



**H**

GO term	P (FDR)	Genes
meiotic nuclear division	0.036	<i>Mei1, Tubgcp6, Mki67, Smc1a, Smc3, Smc1b</i>
lateral element	0.011	<i>Sycp2, Sycp1, Brca1, Smc3, Smc1b</i>

**Figure S2** (Supplement to Figure 1) YTHDC2 binds preferentially to m<sup>6</sup>A-marked RNA transcripts. **(A)** Gel shift assay measuring the dissociation ( $K_d$ , nM, indicated at the upper left corner of the gel) of FLAG-tagged YTHDC2 with methylated and unmethylated RNA probes. Protein concentrations are listed in nM. 4 nmol RNA probe was used. **(B)** *In vitro* probe pulldown assay of positive m<sup>6</sup>A-binding control YTHDF1 and negative m<sup>6</sup>A-binding control HUR in HeLa cells showing that YTHDF1 binds preferentially to probe with m<sup>6</sup>A, while HUR does not. GGYCU Probe sequence: 5'-CGUGGYCUGGCU-B-3' (Y=m<sup>6</sup>A or A, B=biotin) ACYGA Probe sequence: 5'-GAUACYGAGAAG-B-3' (Y=m<sup>6</sup>A or A, B=biotin). **(C)** *In vitro* probe pulldown assay of Ythdc2 in 16 d.p.p. mouse testes showing Ythdc2 binds preferentially to probe with m<sup>6</sup>A on GGACU. **(D)** Consensus motifs of biological replicates of Ythdc2 binding identified by HOMER of CLIP-seq of Ythdc2 in mouse testes. **(E)** Distribution of peak densities in CLIP-seq of Ythdc2 in mouse testes. **(F)** Overlap of Ythdc2 RIP-seq genes and genes containing m<sup>6</sup>A in young mouse testes. Targets of YTHDC2 are defined as genes enriched in the RIP:  $\text{Log}_2(\text{RIP}/\text{input}) \geq 1$ . Non-targets are defined as genes depleted in the RIP:  $\text{Log}_2(\text{RIP}/\text{input}) \leq -1$ . Genes with m<sup>6</sup>A are defined as genes enriched in the m<sup>6</sup>A IP:  $\text{Log}_2(\text{m}^6\text{A IP}/\text{input}) \geq 2$ . **(G)** Overlap of genes common to Ythdc2 RIP-seq in 16 d.p.p. mice and Ythdc2 CLIP-seq in adult mice. 140/144 (97.2%) of genes contain m<sup>6</sup>A, a higher proportion than genes identified by Ythdc2 RIP-seq alone. **(H)** GO analysis of Ythdc2 RIP-seq genes; meiosis-related GO terms.