

Supplemental Information

LincRNA-p21 Suppresses Target mRNA Translation

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INVENTORY of SUPPLEMENTAL INFORMATION

Supplemental text

Figure S1: Complements main Figure 1

Figure S2: Complements main Figure 1

Figure S3: Complements main Figure 2

Figure S4: Complements main Figures 3 and 4

Legends of Supplemental Figures

Table S1

Table S2

Supplemental text

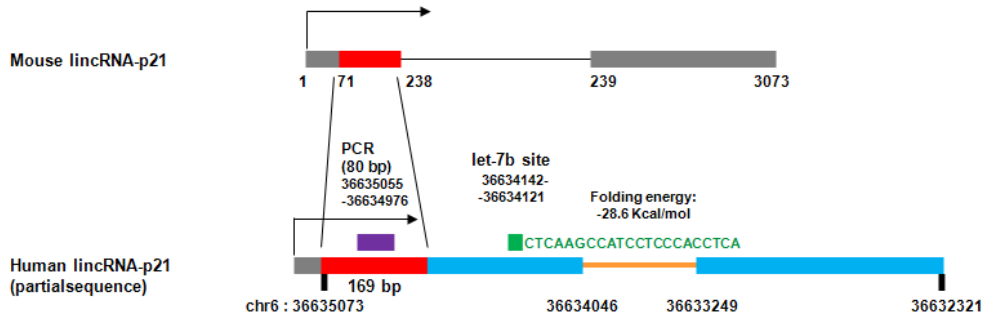
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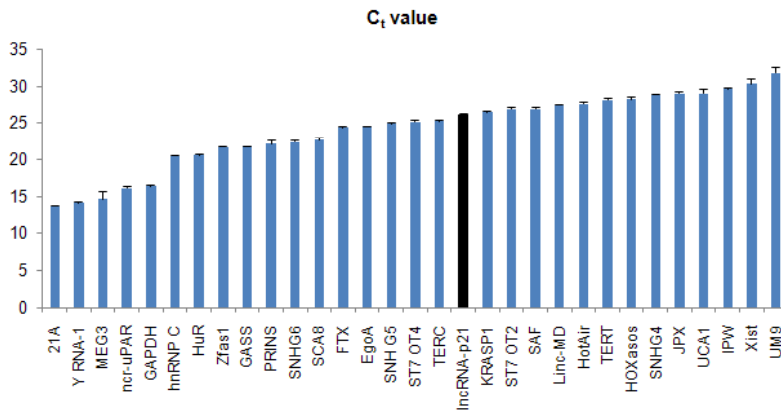
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Rapid Amplification of cDNA Ends (RACE) was performed according to manufacturer's protocol (Invitrogen) using total RNA from HeLa cells.

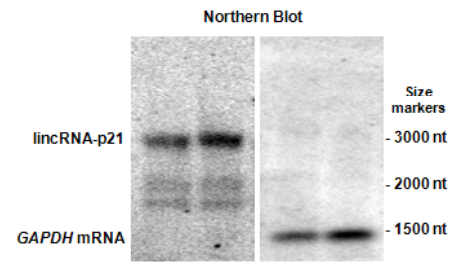
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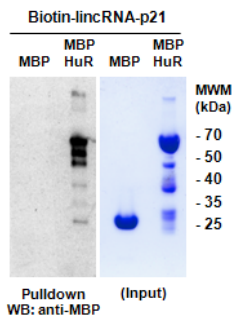
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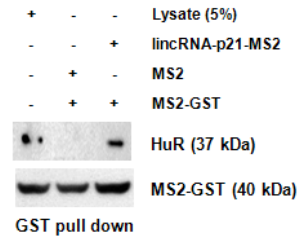
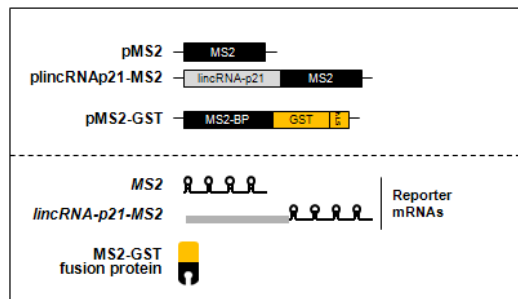
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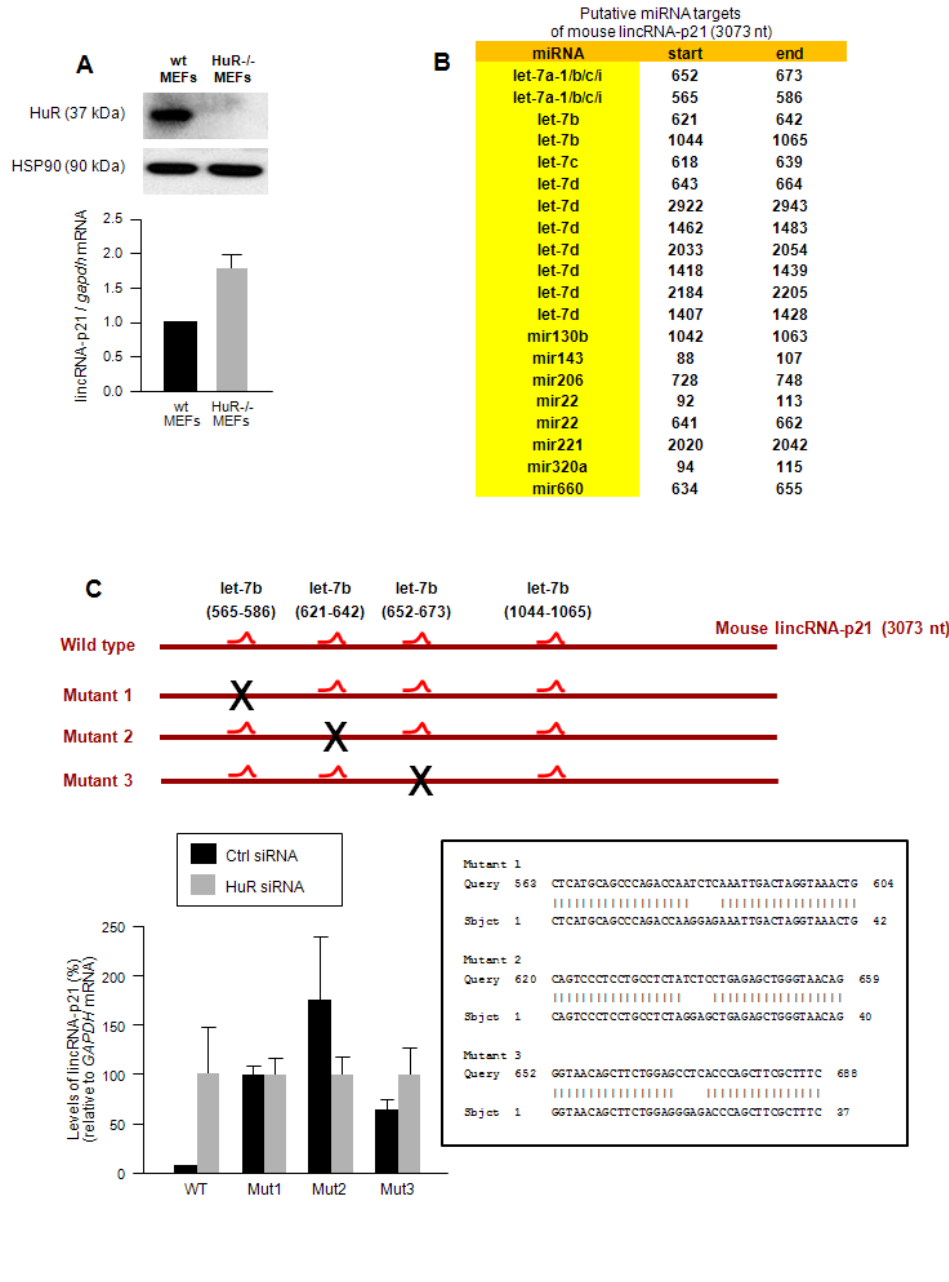


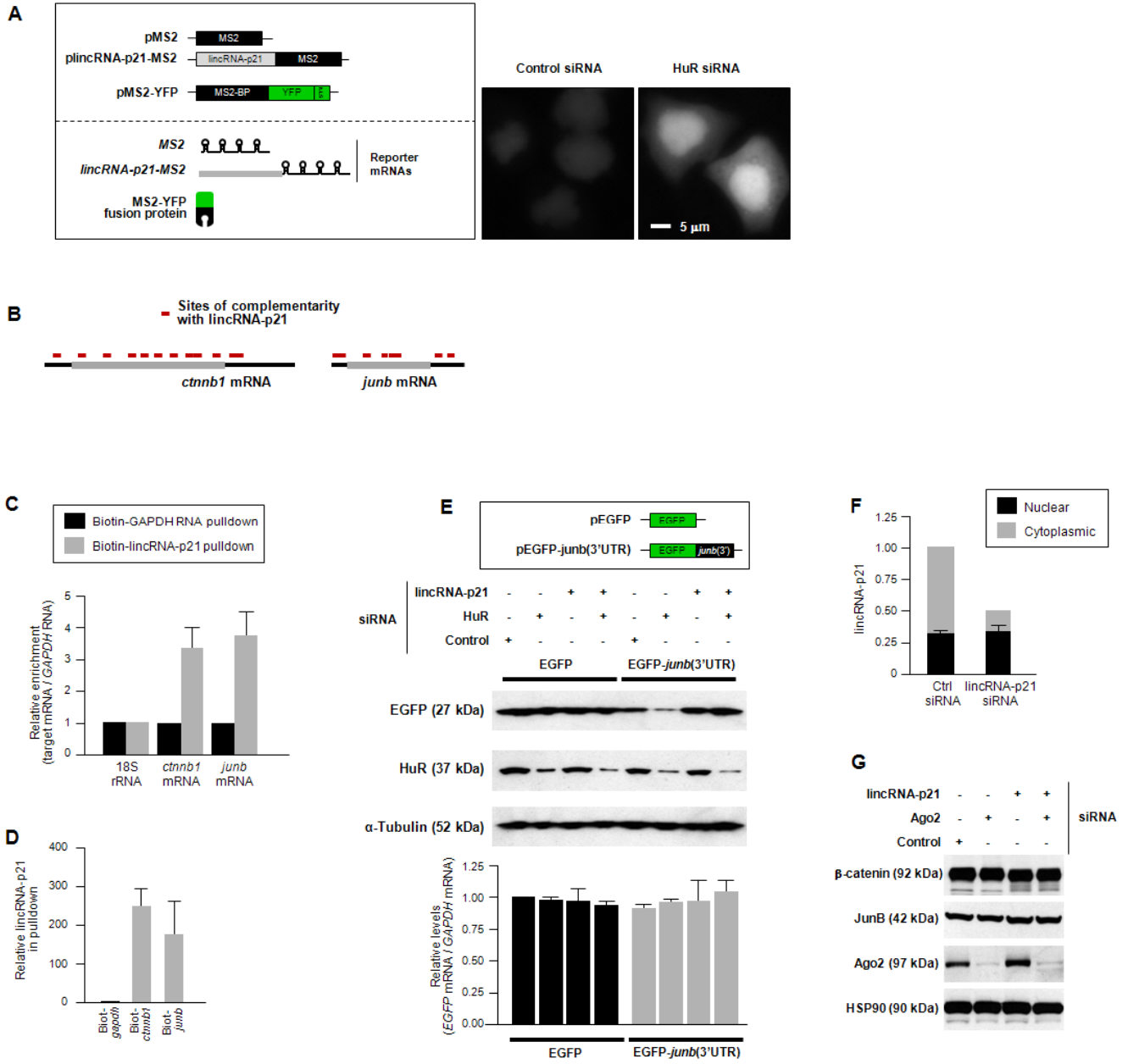
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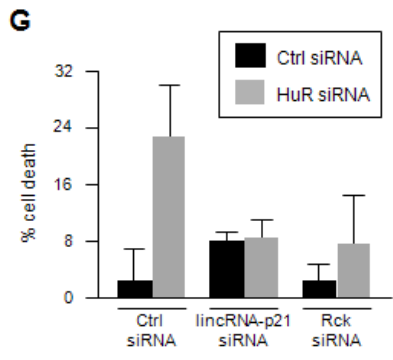
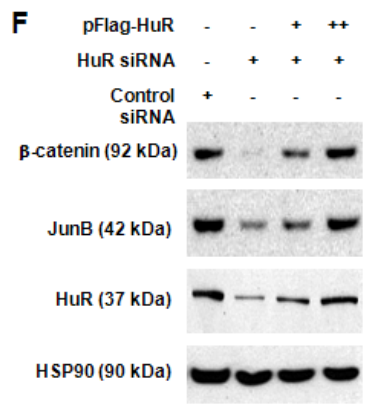
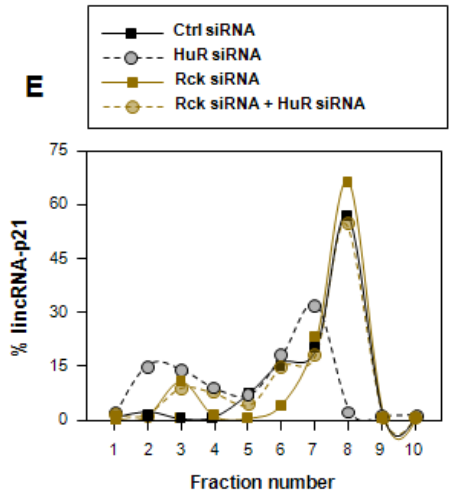
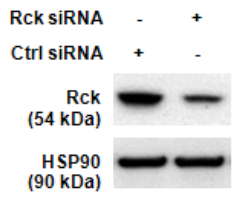
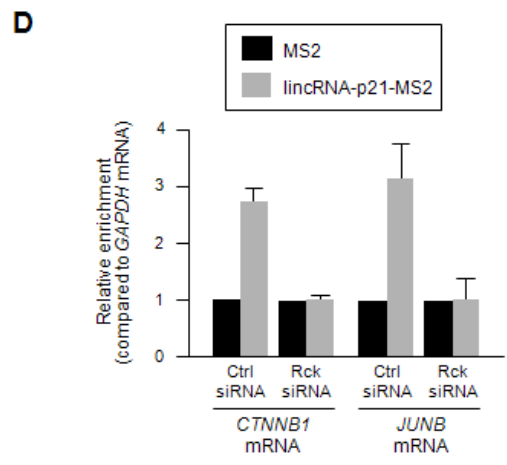
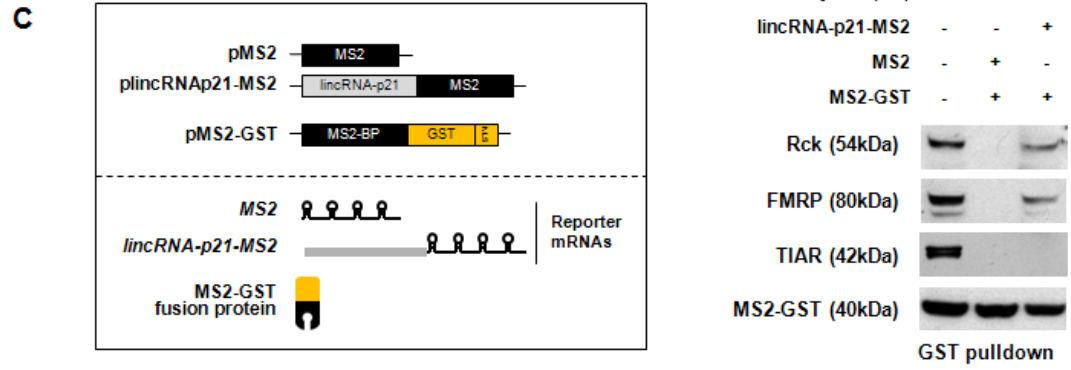
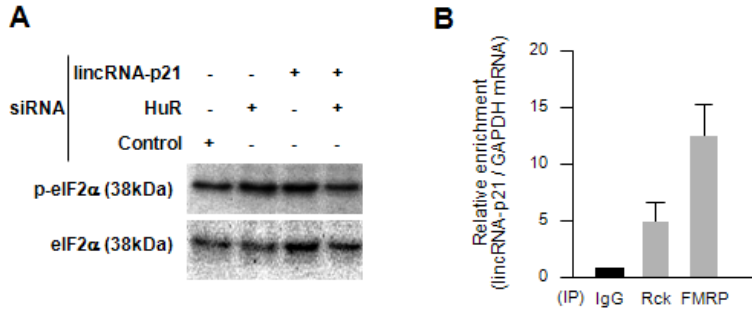


E









Legends of Supplemental Figures

Figure S1 (complements Fig. 1). Additional characterization of mouse and human lincRNA-p21 and their interaction with HuR.

(A) Genomic localization of mouse (*top*) and human (*bottom*) lincRNA-p21. The human lincRNA-p21 was sequenced partially by RACE (Rapid Amplification of cDNA Ends; Invitrogen) using total RNA from HeLa cells (supplemental text). *Red*, region of homology between mouse and human lincRNA-p21. *Purple*, region used to detect human lincRNA-p21 by qPCR. *Green*, let-7b site, with the interaction sequence indicated in green text. (B) Relative abundance of lincRNA-p21 (black) as determined by calculating C_t values among a subset of mRNAs (those encoding hnRNPC and HuR) and lincRNAs, as measured in HeLa cells. (C) Northern blot analysis of lincRNA-p21 expression in HeLa cells. Duplicate samples (20 and 30 μ g of total RNA) were run in agarose/formaldehyde gels; after transfer to nylon membranes, lincRNA-p21 and *GAPDH* mRNA were detected using antisense oligomers (GGGTGGCTCACTCTTCTGGC and GCCAATACGACCAAATCC respectively) that were end-labeled with 32 P and used as probes. Bands were visualized using a Typhoon scanner (GE Healthcare). (D) *In vitro* interaction of maltose-binding protein (MBP) or MBP-HuR (2 μ g each) with biotinylated lincRNA-p21 was assessed by pull-down using streptavidin-coated beads and Western blotting (WB) detection using anti-MBP antibody. Input, 2 μ g recombinant protein. As shown, MBP-HuR selectively interacted with biotinylated mouse lincRNA-p21, while MBP did not. MWM, protein molecular weight markers. (E) HeLa cells were transfected with the plasmids shown in the schematic (*left*). Forty-eight h later, lysates were used in IP reactions employing GSH-agarose beads. The presence of HuR in IP reactions from each transfection group (cells expressing MS2 RNA compared with cells expressing lincRNA-p21 RNA) was assessed by Western blot analysis using an anti-HuR antibody.

Figure S2 (complements Fig. 1). Influence of HuR and let-7 on lincRNA-p21 levels.

(A) In wild-type (wt) and HuR-null (HuR^{-/-}) MEFs, HuR levels were assessed by WB, and lincRNA-p21 by RT-qPCR. (B) Putative microRNAs interacting with mouse lincRNA-p21, as predicted using TargetScan. (C) Predicted let-7 sites on mouse lincRNA-p21. Using a plasmid vector that expressed the wild-type mouse lincRNA-p21 expressed from a CMV promoter (Huarte et al., 2010), site-directed mutagenesis (box) was used to generate the mutants indicated (Mut1, Mut2, Mut3). After transfection of each plasmid into MEFs expressing normal or silenced HuR, the levels of lincRNA-p21 (WT, Mut1, Mut2, Mut3) were quantified by RT-qPCR. Site-directed mutagenesis of mouse lincRNA-p21 was performed with the QuikChange® Site-Directed Mutagenesis Kit (Stratagene). (D) In wild-type (wt) and Ago2-null (Ago2^{-/-}) MEFs, Ago2 levels were assessed by WB (*top*), and lincRNA-p21 by RT-qPCR (*graph*). Data in (A,C,D) represent the means and S.D. (error bars) from 3 independent experiments.

Figure S3 (complements Fig. 2). Translational repression of β -catenin and JunB translation by lincRNA-p21 in mouse cells.

(A) HeLa cells were transfected with the plasmids shown in the schematic. Forty-eight hours later, the localization of the MS2-tagged lincRNA-p21 was assessed by fluorescence microscopy. (B) Regions of predicted interaction between mouse lincRNA-p21 and mouse *ctnmb1* and *junb* mRNAs. (C) Following incubation of mouse biotin-lincRNA-p21 (or control biotin-*GAPDH* RNA) with MEF lysates, RNA was extracted and *ctnmb1* and *junb* mRNAs, as well as normalization control 18S rRNA were assessed by RT-qPCR and represented as fold difference relative to mRNA levels in biotin-*GAPDH* pull-down samples. (D) Biotinylated *GAPDH* mRNA, *ctnmb1* RNA (NM_007614.3, nucleotides 288-2633), and *junb* RNA (NM_008416.3, nucleotides 320-1354) were incubated with MEF lysates; after pull-down, the levels of lincRNA-p21 in beads were assessed by RT-qPCR. (E) MEFs were transfected with the plasmids indicated: pEGFP-*junb*(3'UTR), containing the entire mouse *junb* 3'UTR (amplified from mouse total RNA and cloned into pEGFP-C1), and control plasmid pEGFP, together with the siRNAs shown. Forty-eight h later, the levels of the reporter protein EGFP were detected by Western blot analysis and the levels of *EGFP* or *EGFP*-

junb(3'UTR) mRNAs by RT-qPCR analysis. (F) Forty-eight hours after siRNA transfection in HeLa cells, lincRNA-p21 levels were assessed by RT-qPCR in nuclear and cytoplasmic fractions as explained in main Fig. 2A. (G) Forty-eight hours after transfection with the siRNAs shown, the levels of Ago2, β -catenin, JunB and loading control HSP90 were assessed by Western blot analysis. Data in (C-F) represent the means and S.D. (error bars) from 3 independent experiments. Oligomer sequences are in Table 2.

Microscopy: forty-eight h after transfection of pMS2-YFP together with either pMS2 or pMS2-lincRNA-p21, HeLa cells were fixed and images were acquired with Axio Observer microscope (ZEISS) with Axio Vision 4.7 Zeiss image-processing software or with LSM 510 Meta (ZEISS).

Figure S4 (complements Figs. 3 and 4). Translational inhibition by mouse lincRNA-p21 associated to the recruitment of translational repressor Rck.

(A) HeLa cells were transfected with the siRNAs shown and 48 h later, the levels of eIF2 α and phospho-eIF2 α were assessed by Western blot analysis. (B) RIP analysis of lincRNA-p21 interaction with Rck and FMRP in MEFs. (C) *Left*, plasmids used to study the *in vitro* interaction of lincRNA-p21 with Rck, FMRP, and TIAR present in HeLa cell lysates, as detected by WB analysis in GST pulldown assays (*right*). (D) Mouse lincRNA-p21-MS2 and MS2-GST were expressed in HeLa cells using the plasmids shown in panel (C) in cells expressing normal Rck levels or cells in which Rck was silenced (*right*). RIP analysis was carried out to study if silencing Rck affected the interaction of lincRNA-p21 with endogenous *CTNNB1* and *JUNB* mRNAs. (E) 48 h after transfecting HeLa cells with the siRNAs shown, polysomes were prepared as shown in the main Fig. 4E; the relative distribution of lincRNA-p21 is indicated. (F) In order to establish whether the effects of lincRNA-p21 were directly attributed to HuR, a rescue experiment was devised in which HeLa cells were transfected with siRNA directed to the 3'UTR of HuR or with a plasmid (1 and 2 μ g) to express only the coding region of HuR (pFlag-HuR). Forty-eight h later, the levels of β -catenin and JunB were assessed by Western blot analysis. (G) Percentages of HeLa cell death (as assessed by trypan blue exclusion) 48 h after transfection of the siRNAs shown; cell number and viability were quantified using a TC10 automated cell counter (BioRad). Data in (B,D,G) represent the means and S.D. (error bars) from 3 independent experiments.

Table S1. Putative Regions of interaction of lincRNA-p21 with human *CTNNB1* mRNA, *JUNB* mRNA, and (control) *GAPDH* mRNA.

I. Putative interaction regions of the *CTNNB* mRNA (query) with lincRNA-p21 (subject).

Matched sequence		Identity (%)	Mach Size (bp)	E-value
Query 3295	TTTTTAA--GTCTCTCGTAGTGTAA 3318	76.92	26	54
Sbjct 966	TTTTTAATTGTCCCTGTTAGTGTGAA 941 (intron)			
Query 3441	TTTTGTATAAAATAGACAAATAGAA 3465	80	25	4.4
Sbjct 503	TTTTGACTAAACTA--CAAATAGAA 481			
Query 879	ATACAAATGATGTAGAAACAG 899	80.95	21	4.4
Sbjct 195	ATGCAAAAGCAAGTAGAAACAG 175			
Query 3511	TGGATCTATTTCATGTTTT 3530	80	20	189
Sbjct 2192	TGGATCT-TCTCTGGTTTT 2174			
Query 1353	GAATGCAAGCTT-TAGGACT 1371	80	20	189
Sbjct 2307	GAAAGGAGGCTTCTAGGACT 2288			
Query 3339	AGCAATTCTAATTTTTAA 3357	78.95	19	54
Sbjct 978	AGCCATTGACGTTTTTAA 960 (intron)			
Query 297	TGGACATGGCCATGGAAC 314	83.33	18	15
Sbjct 876	TGGACATGGAACATGGAAC 859			
Query 1509	TAAATGTGGTTCACCTGTG 1526	83.33	18	54
Sbjct 1262	TAAATGT--TCACCCGTG 1247 (intron)			
Query 1350	GTGGAATGCAAGCTTTA 1366	82.35	17	54
Sbjct 806	GTGGAATACAGGCTGTA 790			
Query 3060	AAACTTTTTTGTCTGGT 3076	82.35	17	54
Sbjct 1042	AAACTTTTTTCTTTGGT 1026 (intron)			
Query 2885	CATTGCTGTTTTTAAA 2900	87.5	16	4.4
Sbjct 975	CATTGACGTTTTTAA 960 (intron)			
Query 2173	AGCCACAGCTCCTCTG 2188	87.5	16	54
Sbjct 665	AGCCACAG--CCTCTG 652			
Query 247	TTTTGAAAA-TCCAG 260	93.33	15	15
Sbjct 217	TTTTGAAAAATCCAG 203			
Query 110	GGGAGGCGGAGACGG 124	86.67	15	15
Sbjct 1615	GGGAGGCTGAGGCGG 1601 (intron)			
Query 1812	GAAATCTTGCCCTTT 1826	86.67	15	15
Sbjct 2130	GAAATCTAGCCCTTT 2116			
Query 2913	CCTTCTCTCTTAT 2927	86.67	15	15
Sbjct 2477	CCATTCTCTCTAT 2463			
Query 1409	TGTCTT-TGGACTCT 1422	86.67	15	189
Sbjct 428	TGTCTTTGGATTCT 414			
Query 383	GCCACTA-CCACAGC 396	86.67	15	189
Sbjct 671	GCCACCAGCCACAGC 657			
Query 214	CCCTGAG-GGTATTT 227	86.67	15	189
Sbjct 2084	CCCTGGGTGATTT 2070			
Query 1096	TGGTGGG--GTGAG 1108	86.67	15	189
Sbjct 2499	TGGTGGGCACTGAG 2485			
Query 384	CCACTAC-CACAGCT 397	86.67	15	189
Sbjct 2741	CCACTACGCCAGCT 2727			

II. Putative interaction regions of the *JUNB* mRNA (query) with lincRNA-p21 (subject).

Matched Regions		Identity (%)	Mach Size (bp)	E-value
Query 770	CCCCGGCTGGGCCCCGGGGCCCTACGCCGGCC 802	72.73	33	93
Sbjct 32	CCCCGGCAAGCCAGCGGGAG-CTCTGCCGGCC 1			
Query 1654	TGGGAAGGGGAcccccccccctgcc 1679	80.77	26	0.18
Sbjct 687	TGGGAAGGGGACACAAGCCACCAGCC 662			
Query 1559	AATATA-ATATAT-TTGTGTAT 1578	86.36	22	7.6
Sbjct 1117	AATATATATGTATATTGTGTAT 1096 (intron)			
Query 956	CGGCCGAGCTGGGCTTGG 973	83.33	18	7.6
Sbjct 1767	CGGGCAGCTGGTCTGGG 1750			
Query 1653	TGGGAAGGGGAccccc 1669	82.35	17	27
Sbjct 2175	TTAGGAAGGGGCTCCC 2159			
Query 11	GCCAGCCTCGGAGCCAG 27	82.35	17	27
Sbjct 2532	GCCAGCCTGGGGTCCAG 2516			
Query 1564	AATATATTTGTGTATT 1579	87.5	16	2.2
Sbjct 1117	AATATATATGTATATT 1102 (intron)			
Query 1564	AATATATTTGTGTATT 1579	87.5	16	2.2
Sbjct 1898	AATATATTCCTTGTATT 1883			
Query 1053	CCCCATCA-ACATGGA 1067	87.5	16	27
Sbjct 2208	CCCCACCACACATGGA 2193			
Query 1443	TGGACTCCGGCCCTC 1457	86.67	15	7.6
Sbjct 2672	TGGACTCTGACCTC 2658			

III. Putative interaction regions of the *GAPDH* mRNA (query) with lincRNA-p21 (subject).

Matched Regions		Identity (%)	Mach Size (bp)	E-value
Query 431	CTCATTTCAGGGGGAGCC 450	85.00	20	0.44
Sbjct 2287	CTCATTTCATAGGGGTGCC 2268			
Query 98	ACACCATGGGGAAGG 112	86.67	15	5.4
Sbjct 1316	ACAACATGGGGAGGG 1330 (intron)			
Query 506	TGAACCATGAGAAGT 520	86.67	15	5.4
Sbjct 2012	TGAACCATCATAAGT 2026			

