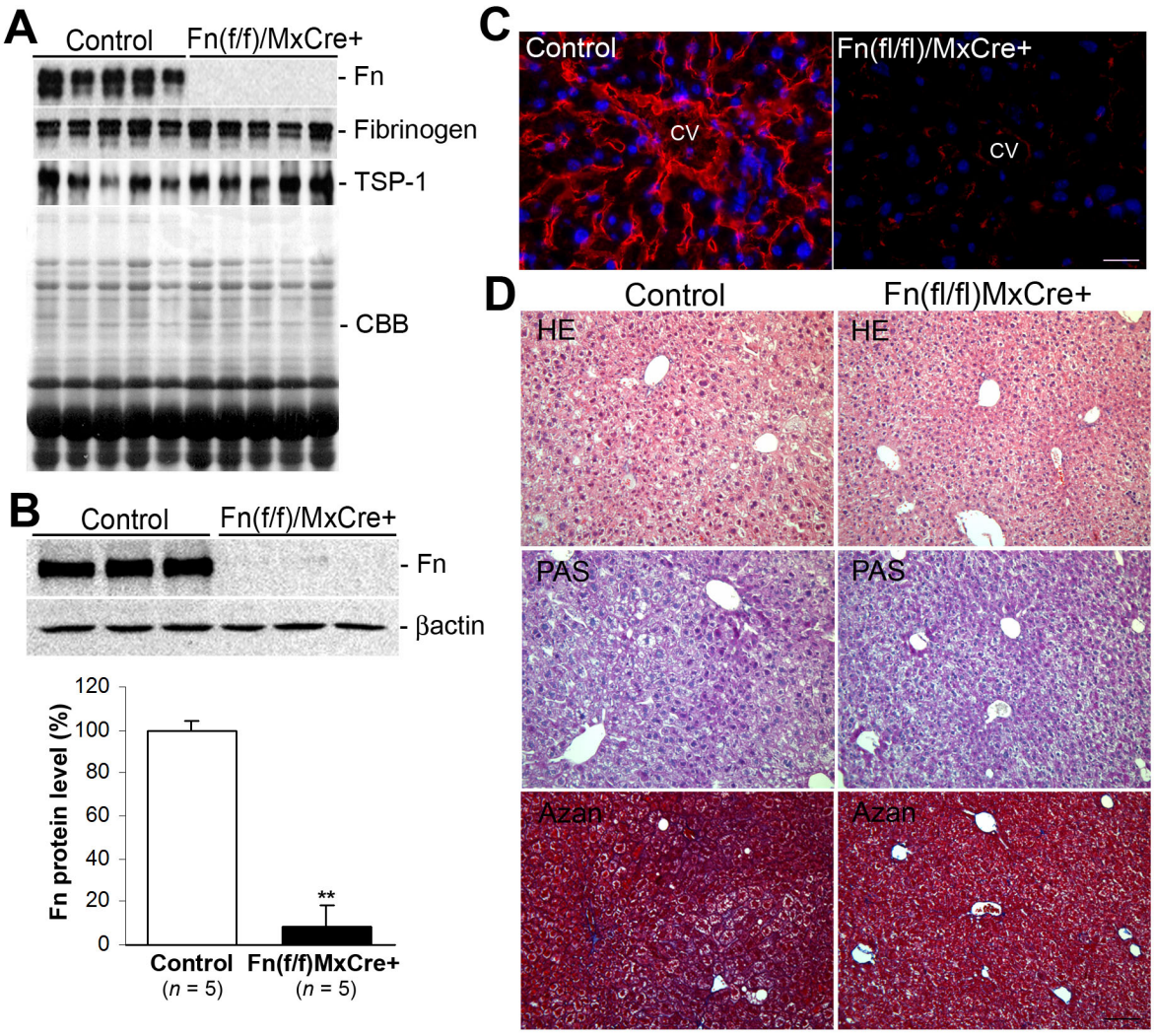
sFig. 8. Western blot analysis of fibronectin in liver tissue lysates from control and mutant mice at 8 weeks of CCl_4 treatment (n = 3 for each group). Note that the fibronectin protein levels in

fibronectin-null livers decreases to ~8.4% of wild type liver levels by densitometric analysis and no apparent induction is observed. **, $P < .01$.

sFig. 9. Serum levels of ALT (as a measure of hepatocyte damage) and total bilirubin (T-Bilirubin, as a measure of hepatic excretory function), and albumin/globulin (A/G) ratio (as a measure of net hepatic damage) at 0 week (untreated) and 8 weeks of CCl_4 treatment (at least $n = 5$ for each group). Note that the levels of all biochemical markers are not significantly different between control and fibronectin-null livers at 0 and 8 weeks, whereas significantly increased ALT and total bilirubin levels and significantly decreased albumin/globulin ratio are observed in both livers at 8 weeks of CCl_4 treatment compared to 0 week. **, $P < .01$.



Supplementary Fig. 1



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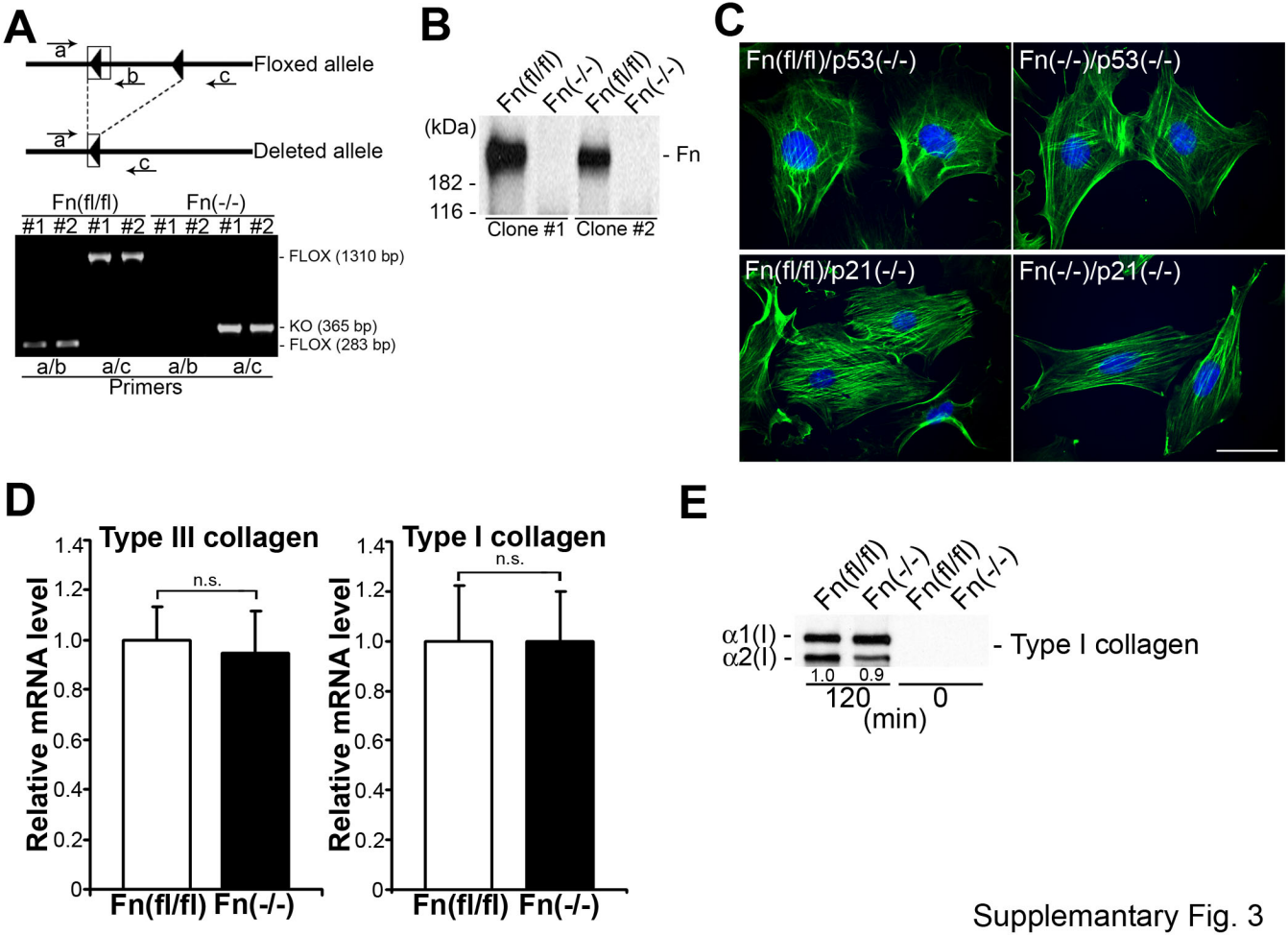

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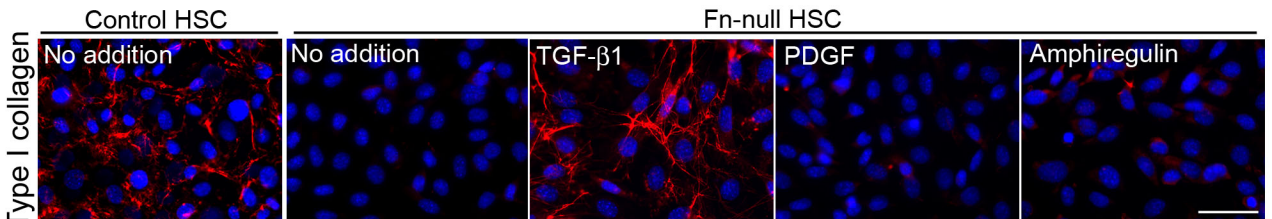

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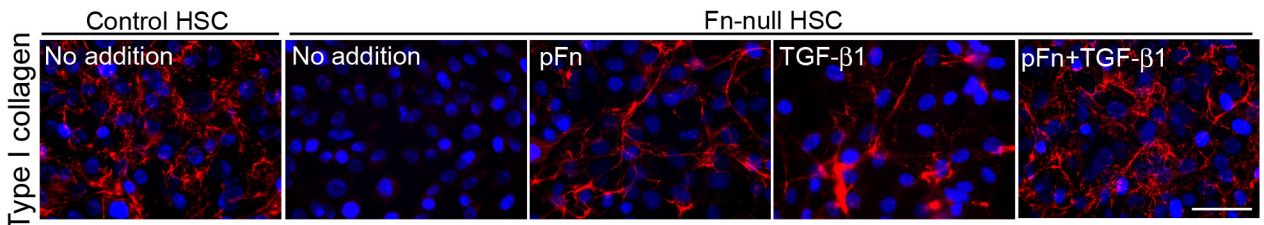

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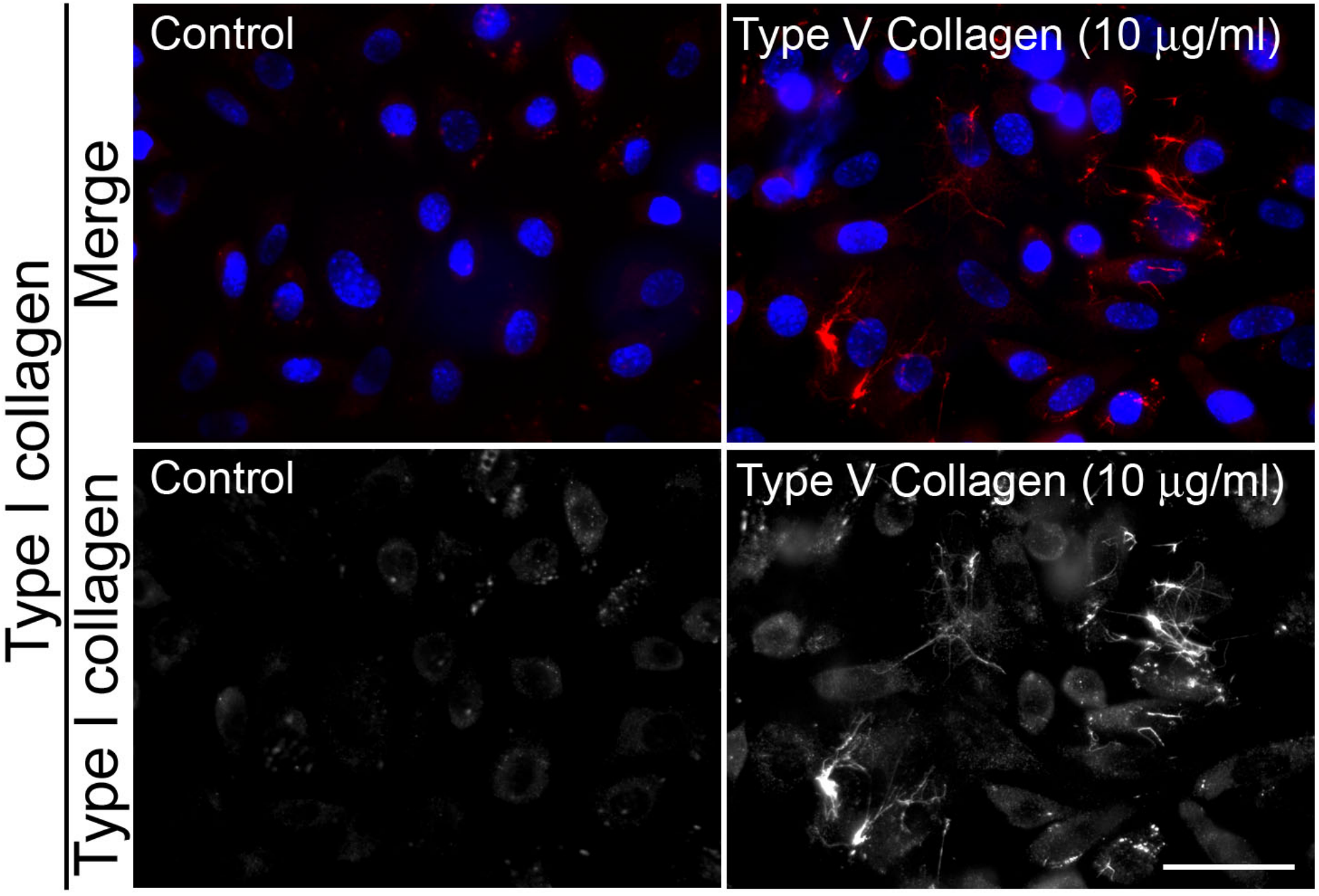

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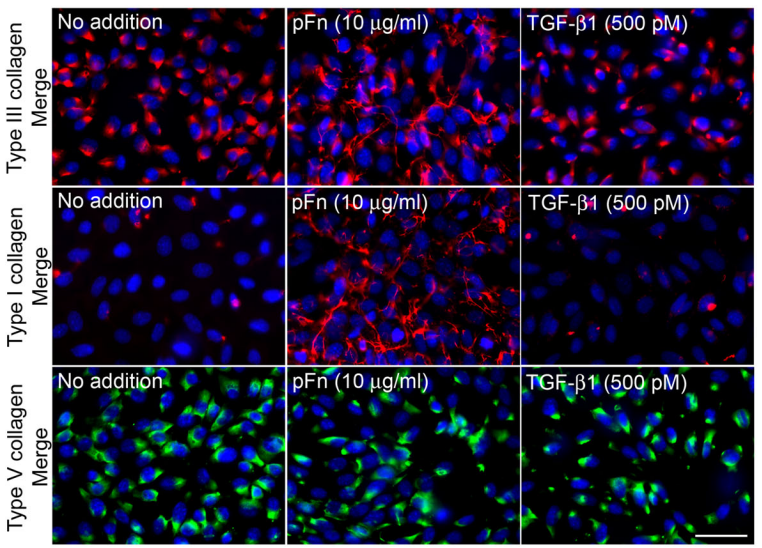
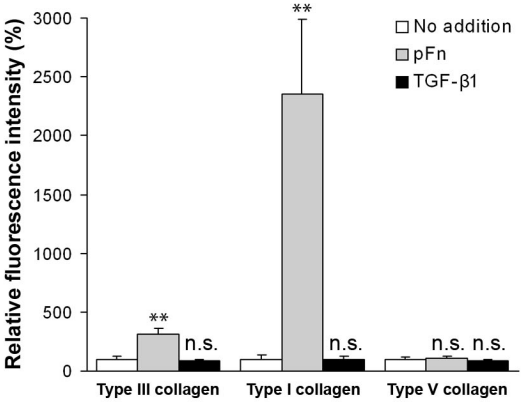
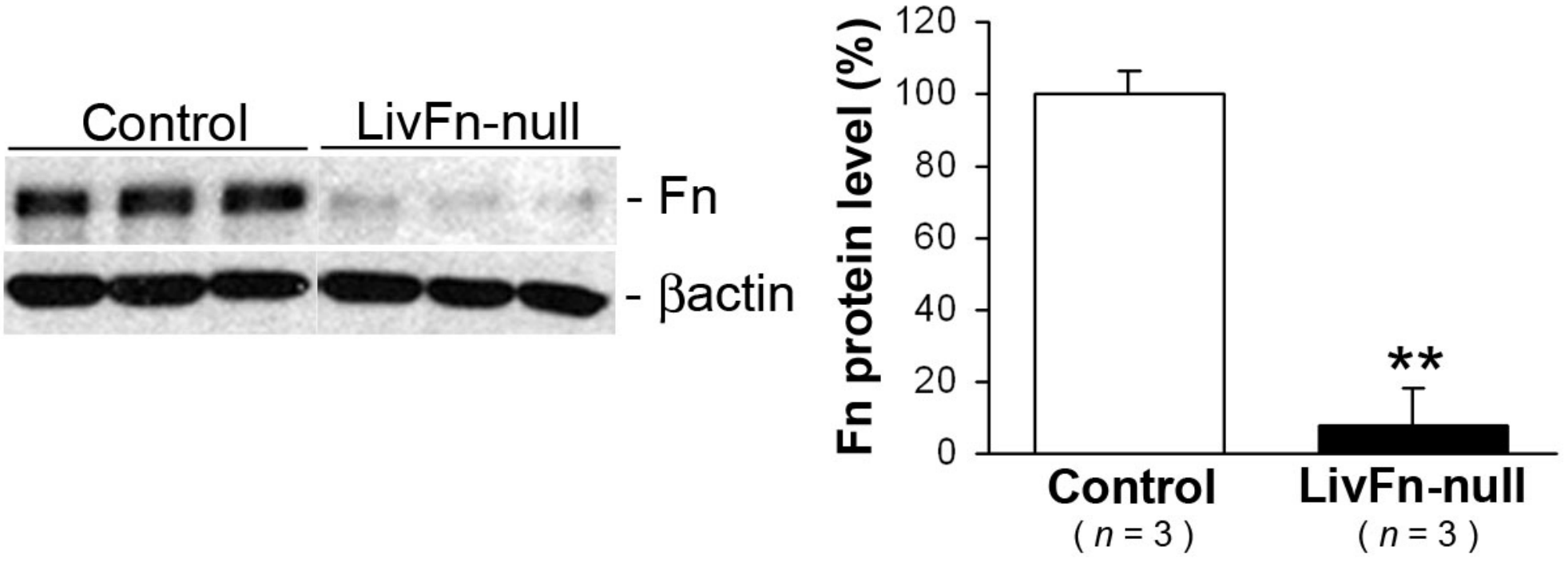
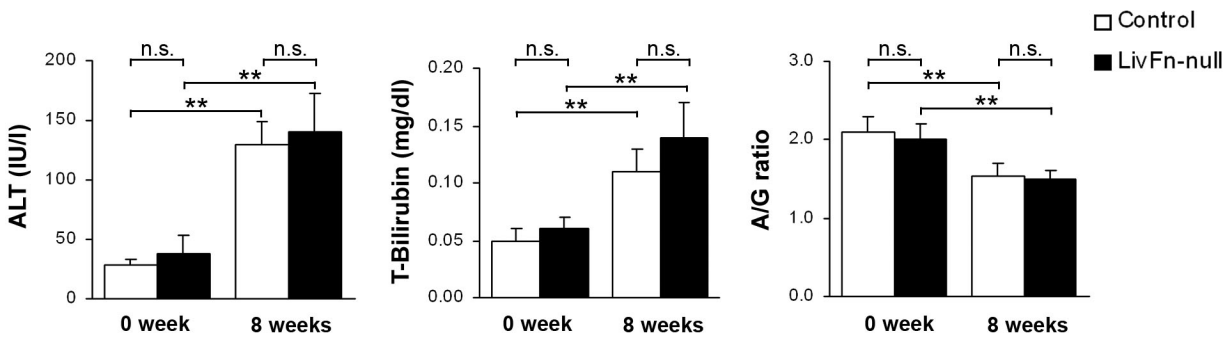




Supplementary Fig. 7



Supplementary Fig. 8



Supplementary Fig. 9

Supplementary Table 1

*Gene	Relative mRNA level		Primer sequence	
	TGF-β1	Fn	Forward primer	Reverse primer
Bgn	1	0.84	5'-ACGAATCCATGACAACCGTATC-3'	5'-GCTCCTGGTTCAAAGCCACT-3'
Bmp1	0.73	0.67	5'-AAAGGACCCGACTCAGCAAG-3'	5'-CCATTGGGATACTCAGGGGAG-3'
Cav1	0.84	0.67	5'-TGTATGACGCGCACACCAA-3'	5'-TGGTTCTGCAATCACATCTTCAA-3'
Col5a1	2.56	1.04	5'-GAGGACCACACAGGGAAGC-3'	5'-CTTGTAGACACTGAGAGCAATTCG-3'
Comp	0.48	0.75	5'-GATAGCCGGGACAACTGC-3'	5'-TTGTCAGCATCGAAGTCACC-3'
Dcn	0.49	0.96	5'-CCCTACCGATGCCAGTGTC-3'	5'-GCAGGTCTAGCAAGGTTGTGT-3'
Egfr	0.98	0.75	5'-CTGGACTGACCTCCATGCTT-3'	5'-AGCCCCAGTGATGTGATGTT-3'
Fmod	0.68	0.99	5'-TTACCTCCAGGGCAACAGG-3'	5'-GCTTGATCTCGTTCCCATCC-3'
Fbn1	1.62	0.73	5'-GGCTCCAGATCCATCCAACAC-3'	5'-GACAGCCACTTTCACAGACAG-3'
Gdnf	0.97	1.06	5'-CGGGCCACTTGGAGTTAAT-3'	5'-ACAGCCACGACATCCCATA-3'
Hsp47	0.92	0.67	5'-ACAACCGTGGCTTCATGG-3'	5'-TCTGCAGCTTCTCCTTCTCG-3'
Lox	1.21	0.76	5'-CAGAGGAGAGTGGCTGAAGG-3'	5'-CTCAATCCCTGTGTGTGTGC-3'
Lum	1.34	2.11	5'-CAACAGTAGTTTGATGTGAAGAGC-3'	5'-ATCCAAGCGCAGATGCTT-3'
Ltbp1	1.1	0.85	5'-CAGATGCTCCTGCAAAATGG-3'	5'-ACTGACAAGGCGGTAACAGG-3'
Pai1	1.28	0.93	5'-ATGGAAGGGCAACATGACC-3'	5'-AGGCATGCCCAACTTCTCC-3'
Plat	0.9	0.95	5'-TTGTAGGGAAACGCTGTGACA-3'	5'-ACTGCTATTCCAGTTGATGCAC-3'
Plaur	1.33	0.94	5'-TGCCTGGTAGAGGAGTGTGC-3'	5'-TGTTGGTCTTTTCGCTGTGG-3'
Plau	0.47	0.71	5'-GCGCCTTGGTGGTGAAAAAC-3'	5'-TTGTAGGACACGCATACACCT-3'
Timp1	1.39	0.76	5'-CTTGGTTCCCTGGCGTACTC-3'	5'-ACCTGATCCCTCCACAAACAG-3'
Tnx	0.43	1.2	5'-ATGCGTCTGCCAAGAAGG-3'	5'-AGCTCGTGTCCACTCTGTCC-3'
Wt1	0.99	0.8	5'-GAGAGCCAGCCTACCATCC-3'	5'-GGGTCCTCGTGTTTGAAGGAA-3'

Real-time PCR analysis of mRNA expression levels in Fn-null HSCs in response to TGF-β1 or plasma Fn. Cells were incubated for 12 hrs in DMEM containing 8% Fn-depleted FBS and with either TGF-β1 (2 pM) or plasma Fn (10 μg/ml). Relative mRNA expression levels (an average taken from multiple experiments/repeats) are shown relative to the control value of 1 (control [no addition]).

*Abbreviations: Bgn, biglycan; Bmp1, bone morphogenetic protein1; Cav1, caveolin1; Col5a1, collagen alpha1(V); Comp, cartilage oligomeric matrix protein; Dcn, decorin; Egfr, epidermal growth factor receptor; Fmod, fibromodulin; Fbn1, fibrillin1; Gdnf, glial cell line derived neurotrophic factor; Hsp47, 47kDa heat-shock protein; Lox, lysyl oxidase; Lum, lumican; Ltbp1, latent TGF-β binding protein1; Pai1, plasminogen activator inhibitor1; Plat, tissue-type plasminogen activator; Plaur, urokinase-type plasminogen activator receptor; Plau, urokinase-type plasminogen activator; Timp1, tissue inhibitor of metalloproteinase1; Tnx, tenascinX; Wt1, Wilm's tumor suppressor gene1