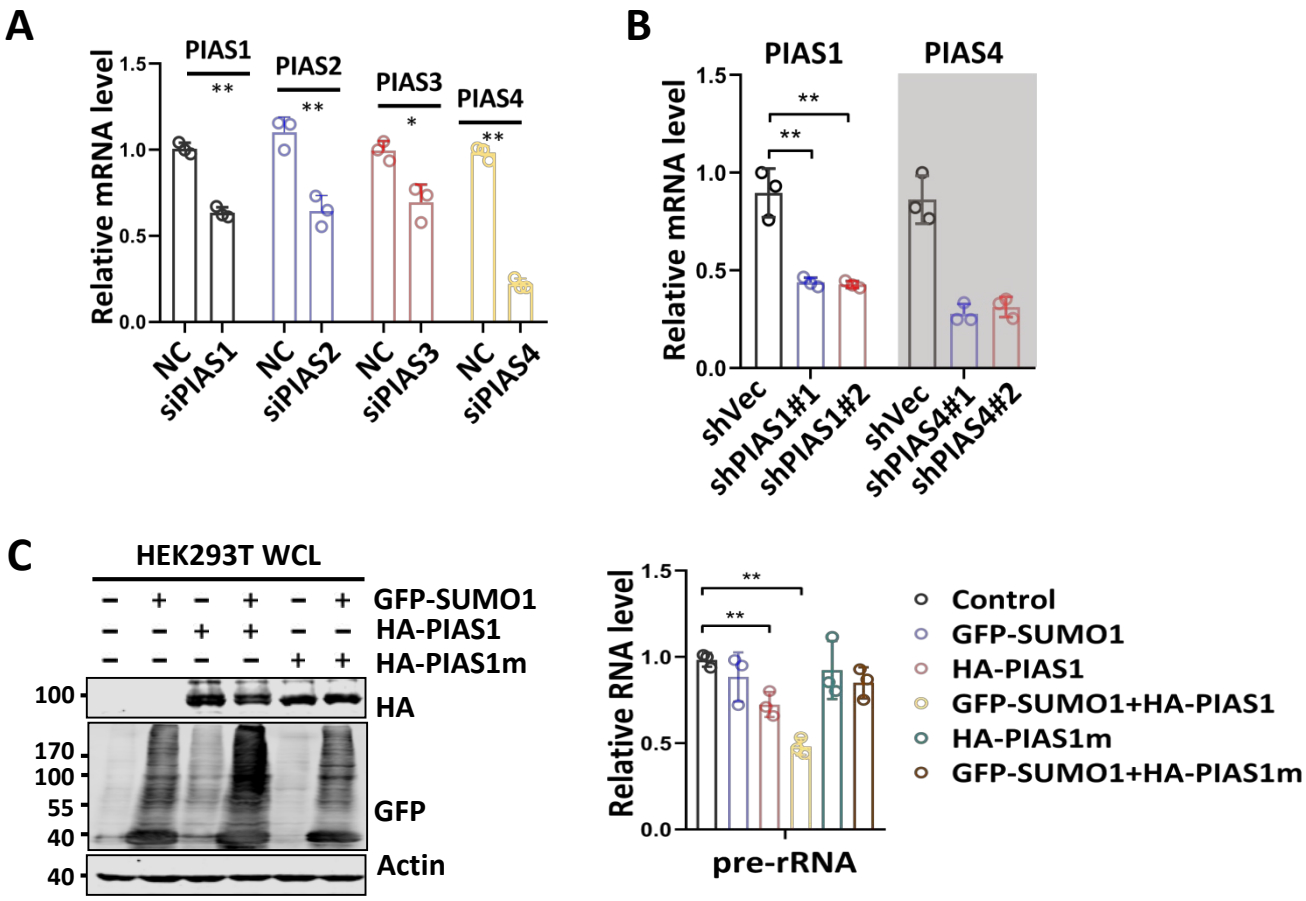
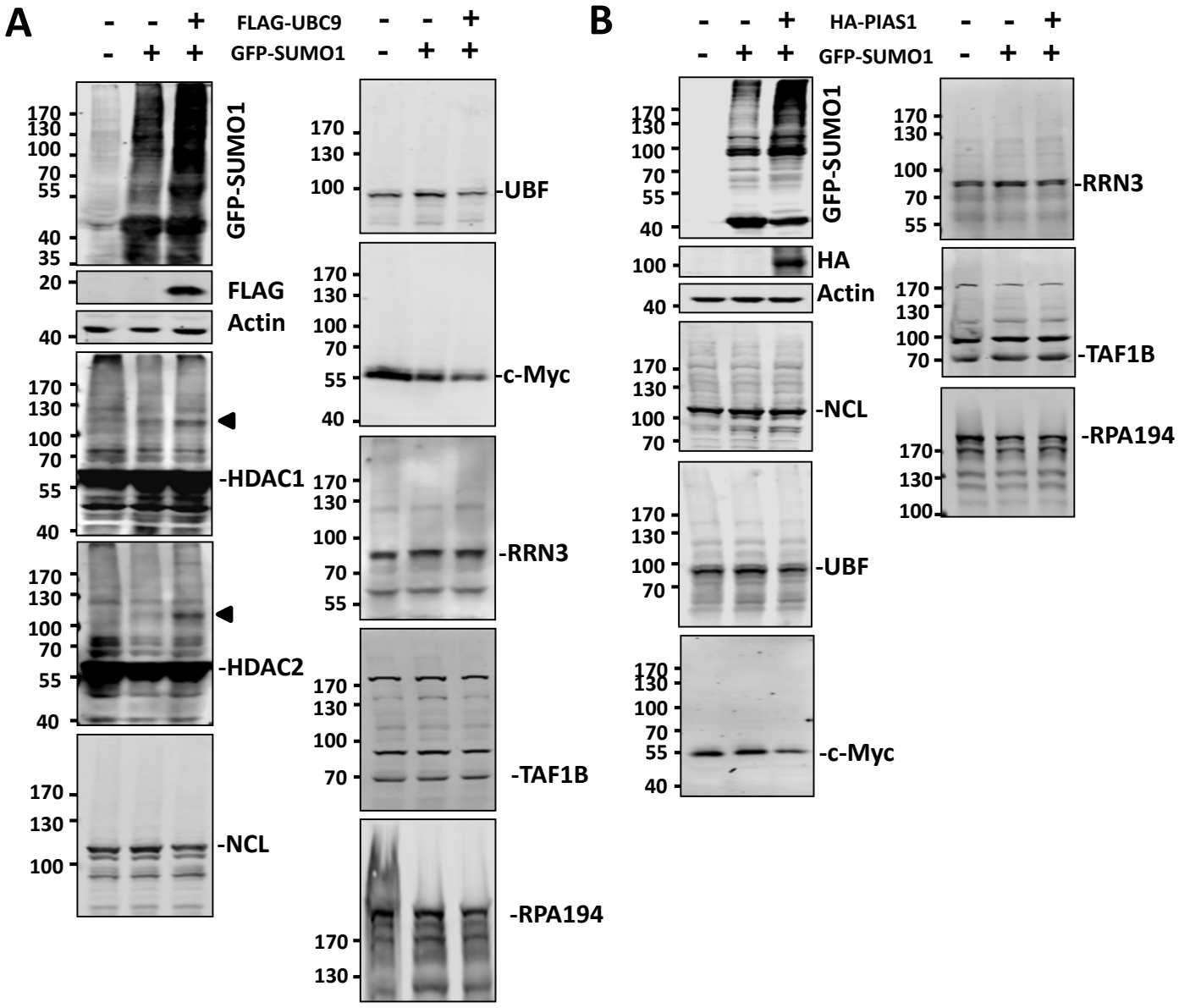
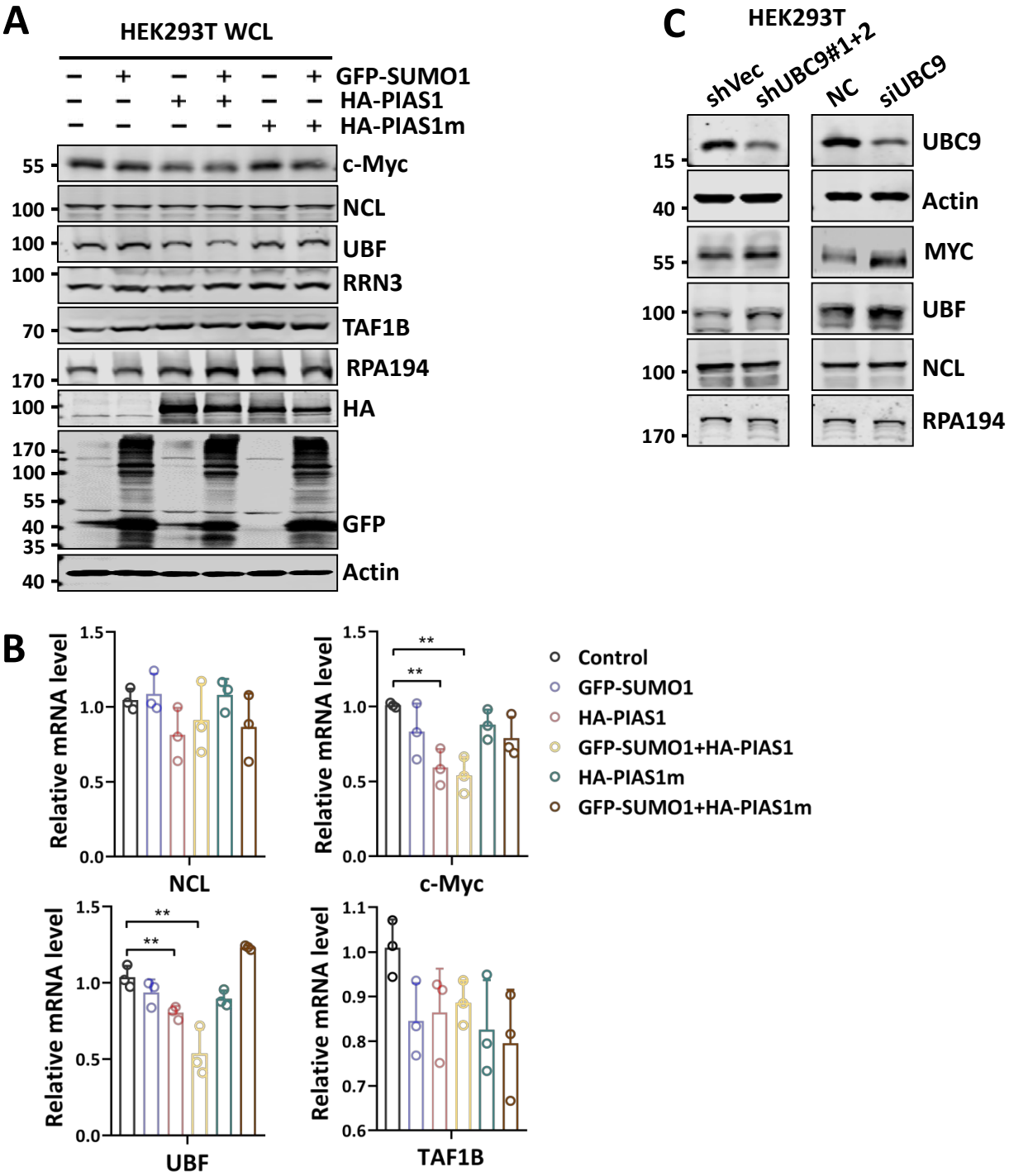
Supporting Information



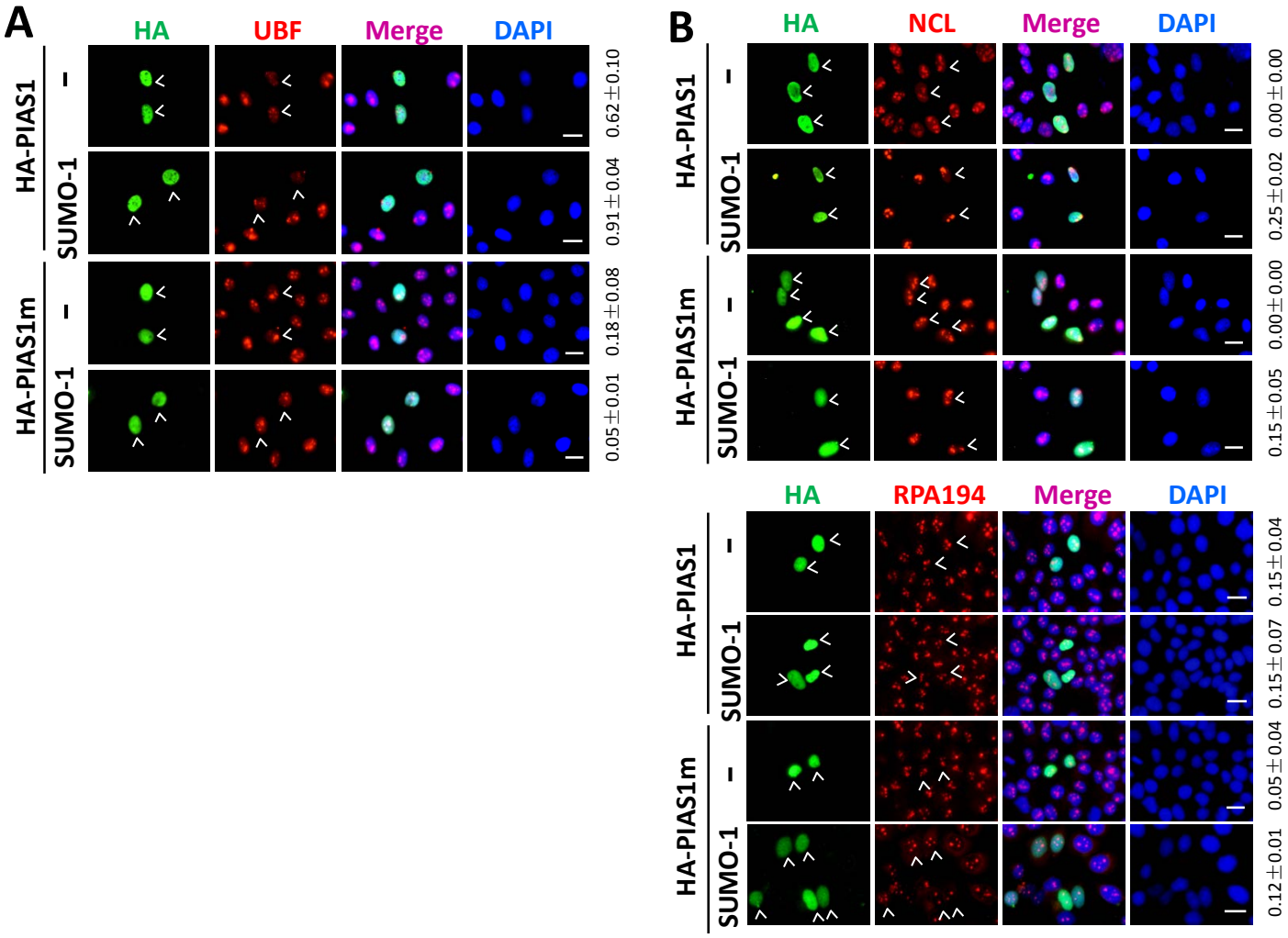
Supplementary Fig. S1. Knockdown of PIAS E3 ligases by siRNAs or shRNAs and repression of rDNA transcription in HEK293 cells by ectopic overexpression of PIAS1 and SUMO. (**A**) HeLa cells were transfected with control scramble (NC) or a smart pool of siRNAs against individual PIAS E3 ligase and two days after transfection the cells were collected for RT-qPCR analysis of mRNA levels for each PIAS. (**B**) HeLa cells were transfected with control shVect or two shRNAs against PIAS1 or PIAS4 as indicated. Two days after transfection the cells were collected for RT-qPCR analysis of mRNA levels for PIAS1 or PIAS4. (**C**). RT-qPCR analyses showing that ectopically expressed PIAS1 repressed rDNA transcription in HEK293 cells in an E3 activity-dependent manner and the repression was augmented by co-expressed SUMO1. Also shown on the left are WB analysis for expression of PIAS1 and PIAS1m and global SUMOylation levels.



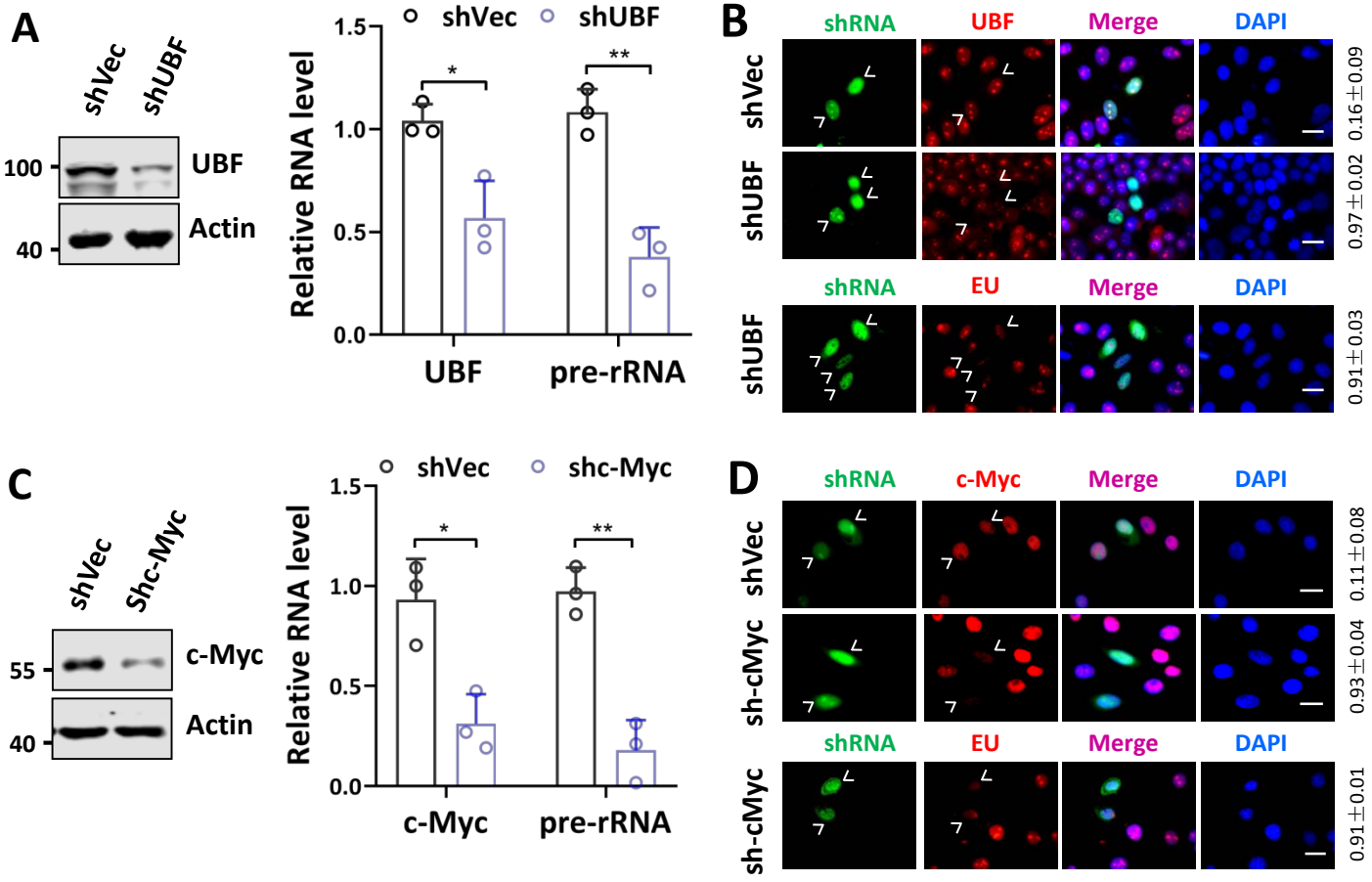
Supplementary Fig. S2. Lack of significant sumoylation on endogenous Pol I transcription factors, regulators and subunit RPA194. (**A** and **B**) HeLa cells were transfected with or without FLAG-UBC9 and/or GFP-SUMO1 (**A**) or with or without HA-PIAS1 and/or GFP-SUMO1 (**B**). Two days after transfection, the cells were collected for western blot analysis using antibodies as indicated. As a positive control, increased sumoylation was observed on HDAC1/2 upon overexpression of FLAG-UBC9/GFP-SUMO1.



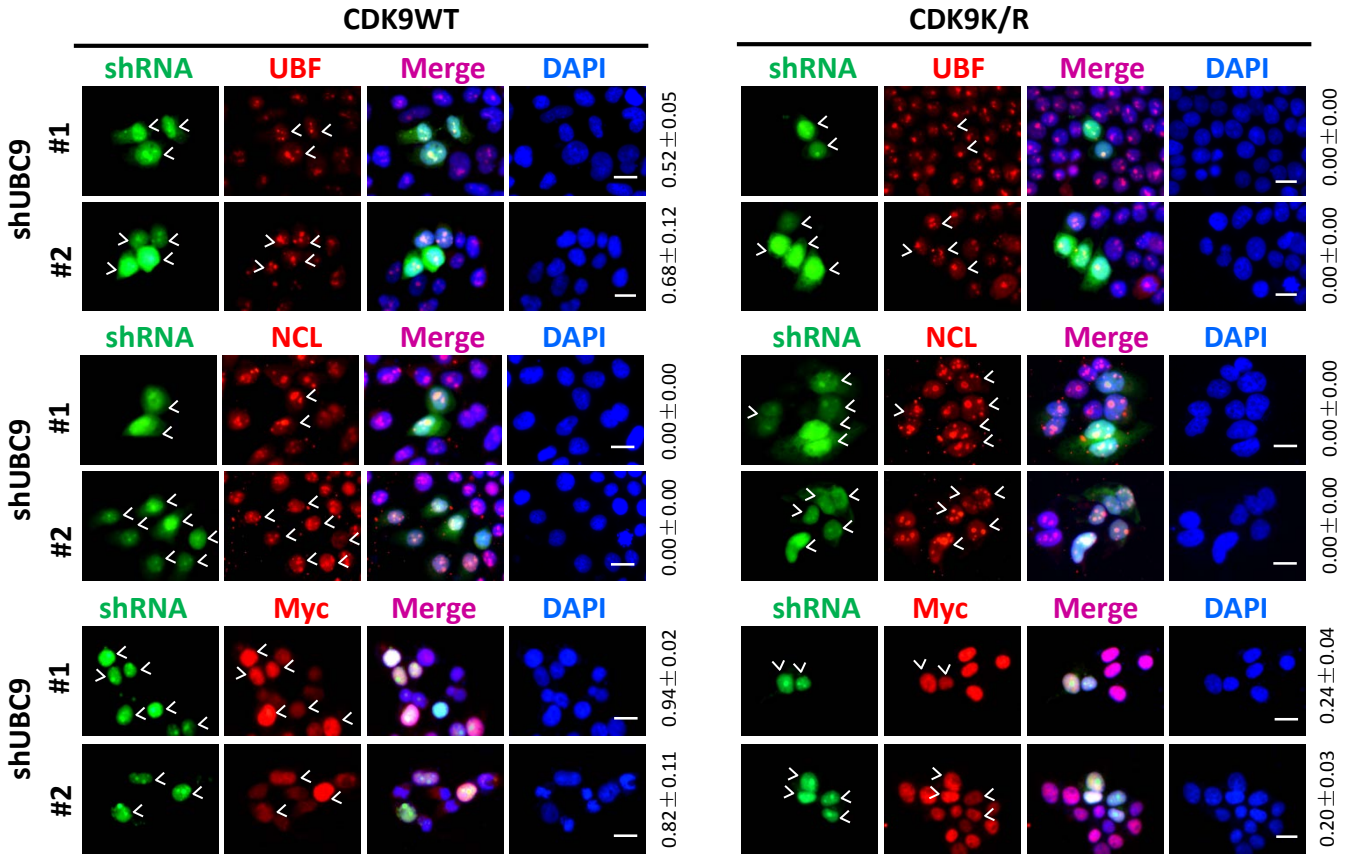
Supplementary Fig. S3. Overexpression and knockdown of the SUMO system oppositely regulate UBF and c-Myc expression in HEK293T cells. (**A** and **B**) HEK293T cells were transfected with or without wild-type PIAS1 or E3 inactive PIAS1 mutant and/or GFP-SUMO1 as indicated. Two days after transfection the cells were collected for Western blot analysis using antibodies as indicated (**A**) and RT-qPCR analysis of the levels of c-Myc, NCL, UBF and TAF1B mRNAs (**B**). (**C**) Western blot analysis showing the effect of knockdown of UBC9 by either shRNA (left panel) or siRNA (right panel) on the levels of various proteins as indicated.



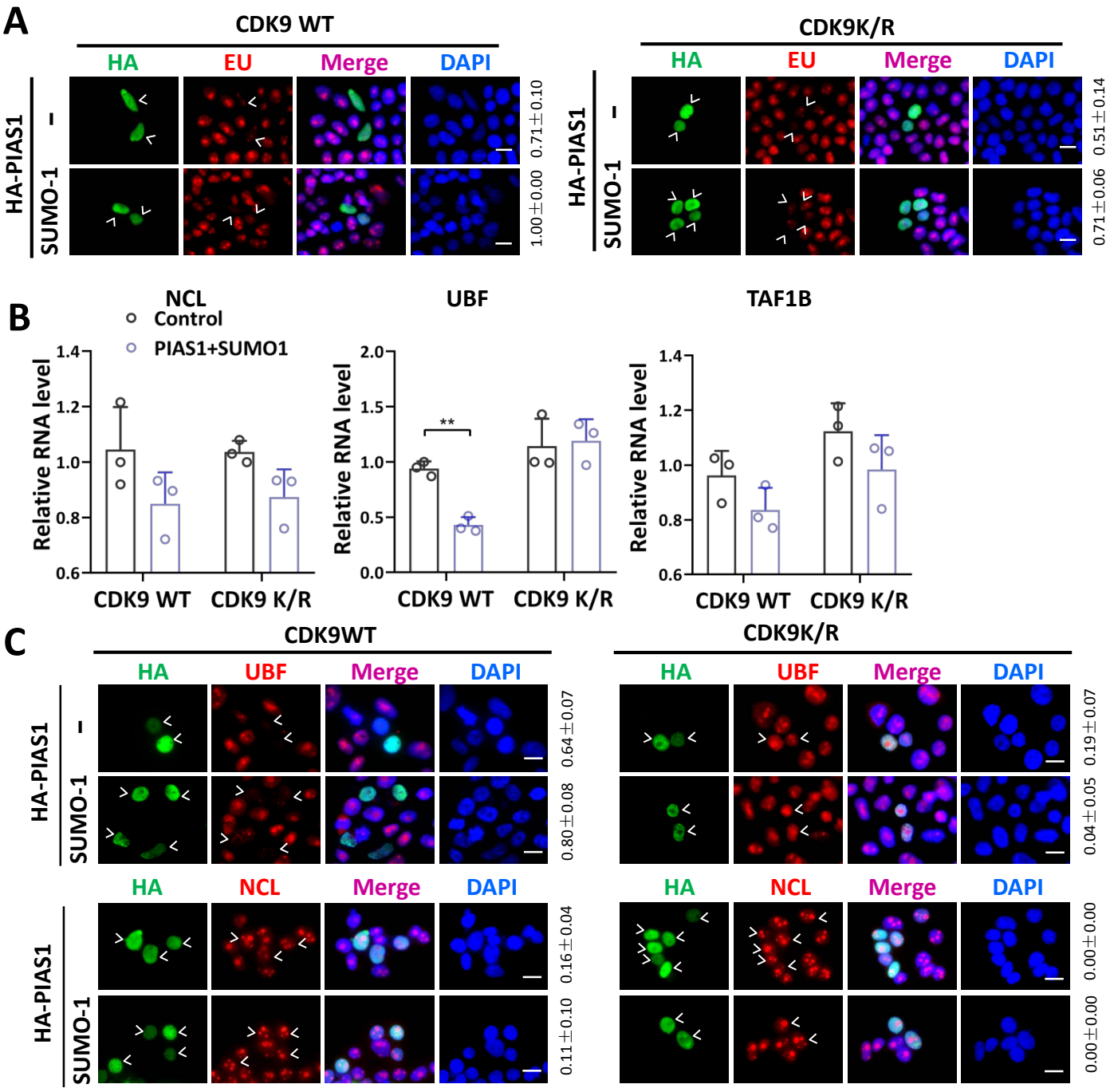
Supplementary Fig. S4. The effect of overexpression of wild-type and mutant PIAS1 with or without SUMO1 on UBF, NCL and RPA194 expression. (**A**) HeLa cells were transfected with HA-PIAS1 or HA-PIAS1m together with or without GFP-SUMO1. Two days after transfection, the cells were processed for double immunofluorescent staining of HA-PIAS1 and UBF. The HA-PIAS1 or HA-PIAS1m-transfected cells were marked by arrows. Scale bar, 20 μm.(**B**) The experiments were performed as in (**A**) except double immunofluorescent staining was performed for HA and NCL or HA and RPA194 as indicated. Scale bar, 20 μm. The immunofluorescent staining experiments were repeated at least three times, with the rate of transfected cells with increased or reduced levels of EU incorporation counted from all 3+ representative experiment (mean±SD).



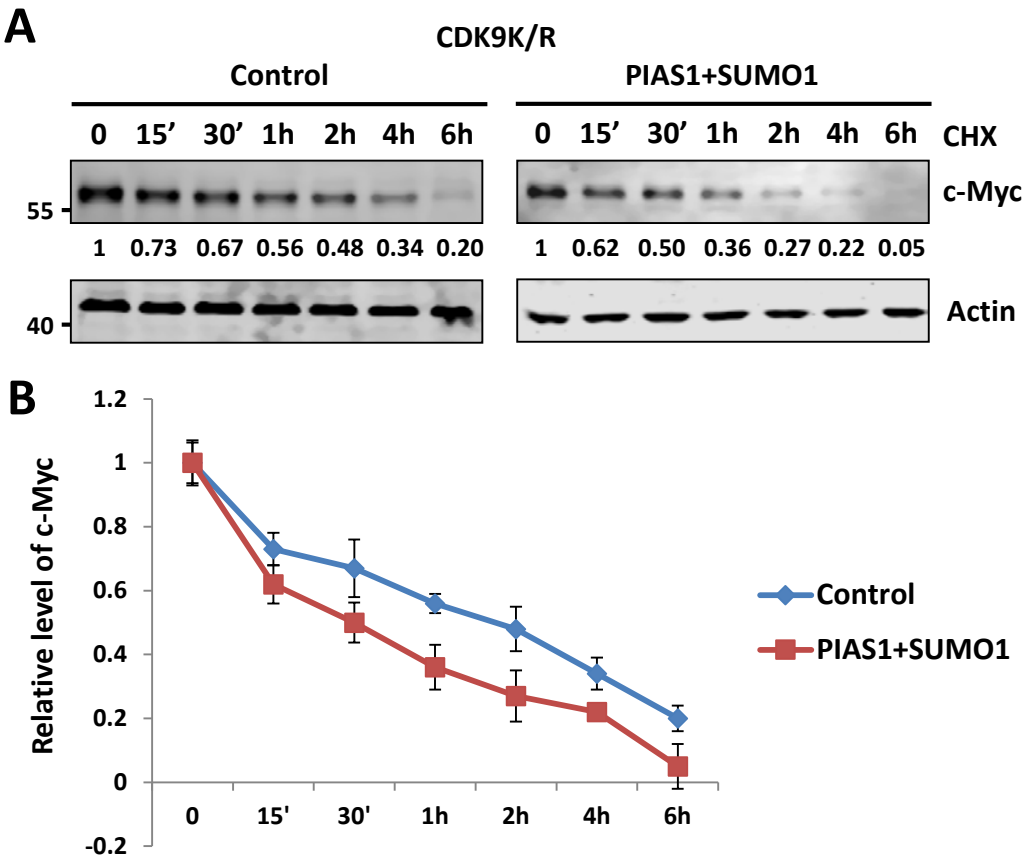
Supplementary Fig. S5. Both UBF and c-Myc are important for rDNA transcription. (**A**) Western blot and RT-qPCR analyses showing that knockdown of UBF impaired rDNA transcription. (**B**) Immunofluorescent staining and EU incorporation assay showing that knockdown of UBF impaired rDNA transcription. Scale bar, 20 μm. (**C**) Western blot and RT-qPCR analyses showing that knockdown of c-Myc impaired rDNA transcription. (**D**) Immunofluorescent staining and EU incorporation assay extra space showing that knockdown of c-Myc impaired rDNA transcription. Scale bar, 20 μm. The immunofluorescent staining experiments were repeated at least three times, with the rate of transfected cells with increased or reduced levels of EU incorporation counted from all 3+ representative experiment (mean±SD).



Supplementary Fig. S6. Knockdown of UBC9 upregulates both the levels of UBF and c-Myc proteins in CDK9WT but not CDK9K/R cells. Both CDK9WT and CDK9K/R cells were transfected with two different shRNAs targeting UBC9 and the cells were processed for immunofluorescent staining of UBF, c-Myc and NCL two days after transfection as indicated. The shRNA-transfected cells (marked by arrows) were revealed by expression of GFP encoded by shRNA vector. Scale bar, 20 μm. The immunofluorescent staining experiments were repeated at least three times, with the rate of transfected cells with increased or reduced levels of EU incorporation counted from all 3+ representative experiment (mean±SD).



Supplementary Fig. S7. Ectopic overexpression of the SUMO system represses rDNA transcription in CDK9 sumoylation-deficient cells. (**A**) EU incorporation assay showing that ectopic overexpression of PIAS1 and SUMO1 repressed rDNA transcription in both CDK9WT and CDK9K/R cells. Scale bar, 20 μm. (**B**) RT-qPCR analysis showing the effect of ectopic overexpression of PIAS1 and SUMO1 on the transcription of NCL, UBF and TAF1B in both CDK9WT and CDK9K/R cells. (**C**) Double immunofluorescent staining showing that ectopic overexpression of PIAS1 and SUMO1 downregulated UBF in CDK9WT but not CDK9K/R cells. As a control, the level of NCL proteins was not affected in either cells. Scale bar, 20 μm. The immunofluorescent staining experiments were also repeated at least three times, with the rate of transfected cells with increased or reduced levels of EU incorporation counted from all 3+ representative experiment (mean±SD).



Supplementary Fig. S8. Ectopic overexpression of the SUMO system promotes c-Myc degradation. (**A**) Western blot analysis showing that ectopic overexpression of PIAS1 and SUMO1 reduced the protein stability of c-Myc in CDK9K/R cells. The CDK9K/R cells were transfected with or without PIAS1 and SUMO1 as indicated. One day after transfection, the cells were treated with cycloheximide (CHX) and collected at the indicated time points. (**B**) Quantitation of the levels of c-Myc proteins in (**A**) was performed using J image program and the results was plotted. Accordingly, the half-life of c-Myc in the control cells was estimated to be ~2h, whereas ectopic overexpression of PIAS1/SUMO1 reduced the half-life of c-Myc to ~30 min.

qRT-PCR primers	
Pre-rRNA	GCCTTCTCTAGCGATCTGAGAG
	CCATAACGGAGGCAGAGACA
UBF	GTCGGCCATGTTCATCTTCT
	CTCAGACAGGTCGTTCCACA
NCL	GCACCTGGAAAACGAAAGAAGG
	GAAAGCCGTAGTCGGTTCTGT
SL1	AAAGAACGCTGTACTCAGTGTG
	CCCCGGTTGAGGGCTTTTA
c-Myc	ACCACCAGCAGCGACTCTGA
	TGCCAGGAGCCTGCCTCTTT
PIAS1	ACAGTGCGGAACTAAAGCAAA
	GGACTTGAATGTACGTTGGGG
PIAS2	ATCCACGAACTCTTGAAGGACT
	TGTGGGCTTAGTATCTTGAAGCA
PIAS3	CTGGGCGAATTAAAGCACATGG
	AAAGCGTCGTCGGTAAAGCTC
PIAS4	CGCTACGCCAAGAAGAACTC
	GTAGAGCACGGGGTAGTCAATA
GAPDH	GGAGCGAGATCCCTCCAAAAT
	GGCTGTTGTCATACTTCTCATGG
siRNA	
UBC9	1# GAGGAAAGCAUGGAGGAAAUU
	2# CCAUCUUAGAGGAGGACAAUU
	3# GGGAAGGAGGCUUGUUUAAUU
PIAS1	CGAAUGAACUUGGCAGAAA
PIAS2	CUUGAAUAUUACAUCUUUA
PIAS3	CCCUGAUGUCACCAUGAAA
PIAS4	CAAGACAGGUGGAGUUGAU
NC	UUCUCCGAACGUGUCACGU
shRNA	
UBC9#1	CCGGAGCAGAGGCCTACACGATTTACTCGAGTAAATCGTGTAGGCCTCTGCTTTTTTG
	AATTCAAAAAAGCAGAGGCCTACACGATTTACTCGAGTAAATCGTGTAGGCCTCTGCT
UBC9#2	CCGGAGAAGTTTGCGCCCTCATAAGCTCGAGCTTATGAGGGCGCAAACTTCTTTTTTG
	AATTCAAAAAAGAAGTTTGCGCCCTCATAAGCTCGAGCTTATGAGGGCGCAAACTQTCT
C-Myc	CCGGCCTGAGACAGATCAGCAACAACTCGAGTTGTTGCTGATCTGTCTCAGGTTTTTG
	AATTCAAAAACCTGAGACAGATCAGCAACAACTCGAGTTGTTGCTGATCTGTCTCAGG
UBF	CCGGGAGATCATGAGAGACTATATCCTCGAGGATATAGTCTCTCATGATCTCTTTTTG
	AATTCAAAAAGAGATCATGAGAGACTATATCCTCGAGGATATAGTCTCTCATGATCTC
PIAS1#1	CCGGGGCAGAAAGAAGAGAACTTTGCTCGAGCAAAGTTCTCTTCTTTCTGCCTTTTTG
	AATTCAAAAAGGCAGAAAGAAGAGAACTTTGCTCGAGCAAAGTTCTCTTCTTTCTGCC
shPIAS1#2	CCGGCCGGATCATTCTAGAGCTTTACTCGAGTAAAGCTCTAGAATGATCCGGTTTTTG
	AATTCAAAAACCGGATCATTCTAGAGCTTTACTCGAGTAAAGCTCTAGAATGATCCGGG
shPIAS4#1	CCGGGCTCTACGGAAAGTACTTAAACTCGAGTTTAAGTACTTTCCGTAGAGCTTTTTG
	AATTCAAAAAGCTCTACGGAAAGTACTTAAACTCGAGTTTAAGTACTTTCCGTAGAGC
shPIAS4#2	CCGGGCCAAGAAGAACTCGGAGCCTCTCGAGAGGCTCCGAGTTCTTCTTGGCTTTTT
	AATTAAAAAGCCAAGAAGAACTCGGAGCCTCTCGAGAGGCTCCGAGTTCTTCTTGGC
shPIAS4#3	CCGGGTCGTCCTGAGAATCTGTTACCTCGAGGTAACAGATTCTCAGGACGACTTTTTG
	AATTCAAAAAGTCGTCCTGAGAATCTGTTACCTCGAGGTAACAGATTCTCAGGACGAC

Supplementary Table S1. The sequence information for qRT-PCR primers, siRNAs and shRNAs.