

Supplemental Material

Nuclear basket subunits Nup211 and Rsm1 influence RNA 3'-processing and transcription termination in fission yeast

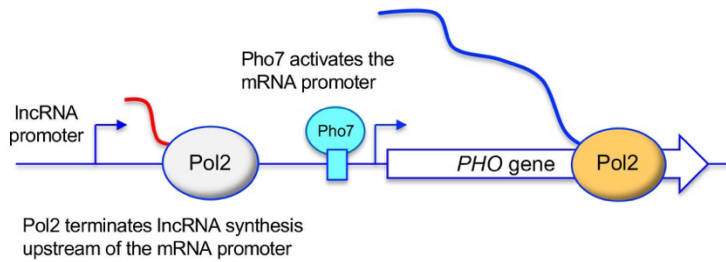
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Figures S1, S2, S3, S4, S5.

Table S4

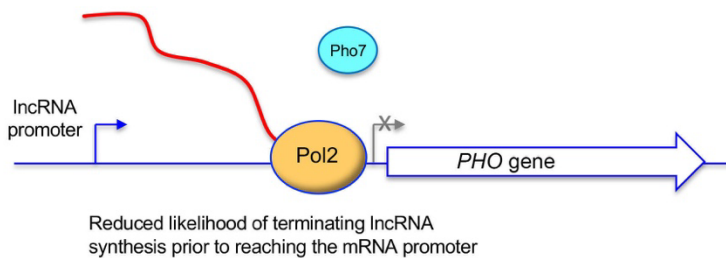
[Tables S1, S2, and S3 are provided as separate .xls files]

A *PHO* gene derepressed in phosphate-replete cells



- Pol2 CTD mutants: S5A, P6A, S7A
- Csk1 and Cdk9 protein kinase mutants
- Inositol pyrophosphatase mutants (increased IP₈)
- Termination factor mutant: *seb1-G476S*
- Rad24 (14-3-3 protein) mutant
- Erh1 mutant

B *PHO* gene hyper-repressed in phosphate-replete cells



- Pol2 CTD mutant T4A; CTD prolyl isomerase Pin1 mutant
- Inositol pyrophosphate kinase mutants (no IP₈)
- Spx1 inositol pyrophosphate sensor mutants
- Cleavage and polyadenylation factor (CPF) subunit mutants: Ssu72, Swd22, Ppn1, Dis2, Msi2, Pfs2, Cft1, Ysh1, Pta1, Ctf1
- Termination factor mutant: *rhn1Δ*

Figure S1. Genetic modulation of *PHO* IncRNA termination can impact *PHO* mRNA repression. (A) Precocious termination of IncRNA synthesis upstream of the mRNA promoter can result in derepression of mRNA transcription. Mutant genetic backgrounds that elicit *PHO* gene derepression are indicated at right. (B) Decreased termination of IncRNA synthesis can increase interference with the mRNA promoter and hyper-repress mRNA expression. Genetic changes that result in *PHO* gene hyper-repression are indicated at right.

Sample	Total Paired Reads	Mapped Reads
<i>WT</i> (1)	27,673,343	27,232,727 (98%)
<i>WT</i> (2)	28,786,900	28,351,350 (98%)
<i>WT</i> (3)	30,660,670	30,191,101 (98%)
<i>nup211-1435*</i> (1)	27,223,040	26,350,090 (97%)
<i>nup211-1435*</i> (2)	27,352,353	26,903,706 (98%)
<i>nup211-1435*</i> (3)	29,828,600	29,316,096 (98%)
<i>rsm1Δ</i> (1)	28,182,110	27,733,697 (98%)
<i>rsm1Δ</i> (2)	26,736,056	26,267,636 (98%)
<i>rsm1Δ</i> (3)	22,961,235	22,610,736 (98%)

Sample pairs	Pearson Coefficient
<i>WT</i> (1) vs (2)	0.987
<i>WT</i> (1) vs (3)	0.987
<i>WT</i> (2) vs (3)	0.987
<i>nup211-1435*</i> (1) vs (2)	0.985
<i>nup211-1435*</i> (1) vs (3)	0.986
<i>nup211-1435*</i> (2) vs (3)	0.989
<i>rsm1Δ</i> (1) vs (2)	0.988
<i>rsm1Δ</i> (1) vs (3)	0.987
<i>rsm1Δ</i> (2) vs (3)	0.986

Figure S2. RNA-seq read counts for triplicate biological replicates (top panel) and data reproducibility between biological replicates (bottom panel).

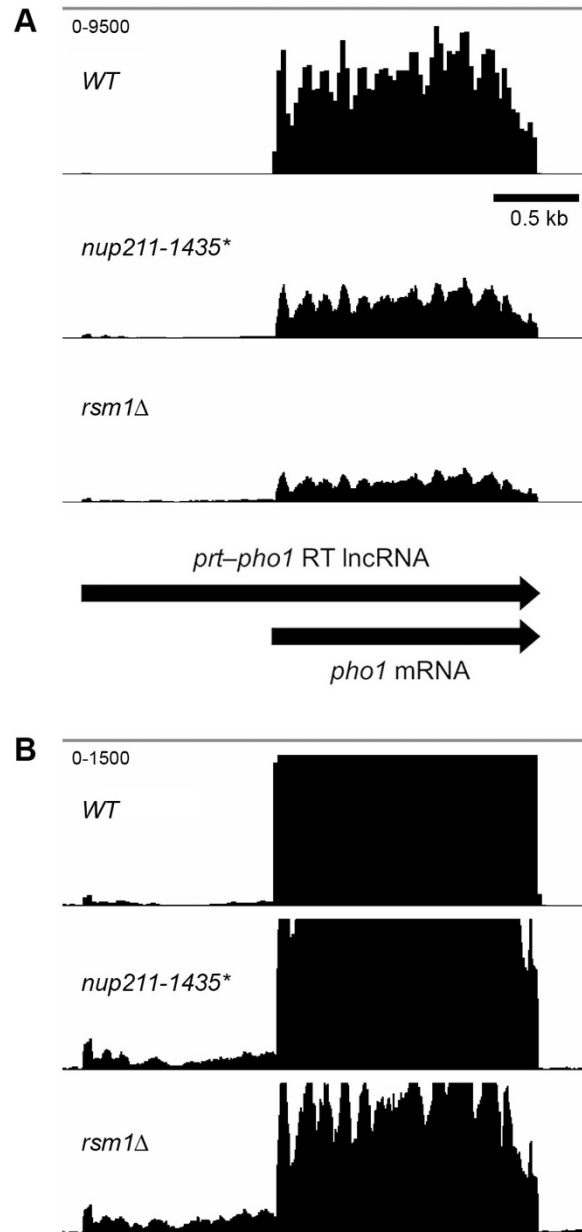


Figure S3. *nup211-1435** and *rsm1Δ* reduce *pho1* mRNA via increased synthesis of interfering *prt* lncRNA. Strand-specific RNA-seq read densities (counts/base/million) are plotted as a function of position across the *prt-pho1* locus for wild-type, *nup211-1435**, and *rsm1Δ* cells. Panels A and B depict the same data at different read density scales (indicated at left in the WT traces), with panel B highlighting the read densities corresponding to the *PHO*-interfering lncRNA. The RNA-seq read densities were determined from cumulative counts of three RNA-seq replicates. The individual lncRNA–mRNA read-through (RT) transcripts and mRNAs are labeled and shown to scale in panel A as black arrows in the direction of their synthesis.

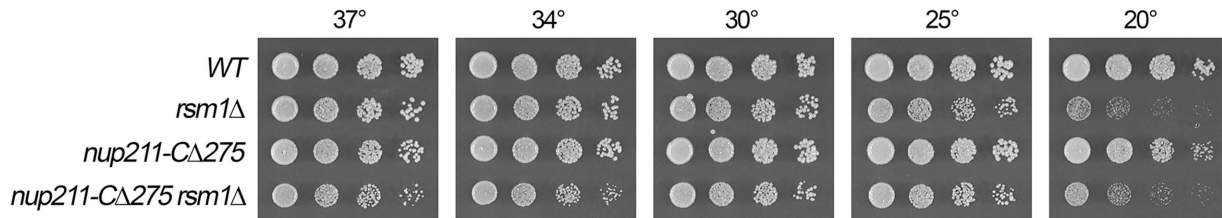


Figure S4. *rsm1Δ* and *nup211-CΔ275* do not synergize with respect to growth. Serial 5-fold dilutions of fission yeast strains specified on the left were spotted to YES agar and tested for growth at the temperatures indicated.

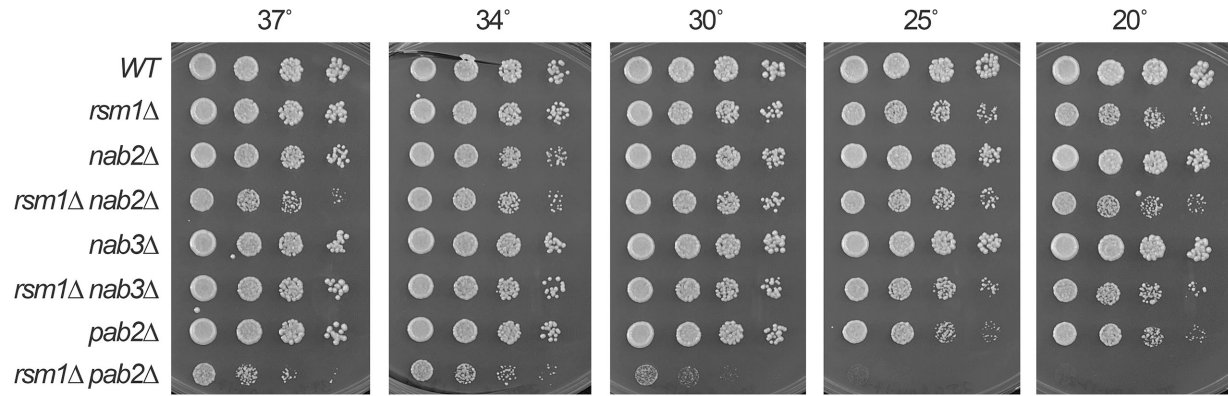


Figure S5. Genetic interactions between Rsm1 and poly(A) binding proteins. Serial 5-fold dilutions of fission yeast strains specified on the left were spotted to YES agar and tested for growth at the temperatures indicated.

Table S4. List of *S. pombe* strains used in this study.

Strain	Alias	Genotype	Source
JS77	WT	<i>h- leu1-32 ura4-D18 his3-D1 ade6-m216</i>	PMC1347026
JS78	WT	<i>h+ leu1-32 ura4-D18 his3-D1 ade6-m210</i>	PMC1347026
BS848	STF6	<i>h+ asp1-W386*::hygMX</i>	PMC10865970
BS853	SST-65	<i>h+ asp1-W386*::hygMX nup211-Q1353*</i>	This study
BS858	STF9	<i>h+ asp1-W493*::hygMX</i>	PMC10865970
IAP37	SST-911	<i>h+ asp1-W493*::hygMX nup211-(+5 fs(L1435))</i>	This study
IAP105	<i>nup211-1435*</i>	<i>h- asp1-WT::kanMX nup211-(+5 fs(L1435))</i>	This study
BS1024	<i>nup211-Q1353*</i>	<i>h- nup211-Q1353*</i>	This study
IAP44	SST-918	<i>h+ asp1-W493*::hygMX rsm1-(+1 fs (S244))</i>	This study
ASY240	<i>rsm1Δ</i>	<i>h- rsm1Δ::natMX</i>	This study
ASY221	<i>rsm1Δ STF6</i>	<i>h+ rsm1Δ::natMX asp1-W386*::hygMX</i>	This study
ASY222	<i>rsm1Δ STF9</i>	<i>h- rsm1Δ::natMX asp1-W493*::hygMX</i>	This study
BS1451	<i>nup211-WT</i>	<i>h- nup211-WT::kanMX</i>	This study
BS1450	<i>nup211-CΔ129</i>	<i>h+ nup211-(1-1708)::kanMX</i>	This study
BS1534	<i>nup211-CΔ199</i>	<i>h+ nup211-(1-1638)::kanMX</i>	This study
BS1533	<i>nup211-CΔ275</i>	<i>h- nup211-(1-1562)::kanMX</i>	This study
BS1414	<i>nup211-CΔ321</i>	<i>h- nup211-(1-1516)::kanMX</i>	This study
BS1569	<i>nup211-CΔ402</i>	<i>h- nup211-(1-1435)::kanMX</i>	This study
BS1530	<i>nup211-CΔ484</i>	<i>h- nup211-(1-1353)::kanMX</i>	This study
BS1445	<i>nup211-CΔ584</i>	<i>h+ nup211-(1-1253)::kanMX</i>	This study
BS1606	<i>nup211-CΔ129 STF6</i>	<i>h+ nup211-(1-1708)::kanMX asp1-W386*::hygMX</i>	This study
BS1608	<i>nup211-CΔ129 STF9</i>	<i>h+ nup211-(1-1708)::kanMX asp1-W493*::hygMX</i>	This study
BS1610	<i>nup211-CΔ275 STF6</i>	<i>h- nup211-(1-1562)::kanMX asp1-W386*::hygMX</i>	This study
BS1612	<i>nup211-CΔ275 STF9</i>	<i>h- nup211-(1-1562)::kanMX asp1-W493*::hygMX</i>	This study
AS612	<i>ssu72-C13S</i>	<i>h+ ssu72-C13S::natMX</i>	PMC6233144
AS1985	<i>ppn1Δ</i>	<i>h- ppn1Δ::natMX</i>	PMC6233144
AS1986	<i>swd22Δ</i>	<i>h- swd22Δ::natMX</i>	PMC6233144
BS1629	<i>nup211-CΔ275 ssu72-C13S</i>	<i>h- nup211-(1-1562)::kanMX ssu72-C13S::natMX</i>	This study
BS1631	<i>nup211-CΔ275 ppn1Δ</i>	<i>h- nup211-(1-1562)::kanMX ppn1Δ::natMX</i>	This study
BS1633	<i>nup211-CΔ275 swd22Δ</i>	<i>h- nup211-(1-1562)::kanMX swd22Δ::natMX</i>	This study
BS1873	<i>nup211-CΔ275 rsm1Δ</i>	<i>h- nup211-(1-1562)::kanMX rsm1Δ::natMX</i>	This study
BS1871	<i>nup211-RDD-AAA</i>	<i>h+ nup211-R1821A-D1822A-D1823A::kanMX</i>	This study
BS1893	<i>nup211-RDD-AAA STF6</i>	<i>h+ nup211-R1821A-D1822A-D1823A::kanMX asp1-W386*::hygMX</i>	This study
BS1894	<i>nup211-RDD-AA STF9</i>	<i>h+ nup211-R1821A-D1822A-D1823A::kanMX asp1-W493*::hygMX</i>	This study
AS1845	<i>nab2Δ</i>	<i>h+ nab2Δ::kanMX</i>	PMC12077094
AS1846	<i>nab3Δ</i>	<i>h- nab3Δ::kanMX</i>	PMC6233144
AS1848	<i>pab2Δ</i>	<i>h- pab2Δ::kanMX</i>	This study
ASY249	<i>rsm1Δ nab2Δ</i>	<i>h+ rsm1Δ::natMX nab2Δ::kanMX</i>	This study
ASY251	<i>rsm1Δ nab3Δ</i>	<i>h+ rsm1Δ::natMX nab3Δ::kanMX</i>	This study
ASY262	<i>rsm1Δ pab2Δ</i>	<i>h- rsm1Δ::natMX pab2Δ:: kanMX</i>	This study

All strains are *leu1-32*, *ura4-D18*, *his3-D1*, and either *ade6-m216* or *ade6-m210*. Mating types were determined by mixing cells with each of the wild-type *h+* and *h-* strains on malt agar and observing tetrads after 24-48 h of incubation.