

SUPPLEMENTARY DATA

Genome-wide function of MCM-BP in *Trypanosoma brucei* DNA replication and transcription

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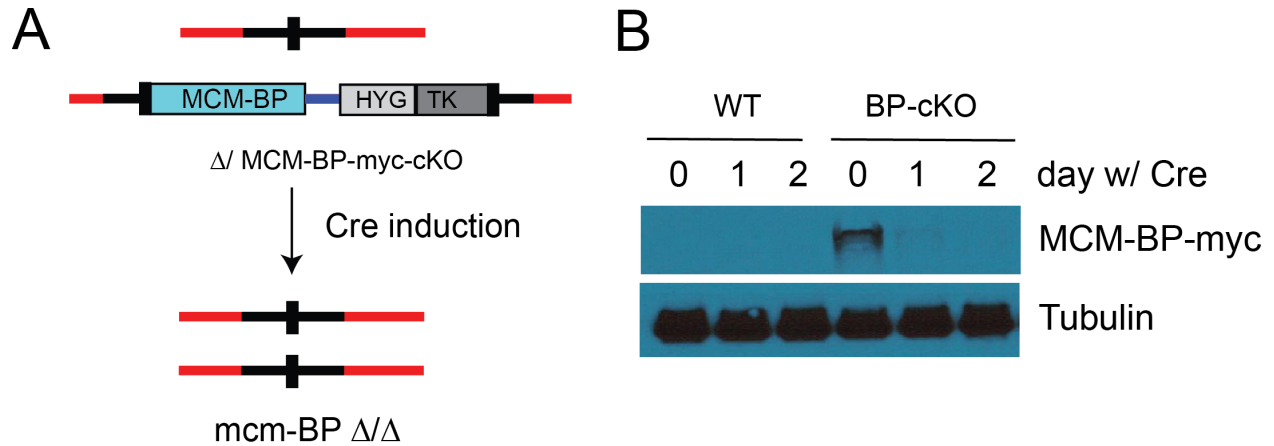
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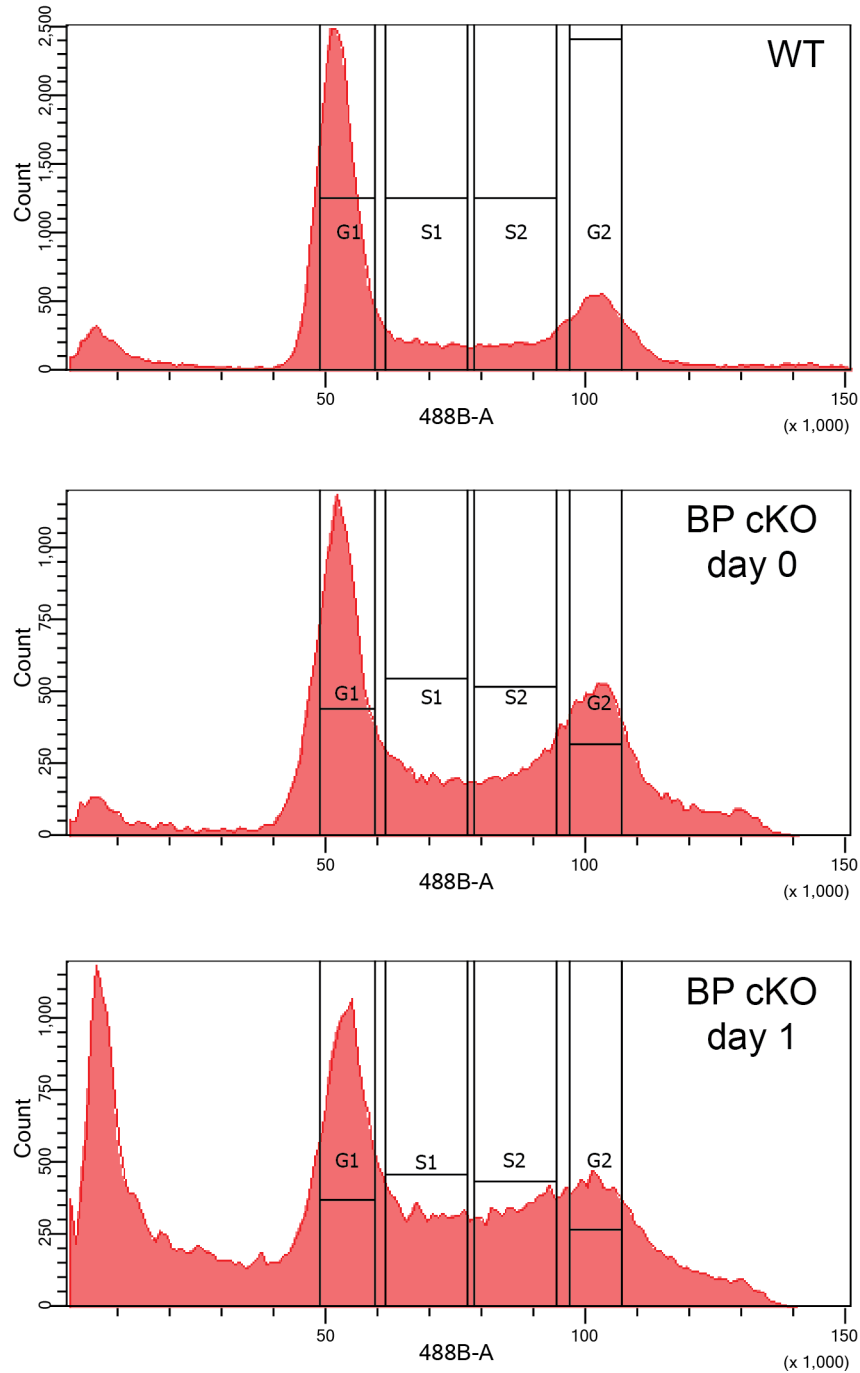
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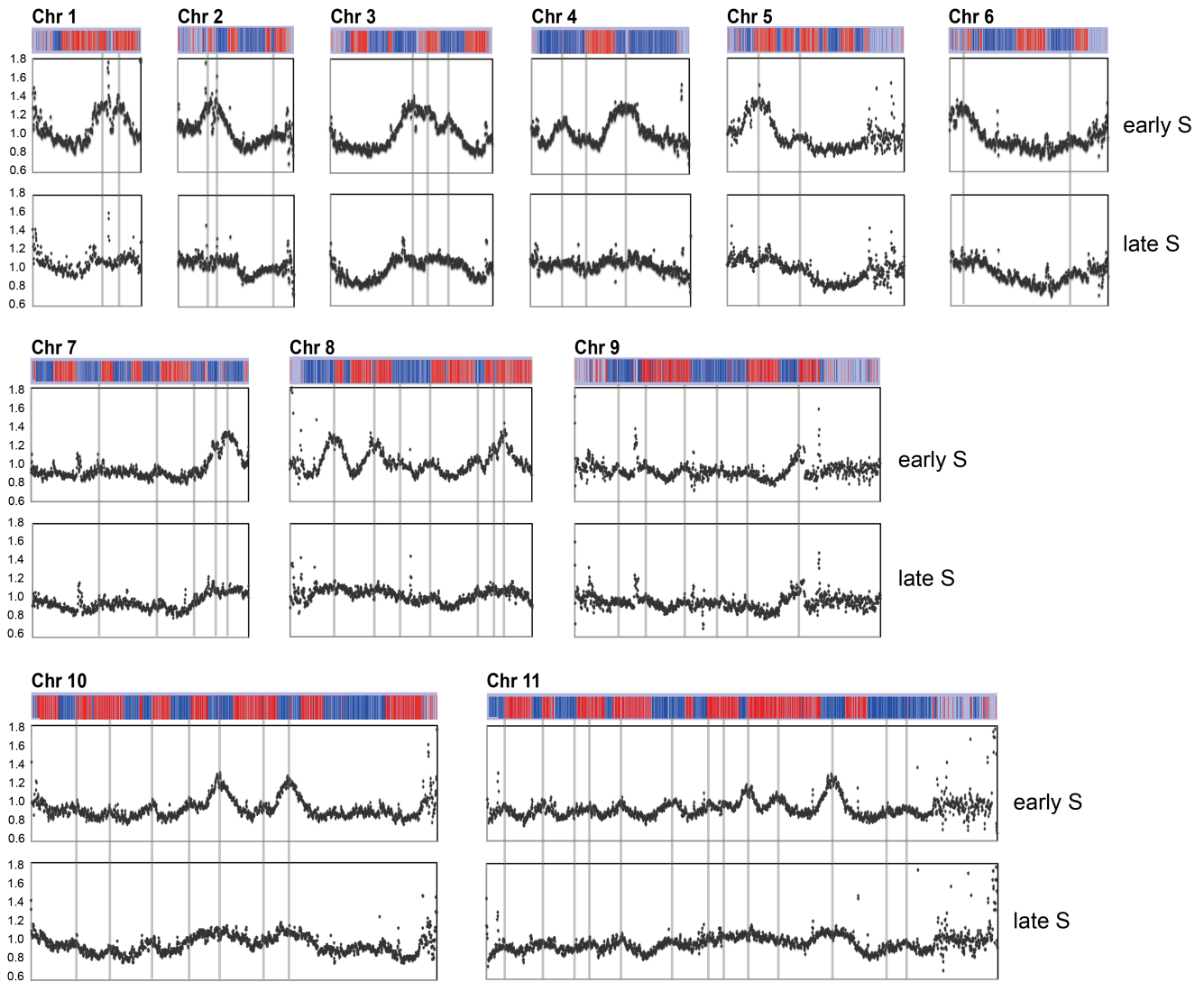
Tel: +1-216-523-7326



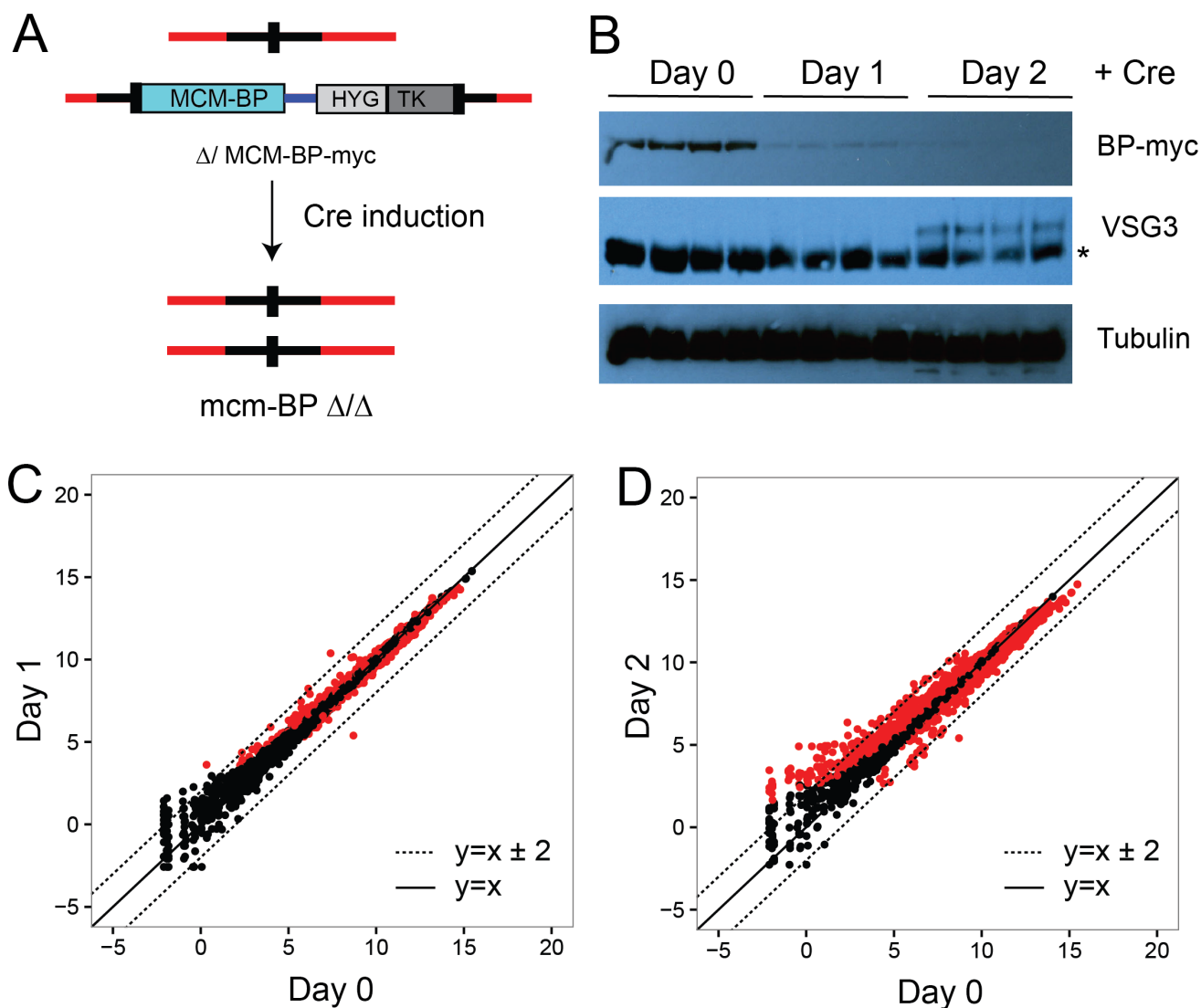
Supplementary Figure S1. *Tb*MCM-BP conditional knock out (cKO) cell line (A) Diagram of *Tb*MCM-BP removal strategy [11]. One allele of *Tb*MCM-BP was removed and the remaining allele was replaced with a loxP-*Tb*MCM-BP-myc-HYG-TK-loxP cassette. Tetracycline addition induces expression of Cre recombinase, which removes sequences between two loxP sites, resulting in a *Tb*MCM-BP knockout. (B) Depletion of *Tb*MCM-BP after tetracycline addition. Cells were collected at day 0, 1, and 2 after tetracycline addition and western blot was performed with mouse anti-myc antibody or mouse anti-tubulin antibody.



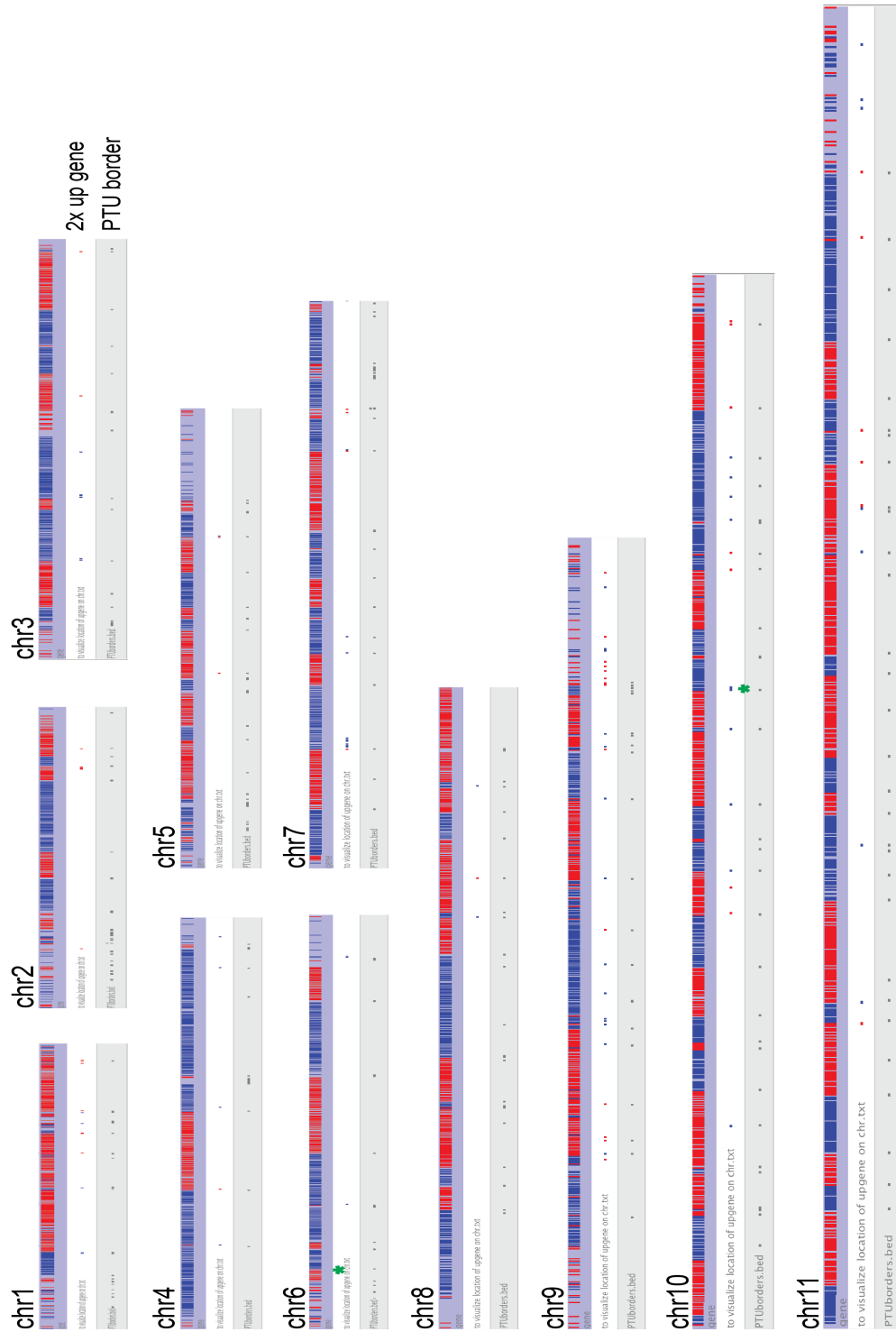
Supplementary Figure S2. Cell sorting by flowcytometry (to perform MFA-seq experiments). Cell population in G1, early S (S1), late S (S2) and G2 phases were sorted by flow cytometry (BD Aria3). Cell cycle profiles for WT, *TbMCM-BP* tet-untreated (day 0) and *TbMCM-BP* cKO tet-treated (day 1) are shown.



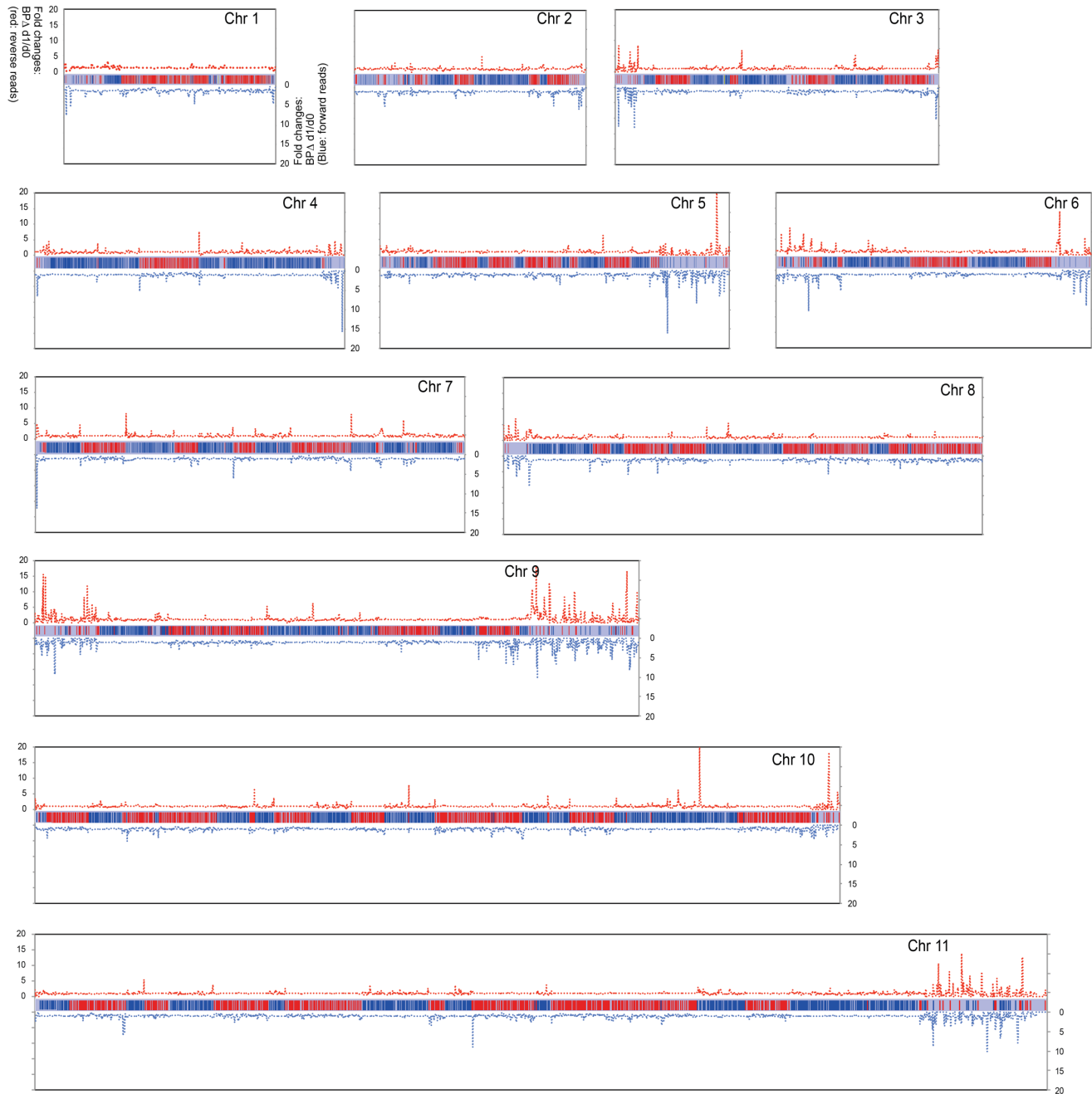
Supplementary Figure S3. Comparison of replication profiles between early S and late S in wild type cells. Replication was examined by the MFA-seq as in Figure 2. Sequence reads from S1 and S2 were compared to G1 phase DNA. Early S profiles are the same as those in Figure 2. Chromosomes were binned at 10 kb resolution with 2.5 kb step. Read count ratio of late S to G1 was plotted for 11 megabase chromosomes. Chromosomes are depicted as bars above each graph. Blue genes are transcribed reverse and red are forward direction. Grey lines indicate the early replicating origins that were identified by the McCulloch lab [7].



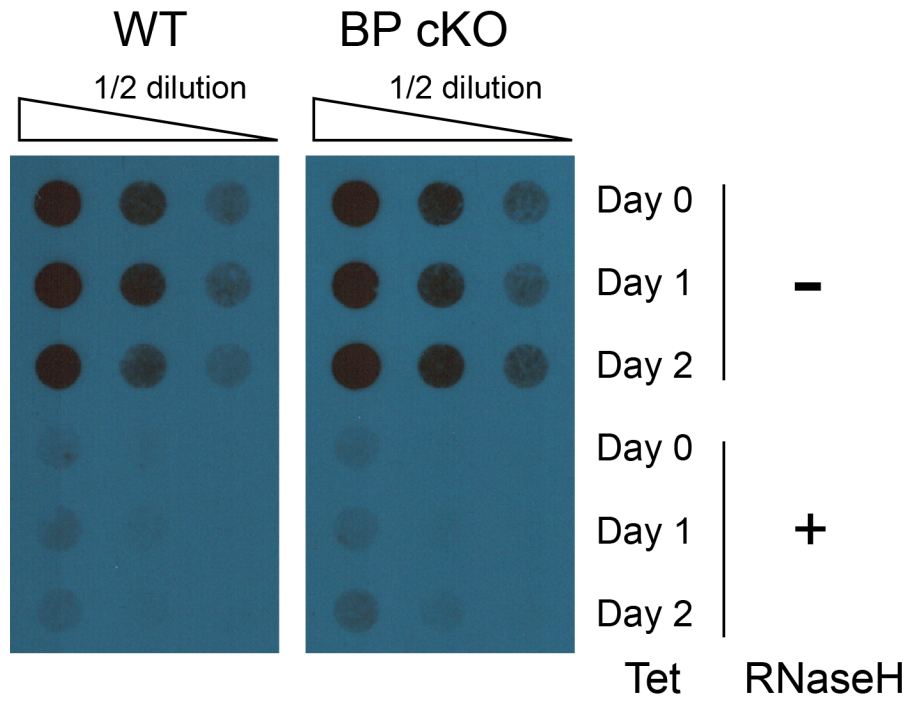
Supplementary Figure S4. Gene expression in the absence of *Tb*MCM-BP by stranded RNA-seq. (A) Diagram of *Tb*MCM-BP removal strategy [11]. (B) Western blot showing *Tb*MCM-BP depletion after tetracycline addition. Two independent cultures from two independent *Tb*MCM-BP cKO strains were analyzed (total 4 samples per day). Expression level of a silent VSG3 increases in the absence of *Tb*MCM-BP. (C & D) Gene expression profiles in the absence of *Tb*MCM-BP. Reads from the RNA-seq experiment were aligned to the Tb927v5 reference genome with Bowtie and analyzed with DESeq. Log_2 (normalized count) values were obtained and graphed in scatter plots. Scatter plots show comparison between day 0 and day 1 (C) or between day 0 and day 2 (D) after *Tb*MCM-BP deletion. Red dots indicate genes whose expression level changed significantly (with $P_{\text{adj}} < 0.01$). Dotted diagonal lines indicate changes that are 4-fold up or down.



Supplementary Figure S5. Genes that are at least > 2-fold up-regulated in the absence of *TbMCM-BP* are located close to borders of transcription. Chromosome diagrams are shown on the top. Chromosomal locations of genes that are at least 2-fold upregulated in the absence of *TbMCM-BP* are shown as dots. Borders of PTUs (locations of TSSs, TTSs, & HTs) are also shown. Locations of procyclin genes on chromosome 6 and 10 are shown as green asterisks.



Supplementary Figure S6. Fold changes of transcription levels between day 0 and day 1 upon *TbMCM-BP* removal. The same analysis as in Figure 4 was performed to compare reads from day 0 and day 1 after *TbMCM-BP* deletion. Reverse and forward reads were analyzed separately. Red lines indicate fold changes from reverse reads (mapping to forward strand, red PTUs) and blue lines indicate fold changes from forward reads (mapping to reverse strands, blue PTUs). Chromosome diagrams are shown in the middle of each graph.



Supplementary Figure S7. R loop analysis by dot blot. Genomic DNA was isolated from WT and *TbMCM-BP* cKO treated with tetracycline for 0, 1, and 2 days. DNA was either treated or untreated with RNase H, 1:2 serially diluted and then spotted on a membrane. R loops were detected using the mouse S9.6 antibody [17].

Supplementary Table S1. DEseq analysis comparing gene expression levels between day 0 and day 2 after *TbMCM-BP* deletion. Genes that are at least 2-fold down or upregulated with *P*_{adj} <0.01 are listed.

Genes that are > 2-fold down regulated					
ID	Name	DEseq count day 0	DEseq count day 2	Fold change	padj value
Tb927.7.1770	TbMCM-BP	414.29	42.31	0.10	7.58E-174
Tb927.8.5120	cytochrome C	1836.13	687.03	0.37	4.06E-60
Tb927.10.3420	NADH-ubiquinone oxidoreductase c	22.10	9.26	0.42	2.66E-03
Tb927.11.14090	DRBD18, double RNA binding domain proein 18	4710.62	2020.80	0.43	5.06E-216
Tb927.7.7510	hypothetical protein	21997.36	9744.99	0.44	8.01E-97
Tb927.10.4560	translation elongation factor 2	28195.76	12605.51	0.45	0.00E+00
Tb927.10.4570	hypothetical protein, conserved	28159.49	12623.48	0.45	3.34E-314
Tb927.9.10400	hypothetical protein, conserved	4459.11	2001.42	0.45	2.06E-200
Tb927.10.10450		430.87	197.54	0.46	3.48E-39
Tb927.10.10540	H2B	879.60	407.34	0.46	1.51E-53
Tb927.10.6060	UMSBP2, universal minicircle sequence binding protein 2	1528.65	718.13	0.47	1.47E-56
Tb927.10.10520	H2B	865.63	409.09	0.47	5.26E-48
Tb927.4.2310	asparaginyl tRNA synthase	4402.78	2102.19	0.48	7.44E-87
Tb927.11.2400	Flabarin-like protein	657.67	314.30	0.48	1.18E-42
Tb927.10.10560	H2B	857.21	410.30	0.48	2.12E-60
Tb927.6.3180	hypothetical protein, conserved	2348.37	1125.86	0.48	1.56E-124
Tb927.10.10480	H2B	865.52	415.32	0.48	4.95E-63
Tb927.10.10550	H2B	867.51	420.72	0.48	6.73E-62
Tb927.10.10590	H2B	1269.57	615.83	0.49	1.43E-57
Tb927.10.10530	H2B	869.99	424.80	0.49	6.94E-61
Tb927.10.10490	H2B	848.86	414.60	0.49	1.88E-59
Tb927.10.10470	H2B	866.06	423.32	0.49	4.89E-58
Tb927.10.10580	H2B	854.34	418.59	0.49	2.49E-59
Tb927.10.10460	H2B	624.62	306.48	0.49	9.77E-38
Tb927.10.540	ATP-dependent RNA helicase SUB2	986.23	485.58	0.49	4.64E-65

Tb927.8.8330	calpain, cysteine peptidase	5336.99	2657.64	0.50	2.68E-124
Tb927.11.890	hypothetical conserved protein	5795.16	2889.66	0.50	2.02E-171
Tb927.4.4860	AATP8, amino acid transporter 8	1518.73	761.52	0.50	2.14E-56
Tb927.9.10310	MCP11, mitochondrial carrier protein 11	2550.58	1280.23	0.50	6.63E-116
Tb927.10.10500	H2B	610.95	307.39	0.50	3.72E-43
Tb927.10.3410	hypothetical protein	22.57	11.38	0.50	7.75E-03
Tb927.10.3400	hypothetical protein, conserved	182.06	92.29	0.51	2.49E-14
Tb927.10.10510	H2B	809.96	417.39	0.52	8.22E-51
Tb927.10.11710	hypothetical protein	647.86	334.01	0.52	7.06E-42
Tb927.11.4180	PSP-1 C-terminal conserved region	1408.52	726.84	0.52	1.24E-26
Genes that are > 2-fold upregulated					
ID	Name	DEseq count day 0	DEseq count day 2	Fold change	padj value
Tb927.3.3700	hypothetical protein	65.05	125.82	1.93	7.11E-10
Tb927.9.8920		12.83	24.83	1.94	1.56E-02
Tb927.9.7430	VSG, related	45.36	88.27	1.95	1.40E-07
Tb927.6.1300	hypothetical protein	10.25	20.02	1.95	1.68E-02
Tb927.7.7550	protein of unknown function (DUF1181)	45.26	89.20	1.97	1.04E-06
Tb927.9.14570	LRRP, leucine-rich repeat protein	562.00	1112.09	1.98	2.77E-32
Tb927.3.2520	ESAG1	23.99	47.91	2.00	1.44E-04
Tb927.2.5310	hypothetical protein, conserved	238.78	479.20	2.01	3.59E-36
Tb927.5.2260	conserved protein	179.41	361.13	2.01	5.81E-20
Tb927.9.3260	hypothetical protein	30.60	61.64	2.01	8.50E-06
Tb927.5.4020	hypothetical protein	383.08	781.92	2.04	1.37E-25
Tb927.11.4080	VSG, related	94.54	193.94	2.05	3.87E-12
Tb927.10.12270	methyltransferase domain containing protein	9.55	19.59	2.05	1.19E-02
Tb927.10.16010	hypothetical protein	19.10	39.28	2.06	2.22E-04
Tb927.2.5340	hypothetical protein, conserved	121.83	250.63	2.06	2.19E-21
Tb927.10.16100	FK506-binding protein (FKBP)-type peptidyl-prolyl isomerase	354.59	731.56	2.06	1.95E-54

Tb927.2.5360	hypothetical protein, conserved	189.50	392.34	2.07	8.18E-32
Tb927.9.9880		9.22	19.09	2.07	1.22E-02
Tb927.3.590	adenosine transporter	143.00	297.99	2.08	2.19E-24
Tb927.9.3190		15.88	33.32	2.10	6.10E-04
Tb927.9.16000		29.80	62.82	2.11	1.12E-06
Tb927.5.297b	hypothetical protein	73.95	156.80	2.12	8.81E-15
Tb927.1.3590		17.25	37.07	2.15	2.14E-04
Tb927.2.5350	hypothetical protein, conserved	133.90	288.11	2.15	1.84E-26
Tb927.6.460	PAG3	14.26	30.72	2.15	3.27E-03
Tb927.11.4410		40.81	87.99	2.16	2.46E-09
Tb927.3.1510	VSG, related	272.15	588.94	2.16	5.66E-50
Tb927.9.3810		11.39	24.95	2.19	4.46E-03
Tb927.1.3760	hypothetical protein, conserved	10.27	22.53	2.19	3.32E-03
Tb927.8.5350	hypothetical protein, conserved	48.05	105.61	2.20	5.02E-11
Tb927.8.7300	VSG, related	140.72	311.10	2.21	1.36E-30
Tb927.10.13690	hypothetical protein, conserved	222.31	491.53	2.21	4.64E-45
Tb927.3.5740	hypothetical protein	16.93	37.49	2.21	4.83E-03
Tb927.5.296b	hypothetical protein	60.02	134.47	2.24	1.17E-14
Tb927.10.13390	hypothetical protein, conserved	228.85	513.93	2.25	1.76E-26
Tb927.4.5090	hypothetical protein	13.47	30.38	2.26	3.66E-04
Tb927.10.6890	hypothetical protein	100.15	229.01	2.29	1.19E-24
Tb927.9.2960		68.01	155.60	2.29	5.45E-17
Tb927.3.3160	PAP1, polynucleotide adenylyltransferase	512.51	1181.47	2.31	5.15E-100
Tb927.1.4850		24.14	56.12	2.32	5.15E-07
Tb927.8.7320	VSG, related	155.25	361.25	2.33	5.92E-38
Tb927.1.4920	hypothetical protein	31.27	73.76	2.36	3.27E-09
Tb927.9.2950		169.86	402.16	2.37	9.66E-44
Tb927.3.1500	VSG, related	451.34	1072.56	2.38	5.94E-74
Tb927.9.760	hypothetical protein	31.45	75.20	2.39	1.29E-09
Tb927.9.7950	hypothetical protein	22.18	53.47	2.41	1.78E-07
Tb927.1.940		9.00	21.80	2.42	1.86E-03
Tb927.8.490	hypothetical protein	244.13	603.72	2.47	1.29E-32
Tb927.11.17030		6.80	17.15	2.52	3.91E-03
Tb927.7.2010	RHS7	82.51	208.30	2.52	6.72E-19
Tb927.7.7560	hypothetical protein	16.28	41.12	2.53	3.40E-03
Tb927.7.2020	RHS7	70.01	177.33	2.53	2.62E-23
Tb927.9.7960	hypothetical protein	26.16	66.47	2.54	8.37E-10
Tb927.1.50		5.50	14.03	2.55	1.12E-02

Tb927.10.1010	hypothetical protein	4.95	12.72	2.57	2.04E-02
Tb927.7.3250	ESAG6	418.83	1092.84	2.61	6.55E-58
Tb927.4.3280	ESAG2	4.84	12.79	2.65	1.30E-02
Tb927.7.6500	VSG	14.68	39.06	2.66	2.57E-06
Tb927.5.170	hypothetical protein, conserved	18.36	49.48	2.69	3.80E-08
Tb927.1.2950		25.54	69.59	2.72	1.26E-09
Tb927.2.5320	hypothetical protein, conserved	74.40	207.12	2.78	9.41E-26
Tb927.9.14620		5.12	14.28	2.79	5.50E-03
Tb927.7.6010	hypothetical protein	4.01	11.20	2.79	1.06E-02
Tb927.11.12000	hypothetical protein, conserved	27.50	77.27	2.81	1.35E-12
Tb927.7.6030	hypothetical protein	8.27	23.26	2.81	1.66E-03
Tb927.11.12100	RBP5, RNA binding protein	531.11	1500.36	2.82	1.55E-169
Tb927.7.3260	ESAG7	381.47	1083.59	2.84	4.25E-50
Tb927.1.900		17.56	49.93	2.84	1.70E-08
Tb927.8.520	hypothetical protein	60.39	178.52	2.96	1.54E-29
Tb927.9.7390	VSG, related	11.26	33.44	2.97	2.08E-06
Tb927.9.770	hypothetical protein, conserved	6.86	20.43	2.98	3.35E-04
Tb927.10.3050	hypothetical protein	7.37	21.98	2.98	4.68E-03
Tb927.7.1970	RHS7	46.36	138.51	2.99	2.29E-23
Tb927.7.2000	RHS7	71.60	215.89	3.02	1.42E-35
Tb927.4.140	hypothetical protein	7.26	22.11	3.05	5.19E-03
Tb927.1.4930		36.13	110.43	3.06	1.40E-19
Tb927.11.11990	hypothetical protein	8.45	26.65	3.15	1.31E-05
Tb927.6.480	EP3-2	37.55	119.29	3.18	1.39E-22
Tb927.6.110	hypothetical protein	5.27	17.04	3.23	4.00E-04
Tb927.11.6730	hypothetical protein	5.40	17.61	3.26	3.95E-04
Tb927.7.1930	nucleoside diphosphatase	67.92	221.53	3.26	8.84E-25
Tb927.7.1980	RHS7	49.29	160.84	3.26	3.11E-30
Tb927.10.14850		11.01	36.77	3.34	7.17E-08
Tb927.2.5720	hypothetical protein	13.46	45.30	3.37	5.52E-10
Tb927.11.11410	trans-sialidase	198.33	680.10	3.43	3.81E-119
Tb927.10.10210	PAG4	53.27	184.11	3.46	1.35E-14
Tb927.9.3200		7.31	25.34	3.46	4.38E-06
Tb927.3.2590	hypothetical protein	38.55	134.68	3.49	8.82E-25
Tb927.9.7440		51.54	181.91	3.53	1.11E-37
Tb927.6.510	GPEET	375.46	1334.72	3.55	1.79E-55
Tb927.10.140	hypothetical protein	6.30	22.44	3.56	9.33E-06
Tb927.6.450	EP3-2	31.41	112.80	3.59	6.21E-24
Tb927.11.16190	hypothetical protein	5.68	20.44	3.60	3.09E-05

Tb927.9.15970		2.17	7.82	3.60	1.57E-02
Tb927.8.480	phosphatidic acid phosphatase protein	168.05	609.14	3.62	4.01E-46
Tb927.8.500	hypothetical protein	41.85	152.46	3.64	6.55E-29
Tb927.7.1950	RHS7	37.41	137.34	3.67	3.95E-30
Tb927.9.14940	SLACS reverse transcriptase	64.30	236.22	3.67	4.25E-45
Tb927.7.1940	hypothetical protein	3.34	12.39	3.71	1.45E-03
Tb927.9.14610		10.12	38.01	3.76	2.29E-08
Tb927.11.17830	hypothetical protein, conserved	9.41	35.41	3.76	7.88E-09
Tb927.5.3990	VSG, atypical	26.46	101.09	3.82	6.13E-06
Tb927.10.10260	EP1	280.96	1095.04	3.90	7.97E-57
Tb927.1.3350	hypothetical protein	7.44	29.07	3.91	1.78E-07
Tb927.8.510	hypothetical protein	39.08	153.23	3.92	8.82E-36
Tb927.10.3060		8.82	35.11	3.98	4.39E-09
Tb927.7.6540	VSG, atypical	12.08	48.13	3.98	4.03E-12
Tb927.2.1290	hypothetical protein	3.12	12.63	4.05	6.67E-04
Tb927.9.14650	SLACS retrotransposabe element (part)	124.84	511.72	4.10	2.31E-112
Tb927.6.520	EP3-3	102.15	431.09	4.22	7.36E-84
Tb927.1.4960		3.54	15.02	4.24	1.90E-04
Tb927.10.10240	PAG1	52.70	227.46	4.32	5.98E-14
Tb927.10.10230	PAG5	74.30	320.78	4.32	1.57E-67
Tb927.8.5930	hypothetical protein	4.89	21.96	4.49	1.27E-06
Tb927.10.12580	hypothetical protein	2.76	12.40	4.50	1.26E-03
Tb927.11.12710	VSG, related	31.99	144.01	4.50	2.78E-25
Tb927.7.1960	RHS7	40.34	182.75	4.53	8.75E-38
Tb927.6.5260	VSG	6.34	28.97	4.57	9.56E-09
Tb927.10.13060	hypothetical protein	1.61	7.38	4.59	8.99E-03
Tb927.9.4880		2.09	9.62	4.60	2.56E-03
Tb927.3.2540	VSG, related	16.85	77.65	4.61	5.50E-21
Tb927.10.10250	PAG1	110.84	511.06	4.61	1.60E-126
Tb927.10.7160	PAG2	15.79	73.47	4.65	1.53E-20
Tb927.9.8930		3.00	14.03	4.69	2.63E-04
Tb927.1.3370		7.66	37.27	4.87	4.49E-11
Tb927.9.3720		6.99	34.09	4.88	1.91E-10
Tb927.9.16070	hypothetical protein	2.11	10.41	4.94	8.81E-04
Tb927.9.3710		19.19	95.61	4.98	6.96E-28
Tb927.9.17410	VSG, atypical	6.23	33.90	5.44	1.92E-11
Tb927.6.5240	VSG, atypical	5.96	32.65	5.48	1.31E-07
Tb927.1.3380		9.58	52.50	5.48	1.50E-16
Tb927.10.12570	hypothetical protein	1.82	10.01	5.48	6.27E-04
Tb927.1.2080		1.95	11.02	5.64	3.99E-04

Tb927.9.3210		4.67	26.75	5.73	2.63E-09
Tb927.9.16490	VSG	2.59	15.03	5.80	1.15E-05
Tb927.9.7340	ESAG9	13.46	79.14	5.88	2.77E-26
Tb927.10.10220	PAG2	69.53	413.00	5.94	1.69E-70
Tb927.9.16460	VSG, atypical	1.07	6.39	5.99	6.06E-03
Tb927.9.11180		4.43	27.05	6.10	1.12E-09
Tb927.11.11420	hypothetical protein	2.24	13.97	6.24	2.38E-05
Tb927.9.9930		1.75	11.12	6.35	1.70E-04
Tb927.9.9910		0.78	5.35	6.89	9.81E-03
Tb927.1.3770		1.25	8.77	7.03	8.16E-04
Tb927.1.910		1.04	7.40	7.12	1.76E-03
Tb927.9.16640	VSG	4.25	30.60	7.20	6.59E-12
Tb927.7.3480	hypothetical protein	1.78	12.93	7.27	1.65E-05
Tb927.9.16200	VSG	1.55	12.46	8.04	1.19E-04
Tb927.9.6810		0.75	6.08	8.06	4.92E-03
Tb927.10.9550	hypothetical protein	7.64	62.04	8.12	2.16E-25
Tb927.3.2560	hypothetical protein, conserved	2.84	23.50	8.26	5.33E-10
Tb927.9.9890		1.53	12.86	8.39	8.72E-06
Tb927.10.9530	hypothetical protein	0.99	8.89	8.98	2.02E-04
Tb927.9.11210		1.01	9.21	9.13	1.81E-04
Tb927.10.14060	hypothetical protein	1.17	10.66	9.14	6.70E-05
Tb927.10.1760	hypothetical protein	5.15	47.35	9.19	1.58E-20
Tb927.11.17720	VSG	1.85	17.84	9.63	2.69E-08
Tb927.4.2090	hypothetical protein	3.08	32.97	10.72	1.73E-15
Tb927.9.3220		0.75	8.27	11.10	2.51E-04
Tb927.9.6820		0.75	8.31	11.15	1.91E-04
Tb927.3.2570	hypothetical protein, conserved	2.12	24.23	11.43	6.64E-12
Tb927.11.13160	hypothetical protein	0.82	9.65	11.74	2.40E-05
Tb927.11.18430	VSG	1.53	18.38	12.01	2.50E-09
Tb05.30F7.410	hypothetical protein	0.76	9.80	12.89	2.33E-05
Tb927.10.6430	hypothetical protein	2.33	30.53	13.10	8.65E-16
Tb927.9.3730		0.49	7.15	14.49	3.47E-04
Tb927.10.8340	hypothetical protein	2.09	30.78	14.71	3.33E-16
Tb927.9.3250		0.56	8.80	15.75	2.74E-05
Tb927.1.920		0.26	4.11	15.76	1.03E-02
Tb927.3.2580	hypothetical protein, conserved	5.07	81.85	16.14	4.61E-43
Tb927.10.9540		0.51	8.29	16.18	3.74E-04
Tb927.3.2550	hypothetical protein, conserved	1.73	29.13	16.81	6.45E-16
Tb927.4.5560	VSG	0.23	4.54	19.45	5.22E-03
Tb927.9.16250	VSG	0.26	5.44	20.63	1.23E-03
Tb927.9.3230		0.50	10.27	20.68	2.99E-06

Tb927.4.1210	hypothetical protein	0.26	5.48	20.77	1.58E-03
Tb927.10.12560	hypothetical protein	0.49	10.38	21.03	2.85E-06
Tb927.9.13160	hypothetical protein	0.26	5.65	21.42	1.12E-03
Tb927.9.11190		0.26	5.60	21.50	1.85E-02
Tb927.1.930		0.50	10.87	21.89	9.74E-06
Tb927.9.11200		0.54	11.90	22.04	2.09E-07
Tb927.9.3240		0.26	5.91	22.42	7.68E-04
Tb927.7.6020	hypothetical protein	0.23	5.30	22.74	1.71E-03
Tb927.10.7170	hypothetical protein	0.26	6.00	22.76	5.64E-04
Tb927.3.370	VSG	0.26	6.21	23.84	3.91E-04
Tb927.9.16320	VSG	1.25	30.07	23.98	7.26E-18
Tb927.3.190	VSG	0.26	6.82	26.19	1.75E-04
Tb927.2.5710	hypothetical protein	0.75	29.77	39.92	2.24E-19
Tb927.9.17600	VSG	0.23	10.95	46.96	2.30E-07

Supplementary Table S2. *P* values generated from Kruskal–Wallis one-way analysis of variance test across samples of *Tb*MCM-BP cKO tetracycline treated for 0, 1, or 2 days and Mann-Whitney U test for transcript levels of TSSs, TTSs, or HT sites comparing between samples.

Statistical analysis for TSSs (for Figure 5)		
Kruskal-Wallis One-Way Analysis of Variance Test		
Kruskal-Wallis chi-squared	20.084	
<i>P</i> -value	4.354e-05	
Mann-Whitney U Test		
	W statistic	<i>P</i> -value
Day 1 to Day 0	4395	0.4748
Day 2 to Day 0	5630	2.789e-05
Day 2 to Day 1	5371	0.0005377
Statistical analysis for TTSs (for Figure 5)		
Kruskal-Wallis One-Way Analysis of Variance Test		
Kruskal-Wallis chi-squared	7.4635	
<i>P</i> -value	0.02395	
Mann-Whitney U Test		
	W statistic	<i>P</i> -value
Day 1 to Day 0	4269	0.3702
Day 2 to Day 0	4867	0.008395
Day 2 to Day 1	4596	0.0647
Statistical analysis for HTs (for Figure 5)		
Kruskal-Wallis One-Way Analysis of Variance Test		
Kruskal-Wallis chi-squared	9.7693	
<i>P</i> -value	0.007562	
Mann-Whitney U Test		
	W statistic	<i>P</i> -value
Day 1 to Day 0	2174	0.5498
Day 2 to Day 0	2669	0.003106
Day 2 to Day 1	2540	0.01917

Supplementary Table S3. *P* values generated from Kruskal–Wallis one-way analysis of variance test across samples of *Tb*MCM-BP cKO tetracycline treated for 0, 1, or 2 days and Mann-Whitney U test for transcript levels of all PTU borders or borders that are replicating early comparing between samples.

Statistical analysis for all PTU borders (for Figure 6)		
Kruskal-Wallis One-Way Analysis of Variance Test		
Kruskal-Wallis chi-squared	32.683	
<i>P</i> -value	7.308e-08	
Mann-Whitney U Test		
	W statistic	<i>P</i> -value
Day 1 to Day 0	31193	0.2143
Day 2 to Day 0	37653	5.307e-08
Statistical analysis for early replicating origins (for Figure 6)		
Kruskal-Wallis One-Way Analysis of Variance Test		
Kruskal-Wallis chi-squared	13.328	
<i>P</i> -value	0.0009937	
Mann-Whitney U Test		
	W statistic	<i>P</i> -value
Day 1 to Day 0	867	0.5234
Day 2 to Day 0	1157	0.0004787

Supplementary Table S4. Expression levels of genes involved in DNA replication are not significantly changed in the absence of *Tb*MCM-BP.

Complex	Gene	Gene ID	DEseq day 0	DEseq day 2	Fold change	padj value
	MCM-BP	Tb927.7.1770	407.97	40.36	0.10	6.14E-177
ORC	ORC1	Tb927.11.7216	179.65	175.32	0.98	7.44E-01
	ORC1B	Tb927.9.2030	549.76	378.04	0.69	6.61E-14
	ORC4	Tb927.10.13380	815.90	832.68	1.02	6.92E-01
	Tb3120	Tb927.9.4530	1436.03	1531.23	1.07	8.86E-02
	Tb7980	Tb927.10.7980	575.44	664.12	1.15	2.24E-03
GINS	SLD5	Tb927.3.4810	204.72	338.06	1.65	8.11E-15
	PSF1	Tb927.9.4990	252.62	302.81	1.20	4.62E-03
	PSF2	Tb927.11.10460	411.74	434.31	1.05	4.10E-01
	PSF3	Tb927.4.4680	138.72	179.01	1.29	2.99E-03
	CDC45	Tb927.11.2710	505.16	369.51	0.73	1.17E-09
MCM	MCM2	Tb927.11.7860	1061.98	1180.73	1.11	7.53E-03
	MCM3	Tb927.2.3930	707.24	604.55	0.85	5.59E-04
	MCM4	Tb927.11.12250	828.56	937.94	1.13	1.90E-03
	MCM5	Tb927.11.5570	586.09	602.82	1.03	6.68E-01
	MCM6	Tb927.11.11640	1096.95	1160.78	1.06	2.93E-01
	MCM7	Tb927.11.16140	819.04	831.24	1.01	7.95E-01
	MCM8	Tb927.10.10410	684.79	661.24	0.97	4.97E-01
	RPA1	Tb927.11.9130	693.41	630.14	0.91	4.07E-02

Supplementary Table S5. DEseq analysis comparing VSG expression levels between day 0 and day 1 after *Tb*MCM-BP depletion. Increased VSGs (over 2-fold) with *P*adj values <0.01 and <0.1 are indicated by ** and *.

Type	ID	DEseq count day 0	DEseq count day 2	Fold change	padj value	
BES	VSG-1	41.71	27.13	0.65	2.87E-02	
BES	VSG-2	729332.60	507927.98	0.70	4.02E-04	
BES	VSG-3	15.63	78.06	5.00	1.63E-17	**
BES	VSG-4	1.11	1.92	1.72	7.05E-01	
BES	VSG-5	1.22	na	na	1.42E-01	
BES	VSG-6	28.87	385.59	13.36	3.52E-50	**
BES	VSG-7	0.28	0.43	1.51	1.00E+00	
BES	VSG-8	173.46	2435.38	14.04	1.86E-25	**
BES	VSG-9	33.21	53.41	1.61	2.41E-02	
BES	VSG-11	55.45	239.79	4.32	2.04E-26	**
BES	VSG-12	0.60	1.00	1.67	8.26E-01	
BES	VSG-13	12.89	75.64	5.87	9.58E-20	**
BES	VSG-14	245.93	429.49	1.75	7.47E-07	
BES	VSG-15	21.01	545.40	25.96	3.15E-57	**
BES	VSG-16	5.29	17.95	3.40	3.16E-04	**
BES	VSG-17	15.72	92.55	5.89	1.91E-22	**
BES	VSG-18	24.29	132.52	5.46	3.61E-26	**
BES	VSG-19	2.01	1.15	0.57	5.07E-01	
BES	VSG-21	16.02	29.07	1.81	6.76E-03	
BES	VSG-22	0.26	na	na	9.07E-01	
BES	VSG-31	na	27.13	0.65	1.00E+00	
MES	VSG-397	59.48	254.70	4.28	8.12E-16	**
MES	VSG-531	130.85	343.52	2.63	5.99E-14	**
MES	VSG-582	5.84	78.25	13.40	6.85E-28	**
MES	VSG-639	0.26	8.73	33.87	3.83E-06	**
MES	VSG-653	73.28	204.50	2.79	1.18E-14	**
MES	VSG-1954	53.35	615.22	11.53	2.33E-48	**
MC	VSG-23	0.28	0.38	1.33	1.00E+00	
MC	VSG-24	2.71	2.51	1.16	1.00E+00	
MC	VSG-25	na	1.38	na	1.27E-01	
MC	VSG-315	0.59	0.15	0.25	8.25E-01	
MC	VSG-365	0.62	0.58	0.93	1.00E+00	
MC	VSG-371	na	0.76	na	5.03E-01	
MC	VSG-374	na	0.49	na	7.50E-01	
MC	VSG-377	2.56	1.48	0.58	5.90E-01	
MC	VSG-416	2.10	2.82	1.34	7.75E-01	
MC	VSG-424	0.28	na	na	9.07E-01	
MC	VSG-430	1.11	0.15	0.13	3.36E-01	

MC	VSG-444	3.38	15.89	4.70	2.98E-05	**
MC	VSG-476	0.59	0.21	0.36	8.28E-01	
MC	VSG-503	na	0.50	na	7.46E-01	
MC	VSG-507	0.26	0.49	1.89	1.00E+00	
MC	VSG-514	4.68	42.72	9.13	3.54E-16	**
MC	VSG-542	0.26	1.29	5.02	3.29E-01	
MC	VSG-567	1.14	0.78	0.68	7.38E-01	
MC	VSG-575	0.28	0.30	1.04	1.00E+00	
MC	VSG-600	0.28	na	na	9.07E-01	
MC	VSG-618	0.62	0.36	0.58	1.00E+00	
MC	VSG-620	0.26	0.42	1.63	1.00E+00	
MC	VSG-622	0.31	na	na	9.07E-01	
MC	VSG-629	0.31	0.21	0.69	1.00E+00	
MC	VSG-634	na	0.30	na	8.14E-01	
MC	VSG-637	0.31	0.36	1.17	1.00E+00	
MC	VSG-643	0.88	0.55	0.62	7.95E-01	
MC	VSG-645	0.90	0.27	0.30	5.29E-01	
MC	VSG-646	0.91	0.15	0.16	5.31E-01	
MC	VSG-647	0.26	na	na	9.07E-01	
MC	VSG-649	0.62	na	na	4.90E-01	
MC	VSG-663	0.31	0.49	1.56	1.00E+00	
MC	VSG-666	1.16	0.64	0.55	7.35E-01	
MC	VSG-671	0.88	na	na	2.67E-01	
MC	VSG-672	0.62	0.86	1.40	1.00E+00	
MC	VSG-717	0.26	0.46	1.77	1.00E+00	
MC	VSG-1963	1.40	0.55	0.39	3.58E-01	
MC	VSG-2075	1.79	2.59	1.44	7.18E-01	
MC	VSG-2114	0.88	0.36	0.41	7.73E-01	
MC	VSG-2348	0.62	0.58	0.93	1.00E+00	
MC	VSG-3338	0.52	0.15	0.29	8.30E-01	

Supplementary Table S6. DEseq analysis comparing VSG expression levels between day 0 and day 2 after *Tb*MCM-BP depletion. Increased VSGs (over 2-fold) with *P*adj values <0.01 and <0.1 are indicated by ** and *. Decreased VSGs (over 2-fold) with *P*adj values <0.01 are indicated by ##.

Type	ID	DEseq count day 0	DEseq count day 2	Fold change	padj value	
BES	VSG-1	50.04	16.47	0.33	9.99E-08	##
BES	VSG-2	873516.06	262904.38	0.30	5.13E-31	##
BES	VSG-3	18.71	181.55	9.70	9.21E-40	**
BES	VSG-4	1.33	3.37	2.54	2.96E-01	
BES	VSG-5	1.48	3.42	2.31	2.82E-01	
BES	VSG-6	34.63	346.09	10.00	3.67E-56	**
BES	VSG-7	0.33	1.45	4.36	4.38E-01	
BES	VSG-8	208.26	1609.04	7.73	5.31E-23	**
BES	VSG-9	39.76	126.31	3.18	2.24E-13	**
BES	VSG-11	66.68	447.58	6.71	7.16E-39	**
BES	VSG-12	0.72	1.99	2.76	4.21E-01	
BES	VSG-13	15.51	111.44	7.19	6.69E-26	**
BES	VSG-14	295.30	655.88	2.22	1.17E-12	**
BES	VSG-15	25.31	662.08	26.16	2.80E-43	**
BES	VSG-16	6.39	60.19	9.42	1.27E-19	**
BES	VSG-17	18.89	130.82	6.93	2.61E-27	**
BES	VSG-18	29.17	193.20	6.62	4.50E-33	**
BES	VSG-19	2.40	1.53	0.64	6.56E-01	
BES	VSG-21	19.26	55.82	2.90	1.18E-07	**
BES	VSG-22	0.30	0.15	0.48	1.00E+00	
BES	VSG-31	1.00	0.44	0.44	7.74E-01	
MES	VSG-397	71.25	638.78	8.97	1.88E-64	**
MES	VSG-531	157.29	695.18	4.42	9.70E-35	**
MES	VSG-582	6.94	71.29	10.27	4.19E-22	**
MES	VSG-639	0.30	20.83	68.83	3.82E-12	**
MES	VSG-653	87.82	440.02	5.01	2.21E-36	**
MES	VSG-1954	64.20	973.12	15.16	2.85E-59	**
MC	VSG-23	0.33	0.36	1.09	1.00E+00	
MC	VSG-24	2.92	2.99	0.95	1.00E+00	
MC	VSG-25	1.00	2.52	2.52	3.90E-02	*
MC	VSG-315	0.71	0.61	0.86	1.00E+00	
MC	VSG-365	0.76	1.65	2.17	5.83E-01	
MC	VSG-371	1.00	2.21	2.21	5.58E-02	*
MC	VSG-374	1.00	1.26	1.26	2.52E-01	
MC	VSG-377	3.04	3.11	1.02	1.00E+00	
MC	VSG-416	2.56	1.38	0.54	5.66E-01	
MC	VSG-424	0.33	1.72	5.17	3.02E-01	

MC	VSG-430	1.31	0.83	0.63	7.88E-01	
MC	VSG-444	4.02	23.34	5.80	4.39E-07	**
MC	VSG-476	0.71	0.34	0.48	8.92E-01	
MC	VSG-503	1.00	2.16	2.16	5.77E-02	*
MC	VSG-507	0.30	0.70	2.33	9.57E-01	
MC	VSG-514	5.56	60.38	10.86	5.96E-20	**
MC	VSG-542	0.30	1.24	4.09	5.47E-01	
MC	VSG-567	1.38	1.46	1.06	1.00E+00	
MC	VSG-575	0.33	1.24	3.71	5.63E-01	
MC	VSG-600	0.33	0.58	1.75	1.00E+00	
MC	VSG-618	0.76	2.18	2.87	3.48E-01	
MC	VSG-620	0.30	1.43	4.73	4.46E-01	
MC	VSG-622	0.39	0.49	1.25	1.00E+00	
MC	VSG-629	0.37	1.00	2.69	8.06E-01	
MC	VSG-634	1.00	1.45	1.45	1.91E-01	
MC	VSG-637	0.37	1.31	3.53	4.55E-01	
MC	VSG-643	1.08	0.90	0.83	1.00E+00	
MC	VSG-645	1.08	1.07	0.99	1.00E+00	
MC	VSG-646	1.11	0.51	0.46	7.60E-01	
MC	VSG-647	0.30	1.04	3.45	6.66E-01	
MC	VSG-649	0.76	0.36	0.48	9.01E-01	
MC	VSG-663	0.39	0.56	1.44	1.00E+00	
MC	VSG-666	1.38	0.70	0.51	6.56E-01	
MC	VSG-671	1.05	1.07	1.01	1.00E+00	
MC	VSG-672	0.74	3.76	5.05	6.37E-02	*
MC	VSG-717	0.30	0.68	2.25	9.62E-01	
MC	VSG-1963	1.66	3.04	1.83	5.01E-01	
MC	VSG-2075	2.16	4.56	2.11	2.37E-01	
MC	VSG-2114	1.05	0.58	0.56	7.60E-01	
MC	VSG-2348	0.76	2.60	3.42	2.09E-01	
MC	VSG-3338	0.61	0.34	0.56	8.79E-01	