Detection of CpG methylation in G-quadruplex Forming Sequences using G-quadruplex ligands

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Native-polyacrylamide gel electrophoresis (Native-PAGE) analysis. Methylated or unmethylated *BCL-2*-87 or polyT-87 were mixed with a 5′-probe, 3′-probe, or both (Table S1). The heat-treated oligonucleotide samples (final concentration (f.c.) 500 nM) were mixed with L1Cy5-7OTD (f.c. 2.5 μM) in TK buffer (10 mM Tris-HCl, 100 mM KCl; pH 7.4). After incubation at RT for 1 h, the mixtures were loaded on 15% polyacrylamide gel, which was separated at a constant current of 20 mA in TBE buffer (89 mM Tris-HCl, 89 mM H_3BO_4, 2 mM EDTA; pH 8.3). L1Cy5-7OTD was visualized by measuring Cy5 fluorescence using Typhoon 8600 (GE Healthcare; Little Chalfont, UK). The oligonucleotides were stained with SYBR Gold Nucleic Acid Gel Stain (Thermo Fisher Scientific) and scanned using a Gel Doc EZ Imager (Bio-Rad Laboratories; Hercules, CA, USA).

Table S1. DNA sequences used in this study. The methylated CpG sites are underlined.

Name	DNA Sequence (5′→3′)
BCL-2 G4	CGGGCGCGGGAGGAAGGGGGCGGGAGC
HRAS2 G4	CGGGGCGGGGCGGGGGCGGGGGCG
VEGF G4	GGGGCGGGCCGGGGGCGGGG
HRAS1 G4	TCGGGTTGCGGGCGCAGGGCACGGGCG
BCL-2-87	GCGGGCGGGCGGGCAGGCGGCGCGGAGGGGCGGGCGCGGGAGGAAGGGGGCGGGAGCGGGGCTGTGGTGCCTGTCCTCTTACTTCAT
PolyT-87	GCGGGCGGGCGGGCAGGCGGCGCGGAGGGGTTTTTTTTTTTTTTTTTTTTTTTTTTTGGGGCTGTGGTGCCTGTCCTCTTACTTCAT
5′-probe	CCCCTCCGCGCCGCCTGCCCGCCCGCCCGC
3′-probe	ATGAAGTAAGAGGACAGGCACCACAGCCCC

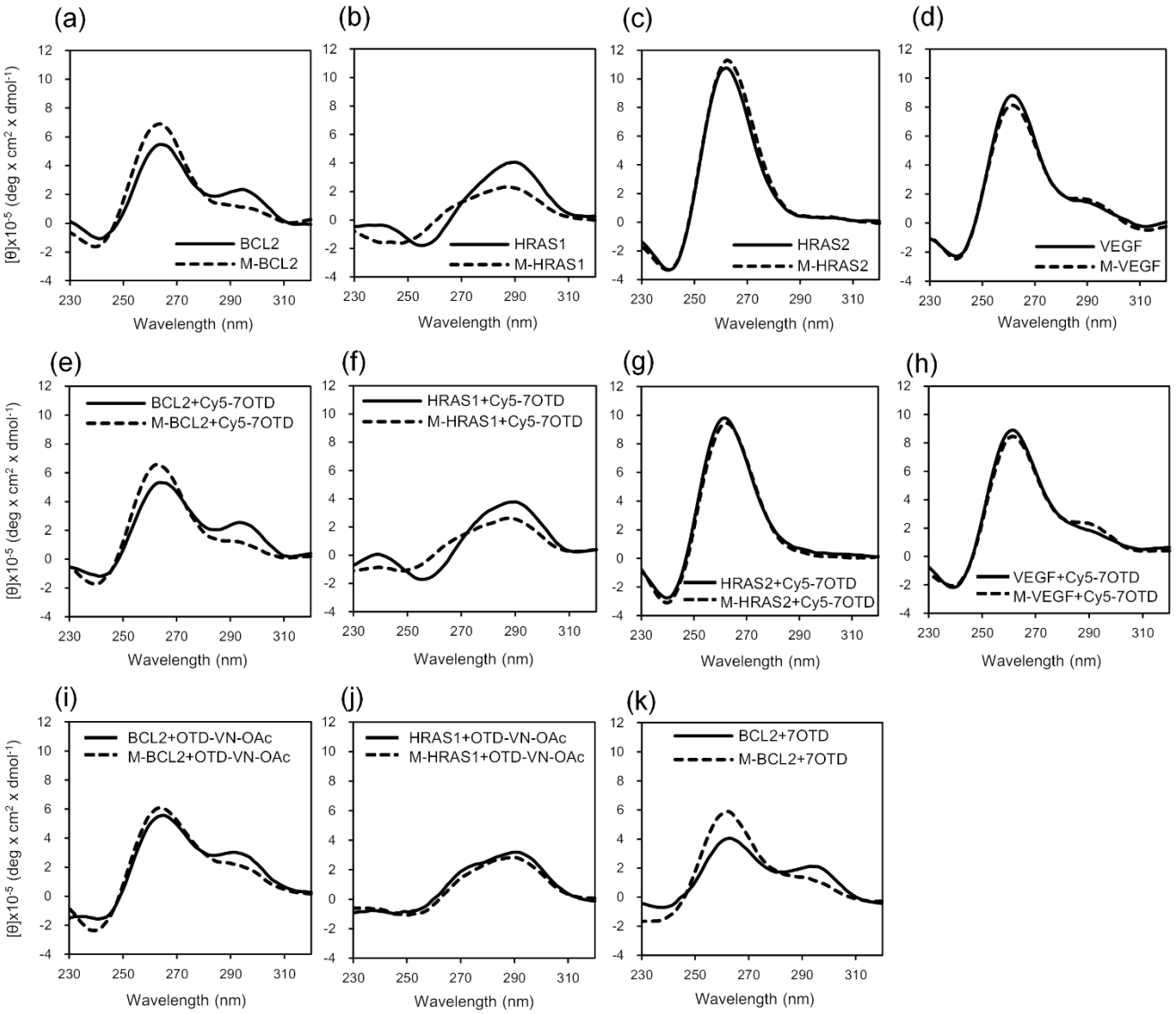

Figure S1. Circular dichroism (CD) spectra of unmethylated (solid line) and methylated (dot line) (a) *BCL-2* G4, (b) *HRAS1* G4, (c) *HRAS2* G4, and (d) *VEGF G4* in 10 mM Tris-HCl, 100 mM KCl, pH 7.4; (e) *BCL-2* G4, (f) *HRAS1* G4, (g) *HRAS2* G4 and (h) *VEGF* G4 in the presence of L1Cy5-7OTD; and (i) *BCL-2* G4, (j) *HRAS1* G4 in the presence of OTD-VN-OAc; and (k) *BCL-2* G4 in the presence of L1H1-7OTD. DNA concentration: 1 µM, L1Cy5-7OTD, OTD-VN-OAc and L1H1-7OTD concentrations: 5 µM.

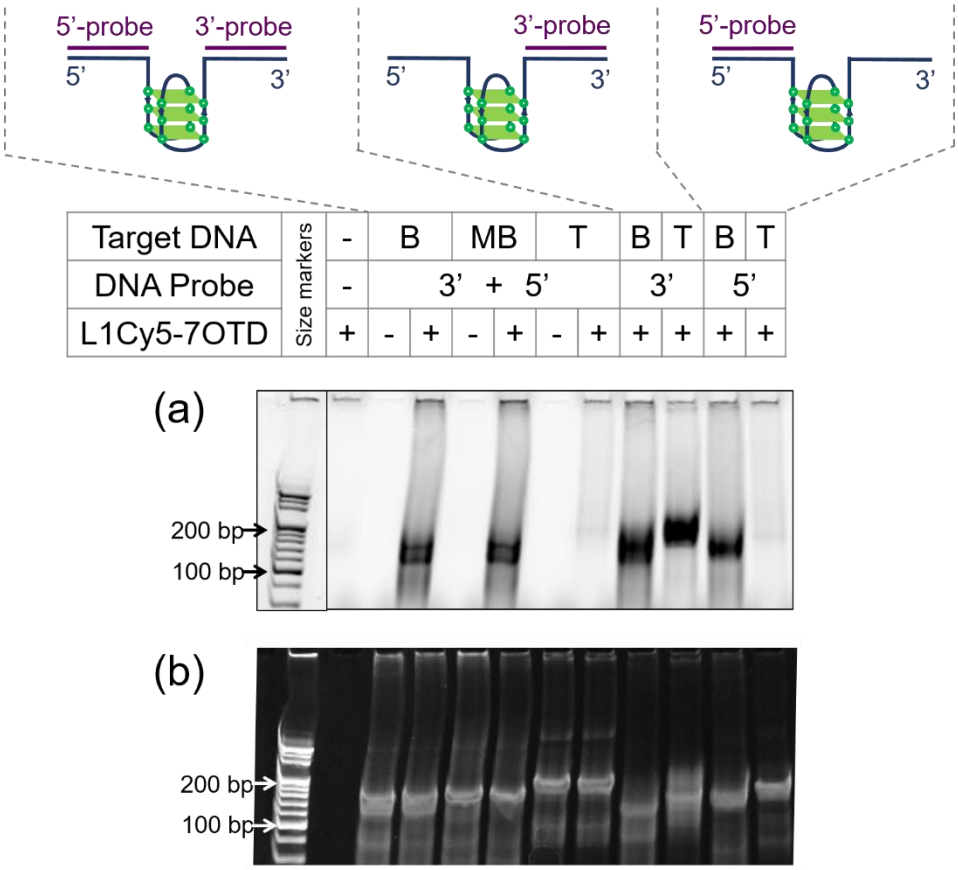

Figure S2. Native-polyacrylamide gel electrophoresis (Native-PAGE) of DNA/L1Cy5-7OTD complex. *BCL-2*-87 (B), methylated *BCL-2*-87 (MB), polyT-87 (T) were hybridized with 3′-probe (3′), 5′-probe (5′), or both probe (3′+5′), and these DNA samples were mixed with L1Cy5-7OTD. (a) Cy5 of L1Cy5-7OTD was detected. (b) DNAs were stained with SYBR Gold and detected. 20 bp ladder pre-mixed with DNA binding fluorescent dye was used as size markers.

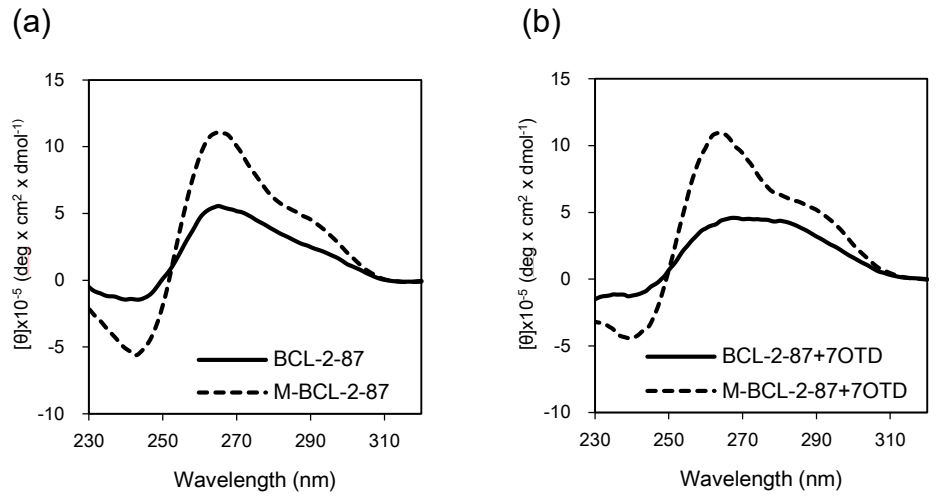

Figure S3. Circular dichroism (CD) spectra of 1 µM *BCL-2*-87 and methylated *BCL-2*-87 (M-*BCL-2*-87) in (a) 10 mM Tris-HCl, 100 mM KCl, pH 7.4 and (b) in the presence of 5 µM L1H1-7OTD. *BCL-2*-87 and M-*BCL-2*-87 were hybridized with 3′-probe and 5′-probe. The CD spectra of the *BCL-2* G4 sequence in *BCL-2*-87 and M-*BCL-2*-87 were determined by subtracting the CD spectrum of a mixed sample consisting of 3′ and 5′ probes and their complementary strands.

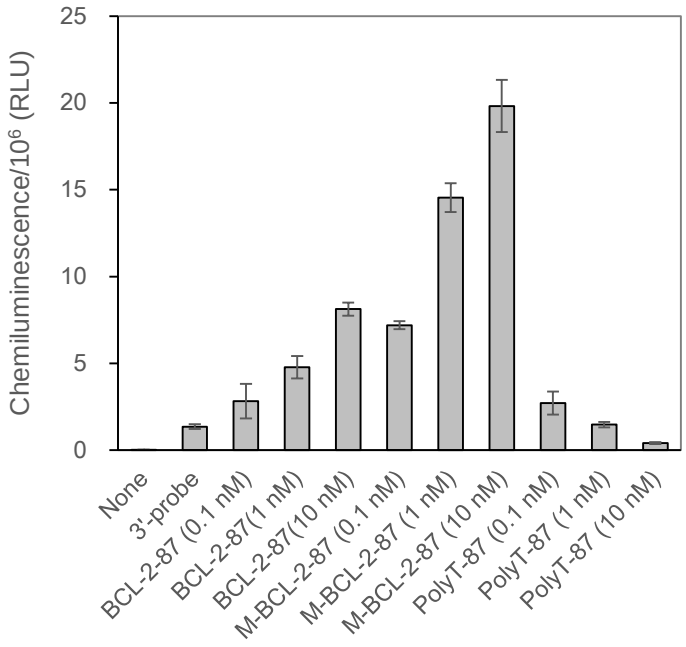

Figure S4. Binding assay of L1Cy5-7OTD to *BCL-2*-87 and methylated *BCL-2*-87 (M-*BCL-2*-87). Binding abilities of L1Cy5-7OTD to target DNAs were determined by chemiluminescence intensities. Target DNAs hybridized with 5′-probe were immobilized on a microplate via 3′-probe, then chemiluminescence was detected after incubation with L1Cy5-7OTD and antibodies. PolyT-87 was used as a negative control.

Table S2. DNA sequence of major depressive disorder (MDD) marker model DNA. The methylated CpG sites are underlined. CpG site reported as MDD marker is shown in bold.

Name	DNA Sequence (5′→3′)
cg26910488_comp	GGGGAGGCCGTGAGCTCGGAAAGGCACCG**CG**GGGCCGGCATTAGGGC