The homologous putative GTPases Grn1p from Fission Yeast and the Human GNL3L are required for growth and play a role in processing of nucleolar Pre-rRNA

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Supplementary Data

1. **Table T1. Plasmids and Yeast strains**
2. **Legends for supplementary figures**
3. **Figures**

Table . Plasmids and Yeast strains

Strains

Strains (YNB)	Parent strain	Plasmid	Marker	Reference
YNB951	YNB484	pBNB475	*Leu2*	This study
YNB952	YNB484	pBNB476	*Leu2*	This study
YNB953	YNB484	pBNB477	*Leu2*	This study

Plasmids

pBNB475	To clone the *Grn1* ORF together with its native promoter (398nts upstream of gene ORF arbitrarily determined to be the promoter), primers NB1149 (promoter sequence) and NB1150 (*Grn1* N-terminal sequence containing an inherent *BamHI* site) were used to PCR-amplify a fragment comprising promoter and ~ 360nts of *Grn1* N-terminal sequence using yeast genomic DNA as the template. The fragment was digested with *SalI* & *BamHI* and cloned into pBNB190. The fragment thus replaced the ~ 360nts of original *Grn1* N-terminal sequence in the new plasmid. Subsequently, promoter-*Grn1*-GFP was released from the above vector using *SacI* and *BglII*, and cloned into pHL1288.	Amp^R	This study
pBNB476	To clone the *Grn1* ΔNLS1 mutant (AA^{6-22}) under its native promoter, a fusion PCR strategy was employed. 5' and 3' end PCR products were amplified by using respective sets of primers NB1149+NB1152 (*Grn1* 5'end sequence with removal of NLS1) and NB1151 (complementary to NB1152) + NB1150, and pBNB475 as the template. The fusion was obtained by using 5' and 3' end PCR products as templates and NB1149+NB1150 as primers. The resulting product was digested with *SacI* & *BamHI* and cloned into pBNB475 treated with the same pair of enzymes. The fragment promoter-*Grn1* ΔNLS1 replaced the original promoter-*Grn1* N-terminal sequence (~360nts).	Amp^R	This study
pBNB477	To clone the *Grn1* ΔNLS2 mutant (AA^{6-36}) under its native promoter, the cloning strategy was similar to that of pBNB476. 5' and 3' end PCR products were amplified by using respective sets of primers NB1149+NB1154 (*Grn1* 5'end sequence with removal of NLS2) and NB1153 (complementary to NB1152) + NB1150, using pBNB475 as template. The fusion PCR was achieved by using the above two PCR products as templates and NB1149+NB1150 as primers. The fragment was digested with *SacI* & *BamHI* and cloned into pBNB475 treated with the same pair of enzymes. The promoter-*Grn1* ΔNLS2 replaced the original sequence.	Amp^R	This study

Legends for supplementary figures

Figure S1.

Alignment of Rpl25 homologs from different sources as indicated- *S. pombe* (Sc), *S. cerevisiae* (Sc), *Humans* (Hs)), *Rattus norvegicus* (Rn), *Mus musculus* (Mm) and *C. elegans* (Ce). Identical residues are shaded black (conservative substitutions are not indicated).

Figure S2

Alignment of the putative GTPases GLN3L, Grn1p and Nucleostemin (NS). Identical residues are shaded black (conservative substitutions are not indicated).

Figure S3

Effect of deleting the putative nucleolar/nuclear targeting domain on Grn1p:GFP localization and on growth. (A) Alignment of the N-terminal domains of GNL3L, Grn1p and NS showing the remarkably conserved pattern of basic residues. The indicated amino acids within Grn1p sequence were deleted. (B) Strains containing the above deletions NLS1Δ, AA5-22 (YNB592) and NLS2Δ, AA5-36 (YNB593) were grown in EMM-leu medium and examined for fluorescence. (C) Growth of strains NLS1Δ, AA6-22 (YNB592) and NLS2Δ, AA6-36 (YNB593) were compared with a wild type strain, WT (YNB591) on EMM-leu medium. (D) Equal amounts of cells from the indicated strains were subjected to western analysis.

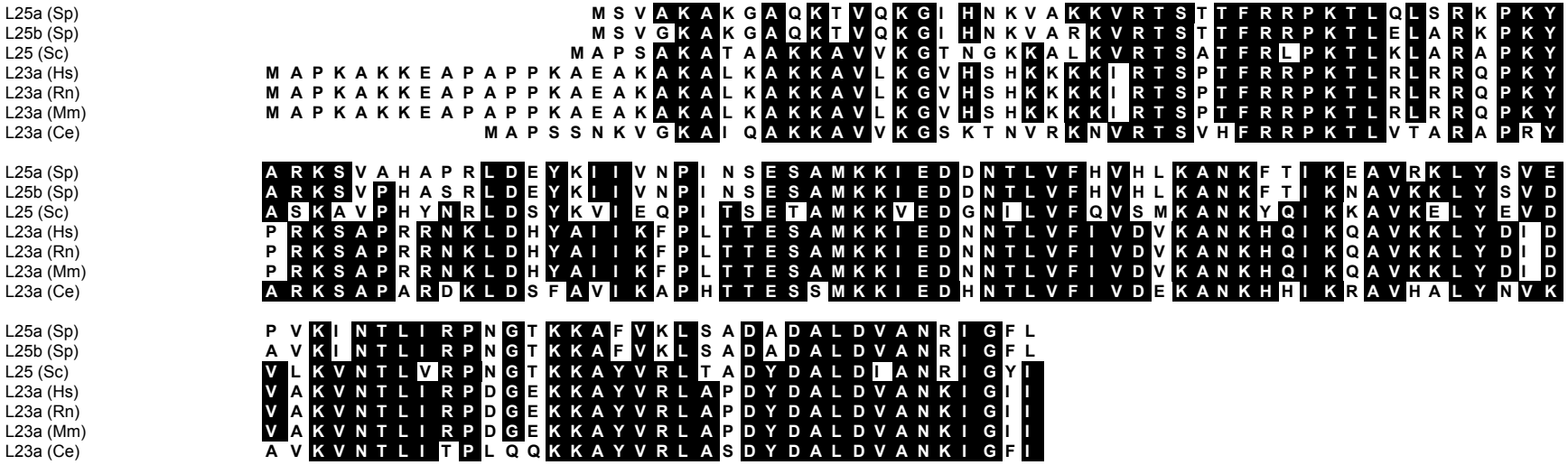
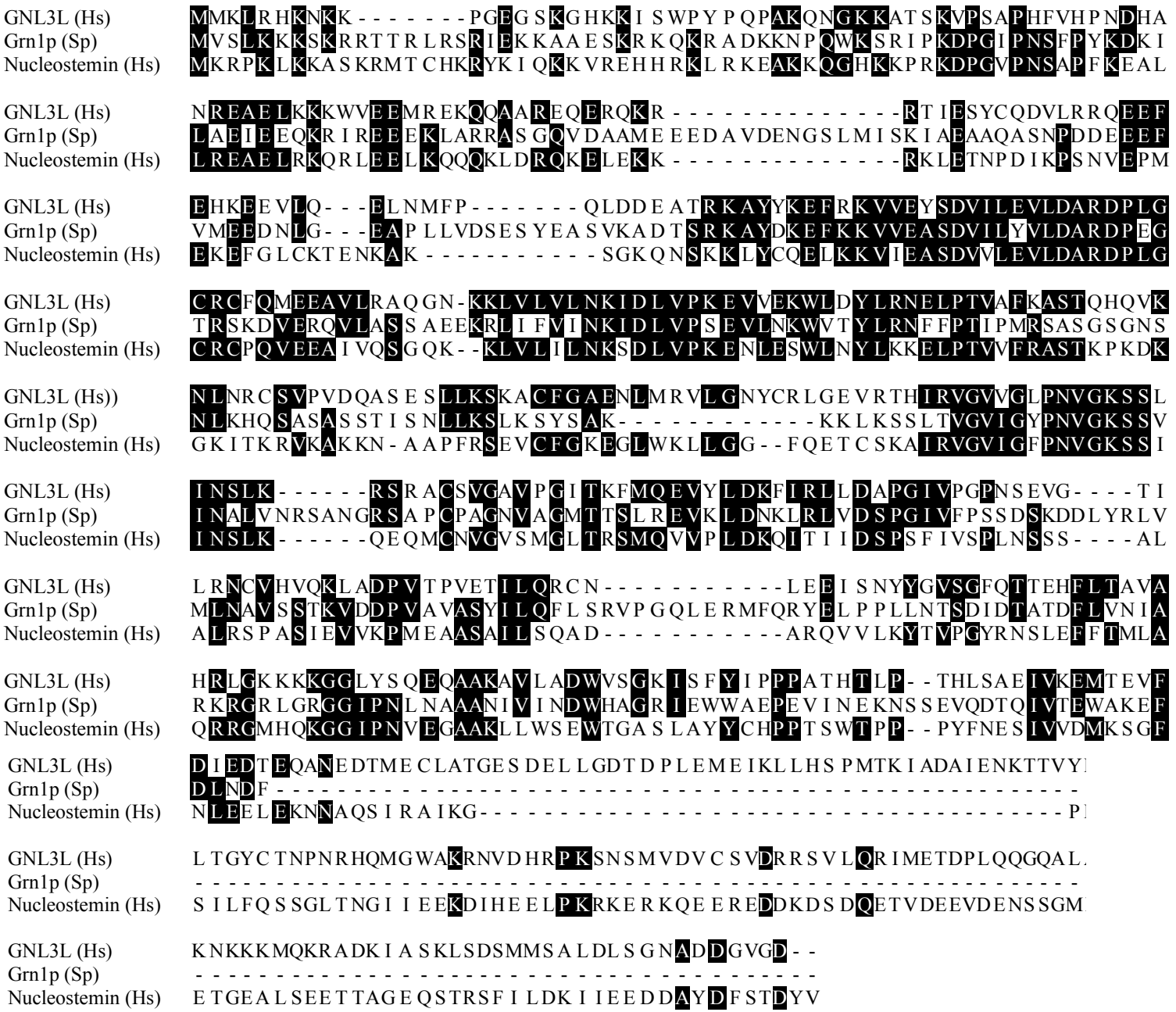

L25a (Sp)
L25b (Sp)
L25 (Sc)
L23a (Hs)
L23a (Rn)
L23a (Mm)
L23a (Ce)
L25a (Sp)
L25b (Sp)
L25 (Sc)
L23a (Hs)
L23a (Rn)
L23a (Mm)
L23a (Ce)
L25a (Sp)
L25b (Sp)
L25 (Sc)
L23a (Hs)
L23a (Rn)
L23a (Mm)
L23a (Ce)

Figure S1


GNL3L (Hs)
Grn1p (Sp)
Nucleostemin (Hs)
GNL3L (Hs)
Grn1p (Sp)
Nucleostemin (Hs)
GNL3L (Hs)
Grn1p (Sp)
Nucleostemin (Hs)
GNL3L (Hs)
Grn1p (Sp)
Nucleostemin (Hs)
GNL3L (Hs))
Grn1p (Sp)
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GNL3L (Hs)
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Nucleostemin (Hs)
GNL3L (Hs)
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Nucleostemin (Hs)
GNL3L (Hs)
Grn1p (Sp)
Nucleostemin (Hs)
GNL3L (Hs)
Grn1p (Sp)
Nucleostemin (Hs)
GNL3L (Hs)
Grn1p (Sp)
Nucleostemin (Hs)

Figure S2

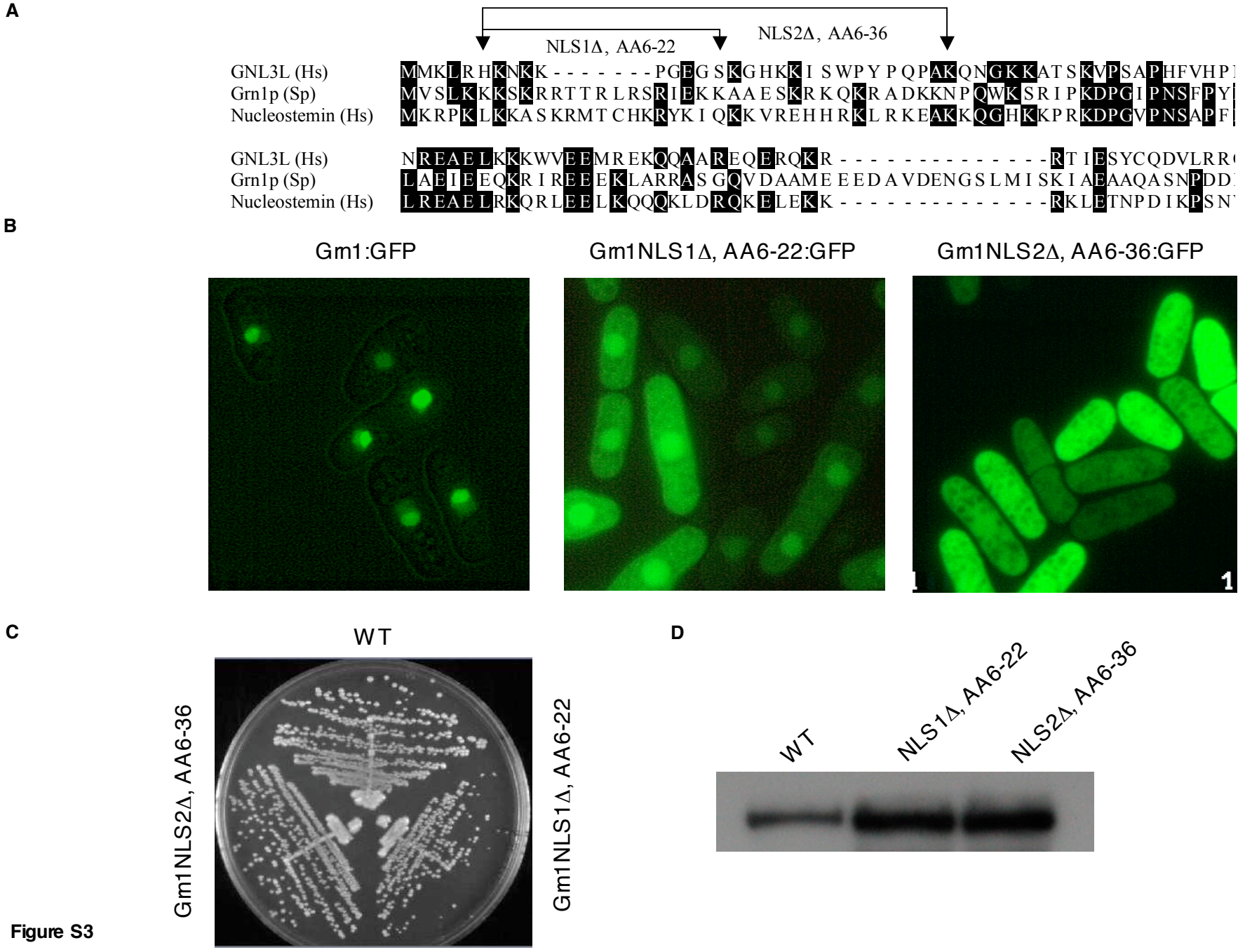
A
NLS1Δ, AA6-22
NLS2Δ, AA6-36
GNL3L (Hs)
Grn1p (Sp)
Nucleostemin (Hs)
B
Gm1:GFP
Gm1NLS1Δ, AA6-22:GFP
Gm1NLS2Δ, AA6-36:GFP
C
WT
Gm1NLS2Δ, AA6-36
Gm1NLS1Δ, AA6-22
D
WT
NLS1Δ, AA6-22
NLS2Δ, AA6-36

Figure S3