

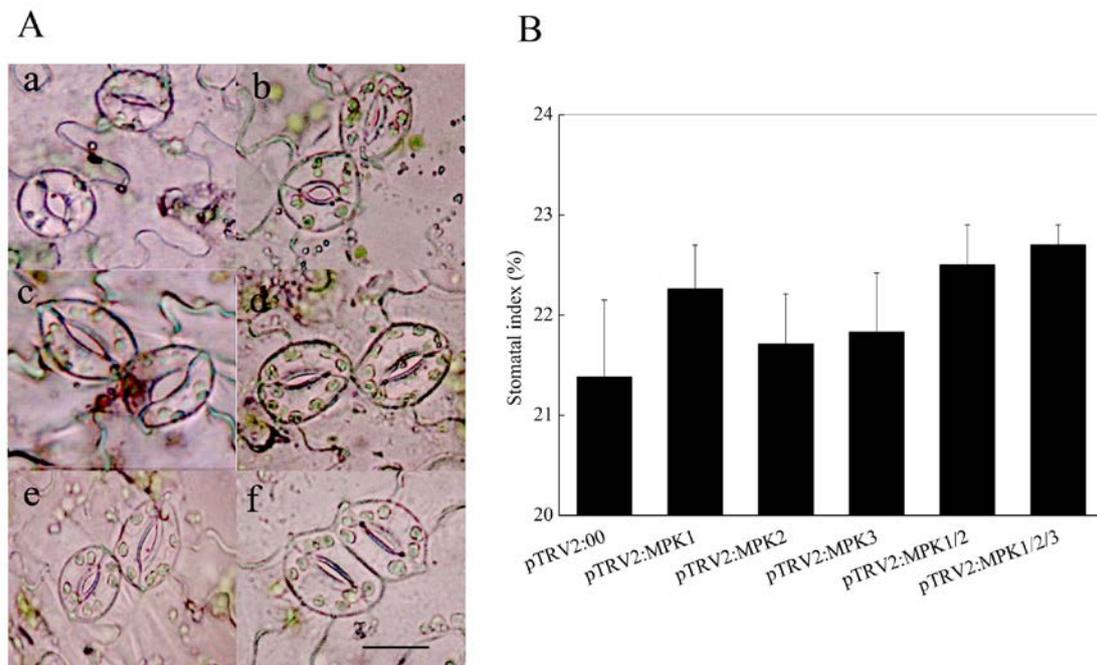
# Supplementary Information

**Table S1.** Primers used in this study.

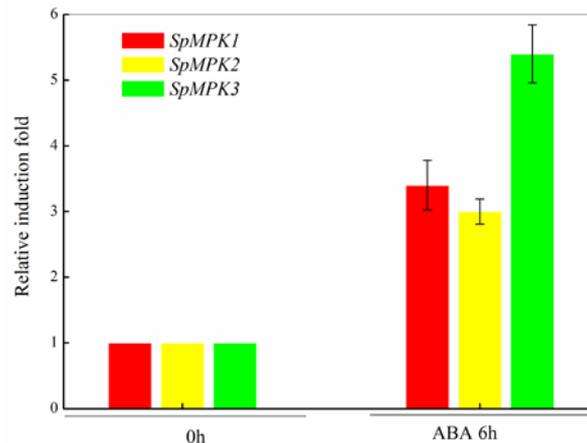
Primer code	Primer sequences (5'–3')	GenBank accession no.	Products lengths (bp)	Objectives
COSM121F	GGCGC <b>GAGCTC</b> CATGGTGGCAGGTTTCATTC	AY261512	577	For cosilencing of <i>SpMPK1/2</i>
COSM121R	CGGCG <b>CTCGAGG</b> CTCAGGTGGACGATACCAT			
SM1F	GGCGC <b>GAGCTC</b> ATAATTGCTGACAGATTGTTGC	AY261512	200	To specifically silence <i>SpMPK1</i>
SM1R	CGGCG <b>CTCGAGC</b> ATTTTCAGTCTAAAATAAAATCCAC			
SM2F	GGCGC <b>GAGCTC</b> GTACTCGCTCGTTTGCTGTT	AY261513	214	To specifically silence <i>SpMPK2</i>
SM2R	CGGCG <b>CTCGAGC</b> ATTTCTGGAATAAAAATACAGAT			
SM3F	GGCGC <b>GAGCTC</b> GCATAAGAGAAATCAGTTCTTCTCT	AK319930	409	To specifically silence <i>SpMPK3</i>
SM3R	CGGCG <b>CTCGAGC</b> ACACCCAAAATTCAAAATGAC			
COSM1231F	GGCGC <b>GAGCTC</b> ACTGATCTCCATCAAATTATTCG	AY261512	252	For cosilencing of <i>SpMPK1/2/3</i>
COSM1231R	<b>GCTCTAGA</b> AGGTGGACGATACCATCTTG			
COSM1233F	AATTGTCACTCATGCTGGACA	AY261514	220	
COSM1233R	CGGCG <b>CTCGAGA</b> AATTTACGGAGCGTCCT			
SPDSF	GGCGC <b>GAGCTC</b> GGCACTCAACTTTATAAACC	NM_001247166	409	To silence <i>SpPDS</i>
SPDSR	CGGCG <b>CTCGAGC</b> TTTCAGTTTTCTGTCAAACC			
EF1 $\alpha$ F	GACAGGCGTTTCAGGTAAGG	X14449	127	To assess the transcription levels of <i>SpEF1<math>\alpha</math></i>
EF1 $\alpha$ R	CCAATGGAGGGTATTCAGC			
qM1F	ATCCCAGAAGGAGAATAACAG	AY261512	228	To assess the transcription levels of <i>SpMPK1</i>
qM1R	ATCAAACCTGcAACAATCTG			
qM2F	ATTCCACCACCTCAACGA	AY261513	196	To assess the transcription levels of <i>SpMPK2</i>
qM2R	TGCTAGGCTTCAAGTCCC			
qM3F	GCAACTCCCACAACATCC	AY261514	233	To assess the transcription levels of <i>SpMPK3</i>
qM3R	TCTGCTCTTCTCCTATCCCT			
qPDSF	ATGCCACGACCAGAAGAT	NM_001247166	200	To assess the transcription levels of <i>SpPDS</i>
qPDSR	TGCTGTAGACAAACCACCC			
qCAT1F	GTGGATTATTTGCCCTCG	NM_001247898	152	To assess the transcription levels of <i>SpCAT1</i>
qCAT1R	GGTTCCCATGATCTGTACCTC			
qCAT2F	TCTGAAGCCAAATCCTAAGTC	NM_001247257	109	To assess the transcription levels of <i>SpCAT2</i>
qCAT2R	CAATATCGTCGAAGAGGAAAGT			

Note: words in red are restriction sites added to Forward primers and Reverse primers.

**Figure S1.** Stomatal Development in control (pTRV2:00) and individual and combined gene-silenced plants. **(A)** The distribution of stomata on the leaves of experimental plants. Control plants follow the one-cell spacing rule (**a**); For gene-silenced plants, clustered stomata are formatted in leaves' lower epidermis (**b–f**); The order is pTRV2:MPK1, pTRV2:MPK2, pTRV2:MPK3, pTRV2:MPK1/MPK2, pTRV2:MPK1/MPK2/MPK3). Bar = 20  $\mu$ m in (**a–f**); and **(B)** Stomatal index of the adaxial surface of experimental leaves. Stomatal index (SI) is calculated using the formula proposed by Salisbury (1927):  $SI = [S/(E + S)] \times 100\%$ , where  $S$  equals the number of stomata per unit of leaf area and  $E$  equals the number of epidermal cells in the same unit area. At least 3 visions from each sample of ten gene-silenced plants were measured. Data represent means  $\pm$  SD. Unexpectedly, there is no significant difference between the 5 types of gene-silenced plants and control plants.



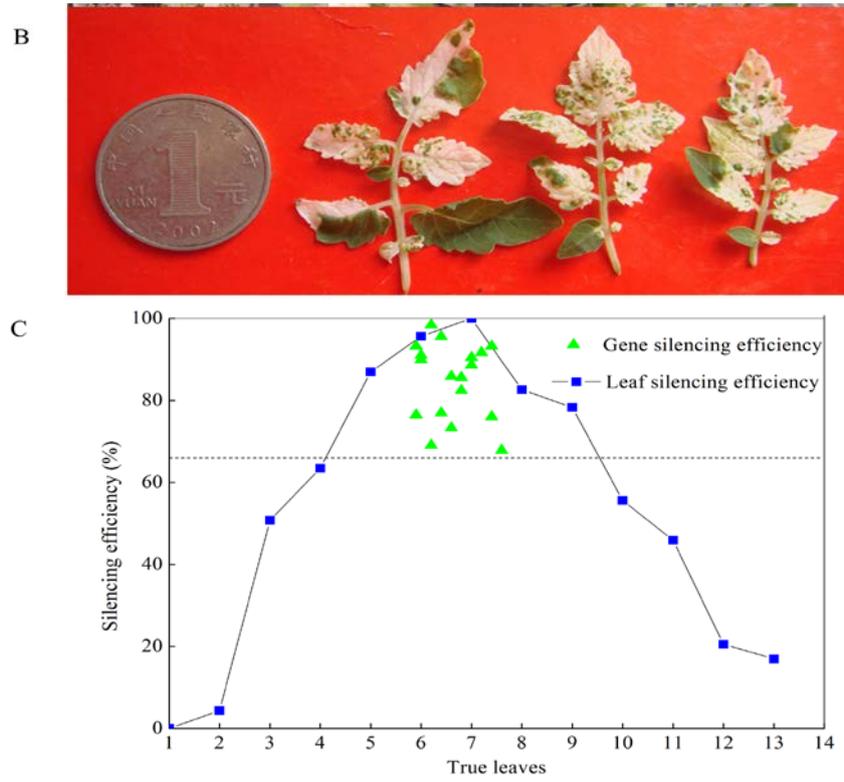
**Figure S2.** Real-time qRT-PCR analysis of ABA induction of *SpMPK1*, *SpMPK2* and *SpMPK3* genes. 40-day-old wild-type plants were treated with 150  $\mu$ M ABA for 6 h. Total RNA was obtained from treated plants and analyzed by qRT-PCR using the gene-specific primers listed in Table S1. The graphs indicate the induction fold of the *SpMPK1*, *SpMPK2* and *SpMPK3* genes in response to ABA (150  $\mu$ M) as compared with that of control (0  $\mu$ M ABA). The mean value of three technical replicates was normalized to the levels of elongation factor 1- $\alpha$  subunit mRNA, an internal control.



**Figure S3.** Silencing efficiency of *SpPDS* in wild-type tomato plants. (A) The photobleaching phenotype induced by pTRV:*PDS* in *Solanum pimpinellifolium*; (B) Leaf samples for RNA analysis come from the fifth to the ninth true leaves; and (C) Qualification of silencing efficiency. Leaf silencing efficiency is calculated by comparing the number of a certain true leaf that showed symptoms with the total number of plants inoculated with pTRV2:*PDS* 40 days post-inoculation. The experiments were representative of all the silenced plants ( $n \geq 50$  at  $OD_{600} = 2.0$ ). Quantitative RT-PCR was performed to evaluate the gene silencing efficiency and it was presented by the reduction of *SpPDS* transcription levels in VIGS plants compared to control plants which were defined as 100%.



Figure S3. Cont.



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