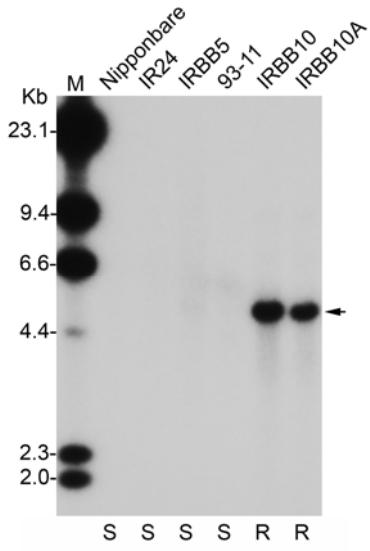
# The Rice TAL Effector-dependent Resistance Protein XA10 Triggers Cell Death and Calcium Depletion in the Endoplasmic Reticulum

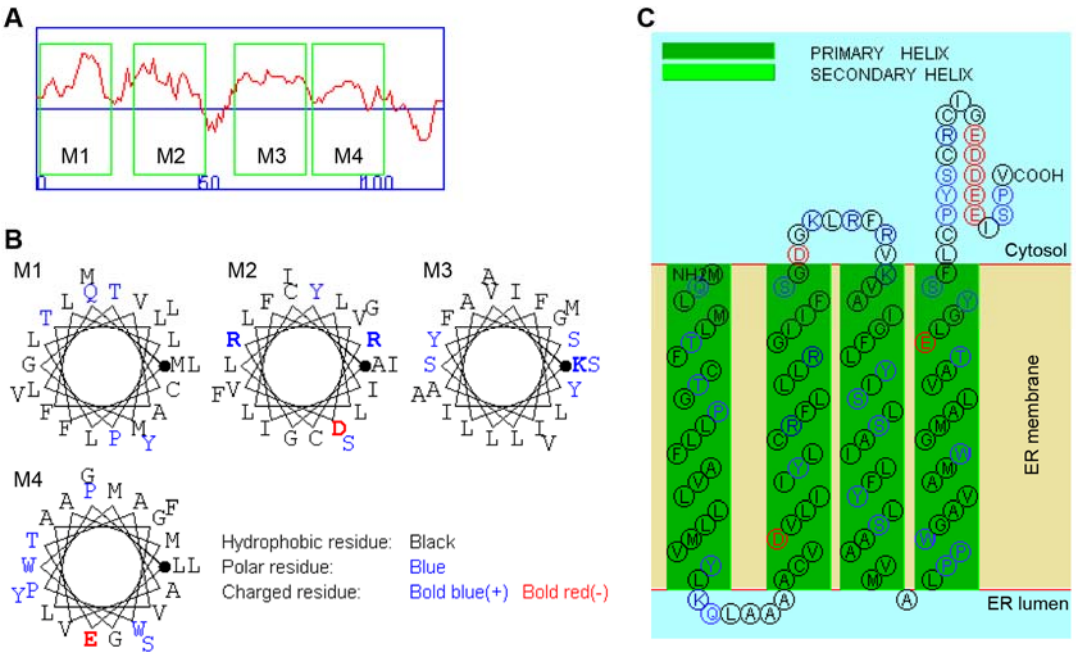
**Dongsheng Tian, Junxia Wang, Xuan Zeng, Keyu Gu, Chengxiang Qiu, Xiaobei Yang, Zhiyun Zhou, Meiling Goh, Yanchang Luo, Maki Murata-Hori, Frank F. White and Zhongchao Yin**

## SUPPLEMENTAL FIGURES




**Supplemental Figure 1.** Detection of the *Xa10* gene in different rice cultivars by DNA gel blot analysis.

Rice genomic DNA was digested with restriction enzyme HindIII. The DNA gelblot was hybridized using a DNA probe amplified from *Xa10* coding region. Arrow indicates the hybridized bands containing *Xa10* gene with the expected DNA size at 5164 bp. The disease phenotype of rice lines at 14 days after inoculation with *X. oryzae* pv. *oryzae* strain PXO99$^{A}$(*avrXa10*) are labeled below. M, DNA marker of λ DNA digested with HindIII; R, resistant; S, susceptible.



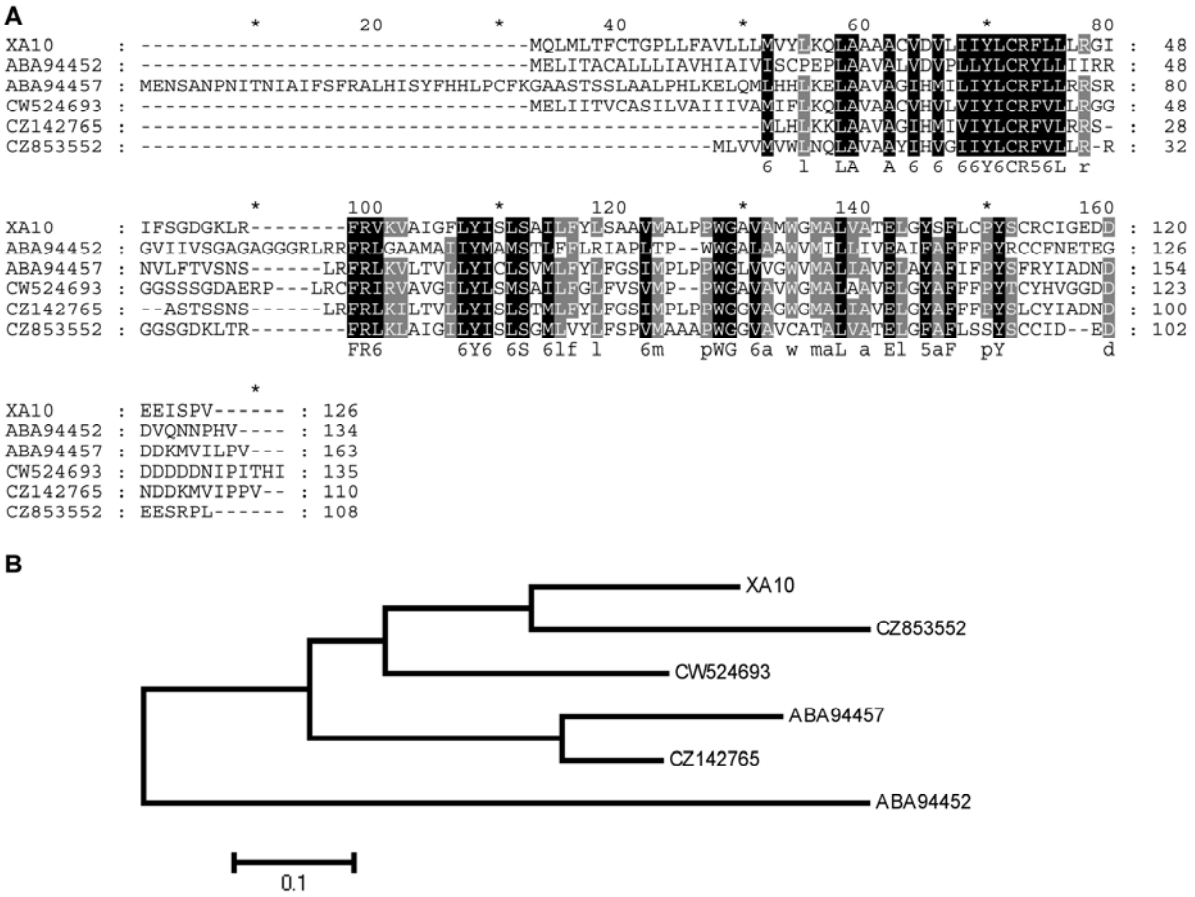

**Supplemental Figure 2.** Transmembrane helix prediction of XA10.

**(A)** Hydropathy profile of XA10. Four transmembrane helices (M1 to M4) were predicted.

(**B**) Helical wheel diagram of predicted transmembrane helices of XA10

**(C)** Topography of XA10 on the ER membrane

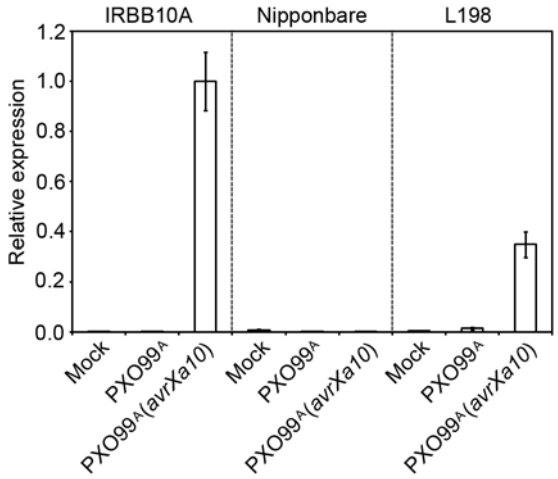
Transmembrane helices were predicted by the SOSUI program (http://bp.nuap.nagoya-u.ac.jp/sosui/sosui_submit.html). The topography of XA10 on the ER membrane was added to the SOSUI output based on the results obtained in this study.




**Supplemental Figure 3.** XA10 and related proteins.

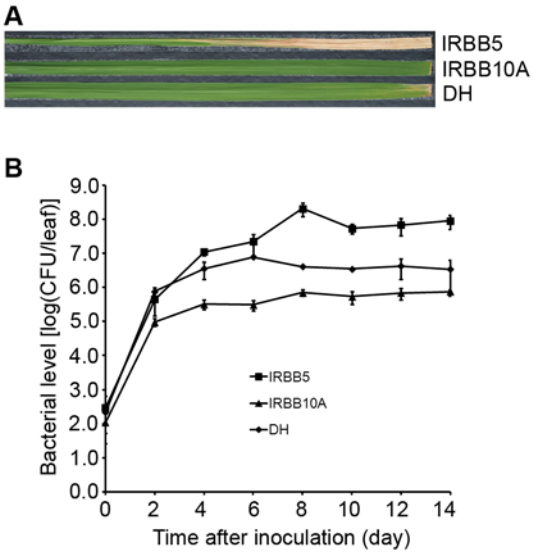
**(A)** Alignment of amino acid sequences of XA10 and putative paralogs/orthologs in cultivar Nipponbare (ABA94452 and ABA94457) and wild rice species *Oryza alta* (CZ142765), *Oryza coarctata* (CZ853552) and *Oryza punctata* (CW524693). The amino acid sequences were aligned using the program Clustal X. The output of the alignment was shaded with GENEDOC software. The identical and conserved amino acids among 6 or 5 proteins are highlighted in black or grey backgrounds, respectively.

**(B)** Phylogenetic tree of XA10 and putative paralogs/orthologs. Un-rooted phylogenetic tree was constructed after neighbor-joining distance analysis using MEGA4.0. The scale bar represents the expected number of changes per amino acid position.




**Supplemental Figure 4.** The expression of *Xa10* in transgenic line L198 after inoculation with *X. oryzae* pv. *oryzae* strains.

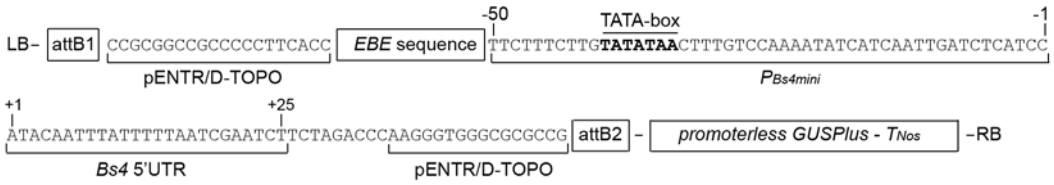
Three-week old rice seedlings were inoculated with water (Mock), $PXO99^{A}$ or $PXO99^{A}$(*avrXa10*). Total RNA was isolated from rice leaves at 24 HAI. *Xa10* transcripts were detected by qRT-PCR. The expression of the rice ubiquitin gene 1 (*Ubi1*) served as the internal control. The average expression level of *Xa10* in IRBB10A at 24 HAI with $PXO99^{A}$(*avrXa10*) was set as "1". The experiments were performed in triplicate and the data are presented as means ± SD.




**Supplemental Figure 5.** The *xa5* allele partially suppresses *Xa10*-mediated resistance to bacterial blight.

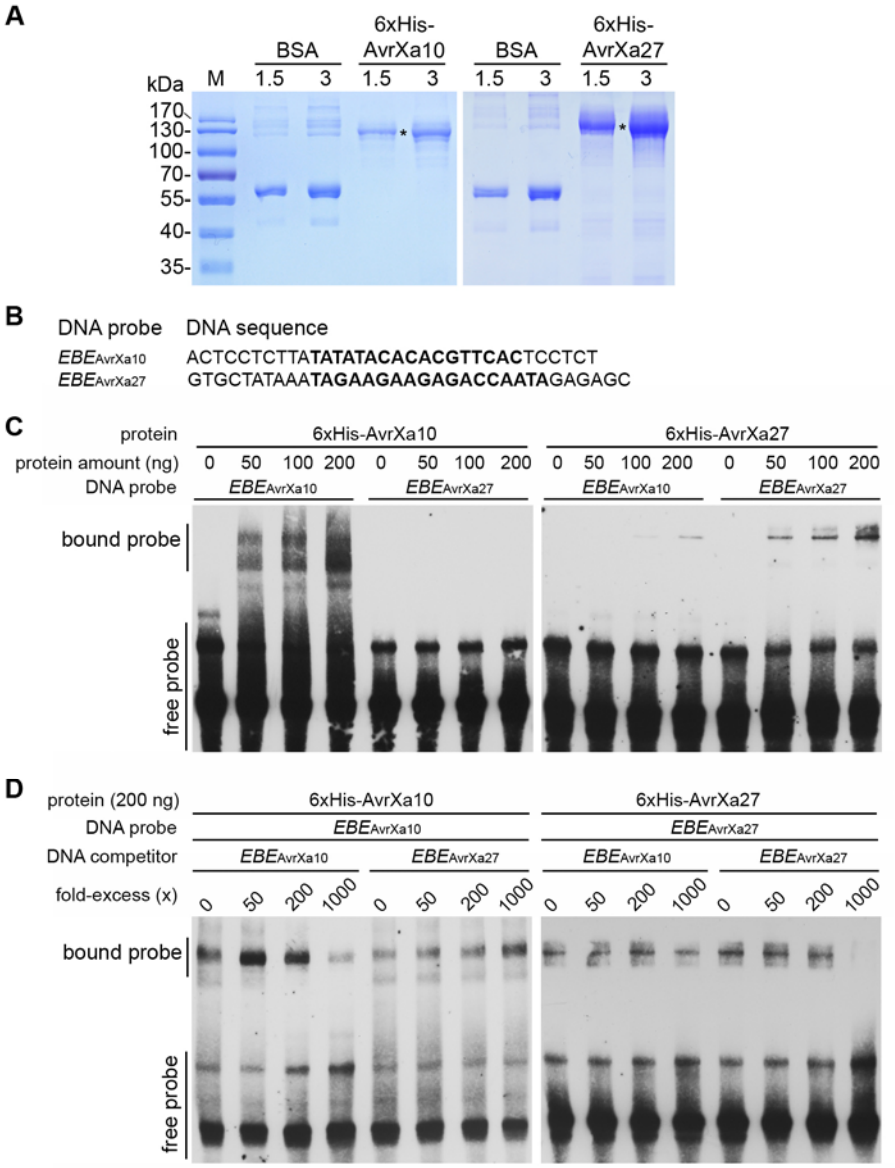
**(A)** Disease phenotype of rice lines IRBB5 (*xa5xa5*), IRBB10A (*Xa10Xa10*), and *xa5* and *Xa10* double homozygous plants (*xa5xa5, Xa10Xa10*) (DH) at 14 days after inoculation with PXO99$^{A}$(*avrXa10*).

**(B)** Bacterial population in leaves of IRBB5, IRBB10A and DH plants over 14 days by leaf-clipped inoculation with PXO99$^{A}$(*avrXa10*).




**Supplemental Figure 6.** GUS reporter constructs.

GUS reporter constructs carrying effector binding elements (*EBE*s) were prepared based on previous report (Boch et al., 2009) with slight modification, in which the *uidA* (β-glucuronidase, GUS) gene was replaced with intron-containing *GUSPlus* gene (see Supplemental Materials and Methods). The *EBE*s were inserted to the 5' of the minimal tomato *Bs4* promoter ($P_{Bs4mini}$; -50 to +25) and transferred by GATEWAY recombination into the *A. tumefaciens* T-DNA vector pCGWGUSint constructing a fusion to an intron-containing *GUSPlus* gene. attB1 and attB2, GATEWAY recombination sites; *promoterless GUSPlus-*$T_{Nos}$, coding sequence of *GUSPlus* and terminator of nopaline synthase (*Nos*) gene from pC1305.1; LB, left border; RB, right border.




**Supplemental Figure 7.** AvrXa10 binds specifically to *EBE*$_{AvrXa10}$

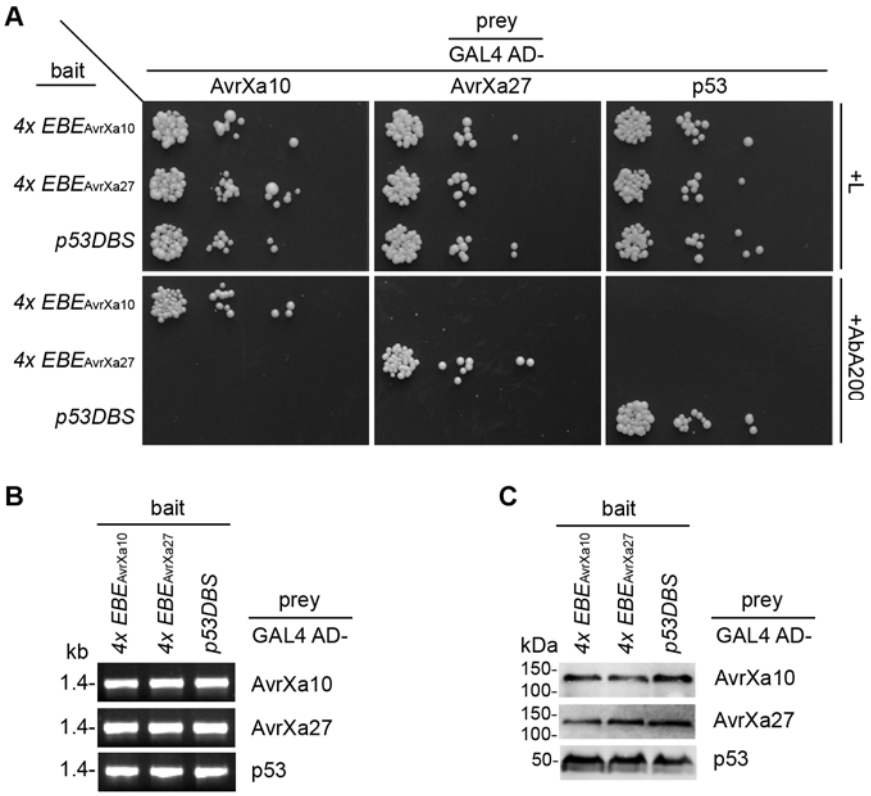
**(A)** Purified 6xHis-AvrXa10 and 6xHis-AvrXa27 proteins used in the electrophoretic mobility shift assay (EMSA). Protein concentrations were determined by Bradford assay. 1.5 and 3 μg purified 6xHis-AvrXa10 (117.5 kDa), 6xHis-AvrXa27 (121.0 kDa) and BSA were separated in an 8% SDS polyacrylamide gel and stained with Coomassie brilliant blue. 6xHis-AvrXa10 and 6xHis-AvrXa27 are marked with asterisks.

**(B)** DNA probes derived from *Xa10* and *Xa27* promoters used in EMSAs. *EBE*$_{AvrXa10}$ and *EBE*$_{AvrXa27}$ are shown in bold letters.

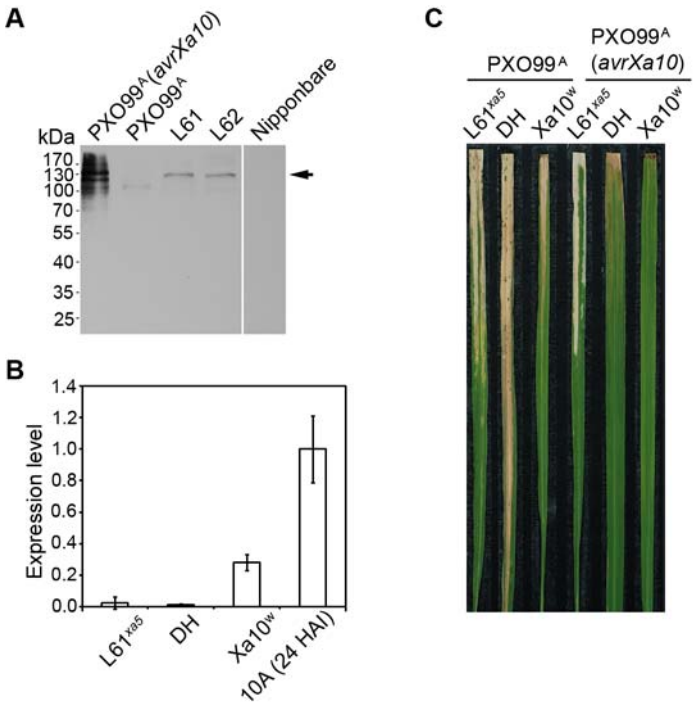
**(C)** AvrXa10 and AvrXa27 bind specifically to the *EBE*$_{AvrXa10}$ and *EBE*$_{AvrXa27}$ probes, respectively.

**(D)** Competition binding assays between biotin-labeled *EBE*$_{AvrXa10}$ probe and different amounts (in fmol) of non-labeled *EBE*$_{AvrXa10}$ or *EBE*$_{AvrXa27}$ competitors to AvrXa10 (left

panel), and between biotin-labeled $EBE_{AvrXa27}$ probe and different amounts (in fmol) of non-labeled $EBE_{AvrXa10}$ or $EBE_{AvrXa27}$ competitors to AvrXa27 (right panel).
The free and bound probes in EMSAs in **C** and **D** were separated in a 6% nondenaturing polyacrylamide gel. Positions of the bound and free probes are indicated on the left. Protein amounts are in nanograms (ng). Each EMSA experiment was repeated three times with similar results.




**Supplemental Figure 8.** The specific interaction between $EBE_{AvrXa10}$ and AvrXa10 in yeast.
**(A)** Yeast growth on selective medium in yeast one-hybrid assay. Four tandem copies of *EBE*s ($4\times EBE_{AvrXa10}$ and $4\times EBE_{AvrXa27}$) in sense were used as baits. TAL effectors (AvrXa10 and AvrXa27) fused to SV40 NLS-GAL4 AD were used as preys. The GAL4-AD fusion of murine p53 protein and a bait containing its target sequence (p53DBS) served as controls. 50 µl of serial dilutions of single transformants in SD liquid medium ($10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$) were dropped on SD medium containing either leucine (+L) or 200 ng/ml aureobasidin A (+AbA200). Two transformants per experiment were analyzed. The experiment was repeated twice with similar results.
**(B)** Verification of DNA baits in yeast genome. The inserts of baits ($EBE_{AvrXa10}$, 1418 bp; $EBE_{AvrXa27}$, 1422 bp; *p53DBS*, 1400 bp) were amplified by yeast colony PCR.
**(C)** Verification of prey proteins in yeast. SV40 NLS-GAL4 AD-HA fused to AvrXa10 (135 kDa), AvrXa27 (141 kDa) and p53 (61 kDa) were detected by immunoblot analysis using an anti-GAL4 AD antibody.

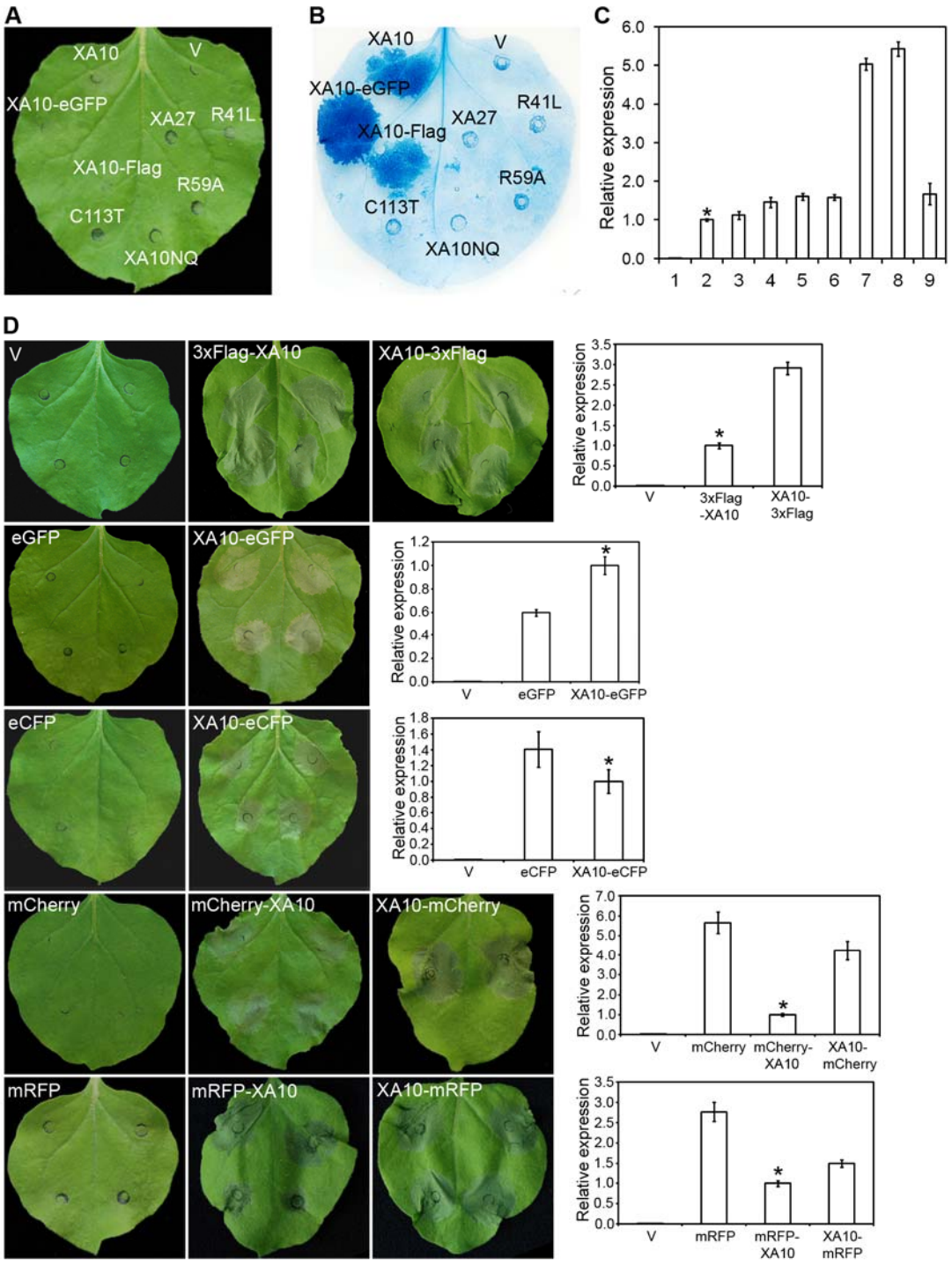



**Supplemental Figure 9**. Generation of *Xa10* weak expression plants (Xa10$^{w}$).

**(A)** Detection of AvrXa10 in $P_{PR1}$*:avrXa10:*$T_{Nos}$ lines by immunoblot analysis. AvrXa10 was detected with anti-AvrXa10 polyclonal antibodies. Arrow indicates the position of AvrXa10. L61, line 61 of $P_{PR1}$*:avrXa10:*$T_{Nos}$; L62, line 62 of $P_{PR1}$*:avrXa10:*$T_{Nos}$.

**(B)** Expression of *Xa10* in rice plants. *Xa10* transcripts in rice plants were detected by qRT-PCR. The expression of rice ubiquitin gene 1 (*Ubi1*) served as control. The average expression level of *Xa10* in IRBB10A at 24 HAI with PXO99$^{A}$(*avrXa10*) was set as "1". The experiments were performed in triplicate and the data are presented as means ± SD. L61$^{xa5}$, L61 in homozygous *xa5* genetic background (genotype: *xa5xa5*, $P_{PR1}$*:avrXa10:*$T_{Nos}$); DH, *xa5* and *Xa10* double homozygous plants (genotype: *xa5xa5, Xa10Xa10*); Xa10$^{w}$, $F_1$ plants of DH x L61$^{xa5}$ (genotype: *xa5xa5, Xa10xa10,* $P_{PR1}$*:avrXa10:*$T_{Nos}$); 10A at 24 HAI, IRBB10A inoculated with PXO99$^{A}$(*avrXa10*) at 24 HAI. HAI, hours after inoculation.

**(C)** Disease phenotypes of Xa10$^{w}$ and parental lines at 14 days after inoculation with PXO99$^{A}$ or PXO99$^{A}$(*avrXa10*).



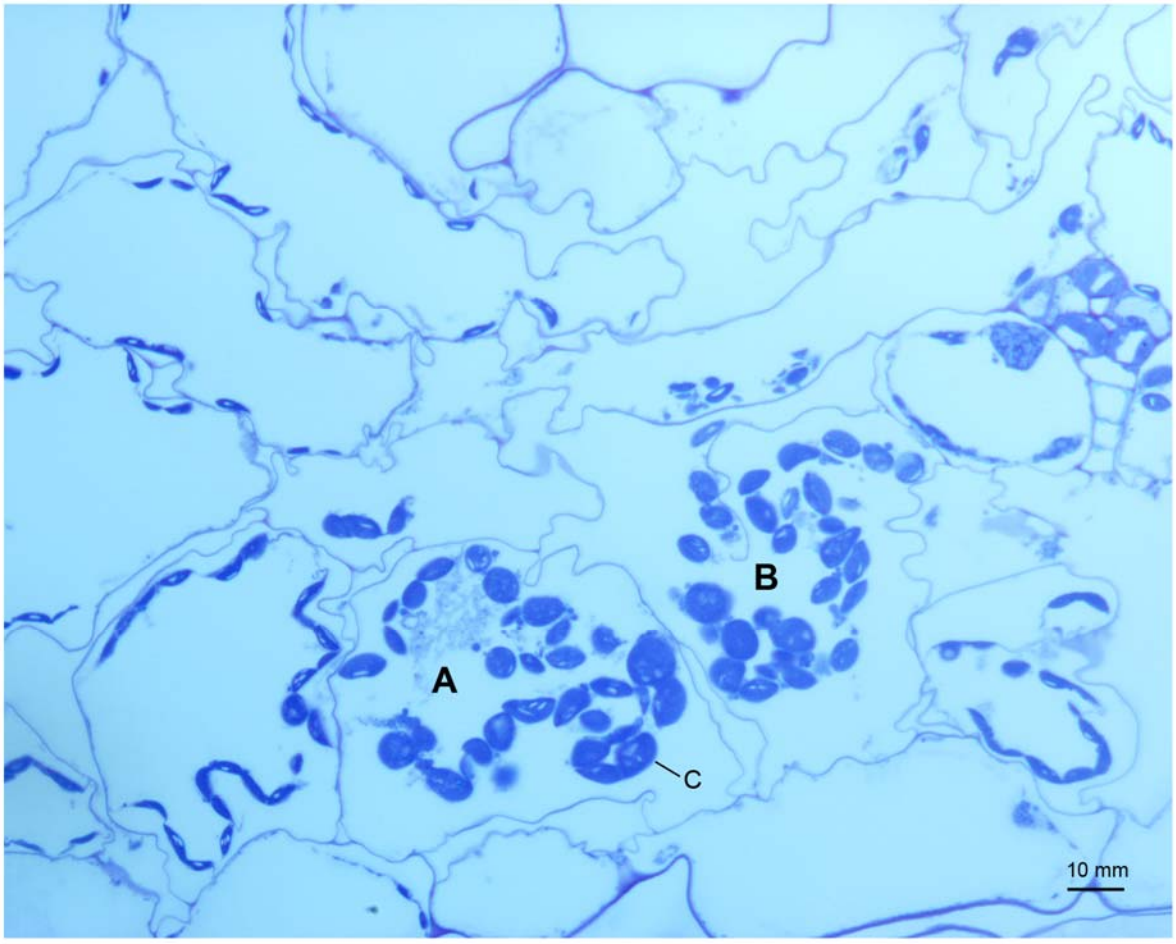

**Supplemental Figure 10**. Phenotype of *N. benthamiana* leaves transiently expressing XA10 or derivatives.

**(A)** Phenotype of *N. benthamiana* leaf at 24 HAI with *A. tumefaciens* harboring *Xa10* or derivatives. XA10, $P_{PR1}$*:Xa10:*$T_{Nos}$; XA10-Flag, $P_{PR1}$*:Xa10-Flag:*$T_{Nos}$; XA10-eGFP, $P_{PR1}$*:Xa10-eGFP:*$T_{Nos}$; R41L, $P_{PR1}$*:Xa10R41L:*$T_{Nos}$; R59A, $P_{PR1}$*:Xa10R59A:*$T_{Nos}$; C113T, $P_{PR1}$*:Xa10C113T:*$T_{Nos}$; XA10NQ, $P_{PR1}$*:Xa10NQ:*$T_{Nos}$; XA27, $P_{PR1}$*:Xa27:*$T_{Nos}$; V, pC1300.

**(B)** Trypan blue staining of leaf tissue of *N. benthamiana* in **(A)**.

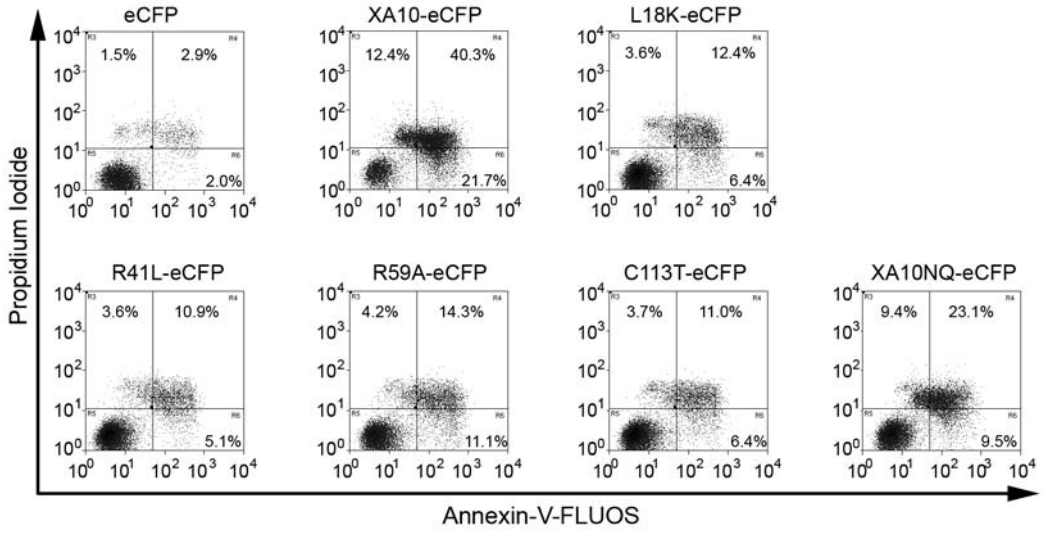
**(C)** Relative expression of transgenes in leaf tissues of *N. benthamiana* at 20 HAI. Gene transcripts were detected by qRT-PCR. The average expression of $P_{PR1}$*:Xa10:*$T_{Nos}$ (sample 2) was set as "1". 1, pC1300; 2, $P_{PR1}$*:Xa10:*$T_{Nos}$; 3, $P_{PR1}$*:Xa10R41L:*$T_{Nos}$; 4, $P_{PR1}$*:Xa10R59A:*$T_{Nos}$; 5, $P_{PR1}$*:Xa10C113T:*$T_{Nos}$; 6, $P_{PR1}$*:Xa10NQ:*$T_{Nos}$; 7, $P_{PR1}$*:Xa10-Flag:*$T_{Nos}$; 8, $P_{PR1}$*:Xa10-eGFP:*$T_{Nos}$; 9, $P_{PR1}$*:Xa27:*$T_{Nos}$.

**(D)** Phenotype of *N. benthamiana* leaf at 48 HAI with *A. tumefaciens* harboring *Xa10* fusion gene constructs and relative expression of transgenes at 20 HAI. Gene transcripts were detected by qRT-PCR. Asterisks indicate genes whose average expression levels were set as "1". V, pC1300; 3xFlag-XA10, $P_{35S}$*:3xFlag-XA10:*$T_{Nos}$; XA10-3xFlag, $P_{35S}$*:XA10-3xFlag:*$T_{Nos}$; eGFP, $P_{35S}$*:eGFP:*$T_{Nos}$; XA10-eGFP, $P_{35S}$*:XA10-eGFP:*$T_{Nos}$; eCFP, $P_{35S}$*:eCFP:*$T_{Nos}$; XA10-eCFP, $P_{35S}$*:XA10-eCFP:*$T_{Nos}$; mCherry, $P_{35S}$*:mCherry:*$T_{Nos}$; mCherry-XA10, $P_{35S}$*:mCherry-XA10:*$T_{Nos}$; XA10-mCherry, $P_{35S}$*:XA10-mCherry:*$T_{Nos}$ ; mRFP, $P_{35S}$*:mRFP:*$T_{Nos}$; mRFP-XA10, $P_{35S}$*:mRFP-XA10:*$T_{Nos}$; XA10-mRFP, $P_{35S}$*:XA10-mRFP:*$T_{Nos}$. The qRT-PCR experiments were performed in triplicate and the data are presented as means ± SD



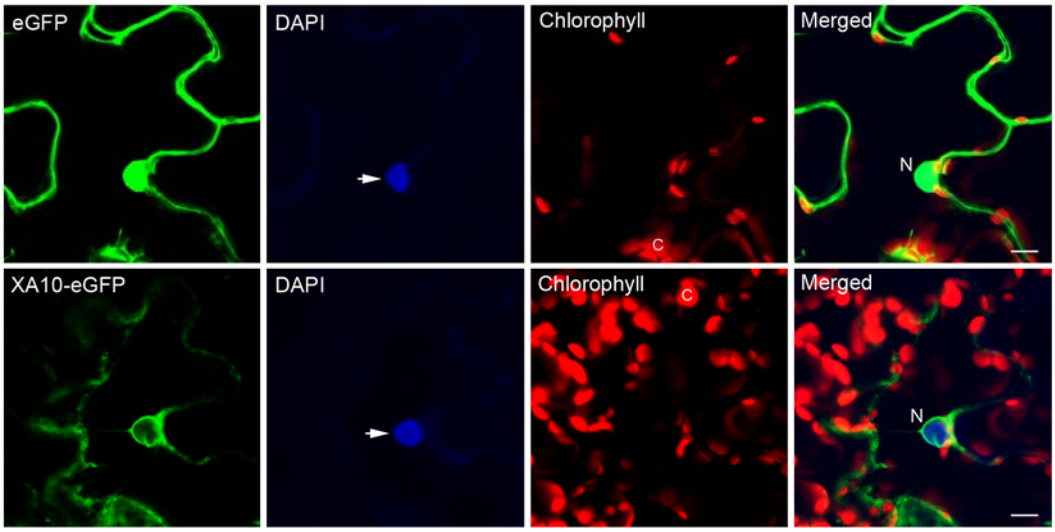

**Supplemental Figure 11.** Chloroplast swelling and cell death in leaf cells of *N. benthamiana* transiently expressing XA10.

Leaf sample was taken at 14 HAI with $P_{PR1}$*:Xa10:*$T_{Nos}$ gene. Two dying leaf cells (A and B) are marked. C, chloroplast.

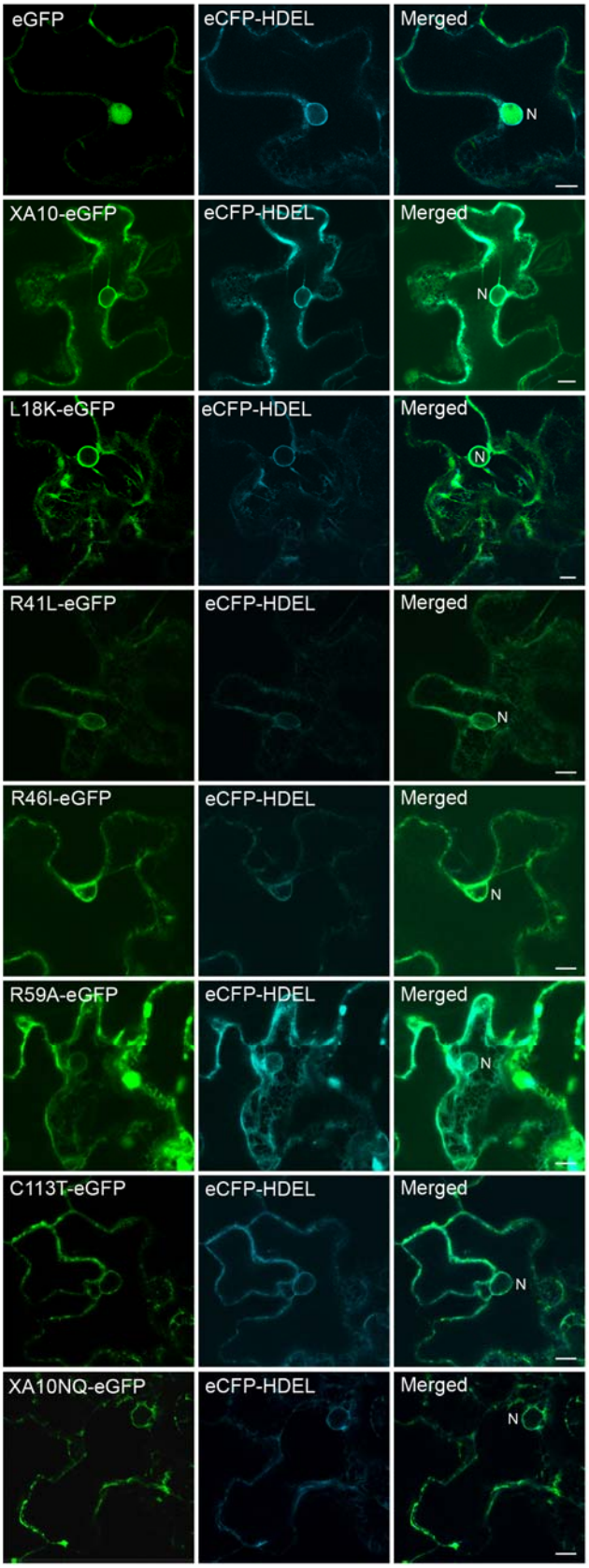



**Supplemental Figure 12**. Flow cytometry analysis of HeLa cells transiently expressing XA10 or variants.

Flow cytometry was conducted with HeLa cells transiently expressing eCFP, XA10-eCFP, XA10L18K-eCFP(L18K-eCFP), XA10R41L-eCFP(R41L-eCFP), XA10R59A-eCFP(R59A-eCFP), XA10C113T-eCFP(C113T-eCFP) and XA10NQ-eCFP, respectively, at 48 HAT.

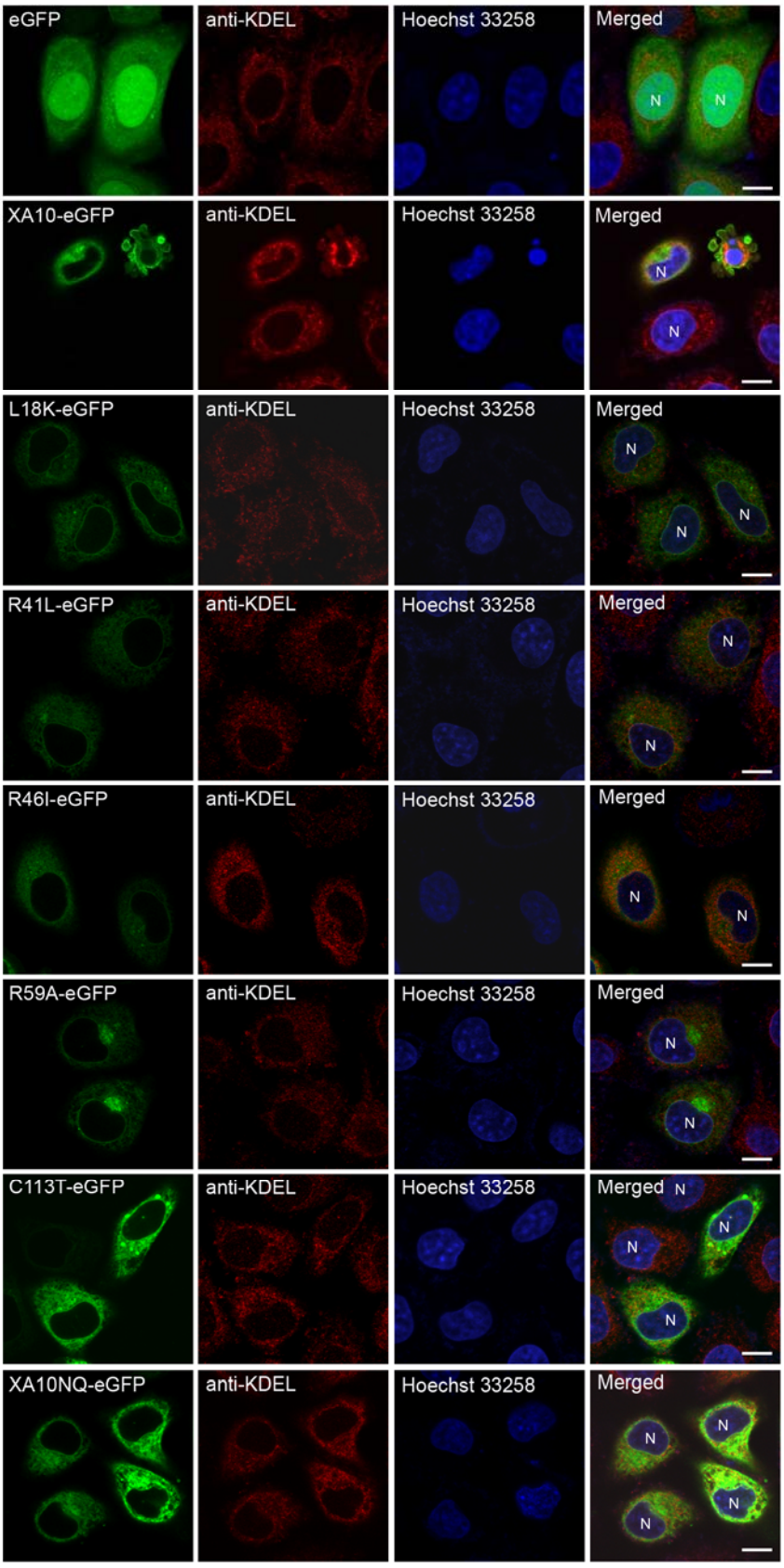



**Supplemental Figure 13**. Subcellular localization of XA10 in *N. benthamiana* leaf cells. XA10-eGFP and eGFP were transiently expressed in *N. benthamiana* leaf cells after infiltration with *A. tumefaciens*. The nuclei of the leaf cells (indicated with arrows) were stained with 4',6-diamidino-2-phenylindole (DAPI) (DAPI channel). Chloroplasts are indicated with the images of chlorophyll fluorescence (chlorophyll channel). Images were taken at 24 HAI. C, chloroplast; N, nucleus.



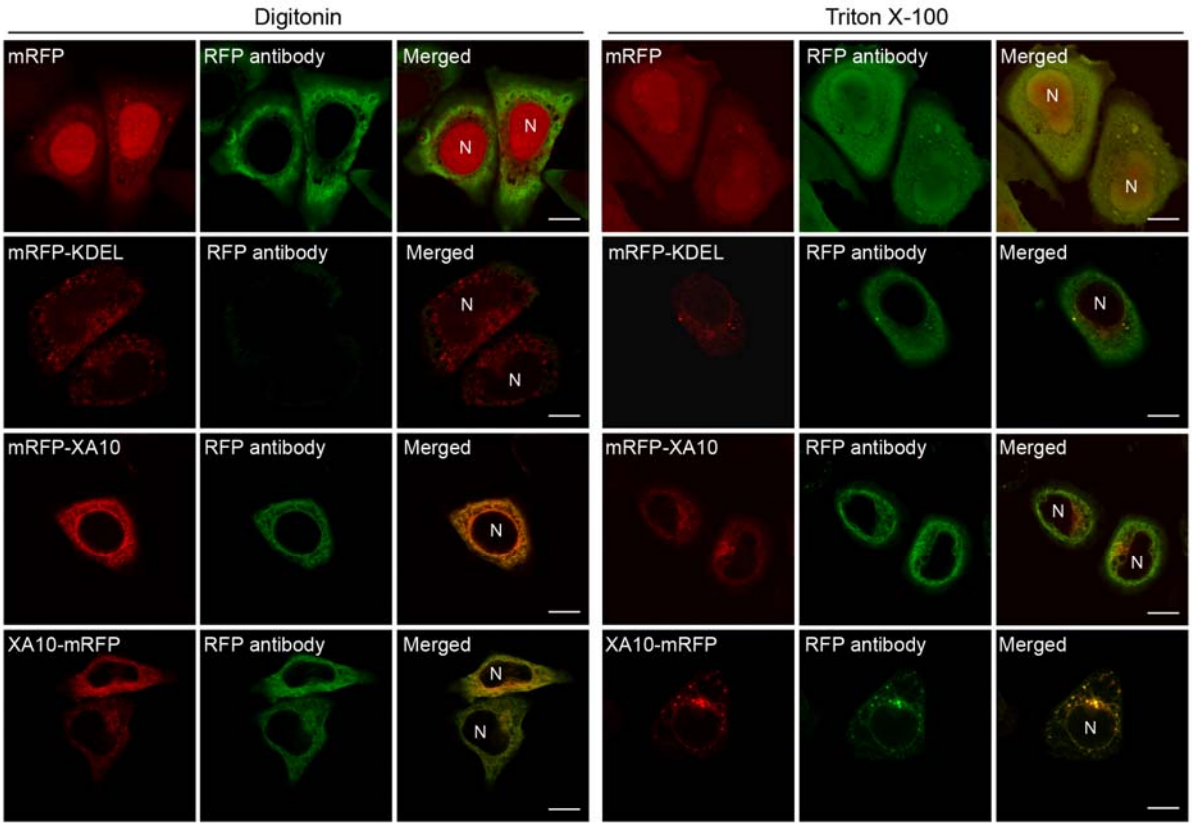

**Supplemental Figure 14.** Subcellular localization of XA10 variants in *N. benthamiana* leaf cells.

XA10 variants fused with eGFP at their C-termini were transiently co-expressed with luminal ER protein eCFP-HDEL in leaf cells of *N. benthamiana*. Images were taken at 24 HAI. N, nucleus. Bars = 10 μm. Abbreviations: L18K-eGFP, XA10L18K-eGFP; R41L-eGFP, XA10R41L-eGFP; R46I-eGFP, XA10R46I-eGFP; R59A-eGFP, XA10R59A-eGFP; C113T-eGFP, XA10C113T-eGFP.

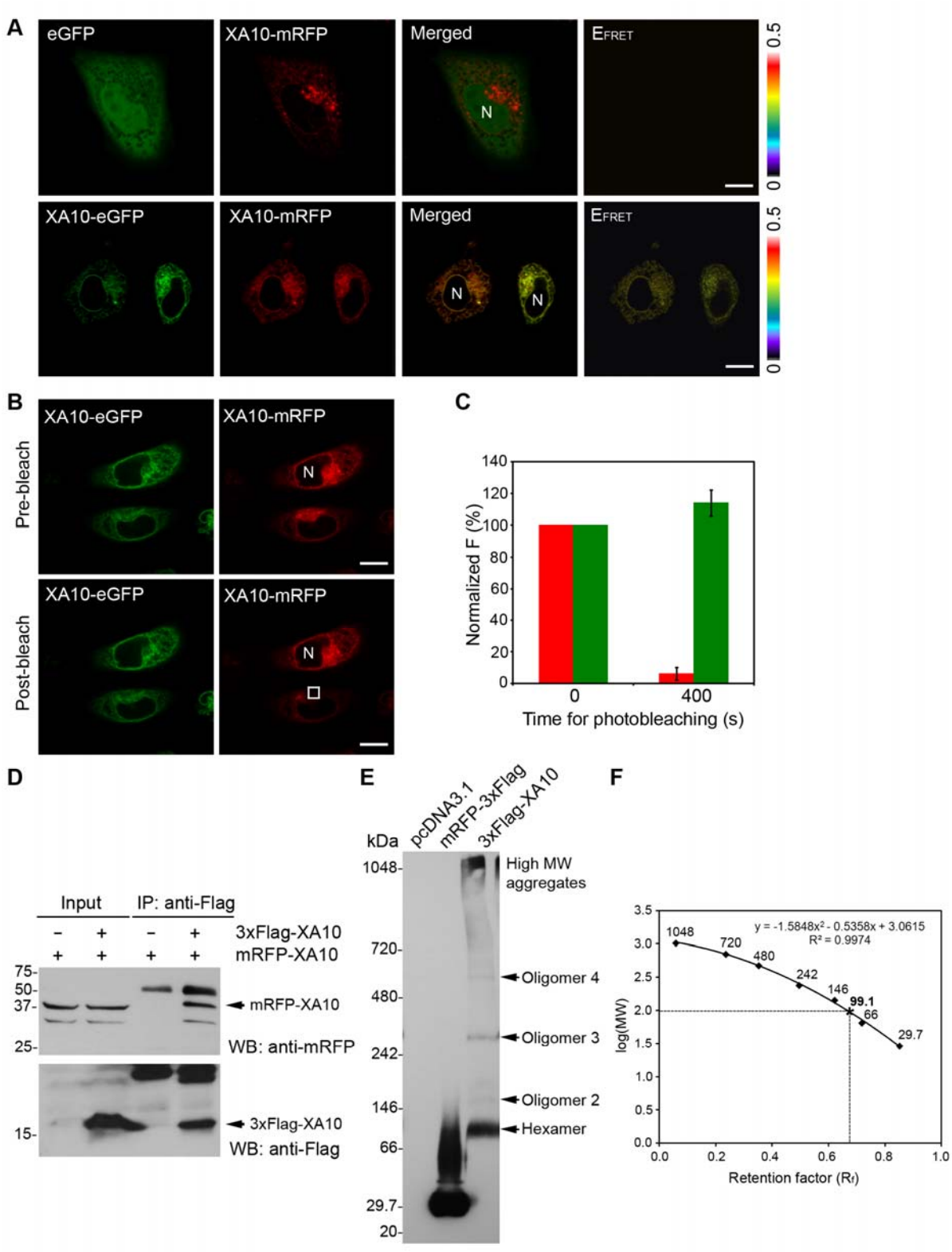



**Supplemental Figure 15.** Subcellular localization of XA10 variants in HeLa cells.

XA10 variants fused with eGFP at their C-termini were transiently expressed in HeLa cells. The ER was labeled with anti-KDEL antibodies. The nuclei of HeLa cells were stained with Hoechst 33258. Samples were prepared at 24 HAT. N, nucleus. Bars = 10 μm. Abbreviations: L18K-eGFP, XA10L18K-eGFP; R41L-eGFP, XA10R41L-eGFP; R46I-eGFP, XA10R46I-eGFP; R59A-eGFP, XA10R59A-eGFP; C113T-eGFP, XA10C113T-eGFP.




**Supplemental Figure 16.** Topography study of XA10 on the ER membrane in HeLa cells**.** HeLa cells transiently expressing mRFP, mRFP-KDEL, mRFP-XA10 and XA10-mRFP were fixed at 18 HAT and permeabilized using either digitonin (left panel) or Triton X-100 (right panel) before immunofluorescence labeling with anti-RFP monoclonal antibody. Bars = 10 μm. N, nucleus.




**Supplemental Figure 17.** XA10 forms oligomers on the ER membrane in HeLa cells.

**(A)** FRET analysis for interaction between XA10-eGFP and XA10-mRFP in HeLa cells. Pseudo-colored bar of FRET is indicated. Bars = 10 μm. N, nucleus.

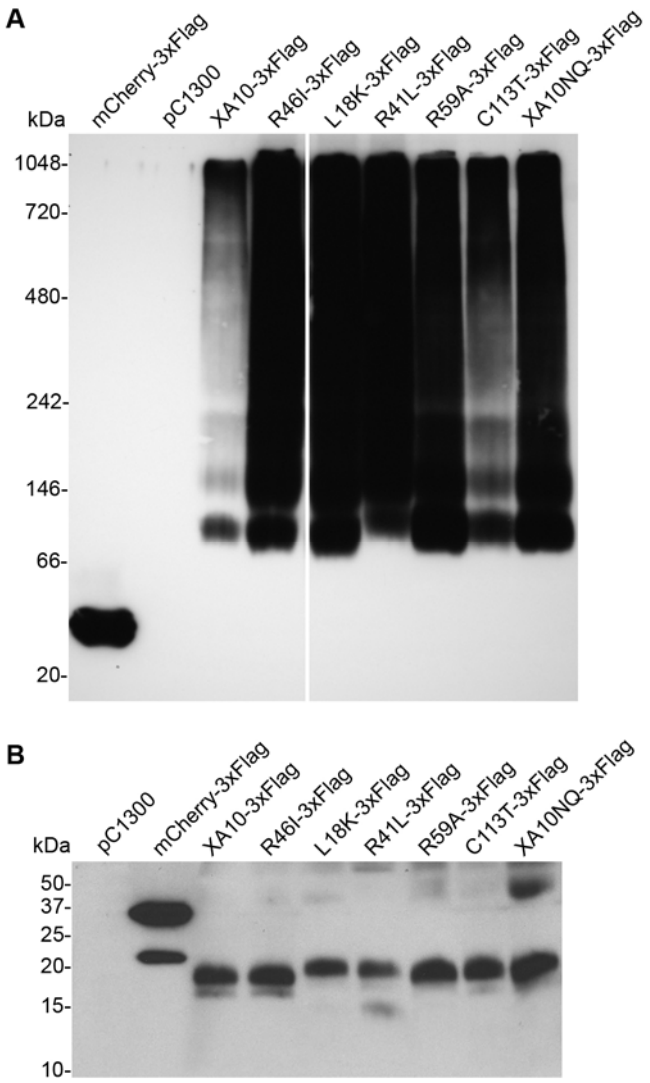
**(B)** Fluorescent images of HeLa cells co-expressing XA10-mRFP and XA10-eGFP before and after photobleaching of mRFP channel. White box indicates the photobleaching area. Bars = 10 μm. N, nucleus.

**(C)** Normalized fluorescence of eGFP (green) and mRFP (red) at the photobleaching area before and after photobleaching of mRFP channel. Number of cells analyzed = 8.

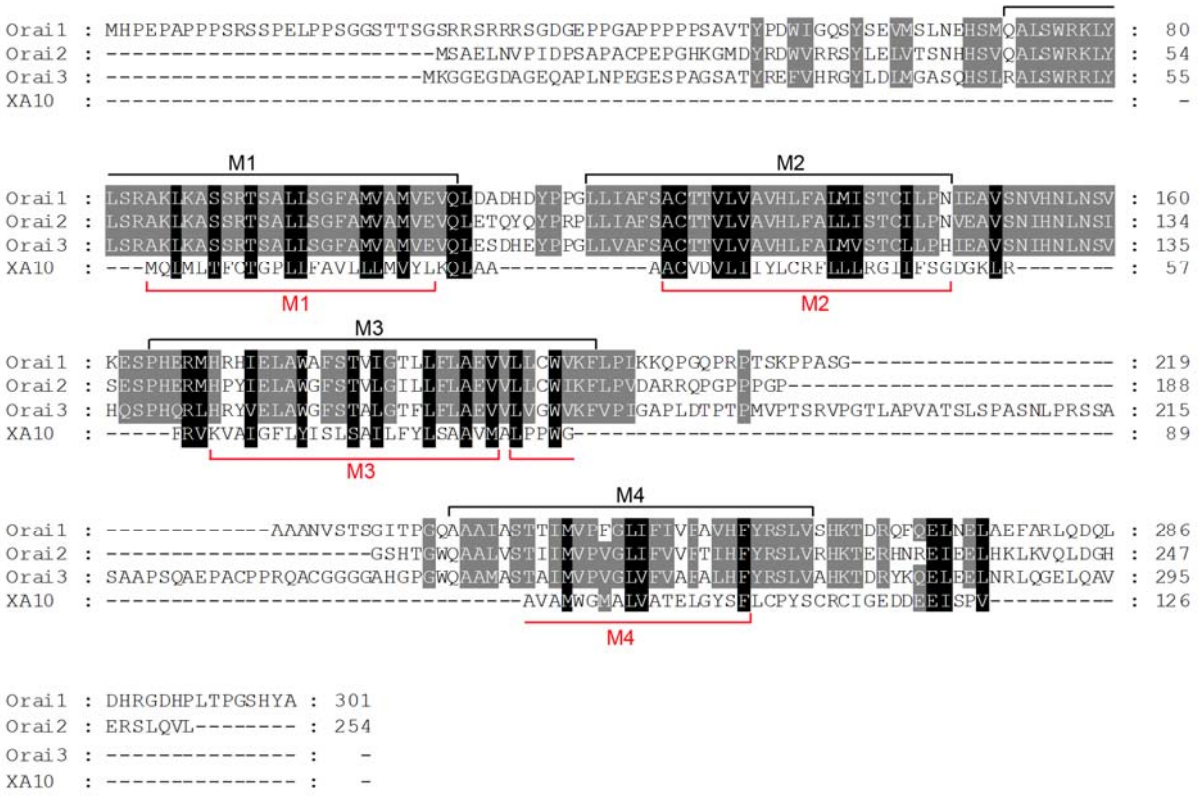
**(D)** Co-IP analysis between mRFP-XA10 and 3xFlag-XA10 co-expressed in HeLa cells. mRFP-XA10 was detected with anti-mRFP antibody, while 3xFlag-XA10 was probed with anti-Flag antibody.

**(E)** Detection of 3xFlag-XA10 oligomers using blue-native polyacrylamide gel-electrophoresis (BN-PAGE). 3xFlag-tagged fusion proteins were detected with anti-Flag antibody. 3xFlag-XA10 oligomers are indicated with arrows. pcDNA3.1, control empty vector. MW, molecular weight.

**(F)** Measurement of molecular size of 3xFlag-XA10 oligomers. The second-order polynomial best-fit was used to plot the Retention factor ($R_f$) values versus log molecular weight. The asterisk denotes the position of 3xFlag-XA10 hexamer on the curve. Experiments were repeated for at least 3 times. The data shown here are the results of one typical experiment.

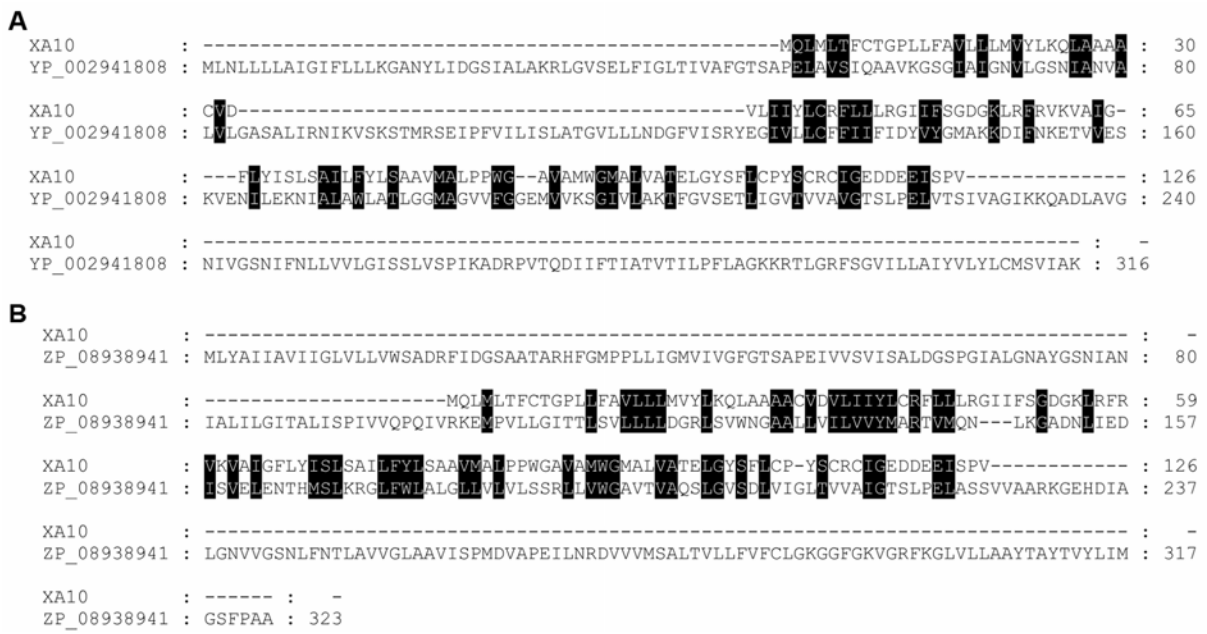



**Supplemental Figure 18.** Detection of XA10 variants expressed in rice protoplasts. Protein samples were isolated from rice protoplasts transiently expressing XA10 or variants and subjected to BN-PAGE (**A**) and SDS-PAGE (**B**) analyses, respectively. Rice protoplasts transformed with pC1300 or mCherry-3xFlag (~29.7kDa) were used as controls. 3xFlag-tagged proteins were detected by anti-Flag antibody. R46I-3xFlag, XA10R46I-3xFlag; L18K-3xFlag, XA10L18K-3xFlag; R41L-3xFlag, XA10R41L-3xFlag; R59A-3xFlag, XA10R59A-3xFlag; C113T-3xFlag, XA10C113T-3xFlag.




**Supplemental Figure 19.** Alignment of amino acid sequences of XA10 and Orai proteins from human being (*Homo sapiens*).

The amino acid sequences of Orai1, Orai2, Orai3 and XA10 were aligned using the program Clustal W (http://www.ebi.ac.uk/clustalw/index.html). The output of the alignment was shaded with GENEDOC software. The identical and conserved amino acids among 4 or 3 proteins are highlighted in black or grey backgrounds, respectively. The transmembrane helices of Orai proteins are marked as M1 to M4 in black color while the transmembrane helixes of XA10 are marked as M1 to M4 in red color.

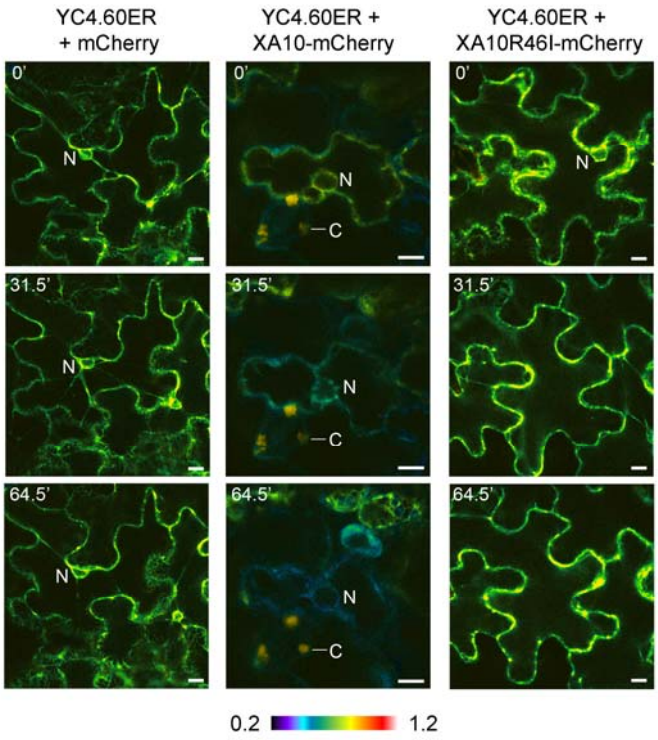



**Supplemental Figure 20.** XA10 and bacterial antiporter proteins.

**(A)** Alignment of amino acid sequences of XA10 and $Na^{+}/Ca^{2+}$ antiporter (YP_002941808) from *Kosmotoga olearid* TBF 19.5.1.

**(B)** Alignment of amino acid sequences of XA10 and CaCA family calcium ($Ca^{2+}$):cation antiporter (ZP_08938941) from *Neisseria wadsworthii* 9715.

The amino acid sequences were aligned using the program Clustal W (http://www.ebi.ac.uk/clustalw/index.html). The output of the alignment was shaded with GENEDOC software. The identical and conserved amino acids between 2 proteins are highlighted in black background.




**Supplemental Figure 21.** XA10 induces ER calcium depletion in *N. benthamiana* cells. Time-lapse images show frames from the beginning (0′), the middle (31.5′) and the end (64.5′) of the time series for constructing Supplemental Movie 1 (YC4.60ER + mCherry), Supplemental Movie 2 (YC4.60ER + XA10-mCherry) and Supplemental Movie 3 (YC4.60ER + XA10R46I-mCherry), respectively. Pseudo-color scale bar indicates the FRET:CFP ratio. N, nucleus; C, chloroplast. Bars = 10 μm.

## SUPPLEMENTAL TABLES

**Supplemental Table 1**. Number of the transgenic $T_0$ plants obtained from rice transformation with subclones at the *Xa10* locus[a].

| Subclone[b] | Total plants | Resistant plants | Susceptible plants |
|---|---|---|---|
| S18901 | 109 | 38 | 71 |
| A14799 | 88 | 50 | 38 |
| ES11947 | 35 | 22 | 13 |
| EA9295 | 65 | 45 | 20 |
| SA4671 | 106 | 7 | 99 |
| PX3834 | 35 | 3 | 32 |
| SX10761 | 70 | 0 | 70 |
| EN6879 | 95 | 0 | 95 |
| B6804 | 98 | 0 | 98 |

[a]Subclones at the *Xa10* locus were used for genetic complementation of susceptible cultivar Nipponbare using *Agrobacterium*-mediated rice transformation and bacterial blight inoculation. Six-week-old transgenic $T_0$ plants were evaluated for resistance to *X. oryzae* pv. *oryzae* strain PXO99$^A$(*avrXa10*) and disease phenotype was scored at two weeks after inoculation.

[b]S18901, 18901-bp SpeI subclone; A14799, 14799-bp AvrII subclone; ES11947, 11947-bp EcoRI-SpeI subclone; EA9295, 9295-bp EcoRV-AvrII subclone; SA4671, 4671-bp SacI-AvrII subclone; PX3834, 3834-bp PmlI-XbaI subclone; SX10761, 10761-bp SpeI-XhoI subclone; EN6879, 6879-bp EcoRV-NruI subclone; B6804, 6804-bp BamHI subclone.

**Supplemental Table 2**. Lesion length and disease phenotype of *Xa10* transgenic plants at two weeks after inoculation with PXO99$^{A}$(*avrXa10*) and PXO99$^{Aa}$.

| Plant | *Xa10* subclone | Lesion length (cm) and disease score | |
|---|---|---|---|
| | | PXO99$^{A}$(*avrXa10*) | PXO99$^{A}$ |
| IR24 | wild-type | 17.3±4.4 (S) | 22.0±3.9 (S) |
| IRBB10A | wild-type | 0.1±0.1 (R) | 26.0±3.6 (S) |
| Nipponbare | wild-type | 10.7±4.8 (S) | 10.9±3.1 (S) |
| L673 | A14799 | 0.1±0.0 (R) | 6.1±2.4 (MS) |
| L58 | ES11947 | 0.5±0.5 (R) | 11.1±2.9 (S) |
| L74 | EA9295 | 0.1±0.0 (R) | 6.6±2.7 (MS) |
| L142 | SA4671 | 0.1±0.0 (R) | 6.3±2.5 (MS) |
| L186 | SA4671 | 0.1±0.1 (R) | N.D. |
| L198 | SA4671 | 0.1±0.0 (R) | 7.8±2.9 (MS) |
| L211 | SA4671 | 0.1±0.1 (R) | 7.2±3.5 (MS) |
| L289 | SA4671 | 0.1±0.1 (R) | N.D. |
| L306 | SA4671 | 0.1±0.1 (R) | N.D. |
| L635 | SA4671 | 0.1±0.0 (R) | 7.1±2.8 (MS) |
| L203 | PX3834 | 0.1±0.0 (R) | 6.4±3.0 (MS) |
| L297 | PX3834 | 0.1±0.0 (R) | 7.9±2.6 (MS) |
| L313 | PX3834 | 0.1±0.1 (R) | 9.1±3.2 (S) |

[a]Six-week-old wild-type plants and $T_2$ plants of *Xa10* transgenic lines that contained single-copy *Xa10* transgene were inoculated with compatible *X. oryzae* pv. *oryzae* strain PXO99$^{A}$ and incompatible strain PXO99$^{A}$(*avrXa10*). Lesion length and disease phenotype of the inoculated plants were scored at two weeks after inoculation. The lesion length and the standard deviation of the mean were the average of 16 infected leaves. For lesion length score: R, resistant, ≤3.0 cm; MS, moderately susceptible, 6.0 cm – 9.0 cm; S, susceptible, >9.0 cm. N.D., not determined.

**Supplemental Table 3**. Summary of transgenic plants transformed with *Xa10* or variants under rice *PR1* gene promoter.

| Gene[a] | Transgenic plants[b] | | | | |
|---|---|---|---|---|---|
| | R | MR | MS | S | Total |
| $P_{PR1}$:*Xa10*:$T_{Nos}$ | 1 | 1 | 12 | 0 | 14 |
| $P_{PR1}$:*Xa10L18K*:$T_{Nos}$ | 0 | 0 | 0 | 53 | 53 |
| $P_{PR1}$:*Xa10R41L*:$T_{Nos}$ | 0 | 0 | 0 | 47 | 47 |
| $P_{PR1}$:*Xa10R46I*:$T_{Nos}$ | 0 | 0 | 0 | 57 | 57 |
| $P_{PR1}$:*Xa10R59A*:$T_{Nos}$ | 0 | 0 | 0 | 52 | 52 |
| $P_{PR1}$:*Xa10C113T*:$T_{Nos}$ | 0 | 0 | 0 | 56 | 56 |
| $P_{PR1}$:*Xa10NQ*:$T_{Nos}$ | 0 | 0 | 0 | 55 | 55 |

[a]The presence of transgenes and their copy number in transgenic plants were validated by DNA gel blot analysis.

[b]$T_0$ and $T_1$ transgenic plants in Nipponbare genetic background were evaluated for resistance to bacterial blight by inoculation with *X. oryzae* pv. *oryzae* strain PXO99$^A$(*avrXa10*). Lesion length and disease phenotype of the inoculated plants were scored two weeks after inoculation. For disease score: R, resistant, lesion length≤3.0 cm; MR, moderately resistant, 3.0 cm<lesion length≤6.0 cm; MS, moderately susceptible, 6.0 cm<lesion length≤9.0 cm; S, susceptible, lesion length>9.0 cm.

**Supplemental Table 4.** Constructs used in this study.

| Construct | Vector | Gene-of-interest | Reference |
|---|---|---|---|
| **I. Constructs for gene expression in *E.coli*** | | | |
| pQE32avrXA10ΔN152 | pQE32 | *6xHis-AvrXa10ΔN152* | This study |
| pQE31avrXa10 | pQE31 | *6xHis-AvrXa10* | This study |
| pQE31avrXa27 | pQE31 | *6xHis-AvrXa27* | This study |
| **II. Constructs for gene expression in *X. oryzae* pv. *oryzae*** | | | |
| pHM1avrXa10 | pHM1 | *avrXa10* | This study |
| pHM1avrXa10AD | pHM1 | *avrXa10AD* | This study |
| pHM1avrXa10NLS | pHM1 | *avrXa10NLS* | This study |
| **III. Constructs for gene expression in rice and *N. benthamiana*** | | | |
| pC1300 | pC1300 | None | CAMBIA |
| pC1305.1 | pC1305.1 | *GUSPlus* | CAMBIA |
| pCPR1avrXa10 | pC1300 | *avrXa10* | This study |
| pCS18901 | pC1300 | *Xa10* genomic subclone, SpeI fragment | This study |
| pCA14799 | pC1300 | *Xa10* genomic subclone, AvrII fragment | This study |
| pCES11947 | pC1300 | *Xa10* genomic subclone, EcoRI-SpeI fragment | This study |
| pCEA9295 | pC1300 | *Xa10* genomic subclone, EcoRV-AvrII fragment | This study |
| pCSA4671 | pC1300 | *Xa10* genomic subclone, SacI-AvrII fragment | This study |
| pCPX3834 | pC1300 | *Xa10* genomic subclone, PmlI-XbaI fragment | This study |
| pCSX10761 | pC1300 | Subclone adjacent to the *Xa10* locus | This study |
| pCEN6879 | pC1300 | Subclone adjacent to the *Xa10* locus | This study |
| pCB6804 | pC1300 | Subclone adjacent to the *Xa10* locus | This study |
| pCGWGUSint | pC1305.1 | Gateway destination vector, *GUSPlus* | This study |
| pCEBGUS-$P_{Bs4mini}$ | pCGWGUSint | *GUSPlus*, minimal *Bs4* promoter | This study |
| pCEBGUS-$P_{Xa10\text{-}220}$ | pCGWGUSint | *GUSPlus*, *Xa10* promoter, minimal *Bs4* promoter | This study |
| pCEBGUS-$EBE_{AvrXa10}$ | pCGWGUSint | *GUSPlus*, $EBE_{AvrXa10}$, minimal *Bs4* promoter | This study |
| pCPR1-Xa27 | pC1305.1 | *Xa27* | This study |
| pCPR1-Xa10 | pC1305.1 | *Xa10* | This study |
| pCPR1-Xa10L18K | pC1305.1 | *Xa10L18K* | This study |
| pCPR1-Xa10R41L | pC1305.1 | *Xa10R41L* | This study |
| pCPR1-Xa10R46I | pC1305.1 | *Xa10R46I* | This study |
| pCPR1-Xa10R59A | pC1305.1 | *Xa10R59A* | This study |
| pCPR1-Xa10C113T | pC1305.1 | *Xa10C113T* | This study |
| pCPR1-Xa10NQ | pC1305.1 | *Xa10EDDEE to QNNQQ* (*Xa10NQ*) | This study |
| pC35S-Xa10-Flag | pC1305.1 | *Xa10-Flag* | This study |
| pC35S-mCherry-3xFlag | pC1305.1 | *mCherry-3xFlag* | This study |
| pC35S-mRFP-3xFlag | pC1305.1 | *mRFP-3xFlag* | This study |
| pC35S-Xa10-3xFlag | pC1305.1 | *Xa10-3xFlag* | This study |
| pC35S-3xFlag-Xa10 | pC1305.1 | *3xFlag-Xa10* | This study |
| pC35S-Xa10L18K-3xFlag | pC1305.1 | *Xa10L18K-3xFlag* | This study |
| pC35S-Xa10R41L-3xFlag | pC1305.1 | *Xa10R41L-3xFlag* | This study |
| pC35S-Xa10R46I-3xFlag | pC1305.1 | *Xa10R46I-3xFlag* | This study |
| pC35S-Xa10R59A-3xFlag | pC1305.1 | *Xa10R59A-3xFlag* | This study |
| pC35S-Xa10C113T-3xFlag | pC1305.1 | *Xa10C113T-3xFlag* | This study |
| pC35S-Xa10NQ-3xFlag | pC1305.1 | *Xa10NQ-3xFlag* | This study |
| pC35S-eGFP | pC1305.1 | *eGFP* | This study |
| pC35S-Xa10-eGFP | pC1305.1 | *Xa10-eGFP* | This study |
| pC35S-Xa10L18K-eGFP | pC1305.1 | *Xa10L18K-eGFP* | This study |
| pC35S-Xa10R41L-eGFP | pC1305.1 | *Xa10R41L-eGFP* | This study |
| pC35S-Xa10R46I-eGFP | pC1305.1 | *Xa10R46I-eGFP* | This study |
| pC35S-Xa10R59A-eGFP | pC1305.1 | *Xa10R59A-eGFP* | This study |
| pC35S-Xa10C113T-eGFP | pC1305.1 | *Xa10C113T-eGFP* | This study |
| pC35S-Xa10NQ-eGFP | pC1305.1 | *Xa10NQ-eGFP* | This study |
| pC35S-eCFP | pC1305.1 | *eCFP* | This study |
| pC35S-Xa10-eCFP | pC1305.1 | *Xa10-eCFP* | This study |
| pC35S-Xa10L18K-eCFP | pC1305.1 | *Xa10L18K-eCFP* | This study |
| pC35S-Xa10R41L-eCFP | pC1305.1 | *Xa10R41L-eCFP* | This study |
| pC35S-Xa10R46I-eCFP | pC1305.1 | *Xa10R46I-eCFP* | This study |
| pC35S-Xa10R59A-eCFP | pC1305.1 | *Xa10R59A-eCFP* | This study |
| pC35S-Xa10C113T-eCFP | pC1305.1 | *Xa10C113T-eCFP* | This study |
| pC35S-Xa10C113T-eCFP | pC1305.1 | *Xa10C113T-eCFP* | This study |

(Continued)

| | | | |
|---|---|---|---|
| pC35S-Xa10NQ-eCFP | pC1305.1 | *Xa10NQ-eCFP* | This study |
| pC35S-mCherry | pC1305.1 | *mCherry* | This study |
| pC35S-Xa10-mCherry | pC1305.1 | *Xa10-mCherry* | This study |
| pC35S-mCherry-XA10 | pC1305.1 | *mCherry-Xa10* | This study |
| pC35S-Xa10R46I-mCherry | pC1305.1 | *Xa10R46I-mCherry* | This study |
| pC35S-mCherry-Xa10R46I | pC1305.1 | *mCherry-Xa10R46I* | This study |
| pC35S-mRFP | pC1305.1 | *mRFP* | This study |
| pC35S-Xa10-mRFP | pC1305.1 | *Xa10-mRFP* | This study |
| pC35S-mRFP-XA10 | pC1305.1 | *mRFP-Xa10* | This study |
| pC35S-Xa10R46I-mRFP | pC1305.1 | *Xa10R46I-mRFP* | This study |
| pC35S-mRFP-Xa10R46I | pC1305.1 | *mRFP-Xa10R46I* | This study |
| pC35S-eCFP-HDEL | pC1305.1 | $SP_{Atchit}$-*eCFP-HDEL* | This study |
| pC35S-eGFP-RcDGAT2 | pC1305.1 | *eGFP-RcDGAT2* | This study |
| pC35S-YC4.60ER | pC1305.1 | *YC4.60ER* | This study |
| pC35S-YC3.60 | pC1305.1 | *YC3.60* | This study |
| **IV. Constructs for gene expression in HeLa cells** | | | |
| pcDNA3.1 | pcDNA3.1 | None | Invitrogen |
| pcDNA3.1-eGFP-Sec61β | pcDNA3.1 | *eGFP-Sec61β* | This study |
| pcDNA3.1-3xFlag-Xa10 | pcDNA3.1 | *3xFlag-Xa10* | This study |
| pcDNA3.1-mRFP-3xFlag | pcDNA3.1 | *mRFP-3xFlag* | This study |
| pcDNA3.1-mRFP-KDEL | pcDNA3.1 | $SP_{Calreticulin}$-*mRFP-KDEL* | This study |
| pEGFP-N1 | pEGFP-N1 | *eGFP* | Clontech |
| pcDNA3.1-Xa10-eGFP | pcDNA3.1 | *Xa10-eGFP* | This study |
| pcDNA3.1-Xa10L18K-eGFP | pcDNA3.1 | *Xa10L18K-eGFP* | This study |
| pcDNA3.1-Xa10R41L-eGFP | pcDNA3.1 | *Xa10R41L-eGFP* | This study |
| pcDNA3.1-Xa10R46I-eGFP | pcDNA3.1 | *Xa10R46I-eGFP* | This study |
| pcDNA3.1-Xa10R59A-eGFP | pcDNA3.1 | *Xa10R59A-eGFP* | This study |
| pcDNA3.1-Xa10C113T-eGFP | pcDNA3.1 | *Xa10C113T-eGFP* | This study |
| pcDNA3.1-Xa10NQ-eGFP | pcDNA3.1 | *Xa10NQ-eGFP* | This study |
| pcDNA3.1-eCFP | pcDNA3.1 | *eCFP* | This study |
| pcDNA3.1-Xa10-eCFP | pcDNA3.1 | *Xa10-eCFP* | This study |
| pcDNA3.1-Xa10L18K-eCFP | pcDNA3.1 | *Xa10L18K-eCFP* | This study |
| pcDNA3.1-Xa10R41L-eCFP | pcDNA3.1 | *Xa10R41L-eCFP* | This study |
| pcDNA3.1-Xa10R46I-eCFP | pcDNA3.1 | *Xa10R46I-eCFP* | This study |
| pcDNA3.1-Xa10R59A-eCFP | pcDNA3.1 | *Xa10R59A-eCFP* | This study |
| pcDNA3.1-Xa10C113T-eCFP | pcDNA3.1 | *Xa10C113T-eCFP* | This study |
| pcDNA3.1-Xa10NQ-eCFP | pcDNA3.1 | *Xa10NQ-eCFP* | This study |
| pcDNA3.1-mRFP | pcDNA3.1 | *mRFP* | This study |
| pcDNA3.1-Xa10-mRFP | pcDNA3.1 | *Xa10-mRFP* | This study |
| pcDNA3.1-mRFP-Xa10 | pcDNA3.1 | *mRFP-Xa10* | This study |
| pcDNA3.1-Xa10R46I-mRFP | pcDNA3.1 | *Xa10R46I-mRFP* | This study |
| pcDNA3-D1ER | pcDNA3 | D1ER | (Palmer and Tsien, 2006) |
| pcDNA3-4mtD3cpv | pcDNA3 | 4mtD3cpv | (Palmer and Tsien, 2006) |
| **IV. Constructs for yeast** | | | |
| pGADT7avrXa10 | pGADT7-Rec | *GAL4 AD-avrXa10* | This study |
| pGADT7avrXa27 | pGADT7-Rec | *GAL4 AD-avrXa27* | This study |
| pGADT7p53 | pGADT7-Rec | *GAL4 AD-p53* | This study |
| pAbAi-4x$EBE_{AvrXa10}$ | pAbAi | *4xEBE*$_{AvrXa10}$ | This study |
| pAbAi-4x$EBE_{AvrXa27}$ | pAbAi | *4xEBE*$_{AvrXa27}$ | This study |
| pAbAi-p53DBS | pAbAi | *p53DBS* | This study |

**Supplemental Table 5.** DNA primers used in this study.

| Primers | DNA sequence (5' to 3') |
|---|---|
| SP1F | TGCCGTCCTCCTACTGATG |
| SP1R | CCTCGTCGTCTTCACCAATG |
| RGP6-F | CCTCGTCGTCTTCACCAATGCA |
| GS4R1 | GACGTGCTCATCATCTACCTC |
| 10RTF2 | GGCATCATCTTCTCCGGCG |
| 10RTR2 | GCAGCTATACGGGCATAAG |
| RBQ3 | CCAGTAAGTCCTCAGCCATG |
| RBQ4 | TTTCAGACACCATCAAACCAG |
| pAbAi-F2 | CCAAGAAGATGTAATGCACCC |
| pAbAi-R2 | CATTACGACCGAGATTCCCG |
| NbGAPDH-F | GGAGGAGGGAACAACAAGAGG |
| NbGAPDH-R | AGATGCCGTCAGTGCCGA |
| TNos-F | CGTTCAAACATTTGGCAATAAAG |
| TNos-R | AAATGTATAATTGCGGGACTCTAATC |

## SUPPLEMENTAL METHODS

### GUS Reporter Constructs

GUS reporter constructs for detection of $EBE_{AvrXa10}$ activity were designed based on the GUS reporter vector published previously (Boch et al., 2009) with a slight modification. In brief, the 35S promoter in pCAMBIA vector pC1305.1 (HindIII-NcoI fragment) was removed and the remaining vector fragment was filled in and self-ligated to produce pC1305.1(-35S). The 2151-bp XhoI-XbaI fragment in pC1305.1(-35S), consisting of the coding sequence of hygromycin resistance gene and another 35S promoter, was replaced with 1787-bp XhoI-SpeI fragment of the *attR* element from pANDA vector pANDA35HK (Miki and Shimamoto, 2004) to generate the Gateway destination vector pCGWGUSint. The minimal tomato *Bs4* promoter (Boch et al., 2009) fused with target DNA elements at its 5' end was amplified by PCR and inserted into pENTR/D-TOPO (Invitrogen, USA). Promoter derivatives were inserted into pCGWGUSint that contained a promoterless and intron-containing *GUSPlus* gene by Gateway technology to generate GUS reporter constructs. The intron-containing *GUSPlus* gene prevents the expression of GUS activity in *A. tumefaciens*.

### DNA Gel Blot Analysis

DNA gel blot analysis was carried out according to standard procedures (Sambrook and Russell, 2001). Approximately 2-5 μg of rice genomic DNA was digested with the appropriate restriction enzymes and then separated in 0.8% Agarose gel. DNA gel blots were hybridized with DNA probes labeled with $^{32}$P-dCTP (GE Healthcare, United Kingdom). The *Xa10* probe was amplified from *Xa10* coding region using DNA primers SP1F and SP1R (Supplemental Table 5).

### Electrophoretic Mobility Shift Assay (EMSA)

EMSA was carried out according to the method described previously (Kay et al., 2007) with slight modification. Polyhistidine-tagged fusion proteins of AvrXa10 (6xHis-AvrXa10) and AvrXa27 (6xHis-AvrXa27) were purified from *E. coli* M15 using a QIAexpressionist™ kit (Qiagen) (Supplemental Figure 7). Protein concentration was determined by Bradford assay (Bio-Rad). Complementary pairs of nonlabeled or 3'-biotin-labeled oligonucleotides (1st BASE, Singapore) were annealed to obtain double-stranded DNA. EMSA was performed with the LightShift® Chemiluminescent EMSA Kit (Pierce, USA) in accordance with the manufacturer's instruction.

**Yeast One-Hybrid Assay**

For protein-DNA interaction studies, the Matchmaker Gold Yeast One-Hybrid Library Screening System (Takara Bio Asia Pacific /Clontech) was used following the manufacturer's protocol. Four copies of bait DNA sequence were cloned into pAbAi vector in tandem to yield bait constructs. The *AvrXa10* gene was cloned into pGADT7-Rec to create prey constructs, in which AvrXa10 is fused to SV40 NLS-GAL4 AD as prey. The GAL4-AD fusion of murine p53 protein and the bait containing its target sequence (p53DBS) served as controls. The bait constructs were digested with BstBI and transformed into yeast strain Y1HGold. The transformants were verified by using a colony PCR. The prey constructs were then transformed into the generated Y1HGold strains that harbored the cognate bait DNA sequences in their genomes. Transformants grown on selective SD medium at 30$^{o}$C were resuspended in 0.9% NaCl with an OD600 of 0.002 or approximately 2000 cells per 100 µl. Serial dilutions in 0.9% NaCl were dropped on SD medium supplemented with Leucine or 200 ng/ml Aureobasidin A (AbA). Identical transformants were inspected for presence of the bait plasmid by colony PCR and for expression of GAL4-AD-fusion proteins by immunoblot analysis.

**Yeast Colony PCR**

Single yeast colonies were resuspended in 20 lyticase buffer [1.2 M sorbitol, 0.1 M sodium phosphate pH7.4, 2.5 mg/ml lyticase (Sigma-Aldrich, INC., Saint Louis USA)], incubated 30 min at 37$^{o}$C and 10 min at 95$^{o}$C. 5 µl of a 1:5 dilution were used for PCR with DNA primers pAbAi-F2 and pAbAi-R2 (Supplemental Table 5).

Immuno**blot Analysis**

Immunoblot analysis for detecting prey proteins in yeast was carried out according to the method described previously (Kay et al., 2007). Single yeast colonies were resuspended in histidine-containing SD liquid medium to an $OD_{600}$ = 0.15 and grown at 30 °C and 150 rpm to $OD_{600}$ = 0.4 to 0.6. Cells of 3 ml culture were resuspended in 30 µl cracking buffer (8 M urea, 5% (w/v) SDS, 40 mM Tris-HCl pH 6.8, 0.1 mM EDTA, 0.4 mg/ml bromophenol blue, and 0.1% β-ME). Samples were incubated together with glass beads (425-600 µm; Sigma #G-8772) at 20 °C and 14,000 rpm for 30s, and set on ice for 30s. This step was repeated three times. Samples were centrifuged at 14,000 rpm and 4 °C for 10 min. The supernatant was denatured at 70 °C for 5 min. 25 µl protein extract was separated on 8% SDS

polyacrylamide gels and subjected to immunoblot analysis with Anti-GAL4 AD (Sigma-Aldrich) and Anti-rabbit IgG (Sigma-Aldrich) antibodies. Reactions were visualized by enhanced chemiluminescence as recommended (Amersham). The AvrXa10 proteins expressed in rice were detected with anti-AvrXa10 polyclonal antibodies and horseradish peroxidase-coupled secondary antibody (Bio-Rad). The anti-AvrXa10 polyclonal antibodies were generated in rabbits using a truncated AvrXa10 with the first 152 amino acids deleted.

SUPPLEMENTAL REFERENCES

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