Supplementary Figure 1 | Specific Venus expression in the cartilage tissues of newborn Col2a1-Venus Tg mice. (a) Schematic diagram of the construct used to generate Col2a1-Venus transgenic (Tg) mice. (b) Rib (left panel), vertebrae (middle panel), and forelimb (left panel) were photographed using fluorescence microscopy. Arrows indicate the articular chondrocytes in the forelimb. Scale bar, 3 mm (rib and forelimb), 1 mm (vertebrae) (c) Venus expression in E13.5 Col2a1-Venus Tg mice. Whole embryo (left panel) and limb (right panel) were photographed under fluorescence microscopy. Scale bar, 2 mm (left panel) and 500 µm (right panel). (d) Protocol for the isolation of Venus-negative and -positive cells followed by microarray analysis. (e) Total RNA was isolated from Venus-negative and -positive cells and the mRNA expression of Venus and β-actin were determined by RT-PCR. M: marker
Supplementary Figure 2 | Immunohistochemical analysis of Foxc1 in growth plate chondrocytes. (a) Paraffin sections of E13.5 mouse digits were subjected to haematoxylin and eosin (H&E) staining and immunohistochemical analyses using anti-Foxc1 and anti-Col2 antibodies. Arrows indicate the expression of Foxc1 in perichondrial cells. Scale bar, 200 μm (lower magnification, ×10) and 50 μm (higher magnification, ×40). (b) Paraffin sections of a newborn mouse tibia were subjected to H&E staining and immunohistochemical analyses using anti-Foxc1 and anti-Col2 antibodies. The boxed areas show higher magnification of resting (upper panels) and proliferating and hypertrophic (lower panels) chondrocyte zones. Scale bar, 200 μm (lower magnification, ×10) and 50 μm (higher magnification, ×40).
Supplementary Figure 3 | Abnormal skeletal development in Foxc1<sup>ch/ch</sup> mice.

(a) Photograph of Alcian Blue/Alizarin Red S-stained skeletal preparations of newborn WT and Foxc1<sup>ch/ch</sup> littermates. (b, c, d) Photographs of rib cages and vertebrae (b), forelimbs (c), and hindlimbs (d) of newborn WT and Foxc1<sup>ch/ch</sup> littermates. Scale bar, 2 mm (b) 5 mm (c, d) (e) Quantitative analysis of the length of femurs and tibias from newborn WT and Foxc1<sup>ch/ch</sup> mice, assessed using stereo microscopy. Note that Foxc1<sup>ch/ch</sup> mice have a shorter tibia and femur compared to WT mice. Data are shown as the mean ± s.d. (n = 5). **p < 0.01 *p < 0.05 (vs. WT); Student’s t-test. (f) Histological analysis of newborn WT and Foxc1<sup>ch/ch</sup> littermate tibias. Note that Alcian blue-positive staining of Foxc1<sup>ch/ch</sup> mice was weaker and the von Kossa positive ossification area was smaller than in WT mice. Scale bar, 500 μm.
Supplementary Figure 4 | Abnormal endochondral ossification in Foxc1<sup>ch/ch</sup> mice.

(a) Photograph of Alcian Blue/Alizarin Red S-stained skeletal preparations of E15.5 WT and Foxc1<sup>ch/ch</sup> littermates. Scale bar, 5 mm. (b) Histological analysis of E15.5 WT and Foxc1<sup>ch/ch</sup> littermate tibias. Paraffin sections of tibias from E15.5 WT and Foxc1<sup>ch/ch</sup> littermate embryos were examined by von Kossa staining. Scale bar, 500 μm. (c) Paraffin sections of tibias from E15.5 WT and Foxc1<sup>ch/ch</sup> littermates were subjected to immunofluorescence analyses using anti-Col2 and anti-Col10 antibodies. Scale bar, 200 μm. (d) Paraffin sections of tibias from E15.5 WT and Foxc1<sup>ch/ch</sup> littermate embryos were examined by in situ hybridization using antisense probes against Col1a1 and Runx2. Arrows indicate the invasion of osteoblasts. The H&E stained images are higher magnification images of those in Fig. 2E. Scale bar, 200 μm.
Supplementary Figure 5 | Decreased chondrocyte proliferation in Foxc1<sup>ch/ch</sup> mice. (a) Representative images of BrdU-staining. Pregnant mice were anesthetized and 1 ml BrdU Labeling Reagent (Life Technologies) per 100 g body weight was injected by intraperitoneal injection. The mice were sacrificed 2 hours after injection, and tibias were collected. Paraffin sections were subjected to a BrdU assay using an Invitrogen BrdU Staining kit. Scale bar, 200 μm. (b) Higher magnification of boxed areas in (a). Scale bar, 100 μm. (c) Quantitative analysis of proliferation rate in columnar chondrocytes. The ratio of BrdU positive nuclei to the total haematoxylin positive nuclei was calculated. BrdU positive nuclei in round and hypertrophic chondrocytes were excluded from the analysis. Data are shown as the mean ± s.d. (n = 5-7) *p < 0.05 (vs. WT); Student’s t-test.
Supplementary Figure 6 | Foxc1 had no effect on Ihh expression. (a) Total RNA was isolated from the hindlimbs of E15.5 Foxc1<sup>ch/ch</sup> and WT littermate foetuses and Ihh mRNA expression was determined by RT-qPCR. Data are shown as the mean ± s.d. (n =3). (b) Effect of Foxc1 and Gli2 on Ihh mRNA expression. Total RNA was isolated from primary chondrocytes infected with adenoviruses as indicated and Ihh mRNA expression was determined by RT-qPCR. Data are shown as the mean ± s.d. (n =3).
Supplementary Figure 7 | Decreased Gli2 binding to the Gli binding element located in the PTHrP, Gli1 and Ptch1 gene promoters in Foxc1<sup>ch/ch</sup> mice. Primary chondrocytes were isolated from WT and Foxc1<sup>ch/ch</sup> littermates and cultured for 3 days. ChIP assays were conducted using an anti-Gli2 antibody and DNA binding was determined by qPCR using primer pairs specific for PTHrP (a), Gli1 (b), and Ptch1 (c) gene promoters, all of which contain the Gli-binding element. Data are shown as the mean ± s.d. (n = 6). **p < 0.01 and *p < 0.05 (vs. WT); Student’s t-test.
Supplementary Figure 8 | Decreased Col10 positive chondrocyte zone in E17.5 Foxc1<sup>ch/ch</sup> tibia. Immunofluorescence analysis of tibial growth plate chondrocytes in E17.5 WT and Foxc1<sup>ch/ch</sup> littermates. Paraffin sections of tibias were subjected to H&E staining and immunofluorescence analyses using anti-Col2 and anti-Col10 antibodies. Scale bar, 200 μm.
Supplementary Figure 9 | Effect of dominant-negative (DN)-Foxc1 on Ihh target genes and Col10a1 expression. (a) Schematic diagram of DN-Foxc1, including the forhead DNA-binding domain (DBD) and transcriptional inhibitory domain (grey box). (b) Expression of DN-Foxc1. 293FT cells were transfected with Flag-Foxc1 and Flag-DN-Foxc1. Cell lysates were immunoblotted with anti-Flag (upper panel) and anti-β-actin (lower panel) antibodies. (c) COS-7 cells were transfected with a
6xFoxc1-BE-Luc construct together with Foxc1, DN-Foxc1, or both. Luciferase activities of cell lysates were measured 48 h after transfection. Data are expressed in relative luciferase units (mean ± s.d., n = 3). **p < 0.01; one-way ANOVA followed by the Tukey–Kramer test. (d, e) Inhibitory effect of DN-Foxc1 on PTHrP (d) and Col10a1 (e) mRNA expression induced by Foxc1 in primary chondrocytes. Data are shown as the mean ± s.d. (n = 3). **p < 0.01; one-way ANOVA followed by the Tukey-Kramer test. (f–i) Inhibitory effect of DN-Foxc1 on PTHrP (f), Gli1 (g), Ptch1 (h), and Col10a1 (i) mRNA expression induced by Ihh in primary chondrocytes. Note that DN-Foxc1 decreased expression of Ihh target genes. Data are shown as the mean ± s.d. (n = 3). **p < 0.01; one-way ANOVA followed by the Tukey–Kramer test.
Supplementary Figure 10 | Functional redundancy between Foxc1 and Foxc2 in chondrocytes. (a) Total RNA was isolated from Venus-negative and -positive cells and analysed by RT-qPCR for Foxc2 expression. Data are shown as the mean ± s.d. (n = 3). *p < 0.01 [vs. Venus (-)]; Student’s t-test. (b) Tissue distribution of Foxc2 mRNA in newborn mouse tissues. Total RNA isolated from indicated tissues was analysed by RT-qPCR. Data are shown as the mean ± s.d. (n = 3). (c) Immunohistochemical analysis of Foxc2 in growth plate chondrocytes. Paraffin sections of a newborn mouse
tibia were subjected to immunohistochemical analyses using an anti-Foxc2 antibody.
Right panel shows high magnification images of boxed areas. Scale bar, 200 μm (low
magnification) and 50 μm (high magnification). (d,e) Primary chondrocytes were
infected with control (Cont) or Foxc2 adenoviruses, and PTHrP (d) and Col10a1 (e)
mRNA expression was analysed by RT-qPCR. Data are shown as the mean ± s.d. (n = 3) **p < 0.01 (vs. Cont); Student’s t-test. (f,g) COS-7 cells were transfected with reporter
constructs containing 6xFoxc1-BE from PTHrP (f) and Col10a1 (g) promoters together
with Foxc2. Data are expressed in relative luciferase units (mean ± s.d., n = 3). **p < 0.01; Student’s t-test. (h,i,j) Synergistic effects of Foxc2 and Gli2 on PTHrP (h), Ptc1 (i),
and Gl1 (j) mRNA expression in primary chondrocytes. Data are shown as the mean ±
s.d. (n = 3). **p < 0.01; one-way ANOVA followed by the Tukey–Kramer test.
Supplementary Figure 11

**Comparative analysis of Foxc1 and Foxc2 expression in chondrocytes.** (a) Haematoxylin and eosin (H&E) staining and *in situ* hybridization analysis of *Col2a1*, *Foxc1* and *Foxc2* in growth plate chondrocytes of E14.5 tibia. Dig-labelled RNA probes specific for *Foxc2* were generated using a DNA fragment of *Foxc2* (nucleotide positions: 2033–2499). To compare the *in situ* hybridization signals of *Foxc1* and *Foxc2* under the same conditions, alkaline-phosphatase signals were developed for the same amount of time. Scale bar, 200 μm (b) Total RNA was isolated from kidney (P0), rib cartilage (P0) and primary chondrocytes and the mRNA expression of *GAPDH* (sense primer: 5’-acatacaagaaggtgtaaggcagg-3’, anti-sense primer: 5’-ctcttgctctcagatctctgtg-3’), *Foxc1* (sense primer: 5’-cccgagagcaagacagtaa-3’, anti-sense primer: 5’-ccgttctttgacatgga-3’) and *Foxc2* (sense primer: 5’-cagaacagaggagagacag-3’, anti-sense primer: 5’-ttaaaggctctggcagaa-3’) were determined by RT-PCR. M: marker.
Supplementary Figure 12 | Foxc1-F112S mutant failed to recruit endogenous Gli2 to the Gli binding element located in PTHrP, Gli1 or Ptch1 gene promoters. Primary chondrocytes were infected with control or F112S adenoviruses and cultured for 3 days. ChIP assays were conducted using an anti-Gli2 antibody and DNA binding to the gene promoter was determined by qPCR using primer pairs specific for PTHrP (a), Gli1 (b), and Ptch1 (c) gene promoters, all of which contain the Gli-binding element. Data are shown as the mean ± s.d. (n = 6).
Supplementary Figure 13 | Proposed mechanism for the role of Foxc1 during endochondral ossification. Foxc1 regulates PTHrP and Col10a1 expression, presumably through direct binding to the Foxc1 binding element (Foxc1-BE). Additionally, Foxc1 physically interacts with Gli2, a major signalling molecule of Ihh, and synergistically enhances the expression of Ihh target genes, including PTHrP, Gli1, Ptch1, and Col10a1.
Supplementary Figure 14

Full western blots and RT-PCR gels performed in this study.
Supplementary Figure 14 (Continued)

Supplementary Figure 1e

Supplementary Figure 11b
Supplementary Table 1 | Microarray analysis of chondrocyte genes.

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<th>Gene Symbol</th>
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<th>Fold Change (Log$_2$ Venus(+)/(-))</th>
<th>Signal intensity of Venus(+)</th>
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<tr>
<td>Col2a1</td>
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<td>Col11a1</td>
<td>collagen, type XI, alpha 1</td>
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<td>Col9a1</td>
<td>collagen, type IX, alpha 1</td>
<td>5.1</td>
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<td>Matn1</td>
<td>matrilin 1, cartilage matrix protein</td>
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<tr>
<td>Comp</td>
<td>cartilage oligomeric matrix protein</td>
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<td>Acan</td>
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<td>------</td>
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| Foxc1 | sense: 5'-CCACTCGGTGCAGGGAAATG-3'  
          antisense: 5'-AGGTGCGTACAGAGACTGA-3'  
          probe: 5'-CGAGTCTCAGAGGATCGGTTAACA-3' |
| Col2a1 | sense: 5'-CCCTCGGCTACTGCGACTGAG-3'  
          antisense: 5'-TGAGGCTCAGGCGCTCTCAGGA-3'  
          probe: 5'-ACGGTCTTCACATCCCAAGG-3' |
| Aggrecan | sense: 5'-CCAGTCTTTACCGCACTTTCC-3'  
            antisense: 5'-ACGTGCGGTACAGAGACTGA-3'  
            probe: 5'-CGAGTCTCAGCGGATCGGCTTGAACA-3' |
| Col11a2 | sense: 5'-CCCTCCGTCTACTGCTCAGGAG-3'  
        antisense: 5'-TGGAGCCCTGGATGAGCAAG-3'  
        probe: 5'-TGAGGTTGCCAGCCGCTTCA-3' |
| Sox5 | sense: 5'-CCTCCGTCTACTGCTCAGGAG-3'  
      antisense: 5'-TGGAGCCCTGGATGAGCAAG-3'  
      probe: 5'-ACGTGCGGTACAGAGACTGA-3' |
| Sox6 | sense: 5'-CCTCCGTCTACTGCTCAGGAG-3'  
      antisense: 5'-TGGAGCCCTGGATGAGCAAG-3'  
      probe: 5'-ACGTGCGGTACAGAGACTGA-3' |
| Sox9 | sense: 5'-CCTCCGTCTACTGCTCAGGAG-3'  
      antisense: 5'-TGGAGCCCTGGATGAGCAAG-3'  
      probe: 5'-ACGTGCGGTACAGAGACTGA-3' |
| PTHrP | sense: 5'-GAACATCAGCTACTGCGACTGAC-3'  
        antisense: 5'-CTCGGTGCACTGCTGAGAGC-3'  
        probe: 5'-ACGTGCGGTACAGAGACTGA-3' |
| Ptch1 | sense: 5'-CCCTCCGTCTACTGCTCAGGAG-3'  
      antisense: 5'-TGGAGCCCTGGATGAGCAAG-3'  
      probe: 5'-ACGTGCGGTACAGAGACTGA-3' |
| Gli1 | sense: 5'-CCTCCGTCTACTGCTCAGGAG-3'  
      antisense: 5'-TGGAGCCCTGGATGAGCAAG-3'  
      probe: 5'-ACGTGCGGTACAGAGACTGA-3' |
| Col10a1 | sense: 5'-CCCTCCGTCTACTGCTCAGGAG-3'  
       antisense: 5'-TGGAGCCCTGGATGAGCAAG-3'  
       probe: 5'-ACGTGCGGTACAGAGACTGA-3' |
| ALPase | sense: 5'-CCCTCCGTCTACTGCTCAGGAG-3'  
      antisense: 5'-TGGAGCCCTGGATGAGCAAG-3'  
      probe: 5'-ACGTGCGGTACAGAGACTGA-3' |
| Runx2 | sense: 5'-CCCTCCGTCTACTGCTCAGGAG-3'  
       antisense: 5'-TGGAGCCCTGGATGAGCAAG-3'  
       probe: 5'-ACGTGCGGTACAGAGACTGA-3' |
| MMP13 | sense: 5'-CCCTCCGTCTACTGCTCAGGAG-3'  
       antisense: 5'-TGGAGCCCTGGATGAGCAAG-3'  
       probe: 5'-ACGTGCGGTACAGAGACTGA-3' |
| Foxc2 | sense: 5'-CCCTCCGTCTACTGCTCAGGAG-3'  
       antisense: 5'-TGGAGCCCTGGATGAGCAAG-3'  
       probe: 5'-ACGTGCGGTACAGAGACTGA-3' |
| β-actin | sense: 5'-CCCTCCGTCTACTGCTCAGGAG-3'  
        antisense: 5'-TGGAGCCCTGGATGAGCAAG-3'  
        probe: 5'-ACGTGCGGTACAGAGACTGA-3' |
### Primer pairs used for ChIP assays.

**SYBR green**

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<tr>
<th>Gene promoter</th>
<th>Primer sequence</th>
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<tr>
<td><strong>PTHrP</strong></td>
<td><strong>sense primer:</strong> 5'-GCGCCAGACAGCAGCATA-3'</td>
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<tr>
<td><strong>Foxc1 binding element</strong></td>
<td><strong>anti-sense primer:</strong> 5'-CACGTTTCAGGCAAGGATTTG-3'</td>
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<td><strong>PTHrP</strong></td>
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<tr>
<td><strong>Gli binding element</strong></td>
<td><strong>anti-sense primer:</strong> 5'-GGACTAAACCTAGCAGGAGGAA-3'</td>
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<td><strong>Gli1</strong></td>
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<td><strong>Gli binding element</strong></td>
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<td><strong>Gli binding element</strong></td>
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