Supplemental Materials

Molecular Biology of the Cell

Mizuno and Sloboda

Supplementary Information

Figure S1. Phylogenetic analysis of PRMTs.

A neighbor-joining phylogenetic tree of PRMTs from various organisms. PRMT genes from *Chlamydomonas reinhardtii*, human, sea urchin (*Strongylocentrotus purpuratus*), *Arabidopsis thaliana*, rice (*Oryza sativa,* Japonica group), and yeast (*Saccharomyces cerevisiae*) were aligned. The numbers on the nodes indicate the bootstrap values (1000 replicates). Protein sequences used in the alignments were as follows. *Chlamydomonas*: Cr-PRMT1 (Cre03.g172550), Cr-PRMT2 (Cre12.g558100), Cr-PRMT3 (Cre16.g685900), Cr-PRMT4 (Cre15.g635350), Cr-PRMT5 (Cre03.g176550), Cr-PRMT7 (Cre02.g141626). Human: Hs-PRMT1 (uniprotkb: Q99873), Hs-PRMT2 (uniprotkb: P55345), Hs-PRMT3 (uniprotkb: O60678), Hs-PRMT4/CARM1 (uniprotkb: Q86X55), Hs-PRMT5 (uniprotkb: O14744), Hs-PRMT6 (uniprotkb: Q96LA8), Hs-PRMT7 (uniprotkb: Q9NVM4), Hs-PRMT8 (uniprotkb: Q9NR22), Hs-PRMT9 (uniprotkb: Q6P2P2), Hs-PCMT1 (uniprotkb: P22061). Sea urchin: Sp-PRMT1 (genbank: XP_011662730), Sp-PRMT2 (genbank: XP_786231), Sp-PRMT3 (genbank: XP_011663239), Sp-PRMT4/CARM1 (genbank: XP_011673927), Sp-PRMT5 (genbank: XP_011660739), Sp-PRMT6 (genbank: XP_011679081), Sp-PRMT7 (genbank: XP_780353), Sp-PRMT8 (genbank: XP_011662730), Sp-PRMT9 (genbank: XP_011672088). *Arabidopsis*: At-PRMT 1 (genbank: NP_179557), At-PRMT 3 (uniprotkb: Q0WVD6), At-PRMT6 (uniprotkb: Q08A71), At-PRMT 10 (uniprotkb: Q9MAT5), At-PRMT11/1b (uniprotkb: Q9SU94), At-PRMT12/1a (uniprotkb: O82210), At-PRMT13/4b (uniprotkb: Q84W92), At-PRMT14/4a (uniprotkb: A3KPF2), At-PRMT15 (uniprotkb: Q8GWT4), At-PRMT16 (uniprotkb: Q944R7). Rice: Os-PRMT 1 (uniprotkb: Q0J2C6), Os-PRMT 3 (uniprotkb: A3BMN9), Os-PRMT4/CARM1 (uniprotkb: Q7XI75), Os-PRMT 5 (uniprotkb: Q6YXZ7), Os-PRMT6a (uniprotkb: Q7XKC0), Os-PRMT6b (uniprotkb: Q75G68), Os-PRMT7 (uniprotkb: Q5VS72), Os-PRMT 10 (uniprotkb: Q9SNQ2). Yeast: ScHMT1 (uniprotkb: P38074), ScHSL7 (uniprotkb: P38274), ScRMT2 (uniprotkb: Q03305). ID for *Chlamydomonas* sequences are from JGI ver5.5 database. Positions for *Chlamydomonas* PRMTs are shown in red on the tree, rice and *Arabidopsis* in blue, yeast in green, sea urchin and human in brown.

Figure S2. Immunoblot analysis of PRMT1 and PRMT10 affinity purified antibodies.

A. Analysis of affinity purified antibodies to PRMT1 and 10, using a gel sample of whole, intact flagella. B. Analysis of affinity purified antibodies to PRMT1 and PRMT10 using flagellar samples from *fla10-1* cells from at the permissive temperature (23 C). The flagella were fractionated as indicated (see Methods for further details on the fractionation). At increased exposure times, as shown here, the PRMT1 antibodies also appear to bind non-specifically to a high molecular weight doublet that is a component of

the axoneme. A BLAST search of the *Chlamydomonas* genome identified five residues of the 15-mer peptide used as antigen in a hypothetical protein having a calculated mass of 265kDa, similar in size, but larger than the bands identified by the PRMT1 antibodies at long exposure times.

Figure S3. Antigen information relative to the antibodies used in this study.
Sequence comparisons of the commercial antibodies for PRMT3 and PRMT5 used in this study. The sequences of each antigenic peptide are proprietary information not disclosed by the vendor.

Figure S4. Sample of control immunofluorescence, second antibody alone.
Top: Immunofluorescence of wt cells using acetylated tubulin antibodies (red). Middle: Control cells, second antibody alone. Non-specific binding of the second antibody alone occurs in the cell body. However, no signal is observed in the flagella. Bottom: Merged image of A and B. Scale bar, 5 μm.

Figure S5. Immunofluorescence microscopy of *fla10-1* cells at the permissive temperature (23°C)
Cells were probed with anti-IFT172 (red, left column) and anti PRMTs (middle, green) as indicated. Flagella outlined with a dashed line box are shown enlarged (4x) in the right-hand column. The localization of IFT172 and PRMTs are sometimes, but not always, identical. Scale bar, 5 μm.

Figure S6. The intensity of Asym24 puncta in the central region of flagella increases during flagellar resorption.
Intensities of each punctate spot were plotted as a function of flagellar length. Note that intensities of spots in the central region are greatly increased in resorbing flagella. (B) Boxplot comparison of Asym24 signal intensities in the central region (positions 0.1-0.9 in relative flagella length) are significantly increased in resorbing flagella, as determined by the Steel-Dwass test.

Table S1. Raw data of PRMT 1 and PRMT 3 intensities for basal puncta during flagellar resorption and regeneration.
Mean ± SEM values from three independent experiments, the numbers of puncta counted, and total flagellar numbers are summarized.

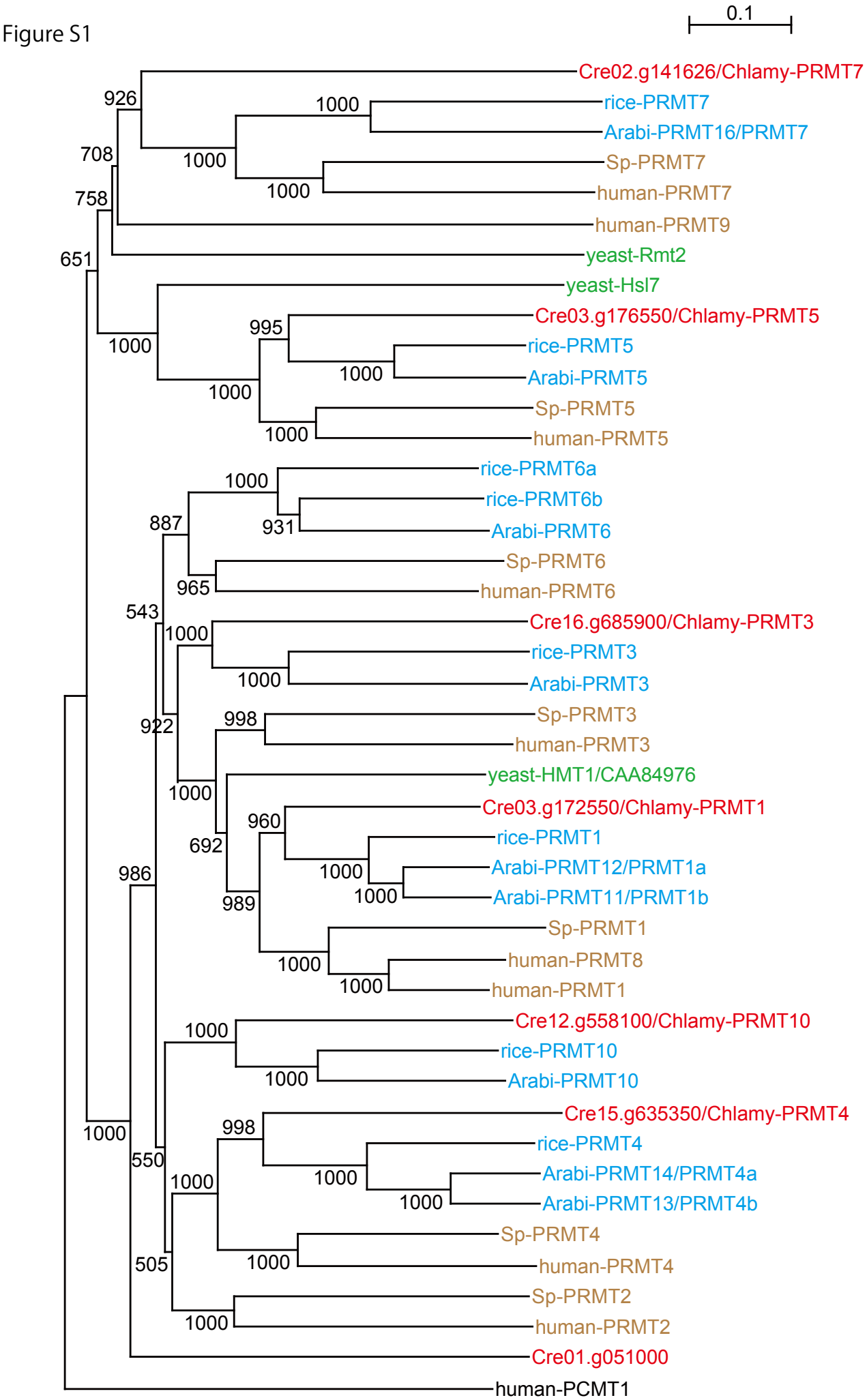
Table S2. Raw data for flagellar PRMTs 1, 3, 5 and 10 puncta intensities from *fla10-1* cells.
(A) Summary of the PRMT 1 and PRMT 3 spot intensities at the flagellar tip and the base. Mean ± SEM values from three independent experiments, the numbers of puncta counted, and total flagellar numbers are summarized.

(B) Summary of PRMT 1, 3, 5, and 10 spots measured along the flagella length from base to tip. Mean ± SEM values from three independent experiments, the numbers of puncta counted, and total flagellar numbers are summarized.

Table S3. Summary of intensities from the base and the tip of resorbing and regenerating flagella labeled with Asym24 antibodies.

Mean ± SEM values from three independent experiments, the numbers of puncta counted, and total flagellar numbers are summarized.

Figure S1


0.1
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rice-PRMT7
Arabi-PRMT16/PRMT7
Sp-PRMT7
human-PRMT7
human-PRMT9
yeast-Rmt2
yeast-Hsl7
Cre03.g176550/Chlamy-PRMT5
rice-PRMT5
Arabi-PRMT5
Sp-PRMT5
human-PRMT5
rice-PRMT6a
rice-PRMT6b
Arabi-PRMT6
Sp-PRMT6
human-PRMT6
Cre16.g685900/Chlamy-PRMT3
rice-PRMT3
Arabi-PRMT3
Sp-PRMT3
human-PRMT3
yeast-HMT1/CAA84976
Cre03.g172550/Chlamy-PRMT1
rice-PRMT1
Arabi-PRMT12/PRMT1a
Arabi-PRMT11/PRMT1b
Sp-PRMT1
human-PRMT8
human-PRMT1
Cre12.g558100/Chlamy-PRMT10
rice-PRMT10
Arabi-PRMT10
Cre15.g635350/Chlamy-PRMT4
rice-PRMT4
Arabi-PRMT14/PRMT4a
Arabi-PRMT13/PRMT4b
Sp-PRMT4
human-PRMT4
Sp-PRMT2
human-PRMT2
Cre01.g051000
human-PCMT1
926
1000
1000
708
1000
758
651
1000
995
1000
1000
1000
1000
887
931
965
543
1000
1000
922
998
1000
960
692
1000
1000
989
986
1000
1000
1000
998
1000
1000
550
1000
505
1000
1000

Figure S2

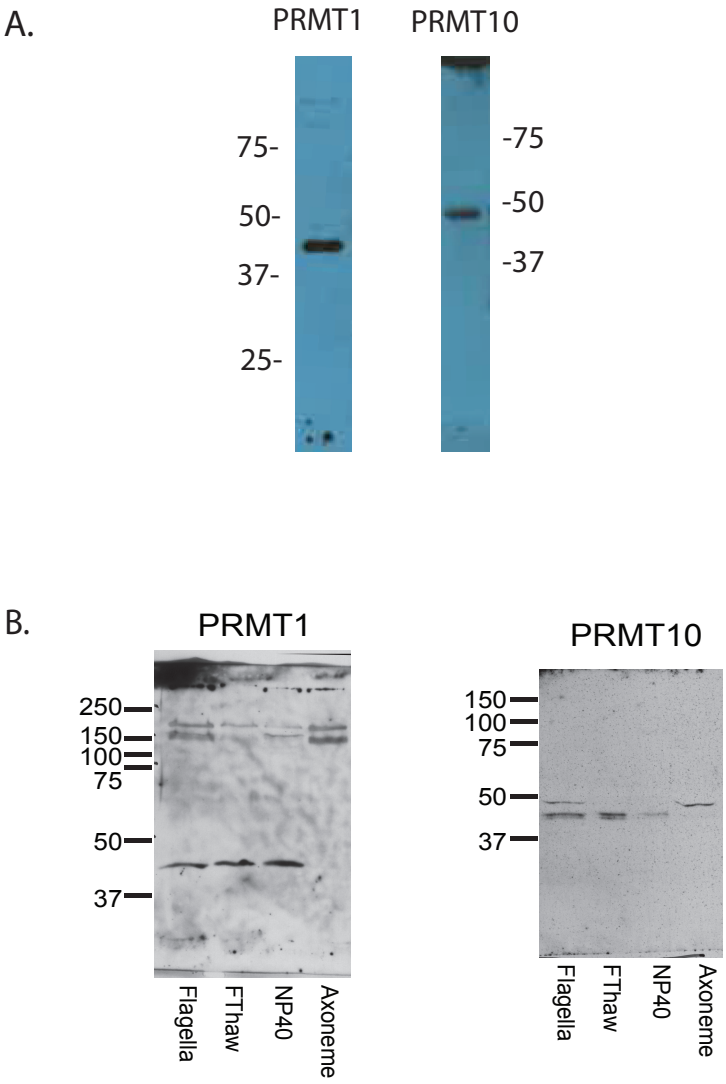

A.
PRMT1
PRMT10
75-
50-
37-
25-
-75
-50
-37
B.
PRMT1
PRMT10
250
150
100
75
50
37
150
100
75
50
37
Flagella
FThaw
NP40
Axoneme
Flagella
FThaw
NP40
Axoneme

Figure S3

Similarity of the *Chlamydomonas* PRMT3 sequence (top row) with the sequence containing the antigen (bottom row) used to produce Abcam antibody ab91430. The synthetic antigenic peptide used was derived from the N-terminal 50 amino acids of human PRMT3 (accession number NP_005779.1).

```
26GREEEWDEWNGDAEDAD
 G E  W+    D +DAD
28GDEAAWE----DEDDAD
```

and

```
132DVSPWADDRYLVPVLEDDALL
  D + W+D        EDDA L
 28DEAAWED--------EDDADL
```

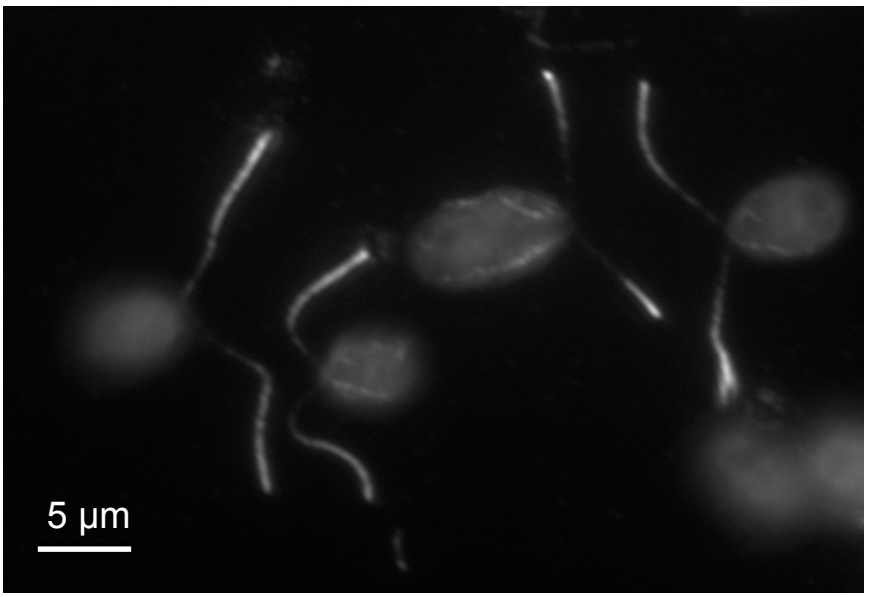
Similarity of the *Chlamydomonas* PRMT5 sequence (top row) with the sequence containing the antigen (bottom) used to produce Abcam antibody ab31751. The synthetic antigenic peptide used was derived from within residues 600 to the C-terminus of human PRMT5 (accession number XM_017020927).

```
545NTVLHGFAGYFETVLYQDITLSIRPETHSPGMFSWFPILFPIKQPITVREGQTICVRFWR
    VLHGFAGYFE  LY+D+ LSI P +HS  MFSWFPI FP+++P+ V  G  + V+ WR
613AAVLHGFAGYFECALYKDVLLSIHPPSHSVNMFSWFPIFFPLREPVYVPAGGEVGVQVWR

605CSNSKKVWYEWAVTAPVCSAIHNPTGRSYTIGL
  C    KVWYEWA++APV   IHN  GRSY +GL
673CCAPHKVWYEWALSAPVAGPIHNVNGRSYWVGL
```

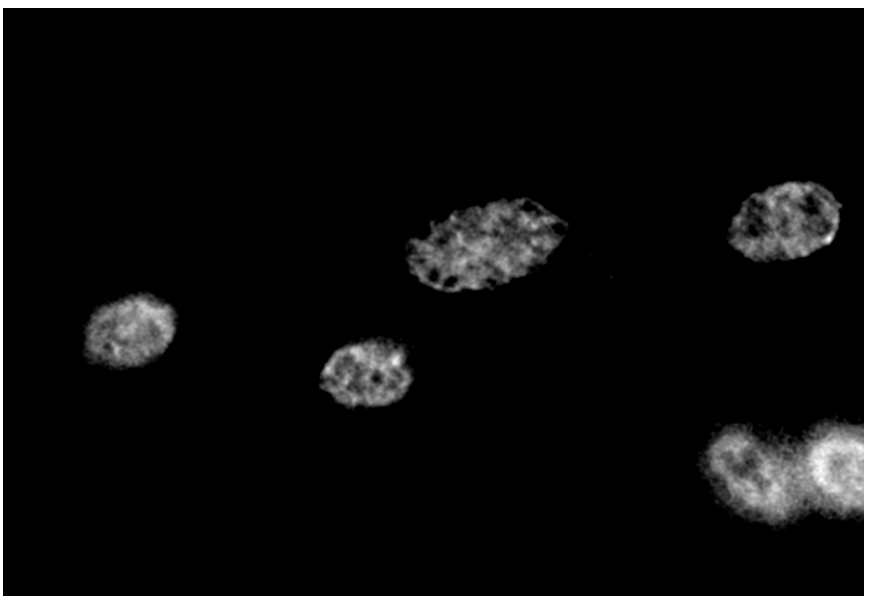
Figure S4

Ac Tubulin



Control

(2nd antibody only)



Merge

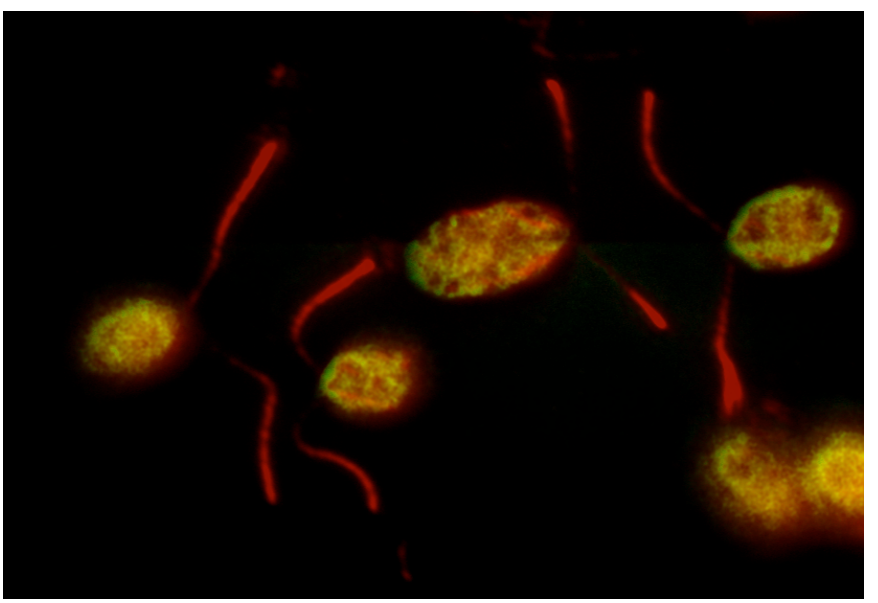
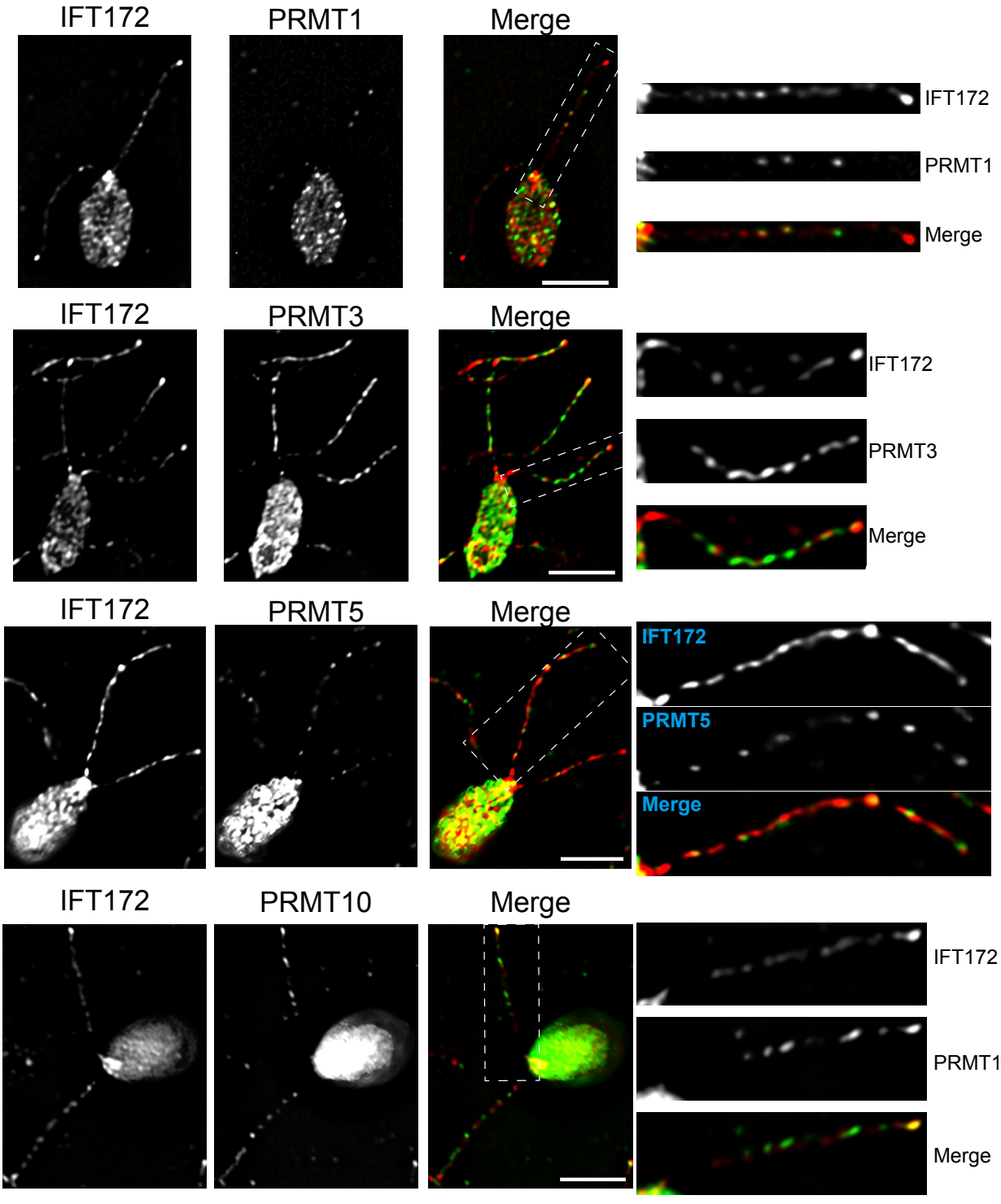


Figure S5


IFT172
PRMT1
Merge
IFT172
PRMT1
Merge
IFT172
PRMT3
Merge
IFT172
PRMT3
Merge
IFT172
PRMT5
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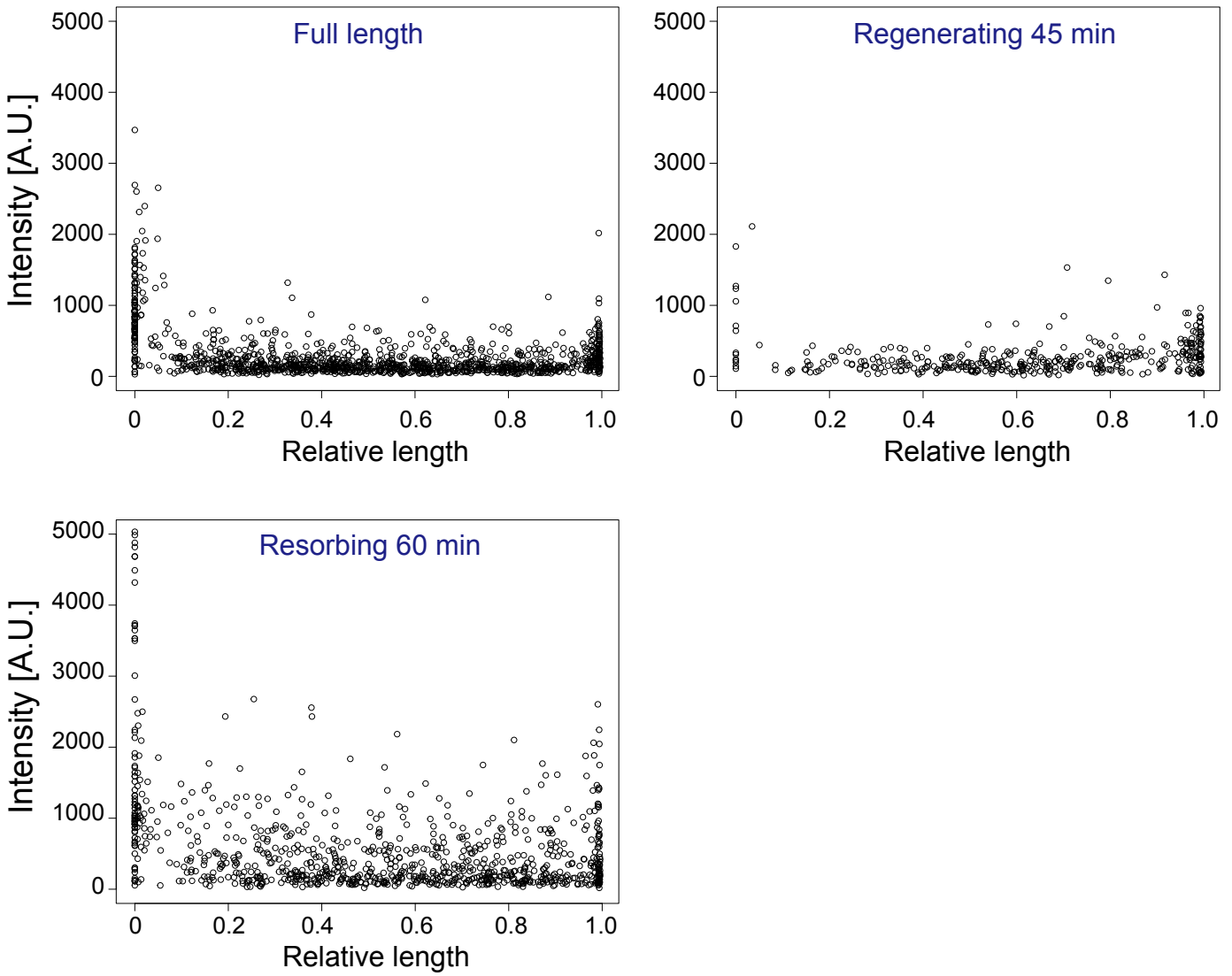
IFT172
PRMT5
Merge
IFT172
PRMT10
Merge
IFT172
PRMT10
Merge

A


Full length
Regenerating 45 min
Resorbing 60 min
Intensity [A.U.]
Relative length
0
1000
2000
3000
4000
5000
0.2
0.4
0.6
0.8
1.0

B

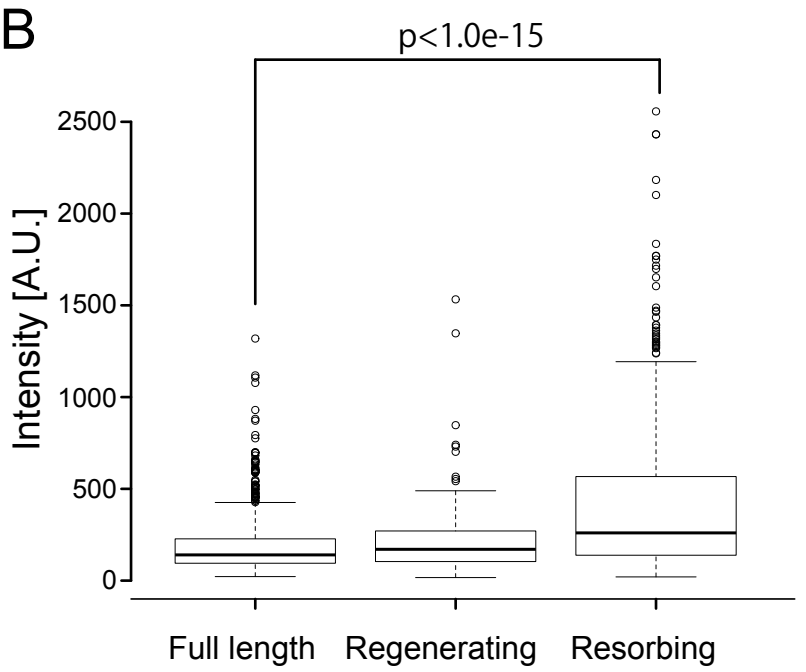

p<1.0e-15
Intensity [A.U.]
0
500
1000
1500
2000
2500
Full length
Regenerating
Resorbing

Figure S6

Table S1

Basal PRMT1 intensity

	time	0	15	30	45	60	90	120
Regenerating	Mean ± SEM	455±54	286±55	199±25	344±62	717±89	379±57	NA
	Spot number	113	22	32	52	68	97	NA
	Flagella number	170	100	112	122	101	139	NA
Resorbing	Mean ± SEM	455±54	539±103	224±28	182±23	544±70	439±56	461±71
	Spot number	113	44	68	62	58	90	66
	Flagella number	170	90	125	163	104	145	73

Basal PRMT3 intensity

	time	0	15	30	45	60	90	120
Regenerating	Mean ± SEM	2042±133	1038±185	891±168	1137±215	921±77	870±92	NA
	Spot number	127	21	26	28	72	76	NA
	Flagella number	148	31	51	70	100	108	NA
Resorbing	Mean ± SEM	2042±133	1176±98	1230±153	663±56	1095±111	858±71	1175±84
	Spot number	127	90	65	81	25	68	77
	Flagella number	148	104	97	114	29	78	100

Tip PRMT1 intensity

	time	0	15	30	45	60	90	120
Regenerating	Mean ± SEM	180±17	182±51	208±26	452±41	419±100	246±28	NA
	Spot number	80	12	36	60	53	62	NA
	Flagella number	170	96	112	121	101	139	NA
Resorbing	Mean ± SEM	180±17	142±14	101±15	94±22	233±23	269±36	122±18
	Spot number	80	45	47	44	52	52	29
	Flagella number	170	90	125	163	104	145	73

Tip PRMT3 intensity

	time	0	15	30	45	60	90	120
Regenerating	Mean ± SEM	1075±76	1307±277	813±83	1113±235	846±73	988±82	NA
	Spot number	123	11	32	44	73	101	NA
	Flagella number	158	29	51	70	100	108	NA
Resorbing	Mean ± SEM	1075±76	697±66	862±78	523±38	930±119	706±70	868±78
	Spot number	123	83	73	88	18	62	57
	Flagella number	158	104	97	114	29	78	100

Spot counts and flagella counts are combined values from three independent experiments.

Table S2

A. Tip and basal intensity of PRMT1 and PRMT3 in *fla10-1*

Tip intensities

	PRMT1		PRMT3	
temperature	23°C	32°C	23°C	32°C
Mean ± SEM	182 ± 38	158 ± 42	643 ± 37	437 ± 40
Spot number	23	14	100	54
Flagella number	94	79	124	75

Base intensities

	PRMT1		PRMT3	
temperature	23°C	32°C	23°C	32°C
Mean ± SEM	947 ± 116	197 ± 28	1584 ± 99	724 ± 94
Spot number	74	34	114	54
Flagella number	94	79	124	75

Spot counts and flagella counts are combined values from three independent experiments.

B. Intensities of PRMT spots along the flagellar length

Intensities

	PRMT1		PRMT3	
temperature	23°C	32°C	23°C	32°C
Mean ± SEM	492 ± 55	172 ± 17	353 ± 29	306 ± 28
Spot number	180	98	458	233
Flagella number	94	79	124	75

	PRMT5		PRMT10	
temperature	23°C	32°C	23°C	32°C
Mean ± SEM	811 ± 22	472 ± 23	311 ± 12	173 ± 12
Spot number	819	339	320	186
Flagella number	67	58	98	71

Table S3

Basal Asym24 intensity

	time	0	15	30	45	60	90	120
Regenerating	Mean ± SEM	971 ± 55	903 ± 94	966 ± 194	684 ± 159	2403 ± 313	1973 ± 247	NA
	Spot number	120	36	16	16	31	57	NA
	Flagella number	172	84	103	107	107	111	NA
Resorbing	Mean ± SEM	971 ± 55	1301 ± 117	1797 ± 98	2803 ± 344	2085 ± 184	1586 ± 164	1186 ± 109
	Spot number	120	74	153	34	118	102	38
	Flagella number	172	113	176	73	140	119	56

Tip Asym24 intensity

	time	0	15	30	45	60	90	120
Regenerating	Mean ± SEM	278 ± 14	260 ± 29	268 ± 19	382 ± 25	500 ± 65	655 ± 85	NA
	Spot number	153	52	92	95	103	109	NA
	Flagella number	172	84	103	107	107	111	NA
Resorbing	Mean ± SEM	278 ± 14	377 ± 30	405 ± 27	501 ± 64	502 ± 43	450 ± 37	345 ± 39
	Spot number	153	105	163	67	124	106	54
	Flagella number	172	113	176	73	140	119	56

Spot counts and flagella counts are combined values from three independent experiments.