Supporting Information for

# Label-free and sensitive detection of Uracil-DNA glycosylase using exponential real-time rolling circle amplification

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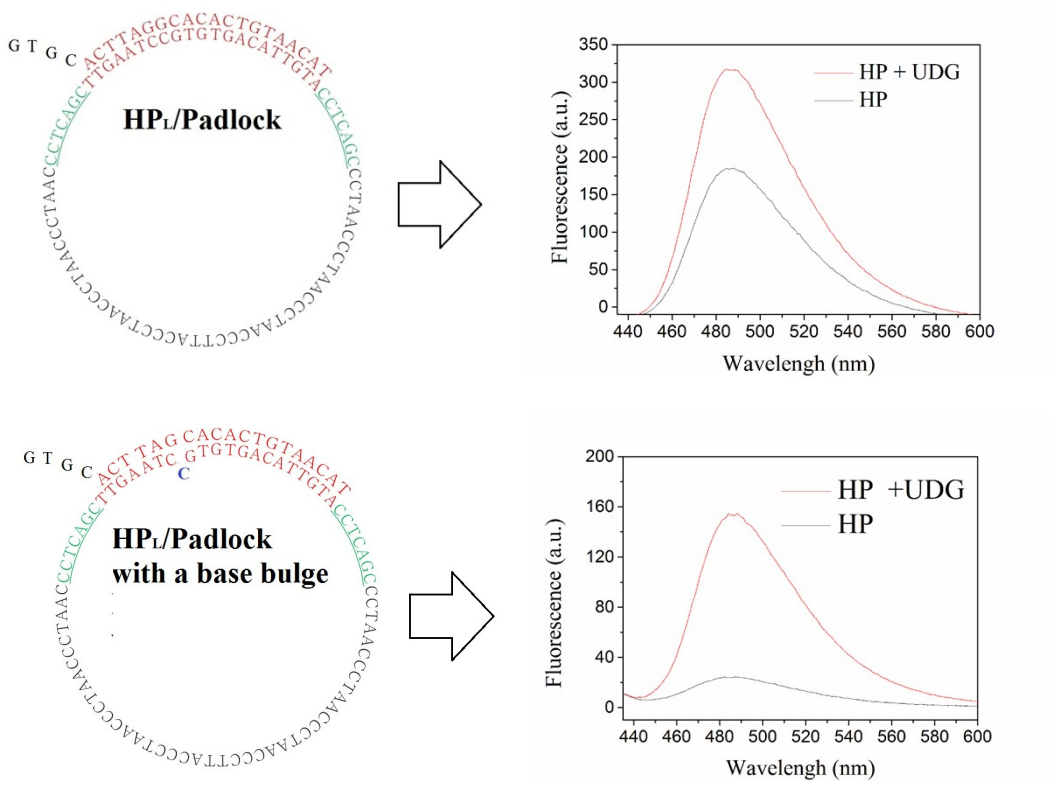
## 1. $HP_L$/Padlock complex with a base bulge




**Scheme S1.** Comparision of $HP_L$/Padlock complexs without or with a base bulge. (Left), Schemetic representation of $HP_L$/Padlock complexs; (Right) Fluorescence signals given by corresponding UDG-sensing systems.

## 2. Optimization of exponential RCA-based UDG-sensing platform

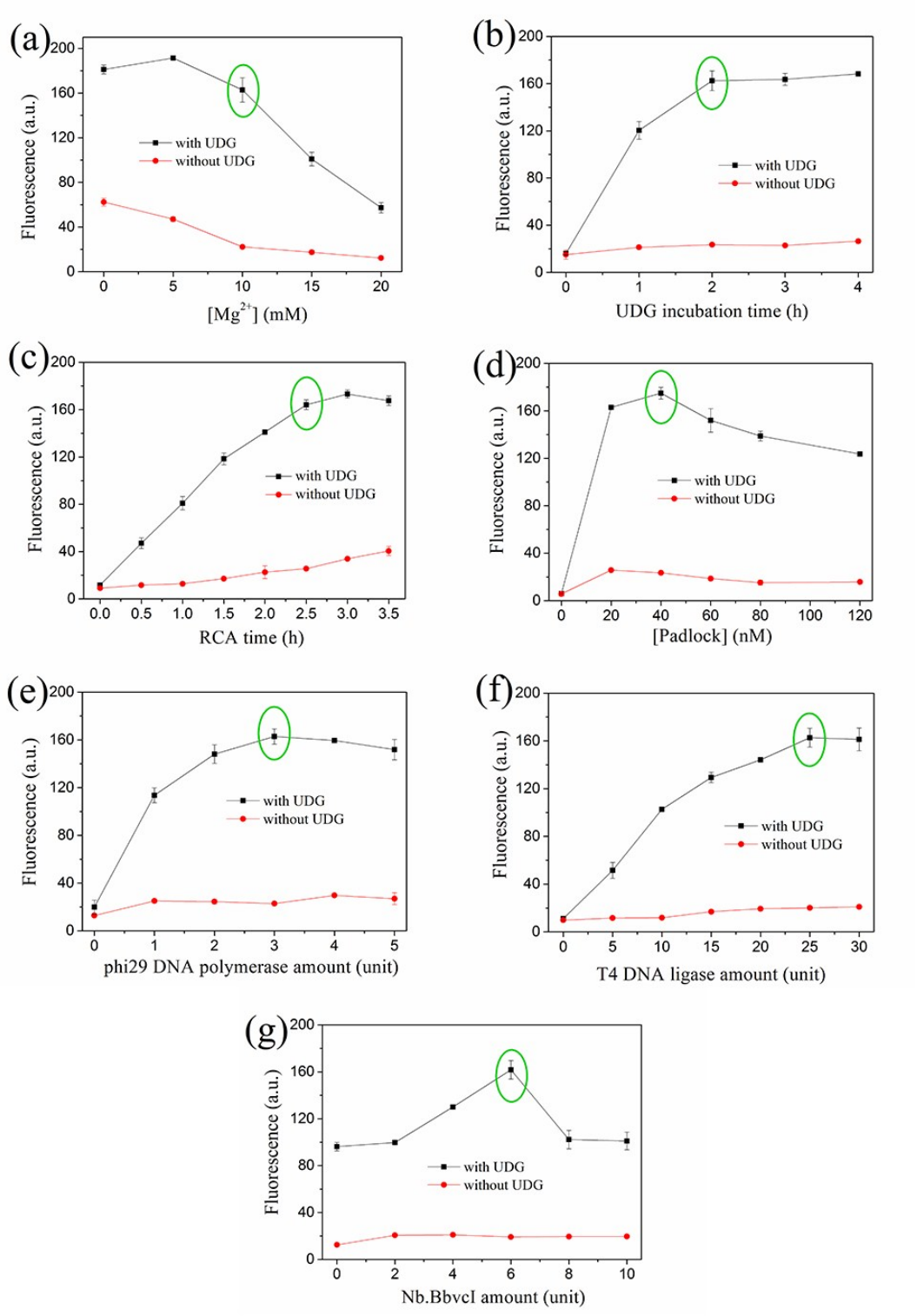



**Fig. S1.** Optimization of $Mg^{2+}$ concentration (a), UDG incubation time (b), RCA reaction time (c), **Padlock** concentration (d) amounts of phi29 DNA polymerase (e), T4 DNA ligase (f) and Nb.BbvCI (g) in exponential RCA-based UDG-sensing system. The data were expressed as mean ± σ (n=3).

## 3. Linear RCA-based UDG-sensing platform

## 3.1 Optimization of experimental conditions

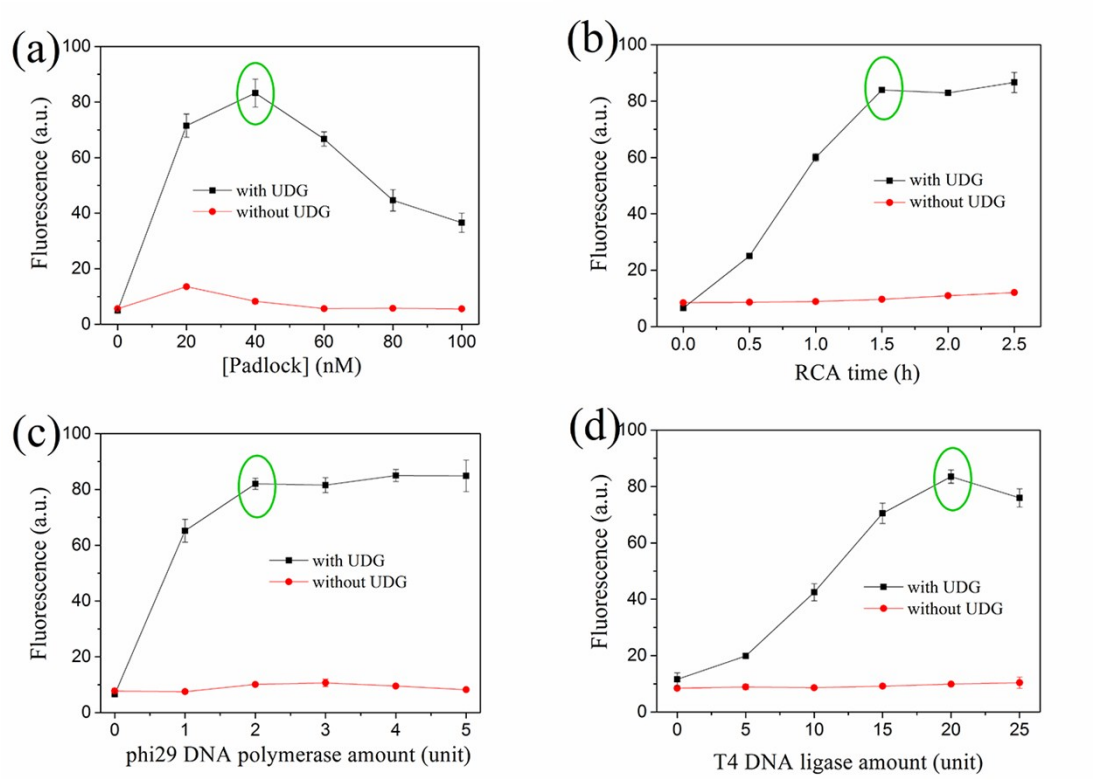



**Fig. S2.** Optimization of **Padlock** concentration (a), RCA reaction time (b), amounts of phi29 DNA polymerase (c) and T4 DNA ligase (d) in linearly amplified RCA-based UDG-sensing system. The data were expressed as mean ± σ (n=3).

## 3.2 UDG activity detection using linear RCA-based sensing platform

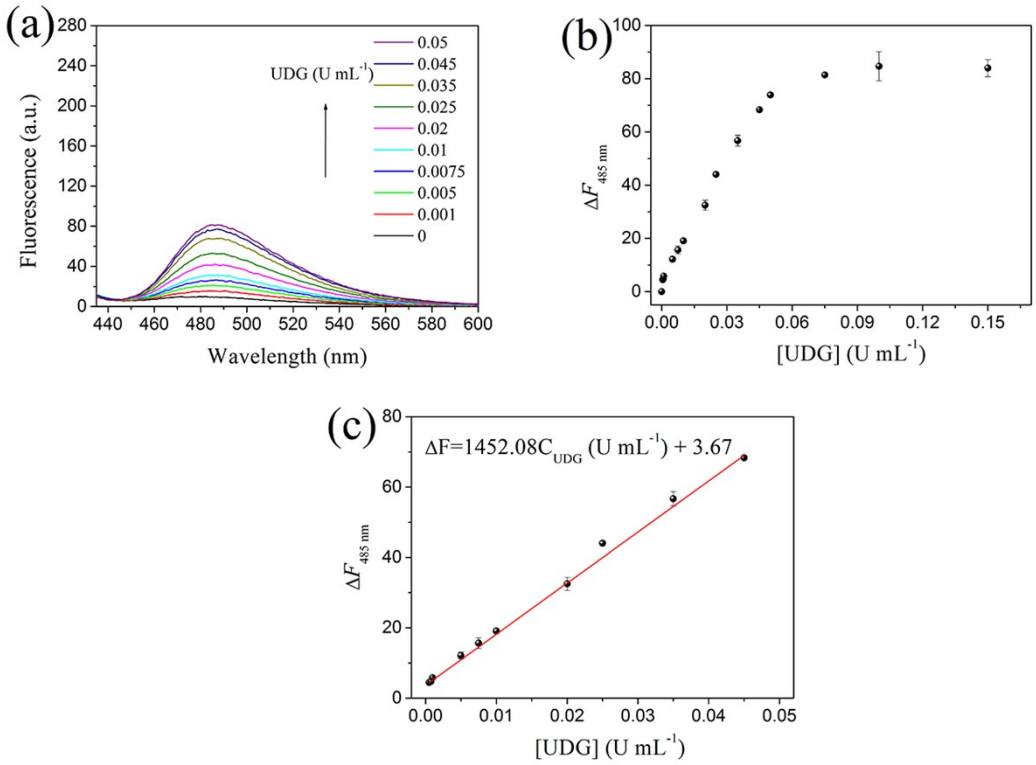



**Fig. S3.** UDG activity detection by linear RCA-based sensing platform. (a) Fluorescence spectra of the sensing systems containing different concentrations of UDG. (b) UDG activity-dependent fluorescence intensity change ($\Delta F$) at 485 nm. (c) The linear relationship between $\Delta F$ and UDG concentration in the range of $1\times10^{-3} \sim 0.045$ U mL$^{-1}$. The data were expressed as mean ± σ (n=3).

## 4. Comparison of our method with other reported ones

**Table S1.** Detection limits and real sample applications of some UDG detection methods

| Method | System | Detection limit (U $mL^{-1}$) | Real sample application | Reference |
|---|---|---|---|---|
| Colorimetric | Formation of a G-quadruplex–cofactor complex | 0.008 | Not given | [12] |
| Colorimetric | Nicking enzyme assisted chain replace amplification | 0.02 | Not given | [13] |
| Luminescent | UDG assisted pyridinium luminescent switch-on molecular probe | 0.005 | Diluted serum samples with spiked UDG | [14] |
| Fluorescence | Enzyme-assisted bicyclic cascade signal | 0.0001 | Cell extract | [16] |
| Fluorescence | Intrinsically fluorescent nucleotides | 0.0025 | Cell extract | [17] |
| Fluorescence | Inserted fluorescence probe | 0.0008 | Cell extract | [18] |
| Fluorescence | Synthesis a G-quadruplex probe | 0.02 | Not given | [19] |
| Fluorescence | Real time monitoring uracil excision via labeled molecular beacons | 0.005 | Cell extract with spiked UDG | [21] |
| Fluorescence | Real-time strand displacement amplification | $6\times10^{-5}$ | Cell extract | [25] |
| Fluorescence | Real-time exponential RCA | $5.5\times10^{-5}$ | Cell extract | This work |