

1 **A small PPR protein interacts with PPR-SMR1 to facilitate the splicing of introns in**
2 **maize mitochondria**

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16 **Supplemental data**

17 **Supplemental Figure S1.** Phenotypes of *spr2-1/+* ear, overexpressed ear *Spr2-OE1*, and
18 complemented ear *Spr2-Com1*.

19 **Supplemental Figure S2.** Linkage analysis of *spr2-1* mutant.

20 **Supplemental Figure S3.** Expression analysis of *Spr2*.

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22 **Supplemental Figure S5.** Expression of *AOX* genes in the *spr2* mutants.

23 **Supplemental Figure S6.** The *spr2* mutant is deficient for mitochondrial *nad1*, *nad2*,
24 *nad4*, *nad5*, and *nad7* mature transcripts.

25 **Supplemental Figure S7.** RT-PCR analysis of the splicing of the *nad1*, *nad2*, *nad4*,
26 *nad5*, and *nad7* introns in the *spr2* mutants.

27 **Supplemental Figure S8.** Splicing efficiency analysis of all 22 mitochondrial introns in
28 overexpression lines, complemented lines, and WT.

29 **Supplemental Figure S9.** Negative controls in BiFC analysis.

30 **Supplemental Figure S10.** PPR-SMR1 has no directly physical interaction with EMP16
31 in Y2H and LCI assays.

32 **Supplemental Figure S11.** SPR2 can form multimer.

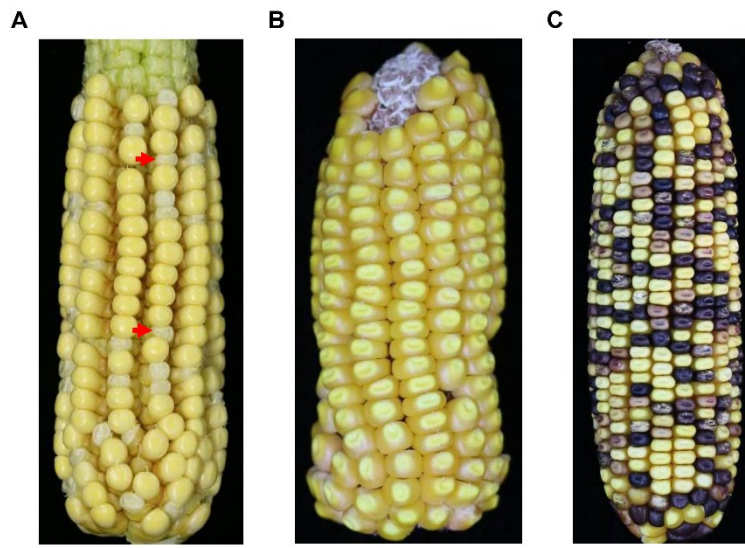
33 **Supplemental Table S1.** Alteration of the respiration rate of WT and *spr2-1*.

34 **Supplemental Table S2.** List of some splicing factors which shared target intron(s) with
35 SPR2.

36 **Supplemental Table S3.** List of the primers used in this study.

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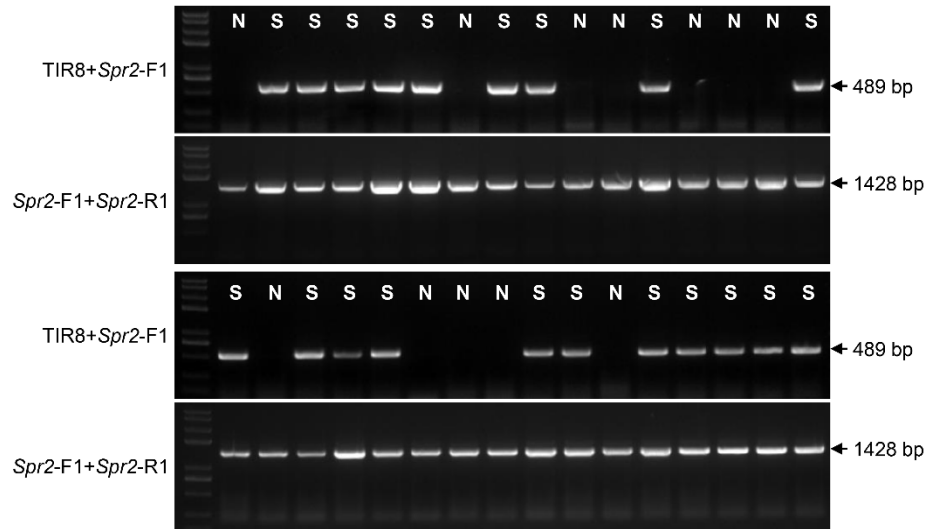
40 **Supplemental Figure S1.** Phenotypes of *spr2-1/+* ear, overexpressed ear *Spr2-OE1*, and
41 complemented ear *Spr2-Com1*.

42 (A) A self-pollinated *spr2-1/+* ear segregating *spr2* and wild-type (WT) kernels at 17
43 days after pollination (DAP). Arrows point to *spr2-1* mutant kernels.

44 (B) A self-pollinated overexpressed ear *Spr2-OE1* (*Ubi::Spr2-HA*).

45 (C) A self-pollinated complemented ear *Spr2-Com1* (*spr2+Ubi::Spr2-HA*).

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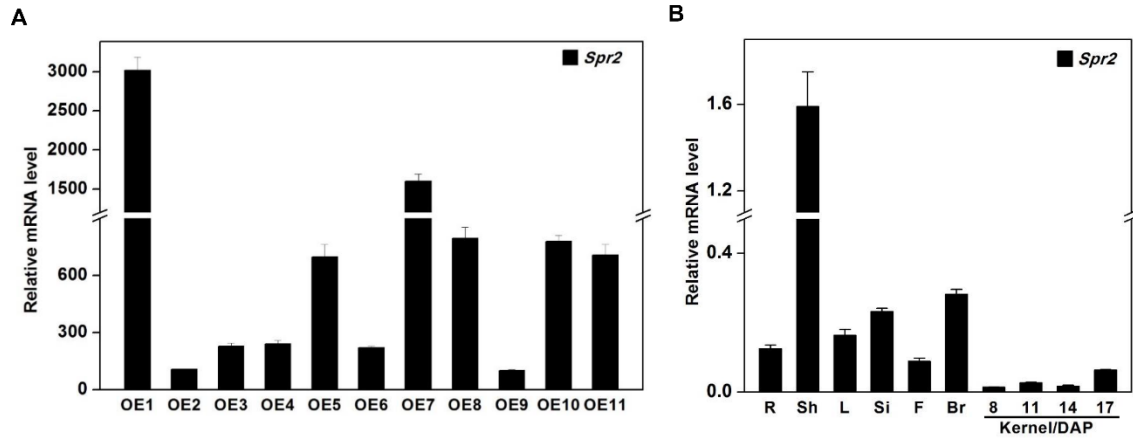
48 **Supplemental Figure S2.** Linkage analysis of *spr2-1* mutant.

49 TIR8 and *Spr2*-F1 are primers used to detect the *Mu* insertion of *spr2-1* mutants, *Spr2*-F1

50 and *Spr2*-R1 are the primers used to detect the *Spr2* gene. N: Non-segregating. S:

51 Segregating.

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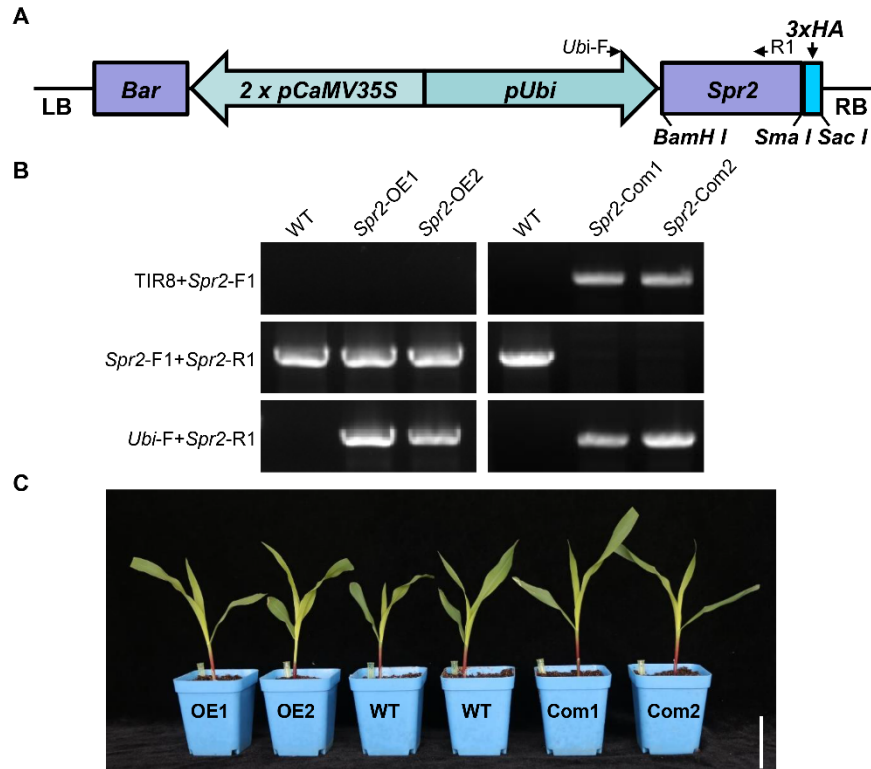
54 **Supplemental Figure S3.** Expression analysis of *Spr2*.

55 (A) Relative expression of *Spr2* in eleven independent *Spr2* overexpression (OE)
56 transgenic lines compared with WT.

57 (B) RT-qPCR analysis on the expression of *Spr2* in major tissues and developing seeds in
58 maize. R: Root; Sh: Shoot; L: Leaf; Si: Silk; F: Flower; Br: Bract. DAP: days after
59 pollination. RNAs were normalized against maize *Actin* gene (*GRMZM2G126010*).

60 Values represent the mean and standard deviation of three biological replicates, \pm SD.

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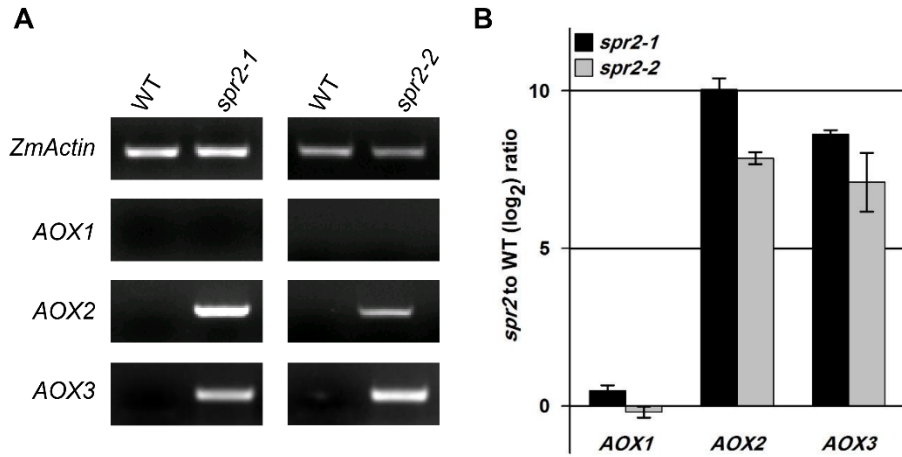
63 **Supplemental Figure S4.** Genetic complementation of maize *spr2-2* with *Spr2*.

64 (A) Transgenic construct for overexpressing *Spr2*. *Ubi-F* and *Spr2-R1* primers were used
 65 in genotyping. LB: left border; RB: right border; 3xHA: triple HA tag.

66 (B) The genotype of *Spr2* in overexpressed lines *Spr2-OE*, complemented lines *Spr2-*
 67 *Com*, and wild type.

68 (C) Seedling phenotype comparison among overexpressed lines *Spr2-OE*, complemented
 69 lines *Spr2-Com*, and wild type. Scale bar, 5 cm.

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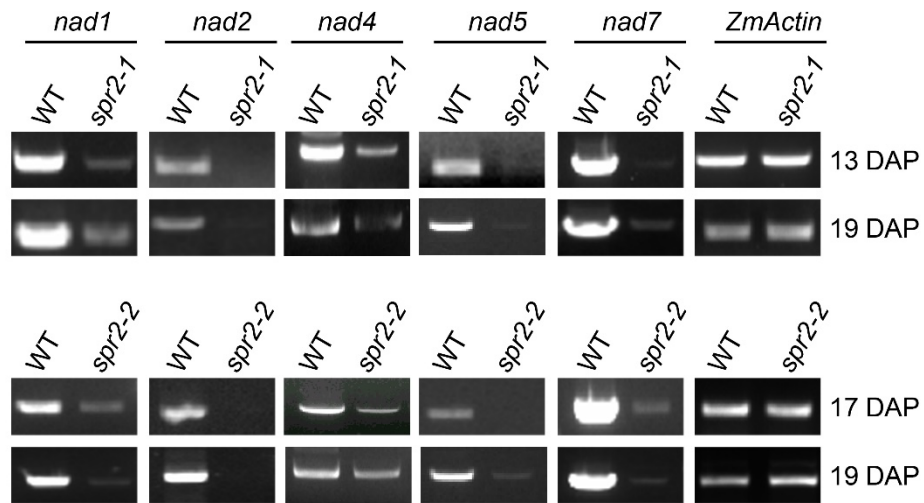
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72 **Supplemental Figure S5.** Expression of *AOX* genes in the *spr2* mutants.

73 (A) RT-PCR analyses of *AOX* genes in WT and *spr2*. RNAs were normalized against
74 *ZmActin* gene (*GRMZM2G126010*).

75 (B) RT-qPCR analyses of *AOX* genes in WT and *spr2*. RNAs were normalized against
76 *ZmActin* gene (*GRMZM2G126010*). Values represent the mean and standard deviation of
77 three biological replicates, \pm SD.

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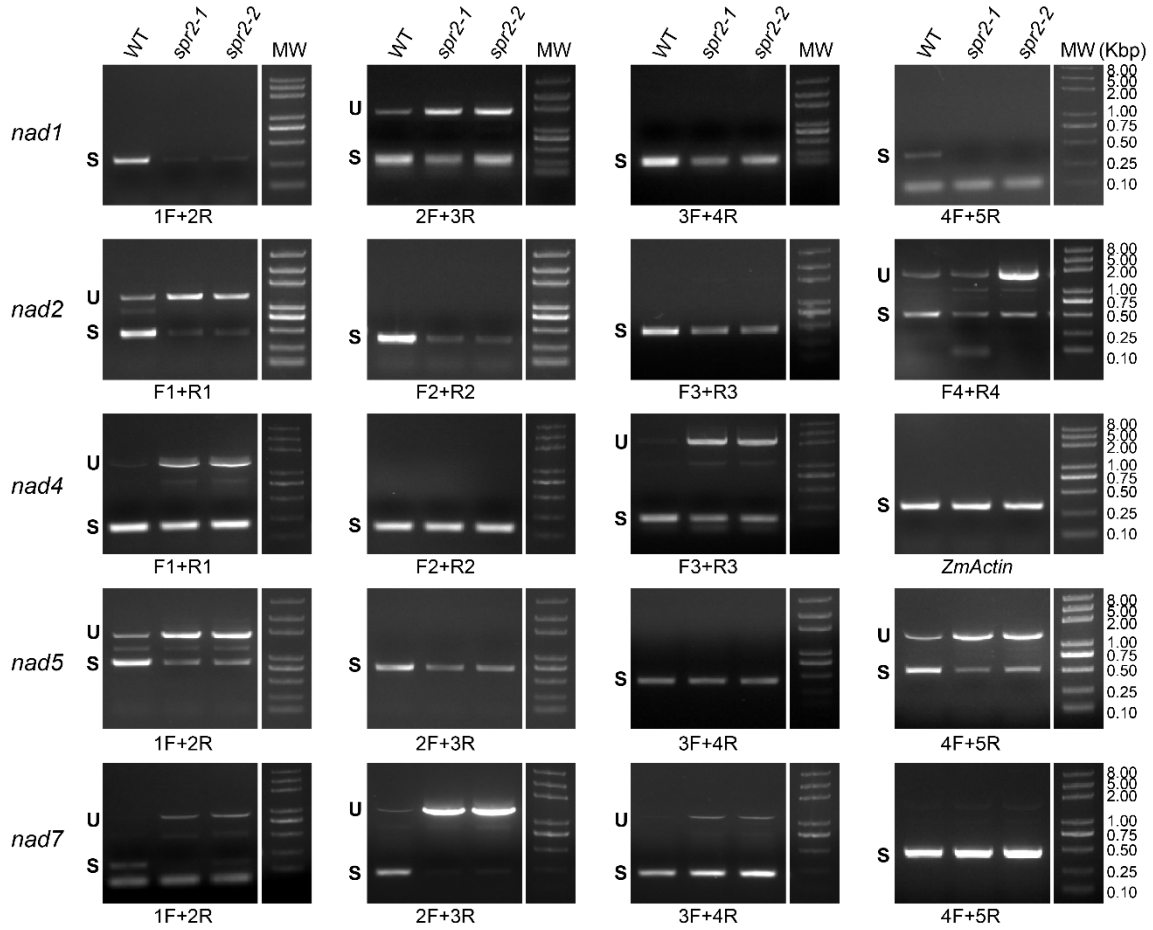


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80 **Supplemental Figure S6.** The *spr2* mutant is deficient for mitochondrial *nad1*, *nad2*,
 81 *nad4*, *nad5*, and *nad7* mature transcripts.

82 The substantial decrease of *nad1*, *nad2*, *nad4*, *nad5* and *nad7* transcripts occur in *spr2*
 83 alleles. Normalization was performed against *ZmActin* gene (*GRMZM2G126010*). The
 84 RNA was isolated from the same ear segregating for WT and *spr2* mutants. DAP: days
 85 after pollination.

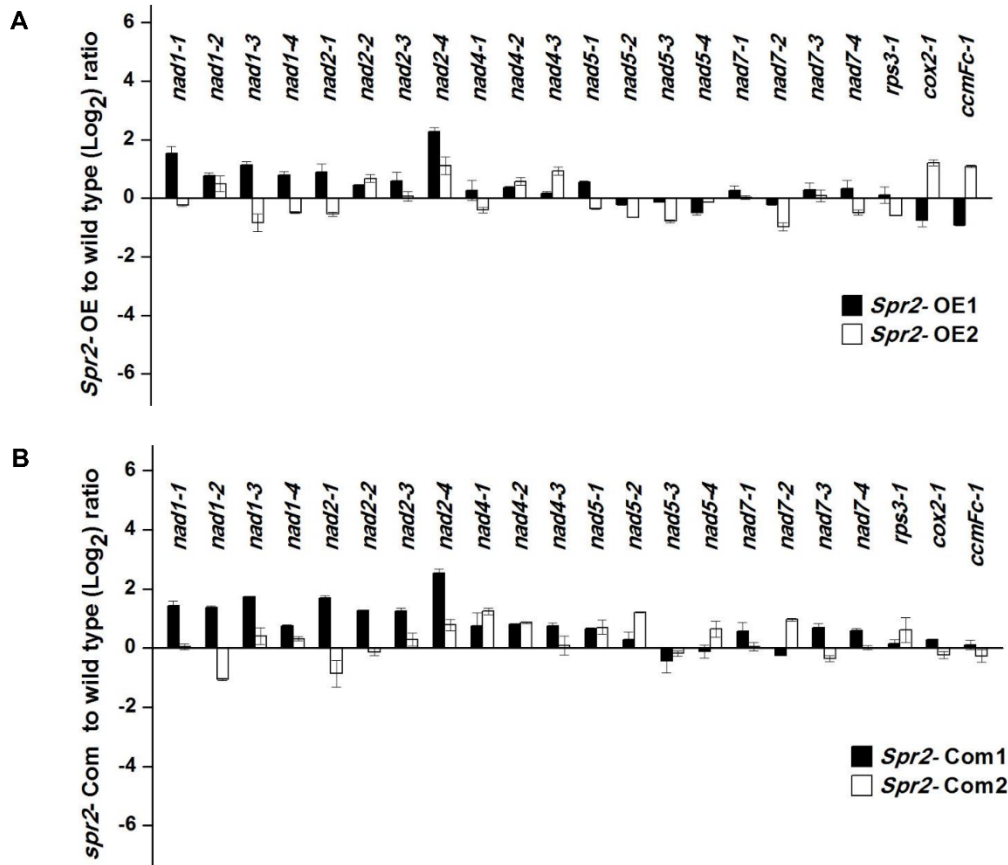
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88 **Supplemental Figure S7.** RT-PCR analysis of the splicing of the *nad1*, *nad2*, *nad4*,
 89 *nad5*, and *nad7* introns in the *spr2* mutants.

90 Normalization was performed against *ZmActin* gene (*GRMZM2G126010*). S, intron
 91 spliced; U, intron unspliced; MW, molecular markers; Kbp, thousand base pair. These
 92 gel images are the same as that in Figure 5B.



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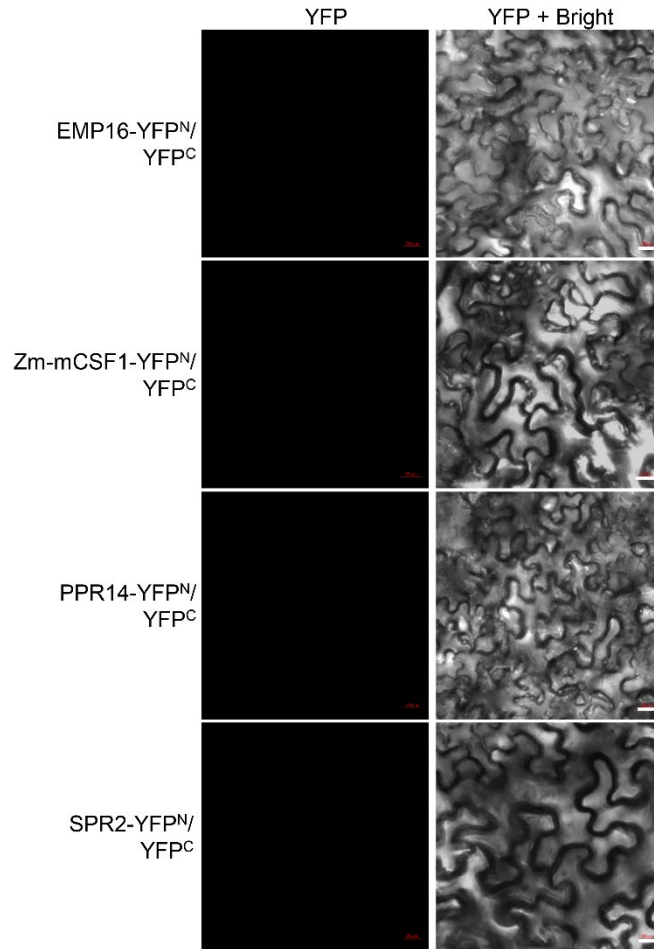
94 **Supplemental Figure S8.** Splicing efficiency analysis of all 22 mitochondrial introns in
 95 overexpression lines, complemented lines, and WT.

96 (A) RT-qPCR analysis of splicing efficiency of all 22 mitochondrial introns in
 97 overexpression lines *Spr2*-OE and WT.

98 (B) RT-qPCR analysis of splicing efficiency of all 22 mitochondrial introns in
 99 complemented *Spr2*-Com and WT.

100 The RNA was isolated from the leaves of *Spr2*-OE, *Spr2*-Com, and WT. Data are means
 101 (\pm SE) of three biological replicates. Normalization was performed against *ZmActin* gene
 102 (*GRMZM2G126010*).

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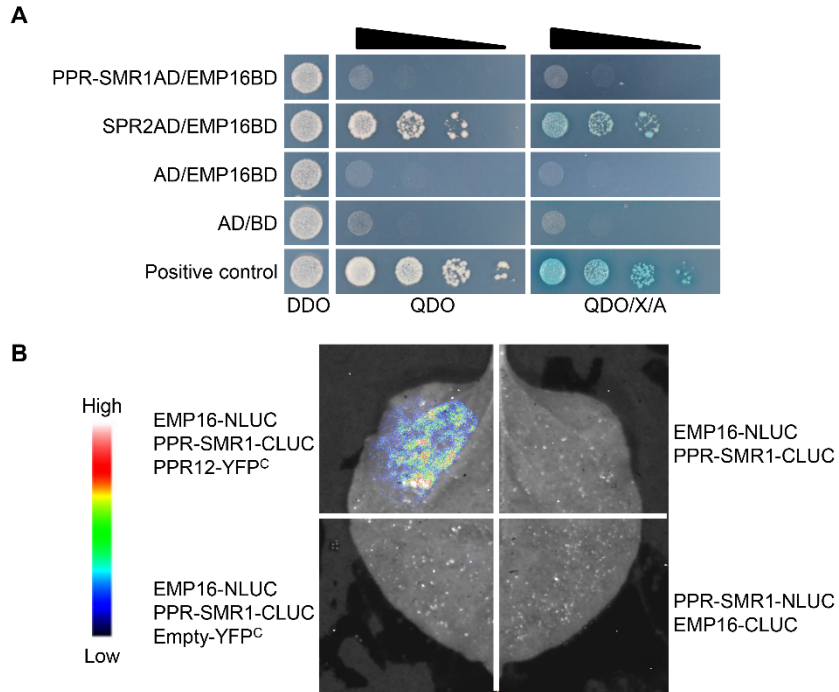


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105 **Supplemental Figure S9.** Negative controls in BiFC analysis.

106 Different combinations of the proteins were tested in *Nicotiana benthamiana* leaves and
 107 detected under a laser confocal microscope. Scale bars, 20 μ m.

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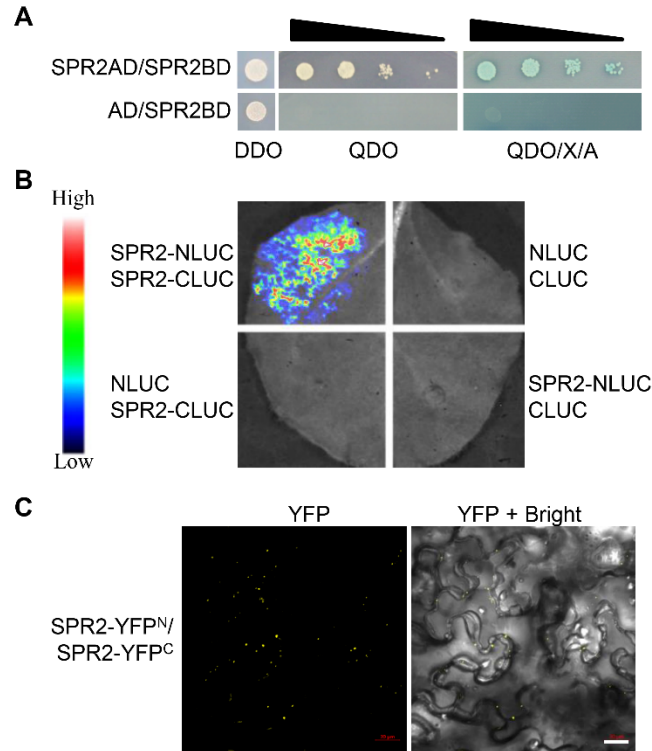
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110 **Supplemental Figure S10.** PPR-SMR1 has no directly physical interaction with EMP16
 111 in Y2H and LCI assays.

112 (A) Y2H assay to determine the interactions between PPR-SMR1 and EMP16. DDO
 113 medium: SD/-Leu/-Trp medium; QDO medium: SD/-Ade/-His/-Leu/-Trp medium;
 114 QDO/X/A: QDO medium with added the X- α -gal and AbA.

115 (B) Determine the interactions between PPR-SMR1 and EMP16 by LCI analysis. An
 116 equal amount of *Agrobacteria* was infiltrated in each test.

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119 **Supplemental Figure S11. SPR2 can form multimer.**

120 (A) Y2H assay to determine SPR2 multimer. DDO medium: SD/-Leu/-Trp medium;

121 QDO medium: SD/-Ade/-His/-Leu/-Trp medium; QDO/X/A: QDO medium with added
122 the X- α -gal and AbA.

123 (B) Determine SPR2 multimer by LCI analysis.

124 (B) Determine SPR2 multimer by BiFC analysis. Scale bars, 20 μ m.

125

126 **Supplemental Table S1.** Alteration of the respiration rate of WT and *spr2-1*.

	Respiration rate (nmol O ₂ min ⁻¹ g ⁻¹ fresh weight)				
	V _t	V _{alt}	V _{cyt}	V _{alt} /V _t (%)	V _{cyt} /V _t (%)
WT	1348.39±11.71	562.10±19.81	908.06±98.4	41.69	67.34
<i>emp36</i>	214.81±9.72	142.22±14.45	104.93±7.65	66.21	48.84

127

128 Mitochondrial total respiration rate (V_t), the capacity of the cytochrome pathway (V_{cyt})
 129 and the alternative pathway (V_{alt}) were measured using a Chlorolab II liquid-phase
 130 oxygen electrode; 2 mM KCN and 2 mM SHAM were used to inhibit the activity of
 131 cytochrome *c* oxidase and alternative oxidase, respectively. Data are mean values ± SEs
 132 from three independent biological samples.

133

134 **Supplemental Table S2.** List of some splicing factors which shared target intron(s) with
 135 SPR2.

Protein	Target transcripts	Reference
EMP16	<i>nad2-int4</i>	(Xiu et al., 2016)
PPR-SMR1	<i>nad1-int1, 2, 3, 4; nad2-int1, 2, 3, 4; nad4-int1, 2, 3; nad5-int1, 3, 4; nad7-int2; rps3-int1</i>	(Chen et al., 2019)
Zm-mCSF1	<i>nad2-int2, 3; nad5-int1, 2; nad7-int3; ccmFc-int1</i>	(Chen et al., 2019)
PPR14	<i>nad2-int3; nad7-int1, 2</i>	(Wang et al., 2020)
SPR2	<i>nad1-int1, 2, 3, 4; nad2-int1, 2, 3, 4; nad4-int1, 3; nad5-int1, 2, 4; nad7-int1, 2</i>	This study

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137 **Supplemental Table S3.** List of the primers used in this study.

Primer name	Primer sequences (5'-3')	Application
Spr2-F1	GAAGCCAGTGGTGAGCATCGC	Genotype <i>spr2-1</i> and <i>spr2-2</i> mutant
Spr2-R1	GAATGGTCGGTCTGATTTTCTCTTGCC	
TIR8.1	CGCCTCCATTTTCGTCGAATCCCCTS	
TIR8.2	CGCCTCCATTTTCGTCGAATCCSCTT	
TIR8.3	SGCCTCCATTTTCGTCGAATCCCKT	
TIR8.4	CGCCTCCATTTTCGTCGAATCACCTC	
Spr2-F2	CACCATGAGCCTGCAGATTCCGG	<i>Spr2</i> cloning into pEntry vector
Spr2-R2	TGATTTCTCATCAATAGCGAAGTTTCTG	
Spr2-F3	ATGAGCCTGCAGATTCCGG	RT-PCR analysis of <i>Spr2</i> gene
Spr2-R3	GAATGGTCGGTCTGATTTTCTCTTGCC	
Spr2-F4	GGTGGACGTGGACGAGATCTTC	RT-qPCR analysis of <i>Spr2</i> gene
Spr2-R4	CCACACAGAACCCGACAGCATC	
Spr2-F5	GGATCCATGAGCCTGCAGATTCCGGT	<i>Spr2</i> -HA cloning into pUNTF vector for overexpression
Spr2-R5	CCCGGGGAATGGTCGGTCTGATTTTCTCTT	
3xHA-pUNTF-F	CCCGGGGGGTTAATTAACATCTTTTACCATA	
3xHA-pUNTF-R	GAGCTCTTAGCTGCACTGAGCAGCGTA	
Ubi-F	CTTGGATGATGGCATATGCAGC	Genotype <i>Spr2</i> -OE and <i>Spr2</i> -Com
Spr2-YTH-F	GAATTCGCAAGGCAAGAGGAGGAGGACG	yeast two-hybrid (Y2H) assay
Spr2-YTH-R	GGATCCGAATGGTCGGTCTGATTTTC	
Emp16-YTH-F	CGCGGAATTCACCGCCGCCGCTTCCACTT	
Emp16-YTH-R	CCCGGGATCCATTAAGTAAATGGTCCCAG	
Spr2-cLUC-F	CCGGGAGCTCATGAGCCTGCAGATTCCGGT	Luciferase complementation imaging (LCI) assay
Spr2-cLUC-R	CGCGGAGCTCGAATGGTCGGTCTGATTTTCTCT	
Spr2-nLUC-F	CCGGGGATCCATGAGCCTGCAGATTCCGGT	
Spr2-nLUC-R	CGCGGTTCGACGAATGGTCGGTCTGATTTTCTCT	
Emp16-nLUC-F	CCGGGGATCCATGCCGCCGGCCAACTCCA	
Emp16-nLUC-R	CGCGGTTCGACATTAAGTAAATGGTCCCAGA	
Spr2-pSPYE-F	tggcgcgccactagtggatccATGAGCCTGCAGATTCCGG	Bimolecular fluorescence complementation (BIFC) analysis
Spr2-pSPYE-R	cccgggagcgggtaccctcgagGAATGGTCGGTCTGATTTTCTCT	
Emp16-pSPYE-F	tggcgcgccactagtggatccATGCCGCCGGCCAACTCCA	

Emp16-pSPYE-R	cccgggagcggtaccctcgagATTAAGTGTAAATGGTCCCAGA
Zm-mCSF1-pSPYE-F	tggcgcgccactagtgatccATGCTCACCTCCCCGGTAC
Zm-mCSF1-pSPYE-R	cccgggagcggtaccctcgagAATTACTTTTGTAATTTGGCAC
PPR14-pSPYE-F	tggcgcgccactagtgatccATGCGTCGCTACTGCCACGT
PPR14-pSPYE-R	cccgggagcggtaccctcgagTTCAAACAGTGTTTGAAATTTC
PPR-SMR1-pSPYE-F	tggcgcgccactagtgatccATGCTGCTCCGCGTTGGC
PPR-SMR1-pSPYE-R	cccgggagcggtaccctcgagCCTAGGCATGCCAAGGGATCT

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