SUPPLEMENTARY DATA

The proofreading exonuclease subunit ε of *Escherichia coli* DNA polymerase III is tethered to the polymerase subunit α via a flexible linker

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Table S1. PCR primers used in this study[1]

975[2]	5'-TTTGAATTCTTATGCTCGCCAGAGGCAAC-3' (29-mer)
974[3]	5'-TTTTTTTTCATATGAGCACTGCAATTACACG-3' (31-mer)
1131	5'-PO_4-TTAGCTGGTCGATCCCGCGAAATTAATACG-3' (30-mer)
1132	5'-PO_4-CCAGCTAACAAAAAACCCCTCAAGACCCG-3' (29-mer)
1133	5'-PO_4-TCGATCCCGCGAAATTAATACG-3' (22-mer)
1134	5'-PO_4-CAAAAAACCCCTCAAGACCCG-3' (21-mer)
1145[2]	5'-TTTTTTTTCATATG**GCTT**CTGCAATTACACGCCAG-3' (35-mer)
1164[3]	5'-TTTGAATTCTTACGCAAAAACAACGCGTAAC-3' (31-mer)
1167[4]	5'-GGAAGGAGAG**G**CACAACAGCAAC-3' (23-mer)
1168[4]	5'-GTTGCTGTTGTG**C**CTCTCCTTCC-3' (23-mer)
1169[5]	5'-GGTGAAGCA**G**CAATTCAGCGC-3' (21-mer)
1170[5]	5'-GCGCTGAATTG**C**TGCTTCACC-3' (21-mer)
1179[6]	5'-CAAACGTCGATGG**G**TTTGCGATGG-3' (25-mer)
1180[6]	5'-CCATCGCAAAAC**C**CATCGACGTTTG-3' (25-mer)
1181[7]	5'-GATGGCTTTTG**G**GATGGAAGGAGAG-3' (25-mer)
1182[7]	5'-CTCTCCTTCCATC**C**CAAAAGCCATC-3' (25-mer)
1183[8]	5'-CAAGGTGAAG**G**AACAATTCAGCGC-3' (24-mer)
1184[8]	5'-GCGCTGAATTGTT**C**CTTCACCTTG-3' (24-mer)
1185[9]	5'-CTCTGGCGAG**G**ATAAATACCTGTG-3' (24-mer)
1186[9]	5'-CACAGGTATTTAT**C**CTCGCCAGAG-3' (24-mer)

1 Codons of mutated amino acids are underlined. Mutated bases are shown in bold.

[2] Forward and reverse primers to generate the S2A/T3S double mutant of ε.

[3] Forward and reverse primers to generate the C-terminal deletion mutant ε217.

[4] Forward and reverse primers to generate the T193A mutant of ε.

[5] Forward and reverse primers to generate the T201A mutant of ε.

[6] Forward and reverse primers to generate the A186G mutant of ε.

[7] Forward and reverse primers to generate the A188G mutant of ε.

[8] Forward and reverse primers to generate the A200G mutant of ε.

[9] Forward and reverse primers to generate the A243G mutant of ε.

Preparation of C-terminally truncated α subunit of the *E. coli* DNA polymerase III

A construct of C-terminally truncated (after residue 917) subunit α with an N-terminal His_6 tag (α917) was prepared by insertion of the corresponding part of the *dnaE* gene as a PCR-generated NdeI-EcoRI fragment between the corresponding sites in the T7 promoter vector pETMCSI (S1), to generate the plasmid pKO1342 that encodes α917.

E. coli cells (BL21::λDE3/plysS) harboring pKO1342 were grown aerobically for two days at room temperature in an auto-induction medium (S2); 2 litres of cell culture yielded about 14 g of cells. The French press lysate was loaded onto a 5 ml column of Ni-NTA resin (Pharmacia) in a buffer of 50 mM Hepes-KOH, pH 7.5, 300 mM NaCl, 5% glycerol, and 20 mM imidazole, Bound α917 was eluted with an gradient of 20–500 mM imidazole in the same buffer. The elute was dialyzed and subjected to chromatography on a DEAE-Toyopearl column (2.6 × 3.5 cm) in a buffer of 20 mM Tris.HCl, pH 7.6, 1 mM EDTA, 1 mM dithiothreitol, and 5% glycerol. The bound α917 was eluted with a gradient of 0–1M NaCl, yielding about 11 mg of pure α917. Its concentrations was determined spectrophotometrically at 280 nm, using the calculated ε_{280} value of 73820 $M^{-1}cm^{-1}$ (S3).

References

S1. Neylon,C., Brown,S.E., Kralicek,A.V., Miles,C.S., Love,C.A. and Dixon,N.E. (2000) Interaction of the *Escherichia coli* replication terminator protein (Tus) with DNA: a model derived from DNA-binding studies of mutant proteins by surface plasmon resonance. *Biochemistry*, **39**, 11989–11999.

S2. Studier,F.W. (2005) Protein production by auto-induction in high-density shaking cultures. *Protein Express. Purif.,* **41,** 207–234.

S3. Gill,S.C. and von Hippel,P.H. (1989) Calculation of protein extinction coefficients from amino acid sequence data. *Anal. Biochem.,* **182**, 319–326.

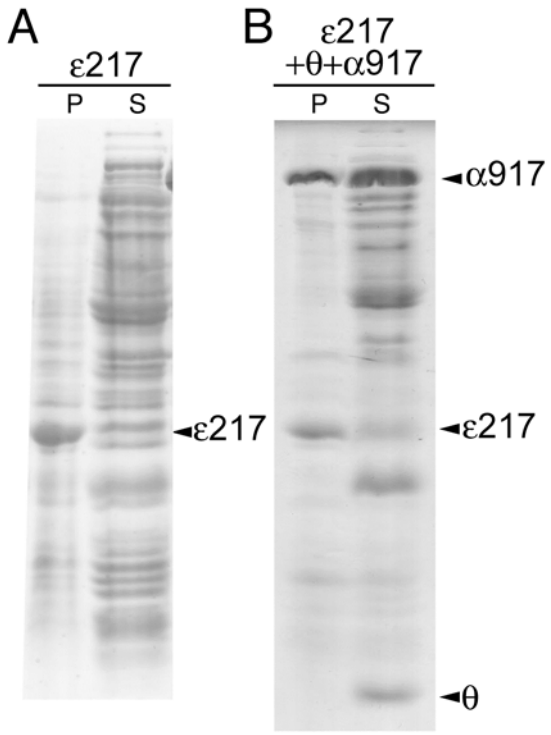

Figure S1. Cell-free synthesis of ε217 in the absence and presence of θ and α917. ε217 was synthesized at 30˚C from PCR-amplified DNA as described in the main text. The gels were stained with Coomassie brilliant blue. P and S denote the insoluble (pellet) and soluble (supernatant) fractions, respectively. **(A)** ε217 synthesized in the absence of θ and α917 is insoluble. **(B)** ε217 synthesized in the presence of 0.5 mg/ml θ and 5 mg/ml α917 results in co-precipitation of α917 with ε217 (lane 1) but also in ε217 in the soluble fraction (presumably in the ε217:θ or α917:ε217:θ complex). The co-precipitation demonstrates binding of ε217 to α917. Whereas the α917:ε:θ complex prepared in the same way could be purified by Ni-NTA chromatography, the α917:ε217:θ complex could not, indicating limited stability (data not shown).