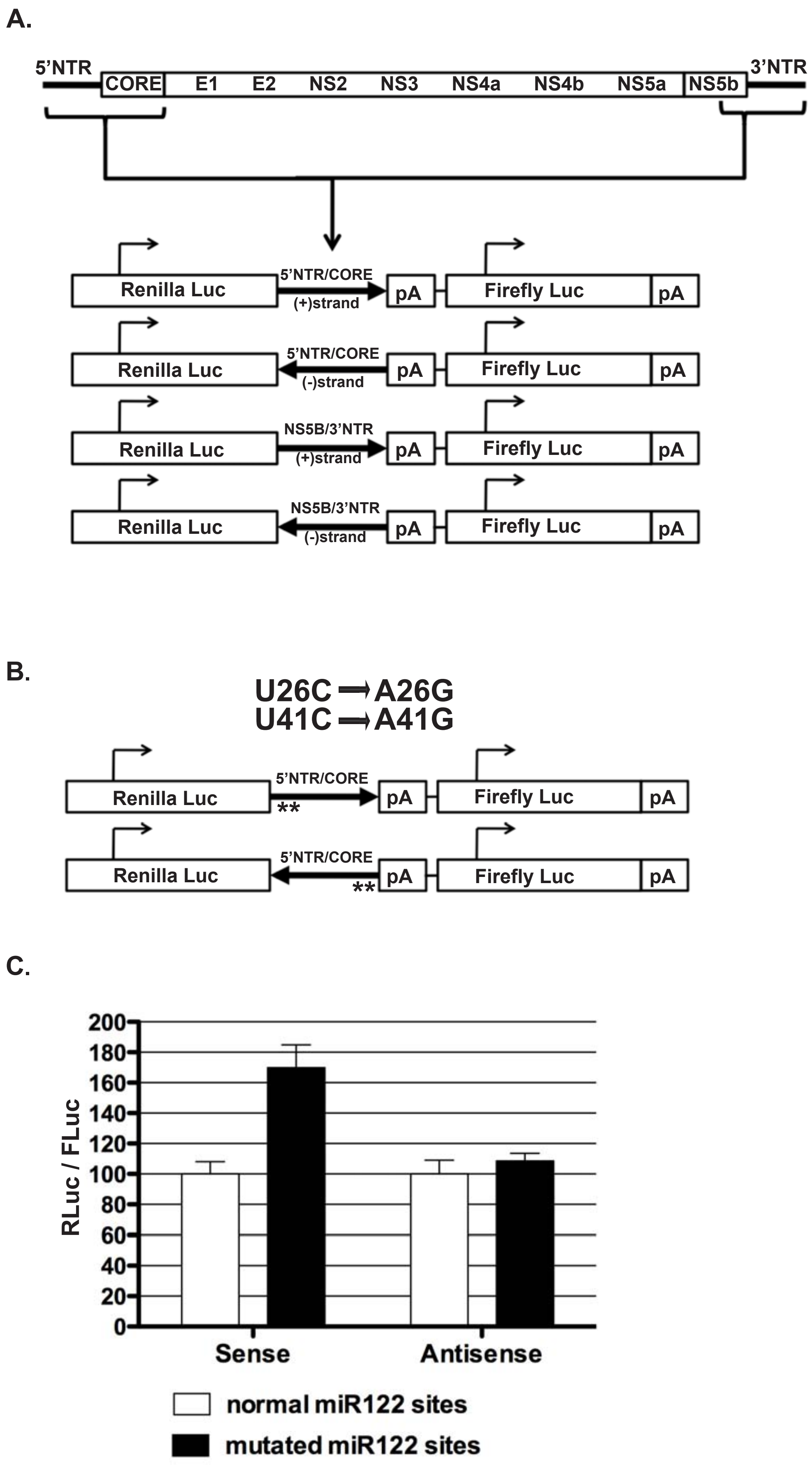
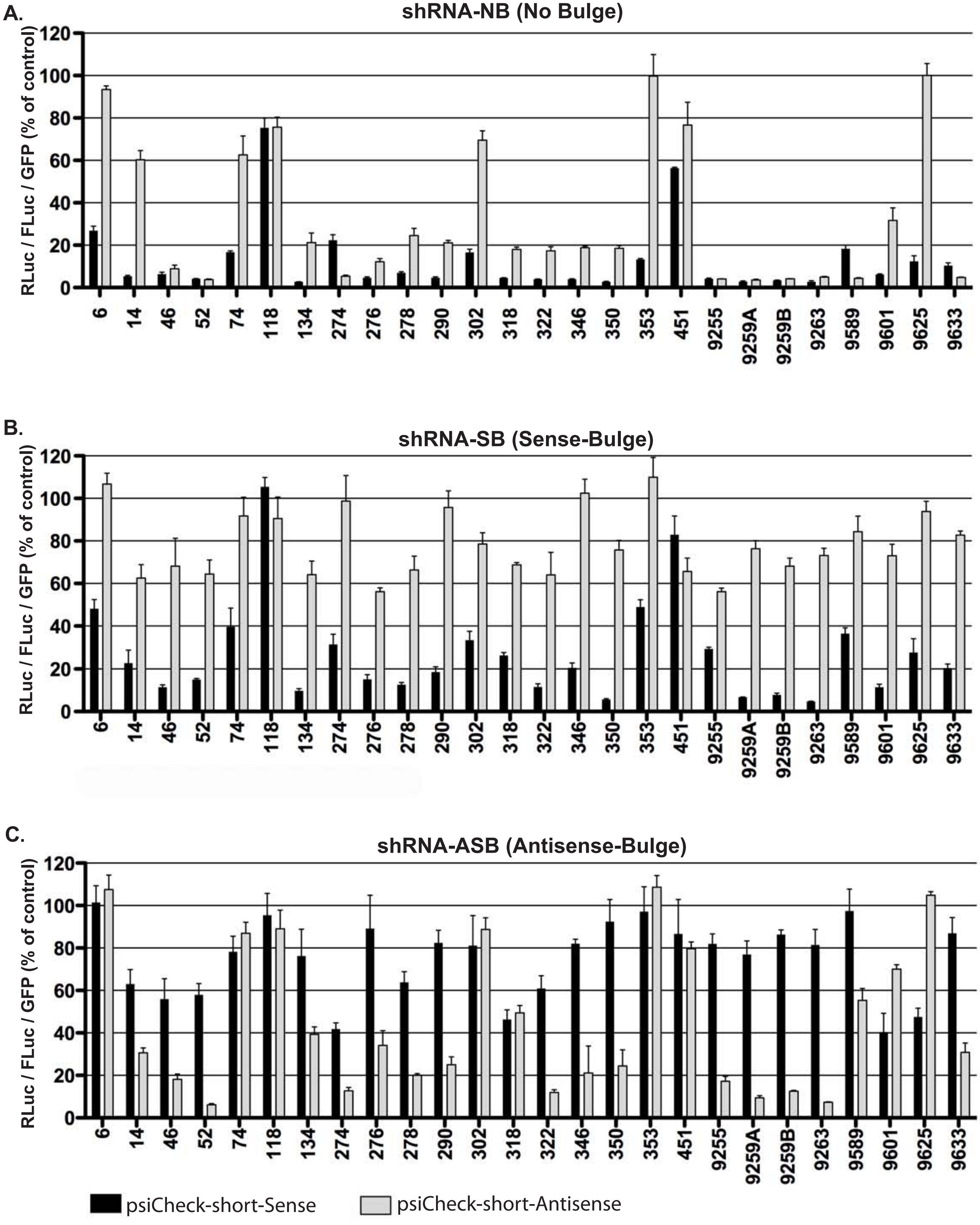
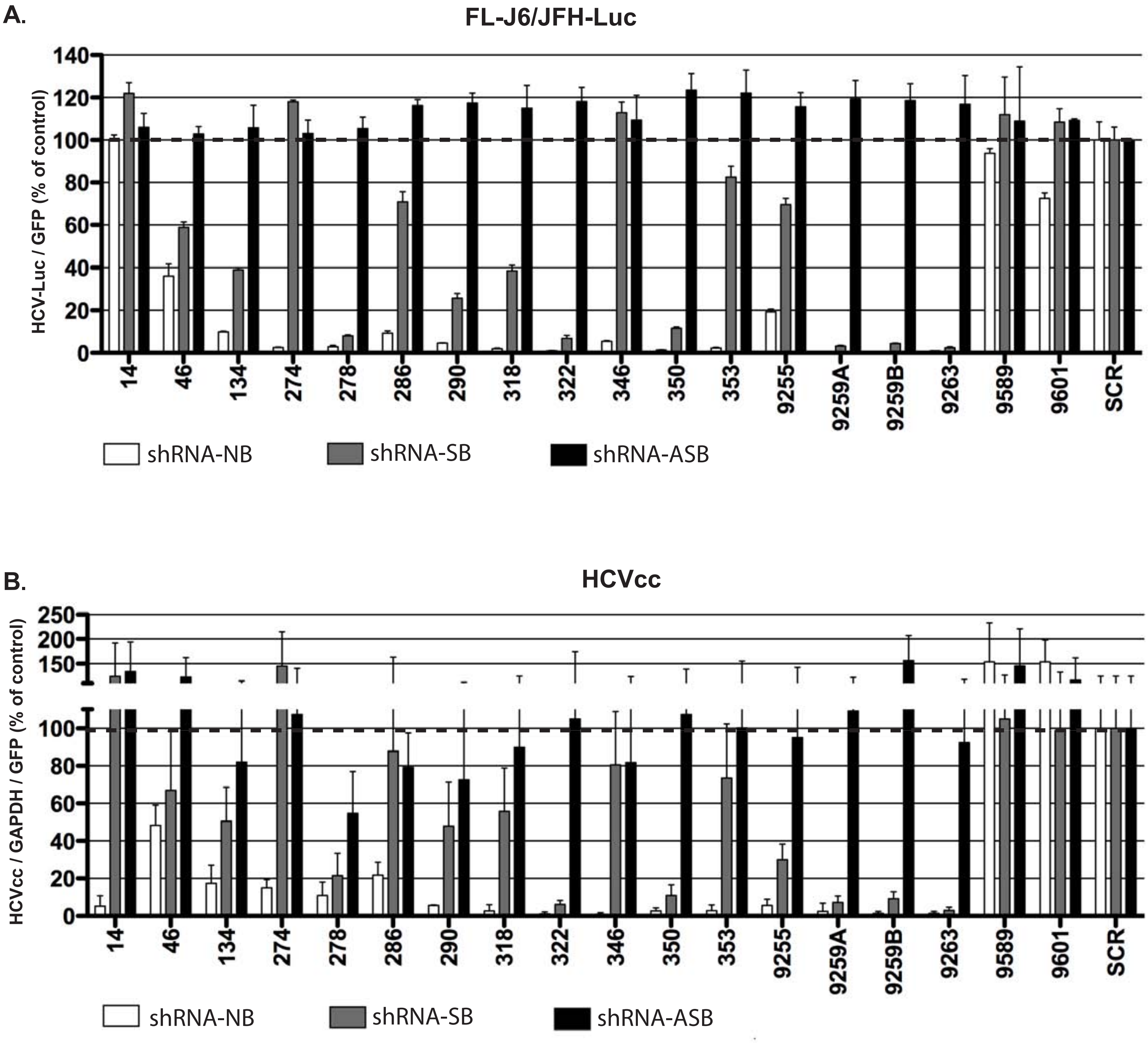


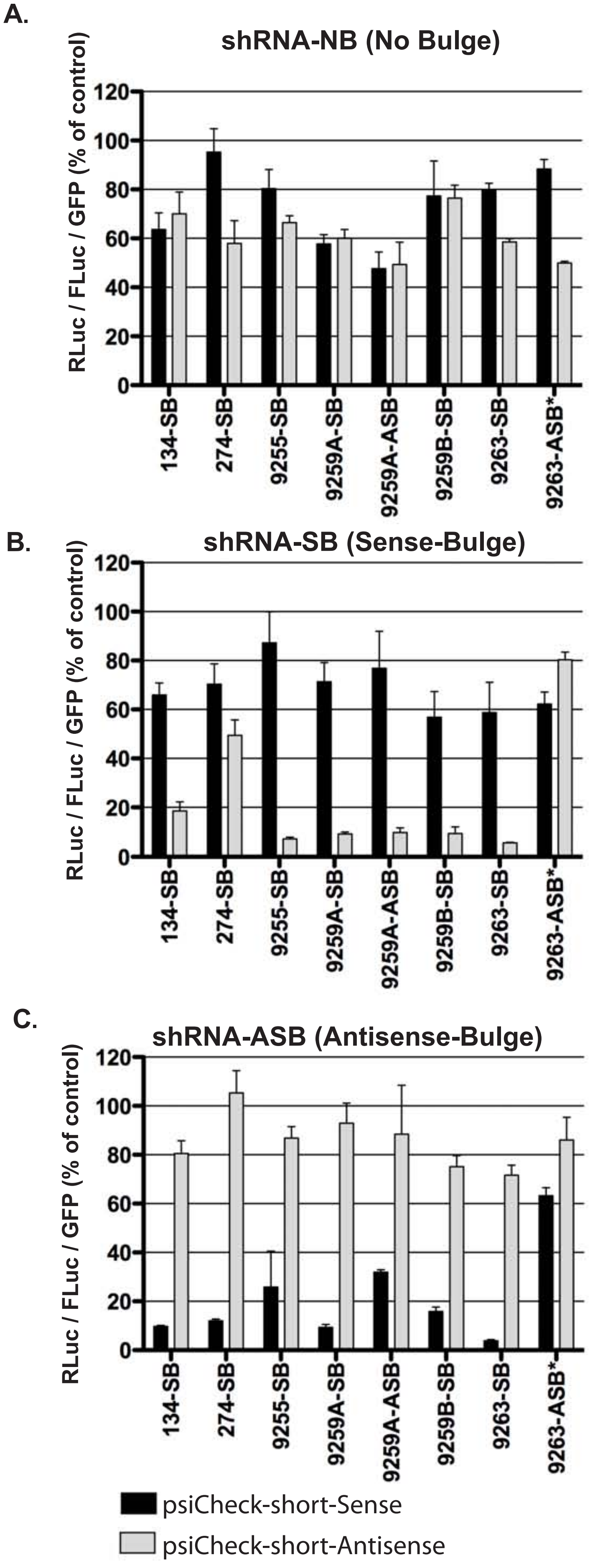
Supplementary Figure 1.



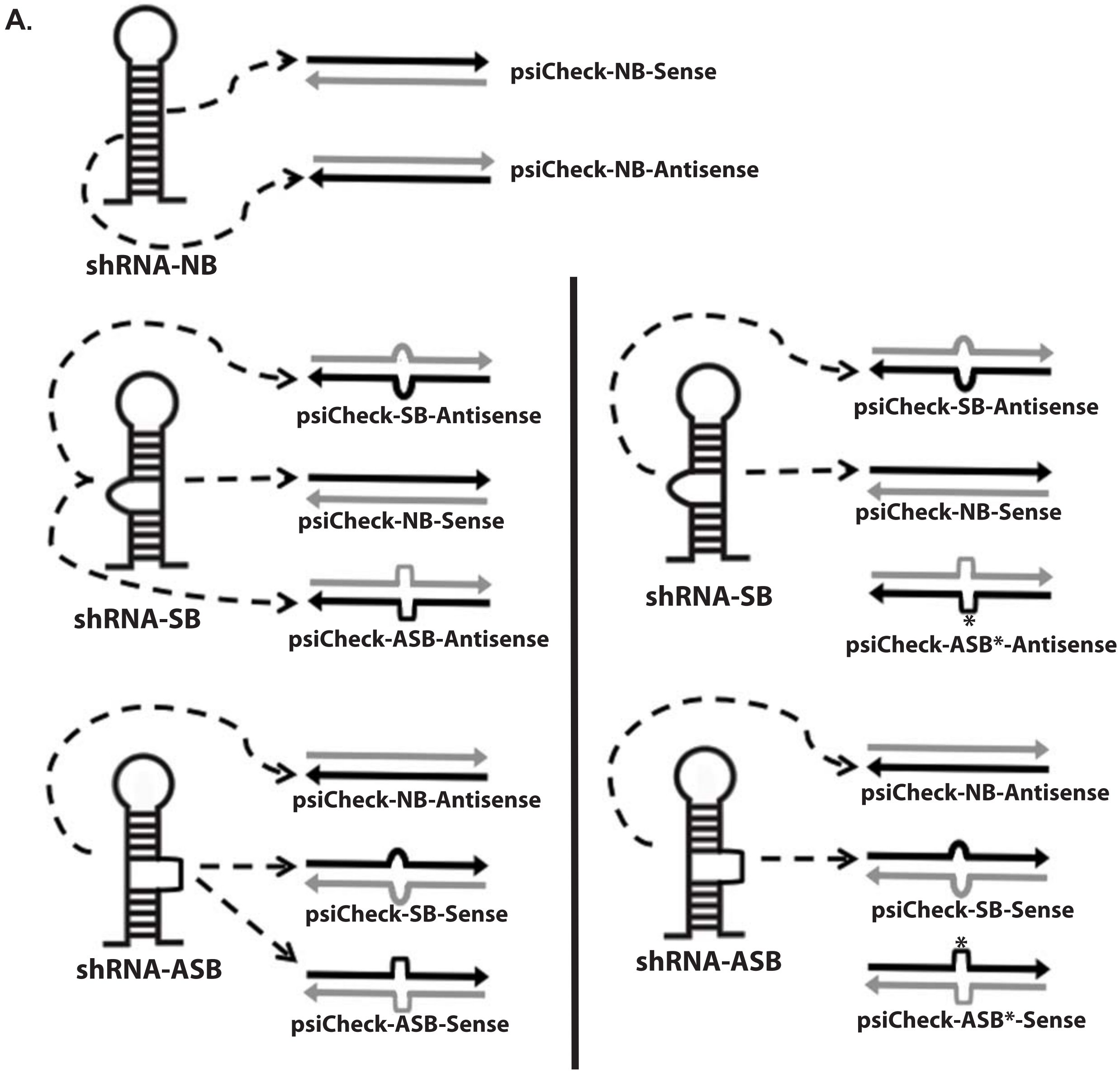
Supplementary Figure 2.



Supplementary Figure 3.



Supplementary Figure 4.



B.

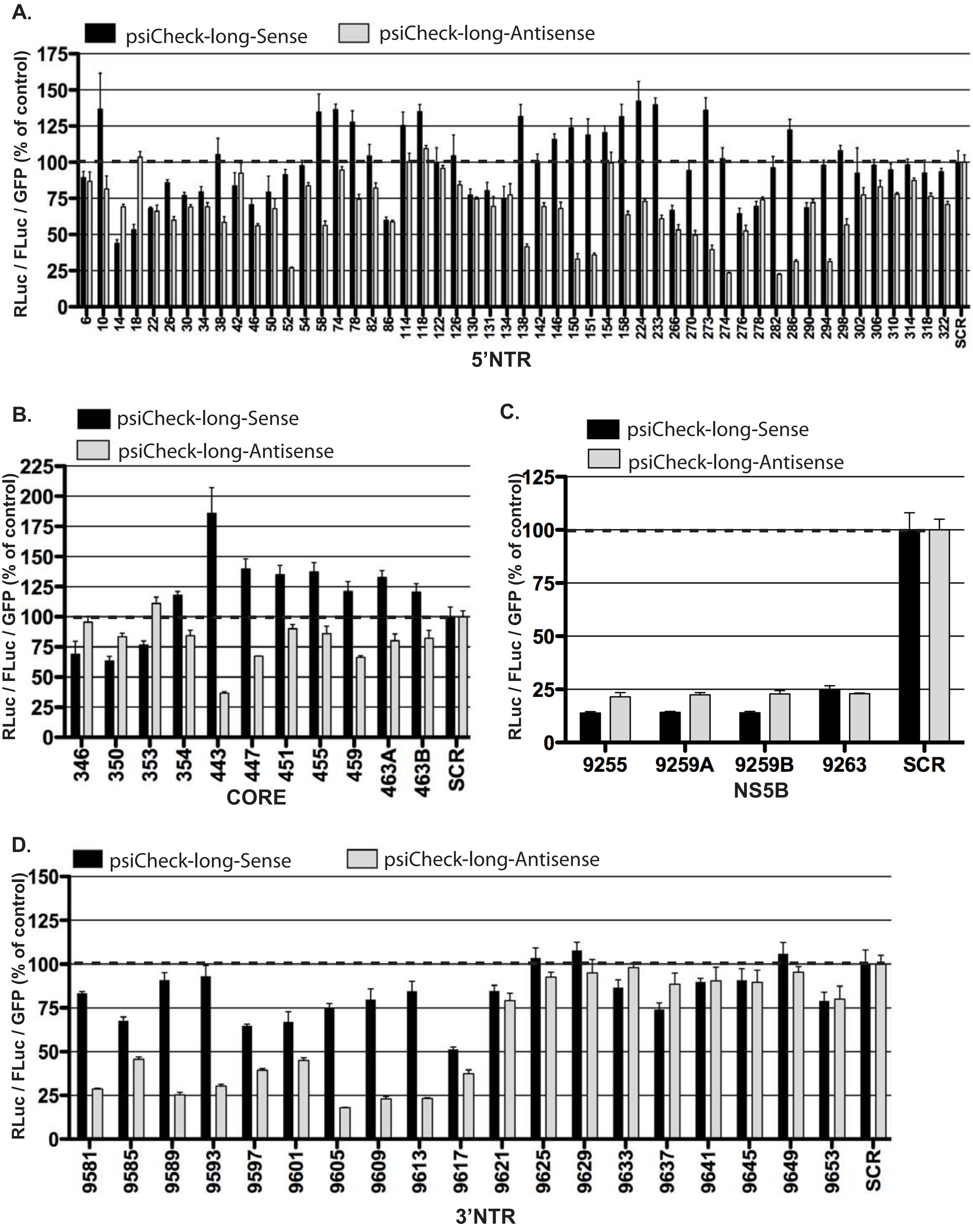
psiCheck-rescue templates

shRNA	Strand	Target	
shRNA-NB	Sense	psiCheck-NB-Antisense	
	Antisense	psiCheck-NB-Sense	
shRNA-SB	Sense	psiCheck-SB-Antisense	psiCheck-ASB-Antisense
	Antisense	psiCheck-NB-Sense	
shRNA-ASB	Sense	psiCheck-NB-Antisense	
	Antisense	psiCheck-SB-Sense	psiCheck-ASB-Sense

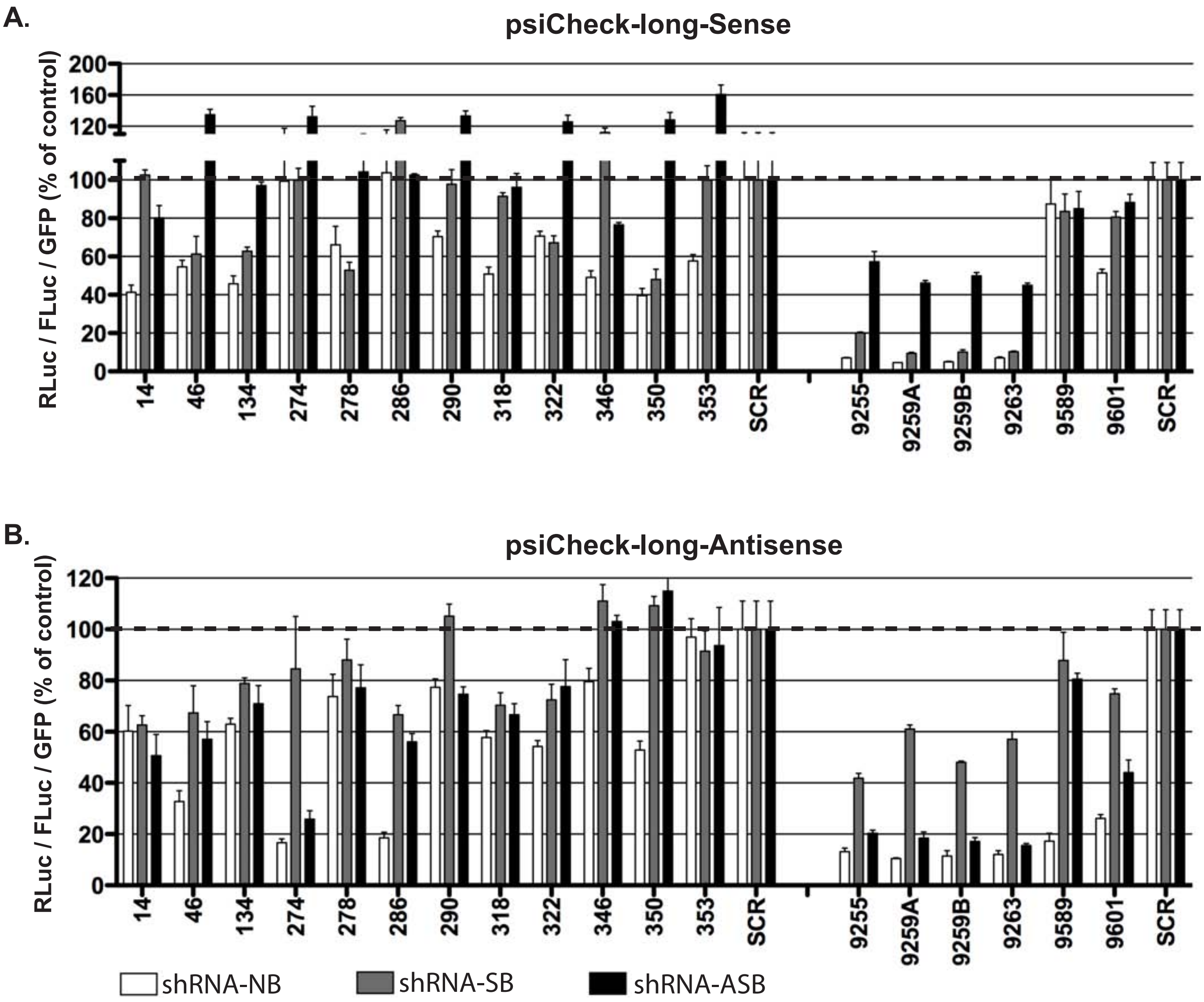
psiCheck-mutated rescue template(*)

shRNA	Strand	Target	
shRNA-NB	Sense	psiCheck-NB-Antisense	
	Antisense	psiCheck-NB-Sense	
shRNA-SB	Sense	psiCheck-SB-Antisense	
	Antisense	psiCheck-NB-Sense	
shRNA-ASB	Sense	psiCheck-NB-Antisense	
	Antisense	psiCheck-SB-Sense	

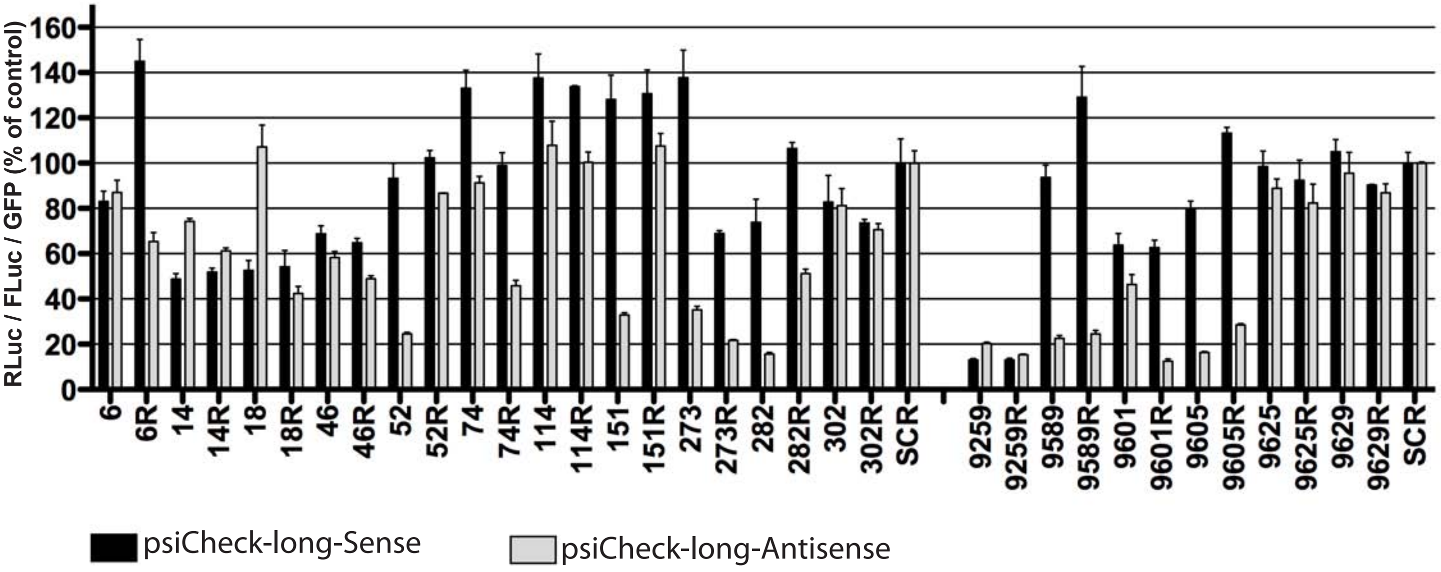
Supplementary Figure 5.



Supplementary Figure 6.



Supplementary Figure 7.



Supplementary Figure 8.

		Sense shRNA strand No Bulge (NB) shRNA	Sense shRNA strand Sense Bulge (SB) shRNA	Antisense shRNA strand Antisense Bulge (ASB) shRNA	Sense shRNA strand Reverse shRNA
5'NTR	**6**	5'-CCCCTAATAGGGGCGACACTCCGC-3'	5'-CCCCTAAATTCGGGCGACACTCCGC-3'	5'-GCGGAGTGTCGCCGGGAATTAGGGG-3'	5'-GCGGAGTGTCGCCCCTATTAGGGG-3'
	10	5'-TAATAGGGGCGACACTCCGCCATG-3'			
	14	5'-AGGGGCGACACTCCGCCATGAATC-3'	5'-AGGGGCGTGGTCTCCGCCATGAATC-3'	5'-GATTCATGGCGGACAACTCGCCCCT-3'	5'-GATTCATGGCGGAGTGTCGCCCCT-3'
	18	5'-GCGACACTCCGCCATGAATCACTC-3'			5'-GAGTGATTCATGGCGGAGTGTCGC-3'
	22	5'-CACTCCGCCATGAATCACTCCCCT-3'			
	26	5'-CCGCCATGAATCACTCCCCTGTGA-3'			
	30	5'-CATGAATCACTCCCCTGTGAGGAA-3'			
	34	5'-AATCACTCCCCTGTGAGGAACTAC-3'			
	38	5'-ACTCCCCTGTGAGGAACTACTGTC-3'			
	42	5'-CCCTGTGAGGAACTACTGTCTTCA-3'			
	46	5'-GTGAGGAACTACTGTCTTCACGCA-3'	5'-GTGAGGAAGAATCTGTCTTCACGCA-3'	5'-TGCGTGAAGACAGATTCTTCCTCAC-3'	5'-TGCGTGAAGACAGTAGTTCCTCAC-3'
	50	5'-GGAACTACTGTCTTCACGCAGAAA-3'			
	52	5'-AACTACTGTCTTCACGCAGAA-3'	5'-AACTACTCAAGTTCACGCAGAA-3'	5'-TTCTGCGTGATCCTCAGTAGTT-3'	5'-TTCTGCGTGAAGACAGTAGTT-3'
	54	5'-CTACTGTCTTCACGCAGAAAGCGT-3'			
	58	5'-TGTCTTCACGCAGAAAGCGTCTAG-3'			
	74	5'-GCGTCTAGCCATGGCGTTAGTATG-3'	5'-GCGCCTAGGGGTTGGCGTTAGTATG-3'	5'-CATACTAACGCCAACCCCTAGGCGC-3'	5'-CATACTAACGCCATGGCTAGACGC-3'
	78	5'-CTAGCCATGGCGTTAGTATGAGTG-3'			
	82	5'-CCATGGCGTTAGTATGAGTGTCGT-3'			
	86	5'-GGCGTTAGTATGAGTGTCGTACAG-3'			
	114	5'-CAGGCCCCCCCTCCCGGGAGAGC-3'			5'-GCTCTCCCGGGAGGGGGGGCCTG-3'
	118	5'-CCCCCCCTCCCGGGAGAGCCATA-3'	5'-CCCCCCCGAAGCCGGGAGAGCCATA-3'	5'-TATGGCTCTCCCGCCCTGGGGGGG-3'	
	122	5'-CCCCTCCCGGGAGAGCCATAGTGG-3'			
	126	5'-TCCCGGGAGAGCCATAGTGGTCTG-3'			
	130	5'-GGGAGAGCCATAGTGGTCTGCGGA-3'			
	131	5'-GGAGAGCCATAGTGGTCTGCGGAA-3'			
	134	5'-GAGCCATAGTGGTCTGCGGAACCG-3'	5'-GAGCCATACAACGTCTGCGGAACCG-3'	5'-CGGTTCCGCAGACGTTGTATGGCTC-3'	
	138	5'-CATAGTGGTCTGCGGAACCGGTGA-3'			
	142	5'-GTGGTCTGCGGAACCGGTGAGTAC-3'			
	146	5'-TCTGCGGAACCGGTGAGTACACCG-3'			
	150	5'-CGGAACCGGTGAGTACACCGGAAT-3'			
	151	5'-GGAACCGGTGAGTACACCGGAA-3'			5'-TTCCGGTGTACTCACCGGTTCC-3'
	154	5'-ACCGGTGAGTACACCGGAATTGCC-3'			
	158	5'-GTGAGTACACCGGAATTGCCGGGA-3'			
	224	5'-ATTTGGGCGTGCCCCCGCAAGAC-3'			
	233	5'-TGCCCCCGCAAGACTGCTAGCCGAGTA-3'			
	266	5'-GGTTGCGAAAGGCCTTGTGGTACT-3'			
	270	5'-GCGAAAGGCCTTGTGGTACTGCCT-3'			
	273	5'-AAAGGCCTTGTGGTACTGCCT-3'			5'-AGGCAGTACCACAAGGCCTTT-3'
	274	5'-AAGGCCTTGTGGTACTGCCTGATA-3'	5'-AAGGCCTTCAACGTACTGCCTGATA-3'	5'-TATCAGGCAGTACGTTGAAGGCCTT-3'	
	276	5'-GGCCTTGTGGTACTGCCTGATA-3'	5'-GGCCTTGTCCCAACTGCCTGATA-3'	5'-TATCAGGCAGTTGGGACAAGGCC-3'	
	278	5'-CCTTGTGGTACTGCCTGATAGGGT-3'	5'-CCTTGTGCAATCTGCCTGATAGGGT-3'	5'-ACCCTATCAGGCACAATCCACAAGG-3'	
	282	5'-GTGGTACTGCCTGATAGGGTGCTT-3'			5'-AAGCACCCTATCAGGCAGTACCAC-3'
	286	5'-TACTGCCTGATAGGGTGCTTGCGA-3'	5'-TACTGCCACCTTAGGGTGCTTGCGA-3'	5'-TCGCAAGCACCCTTAAGAGGCAGTA-3'	
	290	5'-GCCTGATAGGGTGCTTGCGAGTGC-3'	5'-GCCTGATACCCCTGCTTGCGAGTGC-3'	5'-GCACTCGCAAGCAGGGGTATCAGGC-3'	
	294	5'-GATAGGGTGCTTGCGAGTGCCCCG-3'			
	298	5'-GGGTGCTTGCGAGTGCCCCGGGAG-3'			
	302	5'-GCTTGCGAGTGCCCCGGGAGGTCT-3'	5'-GCTTGCGACAACCCCCGGGAGGTCT-3'	5'-AGACCTCCCGGGGGTTGTCGCAAGC-3'	5'-AGACCTCCCGGGGCACTCGCAAGC-3'
	306	5'-GCGAGTGCCCCGGGAGGTCTCGTA-3'			
	310	5'-GTGCCCCGGGAGGTCTCGTAGACC-3'			
	314	5'-CCCGGGAGGTCTCGTAGACCGTGC-3'			
	318	5'-GGAGGTCTCGTAGACCGTGCACCA-3'	5'-GGAGGTCTGCCAAGACCGTGCACCA-3'	5'-TGGTGCACGGTCTTGGCAGACCTCC-3'	
	322	5'-GTCTCGTAGACCGTGCACCATGAG-3'	5'-GTCTCGTACTTGCGTGCACCATGAG-3'	5'-CTCATGGTGCACGCAAGTACGAGAC-3'	
CORE	**346**	5'-CACAAATCCTAAACCTCAAAGAAA-3'	5'-CACAAATGGGAAAACCTCAAAGAAA-3'	5'-TTTCTTTGAGGTTATTCGATTTGTG-3'	
	350	5'-AATCCTAAACCTCAAAGAAAAACC-3'	5'-AATCCTAATGGGTCAAAGAAAAACC-3'	5'-GGTTTTTCTTTGACCCATTAGGATT-3'	
	353	5'-CCTAAACCTCAAAGAAAAACCAA-3'	5'-CCTAAACGAAGAAAGAAAAACCAA-3'	5'-TTGGTTTTTCTTACCTGGTTTAGG-3'	
	354	5'-CTAAACCTCAAAGAAAAACCAAAA-3'			
	443	5'-TACTTGTTGCCGCGCAGGGGCCC-3'			
	447	5'-TGTTGCCGCGCAGGGGCCCCAGGT-3'			
	451	5'-GCCGCGCAGGGGCCCCAGGTTGGG-3'	5'-GCCGCGCACCCCGCCCCAGGTTGGG-3'	5'-CCCAACCTGGGGCGGGGTGCGCGGC-3'	
	455	5'-CGCAGGGGCCCCAGGTTGGGTGTG-3'			
	459	5'-GGGGCCCCAGGTTGGGTGTGCGCG-3'			
	463A	5'-CCCCAGGTTGGGTGTGCGCGCGAC-3'			
	463B	5'-CCCCAGGTTGGGTGTGCGCGCGA-3'			
NS5B	**9255**	5'-TGAAGACCAAGCTCAAACTCACTC-3'	5'-TGAAGACGTTTGCTCAAACTCACTC-3'	5'-GAGTGAGTTTGAGGAAAGGTCTTCA-3'	
	9259A	5'-GACCAAGCTCAAACTCACTCCA-3'	5'-GACCAAGCAGGTAACTCACTCCA-3'	5'-TGGAGTGAGTTACCTGCTTGGTC-3'	5'-TGGAGTGAGTTTGAGCTTGGTC-3'
	9259B	5'-GACCAAGCTCAAACTCACTCCATT-3'	5'-GACCAAGCAGGTAACTCACTCCATT-3'	5'-AATGGAGTGAGTTACCTGCTTGGTC-3'	
	9263	5'-AAGCTCAAACTCACTCCATTGCCG-3'	5'-AAGCTCAATGGACACTCCATTGCCG-3'	5'-CGGCAATGGAGTGACCTTTGAGCTT-3'	
3'NTR	**9581**	5'-GGTGGCTCCATCTTAGCCCTAGTC-3'			
	9585	5'-GCTCCATCTTAGCCCTAGTCACGG-3'			
	9589	5'-CATCTTAGCCCTAGTCACGGCTAG-3'	5'-CATCTTACGGGCTAGTCACGGCTAG-3'	5'-CTAGCCGTGACTACCCCCTAAGATG-3'	5'-CTAGCCGTGACTAGGGCTAAGATG-3'
	9593	5'-TTAGCCCTAGTCACGGCTAGCTGT-3'			
	9597	5'-CCCTAGTCACGGCTAGCTGTGAAA-3'			
	9601	5'-AGTCACGGCTAGCTGTGAAAGGTC-3'	5'-AGTCACGCGGAAGCTGTGAAAGGTC-3'	5'-GACCTTTCACAGCATTCCCGTGACT-3'	5'-GACCTTTCACAGCTAGCCGTGACT-3'
	9605	5'-ACGGCTAGCTGTGAAAGGTCCGTG-3'			5'-CACGGACCTTTCACAGCTAGCCGT-3'
	9609	5'-CTAGCTGTGAAAGGTCCGTGAGCC-3'			
	9613	5'-CTGTGAAAGGTCCGTGAGCCGCAT-3'			
	9617	5'-GAAAGGTCCGTGAGCCGCATGACT-3'			
	9621	5'-GGTCCGTGAGCCGCATGACTGCAG-3'			
	9625	5'-CGTGAGCCGCATGACTGCAGAGAG-3'	5'-CGTGAGCGCCGATGACTGCAGAGAG-3'	5'-CTCTCTGCAGTCAACCGGGCTCACG-3'	5'-CTCTCTGCAGTCATGCGGCTCACG-3'
	9629	5'-AGCCGCATGACTGCAGAGAGTGCC-3'			5'-GGCACTCTCTGCAGTCATGCGGCT-3'
	9633	5'-GCATGACTGCAGAGAGTGCCGTAA-3'	5'-GCATGACTCGGTGAGAGTGCCGTAA-3'	5'-TTACGGCACTCTCACCGAGTCATGC-3'	
	9637	5'-GACTGCAGAGAGTGCCGTAACTGG-3'			
	9641	5'-GCAGAGAGTGCCGTAACTGGTCTC-3'			
	9645	5'-AGAGTGCCGTAACTGGTCTCTCTG-3'			
	9649	5'-TGCCGTAACTGGTCTCTCTGCAGA-3'			
	9653	5'-GTAACTGGTCTCTCTGCAGATCAT-3'			

Supplementary Table 1.

		delta-G	
	HCV target sequence	**Forward**	**Reverse**
	5'NTR-CORE (nt 1- 547 in GenBank: JF343782.1)	-221.8	-225.1
	NS5B-3'NTR (nt 9185-9679 in GenBank: JF343782.1)	-177.6	-168
6	5'-CCCCTAATAGGGGCGACACTCCGC-3'	-8	-8.9
10	5'-TAATAGGGGCGACACTCCGCCATG-3'	-6.9	-6.1
14	5'-AGGGGCGACACTCCGCCATGAATC-3'	-6.9	-6.1
18	5'-GCGACACTCCGCCATGAATCACTC-3'	-2.7	-2.5
22	5'-CACTCCGCCATGAATCACTCCCCT-3'	NA	-1.5
26	5'-CCGCCATGAATCACTCCCCTGTGA-3'	-3.2	-2.9
30	5'-CATGAATCACTCCCCTGTGAGGAA-3'	-3.7	-4.6
34	5'-AATCACTCCCCTGTGAGGAACTAC-3'	-3.7	-4.8
38	5'-ACTCCCCTGTGAGGAACTACTGTC-3'	-1.5	-4.6
42	5'-CCCTGTGAGGAACTACTGTCTTCA-3'	-4.6	-1
46	5'-GTGAGGAACTACTGTCTTCACGCA-3'	-8.8	-2.9
50	5'-GGAACTACTGTCTTCACGCAGAAA-3'	-2.5	-3
52	5'-AACTACTGTCTTCACGCAGAA-3'	-2.5	-3
54	5'-CTACTGTCTTCACGCAGAAAGCGT-3'	-3.6	-4.6
58	5'-TGTCTTCACGCAGAAAGCGTCTAG-3'	-4.1	-6.4
74	5'-GCGTCTAGCCATGGCGTTAGTATG-3'	-4.6	-0.2
78	5'-CTAGCCATGGCGTTAGTATGAGTG-3'	-2.8	-0.2
82	5'-CCATGGCGTTAGTATGAGTGTCGT-3'	-2.4	NA
86	5'-GGCGTTAGTATGAGTGTCGTACAG-3'	-4.5	NA
114	5'-CAGGCCCCCCCTCCCGGGAGAGC-3'	-3.9	-14.7
118	5'-CCCCCCCCTCCCGGGAGAGCCATA-3'	-3.9	-11.9
122	5'-CCCCTCCCGGGAGAGCCATAGTGG-3'	-3.9	-6.4
126	5'-TCCCGGGAGAGCCATAGTGGTCTG-3'	-3.1	-2.5
130	5'-GGGAGAGCCATAGTGGTCTGCGGA-3'	-3.2	-2.5
131	5'-GGAGAGCCATAGTGGTCTGCGGAA-3'	-3.2	-2.5
134	5'-GAGCCATAGTGGTCTGCGGAACCG-3'	-3.4	-3
138	5'-CATAGTGGTCTGCGGAACCGGTGA-3'	-5.1	-3
142	5'-GTGGTCTGCGGAACCGGTGAGTAC-3'	-3.8	-3
146	5'-TCTGCGGAACCGGTGAGTACACCG-3'	-8.8	-7.3
150	5'-CGGAACCGGTGAGTACACCGGAAT-3'	-9.9	-10
151	5'-GGAACCGGTGAGTACACCGGAA-3'	-9.9	-10
154	5'-ACCGGTGAGTACACCGGAATTGCC-3'	-9.9	-10
158	5'-GTGAGTACACCGGAATTGCCGGGA-3'	-6.2	-4.8
224	5'-ATTTGGGCGTGCCCCCGCAAGAC-3'	-3.6	-5.1
233	5'-TGCCCCCGCAAGACTGCTAGCCGAGTA-3'	-1.8	-5.8
266	5'-GGTTGCGAAAGGCCTTGTGGTACT-3'	-3	-0.5
270	5'-GCGAAAGGCCTTGTGGTACTGCCT-3'	-4.1	-3.2
273	5'-AAAGGCCTTGTGGTACTGCCT-3'	-2.9	-3.2
274	5'-AAGGCCTTGTGGTACTGCCTGATA-3'	-3.6	-3.7
276	5'-GGCCTTGTGGTACTGCCTGATA-3'	-2.9	-1.7
278	5'-CCTTGTGGTACTGCCTGATAGGGT-3'	-5.6	-1
282	5'-GTGGTACTGCCTGATAGGGTGCTT-3'	-4.6	-1.4
286	5'-TACTGCCTGATAGGGTGCTTGCGA-3'	-3.5	-1.4
290	5'-GCCTGATAGGGTGCTTGCGAGTGC-3'	-4.2	-1.6
294	5'-GATAGGGTGCTTGCGAGTGCCCCG-3'	-9	-6.9
298	5'-GGGTGCTTGCGAGTGCCCCGGGAG-3'	-8.8	-5.8
302	5'-GCTTGCGAGTGCCCCGGGAGGTCT-3'	-4.1	-2.3
306	5'-GCGAGTGCCCCGGGAGGTCTCGTA-3'	-8.7	-5.7
310	5'-GTGCCCCGGGAGGTCTCGTAGACC-3'	-5.5	-5.3
314	5'-CCCGGGAGGTCTCGTAGACCGTGC-3'	-5.8	-5.5
318	5'-GGAGGTCTCGTAGACCGTGCACCA-3'	-5.8	-5.5
322	5'-GTCTCGTAGACCGTGCACCATGAG-3'	-3.6	-3.6
346	5'-CACAAATCCTAAACCTCAAAGAAA-3'	NA	-0.8
350	5'-AATCCTAAACCTCAAAGAAAAACC-3'	NA	-0.8
353	5'-CCTAAACCTCAAAGAAAAACCAA-3'	NA	NA
354	5'-CTAAACCTCAAAGAAAAACCAAAA-3'	NA	NA
443	5'-TACTTGTTGCCGCGCAGGGGCCC-3'	-3.9	-3.5
447	5'-TGTTGCCGCGCAGGGGCCCCAGGT-3'	-3.9	-5.1
451	5'-GCCGCGCAGGGGCCCCAGGTTGGG-3'	-9.2	-7.9
455	5'-CGCAGGGGCCCCAGGTTGGGTGTG-3'	-5.3	-4.7
459	5'-GGGGCCCCAGGTTGGGTGTGCGCG-3'	-5.1	-4.4
463A	5'-CCCCAGGTTGGGTGTGCGCGCGAC-3'	-4.3	-4.4
463B	5'-CCCCAGGTTGGGTGTGCGCGCGA-3'	-3.8	-4.4
9255	5'-TGAAGACCAAGCTCAAACTCACTC-3'	NA	-4.5
9259A	5'-GACCAAGCTCAAACTCACTCCA-3'	NA	-3.6
9259B	5'-GACCAAGCTCAAACTCACTCCATT-3'	NA	-3.6
9263	5'-AAGCTCAAACTCACTCCATTGCCG-3'	NA	-1.2
9581	5'-GGTGGCTCCATCTTAGCCCTAGTC-3'	-4.6	-5
9585	5'-GCTCCATCTTAGCCCTAGTCACGG-3'	-1.5	-2.9
9589	5'-CATCTTAGCCCTAGTCACGGCTAG-3'	-6.2	-7.5
9593	5'-TTAGCCCTAGTCACGGCTAGCTGT-3'	-6.3	-7.5
9597	5'-CCCTAGTCACGGCTAGCTGTGAAA-3'	-6	-2.9
9601	5'-AGTCACGGCTAGCTGTGAAAGGTC-3'	-6	-2.9
9605	5'-ACGGCTAGCTGTGAAAGGTCCGTG-3'	-4.5	-2.1
9609	5'-CTAGCTGTGAAAGGTCCGTGAGCC-3'	-1.1	-1.7
9613	5'-CTGTGAAAGGTCCGTGAGCCGCAT-3'	-2.3	-0.6
9617	5'-GAAAGGTCCGTGAGCCGCATGACT-3'	-4.5	-1.6
9621	5'-GGTCCGTGAGCCGCATGACTGCAG-3'	-4.9	-5
9625	5'-CGTGAGCCGCATGACTGCAGAGAG-3'	-1.9	-5.1
9629	5'-AGCCGCATGACTGCAGAGAGTGCC-3'	-4.8	-5.1
9633	5'-GCATGACTGCAGAGAGTGCCGTAA-3'	-4.5	-1.7
9637	5'-GACTGCAGAGAGTGCCGTAACTGG-3'	-3.2	-1.7
9641	5'-GCAGAGAGTGCCGTAACTGGTCTC-3'	-4.3	-3.8
9645	5'-AGAGTGCCGTAACTGGTCTCTCTG-3'	-6.5	-3.6
9649	5'-TGCCGTAACTGGTCTCTCTGCAGA-3'	-2.9	-1.7
9653	5'-GTAACTGGTCTCTCTGCAGATCAT-3'	-4.5	-1.8

Supplementary Table 2