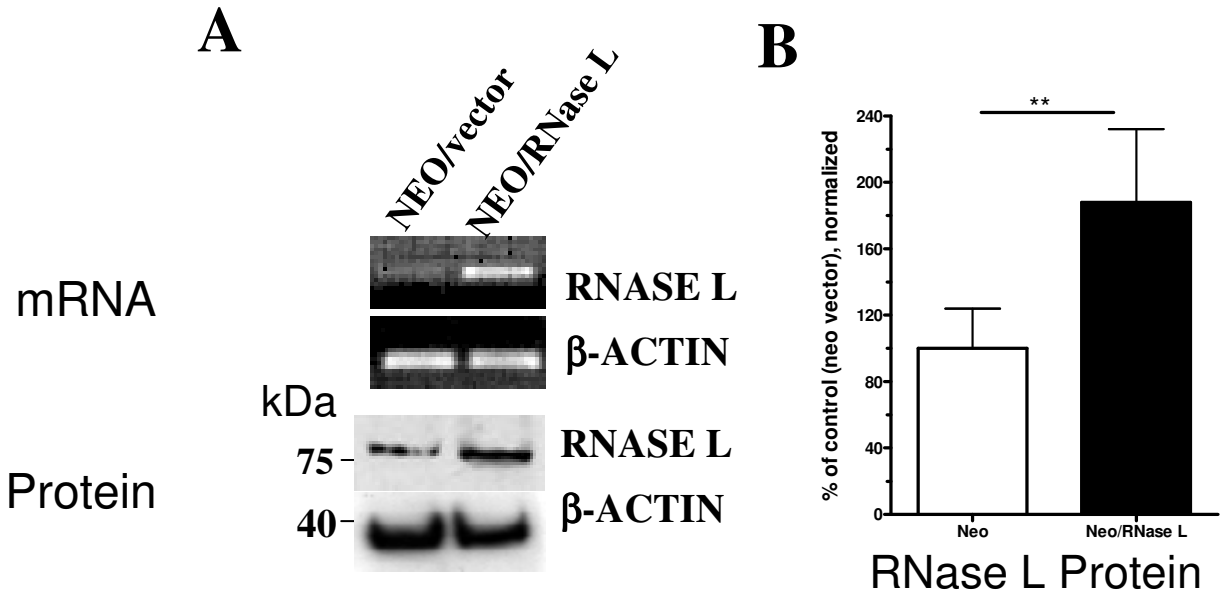
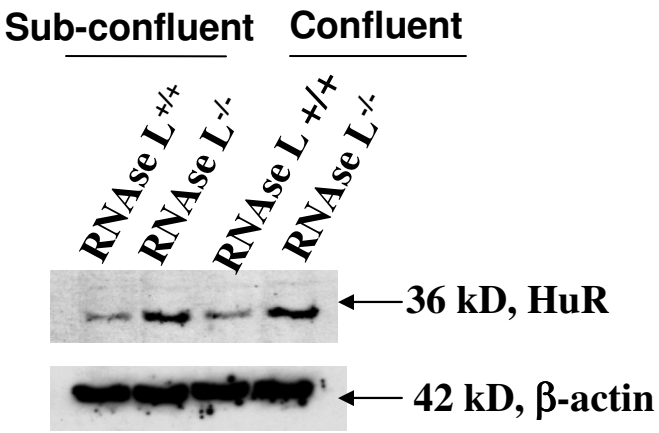
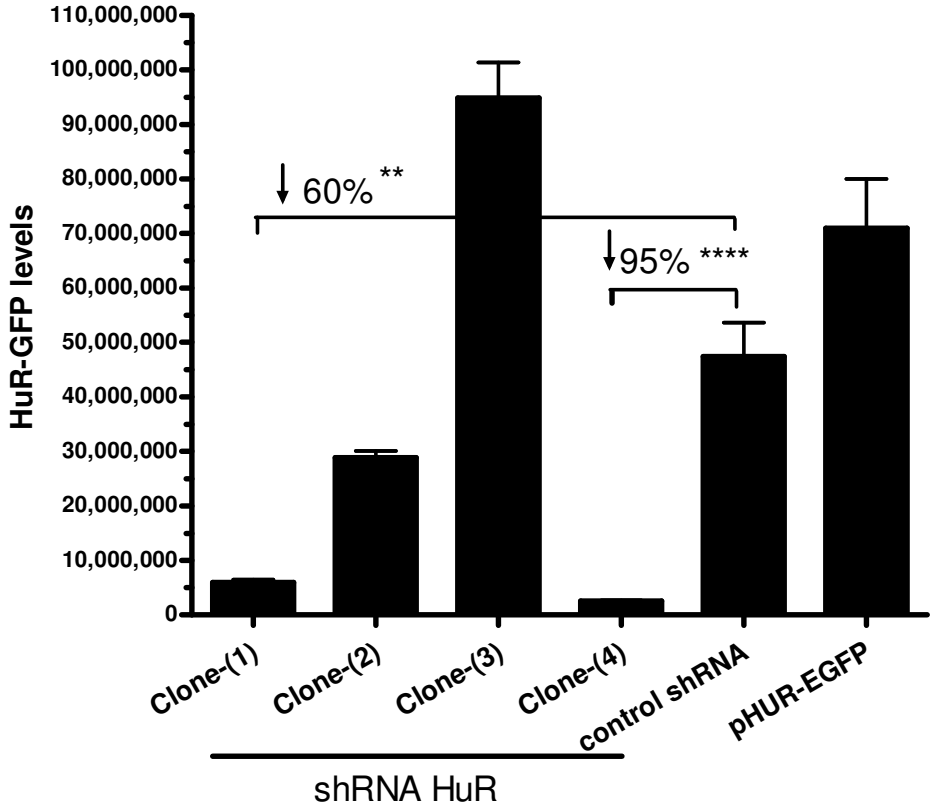
Supplemental Data



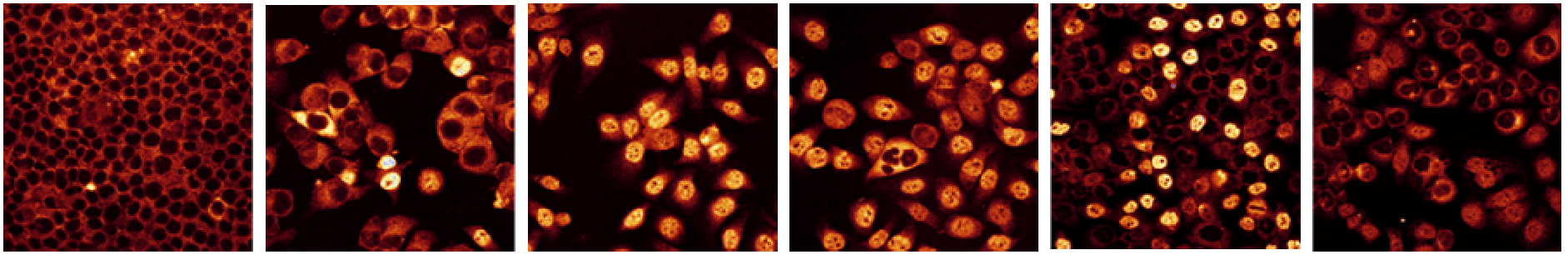
Supplementary Fig.1. Over-expression of RNase L in stable polyclonal cell line



Supplementary Fig.2 HuR protein levels in wild type and RNASEL-null MEF lines at sub-confluent and confluent conditions



Supplementary Fig 3. Functional screening for plasmids coding for short hairpin against HuR-GFP construct.



0	4	8	12	20	24 Hr
HuR: C	C	N	N+C	N+C	C
late G1		S	G2+M	G1/G0	S

serum

G_1/S -arrested by combination of confluence, serum starvation, and aphidocholin

Release to S and other phases by sub-culturing in lower density with 15% serum

Marker	%gated
All	100
Apo	2.56
G1/G0	74.99
S	16.46
G2+M	6.42

Marker	%gated
All	100
Apo	1.64
G1/G0	15.64
S	69.88
G2+M	13.47

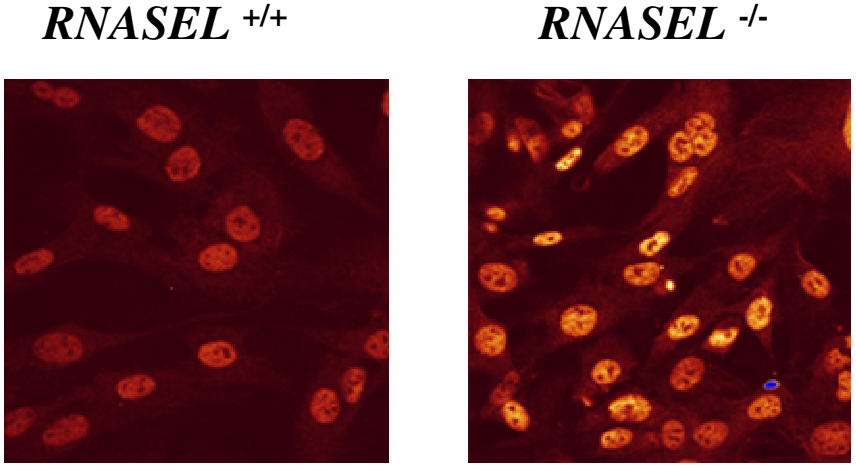
Marker	%gated
All	100
Apo	1.32
G1/G0	7.54
S	82.47
G2+M	8.99

Marker	%gated
All	100
Apo	2.5
G1/G0	10.68
S	20.79
G2+M	66.29

Marker	%gated
All	100
Apo	4.28
G1/G0	63.07
S	19.66
G2+M	13.10

Marker	%gated
All	100
Apo	3.66
G1/G0	45.62
S	36.86
G2+M	14.96

Supplementary Fig.4 Kinetics of HuR translocation. HuR is predominantly nuclear (~16 hr) with shorter period of cytoplasmic location (~ 4 hr). See Materials and Methods for details.



Supplementary Fig.5. Nuclear and cytoplasmic HuR protein in wild type and *RNASEL*-null mouse embryo fibroblasts.

Supplemental Methods:

Microarray expression and analysis

In-house made microarrays were used and contained cDNA probes representing ARE-cDNA clones in which their identities were obtained from the AU-rich element-containing mRNA database (ARED; (Bakheet *et al.*, 2001). The cDNA probes were generated by PCR and spotted at least in duplicate on UltraGAPS II slides (Corning) using the Microgrid spotting robot (Genomic Solutions, San Diego, California). The microarrays were used for co-hybridization, utilizing Genisphere kit (Genisphere, Inc., Hatfield, PA), with cDNA generated from total RNA (20 μg) labeled with Cy3 and Cy5 for control and experiment, respectively; details were previously described (Khabar *et al.*, 2004). Scanning was performed with a ScanArray scanner and the intensity of green and red fluorescent signals from each spotted cDNA sequence on the microarrays was calculated using adaptive circle algorithm and mean intensity of the pixels. Pre-processing, filtering of erroneous signals, normalization procedures, and calculation of intensity ratios were previously described in detail (Khabar *et al.*, 2004).

Semi-quantitative and Real Time RT-PCR

Total RNA was extracted using Trizol method. RT reaction was performed using 200 ng total RNA, 500 ng oligo $dT_{(18-23)}$, 500 mM dNTP mixture, 20 U RNAsin (Pharmacia), 200 U of SuperScript II (Invitrogen). Hot start PCR amplification was performed using Taq DNA polymerase (Qiagen). cDNA was amplified according to an amplification curve, for example, β-actin cDNA was amplified with 28 cycles, whereas, HuR or RNAse L cDNA was amplified with 34 cycles. The amplification curve was determined for each

cDNA of interest by plotting increasing cycle numbers against ethidium bromide stained gel intensity of the amplified products. The optimum cycle number chosen was within the exponential phase of the curve. Cycling was 94° C for 60 s, 60°C for 60 s, and 72°C for 60 s using primers specific to HuR coding region. The RNase L forward primer is: 5′ CGTCATGGAGAGCAGGGAT3′ and the reverse primer is: 5′GAGTCAGCACCCAGGGCT GG3′. The mouse HuR Forward primer is: 5′CCCAAGCTCAGAGGTCATC 3′ and mouse HuR Reverse is 5′GAGTGGTACAGCTGCG AGAG3′. The β-actin forward primer is 5′ATCTGGCACCACACCTTCTACAATGAG CTGCG3′ and the β-actin reverse primer: 5′CGTCATACTCCTGCT TGCTGATCCACA3′. The β-actin primers were designed to span short intronic sequences so that the larger PCR products would be shown in case there was genomic DNA contamination. In all of the above, PCR conditions allowed at least semi-quantitative comparisons of signal strength on agarose gels as the cycle number was chosen in the exponential range which has not reached the plateau.

For real time measurements, primers specific to EGFP that span the CMV promoter intron A –to control DNA contamination- and 6-carboxyfluorescein (6FAM)-labeled TaqMan probe that target CMV exon 1-EGFP (exon 2) junction sequence –control DNA contamination- were custom synthesized by PE Applied Biosystems. The control β-actin probe was labeled with a 5′ reporter dye, tetrachloro-6-carboxyfluorescein (TET). The real time PCR was run on MJ research thermocycler instrument. Standard curves for each gene were generated to determine the relative concentrations of amplifed transcripts. The concentration of each transcript was then normalized to β-actin and the normalized values were used to calculate half-lives as described below.

Statistics and image analysis

For comparison between two groups (columns on figures) the student's paired t test was used. Two-tailed probabilities were reported. Densitometry, band detection, background subtraction, and normalization of images were performed using ImageMaster Software (Amersham). The background-substracted band signals were normalized using β-actin band signals. Background signals of each band was taken from the vicinity of the band. The one-phase exponential decay curve analysis (GraphPad Prism) was used to assess mRNA decay kinetics as previously described (Khabar *et al.*, 2003). y= SPAN X e^{xK} X PLATEAU describes the kinetics of mRNA decay. x is time, and y *is* mRNA levels (β-actin normalized signals) . y begins equal to SPAN + PLATEAU and decreases to PLATEAU with a rate constant K. The half-life of the decay is 0.6932/K. SPAN and PLATEAU are expressed in the same units as the y axis. K is expressed in the inverse of the units used by the x axis. The exponential growth equation Y=Start*exp(K*X) where Y=Start and increases exponentially with rate constant K, was used to assess cellular growth curve fits to allow statistical comparison (F test). In those data with multiple comparisons, the two-way ANOVA with Bonferroni post-test was also used.