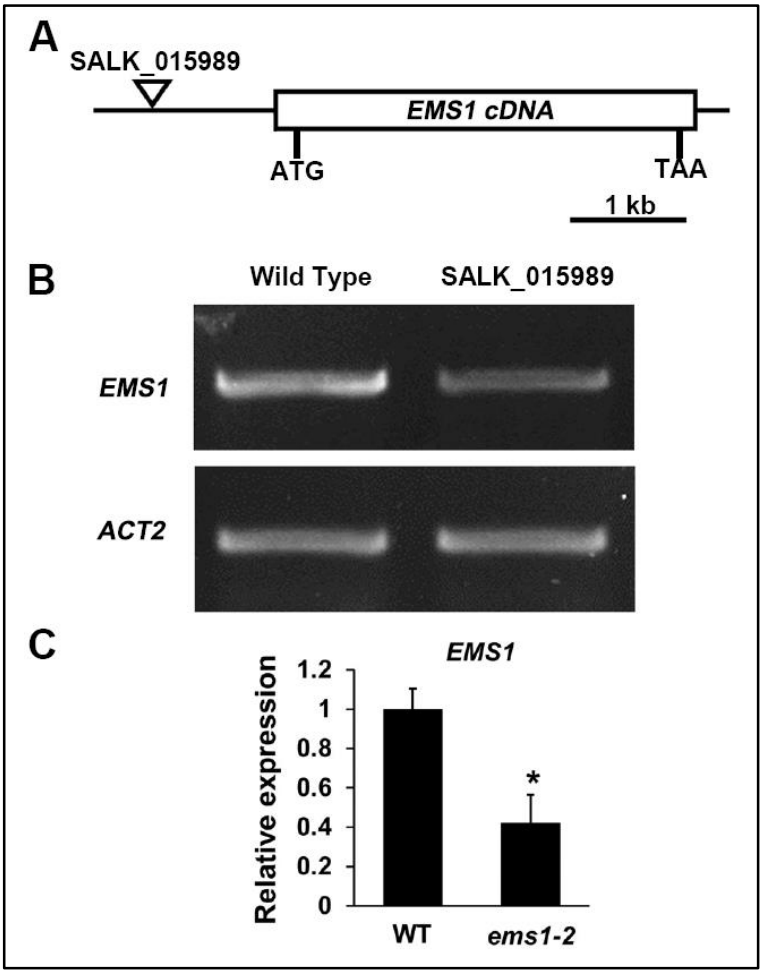
SERK1/2 Acts as a Partner of EMS1 to Control Anther Cell Fate Determination in Arabidopsis

Supplemental Data

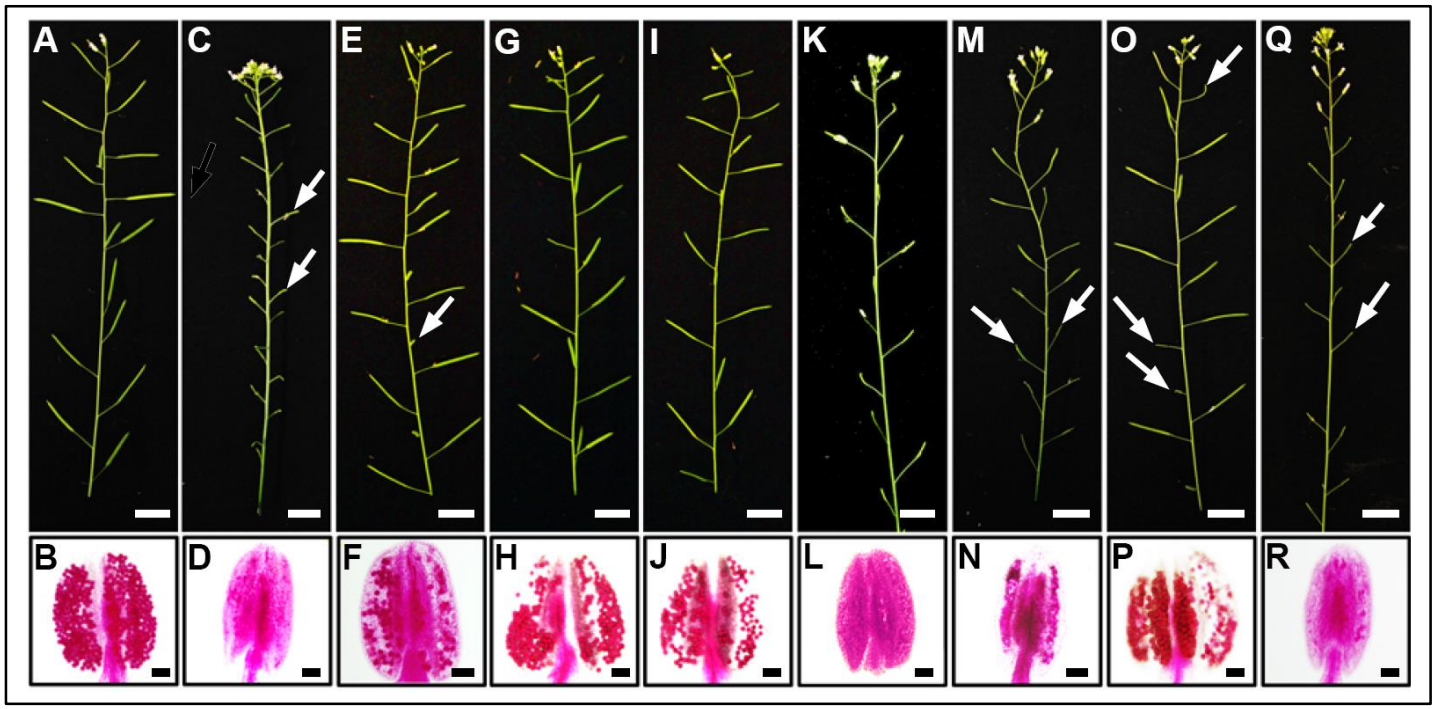
Supplemental Figure S1



Supplemental Figure S1. Identification of the *ems1-2* weak allele.

A, The schematic structure of the *EMS1* gene and the position of T-DNA insertion in the *ems1-2* mutant. The open frame represents the *EMS1* single exon. B, RT-PCR result showing expression of *EMS1* and *ACTIN*2 (*ACT2*, internal control) genes in anthers from wild-type and *ems1-2* (SALK_051989) plants. C, qRT-PCR result showing expression of *EMS1* anthers from wild-type and *ems1-2* (SALK_051989) plants. The *EMS1* gene expression is reduced in *ems1-2* anthers compared with that of wild type.

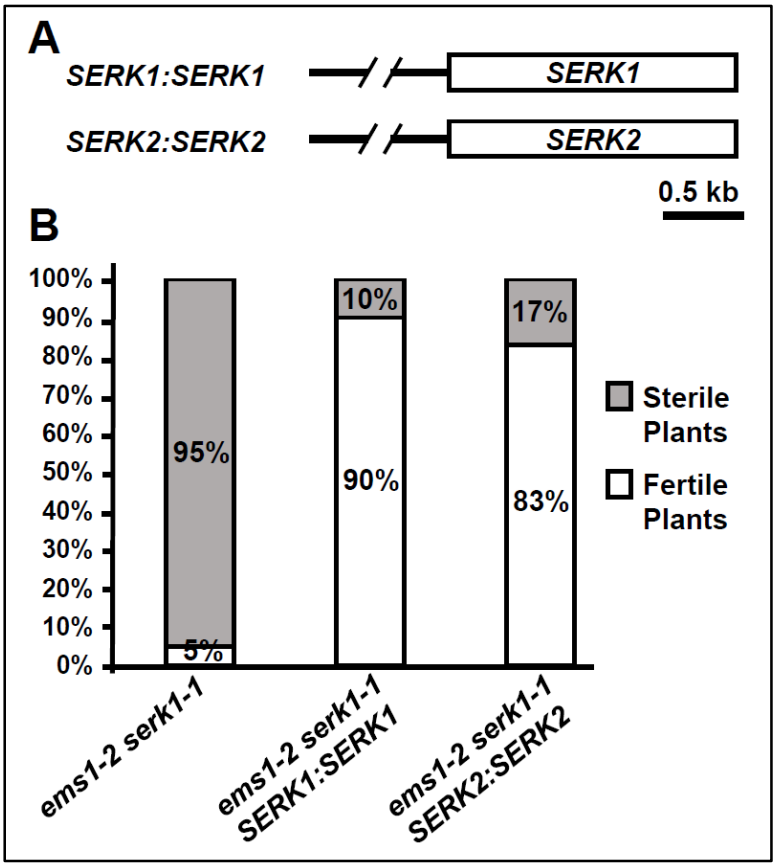
Supplemental Figure S2



Supplemental Figure S2. Fertilities of wild type, *ems1-1*, *ems1-2*, *serk1-1*, *serk2-1*, *serk1-1 serk2-1*, *ems1-2 serk1-1*, *ems1-2 serk2-1*, *ems1-1 serk1-1 serk2-1* plants.

A and B, The wild type plant showing fully elongated siliques (A) and viable pollen grains in the anther (B). C and D, The strong *ems1-1* mutant plant exhibiting short siliques (C, arrows) and no pollen grains (D). E and F, The weak *ems1-2* mutant plant displaying some short siliques (E, arrow) and a reduced amount of pollen grains from partially sterile anthers (F). G-J, The *serk1-1* mutant (G, H) has a similar phenotype to *serk2-1* (I, J), both showing fully elongated siliques (G, I) and viable pollen grains (H, J). K and L, The *serk1-1 serk2-1* double mutant plant exhibiting short siliques (K) and no pollen grains (L). M and N, The *ems1-2 serk1-1* double mutant plant showing that siliques were short (M, arrows) and there was no pollen in sterile anthers (N). O and P, The *ems1-2 serk2-1* double mutant plant exhibiting some short siliques (O, arrows) and a reduced amount of pollen grains (P). Q and R, The *ems1-1 serk1-1 serk2-1* triple mutant plant showing short siliques (Q, arrows) and no pollen grains (R). Scale bars, 1 cm (A, C, E, G, I, K, M, O and Q); 50 μm (B, D, F, H, J, L, N, P and R).

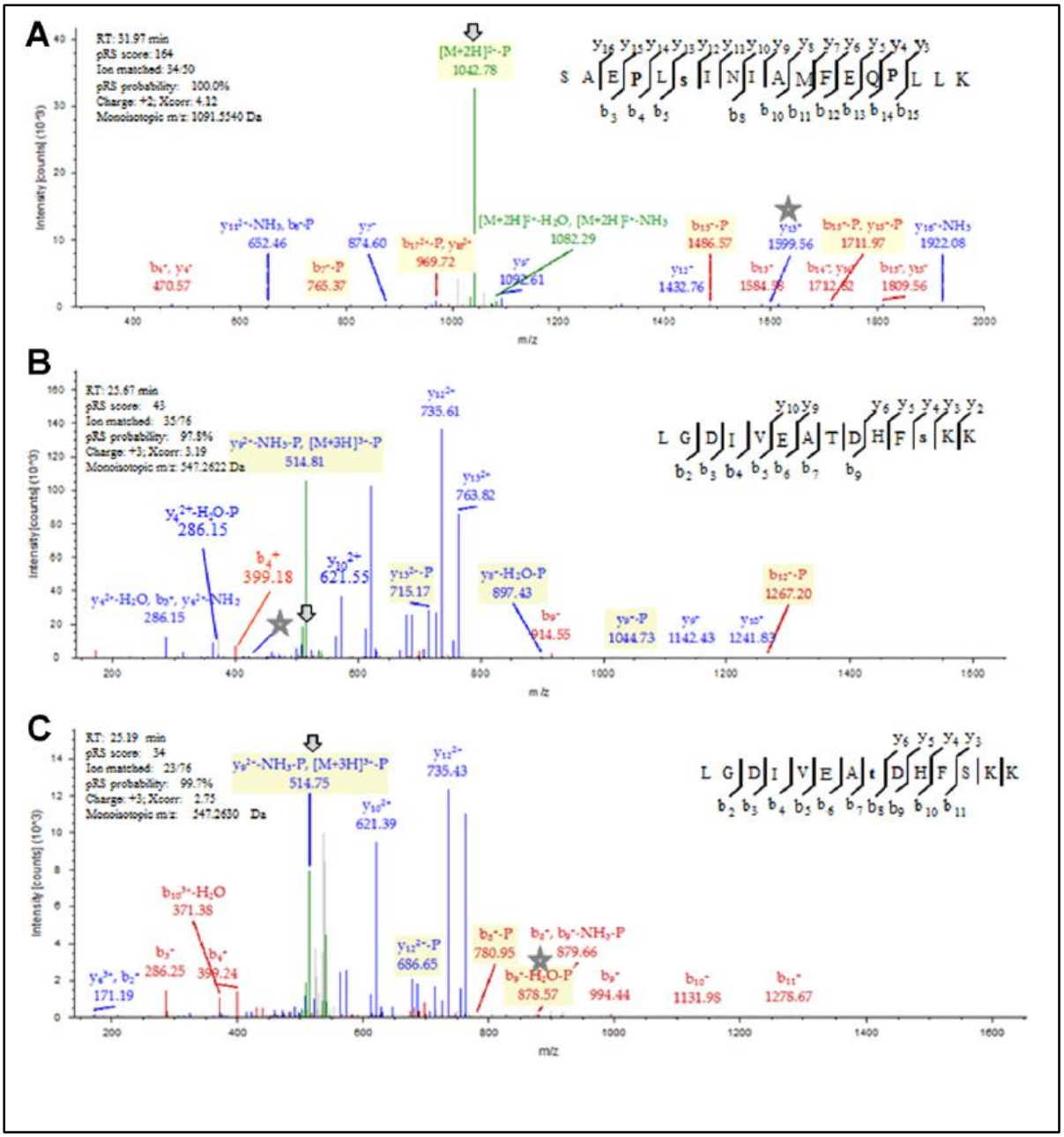
Supplemental Figure S3



Supplemental Figure S3. Functional redundancy of *SERK1* and *SERK2* in rescuing *ems1-2 serk1-1* male sterile phenotype.

A, Schematic structures of constructs used for transforming *ems1-2 serk1-1*$^{+/-}$ double mutant plants. B, Analysis of plant fertility rescued by *SERK1* and *SERK2* in the *ems1-2 serk1-1* double mutant background. Values are the means of 40 independent lines grown under the same condition.

Supplemental Figure S4

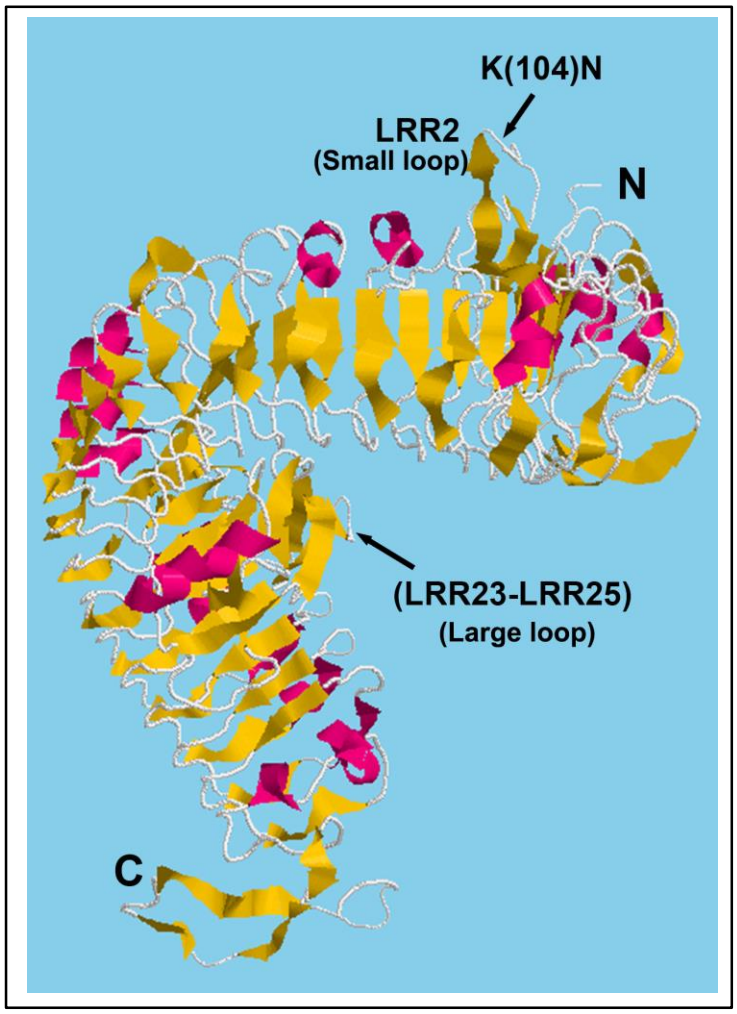


Supplemental Figure S4. Representative MS/MS spectra for identifying *in vitro* auto- and trans-phosphorylation sites in the EMS1 kinase domain.

A, The double-charged phosphopeptide SREPLsINIAMFEQPLLK providing evidence of auto-phosphorylation Ser-892 at the EMS1 kinase domain. B and C, The triple-charged peptide LGDIVEAtDHFsKK corresponding to Thr-914 and Ser-918 providing evidence of trans-phosphorylated sites. Arrow and asterisks indicating the detection of the neutral loss of the

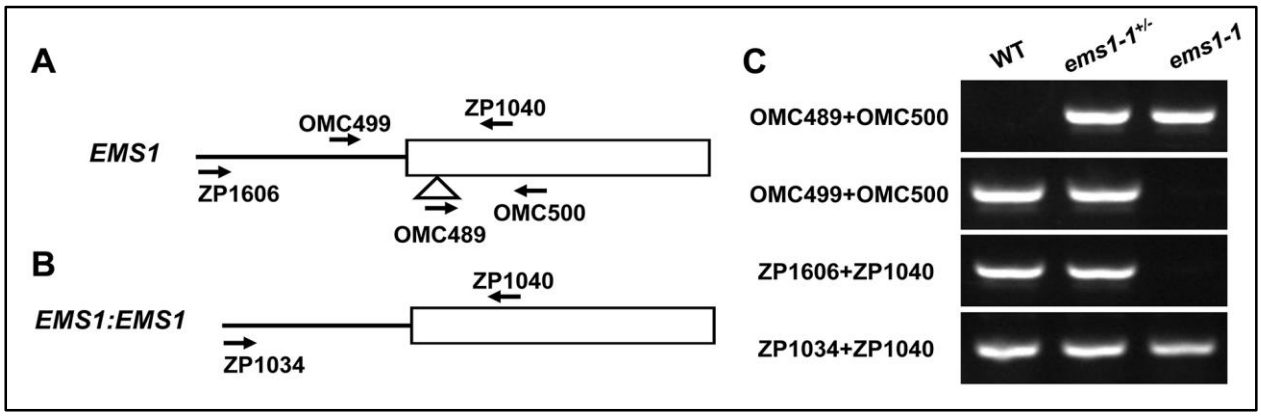
phosphate on fragments or precursor ions and the phosphorylated sites, respectively. The tryptic peptide was fragmented by CID using LTQ XL Orbitrap MS.

Supplemental Figure S5



Supplemental Figure S5. The simulated structure of the EMS1 LRR domain.
The showing structure was generated by RaptorX (http://raptorx.uchicago.edu/). The 3D model of the EMS1 LRR domain was built based on the crystal structure of BRI1 LRR domain. β-sheets are shown in orange and α helices in red. Two loops are formed by LRR2 (the small loop) and LRR23-25 (the large loop), respectively. The *exs-2* mutation [K(104)N] in the small loop results in the same phenotype as the null mutant *ems1-1*.

Supplemental Figure S6



Supplemental Figure S6. Genotyping of *EMS1:EMS1* transgenic plants in the *ems1-1* mutant background.

A and B, Schematic diagrams showing the *EMS1* gene, the *Ds* location (A), the *EMS1* transgene (B), and primers for detecting *Ds* and the *EMS1* transgene. C, PCR results.

Supplemental Figure S7

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                    854             869            883      892
EMS1_CD       LRRWAMTKRVKQRDDPERMEESRLKGFVDQNLYFLSGSRSREPLSINIAMFEQPLLKVRL
BRI1_CD       GREMRKRRRKKEAELEMYAEGHGNSGDRTANNTNWKLTGVKEALSINLAAFEKPLRKLTF
BRL1_CD       ----VRKVQKKEQKREKYIESLPTSG-----SCSWKLSSVPEPLSINVATFEKPLRKLTF
BRL2_CD       -----VRARRRDADDAKMLHSLQAVN----SATTWKIEKEKEPLSINVATFQRQLRKLKF
BRL3_CD       -LYRARKVQKKEKQREKYIESLPTSG-----SSSWKLSSVHEPLSINVATFEKPLRKLTF
FLS2_CD       -----TCCKKKEKKIENSSES----------------------SLPDLDSALKLKRFEP
HAE_CD        ----------------MFIAKCR------------KLRALKS--STLAASKWRSFHKLHF
                    914 918         930        941
EMS1_CD       GDIVEATDHFSKKNIIGDGGFGTVYKACLPGEKTVAVKKLSEAKTQGNRE----------
BRI1_CD       ADLLQATNGFHNDSLIGSGGFGDVYKAILKDGSAVAIKKLIHVSGQGDRE----------
BRL1_CD       AHLLEATNGFSAETMVGSGGFGEVYKAQLRDGSVVAIKKLIRITGQGDRE----------
BRL2_CD       SQLIEATNGFSAASMIGHGGFGEVFKATLKDGSSVAIKKLIRLSCQGDRE----------
BRL3_CD       AHLLEATNGFSADSMIGSGGFGDVYKAKLADGSVVAIKKLIQVTGQGDRE----------
FLS2_CD       KELEQATDSFNSANIIGSSSLSTVYKGQLEDGTVIAVKVLNLKEFSAESD--------KW
HAE_CD        SEH-EIADCLDEKNVIGFGSSGKVYKVELRGGEVVAVKKLNKSVKGGDDEYSSDSLNRDV

EMS1_CD       FMAEMETLGKVKHPNLVSLLGYCS-FSEEKLLVYEYMVNGSLDHWLRNQT--GMLEVLDW
BRI1_CD       FMAEMETIGKIKHRNLVPLLGYCK-VGDERLLVYEFMKYGSLEDVLHDP--KKAGVKLNW
BRL1_CD       FMAEMETIGKIKHRNLVPLLGYCK-VGEERLLVYEYMKWGSLETVLHEKSSKKGGIYLNW
BRL2_CD       FMAEMETLGKIKHRNLVPLLGYCK-IGEERLLVYEFMQYGSLEEVLHGPRTGEKRRILGW
BRL3_CD       FMAEMETIGKIKHRNLVPLLGYCK-IGEERLLVYEYMKYGSLETVLHEKT-KKGGIFLDW
FLS2_CD       FYTEAKTLSQLKHRNLVKILGFAWESGKTKALVLPFMENGNLEDTIHG----SAAPIGSL
HAE_CD        FAAEVETLGTIRHKSIVRLWCCCS-SGDCKLLVYEYMPNGSLADVLHGDR--KGGVVLGW
                                                                      1069
EMS1_CD       SKRLKIAVGAARGLAFLHHGFIPHIIHRDIKASNILLDGDFEPKVADFGLARL---ISAC
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BRI1_CD         STRRKIAIGSARGLAFLHHNCSPHIIHRDMKSSNVLLDENLEARVSDFGMARL---MSAM
BRL1_CD         AARKKIAIGAARGLAFLHHSCIPHIIHRDMKSSNVLLDEDFEARVSDFGMARL---VSAL
BRL2_CD         EERKKIAKGAAKGLCFLHHNCIPHIIHRDMKSSNVLLDQDMEARVSDFGMARL---ISAL
BRL3_CD         SARKKIAIGAARGLAFLHHSCIPHIIHRDMKSSNVLLDQDFVARVSDFGMARL---VSAL
FLS2_CD         LEKIDLCVHIASGIDYLHSGYGFPIVHCDLKPANILLDSDRVAHVSDFGTARILGFREDG
HAE_CD          PERLRIALDAAEGLSYLHHDCVPPIVHRDVKSSNILLDSDYGAKVADFGIAKVG-QMSGS
                      1073  1076 1077
EMS1_CD           ESHVST-VIAGTFGYIPPEYGQSARATTKGDVYSFGVILLELVTGKEPTGP-DFKESEGG
BRI1_CD           DTHLSVSTLAGTPGYVPPEYYQSFRCSTKGDVYSYGVVLLELLTGKRPTDSPDFG-DN--
BRL1_CD           DTHLSVSTLAGTPGYVPPEYYQSFRCTAKGDVYSYGVILLELLSGKKPIDPGEFGEDN--
BRL2_CD           DTHLSVSTLAGTPGYVPPEYYQSFRCTAKGDVYSIGVVMLEILSGKRPTDKEEFG-DT--
BRL3_CD           DTHLSVSTLAGTPGYVPPEYYQSFRCTAKGDVYSYGVILLELLSGKKPIDPEEFGEDN--
FLS2_CD           STTASTSAFEGTIGYLAPEFAYMRKVTTKADVFSFGIIMMELMTKQRPTSLNDEDSQD-M
HAE_CD            KTPEAMSGIAGSCGYIAPEYVYTLRVNEKSDIYSFGVVLLELVTGKQPTDS-ELGDKD--

EMS1_CD           NLVGWAIQKINQGK--AVDVIDPLLVSVALKNSQ---------------LRLLQIAMLCL
BRI1_CD           NLVGWVKQHAK-LR--ISDVFDPELMKEDPALEI-------------ELLQHLKVAVACL
BRL1_CD           NLVGWAKQLYREKR--GAEILDPELVT-DKSGDV-------------ELFHYLKIASQCL
BRL2_CD           NLVGWSKMKAREGK--HMEVIDEDLLKEGSSESLNEKEGFEGGVIVKEMLRYLEIALRCV
BRL3_CD           NLVGWAKQLYREKR--GAEILDPELVT-DKSGDV-------------ELLHYLKIASQCL
FLS2_CD           TLRQLVEKSIGNGRKGMVRVLDMELGDSIVSLKQEE-----------AIEDFLKLCLFCT
HAE_CD            -MAKWVCTALDKCG--LEPVIDPKLDLKFKEEIS---------------KVIHIGLLCT

EMS1_CD           AETPAKRPNMLDVLKALKEI----------------------------------------
BRI1_CD           DDRAWRRPTMVQVMAMFKEIQAGSGIDSQSTIRSIEDGGFSTIEMVDMSIKEVPEGKL
BRL1_CD           DDRPFKRPTMIQLMAMFKEMKADTEEDE-----SLDEFSLKETPLVEESRDKEP----
BRL2_CD           DDFPSKRPNMLQVVASLRELRGS------------ENNSHSHSNSL------------
BRL3_CD           DDRPFKRPTMIQVMTMFKELVQVDTEND-----SLDEFLLKETPLVEESRDKEP----
FLS2_CD           SSRPEDRPDMNEILTHLMKLRG-------------KANSFREDRNEDREV--------
HAE_CD            SPLPLNRPSMRKVVIMLQEVSGAVPCSSPNTS-KRSKTGGKLSPYYTEDLNSV-----
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Supplemental Fig. S7. Alignment of the EMS1 cytoplasmic domain with its four most closely related LRR-RLKs (BRI1, BRL1, BRL2 and BRL3) as well as FLS2 and HAE. Red letters indicate identified phosphorylation sites. Red letters highlighted in green indicate conserved phosphorylation sites. The red letter in cyan indicates S891 in BRI1, FLS2 S938 in blue, and HAE S856 in brown. Numbers indicate positions of identified *in vitro* phosphorylation sites in EMS1.