

Supplementary Information

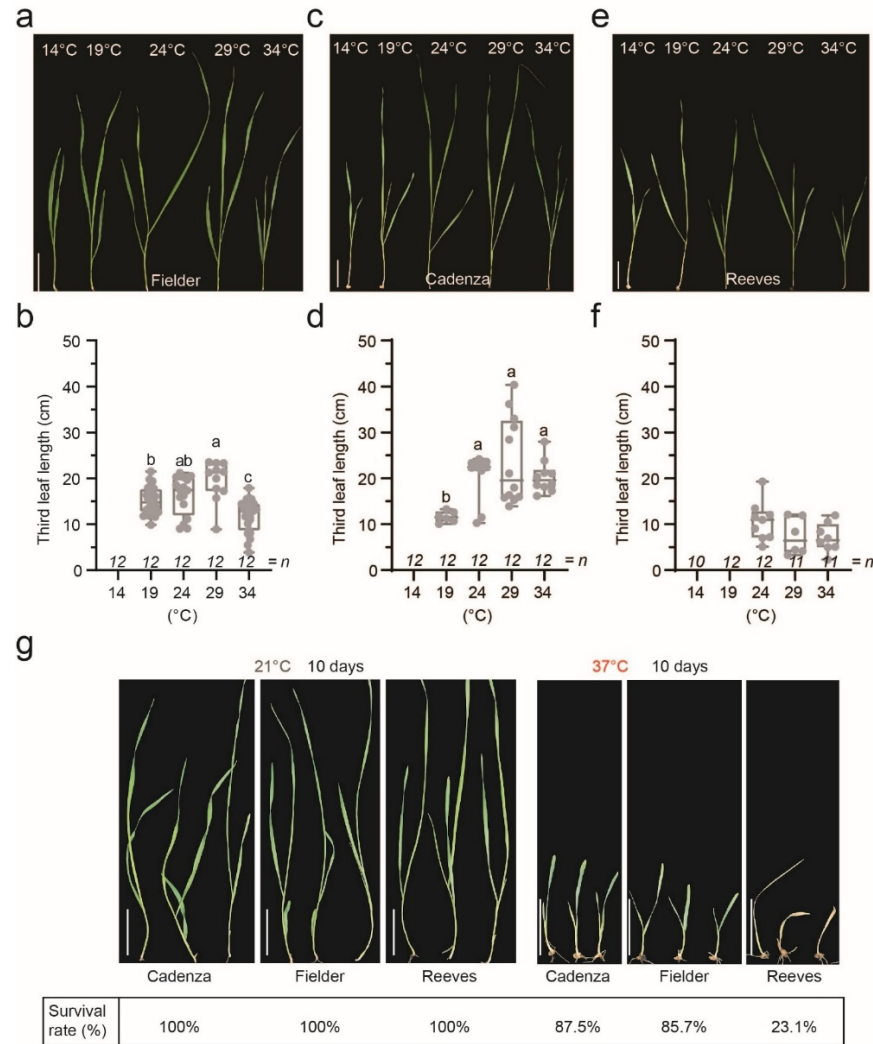
COI1 phosphorylation status drives redox state-mediated thermotolerance in plants

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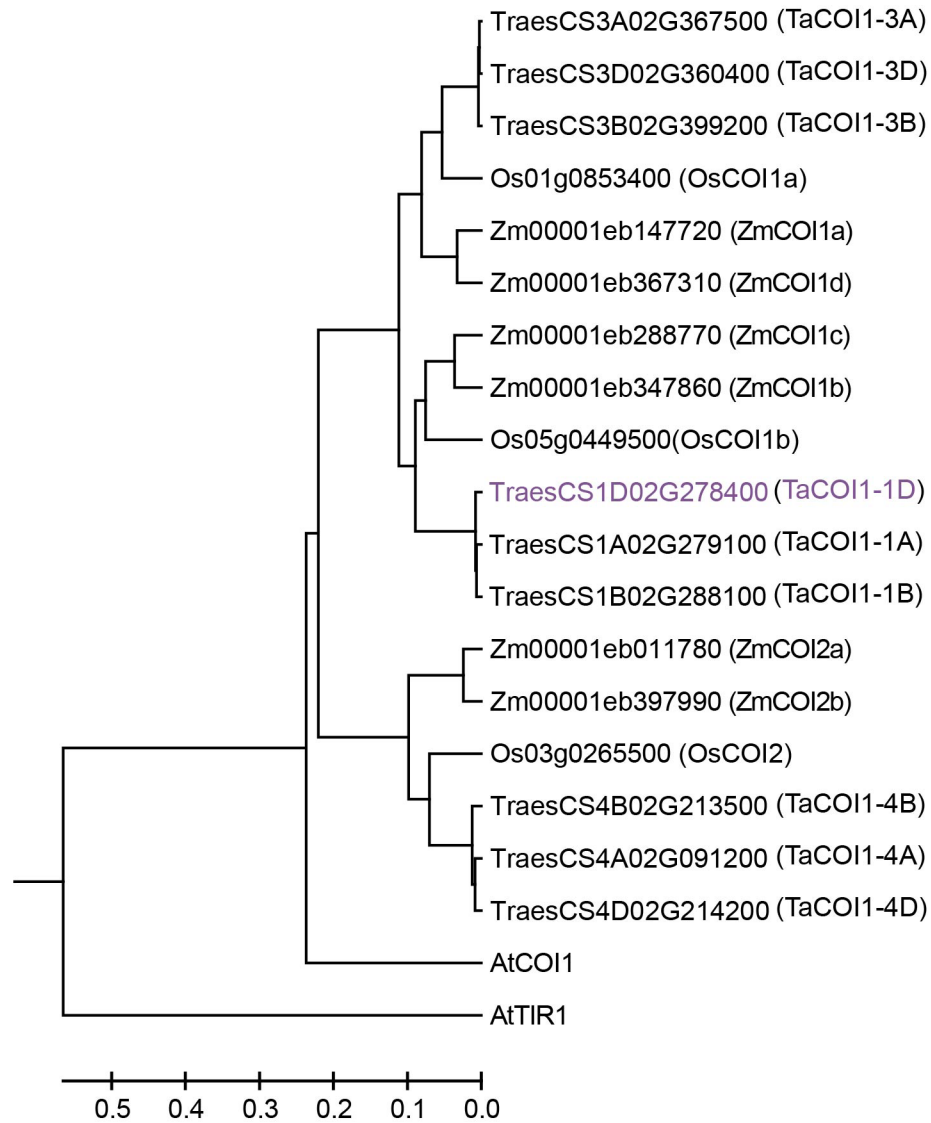
Figs. S1 to S23

Other Supplementary Materials for this manuscript include the following:

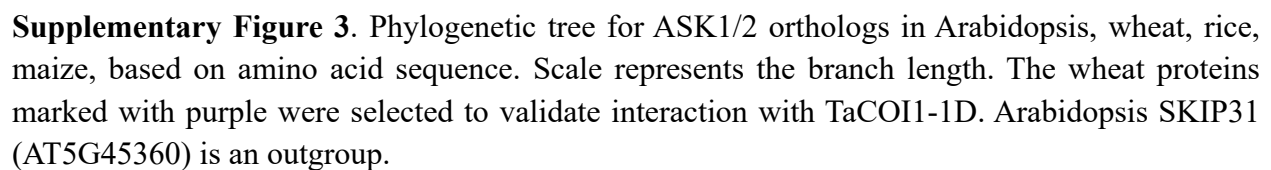
Supplementary Table S1 to S8



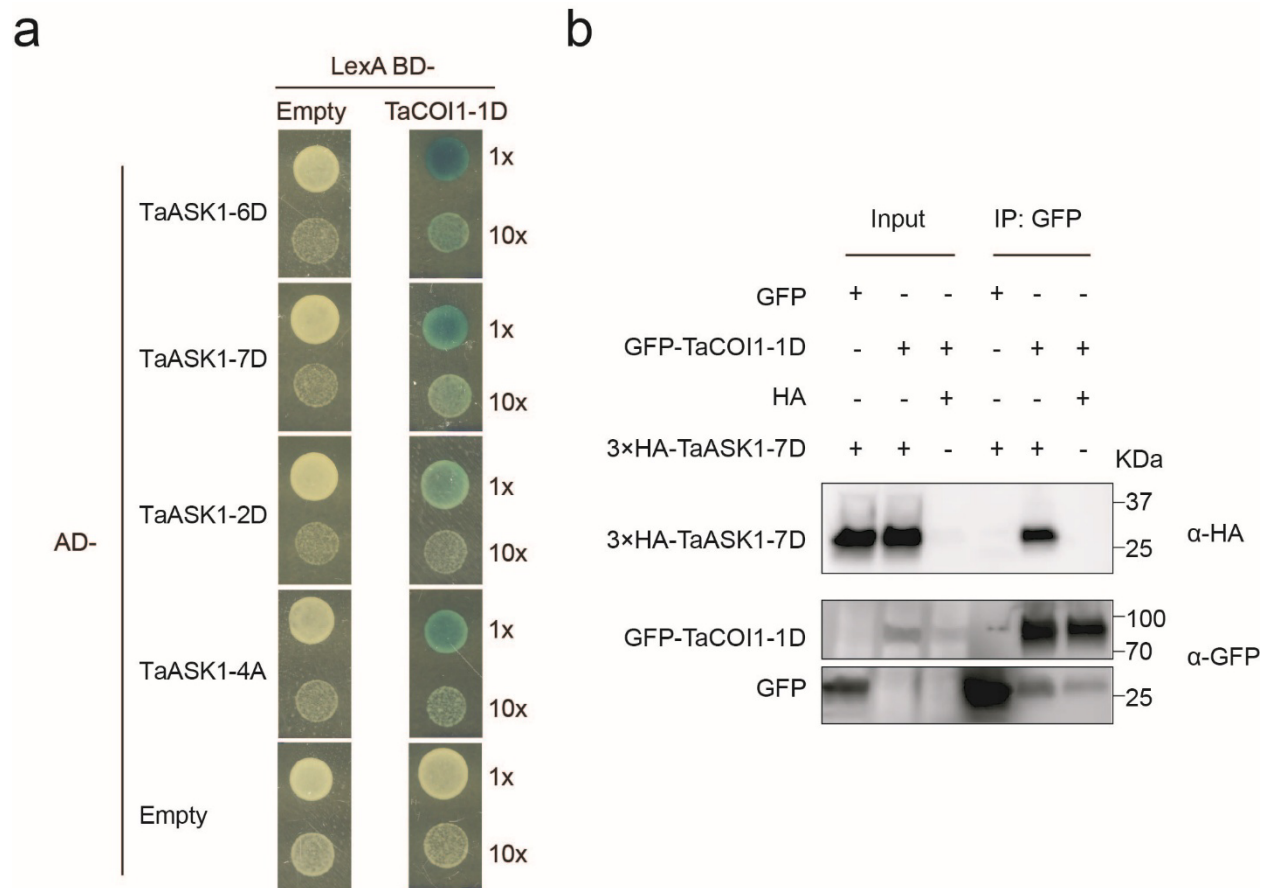
Supplementary Figure 1. (a-f) Representative images of three different wheat varieties along a temperature gradient (a, c, e) and quantification of the length of the third leaf (b, d, f) of 14-days-old ‘Fielder’ (a,b), ‘Cadenza’ (c,d) and ‘Reeves’ (e,f) wheat seedlings at 14°C, 19°C, 24°C, 29°C and 34°C. Scale bar = 5 cm. Box plots with individual data points represent the distribution of all individual measurements, showing the median (central lines) with Tukey-based whiskers and outliers. The box edges show the first and third quartiles. The whiskers represent the range of data within 1.5× the interquartile range from the first and third quartiles. Different letters denote significant differences ($p < 0.05$) based on one-way ANOVA with Tukey’s HSD (b, d, f). The number of individually measured seedlings (n) is indicated above the X axis (b, d, f). (g) Moderately high temperature response of 10-day-old wheat varieties ‘Cadenza’, ‘Fielder’, and ‘Reeves’ wheat seedlings at 21°C or 37°C. Scale bar = 5 cm. The survival percentage was determined for control and moderately high temperature conditions (based on the number of living plants after treatment divided by the total number of plants initially placed under control or moderately high temperature) and shown below the images. At least 12 plants per genotype were examined.



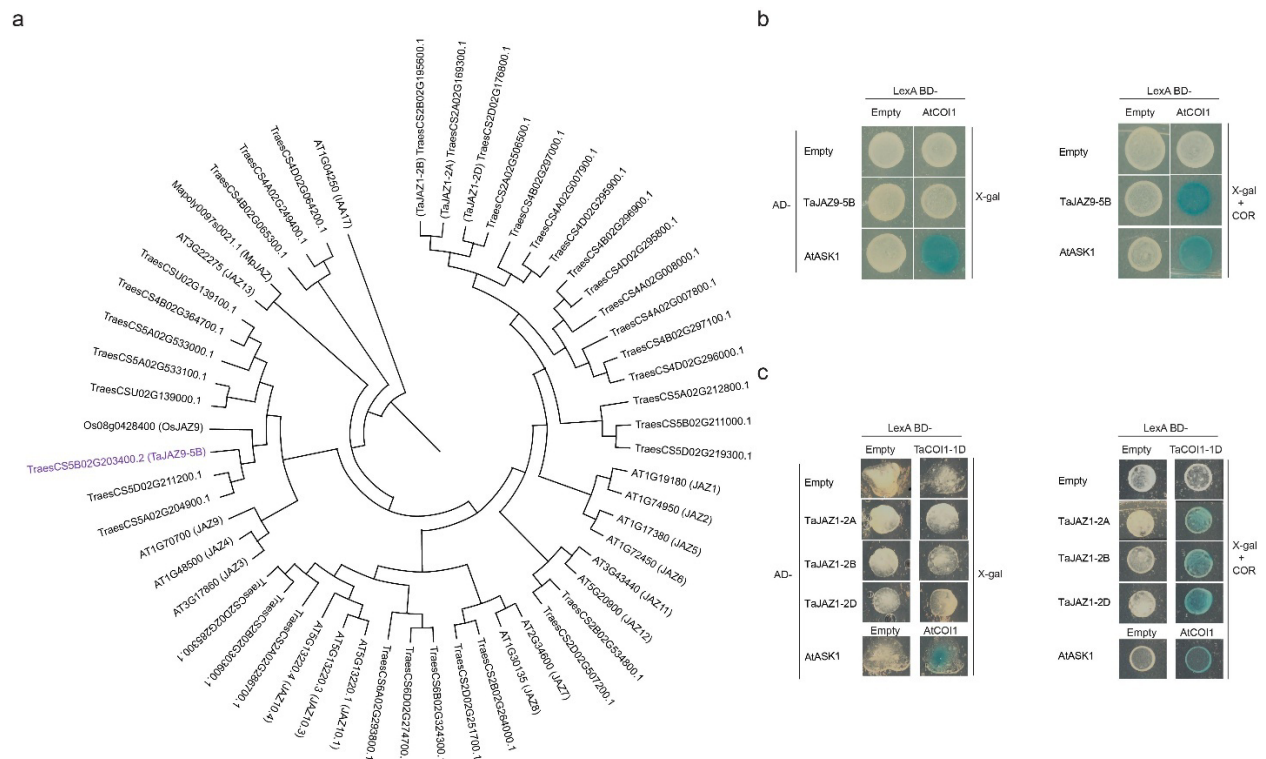
Supplementary Figure 2. Phylogenetic tree for COI1 orthologs in Arabidopsis, wheat, rice, maize, based on amino acid sequence. Scale represents the branch length. The protein with the identified phosphopeptide in TraesCS1D02G278400 (TaCOI1-1D) is marked in purple, and TaCOI1-1D is used in the overall text. Arabidopsis TIR1 (AT3G62980) is an outgroup.



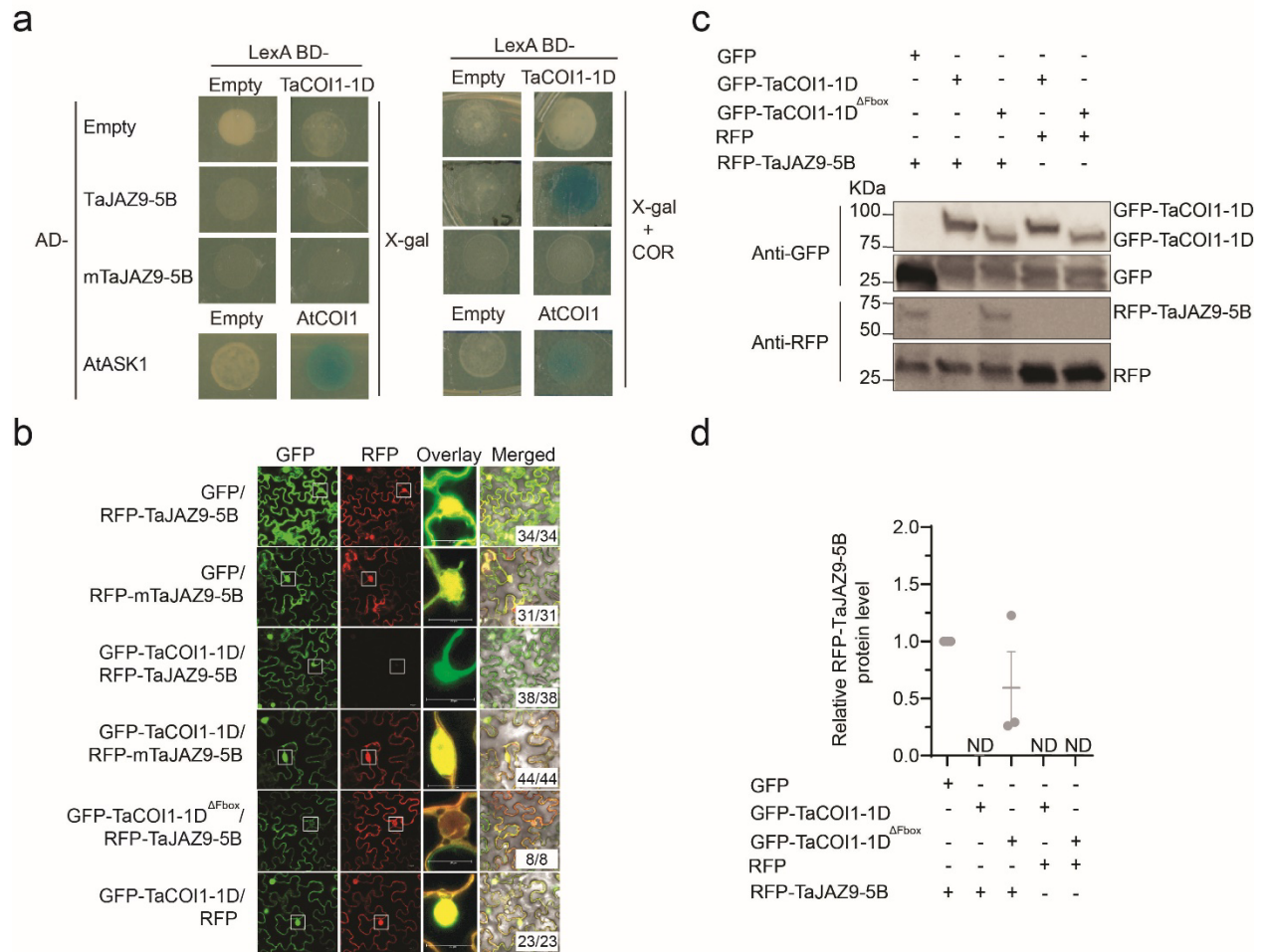
Supplementary Figure 3. Phylogenetic tree for ASK1/2 orthologs in Arabidopsis, wheat, rice, maize, based on amino acid sequence. Scale represents the branch length. The wheat proteins marked with purple were selected to validate interaction with TaCOI1-1D. Arabidopsis SKIP31 (AT5G45360) is an outgroup.



Supplementary Figure 4. (a) Yeast two-hybrid (Y2H) assay of TaASK1-6D, TaASK1-7D, TaASK1-2D, and TaASK1-4A interaction with TaCOI1-1D. 1× and 10× represent the yeast liquid culture that contains the corresponding proteins diluted times. Representative yeast colonies are shown, all independent colonies show similar interaction. **(b)** Representative Western blot of TaCOI1-1D and TaASK1-7D from *Nicotiana benthamiana* leaves transiently co-expressing 35S::GFP or 35S::GFP-TaCOI1-1D with 35S::HA-TaASK1-7D. The immunocomplex and input proteins were analysed by immunoblotting using anti-HA and anti-GFP antibodies. The experiments were independently repeated 3 times with similar results. LexA BD, LexA DNA-binding domain and AD, activation domain.



Supplementary Figure 5. (a) Phylogenetic tree for JAZs orthologs in Arabidopsis, wheat, rice, marchantia, based on amino acid sequence. Scale represents the branch length. TraesCS5B02G203400.2 (TaJAZ9-5B) is marked in purple, and TaJAZ9-5B is used in the overall text. Arabidopsis IAA17 (AT1G04250) is an outgroup. (b) Y2H assay of TaJAZ9-5B interaction with Arabidopsis COI1 in the presence or absence of COR. Blue colony colour indicates positive interaction; white colony colour indicates no interaction in the presence of 50 μ M coronatine (COR), all independent colonies show similar interaction. (c) Y2H assay of TaJAZ1-2A, TaJAZ1-2B, TaJAZ1-2D interaction with TaCOI1-1D, respectively. Blue colony colour indicates positive interaction; white colony colour indicates no interaction in the presence of 50 μ M coronatine (COR), all independent colonies show similar interaction. AtCOI1 and AtASK1 interaction used as a positive control. X-gal, 5-bromo-4-chloro-3-indolyl-beta-D-galacto-pyranoside. COR, coronatine. LexA BD, LexA DNA-binding domain and AD, activation domain.



Supplementary Figure 6. (a) Y2H assay of TaJAZ9-5B or mTaJAZ9-5B with TaCOI1-1D. Representative yeast colonies are shown, and all independent colonies show similar interaction. Blue colony colour indicates positive interaction; white colony colour indicates no interaction in the presence of 50 μ M coronatine (COR). AtCOI1 and AtASK1 interaction used as a positive control. LexA BD, LexA DNA-binding domain and AD, activation domain. **(b)** Representative confocal images of RFP:(m)JAZ9 levels upon transient expression in *N. benthamiana* of 35S::GFP, 35S::GFP-TaCOI1-1D or 35S::GFP-TaCOI1-1D^{ΔF-box} and 35S::RFP-TaJAZ9-5B or 35S::RFP-mTaJAZ9-5B, 35S::RFP in different combinations. GFP: green fluorescent protein, RFP: red fluorescent protein. TaCOI1-1D^{ΔF-box} represents the truncated version of TaCOI1-1D by deleting 2nd – 60th amino acid to delete the F-box domain (21st-60th amino acids) (see Extended Data Fig. 3d). Overlay of GFP and RFP presents the inset of a representative nucleus indicated with white boxes. The number of nuclei showing overlay signal is indicated on the merged image. **(c-d)** Representative Western blot (c) and quantification (d) of GFP, TaCOI1-1D or TaCOI1-1D^{ΔF-box} and TaJAZ9-5B or RFP from *N. benthamiana* leaves transiently co-expressing GFP, GFP-TaCOI1-1D or GFP-TaCOI1-1D^{ΔF-box} and RFP-TaJAZ9-5B or RFP driven by 35S promoter. Anti-GFP and anti-RFP antibodies were used to detect GFP-TaCOI1-1D or GFP-TaCOI1-1D^{ΔF-box} and

RFP-TaJAZ9-5B. Relative RFP-TaJAZ9-5B protein levels were quantified in three independent experiments (dots in the plot) (c). ND, not detected, indicating the protein was not detected in the Western blot.

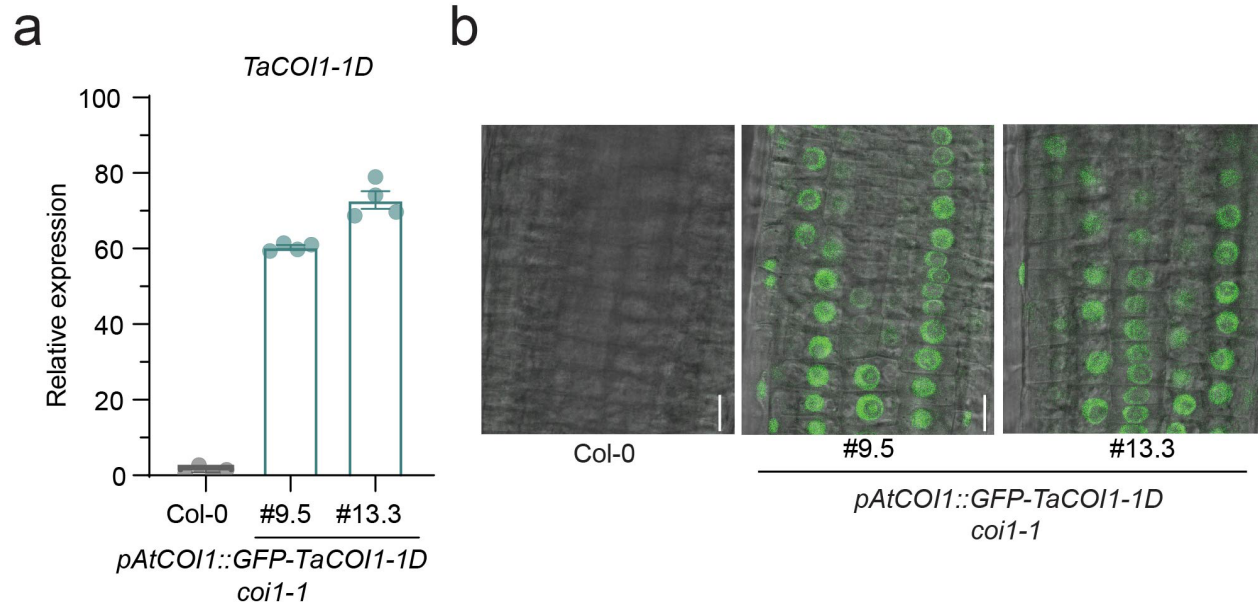
Jas motif

AtJAZ1	P	I	A	R	R	A	S	L	H	R	F	L	E	K	R	K	D	R	V	T	S	K	A	P	Y
AtJAZ9	P	Q	A	R	K	A	S	L	A	R	F	L	E	K	R	K	E	R	L	M	S	A	M	P	Y
TaJAZ9-5B	P	Q	A	R	K	A	S	L	A	R	F	L	E	K	R	K	E	R	V	T	T	T	A	P	Y

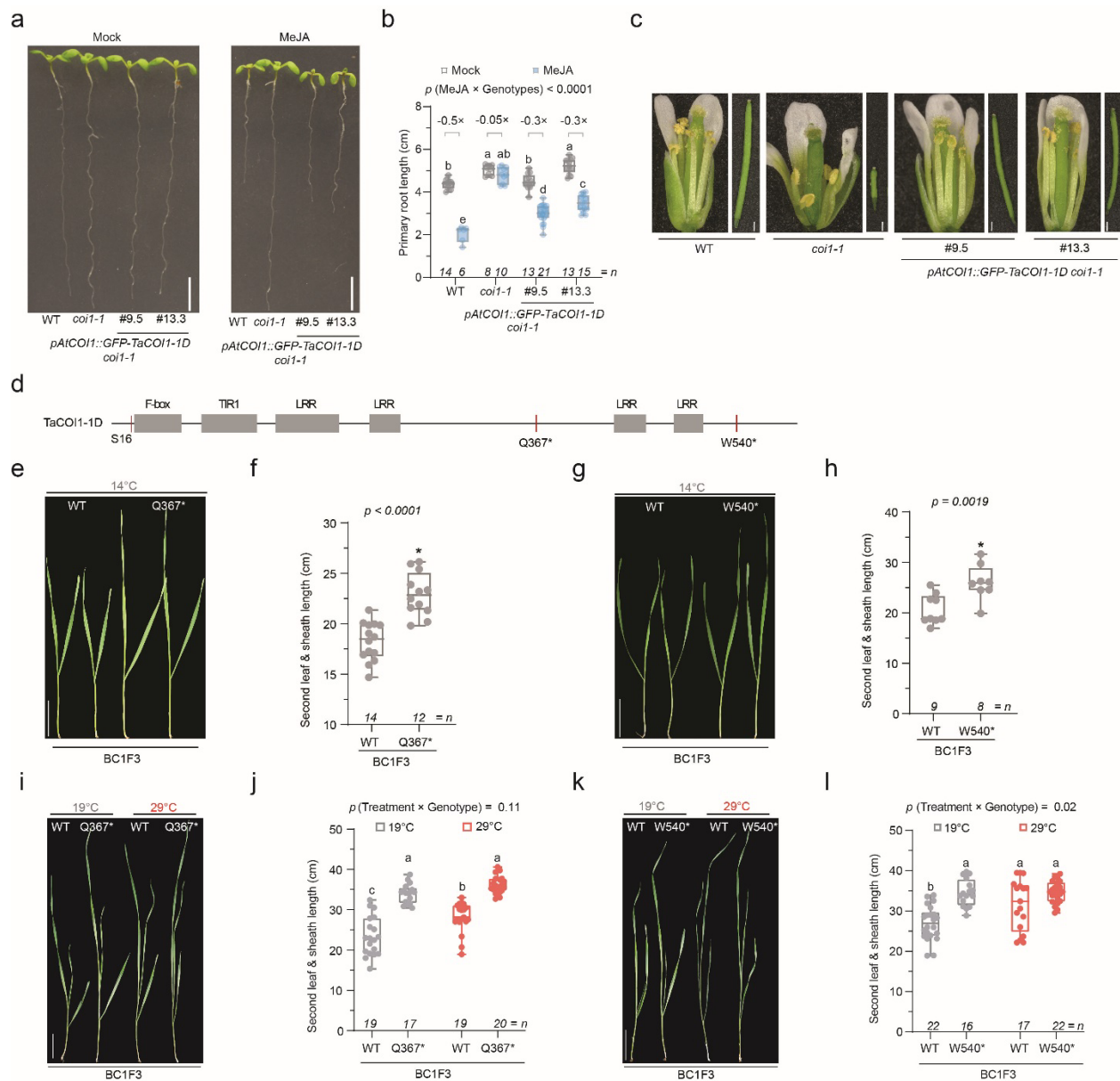
R335K336

mTaJAZ9-5B P Q A A A S L A R F L E K R K E R V T T T A P Y

Supplementary Figure 7. Alignment of Jas motifs in AtJAZ1, AtJAZ9, and TaJAZ9-5B proteins, the two positively charged amino acids highlighted in the box are key amino acids for COI1-JAZ interaction. R335K336 belong to the position of TaJAZ9-5B. mTaJAZ9-5B represents R335K336 mutated to Alanine at the same time. Numbers above the schematic represent amino acid position.

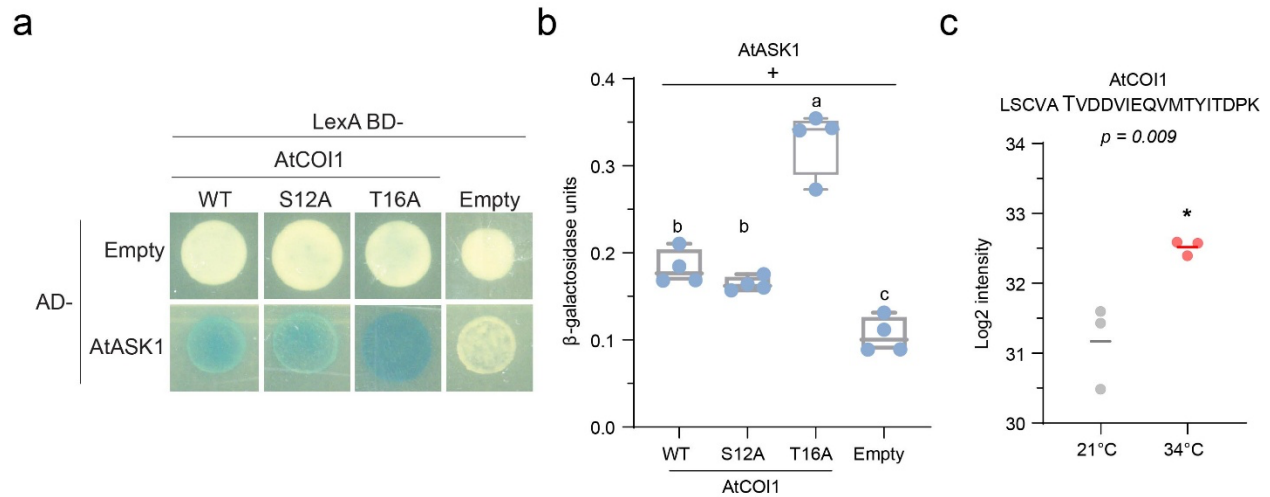


Supplementary Figure 8. (a) Gene expression of *TaCOI1-1D* in wild-type Col-0 and two independent transgenic lines *pAtCOI1::GFP-TaCOI1-1D* (#9.5 and #13.3) in the *coi1-1* background. The forward primer *qTaCOI1-1D* (CACGATTTCGGTTTGGTTCT) and reverse primer *qTaCOI1-1D* (AACCCAACGTCAGTACTGAGTCC) were used. Samples from 7-days-old Arabidopsis seedlings were harvested. Col-0, which exhibits minimal background signal, was assigned a value of 1 to serve as the baseline for relative quantification. (b) Representative confocal image of GFP-TaCOI1-1D localization in the root of *pAtCOI1::GFP-TaCOI1-1D coi1-1* transgenic plants. The GFP signal was observed in root cells (division zone).

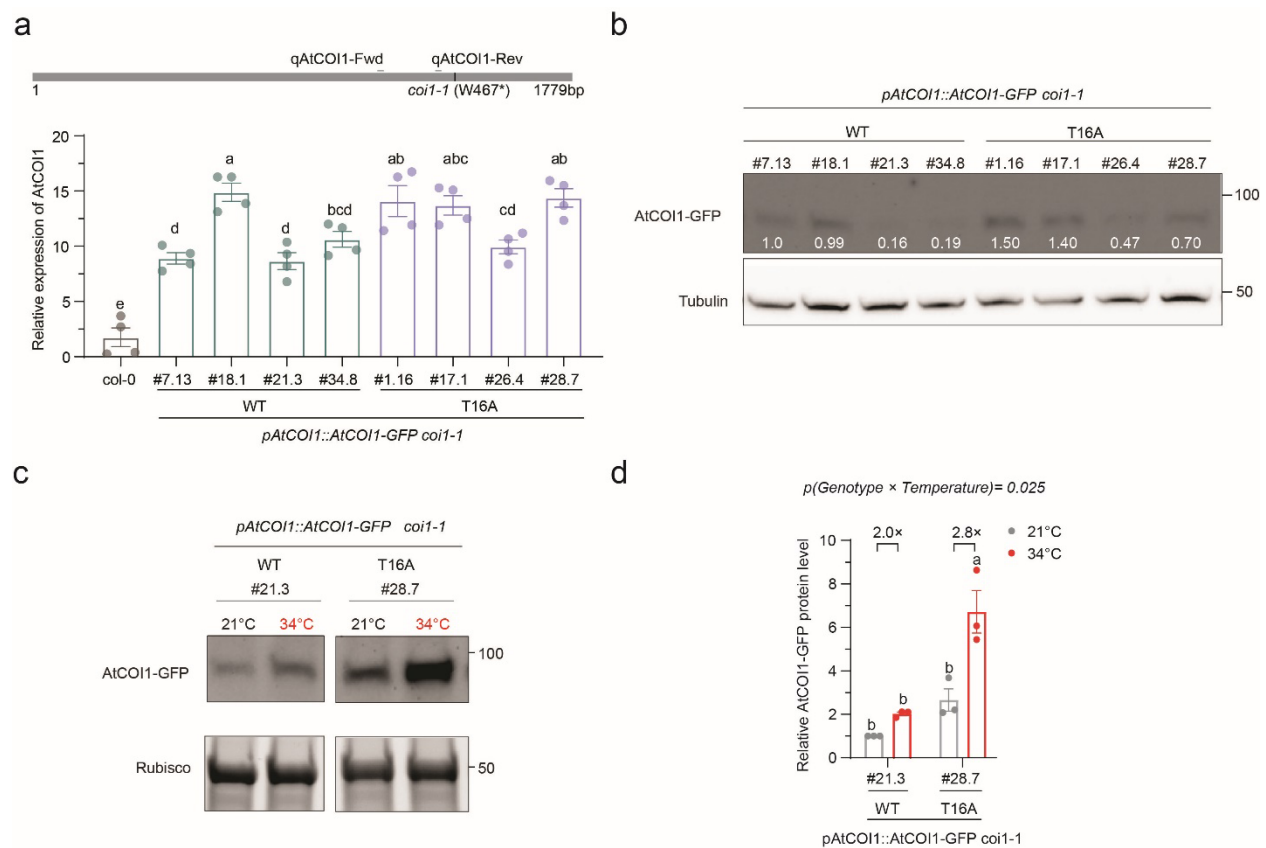


Supplementary Figure 9. (a-b) Representative images (a) and quantification of primary root length (b) of 7-days-old wild type, *coi1-1*, and *pAtCOI1::GFP-TaCOI1-1D* transgenic lines at 21 °C under long-day conditions in the absence or presence of 30 μ M MeJA (b). Scale bar = 5 mm. Box plots with individual data points represent the distribution of all individual measurements, showing the median (central lines) with Tukey-based whiskers and outliers. The box edges show the first and third quartiles. The whiskers represent the range of data within 1.5 $\times$ the interquartile range from the first and third quartiles. Different letters above the box plot denote significant differences ($p < 0.05$) between groups based on a two-way ANOVA with Tukey's HSD post-hoc test. The specific values above the plots indicate the fold-change decrease between the mock and MeJA treatments for each genotype. The number of individually measured seedlings (n) is

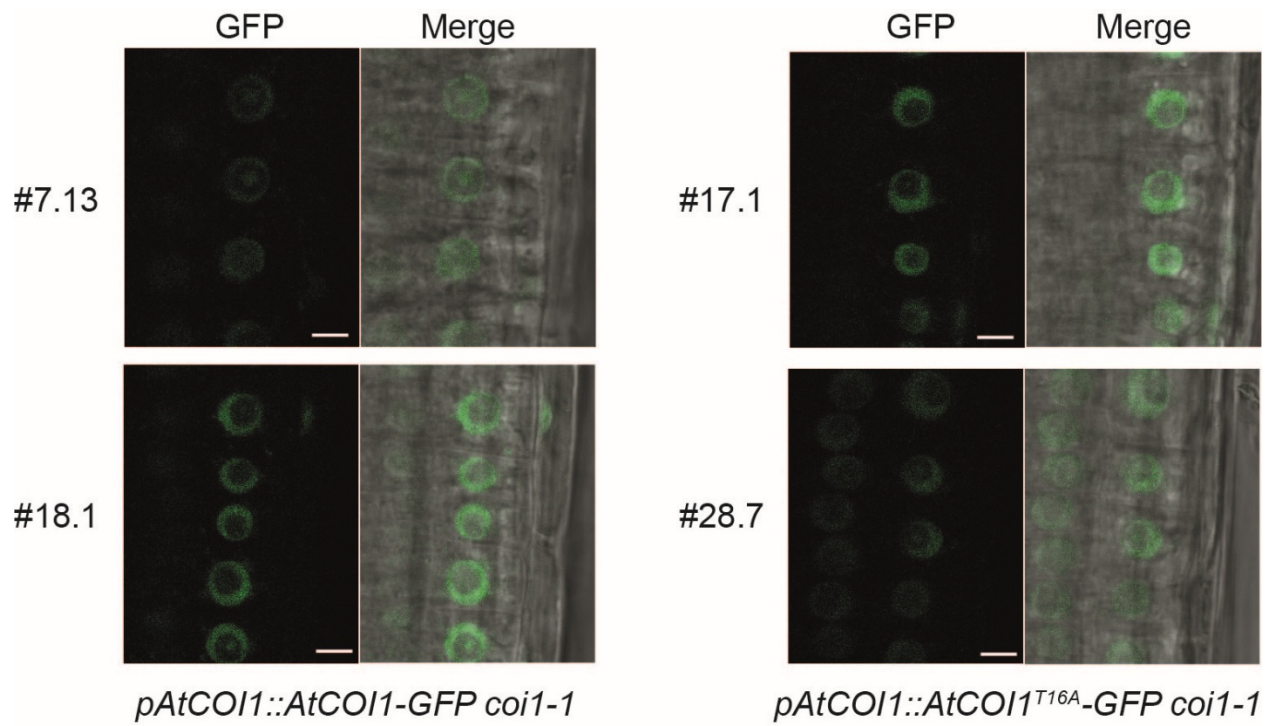
indicated above the X axis (b). The exact p -value for the interaction (MeJA $\times$ Genotypes) (b) is shown at the top. The experiment was repeated two times with similar results. (c) Stamen (left) and silique (right) development in *pAtCOII::GFP-TaCOII-1D coil-1*. Two independent lines (#9.5 and #13.3) were used. Scale bar = 1mm. (d) Schematic of TaCOII-1D protein with F-box, transport inhibitor response 1 (TIR1) domain, and leucine-rich repeat (LRR) domains. Position of Serine 16, position of stop codons (*) in wheat TILLING lines Cadenza0877 (Q367*) and Cadenza1333 (W540*) in protein model for wheat COI1 homolog in D sub-genome. (e-l) Representative images (e, g, i, k) and quantification (f, h, j, l) of the second leaf and sheath length of 14-day-old TILLING lines grown at 14°C (e-h) or 19°C and 29°C (i-l). BC1F3 represents homozygous TILLING mutants that were backcrossed with wild-type once, and segregated wild-type and homozygous mutant lines were obtained in F3 generation. Scale bar = 5 cm. Box plots with individual data points represent the distribution of all individual measurements, showing the median (central lines) with Tukey-based whiskers and outliers. The box edges show the first and third quartiles. The whiskers represent the range of data within 1.5 $\times$ the interquartile range from the first and third quartiles. Different letters denote significant differences ($p < 0.05$) based on a two-way ANOVA with Tukey's HSD post-hoc test. The exact p -value for the interaction (genotype $\times$ temperature) is shown at the top. An * denotes a significant difference ($p < 0.01$) using a student's t-test with two-sample equal variance and one-tailed distribution. The exact p -value is shown at the top. The experiment was repeated two times with similar results.



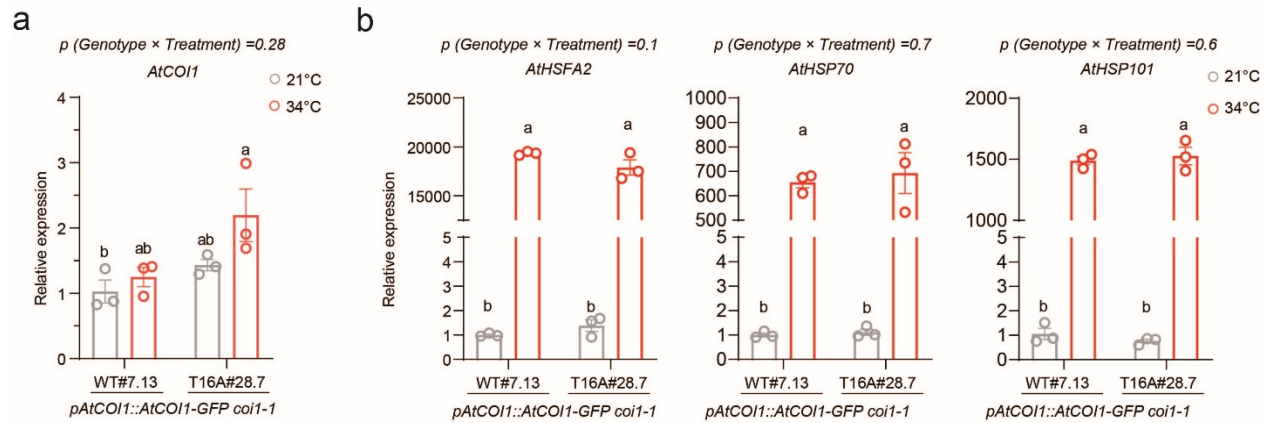
Supplementary Figure 10. (a) Y2H assay of AtCOI1, AtCOI1^{S12A}, or AtCOI1^{T16A} interaction with AtASK1. Representative yeast colonies are shown, all independent colonies shows similar interaction. LexA BD, LexA DNA-binding domain and AD, activation domain. (b) Quantification of β-Galactosidase activity assay showing the interaction intensity between AtCOI1, AtCOI1^{S12A}, or AtCOI1^{T16A} and AtASK1. Box plots with individual data points represent the distribution of all individual measurements, showing the median (central lines) with Tukey-based whiskers and outliers. The box edges show the first and third quartiles. The whiskers represent the range of data within 1.5× the interquartile range from the first and third quartiles. Different letters denote significant differences ($p < 0.05$) based on one-way ANOVA with Tukey's HSD. (c) In immunoprecipitation-mass spectrometry (IP-MS) analysis, the abundance of peptide (LSCVATVDDVIEQVMTYITDPK) containing Thr16 of Arabidopsis COI1 protein from 10-day-old AtCOI1-overexpressing Arabidopsis transgenic whole seedlings was measured at 21°C and 34°C for 1 hour. * denotes significant differences ($p < 0.05$) based on Students' t-test with two-sample equal variance and one-tailed distribution.



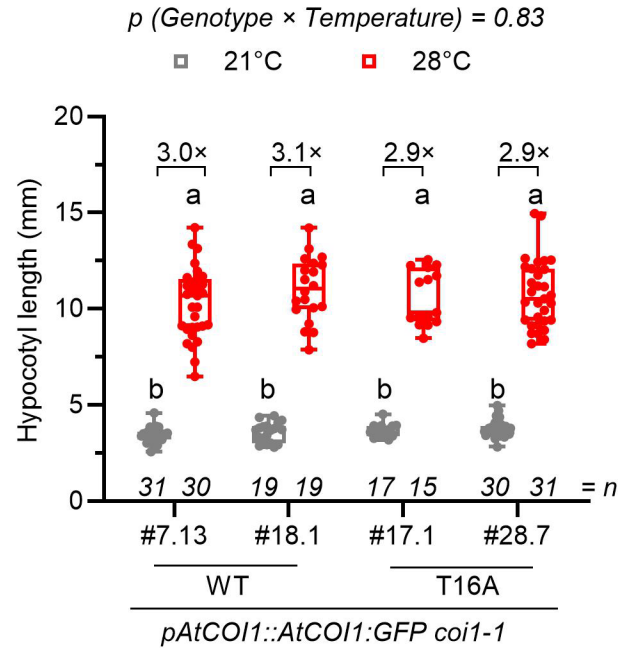
Supplementary Figure 11. (a) *AtCOI1* gene expression and (b) *AtCOI1*-GFP protein level in transgenic lines of *pAtCOI1::AtCOI1-GFP* and *pAtCOI1::AtCOI1^{T16A}-GFP* in the *coi1-1* homozygous background for further analyses. Expression was checked in 7-days-old seedlings of four independent lines for WT (#7.13, #18.1, #21.3, and #34.8) and T16A (#1.16, #17.1, #26.4, and #28.7) grown at 21°C. Different letters denote significant differences ($p < 0.05$) based on one-way ANOVA with Tukey's HSD. A schematic of the forward and reverse primer design for qRT-PCR of *AtCOI1* is shown in the figure panel a. The grey line indicates the full coding sequence of *AtCOI1* (1-1779 bp). Western blot probed with an anti-GFP antibody. Numbers under lanes indicate relative band intensities that were quantified and normalized to the reference protein tubulin for each panel. (c-d) Representative Western blot (c) and quantification (d) showing *AtCOI1*-GFP levels in *pAtCOI1::AtCOI1-GFP coi1-1* (#21.3) and *pAtCOI1::AtCOI1^{T16A}-GFP coi1-1* (#28.7) seedlings treated for 5 hours at 21°C and 34°C and probed with an anti-GFP antibody. Stain-free gel highlighting Rubisco as a loading control. Relative *AtCOI1*-GFP protein levels were plotted using three independent experiments (dots in plot) (d). Bar plots show mean with the error bars ($\pm$ SEM), different letters denote significant differences ($p < 0.05$) based on two-way ANOVA with Tukey's HSD post-hoc test, with fold-change between 21°C and 34°C per genotype. The exact p -value for the interaction (Genotype $\times$ Temperature) (d) is shown at the top.



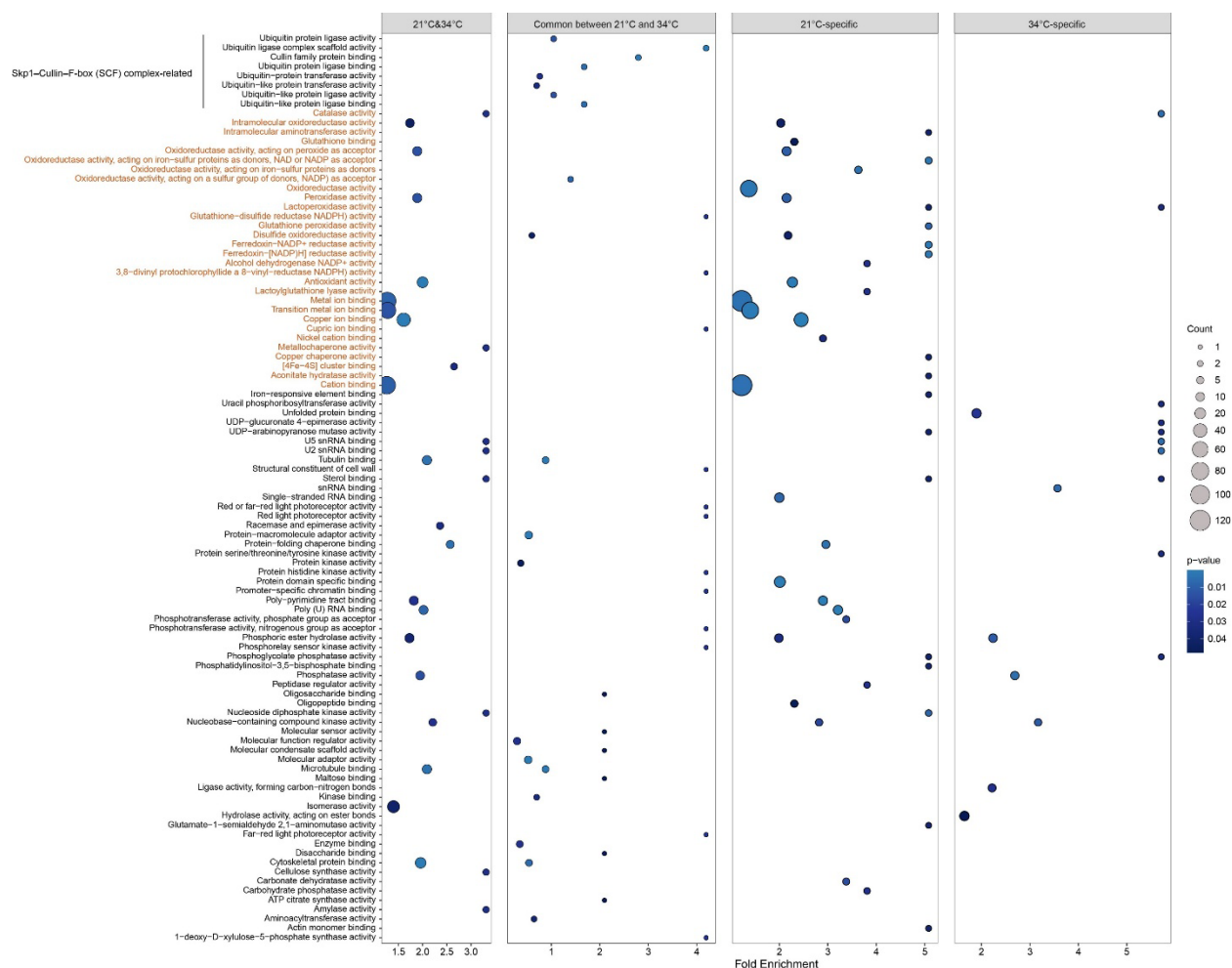
Supplementary Figure 12. Subcellular localization of AtCOI1 protein in the root of representative transgenic lines *pAtCOI1::AtCOI1-GFP* (#7.13 and #18.1) and *pAtCOI1::AtCOI1-GFP^{T16A}* *coi1-1* (#17.1 and #28.7). GFP, green fluorescent protein. The merged view combines DIC and GFP. The different numbers indicate different lines.



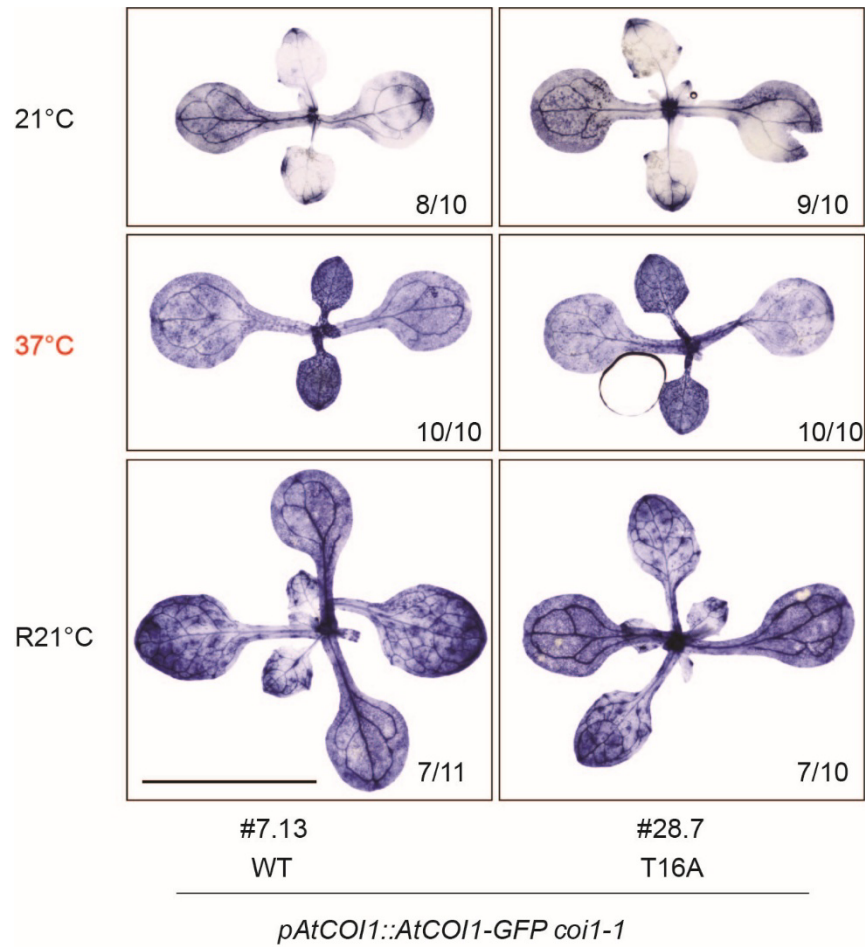
Supplementary Figure 13. (a-b) Relative expression of *AtCOI1* (a) and high temperature marker genes (*AtHSFA2*, *AtHSP70*, *AtHSP101*) (b) in 7-day-old *pAtCOI1::AtCOI1-GFP coi1-1* (#7.13) and *pAtCOI1::AtCOI1^{T16A}-GFP coi1-1* (#28.7) seedlings grown at 21°C and transferred to 21°C or 34°C for 5 h. The graph shows the value of the 3 individual biological replicates (dots) with the error bars ($\pm$ SEM). Different letters denote significant differences ($p < 0.05$) based on two-way ANOVA with Tukey's HSD post-hoc test. The exact p-value for the interaction (Genotype $\times$ treatment) is shown at the top.



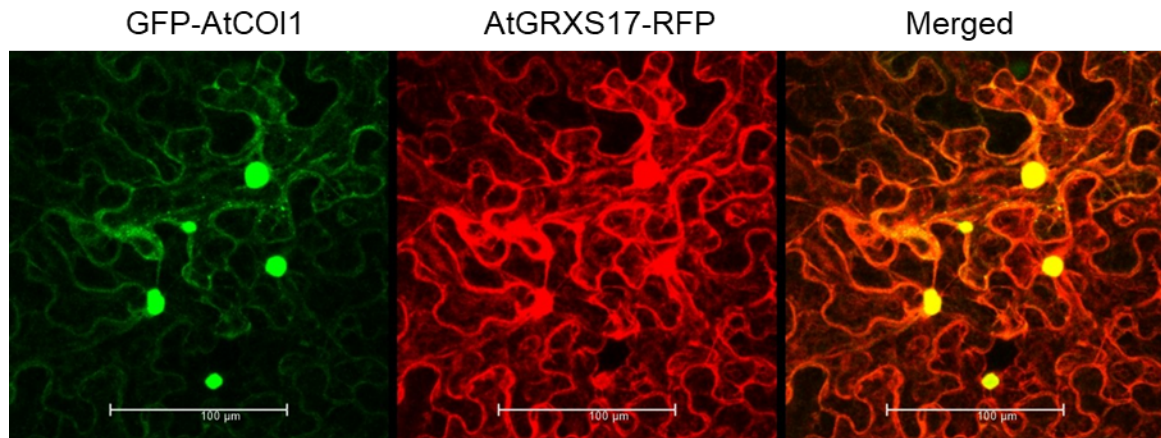
Supplementary Figure 14. Hypocotyl length of 7-day-old two independent lines for *pAtCOI1::AtCOI1-GFP coi1-1* (#7.13 and #18.1) and *pAtCOI1::AtCOI1^{T16A}-GFP coi1-1* (#17.1 and #28.7) transgenic seedlings at 21°C and 28°C. Box plots with individual data points represent the distribution of all individual measurements, showing the median (central lines) with Tukey-based whiskers and outliers. The box edges show the first and third quartiles. The whiskers represent the range of data within 1.5× the interquartile range from the first and third quartiles. Different letters denote significant differences ($p < 0.05$) based on two-way ANOVA with Tukey's HSD post-hoc test. The exact *p-value* for the interaction (genotype × temperature) is shown at the top. The fold change is indicated. The number of individually measured seedlings (*n*) is indicated above the X-axis.



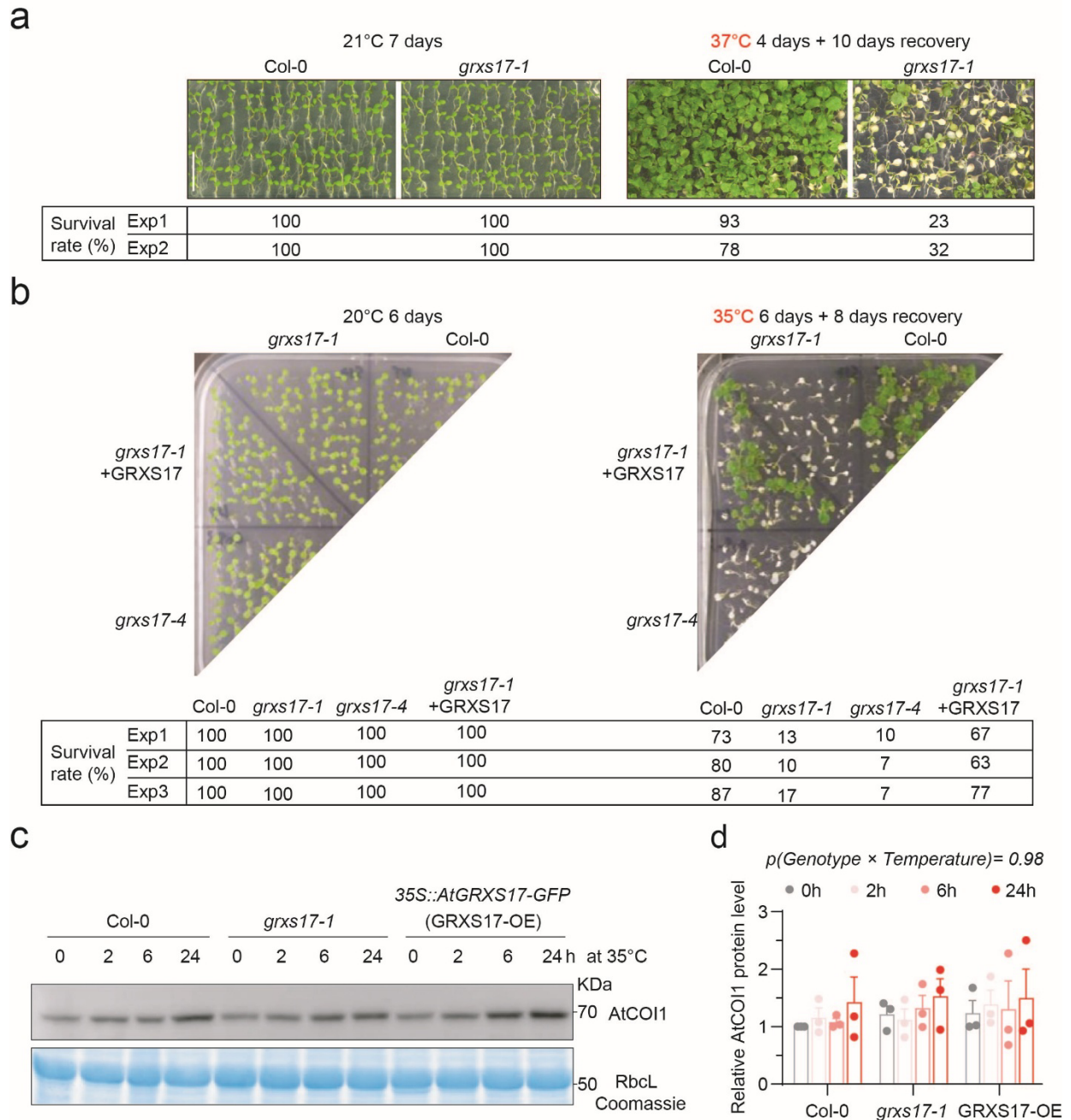
Supplementary Figure 15. GO enrichment (molecular function) of AtCOI1-enriched proteins at 21°C and 34°C is shown. GO enrichment analysis with the use of data from supplementary table S4-5. GO enrichment (molecular function) of proteins commonly enriched by AtCOI1 at 21°C and 34°C, 21°C specifically, or 34°C specifically enriched by AtCOI1. Categories highlighted in orange refer to redox-related proteins. “21°C&34°C” represents the combination of AtCOI1-enriched proteins at 21°C and AtCOI1-enriched proteins at 34°C; “Common between 21°C and 34°C” represents the proteins commonly enriched by AtCOI1 at 21°C and 34°C.



Supplementary Figure 16. Representative images of nitroblue tetrazolium (NBT) staining of 5-day-old *pAtCOI1::AtCOI1-GFP coi1-1* (#7.13) and *pAtCOI1::AtCOI1^{T16A}-GFP coi1-1* (#28.7) seedlings transferred to control (21°C) and heat (37°C) in long-day conditions for 2 days, and recovered for 5 days at 21°C in long-day conditions after 37°C. The number of seedlings that show a similar pattern to the representative image is indicated in the image. Scale bar, 5 mm.

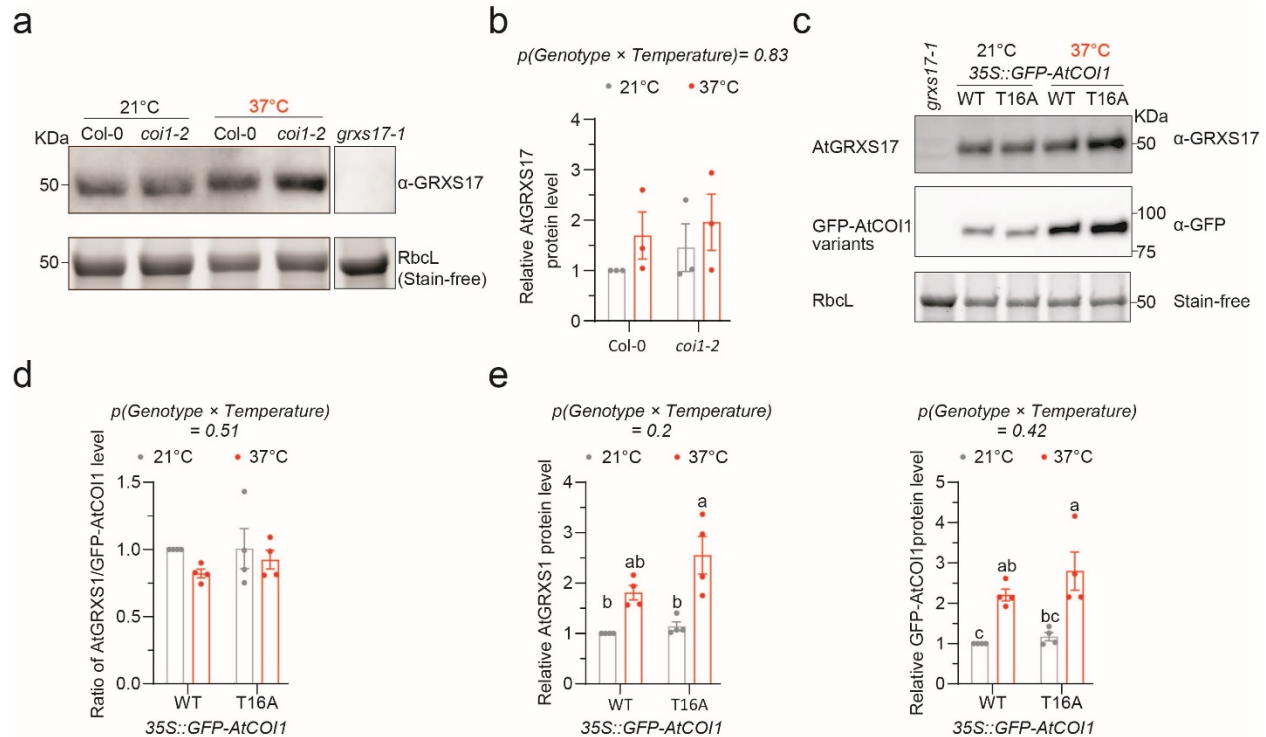


Supplementary Figure 17. Maximal projection of Z-stack images by confocal laser scanning microscopy of GFP-tagged AtCOI1 and RFP-tagged AtGRXS17 that were co-expressed in *Nicotiana benthamiana* leaf cells. The main image is a maximum intensity projection of a Z-stack comprising 15 optical sections with a step size. Merge of green, red, and brightfield channels is shown. Bar, 100 µm.



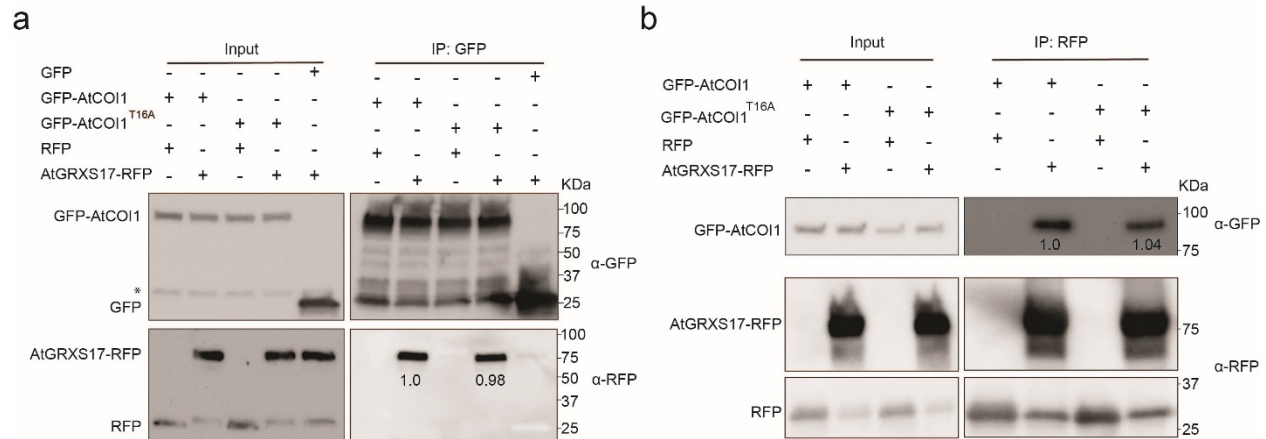
Supplementary Figure 18. (a) Representative images of wild type (Col-0) and *grxs17-1* mutant seedlings under control and prolonged heat treatment. Results from a single representative replicate of two independent biological replicates are shown. Scale bar, 1cm. Quantification of the experiment is shown below the images. The percentage of surviving seedlings was determined for control and moderately high-temperature conditions. In each biological replicate, 30-100 plants per genotype were examined. The moderately high-temperature assay is the same as shown in Figure 3(a). **(b)** Representative images of wild type (Col-0), *grxs17-1*, *grxs17-4* *Cas9* knock-out mutant seedlings and 35S::AtGRXS17-GFP *grxs17-1* (*grxs17-1* + GRXS17) transgenic seedlings

under control and prolonged moderately high temperature followed by 8 days recovery at control temperature. Results from one representative replicate of three independent biological replicates are shown. Quantification of the experiment is shown below the images. The percentage of surviving seedlings was determined for control and moderately high-temperature conditions. In each biological replicate, 40-50 plants per genotype were examined. These experiments were performed using Petri dish setups instead of Jiffy soil, further confirming the robustness of the results. *grxs17-1* is T-DNA line and *grxs17-4* is a CRISPR knock-out mutant. Two distinct, independent mutant alleles of *grxs17* (*grxs17-1* and *grxs17-4*) to genetically validate that the observed phenotypes are strictly due to the disruption of the *GRXS17* gene, rather than any unlinked, second-site background mutations or off-target T-DNA insertions. **(c-d)** Representative Western blot (c) and quantification (d) showing AtCOI1 levels in wild-type Col-0, *grxs17-1* and *35S::AtGRXS17-GFP* transgenic seedlings exposed to 35°C for 0, 2, 6, and 24 hours and probed with an anti-COI1 antibody. Coomassie blue staining highlighting Rubisco large subunit (RbcL) as a loading control. Relative AtCOI1 protein levels were normalized to RbcL and were plotted using three independent experiments (dots in the plot) (d). Bar plots show the mean with the error bars ("± SEM"). The exact *p*-value for the interaction (Genotype × Temperature) (d) is shown at the top.

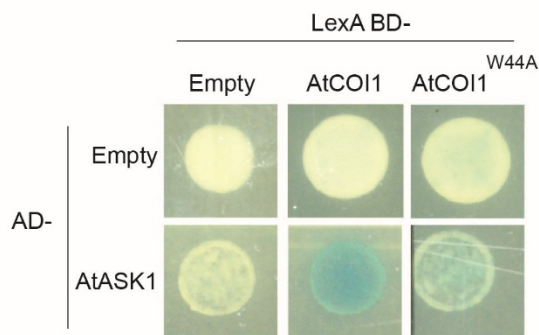
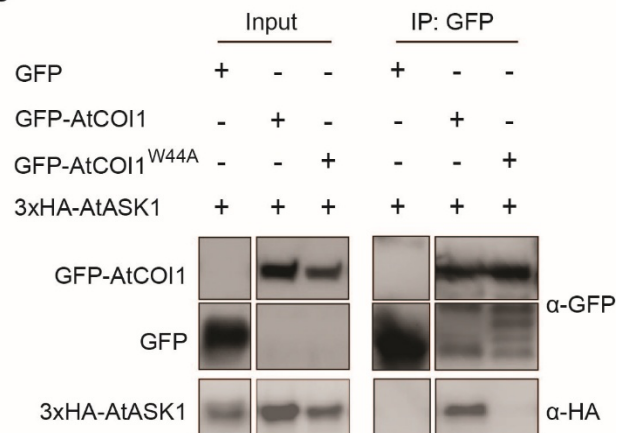


Supplementary Figure 19. (a-b) Representative Western blot (a) and quantification (b) showing AtGRXS17 levels in 7-day-old wild-type Col-0 and *coi1-2* mutant seedlings treated for 1 day at 21°C and 37°C, and probed with an anti-GRXS17 antibody. Stain-free gel showing Rubisco large subunit (RbcL) as a loading control. The experiment was repeated three times with similar results. Relative AtGRXS17 protein levels were normalized to RbcL and were plotted using three independent experiments (b). Bar plots the value of the 3 individual biological replicates (dots) with the error bars (" $\pm$ SEM"). The exact p -value for the interaction (Genotype $\times$ Temperature) (b) is shown at the top. (c) Representative Western blot showing AtGRXS17 protein levels in 7-day-old *35S::GFP-AtCOI1* and *35S::GFP-AtCOI1^{T16A}* transgenic seedlings treated for 1 day at 21°C and 37°C, then probed with anti-GRXS17 and anti-GFP antibodies. Stain-free gel showing Rubisco protein as a loading control. The experiment was repeated four times with similar results. (d) The AtGRXS17/GFP-AtCOI1 ratios were calculated and plotted using quantification data shown in (e). Bar plots the value of the 4 individual biological replicates (dots) with the error bars (" $\pm$ SEM"). The exact p -value for the interaction (Genotype $\times$ Temperature) (d) is shown at the top. (e) Quantification of AtGRXS17 and GFP-AtCOI1 protein levels in 7-day-old *35S::GFP-AtCOI1* and *35S::GFP-AtCOI1^{T16A}* transgenic seedlings after exposure to 21°C or 37°C for 1 day. Proteins were detected using anti-GRXS17 and anti-GFP antibodies, respectively. Band intensities were quantified and normalized to the reference protein Rubisco large subunit (RbcL), as determined from the stain-free gel, for each panel. Relative protein levels were plotted using four independent experiments (c). Bar plots show the mean with the error bars (" $\pm$ SEM"). The exact p -value for the interaction (Genotype $\times$ Temperature) is shown at the top. Different letters denote significant differences ($p < 0.05$) based on two-way ANOVA with Tukey's HSD post-hoc test. The

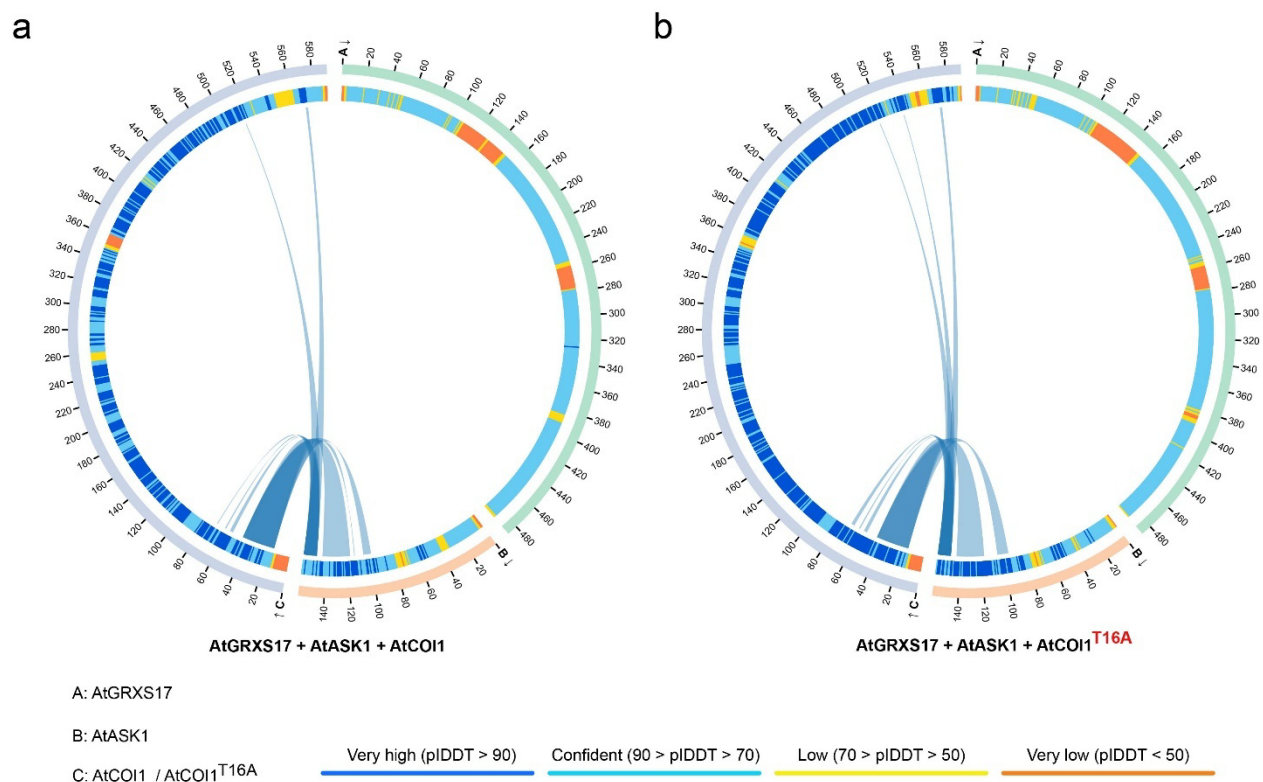
AtGRXS17/GFP-AtCOI1 ratio shown in (d) was calculated from these normalized quantification data.



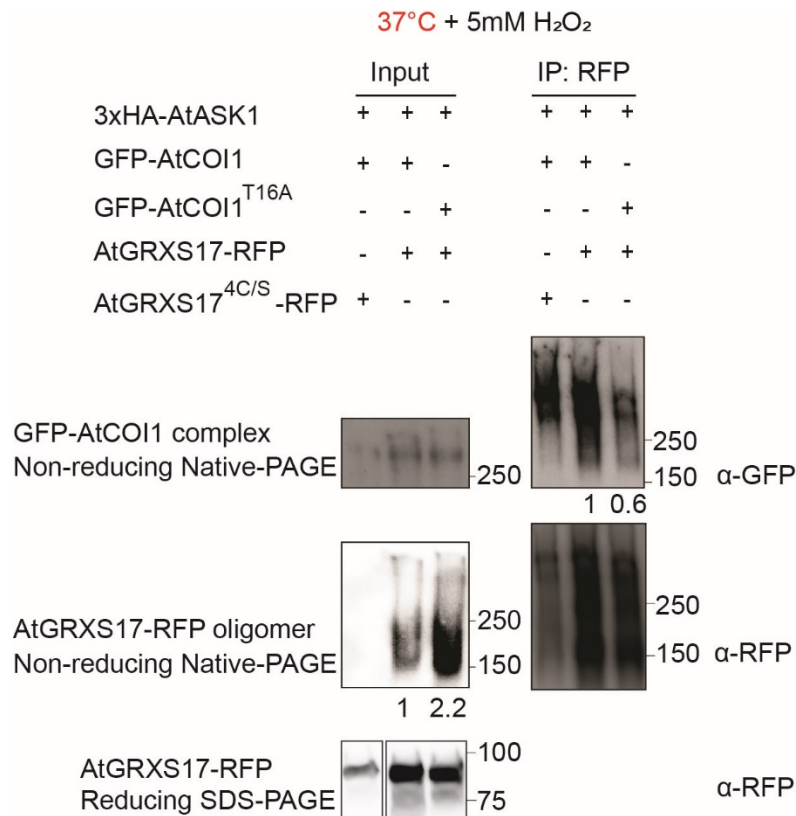
Supplementary Figure 20. Co-immunoprecipitation of AtGRXS17-RFP and GFP-AtCOI1 or GFP-AtCOI1^{T16A} analysed by SDS-PAGE. **(a)** Western blot of Arabidopsis COI1 or COI1^{T16A} and GRXS17 from *Nicotiana benthamiana* leaves transiently co-expressing GFP, GFP-AtCOI1, or GFP-AtCOI1^{T16A} with RFP or AtGRXS17-RFP. **(b)** Western blot of Arabidopsis COI1 or COI1^{T16A} and GRXS17 from *Nicotiana benthamiana* leaves transiently co-expressing GFP-AtCOI1 or GFP-AtCOI1^{T16A} with RFP or AtGRXS17-RFP. The immunocomplex and input proteins were analysed by immunoblotting using anti-GFP and anti-RFP antibodies. Numbers on the blot lanes indicate relative band intensities that AtGRXS17-RFP from precipitated samples was normalized to GFP-Trapped GFP-AtCOI1 variants and input AtGRXS17-RFP protein level or GFP-AtCOI1 variants from precipitated samples were normalized to RFP-Trapped AtGRXS17-RFP and input GFP-AtCOI1 variants protein level. (a) and (b) represent two independent experiments with similar results. * denotes non-specific band.

a**b**

Supplementary Figure 21. (a) Y2H assay of AtCOI1 or AtCOI1^{W44A} interaction with AtASK1. Representative yeast colonies are shown: all independent colonies show similar interaction. Blue colony colour indicates positive interaction; white colony colour indicates no interaction. LexA BD, LexA DNA-binding domain and AD, activation domain. **(b)** Representative Western blot of AtCOI1 or AtCOI1^{W44A} and AtASK1 from *Nicotiana benthamiana* leaves transiently co-expressing GFP, GFP-AtCOI1, or GFP-AtCOI1^{W44A} with 3×HA-AtASK1. The immunocomplex and input proteins were analysed by immunoblotting using anti-HA and anti-GFP antibodies. The experiments were independently repeated 2 times with similar results.



Supplementary Figure 22. Predicted structural interfaces and model confidence for the AtGRXS17-AtASK1-AtCOI1 ternary complexes. AlphaBridge chord diagrams visualize the interfaces and structural interactions within the top-ranked structural models predicted by AlphaFold 3. Plots compare the wild-type ternary complex (a) - comprising AtGRXS17 (Chain A), AtASK1 (Chain B), and AtCOI1 (Chain C) - with the mutant complex (b) containing the AtCOI1^{T16A} variant. The outer circular tracks represent the primary amino acid sequences of each subunit with residue positions indicated. The color-coded inner tracks map the predicted local-distance difference test (pLDDT) per-residue confidence scores: dark blue indicating very high confidence (pLDDT > 90), light blue indicating confident (90 > pLDDT > 70), yellow indicating low confidence (70 > pLDDT > 50), and orange indicating very low confidence (pLDDT < 50). Central ribbons delineate confidently predicted contact links and structural interfaces connecting the subunits.



Supplementary Figure 23. Representative western blot of AtGRXS17-RFP analysed by non-reducing Native PAGE. Proteins from *Nicotiana benthamiana* leaves transiently co-expressing AtGRXS17-RFP with GFP-AtCOI1 or GFP-AtCOI1^{T16A} and 3×HA-AtASK1. AtGRXS17^{4C/S}-RFP used as control. Following confirmation of robust protein expression by confocal laser scanning microscopy, the leaves were infiltrated with 5 mM H₂O₂. The plants were immediately treated with 37 °C for 5 hours. The RFP-trap incubated immunocomplex and input proteins were analysed by immunoblotting using anti-GFP and anti-RFP antibodies. Numbers under lanes indicate relative band intensities that were quantified and normalized to the RFP-trapped AtGRXS17-RFP complex for each panel. The experiment was repeated two times with similar results.