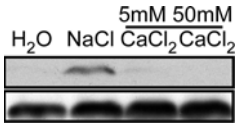
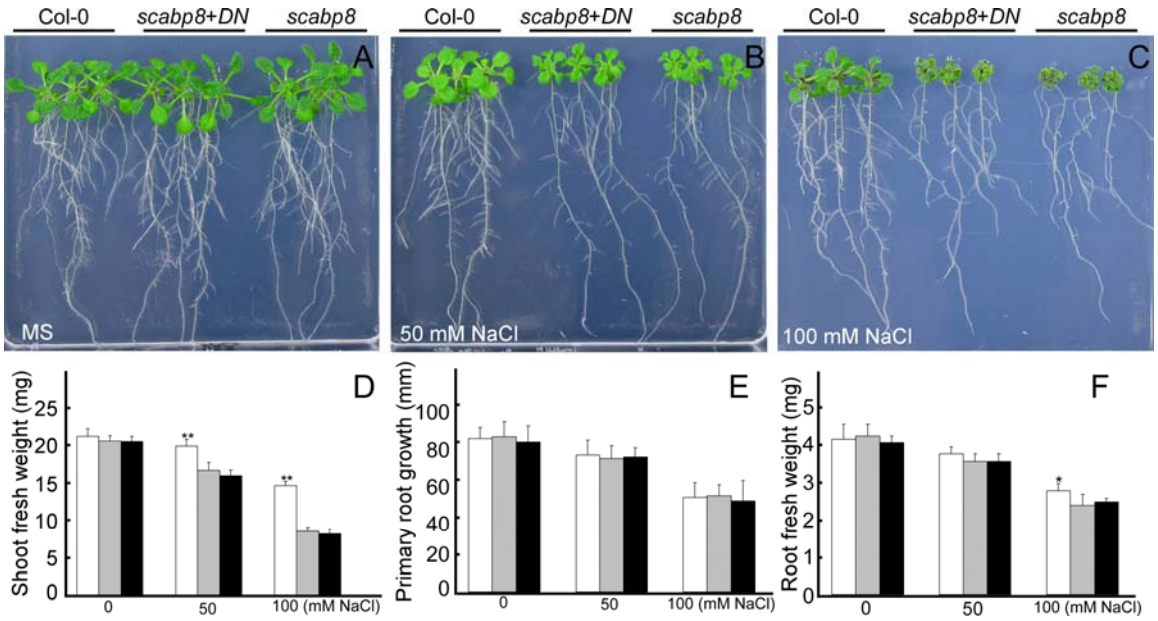
Supplemental Data Lin et al., (2009) Phosphorylation of SOS3-LIKE CALCIUM-BINDING PROTEIN8 by SOS2 protein kinase stabilizes their protein complex and regulates salt tolerance in Arabidopsis.

Supplemental Figure 1. Comparison of the C-terminal sequence of SCaBP/CBL family members, the Serine[237] is conserved in eight SCaBP proteins. The asterisk indicates the conserved Serine.



Supplemental Figure 2. Calcium does not induce the phosphorylation of SCaBP8. Seedlings of wild-type (Col-0) plants expressing *6myc-SCaBP8* were treated with 100 mM NaCl, 5 mM $CaCl_2$, 50 mM $CaCl_2$ or water (H_2O) for 12 hours. Proteins were extracted and analyzed using anti-phosphoserine[237] (top panel) or anti-myc (bottom panel) antibodies.

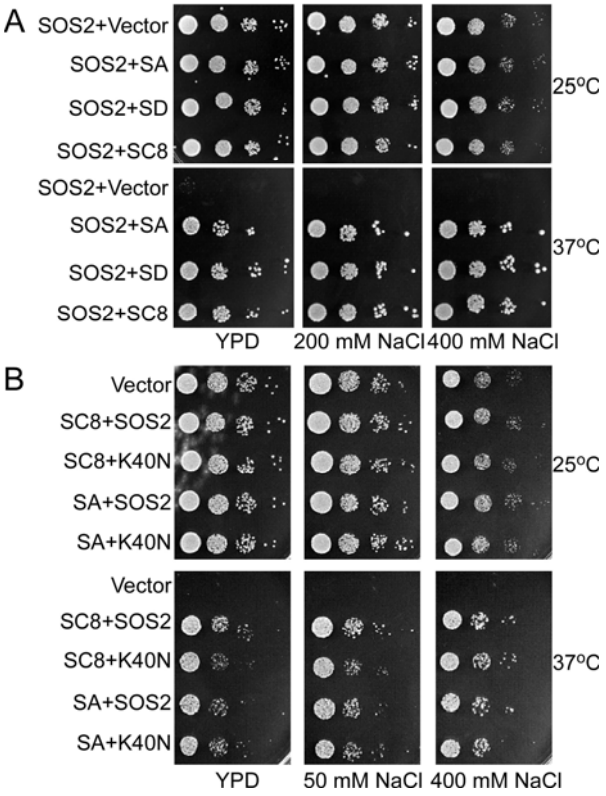


Supplemental Figure 3. A 25 amino-acid hydrophobic peptide in the N-terminus of SCaBP8 is required for salt tolerance.

35S:SCaBP8DN does not complement the *scabp8* mutant. Five-day-old seedlings from wild type (Col-0), the *scabp8* mutant and the *scabp8* mutant expressing *SCaBP8DN*, were transferred onto MS medium (A), MS medium containing 50 (B) or 100 mM (C) NaCl. Shoot fresh weight (D), primary root elongation (E) and root fresh weight (F) were measured 10 days after transfer. Open bars, without NaCl; grey bars, 50 mM NaCl; black bars, 100 mM NaCl.

DN, SCaBP8DN in which a 25-amino acid hydrophobic domain in the N-terminus has been deleted.

Asterisks indicate significant difference from transgenic line and wild type or from transgenic line and *scabp8* (Student's *t* test, $*p<0.05$ and $**p<0.01$).

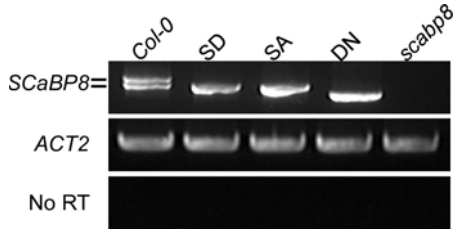


Supplemental Figure 4. serine237 is not required for targeting SCaBP8 to the plasma membrane or for recruitment of SOS2.

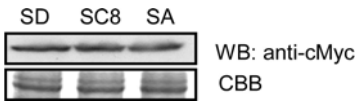
(A) SCaBP8^{S237A} is able to recruit SOS2 to the plasma membrane in yeast. *cdc25-2* mutant yeast cells expressing the reporting protein SOS2:hSos were transformed with plasmids driving the expression SCaBP8^{S237A} (S237A), SCaBP8^{S237D} (S237D), wild-type SCaBP8 or empty vector. Shown are serial decimal dilutions of representative transformants for each combination growing at permissive (25°C) or restrictive (37°C) temperature and with 0, 200 or 400 mM NaCl. Photographs were taken after 4 days.

(B) Phosphorylation of SCaBP8^{S237} does not affect the plasma-membrane localization in yeast. *cdc25-2* mutant yeast cells expressing SCaBP8:hSos or SCaBP8^{S237A}:hSos (S237A), were further transformed with plasmids expressing SOS2 or SOS2^{K40N} (K40N) and grown as described in (A). Representative transformants are shown, including an empty vector control. Photographs were taken after 2 days.

V, vector; K40N.



Supplemental Figure 5. Expression of *SCaBP8* in wide type and *scabp8* mutant, *SCaBP8S/D* in *35S: SCaBP8S/D* transgenic plants (Figure 5), *SCaBP8S/A* in *35S: SCaBP8S/A* transgenic plants (Figure 5), and *SCaBP8DN* in *35S: SCaBP8DN* transgenic plants (Supplemental Figure 3). *Actin 2* was used as a loading control. No PCR product was amplified from RNA samples indicating no DNA contamination.



Supplemental Figure 6. Protein levels in yeast used in Figure 6A.

Proteins were extracted from the yeast with Myc-SCaBP8 (SC8), Myc-SCaBP8S/A (SA), or Myc-SCaBP8S/D (SD) transgene and analyzed using anti-myc (top panel). SDS-PAGE with Coomassie blue-stained total yeast proteins (bottom panel).

Supplemental Table 1: Primers used in this study.

Plasmids	Forward primers	Reverse primers
pGEX-6P-1-SCaBP8L	5'GGGATCCATGGAACAAGTTTCCTCTAG3'	5'AGCGTCGACTCAGACATTCCATTCATCCTTAC3'
pGEX-6P-1- SCaBP8$^{1-201}$	5'GGGATCCATGGAACAAGTTTCCTCTAG3'	5'AGCGTCGACTCAGTCTTTGTCAGAATCTGCATC3'
pGEX-6P-1- SCaBP8$^{1-213}$	5'GGGATCCATGGAACAAGTTTCCTCTAG3'	5'AGCGTCGACTCACACATAGACATTCCATTC3'
pGEX-6P-1- SCaBP8$^{1-234}$	5'GGGATCCATGGAACAAGTTTCCTCTAG3'	5'AGCGTCGACTCATGCTGTCGTCACATCCTTTAG3'
pGEX-6P-1- SCaBP8^{S237A}	5'GGGATCCATGGAACAAGTTTCCTCTAG3'	5'AGCGTCGACTCAGTCTTCAACCTCAGTGTTGAATATAAAGGCTGGGAATGCTGTCGTCAC3'
pGEX-6P-1- SCaBP8^{S237D}	5'GGGATCCATGGAACAAGTTTCCTCTAG3'	5'AGCGTCGACTCAGTCTTCAACCTCAGTGTTGAATATAAAATCTGGGAATGCTGTCGTC3'
pGEX-6P-1- SCaBP8^{T242A}	5'GGGATCCATGGAACAAGTTTCCTCTAG3'	5'AGCGTCGACTCAGTCTTCAACCTCGGCGTTGAATATAAAACTTGG3'
pGEX-6P-1- SCaBP8$^{S237A\ T242A}$	5'GGGATCCATGGAACAAGTTTCCTCTAG3'	5'AGCGTCGACTCAGTCTTCAACCTCGGCGTTGAATATAAAGGCTGGGAATGCTGTCGTCAC3'
pGEX-6P-1-SOS2 FISL motif	5'CGGGATCCATGATGAATGCCTTTGAGATG3'	5'AGCGTCGACTCAGTCAAATAGTGCAGATAAA3'
pGADT7-SOS2	5'TGGAATTCATGACAAAGAAAATGAGAAG3'	5'CGGGATCCTCAAAACGTGATTGTTCTGAG3'
pGBKT7-SCaBP8 / SCaBP8^{S237A}/ SCaBP8^{S237D}	5'TGGAATTCATGGAACAAGTTTCCTCTAG3'	5'AGCGTCGACTCAGTCTTCAACCTCAGTGTT3'