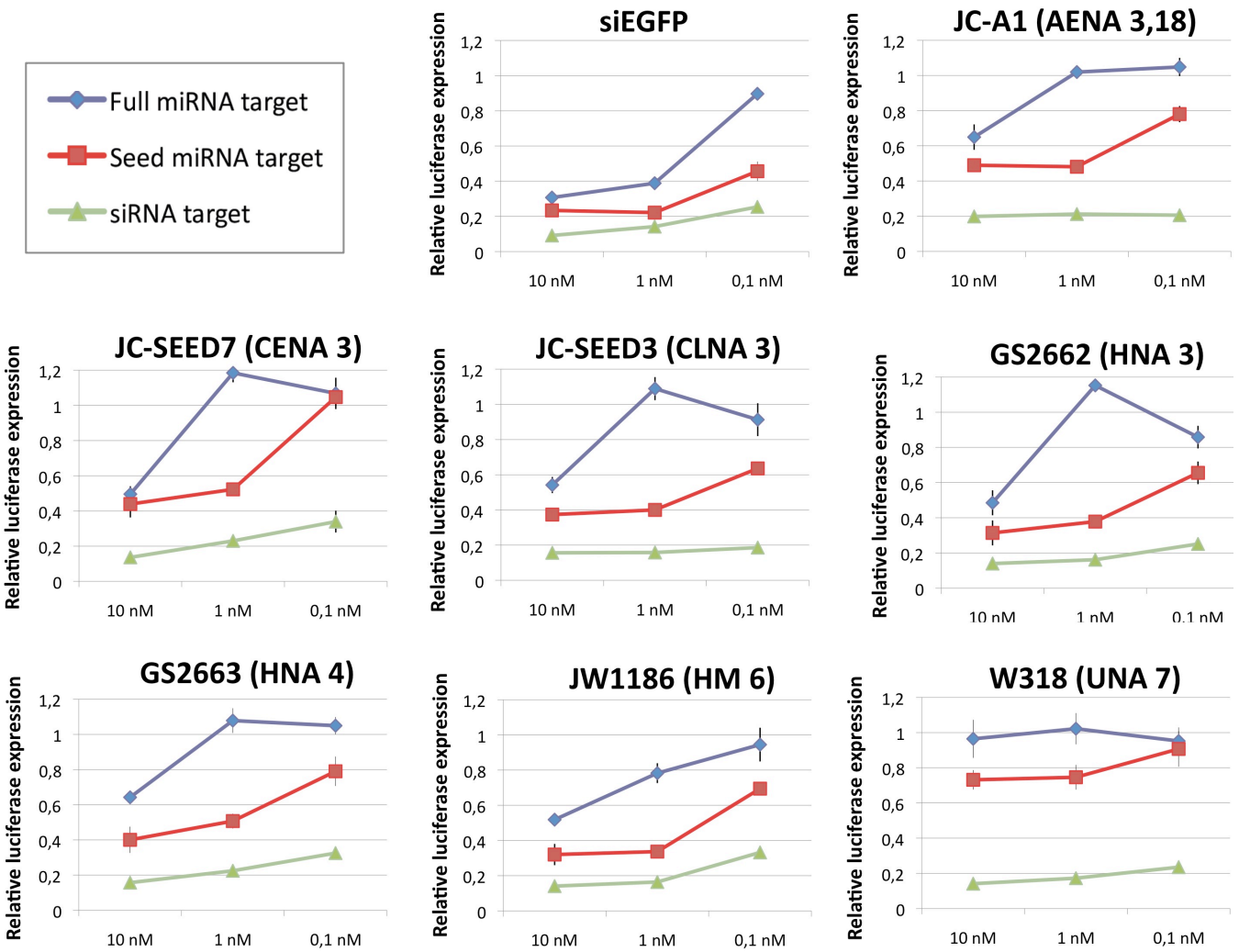
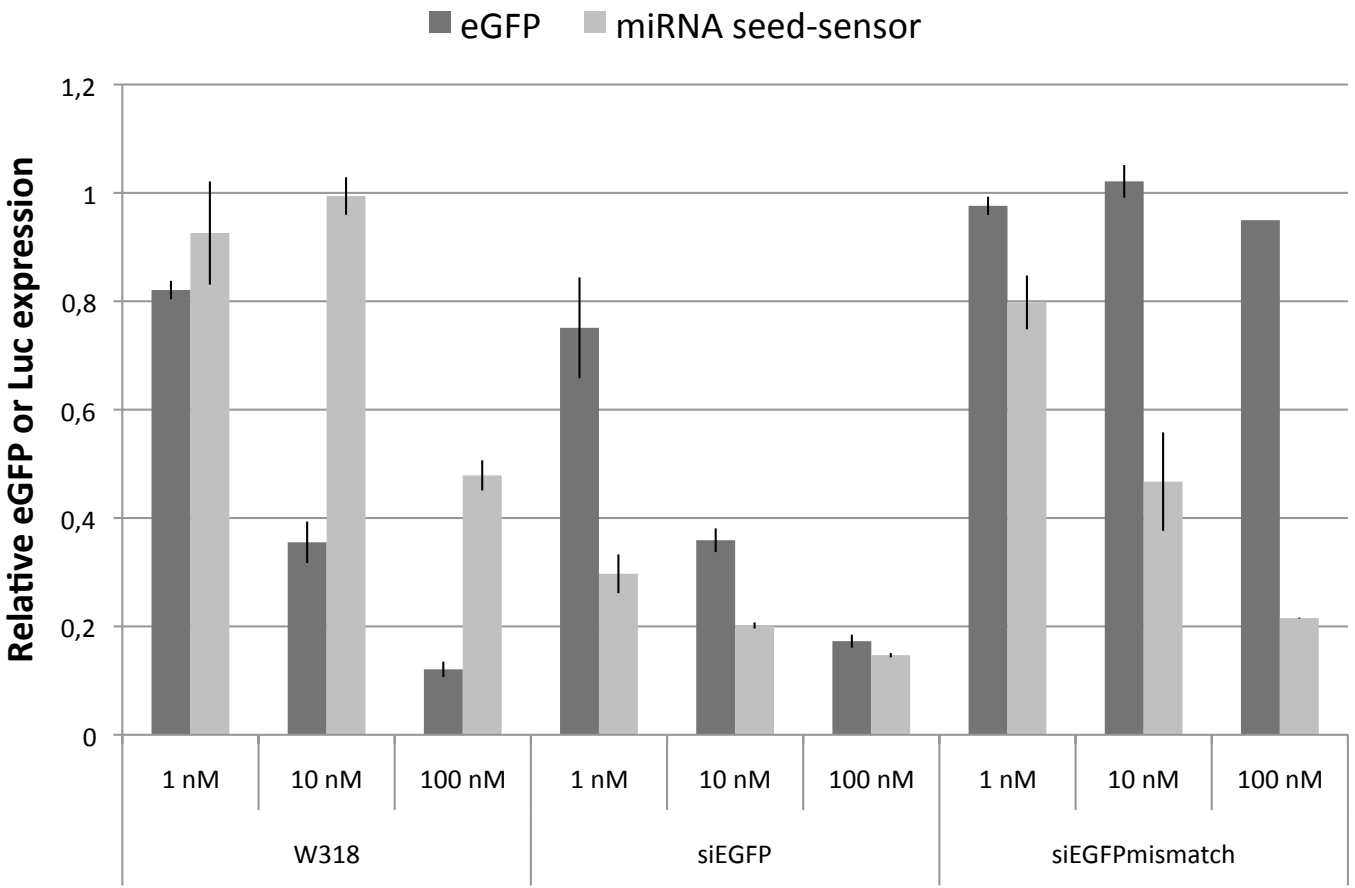
Supplementary figure 1



Supplementary figure 1. UNA-modification of the ASs seed region most dramatically reduces siRNA off-targeting.

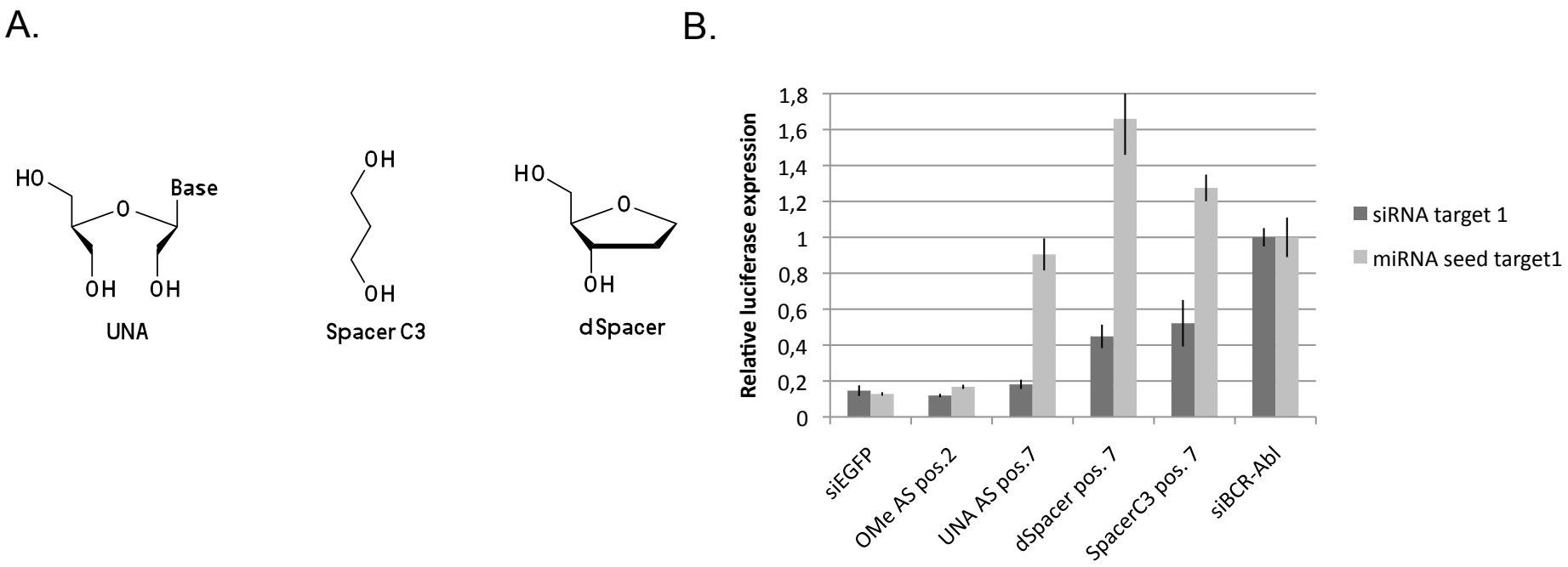
Evaluation of siRNA potency and off-targeting potential of selected ASs at 0,1, 1 and 10 nM concentration using the siRNA sensor1 (green lines) and miRNA seed/full sensor1´s (red/blue lines), respectively. Experiments were performed as in figure 3.

Supplementary figure 2



Supplementary figure 2: Co-evaluation of siRNA potency and off-target effects in the same cell population.
A eGFP-expressing H1299 cell line was cotransfected with siRNAs (siEGFP (W053-W207), W318-W207, siEGFPmismatch and siBCR-ABL) at 1, 10 or 100 nM concentrations and the miRNA seed sensor1 plasmid using TransIT-LT1 and TransIT-KO reagents (both Mirus) according to manufactures instructions. After 48 hours siRNA and off-target effects were monitored in the same cell population by flow cytometry and dual luciferase assays, respectively. eGFP and relative renilla luciferase expression values were normalized to values from cells transfected with the unrelated siRNA, siBCR-ABL. As expected, siEGFP exhibits strong off-target repression of the co-transfected miRNA seed sensor at all concentrations whereas the top-performing AS modified with UNA at position 7 (W318) exhibited a clearly reduced off-targeting profile even at the 100 nM concentration. Experiments were performed in triplicates.

Supplementary figure 3



Supplementary figure 3: SpacerC3 and dSpacer at position 7 dramatically reduced off-targeting potential but also reduces siRNA potencies.

A. Structural overview of the UNA, SpacerC3 and dSpacer modifications incorporated at position 7 of the siEGFP1 AS (5´-ACUUGU**[G/Spacer]**GCCGUUUACGUCGC-3´).

B. Evaluation of siRNA potency and off-target potential for the siEGFP1 AS (W053, table 1) modified at position 7 with UNA, dSpacer (W380, table 1), SpacerC3 (W381, table 1) linkers or OMe at position 2. SiRNAs were transfected at 10 nM concentration into the H1299 stable cell lines “siRNA sensor1” or “miRNA seed sensor1” using lipofectamine 2000 and the relative Renilla/firefly luciferase protein levels were determined after 48 hours using dual luciferase assays. Relative Renilla luciferase levels were normalized to cells transfected with the unrelated siRNA, siBCR-ABL. Experiments were performed in triplicates.