SUPPLEMENTAL INFORMATION INVENTORY

Figure S1, Related to Figure 1. SNI1 and NSE6, ASAP1 and NSE5 Share Limited Sequence Similarity.

(A) Sequence alignments of SNI1, NSE6 and 1B3UA. Alignments were performed using Clustal W. The identical amino acids were shaded black. The identity between SNI1 and NSE6 is 11.6%, NSE6 and 1B3UA is 10.0%.

(B) Sequence alignments of ASAP1, NSE5 and 1JDHA. The identity between ASAP1 and 1JDHA is 12.3%, NSE5 and 1JDHA is 10.6%.

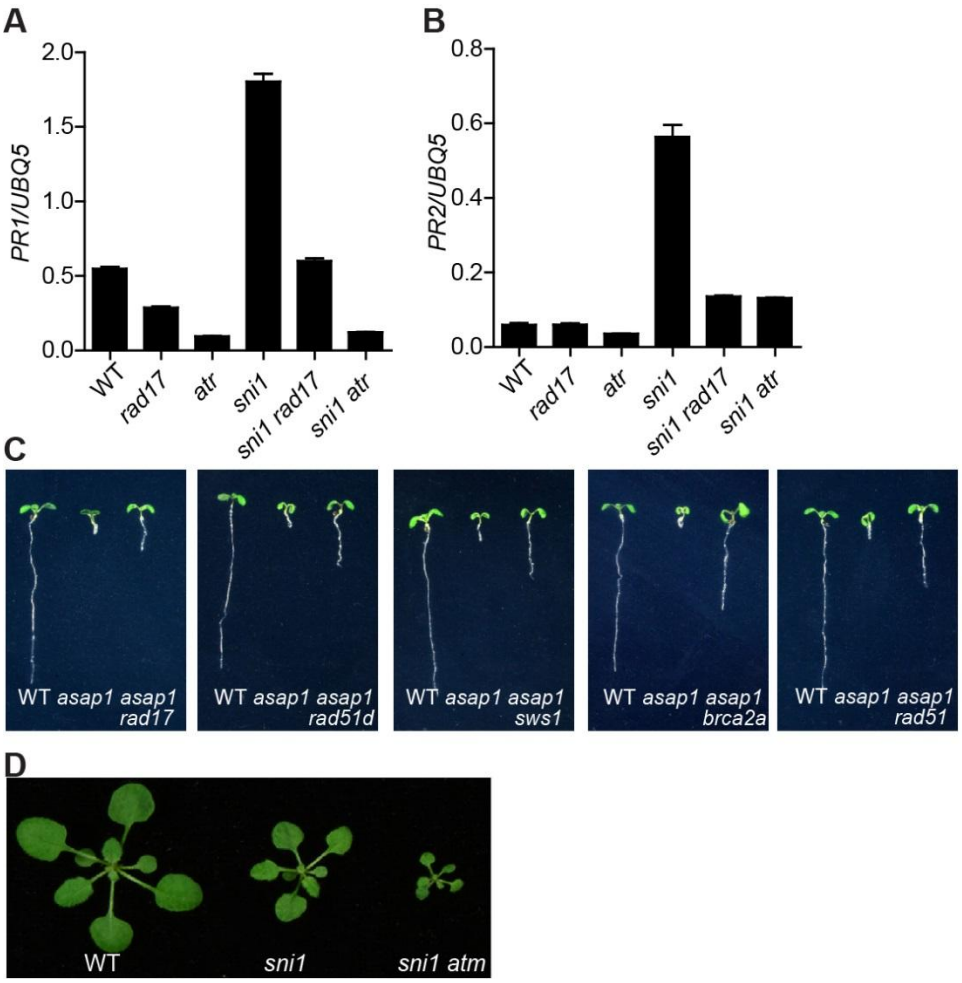

Figure S2, Related to Figure 3. The Effects of Mutations in DDR Genes on the *sni1* and *asap1* Mutants.

(A) and (B) Mutations in *RAD17* and *ATR* suppress the *PR1* and *PR2* gene expression in the *sni1* mutant. The expression level was normalized to the housekeeping gene, *UBQ5*. The data are presented as mean ± SD (n = 3).

(C) Mutations in *RAD17, RAD51D, SWS1, BRCA2A* and *RAD51* suppress the *asap1* mutant.

(D) The mutation in *ATM* cannot suppress the *sni1* phenotype, but enhances it.

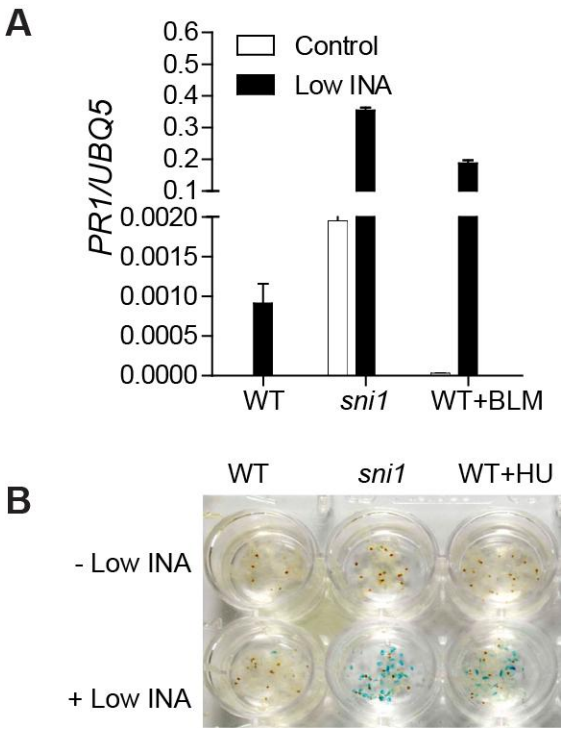

Figure S3, Related to Figure 4. DNA-Damaging Chemicals Enhance INA-Induced Defense Gene Expression.

(A) Plants were grown on medium with 4 μg/mL bleomycin (BLM) and/or low INA (10 μM) for 9 days. *PR1* expression was measured by qRT-PCR. The expression level was normalized to the housekeeping gene, *UBQ5*. The data are shown as mean ± SD (*n* = 3).

(B) Plants were grown on medium with 1 mM hydroxyurea (HU) and/or 10 μM INA for 9 days. The *PR2:GUS* expression pattern was monitored by GUS staining. The blue color indicates the *PR2* promoter activity.

Table S1, Related to Figure 1. Pairwise Structure Alignments

The alignments were performed using TM-align. TM-scores are shown. TM-score > 0.5 means similar protein folding (shown in bold).

	SNI1	NSE6	IB3U	ASAP1	NSE5	1JDH
SNI1	**1**	**0.824**	**0.910**	0.395	0.384	0.481
NSE6	**0.689**	**1**	**0.929**	0.396	0.391	0.450
IB3U	**0.679**	**0.828**	**1**	0.368	0.357	0.420
ASAP1	0.378	0.440	0.450	**1**	**0.690**	**0.863**
NSE5	0.417	0.492	0.497	**0.807**	**1**	**0.919**
1JDH	0.423	0.460	0.471	**0.781**	**0.709**	**1**

Table S2. Primers Used in this Study

Primer Name	Purpose	Sequence
PR1-qPCR-F	qPCR	CTCATACACTCTGGTGGG
PR1-qPCR-R	qPCR	TTGGCACATCCGAGTC
PR2-qPCR-F	qPCR	GCTTCCTTCTTCAACCACACAGC
PR2-qPCR-R	qPCR	CGTTGATGTACCGGAATCTGAC
UBQ5-qPCR-F	qPCR	GACGCTTCATCTCGTCC
UBQ5-qPCR-R	qPCR	GTAAACGTAGGTGAGTCCA
BRCA1-qPCR-F	qPCR	TTGCTCAGGGCTCACA
BRCA1-qPCR-R	qPCR	GGTCCTTTTGCAGGCT
RAD51-qPCR-F	qPCR	ATGAAGAAACCCAGCAC
RAD51-qPCR-R	qPCR	TGAACCCCAGAGGAAC
PARP1-qPCR-F	qPCR	TTGACGCCAGTAGGAA
PARP1-qPCR-R	qPCR	AATACCAGCCCAGTTAG
RAD17-qPCR-F	qPCR	CTTCGTATTTGGCGGA
RAD17-qPCR-R	qPCR	GCACGGGGCTATACTC
RPA-qPCR-F	qPCR	GGTAACTATGTGGGGTGA
RPA-qPCR-R	qPCR	GGAGATACAAGGGACACTC
PR1-pro-F	ChIP	GATCGGTCACCTAGAGT
PR1-pro-R	ChIP	CGCCACATCTATGACG
ACT7-pro-F	ChIP	TTCGCACATGTACTCGTTTCG
ACT7-pro-R	ChIP	CAACAGCGGCAGCAGCTAA
PR1-CDS-F	ChIP	AAGGCTAACTACAACTACGC
PR1-CDS-R	ChIP	ACACCTCACTTTGGCACAT
LB-GABI	Genotyping	ATATTGACCATCATACTCATTGC
LB-SALK	Genotyping	ATTTTGCCGATTTCGGAAC
smc6b-LP	Genotyping	ATGTGGGACTTTGGAACGA
smc6b-RP	Genotyping	AAATGCCTCAAGACGCAAC
smc6a-LP	Genotyping	TTGCATGACTCTGATAGGCAC
smc6a-RP	Genotyping	TCTTTACAACATGCAGGGTCC
atr-LP	Genotyping	GCAGCAAAAATTTCTTGGTTG
atr-RP	Genotyping	ACTTCAAGGGTTCCGATGTTC
atm-LP	Genotyping	ATCCATGTGGTTCAGTCTTGC
atm-RP	Genotyping	TTGGTATCCTGCAGAGGAAAG
asap1-LP	Genotyping	ATCTGCTTGTGACTTTTGGTG
asap1-RP	Genotyping	ATTTTCGTCGCTGTCCTGT
rad51-LP	Genotyping	CTCCCCTTCCAGAGAAATCTG
rad51-RP	Genotyping	ATGCCAAGGTTGACAAGATTG
rad17-2-LP	Genotyping	CAGTCTGGTCAGAAGAGTCCG
rad17-2-RP	Genotyping	ATGTTTTGGCTTGTCACCTTG
sni1-dCAPS-F	Genotyping	TCTTTGTTATGGTGGGGATGGTTG
sni1-dCAPS-R	Genotyping	CCAAGGATCTGCAGAAATCCAAAC
ssn4-F	Genotyping	TGTTTTGGTAGAGATGAGACCCT
ssn4-R	Genotyping	CTAGAAGAAGCTGAAATTGATG

SMC5-attb1	Cloning	ggggacaagtttgtacaaaaaagcaggcttcATGTCTGAACGTCGTGCTAAG
SMC5-attb2	Cloning	ggggaccactttgtacaagaaagctgggtcGGAACATTGACTAGCTTCGGT
SMC6B-attb1	Cloning	ggggacaagtttgtacaaaaaagcaggcttcATGGTAAAATCTGGTGCTCG
SMC6B-attb2	Cloning	ggggaccactttgtacaagaaagctgggtcAGAACGAGGAGCAGCCATT
ASAP1-attb1	Cloning	ggggacaagtttgtacaaaaaagcaggcttcATGGATTTGGATGGACCTCT
ASAP1-attb2	Cloning	ggggaccactttgtacaagaaagctgggtcATTGCTGCTGCAACCTTGA
pRAD17-attb1	Cloning	ggggacaagtttgtacaaaaaagcaggcttcTCTTGTGATACAACGTCTGCCT
cRAD17-attb1	Cloning	ggggacaagtttgtacaaaaaagcaggcttcATGCTTAAGAAAAAACTATCCCTAG
cRAD17-attb2	Cloning	ggggaccactttgtacaagaaagctgggtcCCAGTCTTGTATTTCATCATCTTCA
SNI1-attb1	Cloning	ggggacaagtttgtacaaaaaagcaggcttcATGTCGAAAGAGACGAAGGG
SNI1-attb2	Cloning	ggggaccactttgtacaagaaagctgggtcAGCTTTTGCCTTGAGTACCAT
SNI1-772-F	Cloning	cttggtctcggatccaATGTCGAAAGAGACGAAGGG
SNI1-772-R	Cloning	cttggtctcgtcgactcaAGCTTTTGCCTTGAGTACCAT
RAD17-771-F	Cloning	cttggtctcggatccATGCTTAAGAAAAAACTATCCCTAG
RAD17-771-R	Cloning	cttggtctcgtcgacCCAGTCTTGTATTTCATCATCTTCA

Table S3, Related to Figure 1. The Peptides Matched to SNI1, SMC6B, SMC5 and ASAP1 in the Mass Spectrometry Analysis.

Table S4, Related to Figure 4. Genes Synergistically Induced by BLM and INA.

Table S5, Related to Figure 4. Gene Ontology Enriched in the Genes Synergistically Induced by BLM and INA.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Materials

The mutants *rad17-2* (SALK_009384C), *atr* (SALK_032841) and *atm* (SALK_006953), *smc6b* (SALK_124719C), *smc6a* (SALK_009818C) were ordered from ABRC. The *asap1* mutant (GABI_218F01) was ordered from GABI-Kat. The primers used for genotyping the mutants are listed in Table S2.

SNI1 Complex Purification and Protein Identification

Complex purification was carried out as described (Rubio et al., 2005). Briefly, total protein was extracted in buffer (50 mM Tris–HCl pH 7.5, 150 mM NaCl, 10% glycerol, 0.1% Nonidet P-40, 1% protease inhibitor cocktail and 40 μM MG115) and incubated with IgG Sepharose 6 Fast Flow beads (GE Healthcare). The bound proteins were cleaved using PreScission protease (GE Healthcare) and were further incubated with the Ni-NTA resin (Qiagen). The bound proteins were eluted in buffer containing 250 mM imidazole. The resulting proteins were precipitated by trichloroacetic acid and submitted for LC-MS/MS analysis at Taplin Biological Mass Spectrometry Facility, Harvard Medical School.

In vitro Pull-Down Assay

The recombinant proteins GST, GST-SNI1 (in pGEX-4T-1, GE Healthcare), HisMBP-GFP and HisMBP-SNI1 (in pDEST-HisMBP, Addgene plasmid 11085, Nallamsetty et al., 2005) were expressed in *E. coli* C41. The cells were induced with 0.25 mM IPTG at

20°C for 12 h. GST and GST-SNI1 were purified with Glutathione Sepharose 4B beads (GE Healthcare). HisMBP-GFP and HisMBP-SNI1 were purified using the Ni-NTA beads (QIAGEN). SMC5-MYC, SMC6B-MYC, ASAP1-MYC and RAD17-MYC (in pGBKT7, Clontech) were *in vitro* translated using TNT® Quick Coupled Transcription/Translation Systems (Promega) according to manufacturer's protocol and diluted 6 times in Binding Buffer (50 mM Tris–HCl pH 7.5, 150 mM NaCl, 1 mM EDTA and 0.2% Triton X-100). The beads containing the recombinant proteins were then incubated with the diluted MYC-tagged proteins for 2 h at 4°C. The beads were washed 5 times with Binding Buffer and incubated with the SDS sample buffer containing 100 mM DTT at 95ºC for 10 min. Western blot was performed using the c-MYC antibody [9E10] (Abcam).

Split Luciferase Assay

Split luciferase assay was carried out as described (Chen et al., 2008). Briefly, the genes were either cloned into vectors containing the C-terminal half of luciferase (cLUC) the N-terminal half of luciferase (nLUC). They were transformed into *Agrobacterium tumefaciens* strain GV3101 and then co-expressed in *N. benthamiana.* The images were captured using a CCD camera.

Yeast Two-Hybrid Assay

Yeast two-hybrid analysis was performed using Matchmaker GAL4 Two-Hybrid System following the manufacturer's instructions (Clontech). The coding region of *RAD17* was cloned into pGBKT7 and transformed into the yeast strain Y187 (MATα) and the coding

region of *SNI1* was cloned into pGADT7 and transformed into AH109 (MATa). The interaction was determined by yeast growth on media lacking Trp, Leu, Ade and His.

Real-Time Quantitative RT-PCR

Total RNA was extracted using TRIzol (Invitrogen) and treated with TURBO DNA-free™ (Ambion) to remove residual genomic DNA; first strand cDNA was synthesized using SuperScript® III Reverse Transcriptase (Invitrogen); PCR was performed using FastStart Universal SYBR Green Master (Roche) on Mastercycler ep gradient S (Eppendorf). The expression level was normalized against *UBQ5*.

Comet Assay

Comet assay was performed using CometAssay Kit (Trevigen) with some modifications. Nuclei were isolated by slicing tissues in cold PBS buffer (160 mM NaCl, 8 mM Na_2HPO_4, 4 mM NaH_2PO_4, pH 7.0, 5 mM EDTA) with a new razor blade under dim light. The slides containing the nuclei were treated with lysis buffer for 30 min, high alkali buffer (0.3 M NaOH, 5 mM EDTA, pH 13.5) for 30 min and subjected to electrophoresis (0.7 V/cm) in 1X TBE buffer (90 mM Tris–borate, 2 mM EDTA, pH 8.4). The comets were visualized by staining with SYBR Green I, captured with Zeiss LSM 510 upright confocal microscope at Light Microscopy Core Facility, Duke University and analyzed with CometScore (Tritek).

Trypan Blue Staining

Trypan blue staining was carried out as described (Bowling et al., 1997). Seedlings were vacuum-infiltrated in a solution of phenol, lactic acid, glycerol and water (1:1:1:1) plus 2.5 mg/L trypan blue and then placed in a boiling water bath for 2 min and allowed to cool overnight. The samples were then destained in the chloral hydrate solution.

GUS Staining

GUS staining was described previously (Jefferson et al., 1987). Briefly, the plants were vacuum-infiltrated with the staining solution (50 mM sodium phosphate buffer pH 7.2, 0.2% Triton X-100, 10 mM potassium ferrocyanide, 10 mM potassium ferricyanide, and 1 mM X-gluc) for 15 min and then incubated at 37°C for 12 h. Samples were then washed with 70% ethanol for several times.

Mutant Screen

The screen for suppressor of *sni1* (SSN) was described previously (Durrant et al., 2007). One of the suppressors 38B1 (*sni1 ssn4*) was used for map-based cloning in this study.

Map-Based Cloning

The suppressor 38B1 (in the *Col*-0 ecotype) was crossed with the *Ler* ecotype. In the F2 population, a cleaved amplified polymorphic sequence (CAPS) marker was first used to identify the *sni1* homozygous plants, among which the *sni1 ssn4* homozygous progeny were detected by their wild-type morphology. DNA from 40 *sni1 ssn4* F2 plants was pooled and analyzed using simple sequence length polymorphism (SSLP) markers from

each of the five *Arabidopsis* chromosomes (Lukowitz et al., 2000). There was a clear bias toward *Col*-0 at the marker Ciw10 at the bottom of Chromosome V. More SSLP or CAPS markers were designed using TAIR Polymorphism Search and the Monsanto Arabidopsis Polymorphism Collection (Jander et al., 2002). Further testing located the mutation between MBK-5 (7 recombination) and Cer454879 (2 recombination). For fine mapping, 288 additional F2 plants were used. The locus was mapped to the region between markers Cer454106 and Cer454435. The mutation was identified by sequencing candidate genes in this region.

Complementation Test

The genomic sequence of *RAD17* including the 2061-bp promoter region was cloned into pDONR207 and then introduced into pMDC99 using Gateway cloning (Invitrogen). The resulting construct was transformed into *Agrobacterium tumefaciens* strain GV3101 and then transformed into *sni1 ssn4* using the floral dipping method (Clough and Bent, 1998).

Microarray Analysis

Total RNA was extracted using RNeasy Mini Kit (Qiagen), labeled with MessageAmp Premier RNA Amplification Kit (Ambion) and hybridized with GeneChip Arabidopsis ATH1 Genome Array (Affymetrix) at the Duke Microarray Facility. The microarray data were normalized using Gene-Spring GX Software (RMA algorithm; Agilent). To identify genes that were induced synergistically by the co-treatment of INA and BLM, we applied a Monte Carlo based random sampling strategy. Briefly, the normalized signals from

three biological replicates were assumed normally distributed. 1,000,000 random draws based on the means and variances of these replicates were generated. The following equation was used to calculate the synergistic effect scores: $d_{BLM+INA} + d_{CK} - d_{INA} - d_{BLM}$, where $d_{BLM+INA}$, d_{CK}, d_{INA} (> 0) and d_{BLM} (> 0) are the random draws of BLM+INA-treated, control, INA-treated and BLM-treated, respectively. *P*-values were reported as the percentage of negative synergistic effect scores. The genes with $P < 0.05$ were considered significant.

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