SUPPLEMENTARY MATERIAL

SUPPLEMENTARY FIGURES

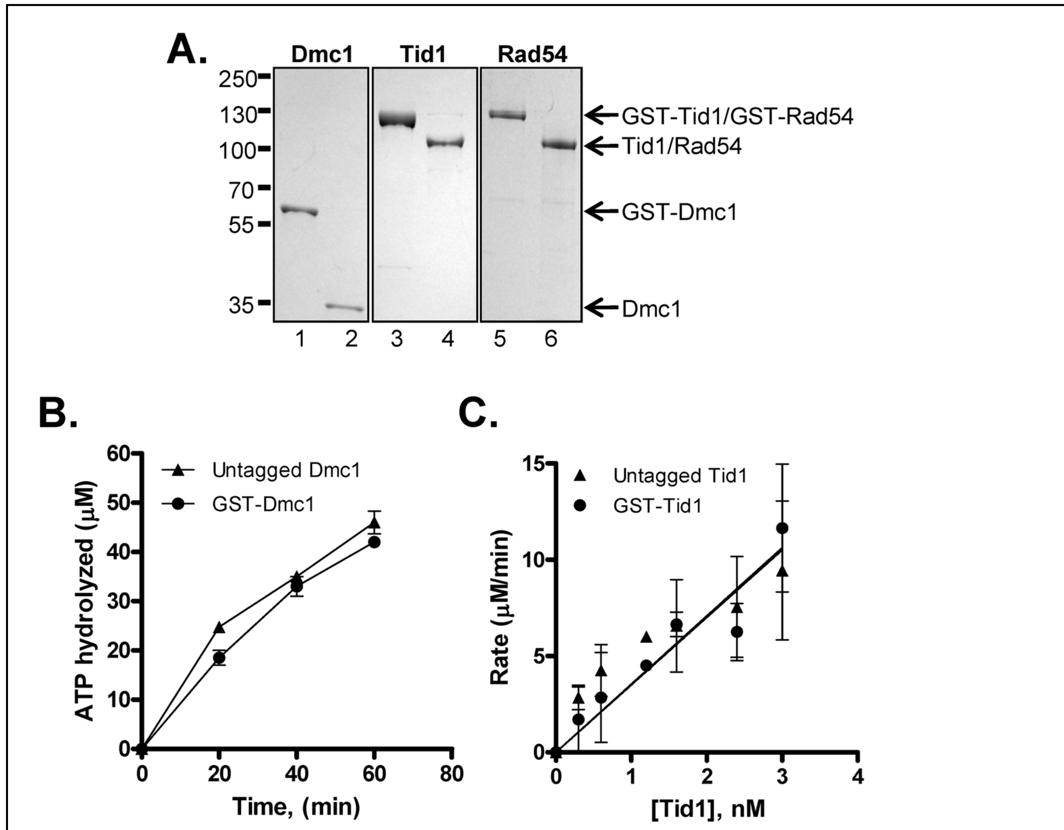

FIGURE S1. The **N-terminal GST-tag does not inhibit the ATPase activity of Dmc1 or Tid1**. *A*, Image showing GST-tagged and untagged versions of Dmc1, Tid1 and Rad54 proteins resolved by 10% SDS-PAGE. *B*, ssDNA-dependent ATPase activity of untagged and tagged Dmc1 (2 μM each) and poly dT (10 μM nt) as a function of time measured using standard conditions. *C*, dsDNA-dependent ATPase activity of untagged and tagged Tid1 with pUC19 scDNA (0.8 μM bp) as a function of protein concentration, measured using standard conditions with the exception that the ATP-regenerating system was omitted. Both experiments are TLC assays and the concentration of ATP was 1 mM. The apparent k_{cat} values were derived from a linear fit to the data points using GraphPad Prism version 5. Error bars indicate standard deviation from 2 independent experiments and are smaller than the symbols when not evident.

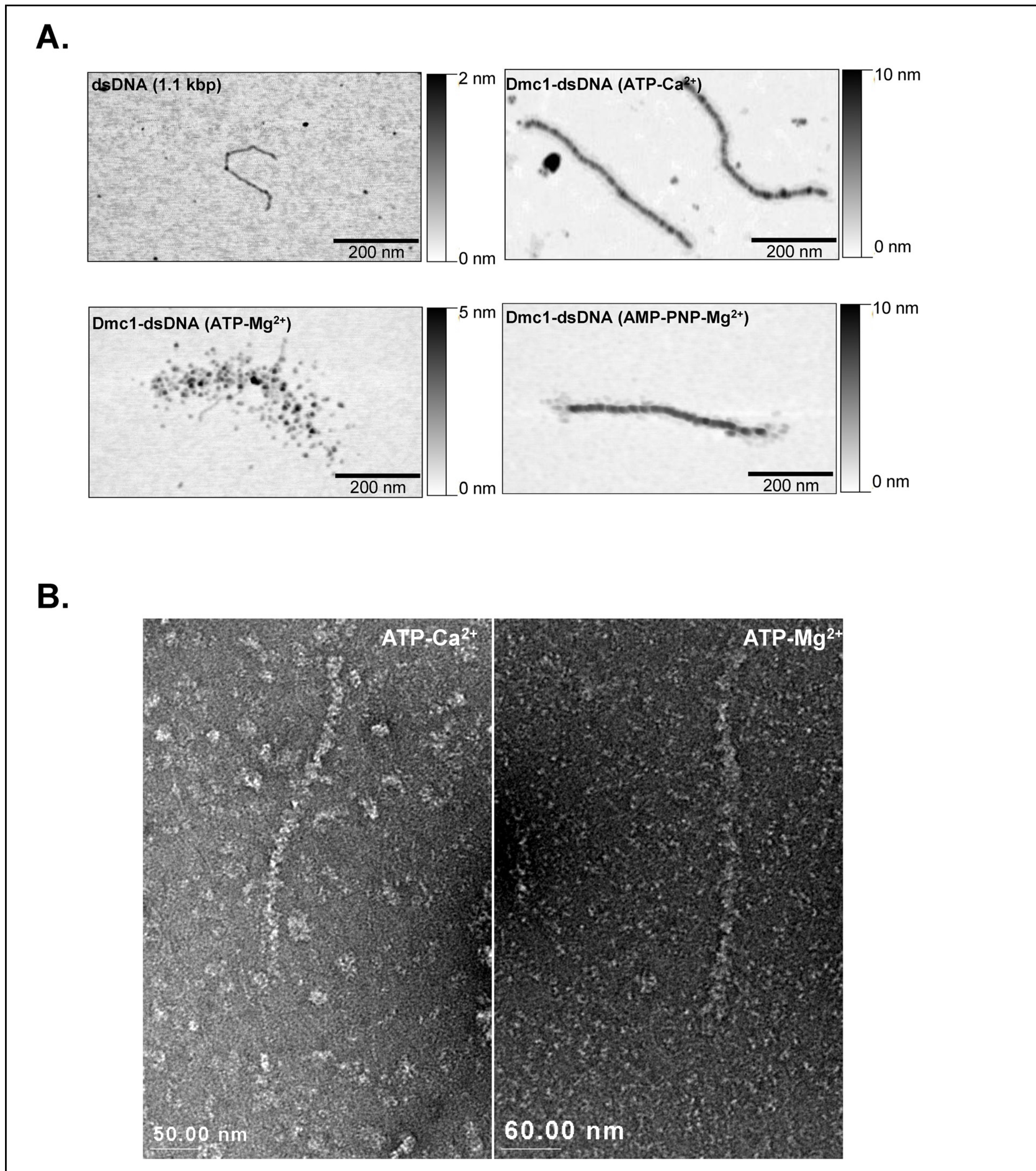

FIGURE S2. **AFM and EM images of Dmc1-dsDNA complexes.** *A*, AFM images showing Dmc1-dsDNA complexes. Representative images of dsDNA and Dmc1-dsDNA (in ATP-Ca^{2+}, ATP-Mg^{2+} and AMP-PNP-Mg^{2+}) are shown. *B*, EM images showing Dmc1-dsDNA complexes formed in ATP-Ca^{2+} and ATP-Mg^{2+}.

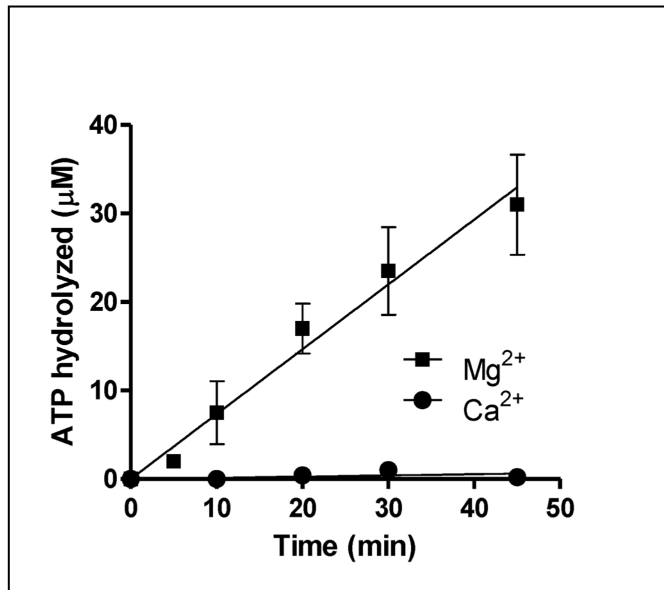

Figure S3. **Dmc1 cannot hydrolyze ATP in Ca^{2+}-containing buffer.** Time course of ssDNA-dependent ATP hydrolysis by Dmc1 in the presence of Ca^{2+} or Mg^{2+}. The concentration of ATP was 1 mM. Error bars indicate standard deviation from at least 3 independent experiments and are smaller than the symbols when not evident.

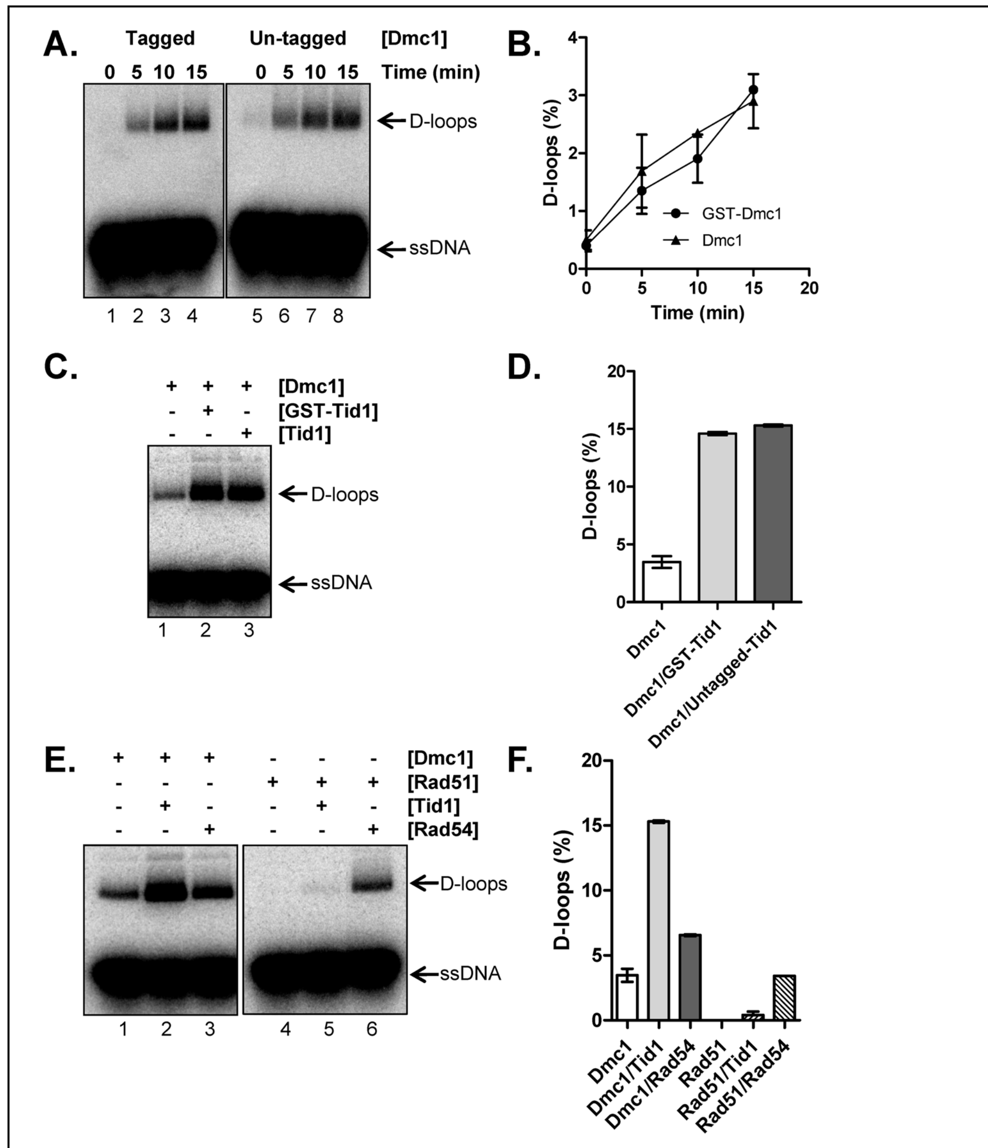

FIGURE S4. **The specificity of stimulation of DNA joint molecule formation is unaltered when untagged proteins are used.** *A*, Time course for D-loop formation catalyzed by GST-tagged and untagged Dmc1. *C*, Formation D-loop by untagged Dmc1 is stimulated by GST-tagged Tid1 to the same extent as by untagged Tid1. *E*, The untagged proteins exhibit similar specificities of stimulation in joint molecule formation as the GST-tagged proteins (Figures 5 and 7). *B*, *D,* and *F*, Graphical representation of data from *A, C,* and *E,* respectively. All reactions were performed in 5 mM Ca^{2+}. The incubation times in panels *C* to *F* were 15 min. The positions of free ssDNA and D-loops are indicated. Error bars indicate standard deviation from 2 independent experiments and are smaller than the symbols when not evident.

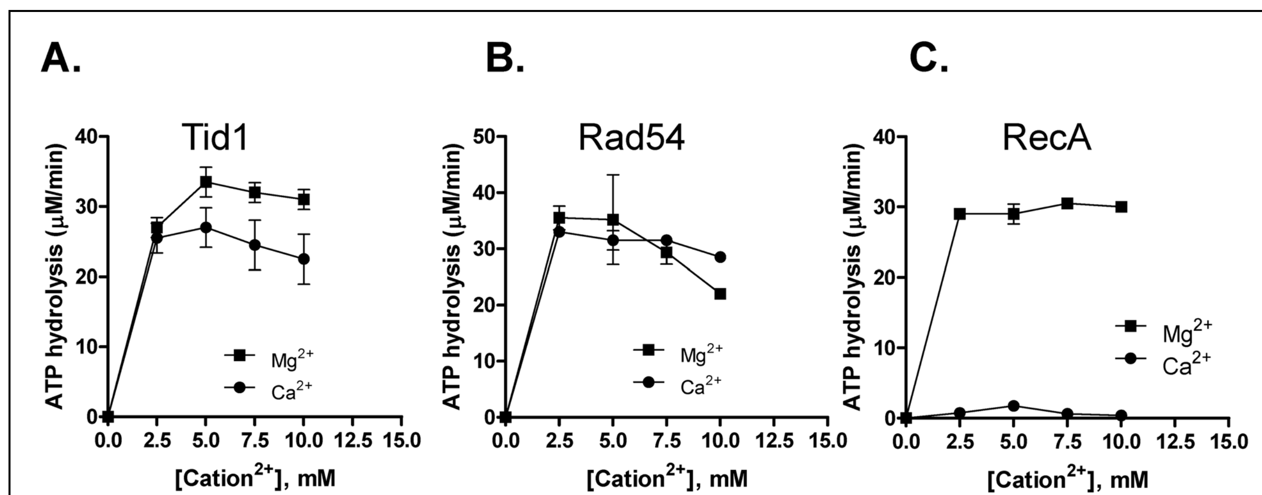

Figure S5. **Tid1 and Rad54 can hydrolyze ATP in Mg^{2+} as well as Ca^{2+}-containing buffers.** ATPase activities of Tid1 (*A*) and Rad54 (*B*) (10 nM each) measured as a function of Mg^{2+} and Ca^{2+}. The concentration of pUC19 scDNA was 2.5 μM bp. *C*, ATPase activity of RecA (1 μM) measured using the same buffers as in *A* and *B*. The concentration of ssDNA was 10 μM nt. EDTA at a final concentration of 200 μM was included in all three experiments. Error bars indicate standard deviation from 2 independent experiments and are smaller than the symbols when not evident.

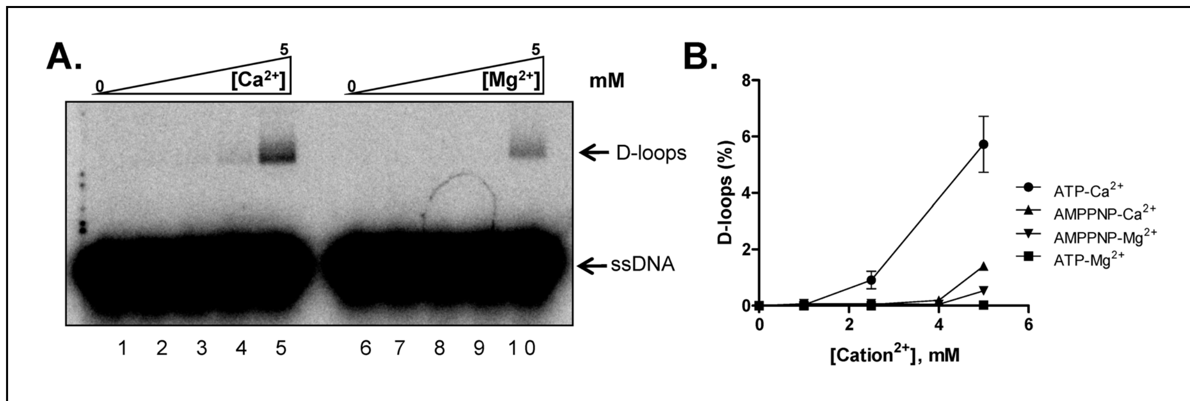

Figure S6. **Ca^{2+} can stimulate Dmc1-dependent joint molecule formation even in the presence of the non-hydrolysable ATP analog, AMP-PNP.** *A*, Formation of D-loops in the presence of AMP-PNP as a function of Ca^{2+} or Mg^{2+}, respectively. The positions of free ssDNA and D-loops are indicated. *B*, Graph showing D-loop formation as a function of either divalent cation in the presence of ATP or AMP-PNP. The positions of free ssDNA and D-loops are indicated. Error bars indicate standard deviation from 2 independent experiments and are smaller than the symbols when not evident.