

Fig. S1 Loss- and gain-of-Ythdf1 function on osteogenesis in mouse BMSCs.

(A and B) Ythdf1 mRNA and protein levels detected 72 h after transfection of pLVX-Ythdf1. *** $P < 0.01$ compared with pLVX-Vector. (C) ALP and Alizarin Red staining to detect osteogenesis of BMSCs after pLVX-Ythdf1 transfection for 7 or 14 days. (D) mRNA expression levels of Alp, Ocn, Osterix and Runx2 detected 72 h after osteogenic induction. * $P < 0.05$, *** $P < 0.01$ compared with the NC and pLVX-Vector groups. (E) ALP and Alizarin Red staining of WT and Ythdf1 KO cells. (F) mRNA expression of osteoblast-specific genes detected in WT and Ythdf1 KO cells 72 h after osteogenic induction (Paired t test, $n=3$, * $P < 0.05$ and *** $P < 0.01$). All experiments were independently performed in triplicate.

Fig. S2 Zfp839 knockdown efficiency verification.

Three shRNAs were generated to suppress Zfp839 expression. (A and B) Zfp839 mRNA and protein expression levels detected 72 h after shRNA transfection. (Paired t test, $n=3$, * $P < 0.05$, *** $P < 0.01$ compared with the NC and NC-shRNA groups). All experiments were independently performed in triplicate.

Fig. S3 Co-localization of Ythdf1 and Zfp839 in bone marrow.

(A) Immunofluorescence staining showing that Ythdf1 and Zfp839 co-localized in bone marrow in WT group, which cannot be shown in Ythdf1 KO group. Scale bar = 20 μm .

Fig. S4 Zfp839 overexpression potentiates mouse BMSCs osteogenesis.

(A and B) Zfp839 mRNA expression and protein levels detected 72 h after transfection of pLVX-Zfp839. (Paired t test, n=3, *** $P < 0.01$ compared with pLVX-Vector). **(C)** mRNA expression of osteogenic-specific genes detected 72 h after osteogenic induction of Zfp839 overexpression, (Paired t test, n=3, * $P < 0.05$, # $P < 0.01$ versus NC or pLVX-Vector samples). **(D)** ALP and Alizarin Red staining performed to detect the osteogenesis of mouse BMSCs after Zfp839 overexpression. All experiments were independently performed in triplicate.