A nucleotide-binding site-leucine-rich repeat receptor pair confers broad-spectrum disease resistance through physical association in rice

Supplementary Figures

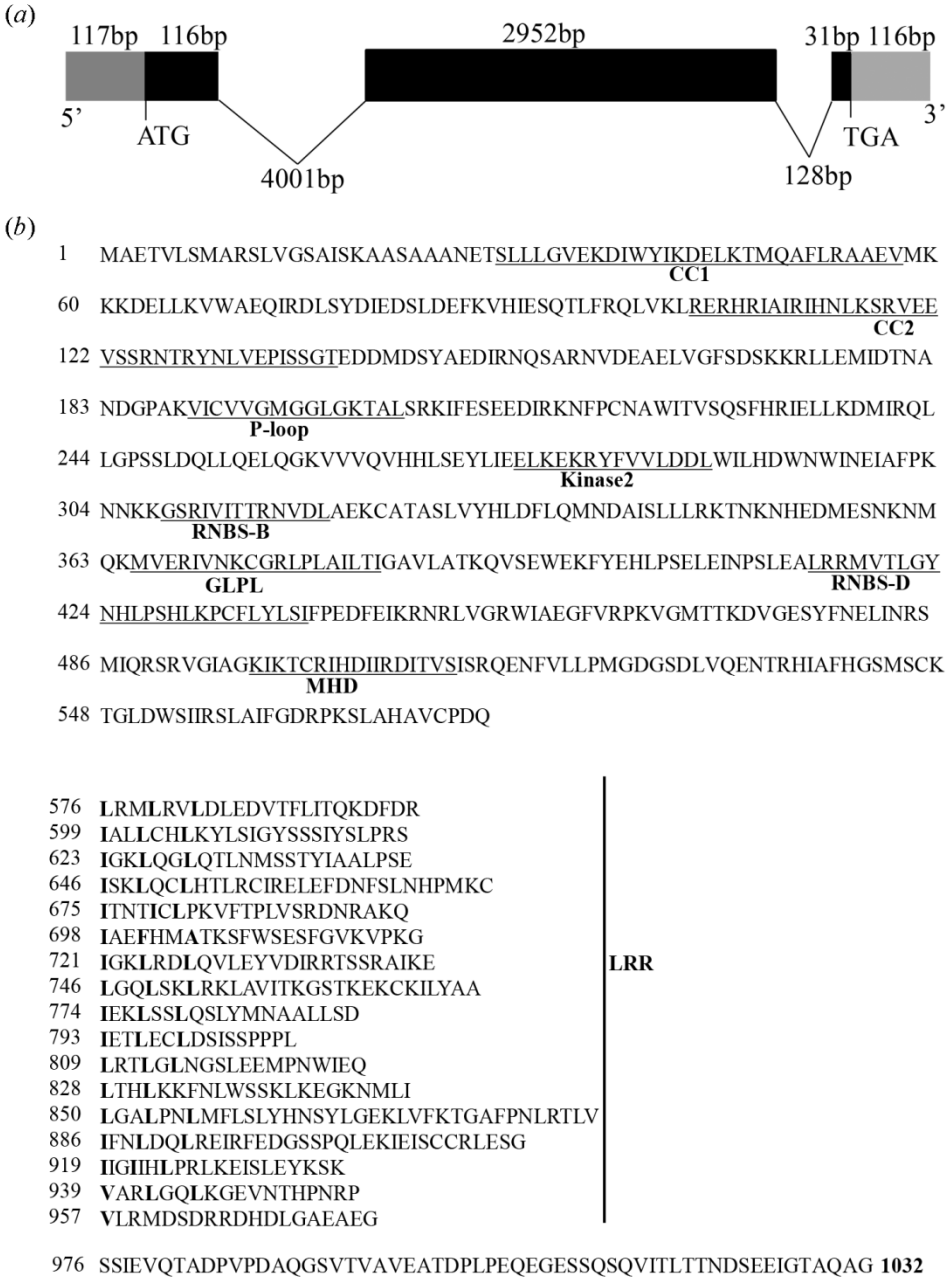

Figure S1. Genomic structure and amino acid sequence of *Pizh-2*

(*a*) Gene structure of *Pizh-2*. The black box represents exon, lines denote introns, and grey boxes indicate 5′ and 3′untranslated regions.

(*b*) Deduced animo acid sequence of the Pizh-2 protein. The two coiled-coil (CC) motifs are underlined. The conserved motifs (P-loop, Kinase2, RNBS-B, GLPL, RNBS-D, MHD) of the nucleotide-binding site (NB-ARC) region are also underlined. The C-terminal leucine-rich repeat (LRR) domain consists of 17 imperfect LRR repeats with the consensus IXX(L)XX(L)XX(L) is shown.

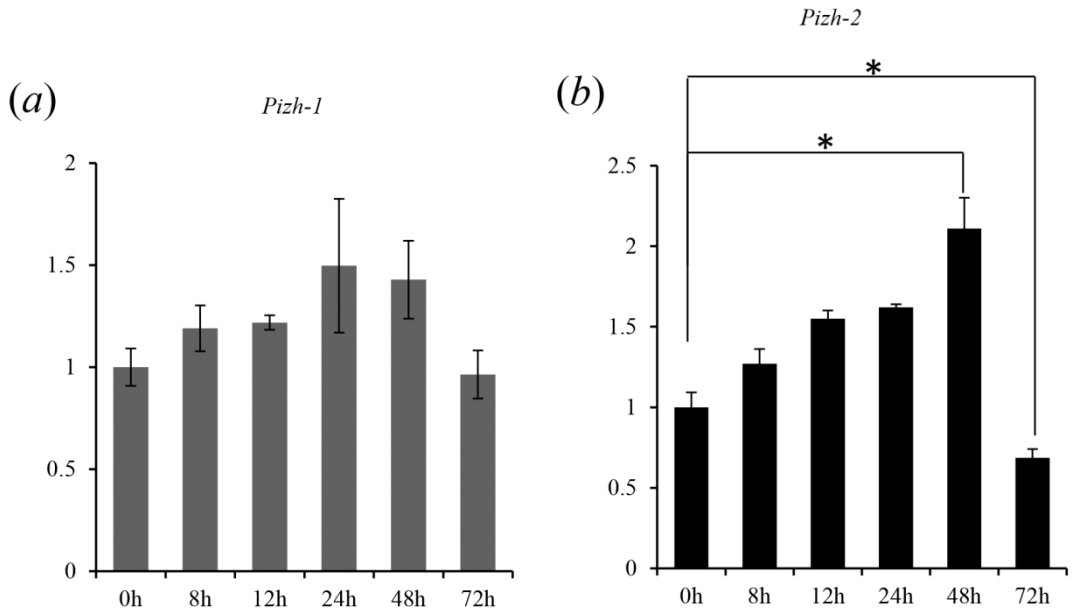

Figure S2. Gene induction analysis of Pizh-1 and Pizh-2 during pathogen infection

qRT-PCR analysis detected transcript levels of *Pizh-1* and *Pizh-2*. Total RNAs were prepared from leaves of ZH11 inoculated with isolate 85-14 in a time course from 0 to 72 h after inoculation. Values are means ± SD of three biological repeats. A Student's *t*-test was used to analyze the difference significance (*p < 0.05).

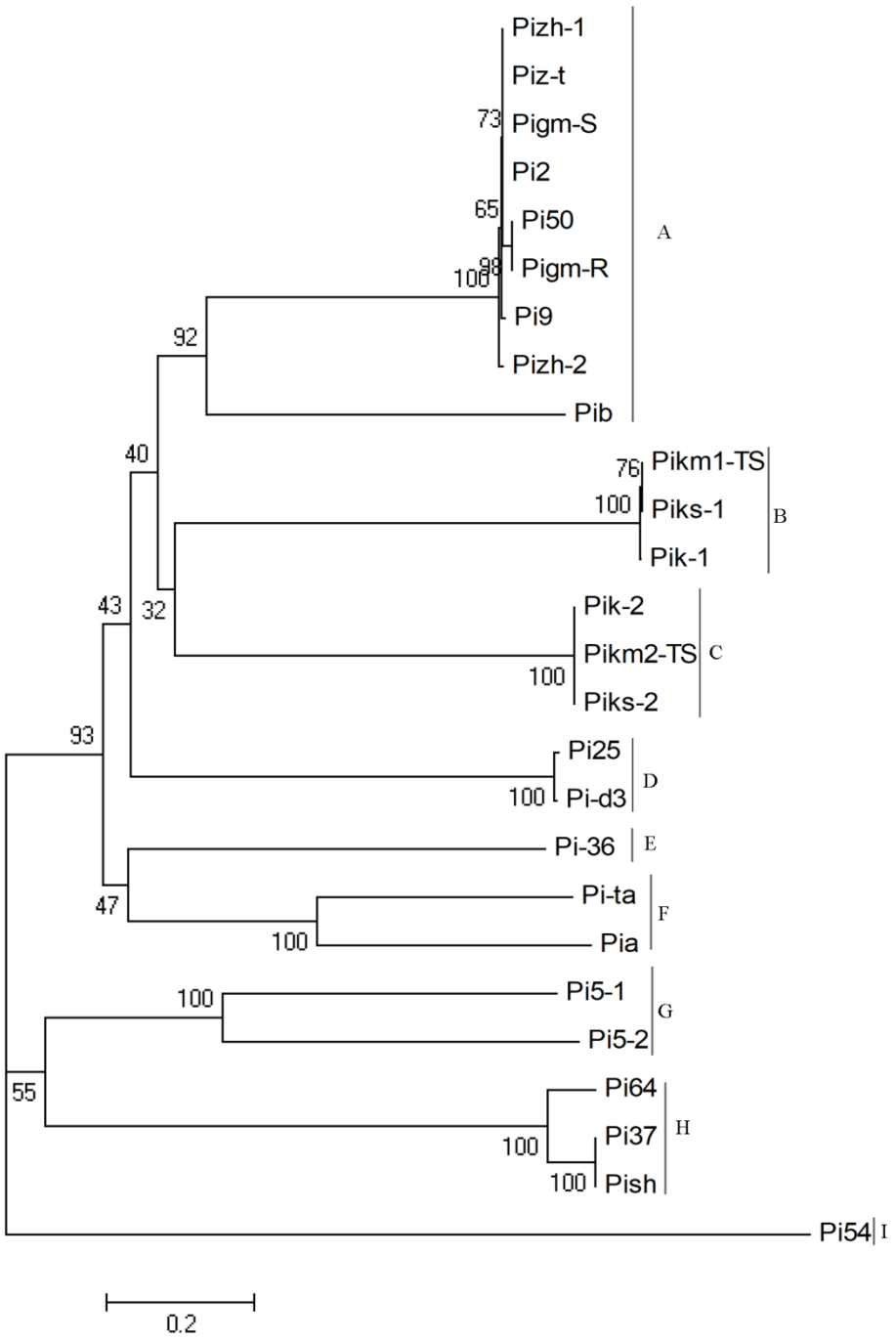

Figure S3. Phylogenetic analysis of Pizh and other NLR proteins in rice

The phylogenic tree is constructed based on full-length amino acid sequences of the selected NLRs, using a neighbor-joining algorithm. The numbers associated with individual branches indicate confidence levels based on 1000 bootstrap replicates, each major group shares >50% similarity at the amino acid level. The unit branch length is equivalent to 0.1 amino acid substitutions per site. A-I represent nine major groups of cloned R proteins.

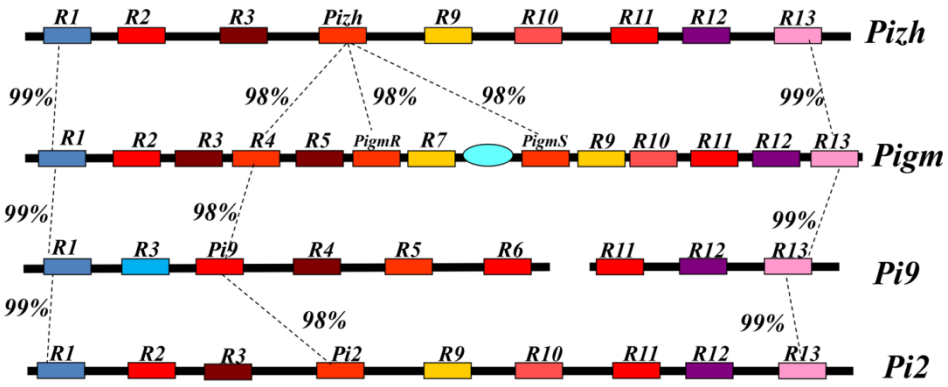

Figure S4. Comparison of genomic structure in the *Pizh* cluster with ellelic loci *Pigm*/*Pi2*/*Pi9*

Copy number variation of *R* genes in the *Pizh* and allelic loci from different rice germplasm. Ortholog and paralog members are indicated with the same color and identity percentages.

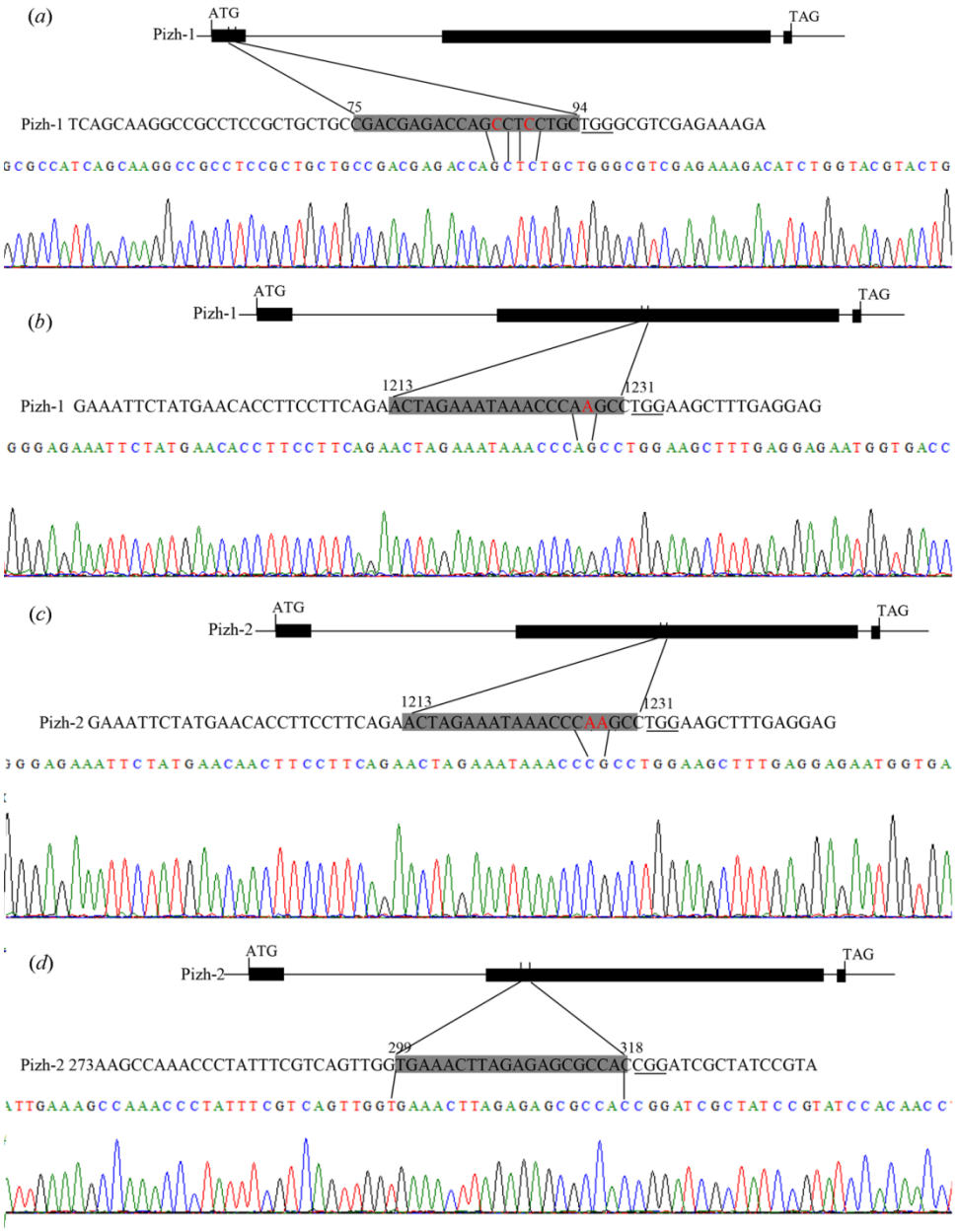

Figure S5. Chromatographs of Sanger sequencing detection of deletion mutations on the *Pizh-1* and *Pizh-2* genes

(*a*) Alignment of the chromatograms to the reference sequence indicating the *pizh-1* mutation (deletion of the two nucleotide 'CC').

(*b*) and (*c*) Alignment of the chromatograms showing the *pizh-1* (deletion of one nucleotide 'A') and *pizh-2* (deletion of two nucleotides 'AA') double mutation.

(*d*) Alignment of the chromatograms to the target sequence, showing no mutation on *Pizh-2*. The spacer is in gray, and the PAM site is underlined.

Supplementary Table

	C101A51(*Pi2*)	75-1-127(*Pi9*)	CO39	ZH11	Zenith	Gumei 4(*Pigm*)
04-52	R	MR	S	R	S	R
ZJ11	R	S	S	R	S	R
01-19	S	R	S	R	S	R
85-14	S	R	S	R	S	R
YN2	R	R	S	R	MR	R
SH188	R	S	S	R	MS	R
CN155	R	R	S	R	R	R
CN97	R	R	S	R	MS	R
CN102	R	R	S	R	MS	R
P06-6	S	S	S	R	MR	R
GD00-1200	S	S	S	R	R	R
GUY11	S	R	S	R	R	R
YJ1-2	R	R	S	R	R	R
2001-054	R	R	S	R	R	R
V13	R	R	S	R	R	R
PH14	S	R	S	R	R	R
TH12	S	R	S	S	S	R
P131	MR	R	S	R	R	R
01-12	R	R	S	R	R	R
CH131	S	R	S	R	MR	R
03-32	R	R	S	R	R	R
03-5	R	R	S	R	R	R
99-188	R	MR	S	R	R	R
05-5-1	R	R	S	R	R	R
CN43	R	R	S	R	MS	R
01-15	R	R	R	R	R	R
99-30-1	MR	MR	S	R	R	R

2001-117	R	R	R	R	R	R
CN199	R	S	S	R	MS	R
CN184	R	R	S	R	R	R
06-3-1	R	R	S	R	S	R

R: Resistance; S: susceptibility; MR: middle resistance; MS: middle susceptibility

Supplementary Table 1: Resistance evaluation of different rice varieties to *M. oryzae* isolates

Isolates	ZH11	NIPB	*Pizh-1* (NIPB)	*Pizh-2* (NIPB)
GD00-1200	R	S	R	S
85-14	R	S	R	S
CN131	R	S	R	S
YJ1-2	R	S	R	S
H14	R	S	R	S
99-128	R	S	R	S

Supplementary Table 2: Resistance evaluation of the transgenic plants expressing *Pizh-1* and *Pizh-2* inoculated with *M. oryzae* isolates

Name	Forward sequence	Reverse sequence	Purpose
RM6836	TTGTTGTATACCTCATCGAC	AGGGTAAGACGTTTAACTTG	For fine mapping
RM3183	GCTCCACAGAAAAGCAAAGC	TGCAACAGTAGCTGTAGCCG	
RM19780	CATGGTGATCAGTGATGGAAACG	TCCAAGATTGGTGAACCTGAAGC	
RM7213	AACAACGAAGAGCAGGGAGAGC	TGTTGGAGCAACAGCAACTAATGG	
RM3330	AGCCAAGCAAGCAAAGCAAACG	GATTTGGGCGAGACGAGAACG	
RM19782	ACCGTGTGCCATGAGAATCTAGC	ATGGCCCTATACGTGTCAGTTGG	
RM19795	TAGTAGTTGGCATCTCCGGTTGC	CAAGCGGCCACTACGTATAGTACC	
RM19800	TACCGGGTGGAACCACAAATCC	CAGCGAAATCGCCTCTACATAAATGG	
RM19804	CAATGATGAAGCCGAGCCATCC	TTGAACTACACCCAATCGGACTCG	
RM19814	GGGTGAGGAAATGGGAGAGAGG	AAGCAACACACTGGAGAAGTGAGG	
RM19819	CAAGGGATACATTGGGTTGTCG	TCCTCACAAATGGGAACTTAGGC	
Indel1	CAAAAGATTCGTCTCGTAGTTTTCA	ACCAGTCAACGGGTTTGGATAA	
Indel2	AATGTAATCTAGGTCCAATTCAAAT	GCCAAAGGAGCAAATAGTGAGT	
Indel3	TTGCATTCTTGAAGTTGTGCCAAGA	CACCTAAGCAGGCTCCTCCATT	
Indel4	TAATTAATTTCCTGTTGTTGTTGTG	TCCGACCAATCACAATCCTCTACCAT	
Indel5	AAAACAGAGTCCTCGGCGTCTAAAC	TTAGAAAGATTATTGGTGTCCC	
Indel6	AATTCGAAATGATGACATGAAAGCT	GTAACTTCCCAATCTTCTATGTC	
OsActin	TGTATGCCAGTGGTCGTACCA	CCAGCAAGGTCGAGACGAA	qRT-PCR detection
Pizh-1	CAGATCCTGTTCCTGATGCC	CTTGAGCTGTGCCTATCTCTTC	
Pizh-2	GTTGACGACGAATGATAGCGAAGAG	ACGACGCTGATGGGGGAGGAGATCG	
R6	CCAAGCGCTACTCAACTGCC	TTTCCAGCCCCACACTGTC	
Pizh1-U6a-f	gccgCGACGAGACCAGCCTCCTGC	aaacGCAGGAGGCTGGTCTCGTCG	gene-editing construct
Pizh2-U3a-f	ggcaTGAAACTTAGAGAGCGCCAC	aaacGTGGCGCTCTCTAAGTTTCA	
2-pizt-u6a-f	gccgCTCCCCTACTGAGGACACTC	aaacGAGTGTCCTCAGTAGGGGAG	
2-pizt-u3-f	ggcaCTAGAAATAAACCCAAGCC	aaacGGCTTGGGTTTATTTCTAG	
35S::*Pizh-1*	CAATGTCGACATGGCGGAGACGGTGCTGAG	ATCAACTAGTTCAGCCAGCTTGAGCTGTG	Clone construct
35S::*Pizh-2*	CAATGTCGACATGGCGGAGACGGTGCTGAG	ATCAACTAGTTCAGCCAGCTTGAGCTGTG	
CLuc- *Pizh-1*	ATCAACTAGTATGGCGGAGACGGTGCTGAG	CCGTGTCGACTCCGCCAGCTTGAGCTGTG	
Pizh-1-NLuc	ATCAACTAGTATGGCGGAGACGGTGCTGAG	CCGTGTCGACTCCGCCAGCTTGAGCTGTG	
CLuc- *Pizh-2*	ATCAACTAGTATGGCGGAGACGGTGCTGAG	CCGTGTCGACTCCGCCAGCTTGAGCTGTG	
Pizh-2-NLuc	ATCAACTAGTATGGCGGAGACGGTGCTGAG	CCGTGTCGACTCCGCCAGCTTGAGCTGTG	
Pizh-1-CDS	CACCATGGCGGAGACGGTGCTGAG	TCAGCCAGCTTGAGCTGTG	Yeast two-hybrid

Pizh-2-CDS	CACCATGGCGGAGACGGTGCTGAG	TCAGCCAGCTTGAGCTGTG	
MgPot2	ACGACCCGTCTTTACTTATTTGG	AAGTAGCGTTGGTTTTGTTGGAT	Analysis of blast infection
OsUbq	GACGGACGCACCCTGGCTGACTAC	TGCCAATTACCATATACCACGAC	
M262	GTTCTCCACTTCACCTCCAT	TTGCTCTACCCAAACCTTTA	Molecular marker

Supplementary Table3: Primers used for mapping, plasmid construction and PCR detection