

Supplemental Figure 1. Meiosis related genes are downregulated in *mil1* anthers.

(A) RT-PCR analysis of *MER3*, *PAIR1*, *AM1*, *SDS*, *SPO11-1*, and *ZEP1* expression in wild-type meiotic panicles and *mil1* panicles at similar development stage (5 cm), using *Ubiquitin* (*UBQ*) as a control. (B) qRT-PCR analysis of *MEL1*, *MEL2* and *REC8* expression in wild-type meiotic panicles and *mil1* panicles at similar development stage (5 cm), with *UBQ* as a control.



Supplemental Figure 2. Isolation of the *MIL1* gene and molecular characterization.

(A) Fine mapping of the *MIL1* locus. BACs: B1, AP004010; B2, AP003824; B3, AP004263; B4, AP003704; B5, AP003847. S1 to S9 are markers developed in this work (Table S1). The *MIL1* locus was located to a 13 Kb region between the two markers S8 and S9. (B) *MIL1* gene structure. The *mil1* genome has insertion in the marked site. (C) Complementation constructs. The plasmid pCMIL contains the entire *MIL1* ORF, the 3870 bp upstream and 1306 bp downstream region. The pCMILC control vector contains only a partial upstream region. (D) The MIL1 protein sequence. The amino acids composing the active site are underlined.



Supplemental Figure 3. RT-PCR analysis shows no detectable *MIL1* transcripts in *mil1* panicles at meiotic stages, with *UBQ* used as a control.



Supplemental Figure 4. Phylogeny of the CC type glutaredoxins.

Phylogenetic tree of CC type glutaredoxins from *Arabidopsis* and rice using MEGA 3.1 based on the alignment given in Supplemental Dataset 1 online. Bootstrap values are percentage of 1000 replicates. The black dot shows the position of MIL1 (Os07g05630).



Supplemental Figure 5. qRT-PCR analysis of *MIL1* expression.

R: root; In: internode; L, leaf; Sh: sheath; P1: 1 cm panicles; P2: 3 cm panicles; P3: 5 cm panicles; P4: 10 cm panicles; P5: booting panicles.



Supplemental Figure 6. Control hybridization of *MIL1* RNA in situ analysis.

Wild-type anthers hybridyzed with *MIL1* sense probe. (A) At the stamen primordial stage.(B) At archesporial cells stage. (C) At the parietal and sporogenous cells differentiation stage. E, epidermis. Scale bars, 10 μm.



Supplemental Figure 7. Structure of the *msp1-5* allele.

Numbering of the coordinates is based on the BAC clone AP003451 containing the *MSP1* gene.



Supplemental Figure 8. Phylogenetic tree of the Arabidopsis and rice TGA proteins.

Phylogenetic analysis of TGA proteins from *Arabidopsis* and rice using MEGA 3.1 based on the alignment given in Supplemental Dataset 2 online. Bootstrap values are percentage of 1000 replicates. The black dot shows the position of the rice TGA1 (Os05g37170).



Supplemental Figure 9. qRT-PCR analysis shows rice *TGA1* having a much lower expression level in *mil1* panicles at meiotic stages, with *UBQ* used as a control.



Supplemental Figure 10. Diagram for MSP1 and MIL1 function in male reproductive cell development.

In rice anthers, MSP1 inhibits the production of extra microsporocytes, and MIL1 regulates the initiation of meiosis in microsporocytes. In *msp1* mutant, multiple microsporocytes are generated; these microsporocytes will enter meiosis but can't complete successfully, and they finally will degenerate with the microsporangium crushed. In *mil1*, initially normal number of SCPs are produced, which couldn't enter meiosis but divide mitotically, leading to a microsporangium filled with parenchyma cells. Due to the absence of MIL1, the excess SCPs generated in *mil1 mspl* double mutant couldn't initiate meiosis; instead, they continue mitosis and produce a *mil1*-like microsporangium. PSC, primary sporogenorous cell; Ms, microsporocyte; Msp, microspore; dMC, degenerated meiocytes.

Marker ^a	Primer Pairs	BAC ^b
S1	F 5'-CGTCGATCGTCAAGACGCA-3'	AP004314
	R 5'-CGAGCTTGAGCTTGCCTGA-3'	
S2	F 5'-GCTAATGCAGCTTCTACCA-3'	AP004010
	R 3'-GTTTCCCAGTTTCTGTCTTC-3'	
S3	F 5'-CAGGAGTTAGCGATCAAAGT-3'	AP003847
	R 3'-CAACATATCTGCAGTAGCTG-3'	
S4	F 5'-GGAGTCTAACACTAATCTGA-3'	AP004307
	R 3'-GGCTAGCCTTTATCTTCGAT -3'	
S5	F 5'-TGATGGGATGTATGCTGTG-3'	AP003824
	R 5'-AACCACAGGAAGAGCCAC-3'	
S6	F 5'-TGGGGAGGTCGGAGTTTTC-3'	AP004263
	R 5'-TGGTCGTGAATCTGCTGGTAGTG-3'	
S7	F 5'-GGGAGGGATGTATCTATTTG-3'	AP003704
	R 5'-AAGCGTGCCATTTTATTTC-3'	
S8	F 5'-GACAGACCAGAATTTGTCAA-3'	AP003704
	R 5'-GGTCATTACAAAGGTTGCAA-3'	
S9	F 5'-TATTAGTGTCAGGTTTTGTGTG-3'	AP003704
	R 5'-AAGAACTTGCTACTGACACTG-3'	

Supplemental Table 1. List of the PCR-based molecular markers developed in this study

^aAll the markers are sequence tagged site markers.

^bThese are the BACs the markers located on.

Primer	Primer Sequence	Description
Name		
3RACE-1	5'-ACATCAATGGCACCCTCGTC-3'	3'-RACE for MIL1
3RACE-2	5'-TCCATCCATCGATCCCTACC-3'	
5RACE-1	5'-TCTCGTACACCGACATCATC-3'	5'-RACE for MIL1
5RACE-2	5'- CCACCACCTGCATCCTCATC-3'	
MIL1GFP-F	5'-GATCCGAGCCAGAGGGCGCCGG-3'	GFP-MIL1 fusion protein
MIL1GFP-R	5'-AAGAATTCATGAGGATGCAGGTGGTGGAG-3'	construct
MIL1RT-F	5'-TCCATCCATCGATCCCTACC-3'	RT-PCR and in situ
MIL1RT-R	5'-GCATTTCAAACTCATCGTCG-3'	hybridization for MIL1
TGAISH-F	5'-GAATGTCAGCCCTACAGGAA-3'	RT-PCR and in situ
TGAISH-R	5'-ATTCAAGAGCCATTGGAACA-3'	hybridization for TGA1
RECISH-F	5'-CTCACTCGCTCATCCATT-3'	<i>in situ</i> hybridization for
RECISH-R	5'-CATCTTTGGTCCCCTTGA-3'	REC8
MER3RT-F	5'-TGGATCCTTTACAGTGGCATCCTGCGT-3'	RT-PCR for MER3
MER3RT-R	5'-TGTCGACACATACTGTTTTCAGGGT-3'	
MEL1RT-F	5'-AAGGAGAGAGTTCGGATGGT-3'	RT-PCR for MEL1
MEL1RT-R	5'-CCAACAATCTCCTCAGCAGT-3'	
MEL2RT-F	5'-GATGCTTCCTGGCTTCAGTA-3'	RT-PCR for MEL2
MEL2RT-R	5'-GCAAAATCGAAGGAGAGTGA-3'	
AM1RT-F	5'-TGGTGTAAAAAGGCACATCG-3'	RT-PCR for AM1
AM1RT-R	5'-CAGCAGCAATTGTTCCTTCA-3'	
REC8RT-F	5'-TCCACTCGTACCTCAAGCTA-3'	RT-PCR for REC8
REC8RT-R	5'-GTTGCTAAAACGCATGCTTG-3'	
SDSRT-F	5'-GCTTTCAAAGTAGGGATCAATAC-3'	RT-PCR for SDS
SDSRT-R	5'-CCCAGAAACACAGTGTAGTACG-3'	
SPO11RT-F	5'-CATCTCGAGGAGAAGGAGACAGTGTTTCAA-3'	RT-PCR for SPO11-1
SPO11RT-R	5'-TGCAGATCTTGCAGATAACGCCTCAATCTCA-3'	
PAIR1RT-F	5'-CCTCGACCCTTGCAACCTTGCAGAC-3'	RT-PCR for PAIR1
PAIR1RT-R	5'-GTTACCATTAATTAGCTAGGATGCGAGC-3'	
UBQRT-F	5'-CAAGATGATCTGCCGCAAATGC-3'	RT-PCR for Ubiquitin
UBQRT-R	5'-TTTAACCAGTCCATGAACCCG-3'	
MIL1BD-F	5'-GCATTTCAAACTCATCGTCG-3'	pLexA-MIL1 and
MIL1BD-R	5'-AAGAATTCATGAGGATGCAGGTGGTGGAG-3'	pMAL-MIL1 construct
TGAAD-F	5'-ATTCAAGAGCCATTGGAACA-3'	pB42AD-TGA1 construct
TGAAD-R	5'-GAATTCATGATCCAAAGTGACGCGTAC-3'	

Supplemental Table 2. The primers for MIL1 molecular characterization.