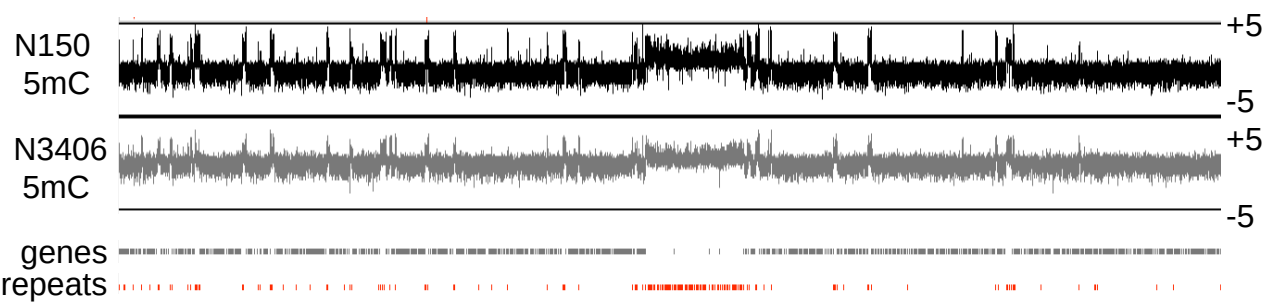
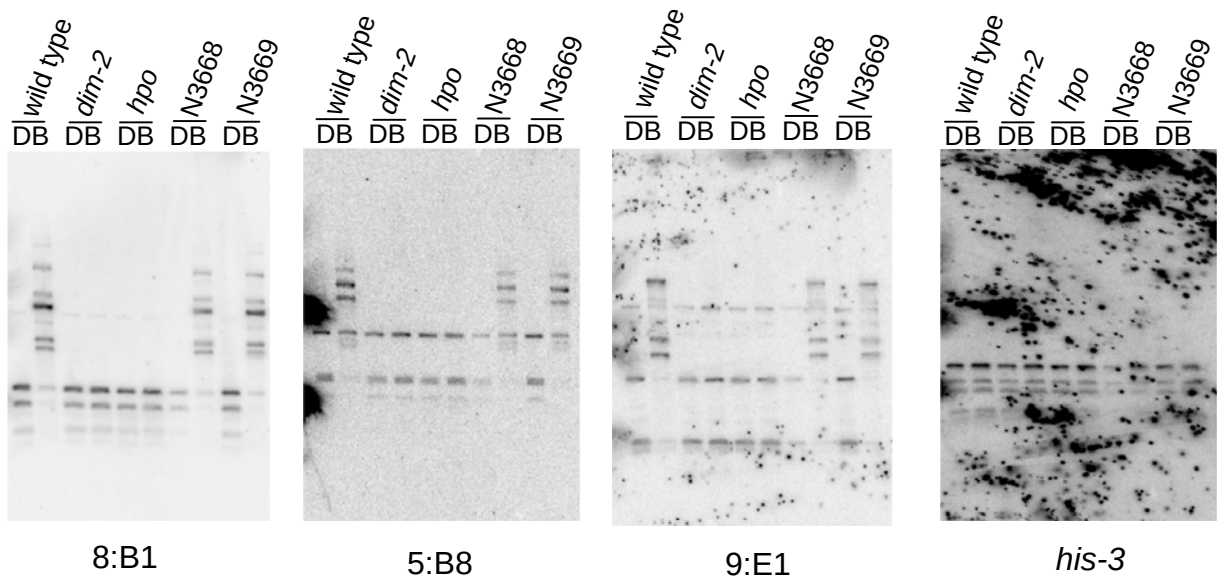


Supplementary Figure 1.



Supplementary Figure 2.



Supplementary Figure 1. DNA methylation in a double argonaute mutant. The distribution of 5mC is shown for the 3.9Mb LGVII. Enrichment values for MeDIP experiments are shown as $\log_2$ values indicated on the y-axis (right). The positions of predicted open reading frames (genes) and repeats are shown in grey and red, respectively. The scale bar on the top right indicates 0.5Mb. Strain N3406 containing null alleles of both *N. crassa* argonaute homologs was obtained from Dr. Yi Liu (UT-Southwestern Medical Center, Dallas, TX).

Supplementary Figure 2. Efficient *de novo* methylation. The wild type strain N3368 was obtained by crossing two strains that lack 5mC but retain H3K9me3, *dim-2* and *hpo*. The wild type strain N3369 was obtained by crossing two strains that lack both H3K9me3 and 5mC, *dim-5* to *dim-8*. Thus, all methylation in these strains is a result of *de novo* methylation. Genomic DNA from the indicated strains was digested with the methylation-sensitive restriction enzyme *Bfu*CI and its methylation-insensitive isoschizomer *Dpn*II. DNA was separated by gel electrophoresis, transferred to Nylon membrane, and probed with the fragment indicated below each blot. The blot was probed with the *his-3* gene, which is not methylated, to verify that the DNA was completely digested.

Supplementary Table 1. Peaks of 5-methylcytosine enrichment.

5mC peak	start	stop	window average	p value	highest window	length	overlapping K9me3 peak	percent overlap
1	252	1452	1.97	5.10E-04	2.93	1200	1	100%
2	49452	51652	1.11	4.15E-04	1.90	2200	2	55%
3	79052	84052	1.88	9.98E-05	2.72	5000	3	98%
4	136052	146952	1.63	9.62E-06	2.84	10900	4	98%
5	179052	187052	1.87	1.16E-05	3.12	8000	5	96%
6	243152	243752	0.87	3.41E-05	1.10	600	6	83%
7	253052	258052	1.86	8.94E-06	2.69	5000	7	100%
8	267252	285752	1.96	4.08E-05	3.50	18500	8	97%
9	435352	448952	1.78	1.80E-05	2.64	13600	9	100%
10	489452	490852	1.03	3.45E-04	1.52	1400	none	0%
11	532752	547152	1.76	2.38E-06	2.89	14400	10	99%
12	625852	627252	0.87	9.01E-04	1.35	1400	11	21%
13	733652	741352	1.71	3.98E-05	3.17	7700	12	99%
14	813452	822652	1.40	6.17E-05	3.05	9200	13	100%
15	920252	941652	1.61	6.37E-05	2.44	21400	14	100%
16	952652	960752	1.44	7.18E-05	2.55	8100	15	91%
17	972452	977652	1.96	1.00E-04	3.23	5200	16	96%
18	1079252	1090152	1.34	2.66E-05	2.29	10900	17	100%
19	1179252	1186152	1.68	1.84E-05	3.01	6900	18	94%
20	1312952	1313552	0.87	3.69E-05	1.17	600	19	83%
21	1369352	1370752	1.97	3.06E-05	2.84	1400	20	50%
22	1507652	1510752	2.11	3.13E-07	2.97	3100	21	81%
23	1563852	1576152	1.97	9.19E-06	3.45	12300	22	98%
24	1617952	1622952	1.82	6.66E-07	2.48	5000	23	100%
25	1806652	1810452	2.01	3.43E-04	3.58	3800	24	95%
26	1816252	1826152	1.57	3.73E-05	2.64	9900	25	99%
27	1832652	1833652	0.90	3.23E-04	1.29	1000	none	0%
28	1843352	1845652	2.02	1.08E-04	3.53	2300	26	87%
29	1855052	1935352	1.48	4.70E-06	3.16	80300	27	100%
30	1935652	2074152	1.01	3.63E-05	2.19	138500	27	44%
30							28	9%
30							29	10%

30							30	20%
30							31	17%
31	2074352	2201952	1.19	1.36E-05	2.44	127600	31	100%
32	2213252	2225152	1.66	6.82E-06	3.10	11900	32	99%
33	2242252	2254952	2.04	3.80E-05	3.39	12700	33	64%
33							34	17%
33							35	11%
34	2286952	2288352	1.33	9.43E-05	2.35	1400	none	0%
35	2294352	2298952	2.27	2.71E-04	3.41	4600	36	72%
36	2357152	2357852	0.98	1.85E-05	1.30	700	none	0%
37	2515152	2530052	1.73	5.39E-05	3.05	14900	37	97%
38	2638552	2650552	1.91	6.56E-06	3.59	12000	38	99%
39	2970452	2972552	2.13	2.50E-05	3.39	2100	39	86%
40	3086552	3094052	2.17	1.98E-05	2.97	7500	40	96%
41	3124752	3144552	1.76	3.19E-05	2.69	19800	41	99%
42	3149752	3153152	1.95	2.97E-06	2.80	3400	42	100%
43	3381752	3385452	1.30	1.99E-04	2.11	3700	43	100%
44	3445652	3446252	1.00	2.86E-04	1.31	600	none	0%
45	3884752	3898252	1.58	8.64E-06	2.72	13500	44	100%
46	3917352	3923152	1.53	6.15E-05	2.50	5800	45	97%

Supplementary Table 2. Peaks of hH3 *tri*-methyl K9 enrichment

K9me3 peak	start	stop	window average	p value	highest window	length	overlapping 5mC peak	percent overlap
1	252	1752	0.72	4.62E-04	1.11	1500	1	80%
2	49852	51052	0.77	3.15E-04	1.00	1200	2	100%
3	78852	83952	0.90	2.35E-04	1.29	5100	3	96%
4	136152	146852	0.88	5.74E-05	1.35	10700	4	100%
5	179352	187152	0.79	1.59E-04	1.23	7800	5	99%
6	243152	243652	0.69	1.88E-05	0.86	500	6	100%
7	252752	258252	0.87	2.18E-04	1.29	5500	7	91%
8	267452	285452	1.00	5.30E-05	1.66	18000	8	100%
9	435152	449652	1.03	1.52E-05	2.62	14500	9	94%
10	532952	547152	0.84	4.63E-05	1.85	14200	11	100%
11	625652	626152	0.43	8.72E-04	0.45	500	12	60%
12	733552	741252	0.78	1.57E-04	1.13	7700	13	99%
13	812252	823852	0.77	9.35E-05	1.41	11600	14	79%
14	919952	941552	0.89	2.53E-05	1.47	21600	15	99%
15	952452	960052	0.83	1.74E-04	1.73	7600	16	97%
16	972652	977752	0.97	3.23E-05	1.44	5100	17	98%
17	1079052	1090152	0.86	1.22E-04	1.75	11100	18	98%
18	1179652	1186152	0.79	1.06E-04	1.29	6500	19	100%
19	1313052	1313552	0.50	1.19E-03	0.62	500	20	100%
20	1369652	1370352	0.48	1.07E-03	0.61	700	21	100%
21	1507852	1510352	0.95	3.01E-04	1.64	2500	22	100%
22	1563852	1575952	0.90	5.96E-05	1.41	12100	23	100%
23	1617752	1622952	0.71	1.05E-04	1.27	5200	24	96%
24	1806852	1810752	0.99	2.42E-04	1.94	3900	25	92%
25	1816252	1826052	0.89	7.78E-05	1.50	9800	26	100%
26	1843652	1845652	0.82	1.85E-04	1.13	2000	28	100%
27	1854552	1996552	0.89	1.53E-05	2.30	142000	29	57%
27							30	43%
28	1996852	2009252	0.79	7.45E-05	1.58	12400	30	100%
29	2009452	2023052	0.75	1.09E-04	1.50	13600	30	100%
30	2023252	2050852	0.74	2.15E-04	1.46	27600	30	100%
31	2051152	2202252	0.83	5.50E-05	1.67	151100	30	15%

31							31	84%
32	2213252	2225052	0.84	9.16E-05	1.54	11800	32	100%
33	2242152	2244352	0.84	9.20E-06	1.22	2200	33	95%
34	2245052	2253152	0.97	7.96E-05	1.63	8100	33	100%
35	2253452	2254852	0.65	3.88E-04	0.83	1400	33	100%
36	2294552	2297852	0.92	1.42E-04	1.31	3300	35	100%
37	2515452	2529952	0.95	9.39E-06	2.23	14500	37	100%
38	2638652	2650652	0.96	2.96E-05	2.37	12000	38	99%
39	2970652	2972452	0.64	6.14E-04	0.87	1800	39	100%
40	3086652	3093852	1.07	1.32E-04	1.60	7200	40	100%
41	3123452	3144352	0.86	1.14E-04	1.35	20900	41	94%
42	3149552	3153152	0.80	2.07E-04	1.14	3600	42	94%
43	3381552	3385852	0.77	2.18E-04	1.14	4300	43	86%
44	3884652	3898352	0.92	3.60E-05	1.84	13700	45	99%
45	3915552	3922952	0.96	2.85E-04	1.87	7400	46	76%

Supplementary Table 3. LGVII sequence contigs

contig	start	stop	orientation
7.251	1	18603	-
7.78	18604	141548	+
7.75	141549	271525	-
7.66	271526	437680	-
7.32	437681	819059	-
7.73	819060	955232	-
7.76	955233	1082351	+
7.84	1082352	1183833	-
7.21	1183834	1822583	-
7.55	1822584	2032610	-
7.249	2032611	2181508	-
7.1	2181509	3128026	-
7.52	3128027	3383926	+
7.23	3383927	3923033	-
7.118	3923034	3938687	+

Table 4. Primers

NAME	SEQUENCE
P1 FP	CGAAGTCGCTAACCTTCTCATC
P1 RP	ATAGCTACGGTTGCGAGGCT
P2 FP	GGTTTTCGTATAGTAAGTTACCCGCTTC
P2 RP	CGACGGAACAAATTACTACTATACACAAC
P3 FP	AGGATGGCTGCAAGGCAGAAC
P3 RP	AGGTTTTTGTGTGTGCAGATG
P4 FP	ACACATTATAGTATCATCTATCCCG
P4 RP	AGTCCACACCCCAAGATTACAAG
P5 FP	GGGCTAGTATTAGTTGGATTAGG
P5 RP	GGTCCACAGCTACCAGCA
P6 FP	GCTATATCGTAATTAACTTTAACGGTAGT
P6 RP	CAATCCGCTAGACCCTATAAACTAATAA
P7 FP	GGATGGCGGATCCTCAAAAATA
P7 RP	TAACCGCCGCTTTTTAAAATTAGGA
hH4 FP	AACCACCGAAACCGTAGAGGGTAC
hH4 RP	ATCGCCGACACCGTGTGTTGTAAC
dim-5 5' flank FP	GCTTTGTTCCCTTTAGTGAGGGTTAATTCGACAAGCAAACCTCTCTGCC
dim-5 5' flank RP	GCTTTGTTCCCTTTAGTGAGGGTTAATTCGACAAGCAAACCTCTCTGCC
dim-5 3' flank FP	GCTCCTTCAATATCATCTTCTGTGACGGGATAGTCAGATCAGGGAATTG
dim-5 3' flank RP	GCGGATAACAATTTACACAGGAAACAGCTCTGCTCCCATCACTCAACC
bar FP	CCGTCGACAGAAGATGATATTGAAGGAGC
bar RP	AATTAACCCTCACTAAAGGGAACAAAAGC