Supplementary information

TFIIB-related factor 1 is a nucleolar protein that promotes RNA polymerase I-directed transcription and tumour cell growth

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Figure S1

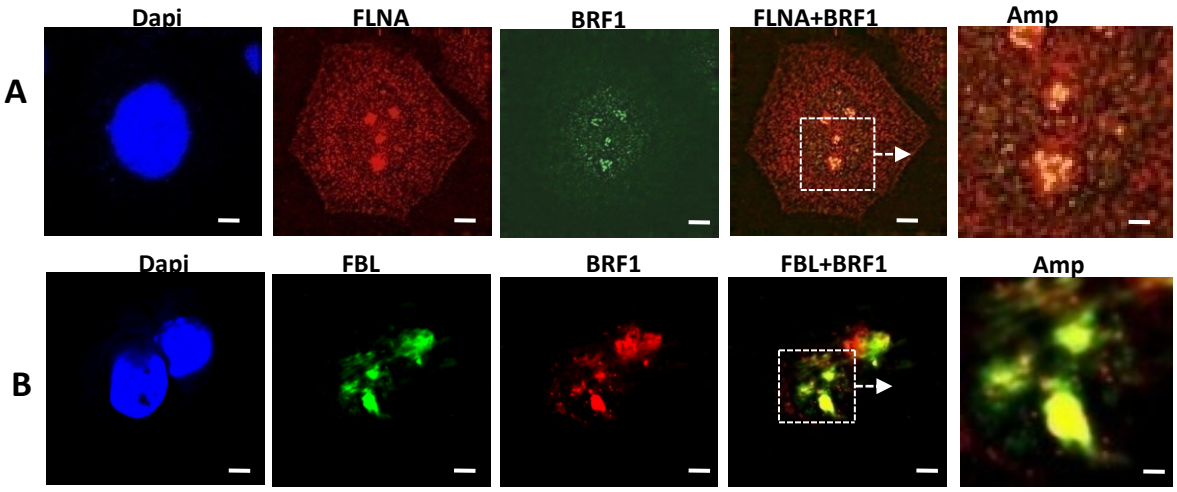


Fig. S1. BRF1 is present in the nucleoli within SaOS2 cells

(**A**) BRF1 and FLNA were colocalized in the nucleoli of SaOs2 cells. (**B**) BRF1 and fibrillarin were colocalized in the nucleoli of SaOS2 cells. IF assays were performed using SaOS2 cells and antibodies against BRF1, FLNA, and FBL. The IF samples in A and B were observed under a DeltaVision fluorescence microscopy and imaged with a 60x objective (Olympus). The scale bars in images represent 1 μm.

Figure S2

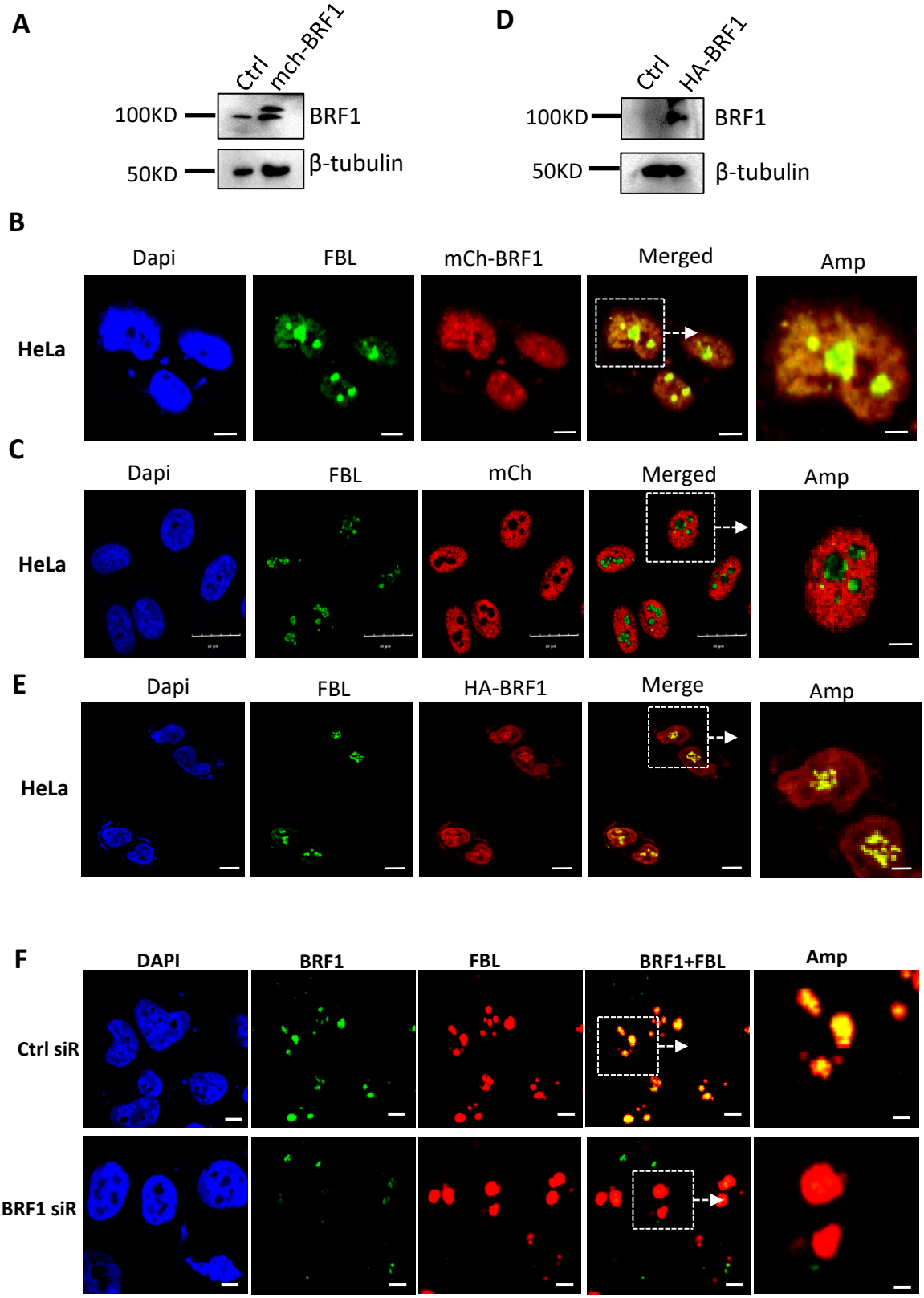

A
Ctrl
mch-BRF1
100KD
BRF1
50KD
β-tubulin
D
Ctrl
HA-BRF1
100KD
BRF1
50KD
β-tubulin
B
Dapi
FBL
mCh-BRF1
Merged
Amp
HeLa
C
Dapi
FBL
mCh
Merged
Amp
HeLa
E
Dapi
FBL
HA-BRF1
Merge
Amp
HeLa
F
DAPI
BRF1
FBL
BRF1+FBL
Amp
Ctrl siR
BRF1 siR

Fig. S2. Alteration of BRF1 expression affected the localization in the nucleoli of HeLa cells
(**A**) Western blot was used to detect mCherry-BRF1 expression in HeLa cells transiently transfected with the vectors expressing mCHerry-BRF1. (**B**) mCherry-BRF1 and fibrillarin (FBL) were colocalized in the nucleoli of HeLa cells transiently transfected with the vectors expressing mCHerry-BRF1. The IF samples were observed under a confocal fluorescence microscopy and imaged with a 60x objective (Olympus). The scale bars represent 5 μm or 2 μm (Amp). Amp : An amplified image from the square area enclosed by white dotted lines. (**C**) mCherry and fibrillarin (FBL) colocalization analysis using HeLa cells transiently transfected with the vectors expressing mCherry. The IF samples were observed under a confocal fluorescence microscopy and imaged with 40x objective (Olympus). The scale bars represent 20 μm or 4 μm (Amp). (**D**) Western blot was used to detect the expression of HA-BRF1 in HeLa cells stably expressing HA-BRF1. (**E**) HA-BRF1 and fibrillarin (FBL) were colocalized in the nucleoli of HeLa cells stably expressing HA-BRF1. The IF samples were observed under a confocal fluorescence microscopy and imaged with a 40x objective (Olympus). The scale bars represent 10 μm or 4 μm (Amp). (**F**) BRF silencing reduced BRF1 localization to the nucleoli of HeLa cells. IF assays were performed using HeLa cells expressing BRF1 siRNA or control siRNA and antibodies against BRF1 and FBL. The IF samples were observed under a confocal fluorescence microscopy and imaged with a 60x objective (Olympus). The scale bars represent 1 μm, the scale bars in amplified images (Amp) represent 0.3 μm.

Figure S3

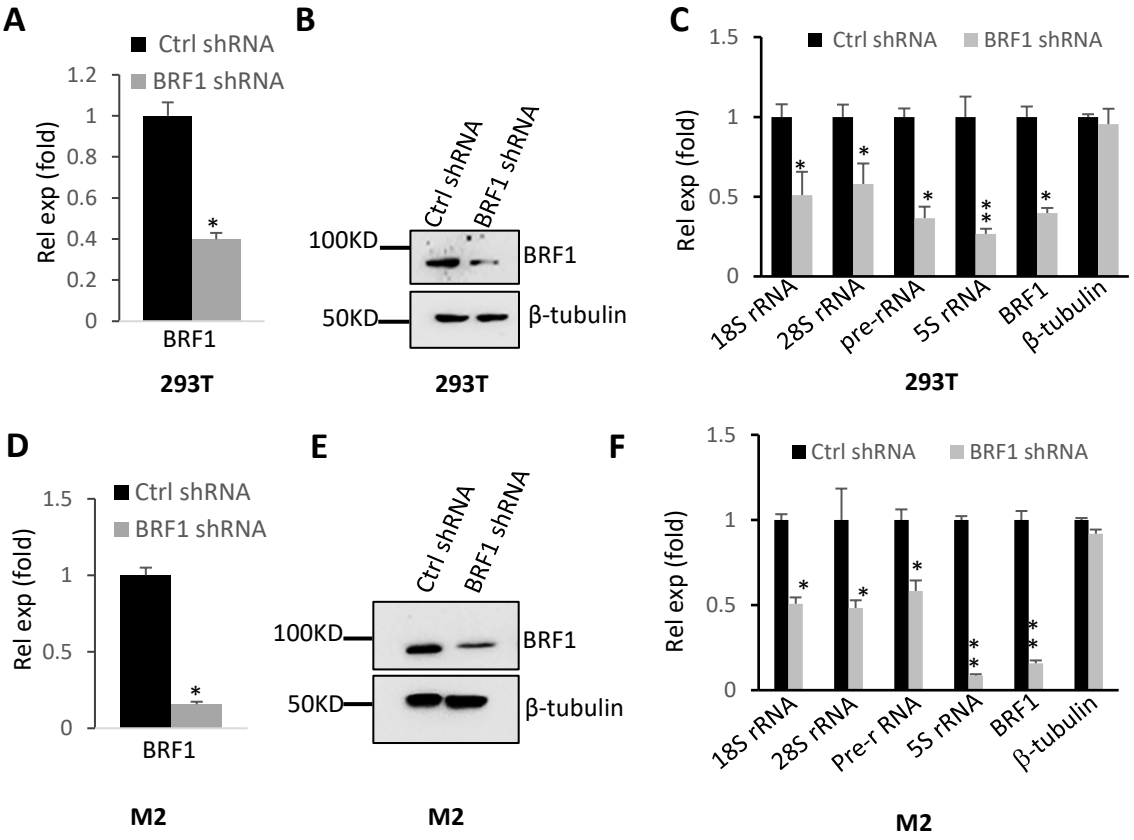


Fig. 3. BRF1 knockdown reduced Pol I-mediated transcription.

(**A**-**C**) Stable expression of BRF1 shRNA reduced Pol I-directed transcription in 293T cells. 293T cell lines stably expressing BRF1 shRNA or control shRNA were generated using a lentiviral transduction system. BRF1 mRNA and protein levels were analyzed by RT-qPCR (A) and Western blot (B), respectively. Ribosomal RNA gene expression was examined by RT-qPCR (C). (**D**-**F**) BRF1 knockdown reduced Pol I-dependent transcription in M2 cells. M2 cell lines stably expressing BRF1 shRNA or control shRNA were generated using a lentiviral transduction system. BRF1 mRNA (D) and protein (E) were detected by RT-qPCR and Western blot, respectively. Ribosomal RNA gene expression (**F**) was analyzed by RT-qPCR. Each column in the bar graphs represents the mean ± SD of three biological replicates. *, $P \leqq 0.05$; **, $P \leqq 0.01$. *P* values for different genes were obtained by Student's *t* test performed with control and treatment groups.

Figure S4

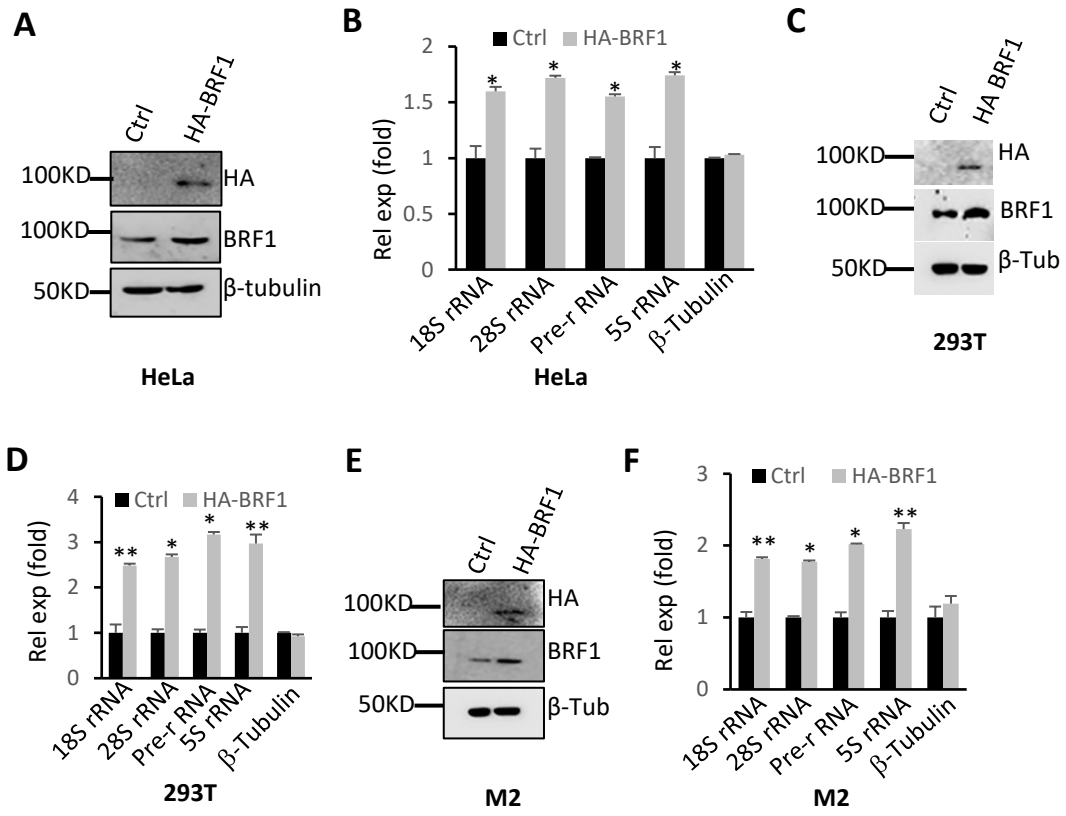


Fig. S4. Effect of BRF1 overexpression on Pol I–directed transcription.

(**A** and **B**) Stable expression of HA-BRF1 in HeLa cells activated Pol I-directed transcription. A HeLa cell line stably expressing HA-BRF1 and the corresponding control cell line were generated and determined by Western blot (A). Ribosomal RNA expression was analysed by RT-qPCR (B). (**C** and **D**) Stable expression of HA-BRF1 enhanced Pol I-directed transcription in 293T cells. A 293T cell line stably expressing HA-BRF1 and the corresponding control cell line were generated as described for HeLa cell lines. HA-BRF1 expression (C) and Pol I transcription (D) was examined using Western blot and RT-qPCR, respectively. (**E** and **F**) Stable expression of HA-BRF1 increased Pol I-mediated transcription in M2 cells. A M2 cell line stably expressing HA-BRF1 and the corresponding control cell line were generated as described for HeLa cells. HA-BRF1 expression (E) and Pol I transcription (F) was examined using Western blot and RT-qPCR, respectively. Each column in B, D, and F represents the mean ± SD of three biological replicates. *, $P \leqq 0.05$; **, $P \leqq 0.01$, *P* values were obtained by Student's *t* test performed with control and treatment groups.

Figure S5

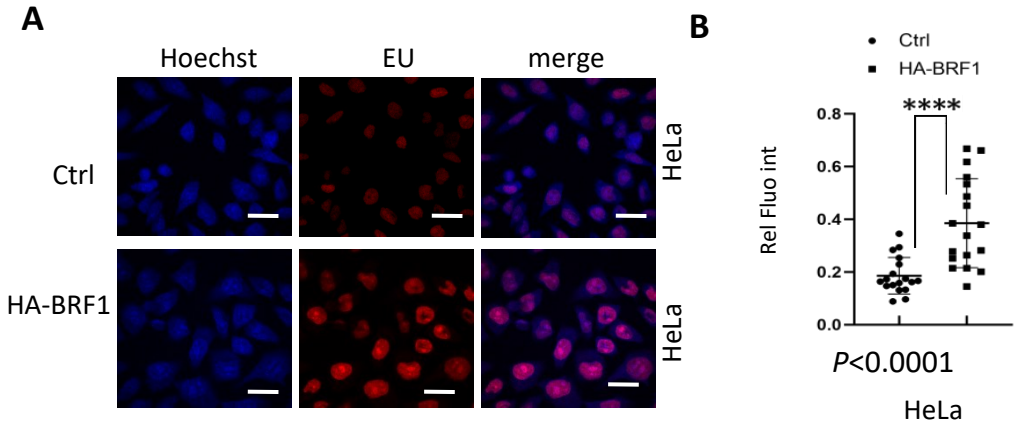


Fig. S5. BRF1 overexpression enhanced rRNA levels newly synthesized in the nulceoli of HeLa cells.

A HeLa cell line stably expressing HA-BRF1 and the corresponding control cell line were labelled with 5-ethynyl urindine (EU). The EU labelled RNA was detected using a EU detection kit. Images (**A**) were captured under a fluorescence microscope (Olympus) and fluorescence intensity (**B**) within nucleoli and nucleoplasm was calculated using the ImageJ software. Scale bars in the images represent 50 µm. The *p* value were obtained by Student's *t* test performed with control and treatment groups.

Figure S6

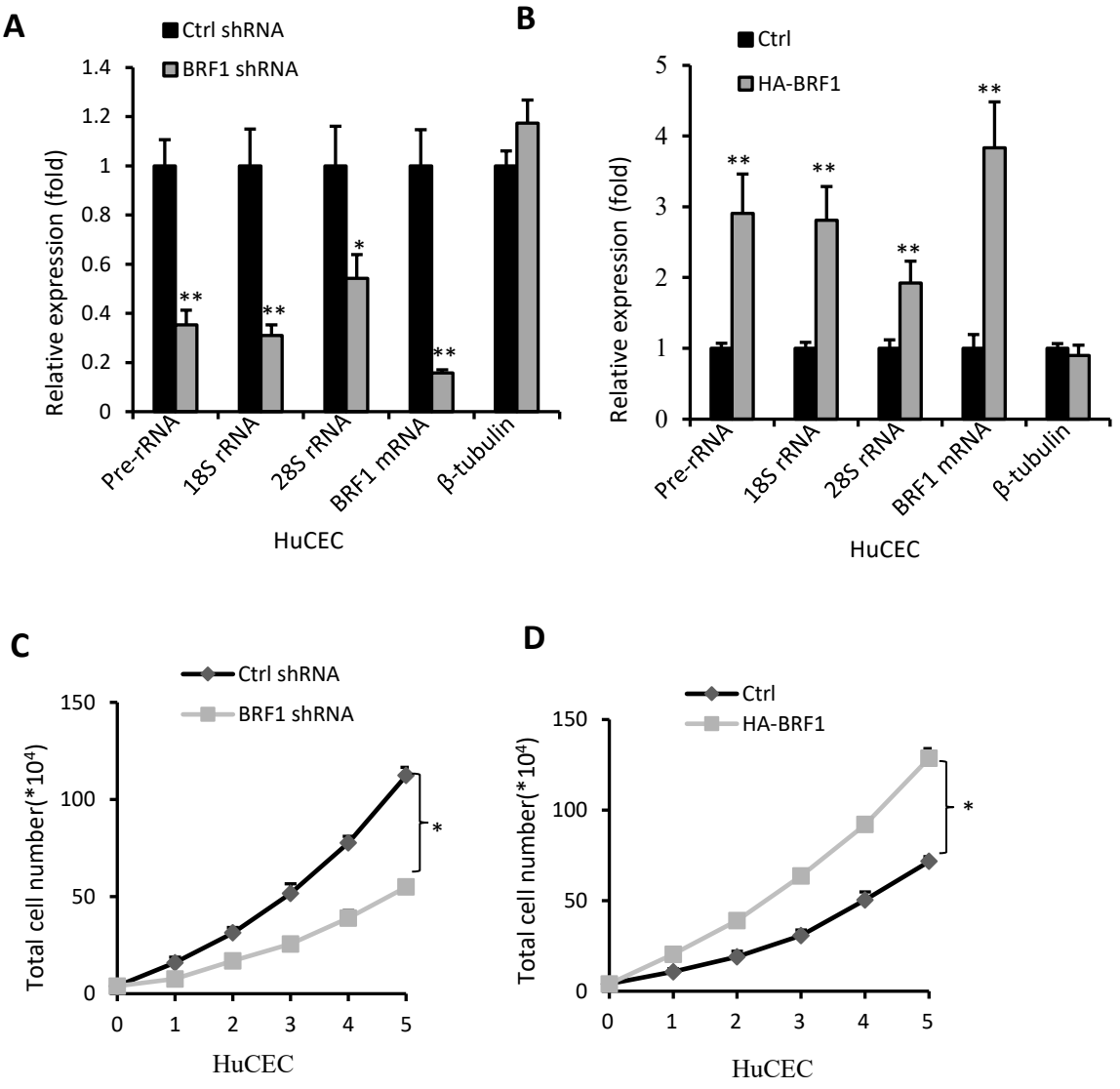


Fig. S6. BRF1 promotes Pol I-mediated transcription and proliferation of HuCEC cells.

(**A**) BRF1 silencing decreased the expression of Pol I products in HuCEC cells. RT-qPCR was performed using the RNA extracted from HuCEC cells stably expressing BRF1 shRNA or control shRNA. (**B**) BRF1 overexpression increased the expression of Pol I products in HuCEC cells. RT-qPCR was performed using the RNA extracted from a HuCEC cells stably expressing HA-BRF1 and its control cell line. (**C**) BRF1 silencing reduced HuCEC cell proliferative activity. Cell counting was performed using HuCEC cell lines stably expressing BRF1 shRNA or control shRNA. (**D**) BRF1 overexpression increased HuCEC cell proliferative activity. Cell counting was performed using a HuCEC cell line stably expressing HA-BRF1 and its control cell line. Each column in the graphs and J represents the mean ± SD of three biological replicates. *, $P \leqq 0.05$; **, $P \leqq 0.01$, *P* values in A and B were obtained by Student's *t* test performed with control and treatment groups; *P* values in C and D were obtained by two-way ANOVA.

Figure S7

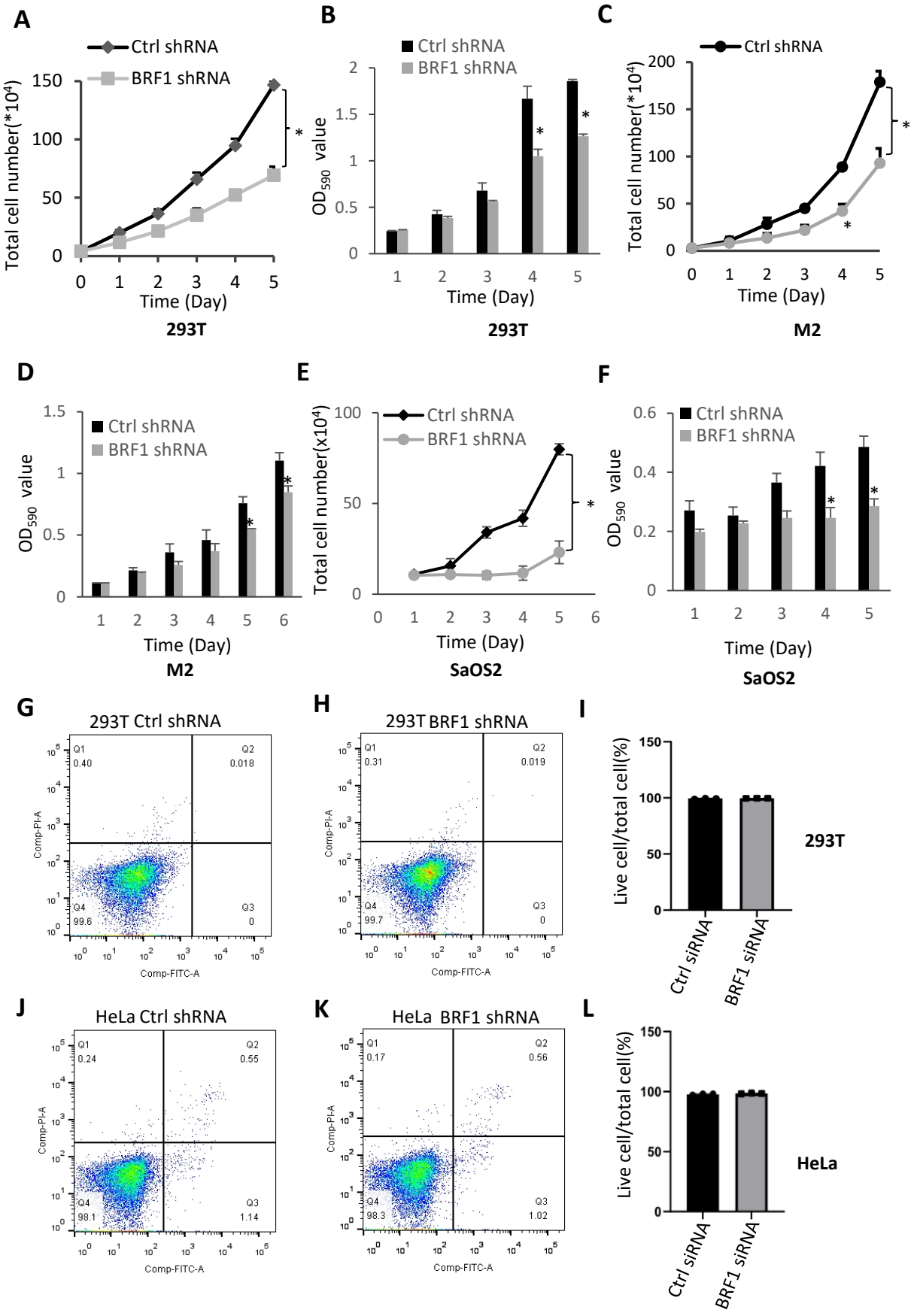

A
Ctrl shRNA
BRF1 shRNA
Total cell number(*10^4)
Time (Day)
293T
B
Ctrl shRNA
BRF1 shRNA
OD590 value
Time (Day)
293T
C
Ctrl shRNA
Total cell number(*10^4)
Time (Day)
M2
D
Ctrl shRNA
BRF1 shRNA
OD590 value
Time (Day)
M2
E
Ctrl shRNA
BRF1 shRNA
Total cell number(x10^4)
Time (Day)
SaOS2
F
Ctrl shRNA
BRF1 shRNA
OD590 value
Time (Day)
SaOS2
G
293T Ctrl shRNA
Q1 0.40
Q2 0.018
Q4 99.6
Q3 0
Comp-PI-A
Comp-FITC-A
H
293T BRF1 shRNA
Q1 0.31
Q2 0.019
Q4 99.7
Q3 0
Comp-PI-A
Comp-FITC-A
I
Live cell/total cell(%)
293T
Ctrl siRNA
BRF1 siRNA
J
HeLa Ctrl shRNA
Q1 0.24
Q2 0.55
Q4 98.1
Q3 1.14
Comp-PI-A
Comp-FITC-A
K
HeLa BRF1 shRNA
Q1 0.17
Q2 0.56
Q4 98.3
Q3 1.02
Comp-PI-A
Comp-FITC-A
L
Live cell/total cell(%)
HeLa
Ctrl siRNA
BRF1 siRNA

Fig. S7. BRF1 knockdown inhibited the proliferative activity of transformed cell lines.

(**A** and **B**) BRF1 knockdown reduced 293T cell proliferation. (**C** and **D**) BRF1 depletion inhibited M2 cell proliferation. (**E** and **F**) BRF1 silence repressed SaOS2 cell proliferation. The results in A, C, and E were obtained by cell counting, whereas the results in B, D, and F were obtained by MTT assays. Each column in the graphs represents the mean±SD of three biological replicates. *, $P \leqq 0.05$; **, $P \leqq 0.01$. *P* values were obtained by two-way ANOVA. (**G**-**I**)The results of cell viability analysis by flow cytometry using 293T cell lines stably expressing ctrl shRNA and BRF1 shRNA. (**J**-**L**) The results of cell viability analysis by flow cytometry using HeLa cell lines stably expressing ctrl shRNA and BRF1 shRNA

Figure S8

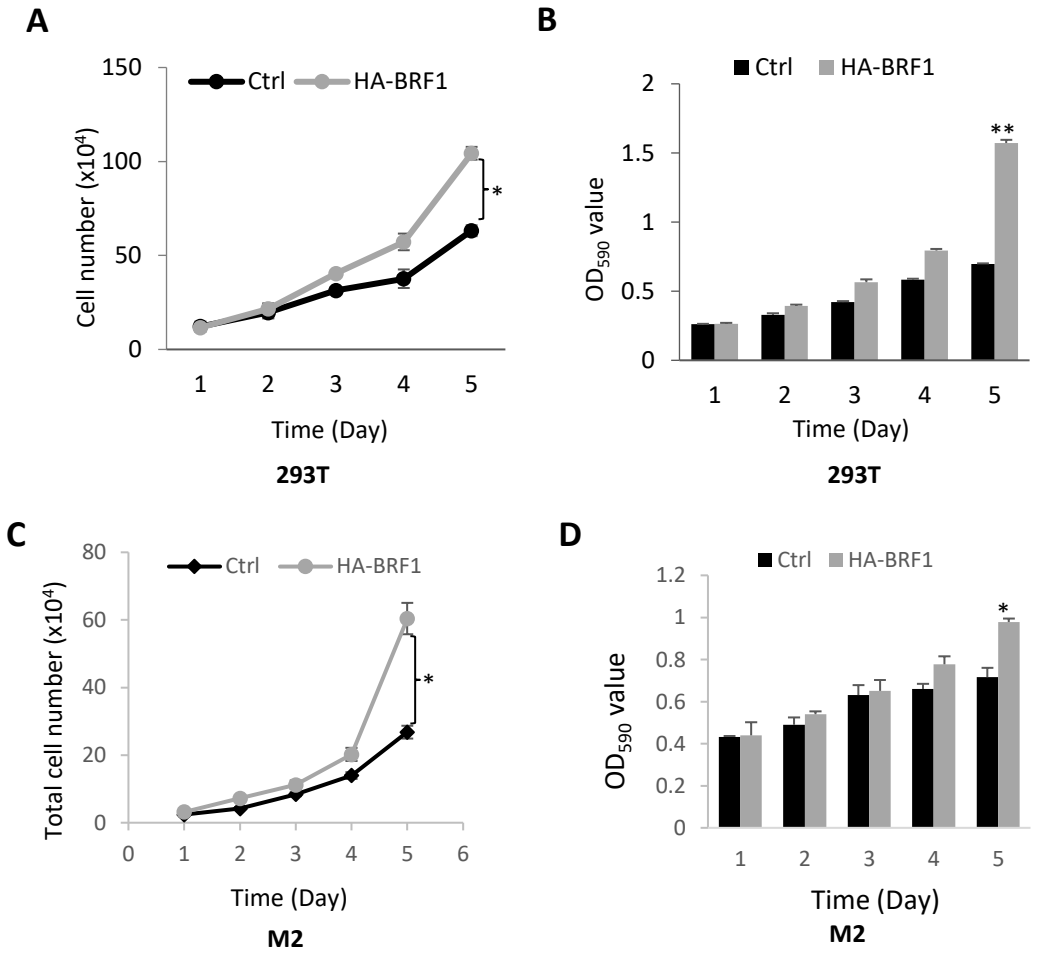


Fig. 8. BRF1 overexpression stimulated the proliferative activity of transformed cell lines

(**A** and **B**) BRF1 overexpression increased 293T cell proliferation; (**C** and **D**) BRF1 overexpression enhanced M2 cell proliferation. The results in A and C were obtained by cell counting, whereas the results in B and D were obtained by MTT assays. Each column in the graphs represents the mean±SD of three biological replicates. *, $P \leqq 0.05$; **, $P \leqq 0.01$, *P* values were obtained by two-way ANOVA.

Figure S9

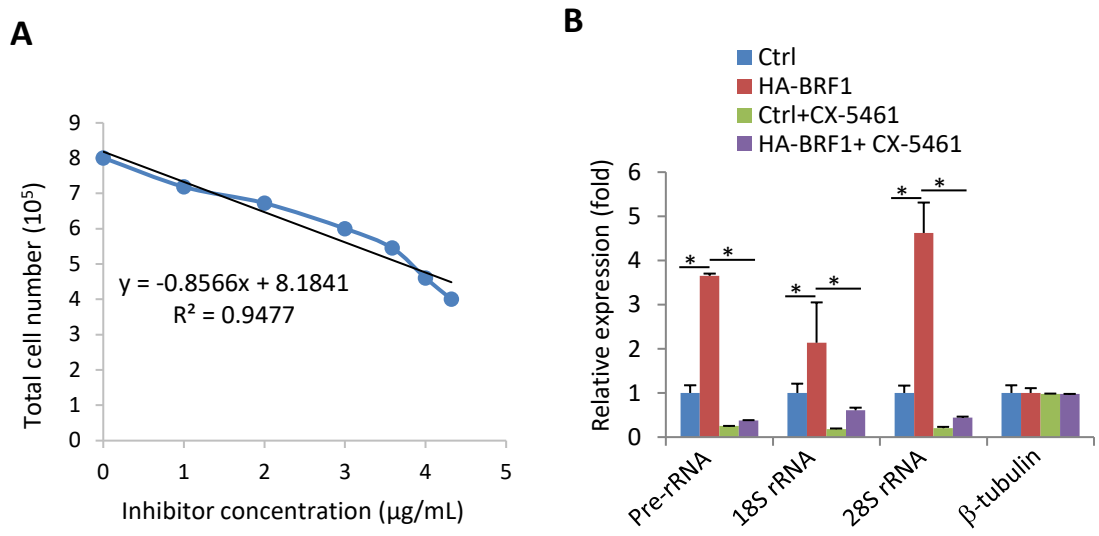


Fig. S9. CX-5461 inhibited the activation of Pol I transcription caused by BRF1 overexpression in HeLa cells.

(**A**) The correlation analysis between the concentrations of the inhibitor CX-5461 and the number of HeLa cells. IC_{50} was determined by a linear regression equation. (**B**) the inhibitor CX-5461 significantly impeded Pol I transcriptional activation caused by BRF1 overexpression. HeLa cells line stably expressing HA-BRF1 or control cells were cultured in 6-well plates for 24 hours. An inhibitor (CX-5461) was added to the HA-BRF1-expressing cells at the final concentration of 4 μg/mL. Cells were harvested for RT-qPCR assays after incubating with the medium containing the inhibitor for 4 hours . *, $P \leqq 0.05$; **, $P \leqq 0.01$, *P* values were obtained by Student *t* test performed with two groups as indicated.

Figure S10

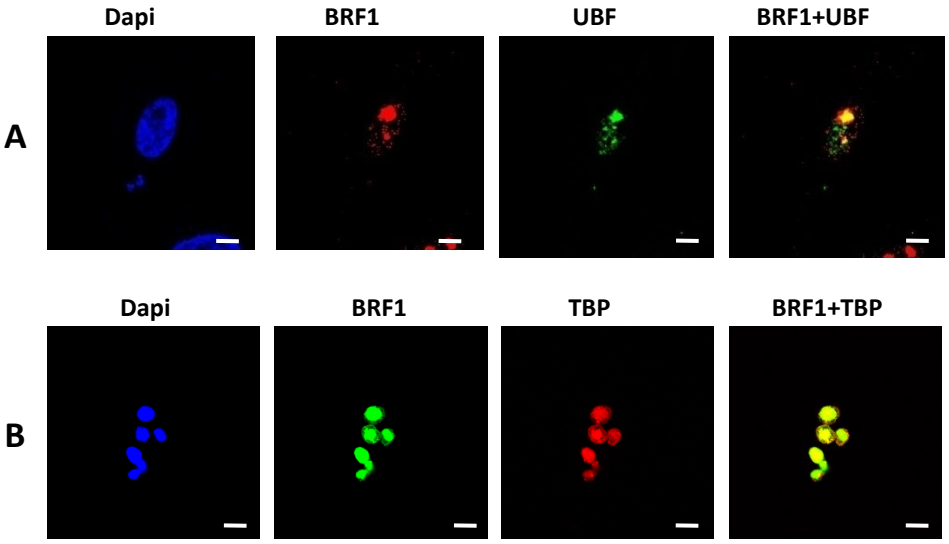
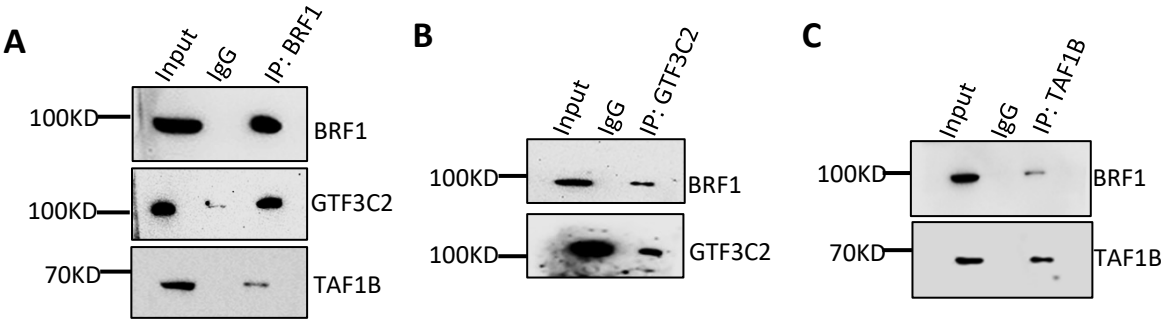


Fig. 10. The colocalization analysis between BRF1 and Pol III transcription factors in HeLa cells.

(**A**) BRF1 and UBF were colocalized in the nucleoli of HeLa cells. Immunofluorescence assays were performed using the HeLa cells and the antibodies against BRF1 and UBF. (**B**) BRF1 and TBP were colocalized in the nucleoli purified from HeLa cells. IF assays were performed using the nucleoli particles purified from HeLa cells and the antibodies against BRF1 and TBP. Scale bars in A and B represent 5 μm.

Figure S11



BRF1 binds to TFIIIC subunit GTF3C2 and SL1 subunit TAF1B

(**A**) The BRF1 IP results showing BRF1 interaction with GTF3C2 and TAF1B. IP assays were performed using an anti-BRF1 antibody and HeLa nuclear extract. IP samples was detected by Western blot using the indicated antibodies. (**B**) GTF3C2 IP result showing GT3C2 interaction with BRF1. IP assays were performed using GTF3C2 antibody and HeLa nuclear extract. IP samples was detected by Western blot using the antibodies as indicated. (**C**) TAF1B IP result showing TAF1B interaction with BRF1. IP assays were performed using an anti-TAF1B antibody and HeLa nuclear extract. IP samples was detected by Western blot using the indicated antibodies.

Table S1. Primer sequences used for qPCR assays in this study

Names of gene products or DNA Fragments	Primer designations	Primer sequences
Pre-rRNA	RTPre-rRNAF	CCGCGCTCTACCTTACCTAC
	RTPre-rRNAR	GAGCGACCAAAGGAACCATA
28S rRNA	RT28S rRNAF	GGAGGATTCAACCCGGCG
	RT28S rRNAR	GGGGCTGTAACACTCGGG
18S rRNA	RT18S rRNAF	GTAACCCGTTGAACCCCATT
	RT18S rRNAR	CCATCCAATCGGTAGTAGCG
5S rRNA	RT5S rRNAF	CTACGGCCATACCACCCT
	RT5S rRNAR	GCCTACAGCACCCGGTATT
BRF1	RTBRF1F	CAGAATGCATGACTTCAGGAGG
	RTBRF1R	TCATCCGCAGCTTCCTCTG
β-tubulin	RTβ-tubulinF	ATGAAGAGGATGACGAGG
	RTβ-tubulinR	TCAGTTGGAGTCAGAGTCTG
rDNA promoter	ChIPrDNAPF	GGTATATCTTTCGCTCCGAG
	ChPrDNAPR	GGTATATCTTTCGCTCCGAG
28S rRNA coding region	ChIP28SCRF	GCGACCTCAGATCAGACGTGG
	ChIP28SCRR	CTGTTCACTCGCCGTTACTGAG
45S rRNA Intergenic Spacer	ChIP45SIGSF	CCTTCCACGAGAGTGAGAAGC
	ChIP45SIGSR	TCGACCTCCCGAAATCGTACA
5S rRNA promoter	ChIP5SrRNAPF	CTACTTTTGTCGTTTTCC
	ChIP5SrRNAPR	ATGGATGGAGAGATAGAA