



**Figure S1** Supporting figure for validation of YTHDF3 partners. **(A)** 293T cells were transfected with Flag-SFB-YTHDF1 or Flag-SFB-YTHDF3 plasmids for 48 h. Cell lysates were precipitated first with Streptavidin sepharose beads and then precipitated with S-protein agarose beads. SDS-PAGE was applied and protein bands were visualized by Coomassie blue staining (Left panel). Western blotting (anti-Flag antibody) showed Flag-SFB-YTHDF1 and Flag-SFB-YTHDF3 pull down efficiency (Right panel). **(B)** PAR-CLIP of YTHDF1 protein in control and YTHDF3-depleted HeLa cells transfected with Flag-SFB-YTHDF1 plasmid. The pull-down RNA products were labeled with biotin at 3' end of RNA (Biotinylation Kit, Thermo) and

then visualized by the Chemiluminescent nucleic acid detection module. **(C)** Coomassie brilliant blue staining of purified GST-YTHDF3, Flag-RPLP0, Flag-RPL3, Flag-RPS2, Flag-RPS3 and Flag-RPS15. Protein size markers were labeled on each gel. **(D)** Different antibodies against RPS3, RPS15, RPLP0, RPL3 and YTHDF3 were used to validate the purity of the FLAG-tagged proteins by western blotting. Protein size markers were labeled on each gel. **(E)** *In vitro* pull-down assay using purified GST-YTHDF3 and Flag-RPLP0, Flag-RPL3 revealed interactions between YTHDF3 and Flag-RPLP0, Flag-RPL3. Protein size markers were labeled on each gel. **(F)** *In vitro* pull-down assay using purified GST-YTHDF3 and Flag-RPS2, Flag-RPS3, Flag-RPS15 revealed interactions between YTHDF3 and Flag-RPS2/3/15. Protein size markers were labeled on each gel.