

HuR (ELAVL1) Stabilizes SOX9 mRNA and Promotes Migration and Invasion in Breast Cancer Cells

Supplementary Datasets

Dataset S1. RNA seq analysis of the gene expression profile of shHuR-1 silenced cells versus shSCR control cells.

Dataset S2. HuR candidate targets selection.

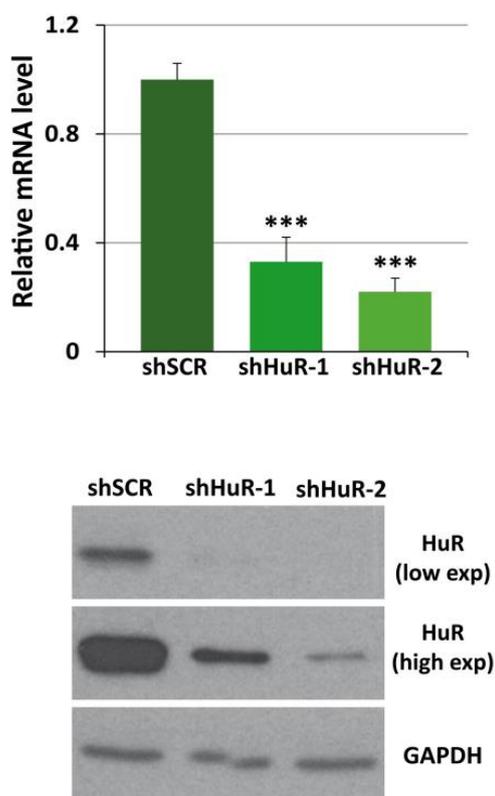
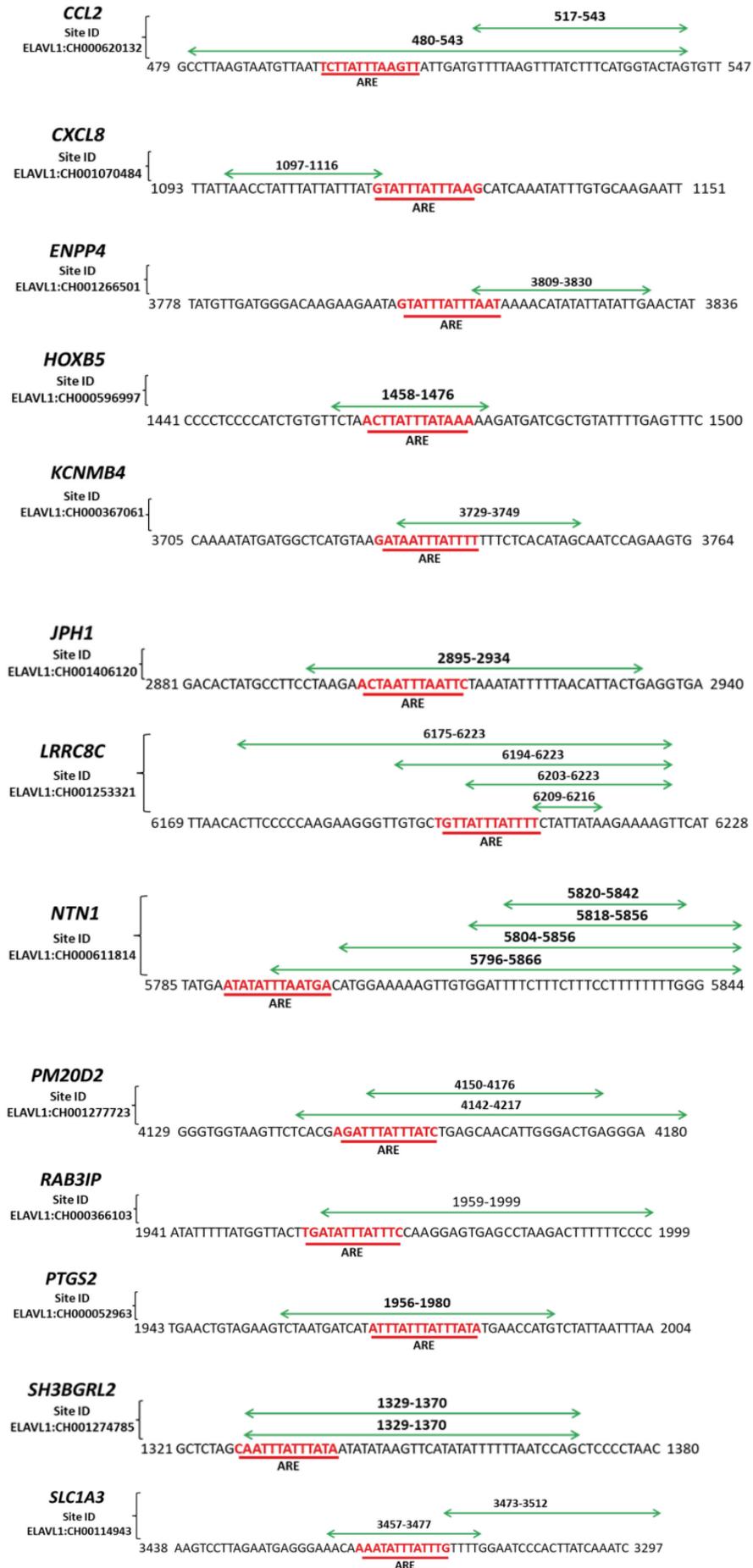


Figure S1. Silencing of HuR in Hs578T cell line. A) HuR silencing using two specific shRNAs (shHuR-1 and shHuR-2) and a scramble control (shSCR) sequence was confirmed by qPCR. Data represent the mean \pm SEM of three independent experiments with triplicate assays. The p-value was calculated using a two-sided unpaired Student's t-test. (***) $p < 0.001$. B) Western blot analysis of HuR levels in control (shSCR) or HuR-silenced (shHuR-1, shHuR-2) cells. GAPDH was used as a loading control.



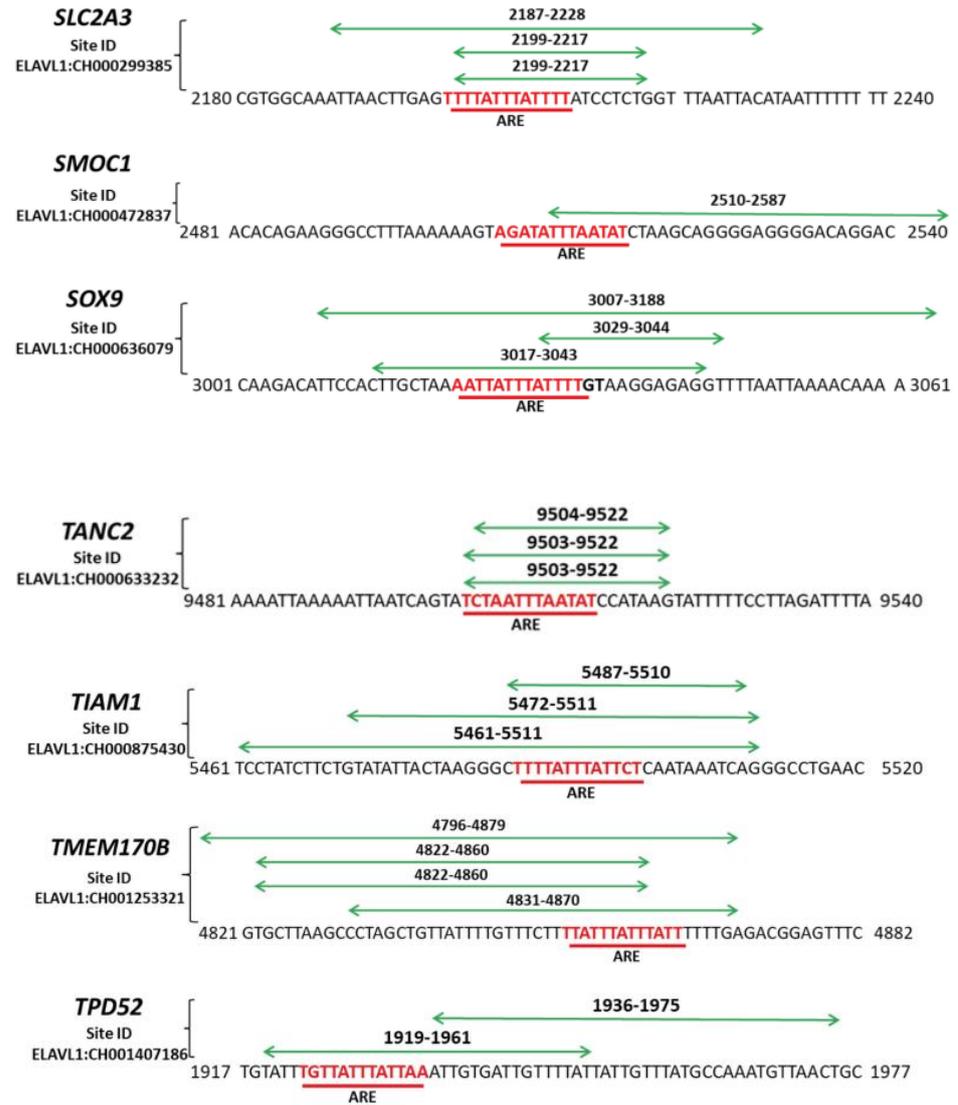


Figure S2. Location of the ARE and HuR binding sites in the 3'UTR of CCL2, CXCL8, ENNP4, HOXB5, KCNMB4, JPH1, LRR8C, NTN1, PM20D2, RAB3IP, PTGS2, SH3BGL2, SLC1A3, SLC2A3, SMOC1, SOX9, TANC2, TIAM1, TMEM170B, and TPD52 genes. ARE sequence in the 3' UTR is highlighted in red. Green arrows denote the coordinate of the HuR binding sites as obtained from ENCORI Site ID indicated on the left.

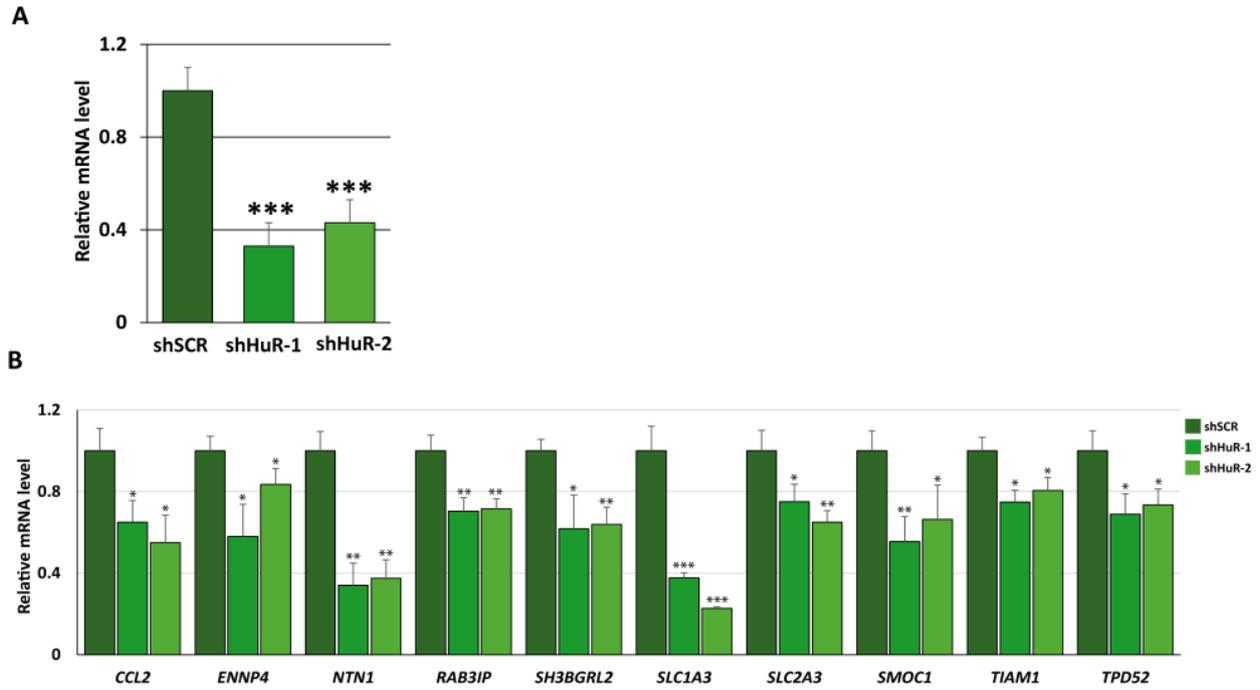


Figure S3. Validation of downregulation of putative targets in HuR-silenced BT549 cells by quantitative RT-PCR. A) Silencing of HuR in BT549 cells using two specific shRNAs (shHuR-1 and shHuR-2) and a scramble control (shSCR) was confirmed by qPCR. B) Validation of downregulation of putative targets in BT549 HuR-silenced cells by quantitative RT-PCR. Data represent the mean \pm SEM of three independent experiments with triplicate assays. The p-value was calculated using a two-sided unpaired Student's t-test. (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

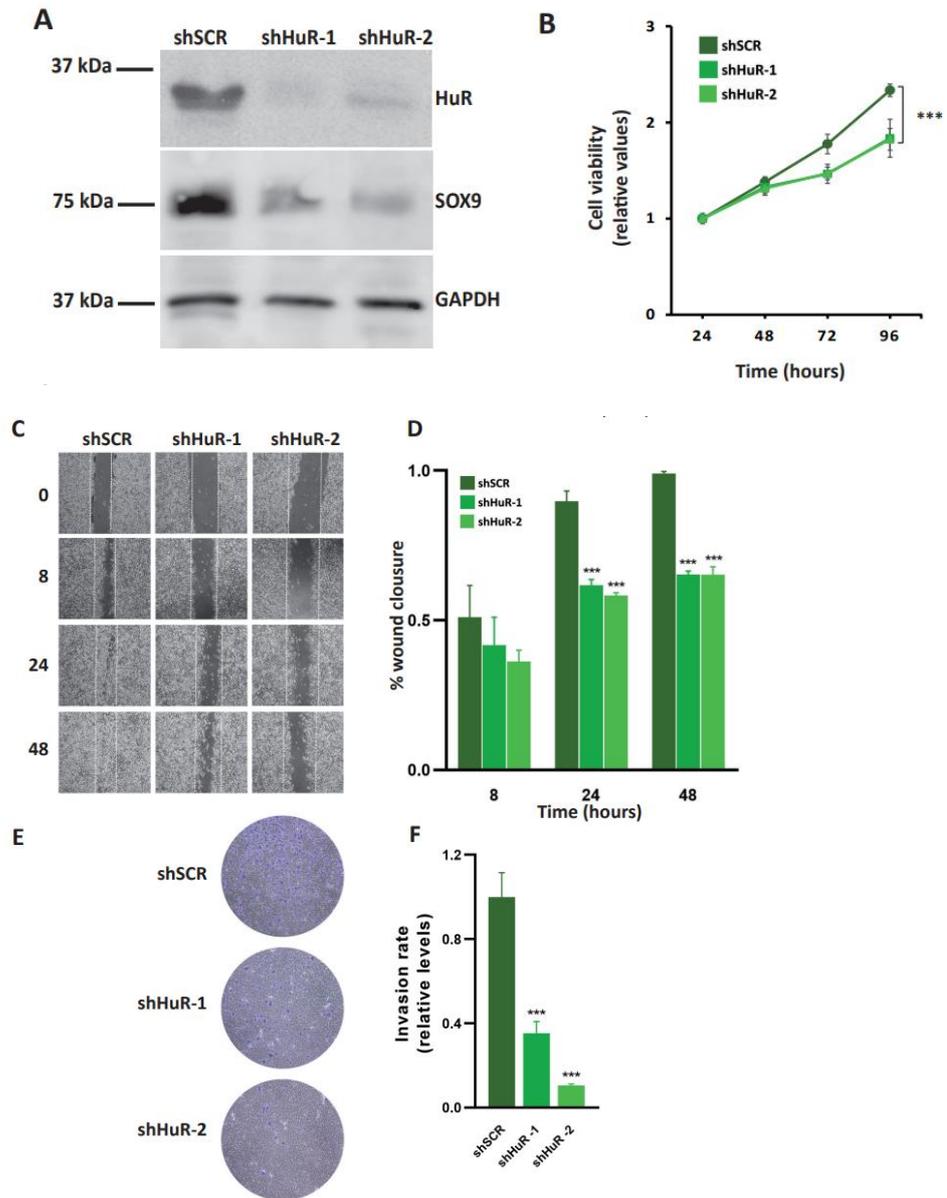


Figure S4. HuR silencing diminishes proliferation, migration, and invasion in BT549 cells. (A) Western blot analysis of HuR and SOX9 levels in control (shSCR) or HuR-silenced (shHuR-1, Scheme 2.) cells. GAPDH was used as a loading control. (B) Cell viability analyses of control (shSCR) or HuR-silenced (shHuR-1, shHuR-2) cells. MTT assays were performed at the indicated time points after seeding. Data were normalized to the value at 24 hours. Error bars represent the mean \pm SEM of three independent experiments assayed in quintuplicate. (C) Cell motility of the indicated clones was analyzed by wound healing assay. Images were taken at 0, 8, 24, and 48 hours (h) after culture scratch (left panel) and area closure was quantified using ImageJ (right panel). Results represent the mean \pm SEM of three independent experiments. (D) Cells were seeded in the upper chambers of Transwell, allowed to migrate for 18 h, and photographed. Left: representative images of the lower chamber (invading cells). Right: percentage of invasiveness by direct measurement with ImageJ. Values represent mean \pm SEM. from three independent experiments performed in triplicate. (***p* < 0.001).

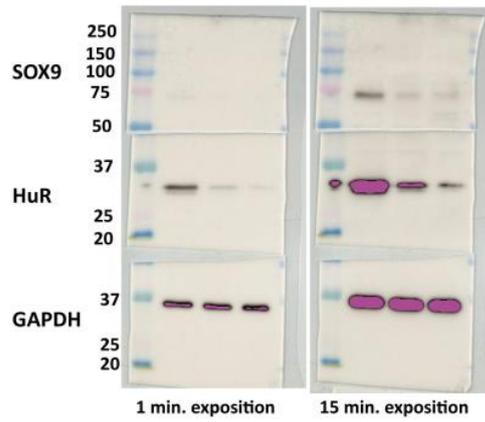


Figure S5. Uncropped Western blot images from Figure 3C.

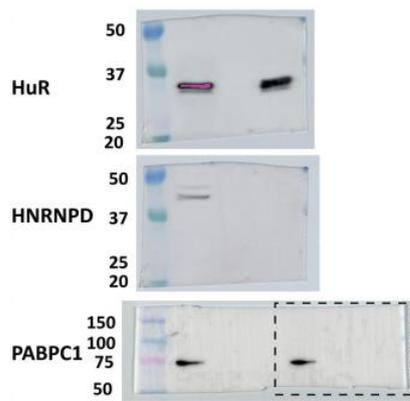


Figure S6. Uncropped Western blot images from Figure 4.

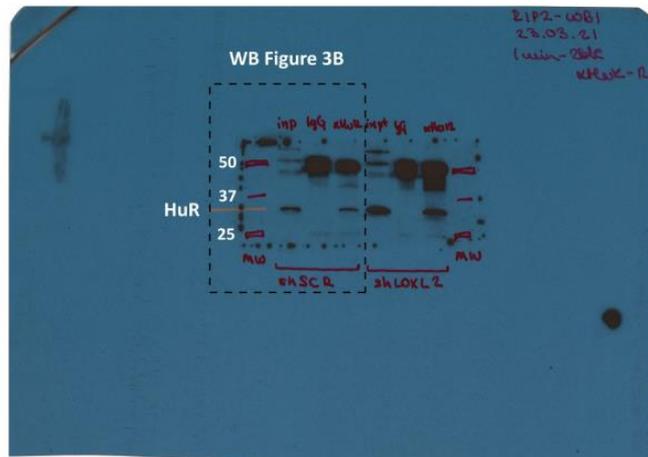


Figure S7. Uncropped Western blot images from Figure 3B.

Table S1. List of primers used for qPCR analysis.

Gene	Forward primer (5'→3')	Reverse primer (5'→3')
<i>ACTB</i>	TTGTTACAGGAAGTCCCTTGCC	ATGCTATCACCTCCCTGTGTG
<i>AKR1C2</i>	CAGAGGTTCTAAAAGTAAAGC	TGTGCAGAATCAATATGGTG
<i>CCL2</i>	AGACTAACCCAGAAACATCC	ATTGATTGCATCCTGGCTG
<i>CLDN1</i>	TTGGCATGAAGTGTATGAAG	ACCTGCAAGAAGAAATATCG
<i>ELAVL1</i>	GATCAGACTACAGGTTTGTC	TTGAAACTGGGTAATTGCCTC
<i>ENPP2</i>	ATAAACAGTACGTGGAAGG	CTACCCATTTTGATTCTGTC
<i>ENPP4</i>	CCTGCATCGATCATTATAC	CATTCATATGAGGGCTACAG
<i>FAM59A</i>	CATCGTCACTAAAAGTACAC	ATAATGGTTAGGCCATGAGAG
<i>GAPDH</i>	CTTCACCACCATGGAGGAGGC	GGCATGGACTGTGGTCTATGAG
<i>ID1</i>	ACTAAGTCACCAGAGACTTTAG	AAATCTGAGAAGCACCAAAAC
<i>ITGA2</i>	GGTGGGGTTAATTCAATATG	ATATTGGGATGTCTGGGATG
<i>LAMA3</i>	GAATCAGTTGCTCAACTACC	TCAGTCAGTTCTTTCCAG
<i>NTN1</i>	CTGCATAAAGATCCCTGTAG	ATGTTAATCTTCAGCTTCCC
<i>PTX3</i>	AGAGAGAGTTGAGACCAATC	AAACAATTGCTCCCTCTGTTC
<i>RAB27B</i>	GAACTGGCTGACAATATGG	GTTTCTACAGCTTCTCCAC
<i>RAB3IP</i>	GTTGATCAGATGTTTTGGGAG	CCTCTTGGAAATAACCCAGC
<i>RNA18S</i>	CGAACGTCTGCCCTATCAACTT	ACCCGTGGTCACCATGGTA
<i>SH3BGRL2</i>	AAGGCAGAACCTTAGAGAAG	CACAGTGATGCATTAGGTAAG
<i>SLC1A3</i>	CCCTTACAAAATCAGAAAATTG	TTTTCTAGGAGGGTCTCTTC
<i>SLC2A3</i>	GGATAACTATAATGGGATGAGC	CCACAATAAACCCAGGGAATG
<i>SMOC1</i>	ATACTTACACTGGGACTGTC	TGAACCTGAACATACAGGAG
<i>SOD2</i>	ATCATACCTAATGATCCAG	AGGACCTTATAGGGTTTTAG
<i>SOX9</i>	CTCTGGAGACTTCTGAACG	AGATGTGCGTCTGCTC
<i>TIAM1</i>	AAGGATGCACGCTATTTTC	GAAGACAAAGTCTTCTTGG
<i>TPD52</i>	AGGTAGAAGAAGAAATCCAGAC	CCTGTAGAGAATTGATCCAAG

Table S2. RNA sequence used in RNA pulldown experiments.

RNA	Sequence (5'→3')
AREwt	GCUAAA <u>UUUUUUUUUUUU</u> UUAAGG
AREmut	GCUAAGCGUAGUGAGUGCGUAAAGG