

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.

```

SCaBP8 (CBL10) 1:MEQVSSRSSSLTVGEQFCVAFIPFFAIIDVLVSSVGGCFDCRSTSPRTCQHADLERLARE 60
SOS3 (CBL4) 1:M-----G--CSVSKKKKKNAMRP----PGYEDPEL---LASV----- 28
SCaBP1 (CBL2) 1:M-----S-QCVDGIKHLCTSVLG---CFDLIDLYKQSGGLGDP-ELLARD 39
SCaBP2 (CBL6) 1:M-----MMQCLDGLKHLALILLT---CCDADPPKVRQ---NPKD-VARG 37
SCaBP3 (CBL7) 1:MDSTNSASSNST--G-CFTDQKKRKALYEVFKLSGV-D-----CQRNEGVVEGV 48
SCaBP4 (CBL5) 1:MGCV-----CSKQLEGRREQDISLLASQTFE----- 28
SCaBP5 (CBL1) 1:M-----G--CFHSAAKEFRGH-----EDPVK---LASE----- 24
SCaBP6 (CBL3) 1:M-----S-QCIDGFKHVCSSFFR---CFDIDIYKQSGGLGDP-ELLARE 39
SCaBP7 (CBL9) 1:M-----G--CFHSTAAREFPDH-----ENPVK---LASE----- 24
SCaBP9 (CBL8) 1:M-----L--AFVKCFSLKRAKHP---RGYEDPHV---LASE----- 28

SCaBP8 (CBL10) 61:SFQSNVEEALYELFKKLSIIDDGLIKKEELRLALFQAPYGENLFLDRVFDLFDKRN 120
SOS3 (CBL4) 29:TFPTVEEVEALYELFKKLSIIDDGLIKKEEFQALAFRRNRNRNLFARIFDFVDFKRN 88
SCaBP1 (CBL2) 40:TVFQSEVEALYELFKKISSAVIDDGLIKKEEFQALAFKTNKKESLFADRVDLFDTRHN 99
SCaBP2 (CBL6) 38:TVFTVNEEALYELFKSISKN---GLIDKEEFQVLEKMMNTRSLFADRVDLFDTRNT 93
SCaBP3 (CBL7) 49:TC-----YY--G-----E-M-NKEDFHVAIFQTDKNESLFSBRVFDLFDTNHD 87
SCaBP4 (CBL5) 29:---A-EVEVHGLFIKLTSCLSNMLLTKKFCFELLNKTKKRSLSARRIFGLFDMRND 83
SCaBP5 (CBL1) 25:TFQSNVEEALYELFKSISSSVVDGLIKKEEFQALAFKSRKRELFARIFDFVDFKRN 84
SCaBP6 (CBL3) 40:TVFQSEVEALYELFKKISSAVIDDGLIKKEEFQALAFKTNKKESLFADRVDLFDTRHN 99
SCaBP7 (CBL9) 25:TFQSNVEEALYELFKSISSSVVDGLIKKEEFQALAFKRNKRELFARIFDFVDFKRN 84
SCaBP9 (CBL8) 29:TFPTVNEEALYELFKKLSIIDDGLIKKEEFQALAFRNGSMQNLFADRVMDFDRRN 88

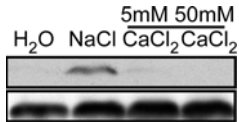
SCaBP8 (CBL10) 121:GVIEEVEEIHSLVFHFYASIQEKTDFARFLYDLRQTGFIEREVEVKQMVSAHLLSEDM 180
SOS3 (CBL4) 89:GVIERGEFVRSLSGVFHFSAFVHKKVFAFKLYDLRQTGFIEREELREMVVALLHESELVL 148
SCaBP1 (CBL2) 100:GILGEEEFARLSVFHFNAPIDDKIHFSQLYDLKQCGFIEREVEVKQMVVAFLAESGMNL 159
SCaBP2 (CBL6) 94:GILDPEEFARLSVFHFNAPFEDKIEFSKLYDLNQQGYIFRVEVKQMVVTRLAESGMNL 153
SCaBP3 (CBL7) 88:GLLGEFEFARLSVFHFNAPIDDKIDLSQLYDLKQCGFIEREQVKQLVVATLAESGM 147
SCaBP4 (CBL5) 84:GAIDGEGEVHITLNFHFNSSPRDKAIFARFLYDTRGTGFIEPEVEKEMIIDVLESELML 143
SCaBP5 (CBL1) 85:GVIDGGDFVRSLSNVFHFNASLEDKIDFTRLYDMDCGYIEREVEVKQMLIALCESEMKL 144
SCaBP6 (CBL3) 100:GILGEEEFARLSVFHFNAPIEDKIDFSQLYDLKQCGFIEREVEVKQMVVAFLAESGMNL 159
SCaBP7 (CBL9) 85:GVIDGDFVRSLSNVFHFNASLEBKIDFTRLYDMDCGYIEREVEVKQMLIALCESEMKL 144
SCaBP9 (CBL8) 89:GVIEEVEFVRSLSVFHFYTPHEHKSAPFVKLFDLHGTGFIEPEELKQMVGALLGETLEL 148

SCaBP8 (CBL10) 181:SEELLTMIDKTFEADSDKDGKISKDEWNVVHKHPSLLKNMTLYLKDITTFPSEIF 240
SOS3 (CBL4) 149:SEDMTEFVMVDRAFVQADRKNQDKIDIDEMKDFVSLNPSILKNMTLYLKDINRTFSPFVS 208
SCaBP1 (CBL2) 160:KDAVTEIDKTFEEDTKHDKGIDKEWRSIVLRHPSLLKNMTLYLKDITTFPSEVF 219
SCaBP2 (CBL6) 154:SDHVTESIDKTFEEDTKIDGKIDKEWRSIVLRHPSLLKNMSTLOHLKDVTKTFPSEVF 213
SCaBP3 (CBL7) 148:SEIIVESIDKTFVQADTKHEGVIDEWEWMDLIVFRHPSLLKNMTLYLKDITTFPSEV 207
SCaBP4 (CBL5) 144:SESIIDSTVSKTFEEDAKKHDGIDLEWENFVATYPLTLKNMTIPELKDIPRIFFFLR 203
SCaBP5 (CBL1) 145:ADETTEIIDLKTFEADAVNQDGKIDKLEWSDVFNKNPSLLKMTLYLKDITTFPSEVF 204
SCaBP6 (CBL3) 160:SEIIVESIDKTFEEDTKHDKGIDKEWRTIVLRHPSLLKNMTLYLKDITTFPSEVF 219
SCaBP7 (CBL9) 145:ADDTIEMILDTFEADAVDRDGGKIDKTEWSNFVKNPSLLKMTLYLKDITTFPSEVF 204
SCaBP9 (CBL8) 149:SESETEAVVEQTMLEVTNNDGKIDEEWKELVAKNPSLLKNMTLYLKEVFLAFPSFVL 208

SCaBP8 (CBL10) 241:NTEVED----- 246
SOS3 (CBL4) 209:SCEEEEEMELQNVSS 222
SCaBP1 (CBL2) 220:HSQVEDT----- 226
SCaBP2 (CBL6) 214:HTIVTDTPELDG- 226
SCaBP3 (CBL7) 208:HSQVEDT----- 214
SCaBP4 (CBL5) 204:----- 204
SCaBP5 (CBL1) 205:HSEVD--E---IAT 213
SCaBP6 (CBL3) 220:HSQVEDT----- 226
SCaBP7 (CBL9) 205:NSEVD--E---IAT 213
SCaBP9 (CBL8) 209:DS--E-VE----- 214

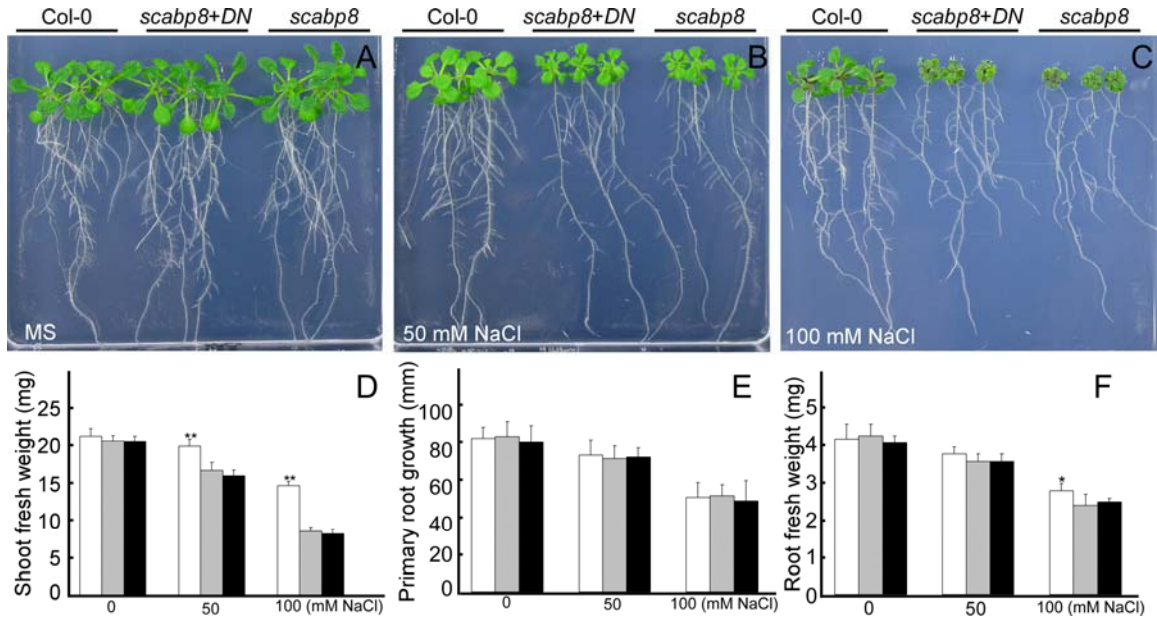
```

Supplemental Figure 1. Comparison of the C-terminal sequence of SCaBP/CBL family members, the Serine<sup>237</sup> 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<sub>2</sub>, 50 mM CaCl<sub>2</sub> or water (H<sub>2</sub>O) for 12 hours. Proteins were extracted and analyzed using anti-phosphoserine<sup>237</sup> (top panel) or anti-myc (bottom panel) antibodies.

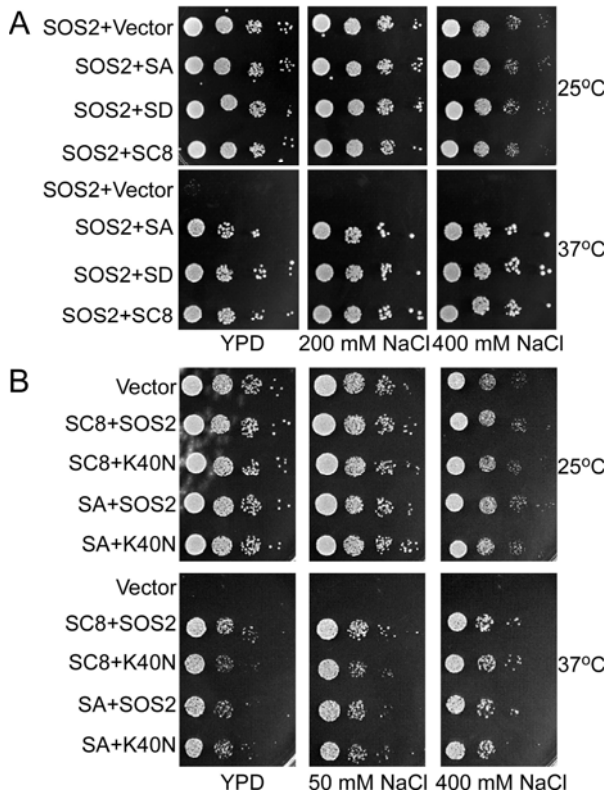


Supplemental Figure 3. A 25 amino-acid hydrophobic peptide in the N-terminus of SCaBP8 is required for salt tolerance.

*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 \*\* $p < 0.01$ ).

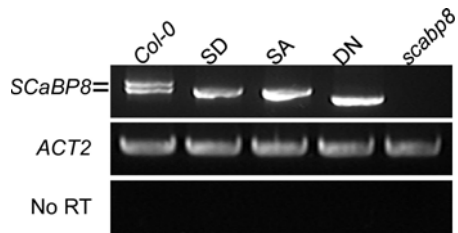


Supplemental Figure 4. serine237 is not required for targeting SCaBP8 to the plasma membrane or for recruitment of SOS2.

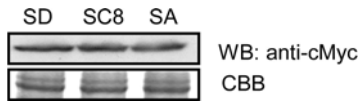
(A) SCaBP8<sup>S237A</sup> is able to recruit SOS2 to the plasma membrane in yeast. *cdc25-2* mutant yeast cells expressing the reporting protein SOS2:hSos were transformed with plasmids driving the expression SCaBP8<sup>S237A</sup> (S237A), SCaBP8<sup>S237D</sup> (S237D), wild-type SCaBP8 or empty vector. 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<sup>S237</sup> does not affect the plasma-membrane localization in yeast. *cdc25-2* mutant yeast cells expressing SCaBP8:hSos or SCaBP8<sup>S237A</sup>:hSos (S237A), were further transformed with plasmids expressing SOS2 or SOS2<sup>K40N</sup> (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'GGGATCCATGGAACAAGTTT CCTCTAG3'	5'AGCGTCGACTCAGACATTCC ATTCATCCTTAC3'
pGEX-6P-1- SCaBP8 <sup>1-201</sup>	5'GGGATCCATGGAACAAGTTT CCTCTAG3'	5'AGCGTCGACTCAGTCTTTGT CAGAATCTGCATC3'
pGEX-6P-1- SCaBP8 <sup>1-213</sup>	5'GGGATCCATGGAACAAGTTT CCTCTAG3'	5'AGCGTCGACTCACACATAG ACATTCCATT3'
pGEX-6P-1- SCaBP8 <sup>1-234</sup>	5'GGGATCCATGGAACAAGTTT CCTCTAG3'	5'AGCGTCGACTCATGCTGTCTG TCACATCCTTTAG3'
pGEX-6P-1- SCaBP8 <sup>S237A</sup>	5'GGGATCCATGGAACAAGTTT CCTCTAG3'	5'AGCGTCGACTCAGTCTTCAA CCTCAGTGTTGAATATAAAGG CTGGGAATGCTGTCGTCAC3'
pGEX-6P-1- SCaBP8 <sup>S237D</sup>	5'GGGATCCATGGAACAAGTTT CCTCTAG3'	5'AGCGTCGACTCAGTCTTCAA CCTCAGTGTTGAATATAAAAT CTGGGAATGCTGTCGTC3'
pGEX-6P-1- SCaBP8 <sup>T242A</sup>	5'GGGATCCATGGAACAAGTTT CCTCTAG3'	5'AGCGTCGACTCAGTCTTCAA CCTCGGCGTTGAATATAAAAC TTGG3'
pGEX-6P-1- SCaBP8 <sup>S237A T242A</sup>	5'GGGATCCATGGAACAAGTTT CCTCTAG3'	5'AGCGTCGACTCAGTCTTCAA CCTCGGCGTTGAATATAAAGG CTGGGAATGCTGTCGTCAC3'
pGEX-6P-1-SOS2 FISL motif	5'CGGGATCCATGATGAATGCC TTTGAGATG3'	5'AGCGTCGACTCAGTCAAATA GTGCAGATAAAA3'
pGADT7-SOS2	5'TGGAATTCATGACAAAAGAA AATGAGAAG3'	5'CGGGATCCTCAAACGTGAT TGTCTGAG3'
pGBKT7-SCaBP8 / SCaBP8 <sup>S237A</sup> / SCaBP8 <sup>S237D</sup>	5'TGGAATTCATGGAACAAGTT TCCTCTAG3'	5'AGCGTCGACTCAGTCTTCAA CCTCAGTGTT3'