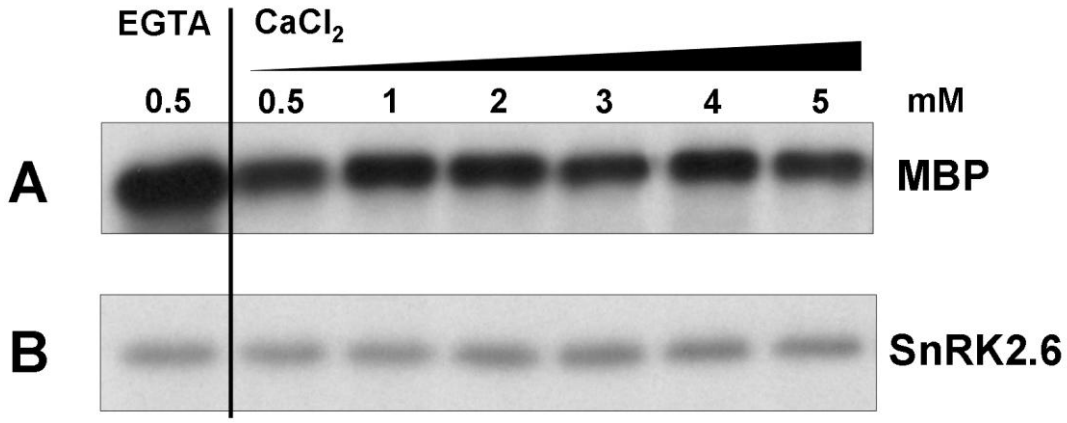
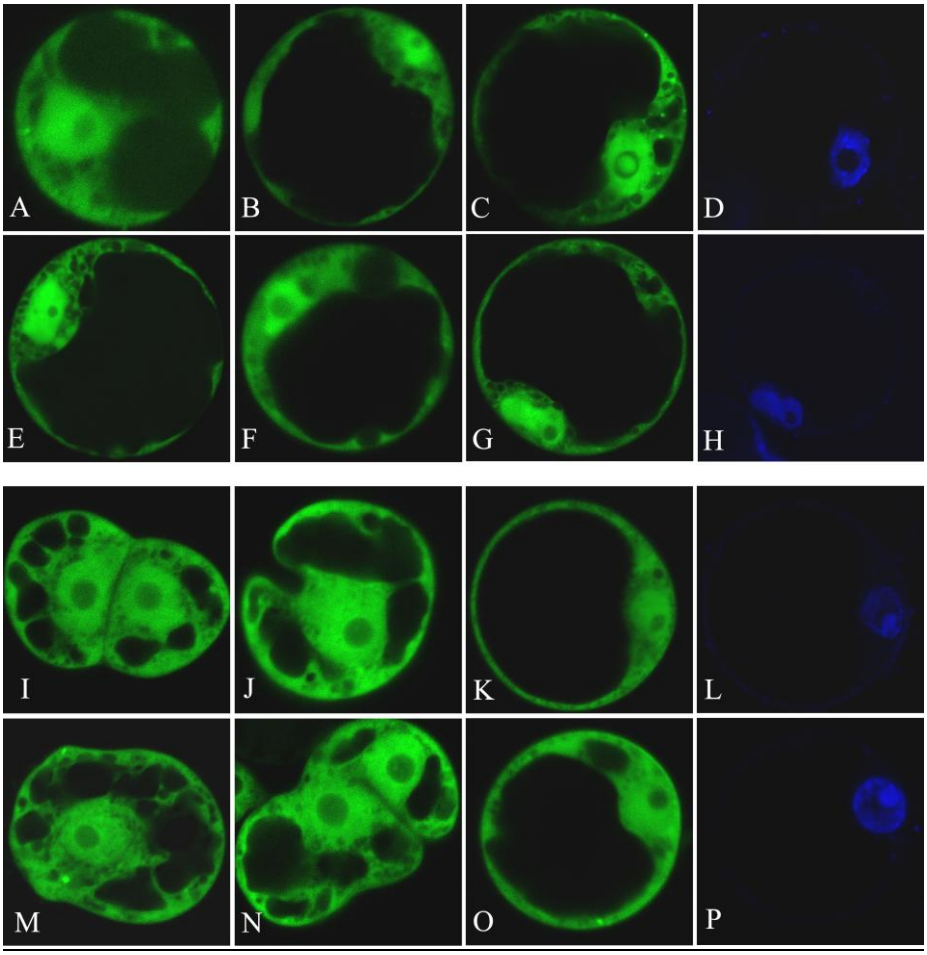
Supplementary data

FIGURE S1



Supplemental Fig. S1
Effect of calcium ions on activity of recombinant SnRK2.6
MBP phosphorylation catalyzed by SnRK2.6 (A) and the kinase autophosphorylation (B) was analyzed in absence (0.5 mM EGTA) or presence of $CaCl_2$ (up to 5 mM).
Kinase activity and autophosphorylation were monitored using MBP and [γ^{32}P]ATP as substrates. Reaction products were separated by SDS-PAGE and MBP or SnRK2.6 phosphorylation was determined by autoradiography.

FIGURE S2



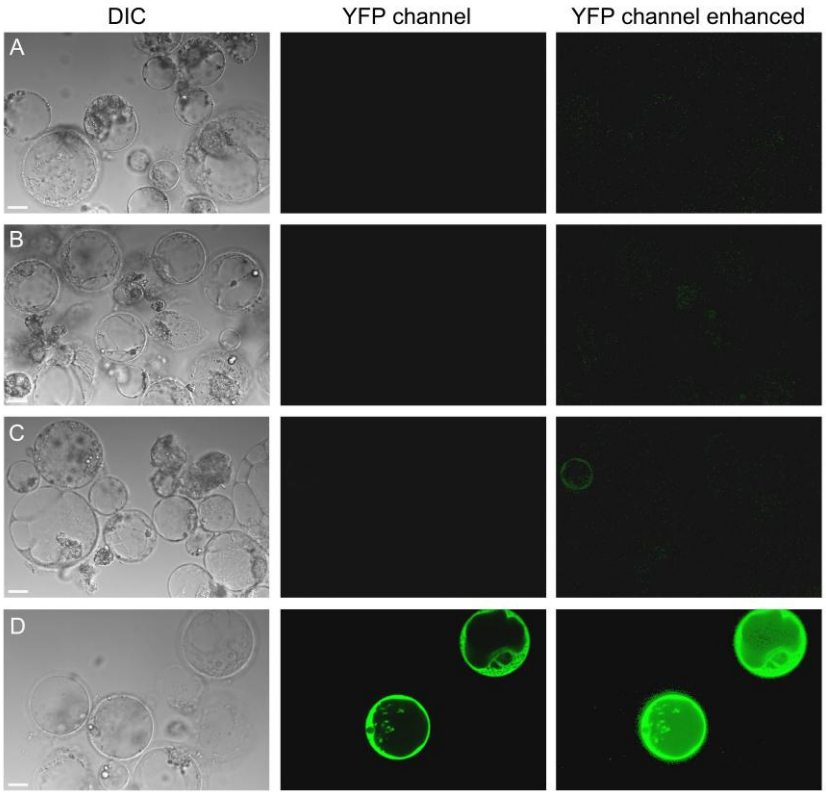
Supplemental Fig. S2

Subcellular localization of SnRK2 kinases and SCS from tobacco and Arabidopsis

Proteins were produced in plant cell protoplast (tobacco proteins in BY-2 protoplasts and Arabidopsis proteins in T87 protoplasts) as EGFP fusion and their localization was monitored by confocal microscopy as it was described in Experimental Procedures. T87 protoplasts expressing: EGFP-SnRK2.4 (A, E); EGFP-SnRK2.6 (B, F); EGFP-SnRK2.8 (C, G); EGFP-AtSCS (K, O) not treated (A,B, C, K) or treated with 300 mM sorbitol (E, F, G, O). BY-2 protoplasts expressing: EGFP-NtOSAK (I, M) or EGFP-NpSCS (J, N) not treated (I, J) or treated with 300 mM sorbitol (M, N). The images of C, G, K, O with nuclei stained using Hoechst 33342 are presented in D, H, J, L, respectively.

In the figure there are shown selected pictures of protoplasts from three independent experiments giving similar results.

FIGURE S3

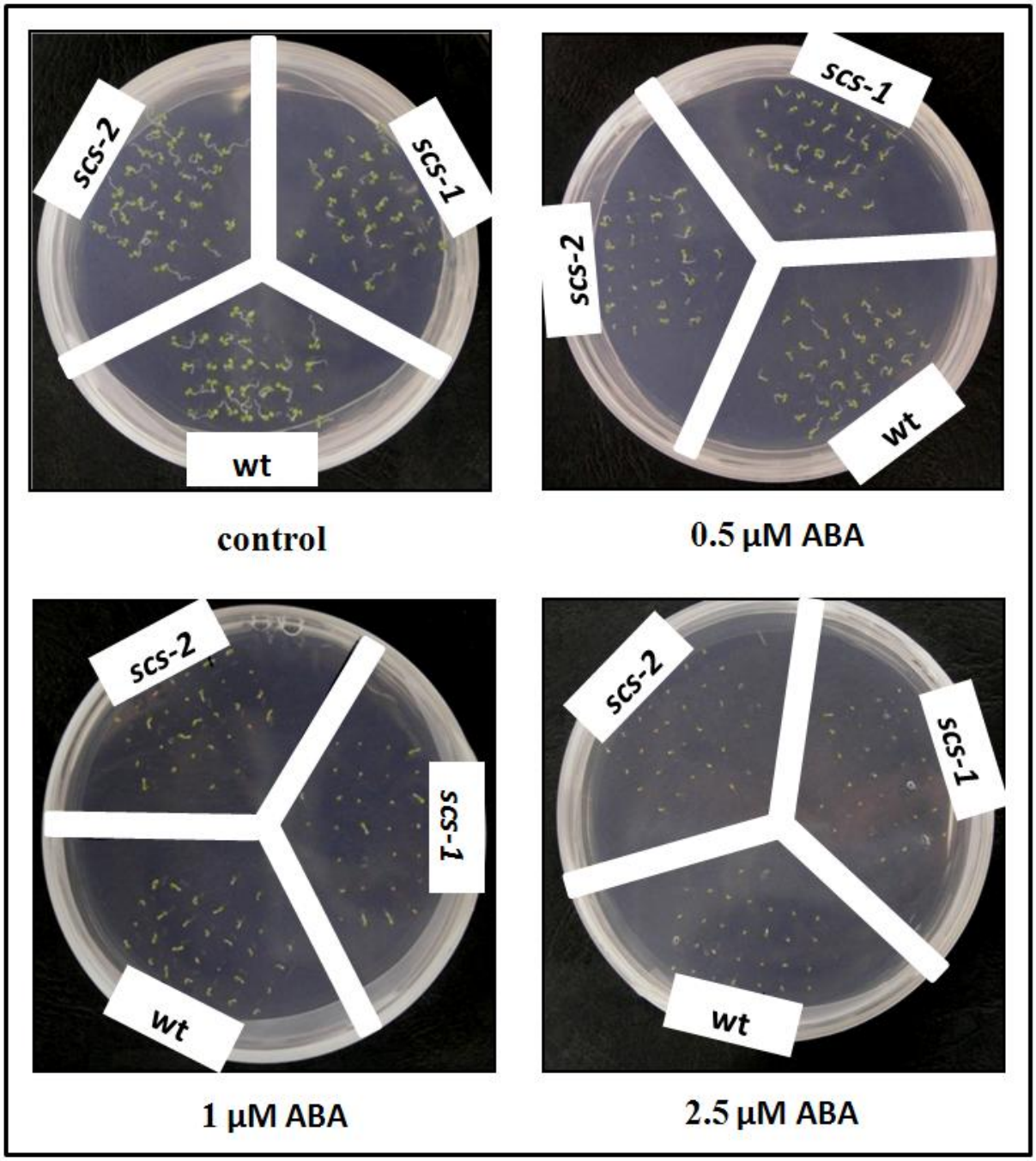


Supplemental Fig. S3

Controls for BiFC assay

Arabidopsis T87 protoplasts were transfected with following constructs: A, pSAT4-nEYFPC1-AtSCS and pSAT4-cEYFPC1-B-AtSCS; B, pSAT4-nEYFPC1-SnRK2.4 and pSAT4-cEYFPC1-B; C, pSAT4-nEYFPC1 and pSAT4-cEYFPC1-B-AtSCS; D, pSAT4-nEYFPC1-SnRK2.4 and pSAT4-cEYFPC1-B-AtSCS as positive result. The first column – DIC image; the second column – image of YFP signal; the third column – image of YFP signal extremely enhanced, so the weak florescence from the background is slightly visible. Bars; 10 μm.

FIGURE S4



Supplemental Fig.S4

The effect of ABA on seedlings green cotyledons formation of scs mutants and wild type Arabidopsis. Surface sterilized *scs-1*, *scs-2*, and wild type (wt) seeds were sawn on Petri dishes containing ½MS medium supplemented with different ABA concentrations or without ABA (control). Seeds were vernalized for five days and grown in a phytothrone in a long day conditions. After 6 days of growing photographs were taken and germinated seeds formed green cotyledons were calculated.

List of primers used in this study

Primers used to amplify cDNAs for constructs used for two hybrid assay:

F:TH-NtOSAK	5’-CCGGAATTCATGGATAAATACGAGCTTGTG-3’
R:TH-NtOSAK	5’-ATTGTCGACTTAGGTGAGACGAACTTCTCC-3’
F:TH- SnRK2.4ΔC	5’-CCGGAATTCATGGACAAGTACGAGCTGGTG-3’
R:TH-SnRK2.4ΔC	5’- AAAGTCGACCTACGGTGTTTTGGCGTCAGC-3’,
F:TH-AtSCS	5’-ATAGGATCCAAATGGACTTGAAAAGCAACA-3’
R:TH-AtSCS	5’-TGCGTCGACTTAGTTTGAAGGCTCCTCTGT-3’

Primers used to amplify cDNAs for constructs used for production of recombinant proteins in *E.coli* :

F: GST-SnRK2.4	5’-CCGGAATTCATGGACAAGTACGAGCTGGTG-3’
R: GST-SnRK2.4	5’-ATCGTCGACTCAACTTATTCTCACTTCTCC-3;
F: GST-SnRK2.6	5’-ACGGAATTCATGGATCGACCAGCAGTGAGT-3’
R: GST-SnRK2.6	5’-ACCGTCGACTCACATTGCGTACACAATCTC-3’
F: GST-SnRK2.8	5’-TCAGAATTCATGGAGAGGTACGAAATAGTG-3’
R: GST-SnRK2.8	5’-TATGTCGACTCACAAAGGGGAAAGGAGATC-3’
F: GST-AtSCS	5’-ACCGGATCCATGGACTTGAAAAGCAACAAC-3’
R: GST-AtSCS	5’-TGCGTCGACTTAGTTTGAAGGCTCCTCTGT-3’
R: GST-AtSCS936	5’-TATGTCGACTTACACCATCTTCAACGCTTCCAT-3’
F: GST-AtSCS331	5’-ATAGGATCCATGGCTGCTGGTCTGAAACGT-3’

Primers used to amplify cDNAs for constructs used for BiFC assay :

F: BiFCNtOSAK	5’-GTCCTCGAGATAAATACGAGCTTGTGAAAG-3’
R: BiFCNtOSAK	5’-ATTGTCGACTTAGGTGAGACGAACTTCTCC-3’;
F: BiFCSnRK2.4	5’-TCCGAATTCGGACAAGTACGAGCTGGTGAAA-3’
R: BiFCSnRK2.4	5’-ATCGTCGACTCAACTTATTCTCACTTCTCC-3’;
F: BiFCSnRK2.6	5’-TCTTCGAATTCGGATCGACCAGCAGTGAGT-3’
R: BiFCSnRK2.6	5’-ACCGTCGACTCACATTGCGTACACAATCTC-3’;
F: BiFCSnRK2.8	5’-TCAGAATTCGGAGAGGTACGAAATAGTG-3’
R: BiFCSnRK2.8	5’-TATGTCGACTCACAAAGGGGAAAGGAGATC-3’;
F: BiFCAtSCS	5’-ACCAGATCTATGGACTTGAAAAGCAACAAC-3’
R: BiFCAtSCS	5’-TGCGTCGACTTAGTTTGAAGGCTCCTCTGT-3’;
F: BiFCNpSCS	5’-ACCAGATCTATGGACGGTTCAGATATAATG-3’
R: BiFCNpSCS	5’-TAGGAATTCCATCTCAGAATTACATGGAGG-3’.

Primers used for selection of homozygous *scs* plants:

LP-104688	5’-GTATGGCTGCTGGTCTGAAAC-3’
RP-104688	5’-TTATTTACCTGGGACACAGCG-3’
LP-051356	5’-TCTTTTGGAATCGTCTCCATG-3’
RP-051356	5’-ATGGAAACCCCAAATTACGTC-3’
LBb1.3	5’-ATTTTGCCGATTTCGGAAC-3’

Primers used for RT-PCR

At4g38810.1f361	5’-GGTGATGAGCAACATCGTAGAACCA-3’
At4g38810.1r350	5’-CCCTCGAGCTGCAACATTATACTCC-3’