Supplementary Material: Tables S

Table S1. Antibodies of TFIIE, TFIIH, RNA polymerase I transcription machinery, Nucleolus and Ribosomal Proteins are listed below.

Antibody	Source	Identifier	Application
TFIIE			
rabbit polyclonal TFIIEα	Abcam	ab28177	ChIP
mouse monoclonal TFIIEα	Santa Cruz	sc-374014 (B-7)	IF
rabbit polyclonal TFIIEβ	GeneTex	GTX105029	WB
rabbit polyclonal TFIIEβ	GeneTex	GTX109005	WB
rabbit monoclonal TFIIEβ	Abcam	ab187143	ChIP, IF
TFIIH			
mouse monoclonal p62	Santa Cruz	sc-25329 (H-10)	WB
mouse monoclonal XPD	Abcam	ab54676	WB
mouse monoclonal cdk7	Santa Cruz	sc-7344 (C-4)	WB
RNA Polymerase I transcription machinery / Nucleolus			
mouse monoclonal UBF	Santa Cruz	sc-13125 (F-9)	WB
mouse monoclonal TAF I p110	Santa Cruz	sc-374551 (C-10)	WB
mouse monoclonal Rrn3	Santa Cruz	sc-390464 (D-9)	WB
rabbit polyclonal RPA194	Santa Cruz	sc-28714 (H-300)	WB
mouse monoclonal RPA194	Santa Cruz	sc-46699 (F-6)	WB, IF
mouse monoclonal RPA135	Santa Cruz	sc-293272 (4H6)	WB
RPA135	homemade		WB, ChIP
rabbit monoclonal Fibrillarin	Abcam	ab166630	IF
mouse monoclonal Fibrillarin	Abcam	ab4566	IF
rabbit monoclonal Nucleolin	Cell Signaling	14574S	IF
mouse monoclonal Nucleolin	Cell Signaling	87792S	IF
Ribosomal proteins			
mouse monoclonal RPL4	Santa Cruz	sc-100838 (RQ-7)	WB
mouse monoclonal RPL5	Santa Cruz	ab137617	WB
Mouse monoclonal RPL 9	Santa Cruz	sc-100828	WB
mouse monoclonal RPL17	Santa Cruz	sc-515904 (C-8)	WB
rsabbit monoclonal RPS15	Abcam	ab157193	WB
rabbit monoclonal RPS20	Abcam	ab133776	WB
mouse monoclonal RPSA	Santa Cruz	sc-101517 (16)	WB

Table S2. Sequences (5’ to 3’) of primers used to amplify rDNA regions are listed below.

Primer	Forward	Reverse	Position (bp)
rDNA1	ACCTAGCGGTCACTGTTACTC	TCAAAGTGGCGATTTCCTAG	-4957
rDNA2	TCTGTCTCTGCGTGGATTC	AGGGAGGGAGAAAGAACAC	-1200
rDNA3 / promoter region	CTGCGATGGTGGCGTTTTTG	ACAGCGTGTCAGCAATAACC	-85
rDNA4 / 47S	TGTCAGGCGTTCTCGTCTC	AGCACGACGTCACCACATC	356
rDNA5	GCTCTGCCTCGGAAGGAAG	CTGCGGTACGAGGAAACAC	1241
rDNA6	GACGACCCATTCGAACGTCT	CTCTCCGGAATCGAACCCTG	4032
rDNA7 / 18S	CGATGCGGCGGCGTTATTCC	GAACGGCCATGCACCACCAC	4796
rDNA8	CGTTGAACCCCATTCGTG	TATGACCCGCACTTACTCG	5281
rDNA9 / ITS1	GCGGAGGTTTAAAGACCCCTTGG	GTCGGAAGGTTTCACACCACGG	6164
rDNA10	TCGTGCGTCGATGAAGAACGCAG	ATTGATCGGCAAGCGACGCTCAG	6671
rDNA11/ 28S	AGTCGGGTTGCTTGGGAATGC	CCCTTACGGTACTTGTTGACT	8260
rDNA12 / 28S beginning	CATCAGACCCCAGAAAAGG	TGATTCGGCAGGTGAGTTG	9900
rDNA13	GGCATGTTGGAACAATGTAGG	CCTTAGAGCCAATCCTTATCC	10781
rDNA14 / 28S middle	CGCCTAGCAGCCGACTTAG	GTTACTCCCGCCGTTTACCC	11590
rDNA15/ 28SETS	ACCTGGCGCTAAACCATTCGT	GGACAAACCCTTGTGTCGAGG	12901
rDNA16 / IGS	CCACAGGTCGAGGCTTCGGT	GGCGCTCTCTGCGTCTCACT	19086

Supplementary Material: Figure S Legends

Figure S1. Co-localization studies of TFIIE, TFIIH and TFIIF within the nucleolus using STED microscopy. (A) STED images of TFIIH (left) and TFIIF (right) with nucleolus marker RNAP1, Fibrillarin and Nucleolin showing strong co-localization of TFIIH with all nucleolus marker and slight co-localization of TFIIF with RNAP1. Scale bar 5 µm. **(B)** STED images of TFIIEβ after specific inhibition of RNA polymerase I transcription by BMH-21 indicating re-organization of RNAP1 and Fibrillarin to nuclear caps and re-distribution of TFIIEβ enrichment from the nucleolus. Scale bar 5 µm. **(C)** Confocal immunofluorescence microscopy (100X) of WT after ActinomycinD treatment indicate re-distribution of TFIIEβ from the nucleolar enrichment and an organization of RNAP1 to nucleolar caps (arrows). Scale bar 5 µm.

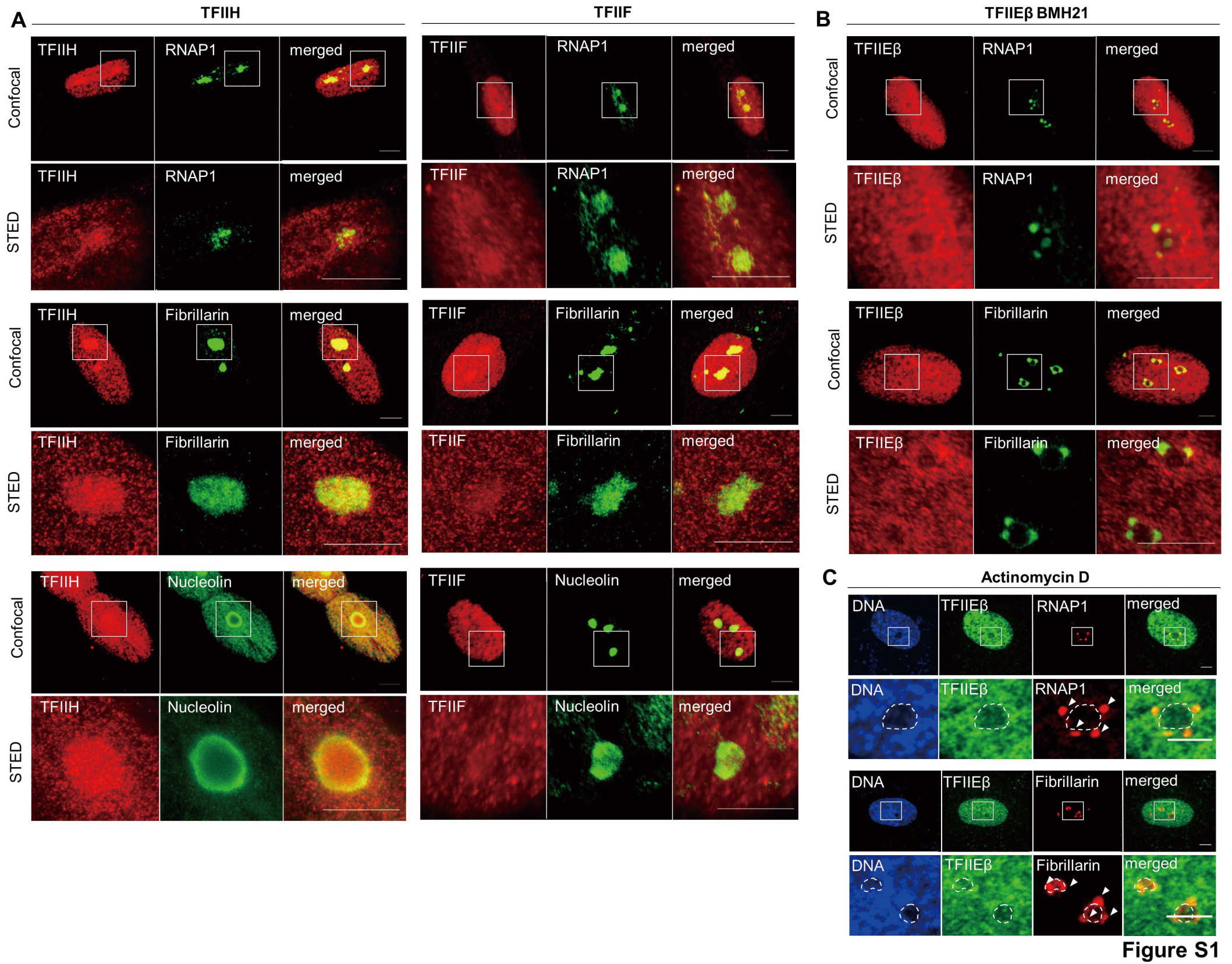
Figure S2. Quantification of co-localization studies. (A-B) Plot Profile of STED images presented in Figure 1A and Figure S1A. Images were analyzed with ImageJ by drawing a line through the nucleolus and measuring the gray value of TFIIEβ (A), TFIIH (B, left) and TFIIF (B, right) with RNAP1, Fibrillarin and Nucleolin. Gray value peaks of TFIIEβ and TFIIH resemble the peaks of RNAP1, Fibrillarin and Nucleolin, while the peaks of TFIIF are more flat indicating less enrichment of TFIIF to the nucleolus compared to TFIIEβ and TFIIH. **(C)** Plot profile of STED images in Figure S1B showing a distinguish plot profile of TFIIEβ and the nucleolus marker RNAP1 and Fibrillarin after BMH-21 treatment. **(D)** Quantification of IF performed in Figure 1D showing no significant changes of TFIIEα localization after inhibition of RNA polymerase I transcription by CX5461.

Figure S3. Binding of TFIIEβ to the same rDNA molecule as RNAP1 and UBF at 28S coding region of the rDNA. (A) Quantitative qPCR analysis of ChIP performed in Figure 2A showing no significant binding of TFIIEβ to the Promotor, 18S coding region, but significant binding to the beginning of the 28S coding region. IGS serves as a negative control. **(B)** Semi-quantitative PCR analysis and of ChIP and sequential ChIP indicating TFIIEβ binds to the same rDNA molecule as RNAP1 and UBF at the 28S coding region. Quantitative qPCR analysis of ChIP (C) and sequential ChIP (D) performed in (B) showing significant binding of TFIIEβ to the same rDNA molecule as UBF at the 28S coding region. **(E)** Full images of Co-immunipercipitation performed in Figure 2E. Data are represented as mean ± SD. ns $p > 0.05$, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$ of three independent experiments.

Figure S4. Reduced TFIIE protein level in TTD cell line. (A) Sequence analysis of TTD218UT (left) and TTD28N (right) compared to WT shows a homozygous mutation (c.559C>A [p.Asp187Tyr]) in both TTD cell lines. **(B)** Immunofluorescence microscopy (100X) of WT cells reveal co-localization of TFIIE and nucleolus marker including Fibrillarin and Nucleolin in WT and reconstituted cells. Reduced fluorescence signals of both TFIIE subunits were observed in TTD cells. **(C)** Western Blot analysis indicates reduced protein abundance of both TFIIE subunits in TTD cells compared to WT and reconstituted cells, whereas the protein abundance of TFIIH is unaffected in TFIIEβ-mutated cells. **(D-E)** Quantification of western blot analysis in Figure S4 C indicate significant reduced protein level of both TFIIE subunits and wild-type protein level of TFIIH.

Figure S5. Disturbed rRNA synthesis in TTD cells. (A) Left: Nuclear Run-On assay shows level of freshly transcribed pre-rRNA in WT, reconstituted and TTD cells. Primer used for Nuclear Run-on analysis are indicated by arrowheads and numbers in the schem of the rRNA subunit. Exact primer sequence are given in Table S2. Nuclear Run-on assay detects in TTD cells significant reduced level of freshly transcribed pre-rRNA starting from 5.8S coding region to 28S coding region of the rDNA indicating a disturbance in the elongation of RNA polymerase I transcription in TTD cells. **Right:** Nuclear Run-on assay in WT, reconstituted and TTD cells detecting mRNA of actin shows no significant differences. **(B)** Quantification of Northern Blot performed in Figure 3C indicating significantly increased mature 5S rRNA level in TTD218UT and increased tendency in TTD28N.

Figure S6. Disturbed rRNA processing and ribosomal composition in TTD cells. (A) Full images of northern blot analysis. Membranes were probed with ITS1 (red), stripped, and re-probed with ITS2 (purple). **(B)** Quantification of 18S-E/47S ratio in WT, reconstituted and TTD cells indicating increased 18S-E level in TTD cells. **(C)** Volcano plot shows a non-significant decreased amount of ribosomal protein of the small 40S ribosome subunit (RPS) and a non-significant increased amount of ribosome protein of the big 60S ribosome subunit (RPL) in TTD cells compared to reconstituted cells. **(D)** Western blot analysis of whole cell lysate and isolated ribosome in WT, reconstituted 218UT TFIIEβ cells and TTD218UT cells. **(E)** Quantification of Western Blots of three ribosomal preparations and selected ribosomal proteins point towards a tendency of over- and underrepresented proteins in lysates and ribosomal preparations. **(F)** Quantification of FISH analysis performed in Figure 4B showing no significant differences in the CTCF of U2 in TTD cells.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TFIIF
B
TFIIEβ BMH21
C
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Confocal
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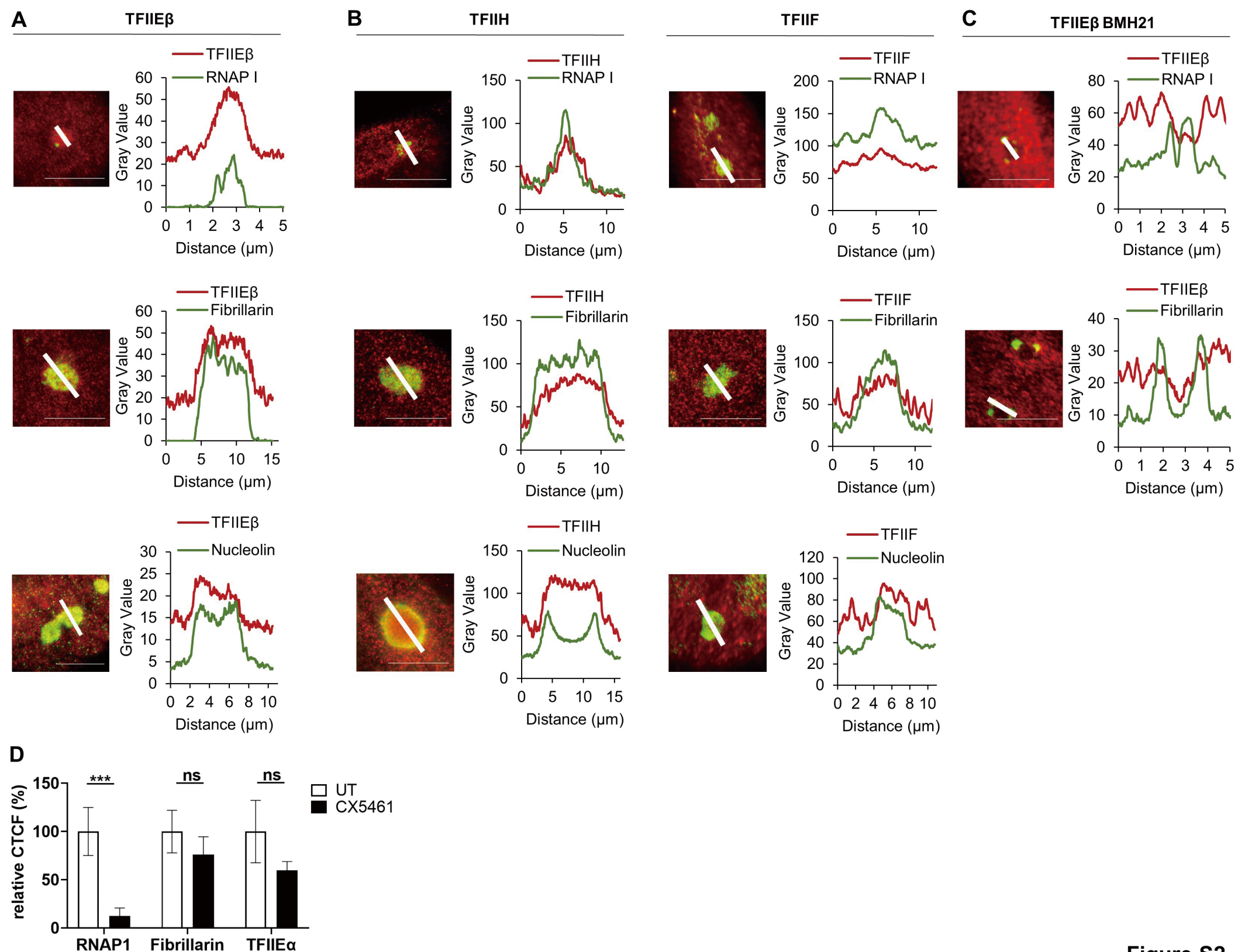
RNAP1
Fibrillarin
Nucleolin
DNA
merged

Figure S1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RNAP I
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Gray Value
Distance (μm)
B
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TFIIH
RNAP I
Gray Value
Distance (μm)
TFIIH
Fibrillarin
Gray Value
Distance (μm)
TFIIH
Nucleolin
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RNAP I
Gray Value
Distance (μm)
TFIIF
Fibrillarin
Gray Value
Distance (μm)
TFIIF
Nucleolin
Gray Value
Distance (μm)
C
TFIIEβ BMH21
TFIIEβ
RNAP I
Gray Value
Distance (μm)
TFIIEβ
Fibrillarin
Gray Value
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relative CTCF (%)
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ns
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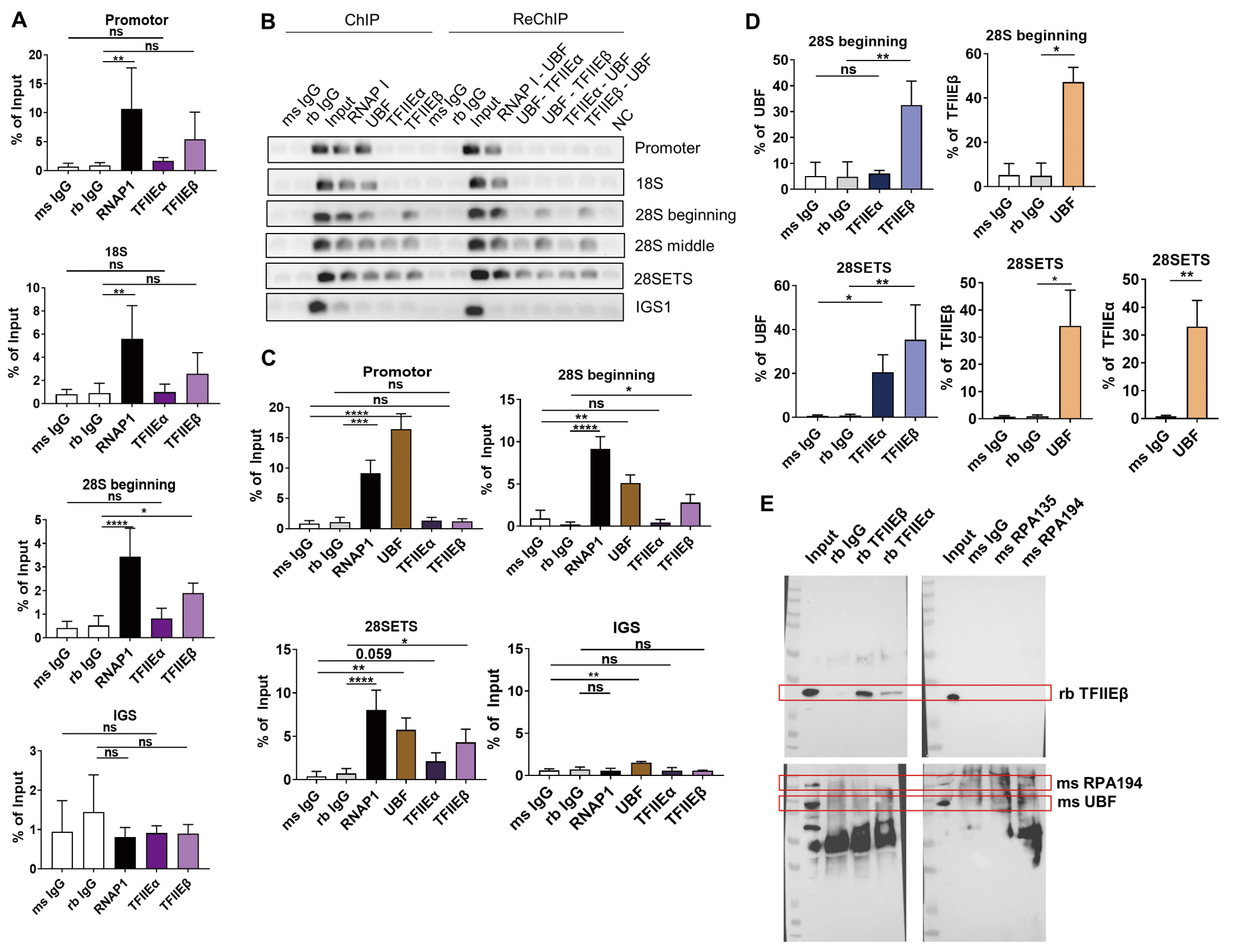
CX5461
RNAP1
Fibrillarin
TFIIEα

Figure S2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IGS
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rb IgG
RNAP1
TFIIEα
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B
ChIP
ReChIP
ms IgG
rb IgG
Input
RNAP I
UBF
TFIIEα
TFIIEβ
RNAP I - UBF
UBF- TFIIEα
UBF - TFIIEβ
TFIIEα - UBF
TFIIEβ - UBF
NC
Promoter
18S
28S beginning
28S middle
28SETS
IGS1
C
Promotor
28S beginning
28SETS
IGS
0.059
D
28S beginning
28SETS
% of UBF
% of TFIIEβ
% of TFIIEα
UBF
E
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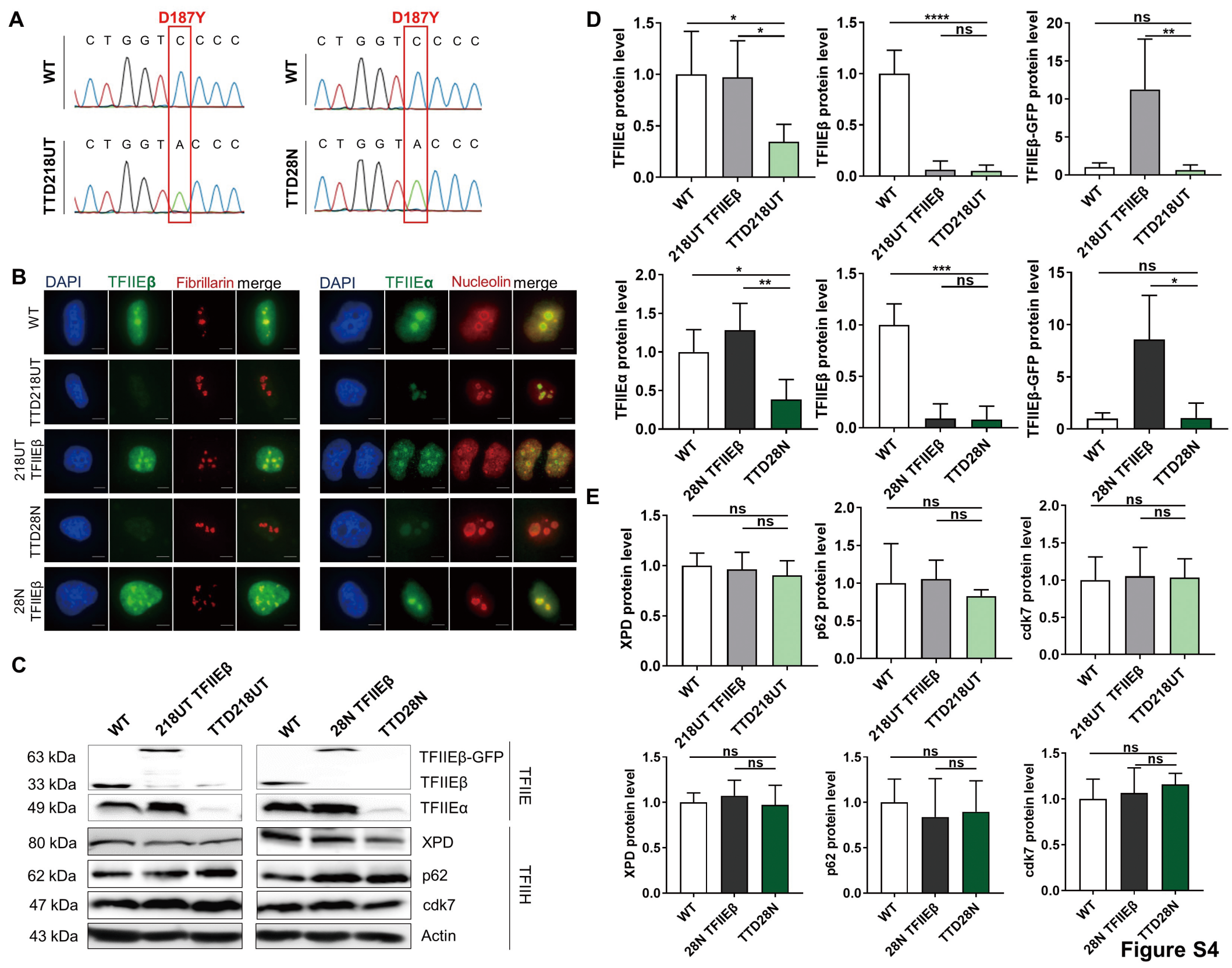
Figure S3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C T G G T A C C C
B
DAPI
TFIIEβ
Fibrillarin
merge
DAPI
TFIIEα
Nucleolin
merge
WT
TTD218UT
218UT TFIIEβ
TTD28N
28N TFIIEβ
C
WT
218UT TFIIEβ
TTD218UT
WT
28N TFIIEβ
TTD28N
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33 kDa
49 kDa
80 kDa
62 kDa
47 kDa
43 kDa
TFIIEβ-GFP
TFIIEβ
TFIIEα
XPD
p62
cdk7
Actin
TFIIE
TFIIH
D
TFIIEα protein level
TFIIEβ protein level
TFIIEβ-GFP protein level
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E
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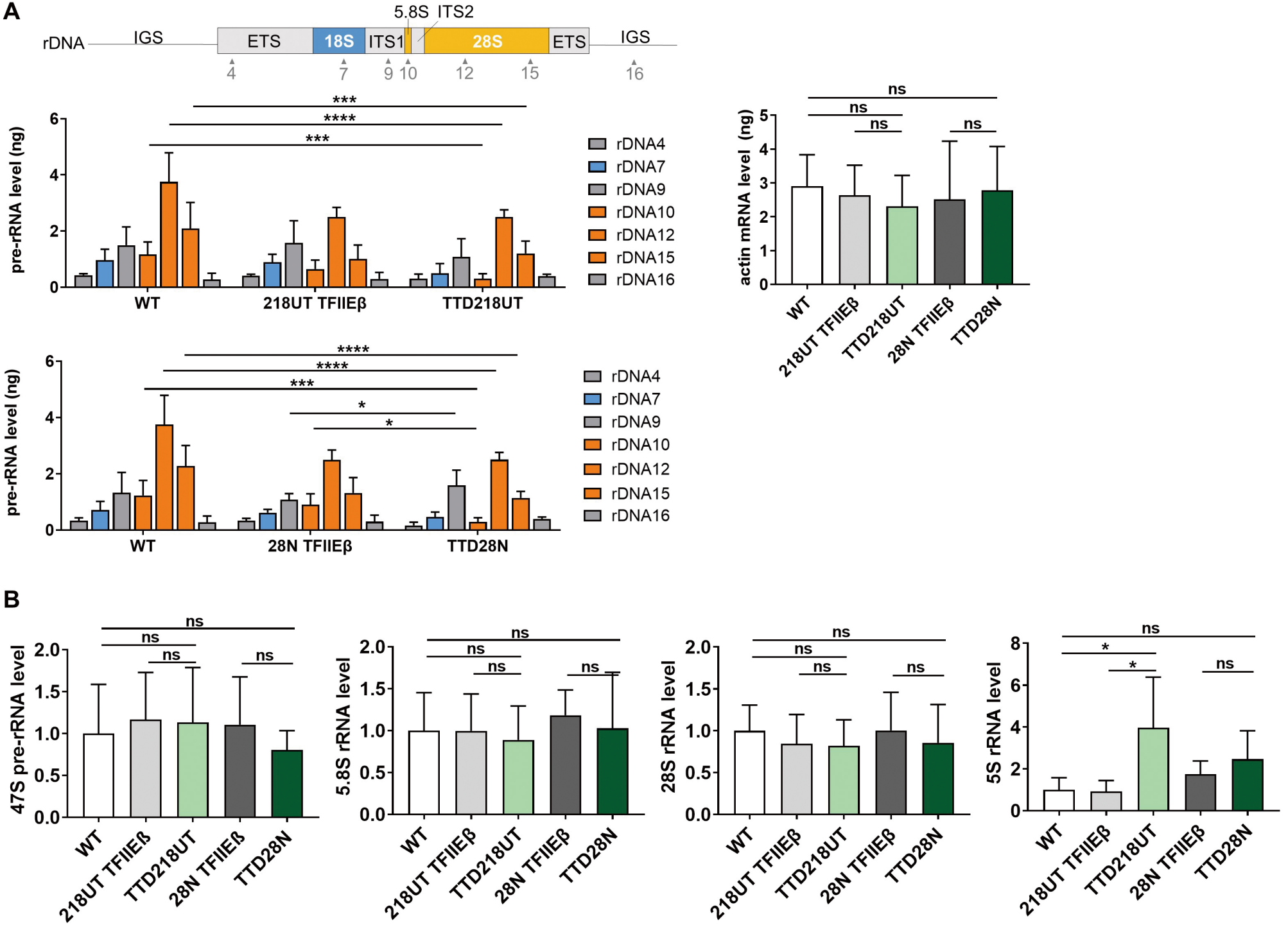
p62 protein level
cdk7 protein level
ns
Figure S4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rDNA
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ETS
18S
ITS1
5.8S
ITS2
28S
ETS
IGS
4
7
9
10
12
15
16
pre-rRNA level (ng)
***
****
***
rDNA4
rDNA7
rDNA9
rDNA10
rDNA12
rDNA15
rDNA16
WT
218UT TFIIEβ
TTD218UT
actin mRNA level (ng)
ns
28N TFIIEβ
TTD28N
****
*
B
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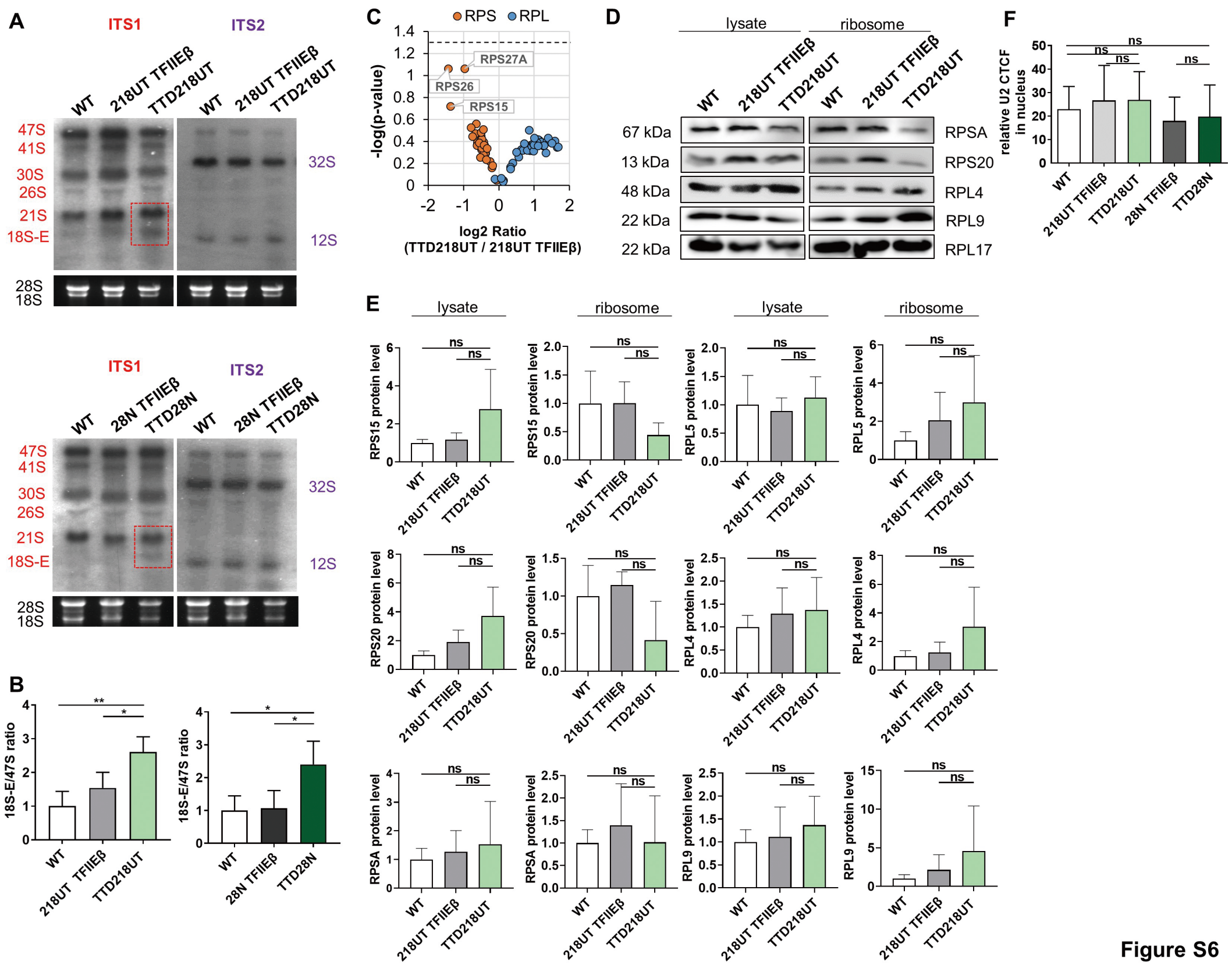
5.8S rRNA level
28S rRNA level
5S rRNA level

Figure S5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ITS1
ITS2
WT
218UT TFIIEβ
TTD218UT
47S
41S
30S
26S
21S
18S-E
32S
12S
28S
18S
28N TFIIEβ
TTD28N
B
18S-E/47S ratio
C
RPS
RPL
-log(p-value)
RPS27A
RPS26
RPS15
log2 Ratio
(TTD218UT / 218UT TFIIEβ)
D
lysate
ribosome
67 kDa
13 kDa
48 kDa
22 kDa
RPSA
RPS20
RPL4
RPL9
RPL17
E
RPS15 protein level
RPS20 protein level
RPL5 protein level
RPL4 protein level
RPSA protein level
RPL9 protein level
ns
F
relative U2 CTCF in nucleus

Figure S6