Supporting Information

Bartlett et al. 10.1073/pnas.1302616110

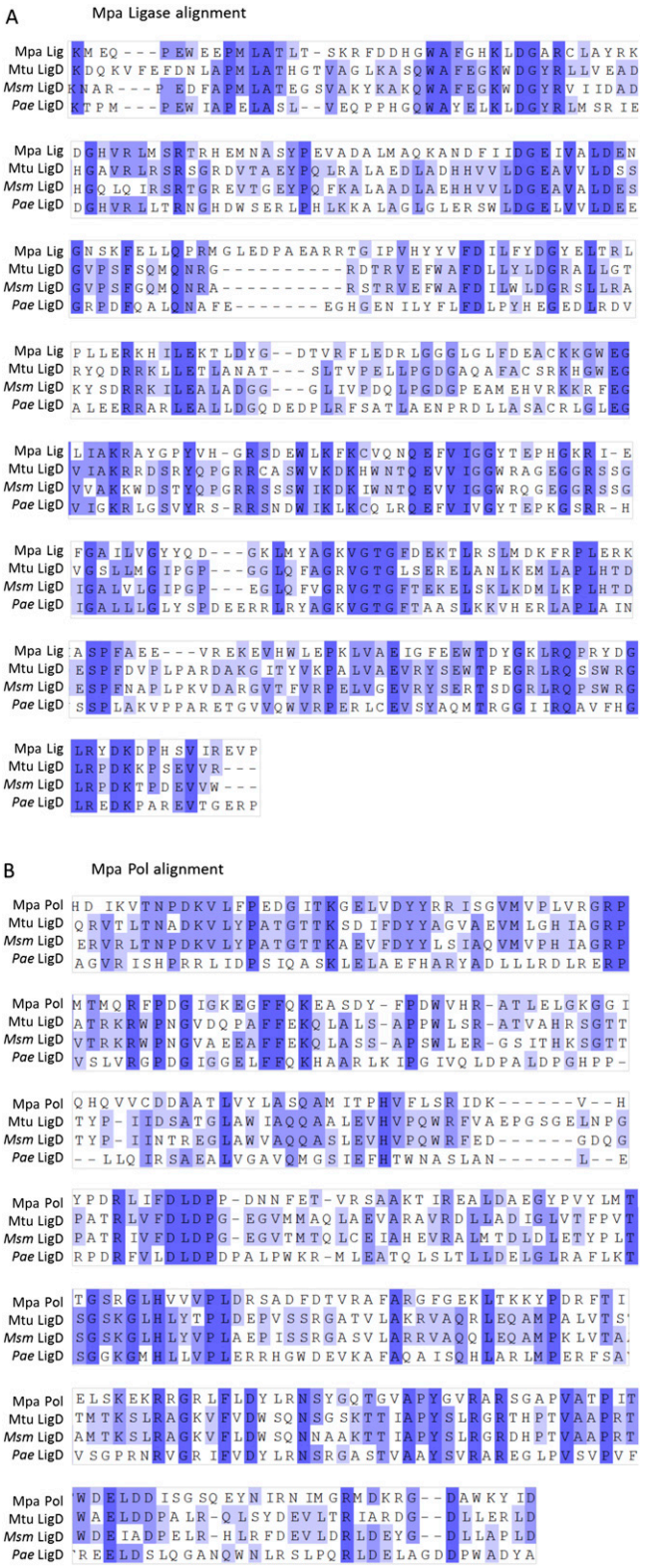

Fig. S1. (Continued)

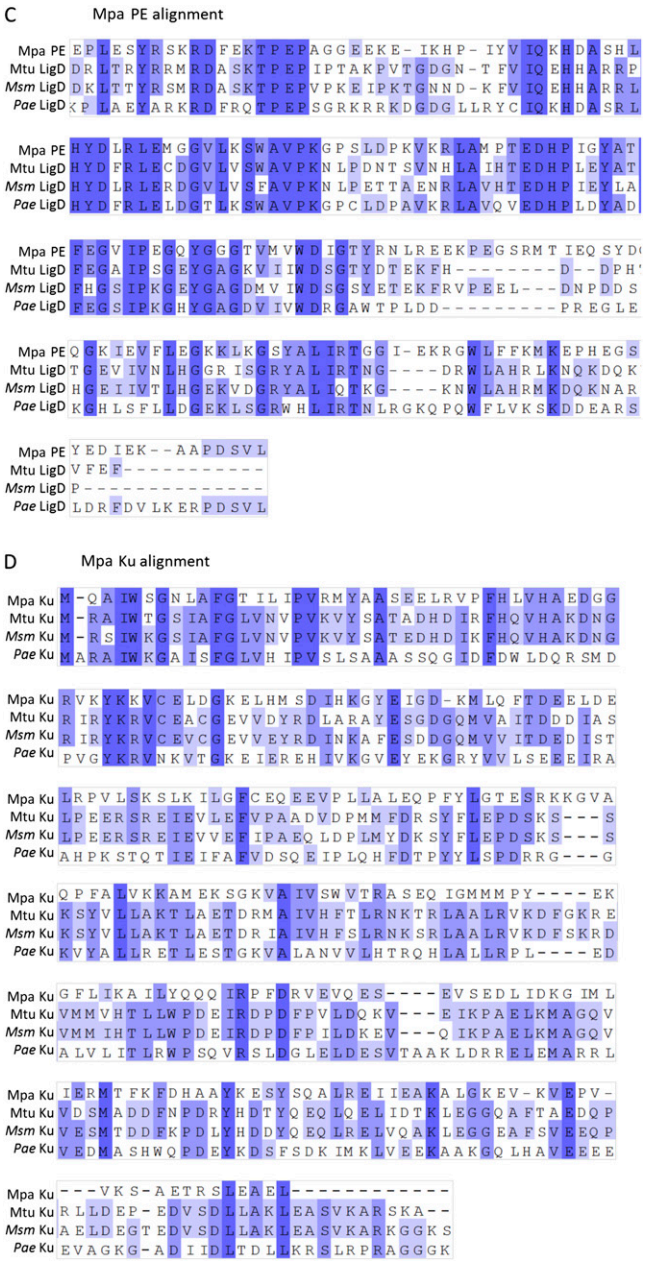

Fig. S1. Protein sequence alignments of *Methanocella paludicola* (Mpa) ligase (Lig), polymerase (Pol), phosphoesterase (PE), and Ku with *Mycobacterium tuberculosis* (Mtu), *Mycobacterium smegmatis* (Msm), *Pseudomonas aeruginosa* (Pae) ligase D (LigD), and Ku. (*A*) Mpa Lig protein sequence alignment with LigD ligase domains (LigDom) of Mtu, Msm, and Pae. (*B*) Mpa Pol protein sequence alignment with LigD Pol domains (PolDom) of Mtu, Msm, and Pae. (*C*) Mpa PE protein sequence alignment with LigD PE domains (PEDom) of Mtu, Msm, and Pae. (*D*) Mpa Ku protein sequence alignment with Ku of Mtu, Msm, and Pae. All alignments were made with TCoffee and edited in JalView.

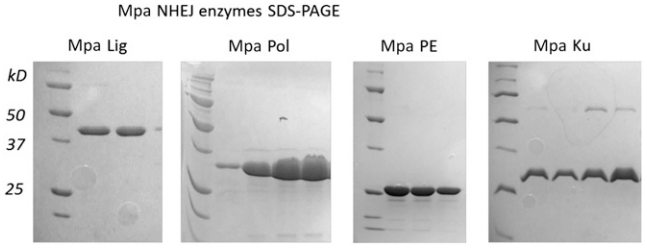

Fig. S2. SDS/PAGE Mpa Lig, Pol, PE, and Ku. SDS/PAGE of gel filtration chromatography fractions of respective purifications of Mpa Lig, Pol, PE and Ku. Migration sizes of the proteins were Mpa Lig ~40; Mpa Pol ~34 kD; Mpa PE ~24 kD; and Mpa Ku ~30 kD (monomer) and ~60 kD (dimer). The gels demonstrate the high purity of the proteins.

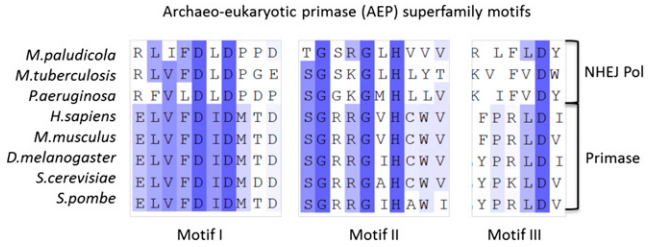

Fig. S3. Archaeo-eukaryotic primase (AEP) superfamily motif sequence alignment. Three key conserved motifs (I, II, and III) confer membership to the AEP superfamily. Here Mpa Pol is aligned with Mtu and Pae nonhomologous end-joining (NHEJ) Pols, and *Homo sapiens*, *Mus musculus*, *Drosophila melanogaster*, *Saccharomyces cerevisiae*, and *Schizosaccharomyces pombe* primase small subunits.

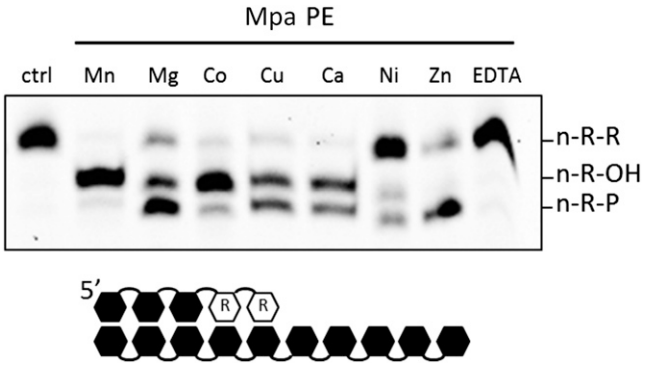

Fig. S4. Mpa PE ion preference. PE reaction mixtures contained 50 mM Tris (pH 7.5), 5 mM $MnCl_2$, $MgCl_2$, $CoCl_2$, $CuCl_2$, $CaCl_2$, $NiCl_2$, or $Zn(O_2CCH_3)_2$ where indicated, 30 nM 5′-fluorescein–labeled 16mer DNA/RNA (D14R2), and 300 nM Mpa PE protein. Reactions were incubated at 37 °C for 1.5 h and electrophoresed on a 15% denaturing polyacrylamide gel. n-R-R, unmodified substrate; n-R-P, removal of the terminal ribonucleotide monophosphate to yield a terminal phosphate group; n-R-OH, product after phosphate removal leaving a terminal OH group.

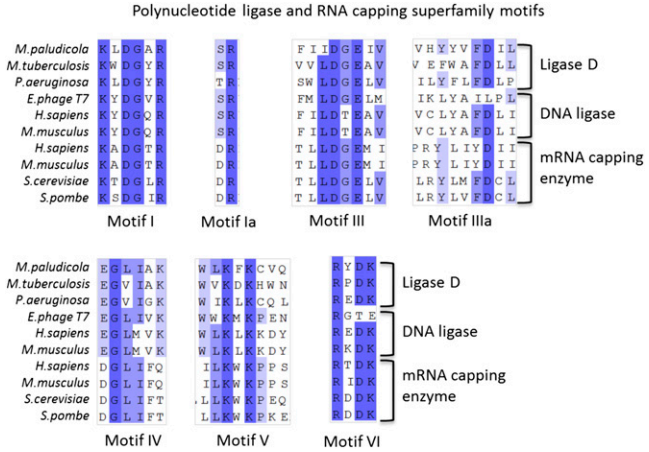

Fig. S5. Polynucleotide ligase and mRNA-capping superfamily motifs. Six key conserved motifs (I, Ia, III, IIIa, IV, and V) confer membership in the polynucleotide ligase and mRNA-capping superfamily. Motif VI also is included with highly conserved residues. Here Mpa Lig is aligned with Mtu and Pae LigD; Enterobacteria phage T7, *H. sapiens*, and *M. musculus* DNA ligase; and *H. sapiens*, *M. musculus*, *D. melanogaster*, *S. cerevisiae*, and *S. pombe* mRNA-capping enzymes.

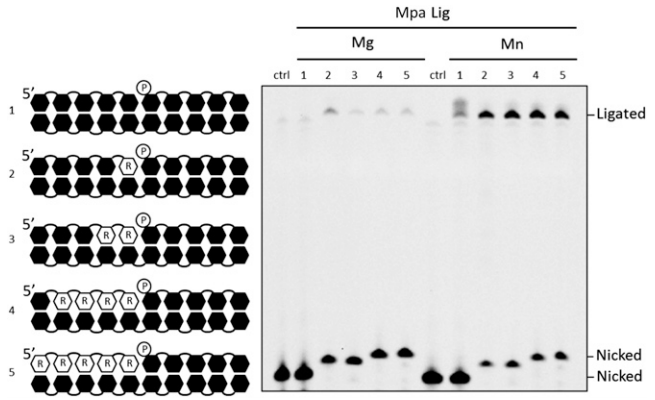

Fig. S6. Mpa Lig preferentially ligates monoribonucleoside containing nicks in the presence of manganese, not magnesium. Lig reaction mixtures contained 50 mM Tris (pH 7.5), 5 mM $MnCl_2$ or Mg Cl_2, 30 nM 5′-fluorescein–labeled 16mer oligomer (D16, D15R, D14R2, D12R4, or D10R6 where indicated), 19mer 5′-phosphorylated DNA, and 300 nM Mpa Lig protein. Reactions were incubated at 37 °C for 2 h and electrophoresed on a 15% denaturing polyacrylamide gel.

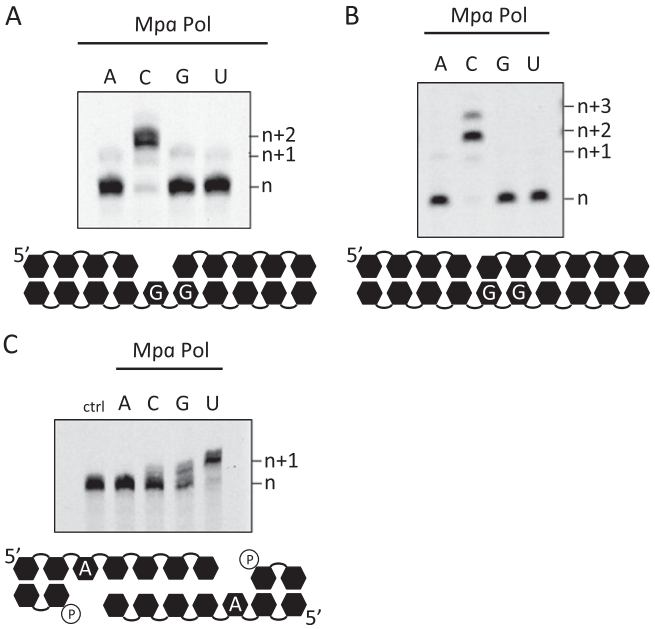

Fig. S7. Mpa Pol gap filling and strand displacement at nicks is template dependent. Pol gap-filling and strand-displacement reaction mixtures contained 50 mM Tris (pH 7.5), 5 mM $MnCl_2$, 62.5 μM of the indicated NTP, 30 nM 5′-fluorescein–labeled 16mer DNA (*A* and *B*) or 47mer DNA (*C*), and 300 nM Mpa Pol protein. Reactions were incubated at 37 °C for 1.5 h and electrophoresed on a 15% denaturing polyacrylamide gel. A and *B* demonstrate template-dependent incorporation of NTPs onto a substrate with a downstream strand. Although Mpa Pol is capable of incorporating NTPs to a DNA primer in a template-independent manner (Fig. S8), these assays indicate that base selection is performed primarily by template dependence even in the presence of an annealed sequence of downstream DNA. *C* demonstrates the template-dependent incorporation of NTPs onto a substrate with a self-annealing microhomology of four base pairs, creating a single-nucleotide gap on either side. Proficient incorporation of UTP required prior formation of the microhomology, because Mpa Pol can extend only from a 3′-hydroxyl, and template-independent incorporation function is limited to a single addition onto a double-stranded blunt DNA terminus. Furthermore, preferential incorporation of UTP indicates strong template dependence.

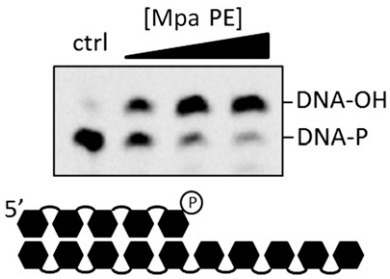

Fig. S8. Mpa PE phosphomonoesterase activity does not require 2′-OH. Phosphatase reaction mixtures contained 50 mM Tris (pH 7.5), 5 mM $MnCl_2$, 30 nM 5′-fluorescein–labeled 16mer DNA with 3′ phosphate, and 250, 500, and 1,000 nM Mpa PE protein. Reactions were incubated at 37 °C for 1.5 h and electrophoresed on a 15% denaturing polyacrylamide gel.

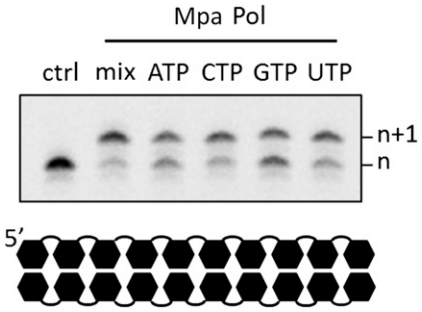

Fig. S9. Mpa Pol incorporation of nontemplated NTP. Pol nontemplated extension reaction mixtures contained 50 mM Tris (pH7.5), 5 mM $MnCl_2$, 62.5 μM ATP, CTP, GTP, UTP, or 250 μM mixed nucleoside triphosphates (NTPs) and, where indicated, 30 nM 5′-fluorescein–labeled 36mer DNA and 300 nM Mpa Pol protein. Reactions were incubated at 37 °C for 1 h and electrophoresed on a 15% denaturing polyacrylamide gel.

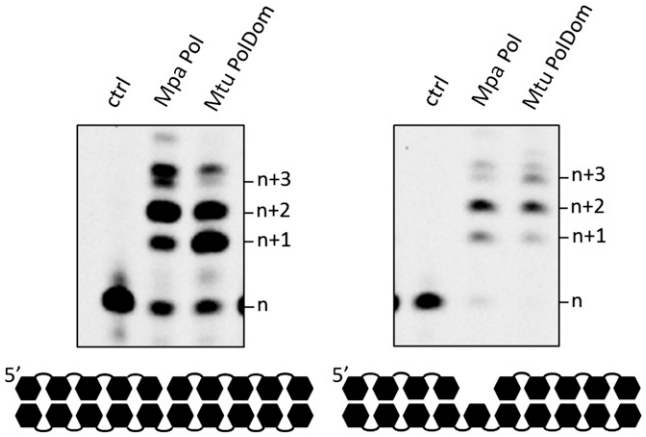

Fig. S10. Mtu Pol domain (PolDom) strand displacement. Polymerase strand displacement reaction mixtures contained 50 mM Tris (pH 7.5), 5 mM $MnCl_2$, 250 μM NTPs, 30 nM 5′-fluorescein–labeled 16mer DNA, and 300 nM Mpa Pol or Mtu PolDom protein where indicated. Reactions were incubated at 37 °C for 1 h and electrophoresed on a 15% denaturing polyacrylamide gel.

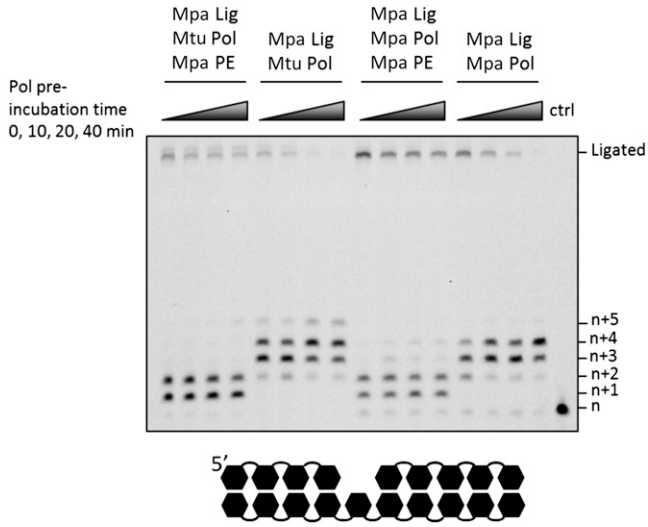

Fig. S11. Mpa PE can rescue NHEJ intermediates formed by Mtu PolDom and Mpa Pol. Gap-filling, PE, and ligation reaction mixtures contained 50 mM Tris (pH 7.5), 5 mM $MnCl_2$, 250 μM NTPs, 30 nM 5′-fluorescein–labeled 16mer DNA, and 300 nM Mpa Pol or Mtu PolDom, 300 nM Mpa Lig, and PE protein where indicated. Reactions were incubated at 37 °C for 1 h and electrophoresed on a 15% denaturing polyacrylamide gel.

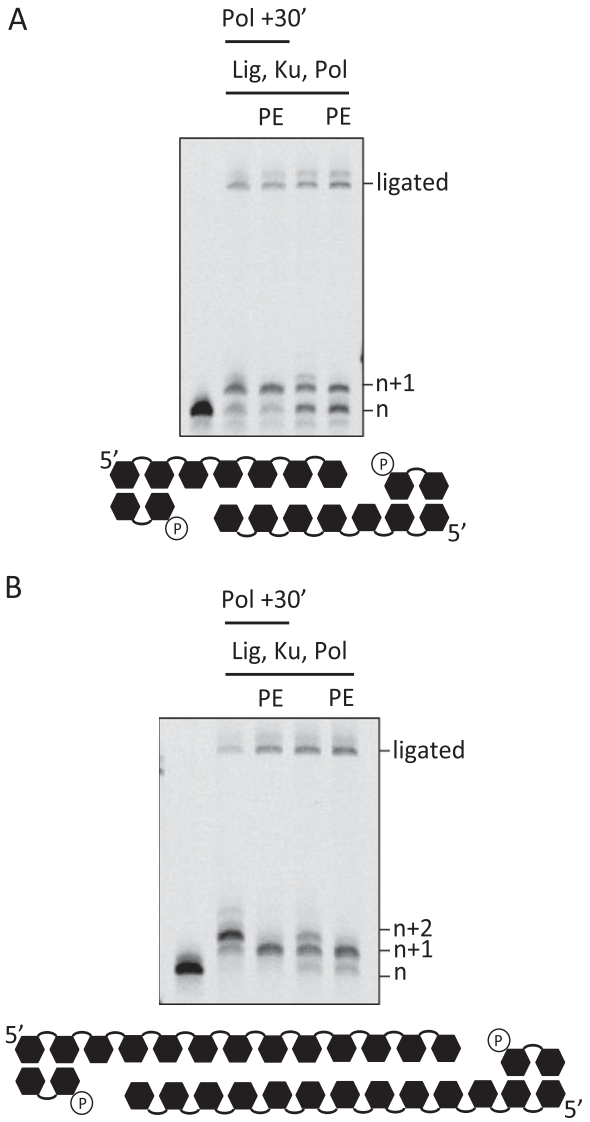

Fig. S12. Mpa PE resection of strand-displacement intermediates during 3′ overhang NHEJ repair. Gap-filling, PE, and ligation reaction mixtures contained 50 mM Tris (pH 7.5), 5 mM $MnCl_2$, 250 μM NTPs, 30 nM 5′-fluorescein–labeled 42mer DNA (*A*) and 48mer DNA (*B*), and 300 nM Mpa Pol, Lig, and PE and 400 nM Ku protein where indicated above the lanes. Mpa Pol was preincubated with the substrate for 30 min where indicated above the lanes before the addition of Mpa Lig, PE and Ku. Electrophoresed on a 15% denaturing polyacrylamide gel.