Supporting Information

Aptamer-mediated universal enzyme assay based on target-triggered DNA polymerase activity

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Table S1. Comparison of our enzyme assay with other methods.

Target enzymes	Key elements	Total analysis time (h)	Detection limit (U/mL)	Reference
DNA-related enzymes	Oligonucleotides with 32P radioisotope	15	-	(Maksimenko et al. 2004)
Exonuclease I	Fluorophore-labeled DNA and graphene oxide	2.5	0.06	(Zhang and Kong 2013)
Exonuclease I	Fluorophore-labeld DNA and porous organic framework	4	0.03	(Song et al. 2014)
Uracil DNA glycosylase	Aminopurine-modified DNA	1.5	0.02	(Lee et al. 2015)
Uracil DNA glycosylase	G-quadruplex–hemin DNAzyme	3	0.02	(Liu et al. 2014)
Exonuclease I and Uracil DNA glycosylase	Detection probes derived from aptamer specific to DNA polymerase	Exo I: 2.4 UDG: 1.9	Exo I: 0.218 UDG: 0.024	This work

Table S2. DNA sequences employed in this work.

Strand name	DNA sequence (5'□ 3')
Exo I detection probe	TTTT TTTT TTTT TTTT CAA TGT ACA GTA TTG
Exo I blocker	AAAA AAAA AAAA AAAA
Control Exo I blocker	AAAA AAAA AAAA AAAA-phosphate
UDG detection probe	TTTT AA TTTT AA TTTT CAA TGT ACA GTA TTG
UDG blocker	AAAA UU AAAA UU AAAA
Control UDG blocker	AAAA TT AAAA TT AAAA
Template DNA	CAG AAA TCT CAG GGA CTC TAA AGC TCA ACT TGC ATA AAC TTC TGA GGA
Primer DNA	TCC TCA GAA GTT TAT GCA
TaqMan probe	FAM-TAG AGT CCC TGA GAT TTC TG-BHQ1

Table S3. Limit of detection (LOD) of Exo I assay. The calibration curve (Y=263.23x + 86.561) in the linear range (0-2 U/mL) was used for estimating LOD.

Concentration (U/mL)	Mean (F_{50}-F_0)	Standard deviation
0	74.38	19.14
0.2	136.6	20.16
0.4	178.11	17.40
0.5	239.44	32.60
1	368.94	20.69
2	601.14	23.20
5	762.22	25.40
10	763.08	30.99

Table S4. Limit of detection (LOD) of UDG assay. The calibration curve (Y = 2474.2x + 9.345) in the linear range (0-0.3 U/mL) was used for estimating LOD.

Concentration (U/mL)	Mean (F_{50}-F_0)	Standard deviation
0	29.90	19.67
0.05	104.89	31.33
0.1	230.34	8.05
0.15	395.53	19.80
0.2	543.16	26.68
0.3	731.57	17.18
0.5	717.23	23.04
0.8	733.71	12.31

Table S5. Determination of UDG in diluted human serum (5 %).

Spiked UDG (U/mL)	Measured UDG (U/mL)	Standard deviation	Coefficient of variation (%)	Recovery (%)
0.15	0.153	0.015	9.96	101.9
0.25	0.256	0.008	3.15	102.3

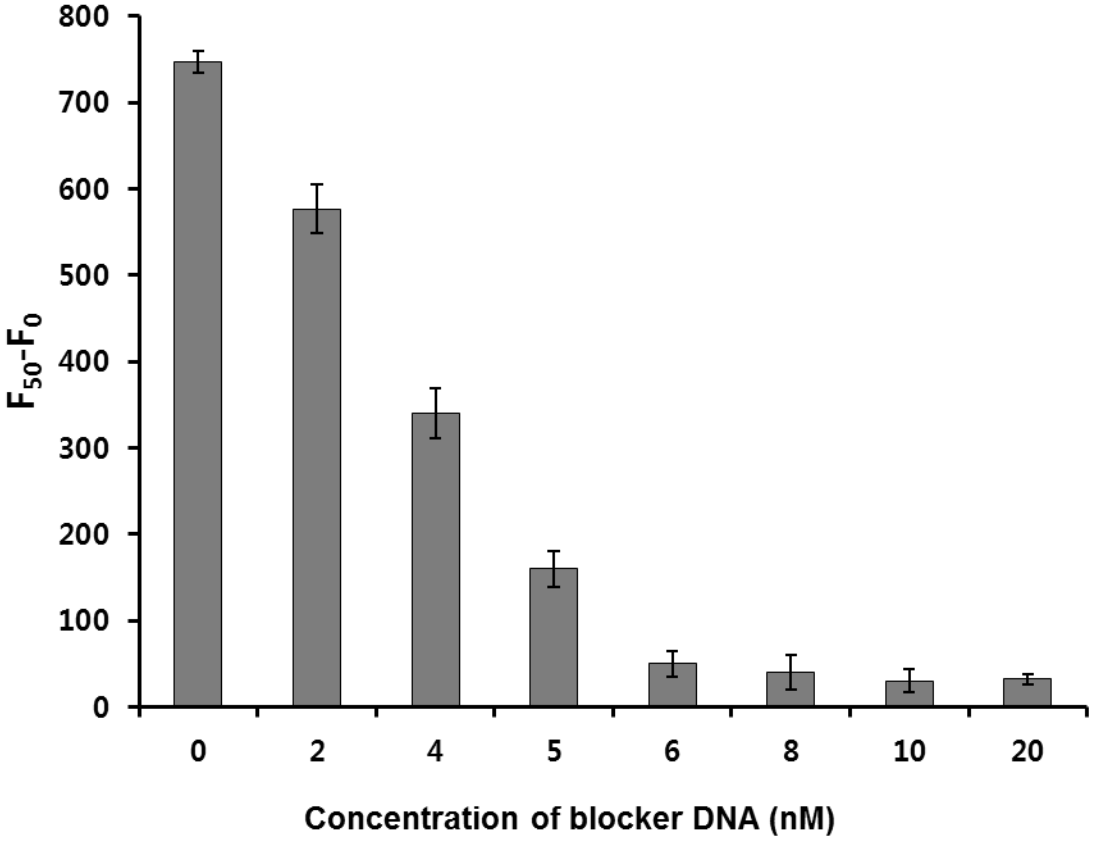

Figure S1. Fluorescence signal change (F_{50}-F_0) in the presence of Exo I blocker at varying concentrations, where F_{50} and F_0 are the fluorescence intensities from TaqMan probe measured at 50 min and 0 min, respectively. The final concentrations of DNA polymerase and Exo I detection probe are 5.5 nM and 200 nM, respectively.

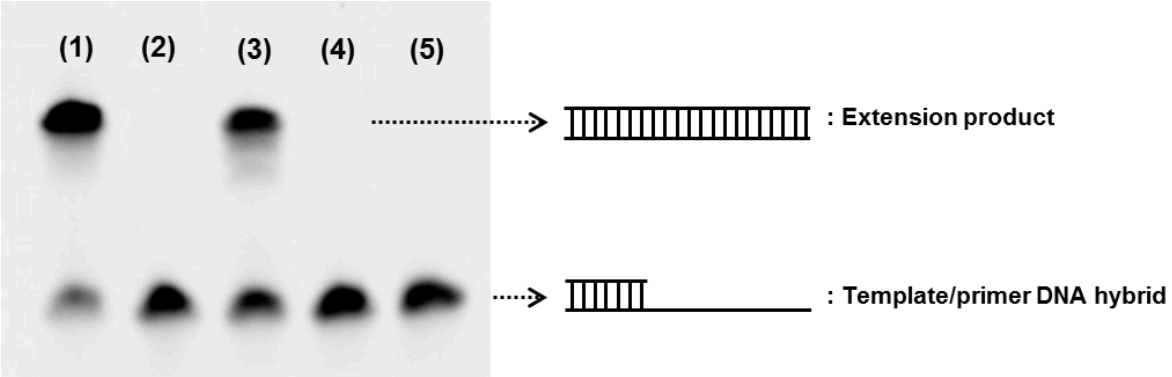

Figure S2. Polyacrylamide gel electrophoresis image to confirm the formation of extension products. The DNA molecule corresponding to the gel electrophoresis image is schematically depicted in the right side. (1) Exo I detection probe, (2) Exo I detection probe + Exo I blocker, (3) Exo I detection probe + Exo I blocker + Exo I, (4) Exo I detection probe + control Exo I blocker, and (5) Exo I detection probe + control Exo I blocker + Exo I. The final concentrations of DNA polymerase, Exo I detection probe, Exo I blocker, and Exo I are 5.5 nM, 200 nM, 100 nM, and 100 U/mL, respectively.

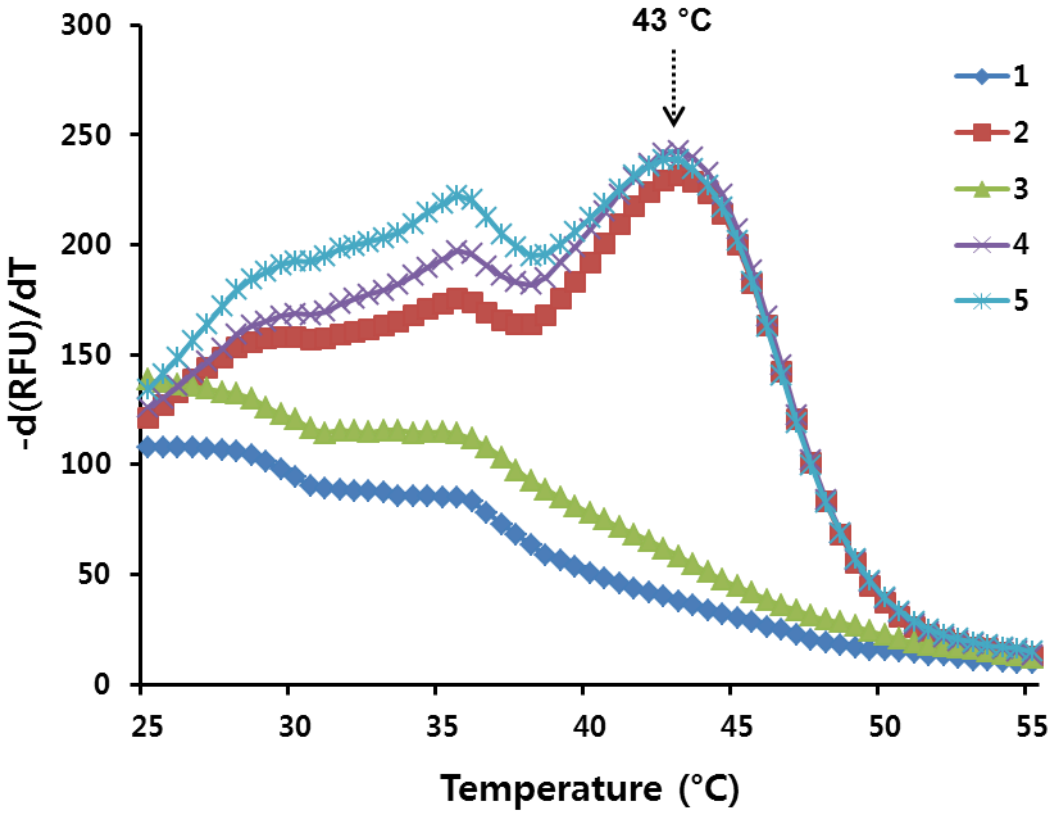

Figure S3. Melting curve analysis for the Exo I detection probe composed of Exo I detection probe and Exo I blocker. (1) Exo I detection probe, (2) Exo I detection probe + Exo I blocker, (3) Exo I detection probe + Exo I blocker + Exo I, (4) Exo I detection probe + control Exo I blocker, and (5) Exo I detection probe + control Exo I blocker + Exo I. The final concentrations of Exo I detection probe, Exo I blocker, and Exo I are 200 nM, 100 nM, and 100 U/mL, respectively.

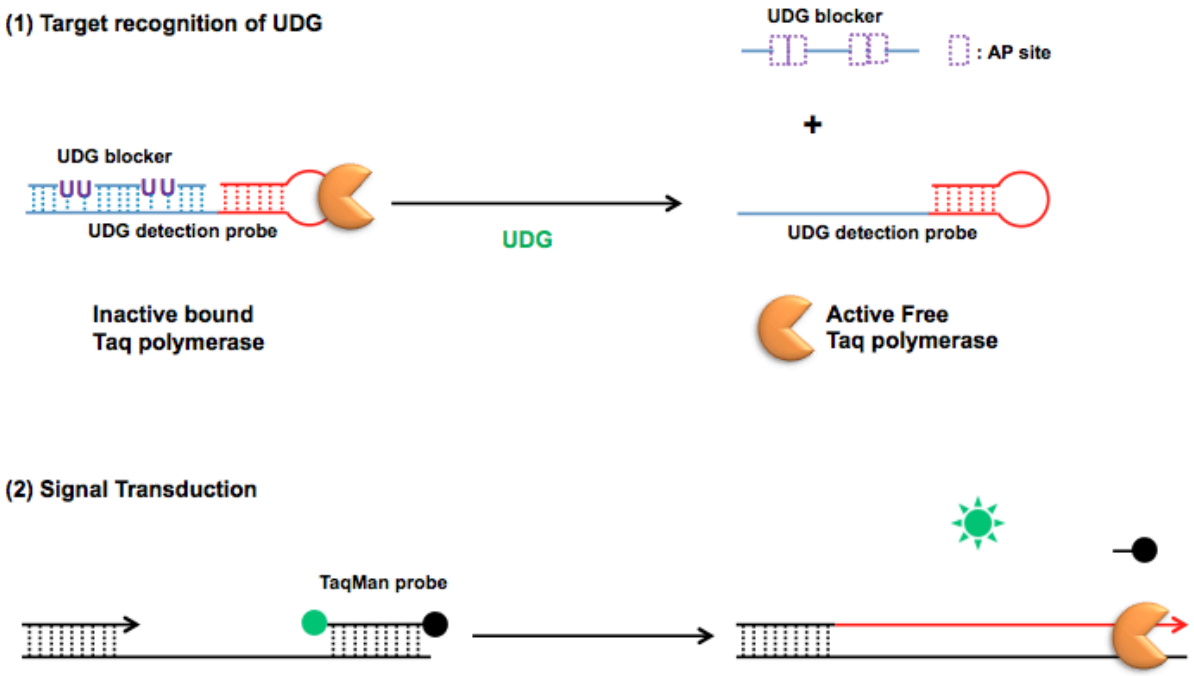

Figure S4. Schematic illustration for UDG assay based on target-triggered polymerase activity.

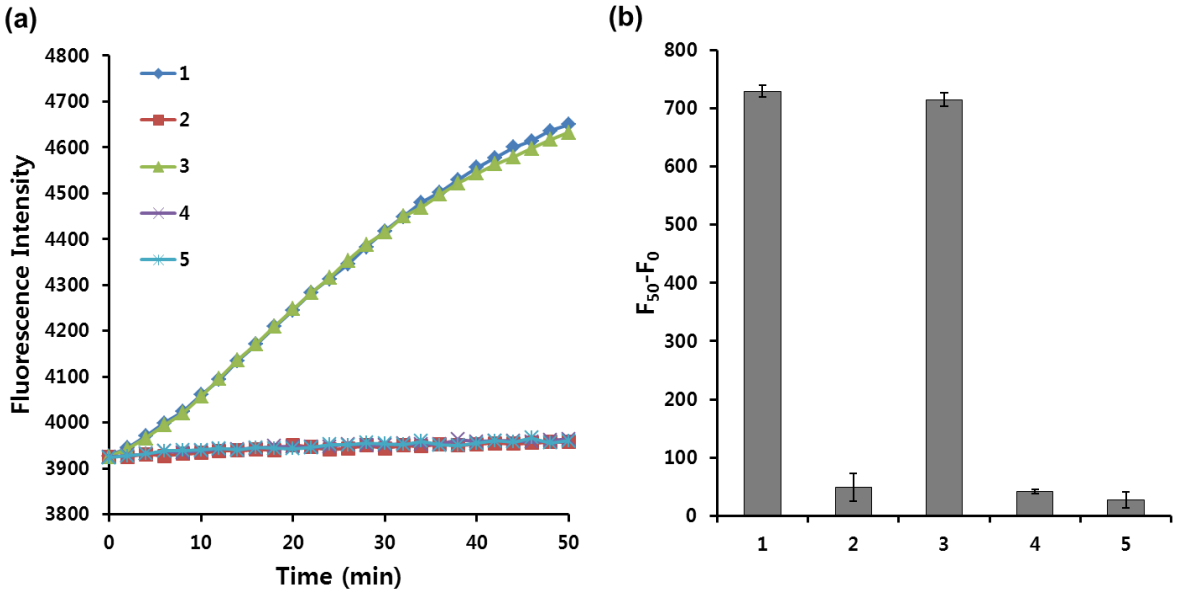

Figure S5. Fluorescence signals produced by target-triggered polymerase activity for UDG assay. (a) Time-dependent fluorescence intensities from TaqMan probe during a primer extension reaction. (b) Fluorescence signal change (F_{50}-F_0), where F_{50} and F_0 are the fluorescence intensities from TaqMan probe measured at 50 min and 0 min, respectively. (1) UDG detection probe, (2) UDG detection probe + UDG blocker, (3) UDG detection probe + UDG blocker + UDG, (4) UDG detection probe + control UDG blocker, and (5) UDG detection probe + control UDG blocker + UDG. The final concentrations of DNA polymerase, UDG detection probe, UDG blocker, and UDG are 5.5 nM, 200 nM, 10 nM, and 0.5 U/mL, respectively.

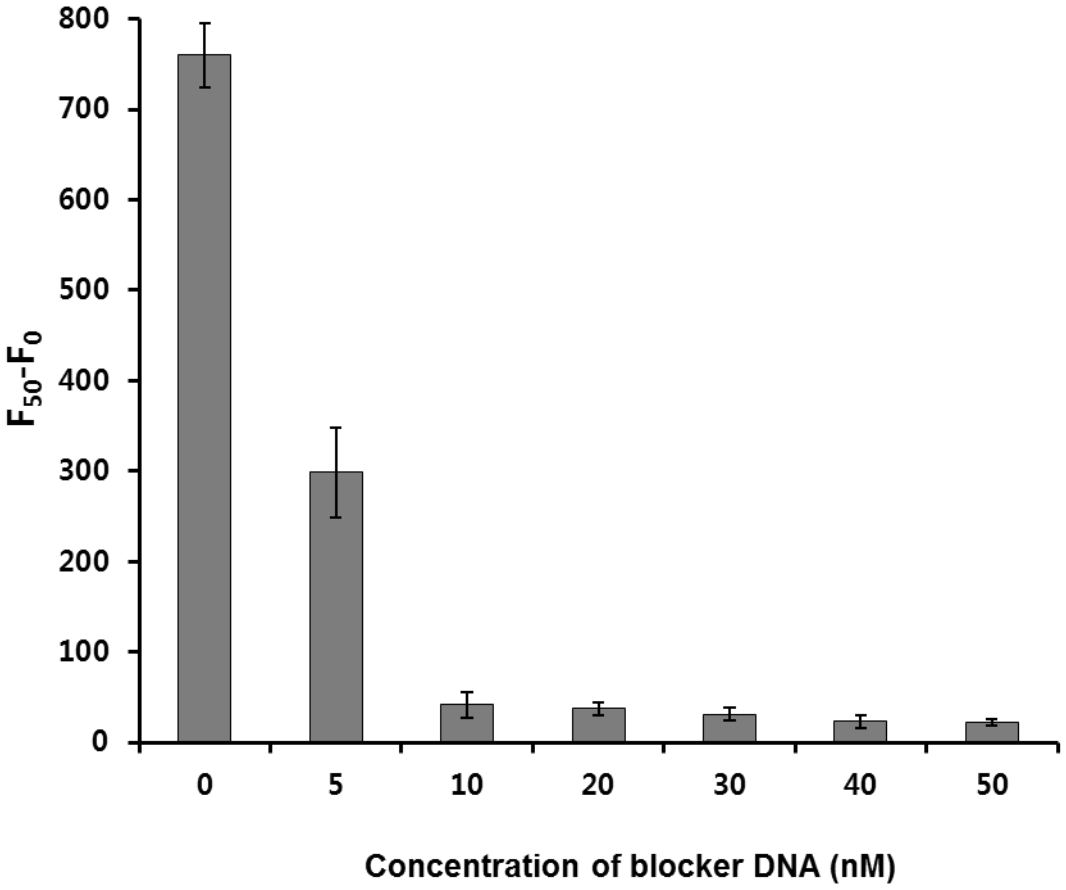

Figure S6. Fluorescence signal change (F_{50}-F_0) in the presence of UDG blocker at varying concentrations, where F_{50} and F_0 are the fluorescence intensities from TaqMan probe measured at 50 min and 0 min, respectively. The final concentrations of DNA polymerase and UDG detection probe are 5.5 nM and 200 nM, respectively.

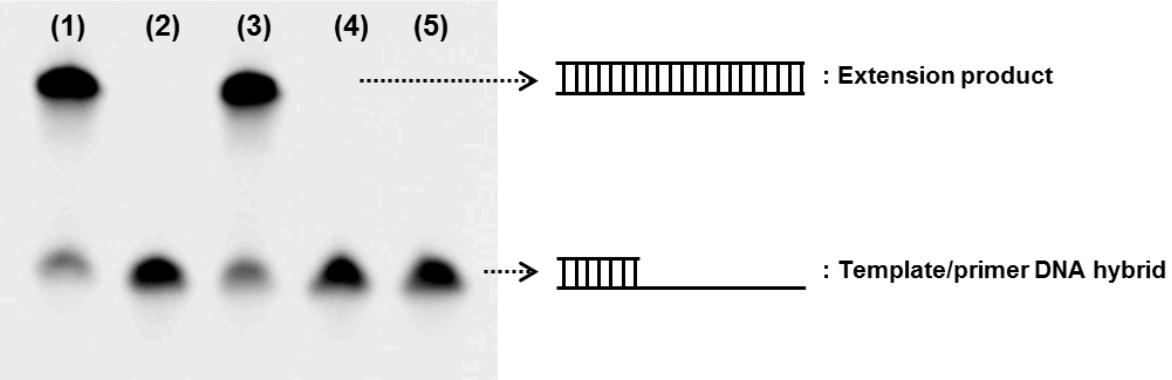

Figure S7. Polyacrylamide gel electrophoresis image to confirm the formation of extension products. The DNA molecule corresponding to the gel electrophoresis image is schematically depicted in the right side. (1) UDG detection probe, (2) UDG detection probe + UDG blocker, (3) UDG detection probe + UDG blocker + UDG, (4) UDG detection probe + control UDG blocker, and (5) UDG detection probe + control UDG blocker + UDG. The final concentrations of DNA polymerase, UDG detection probe, UDG blocker, and UDG are 5.5 nM, 200 nM, 100 nM, and 10 U/mL, respectively.

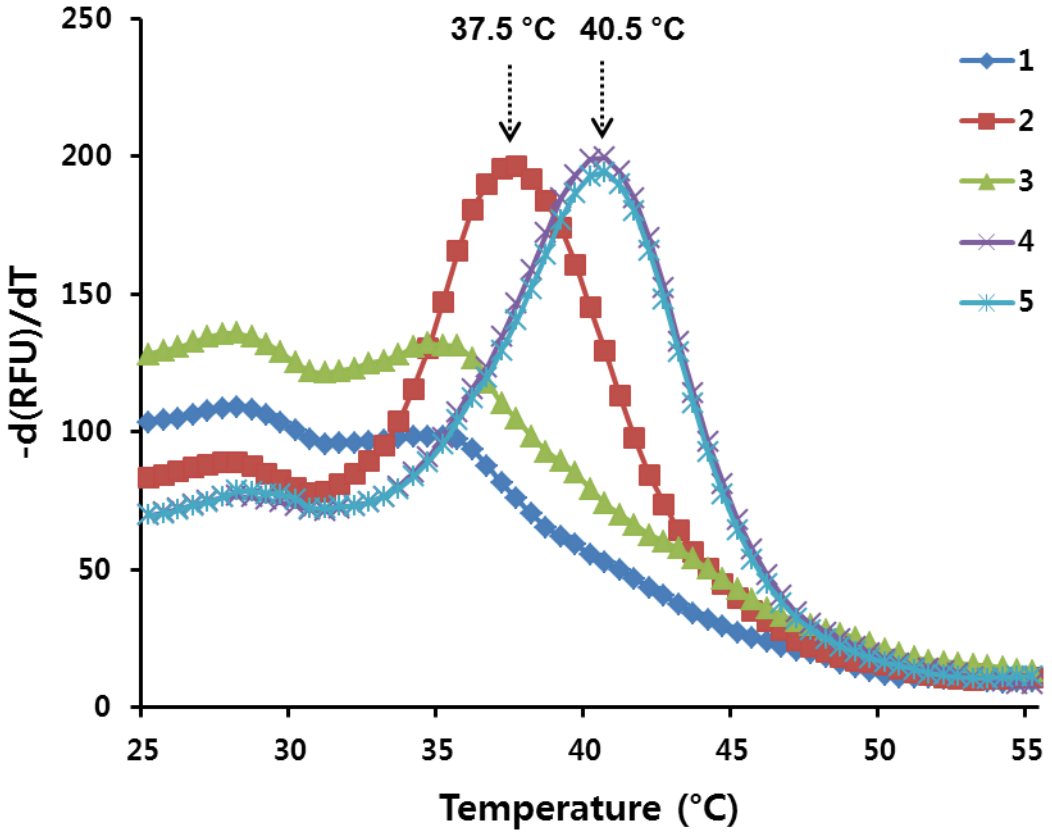

Figure S8. Melting curve analysis for the UDG detection probe composed of UDG detection probe and UDG blocker. (1) UDG detection probe, (2) UDG detection probe + UDG blocker, (3) UDG detection probe + UDG blocker + UDG, (4) UDG detection probe + control UDG blocker, and (5) UDG detection probe + control UDG blocker + UDG. The final concentrations of UDG detection probe, UDG blocker, and UDG are 200 nM, 100 nM, and 10 U/mL, respectively.

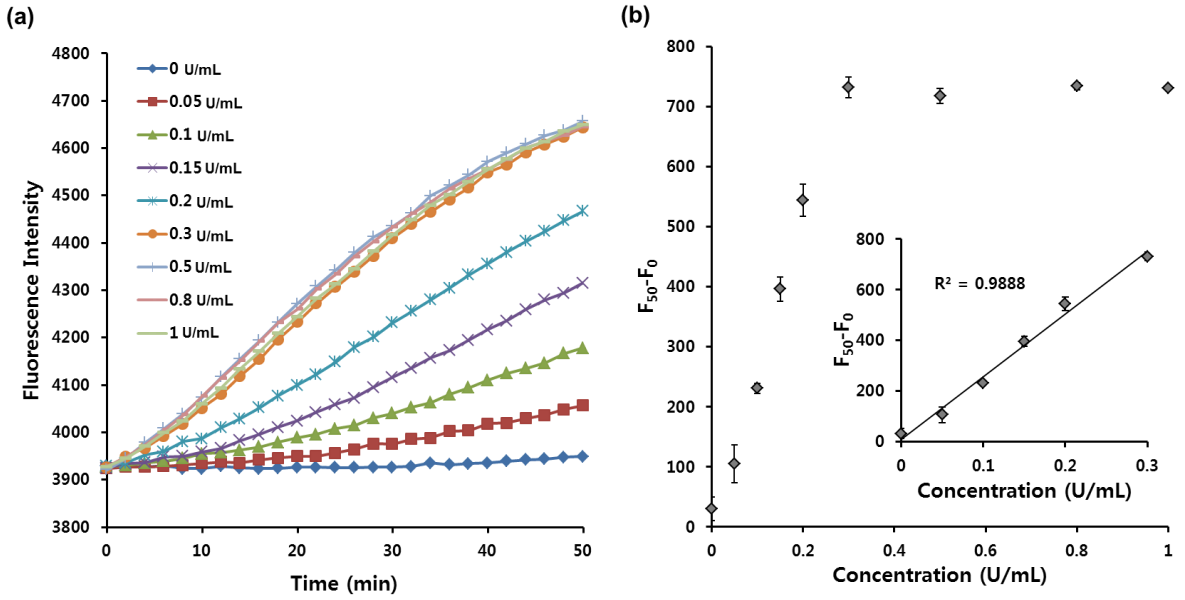

Figure S9. Sensitivity of new UDG assay. (a) Time-dependent fluorescence intensities from TaqMan probe during a primer extension reaction. UDG detection probe and UDG blocker is treated with UDG at varying concentrations and subjected to the assay procedures. (b) Fluorescence signal change (F_{50}-F_0) depending on the concentration of UDG, where F_{50} and F_0 are the fluorescence intensities from TaqMan probe measured at 50 min and 0 min, respectively. Inset in (b): Linear range between (F_{50}-F_0) and UDG (0-0.3 U/mL). The final concentrations of DNA polymerase, UDG detection probe, and UDG blocker are 5.5 nM, 200 nM, and 10 nM, respectively.

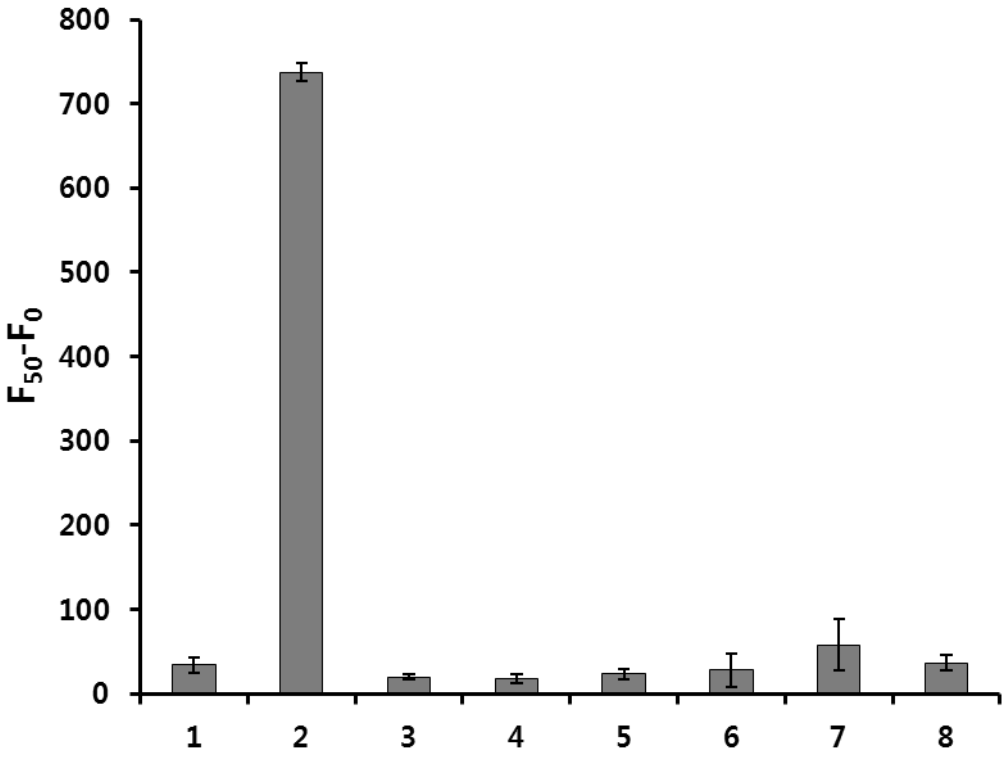

Figure S10. Specificity of new UDG assay. Fluorescence signal change (F_{50}-F_0) without any enzyme treatment (1) and with the treatment of UDG (2), hAAG (3), hOGG1 (4), Fpg (5), BamHI (6), Exo I (7), and Lambda exonuclease (8), where F_{50} and F_0 are the fluorescence intensities from TaqMan probe measured at 50 min and 0 min, respectively. UDG detection probe and UDG blocker is treated with UDG or the other enzymes. The final concentrations of DNA polymerase, UDG detection probe, UDG blocker, UDG, and the other enzymes are 5.5 nM, 200 nM, 10 nM, 0.5 U/mL, and 5 U/mL, respectively.

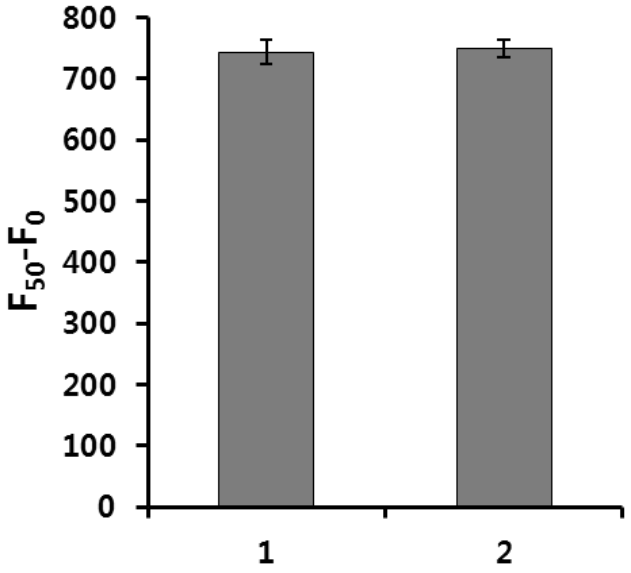

Figure S11. Fluorescence signal change (F_{50}-F_0) in the absence (1) and presence (2) of UGI, where F_{50} and F_0 are the fluorescence intensities from TaqMan probe measured at 50 min and 0 min, respectively. The final concentrations of DNA polymerase and UGI are 5.5 nM and 0.5 U/mL, respectively.

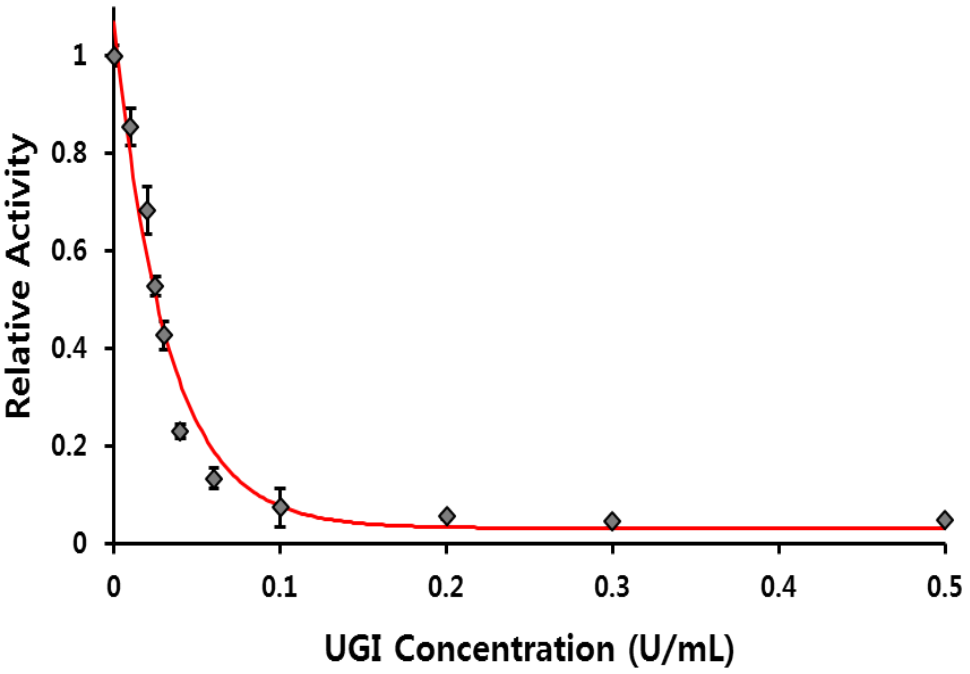

Figure S12. Determination of UDG activity inhibited by the presence of UGI at varying concentrations. The relative activity of UDG is defined as a ratio of the fluorescence signal change (F_{50}-F_0) reduced by the presence of UGI to the initial fluorescence signal change in the absence of UGI, where F_{50} and F_0 are the fluorescence intensities from TaqMan probe measured at 50 min and 0 min, respectively. The final concentrations of DNA polymerase, UDG detection probe, UDG blocker, and UDG are 5.5 nM, 200 nM, 10 nM, and 0.5 U/mL, respectively.

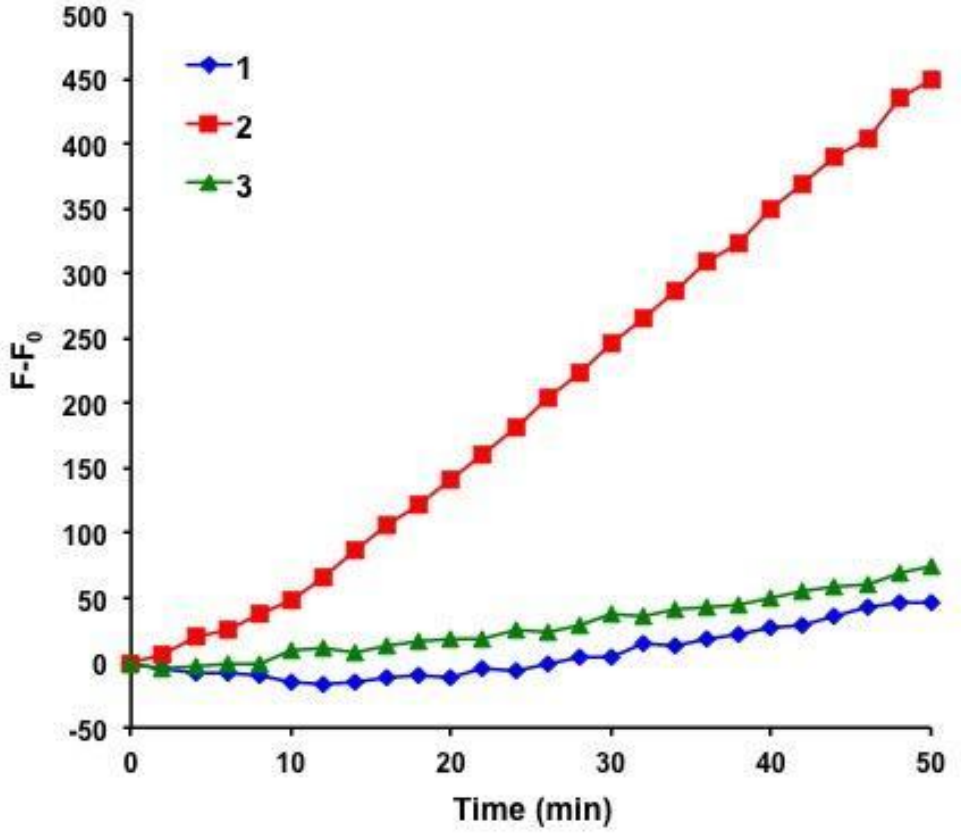

Figure S13. Detection of UDG activity in A549 cells. (1) UDG detection probe and UDG blocker without cell-free extracts, (2) UDG detection probe and UDG blocker with cell-free extracts (2 μg), (3) UDG detection probe and control UDG blocker with cell-free extracts (2 μg). F and F_0 were the fluorescence intensities from Taqman probe measured at different time points and 0 min, respectively. The final concentrations of DNA polymerase, UDG detection probe, UDG blocker and UDG control blocker are 5.5 nM, 200 nM 10 nM, and 10 nM, respectively.

References

Lee, C.Y., Park, K.S., Park, H.G., 2015. Chem. Commun. 51, 13744-13747.

Liu, X., Chen, M., Hou, T., Wang, X., Liu, S., Li, F., 2014. Biosens. Bioelectron. 54, 598-602.

Maksimenko, A., Ishchenko, A.A., Sanz, G., Laval, J., Elder, R.H., Saparbaev, M.K., 2004. Biochem. Biophys. Res. Commun. 319, 240-246.

Song, C., Wang, G.Y., Wang, H.Z., Wang, Y.J., Kong, D.M., 2014. J. Mat. Chem. B 2, 1549-1556.

Zhang, Q., Kong, D.-M., 2013. Analyst 138, 6437-6444.