Supplemental Data

The *Arabidopsis* Chromatin-Modifying

Nuclear siRNA Pathway Involves

a Nucleolar RNA Processing Center

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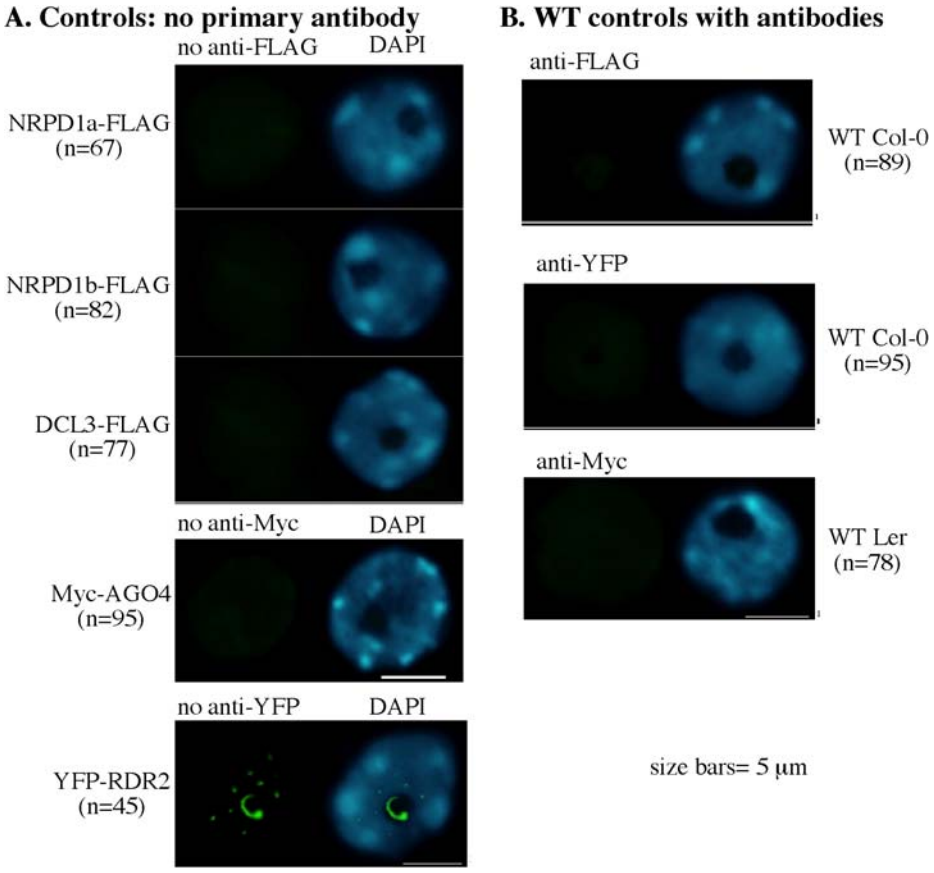

Figure S1. Antibody Specificity Controls

In part A of the figure, nuclei of transgenic lines expressing the indicated epitope tagged proteins were processed for protein immunolocalization as in Figure 3 of the paper except that the primary antibody was omitted prior to incubation with FITC-labeled secondary antibody (green). YFP fluorescence accounts for the YFP-RDR2 signal in the absence of anti-YFP antibody. In part B, non-transgenic, wild-type *A. thaliana* (ecotypes Col-0 or Ler) controls show that no signals are obtained upon immunolocalization using anti-FLAG, anti-YFP or anti-Myc primary antibodies. The images shown are representative of the nuclei observed, with the total number analyzed shown in parentheses. Nuclei were counterstained with DAPI (blue); the size bar corresponds to 5µm.

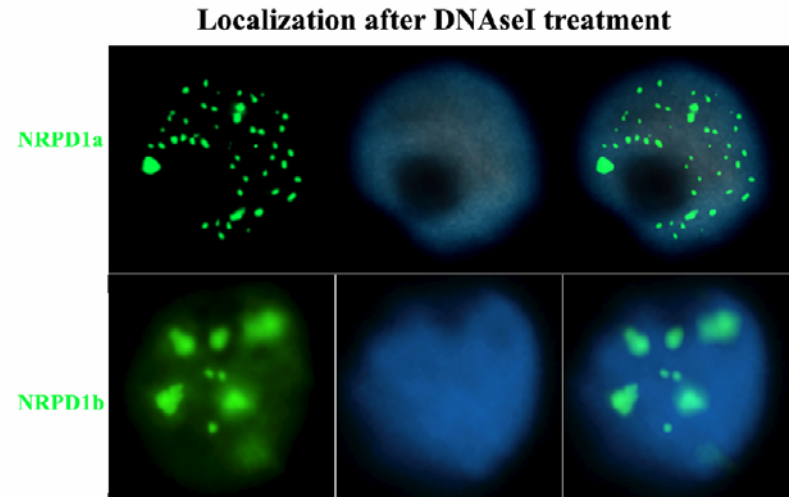

Figure S2. NRPD1a and NRPD1b Immunolocalization Signals Are Not Lost in DNase I-Treated Nuclei

Native NRPD1a and NRPD1b proteins were localized using anti-peptide antibodies in nuclei treated with DNase I as described in Figure 3B of the main paper.

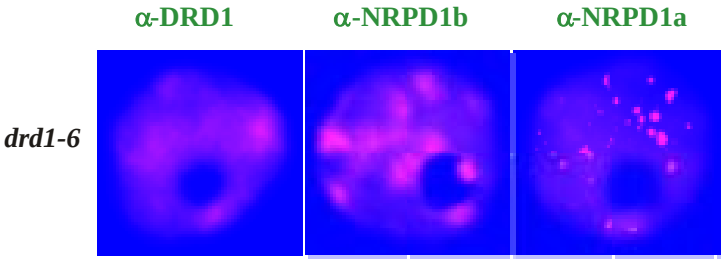

Figure S3. Immunolocalization of DRD1, NRPD1b, and NRPD1a in *drd1-6* Mutant Nuclei

Proteins were detected using anti-peptide antibodies. Note that DRD1 is not detected in the mutant, suggesting that the antibody specifically recognizes DRD1. The *drd1-6* mutation typically does not affect the NRPD1a pattern (85% yield the wild-type pattern for NRPD1a shown below; $n = 90$) but NRPD1b immunolocalization signals are typically more diffuse in drd1-6 (79%; $n = 79$) than in wild-type, suggesting that DRD1 may act upstream, or at the same step, as NRPD1b.

Table S1. Supporting Data for Figure 2A: siRNA Probe Hybridization Patterns and Frequencies

		Frequency (%) of phenotypes observed upon nuclease treatment or in different genetic backgrounds									
RNA probe	**Localization phenotypes**	**Col**	**Ler**	**+RNase A**	**+DNase I**	***nrpd1a***	***nrpd2***	***nrpd1b***	***rdr2-1***	***dcl3-1***	***ago4-1***
45S siR	**Nucleolar dot observed:**	100	100	0	100	0	0	0	0	0	0
	Dispersed nuclear signal:	0	0	0	0	29	13	56	8	9	29
	No signal:	0	0	100	0	71	87	44	92	91	71
	# nuclei observed	n = 75	n = 71	n = 71	n = 63	n = 65	n = 141	n = 132	n = 62	n = 72	n = 76
5S siR	**Nucleolar dot observed:**	100	100	0	100	0	0	0	0	0	0
	Dispersed nuclear signal:	0	0	0	0	0	6	75	11	3	17
	No signal:	0	0	100	0	100	94	25	89	97	83
	# nuclei observed	n = 56	n = 48	n = 62	n = 68	n = 81	n = 127	n = 162	n = 85	n = 62	n = 74
AtSN1	**Nucleolar dot + nucleoplasm:**	74	No data	0	89	No data	0	No data	0	No data	No data
	Nucleoplasm only:	26		0	11		0		0		
	No signal:	0		100	0		100		100		
	# nuclei observed	n = 67		n = 79	n = 85		n = 150		n = 123		
AtCopia4	**Nucleolar dot +nuclear spots:**	100	No data	0	100	No data	0	No data	0	No data	No data
	No signal:	0		100	0		100		100		
	# nuclei observed	n = 85		n = 53	n = 68		n = 103		n = 91		
45S precursor	**Diffuse nucleolar signals:**	100	100	100	100	100	100	100	100	100	100
	# nuclei observed	n = 63	n = 57	n = 64	n = 51	n = 86	n = 79	n = 127	n = 72	n = 74	n = 81

The table is organized as in Figure 2A except that the table includes two columns of data for wild-type nuclei (ecotypes Col-0 and Ler) whereas Figure 2A showed only the Col-0 wild-type control.

Table S2. Supporting Data for Figure 3A: Protein Localization and Effects of RNase

	NRPD1a	**NRPD2**	**NRPD1b**	**RDR2**	**DCL3**	**AGO4**
protein localization	100% of nuclei display pattern shown *n* = 82	100% of nuclei display pattern shown *n* = 245	100% of nuclei show the nucleolar dot. 57% display numerous puncta external to nucleolus, as shown; 43% show <10 puncta *n* = 77	100% of nuclei display pattern shown *n* = 87	100% of nuclei display pattern shown *n* = 125	100% of nuclei display pattern shown *n* = 96
Effect of RNase A	91% , protein not detectable 9% , WT pattern *n* = 85	81% , protein not detectable 19% , WT pattern *n* = 94	65% , protein not detectable 35% , WT pattern *n* = 93	85% , protein not detectable 15% , WT pattern *n* = 62	59% , protein not detectable 41% , WT pattern *n* = 89	72% , protein not detectable 28% , WT pattern *n* = 61

Table S3. Supporting Data for Figure 3C: Pairwise Detection of Nuclear siRNA Pathway Proteins

	Antibodies				
Epitope-tagged lines	**α-NRPD1a**	**α-NRPD2**	**α-NRPD1b**	**α-RDR2**	**α-DCL3**
NRPD1a-FLAG		Majority of the nucleoplasmic signals colocalized *n* = 93			
NRPD1b-FLAG	Few nucleoplasmic signals colocalized *n* = 71	Few nucleoplasmic signals colocalized *n* = 85			
YFP-RDR2	Few nucleoplasmic signals colocalized *n* = 54	Few nucleoplasmic signals colocalized *n* = 48	Nucleolar dot + Few nucleoplasmic signals colocalized *n* = 67		
DCL3-FLAG	Few nucleoplasmic signals colocalized *n* = 76	Few nucleoplasmic signals colocalized *n* = 81	Nucleolar dot + Few nucleoplasmic signals colocalized *n* = 73	Nucleolar dot + Few nucleoplasmic signals colocalized *n* = 86	
Myc -AGO4	Not colocalized *n* = 54	Few nucleoplasmic signals colocalized *n* = 61	Nucleolar dot + Few nucleoplasmic signals colocalized *n* = 58	Nucleolar dot colocalized *n* = 45	Nucleolar dot colocalized *n* = 59

Table S4. Supporting Data for Figure 4: Protein-siRNA Colocalization

		Epitope-tagged lines			
RNA probes		**NRPD1b-Flag**	**YFP-RDR2**	**DCL3-Flag**	**cMyc-AGO4**
45S siR	**Colocalized**	81%	82%	79%	91%
	Not colocalized	19% *n* = 46	18% *n* = 60	21% *n* = 75	9% *n* = 65
siR1003	**Colocalized**	76%	58%	85%	76%
	Not colocalized	24% *n* = 57	42% *n* = 72	15% *n* = 79	24% *n* = 57
AtSN1	**Colocalized**	85%	61%	76%	83%
	Not colocalized	15% *n* = 74	39% *n* = 56	34% *n* = 45	17% *n* = 56
AtCopia4	**Colocalized**	82%	54%	78%	72%
	Not colocalized	18% *n* = 57	46% *n* = 59	22% *n* = 49	28% *n* = 67
45S prec	**Colocalized**	25%	43%	21%	30%
	Not colocalized	75% *n* = 81	57% *n* = 64	79% *n* = 61	70% *n* = 75

Colocalization was considered to be when >50% of the RNA probe signal overlapped >50 % of the protein signal.

Table S5. Supporting Data for Figure 5: Localization of Proteins Relative to NORs and 5S Gene Loci

DNA loci		**NRPD1a**	**NRPD2a**	**NRPD1b**	**RDR2**	**DCL3**	**DRD1**
NORs	**Colocalized**	85%	93%	92%	22%	12%	87%
	Not colocalized	15% *n* = 71	7% *n* = 83	8% *n* = 89	78% *n* = 55	88% *n* = 66	13% *n* = 57
5S gene clusters	**Colocalized**	68%	72%	81%	13%	27%	72%
	Not colocalized	32% *n* = 58	28% *n* = 62	19% *n* = 76	87% *n* = 51	73% *n* = 65	28% *n* = 61

Colocalization was considered to be when at least two NORs and at least four 5S gene *loci* overlapped half of the protein signals outside the nucleolus.

Table S6. Supporting Data for Figure 6: Protein Localization in Various Nuclear siRNA Pathway Mutants

		NRPD2	**NRPD1a**	**NRPD1b**	**RDR2**	**DCL3**
WT	**Col**	100% of nuclei display pattern shown *n* = 245	100% of nuclei display pattern shown *n* = 160	71% of nuclei display pattern shown *n* = 185	77% of nuclei display pattern shown n = 96	100% of nuclei display pattern shown n = 125
Mutants	***nrpd1a***	Reduction in labeling intensity *n* = 181	No signal *n* = 123	WT pattern *n* = 87	Very faint to no signal *n* = 145	Very faint to no signal *n* = 61
	nrpd2	Not detected *n* = 155	Reduction in labeling intensity *n* = 178	Very faint to no signal *n* = 134	Very faint to no signal *n* = 141	Very faint to no signal *n* = 104
	nrpd1b	Very faint to no signal *n* = 138	WT pattern *n* = 67	No signal *n* = 149	Nucleolar dot is not detected *n* = 153	- Very strong reduction in labeling intensity (76%) - Mislocalization of the nucleolar dot to the nucleoplasm (24%) *n* = 84
	nrpd2, nrpd1a	Very faint to no signal *n* = 74	Very faint to no signal *n* = 81	Very faint to no signal *n* = 90	Very faint to no signal *n* = 67	Very faint to no signal *n* = 57
	rdr2-1	Small reduction in labeling intensity *n* = 121	WT pattern *n* = 112	- Nucleolar dot not detected (81%) - Reduction in labeling intensity (19%) *n* = 157	No signal *n* = 61	Very faint to no signal *n* = 87
	dcl3-1	Small reduction in labeling intensity *n* = 130	WT pattern *n* = 74	- Nucleolar dot not detected (78%) - Reduction in labeling intensity (22%) *n* = 72	WT pattern *n* = 89	No signal *n* = 91
	ago4-1	Small reduction in labeling intensity *n* = 109	WT pattern *n* = 65	- Nucleolar dot not detected (92%) - Reduction in labeling intensity (8%) *n* = 133	WT pattern *n* = 122	- WT pattern (67%) - Mislocalization of the nucleolar dot to the nucleoplasm (33%) *n* = 152