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Electronic Supporting Information

A highly selective G-quadruplex-based luminescent switch-on probe for the detection of gene deletion

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Experimental section

Materials

Reagents were purchased from Sigma Aldrich and used as received. Iridium chloride hydrate ($IrCl_3.xH_2O$) was purchased from Precious Metals Online. The Tris buffer used in the experiment consisted of 50 mM Tris-HCl (pH 7.0) containing 20 mM KCl and 150 mM NH_4Ac. All reagents were used without further purification. Milli-Q purified water was used to prepare all solutions. All oligonucleotides were synthesized by Techdragon Inc. (Hong Kong, China), and the sequences of the single-stranded oligonucleotides were as follows:

Table S1. DNA sequences: the **bolded** sequences represent the split G-quadruplex-forming sequences and the underlined represent sequences of the deletion site. Sequences are quoted from the 5′ end. Except for in Fig. S2, all DNA concentrations quoted are on per strand basis.

	Sequences
CCR5-mutant DNA (CCR5-D)	5′-CTCATTTTCCATACATTAAAGATAGTCAT-3′
CCR5-wild DNA	5′-CTCATTTTCCATACAGTCAGTATCAATTCTGGAAGAATTTCCAGACATTAAAGATAGTCAT-3′
CCR5-P1	5′-ATGACTATCTTTAAT**GGGTAGGG**-3′
CCR5-P2	5′-**GGGTTGGG**CGTATGGAAAATGAG-3′
CCR5-D_{m1}	5′-CTCAAAAACCATACATTTTTGATAGTCAT-3′
CCR5-D_{m2}	5′-CTCAAAAACCATACATTAAAGATAGTCAT-3′
Pu22	5′-$GAG_3TG_4AG_3TG_4A_2G$-3′
Random ss DNA	5′-ATGCAGCCTGGGAAAGTCCCCTCAACT-3′
CCR5-$P1_{m1}$	5′-ATGACTATCTTTAATGAATAGAA-3′
CCR5-$P2_{m1}$	5′-AAGTTGAACGTATGGAAAATGAG-3′
CCR5-$P1_{m2}$	5′-ATGACTATCTTTAATGTTTAGTT-3′
CCR5-$P2_{m2}$	5′-TTGTTGTTCGTATGGAAAATGAG-3′
CCR5-P1(a)	5′-ATGACTATCTTTAAT**GGGTTTTGGGG**-3′
CCR5-P2(a)	5′-**GGGGTTTTGGG**CGTATGGAAAATGAG-3′
CCR5-P1(b)	5′-ATGACTATCTTTAAT**GGGGTTTTGGG**-3′
CCR5-P2(b)	5′-**GGGTTTTGGGG**CGTATGGAAAATGAG-3′
CCR5-P1(c)	5′-ATGACTATCTTTAAT**GGGTTTTGGG**-3′
CCR5-P2(c)	5′-**GGGTTTTGGG**CGTATGGAAAATGAG-3′

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CCR5-P1(d)	5′-ATGACTATCTTTAAT**TGGGCGGGTTGGGT**-3′
CCR5-P2(d)	5′-**TGGGT**GTATGGAAAATGAG-3′
CCR5-P1(e)	5′-ATGACTATCTTTAAT**TGGGTAGGGCGGG**-3′
CCR5-P2(e)	5′-**TGGGT**GTATGGAAAATGAG-3′
CCR5-mutant DNA (Δ12)	5′-CTCATTTTCCATACAGTCAGTATCATTTCCAGACATTAAAGATAGTCAT-3′
CCR5-mutant DNA (Δ20)	5′-CTCATTTTCCATACAGTCAGAGACATTAAAGATAGTCAT-3′
CCR5-mutant DNA (Δ26)	5′-CTCATTTTCCATACAGTCACATTAAAGATAGTCAT-3′
CCR5-mutant DNA (Δ29)	5′-CTCATTTTCCATACAGCATTAAAGATAGTCAT-3′
CCR5-mutant DNA (Δ30)	5′-CTCATTTTCCATACAGATTAAAGATAGTCAT-3′
CCR5-mutant DNA (Δ31)	5′-CTCATTTTCCATACAATTAAAGATAGTCAT-3′
CCR5 (lengthened P1)	5′-ATGACTATCTTTAATGTCTGGAAATTCTTCT**GGGATGGG**-3′
CCR5 (lengthened P2)	5′-**GGGTTGGG**TAGAATTGATACTGACTGTATGGAAAATGAG-3′

Synthesis of the $[Ir(ppy)_2(biq)]PF_6$

The $[Ir(ppy)_2(biq)]PF_6$ was prepared according to the literature methods without modification.[1]

DNA extract preparation

HeLa cells were grown in culture flask and total DNA was extracted according to the manufacturer's (QIAamp DNA Mini Kit, Qiagen) instruction. The DNA extract (0.56 μg/ml) was then mixed with annealed DNA mixtures (0.5 μM P1, 0.5 μM P2, 0.5 μM mutant DNA; or 0.5 μM P1, 0.5 μM P2, 0.5 μM wild type DNA), and the luminescence spectra were recorded after the addition of iridium(III) complex **1** and equilibration at 25 °C for 10 min.

Emission measurement

A mixture of P1, P2 (2.5 μL, each 100 μM) and mutant DNA or wild-type DNA (2.5 μL, 100 μM) was mixed in hybridization buffer (97.5 μL, 50 mM Tris-HCl containing 20 mM KCl and 150 mM NH_4Ac, pH 7.0). The mixture was annealed at 95 °C for 10 min, and was slowly cooled to 25 °C. This stock solution containing 2.5 μM duplex DNA was stored at –20 °C for further use. In the emission measurement, 100 μL of the DNA mixture stock solution was diluted with 400 μL of Tris-HCl buffer (50 mM, pH 7.0) with 20 mM KCl and 150 mM NH_4Ac to obtain a 0.5 μM DNA mixture solution in a cuvette, followed by the addition of iridium(III) complex **1** at a final concentration of 1.5 μM. The mixture was allowed to equilibrate at 25 °C for 10 min. Luminescence emission spectra were recorded on a PTI QM-4 spectrofluorometer at 25 °C. The fluorescence emission intensity at 560–730 nm was monitored after excitation of the sample at 390 nm.

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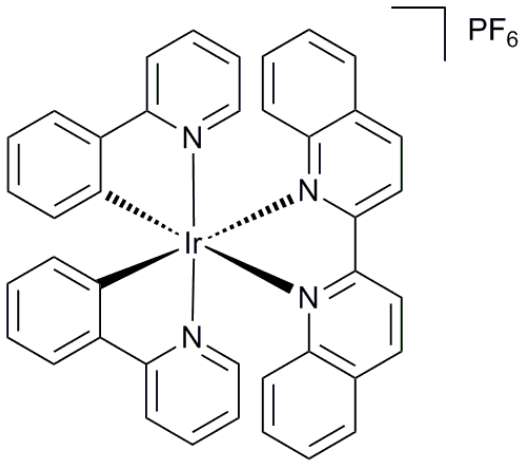

Fig. S1 Chemical structure of the luminescent cyclometallated iridium(III) complex **1**.

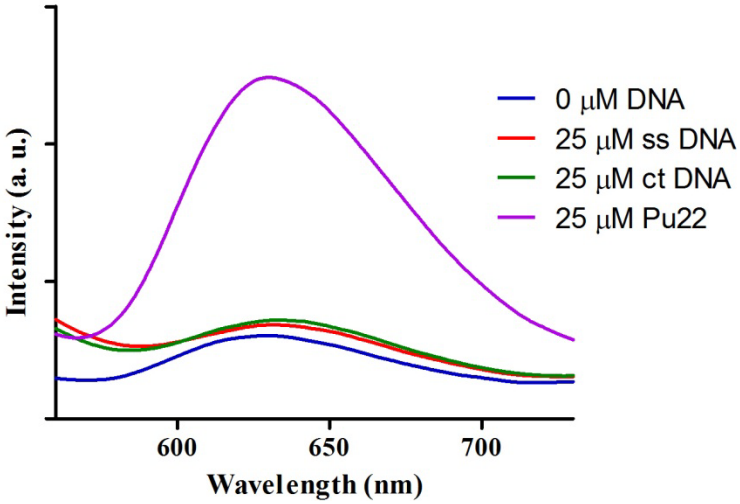

Fig. S2 Luminescence response of complex **1** (1.5 μM) in 50 mM Tris buffer (pH 7.0) in the presence of: 25 μM ct DNA (base concentration); 25 μM ss DNA (base concentration); 25 μM Pu22 (base concentration, 1.1 μM strand concentration). Pu22 was annealed in Tris buffer (50 mM, 100 mM KCl, pH 7.0).

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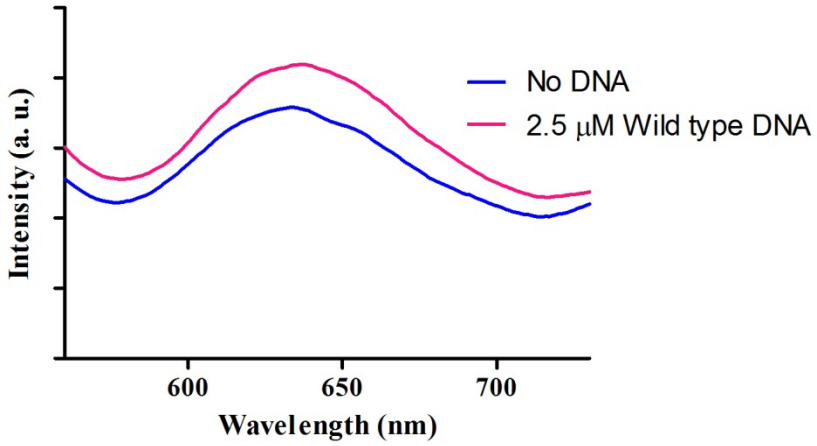
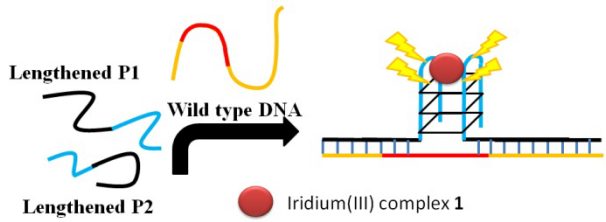


Fig. S3 Luminescence response of complex **1** (1.5 μM) in 50 mM Tris buffer (pH 7.0) containing 20 mM KCl and 150 mM NH_4Ac in the presence of: 2.5 μM wild type DNA and 2.5 μM P1, P2.



CCR5(lengthened P1): 5′-ATGACTATCTTTAATGTCTGGAAATTCTTCT**GGGATGGG**-3′
CCR5(lengthened P2): 5′-**GGGTTGGG**TAGAATTGATACTGACTGTATGGAAAATGAG-3′
CCR5-wild DNA: 5′-CTCATTTTCCATACAGTCAGTATCAATTCTGGAAGAATTTCCAGACATTAAAGATAGTCAT-3′

Scheme S1. Schematic representation of the split G-quadruplex-based luminescent turn-on detection strategy for wild-type DNA sensing using G-quadruplex-selective iridium(III) complex **1**.

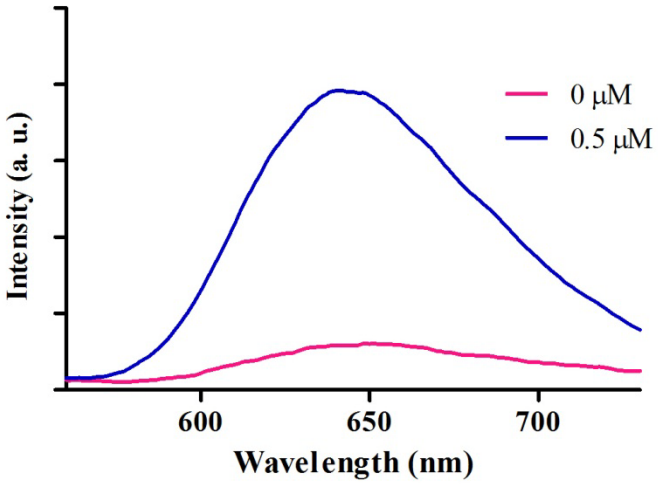


Fig. S4 Emission spectra of complex **1** (1.5 μM) with lengthened P1 and P2 (each 0.5 μM) in the absence (0 μM) or presence (0.5 μM) of the wild-type DNA.

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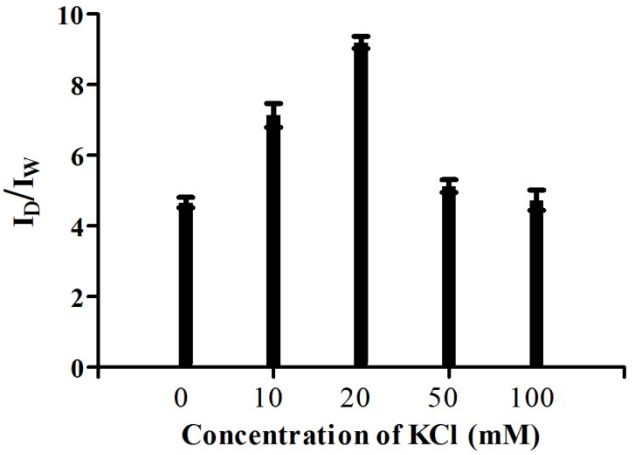

Fig. S5 Effect of the KCl concentration (0, 10, 20, 50 and 100 mM) on the relative luminescence response between mutant DNA and wild-type DNA. Experimental conditions: P1 and P2 (each 0.5 μM), mutant DNA (0.5 μM) or wild-type (0.5 μM) DNA with 1.5 μM complex **1** in 50 mM Tris (pH 7.0) containing 150 mM NH_4Ac.

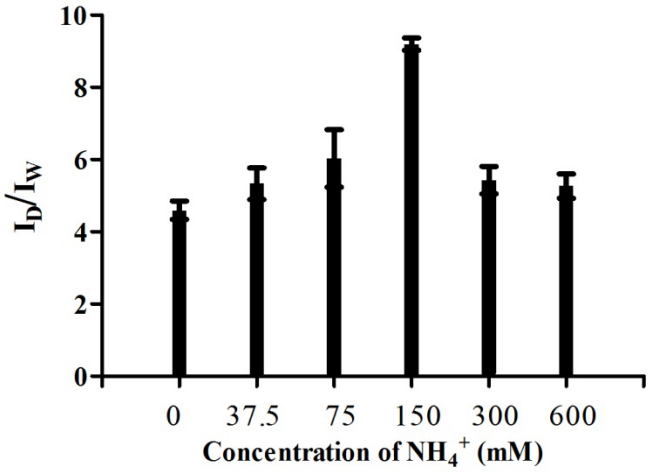

Fig. S6 Effect of the NH_4Ac concentration (0, 37.5, 75, 150, 300 and 600 mM) on the relative luminescence response between mutant DNA and wild-type DNA. Experimental conditions: P1 and P2 (each 0.5 μM), mutant DNA (0.5 μM) or wild-type (0.5 μM) DNA with 1.5 μM complex **1** in 50 mM Tris (pH 7.0) containing 20 mM KCl.

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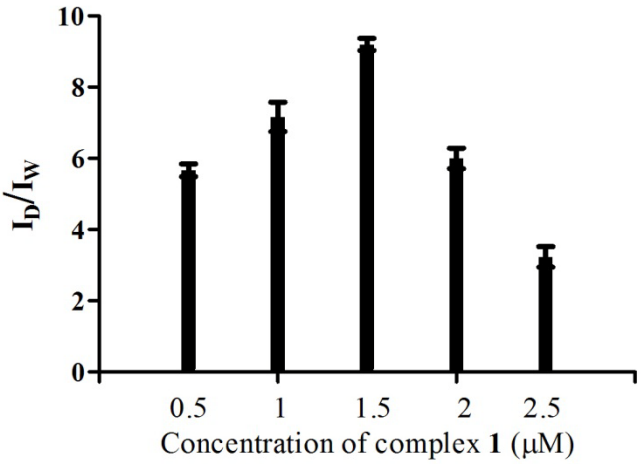

Fig. S7 Relative luminescence response between mutant DNA and wild-type DNA as a function various concentrations of complex **1** (0.5, 1, 1.5, 2 and 2.5 μM). Experimental conditions: P1 and P2 (each 0.5 μM), mutant DNA (0.5 μM) or wild-type (0.5 μM) DNA in 50 mM Tris (pH 7.0) containing 20 mM KCl, 150 mM NH_4Ac.

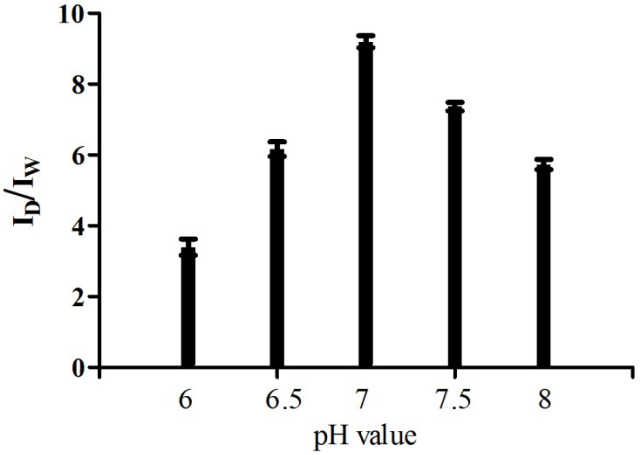

Fig. S8 Relative luminescence response between mutant DNA and wild-type DNA as a function of pH (6, 6.5, 7, 7.5 and 8). Experimental conditions: P1 and P2 (each 0.5 μM), mutant DNA (0.5 μM) or wild-type (0.5 μM) DNA with 1.5 μM complex **1,** 50 mM Tris containing 20 mM KCl, 150 mM NH_4Ac.

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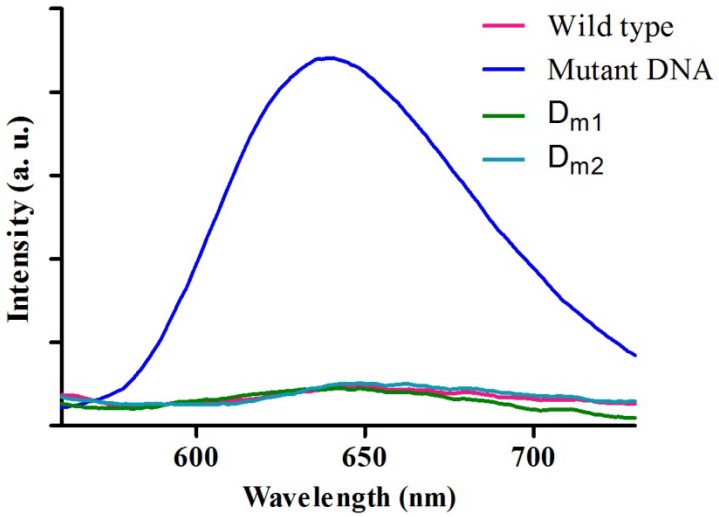


Fig. S9 Luminescence response of complex **1** (1.5 μM) in the presence of mutant DNA (0.5 μM), wild-type gene (0.5 μM), or mutated mutant DNA (0.5 μM). Experimental conditions: P1 and P2 (each 0.5 μM) in 50 mM Tris containing 20 mM KCl, 150 mM NH_4Ac.

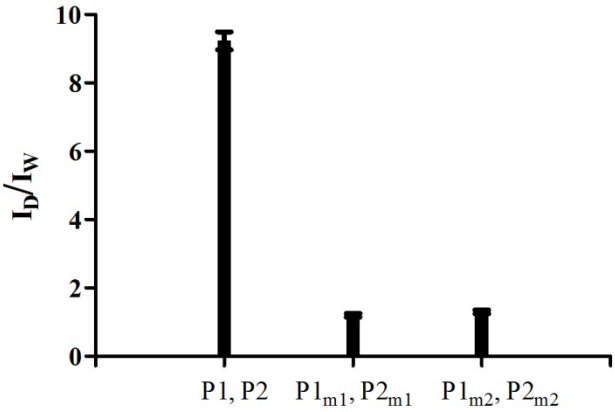


Fig. S10 Relative luminescence response between mutant DNA and wild-type DNA using the P1 and P2, mutated $P1_{m1}$ and $P2_{m2}$ or mutated $P1_{m2}$ and $P2_{m2}$. Experimental conditions: two split G-quadruplex sequences or mutants (each 0.5 μM), mutant DNA (0.5 μM) or wild-type (0.5 μM) DNA, with 1.5 μM complex **1,** 50 mM Tris containing 20 mM KCl, 150 mM NH_4Ac.

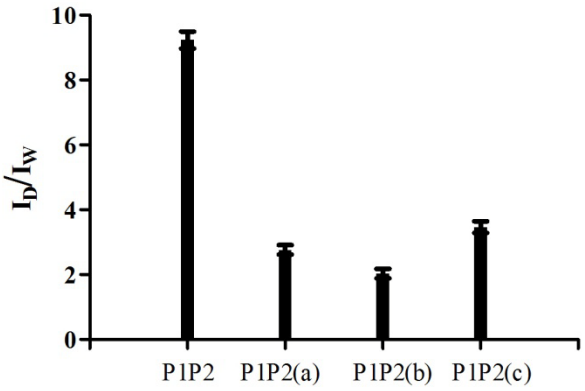
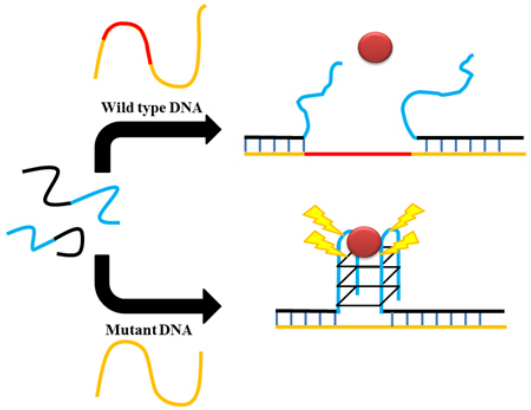


Fig. S11 Relative luminescence response between mutant DNA and wild-type DNA using different split G-quadruplex sequences. Experimental conditions: two split G-quadruplex sequences (each 0.5 μM), mutant DNA (0.5 μM) or wild-type (0.5 μM) DNA with 1.5 μM complex **1,** 50 mM Tris containing 20 mM KCl, 150 mM NH_4Ac.



CCR5-wild DNA : 5′-CTCATTTTCCATACAGTCAGTATCAATTCTGGAAGAATTTCCAGACATTAAAGATAGTCAT-3′
CCR5-mutant DNA (Δ12): 5′-CTCATTTTCCATACAGTCAGTATCATTTCCAGACATTAAAGATAGTCAT-3′
CCR5-mutant DNA (Δ20): 5′-CTCATTTTCCATACAGTCAGAGACATTAAAGATAGTCAT-3′
CCR5-mutant DNA (Δ26): 5′-CTCATTTTCCATACAGTCACATTAAAGATAGTCAT-3′
CCR5-mutant DNA (Δ29): 5′-CTCATTTTCCATACAGCATTAAAGATAGTCAT-3′
CCR5-mutant DNA (Δ30): 5′-CTCATTTTCCATACAGATTAAAGATAGTCAT-3′
CCR5-mutant DNA (Δ31): 5′-CTCATTTTCCATACAATTAAAGATAGTCAT-3′
CCR5-mutant DNA (Δ32): 5′-CTCATTTTCCATACATTAAAGATAGTCAT-3′

Scheme S2. DNA with different deletion lengths used in the investigation of relative luminescence response between mutant DNA and wild-type with different deletion length DNA (deletions of 32, 31, 30, 29, 26, 20 and 12 bases compared to the wild-type, deleted bases are shown highlighted in red).

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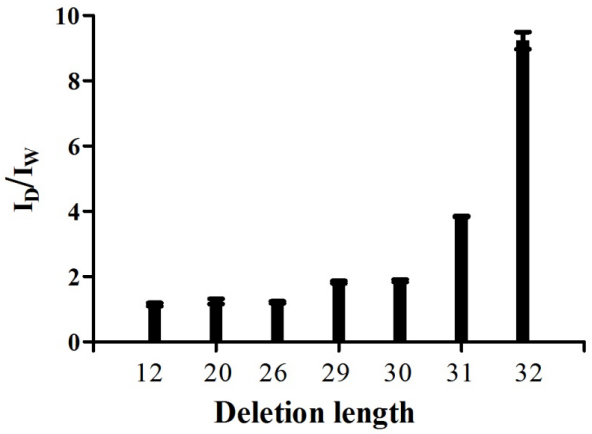

Fig. S12 Relative luminescence response between mutant DNA of different deletion lengths (deletion length of 12, 20, 26, 29, 30, 31 and 32 bases) versus wild-type DNA (no deletion). Experimental conditions: P1 and P2 (each 0.5 μM), mutant DNA with different deletion lengths (0.5 μM) or wild-type DNA (0.5 μM) with 1.5 μM complex **1,** 50 mM Tris containing 20 mM KCl, 150 mM NH_4Ac.

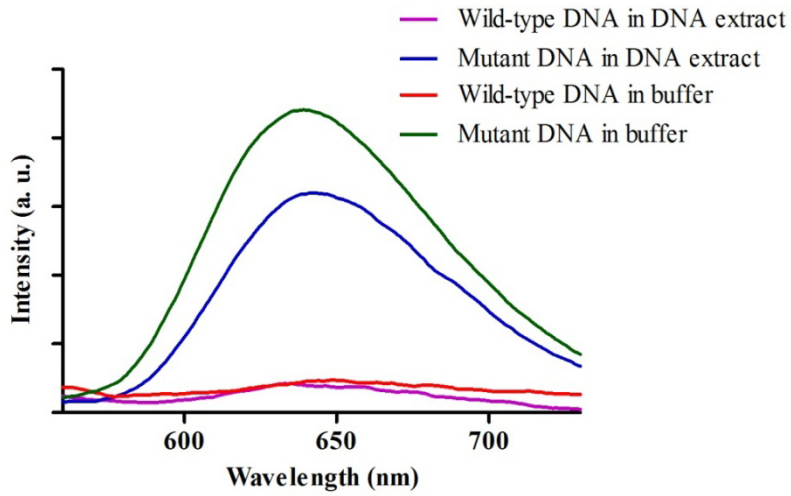

Fig. S13 Emission spectra of the complex **1** (1.5 μM) and CCR5-P1 and P2 (each 0.5 μM) in HeLa DNA extracts (0.56 μg/ml) in the presence of mutant DNA (0.5 μM) or wild-type DNA (0.5 μM). The luminescent response of the system to mutant and wild-type DNA under aqueous buffer conditions are shown together for comparison.

References

1. M. S. Lowry, W. R. Hudson, R. A. Pascal, Jr. and S. Bernhard, *J. Am. Chem. Soc.*, 2004, **126**, 14129-14135.