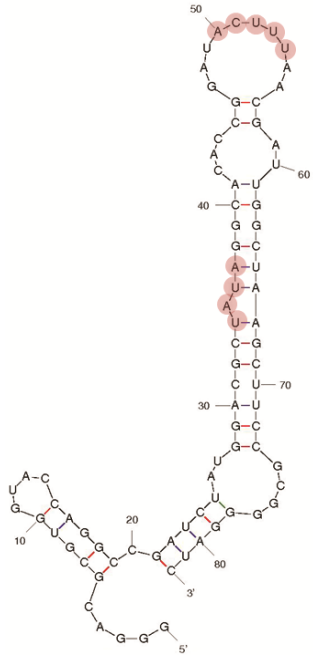
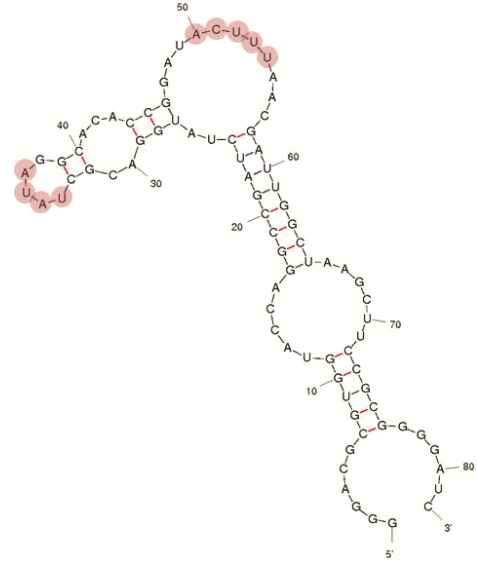
A

dG = -20.40 [Initially -20.40]



B

dG = -19.60 [Initially -19.60]



Supplementary Figure 1. mFOLD prediction of RNA aptamer. (A and B) Nucleotides for the putative β-catenin binding sites are shaded.

A

U-1 (150 nt):

AAGUCUAAUG AUCAU**AUUUA UUUAUUUA**UA UGAACCAUGU CUAUUA**AUUU A**AUU**AUUUA**A UAAU**AUUUA**U AUUAAACUCC
UUAUGUUACU UAACAUCUUC UGUAACAGAA GUCAGUACUC CUGUUGCGGA GAAAGGAGUC AUACUUGUGA

U-2 (188 nt):

AAGUCUAAUG AUCAU**AUUUA UUUAUUUA**UA UGAACCAUGU CUAUUA**AUUU A**AUU**AUUUA**A UAAU**AUUUA**U AUUAAACUCC
UUAUGUUACU UAACAUCUUC UGUAACAGAA GUCAGUACUC CUGUUGCGGA GAAAGGAGUC AUACUUGUGA AGAC**UUUU**AU
GUCACUACUC UAAAGA**UUUU** GCUGUUGC

U-3 (202 nt):

GCGGAGAAAG GAGUCAUACU UGUGAAGAC**U UUU**AUGUCAC UACUCUAAAG A**UUUU**GCUGU UGCUGUUAAG UUUGGAAAAC
AG**UUUUU**AUU CUG**UUUU**AUA AACCAGAGAG AAAUGAG**UUU UG**ACGUC**UUU UU**ACUUGAAU UUCAACUUAU AUUAUAAGGA
CGAAAGUAAA GAUGUUUGAA UACUUAAACA CUAUCACAAG AUGCCAA

U-4 (100 nt):

UGUAACAGAA GUCAGUACUC CUGUUGCGGA GAAAGGAGUC AUACUUGUGA AGAC**UUUU**AU GUCACUACUC UAAAGA**UUUU**
GCUGUUGCUG UUAAGUUUGG

Negative Control(NC, 80 nt):

CAUAGCUCAU UAUACCCUCC UGGGCUCAAG CAAUCCCCCU AACUCUGCCU CCCCAGUAGC UAGGACCACA GGCAUACACC

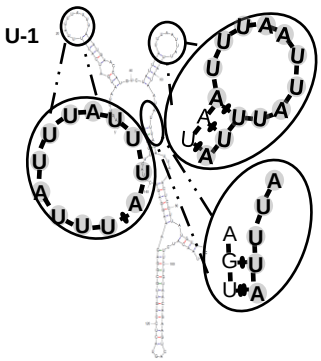
Coding Region (CR, 200nt) :

AGTCAAAGAT ACTCAGGCAG AGATGATCTA CCCTCCTCAA GTCCCTGAGC ATCTACGGTT TGCTGTGGGG CAGGAGGTCT
TTGGTCTGGT GCCTGGTCTG ATGATGTATG CCACAATCTG GCTGAGGGAA CACAACAGAG TATGCGATGT GCTTAAACAG
GAGCATCCTG AATGGGGTGA TGAGCAGTTG TTCCAGACAA

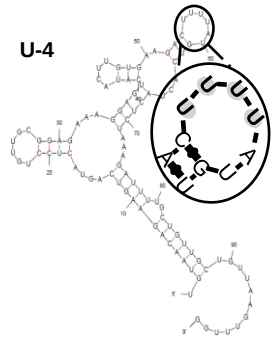
GAPDH 3'UTR (GAPDH, 201 nt) :

AGACCCCTGG ACCACCAGCC CCAGCAAGAG CACAAGAGGA AGAGAGAGAC CCTCACTGCT GGGGAGTCCC TGCCACACTC
AGTCCCCCAC CACACTGAAT CTCCCCTCCT CACAGTTGCC ATGTAGACCC CTTGAAGAGG GGAGGGGCCT AGGGAGCCGC
ACCTTGTCAT GTACCATCAA TAAAGTACCC TGTGCTCAAC C

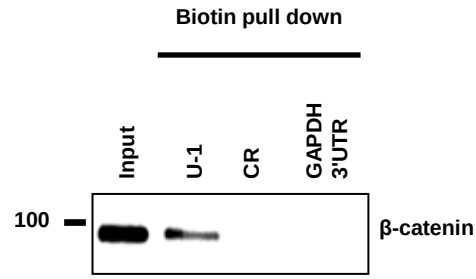
B



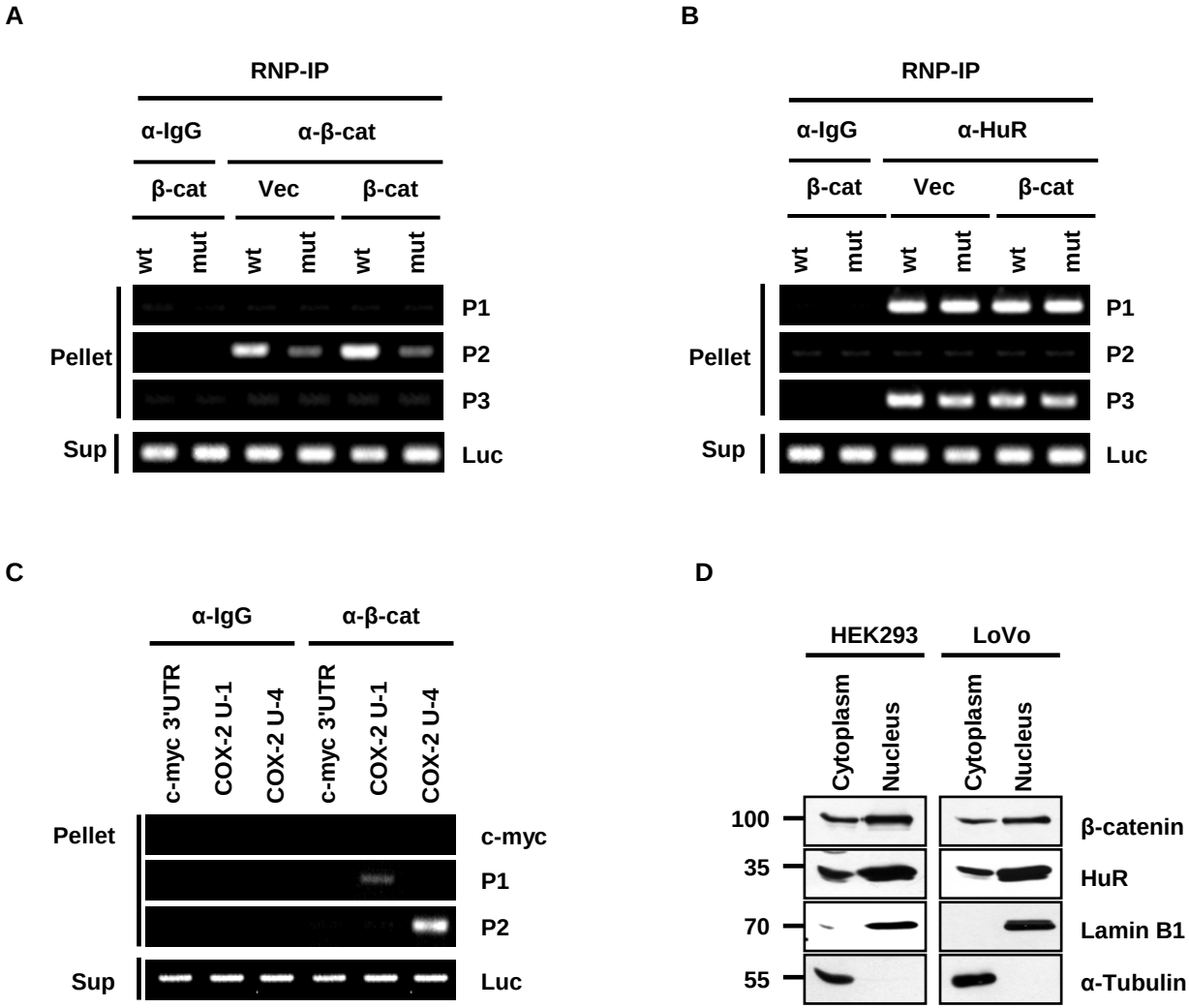
C



D



Supplementary Figure 2. Sequence and predicted structure of COX-2 3'UTR fragments. (A) Sequences of the COX-2 3'UTR fragments, negative control for SPR, coding region of COX-2 mRNA and a GAPDH mRNA 3'UTR. AU-rich elements (Bold) and β-catenin binding elements (Box) are indicated. (B) Predicted secondary structure of the U-1 fragment using the mFold program. (C) Predicted secondary structure of the U-4 fragment using the mFold program. (D) Biotin pull-down analysis of U-1 and negative control RNAs such as a coding region of COX-2 mRNA (CR) and GAPDH mRNA 3'UTR (GAPDH) using HT-29 colon adenocarcinoma cell extracts.. RNA bound pellet fractions were detected by Western blot analysis with anti-β-catenin antibody . Input (10%) was also loaded.



Supplementary Figure 3. Specific binding and collaboration of β-catenin and HuR on COX-2 3'UTR. (A) RNP-IP analysis with anti-β-catenin antibody to test for its binding to wild-type (wt) or mutant (mut) COX-2 3'UTR downstream of the luciferase gene. HEK293T cells were co-transfected either with the vector (Vec) or with 3x Flag-tagged β-catenin in the presence of the wt or mut luciferase reporter. Pellet RNA was analyzed using P1, P2 or P3 primers (as shown in Figure 4A) to test for the binding of the protein to specific sites in COX-2 3'UTR. Expression of the luciferase gene (Luc) in the supernatant (Sup) RNA was analyzed relative to the control. PCR primers are indicated on the right side of the gel. (B) RNP-IP analysis with anti-HuR antibody to test for its binding to wild-type (wt) or mutant (mut) COX-2 3'UTR downstream of the luciferase gene. Analysis was performed as described in (A). (C) RNP-IP with the anti-β-catenin antibody. U-4 and U-1 fragments of COX-2 3'UTR were expressed in LoVo cells. The c-myc 3'UTR downstream of the luciferase gene was expressed as a negative control for β-catenin binding. Analysis was performed as described in (A). (D) Western blot analysis of the subcellular fractionated extracts of HEK293 and LoVo cells. Cytoplasmic and nuclear fractions were prepared as described in the Materials and Methods. Protein levels of the β-catenin and HuR protein were detected along with the cytoplasmic marker (α-Tubulin) and nuclear marker (Lamin B1). Protein size markers are shown on the left side of the gel.

Supplementary Table 1. Sequence of the Oligonucleotides for generation of Plasmids, RT-PCR, site-directed mutagenesis and siRNA.

Assay	PCR primer name	F/R	sequence
In vitro transcription	T7 promoter sequence (T7)		TAA TAC GAC TCA CTA TAG GG
	U-1	Forward	(T7)-AAG TCT AAT GAT CAT ATT
		Reverse	TCA CAA GTA TGA CTC CTT
	U-2	Forward	(T7)-AAG TCT AAT GAT CAT ATT
		Reverse	GCA ACA GCA AAA TCT TTA
	U-3	Forward	(T7)-CGG AGA AAG GAG TCA TAC
		Reverse	CAT CTT GTG ATA
	U-4	Forward	(T7)-TGT AAC AGA AGT CAG TAC
		Reverse	TGT AAC AGA AGT CAG TAC
RT-PCR and qRT-PCR	P1	Forward	GCT CTA GAA AGT CTA ATG ATC ATA TT
		Reverse	AGT CTA GAT CAC AAG TAT GAC TCC TT
	P2	Forward	AGT CTA GAT GTA ACA GAA GTC AGT AC
		Reverse	AGT CTA GAC CAA ACT TAA CAG CAA CA
	P3	Forward	CAT CTA GAA TGA CCT CAT AAA ATA CC
		Reverse	GAT CTA GAG TCT CTT AGC AAA ATG GC
	c-myc	Forward	CGA GAT CTT TTT ATG CTT ACC ATC TTT TTT TTT TC
		Reverse	CGA GAT CTG AAA CAT TGT GTA AAT CTT AAA ATT TT
siRNA	β-catenin	sense	AGC UGA UAU UGA UGG ACA Gtt
	HuR	sense	GCC UGU UCA GCA GCA UUG Gtt

plasmid	PCR primer name	F/R	sequence
For recombinant protein	Arm	Forward	TGC GGA TCC CAC AGA TGC TGA AAC AT
		Reverse	GCG AAT TCA GTC TCA TTC CAA GCC AT
	C-term	Forward	CGG GAT CCA CAA GAA ACG GCT TTC A
		Reverse	GAG AAT TCC AGG TCA GTA TCA AAC CA
pZEO/Luc or pDHFR	U-4	Forward	GCC TCT AGA TGT AAC AGA AGT CAG TAC
		Reverse	AG TCT AGA CCA AAC TTA ACA GCA ACA
Site directed mutagenesis	U6-Aptamer mutant		CTA TAG GCA CAC CGG ATG CGC GAA CGA TTG GCT AAG CTTC
	U-4mut		CAT ACT TGT GAA GCC CCC TAT GTC ACT ACTC

Supplementary Table 2. Binding affinity of β-catenin and HuR on various fragments of COX-2 3'UTR.

COX-2 RNA	Protein (K_D, M)	
	HuR	β-catenin
U-1	$3.34 \pm 0.01 \times 10^{-8}$	$1.00 \pm 0.01 \times 10^{-6}$
U-2	$8.13 \pm 1.58 \times 10^{-8}$	$1.58 \pm 0.16 \times 10^{-7}$
U-3	$7.70 \pm 2.83 \times 10^{-6}$	$2.60 \pm 1.08 \times 10^{-6}$
U-4	$3.51 \pm 1.42 \times 10^{-6}$	$1.31 \pm 1.56 \times 10^{-7}$