Supplemental Data

Structure function analysis of an ADP-ribosyltransferase Type III Effector and its RNA-Binding Target in Plant Immunity

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Table S1. Refinement statistics of HopU1 structure[a]

Structure refinement statistics	
Resolution range (Å)	50.0-2.70
R_{work}/R_{free} (%)[b]	17.87/21.18
R.M.S. Deviation from ideality	
Bonds, (Å)	0.010
Angles, (°)	1.319
Average B factor, (Å^2)	27.49
Ramachandran plot statistics	
Most favored regions (%)	89.7
Allowed regions (%)	8.9
Generously allowed regions (%)	1.4

[a] the Crystallization and preliminary crystallographic analysis has been published in Lin *et al.* (2010. Acta Crystallogr Sect F Struct Biol Cryst Commun 66: 932-934).

[b] $R_{free} = \sum_{Test}||F_{obs}| - |F_{calc}||/\sum_{Test}|F_{obs}|$, where "Test" is a test set of about 5% of the total reflections randomly chosen and set aside prior to refinement for the complex.

Figure S1. Structure comparison of HopU1 and selected ADP-ribosyltransferases. (A) Structure of the HopU1 ADP-RT is shown as ribbon representation and colored in purple blue and L1 and L4 loops are colored yellow **(B-D)** Structures of the rat Ecto-ADP-RT ART2.2 (1GXZ.PDB), the *Clostridium botulinum* C3bot2 ADP-RT (1R45.PDB), and the *L Clostridium limosum* C3 exoenzyme ADP-RT (3BW8.PDB) are shown as ribbon representation and colored in green, purple, and red, respectively.**(E)** Superimposition of the four ADP-RT structures. **(F)** PN loop and ARTT loop are indicated in compared structures. **(E-F)** The structures are colored as in Fig. S1A-D.

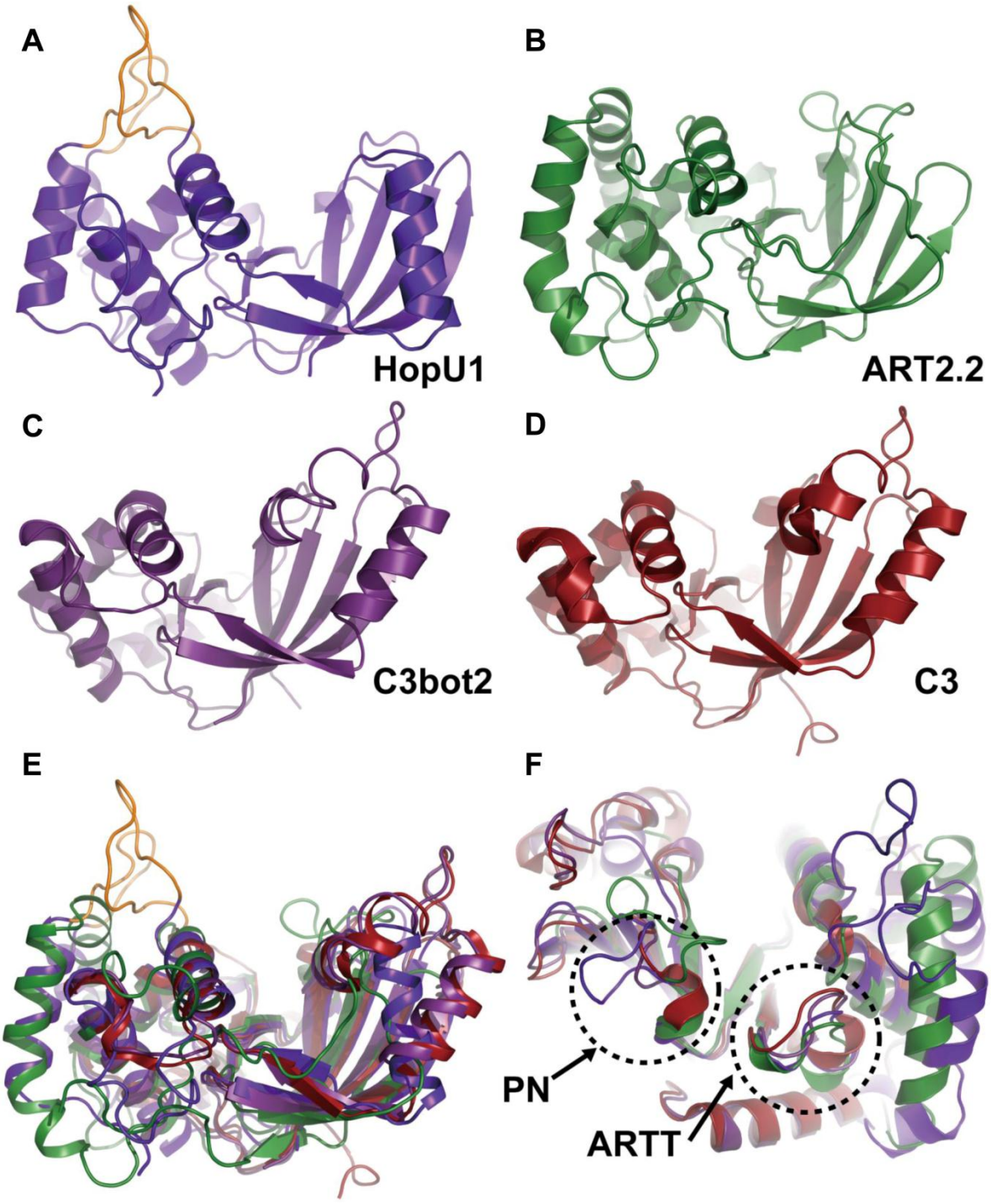
Figure S2. (A) Structures of HopU1 separately indicating the location of mutations M5-M9 and then summarized in central square. The altered residues are highlighted and shown as stick representations. **(B)** Isothermal titration calorimetry of wild type HopU1 and HopU1 mutants M5-M9 with GRP7, which indicates the extent that HopU1 derivatives can interact with GRP7. **(C)** The electrospray ionization-mass spectrometry results of ADP-RT activity for wild type HopU1 and HopU1 derivatives are shown. The peak of substrates and products of each enzymatic reaction is indicated. The molecular mass of GRP7 (1-90, GPHM after 3C cleavage at N terminus) is 11,260 Dalton and GRP7 with an ADP ribose modification is 11,800 Dalton. **(D)** Result summary of the ADP-RT activity and substrate binding affinity for wild type HopU1 and HopU1 mutants M1-M9.

Figure S3. MS/MS spectrum of doubly charged ion of m/z 1029.92 in the tryptic digest of GRP7. The spectrum contains a nearly complete series of y-type ions. Additional ions supporting ADP ribosylation of this peptide observed in the MS/MS spectrum are protonated adenine (m/z 136), AMP (m/z 348) and ADP (m/z 428).

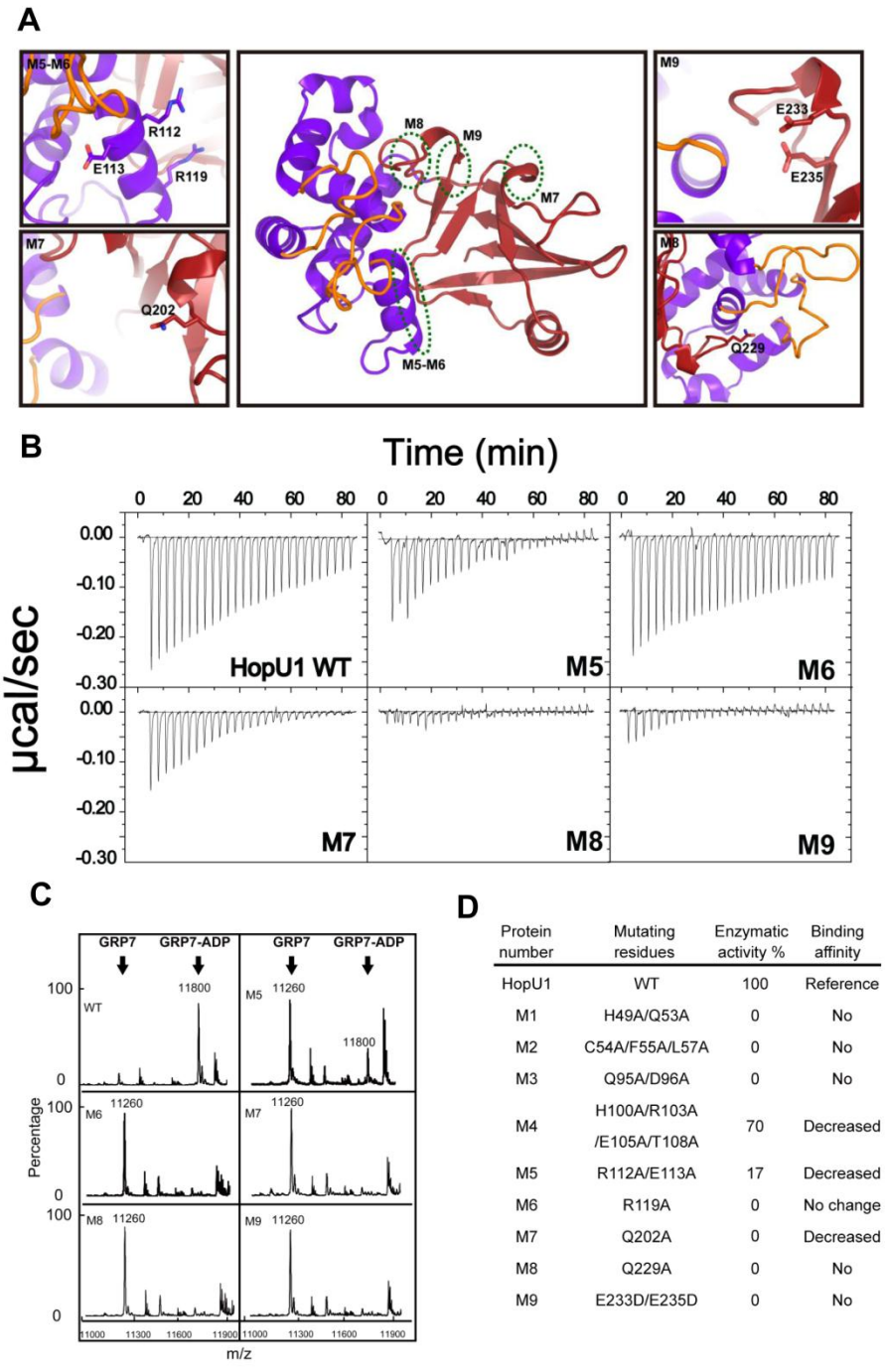
Figure S4. Electrophoretic mobility shift assay of an RNA probe with differing amounts of GRP7-GST after treatment with HopU1 or its catalytic inactive mutant HopU1$_{DD}$. Standard ADP-ribosylation reactions were performed with varying concentrations of GRP7-GST in the presence of HopU1 or HopU1$_{DD}$, and a ^{32}P-labeled probe (ATGRP7 UTR WT) was added to each reaction mix. These were run on native polyacrylamide gels, and exposed to X-ray films.

Figure S5. Expression of GRP7 in different transgenic *A. thaliana* lines. Immunoblot using anti-HA antibodies showing the expression of GRP7-HA and GRP7$_{R49K}$-HA in *A. thaliana* Col-0 and the Col-0 *grp7-1* mutant.

Figure S6. Superimposed HopU1 and AvrPphF structures. Two views (**A, B**) of the HopU1 structure (blue) superimposed with the AvrPphF structure (light green). The root mean square deviation (RMSD) of 2.9 angstrom for 64 Cα atoms indicating that the structures did not superimpose well. The N-termini of both proteins are quite different. The C-terminal catalytic domains of both proteins share a similar fold found in ADP-RTs.

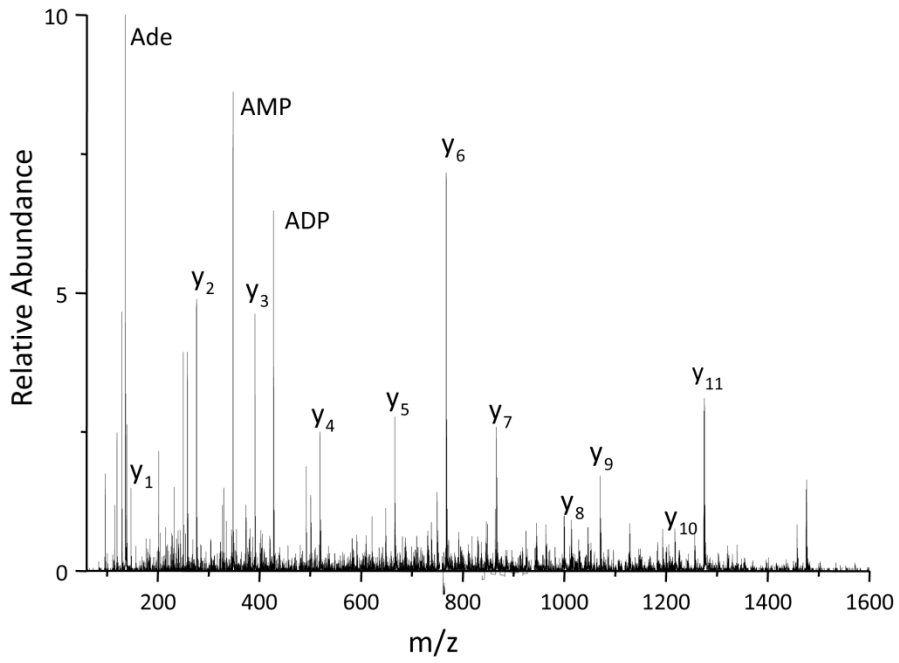


Supplemental Figure 1

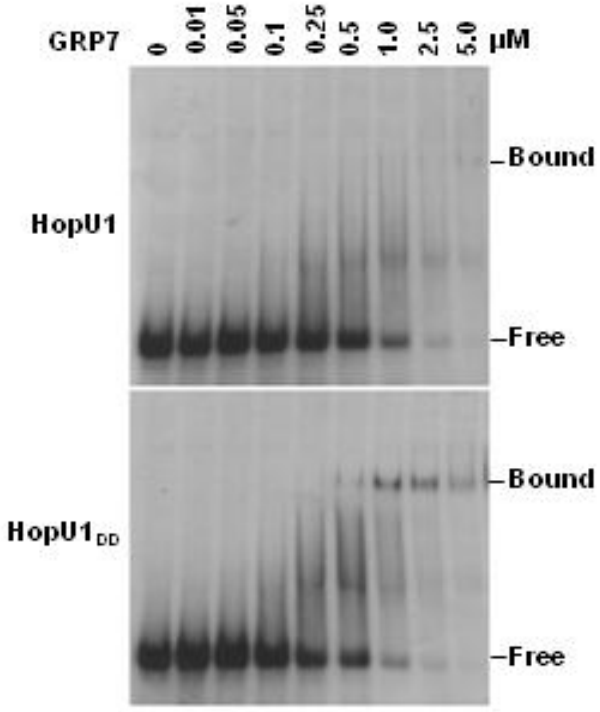


Protein number	Mutating residues	Enzymatic activity %	Binding affinity
HopU1	WT	100	Reference
M1	H49A/Q53A	0	No
M2	C54A/F55A/L57A	0	No
M3	Q95A/D96A	0	No
M4	H100A/R103A /E105A/T108A	70	Decreased
M5	R112A/E113A	17	Decreased
M6	R119A	0	No change
M7	Q202A	0	Decreased
M8	Q229A	0	No
M9	E233D/E235D	0	No

Supplemental Figure 2

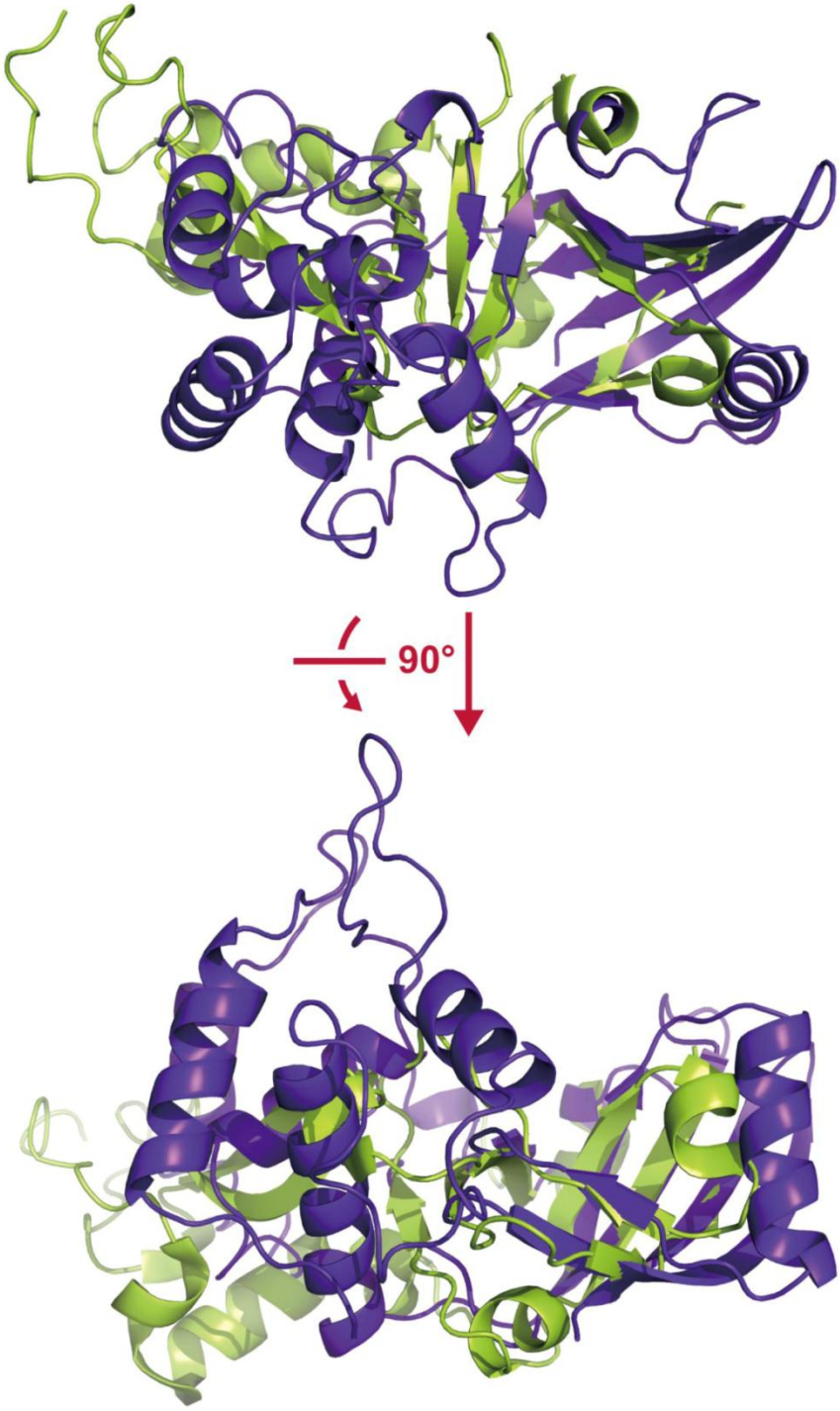


Supplemental Figure 3



Supplemental Figure 4

Supplemental Figure 5



Supplemental Figure 6