

Supplemental Figures

Alphafold-multimer predicts cross-kingdom interactions at the plant-pathogen interface

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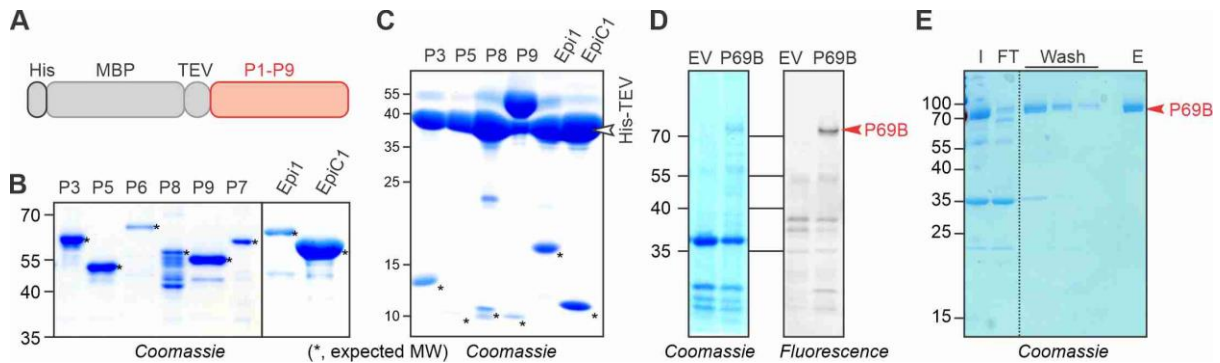


Figure S1 Production of candidate inhibitors and P69B for inhibition assays.

(A) Candidate inhibitors lacking their endogenous signal peptides were codon-optimized for expression in *E. coli* and cloned in-frame with a 6-His purification tag; the maltose binding protein (MBP) and the cleavage site of tobacco etch virus (TEV) protease. (B) Fusion proteins were expressed in *E. coli* and purified on HisPur™ Ni-NTA resin and amylose resin, subsequently. Fusion proteins were detected at their predicted molecular weight (MW, *) by SDS-PAGE and Coomassie staining. (C) Putative inhibitors were released from their purification tags upon incubation with His-TEV protease. (D) P69B-His is efficiently expressed by agroinfiltration. P69B with a C-terminal His tag and an empty vector (EV) control were transiently expressed by agroinfiltration. Apoplastic fluids were isolated, labeled with FP-TAMRA, separated on SDS-PAGE and scanned for fluorescence (right) and stained with Coomassie (left). (E) Purification of P69B-His on Ni-NTA. I, input sample; FT, flow through; E, eluate.

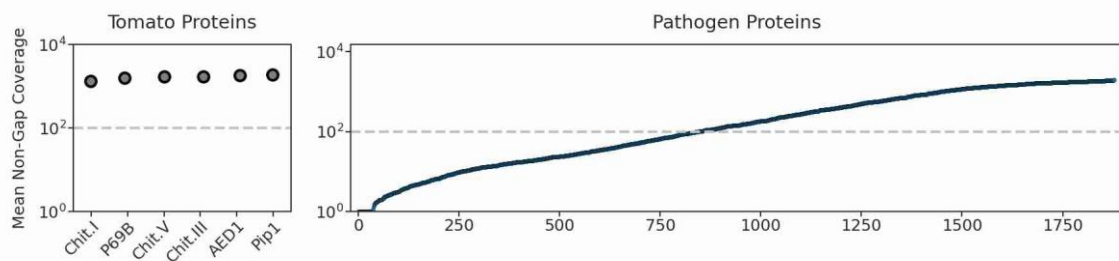


Figure S2 MSA depth of SSPs and hydrolases used in Figure 2.

The mean non-gap multiple sequence alignment (MSA) depth is shown for the tomato hydrolases (left) and the 1,879 SSPs (right), ranked by MSA depth. The dash line indicates the desired minimum of 100 MSA depth.

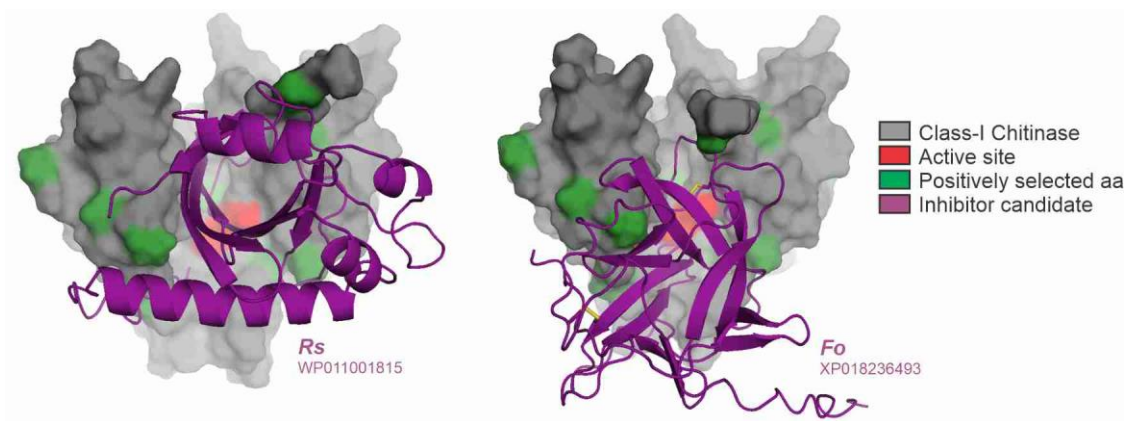


Figure S4 Two candidate inhibitors interact with positively selected sites in Class-I chitinases. Positively selected sites reported previously (Bishop et al., 2000) were plotted onto the AFM-predicted models of tomato Class-I chitinase in complex with inhibitors from *Ralstonia solanacearum* (**A**) and *Fusarium oxysporum f. sp. lycopersici* (**B**).

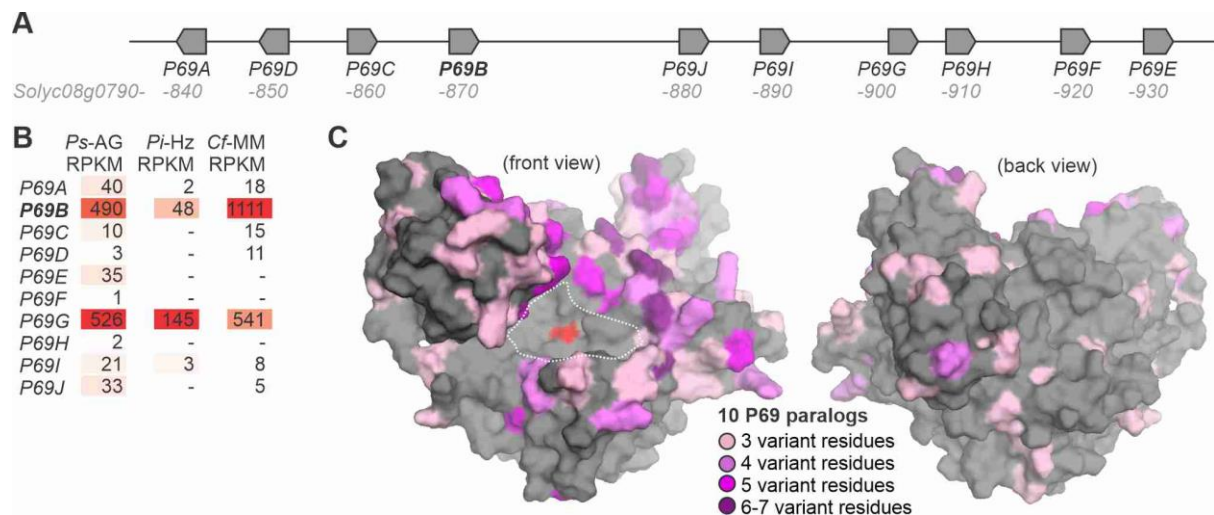


Figure S5 Expression and variation of the ten *P69* paralogs of tomato.

(A) Genomic *P69* cluster contains ten paralogs. Sequence was extracted from the genome of cv. Heinz (Tomato Genome Consortium, 2012). (B) Expression levels in reads per kilobase per million (RPKM) of *P69* genes in three different cultivars infected with *Ps* (cv. Ailsa Graig, Rosli et al., 2013); *Pi* (cv. Heinz, Hwang et al., 2020) and *Cf* (cv. Money Maker, Ilyas et al., 2016). (C) Protein sequences of the ten *P69* paralogs of tomato (*P60A-I*) were aligned and the number of variant residues at each position was plotted on the model of the *P69B* structure. The variation (purple) is surrounding the active site (red) in the substrate binding groove (dashed line) as a ‘ring-of-fire’.