Supporting Information

Song *et al.* 10.1073/pnas.0809201106

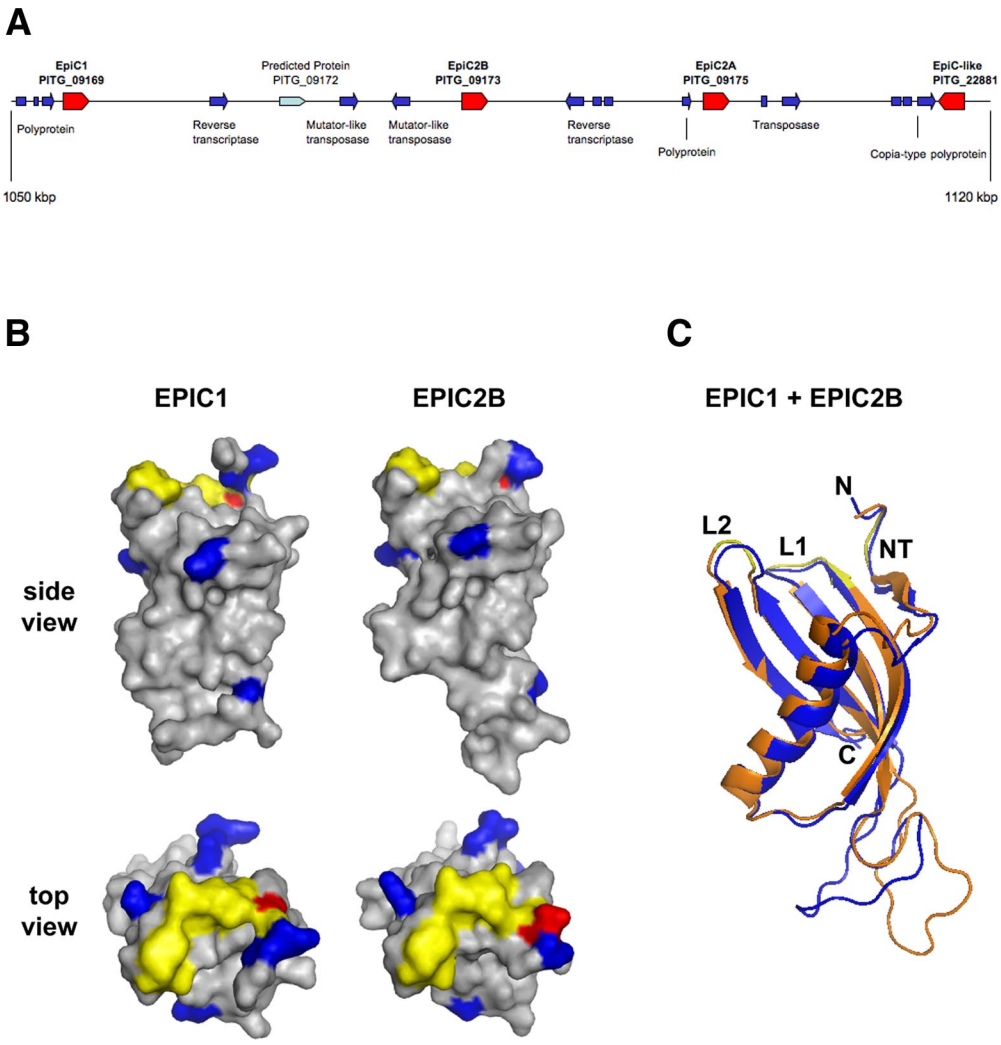

Fig. S1. (*A*) The *epiC* genes are clustered within a 70-kbp region in supercontig 14 of the genome of *P. infestans* T30–4. Graphic view of the cluster, illustrating the cluster with the four genes *epiC1*, *epiC2B*, *epiC2A*, and *epiC1-like*. Other genes are also marked with their PITG identifiers (Broad Institute annotation Web site). Note how the region is interspersed with remnants of transposable elements (blue arrows). There are two additional cysteine protease inhibitor genes in the genome, *epiC3* and *epiC4*, as described by Tian *et al.* (14). (*B*) Surface models of EPIC1 and EPIC2B. Structural prediction with Bioinfo metaserver service (www.bioinfo.pl), modeled on ORYZACYSTATIN-I (pdb: 1eqkA) using MODELLER. The conserved binding regions are yellow, polymorphic residues are blue, and the G>N polymorphism in the N terminus is red. (*C*) Superimposition of EPIC1 (blue) and EPIC2B (orange) ribbon models. Arrows and helical stretches represent b-strands and a-helical regions. N-terminal truck (NT), loop1 (L1) and loop2 (L2) depict conserved regions. N, amino terminus; c, carboxy terminus.

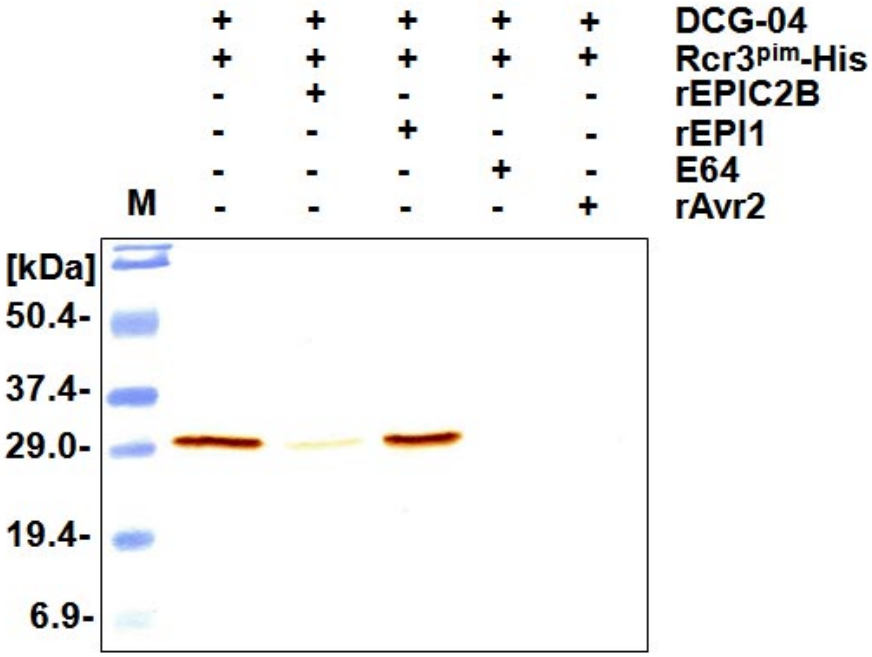

Fig. S2. Avr2 and EPIC2B but not the Kazal-like serine protease inhibitor EPI1 inhibit Rcr3^{pim}. Inhibition of Rcr3^{pim} produced in *N. benthamiana* apoplast by rEPIC2B and rAvr2. Apoplastic fluid was isolated from *N. benthamiana* expressing Rcr3^{pim}-His. Protease activity profiling with 220 nmol/l DCG-04 was performed in the absence (–) of inhibitor or in the presence (+) of E-64, FLAG-EPI1 (rEPI1), FLAG-Avr2 (rAvr2), or FLAGEPIC2B (rEPIC2B). Rcr3^{pim}-His were captured (pulled down) by Ni-NTA beads, electrophoresed on an SDS gel, and detected with streptavidin-HRP. The DCG-04−labeling reactions were stopped at 30 minutes by adding ice-cold acetone into the reaction mix. Detection with streptavidin-HRP reveals that Rcr3^{pim}-His is not biotinylated in the presence of E-64 or rAvr2, whereas biotinylation of Rcr3^{pim} occurs without inhibitor or with rEPI1, indicating that, like E-64 and rAvr2, rEPIC2B inhibits Rcr3^{pim} cysteine protease activity. Approximate molecular weights of the labeled Rcr3^{pim}-His proteins are shown on the left.

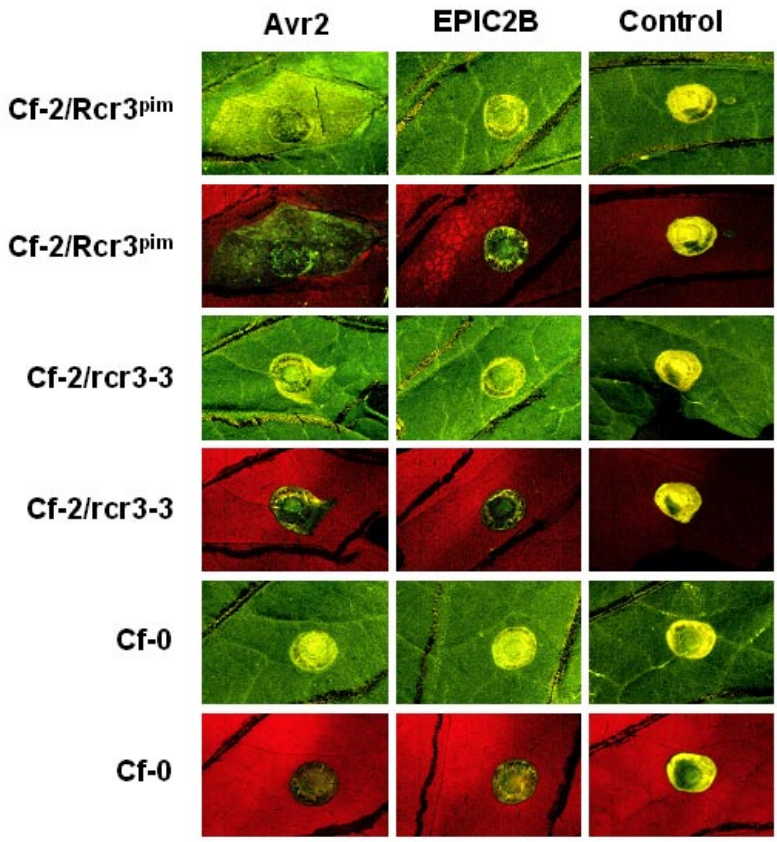

Fig. S3. Fluorescence microscopic detection of the hypersensitive response on tomato plants. Fluorescence microscopic visualization of infiltrated sites on tomato leaves confirmed that rAvr2 triggered HR on Cf-2/Rcr3^{pim} tomato. Tomato leaves with the inoculation area (marked by dark marker pen) were placed under the fluorescence UV light for 40 seconds before the photos were taken (*lower lane of each panel*). Similarly, photos were taken after the leaves were exposed to the normal bright light (*upper lane of each panel*).

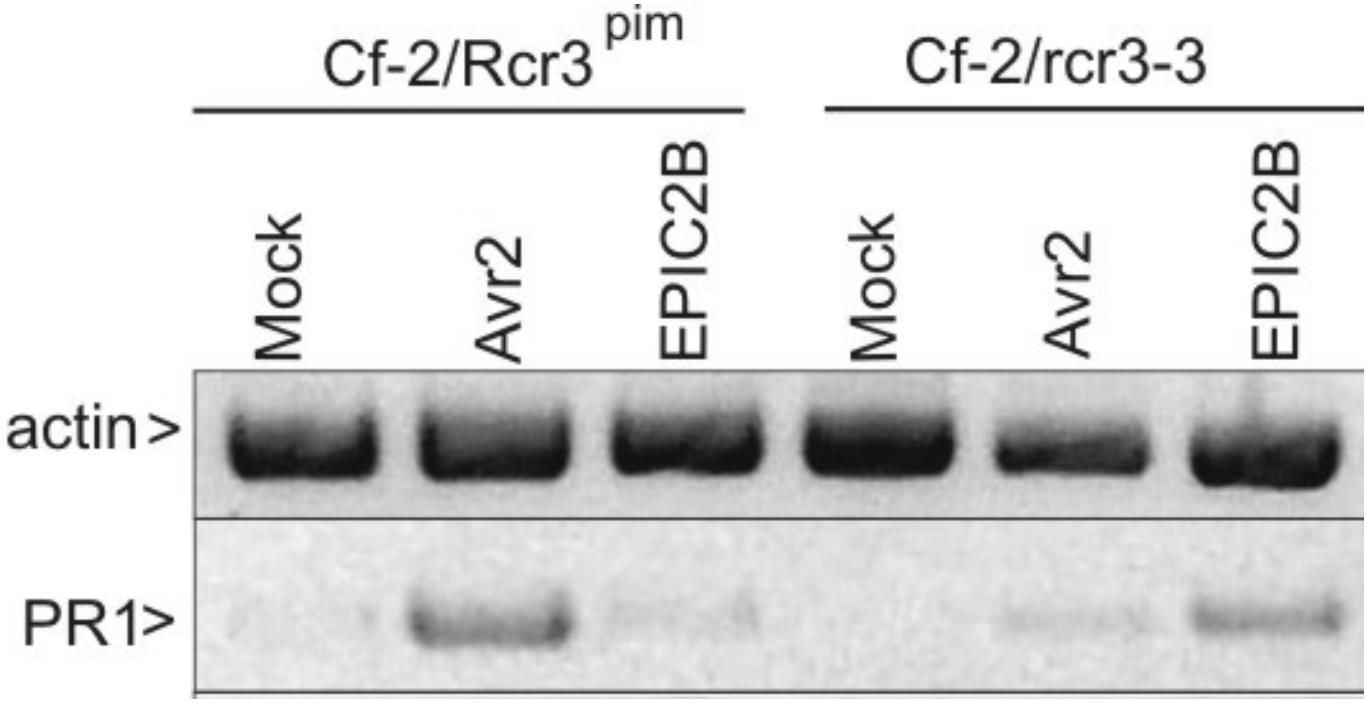

Fig. S4. Unlike Avr2, EPIC2B does not induce the expression of PR1 in Cf-2/Rcr3^{pim} tomato. RT-PCR analysis of pathogenesis-related *PR1* gene in tomato after infiltration with purified rAvr2 and rEPIC2B. Total RNA isolated from infiltrated leaves of Cf-2/Rcr3^{pim} and Cf-2/rcr3–3 tomato was used in RT-PCR amplifications. Amplifications of tomato actin were used as constitutive controls to determine the relative expression of the *PR1* gene.

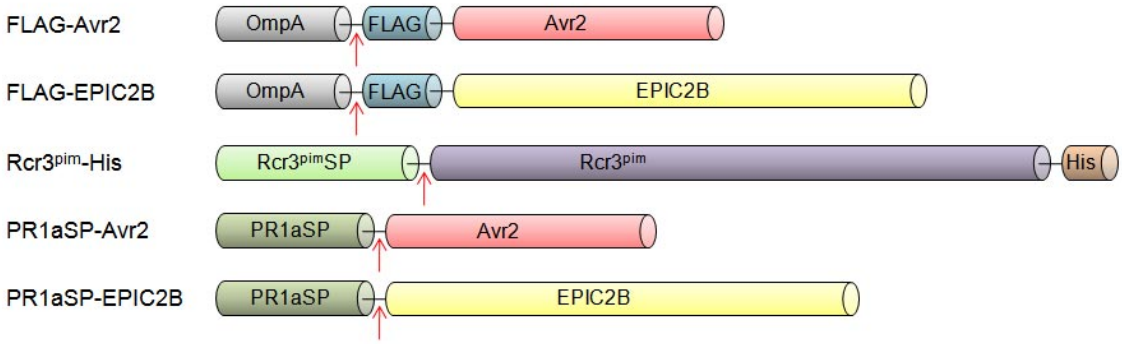

Fig. S5. Graphic view of the recombinant proteins used in this study. Arrow indicates the signal peptide cleavage site.

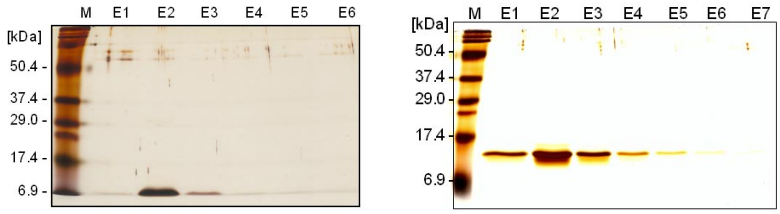

Fig. S6. Purified FLAG-tagged EPIC2B and Avr2 proteins. FLAG-EPIC2B (*left*) and FLAG-Avr2 (*right*) affinity purified using anti-FLAG resin are shown. FLAG-EPIC2B and FLAG-Avr2 were expressed in *E. coli,* supernatant were collected, and protein expression was evaluated by Western blot (data not shown) before affinity chromatography purification. Six elution fractions (E1−E6) were analyzed on 15% SDS/PAGE gel followed by silver staining. The size (kDa) of the molecular weight markers (M) is shown on the left.