

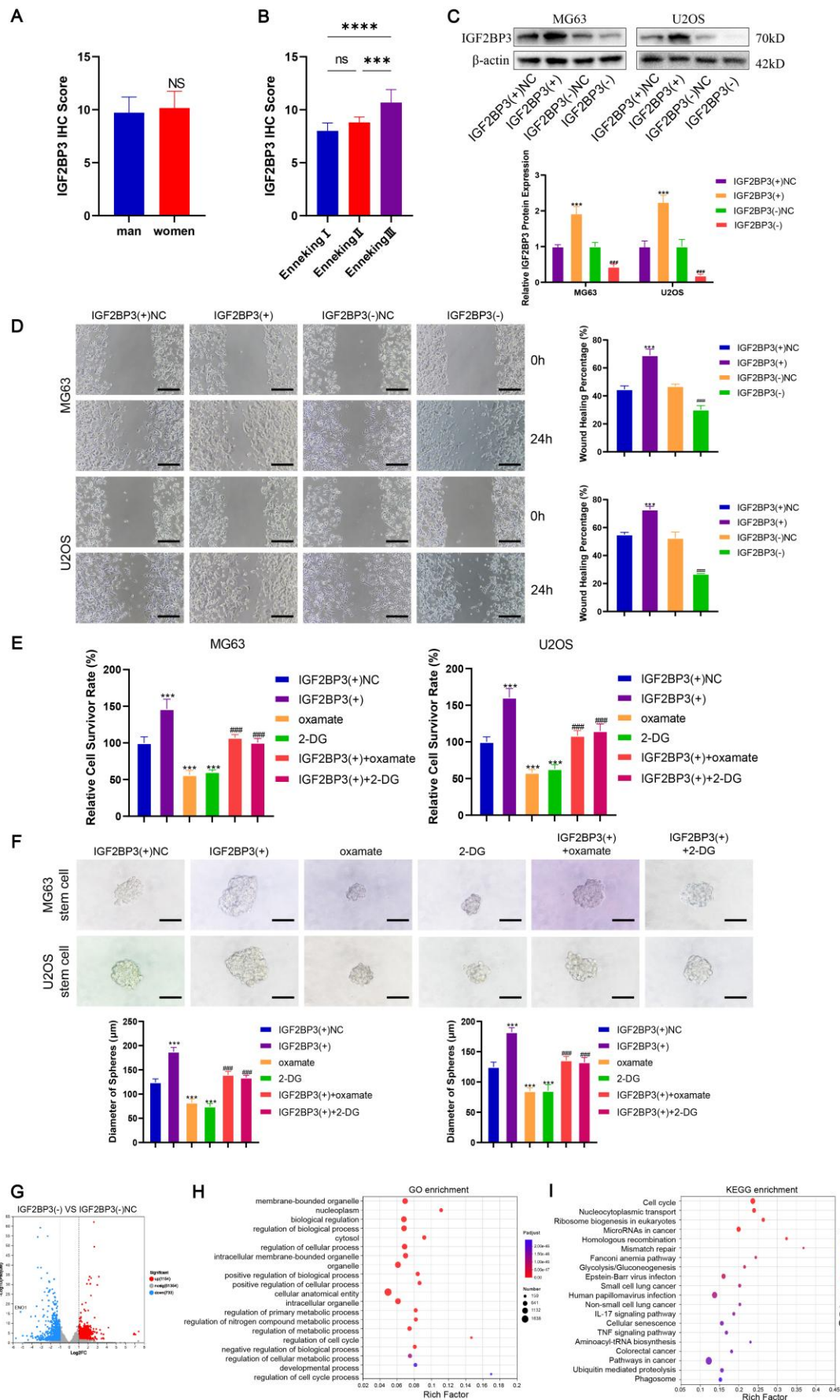


Fig S1 IGF2BP3 promotes the stemness and glycolysis of osteosarcoma. A
Immunohistochemical assessment of IGF2BP3 expression reveals sex-related
differences in osteosarcoma (OS). **B** Expression of IGF2BP3 in Enneking stages I to
III of OS as assessed by immunohistochemistry. **C** The knockdown and
overexpression efficiencies of IGF2BP3 were evaluated by Western blotting. **D** The
cell scratch wound healing assay was used to investigate the effect of IGF2BP3
overexpression or knockdown on cell migration ability (Scale bars, 100µm). **E** The
effect of IGF2BP3 combined with oxamate or 2-DG on cisplatin resistance was
evaluated using the CCK8 assay. **F** Effects of IGF2BP3 combined with oxamate or 2-
DG on self-renewal capacity assessed using serial sphere formation assay (Scale bars,
100µm). **G** Volcano plot of differentially expressed genes comparing IGF2BP3(-) vs.
IGF2BP3(-) NC. **H** GO enrichment analysis of differentially expressed genes
comparing IGF2BP3(-) vs. IGF2BP3(-) NC. **I** KEGG enrichment analysis of
differentially expressed genes comparing IGF2BP3(-) vs. IGF2BP3(-) NC.

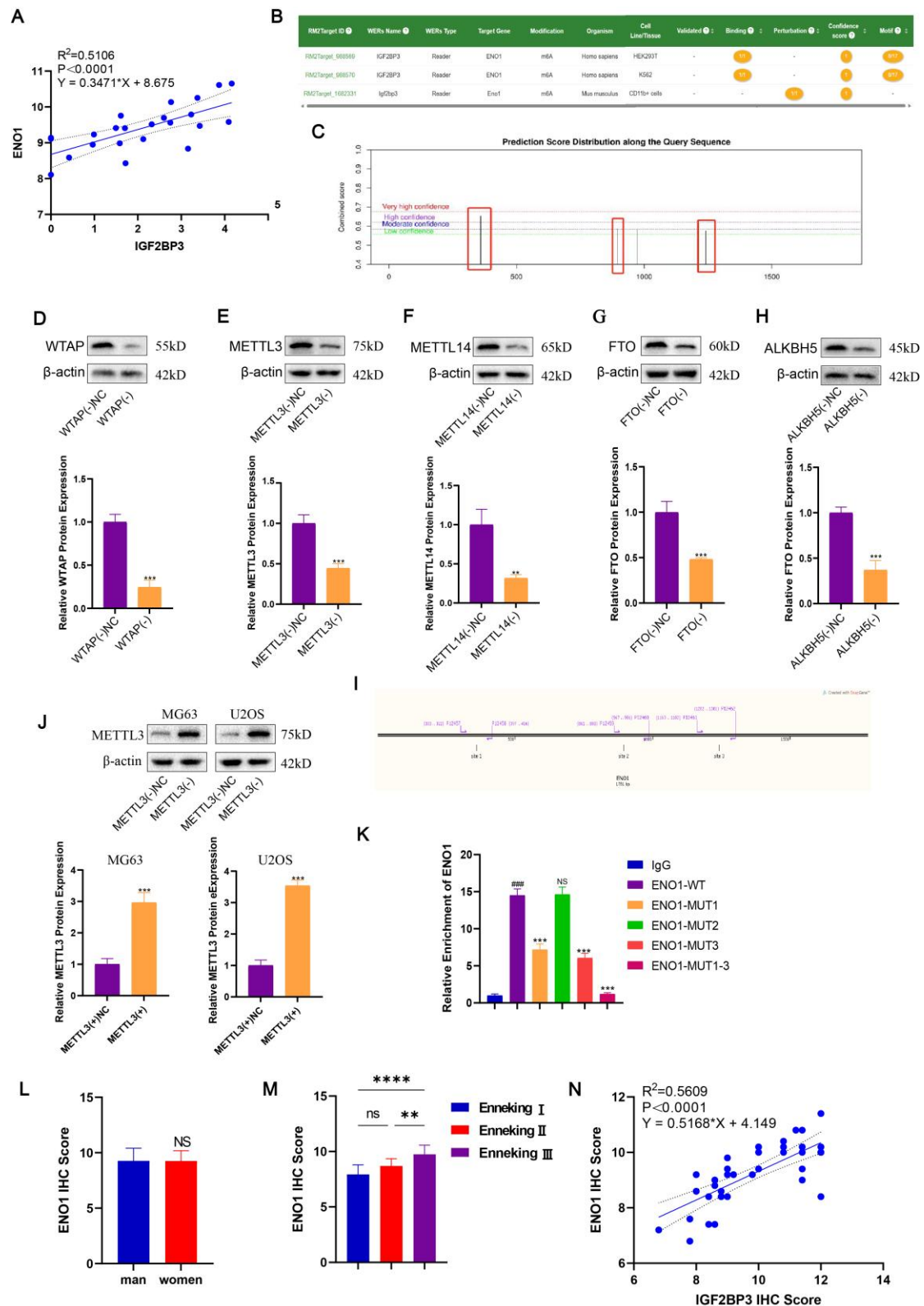


Fig S2 Exploration of m⁶A modification enzymes and readers on ENO1 mRNA.

A Correlation analysis between the expression levels of IGF2BP3 and ENO1 in OS

19 from the GEO database (GSE218035). **B** The RM2Target platform was used to
20 predict the binding relationship between IGF2BP3 and ENO1 mRNA. **C** The enriched
21 and specific m⁶A peak distribution of ENO1 transcripts predicted by SRAMP. Red
22 circles indicate enrichment peaks in m⁶A. **D-H** The knockdown efficiency of WTAP,
23 METTL3, METTL14, FTO and ALKBH5 was evaluated by Western blotting. **I**
24 Specific primers were designed according to the m⁶A enrichment site on the ENO1
25 transcript. **J** The expression of ENO1 in MG63, U2OS, MG63 stem cell and U2OS
26 stem cell detected using western blotting. **K** An enrichment of ENO1-WT and ENO1-
27 MUT in IGF2BP3 immunoprecipitated samples via RNA immunoprecipitation (RIP)
28 assay (n=3) in HEK-293T. **L** Immunohistochemical assessment of IGF2BP3
29 expression reveals sex-related differences in osteosarcoma (OS). **M** Expression of
30 IGF2BP3 in Enneking stages I to III of OS as assessed by immunohistochemistry. **N**
31 Correlation analysis of immunohistochemical scores of IGF2BP3 and ENO1 in
32 clinical specimens.

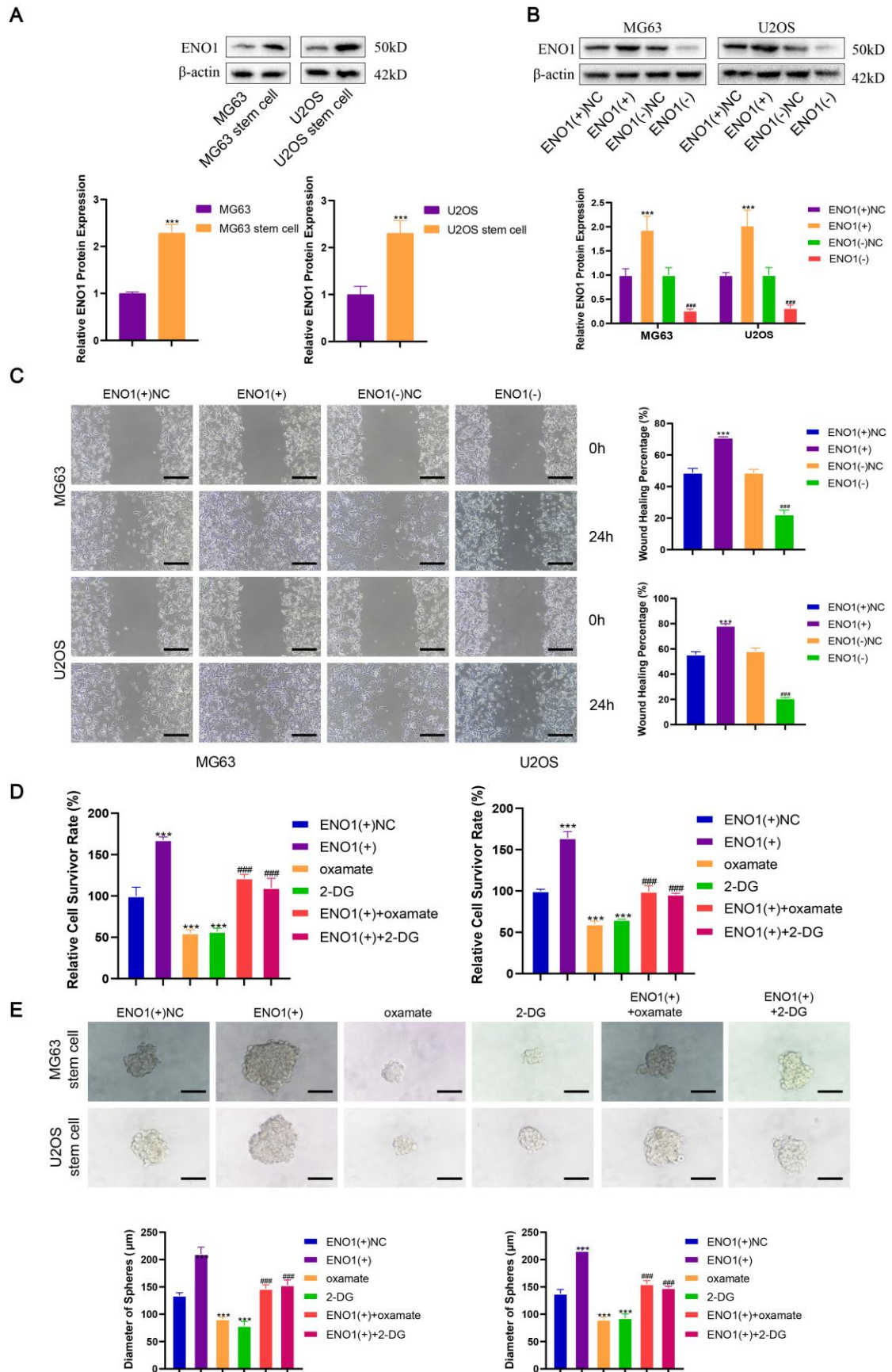


Fig S3 ENO1 promotes the stemness and glycolysis of osteosarcoma. **A** The expression of ENO1 in MG63, U2OS, MG63 stem cell and U2OS stem cell detected using western blotting. **B** The knockdown and overexpression efficiencies of ENO1 were evaluated by Western blotting. **C** The cell scratch wound healing assay was used to investigate the effect of ENO1 overexpression or knockdown on cell migration ability (Scale bars, 100μm). **D** The effect of ENO1 combined with oxamate or 2-DG on cisplatin resistance was evaluated using the CCK8 assay. **E** Effects of ENO1 combined with oxamate or 2-DG on self-renewal capacity assessed using serial sphere formation assay (Scale bars, 100μm).

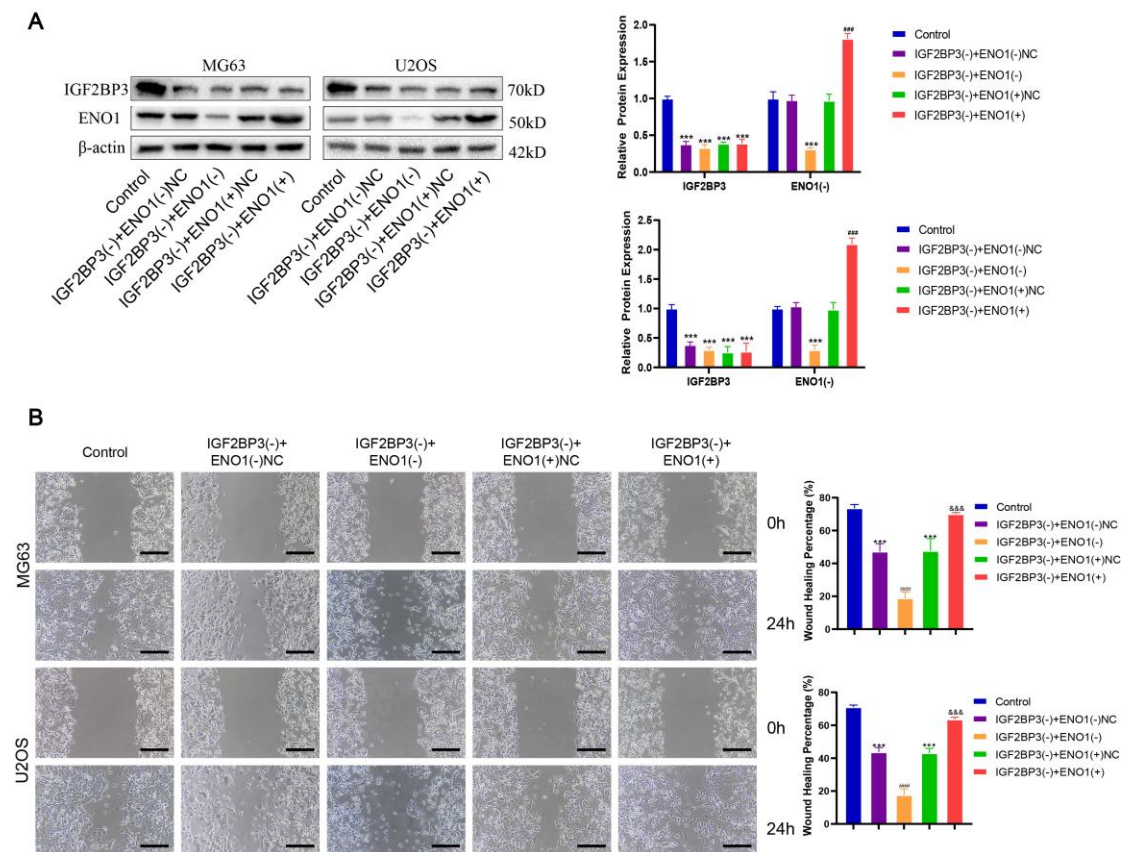


Fig S4 The combined effect of IGF2BP3 and ENO1 on cell migration ability. A

The efficiency of co-transfection of IGF2BP3 and ENO1 was evaluated by Western

46 blotting. **B** The cell scratch wound healing assay was used to investigate the effect of
47 the combined alteration of IGF2BP3 and ENO1 on cell migration ability (Scale bars,
48 100μm).

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