Efficient and specific knock-down of small non-coding RNAs in mammalian cells and in mice

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Supplementary Information

Antisense oligonucleotide probes for human RNAs

U1 snRNA: 5'-ctcccctgccaggtaagtat-3'.
U2 snRNA: XL094,5'-tcggatagaggacgtatcag-3'; XL112, 5'-tcagatattaaactgataagaacag-3', for both human and mouse U2 snRNA.
U4 snRNA: XL019, 5'- attgccagtgccgactatat-3'.
U6 snRNA: XL021, 5'-tggaacgcttcacgaatttgcg-3'.
U3 snoRNA: XL016, 5'-accactcagaccgcgttctctcc-3'.
U16 snoRNA: XL011, 5'-ttgctcagtaagaattttcg-3'; XL104, 5'- gacgcaaattacgacatcat-3', for both human and mouse U16 snoRNA.
U17 snoRNA: XL009, 5'-tgtttcctgcatggtttgtctc-3'.
U18 snoRNA: XL069, 5'-tgtttcagaaacacggacc-3'.
U20 snoRNA: XL100, 5'- ctggatcagaacttgactatc-3'.
U23 snoRNA: XL099, 5'-gaatgtctcacaatacagctaaat-3'; XL051, 5'-gaatccataaactgccactg-3'.
U50 snoRNA: XL103, 5'-ggttcgggataagatcatcaca-3'; XL087, 5'-atctcagaagccagatccgt-3', for both human and mouse U50 snoRNA.
U50B snoRNA: XL098, 5'-cgtacttatttttcttcaggtta-3'; XL111, 5'-gataggtttcatcattgatta-3'.
U80 snoRNA: XL089,5'-gatacatcagataggagcgaa-3'; XL135, 5'-ctgaactatgttatcatcattgt-3'.
U81 snoRNA: XL088, 5'-cagaatatcagatattttattg-3'.
ACA45 snoRNA: XL014, 5'-gctgttggtagataagtaggtct-3'; XL040, 5'-agtcagcttgatttcaaggac-3'.
RPL4 mRNA: XL067, 5'-cagggcagaacagatggcgtatc-3'.
18S rRNA: XL066, 5'-gttatttttcgtcactacctcc-3', used for primer extension to map the modification site targeted by U16 snoRNA. XL096, 5'-gctactggcaggatcaacca-3', used for northern hybridization. XL101, 5'-aaggaaccataactgatttaat-3', used for primer extension to map the U23 RNA target site. XL086, 5'-UmUm dTdGdCdGCmGmCmCmUmGmCmUmGm CmCmUm-3', used for Rnase H cleavage to detect the methylation at site A484. Nm, 2'-O-methylated ribonucleitides. dN, deoxyribonucleotides.
28S rRNA: XL090, 5'-accgacccagcccttagagc-3', used for primer extension to map the modification site targeted by U50 snoRNA. XL091, 5'-tcctctggcttcgccctgcc-3', used for primer extension to map the U80 target site. XL092, 5'-tcttaacggtttcacgccct-3', used for primer extension to map the U81 target site. XL102, 5'-gggaaccagctactagatgg-3', used for primer extension to map the U80 snoRNA target site.

ASO141923: 5'-CmCmUmUmCmcctgaaggttCmCmUmCmCm-3'. Control ASO used for mouse study. Lower case indicates deoxynucleotides. m, 2'MOE.

Supplementary figure legends

Figure S1. Antisense oligonucleotides targeting U16 snoRNA. **(A)** Target positions of ASOs in U16 snoRNA. The snoRNA region (upper case) and flanking sequences (lower case) are shown. Potential C (UGAUGU) and D (CUGA) boxes are indicated. The sequence involved in guiding modification is underlined. The names of the ASOs are given. The active ASOs detected in Fig. 1 that exhibited highest potency are marked with thick lines and the names are in bold letters. **(B)** Composition of the 5-10-5 RNA-DNA chimeric ASOs. All nucleotides are linked by phosphorothioate backbone (PS). R and d, ribonucleotide and deoxyribonucleotide, respectively. m, 2'-O-methyloxylethyl modification (MOE).

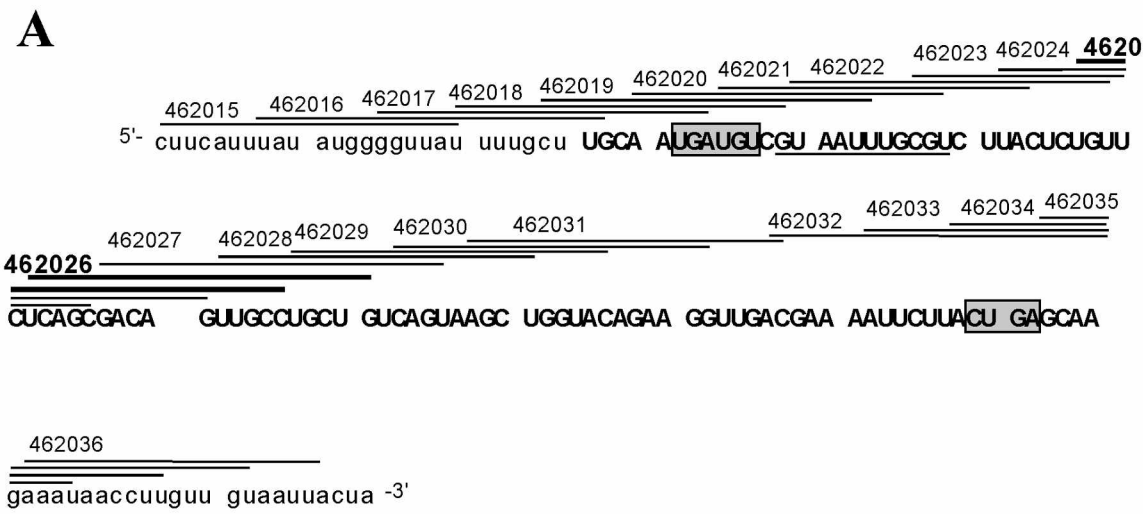
Figure S2. Screening of active ASOs for U80 and U81 snoRNAs. **(A)** The sequence of U80 snoRNA. The C and D motifs are boxed, and the sequence involved in guiding rRNA modification is underlined. The targeted positions of ASOs are indicated. The active ASO (in bold) is marked by a thick line. **(B)** Hela cells were treated for 48 hours with 50 nM of different ASOs for U80 snoRNA. Total RNA was prepared and subjected to northern hybridization using probes specific to U80 or U81 snoRNA. U16 RNA was used as a loading control. The active ASO is boxed. **(C)** The U81 snoRNA ASOs, as in panel A. **(D)** Hela cells were treated with different ASOs for U81 snoRNA, and U81, U80, and U16 snoRNAs were detected by northern hybridization, as in panel B. The active ASOs are boxed.

Figure S3. ASO-mediated snoRNA depletion can be highly specific. **(A)** ASOs targeting U50 snoRNA. The C and D motifs are boxed. The sequences involved in guiding rRNA modification are underlined. The active ASOs are in bold and indicated with thick lines. **(B)** Sequence comparison of U50 and U50B snoRNA was performed using LALIGN server (http://www.ch.embnet.org/software/LALIGN_form.html). The snoRNAs are numbered separately.

Figure S4. **Screening of lead ASOs for U23 H/ACA snoRNA**. **(A)** Positions of ASOs in U23 snoRNA. The H (AnAnnA) and ACA motifs are shown in gray boxes. The active ASOs identified in panel b are indicated by thick lines and the names are in bold. **(B)** Northern hybridization of U23 snoRNA in cells treated for 48 hours with 50 nM of different ASOs, as indicated above lanes. The active ASOs are boxed. 5.8S and 5S rRNAs were detected by ethidium bromide staining. U20 snoRNA encoded in the same host gene as U23 was also determined by northern hybridization. **(C)** The positions of the two active ASOs in the predicted secondary structure of U23 snoRNA are indicated by lines. The snoRNA sequences involved in guiding modification are shown in bold.

Figure S5. Screening of ASOs targeting ACA45 scaRNA. **(A)** Targeted positions of ASOs in ACA45 snoRNA. The H and ACA motifs are boxed. The active ASOs are indicated with thick lines and named with bold letters. **(B)** Northern hybridization of ACA45 RNA in cells treated for 48 hours with different ASOs, in a final concentration of 50 nM. The active ASOs are boxed. U16 and U18 snoRNAs were used as loading controls. **(C)** The secondary structure of ACA45 RNA was predicted using program MFold. The H and ACA motifs are boxed. The targeted positions of active ASOs are marked by lines. The sequences predicted to guide U2 snRNA modification are shown in bold.

Fig-S1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462022
462021
462020
462019
462018
462017
462016
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462035
462034
462033
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462031
462030
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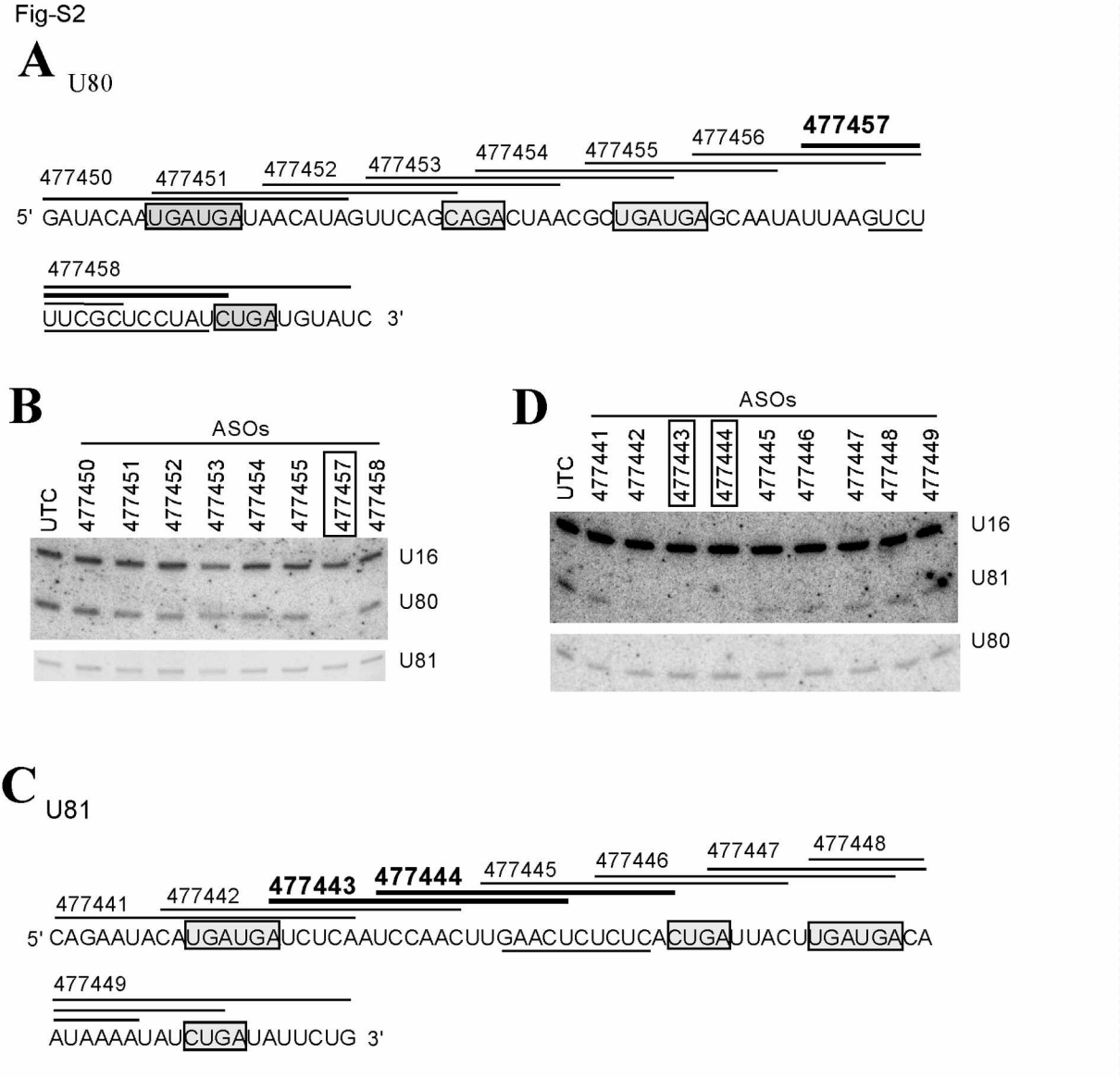
CUCAGCGACA GUUGCCUGCU GUCAGUAAGC UGGUACAGAA GGUUGACGAA AAUUCUUACU GAGCAA
462036
gaaauaaccuuguu guaauuacua -3'
B
5' RmRmRmRmRmddddddddddRmRmRmRmRm 3'

Fig-S2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477451
477452
477453
477454
477455
477456
477457
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477458
UUCGCUCCUAUCUGAUGUAUC 3'
B
ASOs
UTC
477450
477451
477452
477453
477454
477455
477457
477458
U16
U80
U81
D
ASOs
UTC
477441
477442
477443
477444
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477447
477448
477449
U16
U81
U80
C
U81
477441
477442
477443
477444
477445
477446
477447
477448
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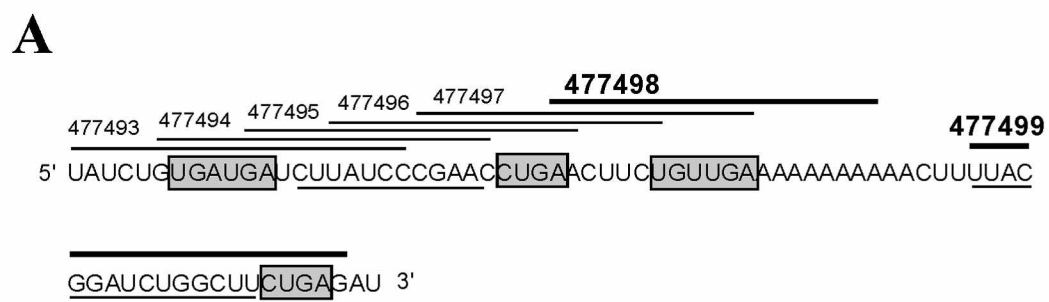
AUAAAAUAUCUGAUAUUCUG 3'

Fig S3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477493
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477495
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477499
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GGAUCUGGCUUCUGAGAU 3'
B
10 20 30 40 50
U50 TA-TCTGTGATGAT-CTTATCCCGAACCTGA--ACTTCTGTTGAAAAAAAAAAACTTTTA
U50B TAATCAATGATGAAACCTATCCCGAAGCTGATAAC--C---TGAAGAAAAATAAGT---A
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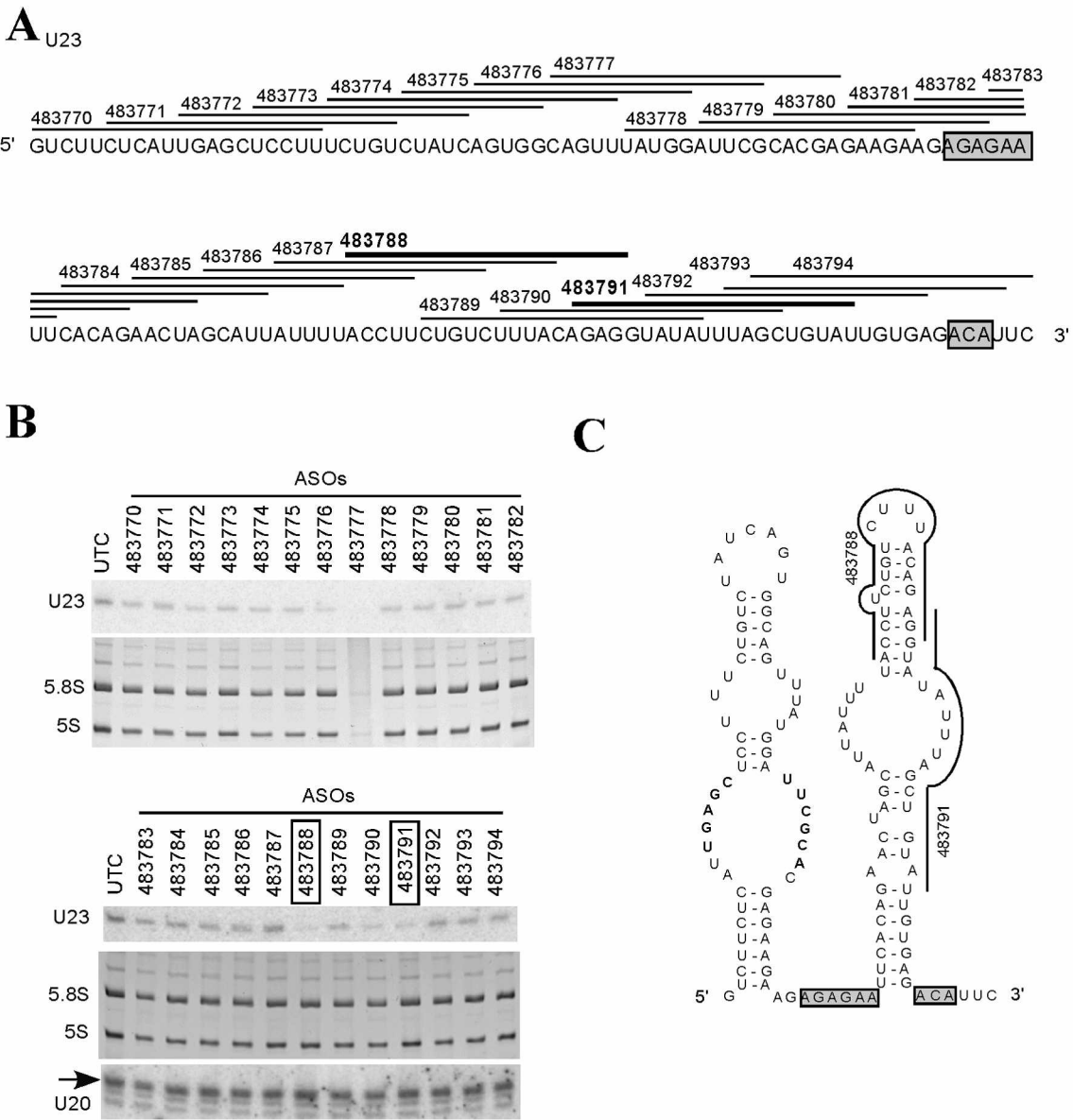
60 70
U50 CGGAT-CTGGCTTCTGAGAT
U50B CGGATTC-GGCTTCTGAGAT
60 70

Fig-S4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483782
483783
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B
ASOs
UTC
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483777
483778
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483780
483781
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U23
5.8S
5S
ASOs
UTC
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483786
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483791
483792
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5S
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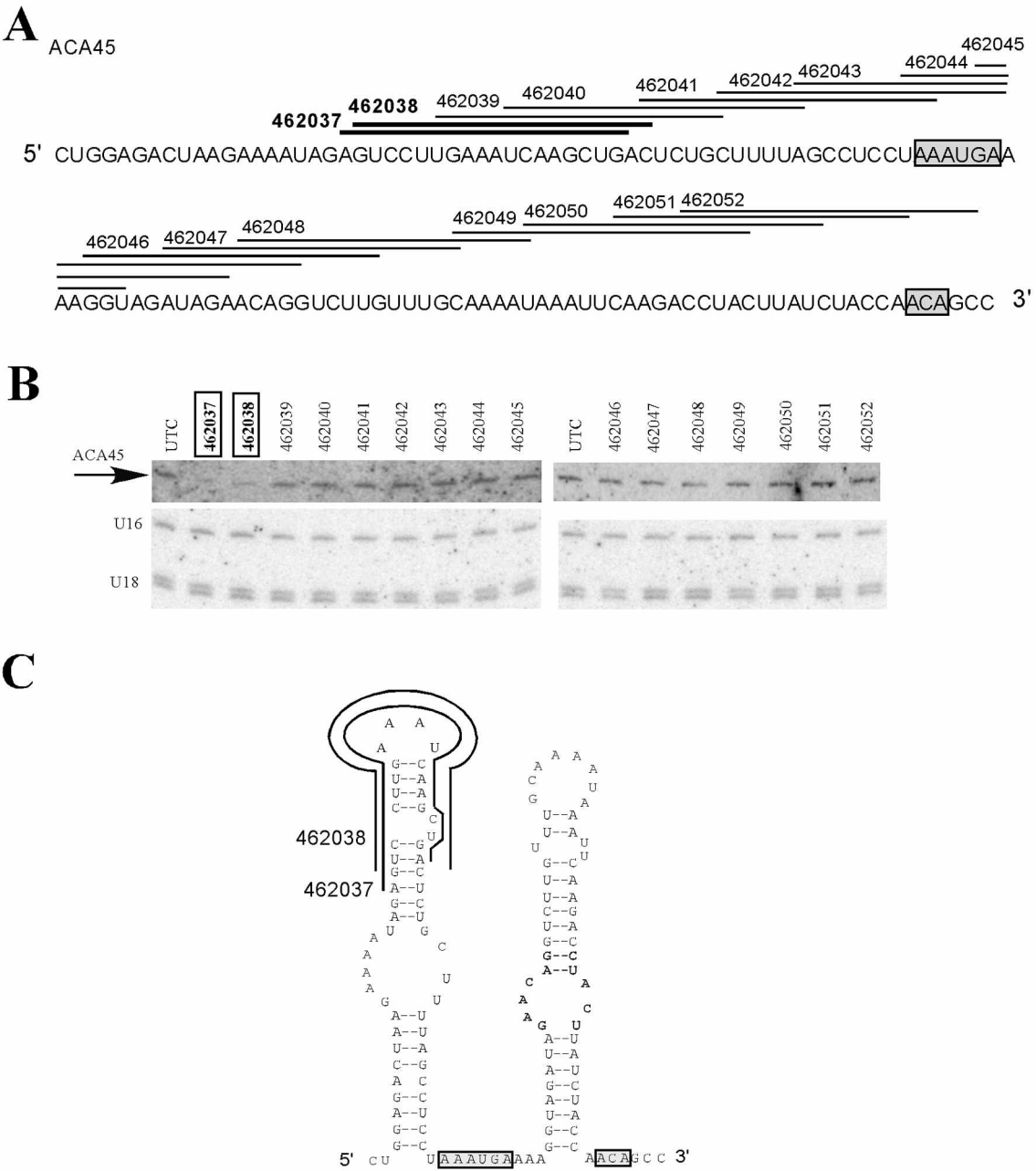
Fig-S5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B
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