

Current Biology

Supplemental Information

Differential Function of *Arabidopsis* SERK Family

Receptor-like Kinases in Stomatal Patterning

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SUPPLEMENTAL FIGURES

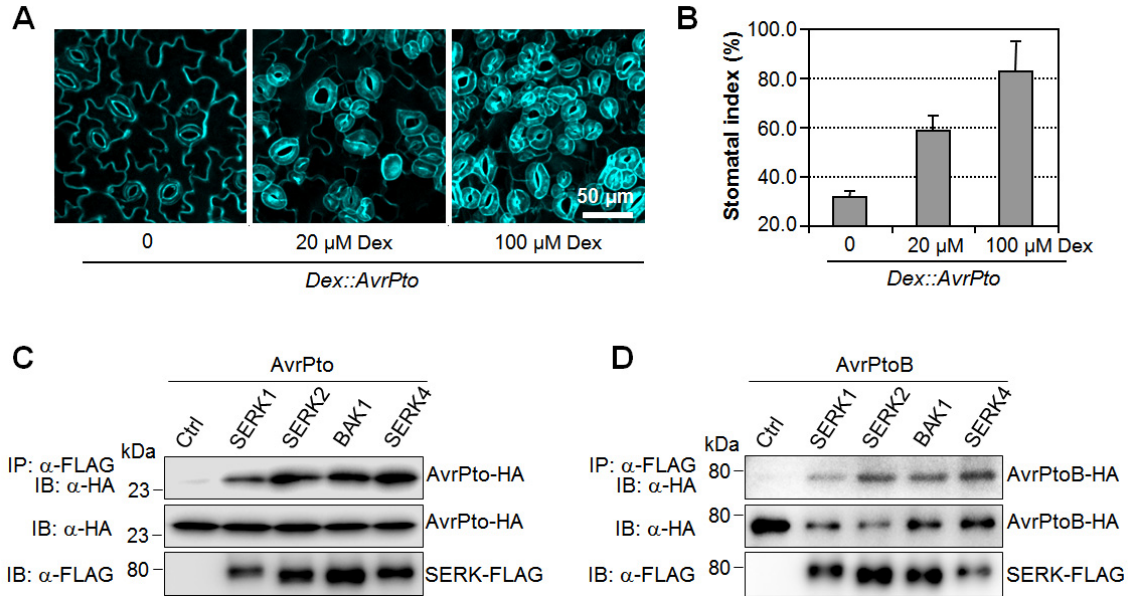


Figure S1, Related to Figure 1. Ectopic expression of AvrPto impairs stomatal patterning and AvrPto/AvrPtoB interact with the SERK family RLKs.

(A) Dex-induced expression of AvrPto in *Arabidopsis* transgenic plants caused severe stomatal clustering. Confocal images were taken on abaxial cotyledon epidermis of 10-day-old *Dex::AvrPto* transgenic seedlings grown on $\frac{1}{2}$ MS medium with (20 or 100 μ M) or without Dex. (B) Abaxial cotyledon stomatal index with the data shown as mean + SD (n=8). (C, D) AvrPto and AvrPtoB interact with SERK1, SERK2, BAK1 and SERK4 in *Arabidopsis* protoplasts. SERK-FLAG proteins were co-expressed with AvrPto-HA (C) or AvrPtoB-HA (D) in protoplasts. Protein extracts were immunoprecipitated with α -FLAG antibody (IP: α -FLAG), and immunoblotted with α -HA antibody (IB: α -HA) (top panel). The protein inputs are shown with immunoblotting before immunoprecipitation (bottom two panels). The experiments were repeated twice with similar results.

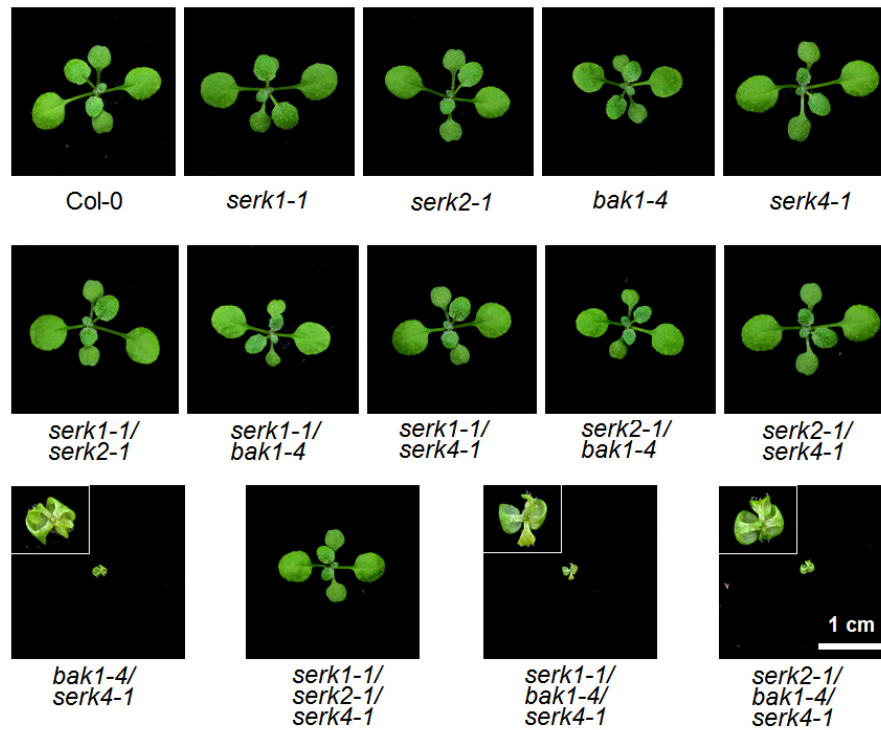


Figure S2, Related to Figure 2. The growth phenotypes of the *serk* single, double and triple mutants.

The *serk1-1/serk2-1/bak1-4* mutant shown in Figure 2D, but not other *serk* mutants shown here, exhibited similar growth morphology as the *er105/erl1-2/erl2-1* mutant (Figure 2D). The *bak1-4/serk4-1*, *serk1-1/bak1-4/serk4-1* and *serk2-1/bak1-4/serk4-1* mutants show seedling lethality. The images were taken on two-week-old seedlings grown on soil.

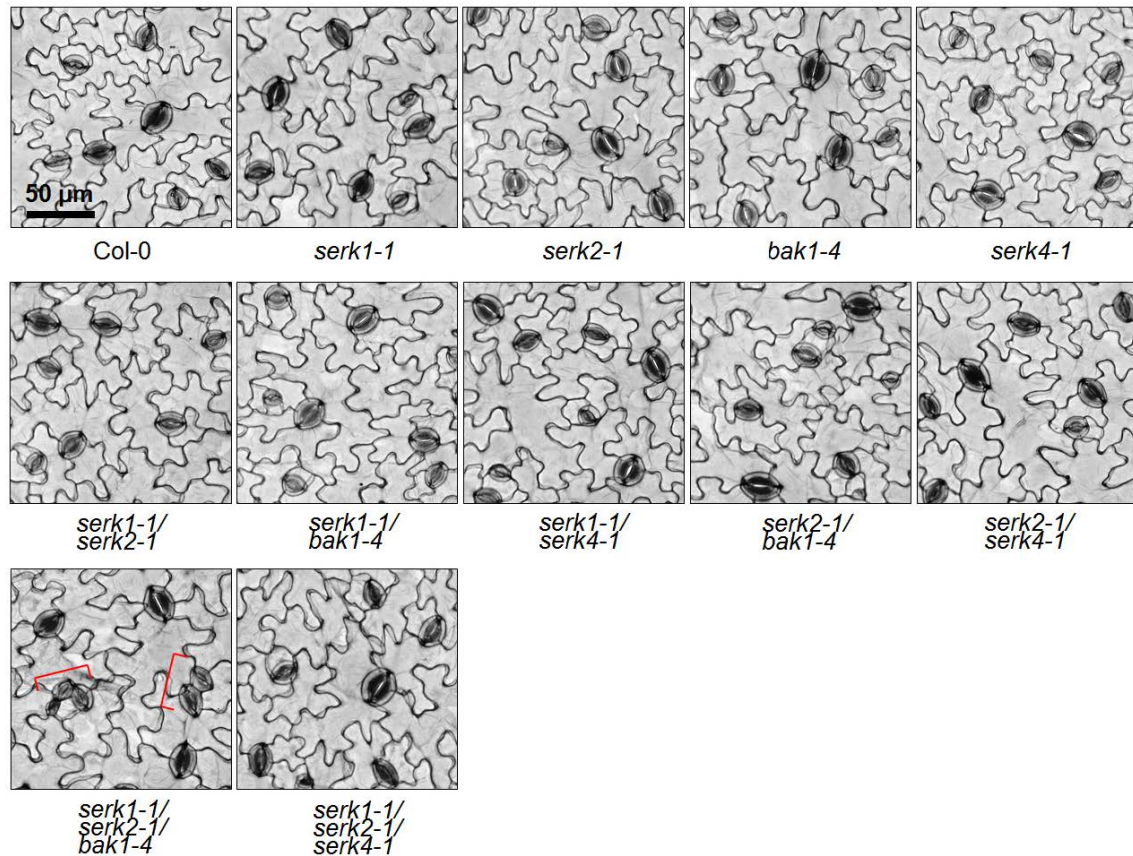


Figure S3, Related to Figure 2. The *serk1-1/serk2-1/bak1-4* mutant shows stomatal clusters in true leaves.

Optical microscopy images of abaxial epidermis of first true leaves from two-week-old seedlings of indicated genotypes grown on ½ MS medium. The *bak1-4/serk4-1*, *serk1-1/bak1-4/serk4-1* and *serk2-1/bak1-4/serk4-1* mutants were not included in the assay because of seedling lethality and no true leaf developed. Cell outlines of peeled epidermal cell layers were visualized with toluidine blue staining. Brackets indicate clustered stomata. The representative images were selected from at least five replicates.

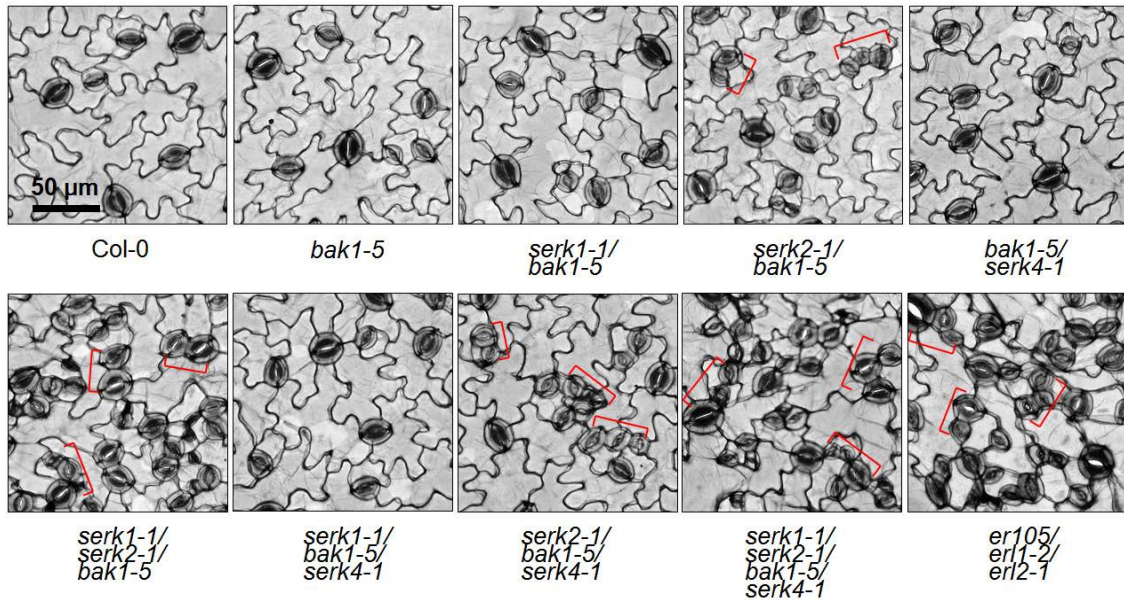


Figure S4, Related to Figure 3. The stomatal clustering phenotypes in true leaves of *serk* higher-order mutants in the *bak1-5* background.

Optical microscopy images of abaxial epidermis of first true leaves from two-week-old seedlings of indicated genotypes grown on ½ MS medium. Cell outlines of peeled epidermal cell layers were visualized with toluidine blue staining. Brackets indicate clustered stomata. The representative images were selected from at least five replicates.

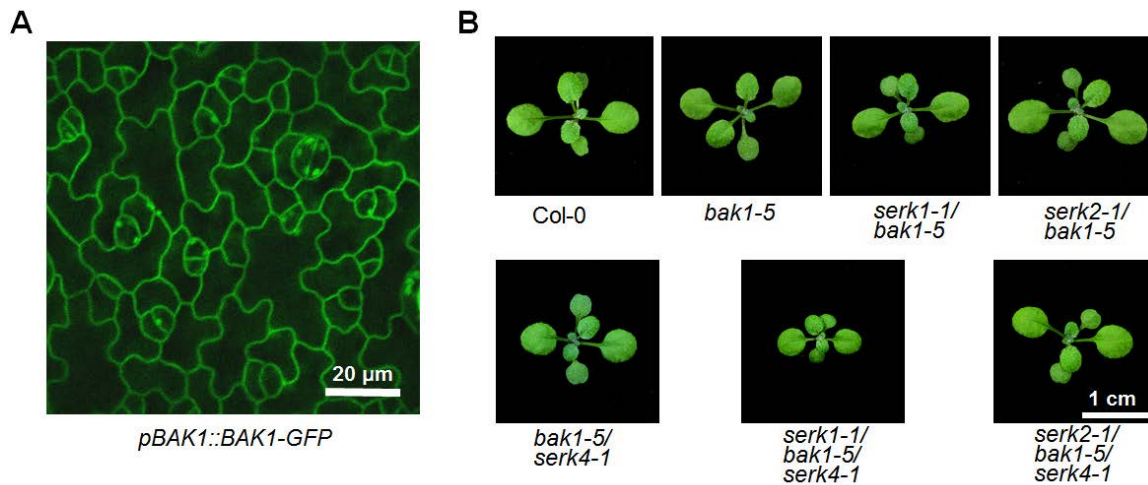


Figure S5, Related to Figure 3. Expression pattern of native promoter-driven BAK1-GFP and growth phenotypes of *serk* mutants in the *bak1-5* background.

(A) Native promoter-driven BAK1-GFP is ubiquitously expressed on the plasma membrane of epidermal cells. The expression of BAK1-GFP under the control of *BAK1* native promoter was observed using a confocal microscope in the abaxial epidermis of first true leaves of one-week-old *pBAK1::BAK1-GFP* transgenic plants. BAK1 is ubiquitously expressed in meristemoid cells, guard mother cells, guard cells and pavement cells. (B) The seedling phenotypes of *serk* single, double and triple mutants in the *bak1-5* background. Notably, the *bak1-5/serk4-1*, *serk1-1/bak1-5/serk4-1* and *serk2-1/bak1-5/serk4-1* mutants did not show seedling-lethal phenotype. The images were taken on two-week-old seedlings grown on soil.

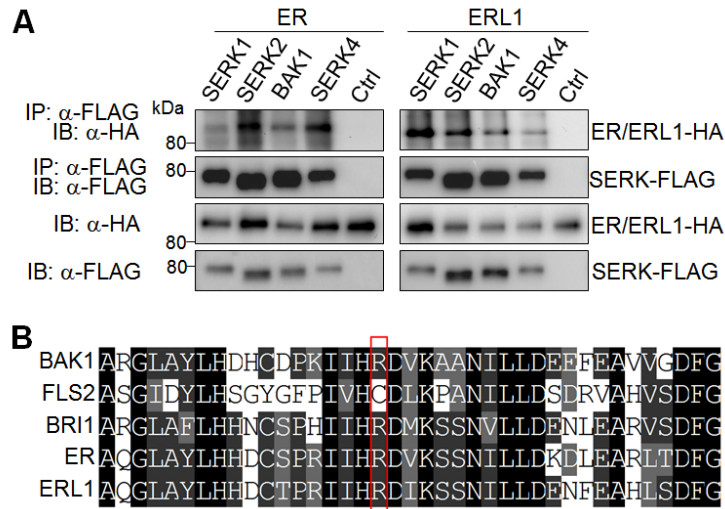


Figure S6, Related to Figure 5 (A) and Figure 6 (B). (A) SERKs associate with ER and ERL1 in *Arabidopsis* protoplasts. SERK-FLAG and ER/ERL1-HA proteins were co-expressed in protoplasts, immunoprecipitated with α -FLAG antibody (IP: α -FLAG), and immunoblotted with α -HA (IB: α -HA) or α -FLAG antibody (IB: α -FLAG) (top two panels). The protein inputs are shown with immunoblotting before immunoprecipitation (bottom two panels). (B) ER and ERL1 are RD-type RLKs. Amino acid sequence alignment of the kinase subdomain VI of BAK1, FLS2, BRI1, ER and ERL1. The dark shading represents identical amino acids among all five RLKs whereas the lightly shaded sequences represent similar amino acids. The red frame indicates presence or absence of the arginine (R) residue preceding the catalytic aspartate (D) in kinase subdomain VI that is used to differentiate RD kinases from non-RD kinases. In general, RD kinases are more active than non-RD kinases.

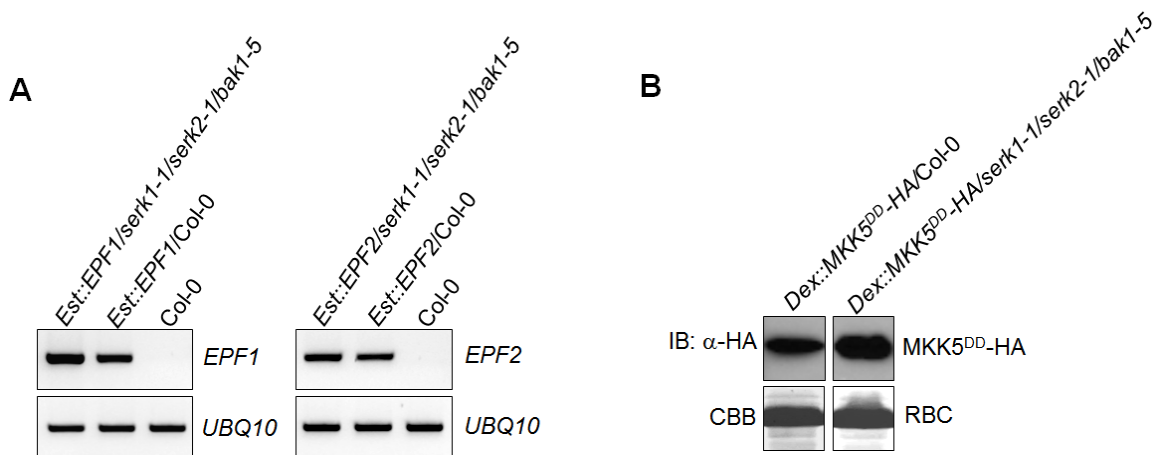


Figure S7, Related to Figure 7. (A) Estradiol-induced expression of *EPF1* and *EPF2* in transgenic plants. Ten-day-old seedlings of the indicated transgenic plants were treated with 10 μ M estradiol for 24 hr. The expression of *EPF1* and *EPF2* genes was analyzed by RT-PCR with *UBQ10* as an internal control. **(B)** Dex-induced expression of *MKK5^{DD}* in transgenic plants. Ten-day-old seedlings of the indicated transgenic plants were treated with 5 μ M Dex for 24 hr. The expression of *MKK5^{DD}-HA* was analyzed using immunoblotting with α -HA antibody and the protein loading is shown by CBB staining of RBC.

SUPPLEMENTAL TABLE

Table S1. Primers used for genotyping and plasmid construction

Primer Name	Sequences
<i>serk1-1-LP</i>	ATACACAAAAGTGAAACGGCG
<i>serk1-1-RP</i>	TTAGACGAAGAATTCGAAGCG
<i>serk2-1-LP</i>	GGAAACTCAGGTGATCCATTAAG
<i>serk2-1-RP</i>	TTAACAGGTGATGCACTGCAC
<i>bak1-4-LP</i>	CAGGGGCTATATGACCAATTG
<i>bak1-4-RP</i>	TCCTATCTCTCCTACACCGCC
<i>bak1-5-dCAPS-F</i>	AAGAGGGCTTGCGTATTTACATGATCAGT
<i>bak1-5-dCAPS-R</i>	GAGGCGAGCAAGATCAAAG
<i>serk4-1-LP</i>	TGGCTCAGAAGAAAACACAG
<i>serk4-1-RP</i>	CTGCTCCACTTCTGTTTCCAC
<i>SERK1-NcoI-F</i>	CATGCCATGGAGTCGAGTTATGTGGTGTT
<i>SERK1-StuI-R</i>	GAAGGCCTCCTTGGACCAGATAACTCAACG
<i>SERK2-NcoI-F</i>	CATGCCATGGGGAGAAAAAGTTTGAAGC
<i>SERK2-StuI-R</i>	GAAGGCCTTCTTGGACCAGACA ACTCCATAG
<i>BAK1-NcoI-F</i>	CATGCCATGGAACGAAGATTAATGATC
<i>BAK1-StuI-R</i>	GAAGGCCTTCTTGGACCCGAGGGGTATTC
<i>SERK4-BamHI-F</i>	CGGGATCCATGACAAGTTCAAAAATGGA
<i>SERK4-StuI-R</i>	GAAGGCCTTCTTGGACCCGAGGGGTAAAT
<i>ER-NcoI-F</i>	CATGCCATGGCTCTGTTTAGAGATAT
<i>ER-StuI-R</i>	GAAGGCCTCTCACTGTTCTGAGAAATAA
<i>ER_{CD}-BamHI-F</i>	CGGGATCCATGCCGCATAATCCTCCTCC
<i>ERL1-BamHI-F</i>	CGGGATCCATGAAGGAGAAGATGCAGCGA
<i>ERL1-StuI-R</i>	GAAGGCCTTATGCTACTTTTGGAGATGACTTCA
<i>MKK5-XhoI-F</i>	CCGCTCGAGATGAAACCGATTCAATCTCCTTC
<i>MKK5-StuI-R</i>	GAAGGCCTAGAGGCAGAAGGAAGAGGACGA
<i>TMM-NcoI-F</i>	CATGCCATGGCACGATATGAATTCTT
<i>TMM-SmaI-R</i>	TCCCCGGGACTAGATATTAGCATAAAAATG