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Supplemental Information

The Ribosome Cooperates with the Assembly Chaperone pICln to Initiate Formation of snRNPs

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Figure S1

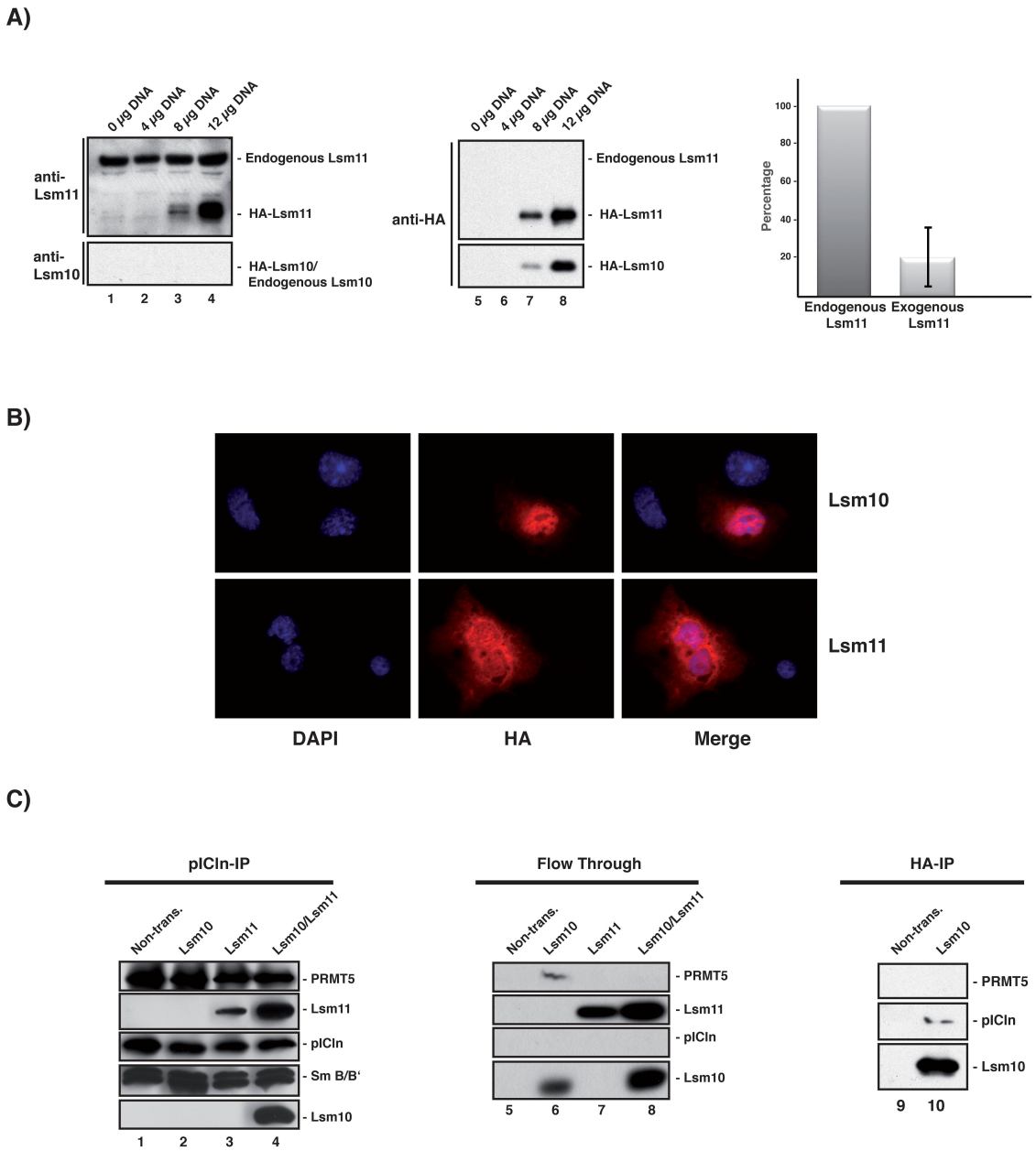

A)
0 µg DNA
4 µg DNA
8 µg DNA
12 µg DNA
anti-Lsm11
- Endogenous Lsm11
- HA-Lsm11
anti-Lsm10
- HA-Lsm10/ Endogenous Lsm10
1 2 3 4
anti-HA
- Endogenous Lsm11
- HA-Lsm11
- HA-Lsm10
5 6 7 8
Percentage
100
80
60
40
20
Endogenous Lsm11
Exogenous Lsm11
B)
Lsm10
Lsm11
DAPI
HA
Merge
C)
pICln-IP
Non-trans.
Lsm10
Lsm11
Lsm10/Lsm11
- PRMT5
- Lsm11
- pICln
- Sm B/B'
- Lsm10
1 2 3 4
Flow Through
Non-trans.
Lsm10
Lsm11
Lsm10/Lsm11
- PRMT5
- Lsm11
- pICln
- Lsm10
5 6 7 8
HA-IP
Non-trans.
Lsm10
- PRMT5
- pICln
- Lsm10
9 10

Figure S2

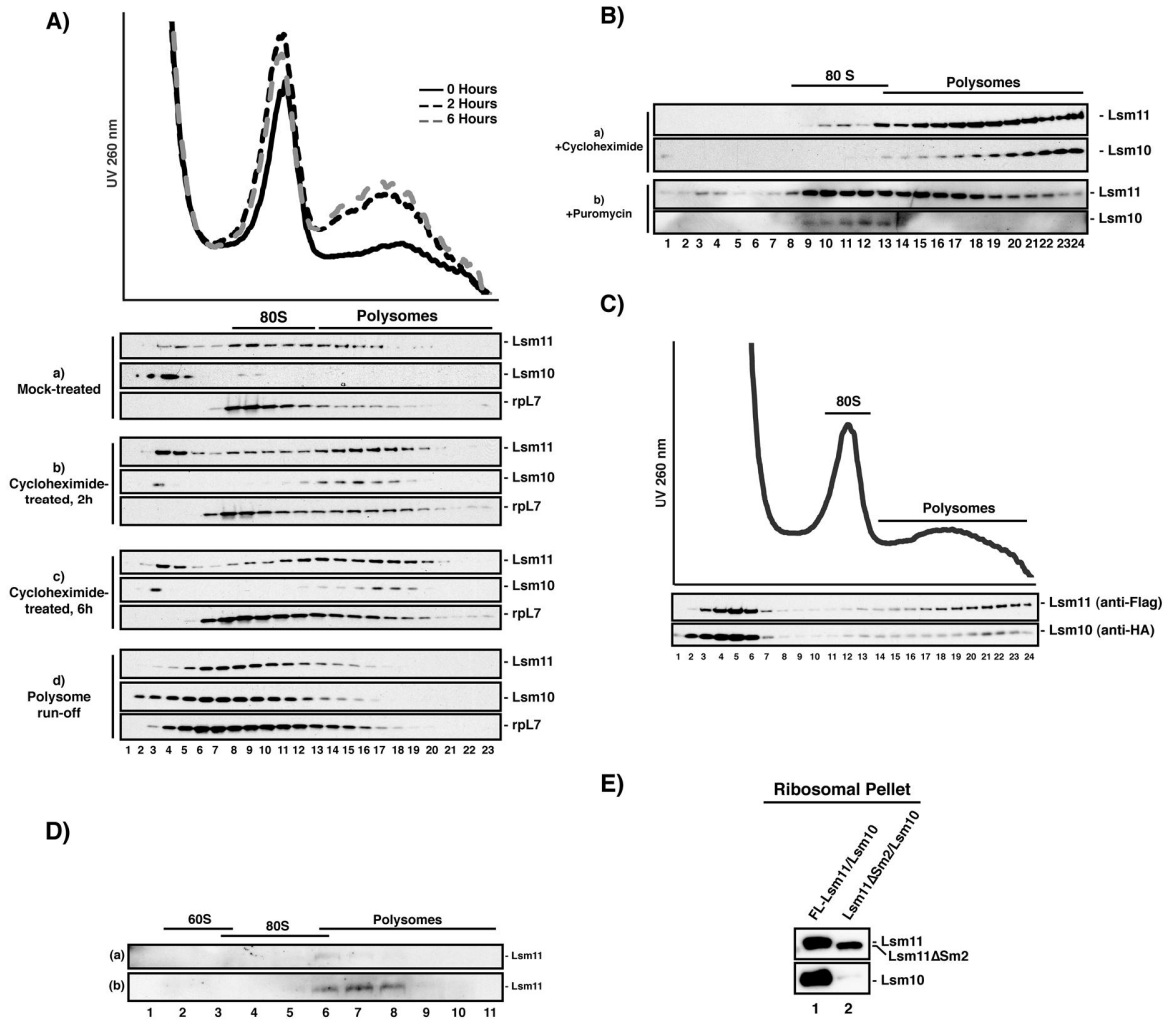
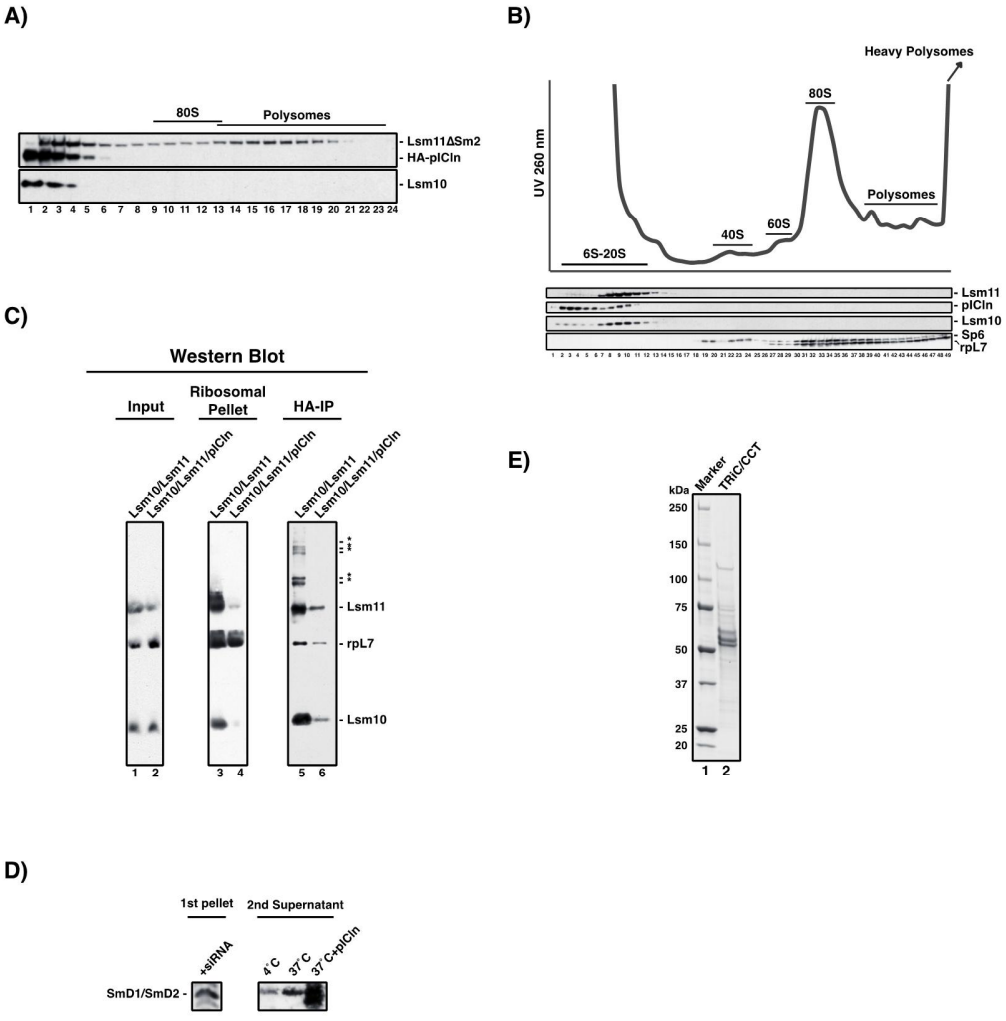

A)
UV 260 nm
0 Hours
2 Hours
6 Hours
80S
Polysomes
a) Mock-treated
b) Cycloheximide-treated, 2h
c) Cycloheximide-treated, 6h
d) Polysome run-off
- Lsm11
- Lsm10
- rpL7
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
B)
80 S
Polysomes
a) +Cycloheximide
b) +Puromycin
- Lsm11
- Lsm10
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 2122 2324
C)
UV 260 nm
80S
Polysomes
- Lsm11 (anti-Flag)
- Lsm10 (anti-HA)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
D)
60S
80S
Polysomes
(a)
(b)
- Lsm11
1 2 3 4 5 6 7 8 9 10 11
E)
Ribosomal Pellet
FL-Lsm11/Lsm10
Lsm11ΔSm2/Lsm10
- Lsm11
Lsm11ΔSm2
- Lsm10
1 2

Figure S3


A)
80S
Polysomes
- Lsm11ΔSm2
- HA-plCln
- Lsm10
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
B)
Heavy Polysomes
80S
UV 260 nm
Polysomes
40S
60S
6S-20S
- Lsm11
- plCln
- Lsm10
- Sp6
- rpL7
C)
Western Blot
Input
Ribosomal Pellet
HA-IP
Lsm10/Lsm11
Lsm10/Lsm11/plCln
- Lsm11
- rpL7
- Lsm10
1 2 3 4 5 6
D)
1st pellet
2nd Supernatant
+siRNA
4˚C
37˚C
37˚C+plCln
SmD1/SmD2 -
E)
kDa
Marker
TRiC/CCT
250
150
100
75
50
37
25
20
1 2

Supplemental Figure Legends

Figure S1. Related to Figure 1: Subcellular localization of exogenous Lsm10 and Lsm11 proteins and characterization of their association with endogenous pICln and PRMT5.

A) Western blot analysis with antibodies specific for Lsm11 to estimate overexpression levels in transfected cells. 100 µg of total protein from either mock-transfected cells (lane 1) or cells co-transfected with 4 µg (lane 2), 8 µg (lane 3) and 12 µg (lane 4) of each Lsm10 and Lsm11 plasmid (left panel) was used for Western blotting. To confirm the migration behavior of exogenous Lsm11, a Western blot was performed on the same samples with anti-HA antibody (middle panel, lanes 5-8). Additionally, the anti-HA Western blot revealed that HA-Lsm10 was present but undetectable by the Lsm10 antibody. Five different samples transfected with 8 µg of each Lsm10 and Lsm11 plasmid were used for quantification. The graph depicts the densitometric analysis of the Western blot signals corresponding to the endogenous and exogenous proteins, respectively (right panel). The expression level of endogenous protein is considered 100% and the expression level of exogenous protein is shown as a percentage of the endogenous protein. Error bars reflect the expression differences of the exogenous protein in different experiments.

B) Immunofluorescence of cells transfected with HA-Lsm10 (upper panel) and HA-Lsm11-FLAG (lower panel). The cells were stained with DAPI to detect nuclei and anti-HA antibodies to detect the exogenous proteins obtained by transfection. Both proteins localize to both the nucleus as well as the cytoplasm as previously reported for their respective endogenous counterparts (Pillai et al., 2003; Pillai et al., 2001).

C) Anti-pICln immuno-precipitation was performed from either cytoplasmic extracts derived from non-transfected 293T cells (lanes 1 and 5) as a control or from 293T cells transfected with either HA-Lsm10 (lanes 2 and 6), HA-Lsm11-FLAG (lanes 3 and 7) or co-transfected with HA-Lsm10 and HA-Lsm11-FLAG (lanes 4 and 8). Under conditions, where endogenous pICln is depleted from the respective extracts, the majority of Lsm proteins are found in the flow-through of the immuno-precipitation (middle panel). This indicates that pICln amounts are limiting in respect to the exogenous Lsm proteins. Anti-HA immuno-precipitation reveals that HA-Lsm10 in light fractions in Figure 1A, panel b is associated with substoichiometric amounts of pICln. Shown is a Western blot analysis using anti-HA, anti-pICln or anti-PRMT5 antibodies of anti-HA immuno-precipitates from either non-transfected cells as a control (lane 9) or cells transfected with HA-Lsm10 (lane 10).

Figure S2. Related to Figure 1 and Figure 2: Characterization of the association of Lsm10 and Lsm11 with ribosomes/polysomes.

Western blot analysis of density gradient fractions using anti-HA and anti-rpL7 antibodies to examine the sedimentation behavior of Lsm10, Lsm11 and ribosomes in extracts from cells co-transfected with Lsm10 and Lsm11 and subjected to various polysome stabilizing/destabilizing conditions.

A) Cells were either mock-treated (panel a) or treated with 100 µg/ml cycloheximide for 2 hrs (panel b) or 6 hrs (panel c). Extracts were then prepared and size-fractionated by 5-45 % sucrose gradients capable of resolving 80S ribosomes and polysomes. In panel d, cells were treated with cycloheximide for 2 hrs as in panel b and extract was

prepared. The extract was then incubated under polysome run-off conditions and size-fractionated by 5-45% sucrose gradients capable of resolving 80S ribosomes and polysomes. Note the gradual increase of rpL7 and Lsm10/Lsm11 in polysomes upon prolonged cycloheximide treatment (compare panels b and c with a), whereas they are shifted to 80S and smaller fractions under polysome run-off conditions (panel d).

B) Western blot analysis of density gradient fractions of ribosomes/polysomes, which were purified from cells co-transfected with Lsm10 and Lsm11 and additionally treated either with 100 µg/ml cycloheximide (panel a) or 5 mM Puromycin (panel b). Anti-HA immunoblots revealed the sedimentation behavior of Lsm10 and Lsm11. Note that sedimentation of Lsm10 and Lsm11 is distinctly shifted from polysomal fractions in cycloheximide treated ribosomes to 80S fractions upon puromycin treatment (compare panel a with panel b).

C) This figure is related to Fig.1B of the manuscript. Extracts from cells co-transfected with HA-Lsm10 and HA-Lsm11-FLAG (i.e. tagged at its N-terminus with an HA tag and at its C-terminus with a FLAG tag) and treated with cycloheximide were fractionated by density gradient centrifugation. The sedimentation pattern of Lsm10 was revealed by a Western blot using anti-HA antibodies and that of Lsm11 by a Western blot using anti-FLAG antibodies. Note that Lsm11 in low-molecular weight, as well as in polysomal fractions is detected by the FLAG antibody. Due to its C-terminal position, detection of the FLAG tag is only possible if translation has proceeded to the 3´-end of the Lsm11 coding sequence, unequivocally proving that it is a full-length protein interacting with polysomes and not a stalled polypeptide.

D) Extracts were prepared from either mock-transfected cells (panel a) or pICln-depleted cells (panel b). The sedimentation behavior of endogenous Lsm11 was investigated using antibodies specific to Lsm11. Note the distinct increase of Lsm11 in polysomes upon pICln knockdown (panel b, lanes 6-8). Only ribosomal fractions are depicted in this figure for reasons of clarity.

E) The Sm2 motif of Lsm11 is necessary for recruitment of Lsm10 to polysomes. Extracts from cells co-transfected either with Lsm10 and full-length (FL) Lsm11 or Lsm10 and Lsm11ΔSm2 were subjected to differential centrifugation. Western blots of the corresponding ribosomal pellets are shown (lanes 1 and 2). Note the reduced presence of Lsm10 in the ribosomal pellet of cells co-transfected with Lsm11ΔSm2 in comparison to full-length (FL) Lsm11.

Figure S3. Related to Figure 3 and Figure 4: Specificity of pICln-dependent ribosomal release of Lsm10/Lsm11

A) Extracts from cells co-transfected with Lsm10, Lsm11ΔSm2 and pICln were fractionated by density gradients. The sedimentation patterns of Lsm10 and Lsm11ΔSm2 were revealed by a Western blot analysis using anti-HA antibody. Note that pICln is incapable of releasing Lsm11ΔSm2 from polysomes.

B) Depicted is a Western blot analysis of a high-resolution density gradient of cytoplasmic extracts from cells co-transfected with Lsm10, Lsm11 and pICln. This gradient relates to Figure 3A, panel c. Note that both Lsm10 and Lsm11 are clearly released from ribosomal and polysomal fractions and co-sediment together with pICln in fractions corresponding to the PRMT5- and 6S-complexes (6S-20S region).

C) Anti-HA immuno-precipitation from purified bulk ribosomes/polysomes of indicated transfected cells. Western blot analysis of the input samples, ribosomal pellets and immuno-precipitates from cells co-transfected either with

Lsm10 and Lsm11 (lanes 1, 3 and 5, respectively) or Lsm10, Lsm11 and pICln (lanes 2, 4 and 6, respectively). The proteins were visualized with anti-HA (Lsm11), anti-rpL7 and anti-FLAG (Lsm10), respectively. Asterisks denote uninterpretable bands.

D) Extract from cells transfected with siRNA against pICln was subjected to differential centrifugation. First ribosomal pellet was incubated at 4°C, 37°C or 37°C in the presence of pICln. This ribosomes were subjected to another round of differential centrifugation. Western blots of the first ribosomal pellet and second supernatants are shown. The release of SmD1/SmD2 occurs only in the presence of pICln at 37°C.

E) SDS-PAGE of the pooled fractions of the TRiC/CCT purification used for the control experiment depicted in Figure 3C, lane 16. Shown is a molecular weight marker (lane 1) and the purified Hela TRiC/CCT (lane 2).

Table S1. Related to Figure 3: Identification of proteins of U7-specific PRMT5- and 6S-complexes by mass spectrometry.

Bio View: Identified Proteins (277) Including 8 Decoys	Accession Number	Mr	PRMT5 complex (Peptide counts)	6S complex (Peptide counts)
U7 snRNA-associated Sm-like protein LSm11 [Homo sapiens],	gi|27735089 (+1)	40 kDa	435	359
Protein arginine N-methyltransferase 5 isoform a [Homo sapiens],	gi|20070220	73 kDa	631	17
U7 snRNA-associated Sm-like protein LSm10 [Homo sapiens],	gi|14249632	14 kDa	254	224
Methylosome protein 50 [Homo sapiens],	gi|13129110 (+1)	37 kDa	319	13
Methylosome subunit pICln [Homo sapiens],	gi|4502891	26 kDa	159	170
Small nuclear ribonucleoprotein E [Homo sapiens] E	gi|4507129	11 kDa	92	117
Small nuclear ribonucleoprotein polypeptide F, isoform CRA_b [Homo sapiens]	gi|119617955 (+1)	8 kDa	42	66
Small nuclear ribonucleoprotein G [Homo sapiens]	gi|4507133	8 kDa	34	62
Small nuclear ribonucleoprotein Sm D2 isoform 1 [Homo sapiens]	gi|4759158	14 kDa	41	_
Small nuclear ribonucleoprotein Sm D1 [Homo sapiens]	gi|5902102	13 kDa	22	_

Table S1: U7-specific PRMT5- and 6S-complexes, purified as in Fig.3E, were subjected to mass spectrometry for the identification of proteins associated with HA-Lsm11-FLAG. The peptide counts of the identified proteins are depicted.

Supplemental Experimental Procedures

Eukaryotic expression vectors, antibodies and chemical reagents

cDNAs encoding Lsm10, Lsm11, Lsm11ΔSm2, SmD1 and pICln were inserted into pcDNA3 plasmid (Invitrogen, Karlsruhe, Germany). N-terminal Hemagglutinin (HA) tag or N-terminal HA- and C-terminal FLAG-tags were appended by standard PCR strategies where appropriate. The anti-pICln, anti-PRMT5 and anti-SmD1/SmD2 antibodies used in this study were described previously (Chari et al., 2008). Additionally, commercial anti-HA and anti-FLAG antibodies were utilized (Sigma Aldrich, Schnelldorf, Germany). Alexa Fluor 594 anti-mouse IgG antibody was purchased from Invitrogen (Karlsruhe, Germany). Anti-Lsm11 and Anti-DNAJC2 antibodies were purchased from Thermo Fischer Scientific (Schwerte, Germany), BSA Fraction V and GroEL as a control for the pICln release reaction (see below) were purchased from Carl Roth (Karlsruhe, Germany) and Mobitec (Goettingen, Germany), respectively. All other chemicals utilized in this study were obtained from Sigma Aldrich (Schnelldorf, Germany), Carl Roth (Karlsruhe, Germany) and Merck (Darmstadt, Germany). A siRNA pool directed against pICln mRNA was obtained from Thermo Scientific Dharmacon (Schwerte, Germany).

Immunocytochemistry

Hela cells were transfected with either HA-Lsm10 or HA-Lsm11-FLAG in pcDNA3. 24 h post-transfection, cells were washed with PBS at room temperature and then fixed with 3.7% formaldehyde in PBS for 10 min at room temperature and subsequently washed with cold PBS for 5 min. Cells were then permeabilized with 0.2% Triton X-100 in cold PBS for 10 minutes and blocked with 1% BSA in PBS for 30 min. Cells were incubated with anti-HA antibody (Sigma Aldrich, Schnelldorf, Germany) for 1 h at room temperature at a 1:500 dilution in PBS containing 1% BSA. Upon completion of incubation, cells were washed twice with cold PBS for 5 min each and then incubated with the secondary antibody (Alexa Fluor 594 anti-mouse IgG (Invitrogen, Karlsruhe, Germany), 1:5000 in PBS containing 1% BSA). Lastly, cells were washed twice with PBS and mounted in Vectashield mounting medium (Eppendorf, Hamburg, Germany) and imaged with Axiovert 200M Microscope (Carl Zeiss AG, Jena, Germany).

Immuno-precipitation assay

Immuno-precipitation assays were performed either with Anti-HA agarose (Sigma Aldrich, Schnelldorf, Germany) in Fig.1C or anti-HA magnetic beads (Thermo Fischer Scientific, Schwerte, Germany) in Fig.3B and Fig.S1C. The pICln-IP in Fig.S1C was performed as described previously (Chari et al., 2008).

Purification of TRiC/CCT

Human TRiC/CCT was purified from Hela cytoplasmic lysates according to a procedure previously reported by Jonathan King and co-workers(Knee et al., 2013).

Supplemental References

Chari, A., Golas, M.M., Klingenhager, M., Neuenkirchen, N., Sander, B., Englbrecht, C., Sickmann, A., Stark, H., and Fischer, U. (2008). An assembly chaperone collaborates with the SMN complex to generate spliceosomal SnRNPs. Cell *135*, 497-509.

Knee, K.M., Sergeeva, O.A., and King, J.A. (2013). Human TRiC complex purified from HeLa cells contains all eight CCT subunits and is active in vitro. Cell Stress Chaperon *18*, 137-144.

Pillai, R.S., Grimmler, M., Meister, G., Will, C.L., Luhrmann, R., Fischer, U., and Schumperli, D. (2003). Unique Sm core structure of U7 snRNPs: assembly by a specialized SMN complex and the role of a new component, Lsm11, in histone RNA processing. Genes Dev *17*, 2321-2333.

Pillai, R.S., Will, C.L., Luhrmann, R., Schumperli, D., and Muller, B. (2001). Purified U7 snRNPs lack the Sm proteins D1 and D2 but contain Lsm10, a new 14 kDa Sm D1-like protein. EMBO J *20*, 5470-5479.