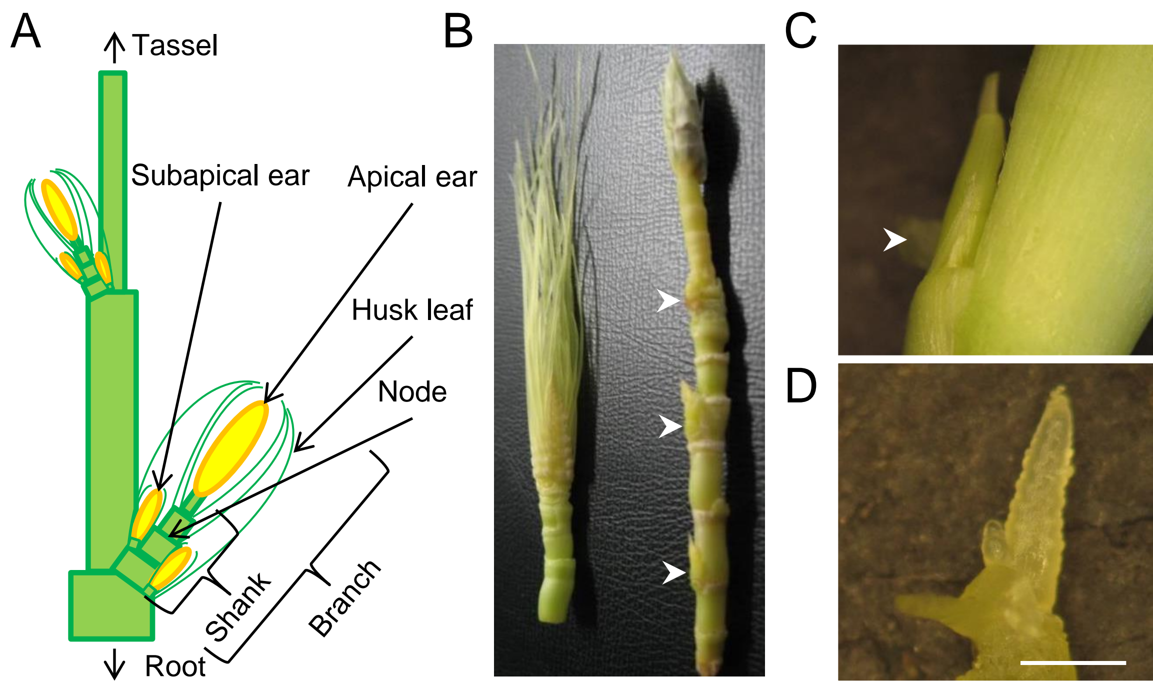

Supporting Figure S1. Loss of apical dominance due to infection by *S. reilianum*.

(A) Schematic view of the maize stem showing outgrowth of subapical ears. (B) Phenotype of female inflorescences of a healthy (left) and an *S. reilianum*-infected maize plant (right). On the shank of the infected ear, outgrowth of subapical ears is apparent (arrowheads). Not all infected ears display long shanks. Ears with short shanks also display outgrowth of subapical ears which come off when removing the husk leaves. See also (Ghareeb et al., 2011). (C) Magnification of a subapical ear covered in husk leaves. (D) Subapical ear from (C) after removal of husk leaves, scale bar = 1 mm.

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  SSSSSSSSSSSSSSSSSSSSMMMMMMMMMMMM
1-MNLLPFKLVLALSLLLLTLSGCHCASGSQSGGGNHNVEVDEQAAQDLRARVAHLERHFKL- 60

                             PPP                   PPPUUUUUUUKUU
61-GPNWFGETRGPHVAQEVARSQLRLTSAKFMVHHDPADYRFNLAYTPVSYRMPDSADWKHG-120

    UUUUU                                      CCCC
121-ILMLRVVPHQPTQVLDLVDFPEGLAQREVEGYWGRFYGAVDKTREDILREYGEPALHLSV-180
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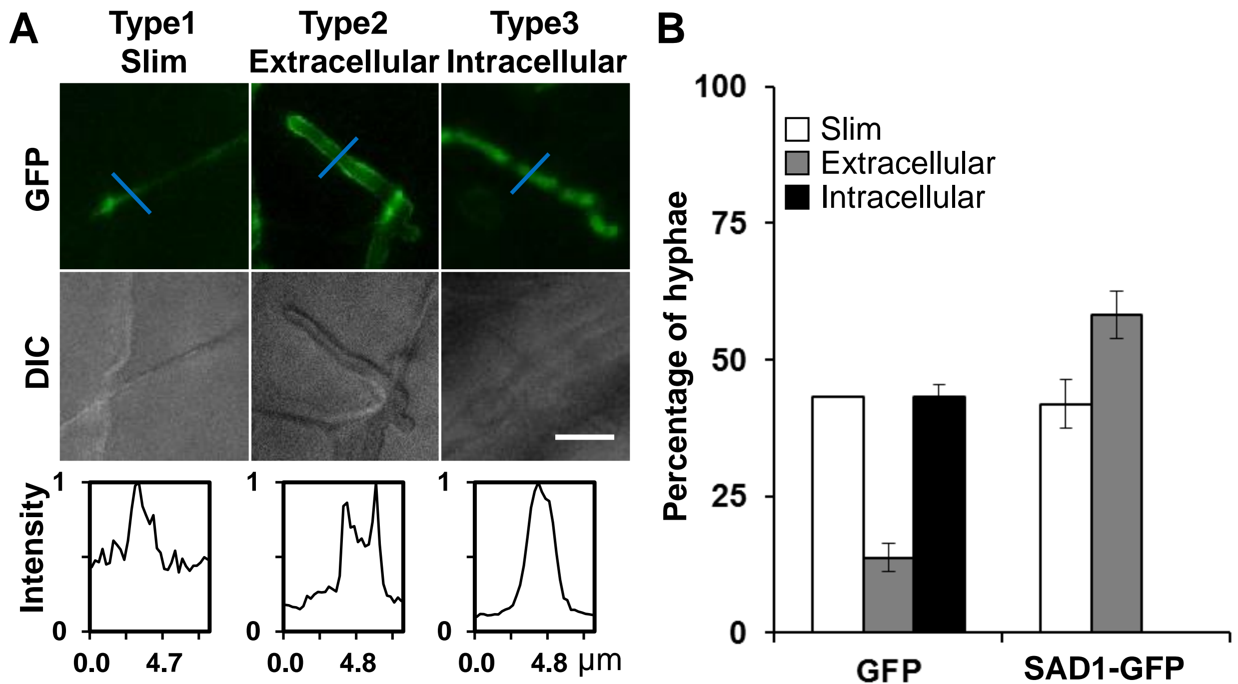
Supporting Figure S2. SAD1 amino acid sequence and domain analysis.

Predicted domains include a secretion signal peptide (S; yellow; SignalP 4.1), two overlapping N-myristoylation sites (M; purple; PROSITE), two protein kinase C phosphorylation sites (P; green; PROSITE), an ubiquitination recognition domain (U; red; BDM-PUB) including a predicted ubiquitination site (K; red; BDM-PUB), and a casein kinase II phosphorylation site (C; olive; PROSITE), as indicated above the amino acid sequence.

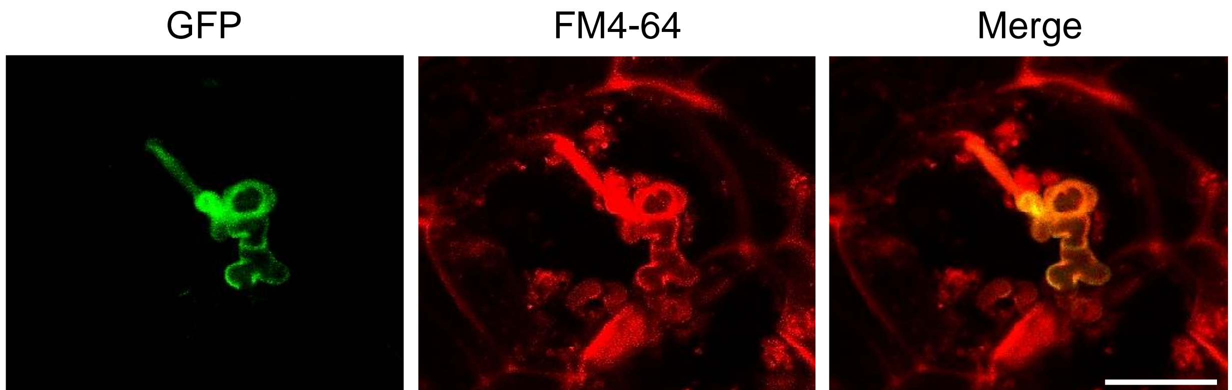


Supporting Figure S3. *S. reilianum* strains expressing SAD1-GFP show extracellular GFP fluorescence.

Maize leaves infected with *S. reilianum* strains expressing GFP or SAD1-GFP.

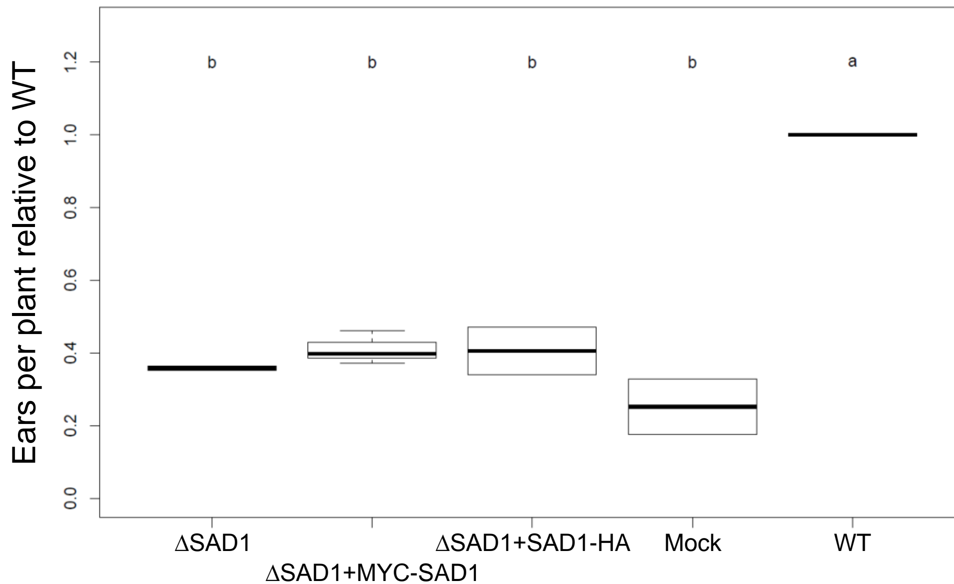
(A) Top panel shows *S. reilianum* hyphae with either extracellular (left) or intracellular (middle) or Slim hyphae with a weak GFP signal (right). Bottom shows the fluorescence intensity profiles associated to the region of interest (ROI, blue) indicated in the GFP pictures.

(B) Quantification of occurrences of the GFP signals represented in (A). GFP-expressing hyphae show mostly intracellular fluorescence signals. Hyphae expressing SAD1-GFP show mostly extracellular GFP signals. Slim hyphae with weak GFP signals made up to 50% of the all hyphae in both strains. 50 – 100 Hyphae were counted in two experiments of 5 different plants. Totally N = 148 hyphae were counted for GFP and N = 128 for SAD1-GFP. Errorbars indicate the SEM. Bars = 10 µm. Pictures are Z-stacks of fluorescence microscope images.



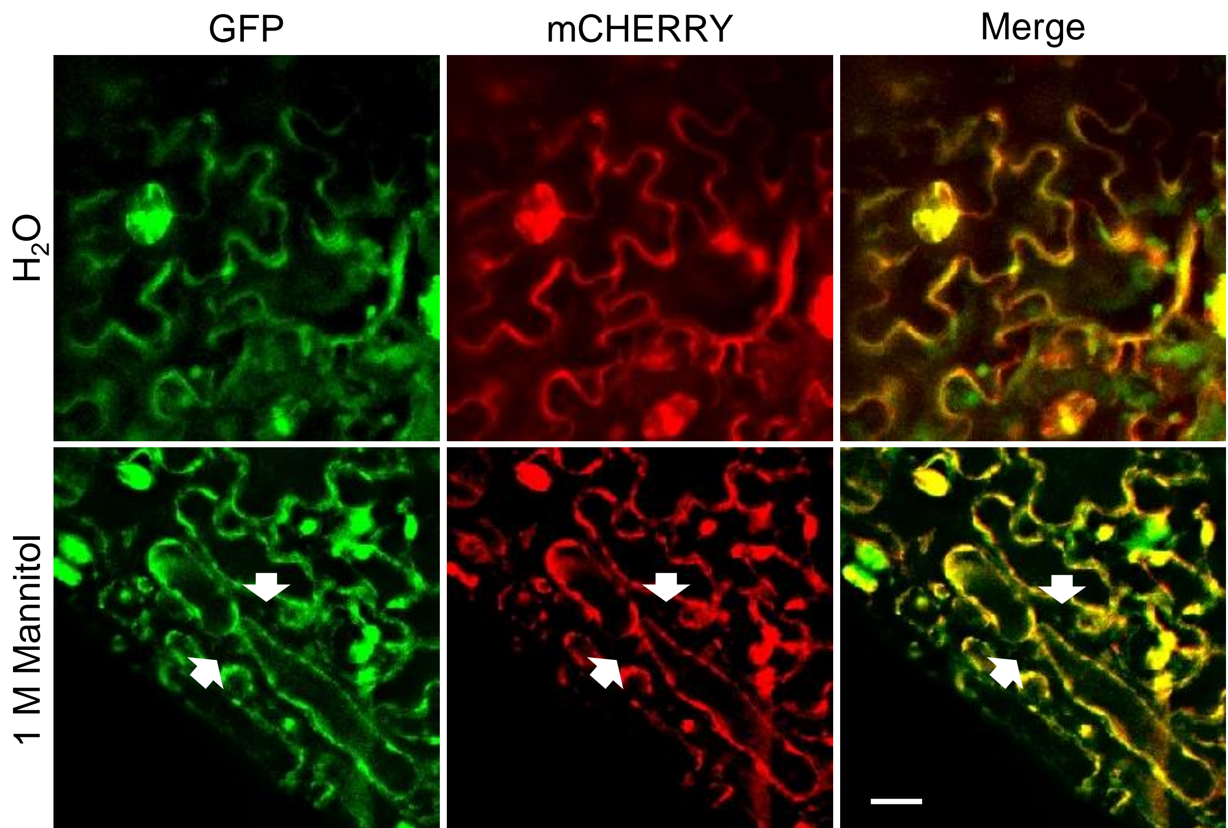
Supporting Figure S4: Colocalization of SAD1-GFP fluorescence with the membrane-staining dye FM4-64.

A hand section of a maize inflorescence colonized by *S. reilianum* expressing SAD1-GFP was stained with FM4-64, and fluorescence was visualized using a confocal microscope (Leica). Left, GFP fluorescence; middle, FM4-64 fluorescence; right, merge. Bar = 10 µm. Pictures are Z-stacks of confocal images.

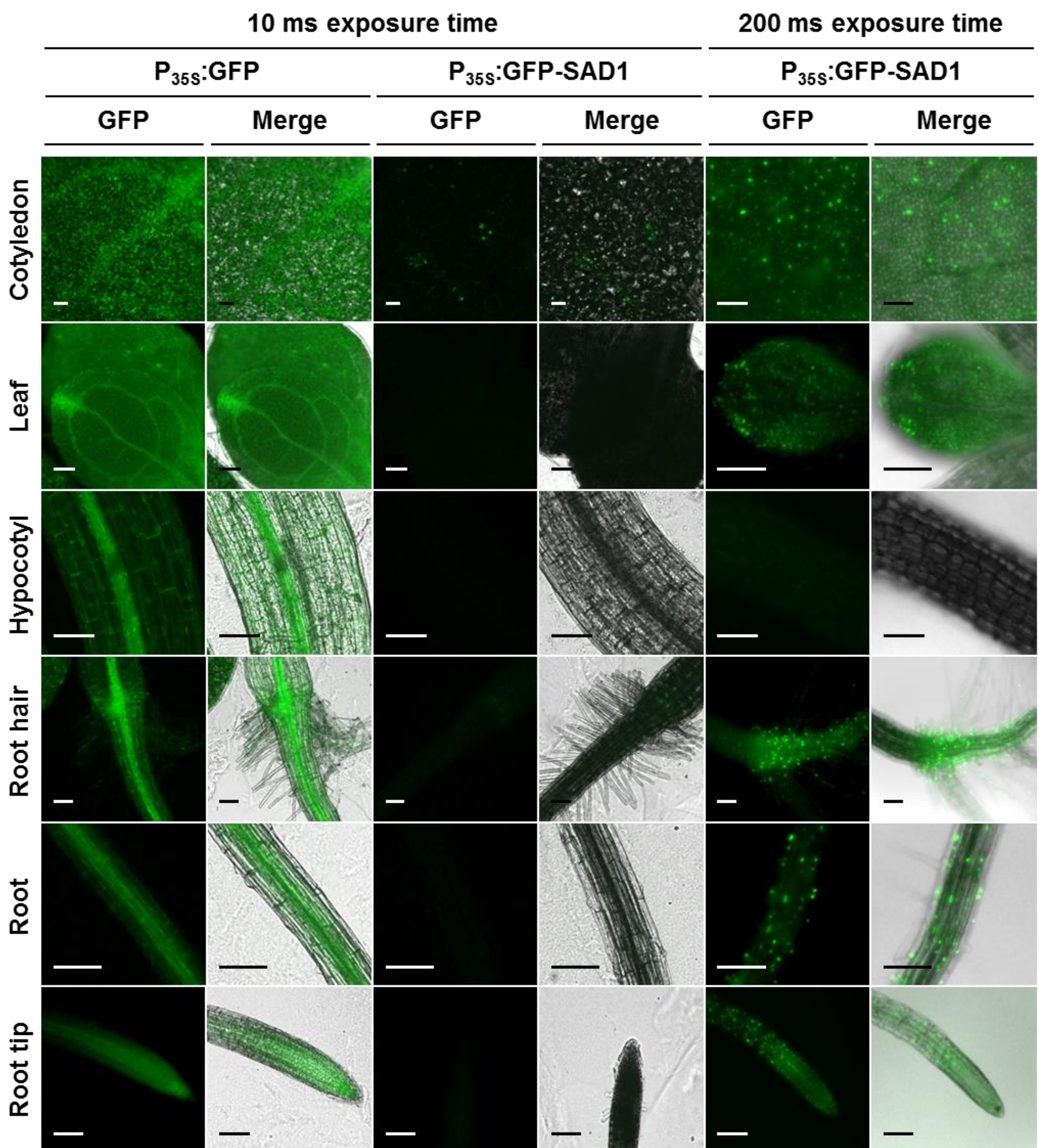


Supporting Figure S5. Number of ears per plant of maize plants inoculated with *S. reilianum* strains expressing SAD1 tagged with MYC or HA.

Boxplot showing ears per plant relative to wild type-inoculated maize plants. Plants were inoculated with water (Mock), wild type *S. reilianum,* or *S. reilianum* strains lacking SAD1 (ΔSAD1), or additionally expressing MYC-SAD1 (ΔSAD1+MYC-SAD1), or SAD1-HA (ΔSAD1+SAD1-HA). Values are means of two individual infection experiments with 25 plants each per *S. reilianum* strain. Significant difference was tested by ANOVA and tukey test with $p < 0.05$. Groups that show significant differences are marked with different letters.

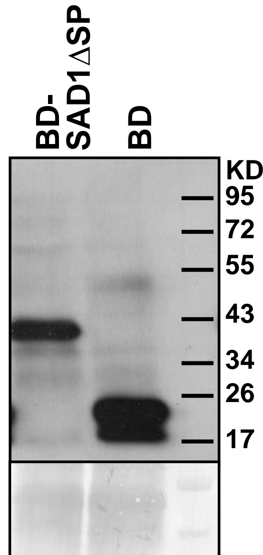


Supporting Figure S6. GFP-SAD1 does not localize to the cell wall in transgenic *A. thaliana* expressing GFP-SAD1 and mCHERRY.
Plasmolysis of *A. thaliana* leaf cells from plants expressing GFP-SAD1 and mCHERRY under the control of the 35S promotor. Samples were incubated for 2 hours in water or 1 M mannitol. After Plasmolysis (bottom panels) signals of GFP-SAD1 (left panels) and mCHERRY (middle panels) do no longer show the interconnected pavement cell structure that is visible for water treated samples (top panels). Instead, cells treated with 1 M Mannitol show a GFP and an mCHERRY signal that co-localize in a globular way (merge, right panels). This indicates that GFP-SAD1 does not localize in the apoplast or the cell wall. Arrows indicate places where the plasma membrane looses contact to the cell wall. Bar = 20 µm.



Supporting Figure S7: Microscopic overview of GFP-SAD1ΔSP distribution in transgenic *A. thaliana* plants.

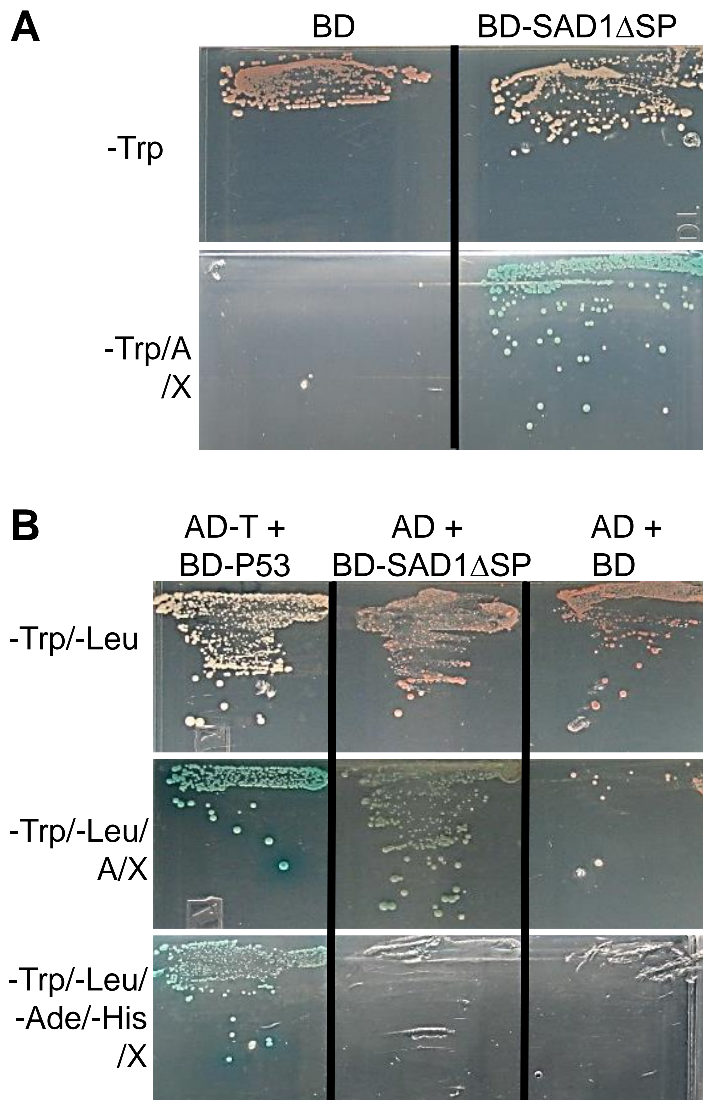
12 day old *A. thaliana plants* expressing GFP (Left) or GFP-SAD1ΔSP (Middle) are shown in the columns on the left side. Microscopic pictures in the left and middle columns were taken with an exposure time of 10 ms. Plants expressing GFP (left) show a fluorescence signal in every part of the plant. Plants expressing GFP-SAD1ΔSP show faint fluorescence signals in the root and strong signals in the guard cells of stomata. At 200 ms exposure time, fluorescence signals of GFP-SAD1ΔSP expressing plants (right) show a different localization than that of GFP expressing plants (left). Fluorescence of GFP-SAD1ΔSP can be detected in the guard cells and the surrounding cells in cotyledons, and in some parts of leaves. In root cells, the GFP signal is present in all cells. No GFP signal was detected in the hypocotyl of GFP-SAD1ΔSP expressing plants. Bars = 100µm. Part of the figure is represented in Figure 6A.



Supporting Figure S8. Immunodetection of BD-SAD1ΔSP and BD in protein extracts of Y2H-Gold strains containing the plasmids pGBKT7-BD-SAD1ΔSP and pGBKT7-BD, respectively, with antibody specific for c-MYC (top).

As a loading control, the membrane was stained with Coomassie Brilliant Blue (bottom). The expected sizes for BD-SAD1ΔSP and BD are 41 and 20 kD, respectively.

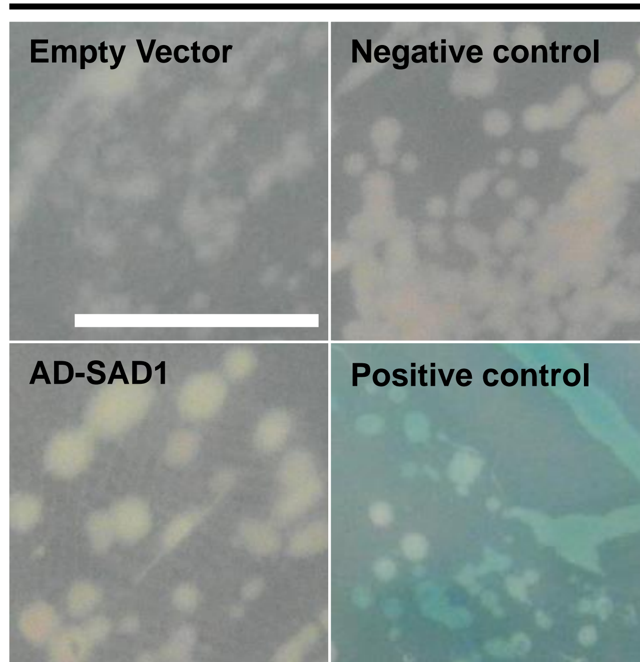
Proteins from yeast were extracted according to the Clontech manual. Proteins were transferred to a PVDF membrane using electro-wet-blotting (Bio-Rad). The membrane was washed for 5 min in TBS-T (10 mM Tris-HCl, 150 mM NaCl, pH 7.5, 0.05% Tween 20) before membranes were blocked for 1 h at room temperature in TBS-T containing 5% blotting grade milk powder (Roth). The membrane was washed briefly with TBS-T, incubated with either anti-GFP polyclonal or anti-c-Myc mouse monoclonal antibodies (1:5000 in TBS-T with 2% milk powder), washed with TBS-T, and incubated for 1 h with a secondary antibody, a horseradish peroxidase-conjugated goat anti-mouse antibody, diluted 1:5000 in TBS-T with 2% milk powder. For detection of chemiluminescence signal the ECL Plus Western Blot detection kit (GE Healthcare) was used. To confirm equal protein loading, the membrane was stained with coomassie brilliant blue solution for 1-2 min and then destained until a good contrast was obtained.



Supporting Figure S9. Transcriptional activation of reporter genes in yeast by SAD1.

(A) Colonies of yeast strains expressing the GAL4 binding domain (BD) were able to grow on medium lacking tryptophan (-Trp) (top left) but not on medium containing Aureobasidin A and α-x-GAL (-Trp/A/X) (bottom left). Colonies of the BD-SAD1ΔSP expressing yeast strain grew on -Trp medium (top right) and showed blue color on medium -Trp/A/X (bottom right) reflecting auto-activation of the reporter genes *AUR1-C* and *MEL1* by SAD1.

(B) Yeast diploids containing plasmids that encode the AD and BD of GAL4 only (right, negative control), or AD and the BD-SAD1ΔSP fusion protein (middle), or the known interacting fusion proteins AD-T and BD-P53 (left, positive control). Growth on medium lacking tryptophan and leucine (-Trp/-Leu) (top) indicates that the diploids contain the two plasmids expressing BD and AD. The diploid expressing BD-SAD1ΔSP + AD could grow on A/X-containing medium (middle) indicating auto-activation of *AUR1-C* and *MEL1* by SAD1 as in (A) but it could not grow on medium lacking adenine and histidine (-Ade/-His) (bottom) reflecting that SAD1 was not able to auto-activate the auxotrophy reporter genes *ADE2* and *HIS3*.



Supporting Figure S10. The fusion protein AD-SAD1 is not able to auto-activate the MEL1 reporter gene in the Yeast-two hybrid strain Y187.

All yeast strains are able to grow on medium lacking leucin and containing X-a-Gal indicating the presence of the pGADT7 plasmid . Yeast colonies that express *MEL1* turn blue in the presence of the X-a-Gal. Yeast containing the empty vector (Top left) and mated strains expressing proteins that are fused to the Gal4 binding domain (BD) or the activation domain (AD) but do not interact (Top right) do not show blue colonies. Yeast expressing two proteins that interact and which are fused to the AD or BD of Gal4 show blue colonies (bottom right). Yeast expressing AD-SAD1 fusion protein do not show blue colonies indicating that AD-SAD1 is not able to autoactivate the transcription of the reporter gene *MEL1* (bottom left). Bar = 1 cm

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                      *        20         *        40         *
ZmPIN1b : ACTTCATCTCCACCAACAACCCCTACACCATGAACCTGCGCTTCATCGCCGCCGACA :  57
ZmPIN1c : ACTTCATCTCCACCAACAACCCCTACACCATGAACCTGCGCTTCATCGCCGCCGACA :  57
ZmPIN1a : ACTTCATCTCCACCAACAACCCCTACACCATGAACATCCGGTTCATCGCCGCCGACA :  57
          ACTTCATCTCCACCAACAACCCCTACACCATGAACcTgCGcTTCATCGCCGCCGACA

           60         *        80         *       100         *
ZmPIN1b : CGCTGCAGAAGCTCATGGTGCTGGCCATGCTCACCGCGTGGAGCCACCTCAGCCGCC : 114
ZmPIN1c : CGCTGCAGAAGCTCATGGTGCTGGCCATGCTCACCGCGTGGGGCCACCTCAGCCGCC : 114
ZmPIN1a : CGCTGCAGAAGCTGATCGTCCTGGCGCTGCTCACTGCATGGAGCTACCTCTCCCGCC : 114
          CGCTGCAGAAGCTcATgGTgCTGGCcaTGCTCACcGCgTGGaGCcACCTCagCCGCC

             120         *        140         *       160         *
ZmPIN1b : GGGG------------CAGCCTGGAGTGGACCATCACGCTCTTCTCCCTCTCCACGC : 159
ZmPIN1c : GGGGGCGGGGCAGCCTCGACCTGGACTGGACCATCACGCTCTTCTCCCTCTCCACGC : 171
ZmPIN1a : GGGG------------CTGCCTCGAGTGGACCATCACGCTCTTCTCCCTGTCGACGC : 159
          GGGG            C gCCTgGAgTGGACCATCACGCTCTTCTCCCTcTCcACGC

                180         *       200         *       220
ZmPIN1b : TGCCCAACACGCTCGTCATGGGCATCCCCCTGCTCAAGGGCATGTACGGCGACTTCT : 216
ZmPIN1c : TGCCCAACACGCTCGTCATGGGCATCCCGCTGCTCAGGGGCATGTACGGCGACTTCT : 228
ZmPIN1a : TGCCCAACACGCTGGTGATGGGCATCCCGCTGCTCAAGGGCATGTACGGCGACTTCT : 216
          TGCCCAACACGCTcGTcATGGGCATCCCgCTGCTCAaGGGCATGTACGGCGACTTCT
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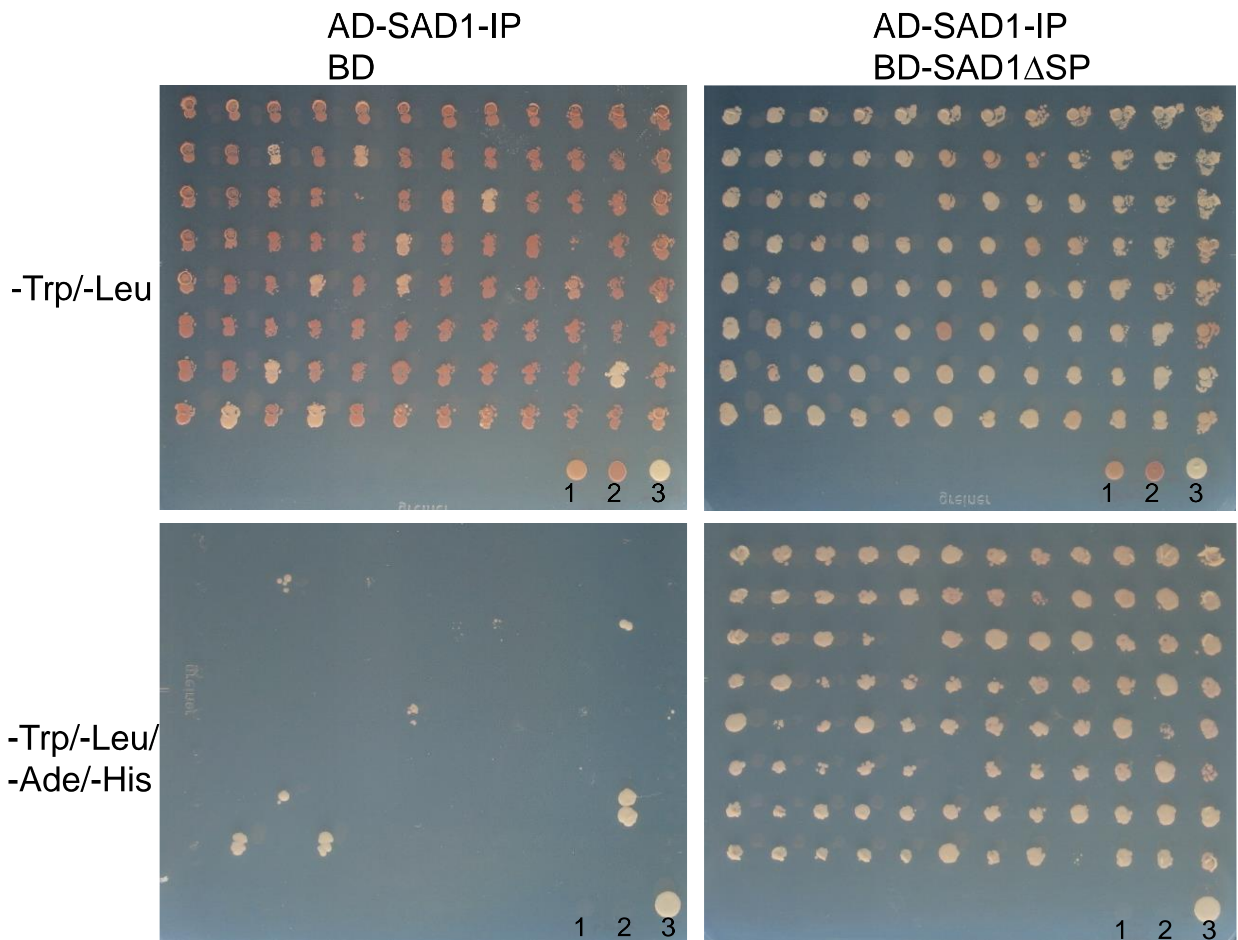
Supporting Figure S11. Nucleotide sequence alignment of qRT-PCR amplicons generated from exon1 of *ZmPIN1a*, *ZmPIN1b* and *ZmPIN1c.*

Aligned amplicons were created with the oligonucleotide primers (oHG252 ACTTCATCTCCACCAACAACCC and oHG253 AGAAGTCGCCGTACATGCCC) that targeted exon1 of the three family members *ZmPIN1a, ZmPIN1b* and *ZmPIN1c*, while *ZmPIN1d* was not amplified. Nucleotides conserved in all three amplicons are shaded in black, those conserved in two of the three sequences are shaded in grey. Below the alignment, the consensus sequence is shown, the primer binding sites used for amplification are shaded in red. The figure was generated by sequence alignment using Clustalx and alignment visualization using GeneDoc.



Supporting Figure S12. Identification of SAD1 interaction partners.

Yeast diploids expressing SAD1 interaction partners (AD-SAD1-IP) and either BD (left) or BD-SAD1ΔSP (right) were inoculated on SD/-Trp/-Leu (top) and SD/-Trp/-Leu/-Ade/-His (bottom) media. Growth on SD/-Trp/-Leu medium shows that the prey and bait plasmids are present, whereas growth on SD-Ade/-His medium shows that prey and bait interact. 1 and 2 are negative controls (1; BD-SAD1 + AD, 2; BD-LAM + AD-T), and 3 is the positive control (BD-P53 + AD-T).