

## Isonucleotide Incorporation into Middle and Terminal siRNA Duplexes Exhibits High Gene Silencing Efficacy and Nuclease Resistance

### Electronic Supplementary Information

Yuan Ma<sup>#, a</sup>, Shuang Liu<sup>#, a</sup>, Yusi Wang<sup>a</sup>, Yuanhe Zhao<sup>a</sup>, Ye Huang<sup>a</sup>, Lijun Zhong<sup>b</sup>, Zhu Guan<sup>a</sup>, Lihe Zhang<sup>a</sup> and Zhenjun Yang<sup>a,\*</sup>

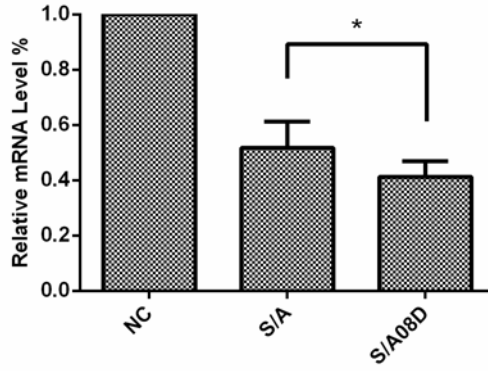
<sup>a</sup> State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, No.38 Xueyuan Road, Haidian District, Beijing 100191, China and <sup>b</sup> Medical and Health Analysis Center, School of Pharmaceutical Sciences, Peking University, No.38 Xueyuan Road, Haidian District, Beijing 100191, China

\*To whom correspondence should be addressed. Tel: +86 10 82802503; Fax: +86 10 82802503; Email: [yangzj@bjmu.edu.cn](mailto:yangzj@bjmu.edu.cn)

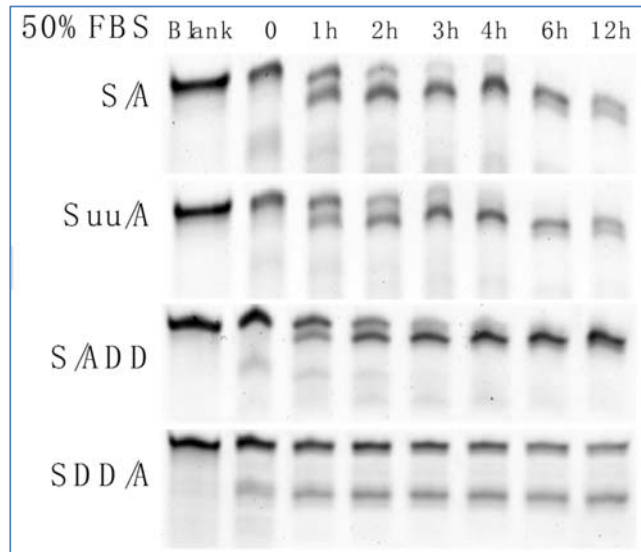
<sup>#</sup> These authors contributed equally to the paper as first authors.

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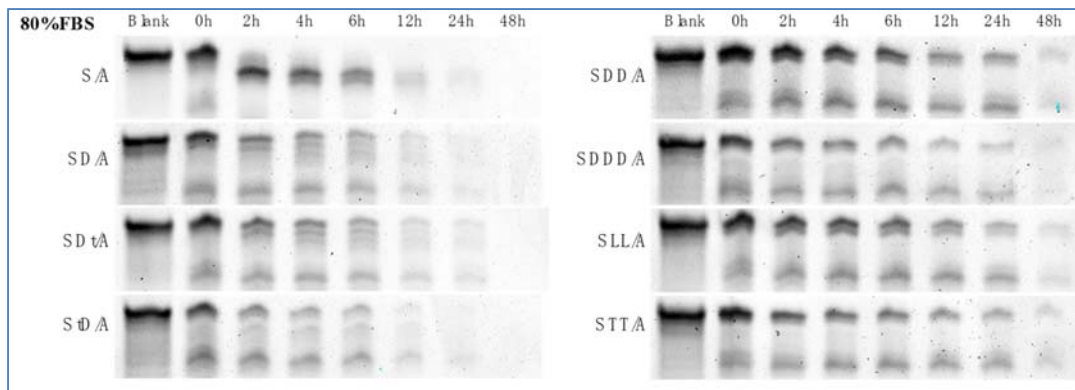
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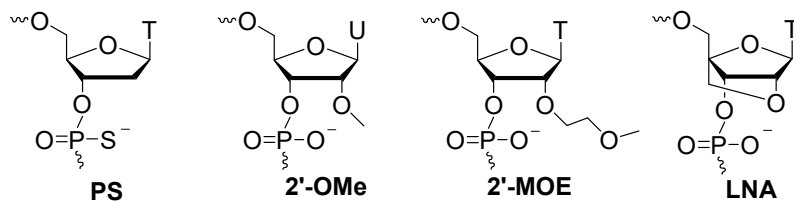
**Figure S1.** Quantitative PCR analysis of Braf-mu mRNA (2 nM). A375 cells were harvested for total RNA 48 h post transfection.



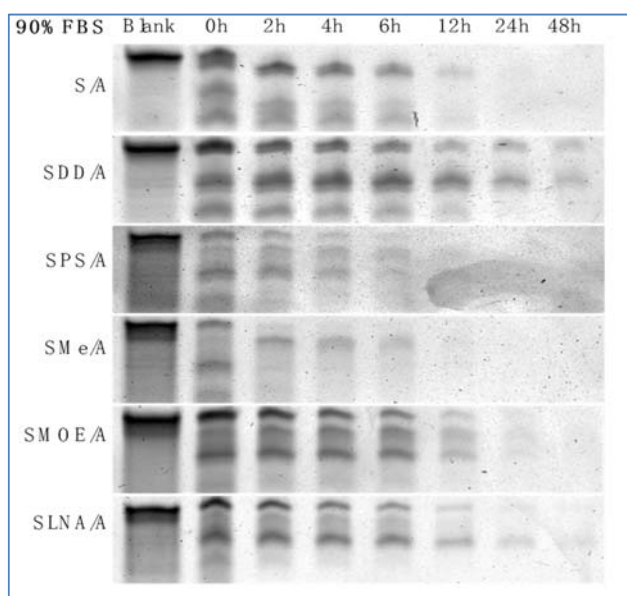
**Figure S2.** Serum stability of modified siRNAs at 3'-overhangs. These siRNAs were incubated in 50% fetal bovine serum at 37 °C and sampled at various time points, followed by separation on 20% PAGE gel with siRNA products visualized by SYBR Gold staining.



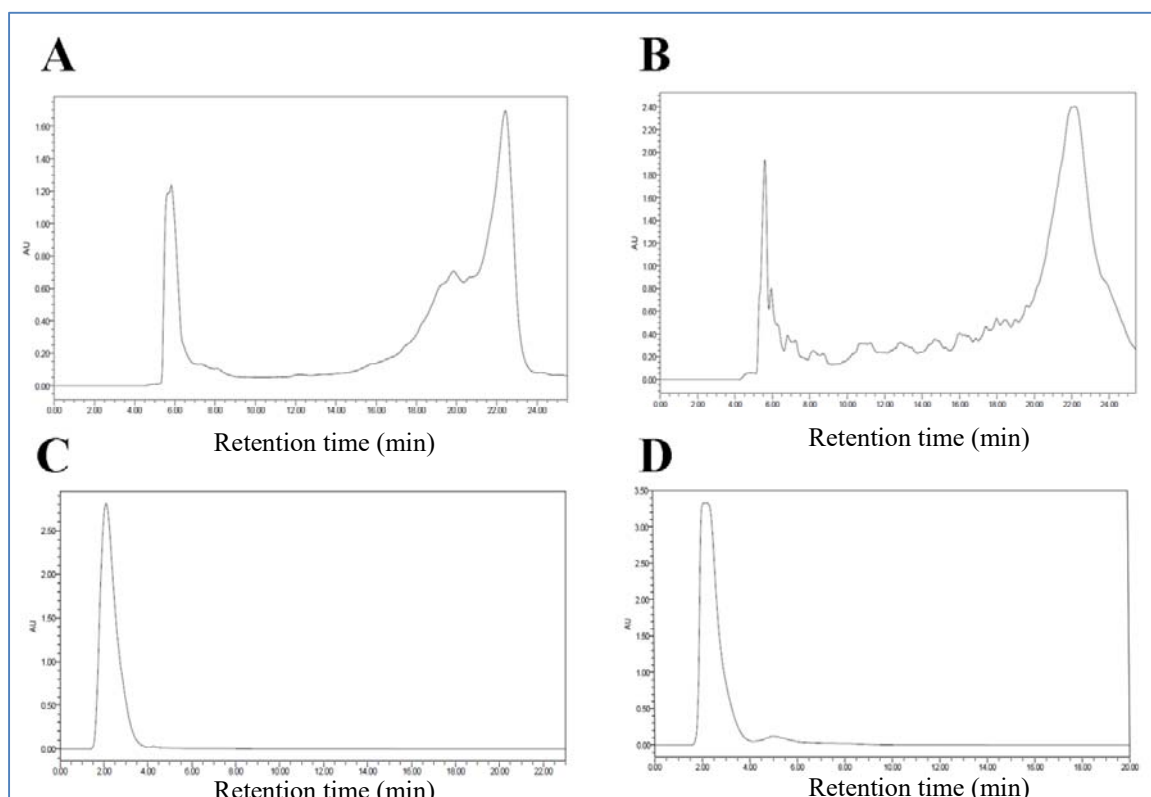
**Figure S3.** Serum stability of D-/L-IsoNAs modified siRNAs at 3'-overhangs of sense strand. These siRNAs were incubated in 50% fetal bovine serum at 37 °C and sampled at various time points, followed by separation on 20% PAGE gel with siRNA products visualized by SYBR Gold staining.



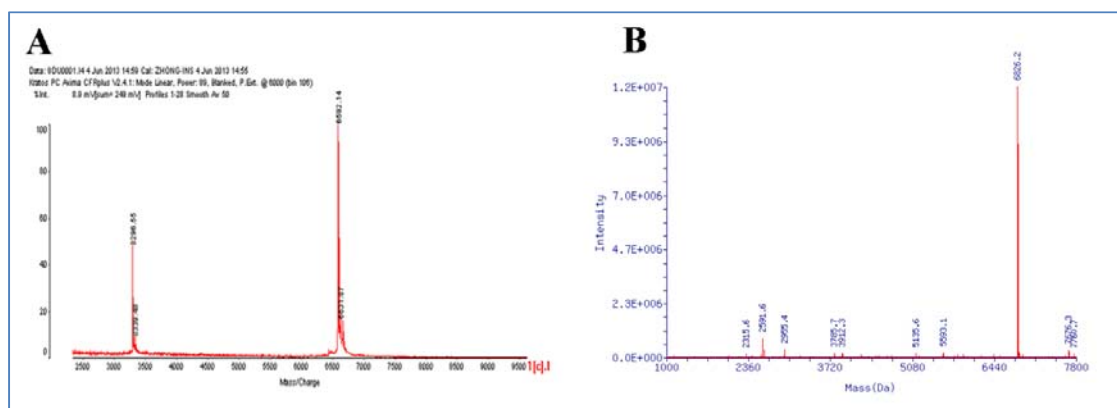
**Figure S4.** The selected structures of chemical modification used in siRNAs 3'-overhangs



**Figure S5.** Serum stability of chemical modified siRNAs at 3'-overhangs of sense strand. These siRNAs were incubated in 50% fetal bovine serum at 37 °C and sampled at various time points, followed by separation on 20% PAGE gel with siRNA products visualized by SYBR Gold staining.



**Figure S6.** HPLC and desalting profiles of single oligonucleotide strand (A-B) Anion exchange HPLC of A08D and SMOE oligonucleotide strand, respectively. (C-D) Desalting images of their oligonucleotide strand, respectively.



**Figure S7.** The MALDI-TOF-MS and ESI-MS of single oligonucleotide strand (A) The MALDI-TOF-MS of A08D strand production. (B) The ESI-MS of SMOE strand production.

**Table S1.** Sequence details of the synthesized oligonucleotide strands

No.	Name	Sequence	Calcd.	Found
1	MB3-SS	5'-GCUACAGAGAAAUCUCGAUtt-3'	6677.1	6678.4
2	S03D	5'-GCU <sub>D</sub> ACAGAGAAAUCUCGAUtt-3'	6661.1	6661.1

3	S03L	5'-GCU $U_L$ ACAGAGAAAUCUCGAUtt-3'	6661.1	6663.5
4	S04D	5'-GCU $A_D$ CAGAGAAAUCUCGAUtt-3'	6661.1	6662.8
5	S04L	5'-GCU $A_L$ CAGAGAAAUCUCGAUtt-3'	6661.1	6662.5
6	S10D	5'-GCUACAGAG $A_D$ AAUCUCGAUtt-3'	6661.1	6662.5
7	S10L	5'-GCUACAGAG $A_L$ AAUCUCGAUtt-3'	6661.1	6662.4
8	S11D	5'-GCUACAGAG $A_D$ AUCUCGAUtt-3'	6661.1	6662.2
9	S11L	5'-GCUACAGAG $A_L$ AUCUCGAUtt-3'	6661.1	6662.0
10	S12D	5'-GCUACAGAGAA $A_D$ UCUCGAUtt-3'	6661.1	6661.4
11	S12L	5'-GCUACAGAGAA $A_L$ UCUCGAUtt-3'	6661.1	6662.5
12	S13D	5'-GCUACAGAGAAA $U_D$ CUCGAUtt-3'	6661.1	6662.5
13	S13L	5'-GCUACAGAGAAA $U_L$ CUCGAUtt-3'	6661.1	6663.0
14	S18D	5'-GCUACAGAGAAAUCUCG $A_D$ Utt-3'	6661.1	6662.2
15	S18L	5'-GCUACAGAGAAAUCUCG $A_L$ Utt-3'	6661.1	6662.3
16	SDD	5'-GCUACAGAGAAAUCUCGAU $U_DU_D$ -3'	6649.1	6650.1
17	Suu	5'-GCUACAGAGAAAUCUCGAUUU-3'	6681.1	6682.7
18	SDDD	5'-GCUACAGAGAAAUCUCGAU $U_DU_DU_D$ -3'	6939.3	6940.2
19	SDt	5'-GCUACAGAGAAAUCUCGAU $U_D$ t-3'	6686.1	6686.7
20	StD	5'-GCUACAGAGAAAUCUCGAUt $U_D$ -3'	6686.1	6687.8
21	SD	5'-GCUACAGAGAAAUCUCGAU $U^D$ -3'	6381.9	6384.4
22	STT	5'-GCUACAGAGAAAUCUCGAU $T_DT_D$ -3'	6677.1	6678.2
23	SLL	5'-GCUACAGAGAAAUCUCGAU $U_LU_L$ -3'	6649.1	6650.1
24	SPS	5'-GCUACAGAGAAAUCUCGAU $t_{st}$ -3'	6693.2	6693.7
25	SMe	5'-GCUACAGAGAAAUCUCGAU $U_{Me}U_{Me}$ -3'	6709.1	6709.9
26	SMOE	5'-GCUACAGAGAAAUCUCGAU $T_{MOE}T_{MOE}$ -3'	6825.1	6826.2
27	SLNA	5'-GCUACAGAGAAAUCUCGAU $T_{LNA}T_{LNA}$ -3'	6733.2	6734.1
28	MB3-AS	5'-AUCGAGAUUUCUCUGUAGCtt-3'	6608.0	6608.9
29	A02D	5'- $A^D$ CGAGAUUUCUCUGUAGCtt-3'	6592.0	6594.3
30	A02L	5'- $A^L$ CGAGAUUUCUCUGUAGCtt-3'	6592.0	6592.7
31	A07D	5'-AUCGAGA $A^D$ UUUCUCUGUAGCtt-3'	6592.0	6591.4
32	A07L	5'-AUCGAGA $A^L$ UUUCUCUGUAGCtt-3'	6592.0	6590.4
33	A08D	5'-AUCGAGA $U^D$ UUCUCUGUAGCtt-3'	6592.0	6592.1
34	A08L	5'-AUCGAGA $U^L$ UUCUCUGUAGCtt-3'	6592.0	6592.5
35	A09D	5'-AUCGAGAU $U^D$ UCUCUGUAGCtt-3'	6592.0	6592.9
36	A09L	5'-AUCGAGAU $U^L$ UCUCUGUAGCtt-3'	6592.0	6592.4
37	A10D	5'-AUCGAGAUU $U_D$ CUCUGUAGCtt-3'	6592.0	6593.5
38	A10L	5'-AUCGAGAUU $U_L$ CUCUGUAGCtt-3'	6592.0	6592.9
39	A16D	5'-AUCGAGAUUUCUCUG $U_D$ AGCtt-3'	6592.0	6593.3
40	A16L	5'-AUCGAGAUUUCUCUG $U_L$ AGCtt-3'	6592.0	6592.9

41	A17D	5'-AUCGAGAUUUCUCUGU <b>A<sub>D</sub></b> GCtt-3'	6592.0	6593.8
42	A17L	5'-AUCGAGAUUUCUCUGU <b>A<sub>L</sub></b> GCtt-3'	6592.0	6592.0
43	ADD	5'-AUCGAGAUUUCUCUGUAGC <b>U<sub>D</sub>U<sub>D</sub></b> -3'	6580.0	6576.4
44	RNA1-SS	5'-AGAAUUGGAUCUGGAUCAUtt-3'	6695.1	6695.2
45	RNA1-AS	5'-AUGAUCCAGAUCCAAUUCUtt-3'	6575.0	6575.2
46	1A08D	5'-AUGAUCCA <b>A<sub>D</sub></b> GAUCCAAUUCUtt-3'	6559.0	6560.2
47	RNA2-SS	5'-AGCAUGAACCAUGAGUUGCtt-3'	6693.1	6694.0
48	RNA2-AS	5'-GCAACUCAUGGUUCAUGCUtt-3'	6607.0	6608.4
49	2A08D	5'-GCAACUCA <b>A<sub>D</sub></b> UGGUUCAUGCUtt-3'	6591.0	6592.6

**Table S2.** Sequences of mismatched siRNAs

	No.	Name	siRNA Sequence
Sense strand(SS)	1	siMB3-S/A	SS: 5'-GCUACAGAGAAAUCUCGAUtt-3'
Antisense strand(AS)			AS: 5'-AUCGAGAUUUCUCUGUAGCtt-3'
	34	S13c/A	SS: 5'-GCUACAGAGAAA <b>c</b> CUCGAUtt-3'/AS
	35	S13a/A	SS: 5'-GCUACAGAGAAA <b>a</b> CUCGAUtt-3'/AS
Sense strand(SS)	36	S13g/A	SS: 5'-GCUACAGAGAAA <b>g</b> CUCGAUtt-3'/AS
Mismatched with	37	S12c/A	SS: 5'-GCUACAGAGAA <b>c</b> UCUCGAUtt-3'/AS
Antisense strand(AS)	38	S12u/A	SS: 5'-GCUACAGAGAA <b>u</b> UCUCGAUtt-3'/AS
	39	S12g/A	SS: 5'-GCUACAGAGAA <b>g</b> UCUCGAUtt-3'/AS

Positions of **mismatch** incorporated are indicated in *Blue, italic and lowercase*