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Supplementary Materials for

Thrombospondin-1 promotes matrix homeostasis by interacting with collagen and lysyl oxidase precursors and collagen cross-linking sites

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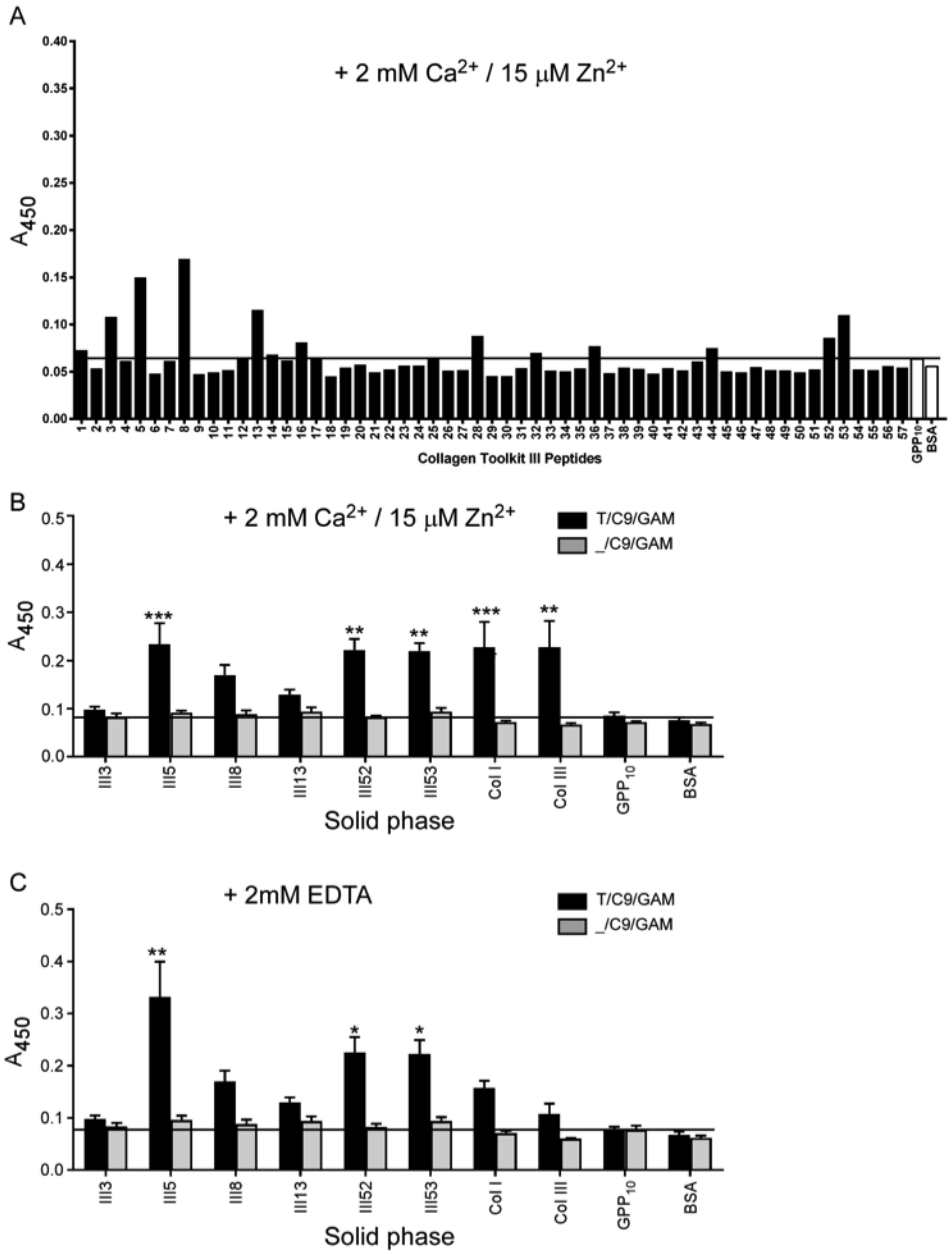

Fig. S1. Identification of specific TSP1-binding peptides within the triple-helical region of collagen III. (**A**) Binding of TSP1 to peptides from the Collagen Toolkit III in the presence of Ca^{2+} and Zn^{2+}. The horizontal line represents the background binding of TSP1 to GPP_{10}. Each bar represents the mean of duplicate samples. (**B**) Binding of TSP1 to the indicated peptides from the Collagen Toolkit III, pepsin-digested collagen I (Col I), and pepsin-digested collagen III (Col III) in sold-phase binding assays. N=3 independent experiments. Each bar represents the mean, and the error bars indicate the s.e.m. (**C**) Binding of TSP1 to the indicated peptides in the presence of the Ca^{2+} chelator EDTA. N= 3 independent experiments. Each bar represents the mean, and the error bars indicate the s.e.m. In all panels, GPP_{10} and BSA were included as negative controls. In B and C, pepsin-digested collagen I (Col I) and collagen III (Col III) were included as positive controls.

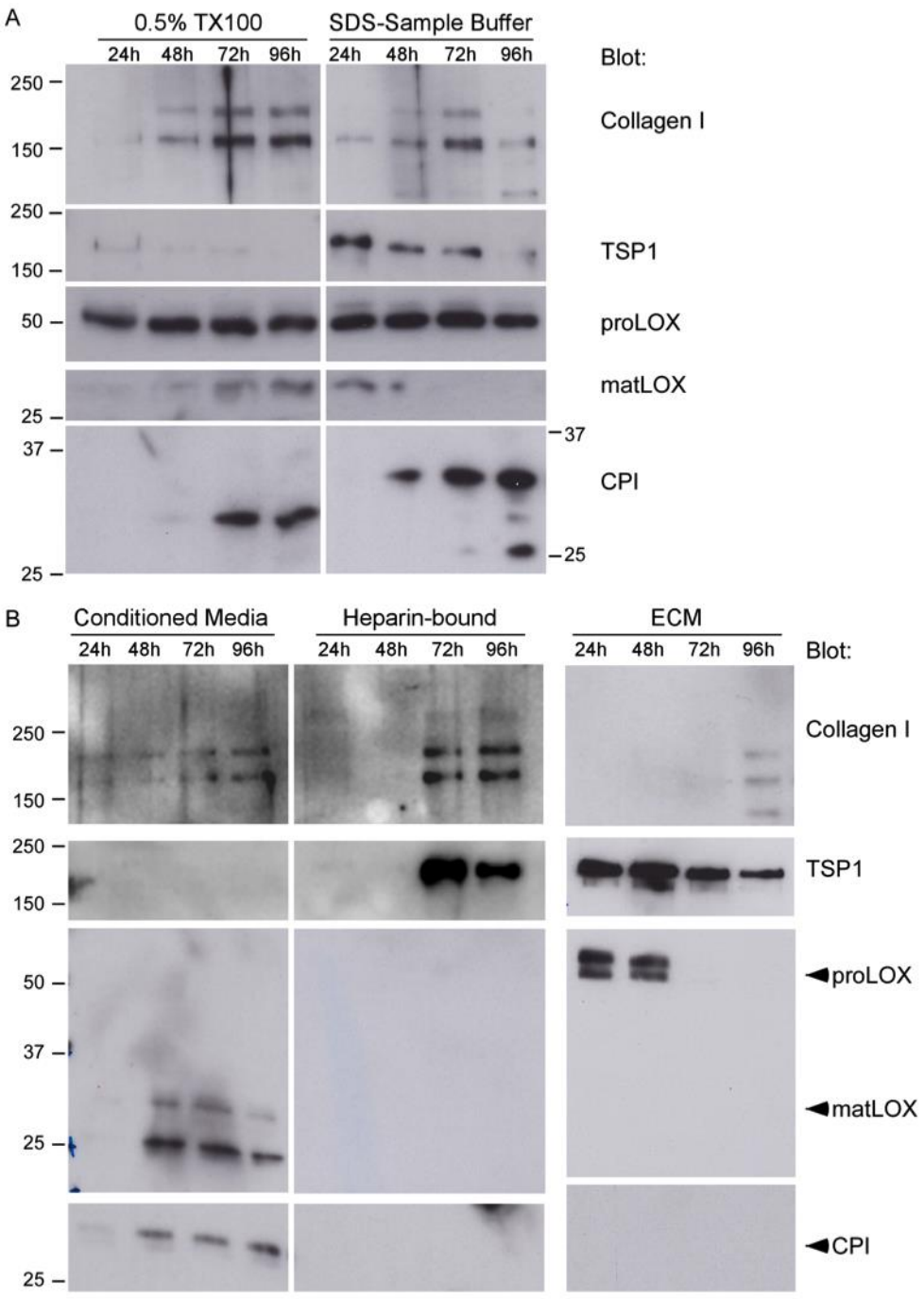

Fig. S2. Detection of collagen I, TSP1, FN, LOX and collagen I C-propeptide in cell fractions, CM, and ECM isolated from HDFs. Samples of human dermal fibroblasts (HDF) were prepared at the indicated timepoints, resolved on 10% SDS-PAGE gels under reducing conditions, and immunoblotted for the indicated proteins. (**A**) Cell lysates were prepared as indicated. In addition to the main CPI band of 30kDa, degradation products of CPI were observed in the sample buffer extracts at 96h. N = 2 independent experiments. (**B**) Conditioned media were analyzed directly (left panels) or after pull-down with heparin-agarose to concentrate heparin-binding proteins (center panels). ECM was isolated from the same cultures (right panels). The blot is representative of duplicate analysis. Non-contiguous lanes are indicated by gaps between panels. Molecular mass markers are indicated to the left of the blots in kDa. TX100, Triton X-100.

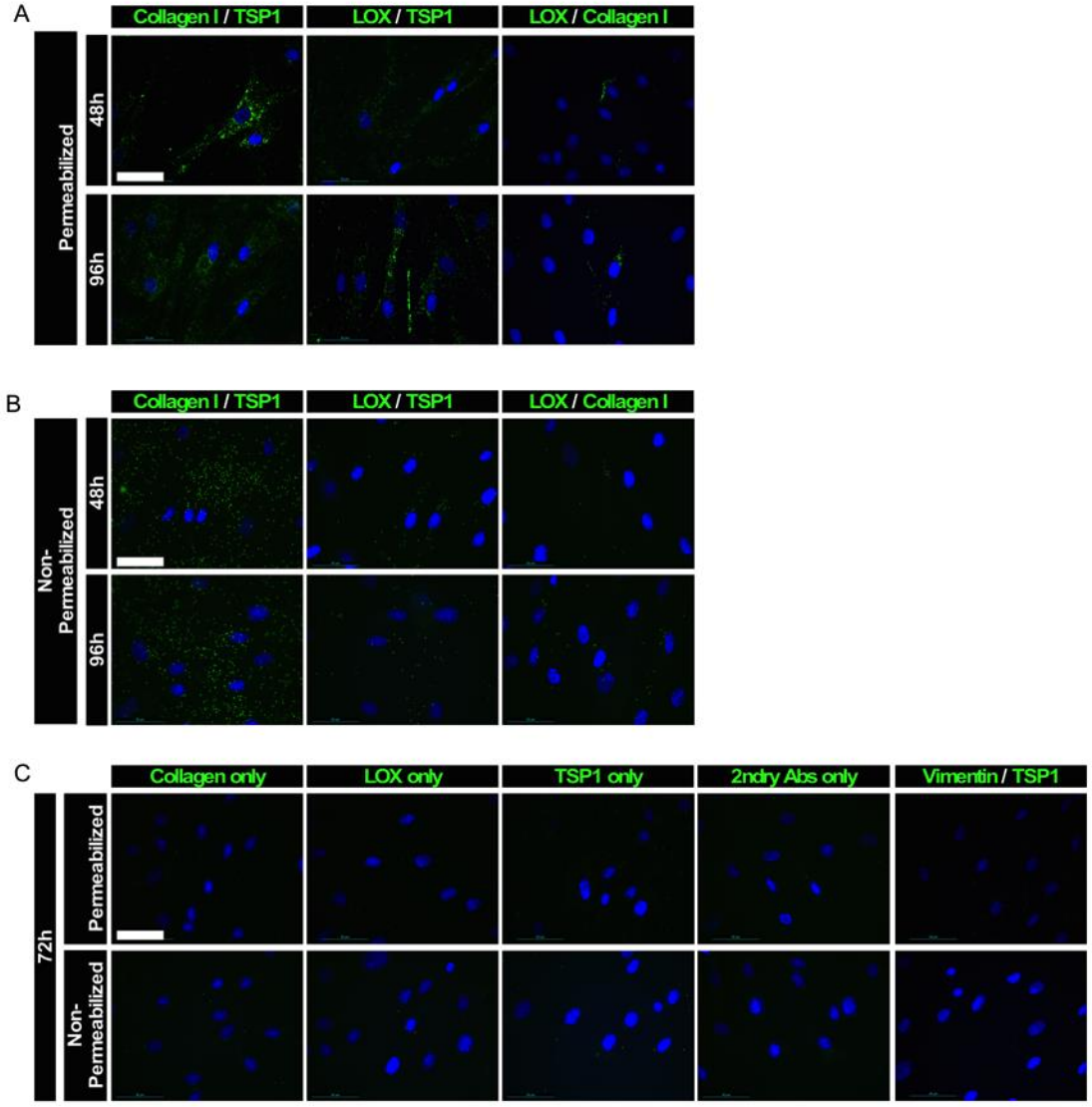

Fig. S3. Detection of the association of TSP1 with collagens I and II and LOX in HDFs by in situ proximity ligation. (**A, B**) In situ proximity ligation for detection of association between the indicated pairs of proteins in permeabilized (A) and non-permeabilized (B) HDF after culture for the indicated times. (**C**) Representative images of control in situ proximity ligation assays with single primary antibodies, secondary antibodies only, or vimentin and TSP1 as a pair of proteins predicted not to associate in HDF, analysed at 72h of culture. All images are representative of N = 4 independent experiments.

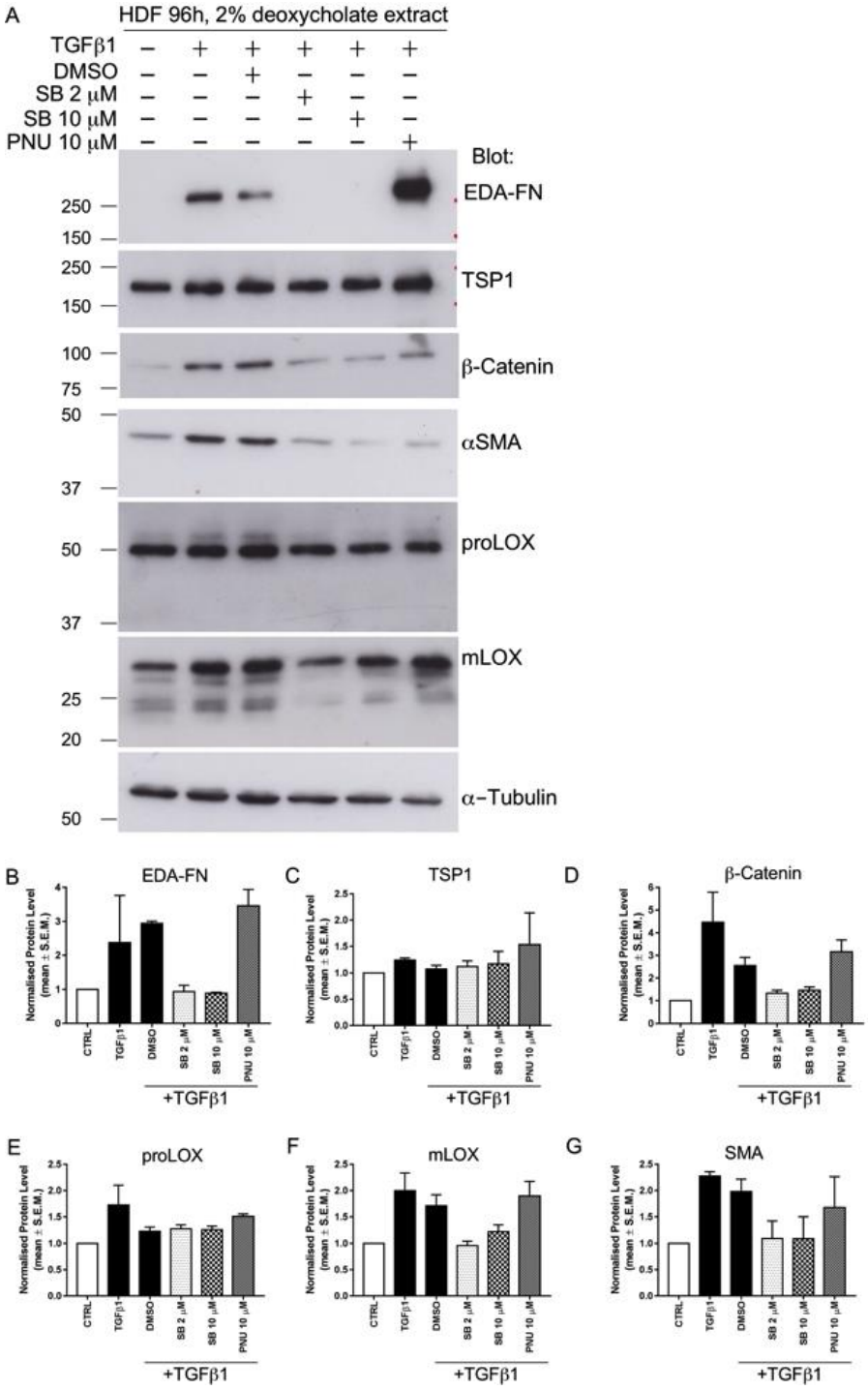

Fig. S4. TGF-β1–mediated induction of αSMA in HDFs. (**A**) HDFs were cultured in the presence or absence of TGF-β1, the TGF-βPR1 inhibitor SB-431542 (SB), or the β-catenin signaling inhibitor PNU-74654 (PNU) as indicated for 96h. Cell extracts were resolved on 10% polyacrylamide gels under reducing conditions and immunoblotted for the indicated proteins. The blot is representative of 3 experiments. Molecular mass markers are indicated to the left of the blot in kDa. (**B-G**) Quantification of EDA-FN (B), TSP1 (C), β-catenin (D), proLOX (E), mLOX (F), and αSMA (G) in the indicated treatment groups as normalized against α-tubulin. Each bar represents the mean from 3 independent experiments; error bars indicate s.e.m.

Table S1. Primary antibodies and the dilutions used in this study.

Antigen	Antibody Isotype	Supplier	Cat. Number #	WB	IF	In situ Proximity Ligation	ELISA
6-His tag	mouse monoclonal IgG2b	Abcam	ab18184				1:1,000
α-smooth muscle actin (SMA)	mouse monoclonal IgG2A	Sigma	A2547	1:1,000	1:1,000		
α-tubulin	rabbit polyclonal IgG	Abcam	ab4074	1:1,000			
Collagen α1(I) C-propeptide (CPI)	rabbit polyclonal IgG	Larry Fisher, NIH	LF41	1:10,000			
Collagen I	mouse monoclonal IgG1	Abcam	ab 90395		1:1,500	1:1,500	
Collagen I	rabbit polyclonal IgG	Novus Biologicals	NB600-408	1:600	1:400	1:400	
EDA-Fibronectin	mouse monoclonal IgG1	Abcam	ab6328	1:1,000			
Lysyl oxidase (aa350 to the C-terminus)	rabbit monoclonal IgG	Abcam	ab174316 [EPR4025]	1:500	1:300	1:300	
Thrombospondin-1 (aa 25-48)	mouse monoclonal IgG1	SCBT	sc-393503	1:200	1:50	1:100	1:200
Vimentin	rabbit monoclonal IgG	Abcam	ab92547			1:1,000	

Key: ELISA = Enzyme-linked immunosorption assay; IF = immunofluorescence; Ig, immunoglobulin; WB = western blot.

Table S2. Secondary antibodies and the dilutions used in this study.

Antigen	Antibody Isotype and Conjugate	Supplier	Cat. Number #	WB	IF	In situ Proximity Ligation	ELISA
mouse IgG	MINUS PLA probe-conjugated donkey Ig	Sigma	DUO92004			1:5	
mouse IgG (Fab specific)	FITC-conjugated goat Ig	Sigma	F8771		1:500		
mouse IgG, IgA, IgM	HRP-conjugated goat IgG	Dako	P0447				1:15,000
mouse IgG, IgA, IgM	HRP-conjugated goat IgG	LI-COR	926-80010	1:100,000			
rabbit IgG	TRITC-conjugated sheep Ig	ThermoFisher Scientific	A16177		1:500		
rabbit IgG	PLUS PLA probe-conjugated donkey Ig	Sigma	DUO92002			1:5	
rabbit IgG, IgA, IgM	HRP-conjugated goat IgG	LI-COR	926-80011	1:100,000			

Key: ELISA = Enzyme-linked immunosorption assay; FITC, fluorescein isothiocyanate; HRP, horseradish peroxidase; IF = immunofluorescence; Ig, immunoglobulin; PLA, proximity ligation; WB = western blot.

Table S3. Chemicals used in this study.

Item	Supplier	Cat. Number #
Acetic Acid (HAc)	Sigma	A0808
Acetone	Fisher Scientific	10745863
Affi-gel® heparin beads	Bio-Rad	153-6173
Amersham ECL	GE Healthcare Life Sciences	RPN2209
Bromophenol Blue	Sigma	B0126
Calcium chloride ($CaCl_2$)	Sigma	449709
Deoxycholic acid (DOCS)	Sigma	D2510
Detection Reagents Green	Sigma	DUO92014
DL-dithiothreitol (DTT)	Sigma	D0632
EDTA	Sigma	ED2SS
Gel Code Blue Stain reagent	ThermoFisher Scientific	24590
Glycerol	Sigma	G5516
Hydrochloric acid (HCl)	Sigma	H1758
L-ascorbic acid	Sigma	A4403
Leupeptin	Sigma	L2884
Methanol	Sigma	322415
N-Ethylmaleimide (NEM)	Sigma	E3876
Paraformaldehyde (PFA) 16% (w/v)	EMS or Alpha Aser	28906
Pepstatin A	Sigma	P5318
Pierce™ TMB Substrate Kit	ThermoFisher Scientific	34021
Sodium Dodecyl Sulfate (SDS)	Fisher Scientific	BP166-100
Triton-X100 (TX100)	Sigma	10789704001
Wash Buffer A	Sigma	DUO82047
Wash Buffer B	Sigma	DUO82048
Zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$)	Sigma	Z0501

Table S4. Chemical inhibitors used in this study.

Target	Inhibitor	Supplier	Cat. Number #	CAS
Interaction between β-catenin and TCF (T cell factor)	PNU-74654	Sigma	P0052	113906-27-7
TGF-β RI Kinases (ALK4 and ALK5)	TGF-β RI Kinase Inhibitor VI, 616461	Calbiochem	SB431542	301836-41-9

Table S5. Proteins used in this study.

Protein	Supplier/Reference	Cat. Number #
Bovine serum albumin (BSA) Fraction V powder	GE Healthcare	K41-001
Recombinant human mini-pro-collagen I alpha 1 (rhProCOL1A1)	R&D	6220-CL-020
Human procollagen C-propeptide I (CPI)	Bourhis et al. 2012 Sharma et al. 2017	
Human procollagen C-propeptide III (CPIII)	Bourhis et al. 2012 Sharma et al. 2017	
Denatured collagen I (bovine)	Devro	NA
Native collagen I fibrils from equine tendon - Kollagen Reagens HORM Suspension	Takeda	NA
Pepsin-digested calf skin-derived collagen I	Sigma	C-3511
Pepsin-digested bovine tracheal cartilage-derived collagen II	Sigma	C-1188
Pepsin-digested human placenta-derived collagen III	Sigma	C-4407
Native purified human thrombospondin-1 (TSP1)	Cell Sciences Inc	CSI19832B
Recombinant human C-terminal MYC/DDK-tagged lysyl oxidase (proLOX)	Origene	TP313323
Recombinant human TGFβ1	R&D	240-B

Table S6. Buffers used in this study.

Buffer	Composition	Experiment
Protease inhibitor cocktail	2 mM NEM, 10 mM leupeptin, 20 mM pepstatin A	Collagen extraction from mouse skin
Incubation Buffer (IB)	Tris-Buffered Saline (TBS), 1 mg/ml BSA, 0.1 % (v/v) Tween-20	Solid phase binding assay
SDS-PAGE sample buffer	100 mM Tris.HCl, pH 6.8, 4 % SDS, 0.2 % bromophenol blue, 20 % glycerol containing 100 mM DTT	WB
DOCS-buffer	2 % deoxycholic acid, 20 mM Tris pH 8.8, 2 mM N-ethylmaleimide	WB
Immunoblot Blocking Buffer (BB)	TBS containing 2 % (w/v) semi-skimmed dried milk and 0.2 % (v/v) Tween 20	WB

Key: WB = western blot.