Supplementary Data to

CARBOXY TERMINAL MODIFICATIONS OF THE P0 PROTEIN REVEAL ALTERNATIVE MECHANISMS OF NUCLEAR RIBOSOMAL STALK ASSEMBLY

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MATERIALS AND METHODS

Table S1. Yeast strains used

Strain	Genotype	Source
W303-1B	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ade2-1, can1-100*.	(39)
W303dGP0	as W303-1B but *RPP0::URA3-*P_{GAL1}*-RPP0*	(28)
W303dM	as W303-1B but *MRT4*::*kanMX4*	(12)
W303dGP0dM	As W303-1B but *RPP0::URA3-*P_{GAL1}*-RPP0, MRT4*::*kanMX4*	(12)
W303D7-GFP	as W303-1B but *RPP0::RPP0D7-GFP/HIS3*	This report
D45	As W303 but *RPP2B::HIS3, RPP2A::URA3*	(25)
D45dM	as D45 but *MRT4*::*natNT2*	This report
AJY1539	*MAT***a** *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 CRM1(T539C)-HA*	(40)
AJY1539dM	as AJY1539 but *MRT4::kanMX4*	(12)
W303Nop7-TAP	as W303-1A but *NOP7::NOP7-TAP/TRP1*	(12)
W303dMNop7-TAP	as W303Nop7-TAP but *MRT4::KanMX4*	(12)
D45Nop7-TAP	as D45 but *NOP7::NOP7-TAP/TRP1*	This report
D45dMNop7-TAP	as D45 Nop7-TAP but *MRT4*::*natNT2*	This report

Table S2. Plasmids used

Plasmid	Description
pFLhisP0-C	(16)
pFLhisP0D7	(16)
pFL37Mrt4/P0	(13)
pFL37P0ΔAB	(24)
YCplac111-Mrt4-eGFP	(12)
YCplac111-P0-eGFP	(12)
pUG23-eEFG	Prof. J. H. Hegemann, Düsseldorf Univ.
pUG23P0-C-GFP	This report
pFL36P0-C-GFP	This report
pUG23P0ΔAB-GFP	This report
pFL36P0ΔAB-GFP	This report
pFL36P0D7-GFP	This report
pUG23Mrt4/P0-GFP	This report
pFL36Mrt4/P0-GFP	This report
pFL39-P2A	(41)
p2B35DU	(27)
PYM44	(18)

Table S3. Oligonucleotides used to construct new plasmids				
Construct	Vector	Template	Oligonucleotide	Sequence
P0-CGFP	pUG23	pUG23-P0	5' P0XbaIGFP	5'ATTCTAGATGACTAGGTTATGAAC3'
			3'P0-C-BamHI-GFP	5'AAGGATCCAGCTGGAGCAGCG3'
	pFL36	pUG23-P0-CGFP	5'P0XbaIGFP	5'ATTCTAGATGACTAGGTTATGAAC3'
			3'GFPSacI	5'TTGAGCTCTTATTTGTACAATTCATCCAT3'
P0ΔAB-GFP	pUG23	pBSP0ΔAB	5' P0XbaIGFP	5'ATTCTAGATGACTAGGTTATGAAC3'
			3'P0BamHI-TAP-GFP	5'AAGGATCCATCGAATAAACCGAA3'
	pFL36	pUG23-P0ΔABCGFP	5' P0XbaIGFP	5'ATTCTAGATGACTAGGTTATGAAC3'
			3'GFPSacI	5'TTGAGCTCTTATTTGTACAATTCATCCAT3'
Mrt4/P0-GFP	pUG23	pFL37-Mrt4/P0	5'XbaIP0GFP	5'ATTCTAGATGACTAGGTTATGAAC3'
			3'P0BamHI-TAP-GFP	5'AAGGATCCATCGAATAAACCGAA3'
	pFL36	pUG23-Mrt4/P0GFP	5'XbaIP0GFP	5'ATTCTAGATGACTAGGTTATGAAC3'
			3'GFPSacI	5'TTGAGCTCTTATTTGTACAATTCATCCAT3'
P0D7-GFP	pFL36	*S.cerevisae* D7GFP Genomic DNA	5' P0XbaIGFP	5'ATTCTAGATGACTAGGTTATGAAC3'
			3'EcoRVGFP	5'TTGATATCTTTGTACAATTCATCC3'
NAT/Mrt4 deletion cassette		pYM17	5'MRT4-nat	5'TACATCACTGTATCGTCCATAAAAGATTTATTATAGTTGAATCATGGGTACCACTCTTGAGGACAC3'
			3'MRT4-nat	5'TATACAATGGTCTATTAAAAAAGGCTTCCAAATAATAGTTCAGCTTTAGGGGCAGGGCATGCT3'

RESULTS

Unusual mobility of the GFP-tagged P0 derivatives in SDS-PAGE.

As shown in Figures 1, 4 and S6, the truncated P0-C-GFP and P0ΔAB-GFP proteins unexpectedly migrated to positions higher than that seen for wild-type P0-GFP, even though the PCR DNA fragment encoding the tagged proteins subcloned in the final vector was of the expected relative size (Fig. S1) and the final constructs were confirmed by DNA sequence. These unexpected results must be due to the different original plasmids used as sources for eGFP: Ycplac111 for wild-type P0 and pUG23 for the truncated proteins. This resulted in amino acid stretches of different lengths linking the protein to GFP, which probably affected differently protein mobility.

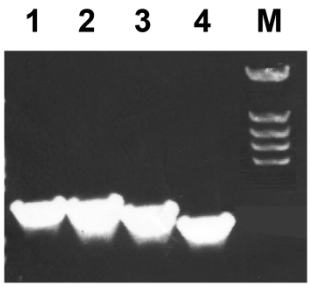

Figure S1. PCR DNA fragments obtained for each tagged protein, which were subsequently cloned into the indicated vector to construct the plasmids used in the Nop7-TAP protein complex purification tests. 1) P0GFP in Ycplac111; 2) P0-C-GFP in pFL36; 3) P0ΔAB-GFP in pFL36; 4) P0D7-GFP in pFL36. M, size markers.

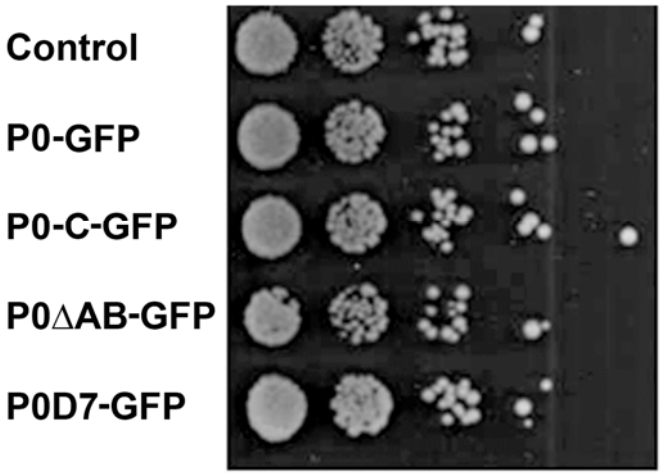

Figure S2. Effect of GFP-tagged P0 truncated proteins on cell growth in *S. cerevisiae*. Serial dilutions of *S. cerevisiae* AJY1539 transformed to express the indicated truncated P0 proteins plated in rich medium and incubated at 30º C for 4 days.

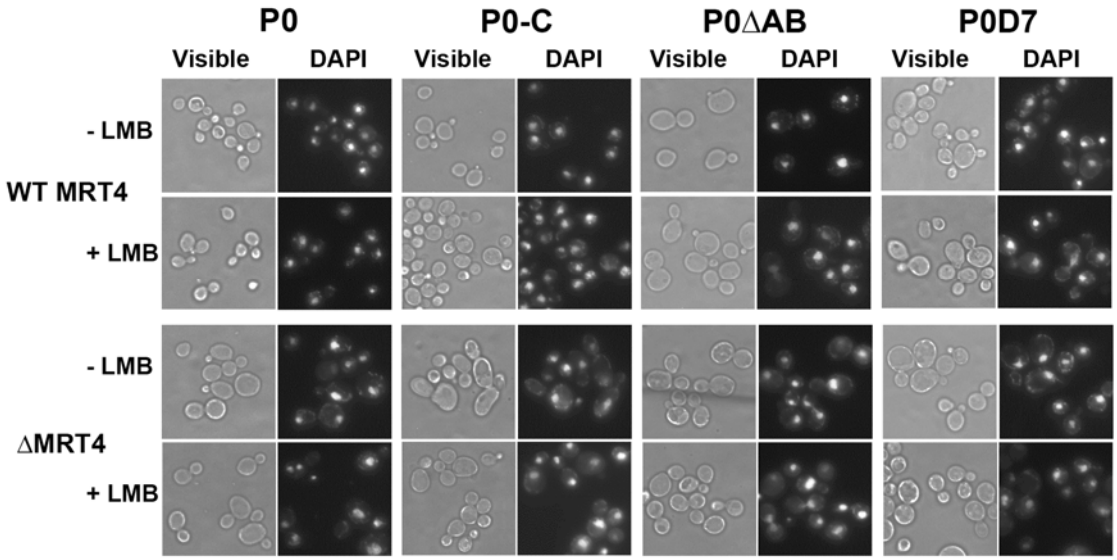

Figure S3. Nuclear location as determined by DAPI staining in cells expressing the GFP-tagged P0 derivatives. The same cell samples of *S. cerevisiae* AJY1539 (WT MRT4) and AJY1539dM (ΔMRT4) expressing the indicated GFP-tagged proteins in the presence and in the absence of LMB shown in Fig. 2 were identified under brightfield illumination and DAPI staining.

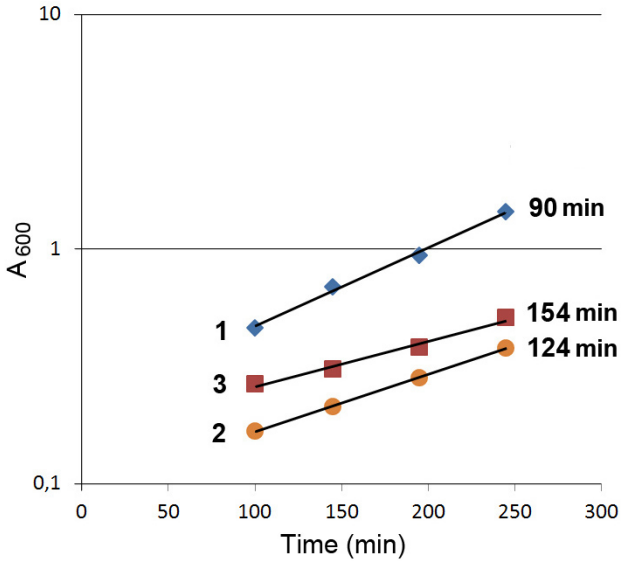

Fig S4. Effect of protein P0D7 on growth rate of a MRT4-deleted yeast strain. *S. cerevisiae* AJY1539dM (3) lacking protein Mrt4 was transformed to express protein P0D7 (2). Both strains, together with the parental AJY1539 (1) as a control were grown in rich lquid medium to estimate the growth rate. The estimated doubling times are indicated.

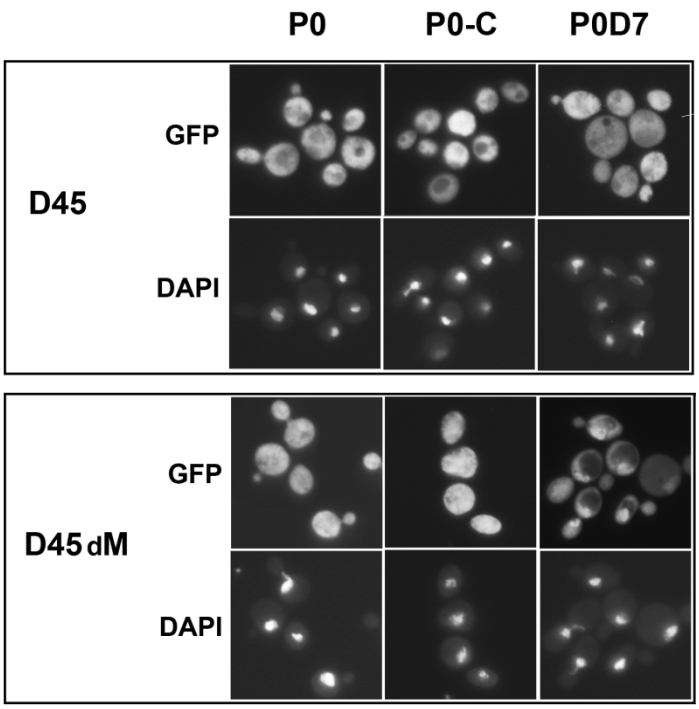

Figure S5. Cellular location of fluorescent P0, P0-C and P0D7 proteins in strains D45 and D45dM. D45 lacks the stalk proteins P1 and P2, and its derivative strain D45dM also lacks Mrt4, and they were transformed using plasmids encoding the indicated GFP-tagged proteins stained with DAPI. Green fluorescence (GFP) and the DAPI stained nuclei are shown in the upper and lower row for each panel.

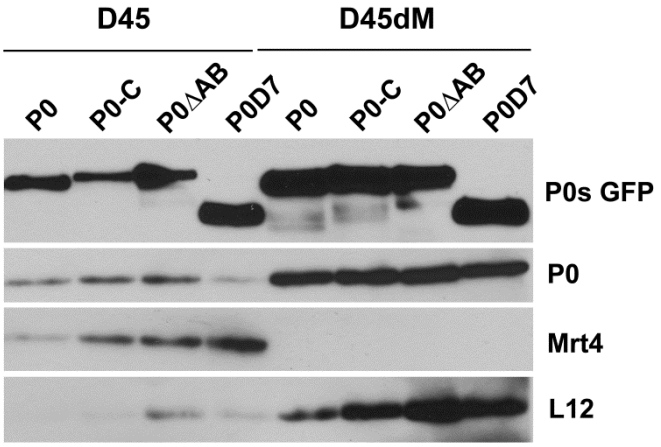

Figure S6. Analysis of Nop7-TAP particles from the D45 *S. cerevisiae* strains. The particles purified from the strains indicated were processed as described in Fig. 4.

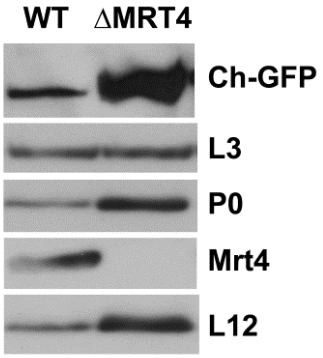

Figure S7. Presence of the Mrt4/P0 chimeric protein in nuclear Nop7-TAP pre-ribosomal particles. Purified particles from parental AJY1539 (WT) and MRT4-deleted AJY1539dM (ΔMRT4) were processed as described in Fig. 4 and Mrt4/P0 was identified at the expected position in western blots using an anti-Mrt4 serum.

References

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