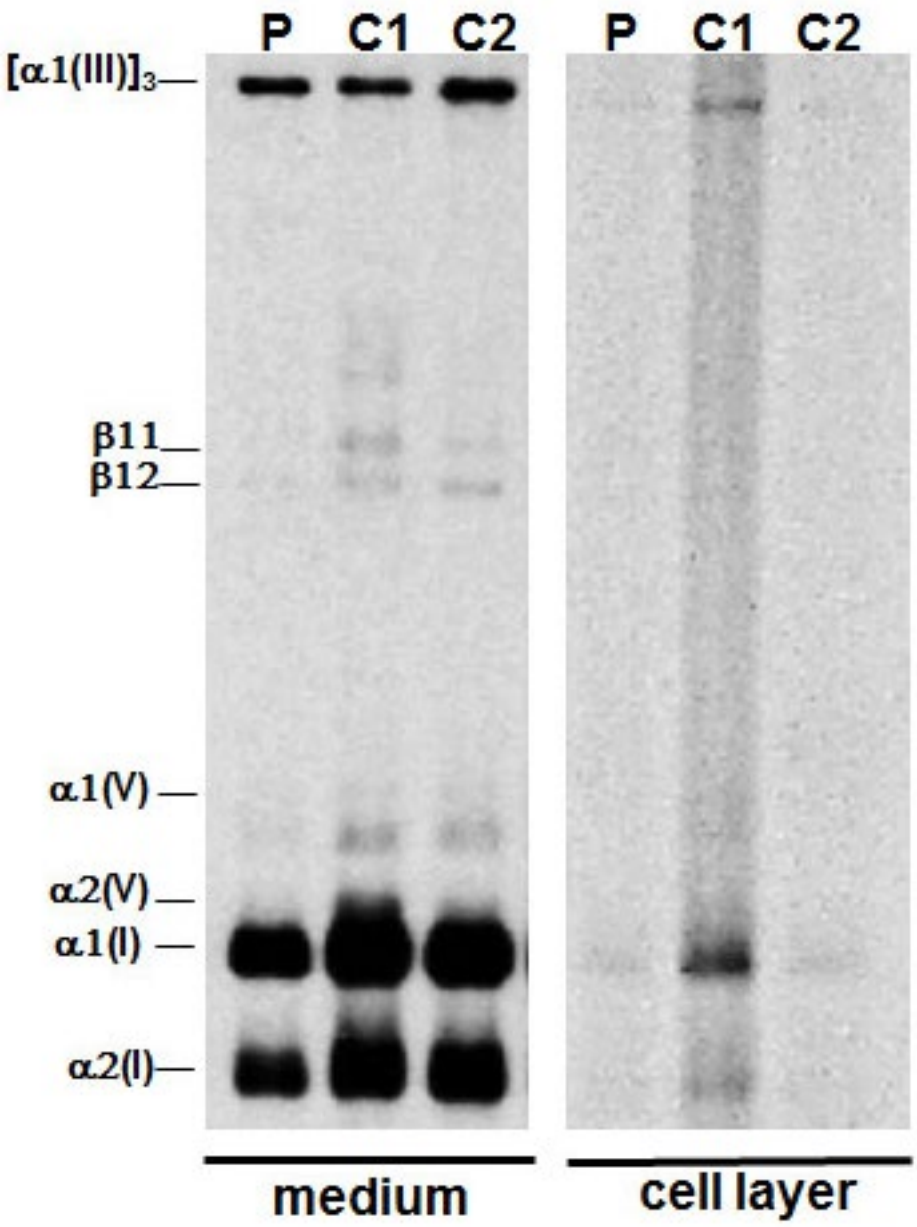
Supplementary data

Supplementary Figure S1: Steady-state analysis of collagen in the medium and the cell layer



Supplementary Table S1: NGS panel for connective tissue disorders

ABCC6	NM_001171	*DSE*	NM_013352	*PPIB*	NM_000942
ACTA2	NM_001613	*DSPP*	NM_014208	*PRDM5*	NM_016599
ACVRL1	NM_000020	*EFEMP2*	NM_016938	*PRKG1*	NM_006258
ADAMTS2	NM_014244	*ELN*	NM_000501	*PYCR1*	NM_006907
ADAMTSL4	NM_019032	*ENG*	NM_000118	*RIN2*	NM_001242581
ALDH18A1	NM_002860	*FBLN5*	NM_006329	*RUNX2*	NM_001024630
ALPL	NM_000478	*FBN1*	NM_000138	*RYR1*	NM_000540
ANO5	NM_213599	*FBN2*	NM_001999	*SEC24D*	NM_014822
ATP6V0A2	NM_012463	*FGFR2*	NM_000141	*SEPN1*	NM_020451
ATP7A	NM_000052	*FGFR3*	NM_001163213	*SERPINF1*	NM_002615
B3GALT6	NM_080605	*FKBP10*	NM_0213939	*SERPINH1*	NM_001235
B4GALT7	NM_007255	*FKBP14*	NM_017946	*SKI*	NM_003036
BMP1	NM_006129	*FLCN*	NM_144997	*SLC25A2*	NM_000112
CASR	NM_001178065	*FLNA*	NM_001456	*SLC2A10*	NM_030777
CBS	NM_000071	*GLA*	NM_000169	*SLC39A13*	NM_001128225
CHRDL1	NM_001143981	*GORAB*	NM_152281	*SMAD3*	NM_005902
CHST14	NM_130468	*IFITM5*	NM_001025295	*SMAD4*	NM_005359
COL11A1	NM_001854	*LEPRE1*	NM_001243246	*SOX9*	NM_000346
(COL11A2)	NM_080680	*LRP5*	NM_002335	*SP7*	NM_001173467
COL12A1	NM_004370	*LTBP2*	NM_000428	*SPARC*	NM_003118
COL1A1	NM_000088	*LTBP4*	NM_0035732	*STAT1*	NM_007315
COL1A2	NM_000069	*MAT2A*	NM_005911	*STAT3*	NM_139278
COL2A1	NM_001844	*MED12*	NM_005120	*TGFB1*	NM_000660
COL3A1	NM_000090	*MFAP5*	NM_003480	*TGFB2*	NM_001135599
COL5A1	NM_000093	*MMP2*	NM_004530	*TGFB3*	NM_003239
COL5A2	NM_000393	*MYH11*	NM_002474	*TGFBR1*	NM_004612
COL6A1	NM_001846	*MYLK*	NM_053025	*TGFBR2*	NM_001024847
COL6A2	NM_001849	*NOTCH1*	NM_017617	*TMEM38B*	NM_018112
COL6A3	NM_004369	*NOTCH3*	NM_000435	*TRIP11*	NM_004239
COL9A1	NM_001851	*P4HB*	NM_000918	*WNT1*	NM_005430
COL9A2	NM_001852	*PLOD1*	NM_000302	*XYLT1*	NM_022166
CREB3L1	NM_052854	*PLOD2*	NM_182943	*XYLT2*	NM_022167
CRTAP	NM_006371	*PLOD3*	NM_001084	*ZDHHC9*	NM_018032
DCHS1	NM_003737	*PLS3*	NM_005032	*ZNF469*	NM_001127464

Supplementary Table S2

Primers and PCR conditions for *TNXB* (NM_0019105.7) Sanger sequencing		
Exons 2-31 PCR: 95 °C, 3'; 95 °C, 30"; 58 °C. 20"; 72 °C, 45"; 72 °C, 7'; 10 °C, ∞; 32 cycles		
Exon 2	**TNXBex2aF**	GATTACTTGTGGGTGGTGGG
	TNXBex2aR	TACCTCTGAAGCAAGGACTGG
	TNXBex2bF	AAGCCCCTCTTCTCAGCTTT
	TNXBex2bR	GGCTCAGATCAAACACACCA
Exon 3	**TNXBex3aF**	GAGCAGACATGGCCTTATGA
	TNXBex3aR	CTCATGCCACAGTCGTCAC
	TNXBex3bF	AATGATCAGGGTCGCTGTG
	TNXBex3bR	CGCAATCGGTTCCAGTGTA
	TNXBex3cF	GGCGAGGACTGTGGTACG
	TNXBex3cR	CCTGAGTAGCCTGCGTCAC
	TNXBex3dF	GAGAACGGCGTGTGTGTTT
	TNXBex3dR	ACAGGCACACTCCTTGCAC
	TNXBex3eF	GCAGGCTACTCAGGGGAAG
	TNXBex3eR	TCCACTCTTCCTCAGGCTCA
Exon 4	**TNXBex4F**	GGGAGATTCTGTGCTCCTGA
	TNXBex4R	GCCATCTGGACTCAACCAAT
Exon 5	**TNXBex5F**	GGACCTGTTTCGTAGGGTTG
	TNXBex5R	ACTCAGGTGGGTGTCTGGTT
Exon 6	**TNXBex6F**	GCTCTTGGAAGGAAAGATGATG
	TNXBex6R	ACAGGACTCCAGGCATCTGA
Exon 7	**TNXBex7F**	CAGGTGAGGGAACCAGAAGT
	TNXBex7R	GCTGAACCTGCAATTCCTTT
Exon 8	**TNXBex8F**	GACCTGAAACCCCCGCAAAGAT
	TNXBex8R	ATGCCCTGTTCTCCAGCCAGAG
Exon 9	**TNXBex9F**	CAGTCCCTCTGTACTCCCTCA
	TNXBex9R	ACTTCTGGGAAGCCTGACAC
Exon 10	**TNXBex10F**	CCCAGAAGTTTAGCACTCGAA
	TNXBex10R	TGGCAAAATGAGCTGAGAAG
Exon 11	**TNXBex11F**	GCTGTCTCAGCCTTCTCCAT
	TNXBex11R	CAGCCTCAAGGTTCCACTG
Exon 12	**TNXBex12F**	TTTACTAACAGCCCCCTACCA
	TNXBex12R	GTCAGGGAACAGAAAGACTGG
Exon 13	**TNXBex13F**	AACTATTCCCCATCTCAGTTCAC
	TNXBex13R	TCAAATGAGACAGAGCAGGTG
Exon 14	**TNXBex14F**	GGAAATATTTTGGGGAAGCAC
	TNXBex14R	CTGGGGCCAAATAATGGTAAT
Exon 15	**TNXBex15F**	GCTGCCATTACCATTATTTGG
	TNXBex15R	GGATGATCGAAAGCAGACAGT
Exon 16	**TNXBex16F**	CTGGTCTTTCGATTGCTGACT
	TNXBex16R	TGGAAAGAGAGGACTGAGGTG
Exon 17	**TNXBex17F**	TTCAGTGATGGGAAGCATGT
	TNXBex17R	AGTGGTCAACCTCACAGGAAG
Exon 18	**TNXBex18F**	GGCCTCTCTGAACTGACCTC
	TNXBex18R	CTGGATTTGCTCTCTCTGTCC
Exon 19	**TNXBex19F**	TTGAGGGACCATAAGGAAGC
	TNXBex19R	CTGGGGCAATACCAAAGTCT
Exon 20	**TNXBex20F**	AGAGAATCCCAGAGTCCCTTG
	TNXBex120R	AGTTCAGAGAGGCCCATTCTT
Exon 21	**TNXBex21F**	GGCCTCTCTGAACTGACCTC
	TNXBex21R	GTGAAAGGGCACAGCAGTAA
Exon 22	**TNXBex22F**	GGATACCTCACCGGCTCTTA
	TNXBex22R	TCTCCATGACATGTCTTTCCA
Exon 23	**TNXBex23aF**	CAATCCAGCAAATGAAGCAA

	TNXBex23R	CAGTCATCACCAAAGAGCAAG
Exon 24	**TNXBex24F**	AGCTGACCCTGGAACTTGTC
	TNXBex24R	TCTTAGCAAGATCCCCAAGC
Exon 25	**TNXBex25F**	CTCTTGGGCACTTTGTGTTTT
	TNXBex25R	CACCCAGGAAGATCTGTCAGT
Exon 26	**TNXBex26F**	CCTCAGAGCTTGTCATGTGTG
	TNXBex26R	AAGCCTGCTGAATCCAAATC
Exon 27	**TNXBex27F**	CAGGGTGAGGGATAGGAAAG
	TNXBex27R	ACCAAAGAGCAAGAGGTGG
Exon 28	**TNXBex28F**	CCCAAGAATGGACTTCTCTGA
	TNXBex28R	TGAGGCAGGATCATTAGCAA
Exon 29	**TNXBex29F**	TCATATGTTGTGCGAGGGTTA
	TNXBex29R	CAATAAATCAGTGGGTGCTGAG
Exon 30	**TNXBex30F**	AGGGGACACTTGCTTTCTTG
	TNXBex30R	ACACAGAGGGACTCACTTTCG
Exon 31	**TNXBex31F**	GTTCTCCCTCATTCCTGTGG
	TNXBex31R	CCTGCTCTGGACTCCTTGAT
Exons 32-44	***Long-range PCR (5440 bp) as template for nested-PCR*** **95 °C, 4'; 95 °C, 30"; 60 °C, 30"; 72 °C, 5'; 72 °C, 10'; 10 °C, ∞; 30 cycles**	
	Intr30F	GTGGCCTTGTCCAGATAGC
	3'UTR-R	ACAGCCCGGGCCAGAG
***Nested-PCR:* 95 °C, 3'; 95 °C, 30"; 58 °C. 20"; 72 °C, 45"; 72 °C, 7'; 10 °C, ∞; 32 cycles**		
Exon 32	**TNXBex32F**	GACTGGGCCTGGACCTATAA
	TNXBex32R	AGCCCATCCATCCTCTCTC
Exon 33	**TNXBex33F**	TGCAAGTCCCTGGTTACAGA
	TNXBex33R	AAGTCGCTCTGCAGATTCCT
Exons 34-37	**TNXBex33F**	CTGACACTGTATGGGCTGCG
	TNXBex37R	CACAGACCCTACCTGTGGTG
Exons 37-38	**TNXBex37F**	TTGTCCTCCACACCAACTACA
	TNXBex38R	ATCTCCTGTGGGACAGACAAG
Exons 39-41	**TNXBex38F**	TACCTGCTCAGCTTCCACAC
	TNXBex41R	TCACAGCCTCTGCTTACCTG
Exon 42	**TNXBex42F**	GCATGGATGGACAGACAGAC
	TNXBex42R	GCGTAGTGGCAGTTCCTGTA
Exon 43	**TNXBex43F**	CTCGGCTGCGGAGTACTACC
	TNXBex43R	ACACTGTGGGGCTGAAACCT
Exon 44	**TNXBex44F**	GAGGGAGCTGGAGTTGATTTA
	TNXBex44R	ACTGCAGTGTCATCCTCAAGA

Supplementary Table S3: Published *TNXB* pathogenic variants

Patient	Family	Sex	Age (years)	Cons.	Status	Allele 1	Type	Allele 2	Type	References
P1	I	F	43	+	Homozygous	Exon 15: c.5362del, p.(Thr1788Profs*100)	Frameshift-PTC	Exon 15: c.5362del, p.(Thr1788Profs*100)	Frameshift-PTC	This report
P2	II	F	69	-	Homozygous	Exon 8: c.3290_3291del, p.(Lys1097Argfs*48)	Frameshift-PTC	Exon 8: c.3290_3291del, p.(Lys1097Argfs*48)	Frameshift-PTC	Schalkwijk *et al*., 2001
P3		F	59							
P4	III	F	60	-	Compound heterozygous	*TNXA* conversion[a]	Frameshift-PTC	*TNXA* conversion[a]	Frameshift-PTC	
P5		F	55							
P6		M	59							
P7	IV	M	57[†]	-	Homozygous	Exon 3: c.2117_2118insGT p.(Glu707*)	Frameshift-PTC	Exon 3: c.2117_2118insGT p.(Glu707*)	Frameshift-PTC	
P8	V	F	49[†]	-	Homozygous	Exon 8: c.3290_3291del, p.(Lys1097Argfs*48)	Frameshift-PTC	Exon 8: c.3290_3291del, p.(Lys1097Argfs*48)	Frameshift-PTC	
P9	VI	M	36	-	Compound heterozygous	*TNXB*/*TNXA* fusion[b]	Frameshift-PTC	*TNXA* conversion[b]	Frameshift-PTC	Voermans *et al*., 2009
P10	VII	F	32	-	Compound heterozygous	Exon 3: c.903del, p.(Tyr301*)	Nonsense	Exon 42: c.12464-1G>A	Splicing	
P11		F	31							
P12	VIII	M	29	+	Homozygous	Exon 8: c.3290_3291del, p.(Lys1097Argfs*48)	Frameshift-PTC	Exon 8: c.3290_3291del, p.(Lys1097Argfs*48)	Frameshift-PTC	
P13	IX	F	27	-	Compound heterozygous	Exon 8: c.3290_3291del, p.(Lys1097Argfs*48)	Frameshift-PTC	*TNXB*/*TNXA* fusion[c]	Frameshift-PTC	Hendriks *et al*., 2012
P14	X	F	12	-	Compound heterozygous	*TNXB*/*TNXA* fusion[d]	Frameshift-PTC	*TNXA* conversion[d]	Frameshift-PTC	
P15	XI	M	42	-	Compound heterozygous	*TNXB*/*TNXA* fusion[e]	Frameshift-PTC	Exon 41: c.12214C>T, p.(Arg4072Cys)	Missense	Pénisson-Besnier *et al*., 2013
P16	XII	F	45	-	Compound heterozygous	Exon 6: c.2539C>T, p.(Arg847*)	Nonsense	Exon 9: c.3574C>T, p.(Gln1192*)	Nonsense	Sakiyama *et al*., 2015
P17	XIII	M	13	-	Compound heterozygous	Exon 9 : c.3637G>A, p.(Val1213Ile)	Missense	Exon 22: c.7774G>A, p.(Gly2592Ser)	Missense	Mackenroth *et al., 2016*
P18	XIV	F	48	-	Compound heterozygous	Exon 43: c.12553C>T, p.(Arg4185*)	Nonsense	Exon 6: c.2590C>T, p.(Gln864*)	Nonsense	Demirdas *et al*., 2017
P19	XV	F	23	-	Compound heterozygous	Exon 6: c.2590C>T, p.(Gln864*)	Nonsense	*TNXB*/*TNXA* fusion[f]	Frameshift-PTC	

P20	XVI	M	16	-	Compound heterozygous	Exon 5: c.2461C>T, p.(Arg821)*	Nonsense	*TNXA* conversion[g]	Frameshift-PTC
P21		M	14						
P22	XVII	F	12	-	Compound heterozygous	Exon 2: c.107_108delinsA, p.(Ala36Aspfs*68)	Frameshift-PTC	Exon 22: c.7826-1G>C	Splicing
P23		M	6						

Note: Exons and mutations numbering are based on transcript NM_019105.6; NP_061978.6; cons. Consanguinity; † patient died. PTC: Premature termination codon.

[a] Patients 4, 5, and 6 were compound heterozygous for the 120 bp deletion [c.11435_11524+30del, p.(Gly3812Phefs*11)] and the "*TNXA*-derived" missense variant [c.12174C>G, p.(Cys4058Trp)], probably due to gene conversion in both alleles;

[b] Patient 9 was compound heterozygous for a *TNXB*/*TNXA* fusion gene in one allele, characterized by the *TNXA*-derived variations: 120bp deletion [c.11435_11524+30del, p.(Gly3812Phefs*11)] and [c.12174C>G, p.(Cys4058Trp)], and a gene conversion in the other allele, characterized by the 120 bp deletion [c.11435_11524+30del, p.(Gly3812Phefs*11)];

[c] Patient 13 was compound heterozygous for a recurrent frameshift deletion in exon 8 in one allele, and a *TNXB*/*TNXA* fusion gene characterized by the *TNXA*-derived variations: 120 bp deletion [c.11435_11524+30del, p.(Gly3812Phefs*11)] and missense variant [c.12174C>G, (p.Cys4058Trp)];

[d] Patient 14 was compound heterozygous for a gene conversion in one allele and a *TNXB*/*TNXA* fusion gene in other allele, characterized both by the "*TNXA*-derived" missense variant [c.12174C>G, p.(Cys4058Trp)];

[e] Patient 15 was compound heterozygous for a *TNXB*/*TNXA* fusion gene in one allele characterized by the 120 bp deletion [c.11435_11524+30del, p(.Gly3812Phefs*11)], and a missense variant in the other allele;

[f] Patient 18 was compound heterozygous for a nonsense variant and a *TNXB*/*TNXA* fusion gene characterized by the *TNXA*-derived variations: 120 bp deletion [c.11435_11524+30del, p.(Gly3812Phefs*11)], and missense variant [c.12174C>G, (p.Cys4058Trp)];

[g] Patients 19 and 20 were compound heterozygous for a nonsense variant in one allele and a gene conversion in the other allele, characterized by the 120 bp deletion [c.11435_11524+30del, p.(Gly3812Phefs*11)].