Figure S1

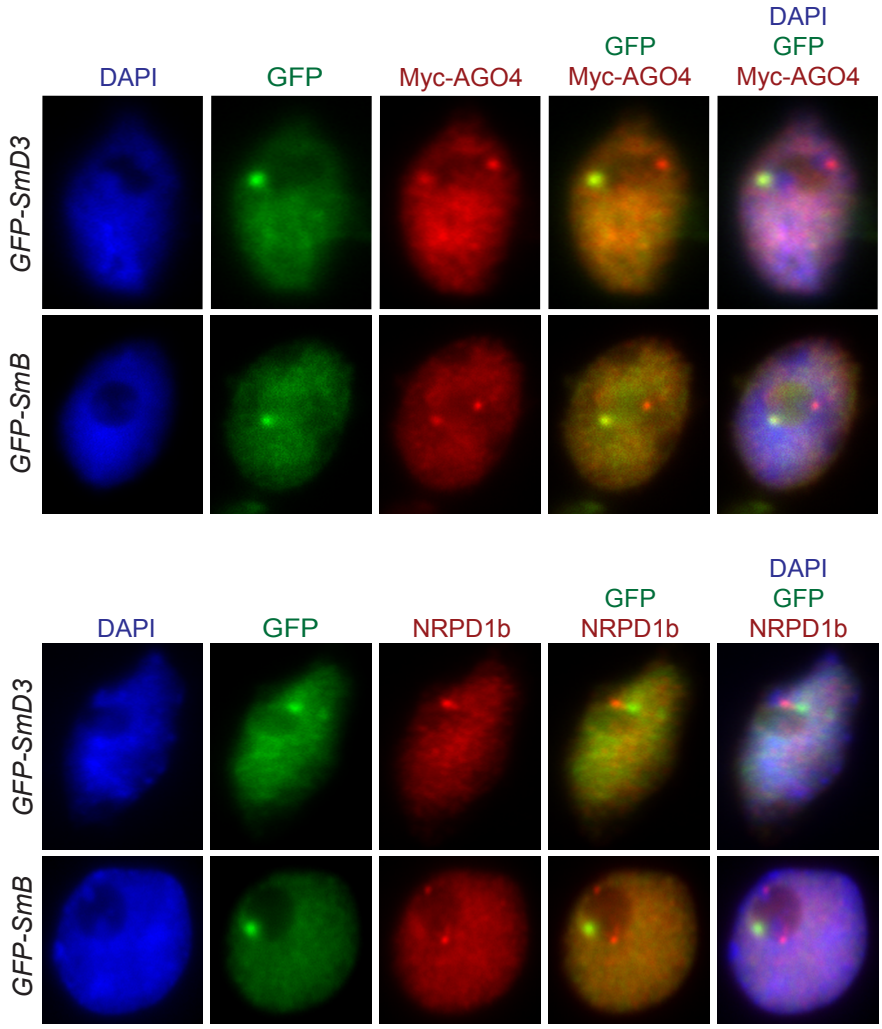
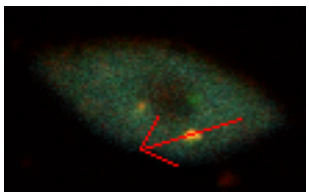
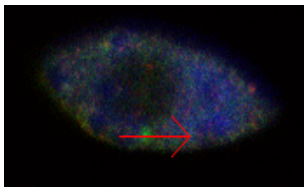
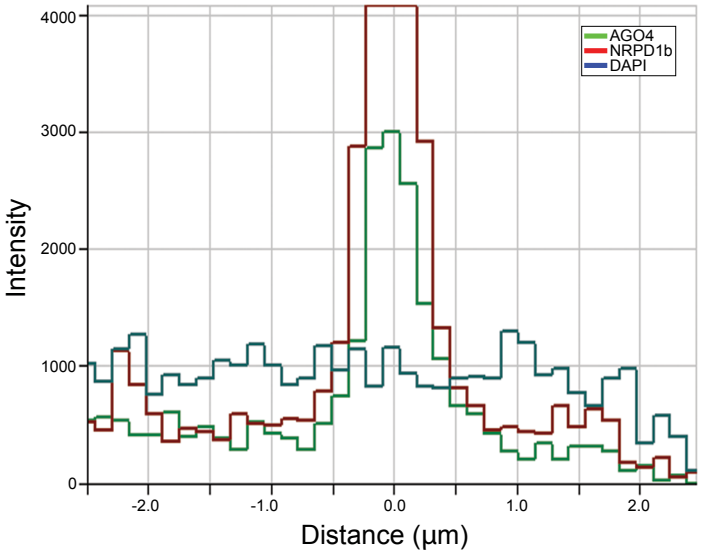

DAPI
GFP
Myc-AGO4
GFP
Myc-AGO4
DAPI
GFP
Myc-AGO4
GFP-SmD3
GFP-SmB
DAPI
GFP
NRPD1b
GFP
NRPD1b
DAPI
GFP
NRPD1b
GFP-SmD3
GFP-SmB

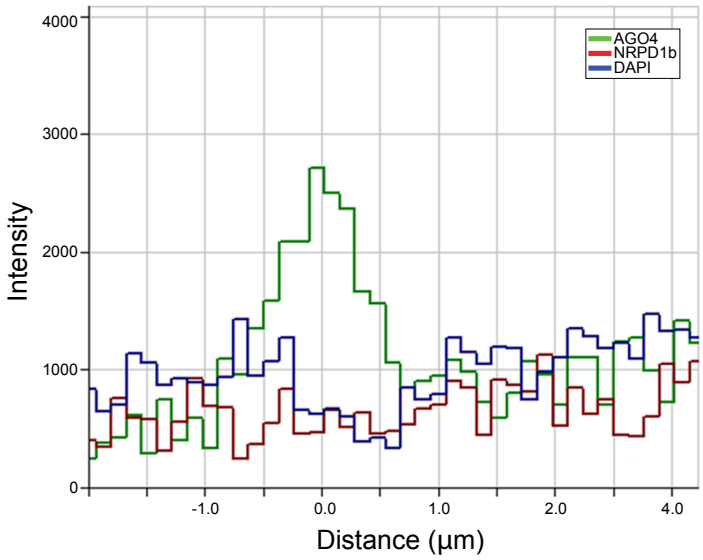
Figure S2

A

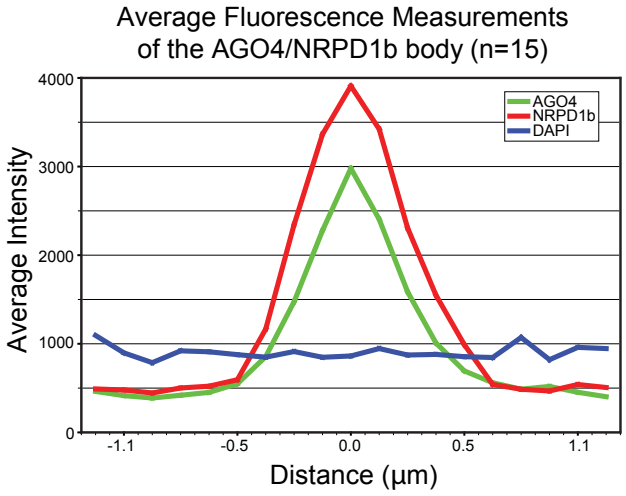





AGO4
NRPD1b
DAPI
Intensity
4000
3000
2000
1000
0
-2.0
-1.0
0.0
1.0
2.0
Distance (µm)


AGO4
NRPD1b
DAPI
Intensity
4000
3000
2000
1000
0
-1.0
0.0
1.0
2.0
4.0
Distance (µm)

B


Average Fluorescence Measurements
of the AGO4/NRPD1b body (n=15)
AGO4
NRPD1b
DAPI
Average Intensity
4000
3000
2000
1000
0
-1.1
-0.5
0.0
0.5
1.1
Distance (µm)

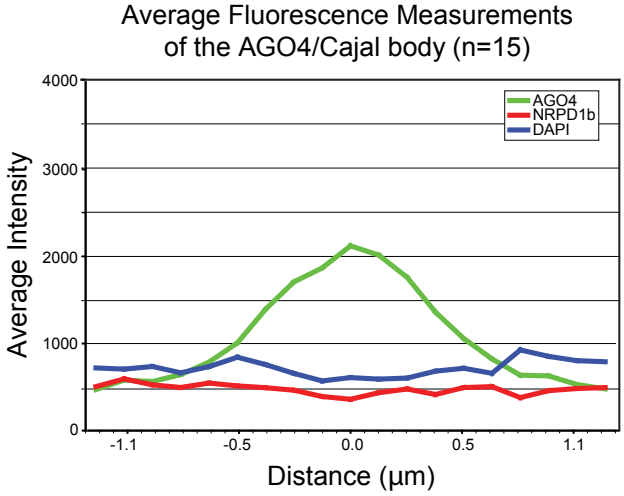

Average Fluorescence Measurements
of the AGO4/Cajal body (n=15)
AGO4
NRPD1b
DAPI
Average Intensity
4000
3000
2000
1000
0
-1.1
-0.5
0.0
0.5
1.1
Distance (µm)

Figure S3

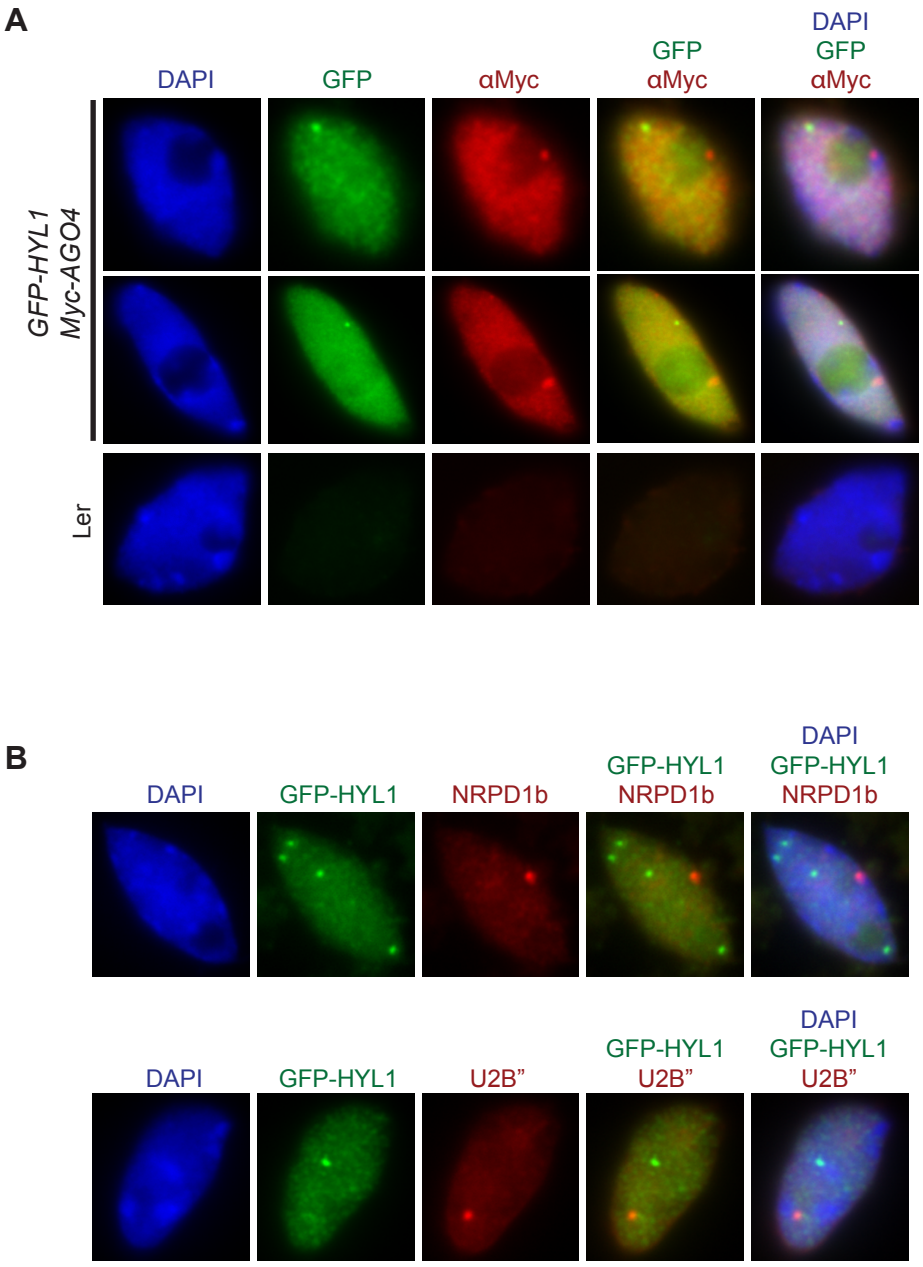

A
DAPI
GFP
αMyc
GFP
αMyc
DAPI
GFP
αMyc
GFP-HYL1
Myc-AGO4
Ler
B
DAPI
GFP-HYL1
NRPD1b
GFP-HYL1
NRPD1b
DAPI
GFP-HYL1
NRPD1b
DAPI
GFP-HYL1
U2B”
GFP-HYL1
U2B”
DAPI
GFP-HYL1
U2B”

Figure S4

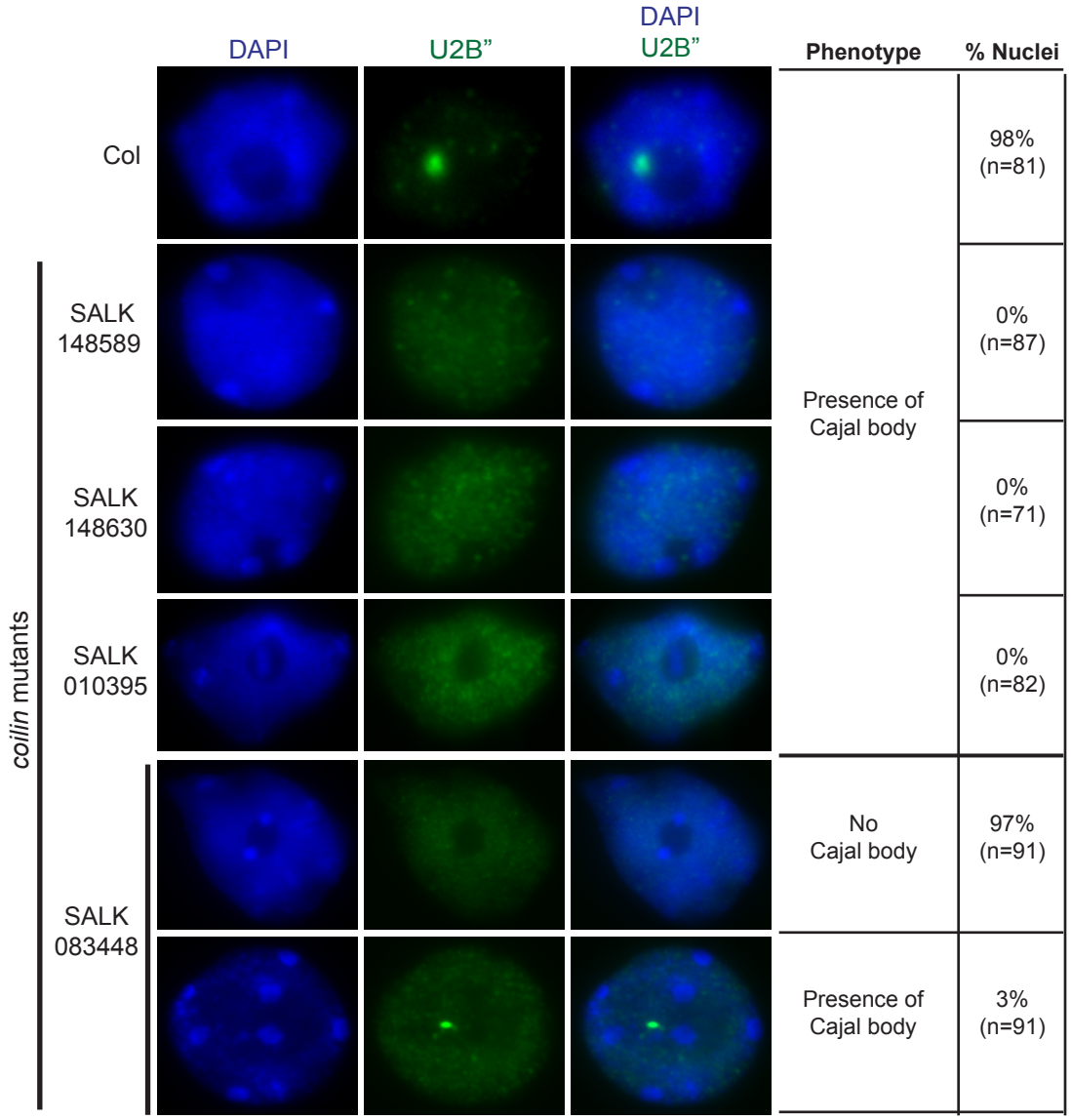

DAPI
U2B"
DAPI
U2B"
Phenotype
% Nuclei
Col
98%
(n=81)
coilin mutants
SALK
148589
0%
(n=87)
Presence of
Cajal body
SALK
148630
0%
(n=71)
SALK
010395
0%
(n=82)
SALK
083448
No
Cajal body
97%
(n=91)
Presence of
Cajal body
3%
(n=91)

Figure S5

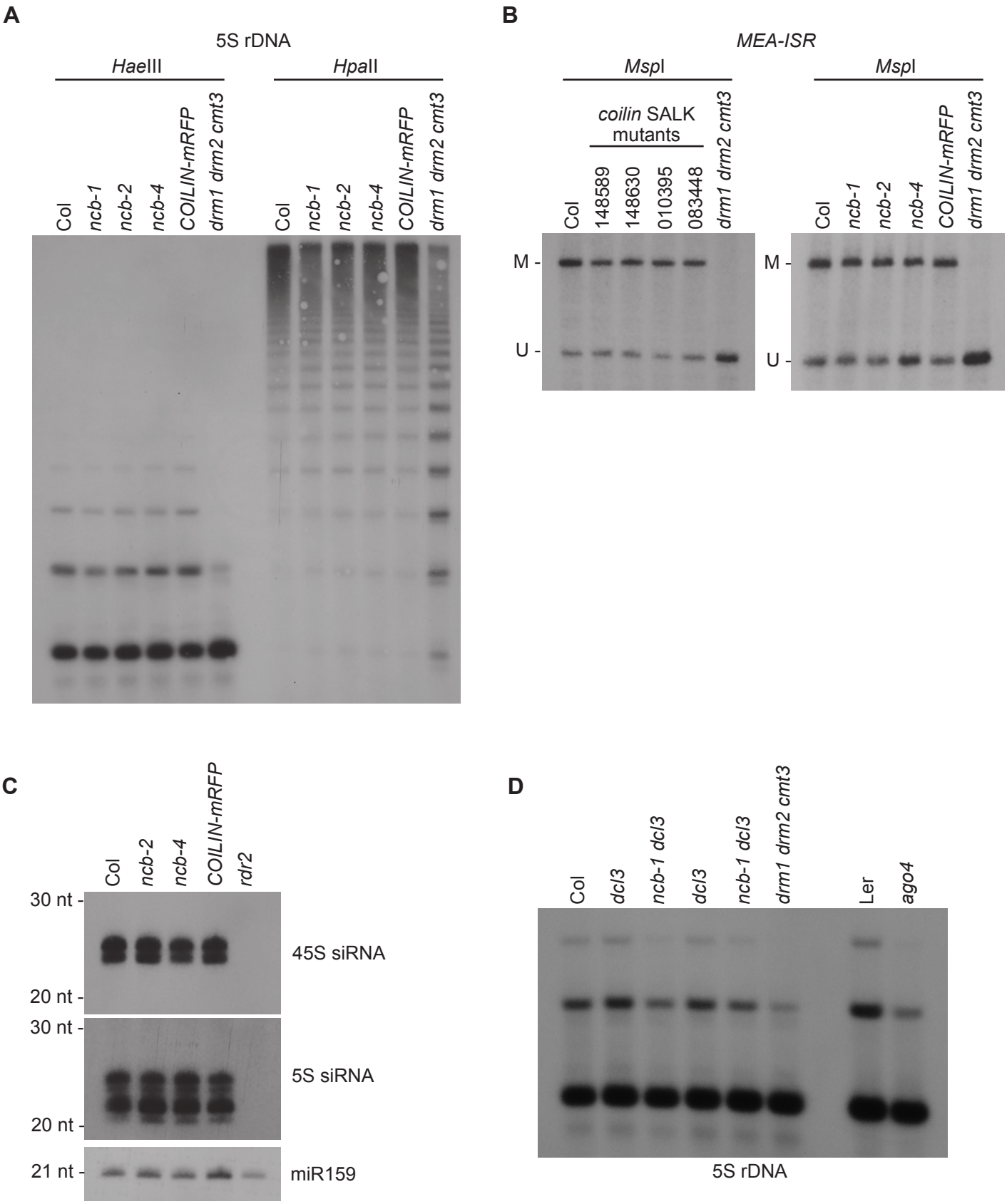
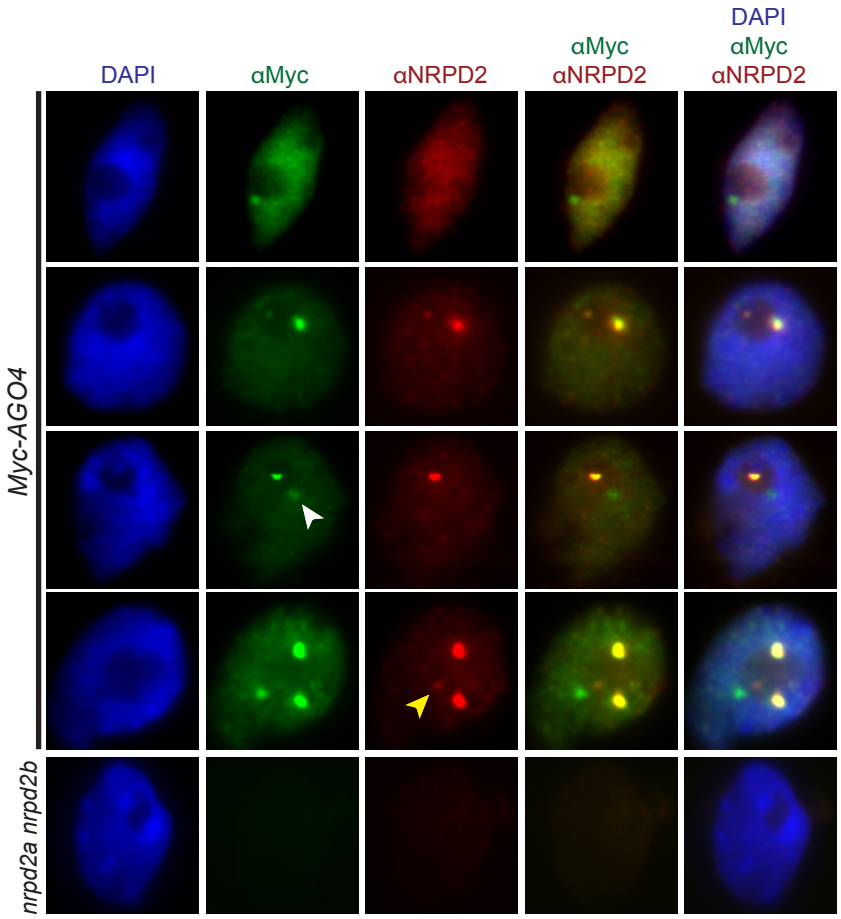

A
5S rDNA
HaeIII
HpaII
Col
ncb-1
ncb-2
ncb-4
COILIN-mRFP
drm1 drm2 cmt3
B
MEA-ISR
MspI
coilin SALK mutants
148589
148630
010395
083448
M
U
C
30 nt
20 nt
45S siRNA
5S siRNA
21 nt
miR159
rdr2
D
dcl3
ncb-1 dcl3
Ler
ago4
5S rDNA

Figure S6



Phenotype	% Nuclei (n=234)
AGO4 body only	89%
AGO4-NRPD2 colocalization	6%
AGO4 colocalized and not colocalized with NRPD2	4%
Non-colocalizing NRPD2	1%

Figure S7

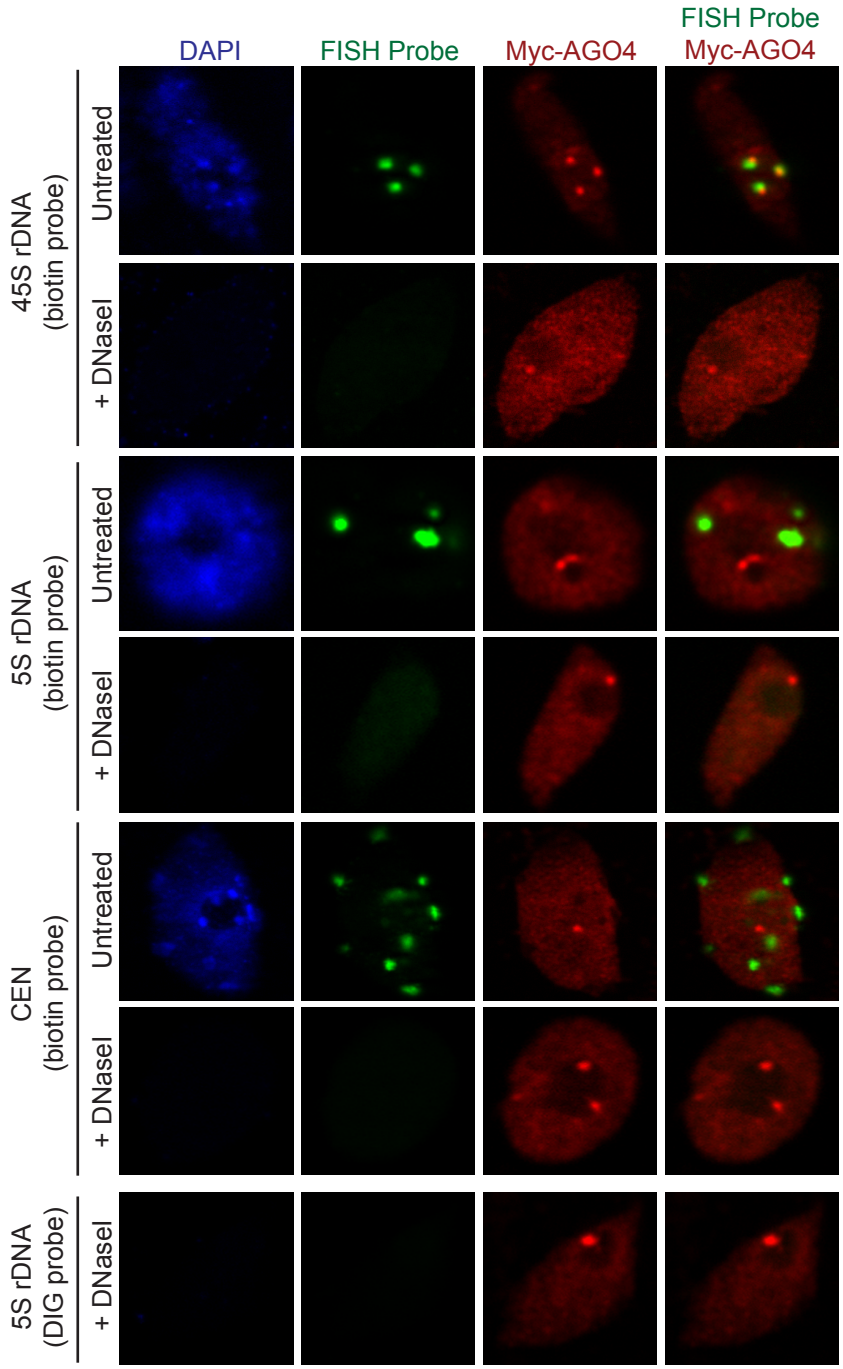
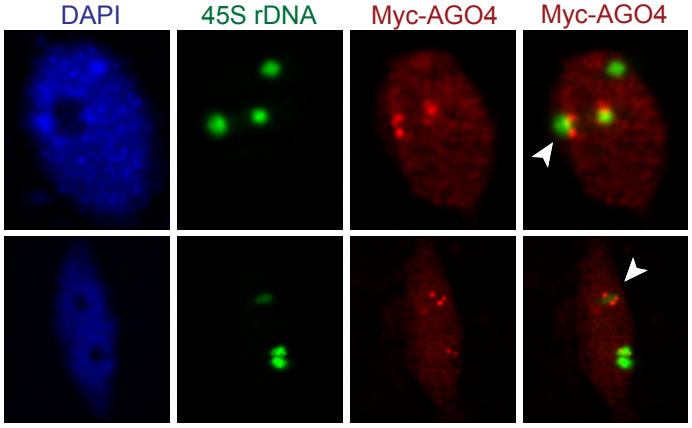

DAPI
FISH Probe
Myc-AGO4
FISH Probe
Myc-AGO4
45S rDNA (biotin probe)
Untreated
+ DNaseI
5S rDNA (biotin probe)
Untreated
+ DNaseI
CEN (biotin probe)
Untreated
+ DNaseI
5S rDNA (DIG probe)
+ DNaseI

Figure S8

A



Phenotype	% Nuclei (n=111)
Two AGO4 foci surrounding one NOR	17%
Three AGO4 foci surrounding one NOR	3%

B

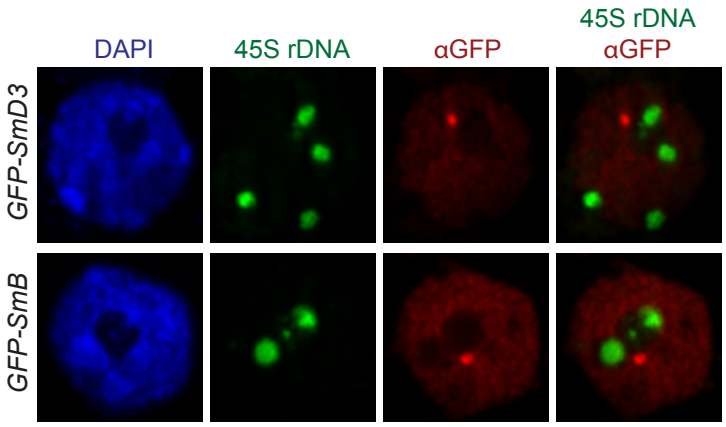
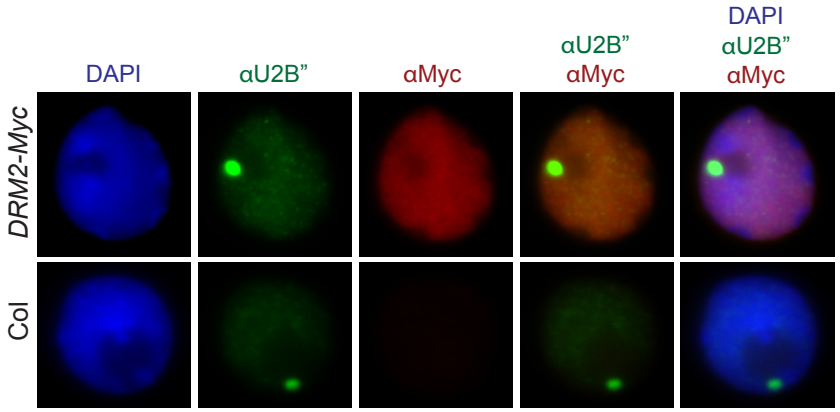


Figure S9



Phenotype	% Nuclei
DRM2-U2B” colocalization	0% (n=112)

Table S1. Nuclei counts for SmD3 or SmB localization relative to AGO4

	Colocalization	Different localization	Colocalization and different localization[#]	Total nuclei* (n)
SmD3 and AGO4	109 (96%)	2 (2%)	2 (2%)	113
SmB and AGO4	147 (95%)	4 (3%)	3 (2%)	154

[#]Nucleus contains two (or more) AGO4 foci in which one AGO4 body colocalizes with the indicated protein (SmD3 or SmB), and a second AGO4 body that is non-colocalizing

*Nuclei containing observable AGO4 nuclear foci were examined

Table S2. Nuclei counts for HYL1 localization

	Colocalization	Different localization	Total nuclei* (n)
HYL1 and AGO4	0 (0%)	99 (100%)	99
HYL1 and NRPD1B	0 (0%)	59 (100%)	59
HYL1 and U2B”	0 (0%)	80 (100%)	80

*Nuclei containing both HYL1 and AGO4/NRPD1b/U2B” nuclear foci were examined

Table S3. Nuclei counts for the *coilin* mutant

	Co-localization	Different localization	Colocalization and different localization*	No AGO4 body	Total nuclei (n)
Wild type:					
U2B" and AGO4	118 (87%)	4 (3%)	10 (7%)	4 (3%)	136
NRPD1b and AGO4	4 (3%)	121 (88%)	9 (7%)	3 (2%)	137
coilin mutant:					
U2B" and AGO4	0 (0%)	28 (13%)	0 (0%)	180 (87%)	208
NRPD1b and AGO4	24 (14%)	0 (0%)	0 (0%)	152 (86%)	176

*Nucleus contains two (or more) AGO4 foci in which one AGO4 body colocalizes with the indicated protein (U2B" or NRPD1b), and a second AGO4 body that is non-colocalizing

Table S4. Nuclei counts for RdDM mutants

	Co-localization	Different localization	Colocalization and different localization*	No AGO4 body/ staining	Total nuclei (n)
Wild type:					
NRPD1b and AGO4	3 (2%)	171 (86%)	18 (9%)	6 (3%)	198
dcl3 mutant:					
NRPD1b and AGO4	8 (4%)	27 (13%)	18 (8%)	163 (75%)	216
Wild type:					
NRPD1b and AGO4	20 (14%)	112 (78%)	7 (5%)	5 (3%)	144
U2B" and AGO4	71 (84%)	9 (11%)	3 (3%)	2 (2%)	85
nprd1b mutant:					
NRPD1b and AGO4	0 (0%)	173 (94%)	0 (0%)	11 (6%)	184
U2B" and AGO4	120 (94%)	0 (0%)	0 (0%)	7 (6%)	127
drm2 mutant:					
NRPD1b and AGO4	0 (0%)	119 (85%)	18 (13%)	3 (2%)	140
U2B" and AGO4	116 (88%)	0 (0%)	12 (9%)	4 (3%)	132

*Nucleus contains two (or more) AGO4 foci in which one AGO4 body colocalizes with the indicated protein (NRPD1b or U2B"), and a second AGO4 body that is non-colocalizing

Table S5. Nuclei counts for NRPD1b localization in *ago4* and *nrpd2* mutants

	Both NRPD1b and U2B" present	Only U2B" present	Total nuclei (n)
Ler:			
NPRD1b and U2B"	18 (10%)	167 (90%)	185
ago4 mutant:			
NPRD1b and U2B"	8 (4%)	194 (96%)	202
Col:			
NRPD1b and U2B"	16 (7%)	219 (93%)	235
nrpd2a nrpd2b mutant:			
NRPD1b and U2B"	0 (0%)	243 (100%)	243

Table S6. Nuclei counts for NRPD2 localization

	Co-localization	Different localization	U2B" present only	Total nuclei (n)
NPRD2 and U2B" in:				
Col	0 (0%)	17 (8%)	201 (92%)	218
nprd1a	0 (0%)	11 (6%)	164 (94%)	175
nrpd1b	0 (0%)	0 (0%)	177 (100%)	177
Ler	0 (0%)	20 (9%)	196 (91%)	216
ago4	0 (0%)	13 (7%)	172 (93%)	185

Table S7. Nuclear foci counts for 45S, 5S, and CEN FISH experiments

	Not adjacent	Adjacent	Overlapping	Total nuclear foci (n)
45S rDNA with AGO4	91 (45%)	100 (50%)	10 (5%)	201
45S rDNA with NRPD1b	0 (0%)	85 (89%)	11 (11%)	96
45S rDNA with SmD3	149 (93%)	8 (5%)	3 (2%)	160
45S rDNA with SmB	137 (89%)	11 (7%)	6 (4%)	154
5S rDNA with AGO4	170 (92%)	12 (7%)	2 (1%)	184
5S rDNA with NRPD1b	65 (90%)	6 (8%)	1 (2%)	72
CEN with AGO4	134 (91%)	8 (6%)	5 (3%)	147
CEN with NRPD1b	64 (89%)	5 (7%)	3 (4%)	72

Table S8. Nuclear foci counts for NOR2 and NOR4

	NOR2 adjacent[1]	NOR4 adjacent[2]	Total nuclear foci (n)
AGO4	27 (60%)	18 (40%)	45*
NRPD1b	25 (64%)	14 (36%)	39

[1]NOR2 is counted as a 45S signal without an adjacent 5S rDNA signal
[2]NOR4 is counted as a 45S signal with an adjacent 5S rDNA signal
*only AGO4 nuclear foci adjacent to a NOR were counted

Table S9. Nuclei counts for DRM2-Myc localization

	Co-localization	NRPD1b/ NRPD2 body only	Diffuse DRM2 staining only, no nuclear foci	Total nuclei (n)
NRPD1b and DRM2 in:				
Wild type	10 (9%)	1 (1%)	102 (90%)	113
ago4 mutant	3 (2%)	12 (9%)	120 (89%)	135
NRPD2 and DRM2	12 (5%)	9 (3%)	239 (92%)	260