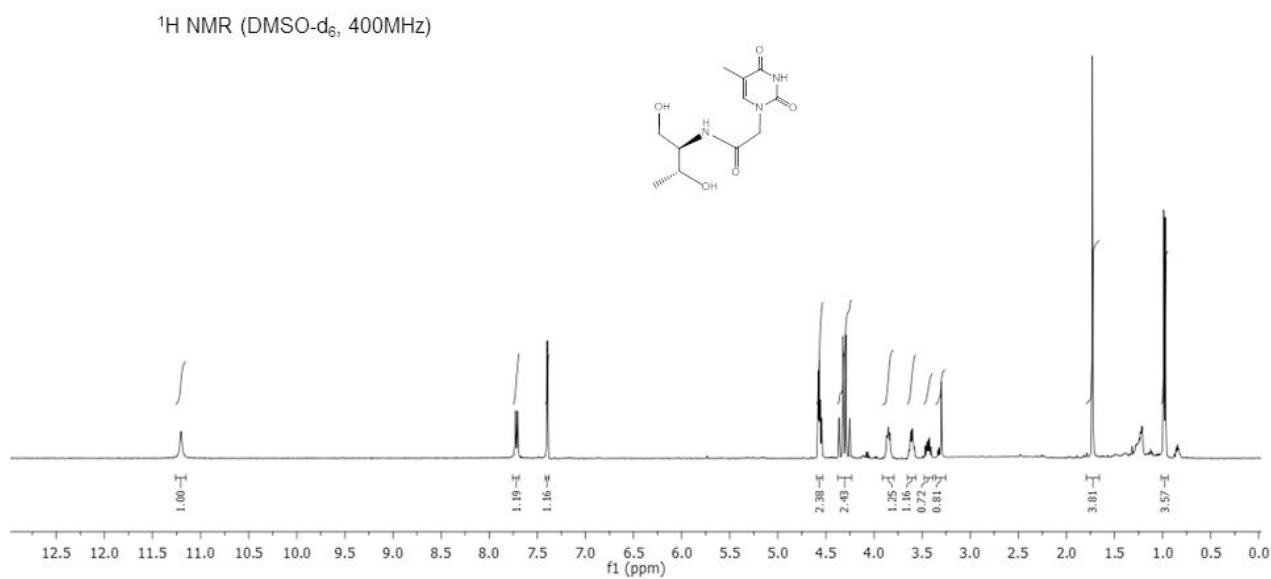


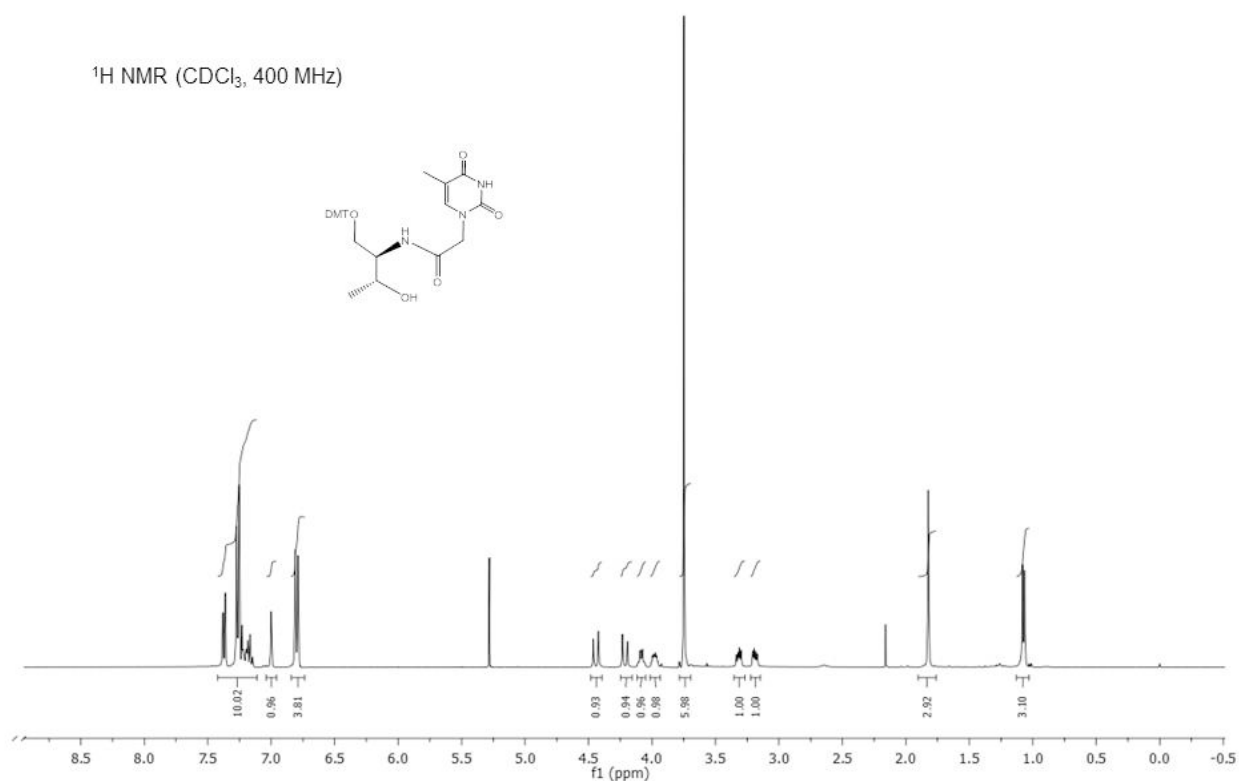
# Supplementary Materials (ESI)

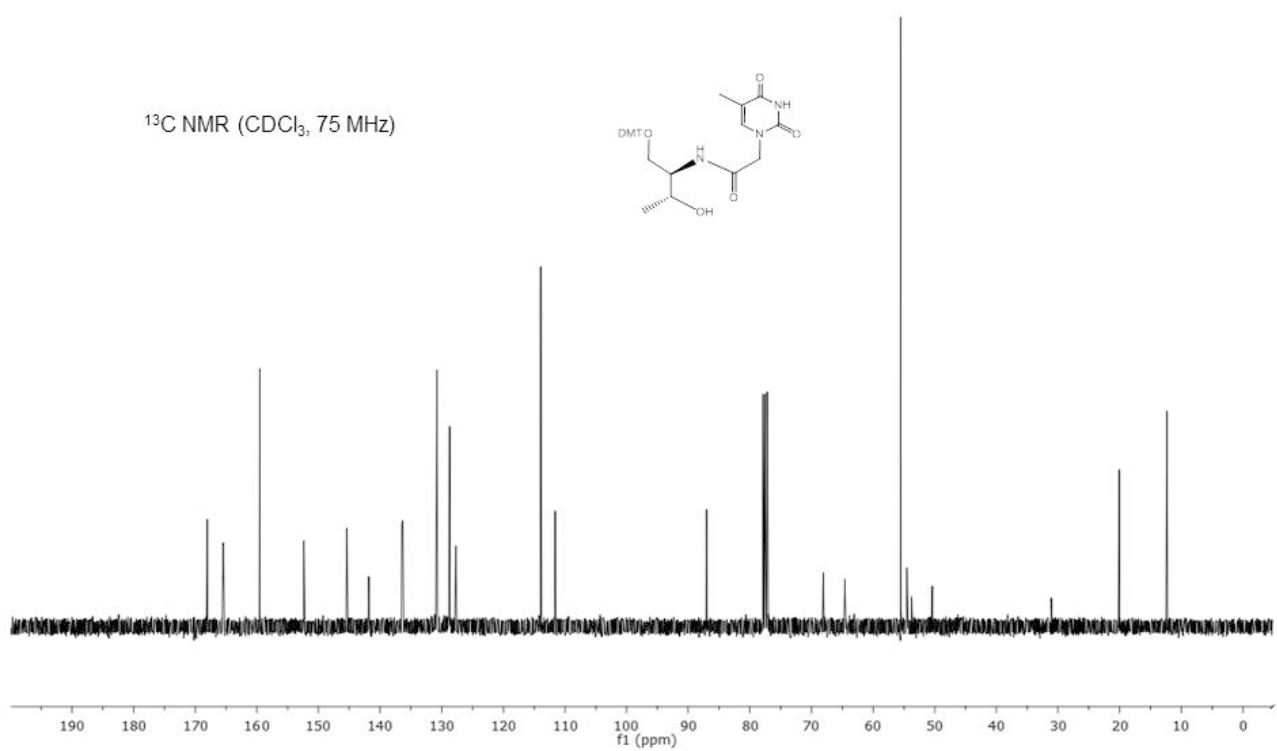
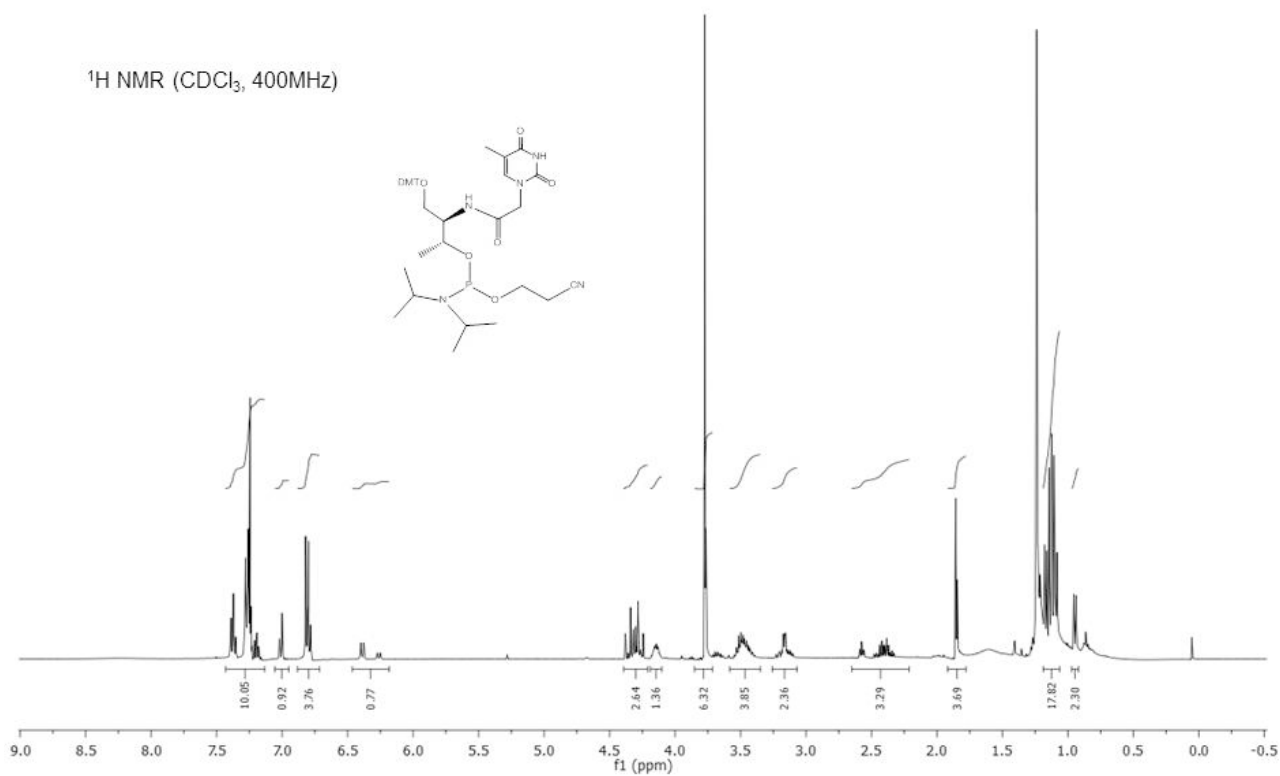
## NMR SPECTRA

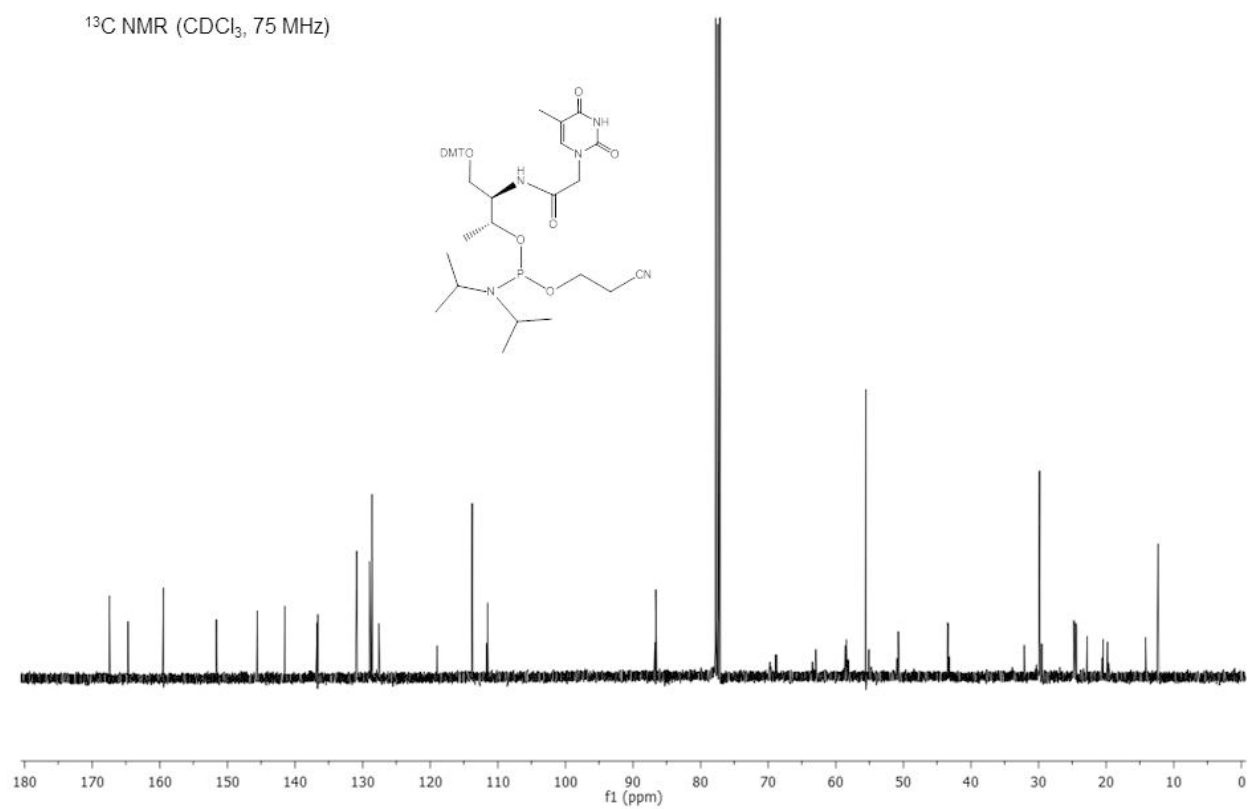
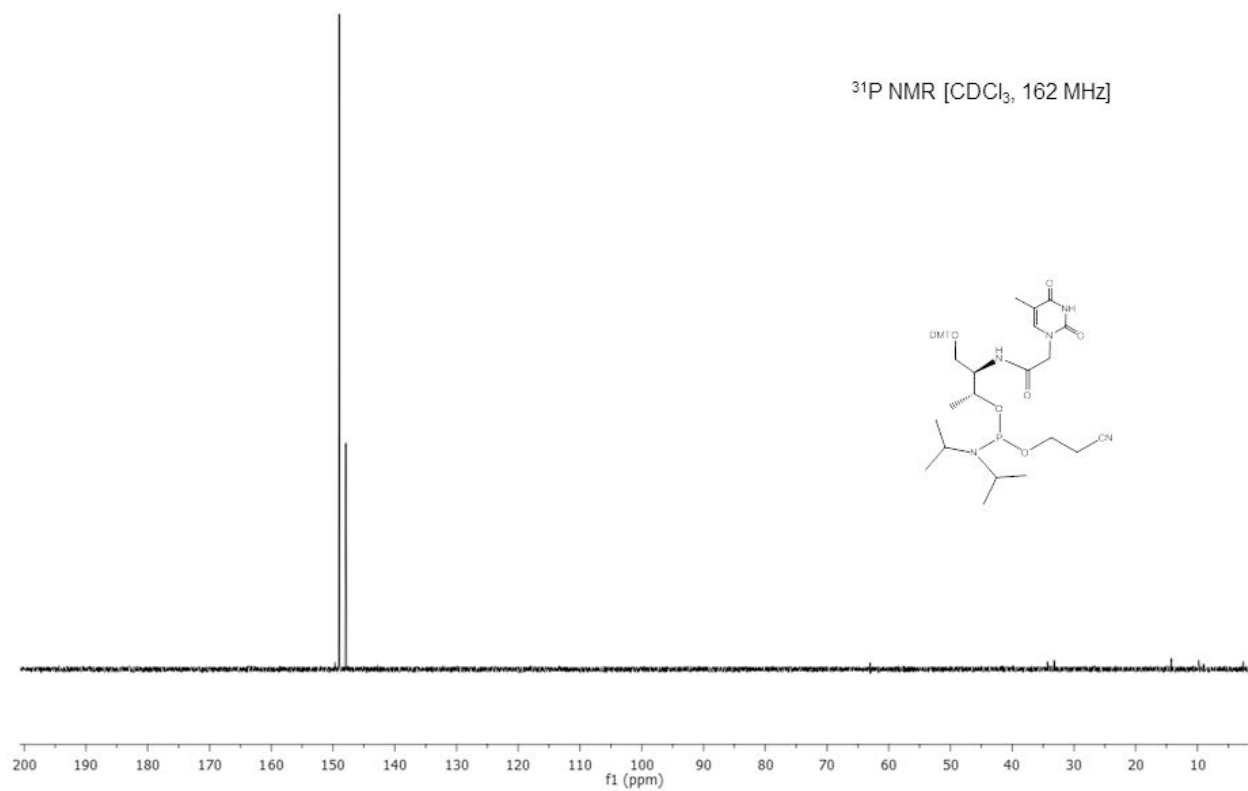
**Figure S1.**  $^1\text{H-NMR}$  spectrum of compound **2**.



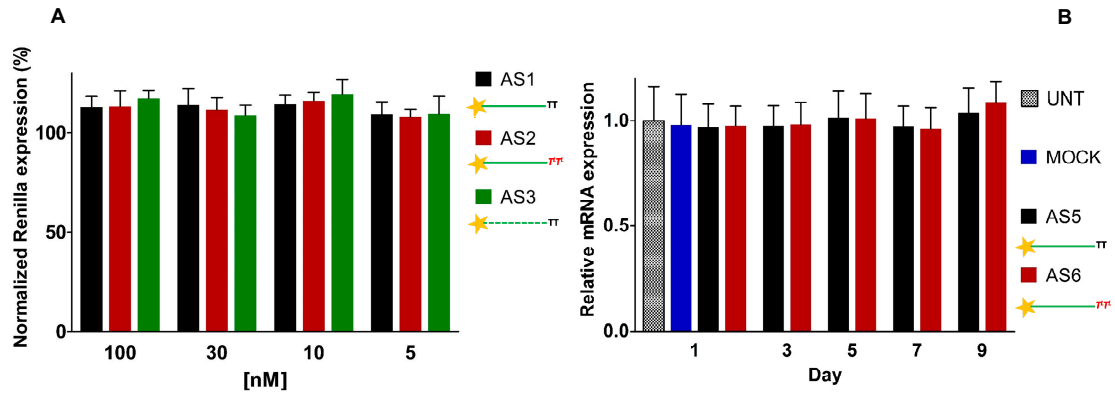
**Figure S2.**  $^1\text{H-NMR}$  spectrum of compound **3**.



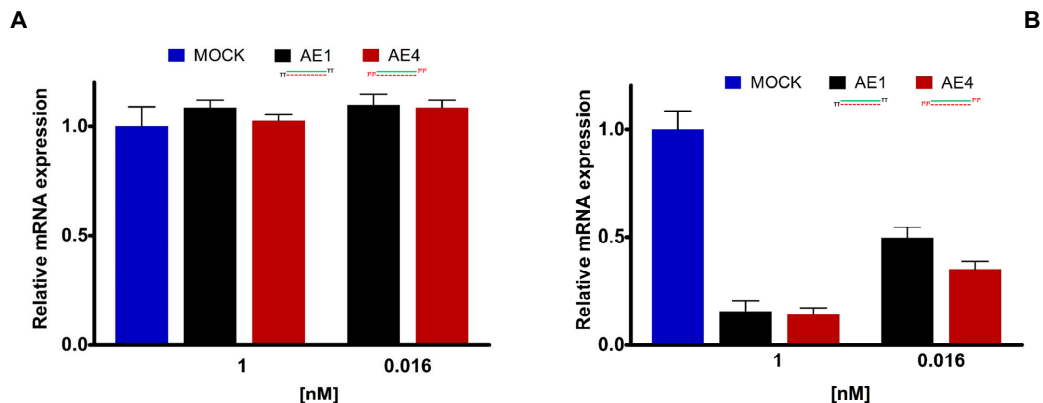
**Figure S3.**  $^{13}\text{C}$ -NMR spectrum of compound **3**.**Figure S4.**  $^1\text{H}$ -NMR spectrum of compound **5**.

**Figure S5.**  $^{13}\text{C}$ -NMR spectrum of compound **5**.**Figure S6.**  $^{31}\text{P}$ -NMR spectrum of compound **5**.

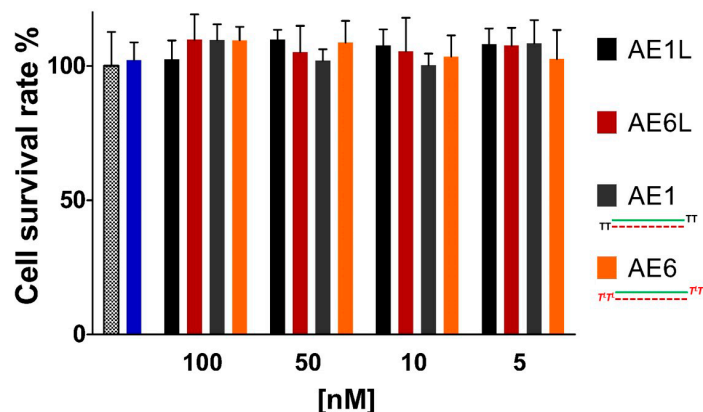
**Figure S7.** RNAi activity of non-phosphorylated ss-siRNAs. (A) Luminescence measurement 24 hours post-transfection of unmodified (AS1), modified (AS2) and scrambled (AS3) ss-siRNAs in HeLa cells. Values are mean  $\pm$ SD, n = 3. (B) *ApoB* mRNA levels in HepG2 cells treated with 60 nM of unmodified (AS5) and modified (AS6) ss-siRNAs. Values are mean  $\pm$ SD, n = 3.



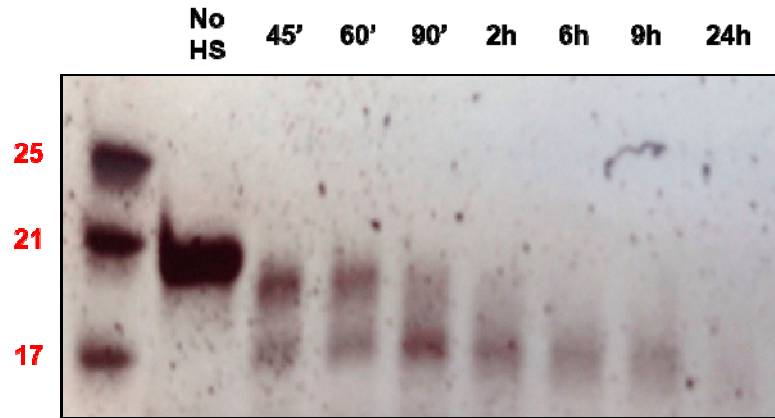
**Figure S8.** Silencing of *Renilla* gene Ago2-mediated. *Renilla* mRNA down-regulation in (A) MEF<sup>Ago2<sup>-/-</sup></sup> and (B) MEF<sup>wt</sup> cells transfected with 1 nM and 0.016 nM of unmodified AE1 and modified AE4. n = 3  $\pm$ SD. For experimental conditions see Material and Methods.



**Figure S9.** Cytotoxic effect of L-threoninol siRNA on HeLa cells survival. MTT assay showing cell proliferation 24 hours post-transfection with unmodified and modified siRNAs with or without transfection reagent (AE1L, AE6L, AE1 and AE6 respectively). Data are shown as mean  $\pm$ SD, n = 5.



**Figure S10.** Native TBE gel of native siRNA (AE1). **Lane 1:** dsRNA ladder; **lane 2:** 300 pmol of AE1 without human serum (No HS); **lane 3-9:** AE1 degradation pattern over the time of 24 hours in presence of 90% of human serum.



**Figure S11.** Quantitative RT-PCR analysis of selected *ISGs*: *ISG-56*, *PKR*, *IFITM1*, *MX1*, *OAS-1*. (A) mRNA levels after HepG2 transfection of unmodified (APO1) siRNA and double-modified (APO6) siRNA. (B) mRNA levels after THP-1 transfection of unmodified (APO1) siRNA and double-modified (APO6) siRNA. Results are presented as mean  $\pm$ SD, n = 3. For experimental conditions see Material and Methods. ns =  $p > 0.05$ ; \* =  $p < 0.05$ ; \*\* =  $p < 0.01$ ; \*\*\* =  $p < 0.001$ .

**A**

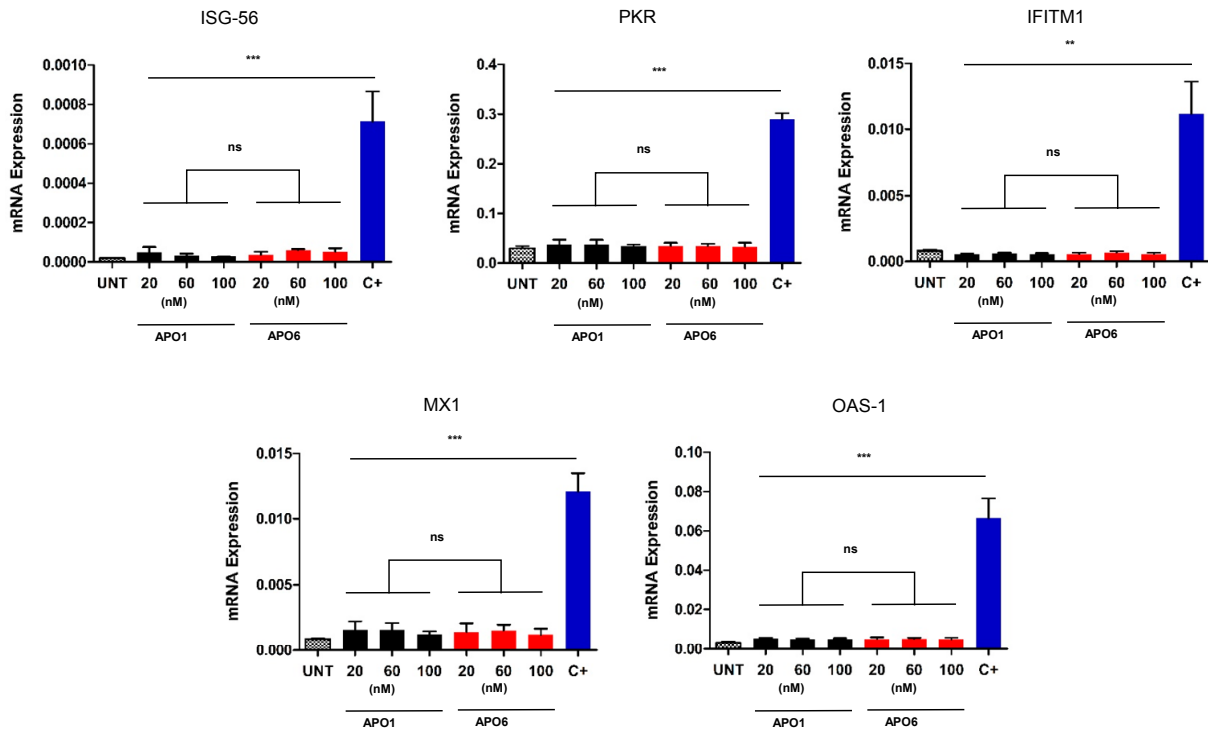


Figure S11. Cont.

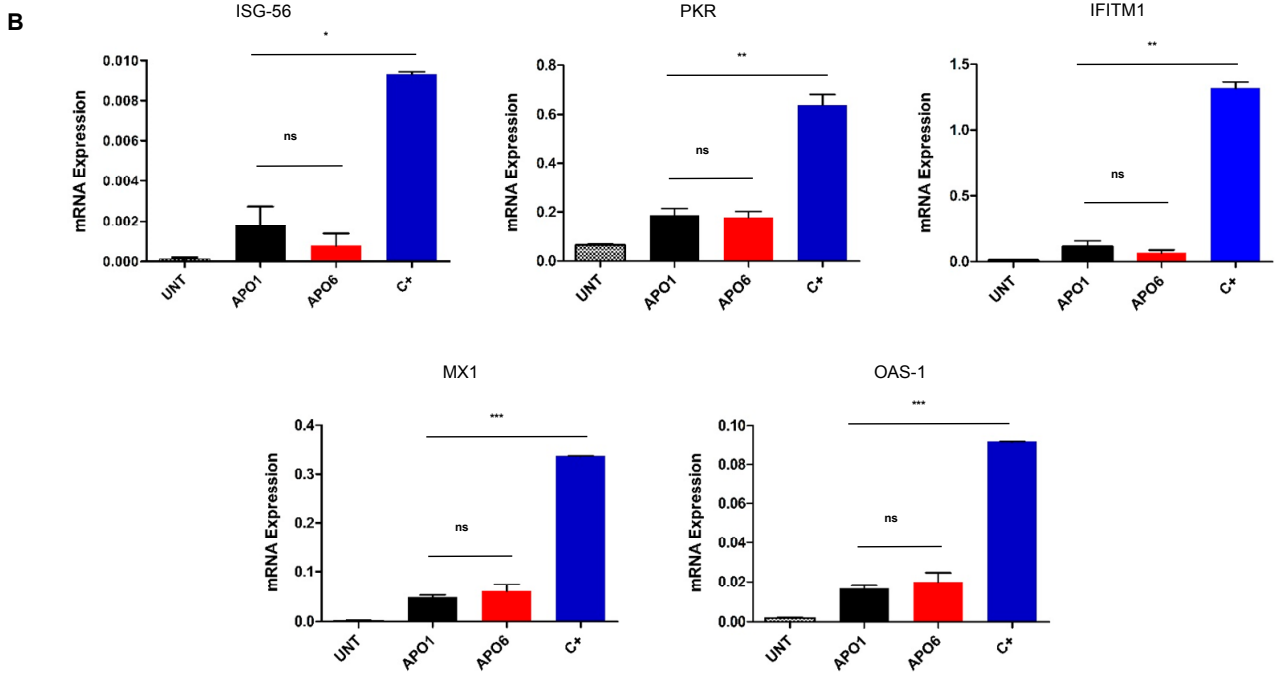


Table S1. List of primers.

	Forward	Reverse
<b>GAPDH</b>	5'-TGCACCACCAACTGCTTAG	5'-GATGCAGGGATGATGTTC
<b>APOB</b>	5'-CAAAGCCACCCTGGA ACTCT	5'-CTGCAATGTCAAGGTGTGCC
<b>PKR</b>	5'-ACTTT TTCCTGGCTCATCTC	5'-ACATGCCTGTAATCCAGCTA
<b>ISG56</b>	5'-TTCGGAGAAAGGCATTAGA	5'-TCCAGGGCTTCATTCATAT
<b>MX1</b>	5'-CAGCACCTGATGGCCTATCA	5'-TGGAGCATGAAGA ACTGGATGA
<b>OAS-1</b>	5'-AGAAGGCAGCTCACGAAACC	5'-CCACCACCCAAGTTTCCTGTA
<b>IFITM-1</b>	5'-GACAGGAAGATGGTTGGCGA	5'-GGTAGACTGTACAGAGCCG
<b>RL-TK</b>	5'-GTGGTGGGCCAGATGTAAAC	5'-CAGGTGCATCTTCTTGCGAA