Supplemental Data

Wild-type allele of *TaHRC* suppresses calcium-mediated plant immune response by hajacking *TaCAXIP4* to trigger FHB susceptibility in wheat

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Materials and methods

Yeast Two-Hybrid (Y2H) screening and co-transformaiton assays

Y2H screening experiment was performed with the ULTImate Y2H screen system by Hybrigenics Services (Paris, France http://www.hybrigenics-services.com). The full-length coding sequences (CDS) of *TaHRC* was amplified from 'Clark' (an *Fhb1* susceptible wheat cultivar) and then constructed into a bait vector (pGBKT7-TaHRC). The construct was verified by Sanger sequencing and used as a bait to screen a wheat cDNA library fused to Gal4. The full-length CDS of *TaCXIP4* from 'Clark' was amplified and constructed into a prey vector (pGADT7-TaCAXIP4), two N-terminal truncated fragments of *TaHRC* (one with a NLS domain, 1-96 aa; another without NLS domain, 1-81 aa) and the C-terminal truncated fragment (without NLS domain, 97-261 aa) were also amplified and cloned into a bait vector as pGBKT7-TaHRC-N, pGBKT7-TaHRC-NΔNLS and pGBKT7-TaHRC-C. The co-transformaiton assays were performed as described by the manufacturer (Clontech Laboratories, CA, USA). All the primers used for vectors construction were listed in Supplemental Table S2.

Mutants generation by CRISPR/Cas9 gene editing

The single-guide RNA (sgRNA) was designed to target three different sites of *TaHRC* using the web-based E-CRISPR program (http://www.e-crisp.org/E-CRISP/). The details for construction of Cas9 and sgRNAs vectors as well as plant transformation, regeneration and selection processes for T*aHRC* mutants were described previously (Su et al., 2019). All primers used for vectors construction and mutants screening are listed in Supplemental Table S2.

FHB evaluation and statistical analysis

The detailed protocol of FHB evaluation for mutant lines was described previously (Su et al., 2019). In brief, the mutant plants were phenotyped for FHB resistance in a growth chamber by injecting a conidial spore suspension of *F. graminearum* (GZ3639) into a central spikelet in a spike at early anthesis using a syringe (Hamilton, Reno, NV). Ten spikes per line were inoculated in each replication and each experiment had three replications. The percentage of symptomatic spikelets (PSS) of FHB symptom in a spike was calculated at 14 days after inoculation. For box plots, boxes indicate the 25th–75th percentile, whiskers indicate the full data range, center lines indicate medians, crosses indicate means and the numbers inside boxes indicate sample size. *P*-

values were generated from two-sided unpaired Student's t-tests of the mean PSS of the mutant lines versus the mean PSS of the non-edited line.

Bimolecular fluorescence complementation (BiFC) assay

The full-length CDS of *TaHRC* and *TaCAXIP4* were amplified from 'Clark' and then cloned into the N-terminus and C-terminus of a split yellow fluorescent protein (YFP) in the expression vector pEarleygate201-YN and pEarleygate202-YC respectively, yielding YN-TaHRC and YC-TaCAXIP4 using the Gateway LR Clonase II enzyme mix kit (Invitrogen) as described previously (Lu et al., 2010). The mixed *Agrobacterium tumefaciens* (GV3101) cultures harboring equal concentrations of YN-TaHRC and YC-TaCAXIP4 vectors (final density OD600, 0.6) were infiltrated into 6-week-old epidermal *Nicotiana benthamiana* leaves. The signal of the reconstituted YFP fluorescence was observed and imaged at 48 hours after infiltration under a Zeiss LSM 880 confocal microscope (Carl Zeiss, Germany). All primers used for vectors construction are listed in Supplemental Table S2.

Subcellular colocalization assay

The full-length CDS of *TaHRC* and *TaCAXIP4* were cloned into in the expression vector pEarleygate103-GFP and pEarleygate102-CFP respectively, resulting in *TaHRC-GFP* and *TaCXIP4-CFP* using the Gateway LR Clonase II enzyme mix kit (Invitrogen) as described previously (Lu et al., 2010). The mixed *Agrobacterium tumefaciens* (GV3101) cultures harboring equal concentrations of *TaHRC-GFP* and *TaCXIP4-CFP* (final density OD600, 0.6) were infiltrated into 6-week-old epidermal *Nicotiana benthamiana* leaves. The GFP and CFP fluorescence signals at 48 hours after infiltration were observed and imaged under a Zeiss LSM 880 confocal microscope (Carl Zeiss, Germany) in two channels and merged using a lookup table with raw data in green and cyan colors, respectively. All primers used for vectors construction are listed in Supplemental Table S2.

Yeast Ca^{2+} supression assay

A Ca^{2+} sensitive yeast strain K667 (hypersensitive to high concentrations of Ca^{2+}) was used for yeast transformation. The full-length CDS of *TaCAX1*, *TaCAXIP4* and *TaHRC* from 'Clark' were cloned into the yeast expression vector pGBKT7 and transformed/cotransformed into K667 yeast

cells. Yeast cells expressing/coexpressing *TaCAX1*, *TaCAXIP4* and *TaHRC* were assayed on a yeast extract/peptone/dextrose (YPD) medium supplemented with and without 200 mM $CaCl_2$, respectively. The detail protocol for yeast transformation was described previously (Cheng et al., 2004). All primers used for vectors construction are listed in Supplemental Table S2.

Reactive oxygen species (ROS) measurement

To examine whether *TaHRC* and *TaCaXIP4* affect ROS production in planta, ROS assay was performed in *N. benthamiana* leaves expressing *GFP* (Control), *TaHRC-GFP*, *TaCaXIP4-GFP* or both using a luminol-based chemiluminescence assay (Hao et al. 2019). *A. tumefaciens* GV3101 strains carrying *TaHRC-GFP* or *TaCaXIP4-GFP* were cultured overnight. The cultures were centrifuged, washed, and suspended in Agromix (10 mM $MgCl_2$, 10 mM MES, and 100 µM acetosyringone). The cell suspension was infiltrated or co-infiltrated into 4- to 5-week-old *N. benthamiana* leaves with an OD600 of 0.4. Two days after infiltration, twelve leaf discs were removed from infiltrated zones with a cork borer and floated on water overnight. On the next day, water was replaced with 200 µL of the assay solution: 17 mM luminol L012, 1 µM horseradish peroxidase (Sigma, St. Louis, MO), and 100 µg/mL crab shell chitin (Sigma, St. Louis, MO). Luminescence was measured for 60 min using the Synergy HT and Gen5 software (BioTek Instruments, Inc. Winooski, VT).

References

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Supplemental Table S1. The results of ULTImate Y2H screen against wheat cDNA libraries using TaHRC as a bait

Number	Clone Name	Type Seq	Contig(s) Name	Gene Name (Best Match)	GenBank ID (NCBI)	Global PBS	Start	Stop	Frame	Sense	% Id 5p/3p	5p Sequence	3p Sequence
1	WHLR_RP1_hgx4861v1_GBKT7_A-69	5p 3p	103592423	Triticum aestivum - Homolog of LOC109771507 (Aegilops tauschii subsp. tauschii) (ubiquitin-like)	https://www.ncbi.nlm.nih.gov/nuccore/XM_020330203.1	A	-121	No Data	IF	Sense	96.7 / 100.0	CACGCTCGAGGTCGAGGGCGGCCACGA	CGCGGATCAGCAGCGCCTCATCTCGCAGGGAAGCA
2	WHLR_RP1_hgx4861v1_GBKT7_A-120	3p	103592383	Triticum aestivum - Homolog of Proteic Match - 50S ribosomal protein L12-2, chloroplastic (Aegilops tauschii subsp. tauschii)	https://www.ncbi.nlm.nih.gov/nuccore/XP_020197796.1	A	393	No Data	IF	Sense	100		TTCCACGGCAATTACCAAGAGGAATACAAAAAGAT
3	WHLR_RP1_hgx4861v1_GBKT7_A-376	5p 3p	103592493	Triticum aestivum - Homolog of Proteic Match - AAA-ATPase ASD, mitochondrial-like(Aegilops tauschii subsp. tauschii)	https://www.ncbi.nlm.nih.gov/nuccore/XP_020181645.1	A	504	No Data	IF	Sense	99.2 / 98.7	CGAGGACATCGACTGCTCCATCGACCTCA	CGAGGACATCGACTGCTCCATCGACCTCACCGGCAA
4	WHLR_RP1_hgx4861v1_GBKT7_A-342	5p	103592427	Triticum aestivum - Homolog of Proteic Match - B3 domain-containing protein Os12g0591400-like isoform X2 (Aegilops tauschii s	https://www.ncbi.nlm.nih.gov/nuccore/XP_020170063.1	A	-1	265	IF	Sense	99.3	CCAGCGGCCGCAGCTTCCCTTTCGGGCTTGGGACGGAGCTCACTGCACGCTGCCCCCTCCCCAGC	
5	WHLR_RP1_hgx4861v1_GBKT7_A-179	5p 3p	103592647	Triticum aestivum - Homolog of Proteic Match - BTB/POZ and MATH domain-containing protein 3-like (Aegilops tauschii subsp. t	https://www.ncbi.nlm.nih.gov/nuccore/XP_020172915.1	B	597	830	IF	Sense	99.9 / 100.0	CACAGACTCATTCCCTGAGATGTACAAGC	GAGTAATGGCGACTAATGGCTGGGAGCATATAAT
6	WHLR_RP1_hgx4861v1_GBKT7_A-188	5p 3p	103592643	Triticum aestivum - Homolog of Proteic Match - COP9 signalosome complex subunit 5b (Zea mays)	https://www.ncbi.nlm.nih.gov/nuccore/NP_001149650.1	B	597	830	IF	Sense	99.4 / 99.7	CCAGCTAGGGTTTTGCCTGCCCGCCCGCC	CTCCGCCGCGCCAGATCTCCGCCCTCGCGCTCCTCAA
7	WHLR_RP1_hgx4861v1_GBKT7_A-84	5p 3p	103592403	Triticum aestivum - Homolog of Proteic Match - DNA-binding protein BIN4(Aegilops tauschii subsp. tauschii)	https://www.ncbi.nlm.nih.gov/nuccore/XP_020155922.1	B	-1	607	IF	Sense	100.0 / 99.5	AGCAGGGCTTCCGCTTCGTTCCCGACGG	GAAAAAATGCAGGACAAGCTTGTGGATATTTCTGT
8	WHLR_RP1_hgx4861v1_GBKT7_A-300	5p 3p	103592462	Triticum aestivum - Homolog of Proteic Match - PREDICTED: CAX-interacting protein 4 (Brachypodium distachyon)	https://www.ncbi.nlm.nih.gov/nuccore/XP_003576706.1	B	-1	607	IF	Sense	100.0 / 99.3	CTCTGATTCTGATATTGATCCCGAGCTGC	CTCTGATTCTGATATTGATCCCGAGCTGGAGAAGA
9	WHLR_RP1_hgx4861v1_GBKT7_A-203	5p	103592350	Triticum aestivum - Homolog of Proteic Match - PREDICTED: SCAR-like protein 2 (Oryza brachyantha)	https://www.ncbi.nlm.nih.gov/nuccore/XP_006643893.2	B	51	709	IF	Sense	100	TGCTGCGGGACTCGTACATCACGGCGCTGAACGTGTGCCAGGCGTACACTCTGAAGAGGATCA	
10	WHLR_RP1_hgx4861v1_GBKT7_A-213	5p 3p	103592609	Triticum aestivum - Homolog of Proteic Match - PREDICTED: serine/threonine-protein kinase RIO1 (Oryza sativa Japonica Group)	https://www.ncbi.nlm.nih.gov/nuccore/XP_015647675.1	B	51	709	IF	Sense	100.0 / 100.0	GTTGGACTTATTAAAGGAAGATTGTTTG	GGATCTATTTAATTTTGTGATTGATCAAAACATTGC
11	WHLR_RP1_hgx4861v1_GBKT7_A-116	5p	103592392	Triticum aestivum - Homolog of Proteic Match - actin cytoskeleton-regulatory complex protein PAN1-like (Aegilops tauschii subsp	https://www.ncbi.nlm.nih.gov/nuccore/XP_020193076.1	B	51	709	IF	Sense	100	CCAACCTGCAACCTGCGACAACATGCTTTGCCACTGGCACAACCACAAGAAGGATTGTCATGGT	
12	WHLR_RP1_hgx4861v1_GBKT7_A-64	5p	103592464	Triticum aestivum - Homolog of Proteic Match - corepressor interacting with RBPJ 1 isoform X1 (Aegilops tauschii subsp. tauschii	https://www.ncbi.nlm.nih.gov/nuccore/XP_020183334.1	B	51	709	IF	Sense	100	CAAAGATAAAATGATGAGAGAGTATAGAGCTCAACTGGATGCTGAAAGGGCCCAAAAGCTTG	
13	WHLR_RP1_hgx4861v1_GBKT7_A-341	5p 3p	103592372	Triticum aestivum - Homolog of Proteic Match - costars family protein (Aegilops tauschii subsp. tauschii)	https://www.ncbi.nlm.nih.gov/nuccore/XP_020179512.1	B	51	709	IF	Sense	98.8 / 99.0	AAAAATCCCCATCAATAAGCATCCCGCCG	AAAAATCCCCATCAATAAGCATCCCGCCGCGGCGCC
14	WHLR_RP1_hgx4861v1_GBKT7_A-164	5p 3p	103592310	Triticum aestivum - Homolog of Proteic Match - dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate deh	https://www.ncbi.nlm.nih.gov/nuccore/NP_001147014.1	B	186	722	IF	Sense	99.7 / 98.0	GGTAATTGAAAAGTTCATTGCCAGTGAA	CTATCGATGATGAAGATACCCACCAAACCAAAAAA
15	WHLR_RP1_hgx4861v1_GBKT7_A-28	5p 3p	103592559	Triticum aestivum - Homolog of Proteic Match - guanine nucleotide-binding protein-like NSN1 (Aegilops tauschii subsp. tauschii)	https://www.ncbi.nlm.nih.gov/nuccore/XP_020165137.1	B	-85	865	IF	Sense	99.1 / 95.4	GTCGCCACCGGCTCTCCCTTCCTCCTCCTC	CAAACGAGGTGGGCCCCTTTCAAAGGAAGGCAGG
16	WHLR_RP1_hgx4861v1_GBKT7_A-329	5p 3p	103592614	Triticum aestivum - Homolog of Proteic Match - histone H2A.Z-specific chaperone chz-1-like (Aegilops tauschii subsp. tauschii)	https://www.ncbi.nlm.nih.gov/nuccore/XP_020151843.1	B	-79	1021	IF	Sense	99.3 / 95.8	CTTAAACCCTAGCTCTCCCTCTCCCTCTCCC	CGGTCTTCCGGAGTATCTTTCCCCCTTCGGACCCCAC
17	WHLR_RP1_hgx4861v1_GBKT7_A-168	5p 3p	103592716	Triticum aestivum - Homolog of Proteic Match - hypothetical protein MELLADRAFT_90063 (Melampsora larici-populina 98AG31)	https://www.ncbi.nlm.nih.gov/nuccore/XP_007413085.1	B	-76	No Data	IF	Sense	99.9 / 98.9	TGAATATTTGCTACTACCACCAAGATCTG	AATCCCATTTTCAGGGCTAGTTCATTCGGCAGGTGA
18	WHLR_RP1_hgx4861v1_GBKT7_A-276	5p 3p	103592611	Triticum aestivum - Homolog of Proteic Match - hypothetical protein RO3G_16591 (Rhizopus delemar RA 99-880)	https://www.ncbi.nlm.nih.gov/nuccore/EIE91880.1	B	-73	No Data	IF	Sense	100.0 / 100.0	CGCACCTGTCGATAAGGCAAATGTGCCA	CGCACCTGTCGATAAGGCAAATGTGCCAAGACCTT
19	WHLR_RP1_hgx4861v1_GBKT7_A-156	5p 3p	103592344	Triticum aestivum - Homolog of Proteic Match - hypothetical protein TRIUR3_00775 (Triticum urartu)	https://www.ncbi.nlm.nih.gov/nuccore/EMS68444.1	B	-52	376	IF	Sense	90.8 / 98.1	CAAGAACCCCATTGTCATCTCATCAGACG	GACGAACGAGGGAGGTAGACAAATGCATTCAGGT
20	WHLR_RP1_hgx4861v1_GBKT7_A-5	5p 3p	103592708	Triticum aestivum - Homolog of Proteic Match - strangulata troponin T, fast skeletal muscle isoforms-like (Aegilops tauschii sub:	https://www.ncbi.nlm.nih.gov/nuccore/APD15308.1	B	-19	388	IF	Sense	99.2 / 95.9	TGAAGATCCTCCTCTAGTCAAATTCAGTG	CTGTCCCCCGGGGAGGTTGACAAGCACCCGCCCCA
21	WHLR_RP1_hgx4861v1_GBKT7_A-51	5p 3p	103592452	Triticum aestivum - Homolog of Proteic Match - mouse DNA EBV homolog 1 (Zea mays)	https://www.ncbi.nlm.nih.gov/nuccore/NP_001151748.1	B	-13	391	IF	Sense	93.8 / 99.3	CCCACAGGGAGCCGCATAGAGCCACCAC	CCCACAGGAGCCGCAGAGAGCCACCACCACCACCCA
22	WHLR_RP1_hgx4861v1_GBKT7_A-325	5p 3p	103592415	Triticum aestivum - Homolog of Proteic Match - nucleolar protein 58-like (Aegilops tauschii subsp. tauschii)	https://www.ncbi.nlm.nih.gov/nuccore/XP_020149764.1	B	279	1189	IF	Sense	100.0 / 100.0	AAAATGGGAACACCGAGTGCAAGGTCC	AAAATGGGAACACCGAGTGCAAGGTCCGGAGAGC
23	WHLR_RP1_hgx4861v1_GBKT7_A-152	5p 3p	103592725	Triticum aestivum - Homolog of Proteic Match - peptidyl-prolyl cis-trans isomerase CYP95 isoform X1 (Aegilops tauschii subsp. ta	https://www.ncbi.nlm.nih.gov/nuccore/XP_020191361.1	B	321	1142	IF	Sense	99.4 / 99.3	CAGAGCCTTCGCTTCGGTACGATCGAAG	CGCCCATGATGACCCGGGGTCTCTTGTCAATGGCA
24	WHLR_RP1_hgx4861v1_GBKT7_A-296	5p	103592529	Triticum aestivum - Homolog of Proteic Match - pre-mRNA-splicing factor SF2 (Zea mays)	https://www.ncbi.nlm.nih.gov/nuccore/NP_001150424.2	B	-155	No Data	OOF2	Sense	100	GTTGAATTCTCCGTCAGTTTCTCCCGTCTCCGCCGCGCTTGCCCCCGCCCGAATCTGTCGGCAACT	
25	WHLR_RP1_hgx4861v1_GBKT7_A-36	5p	103592550	Triticum aestivum - Homolog of Proteic Match - forkhead box protein B2-like (Triticum dicoccoides)	https://www.ncbi.nlm.nih.gov/nuccore/BAK06920.1	B	-1	689	IF	Sense	99.8	CGCCACCACGCCGCCCACCTCCTGCCCGCCGCCGCTCCCTGTGCCCACCACCACCACCACACATCGC	

ULTImate Y2H SCREEN Triticum aestivum - TaHRC (aa 1-261) vs Wheat Head Leave Root_RP1
Summary of PBS categories
A : Very high confidence in the interaction
B : High confidence in the interaction
The total number of screens performed on each organism is taken into account to set this connectivity threshold: 20
interactions to different bait proteins in our entire database for Human, 10 for Mouse, Drosophila and Arabidopsis and 6 for all other organisms.
They can be classified in different categories:

* Prey proteins that are known to be highly connected due to their biological function
* Proteins with a prey interacting domain that contains a known protein interaction motif or a biochemically promiscuous motif
* All the fragments of the same reference CDS are antisense
* The 5p sequence is missing
* All the fragments of the same reference CDS are either all OOF1 or all OOF2
* All the fragments of the same reference CDS lie in the 5' or 3' UTR

Supplemental Table S2. Primer sequences used in this study

Number	Name	Sequence(5'----3')	
1	TaHRC_CDS_F	ATGGATGCCAAGAAGTTCCT	For full-length CDS amplification
2	TaHRC_CDS_R	TTACACGAGTTGCTTCCCGT	
3	TaCAXIP4_CDS_F	ATGGACGACGACATCCAAGCTGGC	
4	TaCAXIP4_CDS_R	TTAGCCCCTCTTATGCTCCTTACT	
5	TaCAX1-CDS_F	ATGGATAGCCAGTCCGCGGTGACG	
6	TaCAX1-CDS_R	TTATGCGACCTGGACAATCATGGG	
7	BK_TaHRC_CDS_F	CATGGAGGCCGAATTCATGGATGCCAAGAAGTTCCTGCAG	For Y2H screen and co-transformation assays
8	BK_TaHRC_CDS_R	GCAGGTCGACTTACACGAGTTGCTTCCCGTCTCT	
9	BK_TaHRC_N_R	GCAGGTCGACCTTGCGCCTCCTGGCACCAT	
10	BK_TaHRC_N△NLS_R	GCAGGTCGACGTGCCTTTTGCGGTCCTTCC	
11	BK_TaHRC_C_F	CATGGAGGCCGAATTCATGCACAGGTCAAAGAGGAGGAG	
12	BK_TaHRC_C_R	GCAGGTCGACTTACACGAGTTGCTTCCCGTCT	
13	AD_TaCAXIP4_CDS_F	GGAGGCCAGTGAATTCATGGACGACGACATCCAAGCTGGC	
14	AD_TaCAXIP4_CDS_R	CGAGCTCGATGGATCCTTAGCCCCTCTTATGCTCCTTACT	
15	TaHRC_gRNA1_F	CTTGGCGACAGTGATCGGAAACAC	For gRNA vectors construction
16	TaHRC_gRNA1_R	AAACGTGTTTCCGATCACTGTCGC	
17	TaHRC_gRNA2_F	CTTGGTGCCAGGAGGCGCAAGCAC	
18	TaHRC_gRNA2_R	AAACGTGCTTGCGCCTCCTGGCAC	
19	TaHRC_gRNA3_F	CTTGGGAAGAAGCACTCGCACAGG	
20	TaHRC_gRNA3_R	AAACCCTGTGCGAGTGCTTCTTCC	
21	TaHRC_CDS_GW_F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGATGCCAAGAAGTTCCT	For BiFC, Subcellular Colocalization, Ca2+ Suppression and ROS assays
22	TaHRC_CDS_GW_R	GGGGACCACTTTGTACAAGAAAGCTGGGTCCACGAGTTGCTTCCCGT	
23	TaHsp_CDS_GW_F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGCATCAAGGGTTGTGTTCTCC	
24	TaHsp_CDS_GW_R	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAGTCGACGTTGACCTCGAAGAC	
25	TaCAXIP4_CDS_GW_F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGACGACGACATCCAAGCTGGC	
26	TaCAXIP4_CDS_GW_R	GGGGACCACTTTGTACAAGAAAGCTGGGTCGCCCCTCTTATGCTCCTTACT	
27	BK_TaCAX1_CDS_F	CATGGAGGCCGAATTCATGGATAGCCAGTCCGCGGTGACG	
28	BK_TaCAX1_CDS_R	GCAGGTCGACGGATCCTTATGCGACCTGGACAATCATGGG	
29	BK_TaCAXIP4_CDS_F	CATGGAGGCCGAATTCATGGACGACGACATCCAAGCTGGC	
30	BK_TaCAXIP4_CDS_R	GCAGGTCGACTTAGCCCCTCTTATGCTCCTTACT	