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Supplemental Data

A Single Recurrent Mutation in the 5-UTR of *IFITM5* Causes Osteogenesis Imperfecta Type V

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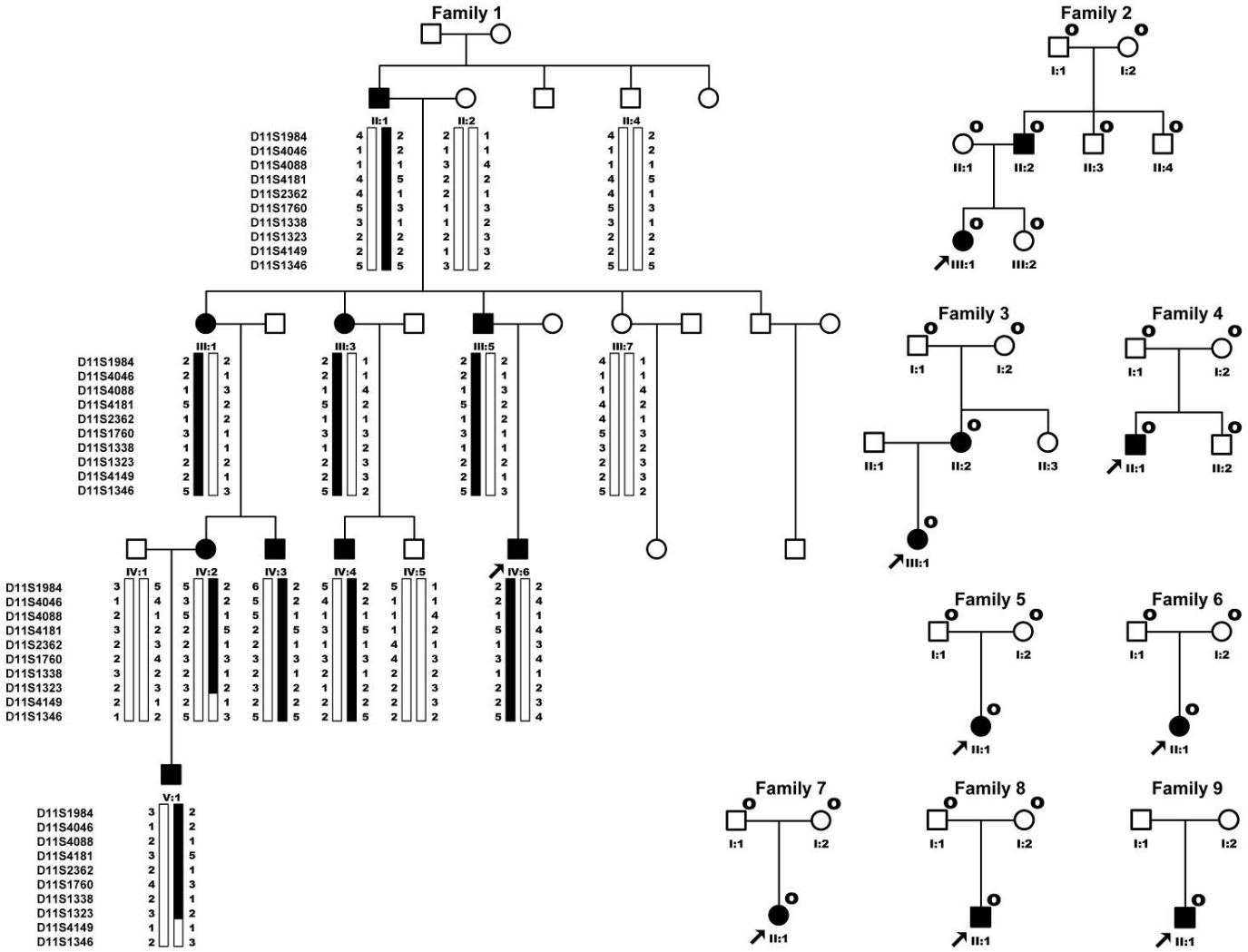

Figure S1. Pedigrees of three familial cases and six simplex individuals. Marker haplotypes on chromosome 11pter-11p15.4 linked to OI type V are indicated by the black bar. In each haplotype pair, paternal haplotypes are to the left and maternal to the right. Mark "**o**" denotes individuals of families 2 to 9 included in this study.

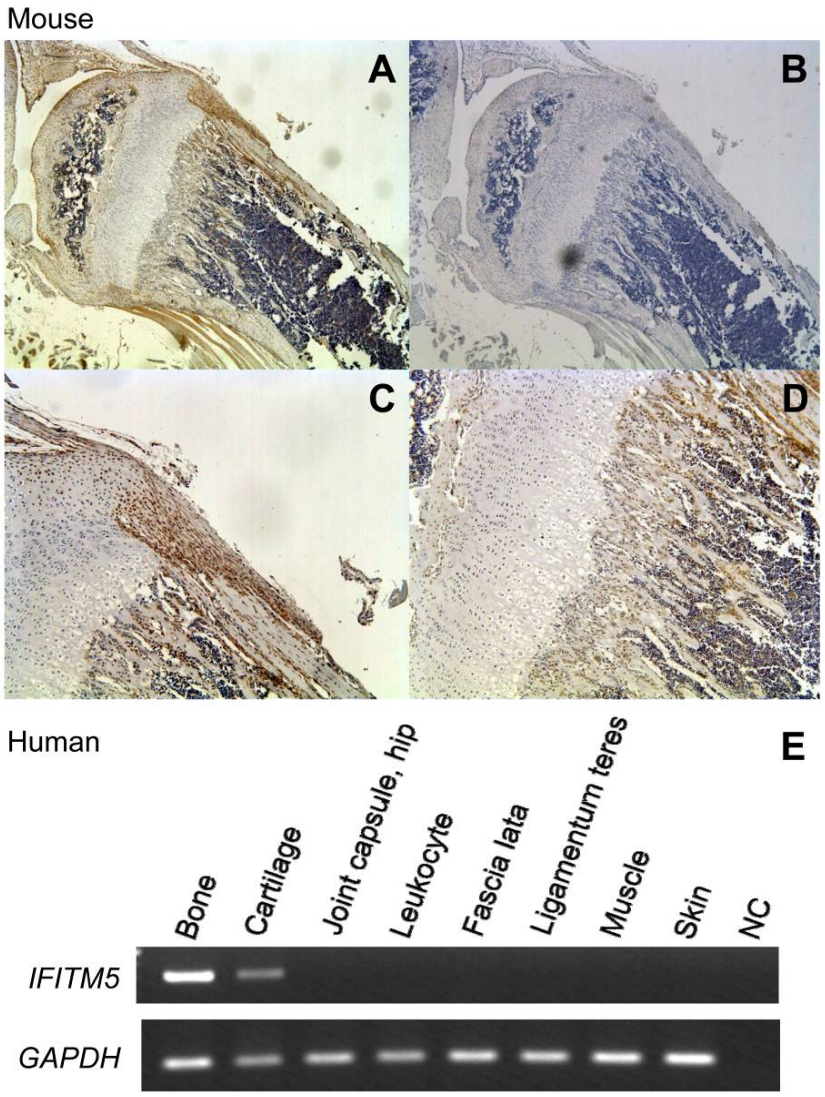

Figure S2. Spatial pattern of the *Ifitm5* expression and protein localization. (A) Immunohistochemical study at the proximal tibia of a 3-week-old BALB/c mouse (x40). (B) Negative control performed without primary antibody (x40). (C) Ifitm5 immunoreactivity was observed at the periphyseal fibro-chondro-osseous structure (the groove of Ranvier and the ring of LaCroix), the superficial layer of articular cartilage, and the deep layer of periosteum (x100). (D) Ifitm5 immunoreactivity was observed at osteoblasts lining the metaphyseal trabeculae and cortical bones, while physeal chondrocytes did not show any immunoreactivity (x100). HRP-conjugated anti-rabbit secondary antibody was used, and the section was counterstained with Mayer's hematoxylin. (E) RT-PCR showed the mRNA expression of *IFITM5* was restricted to bone and cartilage in human tissues. NC: negative control performed without reverse transcription.

Table S1. Two-point LOD scores of the locus associated with family 1.

Marker	Θ at Maximum LOD	Θ						
		0.0	0.05	0.10	0.15	0.20	0.30	0.40
D11S1984	0	1.66	1.53	1.40	1.25	1.08	0.71	0.33
D11S4046	0	2.52	2.34	2.14	1.92	1.68	1.15	0.56
D11S4088	0	1.65	1.54	1.43	1.30	1.16	0.84	0.46
D11S4181	0	2.07	1.91	1.73	1.52	1.31	0.83	0.36
D11S2362	0	1.33	1.19	1.04	0.88	0.71	0.38	0.11
D11S1760	0	1.71	1.57	1.43	1.27	1.10	0.72	0.33
D11S1338	0	1.53	1.41	1.28	1.14	0.99	0.64	0.28
D11S1323	0	0.36	0.34	0.32	0.30	0.27	0.20	0.11
D11S4149	0.2	0.24	0.45	0.53	0.54	0.52	0.38	0.15
D11S1346	0.2	0.12	0.34	0.44	0.47	0.45	0.34	0.14

Table S2. Statistics for exome sequencing.

Exome Capture Statistics	Family 4-II:1	Family 4-I:2	Family 4-I:1	Family 4-II:2
Initial bases on target(bp)	31083979	31083979	31083979	31083979
Total Reads	45981933	56877926	31723002	45836077
Mapped Reads	45701860	56592973	31547247	45590307
Mapping Rate	99.39%	99.50%	99.45%	99.46%
Total effective reads	45519504	56355056	31425950	45371728
Total effective yield(Mb)	4096.76	5071.96	2828.34	4083.46
Read length(bp)	90	90	90	90
Average sequencing depth on target	42.38	52.75	29.24	44.08
Base covered on target(bp)	29558890	29682032	29419875	29633635
Coverage of target region(%)	95.09%	95.49%	94.65%	95.33%
Fraction of target covered with at least 20X	23036590	25111173	19349012	23791778
Fraction of target covered with at least 20X(%)	74.11%	80.78%	62.25%	76.54%
Fraction of target covered with at least 10X	26721632	27653878	25360598	27166414
Fraction of target covered with at least 10X(%)	85.97%	88.97%	81.59%	87.40%
Fraction of target covered with at least 4X	28562051	28927747	28148492	28767057
Fraction of target covered with at least 4X(%)	91.89%	93.06%	90.56%	92.55%

Table S3. Variations unique to the proband in the linked region.

Chrom-osome	Start	End	Referece sequence	Observed sequence	Genotype	Function	Gene	MIM No.
Chr11	299504	299504	G	A	hetero	UTR5	*IFITM5*	
Chr11	574335	574335	G	A	hetero	ncRNA_exonic	*LOC143666*	
Chr11	1253980	1253980	A	G	hetero	exonic	*MUC5B*	600770
Chr11	1687777	1687777	C	-	homo	ncRNA_intronic	*FAM99A*	
Chr11	2720740	2720740	C	G	hetero	ncRNA_exonic	*KCNQ1OT1*	604115
Chr11	5729944	5729947	TTCT	-	hetero	intronic	*TRIM22*	606559
Chr11	8126492	8126492	-	C	hetero	UTR3	*TUB*	601197
Chr11	9111536	9111536	C	T	hetero	intronic	*SCUBE2*	611747

Table S4. Primers used in this study.

Usage	Gene	Direction	Sequence	Amplicon size (bp)	Note
Sanger sequencing	*IFITM5*	F	5'-CCGCAGGCTGTAATTTGTG-3'	452	Exon 1
		R	5'-CCCTCACGGACAAGCAGAG-3'		
	IFITM5	F	5'-AGATTTTGGGTGCAGTAGGG-3'	490	Exon 2
		R	5'-CCTTAGGTGCCCATGTTGG-3'		
	IFITM5	F	5'-TGACGCGGACTATGACTGAC-3'	395	Exon 2
		R	5'-CTGTGGCATTTGGCTTTGG-3'		
Cloning[a]	*IFITM5*	F	5'-GAAGCTTCAGTCTGAGTGTGGAAGAGACG-3'	455	*HindIII*
		R	5'-GGGATCCCCAGCCTGCCAGTCATAGTCCG-3'		*BamHI*
Mutagenesis	*IFITM5*	F	5'-GGCGCTGGAACCCACGGACACGGCGT-3'		
	(c.2T>C)	R	5'-ACGCCGTGTCCGTGGGTTCCAGCGCC-3'		
	IFITM5	F	5'-GTGTGGAAGAGATGGCGCTGGAACCC-3'		
	(c.-14C>T)	R	5'-GGGTTCCAGCGCCATCTCTTCCACAC-3'		
RT-PCR	*IFITM5*	F	5'-TTGATCTGGTCGGTGTTCAG-3'	292	Exon 1-2
		R	5'-GTCAGTCATAGTCCGCGTCA-3'		
	GAPDH	F	5'-ACCGTCAAGGCTGAGAACGGGA-3'	139	Exon 4-6
		R	5'-TGGTGGTGAAGACGCCAGTGGA-3'		

F: forward, R: reverse

[a] *HindIII* and *BamHI* sequences were introduced into the cloned PCR product for subsequent subcloning