

## Supplementary Information

**Table S1.** MutS protein in *T.thermophila*.

Sequences producing significant alignments:		Score (bits)	E Value
TTHERM_00295920	<b>Msh2</b> DNA mismatch repair MutS family DNA-binding domain	462	$1 \times 10^{-130}$
TTHERM_00150000	DNA mismatch repair MutS family DNA-binding domain	199	$8 \times 10^{-51}$
TTHERM_00194810	<b>Msh6</b> MutS domain III protein	196	$6 \times 10^{-50}$
TTHERM_00426230	<b>Msh3</b> DNA mismatch repair MutS family ATPase domain	177	$5 \times 10^{-44}$
TTHERM_00142230	MutS domain III protein	174	$3 \times 10^{-43}$
TTHERM_00857890	MutS domain II family protein	96	$2 \times 10^{-19}$
TTHERM_00763040	MutS domain V protein	70	$7 \times 10^{-12}$

**Table S2.** Similarity comparison of MutS homologs of *T.thermophila*.

	Msh2 (00295920)	Msh6 (00194810)	Msh6L3 (00150000)	Msh3/6L1 (00426230)	Msh3L1 (00142230)	Msh4 (00857890)
Msh6 (00194810)	14.89%					
Msh6L3 (00150000)	13.00%	23.79%				
Msh3/6L1 (00426230)	15.62%	34.07%	21.39%			
Msh3L1 (00142230)	12.34%	27.06%	23.02%	25.61%		
Msh4 (00857890)	8.58%	11.13%	13.35%	10.73%	12.27%	
Msh5 (00763040)	11.01%	11.85%	10.87%	10.23%	12.93%	10.92%

**Table S3.** Primer sequences used in the study

Primer name	Primer sequences
<i>MSH2</i> -5F	GAGCTCAACTCAGCCACTCGTCCATATGTC
<i>MSH2</i> -5R	GCGGCCGCAGATTTTAAAGCAGAAATAATTTTTTATTTTA
<i>MSH2</i> -3F	CTCGAGTCATATTTTAAATATATTTTTTTTGGGAG
<i>MSH2</i> -3R	GGTACCAACTTAATTTATAGTTATAAAATAAATAAATAG
<i>MSH2</i> -MPF	AAAACCAAAGCTCGGATTAATAATTTTG
<i>MSH2</i> -MPR	CAAAATTTTAATCCGAGCTTTGGTTTT
<i>MSH2</i> -KO-5F	CTAGAACTAGTGGATCCATAATGTATTTTATTAAGTTAATGATTG GC
<i>MSH2</i> -KO-5R	GGTACCCGGGGGATCGAGAACCTAGATTTATTTATAATTGTATG CGATACCGTCGACCTCGATCATATTTTAAATATATTTTTTTTGGGA G
<i>MSH2</i> -KO-3F	CCGGGCCCCCCTCGAGCAGAGTAATTCATAGGTTATCAAATAC AAAT
<i>interfer-MSH2</i> -3'F	CTGCAGTTATTAGATAAGTTGCCATATGCGTTC
<i>interfer-MSH2</i> -3'R	CCCGGGCTAAAGTTTCTGCAACAAACAAACCAT
<i>interfer-MSH2</i> -5'F	GGATCCTTATTAGATAAGTTGCCATATGCGTTC
<i>interfer-MSH2</i> -5'R	GTTTAAACCTAAAGTTTCTGCAACAAACAAACCAT
<i>MSH2</i> -3HA-Identify-F	GTTGTTGATCTTGCTTCTCCTATAATG
KO- <i>MSH2</i> -iF	TAAACCTTAATTTAGAATATTTATTAGGC
KO- <i>MSH2</i> -iR	GGAGAAGTACTAAAATGATTTTGAATA
<i>MSH2</i> -3HA-Identify-R	AAGTTCCTCCTAAGATGTGAATAGTG
<i>msh2i</i> -iden-F1	GACATTAATGTGAAAGAAAAGGATAAG
<i>msh2i</i> -iden-R1	TAGACAGTTATAAATCACCTACAATAC
17S-F	CCTGGGAAGGTACGGGTAAT
17S-R	AAGGTTCAACGACCATTCG

**Table S4.** CO-IP-MS data for Msh2-3HA-interacting proteins

Gene Model Identifier	iBAQ MSH2_HA	iBAQ WT	iBAQ WT/iBAQ MSH2_HA
TTHERM_00241700	1206.9	0	0
TTHERM_00765280	952.43	0	0
TTHERM_00564530	49.613	0	0
TTHERM_00558350	2812.2	0	0
TTHERM_00723640	40.276	0	0
TTHERM_00151470	2542.9	0	0
TTHERM_00522600	544.41	0	0
TTHERM_01276420	19.225	0	0
TTHERM_01015890	12.907	0	0
TTHERM_00101330	579.14	0	0
TTHERM_00772030	319.3	0	0
TTHERM_00043890	450.46	0	0
TTHERM_00621340	867.74	0	0
TTHERM_00463450	583.79	0	0
TTHERM_00849320	2393.5	0	0
TTHERM_00387080	21.114	0	0
TTHERM_00622710	1214.4	0	0
TTHERM_00245100	98.361	0	0
TTHERM_00969600	204.98	0	0
TTHERM_00295920	306550	289.87	0.000945588
TTHERM_00850620	38162	346.68	0.00908443
TTHERM_00194810	94985	1504.7	0.015841449
TTHERM_00150000	104330	3084.8	0.029567718
<b>TTHERM_00537060</b>	<b>2318.8</b>	<b>110.39</b>	<b>0.047606521</b>
TTHERM_00600480	347.4	36.242	0.104323546
TTHERM_00773310	24029	3200.7	0.133201548
TTHERM_000088159	5947.4	844.97	0.142073847
...	...	...	...
TTHERM_00648930	325.41	8961.6	27.53941182
TTHERM_00992830	5264.5	2100800	399.0502422
TTHERM_00151488	93.05	52126	560.1934444

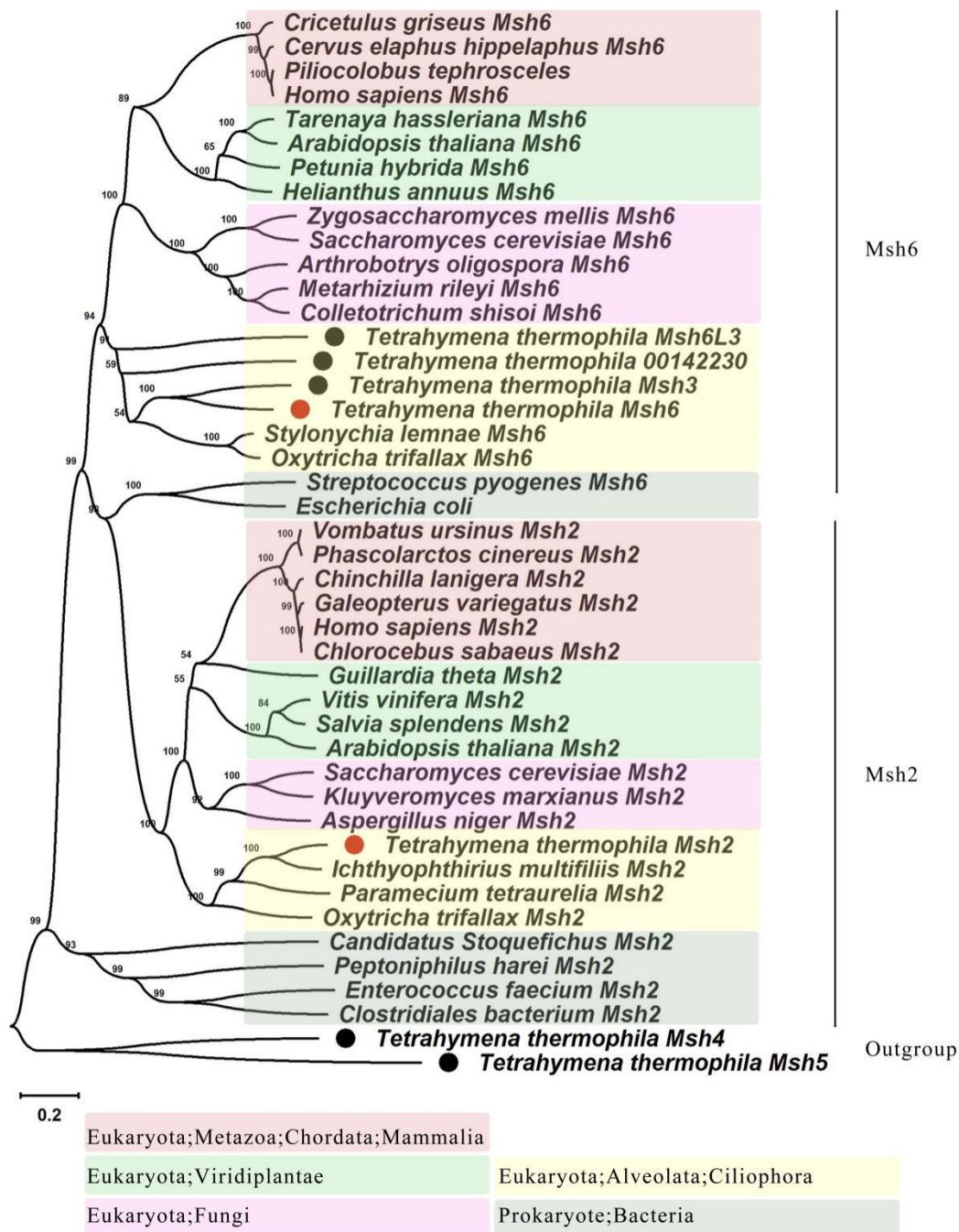
The threshold value of iBAQ WT/iBAQ MSH2\_HA is 0.05.

**Table S5.** qRT-PCR results for relative expression profiles of the *MSH2* gene

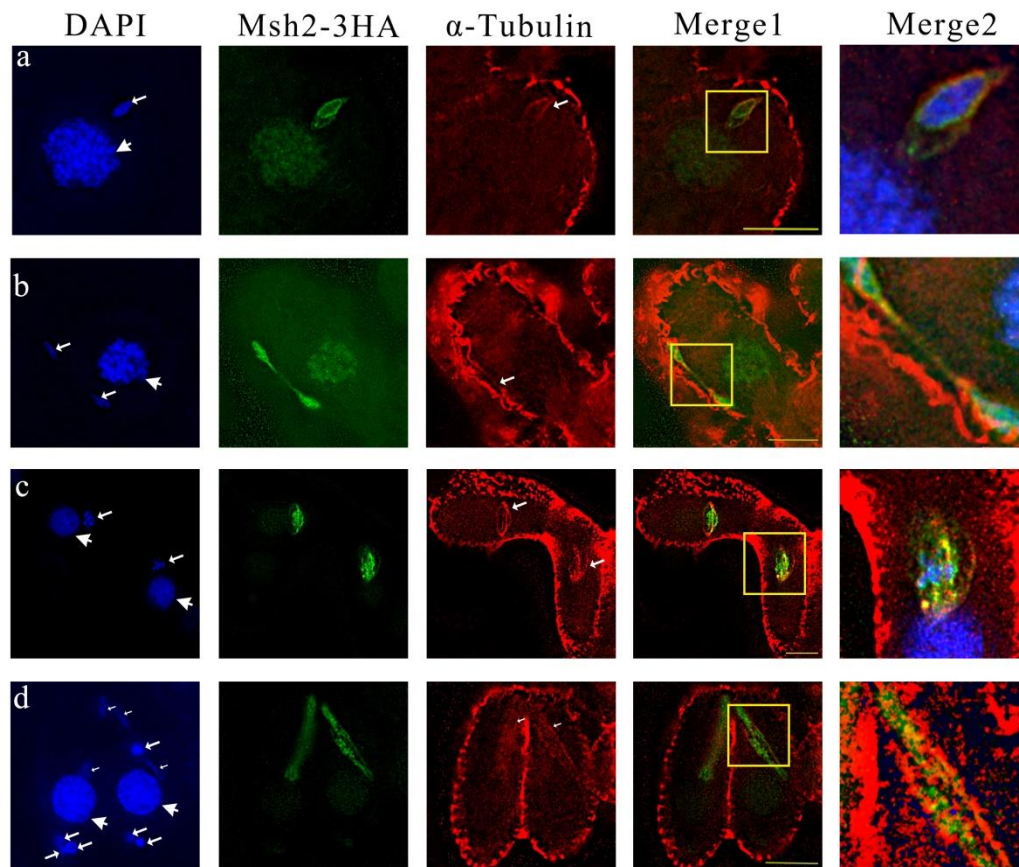
Sample	Expression	Expression SEM	Mean Cq	Cq SEM
Lh	0.4211	0.44712	33.77	1.53024
S0	1	0.0924	33.3	0.09657
C0	0.63151	0.40618	34.72	0.92725
C2	34.65898	10.92061	28.15	0.44249
C4	2.31211	1.72942	27.94	1.01445
C6	1.93963	0.15007	27.73	0.10442
C8	0.39387	0.03013	35.22	0
C10	0.36307	0.40396	35.88	0.43469
C12	0.59112	0.11139	33.08	0.18339
C16	0.86975	0.28795	33.02	0.47214
C18	0.12501	0.17756	35.84	2.04689

**Table S6.** qRT-PCR results for relative expression of *MSH2* in *msh2i* cells

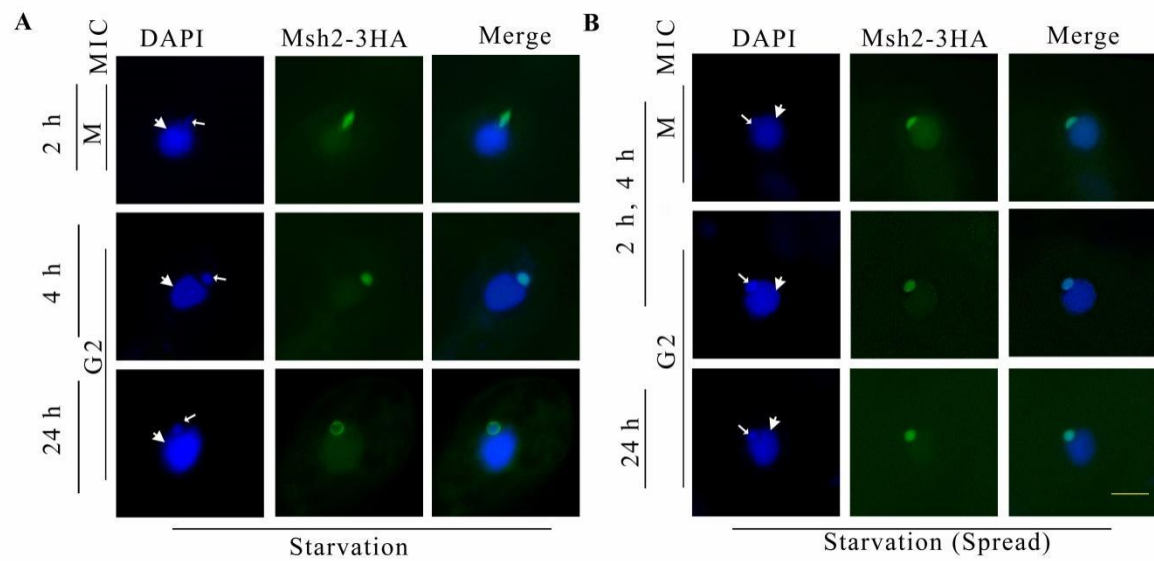
Sample	Expression	Expression SEM	Mean Cq	Cq SEM
WT	1	0.09484	33.15	0.09462
i- <i>MSH2</i> -B2086	0.53937	0.03729	34.18	0.08807
i- <i>MSH2</i> -CU428	0.35041	0.04271	35.26	0.17408



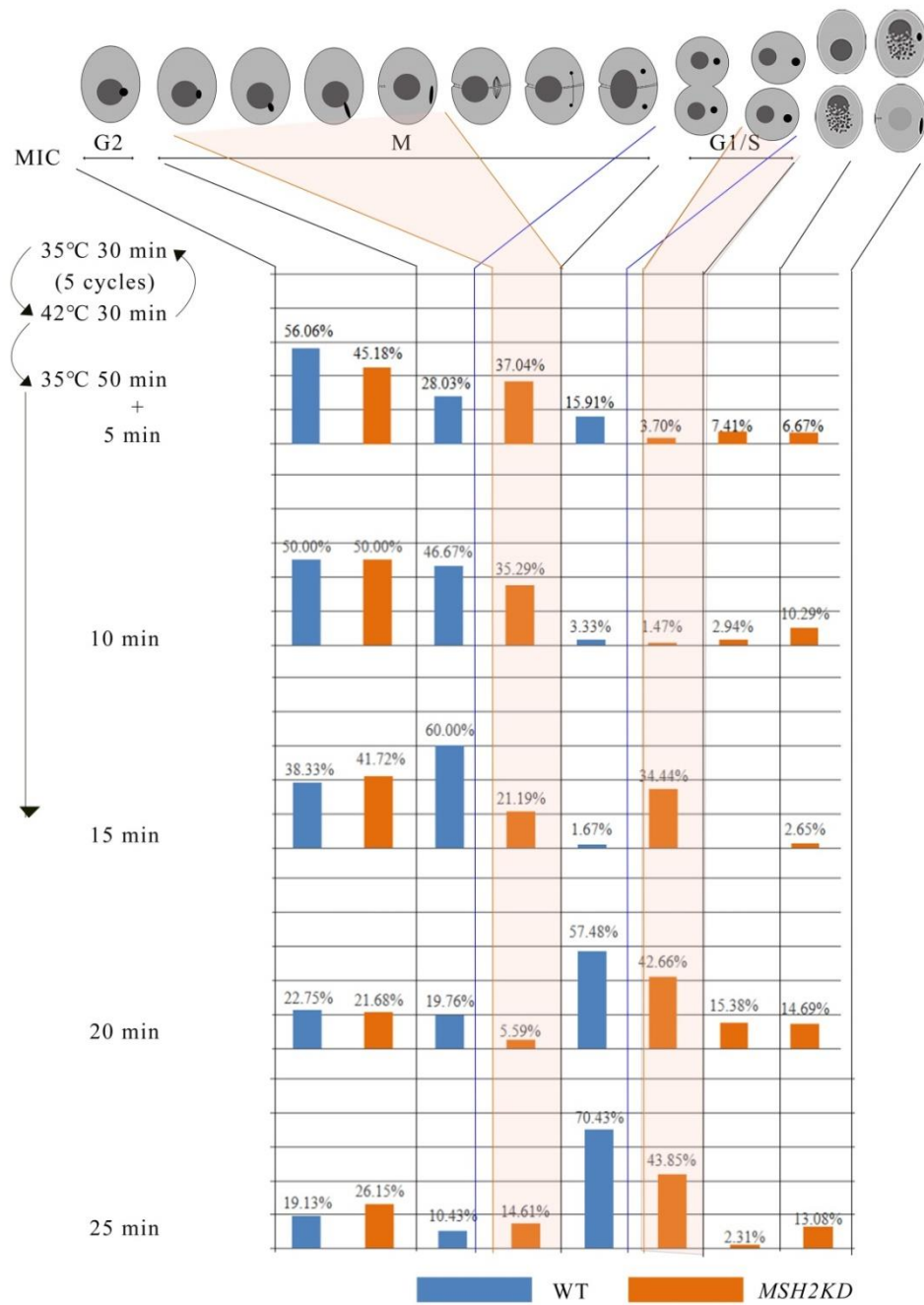
**Supplementary Figure S1.** Phylogenetic tree based on amino acid sequences of Msh2 and Msh6 from different species. Scale bar indicates genetic distance.



**Supplementary Figure S2.** Co-localization of Msh2-3HA and  $\alpha$ -tubulin during vegetative proliferation and conjugation of *Tetrahymena*. The arrow with the larger head indicates the position of the MAC, and the arrow with the smaller head indicates the position of the MIC. Merge2 is a triple magnification of the yellow box in Merge1, incorporating DAPI, Msh2-3HA and  $\alpha$ -tubulin. The scale bar is 10  $\mu$ m.



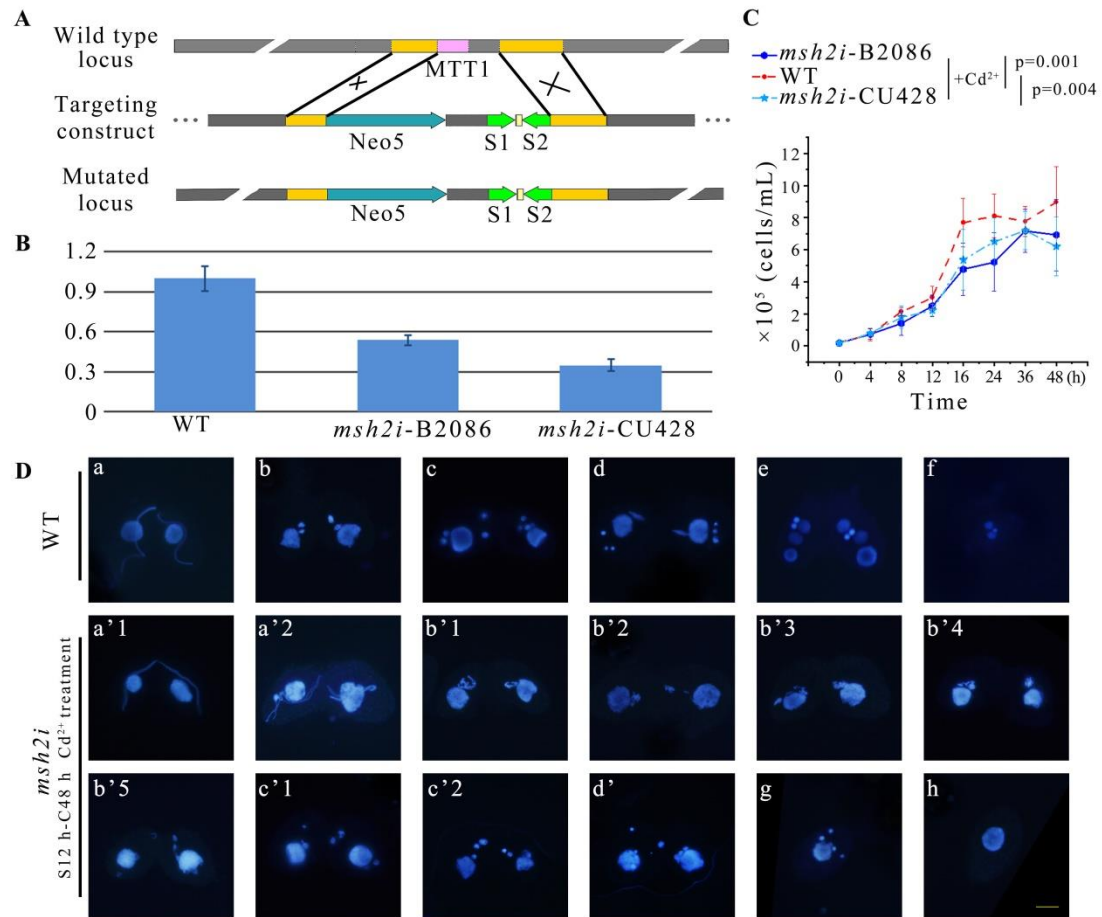
**Figure S3.** Msh2-3HA bound tightly to MIC and MAC chromatin during starvation. **(A)** Immunofluorescence localization of Msh2-3HA; **(B)** Localization of Msh2-3HA on chromatin in spread cells. DAPI stains the nuclei blue, and the localization signal of Msh2-3HA is localized by indirect immunofluorescence via HA tagging (green). The arrow with the larger head indicates the position of the MAC, and the arrow with the smaller head indicates the position of MIC. The scale bar is 10  $\mu\text{m}$ .



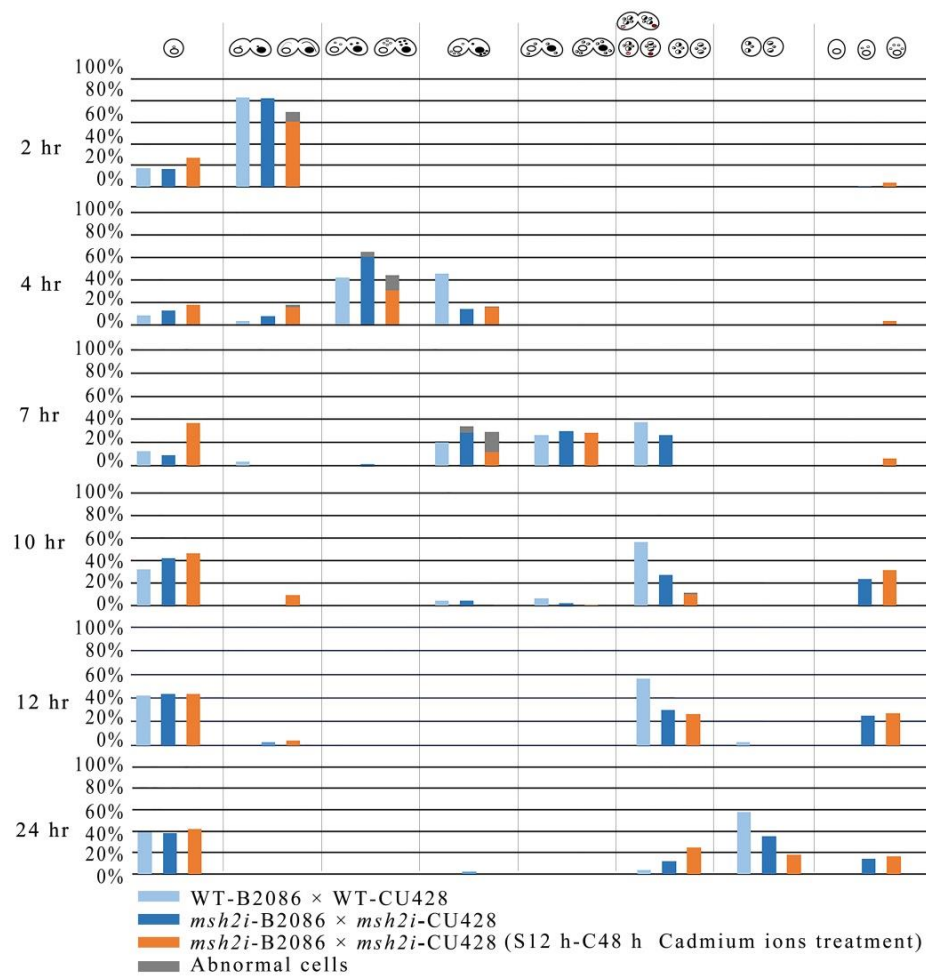
**Figure S4.** Knockdown of *MSH2* affected nuclear division during vegetative proliferation in *T. thermophila*. Nuclear development statistics of the micronucleus of the *MSH2KD* mutant cell line and wild-type control cells after synchronization during vegetative proliferation. The number of cells counted was greater than 120 cells, for each time point, for each cell line. The topmost part of the diagram shows a diagram of the cell development model.







**Figure S6.** Effect of the knockdown of *MSH2* on nuclear division during conjugation in *Tetrahymena* independent of the accumulation of cellular abnormalities during vegetative proliferation. (A) Schematic representation for generating recombinant *msh2i* mutants in *T. thermophile*; (B, C) *msh2i* cells showed a significant decrease in *MSH2* expression and proliferation capacity; (D) Nuclear morphology of *msh2i* mutant and wild-type cells after Cd<sup>2+</sup> induction which was added at late starvation and during conjugation. DAPI stains the nuclei blue. Scale bar is 10 μm. DAPI stains the nuclei blue.



**Supplementary Figure S7.** Nuclear development statistics of the *msh2i* mutant cell line after  $\text{Cd}^{2+}$  induction which was added at late starvation and during conjugation. The number of cells counted was greater than 300 cells, for each time point, for each kind of paired cell. The topmost part of the diagram shows a diagram of the cell development model.