

MATERIALS AND METHODS

Prediction of Intrinsically Unstructured Proteins

Predictions of intrinsic disorder tendency of the proteins were accomplished by specifying the accession numbers of the proteins in the "Enter SWISS-PROT/TrEMBL identifier or accession number" field of IUPred2A(Meszaros et al., 2018) (<https://iupred2a.elte.hu/>). The red line in the result page represents the intrinsic disorder tendency of each protein.

Cell cultures

U2OS cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 100 U/mL Penicillin/Streptomycin at 37 °C with 5% CO₂. E14Tg2a murine embryonic stem cells were cultured in DMEM supplemented with 10% FBS, 1% MEM non-essential amino acid, 55 mM β-Mercaptoethanol, 1000 U/mL LIF (Millipore) and 100 U/mL Penicillin/Streptomycin at 37 °C with 5% CO₂.

Stable cell lines construction

EGFP-tagged murine *Ythdf2* was cloned into the pPB-CAG-IRES-Pac plasmid with N-terminal Flag and HA tags. This plasmid was individually co-transfected with pCMV-PBase plasmid into mESCs using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instruction. Medium was replaced by fresh media with 2 µg/mL Puromycin after 48 hours. After continuous Puromycin selection for 3 days, the survived mESCs were pooled as stable overexpression cell lines.

EGFP-tagged human *YTHDF2* was cloned into the pLenti6.2-V5 plasmid. Lentivirus was made by co-transfection of this vector with VSV-G and psPAX2 in a 3:1:1 ratio into 293T cells. Supernatant at 48 hours post-transfection was collected and concentrated by PEG8000. U2OS cells were seeded in a 6-well plate and infected with lentivirus supernatant in the presence of 5 µg/mL polybrene (Sigma). Medium was replaced by fresh media with 10 µg/mL Blasticidin S at 24 hours post-infection. After continuous Blasticidin S selection for 5 days, survived U2OS cells were pooled as stable infected cell lines.

CRISPR-Cas9 gene targeting was carried out as previously described(Maeder et al., 2013) and the single knockout clones were isolated and then confirmed by Western blot showing undetectable METTL3 and METTL14 protein. Guiding RNA sequences used are: 1) *Mettl3* KO: 5'-GCTTAGGGCCGCTAGAGGTA-3'. 2) *Mettl14* KO: 5'-GTAGCTCAGCAGGTGTGCGG-3'.

Protein Expression and Purification

The different truncated fragments of human *YTHDF2* were subcloned into the

with 488 nm laser. Bleaching was performed over an approximately $1 \mu\text{m}^2$ region using 100% laser power and images were collected every 3.5 seconds (U2OS) or 5.2 seconds (mESC). Fluorescence intensity was measured using the LAS X software. Background intensity was subtracted and values were reported relative to pre-bleaching time points.

References:

Maeder, M.L., Linder, S.J., Cascio, V.M., Fu, Y., Ho, Q.H., and Joung, J.K. (2013). CRISPR RNA-guided activation of endogenous human genes. *NAT METHODS* 10, 977-979.

Meszaros, B., Erdos, G., and Dosztanyi, Z. (2018). IUPred2A: context-dependent prediction of protein disorder as a function of redox state and protein binding. *NUCLEIC ACIDS RES* 46, W329-W337.

Supplementary Fig. 1

- (A) Microscopy images of EGFP-YTHDF2^{aa 230-383} droplets (23 $\mu\text{mol/L}$ YTHDF2, 10% PEG8000) subjected to a NaCl gradient, showing NaCl concentration affects the formation of protein droplets. Scale bar, 10 μm .
- (B) The fusion of YTHDF2^{aa 230-383} droplets (23 $\mu\text{mol/L}$ YTHDF2, 37 mmol/L NaCl, 10% PEG8000) were imaged by microscopy over 120 seconds. Scale bar, 10 μm .
- (C) Predictions of Intrinsic disorder tendency of YTHDF1 and YTHDF3 by IUPred2A(<https://iupred2a.elte.hu/>). Scores above 0.5 indicate disorder.
- (D) Liquid phase separation of YTHDF1^{aa 284-362} (Glutamine rich domain) and YTHDF3^{aa 288-388} (Glutamine rich domain). Scale bar, 10 μm .
- (E) Sequence alignment of the wildtype and Q-to-A mutant of the Glutamine (Q) rich domain in YTHDF2^{aa 230-383}.
- (F) Q-to-A mutation compromised liquid phase separation of YTHDF2^{aa 230-383} (11 $\mu\text{mol/L}$ YTHDF2, 37 mmol/L NaCl, 10% PEG8000). Scale bar, 10 μm .
- (G) Droplet numbers of wildtype and Q-to-A mutated YTHDF2^{aa 230-383} proteins. Data are represented as mean from three replicates. Error bars represent SEM of the number of droplets from three replicates. **P < 0.01, t test.
- (H) Cy5-m⁶A, but not FAM-A RNA oligos induced YTHDF2^{aa 230-579} liquid like droplet formation (13 $\mu\text{mol/L}$ YTHDF2, 37 mmol/L NaCl, 0.74 $\mu\text{mol/L}$ RNA, 10% PEG8000).
- (I) Droplet numbers of Cy5-m⁶A and FAM-A RNA oligos induced YTHDF2^{aa 230-579} proteins. Data are represented as mean from three replicates. Error bars represent SEM of the number of droplets from three replicates. **P < 0.01, t test.
- (J) Western blot showing Mettl3 protein levels in WT, *Mettl3* KO and *Mettl14* protein levels in WT, *Mettl14* KO cell lines.