Supporting Information

Sun et al. 10.1073/pnas.1220162110

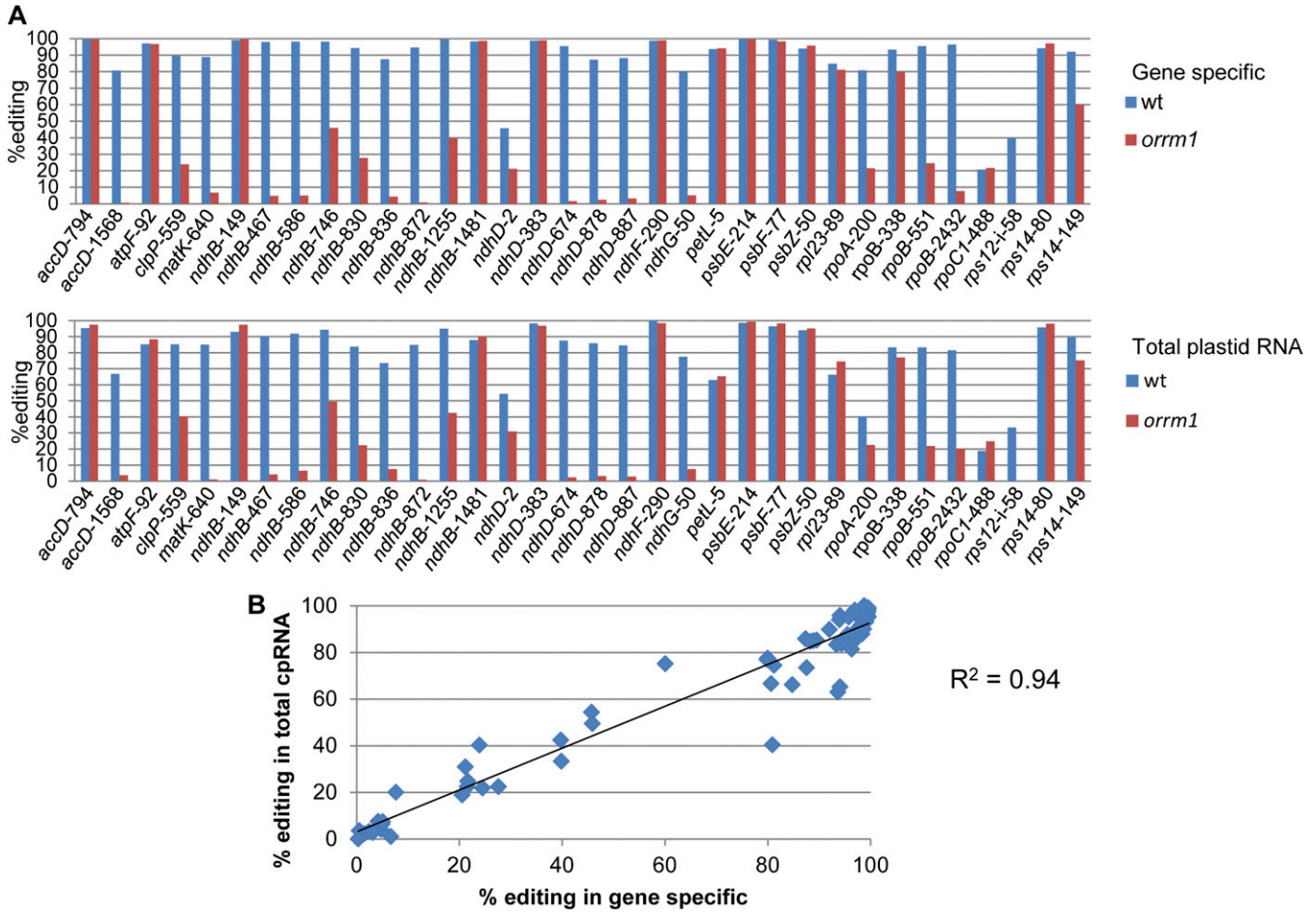

Fig. S1. The reduction of plastid editing extent in the At3g20930 T-DNA insertional mutant is consistently detected in different RNA-seq experiments. (*A*) Comparison of the editing extent of the 34 plastid sites in a wild-type (wt) and *orrm1* mutant plants. The values of editing extent were obtained from gene-specific (*Upper*) or total plastid RNA (*Lower*). (*B*) A correlation >0.9 exists between level of editing extent evaluated on cDNAs corresponding to gene transcripts (gene-specific) and cDNAs corresponding to the whole plastid transcriptome (total plastid RNA).

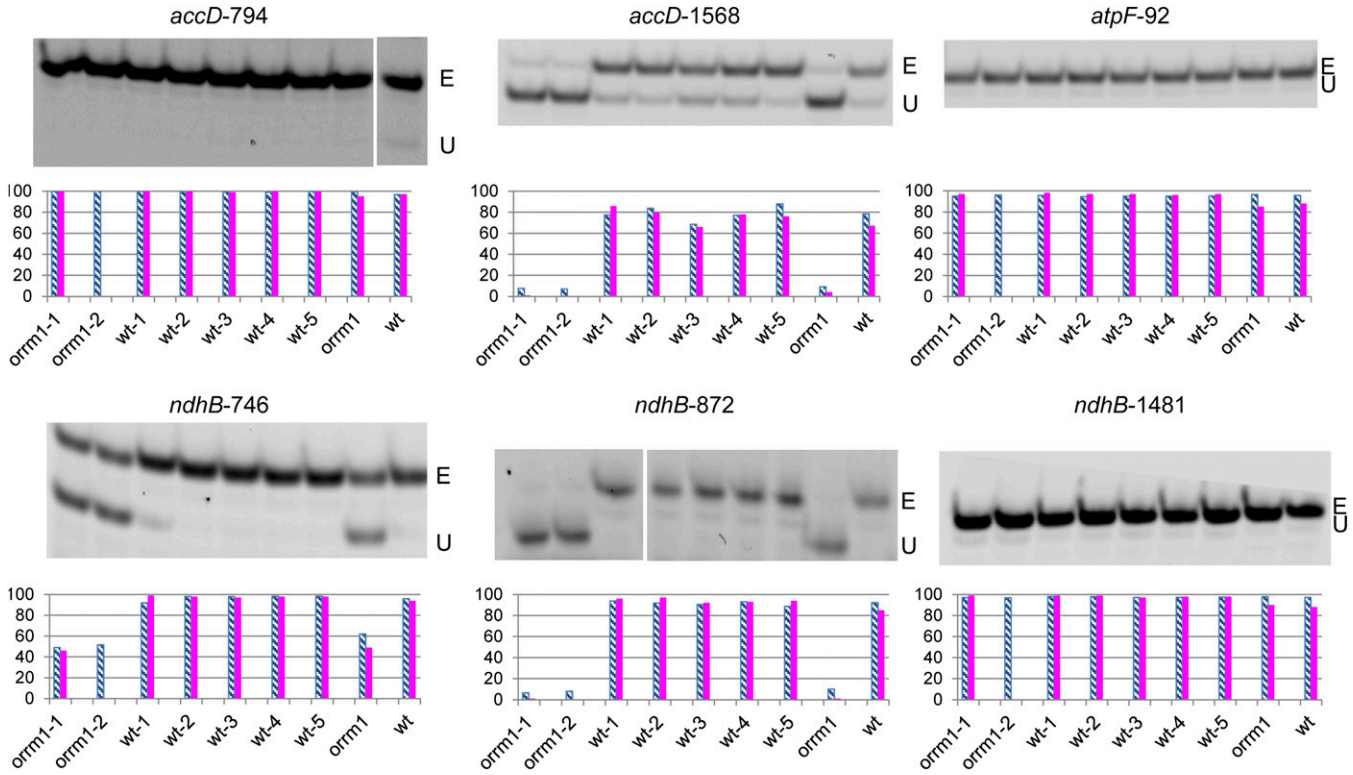

Fig. S2. The poisoned primer extension (PPE) assay confirms the plastid editing defects detected in the *orrm1* mutant by RNA-seq. Acrylamide gels separate the PPE products obtained from samples used in this study. E, edited; U, unedited. The name of the site assayed is given above each gel. The quantification of editing extent derived from the measure of the band's intensity is represented by a bar below each lane of the acrylamide gels (blue diagonal background). As a way of comparison, the editing extent derived from RNA-seq is represented by a magenta bar. RNA-seq was performed on gene specific cDNAs for *orrm1-1*, wt-1, wt-2, wt-3, wt-4, and wt-5, and on total plastid RNA for *orrm1* and wt. *orm1-1* and *orrm1-2* are two homozygous mutant plants.

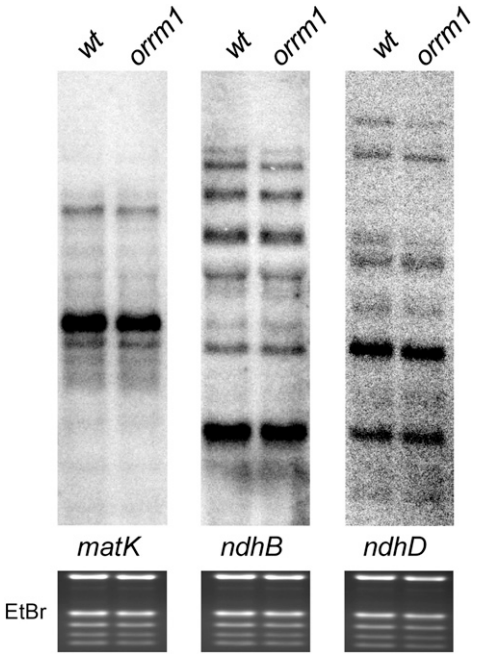

Fig. S3. RNA blots demonstrate the absence of change of transcript abundance in the *orrm1* mutant. Below each blot is given the name of the transcript corresponding to the probe used. Below each blot is shown the EtBr gel as a control for equal loading of the wild-type and *orrm1* RNAs.

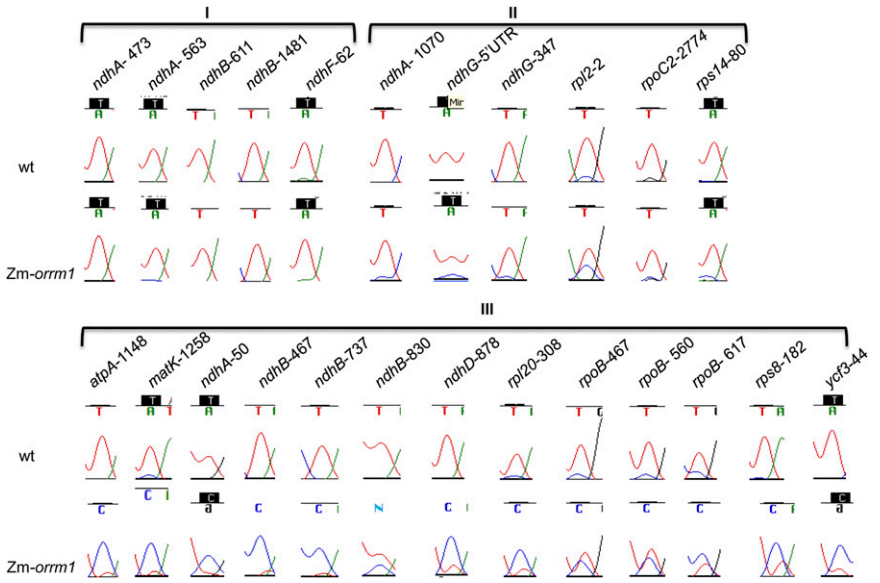

Fig. S4. Plastid editing sites in the Zm-*orrm1* mutant either do not show a reduction of editing extent (I), or show a slight (II), or pronounced (III) reduction of editing extent compared with the wild-type plant. Bulk-sequencing electrophoretograms of RT-PCR products obtained from wild-type (*Upper*), and Zm-*orrm1* (*Lower*) plants. Above each electrophoretogram is given the editing site. Notice the difference in the height of the T (edited, red) and C (unedited, blue) peaks between wild-type plant and the Zm-*orrm1* mutant particularly for editing sites belonging to the III category.

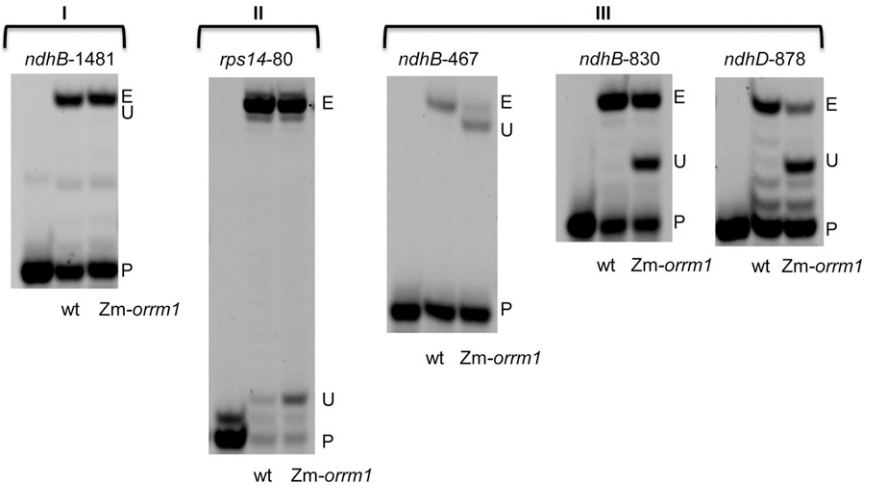

Fig. S5. PPE assay confirms the plastid editing sites in the Zm-*orrm1* mutant to either show an absence of reduction of editing extent (I), or to show a slight (II) or pronounced (III) reduction of editing extent compared with the wild-type plant. Acrylamide gels separate the PPE products obtained from the wild-type (wt) and the the Zm-*orrm1* mutant plant. E, edited; U, unedited; P, primer. The name of the site assayed is given above each gel.

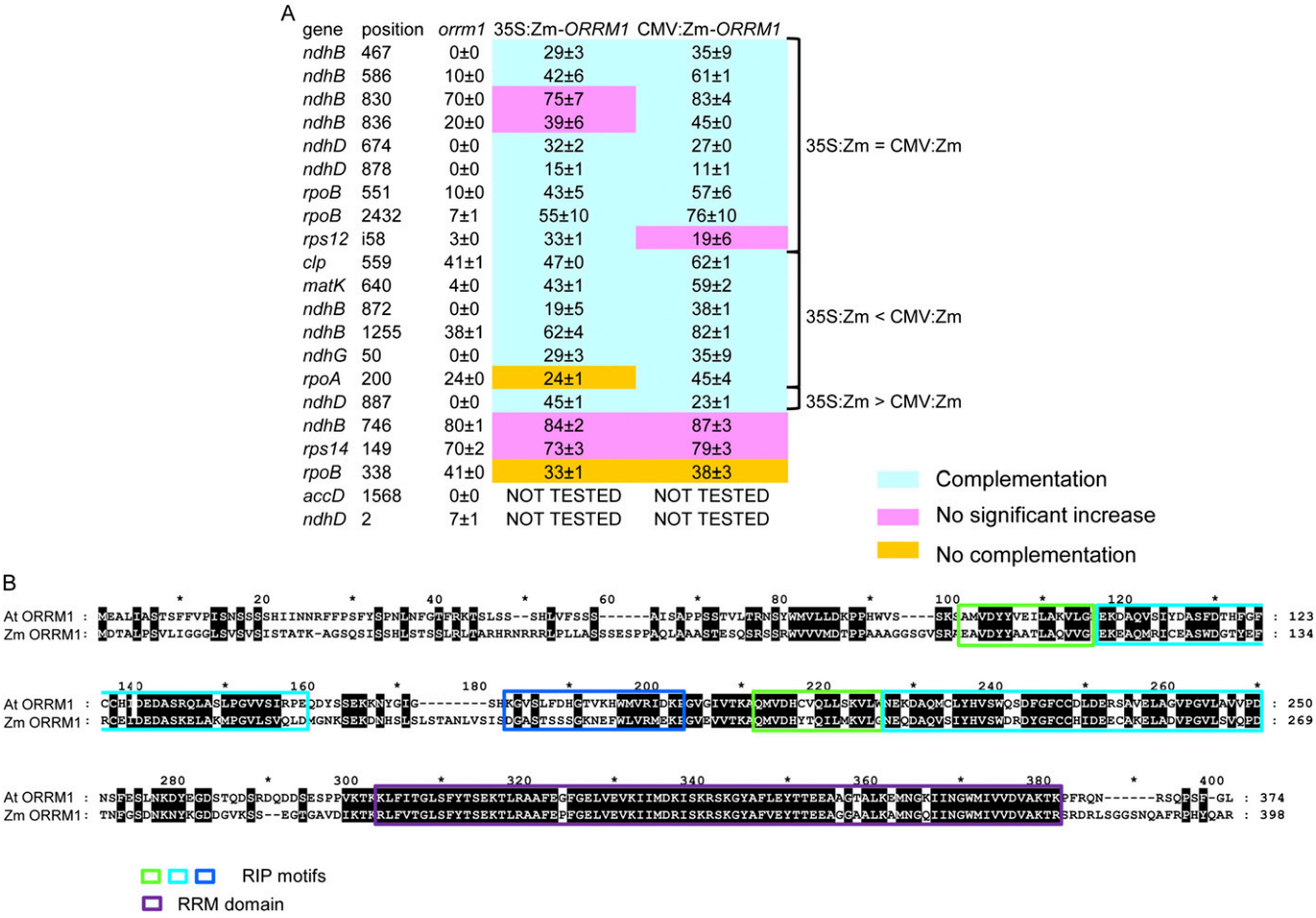

Fig. S6. Zm-ORRM1 is functionally and structurally similar to At-ORRM1. (*A*) Zm-ORRM1 is able to complement *Arabidopsis orrm1* mutant protoplasts when expressed either under a 35S or a CMV promoter. (*B*) Alignment of At-ORRM1 and Zm-ORRM1 shows highly conserved RNA recognition motif (RRM) domains between the two proteins. organellar RRM protein, ORRM.

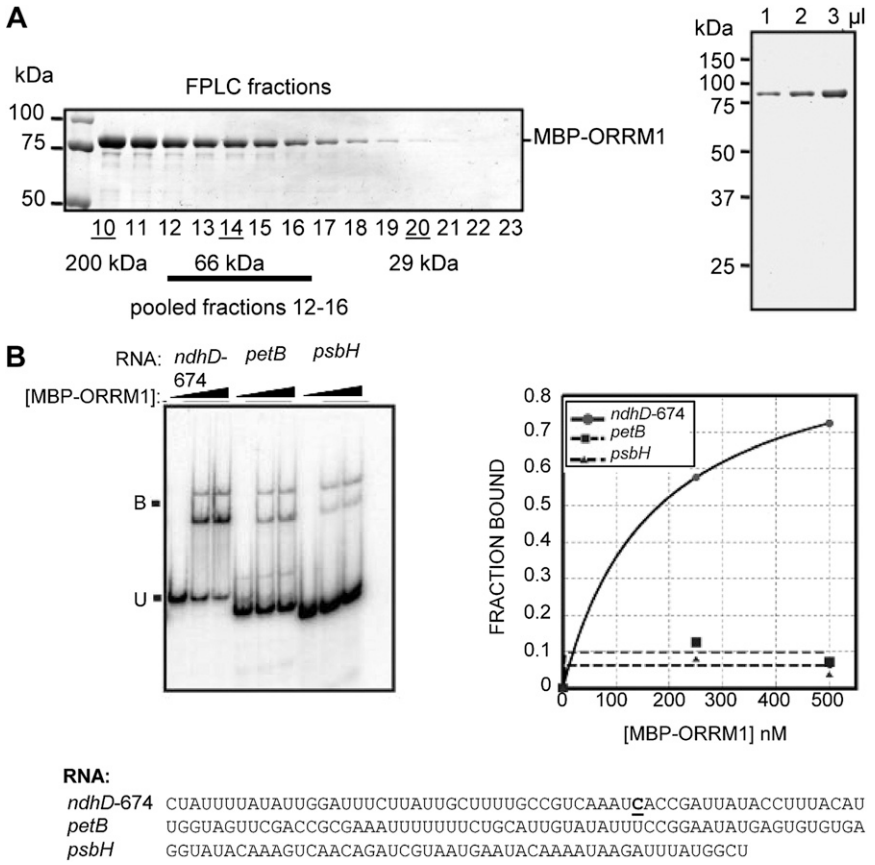

Fig. S7. Additional evidence for binding of ORRM1 to specific RNA substrates. (*A*) Purification of recombinant MBP-ORRM1. MBP-ORRM1 was purified by amylose affinity chromatography followed by size fractionation in a Superdex 200 gel-filtration column. Superdex 200 column fractions were analyzed by SDS/PAGE and staining with Coomassie blue (*Left*). Fractions 12–16 were pooled and dialyzed against storage buffer. The purity of the final preparation is shown in the gel to the right, which was also stained with Coomassie blue. (*B*) Additional gel mobility-shift assays with MBP-ORRM1. Assays were performed as in Fig. 8, except that NaCl was present at 200 mM.

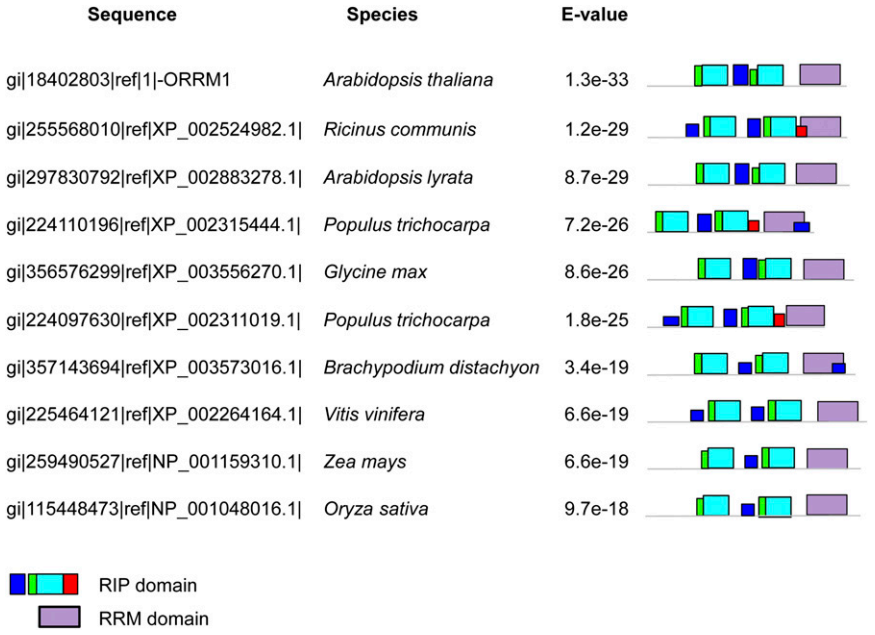

Fig. S8. Known proteins that contain a twin truncated RNA-editing factor interacting protein (RIP)-RIP always carry a RRM domain. The RIP domain defined by MEME was used to interrogate a nonredundant protein database with the MAST search program. All of the significant hits carrying a set of truncated RIP-RIP, like ORRM1, also contain a downstream RRM domain.

Table S1. Effect of the T-DNA insertional mutation in ORRM1 on the editing extent of plastid sites

		Gene specific							Total plastid RNA						
					Number of reads*							Number of reads			
		Editing extent			WT		*orrm1*		Editing extent			WT		*orrm1*	
Gene	Position	WT	*orrm1*	Δ*orrm1*	C	T	C	T	WT	*orrm1*	Δ*orrm1*	C	T	C	T
accD	794	1.00	1.00	0.00	70	17,812	24	5,148	0.95	0.97	−0.02	5	102	3	113
accD	1,568	0.81	0.01	0.99	2,937	12,252	20,671	117	0.67	0.04	0.95	19	38	54	2
atpF	92	0.97	0.97	0.00	1,920	62,144	1,825	54,857	0.85	0.88	−0.04	182	1,057	156	1,194
clpP	559	0.90	0.24	0.73	1,144	9,814	19,229	6,043	0.85	0.40	0.53	21	121	98	66
matK	640	0.89	0.07	0.92	2,633	21,095	573	41	0.85	0.01	0.99	18	102	93	1
ndhB	149	0.99	0.99	0.00	315	36,606	107	17,738	0.93	0.97	−0.05	32	429	24	908
ndhB	467	0.98	0.05	0.95	625	31,831	29,209	1,473	0.91	0.04	0.95	54	515	835	36
ndhB	586	0.98	0.05	0.95	612	33,777	27,689	1,460	0.92	0.06	0.93	40	447	583	40
ndhB	746	0.98	0.46	0.53	375	20,070	13,551	11,499	0.94	0.49	0.48	26	436	336	329
ndhB	830	0.94	0.28	0.71	461	7,731	19,234	7,351	0.84	0.22	0.73	31	159	371	107
ndhB	836	0.88	0.04	0.95	965	6,806	25,408	1,122	0.73	0.08	0.90	30	83	407	33
ndhB	872	0.95	0.01	0.99	685	11,974	27,525	191	0.85	0.01	0.99	23	129	360	3
ndhB	1,255	1.00	0.40	0.60	204	40,709	12,844	8,474	0.95	0.42	0.55	20	376	233	172
ndhB	1,481	0.98	0.99	0.00	268	15,846	320	23,271	0.88	0.90	−0.03	43	310	53	478
ndhD	2	0.46	0.21	0.54	71,538	60,341	11,478	3,086	0.54	0.31	0.43	99	118	230	103
ndhD	383	0.99	0.99	0.00	392	35,163	124	11,839	0.98	0.97	0.01	12	674	21	640
ndhD	674	0.96	0.02	0.98	1,346	28,753	12,917	199	0.88	0.02	0.97	63	446	721	17
ndhD	878	0.87	0.02	0.97	6,529	45,066	15,494	388	0.86	0.03	0.96	112	679	968	32
ndhD	887	0.88	0.03	0.96	6,077	45,838	15,359	504	0.85	0.03	0.97	104	573	723	21
ndhF	290	0.99	0.99	0.00	844	66,974	204	20,210	1.00	0.99	0.01	0	55	1	67
ndhG	50	0.80	0.05	0.94	5,111	20,447	10,297	553	0.77	0.07	0.90	41	141	248	20
petL	5	0.94	0.94	0.00	296	4,371	480	7,642	0.63	0.65	−0.04	10	17	8	15
psbE	214	1.00	1.00	0.00	112	28,930	382	87,546	0.99	0.99	−0.01	33	2,232	26	3,253
psbF	77	0.99	0.98	0.01	12	1,764	213	12,773	0.96	0.98	−0.02	73	1,957	54	3,026
psbZ	50	0.94	0.96	−0.02	5,129	79,997	2,383	53,887	0.94	0.95	−0.01	121	1,906	76	1,479
rpl23	89	0.85	0.81	0.04	1,010	5,632	1,746	7,562	0.66	0.75	−0.13	25	49	26	76
rpoA	200	0.81	0.22	0.73	10,415	44,159	7,017	1,927	0.40	0.22	0.44	111	75	107	31
rpoB	338	0.93	0.80	0.14	1,141	16,137	2,668	10,601	0.83	0.77	0.08	5	25	6	20
rpoB	551	0.96	0.25	0.74	1,086	23,061	6,419	2,086	0.83	0.22	0.74	4	20	25	7
rpoB	2,432	0.96	0.08	0.92	2,608	68,572	4,126	343	0.81	0.20	0.75	8	35	24	6
rpoC1	488	0.21	0.22	−0.05	36,084	9,359	71,377	19,698	0.19	0.25	−0.32	198	46	133	44
rps12	i-58	0.40	0.00	0.99	17,174	11,391	5,454	18	0.33	0.00	1.00	14	7	25	0
rps14	80	0.94	0.97	−0.03	1662	26,479	105	3,384	0.96	0.98	−0.02	75	1,702	99	5,057
rps14	149	0.92	0.60	0.35	1645	18,939	992	1,494	0.90	0.75	0.16	200	1,757	1,081	3,264

Editing extent: EE = T/(C + T). Δ*orrm1*: variation of editing extent in *orrm1* mutant = [EE(wt) − EE (*orrm1*)]/EE(wt). *orrm1* was sequenced twice, the number of reads are the sums of the two sequencing experiments

*C(WT) = C (wt-1) + C (wt-2) + C (wt-3) + C (wt-4) + C (wt-5); T (WT) = T (wt-1) + T (wt-2) + T (wt-3) + T (wt-4) + T (wt-5).

Table S2. Editing extent of the plastid sites in the maize Zm-*orrm1* mutant

Gene	Position in maize cds	Position in *Arabidopsis* cds	Editing extent								
			Bulk			PPE			*Arabidopsis**		
			Zm-*orrm1*	WT	ΔZm-*orrm1*	Zm-*orrm1*	WT	ΔZm-*orrm1*	At-*orrm1*	Col	ΔAt-*orrm1*
atpA	1,148	T in *Arabidopsis*	0.1	1	0.90						
matk	1,258	T in *Arabidopsis*	0.1	0.9	0.89						
ndhA	50	T in *Arabidopsis*	0.1	1	0.90						
ndhA	473	T in *Arabidopsis*	1	1	0.00						
ndhA	563	T in *Arabidopsis*	1	1	0.00						
ndhA	1,070	T in *Arabidopsis*	0.9	1	0.10						
ndhB	467	467	0.1	1	0.90	0.31	0.97	0.68	0.05	0.98	0.95
ndhB	586	586	0	1	1.00	0.07	0.98	0.93	0.05	0.98	0.95
ndhB	611	no editing in *Arabidopsis*	1	1	0.00						
ndhB	737	T in *Arabidopsis*	0.1	1	0.90						
ndhB	830	830	0.6	1	0.40	0.65	0.99	0.34	0.28	0.94	0.71
ndhB	1,481	1481	1	1	0.00	0.99	0.99	0.00	0.99	0.98	0
ndhD	878	878	0.2	1	0.80	0.29	0.96	0.70	0.02	0.87	0.97
ndhF	62	T in *Arabidopsis*	1	1	0.00						
ndhG	347	T in *Arabidopsis*	0.9	1	0.10						
ndhG 5′UTR			0.9	1	0.10						
petB	668	T in *Arabidopsis*	0	1	1.00						
rpl2	2	T in *Arabidopsis*	0.7	0.9	0.22						
rpl20	308	T in *Arabidopsis*	0.2	0.9	0.78						
rpoB	467	T in *Arabidopsis*	0.6	0.9	0.33						
rpoB	545	551	0	0.9	1.00	0.14	0.89	0.84	0.25	0.96	0.74
rpoB	560	T in *Arabidopsis*	0.6	0.9	0.33						
rpoB	617	T in *Arabidopsis*	0.4	0.8	0.50						
rpoC2	2,774	T in *Arabidopsis*	0.9	1	0.10						
rps14	80	80	0.9	1	0.10	0.9	0.97	0.07	0.97	0.94	−0.03
rps8	182	T in *Arabidopsis*	0.3	1	0.70						
ycf3	44	T in *Arabidopsis*	0.2	1	0.80						
ycf3	185	T in *Arabidopsis*	0	1	1.00						

The editing extent values in bulk sequencing are approximate measurements based on the surface of the peaks of unedited (C peak) and unedited. (T peak), editing extent: EE= T/(C + T). Editing extent measurements by PPE come from the measure of the intensity of the extension products bands. Δ*orrm1*: variation of editing extent in orrm1 mutant = [EE (wt) − EE (orrm1)]/(EE(wt).

*Editing extent in *Arabidopsis* corresponds to the gene-specific RNA library.

Table S3. Annotation and subcellular localization of the RRM-containing proteins related to ORRM1

Gene		Protein annotation*	E-value†	Location‡			
				GFP	MS/MS	Predotar	TargetP
ORRM2	At1g73530	RRM similar to S-RBP	5.00E-23		P	P	P
ORRM3	At5g06210	mRBP, S-RBP11	2.00E-20			M	M
ORRM4	At4g20030		4.00E-19		N	P	P
ORRM5	At2g37510	mRBP, S-RBP9	5.00E-19			M	M
ORRM6	At3g46020	S-RBP4	5.00E-19			M	M
ORRM7	At3g23830	mRBP1b, GR-RBP4	8.00E-19	M		M	M
ORRM8	At2g27330		2.00E-18			ER	M
ORRM9	At4g13850	mRBP1a, GR-RBP2	2.00E-18		M	M	M
ORRM10	At3g26420	AtRZ-1A (GR-RBP)	5.00E-17	N	C, Px, Pl M		
	At5g47320	mRBP, RPS19	6.00E-17		M	M	M
ORRM11	At5g54580	mRBP, S-RBP12	1.00E-16	M		M	P
ORRM12	At4g39260	GR-RBP8	3.00E-15	N	N, Px, Pl M, P		
ORRM13	At5g61030	mRBP2b, GR-RBP3	2.00E-14		M	M	M
ORRM14	At5g59860	S-RBP13	3.00E-14				
ORRM15	At1g74230	mRBP2a, GR-RBP5	2.00E-13		M	M	M

*Annotation from Lorkovic and Barta (1) and Vermel et al. (2).
†E-value obtained when the RRM motif from *ORRM1*is used as a query
‡Subcellular location from the SUBA database (3). C, cytosol; ER, endoplasmic reticulum; M, mitochondrion; N, nucleus; P, plastid; Pl M, plasma membrane; Px, peroxisome.

1. Lorković ZJ, Barta A (2002) Genome analysis: RNA recognition motif (RRM) and K homology (KH) domain RNA-binding proteins from the flowering plant *Arabidopsis thaliana*. *Nucleic Acids Res* 30(3):623–635.
2. Vermel M, et al. (2002) A family of RRM-type RNA-binding proteins specific to plant mitochondria. *Proc Natl Acad Sci USA* 99(9):5866–5871.
3. Heazlewood JL, Verboom RE, Tonti-Filippini J, Small I, Millar AH (2007) SUBA: The *Arabidopsis* subcellular database. *Nucleic Acids Res* 35(Database issue):D213–D218.

Table S4. Primers used in this study

Primers	Sequences	Purpose
SALK_072648-LP	TGAACGATTTTATGATTGACGG	Genotyping
SALK_072648-RP	AACCCGAAATGGGTATCAAAG	Genotyping
ORRM1_1F	ATG GAA GCT CTT ATT GCT TCC ACT TC	Complementation
ORRM1_1F_CACC	caccATG GAA GCT CTT ATT GCT TCC ACT TCC	Complementation
ORRM1_822R	cta TGA ATC ATC TTG ATC TCT TGA ATC TTG CGT	Truncation/Y2H/complementation
RecA_1F_CACC	caccATGGATTCACAGCTAGTCTTGTCTCTG	Complementation
RecA_ORRM1-C	CTT TGT CTT TAC GGG AGG AGA CTC GTC GCG ATC GAA TTC AGA ACT GAT TTT GTG GGA G	Complementation
ORRM1_823F	GAGTCTCCTCCCGTAAAGACAAAG	Truncation/Y2H/complementation
ORRM1_R	CTA GAG CCC GAA ACT TGG TTG	Y2H/complementation
OTP84_133F	GCCTCCGCCGTTTCTGGCGCA	Y2H
OTP84_R	TCA CCA ATA GTC TCC ACA GGA GCA	Y2H
CRR28_121F	GCCTCCACCGCCGGTAACCAT	Y2H
CRR28_R	CTA CCA GTA GTC TAA ACA AGA GCA GGA	Y2H
ORRM1_151F	CTCGTCTTCTCATCTTCTGCAATT	Y2H
OTP81_127F	CTCCGACAACTAAAGCAAACCCAT	Y2H
OTP81_R	TCA CCA GAA ATC GTT ACA GGA ACA	Y2H
OTP82-292F	AACCTGTTGATTTGGAACACGATGTTT	Y2H
OTP82-R	CTACCAGTAGTCATTGCAGGAACAAACA	Y2H
RARE1_100F	GGATCCATGTCGAGCACTTCTTCTCCGTCT	Y2H
RARE1_R	TCACCAAGTAATCGTTGCAAGAACA	Y2H
ORRM1_163F_BamHI	tatataggatccTCTTCTGCAATTTCCGCACCGCCT	Protein expression
ORRM1_R_SalI	tatatagtcgacCTA GAG CCC GAA ACT TGG TTG ACT	Protein expression
ndhB-F	TTTTATGTGGTGCTAACGATTTAA	Probe for RNA blot
ndhB-R	AATCGCAATAATCGGGTTCATT	Probe for RNA blot
ndhD-F	CATGTGGGGTGGAAAGAAAC	Probe for RNA blot
ndhD-R	AGCGCCAATAAATCCATGAG	Probe for RNA blot
atpA-maize-F1	GAGCCGCTAAATTAAATTCTCTTT	RT-PCR bulk sequencing
atpA-maize-R1	ATCCTCTCGTTCCGGTATAAATAG	RT-PCR bulk sequencing
ndhA-maize-F1	TAGGGTAGAGGTAGAAACTATCAA	RT-PCR bulk sequencing
ndhA-maize-R1	ATTCTGCCAAAGAAGAAATTAGAA	RT-PCR bulk sequencing
ndhA-maize-F2	TTTGGCAGAATGTGAAAGATTACC	RT-PCR bulk sequencing
ndhA-maize-R2	GAACCCAGTTAGCATAGGGAACAT	RT-PCR bulk sequencing
ndhB-maize-F1	CTATCCGTAGAGTACATTGAATGT	RT-PCR bulk sequencing
ndhB-maize-R1	CTAAAAGAGGGTATCCTGAGC	RT-PCR bulk sequencing
ndhD-maize-F1	TGAACCCGGATTAGATTTAGAAAG	RT-PCR bulk sequencing
ndhD-maize-R1	CCGTTCCCGCCAAGAAA	RT-PCR bulk sequencing
ndhF-maize-F1	ATCAATATGCCTGGGTAATTCCTC	RT-PCR bulk sequencing
ndhF-maize-R1	ATAGGAACACATTCCCACAAGTTC	RT-PCR bulk sequencing
ndhG-maize-F1	GTCAGTTCATGAAAAATTTTATAC	RT-PCR bulk sequencing
ndhG-maize-R1	GAGCCATAGTAATTGCACCTATTA	RT-PCR bulk sequencing
petB-maize-F1	GAGGCCAACTTTGGTTGGTTAATC	RT-PCR bulk sequencing
petB-maize-R1	GGACCCGAAATACCTTGCTTACG	RT-PCR bulk sequencing
rpl2-maize-F1	CAACCGGGTTATTCTATTCCACTT	RT-PCR bulk sequencing
rpl2-maize-R1	TAGCCCCTCTGGGATGTAAAATAT	RT-PCR bulk sequencing
rpl20-maize-F1	GGGGGCTCATTTAAGACTTAA	RT-PCR bulk sequencing
rpl20-maize-R1	TTGGAAATCGTGTAAAGATTATTT	RT-PCR bulk sequencing
rpoB-maize-F1	TATCCGCGAGATTAATTTTTGGTT	RT-PCR bulk sequencing
rpoB-maize-R1	TGTAATTCCTCGCATAAGGACTCC	RT-PCR bulk sequencing
rpoC2-maize-F1	CATATCTTGCCGAGATCCTCATCC	RT-PCR bulk sequencing
rpoC2-maize-R1	CGCGAATTAGATCATTTGTTTTTA	RT-PCR bulk sequencing
rps8-maize-F1	GGCAAGGACACTATTGCTGATTTA	RT-PCR bulk sequencing
rps8-maize-R1	CTCCCCCAATTCTGTTTAGTCG	RT-PCR bulk sequencing
rps14-maize-F1	GGAGAAGAAGCGGCAGAAATT	RT-PCR bulk sequencing
rps14-maize-R1	GTCCGGATAGCCCAAAGTCTC	RT-PCR bulk sequencing
ycf3-maize-F1	ATGCCTAGATCCCGTATAAATGG	RT-PCR bulk sequencing
ycf3-maize-R1	TTCCGAATCGCCCTGTAGAA	RT-PCR bulk sequencing

Y2H, yeast two-hybrid.