SUPPLEMENTAL DATA

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Structural and functional analysis of Sulfolobus solfataricus Y-family DNA polymerase Dpo4-catalyzed bypass of the malondialdehyde-deoxyguanosine adduct

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Figure S1. Comparison of Dpo4-catalyzed incorporation of dCTP and dATP opposite M₁dG and N²-OPdG. A, Dpo4 (5 nM) was incubated with radiolabeled 18/23-mer DNA (50 nM, substrate 1), the indicated dNTP (1 mM), and MgCl₂ (5 mM). Four different conditions, which are described in the materials and methods section, were used to analyze Dpo4 catalysis opposite the ring open (condition 2) and closed (conditions 1, 3, and 4) forms of the lesion. The data was fit by linear regression to calculate the velocity of dNTP incorporation: dCTP condition 1 (■): $v = 2.0 \pm 0.2 \text{ s}^{-1} \times 10^3$, y-intercept = $1.5 \pm 0.3 \text{ nM}$; dCTP condition 2 (□): $v = 1.9 \pm 0.6 \text{ s}^{-1} \times 10^3$, y-intercept = $3.8 \pm 0.7 \text{ nM}$; dCTP condition 3 (●): $v = 2.8 \pm 0.6 \text{ s}^{-1} \times 10^3$, y-intercept = $3.7 \pm 0.7 \text{ nM}$; dCTP condition 4 (○): $v = 1.9 \pm 0.6 \text{ s}^{-1} \times 10^3$, y-intercept = $3.5 \pm 0.6 \text{ nM}$; B, Results for dATP incorporation. dATP condition 1 (■): $v = 11.2 \pm 0.1 \text{ s}^{-1} \times 10^3$; dATP condition 2 (□): $v = 10.6 \pm 0.2 \text{ s}^{-1} \times 10^3$; dATP condition 3 (●): $v = 13.8 \pm 0.4 \text{ s}^{-1} \times 10^3$; dATP condition 4 (○): $v = 11.6 \pm 0.2 \text{ s}^{-1} \times 10^3$. All velocities are normalized to enzyme concentration and reported in units of s⁻¹ × 10³.
Table S1
Observed and theoretical MS fragmentation for Dpo4-catalyzed insertion of C opposite M1dG followed by accurate full-length extension.

5'-pTCCGTGA-3'

| Fragment Assignment | Observed | Theoretical |
|---------------------|----------|-------------|
| 5'-pT (a2-B, -1)    | 481.2    | 481.0       |
| 5'-pTC (a3-B, -1)   | 770.2    | 770.1       |
| 5'-pTCC (a4-B, -1)  | 1059.1   | 1059.1      |
| 5'-pTCCG (a5-B, -1) | 1388.0   | 1388.2      |
| (a5-B, -2)          | 693.6    | 693.6       |
| 5'-pTCCGT (a6-B, -1) | 1691.9  | 1692.2      |
| (a6-B, -2)          | 845.6    | 845.6       |
| p-CCGTGA-3' (w6, -2) | 934.6  | 934.6       |
| p-CGTGA-3' (w5, -1) | 1581.2  | 1581.2      |
| p-GTGA-3' (w4, -1)  | 1292.1   | 1292.2      |
| p-TGA-3' (w3, -1)   | 963.1    | 963.2       |
| p-GA-3' (w2, -1)    | 658.9    | 659.1       |
| p-A-3' (w1, -1)     | 330.0    | 330.0       |
Table S2

Observed and theoretical MS fragmentation for Dpo4-catalyzed insertion of C opposite M1dG followed by accurate full-length extension and blunt-end addition of A.

5’-pTCCGTGAA-3’

| Fragment Assignment       | Observed | Theoretical |
|---------------------------|----------|-------------|
| 5’-pT (a2-B, -1)          | 481.2    | 481.0       |
| 5’-pTC (a3-B, -1)         | 770.1    | 770.1       |
| 5’-pTCC (a4-B, -1)        | 1059.1   | 1059.1      |
| 5’-pTCCG (a5-B, -1)       | 1388.0   | 1388.2      |
| (a5-B, -2)                | 693.6    | 693.6       |
| 5’-pTCCGT (a6-B, -1)      | 1692.0   | 1692.2      |
| (a6-B, -2)                | 845.7    | 845.6       |
| 5’-pTCCGTG (a7-B, -2)     | 1010.2   | 1010.1      |
| p-CCGTGAA-3’ (w7, -2)     | 1091.2   | 1091.1      |
| p-CGTGAA-3’ (w6, -1)      | 1894.0   | 1894.3      |
| (w6, -2)                  | 946.6    | 946.6       |
| p-GTGAA-3’ (w5, -1)       | 1605.1   | 1605.3      |
| p-TGAA-3’ (w4, -1)        | 1276.1   | 1276.1      |
| p-GAA-3’ (w3, -1)         | 972.2    | 972.2       |
| p-AA-3’ (w2, -1)          | 643.2    | 643.1       |
| p-A-3’ (w1, -1)           | 330.2    | 330.0       |
Table S2
Observed and theoretical MS fragmentation for Dpo4-catalyzed insertion of C opposite M1dG followed by accurate full-length extension and blunt-end addition of C.

5'-pTCCGTGAC-3'

| Fragment Assignment | Observed | Theoretical |
|---------------------|----------|-------------|
| 5'-pT (a2-B, -1)    | 481.1    | 481.0       |
| 5'-pTC (a3-B, -1)   | 770.2    | 770.1       |
| 5'-pTCC (a4-B, -1)  | 1059.2   | 1059.1      |
| 5'-pTCCG (a5-B, -1) | 1387.8   | 1388.2      |
| 5'-pTCCGT (a6-B, -1)| 1691.8   | 1692.2      |
| (a6-B, -2)          | 845.6    | 845.6       |
| 5'-pTCCGTG (a7-B, -2)| 1010.0  | 1010.1      |
| p-CCGTGAC-3' (w7, -2)| 1079.0  | 1079.2      |
| p-CGTGAC-3' (w6, -1) | 1869.8  | 1870.3      |
| (w6, -2)            | 934.4    | 934.6       |
| p-GTGAC-3' (w5, -1) | 1581.1   | 1581.2      |
| p-TGAC-3' (w4, -1)  | 1252.0   | 1252.2      |
| p-GAC-3' (w3, -1)   | 948.1    | 948.1       |
| p-AC-3' (w2, -1)    | 619.2    | 619.1       |
| p-C-3' (w1, -1)     | -        | 306.0       |
Table S4
Observed and theoretical MS fragmentation for Dpo4-catalyzed insertion of A opposite M1dG followed by accurate full-length extension.

| Fragment Assignment | Observed | Theoretical |
|---------------------|----------|-------------|
| 5’-pT (a2-B, -1)    | 481.1    | 481.0       |
| 5’-pTC (a3-B, -1)   | 770.1    | 770.1       |
| 5’-pTCA (a4-B, -1)  | 1083.0   | 1083.1      |
| 5’-pTCAG (a5-B, -1) | 1412.2   | 1412.2      |
| (a5-B, -2)          | 705.6    | 705.6       |
| 5’-pTCAGT (a6-B, -1)| 1715.9   | 1716.2      |
| (a6-B, -2)          | 857.7    | 857.6       |
| p-CAGTGA-3’ (w6, -2)| 946.6    | 946.6       |
| p-AGTGA-3’ (w5, -1) | 1605.1   | 1605.3      |
| p-GTGA-3’ (w4, -1)  | 1292.2   | 1292.2      |
| p-TGA-3’ (w3, -1)   | 963.2    | 963.2       |
| p-GA-3’ (w2, -1)    | 659.2    | 659.1       |
| p-A-3’ (w1, -1)     | 330.2    | 330.0       |
Table S5
Observed and theoretical MS fragmentation for Dpo4-catalyzed insertion of A opposite M1dG followed by accurate full-length extension and blunt-end addition of A.

| Fragment Assignment | Observed | Theoretical |
|---------------------|----------|-------------|
| 5'-pT (a2-B, -1)    | 481.1    | 481.0       |
| 5'-pTC (a3-B, -1)   | 770.2    | 770.1       |
| 5'-pTCA (a4-B, -1)  | 1083.1   | 1083.1      |
| 5'-pTCAG (a5-B, -1) | 1411.9   | 1412.2      |
|                    (a5-B, -2) | 705.1    | 705.6       |
| 5'-pTCAGT (a6-B, -1)| 1715.9   | 1716.2      |
|                    (a6-B, -2) | 857.6    | 857.6       |
| 5'-pTCAGTG (a7-B, -2)| 1022.1  | 1022.1      |
| p-CAGTGAA-3’ (w7, -2) | 1103.1  | 1103.2      |
| p-AGTGAA-3’ (w6, -1) | 1918.0  | 1918.3      |
|                    (w6, -2) | 958.6    | 958.6       |
| p-GTGAA-3’ (w5, -1) | 1605.0   | 1605.3      |
| p-TGAA-3’ (w4, -1)  | 1276.1   | 1276.1      |
| p-GAA-3’ (w3, -1)   | 972.0    | 972.2       |
| p-AA-3’ (w2, -1)    | 643.2    | 643.1       |
| p-A-3’ (w1, -1)     | -        | 330.0       |
Table S6
Observed and theoretical MS fragmentation for Dpo4-catalyzed insertion of A opposite M1dG followed by accurate full-length extension and blunt-end addition of G.

5'-pTCAGTGAG-3'

| Fragment Assignment          | Observed | Theoretical |
|-----------------------------|----------|-------------|
| 5'-pT (a2-B, -1)            | 481.2    | 481.0       |
| 5'-pTC (a3-B, -1)           | 770.0    | 770.1       |
| 5'-pTCA (a4-B, -1)          | 1083.2   | 1083.1      |
| 5'-pTCAG (a5-B, -1)         | 1412.2   | 1412.2      |
| 5'-pTCAGT (a6-B, -1)        | 1716.1   | 1716.2      |
| (a6-B, -2)                  | 857.6    | 857.6       |
| 5'-pTCAGTG (a7-B, -2)       | 1022.0   | 1022.1      |
| p-CAGTGAG-3' (w7, -2)       | 1110.8   | 1111.2      |
| p-AGTGAG-3' (w6, -1)        | 1933.9   | 1934.3      |
| (w6, -2)                    | 966.0    | 966.6       |
| p-GTGAG-3' (w5, -1)         | 1621.2   | 1621.3      |
| p-TGAG-3' (w4, -1)          | 1292.1   | 1292.2      |
| p-GAG-3' (w3, -1)           | 988.2    | 988.2       |
| p-AG-3' (w2, -1)            | 659.1    | 659.1       |
| p-G-3' (w1, -1)             | -        | 346.0       |
**Table S7**
Observed and theoretical MS fragmentation for Dpo4-catalyzed insertion of A opposite M1dG followed by insertion of 5’-ATGA-3’.

5’-pTCAATGA-3'

| Fragment Assignment | Observed | Theoretical |
|---------------------|----------|-------------|
| 5’-pT (a2-B, -1)    | 481.1    | 481.0       |
| 5’-pTC (a3-B, -1)   | 770.0    | 770.1       |
| 5’-pTCA (a4-B, -2)  | 541.0    | 541.1       |
| 5’-pTCAA (a5-B, -1) | 1395.8   | 1396.2      |
| (a5-B, -2)          | 697.6    | 697.6       |
| 5’-pTCAAT (a6-B, -1)| 1700.0   | 1700.2      |
| (a6-B, -2)          | 849.7    | 849.6       |
| p-CAATGA-3’ (w6, -2)| 938.7    | 938.6       |
| p-AATGA-3’ (w5, -1) | 1589.1   | 1589.3      |
| p-ATGA-3’ (w4, -1)  | 1276.2   | 1276.2      |
| p-TGA-3’ (w3, -1)   | 963.0    | 963.2       |
| p-GA-3’ (w2, -1)    | 659.2    | 659.1       |
| p-A-3’ (w1, -1)     | 329.9    | 330.0       |
Table S8
Observed and theoretical MS fragmentation for Dpo4-catalyzed insertion of A opposite M1dG followed by insertion of 5'-TGA-3'.

5'-pTCATGA-3'

| Fragment Assignment       | Observed | Theoretical |
|---------------------------|----------|-------------|
| 5'-pT (a2-B, -1)          | 481.1    | 481.0       |
| 5'-pTC (a3-B, -1)         | 770.0    | 770.1       |
| 5'-pTCA (a4-B, -1)        | 1083.2   | 1083.1      |
| 5'-pTCAT (a5-B, -1)       | 1387.7   | 1387.2      |
| (a5-B, -2)                | 693.2    | 693.1       |
| p-CATGA-3' (w5, -1)       | 1565.8   | 1565.3      |
| (w5, -2)                  | 782.6    | 782.1       |
| p-ATGA-3' (w4, -1)        | 1276.1   | 1276.2      |
| p-TGA-3' (w3, -1)         | 963.2    | 963.2       |
| p-GA-3' (w2, -1)          | 659.2    | 659.1       |
| p-A-3' (w1, -1)           | 330.2    | 330.0       |
Table S9
Observed and theoretical MS fragmentation for Dpo4-catalyzed insertion of A opposite M1dG followed by insertion of 5’-CGG-3’.

| Fragment Assignment | Observed | Theoretical |
|---------------------|----------|-------------|
| 5’-pT (a2-B, -1)    | 481.0    | 481.0       |
| 5’-pTC (a3-B, -1)   | 770.2    | 770.1       |
| 5’-pTCA (a4-B, -1)  | 1083.0   | 1083.1      |
| 5’-pTCAC (a5-B, -1) | 1372.1   | 1372.2      |
|                     | 685.7    | 685.6       |
| p-CACGG-3’ (w5, -1) | 1565.9   | 1565.2      |
|                     | 782.6    | 782.6       |
| p-ACGG-3’ (w4, -1)  | 1277.0   | 1277.2      |
| p-CGG-3’ (w3, -1)   | 964.1    | 964.1       |
| p-GG-3’ (w2, -1)    | 675.2    | 675.1       |
| p-G-3’ (w1, -1)     | 346.2    | 346.0       |
Table S10
Observed and theoretical MS fragmentation for Dpo4-catalyzed insertion of A opposite M1dG followed by insertion of 5'-CG-3'.

| Fragment Assignment | Observed | Theoretical |
|---------------------|----------|-------------|
| 5'-pT (a2-B, -1)    | 481.1    | 481.0       |
| 5'-pTC (a3-B, -1)   | 770.5    | 770.1       |
| 5'-pTCA (a4-B, -1)  | 1083.1   | 1083.1      |
| p-CACG-3' (w4, -1)  | 1237.2   | 1237.2      |
| p-ACG-3' (w3, -1)   | 948.1    | 948.2       |
| p-CG-3' (w2, -1)    | 635.2    | 635.1       |
| p-G-3' (w1, -1)     | 346.2    | 346.0       |

Table S11
Observed and theoretical MS fragmentation for Dpo4-catalyzed generation of a -1 frameshift deletion during M1dG bypass.

| Fragment Assignment | Observed | Theoretical |
|---------------------|----------|-------------|
| 5'-pT (a2-B, -1)    | 481.1    | 481.0       |
| 5'-pTC (a3-B, -1)   | 770.1    | 770.1       |
| 5'-pTCG (a4-B, -1)  | 1099.1   | 1099.1      |
| 5'-pTCGT (a5-B, -1) | 1402.9   | 1403.1      |
| (a5-B, -2)          | 701.2    | 701.1       |
| p-CGTGA-3' (w5, -2) | 790.1    | 790.1       |
| p-GTGA-3' (w4, -1)  | 1292.1   | 1292.2      |
| p-TGA-3' (w3, -1)   | 963.1    | 963.2       |
| p-GA-3' (w2, -1)    | 659.2    | 659.1       |
| p-A-3' (w1, -1)     | 330.2    | 330.0       |
Table S12
Observed and theoretical MS fragmentation for Dpo4-catalyzed insertion of T opposite M$_1$dG followed by accurate full-length extension.

| Fragment Assignment | Observed | Theoretical |
|---------------------|----------|-------------|
| 5'-pT (a2-B, -1)    | 481.0    | 481.0       |
| 5'-pTC (a3-B, -1)   | 770.0    | 770.1       |
| 5'-pTCT (a4-B, -1)  | 1073.9   | 1074.1      |
| 5'-pTCTG (a5-B, -1) | 1403.5   | 1403.1      |
| (a5-B, -2)          | 701.2    | 701.1       |
| 5'-pTCTGT (a6-B, -1)| 1707.6   | 1707.2      |
| (a6-B, -2)          | 853.0    | 853.1       |
| p-CTGTGA-3' (w6, -2)| 942.7    | 942.1       |
| p-TGTGA-3' (w5, -1) | 1595.9   | 1596.2      |
| p-GTGA-3' (w4, -1)  | 1292.5   | 1292.2      |
| p-TGA-3' (w3, -1)   | 963.1    | 963.2       |
| p-GA-3' (w2, -1)    | 659.1    | 659.1       |
| p-A-3' (w1, -1)     | 328.6    | 330.0       |
Table S13
Observed and theoretical MS fragmentation for Dpo4-catalyzed insertion of C opposite M1dG followed by accurate full-length extension.

5'-pTCCATGA-3'

| Fragment Assignment | Observed | Theoretical |
|---------------------|----------|-------------|
| 5'-pT (a2-B, -1)    | 481.1    | 481.0       |
| 5'-pTC (a3-B, -1)   | 770.1    | 770.1       |
| 5'-pTCC (a4-B, -1)  | 1059.1   | 1059.1      |
| 5'-pTCCA (a5-B, -1) | 1372.0   | 1372.2      |
| (a5-B, -2)          | 685.7    | 685.6       |
| 5'-pTCCAT (a6-B, -1)| 1676.0   | 1676.2      |
| p-CCATGA-3’ (w6, -1)| 1853.8   | 1854.3      |
| (w6, -2)            | 926.7    | 926.6       |
| p-CATGA-3’ (w5, -1) | 1565.1   | 1565.3      |
| (w5, -2)            | 782.2    | 782.1       |
| p-ATGA-3’ (w4, -1)  | 1276.2   | 1276.2      |
| p-TGA-3’ (w3, -1)   | 963.2    | 963.2       |
| p-GA-3’ (w2, -1)    | 659.2    | 659.1       |
| p-A-3’ (w1, -1)     | 330.1    | 330.0       |
Table S14
Observed and theoretical MS fragmentation for Dpo4-catalyzed insertion of C opposite M1dG followed by accurate full-length extension and blunt-end addition of A.

5'-pTCCATGAA-3'

| Fragment Assignment   | Observed | Theoretical |
|-----------------------|----------|-------------|
| 5'-pT (a2-B, -1)      | 481.1    | 481.0       |
| 5'-pTC (a3-B, -1)     | 770.1    | 770.1       |
| 5'-pTCC (a4-B, -1)    | 1059.0   | 1059.1      |
| 5'-pTCCA (a5-B, -1)   | 1372.0   | 1372.2      |
|                       | (a5-B, -2) | 685.6      | 685.6       |
| 5'-pTCCAT (a6-B, -1)  | 1675.8   | 1676.2      |
|                       | (a6-B, -2) | 837.6      | 837.6       |
| 5'-pTCCATG (a7-B, -2) | 1002.1  | 1002.1      |
| p-CCATGAA-3' (w7, -2) | 1083.2  | 1083.2      |
| p-CATGAA-3' (w6, -1)  | 1877.9   | 1878.3      |
|                       | (w6, -2) | 938.6      | 938.6       |
| p-ATGAA-3' (w5, -1)   | 1589.1   | 1589.3      |
|                       | (w5, -2) | 794.2      | 794.1       |
| p-TGAA-3' (w4, -1)    | 1275.9   | 1276.2      |
| p-GAA-3' (w3, -1)     | 972.2    | 972.2       |
| p-AA-3' (w2, -1)      | 643.2    | 643.1       |
| p-A-3' (w1, -1)       | 330.1    | 330.0       |
Table S15
Observed and theoretical MS fragmentation for Dpo4-catalyzed insertion of C opposite M1dG followed by accurate full-length extension and blunt-end addition of C.

5’-pTCCATGAC-3’

| Fragment Assignment | Observed  | Theoretical |
|---------------------|-----------|-------------|
| 5’-pT (a2-B, -1)    | 481.1     | 481.0       |
| 5’-pTC (a3-B, -1)   | 770.1     | 770.1       |
| 5’-pTCC (a4-B, -1)  | 1058.9    | 1059.1      |
| 5’-pTCCA (a5-B, -1) | 1371.9    | 1372.2      |
| 5’-pTCCAT (a6-B, -2) | 837.4     | 837.6       |
| 5’-pTCCATG (a7-B, -2) | 1002.1    | 1002.1      |
| p-CCATGAC-3’ (w7, -2) | 1071.5    | 1071.2      |
| p-CATGAC-3’ (w6, -2) | 926.4     | 926.6       |
| p-ATGAC-3’ (w5, -1) | 1565.0    | 1565.3      |
| p-TGAC-3’ (w4, -1)  | 1252.1    | 1252.2      |
| p-GAC-3’ (w3, -1)   | 948.0     | 948.2       |
| p-AC-3’ (w2, -1)    | 619.1     | 619.1       |
| p-C-3’ (w1, -1)     | 306.1     | 306.0       |
Table S16
Observed and theoretical MS fragmentation for Dpo4-catalyzed insertion of C opposite M1dG followed by accurate full-length extension and blunt-end addition of G.

5'-pTCCATGAG-3'

| Fragment Assignment               | Observed | Theoretical |
|-----------------------------------|----------|-------------|
| 5'-pT (a2-B, -1)                  | 481.1    | 481.0       |
| 5'-pTC (a3-B, -1)                 | 770.1    | 770.1       |
| 5'-pTCC (a4-B, -1)                | 1058.9   | 1059.1      |
| 5'-pTCCA (a5-B, -1)               | 1371.9   | 1372.2      |
| 5'-pTCCAT (a6-B, -2)              | 837.4    | 837.6       |
| 5'-pTCCATG (a7-B, -2)             | 1002.1   | 1002.1      |
| p-CCATGAG-3’ (w7, -2)             | 1090.9   | 1091.2      |
| p-CATGAG-3’ (w6, -2)              | 946.4    | 946.6       |
| p-ATGAG-3’ (w5, -1)               | 1604.6   | 1605.3      |
| (w5, -2)                          | 802.3    | 802.1       |
| p-TGAG-3’ (w4, -1)                | 1292.2   | 1292.2      |
| (w4, -2)                          | 645.7    | 645.6       |
| p-GAG-3’ (w3, -1)                 | 988.8    | 988.2       |
| p-AG-3’ (w2, -1)                  | 659.2    | 659.1       |
| p-G-3’ (w1, -1)                   | 346.0    | 346.0       |
Table S17
Observed and theoretical MS fragmentation for Dpo4-catalyzed insertion of C opposite M1dG followed by insertion of 5'-GGG-3'.

| Fragment Assignment | Observed | Theoretical |
|---------------------|----------|-------------|
| 5'-pT (a2-B, -1)    | 481.1    | 481.0       |
| 5'-pTC (a3-B, -1)   | 770.1    | 770.1       |
| 5'-pTCC (a4-B, -1)  | 1059.1   | 1059.1      |
| 5'-pTCCG (a5-B, -1) | 1387.2   | 1388.2      |
| p-CCGGG-3' (w5, -1) | 1582.2   | 1582.2      |
| (w5, -2)            | 789.9    | 790.6       |
| p-CGGG-3' (w4, -1)  | 1293.2   | 1293.3      |
| p-GGG-3' (w3, -1)   | 1004.4   | 1004.2      |
| p-GG-3' (w2, -1)    | 675.1    | 675.1       |
| p-G-3' (w1, -1)     | 346.2    | 346.0       |

Table S18
Observed and theoretical MS fragmentation for Dpo4-catalyzed insertion of 5'-AT-3'.

| Fragment Assignment | Observed | Theoretical |
|---------------------|----------|-------------|
| 5'-pT (a2-B, -1)    | 481.0    | 481.0       |
| 5'-pTC (a3-B, -1)   | 770.1    | 770.1       |
| p-CAT-3' (w3, -1)   | 923.2    | 923.2       |
| p-AT-3' (w2, -1)    | 634.2    | 634.1       |
| p-T-3' (w1, -1)     | 321.2    | 321.0       |
Table S19
Observed and theoretical MS fragmentation for Dpo4-catalyzed incorporation of 5’-ATGAA-3’.

| Fragment Assignment | Observed | Theoretical |
|---------------------|----------|-------------|
| 5’-pT (a2-B, -1)    | 481.2    | 481.0       |
| 5’-pTC (a3-B, -1)   | 770.1    | 770.1       |
| 5’-pTCA (a4-B, -1)  | 1083.0   | 1083.1      |
| 5’-pTCAT (a5-B, -1) | 1371.9  | 1387.2      |
|                    (a5-B, -2) | 693.6   | 693.1       |
| 5’-pTCATG (a6-B, -1)| 1715.7  | 1716.2      |
|                    (a6-B, -2) | 857.7   | 857.6       |
| p-CATGAA-3’ (w6, -2)| 938.6  | 938.6       |
| p-ATGAA-3’ (w5, -1) | 1589.2 | 1589.3      |
|                    (w5, -2) | 794.2   | 794.1       |
| p-TGAA-3’ (w4, -1)  | 1276.1  | 1276.2      |
| p-GAA-3’ (w3, -1)   | 972.2   | 972.2       |
| p-AA-3’ (w2, -1)    | 643.2   | 643.1       |
| p-A-3’ (w1, -1)     | 330.0   | 330.0       |
Table S20
Observed and theoretical MS fragmentation for Dpo4-catalyzed incorporation of 5’-ATGGA-3’.

5’-pTCATGGA-3’

| Fragment Assignment | Observed | Theoretical |
|---------------------|----------|-------------|
| 5’-pT (a2-B, -1)    | 481.1    | 481.0       |
| 5’-pTC (a3-B, -1)   | 770.1    | 770.1       |
| 5’-pTCA (a4-B, -1)  | 1083.1   | 1083.1      |
| 5’-pTCAT (a5-B, -1) | 1387.2   | 1387.2      |
|                     (a5-B, -2) | 693.3    | 693.1       |
| 5’-pTCATG (a6-B, -1) | 1715.8   | 1716.2      |
|                     (a6-B, -2) | 857.6    | 857.6       |
| p-CATGGA-3’ (w6, -1) | 1893.9   | 1894.3      |
|                     (w6, -2) | 946.7    | 946.6       |
| p-ATGGA-3’ (w5, -1) | 1605.1   | 1605.3      |
|                     (w5, -2) | 802.0    | 802.1       |
| p-TGGA-3’ (w4, -1)  | 1291.9   | 1292.2      |
| p-GGA-3’ (w3, -1)   | 988.1    | 988.2       |
| p-GA-3’ (w2, -1)    | 659.2    | 659.1       |
| p-A-3’ (w1, -1)     | 330.2    | 330.0       |
Table S21
Observed and theoretical MS fragmentation for Dpo4-catalyzed incorporation of 5’-ATGAAG-3’.

5’-pTCATGAAG-3’

| Fragment Assignment       | Observed | Theoretical |
|---------------------------|----------|-------------|
| 5’-pT (a2-B, -1)          | 481.1    | 481.0       |
| 5’-pTC (a3-B, -1)         | 770.1    | 770.1       |
| 5’-pTCA (a4-B, -1)        | 1083.0   | 1083.1      |
| 5’-pTCAT (a5-B, -2)       | 692.8    | 693.1       |
| 5’-pTCATG (a6-B, -2)      | 857.6    | 857.6       |
| 5’-pTCATGA (a7-B, -2)     | 1014.1   | 1014.1      |
| p-CATGAAG-3’ (w7, -2)     | 1103.6   | 1103.2      |
| p-ATGAAG-3’ (w6, -2)      | 958.6    | 958.7       |
| p-TGAAG-3’ (w5, -1)       | 1606.2   | 1605.3      |
| (w5, -2)                  | 801.9    | 802.1       |
| p-GAAG-3’ (w4, -1)        | 1301.4   | 1301.2      |
| (w4, -2)                  | 650.2    | 650.1       |
| p-AAG-3’ (w3, -1)         | 972.0    | 972.2       |
| p-AG-3’ (w2, -1)          | 659.1    | 659.1       |
| p-G-3’ (w1, -1)           | 346.2    | 346.0       |
Table S22
Observed and theoretical MS fragmentation for Dpo4-catalyzed insertion of A opposite M1dG followed by accurate full-length extension and blunt-end addition of A.

| Fragment Assignment | Observed | Theoretical |
|---------------------|----------|-------------|
| 5’-pT (a2-B, -1)    | 481.1    | 481.0       |
| 5’-pTC (a3-B, -1)   | 770.1    | 770.1       |
| 5’-pTCA (a4-B, -1)  | 1083.0   | 1083.1      |
| 5’-pTCAA (a5-B, -1) | 1396.1   | 1396.2      |
|                     (a5-B, -2) | 697.6    | 697.6       |
| 5’-pTCAAT (a6-B, -1) | 1700.2   | 1700.2      |
|                     (a6-B, -2) | 849.6    | 849.6       |
| 5’-pTCAATG (a7-B, -2) | 1014.1   | 1014.1      |
| p-CAATGAA-3’ (w7, -2) | 1095.1   | 1095.2      |
| p-AATGAA-3’ (w6, -1) | 1902.3   | 1902.3      |
|                     (w6, -2) | 950.8    | 950.7       |
| p-ATGAA-3’ (w5, -1) | 1588.6   | 1589.3      |
|                     (w5, -2) | 794.2    | 794.1       |
| p-TGAA-3’ (w4, -1)  | 1276.0   | 1276.2      |
|                     (w4, -2) | 637.6    | 637.6       |
| p-GAA-3’ (w3, -1)   | 972.1    | 972.2       |
| p-AA-3’ (w2, -1)    | 643.2    | 643.1       |
| p-A-3’ (w1, -1)     | 330.2    | 330.0       |
**Table S23**

Observed and theoretical MS fragmentation for Dpo4-catalyzed insertion of A opposite M1dG followed by accurate full-length extension and blunt-end addition of C.

5'-pTCAATGAC-3'

| Fragment Assignment | Observed | Theoretical |
|---------------------|----------|-------------|
| 5'-pT (a2-B, -1)    | 481.1    | 481.0       |
| 5'-pTC (a3-B, -1)   | 770.1    | 770.1       |
| 5'-pTCA (a4-B, -1)  | 1083.2   | 1083.1      |
| 5'-pTCAA (a5-B, -1) | 1393.1   | 1396.2      |
| (a5-B, -2)          | 697.9    | 697.6       |
| 5'-pTCAAT (a6-B, -2)| 1701.1   | 1700.2      |
| (a6-B, -2)          | 849.4    | 849.6       |
| 5'-pTCAATG (a7-B, -2)| 1014.2  | 1014.1      |
| p-CAATGAC-3' (w7, -2)| 1083.2  | 1083.2      |
| p-AATGAC-3' (w6, -1)| 1877.9  | 1878.3      |
| (w6, -2)            | 938.6    | 938.6       |
| p-ATGAC-3' (w5, -1) | 1565.2   | 1565.3      |
| (w5, -2)            | 782.2    | 782.1       |
| p-TGAC-3' (w4, -1)  | 1252.1   | 1252.2      |
| (w4, -2)            | 625.7    | 625.6       |
| p-GAC-3' (w3, -1)   | 948.2    | 948.2       |
| p-AC-3' (w2, -1)    | 619.2    | 619.1       |
| p-C-3' (w1, -1)     | 306.2    | 306.0       |
Table S24
Observed and theoretical MS fragmentation for Dpo4-catalyzed incorporation of 5'-AGTG-3'.

5'-pTCAGTG-3'

| Fragment Assignment | Observed | Theoretical |
|---------------------|----------|-------------|
| 5'-pT (a2-B, -1)    | 481.1    | 481.0       |
| 5'-pTC (a3-B, -1)   | 770.1    | 770.1       |
| 5'-pTCA (a4-B, -1)  | 1083.0   | 1083.1      |
| 5'-pTCAG (a5-B, -1) | 1412.4   | 1412.4      |
|                     | 705.7    | 705.6       |
| p-CAGTG-3' (w5, -1) | 1582.2   | 1581.2      |
|                     | 789.9    | 790.1       |
| p-AGTG-3' (w4, -1)  | 1292.2   | 1292.2      |
| p-GTG-3' (w3, -1)   | 979.2    | 979.2       |
| p-TG-3' (w2, -1)    | 650.2    | 650.1       |
| p-G-3' (w1, -1)     | 346.2    | 346.0       |

Table S25
Observed and theoretical MS fragmentation for Dpo4-catalyzed incorporation of 5'-ATG-3'.

5'-pTCATG-3'

| Fragment Assignment | Observed | Theoretical |
|---------------------|----------|-------------|
| 5'-pT (a2-B, -1)    | 481.1    | 481.0       |
| 5'-pTC (a3-B, -1)   | 770.1    | 770.1       |
| 5'-pTCA (a4-B, -1)  | 1083.1   | 1083.1      |
| p-CATG-3' (w4, -1)  | 1252.1   | 1252.2      |
| p-ATG-3' (w3, -1)   | 963.1    | 963.2       |
| p-TG-3' (w2, -1)    | 650.2    | 650.1       |
| p-G-3' (w1, -1)     | 345.9    | 346.0       |
Table S26
Observed and theoretical MS fragmentation for Dpo4-catalyzed incorporation of 5’-ATGA-3’.

5’-pTCATGA-3’

| Fragment Assignment     | Observed | Theoretical |
|-------------------------|----------|-------------|
| 5’-pT (a2-B, -1)        | 481.2    | 481.0       |
| 5’-pTC (a3-B, -1)       | 770.1    | 770.1       |
| 5’-pTCA (a4-B, -1)      | 1083.2   | 1083.1      |
| 5’-pTCAT (a5-B, -1)     | 1386.9   | 1387.2      |
|                         | 693.2    | 693.1       |
| p-CATGA-3’ (w5, -1)    | 1565.1   | 1565.3      |
|                         | 782.2    | 782.1       |
| p-CATG-3’ (w4, -1)     | 1276.1   | 1276.2      |
|                         | 637.6    | 637.6       |
| p-ATG-3’ (w3, -1)      | 963.1    | 963.2       |
| p-TG-3’ (w2, -1)       | 659.2    | 659.1       |
| p-G-3’ (w1, -1)        | 330.2    | 330.0       |
### Table S27
Observed and theoretical MS fragmentation for Dpo4-catalyzed insertion of 5’-ATGAGC-3’.

| Fragment Assignment | Observed | Theoretical |
|---------------------|----------|-------------|
| 5’-pT (a2-B, -1)    | 481.0    | 481.0       |
| 5’-pTC (a3-B, -1)   | 770.2    | 770.1       |
| 5’-pTCA (a4-B, -1)  | 1083.0   | 1083.1      |
| 5’-pTCAT (a5-B, -2) | 693.2    | 693.1       |
| 5’-pTCATG (a6-B, -2)| 857.6    | 857.6       |
| 5’-pTCATGA (a7-B, -2)| 1014.5  | 1014.1      |
| p-CATGAGC-3’ (w7, -2) | 1090.9  | 1091.2      |
| p-ATGAGC-3’ (w6, -2) | 946.7   | 946.6       |
| p-TGAGC-3’ (w5, -1)  | 1581.4   | 1581.3      |
| p-GAGC-3’ (w4, -1)   | 1276.9   | 1276.2      |
| p-AGC-3’ (w3, -1)    | 948.1    | 948.2       |
| p-GC-3’ (w2, -1)     | 635.2    | 635.1       |
| p-C-3’ (w1, -1)      | 306.2    | 306.0       |
Table S28
Observed and theoretical MS fragmentation for Dpo4-catalyzed insertion of G opposite M1dG followed by accurate full-length extension and blunt-end addition of C.

5'-pTCGATGAC-3'

| Fragment Assignment            | Observed | Theoretical |
|-------------------------------|----------|-------------|
| 5'-pT (a2-B, -1)              | 481.2    | 481.0       |
| 5'-pTC (a3-B, -1)             | 770.1    | 770.1       |
| 5'-pTCG (a4-B, -1)            | 1099.0   | 1099.1      |
| 5'-pTCGA (a5-B, -1)           | 1412.2   | 1412.2      |
| 5’-pTCGAT (a6-B, -2)          | 857.7    | 857.6       |
| 5’-pTCGATG (a7-B, -2)         | 1022.0   | 1022.1      |
| p-CGATGAC-3’ (w7, -2)         | 1090.9   | 1091.2      |
| p-GATGAC-3’ (w6, -2)          | 946.7    | 946.6       |
| p-ATGAC-3’ (w5, -1)           | 1565.0   | 1565.3      |
| (w5, -2)                      | 782.2    | 782.1       |
| p-TGAC-3’ (w4, -1)            | 1252.1   | 1252.2      |
| p-GAC-3’ (w3, -1)             | 948.2    | 948.2       |
| p-AC-3’ (w2, -1)              | 619.1    | 619.1       |
| p-C-3’ (w1, -1)               | 306.1    | 306.0       |
Table S29
Observed and theoretical MS fragmentation for Dpo4-catalyzed insertion of A opposite M1dG followed by insertion of 5’-GTGAA-3’.

5’-pTCAGTGAA-3’

| Fragment Assignment               | Observed | Theoretical |
|-----------------------------------|----------|-------------|
| 5’-pT (a2-B, -1)                  | 481.1    | 481.0       |
| 5’-pTC (a3-B, -1)                 | 770.1    | 770.1       |
| 5’-pTCA (a4-B, -1)                | 1083.0   | 1083.1      |
| 5’-pTCAG (a5-B, -1)               | 1411.8   | 1412.2      |
| 5’-pTCAGT (a6-B, -2)              | 857.6    | 857.6       |
| 5’-pTCAGTG (a7-B, -2)             | 1022.1   | 1022.1      |
| p-CAGTGAC-3’ (w7, -2)             | 1103.6   | 1103.2      |
| p-AGTGAC-3’ (w6, -2)              | 958.6    | 958.6       |
| p-GTGAA-3’ (w5, -1)               | 1606.3   | 1606.3      |
|                                      (w5, -2) | 802.1    | 802.1       |
| p-TGAA-3’ (w4, -1)                | 1276.1   | 1276.2      |
| p-GAA-3’ (w3, -1)                 | 972.0    | 972.2       |
| p-AA-3’ (w2, -1)                  | 643.1    | 643.1       |
| p-A-3’ (w1, -1)                   | 330.1    | 330.0       |