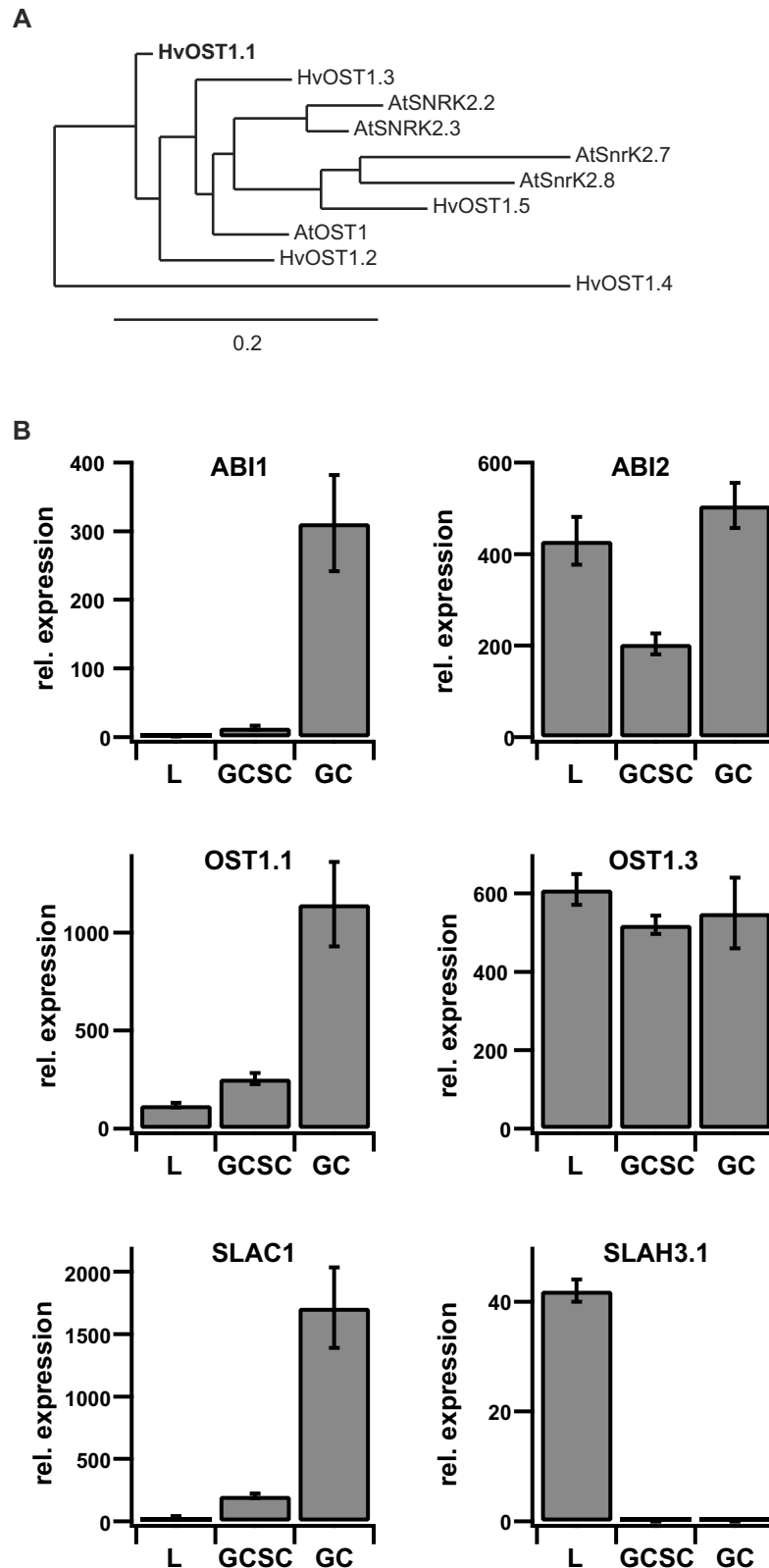


**Current Biology, Volume 28**

**Supplemental Information**

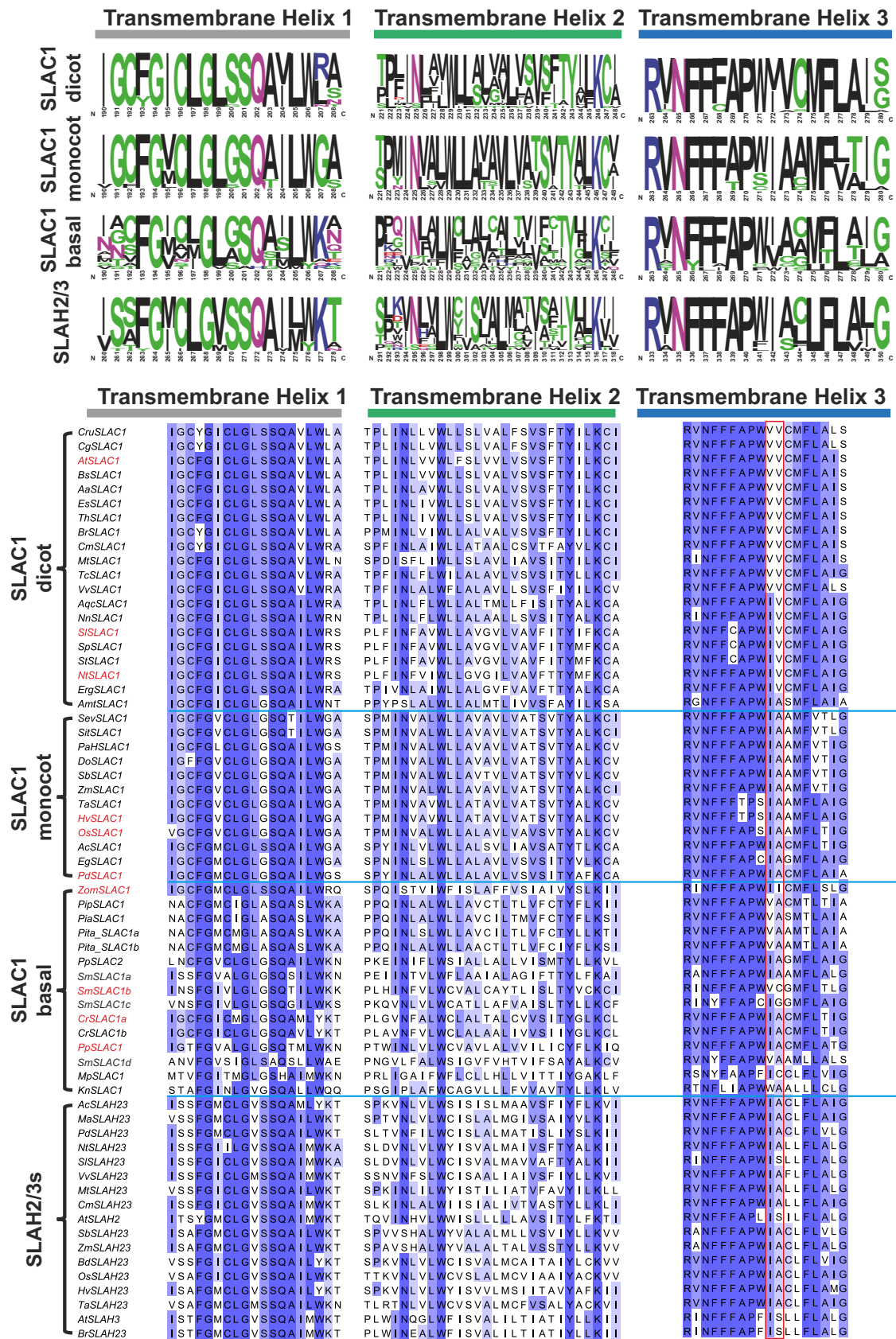
**A Tandem Amino Acid Residue Motif in Guard  
Cell SLAC1 Anion Channel of Grasses Allows  
for the Control of Stomatal Aperture by Nitrate**

**Nadine Schäfer, Tobias Maierhofer, Johannes Herrmann, Morten Egevang  
Jørgensen, Christof Lind, Katharina von Meyer, Silke Lautner, Jörg Fromm, Marius  
Felder, Alistair M. Hetherington, Peter Ache, Dietmar Geiger, and Rainer Hedrich**



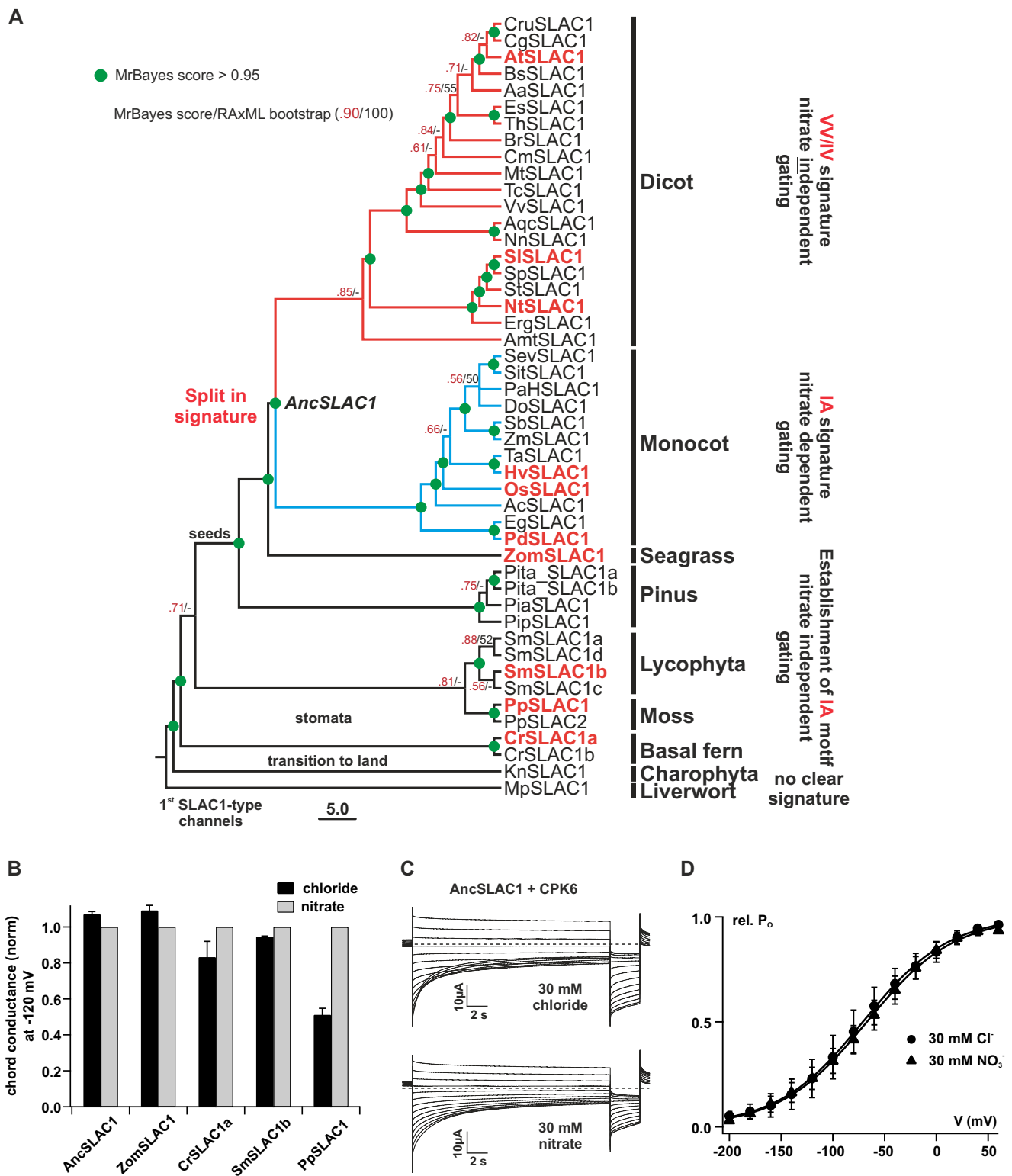
**Figure S1. Phylogenetic analysis of AtOST1 orthologs from barley using the online tool “One Click” at <http://www.phylogeny.fr/> with default settings. Related to Table S1, 2 and 5; (A) In Arabidopsis, OST1, SnRK2.2 and 2.3 represent the major ABA-dependent protein kinases involved in ABA-signalling. Phylogenetic analyses revealed that HvOST1.1, 1.2 and 1.3 are closely related to the Arabidopsis ABA-dependent SnRK2 protein kinase family while HvOST1.4 and 1.5 are SnRK2 protein kinases that lack the ABA-box in their C-terminus. (B) HvSLAC1, HvABI1 and HvOST1.1 transcripts appeared highly guard cell specific. Extended qPCR validation of RNA-seq data with additional samples of subsidiary-free guard cell samples (GC) compared to total leaf (L) and epidermal peels with intact stomatal complexes consisting of guard cells and subsidiary cells (GCSC). Values were normalized to 10.000 molecules of barley actins 4/1. n = 5 mean  $\pm$  SE.**





**Figure S3: Sequence analysis of transmembrane helix 1 to 3 of SLAC1 and SLAH2/3 homologs from selected species. Related to figure 4 and table S4.**

Frequency logos are shown for transmembrane helix 1 to 3 of monocot, dicot and basal SLAC1 homologs as well as SLAH2/3 homologs. Amino acids are colored according to their chemical properties (polar amino acids (G,S,T,Y,C,Q,N) are green, basic (K,R,H) are blue, acidic (D,E) are red and hydrophobic (A,V,L,I,P,W,F,M) amino acids are black). Transmembrane 1 to 3 alignments show a detailed view of the input for the frequency logo generation. The input sequences are deposited in table S4. Blue shading indicates residue conservation at the given position (>80%, >60%, >40%, <40%). The tandem amino acid residue motif is highlighted by a red box.



**Figure S4. The gene family phylogeny suggests that SLAC1 found in dicots and monocots share a common ancestor (AncSLAC1). Related to figure 4.** A) Bayesian inference (MrBayes) tree (s.d. < 0.01) of selected SLAC1 orthologs across the plant kingdom. Green circles at nodes represent a posterior probability of 0.95 or more. Values at nodes separated by a backslash represent MrBayes values below 0.95 in red, followed by RAxML generated bootstrap values in black (only reported when MrBayes value is below 0.95). SLAC1 anion channels depicted in red were used in this study. (B) Chord conductance at -120 mV of kinase activated AncSLAC1, ZomSLAC1, CrSLAC1a and PpSLAC1 as well as of constitutive active SmSLAC1b [S1] in the presence of chloride- or nitrate-based buffers ( $n \geq 4$  from  $\geq 2$  independent experiments, mean  $\pm$  SD). (C) Whole oocyte currents of AncSLAC1 and CPK6 co-expressing oocytes in 30 mM chloride or nitrate (representative currents from 2 independent experiments with  $n = 4$  are shown). (D) Relative open probability (rel. P<sub>o</sub>) of CPK6-activated AncSLAC1 in either 30 mM chloride or 30 mM nitrate. Data points were fitted with a single Boltzmann equation (solid lines,  $n = 4$  from 2 independent experiments, mean  $\pm$  SD).

## Supplemental references

- S1. McAdam, S.A., Brodribb, T.J., Banks, J.A., Hedrich, R., Atallah, N.M., Cai, C., Geringer, M.A., Lind, C., Nichols, D.S., Stachowski, K., et al. (2016). Absciscic acid-controlled sex before transpiration in vascular plants. *Proc Natl Acad Sci U S A* 113, 12862-12867.
- S2. Bauer, H., Ache, P., Lautner, S., Fromm, J., Hartung, W., Al-Rasheid, K.A., Sonnewald, S., Sonnewald, U., Kneitz, S., Lachmann, N., et al. (2013). The stomatal response to reduced relative humidity requires guard cell-autonomous ABA synthesis. *Curr Biol* 23, 53-57.
- S3. Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30, 1312-1313.