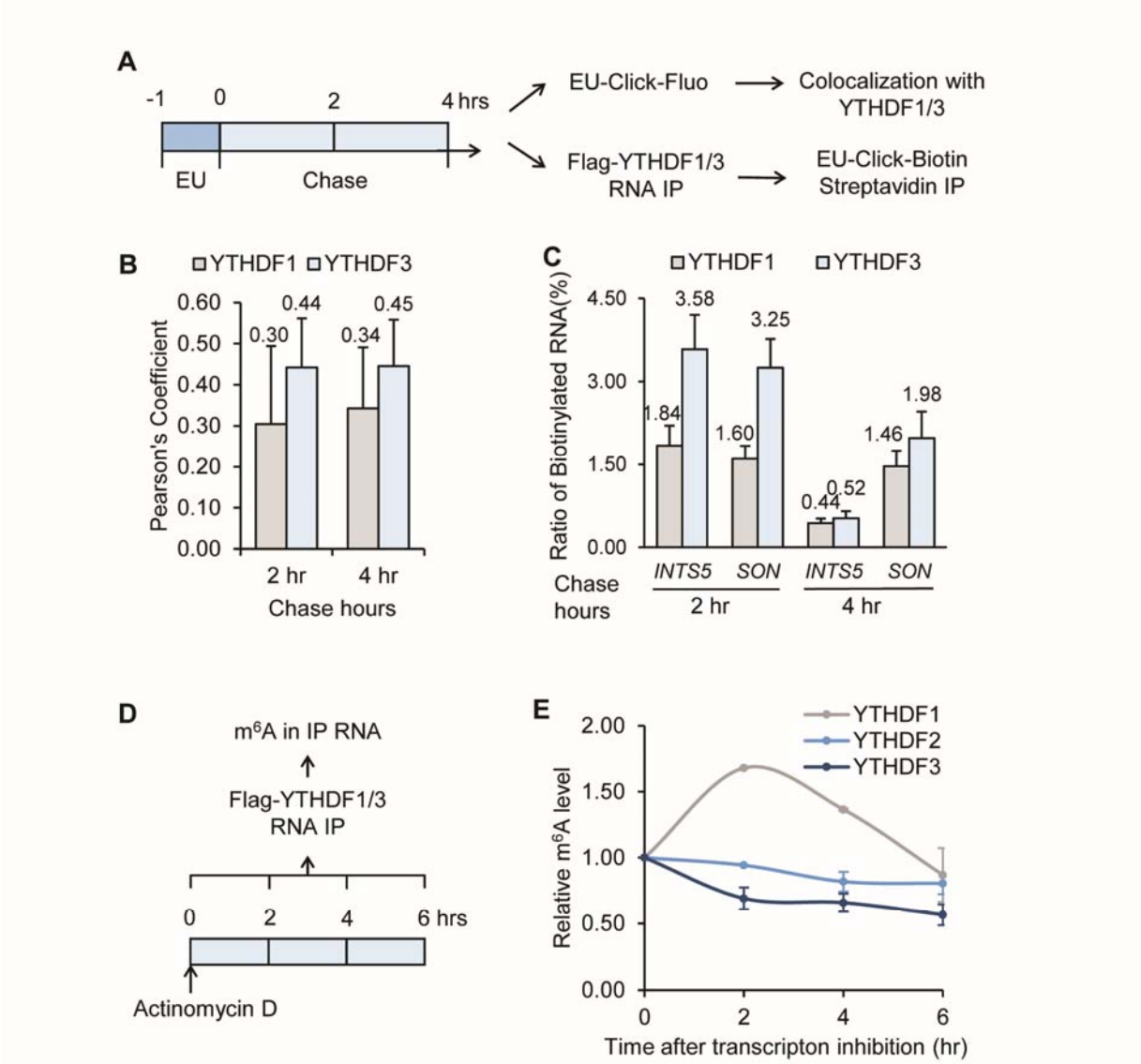

Supplementary information, Figure S5 Temporal order of YTHDFs' interaction with m^{6}A-modified transcripts. **(A)** Scheme of experimental designs for nascent RNA labelling with EU (ethynyl-uridine). HeLa cells were treated with EU for 1 hour. Then 2-hour and 4-hour post labelling, cells were analyzed for either co-localization between EU-labelled RNA and YTHDF proteins, or percentage of a specific nascent transcript in the RNA bounded by each YTHDF, as reported previously[13]. **(B)** Pearson's coefficient quantification of the co-localization between EU-labelled RNA and YTHDF proteins 2-hour or 4-hour post EU-labelling. Error bars, mean ±

s.d., n = 8. **(C)** YTHDF1- or YTHDF3-bound RNAs (input) were first purified with anti-Flag IP, and subjected to Click reaction to label EU with biotin. Nascent (biotinylated) RNAs were captured by Streptavidin-conjugated magnetic beads. For YTHDF1 and YTHDF3 common targets, their amount in the input and the eluent of Streptavidin pull down were determined by RT-qPCR, to obtain the percentage of their nascent transcripts in all their transcripts bound by YTHDF1 or YTHDF3. A higher percentage of the nascent common transcripts was bound by YTHDF3 than YTHDF1 at both time points. Error bars, mean ± s.d., n = 2, technical replicates. **(D)** Scheme of the experimental design for examining the flow of m^6A-modified RNA among YTHDF proteins. HeLa cells were treated with 5 μg/ml actinomycin D (Act D) to stop transcription, and cells were collected 0, 2, 4, 6 hrs post Act D treatment. YTHDF1-3-bound RNAs were purified by anti-Flag IP, and the m^6A level of the bound RNAs was quantified. **(E)** LC-MS/MS quantification of m^6A level in YTHDF1-3-bound RNAs at different time points after transcription inhibition. The m^6A level was calculated as m^6A/G (%) and normalized to the value of 0 hr. Error bars, mean ± s.d., n = 2-4.