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Supplemental Information

**A Protein Complex Required  
for Polymerase V Transcripts and RNA-  
Directed DNA Methylation in *Arabidopsis***

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Figure S1, related to Figure 2

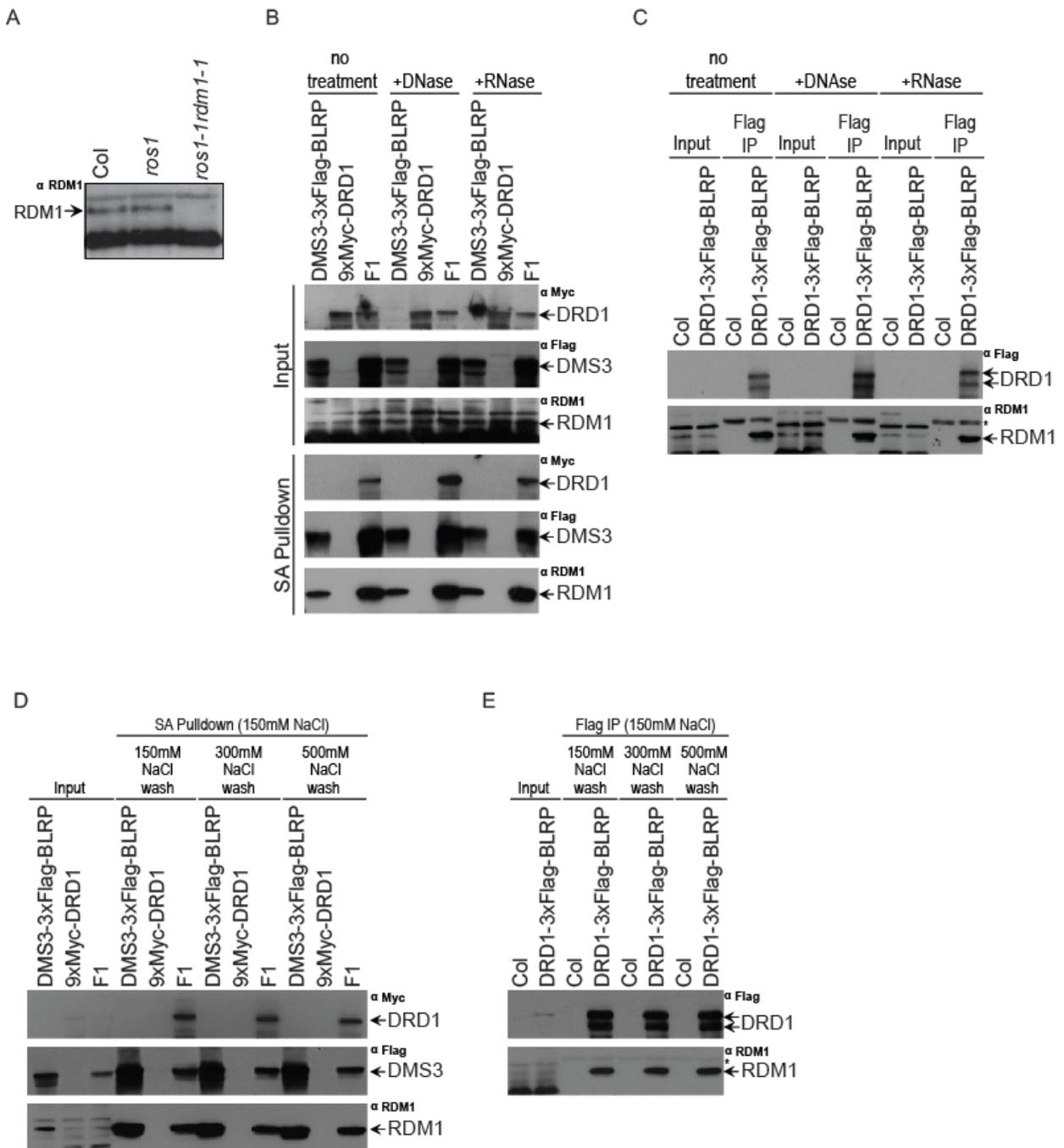


Figure S1, related to Figure 2. DNase, RNase and salt stability of the DDR complex

(A) Western blot demonstrating the specificity of the endogenous RDM1 antibody using total protein extracts from Col, *ros1*, and *ros1-1rdm1-1* seedlings. Non-specific bands both above and below the RDM1

protein band are observed but do not change abundance in the mutant. **(B and C) DNase and RNase stability of the DDR complex.** **(B)** SA pulldown (lower panels) showing the co-purification of DRD1 and RDM1 with DMS3 after no treatment, DNase treatment or RNase treatment as indicated. **(C)** Flag immunoprecipitation showing the co-purification of RDM1 with DRD1 after no treatment, DNase treatment or RNase treatment as indicated. Input lanes confirm expression of the endogenous RDM1 protein and the epitope fusions proteins in the parental lines indicated above each lane (in panel **(C)** the epitope tagged DRD1 protein is not visible due to the exposure length). **(D and E) Salt stability of the DDR complex.** **(D)** SA pulldown showing the association of DMS3 with DRD1 and with RDM1 remains stable through salt washes up to 500mM NaCl. **(E)** Flag immunoprecipitation showing the association of DRD1 with RDM1 remains stable through salt washes up to 500mM NaCl. For each western blot, the antibody used is indicated (upper right) and protein being detected is indicated with an arrow. \* marks a background band that cross reacts with the Flag antibody. F1 represents a cross between the two parental lines. Unlike the 9xMyc-DRD1 shown in **Figure 2 and Figure 3**, the DRD1-3xFlag-BLRP protein is present as a doublet. Since both the 9xMyc and 3xFlag tagged DRD1 fusion proteins are able to complement the *drd1-6* mutation (**Figure 1A**), the reason for the presence of a doublet was not investigated further.

Figure S2, related to Figure 3

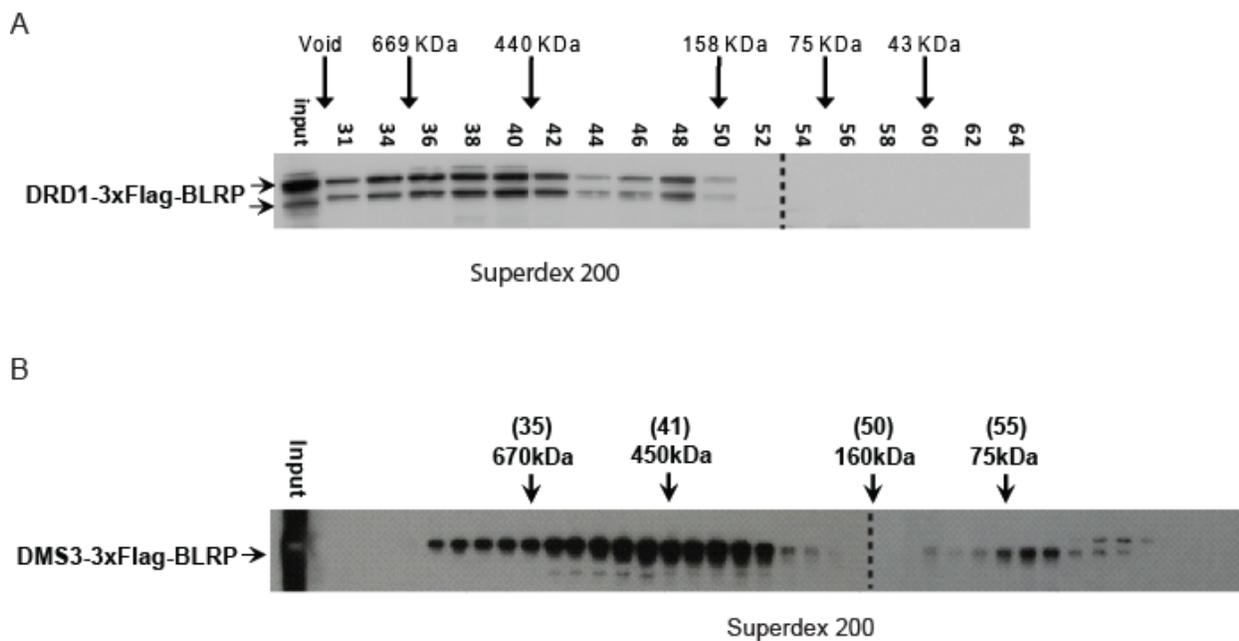


Figure S2, related to Figure 3

**(A)** Elution profiles of DRD1-3xFlag-BLRP and **(B)** DMS3-3xFlag-BLRP following fractionation on a Superdex 200 column. DRD1 and DMS3 were detected by Western blotting using a Flag antibody. Fraction numbers and sizing markers are indicated.

## Supplemental Tables

### Table S1

See Excel file online.

**Table S1, related to Table 1.** Listing of proteins identified in the DRD1 or DMS3 purifications after subtraction of proteins present in purifications using tissue from wild-type Col plants and filtering at 0.04% of DRD1 or 0.0003% of DMS3, respectively.

### Table S2

Primer #	Sequence 5' to 3'
JP4003	CACCGATACAACACAATCGAGGTG
JP4004	CCTTTTGTAGAGAACTCTTATGTC
JP5446	CACCACCGATAAAATGAAAATC
JP5447	TCTGGGTGTGTTTCATTGGCTG
JP4430	TTTCTTGTTTTGTTTATTTGGAGAGTAATGGGAT CCATGGGTTTTGTTTACATTGTGATGACAGG
JP4431	CCTGTCATCACAATGTAACAAAACCCATGGATCCCATTACTCTCCAAATAAACAAAAACAAGAAA
JP2452	TCGTGGTGGTGAGTTTGTTAC
JP2453	CAGCATCATCACAAGCATCC
JP6606	TCCCGAGAAGAGTAGAACAAATGCTAAAA
JP6607	CTGAGGTATTCCATAGCCCCTGATCC
JP3733	CGCGAACGACTATTGCTAAA
JP3734	TGAAATCTAACCGGATTTTGG

**Table S2, related to Figure 1.** Primers used in the cloning of DRD1 and DMS3 as well as the assessment of IGN transcript accumulation by RT-PCR.

**Table S3**

<b>Epitope Tag</b>	<b>Amino Acid Sequence</b>
<b>Carboxy-terminal 3xFlag</b>	DYKDDDDKDYKDDDDKDYKDDDDKGSLEVLFFQGPLEGSMAGGLNDI FEAQRIEWHEDTGGSS
<b>Carboxy-terminal 3xFlag-BLRP</b>	DYKDDDDKDYKDDDDKDYKDDDDKGSLEVLFFQGPLEGSMAGGLNDI FEAQKIEWHEDTGGSS
<b>Amino-terminal 9xMyc</b>	MAGGLNDIFEAQRIEWHEDTGGSSIPGLEVLFFQGPLESRTSGEQKLIS EEDLNGEQKLISEEDLNGEQKLISEEDLNSSRGEQKLISEEDLN KLISEEDLNGEQKLISEEDLNSSRGEQKLISEEDLN GEQKLISEEDLNSTSGSKLTM
<b>Amino-terminal BLRP-9xMyc</b>	MAGGLNDIFEAQKIEWHEDTGGSSIPGLEVLFFQGPLESRTSGEQKLIS EEDLNGEQKLISEEDLNGEQKLISEEDLNSSRGEQKLISEEDLN KLISEEDLNGEQKLISEEDLNSSRGEQKLISEEDLN GEQKLISEEDLNSTSGSKLTM

**Table S3, related to Figure 1.** Amino acid sequences of the epitope tags used in the generation of carboxy-terminal or amino-terminal DRD1 and DMS3 fusion proteins.