

Supplemental Figure 1. Additional phenotypes of *nrb4* in defense. A Responses to *Pto (hrpC-)*, a strain that lacks virulence in Arabidopsis (Deng et al., 1998). 18-day-old plants were inoculated at an OD_{600} of 0.1. Three days after inoculation, the growth of *Pto* was evaluated as Logarithm of colony forming units (cfu) per plant. **B** Inoculations with Plectosphaerella cucumerina. P. cucumerina was provided by Brigitte Mauch-Mani (University of Neuchatel, Switzerland), and used as described (Ton and Mauch-Mani, 2004). C Response to Methyl Jasmonate (MeJA) induced resistance. MeJA was applied by spray at 100 µM in 0.1% DMSO and 0.02% Silwet L-77 one day before Pto inoculation. D Responses to JA-Ile in the length of roots. Plants were grown in Johnson's media (Johnson et al., 1957) with 1 mM KH₂PO₄, with or without 50 µM MeJA (Duchefa). The length of the roots was measured with ImageJ software (MIH, Bethesda, MD, USA). E Responses to coronatine. Pto(cfa⁻) a strain that lacks coronatine (Mittal and Davis, 1995) was inoculated, along Pto, as indicated in A. The experiments were repeated three times with similar results, and the data represent the average, with the error bars plotting the standard deviation of 15 plants in three groups of five. The letters above the bars indicate different homogeneous groups with statistically significant differences (Fisher's LSD Test, P < 0.05). Asterisks indicate statistically significant differences from the mock treatment (P < 0.05 one asterisk, P < 0.050.01 two) using the Student's t test (one tail).





Col-0 npr1-1 nrb4-1 nrb4-2 nrb4-3 nrb4-1 nrb4-2 nrb4-3 npr1-1 npr1-1 npr1-1







Supplemental Figure 2. Epistasis of NRB4 with NPR1. A Three double mutants nrb4 *npr1*-1 and their controls were treated with either mock or 350 µM BTH four times over three weeks, their weights recorded, and the ratio between the BTH and mock treated plants calculated (15 plants in three groups of five). The ratio is expressed as percentage of fresh weight (%FW). B Three double mutants nrb4-1 npr1-70 were tested for its response to SA and BTH upon Pto inoculation. C Confocal image of Arabidopsis 35S:NPR1-GFP in an npr1-1 background in mock conditions. D Same transgenic in an nrb4-2 npr1-1 background in mock conditions. E. The same line as in C, one day after 350 µM BTH treatment. F The same line as in D, one day after 350 µM BTH treatment. G The lines described above were tested for its response to BTH. The overexpression of NPR1, even if it is detected in the nucleus (F), did not complement the mutation nrb4-2. The experiments were repeated three times with similar results, and the data represent the average, with the error bars plotting the standard deviation of 15 plants in three groups of five. The letters above the bars indicate different homogeneous groups with statistically significant differences (Fisher's LSD Test, P < 0.05). Asterisks indicate statistically significant differences from the mock treatment (P < 0.05 one asterisk, P <0.01 two) using the Student's t test (one tail).



Supplemental Figure 3. Additional pictures of *nrb4-4.* **A** Wild type plant, growing seven weeks in short day and six weeks in long day with no treatment or inoculation. **B** *nrb4-4* plant of the same age, growing in the same conditions. **C** *nrb4-4* plant growing seven weeks in short day and eleven weeks in long day, as in A. **D** Detail of the plant in C, note the absence of flowers. **E** *nrb4-4* plant with flowers. **F** Detail of the plant in E. As a reference, the pots have a diameter of 6 cm.



Supplemental Figure 4. Additional pictures of Cryo-SEM. A Section of Col-0. B Section of *nrb4*-2. C Section of *nrb4*-4. D Surface of a leaf from Col-0. E Idem from *nrb4*-2. F Idem from *nrb4*-4. The length of the bar in A, B, and C is 80 μ m, and in D, E, and F is 800 μ m. The leaves were five weeks old for Col-0 and *nrb4*-2 and seven weeks for *nrb4*-4.



Supplemental Figure 5. Stainings of *nrb4.* **A** Trypan blue stains, unveiling cell death and membrane damage in Col-0, *nrb4-2*, and *nrb4-4*. **B** Aniline blue stains under visible light. **C** The same Aniline blue stains under ultraviolet light, which detects callose depositions. Trypan Blue and Aniline Blue staining were performed as described (Tornero et al., 2002; Conrath et al., 1989, respectively). No differences among genotypes were observed with these stains.



Supplemental Figure 6. Characterization of *nrb4* null alleles. A DNA content. Nuclei from the indicated genotypes were extracted, stained with DAPI, and the relative amount of DNA measured with a CyFlow Ploidy Analyzer (Partec GmbH, Münster Germany) following the manufacturer's instructions. At least 5000 nuclei were counted in each measurement, and the same result was obtained in three independent experiments. **B** Phenotypes of *nrb4*-5 in comparison to *nrb4*-4. **C** Phenotypes of a T2 segregating family of *nb4*-4/*NRB4* in MS plates. The arrows point to *nrb4*-4 homozygous plants (confirmed by PCR) Picture taken at two weeks of growing. **D** Plants selected in C were transferred to MS plates with 500 μ M SA, and the picture was taken two weeks after the transfer. **E** Lack of complementation in yeast. Empty vector (E.V.) pAG423 (Alberti et al., 2007) and *NRB4* cloned in pAG423 were introduced in wild type (wt) and *Agal11*. The different strains were grown in liquid and then plated in SD His- plates with or without cycloheximide (0.2µg/ml). The wt and *Agal11* strains were obtained from EUROSCARF (Ref. Y00000 and Y01742, respectively). Supplemental Data. Canet et al. (2012). Plant Cell 10.1105/tpc.111.103028





Supplemental Figure 7. Phenotypes from the transcriptomic analysis. A PR1 immunoblot of Col-0 and nrb4-4 three days after mock or a Pto inoculation and one day after mock or 350 µM BTH. The same blot was probed with anti-RuBisCO for loading and transfer control. The red color indicates saturation of the signal. B Resistance induced by cytokinins. Trans-zeatin (t-zea) 1 µM or a mock solution was applied one day previous to the inoculation with Pto. tga3 (Choi et al., 2010) and ahp1 ahp2 ahp3 (Hutchison et al., 2006, abbreviated as ahp1,2,3) were included as controls. C nrb4-4 did not have a specific phenotype with cytokinins. The controls Col-0 and arr1 arr10 arr12 growing in 5 µM trans-zeatin (left) are the same as in Figure 6B. A T2 family, segregating for *nrb4*-4 (middle) did not produce plants with a different perception to cytokinins. Col-0 and the same T2 family of nrb4-4 plants growing in control conditions (right). In the case of nrb4-4, the space shown at the bottom of the picture was cleared of wild type plants, to check if the *nrb4*-4 homozygous plants can grow in this media. The experiments were repeated three times with similar results, and the data represent the average, with the error bars plotting the standard deviation of 15 plants in three groups of five. Asterisks indicate statistically significant differences from the mock treatment (P < 0.05 one asterisk, P < 0.01 two) using the Student's t test (one tail).



Supplemental Figure 8. Yeast n-hybrid interactions. A Interactions between NRB4 and proteins that have a role in SA perception. The yeasts that had NRB4 and any of these proteins were able to grow in His+ plates, but not in His- plates. Therefore, there was not detectable interaction. TGA7* stands for an additional control with an empty pDEST22, since TGA7 was able to autoactivate the system with no 3AT (the His- plates contained 5 mM 3AT). B As a control, interactions between NPR1 and the rest of proteins that have a role in SA perception. C NRB4 did not alter the interaction between NPR1 and TGA2. NRB4 was cloned in a third vector, and introduced in the first yeast of B. There was no statistical difference between introducing NRB4, respect nrb4-3, or to the empty vector. All the plates were Lys-, Trp-, Leu-, and 100 µM SA. Similar experiments with no SA produced the same results. Similarly, bimolecular fluorescence complementation between NRB4 and NPR1 or TGA2 did not produce a positive result in Nicotiana benthamiana. The pictures were taken 3-5 days after growing at 28°C. NRB4 was cloned in three different versions for detection of interactions in the yeast two hybrid system: a short version, from 1 to 112 aa, included the KIX domain; an intermediate version, from 1 to 670 aa, spanned half of the coding sequence; and a full version. The full version when fused to the GAL4 BD was autoactivated, even with mutated versions of NRB4 that recreated the EMS mutations herein described. The three versions of the wild type protein fused with the GAL4 AD were tested for interaction in yeast with genes described in SA response. The experiment shown in this figure corresponds to the full version of NRB4. The experiments were repeated three times with similar results, and the data represent the average, with the error bars plotting the standard deviation of three colonies. The letters above the bars indicate different homogeneous groups with statistically significant differences (Fisher's LSD Test, P < P0.05).



35S:NRB4-GFP

35S:GFP-NRB4

Supplemental Figure 9. Expression of *NPR1* and *NBR4*. A *NPR1* was detectable in *nrb4*-2 at normal levels. The levels of expression of *NPR1*, measured by RT-qPCR, are normalized to three reference genes and to the level of Col-0. The RNA was extracted from three-week-old plants, from three independent samples of 100 mg each. **B** The nuclear localization did not change with the application of 350 μ M BTH. *35S:NRB4-GFP* (left) and *35S:GFP-NRB4* (right) were infiltrated in *N. benthamiana*. Then, a mock or a 350 μ M BTH was applied one day before these pictures were taken. The controls correspond to the Figure 7C and D, respectively. The experiments were repeated three times with similar results, and the data represent the average, with the error bars plotting the standard deviation of three independent RT-qPCRs. The letters above the bars indicate different homogeneous groups with statistically significant differences (Fisher's LSD Test, P < 0.05).

Supplemental Data. Canet et al. (2012). Plant Cell 10.1105/tpc.111.103028



Supplemental Figure 10. Characterization of NRB4 complementation. A In the stable transgenic lines, GFP was not detectable by immunoblot. A immunoblot with antibody raised against GFP (Roche, Madrid, Spain) was performed in different extracts. The first line of the immunoblot is a *GFP-NRB4* fusion expressed in *E. coli*, which shows partial processing. The rest of lines correspond to plant extracts from the same lines described in Figure 8. The arrows point the position of the weight markers. **B** The complemented *nrb4* alleles express PR1 upon BTH application. The lines described in Figure 8 were treated with BTH and PR1 detected as in Figure 2B. There is a low expression of PR-1 in the lines that contain *35S:NRB4-GFP* (*NRB4F*) in *nrb4-1* and *nrb4-2*, a expression similar to Col-0 in the *35S:NRB4* in *nrb4-3*, and a very strong expression in the lines that overexpress the first 670 AA of NRB4 plus GFP (*NRB4M*) in *nrb4-3*. The red color indicates saturation of signal.

Population	Observed			Expected				
				(3/4)	(1/4)			
	wt	mut	Total	wt	mut	χ^2	<u>d.f.</u>	p
F2 Col-0 x <i>nrb4</i> -1	297	108	405	303.75	101.25	0.600	1	0.44
F2 Col-0 x nrb4-2	248	87	335	251.25	83.75	0.168	1	0.68
F2 Col-0 x nrb4-3	345	104	449	336.75	112.25	0.808	1	0.37
				(1/2)	(1/2)			
F1 <i>nrb4-</i> 4 het x <i>nrb4-</i> 1	25	23	48	24	24	0.083	1	0.77
F1 <i>nrb4</i> -4 het x <i>nrb4</i> -2	26	30	56	28	28	0.286	1	0.59
F1 <i>nrb4</i> -4 het x <i>nrb4</i> -3	28	30	58	29	29	0.069	1	0.79
				(3/4)	(1/4)			
F1 <i>nrb4</i> -5 het x <i>nrb4</i> -4 het	40	11	51	38.25	12.75	0.320	1	0.57

Supplemental Table 1. Evaluation of segregations.

Segregations observed in the indicated populations. The phenotypic classes were evaluated with the χ^2 statistics. The p value gives the probability that any deviation from expected results is due to chance only. Since in all the cases the p value is bigger than the standard value of 0.05, the segregations fit the proposed model.

AGI	NASC	T-DNA	<u>Status</u>	Position	Phenotype
	Col 0				<u>(% wt)</u> 100
	$nnrl_1$		Homoz		0
AT1G07950	N656591	SALK 065283C	Homoz	Exon	100
AT1G11760	N665553	SALK_003265C	Homoz	5'	100
AT1G11760	N678300	SALK_028490C	Homoz	5'	100
AT1G15780	N835429	SAIL 792 F02	Heteroz	Intron <i>nrb4</i> -4	75
AT1G15780	11055425	GABL 955 E02	Heteroz	Intron <i>nrb4</i> -5	75
AT1G16430	N870082	SAIL 9 E04	Heteroz	Exon	100
AT1G23230	N659417	SALK 060062C	Homoz	Exon	100
AT1G23230	N671536	SALK 074015C	Homoz	Exon	100
AT1G25540	N679089	SALK 129555C	Homoz	Exon	100
AT1G25540	N677751	SALK 059316C	Homoz	Exon	100
AT1G26665		No info			
AT1G29940	N876306	SAIL_726_H01	Heteroz	Exon	100
AT1G31360	N661000	SALK_087178C	Homoz	Exon	100
AT1G44910	N521070	SALK_021070	Heteroz	Exon	100
AT1G54250	N679260	SALK_151800C	Homoz	Exon	100
AT1G55080	N529118	SALK_029118	Heteroz	Exon	100
AT1G55325	N861503	SAIL_1169_H11	Homoz	Exon	100
AT1G60850	N655705	SALK_088247	Homoz	Exon	100
AT2G03070	N682656	SALK_092406C	Homoz	Exon	100
AT2G22370	N677657	SALK_027178C	Homoz	Intron	100
AT2G28230		No info			
AT2G29540	N507414	SALK_007414	Heteroz	Exon	100
AT2G38250	N667374	SALK_133090C	Homoz	5'	100
AT2G48110	N671698	SALK_092499C	Homoz	5'	100
AT2G48110	N667838	SALK_015532C	Homoz	Exon	100
AT3G01435		No info			
AT3G04740	N521711	SALK_021711	Heteroz	Exon	100
AT3G09180	N512449	SALK_012449	Heteroz	Exon	100
AT3G10690	N506294	SALK_006294	Heteroz	3'	100
AT3G21350	N662531	SALK_055723C	Homoz	5'	100
AT3G21350	N656864	SALK_110696C	Homoz	5'	100
AT3G23590	N667150	SALK_119561C	Homoz	Exon	100
AT3G23590	N661810	SALK_022477C	Homoz	Exon	100
AT3G25940	N562311	SALK_062311	Heteroz	3'	100
AT3G52860	N685672	SALK_037570C	Homoz	5'	100
AT3G57660	N673273	SALK_116823C	Homoz	3'	100
AT3G57660	N673356	SALK_122465C	Homoz	3'	100
AT3G59600		No info			

Supplemental Table 2. Evaluation of phenotypes in T-DNA insertion lines.

AT4G00450	N678935	SALK_108241C	Homoz	Exon	100
AT4G04780		No info			
AT4G04920	N548091	SALK_048091	Heteroz	Intron	100
AT4G09070	N553156	SALK_053156	Heteroz	3'	100
AT4G25210	N599954	SALK_099954	Heteroz	Exon	100
AT4G25210	N607213	SALK_107213	Heteroz	5'	100
AT4G25630	N682661	SALK_093373C	Homoz	Exon	100
AT5G02850	N622082	SALK_122082	Heteroz	Exon	100
AT5G02850	N683125	SALK_007367C	Homoz	5'	100
AT5G03220	N678464	SALK_049958C	Homoz	5'	100
AT5G03500	N676132	SALK_088220C	Homoz	Intron	100
AT5G12230	N657910	SALK_037435C	Homoz	5'	100
AT5G12230	N658182	SALK_034955C	Homoz	Intron	100
AT5G19480		No info			
AT5G19910	N682219	SALK_035522C	Homoz	Exon	100
AT5G20170	N663678	SALK_111977C	Homoz	Exon	100
AT5G28540	N675173	SALK_054493C	Homoz	3'	100
AT5G28540	N675862	SALK_079156C	Homoz	5'	100
AT5G41010	N549327	SALK_049327	Heteroz	Intron	100
AT5G41910	N663226	SALK_087920C	Homoz	5'	100
AT5G41910	N678994	SALK_115673C	Homoz	5'	100
AT5G42020	N659850	SALK_047956C	Homoz	Exon	100
AT5G42060	N669407	SALK_014079C	Homoz	5'	100
AT5G63480	N654793	SALK_095631C	Homoz	Intron	100
AT5G64680	N685462	SALK_023879	Homoz	5'	100
AT5G67240	N542641	SALK_042641	Heteroz	Exon	100

Phenotypes observed in the indicated populations, either homozygous or heterozygous T-DNA insertions in Arabidopsis genes with homology with Mediator genes. The phenotypic classes were evaluated visually, as described by Canet et al., 2010.

Name	Organism	AA	Max. E value	Identities	Positives
Pdr1p	Yeast	1068	7.7	37/143	58/143
PDR3p	Yeast	976	2.3	27/99	47/99
Oaf1p	Yeast	1047	0.38	24/90	46/90
PPARα	H. sapiens	468	2.3	13/42	24/42
NHR-49	C. elegans	501	0.41	18/62	29/62

Supplemental Table 3. Lack of homologs in Arabidopsis for several nuclear receptors.

The mentioned proteins were used to search in the Arabidopsis genome with BLASTP (TAIR10, <u>www.arabidopsis.org</u>), with the default settings. The column "AA" indicates the number of aa of the original protein. The "Max. E value" indicates the maximal E value obtained with BLASTP, while the "Identities" and "Positives" columns indicate the ratio of aa either identical or similar in the best stretch of homology.

Supplemental Table 4. Primers used

Name	Sequence	Objective		
5249817-NlaIII-F	TGAGCAGCAAGAAAGATGATG	nrb4 mapping		
5249817-NlaIII-R	CTTAGCAGAGGTACGAGGATCA	nrb4 mapping		
5346165-DdeI-F	CACCAAACACCACACTTCTCA	nrb4 mapping		
5346165-DdeI-R	CATATCTTCAAAATCTTTGAGTTGG	nrb4 mapping		
5377218-NlaIII-F	CTGGATTTTGGTCGAGTTAGC	nrb4 mapping		
5377218-NlaIII-R	GTGGCAATAGAGGCACAAGT	nrb4 mapping		
5393430-CauI-F	GAAGAGTGGTTGCAAGCGTA	nrb4 mapping		
5393430-CauI-R	TTTTTGCGAGTCCACGTTC	nrb4 mapping		
5406030-NlaIII-F	AGTTGGTCGGAGCTTTTCCT	nrb4 mapping		
5406030-NlaIII-R	GATTCTCCACACCACCACT	nrb4 mapping		
5425793-SecI-F	AGAACGAGCTCGAACACGAA	nrb4 mapping		
5425793-SecI-R	CTGAAACATTGAATCCCATTTG	nrb4 mapping		
5440252-MseI-F	TGCTTTCAATAATCGTTGTGTT	nrb4 mapping		
5440252-MseI-R	CACACCAAAACAAGCTTCTGC	nrb4 mapping		
5455705-HinfI-F	GAATCTTGATGCTTGCTTGG	nrb4 mapping		
5455705-HinfI-R	CCATGTCCGGGAAACTTATC	nrb4 mapping		
5494532-RsaI-F	GTTGATCGGAAAGGAAAAGTAAAA	nrb4 mapping		
5494532-RsaI-F	AAAAACGGATAACCAAACATGG	nrb4 mapping		
F10B6.1-F	ATTATATTGTTCAACATCAACTGCACAT	nrb4 mapping		
F10B6.1-R	TTTATCTCTTAAACAAGTTCGTAAACCAAC	nrb4 mapping		
T16N11.1-F	AATAGATTAGAAATGAACAGGAGAATTGACT	nrb4 mapping		
T16N11.1-R	TGGCATTTTAATAACATCCTCACC	nrb4 mapping		
15780.1	TAACAAAAAATCCCAATCACGTGTG	NRB4 sequencing		
15780.2	AACAATTGGAGGCCTTCTCTCC	NRB4 sequencing		
15780.3	AAATATTGCACGCCAACAAGCA	NRB4 sequencing		
15780.4	AAGGCGTTCAATAGGCAGCTCA	NRB4 sequencing		
15780.5	TATGCACAGGCCGAGGAAGC	NRB4 sequencing		
15780.6	GCATCTGCGGATTTGTTTGG	NRB4 sequencing		
15780.7	CAAGCCTCTGGTATCCATCAGC	NRB4 sequencing		
15780.8	TCTGTTGGATGCCTGAGCTATTTG	NRB4 sequencing		
15780.9	AATCTATGGATGTGCCATTATTAGCG	NRB4 sequencing		
15780.10	TGCGCAGAATGGAAACACTAAA	NRB4 sequencing		
15780.11	TTCCGGTGGGATTGGCTATT	NRB4 sequencing		
15780.12	GAATGAAATCTACCAGAGAGTTGCA	NRB4 sequencing		
15780.13	TGGTTTGGGACAGCAACGG	NRB4 sequencing		
	GGGGACAAGTTTGTACAAAAAGCAGGCTTCGAAG	NRB4 Cloning in		
NRB4FP2-attb1	GAGATAGAACCATGGATAATAACAATTGGAGGCCT	pDONR221, C-terminal		
	GGGGACCACTTTGTACAAGAAAGCTGGGTCTATGG	NRB4S Cloning in		
NRB4RP1-attb2	ATGTGCCATTATTAGC	pDONR221		
	GGGGACCACTTTGTACAAGAAAGCTGGGTAAGCCA	NRB4M Cloning in		
NRB4RP2-attb2	CCTTATCTTTTAATGC	pDONR221		
	GGGGACCACTTTGTACAAGAAAGCTGGGTGGGAAG	NRB4F Cloning in		
NRB4RP3-attb2	CTGCTACATATTTCTC	pDONR221		
	GGGGACAAGTTTGTACAAAAAGCAGGCTTCATGG	NRB4 Cloning in		
NRB4FP4-attb1	ATAATAACAATTGGAGG	pDONR221, N-terminal		

qRT NPR1.1	GAAGAATCGTTTCCCGAGTTCC	NPR1 RT-qPCR
qRT NPR1.2	CATCACCGGGTGTAAAGATAGCA	NPR1 RT-qPCR
qRT NRB4.3	TTGCCACCTGATTCTCGTCA	NRB4 RT-qPCR
qRT NRB4.4	CTCTGGTCCGGAAAATGGAA	NRB4 RT-qPCR

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