Supplementary Table S1. Targeting sequences of shRNAs used in this study.

target gene	sequence (5'-3')
Thoc2	NN-GGTTATGCCAAGCTGATTG
Thoc1	GGGAGTAACTGAAGGTATT
Thoc5	CCAAATGTTTGGAGTTTAA
Aly	TGGGAAACTGCTGGTGTCCAA
Tex1	GTAAGAACCCTCAGTTTCA
Thoc6	GCCAAAGAGGAAAGTAAGA
Thoc7	GGAGATGATCGGAGAATTA
negative control (Cntl)	AACAGTCGCGTTTGCTACTTT

Supplementary Figures

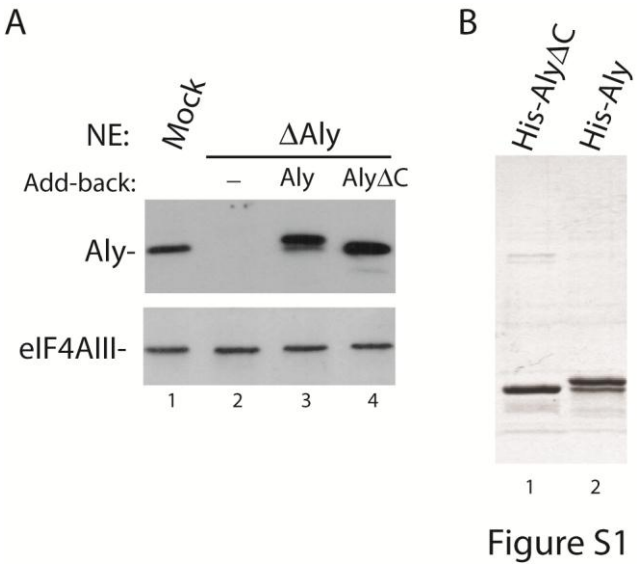


Figure S1

Supplementary Figure S1. The amount of proteins added back to ΔAly extract were equal. Left panel, equal amount of Mock nuclear extract and ΔAly nuclear

extract with or without supplementation of His-Aly or His-AlyΔC were loaded followed by Western using Aly antibody. eIF4A3 served as a loading control. Right panel, equal amount of His-Aly and His-AlyΔC were separated by SDS-PAGE and stained with coomassie blue.

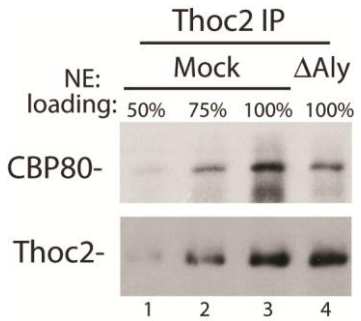
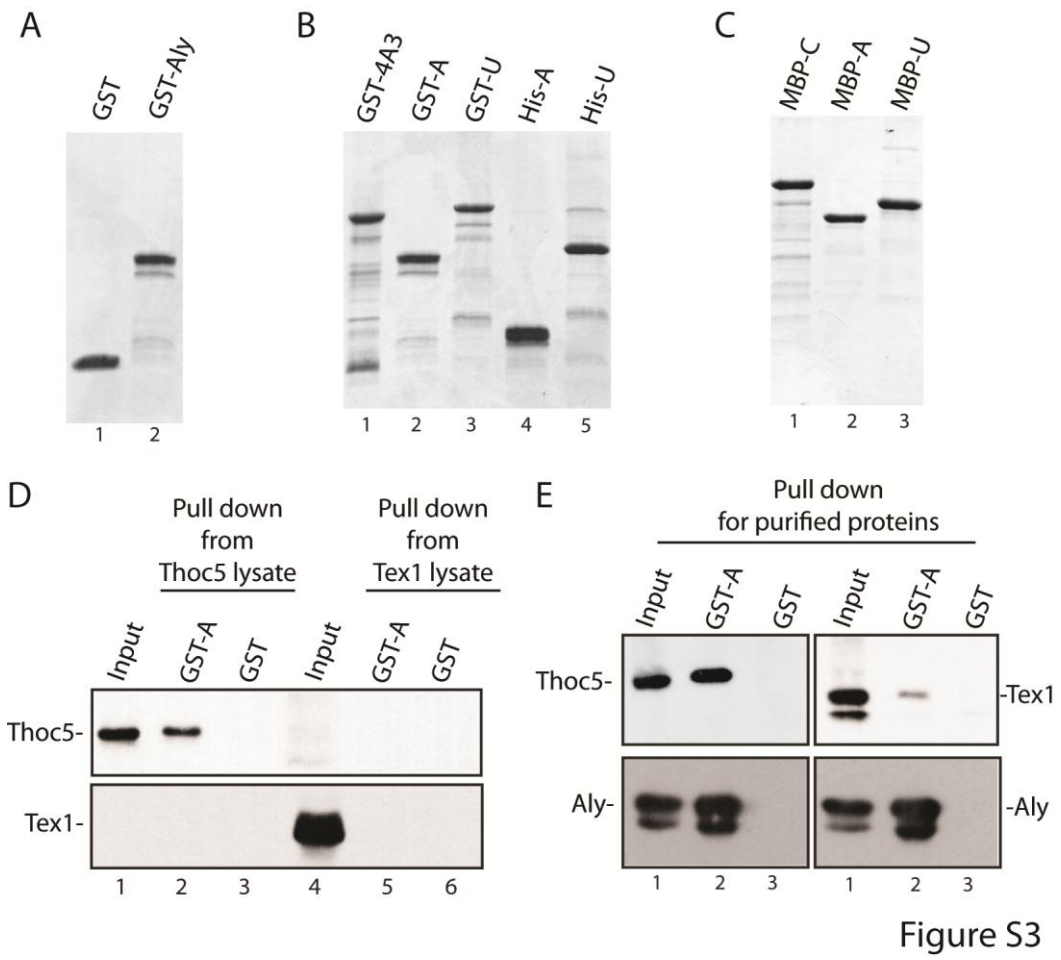


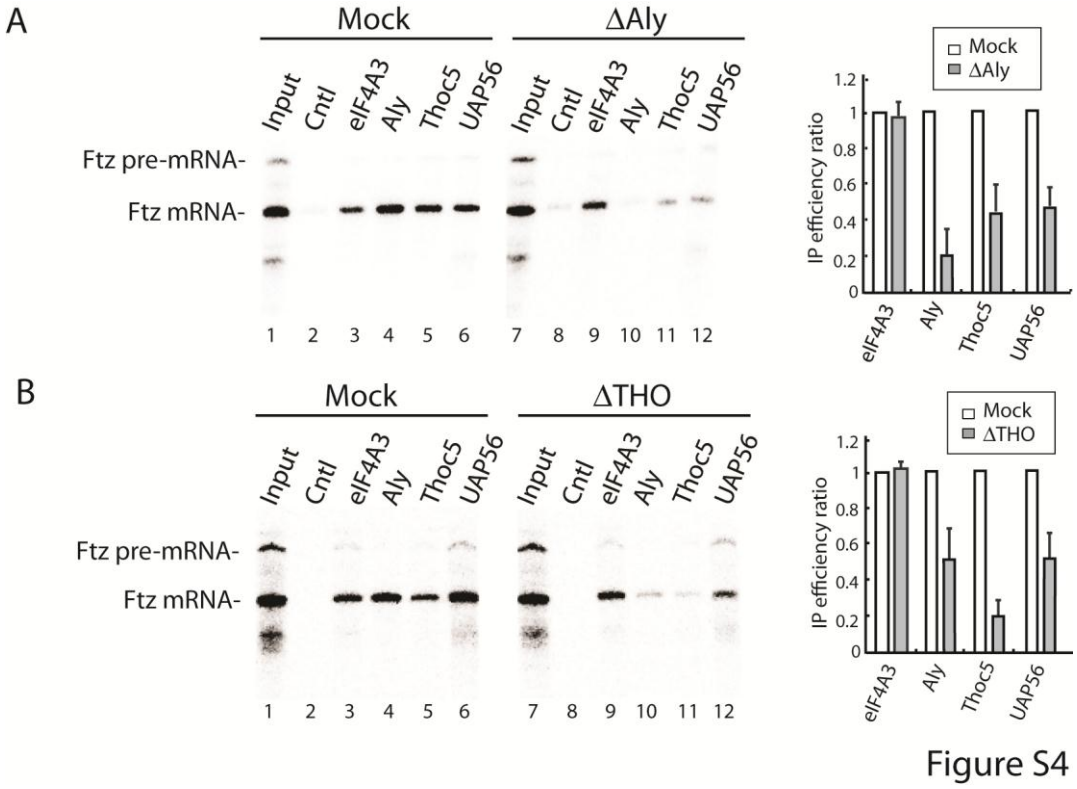
Figure S2

Supplementary Figure S2. The Thoc2-CBP80 interaction mostly remains in the absence of Aly. IPs from Mock and ΔAly extracts with the Thoc2 antibody were carried out. 50%, 75% and 100% of Thoc2 immunoprecipitate in Mock extract and 100% of that in ΔAly extract were loaded on the gel for comparison. Western analyses were carried out using CBP80 and Thoc2 antibodies.

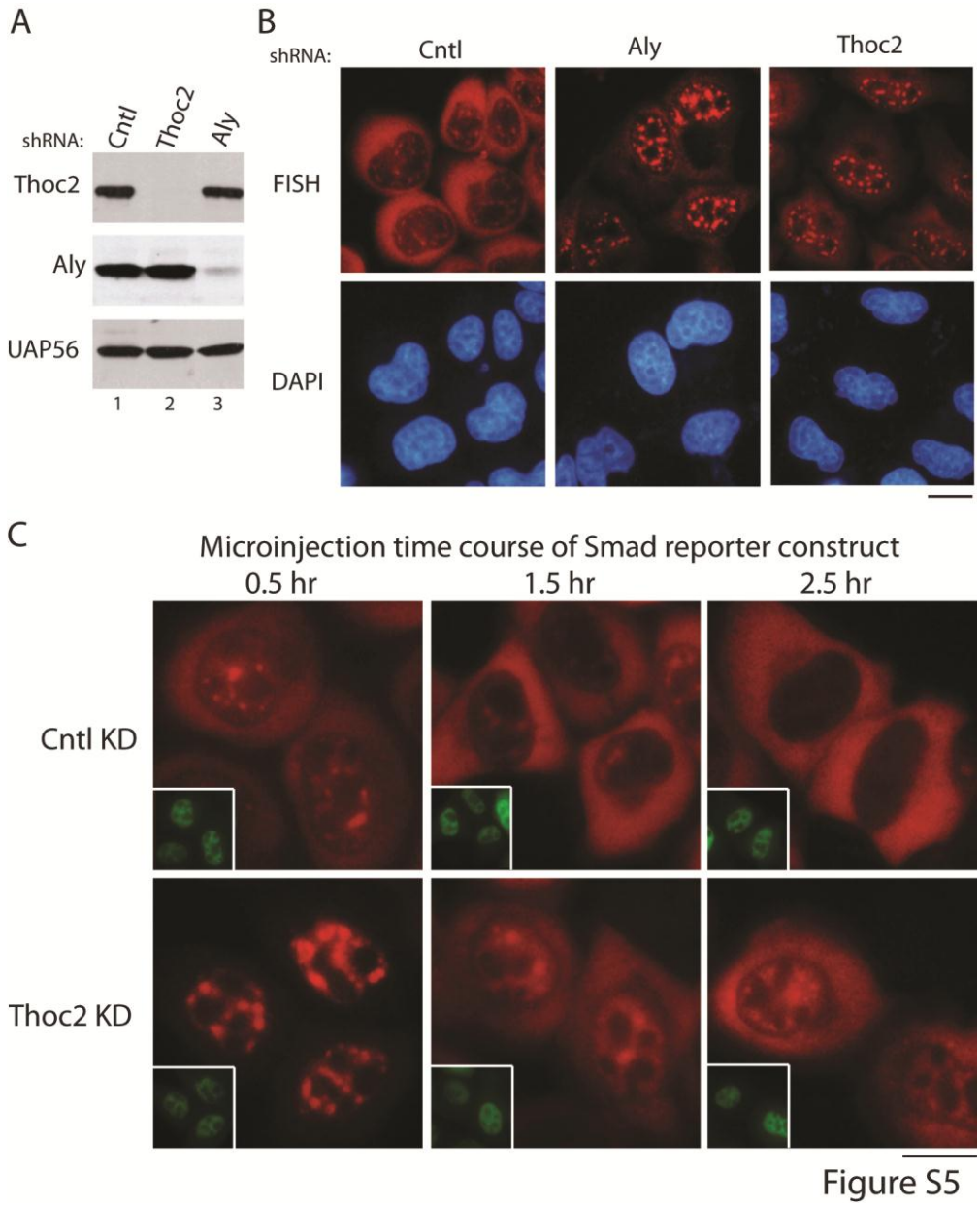


Supplementary Figure S3. Interactions of Aly and UAP56 with THO subunit. (A) Equal amount of GST and GST-Aly were separated on a SDS-PAGE gel and stained with coomassie blue. (B) Equal amount of GST-4A3, GST-Aly, GST-UAP56, His-Aly and His-UAP56 were separated on a SDS-PAGE gel followed by coomassie staining. (C) Equal amount of MBP tagged Mtr4N (MBP-C), Aly (MBP-A) and UAP56 (MBP-U) were separated by SDS-PAGE and stained with coomassie blue. (D) GST pull downs were carried out from insect lysates containing overexpressed Thoc5 or Tex1 using GST-Aly or GST protein followed by western analysis with the Thoc5 and Tex1 antibodies. (E) GST-Aly or GST was used for pull-down of purified Thoc5 and Tex1 followed by western analysis using the Thoc5 and Tex1 antibodies. Aly

served as loading control.



Supplementary Figure S4. Aly and THO are both required for association of TREX components with Ftz spliced mRNA. (A) Ftz pre-mRNA was incubated in Mock or ΔAly nuclear extract under splicing condition followed by mRNA-IPs using indicated antibodies. 25% of the input was loaded. Quantifications of three independent experiments are shown in the right panel. The bars indicate the ratio of IP efficiency in ΔAly extract relative to the corresponding IP efficiency in Mock extract. Error bars represent standard deviations (n=3). (B) Same as (A), except that ΔTHO nuclear extract was used instead of ΔAly nuclear extract.



Supplementary Figure S5. The role of THO in mRNA export. (A) Western analysis of whole cell lysates of control, Thoc2 and Aly knockdown cells with indicated antibodies. UAP56 served as a loading control. (B) FISH for polyA$^+$ RNA were carried out for control, Aly and Thoc2 knockdown cells. (C) Smad construct was microinjected into nuclei of control and Thoc2 knockdown cells, and α-amanitin was added to stop transcription 15 min after injection. FISH of Smad transcripts was carried out at indicated times after addition of α-amanitin. Insets show

FITC-conjugated dextran co-injected as an injection marker. Scale bar, 10 μm.

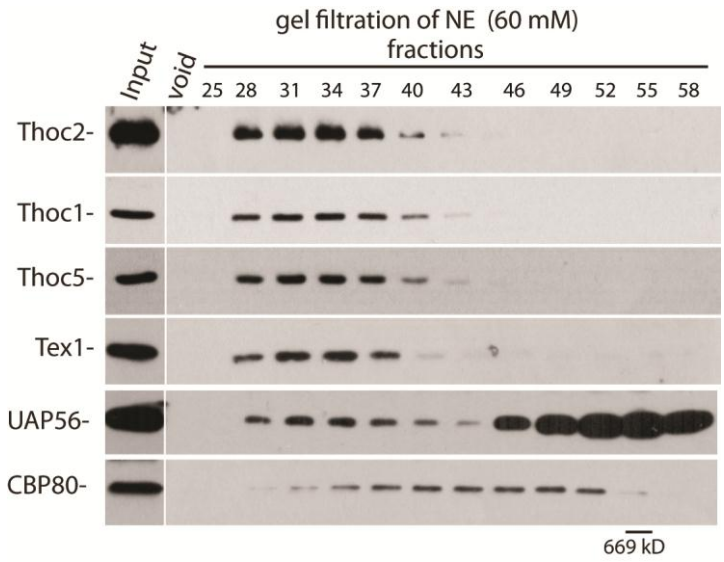


Figure S6

Supplementary Figure S6. Only a small portion of UAP56 co-migrates with THO under low salt condition. HeLa nuclear extracts were separated on a gel filtration column under 60 mM salt concentration followed by western analysis of the fractions using indicated antibodies.

Supplementary methods

DNA microinjection and detection

HeLa cells used for microinjection were plated on coverslip bottom of 35 mm dishes coated with fibronectin. The CMV-Smad construct used for microinjection was described previously (35). Plasmid DNA (50 ng/μl) was coinjected with FITC-conjugated 70-kDa dextran, followed by incubation at 37℃ for 15 min. For each experiment, 50-70 cells were microinjected. Transcription was then terminated

by adding α-amanitin (1 μg/ml, Sigma), and incubation was continued for indicated times. A 70 nt FISH probe was labeled at the 5' end with Alexa Fluor 546 NHS Ester and HPLC purified. FISH was preformed as described (35).

Supplementary reference

35. Valencia, P., Dias, A.P. and Reed, R. (2008) Splicing promotes rapid and efficient mRNA export in mammalian cells. *Proc Natl Acad Sci U S A*, 105, 3386-3391.