SUPPLEMENTARY MATERIAL, Kramara *et al.*

Supplementary Table 1. List of oligonucleotides

Name	Sequence (5‘®3‘)	Application*
10923_InFus_N	TAAGGCCTCTGTCGACTCTCACTTTGGCGATCACGTGGGC	1
10923_Ant_InFus_N	CAGAATTCGCAAGCTTCTACTTTTGATAGTAGTAATTGTTG	1
YlTEL-1x	TTAGTCAGGG	2
YlTEL-1x-C	CCCTGACTAA	2
YlTEL-1.5x	TTAGTCAGGGTTAGT	2
YlTEL-1.5x-C	ACTAACCCTGACTAA	2
YlTEL-2x	TTAGTCAGGGTTAGTCAGGG	2
YlTEL-2x-C	TTAGTCAGGGTTAGTCAGGG	2
YlTEL-3x	TTAGTCAGGGTTAGTCAGGGTTAGTCAGGG	2
YlTEL-3x-C	TTAGTCAGGGTTAGTCAGGGTTAGTCAGGG	2
YlTEL-8REP-Sense	CCCCGAATTC-$(TGACTAACCC)_8$-GGATCCCCCC	2
YlTEL-8REP-Anti	GGGGGGATCC-$(GGGTTAGTCA)_8$-GAATTCGGGG	2
YlTEL-3REP-Sense	CCCC-$(TGACTAACCC)_3$-CCCC	2
YlTEL-3REP-Anti	GGGG-$(GGGTTAGTCA)_3$-GGGG	2
YlTEL_EMSA-C_Sense	CCCCCAGTCGGTTTCAGTCGGTTTCAGTCGGTTTCCCC	2
YlTEL_EMSA-C_Anti	GGGGAAACCGACTGAAACCGACTGAAACCGACTGGGGG	2
YlTEL_EMSA-1_Sense	CCCCTGACTAACCCTGACTAACCCTGACTAACCCCCCC	2
YlTEL_EMSA-1_Anti	GGGGGGGTTAGTCAGGGTTAGTCAGGGTTAGTCAGGGG	2
YlTEL_EMSA-M5_Sense	CCCCACACATTCCCACACATTCCCACACATTCCCCCC	2
YlTEL_EMSA-M5_Anti	GGGGGGGAATGTGTGGGAATGTGTGGGAATGTGTGGGG	2
YlTEL_EMSA-M6_Sense	CCCCTGTATAACAATGTATAACAATGTATAACCCCCCC	2
YlTEL_EMSA-M6_Anti	GGGGGGGTTATACATTGTTATACATTGTTATACAGGGG	2
YLTEL3’ovhng44nt	Phos GTTAGTCAGGGTTAGTCAGGGTTAGTCAGGGTTAGTCAGGGTTAGTCA	3
YlTEL-Sense	GGGGAATTCGGGTTAGTCAGGGTTAGTCAGGGTTAGTCAGGGTTAGTCAGGGTTAGTCAGAATTCCCC	4
YlTEL-Antisense	GGGGAATTCTGACTAACCCTGACTAACCCTGACTAACCCTGACTAACCCTGACTAACCCGAATTCCCC	4
YlModelTEL_A	CCCAAGCTTACCTGCTGACTAACCCTGACTAACCCTGACTAACCCTGACTAACCCTGACTAACCCTGACT AACCCTGACTAACCCTGACTAACCCTGACTAACCCGTCTTCGGATCCCCC	5
YlModelTEL_S2	GGGGGATCCGAAGACGGGTTAGTCAGGGTTAGTCAGGGTTAGTCAGGGTTAGTCAGGGTTAGTCAGGGT TAGTCAGGGTTAGTCAGGGTTAGTCAGGGTTAGTCAGCAGGTAAGCTTGGG	5
3xHA-Sense	ACCAAAATGTACCCATACGATGTTCCTGACTATGCGGGCTATCCCTATGACGTCCCGGACTATGCAGGAT ATCCATATGACGTTCCAGATTACGCTGCT	6
3xHA-Antisense	PhosAGCAGCGTAATCTGGAACGTCATATGGATATCCTGCATAGTCCGGGACGTCATAGGGATAGCCCGCA TAGTCAGGAACATCGTATGGGTACATTTTGGT	6
HA-Amp-S	PhosACCAAAATGTACCCATACGATG	6
Tay1+ATG+Kozak-Sense	PhosACCAAAATGTCTCACTTTGGCGATCACGTG	6
Tay1+STOP-Antisense	PhosCTACTTTTGATAGTAGTAATTGTTGTAGC	6
Tay1_1H_5’P_S	PhosTCTCACTTTGGCGATCACGTGGGC	6,7,9

Supplementary Table 1. List of oligonucleotides (*continued*)		
Tay1_1H_5'P_Anti	PhosCTACTTTTGATAGTAGTAATTGTTGTAGC	6,7
Tay1+ATG+Kozak-S	PhosACCAAAATGTCTCACTTTGGCGATCACGTG	6
YlTEL-4x	TTAGTCAGGGTTAGTCAGGGTTAGTCAGGGTTAGTCAGGG	8
YARLI-T1-4x	GTCAGTCAGTGTCAGTCAGTGTCAGTCAGTGTCAGTCAGT	8
YARLI-T2-4x	TGTGTTTGTGTGTGTTTGTGTGTGTTTGTGTGTGTTTGTG	8
YARLI-T3-4x	GTCTGTGATTGTCTGTGATTGTCTGTGATTGTCTGTGATT	8
Tay1_3utr	TCGACTCTGAGTCTTCCGAGTGGCT	9

*1, InFusion cloning; 2, EMSA; 3, telomeric overhang ligated to the model telomere; 4, construction of pMH25; 5, construction of model telomeres (pYlTEL41 and pYlTEL81); 6, construction of *Y. lipolytica* pINA1312-derived vectors expressing *YlTAY1* with or without 3xHA epitope at the N-terminus; 7, amplification of the *TAY1-YlURA3* discruption cassette; 8, ChIP dot blot; YlTEL-4x contains four Y. lipolytica telomeric repeats; the oligonucleotides used for negative control (YARLI-T1-4x, YARLI-T2-4x, and YARLI-T3-4x) are derived from three independent repetitive regions. Each oligonucleotide contains 4 tandem repeats, whose length (10 bp) and base composition (40-50% of GC-pairs) are similar to the *Y. lipolytica* telomeric repeat. 9, PCR verification of the replacement of *TAY1* gene by the disruption cassette

Supplementary Figure 1. The Myb/SANT domains identified in yeast telomeric DNA-binding proteins Tay1/Mug152, Taz1, and Tbf1 belong to distinct clusters. Unrooted dendrogram was calculated from the sequence alignment shown in Fig. 1B using the MEGA4 package by the neighbor-joining algorithm (1).

Supplementary Figure 2. *Yarrowia lipolytica* model telomere. **(A)** The map of pMH25 used for both EMSA and electron microscopy. The positions of restriction sites used for preparation of various DNA substrates are indicated. **(B)** Plasmid pYlTEL81 carrying 81 *Y. lipolytica* telomeric repeats was constructed as described in Experimental procedures. For the generation of the model telomere the plasmid was digested with *Bfu*AI, leaving the entire telomeric tract at one end of the linear molecule. A 3'-single-stranded telomeric overhang was created by ligation of an oligonucleotide, whose four 5' end bases are complementary to the 5' overhang produced by the *Bfu*AI digestion.

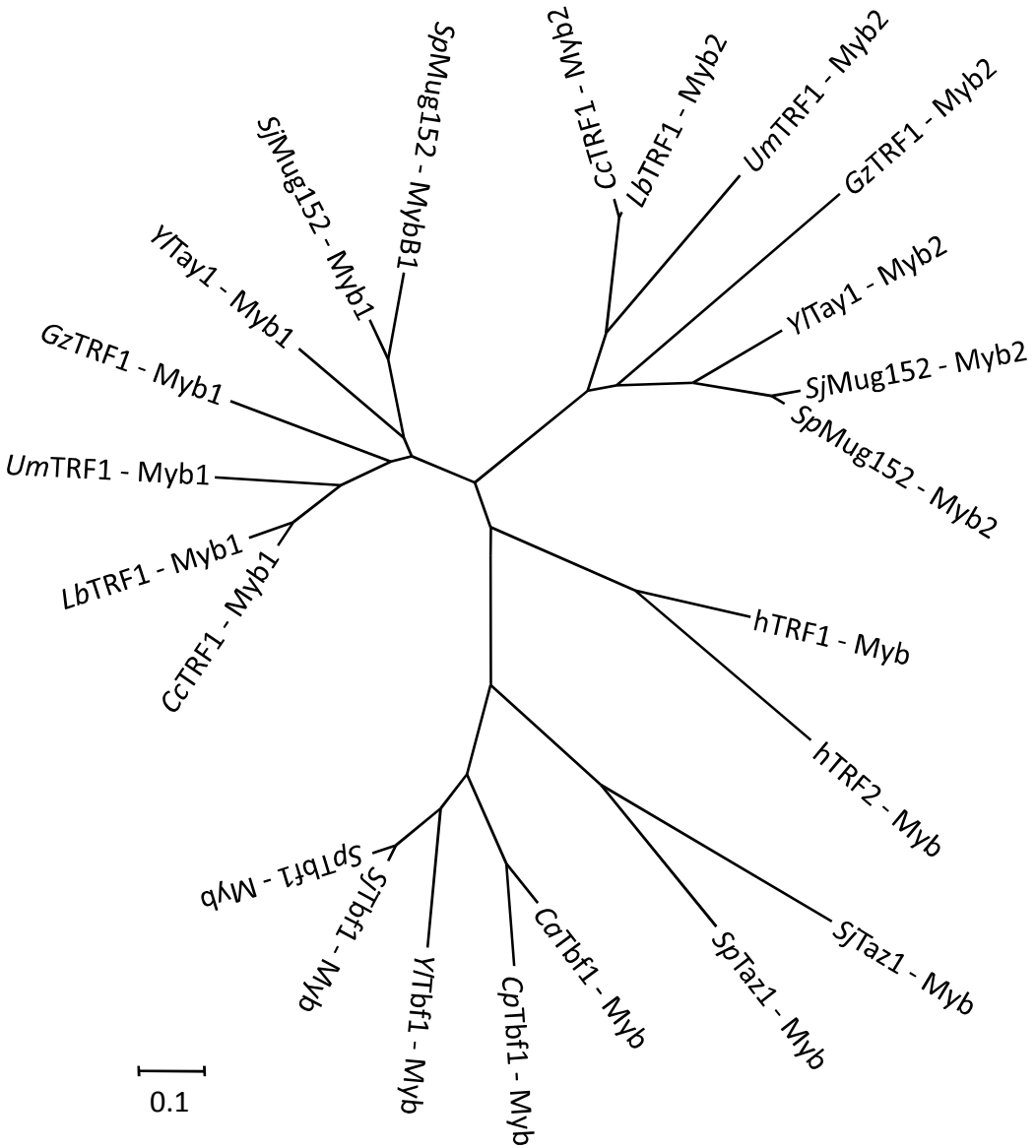
Supplementary Figure 3. Visualization of Tay1p binding to a model *Y. lipolytica* telomere. A model telomere DNA containing 810 bp of *Y. lipolytica* repeats at one end (text, Methods and Materials) was complexed with Tay1p at a ratio of 2.3 micrograms of protein per microgram of DNA and prepared for EM as described in Supplementary Fig. 1. Shown in reverse contrast. **A** and **B**, and **C** and **D** are at the same magnifications.

Supplementary Figure 4. Visualization of Tay1p binding to relaxed circular and supertwisted DNAs containing Tay1p binding sites. A preparation of the plasmid pYlTEL81 containing an 810 bp insert of *Y. lipotytica* telomeric DNA repeats was found to contain an equal number of monomer **(A, C)** and dimer **(B, D)** circles, and both in topologically relaxed **(A, B)** and supertwisted **(C, D)** states. This DNA was complexed with Tay1p at a ratio of 2.3 micrograms of protein per microgram of DNA and the sample prepared for EM as described in the text and legend for Supplementary Fig. 1. The binding of Tay1p is localized at either a single site **(A, C)** or two sites polar to each other in the dimer circles **(B, D)**. Shown in reverse contrast. Bar equals 200 nm for A-D.

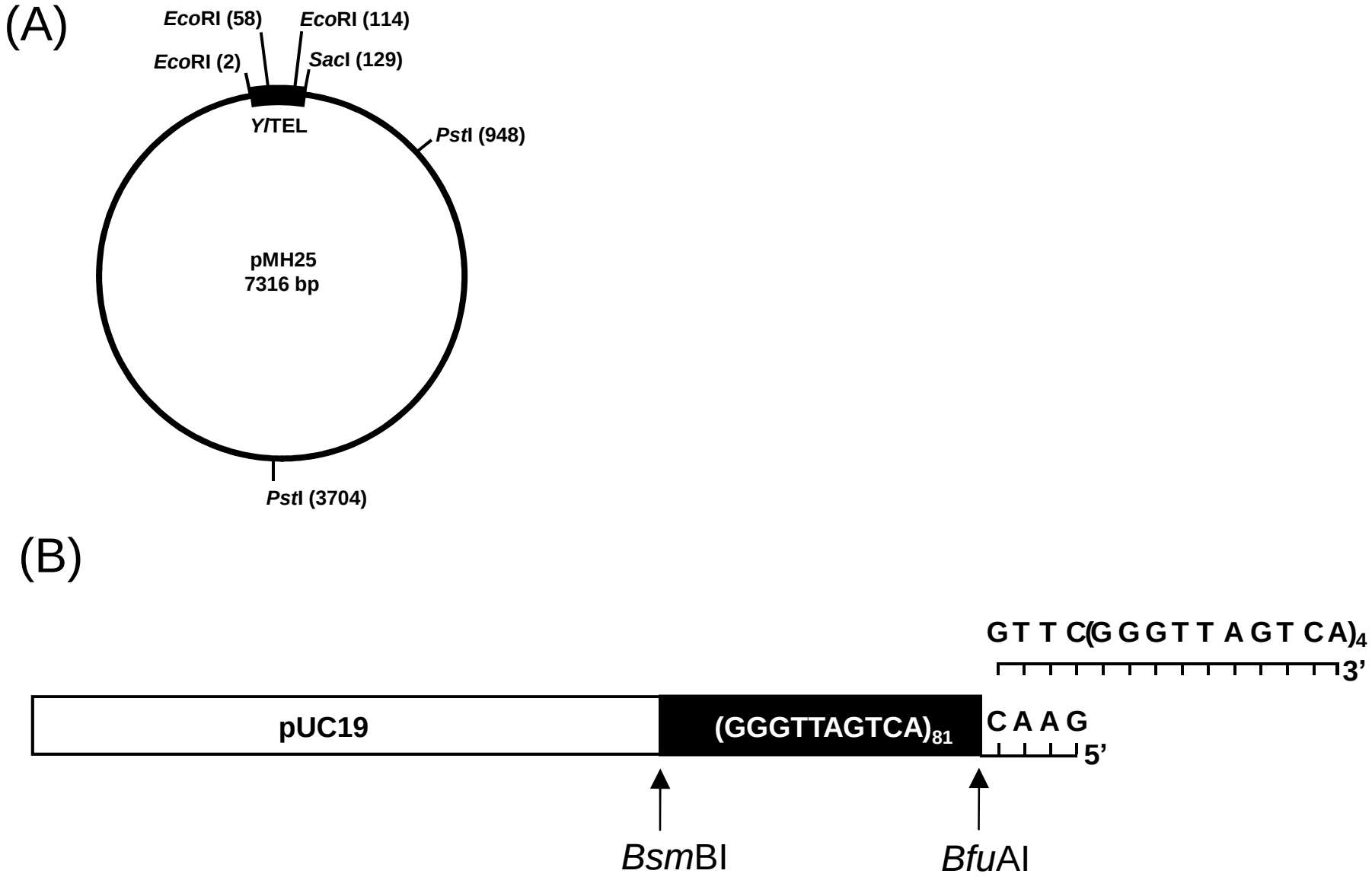
Supplementary Figure 5. Examples of telomere loops generated by Tay1p binding to a model *Y. lipolytica* model telomere. A model *Y. lipolytica* telomere containing 810 bp of telomeric repeats terminated with a 44 nt 3' ssDNA overhang on the G-rich strand was incubated with Tay1p at a ratio of 2.3 micrograms per microgram of DNA and prepared for EM as in Supplementary Fig. 1. **A-D** show the full molecules and **E-H** are enlargements. **H** is an enlargement of the molecule in **A**, **F** an enlargement of the molecule in **B**, and **G** is an enlargement of the molecule in **C**. The telomeric tracts in **A** and **D** (enlarged in **H**) are fully bound by Tay1p while the telomeric tracts in the other examples are only partially covered, but in each case exhibit protein at the base of the loop in the DNA. Shown in reverse contrast. Bar in **C** equals 200 nm for **A-D**, and the bar in **G** equals 100 nm for **E-H**.

REFERENCE

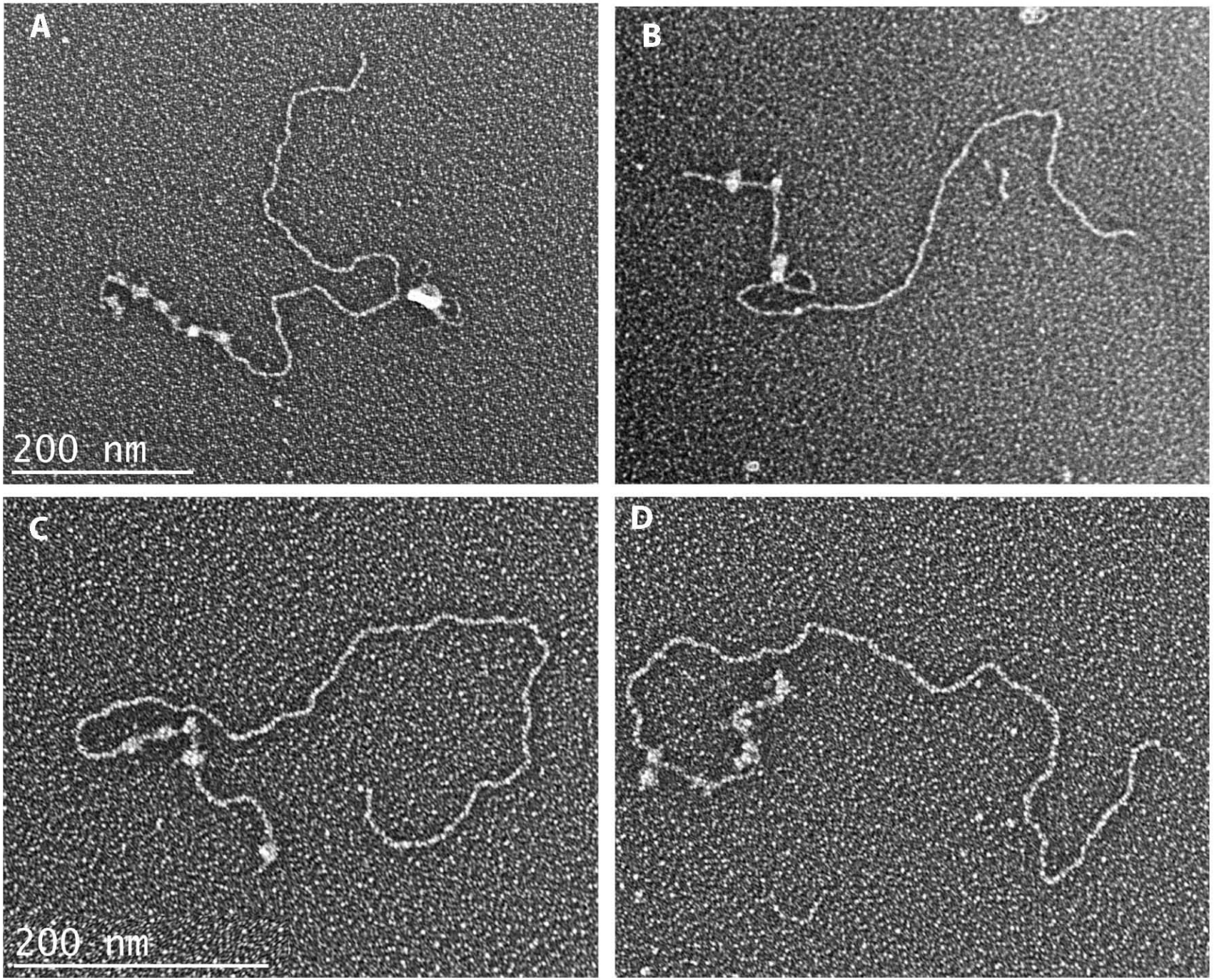
1. Tamura, K., Dudley, J., Nei, M., and Kumar, S. (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol. Biol. Evol.* **24,** 1596-1599



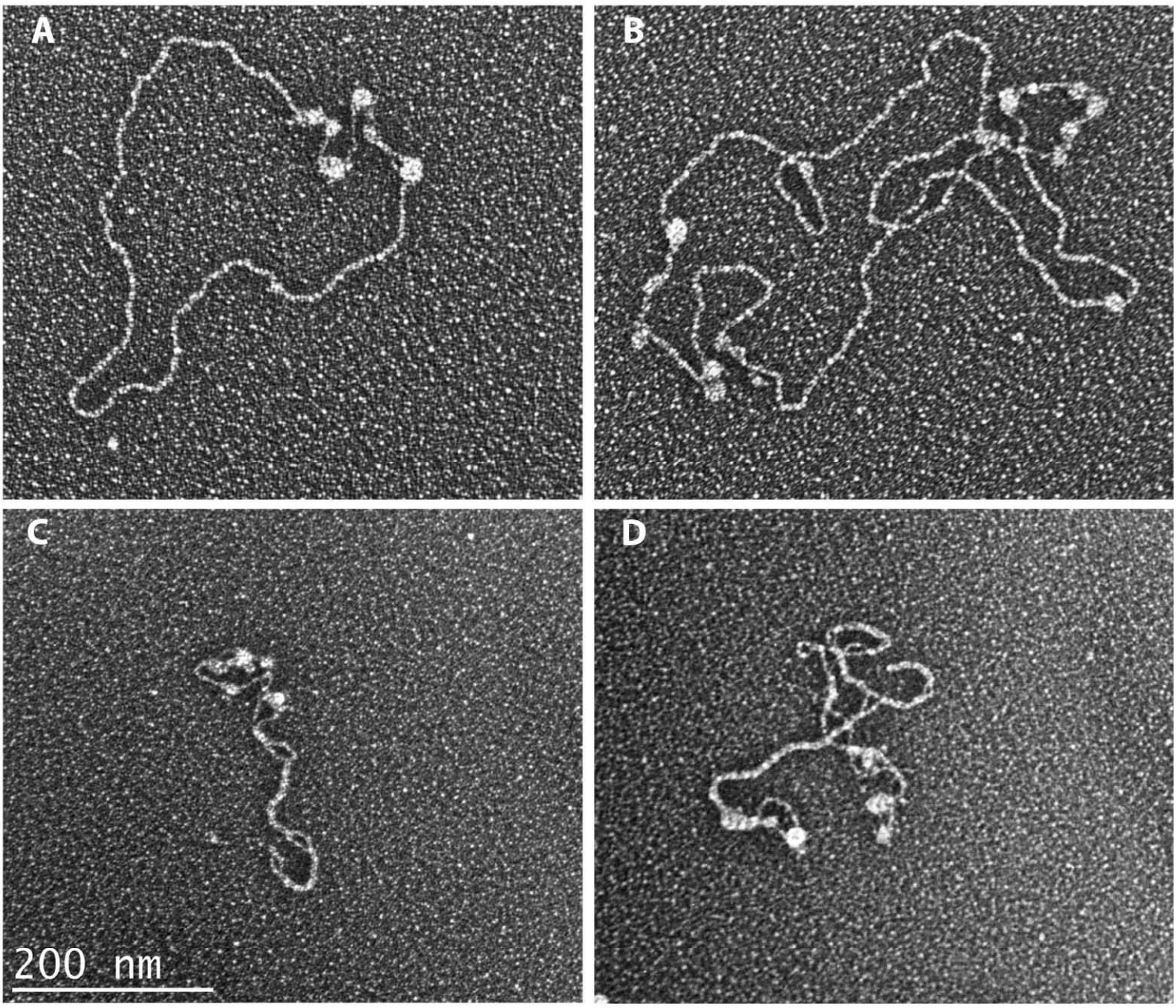
Supplementary Figure 1. Kramara *et al.*



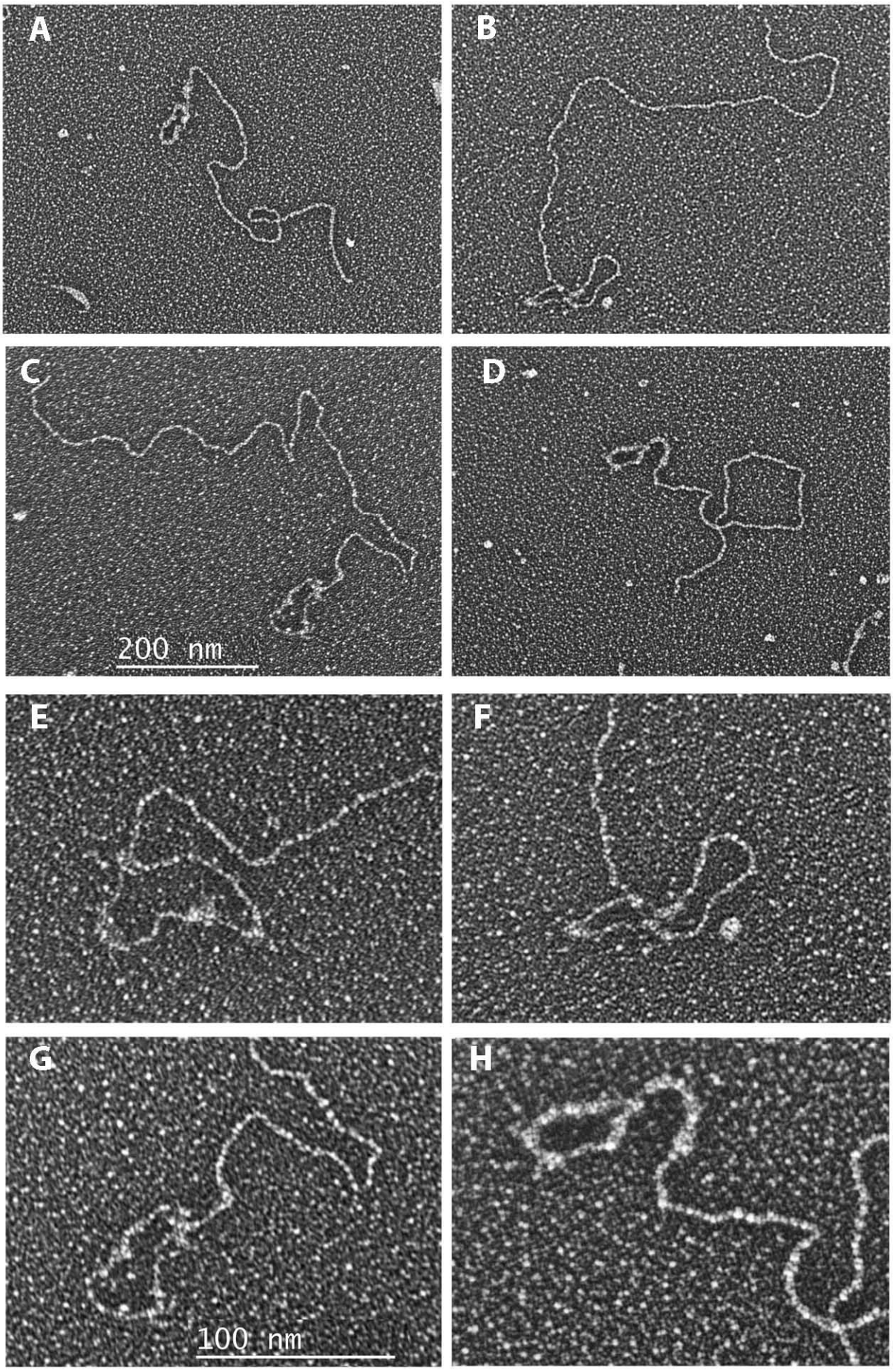
Supplementary Figure 2. Kramara *et al.*



Supplementary Figure 3. Kramara *et al.*



Supplementary Figure 4. Kramara *et al.*



Supplementary Figure 5. Kramara *et al.*