Supporting Online Material

Yeast Rrn7 and human TAF1B are TFIIB-related RNA polymerase I general transcription factors

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This PDF file includes:

Materials and Methods

Homology searches and alignments

Sequence and structure similarity searches were performed by HHpred (http://toolkit.tuebingen.mpg.de/hhpred) using either HMM PDB or genome databases under default settings and thresholds. Multiple sequence alignments were assembled from selected pairwise alignments and converted to clustal format and uploaded to either AlignmentViewer (http://toolkit.tuebingen.mpg.de/alnviz) or Ali2D (http://toolkit.tuebingen.mpg.de/ali2d) to generate images for amino acid residue or secondary structure similarity, respectively. For Ali2D, program settings were changed so that PSIpred (*24*) would predict the secondary structure for each protein sequence. Amino acid and secondary structure conservation was scored manually.

Yeast growth and complementation assay

Yeast strain RLYO5 (*MAT α ade2-1 ade3::hisG ura3-1 his3-11 trp1-1 leu2-112 can1 rrn7::HIS3) (5)* contains the plasmid pNOY103 that expresses the 35S ribosomal RNA transcript under control of the *GAL7* promoter (*25*). The RLYO5 strain was transformed with a 2μ plasmid (pRS425, ARS CEN *LEU2*) that expresses Rrn7 or mutant derivatives from its native promoter and that are tagged with a 2X Flag epitope at the C-terminus. Spot assays were used to examine growth on glucose. Glucose sensitive phenotypes were monitored using five-fold serial dilutions of strains spotted onto plates containing glucose complete media lacking leucine, incubated for 2 days at 30°C, and photographed. Before spotting, cells were grown overnight at 30°C in galactose complete media lacking leucine and washed extensively with water to remove residual galactose. Yeast growth was assessed by colony size relative to wild-type (+++). Rrn7, TFIIB, and Brf1 chimeric proteins were constructed by a modified quick-change mutagenesis method where swapped regions of Rrn7, TAF1B, TFIIB, or Brf1 were amplified by PCR with primers containing *RRN7* homologous ends. The amino acid positions of the Rrn7 domain deletions and Rrn7-TAF1B, -TFIIB, and -Brf1

domain swaps are listed in **Table S1**. PCR products were purified and used in place of primers in the quick-change reaction. KODxtreme (Novagen) enzyme and buffers were used for all PCR amplification and quick-change reactions. Yeast strain SHY98 (*MAT α ade1 ade6 leu2 his4 ura3 delta sua7::HIS4*) containing *SUA7 (TFIIB)* shuffle plasmid (pSH374, ARS CEN *URA3 SUA7*) or SHY284 *(MAT α ade2 his3 leu2 lys2 met15 trp1 ura3 brf1::HIS3)* containing *BRF1* shuffle plasmid (pSH524, ARS CEN *URA3 BRF1*) were transformed with yeast expression vectors (pRS315, ARS CEN *LEU2*) that express either WT or chimeric *SUA7 (TFIIB)-2XFLAG* or *BRF1-3HA* from their native promoters, respectively. Before spotting, cells were grown overnight in glucose media lacking leucine at 30°C, and equivalent cell amounts were washed extensively with water, spotted onto glucose plates lacking leucine but containing 1 g/L 5-Fluoroorotic Acid (FOA), grown for 1 day at 30°C, and photographed.

For Pol I immobilization experiments, we used two *POL I* tagged strains derived from the W3031a parent strain: *RPA135* flag strain (*MAT α ade2-1 ade3::hisG ura3-1 his3-11 trp1-1 leu2-112 can1 RPA135-3XFlag::KanMX*) and *RPA190* deletion strain (*MAT α ade2-1 ade3::hisG ura3-1 his3-11 trp1-1 leu2-112 can1 rpa190::HIS3*) containing pNOY103 (*5*). The *RPA190* deletion strain was transformed with various C-terminal 2x Flag-tagged *RPA190* constructs under control of its native promoter and termination sequences from the 2μ plasmid (pRS424, ARS CEN *TRP1*).

GST-Rrn7 fusion protein purification

Recombinant GST and GST-Rrn7 fusion proteins were expressed by auto-induction in ZY5052 media (25 mM Na2HPO4, 25 mM KH2PO4, 50 mM NH4Cl, 5 mM Na2SO4, 2 mM MgSO4, 0.5 % Glycerol, 0.05 % Glucose, 0. 2% α-lactose, 0.01% tryptone, 0.005% yeast extract) for 16 hrs at 37°C (*26*). Fusion of the GST moiety to Rrn7 considerably enhanced protein expression and solubility. Cells were harvested by centrifugation and washed with 1X PBS buffer (150 mM NaCl, 15 mM NaPO4, pH 7.4) supplemented with 5 mM DTT, 0.5 mM PMSF, and EDTA-free complete protease inhibitor cocktail (Roche). Cells were lysed with 1

mg/ml lysozyme (Sigma) for 30 min on ice followed by sonication. To aid protein solubility and purification, 0.5% Sarkosyl and 2% Triton X-100 were added to the lysates (*27*). Lysates were cleared by centrifugation, and cleared lysates were added to Glutathione beads (GE Healthcare) and mixed for 4 hrs at 4°C. Beads were washed two times each with 1X PBS, 1X PBS with 2M NaCl, and 1X TBS buffer, respectively. GST fusion protein was eluted with three bead volumes of GST elution buffer (50 mM Tris-HCl pH 8.0, 10 mM Glutathione). Eluted protein was dialyzed in GST binding buffer (25 mM Tris-HCl 7.5, 50 mM KCl, 5 mM MgCl2, 10 μM $ZnCl_2$, 0.1% Triton X-100, 2 mM DTT, 10% glycerol) supplemented with protease inhibitor cocktail (Roche).

Whole cell extracts for protein expression levels

Whole cell extracts (WCE) were prepared as described in (*28*) with minor modification. In brief, 50 ml of yeast cells were grown in galactose minimal media lacking leucine to an OD of 0.8 to 1.0. Cells were washed with water and transferred to a 2 ml screw cap tube. Cells were resuspended in 500 μl 20% Trichloroacetic acid (TCA) and ~ 500 μl of ziconia beads. Cells were broken by Bead-beating (Mini Beadbeater-96, Biospec) 3 times for 3 minutes. Lysates, including insoluble material, were transferred to a new tube. Beads were washed once with 500 μl water and the wash was pooled with lysate and frozen at -80°C for 15 min to precipitate protein. Proteins were pelleted by centrifugation, washed with cold acetone, air dried, and resuspended in 2X lithium dodecyl sulfate (LDS) sample buffer (Invitrogen). Samples were heated for 15 min at 70°C, centrifuged, and soluble material was transferred to a new tube. Protein samples were further diluted ~1:5 to 1:10, resolved on 4-12% polyacrylamide gradient gels (Invitrogen) in 1X MOPS buffer, transferred to PVDF, and probed with Flag M2 antibody (Sigma), or HA antibody (sc-7392, Santa Cruz Biotechnology).

RNA polymerase binding assays

Bead-immobilized Flag-Pol I was purified from 500 ml mid-log-phase cultures grown in glucose complete media from either cells expressing Flag-tagged wild-type Rpa135 or Rpa190, wild-type or derivatives. Cells were collected by centrifugation and lysed by bead beating in lysis buffer (100 mM Tris-HCl pH 7.9, 250 mM AmSO4, 1 mM EDTA, 10% glycerol) supplemented with 0.5 mM DTT, 0.5 mM PMSF, and EDTA free complete protease inhibitor cocktail (Roche), and lysates were cleared by centrifugation. Approximately 8 mg of the whole-cell extract was diluted with two volumes of dilution buffer (25 mM HEPES pH 7.5, 50 mM NaCl, 1 mM EDTA) and incubated overnight at 4°C with 100 μl of M2-affinity agarose (Sigma). Beads were washed 5 times with 1 ml 1X TBS containing 0.1% Tween and once with 1X TBS containing protease inhibitor cocktail. Equivalent amounts of GST-fusion protein were added to 10 μl of BSA blocked Flag-beads with or without immobilized Pol I for 2 hr at 4°C. Beads were washed five times in GST-binding buffer and twice with 1X TBS. Proteins were eluted twice with two bead volumes of 1X TBS containing 350 μg/ml Flag-peptide. Proteins were added to LDS sample buffer (Invitrogen), resolved on 4-12% polyacrylamide gradient gels (Invitrogen) in 1X MOPS buffer, transferred to PVDF, and probed with Flag M2 antibody (Sigma) and GST antibody (SCBT, sc-459).

References and Notes

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Table S1. Description of *RRN7* derivatives used in this study.

Construct	Description
RRN7-ZRΔ	Removed *RRN7* DNA sequence encoding a.a.residues 2-33
RRN7-BRΔ	Removed *RRN7* DNA sequence encoding a.a.residues 40-66
RRN7-BLΔ	Removed *RRN7* DNA sequence encoding a.a. residues 67-101
RRN7-BCΔ	Removed *RRN7* DNA sequence encoding a.a. residues 102-317
RRN7-BCNDΔ	Removed *RRN7* DNA sequence encoding a.a. residues 102-210
RRN7-BCCDΔ	Removed *RRN7* DNA sequence encoding a.a. residues 211-317
RRN7-CTDΔ	Removed *RRN7* DNA sequence encoding a.a. residues 317-515
RRN7-BHDΔ	Removed *RRN7* DNA sequence encoding a.a. residues 2-317
RRN7(TAF1B-ZR-BC)	Replaced *RRN7* DNA encoding a.a. residues 1-320 with *TAF1B* DNA encoding residues 1-372
RRN7(TAF1B-ZR)	Replaced *RRN7* DNA encoding a.a. residues 1-41 with *TAF1B* DNA encoding residues 1-40
RRN7(TAF1B-ZR-CTD)	Replaced *RRN7* DNA encoding a.a. residues 1-515 with *TAF1B* DNA encoding residues 1-588
RRN7(TAF1B-CTD)	Replaced *RRN7* DNA encoding a.a. residues 321-515 with *TAF1B* DNA encoding residues 373-588
RRN7(TFIIB-ZR-BC)	Replaced *RRN7* DNA encoding a.a. residues 1-320 with *TFIIB* DNA encoding residues 1-328
RRN7(TFIIB-ZR-BL)	Replaced *RRN7* DNA encoding a.a. residues 1-101 with *TFIIB* DNA encoding residues 1-124
RRN7(TFIIB-ZR-BR)	Replaced *RRN7* DNA encoding a.a. residues 1-66 with *TFIIB* DNA encoding residues 1-89
RRN7(TFIIB-ZR)	Replaced *RRN7* DNA encoding a.a. residues 1-41 with *TFIIB* DNA encoding residues 1-54
RRN7(TFIIB-BR-BL)	Replaced *RRN7* DNA encoding a.a. residues 42-101 with *TFIIB* DNA encoding residues 55-124
RRN7(TFIIB-BR)	Replaced *RRN7* DNA encoding a.a. residues 42-66 with *TFIIB* DNA encoding residues 55-89
RRN7(TFIIB-BL)	Replaced *RRN7* DNA encoding a.a. residues 67-101 with *TFIIB* DNA encoding residues 90-124
RRN7(TFIIB-BC)	Replaced *RRN7* DNA encoding a.a. residues 102-320 with *TFIIB* DNA encoding residues 124-328
RRN7(BRF-ZR-BC)	Replaced *RRN7* DNA encoding a.a. residues 1-320 with *BRF1* DNA encoding residues 1-282
RRN7(BRF-ZR-BL)	Replaced *RRN7* DNA encoding a.a. residues 1-101 with *BRF1* DNA encoding residues 1-78
RRN7(BRF-ZR-BR)	Replaced *RRN7* DNA encoding a.a. residues 1-66 with *BRF1* DNA encoding residues 1-62
RRN7(BRF-ZR)	Replaced *RRN7* DNA encoding a.a. residues 1-41 with *BRF1* DNA encoding residues 1-34
RRN7(BRF-BR-BL)	Replaced *RRN7* DNA encoding a.a. residues 42-101 with *BRF1* DNA encoding residues 35-78
RRN7(BRF-BR)	Replaced *RRN7* DNA encoding a.a. residues 42-66 with *BRF1* DNA encoding residues 35-62
RRN7(BRF-BL)	Replaced *RRN7* DNA encoding a.a. residues 67-101 with *BRF1* DNA encoding residues 63-78
RRN7(BRF-BC)	Replaced *RRN7* DNA encoding a.a. residues 102-320 with *BRF1* DNA encoding residues 79-282
RRN7(BRF-CTD)	Replaced *RRN7* DNA encoding a.a. residues 321-515 with *BRF1* DNA encoding residues 283-596
TFIIB(R7-BL)	Replaced *TFIIB* DNA encoding a.a. residues 90-124 with *RRN7* DNA encoding residues 67-101
BRF1(R7-ZR)	Replaced *BRF1* DNA encoding a.a. residues 1-34 with *RRN7* DNA encoding residues 1-41
BRF1(R7-BL)	Replaced *BRF1* DNA encoding a.a. residues 63-78 with *RRN7* DNA encoding residues 67-101

Knutson and Hahn, Figure S1, HHpred search results for *S.cerevisiae* Rrn7

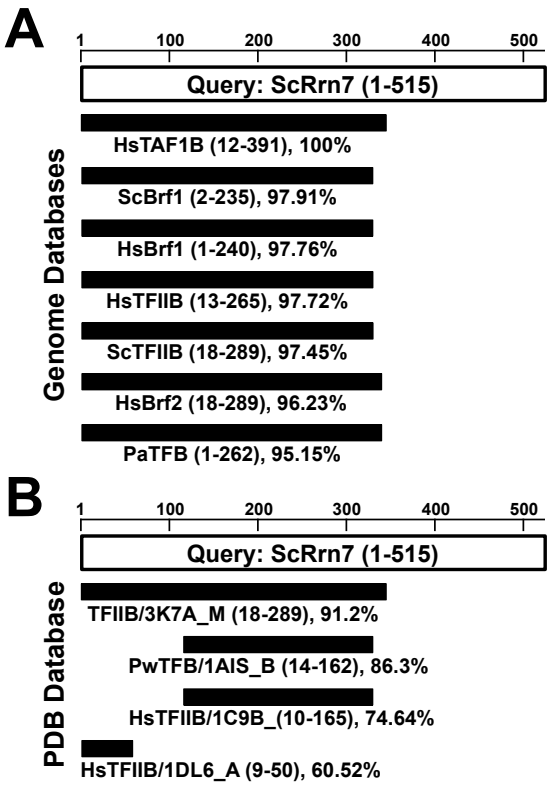


Figure S1. HHpred search results for *S. cerevisiae* Rrn7. Panels **A** and **B** show HHpred results for a search of Rrn7 against the genome and PDB databases, respectively. Black bars indicate homologous regions between query and target proteins. The protein name or PDB entry that matches Rrn7 is indicated below the bar. Hs, *Homo sapiens*; Pa, *Pyrococcus abyssi*; Pw, *Pyrococcus woesei*.

Knutson and Hahn, Figure S2, Multiple sequence and secondary structure alignment of yeast and human TFIIB protein family members

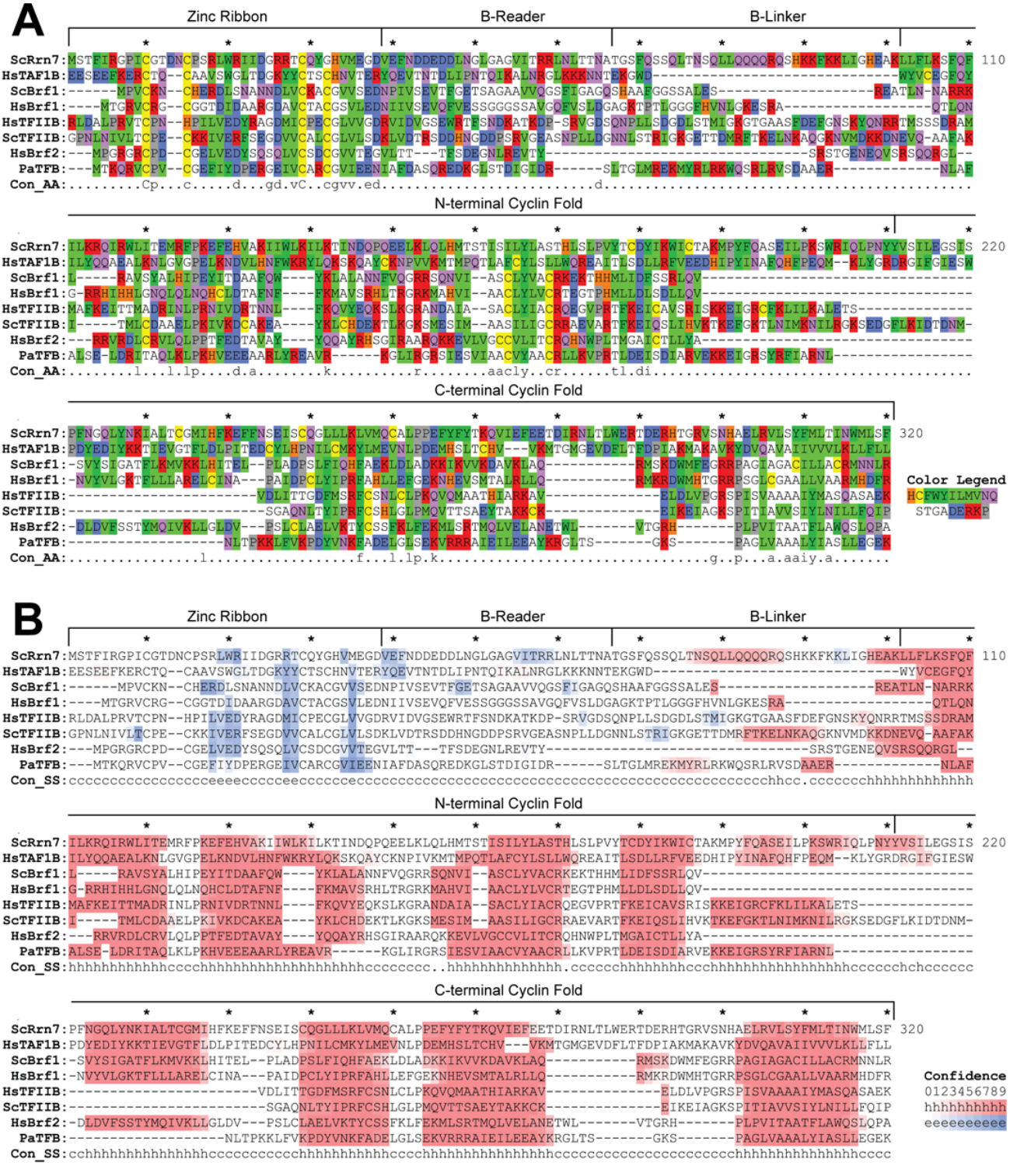


Figure S2. Multiple sequence alignment of yeast and human TFIIB protein family members. Profile HMM alignments generated by HHpred between the BHD of ScRrn7 and various Rrn7 and TAF1B homologs were assembled into Fasta format with minor manual manipulation. Gaps opened in the query protein sequence were also removed. **(A)** Images were created by the Alignment Viewer program. Amino acids are colored based on their biophysical properties as follows: H, orange; C, yellow; P, grey; ILMV (hydrophobic), green; FWY (aromatic hydrophobic), dark green; DE (negative), blue; RK (positive), red; NQ (polar amide), purple; STGA (small), white. Dashes represent gaps in the template alignments. The consensus sequence at the bottom was based on conservation of a residue at any given position in >70% of sequences. Amino acids conserved >70% are shown, 100% conserved amino acids are shown in uppercase letters, and dots represent lack of consensus. **(B)** Alignment images were generated by Ali2D, which uses PSIpred to predict the secondary structure of each protein sequence in the alignment. Alpha helices (h), Beta-sheets (e), and coils (c) are colored in pink, blue, and white, respectively. The confidence of each amino acid prediction is indicated by the color intensity where lighter and dark coloring represents low and high confidence, respectively. A majority secondary structure consensus is shown at the bottom. Sc, *Saccharomyces cerevisiae*; Hs, *Homo sapiens*; Pa, *Pyrococcus abyssi*.

Knutson and Hahn, Figure S3, Multiple sequence alignment of the Rrn7 and TAF1B BHD

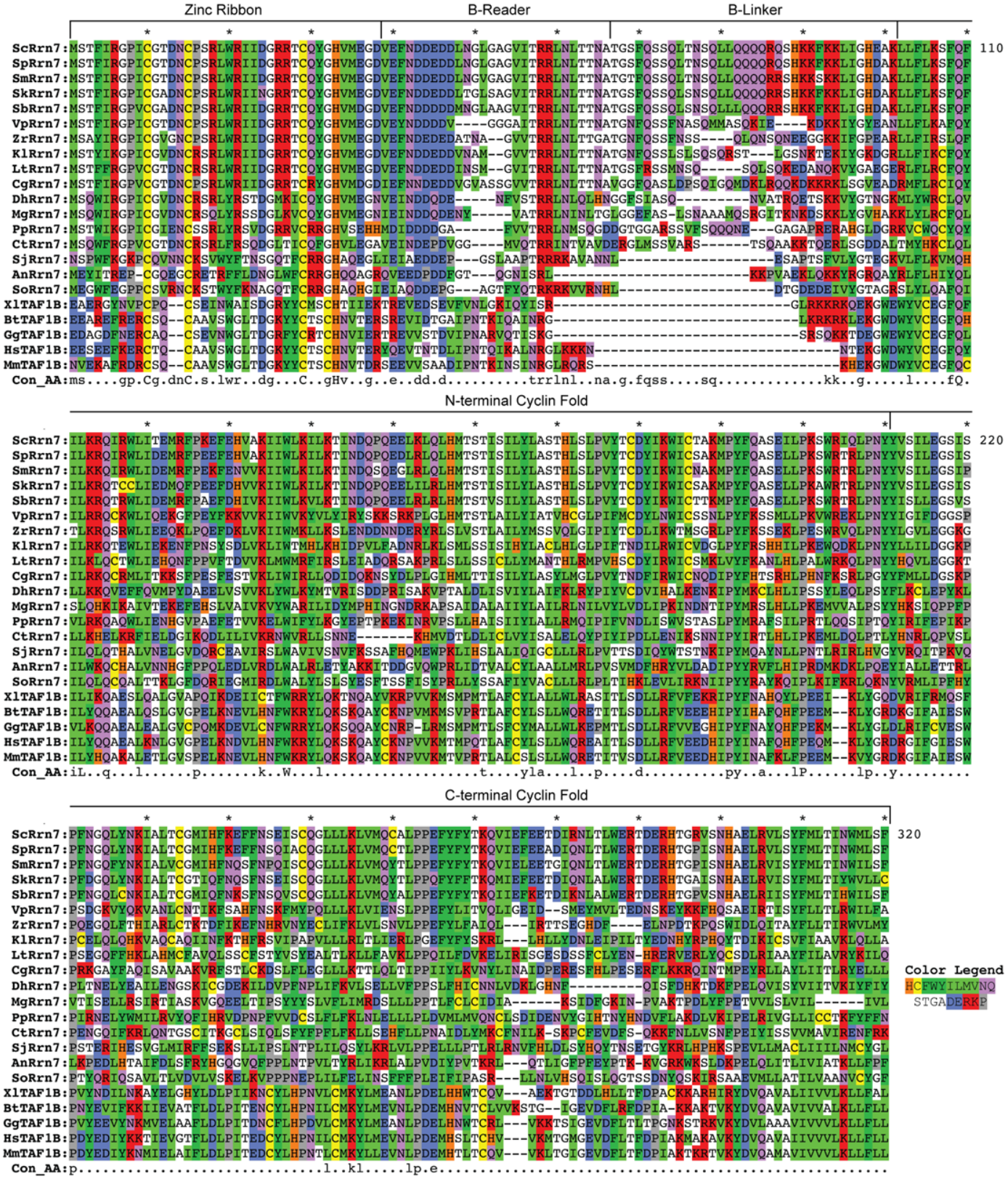


Figure S3. Multiple sequence alignment of the Rrn7 and TAF1B TFIIB homology domains. Multiple sequence alignments were generated as indicated in Figure S2A. Gaps opened in the query protein sequence were removed as in Fig S2. The consensus sequence at the bottom was based on conservation of a residue at any given position in >70% of sequences. Amino acids conserved 70% are shown, 100% conserved amino acids are shown in uppercase letters, and dots represent lack of consensus. Sc, *Saccharomyces cerevisiae*; Sp, *Saccharomyces paradoxus*; Sm, Saccharomyces mikatae; Sk, *Saccharomyces kudriavzevii*; Sb, *Saccharomyces bayanus*; Vp, *Vanderwaltozyma polyspora*; Zr, *Zygosaccharomyces rouxii*; Kl, *Kluyveromyces lactis*; Lt, *Lachancea thermotolerans*; Cg, *Candida glabrata*; Dh, *Debaryomyces hansenii*; Mg, *Meyerozyma guilliermondii*; Pp, *Pichia pastoris*; Ct, *Candida tropicalis*; Sj, *Schizosaccharomyces japonicus*; An, *Aspergillus nidulans*; So, *Schizosaccharomyces pombe*; Xl, *Xenopus laevis*; Bt, *Bos taurus*; Gg, *Gallus gallus*; Hs, *Homo sapiens*; Mm, *Mus musculus*.

Knutson and Hahn, Figure S4, Multiple secondary structure alignment of the Rrn7 and TAF1B BHD

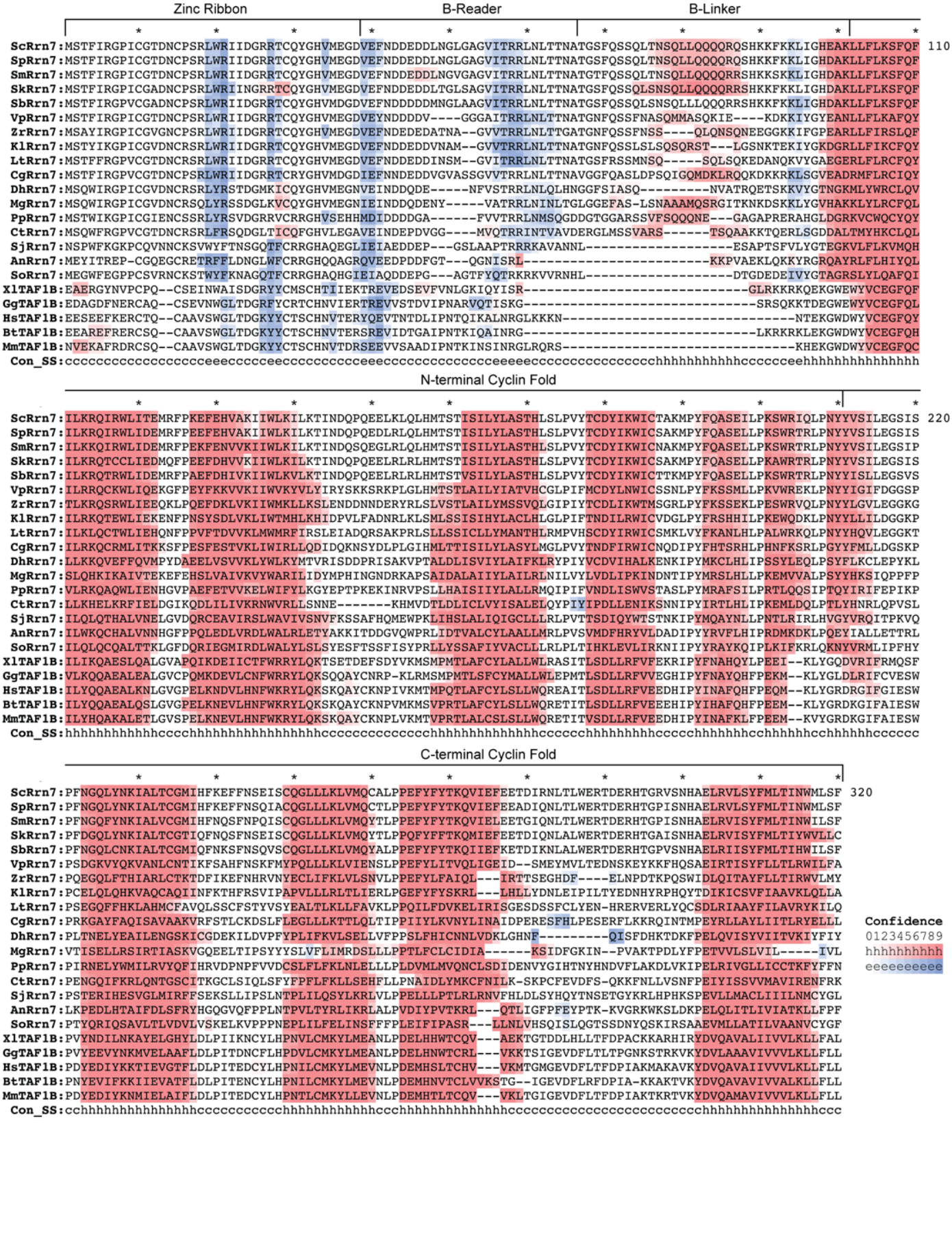
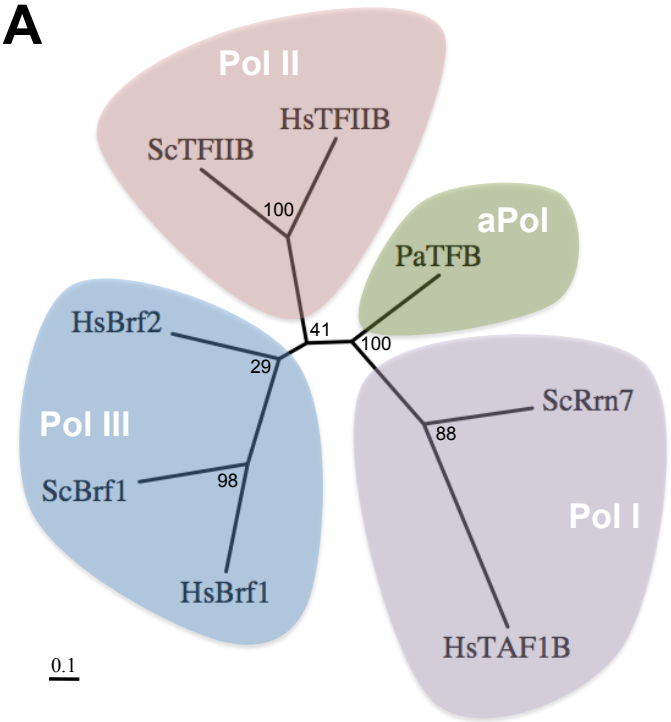


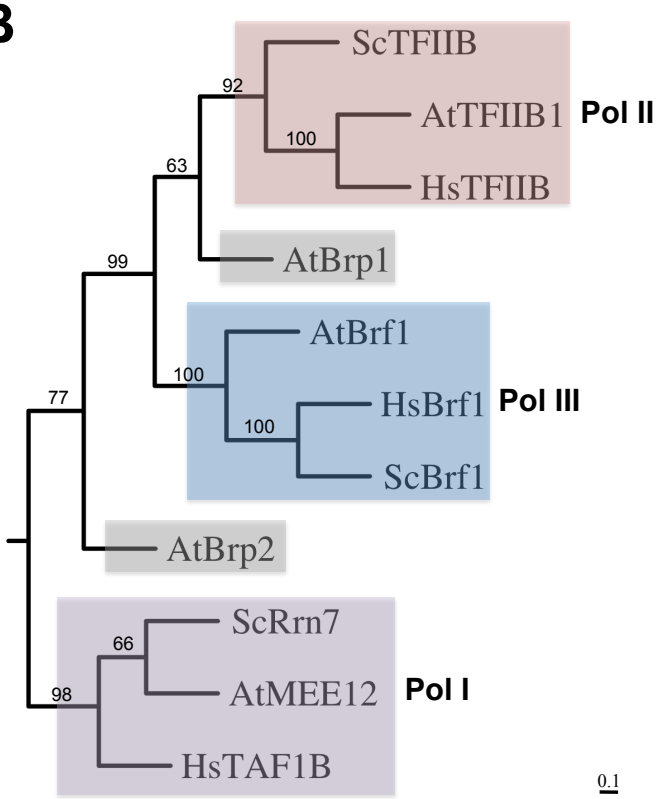
Figure S4. Multiple secondary structure alignment of the Rrn7 and TAF1B TFIIB homology domains. Multiple sequence alignments were generated as indicated in Figure S2B. Gaps opened in the query protein sequence were removed as in Fig S2. Sc, *Saccharomyces cerevisiae*; Sp, *Saccharomyces paradoxus*; Sm, Saccharomyces mikatae; Sk, *Saccharomyces kudriavzevii*; Sb, *Saccharomyces bayanus*; Vp, *Vanderwaltozyma polyspora*; Zr, *Zygosaccharomyces rouxii*; Kl, *Kluyveromyces lactis*; Lt, *Lachancea thermotolerans*; Cg, *Candida glabrata*; Dh, *Debaryomyces hansenii*; Mg, *Meyerozyma guilliermondii*; Pp, *Pichia pastoris*; Ct, *Candida tropicalis*; Sj, *Schizosaccharomyces japonicus*; An, *Aspergillus nidulans*; So, *Schizosaccharomyces pombe*; Xl, *Xenopus laevis*; Bt, *Bos taurus*; Gg, *Gallus gallus*; Hs, *Homo sapiens*; Mm, *Mus musculus*.

Knutson and Hahn, Figure S5, Rrn7 and TAF1B form a distinct Pol I specific clade

A



B



C

Percent identity between Rrn7/TAF1B and *A.thaliana* TFIIB-related proteins

Protein	AtMEE12	AtTFIIB1	AtBrf1	AtBrp1	AtBrp2
ScRrn7	16.95%	7.75%	9.61%	10.48%	10.85%
HsTAF1B	15.00%	8.44%	12.56%	10.80%	9.95%

D

HHpred probability scores between Rrn7/TAF1B and *A.thaliana* TFIIB-related proteins.

Protein	AtMEE12	AtTFIIB1	AtBrf1	AtBrp1	AtBrp2
ScRrn7	100%	97.37%	97.15%	97.74%	97.48%
HsTAF1B	100%	98.07%	98.17%	97.69%	97.14%

Figure S5. Rrn7 and TAF1B form a distinct Pol I specific clade. (A) The unrooted neighbor joining genetic tree was created by comparing the BHD domain of each TFIIB protein family member in *S.cerevisiae* (Sc), *Homo sapiens* (Hs), and *Pyrococcus abyssi* (Pa). Each TFIIB and TFIIB-related clade representing eukaryotic Pol I, II, and III, and archeal Pol are shaded in purple, pink, blue, and green respectively. **(B)** The UPGMA genetic tree was created by comparing the BHD domains of representative TFIIB family proteins in *A.thaliana* (At), *S.cerevisiae* (Sc), and *H.sapiens* (Hs). The plant-specific clades are shaded in grey, and the remaining clades are shaded as described in panel **A**. Both trees in panel **A** and **B** were generated by the program PHYLIP-NEIGHBOR using the JTT matrix model for amino acid replacement and bootstrapping with 100 replicates. The percentage of trees supporting the branching pattern in the bootstrap analysis are indicated at each node. The scale bars denote 0.1 aa substitutions per site relative to the branch length. Percent identity **(C)** and probability **(D)** scores for independent HHpred searches of ScRrn7 and HsTAF1B proteins against the *A.thaliana* genome database.

Knutson and Hahn, Figure S6, Multiple sequence and secondary structure alignment of the Rrn7 CTD

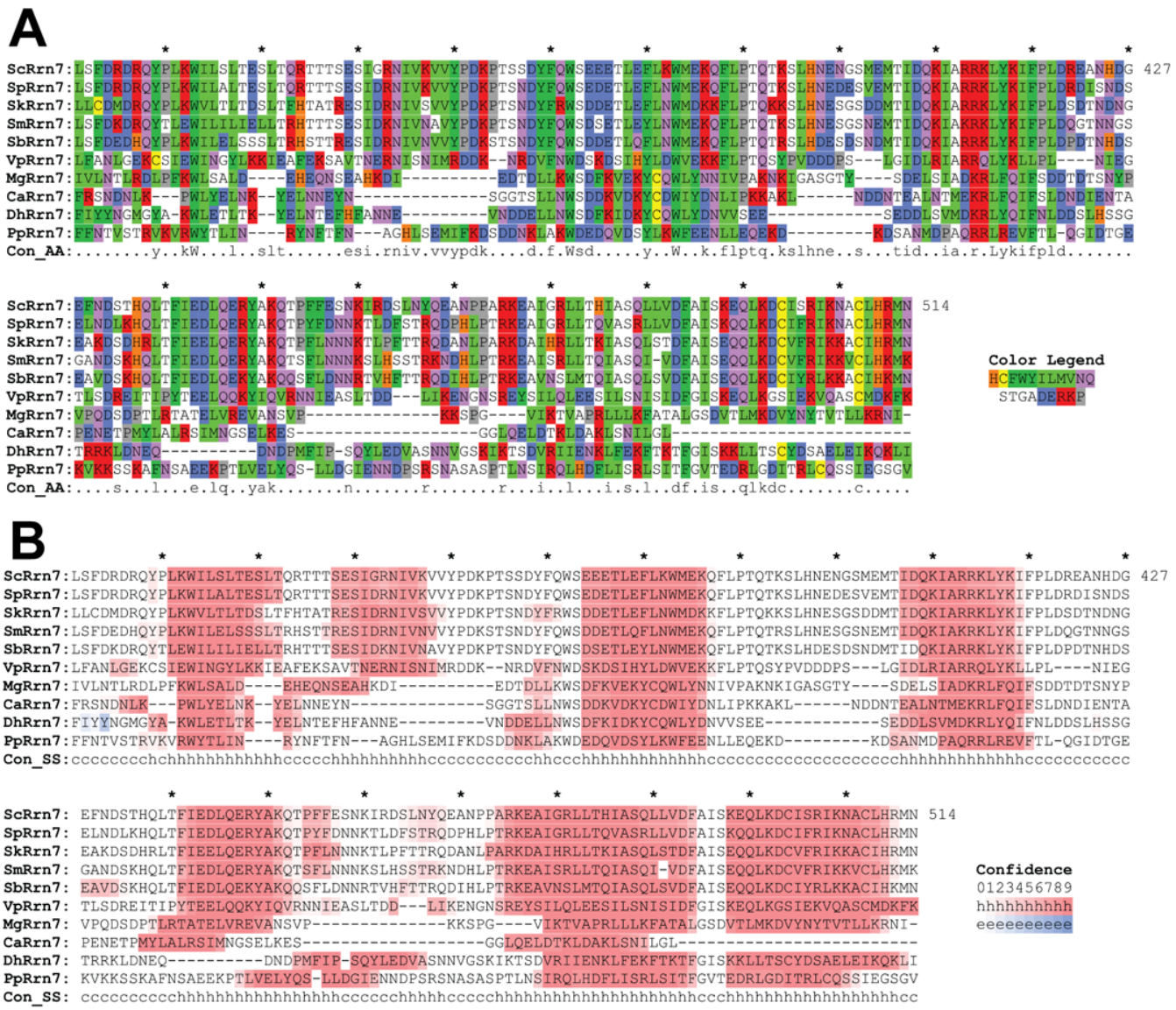


Figure S6. Multiple sequence and secondary structure alignment of the Rrn7 CTD. (A) Multiple sequence alignments were generated as indicated in Figure S2A. **(B)** Multiple secondary structure alignments were generated as indicated in Figure S2b. Gaps opened in the query protein sequence were removed as in Fig S2 Sc, *Saccharomyces cerevisiae*; Sp, *Saccharomyces paradoxus*; Sm, *Saccharomyces mikatae*; Sk, *Saccharomyces kudriavzevii*; Sb, *Saccharomyces bayanus*; Vp, *Vanderwaltozyma polyspora*; Dh, *Debaryomyces hansenii*; Mg, *Meyerozyma guilliermondii*; Pp, *Pichia pastoris*; Ca, *Candida albicans*.

Knutson and Hahn, Figure S7, Multiple sequence and secondary structure alignment of the TAF1B CTD

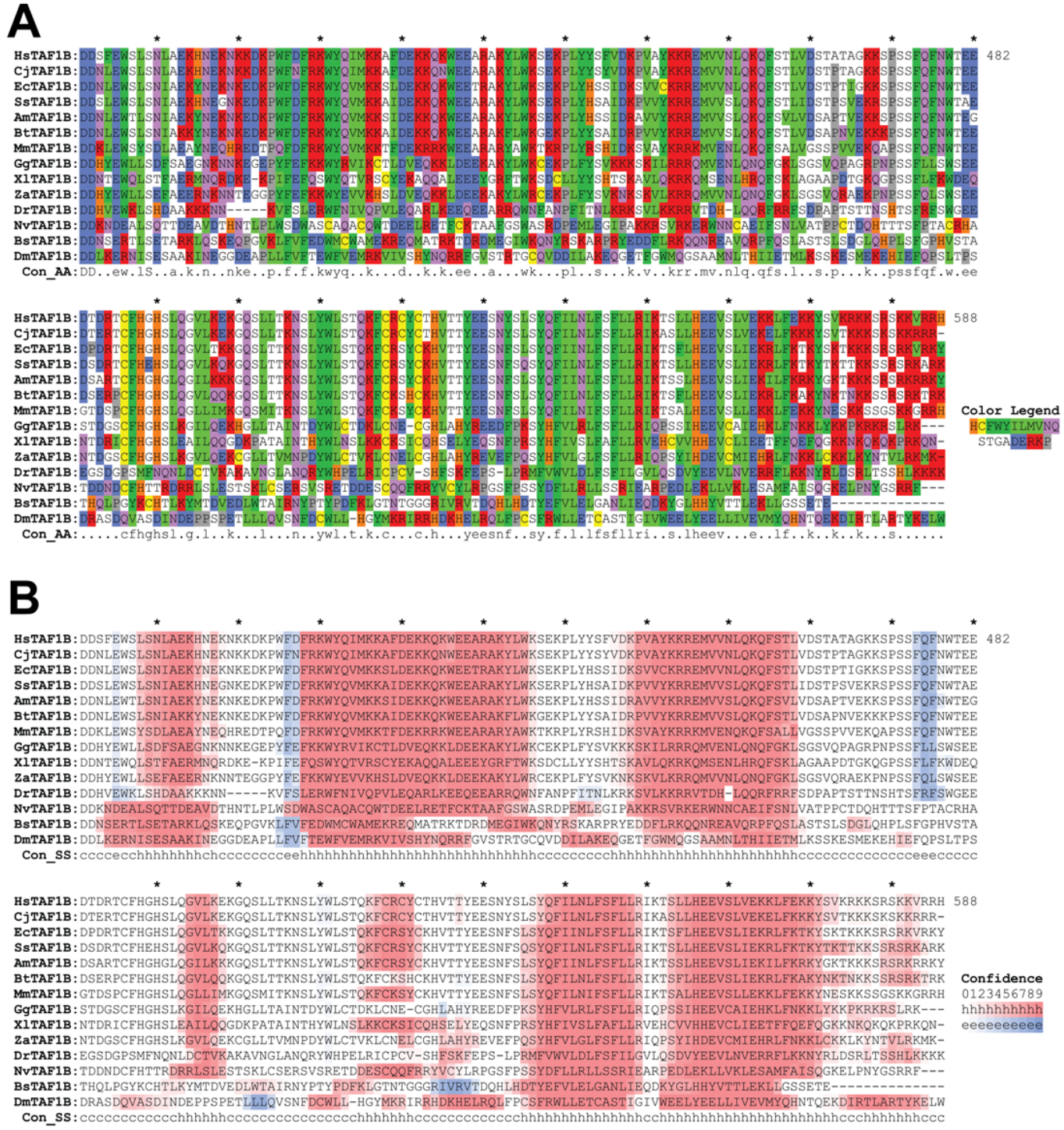


Figure S7. Multiple sequence and secondary structure alignment of the TAF1B CTD. (A) Multiple sequence alignments were generated as indicated in supplemental Figure S2a. **(B)** Multiple secondary structure alignments were generated as indicated in Figure S2B. Gaps opened in the query protein sequence were removed as in Fig S2. Xl, *Xenopus laevis*; Bt, *Bos taurus*; Gg, *Gallus gallus*; Hs, *Homo sapiens*; Mm, *Mus musculus*; Cj, *Callithrix jacchus*; Ec, *Equus caballus*; Ss, *Sus scrofa*; Am, *Ailuropoda melanoleuca*; Za, *Zonotrichia albicollis*; Dr, *Danio rerio*; Nv, *Nematostella vectensis*; Bs, *Bos taurus*; Dm, *Drosophila melanogaster*.

Knutson and Hahn, Figure S8, Rrn7 domains important for viability

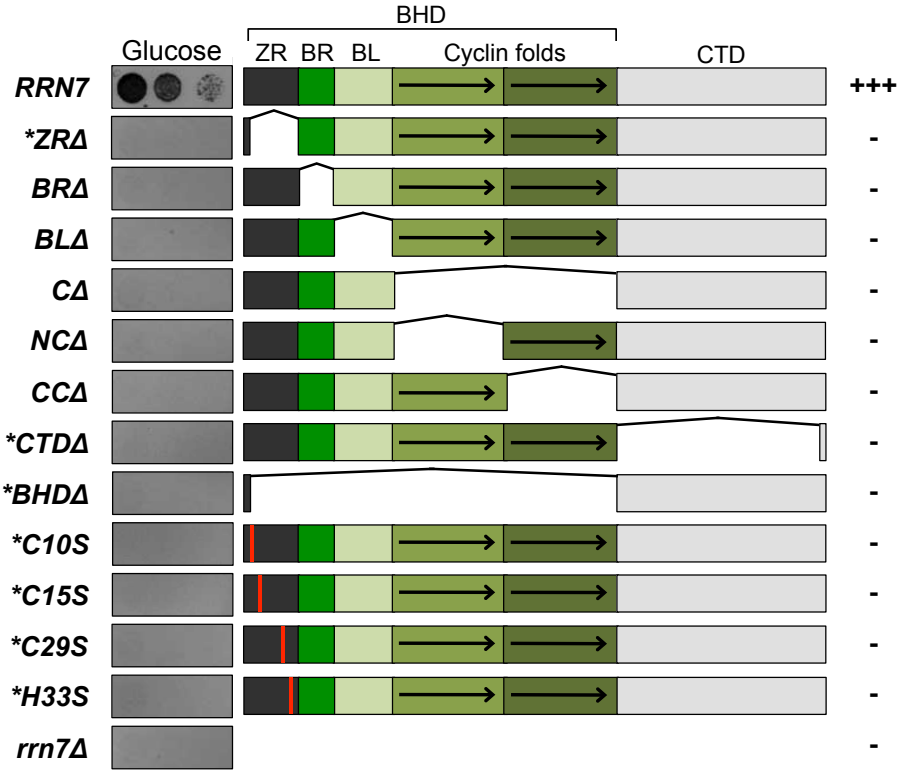


Figure S8. Rrn7 domains important for viability. Growth of yeast strains with the indicated mutations in Rrn7 domains. Rrn7-Flag derivatives were transformed in the strain RLY05 containing *Δrrn7* and plasmid pNOY103 that expresses ribosomal RNA under control of the *GAL7* promoter (*25*). Five-fold serial dilutions of the indicated *RRN7* WT and mutant strains were spotted on glucose plates and grown at 30°C. A schematic and name of the Rrn7 protein derivatives are shown to the right and left of the spot images, respectively. An asterisk before the names denote derivatives that did not stably express in yeast (see **Fig S10**). The domain organization and labels are the same as used in Figure 1b.

Knutson and Hahn, Figure S9, Domain swapping between Rrn7 and TAF1B, TFIIB and Brf1 reveal both functionally conserved and Pol-specific domains

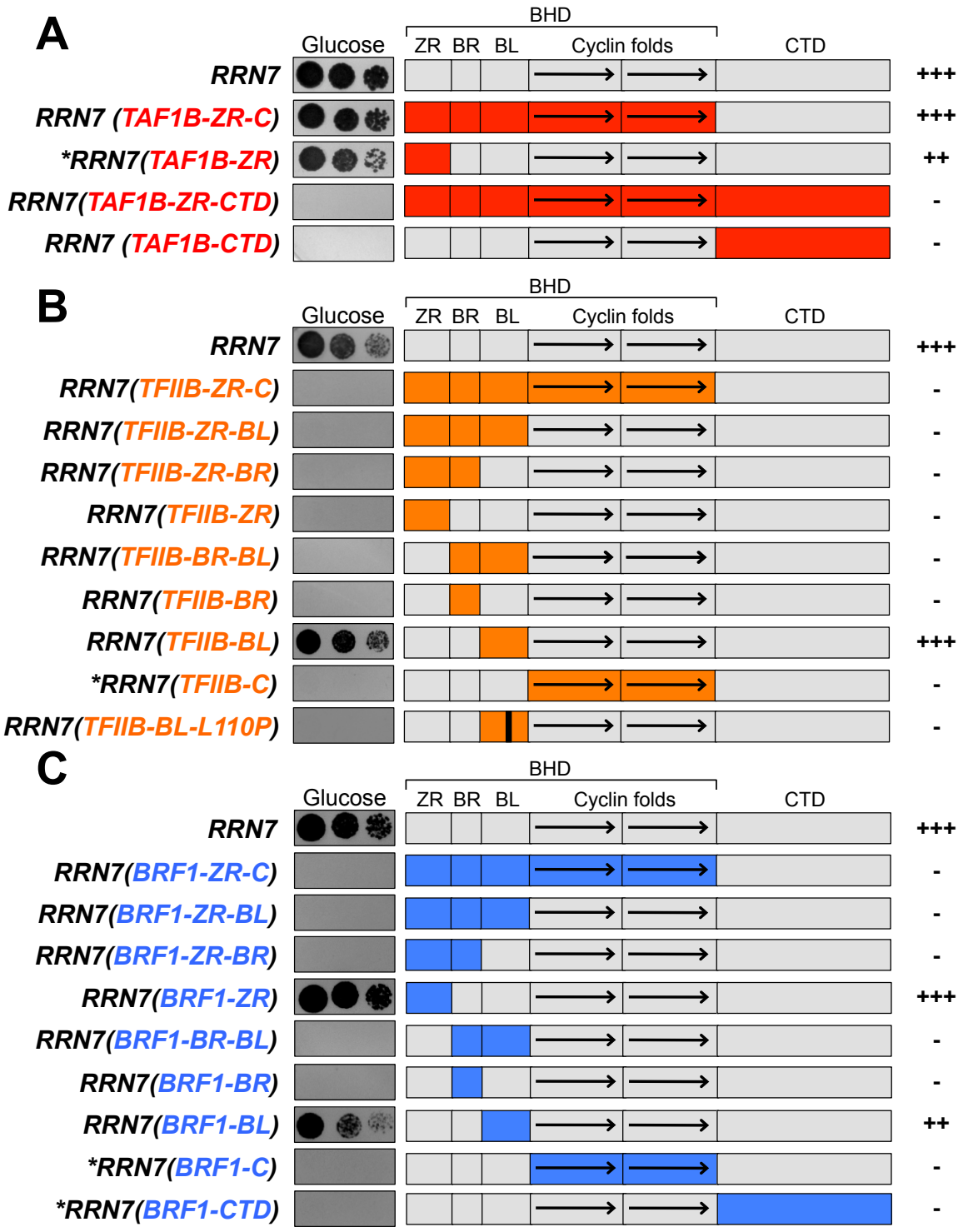


Figure S9. Domain swaps between Rrn7 and TAF1B, TFIIB and Brf1 reveal functionally conserved and Pol-specific domains. *RRN7* complementation assay with Rrn7 derivatives (grey) containing the indicated domains of **(A)** TFIIB (orange) or **(B)** Brf1 (blue). An asterisk before the name denotes chimeras that did not stably express in yeast (see **Fig S10**). The domain organization and labels are the same as used in Figure 1b.

Knutson and Hahn, Figure S10, Yeast protein expression levels of various Rrn7 and Rpa190 derivatives.

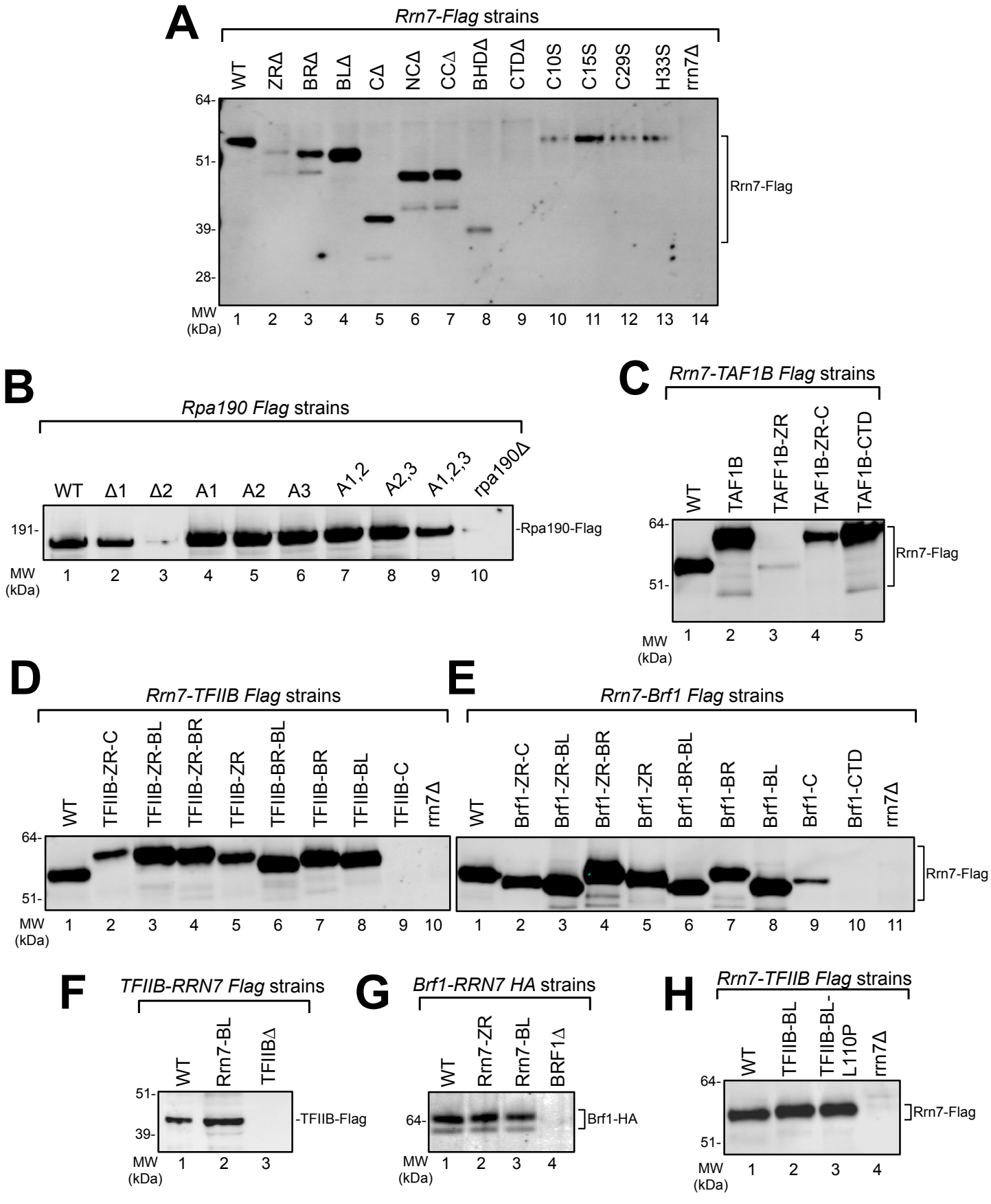


Figure S10. Yeast protein expression levels of various Rrn7 and Rpa190 derivatives. Western blot of whole cell extracts from strains containing indicated Rrn7 derivatives. **(A)** Rrn7 deletion and single substitutions mutants. **(B)** Rpa190 deletion and substitution mutants. **(C)** Rrn7-TAF1B chimeras. Note the low amount of TAF1B-ZR protein that is able to weakly complement growth (**Fig S9**). **(D, H)** Rrn7-TFIIB chimeras. **(E)** Rrn7-Brf1 chimeras. **(F)** TFIIB-Rrn7 chimeras. **(G)** Brf1-Rrn7 chimeras.