Supporting information to:

Activation of Tel1^{ATM} kinase requires Rad50 ATPase and long nucleosome-free DNA, but no DNA ends

Sarem Hailemariam[1], Sandeep Kumar[1,2], and Peter M. Burgers[1]*

[1]Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, Saint Louis, Missouri, USA, [2]current address: Intellia Therapeutics, Cambridge, MA 02139.

*Corresponding author: TEL (314) 362-3772; FAX (314) 362-7183; burgers@wustl.edu

---

DNA substrates used:

20mer: 5'-TTGATAAGAGGTCATTTTT
40mer: 5'-TTGATAAGAGGTCATTTTTGCGGATGGCTTAGAGCTTAAT
60mer: 5'-ACATGTTGAGCTACAGCACCAGATTCAGCAATTAAGCTCTAAGCCATCCGCAAAAATGAC
80mer: 5'-TTGATAAGAGGTCATTTTTGCGGATGGCTTAGAGCTTAATTGCTGAATCTGGTGCTGTAG
CTCAACATGTTTTAAATATG
147mer: 5'-ATCGAGAATCCCGGTGCCGAGGCCGCTCAATTGGTCGTAGACAGCTCTAGCACCGCTTA
AACGCACGTACGCGCTGTCCCCCGCGTTTTAACCGCCAAGGGGATTACTCCCTAGTCTCCAGG
CACGTGTCAGATATATACATCCGAT

Oligonucleotides were hybridized to their complementary sequences in a 1:1 ratio to form blunt-end dsDNAs. The sequence of the 80-mer shown was used as ssDNA in the experiment shown in Fig. 3A. The 147-mer sequence is the Widom 601 sequence (1), and was purchased from EpiCypher, as was the mononucleosome assembled on the same sequence, and used in Fig. 4B,C.

1. Lowary, P.T., and Widom, J. (1998). New DNA sequence rules for high affinity binding to histone octamer and sequence-directed nucleosome positioning. J Mol Biol *276*, 19-42.

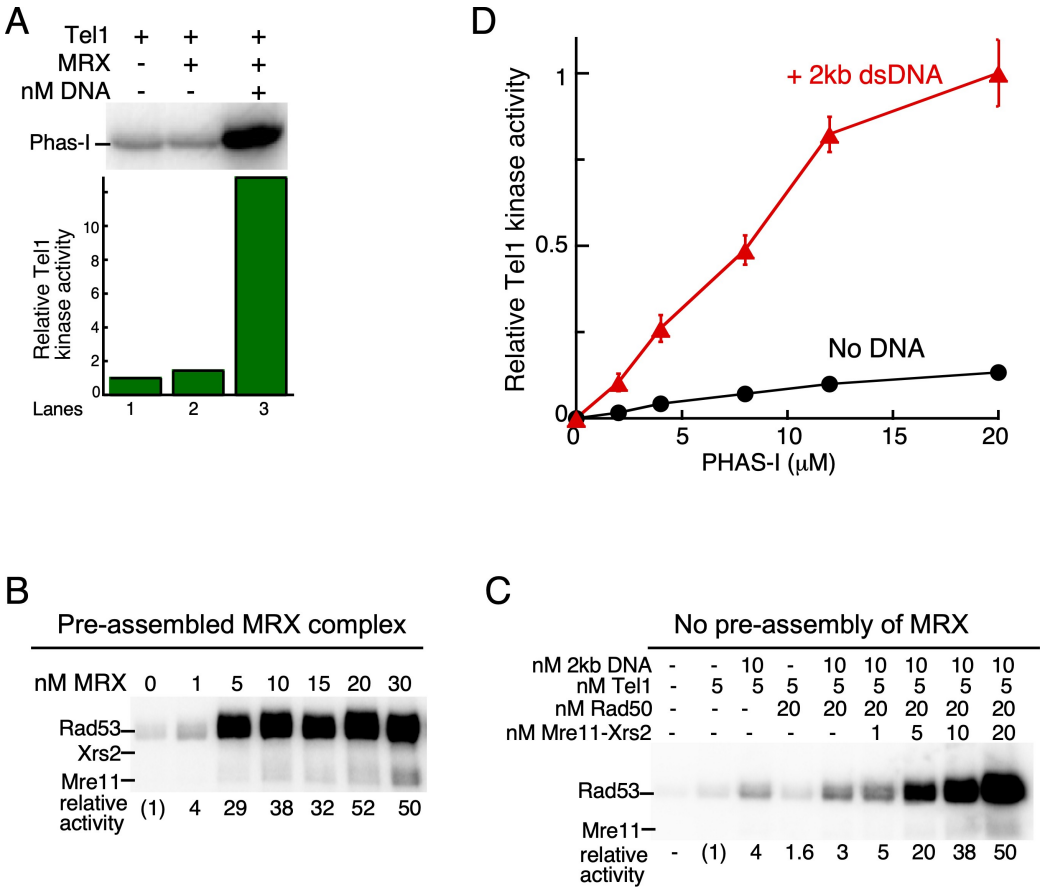

FIGURE S1. **Tel1 activation by MRX and DNA.** *A*, standard kinase reactions contained 2 μM PHAS-I and 50 μM [γ-^{32}P]-ATP in kinase buffer with or without 30 nM MRX and 5 nM 2 kb linear DNA. Kinase reactions were initiated with 5 nM Tel1. Reactions analyzed by 15% SDS-PAGE, followed by phosphorimaging. A representative experiment is shown at top. Bottom, phosphorylation of PHAS-I was quantified and plotted as fold increase of Tel1 kinase activity with lane 1 set at 1. The migration of PHAS-I (15 kDa) is as shown. *B,* standard kinase reactions contained 5 nM Tel1, 10 nM 2kb linear DNA, 200 nM Rad53, and the indicated concentrations of MRX. The MRX used in this experiment was pre-assembled by incubating 300 nM Rad50 with 300 nM Mre11-Xrs2 at 0 ˚C for one hour, prior to dilution into the assay. Rad53 phosphorylation was quantified by setting Tel1 alone reaction (lane 1) to 1. *C*, kinase reactions contained 5 nM Tel1, 10 nM 2kb linear DNA, 200 nM Rad53, 20 nM Rad50. The indicated concentrations of Mre11-Xrs2 was added into the assay at 0 ˚C and the assay incubated at 30 ˚C for 15 min. Rad53 phosphorylation was quantified by setting Tel1 alone reaction (lane 2) to 1. *D,* standard kinase reactions contained 5 nM Tel1, 30 nM MRX, and increasing concentrations of PHAS-I in the presence or absence of 5 nM 2kb linear DNA. In panels B and C the migration of GST-Rad53-kd (118 kDa), Xrs2 (96 kDa), and Mre11 (77 kDa) is as shown.

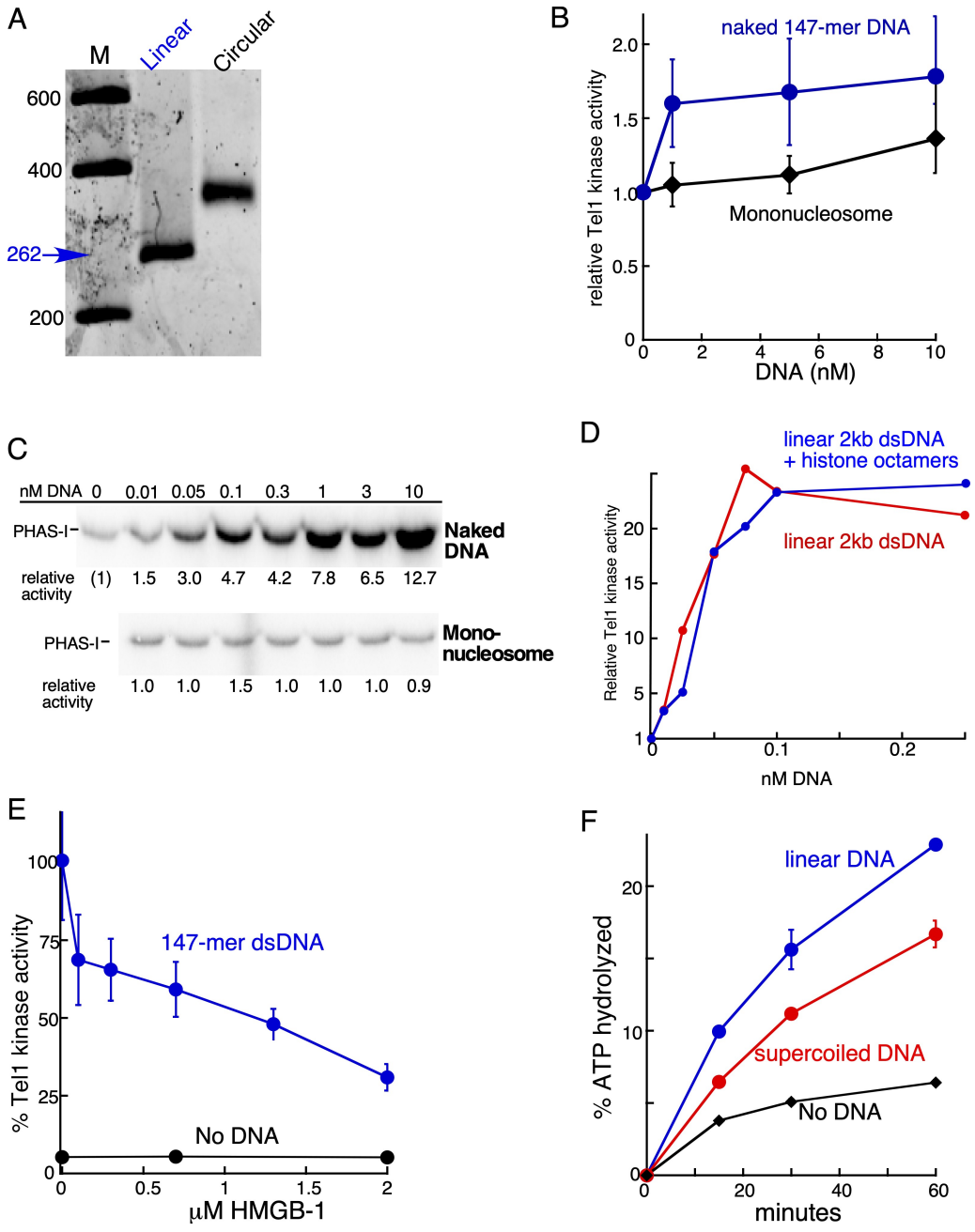

FIGURE S2. **Nucleosome-free DNA, but not DNA ends, is required for Tel1 activation.** *A, preparation of 262-mer circular DNA.* A 262-mer DNA fragment was cut out of a 3 kb plasmid by *Sal*1 and *Xho*1 digestion, and purified by differential PEG_{8000} precipitation in 0.5 M NaCl. The 5-9% w/w cut was >90% pure. It was ligated in a 1 ml ligation reaction containing 20 mM Tris-HCl 7.6, 100 mM Na-acetate, 10 mM Mg-acetate, 1 mM ATP, 1 mM spermidine, 1.5 μg/ml ethidium bromide, 50 nM 262-mer fragment, and 4,000 units of T4 ligase, for 24 h at 13 ˚C. The optimal concentration of ethidium bromide to obtain circularization was previously determined in a test assay with concentrations varying between 0.2-10 μg/ml. The DNA was recovered by ethanol precipitation, and the circular DNA was purified on a 3% preparative Metaphor gel. The

linear DNA was purified on the same gel to remove contaminating plasmid DNA. A comparison of the linear and circular preparations is shown on a 3% Metaphor gel, which was stained with GelRed. *B, Tel1 alone is not stimulated by nucleosomal DNA.* Standard kinase reactions without MRX contained 5 nM Tel1, and increasing concentrations of either 147 bp naked DNA, or the nucleosome assembled on the same DNA. Phosphorylation of Rad53-kd was quantified as fold increase of Tel1 kinase activity, with no DNA set to 1. *C, nucleosomal DNA does not stimulate Tel1 in the MRX-dependent assay using PHAS-I as substrate.* Standard kinase reactions lacking contained 5 nM Tel1, 30 nM MRX, 2 μM PHAS-I instead of Rad53-kd, and increasing concentrations of either 147 bp naked DNA, or the nucleosome assembled on the same DNA. Phosphorylation of PHAS-I was quantified as fold increase of Tel1 kinase activity with lane 1 (on naked DNA gel) set at 1. The migration of PHAS-I (15 kDa) is as shown. *D, free histone octamers do not inhibit Tel1 kinase.* Standard kinase reactions contained 5 nM Tel1, 30 nM MRX, 200 nM Rad53, and increasing concentrations of 2 kb linear DNA with or without increasing concentrations of histone octamers. The concentration of histone octamers required to coat DNA was calculated for each DNA concentration with a single octamer wrapping 147 bp. Relative Tel1 activity was calculated by setting the reaction without DNA to 1. *E, DNA coating by HMGB-1 inhibits Tel1.* Standard kinase reactions contained 5 nM Tel1, 30 nM MRX, 200 nM Rad53-kd, either no DNA or 1 nM 147-mer dsDNA, as indicated, and increasing concentrations of HMGB-1. *F, ATPase activity of MRX is stimulated preferentially by linear DNA.* Standard ATPase reactions contained 50 μM [γ-^{32}P]-ATP, 100 nM MRX, and 1 nM of the indicated 2 kb DNA. Radioactive ATP and ADP were separated by PEI-Cellulose TLC and quantified as described (1).

1. Chen, L., Trujillo, K. M., Van Komen, S., Roh, D. H., Krejci, L., Lewis, L. K., Resnick, M. A., Sung, P., and Tomkinson, A. E. (2005) Effect of amino acid substitutions in the rad50 ATP binding domain on DNA double strand break repair in yeast. *J Biol Chem* **280**, 2620-2627

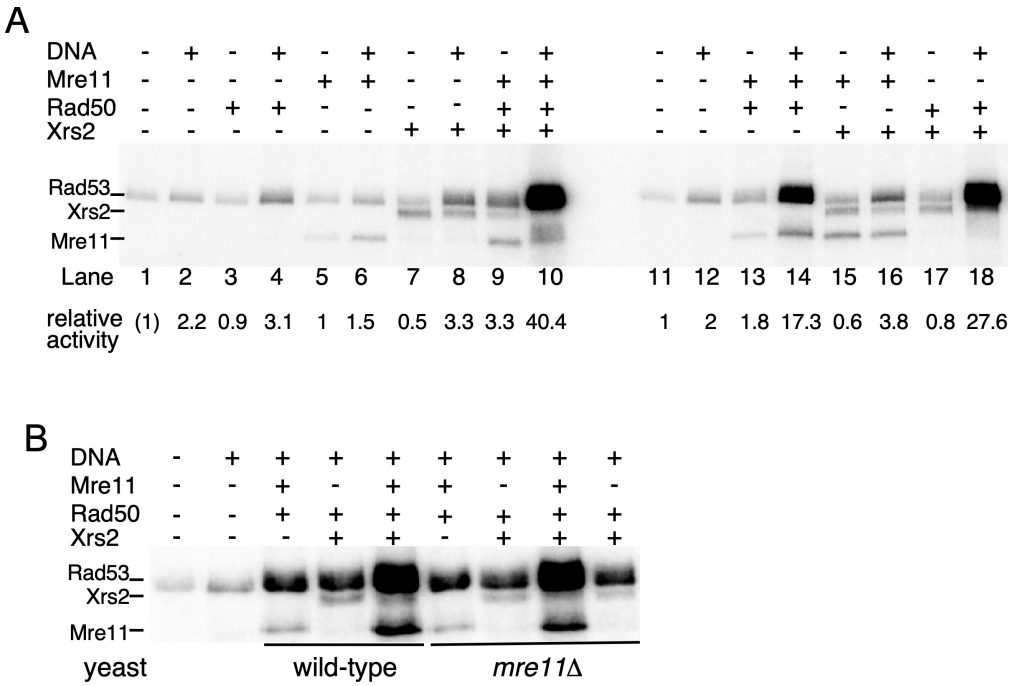

FIGURE S3. *A*, **Single subunits of MRX do not show a substantial activation of Tel1 kinase**. *A*, standard kinase reaction with 5 nM Tel1, 200 nM Rad53, and 30 nM Mre11, Rad50, or Xrs2, with or without 5 nM linear 2 kb DNA (left panel, lanes 1-8). The right panel (lanes 9-18) is the same data presented in FIGURE 5A. Lanes 9 and 10 of the left panel are presented as lanes 9 and 10 in Fig. 5A. Relative Tel1 kinase activity was calculated by setting Tel1 alone (lane 1) to 1. *B*, standard kinase reaction with 5 nM Tel1, 200 nM Rad53, and 30 nM MR, RX or MRX, with 5 nM linear 2 kb DNA. The Rad50 protein used in lanes 3-5 is purified from a wild-type yeast strain while the Rad50 used in the last four lanes is from a yeast *mre11Δ* strain. In all panels, the migration of GST-Rad53-kd (118 kDa), Xrs2 (96 kDa), and Mre11 (77 kDa) is as shown.