The Small Molecule Zaractin Activates ZAR1-Mediated Immunity in Arabidopsis

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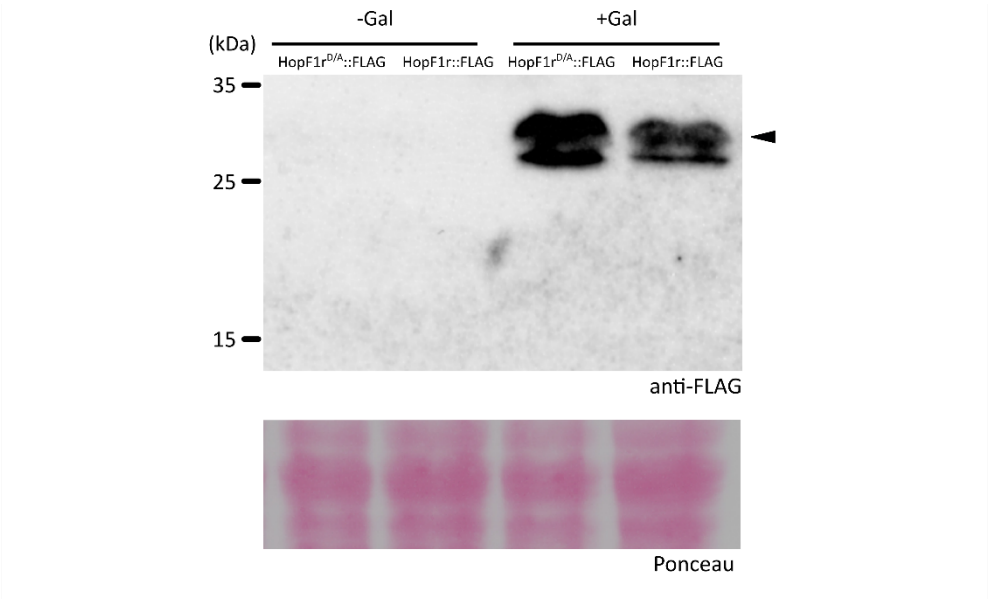
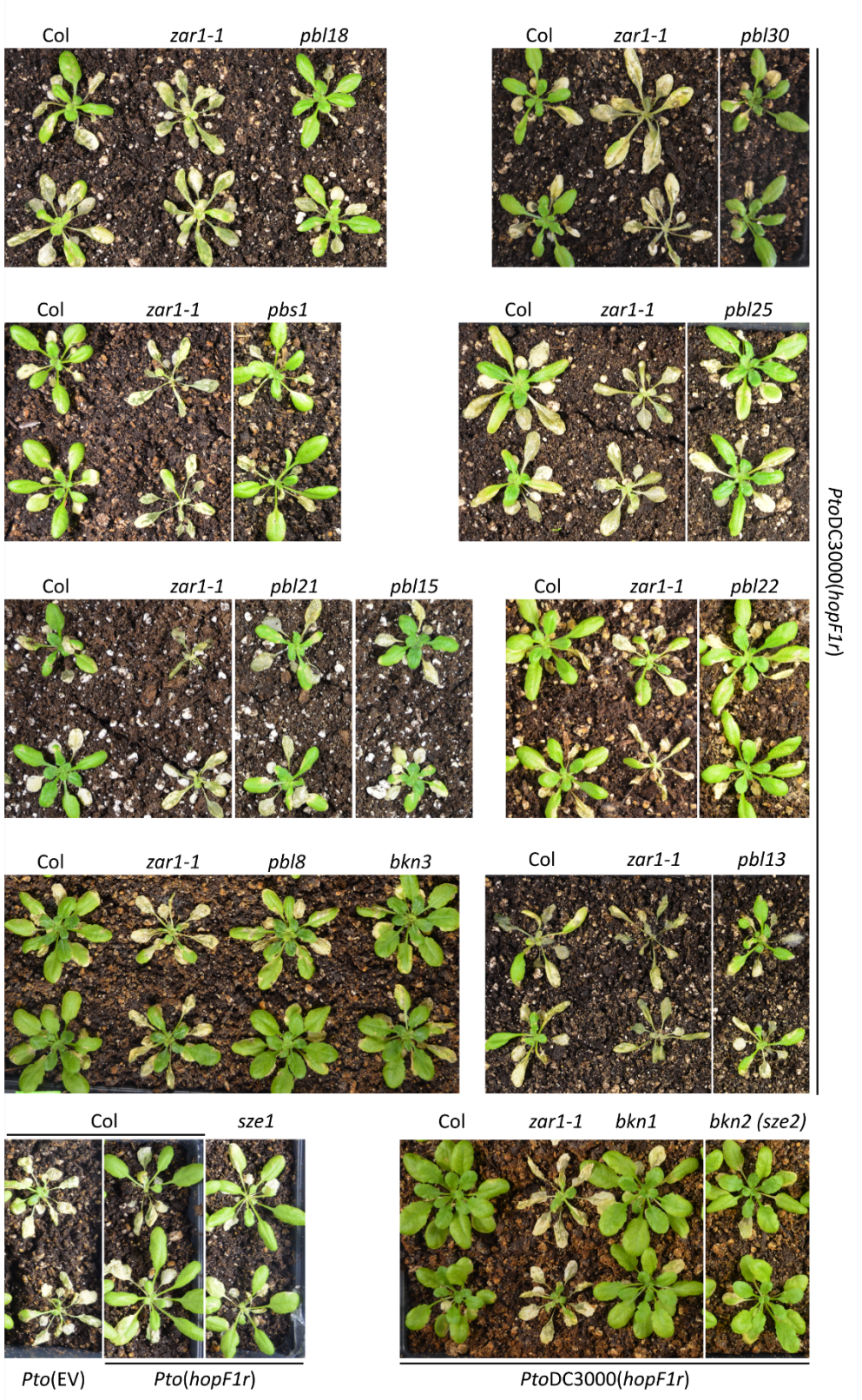
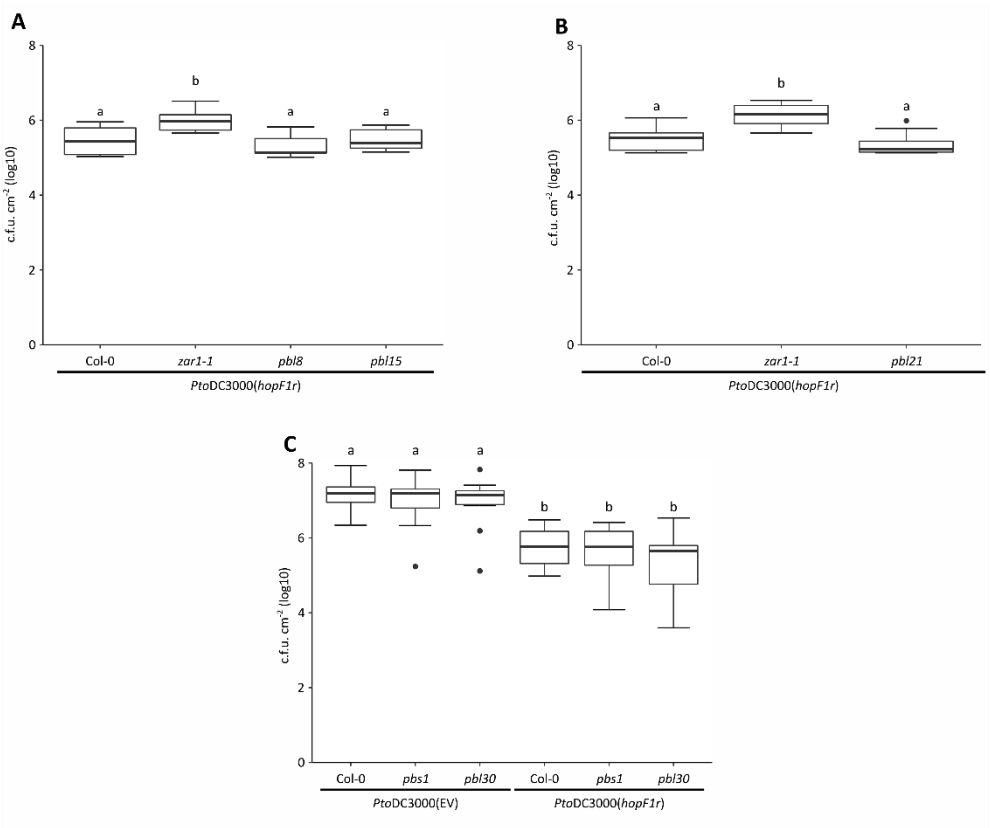
Supporting Information



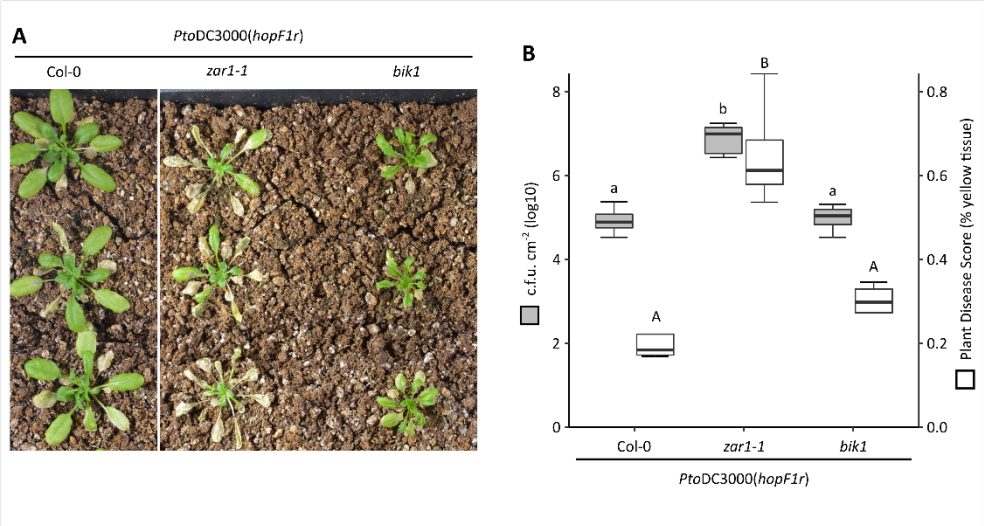
Supplementary Fig. 1 | Genomic expression of HopF1r::FLAG and HopF1r$^{D/A}$::FLAG in yeast. The yeast strain EGY48 containing genomic integrations of *hopF1r* or *hopF1r$^{D/A}$* was grown on YNB supplemented with 2% glucose (first two lanes) or with 1 % raffinose and 2 % galactose (last two lanes) to induce expression of constructs. Expression of FLAG-tagged proteins was detected with anti-FLAG M2 antibody at a dilution of 1/15000 (Sigma). The black arrow indicates size of HopF1r::FLAG (~25 kDa).


Col
zar1-1
pbl18
Col
zar1-1
pbl30
Col
zar1-1
pbs1
Col
zar1-1
pbl25
Col
zar1-1
pbl21
pbl15
Col
zar1-1
pbl22
Col
zar1-1
pbl8
bkn3
Col
zar1-1
pbl13
Col
sze1
Col
zar1-1
bkn1
bkn2 (sze2)
Pto(EV)
Pto(hopF1r)
PtoDC3000(hopF1r)
PtoDC3000(hopF1r)

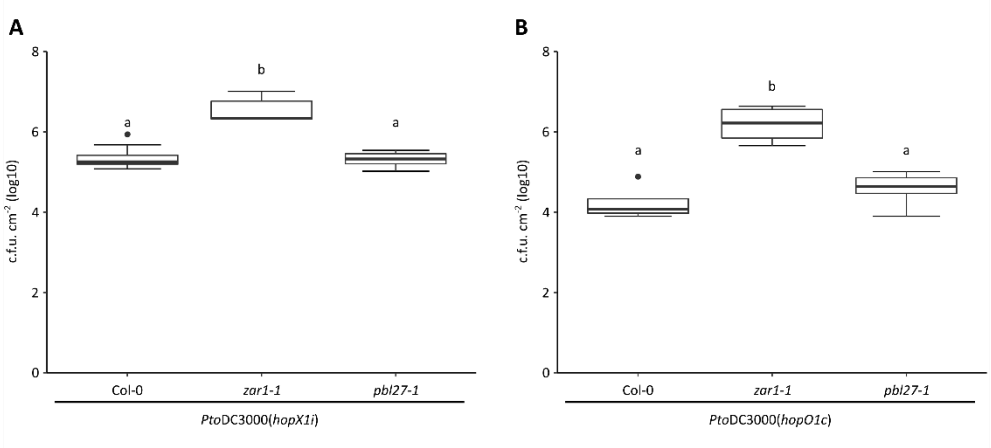
Supplementary Fig. 2 | Multiple Arabidopsis *pbl* knockout lines remain resistant to *Pto*DC3000(*hopF1r*). Disease phenotypes of Col-0, *zar1-1*, or indicated *pbl* knockout Arabidopsis plants after spraying with *Pto*DC3000(*hopF1r*) or *Pto*DC3000(EV) at OD_{600} = 1.0. Symptoms pictured are 14 days post-infection. T-DNA insertion lines are listed in Table S1 and were all genotyped for homozygosity.



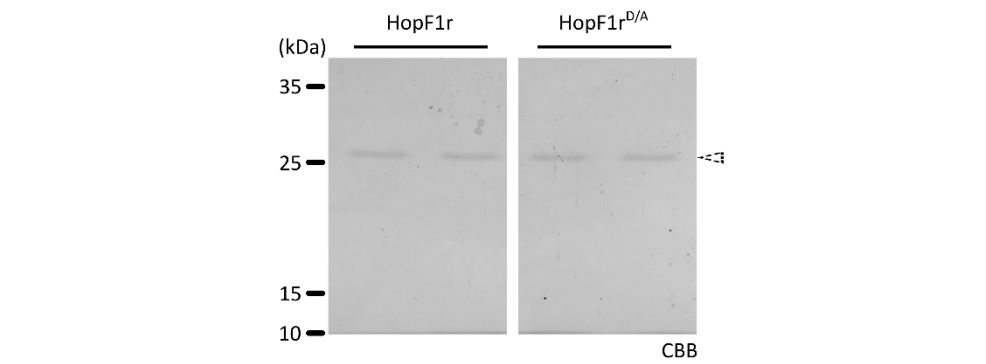
Supplementary Fig. 3 | Multiple Arabidopsis *pbl* knockout lines remain resistant to *Pto*DC3000(*hopF1r*) as demonstrated by bacterial growth assays. Bacterial growth on Arabidopsis Col-0, *zar1-1*, **(A)** *pbl8*, *pbl15*, **(B)** *pbl21,* **(C)** *pbs1*, or *pbl30* plants after spraying with *Pto*DC3000(*hopF1r*) or *Pto*DC3000(EV) as indicated at OD_{600} = 1.0. Letters represent statistically significant differences (Tukey's HSD, $P < 0.05$). T-DNA insertion lines are listed in Table S1 and were all genotyped for homozygosity.



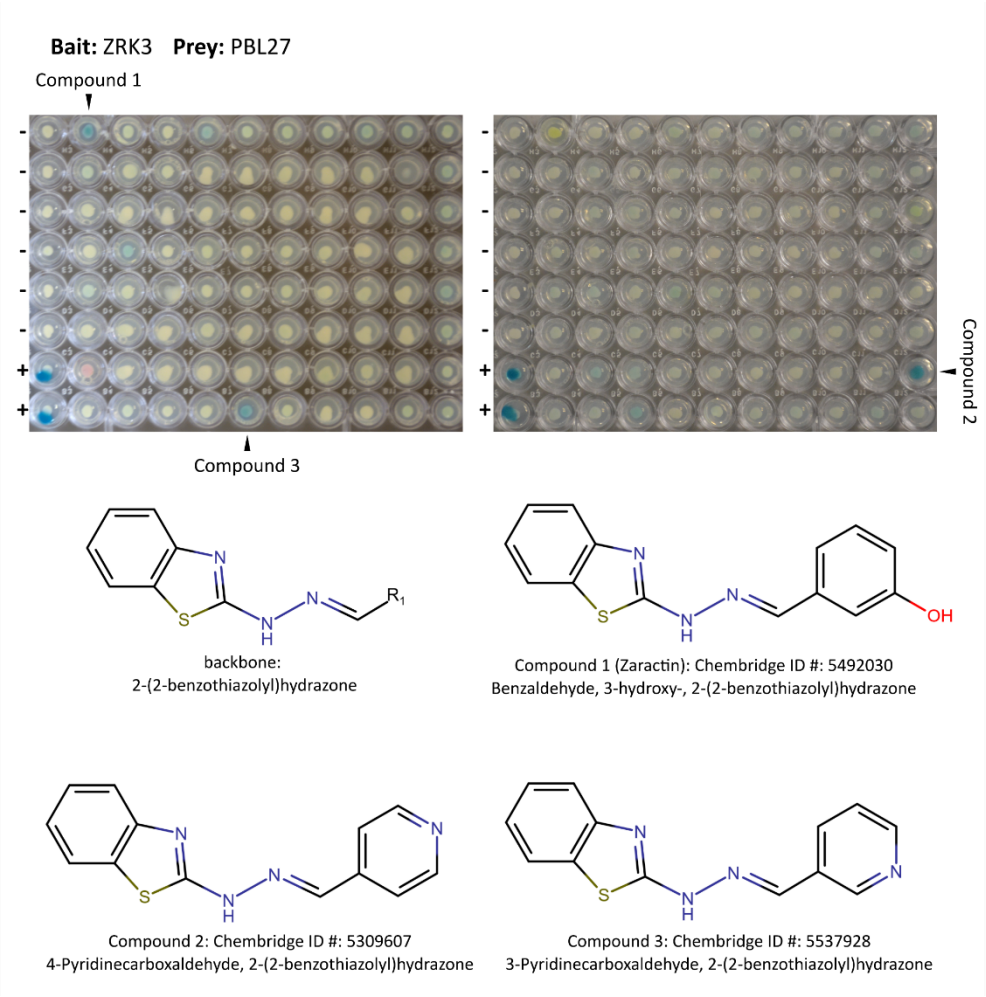
Supplementary Fig. 4 | *BIK1* is not required for HopF1r-triggered immunity in Arabidopsis. (A) Disease phenotypes of Col-0, *zar1-1*, or *bik1* Arabidopsis plants after spraying with *Pto*DC3000(*hopF1r*) at OD_{600} = 1.0. Symptoms pictured are 14 days post-infection. **(B)** Growth of *Pto*DC3000(*hopF1r*) (grey) or PIDIQ disease quantification (white) on Col-0, *zar1-1*, and *bik1* plants. Bacterial counts were taken at 3 days post-infection. Lowercase letters represent statistically significant differences (Tukey's HSD, $P < 0.05$). Experiments were replicated three times with similar results. Capital letters represent statistically significant differences for PIDIQ disease quantification (Tukey's HSD, $P < 0.05$). *bik1* line was genotyped for homozygosity.



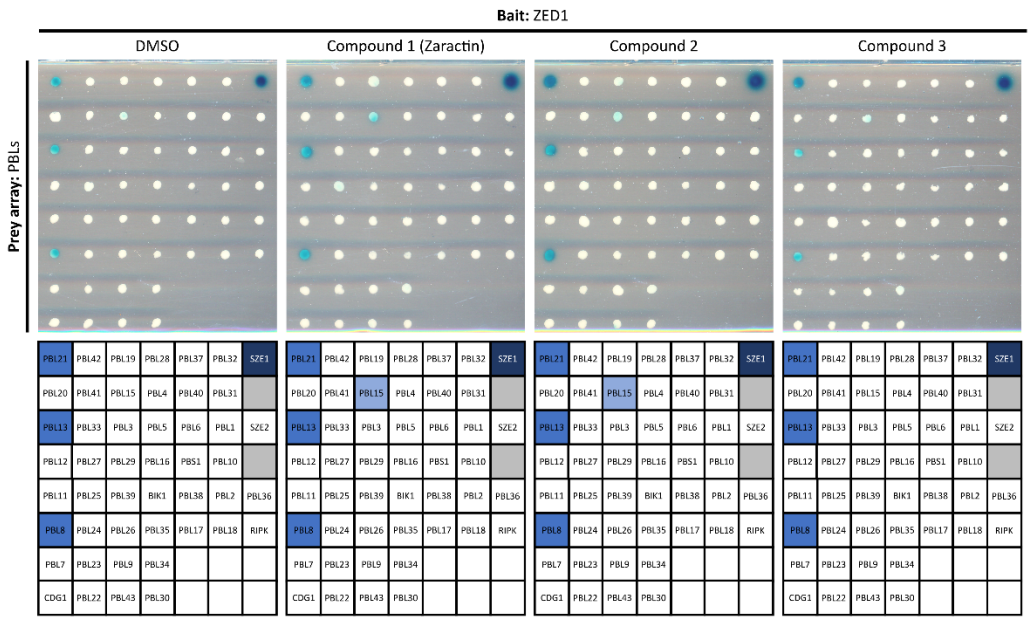
Supplementary Fig. 5 | *PBL27* is not required for HopX1i- and HopO1c-triggered immunity in Arabidopsis. Growth of **(A)** *Pto*DC3000(*hopX1i*) or **(B)** *Pto*DC3000(*hopO1c*) on Col-0, *zar1-1*, or *pbl27-1*, *pbl27-2* plants after spraying at OD_{600} = 1.0. Bacterial counts were taken at 3 days post-infection. Letters represent statistically significant differences (Tukey's HSD, $P < 0.05$). Experiments were replicated three times with similar results.



Supplementary Fig. 6 | Purified HopF1r and HopF1r$^{D/A}$ used for ADP-ribosylation. The same amount of purified His::HopF1r or His::HopF1r$^{D/A}$ used in the ADP-ribosylation assay (Fig. 1D) was run on SDS-PAGE and Coomassie stained (CBB) to indicate size and equal loading of enzyme. Dashed arrow indicates position of His::HopF1r (~25 kDa).

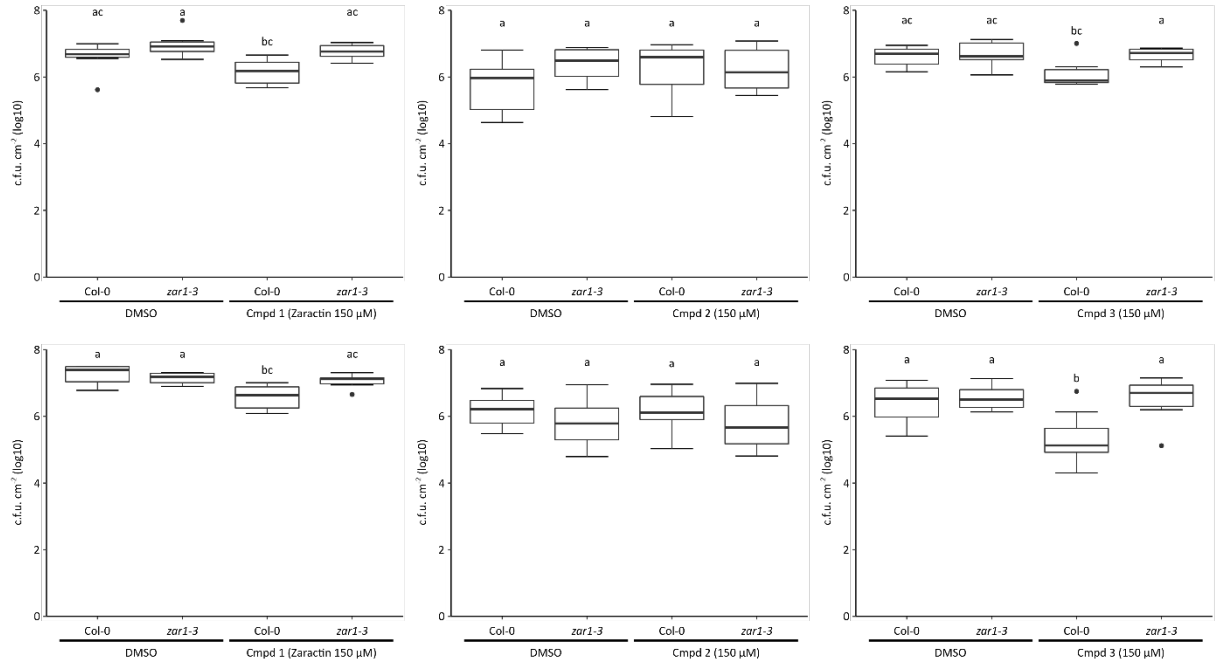


Supplementary Fig. 7 | Chemical screen to induce the ZRK3-PBL27 interaction. Y2H assay screening plates of induced interactions between ZRK3 and PBL27 against a subset of the Yeast-Active chemical library showing the three top hits of the screen. All chemicals were tested at a concentration of 30 µM. The first column of each plate includes two positive control (+) wells [HopZ1a-induced ZED1-PBL15 interaction][6], and 6 negative control (-) wells (DMSO). Structures of chemicals identified as strongest inducers of ZRK3-PBL27 interactions from Y2H chemical screen and their common backbone are displayed below.

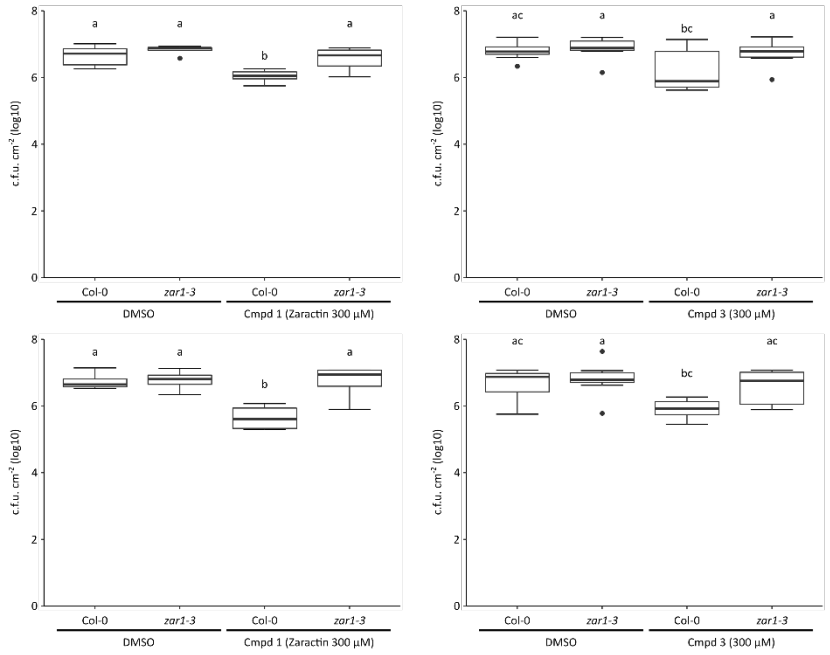


Supplementary Fig. 8 | Zaractin and related chemicals do not induce a ZED1-PBL27 interaction. Y2H assay testing interactions between ZED1 and a PBL array against chemicals that were identified as the strongest inducers of the ZRK3-PBL27 interaction (Fig. S7). Panels from left to right: DMSO, Compound 1 (Zaractin), Compound 2, Compound 3. All chemicals were tested at a concentration of 30 µM. Interaction layouts are depicted on right. Grey boxes are not relevant to this study.

A

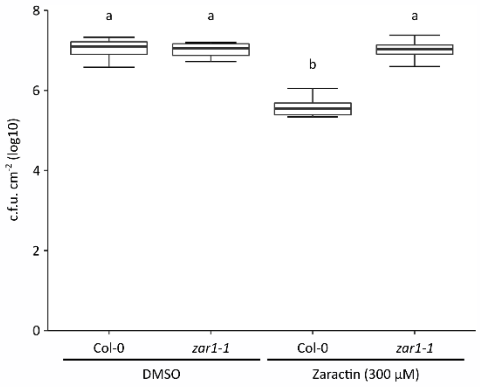

c.f.u. cm⁻² (log10)
Col-0
zar1-3
DMSO
Cmpd 1 (Zaractin 150 µM)
Cmpd 2 (150 µM)
Cmpd 3 (150 µM)

B

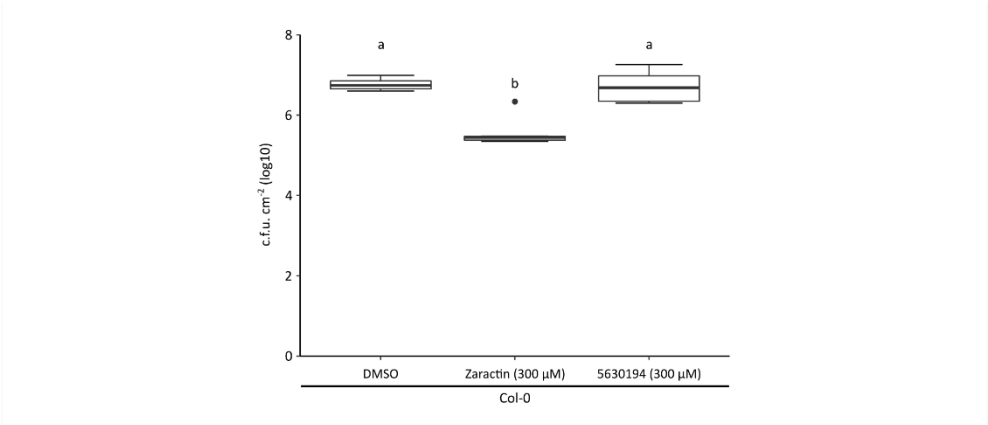

c.f.u. cm⁻² (log10)
Col-0
zar1-3
DMSO
Cmpd 1 (Zaractin 300 µM)
Cmpd 3 (300 µM)

1

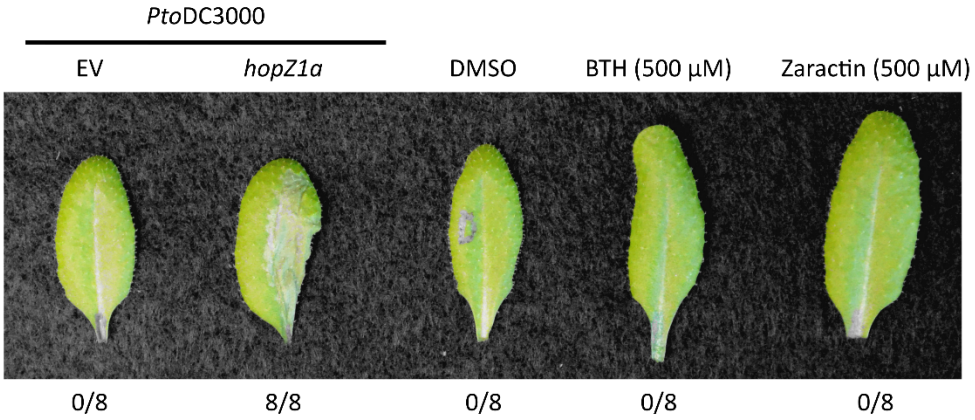
Supplementary Fig. 9 | Immune induction dose-response for the three compounds identified in our chemical screen. **(A)** Growth of *Pto*DC3000 on Col-0 or *zar1-3* Arabidopsis plants treated with DMSO or 150 µM of indicated compound 2 days before infection. Duplicate experiments are shown for Compound 1 (Zaractin; left panels), Compound 2 (middle panels), and Compound 3 (right panels). **(B)** Growth of *Pto*DC3000 on Col-0 or *zar1-3* plants treated with DMSO or 300 µM of indicated compound 2 days before infection. Duplicate experiments are shown for Compound 1 (Zaractin; left panels) and Compound 3 (right panels). Plants were sprayed with *Pto*DC3000 at OD_{600} = 1.0. Letters represent significance groups (Tukey's HSD, $P < 0.05$).



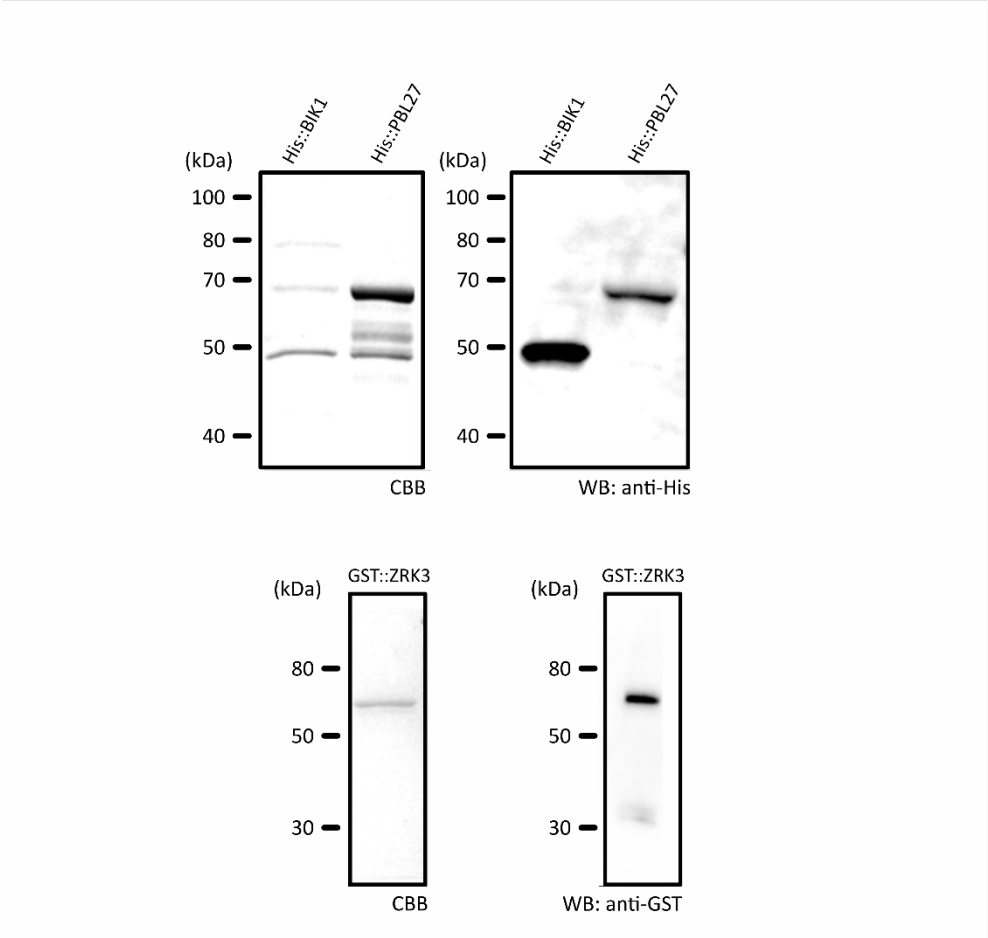
Supplementary Fig. 10 | Zaractin triggers immunity in a *ZAR1*-dependent manner using an independent T-DNA line. Growth of *Pto*DC3000 on Col-0 or *zar1-1* Arabidopsis plants treated with DMSO or 300 µM Compound 1 (Zaractin) 2 days before infection. Plants were sprayed with *Pto*DC3000 at OD_{600} = 1.0. Letters represent statistically significant differences (Tukey's HSD, $P < 0.05$).



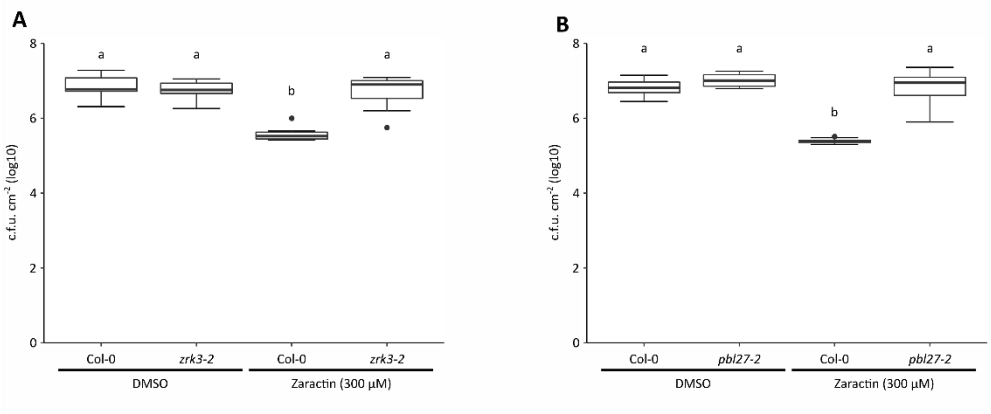
Supplementary Fig. 11 | Modification of the Zaractin R group compromises its activity. Growth of *Pto*DC3000 on Arabidopsis Col-0 plants treated with DMSO, 300 µM Compound 1 (Zaractin), or 300 µM of a closely related compound (Chembridge ID: 5630194) 2 days before infection. Plants were sprayed with *Pto*DC3000 at OD_{600} = 1.0. Letters represent statistically significant differences (Tukey's HSD, $P < 0.05$).



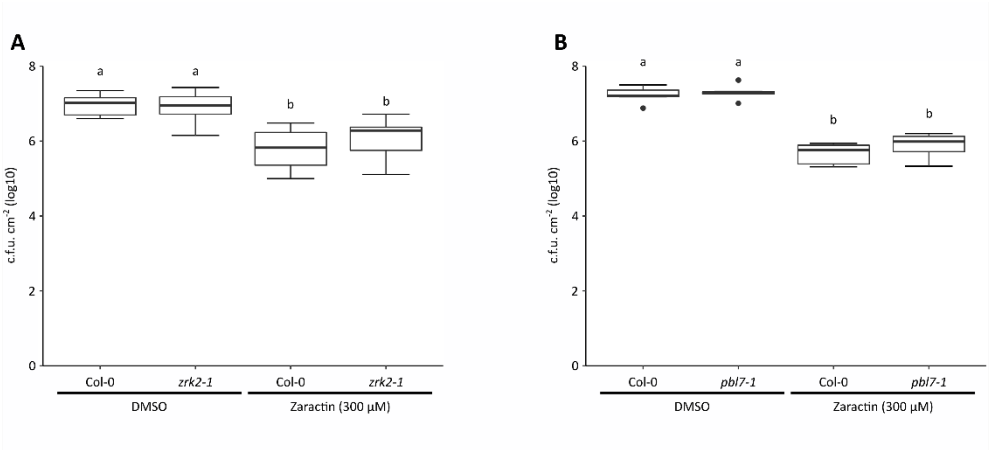
Supplementary Fig. 12 | Zaractin does not trigger a hypersensitive response in Arabidopsis. *Pto*DC3000 (EV), *Pto*DC3000(*hopZ1a*), DMSO, BTH, or Zaractin were infiltrated into half leaves of Arabidopsis Col-0 plants. The images were taken 16 h post-infection (hours post infiltration). The proportion of leaves showing an HR is indicated below each construct.



Supplementary Fig. 13 | Purification of His::BIK , His::PBL27, and GST::ZRK3 tagged proteins. Both His::BIK1 and His::PBL27 proteins were purified by CapturemTM His-tagged purification kit, and GST::ZRK3 proteins were purified with Glutathione Sepharose 4B matrix. The proteins were visualized by Coomassie stain (CBB) and confirmed by Western blot analysis (WB) with His-HRP and GST-HRP conjugated antibodies.



Supplementary Fig. 14 | Zaractin triggers immunity in a *ZRK3*-, and *PBL27*-dependent manner using independent T-DNA lines. Growth of *Pto*DC3000 on Arabidopsis Col-0, **(A)** *zrk3-2* or **(B)** *pbl27-2* plants treated with DMSO or 300 µM Compound 1 (Zaractin) 2 days before infection. Plants were sprayed with *Pto*DC3000 at OD_{600} = 1.0. Letters represent statistically significant differences (Tukey's HSD, $P < 0.05$).



Supplementary Fig. 15 | Zaractin insensitivity is not a general property of T-DNA insertion lines.

Growth of *Pto*DC3000 on Arabidopsis Col-0, **(A)** *zrk2-1*, or **(B)** *pbl7-1* plants treated with DMSO or 300 µM Compound 1 (Zaractin) 2 days before infection. Plants were sprayed with *Pto*DC3000 at OD_{600} = 1.0. Letters represent statistically significant differences (Tukey's HSD, $P < 0.05$).

Table S1: List of ZRK3-PBL Y2H interactions.

ZRK3 interaction type	Gene locus	PBL/Gene name	T-DNA line
Not enhanced by HopF1r	At5g11360	*BKN3*	SALK_092036
	At5g11400	*BKN1*	*bkn1-3* (Daphne Goring: CRISPR)
	At5g11410	*SZE2* (*BKN2*)	*bkn2-2* (Daphne Goring: CRISPR)
HopF1r-enhanced	At1g20650	*PBL21*	SALK_031606
	At1g61590	*PBL15*	SALK_055095
	At1g69790	*PBL18*	SALK_202072
	At1g76370	*PBL22*	SALK_045159
	At2g39660	*BIK1*	SALK_005291 (Jacqueline Monaghan)
	At3g24790	*PBL25*	SALK_036509
	At4g35600	*PBL30*	SALK_099176
	At5g01020	*PBL8*	SALK_044339
	At5g13160	*PBS1*	SALK_023996
	At5g18610	*PBL27*	CS408439 (*pbl27-1*); CS400031 (*pbl27-2*)
	At5g25440	*SZE1*	SALK_083971 (*sze1-3*)
	At5g35580	*PBL13*	SALK_203557

Table S2: List of chemicals used for Y2H experiments

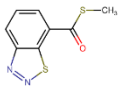
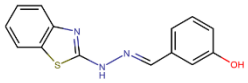
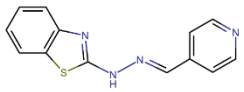
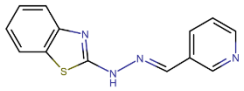
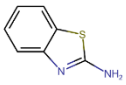
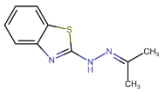
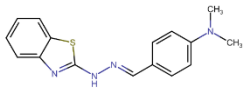
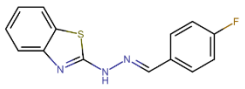
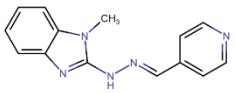
Chemical Structure	Name	Source
	Acibenzolar-S-methyl (benzothiadiazole/BTH deriviative)	MilliporeSigma Product #: 32820
	Compound 1 (Zaractin): Benzaldehyde, 3-hydroxy-, 2-(2-benzothiazolyl)hydrazone	Chembridge ID #: 5492030
	Compound 2: 4-Pyridinecarboxaldehyde, 2-(2-benzothiazolyl)hydrazone	Chembridge ID #: 5309607
	Compound 3: 3-Pyridinecarboxaldehyde, 2-(2-benzothiazolyl)hydrazone	Chembridge ID #: 5537928
	1,3-benzothiazol-2-amine	Chembridge ID #: 5108799
	acetone 1,3-benzothiazol-2-ylhydrazone	Chembridge ID #: 5549542
	4-(dimethylamino) benzaldehyde 1,3-benzothiazol-2-ylhydrazone	Chembridge ID #: 5483044
	4-fluorobenzaldehyde 1,3-benzothiazol-2-ylhydrazone	Chembridge ID #: 5630194
	1-methyl-2-[(2E)-2-[(pyridin-4-yl)methylidene] hydrazin-1-yl]-1H-1,3-benzodiazole	Molport-002-929-064

Table S3: Binding of Zaractin to PBL27 by thermal shift assay

	PBL27	BIK1
Control	49.3 ± 0.42	45.1 ± 0.87
Zaractin (30 nM)	47.6 ± 0.59*	45.3 ± 0.81
BTH (30 nM)	48.3 ± 0.3	45.9 ± 1.8

Values represent mean melting temperature (°C) with standard error. * Represents significant difference from the corresponding control treatment ($P < 0.05$). Data is representative of four independent biological samples with four technical replicates in each experiment.