Supplemental material

Figure S1

A) An overview of the *S. solfataricus* exosome with RNA bound in the central channel. **B)** A sequence alignment of the loop of Rrp41 involved in RNA binding in the channel. The included sequences are: *S. solfataricus* (SULSO), *P. furiosus* (PYRFU), *A. fulgidus* (ARCFU), *H. sapiens* (HUMAN) and *S. antibioticus* (STRAN). Conserved residues contacting RNA are shown in green and putative RNA binding residues for hRrp41 in blue. Numbers in parenthesis denote overall percentage identity of the full-length proteins to the *S. solfataricus* sequence. **C)** Close-up of the density and model at the entrance of the channel. In cyan is the loop of Ss-Rrp41 forming the constriction where the ribonucleotide binds. **D)** Close-up of the entrance of the central channel in the human exosome shown in a similar orientation to that in Panel **C)**. The corresponding loop in Hs-Rrp41 is shown in stick representation.

Figure S2

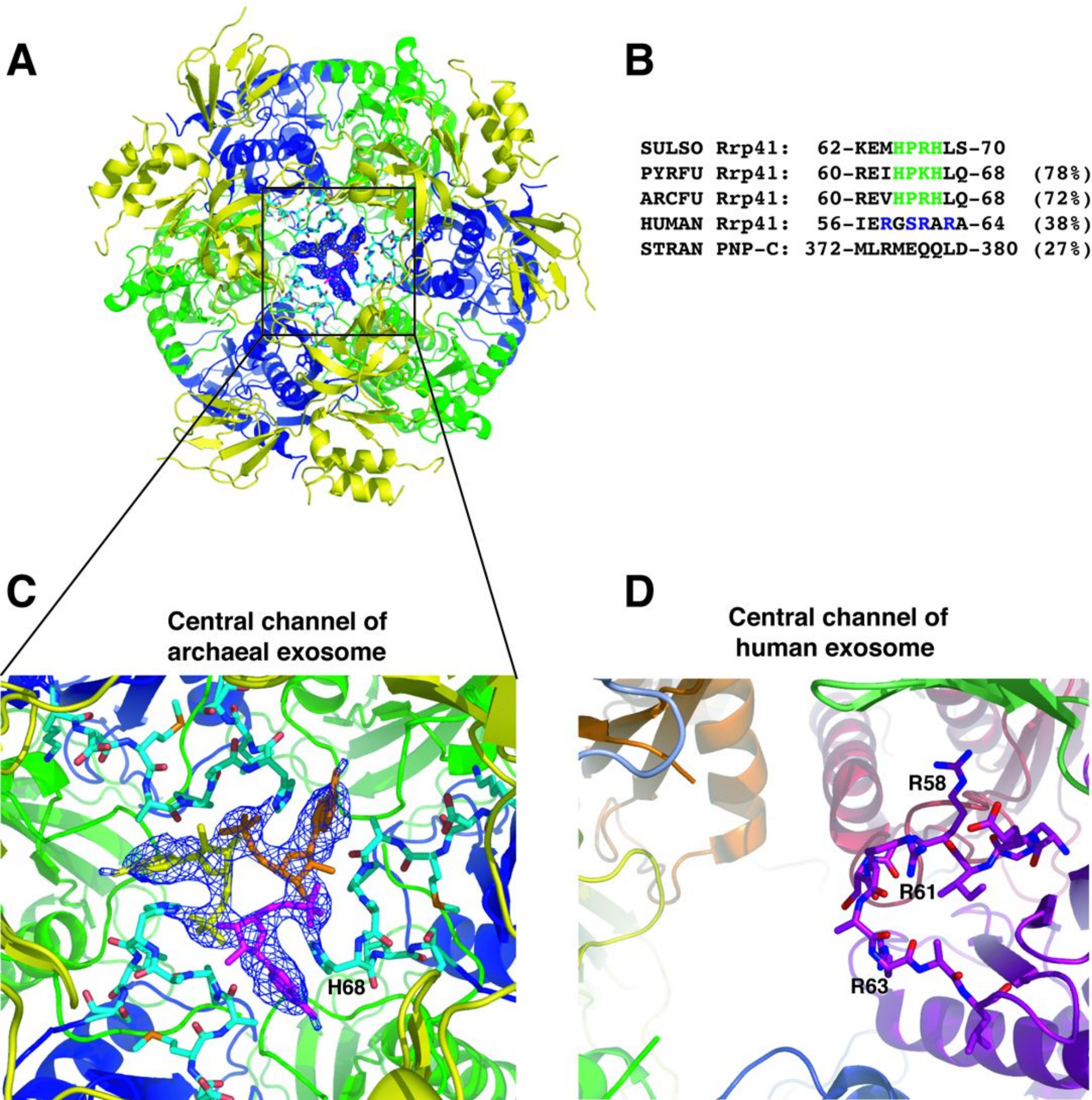
The manganese binding site found in the *S. solfataricus* exosome is not catalytic. To test whether the manganese binding site observed in the structure supports RNase activity, U8 RNA was incubated with the *S. solfataricus* exosome complex in the presence of manganese. Lane 2 shows that the native Rrp41-Rrp42-Rrp4 complex phosphorolytically degrades the U8 RNA molecule. In lanes 4-8 the phosphorolytically dead Rrp41(D182A)-Rrp42-Rrp4 complex was incubated with U8 RNA for 1, 5, 10, 30 and 60 minutes showing no signs of RNase activity.

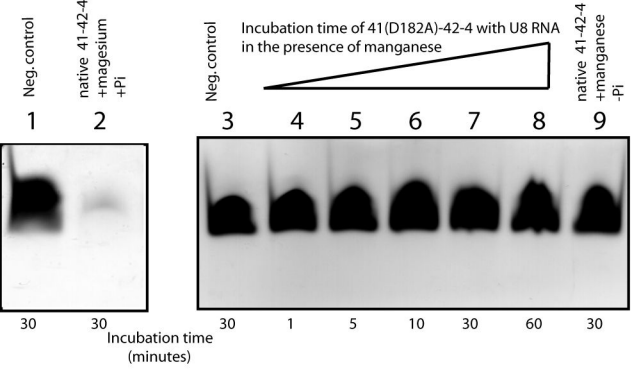
Furthermore, native Rrp41-Rrp42-Rrp4 complex also does not support RNase activity in the presence of $MnCl_2$ when inorganic phosphate is absent (lane 9).

Supplemental experimental procedures

RNA degradation assays

The RNase assays shown in Fig. S2 were carried out by incubating 20 pmol of Ss-Rrp41-Rrp42-Rrp4 wild-type or D182A mutant complexes with 1 nmol of U8 RNA in 40 µl total volume. For the positive control (lane 2), the RNA was incubated with the native protein complex at 70ºC for 30 min in a buffer containing 20 mM Tris-HCl pH 7.6, 60 mM KCl, 10 mM $MgCl_2$, 0.1 mM EDTA, 10% glycerol, 1U/µl RNasin, 10 mM Na_2HPO_4 and 2 mM DTT. To test for manganese supported RNase activity (lanes 3-9), native and D182A mutant complexes were incubated with RNA in a buffer containing manganese instead of magnesium and without inorganic phosphate: 20 mM Tris-HCl pH 7.6, 60 mM KCl, 1 mM $MnCl_2$, 0.1 mM EDTA, 10% glycerol, 1U/µl RNasin and 2 mM DTT. Reaction products were resolved on a 20% polyacrylamide gel containing 8M urea and visualized with toluidine.


A
B
SULSO Rrp41: 62-KEMHPRHLS-70
PYRFU Rrp41: 60-REIHPKHLQ-68 (78%)
ARCFU Rrp41: 60-REVHPRHLQ-68 (72%)
HUMAN Rrp41: 56-IERGSRARA-64 (38%)
STRAN PNP-C: 372-MLRMEQQLD-380 (27%)
C
Central channel of archaeal exosome
H68
D
Central channel of human exosome
R58
R61
R63


Neg. control
native 41-42-4
+magesium
+Pi
Neg. control
Incubation time of 41(D182A)-42-4 with U8 RNA
in the presence of manganese
native 41-42-4
+manganese
-Pi
1
2
3
4
5
6
7
8
9
30
30
30
1
5
10
30
60
30
Incubation time
(minutes)