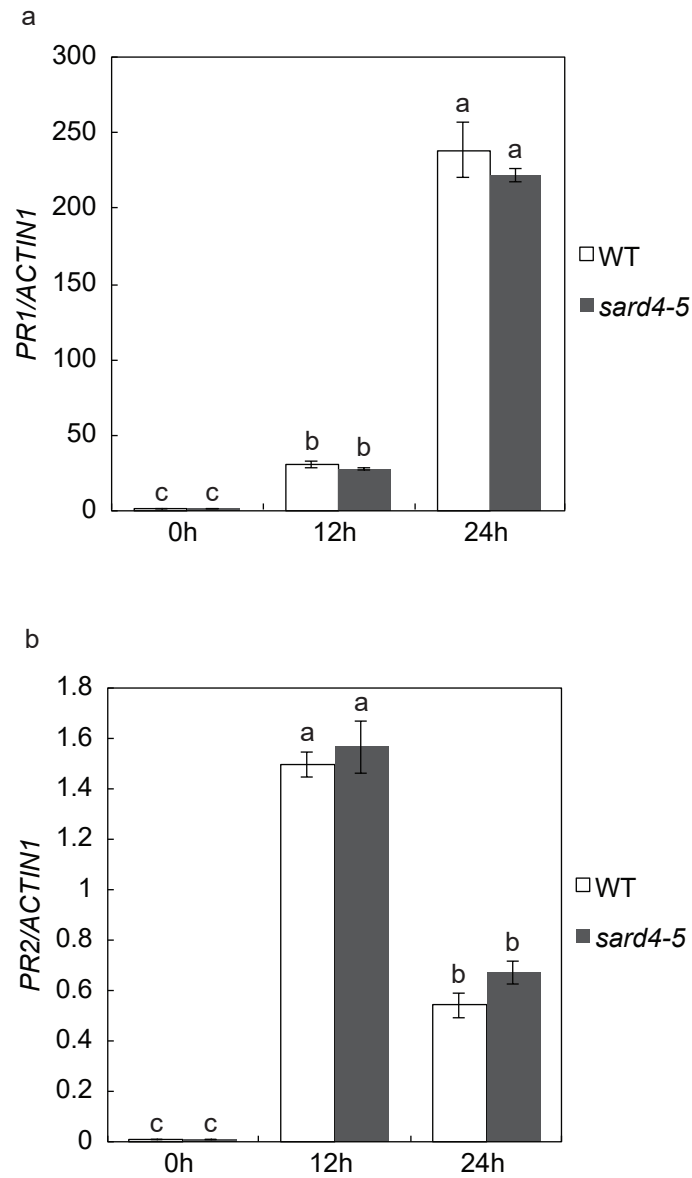
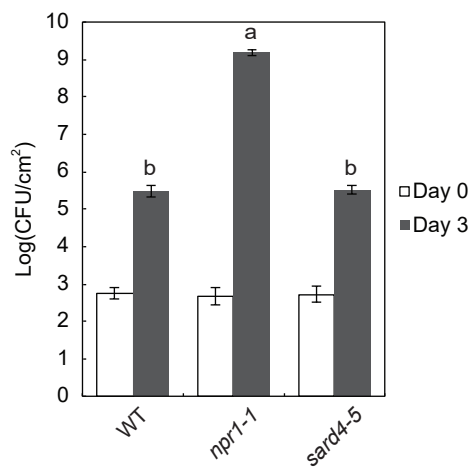


Supplemental Figure 1. SARD4 is not required for local SA accumulation induced by *P.s.m.* ES4326. Leaves of four-week-old plants were infiltrated with *P.s.m.* ES4326 ($OD_{600}=0.005$), and the inoculated leaves were collected 12h and 24h later for SA extraction. Free SA and total SA levels were quantified by high-performance liquid chromatography. Statistical differences between the samples are labeled with different letters ($P < 0.05$, one-way ANOVA; $n = 4$). The experiment was repeated twice with similar results.

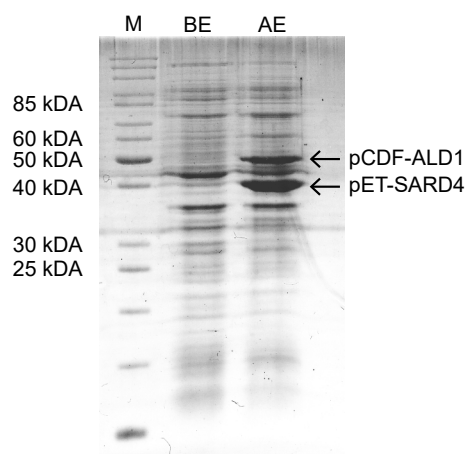


Supplemental Figure 2. SARD4 is not required for local PR gene expression induced by *P.s.m.* ES4326. *PR1* (a) and *PR2* (b) expression in WT and *sard4-5* induced by *P.s.m.* ES4326. Three-week-old plants were infiltrated with *P.s.m.* ES4326 ($OD_{600}=0.005$), and the inoculated leaves were collected 12h and 24h later for RT-qPCR analysis. Values were obtained from abundances of *PR1* and *PR2* transcripts normalized against that of *ACTIN1*. Statistical differences among the samples are labeled with different letters ($P < 0.01$, one-way ANOVA; $n = 3$). Similar results were obtained in three independent experiments.



Supplemental Figure 3. SARD4 is not required for local resistance against *P.s.m.* ES4326.

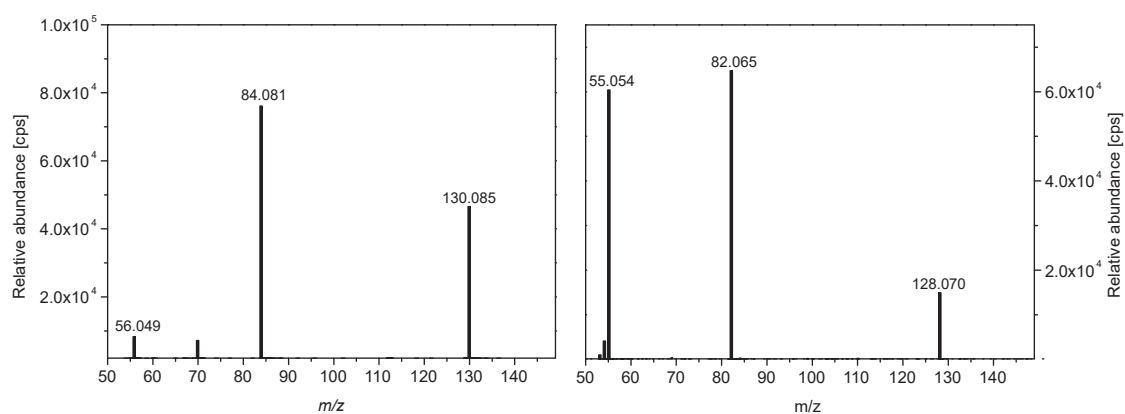
Leaves of four-week-old WT, *npr1-1* and *sard4-5* plants were infiltrated with a *P.s.m.* ES4326 ($OD_{600}=0.0001$). Bacterial growth was determined three days post inoculation. cfu, colony forming unit. Statistical differences between the samples are labeled with different letters ($P < 0.01$, one-way ANOVA; $n = 6$). The experiment was repeated twice with similar results.



Supplemental Figure 4. Heterologous expression of pCDF-ALD1 and pET-SARD4 in a single *E.coli* liquid culture for the in-cell activity assay.

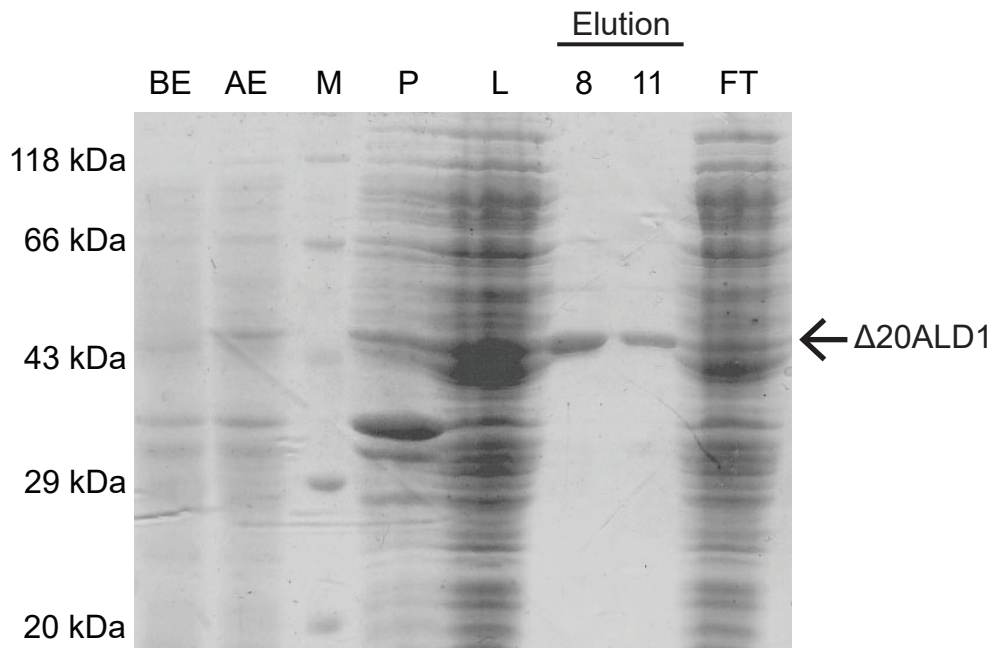
Coomassie brilliant blue-stained SDS-PAGE showing proteins before (BE) and after induction (AE) of protein expression. Proteins of the correct molecular weight (ALD1 in pCDFDuet vector, 51.6 kDa respectively SARD4 in pET24, 38.0 kDa) are marked with black arrows.

Similar results were obtained in two independent experiments.



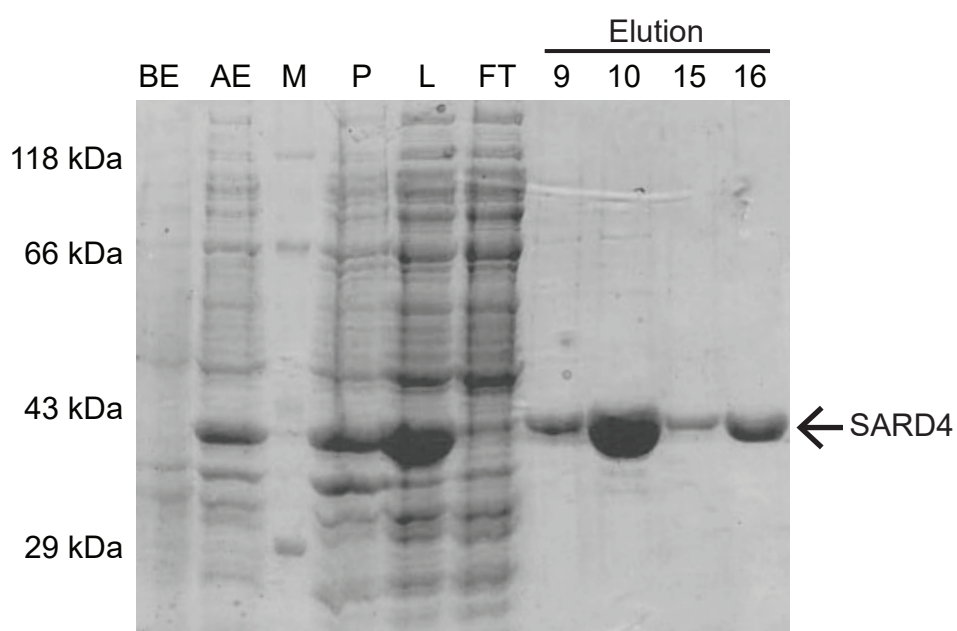
Supplemental Figure 5. MS/MS fragmentation patterns of Pip and P2C from in-cell assay.

MS/MS fragmentation pattern of (a) Pip and (b) P2C detected in the *E. coli* cultures expressing only ALD1 (a), and ALD1 and SARD4 together (b), respectively. The fragmentation of Pip (a) and P2C (b) leads to a loss of the carboxyl group (m/z 84.081 and m/z 82.065 respectively). In Pip, the mass signal of m/z 56.049 represents a C₃H₆N-fragment. In P2C the C=N double bond stays intact so that the mass signal of m/z 55.054 represents a C₄H₇-fragment.



Supplemental Figure 6. SDS-PAGE analysis of heterologous expressed 6×His-tagged $\Delta 20$ ALD1 protein (~ 50 kDa) purified by affinity chromatography.

Coomassie brilliant blue-stained SDS-PAGE gel shows total proteins before (BE) and after induction (AE) of $\Delta 20$ ALD1 expression in *E. coli* and different fractions from the purification process. Protein bands of the correct molecular weight are marked with a black arrow. M, Molecular marker; P, pellet fraction; L, lysate fraction before purification; FT, collected flow through. The results are representative for three independent experiments.



Supplemental Figure 7. SDS-PAGE analysis of heterologously expressed 6×His-tagged SARD4 protein (~ 36.4 kDa) purified by affinity chromatography.

Coomassie brilliant blue-stained SDS-PAGE gel shows total proteins before (BE) and after induction (AE) of SARD4 expression in *E.coli* and different fractions from the purification process. Protein bands of the correct molecular weight are marked with a black arrow. M, Molecular marker; P, pellet fraction; L, lysate fraction before purification; FT, collected flow through. The results are representative for three independent experiments.

Supplemental Table 1. Primers used in this study.

Primer	5'-3' sequence	Purpose
GABI_428E01-F	tacaccgttcatcaccttc	Genotyping
GABI_428E01-R	cccacaaatgtcttctctc	Genotyping
SALK_007673-F	ggtattggtacttacttggag	Genotyping
SALK_007673-R	agaagaaatactctatccggg	Genotyping
RT-SARD4-F	aaggagatttcattcgatagcc	RT
RT-SARD4-R	ctgaggtaagtctcgtgaac	RT
K19E20-F	gacaagaaccacatgagagc	Mapping
K19E20-R	gttatgtgtacacttcaggtc	Mapping
MMN10-F	agctgcaataatgccaagg	Mapping
MMN10-R	gaaccatcaccactggtgag	Mapping
MYN8-F	agtcgtcatgctcatgaacc	Mapping
MYN8-R	ttcacaagggattaagaacc	Mapping
K10D11-F	ctgattcagcacaggtaaca	Mapping
K10D11-R	tctacccaaacggctcacca	Mapping
ALD1-F1	acggaattcatggctcagtctaattgttcttagttc	cloning
ALD1-R1	acgctcgagattggtattagaagtggaagagag	cloning
SARD4-F	cggaattcatggctgcattaccagtatt	cloning
SARD4-R	acgctcgagacaacggctgaggtaagtct	cloning
ALD1-F2	acgggatccatgccaaggctagtttgacttc	cloning
ALD1-R2	acgaagcttctaattggtattagaagtggaag	cloning

Supplemental ANOVA tables**ANOVA for Figure 1B**

	df	SS	MS	F-value	P-value
genotype	3	157.7734	52.5911	34.903	3.31E-06
residuals	12	18.0813	1.5068		

ANOVA for Figure 1C

	df	SS	MS	F-value	P-value
genotype	3	7.2596	2.4199	130.1571	3.97E-07
residuals	8	0.1487	0.0186		

ANOVA for Figure 1D

	df	SS	MS	F-value	P-value
genotype	3	0.0225	0.0075	94.7537	1.37E-06
residuals	8	0.0006	0.0001		

ANOVA for Figure 2B

	df	SS	MS	F-value	P-value
time	1	0	0	2,550.11	9.20E-07
residuals	4	0	0		

ANOVA for Figure 2C

	df	SS	MS	F-value	P-value
genotype	2	574.9904	287.4952	33.7775	6.55E-05
residuals	9	76.6029	8.5114		

Tukey's unequal N HSD groups for Figure 1B

genotype	mean	group
WT	9.0202	a
<i>sard4-4 FMO1-3D</i>	4.5914	b
<i>sard4-3 FMO1-3D</i>	3.8046	b
<i>FMO1-3D</i>	0.1921	c

Tukey's unequal N HSD groups for Figure 1C

genotype	mean	group
<i>FMO1-3D</i>	1.822	a
<i>sard4-3 FMO1-3D</i>	0.048	b
<i>sard4-4 FMO1-3D</i>	0.029	b
WT	0.002	b

Tukey's unequal N HSD groups for Figure 1D

genotype	mean	group
<i>FMO1-3D</i>	0.104	a
<i>sard4-3 FMO1-3D</i>	0.011	b
<i>sard4-4 FMO1-3D</i>	0.002	b
WT	0.001	b

Tukey's unequal N HSD groups for Figure 2B

time	mean	group
24h	0.0019	a
0h	0	b

Tukey's unequal N HSD groups for Figure 2C

treatment	mean	group
WT	16.5376	a
<i>sard4-5 FMO1-3D</i>	13.631	a
<i>FMO1-3D</i>	0.6176	b

ANOVA for Figure 3A, Free SA

	df	SS	MS	F-value	P-value
genotype	1	0.0008	0.0008	6.3293	0.0247
treatment	1	0.0011	0.0011	10.9858	0.0051
genotype × treatment	3	0.002	0.0007	23.3953	2.66E-05
residuals	12	0	0		

Tukey's HSD groups for Figure 3A, Free SA

genotype × treatment	mean	group
WT <i>P.s.m.</i>	0.062	a
<i>sard4-5 P.s.m.</i>	0.04	b
WT mock	0.037	b
<i>sard4-5</i> mock	0.031	b

ANOVA for Figure 3A, Total SA

	df	SS	MS	F-value	P-value
genotype	1	0.6799	0.6799	8.735	0.0104
treatment	1	0.676	0.676	8.6532	0.0107
genotype × treatment	3	1.7485	0.5828	331.403	8.39E-12
residuals	12	0.0211	0.0018		

Tukey's HSD groups for Figure 3A, Total SA

genotype × treatment	mean	group
WT <i>P.s.m.</i>	1.6071	a
WT mock	0.8827	b
<i>sard4-5 P.s.m.</i>	0.8815	b
<i>sard4-5</i> mock	0.7837	b

ANOVA for Figure 3B

	df	SS	MS	F-value	P-value
genotype	1	6.8665	6.8665	1.5402	0.2429
treatment	1	37.7423	37.7423	27.5373	0.0004
genotype × treatment	3	51.2747	17.0916	788.0954	3.18E-10
residuals	8	0.1735	0.0217		

Tukey's HSD groups for Figure 3B

genotype × treatment	mean	group
WT <i>P.s.m.</i>	5.132	a
<i>sard4-5 P.s.m.</i>	2.1285	b
WT mock	0.0944	c
<i>sard4-5</i> mock	0.0721	c

ANOVA for Figure 3C

	df	SS	MS	F-value	P-value
genotype	1	1.0208	1.0208	1.5988	0.2347
treatment	1	5.4675	5.4675	28.2096	0.0003
genotype × treatment	3	7.3849	2.4616	946.7778	1.53E-10
residuals	8	0.0208	0.0026		

Tukey's HSD groups for Figure 3C

genotype × treatment	mean	group
WT <i>P.s.m.</i>	1.9667	a
<i>sard4-5 P.s.m.</i>	0.8367	b
WT mock	0.07	c
<i>sard4-5</i> mock	0.0333	c

ANOVA for Figure 3D

	df	SS	MS	F-value	P-value
genotype	2	2.8929	1.4464	13.9718	4.02E-05
treatment	1	1.5422	1.5422	10.9998	0.0022
genotype × treatment	5	6.2011	1.2402	344.0076	1.11E-16
residuals	30	0.1082	0.0036		

Tukey's HSD groups for Figure 3D

genotype × treatment	mean	group
<i>fmo1 P.s.m.</i>	6.290538667	a
<i>fmo1</i> mock	6.278133167	a
<i>sard4-5</i> mock	6.111863833	b
WT mock	6.1055395	b
<i>sard4-5 P.s.m.</i>	5.882202167	c
WT <i>P.s.m.</i>	5.0809245	d

ANOVA for Figure 4A

	df	SS	MS	F-value	P-value
genotype	2	775,790.78	387,895.39	14.5588	2.93E-05
treatment	1	315,940.38	315,940.38	8.0219	0.0077
genotype × treatment	5	1,578,159.64	315,631.93	123.1973	1.11E-16
residuals	30	76,860.13	2,562.00		

Tukey's HSD groups for Figure 4A

genotype × treatment	mean	group
WT <i>P.s.m.</i>	620.6161583	a
WT mock	104.806915	b
<i>sard4-5 P.s.m.</i>	74.979	b
<i>ald1 P.s.m.</i>	51.0686	b
<i>ald1</i> mock	40.70441667	b
<i>sard4-5</i> mock	39.06668333	b

ANOVA for Figure 4B

	df	SS	MS	F-value	P-value
genotype	2	11,752.47	5,876.24	10.6571	0.0003
treatment	1	5,876.24	5,876.24	8.2997	0.0068
genotype × treatment	5	29,381.18	5,876.24	310.7781	1.11E-16
residuals	30	76,860.13	2,562.00		

Tukey's HSD groups for Figure 4B

genotype × treatment	mean	group
<i>sard4-5 P.s.m.</i>	76.65661667	a
<i>sard4-5</i> mock	0	b
WT mock	0	b
WT <i>P.s.m.</i>	0	b
<i>ald1</i> mock	0	b
<i>ald1 P.s.m.</i>	0	b

ANOVA for Figure 5A

	df	SS	MS	F-value	P-value
genotype	3	0.3484	0.1161	3.7703	0.027
treatment	1	0.26	0.26	7.9508	1.00E-02
genotype × treatment	7	0.82	0.12	119.3415	1.32E-12
residuals	16	0.0157	0.001		

Tukey's HSD groups for Figure 5A

genotype × treatment	mean	group
WT <i>P.s.m.</i>	0.5886	a
<i>fmo1 P.s.m.</i>	0.2376	b
WT mock	0	c
<i>sard4-5</i> mock	0	c
<i>fmo1</i> mock	0	c
<i>ald1</i> mock	0	c
<i>sard4-5 P.s.m.</i>	0	c
<i>ald1 P.s.m.</i>	0	c

ANOVA for Figure 5B

	df	SS	MS	F-value	P-value
genotype	3	809.6088	269.8696	2.0668	0.1368
treatment	1	1,729.02	1,729.02	22.4808	9.87E-05
genotype × treatment	7	3,326.07	475.15	80.0401	2.95E-11
residuals	16	94.983	5.9364		

Tukey's HSD groups for Figure 5B

genotype × treatment	mean	group
<i>fmo1 P.s.m.</i>	30.2538	a
WT <i>P.s.m.</i>	24.5918	a
<i>sard4-5 P.s.m.</i>	13.7923	b
WT mock	0.3667	c
<i>sard4-5</i> mock	0.1743	c
<i>fmo1</i> mock	0.1649	c
<i>ald1</i> mock	0.0352	c
<i>ald1 P.s.m.</i>	0.0055	c

ANOVA for Figure 9A

	df	SS	MS	F-value	P-value
genotype × treatment	4	39.533	9.8832	196.37	1.86E-09
residuals	10	0.5033	0.0503		

Tukey's unequal N HSD groups for Figure 9A

genotype × treatment	mean	group
<i>FMO1-3D</i>	3.34466	a
<i>sard4-4 FMO1-3D</i> Pip	3.307500333	a
<i>sard4-4 FMO1-3D</i>	0.028772	b
WT Pip	0.005109	b
WT	0.003324333	b

ANOVA for Figure 9B

	df	SS	MS	F-value	P-value
genotype × treatment	4	0.0375	0.0094	78.5793	1.63E-07
residuals	10	0.0012	0.0001		

Tukey's unequal N HSD groups for Figure 9B

genotype × treatment	mean	group
<i>FMO1-3D</i>	0.110937333	a
<i>sard4-4 FMO1-3D</i> Pip	0.097814	a
<i>sard4-4 FMO1-3D</i>	0.005183	b
WT Pip	0.001863667	b
WT	0.000936	b

ANOVA for Figure 10A

	df	SS	MS	F-value	P-value
genotype	2	3.612	1.806	514.08	1.95E-07
residuals	6	0.021	0.004		

Tukey's unequal N HSD groups for Figure 10A

genotype	mean	group
<i>FMO1-3D</i>	1.3517	a
WT	0.0102	b
<i>ald1 FMO1-3D</i>	0.0053	b

ANOVA for Figure 10B

	df	SS	MS	F-value	P-value
genotype	2	0.0072	0.0036	164.5865	5.74E-06
residuals	6	0.0001	0		

Tukey's unequal N HSD groups for Figure 10B

genotype	mean	group
<i>FMO1-3D</i>	0.062	a
<i>ald1 FMO1-3D</i>	0.003	b
WT	0.002	b

ANOVA for Figure 10C

	df	SS	MS	F-value	P-value
genotype	2	43.1293	21.5647	69.1852	3.44E-06
residuals	9	2.8053	0.3117		

Tukey's unequal N HSD groups for Figure 10C

genotype	mean	group
<i>ald1 FMO1-3D</i>	4.1858	a
WT	3.8988	a
<i>FMO1-3D</i>	0.0284	b

ANOVA for Supplemental Figure 1, Free SA

	df	SS	MS	F-value	P-value
genotype	1	0.01	0.01	0.0132	0.9096
time	2	12.36	6.18	278.3339	7.77E-16
genotype × time	5	12.41	2.48	107.0406	9.64E-13
residuals	18	0.42	0.02		

Tukey's HSD groups for Supplemental Figure 1, Free SA

genotype × treatment	mean	group
WT 12h	1.790662581	a
<i>sard4-5</i> 12h	1.637603513	a
WT 24h	1.391942233	b
<i>sard4-5</i> 24h	1.368012225	b
<i>sard4-5</i> 0h	0.063219098	c
WT 0h	0.041491404	c

ANOVA for Supplemental Figure 1, Total SA

	df	SS	MS	F-value	P-value
genotype	1	0.04	0.04	0.0005	0.9823
time	2	1,710.66	855.3306	348.4211	1.11E-16
genotype × time	5	1,714.07	342.8144	128.177	2.01E-13
residuals	18	48.1417	2.6745		

Tukey's HSD groups for Supplemental Figure 1, Free SA

genotype × treatment	mean	group
WT 24h	20.26159737	a
<i>sard4-5</i> 24h	19.21905511	a
<i>sard4-5</i> 12h	4.160337996	b
WT 12h	3.373976411	b
<i>sard4-5</i> 0h	0.383617894	c
WT 0h	0.373710726	c

ANOVA for Supplemental Figure 2A

	df	SS	MS	F-value	P-value
genotype	1	193.99	193.99	0.0164	0.8997
time	2	188,471.67	94,235.83	1,230.96	1.11E-16
genotype × time	5	188,903.20	37,780.64	632.4967	4.25E-14
residuals	12	716.7906	59.7326		

Tukey's HSD groups for Supplemental Figure 2A

genotype × treatment	mean	group
WT 24h	238.9839557	a
<i>sard4-5</i> 24h	222.2838741	a
WT 12h	31.78198147	b
<i>sard4-5</i> 12h	28.81623621	b
WT 0h	0.031478838	c
<i>sard4-5</i> 0h	9.78E-05	c

ANOVA for Supplemental Figure 2B

	df	SS	MS	F-value	P-value
genotype	1	0.0188	0.0188	0.0417	0.8409
time	2	7.17	3.59	813.73	5.55E-16
genotype × time	5	7.2042	1.4408	486.056	2.05E-13
residuals	12	0.0356	0.003		

Tukey's HSD groups for Supplemental Figure 2B

genotype × treatment	mean	group
<i>sard4-5</i> 12h	1.571097698	a
WT 12h	1.503378138	a
<i>sard4-5</i> 24h	0.671415652	b
WT 24h	0.545805826	b
<i>sard4-5</i> 0h	0.00239703	c
WT 0h	1.82E-03	c

ANOVA for Supplemental Figure 3

	df	SS	MS	F-value	P-value
genotype	2	53.7031	26.8515	1,822.01	1.11E-16
residuals	15	0.2211	0.0147		

Tukey's unequal N HSD groups for Supplemental Figure 3

genotype	mean	group
<i>npr1-1</i>	9.1647	a
<i>sard4-5</i>	5.5125	b
WT	5.4888	b