In vitro methylation of the U7 snRNP subunits Lsm11 and SmE by the PRMT5/MEP50/pICln methylosome

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SUPPLEMENTARY FIGURE LEGENDS

Fig. S1. Schematic representation of full length Lsm11 and Lsm11 fragments used in the study. Numbers indicate the boundaries of the N-terminal region (amino acids 1-152) and the Sm fold (amino acids 153-206 and 338-360). The internal loop within the Sm fold of Lsm11 encompasses amino acids 207-337. The single α-helix and the five β-strands of the Sm fold are depicted with a horizontal bar and arrows, respectively.

Fig. S2. A HeLa cytoplasmic extract was incubated with the Lsm10/11 heterodimer either in the absence or in the presence of the SmE/F/G heterotrimer (lane 4 and 5, respectively), and bound proteins were purified via MBP attached to Lsm10 on amylose beads. The beads were overnight incubated in a buffer containing ^{3}H SAM and subsequently analyzed by Coomassie Blue staining to identify bound proteins (top panel), and by fluorography to detect protein methylation by endogenous methylosome (bottom panel). Lane 3 shows background proteins bound to amylose beads in the absence of Lsm10/11. No methylation is detected in this sample. Lanes 1 and 2 show methylation of indicated Lsm11 fragments carried out in solution by recombinant methylosome in the presence of the SmE/F/G heterotrimer.

Fig. S3. Methylation of Lsm11 and SmE by recombinant and endogenous PRMT5 methylosome on amylose beads. A. Recombinant methylosome consisting of PRMT5, MEP50 and pICln (lanes 1-4), and endogenous methylosome from HeLa cytoplasmic extract (lanes 5-8) were bound to Lsm10/11ΔL or Lsm10/11ΔNL heterodimers, as

indicated, and immobilized on amylose beads via MBP attached to Lsm10. The beads were overnight incubated in a buffer containing ^{3}H SAM and subsequently analyzed by Coomassie Blue staining to identify bound proteins (top panel), and by fluorography to detect protein methylation (bottom panel). **B.** A fraction of each reaction was also analyzed by silver staining to visualize proteins undetectable by Coomassie Blue staining.

Table S1

Summary of cryo-EM and structure refinement statistics

Structure	Human methylosome-Lsm11/10 complex	Human methylosome-U7 6S complex
Data collection and processing		
Magnification	105,000	105,000
Voltage (kV)	300	300
Electron exposure (e/Å^2)	58.2	51.3
Defocus range (μm)	–0.8 to −2.2	–0.8 to −2.2
Image stacks	4,601	4,662
Initial particle images	1,814,708	3,221,463
Final particle images	754,047	935,088
Symmetry imposed	C1	C1
Map resolution (Å)	2.86	2.69
FSC threshold	0.143	0.143
Map sharpening *B* factor (Å^2)	109.6	113.2
Structure refinement		
No. of protein residues	3,651	
rms deviations		
Bond lengths (Å)	0.009	
Bond angles (°)	0.87	
Clash score	6.2	
Poor rotamers (%)	0.03	
Ramachandran plot		
Favored (%)	97.3	
Allowed (%)	2.7	
Disallowed (%)	0.0	

Fig. S1

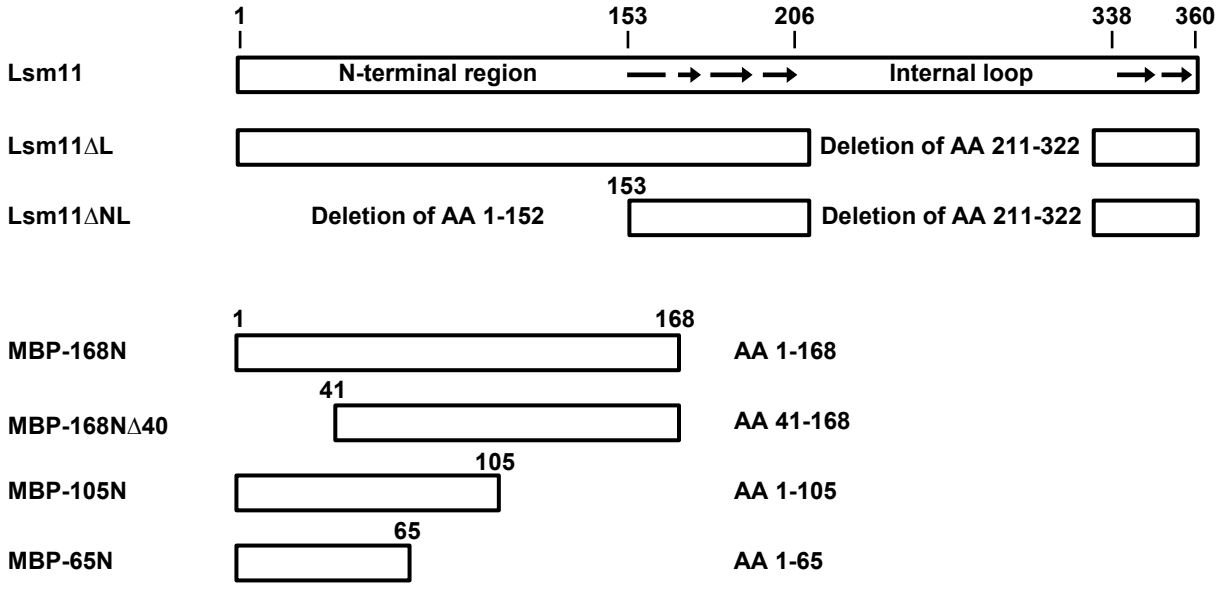

1
153
206
338
360
Lsm11
N-terminal region
Internal loop
Lsm11ΔL
Deletion of AA 211-322
153
Lsm11ΔNL
Deletion of AA 1-152
Deletion of AA 211-322
1
168
MBP-168N
AA 1-168
41
MBP-168NΔ40
AA 41-168
105
MBP-105N
AA 1-105
65
MBP-65N
AA 1-65

Fig. S2

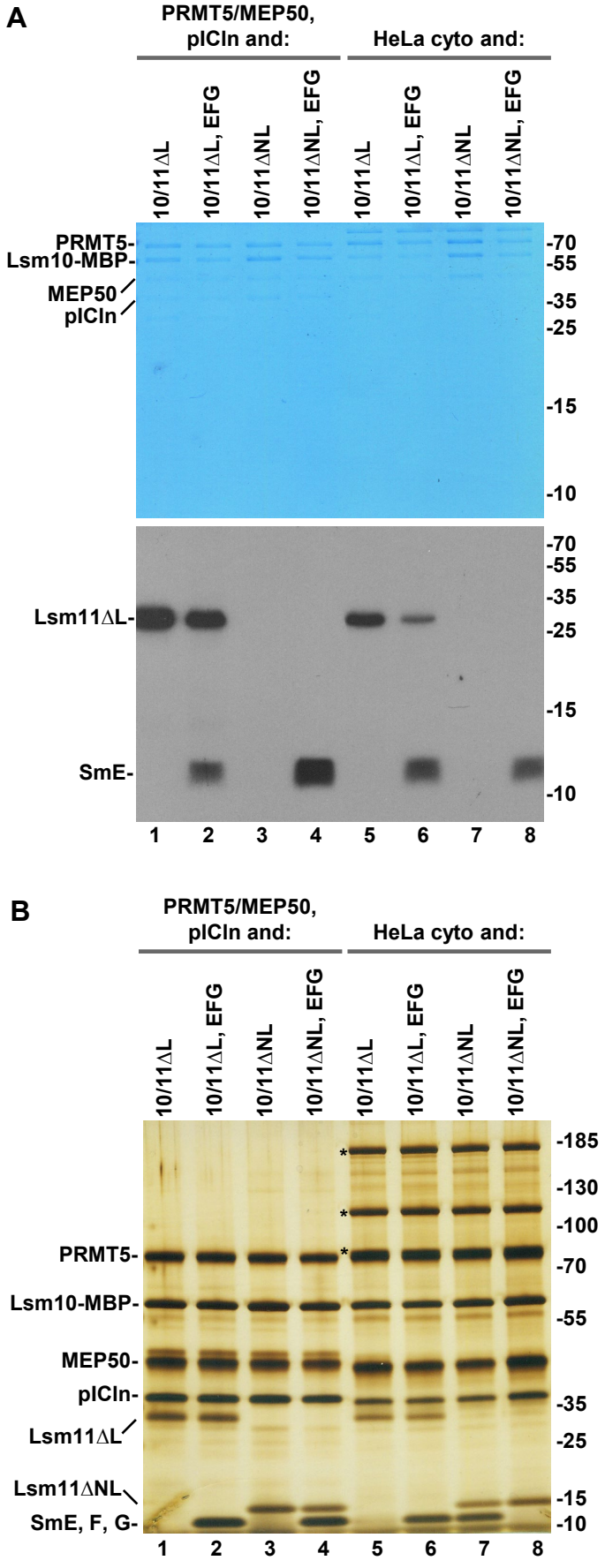
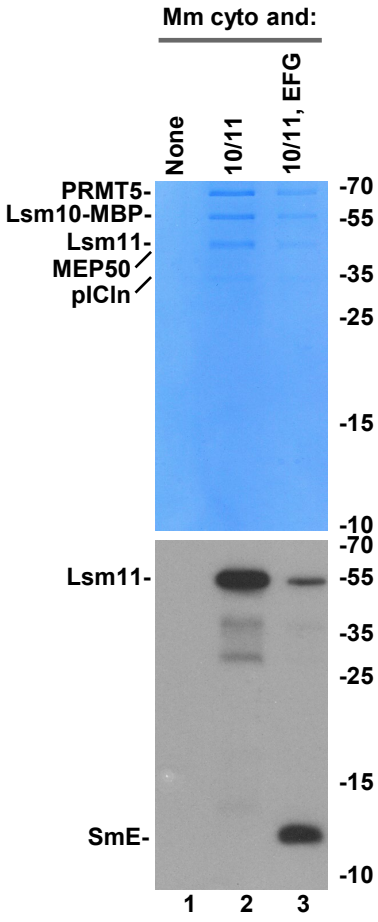

A
PRMT5/MEP50, pICln and:
HeLa cyto and:
10/11ΔL
10/11ΔL, EFG
10/11ΔNL
10/11ΔNL, EFG
PRMT5-
Lsm10-MBP-
MEP50
pICln
Lsm11ΔL-
SmE-
-70
-55
-35
-25
-15
-10
1 2 3 4 5 6 7 8
B
PRMT5/MEP50, pICln and:
HeLa cyto and:
10/11ΔL
10/11ΔL, EFG
10/11ΔNL
10/11ΔNL, EFG
PRMT5-
Lsm10-MBP-
MEP50-
pICln-
Lsm11ΔL
Lsm11ΔNL
SmE, F, G-
*
-185
-130
-100
-70
-55
-35
-25
-15
-10
1 2 3 4 5 6 7 8

Fig. S3


Mm cyto and:
None
10/11
10/11, EFG
PRMT5-
Lsm10-MBP-
Lsm11-
MEP50
pICln
-70
-55
-35
-25
-15
-10
Lsm11-
SmE-
1
2
3