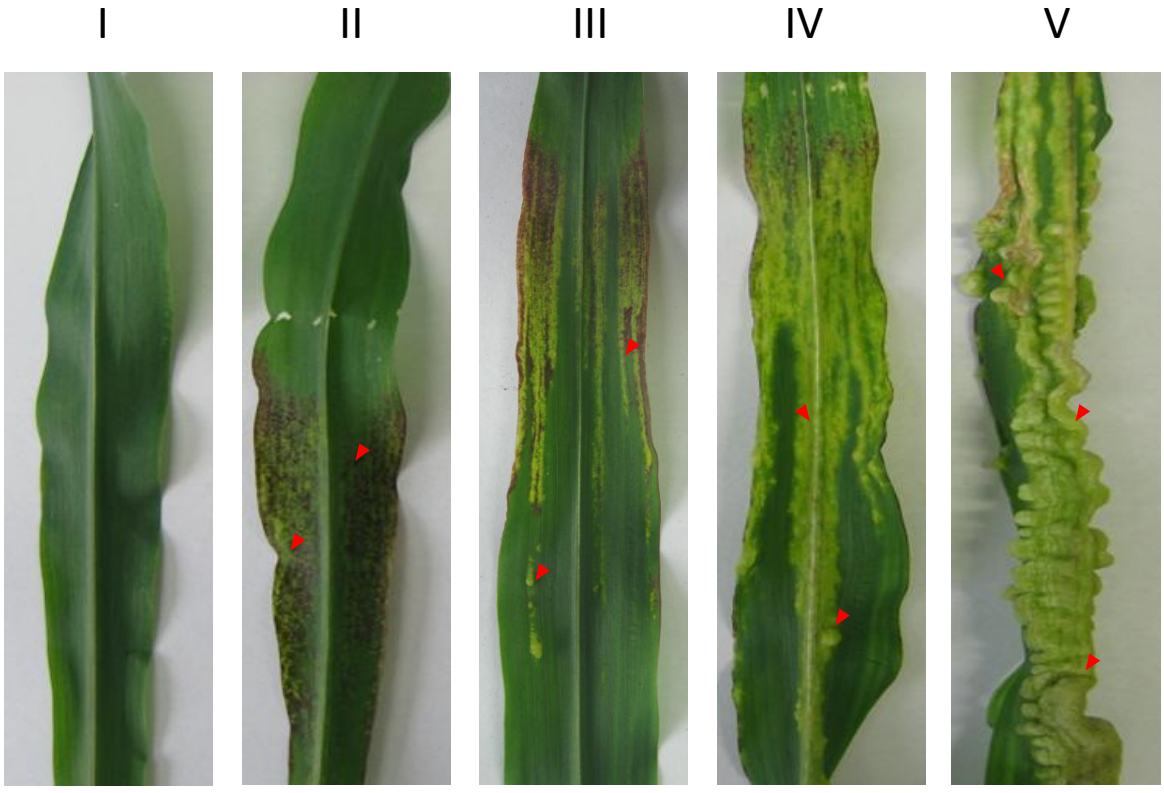
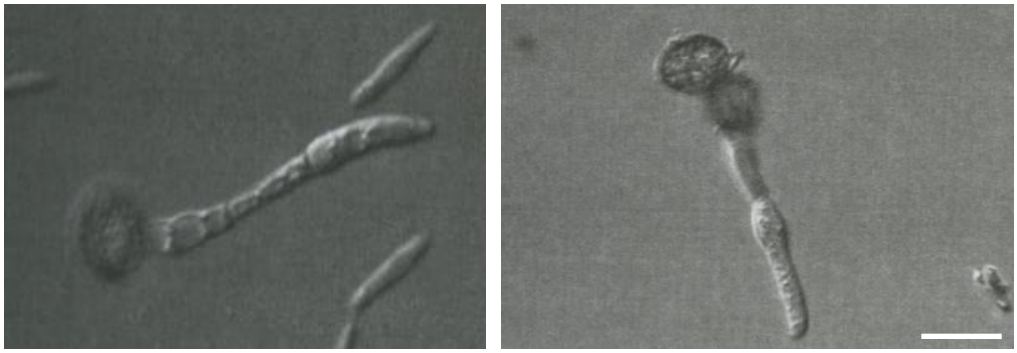


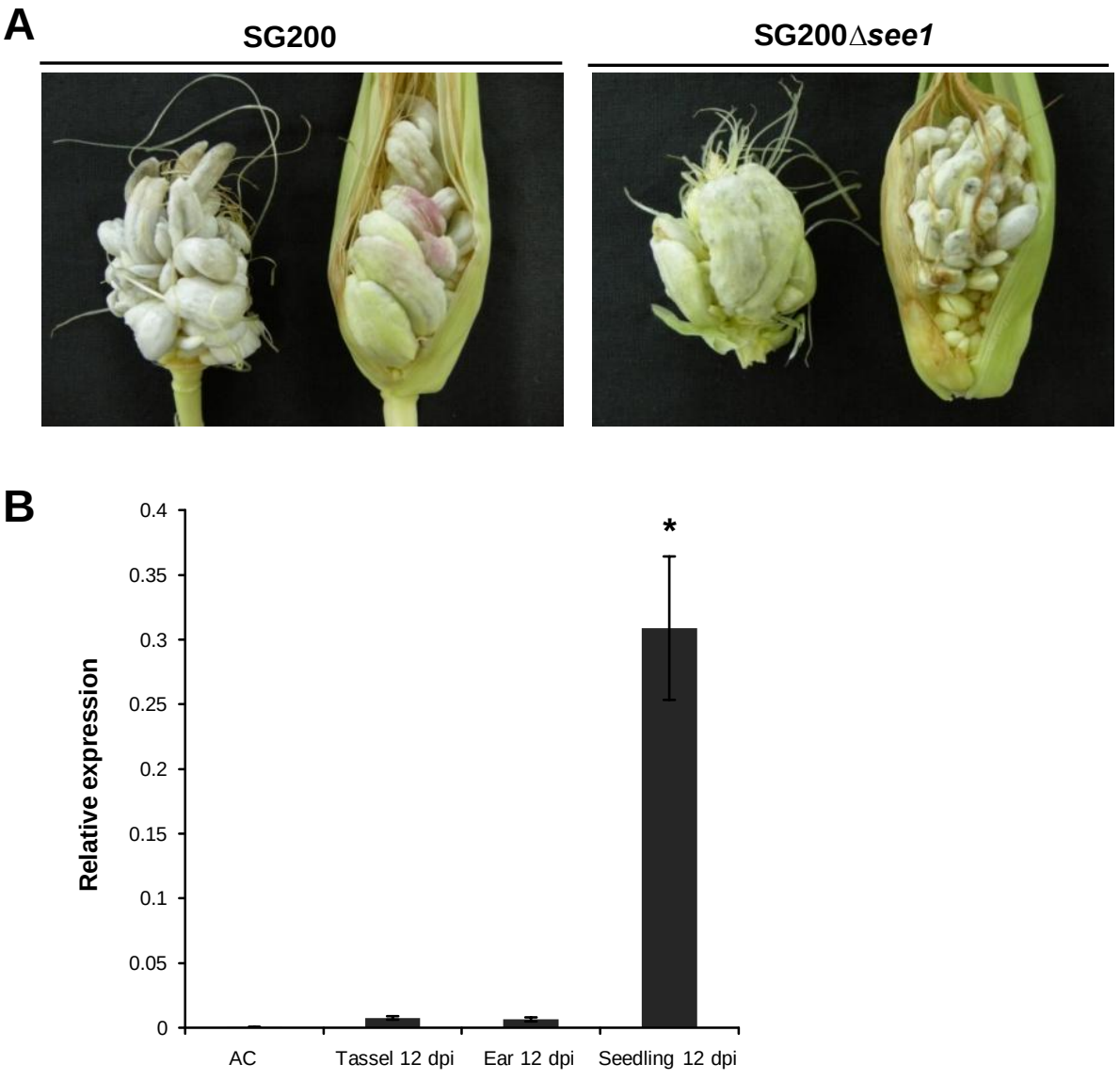
Supplemental Figure 1. Scoring Scheme for Leaf Tumors Based on Symptoms Observed at 12 dpi.

The symptoms were classified into five categories based on increasing symptom severity, and tumor size. I, No symptoms; II, Chlorosis of the leaf; III, Small tumors in range of 1-4 mm in diameter; IV, Tumors of 6-10 mm in diameter; and V, Heavy tumors > 15 mm in diameter resulting in a highly distorted leaf axis. All characteristic symptoms are marked with the red arrowheads.



Supplemental Figure 2. SG200Δ*see1* Mutant Completes its Entire Life Cycle.

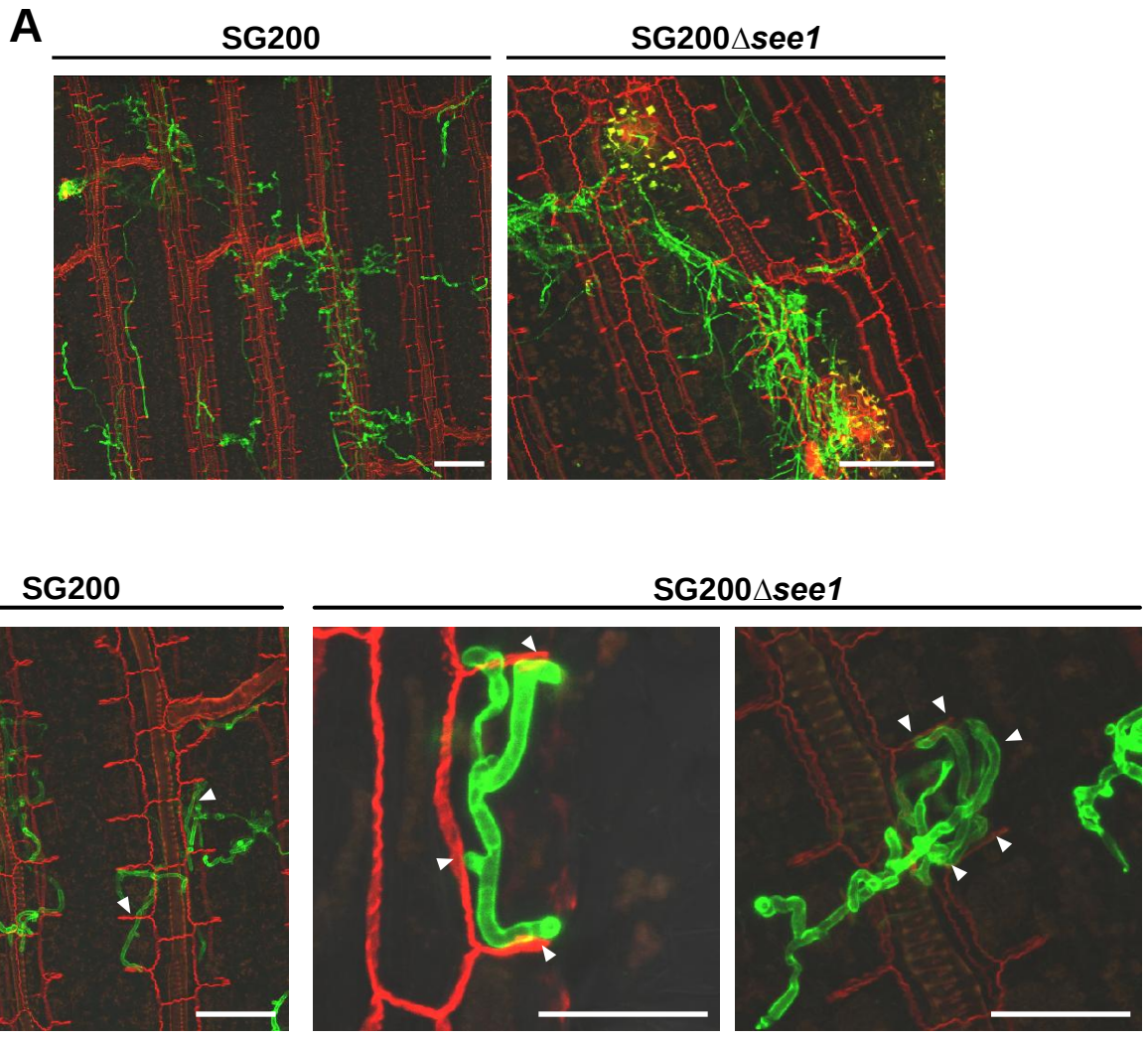
The spores formed in Anther tumors caused by the SG200Δ*see1* mutant germinate as well like the wild-type *U. maydis* strain indicating that *see1* deletion does not result in premature termination of the life cycle.



Supplemental Figure 3. Formation of Ear Tumors in SG200Δ*see1* Demonstrates the Independence of Floral Tumors on See1 Activity.

(A) Comparison of the tumor phenotype in maize ear tissue of maize infected with the wild-type strain, SG200 (left panel) and the SG200Δ*see1* mutant (right panel). Similarly to SG200, the mutant causes development of large and numerous tumors.

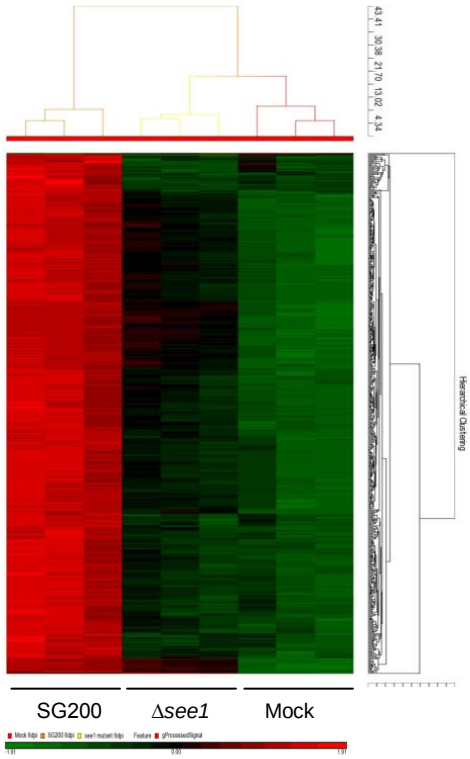
(B) The expression of *See1* was quantified by RT-qPCR to compare leaf, tassel and ear samples 12 dpi after *U. maydis* SG200 infection (for details see Methods). Error bars show SE. *, P ≤ 0.001.



Supplemental Figure 4. Growth of SG200Δ*see1* Mutant is Arrested in the Mesophyll and Vascular Cell Layers of Leaves.

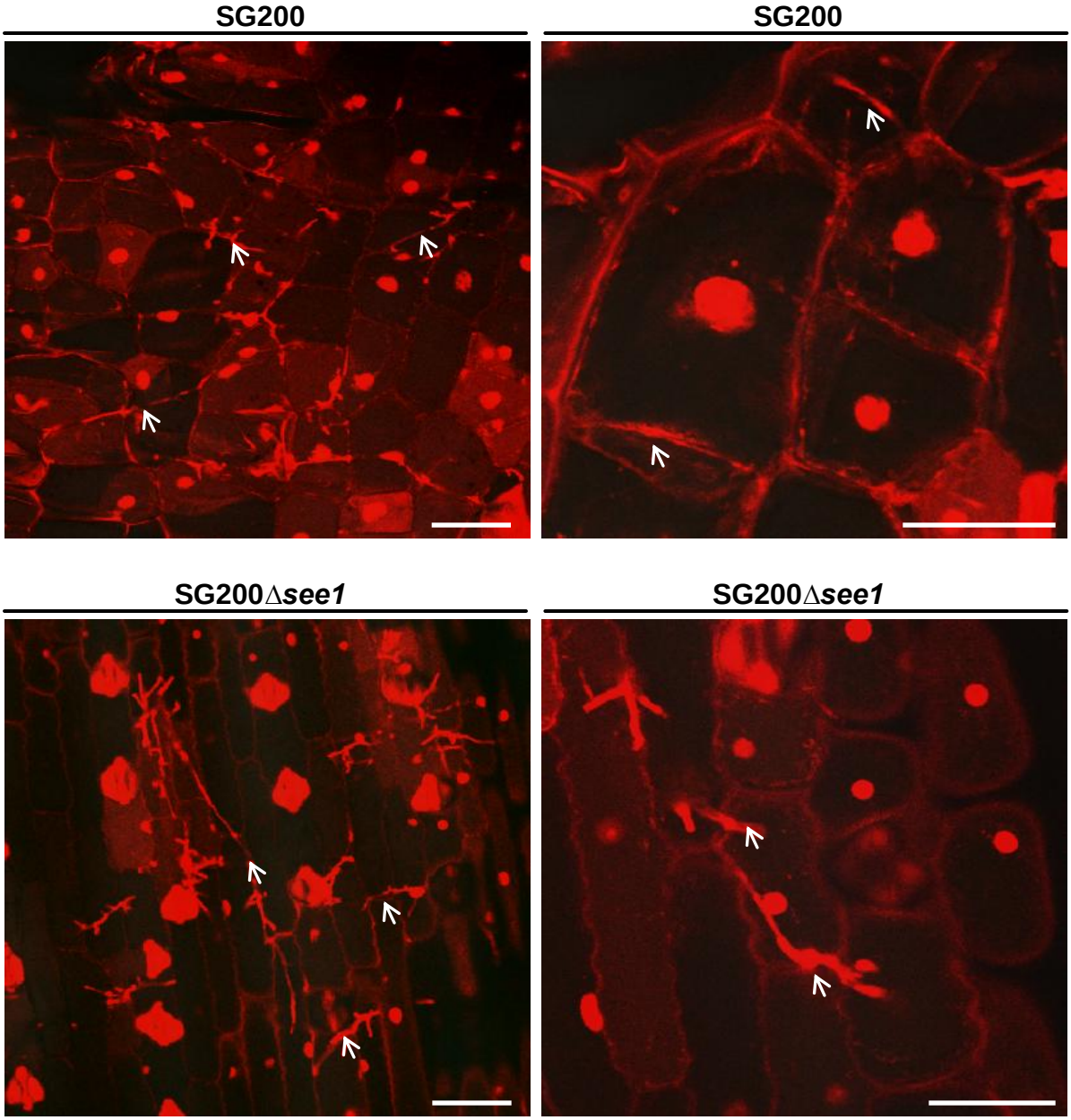
(A) Successful penetration and initial establishment of the SG200Δ*see1* mutant (right panel) in comparison to the wild-type (left panel) at 2 dpi. Bars = 50 µm.

(B) The wild-type strain SG200 proliferates extensively in the mesophyll and the vascular layers of the leaf. Hyphae pass from one cell to another as indicated by white arrowheads. The hyphae of the SG200Δ*see1*-mutant are often arrested in the initial mesophyll or vascular cells contacted; these hyphae reduced in the ability to pass from cell to cell. The SG200Δ*see1* hyphae are frequently trapped in individual cells as indicated by the white arrowheads. Bars = 50 µm.



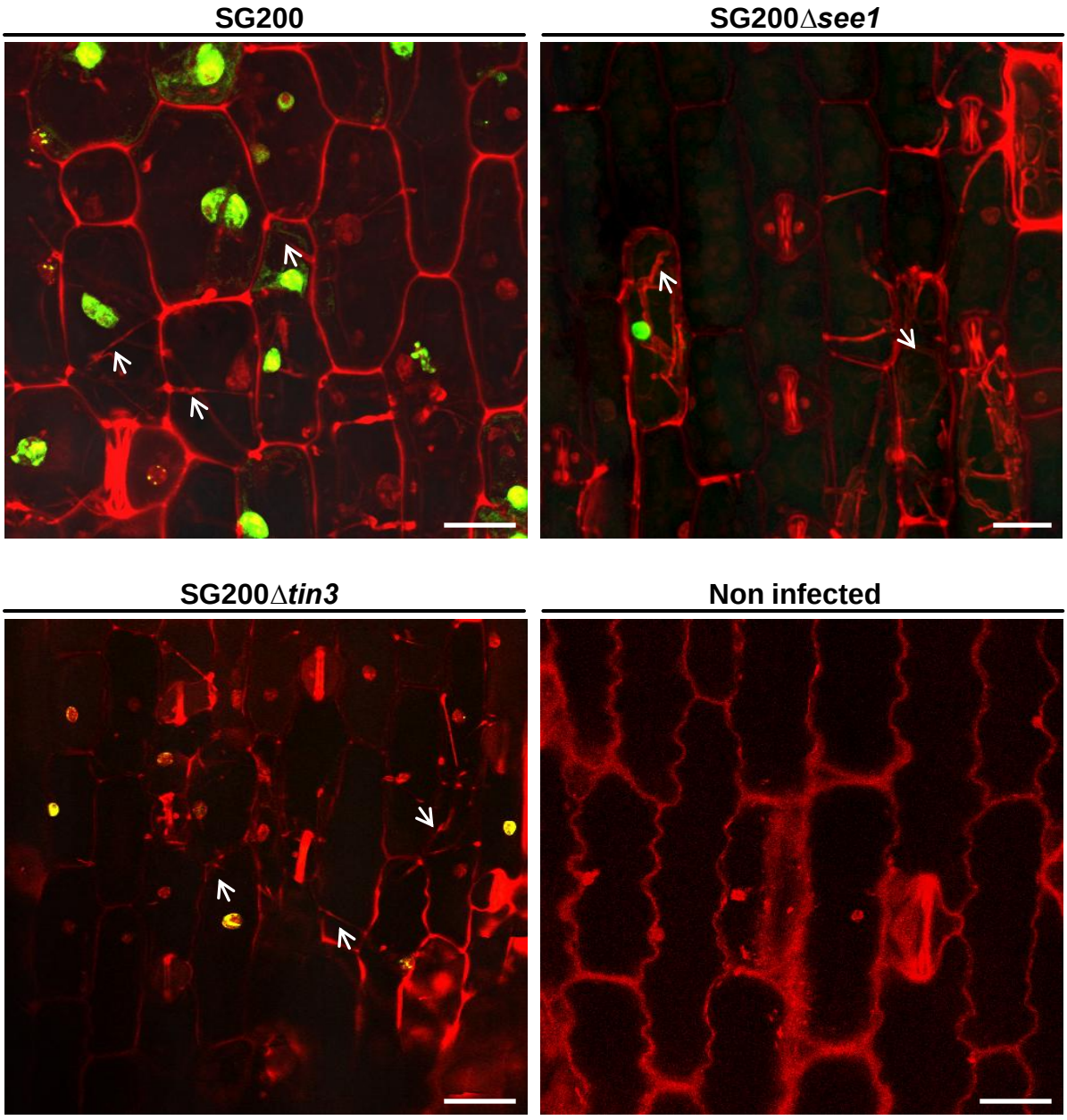
Supplemental Figure 5. Gene Expression During Maize Colonization with SG200 and SG200Δ*see1*.

Plant transcriptome analysis of SG200 (wild-type) and SG200Δ*see1* (mutant) infected tissue using microarray hybridization. Hierarchical clustering was performed by the Partek Genomics Suite version 6.12 to visualize expression of maize genes transcriptionally regulated at 6 dpi by *U. maydis* strain SG200 (left), infection by SG200Δ*see1* (middle), and mock inoculation (right) . The X-axis depicts clustering of the microarray samples for each of the three biological replicates for each treatment. The Y-axis shows clustering of the regulated maize transcripts based on similarity of their expression patterns. red: upregulated genes; green: downregulated genes; black: not significantly altered.



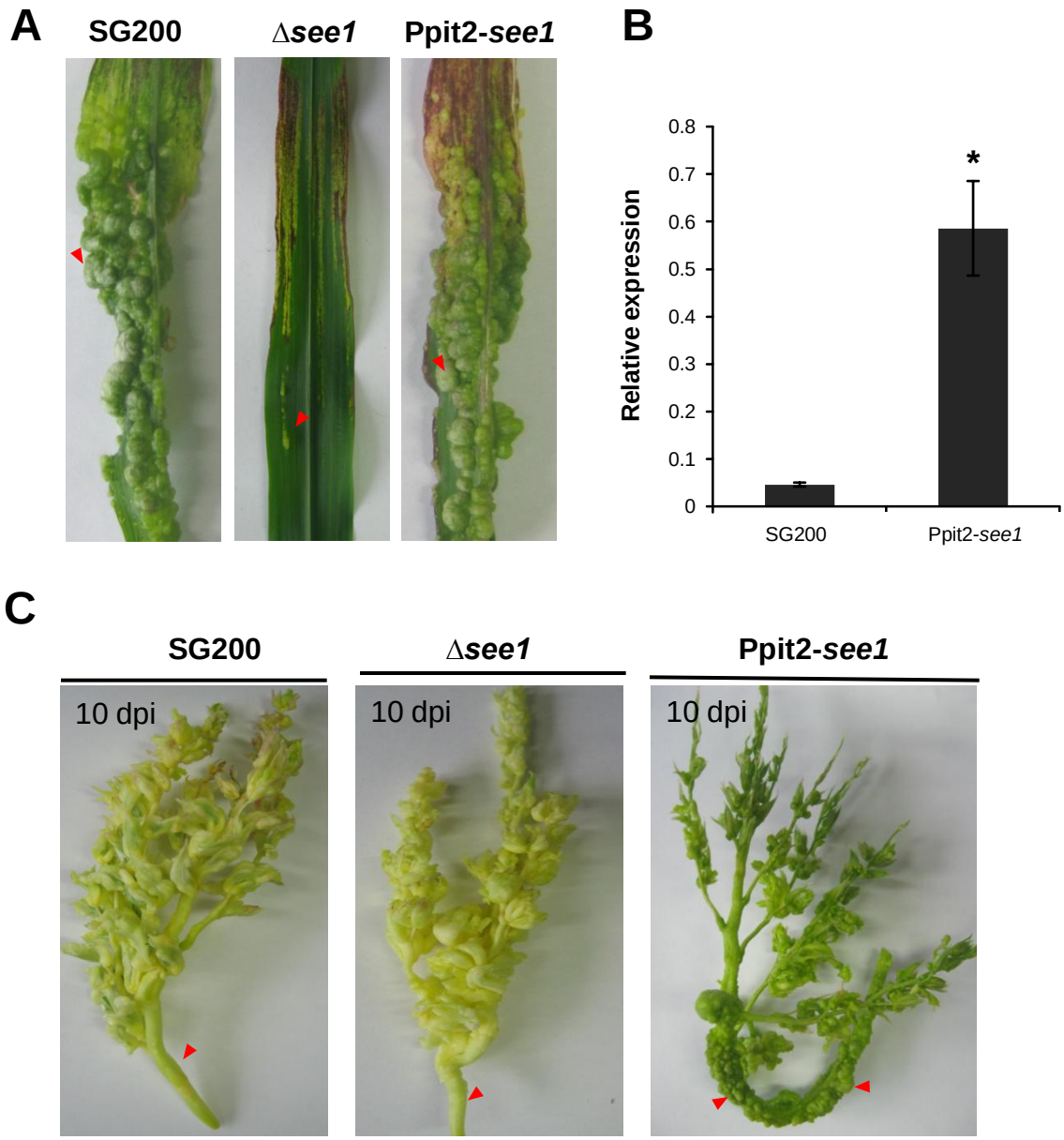
Supplemental Figure 6. Specificity of the EdU labeling assay.

Magnified images of the in vivo DNA synthesis assay in seedling tissue infected with SG200Δ*see1* in comparison to wild-type SG200. DNA synthesis inhibitor hydroxyurea was injected in the infected seedling one day before the EdU treatment. The pretreatment with hydroxyurea did not show any incorporation of EdU. This was also true for the cells infected with the wild-type strain SG200 where some of the cells had already initiated division before the treatment. Fungal hyphae and plant cell walls were visualized by propidium iodide (PI) staining (red), and the EdU labelled host cell nuclei are visualized by AF488 staining (green) which did not show any labeling. The fungal hyphae are marked by the white arrows. Bars = 50 µm.



Supplemental Figure 7. See1 Requirement in Leaf Tumor Formation.

Magnified images of the in vivo DNA synthesis assay in seedling tissue infected with SG200Δ*see1* in comparison to wild-type SG200. Sample infected with SG200Δ*tin3,* which has a similar phenotype to SG200Δ*see1* with respect to tumor size, was used as a control. Non infected sample did not show any DNA synthesis activity. Fungal hyphae and plant cell walls were visualized by propidium iodide (PI) staining (red), and the EdU labelled host cell nuclei are visualized by AF488 staining (green). Non infected samples lacked any DNA synthesis activity. The fungal hyphae are marked by the white arrows. Bars = 25 µm.

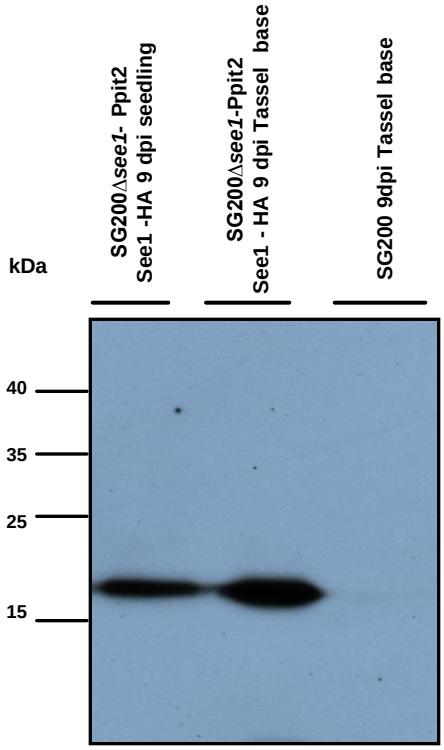


Supplemental Figure 8. Constitutive Overexpression of See1 Results in Tumors on the Vegetative Parts of Tassels.

(A) Overexpression of See1 in leaf tissues leads to a wild-type phenotype with no further increase in tumor size as compared to the wild-type SG200. The infection symptoms are marked with red arrowheads.

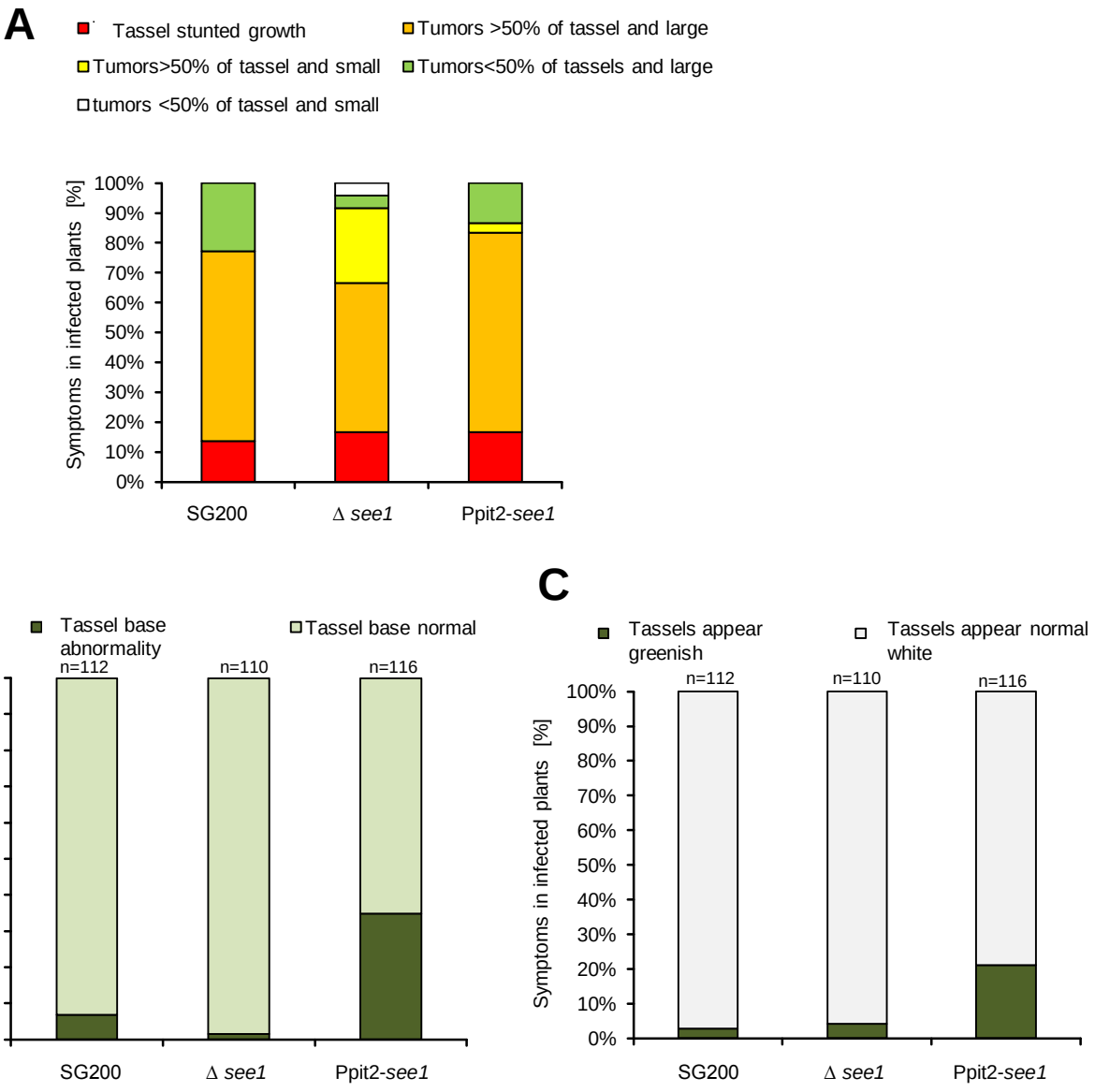
(B) qRT-PCR confirmation of overexpression of Ppit2-*see1* in seedlings. Error bars show SE. *, P ≤ 0.001.

(C) Tassel base abnormality resulting from constitutive overexpression of See1 is observed only in the vegetative parts of the tassels. The tumor symptoms are shown in comparison with the wild-type strain SG200 and SG200Δ*see1*. The tassel appear to be greenish when *see1* is constitutively overexpressed throughout the colonization process. The infection characteristics are marked with red arrowheads.



Supplemental Figure 9. Stable Expression of the Ppit2 overexpressed See1-HA Protein in Infected Seedlings and Tassel base.

Ppit2 overexpressed See1-HA immuno-precipitated from infected plant tissue displays the expected size of 18.81 kDa. Plants infected with SG200Δ*see1*-Ppit2-*see1-HA* were subjected to anti-HA immunoprecipitation at 9 dpi in seedling and tassel base samples. The immunoblot was developed with anti-HA mouse antibodies and demonstrates that full length See1-HA is present in SG200Δ*see1*-Ppit2-*see1-HA* infected tissue whereas no signal can be detected in SG200 infected tassel base which served as negative control.

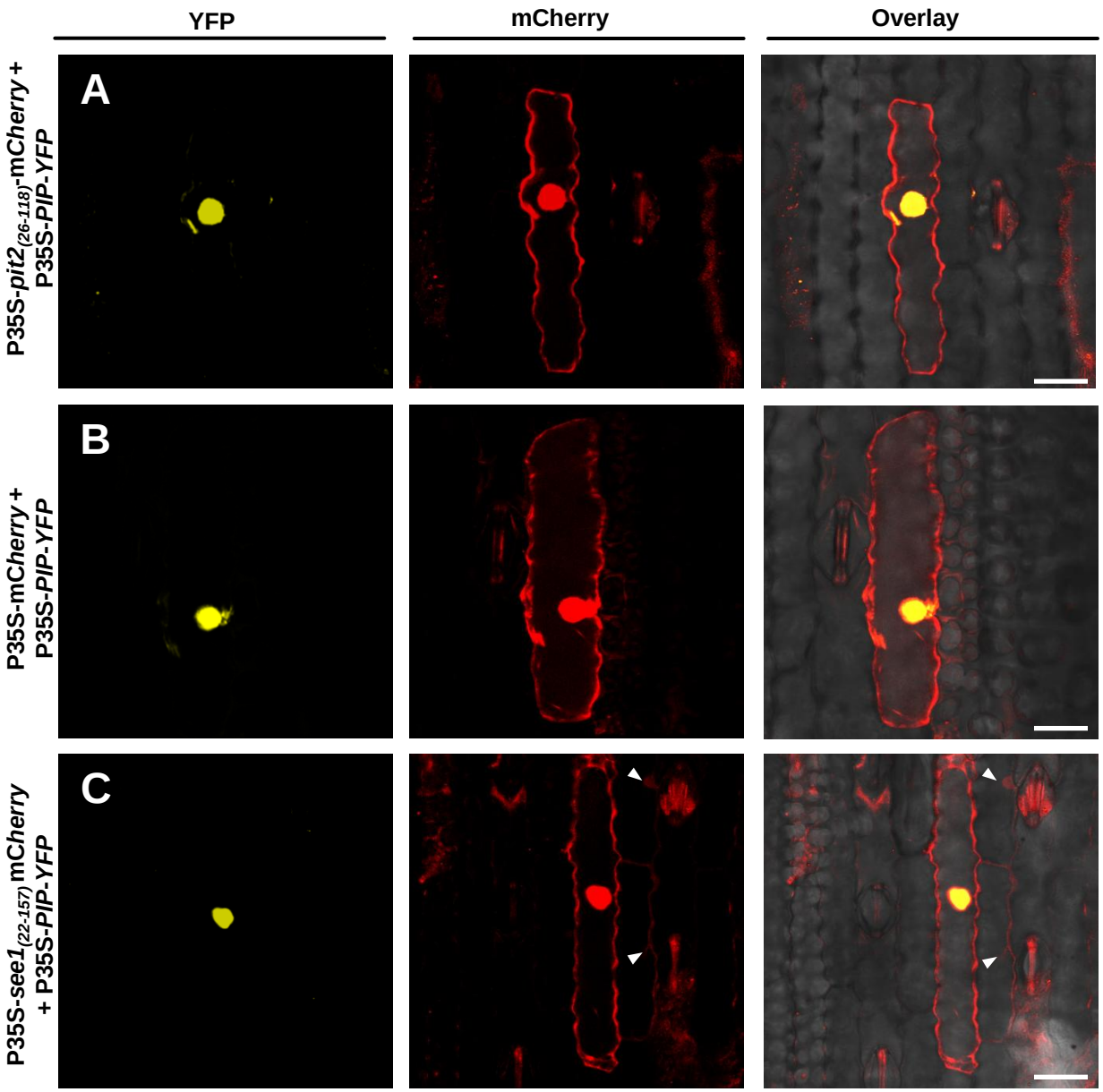


Supplemental Figure 10. Overexpression of See1 Results in Two Abnormalities in Tassels: Tumors at the Tassel Base and Greenish Tassels.

(A) Disease symptoms of tassels infected with See1 over-expressing strain (Ppit2-*see1*) in comparison to the wild-type progenitor strain SG200 and *SG200*Δ***see1***. The symptoms were scored at 14 dpi. The experiment was performed in three independent biological replicates.

(B) The tassel base abnormalities in plants infected with the strain over-expressing *see1* in comparsion to wild-type and the SG200Δ***see1*** strain. The symptoms were scored at 14 dpi. The experiment was performed in three independent biological replicates.

(C) The greenish appearance of the tassel infected by See1 overexpressing strain was quantified as the number of greenish tassels to the total number of tassels infected. The symptoms were scored at 14 dpi. The experiment was performed in three independent biological replicates.

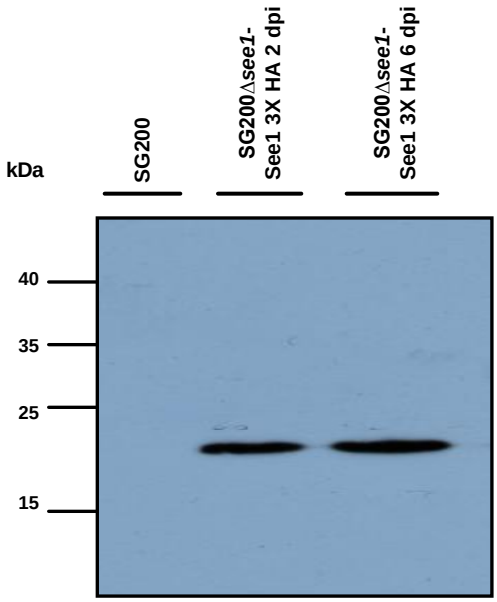


Supplemental Figure 11. See1 is Transferred to Cells Neighboring to the Transformed Cell.

(A) Confocal microscopy of 35S-*pit2*$_{26\text{-}118}$-*mCherry* transiently expressed in maize epidermal cells. The transformed cell (left panel) expresses a PIP-YFP control that is specifically localized to the nucleus. Pit2-mCherry is localized to the cytoplasm and nucleus. Scale bar = 25µm.

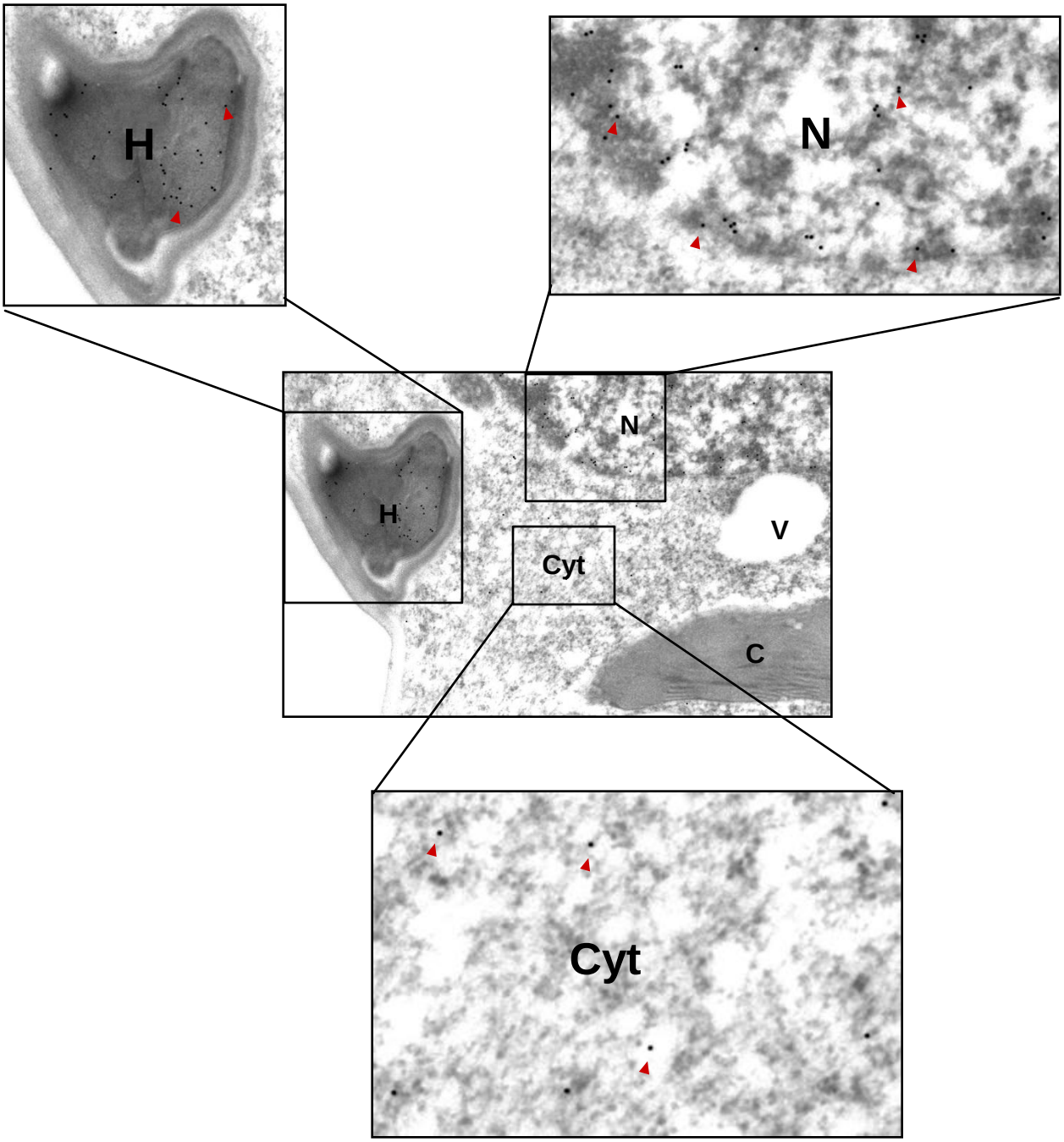
(B) Confocal microscopy of 35S-*mCherry* transiently expressed with 35S-*PIP-YFP* in maize epidermal cells. The PIP-YFP control is specifically localized to the nucleus. mCherry is localized to the cytoplasm and nucleus. Scale bar = 25µm.

(C) Transient expression of 35S-*see1*$_{22\text{-}157}$-*mCherry* in maize epidermal cells with nuclear PIP-YFP control. See1-mCherry is localized to the cytoplasm and nucleus and is transferred to the adjacent neighboring cells as shown by white arrowheads. Scale bar = 25µm.

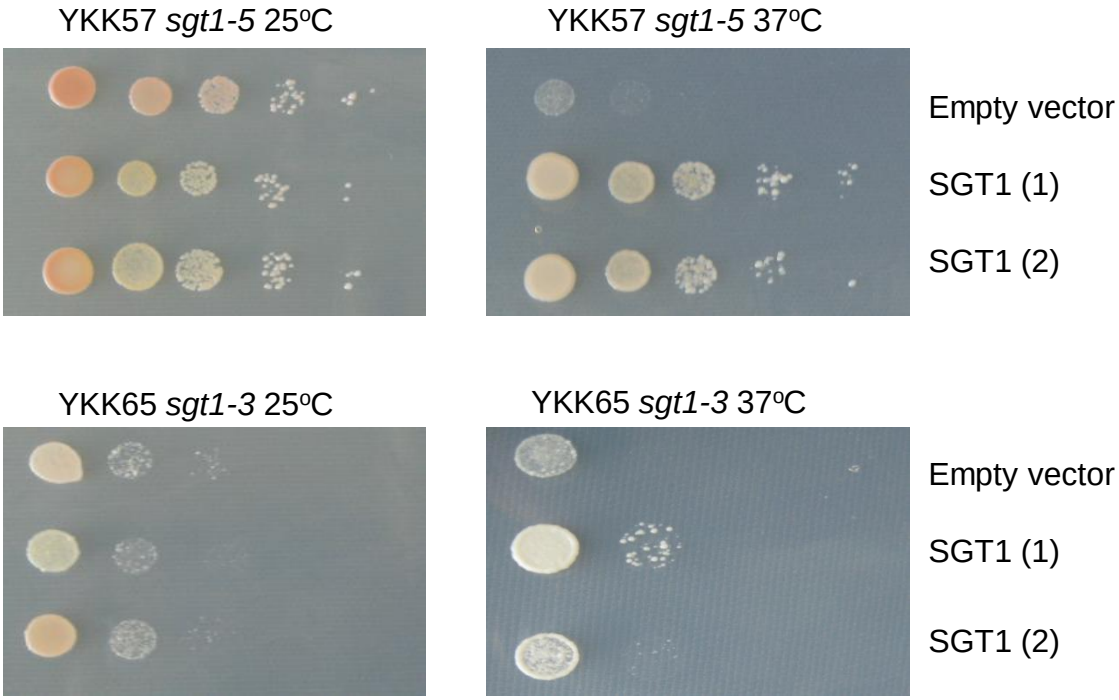


Supplemental Figure 12. Stable Expression of the See1-3xHA Protein in Infected Tissues.

See1-3xHA immuno-precipitated from infected plant tissue displays the expected size of 20.98 kDa. Plants infected with SG200 and SG200Δ*see1-see1-3xHA* were subjected to anti-HA immunoprecipitation 2 and 6 dpi. The immunoblot was developed with anti-HA mouse antibodies and demonstrates that full length See1-3xHA is present in SG200Δ*see1-see1-3xHA* infected tissue whereas no signal can be detected in SG200-infected plants.

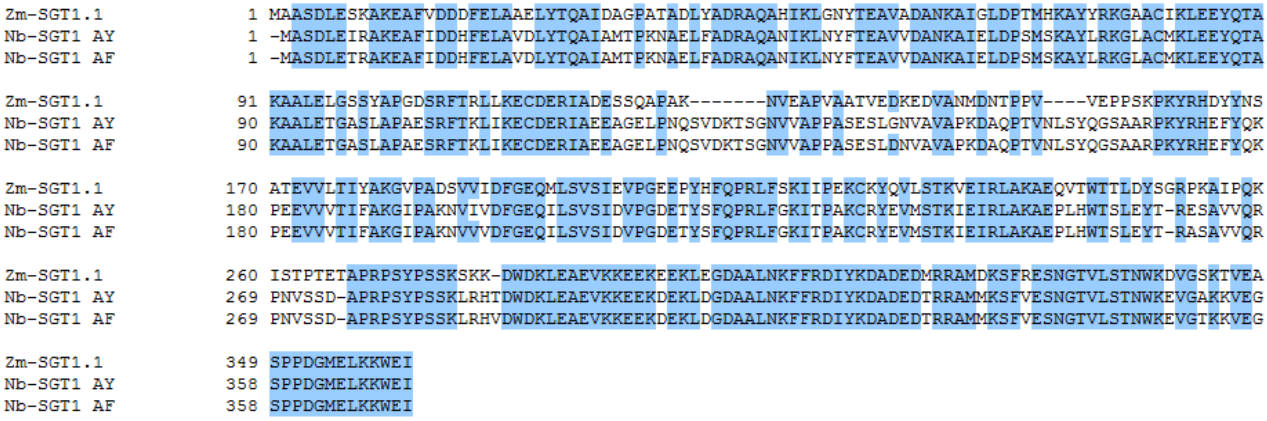


Supplemental Figure 13. See1 is Translocated to the Plant Cell Cytoplasm and Nucleus. Immunogold labeling of See1 could be found in hyphae (H), the cytosol (Cyt) and nuclei (N) as shown by the red arrowheads but not in chloroplasts (C), vacuoles (V), or the cell wall (CW) when the See1 effector was tagged with 3xHA in the strain Psee1-*SPsee1-See1-3xHA*.



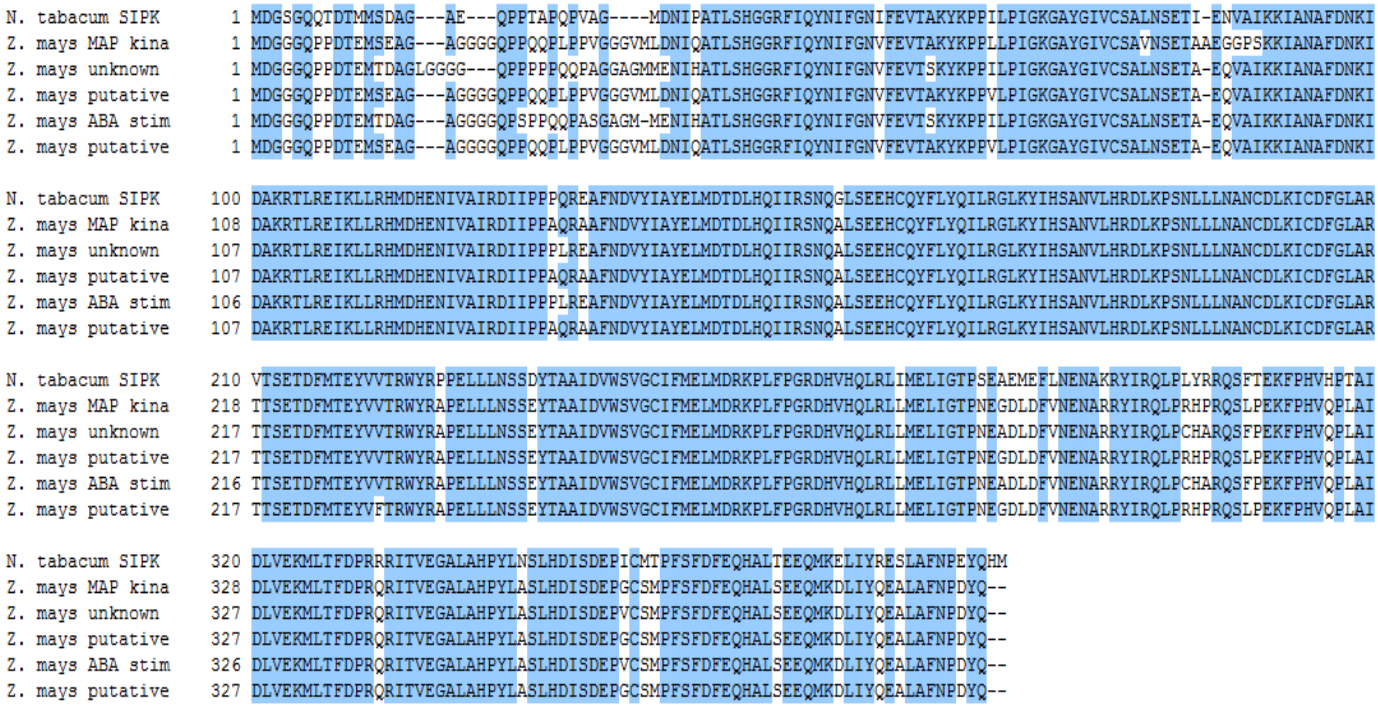
Supplemental Figure 14. Complementation of two Yeast *sgt1* Cell Cycle Temperature Sensitive Mutants with Maize SGT1.

Dilution series of yeast strains YKK57 (*sgt1-5*) and YKK65 (*sgt1-3*) complemented with maize SGT1 or empty vector (EV) after growth at the permissive (25°C) and restrictive (37°C) temperatures. Yeast strains were transformed with maize SGT1, (cloned into pGREG GAL), or by an empty vector. The transformants were selected on Sc-Ura 2% glucose plates. The strains were spread on Sc-Ura 2% galactose plates and incubated for 4 days to test the ability of maize SGT1 to complement the temperature-sensitive *sgt1-5* and *sgt1-3* growth defects. The SGT1 (1) and (2) are two independent clones dropped in the assay.



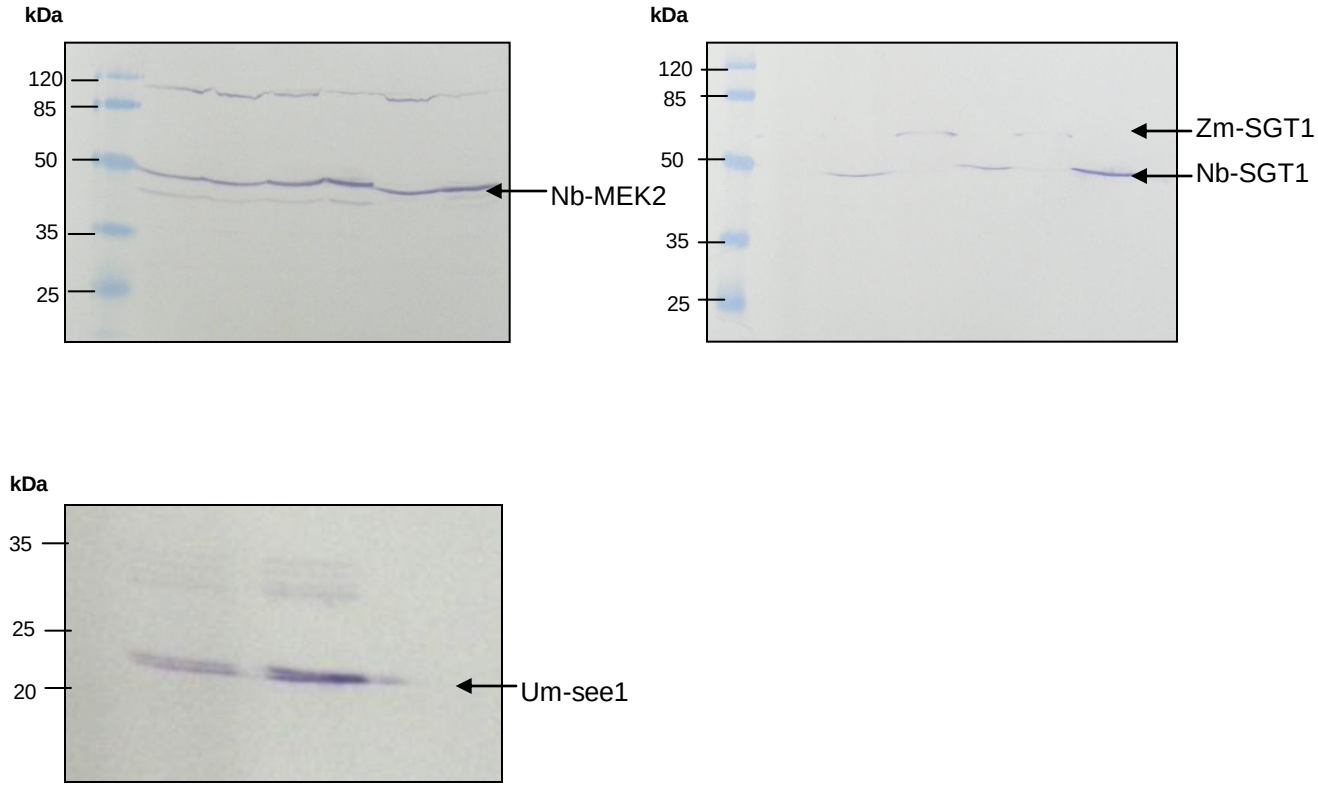
Supplemental Figure 15. The Deduced SGT1 Protein Sequences of *Z. mays* and *N. benthamiana* Showing a Multiple Alignment.

Sequence alignment of SGT1 proteins in *Z. mays* and *N. benthamiana* representing the two isoforms 1 and 2. The blue highlight represents identity at the amino acid level which is seen to be 65 % in comparison to the *Z. mays* SGT1.



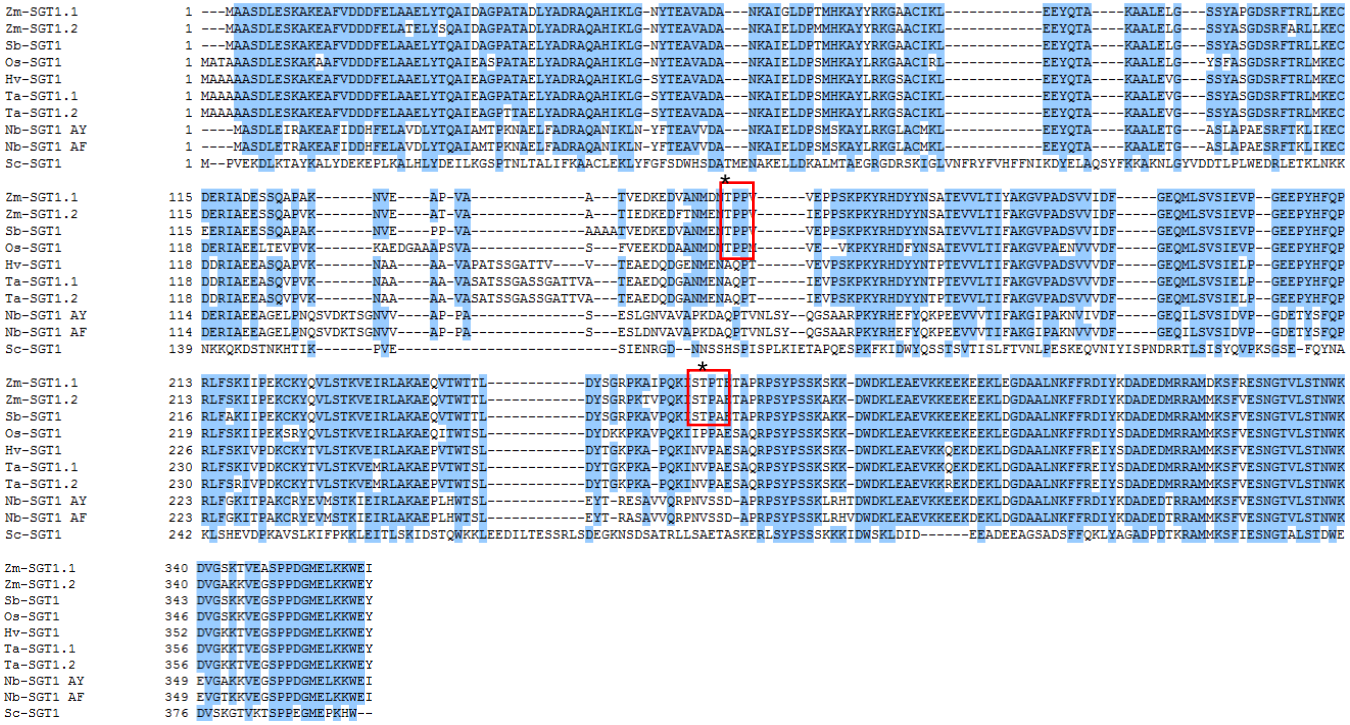
Supplemental Figure 16. The Deduced SIPK Protein Sequences of *N. tabacum* and a Set of Five putative *Z. mays* SIPK Showing a Multiple Alignment.

Sequence alignment of salicylic acid induced protein kinase (SIPK) from *N. tabacum* aligned to a set of 5 putative SIPK's from *Z. mays*. The blue highlight represents identity at the amino acid level which is seen to be 84-86 % in comparison to the *N. tabacum* SIPK.



Supplemental Figure 17. Expression level of the Nt-MEK2, Nb/Zm-SGT1 and Um-See1 Transiently Expressed in *N. benthamiana* Leaves to Assess Zm-SGT1 Phosphorylation In Planta.

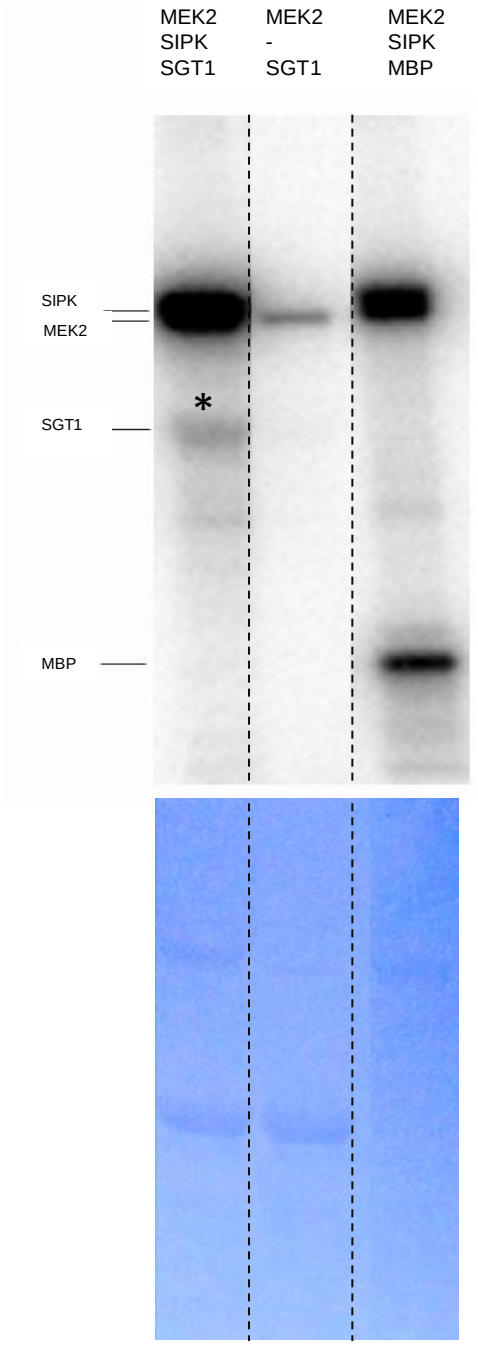
Flag-Nt-MEK2, Nb-SGT1-6xHis-Strep, Zm-SGT1-2xStrep-6xHis and Um-See1-2xStrep-6xHis, were immunodetected with specific anti-Flag or anti-6xHis antibodies in protein extracts from *N. benthamiana* leaf tissue harvested 2 days after agro-infiltration. Bands on the blots correspond to the molecular masses of the full-length proteins, i. e. 42.1 kDa, 43.5 kDa, 46.4 kDa and 24.0 kDa, respectively.



Supplemental Figure 18. The Deduced SGT1 Protein Sequences with Marked Phosphorylation Sites T_{150} and T_{262} within Zm-SGT1.

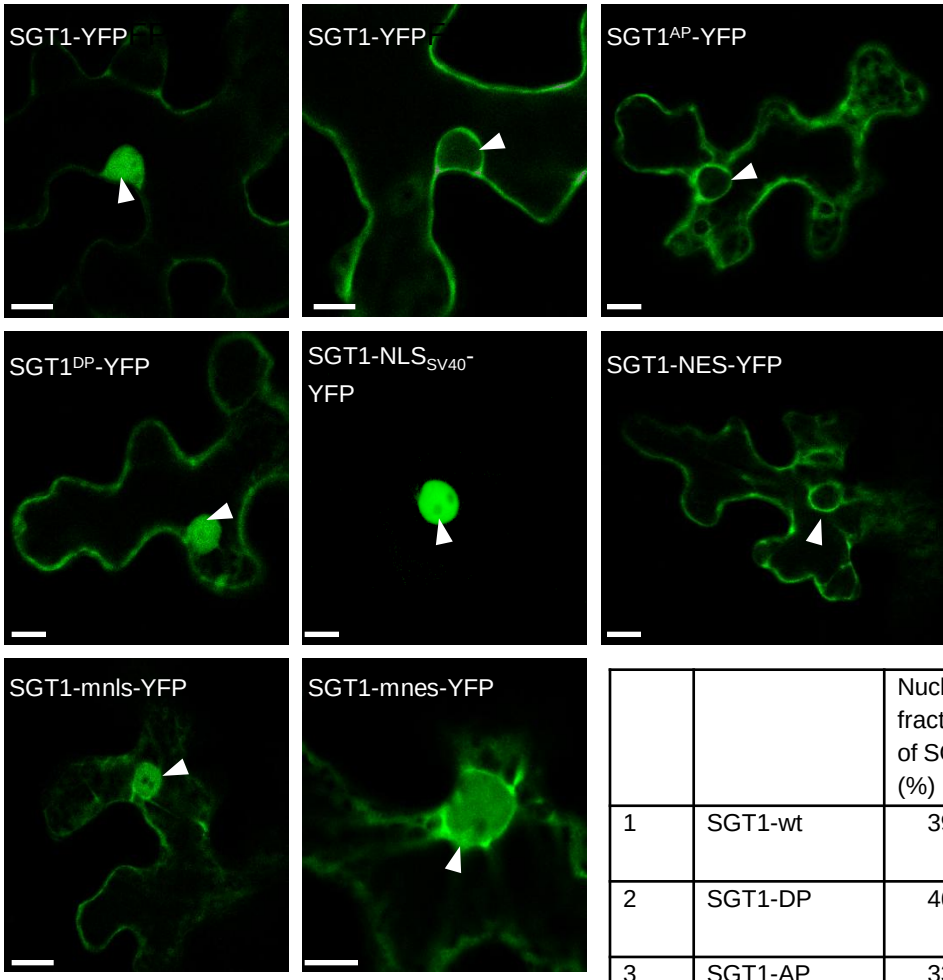
(A) Sequence alignment of SGT1 proteins in various plant species. The blue highlight represents identity at the amino acid level. The phosphorylation sites in the maize SGT1 have been highlighted with red boxes. Both sites lie within variable regions of SGT1 protein The first site comprising T_{150} is conserved in *Z. mays*, *Oryza sativa* and *Sorghum bicolor*. This site might be a specific target for a maize pathogen whereas phosphorylation of the second site seems not to be affected by the pathogen.

Note: Maize SGT1 is present in two isoforms, likely coded by duplicate loci; they are named SGT1.1 and SGT1.2. Neither protein has been annotated in the maize genome. We were unable to amplify transcripts of SGT1.2; we conclude that it is not expressed or expressed at very low levels in the organs examined.

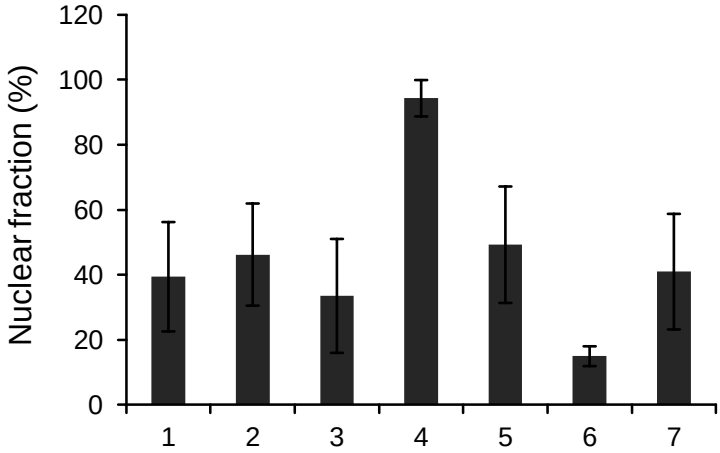


Supplemental Figure 19. Salicylic Acid Induced Protein Kinase (SIPK) Phosphorylates Maize SGT1 In Vitro.

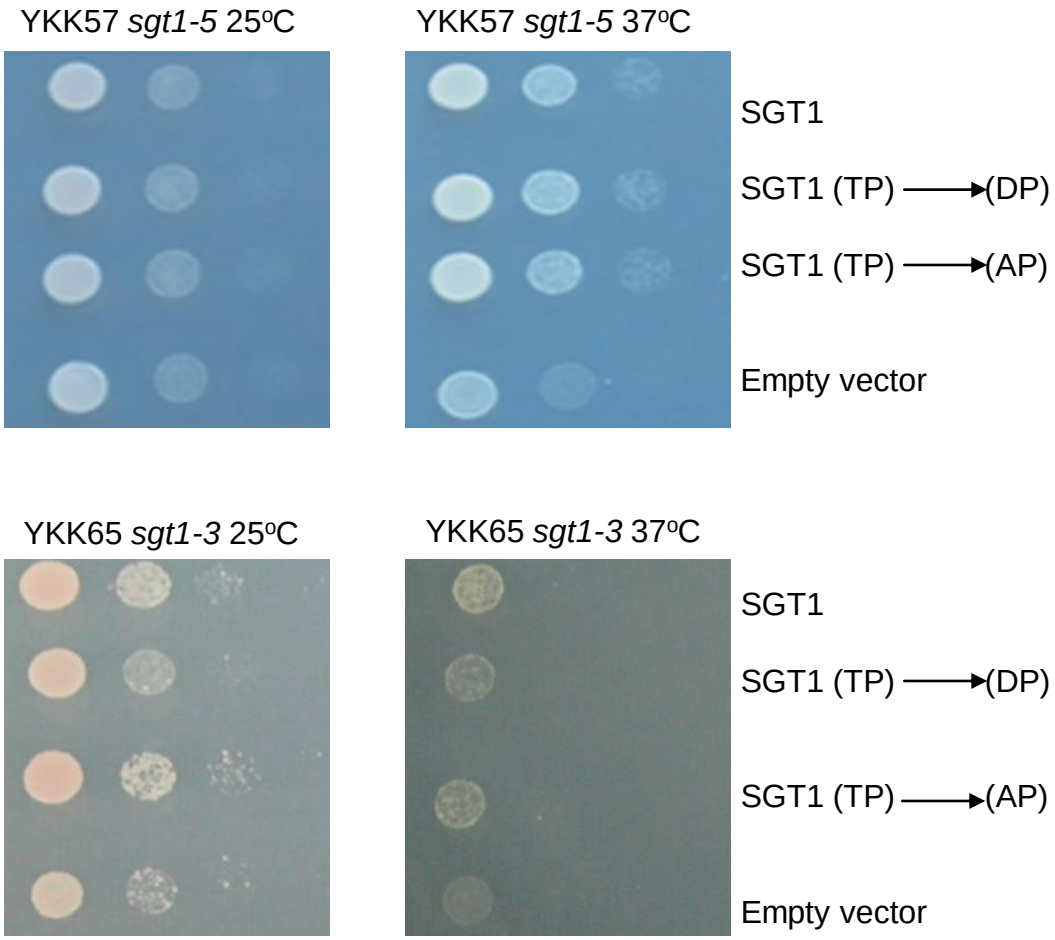
Recombinant maize SGT1 with a N-terminal HIS epitope, as well as SIPK and the upstream kinase MEK2 tagged with N-terminal Glutathione S transferase (GST) epitopes, were incubated with [$Y^{32}P$] ATP, resolved by SDS-PAGE and analyzed by autoradiography. Lane 1 and 2 represents SGT1 as a substrate. Lane 3 includes myelin basic protein (MBP) as a positive control. The band corresponding to phosphorylated SGT1 is designated by an asterisk.



		Nuclear fraction of SGT1 (%)	SD
1	SGT1-wt	39.40	16.81
2	SGT1-DP	46.22	15.70
3	SGT1-AP	33.50	17.51
4	SGT1-wt-NLS	94.28	5.59
5	SGT1-wt-nls	49.25	17.90
6	SGT1-wt-NES	14.96	3.04
7	SGT1-wt-nes	40.95	17.77

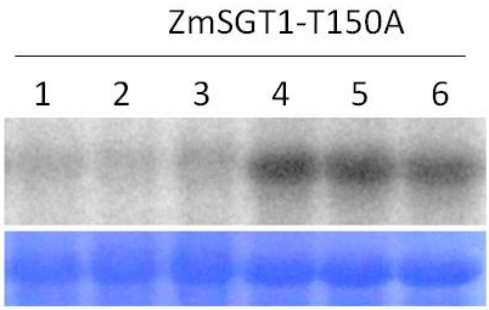


Supplemental Figure 20. Subcellular Localization of Phosphovariants of Maize SGT1.
Confocal images of representative *N. benthamiana* leaf epidermal cells transiently expressing SGT1-eYFP or the phospho-variants SGT1^{AP}- eYFP, SGT1^{DP}- eYFP. As a control localization variants of SGT1-3xHA-NLS-eYFP, SGT1-3xHA-NES-eYFP, SGT1-3xHA-nls- eYFP, SGT1-3xHA-nes-eYFP are shown. Arrowheads mark the nuclei. Bars =10 µm. Wild-type maize SGT1 accumulates in the cytoplasm and nucleus or exclusively in the cytoplasm (Upper panel; left and middle cell, respectively). Fluorescence intensities in the nucleus (IN) and cytoplasm (IC) were determined by ImageJ software in confocal images of 15 cells for each construct. Percentage of nuclear fluorescence intensity in each cell was calculated according to the formula: [IN/(IN+IC)]x100. Mean relative nuclear ratios (+/- SD) are presented in the table and graph.



Supplemental Figure 21. Complementation of Two Yeast *sgt1* Cell Cycle Temperature Sensitive Mutants with Maize SGT1 Wild-type, Phosphomimic and Phosphonull.

Dilution series of yeast strains YKK57 (*sgt1-5*) and YKK65 (*sgt1-3*) complemented with SGT1, SGT1 phosphomimic (T_{150} to D*)*, SGT1 phosphonull (T_{150} to A*)*, or empty vector (EV) after growth at the permissive (25°C) and restrictive (37°C) temperatures. Yeast strains were transformed with SGT1, (cloned into pGREG GAL), or by an empty vector. The transformants were selected on Sc-Ura 2% glucose plates. The strains were spread on Sc-Ura 2% galactose plates and incubated for 4 days to test the ability of maize SGT1 to complement the temperature-sensitive *sgt1-5* and *sgt1-3* growth defects.



Supplemental Figure 22. In planta phosphorylation of maize SGT1^{AP}.

Recombinant SGT1^{AP} (ZmSGT1 with T150A) produced in *E. coli* was incubated in the buffer containing [γ^{32}P] ATP and total proteins extracted from maize seedlings or tassels infected with *U. maydis*. The samples were separated by SDS-PAGE and analyzed with a phosphorimager.

Lanes 1-3: Extracts from seedling leaves 6 dpi infected with: 1- *U. maydis* wild-type SG200; 2, SG200Δ*see1* ; 3, mock-inoculated; Lanes 4-6: extracts from tassel base 9 dpi infected with 4, *U. maydis* wild-type SG200; 5, *U. maydis* overexpressing Ppit2-See1 (single-copy integration); 6, *U. maydis* overexpressing Ppit2-See1 (multiple-copy integration).

Supplemental Table 1. Differentially Expressed Top 30 GO Terms Related to the DNA Synthesis and Cell Differentiation

Gene ID	GO No.	GO Description	Sequence Description	Fold change SG200 vs SG200Δ*see1*
CF919894	41	P:cortical microtubule organization; F:hydrolase activity; P:negative regulation of MAP	dual specificity protein phosphatase phs1	677
TC301470	20	P:translational initiation; P:methylation-dependent chromatin silencing; P:cell-cell signaling	protein argonaute 10-like	578
TC282828	20	P:cellular response to phosphate starvation; F:phosphatidate phosphatase activity; C:pl	phytochrome-associated protein 1	367
TC294593	19	P:myo-inositol transport; F:glucose transmembrane transporter activity; P:mannitol tran	polyol transporter 5-like	327.425
TC307652	17	P:transmembrane transport; P:positive gravitropism; P:leaf formation; C:basal plasma me	auxin efflux carrier	895.397
TC299855	17	P:response to oxidative stress; P:negative regulation of growth; P:oxidation-reduction	peroxidase 15-like	821.735
TC301172	17	P:mitotic cell cycle; P:gene silencing by RNA; F:DNA-dependent ATPase activity; P:respo	dna repair protein rad51 homolog	67.738
TC294810	15	F:DNA-directed DNA polymerase activity; F:metal ion binding; P:double-strand break repa	dna polymerase alpha catalytic subunit	403.239
TC279595	14	C:cytosol; C:nucleosome; P:response to water deprivation; C:mitochondrion; C:nucleolus;	histone h4	749.097
TC279279	14	C:cytosol; C:nucleosome; P:response to water deprivation; C:mitochondrion; C:nucleolus;	histone h4	691.187
TC279246	14	C:cytosol; C:nucleosome; P:response to water deprivation; C:mitochondrion; C:nucleolus;	histone h4	541.567
TC306450	14	F:glucan exo-1,3-beta-glucosidase activity; F:beta-L-arabinosidase activity; F:cellobio	beta-glucosidase 44	506.238
TC310738	13	P:translation; P:endonucleolytic cleavage to	40s ribosomal	417.245

		generate mature 3'-end of SSU-rRNA from (S	protein sa	
TC307447	13	P:regulation of cell adhesion; P:regulation of cell division; P:protein phosphorylation	leucine-rich repeat receptor-like protein kinase family protein	230.113
TC306407	13	C:membrane coat; P:methylation-dependent chromatin silencing; P:chromatin silencing by	microtubule-associated protein tortifolia1-like	155.289
TC311056	13	P:regulation of G2/M transition of mitotic cell cycle; F:RNA polymerase II carboxy-term	cyclin-dependent kinase b2-1-like	118.332
TC283433	12	P:microtubule-based process; P:gene silencing; P:histone modification; C:cytoskeletal p	atp binding	747.103
TC287119	12	C:Cajal body; P:histone H3-K9 methylation; P:defense response to bacterium, incompatibl	Argonaute family protein	251.56
TC279950	11	P:response to glucose stimulus; P:response to sucrose stimulus; P:response to fructose	pyrophosphate--fructose 6-phosphate 1-phosphotransferase subunit alpha-like	674.109
TC294404	11	C:cytoplasm; P:response to nematode; C:plant-type cell wall; P:cell wall modification;	probable pectinesterase pectinesterase inhibitor 40-like	641.189
TC281806	11	C:plant-type cell wall; F:xylan 1,4-beta-xylosidase activity; P:xyloglucan metabolic pr	alpha-xylosidase 1-like	568.093
TC300114	10	F:molecular_function; P:biological_process; P:regulation of transcription, DNA-dependen	ap2-like ethylene-responsive transcription factor at2g41710-like	836.606
TC292250	10	P:mitotic cell cycle; P:stomatal lineage progression; P:negative regulation of cyclin-d	cyclin-dependent kinases regulatory	729.831

			subunit	
TC300266	10	P:mitotic cell cycle; C:cell wall; F:microtubule binding; C:nucleolus; P:thigmotropism;	microtubule-associated protein rp eb family member 3	144.046
TC286934	10	P:meiosis II; P:regulation of cyclin-dependent protein serine/threonine kinase activity	type a-like cyclin	120.597
TC298222	9	C:nucleosome; P:cell proliferation; F:DNA binding; P:nucleosome assembly; C:nucleus; F:	histone h3	862.773
TC298215	9	C:nucleosome; P:cell proliferation; F:DNA binding; P:nucleosome assembly; C:nucleus; F:	histone h3	794.373
TC301787	7	P:regulation of transcription, DNA-dependent; F:core promoter binding; P:transcription,	transcription factor pcf3-like	586.847
TC287426	7	P:regulation of cell cycle; F:identical protein binding; P:histone H3-K9 methylation; P	tetratricopeptide repeat-like superfamily protein isoform 1	152.837
TC298187	7	C:nucleosome; P:cell proliferation; F:DNA binding; P:nucleosome assembly; C:nucleus; F:	histone h3	150.782

Supplemental Table 2. Oligonucleotides Used in this Study.

Name	**Sequence (5'→3')**	**Gene accession No. (NCBI)**	
OSee1-SbfI-fw	GACCTGCAGGGTGTGCACGGTGCTACTG	XP_758386 (See1)	Cloning of See1 in p123 for complementation
OSee1-NotI-rv	GAGCGGCCGCCCCACTCGTGACTGCTAC		
OPpit2_see1-SacII-fw	ATACCGCGGATGCTCTTCACCACCTTCGTTTC		Cloning of See1 and see1-mCherry in p123-Ppit2 for See1 overexpression
OPpit2_see1-HA-XbaI-rv	CCTCTAGATTAAGCGTAATCTGGAACATCGTATGGGTACGTCGTCGGCCCAAATTTATA		
OPpit2_see1-mCherry-XbaI-rv	CGCTCTAGATTACTTGTACAGCTCGTCCA		
Osee1_qPCR-fw	TCAGGTGCAAGGAGAAGG		For quantification of see1 expression during biotrophic colonization and also in the individual parts of tassels upon see1 overexpression
Osee1_qPCR-rv	ACAGAATACTCCGCTTCCC		
Osee1-pGBKT7-NdeI-fw	GCGCATATGCATCCTCTACAATCGTTTCG		Construction of pGBKT7-See1 for yeast two hybrid experiments
Osee1-pGBKT7-BamHI-rv	CGCGGATCCTTACGTCGTCGGCCCAAATT		
OSee1$^{22\text{-}157}$–mCherry-XbaI-fw	CGCTCTAGAATGCATCCTCTACAATCGTTTCG		Cloning of See1$^{22\text{-}157}$ in pGreenII 0029 for transient expression in *N. benthamiana* and *Z. mays*
OSee1$^{22\text{-}157}$-mCherry-SacI-rv	CGGGAGCTCTTACTTGTACAGCTCGTCCA		
Oppi_qPCR_fw	ACATCGTCAAGGCTATCG	XM_754780.1	Amplification of the

Oppi_qPCR_rv	AAAGAACACCGGACTTGG		housekeeping gene ppi from *U. maydis* for qPCR
Osgt1-pGADT7-NdeI-fw	GTATATACCCGGGAATGGAGTACCCATACGAC	AFW83327 (SGT1)	For cloning of the ZmSGT1 in pGADT7 for confirmation of the Y2H interaction
Osgt1-pGADT7-BamHI-rv	GCGCGGATCCTCAAATTTCCCACTTCTTG		
OSee1$^{22-157}$BamHI- BiFC-fw	GCGCGGATCCATGCATCCTCTACAATCGTTTC	XP_758386 (See1)	Cloning in the pSPYNE vector for BiFC
OSee1$^{22-157}$ -xhoI-BiFC-rv	GACTCGAGCGTCGTCGGCCCAAATTT		
OSgt1-BamHI-BiFC-fw	GTATGGATCCATGGCCGCGTCGGATCTG	AFW83327 (SGT1)	Cloning in the pSPYCE vector for BiFC
Osgt1-xhoI-BiFC-rv	GCTCTCGAGGATTCCCTGAATGACTTTG		
OSee1$^{22-157}$XbaI-fw	GCCGGCTCTAGATGCATCCTCTACAATCGTTTC	XP_758386 (See1)	For in planta Co IP: see1-myc in pGreenII
OSee1$^{22-157}$-SacI-Myc_rv	GGCCGCGAGCTCTTAAAGATCCTCCTCAGAAATCAACTTTTGCTCCGTCGTCGGCCCAAATTTATACTCTCC		
Osgt1-XbaI-fw	CATCTAGATGGCCGCGTCGGATCTG	AFW83327 (SGT1)	For in planta Co IP: SGT1-HA in pGreenII
Osgt1-BamHI-HA-rv	CCGGCCGGCGGATCCTTAAGCGTAATCTGGAACATCGTATGGGTAAATTTCCCACTTCTTGAG		
Osgt1-pGREG-EcoRI-fw	GCGAGCTCATGCATCCTCTACAATCGTTTCG	AFW83327 (SGT1)	Cloning of ZmSGT1 into the yeast expression vector pGREG536 with GAL promoter for complementation assay of the yeast cell cycle *sgt1* mutants
OSgt1-pGREG-XhoI-rv	GGCCTCGAGTCAAATTTCCCACTTCTTG		
OSee1$^{22-157}$ pTA7001-SpeI-fw	GCGCATATGAGCATCGAGCTGTTCGAG	XP_758386 (See1)	Cloning of see1 in the DEX inducible pTA7001 vector for
OSee1$^{22-157}$	CTCGAGTTAAGCGTAATCTGGAACAT		

pTA7001-XhoI-rv	CGTATGGGTACGTCGTCGGCCCAAATTTAT		*in planta* phosphorylation assay.
OPsee1_see1_3X HA _SbfI_fw	GGCCTGCAGGGTGGAGTGGAAGCACAAAAT	XP_758386 (See1)	Cloning of the see1 with the promoter and signal peptide and integrated with 3X HA for immune localization using TEM.
OPsee1_see1_3X HA _NotI_rv	GCGGCCGCTTAAGCGTAATCTGGAACATCGTATGGGTAAGCGTAATCTGGAACATCGTATGGGTAAGCGTAATCTGGAACATCGTATGGGTACGTCGTCGGCCCAAATTTAT		
OPsee1_GFP_3X HA _NcoI_fw	TACCATGGATGGTGAGCAAGGGCGAGGA	GFP	Cloning of the GFP with the See1 promoter, without the signal peptide and integrated with 3X HA as a control for immune localization using TEM.
OPsee1_GFP_3X HA _NotI_fw	GGGCGGCCGCTTAAGCGTAATCTGGAACATCGTATGGGTAAGCGTAATCTGGAACATCGTATGGGTAAGCGTAATCTGGAACATCGTATGGGTACTTGTACAGCTCGTCCATGC		
OSPsee1_mcherry_3X HA _NcoI_fw	CCATGGATGCTCTTCACCACCTTCGT	SPsee1 +mcherry	Cloning of the mCherry with the See1 secretion signal and integrated with 3X HA as a control for immune localization using TEM. The PCR product was cloned into p123-Psee1 plasmid.
OSPsee1_Rv with mcherry overhang	TCCTCGCCCTTGCTCACCATAGCAGACACGTGGACAAGAC		
Omcherry_fw	ATGGTGAGCAAGGGCGAGGAGGATA		
OPsee1_SPsee1_mcherry_3X HA _NotI_rv	GCGGCCGCTTAAGCGTAATCTGGAACATCGTATGGGTAAGCGTAATCTGGAACATCGTATGGGTAAGCGTAATCTGGAACATCGTATGGGTACTTGTACAGCTCGTCCATGC		

Osgt1_pENTR_D_TOPO_ZmSGT1_fw	CACCATGGCCGCGTCGGATCTGGA	AFW83327 (SGT1)	For cloning of ZmSGT1 in gateway entry vector and final destination vector pGWB502 for in planta phosphorylation
Osgt1_pENTR_D_TOPO_ZmSGT1 (without stop)_rv	AATTTCCCACTTCTTGAGCTCC		For cloning of ZmSGT1 in gateway entry vector and final destination vector pGWB502 for in planta phosphorylation
Osgt1-point mutation-pGREG- -fw	ATGGCCGCGTCGGATCTGGA	AFW83327 (SGT1)	Additional primer for site directed mutagenesis of ZmSGT1
Osgt1-phosphomimic-T_{150} mutated to D-pGREG- -fw	GATGTCGCAAATATGGATAATGACCC GCCAGTGGTAGAACCC	AFW83327 (SGT1)	For construction of the phosphomimic Zm-SGT1 for yeast complementation
Osgt1-phosphonull-T_{150} mutated to A-pGREG- -fw	GATGTCGCAAATATGGATAATGCACC GCCAGTGGTAGAACCC	AFW83327 (SGT1)	For construction of the phosphonull Zm-SGT1 for yeast complementation
Osgt1- pET15b-NdeI-fw	ATTCATATGGCCGCGTCGGATCTGG A	AFW83327 (SGT1)	For recombinant Zm-SGT1 in *E. coli*
Osgt1- pET15b-BamHI-fw	GGCCGGATCCTCAAATTTCCCACTTC TTGA	AFW83327 (SGT1)	For recombinant Zm-SGT1 in *E. coli*
Osgt1_pENTR_D_TOPO_ZmSGT1_point mutation_fw	AACCCCCAAGCAAACCTAAATATAG	AFW83327 (SGT1)	Additional primer for site directed mutagenesis of SGT1 on pENTR-D-TOPO for localization studies

Osgt1_pENTR_D_TOPO_ZmSGT1_phosphomimic T_{150} to D_fw	CTACCACTGGCGGATCATTATCCTATT	AFW83327 (SGT1)	For construction of the phosphomimic SGT1 in pENTR-D-TOPO for gateway destination for localization studies
Osgt1_pENTR_D_TOPO_ZmSGT1_phosphonull T_{150} to A_fw	CTACCACTGGCGGTGCATTATCCATATT	AFW83327 (SGT1)	For construction of the phosphonull SGT1 in pENTR-D-TOPO for gateway destination for localization studies

Supplemental Table 3. Plasmids Used in the Study.

Plasmid	Description	Reference
$p123P_{see1}see1_{1-157}$	For generation of the complementation strain in the SG200Δsee1 background.	Schilling et al., 2014
pGreen-P35S-$Pit2_{26-118}$-mCherry	For transient expression in maize as shown in Supplemental Figure 10.	Mueller et al., unpublished
p35S-PIP-YFP	For transient expression control in maize which localizes to the nucleus as shown in Figure 6 and Supplemental Figure 10.	Djamei et al., 2011
pTA7001	For a em pty vector control in the in planta phosphorylation assay in *N. benthamiana*	Aoyama and Chua, 1997
pTA7002-Flag-$NtMEK2^{DD}$	For in planta phosphorylation assay	Yang et al., 2001
pTA7002-Flag-$NtMEK2^{KR}$	For in planta phosphorylation assay	Yang et al., 2001
pROK2	Empty vector control for in planta phosphorylation assay	Baulcombe et al., 1986
pROK2-SIPK	For in planta phosphorylation assay	Hoser et al., 2013
pROK2-NbSGT1-6xHis-StrepII	For in planta phosphorylation assay	Hoser et al., 2013
pGEX-6P1-GST-SIPK	For in vitro phosphorylation assay	Hoser et al., 2013
pGEX-6P2-GST-$MEK2^{DD}$	For in vitro phosphorylation assay	Hoser et al., 2013
pGWB502-C-SF-TAP	Destination vector for Zm-SGT1 for in planta phosphorylation assay	Golisz et al., 2013
$p123P_{pit2}see1_{1-157}$	For generation of the overexpression strain with the constitutive expression of *see1* in SG200Δsee1 background.	This Study
$p123P_{pit2}see1_{1-157}$-HA	For generation of the overexpression strain with the constitutive expression of *see1* in SG200Δsee1 background with HA epitope	This Study
$p123P_{see1}$	For generation of the electron microscopy	This Study

	control strain with SPsee1-m-cherry 3X HA and GFP 3X HA in this plasmid only with See1 promoter	
pGBKT7-See1$_{22\text{-}157}$	For yeast two hybrid assay shown in Figure 7.	This Study
pGADT7-ZmSGT1	For yeast two hybrid assay shown in Figure 7.	This study
pGreen-P35S-See1$_{22\text{-}157}$-mCherry	For transient expression in maize as shown in Figure 6.	This study
pGreen-P35S-m-cherry	For transient expression in maize as shown in Supplemental Figure 10.	This study
pTA7001-See1$_{22\text{-}157}$-HA	For the in planta phosphorylation assay to induce See1 under DEX promoter	This Study
p123-P$_{see1}$-GFP-3X HA	As a negative control for electron microscopy with immunogold labeling, without See1 secretion signal	This Study
p123-P$_{see1}$-SP$_{see1}$-mcherry-3X HA	As a negative control for electron microscopy with immunogold labeling, with See1 secretion signal as shown in Figure 6B	This Study
p123-P$_{see1}$-SP$_{see1}$-see1-3X HA	To localize See1 using TEM immunogold labeling approach as shown in Figure 6C	This Study
pGreen_Zmsgt1-HA	For Co-Immunoprecipitation as shown in Figure 7C	This Study
pGreen_See1$_{22\text{-}157}$-Myc	For Co-Immunoprecipitation as shown in Figure 7C	This Study
pGreen-SPYCE-CFP-See1$_{1\text{-}157}$	For Co-Immunoprecipitation as shown in Figure 7C	This Study
pGreen-SPYCE-CFP-SGT2	For Co-Immunoprecipitation as shown in Figure 7C	This Study

pGreen-SPYCE-CFP with ATG	Control plasmid for Co-Immunoprecipitation and BiFC	This Study
pGreen-SPYNE-cherry with ATG	Control plasmid for Co-Immunoprecipitation and BiFC	This Study
pGREG536-7XHIS-ZmSGT1	For complementation of the ZmSGT1 into the yeast cell cycle mutants	This Study
pGREG536-7XHIS-ZmSGT1 (phosphomimic where T_{150} is mutated to D)	For complementation of the phosphomimic ZmSGT1 into the yeast cell cycle mutants	This Study
pGREG536-7XHIS-ZmSGT1 (phosphonull where T_{150} is mutated to A)	For complementation of the phosphonull ZmSGT1 into the yeast cell cycle mutants	This Study
pET 15b-HIS-Zm-SGT1	For the recombinant protein production of Zm-SGT1 in *E. coli* and in vitro phosphorylation assay	This Study
pENTR-D-Topo-ZmSGT1 without stop codon	For cloning of the ZmSGT1 in the pENTR vector and then in the Gateway Compatible Destination vector pGWB502-C-SF-TAP for in planta phosphorylation assay	This Study
pGWB502-ZmSGT1_2xStrep_HIS	For in planta phosphorylation assay	This Study
pGWB441-ZmSGT1-eYFP	For in planta subcellular localization observation	This Study
pGWB441-ZmSGT1-eYFP	For in planta subcellular localization	This Study

(phosphomimic where T_{150} is mutated to D)	observation	
pGWB441-ZmSGT1-eYFP (phosphonull where T_{150} is mutated to A)	For in planta subcellular localization observation	This Study
pGWB414-ZmSGT1-3xHA-NLS-eYFP	For in planta subcellular localization observation	This Study
pGWB414-ZmSGT1-3xHA-nls-eYFP	For in planta subcellular localization observation	This Study
pGWB414-ZmSGT1-3xHA-NES-eYFP	For in planta subcellular localization observation	This Study
pGWB414-ZmSGT1-3xHA-nes-eYFP	For in planta subcellular localization observation	This Study

Supplemental Table 4. *U. maydis* Strains Used in this Study.

Strains	**Genotype**	**Reference**
SG200	a1mfa2bW2 bE1	Kamper et al., 2006
SG200Δ*see1*	a1mfa bW2bE1 Δ*um02239*::hph	Schilling et al., 2014
SG200Δ*see1-see1*	a1mfa2 bW2bE1 Δ*um02239*::hph ip^{r}[Psee1-see1]ip^{s}	Schilling et al., 2014
SG200Δ*tin3*	a1mfa bW2bE1 Δ*um10556*::hph	Brefort et al., 2014
SG200Δ*see1*-Ppit2-see1-HA	a1mfa2 bW2bE1 Δ*um02239*::hph ip^{r}[Ppit2::see1::HA]ip^{s}	This study
SG200Δ*see1*-Psee1-SPsee1-see1-3XHA	a1mfa2 bW2bE1 Δ*um02239*::hph ip^{r}[Psee1:SPsee1:see1::3XHA]ip^{s}	This study
SG200-Psee1-SPsee1-mcherry-3XHA	a1mfa2 bW2bE1 ::hph ip^{r}[Psee1:SPsee1:mcherry::3XHA]ip^{s}	This study
SG200-Psee1-GFP-3XHA	a1mfa2 bW2bE1 ::hph ip^{r}[Psee1:GFP::3XHA]ip^{s}	This study

Supplemental Table 5. Peptides Identified by Mass Spectrometry

1) **ZmSGT1 in absence of See1:** There is phosphorylation detected for the highlighted peptides in the variable regions 1 (T_{150}) and 2 (T_{262}) of the maize SGT1 protein.

MASCOT Search Results

Protein View: gi|670432243

PREDICTED: protein SGT1 homolog [Zea mays]

Database: NCBInr
Score: 15766
Nominal mass (M_r): 40288
Calculated pI: 4.93
Taxonomy: Zea mays

This protein sequence matches the following other entries:

- gi|413950678 from Zea mays

Sequence similarity is available as an NCBI BLAST search of gi|670432243 against nr.

Search parameters

MS data file: \\212.87.29.88\Orbita\DO\1401-styczen\40123130krzy_AR3.raw
Enzyme: Trypsin: cuts C-term side of KR unless next residue is P.
Fixed modifications: Carbamidomethyl (C)
Variable modifications: Carboxymethyl (K), Oxidation (M), Phospho (ST), Phospho (Y)

Protein sequence coverage: 68%

Matched peptides shown in ***bold red***.

```
  1 MAASDLESKA KEAFVDDDFE LAAELYTQAI DAGPATADLY ADRAQAHIKL
 51 GNYTEAVADA NKAIGLDPTM HKAYYRKGAA CIKLEEYQTA KAALELGSSY
101 APGDSRFTRL LKECDECIAE ESSQAPAKNV EAPVAATVED KEDVANMDNT
151 PPVVEPPSKP KYRHDYYNSA TEVVLTIYAK GVPADSVVID FGDQMLSVSI
201 EVPGEEPYHF QPRLFSKIIP EKCKYQVLST KVEIRLAKAE QVTWTTLDYS
251 GRPKAIPQKI STPAETAPRP SYPSSKSKKD WDKLEAEVKK EEKEEKLEGD
301 AALNKFFRDI YKDADEDMRR AMDKSFRESN GTVLSTNWKD VGSKTVEASP
351 PDGMELKKWE I
```

Unformatted sequence string: 361 residues (for pasting into other applications).

Sort peptides by ◉ Residue Number ○ Increasing Mass ○ Decreasing Mass

Show predicted peptides also

Query	Start – End	Observed	Mr(expt)	Mr(calc)	ppm	M	Score	Expect	Rank	U	Peptide
3623	129 – 161	872.6876	3486.7214	3486.7188	0.75	1	64	0.0022	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y
3624	129 – 161	872.6877	3486.7217	3486.7188	0.83	1	52	0.035	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y
3625	129 – 161	872.6877	3486.7217	3486.7188	0.84	1	175	1.8e-14	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y
3630	129 – 161	1163.2480	3486.7222	3486.7188	0.96	1	177	1.3e-14	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y
3636	129 – 161	872.6879	3486.7225	3486.7188	1.07	1	72	0.00039	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y
3640	129 – 161	872.6880	3486.7229	3486.7188	1.18	1	173	3.3e-14	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y
3643	129 – 161	698.3520	3486.7236	3486.7188	1.36	1	80	5.7e-05	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y
3644	129 – 161	872.6882	3486.7237	3486.7188	1.39	1	139	7e-11	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y
3648	129 – 161	872.6884	3486.7245	3486.7188	1.63	1	92	3.8e-06	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y
3649	129 – 161	1163.2488	3486.7246	3486.7188	1.65	1	175	2.1e-14	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y
3653	129 – 161	872.6885	3486.7249	3486.7188	1.76	1	159	7.8e-13	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y
3654	129 – 161	872.6885	3486.7249	3486.7188	1.76	1	189	7.8e-16	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y
3785	129 – 161	1168.5827	3502.7263	3502.7137	3.58	1	79	7.9e-05	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y + Oxidation (M)
3786	129 – 161	876.6889	3502.7263	3502.7137	3.59	1	138	9.1e-11	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y + Oxidation (M)
3866	129 – 161	892.6787	3566.6857	3566.6851	0.15	1	112	6.2e-08	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y + Phospho (ST)
3867	129 – 161	892.6787	3566.6858	3566.6851	0.18	1	119	1.2e-08	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y + Phospho (ST)
3868	129 – 161	892.6789	3566.6867	3566.6851	0.43	1	97	2e-06	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y + Phospho (ST)
3869	129 – 161	892.6792	3566.6876	3566.6851	0.68	1	62	0.0051	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y + Phospho (ST)
2742	260 – 276	633.6344	1897.8812	1897.8822	-0.53	0	105		1		K.ISTPAETAPRPSYPSSK.S + Phospho (ST); [+30.0106 at A5]

Query	Start – End	Observed	Mr(expt)	Mr(calc)	ppm	M	Score	Expect	Rank	U	Peptide
2743	260 – 276	949.9487	1897.8829	1897.8822	0.33	0	125		1		K.ISTPAETAPRPSYPSSK.S + Phospho (ST); [+30.0106 at A5]
2744	260 – 276	949.9493	1897.8840	1897.8822	0.92	0	121		1		K.ISTPAETAPRPSYPSSK.S + Phospho (ST); [+30.0106 at A5]
2745	260 – 276	633.6353	1897.8840	1897.8822	0.95	0	95		1		K.ISTPAETAPRPSYPSSK.S + Phospho (ST); [+30.0106 at A5]
2746	260 – 276	633.6359	1897.8857	1897.8822	1.84	0	53		1		K.ISTPAETAPRPSYPSSK.S + Phospho (ST); [+30.0106 at A5]
2748	260 – 276	949.9502	1897.8859	1897.8822	1.95	0	118		1		K.ISTPAETAPRPSYPSSK.S + Phospho (ST); [+30.0106 at A5]
2749	260 – 276	949.9504	1897.8862	1897.8822	2.08	0	125		1		K.ISTPAETAPRPSYPSSK.S + Phospho (ST); [+30.0106 at A5]
2750	260 – 276	633.6360	1897.8862	1897.8822	2.11	0	97		1		K.ISTPAETAPRPSYPSSK.S + Phospho (ST); [+30.0106 at A5]
2751	260 – 276	633.6360	1897.8863	1897.8822	2.14	0	97		1		K.ISTPAETAPRPSYPSSK.S + Phospho (ST); [+30.0106 at A5]
2752	260 – 276	949.9505	1897.8863	1897.8822	2.17	0	79		1		K.ISTPAETAPRPSYPSSK.S + Phospho (ST); [+30.0106 at A5]
2753	260 – 276	633.6361	1897.8864	1897.8822	2.20	0	98		1		K.ISTPAETAPRPSYPSSK.S + Phospho (ST); [+30.0106 at A5]
1803	345 – 357	687.3347	1372.6548	1372.6544	0.27	0	108	1.4e-07	1	U	K.TVEASPPDGMELK.K
1804	345 – 357	687.3348	1372.6550	1372.6544	0.40	0	103	3.8e-07	1	U	K.TVEASPPDGMELK.K
1805	345 – 357	687.3348	1372.6551	1372.6544	0.53	0	110	8.9e-08	1	U	K.TVEASPPDGMELK.K
1806	345 – 357	687.3349	1372.6553	1372.6544	0.63	0	110	8.2e-08	1	U	K.TVEASPPDGMELK.K
1807	345 – 357	687.3350	1372.6555	1372.6544	0.76	0	104	3.4e-07	1	U	K.TVEASPPDGMELK.K
1808	345 – 357	687.3350	1372.6555	1372.6544	0.79	0	104	3.6e-07	1	U	K.TVEASPPDGMELK.K
1809	345 – 357	687.3354	1372.6562	1372.6544	1.33	0	107	1.5e-07	1	U	K.TVEASPPDGMELK.K
1810	345 – 357	687.3355	1372.6564	1372.6544	1.45	0	110	8.7e-08	1	U	K.TVEASPPDGMELK.K
1811	345 – 357	687.3355	1372.6565	1372.6544	1.52	0	98	1.3e-06	1	U	K.TVEASPPDGMELK.K
1812	345 – 357	687.3356	1372.6567	1372.6544	1.64	0	110	8.3e-08	1	U	K.TVEASPPDGMELK.K
1813	345 – 357	687.3359	1372.6572	1372.6544	2.05	0	104	3.4e-07	1	U	K.TVEASPPDGMELK.K
1814	345 – 357	687.3361	1372.6577	1372.6544	2.38	0	63	0.0038	1	U	K.TVEASPPDGMELK.K
1815	345 – 357	687.3365	1372.6584	1372.6544	2.91	0	91	5.8e-06	1	U	K.TVEASPPDGMELK.K
1816	345 – 357	687.3366	1372.6587	1372.6544	3.14	0	72	0.00049	1	U	K.TVEASPPDGMELK.K
1854	345 – 357	695.3324	1388.6503	1388.6493	0.71	0	67	0.0016	1	U	K.TVEASPPDGMELK.K + Oxidation (M)
1855	345 – 357	695.3325	1388.6504	1388.6493	0.80	0	59	0.0097	1	U	K.TVEASPPDGMELK.K + Oxidation (M)
1856	345 – 357	695.3325	1388.6505	1388.6493	0.87	0	94	3.1e-06	1	U	K.TVEASPPDGMELK.K + Oxidation (M)
1857	345 – 357	695.3326	1388.6507	1388.6493	0.96	0	60	0.0086	1	U	K.TVEASPPDGMELK.K + Oxidation (M)
1858	345 – 357	695.3326	1388.6507	1388.6493	0.96	0	83	4.2e-05	1	U	K.TVEASPPDGMELK.K + Oxidation (M)
1860	345 – 357	695.3329	1388.6513	1388.6493	1.39	0	82	5.1e-05	1	U	K.TVEASPPDGMELK.K + Oxidation (M)
1861	345 – 357	695.3330	1388.6514	1388.6493	1.49	0	84	3.6e-05	1	U	K.TVEASPPDGMELK.K + Oxidation (M)
1862	345 – 357	695.3331	1388.6516	1388.6493	1.61	0	82	4.9e-05	1	U	K.TVEASPPDGMELK.K + Oxidation (M)
1863	345 – 357	695.3331	1388.6516	1388.6493	1.63	0	53	0.041	1	U	K.TVEASPPDGMELK.K + Oxidation (M)
1864	345 – 357	695.3331	1388.6517	1388.6493	1.71	0	83	3.8e-05	1	U	K.TVEASPPDGMELK.K + Oxidation (M)
1866	345 – 357	695.3332	1388.6518	1388.6493	1.75	0	82	5.6e-05	1	U	K.TVEASPPDGMELK.K + Oxidation (M)
1868	345 – 357	695.3337	1388.6529	1388.6493	2.56	0	82	5.2e-05	1	U	K.TVEASPPDGMELK.K + Oxidation (M)

Query	Start – End	Observed	Mr(expt)	Mr(calc)	ppm	M	Score	Expect	Rank	U	Peptide
2115	345 – 358	506.5892	1516.7459	1516.7443	1.07	1	60	0.007	1	U	K.TVEASPPDGMELKK.W + Oxidation (M)

2) **ZmSGT1 in presence of See1:** There is phosphorylation detected for the highlighted peptides only at the unique site TP in the variable region 2 of the maize SGT1 protein (T_{262}).

MASCOT Search Results

Protein View: gi|670432243

PREDICTED: protein SGT1 homolog [Zea mays]

Database: NCBInr
Score: 9381
Nominal mass (M_r): 40288
Calculated pI: 4.93
Taxonomy: Zea mays

This protein sequence matches the following other entries:

- gi|413950678 from Zea mays

Sequence similarity is available as an NCBI BLAST search of gi|670432243 against nr.

Search parameters

MS data file: \\212.87.29.88\Orbita\DO\1401-styczen\40123128krzy_AR1.raw
Enzyme: Trypsin: cuts C-term side of KR unless next residue is P.
Fixed modifications: Carbamidomethyl (C)
Variable modifications: Carboxymethyl (K), Oxidation (M), Phospho (ST), Phospho (Y)

Protein sequence coverage: 63 %

Matched peptides shown in ***bold red***.

```
  1 MAASDLESKA KEAFVDDDFE LAAELYTQAI DAGPATADLY ADRAQAHIKL
 51 GNYTEAVADA NKAIGLDPTM HKAYYRKGAA CIKLEEYQTA KAALELGSSY
101 APGDSRFTRL LKECDECIAE ESSQAPAKNV EAPVAATVED KEDVANMDNT
151 PPVVEPPSKP KYRHDYYNSA TEVVLTIYAK GVPADSVVID FGDQMLSVSI
201 EVPGEEPYHF QPRLFSKIIP EKCKYQVLST KVEIRLAKAE QVTWTTLDYS
251 GRPKAIPQKI STPAETAPRP SYPSSKSKKD WDKLEAEVKK EEKEEKLEGD
301 AALNKFFRDI YKDADEDMRR AMDKSFRESN GTVLSTNWKD VGSKTVEASP
351 PDGMELKKWE I
```

Unformatted sequence string: 361 residues (for pasting into other applications).

Sort peptides by ◉ Residue Number ○ Increasing Mass ○ Decreasing Mass

Show predicted peptides also

Query	Start – End	Observed	Mr(expt)	Mr(calc)	ppm	M	Score	Expect	Rank	U	Peptide
3719	129 – 159	816.4016	3261.5771	3261.5711	1.85	1	78		1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSK.P
3732	129 – 159	1093.5333	3277.5781	3277.5660	3.68	1	90		1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSK.P + Oxidation (M)
3798	129 – 161	1163.2436	3486.7090	3486.7188	-2.82	1	183	3.6e-15	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y
3799	129 – 161	1163.2448	3486.7126	3486.7188	-1.79	1	173	3.2e-14	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y
3800	129 – 161	698.3500	3486.7136	3486.7188	-1.50	1	146	1.7e-11	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y
3802	129 – 161	698.3503	3486.7153	3486.7188	-1.02	1	140	5.8e-11	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y
3803	129 – 161	698.3503	3486.7153	3486.7188	-1.02	1	156	1.7e-12	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y
3805	129 – 161	698.3504	3486.7157	3486.7188	-0.90	1	163	3.5e-13	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y
3807	129 – 161	872.6863	3486.7163	3486.7188	-0.72	1	131	4.9e-10	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y
3808	129 – 161	872.6865	3486.7168	3486.7188	-0.57	1	123	3.2e-09	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y
3809	129 – 161	872.6865	3486.7170	3486.7188	-0.53	1	101	4.7e-07	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y
3810	129 – 161	872.6866	3486.7172	3486.7188	-0.47	1	137	1.4e-10	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y
3811	129 – 161	872.6866	3486.7172	3486.7188	-0.47	1	133	3.5e-10	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y
3812	129 – 161	872.6867	3486.7178	3486.7188	-0.30	1	159	7.9e-13	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y
3813	129 – 161	872.6868	3486.7182	3486.7188	-0.17	1	182	4.1e-15	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y
3817	129 – 161	872.6872	3486.7195	3486.7188	0.21	1	157	1.3e-12	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y

Query	Start – End	Observed	Mr(expt)	Mr(calc)	ppm	M	Score	Expect	Rank	U	Peptide
3818	129 – 161	872.6872	3486.7196	3486.7188	0.23	1	126	1.4e-09	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y
3820	129 – 161	698.3513	3486.7199	3486.7188	0.30	1	156	1.5e-12	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y
3821	129 – 161	872.6873	3486.7200	3486.7188	0.33	1	134	2.4e-10	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y
3822	129 – 161	698.3513	3486.7201	3486.7188	0.38	1	125	1.8e-09	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y
3823	129 – 161	872.6873	3486.7202	3486.7188	0.40	1	138	9.7e-11	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y
3824	129 – 161	1163.2475	3486.7207	3486.7188	0.53	1	194	2.5e-16	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y
3893	129 – 161	876.6878	3502.7220	3502.7137	2.37	1	115	2e-08	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y + Oxidation (M)
3895	129 – 161	876.6880	3502.7229	3502.7137	2.61	1	130	5.8e-10	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y + Oxidation (M)
3896	129 – 161	1168.5830	3502.7272	3502.7137	3.84	1	130	6e-10	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y + Oxidation (M)
3898	129 – 161	1168.5850	3502.7332	3502.7137	5.55	1	144	2.5e-11	1	U	K.NVEAPVAATVEDKEDVANMDNTPPVVEPPSKPK.Y + Oxidation (M)
2212	260 – 276	606.9800	1817.9180	1817.9159	1.16	0	60		1		K.ISTPAETAPRPSYPSSK.S + [+30.0106 at A5]
2213	260 – 276	606.9802	1817.9188	1817.9159	1.59	0	58		1		K.ISTPAETAPRPSYPSSK.S + [+30.0106 at A5]
2214	260 – 276	606.9807	1817.9203	1817.9159	2.42	0	60		1		K.ISTPAETAPRPSYPSSK.S + [+30.0106 at A5]
2321	260 – 276	633.6356	1897.8849	1897.8822	1.41	0	97		1		K.ISTPAETAPRPSYPSSK.S + Phospho (ST); [+30.0106 at A5]
2322	260 – 276	949.9498	1897.8851	1897.8822	1.49	0	128		1		K.ISTPAETAPRPSYPSSK.S + Phospho (ST); [+30.0106 at A5]
2326	260 – 276	633.6358	1897.8856	1897.8822	1.79	0	101		1		K.ISTPAETAPRPSYPSSK.S + Phospho (ST); [+30.0106 at A5]
2327	260 – 276	633.6359	1897.8857	1897.8822	1.84	0	87		1		K.ISTPAETAPRPSYPSSK.S + Phospho (ST); [+30.0106 at A5]
2329	260 – 276	949.9504	1897.8862	1897.8822	2.10	0	93		1		K.ISTPAETAPRPSYPSSK.S + Phospho (ST); [+30.0106 at A5]
2332	260 – 276	949.9514	1897.8882	1897.8822	3.13	0	117		1		K.ISTPAETAPRPSYPSSK.S + Phospho (ST); [+30.0106 at A5]
1451	345 – 357	687.3349	1372.6553	1372.6544	0.63	0	88	1.2e-05	1	U	K.TVEASPPDGMELK.K
1452	345 – 357	687.3351	1372.6556	1372.6544	0.87	0	100	7.2e-07	1	U	K.TVEASPPDGMELK.K
1453	345 – 357	687.3351	1372.6556	1372.6544	0.90	0	113	4.5e-08	1	U	K.TVEASPPDGMELK.K
1454	345 – 357	687.3352	1372.6559	1372.6544	1.10	0	91	6.2e-06	1	U	K.TVEASPPDGMELK.K
1455	345 – 357	687.3356	1372.6566	1372.6544	1.59	0	91	6.7e-06	1	U	K.TVEASPPDGMELK.K
1456	345 – 357	687.3356	1372.6566	1372.6544	1.62	0	99	1.1e-06	1	U	K.TVEASPPDGMELK.K
1457	345 – 357	687.3357	1372.6569	1372.6544	1.81	0	110	8.6e-08	1	U	K.TVEASPPDGMELK.K
1458	345 – 357	687.3358	1372.6570	1372.6544	1.86	0	105	2.3e-07	1	U	K.TVEASPPDGMELK.K
1460	345 – 357	687.3359	1372.6572	1372.6544	2.00	0	105	2.7e-07	1	U	K.TVEASPPDGMELK.K
1461	345 – 357	687.3359	1372.6573	1372.6544	2.08	0	92	5.1e-06	1	U	K.TVEASPPDGMELK.K
1462	345 – 357	687.3365	1372.6584	1372.6544	2.89	0	93	4.1e-06	1	U	K.TVEASPPDGMELK.K
1463	345 – 357	687.3369	1372.6593	1372.6544	3.56	0	71	0.00058	1	U	K.TVEASPPDGMELK.K
1486	345 – 357	695.3320	1388.6495	1388.6493	0.15	0	83	3.9e-05	1	U	K.TVEASPPDGMELK.K + Oxidation (M)
1488	345 – 357	695.3328	1388.6511	1388.6493	1.27	0	80	7.7e-05	1	U	K.TVEASPPDGMELK.K + Oxidation (M)
1489	345 – 357	695.3329	1388.6512	1388.6493	1.32	0	92	5.7e-06	1	U	K.TVEASPPDGMELK.K + Oxidation (M)
1491	345 – 357	695.3329	1388.6513	1388.6493	1.40	0	71	0.00072	1	U	K.TVEASPPDGMELK.K + Oxidation (M)
1492	345 – 357	695.3330	1388.6514	1388.6493	1.46	0	77	0.00016	1	U	K.TVEASPPDGMELK.K + Oxidation (M)

Query	Start – End	Observed	Mr(expt)	Mr(calc)	ppm	M	Score	Expect	Rank	U	Peptide
1493	345 – 357	695.3330	1388.6514	1388.6493	1.52	0	70	0.00086	1	U	K.TVEASPPDGMELK.K + Oxidation (M)

SUPPLEMENTAL METHODS

Supplemental Method 1.

Microscopy and staining with WGA AF488 and Propidium Iodide.

To visualize initial fungal proliferation in tissue infected with Δ*see1*, in comparison to the tissue infected with wild-type SG200 the area approx.1–3 cm below the injection site was excised at 2 and 3 days post infection. The collected leaf samples were fixed in 100% ethanol and then incubated for 3-4 hours in 10% (w/v) KOH at 85ºC. The samples were washed with Phosphate Saline buffer (PBS) repeatedly until the pH reached 7.4. Fungal hyphae were stained with WGA-AF488 (Invitrogen) and the plant cell wall was co-stained with propidium iodide (Sigma-Aldrich). Leaf samples were incubated in 1.5 mL staining solution (1 µg/mL propidium iodide, 10 µg/mL WGA-AF488). Samples were infiltrated 3 times at 250 bar pressure and were then decolorized in PBS and incubated in the dark at 4ºC until microscopy.

Supplemental Method 2.

Mass Spectrometry Analysis.

Gel bands containing the proteins of interest were subjected to a standard proteomic procedure during which proteins were reduced with 100 mM DTT for 30 min at 56°C, alkylated with iodoacetamide in darkness for 45 min at RT and digested overnight with sequencing grade modified trypsin (Promega Corporation, Madison, WI, USA; www.promega.com). The resulting peptides were eluted from the gel with 0.1% TFA and 2% ACN and applied to the SwellGel Gallium-Chelated Discs (Thermo Fisher Scientific Inc., Waltham, MA, USA; www.thermoscientific.com). Phosphopeptide isolation was carried out according to the manufacturer's instructions and finally the phosphopeptide fraction was eluted from Ga(III) resin with 100 mM ammonium bicarbonate. Liquid chromatography (LC) - mass spectrometry (MS) analyses of peptides were carried out using a nano-Acquity (Waters Corp., Milford, MA, USA; www.waters.com) LC system coupled to an Orbitrap Velos (Thermo) mass spectrometer. Spectrometer parameters were as follows: capillary voltage, 2.5 kV; cone, 40 V; N_2 gas flow, 0; range, 300-2000 (m/z). The spectrometer was calibrated on a weekly basis with Calmix (caffeine, MRFA, Ultramark 1621). The

sample was first loaded from the autosampler tray (cooled to 10°C) to the pre-column (Symmetry C18, 180 µm × 20 mm, 5 µm; Waters) with a mobile phase of 100% MilliQ H_2O acidified by 0.1% formic acid. The peptides were then transferred to a nano-UPLC column (BEH130 C18, 75 µm × 250 mm, 1.7 µm; Waters) by a gradient of 5-30% acetonitrile, 0.1% FA in 45 min. The column outlet was directly coupled to the ESI ion source of the Orbitrap Velos (Thermo Fisher Scientific) mass spectrometer working in the regime of data dependent MS to MS/MS switch. A blank run ensuring lack of cross contamination from previous samples preceded each analysis. After preprocessing of the raw data with Mascot Distiller software (version 2.1.1, Matrix Science), output lists of precursor and product ions were compared to NCBInr database using Mascot database search engine (v2.1, Matrix Science). Search parameters included semiTrypsin enzyme specificity, one missed cleavage site, Cys carbamidomethyl fixed modification and variable modifications including Met oxidation and phosphorylation of Ser, Thr or Tyr residues. Protein mass and taxonomy were unrestricted, peptide mass tolerance was 20 ppm and the MS/MS tolerance was 0.8 Da. Proteins containing peptides with Mascot cut-off scores >50, indicating identity or extensive homology ($p<0.05$) of peptide, were considered positive identifications.

Supplemental Method 3.

Microarray Analysis.

Microarray analyses were performed with 200 ng total RNA extracted from *U. maydis* infected seedling tissue at 6 dpi. For this time point, samples from three independent biological replicates were labeled and hybridized according to Agilent's One-Color Microarray-Based Gene Expression Analysis Low Input Quick Amp Labeling protocol (version 6.5). Cye-3-labeled probes were hybridized to 4x44k custom-designed Agilent microarray chips for maize based on a previous 4x44k custom-designed Agilent microarray. The preparation of the one color spike mix labeling, hybridization, microarray washing and scanning was done by following the one color microarray based protocol from Agilent. Microarray image files were analyzed using Agilent's Feature Extraction software v. 10.5 which calculates for each spot a background

corrected signal intensity value (gProcessedSignal) that was used for further analysis. The microarray data obtained in this study were analyzed using the Partek Genomics Suite version 6.12. Expression values were normalized using the RMA method (Irizarry et al., 2003). Criteria for significance were a corrected p-value (per sample) with an FDR of 0.05 and a fold-change of >2. Differentially expressed genes were calculated by a 1-way ANOVA (Bewick et al., 2004).

Supplemental Method 4.

Plasmid Constructs and Nucleic Acid Construction.

Generation of the *U. maydis* ∆*see1* mutant and the complementation strain was done as described previously in Schilling et al., 2014. For See1 ectopic overexpression in *U. maydis*, *um02239* coding sequence was amplified by PCR with primers that added appropriate restriction site using *U. maydis* SG200 DNA as a template. Next, the PCR-product was digested with SacII and XbaI and ligated into plasmid p123-Ppit2 (Mueller et al., 2013) to obtain *p123-Ppit2-See1* where *see1* is under the control of Pit2 promoter. For the electron microscopy constructs, *see1* along with the promoter and signal peptide was amplified from the *U. maydis* SG200 DNA. The reverse primer was integrated with a *3xHA* to make a fusion protein of See1-3xHA. The amplified fragment was digested with SbfI and NotI and ligated into the plasmid p123 (Aichinger et al., 2003) to obtain *p123-Psee1-See1-3xHA*. For generation of the secretory control *mCherry* was amplified from p123-mCherry (Aichinger et al., 2003) and promoter and signal peptide of *see1* was amplified from the previously generated *p123-Psee1-See1-3xHA*. Fusion fragment of *Psee1-SPSee1-mCherry 3xHA* was generated with the overhang ligation coding for signal peptide of See1 and mCherry. The PCR product was digested with SbfI and NotI and ligated into the p123 vector to obtain *p123-Psee1-SPsee1-mcherry-3xHA*. GFP was also amplified from p123, restriction digestion was done with the enzymes NcoI and NotI and ligated into the generated vector p123-Psee1 (this study) to obtain *p123-Psee1-GFP-3xHA*. All *U. maydis* strains used in this study have been listed in the Supplemental Table 4. For transient expression in maize plasmid pGreenII 0029-35S-*See1-mCherry*, was generated by amplifying the sequence of *See1* without its secretion signal from SG200 genomic DNA and integrating it into pGreenII 0029-35S

CP1A-mCherry (Mueller et al., 2013) by replacing *CP1A* with XbaI and SacI fragment. The control 35S- *PIP-YFP* was used from (Djamei et al., 2011). Constructs for the yeast two hybrid interaction were based on the vectors pGBKT7 and pGADT7 (Clontech). To construct pGBKT7-*See1*$_{22\text{-}157,}$ *See1* was amplified from the genomic DNA of *U. maydis*. The PCR products digested with NdeI and BamHI and ligated into plasmid pGBKT7 (Clontech, Mountain View, USA). All the primers used for the amplification of these genes have been mentioned in Supplemental Table 2. To obtain pGADT7 SGT1 full length from maize was amplified from cDNA, digested with NdeI and BamHI and ligated into pGADT7 (Clontech, Mountain View, USA). For expression in *N. benthamiana* and co-immunoprecipitation *See1-Myc* and *SGT1-HA* were cloned from the already generated yeast two hybrid constructs into pGreen000 (Hellens et al., 2000) via restriction sites SacI and XbaI (*See1*) and BamHI and XbaI (*SGT1*). The sequences coding for respective tags were integrated into the reverse primers for PCR amplification. Constructs for the microscopic interaction studies via BiFC were based on modified pUC-SPYNE-35S-*mCherry* and pUC-SPYCE-35S-*CFP* (Hemetsberger et al., 2012). PCR products of *See1* and *SGT1* were cloned into the BiFC-vectors via BamHI and XhoI restriction sites. Created BiFC vectors were transformed into *A. tumefaciens* GV3101 cells by following the protocol of (Sparkes et al., 2006).

For the in planta phosphorylation experiment, *See1*$_{22\text{-}157,}$ was amplified along with a reverse primer that was encoded a *HA*-tag (Supplemental Table 2), thus enabling the expression of gene with a C terminal HA-tag. The PCR product was cloned into DEX inducible vector pTA7001 (Aoyama and Chua, 1997) via the restriction enzymes SpeI and XbaI. Maize *SGT1* was initially cloned into Gateway entry vector pENTR-D-TOPO (Invitrogen) without a stop codon to make C-terminal StrepII-tag fusion and was then transferred into pGWB502-C-SF-TAP (Golisz et al., 2013) via LR clonase to generate 35S-*Zm-SGT1-2xStrepII-HIS*. Primers used to amplify these are included in Supplemental Table 2. All the other destination constructs used in the assay Nb-*SGT1*, salicylic acid induced protein kinase (SIPK), Nb-*MEK2*^{DD}, Nb-*MEK2*^{KR} and pROK empty were used from the previous study of (Hoser et al., 2013).

The Zm-*SGT1* was amplified with the restrictions sited EcoRI and XhoI into the plasmid pGREG536 which is with the GAL4 promoter. This generated construct was used for the complementation assay of the yeast *sgt1* mutants to check for the functionality of the Zm-SGT1 protein. The same plasmid was used for site directed

mutagenesis to perform a complementation assay with the yeast *sgt1* mutants using the phosphovariants of Zm-*SGT1*. For generation of the pET15b-HIS-Zm-*SGT1* for recombinant protein production in *E.coli*, SGT1 from cDNA of maize was amplified from the primers listed in Supplemental Table 2 with the restriction sites NdeI and BamHI. The protein expression vectors for Nt-*SIPK* and Nb-*MEK2* were used from the previous study of Hoser et al., 2013. The previously generated entry vector pENTR-D-TOPO-Zm-SGT1 for the C terminal fusion was used in recombination cloning to generate all the destination vectors for the subcellular localization of the wild-type and the phosphovariants of Zm-*SGT1*.

Supplemental Method 5.

Transient expression in *Z. mays*.

For transient protein expression in maize, ballistic gene transfer method was implemented using the 1.6 µm gold particles (Bio-Rad, München, Germany). The gold particles were coated with the plasmids coding for the indicated genes driven under CaMV 35S promoter (Supplementary Table 2). For loading the plasmid onto the gold particles 2.5 M calcium chloride treatment and several washes of 100% ethanol were performed. The DNA labeled gold particles were resuspended in 100% ethanol and bombardment was performed using a PDS-1000/He system (Bio-Rad) onto 10 days old maize seedling leaves.

Micro-bombardment in *N. benthamiana* .

Leaves from 2- to 4-week-old *N. benthamiana* plants were used to determine the subcellular locations of maize SGT1-eYFP variants. For transient gene expression in epidermal cells, plasmid DNA (3 µg) was adsorbed onto tungsten M17 particles (diameter, 1.1 mm; 350 mg), and then microbombardment was performed at a pressure of 1,100 pound-force per square inch using the Biolistic PDS-1000/He Particle Delivery System (Bio-Rad). Tissues were analyzed 24 h after bombardment.

Supplemental Method 6.

Confocal Laser Scanning Microscopy for SGT1 localization in *N. benthamiana*.

Transient intracellular fluorescence of the wild-type maize SGT1 and the phospho variants was observed by confocal laser scanning microscopy using a Nikon TE2000E EZ-C1 inverted confocal microscope equipped with 609 oil immersion objective lens (numerical aperture = 1.4). YFP was excited at 488 nm from an argon ion laser and the fluorescence signals were detected using the 515/30 emission filter. Scanning was performed in sequential mode to prevent bleed through. Images were collected from a single optical section and processed using the EZ C1 program (Nikon Instruments B.V. Europe, Amstelveen, The Netherlands). Optimal imaging parameters were set up for each experiment and were equal for each image dataset. Quantification of fluorescence intensities in the nuclear and cytoplasmic regions was performed using ImageJ software (Abramoff et al., 2004).

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