Title: The Chromatin Remodeler SPLAYED Negatively Regulates SNC1-Mediated Immunity

Running title: SYD Negatively Regulates SNC1-Mediated Immunity

Corresponding author: Dr. X. Li, Michael Smith Laboratories and Department of Botany, University of British Columbia, 2185 East Mall, Vancouver, V6T 1Z4, Canada

Phone: +1-604-822-3155; Fax: +1-604-822-2114; E-mail: xinli@msl.ubc.ca

Subject areas: (2) environmental and stress responses; (3) regulation of gene expression

Number of figures: Three black and white, two colour

Title: The Chromatin Remodeler SPLAYED Negatively Regulates SNC1-Mediated Immunity

Running title: SYD Negatively Regulates SNC1-Mediated Immunity

Kaeli C. M. Johnson[1,2,5], Shitou Xia[3,5], Xiaoqi Feng[4] and Xin Li[1,2,*]

[1] Michael Smith Laboratories, University of British Columbia, Vancouver, V6T 1Z4, Canada
[2] Department of Botany, University of British Columbia, Vancouver, V6T 1Z4, Canada
[3] Hunan Provincial Key Laboratory of Phytohormones and Growth Development, Hunan Agricultural University, Changsha, 410128, China
[4] John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, United Kingdom
[5] These authors contributed equally to the work described

* Corresponding author

Abbreviations:
ATXR7 – ARABIDOPSIS TRITHORAX-RELATED 7
BRM – BRAHMA
Col-0 – Columbia-0
DDM1 – DECREASED DNA METHYLATION 1
EMS – ethyl methanesulfonate
ET – ethylene
GUS – β-glucuronidase
H.a. – Hyaloperonospora arabidopsidis
JA – jasmonate
LAZ5 – LAZARUS 5
L*er* – Landsberg *erecta*
MOS – MODIFIER OF SNC1
MUSE – MUTANT, SNC1-ENHANCING
NLR – NOD-LIKE RECEPTOR
PR – PATHOGENESIS RELATED
P.s.m. – Pseudomonas syringae pathovar *maculicola*
P.s.t. – Pseudomonas syringae pathovar *tomato*
R – RESISTANCE
RDR2 – RNA-DEPENDENT RNA POLYMERASE 2
RPP4 – RECOGNITION OF PERONOSPORA PARASITICA 4
SA – salicylic acid
SDG8 – SET DOMAIN GROUP 8
SNC1 – SUPPRESSOR OF NPR1, CONSTITUTIVE 1
SWI/SNF – SWITCH/SUCROSE NON-FERMENTABLE
SYD – SPLAYED

Abstract:

SNC1 (SUPPRESSOR OF NPR1, CONSTITUTIVE 1) is one of a suite of intracellular Arabidopsis NOD-like receptor (NLR) proteins which, upon activation, result in the induction of defense responses. However, the molecular mechanisms underlying NLR activation and the subsequent provocation of immune responses are only partially characterized. To identify negative regulators of NLR-mediated immunity, a forward genetic screen was undertaken to search for enhancers of the dwarf, autoimmune gain-of-function *snc1* mutant. To avoid lethality resulting from severe dwarfism, the screen was conducted using *mos4* (*modifier of snc1, 4*) *snc1* plants, which display wild-type-like morphology and resistance. M2 progeny were screened for *mutant, snc1-enhancing* (*muse*) mutants displaying a reversion to *snc1*-like phenotypes. The *muse9 mos4 snc1* triple mutant was found to exhibit dwarf morphology, elevated expression of the *pPR2-GUS* defense marker reporter gene, and enhanced resistance to the oomycete pathogen *Hyaloperonospora arabidopsidis* Noco2. Via map-based cloning and Illumina sequencing, it was determined that the *muse9* mutation is in the gene encoding the SWI/SNF chromatin remodeler SYD (SPLAYED), and was thus renamed *syd-10*. The *syd-10* single mutant has no observable alteration from wild-type-like resistance, although the *syd-4* T-DNA insertion allele displays enhanced resistance to the bacterial pathogen *Pseudomonas syringae* pv. *maculicola* ES4326. Transcription of *SNC1* is increased in both *syd-4* and *syd-10*. These data suggest that SYD plays a subtle, specific role in the regulation of *SNC1* expression and SNC1-mediated immunity. SYD may work with other proteins at the chromatin level to repress SNC1 transcription; such regulation is important for fine-tuning the expression of NLR-encoding genes to prevent unpropitious autoimmunity.

Key Words: Arabidopsis, immunity, resistance, SNC1, SPLAYED, MUSE9

Introduction

To compensate for the vulnerability inherent in being sessile organisms, plants must maintain a tightly regulated innate immune system to ward off pathogenic infection (Dangl et al. 2013). As part of this system, the detection of conserved microbial features by receptors on the plant cell surface induces relatively mild defense responses (Macho and Zipfel 2014). However, successful pathogens are able to deliver effector molecules into the host cell to suppress this immune response and promote infection.

As an additional line of defense, plants possess a suite of intracellular receptors termed RESISTANCE (R) proteins which recognize effectors in a specific manner either directly or through their effects upon other host proteins (Chisholm et al. 2006; Dangl and Jones 2001). Although there are several classes of R proteins, the majority belong to the nucleotide-binding and leucine-rich repeat domain-containing/NOD-like receptor (NLR) class. Upon effector detection, NLR proteins become activated and strong, robust defense responses are induced. NLR protein-mediated immunity is characterized by an accumulation of the defense hormone salicylic acid (SA), increased expression of *PATHOGENESIS-RELATED* (*PR*) defense marker genes, and often a programmed cell death event known as the hypersensitive response (Hammond-Kosack and Jones 1996). While NLR-mediated immunity is a well-documented phenomenon in higher plants, the molecular mechanisms underlying its regulation are only marginally understood.

In the absence of pathogen attack, NLR protein levels must be kept under stringent control in order to prevent growth defects and potential lethality resulting from unwanted activation of autoimmune responses. Upon infection, however, the repression of NLR protein-mediated signaling pathways must be released in order to allow the rapid induction of defense responses.

The regulation of NLR-mediated immunity occurs at the transcriptional, translational, and post-translational levels. At the transcriptional level, a number of positive regulators of *NLR* gene expression have been identified. The histone lysine methyl transferase SDG8 trimethylates H3K36 (Histone 3, Lysine 36) at the NLR-encoding *LAZ5* locus, and this activity is required for the perpetuation of a transcriptionally active chromatin state (Palma et al. 2010). Similarly, MOS9 was shown to function together with the methyl transferase ATXR7 in the methylation of H3K4 at the NLR-encoding *SNC1* and *RPP4* loci, and this methylation is required for the full expression of these genes (Xia et al. 2013). The MOS1 protein, which contains an HLA-B ASSOCIATED TRANSCRIPT 2 domain, is required for full *SNC1* expression and functions antagonistically with the chromatin remodelling factor DECREASED DNA METHYLATION 1 (DDM1) (Li et al. 2010; Li et al. 2011). Although the mechanism of this regulation is not well understood, it is thought to occur at the chromatin level as the expression of transgenic *SNC1* does not require MOS1.

MOS1 and MOS9 were both identified from a forward genetic screen designed to isolate positive regulators of NLR-mediated immunity. The *MODIFIERS OF SNC1* (*MOS*) screen was designed to identify suppressors of the autoimmune mutant *snc1* (*suppressor of npr1, constitutive 1)*, which contains a gain-of-function mutation in an NLR-encoding gene (Li et al. 2001; Zhang et al. 2003). Mutant *snc1* plants display a dwarfed, dark green, curled-leaf morphology, accumulate SA, and exhibit constitutively activated defense responses, although lesions typically associated with the hypersensitive response fail to form. As such, the *snc1* mutant has become a useful genetic background in which to conduct forward genetic screens for regulators of immunity. From the MOS screens, mutants exhibiting a suppression of *snc1*-mediated defense responses were selected and many *mos* mutations were cloned.

As the MOS screens were successful in identifying positive regulators of NLR-mediated immunity (summarized in Johnson et al. 2012), we proceeded to design enhancer screens in the *snc1* background in order to identify negative regulators of immunity. To avoid lethality resulting from dramatic dwarfism the forward genetic screens were conducted by mutagenizing seeds from *mos4 snc1* plants, which are wild-type-like in terms of morphology and resistance levels. As part of the MUTANT, SNC1-ENHANCING (MUSE) screen a number of mutants displaying a reversion back to *snc1*-like morphology and defense outputs were isolated, several of which have been recently published (Huang et al. 2013; Huang et al. 2014a; Huang et al. 2014b; Xu et al. 2015, in press).

This study focuses on the isolation, identification, and characterization of *muse9*. The *muse9 mos4 snc1* triple mutant is dwarfed and displays elevated expression of the *pPR2-GUS* reporter gene. An elevation in resistance against the virulent oomycete strain *Hyaloperonospora arabidopsidis* (*H.a.*) Noco2 was observed in the triple mutant. The *muse9* mutation was found to be a novel allele of *splayed* (*syd-10*), which encodes a SWI/SNF chromatin remodeler. The *syd-10* single mutant exhibits wild-type-like resistance, but the *syd-4* T-DNA insertion allele exhibits enhanced resistance to *Pseudomonas syringae* pv. *maculicola* (*P.s.m.*) ES4326. Double mutant analysis showed that mutations in the *SYD* locus enhance the dwarfism of *snc1*, and SYD is required for modulating transcription at the *SNC1* locus. Thus, we establish that SYD plays a subtle but specific role in repressing *SNC1* expression.

Results

Isolation of *muse9 snc1 mos4*

The *muse9* mutation was isolated from the MUSE forward genetic screen described previously (Huang et al. 2013), which was conducted in the *mos4 snc1* mutant background with ethyl methanesulfonate (EMS) as a mutagen. Mutant lines displaying a reversion to *snc1*-like phenotypes were selected as putative *snc1* enhancers. The *muse9 mos4 snc1* triple mutant displays *snc1*-like morphological phenotypes (Figure 1A).

In *snc1*, a number of *PATHOGENESIS-RELATED* (*PR*) defense marker genes are constitutively expressed. All mutants from the MUSE screens contain a reporter gene construct in which the promoter of *PR2* is fused to the coding region of β-glucuronidase (GUS), allowing for a rapid visualization of defense gene expression. In the wild-type Columbia (Col-0) background no GUS expression is observed (Figure 1B). The *muse9* mutation partially rescues the constitutive expression of the *pPR2-GUS* reporter gene observed in *snc1* but suppressed in *mos4 snc1* (Figure 1B).

The *snc1* mutation confers enhanced resistance against the virulent oomycete pathogen *H.a.* Noco2 (Zhang et al. 2003). Consistent with the observed rescue of *pPR2-GUS* constitutive expression noted above, the *muse9 mos4 snc1* triple mutant showed a moderate but significant enhancement in resistance against *H.a.* Noco2 as compared to the *mos4 snc1* double mutant (Figure 1C). Together, these data indicate that the *muse9* mutation is able to partially enhance *snc1* phenotypes in the *mos4 snc1* background.

Phenotypes associated with *muse9* result from a point mutation in *SYD*

To determine the molecular lesion responsible for the *snc1*-enhancing phenotypes associated with *muse9*, a positional cloning strategy was employed. The *muse9 mos4 snc1* triple mutant in the Col-0 ecotype was crossed with Landsberg *erecta* (L*er*) to generate the mapping

population. Linkage analysis was performed using 24 F2 plants displaying *snc1*-like phenotypes, which revealed that *muse9* showed linkage with markers located between 9.2MB and 13.2MB on chromosome 2 (Figure 2A). Fine mapping using >1000 F3 plants from F2 progeny that were homozygous for *snc1* and *MOS4*, but heterozygous for *muse9*, further narrowed down *muse9* to a region between 10.8MB and 12.4MB on chromosome 2.

To identify the exact mutation responsible for *muse9*, Illumina whole genome sequencing was performed. Comparisons between the mutant sequence and the reference Col-0 Arabidopsis genome indicated that five genes located within this mapped region contained mutations consistent with EMS mutagenesis (Figure 2B). However, four of these are either silent or intronic mutations. The other mutation is in *At2g28290*, and results in an amino acid change; therefore, it was selected as the most likely candidate for *muse9*. *At2g28290* encodes SPLAYED (SYD), a SWI/SNF chromatin remodelling ATPase previously implicated in development as well as jasmonate (JA) and ethylene (ET) signaling pathways (Wagner and Meyerowitz 2002; Walley et al. 2008). The C to T substitution in *muse9* occurs in the last exon of *SYD* in a region of the protein that does not contain any known conserved domains (Figure 2C-D), and results in the substitution of Ala2224 with Val (Figure 2E).

Transgene complementation is commonly employed in verifying positional cloning results. However, the large size of the *SYD* locus (>16 kb) precludes straightforward molecular cloning in binary plasmid vectors. Instead, to verify that the mutation in *SYD* is responsible for the *muse9* phenotypes, an allelism test was carried out between the *muse9* single mutant and *syd-4*, a previously published T-DNA insertion allele (Zhu et al. 2013) that contains an insertion in the conserved helicase domain of SYD (Figure 2D). The *muse9* single mutant was obtained by backcrossing *muse9 mos4 snc1* to Col-0 and selecting F2 lines that were homozygous for the

muse9 mutation and wild-type at the *MOS4* and *SNC1* loci. Both the *muse9* and *syd-4* mutations result in slightly crinkled leaves and a small reduction in stature as compared to wild-type. The F1 progeny resulting from a cross of these two genotypes retain these characteristics (Figure 3A), indicating that *muse9* failed to complement *syd-4* and therefore that *MUSE9* is *SYD.*

As an additional method of verification, the *snc1*-enhancing effects of the two *syd* alleles were compared. The *muse9 snc1* double mutant was isolated from the F2 progeny of the backcross described above, and the *syd-4* mutant was crossed with *snc1* to generate the *syd-4 snc1* double mutant. Both double mutants show a dramatic reduction in size compared to either *muse9* or *syd-4* and *snc1* (Figure 3B). Taken together, we conclude that the phenotypes associated with *muse9* are a result of a mutation in *SYD*; therefore, we renamed *muse9* as *syd-10*.

The *syd-4* single mutant displays enhanced disease resistance

As demonstrated above, *syd-10* was found to enhance *snc1*-associated morphological and disease resistance phenotypes in the *snc1* and *mos4 snc1* genetic backgrounds. As growth of the *syd-10* single mutant is slightly stunted (Figure 3A), and fitness costs including diminished stature and reduced seed production are commonly associated with constitutive activation of NLR-mediated defense responses, it was hypothesized that the single mutant may show enhanced disease resistance independent of the presence of the *snc1* mutation. This hypothesis was tested using a number of infection assays with virulent pathogens.

As noted above, *snc1* displays enhanced resistance to the oomycete *H.a.* Noco2; however, resistance to this pathogen was found to be wild-type-like in both *syd-4* and *syd-10* (Figure 4A). *snc1* also displays enhanced resistance to the virulent bacterial strain *P.s.m.* ES4326 (Zhang et al. 2003). When *syd-10* and *syd-4* plants were challenged with this pathogen the *syd-*

10 single mutant was again found to display wild-type-like resistance, but enhanced resistance was consistently observed in the *syd-4* single mutant (Figure 4B). We found that *PR1* and *PR2* are upregulated in both *syd* alleles (Figures 4C-D), although expression was enhanced to a greater degree in the *syd-4* mutant. Consistent with a previous report which found that expression of the defensin *PDF1.2a*, a marker of intact ET and JA signaling pathways, was reduced in the *syd-2* mutant (Walley et al. 2008), we also observed lower *PDF1.2a* expression in the *syd-4* and *syd-10* mutants (Figure 4E). Since *syd-4* contains an insertion in the conserved helicase domain of SYD while *syd-10* carries a point mutation in the weakly conserved N terminal region of the protein (Figure 2D; Supplementary Figure 1), it is possible that *syd-10* is a weaker allele and therefore exhibits more subtle phenotypes.

Mutations in *SYD* result in elevated transcription of *SNC1*

One mechanism to enhance disease resistance in plants is to increase steady-state levels of NLR proteins through transcriptional up-regulation. As *SYD* encodes an ATP-dependent chromatin remodeler, it was hypothesized that the enhancement of *snc1*-like phenotypes associated with mutations in *SYD* may be a result of altered SYD function and subsequent changes in transcriptional activity at the *SNC1* locus. Using real-time qRT-PCR, it was found that *SNC1* expression is moderately but significantly elevated in both the *syd-10* and *syd-4* single mutants (Figure 4F). However, expression of *RPP4*, another NLR-encoding gene that resides within the same gene cluster as *SNC1*, was unaltered in the *syd* mutants (Figure 4G). These data suggest that *SYD* is responsible for maintaining proper transcript levels of *SNC1* specifically. However, no obvious increase in SNC1 protein was observed in the *syd* single mutants (Supplementary Figure 2). Without SYD function *SNC1* transcription is up-regulated, which can

be amplified in the *snc1* mutant background and result in an enhancement of *snc1*-mediated autoimmunity.

Discussion

Eukaryotic ATP-dependent chromatin remodeling complexes contain a DNA-dependent ATPase subunit which utilizes the energy derived from the hydrolysis of ATP to alter the positions of nucleosomes along the DNA strand (Clapier and Cairns, 2009). The resultant changes to chromatin structure potentially modify the transcriptional activity at affected loci. One extensively studied ATPase in Arabidopsis is SYD, which belongs to the evolutionarily conserved SWI/SNF class of chromatin remodelers and was first identified as a regulator of reproductive development (Wagner and Meyerowitz 2002). Plant SWI/SNF chromatin remodeling complexes have been implicated in many biological processes in addition to development, including hormone signaling and RNA-mediated gene silencing (reviewed in Reyes 2014). In this study, we have determined a novel role for SYD in negatively regulating SNC1-mediated resistance.

SYD was previously shown to be a regulator of JA- and ET-mediated stress signaling pathways and is required for resistance against *Botrytis cinerea*, a necrotrophic fungus with a broad host range (Walley et al. 2008). The same study reported that two mutant alleles of *SYD* conferred wild-type-like resistance to the biotrophic bacterial pathogen *Pseudomonas syringae* pv. *tomato* DC3000, resistance against which is primarily mediated by SA. These results suggested that SYD is specifically involved in the regulation of disease resistance mediated by JA and ET signaling pathways, but not involved in SA-mediated immunity. Consistent with the previously published data, we found that the novel *syd-10* allele also displays wild-type-like

resistance to a different *Pseudomonas syringae* strain, *P.s.m.* ES4326 (Figure 4B). However, the *syd-4* single mutant displays enhanced resistance to this pathogen. Differences in the immune phenotypes of *syd-10* and *syd-4* may be a result of the differing strengths of the mutations: *syd-10* contains a point mutation in the weakly conserved N terminal region of SYD, while *syd-4* carries a T-DNA insertion in the conserved helicase domain (Figure 2D; Supplementary Figure 1). Additionally, the *syd-10* allele confers enhanced resistance to the obligate biotrophic oomycete *H.a.* Noco2 in the *mos4 snc1* genetic background (Figure 1C). The finding that SYD plays a role in mediating resistance to biotrophic pathogens is not wholly unprecedented, as the SA-responsive defense marker gene *PR1* was shown to be upregulated in *syd-2*, although none of the genes upstream in the SA signaling pathway were observed to have altered expression in the mutant (Walley et al. 2008). This supports our postulation that while SYD positively regulates JA- and ET-mediated defense against necrotrophs, it plays a role in the negative regulation of SA-mediated immunity.

From the phenotypic analysis of *syd* mutants, the role SYD plays in regulating SA-mediated defense responses appears to be quite subtle. This study has demonstrated that *syd-10* enhances morphological and resistance phenotypes associated with *snc1*; however, the degree of the enhancement is not as strong as observed for other published *muse* mutants. The presence of the *syd-10* mutation in the *mos4 snc1* background only partially rescues the *H.a.* Noco2 resistance associated with *snc1* (Figure 1C), and the immune phenotypes of the single mutant are almost indistinguishable from wild-type (Figure 4A-B), except for the enhanced resistance phenotype of the *syd-4* single mutant. While SNC1 protein levels appear to be elevated in *syd-10 mos4 snc1* as compared to *mos4 snc1*, SNC1 does not obviously accumulate in the *syd* single mutants (Supplementary Figure 2). As *SNC1* gene expression is only slightly increased in the *syd*

mutants (Figure 4F), the consequent minute protein level change is likely below the detection limit of the western blot method. Given these results, it is unsurprising that *syd* alleles were not identified from any prior known screens for regulators of SA-mediated immunity. The sensitized genetic background used in the MUSE screen has enabled the identification of *syd-10* and other novel components of immune signaling (Huang et al. 2013; Huang et al. 2014a; Huang et al. 2014b; Xu et al. 2015). One possible explanation as to why the defense phenotypes associated with the *syd-10* mutation are only observable in the *snc1* background is that in this background defense responses are constitutively activated; therefore, knocking out negative regulators of this pathway results in a stronger, more quantifiable defense induction. In the wild-type genetic background, knocking out a minor negative immune regulator is insufficient to activate immune responses by itself; perhaps the threshold level of defense gene induction required to confer enhanced resistance cannot be reached.

The mild effects of mutations in *SYD* upon SA-mediated signaling may also be partially explained by redundancy with its close homolog *BRAHMA* (*BRM*). These two ATPases have been demonstrated to act on both shared and unique target genes, and elevated expression of a number of SA-dependent defense response genes including *PR1* has been observed in *brm-101* mutants (Bezhani et al. 2007; Wu et al. 2012).

Other ATP-dependent chromatin remodeling complexes have been shown to repress SA-dependent defense gene expression. Mutations in subunits of the Arabidopsis SWR1 chromatin remodeling complex result in enhanced resistance to *P.s.t.* DC3000 and constitutive expression of genes associated with systemic acquired resistance, a long-lasting broad spectrum defense mechanism that protects against future infection and requires SA (March-Diaz et al. 2008). Such differential gene expression is caused by the loss of H2A.Z (March-Diaz et al. 2008), a histone

variant important for regulating gene expression deposited by SWR1 complexes in plants, yeasts and mammals (Krogan et al. 2003; Kobor et al. 2004; Mizuguchi et al. 2004; Ruhl et al. 2006; Deal et al. 2007). In Arabidopsis, H2A.Z is enriched at genes responsive to environmental and developmental stimuli, such as genes involved in immune and temperature responses, and plays an essential role in controlling their expression (Coleman-Derr and Zilberman 2012; Kumar and Wigge 2010). Knocking out another chromatin-remodeling ATPase, DDM1, has been shown to release the suppression of *SNC1* expression caused by the *mos1* mutation, although expression of *SNC1* in the *ddm1* mutant is comparable to levels observed in wild-type (Li et al. 2010). Taken together, these reports highlight the contribution of chromatin remodeling in defense gene regulation.

ATP-dependent chromatin remodelers are also known to affect DNA methylation, a type of epigenetic mark that can result in modified chromatin accessibility and gene transcription. As such, an examination of the DNA methylation status around the *SNC1* locus was undertaken in *syd* plants. A slight decrease of DNA methylation in the asymmetric CHH (H = A, T or C) context was observed in *syd* at a transposon approximately 3 kb upstream of *SNC1*, as compared to wild type (Supplementary Figure 3). To investigate if this is the cause of *SNC1* transcriptional elevation in *syd*, we took advantage of mutants that exhibit reduced CHH methylation in this transposon (*ddm1* and *rdr2*). No significant alteration of *SNC1* expression was observed in either mutant (Figure 4F), indicating the suppression of *SNC1* by SYD is unlikely to be mediated by DNA methylation at the *SNC1* locus.

A graphic representation of the potential role of SYD in regulating SNC1-mediated immunity is illustrated in Figure 5. SYD acts antagonistically to MOS1 and MOS9, and is required for negatively modulating transcription at the *SNC1* locus. As part of the SWI/SNF

complex, SYD may directly affect the *SNC1* locus (Fig 5A). Alternatively, SYD may alter the chromatin at another locus (or loci), which indirectly results in the down-regulation of *SNC1* transcription (Figure 5B). Although MOS1 and MOS9 also affect *RPP4* transcription (Li et al. 2010; Xia et al. 2013), SYD does not (Figure 4G), indicating that its effects on *SNC1* are more specific.

In summary, we have shown that mutations in the ATPase-encoding gene *SYD* enhance the morphological and resistance phenotypes associated with the gain-of-function *snc1* mutant and result in increased expression of *SNC1*. However, gaining comprehensive insight into the mechanism by which SYD regulates SNC1-mediated immunity requires further investigation.

Materials and Methods

Plant growth conditions and mutant isolation

Soil-grown plants were kept in climate-controlled growth rooms at 22ºC on a 16h light/8h dark cycle. Plate-grown plants were propagated on ½ Murashige and Skoog medium supplemented with 0.5% sucrose and 0.3% phytagel and grown under the above conditions. The MUSE screen was conducted using EMS as described previously (Huang et al. 2013). The *syd-4* (Salk_149549) mutant was obtained from the Arabidopsis Biological Resource Center and genotyped by PCR using the following primers: 5'-TGAAGCTCTGACTTGCTCCTC-3' and 5'-TCAAAGCAACAGACCATCGG-3'.

Expression analysis

Approximately 0.1 g total plant tissue was collected from plate-grown 2-week-old seedlings. RNA was extracted using the Totally RNA Kit (Ambion, now Invitrogen), and Reverse Transcriptase M-MLV (Takara) was used to reverse transcribe 0.4 μg RNA. Primers used for

amplification of *SNC1* and *ACTIN7* were previously described (Zhang et al. 2003; Cheng et al. 2009).

Positional cloning

Positional cloning of *muse9* was performed using markers derived from insertion/deletion and single nucleotide polymorphisms between the Col-0 and L*er* Arabidopsis ecotypes, identified using sequence information available from TAIR (Jander et al. 2002; http://www.arabidopsis.org). After narrowing down the location of the molecular lesion to between 10.8 MB and 12.4 MB, extracted DNA from *muse9 mos4 snc1* was sequenced using the Illumina sequencing platform.

Pathogen assays

Bacterial and oomycete infection assays were performed as previously described (Li et al. 2001). In brief, bacterial infections were conducting using a needleless syringe to infiltrate the abaxial leaf surfaces of 4-week-old soil-grown plants with *P.s.m*. ES4326 (OD_{600} = 0.001). Bacterial growth was quantified using leaf discs (area = 0.38cm^2) collected on the day of infection (day 0) and 2 d later. Oomycete infections were conducted by spray-inoculating 2-week-old soil-grown seedlings with *H.a*. Noco2 (1x10^5 spores mL^{-1}). Sporulation was quantified 7 d post-infection. Total aerial plant tissue was used in the assay. For each genotype, 5 replicates of 5 plants were each suspended in 1mL ddH_20 and vortexed gently, and spores were counted using a hemocytometer. Spore counts were normalized to fresh weight (mg).

Genetic crosses

The *muse9* single mutant was generated by back-crossing *muse9 mos4 snc1* with Col-0 containing the *pPR2-GUS* reporter gene. The F1 progeny were allowed to self-fertilize, and *muse9* single mutants were identified among the F2 progeny by genotyping.

Funding

This work was supported by funds from the National Sciences and Engineering Research Council of Canada [Canada Graduate Scholarship - Doctoral to KJ; Discovery Grant to XL], and the Dewar Cooper Memorial Fund from the Department of Botany at the University of British Columbia to XL.

Disclosures

The authors have no conflicts of interest to declare.

Acknowledgments

The authors would like to thank Dr. Yuelin Zhang and Dr. Yan Li for their assistance with next-generation sequencing, and Mr. Charles Copeland for critical reading of the manuscript.

References

Bezhani, S., Winter, C., Hershman, S., Wagner, J.D., Kennedy, J.F., Kwon, C.S., et al. (2007) Unique, shared, and redundant roles for the Arabidopsis SWI/SNF chromatin remodeling ATPases BRAHMA and SPLAYED. *Plant Cell* 19: 403-416.

Cheng, Y.T., Germain, H., Wiermer, M., Bi, D.L., Xu, F., Garcia, A.V., et al. (2009) Nuclear Pore Complex Component MOS7/Nup88 Is Required for Innate Immunity and Nuclear Accumulation of Defense Regulators in Arabidopsis. *Plant Cell* 21: 2503-2516.

Chisholm, S.T., Coaker, G., Day, B. and Staskawicz, B.J. (2006) Host-microbe interactions: Shaping the evolution of the plant immune response. *Cell* 124: 803-814.

Clapier, C.R. and Cairns, B.R. (2009) The Biology of Chromatin Remodeling Complexes. *Annual Review of Biochemistry* 78: 273-304.

Coleman-Derr, D. and Zilberman, D. (2012) Deposition of Histone Variant H2A.Z within Gene Bodies Regulates Responsive Genes. *Plos Genetics* 8.

Dangl, J.L., Horvath, D.M. and Staskawicz, B.J. (2013) Pivoting the Plant Immune System from Dissection to Deployment. *Science* 341: 746-751.

Dangl, J.L. and Jones, J.D.G. (2001) Plant pathogens and integrated defence responses to infection. *Nature* 411: 826-833.

Deal, R.B., Topp, C.N., McKinney, E.C. and Meagher, R.B. (2007) Repression of flowering in Arabidopsis requires activation of FLOWERING LOCUS C expression by the histone variant H2A.Z. *Plant Cell* 19: 74-83.

Hammond-Kosack, K.E. and Jones, J.D.G. (1996) Resistance gene-dependent plant defense responses. *Plant Cell* 8: 1773-1791. Huang, S., Monaghan, J., Zhong, X.H., Lin, L., Sun, T.J., Dong, O.X., et al. (2014a) HSP90s are required for NLR immune receptor accumulation in Arabidopsis. *Plant Journal* 79: 427-439.

Huang, Y., Chen, X.J., Liu, Y.A., Roth, C., Copeland, C., McFarlane, H.E., et al. (2013) Mitochondrial AtPAM16 is required for plant survival and the negative regulation of plant immunity. *Nature Communications* 4.

Huang, Y., Minaker, S., Roth, C., Huang, S., Hieter, P., Lipka, V., et al. (2014b) An E4 Ligase Facilitates Polyubiquitination of Plant Immune Receptor Resistance Proteins in Arabidopsis. *Plant Cell* 26: 485-496.

Ibarra, C.A., Feng, X., Schoft, V.K., Hsieh, T.F., Uzawa, R., Rodrigues, J.A., et al. (2012) Active DNA demethylation in plant companion cells reinforces transposon methylation in gametes.

Science 337: 1360-1364.

Jander, G., Norris, S.R., Rounsley, S.D., Bush, D.F., Levin, I.M. and Last, R.L. (2002) Arabidopsis map-based cloning in the post-genome era. *Plant Physiology* 129: 440-450.

Johnson, K.C., Dong, O.X., Huang, Y. and Li, X. (2012) A rolling stone gathers no moss, but resistant plants must gather their moses. *Cold Spring Harb Symp Quant Biol* 77: 259-268.

Kobor, M.S., Venkatasubrahmanyam, S., Meneghini, M.D., Gin, J.W., Jennings, J.L., Link, A.J., et al. (2004) A protein complex containing the conserved Swi2/Snf2-related ATPase Swr1p deposits histone variant H2A.Z into euchromatin. *Plos Biology* 2: 587-599.

Krogan, N.J., Keogh, M.C., Datta, N., Sawa, C., Ryan, O.W., Ding, H.M., et al. (2003) A Snf2 family ATPase complex required for recruitment of the histone H2A variant Htz1. *Molecular Cell* 12: 1565-1576.

Kumar, S.V. and Wigge, P.A. (2010) H2A.Z-Containing Nucleosomes Mediate the Thermosensory Response in Arabidopsis. *Cell* 140: 136-147.

Li, X., Clarke, J.D., Zhang, Y.L. and Dong, X.N. (2001) Activation of an EDS1-mediated R-gene pathway in the snc1 mutant leads to constitutive, NPR1-independent pathogen resistance. *Molecular Plant-Microbe Interactions* 14: 1131-1139.

Li, Y., Dong, O.X., Johnson, K. and Zhang, Y. (2011) MOS1 epigenetically regulates the expression of plant Resistance gene SNC1. *Plant Signal Behav* 6: 434-436.

Li, Y.Z., Tessaro, M.J., Li, X. and Zhang, Y.L. (2010) Regulation of the Expression of Plant Resistance Gene SNC1 by a Protein with a Conserved BAT2 Domain. *Plant Physiology* 153: 1425-1434.

Macho, A.P. and Zipfel, C. (2014) Plant PRRs and the Activation of Innate Immune Signaling. *Molecular Cell* 54: 263-272.

March-Diaz, R., Garcia-Dominguez, M., Lozano-Juste, J., Leon, J., Florencio, F.J. and Reyes, J.C. (2008) Histone H2A.Z and homologues of components of the SWR1 complex are required to control immunity in Arabidopsis. *Plant Journal* 53: 475-487.

Mizuguchi, G., Shen, X.T., Landry, J., Wu, W.H., Sen, S. and Wu, C. (2004) ATP-Driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex. *Science* 303: 343-348.

Palma, K., Thorgrimsen, S., Malinovsky, F.G., Fiil, B.K., Nielsen, H.B., Brodersen, P., et al. (2010) Autoimmunity in Arabidopsis acd11 Is Mediated by Epigenetic Regulation of an Immune Receptor. *Plos Pathogens* 6.

Reyes, J.C. (2014) The Many Faces of Plant SWI/SNF Complex. *Molecular Plant* 7: 454-458.

Ruhl, D.D., Jin, J.J., Cai, Y., Swanson, S., Florens, L., Washburn, M.P., et al. (2006) Purification of a human SRCAP complex that remodels chromatin by incorporating the histone variant H2A.Z into nucleosomes. *Biochemistry* 45: 5671-5677.

Wagner, D. and Meyerowitz, E.M. (2002) SPLAYED, a novel SWI/SNF ATPase homolog, controls reproductive development in Arabidopsis. *Current Biology* 12: 85-94.

Walley, J.W., Rowe, H.C., Xiao, Y.M., Chehab, E.W., Kliebenstein, D.J., Wagner, D., et al. (2008) The Chromatin Remodeler SPLAYED Regulates Specific Stress Signaling Pathways. *Plos Pathogens* 4.

Wu, M.F., Sang, Y., Bezhani, S., Yamaguchi, N., Han, S.K., Li, Z.T., et al. (2012) SWI2/SNF2 chromatin remodeling ATPases overcome polycomb repression and control floral organ identity with the LEAFY and SEPALLATA3 transcription factors. *Proceedings of the National Academy of Sciences of the United States of America* 109: 3576-3581.

Xia, S.T., Cheng, Y.T., Huang, S., Win, J., Soards, A., Jinn, T.L., et al. (2013) Regulation of

Transcription of Nucleotide-Binding Leucine-Rich Repeat-Encoding Genes SNC1 and RPP4 via H3K4 Trimethylation. *Plant Physiology* 162: 1694-1705.

Xu, F., Huang, Y., Li, L., Gannon, P., Linster, E., Huber, M., et al. (2015) Two N-terminal acetyltransferases antagonistically control the stability of a Nod-like receptor in plants. *Plant Cell*, in press.

Zemach, A., Kim, M.Y., Hsieh, P.H., Coleman-Derr, D., Eshed-Williams, L., Thao, K., et al. (2013) The Arabidopsis nucleosome remodeler DDM1 allows DNA methyltransferases to access H1-containing heterochromatin. *Cell* 153: 193-205.

Zhang, Y.L., Goritschnig, S., Dong, X.N. and Li, X. (2003) A gain-of-function mutation in a plant disease resistance gene leads to constitutive activation of downstream signal transduction pathways in suppressor of npr1-1, constitutive 1. *Plant Cell* 15: 2636-2646.

Zhu, Y.Y., Rowley, M.J., Bohmdorfer, G. and Wierzbicki, A.T. (2013) A SWI/SNF Chromatin-Remodeling Complex Acts in Noncoding RNA-Mediated Transcriptional Silencing. *Molecular Cell* 49: 298-309.

Legends to Figures

Figure 1. Phenotypic analysis of the *muse9 mos4 snc1* triple mutant.

(A) Morphology of soil-grown Col-0, *snc1*, *mos4 snc1*, and *muse9 mos4 snc1* plants. Photographs were taken 3 weeks post-germination. Scale bar indicates 1 cm.

(B) *PR2* gene expression depicted using the *pPR2-GUS* fusion construct present in all shown genetic backgrounds. Plants were grown for 10 d on MS media.

(C) Growth of *H.a*. Noco2 on indicated genotypes 7 d post-inoculation with $1x10^5$ spores/mL. Values represent the average of 4 replicates of 5 plants each ± SD. Significant difference

between *mos4 snc1* and *muse9 mos4 snc1* indicated by * (P-value < 0.05). The experiment was repeated three times with similar results.

Figure 2. Positional cloning of the *MUSE9* locus on chromosome 2.

(A) A genetic map depicting the region of chromosome 2 that contains the *MUSE9* locus, with markers used for mapping indicated.

(B) Mutations identified within the mapping region of *muse9* using Illumina sequencing.

(C) The gene structure of *SYD*, with the locations of the *syd-4* and *muse9* (*syd-10*) mutations indicated. Boxes and lines represent exons and introns, respectively.

(D) The conserved domain structure of the SYD protein, with the sites of the *syd-4* and *muse9* mutations denoted. Domains were identified using the NCBI Conserved Domain Database.

(E) Sequence comparison between wild-type *SYD* and *muse9*. A nucleotide substitution, indicated by the lower-case bolded 't', results in an A2224V amino acid substitution.

Figure 3. *MUSE9* encodes SPLAYED (SYD), an ATP-dependent chromatin remodeller.

(A) Complementation test between *muse9* and *syd-4*. Morphology of soil-grown Col-0, *snc1*, *mos4 snc1*, *muse9*, *syd-4*, and an F1 plant from a cross between *muse9* and *syd-4*. Photograph was taken 3 weeks post-germination. Scale bar indicates 1 cm.

(B) Morphology of soil-grown Col-0, *snc1*, *muse9*, *muse9 snc1*, *syd-4*, and *syd-4 snc1* plants. Photograph was taken 3 weeks post-germination. Scale bar indicates 1 cm.

Figure 4. The *syd-10* single mutant does not display enhanced disease resistance.

(A) Growth of *H.a*. Noco2 on indicated genotypes 7 d post-inoculation with $1x10^5$ spores/mL. Values represent the average of 4 replicates of 5 plants each ± SD.

(B) Growth of *P.s.m.* ES4326 on indicated genotypes 2 d post-infiltration. Values represent the average of 5 replicates ± SD. Significant difference between Col-0 and *syd-4* indicated by *** (P-value < 0.001).

(C-G) Real-time qRT-PCR analysis of (C) *PR1*, (D) *PR2*, (E) *PDF1.2a*, (F) *SNC1*, and (G) *RPP4* expression in the indicated genotypes. Total RNA was extracted from seedlings grown for 12 d on MS media. Significant differences are indicated by asterisks (* P-value < 0.05, ** P-value < 0.01, *** P-value < 0.001). All experiments were repeated at least once with similar results.

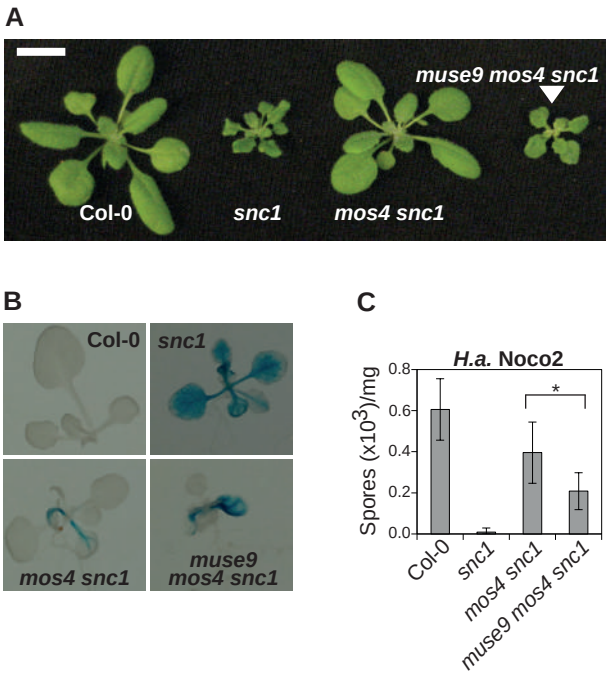
Figure 5. SYD functions antagonistically with MOS1 and MOS9 to regulate SNC1 transcription. The chromatin remodeler SYD is a negative regulator of SNC1-mediated immunity. It may exert its regulatory effects by directly modifying chromatin at the *SNC1* locus, thereby repressing *SNC1* transcription (A). Alternatively, SYD may affect *SNC1* transcription indirectly, by remodeling chromatin at a locus (or loci) elsewhere in the genome, thus affecting the expression of other regulators of *SNC1* expression (B). SYD acts in opposition to previously reported MOS1, ATXR7 and MOS9, which function as positive regulators of endogenous *SNC1* transcription.

Supplementary Figure 1. Alignment of SYD proteins from a number of plant species. BLAST searches using the AtSYD amino acid sequence were performed, and the first hit for each of the indicated species was included in the alignment. A multiple sequence alignment was performed in BioEdit using ClustalW. The regions of the alignment containing the (A) *syd-4* T-DNA

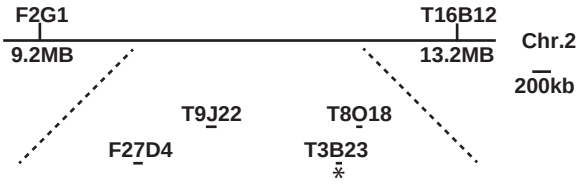
insertion and (B) the *syd-10* mutation are shown, with the sites of the mutations indicated by an asterisk (*).The numbers above the alignment correspond to the amino acid positions of the *Arabidopsis thaliana* sequence. At – *Arabidopsis thaliana*; Al – *Arabidopsis lyrata*; Os – *Oryza sativa*; Zm – *Zea mays*; Gm – *Glycine max*; Fv – *Fragaria vesca*; Pt – *Populus trichocarpa*; Rc – *Ricinus communis*; Tc – *Theobroma cacao*; Vv – *Vitis vinifera*; Sl – *Solanum lycopersicum*; Sm – *Selaginella moelendorffii*; Pp – *Physcomitrella patens*.

Supplementary Figure 2. SNC1 protein levels in the indicated genotypes. *syd-5* is a T-DNA insertion allele (Salk_023209), and *snc1-r1* is a null *SNC1* allele which serves as a negative control. Two biological replicates of *syd-4* are included. Signals detected using Ponceau staining served as internal loading controls.

Supplementary Figure 3. CHH methylation in wild type, *syd-4*, *rdr2*, and *ddm1* plants around the *SNC1* locus. DNA methylation was measured by bisulfite sequencing of genomic DNA from *syd-4* and wild-type 3-week-old seedlings, and analyzed as previously described (Ibarra et al. 2012). *rdr2-1* and *ddm1-2* mutant data were obtained from Zemach et al. 2013. H = A, C, or T.


A
muse9 mos4 snc1
Col-0
snc1
mos4 snc1
B
Col-0
snc1
mos4 snc1
muse9
mos4 snc1
C
H.a. Noco2
Spores (x10^3)/mg
0.8
0.6
0.4
0.2
0.0
*
Col-0
snc1
mos4 snc1
muse9 mos4 snc1

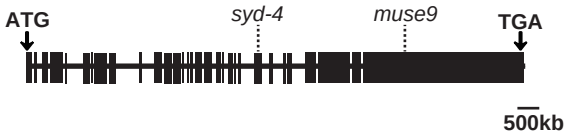
A



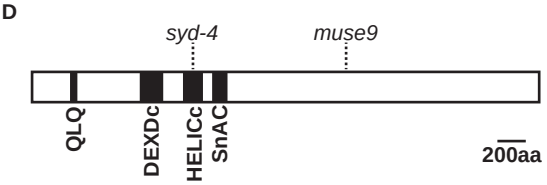
B

Gene	Protein	Mutation
AT2G25440	RLP20	Silent
AT2G26790	PRR superfamily	Silent
AT2G27775	Unknown	Intron
AT2G28290	SYD	A2224 to V
AT2G28810	Zinc finger	Silent

C



D



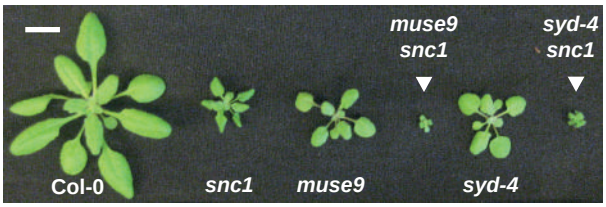
E

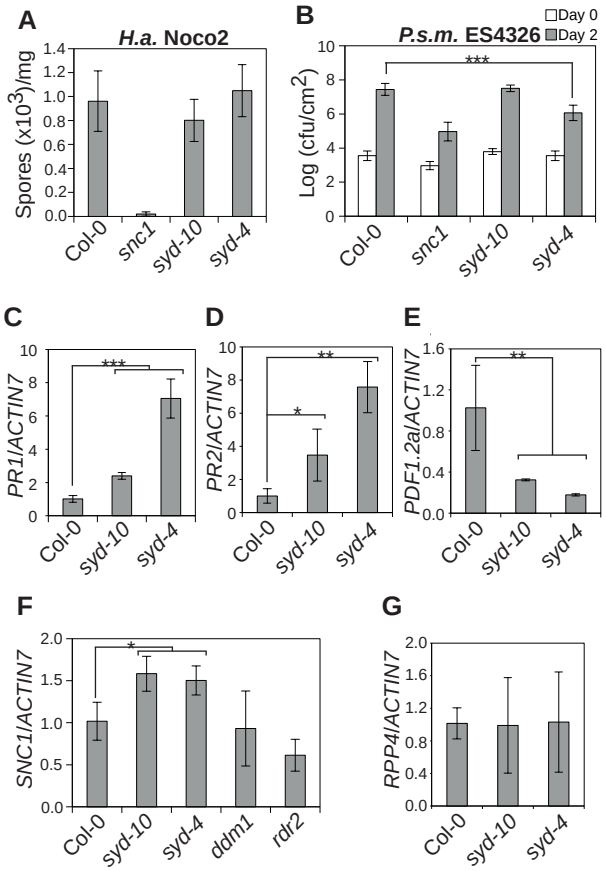
```
     N  D  A  K
SYD  AATGATGCCAAA
muse9 AATGATGtCAAA
     N  D  V  K
```

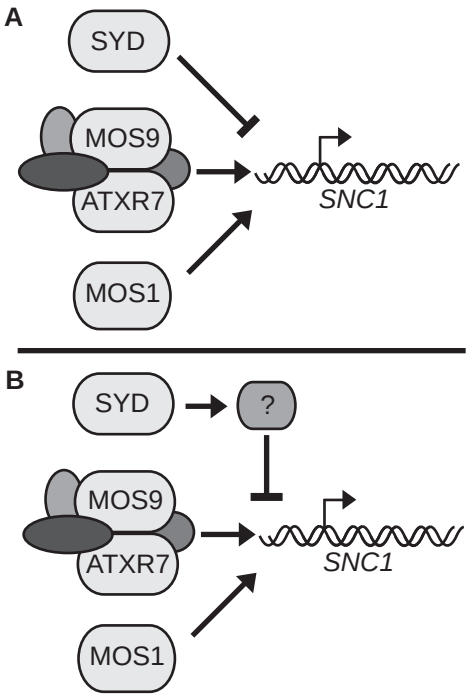
A


F1: muse9/syd-4
Col-0
snc1
mos4 snc1
muse9
syd-4

B


muse9 snc1
syd-4 snc1
Col-0
snc1
muse9
syd-4


A
H.a. Noco2
Spores (x10^3)/mg
Col-0
snc1
syd-10
syd-4
B
P.s.m. ES4326
Day 0
Day 2
Log (cfu/cm^2)
***
C
PR1/ACTIN7
D
PR2/ACTIN7
E
PDF1.2a/ACTIN7
F
SNC1/ACTIN7
ddm1
rdr2
G
RPP4/ACTIN7

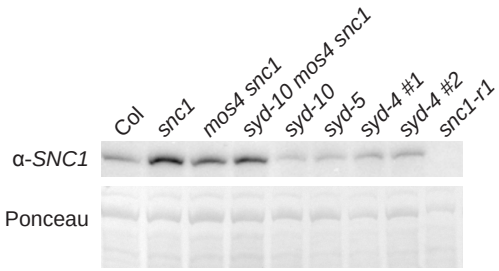

A
SYD
MOS9
ATXR7
SNC1
MOS1
B
SYD
?
MOS9
ATXR7
SNC1
MOS1

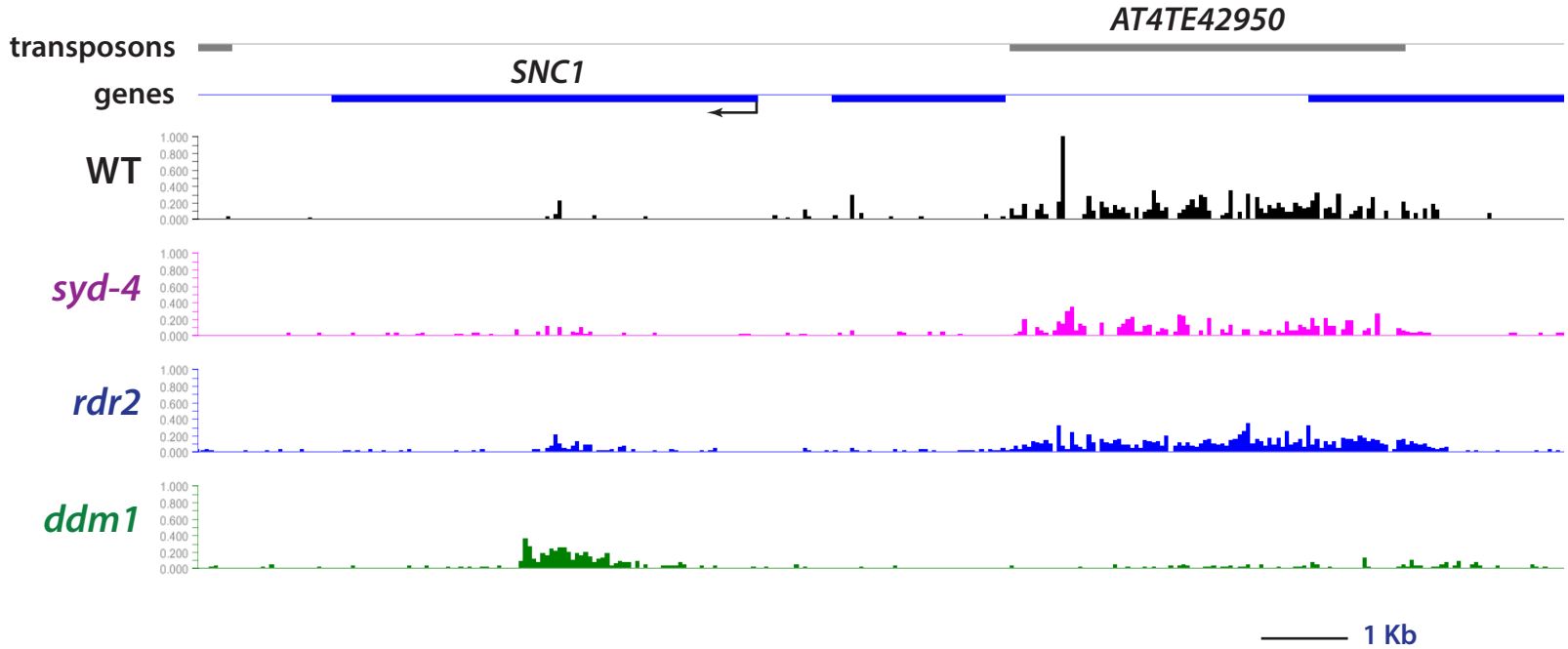
A

```
          1870      1880      1890      1900      1910      1920      1930      1940
          .|....|....|....|....|....|....|....|....|*...|....|....|....|....|....|...
AtSYD     HRVLFFSTMTRLLDVMEDYLTLKGYKYLRLDGQTSGGDRGALIDGFNKSGSPFFIFLLSIRAGGVGVNLQAADTVILFDT
AlSyd     HRVLFFSTMTRLLDVMEDYLTLKGYKYLRLDGQTSGGDRGALIDGFNKSGSPYFIFLLSIRAGGVGVNLQAADTVILFDT
OsSYD     HRVLLFSTMTRLLDVMEDYLVWKKYKYLRLDGHTSGQERGALIDKFNNPNSQAFIFLLSIRAGGVGVNLQAADTVIIFDT
ZmSYD     HRVLLFSTMTRLLDVMEDYLVWKKYKYLRLDGHTSGHERGALIDKFNDPNSQAFIFLLSIRAGGVGVNLQAADTVIIFDT
GmSYD     HRVLFFSTMTRLLDVMEEYLTLKQYRYLRLDGHTSGGDRGALIDLFNQPGSPYFIFLLSIRAGGVGVNLQAADTVILFDT
FvSYD     HRVLFFSTMTRLLDVMEEYLTLKQYKYLRLDGHTSGGDRGSLIDMFNKPDSPFFIFLLSIRAGGVGVNLQAADTVIIFDT
PtSYD     HRVLFFSTMTRLLDVMEEYLTWKQYRYLRLDGHTSGGDRGSLIDSFNQQDSPYFIFLLSIRAGGVGVNLQAADTVIIFDT
RcSYD     HRVLFFSTMTRLLDVMEEYLTMKKYRYLRLDGHTSGNERGALIEQFNKSNSPYFIFLLSIRAGGVGVNLQAADTVIIFDT
TbSYD     HRVLLFSTMTRLLDVMEDYLSLKQYRYLRLDGHTSGNDRGALIDNFNRHDSPFFIFLLSIRAGGVGVNLQAADTVIIFDT
VvSYD     HRVLFFSTMTRLLDVMEEYLHWKQYRYLRLDGHTSGGDRGALIEQFNQPDSPYFIFLLSIRAGGVGVNLQAADTVIIFDT
SlSYD     HRVLLFSTMTRLLDVMEDYLCWKQYKYLRLDGHTCGGDRGALIDKFNQPNSPFFIFLLSIRAGGVGVNLQAADTVIIFDT
SmSYD     HRVLLFSTMTRLLNVLEDYLTWKGYKYLRLDGHTMGSERGSLIDRFNAPDSDAFLFLLSIRAGGIGVNLQAADTVIIFDT
PpSYD     HSVLFFSTMTRLLDVMEDYLEWKGYKYLRLDGSTGGSERGALIQDFNAPQSEAFIFLLSIRAGGIGINLQAADTVIIFDT
```

B

```
                    3130      3140      3150      3160      3170      3180      3190      3200
          ....|....|....|....|....|....|....|....|....|....|....|*...|....|....|....|
AtSYD     LQPLVLVTSNENAMSLDDKDYDPISKSADIEQDPEESVFVQGVGRPKVGTADTQMEDTNDAKLLVGCSVESEEKEKTLQS
AlSyd     MQALVSVTSSENSMSLDQKDDDPISKPADTEQDPEESVSVQGDDRPKVGPDDTRMEDTDDAKHLVGCSVESEEKEKTLQS
OsSYD     A-QYTLQDKIDSSADAGLVPCDK---IASVVIAVDDQDPSNASDKDAPASTEDDGNGLQSECVHVDLVLAKQDNTKVEDT
ZmSYD     AGTEILRDTVEHAACQPEAVCTDGTARQSELSQLDRKAFHDASYKYTHGSTKDDG-ILPSEGTSVDVTGSKQDDVNIVGT
GmSYD     KAEQMQHSVESSTTSCKIALDTTLNAVQKIDGSSERLPTGSALN--DLNIDSSSHQMCSSSGAEPLAVLDRKLKNQSDS-
FvSYD     PAEKDSLMDSTSTSGRVAELDQPVVALAVGSNSVEGSSEAGPVG--SLISQESEHEYKVSDALQVPVTLQEKLCGNMDQP
PtSYD     GSGDGVKMEGDNASDLAKAHISEIDVSTIENNTSHGPIEKMTDT---IQASTKNPITGSYIKVN-HSVFDACEMDNIPS-
RcSYD     GQASCENQVTEGN--MEATCAAPVTVGEEDNVTCNRPVPDQLAG---PSPGSSANVN--STVAS-RDAAEPSEKTNVSS-
TbSYD     GQTTSENSITESN--MEVDSTCPLNAGEKKDVCQGPPGPGGDHTGSRVQPDPPGPMDLPQTAESDKTNIAPVFKESPKA-
VvSYD     ELESTLQEQACKIHGSDVLAGQTLESTEQLNYSAENLKEASSSTTHDCAARLLEKVSVCLEVSEVDASLKSKEETLPTEI
SlSYD     EDQQTMKVQSAASQTPSVHSVGKIPEDIGEVATTKEVLTEDQQT-MEVQSPASQTRSVHSVGKMPEDIVSNEVLPQCDAI
SmSYD     RCLFLREVKAEETSTPEGSDKNGDDRIPENEQAGKSLHASPLAIDGSGDVEMADARGEDVADANVEEGNAERVIMEDAEI
PpSYD     ----------------------------------------------------------------------------
```


Col
snc1
mos4 snc1
syd-10 mos4 snc1
syd-10
syd-5
syd-4 #1
syd-4 #2
snc1-r1
α-SNC1
Ponceau


transposons
AT4TE42950
genes
SNC1
WT
syd-4
rdr2
ddm1
1.000
0.800
0.600
0.400
0.200
0.000
1 Kb