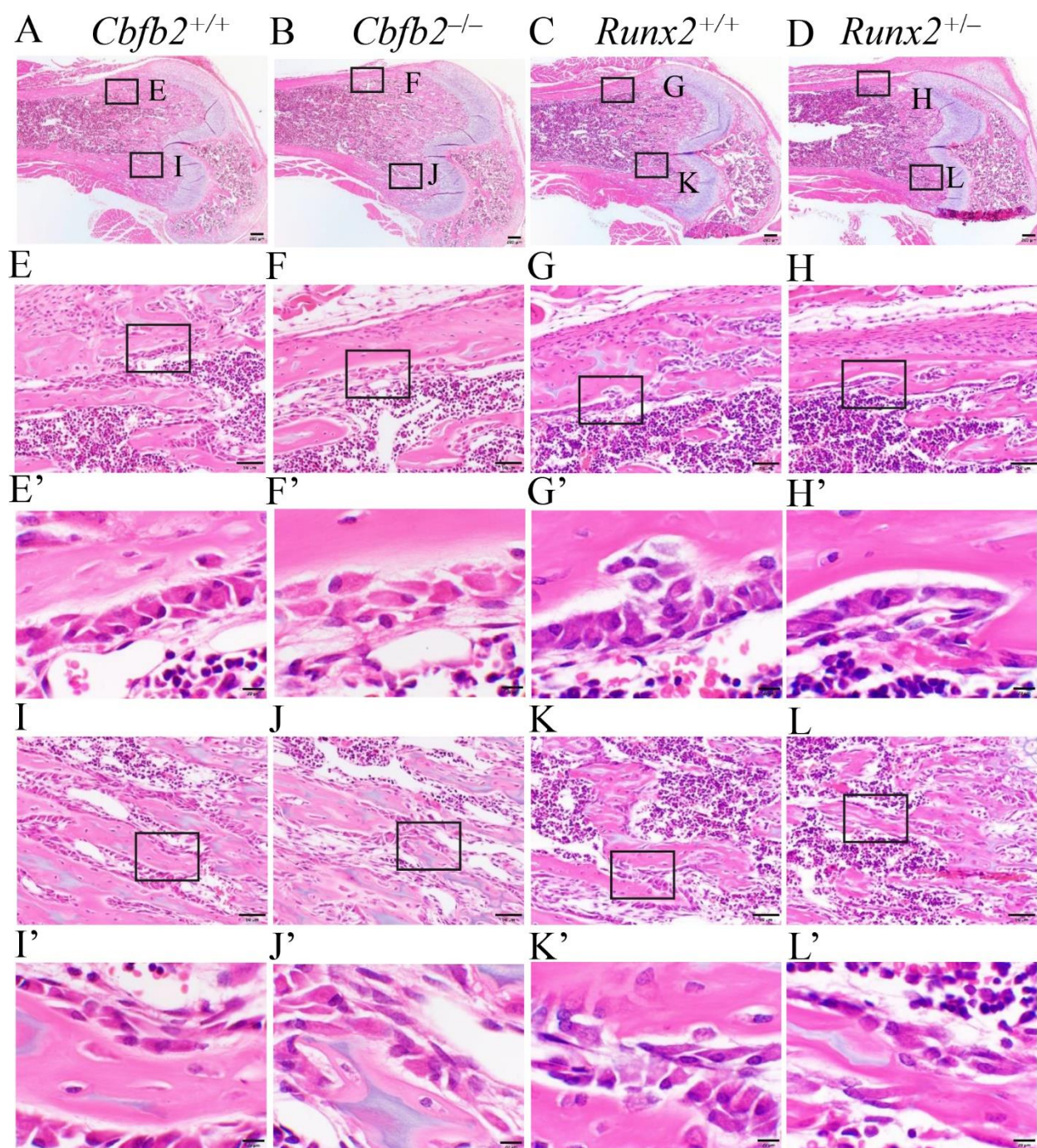


Supplementary Figure S1.

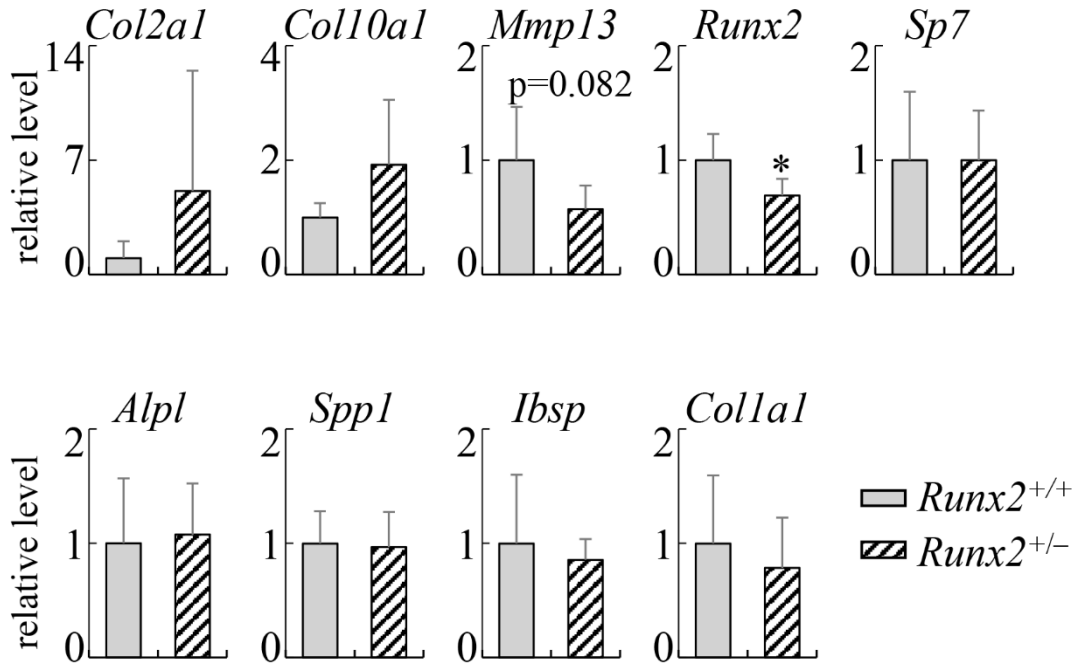
Micro-CT analyses of femurs in $Cbfb1^{+/+}$ and $Cbfb1^{-/-}$ mice at 10 weeks of age. (A, B) X-ray analysis at 10 weeks of age. (C–F) Three-dimensional trabecular bone architecture of distal femoral metaphysis (C, D) and cortical bone at mid-diaphysis (E, F) in femurs. Scale bars: 1cm (A, B) and 1mm (C–F). (G) Quantification of the trabecular bone volume (bone volume/tissue volume, BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), the cortical area (CtAr/TtAr), and cortical thickness (Ct.Th). Seven $Cbfb1^{+/+}$ and $Cbfb1^{-/-}$ mice were analyzed.



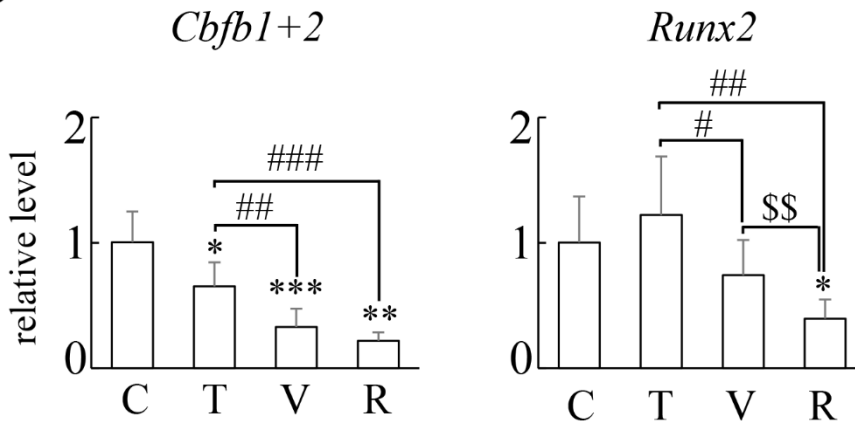
Supplementary Figure S2.

Histological analyses of femurs at 4 weeks of age. H-E staining of femoral sections in *Cbfb2*^{+/+} (A, E, I), *Cbfb2*^{-/-} (B, F, J), *Runx2*^{+/+} (C, G, K) and *Runx2*^{+/-} (D, H, L) mice at 4 weeks of age. The boxed regions in A–D are magnified in E and I, F and J, G and K, and H and L, respectively. The boxed regions in E–L are magnified in E'–L', respectively. Scale bars: 200 μm (A–D), 50 μm (E–L), and 20 μm (E'–L'). The number of mice analyzed: *Cbfb2*^{+/+}, n = 3; *Cbfb2*^{-/-}, n = 3; *Runx2*^{+/+}, n = 3; *Runx2*^{+/-}, n = 4.

A

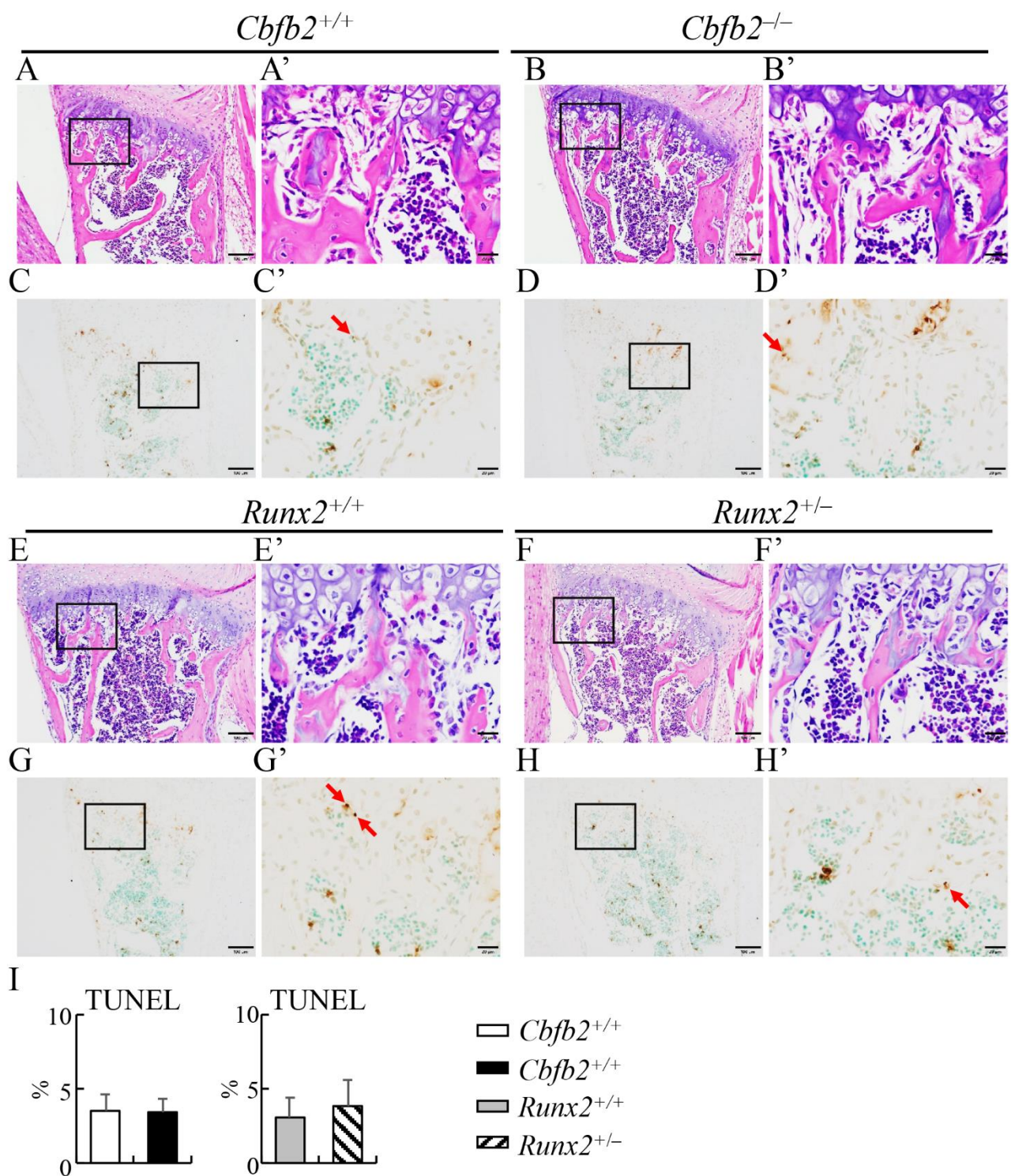


B



Supplementary Figure S3.

(A) Real-time RT-PCR analysis using rib RNA from *Runx2*^{+/+} and *Runx2*^{+/-} mice at 4 weeks of age. The values of *Runx2*^{+/+} mice were defined as 1 and relative levels are shown. The number of mice analyzed: *Runx2*^{+/+}, n = 8; *Runx2*^{+/-}, n = 7. (B) Real-time RT-PCR analysis using RNA from calvarias (C), tibia (T), vertebrae (V) and ribs (R) from wild type mice at 4 weeks of age. The values of calvarias were defined as 1 and relative levels are shown. The number of mice analyzed: C, n = 4; T, n = 8; V, n = 7; R, n = 8. Data are shown as the mean \pm SD. *, #p < 0.05, **, ##, \$\$p < 0.01, and ***, ###p < 0.001.



Supplementary Figure S4.

(A–H) H-E staining (A, B, E, F) and TUNEL staining (C, D, G, H) of the sections of 1st lumbar vertebrae in *Cbfb2*^{+/+} (A, C), *Cbfb2*^{-/-} (B, D), *Runx2*^{+/+} (E, G) and *Runx2*^{+/-} (F, H) mice at 4 weeks of age. The boxed regions in A–H are magnified in A'–H', respectively. Scale bars: 100 μ m (A–H) and 20 μ m (A'–H'). (I) The frequencies of TUNEL-positive osteoblast-like and osteoprogenitor-like cells shown by arrows were counted in C', D', G' and H'. Data are shown as the mean \pm SD. The number of mice analyzed: *Cbfb2*^{+/+}, n = 3; *Cbfb2*^{-/-}, n = 3; *Runx2*^{+/+}, n = 3; *Runx2*^{+/-}, n = 4. Two regions were counted in each mouse.

age	<i>Cbfb2</i> ^{+/+} number (%)	<i>Cbfb2</i> ^{+/-} number (%)	<i>Cbfb2</i> ^{-/-} number (%)
E15.5-E18.5	148 (25)	297 (51)	137 (24)
P0-P2	114 (27)	215 (52)	87 (21)
4w	87 (29)	182 (60)	32 (11)

Supplementary Table S1.

The numbers and percentages of live *Cbfb2*^{+/+}, *Cbfb2*^{+/-} and *Cbfb2*^{-/-} mice obtained in the crossing of *Cbfb2*^{+/-} littermates during the embryonic stage (E15.5-E18.5), newborn stage (P0-P2), and at 4 weeks of age.

Supplementary Table S2.

Primer sequences for real-time RT-PCR :

	Forward	Reverse
	5'-----3'	5'-----3'
<i>Actb</i>	CCACCCGCGAGCACAGCTTC	TTGTCGACGACCAGCGCAGC
<i>Col2a1</i>	ATCTGGTTTGGAGAGACCAT	CTCTACATCATTGGAGCCCT
<i>Col10a1</i>	ATATGCTGCCTCAAATACCC	CTCTTATGGCGTATGGGAT
<i>Mmp13</i>	CTTCTGGCACACGCTTTTCC	ATGGGAAACATCAGGGGCTCC
<i>Runx2</i>	AACAAGACCCTGCCCCGTG	TGAAACTCTTGCCTCGTCCG
<i>Sp7</i>	AGGCACAAAGAAGCCATAC	AATGAGTGAGGGAAGGGT
<i>Alpl</i>	CGCACGCGATGCAACACCAC	TGCCCACGGACTTCCCAGCA
<i>Spp1</i>	GCAGAATCTCCTTGCGCCAC	CGAGTCCACAGAATCCTCGC
<i>Ibsp</i>	TGGAGACGGCGATAGTTC	CTAGCTGTTACACCCGAGAG
<i>Col1a1</i>	CCTGGAATGAAGGGACACCG	CCATCGTTACCGCGAGCACC
<i>Bglap&Bglap2</i>	ACTCCGGCGCTACCTTGAGGCC	GCAGGGTTAAGCTCACACTG
<i>Runx1</i>	CGGCCATGAAGAACCAGGTA	TGGTAGGTGGCAACTTGTGG
<i>Runx3</i>	AACCAAGTGGCCAGGTTCAA	TGATGGCTCGGTGGTAGGTA
<i>Cbfb1</i>	GCTCCCATGATTCTGAATGG	TTGCTGTCTTCTTGCCTCCA
<i>Cbfb2</i>	ATCTCCACAGATTGGATGGT	TGCTGTCTTCTTGCCAGTTA
<i>Cbfb1+2</i>	TTAGAGAGAGAAGCAGGCAA	TCTTCTTCGAGCCTCTTCAA
<i>Runx2(Sup)</i>	TCCACCACGCCGCTGTCT	TCAGTGAGGGATGAAATGCT