Electronic supplementary information (ESI)

The Antisense Oligonuclotides with Oxetane-constrained Cytidine Enahances Heteroduplex Stability, Elicit Satisfactory RNase H Response as well as Show Improved Resistance to Both Exo and Endonucleases

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Autoradiograms of 20% PAGE, showing the cleavage kinetics of $5'_{-}^{32}$ P labeled target RNA (14) by RNase H1 in the native AON (1)/RNA (14) and various oxetane <u>C</u> AONs (5, 6, 9 and 12)/RNA (14) and <u>T</u> modified AONs (2, 3, 4, 8 and 10)/RNA (14) hybrid duplexes.

Figures S1- S3: RNA cleavage kinetics for single <u>C</u> AON (5), double <u>C</u> AON (6) and DPPZ conjugated double <u>C</u> AON (9) in different RNA concentrations (0.008-1 μ M) keeping the same AONs (5 μ M) and RNase H concentration (0.06 U in 30 μ l of reaction mixture).

Figures S4- S8: RNA cleavage kinetics for native 15mer AON (1), single <u>T</u> AON (2), double <u>T</u> AON (3) and triple <u>T</u> AON (4) in different RNA concentrations (0.01-3 μ M) keeping the same AONs (5 μ M) and RNase H concentration (0.06 U in 30 μ l of reaction mixture). The kinetics of the control native 15mer AON (1) and double <u>T</u>

AON (3) with 1 μ M and RNA (14) with 0.1 μ M concentration, used for the calibration of the exact RNase H concentration, were also shown.

Figures S9- S14: RNA cleavage kinetics for native 15mer AON (1), single <u>T</u> AON (2), double <u>T</u> AON (3) and triple <u>T</u> AON (4) in different RNA concentrations (0.01-3 μ M) keeping the same AONs (5 μ M) and RNase H concentration (0.12 U in 30 μ l of reaction mixture). The kinetics of the control native 15mer AON (1) and double <u>T</u> AON (3) with 1 μ M and RNA (14) with 0.1 μ M concentration, used for the calibration of the exact RNase H concentration, were also shown.

Figures S15- S23: RNA cleavage kinetics for native 15mer AON (1), 15-DPPZ AON (7), 15-3<u>T</u>-DPPZ AON (8), 15-3<u>T</u>-Cholest AON (10) and 15-2<u>C</u>-Cholest AON (11) in different RNA concentrations (0.01-1 μ M) keeping the same AONs concentration (5 μ M). The kinetics of the control native 15mer AON (1) with 1 μ M and RNA (14) with 0.1 μ M concentrations, used for the calibration of the exact RNase H concentration, were also shown in Figure S15.