

Supplementary information, Figure S2 (Related to Figure 1) Validation of WTAP and METTL14 as METTL3 associating proteins. (A) Lysates from 293T cells cotransfected with Flag-WTAP and Myc-METTL3 were immunoprecipitated by Flag-beads and blotted with indicated antibodies. (B) 293T cells were cotransfected with Myc-METTL3 and HA-METTL14 constructs as indicated. Cell lysates were subjected to HA-IP and immunoblotted with the indicated antibodies. (C) 293T cells were treated with 0, 10, and 20 mM m6A cycloleucine for 24 hrs. 200 ng mRNA was extracted and spotted onto nylon membrane and m6A levels were detected with anti-m6A antibody. The lower panel is methylene blue staining to verify equal loading of the RNA samples. (D) 293T cells were cotransfected with Myc-METTL3 and Flag-WTAP for 4 hrs and treated with 20 mM cycloleucine for 24 hrs. Cell lysates were immunoprecipitated with Myc-beads and blotted with the indicate antibodies. (E) Lysates from 293T cells cotransfected with Myc-METTL3 and Flag-WTAP were treated with either RNase A or RNAse A inhibitor as indicated, then subjected to immunoprecipitation with myc-beads and immunoblotted with the indicated antibodies.