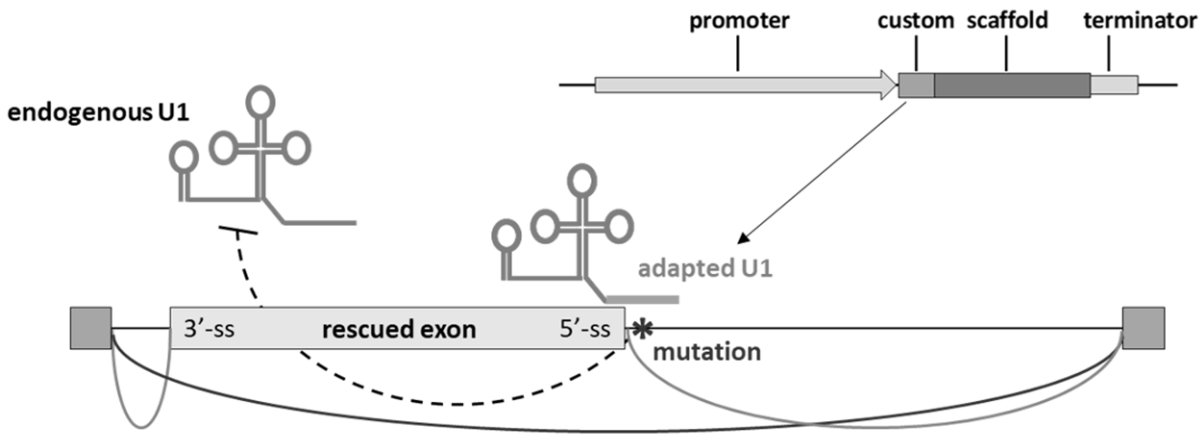
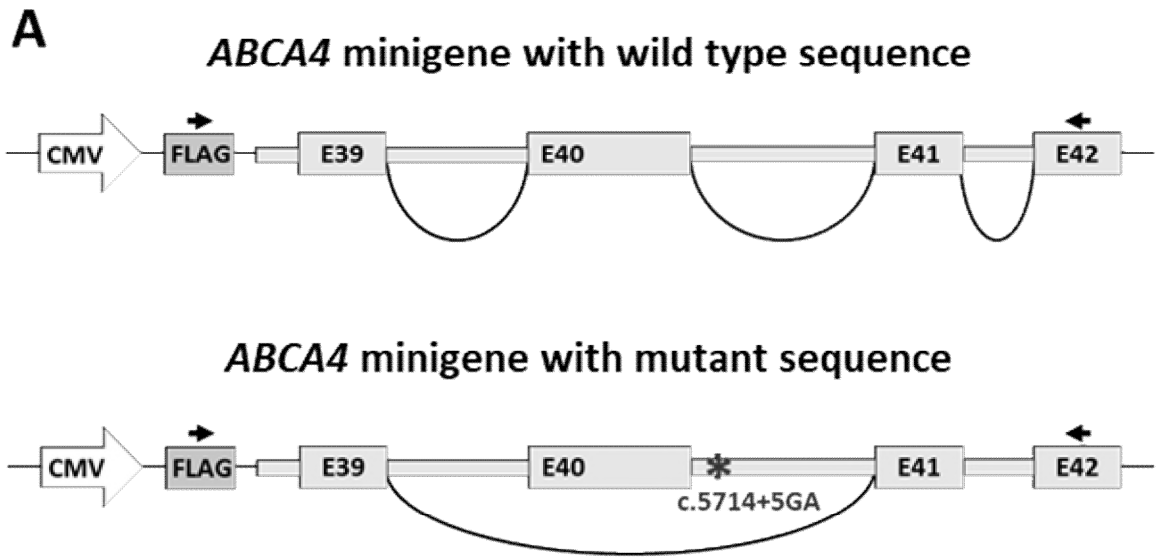

promoter
custom
scaffold
terminator
endogenous U1
adapted U1
3'-ss
rescued exon
5'-ss
mutation

Figure 1


A
ABCA4 minigene with wild type sequence
CMV
FLAG
E39
E40
E41
E42
ABCA4 minigene with mutant sequence
CMV
FLAG
E39
E40
E41
E42
c.5714+5GA

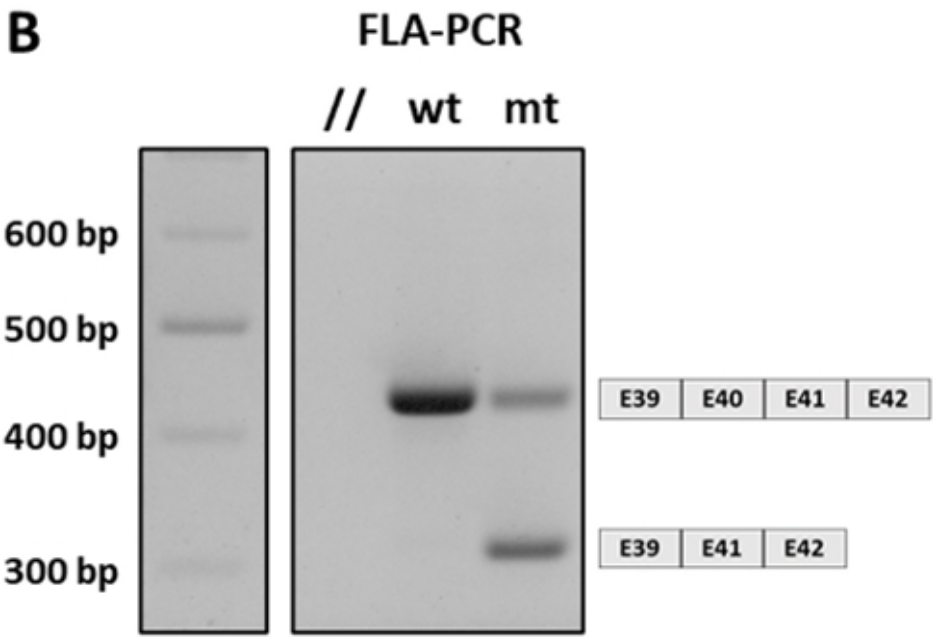

B
FLA-PCR
// wt mt
600 bp
500 bp
400 bp
300 bp
E39 E40 E41 E42
E39 E41 E42

Figure 2

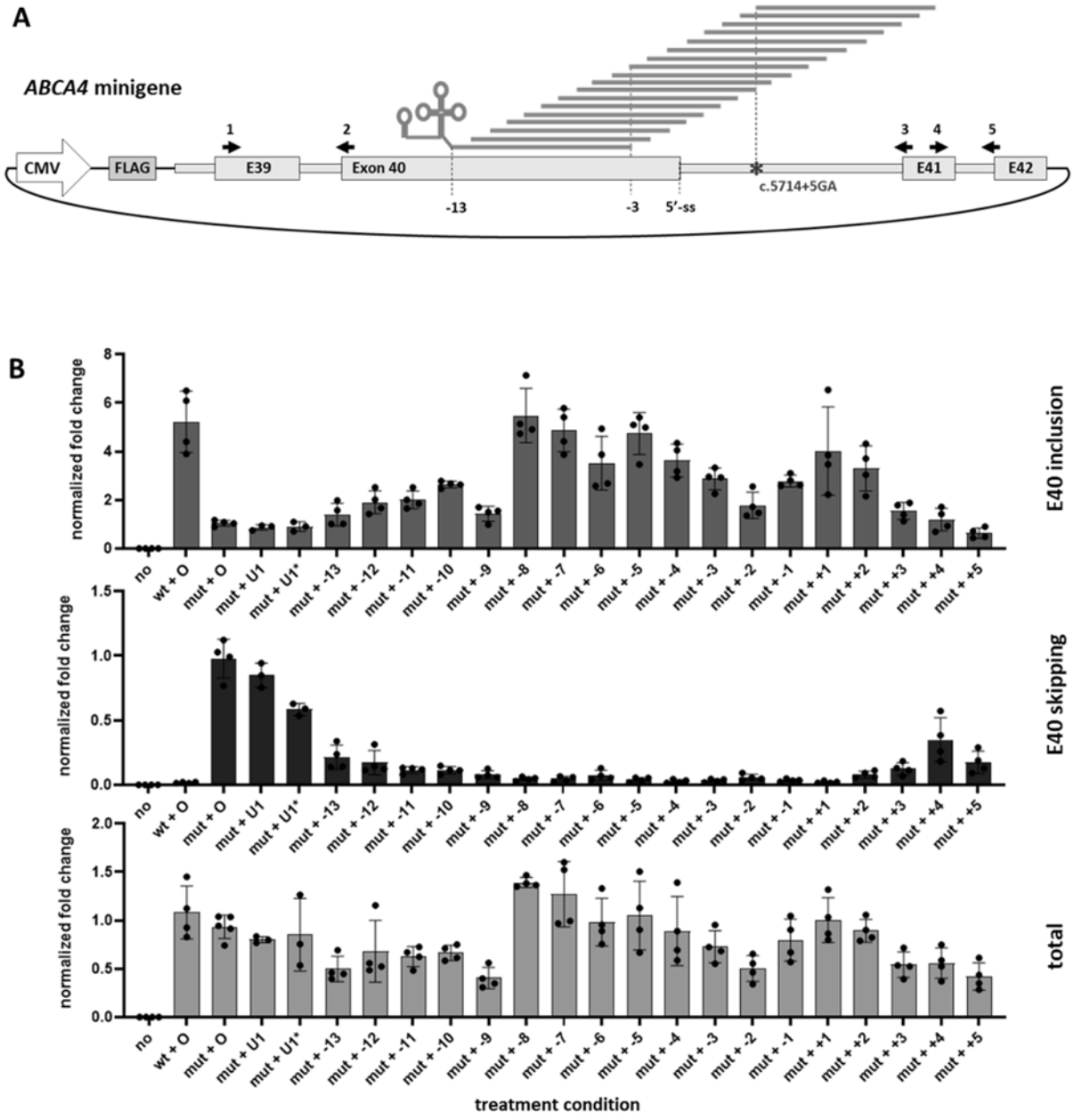

A
ABCA4 minigene
CMV
FLAG
E39
Exon 40
E41
E42
1
2
3
4
5
-13
-3
5'-ss
c.5714+5GA
B
normalized fold change
E40 inclusion
E40 skipping
total
no
wt + O
mut + O
mut + U1
mut + U1*
mut + -13
mut + -12
mut + -11
mut + -10
mut + -9
mut + -8
mut + -7
mut + -6
mut + -5
mut + -4
mut + -3
mut + -2
mut + -1
mut + +1
mut + +2
mut + +3
mut + +4
mut + +5
treatment condition

Figure 3

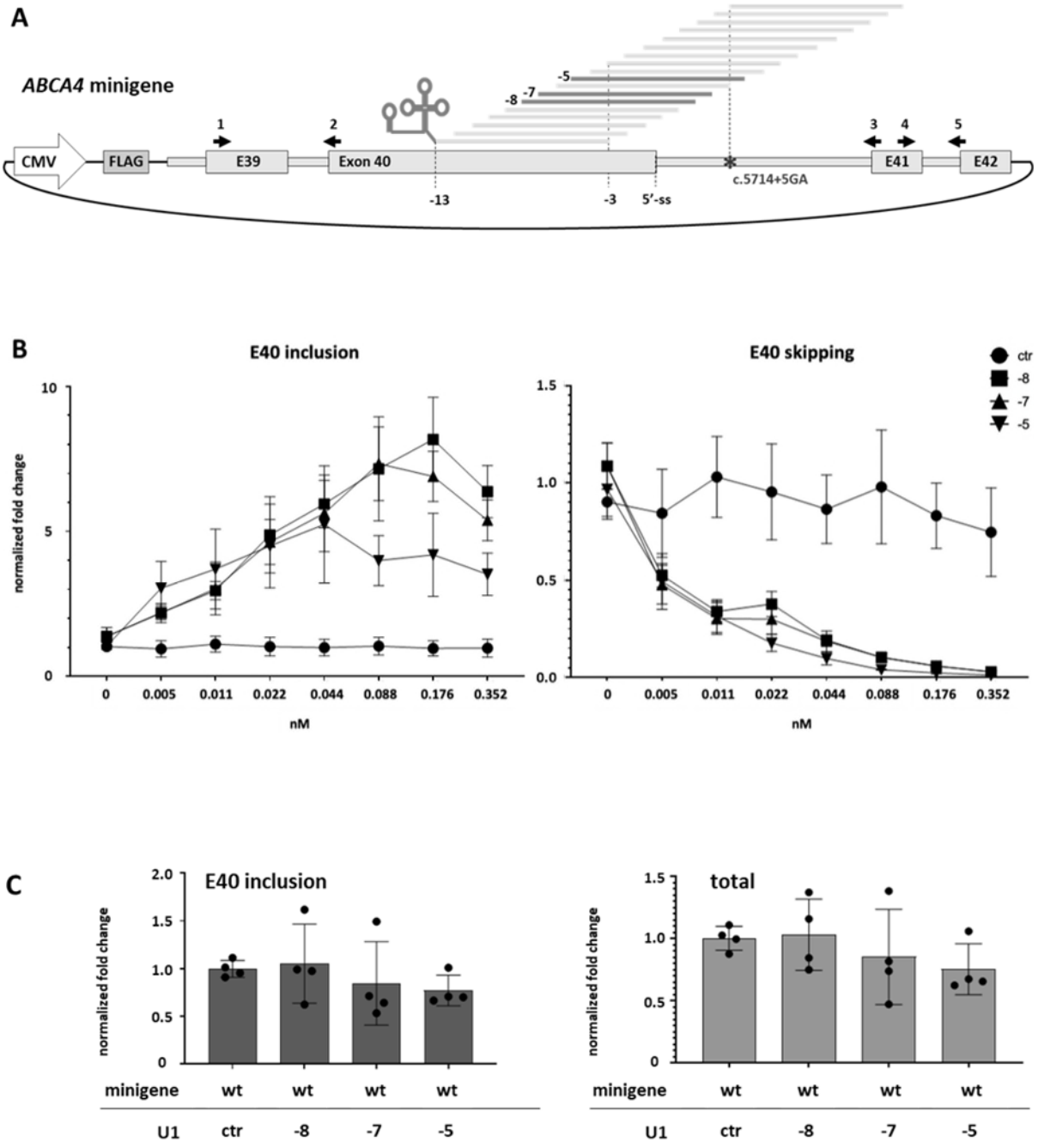

A
ABCA4 minigene
CMV
FLAG
E39
Exon 40
E41
E42
1
2
3
4
5
-5
-7
-8
-13
-3
5'-ss
c.5714+5GA
B
E40 inclusion
E40 skipping
ctr
-8
-7
-5
normalized fold change
nM
0
0.005
0.011
0.022
0.044
0.088
0.176
0.352
C
E40 inclusion
total
normalized fold change
minigene
wt
U1
ctr
-8
-7
-5

Figure 4

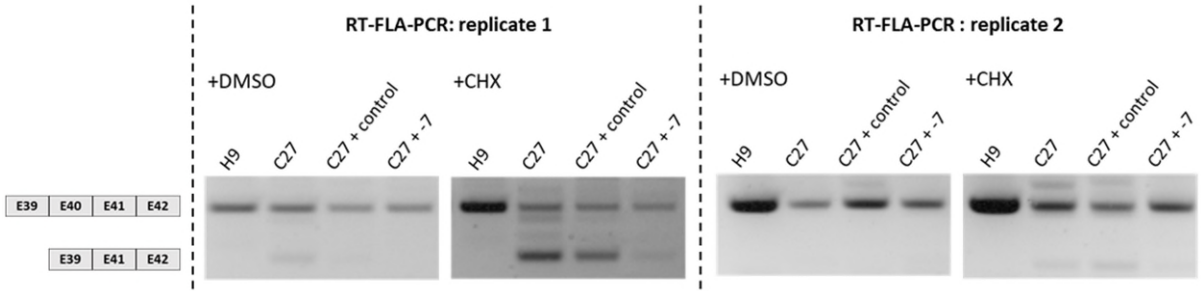

RT-FLA-PCR: replicate 1
RT-FLA-PCR : replicate 2
+DMSO
+CHX
+DMSO
+CHX
H9
C27
C27 + control
C27 + -7
H9
C27
C27 + control
C27 + -7
H9
C27
C27 + control
C27 + -7
H9
C27
C27 + control
C27 + -7
E39
E40
E41
E42
E39
E41
E42

Figure 5

A

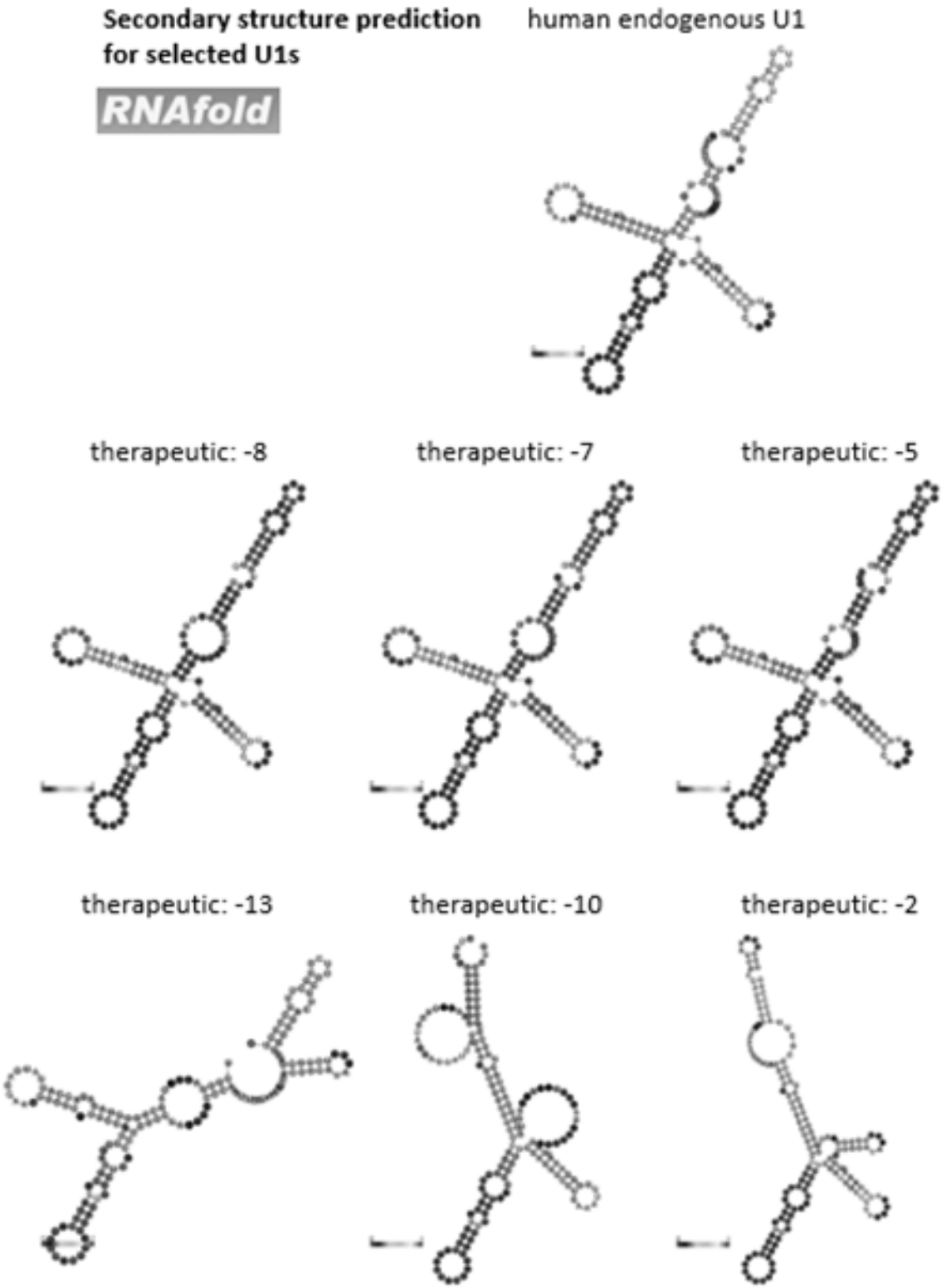

Secondary structure prediction for selected U1s
RNAfold
human endogenous U1
therapeutic: -8
therapeutic: -7
therapeutic: -5
therapeutic: -13
therapeutic: -10
therapeutic: -2

Figure 6

B

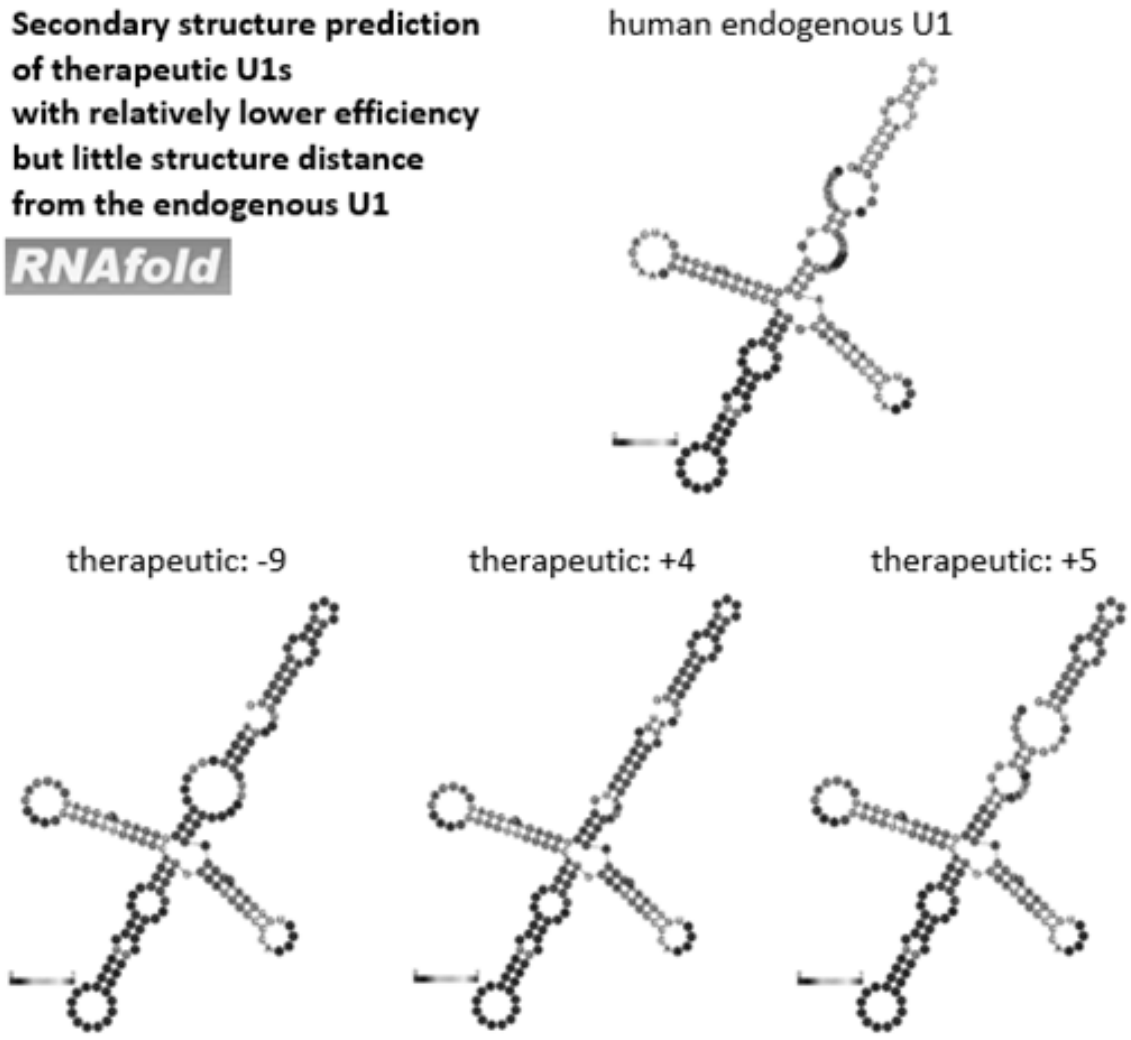

Secondary structure prediction
of therapeutic U1s
with relatively lower efficiency
but little structure distance
from the endogenous U1
RNAfold
human endogenous U1
therapeutic: -9
therapeutic: +4
therapeutic: +5

Figure 6

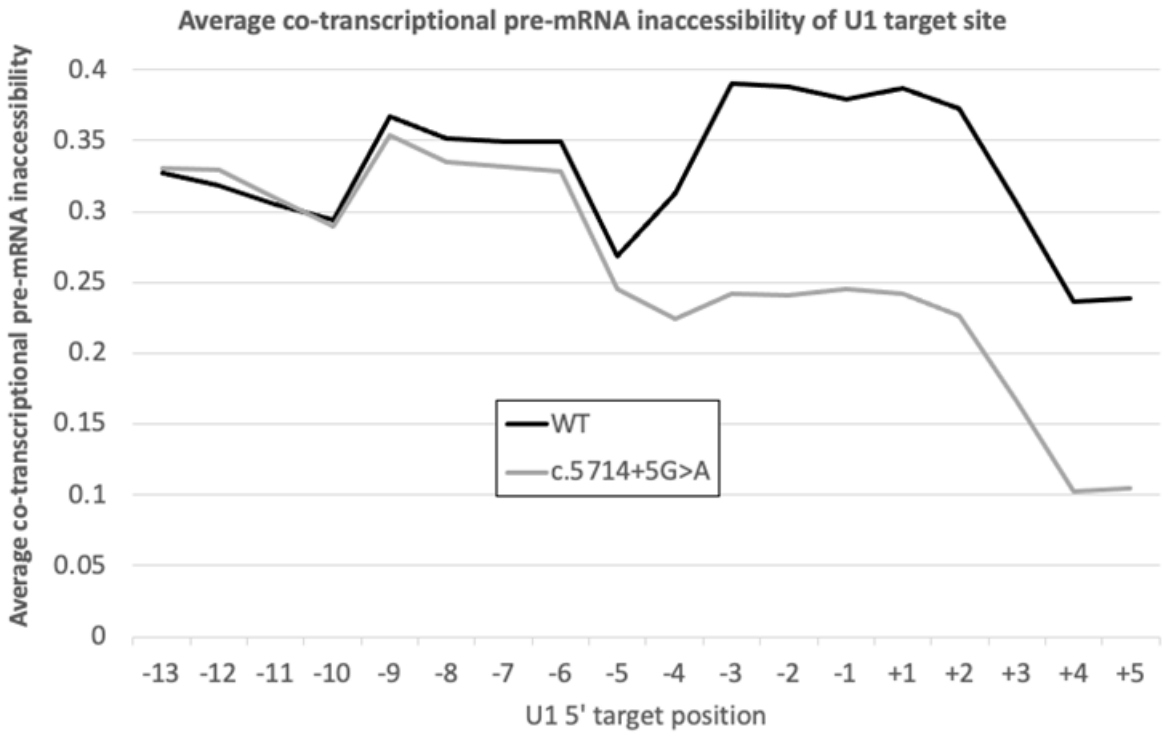

Average co-transcriptional pre-mRNA inaccessibility of U1 target site
Average co-transcriptional pre-mRNA inaccessibility
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
WT
c.5714+5G>A
-13 -12 -11 -10 -9 -8 -7 -6 -5 -4 -3 -2 -1 +1 +2 +3 +4 +5
U1 5' target position

Figure 7