

Supplementary Data

Table S1, related to Experimental Procedures

Oligonucleotides used in this study.

DNA	Sequences
ssDNA	5' T (TTT) ₂₆ T 3' (80dT) or 5' ATGTCCTAGCAAGCCAGAATTCGGC 3' (25 nt)
blunt	5' ATGTCCTAGCAAGCCAGAATTCGGCAGCGTC 3' 5' GACGCTGCCGAATTCTGGCTTGCTAGGACAT 3'
bubble	5' ATGTCCTAGCAAGCCAGAATTCGGCAGCGTCTT(TTT) ₂₆ CCACGTCGGCGTCGCCACGAGC 3' 5' GCTCGTGGCGACGCCGACGTGGTT(TTT) ₂₆ GACGCTGCCGAATTCTGGCTTGCTAGGACAT 3'

Table S2, related to Experimental Procedures

Yeast strains used in this study.

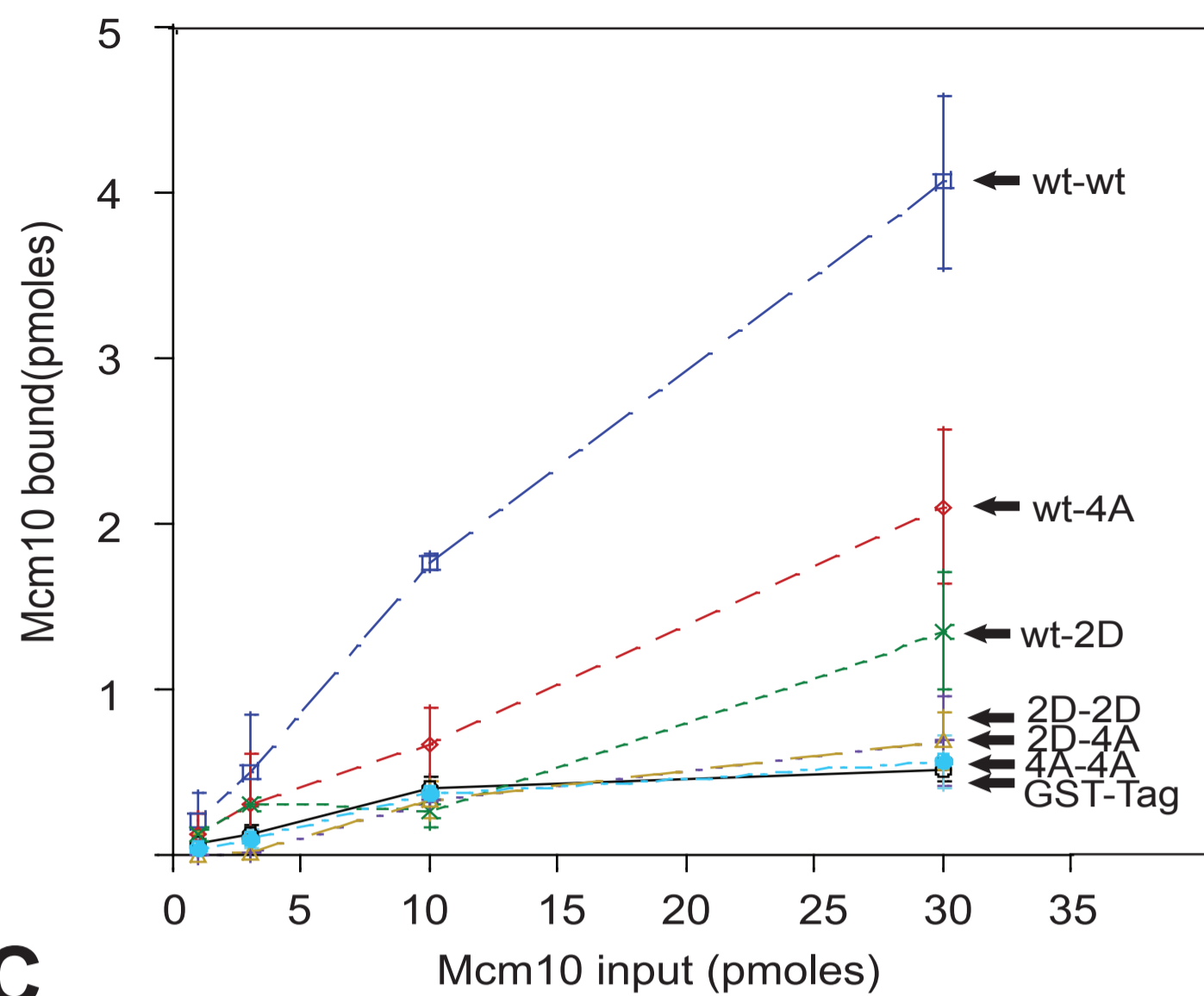
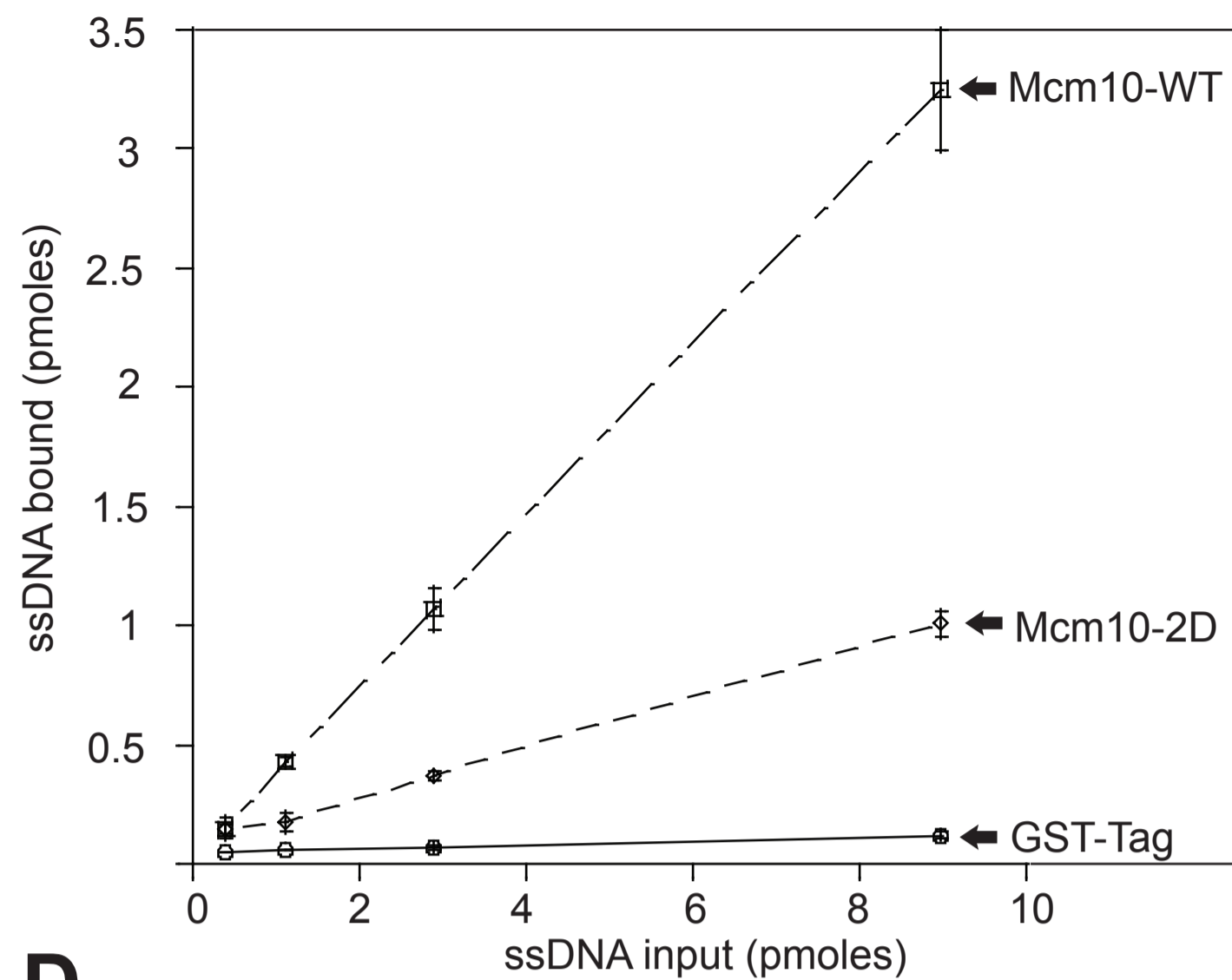
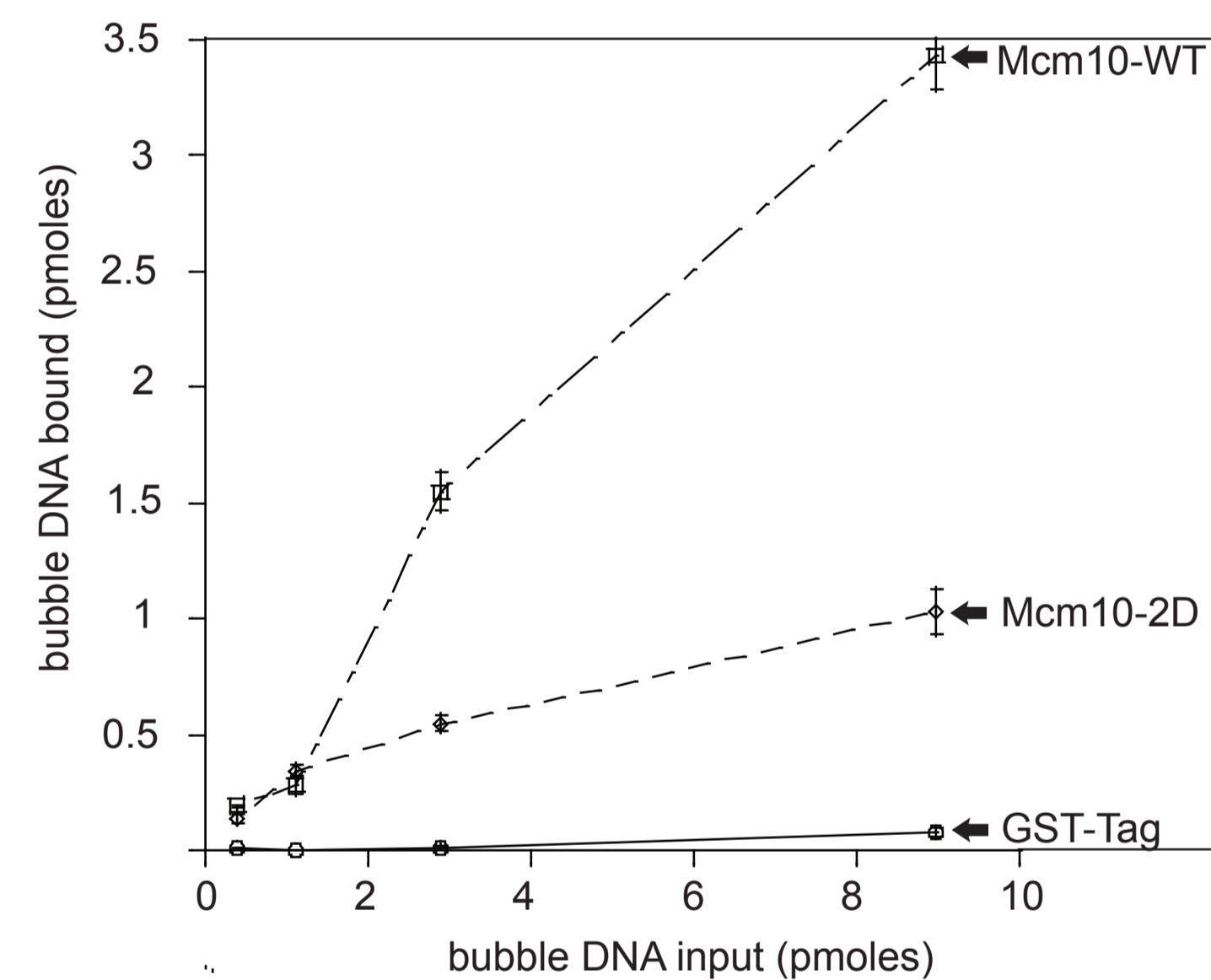
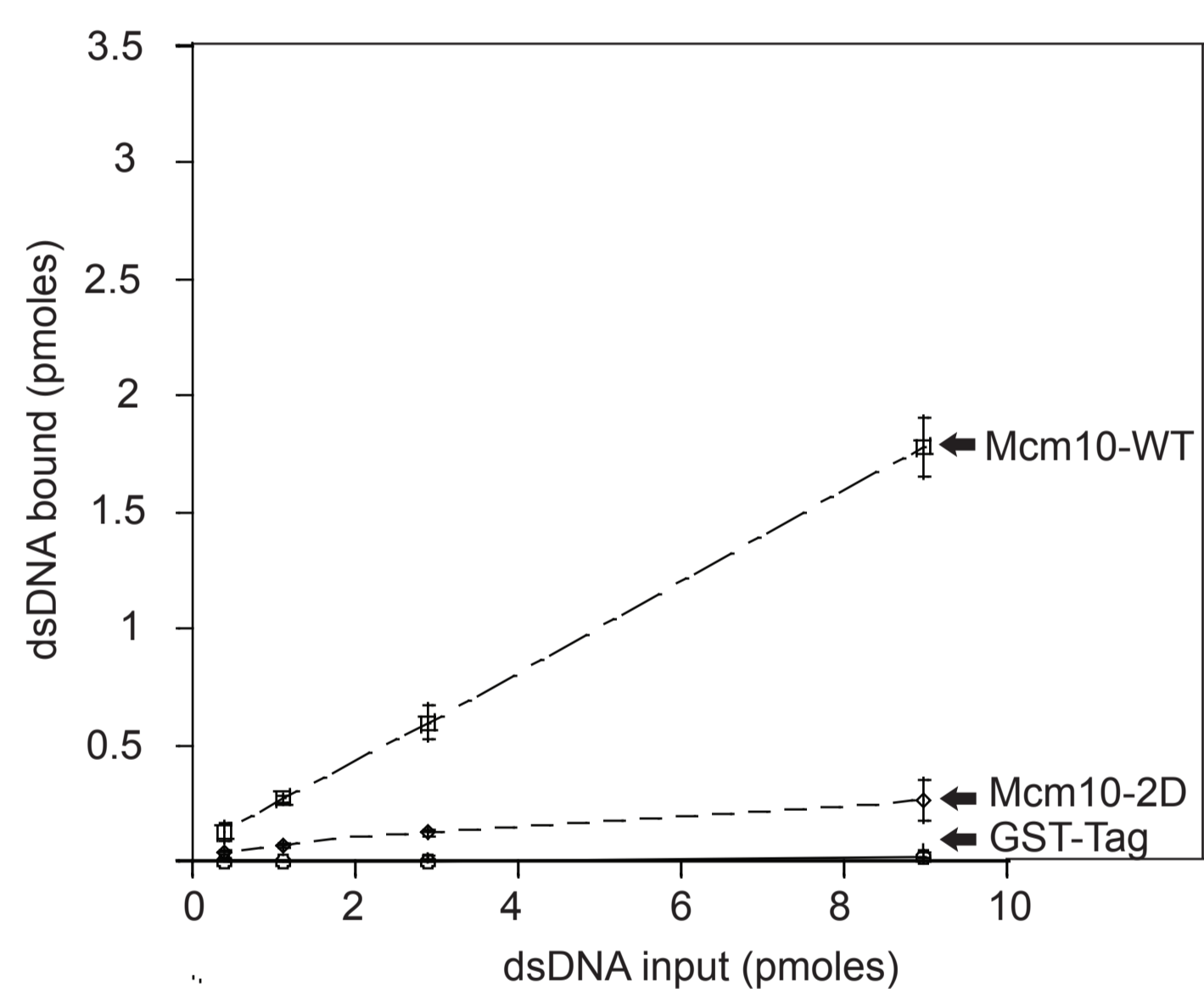
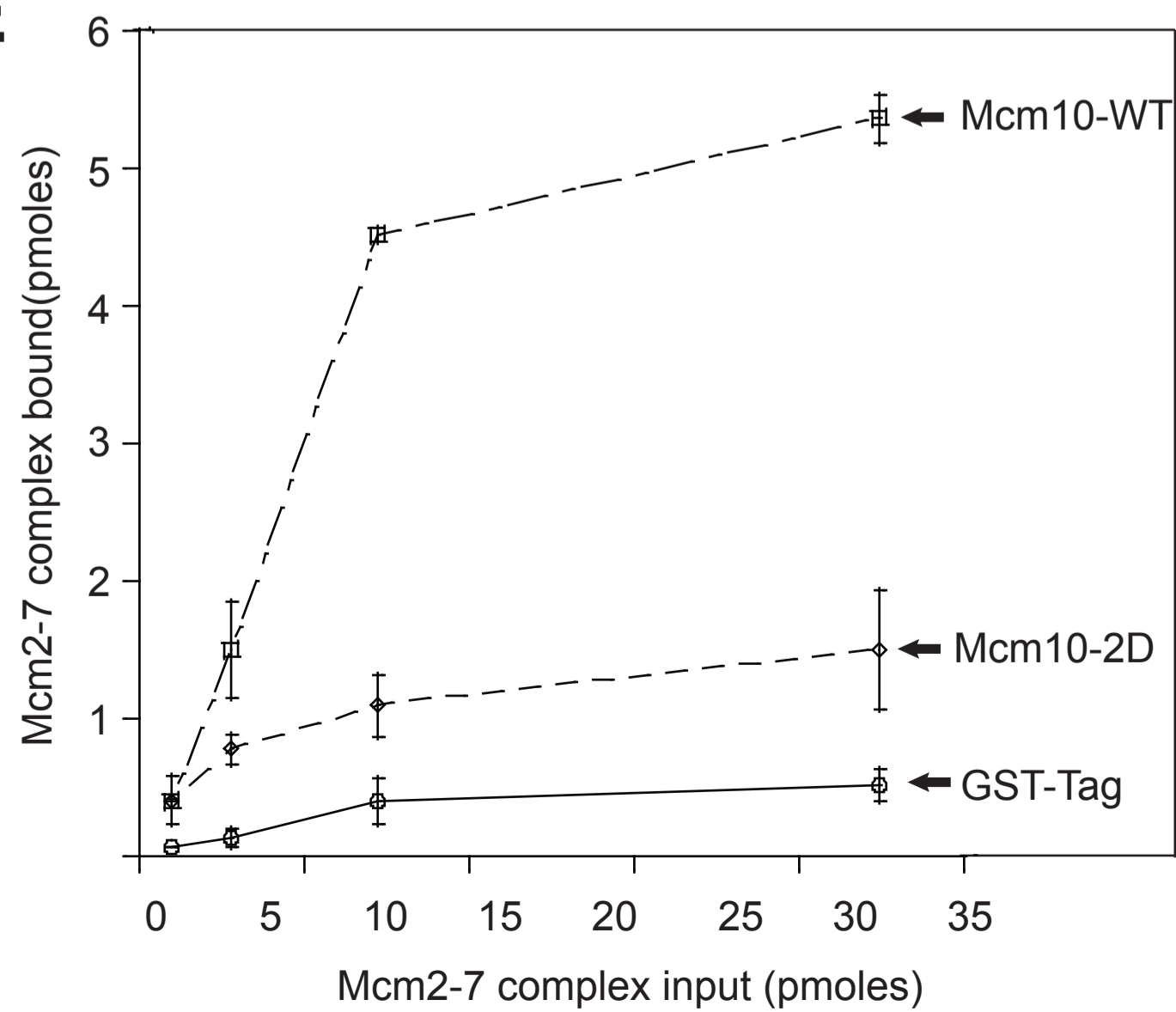
Strain	Genotype	Source
BY25924	<i>MATa ura 3-1::GAL-OsTIR1-9MYC(URA3) mcm10::mcm10-1-aid (KanMX) cdc45::CDC45-5FLAG (hphNT) ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100</i>	Yeast Genetic Resource Center
BY25925	<i>MATa ura 3-1::GAL-OsTIR1-9MYC(URA3) mcm10::mcm10-1-aid (KanMX) psf2::PSF2-5FLAG (hphNT) MCM4::mcm4-6ha (k.l. TRP1) ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100</i>	Yeast Genetic Resource Center
BY25926	<i>MATa ura 3-1::GAL-OsTIR1-9MYC(URA3) mcm10::mcm10-1-aid (KanMX) rfa1::RFA1-5FLAG (HIS3) ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100</i>	Yeast Genetic Resource Center
RSY728	<i>MATa bar1 his6 mcm5-bob1-1 leu2 ura3 lys2 his3-Δ1 cyh2</i>	Sclafani lab (Sclafani, R.A. <i>et al.</i> 2002 Genetics)
PP3278	<i>MATa ura 3-1::GAL-OsTIR1-9MYC(URA3) mcm10::mcm10-1-aid (KanMX) mcm5::mcm5-bob1-1 ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100</i>	Perez-Arnaiz 2016

Figure S1. Mcm10-2D is defective in self-interaction and in DNA, Mcm2-7 and Mcm2 in binding *in vitro*. **A.** 30 pmoles of *wild-type* GST-Mcm10, GST-Mcm10-2D, GST-Mcm10-4A or GST tag was incubated with increasing concentrations of radiolabeled *wild-type* PKA-Mcm10, PKA-Mcm10-2D or PKA-Mcm10-4A at 30°C for 10 minutes in a GST pulldown assay. The products of the pulldown were analyzed by SDS-PAGE followed by phosphorimaging. Results from similar experiments were quantified, averaged and plotted. **B.** 30 pmoles of *wild-type* GST-Mcm10, GST-Mcm10-2D or GST tag was incubated with increasing concentrations of radiolabeled ssDNA at 30°C for 10 minutes in a GST pulldown assay. The products of the pulldown were analyzed by SDS-PAGE followed by phosphorimaging. Results from similar experiments were quantified, averaged and plotted. **C.** 30 pmoles of *wild-type* GST-Mcm10, GST-Mcm10-2D or GST tag was incubated with increasing concentrations of radiolabeled bubble DNA at 30°C for 10 minutes in a GST pulldown assay. The products of the pulldown were analyzed by SDS-PAGE followed by phosphorimaging. Results from similar experiments were quantified, averaged and plotted. **D.** 30 pmoles of *wild-type* GST-Mcm10, GST-Mcm10-2D or GST tag was incubated with increasing concentrations of radiolabeled dsDNA at 30°C for 10 minutes in a GST pulldown assay. The products of the pulldown were analyzed by SDS-PAGE followed by phosphorimaging. Results from similar experiments were quantified, averaged and plotted. **E.** 30 pmoles of *wild-type* GST-Mcm10, GST-Mcm10-2D or GST tag was incubated with increasing concentrations of radiolabeled PKA-Mcm2-7 at 30°C for 10 minutes in a GST pulldown assay. The products of the pulldown were analyzed by SDS-PAGE followed by phosphorimaging. Results from similar experiments were quantified, averaged and plotted. Graphs from (A), (B), (C), (D), and (E) represent mean values from two independent experiments and error bars indicate the standard deviation of the mean.

Figure S2. Plasmid copy of *mcm10* from GalS promoter is expressed at *wild-type* levels. **A.** *mcm10-1-aid* cells (in the absence of IAA) and *mcm10-1-aid* cells expressing *MCM10-WT* under the control of the GalS promoter (in the presence of IAA and Gal) were grown as described in Material and Methods. Whole cell extracts were analyzed by Western blot for the expression of Mcm10. Results from similar experiments were quantified, averaged and plotted. Graph represents mean values from two independent experiments and error bars indicate the standard deviation of the mean.

Figure S3. The presence of *mcm5-bob1* mutation partially suppresses the growth defect observed in cells expressing *mcm2-2A*. **A.** 10-fold serial dilution analysis of budding yeast *mcm10-1-aid* cells expressing *MCM2-WT* and *mcm2-2A* from the GAL-S plasmid inducible promoter system (pRS415). Plates were incubated for 3 days at 25°C. **B.** similar to A, except the cells harbored the *mcm5-bob1* mutation.

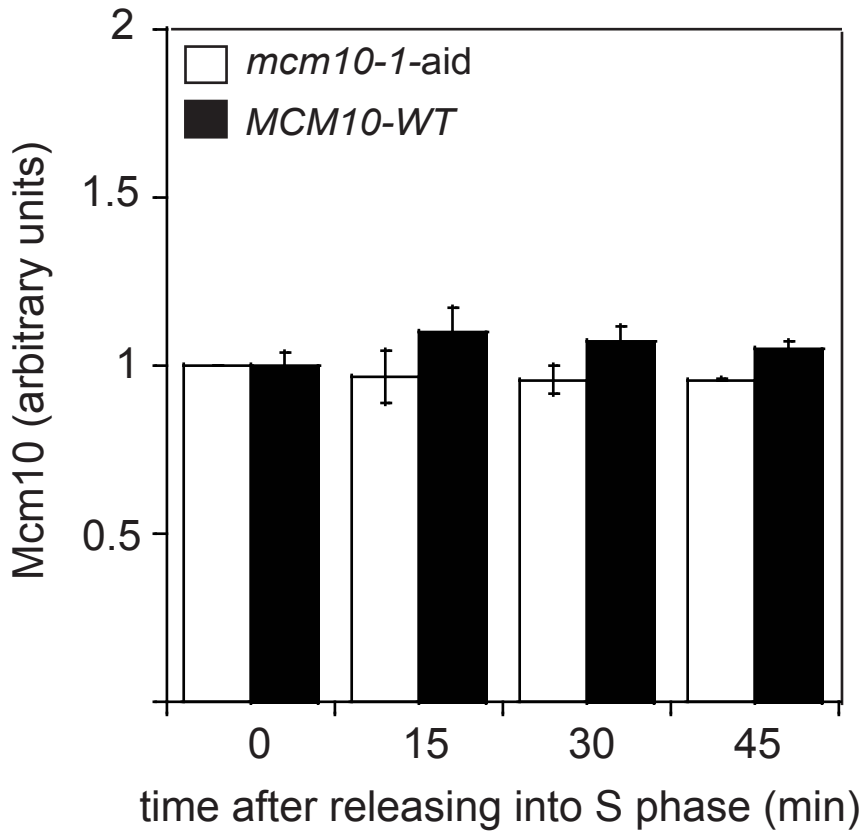
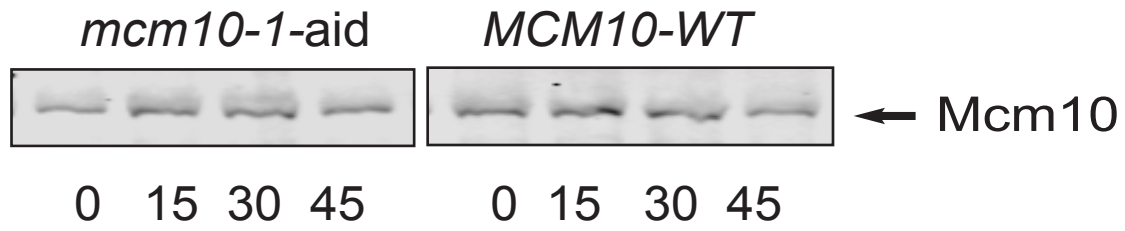
Figure S4. An intact Mcm10 coiled-coil interaction surface is important for cell growth. 10-fold serial dilution analysis of budding yeast *mcm10-1-aid* cells expressing *MCM10-WT*, *mcm10-4A*, vector, or *mcm10-D1-150*. *MCM10* was expressed from either the GAL-S plasmid inducible promoter system (pRS415) or from *MCM10* native promoter. Plates were incubated for 3 days at 25°C.

A**B****C****D****E**

Perez-Arnaiz Figure S2

A

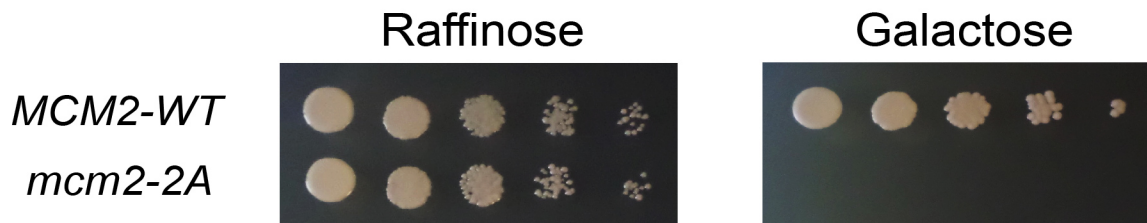
Whole Cell Extracts



Perez-Arnaiz Figure S3

A

mcm10-1-aid MCM5-WT background



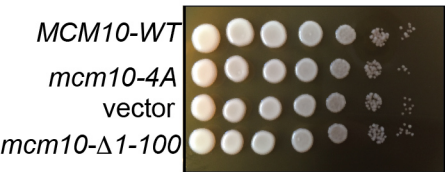
B

mcm10-1-aid mcm5-bob1 background

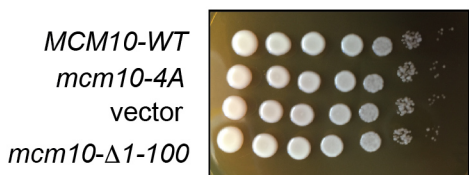


Perez-Arnaiz Figure S4

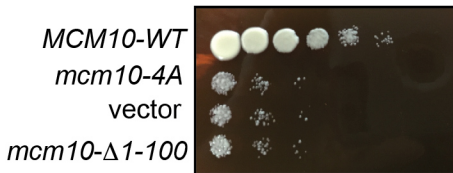
Permissive Conditions
Gal Promoter



Permissive Conditions
Native Promoter



Restrictive Conditions
Gal Promoter



Restrictive Conditions
Native Promoter

