

**Protein binding-protected DNA three-way junction-mediated rolling
circle amplification for sensitive and specific detection of
transcription factors**

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Table S1. Sequences of oligonucleotides used in this study

Name	Sequence (5'→3')
S1-1	GTA GCA CTA GGA <u>GGA AAG TCC CGA TCC</u> CCC CG
S1-2	GTA GCA CTA G <u>GGA AAG TCC CGA TCC</u> CCC CG
S1-3	GTA GCA CTA <u>GGA AAG TCC CGA TCC</u> CCC CG
S2-1	CGG GGG GAT <u>CGG GAC TTT CCA</u> TGT CTC TGT CTC T
S2-2	CGG GGG GAT <u>CGG GAC TTT CCA</u> TGA GT CTC TGT CTC T
S2-3	CGG GGG GAT <u>CGG GAC TTT CCA</u> TGG AGT CTC TGT CTC T
Entire primer	GTA GCA CTA GGA GTC TCT GTC TCT
Padlock probe	<i>TCC TAG TGC TAC</i> CCC AAC CCG CCC TAC CCA AAA CCC AAC CCG CCC TAC CCA AAA CCC AAC CCG CCC TAC CCA <i>AGA GAC AGA GAC</i>

Note: The binding sequence of NF-kB p50 is underlined. The recognition sequence of AlwI is bold. The complementary sequence of the padlock probe to the DNA three-way junction (TWJ) is italic.

Optimization of the reaction conditions

To obtain the best results, we investigated the reaction conditions. The following are the optimal experimental results of the dosage of AlwI and the concentrations of phi29 DNA polymerase, dNTPs and N-methyl-mesoporphyrin IX (NMM).

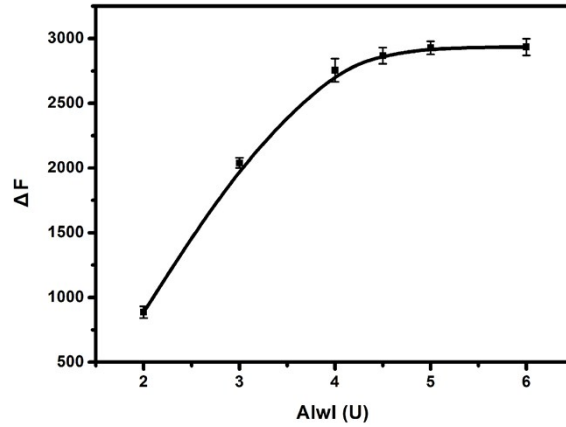


Fig. S1 Effect of Alwi dosage on the TFs detection system. Dosage: 2.0, 3.0, 4.0, 4.5, 5.0 and 6.0 units. The error bars showed the standard deviation of three replicate determinations.

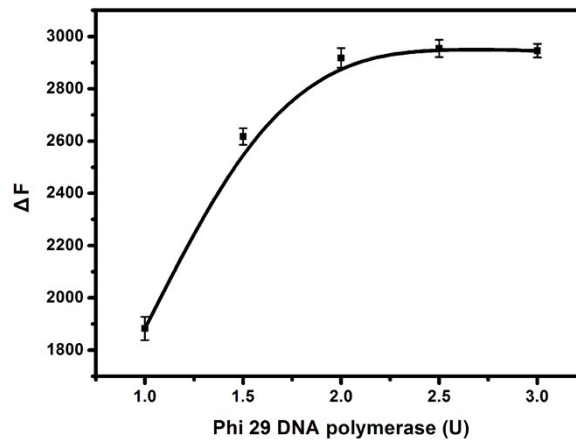


Fig. S2 Effect of phi29 DNA polymerase dosage on the TFs detection system. The phi29 DNA polymerase dosage: 1.0, 1.5, 2.0, 2.5 and 3.0 U. The error bars showed the standard deviation of three replicate determinations.

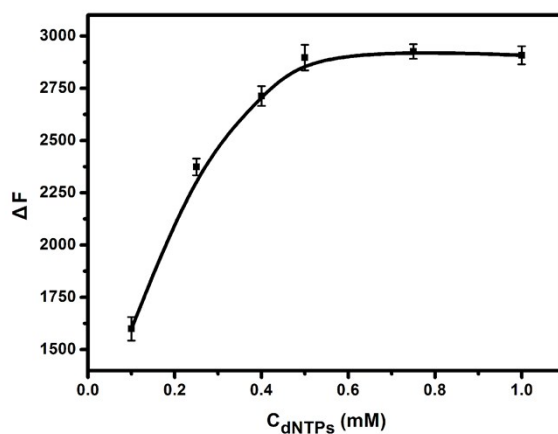


Fig. S3 Effect of the concentrations of dNTPs on the TFs detection system. The concentrations of dNTPs: 0.1, 0.25, 0.4, 0.5, 0.75 and 1.0 mM. The error bars showed the standard deviation of three replicate determinations.

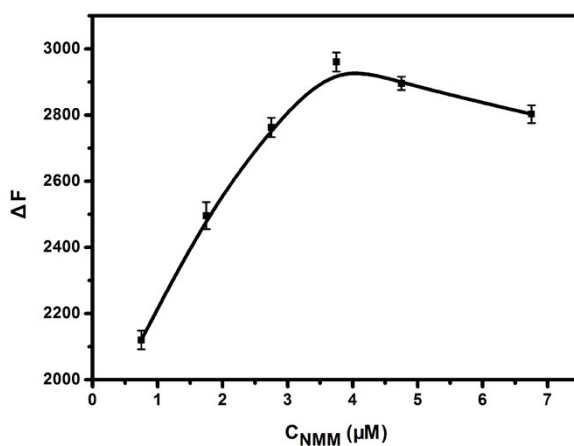


Fig. S4 Effect of different concentrations of NMM on the TFs detection system. NMM concentration: 0.75, 1.75, 2.75, 3.75, 4.75, and 6.75 μM . The error bars showed the standard deviation of three replicate determinations.

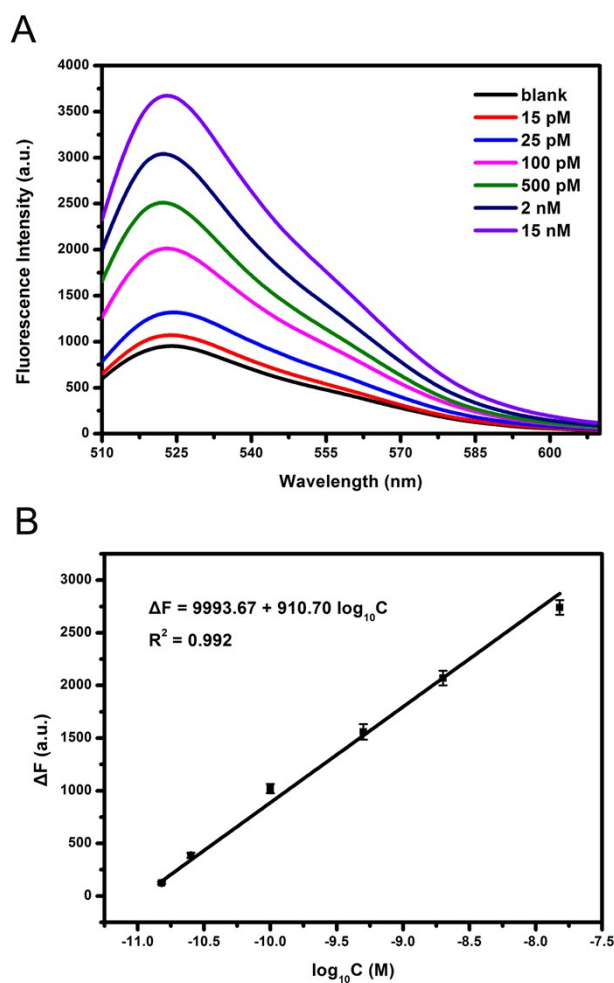


Fig. S5 (A) Fluorescence curves obtained from different NF- κ B p50 concentrations using SYBR Green I as a signal indicator. (B) Linear relationship between the fluorescence enhancement and NF- κ B p50 concentration in SYBR Green I method. Error bars indicate the standard deviation of 3 measurements.

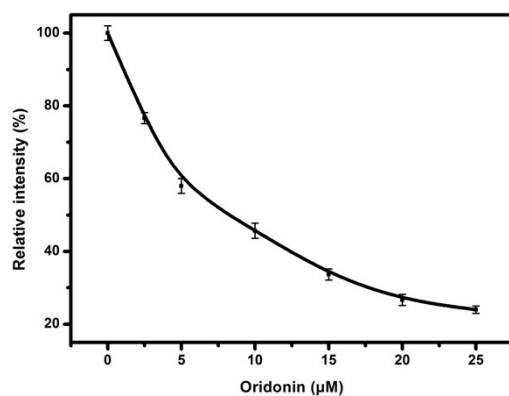


Fig. S6 The relative fluorescence intensity with addition of oridonin with different

concentrations (0, 2.5, 5.0, 10, 15, 20 and 25 μ M). Error bars were the standard deviations three independent measurements.

Table S2. Recovery experiment in this study

Concentration (mol/L)	Sample 1 (mol/L)	Sample 2 (mol/L)	Sample 3 (mol/L)	Recovery	RSD
5.0×10^{-11}	4.7×10^{-11}	4.8×10^{-11}	4.9×10^{-11}	96.0%	2.1%
5.0×10^{-10}	4.9×10^{-10}	5.3×10^{-10}	5.2×10^{-10}	102.7%	4.1%
5.0×10^{-9}	4.8×10^{-9}	5.0×10^{-9}	5.0×10^{-9}	98.7%	2.3%