A novel fluorescence method for activity assay and drug screening

of T4 PNK coupling rGO with ligase reaction

Hongyan Zhou^{1a†}, Chunyi Tong^{1a†}, Wei Zou^{3a†}, Yupei Yang², Yongbei Liu², Bin Li², Yan Qin², Wenya Dang¹, Bin Liu^{1,2*}, Wei Wang^{2*}

¹College of Biology, Hunan University, Changsha, 410082, China

²TCM and Ethnomedicine Innovation & Development International Laboratory, Innovative Material Medical Research Institute, School of Pharmacy, Hunan University of Chinese Medicine,

Changsha, 410208, China

³NHC key laboratory of birth defects research, prevention and treatment, Hunan Provincial Maternal and Child Health Care Hospital, Changsha 410008, PR China

To whom correspondence should be addressed. Tel: +86-731-89720939; Fax: +86-731-89720939;

E-mail: <u>binliu2001@hotmail.com(B. Liu);</u> <u>wangwei402@hotmail.com(W. Wang)</u>

Table.S1 Sequences of Oligonucleotide probes used in this work

| Oligo name | Base sequence (5' to 3') | Bases | 5 'modification | Tm(°C) |
|------------|--------------------------------|-------|-----------------|--------|
| P1 | CACGCCATGTCGAAATTCTTGCGTGCCTAT | 30 | | 76.5 |
| P2 | GCAAGAATTTCGACATGGCGTG | 22 | | 67 |
| P3 | ATAGGCAC | 8 | FAM | <10 |
| P4 | GCAAGAATTTCGACATGGCGTG | 22 | phosphorylation | 67 |
| P5 | ATAGGCAC | 8 | | <10 |

Table. S2 Natural compounds information

| S.no | Source | Name | Structure | Molecular Formula | Molecular Weight |
|------|---------------|----------------|-----------|--|------------------|
| a | Cherokee Rose | Euscaphic acid | | C ₃₀ H ₄₈ O ₅ | 488.70 |

| b | Cherokee Rose | Laevigatanoside A | | C ₃₆ H ₅₈ O ₁₁ | 666.84 |
|---|------------------|-------------------|--|---|----------|
| c | Cherokee Rose | Syringaresinol | H ₁ CO HO H ₂ CO HO H ₃ CO HO H ₁ CO HI OCH ₃ OCH ₃ OCH ₃ OCH ₃ OCH ₃ | C ₂₂ H ₂₆ O ₈ | 418.45 |
| d | kadsura coccinea | Kadsuphilol A | H ₃ CO HO H ₃ CO H ₃ CO O H | C ₂₂ H ₂₆ O ₇ | 402.1679 |
| e | kadsura coccinea | Kadsutherin A | H ₃ CO H ₃ CO HO HO HO O O O Ang | C ₂₆ H ₃₀ O ₈ | 470.1941 |

| f | kadsura coccinea | Abiesatrine J | | C ₃₀ H ₄₆ O ₄ | 470.3396 |
|---|------------------|----------------------|--|---|----------|
| g | kadsura coccinea | Masticadienoic acid | 0 ,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, | C ₃₀ H ₄₆ O ₃ | 454.3447 |
| h | kadsura coccinea | Seco-coccinic acid A | | C ₃₀ H ₄₈ O ₃ | 456.3603 |
| i | kadsura coccinea | Kadsurarin | HO H ₃ CO HO HO HO HO HO HO HO CH ₃ | C ₂₉ H ₃₄ O ₁₀ | 542.2152 |
| j | kadsura coccinea | Schisanlactone B | | C ₃₀ H ₄₂ O ₄ | 466.3083 |



Fig.S1 (A) The effect of pH on the stability of the probe. Reaction buffer's pH is varied from 7.1 to 8.9. The concentration of Mg^{2+} is 10 mM. (B) The effect of cell extract on the stability of the probe. The concentration of Mg^{2+} and pH in reaction buffer is 10mM and 8.0, respectively. (C) The effect of serum on the stability of the probe. The concentration of Mg^{2+} and pH in reaction buffer is 10mM and 8.0, respectively. (C) The effect of serum on the stability of the probe. The concentration of Mg^{2+} and pH in reaction buffer is 10mM and 8.0, respectively. (C) The effect of serum on the stability of the probe. The concentration of Mg^{2+} and pH in reaction buffer is 10mM and 8.0, respectively. (D-F) The effect of various ions including $Na^{+}(D)$, $K^{+}(E)$, $Mg^{2+}(F)$ on the stability of the probe. The pH value of reaction buffer is 8.0.



Fig.S2 (A) The UV-vis spectrum of rGO and GO, [rGO] and [GO] are 20 mg/L, respectively. (B) The ζ-potential of rGO and GO, [rGO] and [GO] are 10 mg/L, respectively. (C) The Infrared Spectroscopy of rGO and GO.



Fig.S3 The feasibility analysis. (A) The quenching effect of rGO on P3. [P3] and [rGO] are 100 nM and 10 mg/L, respectively. (B) The quenching effect of rGO on (P1+P2+P3) and (P1+P4+P3). [P1], [P2], [P3], [P4] and [rGO] are 100 nM and 10 mg/L, respectively.