Supplementary Information for

Molecular Regulation of ZmMs7 Required for Maize Male Fertility and Development of a Dominant Male-sterility System in Multiple Species

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Plant growth conditions. All maize and rice plants were grown in the experimental stations of USTB in Beijing from May to October and in Hainan Province from November to next April. The T₀ transgenic plants of maize and rice were greenhouse-grown from seeding to seed. Arabidopsis plants were grown on soil with a 16-h-light/8-h-dark photoperiod at 22°C in white light of approximately 150 µE x m⁻² x s⁻¹. Transgenic Arabidopsis seeds were germinated on 1/2 MS media with 10 ug/mL BASTA, then BASTA-resistant seedlings were transplanted to soil.

Phenotype observation and fluorescent seed detection. Morphological images of whole plants, tassels, ears and panicles were captured by a Canon EOS 700D digital camera (Canon, Japan). Anthers were photographed with a SZX2-ILLB stereomicroscope (Olympus, Japan). For pollen viability analysis, the pollen grains stained with 1% I₂-KI solution or cultured at 28°C on solid pollen GM [0.01% (w/v) H₃BO₃, 1 mM CaCl₂, 1 mM Ca(NO₃)₂, 1 mM MgSO₄, 18% (w/v) Suc, and 0.5% (w/v) agar, pH 7.0] were imaged by a BX-53F microscope (Olympus, Japan).

Florescent seed detection was performed as described previously (1). The wild-type and p5126-ZmMs7M ears were imaged under bright light, green excitation light with red fluorescence filter I (GREEN.L, China) and red fluorescence filter II (NIGHTSEA, USA), respectively.

Plant genetic transformation. For functional confirmation of ZmMs7, a CRISPR/Cas9 plasmid (pZmMs7-Cas9) was constructed by inserting two knockout targets with 19-bp fragments in the ZmMs7 coding region (5'-CTTCCTCGCCGCTGTCGAC-3' and 5'-TGCTGCACGCGTTGCTGTA-3') into pBUE411 (2). To generate pZmMs7:ZmMS7-SRDX, the SDRX sequence was synthesized and annealed by primers Ms7-SDRXs and Ms7-SDRXantis, the double stranded SDRX oligo was then subcloned into BamHI digested pZmMs7pro::ZmMs7 plasmid (3). To construct the p5126-ZmMs7 plasmid, the p5126 promoter was amplified using primer pairs 5126proF/R from maize B73 genomic DNA, the ZmMs7 CDS fragment was amplified with Ms7F/Ms7R using cDNA from B73 anther, and then the two fragments were fused together and inserted into pCAMBIA3301 by in-fusion method to generate p5126-ZmMs7. A 3.7-kb fragment including p5126 promoter, ZmMs7 CDS and NOS terminator was amplified using primers 5126proF and Ms7Nos-R from p5126-ZmMs7 plasmid and cloned into pCLC vector (3), forming the plasmid p5126-ZmMs7M. To construct p5126-ZmMs7-myc, a 3x myc tag was fused into the C-terminal of ZmMs7 of p5126-ZmMs7 plasmid. Agrobacterium-mediated genetic transformation of maize, rice and Arabidopsis were performed according to the previous procedures, respectively (4-6). The transgenic lines of maize and rice were examined by PCR amplification using Bar gene specific primers. To determine the knockout transformants, target sites of ZmMs7 were amplified and sequenced.
Cytological analysis and microscopy. Fresh anther samples for transverse section, SEM and TEM were fixed at different stages as previously described (1). Transverse section and SEM analyses were done as described previously (7) with minor modifications.

For transverse section assays, the fixed samples were dehydrated in a graded ethanol series (50% 70%, 80%, 90% and 95%) for 30 min each, maintained in 95% ethanol + 0.1% eosin overnight, and then dehydrated twice in 100% ethanol. The samples were dipped into 1:3, 1:1 and 3:1 (v/v) resin/ethanol for 2 h each, and then in 100% resin overnight. The samples were embedded in Spurr resin (SPI-Chem™, America), polymerized at 70°C for sectioning. The anther samples were cut into 2 μm sections using a Leica RM2265 rotary microtome, stained with 0.05% Toluidine Blue O and photographed using the Olympus BX-53 microscope.

For SEM analysis, the fixed materials were dehydrated with an alcohol gradient (50%, 70%, 80%, 90%, and 100%), critical-point dried and coated with gold: palladium (3:2) using an ion sputter coater (JFC-1100; JEOL). SEM images were detected with a HITACHI S-3400N scanning electron microscope.

For TEM analysis, the fixed samples were washed with PBS (0.1 M phosphate buffer) and post-fixed in 1% osmium tetroxide for 2 h, and then rinsed twice in PBS. Following a series of ethanol gradient dehydration, samples were embedded in epoxy resin. Ultra-thin sections (50 to 70 nm) were cut using a Leica UC6 I ultramicrotome and double stained with uranyl acetate and lead citrate. The observation and recording of images were performed with an H-7500 (HITACHI, Japan) transmission electron microscope.

Male-sterility stability analysis of ms7-6007 mutation and p5126-ZmMs7M dominant sterility line under broad genetic backgrounds. For male-sterility stability evaluation of ms7-6007, we used 403 maize inbred lines with broad genetic backgrounds as female parents to cross with heterozygous ZmMs7/ms7-6007 plants. PCR analysis with the primer pairs ZmMs7-TF/TR was used for selecting heterozygous F₁ progenies of the 403 combinations, and F₂ populations were generated by self-pollinating of the selected F₁ plants. The fertility segregation ratios of 403 F₂ populations were investigated, and anthers of three representative sterile individuals in each F₂ population were collected and stained with 1% I₂-KI solution.

To examine the male-sterility stability of p5126-ZmMs7M transgenic lines, 392 maize inbred lines with broad genetic backgrounds were crossed with heterozygous transgenic plants (p5126-ZmMs7M/−). Red fluorescent seeds and normal seeds of 392 F₁ hybrid population were sorted manually under green excitation light, and then planted in USTB experiment station. Fertility observation and evaluation of F₁ plants were conducted as described above.
Evaluation of the effects of *ms7-6007* mutation on maize heterosis and field production. To examine whether *ms7-6007* mutation affects maize heterosis and production, we used *ms7-6007* mutant and its WT sibling line (*ZmMs7/ZmMs7*) as female parents to cross with 31 elite maize inbred lines, respectively. The resultant F₁ hybrids and their corresponding parental lines were grown in three locations, i.e. Pinggu, Beijing (40°10′N, 116°58′E), Zhucheng, Shandong Province (35°58′N, 119°23′E) and Puyang, Henan Province (35°38′N, 115°04′E), in triplicate in the summer of 2019. Experimental units were all five rows with 60 cm row spacing and 2.2 m row length with 24.5 cm plant spacing. Fifty plants were reserved in every plot, and 17 agronomic traits were investigated. Plot yield was calculated and adjusted to a standard moisture of 140 g/kg.

**Real Time Quantitative PCR (RT-qPCR) analysis.** Total RNA was isolated from various maize tissues including shoots, stems, leaves, silks, pollens, cobs and anthers at different stages, using Trizol Reagent (Invitrogen, USA). The cDNA was synthesized using 5X All-In-One RT MasterMix (abm, Canada) as described by supplier. RT-qPCR assays were performed using TB Green™ Premix Ex Taq™ (TaKara, Japan) on the QuantStudio 5 Real-Time PCR System (ABI, USA), with the primers listed in SI Appendix, Table S8. *ZmActin1* was used as the reference gene. Three biological repeats with three technical repeats each were included. The data were analyzed by the 2-ΔΔCt method, and quantitative results were given as means ± SD.

**Transient dual-luciferase assay.** For transcriptional activity analysis of ZmMs7, the coding region of *ZmMs7* was cloned into *Bam HI/Sal I*-digested pRTBD vector by homologous recombination to generate 35S:BD-ZmMs7 used as effector plasmid. The 5x GAL4-TATA:LUC and proAtUbiquitin3: REN were used as reporter and internal control plasmids, respectively. For transactivation assays of ZmMT2C and ZmAP37 promoters, the promoter regions of ZmMT2C (1400 bp before ATG) and ZmAP37 (1591 bp before ATG) were PCR-amplified and cloned into *Kpn I/Bam HI*-digested pEASY-LUC vector by homologous recombination to generate pZmMT2C:LUC and pZmAP37:LUC plasmids used as reporters. For construction of the effector plasmids, the pRTBD vector was digested with *Sac I* and *Xba I* to remove the GAL4 DBD fragment. The *ZmMs7* CDS was cloned into the *Sac I*/*Xba I*-digested pRTBD vector to generate 35S-Ω:*ZmMs7 construct. Next, the 35S-Ω-NF-YB2 CDS-nos fragment was cloned into *Xba I/BamHI* I-digested 35S-Ω:*ZmMs7 vector by homologous recombination to generate 35S-Ω:*ZmMs7-35S-Ω-NF-YB2 construct. Then, the 35S-Ω-NF-YA6 CDS-nos fragment was ligated into *Sph I*-digested 35S-Ω:*ZmMs7-35S-Ω-NF-YB2 vector by homologous recombination to generate 35S-Ω:*ZmMs7-35S-Ω-NF-YB2-35S-Ω-NF-YA6 construct. Finally, the 35S-Ω-NF-YC9 CDS-nos, 35S-Ω-NF-YC12 CDS-nos and 35S-Ω-NF-YC15 CDS-nos fragments were ligated into *Apa I*-digested 35S-Ω:*ZmMs7-35S-Ω-NF-YB2-35S-Ω-NF-YA6 vector respectively by
homologous recombination to generate 35S-Ω:ZmMs7-35S-Ω-NF-YB2-35S-Ω-NF-YA6-35S-Ω-NF-YC9, 35S-Ω:ZmMs7-35S-Ω-NF-YB2-35S-Ω-NF-YA6-35S-Ω-NF-YC12 and 35S-Ω:ZmMs7-35S-Ω-NF-YB2-35S-Ω-NF-YA6-35S-Ω-NF-YC15 constructs. The plasmids were cotransformed into maize leaf protoplasts isolated from 5-d-old etiolated seedlings. The relative LUC activity (LUC/REN) was determined using a Dual-Luciferase Reporter Assay System (Promega, E1960) according to the manufacturer’s instructions.

**Subcellular localization analysis of ZmMs7.** The full-length coding sequence of ZmMs7 was fused with the C-terminus of sGFP and subcloned into pJG053 via in-fusion method and the resulting 35S:GFP-ZmMs7 construct was transformed into maize leaf protoplasts. After 12-16 h of incubation in the dark at 28 °C, GFP signal was excited by the 488 nm laser line and detected at ~510 nm while nuclei were stained by DAPI and fluorescence was excited by the 405 nm laser line and detected at ~460 nm under a confocal microscope (Zeiss LSM780).

**RNA-seq analysis.** Anthers were collected from the WT and ms7-6007 at three stages (stages 8, 9 and 10), and the WT and p5126-ZmMs7M line at six stages (stages 6, 7, 8a, 8b, 9 and 10). Two biological replicates were used for each stage, excepting ms7-6007 anther at stage 8 (one sample) and WT samples in ZmMs7 over-expression experiment at stage 6 (three samples). RNA-seq libraries were constructed using the rRNA-depleted RNA. Paired-end sequencing (2×150 bp) was performed in an Illumina Hiseq 4000 platform. Clean reads were mapped to the maize reference genome (AGPv4) using TopHat2.0 with default parameters (8). Gene expression levels were calculated and normalized into RPKM (reads per kb per million mapped reads) values based on annotated maize gene models (Ensembl_release-37) by Rsubread and edgeR (9, 10). Differentially expressed genes (DEGs) between WT and ms7-6007 anthers were identified with expression level fold changes > 4 and false discovery rate values < 0.01. DEGs between adjacent stages in WT as well as DEGs between WT and p5126-ZmMs7M-01 line at each stage were identified using thresholds of expression level fold changes > 4 and false discovery rate values < 0.01. Gene co-expression analysis was performed using K-means (K = 8) clustering. GO enrichment analyses were implemented by agriGO (v2.0) online service (11).

**Analysis of anther wax, cutin, and internal lipid.** Wax, internal fatty acids and cutin of anthers were analyzed as described previously (12) with minor modification. Measurement of anther surface areas was also as previously described (13). To extract wax, 50-60 mg of freeze-dried anthers were submersed in 3 mL of chloroform for 1 min. Extraction was repeated once, and the resulting chloroform extracts were combined, 10 µg of nonadecanoic acid was added as an internal standard. The solvents were evaporated under nitrogen gas. Compounds containing free hydroxyl and carboxyl groups were converted to their trimethylsilyl ethers with 100 mL of N-
methyl-N- (trimethylsilyl) trifluoroacetamide for 1 h at 50°C. These samples were analyzed by GC-MS (Agilent gas chromatograph coupled to an Agilent 5975C quadrupole mass selective detector).

To extract internal fatty acids, anthers that had been used in the wax extraction were re-extracted in freshly added 3 mL of chloroform/methanol (1:1 [v/v]) for 36 h. This extraction step was repeated once, and combined lipid extracts were evaporated under nitrogen gas. The residue was transesterified in 1mL of 1 N methanolic HCl (containing 50 ug of Nonadecanoic acid as an internal standard) for 2 h at 80 °C, added 1.5 mL of 0.9% (w/v) NaCl and 1.5 mL of hexane, and then evaporated under nitrogen gas. The remaining sample was dissolved in 100 uL of hexane and then analyzed by GC-MS as described above.

To extract cutin, the remaining delipidated anthers were freeze-dried, and depolymerized using transesterification in 1 mL of 1 N methanolic HCl for 2 h at 80 °C with 50 ug of Nonadecanoic acid as an internal standard. After the addition of 2 mL of 10% NaCl solution and 1mL of hexane, the hydrophobic monomers were subsequently extracted three times with1 mL of hexane. The combined organic phases were evaporated under nitrogen gas, and then dried under vacuum for 4 h. The remaining samples were derivatized and analyzed by GC-MS as described above.

**Y2H assays.** The maize anther cDNA library was constructed into the pGADT7 vector using CloneMiner cDNA Library Construction Kit (Invitrogen) and transformed into yeast strain Y187. The ORF of ZmMs7N (1-358 aa) was amplified and cloned into pGBK7T7 vector. The resulted pGBK7T7-ZmMs7N was transfected into the yeast strain Y2HGold to generate a bait clone. The constructed yeast library was then screened by the bait ZmMs7N via yeast mating method according to the instruction of the Matchmaker Goldyeast two-hybrid system (Clontech). Plasmids of positive clones were extracted and sequenced. To confirm the specific protein-protein interactions, the ORFs of ZmMs7N (1-358 aa), ZmNF-YA6, ZmNF-YB2, ZmNF-YB10, ZmNF-YC9, ZmNF-YC12 and ZmNF-YC15 were amplified and cloned to both the bait vector pGBK7T7 and the prey vector pGADT7, respectively. A pGBK7T7/bait plasmid and a pGADT7/prey plasmid were co-transfected into the yeast strain Y2HGold and verified on selective media as described in Matchmaker Goldyeast two-hybrid system (Clontech).

**BiFC assays.** For investigating protein-protein interaction among ZmMs7, NF-YA6 and NF-YC9/12/15, we performed BiFC assays as previously described (14) with minor modifications. Full-length ZmNF-YA6, ZmNF-YB2, ZmNF-YC9/12/15 coding sequences were fused to the N-terminal half (nYFP: 1-155 aa), respectively. ORFs of ZmMs7, ZmNF-YA6 and ZmNF-YB2 were fused to the C-terminal half (cYFP: 156-239 aa), respectively. BiFC constructs were co-
transformed into maize protoplasts as described above. Yellow fluorescent protein (YFP) was imaged using a confocal laser-scanning microscope (Zeiss LSM780).

**Co-IP assays.** For coimmunoprecipitation with anti-c-Myc affinity gel, we created a 35S-Ω-Myc vector by ligating 4x Myc sequence into the Sac I/Xba I-digested pRTBD vector. NF-YA6-coding sequence was cloned into Sac I/Sma I-digested 35S-Ω-Myc vector by homologous recombination to generate 35S-Ω:NF-YA6-Myc construct. NF-YC12-coding sequence was cloned into Sac I/Xba I-digested 35S-Ω-Myc vector by homologous recombination to generate 35S-Ω:NF-YC12-Myc construct. The plasmid DNAs in various combinations were cotransformed into maize protoplasts. The transformed protoplasts were incubated at 28°C for 12-16 h in the dark. Protein isolation and immunoprecipitation with anti-c-Myc affinity gel (E6654, Sigma-Aldrich) were performed as described previously (15). The eluted immunoprecipitates were detected by Western blot analysis with anti-c-Myc (9E10, sc-40, Santa Cruz, 1:1000) and anti-FLAG (FLA-1, M185-3, MBL, 1:10,000) antibodies. Secondary goat anti-mouse-IgG-HRP antibody was used at 1:10,000 dilutions in PBS. The signals were detected using a SuperSignal West Pico kit (34080, Thermo Scientific).

**EMSA.** The recombinant MBP-ZmNF-YA6 protein purified from *Escherichia coli* was used for EMSA assay. Briefly, the ORFs of ZmNF-YA6 was fused with MBP and inserted in-frame into the vector pMCSG7. The construct was transformed into *Escherichia coli* BL21 (DE3) cells and the expression of recombinant proteins was induced overnight at 16 °C by the addition of 0.2 mM isopropylthio-β-galactoside. Cells were harvested and purified using Ni-charged resin (Bio-red) according to the manufacture instructions. The purified proteins were desalted using PD-10 Desalting Columns (GE Healthcare). The biotin-labeled ZmMT2C and ZmAP37 promoter probes were generated by annealing of primer pairs MT2CSbio/ MT2CantiS and AP37Sbio/AP37antiS, respectively. A total of 1 µg of desalted recombinant protein was incubated with 0.02 pmol of the biotin-labeled promoter probes in 1× EMSA/Gel-Shift Binding Buffer (Beyotime, GS005) in a total volume of 10 µl. The reaction products were analyzed on 6% (w/v) polyacrylamide gels under non-denaturing conditions. To show the specificity of the DNA-protein interaction, a 100- or 1000-fold excess of the unlabeled DNA probe or 1000-fold excess core motif-mutated probes were added and the purified MBP protein was used as a negative control.

**TUNEL assay.** Wild type and ms7-6007 mutant anthers were collected at different developmental stages. DNA fragmentation was detected by TUNEL assay using a TUNEL kit (DeadEnd™ Fluorometric TUNEL System, Promega) according to the manufacturer’s instructions. Samples were analyzed under a fluorescence confocal scanner microscope (TCS-SP8, Leica).
**Chromatin immunoprecipitation (ChIP).** ChIP assay was performed as described previously with minor modifications (16). Briefly, a total of 1-1.5g collected tassels were chopped with a razor blade and cross-linked in the buffer containing 1% formaldehyde for 30 min. The nuclei were isolated and sheared by an ultrasonicator (Bioruptor Plus UCD-300) to an average a size of 200-1000bp. After pre-clearing with protein G magnetic beads (Invitrogen) for 1h, 1/100 volume of the supernatant were collected as input, the left was equal divided into two 2 ml tubes. One tube was incubated with antibody against Myc (9E10, sc-40, Santa Cruz) at 4 °C overnight with 1:100 dilution while the other was incubated without antibody. Forty microlitres of equilibrated magnetic beads protein G were added to precipitate the antibody–protein/DNA complexes. The DNA fragments were eluted after reverse crosslinking and purified following the manufacturer’s instructions. Real-time PCR were performed as described above. All PCR experiments were performed as the following conditions: 95 °C for 5 min, 40 cycles of 95 °C for 10s, 60 °C for 25s and 72 °C for 20s. % input which represents the percentage of DNA being precipitated by anti-Myc antibody was calculated as 100*2^ (Ct Input- 6.644) - Ct (IP).
**Fig. S1:** Phenotypes of tassels, anthers and pollen grains in four knockout lines of ZmMs7 generated by a CRISPR/Cas9 system. (A) Gene structure of ZmMs7 and four types of knockout lines (Cas9-Ms7-1, 2, 3 and 4). (B) DNA sequence analysis of targeted mutation sites or fragments among WT and the four knock-out lines. (C) Phenotypes of tassels, anthers and pollen grains stained with I$_2$-KI in WT (C1) and the four knock-out lines (C2-C5). Bars = 1 mm for anther and 200 μm for pollen grains, respectively.
Fig. S2: SEM analysis of cuticle and inner surface of anther wall in WT, ms7-6007 mutant and p5126-ZmMs7M-01 dominant male-sterility line during sequential anther developmental stages 6 to 13. Bars = 5 μm.
Fig. S3: Transverse section and SEM analyses of anther and pollen in WT, ms7-6007 mutant and p5126-ZmMs7M-01 dominant male-sterility line during maize anther development (Stages 6 to 13). CMsp, Collapsed microspore; Dy, Dyad; E, Epidermis; En, Endothecium; ML, Middle layer; MMC, Microspore mother cell; MP, Mature pollen; Msp, Microspore; PMC, Pollen mother cell; T, Tapetum; Tds, Tetrads. Bars = 50 μm for transverse section images and 20 μm for SEM images, respectively.
Fig. S4: TEM analysis of anther wall, microspore, Ubisch body, and exine in WT, ms7-6007 mutant and p5126-ZmMs7M-01 dominant male-sterility line from stages 7 to 11 during maize anther development. Bars = 10 μm (anther wall and microspore) and 0.5 μm (Ubisch body and exine), respectively. Ba, Bacula; CMsp, Collapsed microspore; Dy, Dyad; E, Epidermis; En, Endothecium; Ex, Exine; F, Foot layer; LD, Lipid droplet; MC, Meiotic cell; ML, Middle layer; MP, Mature pollen; Msp, Microspore; Ta, Tapetum; Tds, Tetrads; Te, Tectum; Ub, Ubisch body.
Fig. S5: Transcriptomics analysis of WT and ms7-6007 mutant anthers. (A) WT and ms7-6007 mutant anthers at stages 8, 9 and 10 were collected for RNA-seq, respectively. (B) Principal component analysis (PCA) of transcriptomes from WT and ms7-6007 mutant anthers at stages 8, 9 and 10. (C) A total of 2937 DEGs between WT and ms7-6007 mutant anthers were identified. (D) A total of 2345 DEGs between different anther developmental stages in WT were identified. (E) Expression patterns of the 2345 DEGs were clustered into eight groups. (F) A total of 1143 shared DEGs could be considered as the predicted down-stream genes of ZmMs7 based on WT and ms7-6007 anther transcriptomes. (G) Go enrichment analysis result of 1143 shared DEGs. Red frames indicate seven significantly enriched biological processes.
**Fig. S6**: Comparison of anther cutin, wax, and internal lipid contents between WT and *ms7-6007* mutant. (A) Cutin monomer amount per unit surface area between WT and *ms7-6007* mutant anthers. (B) Wax monomer amounts per unit surface area between WT and *ms7-6007* mutant anthers. (C) Internal lipid monomer amount per unit dry weight between WT and *ms7-6007* mutant anthers. Values are means ± SD of five biological replicates, by Student’s t test (* P < 0.05; ** P < 0.01; *** P < 0.001).
Fig. S7: Phylogenetic analysis of maize NF-YA (A), NF-YB (B) and NF-YC (C) proteins and expression analysis of five maize NF-Y type genes (ZmNF-YA6, ZmNF-YB2, and ZmNF-YC9/12/15) (D). (A-C) Neighbor-joining trees were constructed using MEGA6.0 program with a bootstrap setting of 1000. The ZmMs7 interacting proteins were underlined. (D) Expression of ZmNF-YA6, ZmNF-YB2, and ZmNF-YC9/12/15 genes in maize anthers as revealed by RT-qPCR analysis. S6-S10 indicate different stages of anther development. Data are means ± SD, n=3.
**Fig. S8:** Y2H assay detecting interaction of NF-YB10 with ZmMs7, NF-YA6 and NF-YC9/12/15. Yeast cells transformed with the combined plasmids were grown on the double dropout medium DDO (SD/-Trp/-Leu) and quadruple dropout medium QDO (SD/-Trp/-Leu/-His/-Ade) containing 40 mg/L X-gal. The combinations containing empty prey or bait vectors were used as negative controls.
**Fig. S9:** Male-sterility stability assay of *ms7-6007* mutation under wide maize genetic backgrounds. (A) Clustering graph of 403 maize lines with wide genetic backgrounds based on their genetic distances. (B) Distribution diagram of ratios of fertile to sterile individuals among the 403 F\textsubscript{2} populations with F\textsubscript{1} genotype as *ZmMs7/ms7-6007* under different genetic backgrounds. (C) Genotyping of individuals from four representative F\textsubscript{2} populations with ratios of fertile number to sterile number far from theoretical value of 3:1. The sterile individuals were marked with red boxes. (D) Eight representative male-sterility tassel types and I\textsubscript{2}-KI-stained pollen grains with genotype of *ms7-6007/ms7-6007* under 403 different genetic backgrounds.
Fig. S10: Field plot experiment of the two representative hybrid combination pairs with male lines as Liangyu88M (A) and 4132 (B) in Pinggu, Beijing; Zhucheng, Shandong province; and Puyang, Henan province in China, respectively.
**Fig. S11**: Ear morphology for heterosis assay of ms7-6007 mutation. (A-D) Comparison of ears of four representative hybrid combination pairs with male lines as Liangyu88M (A), 4132 (B), Dedan5M (C), and, Wu109 (D) respectively. All the images were collected from the plants cultivated in three locations (Pinggu, Beijing; Zhucheng, Shandong province; and Puyang, Henan province in China, respectively).
**Fig. S12:** Five agronomic trait comparison of 13 representative hybrid combination pairs (\textit{ms7-6007/ms7-6007} \times \text{male line} and \textit{ZmMs7/ZmMs7} \times \text{male line}) and two parents in three locations for heterosis assay of \textit{ms7-6007} mutation. (A-E) Analyses of plot yield \textit{(A)}, 100-seed weight \textit{(B)}, plant height \textit{(C)}, grain number per ear \textit{(D)} and mature period \textit{(E)} of the thirteen representative hybrid combination pairs from the crosses between male sterile \textit{ms7-6007} line or WT and thirteen maize male lines. Values are means ± SD of three biological replicates, by Student’s t test (*\textit{P} < 0.05; **\textit{P} <0.01). All the data were collected from the plants cultivated in three locations (Pinggu, Beijing; Zhucheng, Shandong province; and Puyang, Henan province in China, respectively).
Fig. S13: The p5126-ZmMs7M construct map (A), ear phenotypes of the four dominant male-sterility lines (p5126-ZmMs7M-02 to -05) under bright light and green excitation light with two different red fluorescence filters (B) and schematic diagram for the DMS system and its utilization strategies (C), respectively.
**Fig. S14:** Transcriptomics analysis of WT and the dominant male-sterility line *p5126-ZmMs7M-01* anthers. (A) PCA of transcriptomes from WT and the dominant male-sterility line *p5126-ZmMs7M-01* anthers at stages 6 to 10. (B) The numbers of DEGs between WT and the dominant male-sterility line *p5126-ZmMs7M-01* anthers at stages 6 to 10. (C) A total of 492 shared DEGs were identified as the predicted down-stream genes of ZmMs7 based on *ms7-6007* mutant and *p5126-ZmMs7M-01* anther transcriptomes. (D) Go enrichment analysis of the 492 shared DEGs. The significantly enriched GO terms were finally pointed to the biological process of "pollen exine formation".
Fig. S15: Dominant male-sterility stability assay of p5126-ZmMs7M-01 line under wide maize genetic backgrounds. (A) Clustering graph of 392 maize lines with wide genetic backgrounds based on their genetic distances. (B-D) Four pairs of representative male-fertile and dominant male-sterility tassel types (B), anthers (C) and pollen grains stained with I$_2$-KI (D) with genotypes of p5126-ZmMs7M-01/− (transgenic positive sibling, P) and −/− (transgenic negative sibling, N).
Fig. S16: Expression patterns of the 126 shared DEGs between ms7-6007 mutant and p5126-ZmMs7 line. In maize anther of ms7-6007 mutant, the 126 DEGs show down-regulated expression, but these DEGs show up-regulated expression at stages 7 and/or 8a in maize anther of p5126-ZmMs7 line. (The supplemental data correspond to Figure 6A and 6B.)
Continued
| Gene 81 | Gene 82 | Gene 83 | Gene 84 | Gene 85 |
|--------|--------|--------|--------|--------|
| ![Gene 81 Graph] | ![Gene 82 Graph] | ![Gene 83 Graph] | ![Gene 84 Graph] | ![Gene 85 Graph] |
| Gene 86 | Gene 87 | Gene 88 | Gene 89 | Gene 90 |
| ![Gene 86 Graph] | ![Gene 87 Graph] | ![Gene 88 Graph] | ![Gene 89 Graph] | ![Gene 90 Graph] |
| Gene 91 | Gene 92 | Gene 93 | Gene 94 | Gene 95 |
| ![Gene 91 Graph] | ![Gene 92 Graph] | ![Gene 93 Graph] | ![Gene 94 Graph] | ![Gene 95 Graph] |
| Gene 96 | Gene 97 | Gene 98 | Gene 99 | Gene 100 |
| ![Gene 96 Graph] | ![Gene 97 Graph] | ![Gene 98 Graph] | ![Gene 99 Graph] | ![Gene 100 Graph] |
Legends for Dataset Tables S1 to S8

**Table S1:** Gene expression levels in *ms7-6007* and WT anther transcriptomes

**Table S2:** Internal lipids, cutin and wax amounts in WT and *ms7-6007* anthers

**Table S3:** Genotypes of 403 lines and segregation ratios of fertile to sterile plants in the 403 F2 populations

**Table S4:** The 17 agronomic trait investigation for testing the effects of *ms7-6007* mutation on maize heterosis and field production

**Table S5:** Gene expression levels in *p5126-ZmMs7M-01* and WT anther transcriptomes

**Table S6:** Expression levels of the shared 126 genes in *ms7-6007* and *p5126-ZmMs7M-01* anther transcriptomes

**Table S7:** Genotypes of 392 lines for examining dominant male-sterility stability of *p5126-ZmMs7M-01* line

**Table S8:** List of primers used in this study.

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