SUPPLEMENTAL FIGURES FOR

Only One ATP-Binding DnaX Subunit is Required for Initiation Complex Formation by the *E. coli* DNA Polymerase III Holoenzyme

Anna Wieczorek, Christopher D. Downey, H. Garry Dallmann and Charles S. McHenry

From the Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO 80309

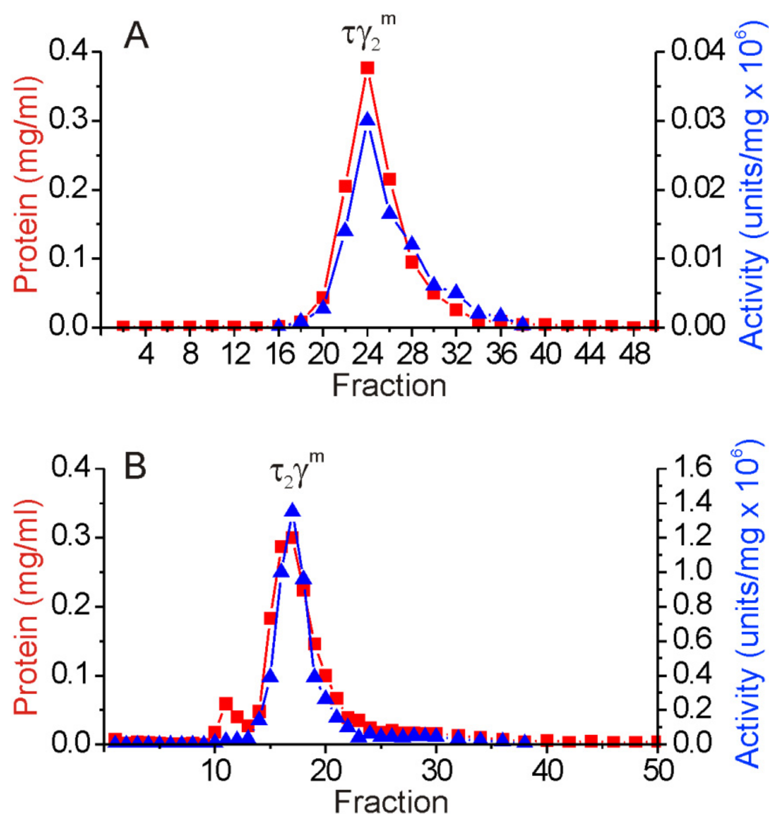

FIGURE S1. Rechromatography of DnaX complexes. Complexes containing a mixture of K51E and wild-type DnaX protomers (obtained as shown in Figure 1 of the main paper) were rechromatographed on the Mono-S column to ensure purity. Representative purifications are shown. *A*, Rechromatography of $\tau\gamma^m{}_2$ complex was performed using a shallow 20-column volume gradient of 0.08–0.17 mM NaCl with other components and procedures as described for Mono S chromatography under *Experimental Procedures* in the main paper. *B*, Rechromatography of $\tau_2\gamma^m$ complex was done in the 20-column volume gradient of 0.14–0.19 mM NaCl.

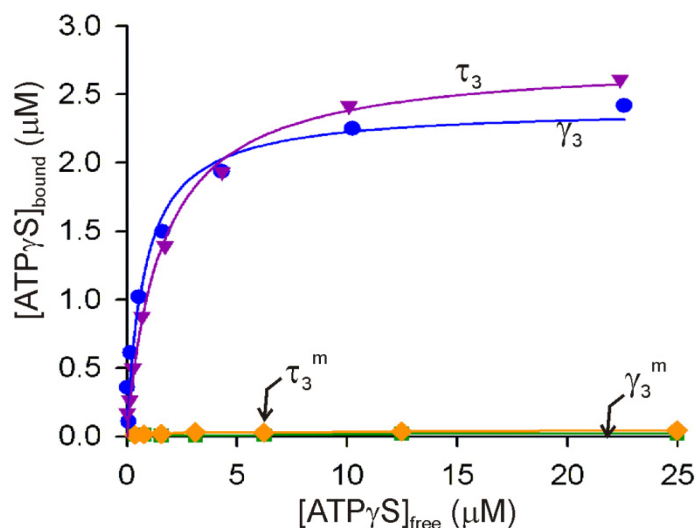

FIGURE S2. A K51E change in the DnaX Walker A motif eliminates ATP binding. For each data point, 1.0 μM of the indicated DnaX complex was incubated at room temperature with 0–25 μM [^{35}S] ATPγS (specific activity ~1000 cpm/pmol) in binding buffer (50 mM HEPES (pH 7.5), 100 mM K^+ glutamate, 10 mM Mg^{2+} acetate, 5% glycerol, 5 mM DTT) in a volume of 12.5 μL. A 25 mm nitrocellulose filter (Millipore) pre-soaked in wash buffer (50 mM HEPES (pH 7.5), 100 mM K^+ glutamate, 10 mM Mg^{2+} acetate) was placed in a 10 mL glass vacuum manifold (Millipore); vacuum was applied, and the filter was washed with 1.5 mL ice-cold wash buffer. With the vacuum off, 10.0 μL of sample was spotted onto the filter. The vacuum was reapplied and the samples washed with 1.0 mL cold wash buffer dripped on at a rate of ~0.2 mL/s. The filters were dried and counted by liquid scintillation. The counted values were corrected for filtration efficiency, which was determined as the fraction of the total ^{35}S counts retained on the filter with limiting ATPγS and saturating DnaX complex. The efficiency was 0.69 for γ_3 complex and 0.77 for τ_3 complex. The free ATPγS concentration was determined as total ATPγS minus bound ATPγS. The γ_3 and τ_3 data were fit to a binding isotherm to determine the dissociation constant (K_d) and apparent total ATP receptor concentration (R_t):

$$[\text{ATP}\gamma\text{S}]_{\text{bound}} = \frac{R_t\,[\text{ATP}\gamma\text{S}]_{\text{free}}}{K_d + [\text{ATP}\gamma\text{S}]_{\text{free}}}$$

The curve fits yielded K_d = 1 μM and R_t = 2.4 μM for the γ_3 complex and K_d = 2 μM and R_t = 2.7 μM for the τ_3 complex.

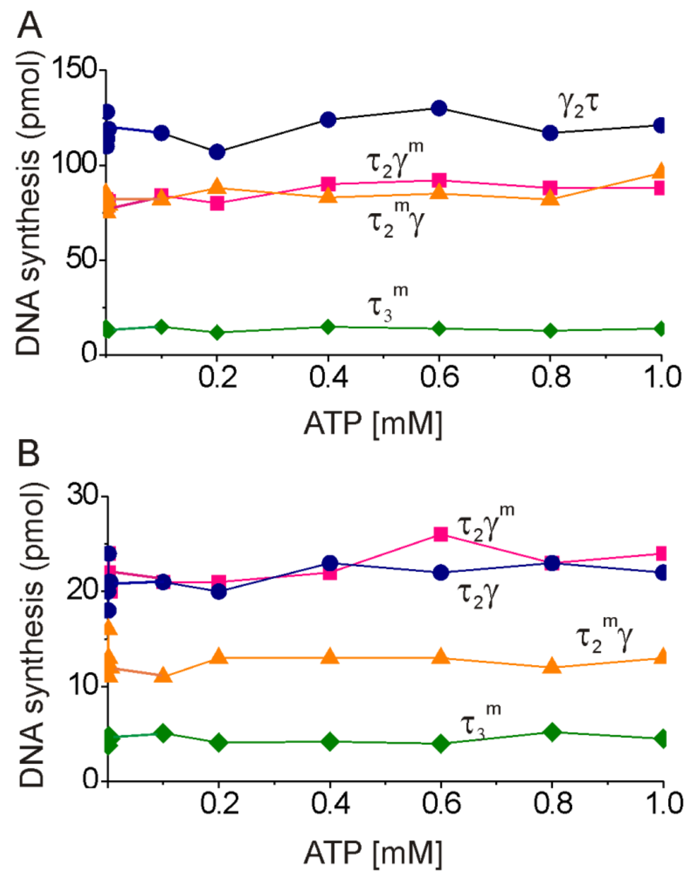

FIGURE S3. High concentrations of ATP do not overcome defects observed in DnaX complexes containing K51E γ or τ subunits. ATP was titrated under two sets of conditions using the reconstitution assay as described under *Experimental Procedures* in the main paper. Representative protein complexes containing two or three τ subunits but at least one wild-type subunit were used. *A*, Assays were performed at a DnaX complex concentration corresponding to 50% of the amount required for maximal activity in the presence of saturating β_2 (7 x K_d). *B*, Assays were performed with the DnaX complex concentration set at 0.8 nM and β_2 set at one-half of the K_d of each complex (Table 1). dATP will substitute for ATP in supporting initiation complex formation by the Pol III HE. The 48 μM dATP present in the elongation mix is adequate to support full DnaX activity in the absence of ATP in all cases.