

**Supplementary information, Figure S4** YTHDF3 regulates binding specificity and affinity of YTHDF1 and YTHDF2 towards target RNAs. (**A**) Quantification of Flag-HA YTHDF1 (top) and Flag-HA YTHDF2 (bottom) RNA immunoprecipitation efficiency upon depletion of endogenous YTHDF3. Representative target transcripts co-immunoprecipitated with Flag-HA-YTHDF1 or Flag-HA-YTHDF2 were quantified with RT-qPCR and normalized to their amounts in input RNA. Error bars, mean  $\pm$  s.d., n = 2, technical replicates. (**B**) Western blotting showing that knockdown of YTHDF3 leads to upregulated protein levels of YTHDF1 and YTHDF2 in wild-type HeLa cells. (**C**) Total RNAs bound by YTHDF3 quantified with PAR-CLIP followed by  $5^{\circ}$ - $^{32}$ P labelling in the control HeLa cells and cells depleted of either YTHDF1 or YTHDF2 (left panel). Western blot showing knockdown efficiency of the siYTHDF1 and siYTHDF2 oligos (right panel).