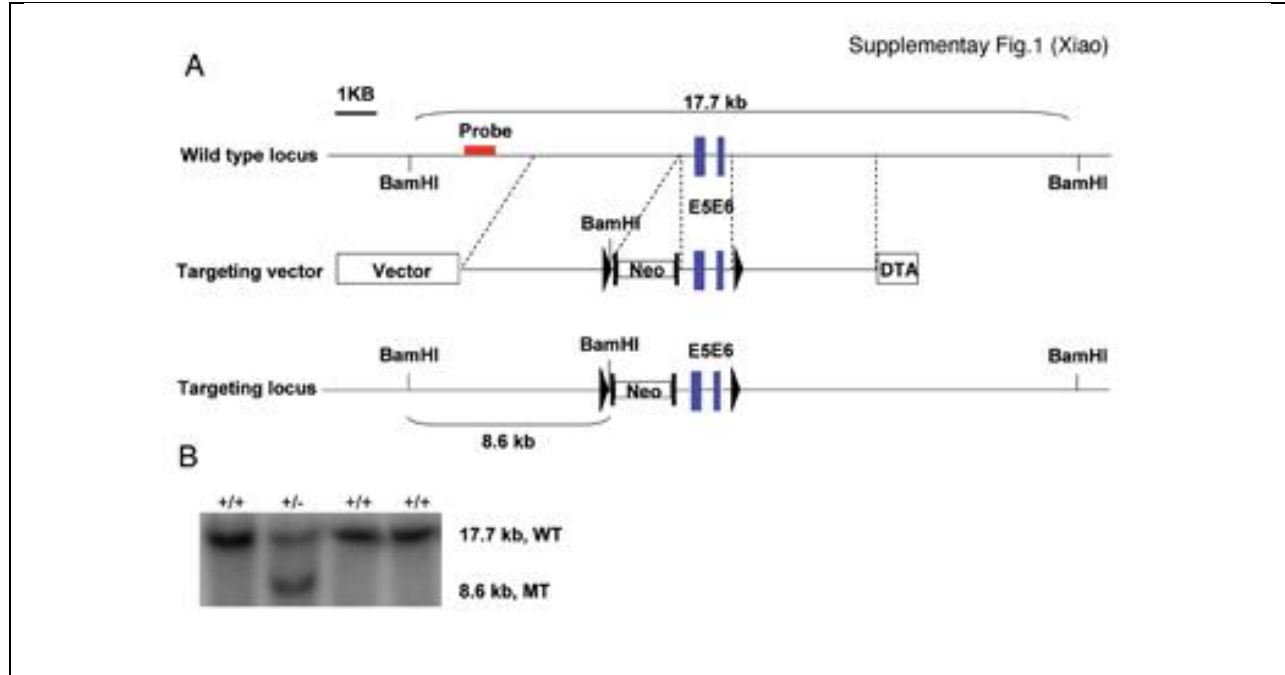
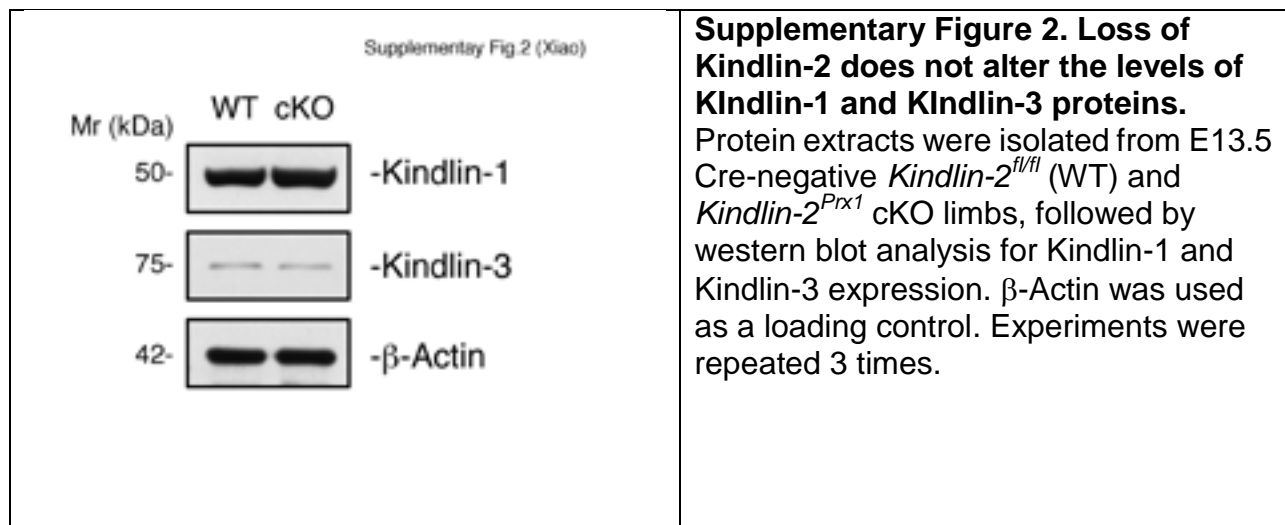


SUPPLEMENTARY INFORMATION

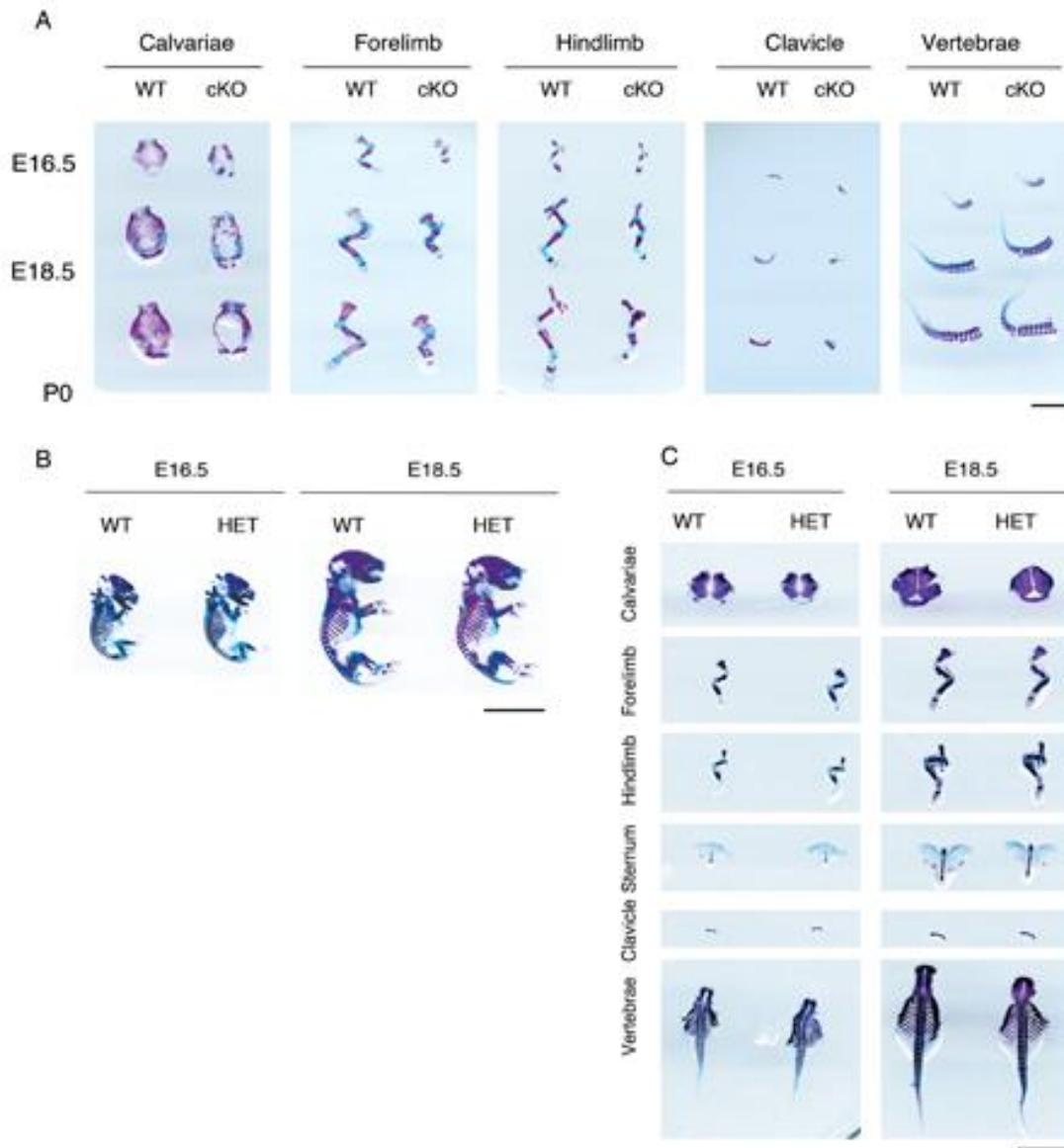


Supplementary Figure 1. Generation of a conditional allele of the *Kindlin-2* gene.

(A) A restriction map of the relevant genomic region of *Kindlin-2* (top), the targeting construct (middle), and the mutated locus after recombination (bottom) are shown. The blue rectangles indicate the targeted exons 5 and 6; black triangles indicate *LoxP* sites, and blue rectangles indicate *frt* sites; DTA, Diphtheria Toxic A chain gene; Neo, Neomycin resistance gene. (B) Detection of WT and mutated alleles (MT). DNA from electroporated embryonic stem (ES) cells was digested with *Bam*HI and analyzed by Southern blot with the probe shown in A. The 17.7 kb and 8.6 kb bands represent WT and mutated alleles (MT), respectively.

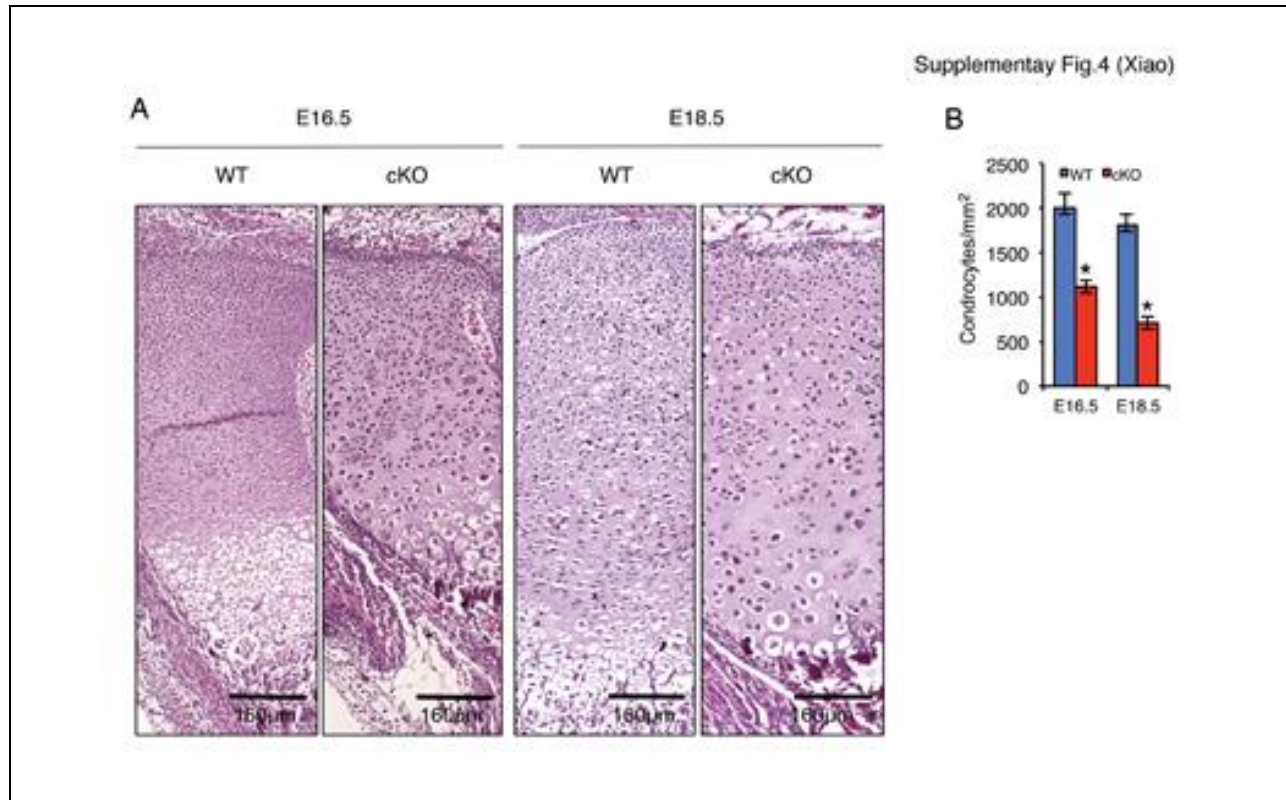


Supplementary Fig. 3



Supplementary Figure 3. Staining of *Kindlin-2^{Prx1} cKO*, *Prx1-Cre; Kindlin-2^{fl/+}*, and WT skeletons. (A) Alizarin red and alcian blue double stain of *Kindlin-2^{Prx1} cKO* (cKO) and Cre-negative *Kindlin-2^{fl/fl}* (WT) mice showing lack of skull vault, shortened and broadened limbs and sternum, hypoplastic clavicle, and impaired elongation of distal

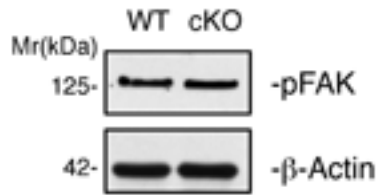
digits in *Kindlin-2^{Prx1}* cKO mice. N=5. Scale bar: 0.5 cm. **(B)** Alizarin red and alcian blue double stain of E16.5 and E18.5 *Prx1-Cre; Kindlin-2^{fl/+}* (HET) and WT skeletons, showing no marked differences between the two genotypes. N=5. Scale bar: 1 cm. **(C)** Calvaria, forelimb, hindlimb, sternum, and clavicle from E16.5 and E18.5 *Prx1-Cre; Kindlin-2^{fl/+}* (HET) and WT embryos. N=5. Scale bar: 1 cm.



Supplementary Figure 4. Reduced cellularity, abnormal cell morphology, and disrupted column formation in *Kindlin-2^{Prx1}* cKO tibial growth plates.

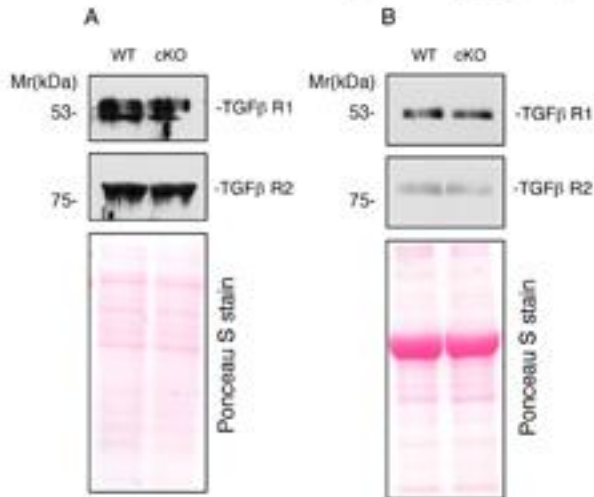
(A) Hematoxylin staining of E16.5 and E18.5 tibiae from of *Kindlin-2^{Prx1}* cKO (cKO) and Cre-negative *Kindlin-2^{fl/fl}* (WT) mice. Representative images from each group are shown. Original magnification: 100x. Scale bar: 160 μm. **(B)** Quantitation of chondrocyte densities of E16.5 and E18.5 tibial growth plates. N=4, *p<0.05, versus WT, Student's *t*-test. Results were expressed as mean ± standard deviation.

Supplementary Fig.5 (Xiao)



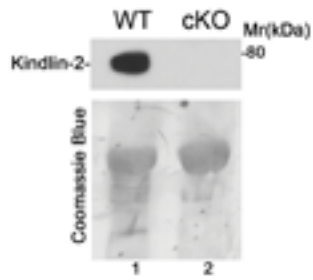
Supplementary Figure 5. Loss of Kindlin-2 does not reduce the level of pFAK protein in limbs. Protein extracts were isolated from E13.5 Cre-negative *Kindlin-2^{fl/fl}* (WT) and *Kindlin-2^{Prix1}* cKO limbs, followed by western blot analysis for Kindlin-1 and Kindlin-3 expression. beta-Actin was used as a loading control. Experiments were repeated 3 times.

Supplementary Fig.6 (Xiao)



Supplementary Figure 6. Loss of Kindlin-2 does not reduce the levels of TGF-beta R1 and TGF-beta R2 proteins. Protein extracts were isolated from limbs (A) or primary limb mesenchymal progenitors (B) of E13.5 Cre-negative *Kindlin-2^{fl/fl}* (WT) and *Kindlin-2^{Prix1}* cKO mice, followed by western blot analysis for TGF-beta R1 (top) and TGF-beta R2 (middle) expression. Ponceau S stain of the blots immediately after transferring (bottom). Experiments were repeated 3 times.

Supplementary Fig. 7 (Xiao)



Supplementary Figure 7. Kindlin-2 protein is essentially abolished in primary mesenchymal progenitors from E12.5 *Kindlin-2^{Prx1}* cKO limbs.

Protein extracts were isolated from primary mesenchymal progenitors of E12.5 Cre-negative *Kindlin-2^{fl/fl}* (WT) and *Kindlin-2^{Prx1}* cKO limbs, followed by western blot analysis for Kindlin-2 expression. Experiments were repeated 3 times.

Figure 1B

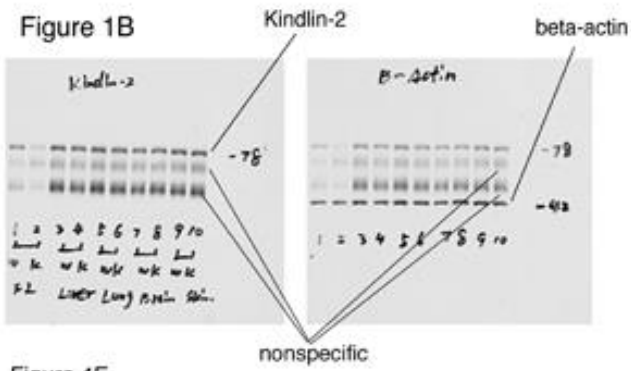


Figure 4C

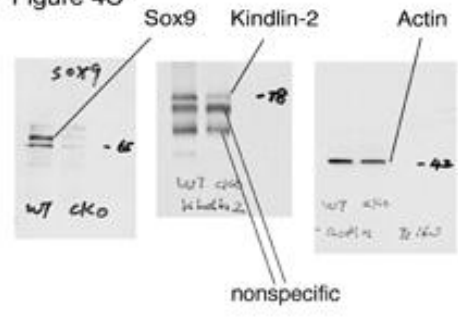


Figure 4E

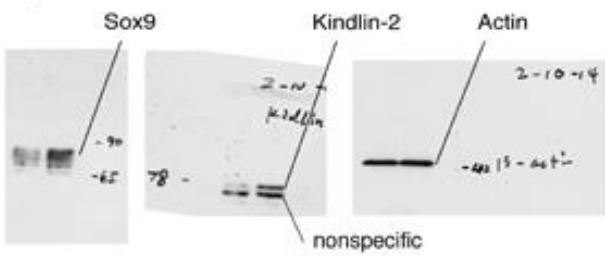


Figure 4I

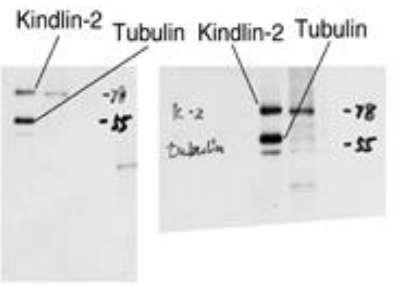
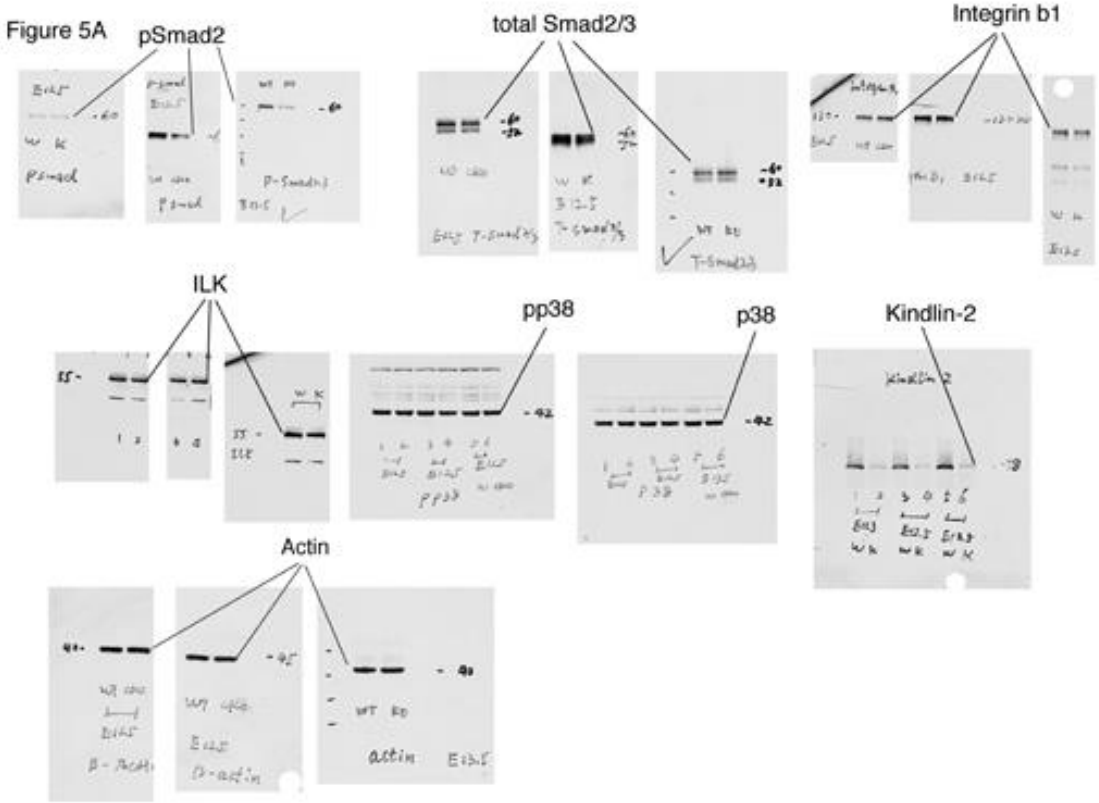
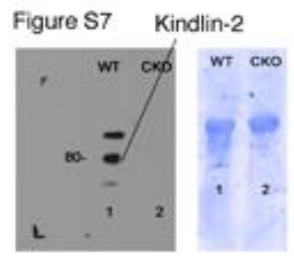
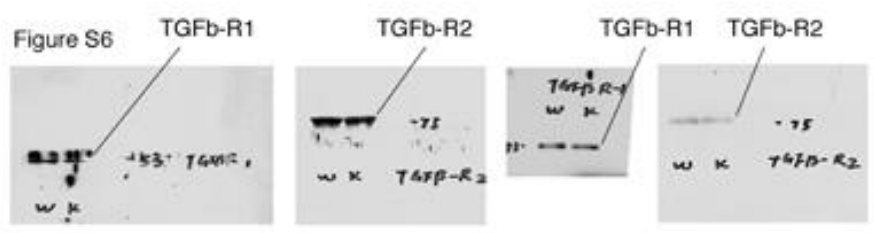
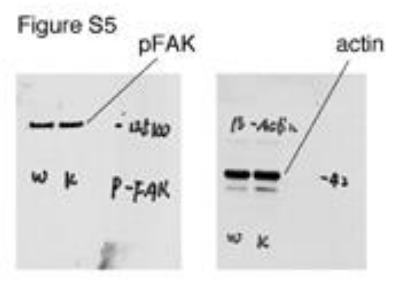
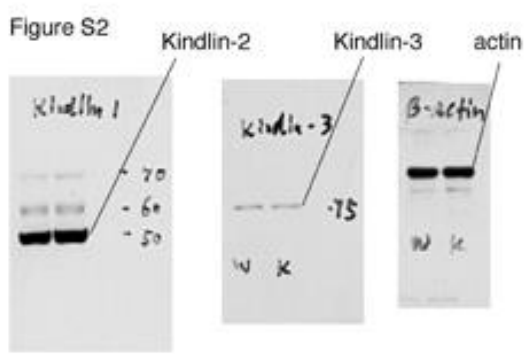
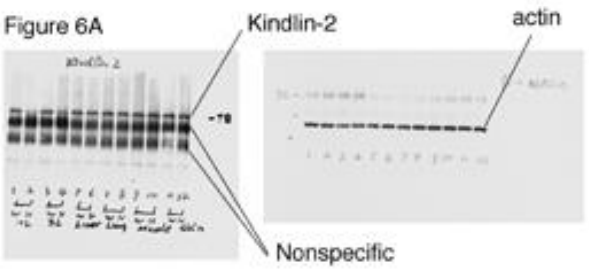


Figure 5A



Supplementary Figure 8. Western blots (1)



Supplementary Figure 9. Western blots (2)

Supplementary Table 1 : Real-time Polymerase Chain Reaction (PCR) Primers

Name	5' primer	3' primer
<i>Actin</i>	TCCTCCTGAGCGCAAGTACT CT	CGGACTCATCGTACTCCTGCT T
<i>Aggrecan</i>	AGGACCTGGTAGTGCGAGTG	GCGTGTGGCGAAGAA
<i>Cadherin</i>	AGCGCAGTCTTACCGAAGG	TCGCTGCTTTCATACTGAACTTT
<i>Ap2</i>	GATGAAATCACCGCAGACGA CAGGA	CACCACCAGCTTGTACCATCTC G
<i>Col2a1</i>	CCACACCAAATTCCTGTTCA	ACTGGTAAGTGGGGCAAGAC
<i>Gapdh</i>	CAGTGCCAGCCTCGTCCCGT AGA	CTGCAAATGGCAGCCCTGGTGAC
<i>Ihh</i>	TGGACTCATTGCCTCCCAGA	CAAAGGCTCAGGAGGCTGGA
<i>Kindlin-2</i>	TGGACGGGATAAGGATGCCA	TGACATCGAGTTTTTCCACCAAC
<i>Runx2</i>	TAAAGTGACAGTGGACGGTC CC	TGCGCCCTAAATCACTGAGG
<i>Sox9</i>	CCACGGAACAGACTCACATC TCTC	CTGCTCAGTTCACCGATGTCCAC G
<i>Tgf-β1</i>	GGATACCAACTATTGCTTCAG CTCC	AGGCTCCAAATATAGGGGCAGGG TC

Ap2=adipocyte protein 2; Ihh=Indian hedgehog; TGF=transforming growth factor