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Supplemental Information

Investigation of N-Terminal Phospho-Regulation of Uracil DNA Glycosylase Using Protein Semisynthesis

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SUPPORTING MATERIAL

Investigation of N-terminal Phospho-Regulation of Uracil DNA Glycosylase (UNG2) Using Protein Semisynthesis

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Figure S1. MALDI-MS of peptide UNG2(a.a.1-19). Sequence: MIGQKTLYSFFSPSPARKR; predicted monoisotopic mass = 2213.2 Da.

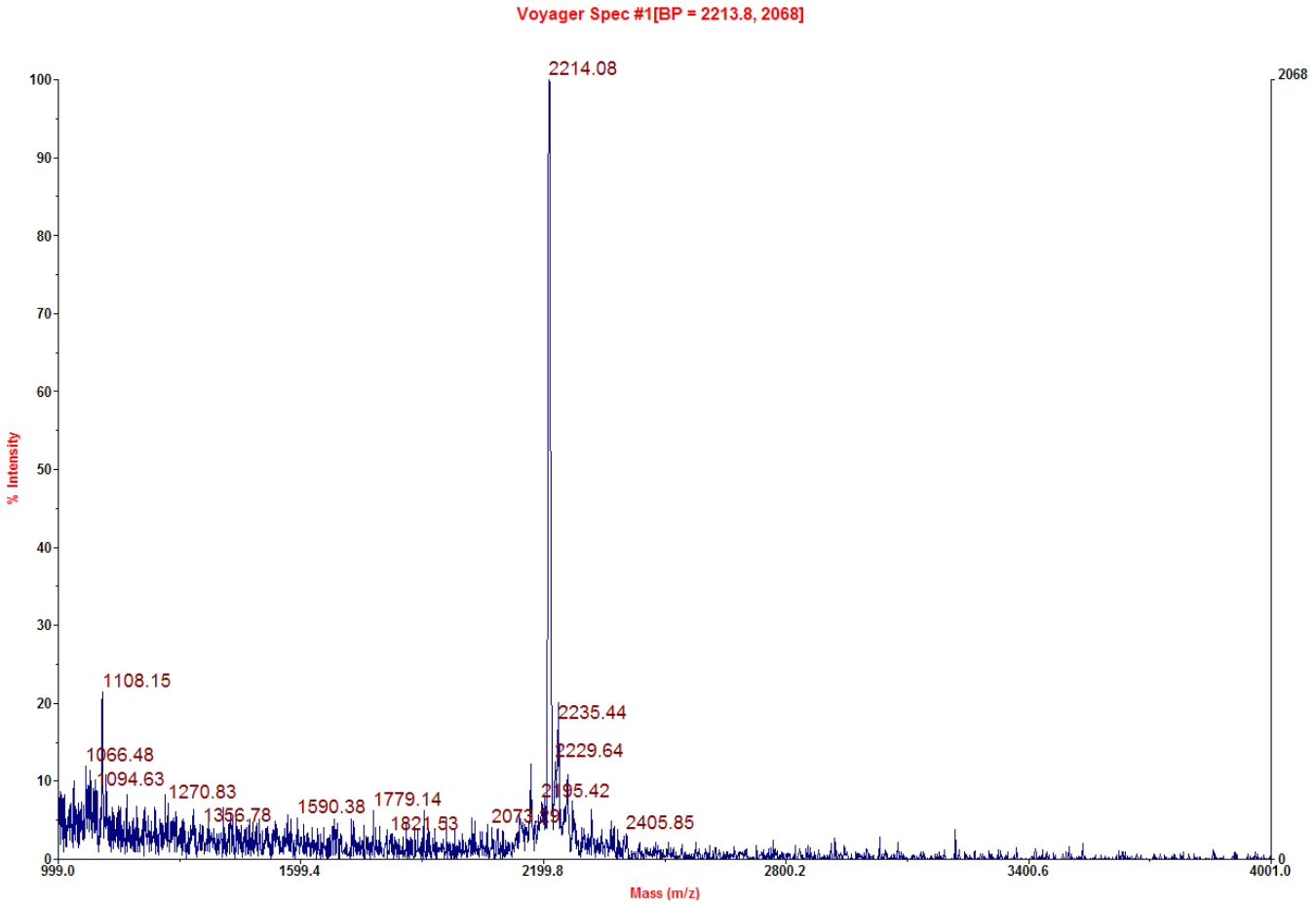

Figure S2. MALDI-MS of peptide pThr6-UNG2(a.a.1-19). Sequence: MIGQKpTLYSFFSPSPARKR; predicted monoisotopic mass = 2293.2 Da.

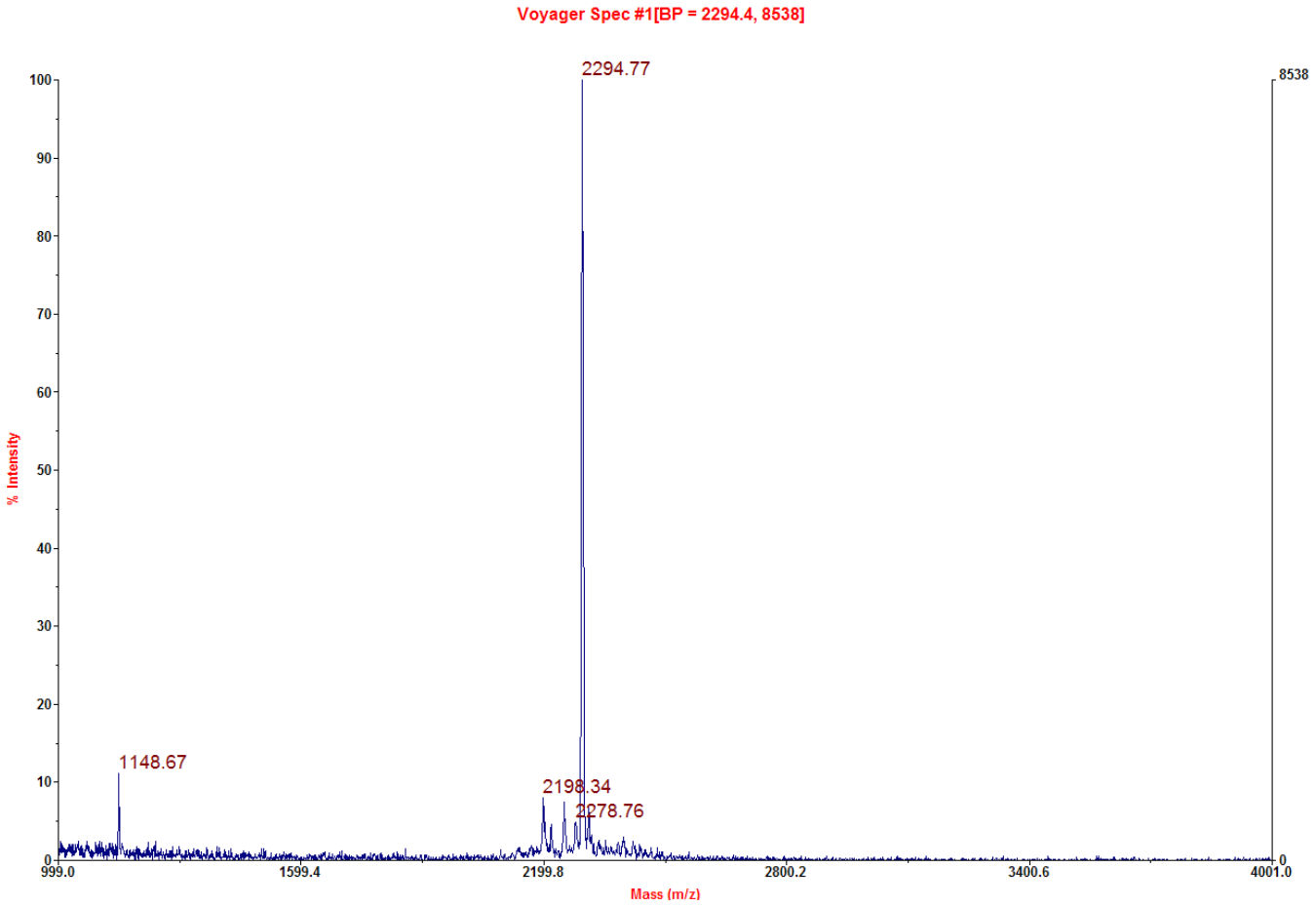

Figure S3. MALDI-MS of peptide pTyr8-UNG2(a.a.1-19). Sequence: MIGQKTLpYSFFSPSPARKR; predicted monoisotopic mass = 2293.2 Da.

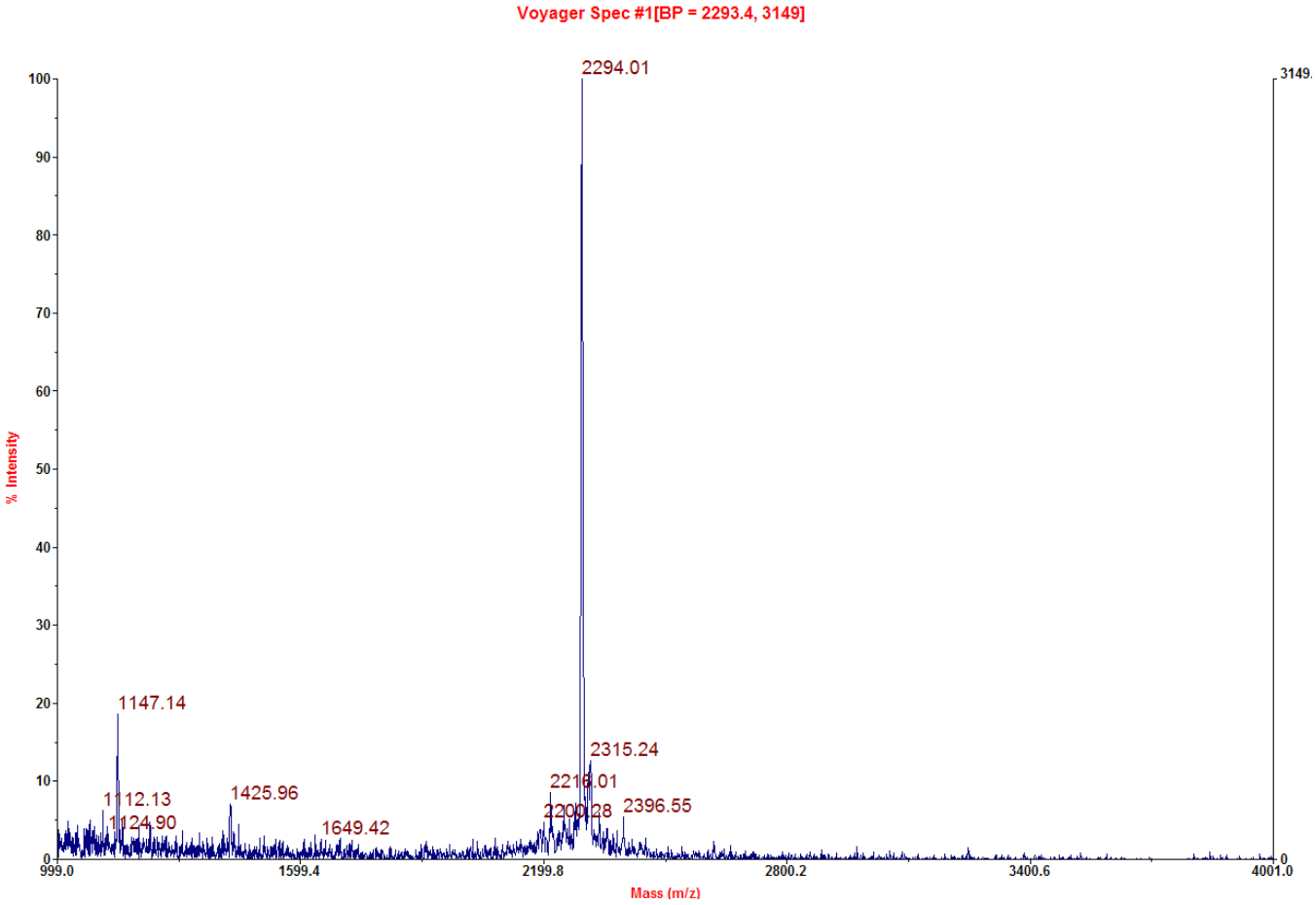

Figure S4. MALDI-MS of 11mer peptide pThr6-UNG2(a.a.1-11)-Nbz. Sequence: MIGQKpTLYSFF-Nbz; predicted monoisotopic mass = 1572.7 Da.

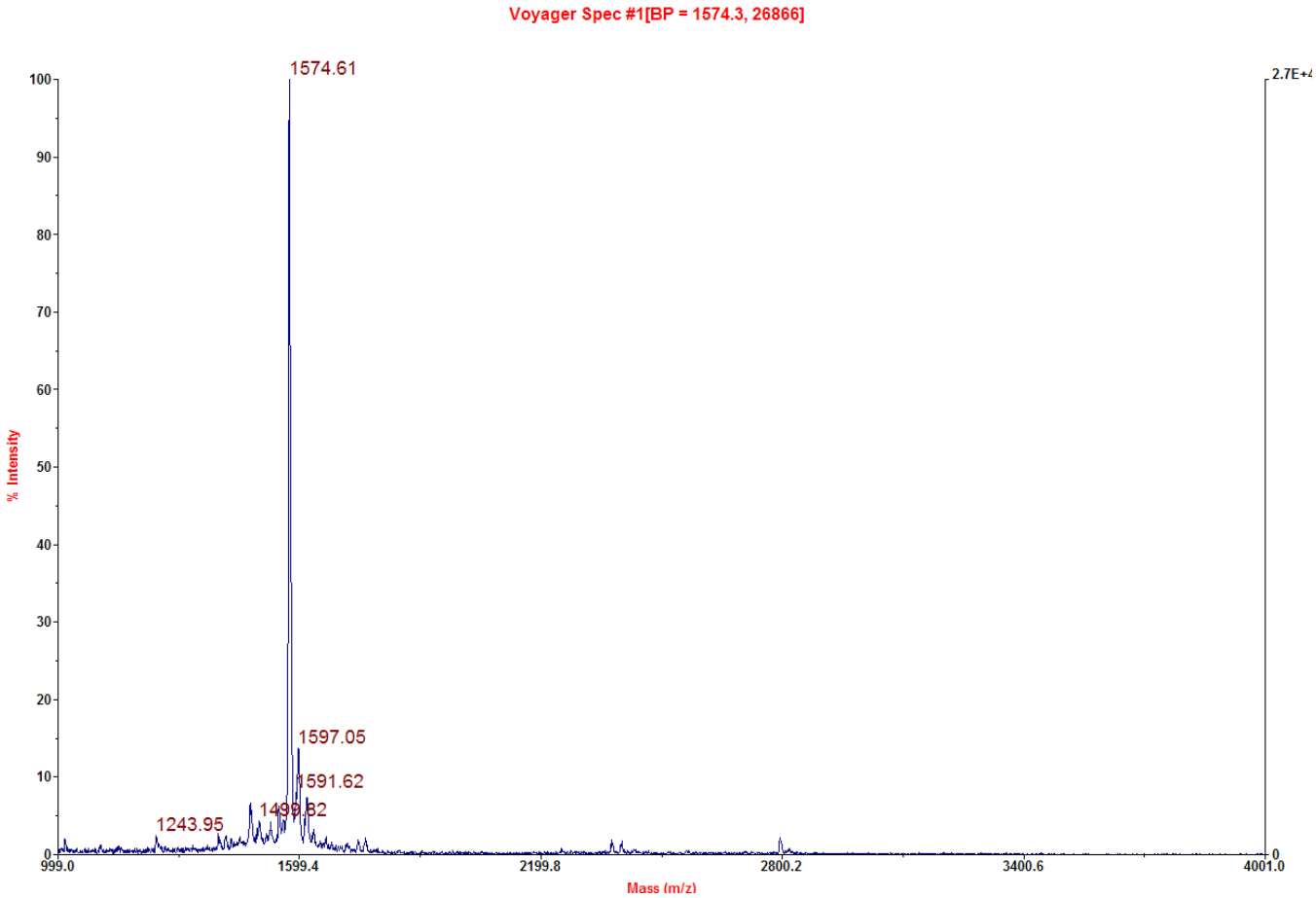

Figure S5. MALDI-MS of 29mer peptide His-TEV-pTyr8-UNG2(a.a.1-11)-Nbz. Sequence: MHHHHHHKRKGENLYFQGMIGQKTLpYSFF-Nbz; predicted monoisotopic mass = 3846.8 Da.

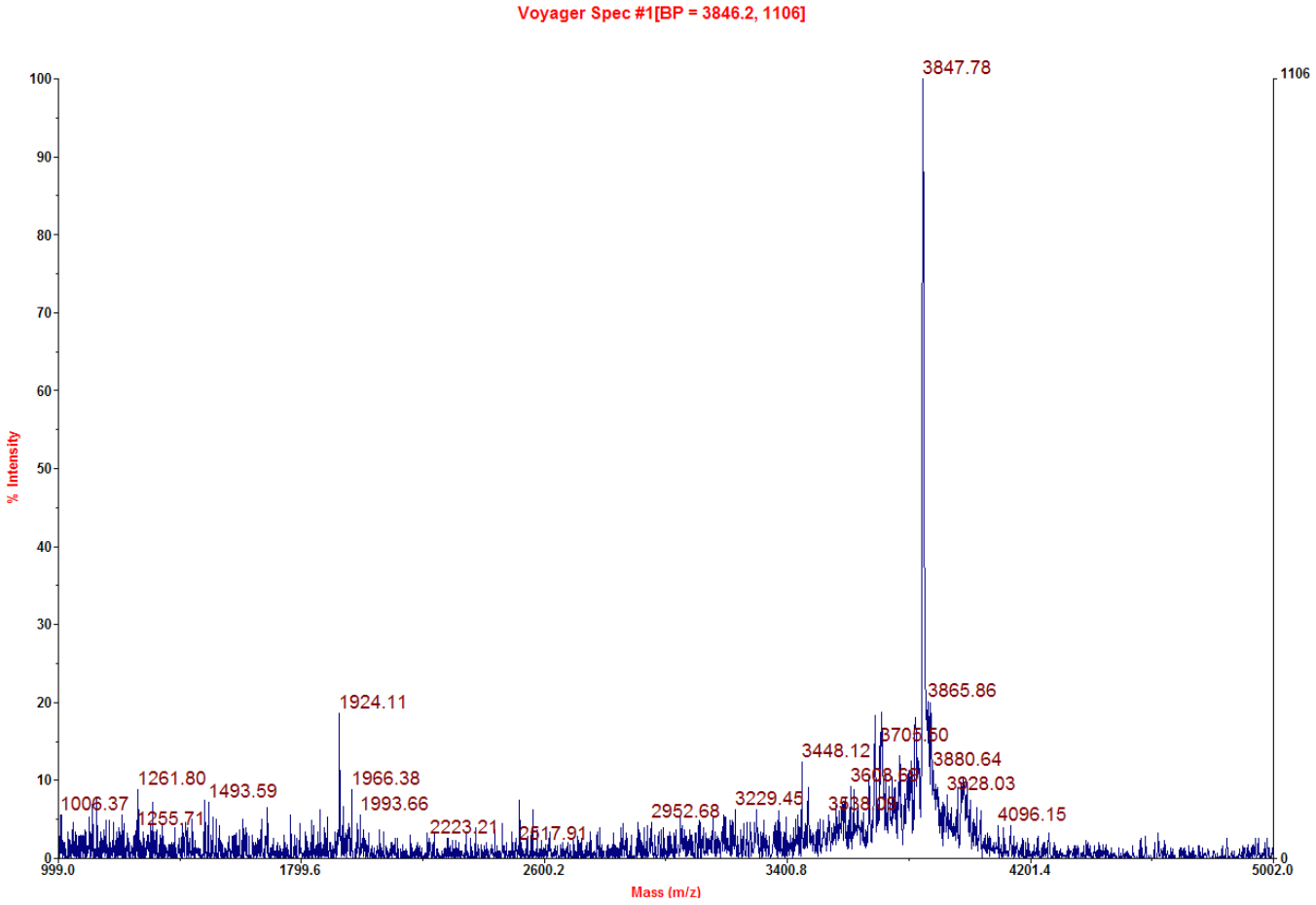

Figure S6. MALDI-MS of peptide PogoLigase. Sequence: Fluorescein-Ahx-SAVLQKKITDYFHPKK; predicted monoisotopic mass = 2373.2 Da.

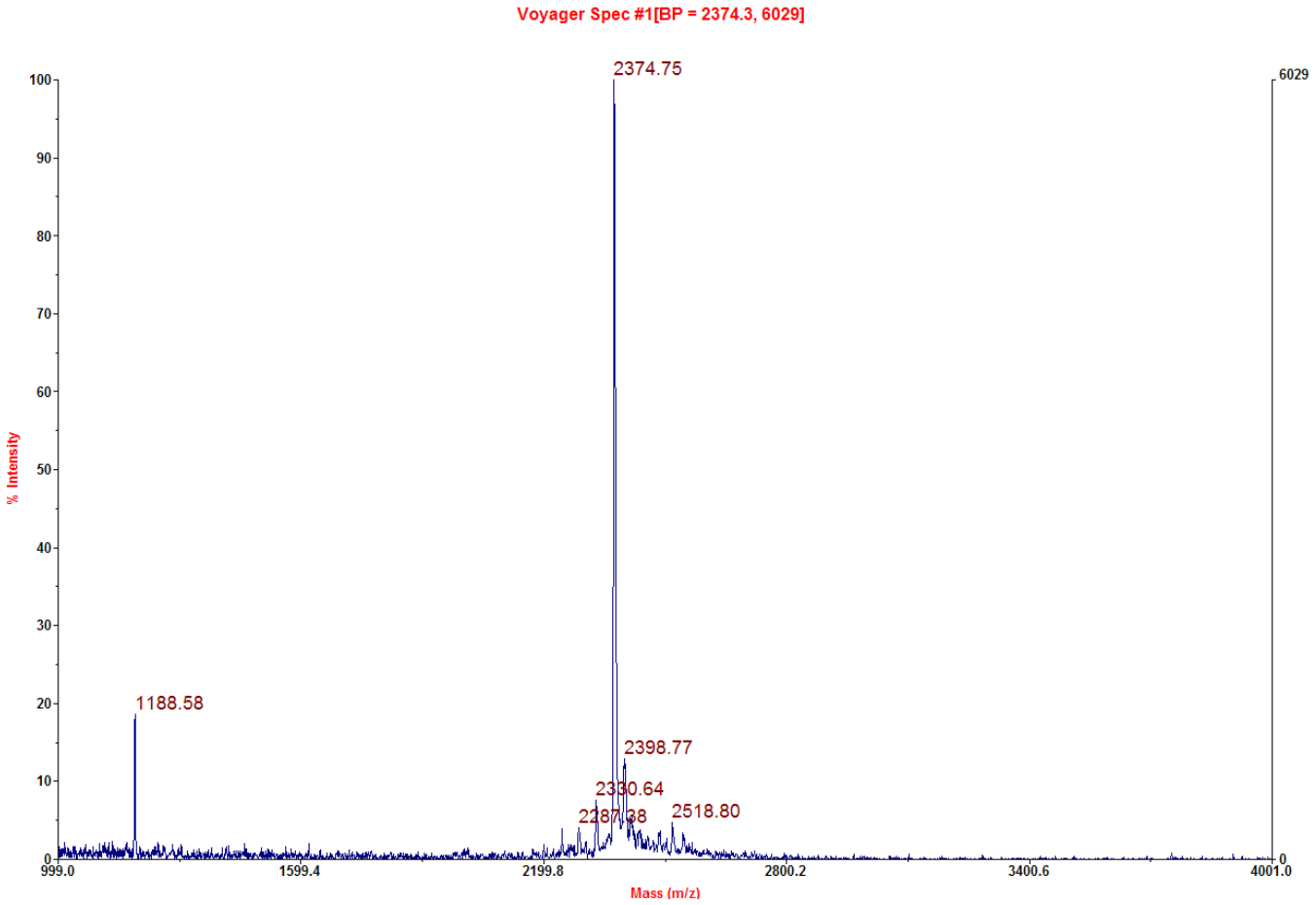

Figure S7. MALDI-MS of peptide SMARCAL(a.a.5-30) (SMARCAL). Sequence: Fluorescein-Ahx-LTEEQRKKIEENRQKALARRAEKLLA; predicted monoisotopic mass = 3591.9 Da.

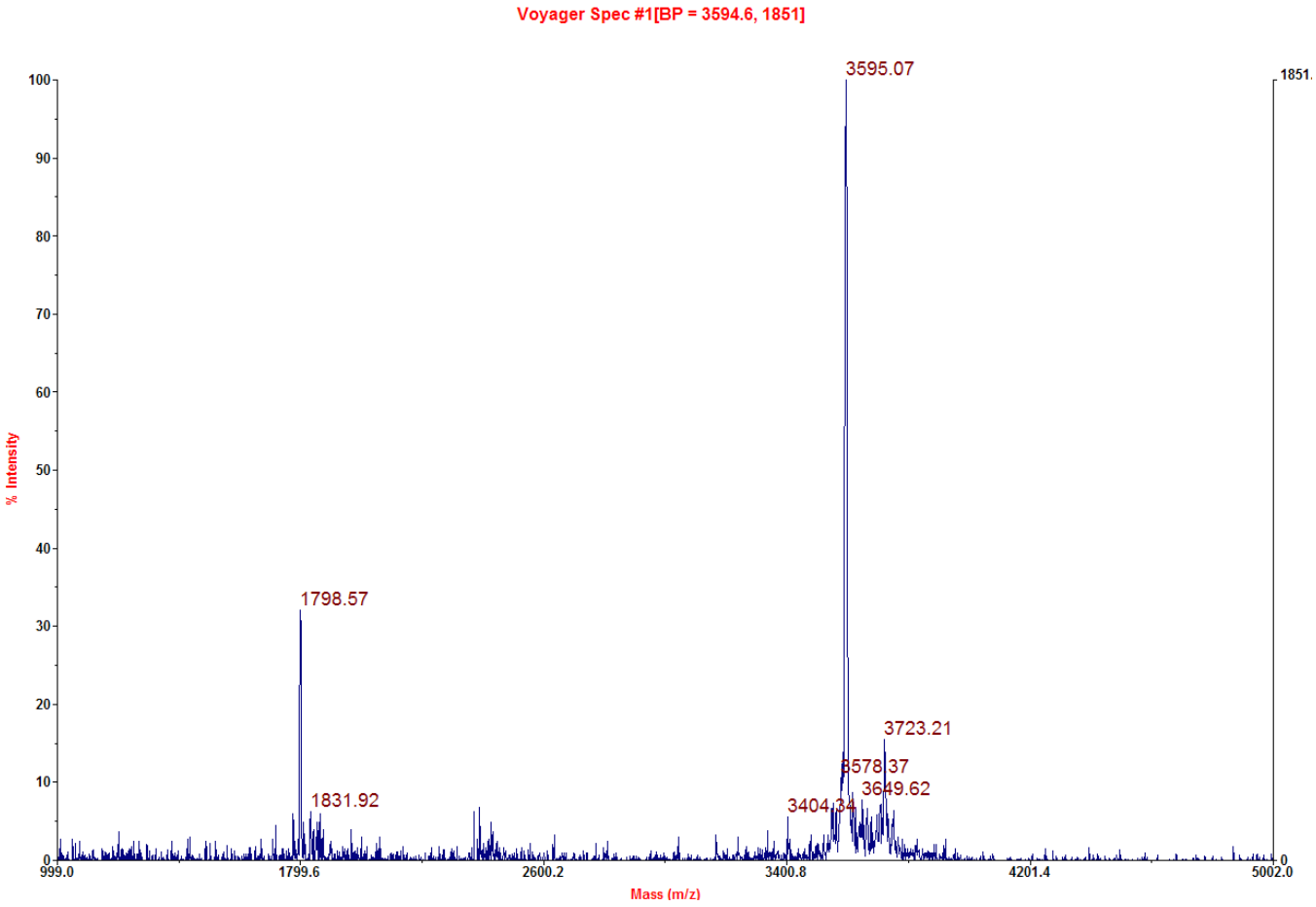

Figure S8. ESI-MS of UNG catalytic domain, which was used as a standard control to test instrument accuracy. Predicted monoisotopic mass = 25489.0 Da; observed mass = 25494.6 ± 4.0 Da (mean ± SD).

	Pred.	Obs.
z	*m/z*	*m/z*
+22	1159.6	1160.2
+23	1109.2	1109.7
+24	1063.0	1063.4
+25	1020.6	1020.9
+26	981.4	981.6
+27	945.0	945.3
+28	911.3	911.5
+29	879.9	880.1
+30	850.6	850.8
+31	823.2	823.3
+32	797.5	797.6
+33	773.4	773.4
+34	750.7	750.7

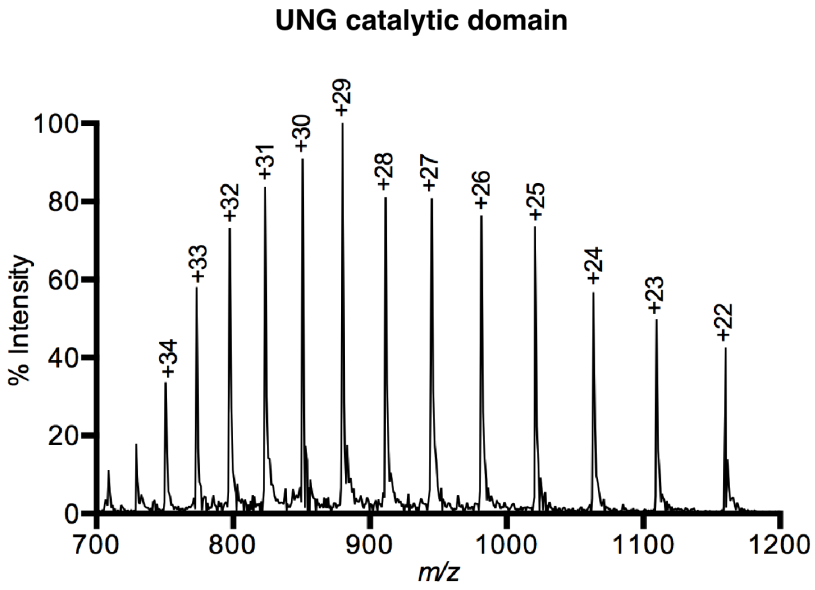

Figure S9. Representative trace from the purification of pThr6-UNG2 from UNG2(a.a.12-313, S12C) using Mono S chromatography. The fractions indicated by the dashes beginning at ~200 min were separated on SDS-PAGE, and the gel was then stained with Coomassie.

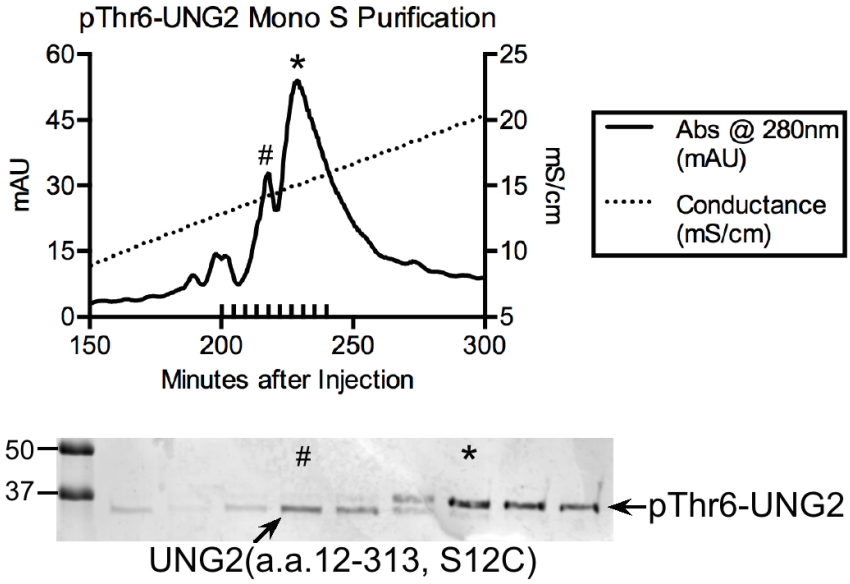

Figure S10. Intermediates and final product during pTyr8-UNG2 production. Shown on the Coomassie-stained gel are the starting material UNG2(a.a.12-313, S12C), the His-TEV-pTyr8-UNG2 construct after Ni^{2+} column purification, and the final pTyr8-UNG2 after TEV cleavage.

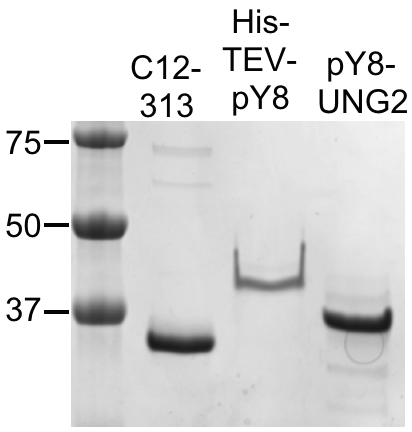

Figure S11. Steady-state kinetics data for UNG2, UNG2(S12C), and phosphorylated semisynthetic proteins.

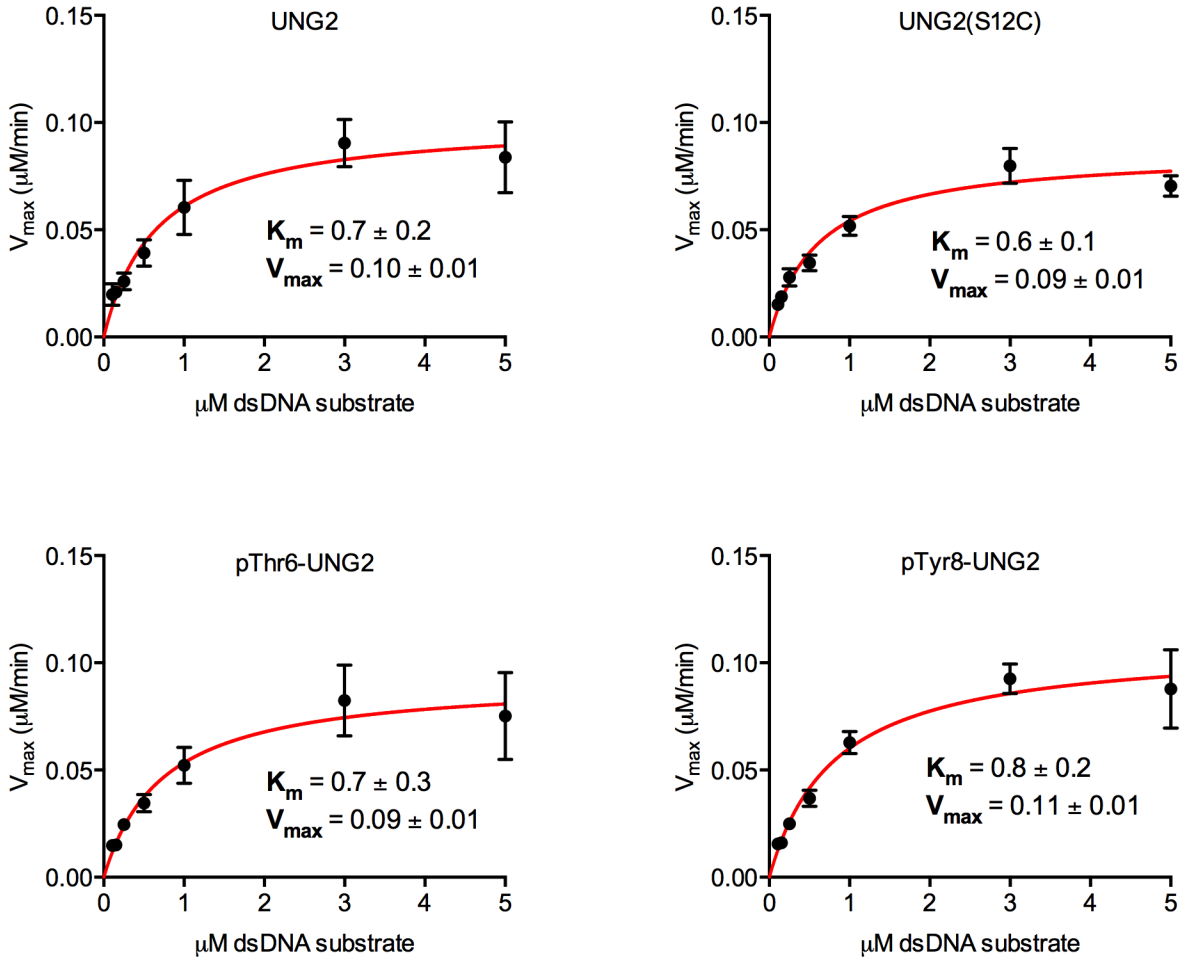

Figure S12. Displacement of PogoLigase peptide from PCNA using UNG2 or UNG2(S12C). Note that the UNG2 data is also shown in Fig. 2C of the main text.

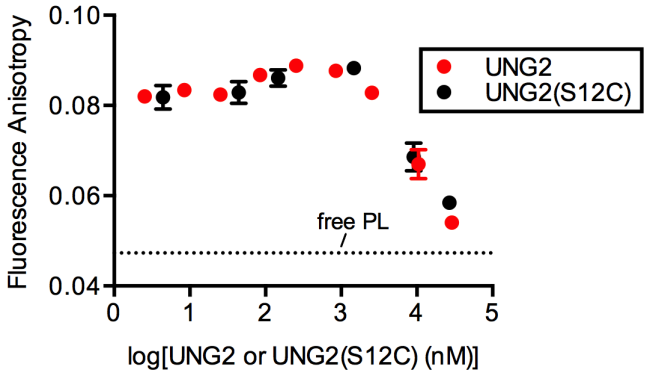

Figure S13. (A) Binding data obtained by equilibrating SMARCAL peptide with increasing concentrations of RPA that was pre-bound to dT(31) ssDNA. (B) Competition experiment during which UNG2 was used to displace SMARCAL peptide from dT(31)-bound RPA. The Hill slope for the curve in (B) was -1.7.

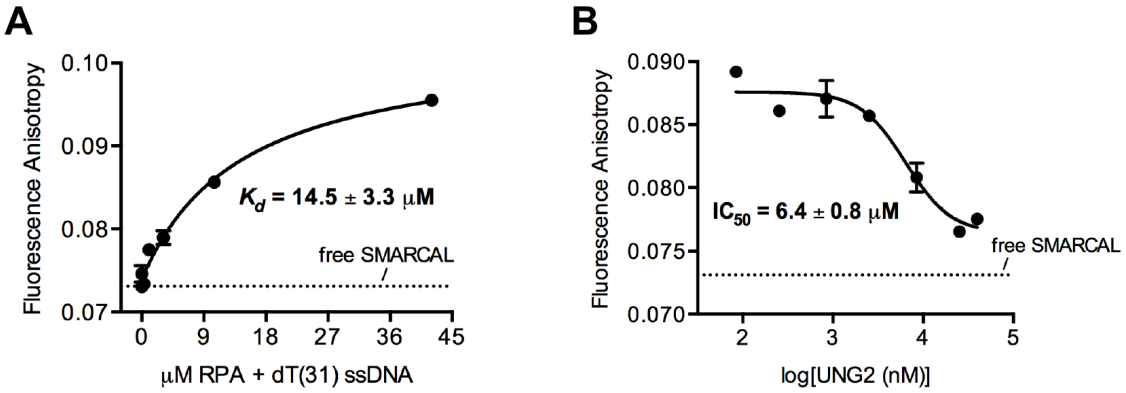

Figure S14. (A) Steady-state kinetics data for the mutant UNG2 construct that has an N-terminal cysteine before Met1 and also has the wild-type cysteines mutated to alanines. The substrate used was the same as that in Table 1 and Fig. S11. (B) *Left,* Image of a fluorescence scanned (Ex/Em: 495/520) SDS-PAGE gel containing UNG2(Fluor). *Right,* Image of the same gel that was stained with Coomassie after obtaining the fluorescence scan. On both gels, the MW of specific ladder bands are shown, and the arrow is indicating UNG2(Fluor).