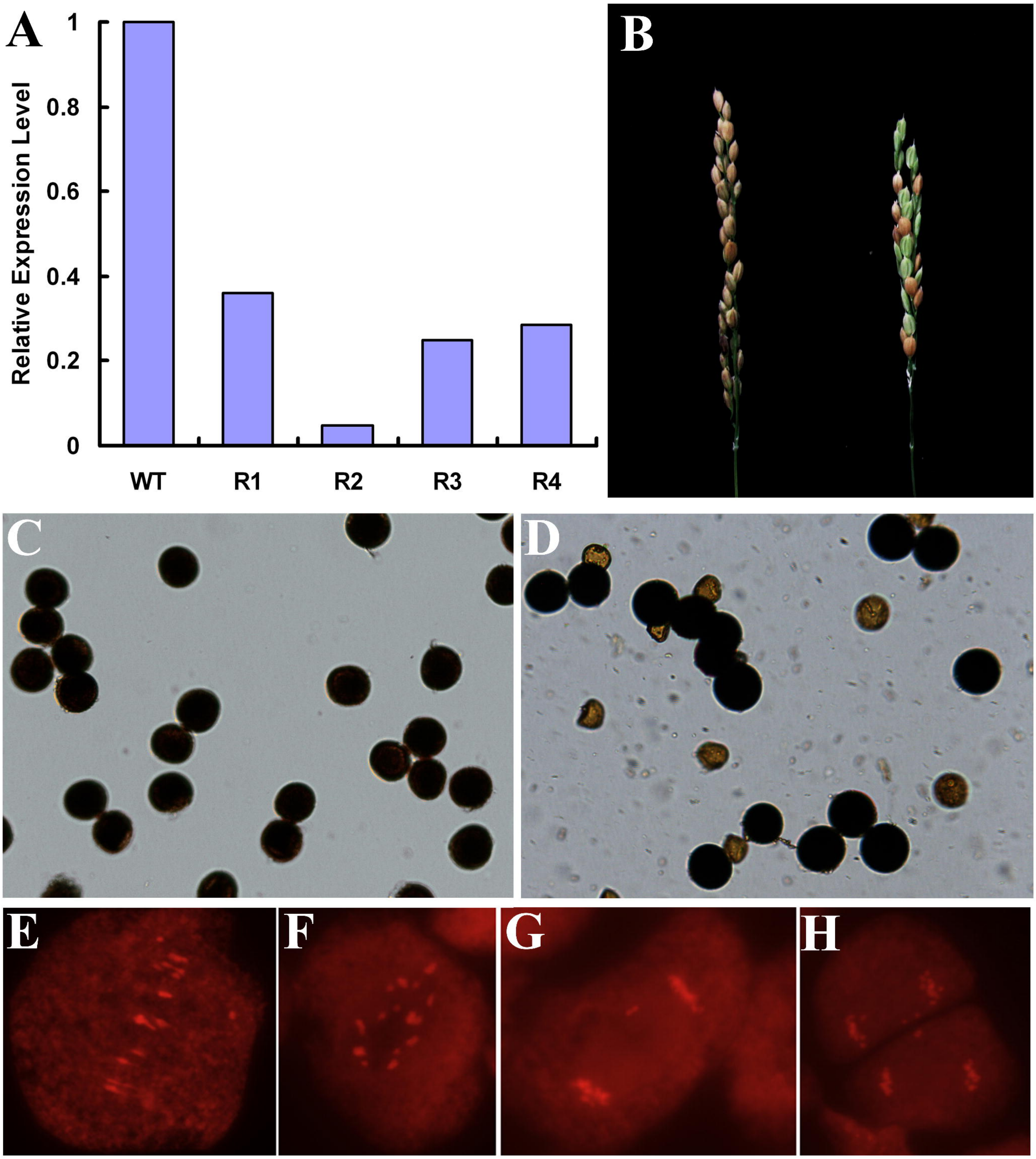
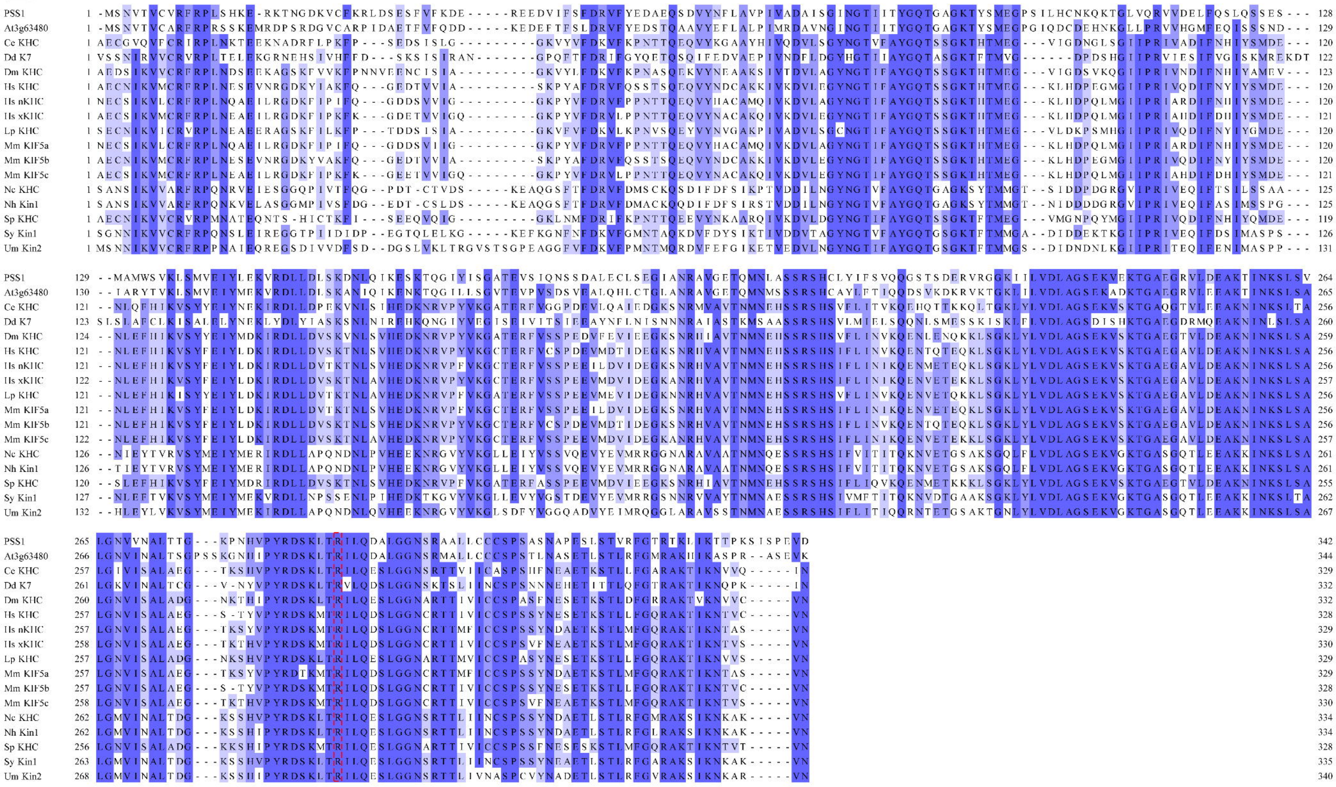


Supplemental figure 1. PSS1 RNAi plants Show a Similar Phenotype to the *pss1* Mutant.
(A) Real-time PCR analysis showing that reduced mRNA accumulation in four independent RNAi lines (R1, R2, R3 and R4). WT, the wild type cultivar Kitaake.
(B) The spikelet fertility of the RNAi line R2 (right) is about 40% to the wild type (left).
(C) KI-I_2 staining of the wild type pollen grains.
(D) KI-I_2 staining of the pollen grains of RNAi line R2.
(E) to **(H)** Chromosome dynamics in the RNAi line R2.

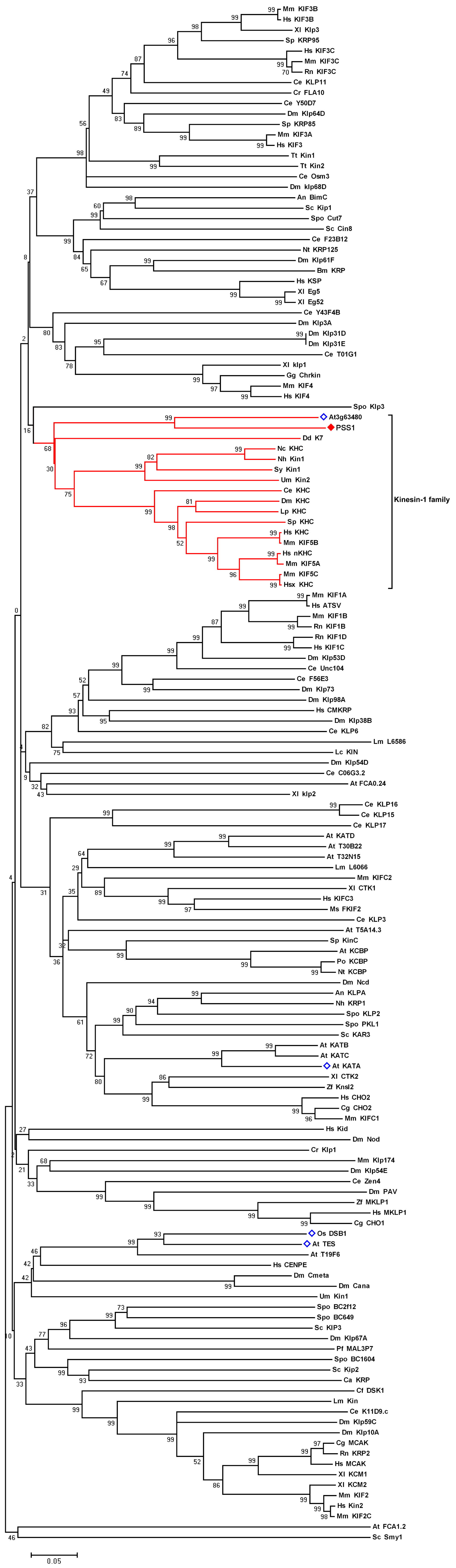
A

1 MSNVTVCVRF RPLSHKERKT NGDKVCFKRL DSESFVFKDE REEDVIFSFD RVFYEDAEQS DVYNFLAVPI VADAISGING TIITYGQTGA
91 GKTYSMEGPS ILHCNKQKTG LVQRVVDELF QSLQSSESMA MWSVKLSMVE IYLEKVRDLL DLSKDNLQIK ESKTQGIYIS GATEVSIQNS
181 SDALECLSEG IANRAVGETQ MNLASSRSHC LYIFSVQQGS TSDERVRGGK IILVDLAGSE KVEKTGAEGR VLDEAKTINK SLSVLGNVVN
271 ALTTGKPNHV PYRDSKLTRI LQDALGGNSR AALLCCCSPS ASNAPESLST VRFGTRTKLI KTTPKSISPE VDSIKKPIPD SHGQNDLRDR
361 ILNKLRLSLK EEDVDLLEEL FVQEGIIFDP NYSVADIDSA CQDAASQEVS LLTQAVEELK ETVEELTDEN ERLRGELELA QEAAAAAAAA
451 RADGALLGFV PAVAISSLLR PFGFVPD

B

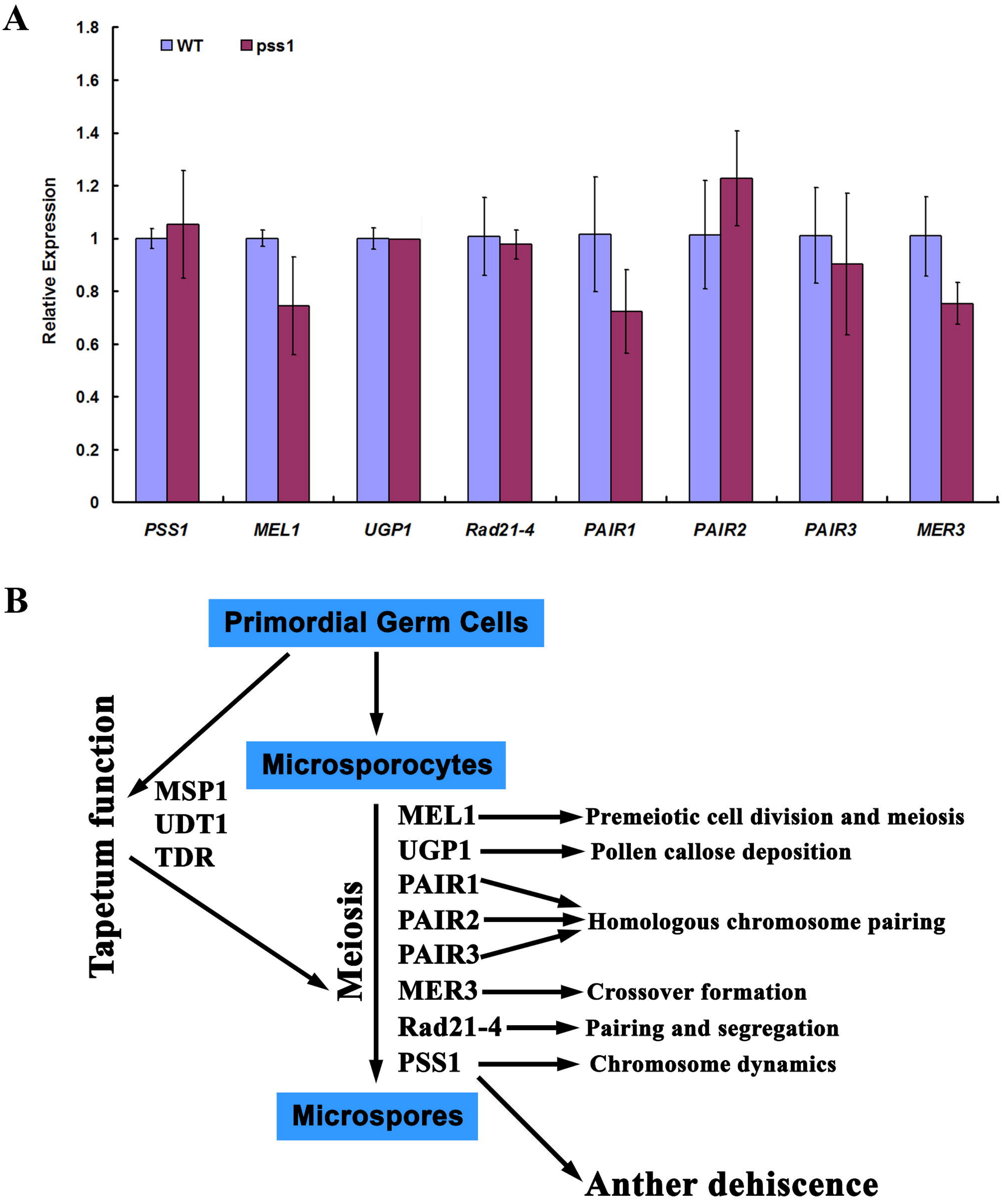


Supplemental figure 2. Sequence Analysis of the Predicted PSS1 and Related Proteins.
(A) Deduced amino acid sequences of PSS1.
The motor domain is underlined and the highlighted red “R” indicates the mutated amino acid (R289) causing the mutant phenotype.
(B) Amino acid sequences alignment of the motor domain of kinesin-1 family proteins.
The boxed “R” indicates the mutated amino acids in *pss1* mutant, which is conserved in all kinesin-1 family members.



Supplemental figure 3. Neighbor-joining Tree of the Kinesin Superfamily Proteins

Bootstrap neighbor-joining phylogenetic tree was constructed using MEGA 4.0 and with 1000 bootstrap replicates. Kinesins from the following organisms were used: At, *Arabidopsis thaliana*; An, *Aspergillus nidulans*; Bm, *Bombyx mori*; Ca, *Candida albicans*; Ce, *Caenorhabditis elegans*; Cr, *Chlamydomonas rheinhardtii*; Cg, *Cricetulus griseus*; Cp, *Cyanophora paradoxa*; Cf, *Cylindrotheca fusiformis*; Zf, *Danio rerio*; Dd, *Dictyostelium discoideum*; Dm, *Drosophila melanogaster*; Dy, *Drosophila yakuba*; Gg, *Gallus gallus*; Hs, *Homo sapiens*; Lc, *Leishmania chagasi*; Lm, *Leishmania major*; Lp, *Loligo pealii*; Ld, *Lymantria dispar*; Md, *Monodelphis domestica*; Ms, *Morone saxatilis*; Mm, *Mus musculus*; Nh, *Nectria haematococca*; Nc, *Neurospora crassa*; Nt, *Nicotiana tabacum*; Os, *Oryza sativa*; Pl, *Paracentrotus lividus*; Pf, *Plasmodium falciparum*; Rn, *Rattus norvegicus*; Sc, *Saccharomyces cerevisiae*; Spo, *Schizosaccharomyces pombe*; Po, *Solanum tuberosum*; Sp, *Strongylocentrotus purpuratus*; Sy, *Syncephalastrum racemosum*; Tt, *Tetrahymena thermophila*; Tr, *Trypanosoma brucei*; Um, *Ustilago maydis*; Vc, *Volvox carteri* and Xl, *Xenopus laevisare*. The proteins are named by the abbreviations of the specie name with the gene name or the locus tag in the Arabidopsis Information Resource (TAIR). The accession numbers of the genes are as follows: BAB32972 (Os DSB1), M32075 (An BimC), X64603 (An KLPA), AF193768 (At FCA0.24), Z97336 (At FCA1.2), D11371 (At KATA), D21137 (At KATB), D21138 (At KATC), AF080249 (At KATD), L40358 (At KCBP), AL109619 (At T19F6), AC002535 (At T30B22), AC002534 (At T32N15), AC005223 (At T5A14.3), At3g43210 (At TES), At3g63480, D21206 (Bm KRP), AL033502 (Ca KRP), U61947 (Ce C06G3.2), Z77659 (Ce F23B12), U41536 (Ce F56E3), Z92807 (Ce K11D9.c), L19120 (Ce KHC), Z68161 (Ce KLP11), U80450 (Ce KLP15), Z81048 (Ce KLP16), Z66521 (Ce KLP17), Z36753 (Ce KLP3), U23515 (Ce KLP6), D38632 (Ce Osm3), Z92811 (Ce T01G1), M58582 (Ce Unc104), AL021481 (Ce Y43F4B), NP_497178 (Ce Y50D7), U61955 (Ce Zen4), U51680 (Cf DSK1), X83575 (Cg CHO1), X83576 (Cg CHO2), U11790 (Cg MCAK), L33697 (Cr FLA10), X78589 (Cr Klp1), U41289 (Dd K7), AF220354 (Dm Cana), AF220353 (Dm Cmeta), M24441 (Dm KHC), AE003485 (Dm Klp10A), AF247500 (Dm Klp31D), AF247500 (Dm Klp31E), X99617 (Dm Klp38B), AF132186 (Dm Klp3A), AE003805 (Dm Klp53D), AE003802 (Dm Klp54D), AE003802 (Dm Klp54E), AE003459 (Dm Klp59C), U01842 (Dm Klp61F), AE003566 (Dm Klp64D), U89264 (Dm Klp67A), U15974 (Dm klp68D), U81788 (Dm Klp73), AE003761 (Dm Klp98A), X52814 (Dm Ncd), M36195 (Dm Nod), AJ224882 (Dm PAV), U18309 (Gg Chrkin), X90840 (Hs ATSV), Z15005 (Hs CENPE), AL021366 (Hs CHO2), D26361 (Hs CMKRP), X65873 (Hs KHC), AB017430 (Hs Kid), AB014606 (Hs KIF1C), AF041853 (Hs KIF3), AB002357 (Hs KIF3B), AF035621 (Hs KIF3C), AF071592 (Hs KIF4), BC001211 (Hs KIFC3), Y08319 (Hs Kin2), U37426 (Hs KSP), U63743 (Hs MCAK), X67155 (Hs MKLP1), U06698 (Hs nKHC), AB011103 (Hs xKHC), L07879 (Lc KIN), AC003011 (Lm Kin), AL157416 (Lm L6066), AL049768 (Lm L6586), J05258 (Lp KHC), D29951 (Mm KIF1A), D17577 (Mm KIF1B), D12644 (Mm KIF2), AB054029 (Mm KIF2C), D12645 (Mm KIF3A), D26077 (Mm KIF3B), AF013116 (Mm KIF3C), D12646 (Mm KIF4), AF067179 (Mm KIF5A), U86090 (Mm KIF5B), AF067180 (Mm KIF5C), D49544 (Mm KIFC1), D49545 (Mm KIFC2), Y09632 (Mm Klp174), U64819 (Ms FKIF2), L47106 (Nc KHC), U86521 (Nh Kin1), AF102992 (Nh KRP1), U52078 (Nt KCBP), D83711 (Nt KRP125), AL034559 (Pf MAL3P7), L46702 (Po KCBP), AK287457 (PSS1), AF083331 (Rn KIF1B), AJ000696 (Rn KIF1D), AJ223599 (Rn KIF3C), U44979 (Rn KRP2), M90522 (Sc Cin8), M31719 (Sc KAR3), Z11962 (Sc Kip1), Z11963 (Sc Kip2), Z 72739 (Sc KIP3), M69021 (Sc Smy1), X56844 (Sp KHC), AF191095 (Sp KinC), L16993 (Sp KRP85), U00996 (Sp KRP95), AL034433 (Spo BC1604), Z97211 (Spo BC2f12), AL023587 (Spo BC649), X57513 (Spo Cut7), SPAC664.10 (Spo KLP2), AF154055 (Spo Klp3), U63916 (Spo PKL1), AJ225894 (Sy Kin1), AJ244020 (Tt Kin1), AJ244021 (Tt Kin2), U92844 (Um Kin1), U92845 (Um Kin2), X82012 (Xl CTK1), U82809 (Xl CTK2), X54002 (Xl Eg5), X71864 (Xl Eg52), U36485 (Xl KCM1), U36486 (Xl KCM2), X82012 (Xl klp1), X94082 (Xl klp2), AJ009839 (Xl Klp3), AF181248 (Zf Knsl2) and AF139990 (Zf MKLP1). The scale bar represents 0.05 amino acid substitutions per residue.

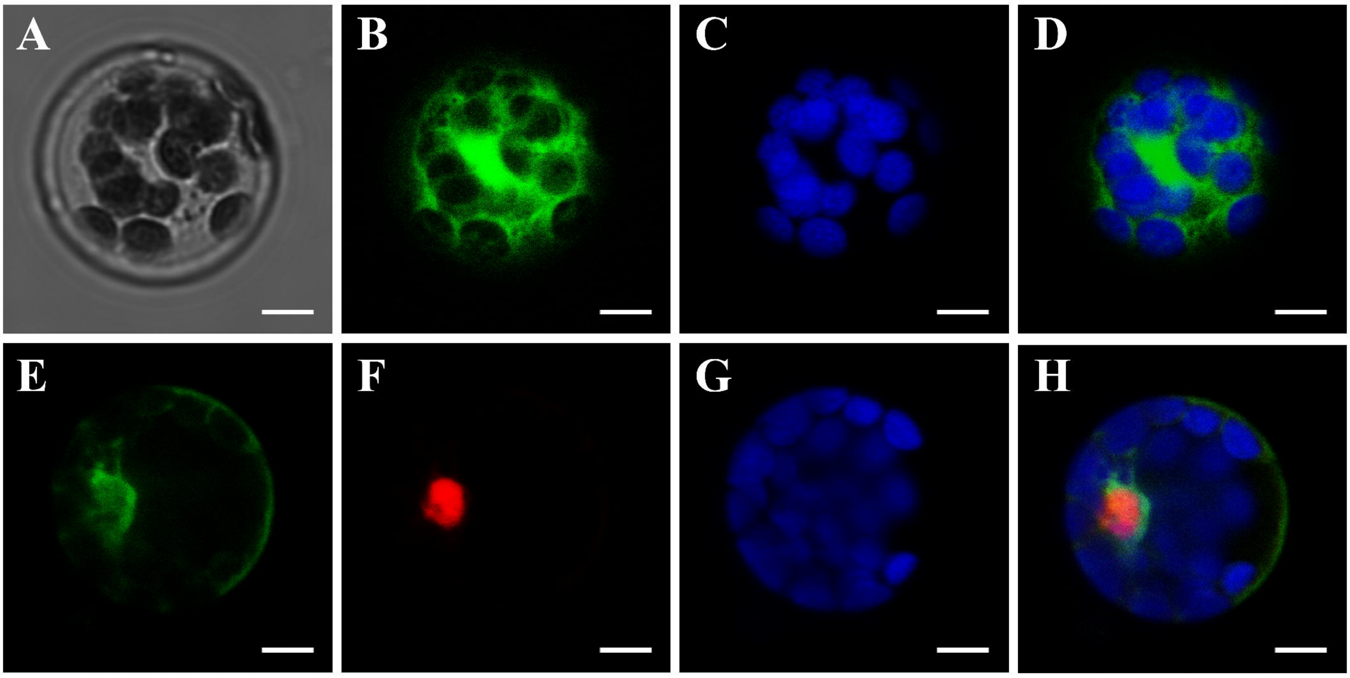


Supplemental figure 4. Transcriptional Analysis of Male Meiotic Genes in *pss1* Mutant and a Putative Genetic Pathway for Microsporegenesis.

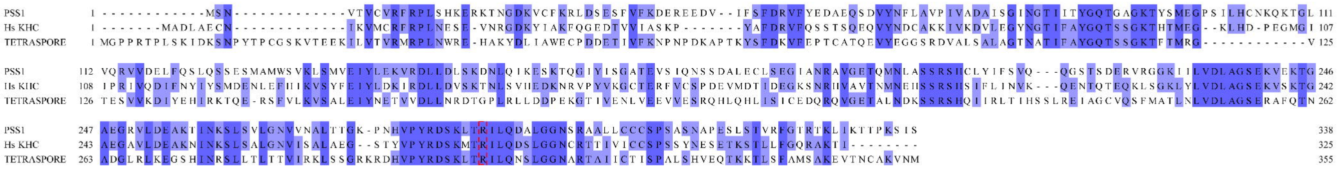
(A) Transcription level of known genes regulating male meiosis process in P5 stage panicles was compared between wild type and the *pss1* mutant, including *MEL1, UGP1, Rad21-4, PAIR1, PAIR2, PAIR3* and *MER3*. Error bars indicate SD of three independent samples.

(B) A Summarized Putative Genetic Pathway for Microsporegenesis.

MSP1, UDT1 and TDR affect both tapetum function and meiosis. MEL1 affects premeiotic cell division and meiosis; UGP1 affects the pollen callose deposition during PMC meiosis; PAIR1, PAIR2 and PAIR3 regulate homologous chromosome pairing; MER3 is required for normal meiotic crossover formation; Rad21-4 is essential for efficient meiosis including homologous chromosome pairing and segregation. PSS1 is essential for male meiotic chromosomal sorting and anther dehiscence.



Supplemental figure 5. Sub-cellular Localization of PSS1 Protein in Arabidopsis protoplasts.
(A) to **(D)** Expressing of the GFP empty construct in Arabidopsis protoplast cells,
(A) is the bright field; **(B)** shows GFP fluorescent signal constitutively both in the nucleus and cytoplasm;
(C) is the chloroplast ; and **(D)** is the merged image of **(B)** and **(C)**.
(E) to **(H)** Expressing of the PSS1-GFP construct in Arabidopsis protoplast cells.
(E) PSS1-GFP is predominantly detected in the cytoplasm, especially in the nucleus surrounding area.
(F) Nuclear localization of RPBF-mCherry (a nuclear marker).
(G) is the chloroplast ; and **(H)** is the merged image of **(E)** , **(F)** and **(G)**.
Bars=5 μm.



```
PSS1        1   -------------MSN-------------VTVCVRFRPLSHKERKTNGDKVCFKRLDSESFVFKDEREEDV--IFSFDRVFYEDAEQSDVYNFLAVPIVADAISGINGTIITYGQTGAGKTYSMEGPSILHCNKQKTGL 111
Hs KHC     1   -------MADLAECN-------------IKVMCRFRPLNESE-VNRGDKYIAKFQGEDTVVIASKP-------YAFDRVFQSSTSQEQVYNDCAKKIVKDVLEGYNGTIFAYGQTSSGKTHTMEG--KLHD-PEGMGI 107
TETRASPORE  1   MGPPRTPLSKIDKSNPYTPCGSKVTEEKILVTVRMRPLNWRE-HAKYDLIAWECPDDETIVFKNPNPDKAPTKYSFDKVFEPTCATQEVYEGGSRDVALSALAGTNATIFAYGQTSSGKTFTMRG------------V 125

PSS1      112   VQRVVDELFQSLQSSESMAMWSVKLSMVEIYLEKVRDLLDLSKDNLQIKESKTQGIYISGATEVSIQNSSDALECLSEGIANRAVGETQMNLASSRSHCLYIFSVQ---QGSTSDERVRGGKIILVDLAGSEKVEKTG 246
Hs KHC   108   IPRIVQDIFNYIYSMDENLEFHIKVSYFEIYLDKIRDLLDVSKTNLSVHEDKNRVPYVKGCTERFVCSPDEVMDTIDEGKSNRHVAVTNMNEHSSRSHSIFLINVK---QENTQTEQKLSGKLYLVDLAGSEKVSKTG 242
TETRASPORE 126 TESVVKDIYEHIRKTQE-RSFVLKVSALEIYNETVVDLLNRDTGPLRLLDDPEKGTIVENLVEEVVESRQHLQHLISICEDQRQVGETALNDKSSRSHQIIRLTIHSSLREIAGCVQSFMATLNLVDLAGSERAFQTN 262

PSS1      247   AEGRVLDEAKTINKSLSVLGNVVNALTTGK-PNHVPYRDSKLTRILQDALGGNSRAALLCCCSPSASNAPESLSTVRFGTRTKLIKTTPKSIS 338
Hs KHC    243   AEGAVLDEAKNINKSLSALGNVISALAEG--STYVPYRDSKMTRILQDSLGGNCRTTIVICCSPSSYNESETKSTLLFGQRAKTI-------- 325
TETRASPORE 263 ADGLRLKEGSHINRSLLTLTTVIRKLSSGRKRDHVPYRDSKLTRILQNSLGGNARTAIICTISPALSHVEQTKKTLSFAMSAKEVTNCAKVNM 355
```

Supplemental figure 6. Amino Acid Sequences Alignment of the Kinesin Motor Domain of rice PSS1, human KHC, and Arabidopsis TETRASPORE (TES).
The boxed “R” indicates the R289 in rice PSS1, R284 in human KHC and R306 in Arabidopsis TES.

Supplemental figure 7. A Ribbon Diagram of the Human KHC Motor.
The marked “α5” indicates a highly conserved helix in human kinesin heavy chain, which is part of the core microtubule-binding interface. R284 (Yellow residue in the ribbon diagram) is located in the α5 region of the Human KHC, and rice PSS1 R289 is the homologous residue of Human KHC R284, which is one of the key residues involved in microtubule interaction.

Supplemental Tables

Supplemental Table 1. Statistics of abnormal chromosomal behavior in RNAi line2.

Stages	**Cells observed**	**Normal cells/Frequency**	**Abnormal cells**			
			Chromosome behavior	**Cells/Frequency**	**Chromosome behavior**	**Cells/Frequency**
Metaphase I	30	12/40%	**Univalents**	18/60%	**-**	**-**
Anaphase I	28	10/36%	**Chromosome bridge**	10/36%	**Delayed univalents**	8/28%
Anaphase II	32	11/34%	**Micronucleus**	9/28%	**Delayed univalents**	12/38%
Tetrad	50	22/44%	**Micronucleus**	28/56%	**-**	-

Supplemental table 2. List of the primers used for vectors construction, transgenic plants identification and gene expression analysis.

Primer Name	**Sequence**
W1f	5'-ggggacaagtttgtacaaaaaagcaggctagtgattgattgattcttttctgcgagtcc-3'
W1r	5'-ggggaccactttgtacaagaaagctgggtacataagcaaagcaaacaaacgattagggt-3'
W2r	5'- tcaccaaccagtgcatcttgaagaatt-3'
W2f	5'-aacatcaacagaaggtcaccctcacg-3'
W3f	5'-tctctcgagctaacaactggtaaaccgaatc-3';
W3r	5'-ttggtaccgtcttggcaggctgagtc-3';
W4f	5'-tctggatccgtcttggcaggctgagtc-3'
W4r	5'-cctctagactaacaactggtaaaccgaatc-3'
W5f	5'-gattgaacaagatggattgcacgcaggtt-3'
W5r	5'-cagaagaactcgtcaagaaggcgatagaa-3'
W6f	5’-tctcccgaagtggatagca-3’
W6r	5’-ttcagtctggaacgaaccc-3’
W7f	5′-agaccaccaagtactactgcac-3′
W7r	5′-ccaccgatcttgtacacgtcc-3′
ubif	5’-accctggctgactacaacatc-3’
ubir	5’-agttgacagccctagggtg-3’
pss1f	5’-tactccgtggcagacatc-3’
pss1r	5’-caacctctcgttctcatcg-3’
mel1f	5’-ttctgtggtgacctgattc-3’
mel1r	5’-ctccctttccctctctgg-3’
ugp1f	5’-ggctgctcacggaaaccttc-3’
ugp1r	5’-gccctggctgccgaatgc-3’
rad21-4f	5’-aatcttattcggtcaatcaa-3’
rad21-4r	5’-ttcagtacattccttcca-3’
pair1f	5’-caccagtcagcataatcatc-3’
pair1r	5’-ctctcctcctcctcttcc-3’
pair2f	5’-acaagcgattcaggaagg-3’
pair2r	5’-cggaactaggaataggatgg-3’
pair3f	5’-gataatacgccagcaccttg-3’
pair3r	5’-cctcctccttgaacttctcg-3’
mer3f	5’-ctctcaaggttcctctcatcag-3’
mer3r	5’-ttcattgtcaccagtcatctcc-3’
promoter-f	5’-tagcccaccacctgctgcttcat-3'
promoter-r	5’-cctgccagccaaccaaatacg-3'
motor-f	5’-atgtccaacgtgactgtatgt-3'
motor-r	5’-tcaatcagggatgggcttctt-3'