Supplementary Material

A conserved EAR motif is required for avirulence and stability of the *Ralstonia solanacearum* effector PopP2 in planta

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The Supplementary Material for this article includes 5 figures, 1 table and references.



Supplementary Figure 1. Regional map of the Republic of Korea with location of strain isolation. Strains labelled in pink did not harbor *popP2* (Pe_4, Pe_57, To_52). Strains labelled in blue harbored the "Pe_1" allele (Pe_1, Pe_26, Pe_28, Pe_40 and To_63). Strains labelled in black harbored the "Pe_2" allele (Pe_2, Pe_3, Pe_18, Pe_24, Pe_27, Pe_45, Pe_56, To_1, To_7 and To_42). Pe_13 is labelled in grey.



Supplementary Figure 2. PopP2 does not interact with known transcriptional corepressors in yeast. Growth of yeast cells expressing LexA-DNA-binding domain (DBD) fusion (bait) and B42-activation domain (AD) fusion (prey) as indicated. Yeast cells were grown on non-selective media lacking histidine, tryptophan and uracil (-H/-T/-U) or selective media also lacking leucine (-H/-T/-U/-L) and supplemented with X-Gal. TPL, TPR4 and SAP18 B42-AD fusions are weakly auto-active as indicated by the growth of yeast cells expressing LexA-DBD alone and B42-AD protein fusions. This experiment was conducted twice with similar results.



Supplementary Figure 3. The SRDX motif partially restores PopP2^{LAAL} **avirulence activity in Arabidopsis.** PopP2 variants C-terminally tagged with 6xHA were delivered by *Pseudomonas fluorescens* Pf0-1(T3S) into Ws-2 leaves and photographs were taken 1 day after infiltration. A red asterisk indicates visible cell death. This experiment was conducted 3 times with similar results.

Ws-2



Supplementary Figure 4. The EAR motif is required for PopP2-mediated inhibition of *P. fluorescens* **Pf0-1-induced PTI in** *N. benthamiana.* 3x10⁸ cfu/ml of *P. fluorescens* Pf0-1 carrying empty vector or PopP2 variants (WT, C321A, LAAL, LAAL-SRDX or LAAL-srdx) were infiltrated in 5-week old *N. benthamiana* leaves (in indicated circles as 'Pf0-1'). After 8hours, 2x10⁷ cfu/ml of *Pto* DC3000 was infiltrated (in indicated circles as 'DC3000'). *Pto* DC3000-triggered tissue collapse was scored at 48hpi after *Pto* DC3000 infiltration. This experiment was conducted 4 times with similar results.



Supplementary Figure 5. In planta processing of Pto DC3000-delivered AvrRps4N:PopP2:6xHA variants in N. benthamiana. The AvrRps4N:PopP2 variants C-terminally tagged with 6xHA were delivered by Pto DC3000 $(1x10^{9}$ cfu/ml) in N. benthamiana. N. benthamiana leaf samples were harvested at 10 hours post infiltration. Protein accumulation of PopP2 variants was confirmed by immunoprecipitation followed by immunoblot analysis using α -HA antibody. Protein loading was confirmed with Ponceau staining.

Corepressor	Chromatin modifier (HDAC)	Repressor recruitment	Cofactor recruitment	References
HOS15	HOS15	Unknown		Zhu et al., 2008
LUG	HDA19	-		Gonzalez et al., 2007
LUH	Unknown	-		Shrestha et al., 2014
SAP18	HDA19	EAR motif		Song & Galbraith, 2006; Hill et al., 2008
SEU	- LUG - HDA19 - LUH - Unknown	Unknown	LUFS domain of LUG to form corepressor complex	Sridhar et al., 2004; Sitaraman et al., 2008; Gonzalez et al., 2007; Shrestha et al., 2014
SLK1	- LUG - HDA19	Unknown		Gonzalez et al., 2007
	- LUH - Unknown			Stahle et al., 2009; Shrestha et al., 2014
SLK2	- LUG - HDA19	Unknown		Gonzalez et al., 2007
	- LUH - Unknown			Stahle et al., 2009; Shrestha et al., 2014
TPL	HDA19	EAR motif		Long et al., 2006; Szemenyei et al., 2008; Causier et al., 2012; Krogan et al., 2012
TPR1	HDA19	EAR motif		Zhu et al., 2010; Causier et al., 2012; Lee et al., 2016
TPR2	Unknown, likely HDA19	EAR motif		Causier et al., 2012; Lee et al., 2016
TPR3	Unknown, likely HDA19	EAR motif		Causier et al., 2012; Lee et al., 2016
TPR4	Unknown, likely HDA19	EAR motif		Causier et al., 2012; Lee et al., 2016

Supplementary Table 1. Co-repressor library screened in the Y2H assay. Details of the co-repressor library screened for interaction with PopP2 in the yeast-two-hybrid (Y2H) assay.

Supplementary References

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