Structure and RNA-binding properties of the Not1–Not2–Not5 module of the yeast Ccr4–Not complex

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Supplementary Information

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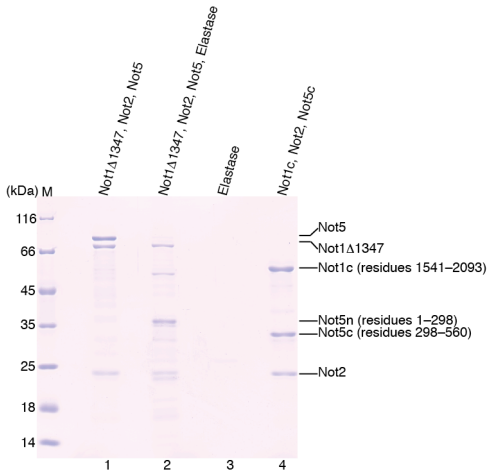

Supplementary Figure 1 Identification of the core of the *S. cerevisiae* Not1–Not2–Not5 interaction. The 12% SDS PAGE gel shows in lane 1 the larger complex that we initially purified (Not1 starting at residue 1348, Not2 f.l. and Not5 f.l.). Lane 2 shows the result of the limited proteolysis of the complex in lane 1 with elastase. Lane 3 shows the protease alone as a control. Lane 4 shows the complex reconstituted with the minimal interacting regions of Not1, Not2 and Not5 that yielded diffracting crystals (Not1c, Not2 and Not5c).

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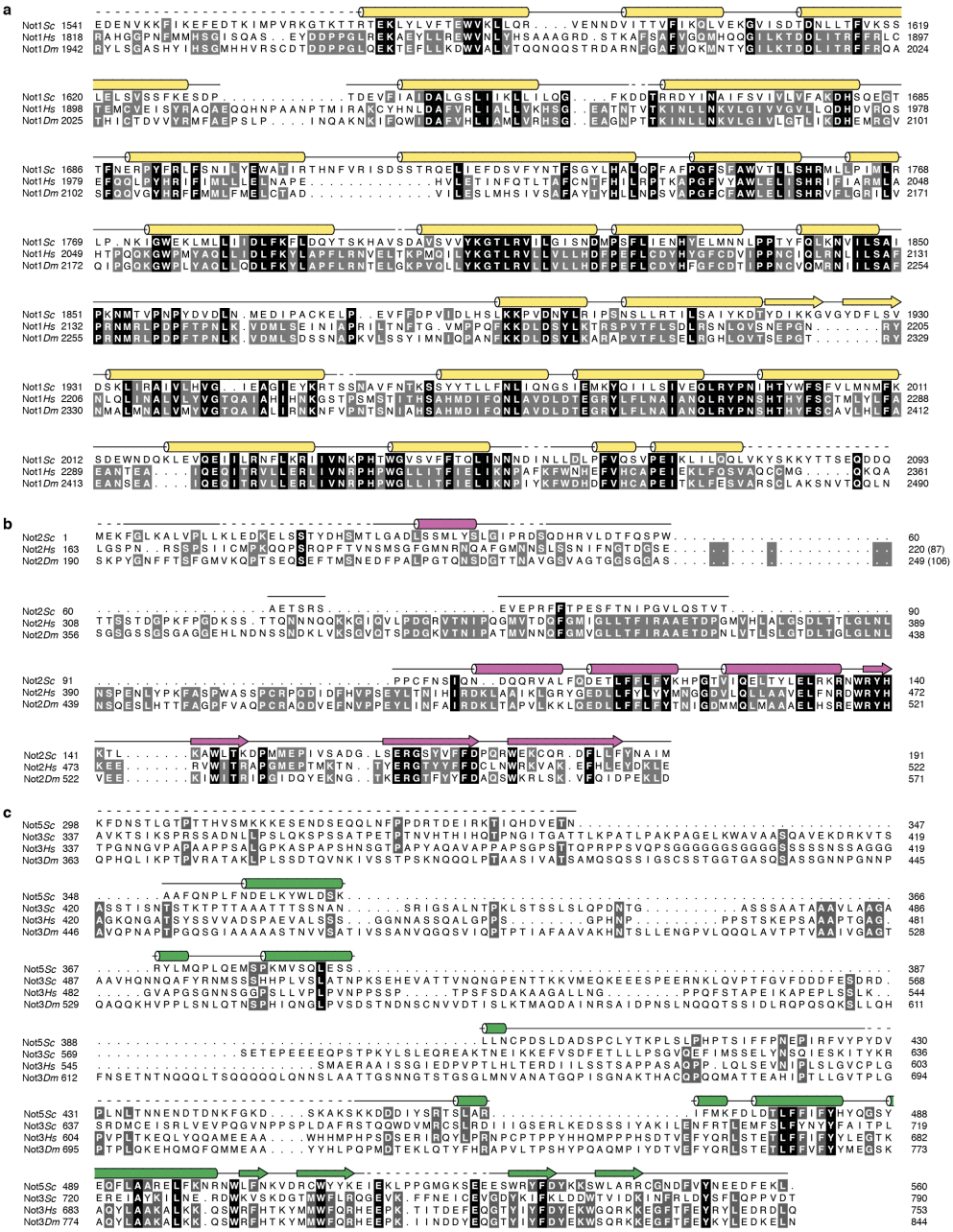
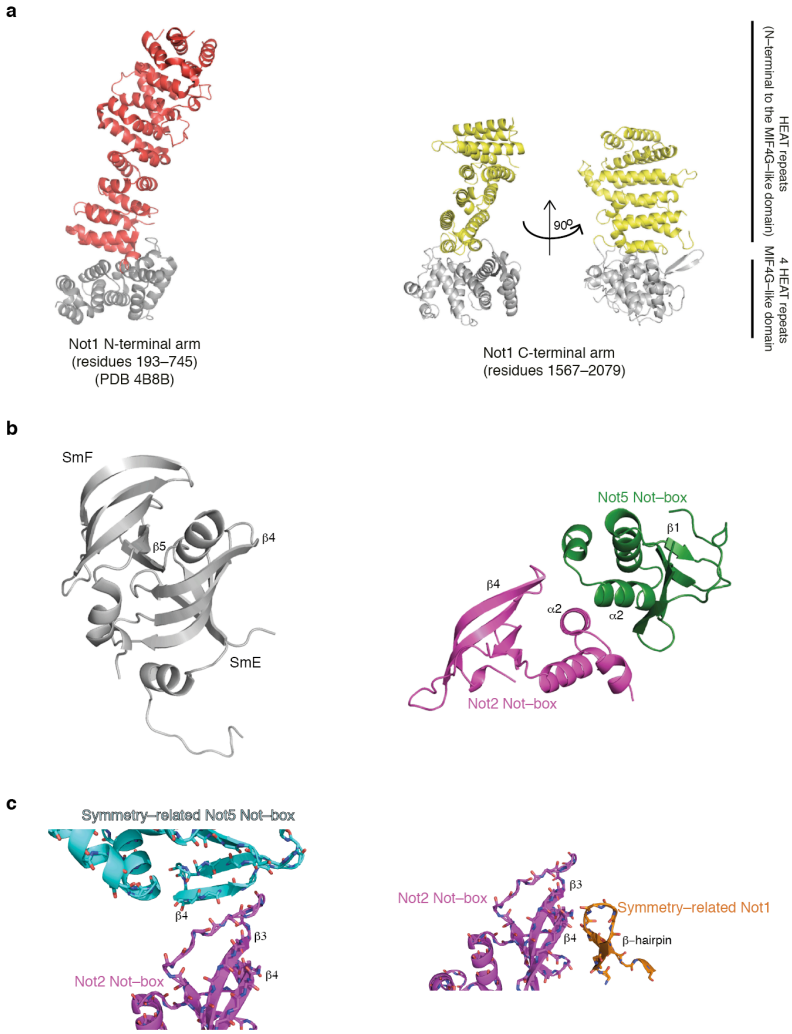
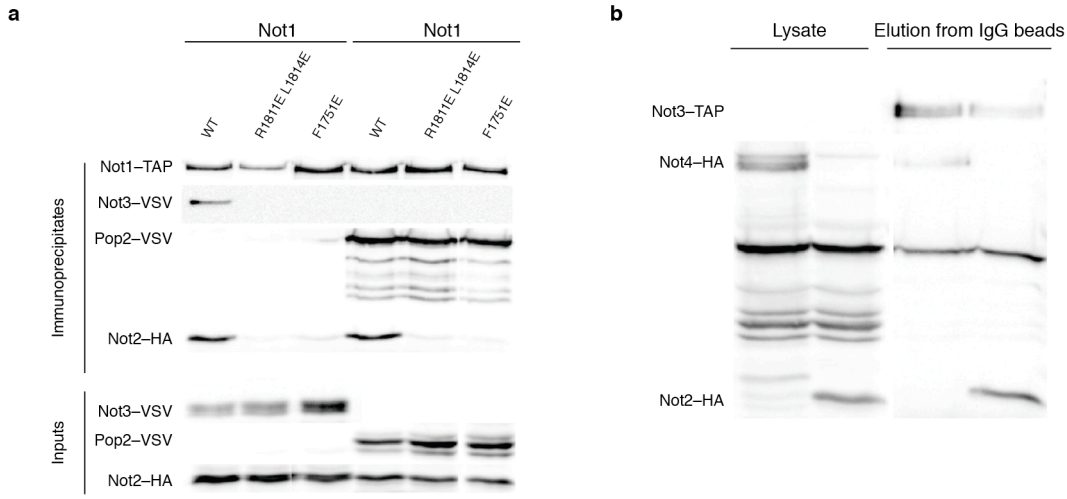


Supplementary Figure 2 Structure–based sequence alignments of Not1c, Not2 and Not5c. The sequence alignments include the polypeptides of *S. cerevisiae* (*Sc*) Not1, Not2 and Not5 we crystallized in a complex and their orthologues from *H. sapiens* (*Hs*) and *D. melanogaster* (*Dm*). Not5*Sc* is a homologue of Not3. Secondary structure elements are shown above the sequences and colored in yellow for Not1 **(a)**, magenta for Not2 **(b)** and green for Not5 (**c**). Straight lines refer to extended regions and dotted lines refer to disordered regions in the structure of the *S. cerevisiae* complex. Sequence conservation is highlighted in shades of gray.



Supplementary Figure 3 The HEAT and Sm folds of Not1–Not2–Not5. **(a)** Comparison of the HEAT–repeat architecture in the C–terminal arm of Not1 (on the right) and the N–terminal arm of Not1 (on the left, PDB code 4B8B[1]). The MIF4G–like folds are shown in gray. The longer HEAT–repeat units perpendicular to the MIF4G–like folds are shown in yellow and red for the C-terminal and N-terminal arms, respectively. **(b)** Dimerization properties of Not-box domains. The subcomplex of SmF and SmE (PDB code 2Y9A[2]) is shown on the left in gray. The β4 strand of one monomer (SmE) interacts with strand β5 of the other monomer (SmF). On the right, dimerization of Not2–Not5 leaves strand β4 exposed to solvent. **(c)** Lattice contacts are reminiscent of Sm–Sm interactions. In the upper panel, the loop between strands β2 and β3 of a Not2 molecule (in magenta) has an extended conformation and interacts both with the β4 strand of a symmetry–related Not5 molecule (in cyan). In the lower panel, the β4 strand of a Not2 molecule (in magenta) interacts with the β–hairpin of a symmetry–related Not1 molecule (in orange).



Supplementary Figure 4 *In vivo* interactions of Not proteins **(a)** Immunoprecipitation of Not1 from yeast strains harbouring a TAP tagged Not1 wild-type (WT) or indicated mutants and carrying tagged chromosomal variant of Not2 and Not3 (BSY1230), or Not2 and Pop2/Caf1 (BSY1231). Coimmunoprecipitation of Not2–HA, Not3–VSV or Pop2–VSV was assayed by western blotting. As a control, the presence of the tagged protein in the starting extracts was also assayed. **(b)** Immunoprecipitation of Not3 from yeast strains carrying tagged chromosomal variant of Not3, Pop2/Caf1 and Not4 (BSY1240), or Not3, Caf1/Pop2 and Not2 (BSY1242). Coimmunoprecipitation of Not2–HA or Not4–HA was assayed by western blotting. As a control, the level of the tagged protein in the starting extracts was also assayed.

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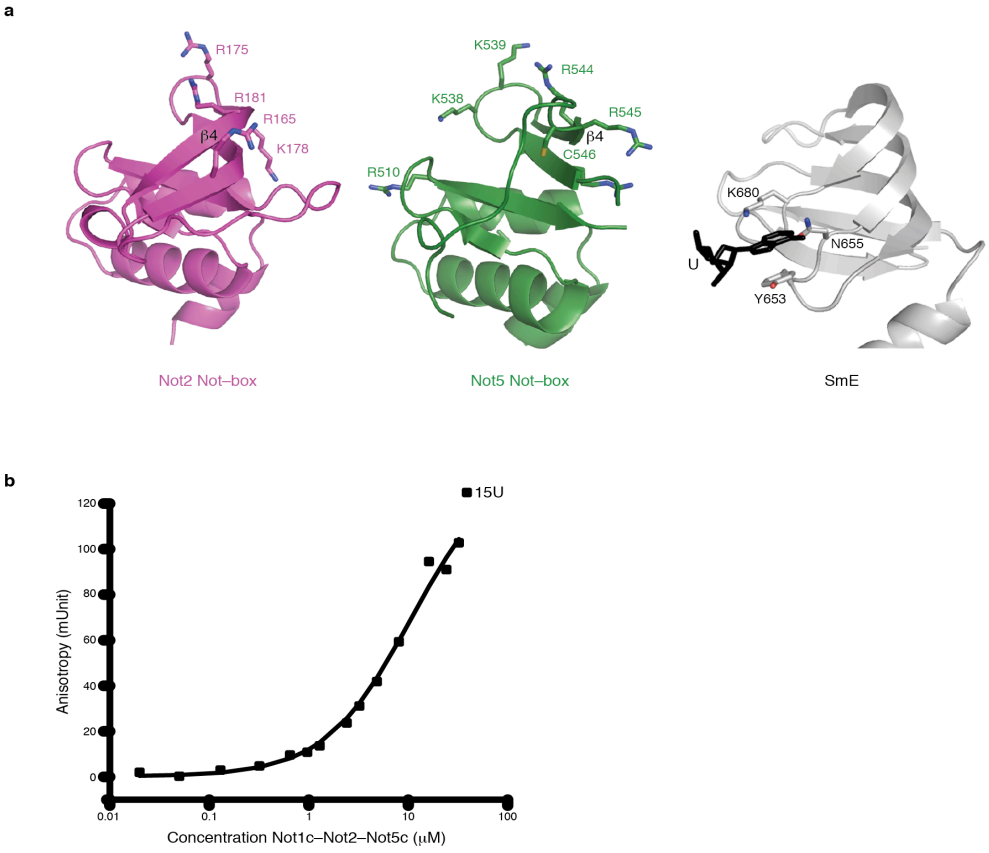

Supplementary Figure 5 Protein and RNA interactions at the Not-boxes **(a)** Surface features of the Not2 and Not5 Not-boxes. On the left is the structure of Not2, showing Arg165 (putative ADA2–binding residue) as well as positively charged residues at a similar position as in Not5. In the central panel is the Not-box of Not5, in the same orientation, showing the uridine–crosslinked residue Cys546 as well as the surrounding positively charged residues (as in **Fig. 5d**). On the right is the structure of RNA–bound SmE (U4 snRNP, PDB code 2Y9A[2]) oriented in a similar view as the structures in the left and central panels (after optimal superimposition), showing a bound uridine nucleotide (in black). **(b)** Quantification of the RNA–binding properties of Not1c–Not2–Not5c by fluorescence anisotropy. The K_d of the Not1c–Not2–Not5c complex to 6–FAM–labeled U_{15} RNA under these conditions was found to be 9.47±0.95 μM.

Reference

1 Basquin, J. *et al.* Architecture of the Nuclease Module of the Yeast Ccr4–Not Complex: the Not1–Caf1–Ccr4 Interaction. *Mol Cell* **48**, 207–218 (2012).

2. Leung, A. K. W., Nagai, K. & Li, J. Structure of the spliceosomal U4 snRNP core domain and its implication for snRNP biogenesis. *Nature* **473**, 536–539 (2011).

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