SUPPLEMENTAL INFORMATION

Monosome Formation during Translation Initiation Requires the SR Protein Npl3

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Running title: SR-protein Npl3 is important for monosome formation

TABLE S1. Yeast strains

Strain number	Genotype	Source
HKY36	*MATα ura3-52 leu2Δ1 his3-200*	Fred Winston,Boston
HKY45	*MATa ura leu ade2 xpo1::LEU2 + p CEN HIS3 xpo1-1*	this study
HKY77	*MATa ura leu his trp rpb1-1*	(1)
HKY124	*MATα ura3-52 leu2Δ1 his3Δ200 rat7-1*	(2)
HKY157	*MATa ura3 leu2 his3 ade npl3::HIS3 + p CEN URA3 NPL3-myc*	(3)
HKY160	*MATa ura3-1 leu2 his3 lys2 ade2 ade3 npl3::HIS3 + p CEN LEU2 npl3-17*	(4)
HKY265	*MATa ura3-52 leu2-3,112 trp1-901 his3Δ200 gal4Δ gal80Δ*	Clontech
HKY270	*MATα ura leu ade trp1 mtr10::HIS3 +p CEN TRP1 mtr10-7*	(5)
HKY380	*MATa ura3Δ0 leu2Δ0 his3Δ1 met15Δ0 npl3::kanMX4*	Euroscarf
HKY381	*MATα ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0*	Euroscarf
HKY408	*MATα ura3 leu2 trp1 his3 prt1-1*	Mark Ashe, Manchester
HKY705	*MATa ura3 leu2 trp1 his3 rix1-1*	(6)
HKY611	*MATα ura3 leu2 his3 npl3::kanMX4 rat7-1*	this study
HKY644	*MATα ura3 leu2 trp1 his3 ade2 mex67::HIS3 + p CEN LEU2 mex67-5*	(7)
HKY749	*MATa ura3Δ0 leu2Δ0 his3Δ1 npl3::kanMX4 fun12::kanMX4 + p CEN URA3 NPL3*	this study
HKY802	*MATα ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0 RPL25-3xmyc:HIS3MX6*	(8)
HKY805	*MATa ura3Δ0 leu2Δ0 his3Δ1 met15Δ0 npl3::kanMX4 RPL25-3xmyc:HIS3MX6*	(8)
HKY812	*MATa ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0 lsg1::kanMX4 + p CEN LEU2 lsg1-2*	this study
HKY816	*MATa ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0 lsg1::kanMX4 + p CEN LEU2 lsg1-3*	this study
HKY858	*MATa ura3Δ0 leu2Δ0 his3Δ1 rpl10::kanMX4 + p CEN URA3 RPL10-GFP*	this study
HKY863	*MATα ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0 rpl10::kanMX4 + p CEN URA3 rpl10(G161D)-GFP*	this study
HKY890	*MATa ura3 leu2 his3 lys2 mtr2::kanMX4 + p CEN TRP1 mtr2-21 + p CEN URA3 MTR2*	(6)
HKY892	*MATa ura3 leu2 his3 lys2 mtr2::kanMX4 + p CEN TRP1 mtr2-33 + p CEN URA3 MTR2*	(6)
HKY894	*MATα ura3 leu2 his3 lys2 nmd3::kanMX4 + p CEN TRP1 nmd3-2 + p CEN URA3 NMD3*	(6)
HKY906	*MATα ura3Δ0 leu2Δ0 his3Δ0 trp1::kanMX4 mtr2::kanMX4 + p CEN TRP1 mtr2-21+ p CEN URA3 MTR2*	this study
HKY918	*MATα ura3Δ0 leu2Δ0 his3Δ1 trp1::kanMX4 fun12::kanMX4 mtr2::kanMX4+ p CEN TRP1 mtr2-21 + p CEN URA3 MTR2*	this study
HKY931	*MATa ura3Δ0 leu2Δ0 his3Δ1 trp1::kanMX4*	this study
HKY934	*MATa ura3Δ0 leu2Δ0 his3Δ1 npl3::kanMX4 trp1::kanMX4*	this study
HKY949	*MATa ura3Δ0 leu2Δ0 his3Δ1 met15Δ0 fun12::kanMX4*	Euroscarf
HKY991	*MATα rpl10::kanMX4 trp1::kanMX4 + p CEN URA3 RPL10-GFP*	this study
HKY994	*MATα rpl10::kanMX4 npl3::kanMX4 trp1::kanMX4 + p CEN URA3 RPL10-GFP*	this study
HKY1047	*MATa ura3 leu2 his3 trp1 rio2::kanMX4 + p CEN LEU2 rio2-1*	(9)

HKY1056	*MATα ura3 leu2 his3 lys2 trp1 fun12::kanMX4 rix1-1*	this study
HKY1058	*MATa ura3 leu2 his3 lys2 trp1 fun12::kanMX4 rio2::kanMX4 + p CEN LEU2 rio2-1*	this study
HKY1243	*MATα ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0 rpl10::kanMX4 + p CEN HIS3 rpl10(G161D)*	this study

TABLE S2. Plasmids

Plasmid number	Features	Source
pHK12	*CEN URA3 P_{ADH}-NLS-NES-GFP-GFP*	(10)
pHK25	*CEN LEU2 NPL3*	this study
pHK87	*CEN LEU2*	(11)
pHK88	*CEN URA3*	(11)
pHK103	*2μ LEU2*	(12)
pHK104	*2μ URA3*	(12)
pHK119	ampR GST(pGEX4T-1)	Pharmacia
pHK144	*2μ URA3 P_{GAL}-GFP-NPL3*	(4)
pHK145	*2μ URA3 P_{GAL}-GFP-C-NPL3*	this study
pHK154	*CEN LEU2 npl3-17*	(4)
pHK195	*2μ URA3 NPL3*	(4)
pHK230	*2μ URA3 P_{GAL}-GFP*	(4)
pHK285	*2μ LEU2 MTR2*	(13)
pHK391	*2μ TRP1 GAL4 DNA-BD*	Clonetech
pHK392	*2μ LEU2 GAL4 AD*	Clonetech
pHK418	*CEN LEU2 GFP-NPL3*	(14)
pHK501	*2μ TRP1 P_{ADH}-GAL4-BD-myc-NPL3*	this study
pHK598	*2μ LEU2 ADE3 NPL3*	this study
pHK697	*CEN URA3 RPS2-GFP*	(15)
pHK698	*CEN URA3 RPL25-GFP*	(16)
pHK718	*2μ ADE3 URA3 NMD3*	(17)
pHK719	*2μ LEU2 NMD3*	(17)
pHK747	*CEN URA3 RPL10-GFP*	this study
pHK758	*CEN TRP1 rpl10(G161D)-GFP*	this study
pHK763	*2μ TRP1 P_{ADH}-GAL4-BD-NPL3*	this study
pHK764	*2μ LEU2 P_{ADH}-GAL4-AD-NPL3*	this study
pHK765	*CEN URA3 GFP-NPL3*	(8)
pHK777	*CEN LEU2 6xmyc-NPL3*	this study
pHK805	*2μ LEU2 P_{ADH}-GAL4-AD-npl3ΔC* (Δaa276-414)	this study
pHK806	*2μ LEU2 P_{ADH}-GAL4-AD-npl3ΔN* (Δaa1-125)	this study
pHK809	*2μ LEU2 P_{ADH}-GAL4-AD-npl3ΔNΔC* (Δaa1-113, 275-414)	this study
pHK810	*2μ LEU2 P_{ADH}-GAL4-AD-npl3ΔNΔRRM1* (Δaa1-195)	this study
pHK812	*2μ TRP1 P_{GAL}-GFP-C-NPL3*	this study
pHK829	*2μ LEU2 P_{ADH}-GAL4-AD-npl3Δ25* (Δaa390-414)	this study
pHK830	*2μ LEU2 P_{ADH}-GAL4-AD-npl3Δ50* (Δaa365-414)	this study
pHK831	*2μ LEU2 P_{ADH}-GAL4-AD-npl3Δ75* (Δaa340-414)	this study
pHK832	*2μ LEU2 P_{ADH}-GAL4-AD-npl3Δ100* (Δaa315-414)	this study
pHK833	*2μ LEU2 P_{ADH}-GAL4-AD-npl3Δ125* (Δaa290-414)	this study
pHK835	*CEN LEU2 npl3Δ50* (Δaa365-414)	this study
pHK836	*CEN LEU2 npl3Δ75* (Δaa340-414)	this study
pHK837	*CEN LEU2 npl3Δ100* (Δaa315-414)	this study
pHK838	*CEN LEU2 npl3Δ125* (Δaa290-414)	this study
pHK845	*6xHIS-NPL3*	(8)
pHK883	*CEN URA3 P_{ADH}-GFP-GFP*	this study

pHK1227	*2μ URA3 P_{GAL}-GFP-NES-C-NPL3*	this study
pHK1234	*2μ URA3 FLAG-FUN12*	(18)
pHK1274	*CEN LEU2 NLS-npl3Δ100*	this study
pHK1276	*GST-NPL3*	this study
pHK1291	*CEN HIS3 rpl10(G161D)*	this study
pHK1302	*2μ URA3 RPL10*	this study
pHK1303	*CEN URA3 P_{ADH}-GFP-NPL3*	this study
pHK1304	*CEN URA3 P_{ADH}-GFP-npl3Δ100*	this study
pHK1305	*CEN URA3 P_{ADH}-GFP-NLS-npl3Δ100*	this study
pHK1321	*CEN URA3 N-GFP-NPL3*	this study
pHK1322	*CEN LEU2 C-GFP-NPL3*	this study
pHK1367	*CEN URA3 N-GFP-npl3Δ100*	this study

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STRAINS AND PLASMIDS USED IN ALL FIGURES

FIG 1.
A: *E. coli BL21(DE3)*, pHK119, pHK845, pHK1276
C: HKY265, pHK763, pHK764, pHK829-833
D: HKY157, pHK25, pHK87, pHK835-838, pHK1274
E: HKY157, pHK25, pHK894, pHK835-837
F: HKY36, pHK1303-1305
G: HKY380, HKY611, pHK25, pHK894, pHK835-837
H: HKY157, pHK25, pHK835-837, pHK1274, HKY160, HKY644
I: HKY157, pHK25, pHK835-837, HKY160, HKY45, pHK698

FIG 2.
A: HKY36, pHK87, HKY408, HKY157, pHK835-837, HKY160
B: HKY157, pHK25, pHK837, HKY949, HKY863, HKY160, HKY890 (selected on FOA), HKY892 (selected on FOA), HKY894 (selected on FOA), HKY124

FIG 3.
A: HKY36, HKY157, pHK25, pHK837, HKY863
B: HKY802, HKY805, pHK835, pHK837, HKY36
C: HKY36, HKY157, pHK25, pHK837, HKY894 (selected on FOA)
D: HKY157, pHK25, pHK837, HKY36

FIG 4.
A: HKY934, pHK777, pHK765, pHK812
B: HKY36, pHK144, pHK145, pHK1227
C: HKY36, pHK144, pHK145, pHK1227
D: HKY36, HKY270, pHK1321, pHK1322, pHK1367
E: HKY36, pHK144, pHK145, pHK230, pHK1227

FIG 5.
A: HKY381, HKY380, pHK837, pHK154, HKY994, pHK758, HKY949, HKY890, HKY705, HKY1047, HKY749, HKY918, ,HKY1056, HKY1058
B: HKY36, pHK104, pHK195, HKY812, HKY863, pHK103, pHK598, pHK285, pHK719, HKY816, HKY949
C: HKY863, pHK103, pHK719, pHK598
D: HKY1243, pHK698, pHK103, pHK598
E: HKY157, pHK837, pHK104, pHK1234, pHK1302, pHK718

FIG 6.
A: HKY36, pHK697
B: HKY157, pHK25, pHK837, pHK747
C: HKY36, pHK1303, pHK1304
D: HKY36, pHK1303, pHK1304

FIG S1.
HKY265, pHK763, pHK764, pHK391, pHK392, pHK806, pHK805, pHK809, pHK810
FIG S2.
HKY157, pHK25, pHK837, pHK836, pHK835
FIG S3.
HKY36, pHK144, pHK145, pHK1227, pHK812

FIGURES

Figure S1

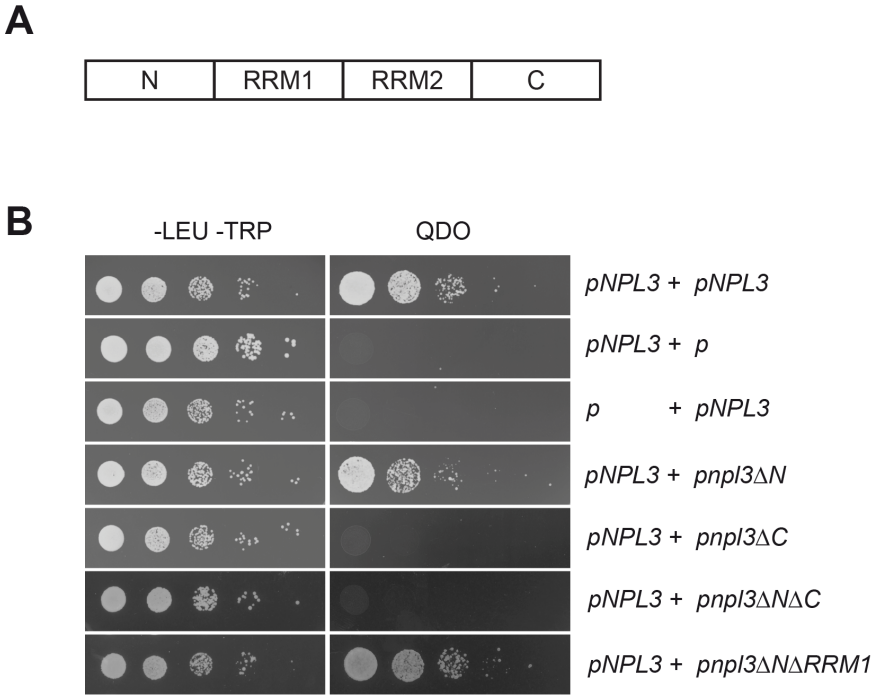

FIG S1. The C-terminal domain of Npl3 is crucial for homodimerization. (A) Schematic overview of the domain organization of Npl3. (N) N-terminal domain, (RRM1 and RRM2) RNA recognition motifs, (C) C-terminal domain. (B) Cells carrying full length *NPL3* in combination with either truncated versions of *NPL3* or empty vectors were spotted in serial dilutions onto quadruple drop out plates (QDO) for interaction studies in the Yeast-two-hybrid system. The growth was verified on plates lacking tryptophan and leucin (-Trp -Leu) upon incubation at 25°C for 3 days.

Figure S2

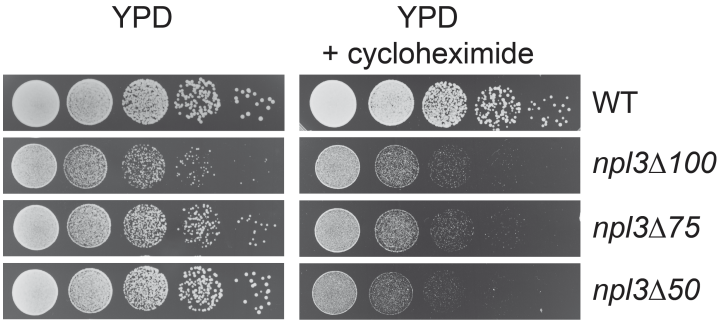

FIG S2. The C-terminal domain of Npl3 is important for the growth in the presence of cycloheximide. Serial dilutions of the indicated strains were spotted onto YPD plates without or with 0,04 μg/ml cycloheximide. The plates were incubated for 3 days at 25°C.

Figure S3

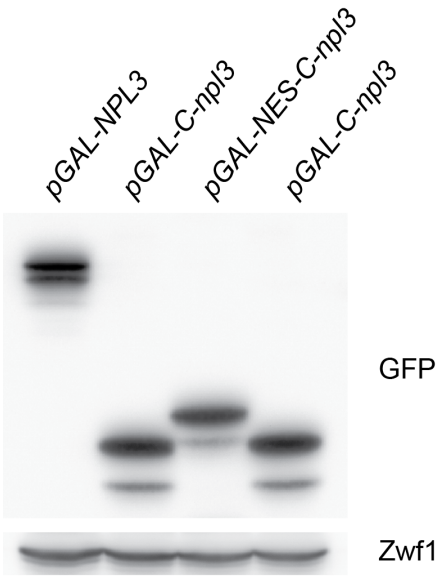

FIG S3. High copy plasmids encoding *NPL3* lead to a similar protein production. Expression levels of multicopy plasmids used in FIG 4 were determined in wildtype cells by western blot analyses with anti-GFP antibodies. Equal loading is shown by the detection of Zwf1.