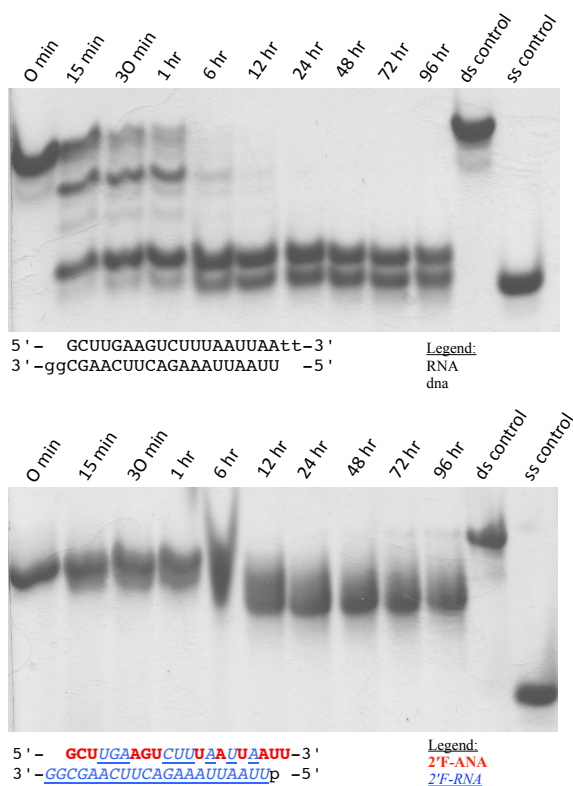


Synergistic Effects Between Analogues of DNA and RNA Improve the Potency of siRNA-Mediated Gene Silencing

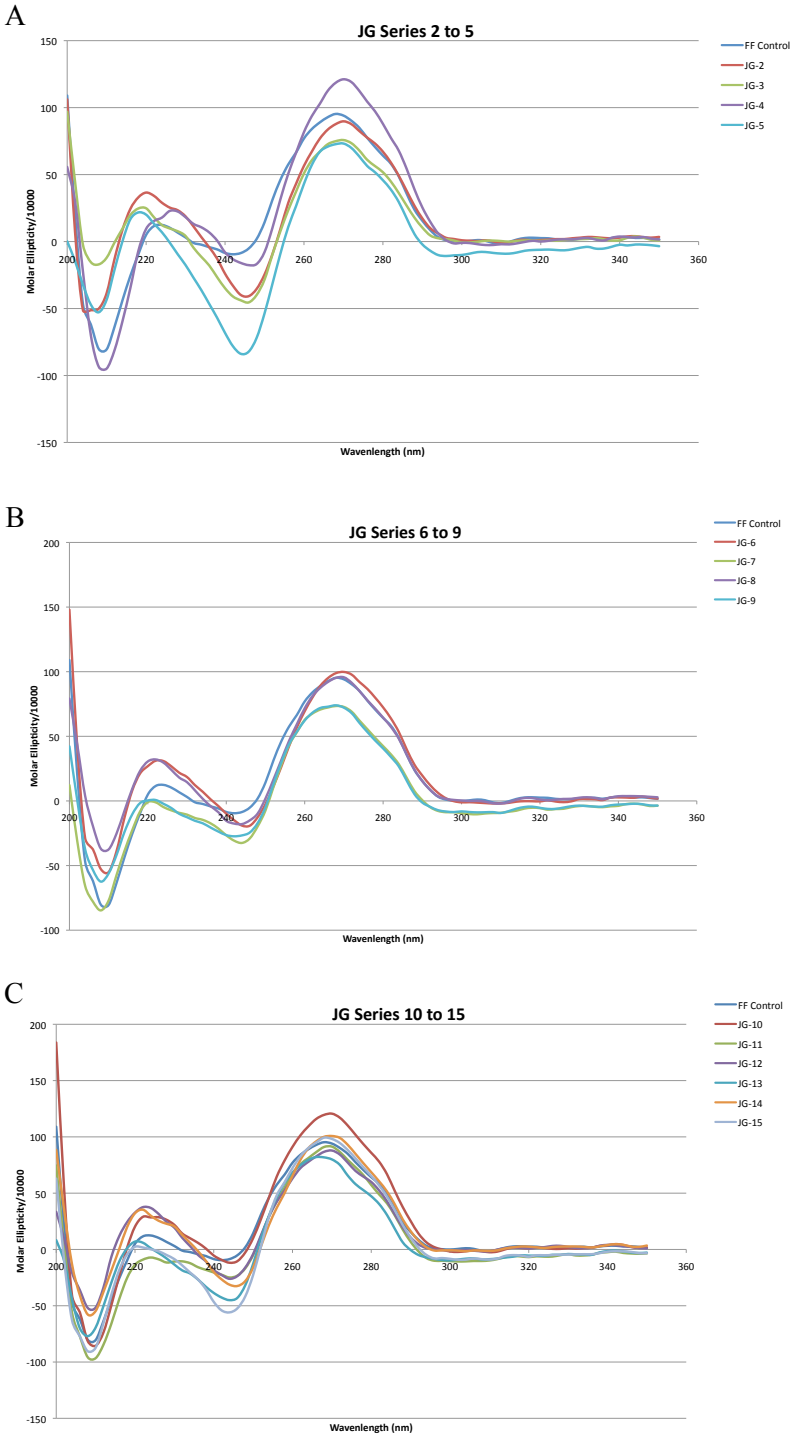
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Supplementary Information



Supplementary Figure S.1. FBS stability assays of unmodified FF Control (a) and modified FFC-611 (b) siRNAs. Assays spanned a 4 day period, and were conducted in 10% FBS in DMEM at 37°C. ds control is the double-stranded siRNA, ss control is the single stranded control (the sense strand of the siRNA duplex).



Supplementary Figure S.2: Circular dichroism spectra of siRNAs from the JG series. **(A)** CD spectra of JG-2 thru JG-5, compared with the dsRNA FF Control. JG-5, a 2F-ANA duplex, appears much more B-form than the 2F-RNA and 2F-ANA/2F-RNA duplexes. **(B)** FF Control siRNA compared with 2F-ANA/2F-RNA duplexes JG 6-9, all of which maintain an A-form conformation. **(C)** FF Control siRNA compared with the A-form 2F-RNA/RNA duplexes JG-10 and 11, 2F-ANA/RNA duplexes JG-12 and 13, and 2F-ANA/2F-RNA duplexes JG-14 and 15.

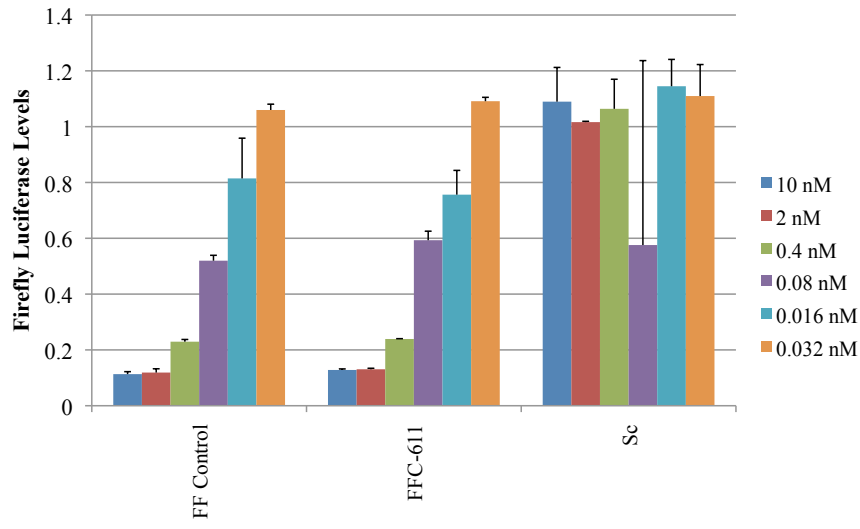


Figure S.3: Comparison of firefly luciferase gene silencing activity of unmodified FF Control siRNA and the fully 2F-ANA/2F-RNA modified siRNA FFC-611, which has modifications analogous to the potent ff6-11 siRNA. mRNA target is firefly luciferase. n=2. Firefly luciferase levels were normalized to total cellular protein and luciferase counts of cells treated with scrambled (non-targeting) siRNA. Bars indicate standard deviation.

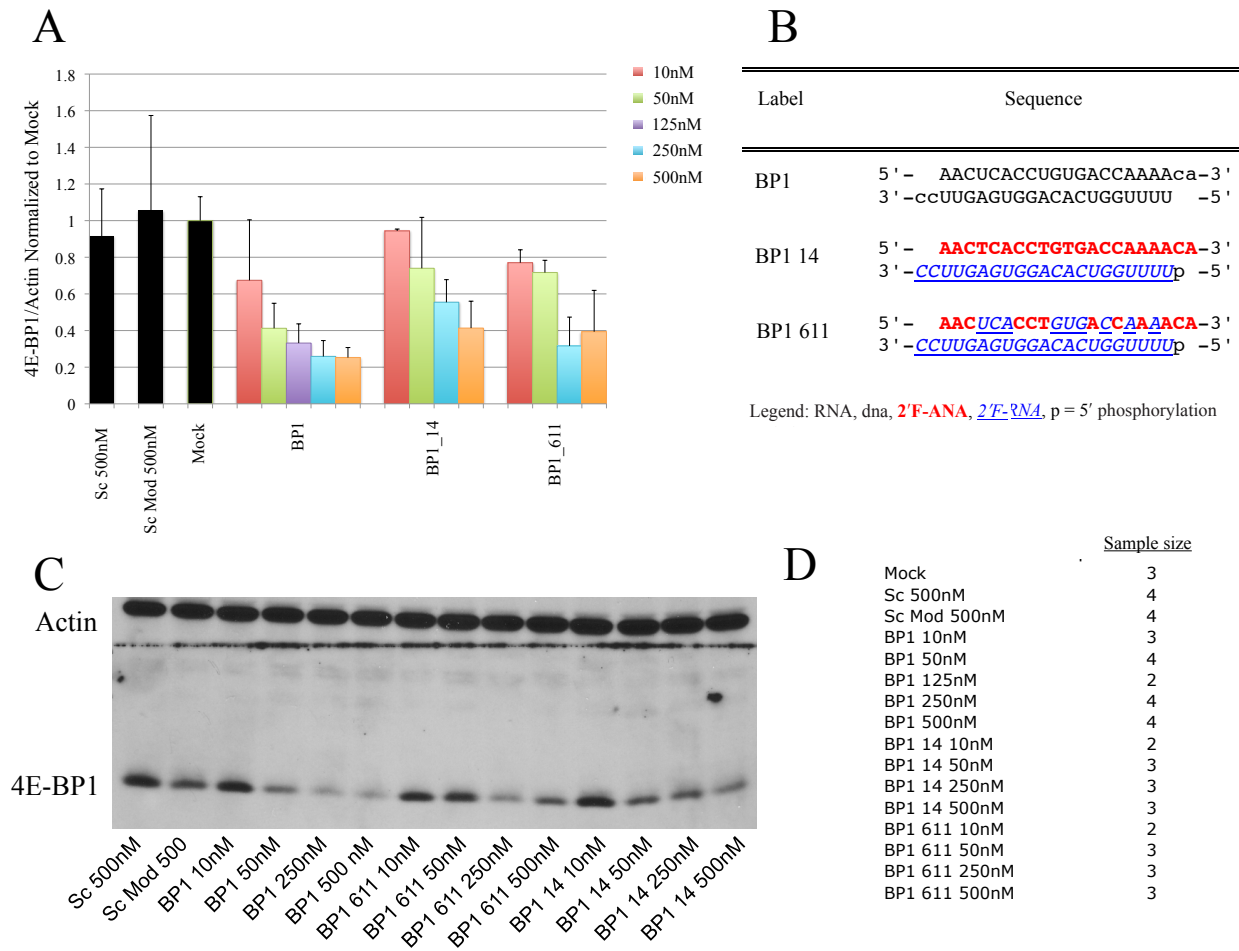
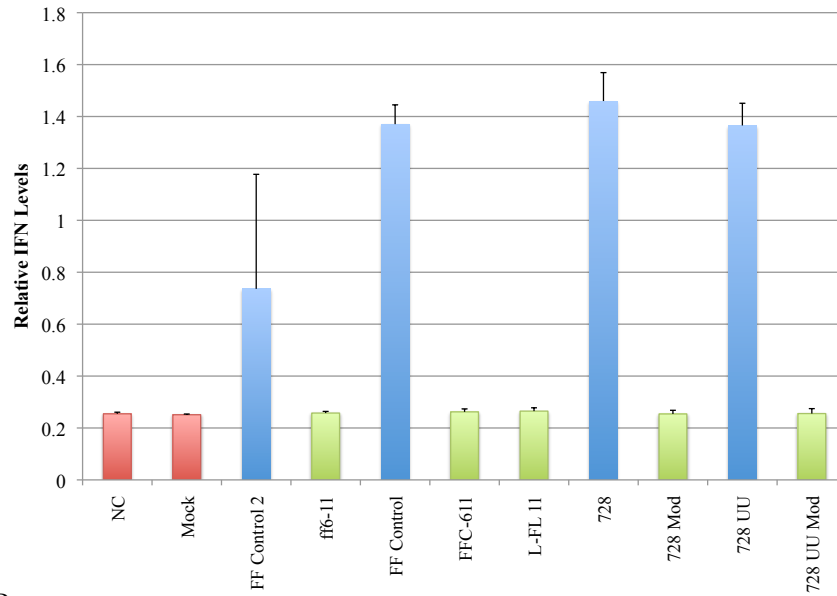


Figure S.4. (A) Densitometry analysis of Western blot results following cell treatment with siRNAs modified with 2'F-ANA and 2'F-RNA (sequences shown in **(B)**). mRNA target is *4E-BP1* in human HEK293T cells. siRNA concentrations ranged from 10 to 500 nM. A representative Western blot is shown in **(C)**. Bars represent standard deviation of replicates (sample size shown in **(D)**).

A



B

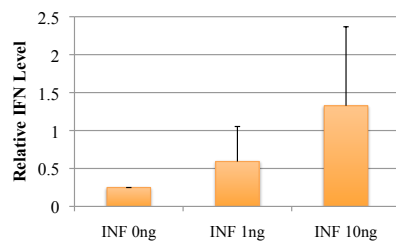


Figure S.5. (A) IFN levels in PBMC cells 24 hours after treatment with siRNAs transfected using Lipofectamine 2000, as measured by the HEK-Blue IFN assay. IFN levels in response to unmodified siRNAs are shown in blue, modified siRNAs in green, and control treatments in red. NC is the negative control (cells alone). Mock treatment was transfection without siRNA. Data was collected in duplicate for each of two donors. Bars indicate standard deviation. (B) IFN quantification by the HEK-Blue IFN assay following incubation with purified IFN is shown.

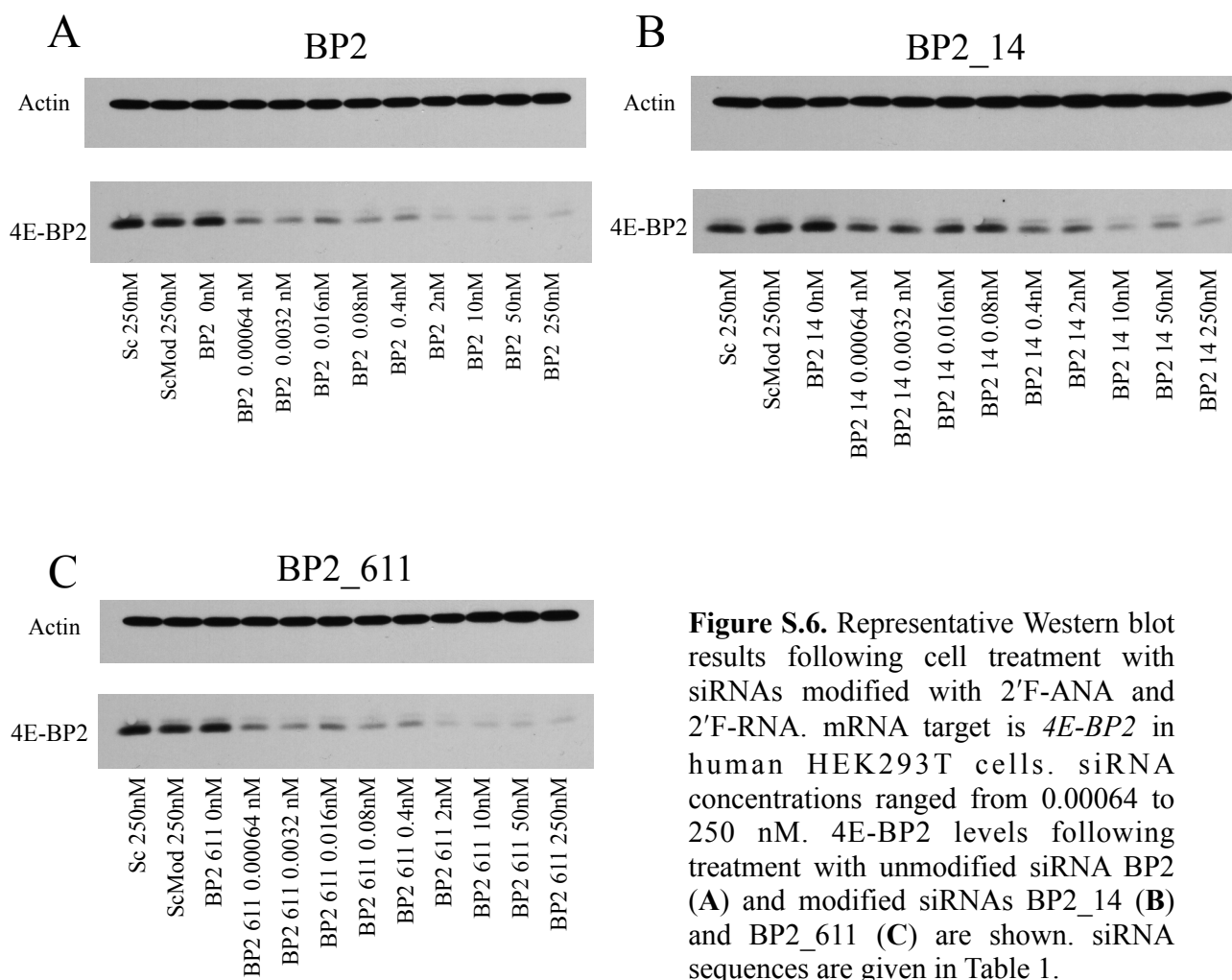


Figure S.6. Representative Western blot results following cell treatment with siRNAs modified with 2'F-ANA and 2'F-RNA. mRNA target is *4E-BP2* in human HEK293T cells. siRNA concentrations ranged from 0.00064 to 250 nM. 4E-BP2 levels following treatment with unmodified siRNA BP2 (**A**) and modified siRNAs BP2_14 (**B**) and BP2_611 (**C**) are shown. siRNA sequences are given in Table 1.