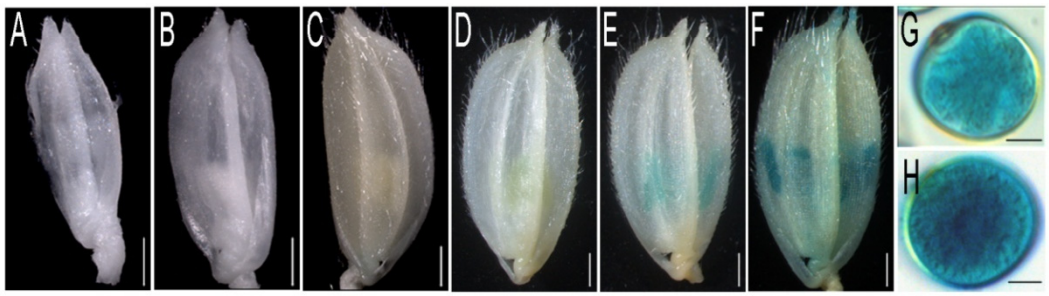
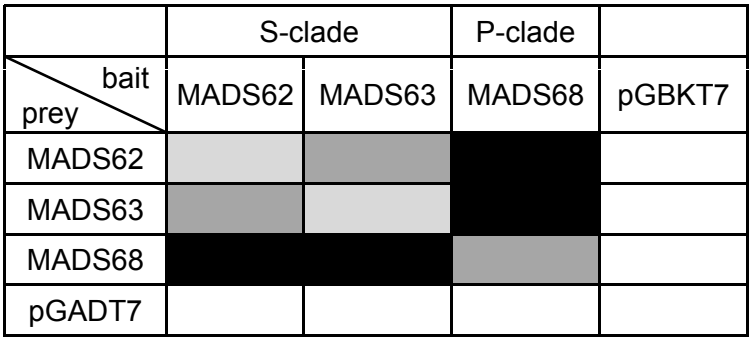
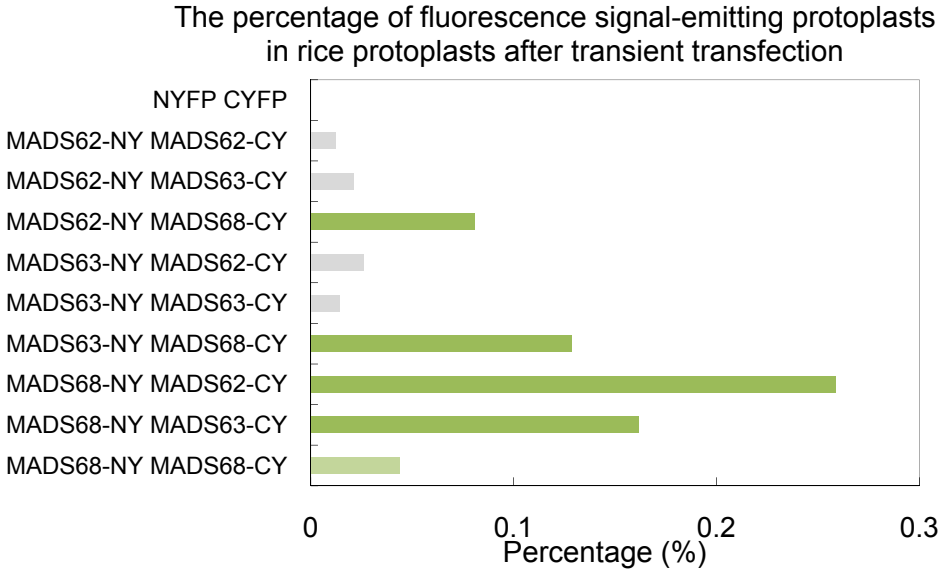


Supplemental Figure 1. GUS staining analyses of florets and pollen grains in *MADS62*$_{pro}$:*GUS* transgenic plants.
A stained floret with anther before stage 8 **(A)**, at stage 8 **(B)**, at stage 9 **(C)**, at stage 10 **(D)**, at stage 11 **(E)** and a magnified pollen grain **(G)**, at stage 12 **(F)** and a magnified pollen grain **(H)**.
Bars = 1 mm in **(A)** to **(F)**, and 10 μm in **(G)** and **(H)**.



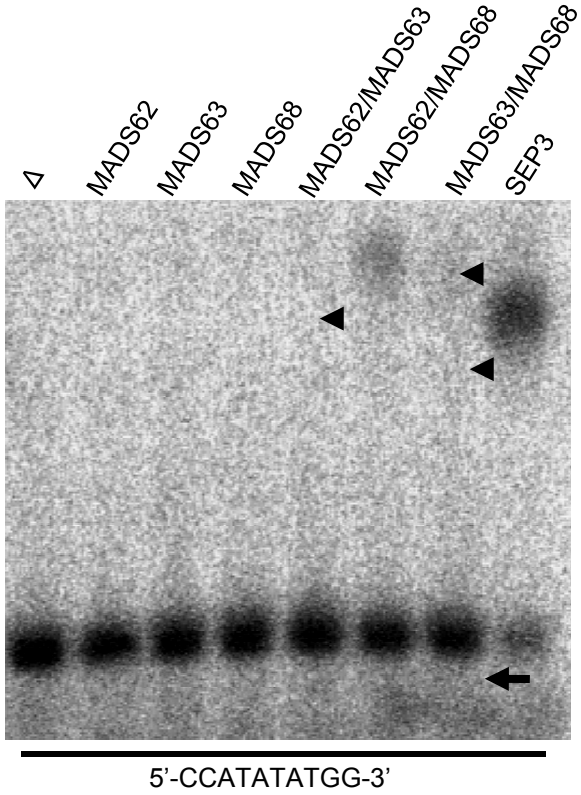
Supplemental Figure 2. Schematic depiction of rice MIKC*-type fusion protein interactions as revealed by Y2H analyses.

Each MIKC*-type protein was fused to the activation domain (AD) as a prey and the DNA-binding domain (BD) as a bait. Color intensity corresponds to interaction strength and a darker color of the boxes represents stronger interactions. Empty vectors pGADT7 and pGBKT7 were used as a negative control.



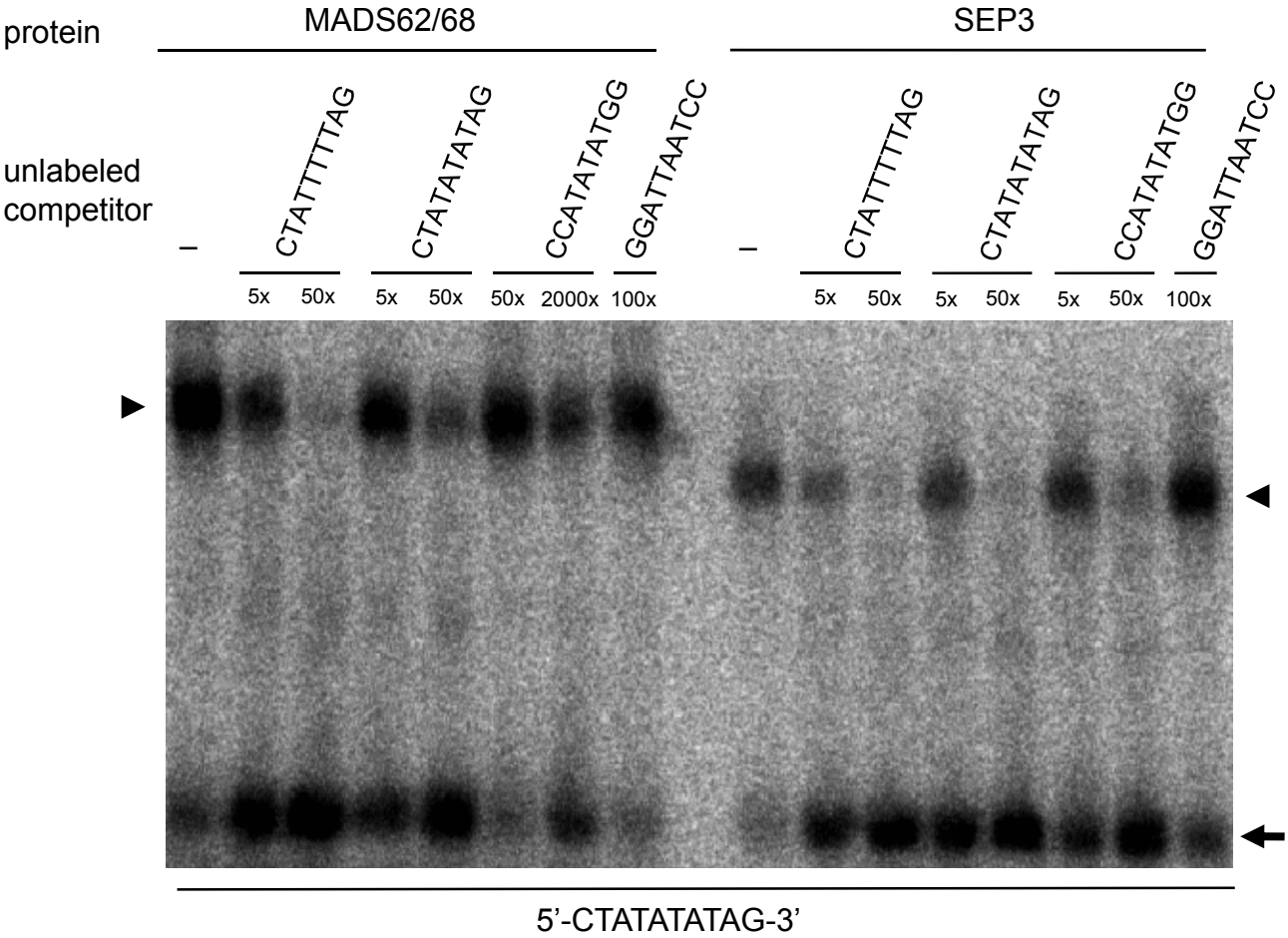
Supplemental Figure 3. The percentage of BiFC fluorescence signal-emitting protoplasts in rice protoplasts after transient transfection.

The mean of six independent experiments for each combination are shown. Empty NYFP and CYFP were used as negative control.



Supplemental Figure 4. EMSA assay for rice MIKC*-type heterodimeric complexes binding SRE-type CArG-box DNA.

A probe containing a SRE-type CArG-box (5'-CCATATATTGG-3') was incubated with *in vitro* translated MADS62, MADS63, MADS68 and combinations of these proteins. Free DNA is indicated by an arrow, shifted complexes by arrowheads. *In vitro* translation with SEP3 and an empty vector (Δ) served as positive and negative control, respectively.

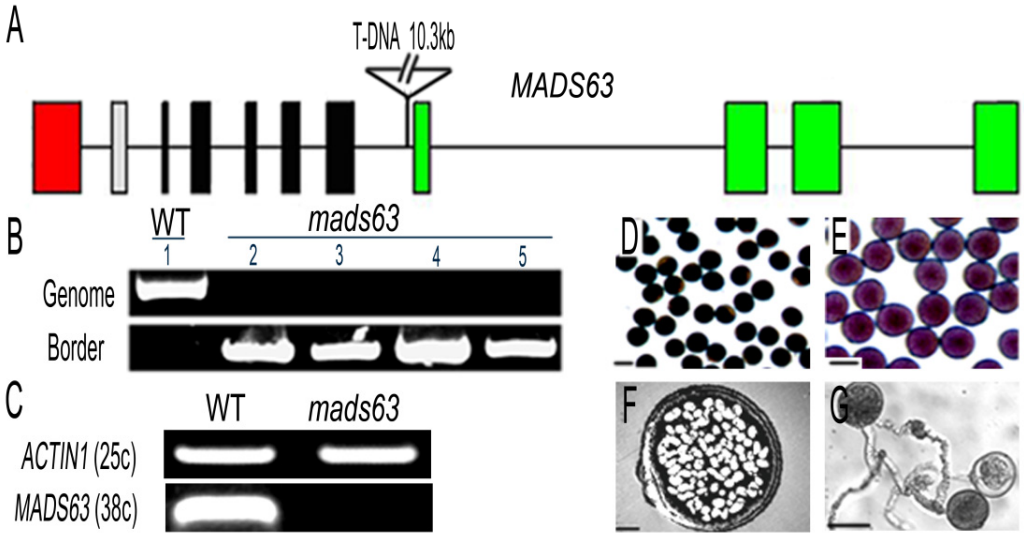


Supplemental Figure 5. Competetive EMSA for MIKC*-type heterodimers and SEP3.

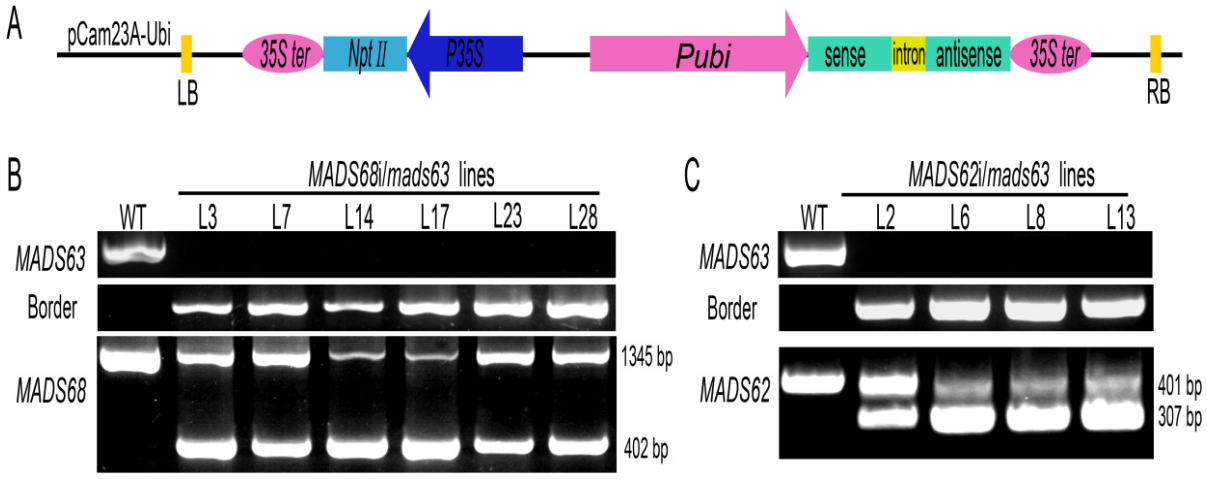
In vitro co-translated MADS62/MADS68 or SEP3 were incubated with a labeled containing a N10-type CTATATATAG probe (-), and the different competitor probes with the excess at which they were used in each lane. Free DNA is indicated by an arrow, shifted complexes by arrowheads.

Left panel: An unlabeled N10-type CTATTTTTAG probe successfully competes with a labeled N10-type CTATATATAG probe for binding to the MADS62/MADS68 protein complex (complete competition at 50-fold excess) and another unlabeled N10-type CTATATATAG probe compete to a lesser extent. In contrast, almost no competition was observed with an unlabeled probe containing the SRE-type motif CCATATATGG, even when a 2000-fold excess was supplied. Similarly, a 100-fold excess of an unlabeled probe containing the derived motif GGATTAATCC was not able to compete for binding.

Right panel: For N10-type competitor probes, SEP3 showed competition behavior similar to the MADS62/MADS68 heterodimer. But the binding of SEP3 to N10-type probe could be almost completely outcompeted with only 50-fold excess of the SRE-type competitor. An unlabeled probe containing the derived motif GGATTAATCC was used for a negative control.



Supplemental Figure 6. The identification and microscopic analyses of pollen of *MADS63* T-DNA insertion mutants.
(A) Schematic diagram of the genomic structure of *MADS63* and T-DNA insertion position. Red box corresponds to exon encoding MADS-box domain, whereas grey box represents exon encoding I region; black boxes, K domain and light green boxes, C terminal, with lines indicating introns. The 10.3 kb T-DNA insertion disrupts the seventh intron of *MADS63* in the mutant line 2D-10691.
(B) The molecular identification of *mads63* T-DNA mutant. Four homozygous plants from F3 generation (lane 2-5) were determined by genomic PCR using T-DNA border primer combined with *MADS63* specific primer pairs (listed in Supplemental Table 3 online).
(C) RT-PCR analyses of *MADS63* transcript in homozygous mutant. Total RNA samples were prepared from anthers at stage 13 of wild-type and *mads63* homozygous mutant of F3 generation. The *ACTIN1* serves as standard control. The cycle number of PCR is shown in parentheses.
(D) to **(G)** The *mads63* pollen phenotypes investigated by I_2-KI staining **(D)**, Alexander staining **(E)**, transmission electron microscopy **(F)** and the germination assays *in vitro* **(G)**. Scale bars = 50 μm in **(D)**, **(E)** and **(G)**, and 3 μm in **(F)**.

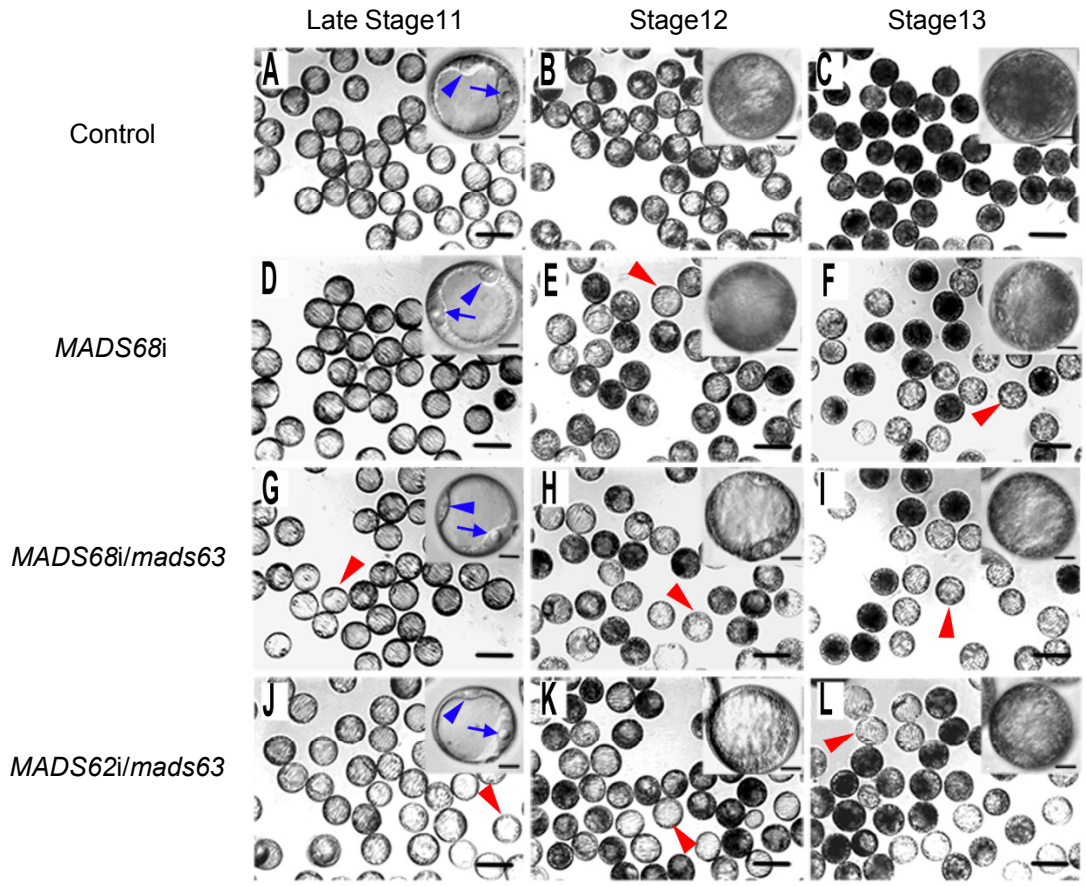


Supplemental Figure 7. Schematic diagram of RNAi constructs and identification of *MADS68*i/*mads63* and *MADS68*i/*mads63* transgenic plants.

(A) Schematic representation of the *MADS62* and *MADS68* RNAi constructs for transformation into *mads63* background rice. The dsRNAi cassette containing two oppositely orientated fragments and an intron was introduced into pCam23A-Ubi bone vector. *Pubi*, Maize *UBIQUITIN* promoter. *Npt II,* G418-resistant selectable marker gene. *35S ter*, CaMV35S terminator. *P35S*, CaMV35S promoter. LB, left border. RB, right border.

(B) The molecular identification of *MADS68*i/*mads63* transgenic lines. The homozygous status for *MADS63* allele in *MADS68*i/*mads63* plants were determined by genomic PCR using T-DNA border primer combined with *MADS63* specific primer pairs (see Methods). The *MADS68* RNAi positive lines were identified by genomic PCR using *MADS68* specific primer pairs (*MADS68*-F and *MADS68*-R, see Supplemental Table 3 online; the length of the foreign RNAi fragment and the corresponding endogenous genomic sequence is 402 and 1345 bp, respectively). Six selected lines L3, L7, L14, L17, L23 and L28 were showed.

(C) The molecular identification of *MADS62*i/*mads63* transgenic lines. The homozygous status for *MADS63* allele in *MADS62*i/*mads63* plants were determined by genomic PCR using T-DNA border primer combined with *MADS63* specific primer pairs. The *MADS62* RNAi positive lines were identified by genomic PCR using *MADS62* specific primer pairs (*MADS62*-F and *MADS62*-R, see Supplemental Table 3 online; the length of the foreign RNAi fragment and the corresponding endogenous genomic sequence is 307 and 401 bp, respectively). Four selected lines L2, L6, L8, and L13 were showed.



Supplemental Figure 8. Delay and arrest of pollen development occurs from bicellular stage.

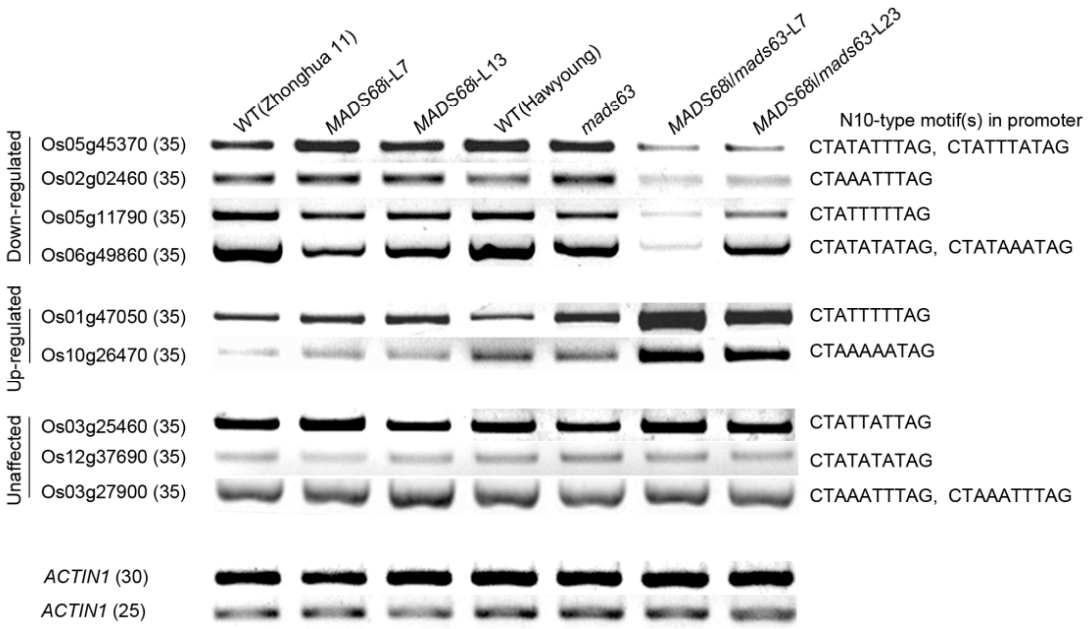
(A) to **(C)** The development process of pollen from anther at late stage 11 to 13 of a control plant.

(D) to **(F)** The development process of pollen from anther at late stage 11 to 13 of line *MADS68* RNAi L7.

(G) to **(I)** The development process of pollen from anther at late stage 11 to 13 of line *MADS68*i/*mads63* L7.

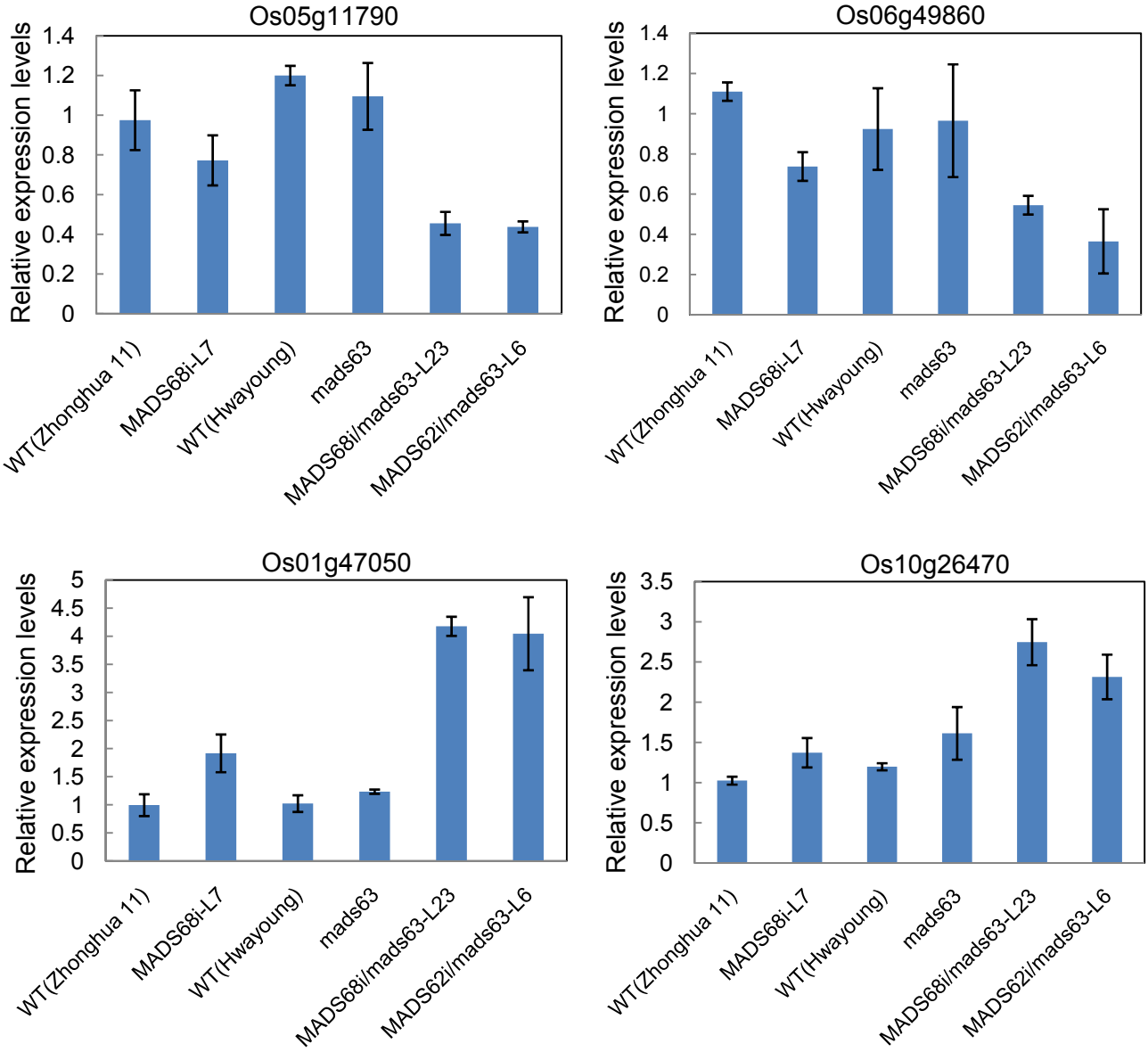
(J) to **(L)** The development process of pollen from anther at late stage 11 to 13 of line *MADS62*i/*mads63* L13.

The pollen were collected and prepared from anthers at the late stage 11 (**[A], [D], [G],** and **[J]**), when starch granules started to accumulate in bicellular pollen of control plants; anthers at the stage 12 (**[B], [E], [H],** and **[K]**), when starch were filled in the most space of pollen in control plants; and anthers at the stage 13 (**[C], [F], [I],** and **[L]**), when pollen achieved maturation with three nuclei in control plants. Insets show magnified pollen with apparent defects at the corresponding panel. Red arrowheads indicate abnormal pollen from *MADS68* RNAi, *MADS68*i/*mads63*, and *MADS62*i/*mads63* lines. Blue arrows indicate the vegetative nucleus and arrowheads indicate generative cell in insets (**[A], [D], [G],** and **[J]**). Scale bars = 100 μm in **(A)** to **(L)**, and 12.5 μm in the corresponding insets.



Supplemental Figure 9. RT-PCR analyses for a selection of nine *in silico* predicted target genes of the rice MIKC*-type protein complexes.

Total RNAs were pooled from anthers at stage 13 of wild-type (Zhonghua 11 and Hwayoung), *mads63* mutant, *MADS68* RNAi, and *MADS68*i/*mads63* lines. *ACTIN1* is amplified as the standard control. For each of the putative target genes, N10-type motif(s) present in the promoter region are listed in right. The cycle number of PCR is shown in parentheses for each gene.



Supplemental Figure 10. qRT-PCR analyses of the expressions of putative target genes of the rice MIKC*-type protein complexes.
Total RNAs were pooled from anthers at stage 13 of wild-type (Zhonghua 11 and Hwayoung), *mads63* mutant, *MADS68* RNAi, *MADS68*i/*mads63*, and *MADS62*i/*mads63* lines. The samples were quantified using *UBIQUITIN* as a reference gene and the data are presented as mean ± SD (standard deviation, *n* = 3).

Supplemental Table 3. Gene-specific primers used in this study.

Gene name	Primer name	Sequences (5' to 3')	Enzyme
RNAi constructs (restriction sites are underlined)			
MADS62	*MADS62*-F	CAGAAGCTT GAATTC GCCGGAGGCCACGCCG	*Hind* III/*Eco* RI
	MADS62-R	GACCTCGAG CCCGGG TAGGTTAGTTAGGTGAGGT	*Xho* I/*Sma* I
MADS68	*MADS68*-F	CAGAAGCTT GAATTC TCACAGCAGCATAGAGGATGTC	*Hind* III/*Eco* RI
	MADS68-R	GACCTCGAG CCCGGGAGTGGGAGCTTCATGTCGTTCT	*Xho* I/*Sma* I
mads63 mutant identification			
	Right border primer	ACCGTGGTAGTAAGAATGGA	
	M63-F	TTTGATCAACATGCTCACC	
	M63-R	CTGCATGGCAGATGTTGAC	
Promoters amplification (restriction sites are underlined)			
MADS62	P62-F	CAGCTGCAGCGGAGTGATCAGTAGTTCTTG	*Pst* I
	P62-R	GACCCCGGGCCTTATCCCTCGCCGCCGA	*Sma* I
MADS63	P63-F	CAGCTGCAGAAGGCCTTGCAGCTCACCATAC	*Pst* I
	P63-R	GACTCTAGAGGCCGCCGCCGGCGATTCA	*Xba* I
MADS68	P68-F	CAGTCTAGAGGAACTCACCGGCTAGCCATC	*Xba* I
	P68-R	GACCCCGGGGGCGGAGGCAGAATCCCCTCT	*Sma* I
Y2H constructs (restriction sites are underlined)			
MADS62	Y62-F	GGAATTCCATATGATGGGGAGGGTGAAGCTGCC	*Nde* I
	Y62-R	CAGGAATTCGGCGATGTTCGCCGGCGGCG	*Eco* RI
MADS63	Y63-F	GACGAATTCATGGGACGGGTGAAGCTGCA	*Eco* RI
	Y63-F	CAGGGATCCACCAACGTTAACCGGAGCAA	*Bam* HI
MADS68	Y68-F	GGAATTCCATATGATGGGGAGGGTCAAGCTC	*Nde* I
	Y68-R	CAGGAATTCAATCATGAGCTGCCGGTGTG	*Eco* RI
BiFC constructs (restriction sites are underlined)			

MADS62	B62-F	GACACTAGTATGGGGAGGGTGAAGCTGCC	*Spe* I
	B62-R	CAGCTCGAGGGCGATGTTCGCCGGCGG	*Xho* I
MADS63	B63-F	GACACTAGTATGGGACGGGTGAAGCTGCA	*Spe* I
	B63-R	CAGCTCGAGACCAACGTTAACCGGAGCAAT	*Xho* I
MADS68	B68-F	GACACTAGTATGGGGAGGGTCAAGCTCAA	*Spe* I
	B68-R	CAGCTCGAGCATGAGCTGCCGGTGTGGT	*Xho* I
In situ expression analyses			
MADS62	62i-F	GACGCAGATGTACGTGAGCC	
	62i-R	GTTGCTGTCGTACGCCATGA	
	T762i	TAATACGACTCACTATAGGGGTTGCTGTCGTACGCCATGA	
MADS63	63i-F	TTGGAAGCGATGAGGTGGC	
	63i-R	GAGCAATGTCCTCAGCTGC	
	T763i	TAATACGACTCACTATAGGGGAGCAATGTCCTCAGCTGC	
MADS68	68i-F	TCACAGCAGCATAGAGGATGTC	
	68i-R	AGTGGGAGCTTCATGTCGTTCT	
	T768i-F	TAATACGACTCACTATAGGGTCACAGCAGCATAGAGGATGTC	
	T768i-R	TAATACGACTCACTATAGGGAGTGGGAGCTTCATGTCGTTCT	
RT-PCR analyses			
MADS62	R62-F	AGGGTGAAGCTGCCGATCA	
	R62-R	AGGTCAGGCGATGTTCGC	
MADS63	R63-F	ACGGGTGAAGCTGCAGATC	
	R63-R	TCTAATTAACCAACGTTAACCG	
MADS68	R68-F	GGAGGGTCAAGCTCAAGATA	
	R68-R	TCAAATCATGAGCTGCCGGT	
LOC_Os05g45370	R05-F1	TCGCTCATCGGCATCATCTT	
	R05-R1	TGCCAAACTTAATGGTCCTGTC	
LOC_Os03g25460	R03-F1	TAACCTCAACAACCCTGCT	
	R03-R1	GTCTCGTCTCCTCCCATCC	
LOC_Os01g47050	R01-F	TCTTCTTGCGGATTAGTTTGTT	
	R01-R	ACCTACCTCTTGCCATTGTTT	

LOC_Os05g11790	R05-F3	CCAAACCCTACAACTCGCATCA
	R05-R3	GCCCTTTCCTCCCTTCCTT
LOC_Os03g27900	R03-F2	GGAGGCCAAGGAGAAGATGG
	R03-R2	TGCTCGAACACCAGCACCT
LOC_Os12g37690	R12-F	TGCGATGCCAACAGTGCTA
	R12-R	CTCGGAAATGCCCTCAGAAAA
LOC_Os02g02460	R02-F	ATGTTCGTCAGCCAGGTGC
	R02-R	AGGAGCCGAGGAAGAAAGG
LOC_Os10g26470	R10-F	GCGGCACGACGATGGAGAT
	R10-R	TCGACACCGACTGGATGGG
LOC_Os06g49860	R06-F	AGATGATCGGCAGCGTGTCC
	R06-R	GACCTCCAACGAGTGCTACGAG
ACTIN1	Act-F	CCAATCGTGAGAAGATGACCCA
	Act-R	CCATCAGGAAGCTCGTAGCTCT
qRT-PCR analyses		
MADS62	q62-F	AGGACCGCCTTAGGATGTTCG
	q62-R	GACTCGGGTCAGCAATTCCAT
MADS63	q63-F	GTCGGTGGCGTCGATATTC
	q63-R	GCATCTTGTCTCATCGTGTCGT
MADS68	q68-F	CGGATTCATAAGGAGAACTTTGC
	q68-R	GAAGTGTTCGGGTCACCTGTTAG
LOC_Os01g47050	q01-F	AATCGTCGATCCCAGCACC
	q01-R	CAATCCCACCAACCAGAACC
LOC_Os05g11790	q05-F	CAATGGCGAGTTCAAGGTCC
	q05-R	GATGCGAGTTGTAGGGTTTGG
LOC_Os06g49860	q06-F	GCGAGTCATTCAGCACCTACC
	q06-R	CCTCACGATCCTGTCCACCT
LOC_Os10g26470	q10-F	TCCACCAATGACTGGCACAA
	q01-R	CCAAGAAGGCCGCTTTGAGA

ACTIN1	qAct-F	TGCTATGTACGTCGCCATCCAG	
	qAct-R	AATGAGTAACCACGCTCCGTCA	
UBIQUITIN	qUbi-F	CACCCTGGCTGACTACAACA	
	qUbi-R	TTCTTCTTGCGGCAGTTGAC	
EMSA assays (restriction sites are underlined)			
MADS62	E62-F	ATCGATGAATTCGCCGCCATGGGGAGGGTGAAGCTGC	*Eco* RI
	E62-R	ATCGATCTAGATCAGGCGATGTTCGCCGGC	*Xba* I
MADS63	E63-F	ATCGATGAATTCGCCGCCATGGGACGGGTGAAGCTGCAG	*Eco* RI
	E63-R	ATCGATCTAGATTAACCAACGTTAACCGGAGCAATG	*Xba* I
Protoplast transient transfection assays (restriction sites are underlined)			
MADS62	LUC62-F	ATGGGGAGGGTGAAGCTGC	
	LUC62-R	TCAGGCGATGTTCGCCGG	
MADS63	LUC63-F	ATGGGACGGGTGAAGCTGC	
	LUC63-R	TTAACCAACGTTAACCGGAGCA	
MADS68	LUC68-F	ATGGGGAGGGTCAAGCTCAA	
	LUC68-R	TCAAATCATGAGCTGCCGGT	
LOC_Os05g11790	LUC05-F	CAGAAGCTTGTTCACTAATGATGCAACGGAA	*Hind* III
	LUC05-R	CTGACTAGTCCGGATTAGTGTATGTTCACG	*Spe* I
LOC_Os06g49860	LUC06-F	CAGAAGCTTCATCACCATCTTGTCTCATCAG	*Hind* III
	LUC06-R	GACGGATCCTGATCGGACAAGGACGAGTA	*Bam* HI

Supplemental Table 2. Genetic analyses of the *mads63* mutant.
+/+, +/−and −/− represents wild-type (WT), heterozygous and homozygous genotype, respectively.

Self-cross	the number of each genotype (precentage)			
+/−(♀) × +/−(♂)	+/+	+/−	−/−	total
	15 (21 %)	40 (55 %)	17 (24 %)	72
Test-cross	the number of each genotype (precentage)			
+/−(♀) × +/+(♂)	+/+	+/−	−/−	total
	9 (42.9 %)	12 (57.1 %)	0	21
back-cross	the number of each genotype (precentage)			
−/−(♀) × +/+(♂)	+/+	+/−	−/−	total
	0	18 (100 %)	0	18

Supplemental Table 1. Detailed description of MIKC*-type genes used for alignment and phylogenetic analyses.

Class	Order	Species	Gene name	Accesion number of mRNA / EST / PUT
bryophyte MIKC* sequences				
liverwort (the most ancient land plant family)	Marchantiales	*Marchantia polymorpha*	Mp *MADS1*	GQ334454
mosses	Sphagnales	*Sphagnum subsecundum*	Ss *MADS1*	GQ334455
	Sphagnales	*Sphagnum subsecundum*	Ss *MADS2*	GQ334456
	Sphagnales	*Sphagnum subsecundum*	Ss *MADS3*	GQ334457
	Sphagnales	*Sphagnum subsecundum*	Ss *MADS4*	GQ334458
	Funariales	*Funaria hygrometrica*	Fh *MADS1*	GQ334460
	Funariales	*Funaria hygrometrica*	Fh *MADS2*	GQ334461
	Funariales	*Funaria hygrometrica*	Fh *MADS3*	GQ334462
	Funariales	*Funaria hygrometrica*	Fh *MADS4*	GQ334463
	Funariales	*Funaria hygrometrica*	Fh *MADS5*	GQ334464
	Funariales	*Funaria hygrometrica*	Fh *MADS6*	GQ334465
	Funariales	*Funaria hygrometrica*	Fh *MADS7*	GQ334466
	Funariales	*Funaria hygrometrica*	Fh *MADS8*	GQ334467
	Funariales	*Funaria hygrometrica*	Fh *MADS9*	GQ334468
	Funariales	*Funaria hygrometrica*	Fh *MADS10*	GQ334469
	Funariales	*Funaria hygrometrica*	Fh *MADS11*	GQ334470
lycophyte MIKC* sequences				
lycophyte	Selaginellales	*Selaginella moellendorffii*	Sm *MADS4*	FM999805
	Selaginellales	*Selaginella moellendorffii*	Sm *MADS10*	FM999806
	Selaginellales	*Selaginella moellendorffii*	Sm *MADS2*	FM999807
monilophyte MIKC* sequences				

fern	Polypodiales	*Ceratopteris richardii*	Cr *CRM13*	FM995267
	Polypodiales	*Ceratopteris richardii*	Cr *CRM14*	FM995269
	Polypodiales	*Ceratopteris richardii*	Cr *CRM15*	FM995271
	Polypodiales	*Ceratopteris richardii*	Cr *CRM16*	FM995273
seed plant MIKC* sequences				
gymnosperm	Pinales	*Picea glauca*	Pigla *MADS1*	PUT-163a-Picea_glauca-52072
basal eudicots	Ranunculales	*Eschscholzia californica*	Ec *MADS1*	FM958508
	Ranunculales	*Eschscholzia californica*	Ec *MADS2*	FM958509
	Ranunculales	*Aquilegia coerulea*	Ac_*v1.025702*	scaffold_14:3,009,710..3,011,727
	Ranunculales	*Aquilegia coerulea*	Ac_*v1.007645*	scaffold_2:5,042,310..5,047,097
eudicots	Brassicales	*Arabidopsis thaliana*	At *AGL30*	NM_001084404
	Brassicales	*Arabidopsis thaliana*	At *AGL65*	NM_101733
	Brassicales	*Arabidopsis thaliana*	At *AGL94*	NM_105623
	Brassicales	*Arabidopsis thaliana*	At *AGL66*	NM_106447
	Brassicales	*Arabidopsis thaliana*	At *AGL67*	NM_106444
	Brassicales	*Arabidopsis thaliana*	At *AGL104*	NM_102063
	Malpighiales	*Populus trichocarpa*	POPTR_*0008s08780*	XM_002311272
	Malpighiales	*Populus trichocarpa*	POPTR_*0002s09290*	XM_002300999
	Malpighiales	*Populus trichocarpa*	POPTR_*0007s05980*	XM_002310547
	Malpighiales	*Populus trichocarpa*	POPTR_*0010s17450*	XM_002316094
	Vitales	*Vitis vinifera*	GSVIVG *01007989001*	XM_002281925
	Vitales	*Vitis vinifera*	GSVIVG *01022182001*	XM_002276798
	Graminales	*Brachypodium distachyon*	Bradi *3g39177*	XM_003572285
	Graminales	*Zea mays*	GRMZM *2G152415*	EU975181
	Graminales	*Zea mays*	GRMZM *2G334225*	BT066884
	Graminales	*Sorghum bicolor*	Sb *10g007810*	XM_002436689
	Graminales	*Brachypodium distachyon*	Bradi *4g11097*	XM_003577224

monocots	Graminales	*Sorghum bicolor*	Sb *05g025970*	XM_002449891
	Graminales	*Zea mays*	GRMZM *2G441115*	Chr4:2,887,800-2,890,099
	Graminales	*Oryza sativa*	Os *MADS68*	FM956505
	Graminales	*Oryza sativa*	Os *MADS62*	FM956504
	Graminales	*Oryza sativa*	Os *MADS63*	FN663130
	Graminales	*Hordeum vulgare*	Hovul *MADS1*	AK373111
	Graminales	*Hordeum vulgare*	Hovul *MADS2*	AC239041 30785-32897
	Graminales	*Panicum virgatum*	Pavirv *00040498m.g*	sg0.contig58690: 2292 - 4176
	Graminales	*Panicum virgatum*	Pavirv *00060579m.g*	sg0.contig155006: 367 - 2175
	Graminales	*Panicum virgatum*	Pavirv *00018936m.g*	sg0.contig49821: 1269 - 4636
	Graminales	*setaria italica*	Si *013985m.g*	scaffold_6: 31708741 - 31711109
	Graminales	*setaria italica*	Si *008439m.g*	scaffold_4: 7726121 - 7728329
	Graminales	*setaria italica*	Si *028357m.g*	scaffold_8: 37418473 - 37421603

Supplemental Table 4. Sequences of EMSA probes used in this study. CArG-boxes are underlined.

probe name	Sequences (5' to 3')
MEF2-1 probe	AATTCATCGATCGTTTACTATTTTTAG AAATATCGATCGG
MEF2-2 probe	AATTCATCGATCGTTTACTATATATAGAAATATCGATCGG
SRF probe	AATTCATCGATCGTTTACCATATATGGAAATATCGATCGG
competetive randomized probe	AATTCATCGATCGTTTAGGATTAATCCAAATATCGATCGG
probe for LOC_Os10g26470	TAATCATTTGGCTGGATAAACCCACTAAAAATAGGGATGCTCCAATGAGATCATGCGG
probe for LOC_Os05g11790	GCTAAATTTGTATCGTCTTATATCCTATTTTTAGGCAGTTCACAAATGGTGAAATTTA
probe for LOC_Os01g47050	ATATACTGAGTAACACTAGTAAGACTATTTTTAGAGAGAGAACGATTCAATGATTCAT
probe for LOC_Os06g49860	TTTCAGTTCCCTGCAACATTAGATCTATATATAGTGAAACATTGTGAGTACACTATGC