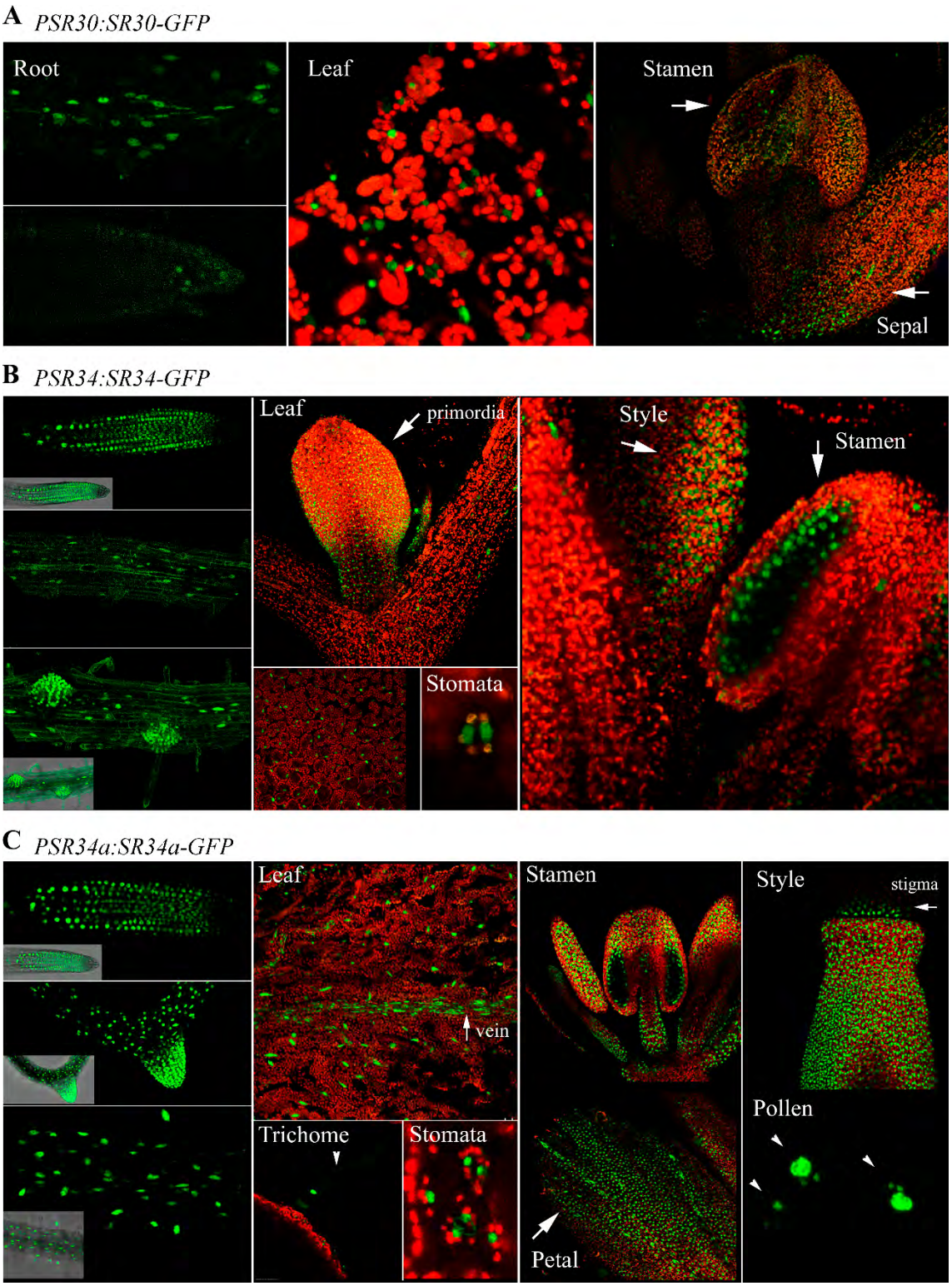
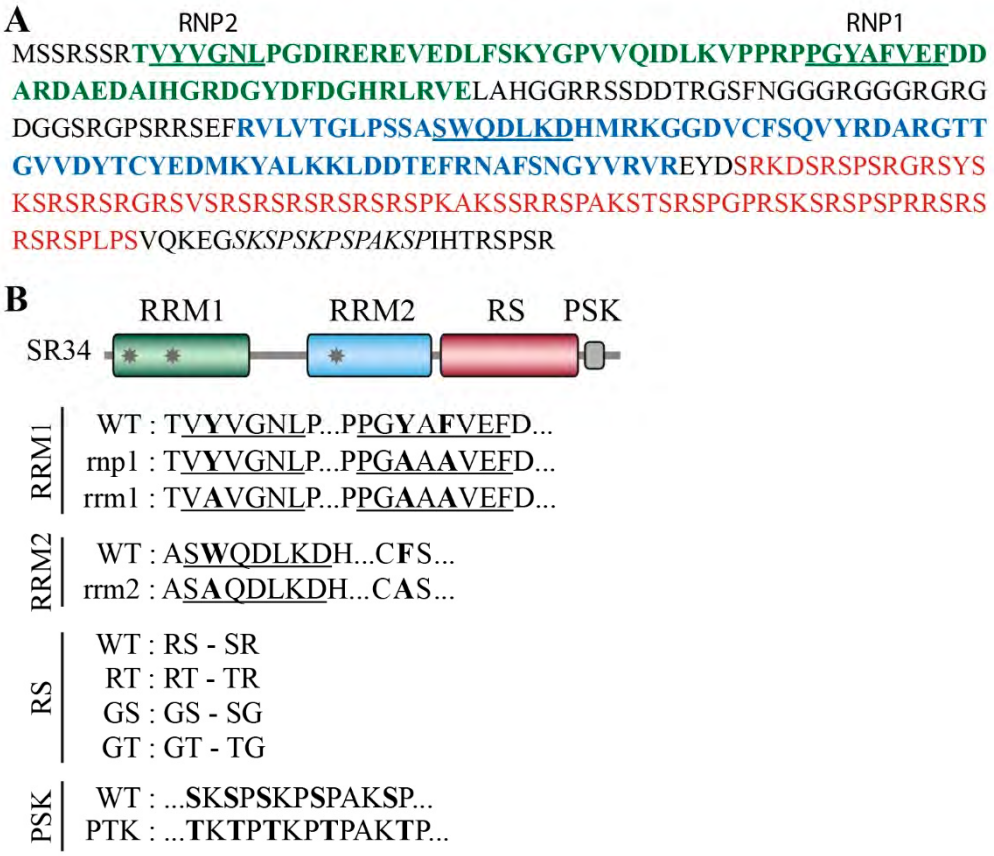
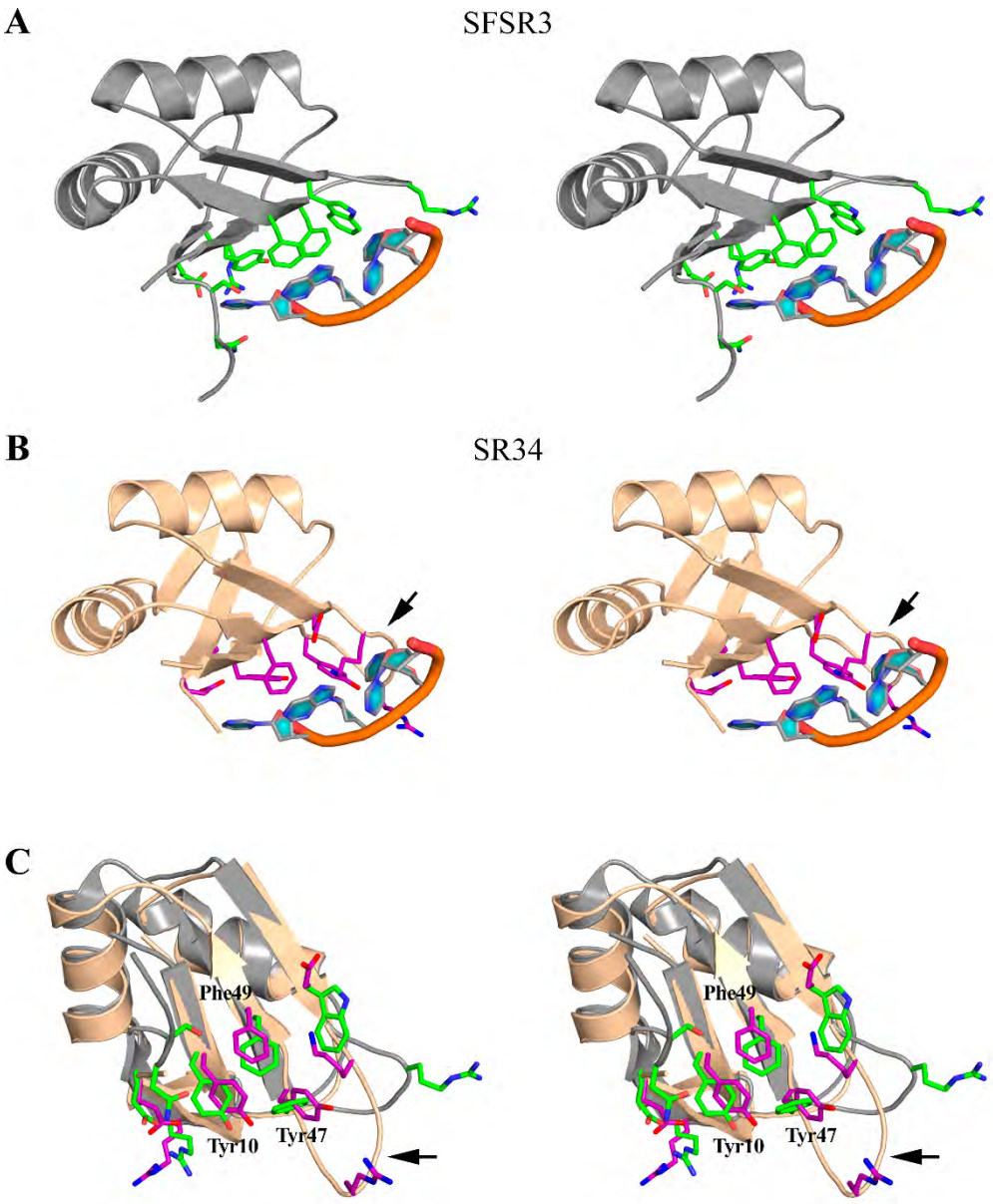


Supplemental Figure S1. Expression pattern and subcellular localization of SR30-GFP (A), SR34-GFP (B) and SR34a-GFP (C) fusion proteins in root, leaves and reproductive tissues in Arabidopsis transgenic plants. White arrows point to the specified organ or cell-types.



Supplemental Figure S2. Sequence of SR34 (A) and diagram depicting the domains of SR34 and generated mutant derivatives (B). SR34 contains an N-terminal RRM1 domain (green) followed by an internal RRM2 (light blue). The RRM1 is characterized by two highly conserved motifs of six and eight amino acid residues called RNP2 and RNP1, respectively (stars within the box; see sequences). The RRM2 contains the conserved motif SWQDLKD (star). The RS domain is boxed in red and the PSK in grey. The mutagenized residues within the domains are indicated in bold and underlined letters.

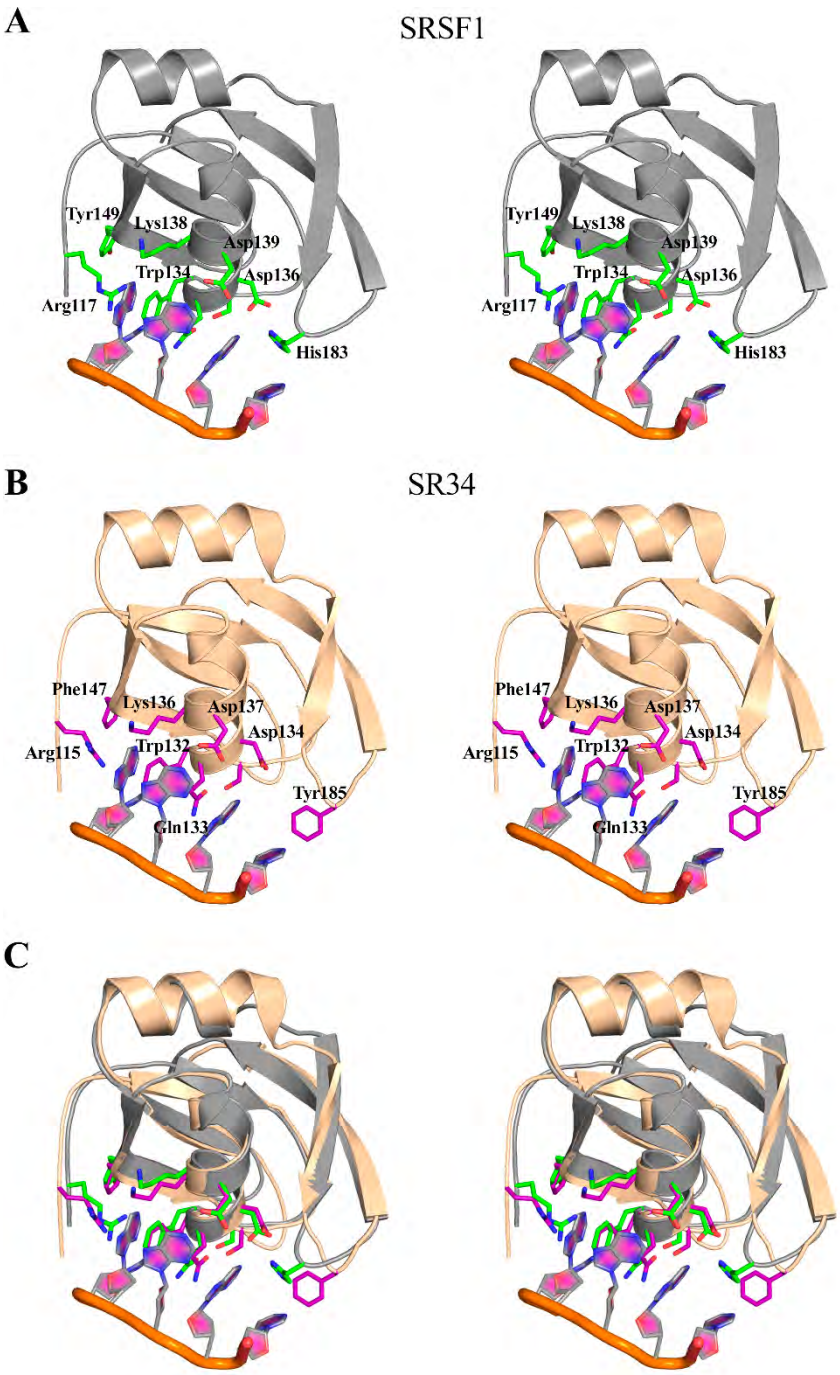


Supplemental Figure S3. Model of SR34 RRM1 domain.

(A) Cartoon representation of the RRM domain of SRp20 bound to the RNA CAUC (pdb 2I2Y). Important residues interacting with RNA are shown as green sticks.

(B) Cartoon representation of the model of SR34 RRM1. The nucleotides CAUC indicate residues (shown as pink sticks) potentially interacting with RNA by homology with the SRp20:RNA complex. Residues are not labelled for clarity. From left to right, Pink sticks represent E78, Y10, F49, D37, K39, and R43.

(C) Superposition of SR34 RRM1 model with the RRM domain of SRp20. The color code is identical to A and B but the orientation is slightly different. Mutated residues (Tyr10, Tyr47, Phe49, see text) are labelled.

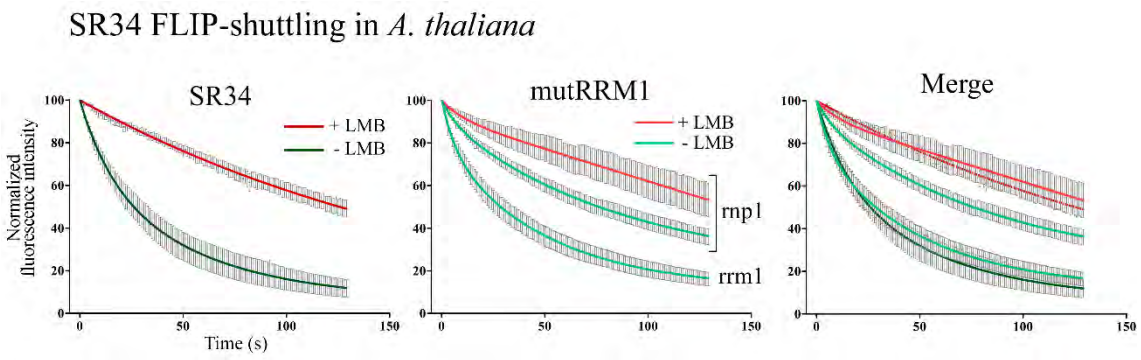


Supplemental Figure S4. Model of SR34 RRM2 domain.

(A) Cartoon representation of the human SRSF1 pseudo-RRM bound to RNA (2M8D). Important residues interacting with RNA are shown as green sticks.

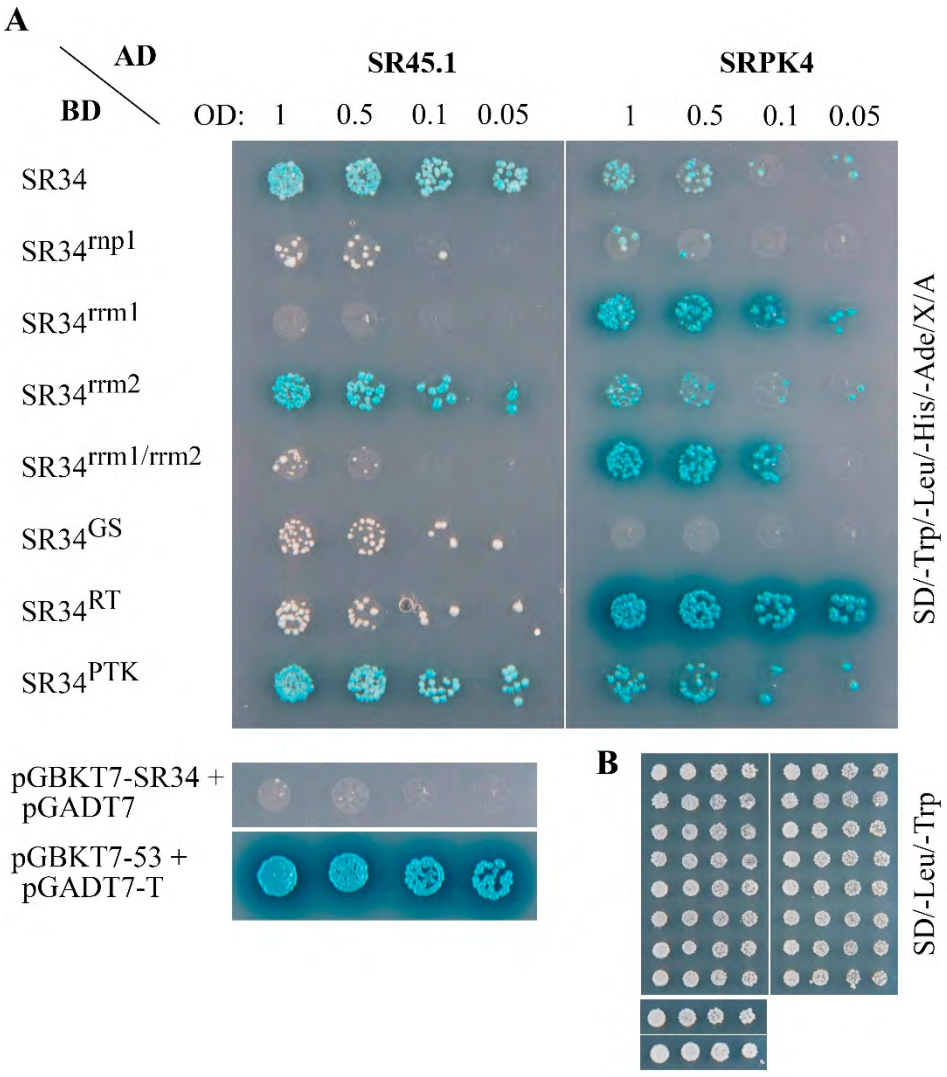
(B) Cartoon representation of the model of SR34 RRM2. The nucleotides indicate residues (shown as pink sticks) potentially interacting with RNA by homology with the SRSF1:RNA complex.

(C) Superposition of SR34 RRM2 model with the pseudo-RRM domain of human SRSF1. The color code is identical to A and B.



Supplemental Figure S5. Nucleocytoplasmic shuttling of SR34, SR34^{rnp1} and SR34^{rrm1} mutant proteins in Arabidopsis leaf cells.

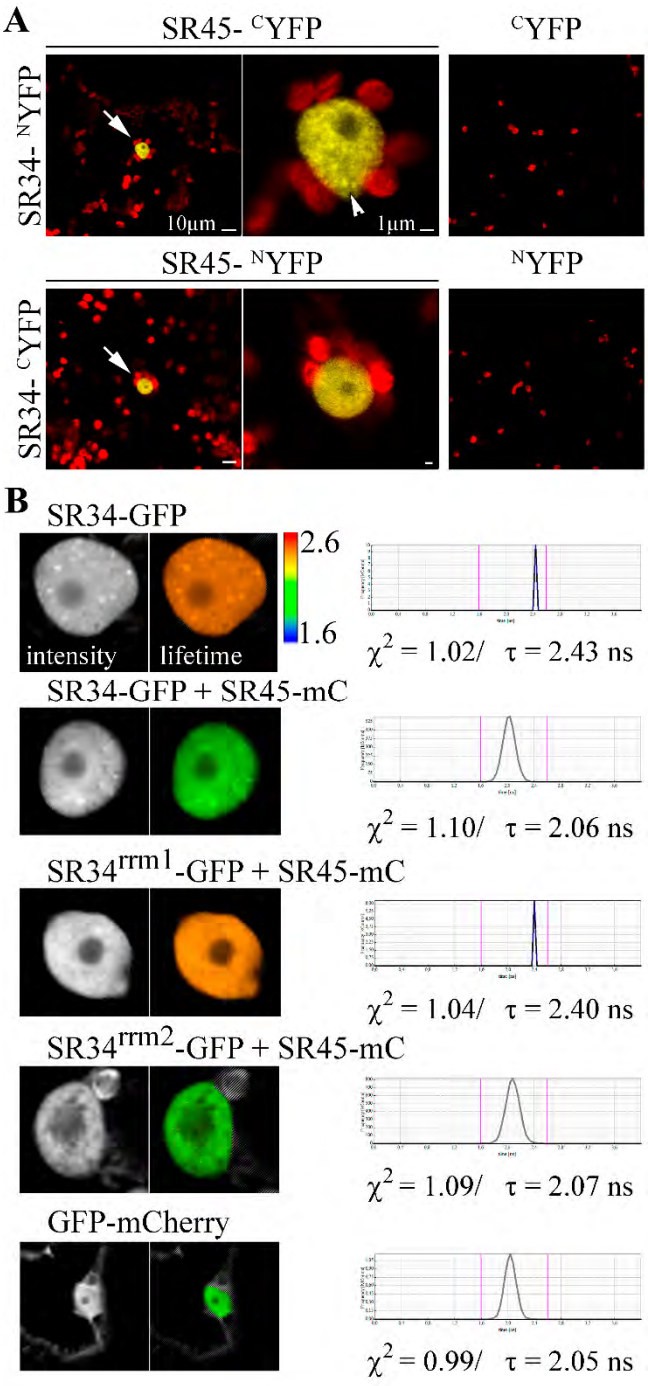
Flip-shuttling was monitored in the absence (-LMB) and upon LMB (+LMB) treatment. Values are means +/- SEM for at least 8 nuclei. The differences observed in the FLIP curves between untreated and LMB treated cells are statistically different for SR34 ($P < 0.0001$) and SR34^{rnp1} ($P < 0.05$).



Supplemental Figure S6. Identification of SR34 mutations that influence interaction with SR45 and SRPK4 in an Y2H assay.

(A) Interaction of SR34 and SR34 mutants (baits) and either SR45.1 or SRPK4 (preys). From the mated culture, dilutions to an OD_{600} of 1, 0.5, 0.1 and 0.05 were spotted on synthetic dropout (SD)/-Trp/ -Leu/ -His/-Ade/X-α-Gal/AurA agar plates. Positive interactions were confirmed by growth and turning blue.

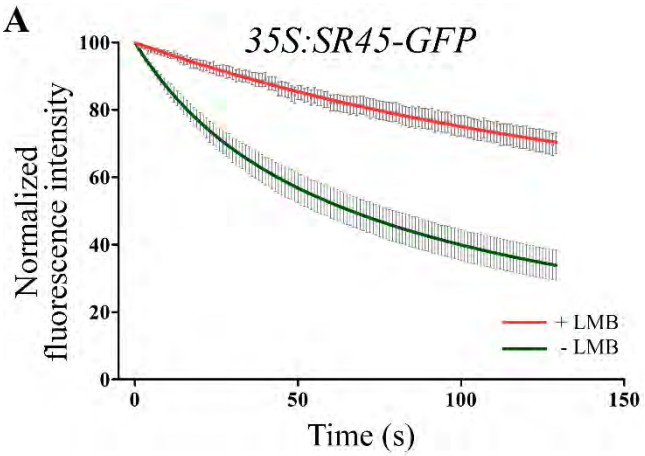
(B) Yeast cultures on SD/-Leu /-Trp control plates confirm the presence of both plasmids.



Supplemental Figure S7. SR34 interacts with SR45 in plant cells.

(A) Transient expression assay was used to coexpress SR34 fused to the N-terminal half of YFP (SR34-^{N}YFP) and SR45 fused to the C-terminal half of YFP (SR45-^{C}YFP), or SR34 fused to the C-terminal half of YFP (SR34-^{C}YFP) and SR45 fused to the N-terminal half of YFP (SR45-^{N}YFP). Selected images of nuclear fluorescence reveals the interaction of SR34 and SR45 in the nucleus (arrows) and in speckles (arrowhead). No fluorescence was obtained when SR34 fused to one half of YFP was coexpressed with the other half part of YFP.

(B) FLIM-FRET analysis of SR34-GFP and SR45-mCherry. Fluorescence intensity and lifetime imaging of the donor (GFP) are shown (left) in cells expressing SR34-GFP alone, SR34-GFP in the presence of SR45-mCherry, and RRM1/RRM2 mutant variants of SR34 in the presence of SR45-mCherry. Pseudocolored images show shortening of GFP fluorescence lifetime (tau, expressed in ns) with histogram curves ranging from 1.6 ns and 2.6 ns (histograms shown at the right). The $\chi 2$ and τ are specified for these individual experiments.



Supplemental Figure S8. Nucleocytoplasmic shuttling of SR45 in transient expression assays in tobacco leaf cells.

FLIP-shuttling of SR45 was monitored in the absence (-LMB) and upon LMB (+LMB) treatment in leaf epidermal cells. One hundred percent fluorescence indicates pre-bleach fluorescence intensity. Values are means+/-SEM for at least 10 nuclei. The curves show a significant inhibitory effect of LMB on shuttling ($P < 0.0001$).

Supplemental Table S1. List of primers used in the study.

A. Construction of binary vectors

Target	Fwd	Rev	Constructs
PSR34	TATAAAGCTTACGGAGTTACCAAGAGCACCG	TATAGGATCCTCTTCCTTTATCAAATCCAAAAACAC	GFP/GUS
SR30	CGGGATCCATGAGTAGCCGATGGAATCGTACG	AGGGTACCACCACCACCAGATATCACAGGTGAAACTGGAC	GFP fusion
PSR30	TATAAAGCTTCGTTGAACAGATGCCTTCACTT	ATAGGATCCCTGATACCTCAGAGCAGAAAAT	GFP/GUS
SR34a	CGGGATCCATGAGTGGGCGATTTTCTCGGT	AGGGTACCACCACCCACACTGCCTTCGCGAACCTT	GFP fusion
PSR34a	TATAAAGCTTCGATTAAAGTTTTTGGAAGA	TATAGGATCCTGTTTGCAAAGGTTAAAAAG	GFP/GUS
SR34b	TATAGGATCCATGAGCAGCCGTTCGAGTAGA	TATAGGTACCACCACCTATCAATGCGATCCAATGTCTCTCC	GFP fusion
PSR34b	TATAGGCGCCAGAATTTCGTCTCACCAGACATCC	TACGGGATCCTTATTTCTTTCCTATACCAAAATCAAAAT	GFP/GUS
SR45.1	CGGGATCCATGGCGAAACCAAGTCGTGGC	CCGGTACCTTAAGTTTTACGAGGTGGAGGTGGTGG	GFP fusion
SR34^{rnp1}	CAAGGCCTCCTGGT**GCT**GCA**GCC**GTTGAGTTTGATG	CATCAAACTCAAC**GGC**TGC**AGC**ACCAGGAGGCCTTG	Domain mutants
SR34^{rnp2}	CGAGTAGAACCGTG**GCC**GTCGGAAACCTTCCTG	CAGGAAGGTTTCCGA**CGG**CCACGGTTCTACTCG	Domain mutants
SR34^{rrm2} W_A	TGCCTTCATCTGCTTCT**GC**GCAAGATCTCAAGG	CCTTGAGATCTTGC**GC**AGAAGCAGATGAAGGCA	Domain mutants
SR34^{rrm2} F_A	GAGGCGATGTCTGT**GC**CTCGCAAGTGTACCG	CGGTACACTTGCGAG**GC**ACAGACATCGCCTC	Domain mutants
SR34^{PTK} part1	TCAGAAGGAAGGAA**C**CAAGA**C**CCCTA**C**CAAGCCAAGTCCAG	CTGGACTTGGCTTG**G**TAGGG**G**TCTTG**G**TTCCTTCCTTCTGA	Domain mutants
SR34^{PTK} part2	CCCTA**C**CAAGCCAA**C**TCCAGCCAAGA**C**TCCTATCCACACTAG	CTAGTGTGGATAGGA**G**TCTTGGCTGGA**G**TTGGCTTG**G**TAGGG	Domain mutants
YFP^{C}	CCGGTACCCGCCCGGCCTGCAAGATCCCGAACGACCTGAAACAGAAG GTCATGAACCACGGCAGCGTGCAGCTCG	CCGAGCTCTCACTTGTACAGCTCGTCCATGCC	BiFC
YFP^{N}	CCGGTACCCGCTCCATCGCCACGATGGTGAGCAAGGGCGAGGAGCTG TTCACCG	CCGAGCTCTCAGTCCTCGATGTTGTGGC	BiFC
mCherry SR45 fusion	CGGGATCCATGGTGAGCAAGGGCGAGGAG	TCGAGCTCCTATCTAGAGAATTCCTTGTACAGCTCGTCC	FLIM-FRET
mCherry GFP fusion	CGGGATCCATGGTGAGCAAGGGCGAGGAG	GGGGTACCTCCGCCACCCCCGCCTCTAGAGAATTCCTTGTACA	FLIM-FRET

Restriction sites used for cloning are underlined. Linkers for cloning in frame to GFP or YFP are in green font. Mutagenic bases are in bold.

B. Construction of yeast vectors

Target	Fwd	Rev	Constructs
SR34 and variants	GAATTCATGAGCAGTCGTTCGA	AGGGATCCCCTCGATGGACTC	pGBKT-7
SR34a	GGGCCATGGGGATGAGTGGGCGATTTTCTCGGTC	AGGGATCCCACACTGCCTTCGC	pGBKT-7 - pGADT7-AD
SR30	ggggccatggggatgagtagccgatggaatcgtacg	GGATCCACCAGATATCACAGGTGAAAC	pGBKT-7 - pGADT7-AD
SR45.1	CGGGATCCGGATGGCGAAACCAAGTCGTGGC	CCGAGCTCTTAAGTTTTACGAGGTGGAGGTGGTGG	pGADT7-AD
CypRS64	CGGAATTCATGACTAAAAAGAAGAATCCTAATGTTTT	TATAGAGCTCTCAATCCGCATAGCTAACCAG	pGADT7-AD
SRPK4	GGGAATTCATGGAGGCGGAGAAGTGGAACAG	CGGGATCCAATTGCTAGCTTAAGAGTGGAGGAGCTT	pGADT7-AD

Restriction sites used for cloning are underlined.

C. Quantitative RT-PCR primers

Target	Fwd	Rev	Primer Efficiency
SR34	TCCGTGAGAGAGAGGTCGAAGA	ATGCATAACCAGGAGGCCTTG	1,89 ± 0.03
SR34a	GCCGTGATGGCTATAATTTGGA	CACCACGACGATCACTTGAAGA	1,92 ±0.06
SR34b	TCGCCTGCAAAATCTACATCG	TCTCCACTGTTACCCATCCTCG	1,83 ± 0.09
SR30	AGATCCTCGTGATGCAGACGAT	TGTGCAATCTCAACCCGAAGT	1,72 ± 0.08
EF1a	TGAGCACGCTCTTCTTGCTTTCA	GGTGGTGGCATCCATCTTGTTACA	1,92 ± 0.04
UBQ10	GGCCTTGTATAATCCCTGATGAATAAG	AAAGAGATAACAGGAACGGAAACATAGT	1,85 ± 0.05
At1g58050	CCATTCTACTTTTTGGCGGCT	TCAATGGTAACTGATCCACTCTGATG	1,91 ± 0.04
At1g62930	GAGTTGCGGGTTTGTTGGAG	CAAGACAGCATTTCCAGATAGCAT	1,86 ± 0.03