Title: Male gametophyte-specific WRKY34 transcription factor mediates cold sensitivity of mature pollen in *Arabidopsis*

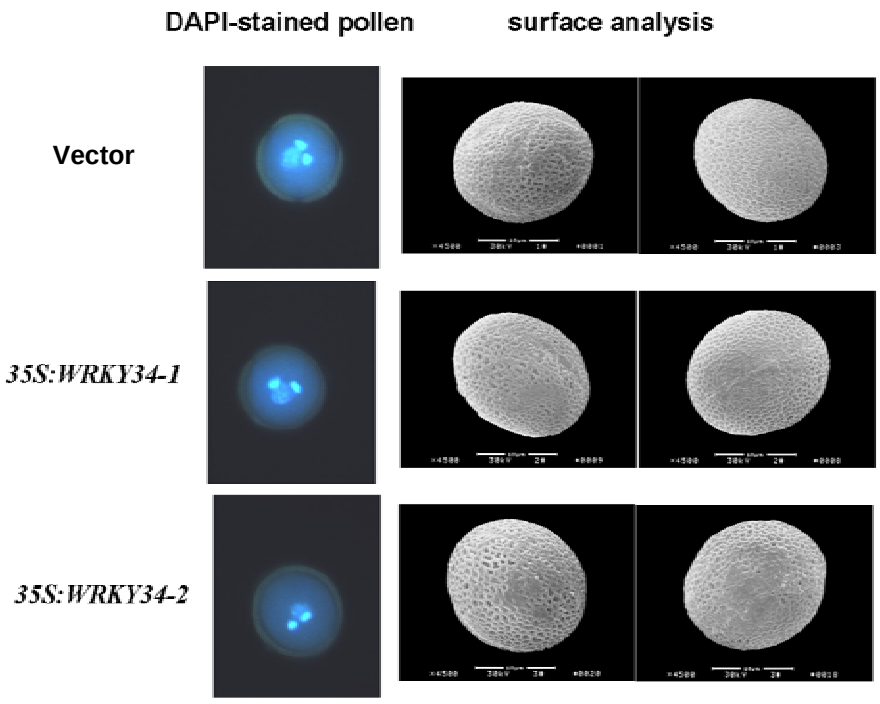
Authors: Changsong Zou[1,2], Wenbo Jiang[1] and Diqiu Yu[1],*

Supplementary Table S1. List of quantitative RT-PCR primer sequences

Gene Name	AGI	Primer forward (5'->3')	Primer reverse (5'->3')
AGL65	AT1G18750	GAGCACAAGCAACAGGCAAG	CGGTAGGGGAAAACATAAGAAGG
AGL30	AT2G03060	CTCCTCCTCTTCCTCTTACTCTTCC	GGTTTGGTTGGTGTTGTCGTT
WRKY34	AT4G26440	GCACGCACCCGAATTGT	CGGTTAGGTGGAGGTTTGCT
AGL104	AT1G22130	ACTTTCCTGACCAGAATAGACG	TCTGCCATTTGGAGTTGTTG
AGL66	AT1G77980	CAGCAACTCAAGGCTGAGAA	CACAAGTTTCGTACTCTTCCATTG
AGL94	AT1G69540	ACAGTTGAGGTGCTTAGCGA	AGTTGTTGCTGCTGACGTTG
CBF2	AT4G25470	CTCACGACGTCGCCGCCATA	GCTTCAGCCGCCGCCTTTTG
CBF1	AT4G25490	TGAAGTGAGAGAGCCAAACAAGA	CCGAGTCAGCGAAGTTGAGA
CBF3	AT4G25480	GGCGGAACAGAGCGAAAA	GGAAGCGGCAAAAGCATC
ZAT6	AT5G04340	ATATCCGGTGGCGGGGGAGG	TTGTGGCCGCCGAGAGCTTG
T1B8.13	AT2G33830	CCTTGGCCGCCTCCGCAATA	TTCCAGGACTCCCGGCCACC
MMI9.21	AT5G62350	GCCGTGACACTAAGCCGAGCC	TGGCTTCGACCTCGCGCTTC
Xero2	AT3G50970	GCTGCCAGGTCATCATGGTGCT	GGCAGCTGCTCCATAACTTTTTCCGT
RD29A	AT5G52310	AGGTCAACGTCGAGACCCCG	TCAGCAATCTCCGGTACTCCTCCA
F16B3.11	AT3G02480	TGGACAACAAGCAAAACGCGAGCTA	CTTGTCCATCATTCCACCGGCCTTC
F3I6.3	AT1G24110	GCACGGTTACGGGTTGCTCCA	AACGCCGTTTCGTCCTCCGC
SRF5	AT1G78980	GGATCTGCGCAAGAGCTTCGTGT	AGACATCACACGCGACGAAACA
T19N8.6	AT3G28770	GAGAGAGCATCGGAGACTCGACTAA	GCCTCTTGATGCCTCTCCTTCGT
ACT2	AT3G18780	TGTGCCAATCTACGAGGGTTT	TTTCCCGCTCTGCTGTTGT

Supplementary Table S2. Mircoarray data for expression of 147 mature pollen grains (MPG) specific genes.

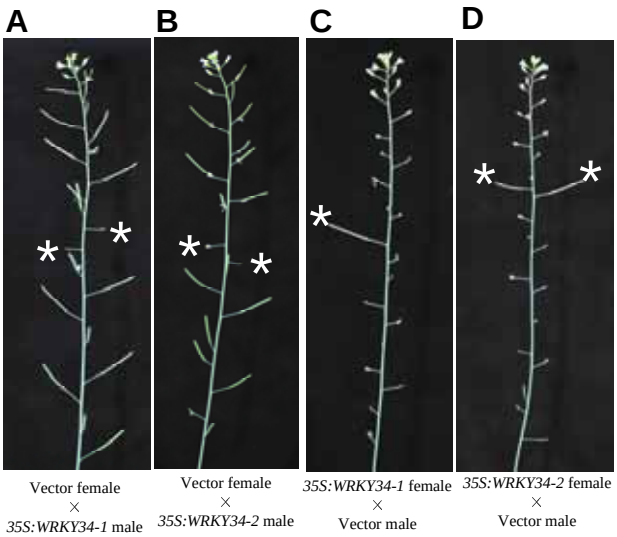
MPG-specific genes mean that their expression levels have at least 5-fold higher expression in MPG than in any of the non-pollen tissues, and at least 2-fold higher expression in MPG than in bicellular pollen (BCP). RNA was extracted from the mature pollen grain of wrky34-1 mutant and wild type plants with 48 hours at 4^{o}C.



Supplementary Fig. S1. Nuclear staining and pollen surface of vector and *35S:WRKY34* plants.

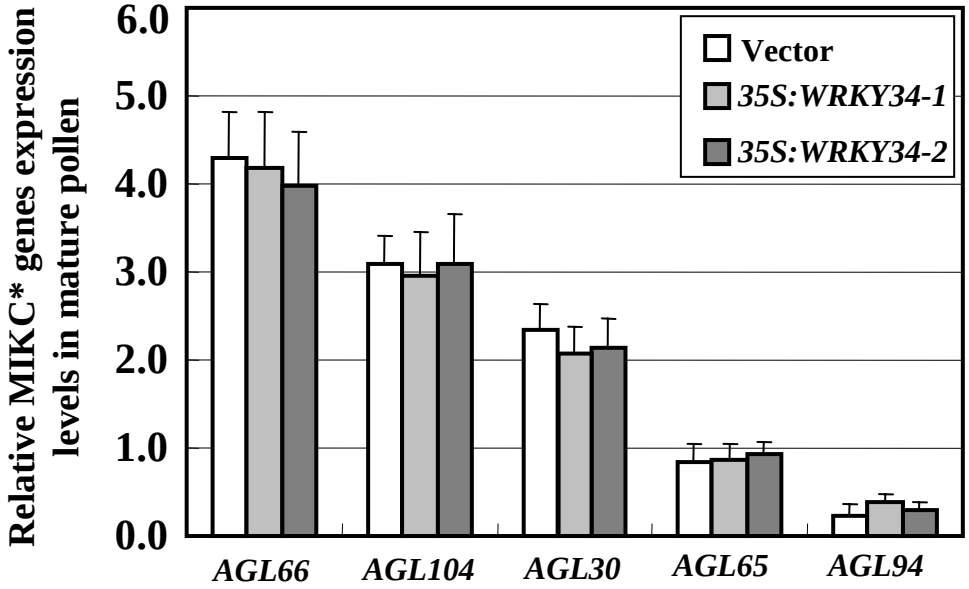
Nuclear staining of wild type and 35S:WRKY34 plants by DAPI (left)

Pollen surface of wild type and 35S:WRKY34 plants by scanning electron microscopy (right)



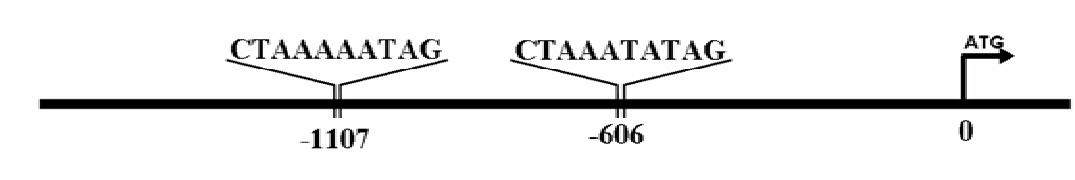
Supplementary Fig. S2. Example of the reciprocal crosses between *35S:WRKY34* and vector plants.

A and B, Wild-type plants (maternal parent) used in crosses with *35S::WRKY34* plants (paternal parent). C and D, *35S::WRKY34* plants (maternal parent) used in crosses with the WT (paternal parent). The crosses position is shown by asterisks.

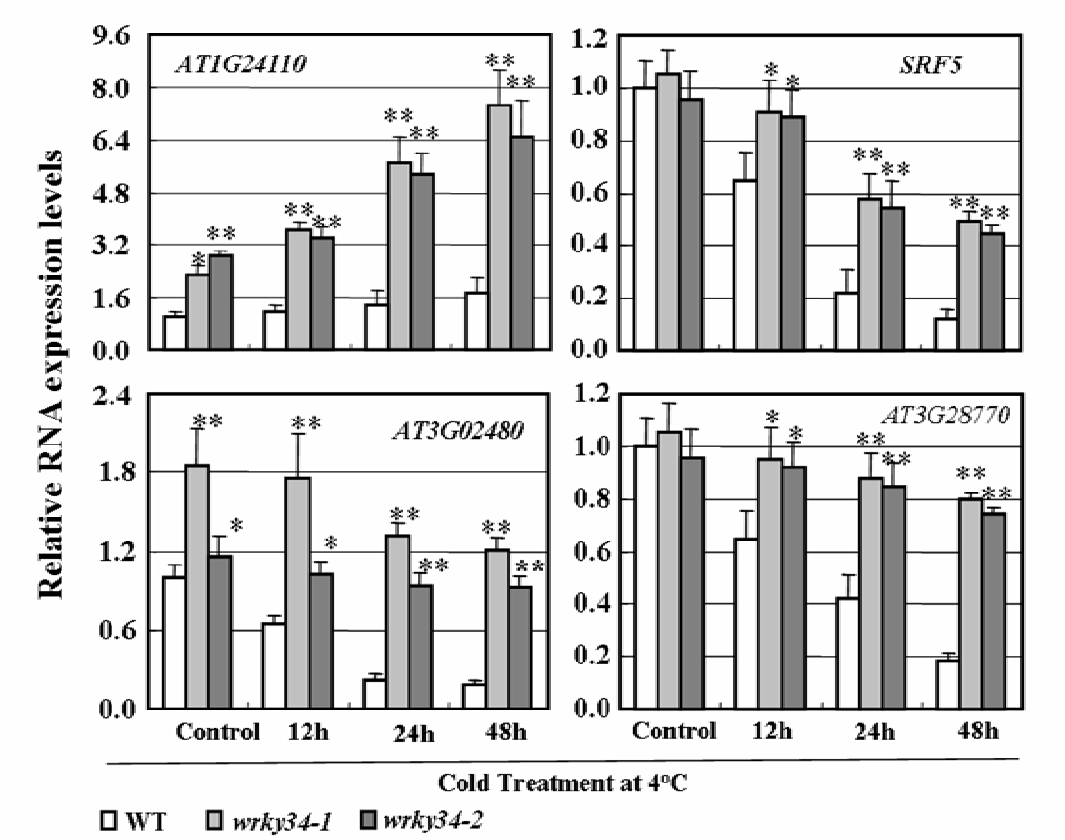


Supplementary Fig. S3. Levels of MIKC* RNA in mature pollen of vector and *35S:WRKY34* plants.

Relative RNA levels were analyzed using gene-specific primers by real-time PCR. Three independent experiments are shown by re-extracting RNA from the vector and *35S:WRKY34* plants samples. Each experiment was repeated three times. Error bars indicate standard deviations of three independent biological samples.



Supplementary Fig. S4. Positions of two MEF2 motifs in the putative promoter of WRKY34.



Supplementary Fig. S5. RNA levels of mature pollen specific genes after cold stress.

RNA was extracted from mature pollen treated with 4°C for 12 h, 24 h or 48 h at the end of cold-stress treatment and from the unstressed control. Relative RNA levels were analyzed using gene-specific primers by real-time PCR. Three independent experiments are shown by re-extracting RNA from different samples. Each experiment was repeated three times. Error bars indicate standard deviations of three independent biological samples. Differences between the wild-type (WT) and *wrky34* mutants after cold stress are significant at the 0.05 > P > 0.01 (*) or P < 0.01 (**)