Myokine Irisin promotes osteogenesis by activating BMP/SMAD signaling via $\alpha V$ integrin

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Supplementary Figure 1: Genotype identification of FNDC5<sup>−/−</sup> mice. HO stood for homozygous, 481bp; WT stood for wild type, 1210bp; HE stood for heterozygote, 481bp&1210bp.

Supplementary Figure 2: Effects of different concentrations of r-irisin on the proliferation of BMSCs. (A)BMSCs were cultured under the intervention of 0.1, 1, 5, 10, 20ng/mL r-irisin, respectively, and cell viability was measured by CCK-8 on the 1st, 3rd, and 5th days. (B)Live/Dead staining on the 3rd day.
Supplementary Figure 3: (A) Western blot of OPN and RUNX2 after the intervention of recombinant irisin on osteogenic induction of BMSCs in WT mice for 7 days. (B) Quantitative analysis of Western blot. (n=5, **$P<0.01$, ***$P<0.001$, ****$P<0.0001$).

Supplementary Figure 4: Western blot analysis of RUNX2 and OPN after osteogenic induction for 7 days in BMSCs of WT and FNDC5−/− mice. (B) Quantitative analysis of Western blot. (n=5, *$P<0.05$, **$P<0.01$, ****$P<0.0001$).
Supplementary Figure 5: (A) Quantitative analysis of WB of Protein expression levels of integrin αV, p-Erk1/2, p-STAT3, BMPR2, p-Smad1/5/9, and Smad4 of BMSCs induced into osteoblasts for 3 days in the presence of r-irisin and SB273005. (B) Quantitative analysis of WB of Protein expression levels of BMP2 and Smad4 after 3 days of osteogenic induction of BMSCs treated with r-irisin. (n=5, *P < 0.05, **P < 0.01, ***P <0.001, ****P <0.0001).