Table I. Primers used in quantitative real-time PCR for detecting gene expressions in mouse liver tissues

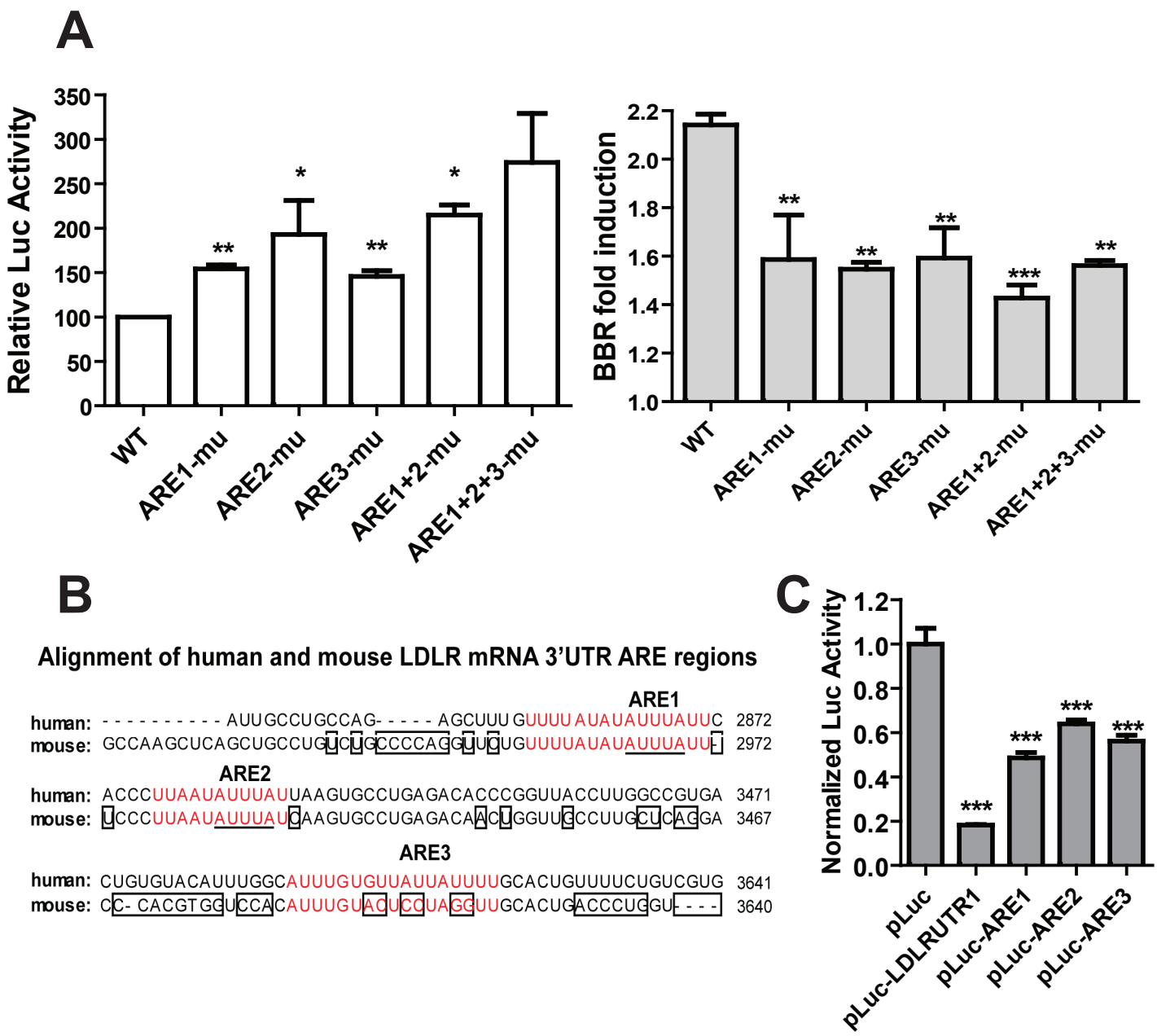
Gene name	Forward primer 5' to 3'	Gene name	Reverse primer 5' to 3'
ACACA_F	TGACACCATGTTGGGAGTTG	**ACACA_R**	TGTGAGCAGGAAGGACTTGA
ACACB_F	ACTATGAGGCCCAGCATGTC	**ACACB_R**	TGACCCTATTGCCTCCAAAG
Albumin_F	AGATGACAGGGCGGAACTTG	**Albumin_R**	GCACACTTCCTGGTCCTCAA
FASN_F	GGCATCATTGGGCACTCCTT	**FASN_R**	GCTGCAAGCACAGCCTCTCT
GAPDH_F	AACTTTGGCATTGTGGAAGG	**GAPDH_R**	GGATGCAGGGATGATGTTCT
HMGCR_F	CTTTCAGAAACGAACTGTAGCTCAC	**HMGCR_R**	CTAGTGGAAGATGAATGGACATGAT
HMGCS1_F	TTTGATGCAGCTGTTTGAGG	**HMGCS1_R**	CCACCTGTAGGTCTGGCATT
HMGCS2_F	CCCCTGAGGAATTCACAGAA	**HMGCS2_R**	TGCATCTCATCCACTCGTTC
HNF1a_F	GCACCAGAGACCCACGTGCC	**HNF1a_R**	GGCTTCCCCTCAGCTCCCGA
HNRNP-D_F	AGTCGGAGAGTGTAGATAAGGTC	**HNRNP-D_R**	GGCCCTTTTAGGATCAATGACTT
HNRNP-I_F	AGCCAATGGAAACGATAGCAA	**HNRNP-I_R**	GCGCCACCGATGTATAGTAGT
KSRP_F	ACTGGAGCACCTGAGTCTGT	**KSRP_R**	CGTTGTCGTGAAACTGTCCT
LDLR_F	ACCTGCCGACCTGATGAATTC	**LDLR_R**	GCAGTCATGTTCACGGTCACA
Luc2_F	CGCACATATCGAGGTGGACA	**Luc2_R**	GCAAGCTATTCTCGCTGCAC
PCSK9_F	TTGCAGCAGCTGGGAACTT	**PCSK9_R**	CCGACTGTGATGACCTCTGGA
SREBP1c_F	CAAGGCCATCGACTACATCCG	**SREBP1c_R**	CACCACTTCGGGTTTCATGC
SREBP2_F	CCAAAGAAGGAGAGAGGCGG	**SREBP2_R**	CGCCAGACTTGTGCATCTTG

Table II. miRNA microarray analysis to identify BBR regulated micro RNAs in HepG2 cells with target sites in LDLR 3'UTR.

Column #	Column ID	Fold change (BBR 4 h vs. DMSO 4 h)	Fold change (BBR 8 h vs. DMSO 8 h)	Fold change (BBR 24 h vs. DMSO 24 h)
13360	mml-miR-19a	-1.32	-1.04	2.21387
11747	hsa-miR-130b	-1.44	2.12	1.41
12543	hsa-miR-615-5p	-2.03	1.43	1.22
12605	hsa-miR-671-5p	-2.06	-2.24	1.09
12619	hsa-miR-760	-1.45	-1.15	-2.15
11633	hsa-miR-1201	-6.84	1.89	2.99
11662	has-miR-1244*	-10.92	1.11	1.20
11881	hsa-miR-1979*	-3.06	-1.79	-1.76
11829	hsa-miR-1826	-2.03	-1.23	1.01
6145	crm-miR-2231	-2.76	-1.64	-2.30

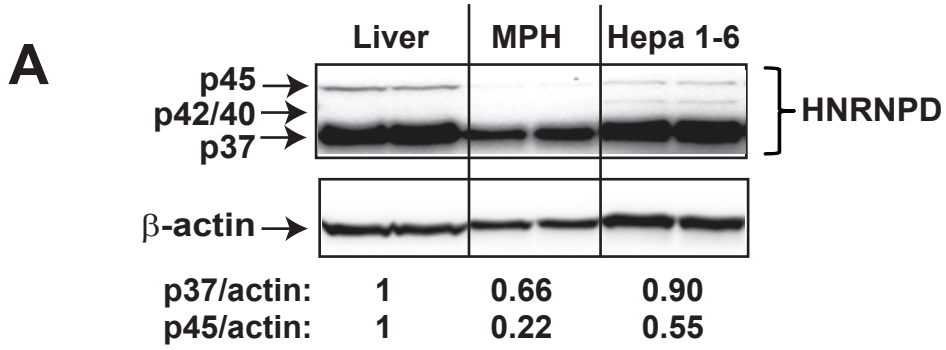
* miRNA target site is not present in LDLR 3'UTR

Supplemental Figure I



Supplemental Figure I: Identification of three destabilizing ARE motifs in mouse LDLR 3'UTR.
(A) HepG2 cells were transfected with individual luciferase-UTR reporter plasmid along with pRL-SV40 as the normalizing transfection vector for 48 hours. BBR was added to cells for 8 hours before cell lysis. The stimulatory activity of BBR on the wild-type UTR reporter was defined as 100%, and activities of BBR on mutated vectors were plotted relative to that value. The data shown are mean ± SEM of three separate transfections in which triplicate wells were used for each reporter.
(B) Comparison of human and mouse LDLR mRNA 3'UTR sequences. Segments of ARE containing sequences are shown. Mouse nucleotide sequences that differ from those of the human sequence are boxed. The sequences of AREs are in red color and the AUUUA motifs are underlined.
(C) LDLR3'UTR reporter plasmids or control vector pLuc were cotransfected with pRL-SV40 into Hepa 1-6 cells. Two days post-transfection, cell lysates were prepared. Dual luciferase activities were measured and expressed as normalized luciferase activity. Each value represents the mean ± SEM of four wells per condition. *** $p < 0.001$ as compared to pLuc. The graph shown is representative of three separate experiments.

Supplemental Figure II



B

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shRNA:                                                    CGGAGAGTGTAGATAAGGTCA
human HNRNPD V1: TCACAGGGCGATCAAGGGGTTTTGGCTTTGTGCTATTTAAAGAATCGGAGAGTGTAGATAAGGTCATGGATCAAAAAGAA
mouse HNRNPD V1: TCACAGGGCGATCAAGGGGTTTTGGCTTTGTGCTATTTAAAGAGTCGGAGAGTGTAGATAAGGTCATGGATCAGAAAGAA
mouse HNRNPD V2: TCACAGGGCGATCAAGGGGTTTTGGCTTTGTGCTATTTAAAGAGTCGGAGAGTGTAGATAAGGTCATGGATCAGAAAGAA
mouse HNRNPD V3: TCACAGGGCGATCAAGGGGTTTTGGCTTTGTGCTATTTAAAGAGTCGGAGAGTGTAGATAAGGTCATGGATCAGAAAGAA
mouse HNRNPD V4: TCACAGGGCGATCAAGGGGTTTTGGCTTTGTGCTATTTAAAGAGTCGGAGAGTGTAGATAAGGTCATGGATCAGAAAGAA
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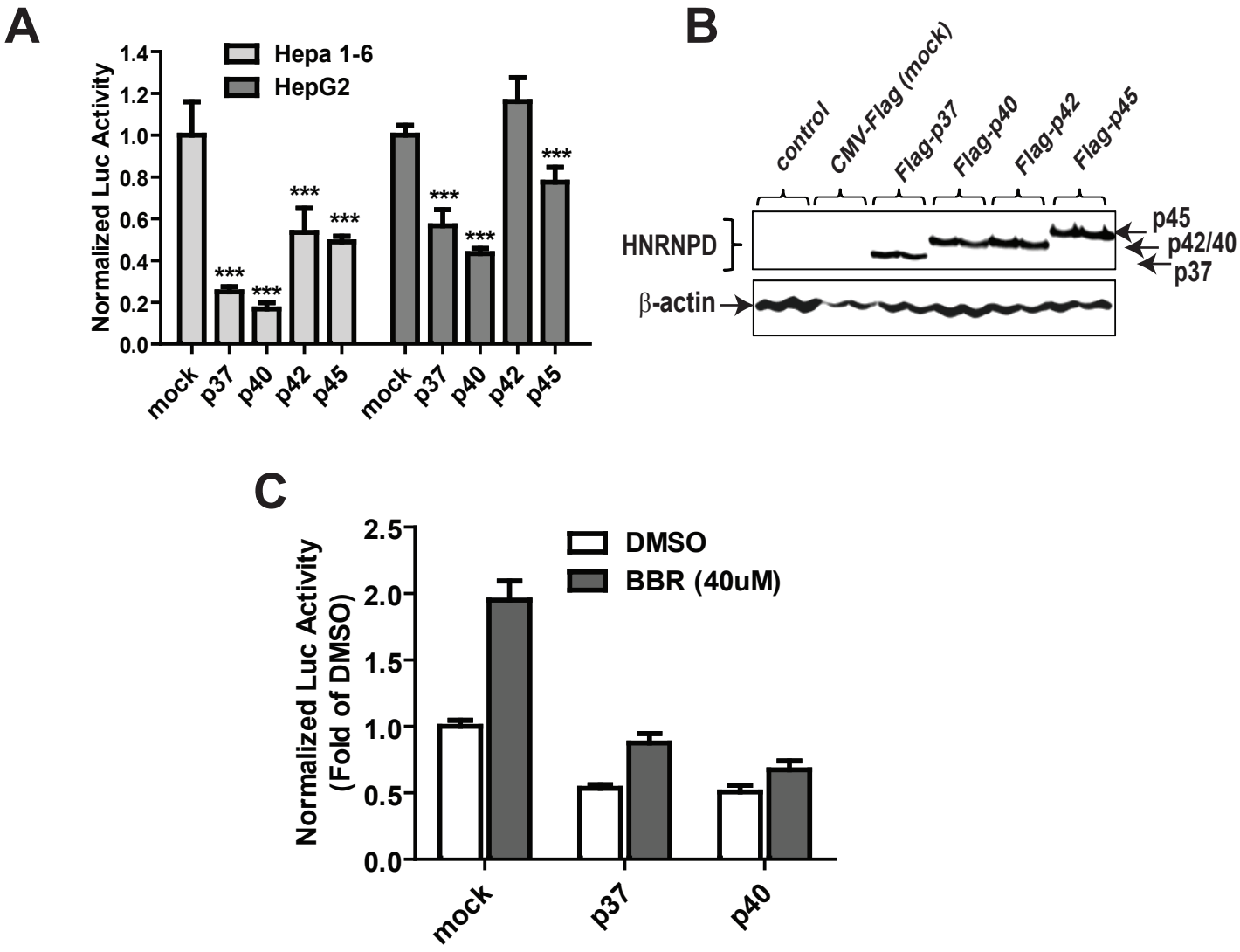
Supplemental Figure II: Characterization of mouse hnRNP D protein isoforms and hnRNPD shRNA sequence alignment with hnRNPD cDNAs.
(A) Western blot analysis of hnRNPD isoforms in mouse liver tissue, mouse primary hepatocytes (MPH) and hepatoma cells. Protein abundances of hnRNP D p37/p45 were quantified using the Alpha View Software with normalization by signals of β-actin. Values are average of two samples. The relative signal intensity of hnRNP D in liver is expressed as 1.
(B) hnRNPD shRNA shows 100% alignment with all mouse hnRNP D isoforms and human hnRNP D V1.

Supplemental Figure III



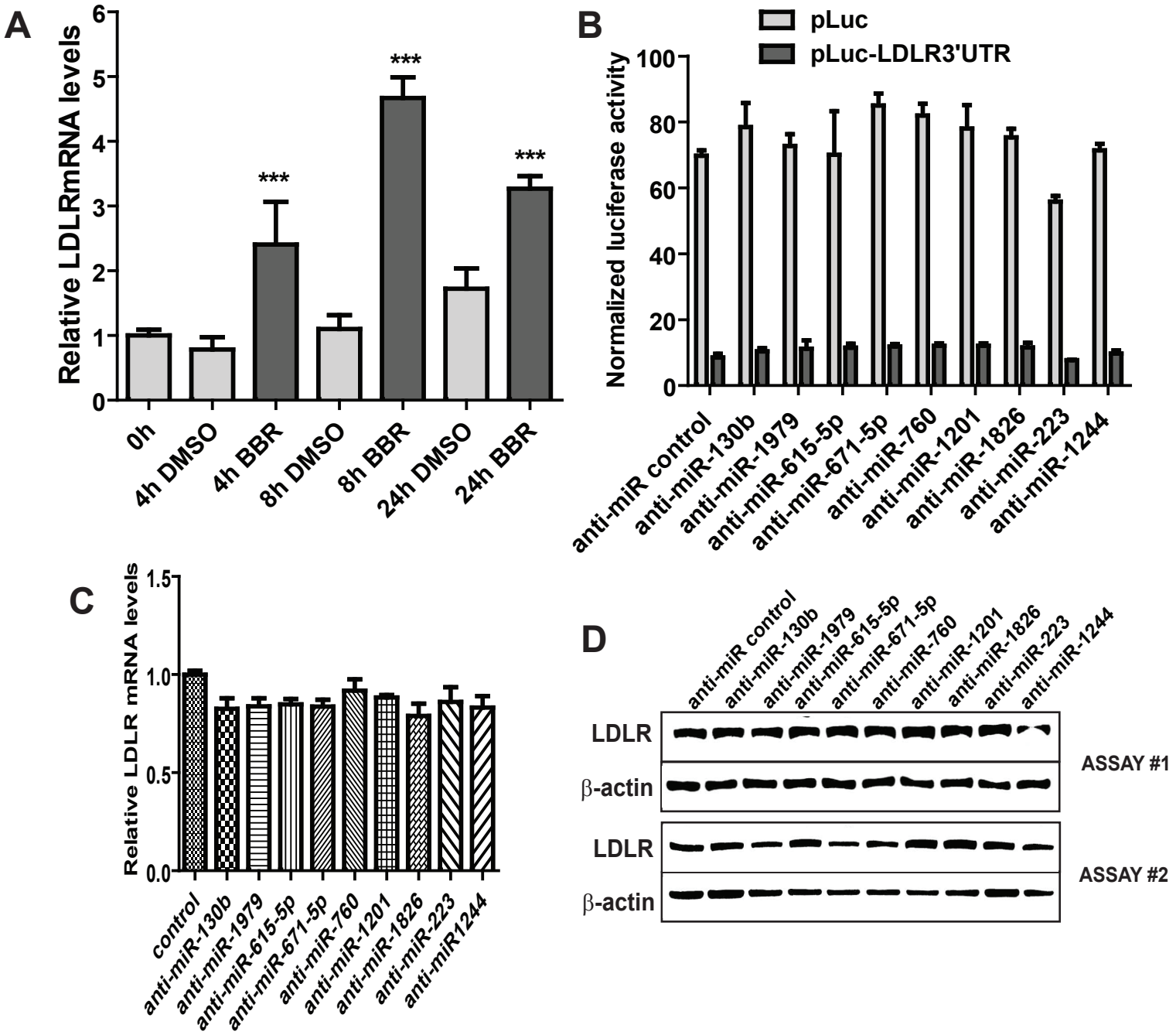
Supplemental Figure III: Exogenous expression of hnRNP D isoforms reduced LDLR3'UTR reporter activity in hepatic cells.

(A) Hepa 1-6 and HepG2 cells were cotransfected with LDLR3'UTR luciferase reporter and indicated hnRNP D isoform expression plasmids or the control vector (mock). pRL-SV40 was cotransfected in each condition for normalization of transfection efficiency. In each cell line, the normalized luciferase activity in mock-transfected cells is expressed as 1. *** $p<0.001$ as compared to the mock transfection. The graph is representative of two independent transfection experiments.

(B) Huh7 cells were transfected with flag-tagged hnRNP D isoforms or the empty vector. Cell lysates from transfected or untransfected control cells were analyzed for isoform expression using anti-flag antibody.

(C) HepG2 cells were cotransfected with LDLR3'UTR luciferase reporter and indicated hnRNP D isoform expression plasmids or the control vector (mock). One day post transfection, cells were treated with BBR or DMSO for 24 h before cell lysis. pRL-SV40 was cotransfected in each condition for normalization of transfection efficiency. The normalized luciferase activity in mock-transfected cells without BBR treatment is expressed as 1. The graph is representative of two independent transfection experiments in which six wells were used for each condition.

Supplemental Figure IV



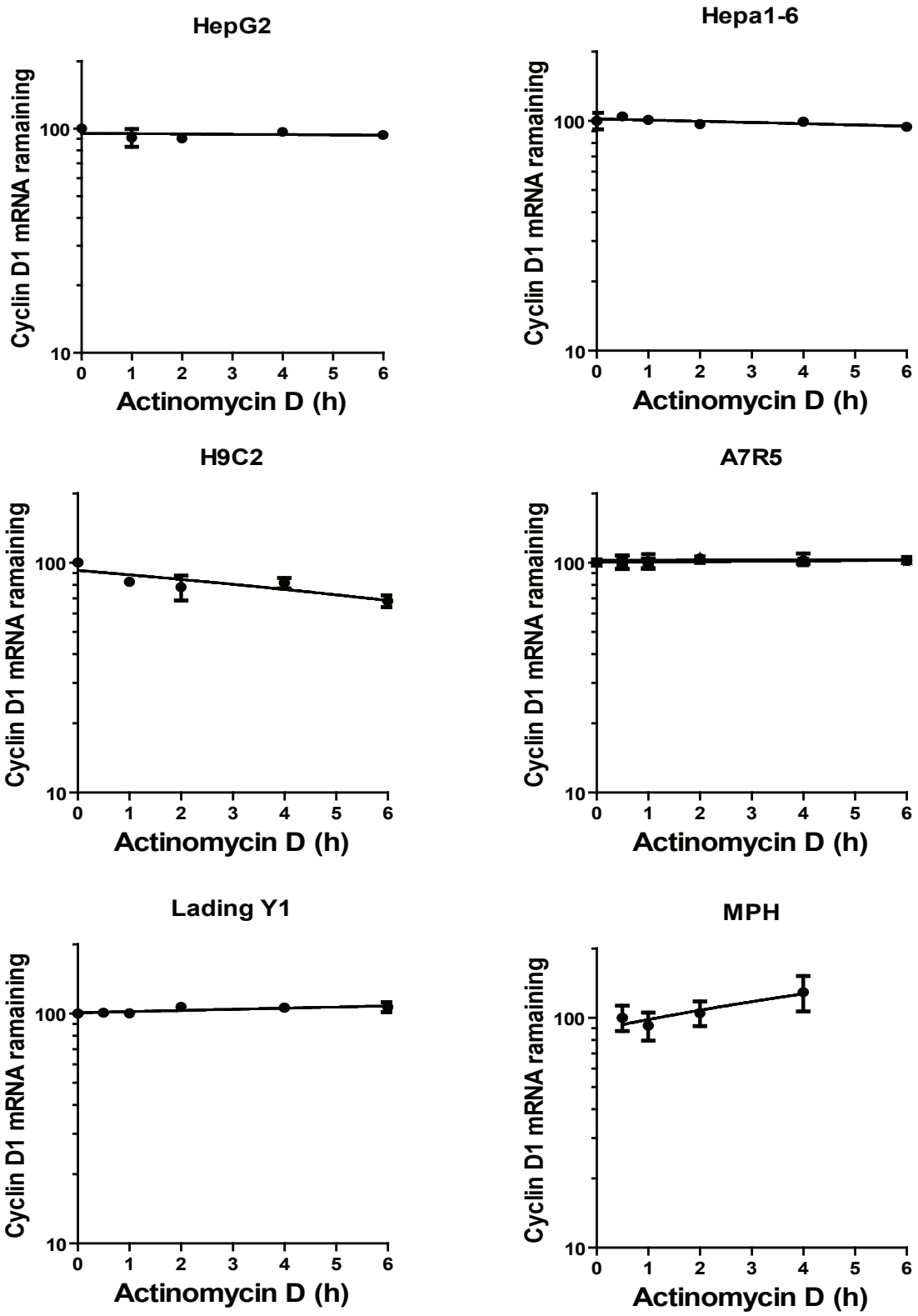
Supplemental Figure IV: Evaluation of miRNAs in LDLR3'UTR reporter activity, LDLR mRNA and protein expression in HepG2 cells

(A) HepG2 cells were treated with BBR for indicated times. Total RNA was isolated for qPCR analysis of LDLR and GAPDH mRNA expressions. After normalization with GAPDH mRNA levels, the relative levels are presented, and the results are means ± SE of triplicate measurements for each cDNA sample. *** $p < 0.001$ compared to DMSO.

(B) HepG2 cells were cotransfected with pLuc control vector or LDLR3'UTR luciferase reporter and indicated anti-miRNA oligos or the control oligo (mock). pRL-SV40 was cotransfected in each condition for normalization of transfection efficiency. The graph is representative of two independent transfection experiments in which six wells were used for each condition.

(C-D) HepG2 cells were transfected with indicated anti-miRNA synthetic oligonucleotides for three days before isolation of total RNA or toaly cell lysates for analyses of LDLR mRNA and protein expression levels.

Supplemental Figure V



Supplemental Figure V: Measurement of cyclin D1 mRNA decay rates in various cell lines and mouse primary hepatocytes (MPH). Cells were treated with actinomycin D (5 μg/ml) for different intervals. Total RNA was isolated and analyzed for the amounts of cyclin D1 mRNAs by qRT-PCR. The cyclin D1 mRNA levels were plotted as the percentage of the mRNA remaining. Decay curves were plotted versus time.