Supplementary material

Fig. S1 Amino acid sequence alignment of the exonuclease domain of AtPolIs versus KF-DNAP I. The conserved exonuclease motifs I, II, and II are underlined. Amino acid involved in the two-metal ion metal ion mechanism for exonucleolysis in KF-DNAP I are indicated by an arrow.

Fig. S2 Amino acid sequence alignment of the polymerase domain of AtPolIs versus –DNAPs I from *E. coli*, *T. aquaticus*, and *G. stearothermophilus*. The subdomain organization is indicated by color arrows as follow: thumb, green; palm, brown; and fingers, purple. The three unique amino acid insertions in AtPolIs are indicated by blue, red and yellow boxes respectively.

Fig. S3 Amino acid sequence alignment of the polymerase domain of representative POPs from plants, protists and algae in comparison to KF-DNAP I. Three insertion are indicated by a black line.

Fig. S4 Deletion of the disorder region in AtPoIIs increase protein purification yields with out altering polymerization, exonuclease and TLS activities A) SDS-PAGE gel showing purified AtPoIIs result of the removal of the N-terminal disorder region (residues 31 to 257). These constructs resemble the Klenow fragment of *E. coli* DNAP I and are dubbed KF-AtPoIIs **B**) **Comparison between polymerization and exonuclease activities of full-length and KF-AtPoIs.** Time course reaction from 15 to 60 sec showing the exonuclease (lanes 1 to 12) and the polymerase (lanes 13 to 24) activity of AtPoIIs in comparison to KF-AtPoIIs. Exonucleolysis started by the addition of 2mM MgCl₂, whereas primer extension started by addition of 2mM MgCl₂ and 50 μM of each dNTP. **C**) Primer extension reactions by by full-length and KF-AtPoIIs on a primer-template contain an AP site (tetrahydrofuran) in the templating position located immediately after the 3'-OH end of the primer. Time points were taken at 5, 10, 20, 40 and 80 sec after the addition of MgCl₂ Both full-length and Klenow fragment AtPoIIs bypass an AP site (lanes 1 to 20) The migration of the 19-mer primer and full length extension 28-mer product are labeled. **Fig. S6 SDS-PAGE showing the purification of KF AtPolIB deletions.** 10%SDS-PAGE gel showing the purification of KF-AtPolIB deletions with molecular weight between 85 to 95 KDa.

Fig. S7 Amino acid sequence alignment of the polymerase domain of AtPolIs in comparison to POLQ. Three insertions in POLQ and AtPolIs are indicated by a red and green line respectively.

Table S1 Oligonucleotides used in this study

Fig S1

| AtPoliA [1] AtPoliB [1] KF-DNAP1 [1] | INTS |
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| AtPolia [73] AtPolia [79] KF-ONAP I | HSVNORSSVVYNOEMELASESNINVERVYKTIKVGI(ZEVAETROVFCTVGAMEEMII-ELBERNOGIARILDDROYFNOE |
| AtPolia [156] AtPolia [156] KF-DNAP1 [324] | ISSAULTERORIDIERKURGTVESTENTLERELGTVGE |
| AtPolia [242] AtPolib [235] KF-DNAP I [332] | A 1 SRVERSTER SINVERILLSKTY DRVLLTVDNVQA-KEDT VALEVNQTHRI MILECTER VALEVNDISEL LEVST 1 VEDPLAP VANE T 1 SRVERSTER SINVERSILLSKTY DRVLLTVDNVQA-KEDT VALEVNQTHRI MILECTER VALEVNDISEL LEVST 1 VEDPLAP VANE T LEVET |
| AtPolia (332) AtPolii (325) KF-DNAP1 (387) | СТИМОЛОВИЕИТЕЛЕКТИКЕ КАЛОПИТИКОТИ ОТ ПОЛИТИКАТИ ОТ ОТ ПОЛИТИКАТИ ОТ ПОЛИТИКАТИ ОТ ПОЛИТИКАТИ ОТ |
| AtPolia [421] AtPolib [414] RF-DNAP I [459] | EXO III REARING IIMATI VARAKARDOSEGRIVVI PIVERLAREBRANI STALASI STALASI STALASI SAMADA ANALASI SAMADA ANALA |
| AtPolia (508) AtPolia (501) KF-DNAP I (525) | DIVINETWEIVVELLUWGENE DIVIDETWEIVVELLUWGENE -VYENTIMETUVVLUELENI |

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| | | | Palm |
| AtPolIA AtPolIB KF.DNAP I G. stearothermophilus Kien Tag | [702] [701] [397] [566] [521] | YVAFGGGERGEGACHALASLCEVCSIDGITAPTLAFCGGNVGGNVGGRDGRVT FDAFGGESGGRACHALALCEVCSIDGITAPTLAFCGSNVGGRDGRVT CONTRACTION AND AND AND AND AND AND AND AND AND AN | SLNI-NOR DEL ARRENICO Q'ALEKDRYKTER VAS SLNI-NOR SLA ARRENICO Q'ALEKDRYKTEON TAS SYRQAVANERL STDINION I VRNEGRETON TA- FRQAN ANDRIS STRINCT I VRNEGRETON VRS RENQTA ANDRIS SEDINICE I VRTPLOQUIE RATA- |
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Fig S7

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| PolQ [2422] GINGENTETVRICKRDGEVQTILGERRYLPGIKONNPERKAHAERQAINTIVQGSAADIVKIATVNIQKQLETEHSTEKSBOHRECH | MLQ |
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Table S1 Oligonucleotides used in this study

A Primers used for subcloning

| A1- N terminal | 5'-GGGAAGGGCATATGAGGCTTAGCTCTAGTCCATTACC-3' |
|----------------|---|
| | |
| AI- C terminal | 5-GUAUUAUUAUUIIAIIIAUUUUAIAUU-3 |
| B1- N terminal | 5'-GGGAAGGGCATATGCCACGCCGTCGTATCCTGTGTAC-3' |
| B1- C terminal | 5'-GGGAAGGG GGATCCTTATTTGCCCGCATACC-3' |

B Primers used for Site Directed mutagenesis

| mutA3-F | 5'-GGGAAGGGCATATGCTCGGGAAAATCTATGATAAA-3' |
|---------|--|
| mutA3-R | 5'-GGAGGAGGGATCCTTATTTAGCTGCATACCAATT-3' |
| mutB3-F | 5'-GGGAAGGGCATATGCTGAAGAAAATCTACAATCGCGTTC GTGTTGTGGATAATG-3' |
| mutB3-R | 5'-GGGAAGGG GGATCCTTATTTGCCCGCATACC-3' |
| AExo-F | 5'-GGCAGTGAGCGGCATCGAAGTT-3' |
| AExo-R | 5'-GTAGCGCAAGAATGCACATGATT-3' |
| BExo-F | 5'-GGCAGTGTCACGCATCGATGTTAAAAC-3' |
| BExo-R | 5'-GTTGCGCAAGCATGAACCAAGTTAC-3' |

C Primers used in DNA polymerization assays

| Assay | Polymerase and exonuclease |
|-------------------|---|
| oligo 24 labeled | 5' CGC AGC CCA CCT GCC CAC CTA ACT 3' |
| oligo 45 | 5' CCT TGG CAC TAG CGC AGG GCC AGT TAG GTG GGC AGG TGG GCT GCG 3' |
| | Strand-displacement |
| oligo 24 labeled | 5' CGC AGC CCA CCT GCC CAC CTA ACT 3' |
| oligo 65 | 5' CCT TGG CAC TAG CGC ACG ATG CCG CTA AGA ACC TCA GGG CCA GTT AGG TGG GCA GGT GGG CTG CG 3' |
| oligo 15 blocking | 5' CGC AGC CCA CCT GCC 3' |
| | Abasic site extension |
| Oligo 21 labeled | 5'CGA AGTACT AGT AAC GAC CCT 3' |
| Positive control | 5' ATA TAG GGT ACC GAC TGA AGC ACT GGA CGC CGT AAG TCA GGG TCG TTA CTA GTA CTT CG 3' |

| Abasic fidelity | 5' ATA TAG GGT ACC GAC TGA AGC ACT GGA CGC CG [abasic site] AAG TCA GGG TCG TTA CTA GTA CTT CG 3' |
|------------------|--|
| | Nucleotide insertion opposite canonical an abasic site and |
| | Steady-state kinetics |
| Oligo 24 labeled | 5' CGC AGC CCA CCT GCC CAC CTA ACT 3' |
| FideA | 5' CCT TGG CAC TAG CGC AGG GCA AGT TAG GTG GGC AGG TGG GCT GCG 3' |
| FideT | 5' CCT TGG CAC TAG CGC AGG GCT AGT TAG GTG GGC AGG TGG GCT GCG 3' |
| FideG | 5' CCT TGG CAC TAG CGC AGG GC G AGT TAG GTG GGC AGG TGG GCT GCG3' |
| FideC | 5' CCT TGG CAC TAG CGC AGG GGC AGT TAG GTG GGC AGG TGG GCT GCG 3' |
| Oligo 19 labeled | 5' TGT TAG CAG ACA AGC CGA T 3' |
| Abasic 28 | 5' AAG AGT AC [abasic site] ATC GGC TTG TCT GCT AAC A 3' |
| Long_8A labeled | 5' CGC AGC GGA CCT GCC CAC CTA ACT GAT ATA 3' |
| Abasic45 | 5' CCT TGG CAC TAG CGC [abasic site]AT ATC AGT TAG GTG GGC AGG TCC GCT GCG 3' |
| | Non-damaged and damaged template extension by deletions |
| Long8 labeled | 5' CGC AGC GGA CCT GCC CAC CTA ACT GAT AT 3' |
| C45 | 5' CCT TGG CAC TAG CGC TAT ATC AGT TAG GTG GGC AGG TCC GCT GCG 3' |
| Long_8A labeled | 5' CGC AGC GGA CCT GCC CAC CTA ACT GAT ATA 3' |
| Abasic45 | 5' CCT TGG CAC TAG CGC [abasic site]AT ATC AGT TAG GTG GGC AGG TCC GCT GCG 3' |