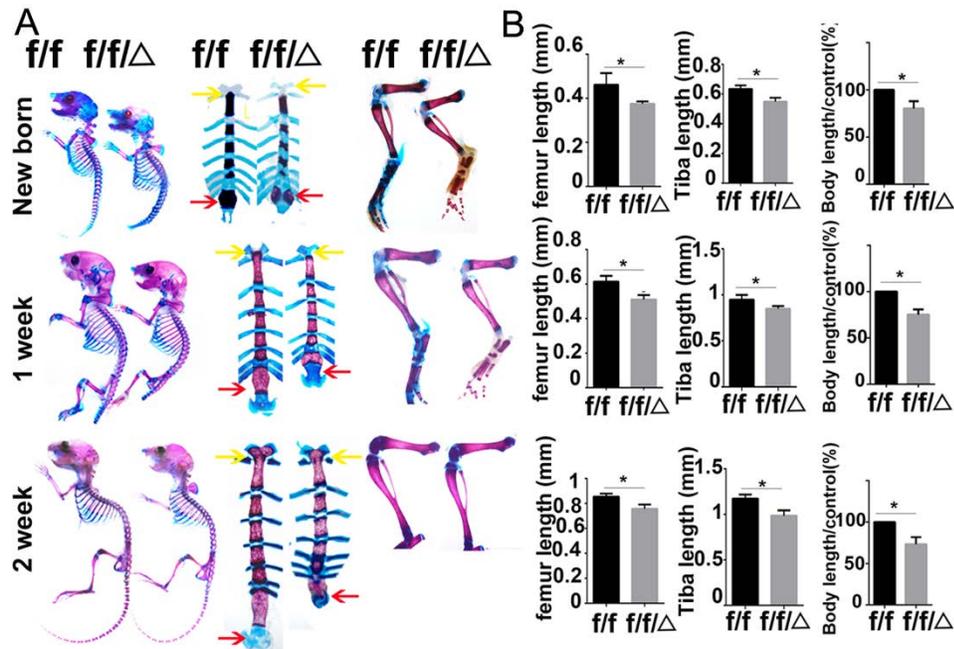


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Supplemental Materials

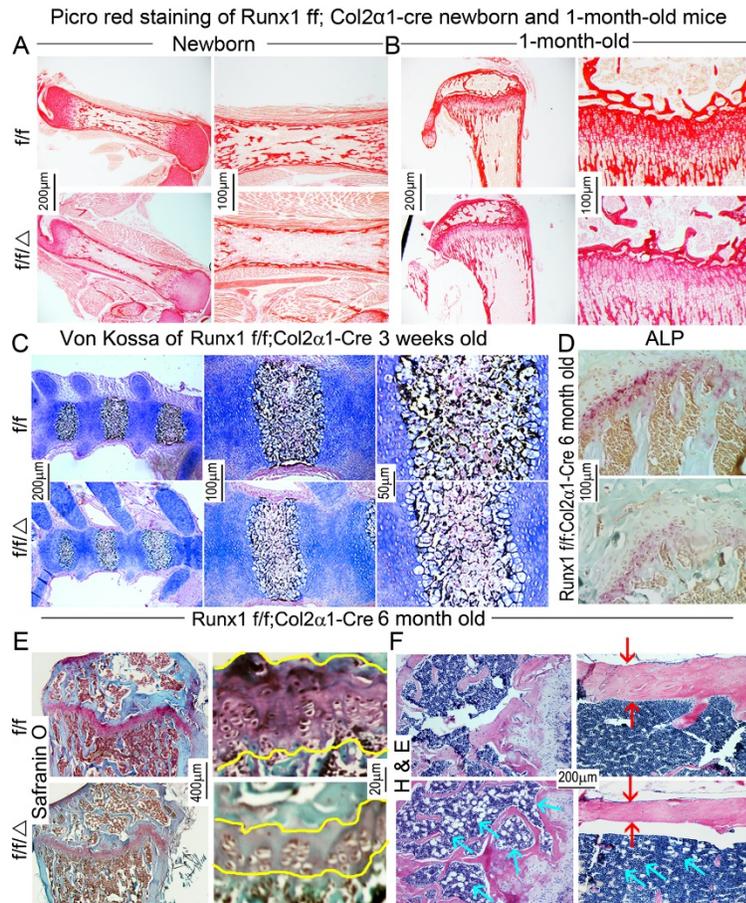
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790 Supplementary Figure legends



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792 **Figure S1. Alizarin Red and Alcian blue staining.** (A) Alizarin red and alcian blue staining of
793 newborn, 1-week and 2-week old mutant and control mice skeletons. Yellow arrows and red
794 arrows illustrate the deformed xiphoid process in mutant mice. (B) Lengths of femur, tibia and
795 body were evaluated in mice of newborn, 1-week, and 2-week old mice. All data are presented as
796 mean \pm SD, n=4, NS denotes not significant, *p<0.05.



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798 **Figure S2. Picro Sirius Red, ALP, Von Kossa, H&E and Safranin O staining for different**

799 **ages of *Runx1^{ff}* and *Runx1^{ff}col2α1-cre* mice.** (A) Picro Sirius Red staining of *Runx1^{ff}* and

800 *Runx1^{ff}col2α1-cre* newborn mice femur and (B) 1-month-old mice tibia. (C) Von Kossa staining

801 of *Runx1^{ff}* and *Runx1^{ff}col2α1-cre* 3-week-old mice vertebra. (D) ALP staining of *Runx1^{ff}* and

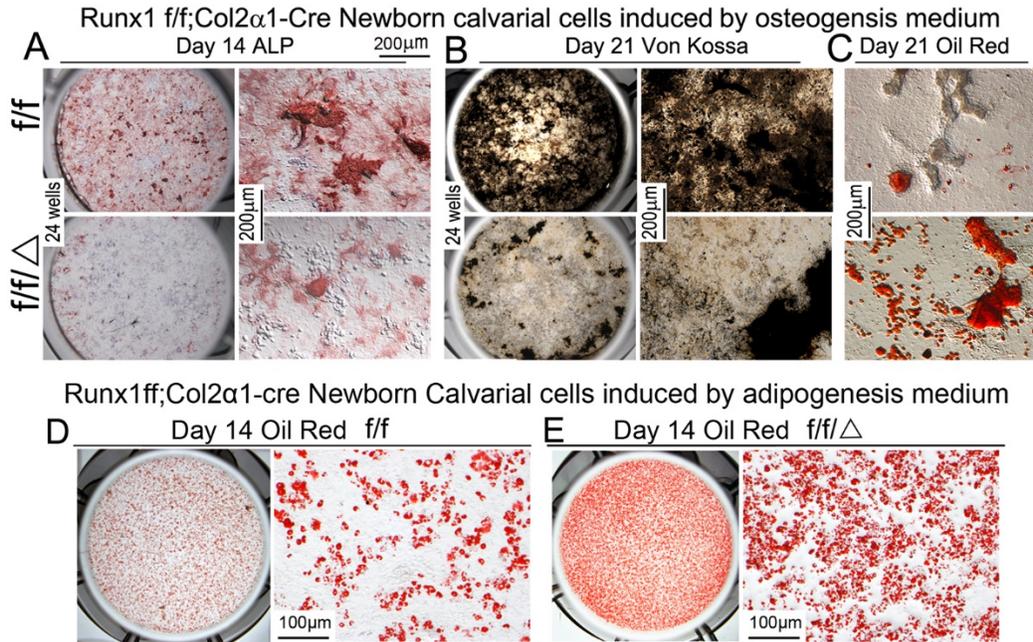
802 *Runx1^{ff}col2α1-cre* 6-month-old male mice femurs. (E) Safranin O and (F) H&E staining of

803 *Runx1^{ff}* and *Runx1^{ff}col2α1-cre* 6-month-old male mice femurs. Yellow areas illustrate the

804 *Runx1^{ff}* and *Runx1^{ff}col2α1-cre* chondrocyte zone, red arrows indicate the cortical bone thickness,

805 and blue arrows indicate adipocytes in *Runx1^{ff}col2α1-cre* mice femurs.

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808 **Figure S3. ALP, Von Kossa, and Oil-Red staining of newborn *Runx1^{ff}col2a1-cre* mice**

809 **calvarial cells cultured in osteogenesis or adipogenesis medium for 14 or 21 days. (A) ALP**

810 **staining of *Runx1^{ff}* and *Runx1^{ff}col2a1-cre* newborn mice calvarial cells after osteogenesis**

811 **induction for 14 days. (B, C) (B) Von Kossa staining and (C) Oil-Red staining of *Runx1^{ff}* and**

812 ***Runx1^{ff}col2a1-cre* newborn mice calvarial cells osteogenesis induction for 21 days. (D, E) Oil-**

813 **Red staining of (D) *Runx1^{ff}* and (E) *Runx1^{ff}col2a1-cre* newborn mice calvarial cells**

814 **adipogenesis induction for 14 days.**

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822 **Supplementary Tables**823 **Table S1. Primers used for qPCR**

Gene	Forward primer	Reverse primer
<i>Runx1</i>	GATGGCACTCTGGTCACCG	GCCGCTCGGAAAAGGACAA
<i>Runx2</i>	AGAGACCACAATAACCAGCACG	GGCGGCCATATGACTACAAAG
<i>Osx</i>	ATGGCGTCCTCTCTGCTTG	TGAAAGGTCAGCGTATGGCTT
<i>Ocn</i>	GAACAGACAAGTCCCACACAG	GAGCTGCTGTGACATCCATAC
<i>Col2a1</i>	CGGCTTCCACACATCCTTAT	CTGTCCTTCGGTGTCAAGG
<i>Coll0a1</i>	TGCTGCCTCAAATACCCTTTCT	TGGCGTATGGGATGAAGTATTG
<i>Mmp13</i>	TCCTCGGAGACTGGTAATGG	TGATGAAACCTGGACAAGCA
<i>Ihh</i>	CCACTTCCGGGCCACATTTG	GGCCACCACATCCTCCACCA
<i>Pth1r</i>	GTGAACGGGAGGTGTTTGA	CCCGGACGATATTGATGAAG
<i>Gapdh</i>	CAATGACCCCTTCATTGACC	GACAAGCTTCCCGTTCTCAG
<i>Alp</i>	CCTAGTTATTGCCCTTTGGCC	TGCCTGCCCAAGAGAGAAA
<i>Fabp4</i>	AGCACCATAACCTTAGATGGGG	CGTGGAAGTGACGCCTTTCA
<i>C/ebpa</i>	CAAGAACAGCAACGAGTACCG	GTCACTGGTCAACTCCAGCAC
<i>Pparγ</i>	GGAAGACCACTCGCATTTCCTT	GTAATCAGCAACCATTGGGTCA

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829 **Supplementary Tables**

830 **Table S2. Primers used for ChIP assay**

Gene	Forward primer	Reverse primer
<i>Ihh 1</i>	CCCAGCACCATTTGGCATTAG	CCCTCACAGCTAGGCCATTT
<i>Ihh 2</i>	GCAAATGGAGTCCCAGGTGA	GAAGAGAGTGCTGGGTGCTT
<i>Ihh 3</i>	TGCAGGCACTGAAGATGTGT	CCACCAGCAACCTATGCTCA
<i>Ihh 4</i>	TCCTTTGGTAGAGGAAGGTGC	TCTTCCTCCCTCACCCCTCAC
<i>Ihh 5</i>	CAGGGACATGGATAGGGTGC	GGGTCGAGTGAGAGGGGAAA

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834 **Supplemental Tables**

835 **Table S3. Primers used for subcloning**

Gene	Primer
<i>Ihh F1</i>	CCGACGCGTAAGCTTCCCAGCACCATTTGGCATTAG
<i>Ihh F2</i>	CCGACGCGTAAGCTTCTACTGTGTAGACCAGGCTAGC
<i>Ihh F3</i>	CCGACGCGTAAGCTTGCAAATGGAGTCCCAGGTGA
<i>Ihh F4</i>	CCGACGCGTAAGCTTTGCAGGCACTGAAGATGTGT
<i>Ihh F5</i>	CCGACGCGTAAGCTTCTTGTAAGTGCGCGTAAACCC
<i>Ihh R</i>	CCGCTCGAGCGCCTCGACTCTGAGCTG

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