Supplemental Material

Supplementary Figure Legends.

Figure S1. Dim2ΔC does not bind RNA but does bind Nob1. (A) A C-terminal truncation of Dim2 (Dim2ΔC), in which the KH domain is deleted, does not bind RNA, as seen before (Vanrobays et al. 2008). (B) Dim2ΔC binds Nob1.

Figure S2. Structure of Dim2's KH and KH-like domains. (A) Alignment of different KH domains with Dim2's KH and KH-like domains. The conserved secondary structure elements of the KH domain are indicated above the alignment and the conserved GxxG loop is highlighted in bold red as well as below the alignment. Other highly conserved residues are indicated in red. Hydrophobic signature residues conserved within the KH fold are highlighted in yellow and charged conserved residues are highlighted in blue. The mutated residues (HR/E and DDD/K) are highlighted in green. (B) Predicted structure of Dim2's KH-like and KH domains based on the structure of archeal Dim2 (pdb ID 2E3U, (Jia et al. 2007)). The NSWT sequence, which replaces the canonical GxxG loop, is shown in brown, the three aspartates mutated to lysines are shown in green, and the positively charged histidine and arginine are shown in purple. (C) The structure of archeal Dim2 bound to RNA [(Jia et al. 2010), pdb ID 3AEV]. As in (B) the residues that correspond to the GxxG, HR/E and DDD/K mutations in yeast Dim2 are highlighted in brown, purple, and green, respectively.

Figure S3. The HR/E, DDD/K and GxxG mutants bind rRNA. Gel-shift experiments were quantified and fit with the Michaelis-Menten Equation to give $K_{1/2}$ values of 0.8 μM for wild type Dim2 (●), 0.6 μM for the DDD/K mutant (●), 0.8 μM for the HR/E mutant (●) and 1.4 μM for the GxxG mutant (●), respectively. Note that the two-fold higher $K_{1/2}$ value for the GxxG mutant can be explained, at least partially, by the additional degradation products seen in that mutant (Figure 3), which contribute to the determined protein concentration but likely not to RNA binding.

Supplemental References

Jia, M.Z., Horita, S., Nagata, K., and Tanokura, M. 2010. An archaeal Dim2-like protein, aDim2p, forms a ternary complex with a/eIF2 alpha and the 3' end fragment of 16S rRNA. *J Mol Biol* **398**(5): 774-785.

Jia, M.Z., Ohtsuka, J., Lee, W.C., Nagata, K., and Tanokura, M. 2007. Crystal structure of Dim2p: a preribosomal RNA processing factor, from Pyrococcus horikoshii OT3 at 2.30 A. *Proteins* **69**(2): 428-432.

Vanrobays, E., Leplus, A., Osheim, Y.N., Beyer, A.L., Wacheul, L., and Lafontaine, D.L. 2008. TOR regulates the subcellular distribution of DIM2, a KH domain protein required for cotranscriptional ribosome assembly and pre-40S ribosome export. *RNA* **14**(10): 2061-2073.

Figure S1

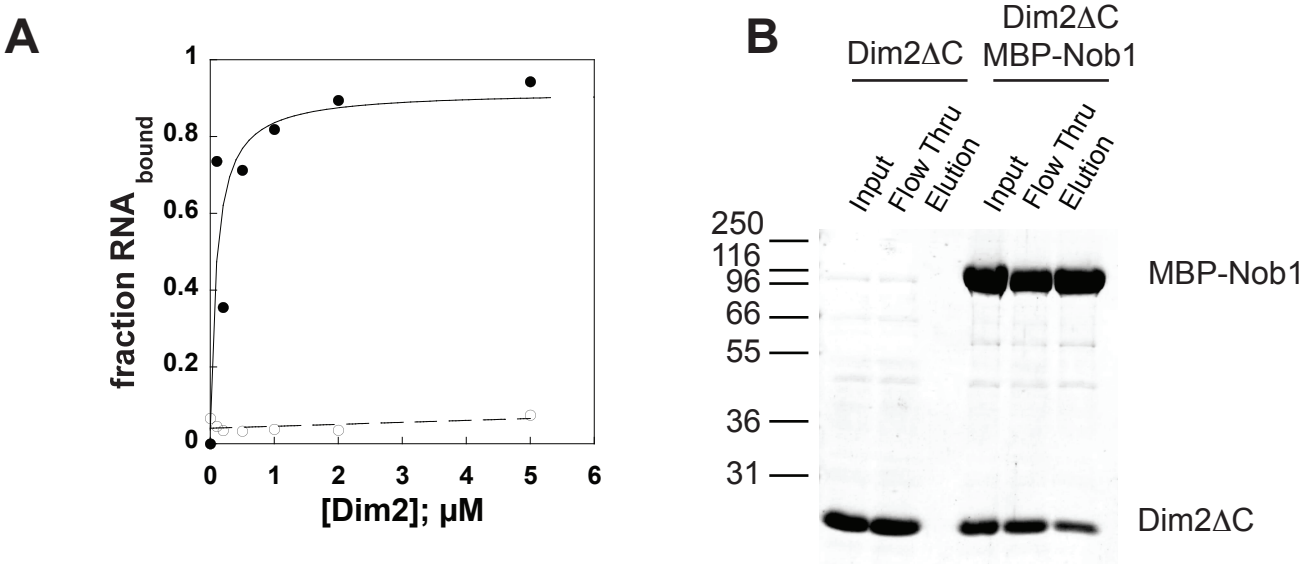
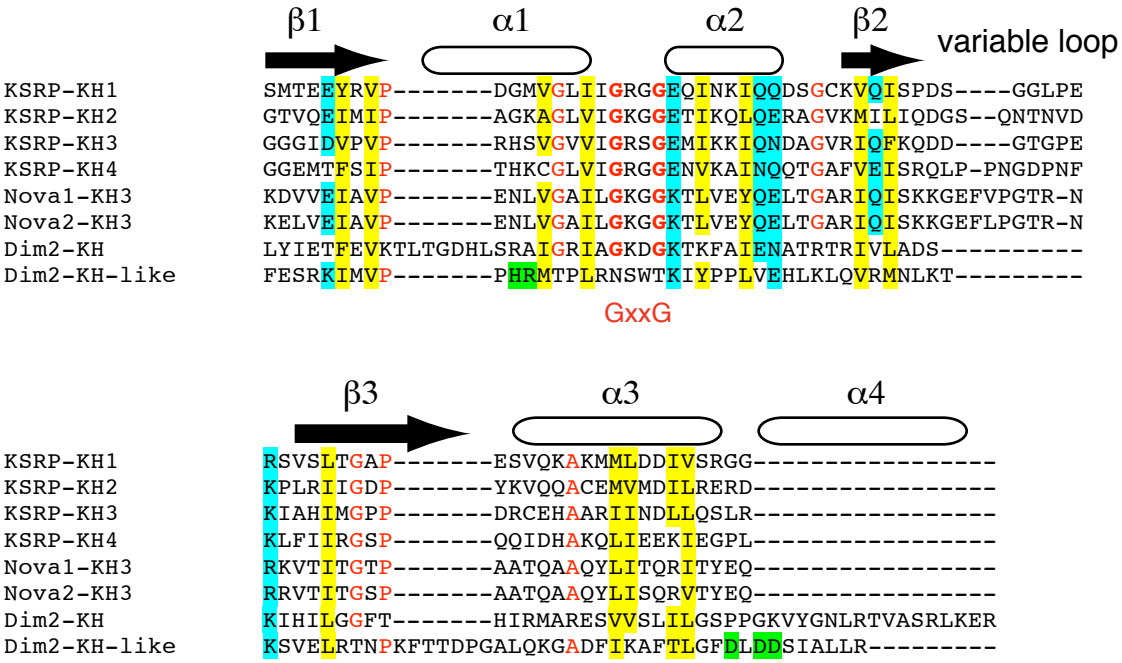

A
fraction RNA bound
1
0.8
0.6
0.4
0.2
0
0
1
2
3
4
5
6
[Dim2]; μM
B
Dim2ΔC
Dim2ΔC
MBP-Nob1
Input
Flow Thru
Elution
Input
Flow Thru
Elution
250
116
96
66
55
36
31
MBP-Nob1
Dim2ΔC

Figure S2

A



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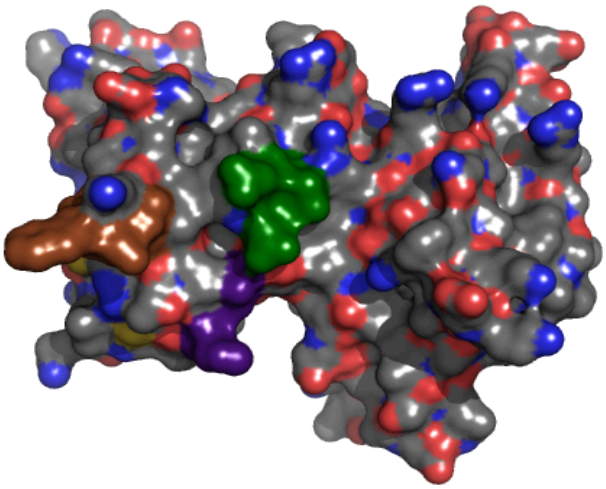

C

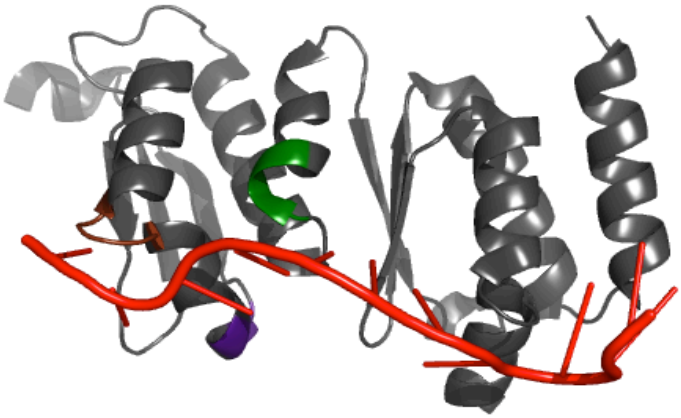

Figure S3