

Appendix of the manuscript entitled:

The key protein of endosomal mRNP transport Rrm4 binds translational landmark sites of cargo mRNAs

**Lilli Olgeiser¹, Carl Haag¹, Susan Boerner², Jernej Ule^{3,4}, Anke Busch⁵, Janine Koepke⁶,
Julian König⁵, Michael Feldbrügge^{1, \$} and Kathi Zarnack^{2, \$}**

\$ shared corresponding authorship

¹ Heinrich Heine University Düsseldorf, Institute for Microbiology, Cluster of Excellence on Plant Sciences, 40204 Düsseldorf, Germany

² Buchmann Institute for Molecular Life Sciences (BMLS), Goethe University Frankfurt, Max-von-Laue-Str. 15, 60438 Frankfurt am Main, Germany

³ The Francis Crick Institute, Midland Road 1, Kings Cross, London NW1 1AT, UK

⁴ Department of Molecular Neuroscience, UCL Institute of Neurology, Queen Square, London, WC1N3BG, UK

⁵ Institute of Molecular Biology gGmbH, Ackermannweg 4, 55128 Mainz, Germany

⁶ Medical Clinic II (Molecular Pneumology), Justus Liebig University of Gießen, Excellence Cluster Cardio-Pulmonary System, Aulweg 130, 35392 Gießen, Germany

Table of content:

Appendix Table S1.....	02
Appendix Table S2.....	03
Appendix Table S3.....	04
Appendix Table S4	05

Appendix Table S1: *U. maydis* strains used in this study.
eGfp, enhanced Gfp. UMa, internal reference number.

Strain	Relevant genotype	Short description	Reference	UMa
AB33	<i>a2 Pnar:bW2bE1</i>	expression of active b heterodimer under control of the P _{nar1} promoter; strain grows filamentously upon changing the nitrogen source	Brachmann et al., 2001	133
AB33rrm4Δ	<i>rrm4Δ</i>	carries a deletion of <i>rrm4</i>	Becht et al., 2006	273
AB33rrm4G	<i>rrm4G</i>	expresses Rrm4 C-terminally fused to eGFP	Becht et al., 2006	274
AB33pab1G	<i>pab1G</i>	expresses Pab1 C-terminally fused to eGfp	König et al., 2009	389
AB33grp1Δ	<i>grp1Δ</i>	carries a deletion of <i>grp1</i>	This study	380
AB33grp1G	<i>grp1G</i>	expresses Grp1 C-terminally fused to eGfp	This study	911
AB33rrm4tR/grp1G	<i>grp1G/rrm4Δ</i>	co-expresses Rrm4 C-terminally fused to tagRfp and Grp1 C-terminally fused to eGfp	This study	2169
AB33pab1mC/grp1G	<i>pab1mC/grp1G</i>	co-expresses Pab1 C-terminally fused to mCherry and Grp1 C-terminally fused to eGfp	This study	2200
AB33pab1mC/rrm4G	<i>pab1mC/rrm4G</i>	co-expresses Pab1 C-terminally fused to mCherry and Rrm4 C-terminally fused to eGfp	König et al., 2009	554
AB33rrm4G/grp1Δ	<i>rrm4G/grp1Δ</i>	expresses Rrm4 C-terminally fused to eGfp and carries a <i>grp1</i> deletion	This study	880
AB33grp1G/rrm4Δ	<i>grp1G/rrm4Δ</i>	expresses Grp1 C-terminally fused to eGfp and carries a <i>rrm4</i> deletion	This study	1050
AB33pab1G/rrm4Δ	<i>pab1G/rrm4Δ</i>	expresses Pab1 C-terminally fused to eGfp and carries a <i>rrm4</i> deletion	König et al., 2009	472
AB33gfp	<i>gfp</i>	expresses <i>gfp</i> under control of the constitutive P _{ter} -promotor. The <i>gfp</i> construct is integrated ectopically into the <i>ipS</i> -locus.	Koepke et al., 2011	487
AB33rrm4mR123tR/grp1G	<i>grp1G/rrm4Δ</i>	co-expresses Rrm4 ^{mR123} C-terminally fused to tagRfp and Grp1 C-terminally fused to eGfp	This study	2012
AB33rrm4mR123tR/pab1G	<i>pab1G/rrm4Δ</i>	co-expresses Rrm4 ^{mR123} C-terminally fused to tagRfp and Pab1 C-terminally fused to eGfp	This study	1200
AB33 rrm4G/pab1mC/grp1Δ	<i>rrm4GT/pab1mC/grp1Δ</i>	co-expresses Rrm4 C-terminally fused to eGfp-tandem affinity purification tag and Pab1 C-terminally fused to mCherry, while carrying a <i>grp1</i> deletion	This study	2156

Appendix Table S2: *U. maydis* strains generated in this study.

UMa and pUMa, internal reference numbers for strains and plasmids, respectively.

Strain	UMa	Relevant genotype	Transformed plasmid	Locus	Progenitor strain
AB33grp1Δ	380	<i>grp1Δ</i>	pGrp1Δ-HygR (pUMa 848)	<i>grp1</i>	AB33
AB33grp1G	911	<i>grp1G</i>	pGrp1G-NatR (pUMa 1595)	<i>grp1</i>	AB33
AB33rrm4tR/grp1G	2169	<i>rrm4tR/grp1G</i>	pRrm4tR_G418R (pUMa 3004)	<i>rrm4</i>	AB33grp1G/rrm4Δ
AB33pab1mC/grp1G	2200	<i>pab1mC/grp1G</i>	pPab1mC_HygR (pUMa1208)	<i>pab1</i>	AB33grp1G
AB33rrm4G/grp1Δ	880	<i>rrm4G/grp1Δ</i>	pGrp1Δ-HygR (pUMa848)	<i>grp1</i>	AB33rrm4G
AB33grp1G/rrm4Δ	1050	<i>grp1G/rrm4Δ</i>	pRrm4Δ-HygR (pUMa1391)	<i>rrm4</i>	AB33grp1G
AB33rrm4mR123tR/grp1G	2012	<i>rrm4mR123tR/grp1G</i>	pRrm4mR123tR_G418R (pUMa2849)	<i>rrm4</i>	AB33grp1G/rrm4Δ
AB33rrm4mR123tR/pab1G	1200	<i>rrm4mR123tR/pab1G</i>	pRrm4mR123tR_G418R (pUMa2849)	<i>rrm4</i>	AB33pab1G
AB33 rrm4G/pab1mC/grp1Δ	2156	<i>rrm4G/grp1Δ/pab1mC</i>	pPab1mC-G418R (pUMa3052)	<i>pab1</i>	AB33 rrm4G/grp1Δ

Appendix Table S3: Plasmids generated in this study for *U. maydis*.

pUMa, internal plasmid reference number.

Plasmid	pUMa	Resistance cassette	Short description
pGrp1Δ-HygR	pUMa 848	HygR (SfiI-insert of MF1hs)	Plasmid for generating deletion mutants of <i>grp1</i> . The hygromycin resistance cassette is flanked by the regions 0.9 kb upstream and 0.8 kb downstream of <i>grp1</i> . The flanking regions were amplified by PCR (UF: oSL217 ATGGTCTCGACTTGCGCG, oSL218 ttcggccatctaggccACGTAAACTTTGGCGGCC), (DF: oSL219, oSL220) using UM521 wild type DNA.
pGrp1G-NatR	pUMa 1595	NatR (SfiI-insert of pMF5-1n)	Plasmid for generating eGfp-fusions of <i>grp1</i> . The eGfp cassette, containing the T _{nos} terminator and the nourseothricin resistance cassette is flanked by a 2.3 kb upstream region, containing the entire <i>grp1</i> ORF, and a 0.8 kb downstream region. Both regions were amplified by PCR using genomic UM521 wild type DNA as template (UF: oSL865 GTCATTTAAATGGTCTCGACTTGCGCG, oSL863 CCATGGTGGCCGCTTGGCCGCCTGGCTCTGTCCGTTGTATC; 2.3 kb); DF (oSL864 TGAGGCCTGAGTGGCCACGACTCTTGTCTCGGGGC, oSL866 GCTATTTAAATGTAGCGTTGCCACTCAGC; 0.8 kb).
pRrm4tR_G418R	pUMa 3004	G418R (SfiI-insert of pMF1-g)	Plasmid for generating tagRfp-3x myc tag fusions of tagRfp of <i>rrm4</i> . The tagRfp cassette contains the T _{nos} terminator and a G418 resistance cassette. It is flanked by a 3.2 kb upstream region, containing the entire 2.4 kb <i>rrm4</i> ORF, and the 2 kb immediately downstream of <i>rrm4</i> . The plasmid is a derivate of pRrm4G-NatR (Becht et al., 2006), with eGfp being exchanged for tagRfp.
pRrm4mR123tR_G418R	pUMa2849		Plasmid for generating tagRfp-3x myc tag fusions of <i>rrm4</i> ^{mR123} . pRrm4tR_G418R, but carrying mutations in the RNP1 region of each RRM.
pPab1mK-G418R	pUMa3052		Plasmid for generating mKate ₂ -HA tag fusions of <i>pab1</i> . The mKate ₂ cassette contains the T _{nos} terminator and a G418 resistance cassette. The cassette is flanked by a 3 kb upstream region, containing the 2 kb <i>pab1</i> ORF, and the 1 kb downstream of <i>pab1</i> . The plasmid is a derivate of pPab1G-NatR (König et al., 2009).

Appendix Table S4: Plasmids used for yeast three-hybrid system.

pUMa, internal plasmid reference number.

Plasmid	pUMa	Gene	Short description
pACTII-Ade	399		Plasmid for the expression of hybrid proteins. Contains a Gal4 activation domain, to which sequences of interest can be fused. For positive selection this plasmid carries the <i>LEU2</i> and <i>ADE2</i> auxotrophy markers. Served as vector control
pRrm4-Gfp-pACTII-Ade	427	<i>rrm4</i>	Plasmid for expressing an Rrm4 hybrid protein, fused N-terminally to a Gal4 activation domain and C-terminally to eGfp. The plasmid is derived from pACTII-Ade (König et al., 2007).
pIII MS2-5	494		Plasmid for expressing RNA-hybrids. Contains a <i>RPR1</i> leader, followed by two MS2 coat protein binding sites, a junk sequence flanked by a ClaI & AscI restriction sites, and a <i>RPR1</i> terminator. For positive selection this plasmid carries the <i>URA3</i> auxotrophy marker. Sequences can be cloned via the ClaI/ AscI restriction sites. Served as vector control.
pRrm4-mR3-Gfp-pACTII-Ade	526	<i>rrm4</i>	Plasmid for expressing the Rrm4 ^{mR3} hybrid protein. Similar to pRrm4-gfp-pACTII-Ade, but carries a mutation in the RNP1 region of RRM3 (König et al., 2007)
pIII-MS2-5-Sel13	550		Plasmid for expressing the RNA-hybrid containing the SELEX aptamer A1. SELEX library sequences were inserted into pIII MS2-4 via ClaI/AscI (König et al., 2007).
pRrm4-mR1-Gfp-pACTII-Ade	581	<i>rrm4</i>	Plasmid for expressing the Rrm4 ^{mR1} hybrid protein. Similar to pRrm4-gfp-pACTII-Ade, but carries a mutation in the RNP1 region of RRM1 (König et al., 2007).
pRrm4-mR12-Gfp-pACTII-Ade	591	<i>rrm4</i>	Plasmid for expressing the Rrm4 ^{mR12} hybrid protein. Similar to pRrm4-gfp-pACTII-Ade, but carries mutations in the RNP1 regions of RRM1 and RRM2.
pRrm4-mR2-Gfp-pACTII-Ade	594	<i>rrm4</i>	Plasmid for expressing the Rrm4 ^{mR2} hybrid protein. Similar to pRrm4-gfp-pACTII-Ade, but carries a mutation in the RNP1 region of RRM2 (König et al., 2007)
pIII MS2-5 A1- mUAUG	1937		Plasmid for expressing the RNA-hybrid containing SELEX aptamer A1 ^{mUAUG} . TATGC was mutated to TCTCA by inverse PCR using oligos oRL750 GTGTGAAAGATCCGAAGTTTCTCACGGCAGCGCTGGCGCTG & oRL751 CAGCGCCAGCGGTGCCGTGAGAACTTCGGATCTTTCACAC and pIII-MS2-5-Sel13 as template.