SUPPLEMENTARY INFORMATION

A Plant 5S Ribosomal RNA Mimic Regulates Alternative Splicing of Transcription Factor IIIA Pre-mRNAs

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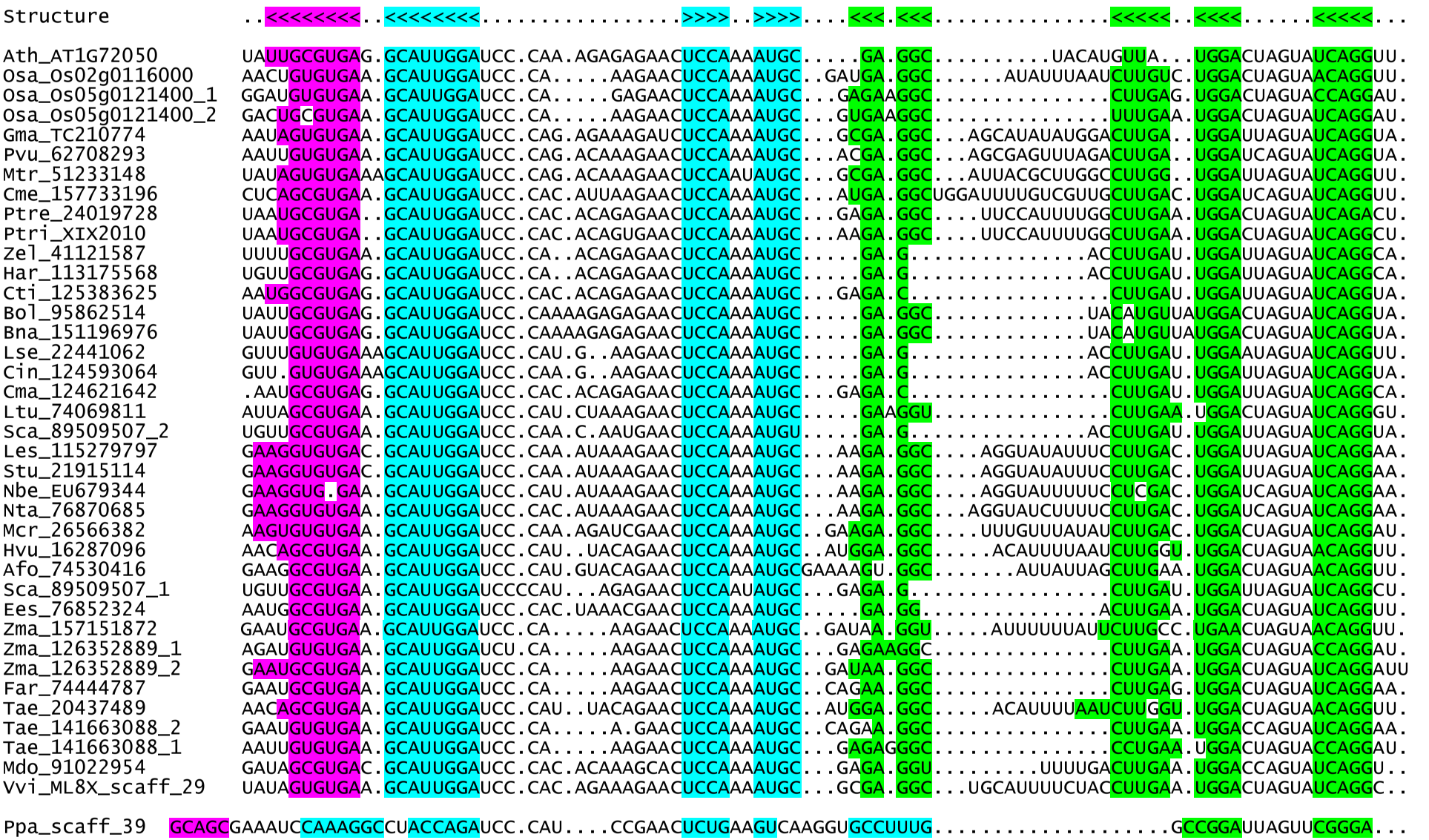
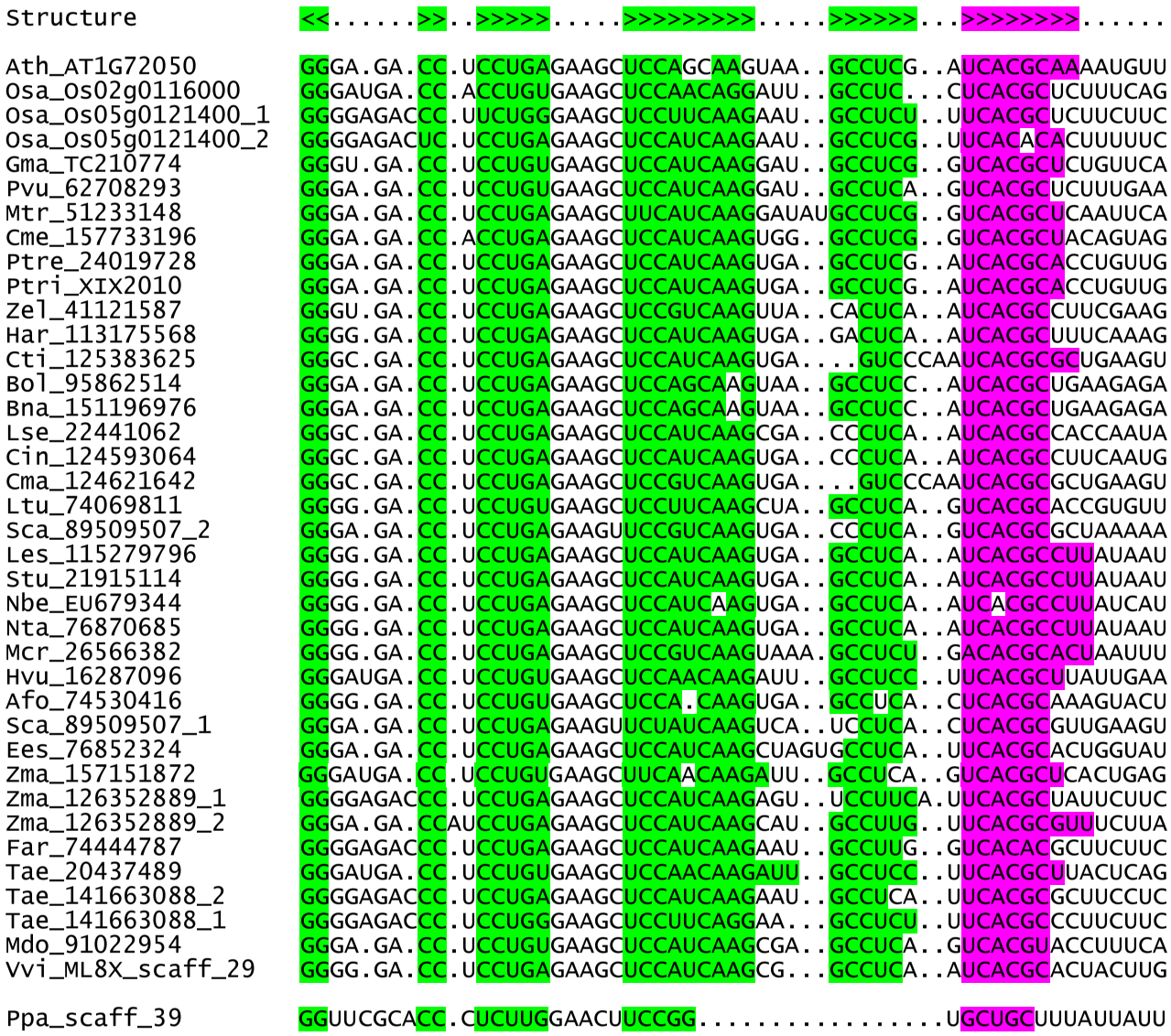
Supplementary Figure 1. Representative sequences for plant 5S rRNA mimic





ORGANISM CODE LEGEND:

Afo	*Aquilegia formosa x Aquilegia pubescens*
Ath	***Arabidopsis thaliana***
Bol	*Brassica oleracea*
Bna	*Brassica napus*
Cin	*Cichorium intybus*
Cma	*Centaurea maculosa*
Cme	*Cucumis melo*
Cti	*Carthamus tinctorius*
Ees	*Euphorbia esula*
Far	*Festuca arundinacea*
Gma	*Glycine max*
Har	*Helianthus argophyllus*
Hvu	*Hordeum vulgare*
Les	*Lycopersicon esculentum*
Lse	*Lactuca serriola*
Ltu	*Liriodendron tulipifera*
Mcr	*Mesembryanthemum crystallinum*
Mdo	*Malus domestica*
Mtr	*Medicago truncatula*
Nbe	*Nicotiana benthamiana*
Nta	*Nicotiana tabacum*
Osa	***Oryza sativa***
Ppa	***Physcomitrella patens***
Ptre	*Populus tremula x Populus tremuloides*
Ptri	***Populus trichocarpa***
Pvu	*Phaseolus vulgaris*
Sca	*Senecio cambrensis*
Stu	*Solanum tuberosum*
Tae	*Triticum aestivum*
Zel	*Zinnia elegans*
Zma	*Zea mays*
Vvi	***Vitis vinifera***

Bold = Genome sequenced

Sequence alignment of plant 5S rRNA mimic representatives from various plant species shows the conservation of sequence and structure. Nucleotides forming pairing interactions P1 through P3 are highlighted in color. Each sequence is annotated with the organism code (see Legend) and the corresponding NCBI gi accession number (for ESTs / cDNAs) or gene locus id (for sequenced genomes). The *Physcomitrella patens* (moss) sequence is displayed on a separate line because it is significantly diverged from the other sequence examples, which are from angiosperms. All sequences except for the example from *P. patens* were used to calculate conservation of nucleotide identity and presence, and covariation or compatible mutations in base-pairing interactions (**Fig. 1a**), as described[1].

We acknowledge the following sources for genomic data: TAIR for *Arabidopsis thaliana*[2], RAP-DB for *Oryza sativa*[3], DoE Joint Genome Institute (JGI) and Poplar Genomic Consortium for *Populus trichocarpa*, Genoscope for *Vitis vinifera*[4], and Cosmoss for *Physcomitrella patens*[5].

Supplementary Figure 2. Variant P5SM representative in the moss *Physcomitrella patens*

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CLUSTAL 2.0.3 multiple sequence alignment[6]

Lhe_5S          GGAUGCGGUCAUA-CCAA-GGCUACUACACCAGAUCCCAUCAGAACUCUGCAGUUAAGC-GCCCUUUGGGCCGGAAUAGUACUGGGAUGGGUG--ACCUCCCGGGAAG
Mpo_5S          GGAUGCGGUCAUA-CCAG-GGCUACUACACCAGAUCCCAUCAGAACUCUGCAGUUAAGC-GCC-CUUGGGCCGGAAUAGUACUGGGAUGGGUG--ACCUCCCGGGAAG
Apu_5S          -GGUGCGGUCAUA-CCAG-GGCUACUACACCGGAUCCCAUCAGAACUCCGUAGUUAAGC-GCC-CUUGGGCCGGAUCAGUACUGGGAUGGGUG--ACCUCCCGGGAAG
Plag_5S         GGAUGCGGUCAUA-CCAA-GGCUACUACACCAGAUCCCAUCAGAACUCUGAAGUUAAGC-GCC-UUUGGGCCGGAAUAGUACUGGGAUGGGUG--ACCUCCCGGGAAG
Ppa_P5SM        CUAUGCAGCGAAAUCCAAAGGC--CU--ACCAGAUCCCAUCCGAACUCUGAAGUCAAGGUGCC-UUU-GGCCGGAUUAGUUC-GGGA-GGUUCGCACCCUCUUGGAAC
                   *** *  * * ***  ***  **  *** ********* ****** * *** ***  ***  ** *******  *** * **** ** *   ***  *  ****

Lhe_5S          UCCCGGUGCUGCAUCCA
Mpo_5S          UCCCGGUGCUGCAUCCA
Apu_5S          UCCCGGUGCUGCACCCU
Plag_5S         UCCCGGUGCUGCAUCCA
Ppa_P5SM        UUCCGGUGCUGCUUUAU
                * **********
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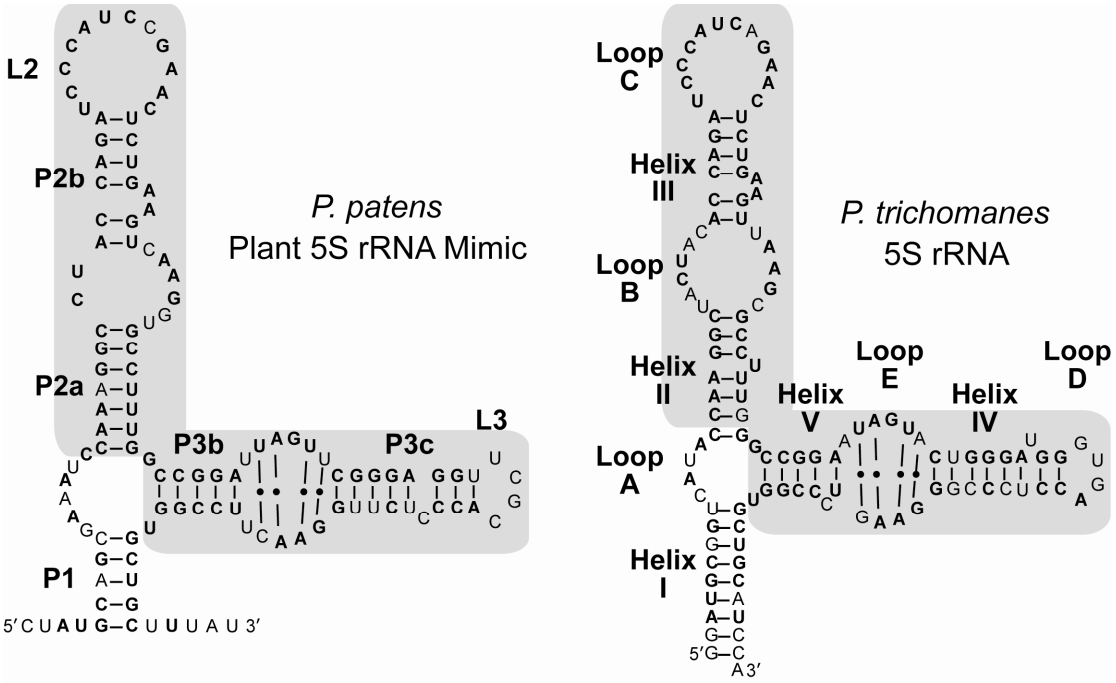
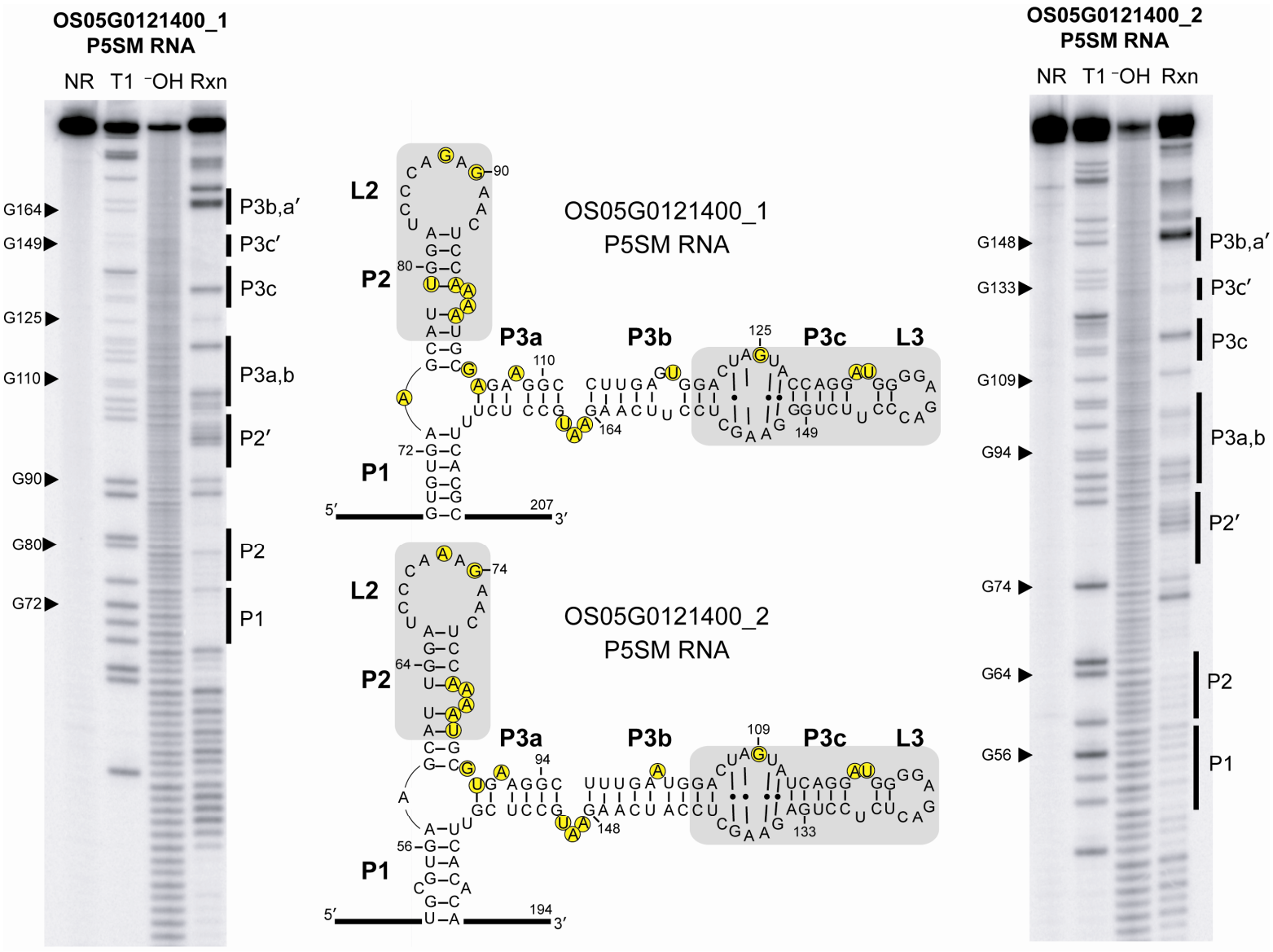


Through tBLASTn searches of the *Physcomitrella patens* genome using the amino acid sequence for zinc fingers 2 and 3 of *At*TFIIIA, we identified a variant of P5SM in a putative TFIIIA gene of the moss *P. patens*. Since the 5S rRNA sequence for this organism is not yet available, we aligned the *P. patens* P5SM sequence to the 5S rRNA sequences for four Bryophyta species: *Marchantia polymorpha* (Mpo, liverwort), *Lophocolea heterophylla* (Lhe, liverwort), *Plagiomnium trichomanes* (Plag, moss), and *Anthoceros punctatus* (Apu, hornwort)[7]. Asterisks indicate conserved nucleotides across the five sequences. The secondary structure model for the *P. patens* P5SM was manually determined based upon comparisons to *P. trichomanes* 5S rRNA (shown) and *A. thaliana* P5SM (**Fig. 1c**). Nucleotides in bold are identical between moss 5S rRNA and P5SM, and were used to calculate the percentage of the 5S rRNA sequence maintained in the P5SM sequence.

Supplementary Figure 3. In-line probing analysis of *Oryza sativa* P5SM RNAs



The structure of two *O. sativa* representatives of P5SM were analyzed by in-line probing ("1" consists of nucleotides 903-1109 and "2" consists of 1500-1693 of OS05G0121400 gene, NCBI gi 115465852). Nucleotides spontaneously cleaved under in-line reaction conditions are circled in the secondary structure models of the rice P5SM elements. Labeled G nucleotides correspond to the same positions labeled in the *A.thaliana* representative of P5SM (**Fig. 1b**).

Supplementary Figure 4. Sequences of TFIIIA gene, protein, and splice products

LEGEND: Primer annealing site, UTR, coding sequence, cassette sequence, stop codon, splice site, zinc finger, cassette protein sequence, **!** = translation termination, XhoI

AT1G72050: NCBI gi 42592260 / NC_003070 region 27118686..27121132

Precursor mRNA sequence | **Protein sequence**

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 151 AAACUAUCUC AUCACUAAAC ACAUCCAAUC UCAUCAUCAG GUUUGAGAUC      NYLITKHIQSHHQ
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 951 CAAUAGGCAU CUUCUUACAC AUAAAGGGAA GCUCUUUAAG UGUCCGAAGG      NRHLLTHKGKLFKCPKE
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RT-PCR products (from primers a and b) were cloned into TOPO vector and clones were sequenced. Primer sites are underlined. Overhang and restriction sites in primers are in lowercase.

Primer a: 5´-atgcggatccGTGCGGCGTCTTGATGGA
Primer b: 5´-ACTCTTGCAGTTCTCCTTCG

AT1G72050 PRECURSOR

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AT1G72050 SPLICE PRODUCT II

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 601 AGUGUCCGAA GGAGAACUGC AAGAGU
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OS02G0116000

Precursor mRNA sequence

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Protein sequence

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RT-PCR products (from primers c and d) were cloned into TOPO vector and clones were sequenced.

Primer c: 5´-TGATGGAGACATGAGGGTTG

Primer d: 5´-TGTTACAGCCCTCCTCCTTG

OS02G0116000 SPLICE PRODUCT I

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OS02G0116000 SPLICE PRODUCT II

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OS05G0121400

First cassette sequence, second cassette sequence

Precursor mRNA sequence

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 301 CUGCUAGGGA UAUUAGGCGG UACAAGUGCG AUUUCUGCAG UGUCGUCCGU
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 701 UGAAUUUCGA UACAUGUUCA CUGAAAUGAU CAAGUUCUUU GGCGCAUGUG
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Protein sequence

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                                                    CKEMDHDCKVCGASFKK
                                                    PAHLRQHMQSHSLE

                                                    TIS
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                                                    NARRP!

                                                    TIYD
                                                    CVKHWIPKNSKMREGFE
                                                    WTSIRMGRLS!

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                                                    KYASKLQKHEESH

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RT-PCR products (from primers f and g) were cloned into TOPO vector and clones were sequenced.

Primer f: 5´-ATGATTACTTGGGACGTGGT

Primer g: 5´-GTCTTCCCACAGTTTTCC

OS05G0121400 SPLICE PRODUCT I

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 201 ACACAUCAAG GAAAGCUAUU UGCAUGCCCC AUGGAAGGAU GCAACCGUAA
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 351 UGUGGGAAGA C
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OS05G0121400 SPLICE PRODUCT II

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OS05G0121400 SPLICE PRODUCT III

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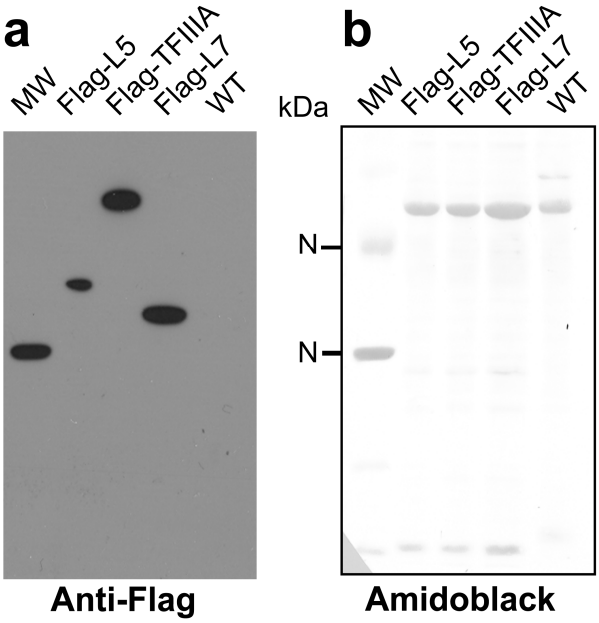
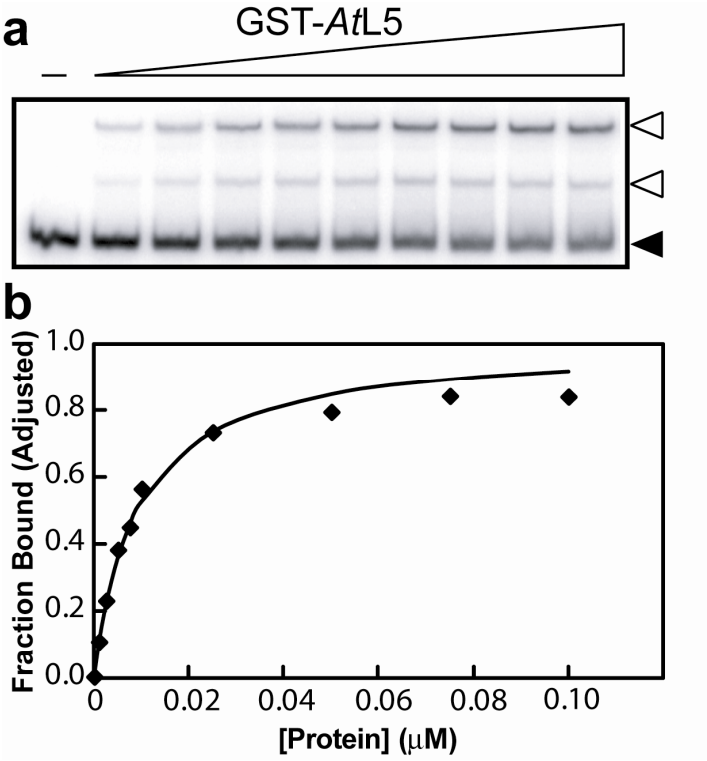
Supplementary Figure 5. Expression of Flag-tagged versions of L5, TFIIIA, and L7 proteins



(**a**) Western blot analysis of crude extracts from *N. benthamiana* infiltrated with Flag-L5, Flag-TFIIIA, Flag-L7 coding sequence constructs, or none (WT). A Flag peptide sequence was inserted at the N-terminus after the start codon and does not change the effect on Pre-EGFP reporter splicing (data not shown). The immunoblot was probed with anti-Flag antiserum. The 30 kDa molecular weight marker (MW) protein cross-reacts with the antibody. The molecular weights for the detected proteins were estimated using a standard curve for all marker bands (expected sizes in parentheses): Flag-L5 32 kDa (36 kDa), Flag-TFIIIA 50 kDa (48 kDa), Flag-L7 28 kDa (30 kDa).
(**b**) Amidoblack staining of the western blot shown in **a**, which visualizes all proteins present on the blot. The major band in the plant extracts corresponds to Rubisco.

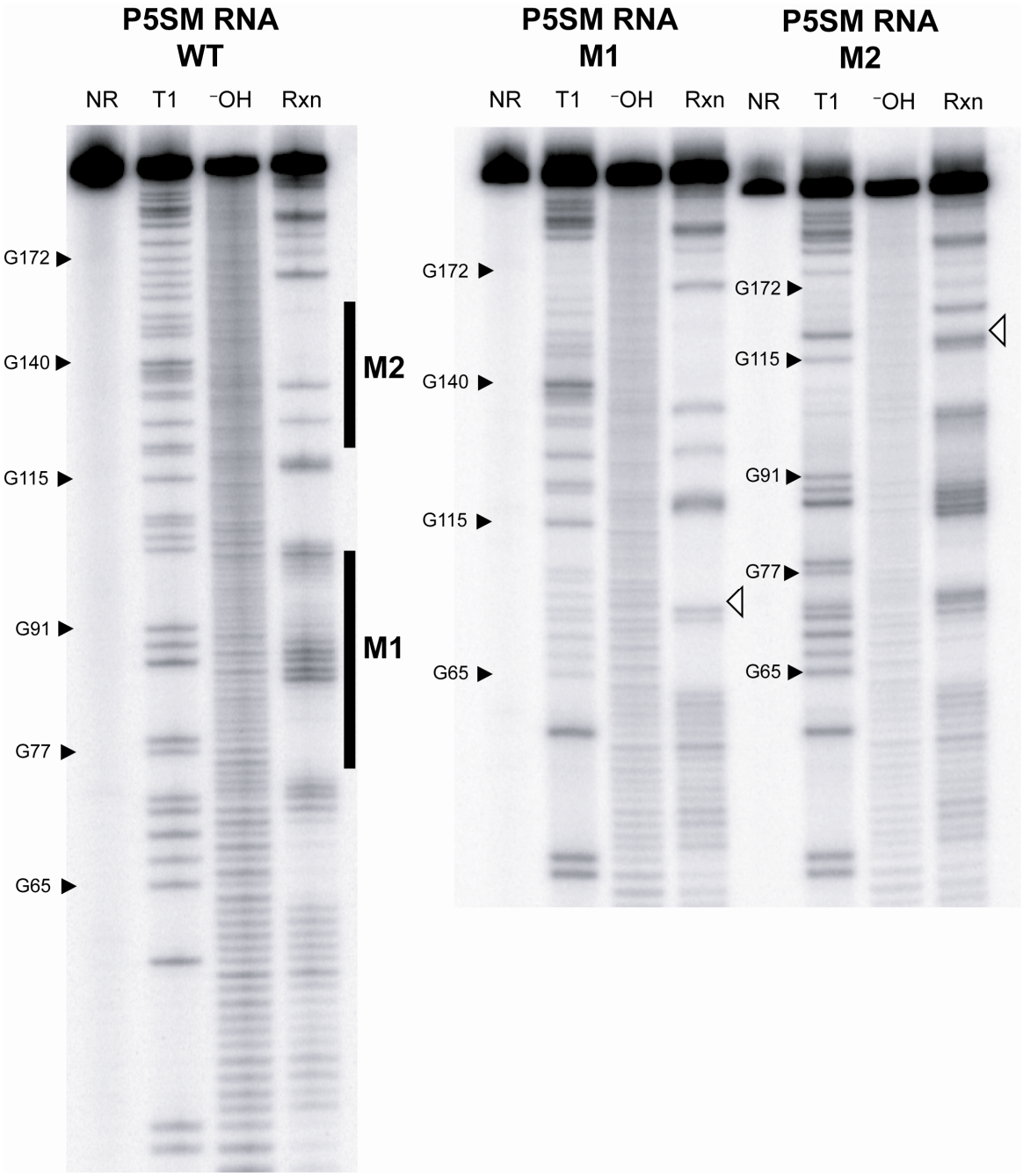
The expression of all three proteins tested for an effect on TFIIIA reporter was confirmed by western blot analysis. In general, somewhat less L5 is expressed than TFIIIA or L7, but it alone has an effect on reporter splicing and expression (**Fig. 3a, c**). No protein was detected by anti-Flag probing of crude extracts from wild-type *N. benthamiana* plants. Roughly equal loading of crude extracts in all lanes is shown by amidoblack staining and comparison of the main band corresponding to the highly abundant protein Rubisco (large subunit).

Supplementary Figure 6. GST-*At*L5 fusion protein binds to 5S rRNA *in vitro*

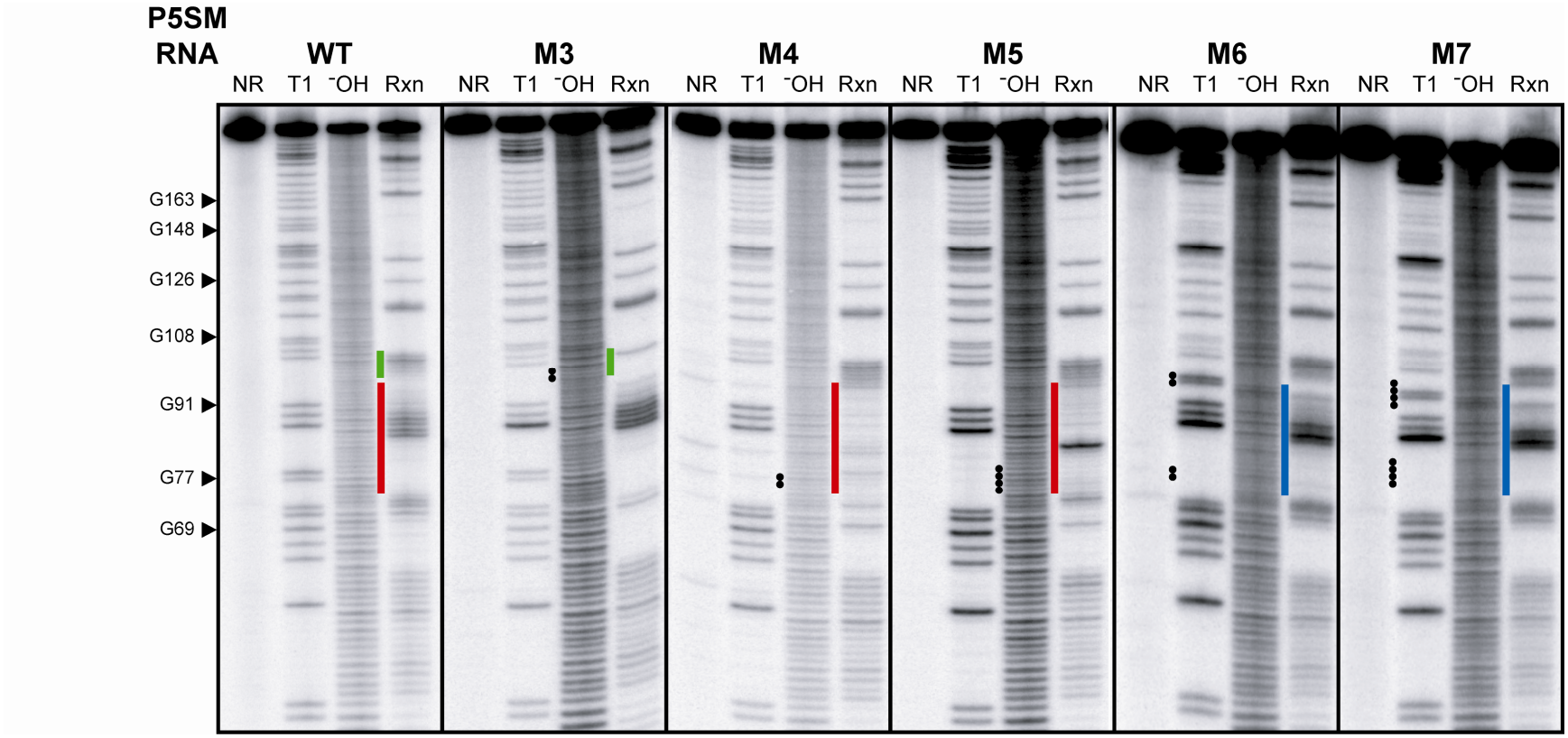


(**a**) *In vitro* binding analysis for 5S rRNA with GST-*At*L5. Sequence used is the major 5S transcript identified in *A. thaliana*[8]. Radiolabeled RNA was incubated at 25ºC in binding buffer in the absence or presence of protein (0-0.1 μM). RNA-protein complex formation was analyzed by non-denaturing PAGE. Unbound RNA (filled arrowhead) and RNA-protein complexes (open arrowhead) are indicated. Similar to previous data[9], two bands corresponding to RNA-protein complexes are observed which have similar binding characteristics. This suggests that the RNA fold may be heterogenous but still bind the protein. Additionally, ~50% of the RNA remains unbound at saturating concentrations of protein, even with optimized renaturation protocols.
(**b**) Representative plot used to determine the apparent K_D for the interaction between 5S rRNA and GST-*At*L5 protein. Maximal binding observed at 0.5 μM protein was normalized to 1. Graphed line corresponds to the best-fit curve for a two-state binding model with 1:1 stoichiometry and K_D of 9 nM. Analysis of either of the two RNP signals or a summation of both give similar results for the apparent K_D.

Supplementary Figure 7. In-line probing of *A. thaliana* P5SM RNA mutants



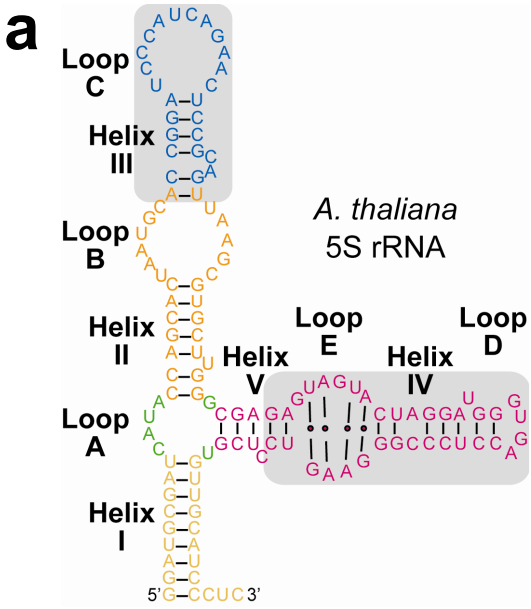
Comparison of in-line patterns between wild-type P5SM RNA (nucleotides 603-810 of *At*TFIIIA gene, NCBI gi 42592260) and truncated constructs show that deletion of the designated regions (M1 and M2, **Fig. 5a**) of the RNA do not perturb the other parts of the RNA. A white arrowhead indicates the location of each truncation. The unperturbed portions of the RNAs remain well-folded and have similar patterns of spontaneous cleavage compared to WT except missing the indicated deletion region.

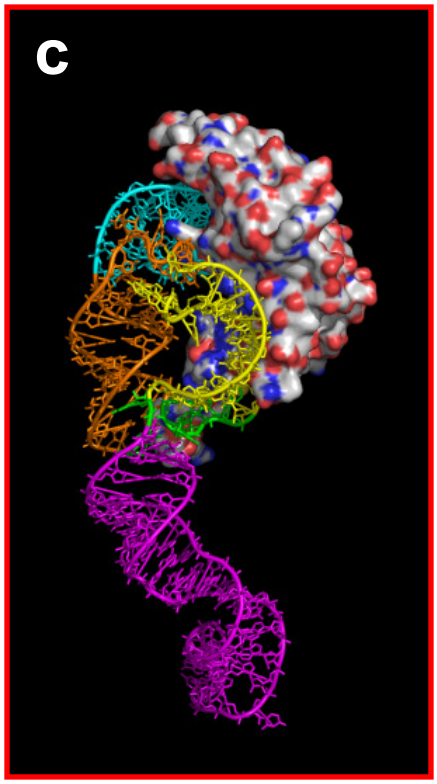


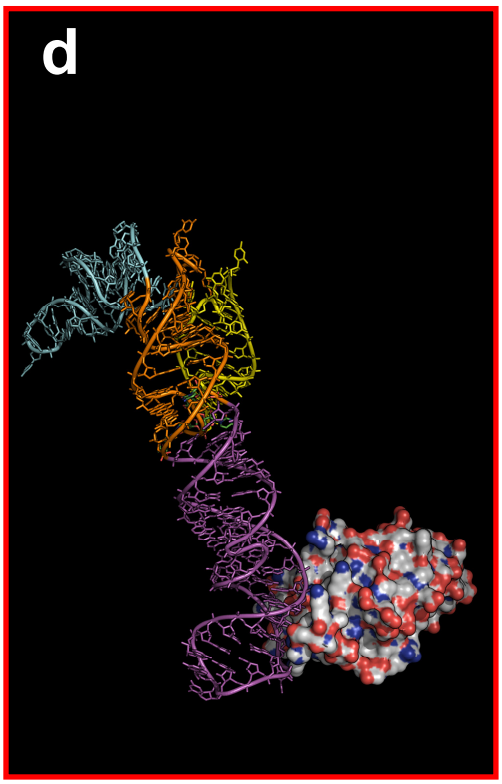
Comparison of in-line patterns between wild-type P5SM RNA and mutant constructs with nucleotide changes in the P2 stem (M3 through M7, **Fig. 5a**) show expected changes in structure confined to the P2 stem. Positions of labeled G nucleotides are the same as shown for the shorter construct in **Fig. 1b**. Nucleotide deletions or substitutions are mapped by black dots. Colored bars indicate regions for comparison between WT and a corresponding mutant.

Deletion of the dinucleotide bulge (M3) results in a more stable hairpin structure, indicated by reduced spontaneous phosphoester cleavage in the stem region surrounding the deleted AA (green bars). In contrast, nucleotide substitutions that disrupt base pairing (M4, M5) destabilize the normal P2 stem structure, indicated by including increased spontaneous cleavage overall in the stem region and alterations in the cleavage pattern (red bars). Finally, nucleotide substitutions that compensate for base pairing (M6, M7) restore very similar in-line patterns to WT (blue bars).

Supplementary Figure 8. The interaction of 5S rRNA with ribosomal proteins L18 (L5 homolog) and L30 (L7 homolog) in the *Haloarcula marismortui* large ribosomal subunit





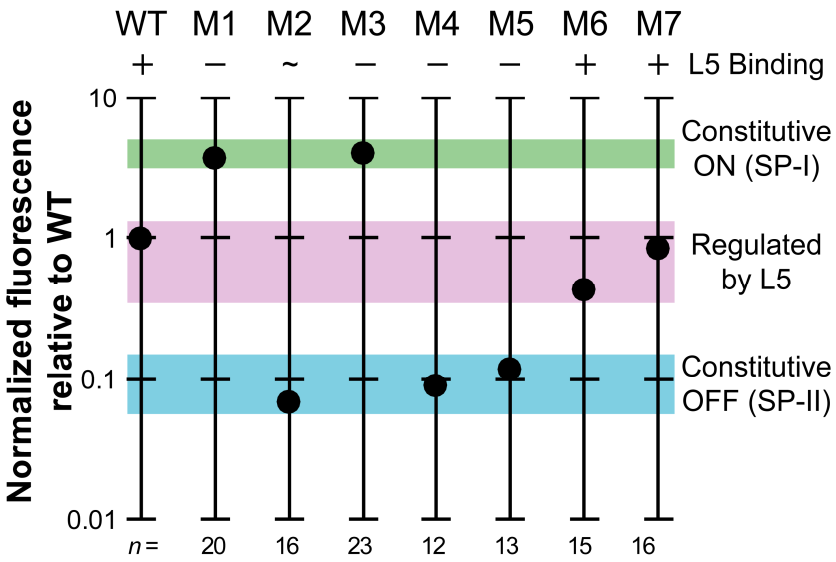


Atomic coordinates used to make figures are from the crystal structure of the large ribosomal subunit (pdb accession code 1QVG, chain 9 = 5S rRNA, chain M = L18P, the homolog to L5, chain V = L30P, the homolog to L7)[10].

(**a**) Conventional 5S rRNA structure model colored in accordance with panels and with regions homologous to P5SM boxed in grey; (**b**) view highlighting the 5S rRNA helix III interaction with L5; (**c**) second view highlighting the 5S rRNA helix I interaction with L5 and showing that helix II is not in contact with the protein; (**d**) third view highlighting the 5S rRNA loop E interaction with L7.

P5SM does not have a region strongly homologous to helix I, which may account for lower affinity in the P5SM-L5 interaction. Other structural features, such as the conserved but distinct P1 (**Fig. 1c**), may partially substitute for helix I, whose length has been shown to be important for complex formation in yeast[11], but allow L5 to bind P5SM in the context of a long pre-mRNA.

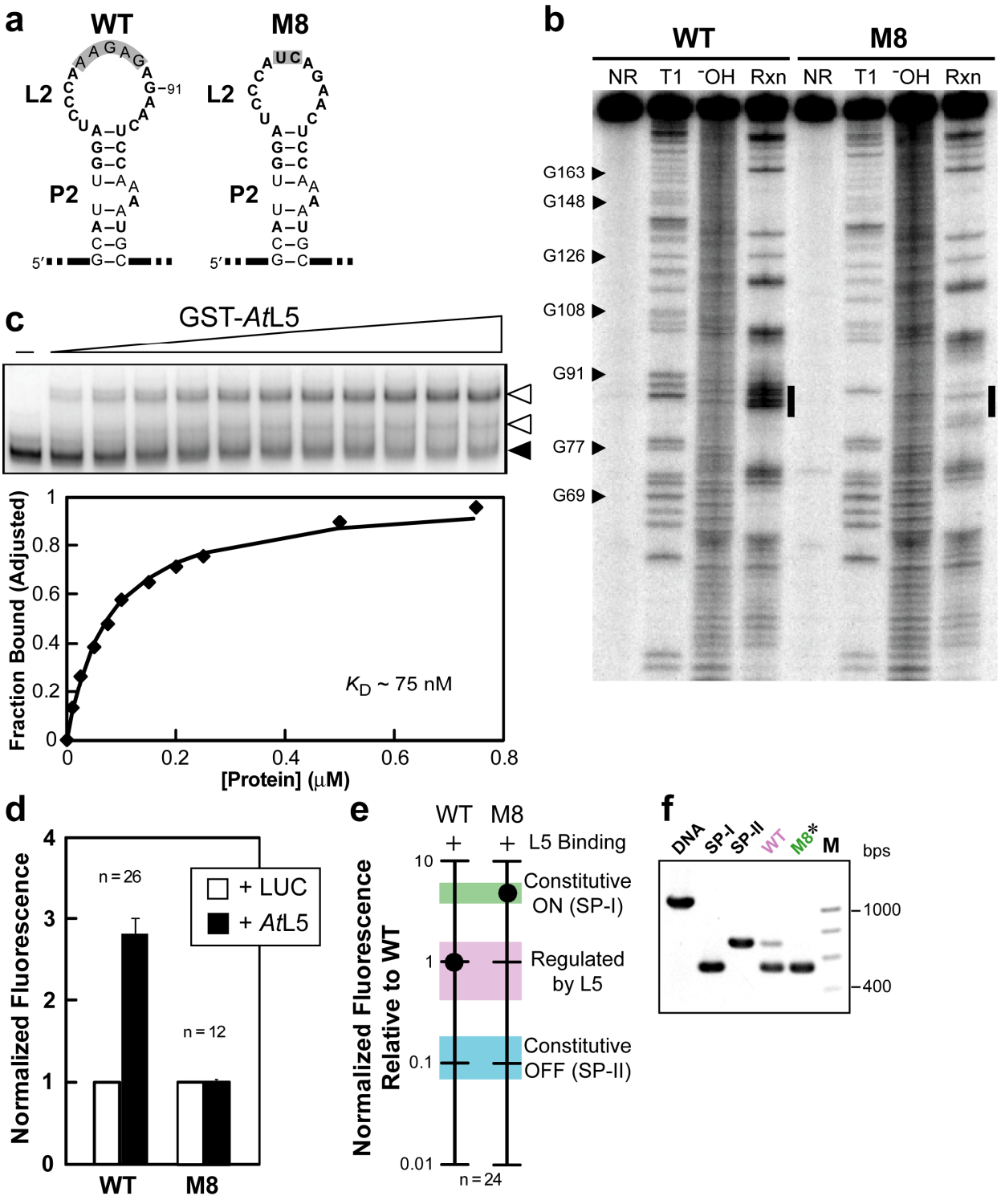
Supplementary Figure 9. The relative protein expression of P5SM mutant reporters reflects their splicing patterns



Reporter fluorescence for mutants normalized to WT Pre-EGFP are shown on a semi-log plot. WT and each mutant reporter construct was transformed on half of the same leaf to ensure near identical conditions for comparison. Effects from varying endogenous L5 levels were minimized by measuring reporter fluorescences upon constitutive *At*L5 expression. Thus, the data from this figure corresponds to the filled bars in **Fig. 5d**, except that the former is normalized to WT + *At*L5 and the latter is normalized to + LUC for each construct separately. Numbers of independent leaf samples (*n*) measured are shown. Error bars representing SEM are within the diameter of the symbols.

The observed protein expression relative to WT corresponds to the three splicing pattern types labeled in the different colors, which are also shown for a representative leaf sample in **Fig. 5d**.

Supplementary Figure 10. Replacement of the purine-rich loop sequence in L2 causes constitutive exon skipping without loss of L5 binding



(a) Partial sequence of the L2 mutant (M8) compared to wild-type (WT) P5SM RNA with the altered nucleotides shaded. Bolded nucleotides are identical to the *A. thaliana* 5S rRNA sequence.

(b) Comparison of RNA cleavage patterns from in-line probing between WT and M8 P5SM constructs show that changes in structure are confined to L2. The labeled G nucleotides correspond to the same positions labeled for the shorter construct in **Fig. 1b**. Black bars indicate the location of nucleotide changes. Note that the pattern for M8 is shifted down from the adjacent pattern for WT above this region because these 5′ cleavage products include the deletion, which shortens the length of M8 relative to WT.

(c) *In vitro* binding analysis for the M8 P5SM RNA with GST-*At*L5. The same methods were used as described in **Fig. 4**. As observed for 5S rRNA in **Supplementary Fig. 6**, two bands corresponding to RNA-protein complexes are observed, suggesting that the RNA fold may be heterogenous but still binds the protein. Also shown is a representative plot used to determine the apparent K_D for the interaction between M8 P5SM RNA and GST-*At*L5 protein. Maximal binding observed at 1 μM was normalized to 1. Graphed line corresponds to the best-fit curve for a two-state binding model with 1:1 stoichiometry and K_D of 75 nM.

(d) *In vivo* expression analysis for the Pre-EGFP reporter construct incorporating the M8 mutation in P5SM with co-expression of AtL5. Data for the Pre-EGFP WT construct (from **Fig. 3c**) is shown for reference. For each construct, the EGFP fluorescence measured with expression of luciferase (LUC) was set to a value of 1. Number of independent leaf samples (*n*) measured are shown. Error bars represent SEM.

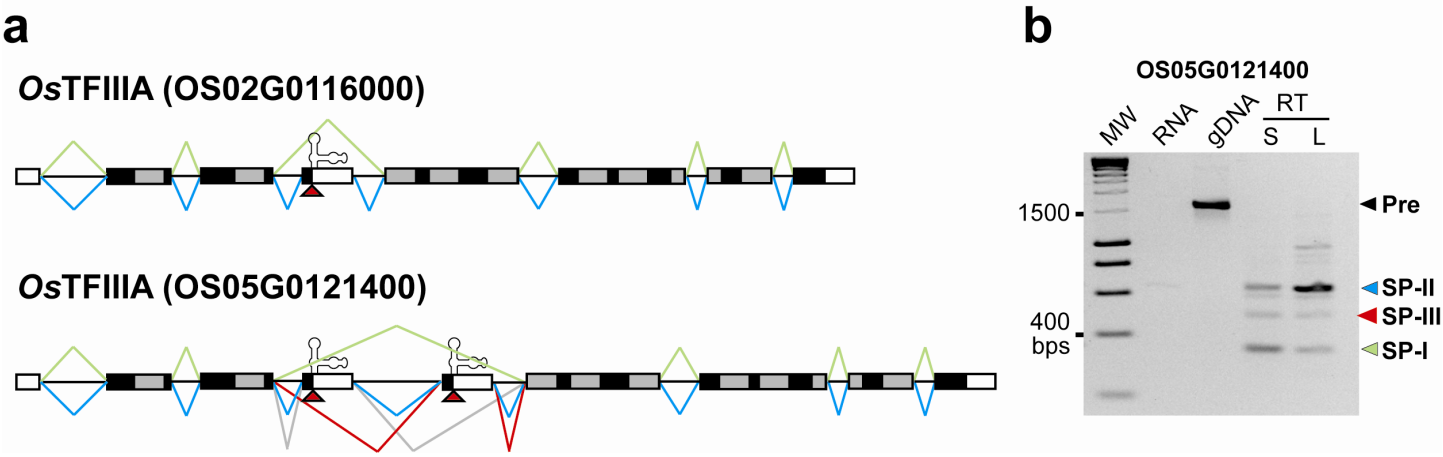
(e) Reporter fluorescence for the M8 mutant normalized to WT Pre-EGFP shown on a semi-log plot. WT and M8 were transformed on half of the same leaf to ensure near identical conditions for comparison. Effects from varying endogenous L5 levels were minimized by measuring reporter fluorescences upon constitutive *At*L5 expression. Number of independent leaf samples (*n*) measured are shown. Error bars representing SEM are within the diameter of the symbols.

(f) RT-PCR detection of splice products arising from splicing of WT versus M8* Pre-EGFP reporter constructs. The M8* construct is identical in sequence to the M8 construct analyzed in **d** and **e**, except for two single-nucleotide mutations in the constitutive intronic region. These additional mutations do not affect reporter splicing, as the protein expression results for M8* were the same as for M8. This set consists of WT and M8* from a representative leaf sample, in which *At*L5 was over-expressed. The color coding corresponds to the splicing pattern types labelled in **e**. Also shown are PCR products corresponding to unspliced precursor, SP-IE, and SP-IIE derived from DNA templates.

Based upon a displacement model for splicing regulation by ribosomal protein L5, the purine-rich insertion in the L2 loop of P5SM was postulated to be an exon splicing enhancer (ESE) that binds a splice factor to favor exon definition and splicing to SP-II. To test its proposed role as an ESE with minimal perturbations to other functions of the RNA, the five purine nucleotides were replaced with the UC sequence from Loop C of 5S rRNA. As expected, the replacement mutant M8 still binds L5, with comparable affinity as the WT P5SM and changes to the structure are confined to the L2 region. However, unlike all other previously tested P5SM reporter constructs that bound L5 (WT, M6, M7), loss of the purine-rich sequence causes loss of L5 activation and constitutive splicing to SP-I, as observed by reporter fluorescence and RT-PCR. These results support a role for this sequence in exon definition, possibly as an ESE that recruits an exonic splice factor.

Supplementary Figure 11. The two TFIIIA genes in *O. sativa*, *Zea mays*, and *Triticum aestivum* may be differentially regulated by a single or tandem arrangement of the P5SM element

(**a**) Two TFIIIA genes (OS02G0116000 and OS05G0121400) were identified in the *O. sativa* genome, shown is the comparison of the annotated splicing models; (**b**) RT-PCR analysis of OS05G0121400, the rice TFIIIA gene containing the tandem P5SM arrangement detected three main splice types (SP-I, SP-II, and SP-III) but not the fourth predicted splice type (splice reaction in grey); see Figure S4 for sequences of the gene, the translated protein, and the splice products detected by RT-PCR for OS02G0116000 and OS05G0121400; (**c**) sequences of SP-II type transcripts for the homologous two TFIIIA genes in *Zea mays* and *Triticum aestivum* (from EST or cDNA data). Cassette exon is highlighted in blue; P5SM sequences are underlined.



c

Tae_20437489 (corresponds to SP-II type transcript)
Contains single P5SM

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 121 GAGATGAGAA GTCTGAAGAG ACACATGGCA AAGATATTAG ACGCATCAAG TGTGAATTTT
 181 GCACTGTTGT TAGGTCCAAG ATGTATCTCA TACGAGCTCA CATGGTAGCT CAGCACAAGG
 241 ATGAGTTGGA CGCATCAGAA ATCTATGACT CAAATGGTGA AAAGGTTGTT TATGGGGTTG
 301 GACACACATG TGAAGAGTGT GGAGCTTGTT TCCGGAAGCC AGCTCATCTG AAGCAGCATA
 361 TGCAAAGTCA TTCCAAAGAG GGTTTAGATA ATAGATACTT AACAGCGTGA AGCATTGGAT
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 481 GGTTGGGATG ACCTCCTGTG AAGCTCCAAC AAGATTGCCT CCTTCACGCT TACTCAGGAG
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Tae_141663088 (corresponds to SP-II type transcript)
Contains tandem P5SM

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Zma_157151872 (Chr 8 contig)
Contains single P5SM

Chr 8 Contig 27601..37898
Exons are based upon manual alignment of available ESTs to genomic sequence
Sequences highlighted in red are inconclusive from available ESTs

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Zma_126352889 (Chr 6 contig)
Contains tandem P5SM

Chr 6 Contig 74014..86960
Exons are based upon manual alignment of available ESTs to genomic sequence

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SUPPLEMENTARY TABLE 1. Sequences of DNA primers

DNA templates for *in vitro* transcription		
	A. thaliana P5SM, minimal (nucleotides 649-793 of AT1G72050)	
DNA1	5′-*TAATACGACTCACTATA*GGTCTTACCTATGTTATTGCG	For, T7 promoter
DNA2	5′-CTCAAACATTTTGCGTGATCG	Rev
	A. thaliana P5SM RNA (nucleotides 603-810 of AT1G72050)	
DNA3	5′-*TAATACGACTCACTATAG*GTTTATACATTTGCAGGAAATAA	For, T7 promoter
DNA4	5′-GCTATTGAACATCAGACCTC	Rev
	O. sativa P5SM RNA_1 (nucleotides 903-1109 of Os05g0121400)	
DNA5	5′-*TAATACGACTCACTATA*GGTTGGGTAGGTTTGTTGATCTA	For, T7 promoter
DNA6	5′-GCAACAGAAGAAATCACCTGAAG	Rev
	O. sativa P5SM RNA_2 (nucleotides 1500-1693 of Os05g0121400)	
DNA7	5′-*TAATACGACTCACTATA*GGCCTATTGTGGTTTCTGATATATT	For, T7 promoter
DNA8	5′-TTTGAAATGAACAAGCAAACCTG	Rev
	A. thaliana 5S gene (CIC YAC 6A1, [8])	
DNA9	5′-CGTGATTTGGGCTATATTACG	For
DNA10	5′-CAGTCTACAAGTTATCGAGTCATA	Rev
	A. thaliana 5S rRNA	
DNA11	5′-*TAATACGACTCACTATA*GGATGCGATCATACCAGC	For, T7 promoter
DNA12	5′-GAGGGATGCAACACGAG	Rev
RT-PCR analysis		
	TFIIIA (AT1G72050) from *A. thaliana*	
a (DNA13)	5´-atgc**ggatcc**GTGCGGCGTCTTGATGGA	For, BamHI
b (DNA14)	5´-ACTCTTGCAGTTCTCCTTCG	Rev
e (DNA15)	5´-GGCACGGGCAGCTTACCGGTGGTGCATATGAACTTCAGGGT	Rev (EGFP)
	TFIIIA (Os02G0116000) from *O. sativa*	
c (DNA16)	5´-TGATGGAGACATGAGGGTTG	For
d (DNA17)	5´-TGTTACAGCCCTCCTCCTTG	Rev
	TFIIIA (Os05G0121400) from *O. sativa*	
DNA18	5′-ATGATTACTTGGGACGTGGT	For
DNA19	5′-GTCTTCCCACAGTTTTCCTC	Rev
qRT-PCR analysis		
	TFIIIA transcripts retaining exon (SP-II and unspliced*)	
DNA20	5′-TTATTGCGTGAGGCATTGGA	For
DNA21	5′-TCTCAGGAGGTCTCCCAACCT	Rev
	TFIIIA transcripts skipping exon (SP-I)	
DNA22	5′-TGTCAAGAATGTGGTGCTGA	For
DNA23	5′-GTAAAAGATCTCTCGAGCGAATG	Rev
	DsRED transcripts (reference)	
DNA24	5′-AGACCCACAAGGCCCTGAA	For
DNA25	5′-CAGCTGCACGGGCTTCTT	Rev
Cloning of reporter constructs		
	5' fragment of TFIIIA (Pre-EGFP, I-EGFP, II-EGFP)	
DNA26	5′-atgc**ggtacc**GTGCGGCGTCTTGATGGA	For, KpnI
DNA27	5′-agct**tctaga**ATCCACATAGCAAGTAAAAGA	Rev, XbaI
	EGFP without start codon	

DNA28	5′-agct**tctaga**GTGAGCAAGGGCGAGGA	For, XbaI
DNA29	5′-agct**gtcgac**TTACTTGTACAGCTCGTCCATGC	Rev, SalI
	Flag tag insertion in frame within N-terminal coding region	
DNA30	atgc**ctcgag**GACTACAAAGACGATGATGACAAG**ctcgag**atgc	For, XhoI, XhoI
DNA31	gcat**ctcgag**CTTGTCATCATCGTCTTTGTAGTC**ctcgag**gcat	Rev, XhoI, XhoI
Cloning of protein coding sequences		
	TFIIIA cDNA (AT1G72050) from *A. thaliana*	
DNA32	5′-gac**ggatcc**ATGGCGGAAGAAGCTAAAG	For, BamHI
DNA33	5′-gac**gtcgac**CTAGCAAGTTTCGTGTTCTTC	Rev, SalI
	L5 cDNA (AT3G25520) from *A. thaliana*	
DNA34	5′-gac**ggatcc**ATGGTGTTTGTGAAGTCCACC	For, BamHI**
DNA35	5′-gac**gtcgac**TAAAGAAGGCTTGACTGATTTACTCTTC	Rev, SalI
DNA36	5′-atg**cagatc**tATGGTGTTTGTGAAGTCCACC	For, BglII
	L7A cDNA (AT1G80750) from *A. thaliana*	
DNA37	5′-atgc**ggatcc**ATGGCTGAGGAAGAAGCTAA	For, BamHI
DNA38	5′-atgc**gtcgac**CTAATTCATTTTGCTGATGAGA	Rev, SalI
	L7B cDNA (AT2G01250) from *A. thaliana*	
DNA39	5′-atgc**ggatcc**ATGGTTGAGTCAAAGGTTGT	For, BamHI
DNA40	5′-atgc**gtcgac**CTAATTCATCCTCCTGATAAGC	Rev, SalI
	N-terminal Flag tag w/ start codon and BamHI overhangs	
DNA41	**gatcc**ATGGACTACAAAGACGATGATGACAAG**g**	For, BamHI, Flag
DNA42	**gatcc**CTTGTCATCATCGTCTTTGTAGTCCAT**g**	Rev, BamHI, Flag
	Primer containing N-terminal Flag tag w/ start codon and overlap 5' end of L5 cDNA (internal BamHI site prevents use of above primers)	
DNA43	atgc**agatct**ATGGACTACAAAGACGATGATGACAAG ATGGTGTTTGTGAAGTCC	For, BglII, Flag
P5SM mutant constructs by two-piece PCR ligation		
	M1 (template TOPO-DNA26/27 PCR)	
DNA44	5′-GTAGCCTCGCCTCACGCAATAACATAGG	5' seg, w/ DNA3
DNA45	5′-GCGTGAGGCGAGGCTACATGTTATGGAC	3' seg, w/ DNA4
	M2 (template TOPO-DNA26/27 PCR)	
DNA46	5′-GCTTACTTGCTATAACATGTAGCCTCGCATTTTGG	5' seg, w/ DNA3
DNA47	5′-GCTACATGTTATAGCAAGTAAGCCTCGATCACG	3' seg, w/ DNA4
	M3 (template TOPO-DNA26/27 PCR)	
DNA48	5′-CGCATTGGAGTTCTCTCTTTGGGATCC	5' seg, w/ DNA3
DNA49	5′-CTCCAATGCGAGGCTACATGTTATGGAC	3' seg, w/ DNA4
	M4 (template TOPO-DNA26/27 PCR)	
DNA50	5′-GGGATGGAATGCCTCACGCAATAACATAGG	5' seg, w/ DNA3
DNA51	5′-CATTCCATCCCAAAGAGAGAACTCCAAAATGC	3' seg, w/ DNA4
	M5 (template TOPO-DNA26/27 PCR)	
DNA52	5′-GGGAAGGTATGCCTCACGCAATAACATAGG	5' seg, w/ DNA3
DNA53	5′-CATACCTTCCCAAAGAGAGAACTCCAAAATGC	3' seg, w/ DNA4
	M6 (template TOPO-M4)	
DNA54	CATTTTCCAGTTCTCTCTTTGGGATGGAATGC	5' seg, w/ DNA3
DNA55	GAACTGGAAAATGCGAGGCTACATGTTATGG	3' seg, w/ DNA4
	M7 (template TOPO-M5)	
DNA56	CATTTACCTGTTCTCTCTTTGGGAAGGTATGC	5' seg, w/ DNA3

DNA57	GAACAGGTAAATGCGAGGCTACATGTTATGG	3’ seg, w/ DNA4
	M8 (template TOPO-DNA26/27 PCR)	
DNA58	GAGTTCTGATGGGATCCAATGCCTCACG	5’ seg, w/ DNA3
DNA59	GATCCCATCAGAACTCCAAAATGCGAGGC	5’ seg, w/ DNA4

Italicized sequence includes T7 promoter sequence for *in vitro* transcription; lowercase sequence includes overhang and restriction digest sites (bold) indicated

*The unspliced pre-mRNA is not observed by RT-PCR, and so should contribute negligibly to the transcript abundance measured by qRT-PCR.
**The L5 cDNA sequence contains a BamHI site at nucleotide position 104; a partial digest with BamHI could be performed and the larger DNA product isolated, or the restriction site could be switched to BglII (DNA34), which results in a digested end compatible with the BamHI cloning site.

SUPPLEMENTARY METHODS

Cloning of DNA constructs

Reporter constructs containing the 5' region of either the unspliced pre-mRNA (Pre-EGFP) or the two splice variants (I-EGFP and II-EGFP) of TFIIIA from *A. thaliana* fused to the cDNA of enhanced green fluorescent protein (EGFP) were cloned into the binary vector pBinAR[12]. The TFIIIA 5' regions starting from the 5' UTR and extending to the exon downstream of P5SM were PCR amplified with primers DNA26 and DNA27 from *A. thaliana* genomic DNA or cDNA, then the resulting DNA products were subjected to restriction digest with KpnI and XbaI. EGFP cDNA was PCR amplified with primers which skip the start codon of EGFP, and the resulting DNA product was subjected to restriction digest with XbaI and SalI. To generate in-frame fusion constructs, the different TFIIIA fragments were ligated with EGFP cDNA through the common XbaI ends. The resulting products were cloned into the KpnI/SalI sites of pBinAR. For insertion of a Flag tag into these reporter constructs, two complementary oligonucleotides encoding the Flag peptide sequence (DYKDDDDK) flanked by XhoI sites were annealed, subjected to restriction digest, and cloned in-frame into a natural XhoI site within the 5' part of the TFIIIA coding sequence (annotated in **Supplementary Fig. 4**).

For cloning of Pre-EGFP reporter constructs with mutations in P5SM, first the 5' region of wild-type TFIIIA gene was PCR amplified with primers DNA26 and DNA27. The resulting product was cloned into EcoRV linearized vector pBluescript II SK (Stratagene) via blunt end ligation. PCR mutagenesis was performed on the plasmid containing the TFIIIA fragment and, after sequence confirmation, the fragment was released by restriction digest with KpnI and SalI for use in cloning with EGFP into pBINAR as described above.

Various proteins were co-expressed with TFIIIA reporter constructs to assess effects on reporter activity. Their respective sequences were amplified by PCR from *A. thaliana* cDNA and after restriction digest, cloned into the BamHI/SalI sites of pBINAR. Cloning of control constructs containing luciferase (LUC) or DsRED2 were described previously[13]. For introduction of an N-terminal Flag tag, two complementary oligonucleotides encoding a start codon followed by the Flag peptide sequence flanked by sequences with BamHI-compatible overhangs were annealed and cloned in-frame into the BamHI site immediately 5' of the protein coding region. Since the L5 coding region contains a BamHI site, the Flag tag was instead introduced as an extended overhang to the 5' primer complemenary to L5 (DNA43).

Additional notes on non-denaturing gel shift assays

Renaturation of the RNA before performing the assay was found to be important to reduce the amount of alternatively folded forms, as sometimes observed by the appearance of multiple RNA bands in the absence of added protein. Gels below 6% acylamide are not able to resolve these multiple bands, and it is possible that even at 10% acrylamide there are still unresolved bands. Some of these alternatively folded P5SM RNAs apparently do not bind GST-*At*L5 and therefore are unaffected by addition of protein. We observe with the renaturation protocol that saturated binding of the RNA by protein usually is reached with ~60-70% of P5SM RNA bound and shifted. To calculate the dissociation constant for the RNA-protein interaction, the percentage bound was normalized to this empirically observed maximal binding. We and others[9] have observed a similar effect for *A. thaliana* 5S rRNA, in which saturated binding is reached with some of the RNA remaining unshifted.

References

1. Weinberg, Z. et al. Identification of 22 candidate structured RNAs in bacteria using the CMfinder comparative genomics pipeline. *Nucleic Acids Res* **35**, 4809-19 (2007).

2. Swarbreck, D. et al. The *Arabidopsis* Information Resource (TAIR): gene structure and function annotation. *Nucleic Acids Res* **36**, D1009-14 (2008).

3. Tanaka, T. et al. The Rice Annotation Project Database (RAP-DB): 2008 update. *Nucleic Acids Res* **36**, D1028-33 (2008).

4. Jaillon, O. et al. The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. *Nature* **449**, 463-7 (2007).

5. Lang, D., Eisinger, J., Reski, R. & Rensing, S.A. Representation and high-quality annotation of the *Physcomitrella patens* transcriptome demonstrates a high proportion of proteins involved in metabolism in mosses. *Plant Biol (Stuttg)* **7**, 238-50 (2005).

6. Chenna, R. et al. Multiple sequence alignment with the Clustal series of programs. *Nucleic Acids Res* **31**, 3497-500 (2003).

7. Katoh, K., Hori, H. & Osawa, S. The nucleotide sequences of 5S ribosomal RNAs from four *Bryophyta* species. *Nucleic Acids Res* **11**, 5671-5674 (1983).

8. Cloix, C. et al. Analysis of the 5S RNA pool in *Arabidopsis thaliana*: RNAs are heterogeneous and only two of the genomic 5S loci produce mature 5S RNA. *Genome Res* **12**, 132-144 (2002).

9. Mathieu, O. et al. Identification and characterization of transcription factor IIIA and ribosomal protein L5 from *Arabidopsis thaliana*. *Nucleic Acids Res* **31**, 2424-2433 (2003).

10. Schmeing, T.M., Moore, P.B. & Steitz, T.A. Structures of deacylated tRNA mimics bound to the E site of the large ribosomal subunit. *RNA* **9**, 1345-52 (2003).

11. Lee, Y. & Nazar, R.N. Terminal structure mediates 5 S rRNA stability and integration during ribosome biogenesis. *J Biol Chem* **278**, 6635-41 (2003).

12. Hofgen, R. & Willmitzer, L. Biochemical and genetic analysis of different patatin isoforms expressed in various organs of potato (*Solanum tuberosum*). *Plant Sci* **66**, 221-230 (1990).

13. Wachter, A. et al. Riboswitch control of gene expression in plants by splicing and alternative 3' end processing of mRNAs. *Plant Cell* **19**, 3437-50 (2007).