Supplemental Tables and Figures

The metalloproteinase-proteoglycans ADAMTS7 and ADAMTS12 provide an innate, tendon-specific protective mechanism against heterotopic ossification

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Supplemental Table 1.

Antibodies used for immunofluorescence and western blots.

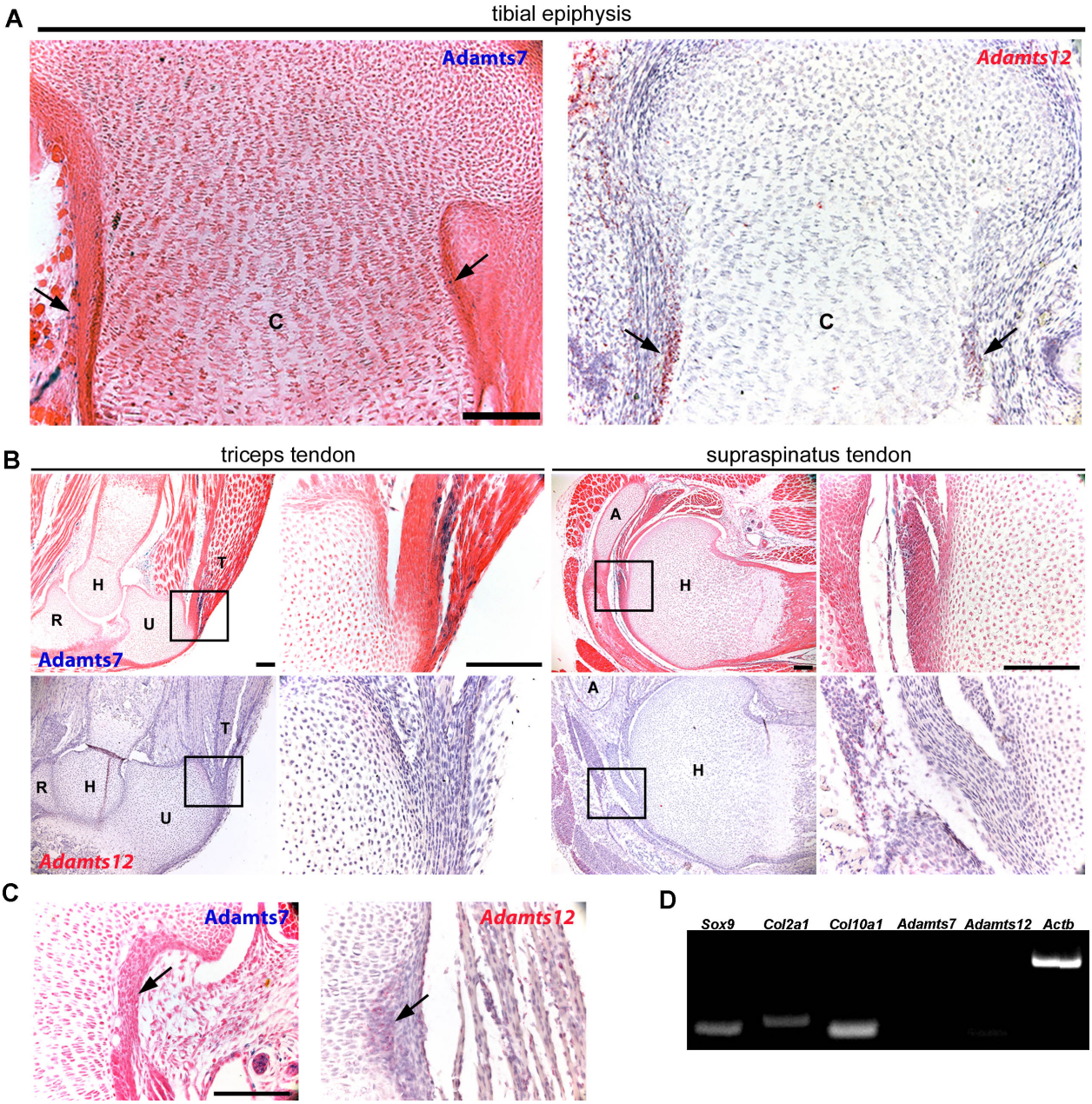
Antibody	Product #	Source	Dilution
anti-biglycan	LF-159	Dr. Larry Fisher, NIH	1:200
anti-fibromodulin	LF-150	Dr. Larry Fisher, NIH	1:200
anti- decorin	LF-114	Dr. Larry Fisher, NIH	1:200
anti-aggrecan	AB1031	EMD-Millipore	1:500
anti-collagen X	ab58632	Abcam	1:1000
anti-pSMAD159	#13820	Cell Signaling	1:200
anti-GAPDH	MAB374	EMD-Millipore	1:5000
anti-ADAMTS7	AB45044	Abcam	1:100
anti-ADAMTS12	24934-1-AP	Proteintech	1:50

Supplemental Table 2.

Quantitative Real-Time PCR primer pairs

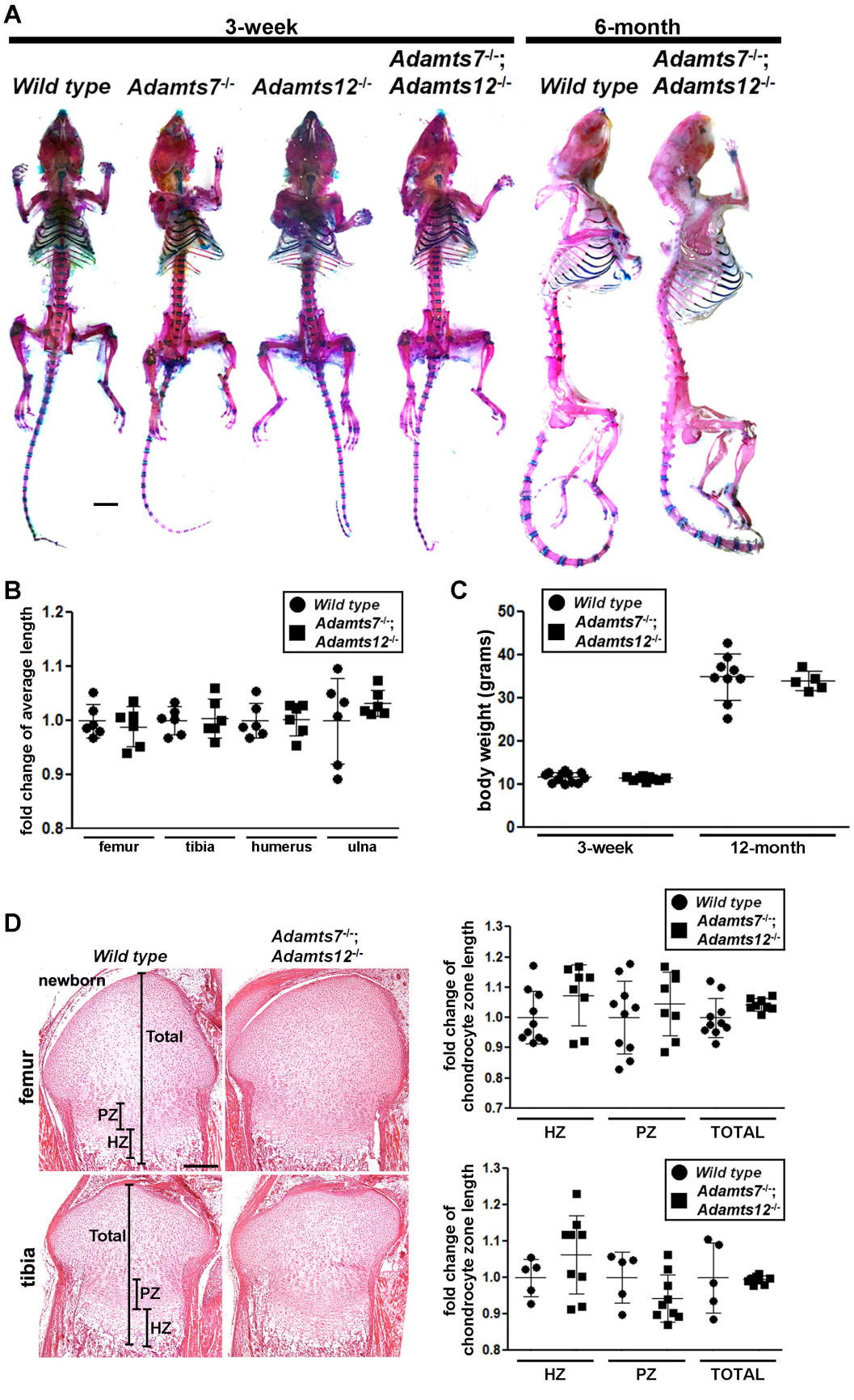
Gapdh	Forward: 5'-TGGAGAAACCTGCCAAGTATGA-3'
	Reverse: 5'-CTGTTGAAGTCGCAGGAGACA-3'
Adamts7	Forward: 5'-GGAGTGAGGACCCAGATAAGTA-3'
	Reverse: 5'-CGTGCATAGGTGAAGGTAGTG-3'
Adamts12	Forward: 5'-CCAAAGGTGCGAGGGATATAAG-3'
	Reverse: 5'-ACCCTCCGTTGAGGTAGTATT-3'
Scx	Forward: 5'-GCACCTTCTGCCTCAGCAAC-3'
	Reverse: 5'-TTCTGTCACGGTCTTTGCTCA-3'
Mkx	Forward: 5'-ACAATCCACACACAGGGCCG-3'
	Reverse: 5'-GGTCTGCCGCCAGCTTTTATC-3'
Tnm	Forward: 5'-CTACAGCAATGGCGAGAAGAAGAAG-3'
	Reverse: 5'-GACCTACAAAGTAGATGCCAGTGTATC-3'
Col1a1	Forward: 5'-GTCCGAGGTCCTAATGGAGATGC-3'
	Reverse: 5'-GGTCCAGGGAATCCGATGT-3'
Col3a1	Forward: 5'-GAGGAATGGGTGGCTATCCT-3'
	Reverse: 5'-GGTATCCAGGAGAACCAGGAG-3'
Acan	Forward: 5'-CTGTCTATCTGCACGCCAACC-3'
	Reverse: 5'-CCTCTTCACCACCCACTCCGA-3'
Col10a1	Forward: 5'-CCAGGACACAATACTTCATCCCATACC-3'
	Reverse: 5'-CCAGGAATGCCTTGTTCTCCTCTTAC-3'
Runx2	Forward: 5'-CCACAGAGCTATTAAAGTGACAGTG-3'

	Reverse: 5'-AACAAACTAGGTTTAGAGTCATCAAGC-3'
Sp7	Forward: 5'-CTCTCCATCTGCCTGACTCC-3'
	Reverse: 5'-CCAAATTTGCTGCAGGCT-3'
Bgn	Forward: 5'-ATTGCCCTACCCAGAACTTGAC-3'
	Reverse: 5'-GCAGAGTATGAACCCTTTCCTG-3’
Fmod	Forward: 5'-CAAGGCAACAGGATCAATGAG-3'
	Reverse: 5'-CTGCAGCTTGGAGAAGTTCA-3'
Dcn	Forward: 5'-GACTCCACGACAATGAGATCACC-3'
	Reverse: 5'-GTTGCCATCCAGATGCAGTTC-3'
ADAMTS7	Forward: 5'-CTTCTGCGAGGACATGGATAAT-3'
	Reverse: 5'-CCCACTGAGACACCACTTATTC-3'
ADAMTS12	Forward: 5'-TGGGAAACAGTGGCAAGATAG-3'
	Reverse: 5'-TGCTCAAGGATTGGGAAGTG-3'
BGN	Forward: 5'-AACTAGTCAGCCTGCGCCT-3'
	Reverse: 5'-GTCCCAGAAGCCTCTCTGCT-3'
FMOD	Forward: 5'-AGCAGCCTCCTTGAGCTAGA-3'
	Reverse: 5'-CAGAAGCTGCTGATGGAGAA-3'
DCN	Forward: 5'-AATGCCATCTTCGAGTGGTC-3'
	Reverse: 5'-AGCAATGCGGATGTAGGAGA-3'
GAPDH	Forward: 5'-AGCCTCAAGATCATCAGCAATG-3'
	Reverse: 5'-CTTCCACGATACCAAAGTTGTCAT-3'

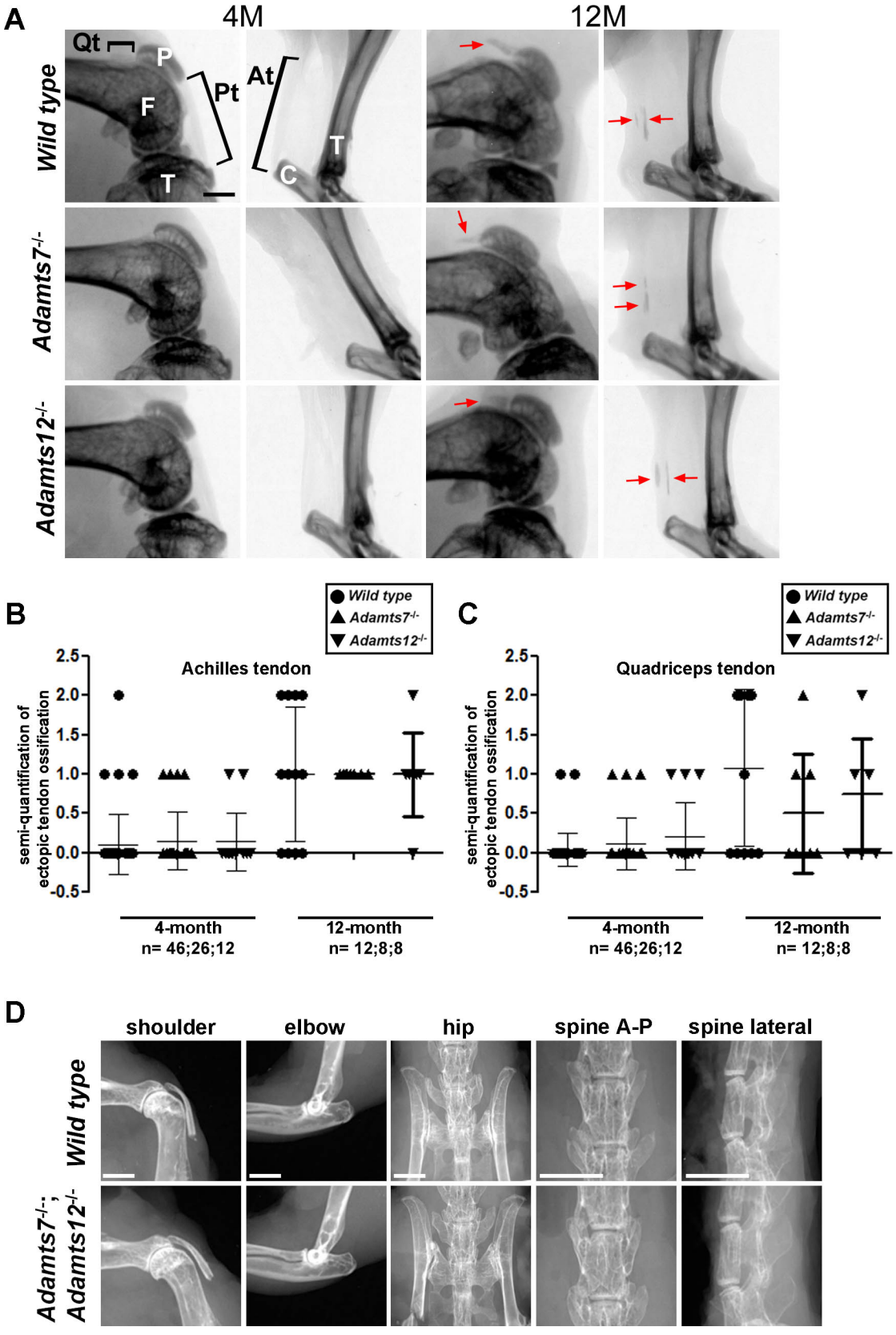


Supplemental Figure 1. *Adamts7* and *Adamts12* are not expressed in growth plate cartilage or forelimb tendons. (A) 18.5 day-old embryonic tibia shows lack of *Adamts7* (β-gal staining, blue nuclei, eosin counterstain is red) and *Adamts12* expression (RNA in situ hybridization (red signal) in the growth plate cartilage (C), but both are expressed in the perichondrial groove of Ranvier (arrows). The data are representative of n=5. **(B)** 18.5 day-old embryo forelimbs show sparse *Adamts7* (β-gal staining, blue nuclei, eosin counterstain is red) and *Adamts12* expression (RNA in situ hybridization (red signal)) respectively in triceps and supraspinatus tendons. The data are representative of n=6. R; radius; H, humerus; U, ulna; T, triceps; A, acromion. **(C)** 18.5 day-old mouse embryo humeral head shows *Adamts7* and *Adamts12* expression in the perichondrial groove of Ranvier (arrows). The data are representative of n=4. **(D)** RT-PCR of

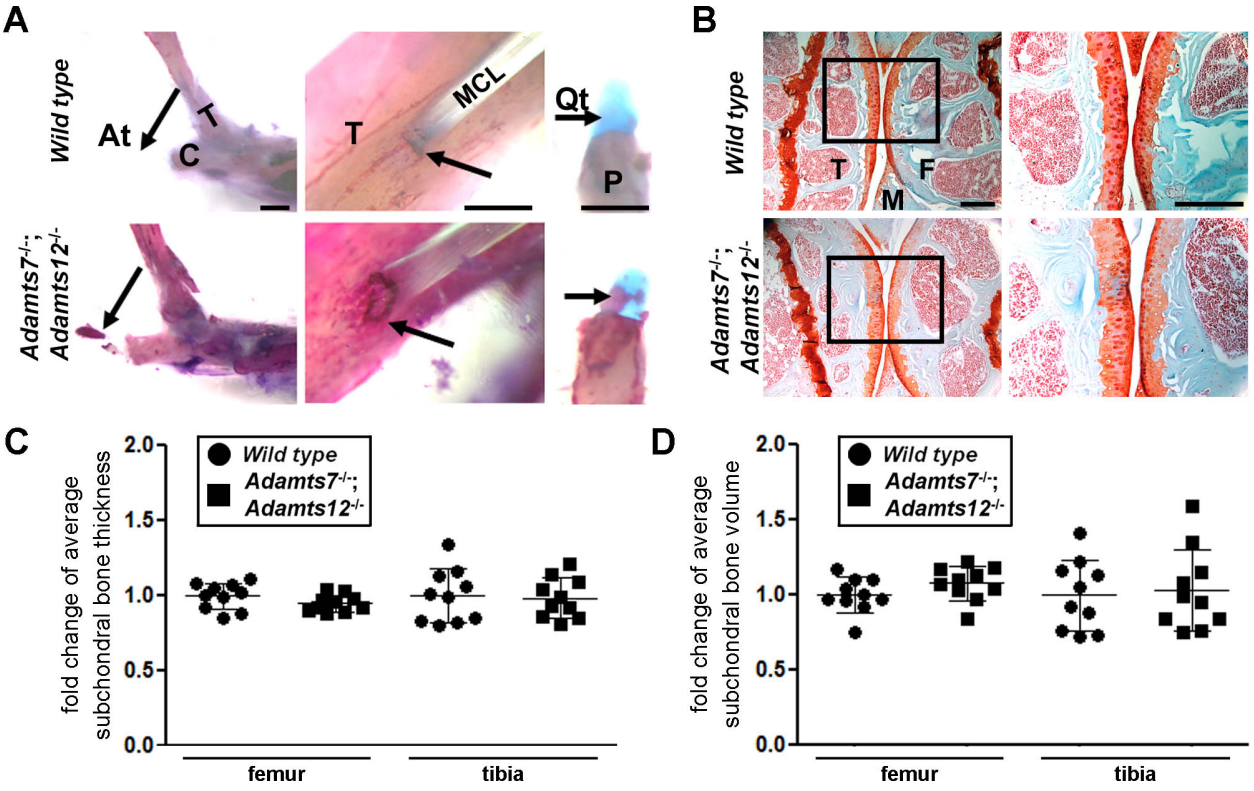
18.5 day-old embryo distal femoral cartilage reveals expression of chondrocyte markers *Sox9*, *Col2a1* and *Col10a1*, but neither *Adamts7* nor *Adamts12*. n=3. Scale bars: 100μm.


A
3-week
6-month
Wild type
Adamts7-/-
Adamts12-/-
Adamts7-/-; Adamts12-/-
Wild type
Adamts7-/-; Adamts12-/-
B
fold change of average length
1.2
1.1
1.0
0.9
0.8
femur
tibia
humerus
ulna
Wild type
Adamts7-/-; Adamts12-/-
C
body weight (grams)
50
40
30
20
10
0
3-week
12-month
Wild type
Adamts7-/-; Adamts12-/-
D
Wild type
Adamts7-/-; Adamts12-/-
newborn
femur
tibia
Total
PZ
HZ
fold change of chondrocyte zone length
1.3
1.2
1.1
1.0
0.9
0.8
0.7
HZ
PZ
TOTAL
Wild type
Adamts7-/-; Adamts12-/-

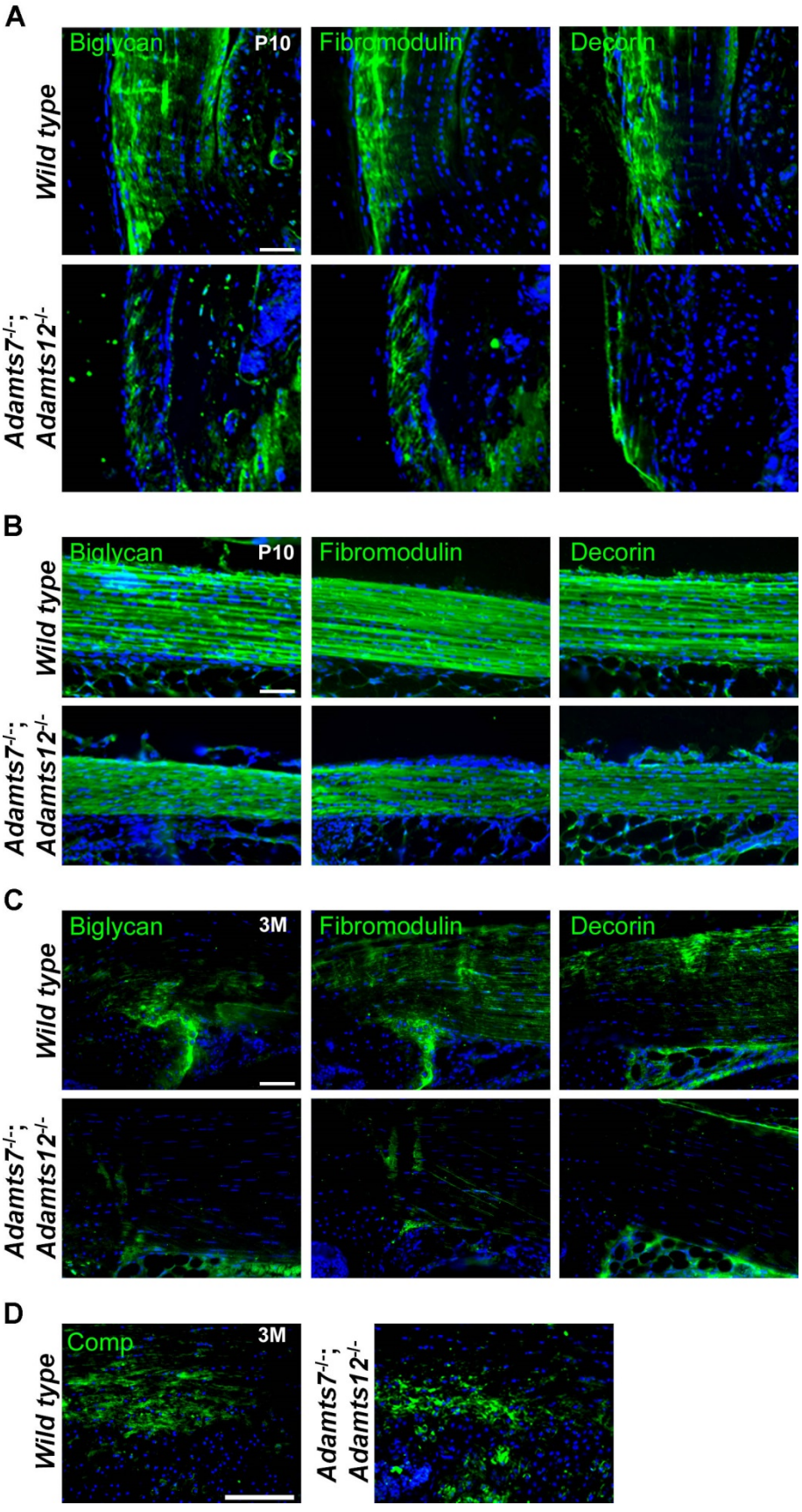
Supplemental Figure 2. *Adamts7*$^{-/-}$;*Adamts12*$^{-/-}$ mice have normal skeletal patterning and growth. (A) Alizarin red-Alcian blue stained skeletal preparations of 3 week-old wild type, *Adamts7*$^{-/-}$, *Adamts12*$^{-/-}$ and *Adamts7*$^{-/-}$;*Adamts12*$^{-/-}$ mice reveals no skeletal patterning, structural or growth abnormalities. Data are representative of n=5. At 6 months of age, Alizarin red-Alcian blue stained skeletal preparations show that *Adamts7*$^{-/-}$;*Adamts12*$^{-/-}$ mice have normal skeletal dimensions and maturity. Data are representative of n=6. **(B)** No difference was observed in the femoral, tibial, humeral and ulnar length in 6 month-old mice. n=6. **(C)** Comparable body weights of wild type and *Adamts7*$^{-/-}$;*Adamts12*$^{-/-}$ mice at 3 weeks and 12 months of age. n=12 at 3 weeks, n=9 at 12 months. **(D)** Representative H&E-stained sections of newborn femur and tibia showing no change in growth plate dimensions. HZ, hypertrophic zone; PZ, pre-hypertrophic zone; Total, total growth plate. Femur: n=10, 8, respectively. Tibia: n=5, 9, respectively. Scale bars: 1cm in A; 200μm in D. Error bars represent ± SEM. Significance was determined by the Student *t*-test.


A
4M
12M
Wild type
Adamts7-/-
Adamts12-/-
Qt
P
F
T
Pt
At
T
C
B
Wild type
Adamts7-/-
Adamts12-/-
Achilles tendon
semi-quantification of ectopic tendon ossification
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
4-month
n= 46;26;12
12-month
n= 12;8;8
C
Wild type
Adamts7-/-
Adamts12-/-
Quadriceps tendon
semi-quantification of ectopic tendon ossification
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
4-month
n= 46;26;12
12-month
n= 12;8;8
D
shoulder
elbow
hip
spine A-P
spine lateral
Wild type
Adamts7-/-; Adamts12-/-

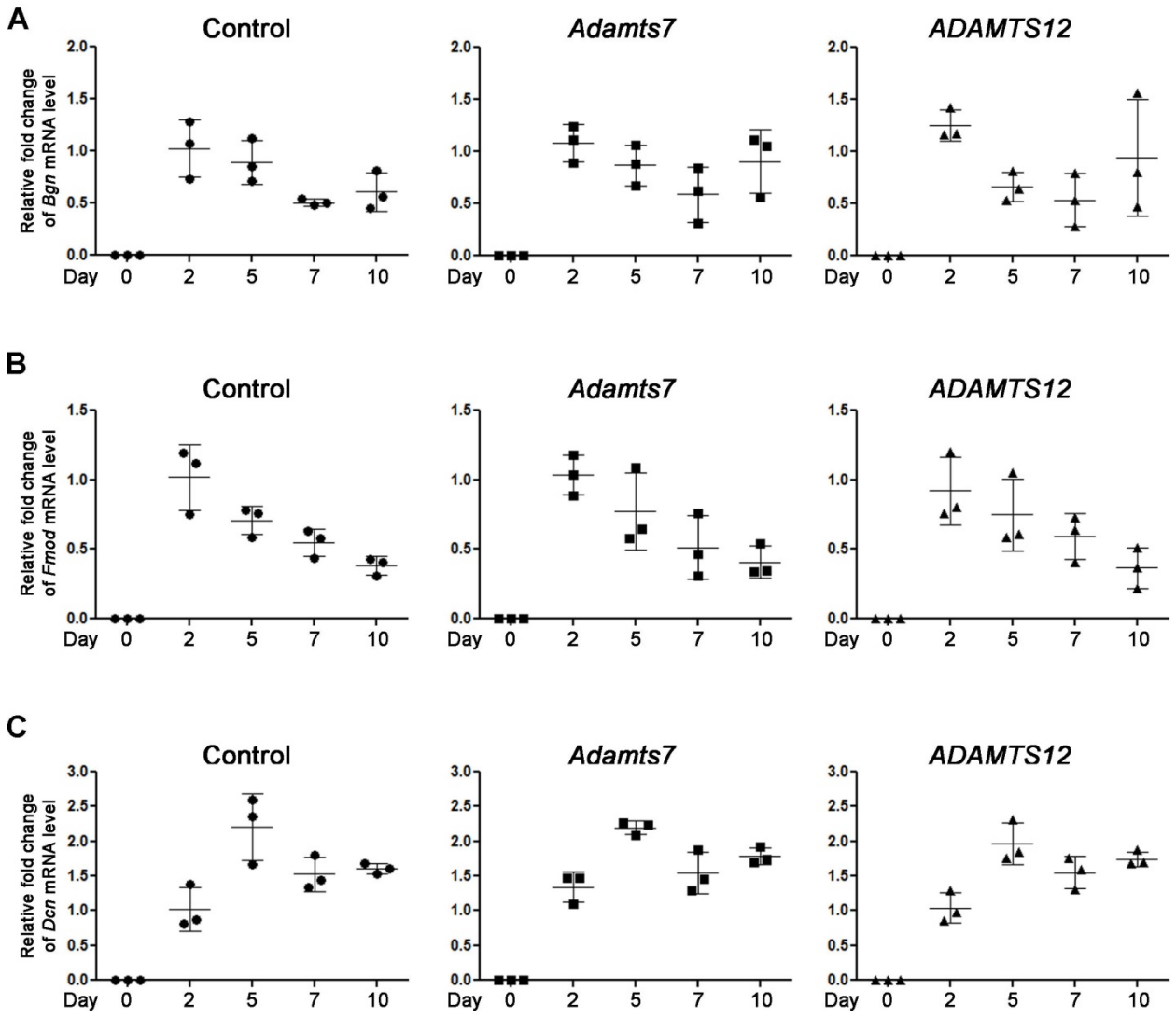
Supplemental Figure 3. Absence of ectopic ossification in *Adamts7*$^{-/-}$ and *Adamts12*$^{-/-}$ mice. **(A)** Lateral radiographs of 4 month- and 12 month-old hindlimbs reveal no radiopacities in *Adamts7*$^{-/-}$, *Adamts12*$^{-/-}$ and wild type tendons at 4 months. At 12 months, wild type, *Adamts7*$^{-/-}$ and *Adamts12*$^{-/-}$ mice have minimal radiopacity (red arrows) within the quadriceps and Achilles tendon. P, patella; F, femur; T, tibia; C, calcaneus, At, Achilles tendon; Pt patellar tendon. **(B-C)** No progression of radiopacity in *Adamts7*$^{-/-}$ and *Adamts12*$^{-/-}$ Achilles and quadriceps tendons as compared to control. **(D)** No soft tissue radiopacities are evident in forelimb, pelvis and spine tendons in 18 month-old wild type and *Adamts7*$^{-/-}$;*Adamts12*$^{-/-}$ mice. Data are representative of n=6. Scale bars: 1 mm. Error bars represent ± SEM. Significance was determined by the Student *t*-test.



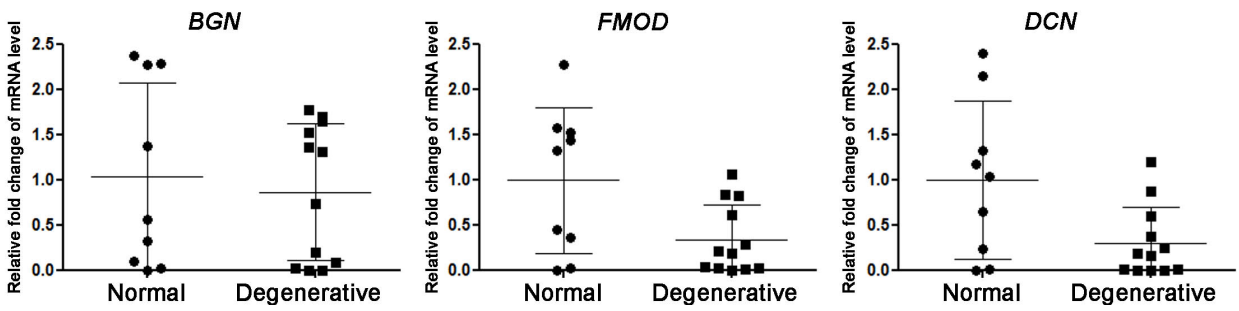
Supplemental Figure 4. Heterotopic ossification, no arthritic change and normal subchondral bone in *Adamts7*$^{-/-}$;*Adamts12*$^{-/-}$ hindlimbs. (A) Alizarin red-alcian blue stained skeleton preparations of 6 month-old wild type and *Adamts7*$^{-/-}$;*Adamts12*$^{-/-}$ hindlimbs reveal ectopic ossification in Achilles tendon (At), medial collateral ligament (MCL) and quadriceps tendon (qt). Data are representative of n=8. **(B)** Safranin O stained 18 month-old *Adamts7*$^{-/-}$;*Adamts12*$^{-/-}$ hindlimbs shows normal articular cartilage on the femoral and tibial surfaces. M, meniscus; F, femur; T, tibia; P, patella. Images are representative of n=5. **(C-D)** No change in subchondral bone thickness (C) or volume (D) of 18 month-old wild type and *Adamts7*$^{-/-}$;*Adamts12*$^{-/-}$ femur and tibia. n=10. Scale bars: 1 mm in A; 200μm in B. Error bars represent ± SEM. Significance was determined by the Student *t*-test.


A
Biglycan
P10
Fibromodulin
Decorin
Wild type
Adamts7-/-; Adamts12-/-
B
Biglycan
P10
Fibromodulin
Decorin
Wild type
Adamts7-/-; Adamts12-/-
C
Biglycan
3M
Fibromodulin
Decorin
Wild type
Adamts7-/-; Adamts12-/-
D
Comp
3M
Wild type
Adamts7-/-; Adamts12-/-

Supplemental Figure 5. Reduction of small leucine-rich proteoglycans in *Adamts7*$^{-/-}$;*Adamts12*$^{-/-}$ patellar tendons. (A-B) Reduced staining of biglycan, decorin and fibromodulin (green) in 10 day-old patellar tendons near the insertion site **(A)** and mid-substance **(B)**. Sections were counterstained with DAPI (blue). Data are representative of n=3. **(C)** Reduced biglycan, decorin and fibromodulin (green) staining in 3-month-old (3M) *Adamts7*$^{-/-}$;*Adamts12*$^{-/-}$ patellar tendons. Data are representative of n=3. **(D)** No change in cartilage oligomeric matrix protein (Comp) (green) staining in 3-month-old (3M) *Adamts7*$^{-/-}$;*Adamts12*$^{-/-}$ quadriceps tendons. Sections were counterstained with DAPI (blue). Data are representative of n=3. Scale bars: 100μm.



Supplemental Figure 6. No change in small leucine-rich proteoglycan mRNA in 10T1/2 pellet cultures. (A-C) qRT-PCR reveals no significant reduction of *Bgn*, *Fmod* or *Dcn* mRNA in differentiated 10T1/2 cells subjected to chondrogenic culture conditions as compared to control. n=3. Error bars represent ± SEM. Significance was determined by a Student *t*-test.



Supplemental Figure 7. No change in small leucine-rich proteoglycan mRNA in degenerative adult human biceps tendons. qRT-PCR reveals no significant change of *BGN*, *FMOD* or *DCN* mRNA in degenerative samples. n=9, 12 respectively. Error bars represent ± SEM. Significance was determined by a Student *t*-test.