## Supplementary materials for:

# The 68 kDa subunit of mammalian cleavage factor I interacts with the U7 small nuclear ribonucleoprotein and participates in 3' end processing of animal histone mRNAs

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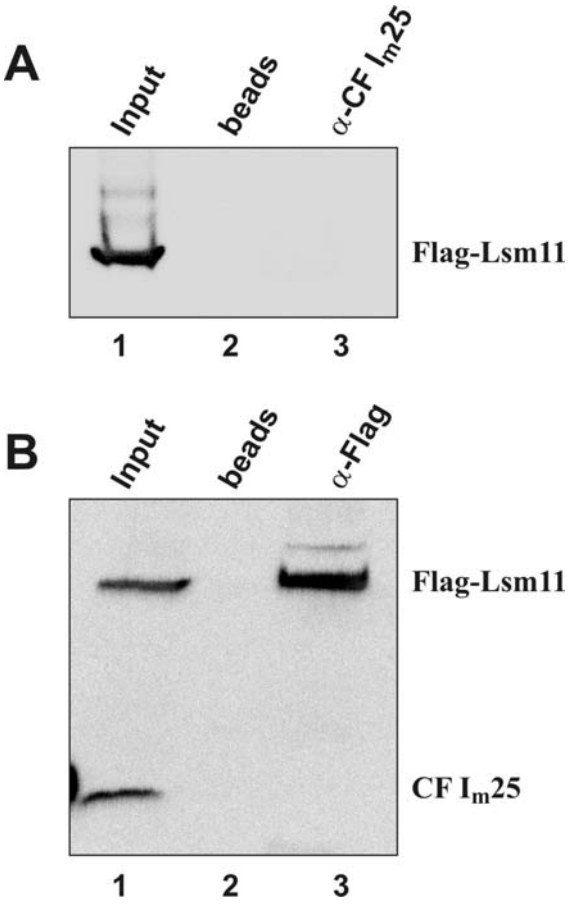
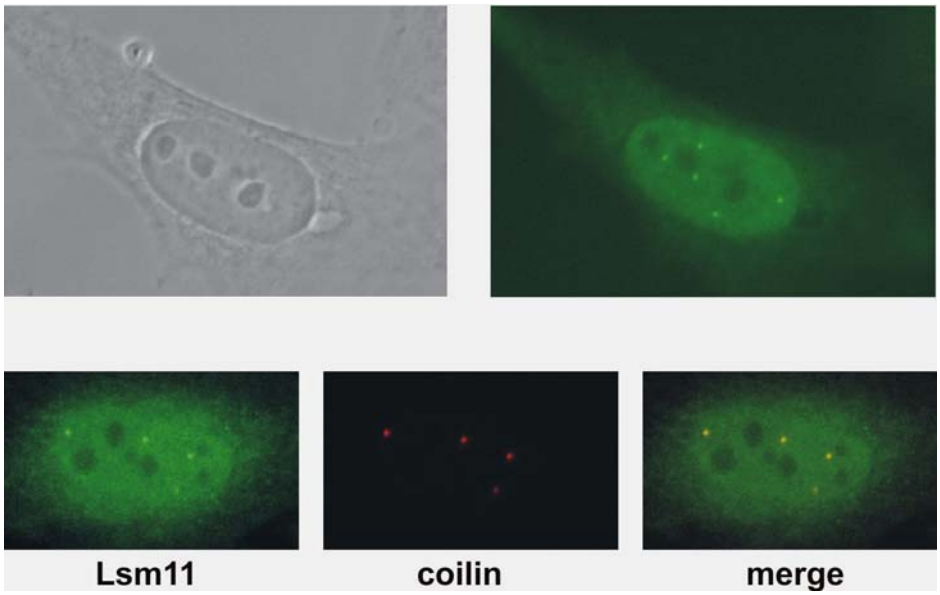
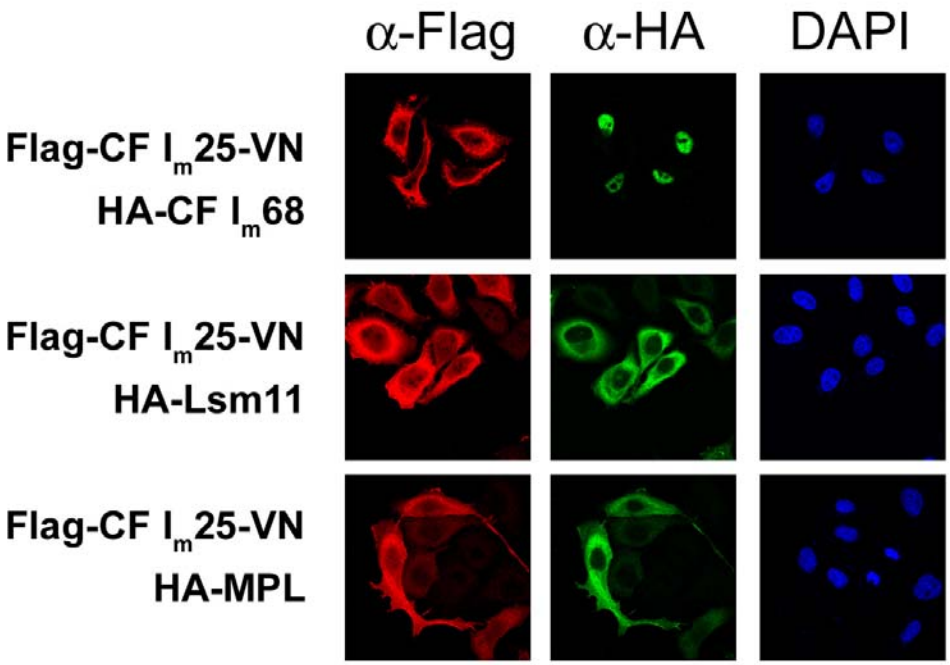


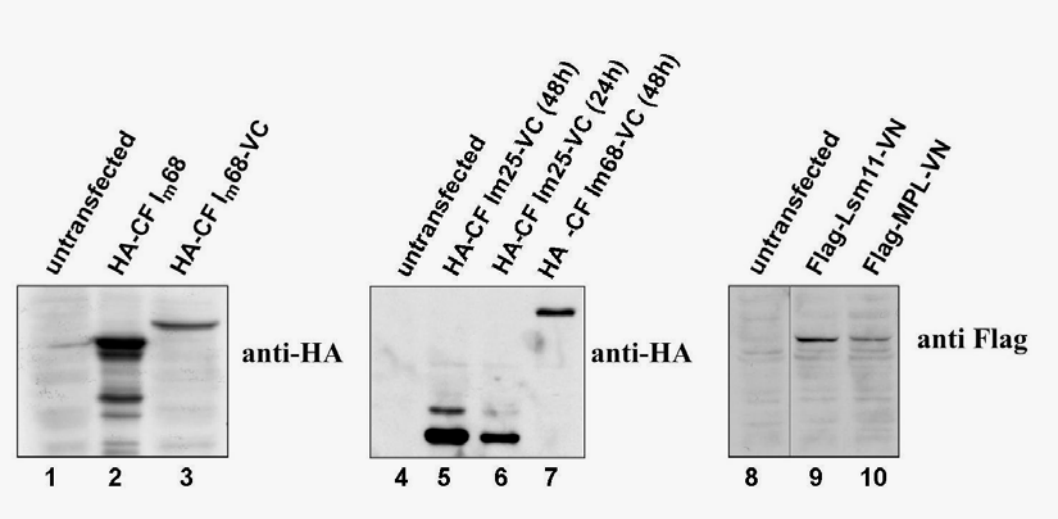

**Supplementary Figure S1.** Lack of evidence for an interaction of CF $I_m25$ with the U7 snRNP. Flag-tagged Lsm11 was expressed in human 293-T cells and its ability to interact with CF $I_m25$ was assessed by immunoprecipitation with anti-CF $I_m25$ **(A)** or anti-Flag **(B)** antibodies. Relevant proteins were revealed by anti-Flag HRP (A and B, top) and anti-CF $I_m25$ (B, bottom). Negative controls (lanes 2), beads incubated with bovine serum albumin. Input, 1/30 of the amount used in the co-immunoprecipitation. Note that additional experiments also did not reveal any co-precipitation of Flag-tagged CF $I_m25$ by anti-Lsm11 antibody (data not shown).



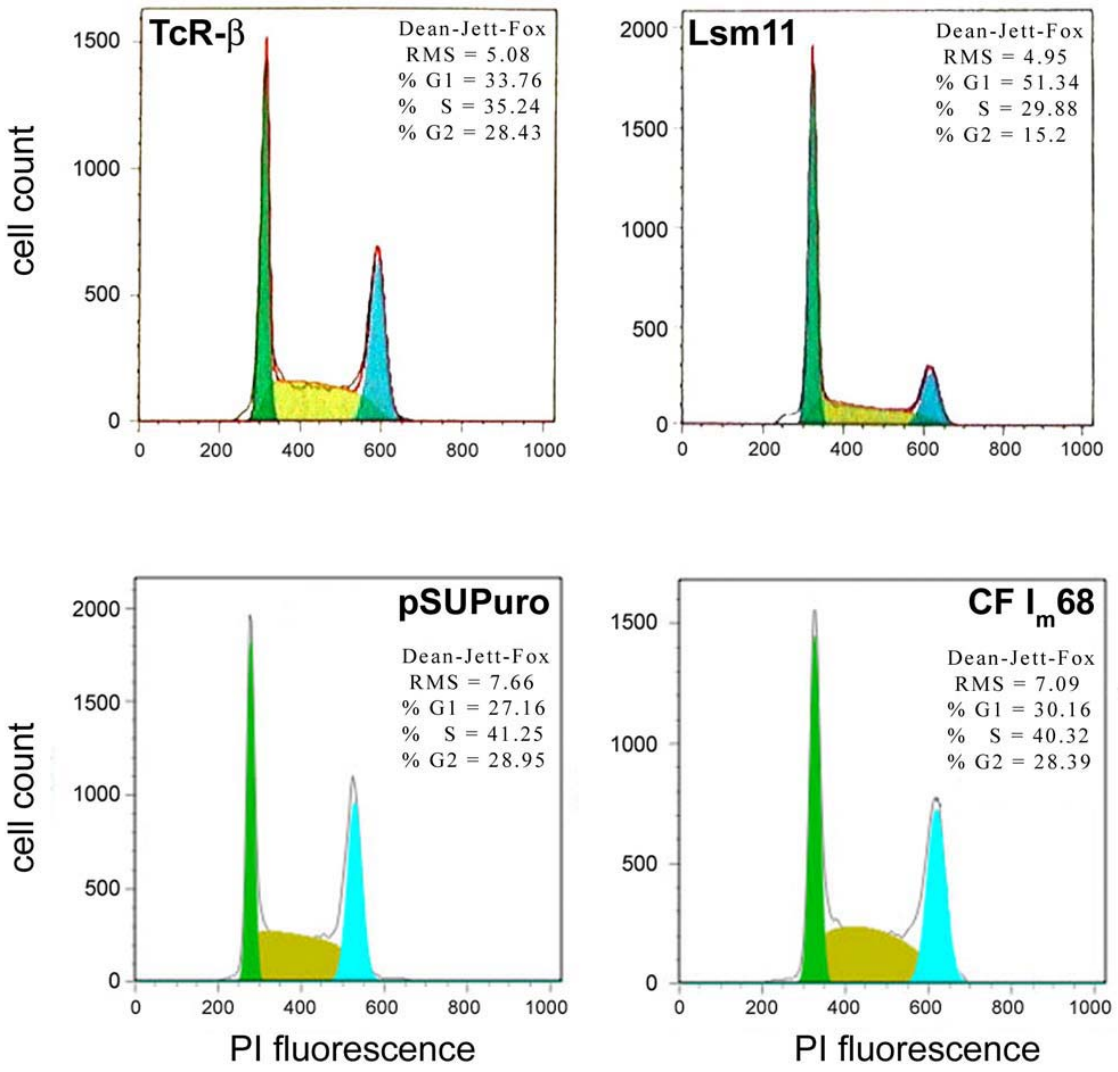

**Supplementary Figure S2.** Immunolocalisation of Lsm11. Lsm11 (green) and coilin (red) were revealed in HeLa cells by indirect immunofluorescence: Methods were as described (1), except that Lsm11 was detected with affinity-purified rabbit polyclonal anti-Lsm11 antibodies (1:66).



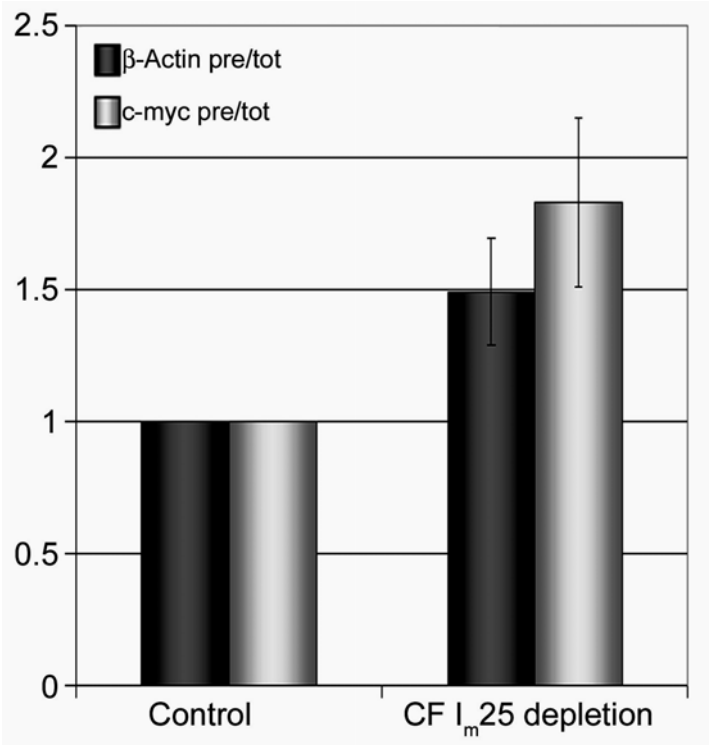

**Supplementary Figure S3.** Co-expression and immunofluorescence localisation of Flag-CF $I_m$25-VN with HA-CF $I_m$68 (top row), HA-Lsm11 (middle) or HA-MPL (bottom) in HeLa cells. The columns show (from left to right): the red fluorescence of of Flag-CF $I_m$25-VN detected with anti-Flag antibody, the green fluorescence of the respective HA-tagged protein detected with anti-HA antibody, and the blue fluorescence of the nuclear DAPI staining.




**Supplementary Figure S4.** Expression of fusion proteins in the same cell batches determined by immunoblotting with anti-Flag and anti-HA antibodies. Note that a lane between lanes 8 and 9 has been omitted from the right hand panel.




**Supplementary Figure S5.** Cytofluorometric analysis of HeLa cells depleted of Lsm11 (top right) or CF Im68 (bottom right) by shRNA expression (see Materials and Methods of main manuscript). As controls cells were depleted from T-cell receptor β (top left) or transfected with empty pSUPuro vector (bottom left), respectively. Four (top) or five (bottom) days after transfection, the cells were trypsinised and washed with phosphate buffered saline (PBS, 137 mM NaCl, 2.7 mM KCl, 10 mM $Na_2HPO_4$, 2 mM $KH_2PO_4$, pH 7.4) followed by resuspension in 400 µl PBS. For fixation, the cell suspension was added dropwise into 3ml precooled 70% EtOH under constant swirling. The fixed cells were then washed twice with PBS followed by incubation in 1 ml propidium staining solution (PBS supplemented with 0.1% Triton-X-100, 2mg/ml RNAse A, 0.2 mg/ml propidium iodide) for 30 minutes at room temperature. Cell cycle stages were measured by cytofluorometry (Becton Dickinson BD FACS Calibur), and data were analysed by using FlowJo software. The peaks corresponding to G1 and G2 are coloured in green and cyan, respectively. Cells in S-phase are coloured in yellow.




**Supplementary Figure S6.** Effect of CF $I_m25$ depletion on apparent in vivo 3' processing of b-actin and c-myc pre-mRNAs. Similar qRT-PCR assays as described for H3C in the main paper were used to determine the levels of pre-mRNAs (by using PCR probes spanning the polyadenylation site) or total mRNAs (with probes near the initiation codon). Details are available on request. Number of experiments: 2.

**Supplementary Table S1.** Sequences of oligonucleotides used for reverse-transcription-PCR.

| **Regular oligonucleotides** | |
|---|---|
| Lsm11 forward primer: | 5'-AAGTTGGCTTCAGTGTGGGGAAG-3' |
| Lsm11 reverse primer: | 5'-TCACTGTGCAAGATGAACCAGC-3' |
| U6 snRNA forward primer | 5'-CTCGCTTCGGCAGCACA-3' |
| U6 snRNA reverse primer | 5'-AACGCTTCACGAATTTGCGT-3' |
| CF $I_m25$ forward primer | 5'-GCACCAGGATATGGACCCATCATTTC-3' |
| CF $I_m25$ reverse primer | 5'-TAGCTGTGCTCACAGAGACAAG-3' |
| **TaqMan oligonucleotides for quantitative RT-PCR [a]** | |
| H3 pre *Taq*Man probe: | 5'-FAM-CACCCACATCAGCACTT-NFQ-3' |
| H3 pre forward primer: | 5'-TTCCATCGTATCCAAAAGGCTCTT-3' |
| H3 pre reverse primer: | 5'-CAAGCGGTACAGCTTCTTCC-3' |
| H3 tot *Taq*Man probe: | 5'-FAM-TCGCTATGGCCCGTACTAA-NFQ-3' |
| H3 tot for: | 5'-GCTGGTAAGCCTGTGTTTTGG-3' |
| H3 tot rev: | 5'-GCCGCCGGTCGACTT-3' |

Abbreviations: FAM, 6-carboxyfluorescein; NFQ, non-fluorescent quencher.

[a] The assays were designed by using File Builder of Applied Biosystems.

## Reference

1. Pillai,R.S., Will,C.L., Lührmann,R., Schümperli,D. and Müller,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.