# Supporting Information for

# Topological effect of intramolecular split G-quadruplex on Thioflavin T binding and fluorescence light-up

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## Experimental section

**Asymmetric PCR amplification (A-PCR).** The gene sequences of Hepatitis B (GenBank accession number AB116094), Ebola (GenBank accession number KY786026.1) and Nova (GenBank accession number KB270442.1) viruses can be retrieved from the GenBank database of the National Center for Biotechnology Information. A-PCR primers including a forward primer (*FP*) and a reverse primer (*RP*) were designed for HBV fragment (*HF*) using the Snap gene sequence analysis and design software.

As for the developed LABP (Figure 6D-E), an optimum molar ratio of 10:1 was adopted. Each A-PCR sample (20 μL) was prepared by mixing 10 μL of Taq PCR Master Mix consisting of Taq DNA polymerase, dNTPs mix and $MgCl_2$, 3.6 μL of *FP* (20 μM), 1.8 μL of *RP* (4 μM), and 5 μL of target template (*HF*, *EF* or *NF*) of different concentrations. The template was replaced by buffer as a negative control. All the reaction mixtures were subjected to repeated cycles of 10 s at 98℃, 15 s at 60℃, and 15 s at 72℃, on an Applied Biosystems Veriti Thermal Cycler (Life Technologies Holdings Pte Ltd., Singapore). The A-PCR conditions used included an initial denaturation step at 98℃ for 30 s, followed by 35 cycles of denaturation at 98℃ for 10 s, annealing at 60℃ for 15 s, and an extension at 72℃ for 15 s. In the end, there was a final extension step at 72℃ for 7 min. The A-PCR amplicons were subjected to subsequent ligation and RCA reaction.

**Ligation and RCA reaction.** 20 μl of A-PCR product was mixed with 2 μl of T4 DNA ligase reaction buffer (400 mM Tris-HCl, pH 7.8, 100 mM $MgCl_2$, 100 mM DTT, 5 mM ATP and 50% PEG4000), 2.5 μl of padlock (*Pa*, 3.2 μM) and 0.75 μl of $ddH_2O$.

After annealing for 5 min at 75°C, 2 μl of T4 DNA ligase (4 U/μl) was added into the mixture, and the ligation reaction proceeded for 2 h at 37°C. Subsequently, the polymerization reaction was initiated by adding 1.75 μL of dNTPs (10 mM), 2 μL of phi29 DNA polymerase (5 U/μL) and 4 μL of phi29 buffer (330 mM Tris-HCl, 660 mM KCl, 100 mM $MgCl_2$, 1% (v/v) Tween20 and 10 mM DTT, pH 7.5) into the ligation solution. After 2 h at 37°C, it was terminated through a thermal treatment for 5 min at 95°C.

**Signal transduction.** 44 μL of Tris/Mg buffer (25 mM Tris-HCl buffer, 10 mM $Mg^{2+}$, pH 8.0) was added to each RCA sample. After sonication for 10 min at room temperature, *S8Ra'* (10 μL, 9 μM) in Tris/K buffer and *Rv*/*BL* conversion probe (5 μL, 24 μM) in Tris/Mg buffer were added and incubated for 1 h at room temperature. Finally, the solution was mixed with ThT (6 μL, 250 μM) and 204 μL of Tris/Mg buffer with 30 mM $K^+$, and then fluorescence spectra were collected after 20 min of incubation at room temperature.

**Gel electrophoresis**. As for agarose gel electrophoresis in Figure 6A, an agarose gel (3% (w/v)) was prepared in 1×TAE buffer (40 mM Tris-acetic acid, 2 mM EDTA, pH 7.5).10 μL of each A-PCR sample was mixed with 4 μL of loading buffer (1×TAE with 36% glycerol) and loaded into the gel. As for denaturing polyacrylamide gel electrophoresis in Figure 6B, a polyacrylamide gel (12% (w/v)) was prepared within 1×TBE buffer with urea (89 mM Tris, 89 mM boric acid, 2 mM EDTA and 7 M urea, pH 8.3). 5 μL of each ligation sample was mixed with 2 μL of loading buffer (1×TBE with 7 M urea and 36% glycerol, pH 8.3) and loaded into the gel. As for native polyacrylamide gel electrophoresis in Figure 6C, a polyacrylamide gel (12% (w/v)) was prepared within 1×TBE buffer. 5 μL of each sample was mixed with 2 μL of loading buffer (1×TBE with 36% glycerol, pH 8.3) and loaded into the gel. All the gels were run at 60 V for 80-100 min, stained with Gel-Red (0.2‰) for 40 min and photographed using a fluorescence imaging system, iBrightTM FL1000 (Thermo Fisher Scientific, USA).

**Table S1.** Oligonucleotides used in this study. (Note: Spacer sequences are indicated by italic letter. The mutated bases were indicted by red letters.)

| Oligo | Sequence (5' to 3') |
|---|---|
| *T30695* | GGGTGGGTGGGTGGGT |
| *S1* | G *TTCTTTTCTTTTCTTTTCTT* GGTGGGTGGGTGGGT |
| *S2* | GG *TTCTTTTCTTTTCTTTTCTT* GTGGGTGGGTGGGT |
| *S3* | GGG *TTTCTTTTCTTTTCTTTTCTT* GGGTGGGTGGGT |
| *S4* | GGGTG *TTCTTTTCTTTTCTTTTCTT* GGTGGGTGGGT |
| *S5* | GGGTGG *TTCTTTTCTTTTCTTTTCTT* GTGGGTGGGT |
| *S6* | GGGTGGGT *TTCTTTTCTTTTCTTTTCTT* GGGTGGGT |
| *S7* | GGGTGGGTG *TTCTTTTCTTTTCTTTTCTT* GGTGGGT |
| *S8* | GGGTGGGTGG *TTCTTTTCTTTTCTTTTCTT* GTGGGT |
| *S9* | GGGTGGGTGGGT *TTCTTTTCTTTTCTTTTCTT* GGGT |
| *S10* | GGGTGGGTGGGTG *TTCTTTTCTTTTCTTTTCTT* GGT |
| *S11* | GGGTGGGTGGGTGG *TTCTTTTCTTTTCTTTTCTT* GT |
| *C'* | TTCTTTTCTTTTCTTTTCTT |
| *C* | AAGAAAAGAAAAGAAAAGAA |
| *S8Ha'* | GGGTGGGTGG *TCCTAGGAATCCTGATGTGA* GTGGGT |
| *Ha* | TCACATCAGGATTCCTAGGA |
| *S8Hb'* | GGGTGGGTGG *TAAGAAGATGAGGCATAGCA* GTGGGT |
| *Hb* | TGCTATGCCTCATCTTCTTA |
| *S8Hc'* | GGGTGGGTGG*AGAAGTCCACCACGAGTCTA*GTGGGT |
| *Hc* | TCCTCACAATACCGCAGAGT |
| *S8Hd'* | GGGTGGGTGG*TCCTGGTTATCGCTGGATGT* GTGGGT |
| *Hd* | ACATCCAGCGATAACCAGGA |
| *5HT1* | tTCACATCAGGATTCCTAGGA |
| *5HT2* | ttTCACATCAGGATTCCTAGGA |
| *5HT4* | ttttTCACATCAGGATTCCTAGGA |
| *5HT6* | tttttTCACATCAGGATTCCTAGGA |
| *5HT8* | ttttttttTCACATCAGGATTCCTAGGA |

| | |
|---|---|
| *5HT10* | ttttttttttTCACATCAGGATTCCTAGGA |
| *3HT1* | TCACATCAGGATTCCTAGGAt |
| *3HT2* | TCACATCAGGATTCCTAGGAtt |
| *3HT4* | TCACATCAGGATTCCTAGGAtttt |
| *3HT6* | TCACATCAGGATTCCTAGGAtttttt |
| *3HT8* | TCACATCAGGATTCCTAGGAtttttttt |
| *3HT10* | TCACATCAGGATTCCTAGGAttttttttt |
| *5HA1* | aTCACATCAGGATTCCTAGGA |
| *5HA2* | aaTCACATCAGGATTCCTAGGA |
| *5HA4* | aaaaTCACATCAGGATTCCTAGGA |
| *5HA6* | aaaaaaTCACATCAGGATTCCTAGGA |
| *5HA8* | aaaaaaaaTCACATCAGGATTCCTAGGA |
| *5HA10* | aaaaaaaaaaTCACATCAGGATTCCTAGGA |
| *3HA1* | TCACATCAGGATTCCTAGGAa |
| *3HA2* | TCACATCAGGATTCCTAGGAaa |
| *3HA4* | TCACATCAGGATTCCTAGGAaaaa |
| *3HA6* | TCACATCAGGATTCCTAGGAaaaaaa |
| *3HA8* | TCACATCAGGATTCCTAGGAaaaaaaaa |
| *3HA10* | TCACATCAGGATTCCTAGGAaaaaaaaaaa |
| *5ST1* | tAAGAAAAGAAAAGAAAAGAA |
| *5ST2* | ttAAGAAAAGAAAAGAAAAGAA |
| *5ST4* | ttttAAGAAAAGAAAAGAAAAGAA |
| *5ST6* | tttttAAGAAAAGAAAAGAAAAGAA |
| *5ST8* | ttttttttAAGAAAAGAAAAGAAAAGAA |
| *5ST10* | ttttttttttAAGAAAAGAAAAGAAAAGAA |
| *3ST1* | AAGAAAAGAAAAGAAAAGAAt |
| *3ST2* | AAGAAAAGAAAAGAAAAGAAtt |
| *3ST4* | AAGAAAAGAAAAGAAAAGAAtttt |
| *3ST6* | AAGAAAAGAAAAGAAAAGAAttttt |

| | |
|---|---|
| *3ST8* | AAGAAAAGAAAAGAAAAGAAttttttt |
| *3ST10* | AAGAAAAGAAAAGAAAAGAAttttttttt |
| *M1* | ACACATCAGGATTCCTAGGA |
| *M2* | TGACATCAGGATTCCTAGGA |
| *M3* | TCTCATCAGGATTCCTAGGA |
| *M4* | TCAGATCAGGATTCCTAGGA |
| *M5* | TCACTTCAGGATTCCTAGGA |
| *M6* | TCACAACAGGATTCCTAGGA |
| *M11* | TCACATCAGGTTTCCTAGGA |
| *M16* | TCACATCAGGATTCCAAGGA |
| *M20* | TCACATCAGGATTCCTAGGT |
| *R6* | TCACAT |
| *R7* | TCACATC |
| *R9* | TCACATCA |
| *R10* | TCACATCAG |
| *R11* | TCACATCAGG |
| *R16* | TCACATCAGGATTCC |
| *R19* | TCACATCAGGATTCCTAGG |
| *L6* | CTAGGA |
| *L7* | CCTAGGA |
| *L9* | TTCCTAGGA |
| *L10* | ATTCCTAGGA |
| *L11* | GATTCCTAGGA |
| *L16* | ATCAGGATTCCTAGGA |
| *L19* | CACATCAGGATTCCTAGGA |
| *RT5* | TCACATCAGGttttt |
| *RT10* | TCACATCAGGtttttttttt |
| *RT20* | TCACATCAGGtttttttttttttttttttt |
| *RT30* | TCACATCAGGttttttttttttttttttttttttttttt |

| | |
|---|---|
| *S8Ra'* | GGGTGGGTGG*TTAATAATATCCAGATAGTT*GTGGGT |
| *Ra* | AACTATCTGGATATTATTAA |
| *S8Rb'* | GGGTGGGTGG*TCCTGAGACACCTATCTCAT*GTGGGT |
| *Rb* | ATGAGATAGGTGTCTCAGGA |
| *S8Rc'* | GGGTGGG*TGGTAATAATATACCTATCTCAT*GTGGGT |
| *Rc* | ATGAGATAGGTATATTATTA |
| *S8Rd'* | GGGTGGGTGG*TCCTATGACTCTCATTGTGA*GTGGGT |
| *Rd* | TCACAATGAGAGTCATAGGA |
| *S8Re'* | GGGTGGGTGG*TCATTACAGTCCACATAGTT*GTGGGT |
| *Re* | AACTATGTGGACTGTAATGA |
| *S8Rf'* | GGGTGGGTGG*TCCTATGACTTAGTTTGTGA*GTGGGT |
| *Rf* | TCACAAACTAAGTCATAGGA |
| *5RT1* | tAACTATCTGGATATTATTAA |
| *5RT2* | ttAACTATCTGGATATTATTAA |
| *5RT4* | ttttAACTATCTGGATATTATTAA |
| *5RT6* | tttttAACTATCTGGATATTATTAA |
| *5RT8* | tttttttAACTATCTGGATATTATTAA |
| *5RT10* | ttttttttttAACTATCTGGATATTATTAA |
| *3RT1* | AACTATCTGGATATTATTAAt |
| *3RT2* | AACTATCTGGATATTATTAAtt |
| *3RT4* | AACTATCTGGATATTATTAAtttt |
| *3RT6* | AACTATCTGGATATTATTAAtttttt |
| *3RT8* | AACTATCTGGATATTATTAAtttttttt |
| *3RT10* | AACTATCTGGATATTATTAAtttttttttt |
| *5RA1* | aAACTATCTGGATATTATTAA |
| *5RA2* | aaAACTATCTGGATATTATTAA |
| *5RA4* | aaaaAACTATCTGGATATTATTAA |
| *5RA6* | aaaaaaAACTATCTGGATATTATTAA |
| *5RA8* | aaaaaaaaAACTATCTGGATATTATTAA |

| | |
|---|---|
| *5RA10* | aaaaaaaaaaAACTATCTGGATATTATTAA |
| *3RA1* | AACTATCTGGATATTATTAAa |
| *3RA2* | AACTATCTGGATATTATTAAaa |
| *3RA4* | AACTATCTGGATATTATTAAaaaa |
| *3RA6* | AACTATCTGGATATTATTAAaaaaaa |
| *3RA8* | AACTATCTGGATATTATTAAaaaaaaaa |
| *3RA10* | AACTATCTGGATATTATTAAaaaaaaaaaa |
| *MR1* | TACTATCTGGATATTATTAA |
| *MR2* | ATCTATCTGGATATTATTAA |
| *MR3* | AAGTATCTGGATATTATTAA |
| *MR4* | AACAATCTGGATATTATTAA |
| *MR5* | AACTTTCTGGATATTATTAA |
| *MR6* | AACTAACTGGATATTATTAA |
| *MR7* | AACTATGTGGATATTATTAA |
| *MR9* | AACTATCTCGATATTATTAA |
| *MR10* | AACTATCTGCATATTATTAA |
| *MR11* | AACTATCTGGTTATTATTAA |
| *MR16* | AACTATCTGGATATTTTTAA |
| *MR20* | AACTATCTGGATATTATTAT |
| *D6* | AACTAT |
| *D7* | AACTATC |
| *D9* | AACTATCTG |
| *D10* | AACTATCTGG |
| *D11* | AACTATCTGGA |
| *D16* | AACTATCTGGATATTA |
| *D19* | AACTATCTGGATATTATTA |
| *RD5* | AACTATCTGGAttttt |
| *RD10* | AACTATCTGGAttttttttt |
| *RD20* | AACTATCTGGAttttttttttttttttttt |

| | |
|---|---|
| *RD30* | AACTATCTGGAttttttttttttttttttttttttttttt |
| *Rv* | AACTATCTGGAATGAT |
| *FP* | ACCGAACATGGAGAACATCACA |
| *RP* | GAGGACAAACGGGCAACATAC |
| *SC* | GGACCCTGCACCGAACATGGAGAACATCACATCAGGATTCCTAGG<br>ACCCCTGCTCGTGTTACAGGCGGGGTTTTTC |
| *Pa* | Phosphate-<br>CTGATGTGATGTTCTTTCCTTTCTCATGTTTAGATAGTTCGAGACAC<br>TGACATTTGGTCCTAGGAATC |
| *BL* | ATCATTGTAGATAGTTCGAGACACTGACA |

**Table S2.** The sequences of three double-stranded DNA targets (*HF*, *EF* and *NF*) derived from genes of Hepatitis B, Ebola and Nova viruses, respectively.

| Name | Information (5′ to 3′) |
|---|---|
| *HF* | AATCTCCTCGAGGACTGGGGACCCTGCACCGAACATGGAGAA<br>CATCACATCAGGATTCCTAGGACCCCTGCTCGTGTTACAGGCG<br>GGGTTTTTCTTGTTGACAAGAATCCTCACAATACCGCAGAGTC<br>TAGACTCGTGGTGGACTTCTCTCAATTTTCTAGGGGGATCACC<br>CGTGTGTCTTGGCCAAAATTCGCAGTCCCCAACCTCCAATCAC<br>TCACCAACCTCCTGTCCTCCAATTTGTCCTGGTTATCGCTGGAT<br>GTGTCTGCGGCGTTTTATCATATTCCTCTTCATCCTGCTGCTAT<br>GCCTCATCTTCTTATTGGTTCTTCTGGATTATCAGGGTATGTTG<br>CCCGTTTGTCCTCT |
| *EF* | CTTCAGCTCACACCCCTTGAGAGAGCCGGTCAATGCAACGGA<br>GGACCCGTCCAGTGGCTACTATTCTACCACAATTAGATATCAG<br>GCTACCGGTTTTGGAACCAATGAGACGGAGTACTTGTTCGAG<br>GTTGACAATTTGACCTACGTCCAACTTGAATCAAGATTCACGC<br>CACAGTTTTTGCTCCAGCTGAATGAGACAATATATGCAAGTGG<br>GAAAAGGAGCAACACCACGGGAAAACTAATTTGGAAGGTCA<br>ACCCCGAAATTGATACAACAATCGGGGAGTGGGCCTTCTGGG |

| | |
|---|---|
| | AAACTAAAAAAACCTCACTAGAAAAATTCGCAGT |
| *NF* | TACTCCTGATTTTGAGTTCACTTATTTAGTGCCACCTTCTGTTG<br>AATCTAAAACCAAGCCTTTTTCCTTACCTATTTTAACCCTTTCT<br>GAGCTCACAAATTCGAGGTTCCCAGTCCCCATCGATTCGCTTT<br>TCACCGCCCAGAATAATGTGTTGCAGGTGCAGTGTCAAAATG<br>GCAGGTGTACACTTGATGGTGAGTTACAAGGCACAACCCAGT<br>TGCTCCCATCTGGCATCTGTGCATTCAGAGGACGGGTGACAGC<br>ACAAATTAACCAACGTGACAGGTGGCACATGCAACTGCAAAA<br>CCTCAATGGTACAACATATGACCCAACTGATGATGTGCCAGC<br>CCCGCTGG |

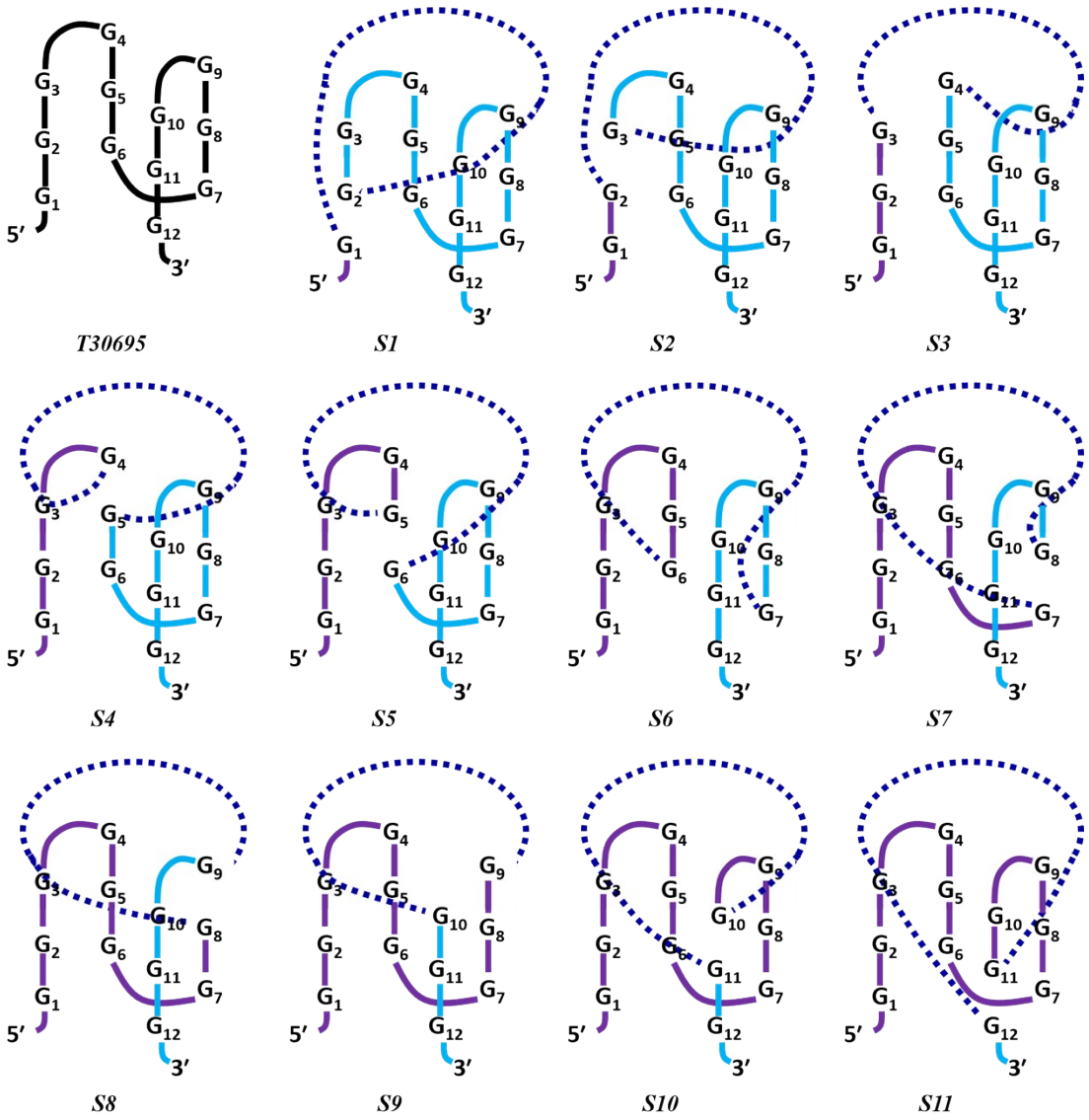


**Figure S1.** Eleven Intra-SG structures (*S1-S11*) derived from *T30695* G-quadruplex.

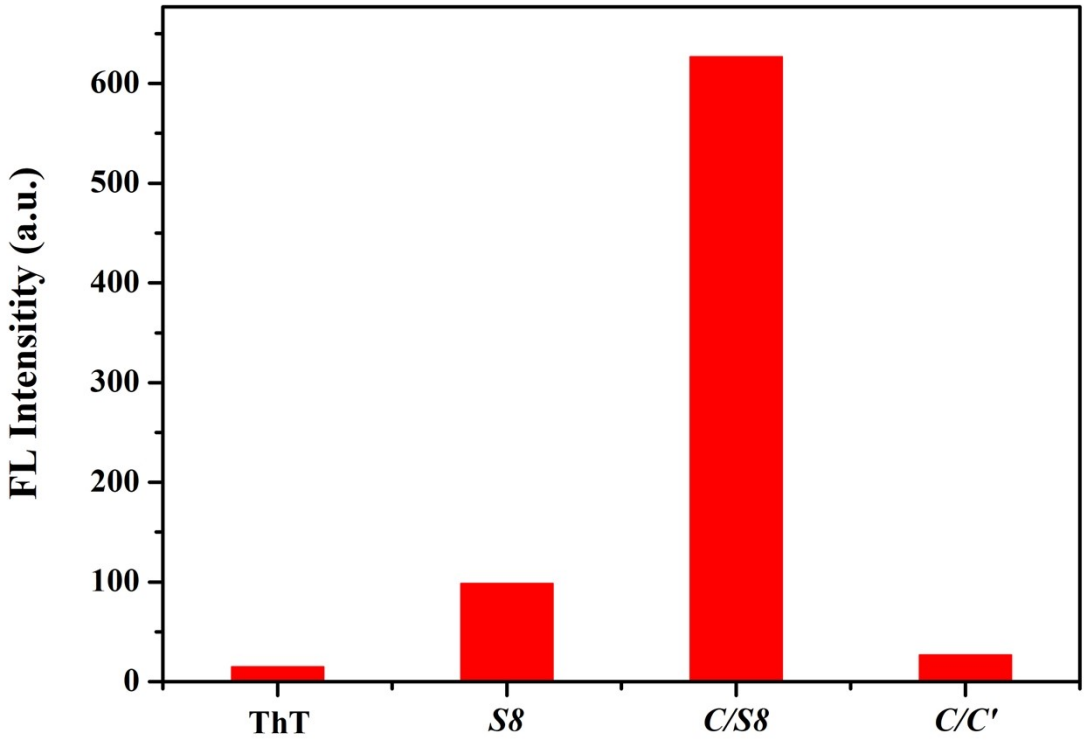


**Figure S2.** Fluorescence responses of ThT with different types of DNA strands (*S8*, *C*/*S8* or *C*/*C'*). The concentration of each DNA strand and ThT was 300 nM and 5 μM, respectively, in Tris/K buffer.

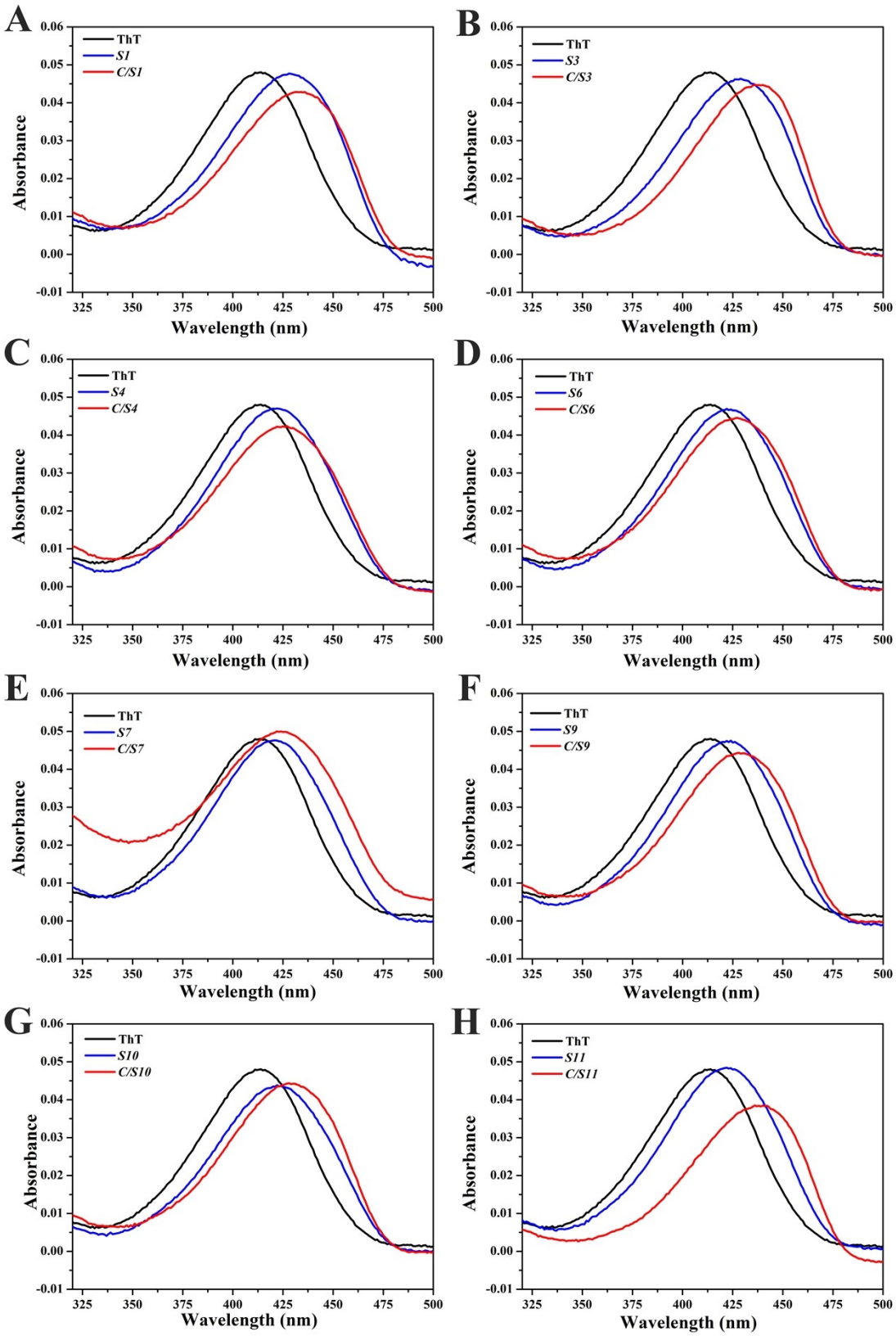


**Figure S3.** (A-H) UV-vis spectra of ThT incubated with different Intra-SG strands (blue curves) or *C*/Intra-SG hybrids (red curves). The black curves represent ThT only. The final concentrations of ThT and DNA were 2.5 μM and 5 μM, respectively, in

Tris/K buffer.

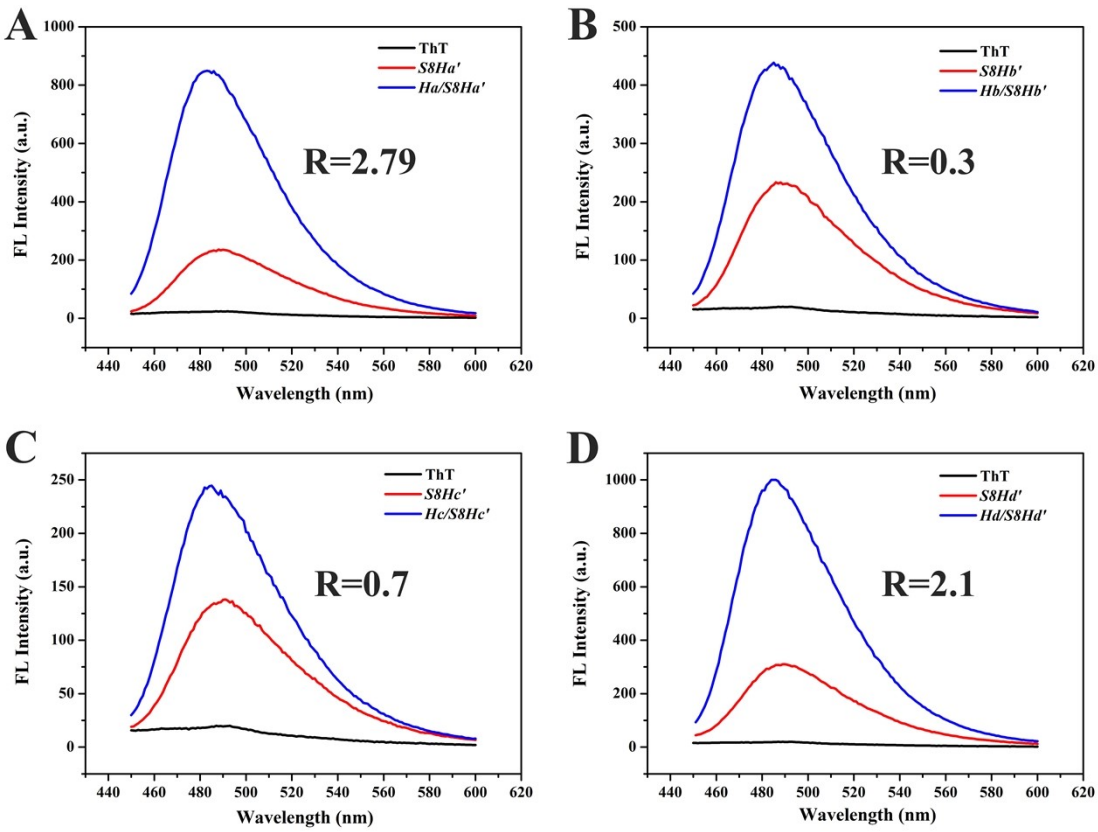


**Figure S4.** Fluorescence spectra of different ThT/Intra-SG systems. Four new Intra-SG structures (*S8Ha'*, *S8Hb'*, *S8Hc'* and *S8Hd'*) derived from *S8* were designed for recognizing four short DNA segments (*Ha*, *Hb*, *Hc* and *Hd,* respectively) from HBV gene. The calculated R values were indicated in the graphs. The concentration of each DNA strand and ThT in Tris/K buffer were 300 nM and 5 μM, respectively.

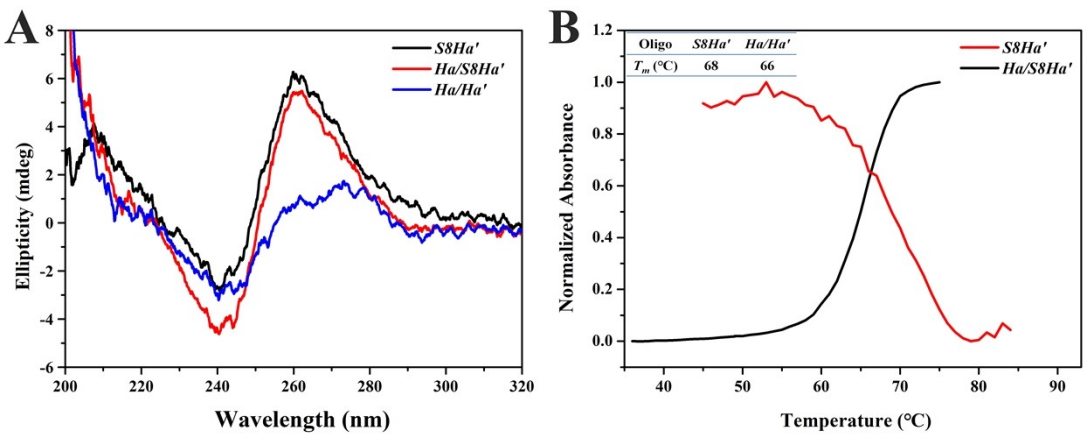


**Figure S5.** (A) CD spectra of *S8Ha′* without (black curve) or with (red curve) *Ha*. The blue curve represents the duplex *Ha/Ha′*. The final concentration of each indicated strand in Tris/K buffer was 7 μM. (B) Normalized UV-melting profiles of *S8Ha′* at 295 nm (red curve) and duplex *Ha*/*Ha′* at 260 nm (black curve). The corresponding $T_m$ values were shown in the inserted table. The concentration of each DNA strand in Tris/K buffer was 2 μM.

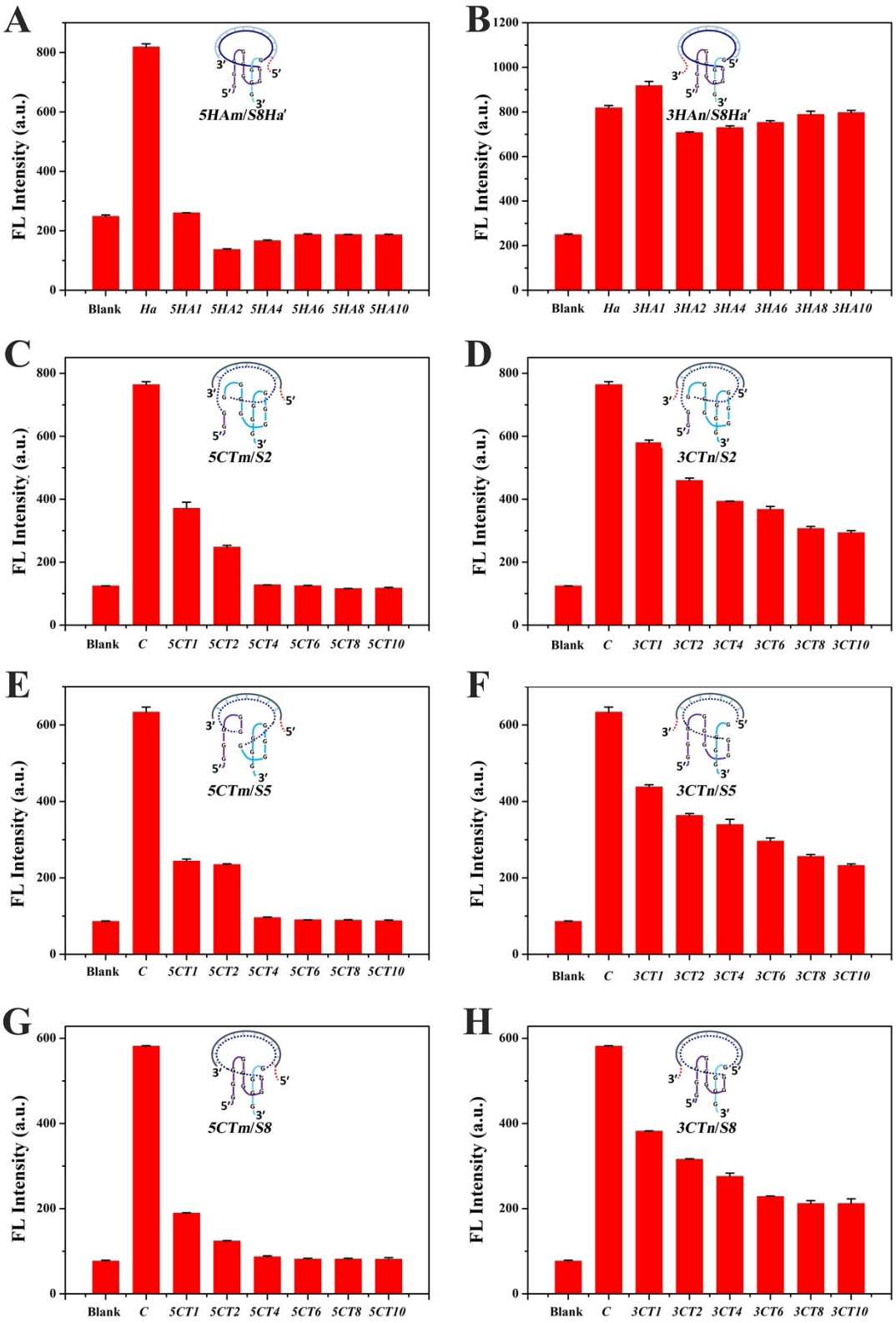


**Figure S6.** (A-B) The fluorescence intensity of ThT/*S8Ha′* (Blank) in the presence of *Ha* or different *Ha* variants generated through grafting different numbers (*m*, *n*) of A bases at the 5′ (*5HAm*, A) or 3′ end (*3HAn*, B). The value. (C-H) The fluorescence intensity of ThT/*S2* (Blank, C-D), ThT/*S5* (Blank, E-F), and ThT/*S8* (Blank, G-H), in

the presence of *C* or different *C* variants generated through grafting different numbers (*m*, *n*) of T bases at the 5′ (*5CTm*, C, E and G) or 3′ end (*3CTn*, D, F and H). The concentrations of ThT and each DNA strand were 5 μM and 300 nM, respectively, in Tris/K buffer.

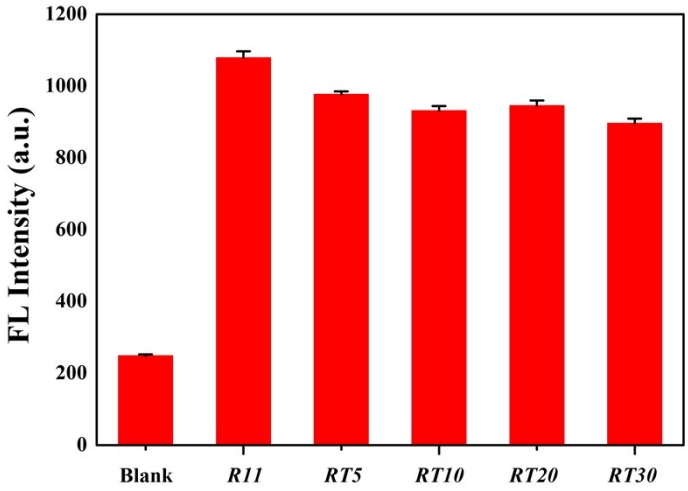


**Figure S7.** The fluorescence intensity of ThT/*S8Ha′* with *R11* or *R11* variants generated through grafting different numbers (*k*) of T bases at the 3′ terminus (*RTk*). The concentration of each DNA strand and ThT was 300 nM and 5 μM, respectively, in Tris/K buffer.

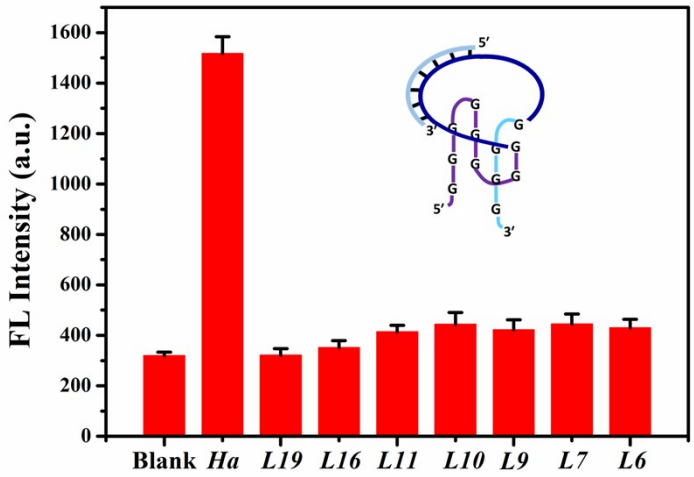


**Figure S8.** The fluorescence intensity of ThT/*S8Ha′* (Blank) in the presence of *Ha* or different *Ha* variants generated through tailoring different numbers of bases from the 5′ end (*Ly*). The letter "*y*" in *Ly* represents base numbers of target DNA after tailoring. A schematic diagram is inserted in each figure for illustrating the target DNA tailoring. The concentration of each DNA strand and ThT was 300 nM and 5 μM, respectively,

in Tris/K buffer.

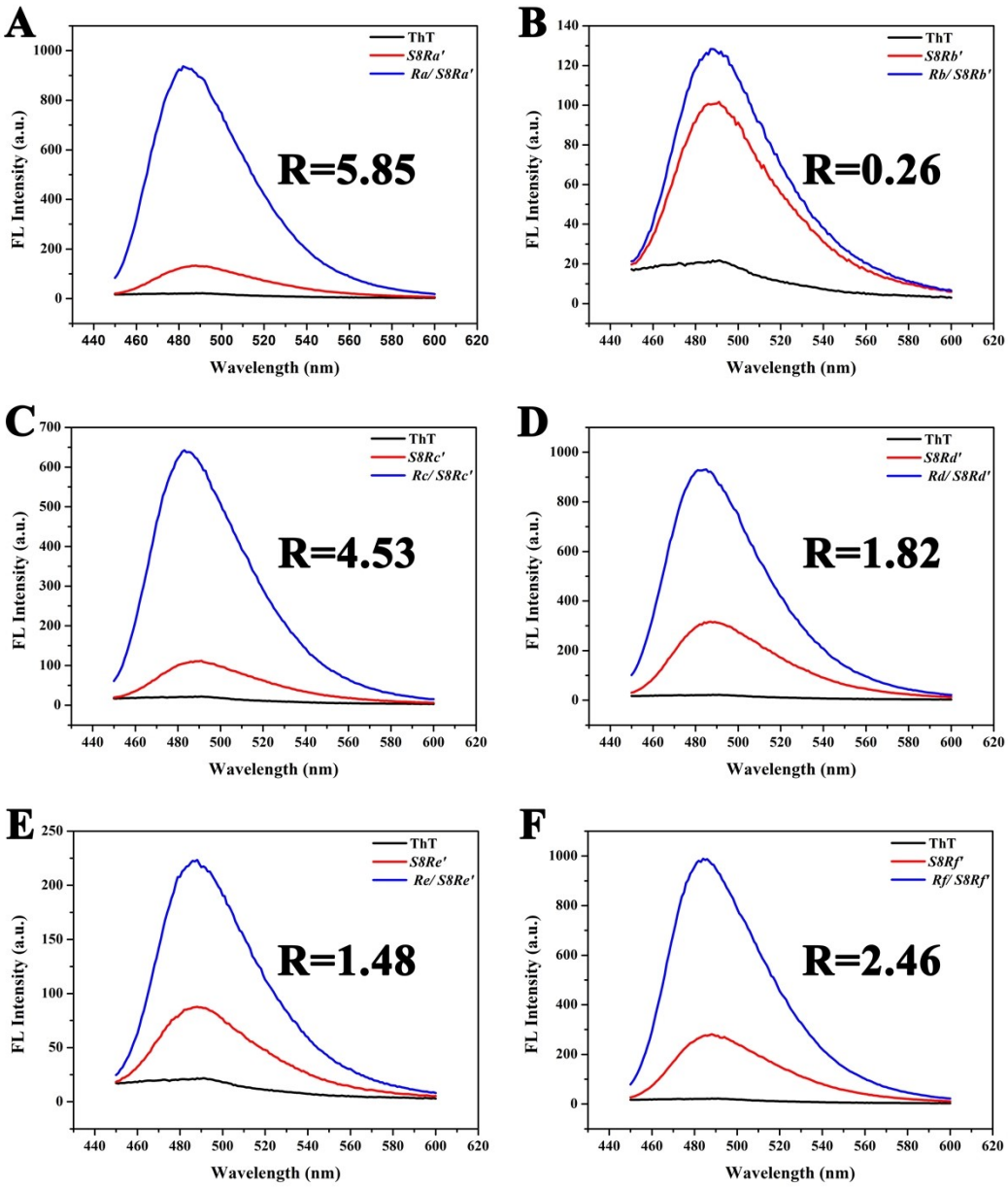


**Figure S9.** Fluorescence spectra of different ThT/Intra-SG systems. These new six Intra-SG structures (*S8Ra'*, *S8Rb'*, *S8Rc'*, *S8Rd'*, *S8Re'* and *S8Rf'*) derived from *S8* were designed for recognizing six random DNA target strands, *Ra*, *Rb*, *Rc*, *Rd*, *Re* and *Rf*, respectively. The calculated R values were indicated in the graphs. The concentration of each DNA strand and ThT was 300 nM and 5 μM, respectively, in Tris/K buffer.

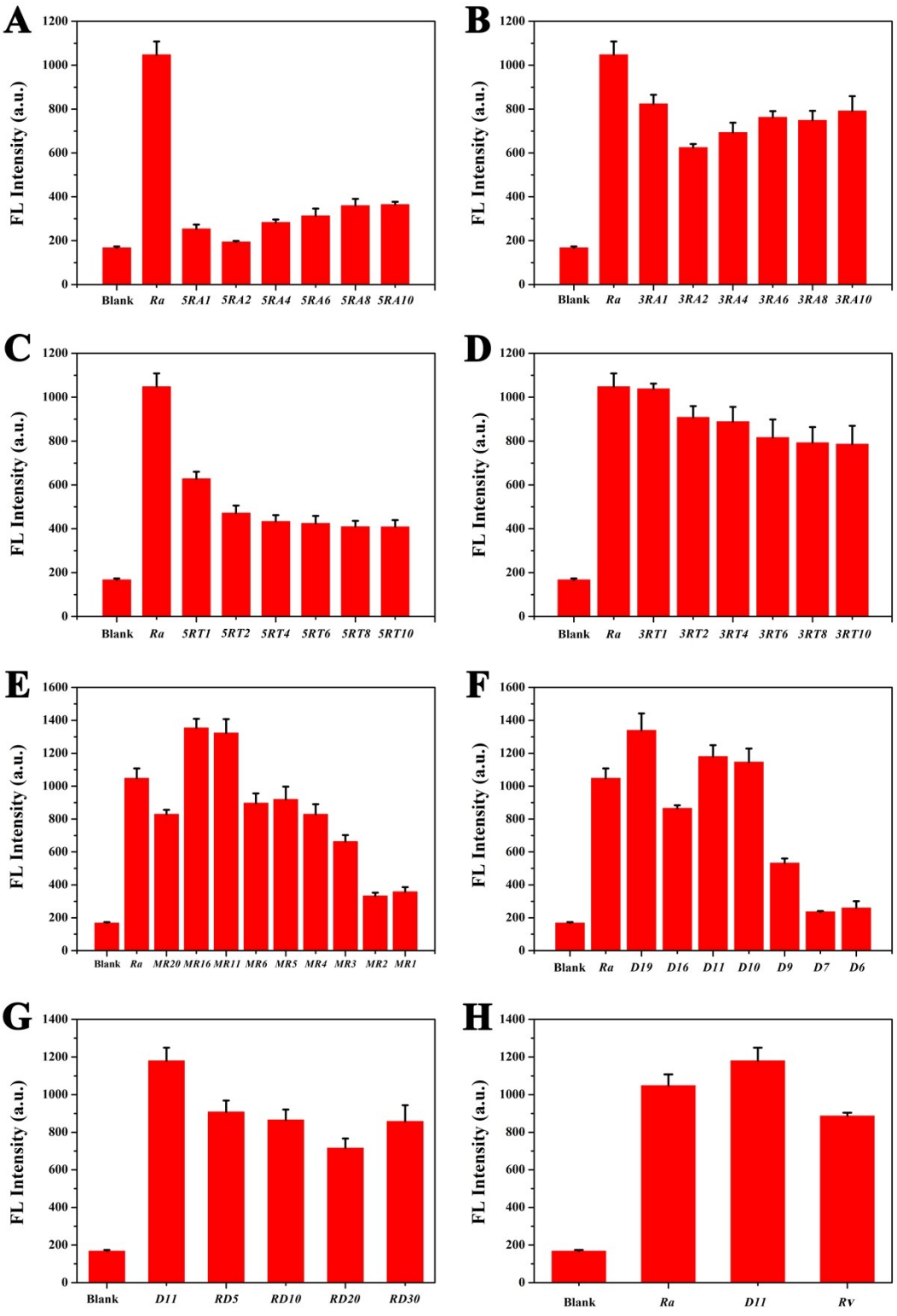


**Figure S10.** (A-D) The fluorescence intensity of ThT/*S8Ra′* (Blank) in the presence of *Ra* or different *Ra* variants generated through grafting different numbers (*m*, *n*) of A bases (A-B) or T bases (C-D) at the 5′ (*5RAm*, A and C) or 3′ end (*3RAn*, B and D), or single-base mutation (*MRx,* E), or tailoring different numbers of bases from the 3′ end

(*Dy*, F). The letter "*x"* in *MRx* represents the mutation site from the 5′ to 3′ end. The letter "*y*" in *Dy* represents base numbers of target DNA after tailoring. (G) The fluorescence intensity of ThT/*S8Ra′* with *D11* or *D11* variants generated through grafting different numbers (*k*) of T bases at the 3′ terminus (*RDk*). (H) Fluorescence intensity of ThT/*S8Ra′* without or with different targets (*Ra*, *D11* or *Rv*). The concentrations of each DNA strand and ThT were 300 nM and 5 μM, respectively.