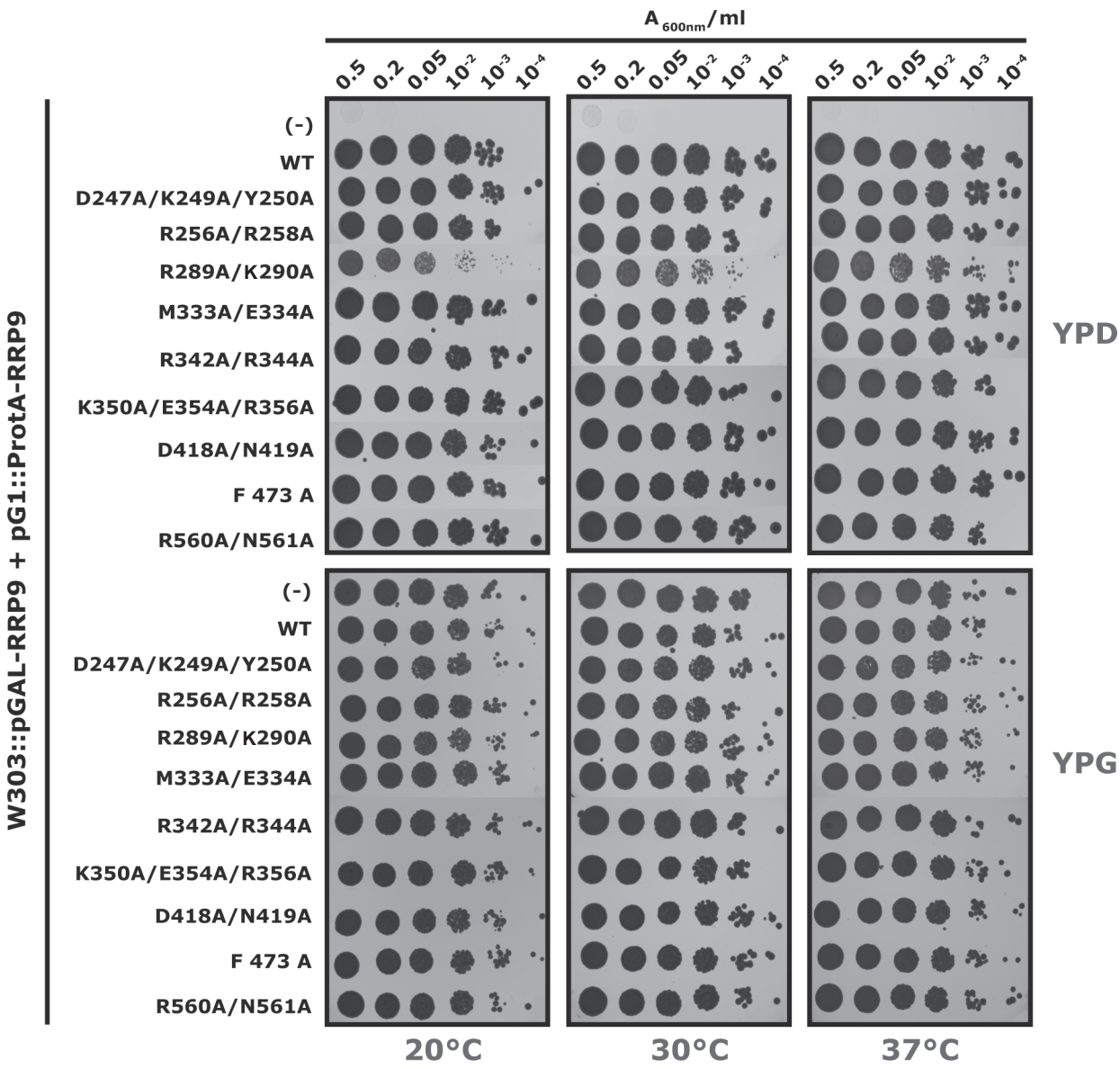
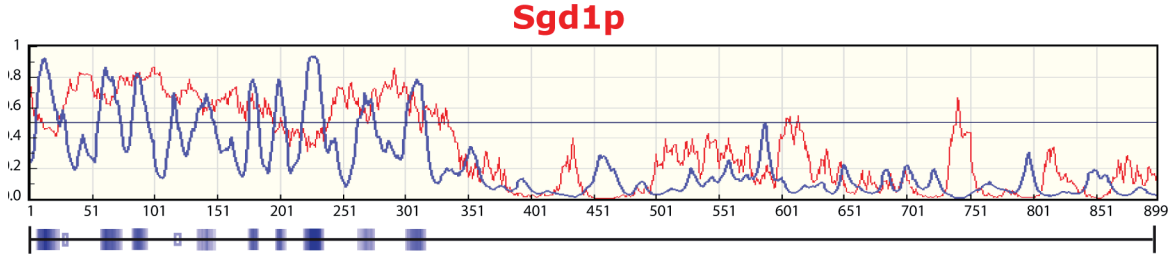
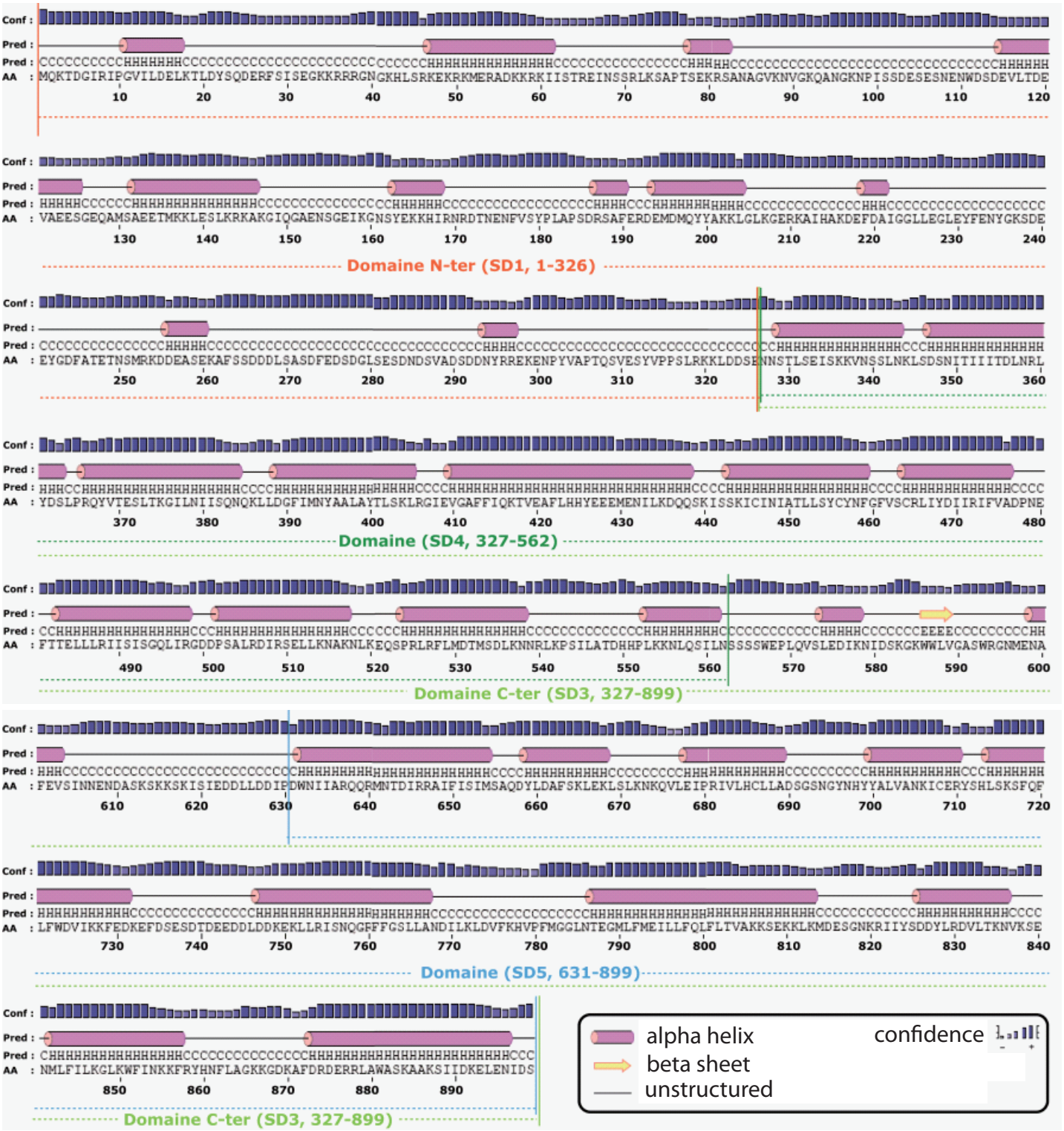


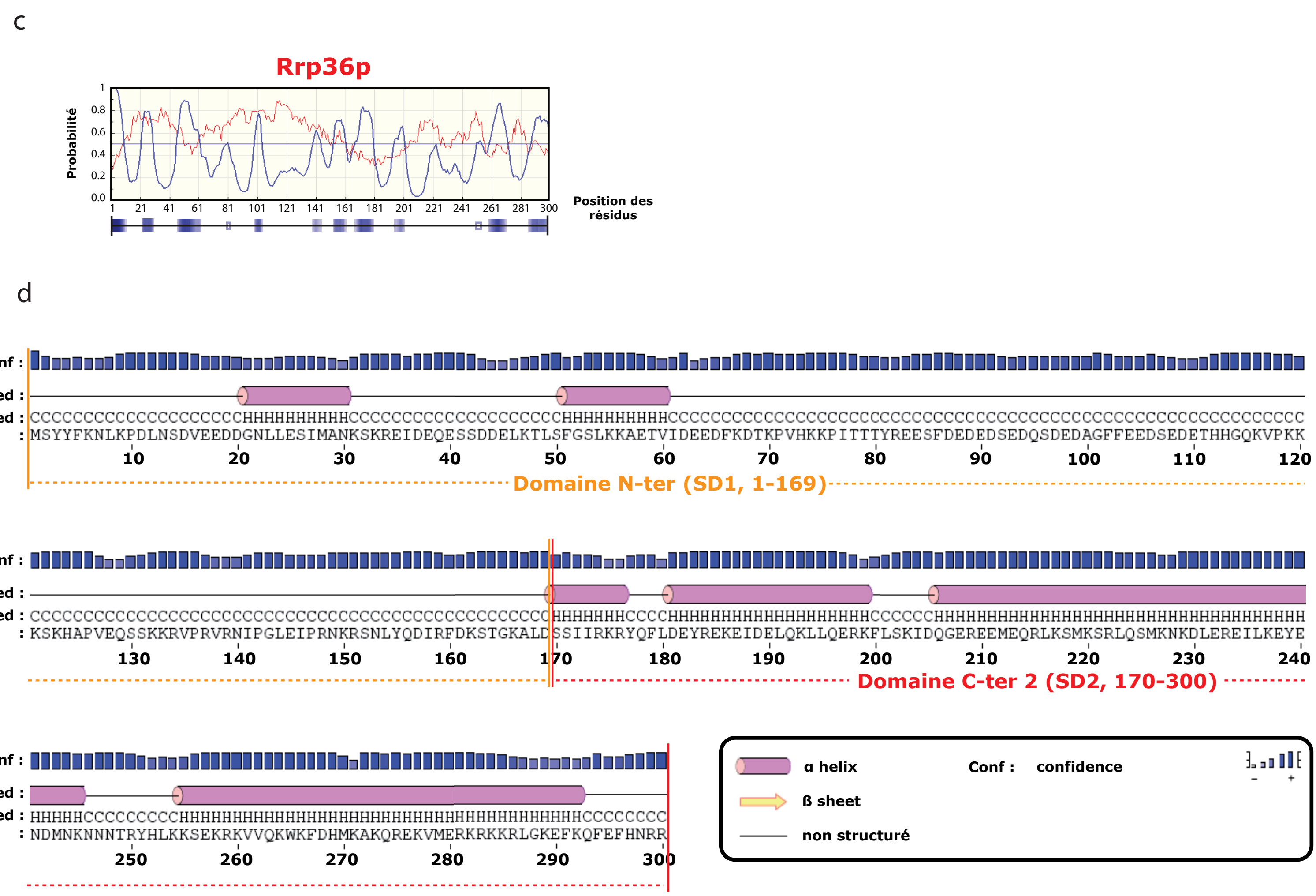
SUPP. FIGURE 1

a


Sgd1p

b

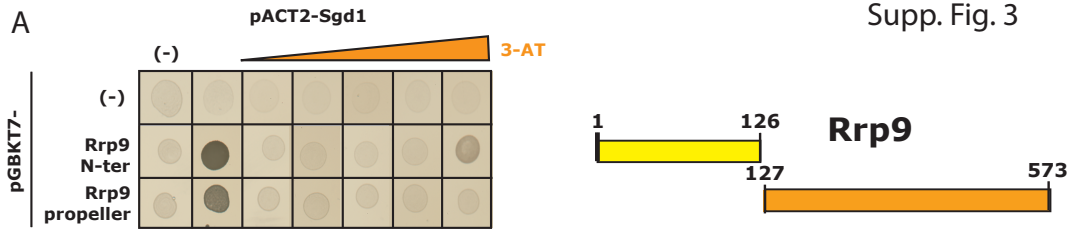

Domaine N-ter (SD1, 1-326)
Domaine (SD4, 327-562)
Domaine C-ter (SD3, 327-899)
Domaine (SD5, 631-899)
Domaine C-ter (SD3, 327-899)
alpha helix
beta sheet
unstructured
confidence



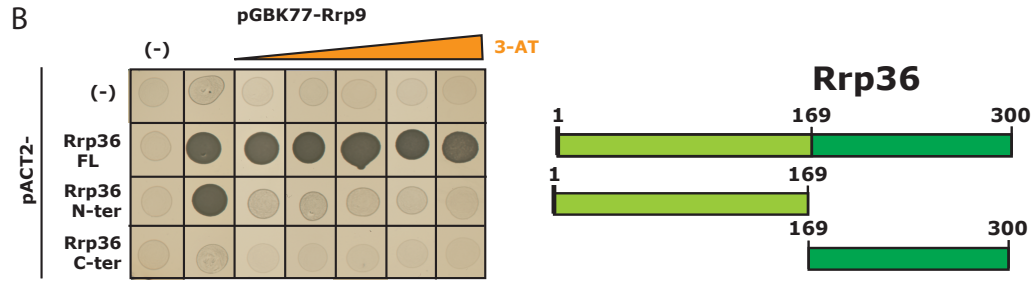
c
Rrp36p
Probabilité
1
0.8
0.6
0.4
0.2
0.0
1 21 41 61 81 101 121 141 161 181 201 221 241 261 281 300
Position des résidus
d
Conf :
Pred :
Pred : CCCCCCCCCCCCCCCCCCCCHHHHHHHHHHCCCCCCCCCCCCCCCCCCCCHHHHHHHHHHCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
AA : MSYYFKNLKPDLNSDVEEDDGNLLESIMANKSKREIDEQESSDDELKTLSFGSLKKAETVIDEEDFKDTKPVHKKPITTTYREESFDEDEDSEDQSDEDAGFFEEDSEDETHHGQKVPKK
10 20 30 40 50 60 70 80 90 100 110 120
Domaine N-ter (SD1, 1-169)
Conf :
Pred :
Pred : CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCHHHHHHHCCCHHHHHHHHHHHHHHHHHHHCCCCCHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH
AA : KSKHAPVEQSSKKRVPRVRNIPGLEIPRNKRSNLYQDIRFDKSTGKALDSSIIRKRYQFLDEYREKEIDELQKLLQERKFLSKIDQGEREEMEQRLKSMKSRLQSMKNKDLEREILKEYE
130 140 150 160 170 180 190 200 210 220 230 240
Domaine C-ter 2 (SD2, 170-300)
Conf :
Pred :
Pred : HHHHHCCCCCCCCCHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHCCCCCCCC
AA : NDMNKNNNTRYHLKKSEKRKVVQKWKFDHMKAKQREKVMERKRKKRLGKEFKQFEFHNRR
250 260 270 280 290 300
α helix
ß sheet
non structuré
Conf : confidence
–
+

SUPP. FIG. 2

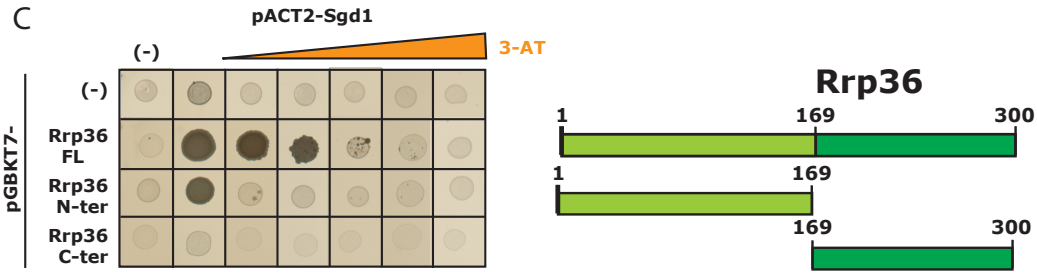
A


pACT2-Sgd1
(-)
3-AT
pGBKT7-
(-)
Rrp9 N-ter
Rrp9 propeller
Rrp9
1
126
127
573

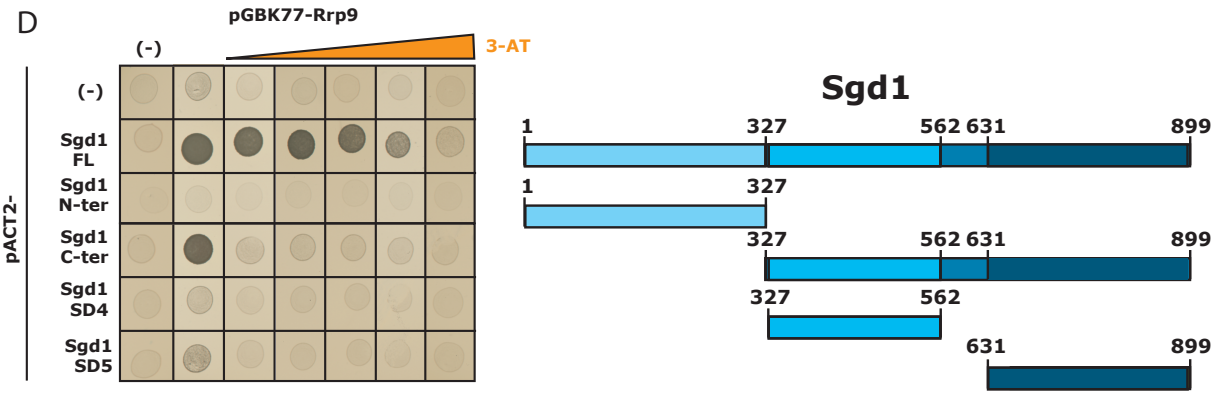
B


pGBK77-Rrp9
(-)
3-AT
pACT2-
(-)
Rrp36 FL
Rrp36 N-ter
Rrp36 C-ter
Rrp36
1
169
300
1
169
169
300

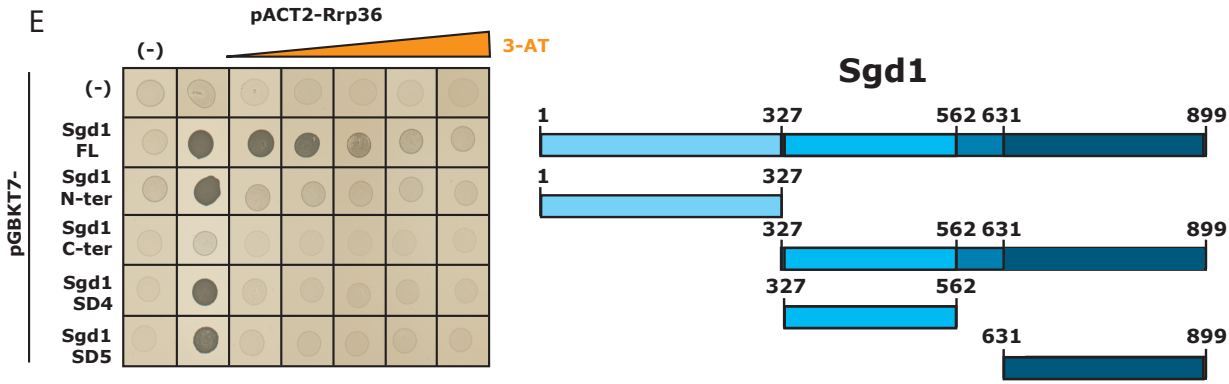
C


pACT2-Sgd1
(-)
3-AT
pGBKT7-
(-)
Rrp36 FL
Rrp36 N-ter
Rrp36 C-ter
Rrp36
1
169
300
1
169
169
300

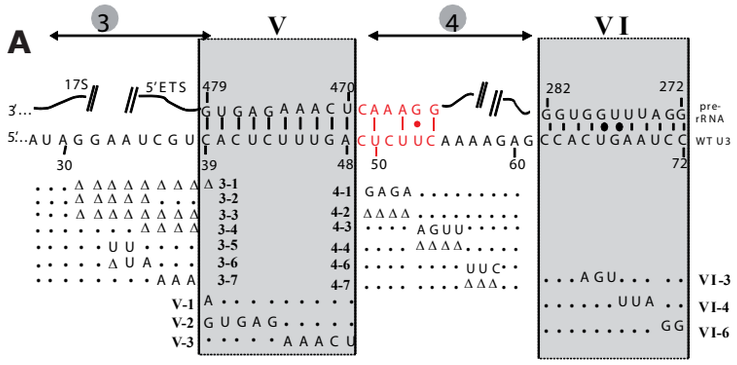
D

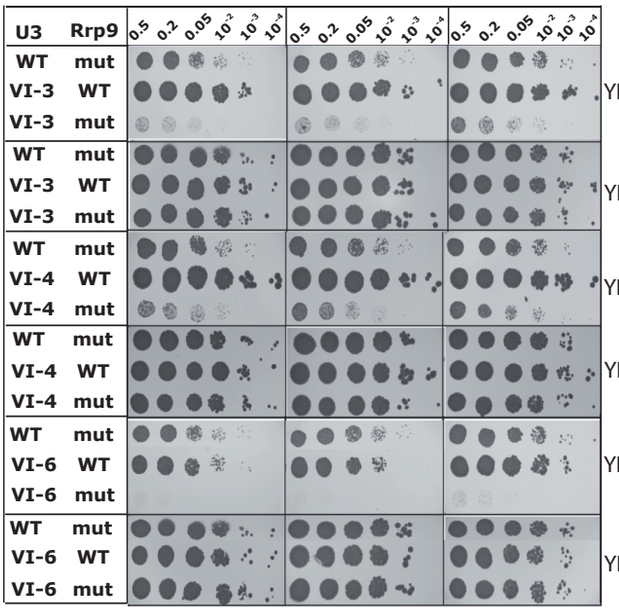

pGBK77-Rrp9
(-)
3-AT
pACT2-
(-)
Sgd1 FL
Sgd1 N-ter
Sgd1 C-ter
Sgd1 SD4
Sgd1 SD5
Sgd1
1
327
562
631
899
1
327
327
562
631
899
327
562
631
899

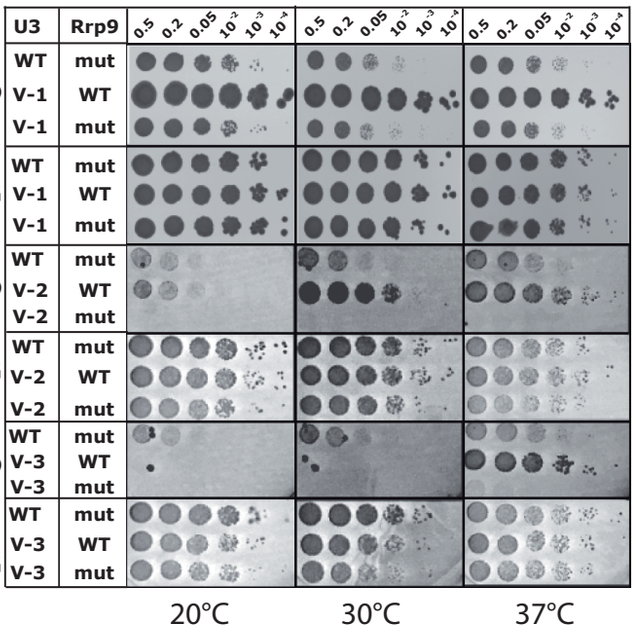
E

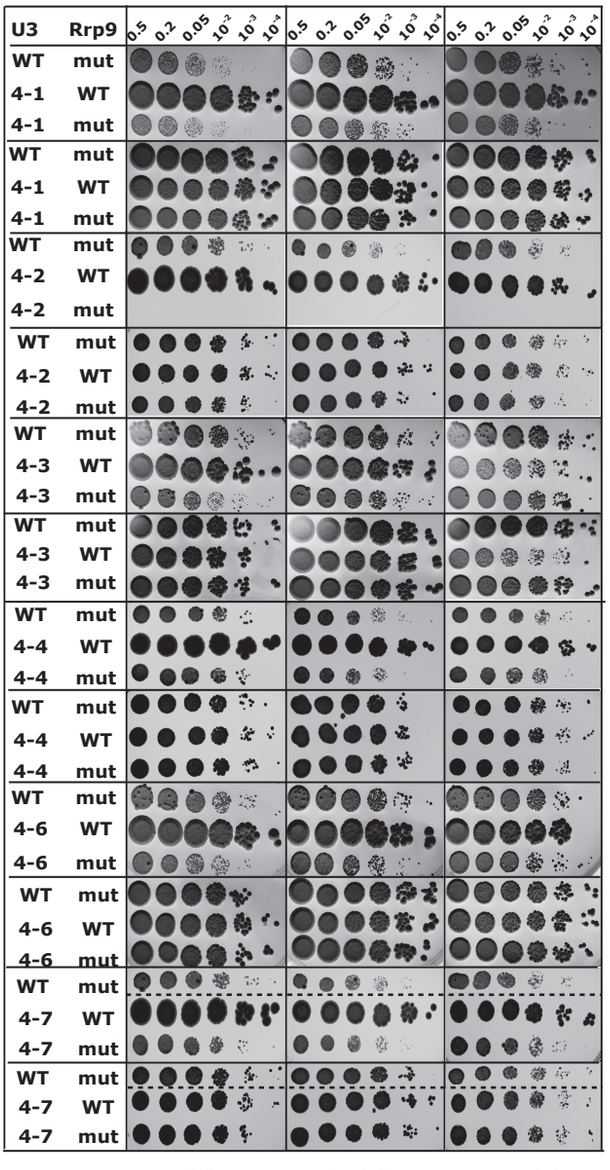

pACT2-Rrp36
(-)
3-AT
pGBKT7-
(-)
Sgd1 FL
Sgd1 N-ter
Sgd1 C-ter
Sgd1 SD4
Sgd1 SD5
Sgd1
1
327
562
631
899
1
327
327
562
631
899
327
562
631
899

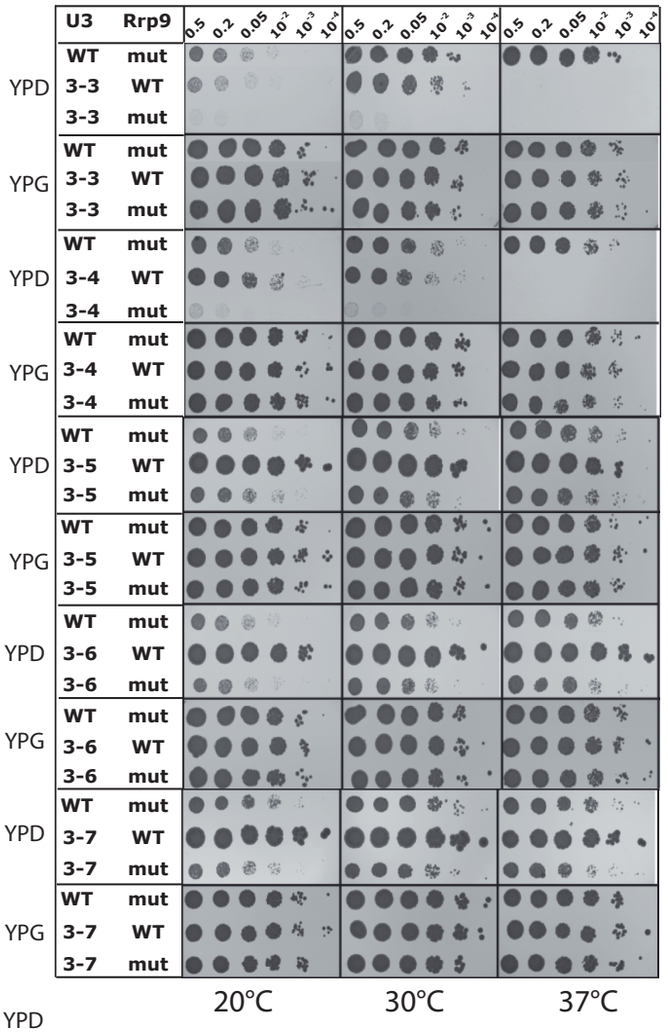
SUPP. FIG. 4

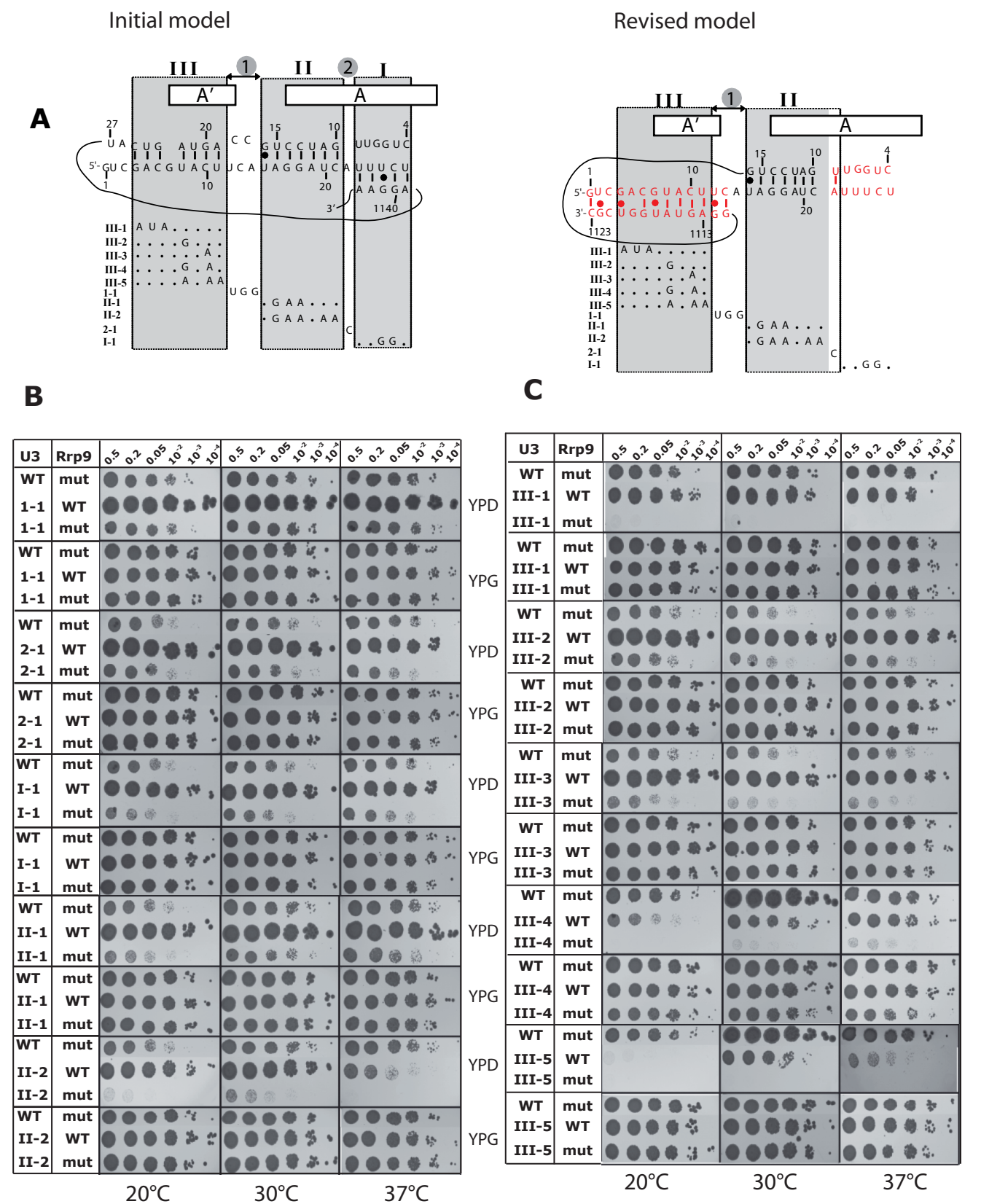

A
3
V
4
VI
17S
5'ETS
pre-rRNA
WT U3
V-1
V-2
V-3
VI-3
VI-4
VI-6


B
U3
Rrp9
WT mut
VI-3 WT
VI-3 mut
VI-4 WT
VI-4 mut
VI-6 WT
VI-6 mut
0.5 0.2 0.05 10-2 10-3 10-4
20°C
30°C
37°C


C
U3
Rrp9
WT mut
V-1 WT
V-1 mut
V-2 WT
V-2 mut
V-3 WT
V-3 mut
YPD
YPG
0.5 0.2 0.05 10-2 10-3 10-4
20°C
30°C
37°C


D
U3
Rrp9
WT mut
4-1 WT
4-1 mut
4-2 WT
4-2 mut
4-3 WT
4-3 mut
4-4 WT
4-4 mut
4-6 WT
4-6 mut
4-7 WT
4-7 mut
0.5 0.2 0.05 10-2 10-3 10-4
20°C
30°C
37°C

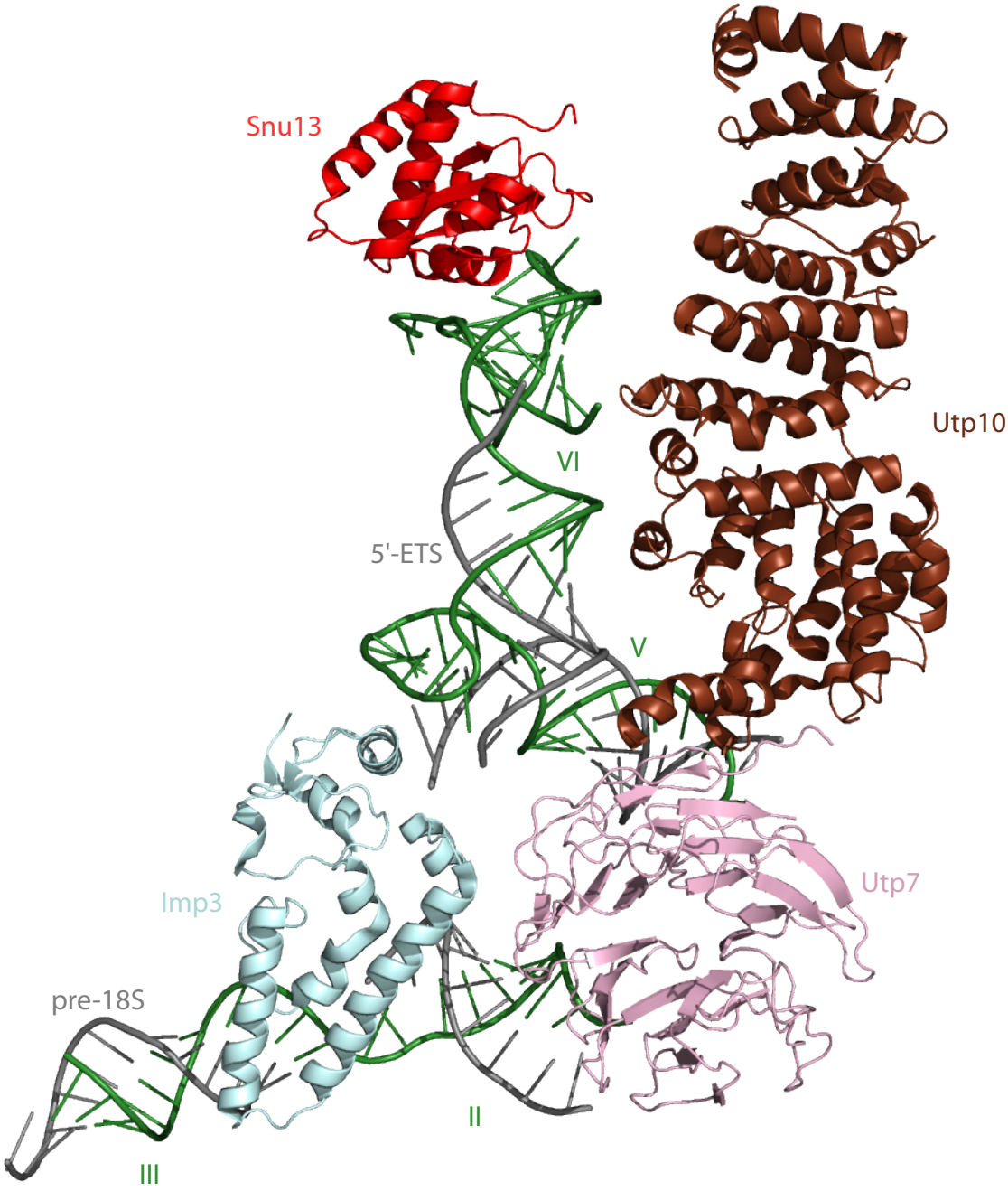

E
U3
Rrp9
WT mut
3-3 WT
3-3 mut
3-4 WT
3-4 mut
3-5 WT
3-5 mut
3-6 WT
3-6 mut
3-7 WT
3-7 mut
YPD
YPG
0.5 0.2 0.05 10-2 10-3 10-4
20°C
30°C
37°C


Initial model
Revised model
A
B
C
U3
Rrp9
WT
mut
1-1
2-1
I-1
II-1
II-2
III-1
III-2
III-3
III-4
III-5
YPD
YPG
20°C
30°C
37°C

SUPP. FIG. 5


Utp10
Snu13
Utp10
VI
5'-ETS
Nop1
Nop58
Utp7
Utp11
V
Nop56
U3
Imp3
Nop1
Sgd1
Snu13
II-III
Rrp36
R289
pre-18S
Rrp9
Utp24

Supp. Fig. 6



Supp. Fig. 7

112	GGGTACAAAGGTTAT	Primer used for the primer extension experiments in the in vivo 3D structure analysis.
41	ACTTGTCAGACTGCC	Northern-Blot probe used to detect the U3 snoRNA
001	CCAGTTACGAAAATTCTTG	Northern-Blot probe used to detect the 35S and the 27SA2 pre-rRNAs (hybridization on ITS1 between the A3 cleavage site and the 5,8S rRNA)
003	TGTTACCTCTGGGCCC	Northern-Blot probe used to detect the 35S, 23S and the 22S pre-rRNAs (hybridization on ITS1 between the A2 and A3 cleavage sites)
007	CTCCGCTTATTGATATGC	Northern-Blot probe used to detect the 25S rRNA
008	CATGGCTTAATCTTTGAGAC	Northern-Blot probe used to detect the 18S rRNA
013	GGCCAGCAATTTCAAGTTA	Northern-Blot probe used to detect the 35S, 27SA2 and the 27SA3 pre-rRNAs (hybridization on ITS2)
013b	CGCCTAGACGCTCTCTTCTTA	Northern-Blot probe used to detect the 35S, 27SA2 and the 27SA3 pre-rRNAs (hybridization on ITS2)
002	AAAGCTCTCATGCTCTTGCC	Northern-Blot probe used to detect the 35S, 23S, 22S and the 20S pre-rRNAs (hybridization on ITS1 between the D and A2 cleavage sites)
000	CGCTGCTCACCAATGG	Northern-Blot probe used to detect the 35S and 23S pre-rRNAs (hybridization on 5'-ETS upstream of the A0 cleavage site)
9660	GTCCACGAAGGGTTACTTCGCG	Northern-Blot probe used to detect the U6 snRNA
026	CCAGATAACTATCTTAAAAG	
026b	AGCAGTAAAAAAGAAAGAAACCGAAATCTC	Reverse Transcription primer used to identify the 5'-end of the pre-rRNA maturation intermediate corresponding to the A0 cleavage site (22S) and Northern-Blot probe used to detect the 35S, 23S and 22S intermediates (Hybridization between the A0 and A1 sites)
7211	GTTTTATCGTTGGCTTTTGCAGCAAATTCTGATCAGT TATAT	Mutagenesis oligonucleotide used to generate the R289A/K290A Rrp9p mutant
7212	ATATAACTGATCAGAATTTGCTGCAAAAGCCAACGAT AAAAC	Mutagenesis oligonucleotide used to generate the R289A/K290A Rrp9p mutant
8663	GTTTTATCGTTGGCTTTTGCAAAAAATTCTGATCAGT TATAT	Mutagenesis oligonucleotide used to generate the R289A Rrp9p mutant
8664	ATATAACTGATCAGAATTTTTTTGCAAAAGCCAACGAT AAAAC	Mutagenesis oligonucleotide used to generate the R289A Rrp9p mutant
8665	GTTTTATCGTTGGCTTTTAGAGCAAATTCTGATCAGT TATAT	Mutagenesis oligonucleotide used to generate the K290A Rrp9p mutant
8666	ATATAACTGATCAGAATTTGCTCTAAAAGCCAACGAT AAAAC	Mutagenesis oligonucleotide used to generate the K290A Rrp9p mutant
7835	CATCAATTCAAAGTTCTTTATTTCTTCTTTAGCATTTC AAAATTATAGAACTCTTGGCCTCCTCTAG	Insertion of the GAL10 regulatory element upstream of the RRP9 gene
7836	GATCTTTTCCTCTTTTTCTGTTGGGTAACATCTGACA TCTTTTCTAGATTCGAATTCCTTGAATTTTCAAA	Insertion of the GAL10 regulatory element upstream of the RRP9 gene
1031	ATCCTATGAATTCCGTCGACCTGC	Mutagenesis oligonucleotide used to generate U3 mutant III-4
1032	CCTATAGAAAGGATCCTATGA	Mutagenesis oligonucleotide used to generate U3 mutant 2-1
1033	GAGTCAAAGAGTGTATAGAAATGATCC	Mutagenesis oligonucleotide used to generate U3 mutant 3-3
1034	CAAAGAGTGACGTACCTATAGAAATG	Mutagenesis oligonucleotide used to generate U3 mutant 3-6
1035	CAAAGAGTGACGAAACCTATAGAAATG	Mutagenesis oligonucleotide used to generate U3 mutant 3-5
1036	AAATGATCCTACCAAGTACGTCGA	Mutagenesis oligonucleotide used to generate U3 mutant 1-1
1187	CTTAAAATCTGTGTCATATACTTCATAGGATC	Mutagenesis oligonucleotide used to generate U3 mutant III-1
1188	GAGTCAAAGAGTTACGATTCCTATAG	Mutagenesis oligonucleotide used to generate U3 mutant V-1
1189	CCTATAGAAATGATTTCATGAAGTACGTC	Mutagenesis oligonucleotide used to generate U3 mutant II-1
1190	GATTCCTATAGAAATTTTTTCATGAAGTACGTCGAC	Mutagenesis oligonucleotide used to generate U3 mutant II-2
1191	GACGATTCCTATACCAATGATCCTATGAAG	Mutagenesis oligonucleotide used to generate U3 mutant I-1
1192	GAAATGATCCTATGATTTTCGTCGACCTATAG	Mutagenesis oligonucleotide used to generate U3 mutant III-5
1193	GAAGAGTCAAAGAGTGTTCCTATAGAAATGATC	Mutagenesis oligonucleotide used to generate U3 mutant 3-4
1362	GAGTCAAAGAGTGTTTATTCCTATAGAA	Mutagenesis oligonucleotide used to generate U3 mutant 3-7

1972	GATCCTATGAATTACGTCGACCTA	Mutagenesis oligonucleotide used to generate U3 mutant III-3
1973	GATCCTATGAAGTCCGTCGACCTA	Mutagenesis oligonucleotide used to generate U3 mutant III-2
C310	CTCTTTTGAAGAGTCAAACTCACACGATTCCTATAG	Mutagenesis oligonucleotide used to generate U3 mutant V-2
C311	CTATAGGAATCGTGTGAGTTTGACTCTTCAAAAGAG	Mutagenesis oligonucleotide used to generate U3 mutant V-2
C312	CTCTTTTGAAGAGAGTTTGAGTGACGATTCCTATAG	Mutagenesis oligonucleotide used to generate U3 mutant V-3
C313	CTATAGGAATCGTCACTCAAACTCTCTTCAAAAGAG	Mutagenesis oligonucleotide used to generate U3 mutant V-3
C314	CTCTTTTGAAGAGAGTTTCTCACACGATTCCTATAG	Mutagenesis oligonucleotide used to generate U3 mutant V-4
C315	CTATAGGAATCGTGTGAGAAACTCTCTTCAAAAGAG	Mutagenesis oligonucleotide used to generate U3 mutant V-4
C316	CTCTTTTGAAGAGTCTTTCTCTGACGATTCCTATAG	Mutagenesis oligonucleotide used to generate U3 mutant V-5
C317	CTATAGGAATCGTCAGAGAAAGACTCTTCAAAAGAG	Mutagenesis oligonucleotide used to generate U3 mutant V-5

Supplementary Table 1 | Oligonucleotide probes and primers used in this study.

SUPPLEMENTARY TABLE 2

A.

pACT / pAS	Utp4	Utp5	Sof1	Utp3	Utp14	Rrp36	Utp7	Utp25	Utp6	Utp1	Utp12	Utp13	Utp18	Utp21	Utp16
Rrp9	**NI**	**NI**	**NI**	**NI**	**NI**	**Strong**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**

pACT / pAS	Mpp10	Imp3	Imp4	Nop1	Nop56	Nop58	Rrp9	Snu13	Sgd1	Bfr2	Kri1	Rrp5	Nsr1	Utp10	Utp20
Rrp9	**NI**	**NI**	**NI**	**NI***	**NI**	**NI**	**NI**	**NI***	**Strong**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**

B.

pAS / pACT	Utp4	Utp5	Sof1	Utp3	Utp14	Rrp36	Utp7	Utp25	Utp6	Utp1	Utp12	Utp13	Utp18	Utp21	Utp16
Rrp9	**NI**	**NI**	**NI**	**NI**	**NI**	**Strong**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**

pAS / pACT	Mpp10	Imp3	Imp4	Nop1	Nop56	Nop58	Rrp9	Snu13	Sgd1	Bfr2	Kri1	Rrp5	Nsr1	Utp10	Utp20
Rrp9	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**Strong**	**NI**	**NI**	**Weak**	**NI**	**Weak**	**Weak**

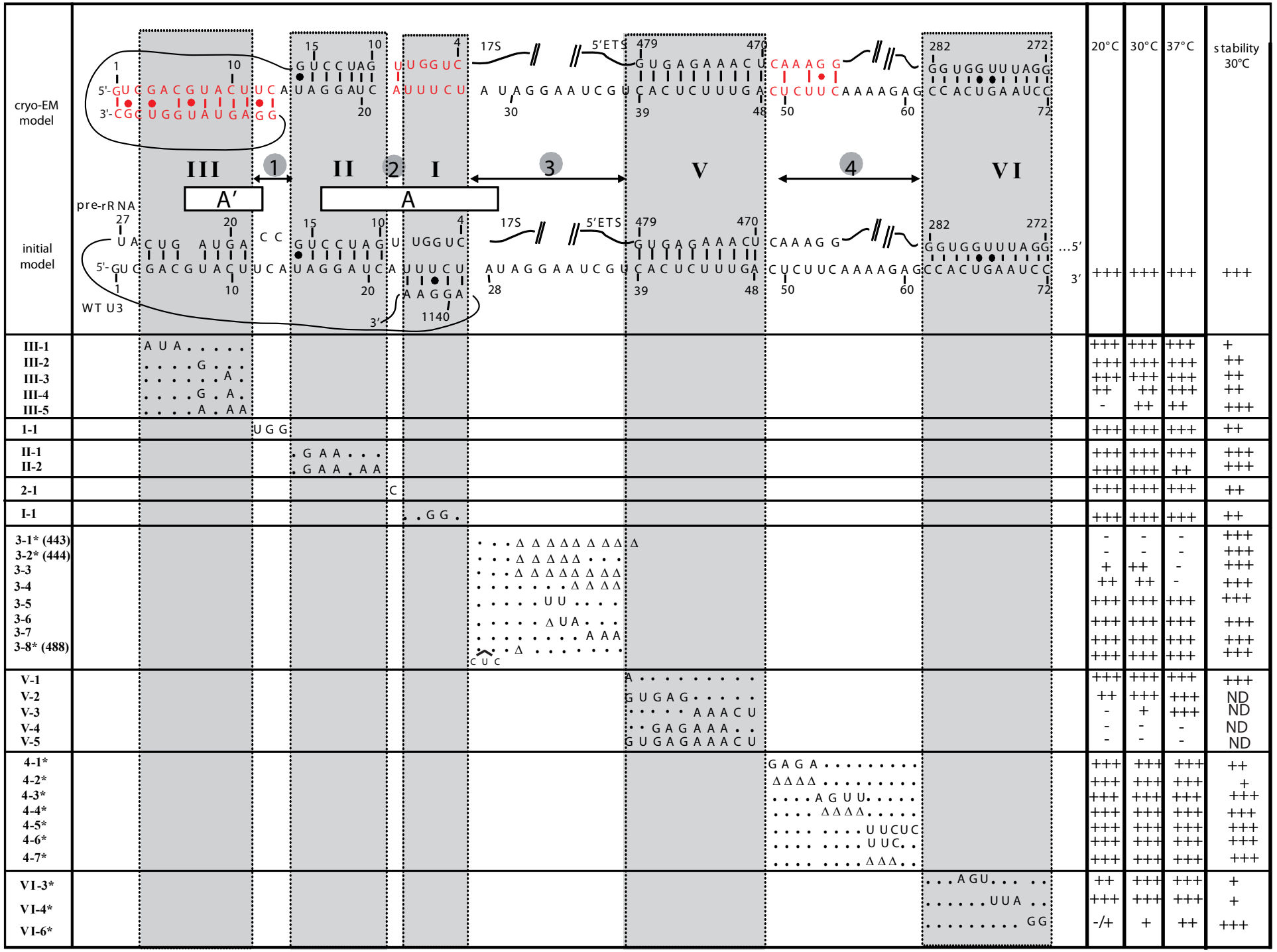
NI : No Interaction ; ND : Not Determined

C.

pACT / pAS	Rrp9	Rrp9 R289A	Rrp36	Sgd1	Rrp5	Utp10	Utp20
Rrp9	**ND**	**ND**	**Strong**	**Strong**	**NI**	**NI**	**NI**
Rrp9 R289A	**ND**	**ND**	**Weak**	**Strong**	**ND**	**NI**	**NI**
Rrp36	**Strong**	**Weak**	**ND**	**Strong**	**Strong**	**Strong**	**NI**
Sgd1	**Strong**	**Strong**	**Strong**	**ND**	**Strong**	**Weak**	**NI**
Rrp5	**Weak**	**ND**	**Strong**	**Strong**	**ND**	**NI**	**NI**
Utp10	**Weak**	**NI**	**Strong**	**NI**	**NI**	**ND**	**ND**
Utp20	**Weak**	**NI**	**Strong**	**NI**	**Weak**	**ND**	**ND**

NI : No Interaction ; ND : Not Determined

Supp. Table 3


cryo-EM model
initial model
pre-rRNA
WT U3
III
II
I
V
VI
A′
A
1
2
3
4
17S
5′ETS
20°C
30°C
37°C
stability 30°C
III-1
III-2
III-3
III-4
III-5
1-1
II-1
II-2
2-1
I-1
3-1* (443)
3-2* (444)
3-3
3-4
3-5
3-6
3-7
3-8* (488)
V-1
V-2
V-3
V-4
V-5
4-1*
4-2*
4-3*
4-4*
4-5*
4-6*
4-7*
VI-3*
VI-4*
VI-6*

Supp. fig. legends

Supp. Fig. 1. Growth of yeast cells expressing the various Rrp9 mutants produced in the study. Growth of the W303::pGAL-RRP9 cells expressing the mutant proteins was monitored under permissive galactose (YPG) or repressive glucose (YPD) medium at 3 different temperatures (20°C, 30°C, and 37°C) using 6 increasing dilutions. The generated mutations are indicated on the left side.

Supp. Fig. 2. 2D structure predictions and subdomains delineation for proteins Rrp36 and Sgd1. Panels a and c were performed thanks to the web server IUPred (79) and panels b and d were carried out by the web server PSIPRED V3.3 (78).

Supp Fig. 3. Search for interacting domains within the Rrp9 protein-protein interaction network (complementary results of data shown in Figure 4). Y2H assays were performed with full-length (FL) proteins or protein sub-fragments. The sub-fragments used are depicted on the right-side, with the C-ter and N-ter extremities indicated. 3-amino-triazole concentrations comprised between 0 and 40 mM were used to evaluate the strength of the detected interactions.

Supp. Fig. 4. Comparison of growth on YPD and YPG medium of cells expressing U3 snoRNAs mutated in the segments involved in the interaction with the 5'-ETS sequence of the pre-rRNA and their linking segments. A. Representation of the U3 snoRNA/pre-rRNA base-pair interaction. Helices V and VI and segments 3 and 4 are represented, together with their respective mutations. **B-E.** Yeast cells were transformed with plasmid expressing Rrp9 (Wild type WT or the R289A mutant mut) and U3 snoRNA (Wild-type WT or mutants 3-1 to 3-7, 4-1 to 4-7, V-1 to V-3 and VI-3, VI-4, VI-6). They were grown on YPD and YPG media after serial dilutions (2, 5, 10, 10^2, 10^3 or 10^4 X) at 20, 30 and 37°C (left, middle and right panels, respectively; for details see methods section).

Supp. Fig. 5. Comparison of growth on YPD and YPG medium of cells expressing U3 snoRNAs mutated in the heterologous interaction with the 18S and their linking segments. A. Representation of the U3 snoRNA/pre-rRNA base pair interaction. The different helices (I, II, III) and segments (1, 2), together with their respective mutations are represented on each of the two interaction models (initial and new cryo-EM model) (10,17,28,30,40,44). **B, C.** Yeast cells were transformed with plasmid expressing Rrp9 (Wild type WT or R289A mutant mut) and U3 snoRNA (Wild-type WT or mutants 1-1, 2-1, I-1, II-1, II-2, III-1 to III-5). They were grown on YPD and YPG media after serial dilutions (2, 5, 10,

10^2, 10^3 or 10^4 X) at 20, 30 and 37°C (left, middle and right panels, respectively; for details see methods section).

Supp. Fig. 6/7. View in two different orientations of the U3 snoRNA and surrounding proteins based on the Cryo-EM data from Barandun et al. (40) **and the corresponding atomic coordinates deposited in the Protein Data Bank under PDB 5WLC** (40)**.** The U3 snoRNA is represented in green, and the 5'-ETS and 18S pre-rRNA in grey. The putative positions of Rrp36 and Sgd1 are indicated. Rrp9 residue R289 is highlighted.

Supp. Table 1. Sequences of oligonucleotide probes and primers used in this study.

Supp. Table 2. The overall proteins tested in our yeast two hybrid screens and the results obtained. A. Rrp9 was tested with different proteins, in both possible Y2H orientations. These proteins included members of the various SSU-processome sub-complexes. Some of the proteins were selected because of their early recruitment into the SSU-processome, recent kinetics studies of SSU-processome assembly on pre-rRNA or proximity of Rrp9 determined from the cryo-EM 3D structures, as well as recent studies (24,40–45,52,53,74–76). **B.** Some Rrp9 identified interactants were also tested for possible interactions with each other.

Supp. Table 3. Global representation of all the U3 snoRNA mutants produced together with their stability and induced growth phenotype. The different heterologous helices (I, II, III, V, VI) formed with the pre-rRNA and the linking segments (1, 2, 3, 4) are represented according to both pairing models (10,17,28,30,40,44). The phylogenetically conserved A and A' boxes are indicated. Growth of cells was tested at 20, 30 and 37°C (+++, ++, +, -: normal, medium, highly reduced and no growth). U3 stability was measured for cells grown at 30°C. Mutants previously characterized are indicated with an * (with their initial published name for mutants 3-1, 3-2 and 3-8) (10,28). The other variants were generated and characterized in the present work.