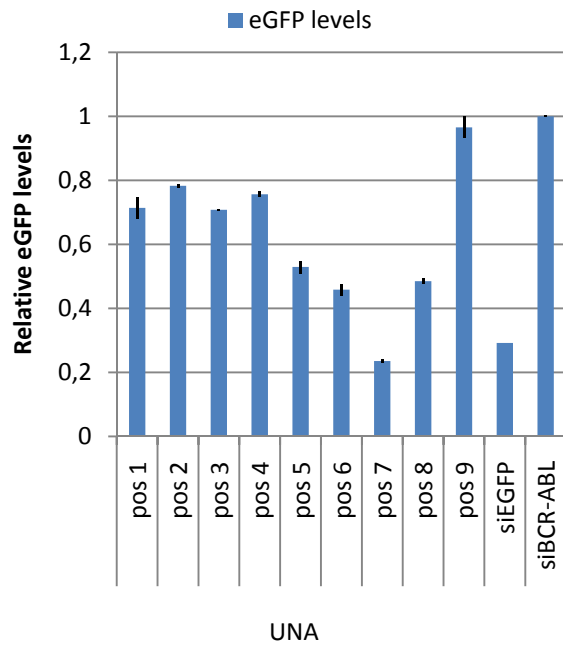
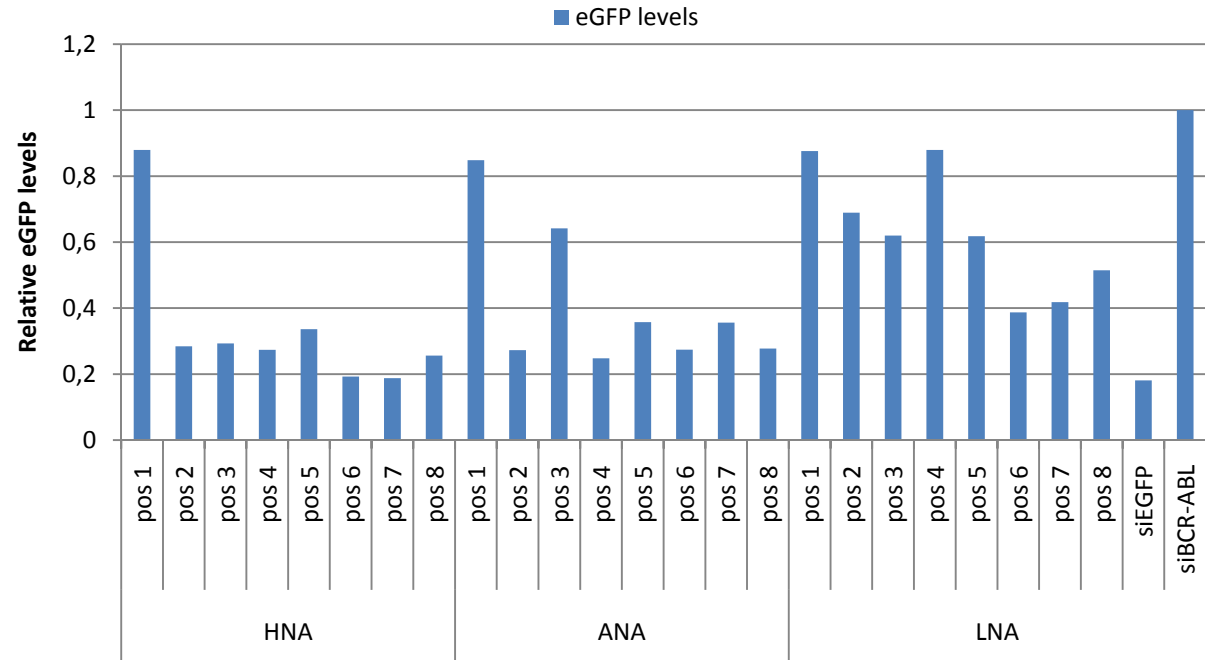


a

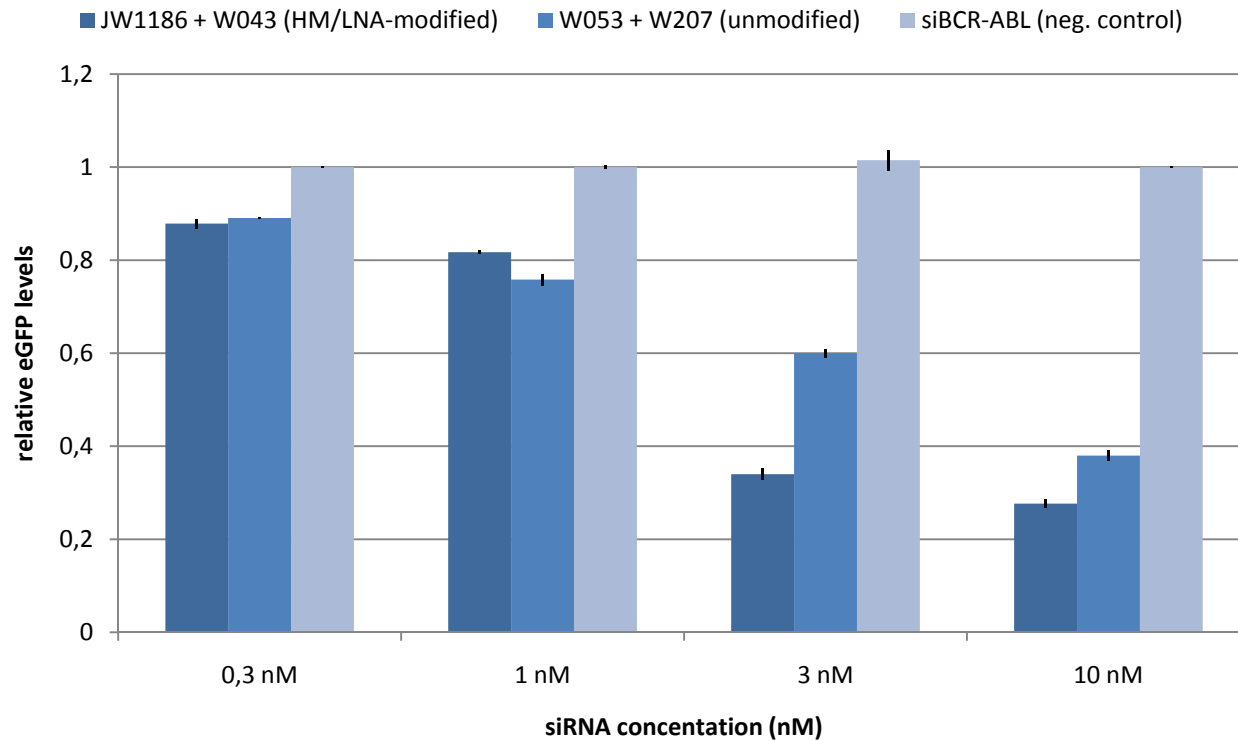


b



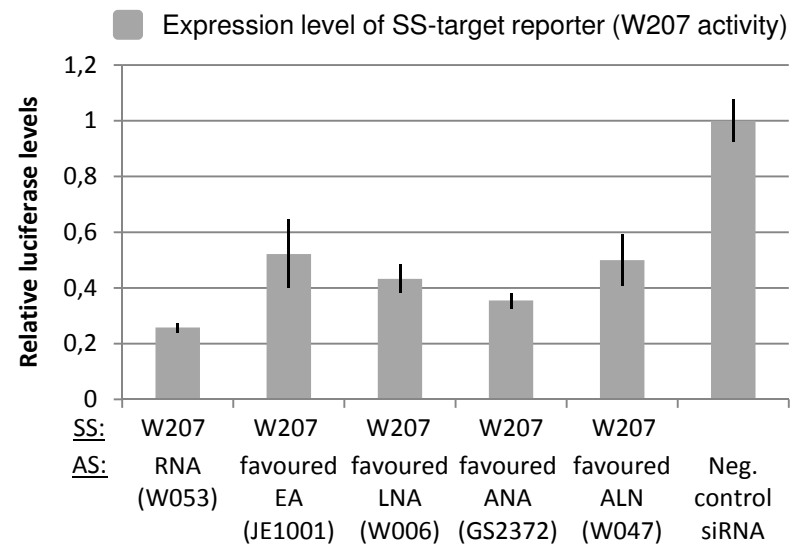
Supplementary figure 1: Silencing activity of siRNAs having single UNA, HNA, ANA or LNA modification in the AS seed region

H1299 cells stably expressing eGFP (half-life 2 hours) were transfected with the indicated siRNAs using TransIT-TKO (Mirus Corp.) at a final siRNA concentration of 10 nM. eGFP levels were measured 48 hours post transfection using either a Flourstar (BMG labtech) (suppl. figure 1a) or by flow cytometry using a FACSCalibur (BD biosciences) (suppl. figure 1b). The positions of the modification (pos.) is counted from the 5' end of the AS. siEGFP corresponds to the unmodified siRNA W053-W207. siBCR-ABL is a non-related siRNA used as negative control.



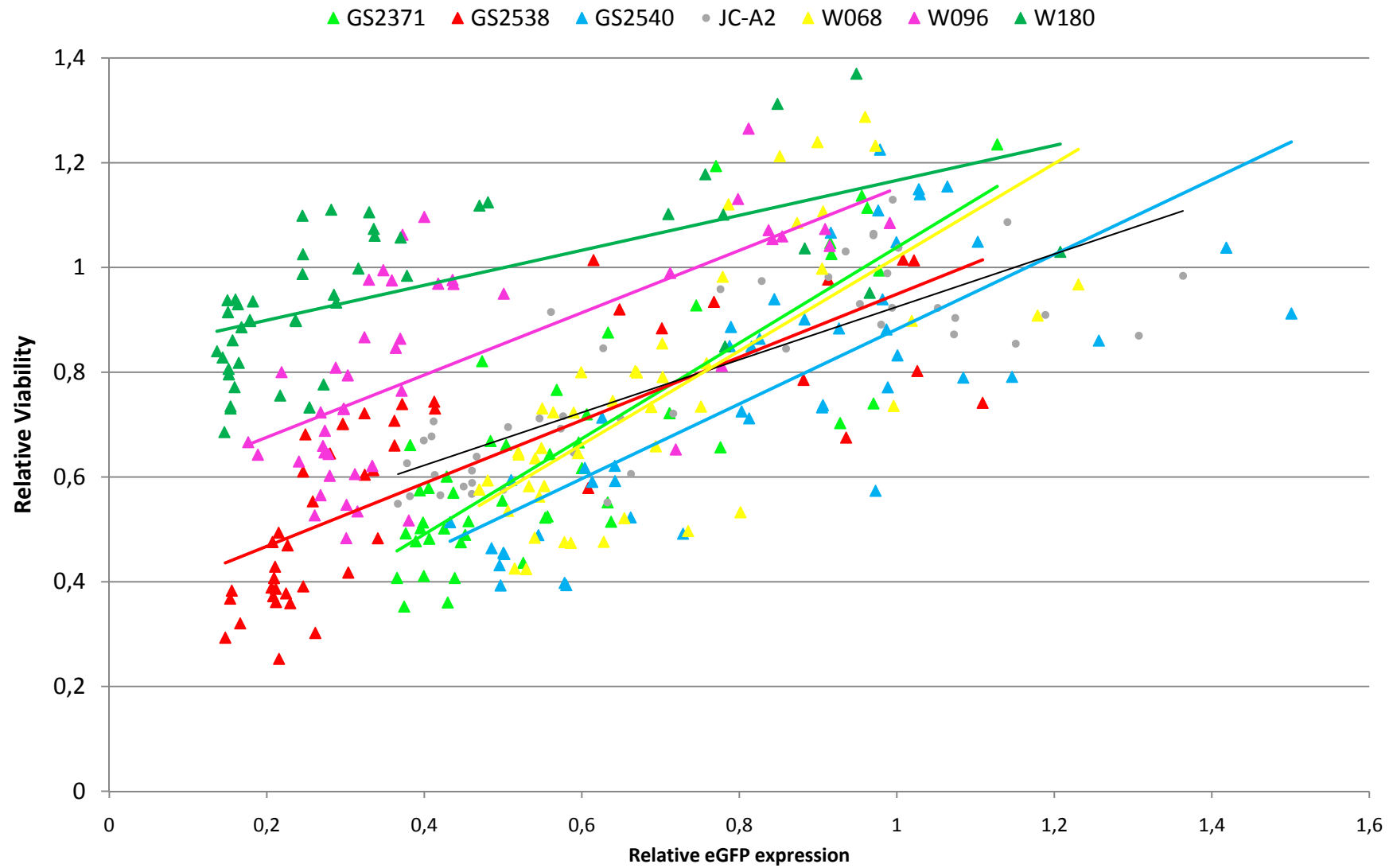
Supplementary figure 2: Modified siRNAs have improved activity at low siRNA concentrations

H1299 cells stably expressing destabilised eGFP (half-life 2 hours) were transfected with the indicated siRNAs using TransIT-TKO (Mirus Corp.) at the indicated concentrations. eGFP levels were measured 48 hours post transfection by flow cytometry. Experiment was performed in triplicates and results were normalised to an unrelated siRNA, siBCR-ABL.



Supplementary figure 3: Modified AS 3' overhang can lower SS activity.

ASs modified in their 3' overhang by EA (JE1001), LNA (W006), ANA (GS2372) and ALN (W047) significantly lowers the activity of the unmodified SS (W207) on the SS-target reporter as compared to the unmodified AS (W053).



Supplementary figure 4: Cell viability is reversely correlated with siRNA activity for many ASs
 Scatter plot of cell viability and eGFP expression for the seven indicated ASs in combination with all 45 SSs. Trend lines are given for each AS.