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Supplemental information

CircHGF suppressed cell proliferation

and osteogenic differentiation of BMSCs in ONFH

via inhibiting miR-25-3p binding to SMAD7

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Supplemental Material

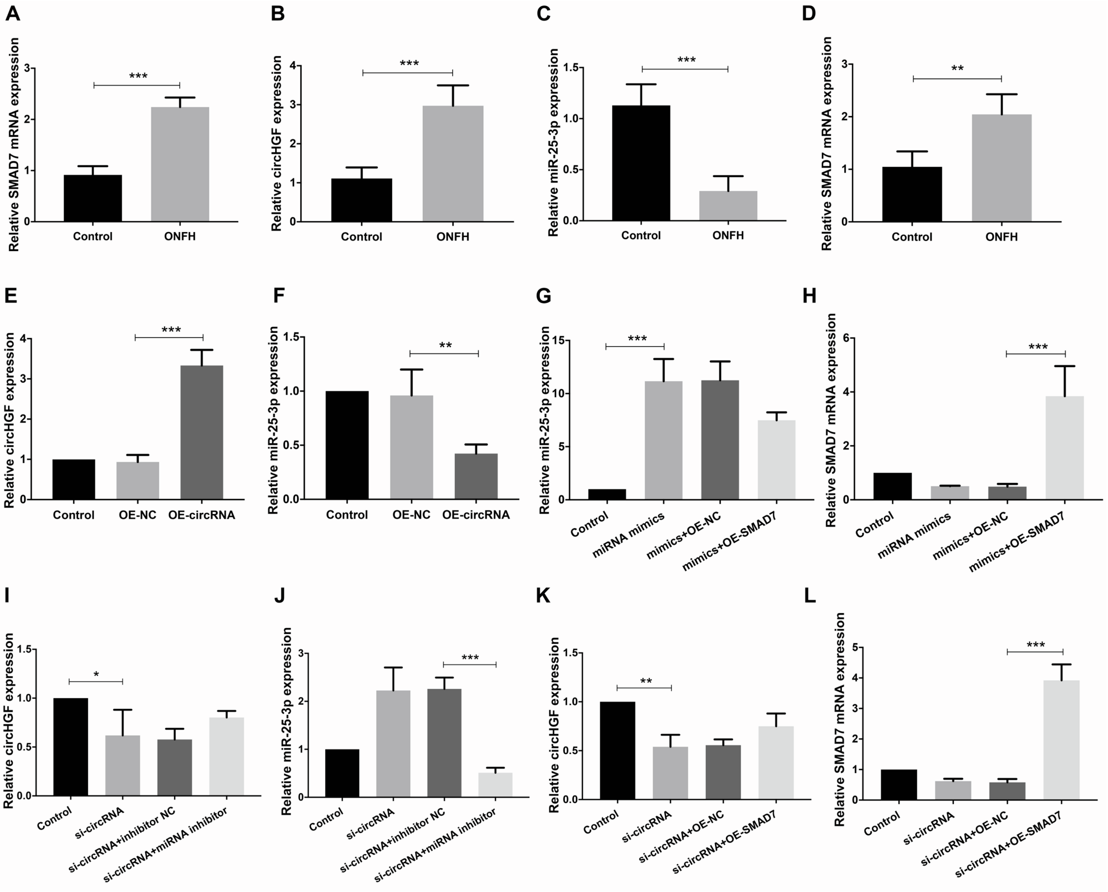

Figure S1. CircHGF functioned in BMSCs by targeting miR-25-3p and SMAD7

(A) The relative expression levels of SMAD7 mRNA in ONFH-BMSCs and control BMSCs samples were assessed by RT-qPCR analysis. $^{***}p < 0.001$, n=3. (B-D) The relative expression levels of circHGF, miR-25-3p, and SMAD7 mRNA in the blood samples of ONFH group and femoral neck fracture group (control) were assessed by RT-qPCR analysis, $^{**}p < 0.01$, $^{***}p < 0.001$, n=5. (E-F) The BMSCs were treated with OE-circRNA or corresponding controls, and the relative expression levels of circHGF and miR-25-3p in each group were determined by RT-qPCR analysis. $^{**}p < 0.01$, $^{***}p < 0.001$, n=3. (G-H) The BMSCs were treated with miR-25-3p mimics, mimics +

OE-NC, or mimics + OE-SMAD7, and the relative expression levels of miR-25-3 and SMAD7 mRNA in each group were examined by RT-qPCR analysis. $^{***}p < 0.001$, n=3. (I-J) The BMSCs were treated with si-circRNA, si-circRNA + inhibitor NC, or si-circRNA + miRNA inhibitor, and the relative expression levels of circHGF and miR-25-3p in each group were determined by RT-qPCR analysis. $^{*}p < 0.05$, $^{***}p < 0.001$, n=3. (K-L) The BMSCs were treated with si-circRNA, si-circRNA + OE-NC, or si-circRNA + OE-SMAD7 vector, and and the relative expression levels of circHGF and SMAD7 mRNA in each group were determined by RT-qPCR analysis. $^{**}p < 0.01$, $^{***}p < 0.001$, n=3. Data between two groups were analyzed by Student's t test. Data among multiple groups were analyzed by one-way ANOVA test.

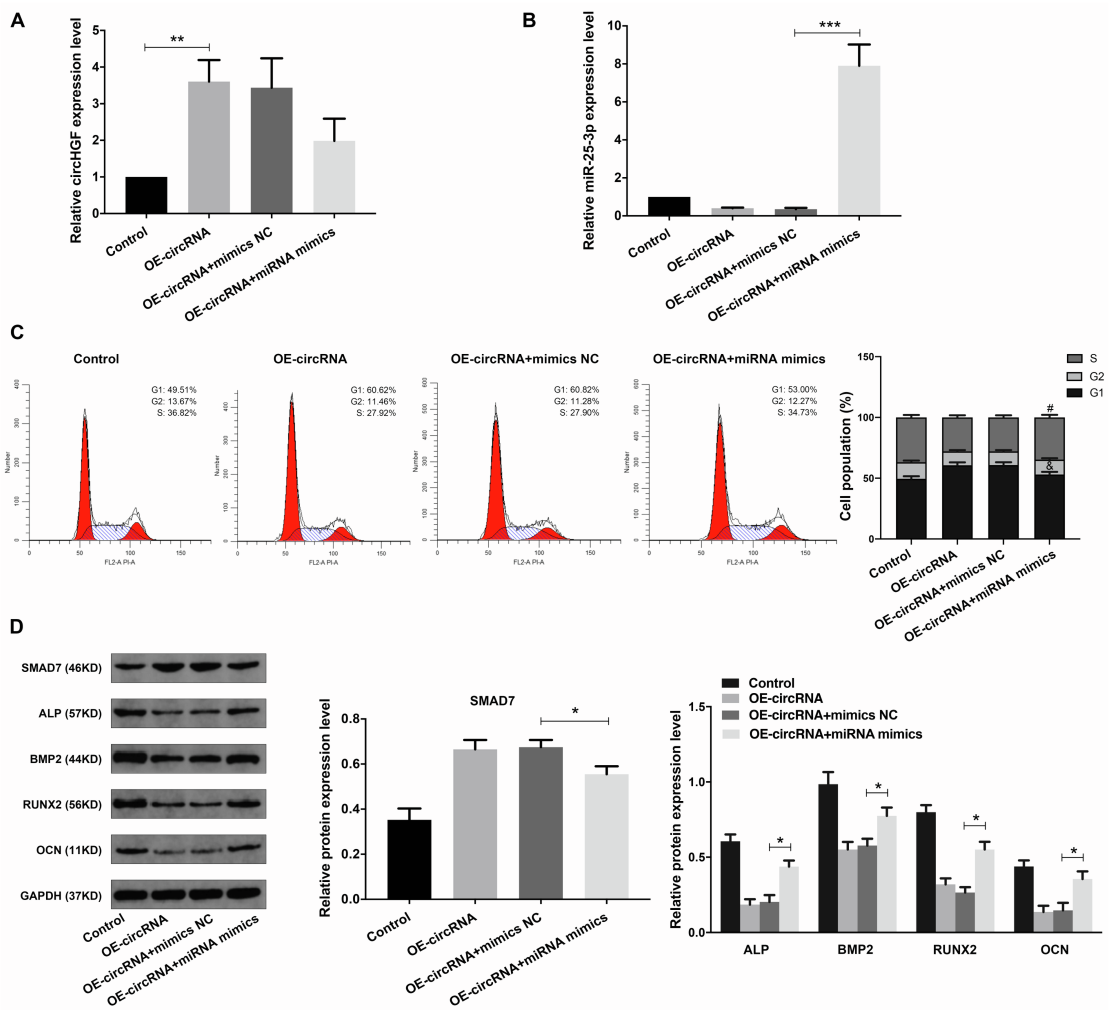

Figure S2. CircHGF functioned in BMSCs by targeting miR-25-3p

(A) The BMSCs were treated with OE-circRNA, OE-circRNA + mimics NC, or OE-circRNA + miR-25-3p mimics, and the relative expression levels of circHGF were examined by RT-qPCR analysis. (B) The BMSCs were treated with OE-circRNA, OE-circRNA + mimics NC, or OE-circRNA + miR-25-3p mimics, and the miR-25-3p expression level in each group was determined by RT-qPCR analysis. (C) The cell proliferation ability of BMSCs in each group was determined by the cell cycle analysis, with the cell population of G1, G2 and S cell cycle phase indicated. $^{\#}p < 0.05$ versus the OE-circRNA + mimics NC group for comparing cell percentage in S phase, $^{\&}p < 0.05$ versus the OE-circRNA + mimics NC group for comparing cell percentage in G1 phase, n = 3. (D) The protein expression levels of SMAD7, ALP, BMP2, RUNX2 and OCN in each group were evaluated by western blot analysis. The quantitative analysis showed the SMAD7 expression level was decreased, and the expression levels of ALP, BMP2, RUNX2 and OCN were increased, in OE-circRNA + miR-25-3p mimics group compared with OE-circRNA + mimics NC group. Data were shown as mean ± SD. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, n=3. Data among multiple groups were analyzed by one-way ANOVA test.

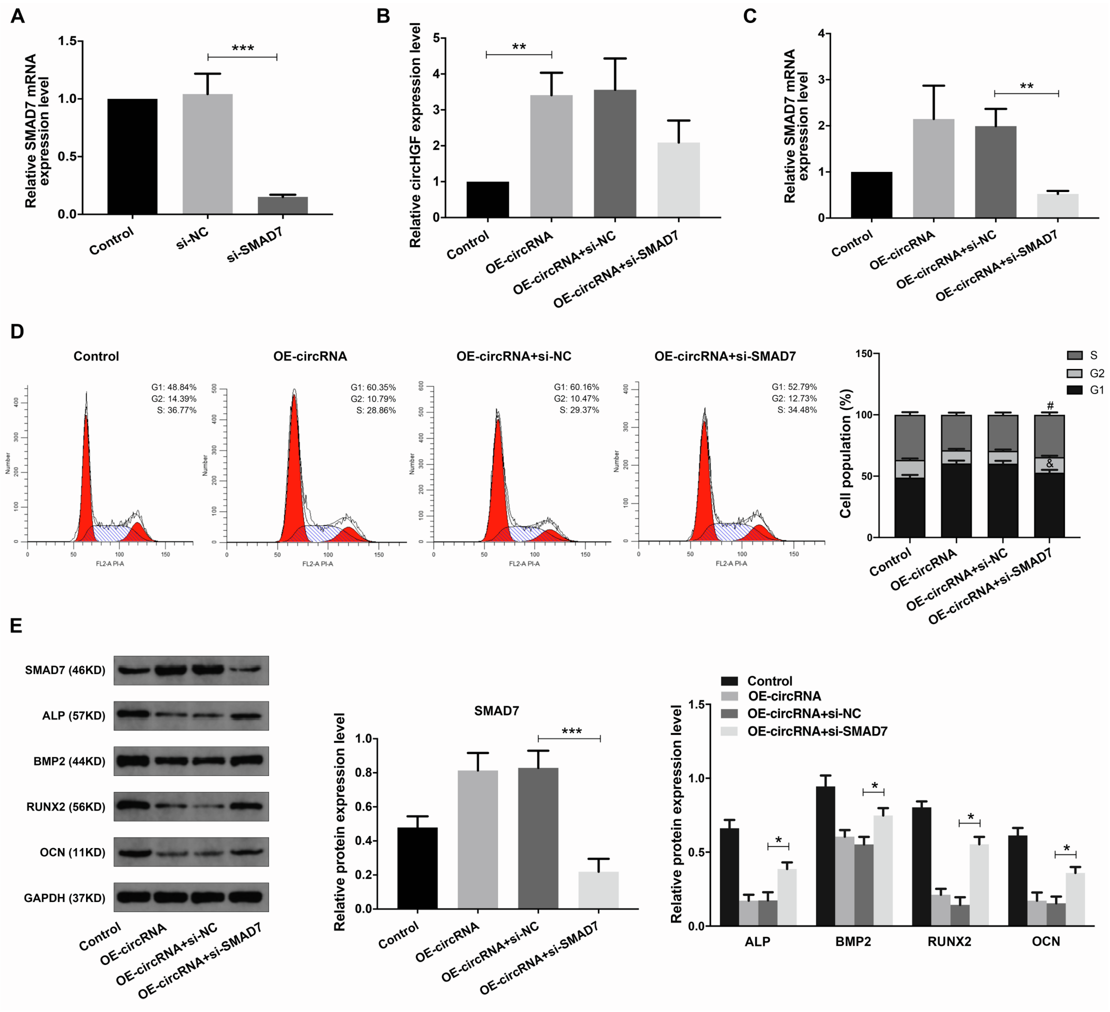

Figure S3. CircHGF functioned in BMSCs by targeting miR-25-3p and SMAD7

(A) The SMAD7 mRNA expression level in BMSCs treated with SMAD7 siRNA was validated by RT-qPCR analysis. (B) The BMSCs were treated with OE-circRNA, OE-circRNA + si-NC, or OE-circRNA + si-SMAD7, and the relative expression levels of circHGF were examined by RT-qPCR analysis. (C) The mRNA expression level of SMAD7 in each group was determined by RT-qPCR analysis. (D) The cell proliferation ability of BMSCs in each group was determined by cell cycle analysis, with the cell population of G1, G2 and S cell cycle phase indicated. $^{\#}p < 0.05$ versus the OE-circRNA + si-NC group for comparing cell percentage in S phase, $^{\&}p < 0.05$ versus the OE-circRNA + si-NC group for comparing cell percentage in G1 phase, n = 3. (E) The protein expression levels of SMAD7, ALP, BMP2, RUNX2 and OCN in each group were evaluated by western blot analysis. Quantitative analysis showed the SMAD7 protein expression was decreased, and the expression levels of ALP, BMP2, RUNX2 and OCN were increased in OE-circRNA + si-SMAD7 group compared with OE-circRNA + si-NC group. Data were shown as mean ± SD. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, n=3. Data among multiple groups were analyzed by one-way ANOVA test.