Supporting Information (SI) Appendix

SI Materials and Methods

RP4Htn1 BAC-library construction

A BAC library for the resistant line RP4Htn1 (RP4 with *Htn1* introgression from B37Htn1) was constructed at Amplicon Express (http:// ampliconexpress.com/). Seeds were germinated in the greenhouse and leaf material of very young maize plants (3 to 4-leaf stage) was collected and frozen in liquid nitrogen. In total 99,456 clones were obtained in the vector pCC1BAC (Epicentre, http://www.epibio.com/) with the cloning enzyme *Hin*dIII. The clones were arranged in 259 384-plates with an average insert size of 120 kb, 2% of blue clones and 39 missing wells. The storage medium of the clones in the 384-well plates consists of 36 mM KH_2HPO_4, 13.2 mM KH_2PO_4 1.7 mM sodium citrate, 0.4 mM $MgSO_4$, 6.8 mM $(NH_4)_2SO_4$, 4.4% glycerol, 12.5 µg/mL chloramphenicol.

Multidimensional BAC DNA pools were constructed by Amplicon Express according to the company protocol. The BAC library is separated into sequential Superpools of seven 384-well plates. Each Superpool of 7 plates is further separated into 7 Plate pools, 16 Row pools and 24 Column pools. As part of the Quality Assurance/Quality Control (QA/QC), a missed well in one of the Superpools of each BAC library was chosen and the missed well was replaced by a positive control BAC clone. The Superpool, plate and well location of this positive control is different for each library. This positive clone is a BAC clone of approximately 130 kb from *Anaplasma marginale*. The Internal Standard for RP4Htn1 was placed in library plate 028, well F04, which is identified as Superpool 4, plate 07, row F, column 04. BAC clones were grown at 37°C in LB or 2x YT containing 12.5 µg/mL chloramphenicol. The BAC library and pools were stored at Keygene.

Screening of the BAC-library pools was performed at Keygene (http://www.keygene.com/). The reference sequence for primer pair design was the B73 AGPv01 genome (FPC Dec 2008 at www.maizesequence.org) on chromosome 8 from 149,957,158 bp to 152,977,351 bp. The sequence was masked for repetitive sequences with the software Repeatmasker. Afterwards the software Primer3 was used for selection of primer pairs. The settings for Primer3 were: average GC-content (~50%), primer length is 20 to 25 bp, melting temperature between 60 and 70 °C and amplicon length between 70 and 80 bp. In order to select the unique primers, all primers which came out of the primer design were submitted for a Blast analysis against the whole, unmasked, maize genome sequence. A second filtering was done on the basis of the presence of multiple N's in the primer sequence: primer pairs containing a primer with multiple N's were removed from the list. This resulted in a final list of 907 unique primer pairs

spread over the physical region of interest. For screening, primer pairs with an average distance on the reference sequence of 10 kb were tested on the genotypes B73, RP4Htn1, RP1 and RP1Htn1 for amplification via RT-PCR. RT-PCR of 47 primer pair result in 26 positive BAC-clones for the region of interest.

BAC library screening

A BAC library for the resistant line RP4Htn1 was constructed at Amplicon Express (http:// ampliconexpress.com/). Screening of the BAC-library pools was performed at Keygene (http://www.keygene.com/) (Table S6). The reference sequence for primer pair design was the B73 AGPv01 genome sequence (FPC Dec 2008 at www.maizesequence.org) on chromosome 8 from 149,957,158 bp to 152,977,351 bp. BAC clone sequencing of 13 BACs was performed by Amplicon Express using GS-FLX Titanium sequencing (Roche). The obtained total sequence amount was on average 12,000 reads per BAC with 400 bp length. Sequences were cleaned for *E. coli* contamination. The raw sequences were automatically assembled using the software Newbler (454 runAssembly software, software release 2.3). The resulting sequence contigs per BAC were manually ordered. Genes were annotated manually using the coding sequences of the B73 maize genome (http://maizegdb.org/gene_model.php). The coding sequences were aligned with the RP4Htn1 scaffold using DotPlot (http://www.dotplot.org/) for verification of exon and intron positions of possible candidate genes.

Marker development for genetic mapping

SSR-Marker (umc1121, umc2210, bnlg1782, bnlg1152 and bnlg240) sequences were selected from National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/) and primer pairs were ordered with according modifications for detection. PCR-products were separated on the ABI3730xl DNA analyzer (Life Technologies) according to manufacturer protocol with a capillary length of 36 cm and the GeneScan™ 400 HD ROX™ Size Standard.

SNP-markers (Table S7) were established as KASP-Assays (LGC Genomics) from different resources. Public markers were selected from the Maize Community 50K-Illumina-Chip (1) based on the SNP and their physical position on B73 AGPv02. Furthermore, comparative sequencing of PCR-amplicons on donor lines and recurrent parents was performed. In addition, SNP calling from the RP4Htn1 BAC sequence against the B73 AGPv02 reference sequence was performed with the Blast-Algorithm (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The identified homologous sequences were assembled in the software Lasergene Seqman (DNASTAR) and SNPs called with the software default settings. Oligo-Design for KASP-primer development was conducted with the Kraken™ Software (LGC Genomics) according to the manufacturer protocol. Evaluation of the marker assay results was performed with the Kraken™ Software (LGC Genomics) according the manufacturer protocol.

Development and screening of a TILLING mutant population

The development of a TILLING mutant population of KWS line RP3Htn1 was performed according to Kato (2). Pollen was harvested from field-grown RP3Htn1 plants and treated with 0.1% EMS solution for 45 min. Silks of individual plants were then pollinated and emerging ears bagged. From 436 pollinated M0 plants seeds were harvested. An additional propagation and selfing led to 10,084 individual M1 plants. Leaf material from these M1 plants was collected for DNA isolation. DNA of dried leaf samples (10 leaf discs bunches/sample) was isolated from 10,000 M1 individuals with the CTAB extraction method at Traitgenetics (Gatersleben, Germany). DNA was aliquoted to 100µl with 20ng/µl. Primer development for mutant screening was performed at Traitgenetics (Table S3). The amplification assay consisted of 20 ng/µl DNA, 5x GoTaq-Buffer, 25 µM dNTPs, 10 µM forward Primer, 10 µM reverse Primer, 5 Units/µl GoTaq. After denaturation for 300 s at 94°C the amplification cycles were performed with 35 cycles of 60 s at 94°C, 60 s at 60°C and 60 s at 72°C followed by a final elongation time for 600 s at 72°C. The Sanger-sequencing of PCR products was performed at Traitgenetics according to the company protocol.

The sequences were assembled in the software Lasergene Seqman NGen (DNASTAR) and heterozygote SNPs called with the software default settings. Positive mutant plants were sequenced again with the Sanger-method in order to confirm the polymorphism.

Southern blot analysis

Isolations of genomic DNA from leaf material and Southern hybridization were performed as described (3, 4). Genomic DNA was digested with the restriction enzyme DraI. One probe each was designed by PCR amplification from the exctracellular domains of *ZmWAK-RLK1* and *ZmWAK-RLK2*, respectively. The same forward primer GH119 (5'-GCTACCCGTTCTATCTTGCC-3') and reverse primer GH196 (5'-CTGCTCCTCTTGTTCGTCAA-3') could be used. These two probes were mixed together 1:1 for hybridization.

Expression analysis of *ZmWAK-RLK1*, *ZmWAK-RLK2* and *ZmWAK-RLP1*

First strand cDNA was synthesized for lines RP1, RP1Htn1, RP3, RP3Htn1, RP4, RP4Htn1, W22Htn1, B37Htn1 and B37Ht2 from 1 mg of total RNA using 2 mM oligo-dT-primer (5'dT20NV-3') complemented with 100 nM of the reverse primers GH279 and GH287, the reverse transcriptase SuperScriptIII (18080-044, Life Technologies) and RNaseOUT™ Recombinant RNase Inhibitor (10777-019, Life Technologies) according to the manufacturer's protocol. Full-length cDNA amplification for *ZmWAK-RLK1* from these lines was performed using the forward primer GH278 (5'-GTGAACCCAGCCCCACCTC-3') and the reverse primer GH279 (5'- GTTAATGGGCACTCGTCTCCAC-3'), except for RP1 where the reverse primer GH287 (5'- TGGGCACTCGTACGTCTCCAC-3') was used. In addition, a nested PCR was

performed for lines W22Htn1 and B37Ht2 with the forward primer GH184 (5'-CGTCTACCACGTCTTCTCCC-3') and the reverse primer GH185 (5'-TGCTCCCTTCCAACATCTCG-3'). For PCR amplification, the KAPA HiFi HotStart Polymerase (KK2502, Kapa Biosystems) was used with an annealing temperature of 63° C. In line RP1Htn1 a 2,128 bp fragment was amplified and confirmed by sequencing as the *ZmWAK-RLK1.1* transcript. From line RP1 two fragments were amplified (Fig. 4*A*). The smaller 2,076 bp long fragment was identified by cloning and sequencing as the *ZmWAK-RLK1.1* transcript and the bigger 2,357 bp long fragment as the *ZmWAK-RLK1.2* transcript, which retained the second intron.

Expression of *ZmWAK-RLK2* in line RP1Htn1 was verified by full-length cDNA amplification of a 2,251 bp long amplicon using the forward primer GH191 (5'-AAAGCAGCTAGATGAAGGGTG-3') and the revers primer GH192 (5'-AGTCACACAGCAGCAATACAGA-3') and using cDNA produced as described above. The KAPA HiFi HotStart polymerase was used and an annealing temperature of 62° C.

Expression of *ZmWAK-RLP1* in line RP1Htn1 was verified by a 3' race PCR. Reverse transcription was made with SMARTer™ RACE cDNA Amplification Kit (634923; Clontech) according to the protocol using 30 ng of Oligotex (72022; Qiagen) purified mRNA. First 3' race touchdown PCR was performed according to the SMARTer™ RACE cDNA Amplification Kit with reverse primer Universal Primer long (SMARTer RACE KIT) and the *ZmWAK-RLP1* specific forward primer GH222 (5'- ACGCTGACCCTCCCCTACATGTCCCACAGA -3') with KAPA HiFi HotStart Polymerase (KK2502, Kapa Biosystems) and 2 µl of 1:10 diluted cDNA in 25 µl. A nested PCR was made with 1 µl of the obtained PCR product with KAPA HiFi polymerase using forward primer Nested Universal A (SMARTer RACE KIT) together with *ZmWAK-RLP1* specific forward primer GH168 (5'- CGACTACAAGACGCGTACC-3') at 63° C annealing temperature.

SI Figures

Fig. S1. Example of resistant and susceptible progeny of the RP1 x RP1Htn1 mapping population in a field infection test. These plants were scored as 3 to 4 scoring units for the resistant recombinant plants and as 8 for the susceptible recombinant plants.

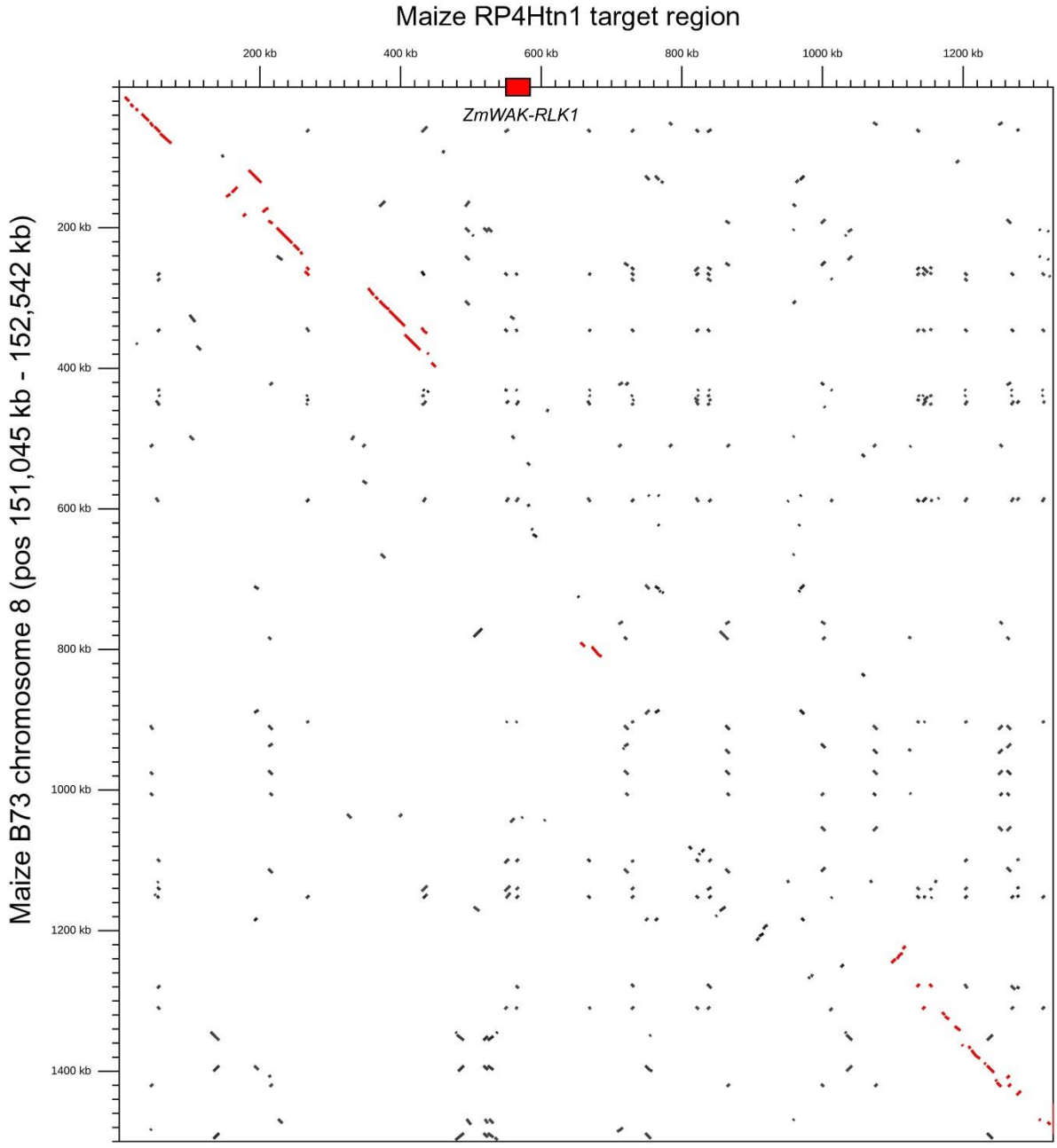

Fig. S2. Dotplot alignment of the *Htn1* target region in maize cultivars RP4Htn1 and B73 (vertical). The Dotplot was generated from a blast output of the two sequences against each other by plotting all blast alignments that are longer than 1,500 bp and show at least 95% sequence identity. The very high content of transposable elements generates many non-specific signals. The blast hits reflecting sequence colinearity between RP4Htn1 and B73 are depicted in red. Note that the central part of the region (which includes the *Htn1* candidate genes) shows almost no sequence conservation while the ends show relatively strong sequence colinearity.

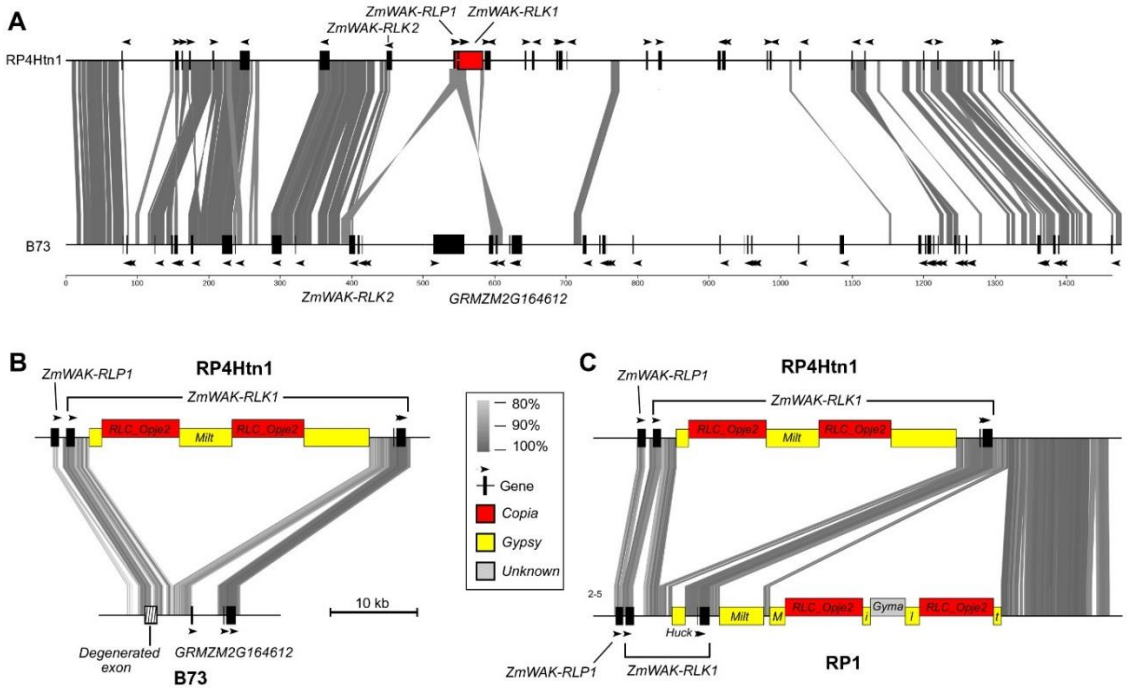

Fig. S3. Sequence alignment of the *Htn1* region in maize genotypes RP4Htn1, B73 and RP1. Conserved sequences are connected by shaded areas. (*A*) The two candidate genes for *Htn1* (*ZmWAK-RLP1* and *ZmWAK-RLK1*) are highlighted in red. (*B*) and (*C*) Detailed comparisons of the RP4Htn1 *ZmWAK-RLK1* region with its counterparts in B73 and RP1.

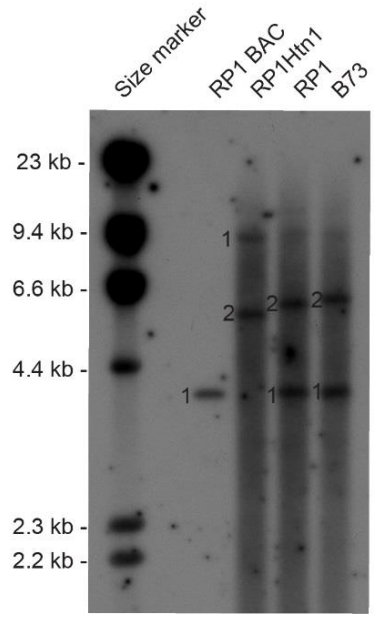

Fig. S4. Southern blot analysis with probes derived from the conserved extracellular domains of *ZmWAK-RLK1* and *ZmWAK-RLK2*. Genomic DNA was digested with the restriction enzyme *Dra*I. Blots were hybridized with a mixture of two probes amplified from the *ZmWAK-RLK1* and *ZmWAK-RLK2* extracellular domains. A BAC clone from the susceptible genotype (RP1) carrying the *ZmWAK-RLK1* gene was used as control. 1, DNA fragment with *ZmWAK-RLK1*; 2, DNA fragment with *ZmWAK-RLK2*.

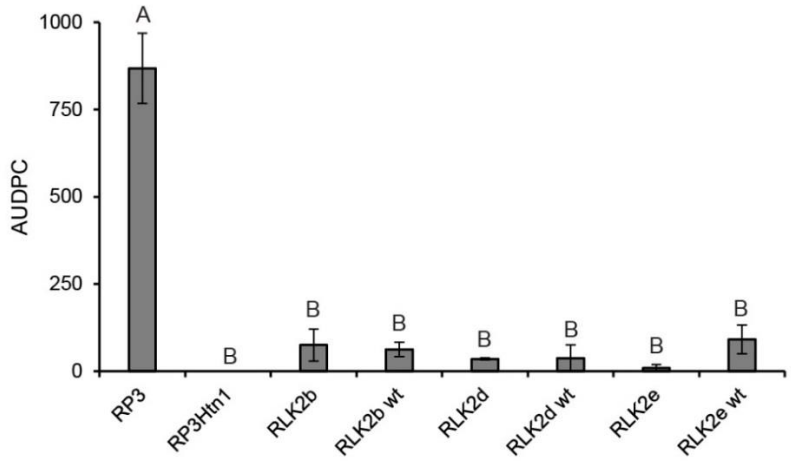

Fig. S5. Amino acid changes in the *ZmWAK-RLK2* gene have no effect on resistance to *Exserohilum turcicum* in greenhouse infection tests. The mutant lines RLK2b, RLK2d and RLK2e were as resistant as RP3Htn1 and the corresponding wild-type segregants. Plants were scored for resistance and susceptibility from 11 to 25 days post infection approximately every second day and AUDPC values were then calculated. AUDPC values represent the means for two to three biological replicates and error bars represent standard errors (SE). Different letters on top of bars denote a significant difference in AUDPC values (Student's *t-test*, $P < 0.05$).

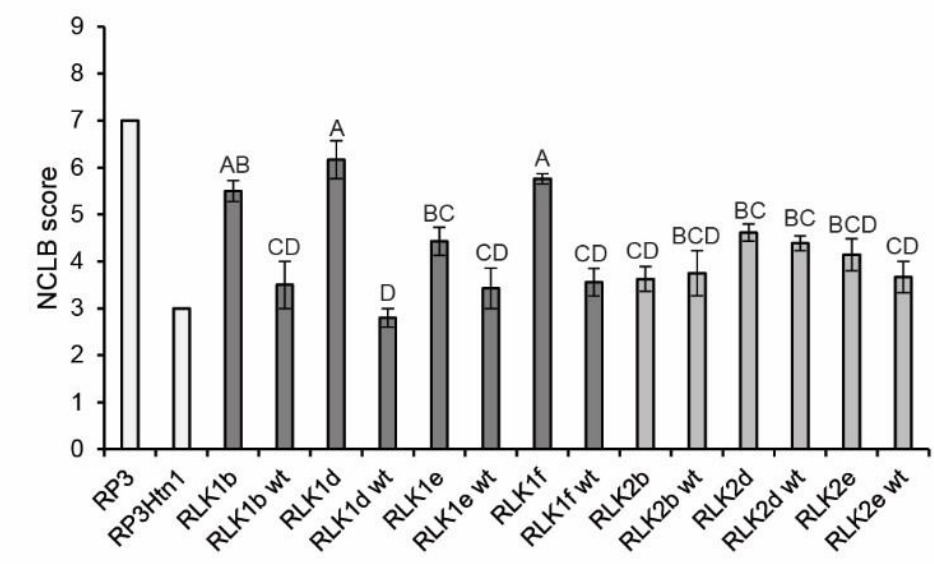

Fig. S6. RLK1 mutants showed increased NCLB susceptibility in field infection tests. RLK1 mutants (RLK1b, RLK1d, RLK1f) were scored two to three NCLB disease scores higher than their corresponding wild-type lines. The RLK2 mutants (RLK2b, RLK2d and RLK2e) showed no increase. Mean NCLB scores were calculated for 20 plants per line in the field. The values presented here for the mutants are the means of at least four such replicates and the standard error (SE) is given. Since for RP3 no replicate was produced and for RP3Htn1 only one, we excluded these lines from the statistical analysis. On top of the bars, different letters denote a significant difference in NCLB scores (Tukey's honestly significant difference test, $\alpha = 0.050$).

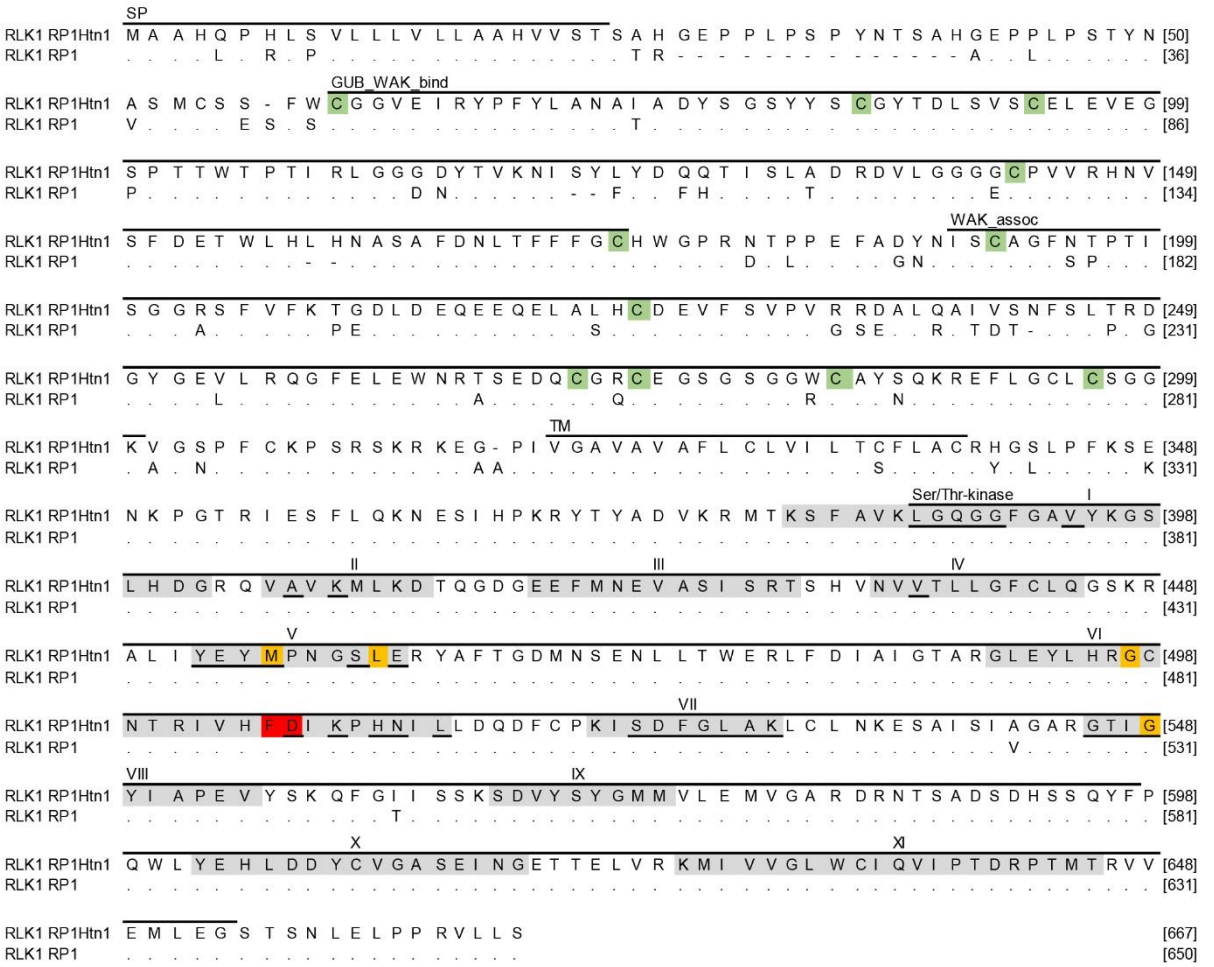

Fig. S7. Amino acid sequence alignment of the ZmWAK-RLK1 (RLK1) protein from the resistant cultivar RP1Htn1 and the susceptible cultivar RP1. The reference sequence RLK1 from RP1Htn1 is shown completely, while for RLK1 of RP1 only polymorphic amino acids are shown (dashes represent deletions). The protein domains are indicated on top of the sequence: SP, signal peptide; GUB_WAK, wall-associated receptor kinase galacturonan-binding; WAK_assoc, wall-associated receptor kinase C-terminal; TM, transmembrane domain; Ser/Thr-kinase, Catalytic domain of the Serine/Threonine kinases and Interleukin-1 receptor associated kinases. Amino acids in yellow represent residues which are mutated in the ZmWAK-RLK1 TILLING mutants (from top to bottom: RLK1b, RLK1e, RLK1d, RLK1f). Underlined amino acids in the serine/threonine-kinase domain represent conserved domains and sites (ATP binding site, active site, polypeptide substrate binding site and/or activation loop (A-loop) (cd14066, (5)). The two residues in red indicate the RD motif. Both resistant and susceptible alleles belong to the non-RD class of kinases due to the lack of the conserved arginine at the first position. In green, the cysteines that are typically found in the GUB_WAK and WAK_assoc. domains are indicated. Roman numerals on top of amino acids overlaid in grey indicate the conserved kinase subdomains (6).

SI Tables

Table S1. Disease scores for phenotyping northern corn leaf blight on maize plants in field experiments after natural or artificial *Exserohilum turcicum* inoculation.

score	phenotype
1	Plants show no disease symptoms 0%
2	Disease starts and first small lesions (smaller than 2cm) are visible on few plants per row. Less than 5% of leaf surface is affected.
3	Few lesions on one particular leaf level and on several plants per row. Between 5-10% of the leaf surface is affected.
4	10-20% of leaf surface is affected. Clear lesions on several leaf levels.
5	20-40% of leaf surface is affected. Lesions start merging.
6	40-60% of leaf surface is affected. Systematic disease on leaves is visible.
7	60-80% of leaf surface is affected. Half of the leaf material is destroyed and dried down because of the disease infection.
8	80-90% of leaf surface is affected. More than half of the leaf material is destroyed and dried down because of the disease infection.
9	90-100% of leaf surface is affected. Plants are nearly completely dry.

Table S2. Phenotypic field scores of the three pairs of near isogenic lines used for map-based isolation of *Htn1.*

genotype	without *Htn1*	with *Htn1* introgression
RP1	7 to 9	3 to 4
RP3	4 to 6	2 to 3
RP4	6	2 to 3

Table S3. Primer pairs used to screen of the TILLING population. RLK1, *ZmWAK-RLK1*; RLK2, *ZmWAK-RLK2*

gene	primer pair 1 primer pair 2	sequence (5'-3')	melt-temp °C	amplicon size (bp)
RLK1 – exon 3	RLK1-exon3.for	CTTCCTACAGAAGAACGAGAGT	60	804
	RLK1-exon3.rev	TTCCTCACGAGCTCTGTGGTC		
RLK2 – exon 3	RLK2-exon3.for	TGTTTCAGGAATCACGCAACTGGA	55	367
	RLK2-exon3.rev	GCACCACGCCATGACCAACATC		
RLK2 – exon 4	RLK2-exon4.for	AGTACAAGTGATCTCTGGATTTG	55	648
	RLK2-exon4.rev	GGCAAACAATGGTCTGGTGG		

Table S4. Position of the nucleotide exchanges in the TILLING mutants of *ZmWAK-RLK1* (RLK1) and *ZmWAK-RLK2* (RLK2).

mutated gene	mutant line	position of mutation (bp)*	base pair change	amino acid change
RLK1	RLK1b	1365	G > A	M > I
RLK1	RLK1d	1490	G > A	G > E
RLK1	RLK1e	1378	C > T	L > F
RLK1	RLK1f	1642	G > A	G > R
RLK2	RLK2b	965	C > T	A > V
RLK2	RLK2d	977	G > A	G > D
RLK2	RLK2e	1159	G > A	E > K

***** based on the cDNA sequence of the *ZmWAK-RLK1* and *ZmWAK-RLK2* gene, respectively.

Table S5. Marker analysis reveals a common haplotype at the *Htn1* locus in *Htn1* introgression lines.

Marker/ line	umc1121	MA0023	MA0003	MA0004	MA0005	**MA0024**	**MA0013**	MA0025	PZE-108096011	umc2210	MA0020	bnlg1782	bnlg1152	bnlg240
B37	149	C	A	C	A	**C**	**G**	G	G	079	A	232	176	132
W22	n.d.	T	A	n.d.	A	**C**	**A**	G	G	089	C	220	n.d.	n.d.
RP1	148	T	A	C	A	**C**	**A**	G	G	089	C	230	180	126
RP3	156	C	A	C	A	**C**	**A**	G	G	091	C	220	n.d.	128
RP4	156	C	A	C	A	**n.d.**	**A**	G	G	091	C	220	151	128
B37Htn1	149	C	C	A	C	**T**	**G**	A	A	079	A	228	153	134
W22Htn1	149	C	C	A	C	**T**	**G**	A	A	079	A	228	153	134
RP1Htn1	149	C	C	A	C	**T**	**G**	A	A	079	A	228	153	134
RP3Htn1	156	C	C	A	C	**T**	**G**	A	A	079	A	228	n.d.	134
RP4Htn1	156	C	C	A	C	**T**	**G**	A	A	079	A	228	n.d.	134

n.d. = not determined

Table S6. Primers used for selection of RP4Htn1 BAC clones. Two primer pairs were used per BAC clone to compensate for primer failures due to possible sequence differences between RP4Htn1 and B73.

BAC clone ID	primer pair 1 primer pair 2	sequence (5'-3')	CP-value (Cycle when exponential phase starts)	amplicon size (bp)
144N24	579ZMPM0_5F; 579ZMPM0_5R	GGCATTATTAGCTAGGCGCA TTGGGAAACTCAGGTTCTGC	27.09	76
	579ZMPM0_17F; 579ZMPM0_17R	TGTACCCCAGCTACGACGTT AACCTTCACGCAAAGAATCG	25.53	78
219G11	579ZMPM0_16F; 579ZMPM0_16R	AAACATATGCGTGATCGGCT ATGGCTCGTTTCTTCAGGTG	25.96	78
	579ZMPM0_25F; 579ZMPM0_25R	TTGGACCAAACACTATCGATCC CGTTGGCAAAACCTAGGAATC	26.09	80
119F13	579ZMPM0_22F; 579ZMPM0_22R	ACTGGAACTGCAGGAAGGTG GACGTTTAACCGGCAGTCAG	25.98	73
	579ZMPM0_34F; 579ZMPM0_34R	TGAATTGCAAGCCCACACTA CCTGGTTTGCTGCTCTTCAT	24.43	76
86N21	579ZMPM0_35F; 579ZMPM0_35R	CCAAATGAACACGAACACCA GGCGTGGTGACTTTTTGTCT	25.27	70
	579ZMPM0_38F; 579ZMPM0_38R	CCCAAGATGAAGATCCGATG CAAACCAAAGAACTCGAGCG	26.01	71
16B6	579ZMPM0_37F; 579ZMPM0_37R	GTAATGGGGCAGATGTTTGG GCGACTCTTCGCTACACACC	25.71	80
	579ZMPM0_41F; 579ZMPM0_41R	NNNCCCTGTTCATGTAACTTCAAT TGCACACGATAAGGACATGC	26.6	74
84L18	579ZMPM0_41F; 579ZMPM0_41R	NNNCCCTGTTCATGTAACTTCAAT TGCACACGATAAGGACATGC	26.6	74
	579ZMPM0_46F; 579ZMPM0_46R	TCAAGAGAACTCTGGGTGGC GGCCAACAATGACGAGAGTC	25.68	78
128D2	579ZMPM0_180F; 579ZMPM0_180R2	GGGAGGGTTGTTCTGGTTTT GGTCCTTGTCAATGTCACCC	25.99	77
	579ZMPM0_48F; 579ZMPM0_48R	ATGGACCCCCGTTGTTATCT GCCTGCAGACAAATTCCTGT	25.33	77

25M23	579ZMPM0_48F; 579ZMPM0_48R	ATGGACCCCCGTTGTTATCT GCCTGCAGACAAATTCCTGT	25.33	77
	579ZMPM0_56F; 579ZMPM0_56R	CCTGTCATGGTGGGAACAAT CTCATCAGCGAAGCGAAAAA	29.12	79
19J24	579ZMPM0_51F; 579ZMPM0_51R	ACCCTCTCCTTGCTATTGGC CTCCAGCTCTTCGTTCGTTC	27.75	77
	579ZMPM0_199F; 579ZMPM0_199R	GCAGGCTGGACAAAAGTGTT TTCTTTTTGCGGCCTATCTG	26.56	79
96H10	579ZMPM0_63F; 579ZMPM0_63R	ATTTGCTTGGCGTAATCCTG CAGCCGTGTTTTTCTTTGCT	26.08	63
	579ZMPM0_208F; 579ZMPM0_208R	CGCACGGATCAAGAAGAGTT CAATCGCCATGCATACTTTG	26.84	79
136A1	579ZMPM0_206F; 579ZMPM0_206R	ATGGTACAAGTGTCGATCCCTC AATGAATCGATGTCGCTGGT	32.09	70
	579ZMPM0_86F; 579ZMPM0_86R	CACAACTAAAGAGGAAACCGGA GGCTGACGGTCTAGTCTTCG	30.07	71
135F7	579ZMPM0_79F; 579ZMPM0_79R	AACCAAATGGGGTCTTAGCC ATCCGCCACTGGTCAAAATA	25.43	72
	579ZMPM0_278F; 579ZMPM0_278R	CATCGCAACATCAGCAACAT ACGTTTGTTCCCTTCATCCA	22.69	78
75H6	579ZMPM0_209F; 579ZMPM0_209R	CTGTGCTTCTGGTGCTGAAA CTTTCCCGCCTGTAAATGAA	24.93	77
	579ZMPM0_86F; 579ZMPM0_86R	CACAACTAAAGAGGAAACCGGA GGCTGACGGTCTAGTCTTCG	30.07	71
117O2	579ZMPM0_87F; 579ZMPM0_87R	CTCACCCCACCCTACCCTAT GGGGAGCTGTTGAAGGAAAT	27.7	76
	579ZMPM0_91F; 579ZMPM0_91R	ACGTCGATCTGCTTGCTACC GAGACCTAGCGATCCAACGA	26.93	75
173H23	579ZMPM0_216F; 579ZMPM0_216R	CTCCATAGTGTTTCGGCCTTT GCCCTCAGGACTTACCGACT	25.76	80
	579ZMPM0_95F; 579ZMPM0_95R	GTCACTATACGGAGACGGCG CTCGGCCTTCAATTTGTGAT	24.97	73

Table S7. SNP markers used for *Htn1* mapping.

SNP-marker	marker position in AGPv02 on maize chromosome 8 [bp]	primer allele RP1Htn1 (5‘-3‘)	primer allele RP1 (5‘ -3‘)	common primer (5‘-3‘)
MA0003	151346184	GAAGGTCGGAGTCAACGGATTCGTGATGCGCCTTGCCGT**C**	GAAGGTGACCAAGTTCATGCTCCGTGATGCGCCTTGCCGT **A**	GCACATCAATCGACTCAGCC CTAT
MA0004	151688652	GAAGGTGACCAAGTTCATGCTGCCTCGTCCGCACTTCACG **T**	GAAGGTCGGAGTCAACGGATTCCTCGTCCGCACTTCACG**G**	GTGTCAACGCCGGATACGGG AT
MA0005	151831049	GAAGGTGACCAAGTTCATGCTCCAAAATTTTAGAATCACAAACAGATTTA C**G**	GAAGGTCGGAGTCAACGGATTCCAAAATTTTAGAATCACAAACAGATTTA C**T**	AAACGCCAGTATCAAGGAGTTATTAGTATT
MA0013	152133057	GAAGGTCGGAGTCAACGGATTCTTCCGACAAATAGCACAG ATC**G**	GAAGGTGACCAAGTTCATGCTCCTTCCGACAAATAGCACAGATC **A**	GACACGGTGTGTGCCAGTTTGTAAT
MA0020	152753635	GAAGGTCGGAGTCAACGGATTAATTTTGCCAGCATCTCTC TGCTC**A**	GAAGGTGACCAAGTTCATGCTTTGCCAGCATCTCTCTGCT C**C**	GTGAATCGGAGACCAAGGATTGCTT
MA0023	147689178	GTCATTACTAGCTCTGTCTTCAGTTACAA (Forward Primer)	GCAAAAGCTCGGATTGACTATCCAT (Reverse Primer)	CTGAGACA**(A/C)**TATATCG
MA0024	151914087	GAAGGTCGGAGTCAACGGATTCCTGCGAACTCGAGGTCGA**A**	GAAGGTGACCAAGTTCATGCTCCTGCGAACTCGAGGTCGA**G**	CGCCGCCGAGACGGATGGTA
MA0025	152303277	GAAGGTCGGAGTCAACGGATTCCTTCTGTTGCAAGAAATAAGCTCT**A**	GAAGGTGACCAAGTTCATGCTCCTTCTGTTGCAAGAAATAAGCTCT**G**	CATAGAGGTAGGTCCTCTTTAGCTATTAA
PZE-108096011	152435855	GAAGGTGACCAAGTTCATGCTCCACTAATGCAGAGATGGA GACT**A**	GAAGGTCGGAGTCAACGGATTCACTAATGCAGAGATGGAG ACT**G**	CATTTACACACTTTGCAAGGGCCCTA

Red color represents marker tail sequence and nucleotides in bold and uderlined the allele specific SNP.

Table S8. Setup of RT-qPCR assay for *ZmWAK-RLK1*.

gene name / transcript variant	5'-3' primer sequence	qPCR master mix	primer nM	PCR efficiency (E) r^2 of calibration curve slope	amplicon length bp	reference
ZmWAK-RLK1.1	F TATTGTTGGTGCTGTTGCCG R GGACTCAATCCTTGTCCCTG	KAPA SYBR® FAST qPCR Master Mix (KK4601; Kapa Biosystems)	250 250	E = 102% r^2 = 0.978 Slope = -3.272	121	this work
ZmWAK-RLK1.2	F TTGTGCAGCGGAGGGAAG R GCTTTTCTTCTGCTCTTTTAGACG	KAPA SYBR® FAST qPCR Master Mix (KK4601; Kapa Biosystems)	250 250	E = 96.7% r^2 = 1.00 Slope = -3.403	RP1Htn1, 133; RP1, 136; RP3 and RP4, 142	this work
FPGS	F ATCTCGTTGGGGATGTCTTG R AGCACCGTTCAAATGTCTCC	SsoFast™Eva Green Supermix (172-5201, Bio-Rad)	400 400	E = 109% r^2 = 0.989 Slope = -3.119	133	(7)

SI References

1. Ganal MW, et al. (2011) A large maize (*Zea mays* L.) SNP genotyping array: development and germplasm genotyping, and genetic mapping to compare with the B73 reference genome. *PLoS ONE* 6(12):e28334.
2. Kato (2000) *The maize handbook*, eds Freeling M & Walbot V (Springer, NY), pp 212-219.
3. Stein N, Herren G, Keller B (2001) A new DNA extraction method for high-throughput marker analysis in a large-genome species such as *Triticum aestivum*. *Plant Breed* 120(4):354-356.
4. Travella S, Klimm TE, Keller B (2006) RNA interference-based gene silencing as an efficient tool for functional genomics in hexaploid bread wheat. *Plant Physiol* 142(1):6-20.
5. Marchler-Bauer A, et al. (2015) CDD: NCBI's conserved domain database. *Nucleic Acids Res* 43(Database issue):D222-D226.
6. Hanks SK, Quinn AM, Hunter T (1988) The protein kinase family: conserved features and deduced phylogeny of the catalytic domains. *Science* 241(4861):42-52.
7. Manoli A, Sturaro A, Trevisan S, Quaggiotti S, Nonis A (2012) Evaluation of candidate reference genes for qPCR in maize. *J Plant Physiol* 169(8):807-815.