

**TALEN-based editing of *TFIIA5* changes rice response to *Xanthomonas oryzae* pv.
*Oryzae***

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Supplementary Information

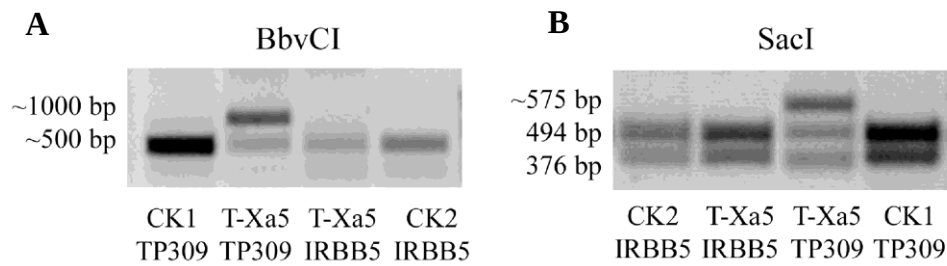


Figure S1. Gene editing of TALEN-Xa5 on *Xa5* and *xa5* in rice protoplasts through transiently expression.

The detection was performed using PCR/RE. The primers of Xa5F and Xa5R (shown in Supp. Table S1) were used to amplify the target region, and two restriction endonucleases, *BbvCI* (A) and *SacI* (B), were selected to detect the sequence changes in the target region of TALEN-Xa5. T-Xa5 indicates the cell was transformed with TALEN-Xa5 plasmid, while the cell of CK1 and CK2 use a GFP reporter plasmid as a positive control of transformation and a negative control of gene editing respectively. Rice TP309 possesses the homozygous *Xa5* gene; however IRBB5 contains the homozygous *xa5* gene. The extra bands in the PCR product digested by *BbvCI* (A) or *SacI* (B) indicated the sequence changes in target region of TALEN-Xa5. Gene editing only takes place in TP309 protoplasts transformed with TALEN-Xa5.

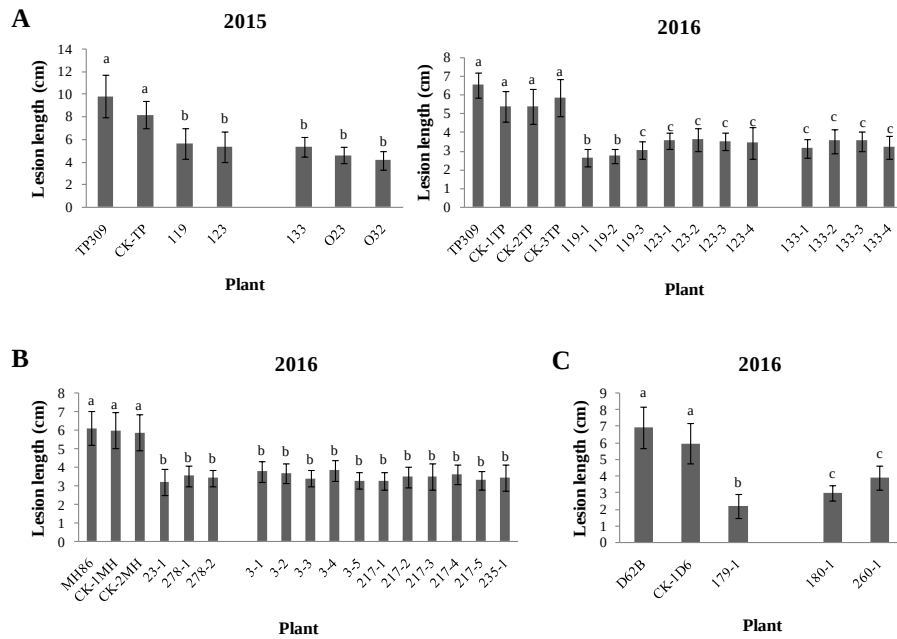


Figure S2. The lesion length of the knockout mutants and in-frame mutants derived from TP309 (A), MH86 (B) and D62B (C). The experiments were conducted in Beijing on August 2015 and in Hainan on February 2016. The investigation of lesion length is carried out at 14 days after inoculation with Xoo strain PXO86. Rice varieties TP309, MH86, and D62B possess the homozygous *Xa5* gene. CK-TP is a T₂ plant from TP309 with pCambia1300 plasmid; CK-1TP, CK-2TP, and CK-3TP are the T₃ plants of the CK-TP; 119 and 123 are knockout mutants of *Xa5* from TP309; 133, O23, and O32 are T₂ in-frame mutant plants, from TP309 and have the same mutation of the 32nd amino acid deletion in the *Xa5* protein; 119-1, 119-2 and 119-3 are T₃ plants from 119; Similarly, 123-1, 123-2, 123-3, 123-4 are T₃ plants from 123; 133-1, 133-2, 133-3, 133-4 are T₃ plants from 133. CK-1MH and CK-2MH are homozygous T₃ plants from MH86; 23-1, 278-1, and 278-2 are T₃ knockout mutants in MH86 background; 3-1, 3-2, 3-3, 3-4, 3-5 are homozygous T₃ plants in MH86 background, they are all have the same mutation with 133, O23, and O32; 217-1, 217-2, 217-3, 217-4, 217-5 are homozygous T₃ plants, they are lack 4 amino acids from the 29th to 32nd in *Xa5* protein; 235-1 are homozygous T₃ plant, in which the amino acid sequence of *Xa5* from the 32nd to 40th site is replaced by a different 5- amino acids sequence. CK-1D6 is a homozygous T₃ plant from D62B; 179-1 is a T₃ homozygous knockout plant in D62B background; 180-1 is a T₃ homozygous mutant in D62B background, which have three amino acids deletion from 32nd to 34th amino acid in the *Xa5* protein; 260-1 is a T₃ homozygous mutant in D62B background that has an amino acid insertion between the 32nd and the 33th amino acid in the *Xa5* protein. Bars represent the average $\pm$ SD of three biological repeats. Different letters above columns indicate significant differences at $P < 0.05$ as determined by a one-way ANOVA followed by post hoc Tukey honest significant difference (HSD) analysis.

Figure S3. Homology analysis between TFIIA γ 1 and TFIIA γ 5 (Xa5)

The comparison of *TFIIA γ 1* to *TFIIA γ 5* (*Xa5*) gene in amino acid sequence (A) and cDNA sequence (B).

A

Protein	MATFELYRRSTIGMCLDTLDDMVSSGALSPELAIQVLVQFDKSM TSALENQVKS KVSVK
	10 20 30 40 50 60
TFIIA γ 1	MATFELYRRSTIGMCLDTLDDMVSSGALSPELAIQVLVQFDKSM TSALEHQVKS KVTVK
Xa5	MATFELYRRSTIGMCLTETLDEMVS SGTLSPELAIQVLVQFDKSM TEALENQVKS KVS IK
Protein	GHLHTYR FCDNVWTFILTD AIFKNEEITETV GKVKIVACDSKLLSTKEE-
	70 80 90 100 11
TFIIA γ 1	GHLHTYR FCDNVWTFILTD AIFKNEEITETINKVKIVACDSKLLSTKEE.
Xa5	GHLHTYR FCDNVWTFILTEASFKNEETTEQVGKVKIVACDSKLLSQ.

B

cDNA	ATGGCCACCTTCGAGCTGTACCGGAGGTCCACCATTGGGTATGTGCCTCACTGAGACGCTG
	10 20 30 40 50 60
TFIIA γ 1	ATGGCCACCTTCGAGCTGTACCGGAGGTCCACCATTGGGTATGTGCCTCACTGAGACGCTG
Xa5	ATGGCCACCTTCGAGCTCTACCGGAGGTCCACCATTGGCATGTGCCTCACTGAGACGCTC
cDNA	GACGAGATGGTCTCCAGTGGGGCGCTCAGCCCGAGCTCGCCATTCAAGTCTTGTGCAG
	70 80 90 100 110 120
TFIIA γ 1	GACGACATGGTCTCCAGTGGGGCGCTCAGCCCCGAGCTCGCCATCCAAGTCTCTGTGCAG
Xa5	GACGAGATGGTCTCCAGCGGCACCCCTCAGCCCGAGCTCGCCATTCAAGTCTTGTCCAG
cDNA	TTTGATAAGTCTATGACTGGCGCTTTGGAGCATCAGGTGAAGAGCAAGGTTTCTGTCAAG
	130 140 150 160 170 180
TFIIA γ 1	TTTGACAAGTCCATGACTAGCGCTTTGGAGCATCAGGTGAAGAGCAAGGTTACTGTCAAG
Xa5	TTTGATAAGTCTATGACGGAAGCCTTGGAGAACCAAGTCAAGAGCAAGGTTTCTATCAAG
cDNA	GGCCATCTGCACACTTACAGGTTCTGTGACAATGTGTGGACTTTCATCTTGACTGATGCA
	190 200 210 220 230 240
TFIIA γ 1	GGCCATCTGCACACCTACAGGTTCTGCGACAATGTGTGGACTTTCATCCTAACAGATGCA
Xa5	GGCCACCTGCACACTTACAGGTTCTGTGACAATGTATGGACATTCATCTTGACTGAAGCA
cDNA	TTTTTCAAGAACGAGGAGATTACAGAGCCAGTTGGCAAGGTGAAGATTGTGGCCTGTGAT
	250 260 270 280 290 300
TFIIA γ 1	ATTTTCAAGAACGAAGAGATTACAGAGACAATAACAAGGTGAAGATCGTGGCCTGCGAT
Xa5	TCATTCAAGAACGAGGAGACTACAGAACAAGTTGGCAAGGTGAAGATTGTGGCCTGTGAT
cDNA	TCCAAATTGCTGGGGCCTTAAXXXXXXXXXXX
	310 320 33
TFIIA γ 1	TCCAAATTGCTGGAGACTAAAGAAGAGTAA
Xa5	TCCAAACTACTCAGCCAATAA

Table S1. Primer sequences.

Oligonucleotide	Sequence (5'-3')
Xa5F	AGAGAAATCTGGCGTCTCGTC
Xa5R	TACGTGTCGGACGTGAATGG
TF II A-gamma-1F	GAGGGAGGGATGCGATACTTG
TF II A-gamma-1R	AGCCTATGAAGCAAGCAGGAA
Hpt-F	CGCCGATGGTTTCTACAA
Hpt-R	GGCGTCGGTTTCCACTAT
Fok-F	CTACAGGGGAAAGCACCTGG
Fok-R	ATAGGCAGATTGTAGCCGCC

Table S2. The mutation types of the gene *TFIIA γ 1*.

<i>TFIIAγ1</i>	<u>TGGTCTCCAGTGGGGCGCTCAGCCCCGAGCTCGCCATCCAAGTCCTCGTGCA</u>	
148T0-1	TGGTCTCCAGTGGGGCGCTCAGCCC ———TCGCCATCCAAGTCCTCGTGCA	-5
148T0-2	TGGTCTCCAGTGGGGCGCTCAGCCC ———GCTCGCCATCCAAGTCCTCGTGCA	-3
148T0-3	TGGTCTCCAGTGGGGCGCTCAGCC ———CCTCGTGCA	-19
<i>TFIIAγ1</i>	<u>TGGTCTCCAGTGGGGCGCTCAGCCCCGAGCTCGCCATCCAAGTCCTCGTGCA</u>	
207T0-1	TGGTCTCCAGTGGGGCGCTCAGCCCC ———TCGCCATCCAAGTCCTCGTGCA	-4
207T0-2	TGGTCTCCAGTGGGGCGCTCAGCCCC ———CGCCATCCAAGTCCTCGTGCA	-5
207T0-3	TGGTCTCCAGTGGGGCGCTCAGCCCC ———CTCGCCATCCAAGTCCTCGTGCA	-3
<i>TFIIAγ1</i>	<u>TGGTCTCCAGTGGGGCGCTCAGCCCCGAGCTCGCCATCCAAGTCCTCGTGCA</u>	
221T0-1	TGGTCTCCAGTGGGGCGCTCAGCCCC ———TCGCCATCCAAGTCCTCGTGCA	-4
221T0-2	TGGTCTCCAGTGGGGCGCTCAGCCCC — GCTCGCCATCCAAGTCCTCGTGCA	-2
<i>TFIIAγ1</i>	<u>TGGTCTCCAGTGGGGCGCTCAGCCCCGAGCTCGCCATCCAAGTCCTCGTGCA</u>	
237T0-1	TGGTCTCCAGTGGGGCGCT ———CTCGCCATCCAAGTCCTCGTGCA	-10
237T0-2	TGGTCTCCAGTGGGGCGCTCAGCCC ———GCCATCCAAGTCCTCGTGCA	-7
237T0-3	TGGTCTCCAGTGGGGCGCTCAGCCC ———CTCGCCATCCAAGTCCTCGTGCA	-4
237T0-4	TGGTCTCCAGTGGGGCGCTCAGCCCC ———CTCGCCATCCAAGTCCTCGTGCA	-3