Supporting Information

Zhu et al. 10.1073/pnas.1002828107

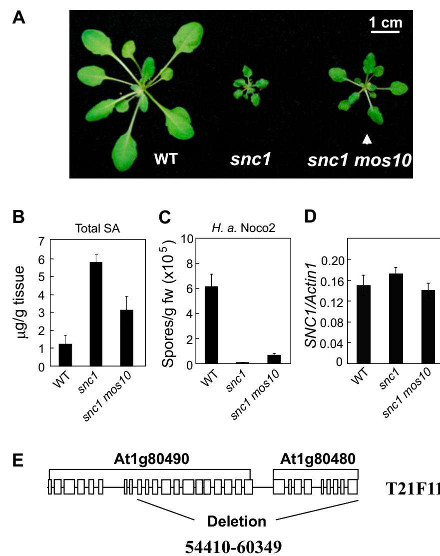

Fig. S1. Partial suppression of *suppressor of npr1-1, constitutive 1* (*snc1*) mutant phenotypes by *modifier of snc1, 10* (*mos10*)/*topless-related 1* (*tpr1*). (*A*) Morphology of 4-wk-old Columbia (Col-0) wild-type, *snc1*, and *snc1-mos10* plants. (*B*) Total salicylic acid (SA) levels in wild-type, *snc1*, and *snc1-mos10* plants. Soil-grown 4-wk-old plants were collected for SA extraction. SA levels were measured with HPLC as previously described (1). Values are averages of four replicates ± SD. (*C*) Growth of *Hyaloperonospora arabidopsidis* (*H. a.*) Noco2 on wild-type, *snc1*, and *snc1-mos10*. Two-wk-old seedlings were sprayed with *H. a.* Noco2 at a concentration of 50,000 spores/mL water. Infection was scored 7 d after inoculation by counting the number of spores per gram of leaf fresh weight (fw). Error bars represent SD from three measurements. (*D*) Real-time RT-PCR analysis of *SNC1* expression in wild-type, *snc1*, and *snc1-mos10* plants. (*E*) Sequences between nucleotides 54410 and 60349 on BAC clone T21F11 (AC018849.2) were deleted in *mos10*.

1. Li X, Zhang Y, Clarke JD, Li Y, Dong X (1999) Identification and cloning of a negative regulator of systemic acquired resistance, SNI1, through a screen for suppressors of *npr1-1*. *Cell* 98: 329–339.

Fig. S2. *Enhanced disease susceptibility 1* (*EDS1*) and *phytoalexin deficient 4* (*PAD4*) are required for activation of defense responses mediated by *Topless-related 1* (*TPR1*) overexpression. (*A*) TPR1-HA protein levels in *TPR1-HA* transgenic lines #3 and #11. Western blot analysis was carried out on total protein extracts from the *TPR1-HA* transgenic lines using an HA antibody. Equal loading was monitored with a Bradford assay. (*B*) Suppression of the dwarfism of *TPR1-HA* overexpression line #3 by *eds1-2* and *pad4-1*. (*C* and *D*) Suppression of constitutive *pathogenesis-related 1* (*PR1*) (*B*) and *pathogenesis-related 2* (*PR2*) (*C*) expression in *TPR1-HA* overexpression line #3 by *eds1-2* and *pad4-1*. (*E*) Suppression of enhanced resistance to *H. a.* Noco2 in *TPR1-HA* overexpression line #3 by *eds1-2* and *pad4-1*.

Fig. S3. *TPR1* and its close homologs are required for *resistance to Peronospora parasitica 2* (*RPP2*)-mediated immunity. Infection phenotypes of leaves inoculated with avirulent *H. a.* Cala2. Two-wk-old seedlings of the indicated genotypes were sprayed with *H. a.* Cala2 (40,000 spores/mL water) Leaves were stained with lactophenol trypan blue 7 d after inoculation to visualize pathogen hyphae and plant cell death. h, hyphae; HR, hypersensitive response; TN, trailing necrosis; *tpl*, *topless*; *tpr4*, *topless-related 4.*

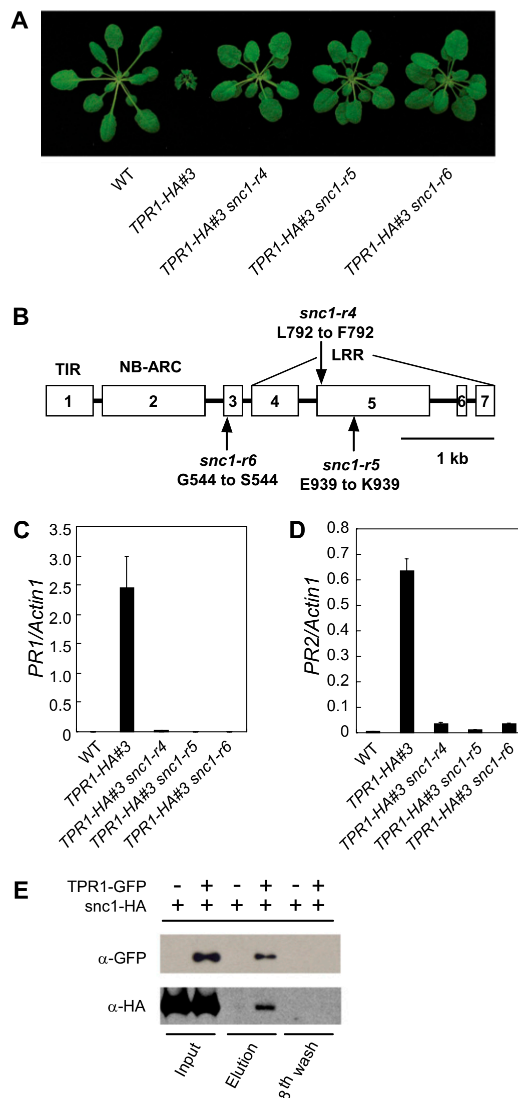

Fig. S4. Analysis of suppressor mutants of the *TPR1-HA* overexpression line #3 and association of SNC1 and TPR1 in nucleus. (*A*) Suppression of the stunted morphology of *TPR1-HA* overexpression line #3 by three suppressor mutants, *snc1-r4*, *snc1-r5*, and *snc1-r6*. (*B*) Mutations in SNC1 identified from the three suppressor mutants. (*C* and *D*) Suppression of constitutive *PR1* (*C*) and *PR2* (*D*) expression in the *TPR1-HA* #3 overexpression line by the three suppressor mutants. (*E*) Association of SNC1 and TPR1 in nucleus. Coimmunoprecipitation of snc1-HA with TPR1-GFP in protein extracts of *TPR1-GFP* and *snc1-HA* double-tagged transgenic plants. Nuclear protein extracts were subjected to immunoprecipitation with anti-GFP magnetic beads. Crude lysates (input) and immunoprecipitated proteins (elution) were detected with anti-GFP or anti-HA antibodies.

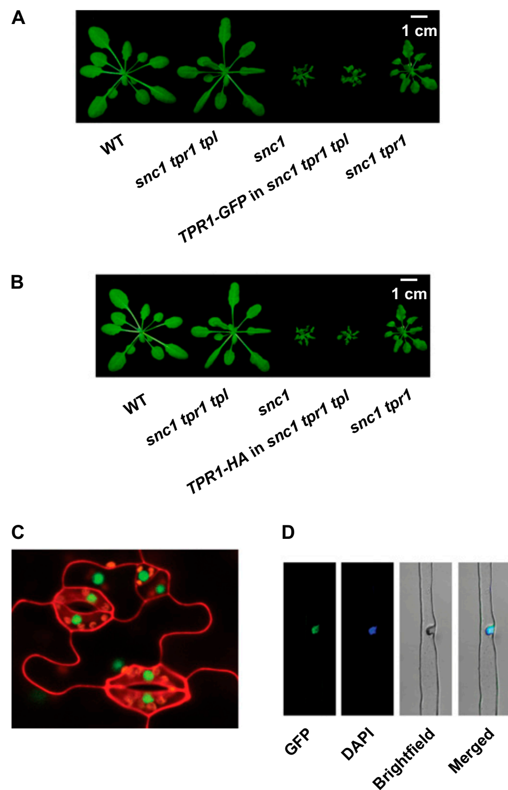

Fig. S5. Complementation of *snc1-tpr1-tpl* mutant morphology by *TPR1-GFP* and *TPR1-HA* and localization of TPR1-GFP. (*A*) Complementation of *snc1-tpr-tpl* mutant morphology by *TPR1-GFP*. A construct expressing the TPR1-GFP fusion protein under the control of its native promoter was transformed into the *snc1-tpr1-tpl* mutant. The picture was taken from 4-wk-old soil-grown plants. (*B*) Complementation of *snc1-tpr1-tpl* mutant morphology by *TPR1-HA*. A construct expressing the TPR1-HA fusion protein under the control of its native promoter was transformed into the *snc1-tpr1-tpl* mutant. The picture was taken from 4-wk-old soil-grown plants. (*C*) Localization of TPR1-GFP protein in leaf pavement and guard cells of *TPR1-GFP* transgenic plants. Plant cell walls were stained with 5 mg/mL propidium iodine (red). (*D*) Localization of TPR1-GFP protein in root hair cells of *TPR1-GFP* transgenic plants. The nuclei were stained with DAPI.

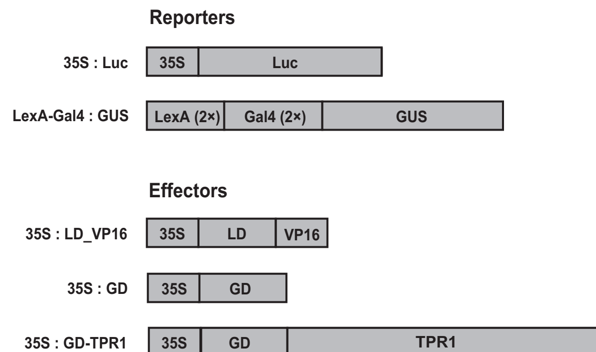

Fig. S6. Reporter and effector constructs used in the transfection assays. Luc, luciferase; GUS, GUS: β-glucuronidase.

Target	Fold enrichment	Annotation
At5g15410	8.5 ± 1.7	DND1 (Defense No Death 1)
At3g20820	11.5 ± 2.2	Leucine-rich repeat family protein
At1g51940	18.6 ± 2.2	Protein kinase family protein
At4g21960	3.1 ± 1.1	Peroxidase 42
At1g14720	7.2 ± 1.2	XRT2
At5g54250	5.4 ± 3.3	DND2 (Defense, No Death 2)
At5g56040	3.8 ± 1.6	Leucine-rich repeat protein kinase
At4g36540	7.6 ± 1.4	BR Enhanced Expression 2
At3g25500	3.9 ± 1.5	Formin Homolog 1
At5g07240	4.9 ± 1.4	IQ-domain 24; calmodulin binding
At5g61420	8.6 ± 3.1	AtMYB28
At3g19000	6.0 ± 1.7	2OG-Fe(II) oxygenase family protein

Fig. S7. List of target genes of TPR1 identified by ChIP followed by real-time PCR analysis. Transgenic plants expressing *TPR1-HA* were subjected to ChIP analysis using an anti-HA antibody. *TPR1-GFP* transgenic plants were used as negative controls. The fold of enrichment of a target promoter was calculated by dividing the amount of DNA immunoprecipitated from the *TPR1-HA* transgenic plants by that from the negative control plants.

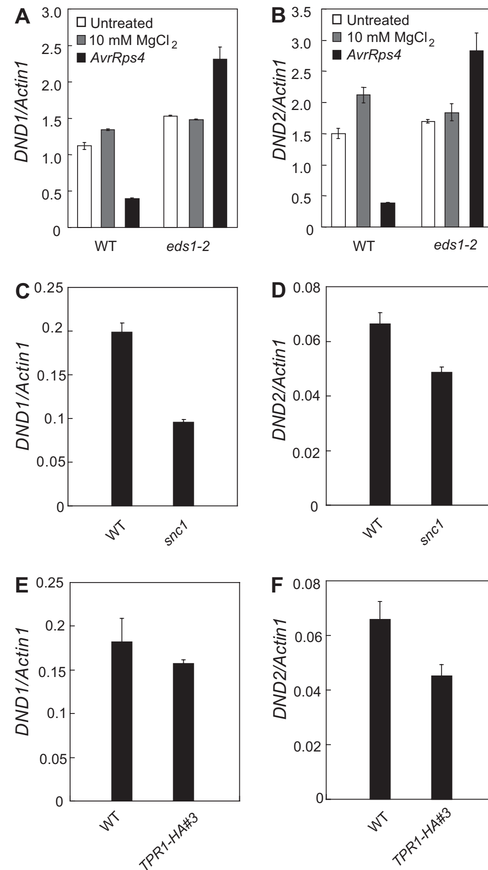

Fig. S8. Real-time RT-PCR analysis of *Defense no Death 1* (*DND1*) and *Defense no Death 2* (*DND2*) expression. (*A* and *B*) Expression of *DND1* (*A*) and *DND2* (*B*) in wild-type and *eds1-2* plants in response to *Pseudomonas syringae* pv. *tomato* (*P.s.t.*) DC3000 *AvrRps4*. Plants were infiltrated with or without *P.s.t.* DC3000 *AvrRps4* (OD_{600} = 0.001) in 10 mM $MgCl_2$. Samples were taken 12 h after inoculation. (*C* and *D*) Expression of *DND1* (*C*) and *DND2* (*D*) in wild-type and *snc1* plants. (*E* and *F*) Expression of *DND1* (*E*) and *DND2* (*F*) in wild-type and *TPR1-HA* overexpression line #3.

Table S1. Primers used for real-time PCR analysis of the promoters of target genes of TPR1

Gene	Forward primer	Reverse primer
At2g24762	gatatcatgctatcactgac	gcctacaagaactaccgcag
At5g15410	agtgaatgtacgatttaagc	agtatcaattgacggcaaac
At2g18940	caccaatttgatcacttaac	ggaggaatcggcatttacgg
At1g60990	tcatcttcatctctcacatc	ggcacaatatcaagagcagc
At1g24450	tgttaccgtaactggaacac	atcaagtactcgttgtagac
At4g17770	agagaaagatcggagaaaag	agatcagaacacacagctgg
At2g45340	catgagtcttttgttttcc	accacttgactttttatcac
At2g36870	tccaccaccagctttatctc	gtaaagattgaccaaacacg
At2g37450	ttagatgtgactttgtaccc	tcacaaccacataacaaacg
At3g20820	ccaaccggcaagtcgcatcg	tgtagcaataatatggccac
At4g20270	agtgcacaaataatctgctg	aacatagtcacatagtctcc
At1g62560	tcgatggatgtaatagtagc	aactagtgaaatccagtagg
At1g51940	ggttgaaagtttaagaacttcg	attctccctttcatttacac
At5g57180	gaatgaacgatcgtcgctac	agcgatatgattgtggcaac
At1g68520	ctcgtgattatgtggaaagc	ctttagtcctaataattggg
At3g62550	agctcttcacgtatttgggc	gcatacagataccttccaac
At4g21960	actcaaatgtatgctagacg	ctatctaacaagccatccac
At2g33330	ttggtgtgtgtattgcagtc	taattattgtcgaaaacgtg
At1g78020	ctgtaactttaatgaatggg	ttcttttatagacggaaagg
At3g59400	ttgacctatctataatgggc	aaaatcgctctcagtgattg
At1g14720	gatcagatctttattagtggg	ttagtgactctacctgtgac
At5g54250	cttgaaatacaaatgagtcc	acgattctacgaatgagacc
At5g56040	tagccacttcagactctccc	gacttctagacttctagttac
At5g52010	catgaatgtagtacttgacac	atggcttccgcttctctctg
At3g53950	aaatgaattgagctgtagag	gaaactttggagtatacggg
At2g29320	ttggagaaatgtcacaaacc	cttgcttccttccctatatg
At1g14345	tagcagttacttctccagcg	gccagttattcatgatcagc
At5g39210	ttcgagtataggagtttagg	ccatagttgtaccacacttg
At2g28720	acgatgtcgacttagatcac	tattgcatactcattgtcgg
At4g30020	aggtcccctcaaagattatg	tttggttttggaagaaatgg
At5g64330	gaactaatcacattggaaac	atcatctctatgtacattgc
At1g17970	ggaaaggaaaacggttcagg	gaagagacacaaagtccagc
At4g36540	agtaatcatacgtcacaagg	agagagagagagagagaggg
At1g75500	ataaacgattgtgttttcgg	ttatctcgttttggtaacgg
At5g62920	tgaattgtggaagttcacgc	tttggtgaatgaaaaggtgg
At3g25500	tgtgctttcgccgaatgctg	gcatgaaccatgatgtagcc
At2g21210	aactacagttacaacaaccc	agatgtcaaacgcttcacac
At1g01320	ggtgtgagagagagacacag	tcaaggtgaaacaaggaagg
At5g07240	gtgaactgtgcaggcataag	atctgtcataagtattgaac
At2g03550	gattctctatttttgtaggg	ctagtgatttctccaagtttc
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At3g28200	tataacccattacaagacac	atggtcttaggttggatccg
At3g19000	gtagggtgaagaatatagac	cgttatagcatatgtgatgtg
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At5g23310	atgagggactttcggtgcag	tctgtttgattccactgtcg