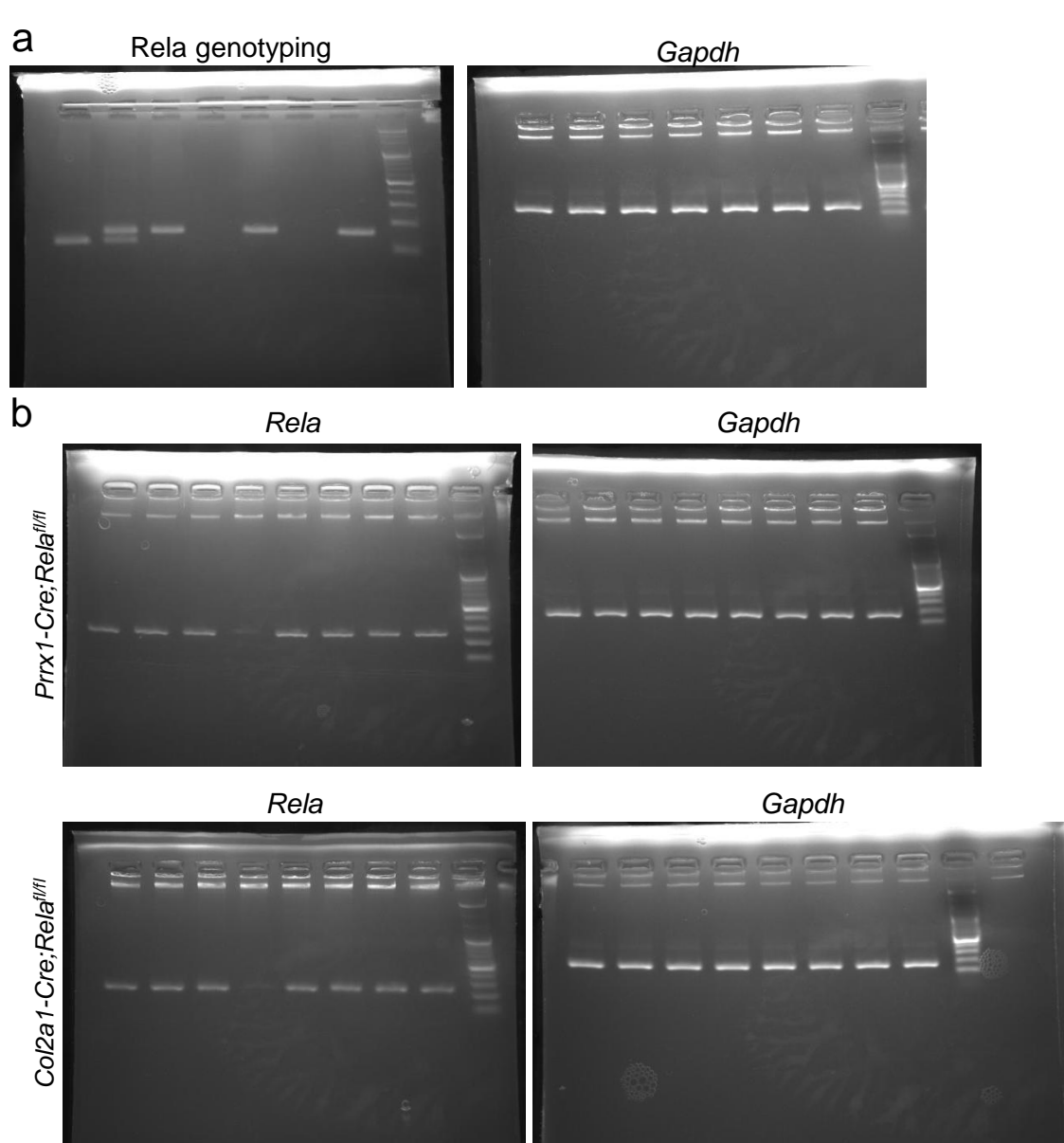


### Supplementary Figure 1

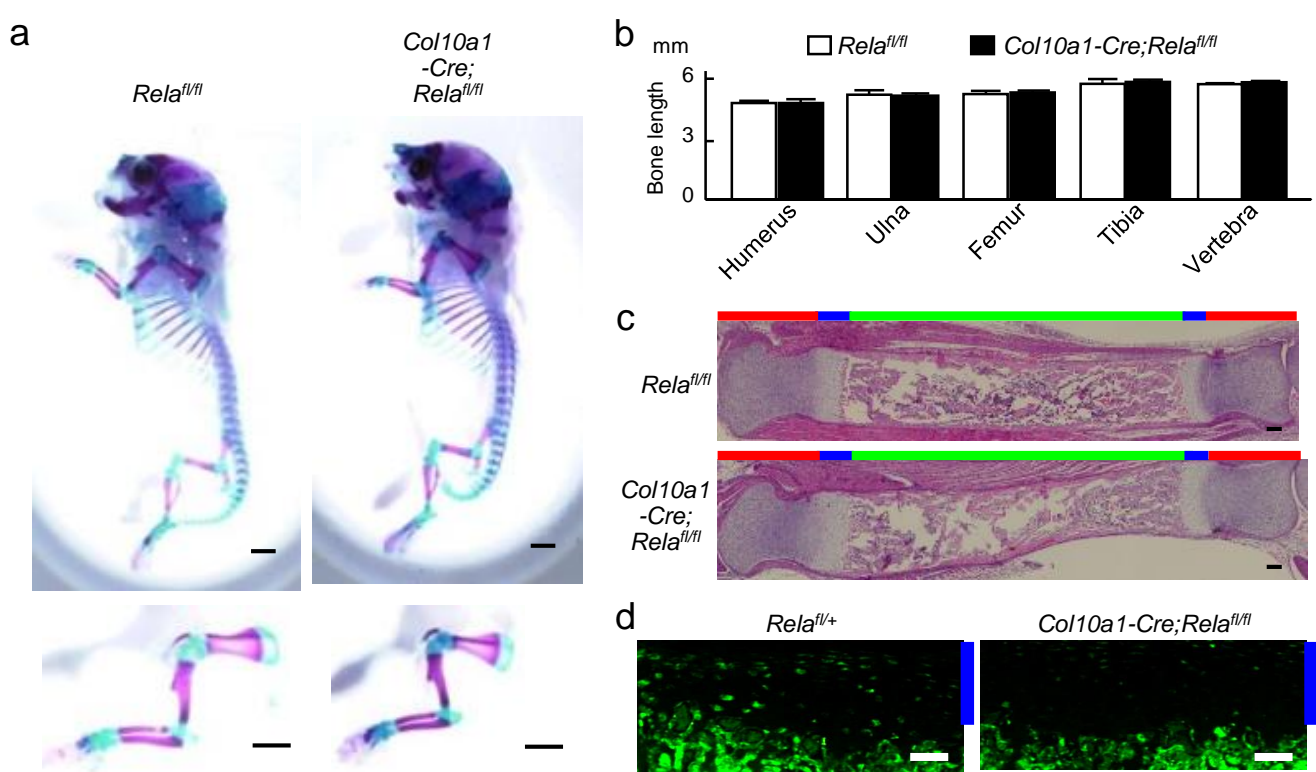
Cre mice used for *in vivo* analyses of *Rela* during skeletal development. **(a)** Scheme of Cre expression in the three mouse strains during chondrocyte generation and endochondral ossification. **(b)** Genotyping of *Rela*-flox mice. A primer set for genotyping was designed to detect the latter loxP site. WT or floxed alleles were not detected in genomic samples obtained from epiphyseal cartilage of *Prrx1-Cre;Rela*<sup>fl/fl</sup> and *Col2a1-Cre;Rela*<sup>fl/fl</sup> mice, indicating tissue-specific recombination in both mouse lines. **(c)** RT-PCR analysis of *Rela* using mRNA samples obtained from various tissues of *Prrx1-Cre;Rela*<sup>fl/fl</sup> and *Col2a1-Cre;Rela*<sup>fl/fl</sup> mice, indicating tissue-specific knockdown of *Rela* in both mouse lines.



**Supplementary Figure 2**

Original images of the agarose gel electrophoresis shown in Supplementary Fig.1b, and c.

(a) Original images of Supplementary Fig.1b. (b) Original images of Supplementary Fig.1c.



### Supplementary Figure 3

Skeletal development of *Col10a1-Cre;Rela<sup>fl/fl</sup>* mice. **(a)** Double staining with Alizarin red and Alcian blue of the whole skeleton (left) and upper extremities (right) of *Rela<sup>fl/fl</sup>* and *Col10a1-Cre;Rela<sup>fl/fl</sup>* littermate embryos (E18.5). Scale bars, 1 mm. **(b)** Length of long bones and vertebrae (first to fifth lumbar spines) of *Rela<sup>fl/fl</sup>* and *Col10a1-Cre;Rela<sup>fl/fl</sup>* littermate embryos (E18.5). Data are expressed as the means  $\pm$  s.d. of five mice per group. **(c)** H&E staining of whole tibias of *Rela<sup>fl/fl</sup>* and *Col10a1-Cre;Rela<sup>fl/fl</sup>* littermate embryos (E18.5). Scale bars, 100  $\mu$ m. Upper bars indicate lengths of the proliferative zone (red), hypertrophic zone (blue), and bone area (green). **(d)** Immunofluorescence of *Rela* in the proximal tibia of *Rela<sup>fl/fl</sup>* and *Col10a1-Cre;Rela<sup>fl/fl</sup>* littermate embryos (E18.5). Right blue bars indicate the hypertrophic zone. Scale bars, 50  $\mu$ m.

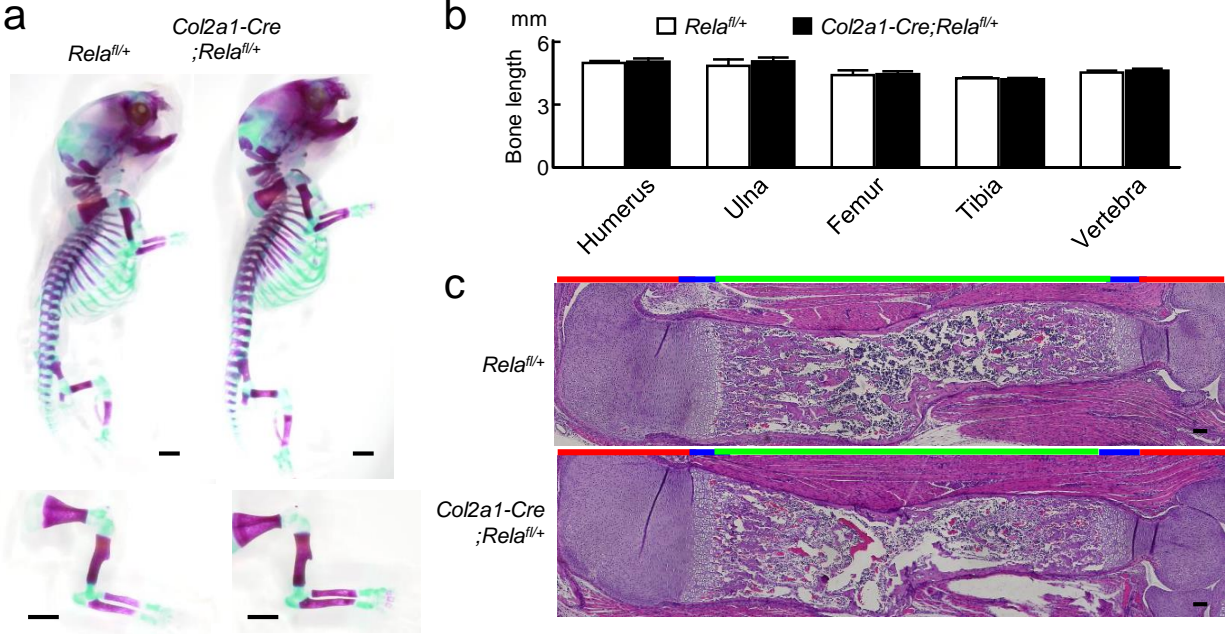
*Rela<sup>fl/fl</sup>*

*Col2a1-Cre<sup>ERT</sup>;  
Rela<sup>fl/fl</sup>*



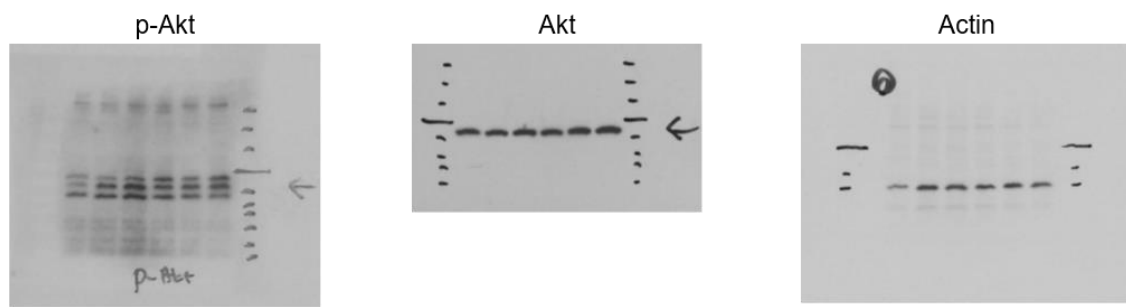
**Supplementary Figure 4**

Skeletal development of *Col2a1-Cre<sup>ERT</sup>;**Rela<sup>fl/fl</sup>* mice. Plain radiograph of *Rela<sup>fl/fl</sup>* and *Col2a1-Cre<sup>ERT</sup>;**Rela<sup>fl/fl</sup>* littermates (7 weeks old, without tamoxifen induction). Scale bars, 10 mm.



**Supplementary Figure 5**

Skeletal development of *Col2a1-Cre;Rela<sup>fl/+</sup>* mice. **(a)** Double staining with Alizarin red and Alcian blue of the whole skeleton (top) and upper extremities (bottom) of *Rela<sup>fl/+</sup>* and *Col2a1-Cre;Rela<sup>fl/+</sup>* littermate embryos (E18.5). Scale bars, 1 mm. **(b)** Length of long bones and vertebrae (first to fifth lumbar spines) of *Rela<sup>fl/+</sup>* and *Col2a1-Cre;Rela<sup>fl/+</sup>* littermate embryos (E18.5). Data are expressed as the means  $\pm$  s.d. of three *Rela<sup>fl/+</sup>* mice and five *Col2a1-Cre;Rela<sup>fl/+</sup>* mice. **(c)** H&E staining of whole tibias of *Rela<sup>fl/+</sup>* and *Col2a1-Cre;Rela<sup>fl/+</sup>* littermate embryos (E18.5). Scale bars, 100  $\mu$ m. Upper bars indicate lengths of the proliferative zone (red), hypertrophic zone (blue), and bone area (green).



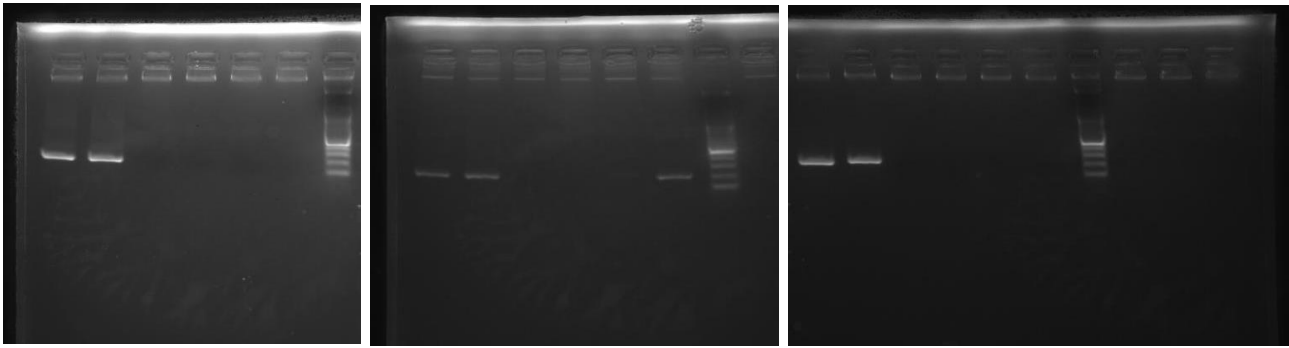
**Supplementary Figure 6**

Original images of the immunoblots shown in Fig. 5d.

N-1

N-2

NC



**Supplementary Figure 7**

Original images of the agarose gel electrophoresis of ChIP-PCR shown in Fig. 6e.

**Supplementary Table 1.** Primers used for genotyping.

<i>Rela</i>	F	gaagctggtctgccaagaac
	R	catcaaagtcagccaggtca
<i>Cre</i>	F	gcggtctggcagtaaaaactatc
	R	gtgaaacagcattgctgtcactt
<i>Gapdh</i>	F	tgcaccaccaactgcttagc
	R	accctaaaaggacacctaca



**Supplementary Table 2.** Primers used for real-time RT-PCR.

<i>Rela</i>	F	aggtgtatttcacgggacca
	R	cataggtcctttgcgcttc
<i>Col2a1</i>	F	gccaaacctgaaactctgc
	R	gccatagctgaagtggaagc
<i>Acan</i>	F	caaaccagcctgacaactt
	R	tctagcatgctccaccactg
<i>Sox9</i>	F	cgactacgctgaccatcaga
	R	agactggttgttcccagtgc
<i>Cdkn1c</i>	F	aggagcaggacgagaatcaa
	R	acgtttggagagggacacc
<i>Ccnd1</i>	F	agtgcgtgcagaaggagatt
	R	cacaacttctcggcagtcaa
<i>Traf2</i>	F	gccagattttgagcagaag
	R	ccagctgttcaccttgta
<i>Cflar</i>	F	acagagtgaggcggttgac
	R	ccttggtatcttgcctctg
<i>Birc2</i>	F	gtcagaatcaaaggccaag
	R	caccaggctcctactgaagc
<i>Pik3ca</i>	F	actgttcagagaggccagga
	R	cggttgctactggttcaat
<i>Birc3</i>	F	ggggacagtctgttgaaa
	R	ggctagagcacaggttgag
<i>Map3k14</i>	F	caaatcctctctgcctcag
	R	tgttcgccattgagagactg
<i>Pik3r1</i>	F	agccgccagctctgataata
	R	tctcccagtaccattcagc
<i>Ikbkg</i>	F	ggtggagagactgagcttgg
	R	ctaaagcttgccgatccttg
<i>Ppp3cc</i>	F	gaaccaagcaactggcttc
	R	tccaacaaaaggcaaagacc
<i>Adamts5</i>	F	gctactgcacaggaagagg
	R	tgcatatttgggaaccatt
<i>Hif2a</i>	F	tgagttggctcatgagttgc
	R	ctcacggatctcctcatggt
<i>Actb</i>	F	agatgtggatcagcaagcag
	R	gcgcaagttaggtttgtca
<i>Gapdh</i>	F	tgaccaccaactgcttagc
	R	ggatgcagggatgatgttct

**Supplementary Table 3.** Primers used for ChIP-PCR

N-1	F	ctccggttcaccctttcac
	R	accccaggcttttcgag
N-2	F	ggacgtgtagagccagagca
	R	gagcccacaccaaccaga
NC	F	aggctagctgtcggagacg
	R	tgccagtgacaggaagagag