### Supplementary Material

#### Supplementary Tables

#### Supplementary Table S1. Strains and plasmids used in this study

| Strains     | Genotype             | Source                               |
|-------------|----------------------|--------------------------------------|
| E233S       | ΔpyrEFΔlacS          | Deng et al., 2009 (Deng et al., 2009) |
| Δdpo2       | ΔpyrEFΔlacSΔdpo2     | This work                            |
| Δdpo3       | ΔpyrEFΔlacSΔdpo3     | This work                            |
| Δdpo4       | ΔpyrEFΔlacSΔdpo4     | This work                            |

| Plasmids    | Features                                                        |
|-------------|-----------------------------------------------------------------|
| pSeSD       | A *Sulfolobus-E.coli* shuttle vector carrying an expression cassette controlled under a synthetic strong promoter ParaS-SD |
| pSeSD_dpo2  | pSeSD carrying Dpo2 encoding sequence                           |
| pSe-Rp      | The plasmid contains a DNA fragment of two tandem copies of CRISPR repeat sequences for the construction of the artificial mini-CRISPR array |
| pAC-dpo1, -2, -3, -4 | pSe-Rp carrying a spacer matching to the protospacer in the coding region of *dpo1*, -2, -3, -4 gene correspondingly in genome |
| pGE-dpo1, -2, -3, -4 | The genome-editing plasmid derived from pAC-dpo1, -2, -3, -4 respectively, with the corresponding |
| donor DNA inserted between SphI and XhoI |   |
**Supplementary Table S2. Oligos used in this study**

| Oligos | Sequence |
|--------|----------|
| **Construction of dpo mutant** |
| KDp1Larm-F | tttgcatgcCATAGAATTGAATAAGGAGCTTCTGG |
| KDp1SOE-R | CCTCCTGTGAAAGGAATATCAAATAAGGTAAGTTGCT |
| KDp1SOE-F | TTGATATTCCTTTCACAGGAGGAAAAGGGAATAATCA |
| KDp1Rarm-R | tttctcgagTGGAATATGAACCACGATGTC |
| KDp1spf | aagTTATTGGGTATATCAAAAGTTAAGGTGGAATACGCTAT |
| KDp1spr | ageATATTAGCGTATCCACCTTAACTTTGATATACCCAATA |
| KDp2 F | AGGATTTAGGGGATTGGA |
| KDp2 R | TGGAGGGGAACCATCGCC |
| *dpo* inner F | CCCACTTACGGAGATAGCCT |
| *dpo* inner R | CCTCCCTTATCCGCCATCAT |
| KDp2Larm-F | tttgcatgcGGAAGAACACAGCCATCATCA |
| KDp2SOE-R | CCTCCTCAAGTACTCCTCCATTTTCTCGCATTC |
| KDp2SOE-F | GAAATGGAGGTGACTTGGAGGAGGTTATGAC |
| KDp2Rarm-R | tttctcgagACCTCCATCATACCTTTA |
| KDp2spf | aagTGATTGGTAAATACACATTTCAGGGAACCGTTGACG |
| KDp2spr | ageCGTCAACCGTTTCAGGGGATGTTGATTTTACAATCAA |
| KDp3 F | CTAGTGGCCGATGACGCT |
| Code  | Primer Sequence                  |
|-------|----------------------------------|
| KOdpo3 R | TGAGAAAGTTCAAGTGCGAGA           |
| dpo3 inner F | TTCTTTCGCACTATGAGGGT          |
| dpo3 inner R | AGATCATCCATGCTTTTCGCTCT       |
| KOdpo3Larm-F | ttgcatgcCATGCATGCTCCGAGAGTATCTTTATCCCT |
| KOdpo3SOE-R | ATTTCTTCTTAGA ACTACCAAATGACTGGCT |
| KOdpo3SOE-F | TCA T TGGTTAGT TCTAAGAGAAATAATGTCA GCTAAA |
| KOdpo3Rarm-R | tttctegacCGCTCGAGTTAGACAGGATTGAGACTGC |
| KOdpo3spf | aagCTAATTTACATTTGGAGCATTGATGATGAAGGTAAACAGTT |
| KOdpo3spr | ageA ACTGT TTTCACTCATCAAT GCTCCAAATGTA AAT TAG |
| KOdpo4 F | CTCTCTCTCCAGCGAAATCAG          |
| KOdpo4 R | ATGGGCAAGAAAGGGGCAAA          |
| dpo4 inner F | ATGGGCAAGGCAAATGGGAT          |
| dpo4 inner R | TGGCTTTAGCCTCACCAATTA          |
| KOdpo4Larm-F | ttt gc atgc CATGCATGCTATC TTCT CTCTCCACCTT |
| KOdpo4SOE-R | CTAAACCTTACTCCGCT AAAAGTAGTC AAAATCAACGA |
| KOdpo4SOE-F | GACTACTTTTACCGGGAGT AAGGT TTAGCA AATTCATC |
| KOdpo4Rarm-R | tt tct egacCGCTCGAGCATG TGAAGACCTTTGG |
| KOdpo4spf | aagATAGTTGAAGGCAA AAATTTTACCTAATGCAGTTTACT |
| KOdpo4spr | ageAGTAAA ACTGCATTAGGTAA AATTTTTTCTTTGCTTC AACATAT |
### Overexpression of *dpo2*

|              | Sequence                      |
|--------------|-------------------------------|
| *dpo2*-NdeI-F | TCCACCTcatatgCGAGAAATGGAGGAGTACGT |
| *dpo2*-SalI-R | ATTTgtcgacACATCTAGAGATCACCTCT    |

### Others

|              | Sequence                      |
|--------------|-------------------------------|
| Sisapt-F     | TACCCGGATCATATAACCCAG          |
| Sisapt-R     | AAGGTTTTTTGTGGTGGTGAT          |
Supplementary Table S3. Dpo2 homologues in crenarchaeal species

| Dpo2 Homologue | Species                          | Size (aa) | Identity to SisDpo2 (%) | Similarity to SisDpo2 (%) |
|----------------|----------------------------------|-----------|-------------------------|---------------------------|
| Sis            | *Sulfolobus islandicus* Rey15A   | 555       | 100                     | 100                       |
| Sso            | *Sulfolobus solfataricus* P2     | 561       | 91                      | 96                        |
| Sto            | *Sulfolobus tokodaii* str. 7     | 540       | 68                      | 81                        |
| Sac            | *Sulfolobus acidocaldarius* DSM639 | 582       | 54                      | 72                        |
| Ahos           | *Acidianus hospitalis* W1        | 554       | 56                      | 73                        |
| Mese           | *Metallosphaera sedula* DSM5348  | 562       | 53                      | 71                        |
| Mecu           | *Metallosphaera cuprina* AR-4    | 562       | 53                      | 73                        |
| Ffo            | *Fervidicoccus fontis* Kam940    | 541       | 31                      | 52                        |
| Calag          | *Caldisphaera lagunensis* DSM 15908 | 624       | 29                      | 46                        |
| Aca            | *Aeropyrum camini* SY1           | 636       | 30                      | 47                        |
| Ape            | *Aeropyrum pernix* K1            | 633       | 32                      | 48                        |
| Tagg           | *Thermosphaera aggregans* DSM 11486 | 636       | 32                      | 51                        |
| Tcal           | *Thermogladius calderae* 1633    | 644       | 29                      | 45                        |
| Smar           | *Staphylothermus marinus* M1     | 648       | 34                      | 55                        |
| Shell          | *Staphylothermus hellenicus* DSM 12710 | 648       | 36                      | 59                        |
Supplementary Figure S1. Effect of NQO on cell growth and expression of DNA polymerases in S. islandicus

(A) Growth curve of the wild-type strain of S. islandicus E233S in the presence of NQO. NQO was added to exponential growth cultures (A600nm=0.2) at the concentration of 0, 1, 2 and 3 μM, and incubated for 24 h during which cell samples were taken for monitoring their A600 values.

(B) Expression profiles of the four DNA polymerases revealed by western analysis. 10 μg of total cell extracts of NQO-treated samples (1, 2, 3 μM) and the untreated reference (CK) were used for the immunoblotting analysis using antibodies against each DNA polymerase. PCNA1, which has a constant expression upon DNA damage, serves as a loading control.

(C) Quantification of relative amounts of Dpo2 in samples taken from cultures incubated with different concentrations of NQO.
Supplementary Figure S2. Cell growth and western blotting analysis of dpo2-overexpression strain and its reference

(A) Exponentially growing cultures (A600nm=0.2) were incubated with 0, 1, 2 and 3 μM NQO for 24 hours. The A600nm value of each culture at 0h and 24 hour after NQO addition was plotted. Three independent experiments were performed with the standard deviation shown in the error bar. Unpaired t test was performed for each group of data, with p values indicated in the graph.

(B) Cell samples were taken at 6 hours after NQO addition and cell extracts were obtained by sonication and centrifugation. 10 μg of cell extracts of NQO-treated and control (CK) samples were used for immunoblotting analysis using Dpo2 and Penta-His Tag antibodies. To estimate the relative amounts of overexpressed Dpo2, samples of overexpression strain were diluted by 20 times individually and used for the western blot analysis. PCNA1 serves as an internal control. 1N, 2N and 3N refers to the sample incubated with 1, 2 and 3 μM NQO respectively.
**Supplementary Figure S3. Expression of each DNA polymerase in different strains upon NQO treatment**

Exponentially growing cultures (A600nm=0.2) were incubated with 2 μM NQO for 6 hours and samples were taken for the preparation of cell extracts. Equal amount of cell extracts (10 μg) for each sample were used in western blotting assay using antibodies against each DNA polymerase. PCNA1 serves as an internal control.
Supplementary Figure S4. Expression of each DNA polymerase in different strains post UV irradiation

Exponentially growing cultures (A600nm=0.2) were exposed to 50 J/m² UV-C light, then, the treated cultures were allowed to recover for 6 h under the dark condition with shaking. Cell extracts were prepared and equal amounts of cell extracts (10 μg) for each sample were used for the western blotting assay using antibodies against each DNA polymerase. PCNA1 serves as an internal control.
Supplementary Figure S5. Dpo2 homologues carry a substitution at the PolC motif

Structure-based sequence alignment of the PolC motif of Dpo2 homologues. The mutated aspartate in the PolC motif (YGDTDS) was indicated by the asterisk symbol. The structure of *Thermococcus gorgonarius* PolB (1tgo) was used as the template for the structure-based sequence alignment, which was performed using PROMALS3D webserver (Pei et al., 2008) and depicted using Espript 3 (Robert and Gouet, 2014). SisDpo1 harboring the canonical PolC motif was shown as the control.
Supplementary Figure S6. Sequence alignment of conserved regions of Dpo2 homologues

The structure of *Thermococcus gorgonarius* PolB (1tgo) was used as the template for the structure-based sequence alignment, which was performed using PROMALS3D webserver (Pei et al., 2008) and depicted by Espript 3 (Robert and Gouet, 2014). SisDpo1 belonging to PolB1 family was shown as a control. Framed sequences indicates a 12 aa sequence insertion in *Thermogladius calderae* PolB2 (Tcal).
Supplementary Figure S7. Phylogenetic tree of Dpo2 homologues

The tree was constructed using sequences of Dpo2 homologs extracted from NCBI. These sequences were first aligned using MUSCLE, then the poorly aligned regions were removed by Gblocks program (v0.91b) using the default setting. The phylogenetic tree was constructed using the trimmed sequences with the PhyML program (v3.0) and the tree was visualized by using the TreeDyn program (v198.3). Sis_Dpo1 and Sis_Dpo3 sequences are used as the outgroup.
Supplementary Figure S8. Phylogenetic tree of crenarchaeal species

Phylogenetic trees of representative crenarchaeal species were constructed using their 16S rDNA sequences retrieved from the NCBI databases. The 16S rDNA sequences were first aligned using MUSCLE program, then, poorly aligned regions were removed by Gblocks program (v0.91b) using the default setting. The phylogenetic tree was constructed using the trimmed sequences with the PhyML program (v3.0) and visualized by the TreeDyn program (v198.3). The 16s rDNA sequence of *Haloferax volcanii* DS2 was used as the outgroup.
Supplementary Figure S9. Sequence alignment of YxGG/A and PolA motif of archaeal PolB2 homologs

The structure of *Thermococcus gorgonarius* PolB (1tgo) was used as the template to conduct structure-based sequence alignments using PROMALS3D webserver (Pei et al., 2008), and the resulting data were depicted by Espript 3 (Robert and Gouet, 2014). Conserved residues are highlighted by yellow background and identical ones are in the red. Variations in the YxGG/A and PolA motifs of PolB2s from *Aeropyrum pernix K1* (Ape) and *Aeropyrum camini* SY1 are framed and indicated by red arrows.
Supplementary Figure S10. Spontaneous mutation spectra of apt3 locus in WT and Δdpo4

Mutated bases are shown in red on the top of original ones. Single base deletions are indicated with blue “−” signs above the deleted bases, and single base insertions are indicated by green “+” signs beneath the bases immediately before the insertion positions with the inserted shown in green. Large insertions (>2bp) are shown with black triangle signs. Numbers in the bracket indicate the sample size (total number of analyzed mutants).
Supplementary Figure S11. DNA damage-induced mutation hotspots in the apt3 locus

Only mutation hotspots are shown with their locations in the apt3 locus indicated. The mutated bases of treated and reference samples are shown on the top of and under the original ones respectively. Tandem mutations are double underlined. Single base deletions are indicated with blue “−” signs above/beneath the deleted bases, and single base insertions are indicated by green “+” signs above/beneath the bases immediately before the insertion positions with the inserted shown in green. Large insertions (>2bp) are shown with black triangle signs. Numbers in the bracket indicate the sample size (total number of analyzed mutants).
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