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Supplementary Materials for

High-impact *FN1* mutation decreases chondrogenic potential and affects cartilage deposition via decreased binding to collagen type II

Marcella van Hoolwerff, Alejandro Rodríguez Ruiz, Marga Bouma, H. Eka D. Suchiman, Roman I. Koning, Carolina R. Jost, Aat A. Mulder, Christian Freund, Farshid Guilak, Yolande F.M. Ramos, Ingrid Meulenbelt*

*Corresponding author. Email: i.meulenbelt@lumc.nl

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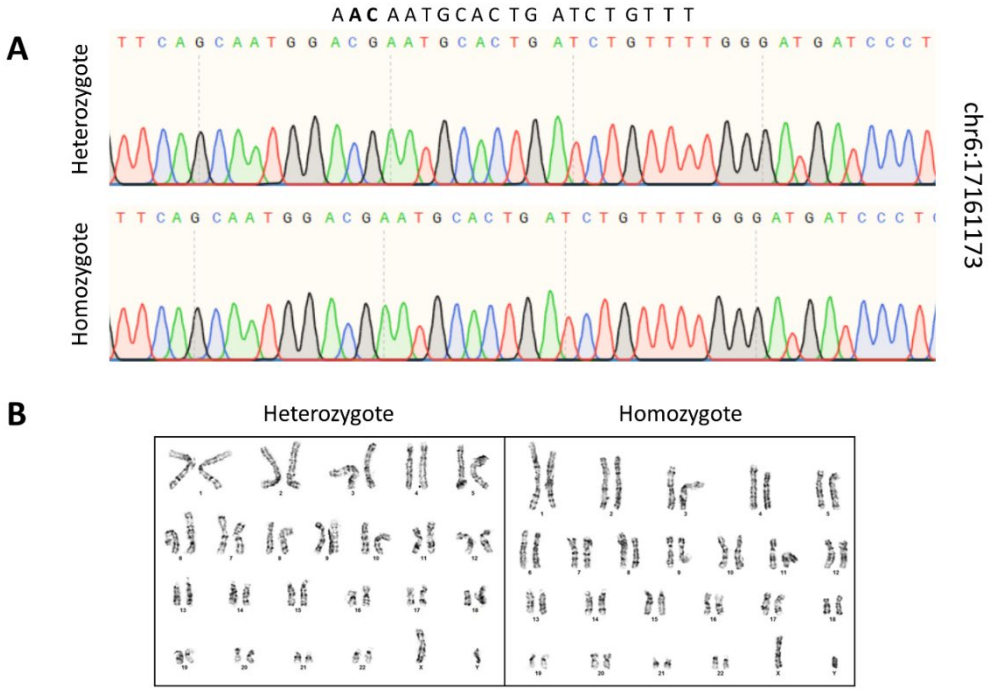

Fig. S1.

(A) Sanger sequencing results of *in silico* predicted off-target site at chr6:17161173 allowing 2 mismatches for the heterozygote and homozygote *FN1* clone. Sequence of gRNA is depicted above, where mismatches are depicted in bold. **(B)** Karyotypes of isogenic hiPSC lines heterozygous and homozygous for FN1 mutation, showing absence of chromosomal abnormalities.

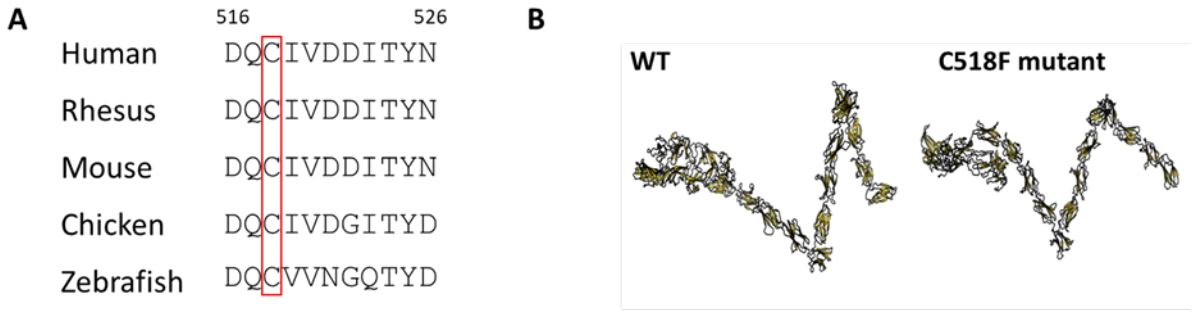

Fig. S2.

Investigation of C518F fibronectin mutation. **(A)** Alignment of the amino acid sequence around C518 of FN1, where the identified mutation is located. Mutated residue is outlined in red. **(B)** Structure prediction of wildtype (WT) and C518F mutant fibronectin by RaptorX of the 1,980 N-terminal amino acid residues.

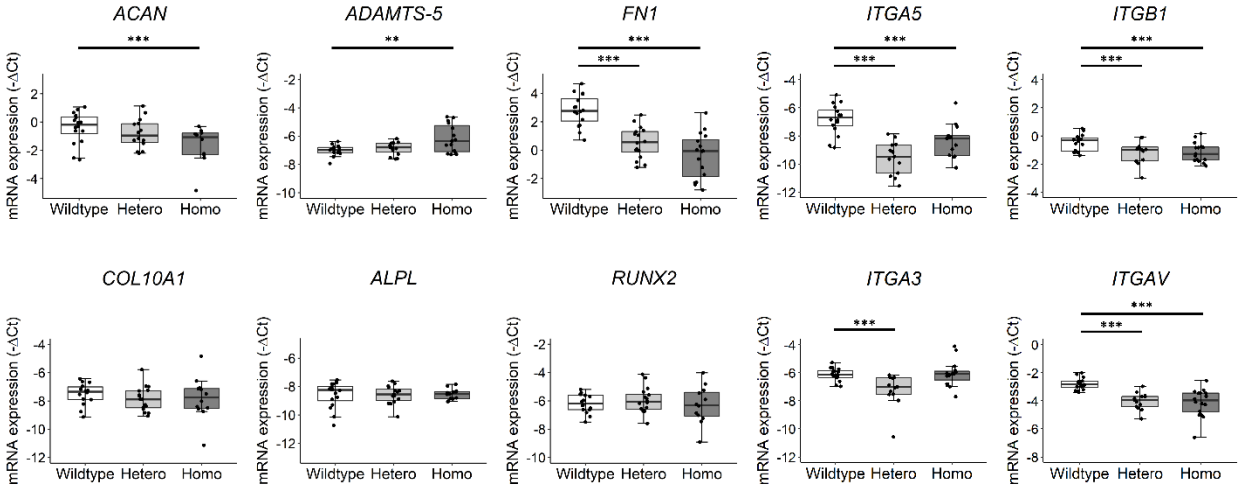

Fig. S3.

Boxplots of –ΔCt values of *ACAN*, *ADAMTS-5*, *FN1*, *ITGA5*, *ITGB1*, *COL10A1*, *ALPL*, *RUNX2*, *ITGA3*, *ITGAV* in wildtype, C518F FN1 hetero- (Hetero) and homozygous (Homo) chondrogenic pellets. –ΔCt values shown were corrected for *GAPDH* and *SDHA* expression levels. The boxplots represent 25th, 50th and 75th percentiles, and whiskers extend to 1.5 times the interquartile range. Independent samples are depicted by black dots in each graph. P values were determined by generalized estimation equations, with gene expression levels (-ΔCt) as dependent variable and genotype as factor. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$

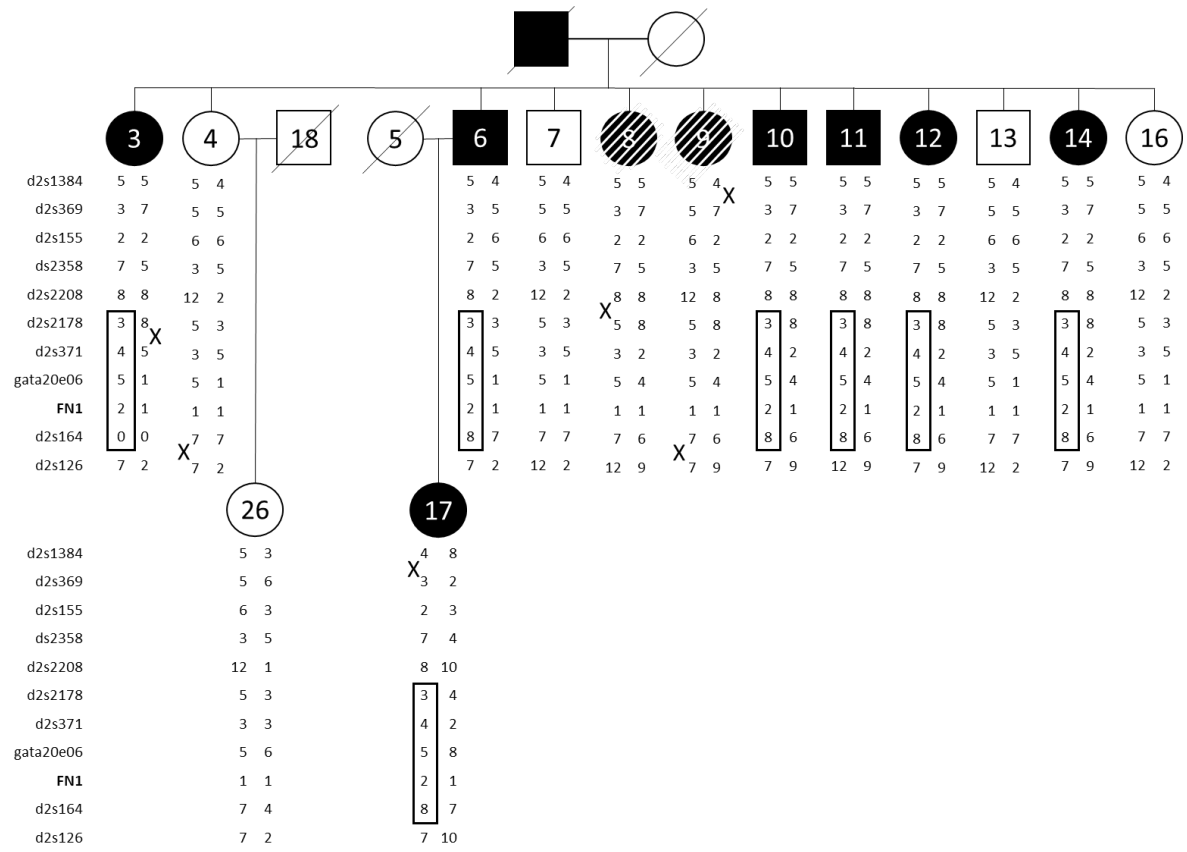

Fig. S4.

Pedigree of investigated early-onset osteoarthritis (OA) family, where co-segregating haplotypes among affected individuals are depicted as squared boxes and recombination as crosses. Individual 10 was used for exome sequencing. Circles represent females, squares represent males. Closed and open shapes represent affected respectively healthy individuals; striped shapes represent uncertain phenotypes.

Table S1.

Radiographic and clinical abnormalities in members of a family with early-onset OA.

Patient #	Sex	Age of onset	DIJ	PIJ	MCJ	CMC	Elbow	Shoulder	Hip	Knee	Foot	Ankle	CS	LS	Confirmed ROA
3	F	45	+	+		OA				OA		OA			Yes
4	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	M	40	+	+						OA	+	+			Yes
7	M	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	F	50	+	+						+			+		No
9	F	40	+	+						+	+	+			No
10	M	43	OA	OA	OA		+	+		+		OA			Yes
11	M	40	+	+							+	OA	OA	OA	Yes
12	F	39	OA	OA	OA				OA	+	+	+			Yes
13	M	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	F	28	OA	OA	OA					OA		OA		OA	Yes
15*	F	30	+	+	+					+					No
16	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	F	23	H	B		OA		-	-	OA	-	-			Yes

F = female, M = male, OA = Radiographic osteoarthritis, Kellgren Lawrence grade 2 or higher in one or both joints, + = joint complaints, - = no x-ray available or no joint complaints, H = Heberden nodes, B = Bouchard nodes, DIJ = distal interphalangeal joints, PIJ = proximal interphalangeal joints, MCJ = metacarpophalangeal joint, CMC = carpometacarpal joint, CS = cervical spine, LS = lumbar spine, confirmed ROA = confirmed radiological OA.

* patient 15 is a monozygotic twin with patient 14, since there was no confirmed ROA, patient 15 was removed from the linkage analysis

Table S2.
Variant prioritization scheme applied after exome sequencing.

	Subject 10
Total number of variants detected	73,407
Exonic including 5'UTR and 3'UTR	20,062
Missense, nonsense, readthrough, frame error, splice site	7,907
Novel	1,032
Missense damaging, nonsense, readthrough, frame error, splice site	122

Table S3.

List of damaging missense variants in an affected family member of an early-onset OA family after our applied prioritization scheme. Base change is base of the sense strand of the DNA.

Chromosome	Gene Name	Ensembl ID	Base change	Base position	Codon change	Substitution
1	CPSF3L	ENSG00000127054	A/G	1250997	GTA-GcA	V46A
1	RBMXL1	ENSG00000213516	T/G	89448812	GAT-GcT	D233A
1	ITGA10	ENSG00000143127	C/T	145530939	ACG-AtG	T224M
1	NBPF14	ENSG00000122497	C/A	148004625	GTG-tTG	V897L
1	ADAMTSL4	ENSG00000143382	G/A	150531177	GAC-aAC	D871N
1	LINGO4	ENSG00000213171	G/A	151774330	TCC-TtC	S284F
1	SELE	ENSG00000007908	A/T	169697065	GTG-GaG	V428E
1	C1orf129	ENSG00000117501	G/A	170961360	GTG-aTG	V362M
1	HEATR1	ENSG00000119285	A/G	236722383	GTG-GcG	V1527A
1	ZP4	ENSG00000116996	G/A	238050846	ACC-AtC	T190I
2	GPR113	ENSG00000173567	G/A	26534245	GCG-GtG	A784V
2	MOBKL1B	ENSG00000114978	A/G	74399827	TCT-cCT	S23P
2	DNAH6	ENSG00000115423	A/T	84899498	ATG-tTG	M2168L
2	MMADHC	ENSG00000168288	C/T	150436081	GGT-GaT	G79D
2	ALS2	ENSG00000003393	A/C	202590176	TAT-gAT	Y1084D
2	FN1	ENSG00000115414	C/A	216285518	TGC-TtC	C518F
2	ABCB6	ENSG00000115657	G/C	220075737	CGT-gGT	R688G
3	DNAH12	ENSG00000174844	G/C	57401178	GCA-GgA	A1924G
3	NEK11	ENSG00000114670	G/C	130748679	GTC-cTC	V43L
3	PAK2	ENSG00000180370	G/C	196529902	CAG-CAc	Q101H
4	MFSD10	ENSG00000109736	A/C	2935381	TAT-gAT	Y61D
4	MAN2B2	ENSG00000013288	T/A	6596307	ATG-AaG	M302K
4	TMPRSS11	ENSG00000198092	G/A	68930465	TCT-TtT	S318F
4	FAM13A	ENSG00000138640	A/C	89660254	GTG-GgG	V830G

4	GPRIN3	ENSG00000185477	T/G	90170862	ACC-cCC	T134P
4	NHEDC1	ENSG00000164037	A/C	103832643	CTG-CgG	L294R
4	SEC24D	ENSG00000150961	G/A	119678880	CCA-tCA	P473S
4	FRG1	ENSG00000109536	C/T	190874234	CCT-tCT	P91S
4	FRG1	ENSG00000109536	C/A	190878563	GCT-GaT	A148D
5	CDC20B	ENSG00000164287	G/A	54423155	CGG-tGG	R307W
5	PPIC	ENSG00000168938	C/T	122361517	GGC-aGC	G158S
5	LECT2	ENSG00000145826	C/T	135287013	GGA-GaA	G63E
5	PCDHB8	ENSG00000120322	G/A	140558064	GGG-GaG	G150E
5	ZNF354C	ENSG00000177932	A/G	178507004	TAT-TgT	Y524C
6	ZNF318	ENSG00000171467	G/A	43307399	CCA-CtA	P1446L
7	SDK1	ENSG00000146555	G/A	4002375	GGA-aGA	G441R
7	HBP1	ENSG00000105856	G/C	106836335	CGC-CcC	R375P
7	SLC26A3	ENSG00000091138	T/G	107427841	AAA-AAc	K283N
7	CFTR	ENSG00000001626	C/T	117188750	TCT-TtT	S422F
7	OR9A2	ENSG00000179468	A/C	142723621	GTT-GgT	V200G
7	GALNT11	ENSG00000178234	T/C	151810377	GTA-GcA	V376A
7	MLL3	ENSG00000055609	A/G	151919751	TGT-cGT	C1114R
8	PABPC1	ENSG00000070756	G/A	101721812	CGC-tGC	R374C
8	PABPC1	ENSG00000070756	T/C	101721817	GAA-GgA	E372G
8	PABPC1	ENSG00000070756	C/A	101721839	GTA-tTA	V365L
8	FER1L6	ENSG00000214814	C/T	124989741	CGG-tGG	R319W
8	PLEC	ENSG00000178209	A/G	145003292	CTG-CcG	L1217P
8	ZNF251	ENSG00000198169	T/C	145979657	GAG-GgG	E28G
9	EXOSC3	ENSG00000107371	G/C	37782053	CGA-gGA	R186G
9	NOTCH1	ENSG00000148400	T/C	139390627	AGC-gGC	S2522G
10	IL15RA	ENSG00000134470	G/A	6008296	ACG-AtG	T32M
10	ARMC3	ENSG00000165309	A/G	23250814	GAT-GgT	D180G
10	PLAU	ENSG00000122861	C/T	75673437	CGG-tGG	R165W
10	LIPM	ENSG00000173239	C/A	90579993	ACT-AaT	T336N

10	TM9SF3	ENSG00000077147	C/A	98292928	TGT-TtT	C402F
10	CPXM2	ENSG00000121898	C/G	125521390	GGA-GcA	G592A
11	OR51S1	ENSG00000176922	C/A	4870162	GCC-tCC	A93S
11	DNHD1	ENSG00000179532	C/T	6585157	CGC-tGC	R3363C
11	OLFML1	ENSG00000183801	C/T	7509386	ACG-AtG	T53M
11	SAA4	ENSG00000148965	T/A	18254071	GAC-GtC	D34V
11	LGR4	ENSG00000205213	A/T	27390683	TTT-TTa	F529L
11	BTBD18	ENSG00000233436	A/G	57513573	TTC-cTC	F58L
11	ATM	ENSG00000149311	T/C	108214007	ATT-AcT	I2776T
11	CRYAB	ENSG00000109846	C/A	111781098	GTG-tTG	V93L
12	FOXM1	ENSG00000111206	T/C	2967952	AAT-AgT	N753S
12	PZP	ENSG00000126838	G/A	9307415	CGC-tGC	R1191C
12	SLC11A2	ENSG00000110911	G/C	51384724	CGG-gGG	R477G
12	NUAK1	ENSG00000074590	C/A	106460782	CGC-CtC	R595L
13	CCNA1	ENSG00000133101	G/A	37014187	CGA-CaA	R322Q
13	KIAA1704	ENSG00000133114	G/T	45589633	GGT-tGT	G33C
14	HEATR5A	ENSG00000129493	G/A	31762650	TCT-TtT	S1995F
14	RALGAPA1	ENSG00000174373	A/G	36103797	CTC-CcC	L1534P
14	MTHFD1	ENSG00000100714	A/C	64884707	ACC-cCC	T194P
14	PLEKHG3	ENSG00000126822	C/T	65198849	CAC-tAC	H332Y
14	DIO2	ENSG00000211448	T/C	80669078	GAG-GgG	E259G
14	DDX24	ENSG00000089737	A/C	94521529	GTC-GgC	V664G
14	CDC42BPB	ENSG00000198752	C/G	103410810	GCT-cCT	A1276P
14	PACS2	ENSG00000179364	A/G	105821407	AGG-gGG	R106G
15	HERC2	ENSG00000128731	G/A	28483865	CGC-tGC	R1211C
15	ARHGAP11	ENSG00000198826	G/C	32921855	GAT-cAT	D333H
15	RYR3	ENSG00000198838	G/T	34152828	GAC-tAC	D4774Y
15	KIF23	ENSG00000137807	C/A	69709740	CCA-aCA	P34T
15	AKAP13	ENSG00000170776	G/T	86270411	GTC-tTC	V2317F
16	RGS11	ENSG00000076344	T/C	323545	CAG-CgG	Q152R

16	TBL3	ENSG00000183751	A/G	2024410	AGC-gGC	S75G
16	TSC2	ENSG00000103197	T/C	2130361	CTG-CcG	L1198P
16	ACSM5	ENSG00000183549	C/T	20429546	CGG-tGG	R124W
16	ABCC11	ENSG00000121270	C/T	48218359	GTG-aTG	V1084M
16	SALL1	ENSG00000103449	C/T	51171034	GTC-aTC	V1322I
16	GLG1	ENSG00000090863	T/C	74514287	GAC-GgC	D560G
16	KIAA1609	ENSG00000140950	A/G	84531559	TTC-TcC	F45S
17	FAM57A	ENSG00000167695	G/A	644611	CGG-CaG	R160Q
17	ALOX12B	ENSG00000179477	T/C	7977033	TAT-TgT	Y566C
17	CCDC144A	ENSG00000170160	G/A	16664922	GAA-aAA	E1186K
17	LGALS9C	ENSG00000171916	G/A	18397608	CGC-CaC	R333H
17	TRAF4	ENSG00000076604	C/T	27076578	CGG-tGG	R466W
17	SLFN11	ENSG00000172716	A/G	33680081	ATT-AcT	I667T
17	CDC27	ENSG00000004897	A/C	45216160	GTT-GgT	V550G
17	CDC27	ENSG00000004897	G/A	45216182	CTT-tTT	L543F
17	ABCC3	ENSG00000108846	G/A	48762223	GGG-aGG	G1423R
17	PYCR1	ENSG00000183010	G/A	79893008	CGG-tGG	R112W
18	DSG1	ENSG00000134760	C/T	28934644	CCT-tCT	P829S
19	MATK	ENSG00000007264	C/G	3779073	GGC-cGC	G372R
19	SH2D3A	ENSG00000125731	T/C	6755228	AGG-gGG	R199G
19	XAB2	ENSG00000076924	A/G	7685456	TGC-cGC	C691R
19	HAUS8	ENSG00000131351	C/T	17170885	GGA-aGA	G83R
19	ISYNA1	ENSG00000105655	C/T	18546943	GCG-aCG	A90T
19	ZNF98	ENSG00000197360	T/C	22574988	TAC-TgC	Y350C
19	MEGF8	ENSG00000105429	T/C	42855627	TGG-cGG	W968R
19	ZNF285	ENSG00000062370	C/T	44890654	GAG-aAG	E585K
19	ZNF285	ENSG00000062370	G/C	44891010	GCG-GgG	A466G
19	PPP1R13L	ENSG00000104881	G/A	45885802	CCG-tCG	P385S
19	DMWD	ENSG00000185800	T/C	46290117	AAG-gAG	K213E
19	ZNF845	ENSG00000213799	T/C	53856761	TGT-cGT	C861R

19	ZNF813	ENSG00000198346	T/C	53995143	TGT-cGT	C553R
19	ZNF543	ENSG00000178229	C/T	57839675	CCT-CtT	P282L
20	XKR7	ENSG00000101321	C/G	30556211	GCG-GgG	A78G
21	TSPEAR	ENSG00000175894	T/C	45941975	AGT-gGT	S453G
22	SOX10	ENSG00000100146	T/C	38374107	GAG-GgG	E155G
22	L3MBTL2	ENSG00000100395	G/C	41615519	GCC-cCC	A233P
22	SCUBE1	ENSG00000159307	C/T	43604155	CGC-CaC	R886H
X	MAGEA6	ENSG00000197172	A/C	151870145	ATT-cTT	I279L

Table S4.

Genotyping of the detected FN1 variant in FOA family members.

Patient #	Status	FN1 - C518F
3	Affected	C A
4	Unaffected	C C
6	Affected	C A
7	Unaffected	C C
8	Diagnostic uncertain	C C
9	Diagnostic uncertain	C C
10	Affected	C A
11	Affected	C A
12	Affected	C A
13	Unaffected	C C
14	Affected	C A
16	Unaffected	C C
17	Affected	C A

Table S5.

Design of CRISPR/Cas9 system. In the single-strand oligonucleotide template the introduced silent mutation to give a HincII restriction site is depicted in italic font and the restriction site is shown in grey, the wanted FN1 mutation is depicted in bold font, and the mutated PAM site is depicted underlined.

guide RNA	5'- AACAAUGCACUGAUCUGUUU-3'
single-strand oligonucleotide template	5'-CAG CAT GTG CCC CTC TTC ATG ACG CTT GTG GAA TGT GTC GTT CAC ATT GTA AGT GAT GTC *GTC AAC* AAT G**A**A CTG ATC TGT TTA CGA AAC AGG TGG GTG AGT GAG AAA CTT TTT AAA GTT CCA TTG ATG AAA GAA AAA AGG-3'

Table S6.

Primer sequences to measure mRNA expression levels, amplify region of interest around FN1 mutation (exon 11), and amplify off-target region.

Region of interest	**Forward primer (5' --> 3')**	**Reverse primer (5' --> 3')**
ACAN	AGAGACTCACACAGTCGAAACAGC	CTATGTTACAGTGCTCGCCAGTG
ADAMTS-5	GTGGTGAAGGTGGTGGTGCT	CTCATGGTCATCTCCCAGCTG
ALPL	CAAAGGCTTCTTCTTGCTGGTG	CCTGCTTGGCTTTTCCTTCA
COL1A1	GTGCTAAAGGTGCCAATGGT	ACCAGGTTCACCGCTGTTAC
COL2A1	CTACCCCAATCCAGCAAACGT	AGGTGATGTTCTGGGAGCCTT
COL10A1	GGCAACAGCATTATGACCCA	TGAGATCGATGATGGCACTCC
FN1	CCGACCAGAAGTTTGGGTTC	CACGACCATTCCCAACACAC
GAPDH	TGCCATGTAGACCCCTTGAAG	ATGGTACATGACAAGGTGCGG
ITGA5	GTCGGGGGCTTCAACTTAGAC	AGCACACTGACCCCGTCTG
ITGA3	TGCCTACAACTGGAAAGGAAAC	CTGCCTACCTGCATCGTGTA
ITGAV	TCTCTCGGGACTCCTGCTAC	AAGAAACATCCGGGAAGACGC
ITGB1	AGCGAAGGCATCCCTGAAAG	AATGTCTACCAACACGCCCT
MCAM	GGAAGTCACCGTCCCTGTTT	ATGCTGAAGTGTGGTGGAGG
PDGRFβ	GGACATACCCCCGCAAAGAA	CTCTCCGTCACATTGCAGGT
SDHA	TGGGAACAAGAGGGCATCTG	GCCTACCACCACTGCATCAA
RUNX2	CTGTGGTTACTGTCATGGCG	AGGTAGCTACTTGGGGAGGA
FN1 (exon 11)	TGGCTTCTCACAGGAAAGTGT	CCAGACGACTACCAATATCGAG
gRNA off-target	AGTGAAGGAGATGTGGCTGC	AAGAGCAACGGGGTGAACAA