Expanded View Figures

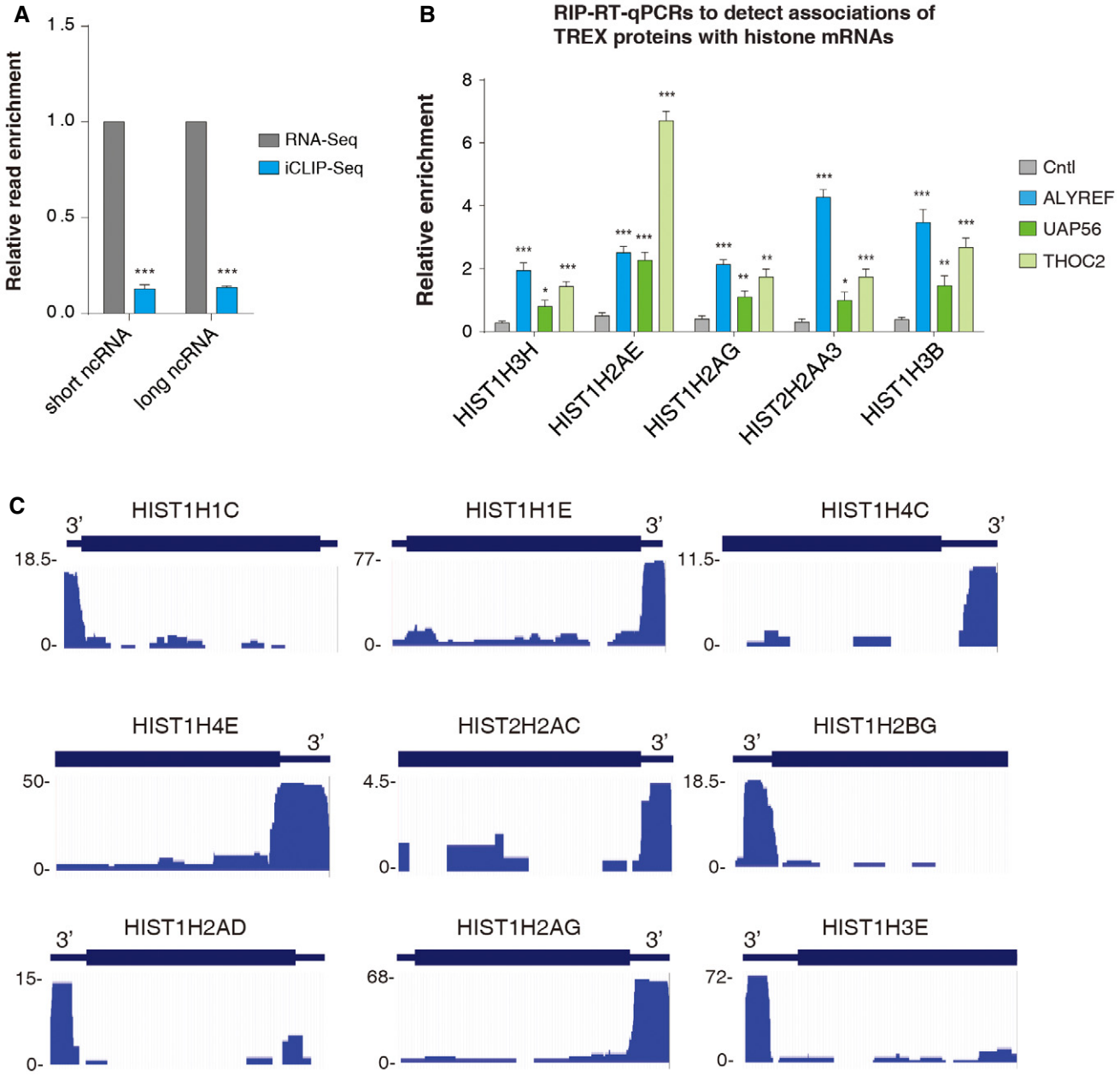

Figure EV1. TREX associates with histone mRNAs.

A The ratios of unique histone iCLIP-seq read population to unique short or long ncRNA RNA-seq read population were calculated and are shown, with the unique histone RNA-seq read population set as "1". Statistical analysis was performed based on three replicates of ALYREF iCLIP data using Student's *t*-test. ***$P < 0.001$.

B ALYREF, UAP56, and THO RIP–qPCRs to examine their binding on multiple histone mRNAs. Relative RIP efficiencies are shown.

C Screenshots of several histone mRNAs with ALYREF iCLIP reads.

Data information: In (A and B), error bars represent standard deviations from biological repeats ($n = 3$). Statistical analysis was performed using Student's *t*-test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

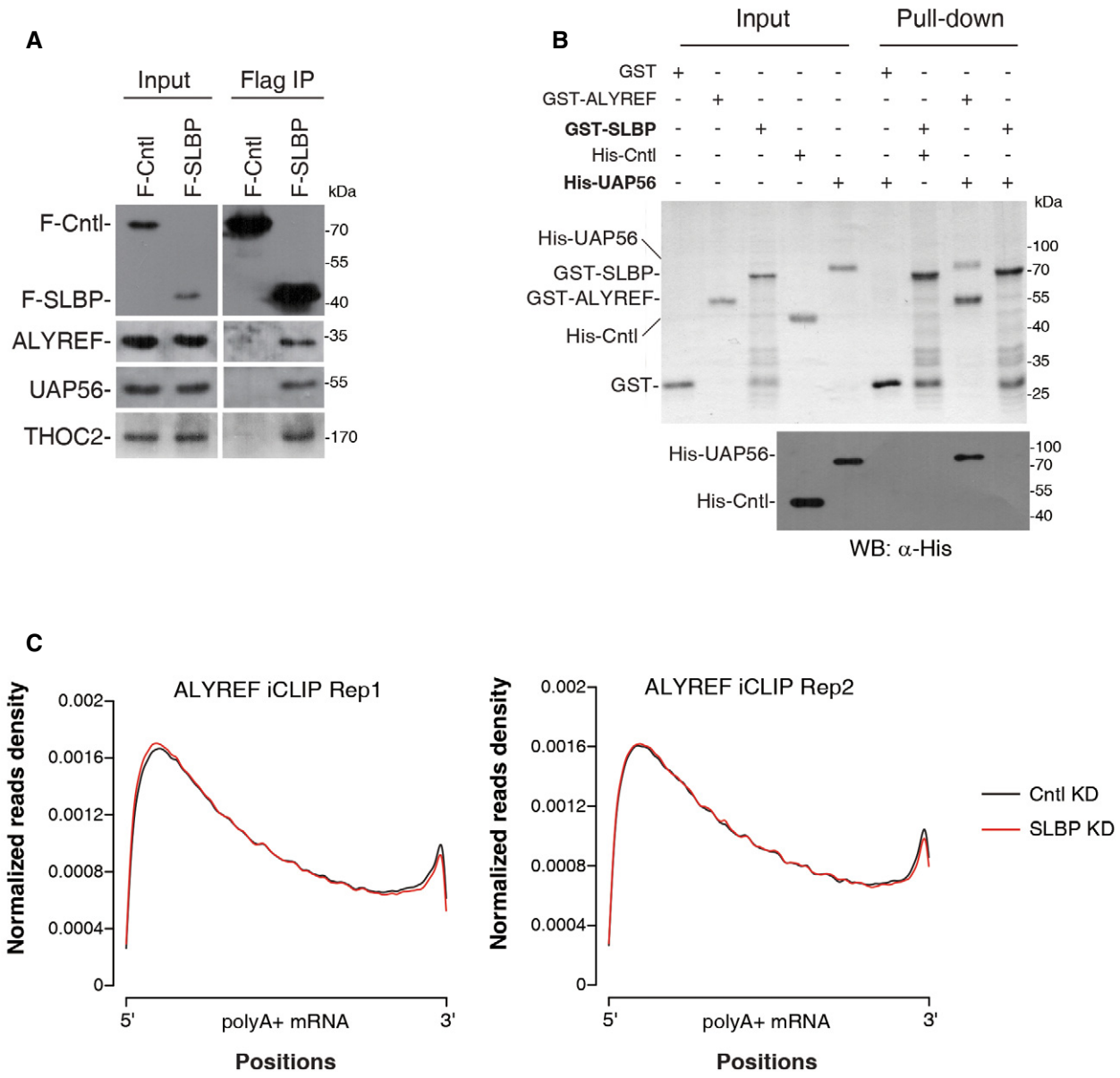

Figure EV2. TREX associates with SLBP.

A Flag IPs from RNase A-treated HeLa cell lysate expressing Flag-Cntl (DDX3) or Flag-SLBP, followed by Western blotting using the indicated antibodies. 3% of input was loaded.

B GST-SLBP, GST-ALYREF, and GST were used for pull-down of purified His-UAP56 or His-Cntl (PGK1) in the presence of RNase A. Proteins pulled down were separated by SDS–PAGE, followed by Coomassie staining and Western blotting. 18.75% of input was loaded.

C Metagene analysis of normalized ALYREF signals on polyadenylated mRNAs in Cntl and SLBP KD cells based on two replicates of iCLIP-seq data. iCLIP-seq signal at each position of an mRNA is divided by the sum of signal of the mRNA to normalize each mRNA's contribution to the plot.

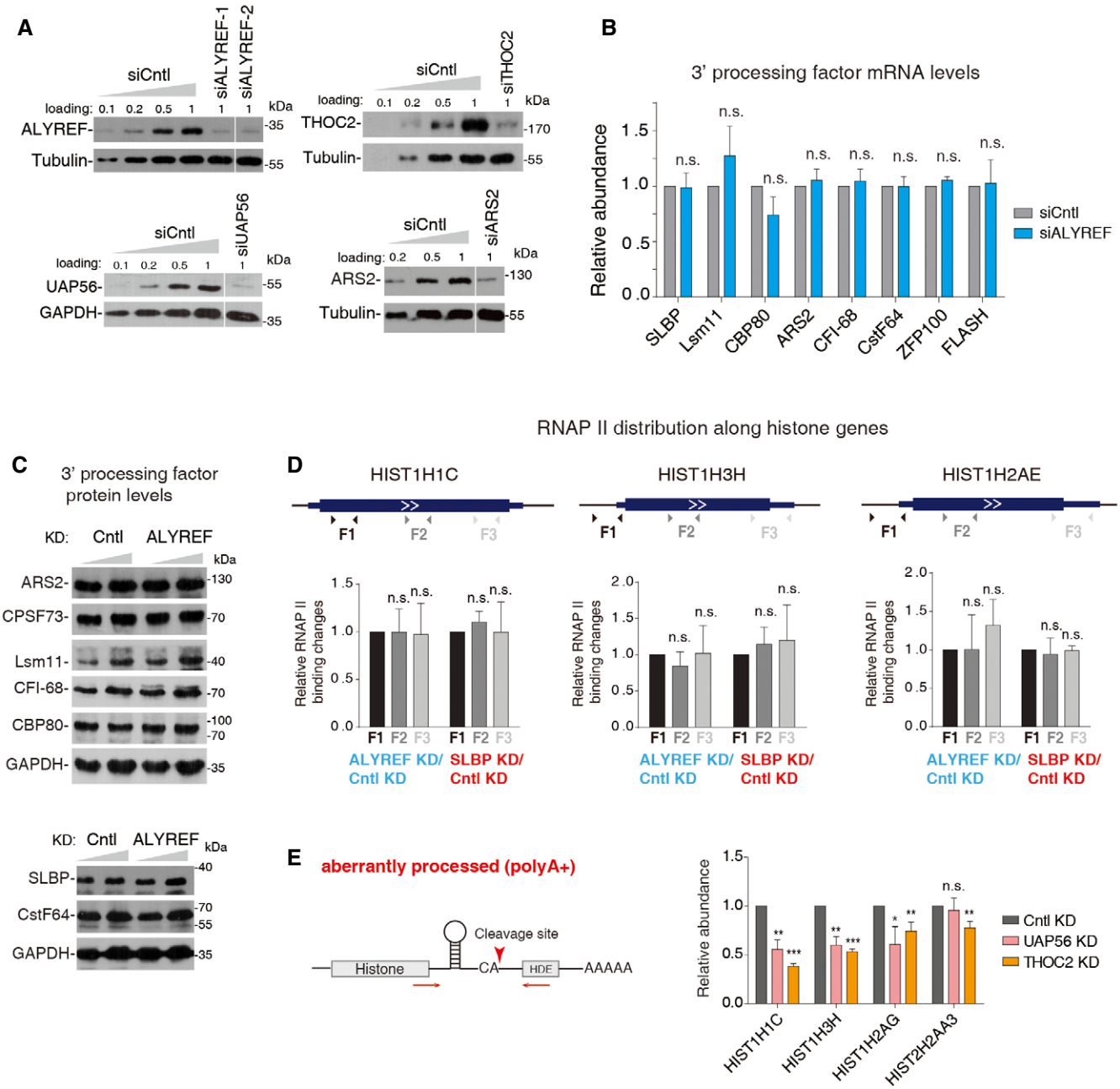

Figure EV3. Depletion of UAP56 and THOC2 did not result in enhanced level of polyA$^+$ histone transcripts.

A Western blotting to examine the KD efficiencies of ALYREF, UAP56, THOC2, and ARS2. Tubulin or GAPDH was used as a loading control. Different amounts of Cntl KD samples were loaded to estimate the KD efficiencies. The white line delineates the boundary where irrelevant lanes have been removed from the same blot.

B RT–qPCRs to examine mRNA levels of histone 3′-end processing factors in Cntl and ALYREF KD cells.

C Western blot analysis to examine protein levels of histone 3′-end processing factors in Cntl and ALYREF KD cells. GAPDH was used as a loading control.

D (Top) Illustration of the locations of primer sets. (Bottom) ChIP–PCR to examine RNAP II distribution change along several histone genes in cells treated with Cntl, ALYREF, or SLBP siRNAs. RNAP II binding change (KD/Cntl) at F1 was set as "1", and the relative ratios of F2/F1 and F3/F1 were calculated and are shown.

E RT–qPCRs to detect polyA$^+$ histone mRNAs in cells depleted of Cntl, UAP56/URH49, or THOC2. The arrowhead and arrows indicate the correct cleavage site and the location of primers, respectively. The bars show the relative abundance of polyA$^+$ forms to the actin mRNA.

Data information: In (B, D, and E), error bars represent standard deviations from biological repeats ($n = 3$). Statistical analysis was performed using Student's t-test. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, n.s.: not significant.

Source data are available online for this figure.

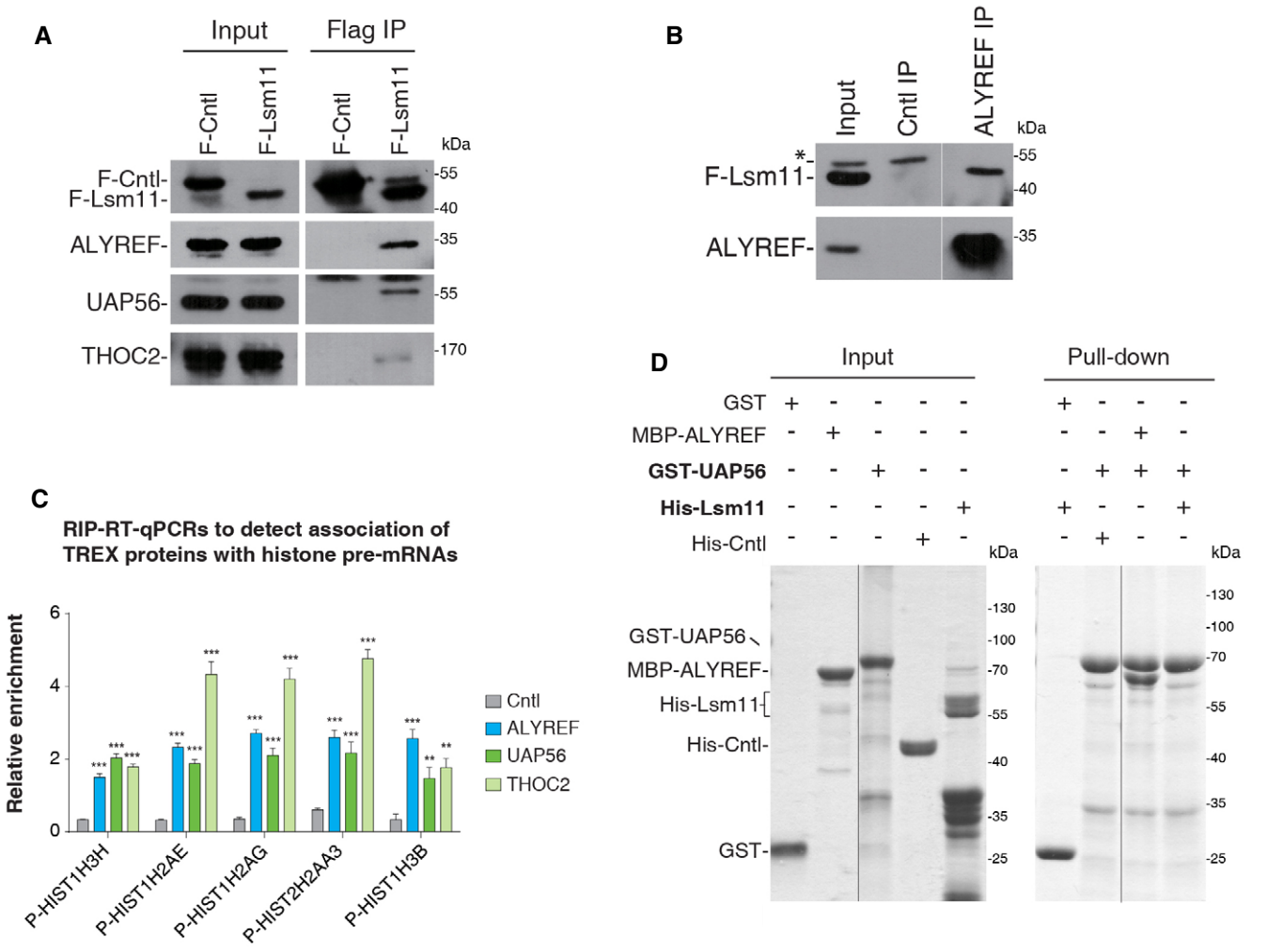

Figure EV4. TREX associates with Lsm11 and histone pre-mRNAs.

A Flag IPs from RNase A-treated HeLa cell lysate expressing Flag-Cntl (eIF4A3) or Flag-Lsm11, followed by Western blotting with the indicated antibodies. 2.5% of input was loaded.

B IPs with IgG or the ALYREF antibody from RNase A treated Flag-Lsm11 stable expression cells, followed by Western blotting using the Flag and ALYREF antibodies. 3% of input was loaded. * indicates a nonspecific band. The white line delineates the boundary where irrelevant lanes have been removed from the same blot.

C ALYREF, UAP56, and THO RIP–qPCRs to examine their binding on multiple histone pre-mRNAs. Relative RIP efficiencies are shown. Error bars represent standard deviations from biological repeats (n = 3). Statistical analysis was performed using Student's t-test. $^{**}P < 0.01$, $^{***}P < 0.001$.

D GST-UAP56 and GST were used for pull-down of purified His-Lsm11, His-Cntl (PGK1), or MBP-ALYREF in the presence of RNase A. Proteins pulled down were separated by SDS–PAGE, followed by Coomassie staining. 37.5% of input was loaded. The black line delineates the boundary where irrelevant lanes have been removed from the same gel.

Source data are available online for this figure.

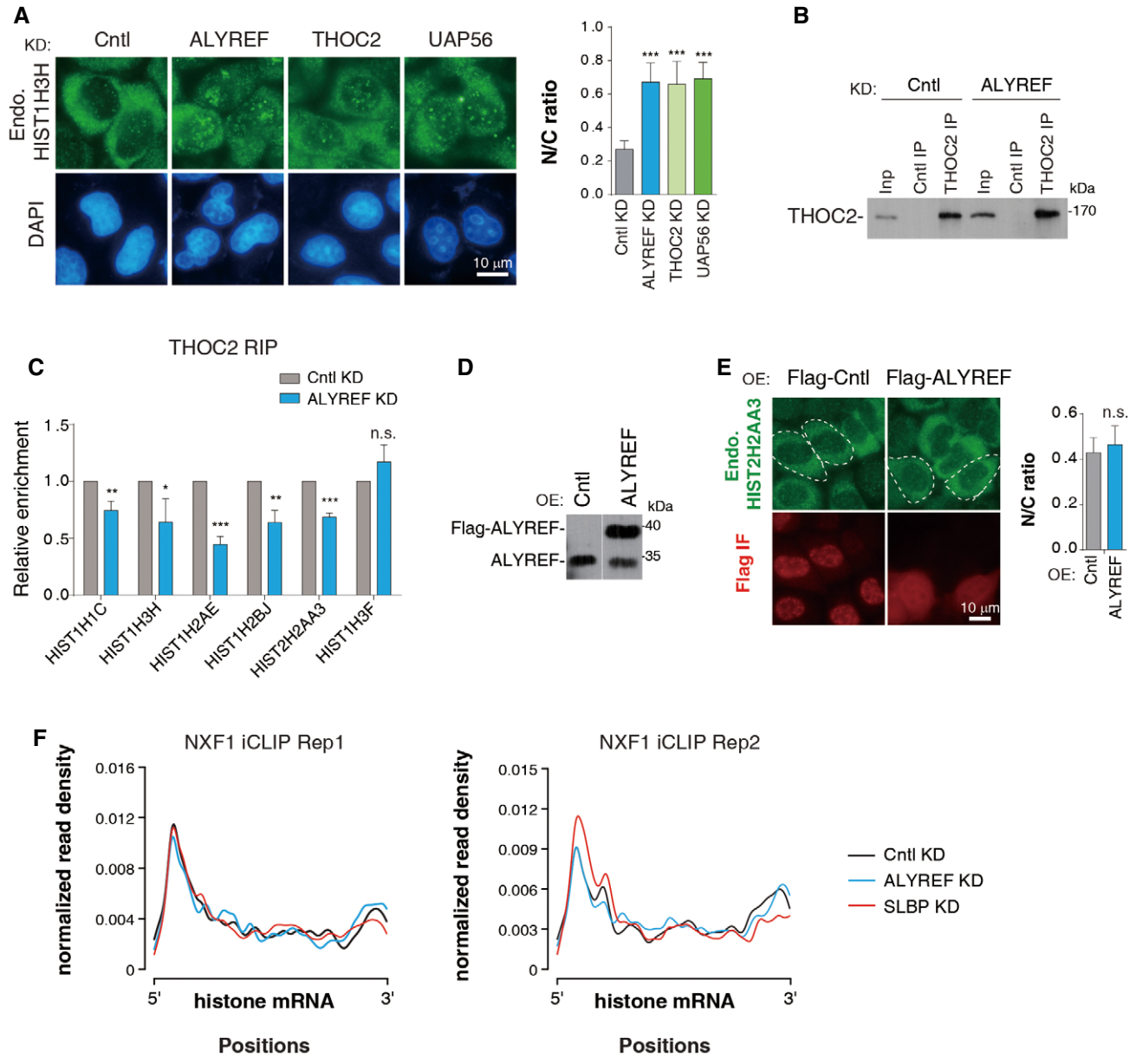

Figure EV5. TREX functions in histone mRNA export.

A Distribution of the endogenous HIST1H3H mRNA was detected with the transcript-specific probe in HeLa cells depleted of Cntl, ALYREF, THOC2 (THO), and UAP56. DAPI staining served as a nuclear marker. N and C indicate nuclear and cytoplasmic FISH signals, respectively. N/C ratios were determined for 20 cells in each experiment.

B, C Cntl- or ALYREF siRNA-treated HEK293 cells were used for IPs with IgG or the THOC2 antibody. The immunoprecipitates were subjected to Western blot analysis (B) and RT–qPCRs (C).

D Western blot analysis to examine the overexpression of Flag-ALYREF. The white line delineates the boundary where irrelevant lanes have been removed from the same blot.

E Distribution of endogenous HIST2H2AA3 mRNA was detected with the transcript-specific probe in Cntl (eIF4A3) and ALYREF overexpression cells. N/C ratios were quantified the same as (A).

F Metagene analysis of normalized NXF1 signals on histone mRNAs in Cntl, ALYREF, and SLBP KD cells based on iCLIP-seq data. iCLIP-seq signal at each position of an mRNA is divided by the sum of signal of the mRNA to normalize each mRNA's contribution to the plot.

Data information: In (A, C, and E), error bars represent standard deviations from biological repeats ($n = 3$). Statistical analysis was performed using Student's t-test. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, n.s.: not significant.
Source data are available online for this figure.