Conditional knockout of Cdc20 impairs bone formation and blunts bone regeneration, related to Fig 1.

A The design strategy of conditional deletion of Cdc20 gene.
B Representative image of PCR genotypes of indicated mice. Sp7-Cre:Cdc20f/f mice were in experimental groups, Cdc20f/f mice were in control groups.
C Representative micro-CT images and H&E staining of trabecular bone from the femoral metaphysis of 12-week-old male Sp7-Cre:Cdc20f/f and littermate control mice. Scale bar, 500 µm.
D Histomorphometric analyses of 12-week-old male femurs (n = 6).
E Representative micro-CT images and H&E staining of trabecular bone from the femoral metaphysis of 12-week-old female Sp7-Cre:Cdc20f/f and littermate control mice. Scale bar, 500 µm.
F Histomorphometric analyses of 12-week-old female femurs (n = 6).

Data information: Data are displayed as mean ± SD and show one representative of n ≥ 3 independent experiments with three biological replicates. Statistical significance was calculated by a two-tailed unpaired Student’s t-test and defined as *P < 0.05; **P < 0.01.
Figure EV1.
Figure EV2. CDC20 modulates osteogenic differentiation of BMSCs, related to Fig 2.

- Western blot analyses (A) and qRT–PCR (B–D) of the expression of CDC20 and osteogenic marker RUNX2, OCN. Cells were cultured in osteogenic medium for 7 and 14 days (n = 6).
- The knockout efficiency of Cdc20 (E) and the expression of osteogenic marker Runx2 (F) in BMSCs of Sp7-Cre;Cdc20fl/fl and Cdc20fl/fl mice determined by qRT–PCR (n = 5).
- Representative images of light and fluorescence of lentivirus infected NC and CDC20sh hBMSCs. Scale bar: 500 μm.
- The knockdown efficiency of CDC20 in NC and CDC20sh hBMSCs determined by qRT–PCR (n = 5).
- The expression of RUNX2 in NC and CDC20sh hBMSCs after 7 days osteogenic differentiation determined by qRT–PCR (I) and Western blot analyses (J) (n = 5).
- Western blot analyses of Myc-CDC20, Myc-CDC20 171–499 fragment (containing WD40 domain), Myc-CDC20 1–170 fragment (lacking WD40 domain) plasmids expression in HEK293T cells.
- Western blot analyses of the degradation of the substrate Cyclin B1 under the overexpression of truncated fragments of CDC20.

Data information: Data are displayed as mean ± SD and show one representative of n ≥ 3 independent experiments with three biological replicates. Statistical significance was calculated by a two-tailed unpaired Student’s t-test or one-way ANOVA followed by a Tukey’s post hoc test and defined as ***P < 0.001.
Figure EV2.
Figure EV3. CDC20 induces proteasome-dependent degradation of p65 and CDC20 interacts with p65, related to Figs 3 and 4.

A–D  The expression of CDC20 (A) and NF-kB pathway downstream genes IL-8, IL-6, and ICAM1 (B–D) of NC and CDC20si HEK293T cells after TNF-α stimulation determined by qRT-PCR (n = 6).

E  Western blot analyses of the degradation of endogenous p65 protein in NC and CDC20si HEK293T cells.

F  Western blot analyses of the degradation of p65 protein after the overexpression of Myc-CDC20. HEK293T cells were transfected with Vector and Myc-CDC20 plasmids for 36 h, cells were treated with or without 10 µM MG132 (the proteasome inhibitor) for 6 h before collected.

G, H  Co-immunoprecipitation of endogenous CDC20 with endogenous p65 in hBMSCs.

I, J  Co-immunoprecipitation of endogenous CDC20 with endogenous p65 in mBMSCs.

K  The co-localization of CDC20 and p65 in hBMSCs. Scale bar: 20 µm.

Data information: Data are displayed as mean ± SD and show one representative of n ≥ 3 independent experiments with three biological replicates. Statistical significance was calculated by one-way ANOVA followed by a Tukey's post hoc test and defined as ***P < 0.001.
Figure EV4. CDC20 regulates p65 degradation in an APC11-dependent manner, related to Fig 6.

A, B No interaction was found in the immunoprecipitation of p65 and APC2 in HEK293T cells.

C, D The expression of p65 remained stable under the knockdown (C) or overexpression (D) of APC2 in HEK293T cells determined by Western blot analyses.

E, F No change of p65 protein (E) and CDC20 protein (F) were seen in NC and CDH1sh HEK293T cells.

G The interaction of APC11 and p65 remained stable in NC and CDH1sh HEK293T cells.

H The knockdown efficiency of APC11 in hBMSCs was determined by qRT–PCR (n = 6).

Data information: Data are displayed as mean ± SD and show one representative of n ≥ 3 independent experiments with three biological replicates. Statistical significance was calculated by one-way ANOVA followed by a Tukey’s post hoc test and defined as ***P < 0.001.
Figure EV5. CDC20 regulated osteogenic differentiation of BMSCs in a p65-dependent manner, related to Fig 8.

A  Fluorescent staining of lentiviruses injected from tail intravenously.
B, C  The efficiency of p65 knockdown determined by qRT–PCR (B) and Western blot (C) of BMSCs in Sp7-Cre;Cdc20<sup>f/f</sup> mice (n = 6).
D, E  The ALP staining (D) and ALP quantification (E) of control and CDC20 knockdown hBMSCs after 7 days osteogenic differentiation treated with negative control or p65si RNA (n = 5).
F, G  The ALP staining (F) and ALP quantification (G) of NC and CDC20sh hBMSCs after 7 days osteogenic differentiation treated with BAY 11–7082 (n = 6).
H  The expression of RUNX2 in BMSCs from Cdc20<sup>f/f</sup> mice and Sp7-Cre;Cdc20<sup>f/f</sup> mice after 7 days osteogenic differentiation treated with negative control or p65si, determined by qRT–PCR (n = 5).

Data information: Data are displayed as mean ± SD and show one representative of n ≥ 3 independent experiments with three biological replicates. Statistical significance was calculated by one-way ANOVA followed by independent two-tailed Student’s t-tests or a Tukey’s post hoc test and defined as *P < 0.05, **P < 0.01, ***P < 0.001.