Wilson et al., http://www.jcb.org/cgi/content/full/jcb.201007162/DC1

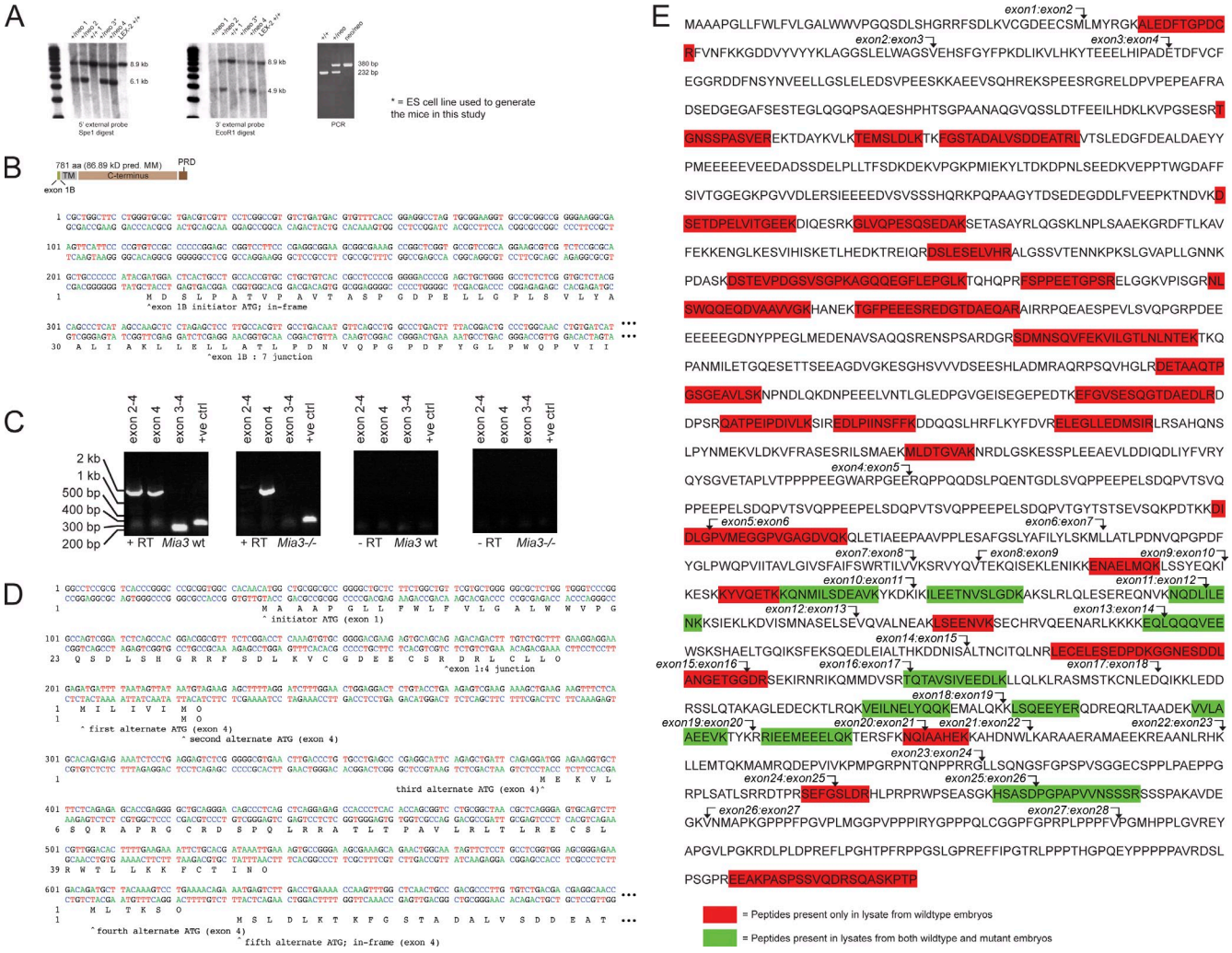


Figure S1. **Characterization of *Mia3* isoforms and validation of targeting vector construction.** (A) Southern blots using 5′ and 3′ probes demonstrate correct targeting of the *Mia3* locus in embryonic stem (ES) cells, and PCR genotyping distinguishes all three resulting genotypes. neo, neomycin. (B) Exon1B–7 fusion transcripts confirmed by RT-PCR contain an in-frame initiator methionine with the potential to produce a truncated Mia3 protein (781 amino acids with a 86.89-kD predicted molecular mass [pred. MM]; deduced amino acid sequence underlies the DNA sequence). The likelihood of this sequence encoding a functional signal peptide is very low (SignalP 3.0 algorithm; ExPASy Server), suggesting that this variant is not inserted into the lumen of the ER. TM, transmembrane domain; PRD, proline-rich domain. (C) RT-PCR electrophoresis using RNA generated from wt and *Mia3*-null embryos confirms deletion of exons 2 and 3 but maintenance of exon4 transcripts in *Mia3* knockouts. (D) Exon1–4 fusion transcripts confirmed by RT-PCR and potential translation products demonstrate an absence of full-length functional Mia3 protein in targeted cells. Nucleotides are color coded for clarity, with the amino acid sequence in black lettering underneath. The three dots represent further downstream sequences. (E) Mass spectrometry of immunoprecipitated Mia3 protein confirms presence of an alternate exon1B promoter and absence of a full-length isoform after gene targeting.

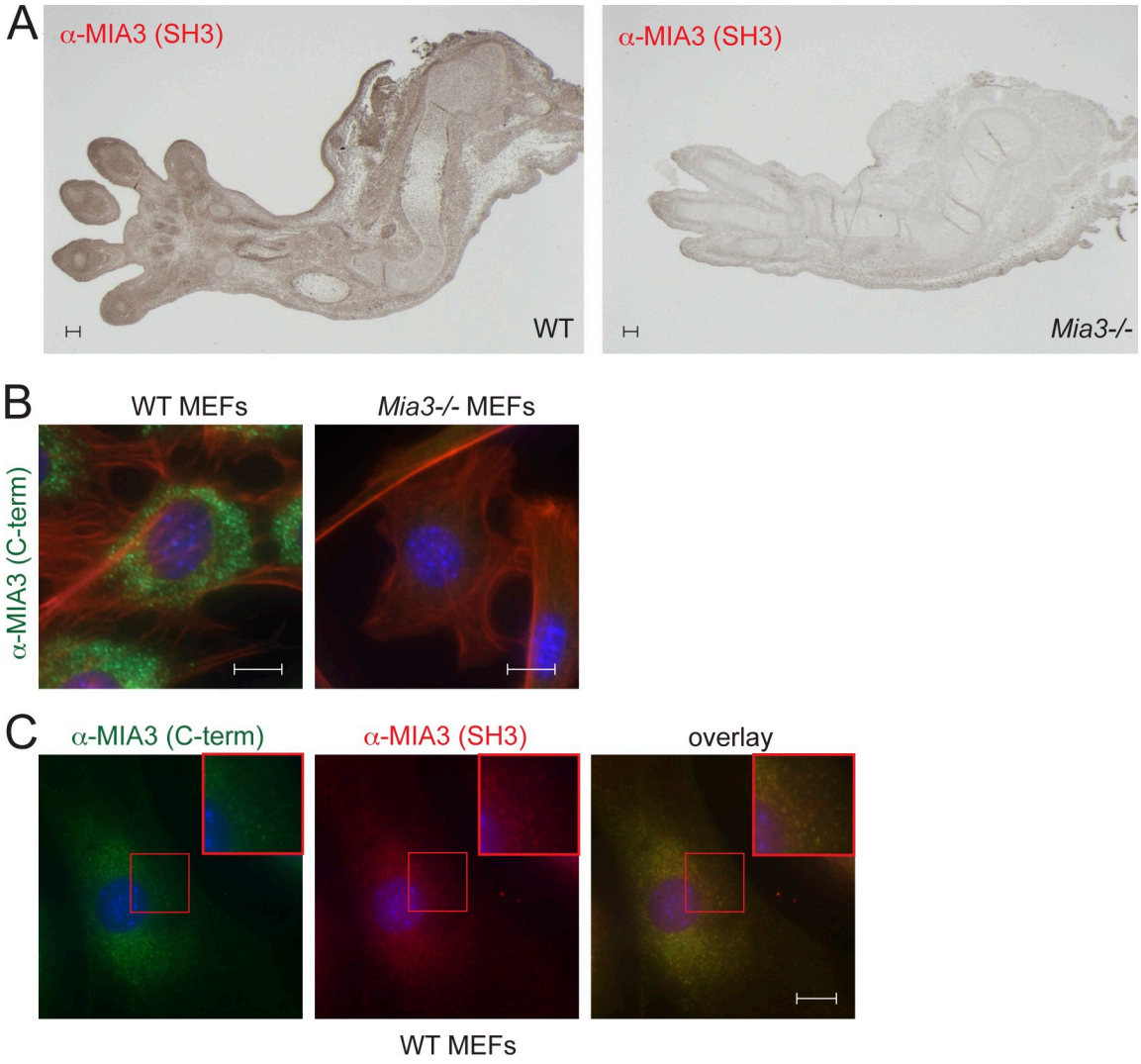


Figure S2. **Validation of α-Mia3 rabbit polyclonal antibodies.** (A) Immunohistochemistry of wt and *Mia3*$^{-/-}$ limbs using polyclonal antibodies directed against the SH3 domain highlights ubiquitous staining in all tissues that is absent from the knockout mouse. (B) α-Mia3 C-terminal (C-term) polyclonal antibody staining is absent in the ER of Mia3-null MEFs. (C) Colocalization analysis in wt MEFs reveals a 1:1 correspondence between α-Mia3 SH3 and α-Mia3 C-terminal antibodies within ER puncta. Boxed regions are enlarged in inset panels. Bars: (A) 100 µm; (B and C) 10 µm.

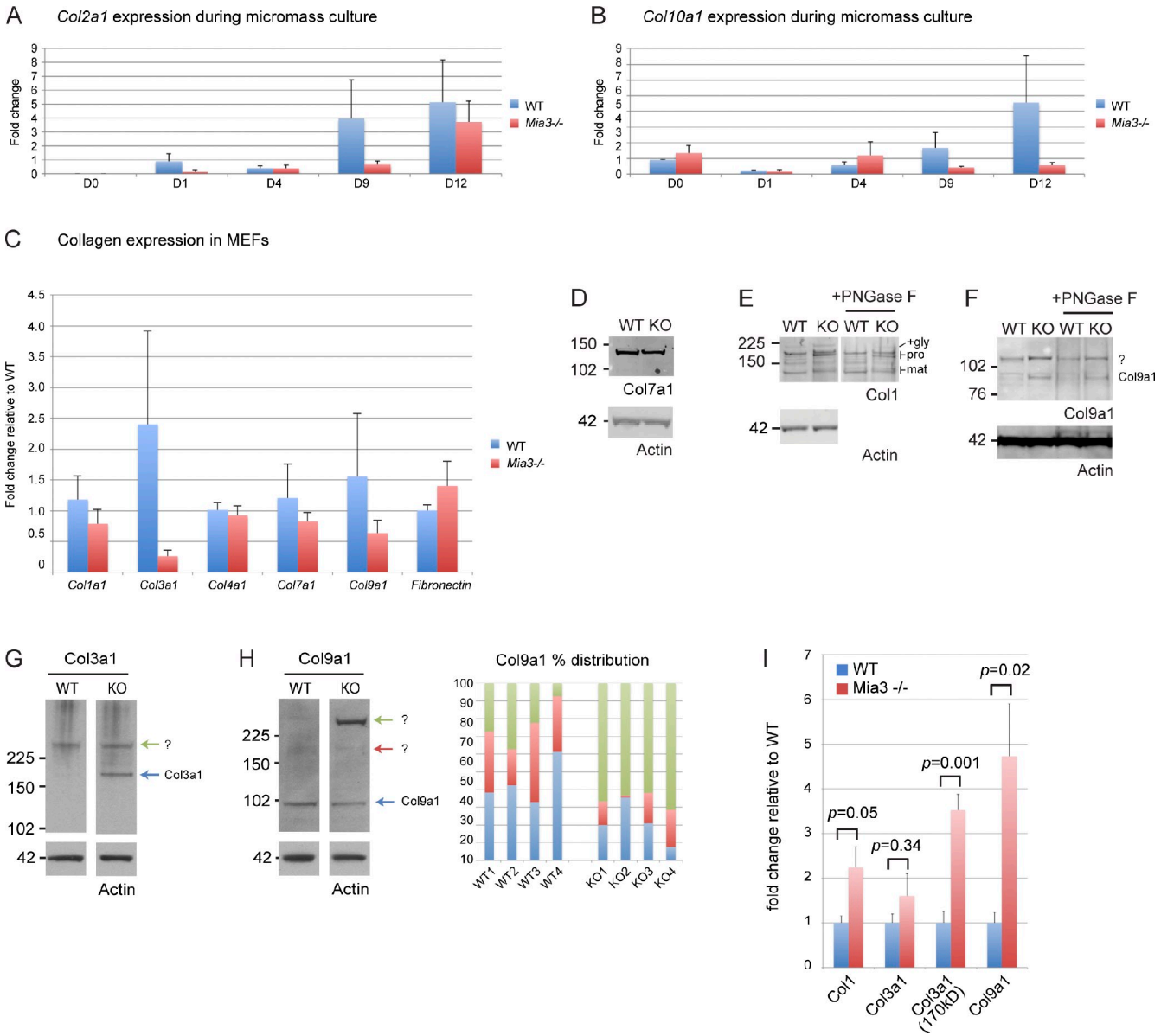


Figure S3. **Ex vivo culture of wt and *Mia3*-null MEFs and chondrocytes reveals a primary requirement for Mia3 in the progression of chondrogenesis and collagen secretion.** (A and B) TaqMan quantitative PCR analysis of *Col2a1* and *Col10a1* from primary MEF micromasses cultured for 12 d under chondrogenic conditions reveals a primary requirement for Mia3 in the timely onset of collagen marker expression. $n = 6$ samples per genotype. (C) TaqMan analysis for multiple collagens and fibronectin in confluent MEFs reveals no significant changes in expression between wt and *Mia3*-null cells before differentiation. Relative fold change is plotted ± SEM. $n = 3$ samples per genotype. (D–F) Col1 and Col9a1 display mobility shifts consistent with altered maturation and/or degradation in knockout (KO) embryonic tissues. Deglycosylation with PNGase F failed to resolve these shifts. +gly, glycosylated form; pro, procollagen; mat, mature collagen. (G and H) Col3a1 and Col9a1 are enriched in *Mia3*-null MEF lysates. Although the identities of the high molecular mass bands remain unresolved, the ∼250-kD Col9a1 band is highly enriched in *Mia3*-null cells (see distribution plot). (I) Quantitation of cell-associated collagen reveals significant enrichment in total Col1 and Col9a1 signal as well as the ∼170-kD Col3a1 isoform in *Mia3*-null MEFs. Values are ± SEM. $n = 4$ per genotype. Molecular masses are given in kilodaltons.

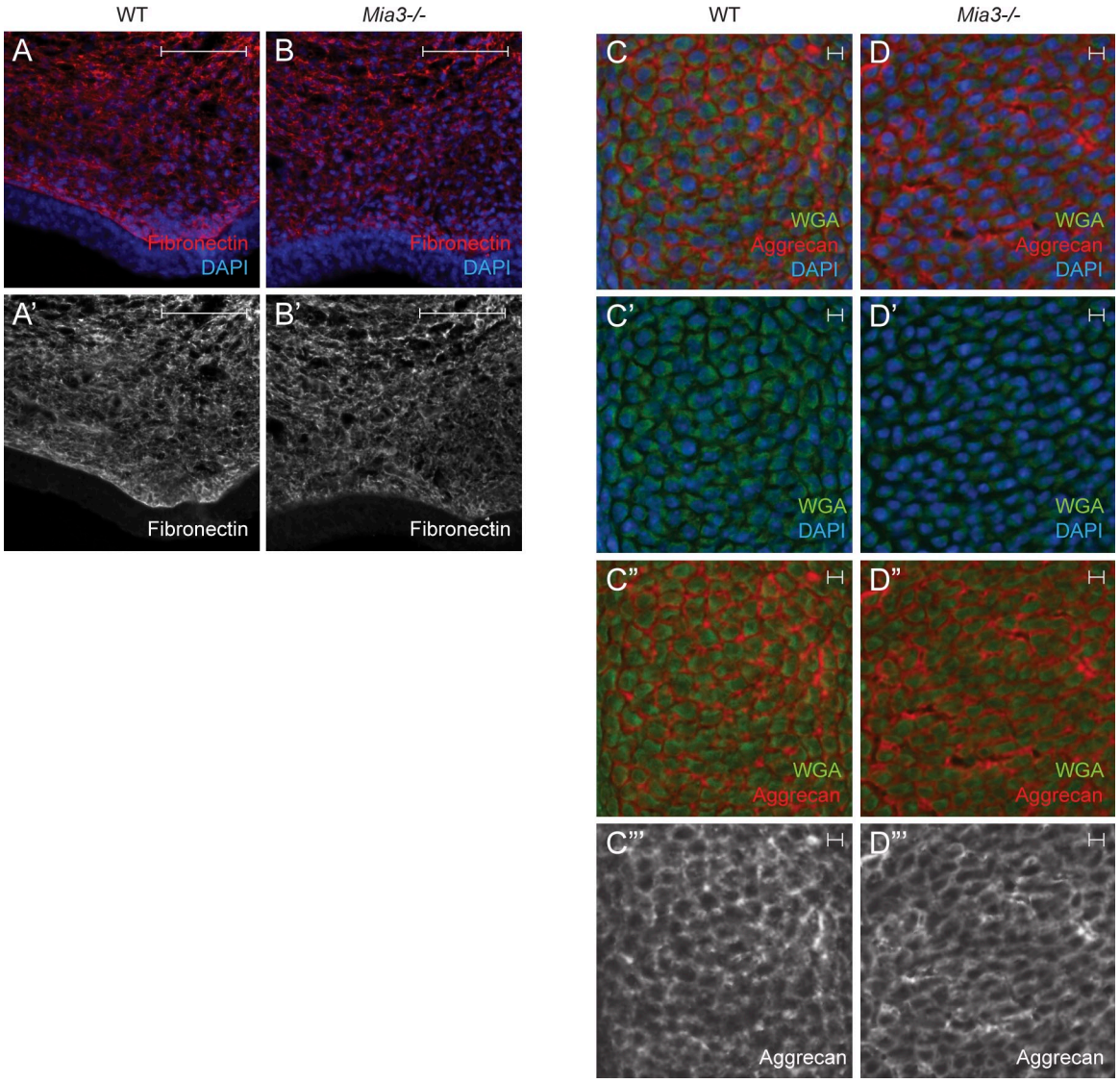
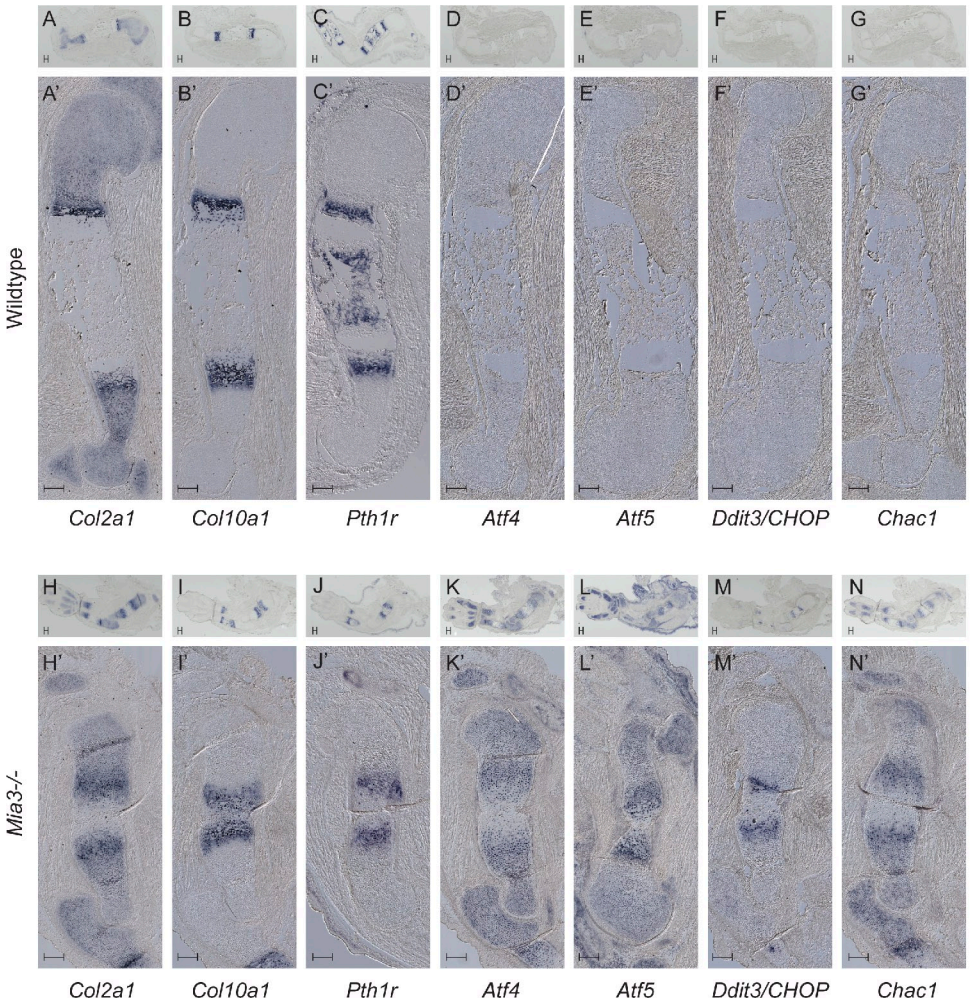


Figure S4. **Fibronectin and aggrecan are deposited normally in the *Mia3*$^{-/-}$ embryo ECM at 14.5 dpc.** (A and B) Fibronectin staining is predominantly extracellular in wt and *Mia3*-null subdermal mesenchyme. (C and D) Costaining of aggrecan, WGA, and DAPI in wt and *Mia3*$^{-/-}$ phalangeal chondrocytes also reveals predominantly extracellular deposition and an absence of intracellular accumulation. Single/double channel immunofluorescence data are denoted by A′–D′. Bars: (A–B′) 100 µm; (C–D′) 10 µm.



O. 16.5 dpc UPR PCR array hits

Gene	Fold change
Ddit3/CHOP	3.99
Atf4	3.08
Serp1	2.79
Herpud1	2.60
Eif2ak3	2.46
H47	1.85
Tor1a	1.63
Sec62	1.60
Mapk9	1.56
Prkcsh	1.55
Os9	1.52
Hspa5/Grp78/Bip	1.48
Sil1	1.45
Rpn1	1.44
Canx	1.41
Nploc4	1.37
Pfdn2	1.37
Edem1	1.34
Syvn1	1.31
Htra2	1.30
Xbp1	1.30
Creb3l3	1.28
Ganab	1.26
Ubxn4	1.22
Nucb1	1.20
Rnf139	1.08

p<0.05 using Student's T-Test
N=3 samples per genotype

Figure S5. **UPR and ERAD genes are highly up-regulated primarily in dermal fibroblasts and chondrocytes of *Mia3*$^{-/-}$ limbs.** (A–N) Section in situ hybridization of 16.5-dpc wt and *Mia3*-null humeri using probes against *Col2a1*, *Col10a1*, *Pth1r*, *Atf4*, *Atf5*, *Ddit3*, and *Chac1* demonstrates sustained UPR activation throughout all chondrocytes. *Ddit3* is up-regulated within the prehypertrophic chondrocytes in correspondence with *Pth1r*. *Atf5* is also significantly up-regulated in the fibroblasts underneath the skin. A′–N′ denote close-ups from humeri shown in A–N. (O) Differential expression of several genes with relevance to UPR. Bars, 100 µm.

Table S1. **Antibodies used in this study**

Antibody	Immunogen	Vendor	Catalog no.	Dilution	Use
Aggrecan	Rat aggrecan	DHSB	12/21/1-C-6	1:50	FFPE
CD-31 (PECAM)	Mouse endothelioma	BD	550274	1:50	CRYO
Collagen I	Mouse type I collagen (skin)	Millipore	AB765P	1:200	CRYO
Collagen II	Human type II collagen peptide	Abcam	ab53047	1:500	CRYO
Collagen II	Human type II collagen peptide	Abcam	ab53047	1:250	WB
Collagen III	Human type III collagen (absorbed against I, II, IV, V, and VI)	SouthernBiotech	1330-08	1:20	CRYO
Collagen IV	Mouse type IV collagen	Cosmo Bio	LB1403	1:10,000	FFPE
Collagen VII	Human type VII peptide	Everest Biotech	EB06781	1:500	CRYO
Collagen VII	Human type VII peptide (C terminus)	Millipore	CBL193	1:200	WB
Collagen IX	Human type IX peptide (middle)	Abcam	ab75807	1:500	CRYO
Collagen IX	Human type IX peptide (middle)	Abcam	ab75807	1:1,000	WB
Fibronectin	Mouse fibronectin	Neomarkers	RB-077-A1	1:200	FFPE
IGFBP6	Mouse Igfbp6	R&D Systems	AF776	1:50	FFPE
Ki67	Human KI67 peptide	Thermo Fisher Scientific	sc-RM-9106-R7	1:100	FFPE
Meca-32	Mouse lymph node stroma	BD	553849	1:200	FFPE
Mia3 C-terminal	Mouse Mia3 peptide	In house	4450	1:500	WB and IP
Mia3 SH3	Mouse Mia3 SH3 domain	In house	4667	1:500	WB and IP
Mmp9	Mouse Mmp9	Abcam	Ab38898	1:500	FFPE
Phospho-CREB	Human CREB phosphopeptide	Cell Signaling Technology	9198	1:50	FFPE
Runx2	Human RUNX2	R&D Systems	AF2006	1:200	FFPE
Sox9	Human SOX9 peptide	Millipore	AB5535	1:100	FFPE
WGA	WGA	Invitrogen	W11262	1:200	CRYO

CRYO, cryoembedded tissue; DHSB, Developmental Studies Hybridoma Bank; FFPE, formalin-fixed, paraffin-embedded tissue; IP, immunoprecipitation; PECAM, platelet/endothelial cell adhesion molecule; WB, Western blot.

Table S2. **In Situ probe DNAs and syntheses**

Clone source	Gene	Antisense polymerase
MGC IRAV no. 6314986	*Collagen 9*	T7
Full-length cDNA	*Chac1*	T7
Full-length cDNA	*Pthr1*	T7
Full-length cDNA	*Tribbles3*	T7
Full-length cDNA	*Ddit3/CHOP*	T7
MGC IRAV no. 30551592	*Atf4*	T3
IMAGE no. 5135000	*Atf5*	T7

MGC IRAV, Mammalian Gene Collection full-length clone; IMAGE, Integrated Molecular Analysis of Genomes Consortium.

Table S3. **Quantitative PCR primer/probe sets**

Gene	Catalog number
Col1a1	Mm00801666_g1
Col3a1	Mm01254476_m1
Col4a1	Mm01210125_m1
Col7a1	Mm00483818_m1
Col9a1	Mm00483834_m1
Fibronectin 1	Mm01256744_m1