**Supplementary Information**

**Design of 2'-*O*-methyl RNA and DNA double-stranded oligonucleotides: naturally-occurring nucleotide components with strong RNA interference gene expression inhibitory activity**

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This paper is dedicated to Dr. Akira Matsuda, Emeritus Professor of Hokkaido University, on the occasion of his 70th birthday.



**Figure S1.** Sequences of unmodified siRNA and MED-siRNA. The black, blue, and red letters represent RNAs, DNAs, and 2'-OMe RNAs, respectively. “p” represents a phosphate group.



**Figure S2.** Representation of modified siRNAs and western blot analysis to detect the inhibitory activities of various double-stranded oligonucleotides on the expression of the human -catenin gene (CTNNB1). “Actin” represents the expression of -actin proteins used as a control. The sky-blue circles, the open circles, and the red circles represent RNAs, DNAs, and 2'-OMe RNAs, respectively. “p” represents a phosphate group. The chain lengths of the passenger and guide strands are indicated, such as e.g. 19/21mer (passenger/guide). The black, blue, and red letters represent RNAs, DNAs, and 2'-OMe RNAs, respectively. “p” represents a phosphate group.



**Figure S3.** Representation of modified siRNAs and western blot analysis to detect the inhibitory activities of various double-stranded oligonucleotides on the expression of the human -catenin gene (CTNNB1). “Actin” represents the expression of -actin proteins used as a control. The sky-blue circles, the open circles, and the red circles represent RNAs, DNAs, and 2'-OMe RNAs, respectively. “p” represents a phosphate group. The chain lengths of the passenger and guide strands are indicated, such as e.g. 17/21mer (passenger/guide). The black, blue, and red letters represent RNAs, DNAs and 2'-OMe RNAs, respectively. “p” represents a phosphate group.

**Table S1.** Melting temperatures (*T*m) of duplexes between the target RNA and the guide strand of unmodified siRNA or MED-siRNA.

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Guide strand Type Guide sequences (3'-5') *T*m (°C) *T*m (°C)

CT-041 unmodified UUCGUGUUCUUACCUAGUGUU 74.5

CT-157 MED UtCgUgUtCtUaCcUaGtGtUp 70.5 -4.0

Uppercase letters: RNA; underlined: 2'-OMe RNA; lowercase letters; DNA; p: phosphate. The target RNA sequence of -catenin: 5'-GCACAAGAAUGGAUCACAAUU.

**Material and Methods**

*Measurement of melting temperature*

The melting temperature (*T*m)of the duplexes of the target RNA (5'-GCA CAA GAA UGG AUC ACA AUU-3' for CTTNB1) and the guide strand of unmodified siRNA or MED-siRNA was measured using SYBR Green I (Invitrogen) and Mx4000 (Strategene) [1]. A solution containing 5 M of duplex, 5 × SYBR Green I, and 1 × PBS buffer was run using Mx4000. The duplex-SYBR mixture was denatured at 95°C for 30 s, then subjected to a 25°C for 8 min. The samples were heated incrementally by 1°C with 30 s holds from 25°C to a final temperature of 90°C. The software attached to Mx4000 performed the melting curve analysis and determined *T*m.

**References**

1. Gudnason, H.; Dufva, M.; Bang, D.D.; Wolff, A. Comparison of multiple DNA dyes for real-time PCR: effects of dye concentration and sequence composition on DNA amplification and melting temperature. *Nucleic Acids Res.,* **2007**, *35*, e127.