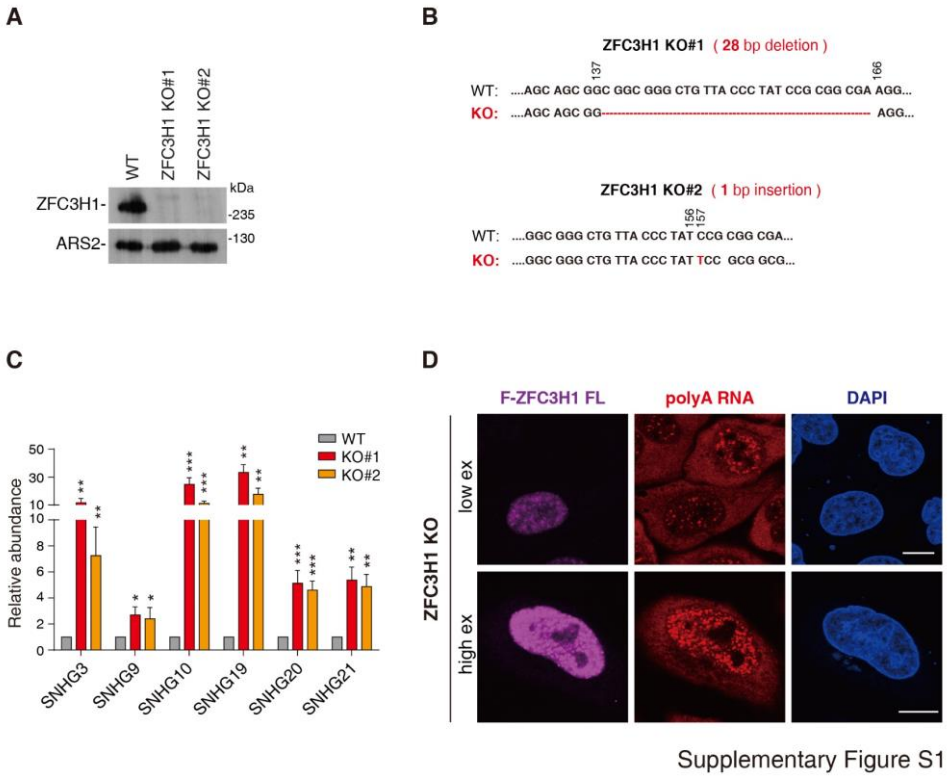
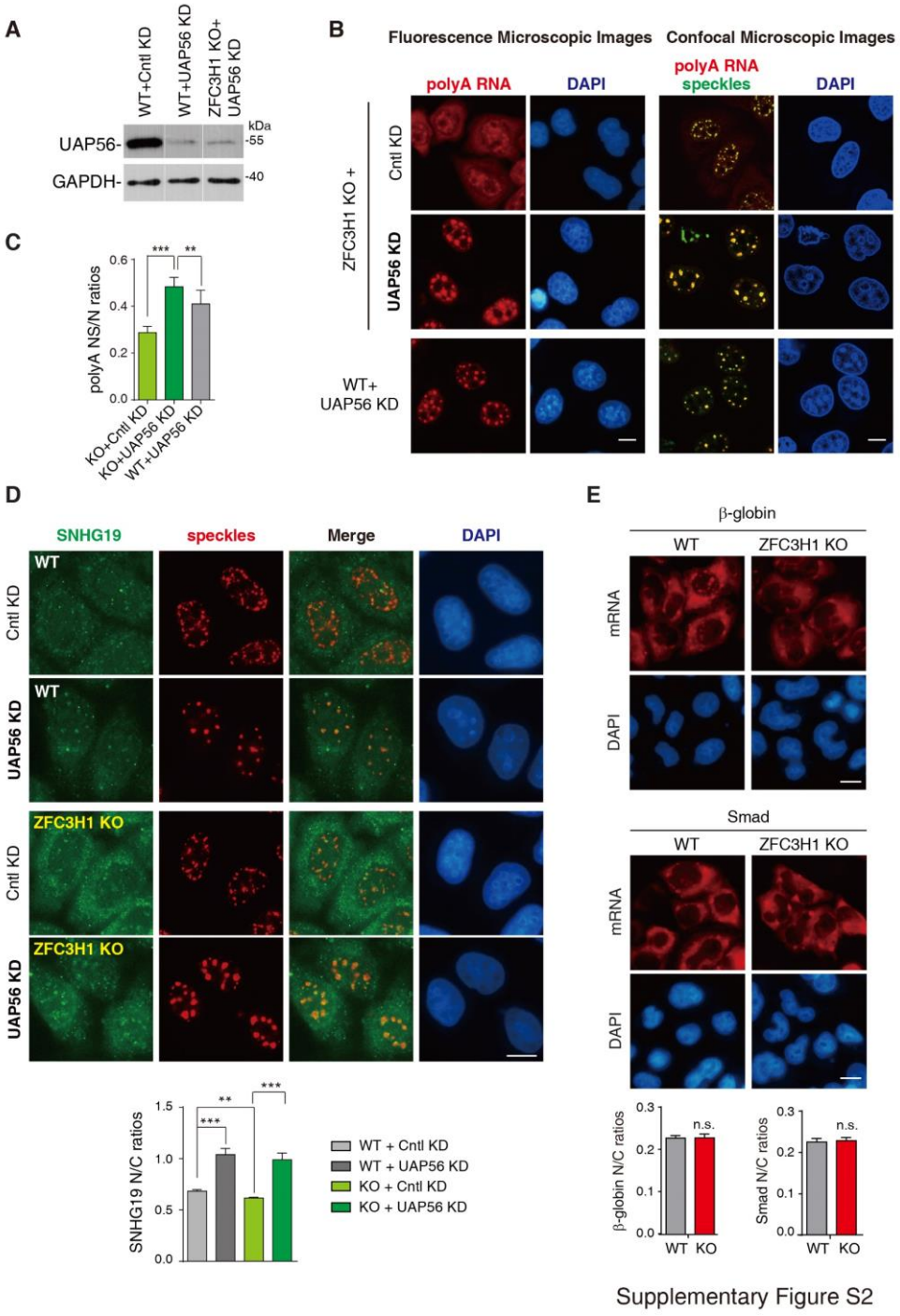
Supplementary Information

Supplementary Figures



Supplementary Figure S1

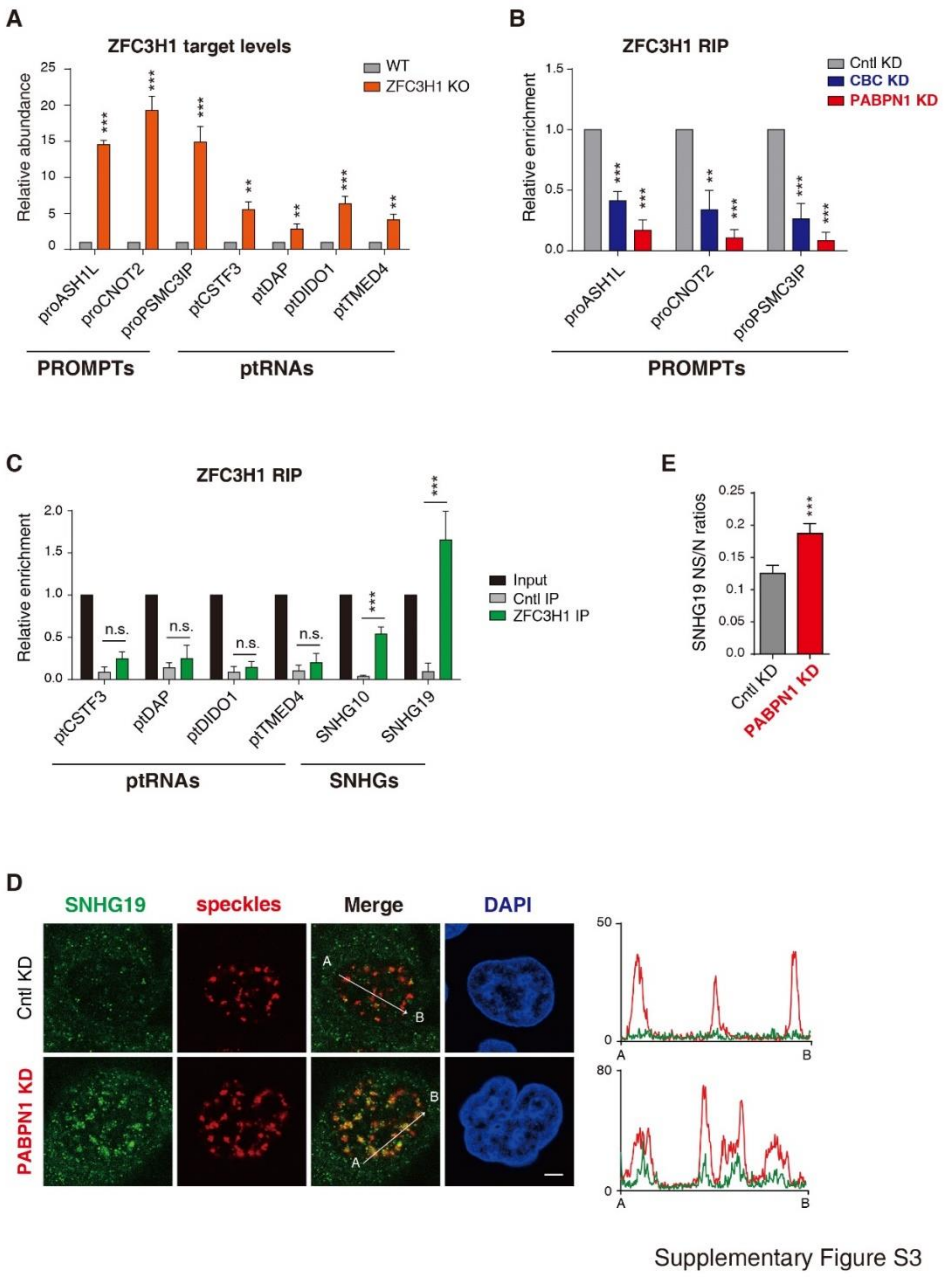
Supplementary Figure S1. Identification of ZFC3H1 KO cells. (**A**) Western blots to examine ZFC3H1 expression in WT and ZFC3H1 KO cells. ARS2 was used as a loading control. (**B**) Genomic sequences of the ZFC3H1 KO cells. The mutated sites are shown in red. (**C**) RT-qPCRs to examine SNHG RNA levels in WT and ZFC3H1 KO cells. The bars show RNA levels relative to 18S rRNA. Error bars, standard deviations (n=3). Statistical analysis was performed using Student's t test. *P < 0.05, **P < 0.01, ***P < 0.001. (**D**) Confocal microscopic imaging to detect the distribution of polyA RNAs and Flag-ZFC3H1 at different levels in ZFC3H1 KO cells. FISH with an oligo (dT) probe and IF using the Flag antibody were carried out. Scale bar, 10 μm.



Supplementary Figure S2

Supplementary Figure S2. ZFC3H1 prevents its target RNAs trafficking into NSs. (**A**) Western blots to examine the KD efficiency of UAP56. GAPDH was used as a loading control. The white line delineates the boundary where irrelevant lanes have been removed from the same blot. (**B**) (Left) Fluorescence microscopic

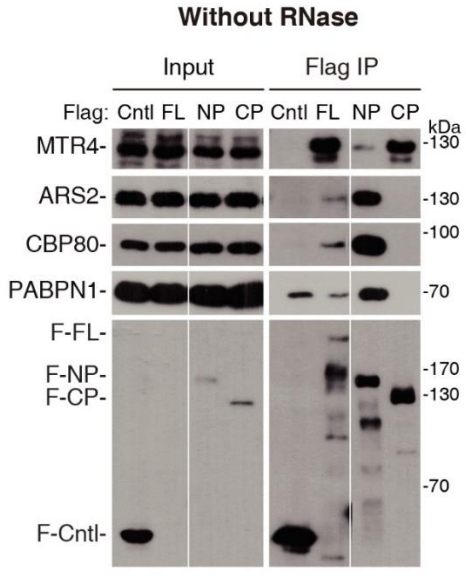
imaging to show distribution of polyA RNAs in WT and ZFC3H1 KO cells treated with Cntl or UAP56/URH49 siRNAs. Images were captured with a DP72-CCD camera (Olympus). Scale bar, 10 μm. (Right) Confocal microscopic imaging to examine colocalization of polyA RNAs with NSs in the same set of cells as shown in (Left). FISH with an oligo (dT) probe and IF using the SC35 (as a NS marker) antibody were carried out. Confocal imaging was performed on a Leica TCS SP8 WLL. Scale bar, 10 μm. (**C**) Quantification of polyA RNA NS/N ratios in (B). Error bars, standard deviations (n=10). (**D**) (Top) Confocal microscopic imaging to examine the distribution of SNHG19 RNAs and NSs in WT and ZFC3H1 KO cells treated with Cntl or UAP56/URH49 siRNAs. FISH with an SNHG19-specific probe and IF using the SON (as a NS marker) antibody were carried out. Scale bar, 10 μm. (Bottom) Quantification of nuclear (N) and cytoplasmic (C) signals of SNHG19 RNA. N/C ratios of 30 cells were calculated in each experiment. Error bars, standard deviations (n=3). (**E**) (Top) FISH with a vector probe to detect the distribution of corresponding RNAs in WT and ZFC3H1 KO cells 19 hr after transfection with spliced β-globin or Smad reporter construct. Scale bar, 20 μm. (Bottom) Quantification of N/C ratios of corresponding mRNAs. N/C ratios of 40 cells were calculated in each experiment. Error bars, standard deviations (n=3). Statistical analysis was performed using Student's t test. *P < 0.05, **P < 0.01, ***P < 0.001, n.s., not significant.



Supplementary Figure S3

Supplementary Figure S3. PABPN1 facilitates ZFC3H1 recruitment to polyA+ PROMPTs. (**A**) RT-qPCRs to detect the levels of polyA+ PROMPTs and ptRNAs in WT and ZFC3H1 KO cells. The bars show RNA levels relative to 18S rRNA. Error bars, standard deviations (n = 3). (**B**) RNA-IP analysis to examine the association of ZFC3H1 with polyA+ PROMPTs upon CBC or PABPN1 KD. IPs were carried out with a ZFC3H1 antibody in cells treated with Cntl, CBC or PABPN1 siRNAs, followed by RT-qPCRs to detect RNAs. Error bars, standard deviations

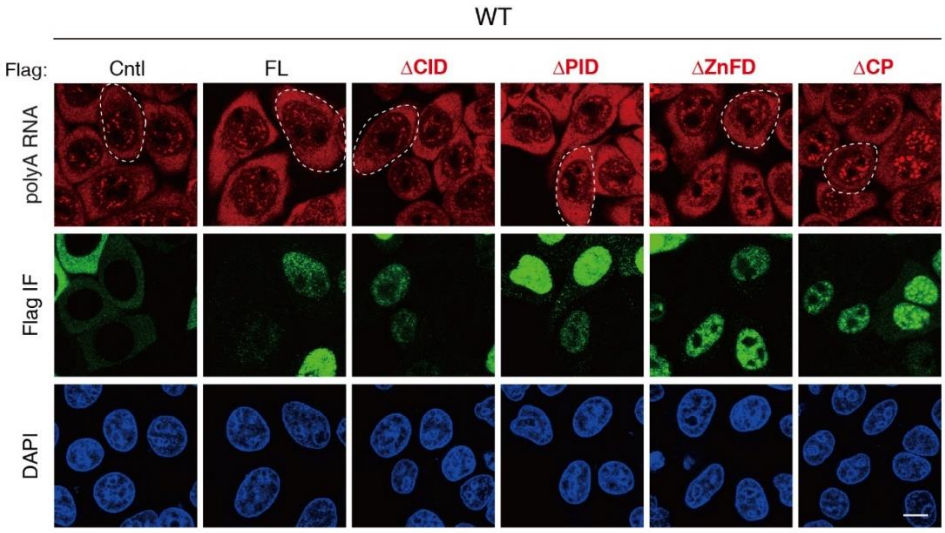
(n=3). (**C**) RNA-IP analysis to examine the association of ZFC3H1 with ptRNAs. IPs were carried out with the Cntl or ZFC3H1 antibody in normal cells, followed by RT-qPCRs to detect RNAs. Error bars, standard deviations (n=3). (**D**) Confocal microscopic imaging to examine co-localization of SNHG19 RNA with NSs in Cntl and PABPN1 KD cells. FISH with an SNHG19-specific probe and IF using the SON (as a NS marker) antibody were carried out. The green and red lines in the graphs show the intensity of FISH and SON IF signals along the freely positioned arrow indicated from A to B, respectively. Scale bar, 5 μm. (**E**) Quantification of SNHG19 RNA NS/N ratios in (D). Error bars, standard deviations (n=10). Statistical analysis was performed using Student's t test. *P < 0.05, **P < 0.01, ***P < 0.001, n.s., not significant.



Supplementary Figure S4

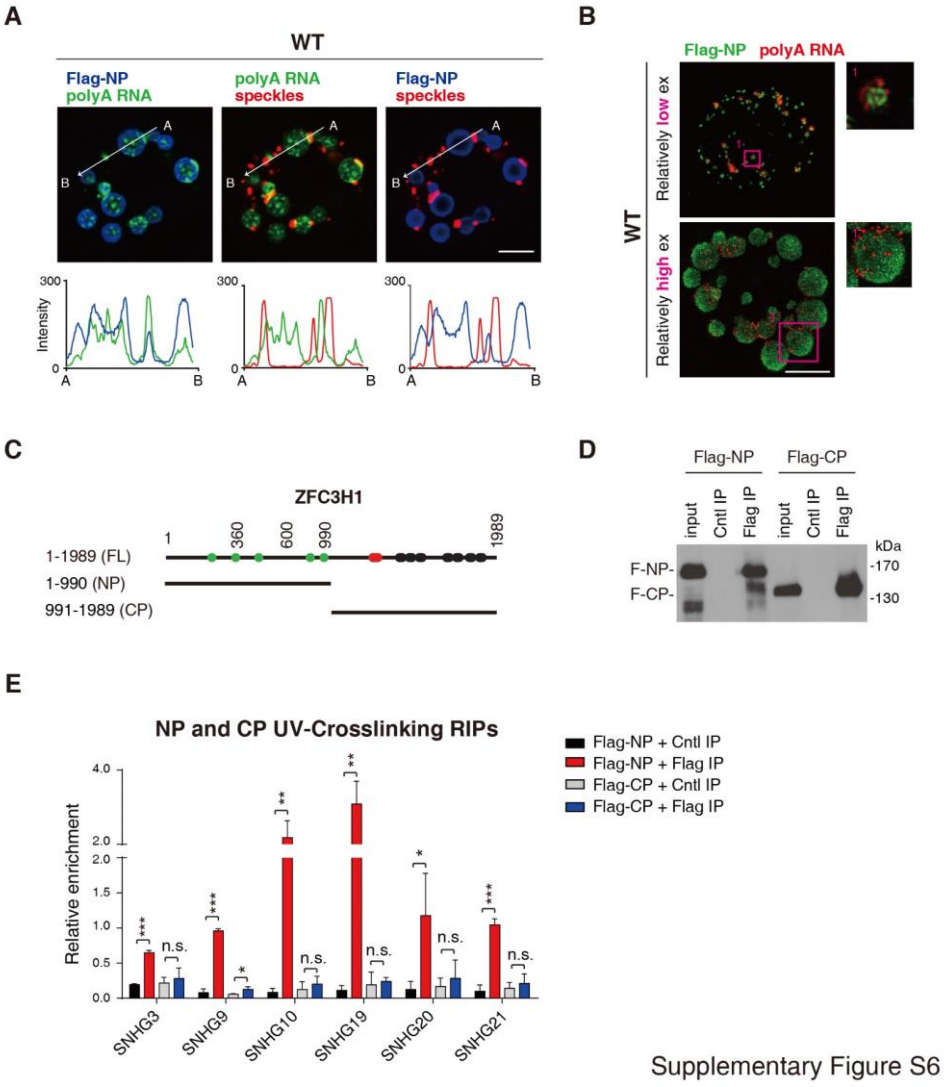
Supplementary Figure S4. The NP of ZFC3H1 associates with CBC and PABPN1, and the CP interacts with MTR4. Flag IP-WB to examine the

associations of ZFC3H1 fragments with CBC, PABPN1 and MTR4 in the absence of RNase A. The white line delineates the boundary where irrelevant lanes have been removed from the same blot.



Supplementary Figure S5

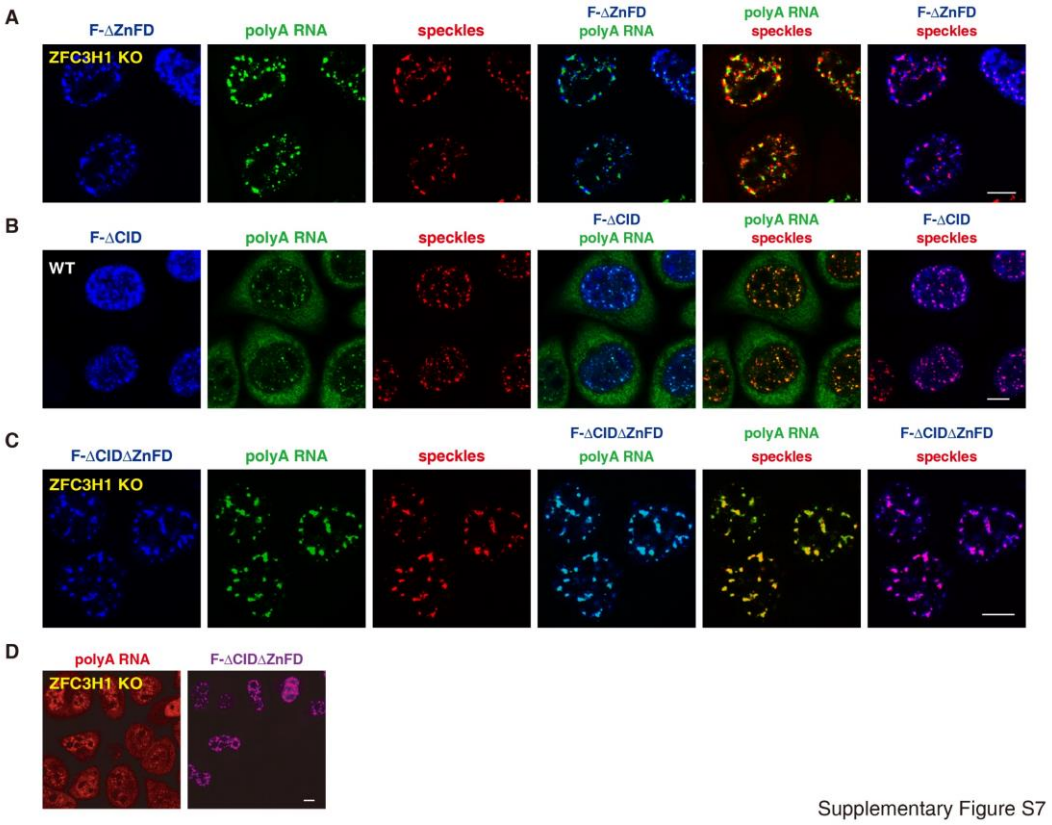
Supplementary Figure S5. The effect of ZFC3H1 FL and mutants on polyA RNA distribution in WT cells. Confocal microscopic imaging to detect the distribution of polyA RNAs in WT cells expressing indicated ZFC3H1 mutants. FISH with an oligo (dT) probe and IF using the Flag antibody were carried out. The white dashed lines indicate the cells with similarly low expression of ZFC3H1 FL and mutants. Scale bar, 10 μm.



Supplementary Figure S6

Supplementary Figure S6. ZFC3H1-NP with RNA binding ability forms condensates in nucleoplasmic. (**A**) Confocal microscopic imaging to examine the localization of polyA RNAs, ZFC3H1-NP and NSs in WT cells. FISH with an oligo (dT) probe and IF using the SON (as a NS marker) and Flag antibodies were carried out. The green, blue and red lines in the graphs show the intensity of the FISH, Flag IF and SON IF signals along the freely positioned arrow indicated from A to B, respectively. Scale bar, 5 μm. (**B**) SIM imaging to detect the distribution of polyA RNAs and ZFC3H1 in cells expressing Flag-ZFC3H1-NP at different expression levels. FISH with an oligo (dT) probe and IF using the Flag antibody were carried

out. Higher magnification of the boxed regions is shown. Scale bar, 5 μm. (**C**) Domain schematic representation of ZFC3H1. The green, red and black bars indicate coiled-coil, zinc finger and TPR repeat domains, respectively. (**D, E**) UV-crosslinking RNA-IP analysis to examine the association of ZFC3H1 fragments with SNHG RNAs. IPs were carried out with Cntl or Flag antibody in cells expressing Flag-NP or CP, followed by western blot to detect ZFC3H1 fragments (D) and RT-qPCRs to detect RNAs (E). Error bar, standard deviations (n=3). Statistical analysis was performed using Student's test. * P<0.05, **P<0.01, ***P<0.001, n.s., not significant.



Supplementary Figure S7. The condensation activity of ZFC3H1 is required for retaining target RNAs in the nucleoplasm. (**A-C**) Confocal microscopic imaging

to examine multiple cells shown in **Figure. 6 A, C, E**. FISH with an oligo (dT) probe and IF using the SON (as a NS marker) and Flag antibodies were carried out. Scale bar, 10 μm. (**D**) Confocal microscopic imaging to examine the localization of polyA RNAs and ΔCIDΔZnFD mutant in ZFC3H1 KO cells. FISH with an oligo (dT) probe and IF using the Flag antibody were carried out. Scale bar, 10 μm.

Supplementary tables

Supplementary Table S1 siRNAs and sgRNA used in this study

siRNAs used in this study	
Target gene	**Targeting sequence (5' to 3')**
Cntl	CGTACGCGGAATACTTCGA
ARS2	CGACCGCAGTGTTAACATT
CBP80	GCUGAUCUUCCUAACUACA
PABPN1	AGTCAACCGTGTTACCATA
UAP56	AAGGGCTTGGCTATCACATTT
URH49	AAAGGCCTAGCCATCACTTTT
sgRNA used in this study	
ZFC3H1	GCGGGCTGTTACCCTATCCG

Supplementary Table S2 Primers used in this study

RT-qPCR primers used in this study		
SNHG3	Forward	CTTCGCATTTTGGCATTGAC
SNHG3	Reverse	TTGCTCCAAGTCTGCCAAAG
SNHG9	Forward	TCCTCTTCACTTAGGACACTGG
SNHG9	Reverse	TTGAAAGACGTGGGACAGC
SNHG10	Forward	GTTATTGACTTCCTACCCAGCA
SNHG10	Reverse	CTGGAATCAATGAATCACGTTC
SNHG19	Forward	CGTCCAGGCCTGGCCTAC
SNHG19	Reverse	GCTCGCGACGAAACCTGC
SNHG20	Forward	GGCATCCAGGTCAGTTTCTG
SNHG20	Reverse	TGGTCAAGAACTAAACCAAACA
SNHG21	Forward	CCACGGAGCTGAGGTATATGA
SNHG21	Reverse	AATTGAAGGCAGGGTTTGTG
proASH1L	Forward	TAGGGAGTGAGGCCAGTAGGA
proASH1L	Reverse	TCCCAGGTTGGCAACTCAAC
proCNOT2	Forward	GTGGTTTCATCAGAGGGGAACT
proCNOT2	Reverse	TGTGCGCGTACTTCAGGG
proPSMC3IP	Forward	TTTCGAGACCCAGTTCCAGC

proPSMC3IP	Reverse	GTCCCTGTGGGGTAACCTTG
ptCSTF3	Forward	CTGGAAACTGTACATTGAAGCAGAG
ptCSTF3	Reverse	TCCTCCTTGTCCTAATTGGCTAT
ptDIDO1	Forward	CCAAACTCTTGCCCTTTGAG
ptDIDO1	Reverse	TCCTAACTCCTGCTCCCAGA
ptDAP	Forward	CGGCCCACTACACTAAAGGA
ptDAP	Reverse	TGAGCATTGAGGCACAAGTC
ptTMED4	Forward	CAGTTGCTTGATCAGGTGGA
ptTMED4	Reverse	AGCTGATCTCCCAAGCAGAA
18S-rRNA	Forward	CATGGCCGTTCTTAGTTGGT
18S-rRNA	Reverse	CGCTGAGCCAGTCAGTGTAG

Supplementary Table S3 Probes used in this study

probe used in this study	
Name	**Targeting sequence**
Vector Probe	AAGGCACGGGGGAGGGGCAAACAACAGATGGCTGG CAACTAGAAGGCACAGTCGAGGCTGATCAGCGGGT
SNHG19 probe	Antisense sequence of human SNHG19 exon region (1-357 nt)