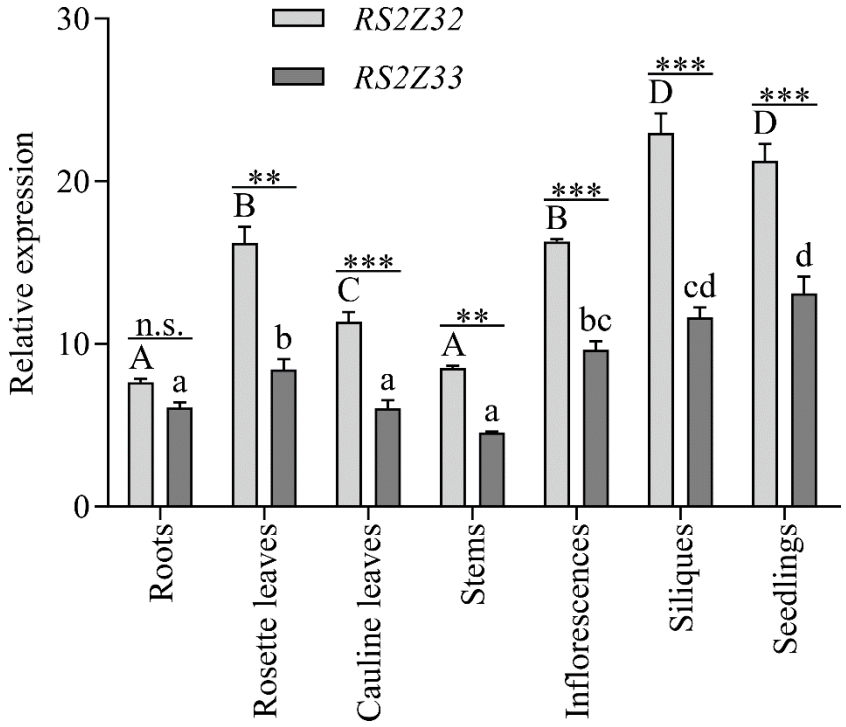
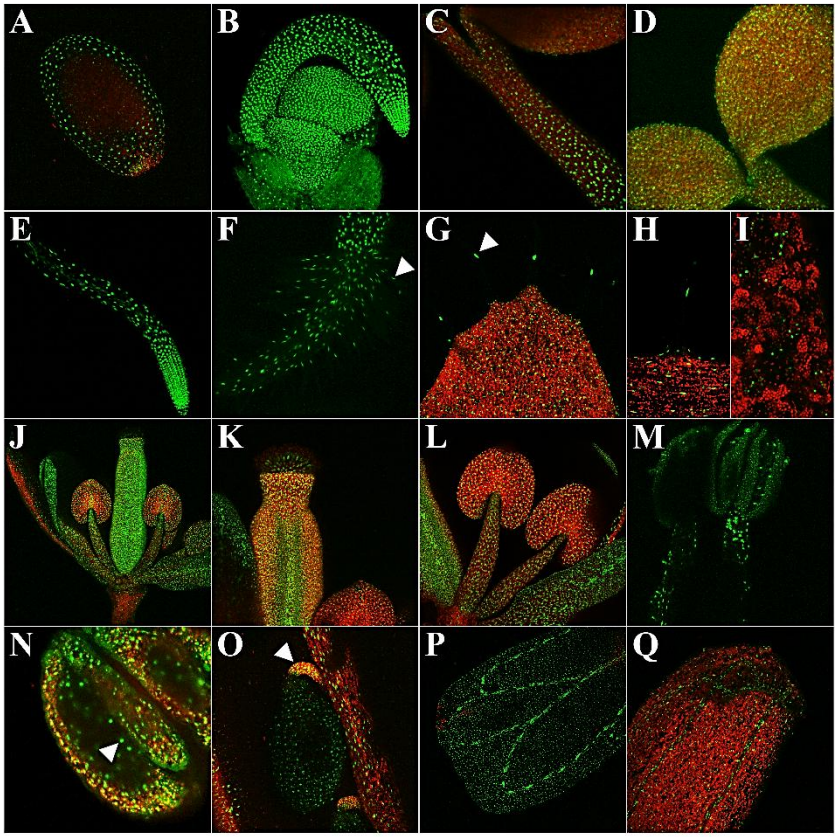
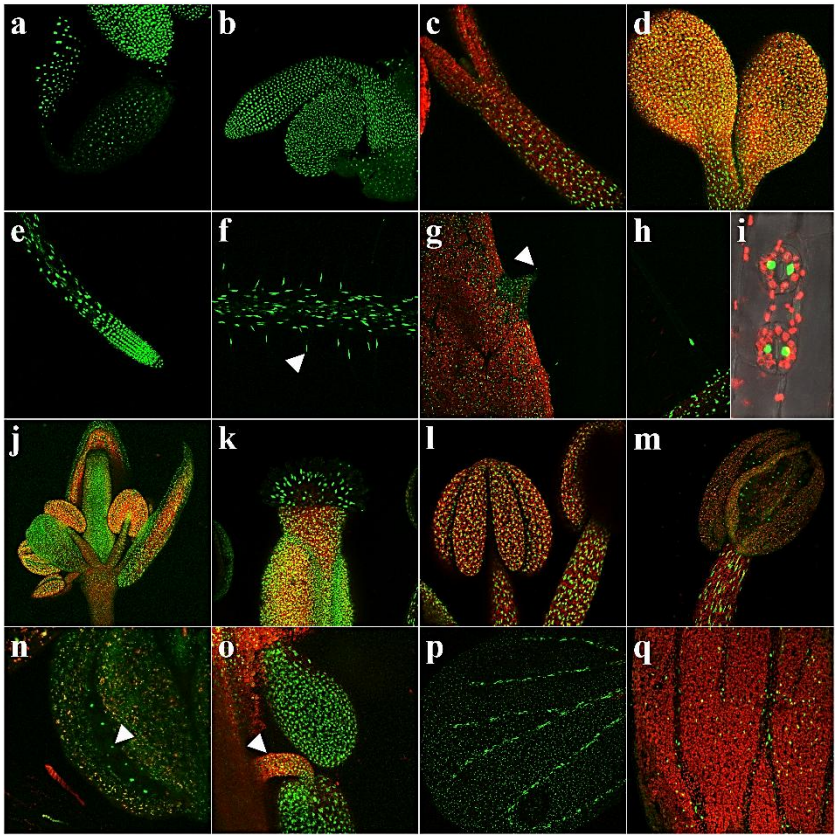
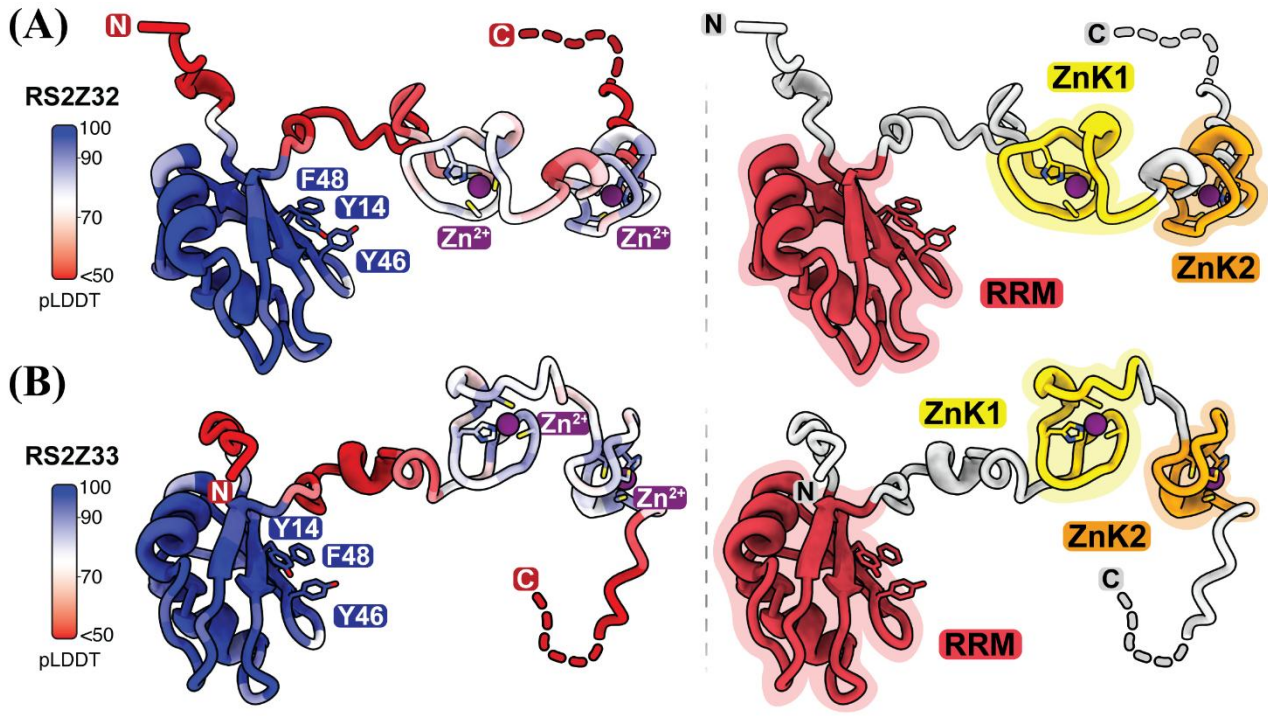

RS2Z32
RS2Z33
Relative expression
30
20
10
0
n.s.
A
a
**
B
b
***
C
a
**
A
a
***
B
bc
***
D
cd
***
D
d
Roots
Rosette leaves
Cauline leaves
Stems
Inflorescences
Siliques
Seedlings

pRS2Z32:RS2Z32-EGFP

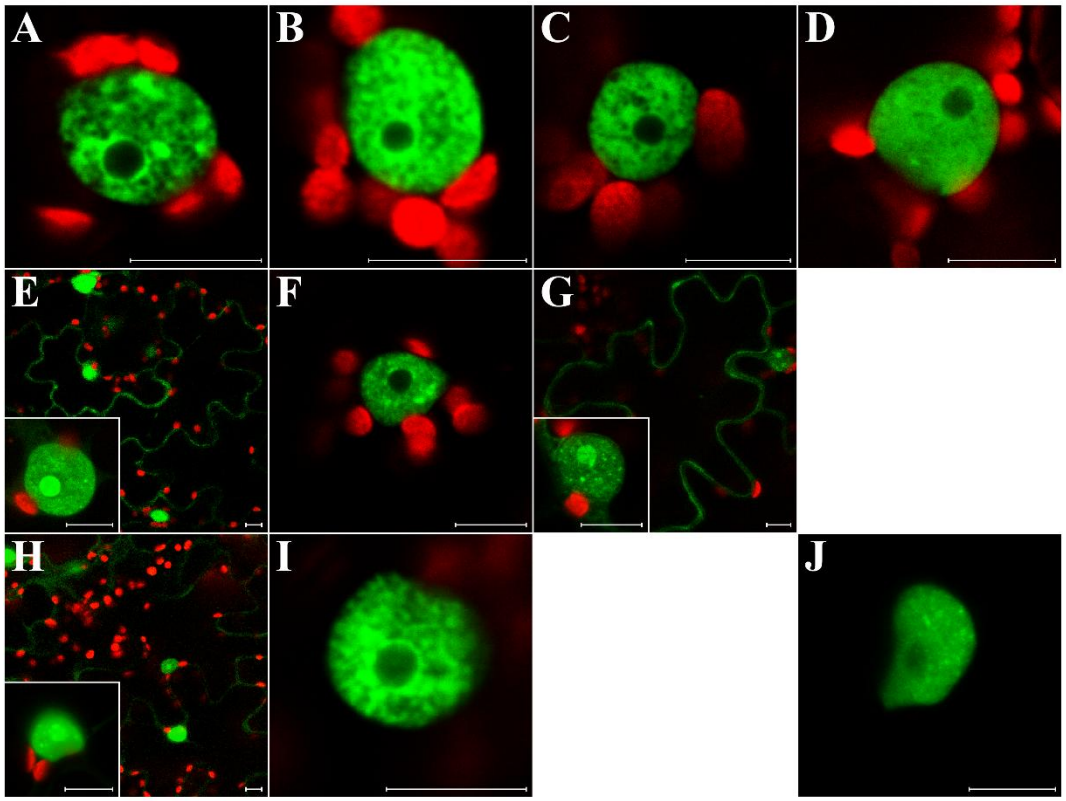


pRS2Z33:RS2Z33-EGFP

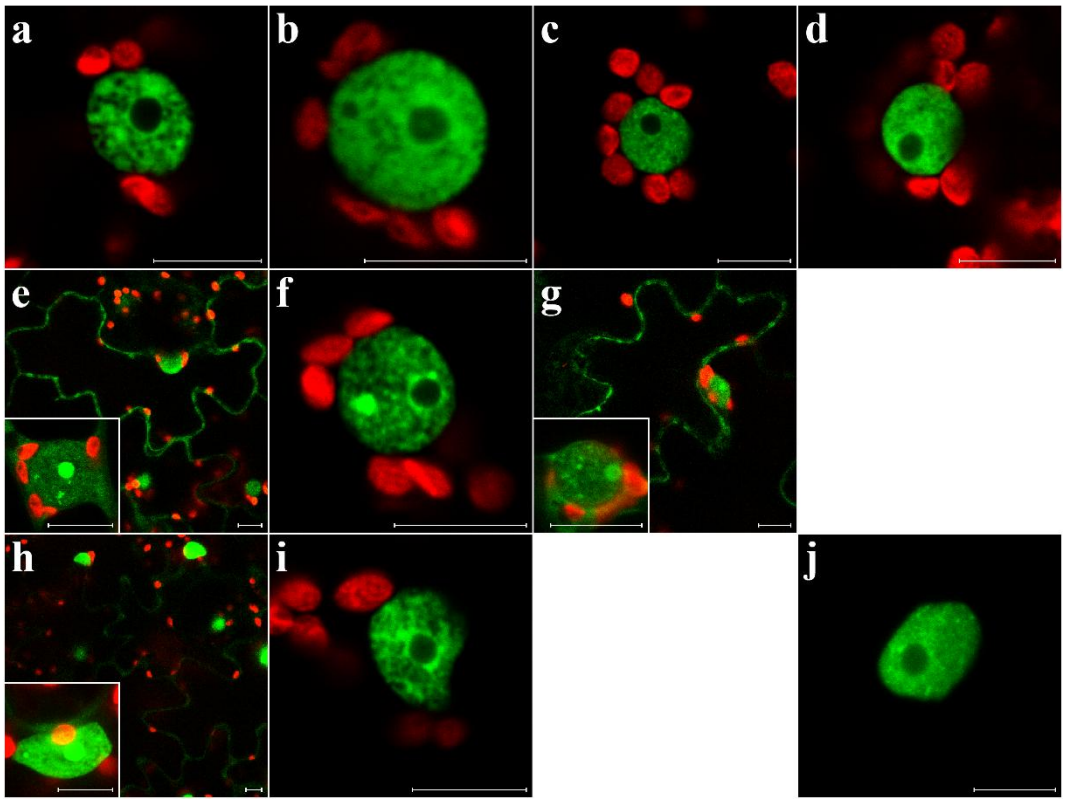


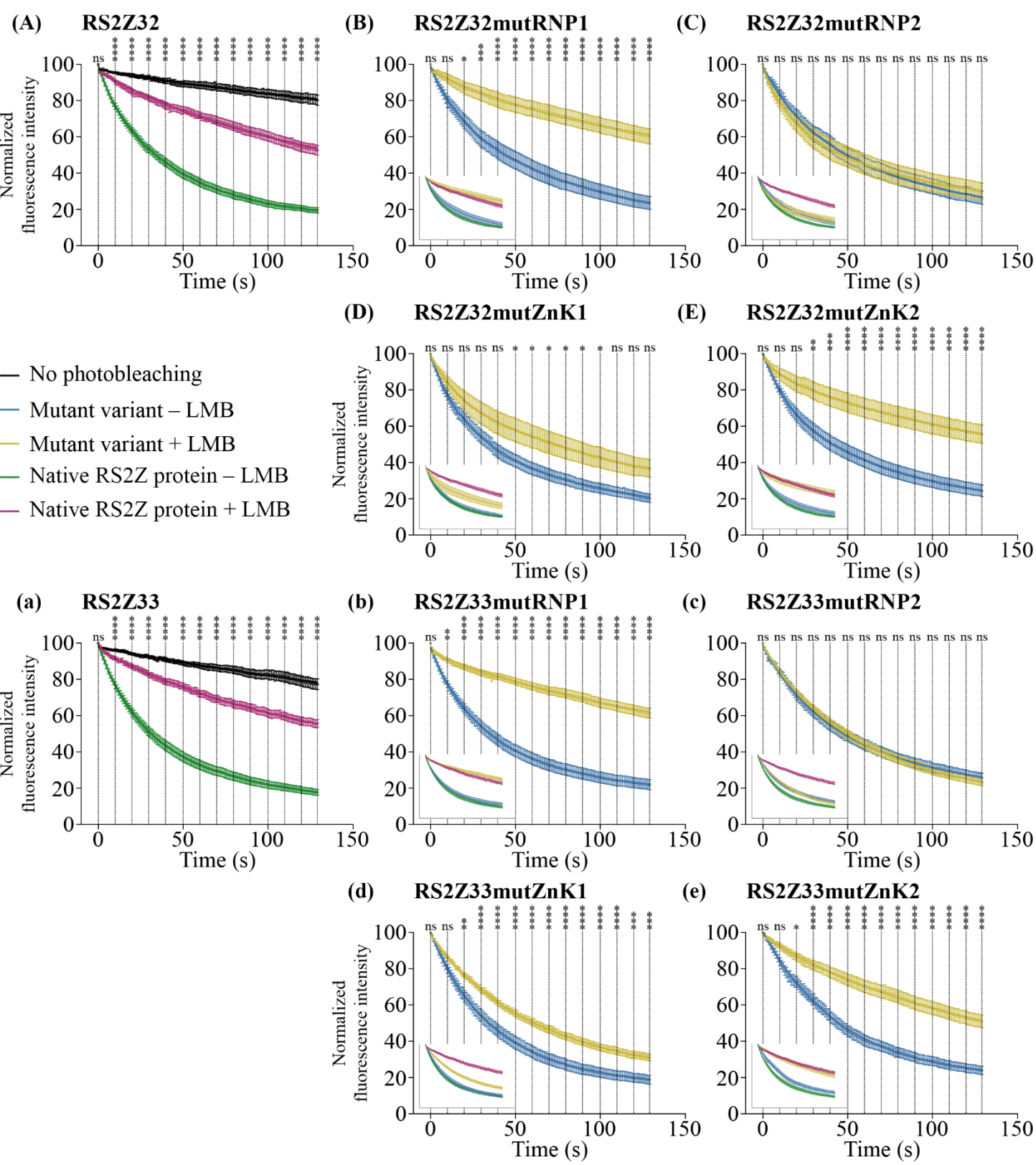

(A)
RS2Z32
100
90
70
<50
pLDDT
N
C
F48
Y14
Y46
Zn2+
Zn2+
N
C
ZnK1
ZnK2
RRM
(B)
RS2Z33
100
90
70
<50
pLDDT
N
Y14
F48
Y46
Zn2+
Zn2+
C
ZnK1
N
ZnK2
C
RRM

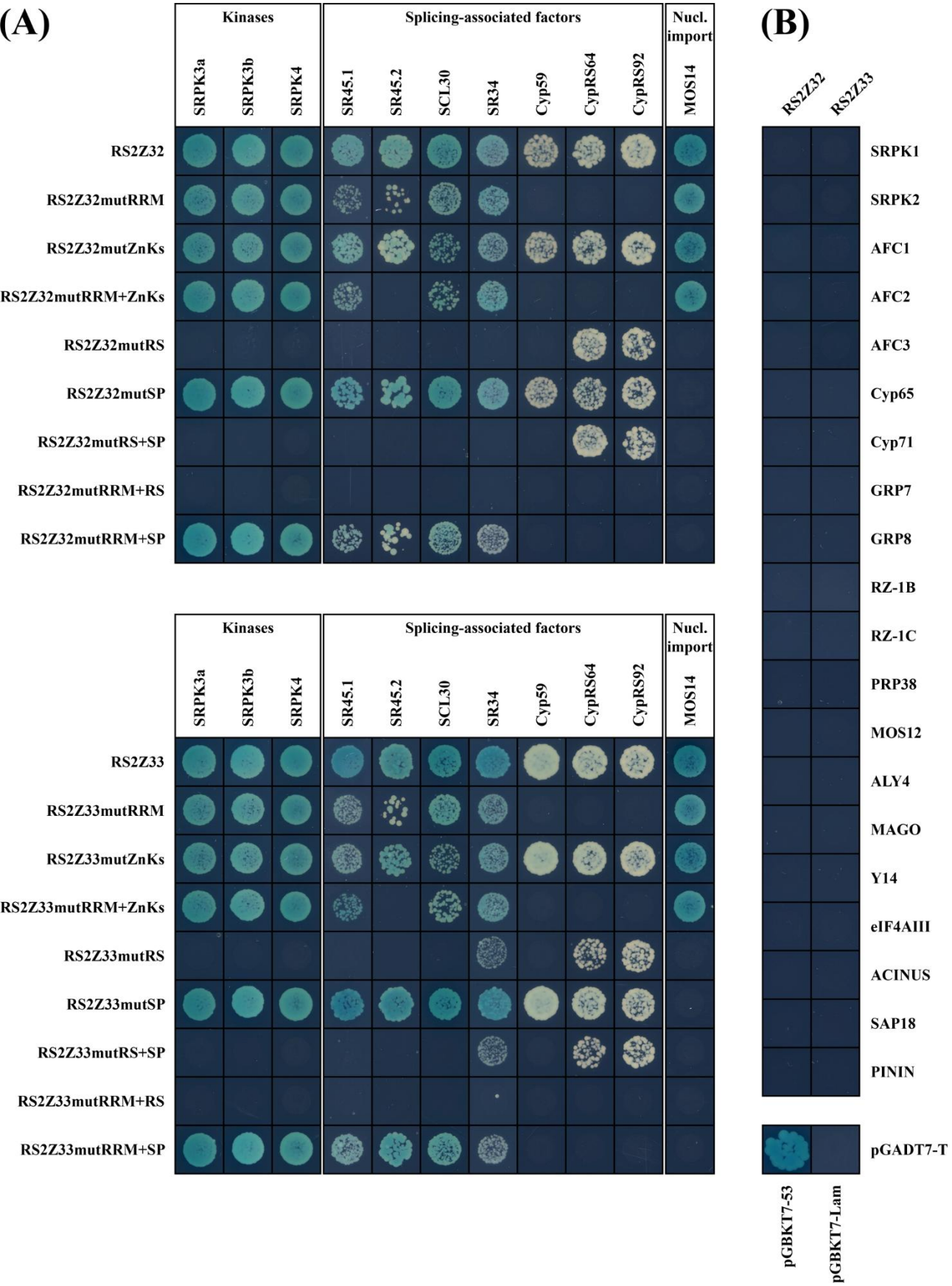
RS2Z32 and mutant variants



RS2Z33 and mutant variants




(A) RS2Z32
(B) RS2Z32mutRNP1
(C) RS2Z32mutRNP2
(D) RS2Z32mutZnK1
(E) RS2Z32mutZnK2
(a) RS2Z33
(b) RS2Z33mutRNP1
(c) RS2Z33mutRNP2
(d) RS2Z33mutZnK1
(e) RS2Z33mutZnK2
Normalized fluorescence intensity
Time (s)
No photobleaching
Mutant variant – LMB
Mutant variant + LMB
Native RS2Z protein – LMB
Native RS2Z protein + LMB


(A)
Kinases
Splicing-associated factors
Nucl. import
SRPK3a
SRPK3b
SRPK4
SR45.1
SR45.2
SCL30
SR34
Cyp59
CypRS64
CypRS92
MOS14
RS2Z32
RS2Z32mutRRM
RS2Z32mutZnKs
RS2Z32mutRRM+ZnKs
RS2Z32mutRS
RS2Z32mutSP
RS2Z32mutRS+SP
RS2Z32mutRRM+RS
RS2Z32mutRRM+SP
RS2Z33
RS2Z33mutRRM
RS2Z33mutZnKs
RS2Z33mutRRM+ZnKs
RS2Z33mutRS
RS2Z33mutSP
RS2Z33mutRS+SP
RS2Z33mutRRM+RS
RS2Z33mutRRM+SP
(B)
RS2Z32
RS2Z33
SRPK1
SRPK2
AFC1
AFC2
AFC3
Cyp65
Cyp71
GRP7
GRP8
RZ-1B
RZ-1C
PRP38
MOS12
ALY4
MAGO
Y14
eIF4AIII
ACINUS
SAP18
PININ
pGADT7-T
pGBKT7-53
pGBKT7-Lam

RS2Z32:CYFP
RS2Z33:CYFP
SR45.1:NYFP
SCL30:NYFP
SR34:NYFP
CypRS64:NYFP

(A) Native RRM of RS2Z32

5’ *E*-value: 2.9e-5 3’

(C) Mutated RRM of RS2Z32

#1

5’ *E*-value: 3.4e+1 3’

#2

5’ *E*-value: 7.2e+0 3’

(B) Native RRM of RS2Z33

5’ *E*-value: 4.0e-9 3’

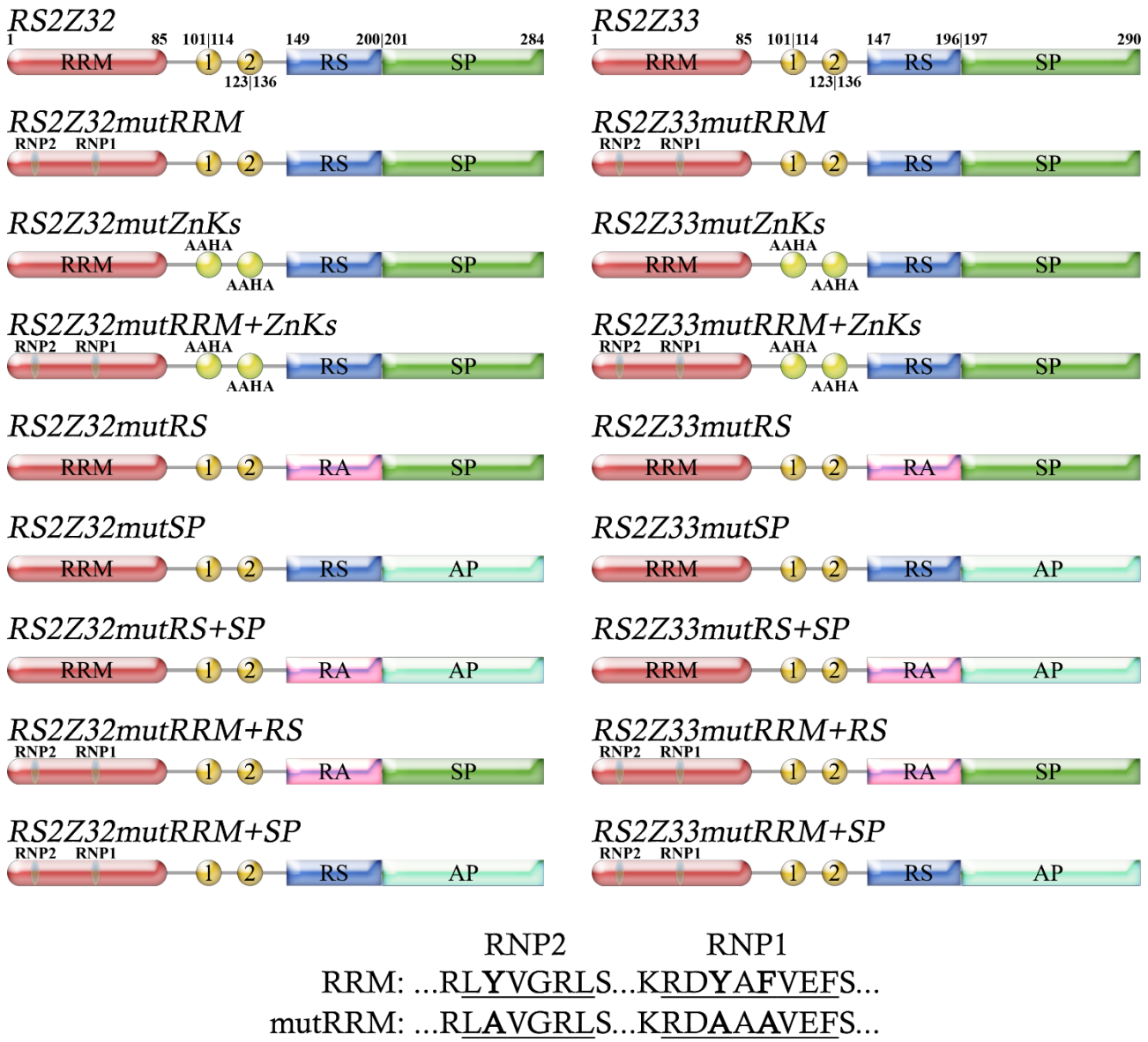
(D) Mutated RRM of RS2Z33

#1

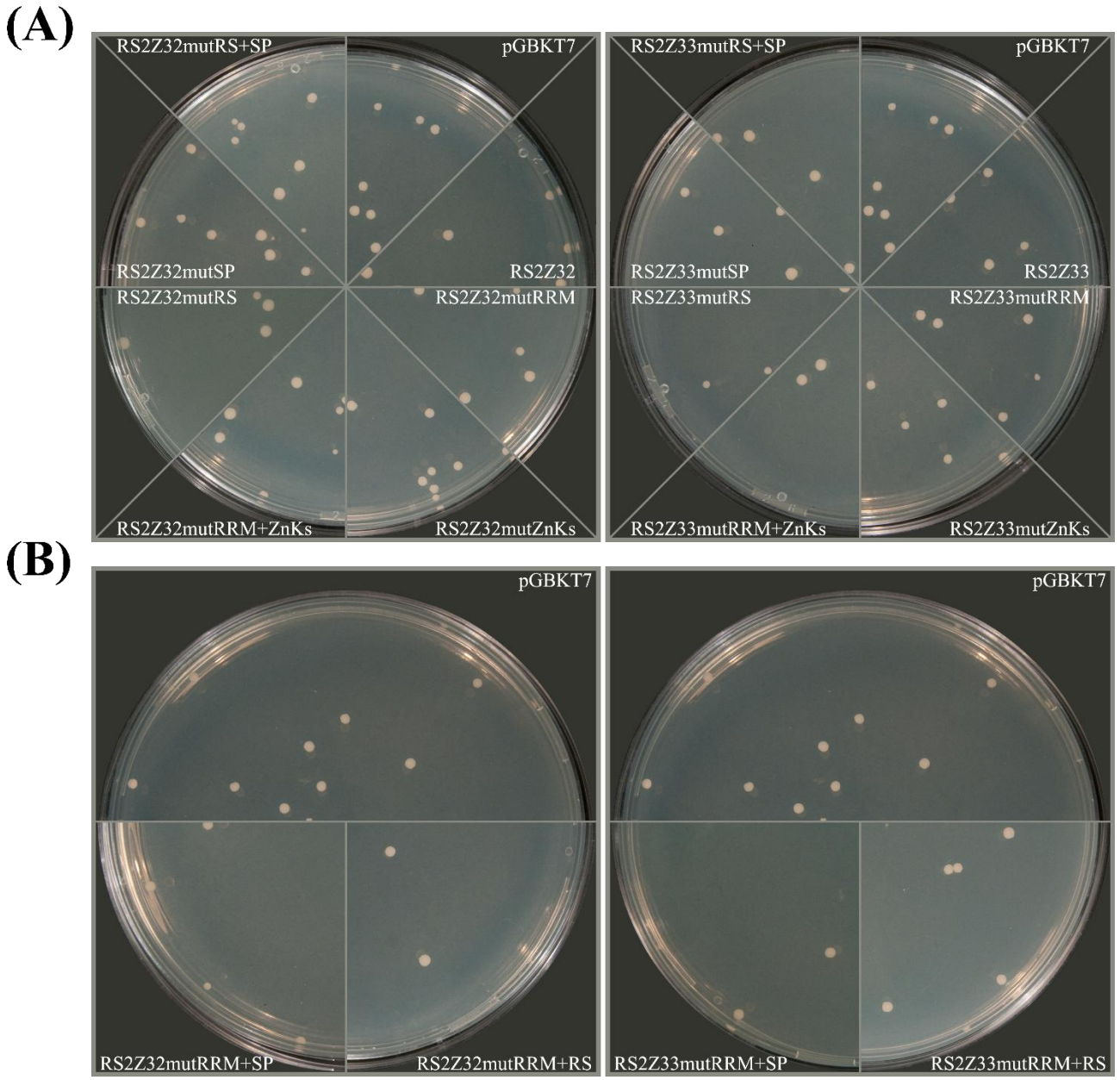
5’ *E*-value: 9.1e-1 3’

#2

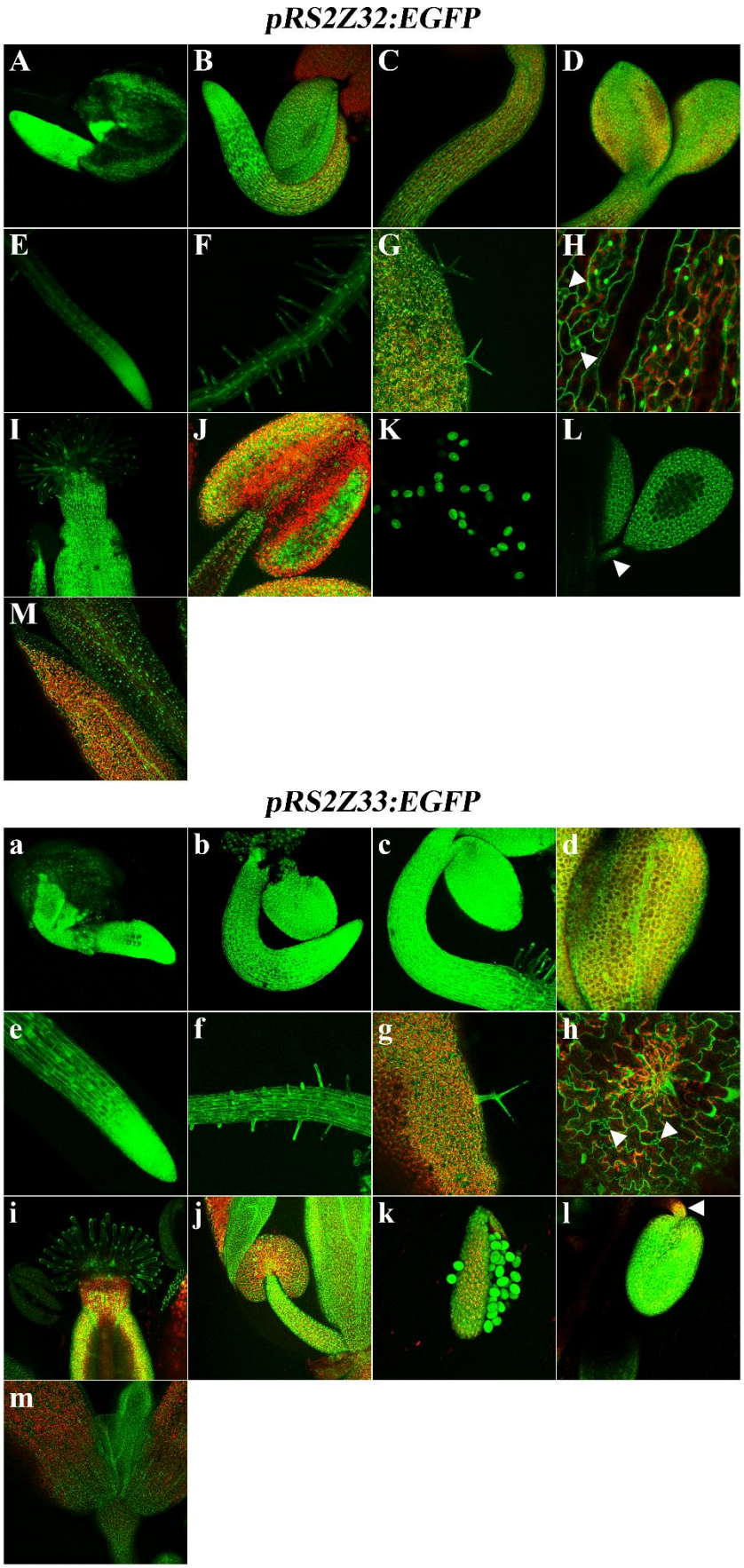
5’ *E*-value: 6.3e+0 3’



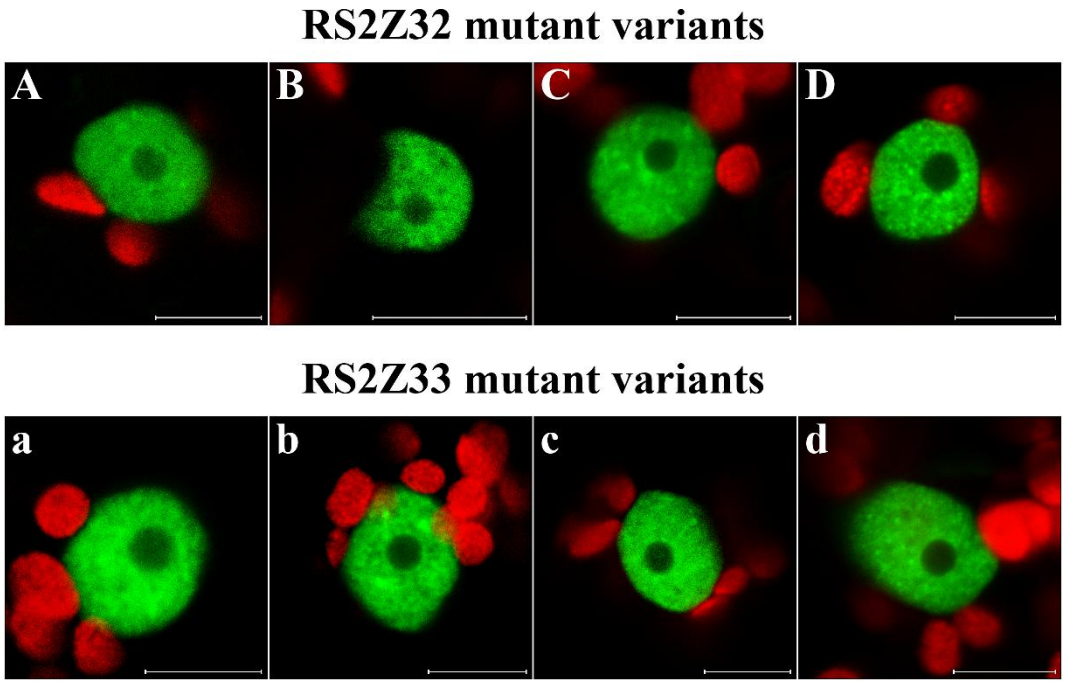
Supplementary Figure S1. Scaled schemes depicting the native RS2Z proteins and their corresponding mutant variants. Domains named '1' and '2' correspond to ZnK1 and ZnK2, respectively. Domains named RA and AP variants correspond to the RS and SP domain, respectively, in which all the serine residues (17 in both genes for the RS domain, or 15/16 in RS2Z32/RS2Z33 for the SP domain) were substituted in alanine residues as shown in **Supplementary Table S5**. Critical aromatic amino acids (bold underlined) connecting the RNA in RNP2 and RNP1 motifs (Y14, Y46, F48) were substituted in alanines (Maris *et al.*, 2005; Califice *et al.*, 2012; Stankovic *et al.*, 2016). 1-284: RS2Z32 protein size in amino acids. 1-290: RS2Z33 protein size in amino acids.



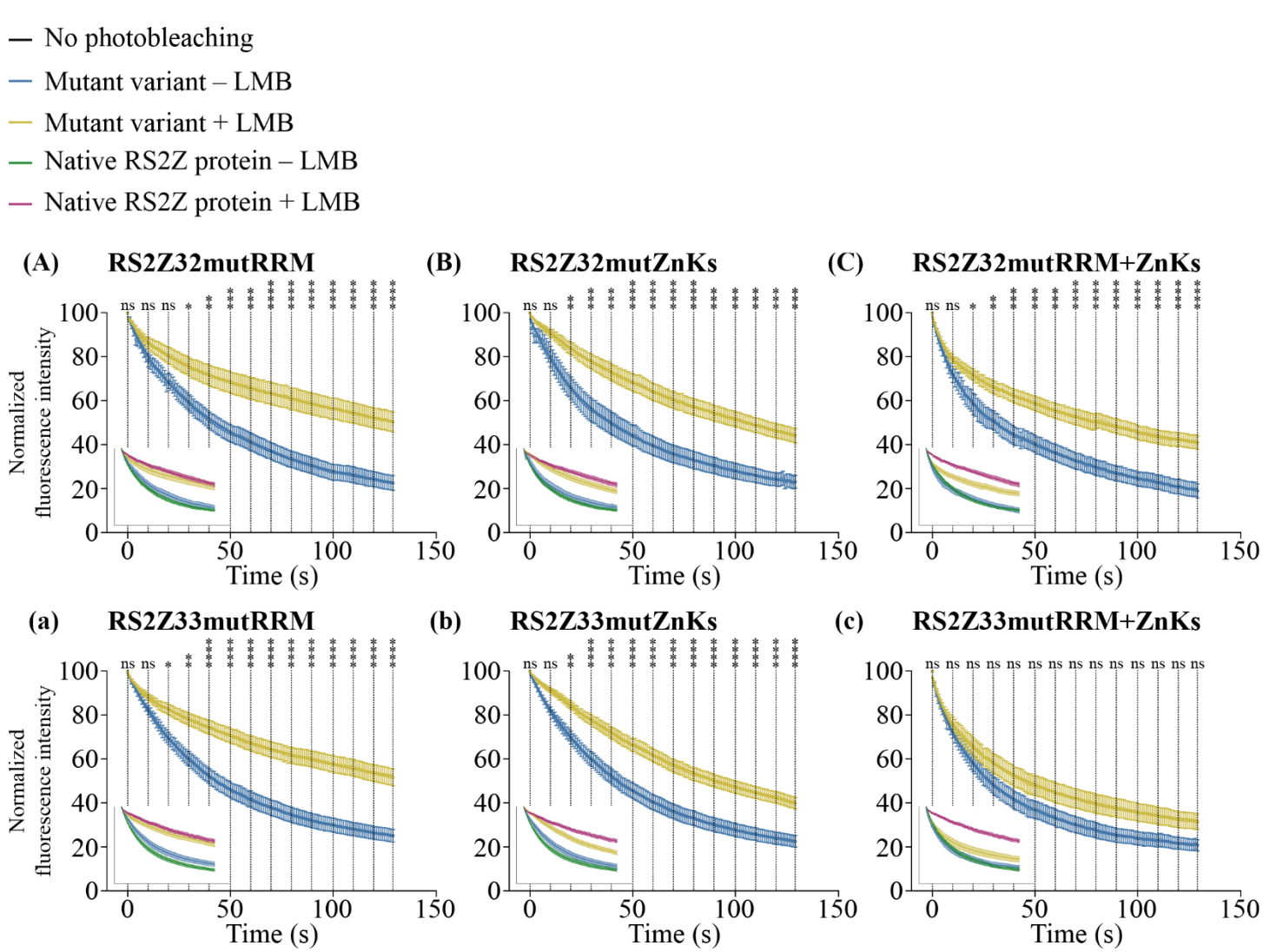
Supplementary Figure S2. Toxicity and autoactivation assays of the native RS2Z32 and RS2Z33 and their corresponding mutant variants on a –Trp/X-a-gal medium. **(A)** Yeast transformants obtained using 100 ng of the following plasmids (clockwise): empty pGBTK7, pGBKT7:RS2Z, pGBKT7:RS2ZmutRRM, pGBKT7:RS2ZmutZnKs, pGBKT7:RS2ZmutRRM+ZnKs, pGBKT7:RS2ZmutRS, pGBKT7:RS2ZmutSP, and pGBKT7:RS2ZmutRS+SP. **(B)** Yeast transformants obtained using 100 ng of the following plasmids (clockwise): empty pGBTK7, pGBKT7:RS2ZmutRRM+RS, and pGBKT7:RS2ZmutRRM+SP. Similar results were obtained for pGADT7 constructs.



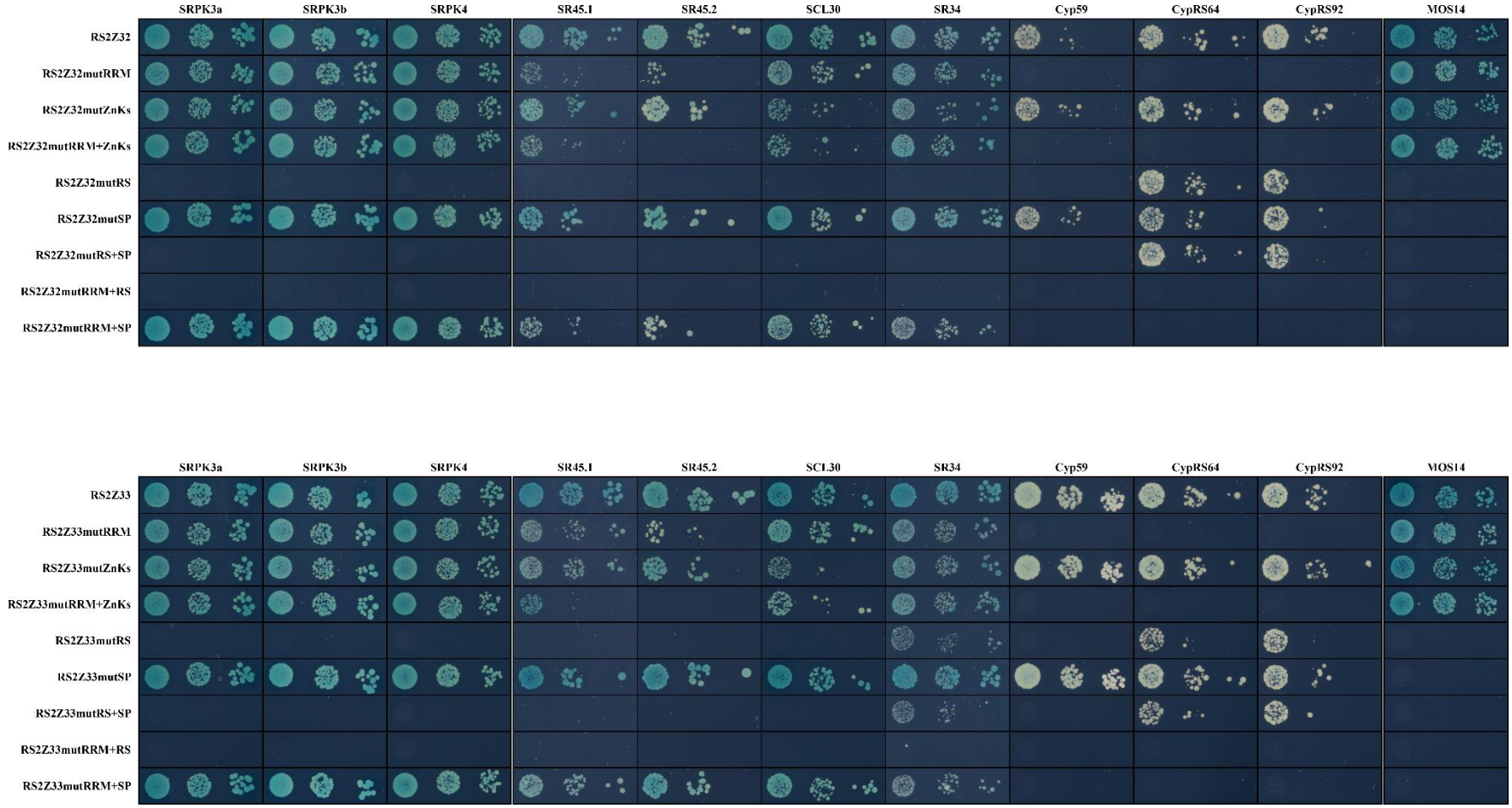
Supplementary Figure S3. Reporter lines expressing *pRS2Z32:EGFP* and *pRS2Z33:EGFP*. Both promoters are active in the seed coat and radicle **(A/a**), embryo **(B/b)**, hypocotyl **(C/c)** and cotyledons **(D/d),** two-week-old root epidermis **(E/e)** including root hairs **(F/f)**, leaf epidermis **(G/g-H/h)** including trichomes **(G/g)** and stomata [**(H/h)**, arrows], gynoecium (valves, stigma and style) **(I/i)**, androecium (anther and filament) **(J/j)**, pollen grains **(K/k)**, ovules **(L/l)** and funiculi [**(L/l)**, arrow], and petals and sepals **(M/m)**. Red signals represent chlorophyll autofluorescence. At least three independent T3 homozygous lines were generated and analyzed, depicting similar fluorescence profiles.



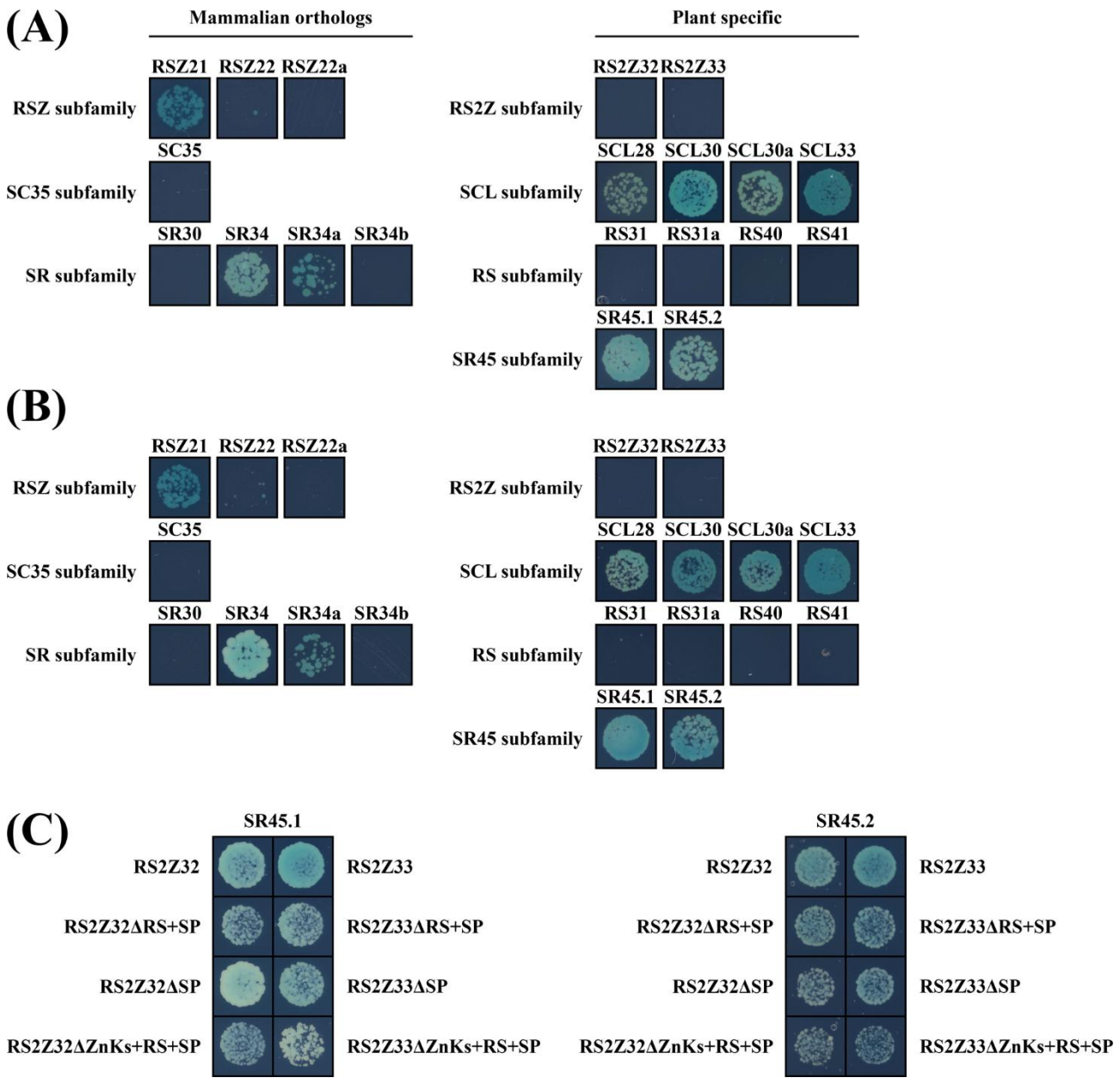
Supplementary Figure S4. Neither RNP motifs nor ZnK domains control the nuclear distribution of RS2Z proteins. Subcellular fluorescence distribution in transient expression assays in tobacco leaf cells upon N-terminal EGFP-tagging of the mentioned mutant variants: RS2ZmutRNP1 **(A/a)**, RS2ZmutRNP2 **(B/b)**, RS2ZmutZnK1 **(C/c)** and RS2ZmutZnK2 **(D/d)**. Scale bars = 10 µm. Red signals represent chlorophyll autofluorescence. At least three independent transient events were generated and analyzed, depicting similar fluorescence profiles.



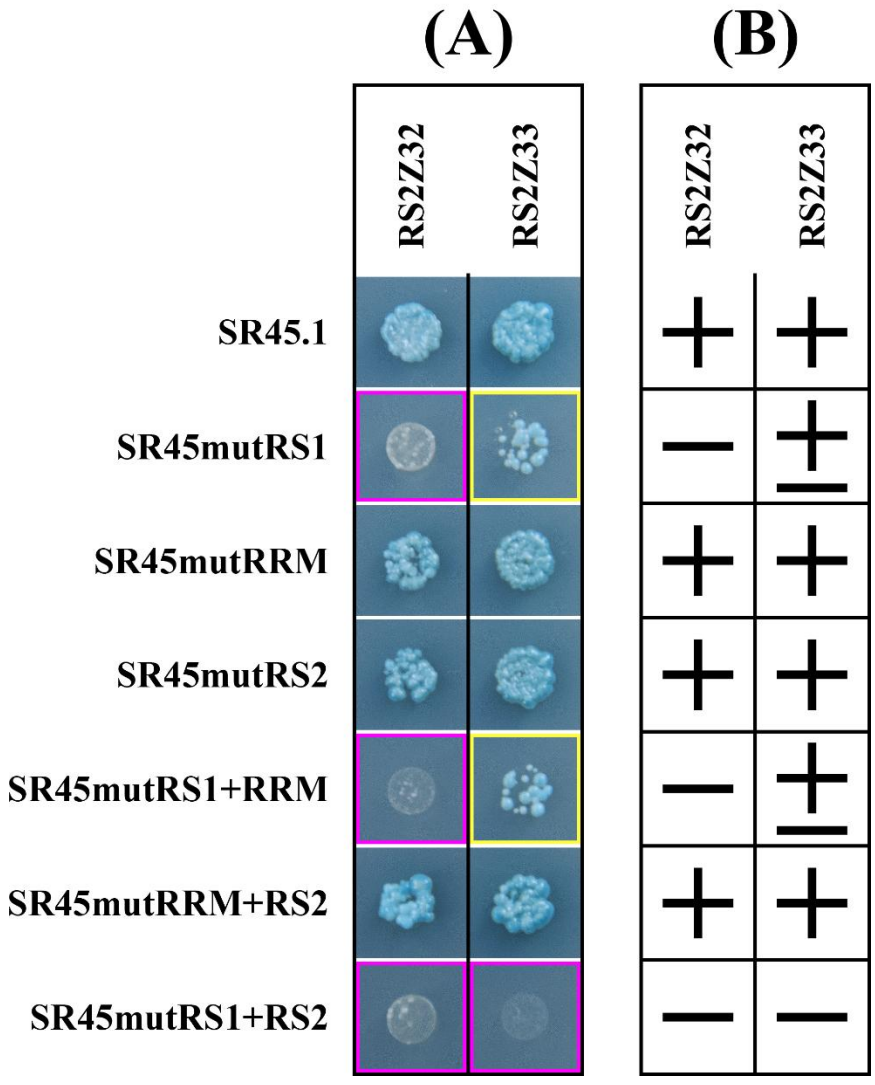
Supplementary Figure S5. Nucleocytoplasmic shuttling of mutant variants altered in the RRM, ZnKs or RRM+ZnKs. FLIP-shuttling was assessed without (−LMB) or with LMB (+LMB) treatment in tobacco epidermal leaf cells. One hundred percent fluorescence indicates prebleach fluorescence intensity. Insets show the overlay of wild-type and mutant curves. Values are means ± SEM for at least 12 nuclei. A significant inhibitory effect of LMB is indicated by asterisks (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001). n.s., not significant.



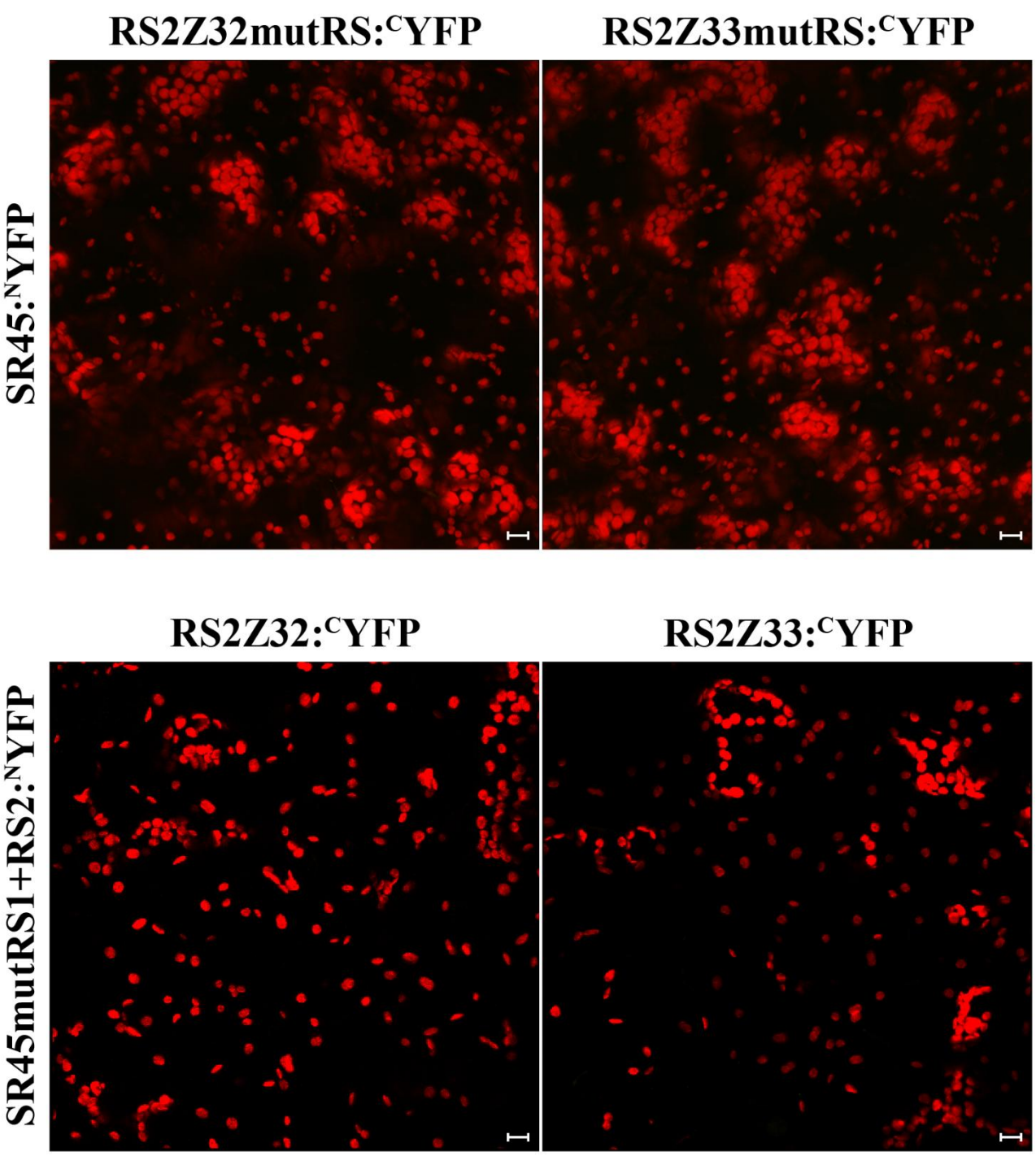
Supplementary Figure S6. Interactions of RS2Z32 and RS2Z33 with kinases, splicing-associated factors, and the nuclear import factor upon serial dilutions. From the initial culture, dilutions to an OD600 of 0.25, 0.025 and 0.0025 were spotted on -Trp/-Leu/-His/X-α-Gal/AurA agar plates. Companion to **Figure 6**.



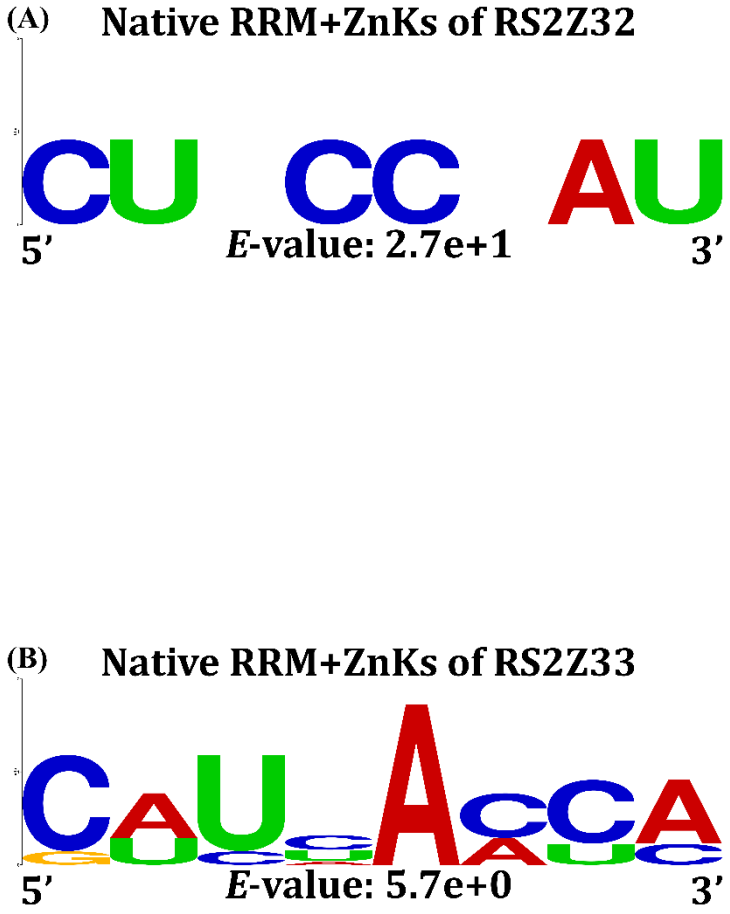
Supplementary Figure S7. Full-length RS2Z proteins strongly interact with only few Arabidopsis SR proteins. RS2Z32 **(A)** and RS2Z33 **(B)** interactions with RSZ, SC35 and SR subfamilies (mammalian orthologs), and RS2Z, SCL, RS and SR45 subfamilies (plant specific). **(C)** Interactions established between SR45 isoforms and truncated RS2Z32 or RS2Z33 variants lacking both RS and SP domains (ΔRS+SP), or the SP domain (ΔSP), or all domains but the RRM (ΔZnKs+RS+SP). From the initial culture, dilutions to an OD600 of 0.25 were spotted on -Trp/-Leu/-His/X-a-Gal/AurA agar plates. Positive interactions were confirmed by growth and blue staining. The absence of interaction is characterized at most by a shadow of dead cells.



Supplementary Figure S8. RS2Z proteins associate to SR45, partly through its RS1 domain. **(A)** Observed RS2Z interactions with SR45 mutant variants in yeast cells. From the mated culture, dilutions to an OD600 of 0.5 were spotted on -Trp/-Leu/-His/X-a-Gal/AurA agar plates. Positive interactions were confirmed by growth and blue staining. Yellow and purple squares are indicative of a weakened or abolished interaction, respectively, upon substitutions within the corresponding SR45 domain. A weakened interaction is characterized by the ability of several single colonies to grow, while an abolished interaction is characterized at most by a white halo of dead cells. **(B)** Interpretive summary of the interactions of RS2Z32 and RS2Z33 with SR45 mutant variants. Signs: +, positive interaction; -, absence of interaction; ±, weakened interaction.



Supplementary Figure S9. The *in planta* interaction between RS2Z proteins and SR45 is dependent on their respective phosphorylatable RS domain(s). Bimolecular fluorescence complementation (BiFC) through co-expression of RS2Z32(mutRS) or RS2Z33(mutRS) fused to the C-terminal half of YFP [RS2Z32(mutRS):^{C}YFP or RS2Z33(mutRS):^{C}YFP] and SR45 or SR45mutRS1+RS2 fused to the N-terminal half of YFP [SR45(mutRS1+RS2):^{N}YFP]. Representative images of fluorescence reveal the absence of interaction of either RS2Z32mutRS or RS2Z33mutRS with SR45, or RS2Z32 or RS2Z33 with SR45mutRS1+RS2 in transient expression assay in tobacco leaf cells. At least three independent transient events were generated and analyzed, depicting similar fluorescence profiles. Scale bars = 10 µm. Red signals represent chlorophyll autofluorescence.



Supplementary Figure S10. The RRM and zinc-knuckles of the RS2Z proteins do not conjointly provide a specific RNA motif binding. The non-significant RNA motifs were identified through 4 rounds of SELEX selection with the native RRM and the two ZnKs of RS2Z32 **(A)** and RS2Z33 **(B)**. The statistical significance (*E*-value) is indicated at the bottom of each consensus. Logos were redesigned using WebLogo (Crooks *et al.*, 2004). All RNA motifs were discovered using the MEME tool (version 5.5.1) with 30 sequences.

Target	Forward (5’ – 3’)	Reverse (5’ – 3’)	Construct	Literature
SR proteins				
RS2Z32	GGGAATTCATGCCTCGCTATGATGATCGC	GGGGATCCTCAAGGTGACTCACTGCCTTTAGG	pGADT7 / pGBKT7	(Fanara *et al.*, 2024)
RS2Z32ΔSP	GGGAATTCATGCCTCGCTATGATGATCGC	GGGGATCCTCATGGACTGCGTGATCT	pGADT7 / pGBKT7	This study
RS2Z32ΔRS+SP	GGGAATTCATGCCTCGCTATGATGATCGC	GGGGATCCTCATCCACCCTGCCTGGC	pGADT7 / pGBKT7	This study
RS2Z32ΔZnKs+RS+SP	GGGAATTCATGCCTCGCTATGATGATCGC	GGGGATCCTCAGCGACCAGAACCAGGAG	pGADT7 / pGBKT7	This study
RS2Z33	GGGAATTCATGCCTCGCTATGATGATCGC	GGATCCAGGAGACTCACTTCCTCTAGG	pGADT7 / pGBKT7	(Fanara *et al.*, 2024)
RS2Z33ΔSP	GGGAATTCATGCCTCGCTATGATGATCGC	GGGGATCCTCATGGACTGCGTGATCT	pGADT7 / pGBKT7	This study
RS2Z33ΔRS+SP	GGGAATTCATGCCTCGCTATGATGATCGC	GGGGATCCTCATCCACTGCGCCTAAGCT	pGADT7 / pGBKT7	This study
RS2Z33ΔZnKs+RS+SP	GGGAATTCATGCCTCGCTATGATGATCGC	GGGGATCCTCAGCGACCAGCTCCAGG	pGADT7 / pGBKT7	This study
RS31	GGGAATTCATGAGGCCAGTGTTCGTCG	GGGGATCCTCAAGGTCTTCCTCTTGGGACT	pGADT7	(Fanara *et al.*, 2024)
RS31a	GGGAATTCATGAGACATGTGTACGTTGGGAATT	GGGGATCCTCAACCTCTTGCTCTTTGAATCG	pGADT7 / pGBKT7	(Fanara *et al.*, 2024)
RS40	GGGAATTCATGAAGCCAGTCTTCTGTGGG	GGGGATCCTCACTCGTCAGCTGGTGGC	pGBKT7	(Fanara *et al.*, 2024)
RS41	GGGAATTCATGAAGCCTGTCTTTTGCGG	GGGGATCCTCATTCCTCTGCTGGCGG	pGADT7 / pGBKT7	(Fanara *et al.*, 2024)
RSZ21	GGCCCGGGTATGACGAGGGTTTATGTCGGG	GGGGATCCTCACACCCCATTGGCATATG	pGADT7	(Fanara *et al.*, 2024)
RSZ22	GAATTCATGTCACGTGTGTACGTCG	GGATCCTCAGCTCCTGCTTCTGC	pGADT7 / pGBKT7	(Fanara *et al.*, 2024)
RSZ22a	GGGAATTCATGTCGCGTGTGTATGTTGGTAAT	GGCCCGGGTCAGCTCCGGCTTCTGC	pGADT7 / pGBKT7	(Fanara *et al.*, 2024)
SC35	GGGAATTCATGTCGCACTTCGGAAGGTC	GGGGATCCTCATTCCGCAGCATAAGGAGA	pGADT7 / pGBKT7	(Fanara *et al.*, 2024)
SCL28	GGGAATTCATGGCTAGAGCGAGAAGCCG	GGGGATCCTCAACGACTTAAGGATCGAGAACG	pGBKT7	(Fanara *et al.*, 2024)
SCL30	GGGAATTCATGAGGAGATACAGTCCGCCTTATTA	GGCTGCAGTCATCTTGGAGATACCTCCACAGAC	pGBKT7	(Fanara *et al.*, 2024)
SCL30	GGGAATTCATGAGGAGATACAGTCCGCCTTATTA	GGGAGCTCTCATCTTGGAGATACCTCCACAGAC	pGADT7	(Fanara *et al.*, 2024)
SCL30a	GGGAATTCATGAGAGGAAGGAGCTACACGC	GGCCCGGGTCACTGGCTTGGAGAACGG	pGADT7 / pGBKT7	(Fanara *et al.*, 2024)
SCL33	GGGAATTCATGAGGGGAAGGAGCTACACTCC	GGGGATCCTCACTGGCTTGGTGAACGG	pGADT7 / pGBKT7	(Fanara *et al.*, 2024)
SR30	GGGGCCATGGGGATGAGTGGGCGATTTTCTCGGTC	GGATCCACCAGATATCACAGGTGAAAC	pGADT7 / pGBKT7	(Stankovic et al., 2016)
SR34	GAATTCATGAGCAGTCGTTCGA	AGGGATCCCCTCGATGGACTC	pGADT7 / pGBKT7	(Stankovic et al., 2016)
SR34a	GGGCCATGGGGATGAGTGGGCGATTTTCTCGGTC	AGGGATCCCACACTGCCTTCGC	pGADT7 / pGBKT7	(Stankovic et al., 2016)
SR34b	GAATTCATGAGCAGCCGTTCG	GGATCCTATCAATGCGATCCAATG	pGBKT7	(Stankovic et al., 2016)
SR45.1 / SR45.2	CGGGATCCGGATGGCGAAACCAAGTCGTGGC	CCGAGCTCTTAAGTTTTACGAGGTGGAGGTGGTGG	pGADT7	(Stankovic et al., 2016)
SR45.1 / SR45.2	CGGGATCCGGATGGCGAAACCAAGTCGTGGC	GGCTGCAGAGTTTTACGAGGTGGAGG	pGBKT7	(Stankovic et al., 2016)

SR45mut(RRM+)RS2	GGGGCGCGCCATGGCGAAACCAAGTCGTG	GGGGATCCAGTTTTACGAGGTGGAGGTGGTGGTGG	pGADT7(+) / pGBKT7(+)	(Fanara *et al.*, 2024)
SR45mutRS1(+RRM+RS2)	GGGGCGCGCCATGGCGAAACCAGCTCGT	GGGGATCCAGTTTTACGAGGTGGAGGTGGTGGTGG	pGADT7(+) / pGBKT7(+)	(Fanara *et al.*, 2024)
hnRNP-like				
GRP7	GGGAATTCATGGCGTCCGGTGATGTTG	GGGGATCCTTACCATCCTCCACCACCACC	pGADT7	(Fanara *et al.*, 2024)
GRP8	GGGAATTCATGTCTGAAGTTGAGTACCGGTGC	GGGGATCCTTACCAGCCGCCACCAC	pGADT7 / pGBKT7	(Fanara *et al.*, 2024)
RZ-1B	CCGAATTCATGAAAGATAGAGAAAACGATGGAAATC	GGGGATCCCTACCAACGTTCATATGATGAAGGTC	pGBKT7	(Fanara *et al.*, 2024)
RZ-1C	CCGAATTCATGGCTGCAAAAGAAGGTAGTAGG	GGGGATCCTTAATAACGGTCAAAAGTGGACG	pGBKT7	(Fanara *et al.*, 2024)
PRPs				
PRP38	GGGGATCCTTATGGCAAACAGAACAGATCCG	GGCTCGAGGTCCCTGAGGGGTTTCATTCC	pGADT7	(Fanara *et al.*, 2024)
Kinases				
AFC1	GGCCCGGGTATGCAAAGCAGTGTGTATCGTGATAA	GGGGATCCTTAGTTCTTTTGGTTGTATAAAAATGGATGAG	pGADT7	(Fanara *et al.*, 2024)
AFC2	GGCATATGATGGAGATGGAGCGTGTGC	GGGGATCCCTATCTTCTCCTTGCGAAAAACG	pGADT7 / pGBKT7	(Fanara *et al.*, 2024)
AFC3	GGGAATTCATGATAGCTAACGGATTCGAGAGTATG	GGGGATCCTCAACTTGAGCTCTTAAAGAAAGGATG	pGADT7	(Fanara *et al.*, 2024)
SRPK1	GGGAATTCATGTCTTGTTCATCTTCTTCCGGAT	GGGGATCCTCACCCTTTGATATGCAAGTTGTTC	pGADT7	(Fanara *et al.*, 2024)
SRPK2	GGGAATTCATGTCGTGTTCATCCTCATCTGG	GGCCCGGGTCAAGAACATGAACCTTTGATCTGC	pGADT7	(Fanara *et al.*, 2024)
SRPK3a	GGGAATTCATGGCGGATGAGAAGAACGG	GGGGATCCTTAAGTACGAAGATCACGAGCTGATTG	pGADT7 / pGBKT7	(Fanara *et al.*, 2024)
SRPK3b/SRPK5	GGGAATTCATGGCGGAGGACAAAAACAAC	GGGGATCCTCACTTCGGTTCAGAGACATCAATAG	pGADT7	(Fanara *et al.*, 2024)
SRPK4	GGGAATTCATGGAGGCGGAGAAGTGGAACAG	CGGGATCCAATTGCTAGCTTAAGAGTGGAGGAGCTT	pGADT7 / pGBKT7	(Stankovic et al., 2016)
Cyclophilines				
Cyp59	GGGAATTCATGTCAGTTCTTATTGTGACGAGCC	GGGGATCCTCATCTATCCCTTCTCTCATGTCTAGCT	pGADT7	(Fanara *et al.*, 2024)
CypRS64	CGGAATTCATGACTAAAAAGAAGAATCCTAATGTTTT	TATAGAGCTCTCAATCCGCATAGCTAACCAG	pGADT7	(Stankovic et al., 2016)
Cyp65	GGGAATTCATGGGGAAGAAACAACACAGCA	GGGGATCCTTACCAGCTAGAGAAATCTTTAAACCCTG	pGADT7	(Fanara *et al.*, 2024)
Cyp71	GGGAATTCATGGAGGAAGAATCTAAGAATGGCG	GGCCCGGGCTAAGATTTCGGAACGGTGACATTAA	pGADT7	(Fanara *et al.*, 2024)
CypRS92	GGGAATTCATGGCAAAAAAGAAGAATCCACAGG	GGCCCGGGTTAATCATAGGCTACTAATCCCTTTTTCCC	pGADT7	(Fanara *et al.*, 2024)
EJC(-related)				
ACINUS(RRM)	CCGAATTCATGCTTCCTGCTAATGATCAAGAAGC	CCGGATCCTCACTTGTTATTATTCGCTGCAAGTTTAG	pGADT7 / pGBKT7	(Fanara *et al.*, 2024)
ALY4	CCGAATTCATGTCTGGAGCATTGAATATGACTCTTG	CCGGATCCTCAAGAGGTGTTCATGGCATCAGC	pGADT7 / pGBKT7	(Fanara *et al.*, 2024)
eIF4AIII	CCGAATTCATGGCGACAGCGAATCCTGG	CCGGATCCTCAGATAAGATCAGCTACATTCATTGGC	pGADT7 / pGBKT7	(Fanara *et al.*, 2024)
MAGO	GGGAATTCATGGCCGCGGAAGAAGC	GGGGATCCCTAGATAGGCTTGATTTTGAAGTGCAG	pGADT7 / pGBKT7	(Fanara *et al.*, 2024)

PININ	GGCATATGATGGGAGACACCGCCTTG	CCGAATTCTTAGAGAACCTCATGTTTAATATCTTCC	pGADT7 / pGBKT7	(Fanara *et al.*, 2024)
SAP18	CCGAATTCATGGCTGAAGCAGCGAGAAGAC	CCGGATCCTCAGTAAATTGCCACATCCAGATAATC	pGBKT7	(Fanara *et al.*, 2024)
Y14	CCGAATTCATGGCGAACATAGAATCAGAAGCAGTC	CCGGATCCTCAGTAACGTCTTCTCGGACTTCTTG	pGADT7 / pGBKT7	(Fanara *et al.*, 2024)
MOSes				
MOS12	GGCATATGATGATTTACACTGCTATCGACAATTTTTAC	GGGGATCCTTAATGGTGCCTACGACGGTCT	pGBKT7	(Fanara *et al.*, 2024)
MOS14	GGCCCGGGTATGGAGCATCAGAACGCGG	GGGGATCCTGATACAGGAGCAGTAACCAGATTCA	pGADT7	(Fanara *et al.*, 2024)

Supplementary Table S1: Primers used to amplify coding sequences of potential interactors used in directed yeast two-hybrid assays. Restriction sites used are underlined. Bold blue bases are used to preserve the open reading frame in final construct. Cells colored in light green or light orange represent, respectively, interactors identify through yeast two-hybrid screens using either a commercially available Arabidopsis cDNA library (Mate and Plate Library-Universal Arabidopsis, Clontech) or a custom cDNA library (Make Your Own “Mate & Plate” Library System, Clontech).

Expression profiling and protein localization			
Target	**Forward (5’ – 3’)**	**Reverse (5’ – 3’)**	**Construct** ***Remark***
pRS2Z32	GGCCTGCAGGTCTTTAATGGGCTTTAGAGTTGATATAAGG	CCGGTACCTGTCAAGCTGCAAAATATTTT	pMDC32:pRS2Z32:RS2Z32:EGFP pMDC32:pRS2Z32:EGFP
RS2Z32	CGGGCGCGCCATGCCTCGCTATGAT	CCTTAATTAAAGGTGACTCACTGCCTTTAGG	pMDC32:pRS2Z32:RS2Z32:EGFP
pRS2Z33	GGCCTGCAGGAATAATAAAGTCATAATTTATGAA	CCGGTACCTGTCAAGCTACAAAATCAAAAA	pMDC32:pRS2Z33:RS2Z33:EGFP pMDC32:pRS2Z33:EGFP
RS2Z33	TTGGCGCGCCATGCCTCGCTATGATGATCGCTATG	CCTTAATTAAAGGAGACTCACTTCCTCTAGGGGAAGTG	pMDC32:pRS2Z33:RS2Z33:EGFP
EGFP	CCTTAATTAA**C**ATGGTGAGCAAGGGCGAGGAG	CCGAGCTCTTACTTGTACAGCTCGTCCATGC	pMDC32:pRS2Z32:RS2Z32:EGFP pMDC32:pRS2Z33:RS2Z33:EGFP
EGFP	CCGGCGCGCC**C**ATGGTGAGCAAGGGCGAGGAG	CCGAGCTCTTACTTGTACAGCTCGTCCATGC	pMDC32:pRS2Z32:EGFP pMDC32:pRS2Z33:EGFP

Supplementary Table S2: Primers used for constructs for expression profiling and protein localization *in planta*. Restriction sites used are underlined. Bold blue bases are used to ensure the open reading frame in final constructs.

PCR-based point mutagenesis			
Gene	**Forward (5' – 3')**	**Reverse (5' – 3')**	**Mutant**
RS2Z32	GATGTGGATATGAAGCGTGATGCTGCCGCTGTTGAATTTAGTGATCCTCG	CGAGGATCACTAAATTCAACAGCGGCAGCATCACGCTTCATATCCACATC	RS2Z32mutRNP1
RS2Z32	TGGAAACACTCGCCTCGCTGTTGGTCGCTTATCA	TGATAAGCGACCAACAGCGAGGCGAGTGTTTCCA	RS2Z32mutRNP2
RS2Z32	GGTTCTGGTCGCGCTTTTAATGCTGGTGTCGATGGC	GCCATCGACACCAGCATTAAAAGCGCGACCAGAACC	RS2Z32mutZnK1.1
RS2Z32	ACTGGGCCCGAGACGCCACAGCAGGAGACT	AGTCTCCTGCTGTGGCGTCTCGGGCCCAGT	RS2Z32mutZnK1.2
RS2Z32	ACTGGAAGAATAAAGCTTACCGCGCTGGTGAAAGAGGACA	TGTCCTCTTTCACCAGCGCGGTAAGCTTTATTCTTCCAGT	RS2Z32mutZnK2.1
RS2Z32	AGAGGACACATTGAGAGAAACGCCAAAAACAGTCCTAGTCCAAA	TTTGGACTAGGACTGTTTTTGGCGTTTCTCTCAATGTGTCCTCT	RS2Z32mutZnK2.2
RS2Z33	TGGATATGAAGCGAGATGCTGCTGCCGTTGAATTTGGTGATCCCC	GGGGATCACCAAATTCAACGGCAGCAGCATCTCGCTTCATATCCA	RS2Z33mutRNP1
RS2Z33	GCTATGGGAACACTCGTCTTGCCGTTGGCCGATTATCATCGA	TCGATGATAATCGGCCAACGGCAAGACGAGTGTTCCCATAGC	RS2Z33mutRNP2
RS2Z33	GAGCTGGTCGCGCTTTTAACGCTGGTGTAGATGG	CCATCTACACCAGCGTTAAAAGCGCGACCAGCTC	RS2Z33mutZnK1.1
RS2Z33	ATTGGGCTCGTGACGCCACAGCAGGGGACT	AGTCCCCTGCTGTGGCGTCACGAGCCCAAT	RS2Z33mutZnK1.2
RS2Z33	TGGAAGAACAAGGCTTACCGTGCTGGAGAGAGAGGACACA	TGTGTCCTCTCTCTCCAGCACGGTAAGCCTTGTTCTTCCA	RS2Z33mutZnK2.1
RS2Z33	AGGACACATTGAGAGAAACGCCAAAAACCAGCCCAAGAAG	CTTGGGCTGGTTTTTGGCGTTTCTCTCAATGTGTCCT	RS2Z33mutZnK2.2

Supplementary Table S3: Primers used for PCR-based point mutagenesis in the RRM and in ZnK1/ZnK2 domains of *RS2Z32* and *RS2Z33*. Critical aromatic amino acids connecting the RNA in RNP2 and RNP1 motifs were substituted by alanine residues (Maris *et al.*, 2005; Califice *et al.*, 2012; Stankovic *et al.*, 2016). The substituting codons are underlined in the sequence.

Primers	Sequence (5’ – 3’)	
	RS2Z32	RS2Z33
1	GGGAATTCATGCCTCGCTATGATGATCGC	GGGAATTCATGCCTCGCTATGATGATCGC
2	GGGGATCCTCAAGGTGACTCACTGCCTTTAGG	GGATCCAGGAGACTCACTTCCTCTAGG
3	GGGGATCCTCAAGGTGCCTCAGCGCC	GGGGATCCTTAAGGAGCCTCAGCTCCTCTAGG
4	GGCGCTGAGGCACCTTGAGGATCC	CCTAGAGGAGCTGAGGCTCCTTAAGGATCC
5	CGGGCCCAGTGGCCATCGACACCACAATTAAAACAGCG	ATCCCGAGAACCACGAGGTGCCCCTCGTGAAAACTCCACAGT
6	TGGCCACTGGGCCCG	CACCTCGTGGTTCTCGGGAT
7	TCAAGGTGCCTCAGCGCC	TTAAGGAGCCTCAGCTCCTCTAGG
8	GGACAGAGCACGCGCTCCTAAGGCAATGGAGCGATCTGTATC	GGAGGAGAGATCACGCAGTCCAAAGCGGATGGATGACGCTCTA
9	GGAGGACAGATCACGCAGTCCTAAGGCAATGGAGCGAGCTGTA	GGAGGACAGATCACGCAGTCCTAAGGCAATGGAGCGAGCTGTA

Supplementary Table S4: Primers used to construct *RS2Z32* and *RS2Z33* mutant variants. Restriction sites used (*Eco*RI et *Bam*HI) are underlined.

Synthetic genes	
Mutant variant	**Coding sequence**
RS+SP domains to RA+AP domains of *RS2Z32* (411 bp, 32 S-to-A substitutions)	GGA**GCA**TAT**GCC**AGG**GCA**CCAGTCAAA**GCC**CGC**GCC**CCTCGTCGCCGAAGG**GCA**CCA**GCA**CGT**GCA**CGT**GCT**TAC**GCT**CGAGGT CGC**GCA**TAC**GCT**CGA**GCC**CGA**GCC**CCAGTGAGAAGAGAGAAA**GCA**GTGGAGGACAGA**GCA**CGC**GCT**CCTAAGGCAATGGAGCGA **GCT**GTA**GCT**CCCAAAGGTAGGGACCAA**GCA**CTG**GCT**CCAGACCGAAAAGTGATAGATGCA**GCA**CCAAAGCGTGGA**GCA**GACTAT GATGGT**GCA**CCAAAAGAGAATGGTAATGGCAGGAAC**GCT**GCG**GCT**CCCATTGTTGGAGGTGGTGAA**GCT**CCTGTTGGACTTAAT GGTCAAGACAGG**GCA**CCGATTGATGATGAGGCTGAGCTT**GCA**CGTCCT**GCC**CCTAAAGGC**GCT**GAG**GCA**CCTTGA
RS+SP domains to RA+AP domains of *RS2Z33* (435 bp, 33 S-to-A substitutions)	GGA**GCA**TAC**GCC**AGG**GCA**CCTGTAAGA**GCC**CGT**GCT**CCTCGTCGTAGAAGA**GCA**CCA**GCA**CGG**GCT**CTT**GCA**CGT**GCA**CGA**GCA** TAC**GCA**CGA**GCA**CGA**GCC**CCGGTGAGAAGAAGAGAGAGG**GCT**GTGGAGGAGAGA**GCA**CGC**GCT**CCAAAGCGGATGGATGAC**GCT** CTA**GCC**CCAAGAGCCAGAGATCGT**GCT**CCGGTTCTTGATGATGAAGGC**GCC**CCAAAGATCATAGACGGG**GCA**CCACCACCA**GCA** CCAAAGCTTCAAAAGGAAGTCGGA**GCT**GACCGTGACGGTGGT**GCC**CCCCAAGACAATGGCAGAAAC**GCT**GTTGTC**GCT**CCTGTT GTAGGAGCCGGTGGTGAC**GCTGCC**AAAGAGGACCGG**GCA**CCTGTTGATGATGATTACGAGCCAAACCGCACT**GCC**CCTAGAGGA **GCT**GAG**GCT**CCTTAA

Supplementary Table S5: Synthetic genes provided by GenScript where serine codons within RS and SP domains of *RS2Z32* and *RS2Z33* genes were substituted by alanine codons (red and bold).

BiFC			
Target	**Forward (5' – 3')**	**Reverse (5' – 3')**	**Construct**
RS2Z32(mutRS)	CGGGATCCATGCCTCGCTATGAT	GTGGTACCAGGTGACTCACTGCCTT	pBI121:35S:RS2Z32(mutRS):^{N}YFP pBI121:35S:RS2Z32(mutRS):^{C}YFP
RS2Z33(mutRS)	CGGGATCCATGCCTCGCTATGAT	GTGGTACCAGGAGACTCACTTCCTC	pBI121:35S:RS2Z33(mutRS):^{N}YFP pBI121:35S:RS2Z33(mutRS):^{C}YFP
SR45.1	GGCGGGATCCGGATGGCGAAACCAAGTCGTGGCCG	CCGGTACCAGTTTTACGAGGTGGAGGTGG	pBI121:35S:SR45.1:^{N}YFP
SCL30	GGGGATCCTTATGAGGAGATACAGTCCGCCTTATTA	GGGGTACCTCTTGGAGATACCTCCACAGACCT	pBI121:35S:SCL30:^{N}YFP
SR34	CCGGATCCGGATGAGCAGTCGTTCGAGTAGAACCG	GGGGTACCCCTCGATGGACTCCTAGTGTGGATAG	pBI121:35S:SR34:^{N}YFP
CypRS64	GGGGCGCGCCATGACTAAAAAGAAGAATCCTAATG	GGTTAATTAAATCCGCATAGCTAACCAG	pBI121:35S:CypRS64:^{N}YFP

Supplementary Table S6: Primers used to confirm protein-protein interactions *in planta* (BiFC experiments). Restriction sites used are underlined.

SELEX			
Synthesis of the DNA initial library			
Randomized initial library		***T7* promoter**	
TCCCGCTCGTCGTCT*NNNNNNNNNNNNNNNNNNNNNNNNN*CCGCATCGTCCTCCCT		GAAATTAATACGACTCACTATAGGGAGGACGATGCGG	
Recombinant gene construction			
Target	**Forward (5' – 3')**	**Reverse (5' – 3')**	**Construct**
RS2Z32 (native & mutated RRM)	GGGGATCCATGCCTCGCTATGATGATCGC	GGGAATTCTCAGCGACCAGAACCAGGAG	pGEX6P1
RS2Z32 (native RRM+ZnKs)	GGGGATCCATGCCTCGCTATGATGATCGC	GGGAATTCTCATCCACCCTGCCTGGC	pGEX6P1
RS2Z33 (native & mutated RRM)	GGGGATCCATGCCTCGCTATGATGATCGC	GGGAATTCTCAGCGACCAGCTCCAGG	pGEX6P1
RS2Z33 (native RRM+ZnKs)	GGGGATCCATGCCTCGCTATGATGATCGC	GGGAATTCTCATCCACTGCGCCTAAGCT	pGEX6P1

Supplementary Table S7: Primers used for SELEX experiments (De Franco *et al.*, 2019). Red bases correspond to the *T7* promoter. Restriction sites used are underlined.

qRT-PCR				
Gene ID	**Symbol**	**Forward (5' – 3')**	**Reverse (5' – 3')**	**Reference**
AT3G53500	RS2Z32	CAAATCGCTACCGTTGAATCTC	AAGGAAGTGAACCGCGGAT	This study
AT2G37340	RS2Z33	AACAGCCCCAAGAAGCTTAGG	AGCTTCGGCTACGGCTAAGACT	This study
AT1G58050	AT1G58050	CCATTCTACTTTTTGGCGGCT	TCAATGGTAACTGATCCACTCTGATG	(Rausin *et al.*, 2010)

Supplementary Table S8: Primers used for mRNA levels analysis (qRT-PCR).

Comparison	Estimate	Standard error	DF	t Value	Pr > |t|	Summary
RS2Z32: −LMB vs +LMB	-29.9254	1.0993	11E3	-27.22	<.0001	****
RS2Z32mutRNP1: −LMB vs +LMB	-28.5700	1.3669	11E3	-20.90	<.0001	****
RS2Z32mutRNP2: −LMB vs +LMB	0.3087	1.2628	11E3	0.24	0.8069	n.s.
RS2Z32mutRRM: −LMB vs +LMB	-21.0303	1.3191	11E3	-15.94	<.0001	****
RS2Z32mutZnK1: −LMB vs +LMB	-14.4225	1.2941	11E3	-11.14	<.0001	****
RS2Z32mutZnK2: −LMB vs +LMB	-24.7687	1.2855	11E3	-19.27	<.0001	****
RS2Z32mutZnKs: −LMB vs +LMB	-20.2759	1.4118	11E3	-14.36	<.0001	****
RS2Z32mutRRM+ZnKs: −LMB vs +LMB	-16.4865	1.6691	11E3	-9.88	<.0001	****
RS2Z33: −LMB vs +LMB	-33.1373	0.9694	11E3	-34.18	<.0001	****
RS2Z33mutRNP1: −LMB vs +LMB	-32.6177	1.2654	11E3	-25.78	<.0001	****
RS2Z33mutRNP2: −LMB vs +LMB	-1.1055	1.2112	11E3	-0.91	0.3614	n.s.
RS2Z33mutRRM: −LMB vs +LMB	-20.8057	1.0937	11E3	-19.02	<.0001	****
RS2Z33mutZnK1: −LMB vs +LMB	-12.6618	1.3191	11E3	-9.60	<.0001	****
RS2Z33mutZnK2: −LMB vs +LMB	-22.4074	1.0497	11E3	-21.35	<.0001	****
RS2Z33mutZnKs: −LMB vs +LMB	-16.9740	1.2766	11E3	-13.30	<.0001	****
RS2Z33mutRRM+ZnKs: −LMB vs +LMB	-9.9068	1.2767	11E3	-7.76	<.0001	****
−LMB: RS2Z32 vs RS2Z32mutRNP1	-6.1899	1.2039	11E3	-5.14	<.0001	****
−LMB: RS2Z32 vs RS2Z32mutRNP2	-8.9960	1.1731	11E3	-7.67	<.0001	****
−LMB: RS2Z32 vs RS2Z32mutRRM	-5.1025	1.1880	11E3	-4.29	<.0001	****

−LMB: RS2Z32 vs RS2Z32mutZnK1	-1.9440	1.1332	11E3	-1.72	0.0863	n.s.
−LMB: RS2Z32 vs RS2Z32mutZnK2	-5.4579	1.1213	11E3	-4.87	<.0001	****
−LMB: RS2Z32 vs RS2Z32mutZnKs	-4.7918	1.2588	11E3	-3.81	0.0001	****
−LMB: RS2Z32 vs RS2Z32mutRRM+ZnKs	-1.1198	1.5066	11E3	-0.74	0.4573	n.s.
−LMB: RS2Z33 vs RS2Z33mutRNP1	-4.7573	1.1307	11E3	-4.21	<.0001	****
−LMB: RS2Z33 vs RS2Z33mutRNP2	-9.8165	1.0039	11E3	-9.78	<.0001	****
−LMB: RS2Z33 vs RS2Z33mutRRM	-8.7671	1.0694	11E3	-8.20	<.0001	****
−LMB: RS2Z33 vs RS2Z33mutZnK1	-3.4271	1.1940	11E3	-2.87	0.0041	**
−LMB: RS2Z33 vs RS2Z33mutZnK2	-8.9803	1.0299	11E3	-8.72	<.0001	****
−LMB: RS2Z33 vs RS2Z33mutZnKs	-8.1030	1.1452	11E3	-7.08	<.0001	****
−LMB: RS2Z33 vs RS2Z33mutRRM+ZnKs	-1.3723	1.1043	11E3	-1.24	0.2140	n.s.
+LMB: RS2Z32 vs RS2Z32mutRNP1	-4.8345	1.2891	11E3	-3.75	0.0002	***
+LMB: RS2Z32 vs RS2Z32mutRNP2	21.2380	1.2057	11E3	17.62	<.0001	****
+LMB: RS2Z32 vs RS2Z32mutRRM	3.7925	1.2522	11E3	3.03	0.0025	**
+LMB: RS2Z32 vs RS2Z32mutZnK1	13.5588	1.2699	11E3	10.68	<.0001	****
+LMB: RS2Z32 vs RS2Z32mutZnK2	-0.3012	1.2699	11E3	-0.24	0.8125	n.s.
+LMB: RS2Z32 vs RS2Z32mutZnKs	4.8577	1.2891	11E3	3.77	0.0002	***
+LMB: RS2Z32 vs RS2Z32mutRRM+ZnKs	12.3190	1.3328	11E3	9.24	<.0001	****
+LMB: RS2Z33 vs RS2Z33mutRNP1	-4.2376	1.1645	11E3	-3.64	0.0003	***
+LMB: RS2Z33 vs RS2Z33mutRNP2	22.2153	1.1980	11E3	18.54	<.0001	****

+LMB: RS2Z33 vs RS2Z33mutRRM	3.5645	1.0171	11E3	3.50	0.0005	***
+LMB: RS2Z33 vs RS2Z33mutZnK1	17.0484	1.1645	11E3	14.64	<.0001	****
+LMB: RS2Z33 vs RS2Z33mutZnK2	1.7496	1.0086	11E3	1.73	0.0828	n.s.
+LMB: RS2Z33 vs RS2Z33mutZnKs	8.0604	1.1645	11E3	6.92	<.0001	****
+LMB: RS2Z33 vs RS2Z33mutRRM+ZnKs	21.8583	1.1980	11E3	18.25	<.0001	****

Supplementary Table S9: Statistical analyses of FLIP-shuttling assays of native proteins or mutant variants in the absence (−LMB) and upon leptomycin B (+LMB) treatment. Cells colored in light blue, light pink or light green represent, respectively, effect of leptomycin B treatment on the nucleocytoplasmic shuttling activity; effect of mutations on the nucleocytoplasmic shuttling activity, in absence of leptomycin B (−LMB); and effect of mutations on the nucleocytoplasmic shuttling activity, in presence of leptomycin B (+LMB). For statistical analysis of FLIP-shuttling assays, percentages (relative to the initial level) level of fluorescence have been analyzed using mixed linear models (MIXED procedure) at thirteen given time points (every 10 sec). The differences observed between the two compared groups were considered to be statistically significant if *p*-values were at least < 0.05, as indicated by asterisks ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$). n.s., not significant.

Native RRM of RS2Z32				
#	**Sequence**	**Length (bp)**	**Purine (%)**	**Pyrimidine (%)**
1	UAAGACCGGUAUCCCAACCCCGUA	24	45.83	54.17
2	UAAACAGACCGGUAUCCCUUAGCA	24	50.00	50.00
3	UAAGUCCGGUACCCCCCUAAA	21	42.86	57.14
4	ACCAGCAUCGAUAGAGCUACUCCA	24	50.00	50.00
5	CCGAGCGGUAUCCCACAUGAU	21	47.62	52.38
6	UCCCCUCGUCUCUAUAUAUUCCGCUAUUCUCAUAUGCC	38	23.68	76.32
7	GGGCGGGUUUAGGCCGUUGGGAAC	24	62.50	37.50
8	GAACUUAUGAAGAGUAAAAAUAUCGAGACGAGGGG	35	74.29	25.71
9	GAUGAUCAUCCGUGGAAUUGGAA	23	60.87	39.13
10	AUAUCGACAAAUAGGGAG	18	72.22	27.78

Supplementary Table S10: List of sequences submitted to MEME to find a significant consensus for the native RRM of RS2Z32.

Native RRM of RS2Z33				
#	**Sequence**	**Length (bp)**	**Purine (%)**	**Pyrimidine (%)**
1	AAGUAGCUUCCGGUAUCCCUUA	22	40.91	59.09
2	UGUAGAGCCGGUAUCCCAGCUGUC	24	45.83	54.17
3	AGAGCAAUCUGCUAACUCCCUGG	23	47.83	52.17
4	UACAAUCUGCUACUCCCUGUAC	22	31.82	68.18
5	UUACAACCGGUAUCCCAGCUACCG	24	41.67	58.33
6	CUGAUCCGGUAUCCUAAACCGACA	24	45.83	54.17
7	AUGAUCCGGUAUCCUAACCCGACA	24	45.83	54.17
8	GCGACGGGGGACAAGAGGGUGUGA	24	79.17	20.83
9	GCAUAAUAAAGGAAGGCGGGGAUA	24	79.17	20.83
10	GGAGAAAUUAAUACGACUCACUAUAGGGAG	30	66.67	33.33

Supplementary Table S11: List of sequences submitted to MEME to find a significant consensus for the native RRM of RS2Z33.

Mutated RRM of RS2Z32				
#	**1st set of sequences (Motif #1)**	**Length (bp)**	**Purine (%)**	**Pyrimidine (%)**
1	GCGGGCGGGUUAUCA	15	60.00	40.00
2	AAAGAGGGGUGC	12	83.33	16.67
3	AUAUCGACAAAUAGGGAG	18	72.22	27.78
4	GACGAUGCGCAUAAACAAGAUGGUG	25	68.00	32.00
5	GAAAUGUGGGCUCAGGGAGUUGGUA	25	68.00	32.00
6	GCCACAGUAUUUGAGAGA	18	61.11	38.89
7	AACUGACAACGCAUCUUAACGAAUA	25	56.00	44.00
8	UAAGUCCGGUACCCCCCUAAA	21	42.86	57.14
9	GGAAAAUUAAUACGACUCACUAUAGGGAG	29	65.52	34.48
10	GCGGGCUAUAAAGUAUGAAGCGGCU	25	64.00	36.00

Mutated RRM of RS2Z32				
#	**2nd set of sequences (Motif #2)**	**Length (bp)**	**Purine (%)**	**Pyrimidine (%)**
1	CGGGAAAGAGGGUUAC	16	75.00	25.00
2	CAAACGGGUACUACAUAUUGUGUA	24	54.17	45.83
3	CGGGGGUAGCGGGA	14	78.57	21.43
4	GAGGGGAACCGGGACGGGAGUGAA	24	83.33	16.67
5	GACAGAAGACGAGGGAG	17	88.24	11.76
6	GACAGACGACGAG	13	76.92	23.08
7	GAAAGGUUAAGGGGGGGUUUGAUC	24	70.83	29.17
8	AUAGUGAGUCGUAAUAAUAUCCGACUCACUAUA	33	51.52	48.48
9	GGAGGGAGGACGAUGCGGGG	20	85.00	15.00
10	GUUAAAGCAUGGUGCCACUGCGAA	24	58.33	41.67

Supplementary Table S12: List of sequences (two sets of ten sequences) submitted to MEME for the mutated RRM of RS2Z32.

Mutated RRM of RS2Z33				
#	**1st set of sequences (Motif #1)**	**Length (bp)**	**Purine (%)**	**Pyrimidine (%)**
1	GACGGGCCUUAGUGAGUGAUGCU	23	56.52	43.48
2	GCGGGCCAUGAUACAAAGCGCGCU	24	58.33	41.67
3	CGGGGAAUUGAGCGAGGGGCAGA	23	78.26	21.74
4	AAAAAACAAGGGCGUAAGGCGGG	23	82.61	17.39
5	UACAAGACACCAUUUGUGGCCCGC	24	45.83	54.17
6	AGGGGGGGAGGAUAUCAAAUGGG	23	82.61	17.39
7	UCCUGACACACUAUGGGAGGG	21	57.14	42.86
8	AGGACGAUGCGGUCCUGA	18	61.11	38.89
9	GGUUAUCGAAAACACUAGGGGGGG	24	70.83	29.17
10	UAUAAUUUAAGCUACCUGUAGCUA	24	45.83	54.17

Mutated RRM of RS2Z33				
#	**2nd set of sequences (Motif #2)**	**Length (bp)**	**Purine (%)**	**Pyrimidine (%)**
1	GCCGGGCAGGGGAACGGGUGAAUA	24	75.00	25.00
2	CUCACUAUAGGGAGGACGAUGCGAGC	26	61.54	38.46
3	AAAAACCGAAACCAACAAAAAAAAC	25	76.00	24.00
4	AGCAGAUACCGACGGAGGACAAGGCGGGG	29	75.86	24.14
5	GGAAGAUUUUGGAAGAAGGGGUCA	24	75.00	25.00
6	GCGGGAAAAUUGAGGAAAUACGUAC	25	72.00	28.00
7	GGGAAAGAAUCAGGCGGGAAUUAA	24	79.17	20.83
8	AUUAUACCUCCAGGGUUAUAGAAUU	25	48.00	52.00
9	ACGAAGGGGAGGACGAUGAA	20	85.00	15.00
10	CAAACAAGGGUGGGUAAACGGAGA	24	79.17	20.83

Supplementary Table S13: List of sequences (two sets of ten sequences) submitted to MEME for the mutated RRM of RS2Z33.

Native RRM+ZnKs of RS2Z32				
#	**Sequence**	**Length (bp)**	**Purine (%)**	**Pyrimidine (%)**
1	CAAAAAAUCACAGGAGGUGAAGGA	24	79.17	20.83
2	GGUAAGGGUGUGAAAUAAGUGCUC	24	66.67	33.33
3	GCGAUGAUGAUGUAUCGCCAAACU	24	54.17	45.83
4	UUAAAAGAGGUGGCAUGUUUAAGA	24	66.67	33.33
5	CUUACGAGAGUGGUGAACCUGGCU	24	54.17	45.83
6	GCAGUGGGCUAAGGGAG	17	76.47	23.53
7	AGGAGCCACAUCAGAAUAAGCG	22	68.18	31.82
8	UGUGGAGGAUGUGCAGUUAGCCCG	24	58.33	41.67
9	GGUGGGAUUGAGUCACGUUUAACA	24	58.33	41.67
10	GACUGAAUGUUGGCAUGUUAG	21	57.14	42.86
11	GCAUACACAAAGGUGAAGUGUAACUAGACG	30	66.67	33.33
12	GAAUUGAUAUCAGAGGGAGGCGAUGCGGGAAUUGAUAU	38	68.42	31.58
13	UGAGGAGGGUUUGACAGCAGUA	22	68.18	31.82
14	GUGGGACUAUGAAAGAUAUACGGC	24	66.67	33.33
15	UAGAAGUGAAGGUUGAUAAAGGU	23	73.91	26.09
16	CGGGAGGGAUACGGAGAUAUCUCG	24	66.67	33.33

17	GCCAGGGAGGAGUAGAUGUAGUGA	24	75.00	25.00
18	AAGAUAUGGGAGGGCAGGAUUAGA	24	79.17	20.83
19	GGGAGCAUGGGGAAAUGGGCGCU	23	73.91	26.09
20	CUCCCUAUAGUGAGUCGUAUUAAUUUCG	28	39.29	60.71
21	AAAACAAAGUGUGGAAUGAUGGCU	24	70.83	29.17
22	UUGUGGCAUGUCCUCCGGCUUUAA	24	37.50	62.50
23	AUCUGGGCGGGGUUAUGGGUAUAA	24	62.50	37.50
24	UAAAAUGGUGAGCCCGCAAUGCAU	24	58.33	41.67
25	GGGGGUUCGUAGCAGGGGGCUAAU	24	66.67	33.33
26	UGGGUAAGACGGAUACGGGAGGUU	24	70.83	29.17
27	AUAAUGUACGUUAGAAGUAAAGGU	24	66.67	33.33
28	CGGGAGGGUGAAAGGAUGGGGUAA	24	83.33	16.67
29	CCGAUGUAUGAACAGAUCCGAUUG	24	54.17	45.83
30	UGCCUACCCAUGGGAGGACUCG	22	50.00	50.00

Supplementary Table S14: List of sequences submitted to MEME for the native RRM+ZnKs of RS2Z32.

Native RRM+ZnKs of RS2Z33				
#	**Sequence**	**Length (bp)**	**Purine (%)**	**Pyrimidine (%)**
1	GACAUCAACAAAGGGUCAUAUUGA	24	62.50	37.50
2	GAUGCUCAUAACACAAGGGGGAAC	24	66.67	33.33
3	GAGGGAAUGUAGAUGAGUAGGGG	23	82.61	17.39
4	AAGGCGAGGAGAAGUAUGUGCAACU	25	72.00	28.00
5	GCGGGAGAGGGAGGACGAUG	20	85.00	15.00
6	GCGGGAGAUAUAAGAGCGUUUGG	23	69.57	30.43
7	UCAAGUAGCAUCACCAAUGAAAUU	24	54.17	45.83
8	GGUGUGGGAAGAAAUGAGAGG	21	85.71	14.29
9	AUGACACAAAAUAUAAUGUGAUUU	24	58.33	41.67
10	AGUUAACAUAACCAGGGAUGCAAU	24	62.50	37.50
11	AGAUUUAGAGAGGAUUACCCA	21	61.90	38.10
12	UAUUAAAGGCUGCCUCCACCACCA	24	41.67	58.33
13	GAAGAGGGAAGACAGGAGGGUAAG	24	91.67	8.33
14	AUCAUCAAUACGAUAAUAUUAUG	23	52.17	47.83
15	ACUACGGUUCCGCAGAGGGGAAU	23	60.87	39.13
16	GGGAAUCCGAACCUAAGAGGAGC	23	69.57	30.43

17	UUAGACGAGAGCGGGA	16	75.00	25.00
18	GCCAACGGGCCAAUUGAGAGACCG	24	62.50	37.50
19	GGAAAGAGCGAAACAGGGCGCUCA	24	75.00	25.00
20	UAACCGCUACAUGCACAGACGGGA	24	58.33	41.67
21	GAGGAACCUGACGCAGACAGAACG	24	70.83	29.17
22	ACGAAUGAAAGGGUCUAGAUUCAG	24	66.67	33.33
23	AUAGCGACUGGCGGAGGGUGUCAA	24	66.67	33.33
24	ACGGAGAAUGCUAACUUUACCCCG	24	50.00	50.00
25	CUUUAAUAGCAACCUCCGAUAGUA	24	45.83	54.17
26	AAAAGAGGGGUAACCAGUGAGGG	23	82.61	17.39
27	UUAGACGACGGGGA	14	71.43	28.57
28	ACAGACGACGAGCGGGACAGACG	23	73.91	26.09
29	GUGGCAUGACGUUAGGGGAGGAUU	24	66.67	33.33
30	CUAGGCAAGCCAUCAGUUAGAGCA	24	58.33	41.67

Supplementary Table S15: List of sequences submitted to MEME for the native RRM+ZnKs of RS2Z33.