Supplementary legends

Supplementary figure 1: **UBF1 expression results in an increased nucleolar DFC.**

The internal nuclear structure was analyzed using cryo-fixation so as to circumvent the large structural alterations resulting from chemical fixation. Yeast nucleolus was described to contain three distinct sub domains: the fibrillar centers (FCs), the dense fibrillar component (DFC) and the granular component (GC) (51). In human cells, UBF is enriched in both the DFC and the FC (44). The existence of distinct FCs in yeast was questioned, suggesting that FC and DFC would be one single fibrillar structure (52). Moreover, FCs are rarely observed in sections, and were not taken into account in our analysis. DFC corresponds to nascent precursor ribosomal RNAs, with a high concentration of Nop1, (51). The GC comprises late ribosomal precursors as shown by Rlp7 localization (53). **A** Ultrastructural study of cryo-fixed, cryo-substituted wild-type budding yeast cells with or without UBF1 expression. UBF1 expression in yeast increases nucleolar size (see also Figure 1) and DFC sub-nucleolar compartment. Representative sections of the nuclei are depicted, with manual segmentation of nucleus (black line), nucleolus bounded by blue line and Dense Fibrillar Component (DFC) by red line. **B** Quantification of ultrastructural morphological alteration upon UBF1 expression. Nucleolar area normalized by nuclear surface is estimated by manual segmentation on EM sections (n=20). Nucleolar expansion caused by UBF1 expression appears to be largely a consequence of DFC enlargement.

Supplementary figure 2: **Expression of UBF1 and Hmo1 deletion constructs in budding yeast.**

A: Detection of UBF1 C-terminal truncation from a yeast extract. Western analysis was performed on crude yeast extract of wild type strain containing pRS425 derivatives: empty

plasmid (-), UBF1 box1 or UBF1 box1-2. PGK detection is used as loading control. **B** Detection of Hmo1 and derivatives. Whole cell extracts were prepared from *hmo1Δ* strains expressing the different Hmo1 variants. Note that using polyclonal antibody against UBF1 and Hmo1 proteins, the relative signal intensity does not directly reflect abundance of the different deletion constructs.

Supplementary figure 3 **Localization of untagged Hmo1 and Hmo1 lacking C-terminal domain**

hmo1Δ mutant strains carrying plasmid expressing either Hmo1 (upper Panel), the truncated form Hmo1ΔC (mid panel) or containing the empty vector (lower panel) were fixed and incubated with antibody serum against Hmo1. Fluorescence detection was performed using Texas Red conjugated secondary antibody. Nucleoplasm DNA was stained with DAPI. Scale Bar = 5 μm.

Supplementary tables

Supplementary table 1: Yeast strains used in this study

Refered as	strain	Genotype	source
Wild type (Figure 1A)	BMA64-1a	*MATa leu2-3,112 his3-11,15 trp1 ade2-1 ura3-1*	(54)
Wild-type (Figure 1B)	*OGP069-1a*	*MATa leu2-3, 112 his3-11,15 trp1-Δ can1-100, ade2-1, ura3-52 HMO1 ::CFP-TRP1 + pUN100-mRFP-NOP1 (mRFP-NOP1 LEU2 CEN)*	(14)
Wild-type (Figure 1C)	*TMS5-8d*	*MATα leu2Δ0, ura3Δ0, ade2-801, lys2-801, LYS2::TETR-GFP, nup49-Δ::HPH-MX6 +pASZ11-NupNop(ADE2 GFP-NUP49 mCherry-NOP1)*	This work
Wild-type (Figure 2B)	BY4741	*MATa his3-1 leu2-0 met15-0 ura3-0*	euroscarf
hmo1Δ rpa49Δ	YAB9-1A	*MATα his31 leu20 met150 ura30 hmo1::PrNAT-TetR-VP16 rpa49-::KANMX4 + pGID-HMO1 (TetO-CEN URA3 MET15 HMO1)*	(14)
hmo1Δ rps23bΔ	YAB5-1A	*MATα his31 leu20 met150 ura30 hmo1::PrNAT-TetR-VP16 rps23b-::KANMX4* + pGID-*HMO1* (*TetO-CEN URA3 MET15 HMO1*)	(24)
Sp-Wild type	TG11	*ade6-M210 ura4-Δ18 leu1-32, mating type h-*	(55)
Sp-rpa49Δ	BENSP2-1A	*ade6-M210 ura4-Δ18 leu1-32, rpa51::URA4 h+*	This work
Sp-hmo1Δ	BENSP3-1A	*ade6-M210 ura4-Δ18 leu1-32, SPBC28F2.11::KANMX44 h-*	This work
hmo1Δ	Hmo1-Δ	*MATa his3-1 leu2-0 met15-0 ura3-0 Hmo1::KAN*	euroscarf
hmo1Δ rpa34Δ	SL7-4A	*MATa rpa34-D::HIS3 ura3 ade2-1 lys2-801 his3-D200 trp1-D hmo1-D::URA3 leu2*	(14)
wild type	TG12	*ade6-M210 ura4-Δ18 leu1-32, mating type h+*	(55)
rpa49Δ-25 copies	BEN26	*MATa leu2-3,112 ura3-1 his3-11 trp1-1 ade2-1 can1-100 fob1-Δ::HIS3 rpa49::HPH rDNA copy number 25*	This work

Supplementary table 2: Plasmids used in this study

Name	Description	Source
pFL44-Hmo1	Hmo1 over-expression	(14)
pUN100-mCherry nop1	Expression of mCherry Nop1 fusion protein	(16)
pASZ11-CFP-NUP49	Expression of CFP-Nup49 fusion protein	(16)
pUN100-CFP-NOP1	Expression of CFP Nop1 fusion protein	(53)
pRS423-pPGK	Gateway destination vector, *HIS3* gene	(16)
pRS425-pPGK	Gateway destination vector, *LEU2* gene	(16)
mRFP-NOP1	pUN100-mRFP-NOP1 was constructed by allowing homologous recombination of the PCR-product derived pFA6-mRFP-KANMX6 with the SphI-cut vector pUN100-CFP-NOP1	This work
pEYFP-C1-UBF1	UBF1 cDNA	Gift From M.F. O'Donohue
pEYFP-C1-UBF2	UBF2 cDNA	Gift from M.F. O'Donohue
pENTR- eYFP-hUBF1	pEYFP-C1-UBF1 was digested with *Afe*I and *Avr*II generating an eYFP-UBF1 fragment which was cloned into *Hinc*II-*Xba*I digested pENTR3C vector (invitrogen). Secondly this plasmid was shortened by the *Sfi*I-*Bam*HI fragment, generating pENTR3C-EYFP-C1-UBF1	This work
pENTR- eYFP-hUBF2	pENTR3C-EYFP-C1-UBF2 was constructed as pENTR- eYFP-hUBF, but using pEYFP-C1-UBF2	This work
pENTR-UBF1	pENTR3C-UBF1 was generated by cutting out the coding sequence of eYFP from pENTR- eYFP-hUBF1 using *Hinc*II and *Xmn*I	This work
pENTR-UBF2	pENTR3C-UBF2 was generated by cutting out the coding sequence of eYFP from pENTR- eYFP-hUBF2 using *Hinc*II and *Xmn*I	This work
pENTR-UBF1-box1	BP reaction with pDONR201 and PCR-generated fragment amplified using oligonucleotides 883-888 and pENTR-UBF1 as matrix.	This work
pENTR-UBF1-box1-2	BP reaction with pDONR201 and PCR-generated fragment amplified using oligonucleotides 883-887and pENTR-UBF1 as matrix.	This work
pRS425pPGK-UBF1	LR gateway reaction with prs425-pPGK and pENTR-UBF1	This work
pRS425pPGK-UBF2	LR gateway reaction with prs425-pPGK and pENTR-UBF2	This work
pRS425pPGK-UBF1-box1	LR gateway reaction with prs425-PGK and pENTR-UBF1 box1	This work
pRS425pPGK-UBF1-box1-2	LR gateway reaction with prs425-PGK and pENTR-UBF1 box1-2	This work
Peb5-YFP-UBF1-box1	LR gateway reaction with peb5 and pENTR-UBF1 box1	This work
Peb5-YFP-UBF1-box1-2	LR gateway reaction with peb5 and pENTR-UBF1 box1-2	This work
Peb5-YFP-UBF1	LR gateway reaction with peb5 and pENTR-UBF1	This work
Peb5-YFP-UBF2	LR gateway reaction with peb5 and pENTR-UBF2	This work
pENTR-Hmo1	BP reaction with pDONR201 and PCR-generated fragment amplified using oligonucleotides 899-898 and pfl44-Hmo1.	This work
pENTR-BoxA	BP reaction with pDONR201 and PCR-generated fragment amplified using oligonucleotides 899-879 and pfl44-Hmo1.	This work
pENTR-BoxAB	BP reaction with pDONR201 and PCR-generated fragment amplified using oligonucleotides 899-880 and pfl44-Hmo1.	This work
pENTR-Hmo1ΔC	BP reaction with pDONR201 and PCR-generated fragment amplified using oligonucleotides 899-881and pfl44-Hmo1.	This work
pENTR-Hmo1ΔCR	PCR Mutagenesis by overlap extension was achieved using PCR-generated fragment amplified using oligonucleotides 877-878 and 899-898 with pfl44-Hmo1 as template. Two overlapping fragments are fused together in a subsequent extension reaction using oligonucleotides 899-898, and cloned by BP reaction with pDONR201.	This work
pENTR-Hmo1ΔBoxA	BP reaction with pDONR201 and PCR-generated fragment amplified using oligonucleotides 886-898 and pfl44-Hmo1.	This work
pENTR-chimHMO1	PCR Mutagenesis by overlap extension was achieved using PCR-generated fragment amplified using oligonucleotides 885-886 with pENTR-UBF1 as template and 886-898 with pfl44-Hmo1 as template. Two overlapping fragments are fused together in a subsequent extension reaction using oligonucleotides 885-898, and cloned by BP reaction in pDONR201.	This work
pRS423- pPGK-Hmo1	LR reaction between prs423-pPGK and pENTR201-Hmo1	This work
pRS423-pPGK-BoxA	LR reaction between prs423-pPGK and pENTR201-BoxA	This work
pRS423-pPGK-BoxAB	LR reaction between prs423-pPGK and pENTR201-BoxAB	This work
pRS423- pPGK-Hmo1ΔC	LR reaction between prs423-pPGK and pENTR201-Hmo1ΔC	This work
pRS423- pPGK-Hmo1ΔCR	LR reaction between prs423-pPGK and pENTR201-Hmo1ΔCR	This work
pRS425- pPGK-Hmo1ΔBoxA	LR reaction between prs425-pPGK and pENTR201-Hmo1ΔBoxA	This work
pRS423-pPGK-chimHMO1	LR gateway reaction with prs423-pPGK and pENTR201-chimHMO1	This work
peb5-Hmo1	LR gateway reaction with peb5 and pENTR201-Hmo1	This work
peb5-BoxA	LR gateway reaction with peb5 and pENTR201-BoxA	This work
peb5-BoxAB	LR gateway reaction with peb5 and pENTR201-BoxAB	This work
peb5-Hmo1ΔC	LR gateway reaction with peb5 and pENTR201-Hmo1ΔC	This work
peb5-Hmo1ΔCR	LR gateway reaction with peb5 and pENTR201-Hmo1ΔCR	This work
peb5-Hmo1ΔBoxA	LR gateway reaction with peb5 and pENTR201-Hmo1ΔBoxA	This work
pENTR-SPBC28F2	BP reaction with pDONR201 and PCR-generated fragment amplified using oligonucleotides 868-900 and genomic DNA of *S. pombe*.	This work

pENTR-SPAC57	Splicing by overlap extension was achieved using PCR-generated fragment amplified using oligonucleotides 869-901 and 870-903 with genomic DNA of *S. pombe* as template. Two overlapping fragments are fused together in a subsequent extension reaction using oligonucleotides 869-903, and cloned by BP reaction in pDONR201.	This work
pENTR-SPC4G9	BP reaction with pDONR201 and PCR-generated fragment amplified using oligonucleotides 866-867 and genomic DNA of *S. pombe*.	This work
pENTR-SPAC10F6	Splicing by overlap extension was achieved using PCR-generated fragment amplified using oligonucleotides 862-863 and 864-865 with genomic DNA of *S. pombe* as template. Two overlapping fragments are fused together in a subsequent extension reaction using oligonucleotides 862-865, and cloned by BP reaction in pDONR201.	This work
peb5-SPBC28F2	LR gateway reaction with peb5 and pENTR-SPBC28F2	This work
peb5-SPAC57	LR gateway reaction with peb5 and pENTR-SPAC57	This work
peb5-SPC4G9	LR gateway reaction with peb5 and pENTR-SPC4G9	This work
peb5-SPAC10F6	LR gateway reaction with peb5 and pENTR-SPAC10F6	This work
pRS425-SPBC28F2	LR gateway reaction with prs425pPGK and pENTR- SPBC28F2	This work
pRS425-SPAC57	LR gateway reaction with prs425pPGK and pENTR-SPAC57	This work
pRS425-SPC4G9	LR gateway reaction with prs425pPGK and pENTR-SPC4G9	This work
pRS425-SPAC10F6	LR gateway reaction with prs425pPGK and pENTR-SPAC10F6	This work
pENTR-HA-SBC28F2	BP reaction between PCR product oligo 868-882 and pDnor201	This work
pYN1003usds	Deletion of SpRPA49 (RPA51)	(43)
Rep41	*S. pombe* vector	(16)
Rep41-HA-SPBC28F2	LR reaction between pENTR-HA-SPBC28F2 and rep41	This work

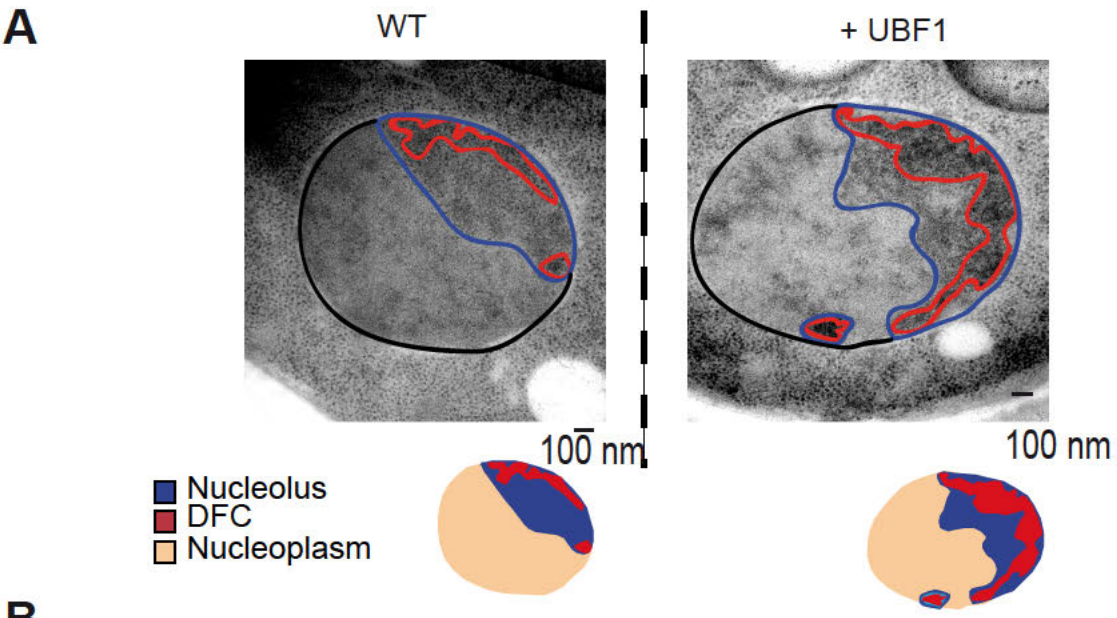
Supplementary table 3: Oligonucleotides used in this study

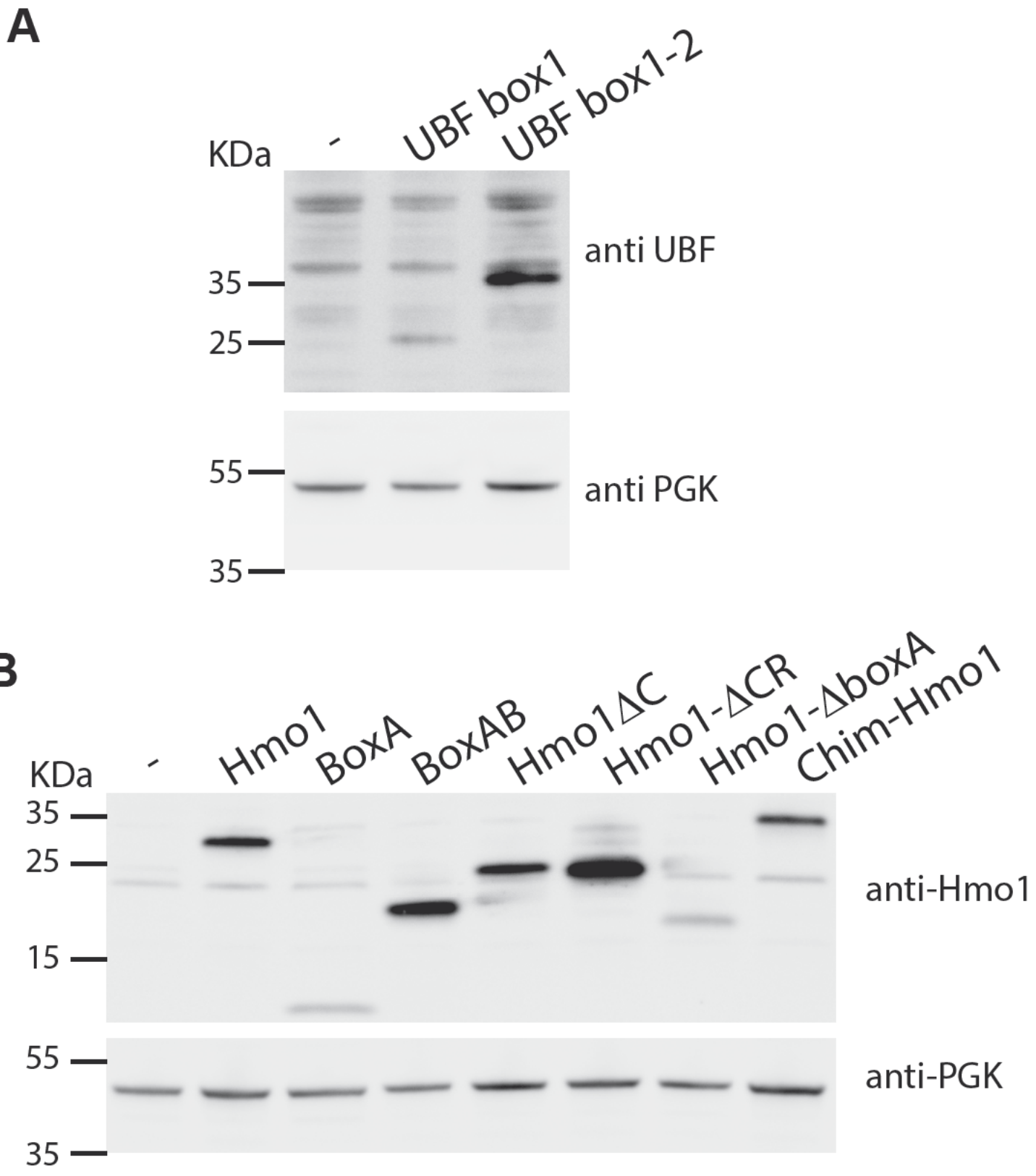
Name	Sequence	N°
attB1(10F6.08c)1	ggggacaagtttgtacaaaaaagcaggcttaatggaaaaccctccataccatgtatctatttccaaatcggaagaaattaaatatcgccaaaagtgcaag	862
intron2(10F6.08c)1	cgttgatattttgattttaatacctttcgggaggaggaggaggagatggctcagagcgctgtccaaattcg	863
Intron2(10F6.08c)2	cgaatttggacagcgctctgagccatctcctcctcctcctcccgaaggtattaaaatcaaaatatcaacg	864
attB2(10F6.08c)2	ggggaccactttgtacaagaaagctgggtgttaagcattagaggatctattaacaacggtg	865
AttB1(4G9.11c)1	ggggacaagtttgtacaaaaaagcaggcttaatgcgtctgtttgacgcaatgcctccttacc	866
AttB2(4G9.11c)1	ggggaccactttgtacaagaaagctgggtgtcatcgaaatccggcttctttcataaacttatcg	867
AttB1(28f2.11)1	ggggacaagtttgtacaaaaaagcaggcttaatggctcaaaactcaacccaactggaaaagatttctggttctttcacaaggttggcggaagc	868
AttB1(57A10.09c)1	ggggacaagtttgtacaaaaaagcaggcttaatgcctagagccgcaaaatccagccgt	869
Intron2(57A10.09c)2	gccagctaggatctttgttgggcaaaagatggaaagagcttacaagcacagaacgtgagccatacgaggaaaaagctcgtcaggacaaag	870
Hmoshort_for	aattaccaaagagagaaatccaaatacttacacgatgatgatggatcttcggaaaagaag	877
Hmoshort_rev	cttcttttccgaagatccatcatcatcgtgtaagtatttggatttctctctttggtaatt	878
boxA_Hmo	ggggaccactttgtacaagaaagctgggtgtcaagcagcgataatagcatctttatcatcatc	879
boxAB_Hmo	ggggaccactttgtacaagaaagctgggtgtcataagtatttggatttctctctttggtaatt	880
boxABlong_hmo	ggggaccactttgtacaagaaagctgggtgtcagtgctgtaaactgaaaggaattggaaccgg	881
C_HA_Spbc28F211	ggggaccactttgtacaagaaagctgggtgagcgtaatctggaacatcgtatgggtacatagcgtaatctggaacatcgtatgggtaattagcaa	882
O_attb1_UBF1	ggggacaagtttgtacaaaaaagcaggcttaatgaacggagaagccgactgccccacagac	883
O_UBF1DIM_attb2	ggggaccactttgtacaagaaagctgggtgtttcttgagttttttgcctttgtaaggatttttaacatgttcctgagcat	884
O_UBF1DIM	tggcttctttggagcatttggatcacgttcgattttcttgagttttttgcctttgtaaggatttttaacat	885
O_hmoDimless	atgttaaaaatccttacaaaggcaaaaaactcaagaaaatcgaacgtgatccaaatgctccaaagaagcca	886
UBF1-12_attb2	ggggaccactttgtacaagaaagctgggtgtcactcactgatgttcagctctgggtgctt	887
UBF1-1_attb2	ggggaccactttgtacaagaaagctgggtgtcatttcttggcattctggattaggtcggg	888
KAN_SPB28F2_for	cccgccatcaccccggtaggtcagccaccatttcctgtatttgccatcgtattgaagctttagttgtattagaaactttgaatgtaaaacgacggccagt	889
KAN_SPBC28F2_rev	aagcaatatttacagacattagcaacctgagtaataatagagagcactcaaatcagcaatacaagcaaacacttgttgccaggaaacagctatgaccatg	890
o_35S_sp	ctcctttcaaccaccactcc	891
o_18S_sp	catggcttaatctttgagac	892
o_ITS1_sp	tgatatgcttggcatgcaac	893
o_5,8S_sp	gcgttcttcatcgatgc	894
o_28S_sp	tcctgcgcttattgatatgc	895
o_ITS2_sp	tcctgcgcttattgatatgc	895
Attb2_Hmo1	ggggaccactttgtacaagaaagctgggtgtcaaatagaagagttggatttgtccttctt	898
Attb1_Hmo1	ggggacaagtttgtacaaaaaagcaggcttaatgactacagatccttctgtcaaattgaag	899
AttB2(28f2.11)1	ggggaccactttgtacaagaaagctgggtgtcaattagcaactttggcggtaggggagtgg	900
intron1(57A10.09c)1	ctcacgttctgtgcttgtaagctctttccatcttttgcccaacaaagatcctagctggccaaaggtggcatcagggttgtctgttttca	901
AttB2(57A10.09)2	ggggaccactttgtacaagaaagctgggtgttattcctctttggcggcagcagcagcagcagg	903
Rpa49(-504)Fo	cat agt ttc tgg cat gac cc	649
Rpa49(1701)Re	gaa gaa tcc tgg tag tat gg	650
1am	tcgcgtatggtcacccactac	291
1av	gttgcggccatatctaccaga	292
3am	gcagtattgagaccatgagagtagca	295
3av	tccaaatgtaaaatggcctatcg	296
6am	aaagcagttgaagacaagttcgaa	301
6av	gactctctccaccgtttgacg	302
10am	aatattaaaaactttcaacaacggatctct	309
10av	cgatgattcacggaattctgc	310
11am	ggtggtaaattccatctaaagctaaatatt	311
11av	cacgtacttttcactctcttttcaaa	312
12am	tatgaggtaaagcgaatgattagagg	313
12av	cacgttcaattaagtaacaaggacttct	314
13am	ggaggagttatcttttcttcttaacagct	315
13av	aaggtgctggcctcttcca	316
16am	atcatttgtatacgacttagatgtacaacg	321
16av	aacaaatcagacaacaaaggcttaatc	322
17am	tacgatgaggatgatagtgtgtaagagtg	323
17av	tctctttcaacccatctttgcaa	324
18am	ctcatttcctatagttaacaggacatgc	325
18av	ttcacttgtctcttacatctttcttgg	326
19am	taacagatatggaatggttggcg	327
19av	tgccgcattacactatatgatcg	328
20am	gtacatatcaagtagtagcaacccaatgag	329
20av	accattcgattcagaaaaattcg	330

Supplementary references :

14. *Gadal, O., Labarre, S., Boschiero, C. and Thuriaux, P. (2002) Hmo1, an HMG-box protein, belongs to the yeast ribosomal DNA transcription system. Embo J,* ***21****, 5498-5507.*
16. *Albert, B., Leger-Silvestre, I., Normand, C., Ostermaier, M.K., Perez-Fernandez, J., Panov, K.I., Zomerdijk, J.C., Schultz, P. and Gadal, O. (2011) RNA polymerase I-specific subunits promote polymerase clustering to enhance the rRNA gene transcription cycle. J Cell Biol,* ***192****, 277-293.*
24. *Berger, A.B., Decourty, L., Badis, G., Nehrbass, U., Jacquier, A. and Gadal, O. (2007) Hmo1 Is Required for TOR-Dependent Regulation of Ribosomal Protein Gene Transcription. Mol Cell Biol,* ***27****, 8015-8026.*
43. *Nakagawa, K., Hisatake, K., Imazawa, Y., Ishiguro, A., Matsumoto, M., Pape, L., Ishihama, A. and Nogi, Y. (2003) The fission yeast RPA51 is a functional homolog of the budding yeast A49 subunit of RNA polymerase I and required for maximizing transcription of ribosomal DNA. Genes Genet Syst,* ***78****, 199-209.*
44. *Roussel, P., Andre, C., Masson, C., Geraud, G. and Hernandez-Verdun, D. (1993) Localization of the RNA polymerase I transcription factor hUBF during the cell cycle. J Cell Sci,* ***104 (Pt 2)****, 327-337.*
51. *Léger-Silvestre, I., Trumtel, S., Noaillac-Depeyre, J. and Gas, N. (1999) Functional compartmentalization of the nucleus in the budding yeast Saccharomyces cerevisiae. Chromosoma,* ***108****, 103-113.*
52. *Thiry, M. and Lafontaine, D.L. (2005) Birth of a nucleolus: the evolution of nucleolar compartments. Trends Cell Biol,* ***15****, 194-199.*
53. *Gadal, O., Strauss, D., Petfalski, E., Gleizes, P.E., Gas, N., Tollervey, D. and Hurt, E. (2002) Rlp7p is associated with 60S preribosomes, restricted to the granular component of the nucleolus, and required for pre-rRNA processing. J Cell Biol,* ***157****, 941-951.*
54. *Baudin, A., Ozier-Kalogeropoulos, O., Denouel, A., Lacroute, F. and Cullin, C. (1993) A simple and efficient method for direct gene deletion in Saccharomyces cerevisiae. Nucleic Acids Res,* ***21****, 3329-3330.*
55. *Pelloquin, L., Belenguer, P., Menon, Y., Gas, N. and Ducommun, B. (1999) Fission yeast Msp1 is a mitochondrial dynamin-related protein. J Cell Sci,* ***112 (Pt 22)****, 4151-4161.*

Albert_Suppl. Fig. 1




A
KDa
-
UBF box1
UBF box1-2
35
25
anti UBF
55
anti PGK
35
B
KDa
-
Hmo1
BoxA
BoxAB
Hmo1ΔC
Hmo1-ΔCR
Hmo1-ΔboxA
Chim-Hmo1
35
25
15
anti-Hmo1
55
anti-PGK
35

Albert_Suppl. Fig. 3