# Secondary-structure switch regulates the substrate binding of a YopJ family acetyltransferase

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Supplementary Figure 1. Structural comparison of apo-PopP2 and InsP6-42 bound PopP2 in the InsP6 binding pocket. The structure of apo-PopP2 is 43 colored in cyan and the InsP6-bound in light blue. The InsP6 molecule is shown 44 in stick-and-ball model. The hydrogen-bonding interactions are shown as dashed 45 46 lines.





Supplementary Figure 3. The flexibility study of PopP2 in apo (a, c, e) form and in complex with InsP6 (b, d, f). (a) and (b): PCA analysis of PopP2. The length of arrow is correlated to the flexibility of corresponding region. (c) and (d): the RMSD of PopP2 during all-atom MD simulations. Each simulation was repeated three times which were represented by green, blue and orange lines. (e) and (f): the binding pocket surface of PopP2.



102 Supplementary Figure 4. Residues selected for mutation study in the fold-

103 **switching motif.** Fold-switching motif in apo (a) and InsP6-bound (b) structures

104 are colored in cyan, with the side chains of the residues selected for mutation

105 study are shown as sticks.



Supplementary Figure 5. PopP2 mutants L369P/V370P, L371P/D372P and 120 121 K383A are affected in their autoacetylation activity. Recognition of acetylated wild-type PopP2 and R380A mutant, but not PopP2-C321A, PopP2-122 L369P/V370P, PopP2-L371P/D372P or PopP2-K383A, by an anti-acetyl-lysine 123 124 antibody. The different PopP2 variants were N-terminally tagged with a 125 6xhistidine and expressed in Rosetta cells. Recombinant proteins were analyzed 126 by immunoblot using an antibody that recognizes 6xhistidine tagged proteins (a-His6-HRP) or acetylated lysines ( $\alpha$ -AcK). 6his-tagged PopP2 proteins are shown 127 128 after Ponceau staining (top). This experiment was conducted three times with 129 similar results.



142 Supplementary Figure 6. Representative binding curves of PopP2 variants

### 143 to RRS1<sub>WRKY</sub> in the presence of InsP6 examined by BLI assays. The

144 concentrations of PopP2 variants are labeled in the first panel. Representative

145 curves are from one of two independent experiments.



Supplementary Figure 7. PopP2 L369P/V370P, L371P/D372P and K383A 148 149 mutants are unable to physically interact in planta with the C-terminal 150 portion of RRS1-R containing the WRKY domain (RRS1-R<sub>Cterm</sub>). CFP lifetime distribution of the different PopP2 variants fused with CFP and expressed either 151 alone or with RRS1-R<sub>Cterm</sub>-YFPv. Histograms shows the distribution of nuclei 152 153 (number) according to PopP2-CFP lifetime classes in the absence (blue bars) or presence (orange bars) of RRS1-R<sub>Cterm</sub>-YFPv. The scanned nuclei correspond to 154 155 those used for FRET-FLIM measurements presented in Table1. This experiment 156 was conducted twice with similar results.

157



Supplementary Figure 8. Immuno-detection of the different PopP2 variants fused to CFP and expressed either alone or with RRS1-R<sub>Cterm</sub>-YFPv in *N. benthamiana.* The CFP- and YFPv-tagged proteins (crude extracts) are detected with an anti-GFP antibody. Ponceau S staining of total protein demonstrates equal loading of the samples. The star indicates the signal corresponding to RRS1-R<sub>Cterm</sub>-YFPv fusion protein. This experiment was conducted twice with similar results.

166



Supplementary Figure 9. Immuno-detection of the different PopP2 variants
delivered by *Pf0-1* in the *rrs1-1* null mutant. Leaf samples were harvested 7
hours post infiltration (hpi). HA-tagged proteins were detected using an anti-HAHRP antibody (from crude extracts (CE) and immunoprecipitated proteins).
Ponceau staining indicates similar protein amounts loaded in the different lanes.
This experiment was conducted three times with similar results.



196 Supplementary Figure 10. Phylogeny of representative CE clan of proteases. The phylogenetic tree was generated with MEGA7 using the 197 198 sequences of catalytic regions. The GenBank accession numbers of effectors 199 used for this analysis are as follows: AAD39255 for AvrBsT (Residues 61-350), AAR02168 for HopZ1a (Residues 31-369), CAD14570 for PopP2 (Residues 151-200 201 488), AKN09807 for YopJ (Residues 1-288), AAL21745 for AvrA (Residues 1-202 288), B0B9A0.1 for ChlaDUB (Residues 150-401), Q8ZNG2.2 for SseL 203 (Residues159-340) and Q8XCY9.1 for ElaD (Residues 201-407). YopJ effectors 204 produced by plant and animal pathogens are colored in red and green, 205 respectively.

207	PopP2	151				
208	HopZ1a AvrBst YopJ AvrA Ch1aDUB1	31 61 1 150	. TPDRRQRAAGDA.ERTQSMRLQQKINOLKPYVRHARGPIKAYGQAALDRAS.GKKTSVSFA AIALEESLNSSNNIPYEMRMYAEAALSAAKHGSSEAITKA MIGPISQINISGGLSEKETSSLI.SNEELKNIITQ.LETDISDGSWFHKNYS MLSPTTRNMGASLSPQPDVSGEL.NTEALTCIVER.LESEIIDGSWIHISYE EDAWRQTIEALVPANDS			
209	SseL ElaD	0.00				
210						
211	PopP2 HopZ1a AvrBst YopJ	192 90 101 51	DVDRAIMPLLTVAENARNPGLNLVPLHMDMAED.EEVRTQPPMAG ELDATHLDAMVYIENQRNPGLNLKHFRDHK DVEN.KYYLAHAYNERPPELHLS.CH.D.SASA RMDVEVMPALVIQANNKYPEMNLNLVTSPLD.LS.			
212	ChlaDUB1 SseL ElaD	168 159	DIFKCLCYFHILYPGLUILETFPPATIFFFKQKIISILEDKKAVLRGEPIKGPLPICCSKENYRRHLQRT MLS.SDELAAATQGLVQESPLLSVNYPI			
213						
214	PopP2 HopZ1a AvrBst YopJ	236 120 128 84	SRHIAEFVASARPGRYRAVIDDGSHTRAADIRKDASGTSV.IVVD ELIQALQSDGPSAFRAIFPQTCPETGQTLKHHVMADVRLHQGGAPTIIITE OSFFSEFMTSEKQAWRSIVRLSPSSMHAAIDVRFKDGKRTM.LVIE IEIKN.VIDNGV.RSSRFIINMGEGGIHFSVIDYKHINGKTSLILFE			
215	AvrA Ch1aDUB1 SseL E1aD	84 239 187 201	SSIKE.TRMEQUESARFLVNMGSSGIHISVVDFRVMDGKTSV.ILFE TLLPVFMWYHPTPKTLSDTMQTMKQLAIKGSVGASHWLLVIVDIQARR.LVYFD GLIHPTTKENILSTQLLEKI.AQSGLSHNEVFLVNTGDHWLLCLFYKLAEKIK.CLIFN TYLEVALNDNIKSDPSWPKEVQLFPINTGGHWLLVSLQKIVNEKNNTQQIK.CIIFN			
216						
217	PopP2 HopZ1a AvrBst YopJ	280 171 174 129	PERKEKDESAYVDYADNVNMEFGEHAKCÄFTPMDTOK.SFFDCRTISLSLÄLKMHDK PAVIVGARYQQLQRHN.LTLE.DLSESGVPLSQVATTETQAOK.TSDDCVMYSLNYATKAHKN PALAYGMKDGETKVMAGVETLGKNVQNCLGENGDMAVIJQLGAOK.SIFDCVTFSLMMALCAYQK PANFNSMGPAMLAIRKTATERYQDPCCHFSMVEMDTOR.SISCCGFFSLAMALVIE			
218	AvrA Ch1aDUB1 SseL E1aD	129 292 244 257	PÅACSAFGPALLALRTKAALEREQLPDCYFÄMVUELDIGR.GSSECGIFSLALAKKLQLE SLYNYVMPPENMKE.LQSFAQQ.LDQVYPAYDSKKFSVKTAAKEVIGRGSGSSCGAMCCQFLH.WYLK TYYDLNENTKQEIIEAAKIAGISESDEVNFIEMNLONNVPNGCGLFCYHTIQ SLRALGHEKENSLKRIINSFNSFNCDPTRETPNNKNITDHLTEPEILFLHADLQYYLSQSCGAFVCMAAQ.EVIE			
219						
220	PopP2	336	Fold-switching motif Regulatory domain			
221	HopZ1a AvrBst YopJ AvrA Ch1aDUB1	231 237 187 187 358	AAQFDDIHHGLQHGTLSTESESRARTILGALEAS.SYSYMHEGAHAAFGADVLPVDFYKHGASLTQÅNQLMKRP DSVFDNLHDCLRRNVRCFSSGERX.S.ILHKNIEFIEGDKELPPIFYKHSHSRGVVGEFISNQ RDSLLKIHEDNIKGILSDGENPLP			
222	SseL ElaD	296 331	LLSNAGONDPATTLREFAERFLTLSV.EEQAFNTOIRROIYEYSLO QMESNSDSAPYTLLKNYADRFKKYSA.EEQYEIDFQHRLENRNCYLDKYGDANINHYYRNL			
223			Regulatory domain			
224	PopP2 HopZ1a AvrBst YopJ AvrA	399 305 298 240 240	PEQSTVPVNKRREILGERTTRHLVRRKVRRADSEGRVTSGETKEITFSNSVEQKRIALLNRAASYVNSAP DGRMAGRVNSEGHSEAENLVQRNQAFRVKRELLDDETPSNTQFSAIDGFRLQEIKRVLAEEQR. PEYAHKNVSIGRTNPSEDLSERVENFRVR. PQGVGIVVNKKREIVNREDNNKSIVGKELSVSVIKKRIAESTLRKIES PAAGSSIVNKKREIVNREDNNKSI			
225	Ch1aDUB1 3 SseL E1aD 3	396 391	EIKNSHPKNRAS.			
226	Supple	mei	ntary Figure 11. Sequence alignment of representative CE clan			
of proteases. The same sequences were used to perform phylogenetic analysis						
228	and se	que	nce alignment. The fold switching motif and regulatory domain are			

marked by red and blue boxes, respectively. 229

	apo-PopP2
	(7F3N)
Data collection	
Space group	P 3 <sub>2</sub> 2 1
Cell dimensions	
a, b, c (Å)	89.4,89.4, 79.9
$\alpha, \beta, \gamma(^{\circ})$	90.00, 90.00,120.00
Resolution (Å)	50.00-2.35(2.43-2.35)
R <sub>merge</sub>	0.136(0.702)
$I/\sigma(I)$	15.9(2.6)
$CC_{1/2}$	0.984(0.898)
Completeness (%)	100.0(100.0)
Redundancy	9.9(10.2)
Refinement	
Resolution (Å)	50.00-2.35
No. reflections	15654
$R_{ m work}$ / $R_{ m free}$	0.193/0.231
No. atoms	
Protein	1853
Water	34
B factors	
Protein	68.0
Water	55.4
R.m.s.deviations	
Bond lengths (Å)	0.004
Bond angles (°)	0.78

#### 231 Supplementary Table 1. Data collection and refinement statistics

<sup>a</sup>Values in parentheses are for highest-resolution shell.

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# 236 Supplementary Table 2. Energies of RRS1<sub>WRKY</sub> binding to apo- and InsP6-

	$\Delta$ G (kcal/mol)	$\Delta$ H (kcal/mol)	-T $\triangle$ S (kcal/mol)
apo-PopP2	$-3.8 \pm 3.8$	$-59.7 \pm 5.9$	$55.9 \pm 8.7$
InsP6-bound PopP2	$-9.1 \pm 4.3$	$-85.3 \pm 8.1$	$76.2 \pm 10.5$

237 bound PopP2. All terms are in kcal/mol.

## 242 243 Supplementary Table 3. Primers used in this study.

Primer name	sequence
AttB1-PopP2	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGAAGGTCAGTAGCGCA
AttB2-PopP2	GGGGACCACTTTGTACAAGAAAGCTGGGTCGTTGGTATCCAATAGGGAAT
PopP2-Fw	ATGAAGGTCAGTAGCGCAAACGC
PopP2-Rev	GTCGTTGGTATCCAATAGGGA
PopP2-C321A-Fw	TCCTTCTTCGATGCCCGGATACTCTCCCTGTCACT
PopP2-C321A-Rev	GAGAGTATCCGGGCATCGAAGAAGGACTTCTGAAT
P2-L369-V370P-Fw	AGGAACTTGGCGCTACCCCACCGCTTGATGGTGCGCCACTGGT
P2-L369-V370P-Rev	TGGCGCACCATCAAGCGGTGGGGTAGCGCCAAGTTCCTCCGT
P2-L371-D372P-Fw	GGCGCTACCCTTGTGCCTCCAGGTGCGCCACTGGTCGA
P2-L371-D372P-Rev	ACCAGTGGCGCACCTGGAGGCACAAGGGTAGCGCCAAGT
P2-R380A-Fw	TGGTCGACGCCGCTATGATGAAACATGGTCAAGCCGCA
P2-R380A-Rev	ACCATGTTTCATCATAGCGGCGTCGACCAGTGGCGCAC
P2-K383A-Fwd	GTATGATGGCACATGGTCAAGCCGCA
P2-K383A-Rev	CTTGACCATGTGCCATCATACGGGCGT