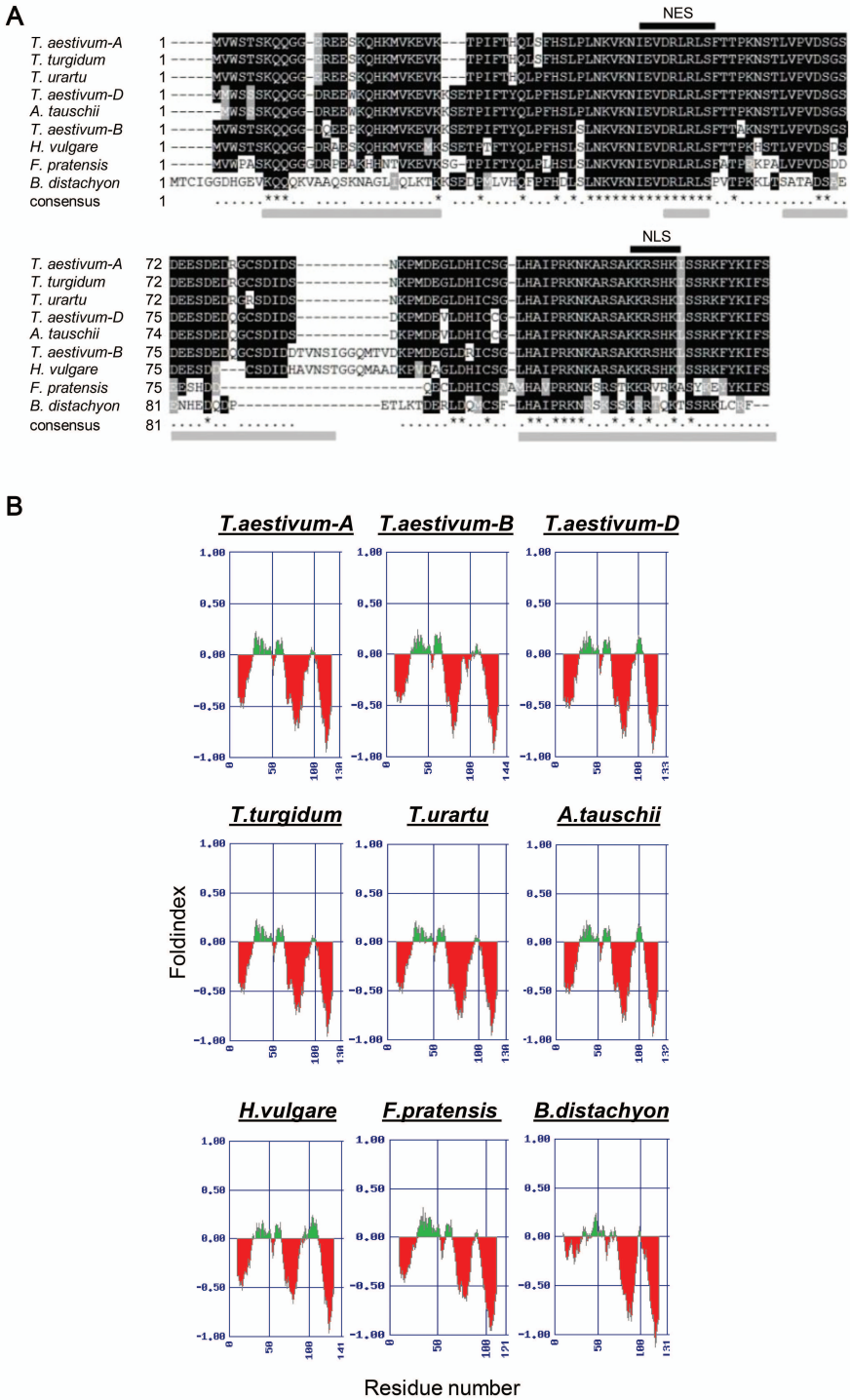

Figure S1. Characteristics of FROG protein sequences. A, Multiple sequence alignment of wheat TaFROG proteins and homologs. Identical and conservative amino acid residues are respectively marked with asterisks and dots. Bars represent the conserved position of predicted nuclear localization signal (NLS) or nuclear export signal (NES) in black and the disordered protein regions in grey. B, Fold index profiles of TaFROG (*T.aestivum-A*) its' homeologs from the chromosome B and D (*T.aestivum-B*, *T.aestivum-D*) and other *Pooideae* variants were determined using the FoldIndex prediction tool.

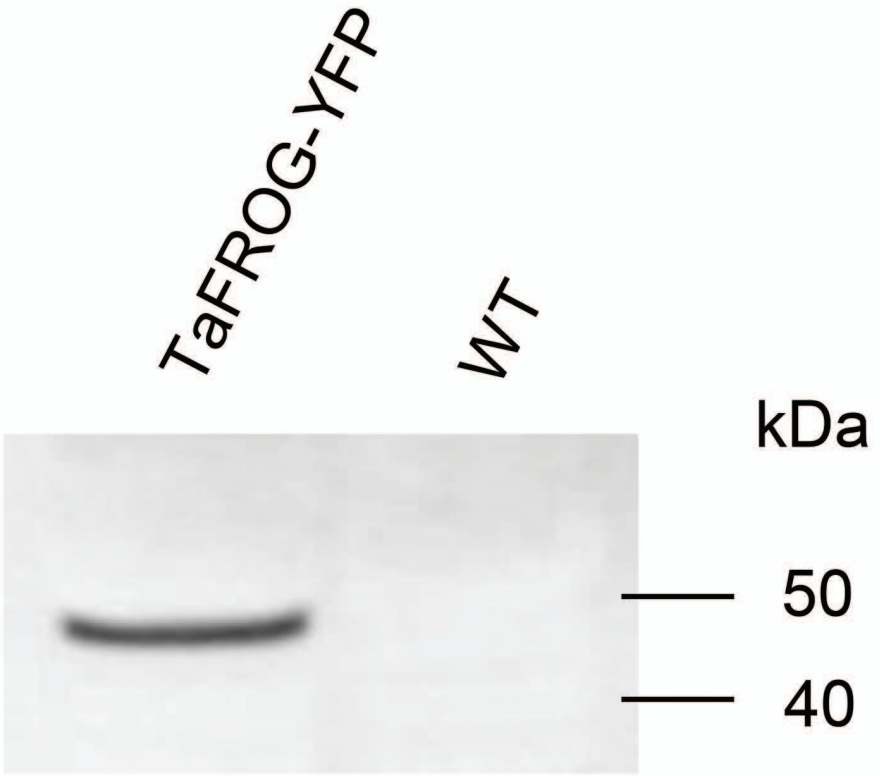

Figure S2. Immunoblot analysis with GFP antibody using the total proteins extracted from wild type (WT) or TaFROG-YFP *Arabidopsis* leaves. The TaFROG-YFP product is 47 kDa.

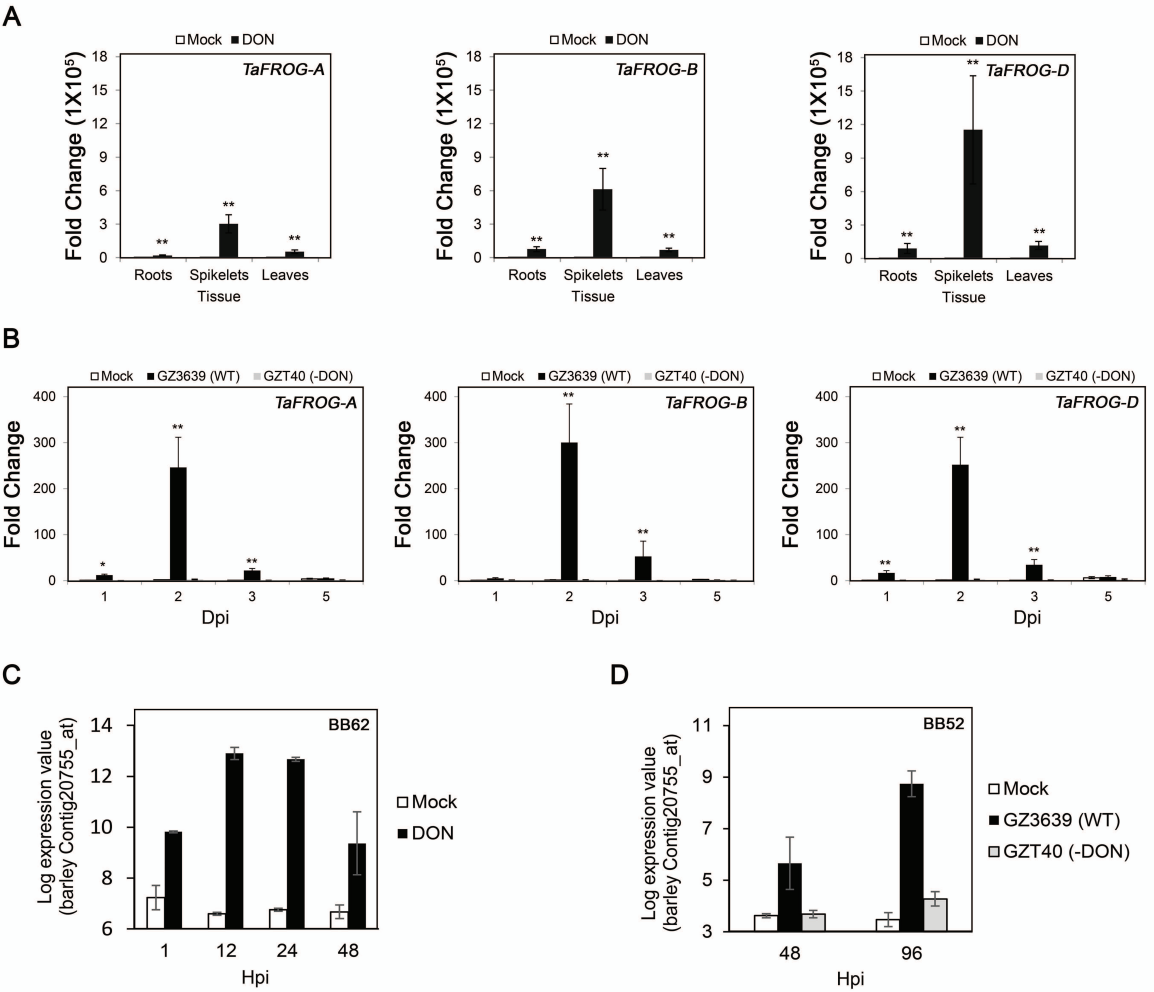

Figure S3. Expression of *TaFROG* homeologs and its barley homolog in different tissues and in response to DON or *F. graminearum*. A and B, *TaFROG-A* (chromosome 4A homeolog), *TaFROG-B* (chromosome 4B homeolog) and *TaFROG-D* (chromosome 4D homeolog) gene expression was assessed *via* qPCR. Fold change were calculated relative to (A) mock-treated roots or (B) mock-treated heads at 1 dpi using the formula $((E_{target})^{\Delta Ct\ target\ (control - sample)})/(E_{housekeeping})^{\Delta Ct\ housekeeping\ (control - sample)}$) with the wheat *alpha-tubulin* gene used as a housekeeping gene. C and D, time course expression profiling of the *TaFROG* barley homolog (Contig20755_at) in heads after DON or *F. graminearum* treatment, respectively. RMA expression profile were extracted from PLEXdb microarray experiment BB52 and BB62 and plotted in graphs. Error bars indicate ± SEM (A, B: $n = 8$; C, D: $n = 3$). Asterisks show significant differences between treatments and mock (Kruskal-Wallis test; *, $P<0.05$; **, $P<0.01$).

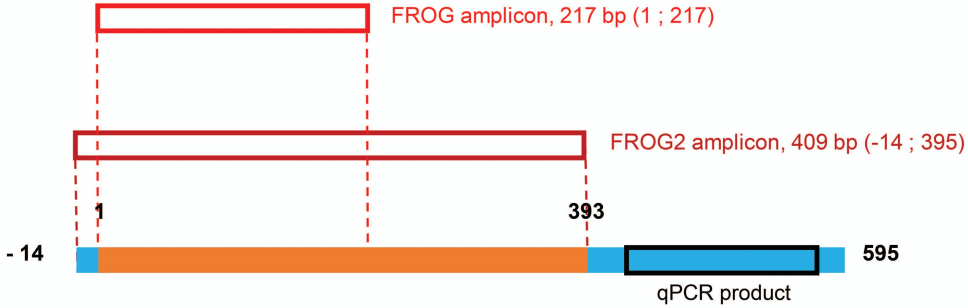

Figure S4. Schematic representation of the position of the two VIGS gene fragments (FROG and FROG2) and the qPCR target region within the *TaFROG* gene. The open reading frame is coloured in orange and the untranslated regions at the 5' and 3' end are coloured in blue. Numbers indicate base pair positions in the *TaFROG* mRNA sequence.

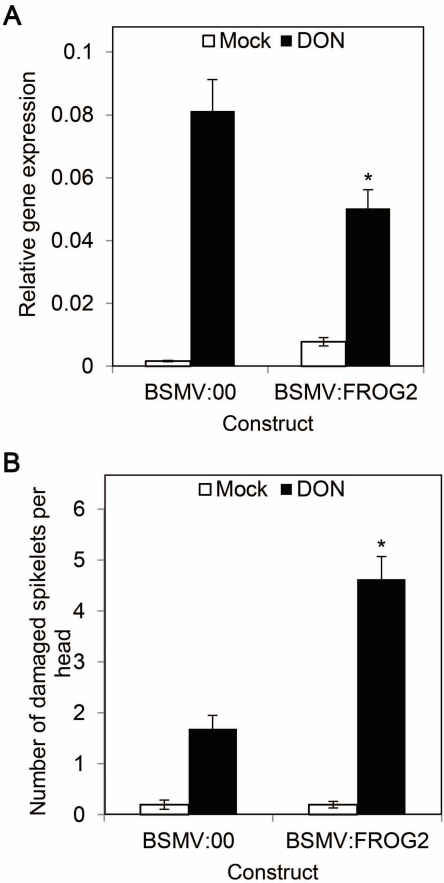

Figure S5. Effect of *TaFROG* silencing on DON tolerance. A and B, in the VIGS experiment, (A) gene silencing of *TaFROG* in wheat spikelets was quantified by qPCR analysis and (B) the phenotypic response to DON was assessed at 14 days post toxin treatment. VIGS constructs used were empty vector BSMV:00 or the construct BSMV:FROG2 that targets the *TaFROG* gene for silencing. Asterisks indicate significant differences between DON-treated BSMV:00 and DON-treated BSMV:FROG2 plants (Mann-Whitney *U* test; *, $P<0.05$). Error bars indicate ± SEM (A, B: $n = 20$-30).

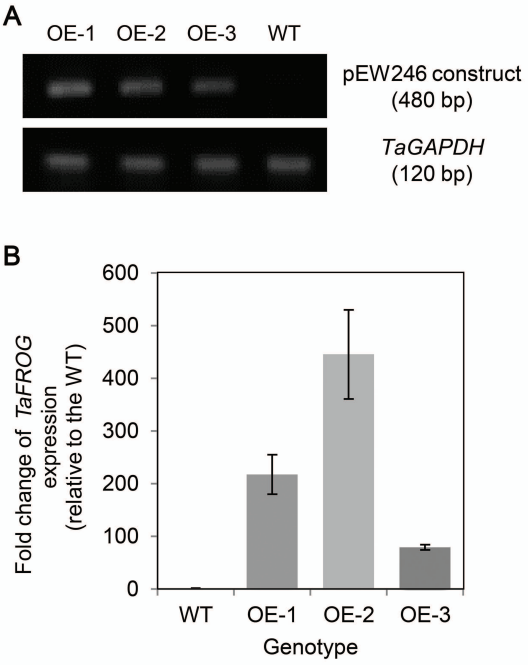

Figure S6. Molecular characterization of transgenic wheat cv. Fielder overexpressing *TaFROG* under the control of a rice *actin* promoter. A, Verification by PCR of the T-DNA insertion in transgenic lines OE-1, OE-2, OE-3 using primers targeting the *Actin* promoter and *TaFROG* coding sequence (forward and reverse primers respectively). The wild type plant (WT) and a pair of primers targeting the endogenous gene *TaGAPDH* were used as a PCR negative and positive controls, respectively (primers are detailed in Supplementary Table 1). For each line, the T-DNA copy number was determined using a qPCR assay (Craze *et al*. in preparation) and results are indicated in parenthesis. B, The transcript level of *TaFROG* in transgenic lines and wild type plants grown under normal plant growth conditions was determined by qPCR. Error bars indicate ± SEM (n = 6).

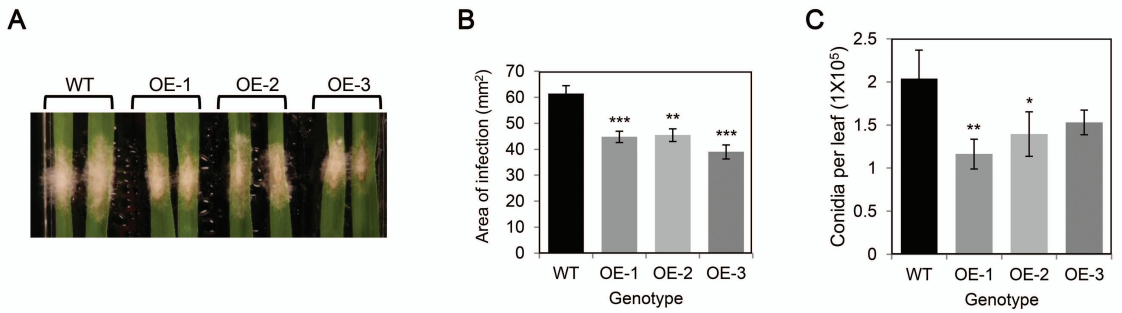

Figure S7. Effect of *TaFROG* overexpression on wheat leaf resistance to *F. graminearum*. A, B and C, *TaFROG* overexpressor lines (OE-1, OE-2 and OE-3) and control plants (WT) were used for phenotypic analysis. (A) Representative leaf symptoms, (B) diseased leaf area and (C) conidia production were determined 4 days post-treatment of detached wheat leaves with *F. graminearum* plus DON (75 µM). Error bars indicate ± SEM (B, C: $n = 36$). Asterisks show significant differences compared to the WT ((B) Tukey's HSD test; (C) Mann-Whitney *U* test; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$).

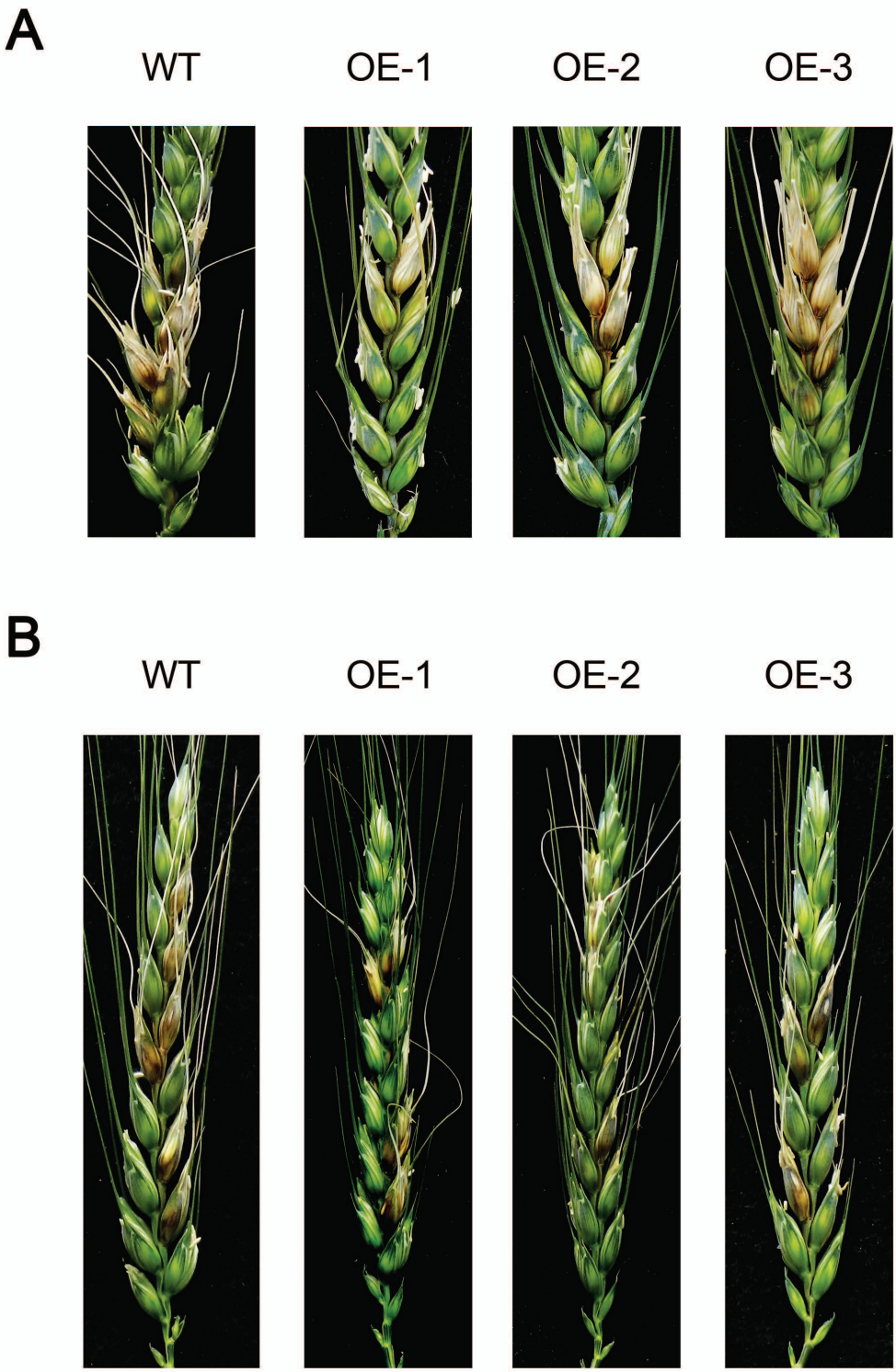

Figure S8. Symptoms of FHB on the *TaFROG* overexpression lines. At mid-anthesis, wheat ears from overexpression lines (OE-1, OE-2 and OE-3) or control plants (WT) were (A) point-inoculated or (B) spray-inoculated with *F. graminearum*. Representative head symptoms at (A) 21 days after point inoculation or (B) 10 days after spray-inoculation.

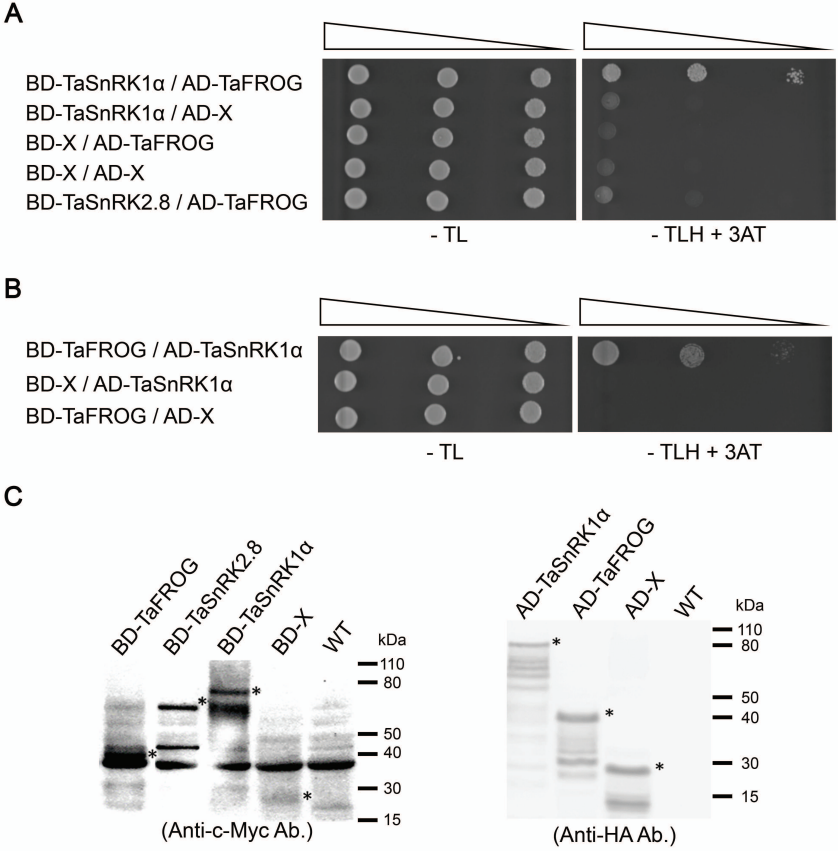

Figure S9. Yeast two-hybrid analysis. A, Serial dilutions of the yeast two-hybrid analysis depicted in Fig. 6a. B, Domain swapping of TaSnRK1α and TaFROG. Yeast was grown on selective Trp/Leu/His/Ade drop-out medium in the presence of 3-AT (-TLHA + 150 mM 3-AT) or non-selective Trp/Leu drop-out medium (-TL) conditions. C, Immunoblot analysis of the total proteins extracted from the yeast used in the protein-protein interaction assays. Proteins fused to the Gal4 activating domain (AD) was detected with an anti-HA antibody. In case of proteins fused to the Gal4 binding domain (BD) an anti-c-Myc antibody was used. The nature of the fusion protein present in each protein extract is indicated above each lane. The position of the Gal4 domains and Gal4 domain fusion proteins are indicated with an asterisk (other bands represent protein degradation products; in the c-Myc immunoblot there is also a non-specific product between 40 and 30 kDa). The molecular weight are indicated on the right of the blot. The protein size expected are: 37 kDa (BD-TaFROG), 62kDa (BD-TaSnRK2.8), 80 kDa (BD-TaSnRK1α), 22 kDa (BD-X), 82 kDa (AD-TaSnRK1α), 39 kDa (AD-TaFROG), 24 kDa (AD-X).

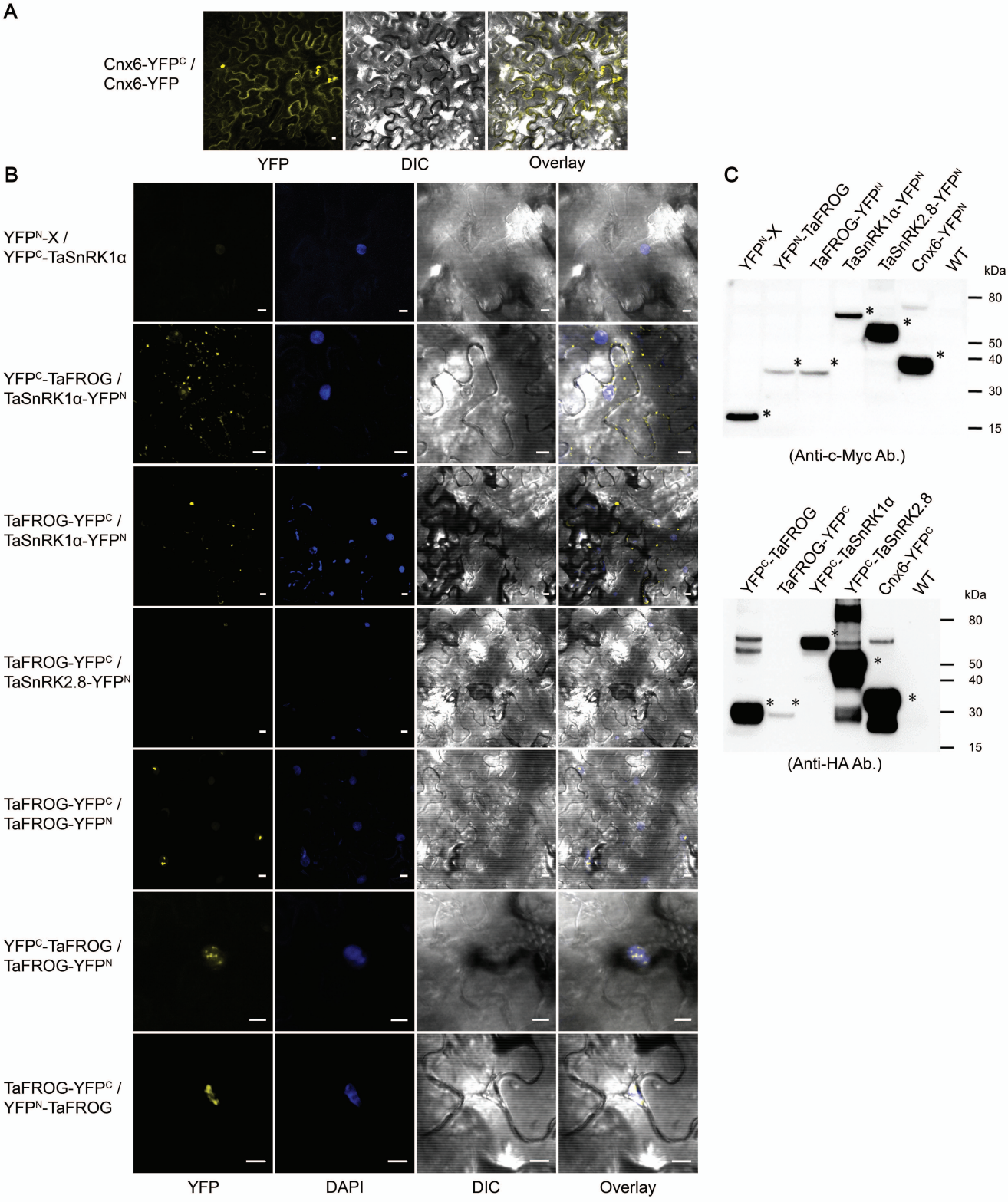

Figure S10. Results for positive and negative controls included within the Bimolecular Fluorescence Complementation analysis. A and B, Confocal microscopy images of representative *N. benthamiana* epidermal leaf cells expressing proteins fused to either the N- or C-terminal part of the YFP, as indicated. A, Cnx6 homodimerization *in planta* was used as a BiFC positive control. B, Combinations of TaSnRK1α/TaFROG (other than those shown in Fig. 6b) giving an *in planta* interaction and of BiFC empty vector (X) or TaSnRK2.8 used as a negative controls are shown. YFP, DAPI fluorescence and Differential Interference Contrast (DIC) images are merged in the overlay. Scale bar indicates 10 µm. C, Immunoblot analysis on the total proteins extracted from tobacco leaves used in the protein-protein interaction assays. Proteins fused to the N part of the YFP (YFP^N) was detected with an anti-HA antibody. In the case of proteins fused to the C part of the YFP (YFP^C) an anti-c-Myc antibody was used. The nature of the fusion protein present in each protein extract is indicated above each lane, and the position of the full-length fusion protein is shown by an asterisk. The molecular weight are indicated on the right of the blot.

Supplemental Table S1. Primer sets used in this study.

Primers	Primer sequence (5' to 3')	Application*	Reference
TaFROG-GSP1 for	CACACACAGAAAGAGAGAGTGCCTGAAGTG	RACE-PCR	This study
TaFROG-GSP1 rev	CCCTTCGTCCATAGGCTTGTTAGAATCG		
TaFROG-GSP2 for	GCAAGCAACAAGGAGGAGAGCGAGAAGAAT		
TaFROG-GSP2 rev	GATTCTTCTCGCTCTCCTCCTTGTTGCT		
TaFROG M1 for GWY	GGAGATAGAACCATGGTGTGGTCTACCAGCAAG	Cloning TaFROG into pDONR207	
TaFROG Stop rev GWY	CAAGAAAGCTGGGTCTCAACTGAATATTTTGTAGAATTTCC	Cloning TaFROG into pDONR207 and pSc4ActR1R2	
TaSnRK1α M1 for GWY	GGAGATAGAACCATGGACGCAGCAGGCAGAG	Cloning TaSnRK1α into pDONR207	
TaSnRK1α Stop rev GWY	CAAGAAAGCTGGGTCTCAAAGGACTCTCAGCTGG		
TaSnRK2.8 M1 for GWY	GGAGATAGAACCATGGCAGGGGCGGCGC	Cloning TaSnRK2.8 into pDONR207	
TaSnRK2.8 Stop rev GWY	CAAGAAAGCTGGGTCTCACATCGCATACACGATCTCTCC		
attB1	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAGGAGATAGAACCATG	attB extension for subcloning into pDONR207	
attB2	GGGGACCACTTTGTACAAGAAAGCTGGGTC		
pDONR207 for	TCGCGTTAACGCTAGCATGGATCTC	Sequencing pDONR207	
pDONR207 rev	GTAACATCAGAGATTTTGAGACAC		
TaFROG M1 monocot for GWY	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCACCATGGTGTGGTCTACCAGCAAG	Cloning TaFROG into pSc4ActR1R2	
TaGAPDH for	CCTTCCGTGTTCCCACTGTTG	Control DNA contamination in RNA samples	
TaGAPDH rev	ATGCCCTTGAGGTTTCCCTC		
TaGAPDH2 for	TCACCACCGACTACATGACC	Confirmation T-DNA insertion	This study
TaGAPDH2 rev	ACAGCAACCTCCTTCTCACC		
pEW246 Actin for	ATCAGGAAGAGGGGAAAAGG		
pEW246 TaFROG rev	CTCGATCTTCGTCACTCTCT		
TaFROG VIGS PACI for	CGATTAATTAAATGGTGTGGTCTACCAGCAAG	Cloning VIGS construct BSMV:FROG	
TaFROG VIGS NOTI rev	CGAGCGGCCGCCATCAGAACCGGAATCAACG		
TaFROG2 VIGS PACI for	CTTAATTAAGAGTGCCTGAAGTGATGGTGTGG	Cloning VIGS construct BSMV:FROG2	
TaFROG2 VIGS NOTI rev	CGCGGCCGCCGGAAGCACGAGGTCAACTGAA		
TaFROG-CDS for	GACGAAGATCGAGGCTGTTCGGA	qPCR (overexpressor lines) of *TaFROG*	
TaFROG-CDS rev	GAATTTCCTAGAGCTGATCTTATGG		
TaFROG for	TATGGGATCTCGAGGACTGG	sqRT-PCR/qPCR of *TaFROG*	
TaFROG rev	TTGCCCAAAACGTAATAATGA		
TaFROG-B for	GAGGGGCCTTTTTATTGGAG	qPCR of *TaFROG* (chromosome B homeolog)	
TaFROG-B rev	TTGCTAAGTAATGACGATTACATTCA		
TaFROG-D for	ACAAGGGATCTCGAGGACTG	qPCR of *TaFROG* (chromosome D homeolog)	
TaFROG-D rev	TTGCCAAACAAGATGATTACTATTC		
TaPR1 for	AACAACCGCGGCGTCTT	qPCR	
TaPR1 rev	CAGTTAGTATGGTTTCTGTCCAATGAC		
TaAlpha-tubulin for	ATCTCCAACTCCACCAGTGTCG	sqRT-PCR/qPCR	(Xiang et al., 2011)
TaAlpha-tubulin rev	TCATCGCCCTCATCACCGTC		
FgActin for	ATGGTGTCACTCACGTTGTCC	qPCR	(Brown et al., 2011)
FgActin rev	CAGTGGTGGAGAAGGTGTAACC		
FgTri5 for	GATGAGCGGAAGCATTTCC	qPCR	
FgTri5 rev	CGCTCATCGTCGAATTCC		

*Unless otherwise stated, primers for *TaFROG* are specific to the chromosome A homeolog.