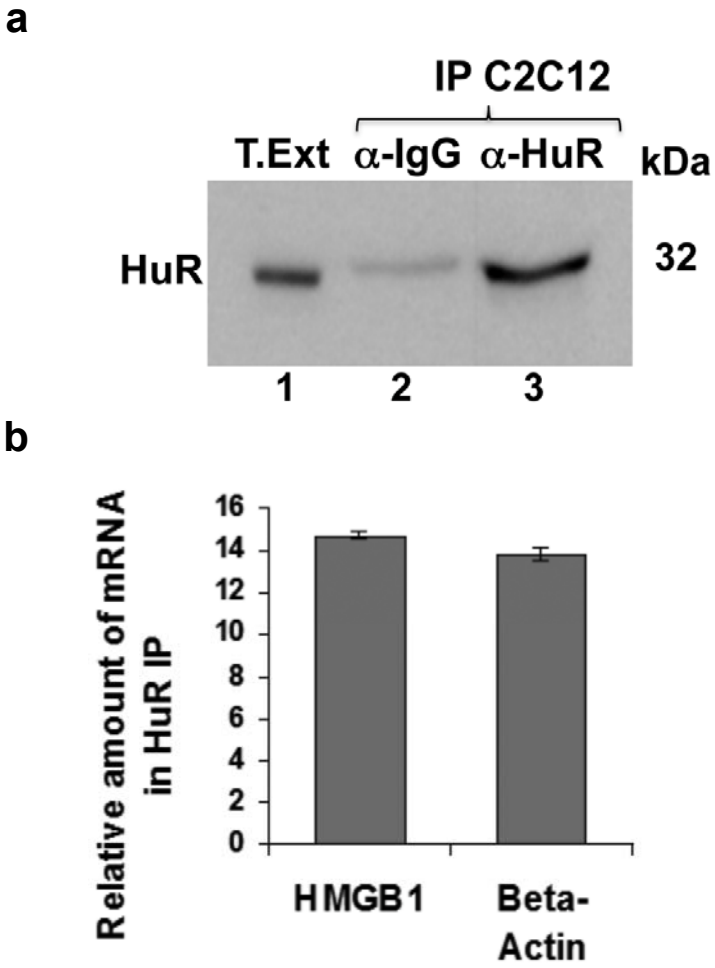
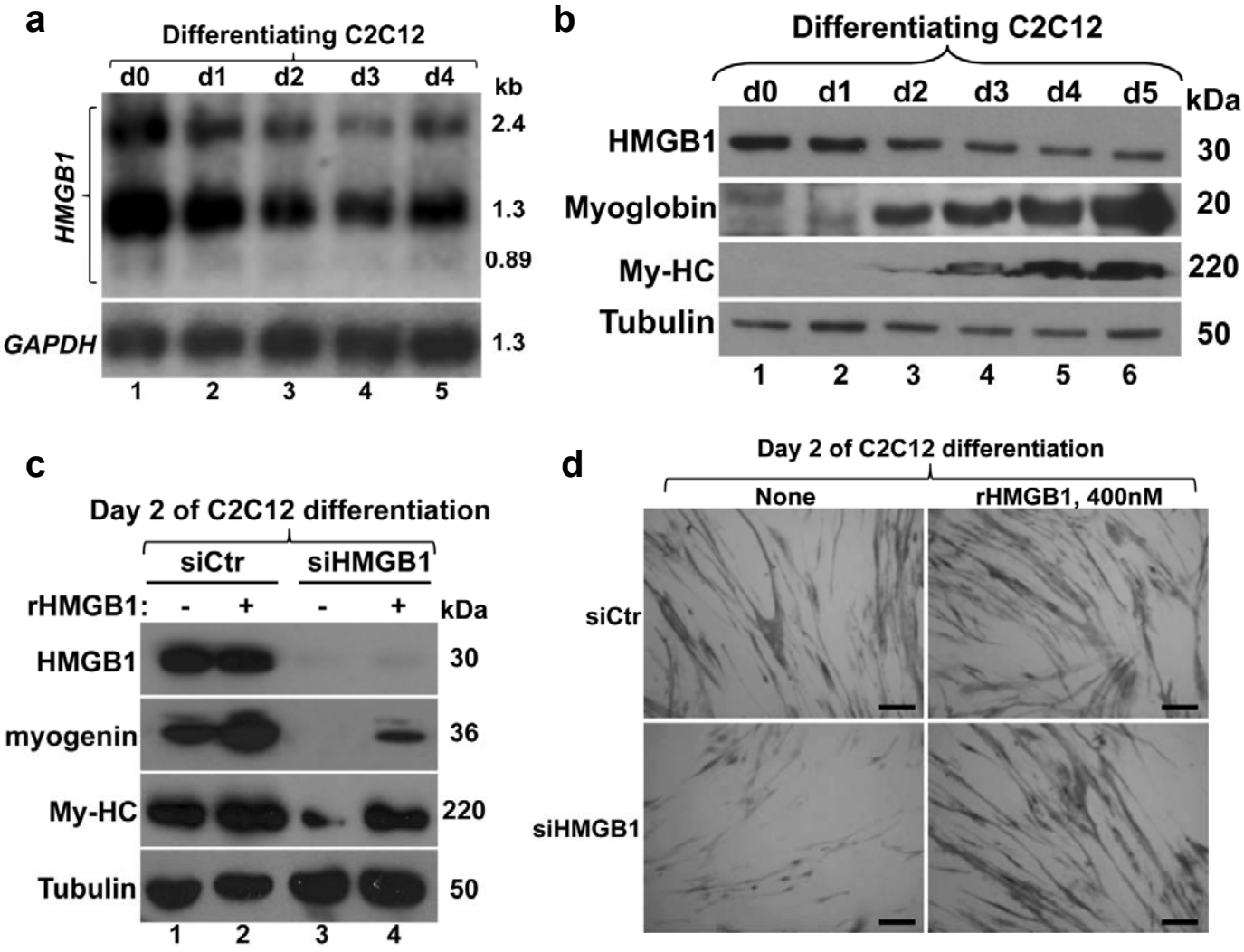
Supplementary Figure S1: *HuR associates with HMGB1 mRNA.*



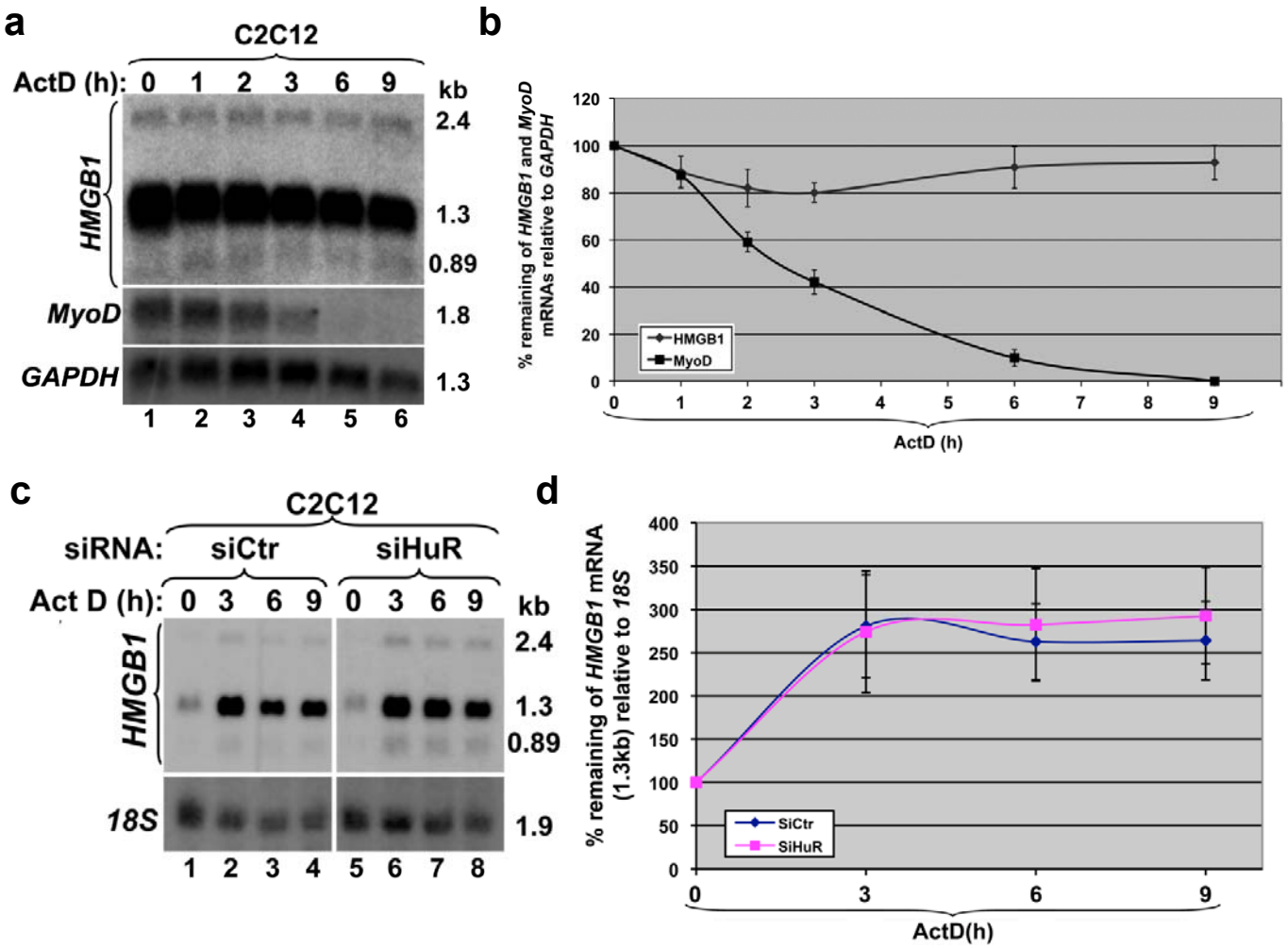
(a) Total cell extracts from C2C12 cells were used for immunoprecipitation (IP) experiment with an anti-IgG antibody and as a control with an anti-HuR antibody that was also used for western blotting. **(b)** RNA was isolated from the IP in (a), and quantitative RT-PCR was performed using primers specific to *HMGB1* and *β-Actin*. The non-specifically associated *GAPDH* mRNA was used as a reference message to normalize mRNA levels in our immunoprecipitate. The level of *HMGB1* and *β-Actin* mRNAs in HuR IP was determined relative to their level in the IgG IP. The relative amounts of these messages were plotted +/- the standard error of the mean (S.E.M) of four independent experiments.

Supplementary Figure S2: *HMGB1 mRNA and protein are highly expressed in undifferentiated C2C12 while their levels decrease in mature muscle fibers.*



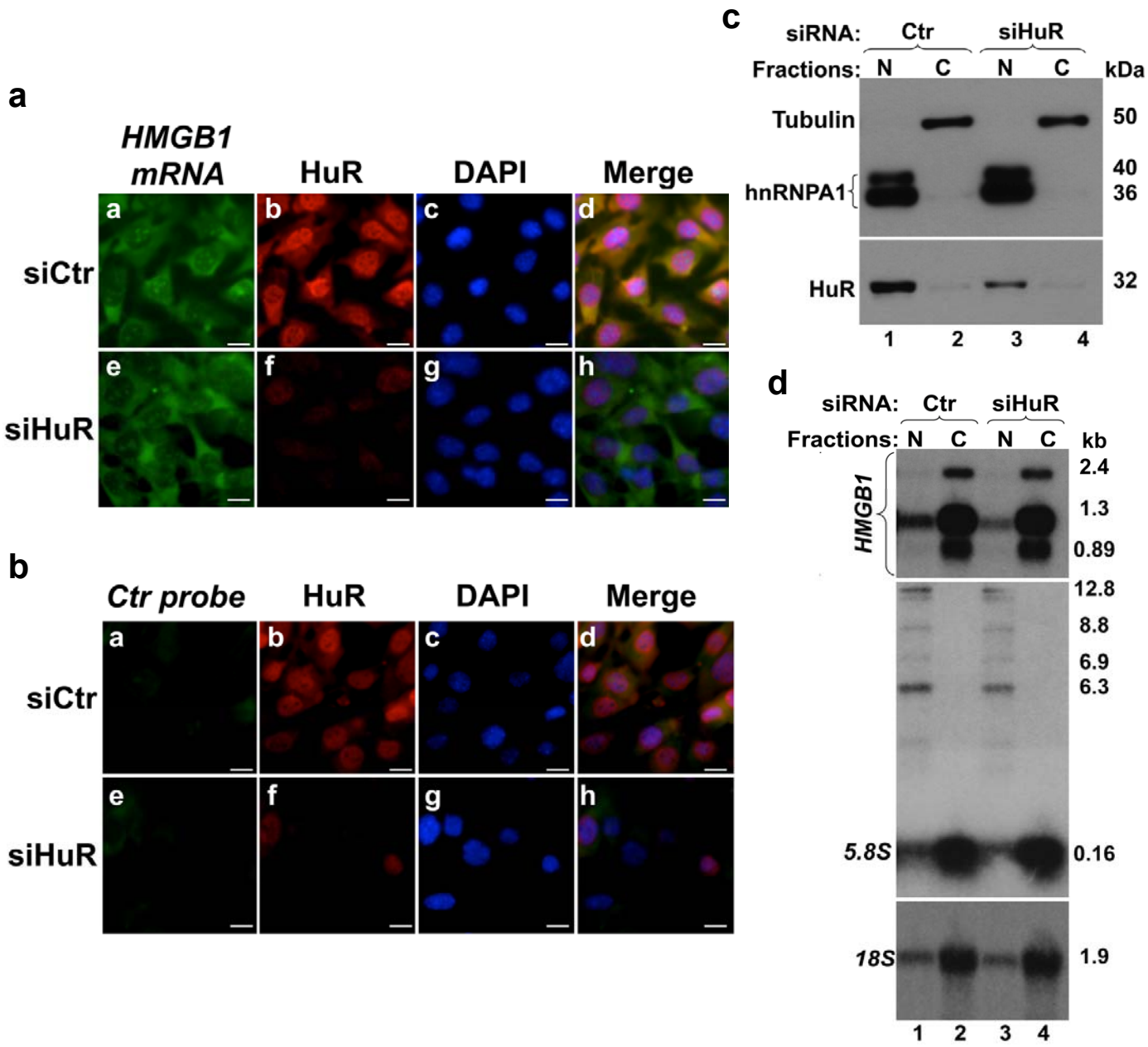
(a) Total RNA was isolated from confluent cells (d0) and during the differentiation process (d1 to d4). Northern blotting was performed using radiolabeled probes against *HMGB1* and *GAPDH* as a loading control. **(b)** Total cell extracts were prepared from differentiating C2C12 cells as indicated for a. Western blotting on these extracts was performed using a polyclonal antibody against HMGB1. Myoglobin and Myosin heavy chain (My-HC) were used to control differentiation progression and α-tubulin as a loading control. Blots shown in a and b are representative of three independent experiments. **(c, d)** *Exogenous HMGB1 promotes myogenic differentiation in myoblasts knocked down for endogenous HMGB1.* C2C12 cells depleted of HMGB1 were induced for differentiation DM in the absence or presence of 400nM of recombinant HMGB1 (rHMGB1) protein. (c) Western blot analysis was performed using antibodies against HMGB1, myogenin and My-HC, and tubulin as a loading control. The blot is representative of three independent experiments. Note that silencing of HMGB1 results in reduced myogenin and My-HC expression; however, treatment of HMGB1-siRNA-treated myoblasts with rHMGB1 rescues their myogenic potential. (d) Immunohistochemistry analysis of cells treated as described in (c) using an anti-My-HC antibody. Representative images from three independent experiments are shown. *Scale bars, 100 μm*.

Supplementary Figure S3: *The long half-life of HMGB1 mRNA is independent of HuR.*



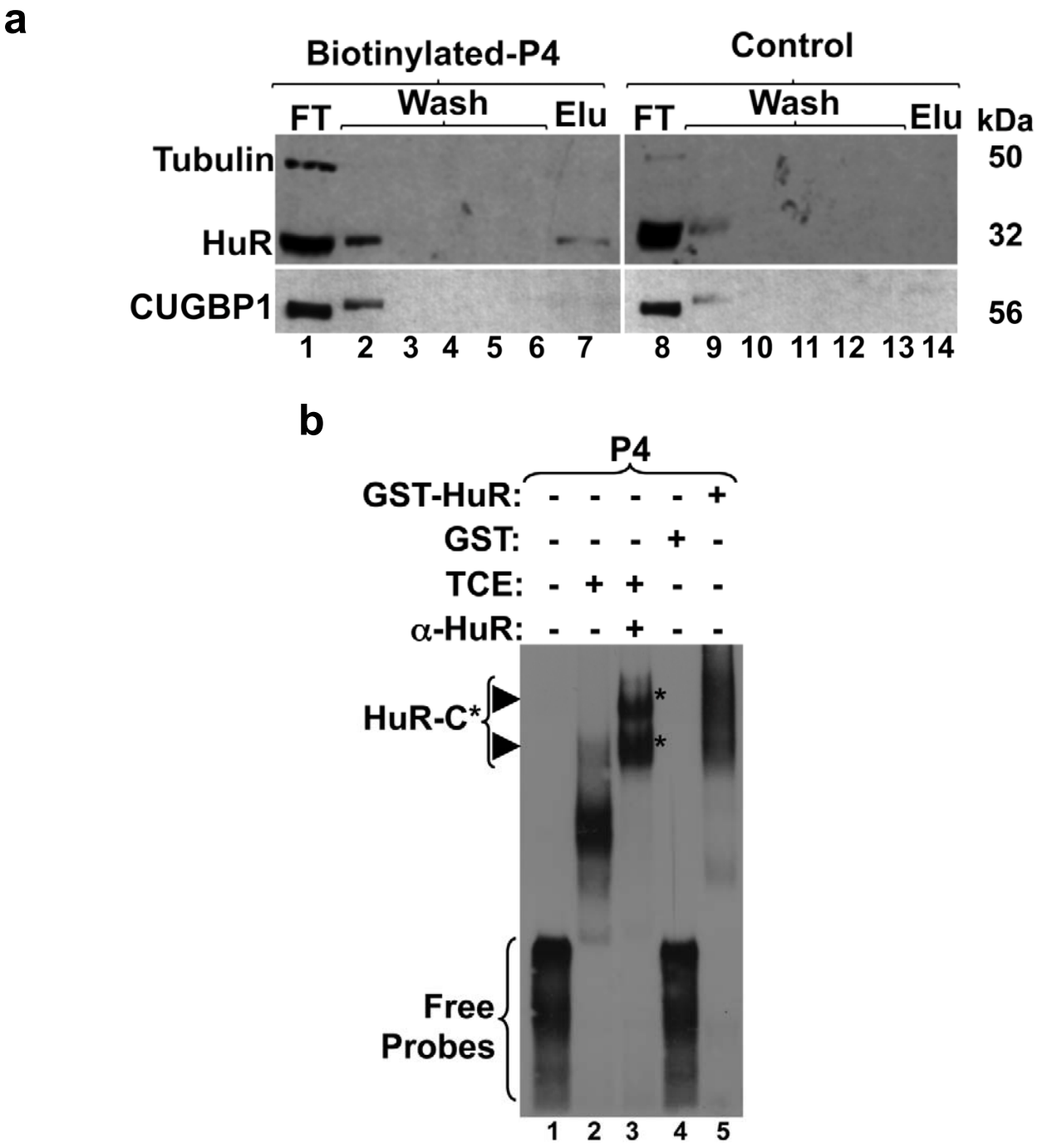
(a) Exponentially growing C2C12 cells were treated with ActD for 0, 1, 2, 3, 6 or 9 hours. The mRNAs of *HMGB1*, *MyoD* and *GAPDH* (loading control) were detected by northern blot. (**b**) The amount of *MyoD* message and the 1.3kb isoform of *HMGB1* mRNA were quantified using the ImageQuant software. mRNA stability was determined by normalizing the amount of *HMGB1* and *MyoD* mRNAs for each time point against the corresponding quantity of *GAPDH* mRNA. Each time point was then normalized against the abundance of mRNA at 0h of ActD treatment, which is considered 100%. These percentages were then plotted +/- S.E.M of three independent experiments. **(c, d)** The stability experiments and northern blotting (c) as well as the statistic analysis (d) were performed as described for (a-b) except that we used total RNAs prepared from siCtr- and siHuR-treated C2C12 cells.

Supplementary Figure S4: *HuR is not involved in the cellular movement of HMGB1 mRNA*.



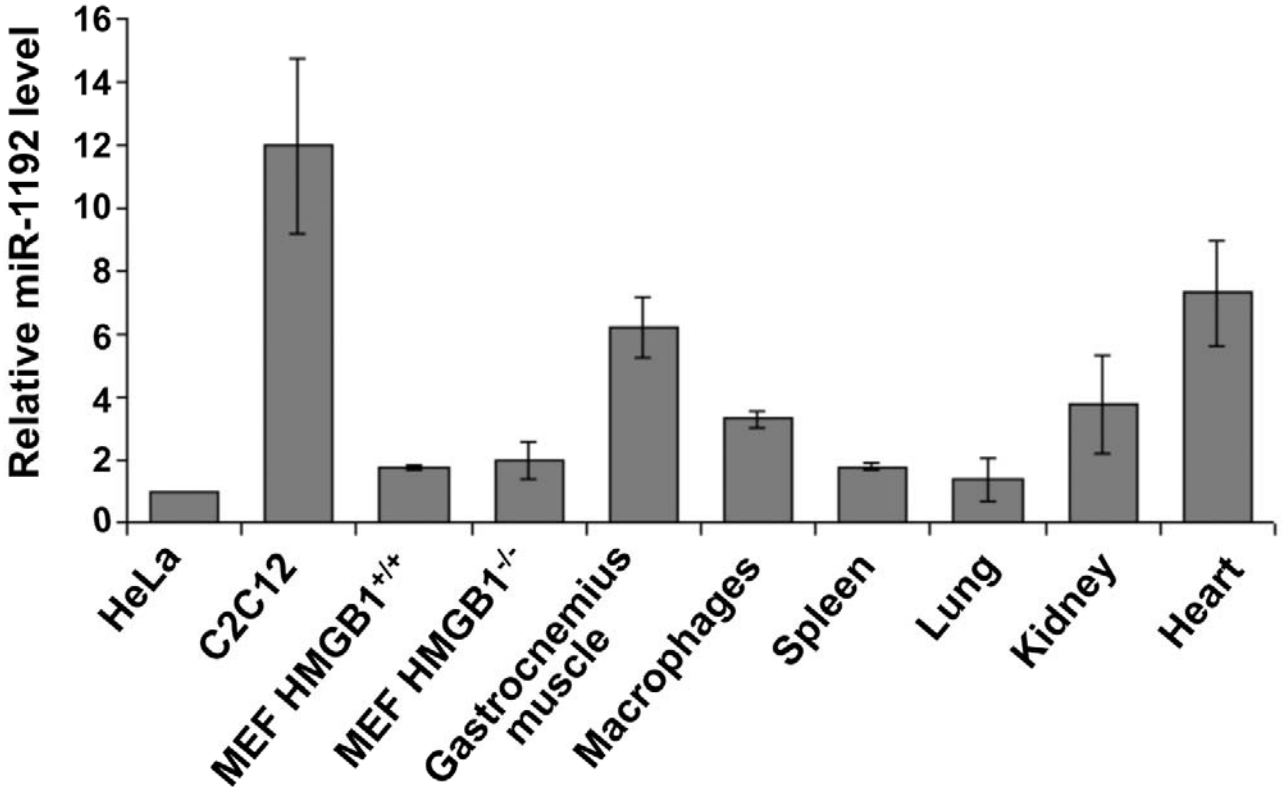
(a, b) C2C12 cells transfected with siHuR or siCtr were fixed, permeabilized, and incubated with digoxigenin-labeled *in vitro* transcribed antisense probe to detect *HMGB1* mRNA (*a, panels a, e*) and with sense RNA probe (*b, panels a, e*) as a control (*Ctr probe*). Detection of HuR with anti-HuR antibody enabled the visualization of the knockdown of HuR in these cells. A single representative field for each cell treatment is shown. *Scale bars, 20 μm*. **(c)** Exponentially growing C2C12 cells were transfected with siCtr or siHuR. 48 hours after transfection cells were fractionated into nuclear and cellular fraction that were used for western blotting. Membranes were probed with anti-HuR, -α-tubulin (as a control for cytoplasmic fractions) -and hnRNP A1 (as a control for nuclear fractions) antibodies. **(d)** Total RNA prepared from the same fractions described in (c) were used for northern blotting. The membrane was probed with specific radiolabeled probes against *HMGB1*, *18S* (as a loading control) and 5.8S (as a control for fractionation quality). All blots shown in c and d are representative of three independent experiments.

Supplementary Figure S5: *HuR binding site in the HMGB1-3'UTR*.



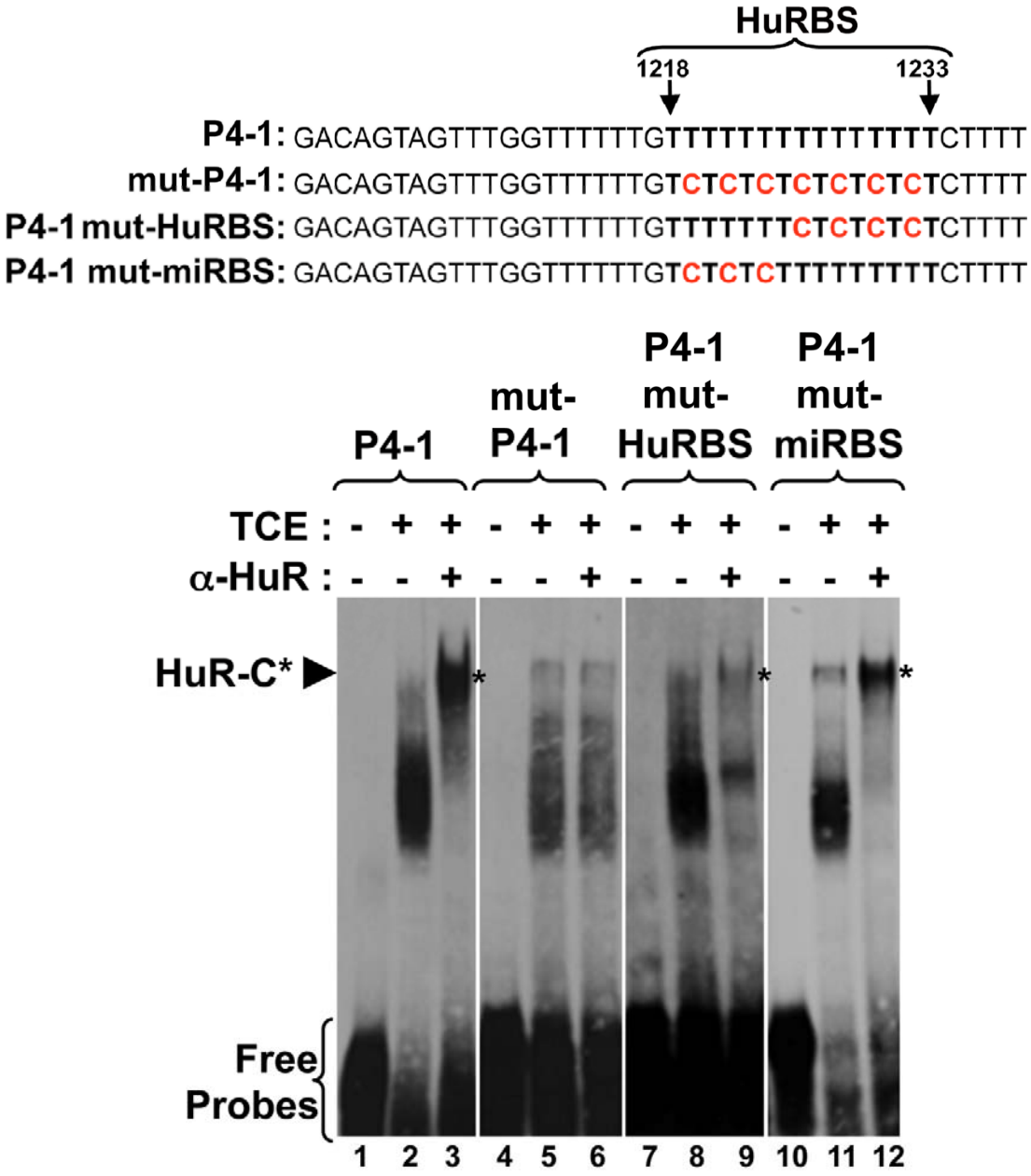
(a) *Biotinylated P4 probe associates with HuR but not with CUGBP1.* The P4 probe described in Figure 5a was biotinylated and used for a pull-down assay. Total extracts from C2C12 cells were incubated with Streptavidin beads coated or not with Biotynilated P4. Then, the beads were washed four times before elution with 1M NaCl. The four wash as well as the elution products were analysed by western blot using the anti-HuR, -CUGBP1 and α-tubulin antibodies. **(b)** *HuR directly associates with a U-rich element within HMGB1 3'UTR*. Gel-shift binding assay was performed by incubating total cell extracts (TCE) prepared from C2C12 cells with the radiolabelled cRNA probe spanning the P4 region or by incubating 300 ng of purified GST or GST-HuR protein. This blot is a representation of three independent experiments.

<u>Supplementary Figure S6</u>: *miR-1192, a novel miRNA that associates with HuR, is expressed in C2C12 cells and muscle tissues*.



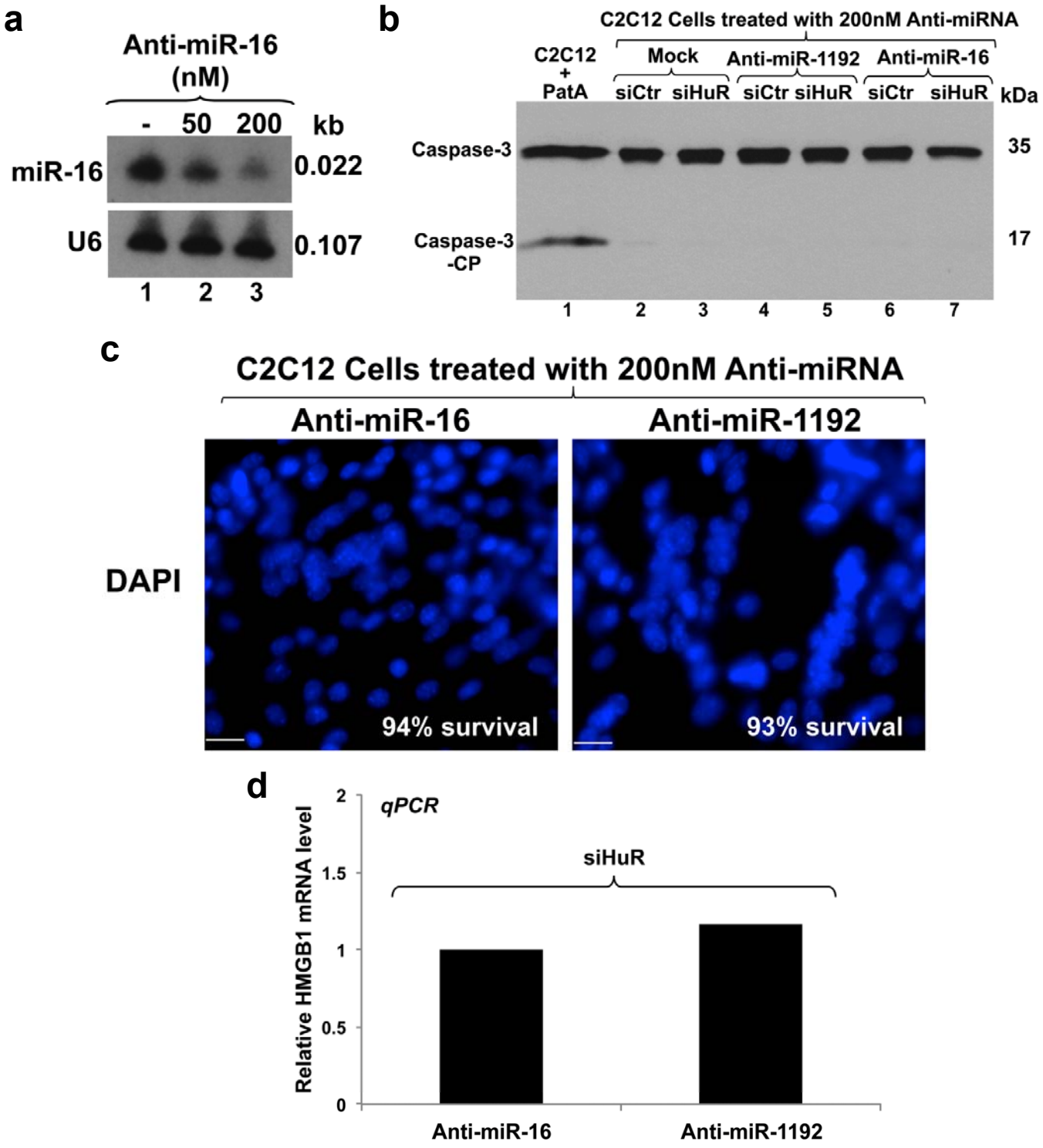
Total extracts from cells [HeLa, C2C12, macrophages and murine embryonic fibroblasts (MEFs) expressing or not HMGB1] or mice tissues (gastrocnemius muscle, spleen, lung, kidney and heart) were prepared and used for RT-qPCR to assess the expression levels of miR-1192. The level of the miR-1192 was determined relative to the level of the miR-16 in each sample and plotted as a relative percentage +/- the S.E.M. of three independent experiments.

Supplementary Figure S7: *A U_8 element (nts 1226-1233) is sufficient to mediate HuR binding to the HMGB1-3'UTR.*



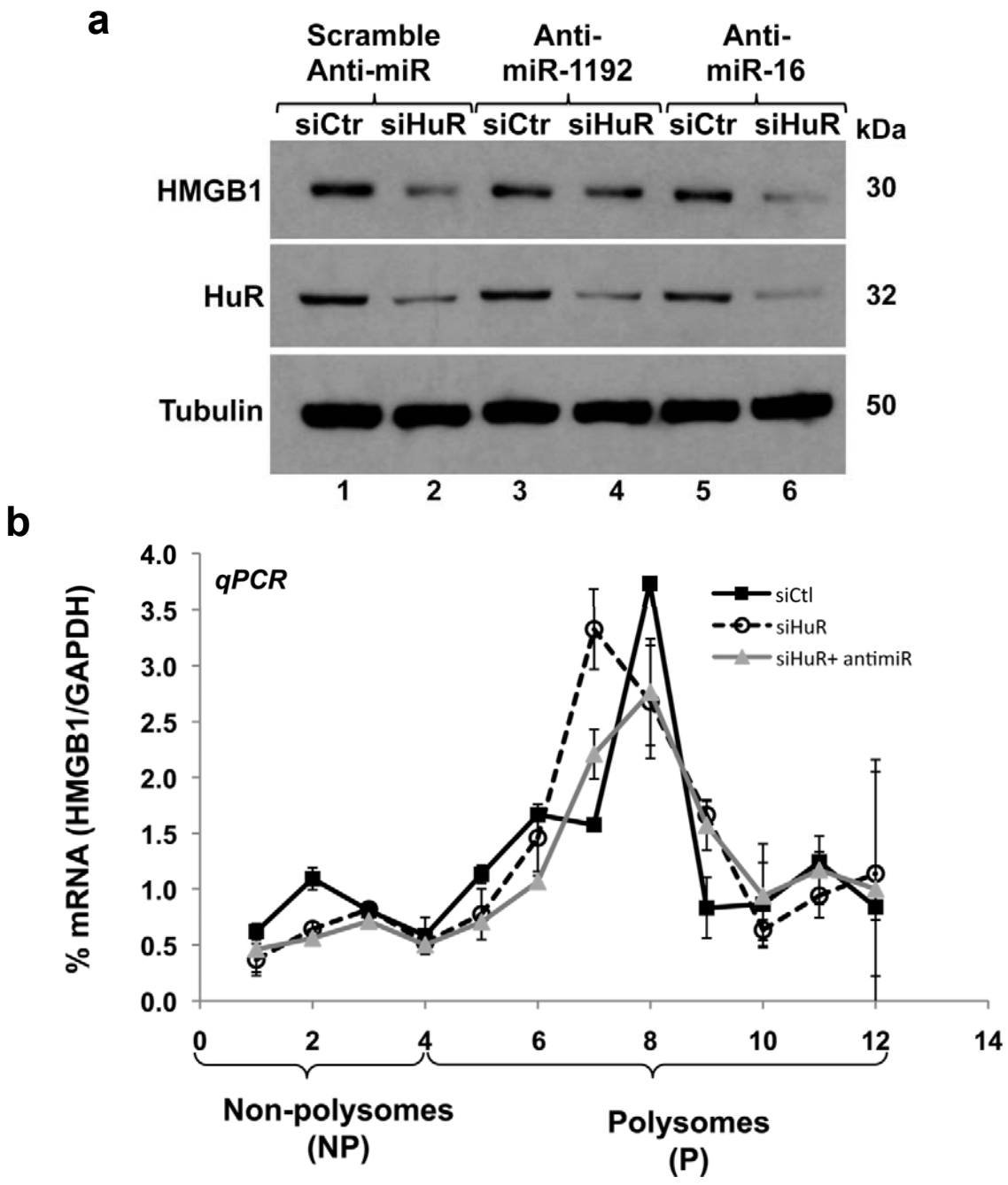
Nucleotide sequence of the HuR binding site (HuRBS) within the P4-1 region showing the two new probes (P4-1 mut-HuRBS and P4-1 mut-miRBS) that were generated by mutatiting the right part of the HuRBS or the miR-1192 seed element respectively (upper panel). Gel-shift binding assay was performed by incubating total cell extracts (TCE) from C2C12 cells with radiolabeled probes P4-1, mut-P4-1, P4-1 mut-HuRBS and P4-1 mut-miRBS. Supershift analysis was carried out where indicated (+ αHuR). Asterisks indicate supershifted complexes (lower panel). This blot is a representation of three independent experiments.

Supplementary Figure S8: *Silencing miR-16 in C2C12 cells does not induce cell death nor affect the steady state level of HMGB1 mRNA.*



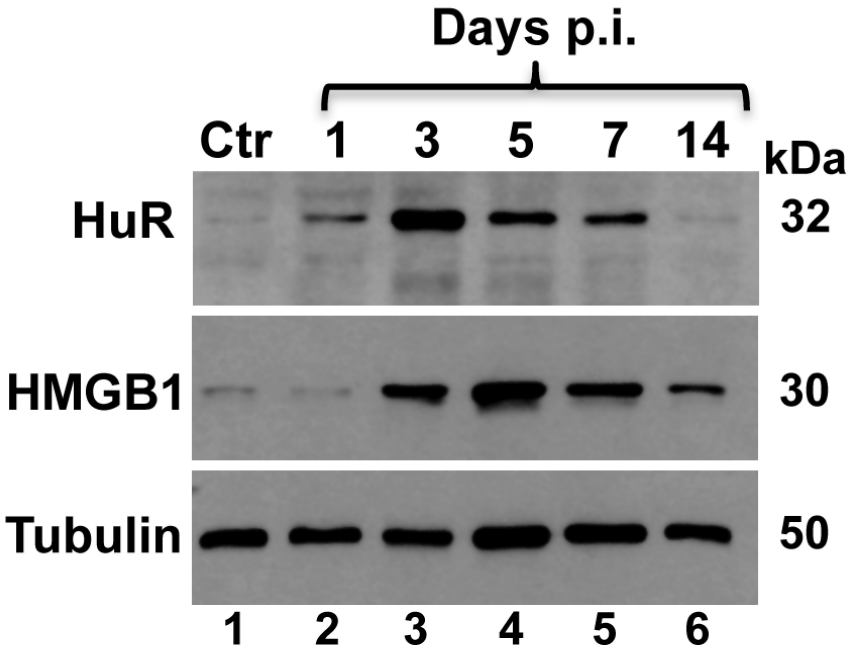
(a) 50 or 200 nM miR-16 antagomir (Anti-miR-16) were transfected in myoblasts. 48h later, RNA was extracted and northern blot was performed using Starfire-probe for miR-16 and a U6 probe as a control. This blot is a representation of two independent experiments. **(b)** Extracts from C2C12 treated with the translation inhibitor Pateamine A (PatA) (a known inhibitor of inducer of apoptosis [64-66]) as well as the same extracts described in Figure 8a, were used for western blot with anti-Caspase-3 antibody. **(c)** C2C12 cells treated with 200nM anti-miR-1192 or -16 and used for DAPI staining to assess cell viability. *Scale bars, 20μm*. **(d)** C2C12 depleted of endogenous HuR were treated as described in C. Total RNA was prepared and subjected to RT-qPCR analysis using specific primers for *HMGB1* and *GAPDH* mRNAs.

Supplementary Figure S9: *Anti-miR-1192 recovers HMGB1 translation in HuR-depleted C2C12 cells.*



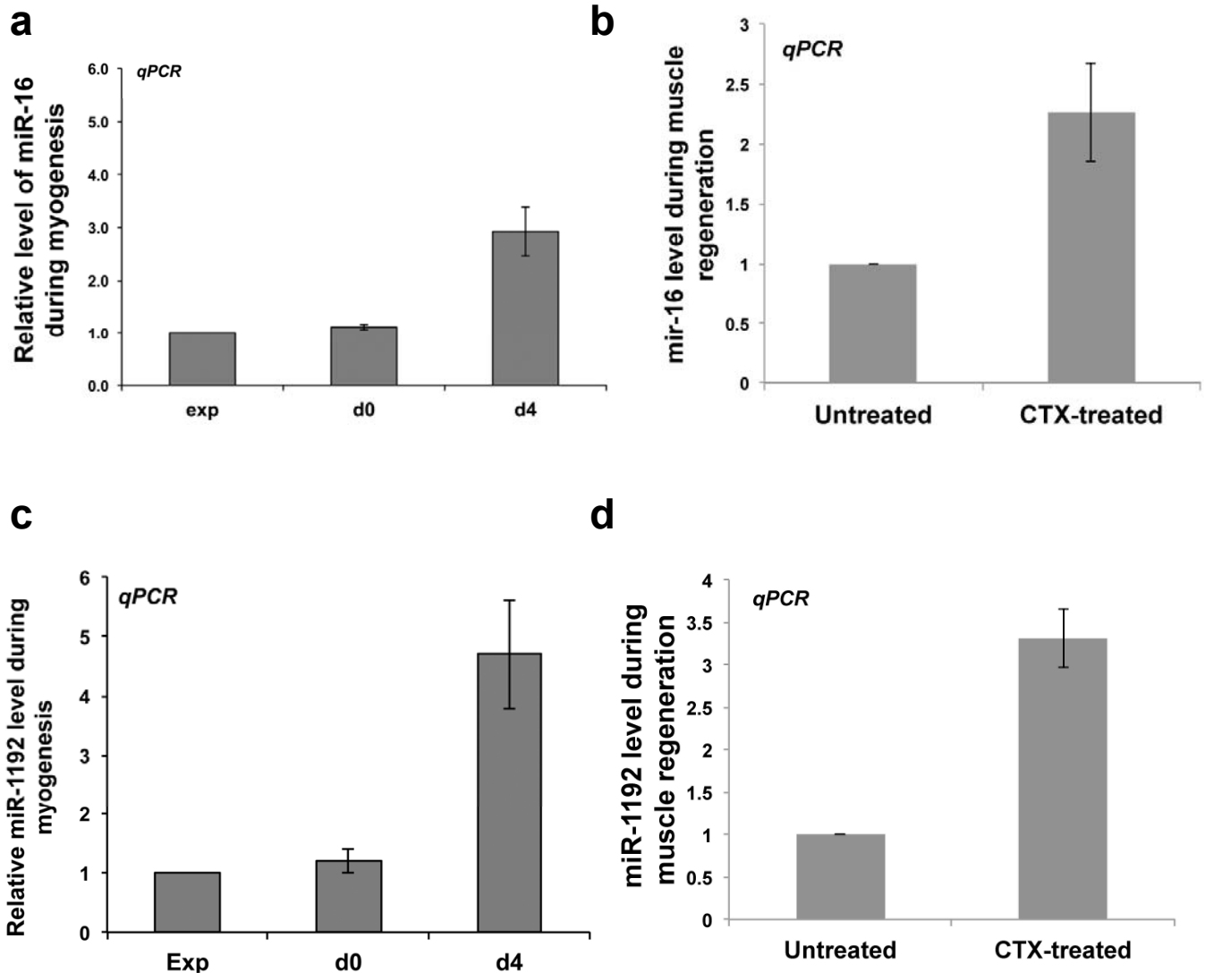
(a) C2C12 cells were treated as described in Figure 8a except that scramble anti-miRNA were used instead of the Mock treated cells. **(b)** C2C12 cells treated with siHuR alone or with both siHuR and an Anti-miR-1192 were subjected to sucrose fractionation and analyzed as described in Figure 4F. Error bars represent S.E.M. from two independent experiments.

Supplementary Figure S10: *The expression of HuR and HMGB1 increases during muscle regeneration in vivo.*



Western blot analysis of HuR and HMGB1 protein levels during muscle regeneration. GAPDH protein levels are included as a loading control.

Supplementary Figure S11: *The expression profile of miR-1192 and miR-16 during myogenesis in vitro and muscle regeneration in vivo.*

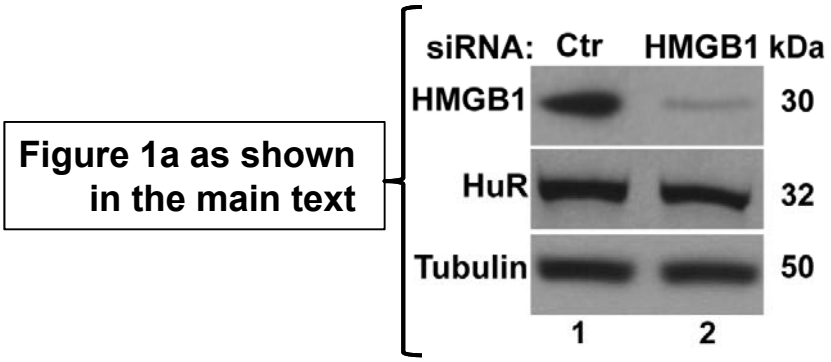


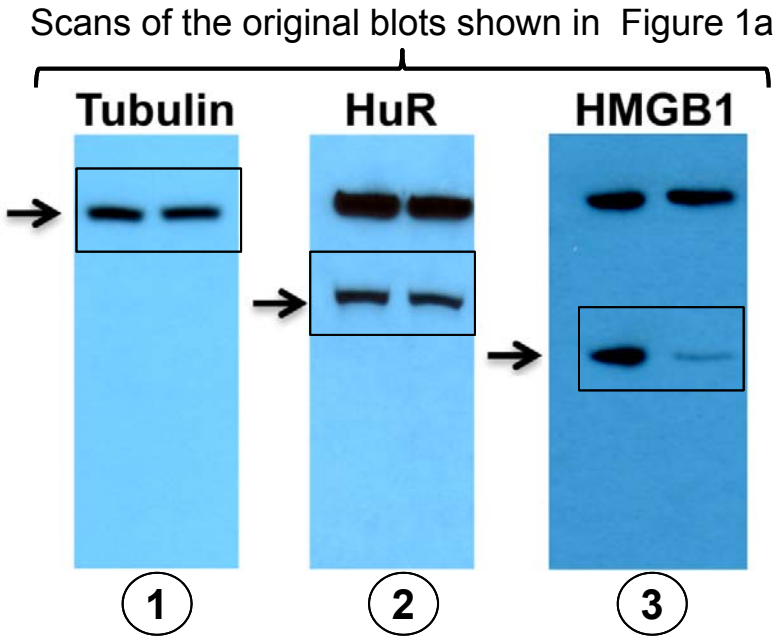
Total RNA extracts prepared from differentiating C2C12 cells (**a** and **c**) or from regenerating Tibialis muscle as described in Figure 3 (**b** and **d**) were subjected to RT-qPCR analysis using specific primers for miR-16 or miR-1192 as described in Supplementary Figure S6. Error bars represent S.E.M. from two independent experiments (a and c) and three Tibialis muscles (b and d).

<u>Supplementary Figure S12</u>: Scans of the blots shown in the main and Supplementary Figures .

Each panel is shown in a separate page. The full panel as shown in Figures or Supplementary Figures is presented at the top, followed by the full blot of each sub-panel. A legend is also provided at the bottom of each page.

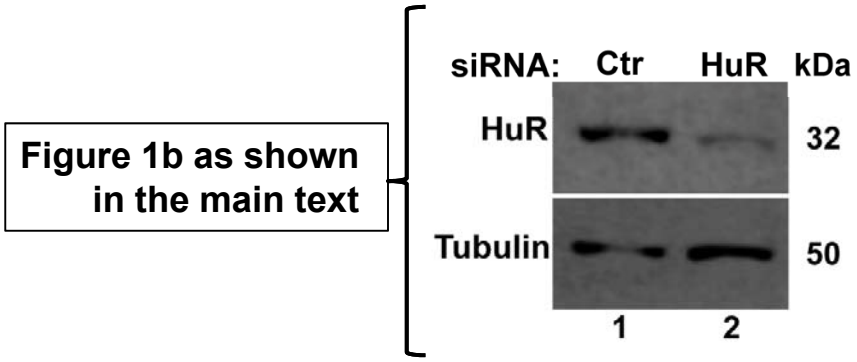
Supplementary Figure S12a



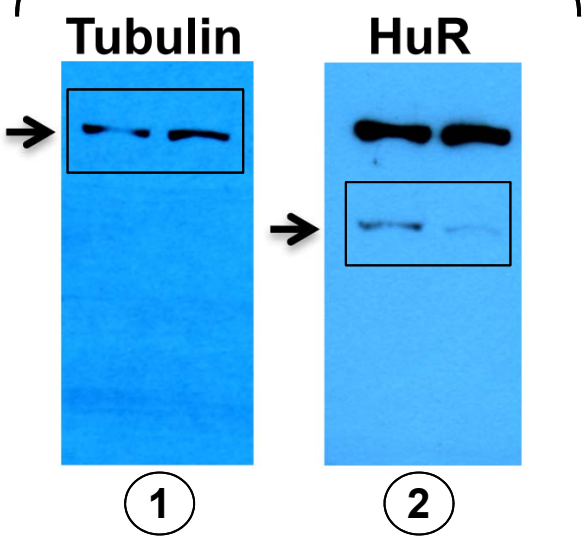


Scans 1 and 2 represent the same blot that was first probed with an anti-Tubulin antibody (1) then with with anti-HuR antibody (2). Since HMGB1 and HuR have a very close molecular weight (30 and 32 respectively) the same samples were run on a separate gel (3) and probed with both anti-Tubulin and anti-HMGB1 antibodies. The boxes highlight the area of each blot used to mount Figure 1a.

Supplementary Figure S12b

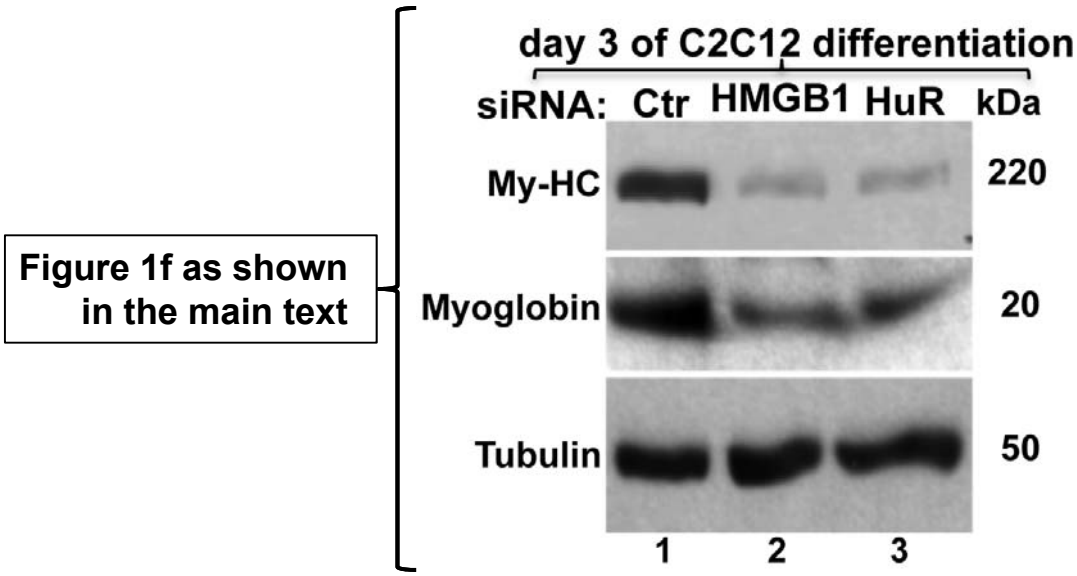


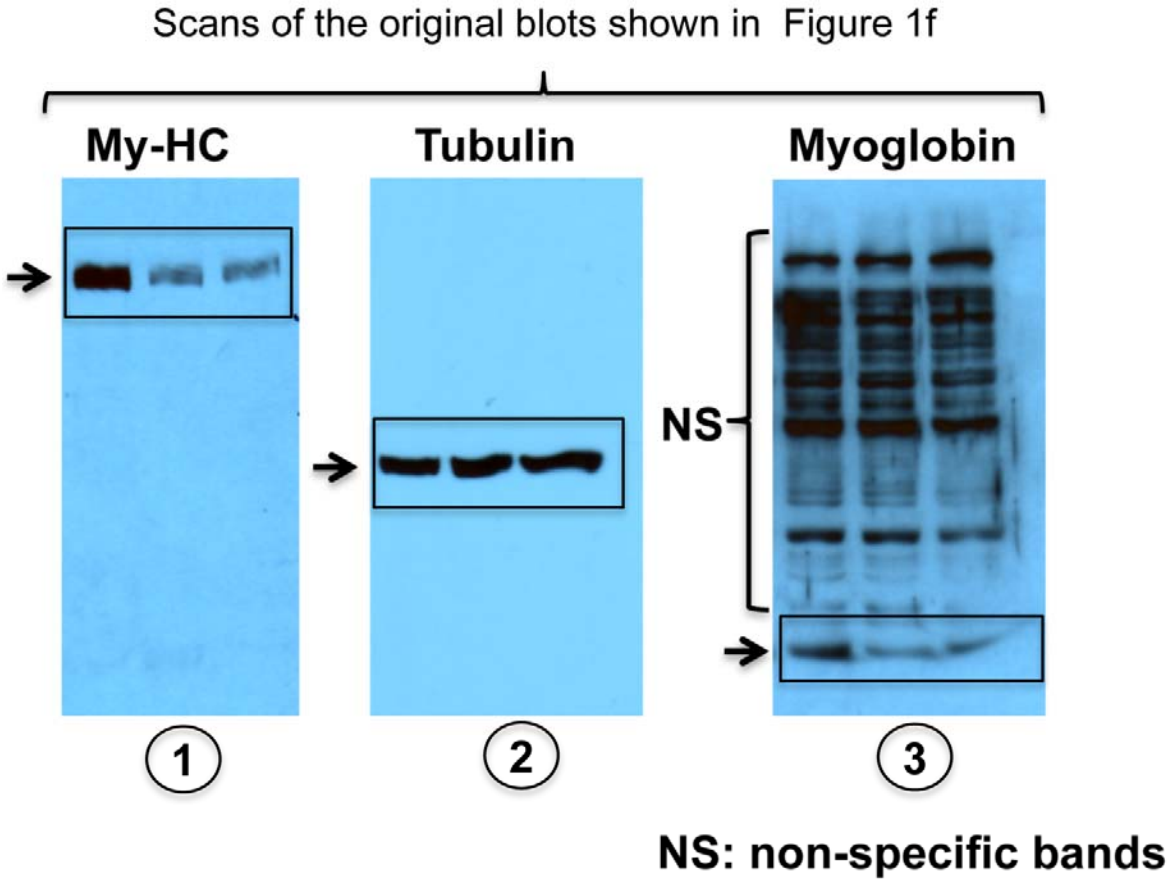
Scans of the original blots shown in Figure 1b



Scans 1 and 2 represent the same blot that was first probed with anti-Tubulin (1) then with anti-HuR (2) antibodies. The boxes highlight the area of each blot used to mount Figure 1b.

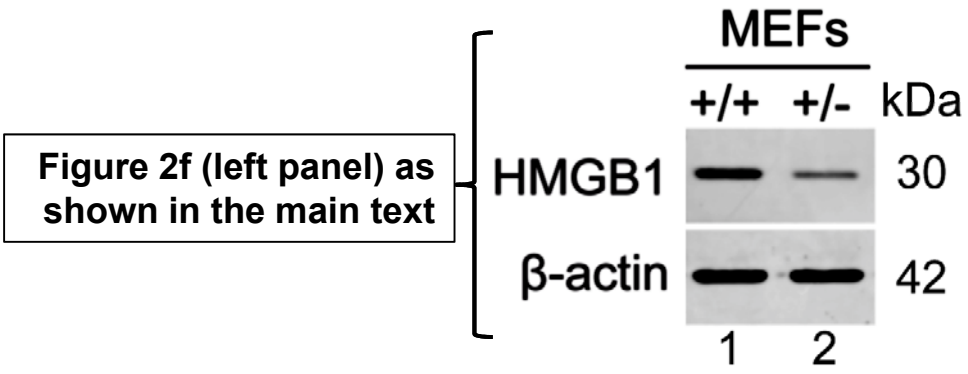
Supplementary Figure S12c



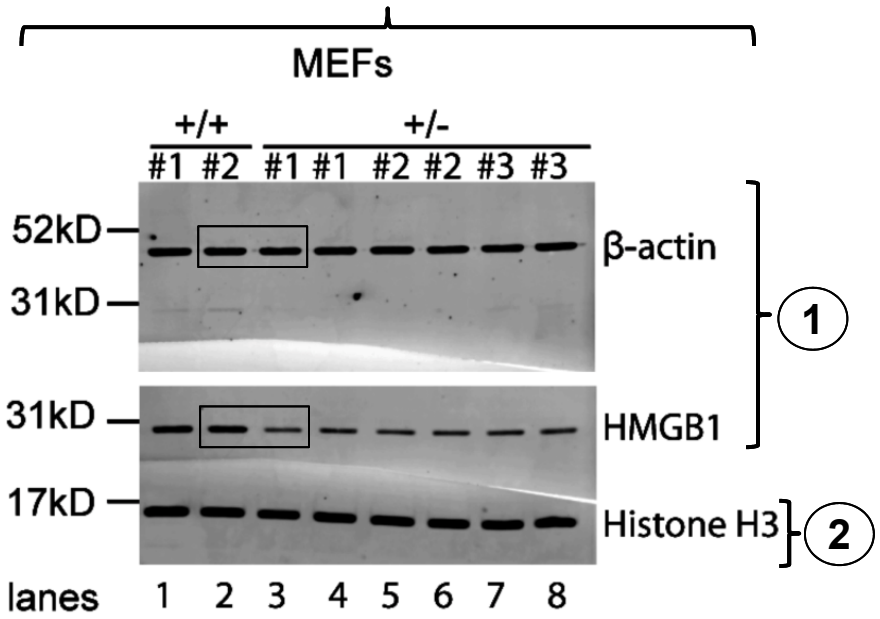


Scans 2 and 3 represent the same blot that was first probed with anti-Myoglobin (3) then (after being stripped) with an anti-Tubulin (2) antibodies. Since the molecular weight of Myosin heavy chain (My-HC) is very high (220kDa) the same extracts were run on a separate gel (1) and probed with the anti-Myosin Heavy chain anti-body. Of note the anti-Myoglobin antibody also detects several non-specific bands (NS). The boxes highlight the area of each blot used to mount Figure 1f.

Supplementary Figure S12d1

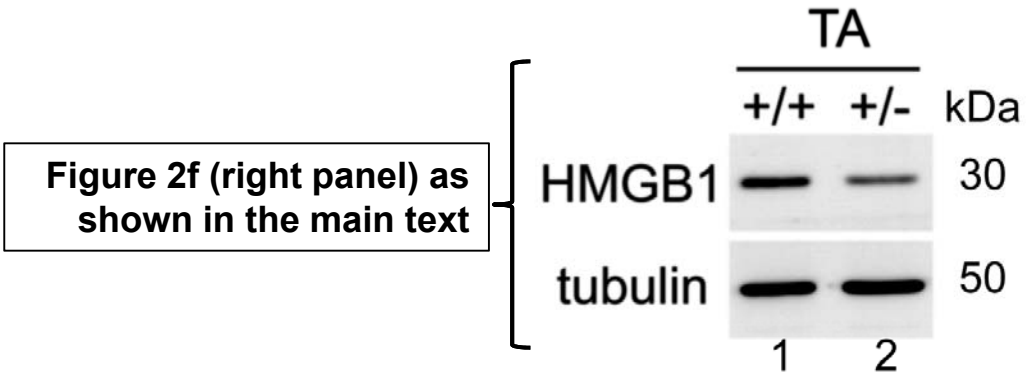


Scans of the original blots shown in Figure 2f (left panel)

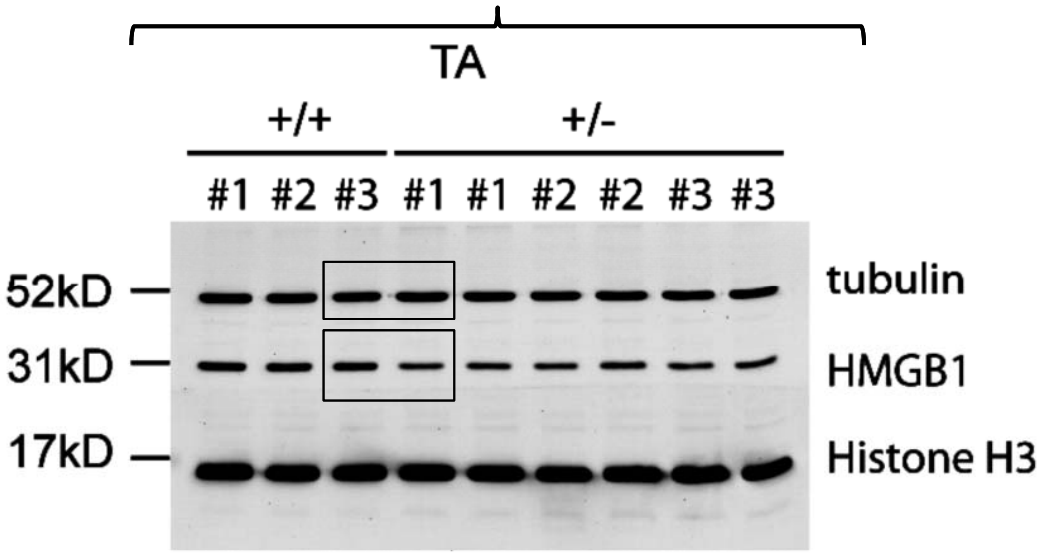


The original membrane was cut into two parts (1 and 2) and respectively probed with anti-HMGB1, anti-β-actin antibodies (part 1) and anti-Histone H3 antibody (part 2). H3 was used as another control but is not shown in the main Figure 2f. The boxes highlight the area of each blot that was used to mount Figure 2f (left panel).

Supplementary Figure S12d2

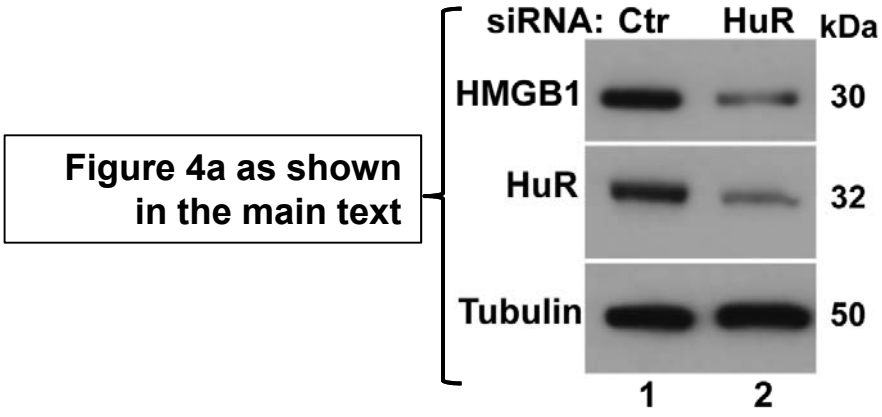


Scans of the original blots shown in Figure 2f (right panel)

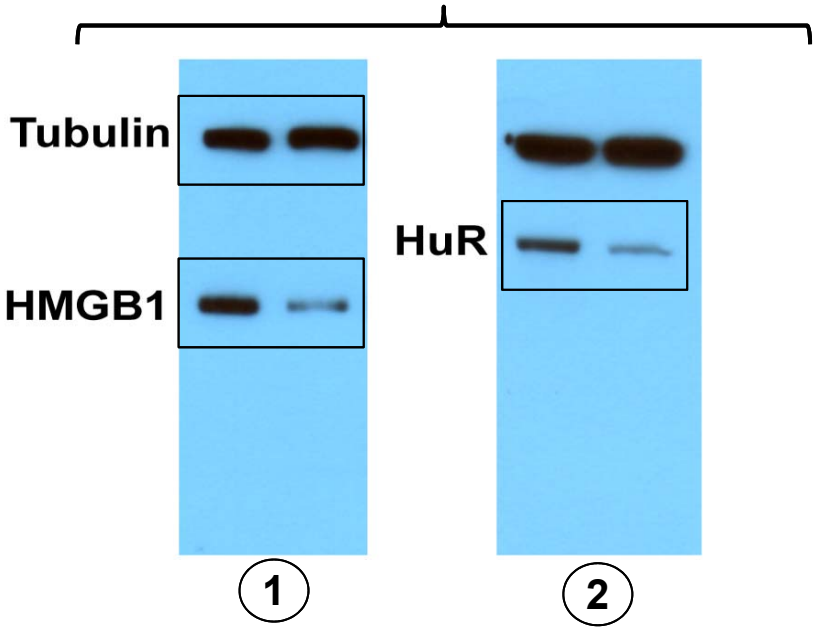


Here the blot was simultaneously probed with anti-HMGB1, -Tubulin and -Histone H3 antibodies. H3 was used as another control but is not shown in the main Figure 2f (right panel). The boxes highlight the area of the blot that were used to mount Figure 2f (right panel).

Supplementary Figure S12e

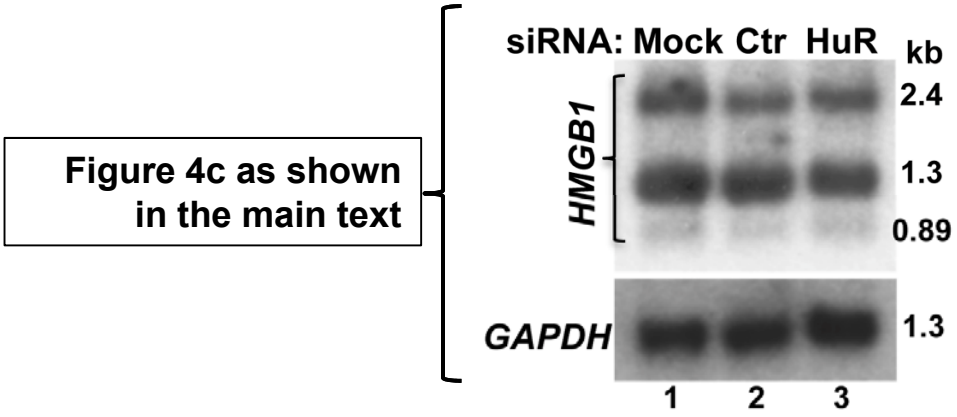


Scans of the original blots shown in Figure 4a

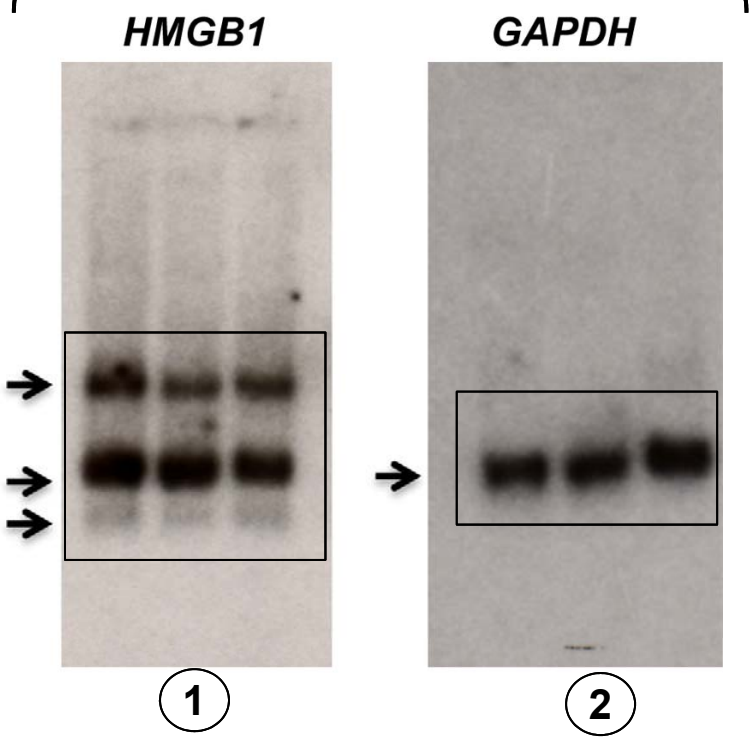


Since HMGB1 and HuR have very close molecular weight (30 and 32 respectively) the same samples were run on two separate gels and the blots are indicated as (1) and (2). Blot (1) was probed with both anti-Tubulin and anti-HMGB1 antibodies, while blot (2) was probed with both anti-Tubulin and anti-HuR antibodies. The boxes highlight the area of each blot used to mount Figure 4a.

Supplementary Figure S12f

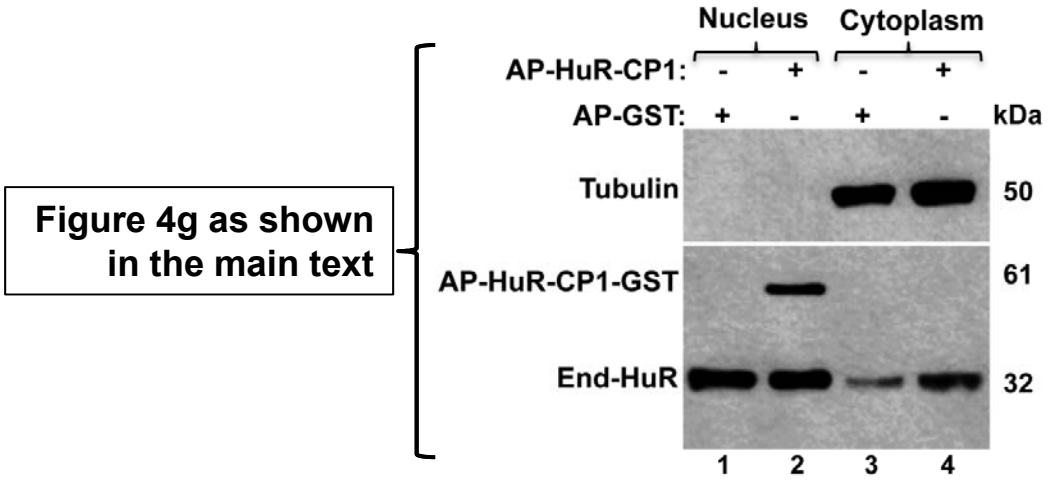


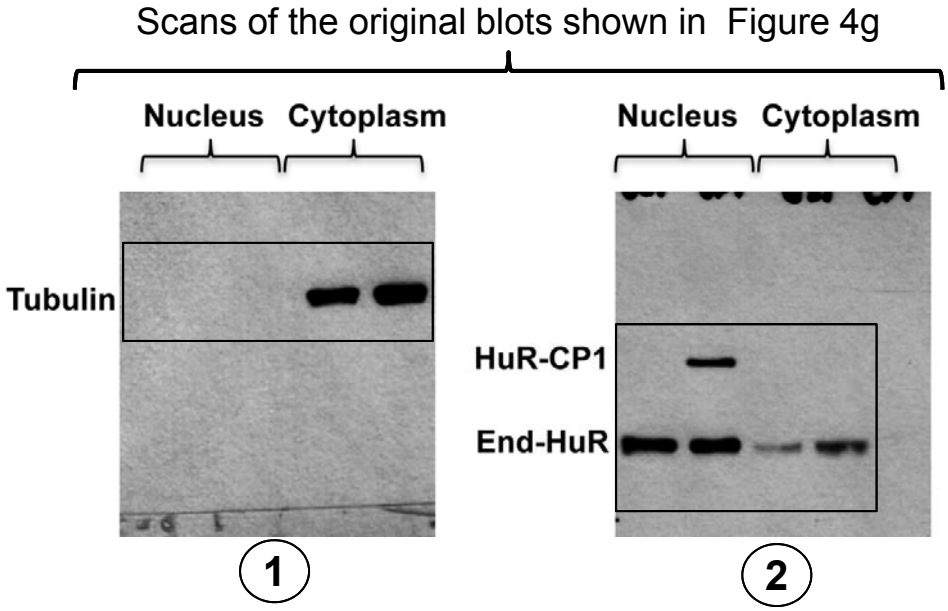
Scans of the original blots shown in Figure 4c



The same membrane was probed first with a specific anti-sense probe specific for *HMGB1 mRNA*, then with an anti-sense probe specific for *GAPDH.* The boxes highlight the area of the blots that were used to mount Figure 4c.

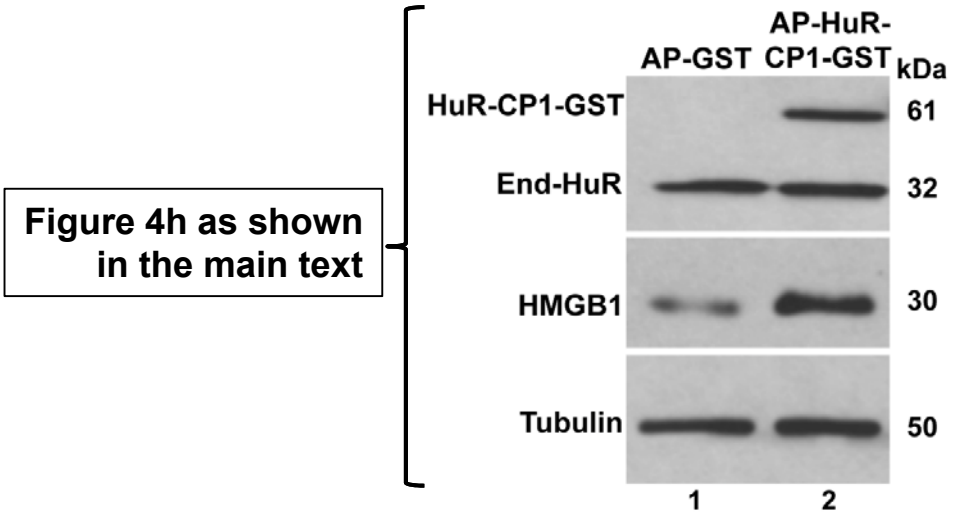
Supplementary Figure S12g

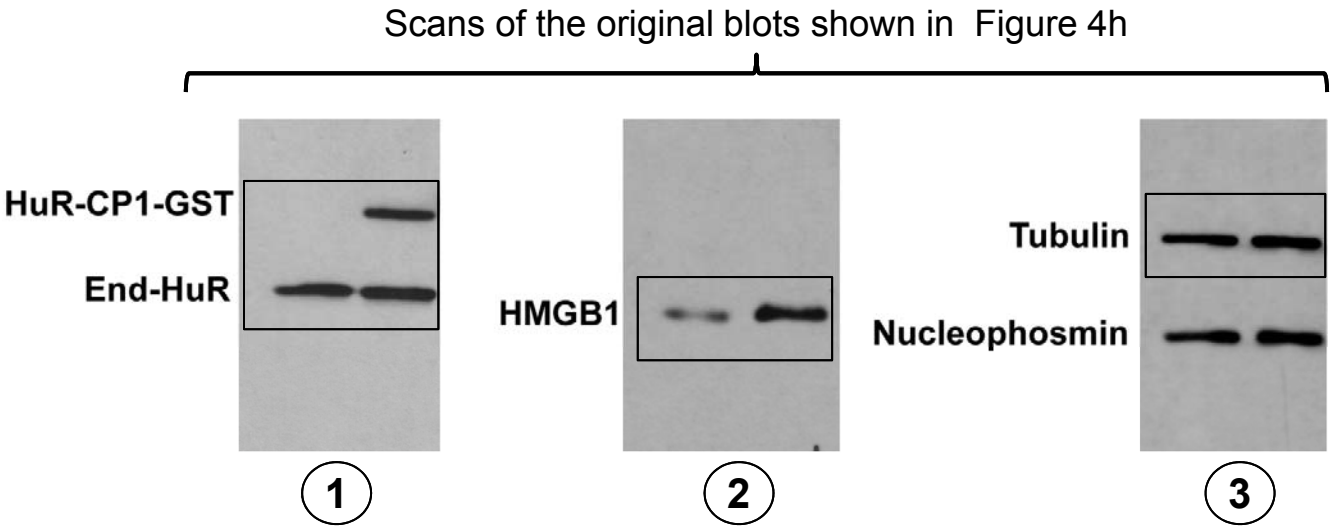




Scans 1 and 2 represent two gels that were run using the same samples. Blot (1) was probed with an anti-Tubulin antibody. Blot (2) was probed with anti-HuR antibody (2). The boxes highlight the area of each blot used to mount Figure 4g.

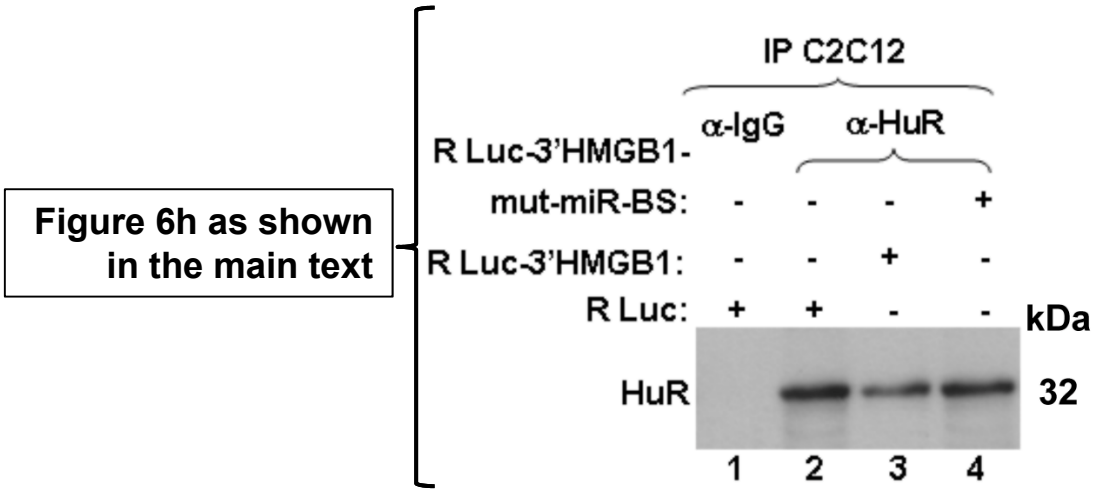
Supplementary Figure S12h



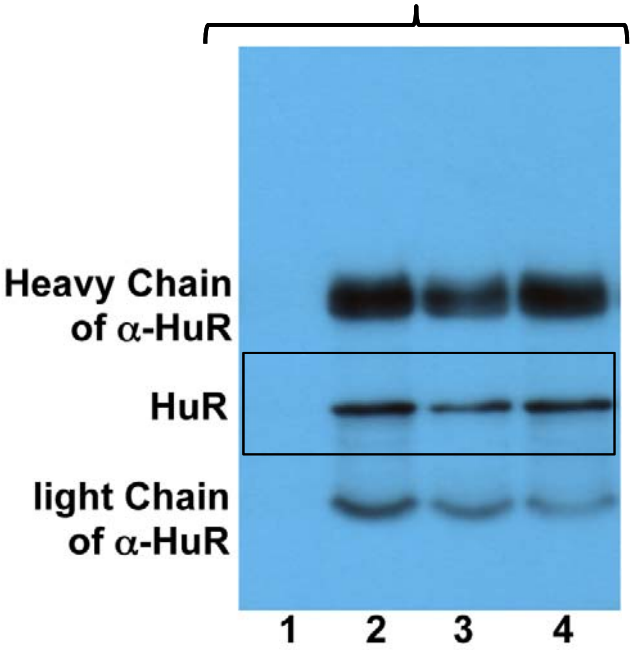


Scans 1, 2 and 3 represent three different gels that were run using the same samples. Blot (1) was probed with an anti-HuR antibody. Blot (2) was probed with an anti-HMGB1 antibody. Blot (3) was probed with both anti-Tubulin and Nucleophosmin antibodies (3). Nucleophosmin was used as another control, but was not included in Figure 4h. The boxes highlight the area of each blot used to mount Figure 4h.

Supplementary Figure S12i



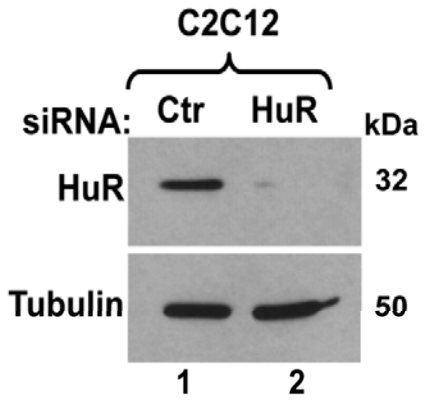
Scans of the original blots shown in Figure 6h



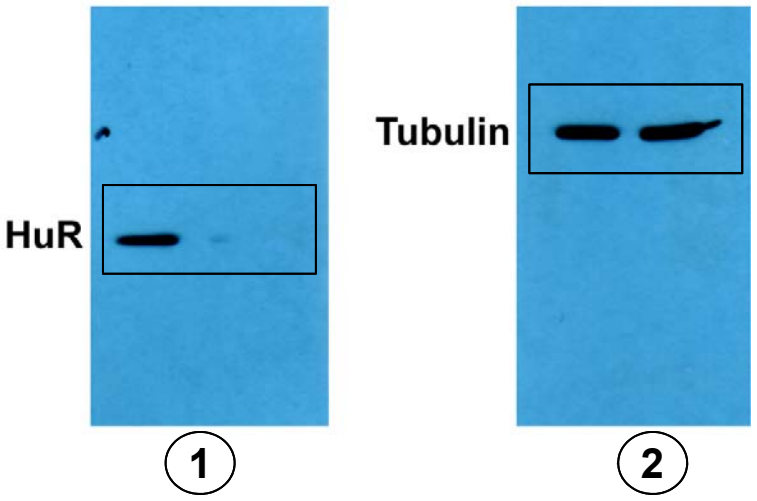
The scan shown represents the original blot that was probed with anti-HuR antibody. Due to cross-reactivity between the anti-HuR antibody (3A2) and the secondary antibody used in the western blot, the heavy and light chains of the 3A2 antibody were also revealed. The boxes highlight the area of each blot used to mount Figure 6h.

Supplementary Figure S12k

Figure 7a as shown in the main text



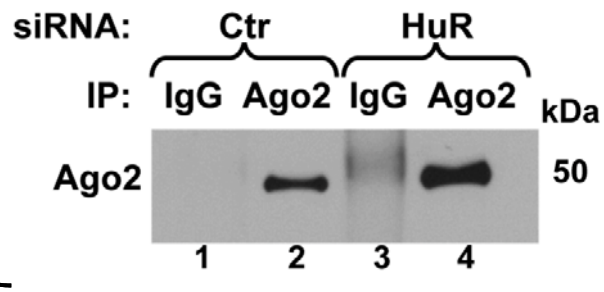
Scans of the original blots shown in Figure 7a



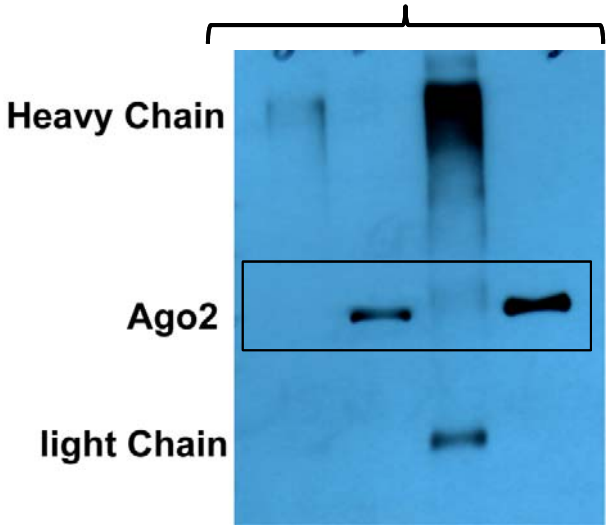
Scans 1 and 2 represent the same blot that was first probed with anti-HuR antibody (1) then with anti-Tubulin antibody (2). The boxes highlight the area of each blot used to mount Figure 7a.

Supplementary Figure S12l

Figure 7b as shown in the main text



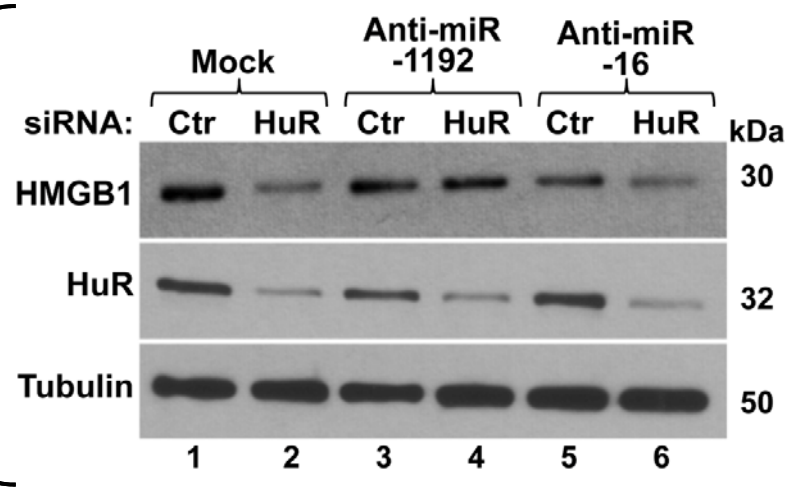
Scan of the original blot shown in Figure 7b



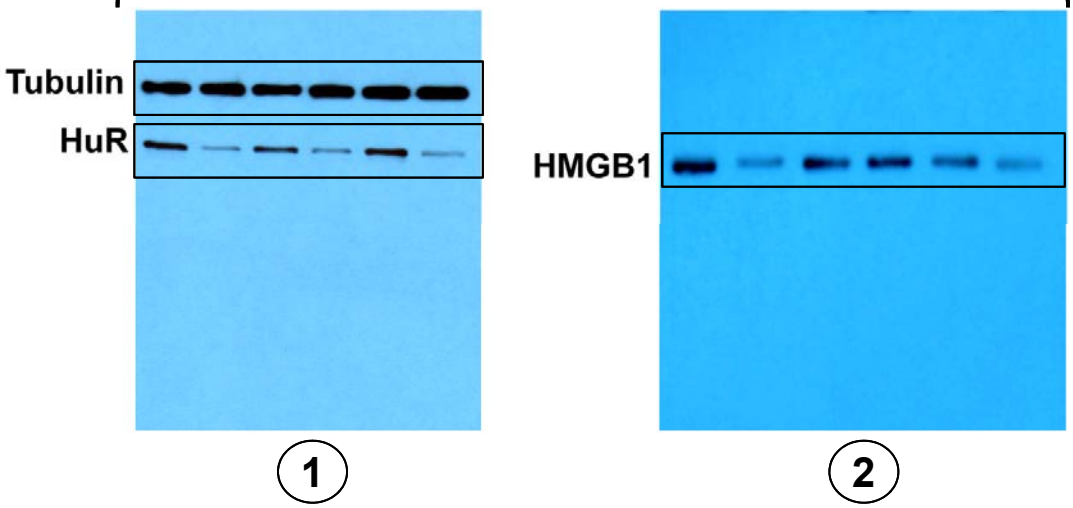
The scan shown represents the original blot that was probed with anti-Ago2 antibody. Due to cross-reactivity between the anti-Ago2 antibody and the secondary antibody used in the western blot, the heavy and light chains of the anti-Ago2 antibody were also revealed. The box highlight the area of the blot used to mount Figure 7b.

Supplementary Figure S12m

Figure 8a as shown in the main text

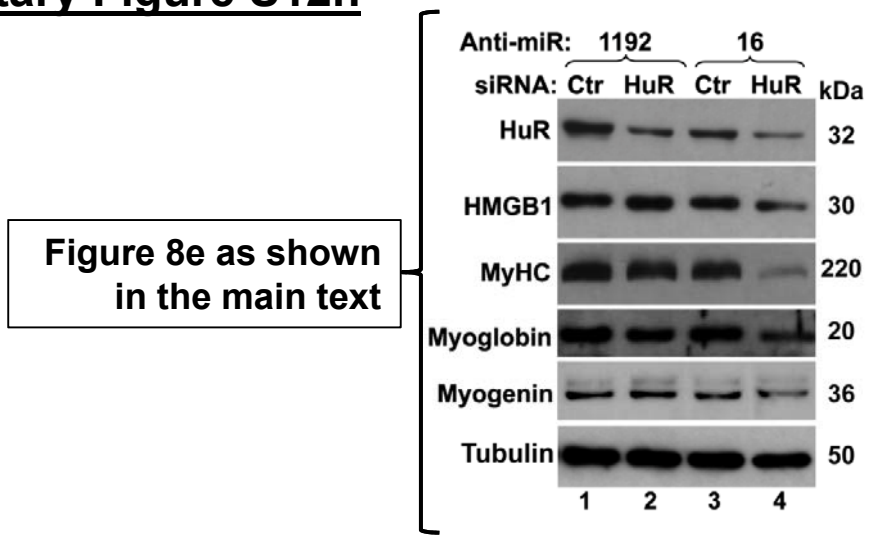


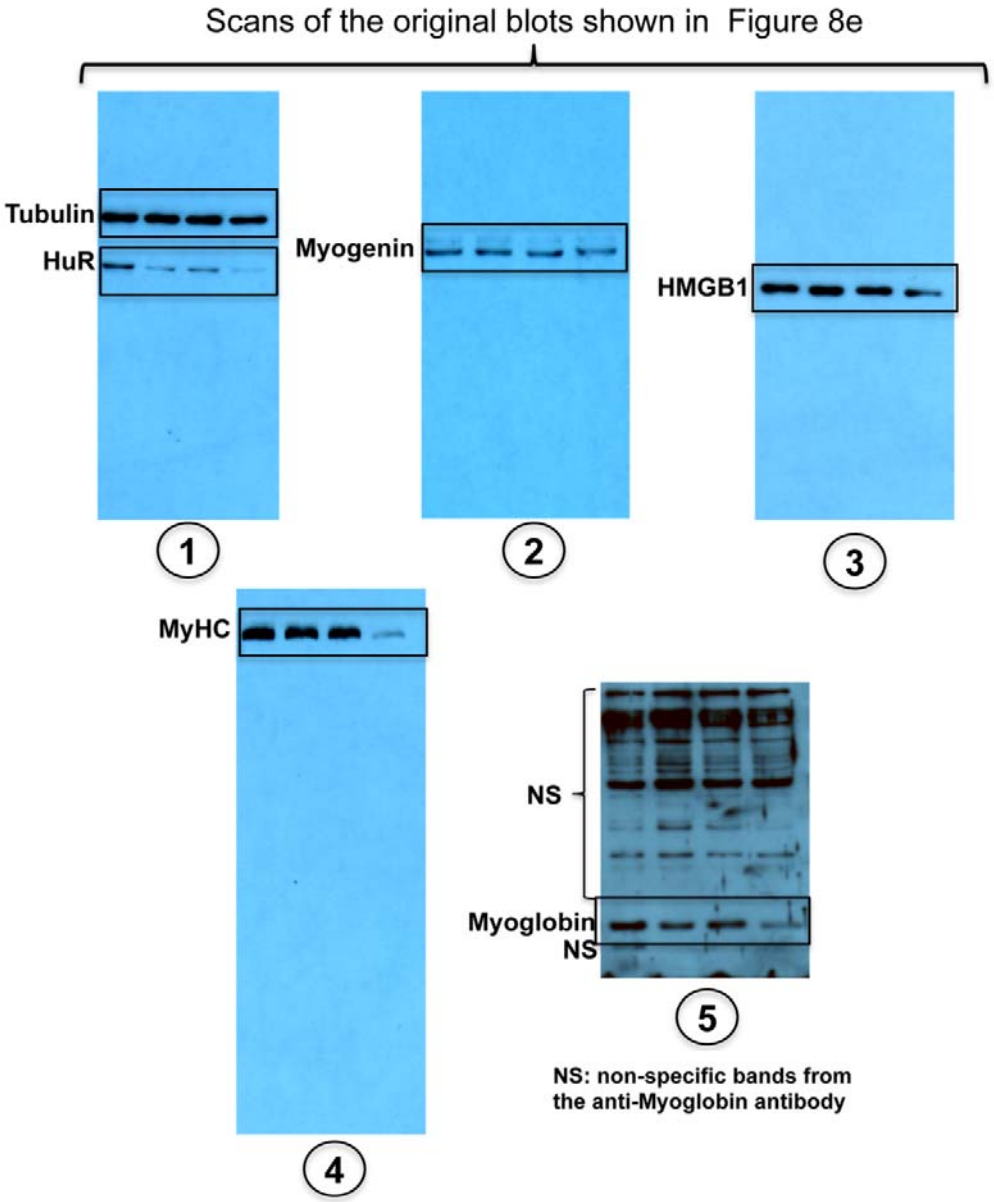
Scans of the original blots shown in Figure 8a



Since HMGB1 and HuR have very close molecular weights (30 and 32 respectively) the same samples were run on two separate gels (1 and 2). Blot (1) was probed with both anti-HuR and anti-Tubulin antibodies. Blot (2) was probed with anti-HMGB1 antibody (2). The boxes highlight the area of each blot used to mount Figure 8a.

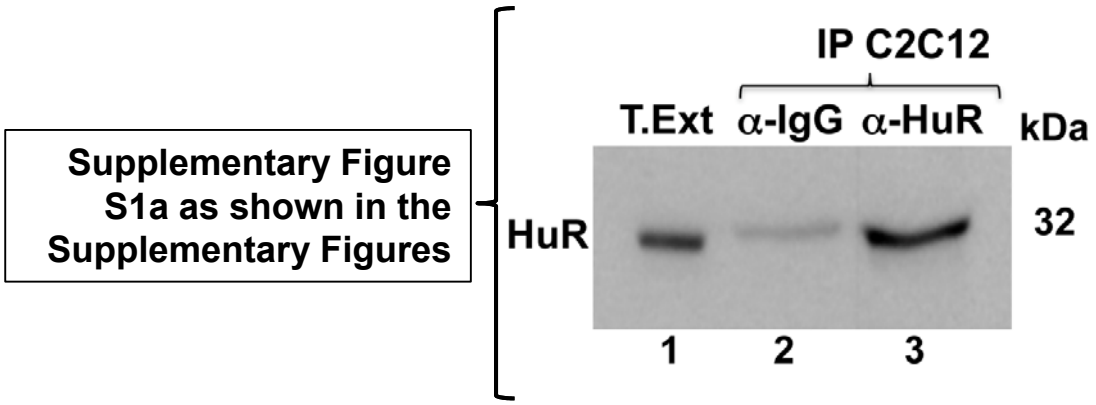
Supplementary Figure S12n



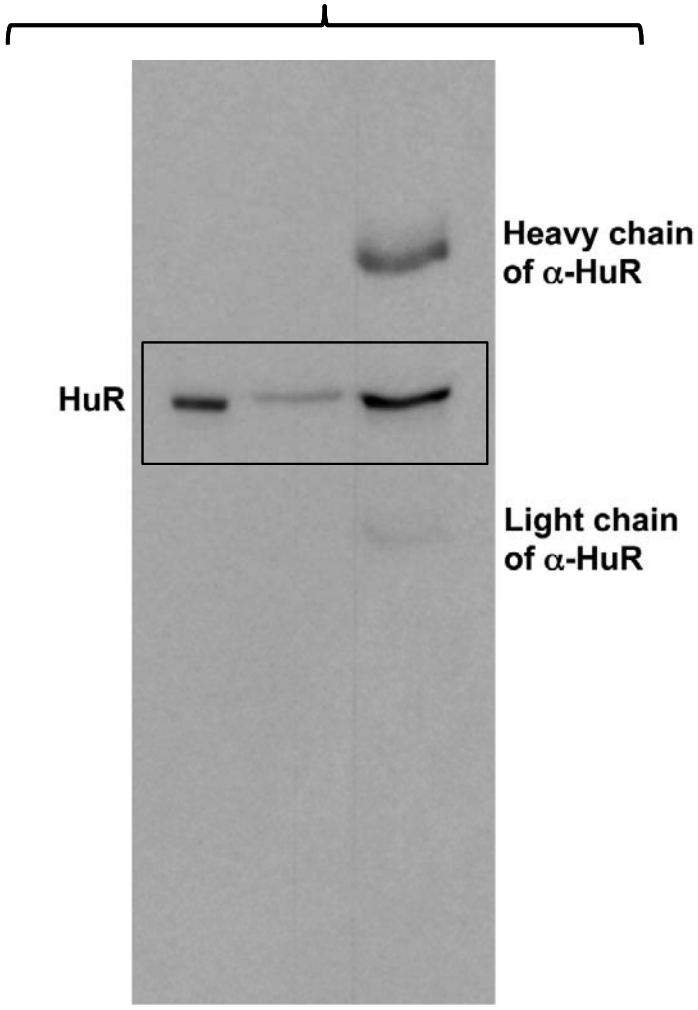


The scans represent different gels that were run using the same samples from the same experiment. Scan (1) represents the blot that was probed with both anti-HuR and anti-Tubulin antibodies. Scans, (2), (3), (4) and (5) represent blots that were respectively probed with an anti-Myogenin (2), anti-HMGB1 (3), anti-Myosin Heavy Chain (4) and anti-Myoglobin (5) antibodies. NS in blot (5) indicates non-specific bands detectes by the anti-Myoglobin antibody. The boxes highlight the area of each blot used to mount Figure 8e.

Supplementary Figure S12o

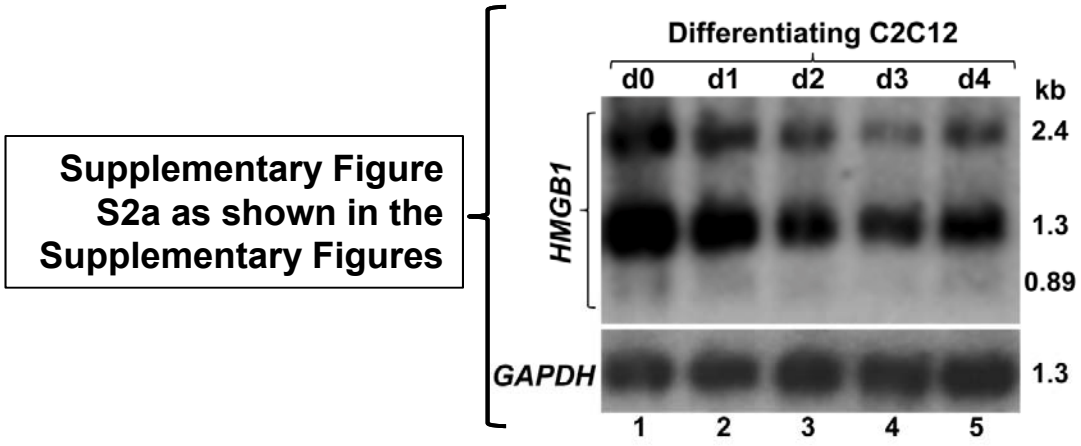


Scan of the original blot shown in Supplementary Figure S1a

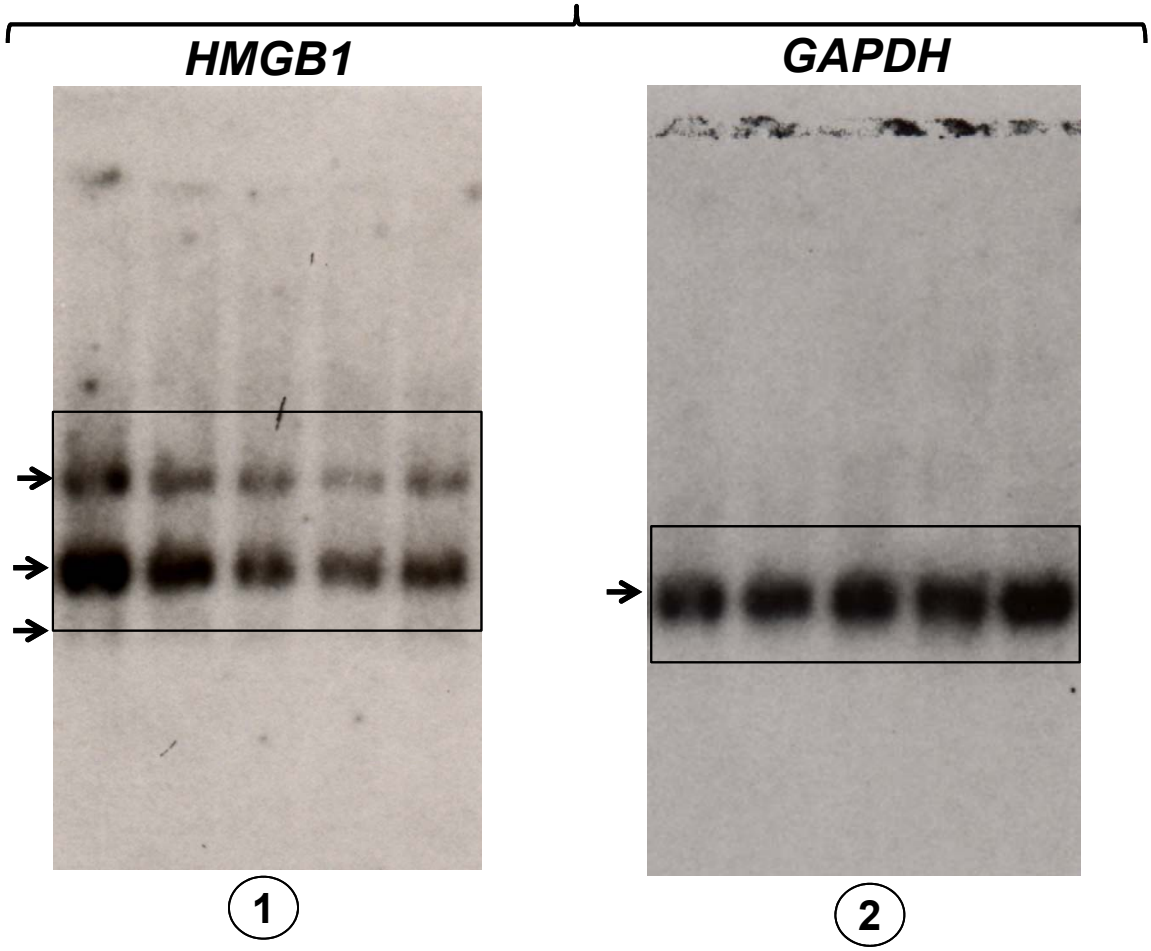


The scan shown represents the original blot that was probed with anti-HuR antibody. Due to cross-reactivity between the anti-HuR antibody (3A2) and the secondary antibody used in the western blot, the heavy and light chains of the 3A2 antibody were also revealed. The box highlights the area of each blot used to mount Supplementary Figure S1a.

Supplementary Figure S12p

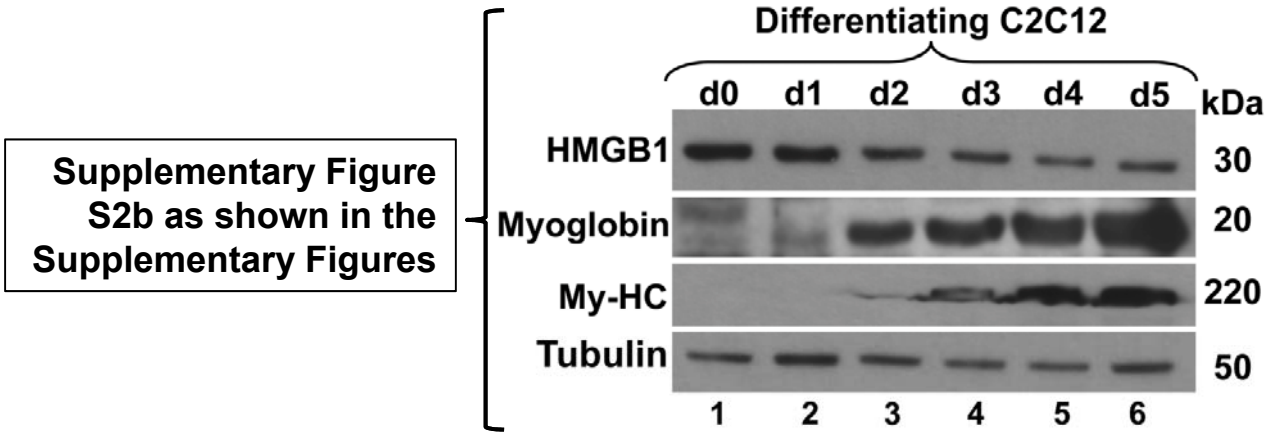


Scans of the original blots shown in Supplementary Figure S2a

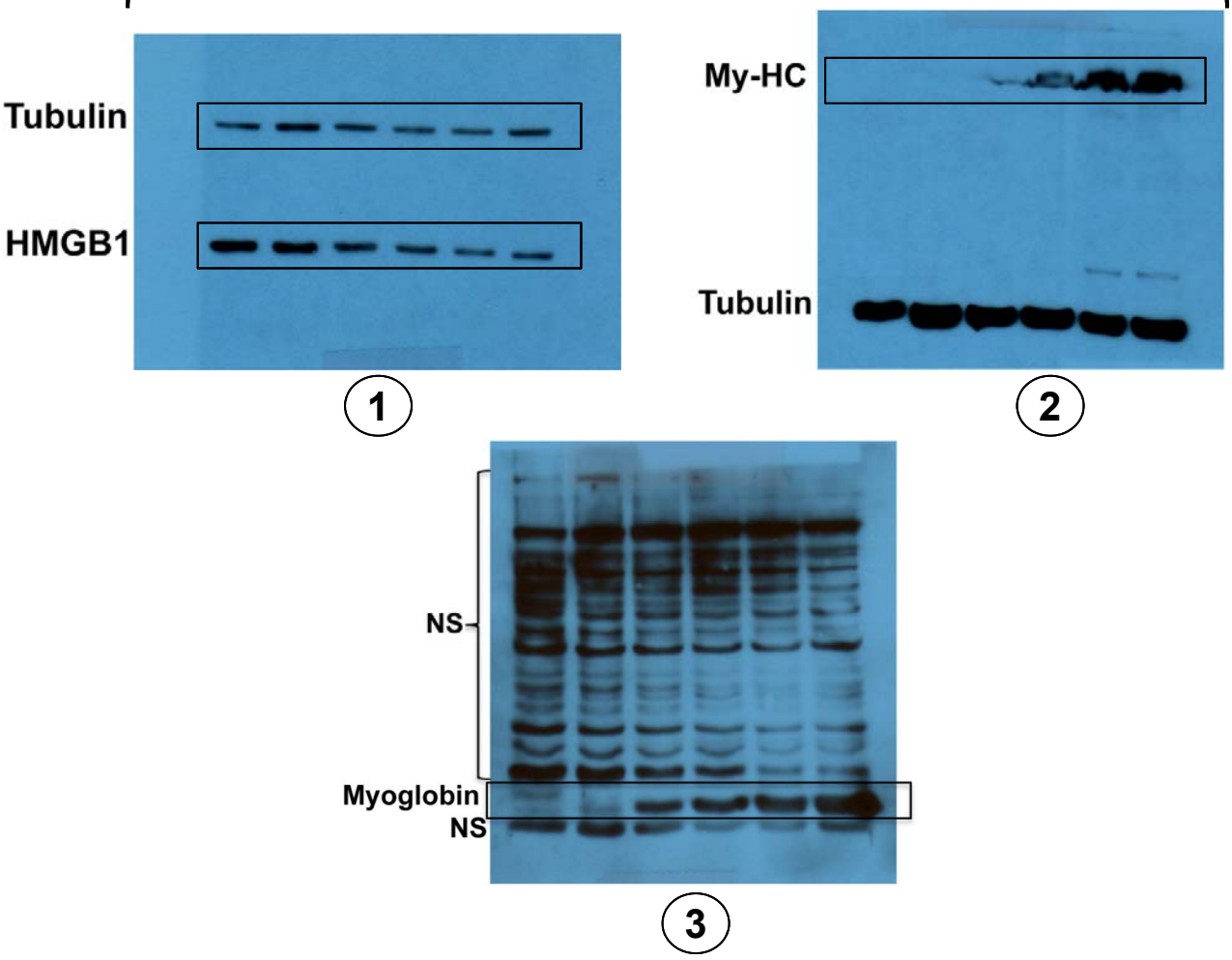


The same membrane was probed first with a specific anti-sense probe specific for *HMGB1 mRNA* (1), then the membrane was stripped and probed with an anti-sense probe specific for *GAPDH* (2). The boxes highlight the area of the blots that were used to mount Supplementary Figure S2a.

Supplementary Figure S12q



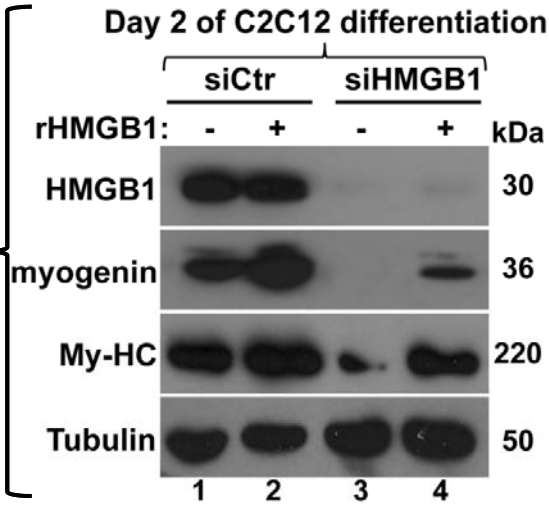
Scans of the original blots shown in Supplementary Figure S2b



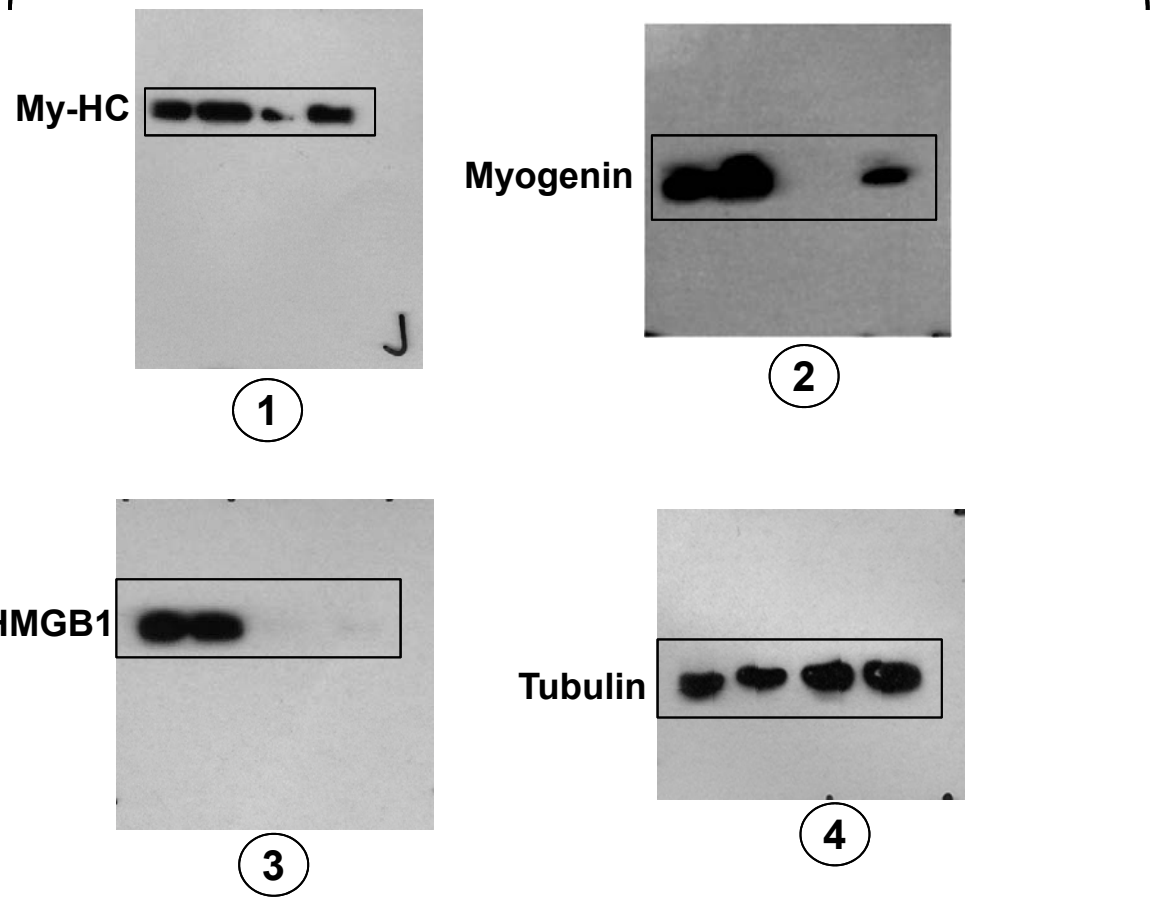
The three scans shown represent three different gels that were run using the same samples from the same experiment. Scan (1) represents a blot that was probed with both anti-HMGB1 and anti-Tubulin antibodies. Scan (2) represents a blot that was probed with both the anti-Tubulin and anti-Myosin Heavy Chain. Scan (3) represents a blot that was probed with the anti-Myoglobin antibody. NS in blot (3) indicates non-specific bands detected by the anti-Myoglobin antibody. The boxes highlight the area of each blot used to mount Supplementary Figure S2b.

Supplementary Figure S12r

Supplementary Figure S2c as shown in the Supplementary Figures



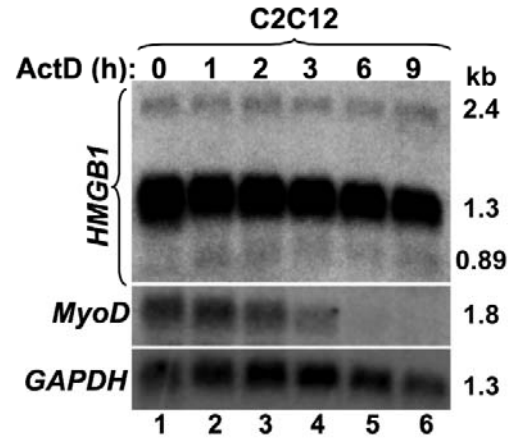
Scans of the original blots shown in Supplementary Figure S2c



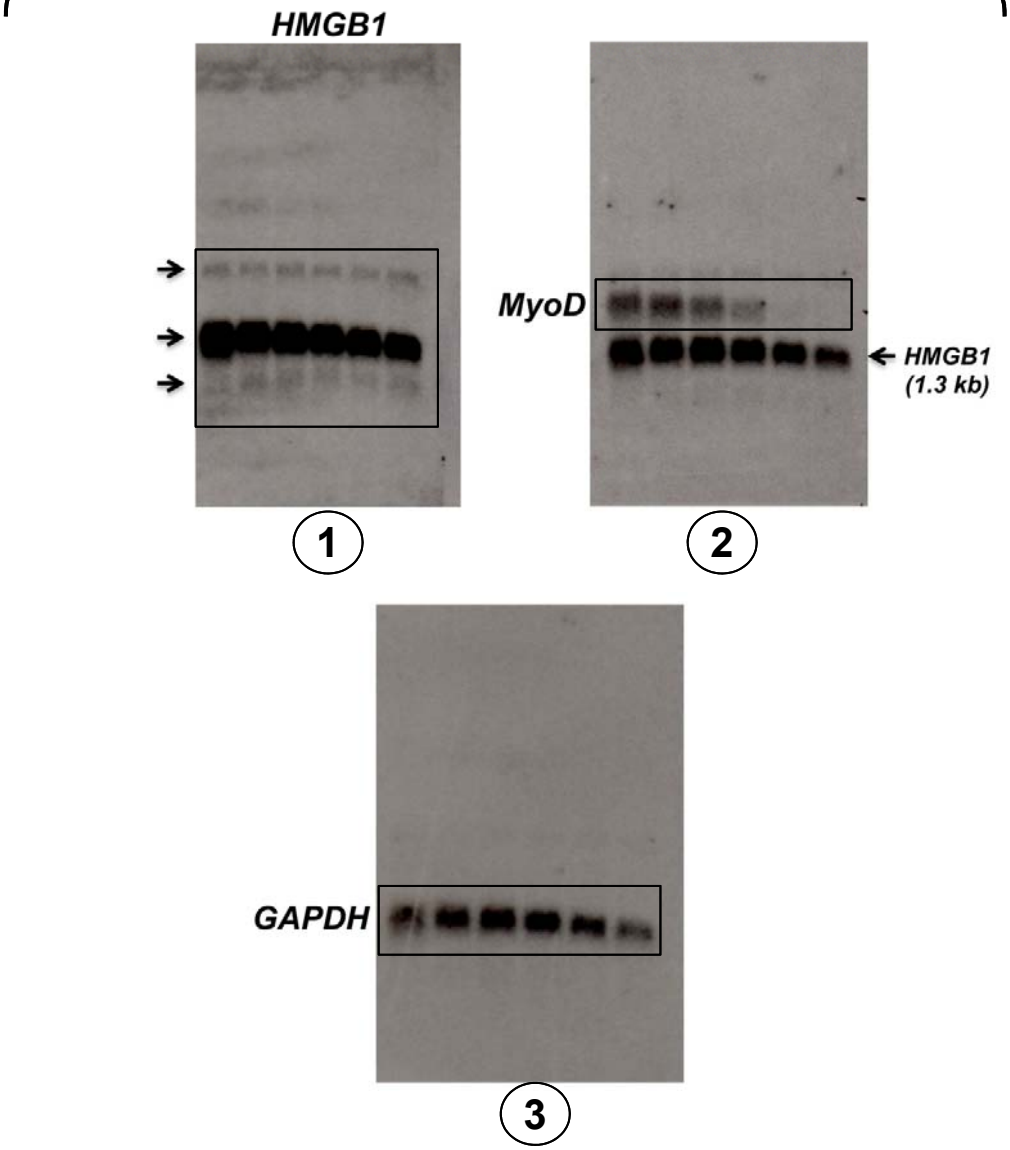
The four scans shown represent three different gels that were run using the same samples from the same experiments. Scan (1) represents the blot that was probed with the anti-Myosin heavy chain antibody. Scans (2), (3) and (4) respectively represent blots that were probed with anti-Myogenin (2), anti-HMGB1 (3) and with anti-Tubulin (4) antibodies. The boxes highlight the area of each blot used to mount Supplementary Figure S2b.

Supplementary Figure S12s

Supplementary Figure S3a as shown in the Supplementary Figures



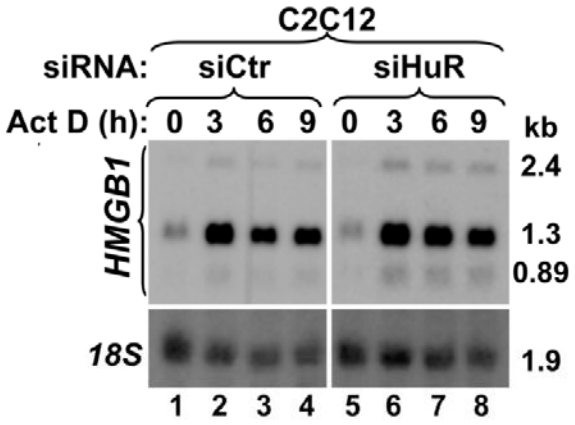
Scans of the original blots shown in Supplementary Figure S3a

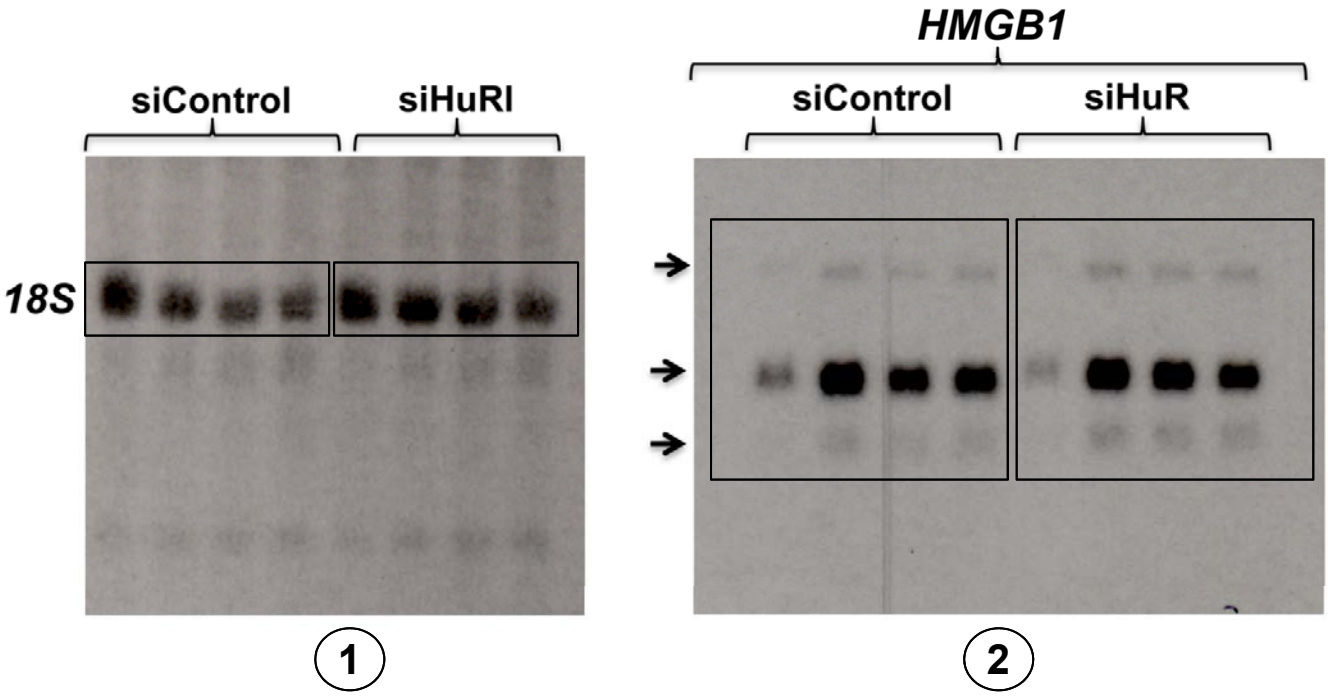


The same membrane was probed first with an anti-sense probe specific for *HMGB1 mRNA* (1), then with anti-sense probe specific for MyoD (2). The blot was then probed with an anti-sense probe specific for *GAPDH* (3). The boxes highlight the area of the blots that were used to mount Supplementary Figure S3a.

Supplementary Figure S12t

Supplementary Figure S3c as shown in the Supplementary Figures

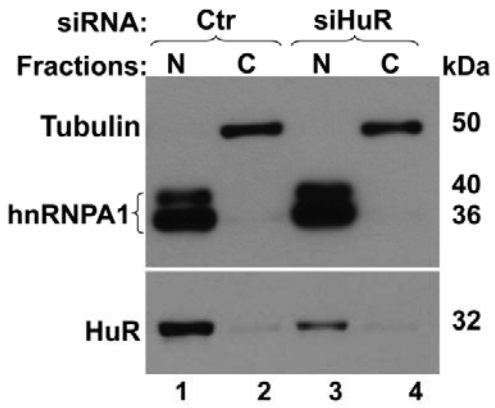




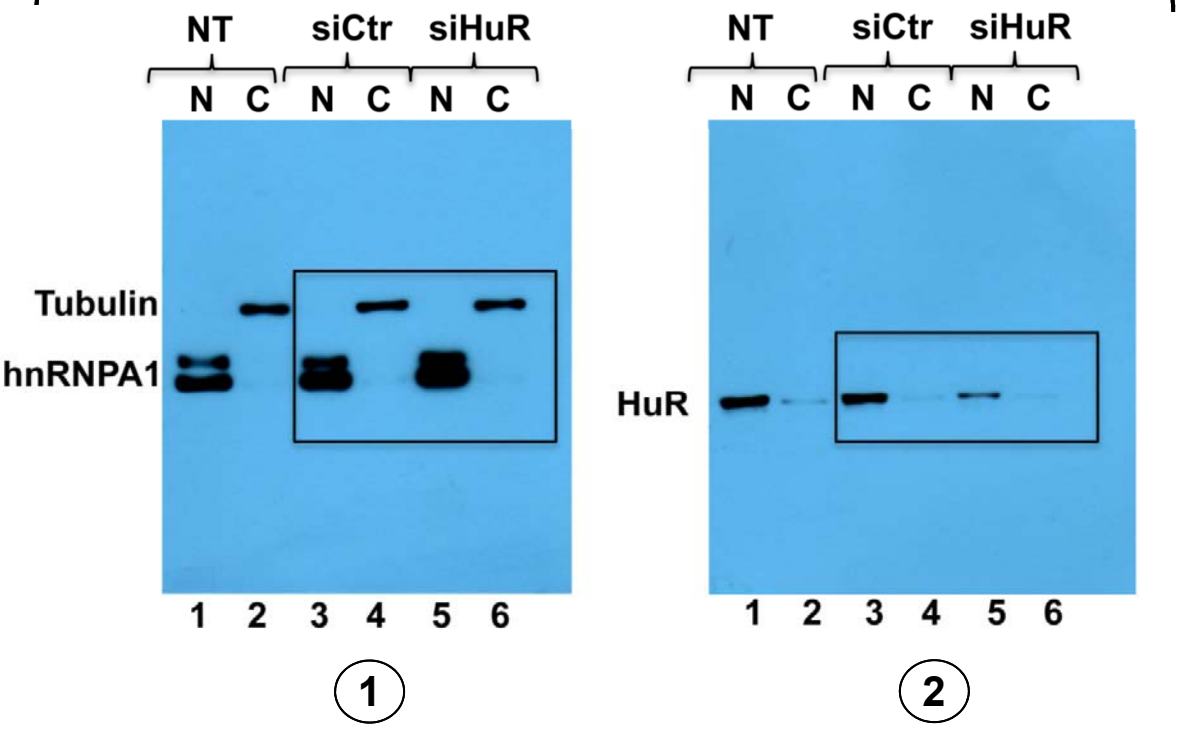
The same membrane was probed first with an anti-sense probe specific for *HMGB1 mRNA* (2), the membrane was then stripped and probed with anti-sense probe specific for *18S RNA* (1). The boxes highlight the area of the blots that were used to mount Supplementary Figure S3c.

Supplementary Figure S12u

Supplementary Figure S4c as shown in the Supplementary Figures



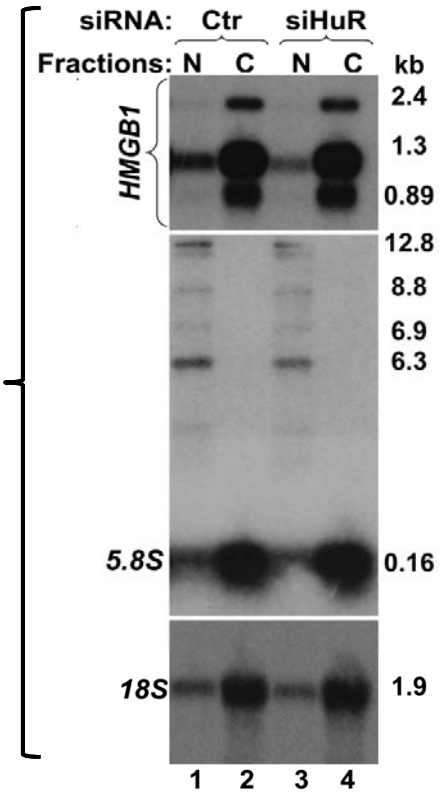
Scans of the original blots shown in Supplementary Figure S4c



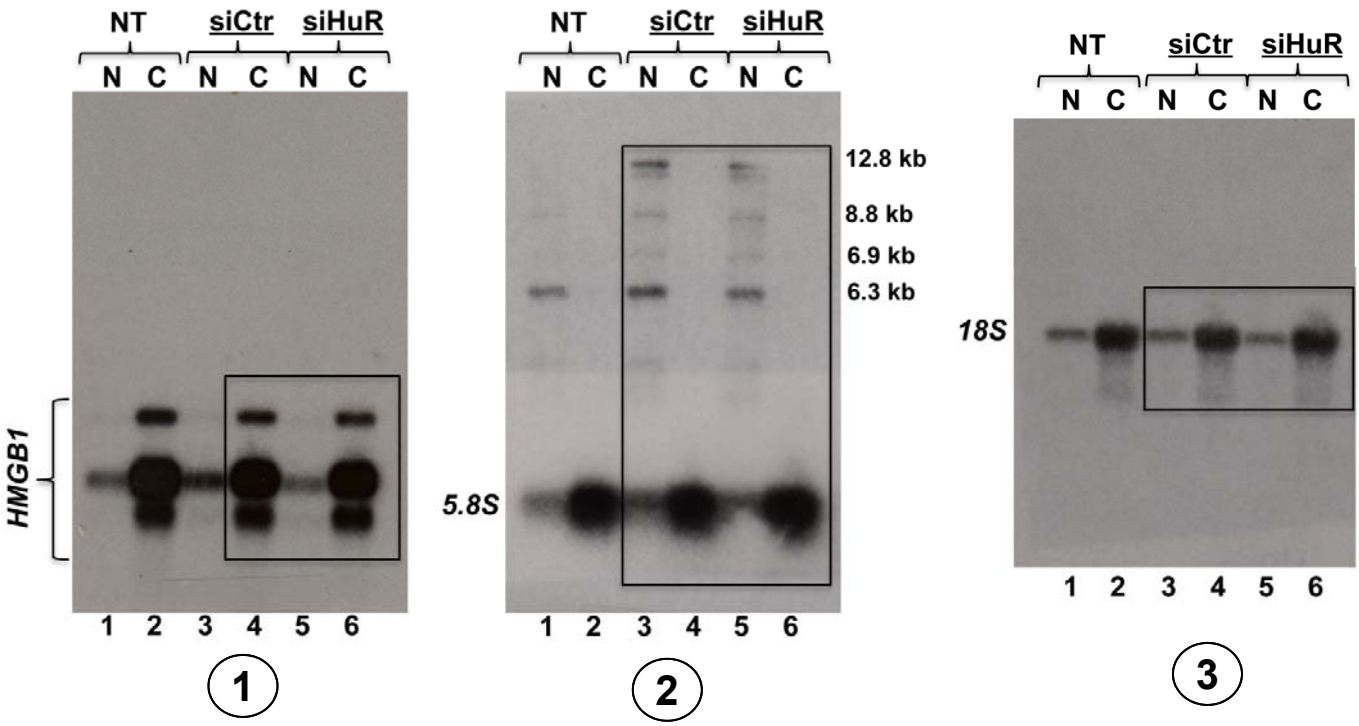
The two scans represent the same membrane that was first probed with the anti-HuR antibody (2). The membrane was then stripped and probed with both anti-Tubulin and anti-hnRNPA1 antibodies (1). Only lanes 3 to 6 of the gel are shown in Supplementary Figure S4c. Lanes 1-2 show Tubulin, hRNPA1 and HuR in Nuclear and Cytoplasmic fraction in cells not treated with siRNAs. These two lanes were not shown in Supplementary Figure S4c. The boxes highlight the area of the blots that were used to mount Supplementary Figure S4c

Supplementary Figure S12v

Supplementary Figure S4d as shown in the Supplementary Figures



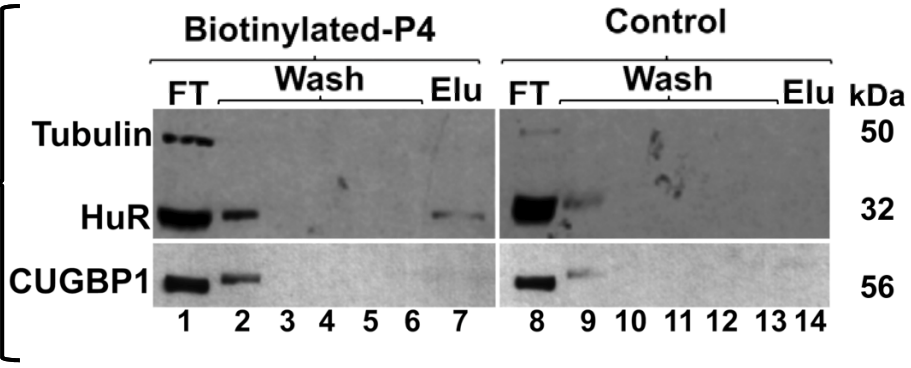
Scans of the original blots shown in Supplementary Figure S4d



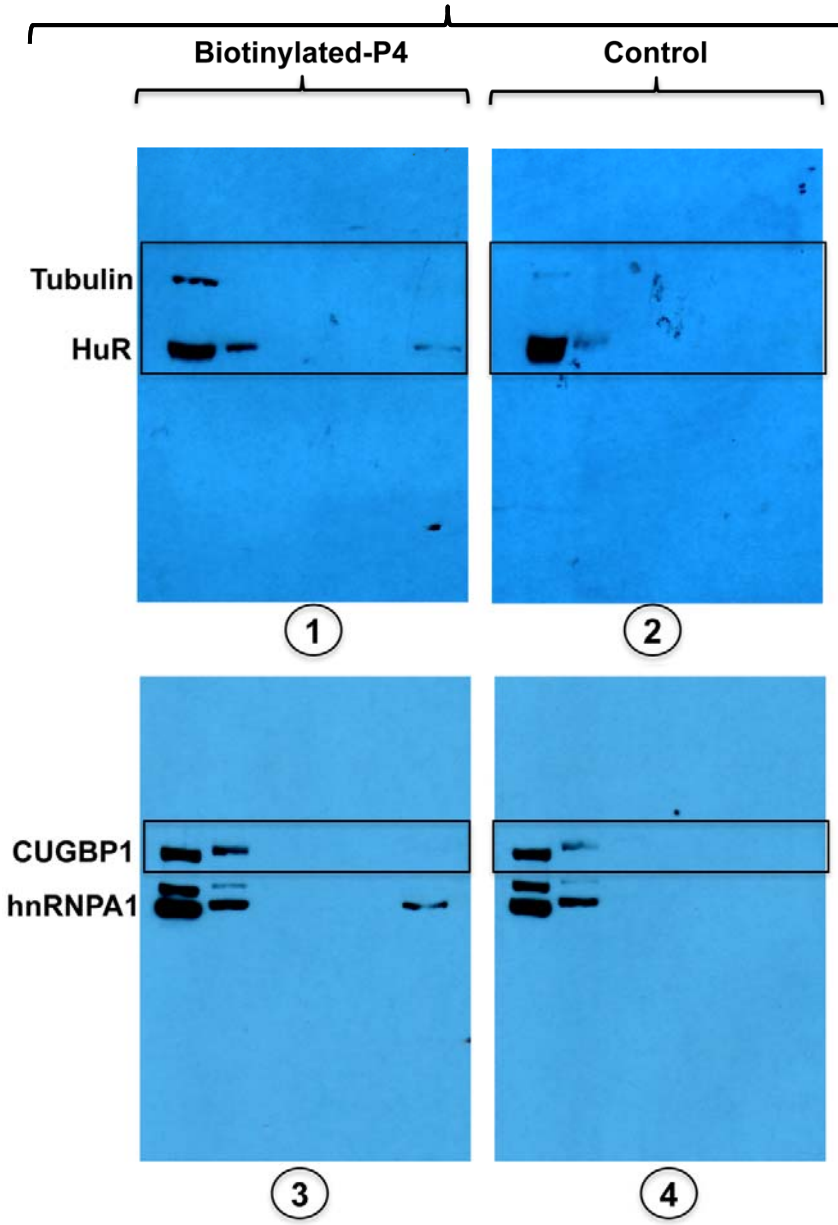
The scans represent the same membrane that was probed with different anti-sense probes. The membrane was first probed with an anti-sense probe specific for *HMGB1 mRNA* (1), then it was stripped and probed with anti-sense probe specific for the 5.8S RNA and its precursors (2). The membrane was then stripped again and probed with an anti-sense probe specific for the 1*8S RNA* (3). Only lanes 3 to 6 of the gel are shown in Supplementary Figure S4d. Lanes 1-2 show 18S (3), 5.8S and its precursors (2) and HMGB1 (1) RNAs in the Nuclear and Cytoplasmic fractions in cells not treated with siRNAs. Lanes 1 and 2 were not shown in Figure Supplementary Figure S4d. The boxes highlight the area of the blots that were used to mount Supplementary Figure S4d.

Supplementary Figure S12w

Supplementary Figure S5a as shown in the Supplementary Figures

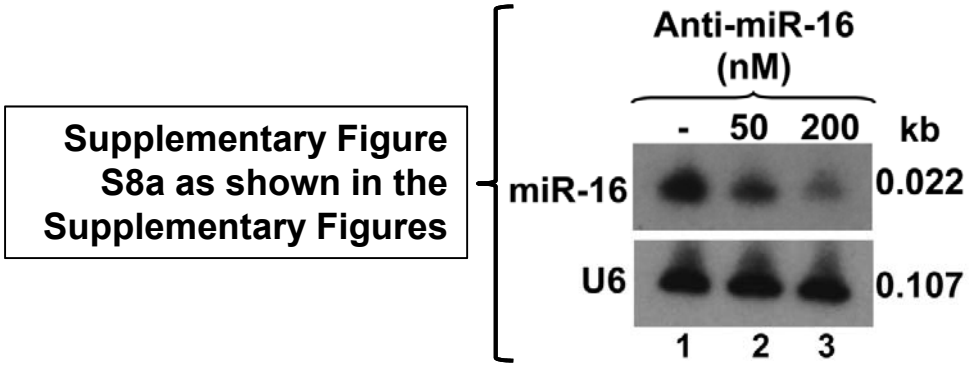


Scans of the original blots shown in Supplementary Figure S5a

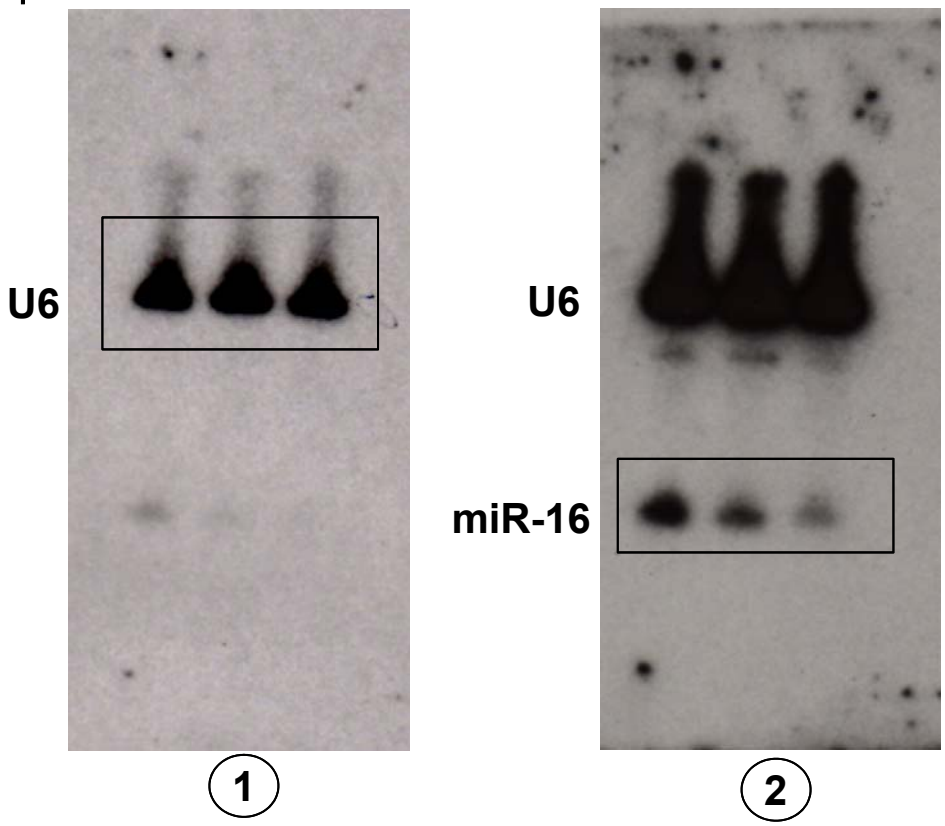


Scans (1) and (3) represent the membrane of the same biotinylated-P4 pull-down experiment that was first probed with both anti-HuR and anti-Tubulin antibodies (1), then stripped and probed with both anti-CUGBP1 and anti-hnRNP A1 antibodies (3). The control experiments (2 and 4) was run on a separate gel and the membrane was respectively probed as described for (1) and (3). The hnRNP A1 was used as a positive control but was not included in the final Supplementary Figure S5a. The boxes highlight the area of each blot used to mount Supplementary Figure S5a.

Supplementary Figure S12x



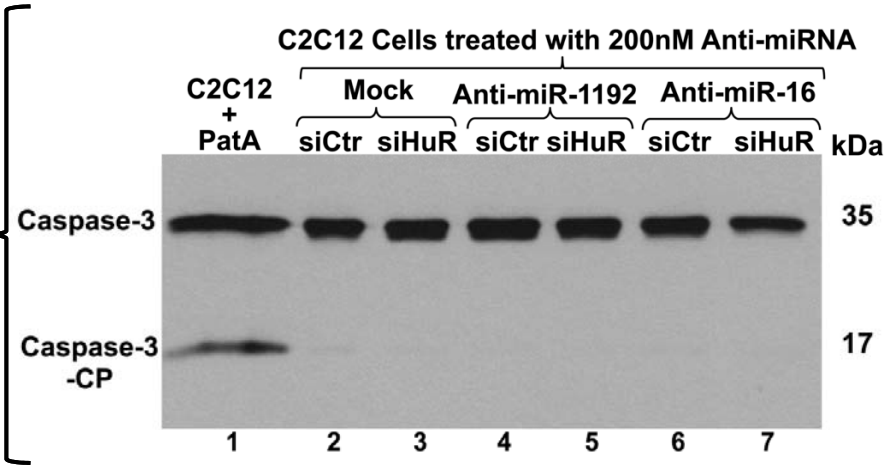
Scans of the original blots shown in Supplementary Figure S8a



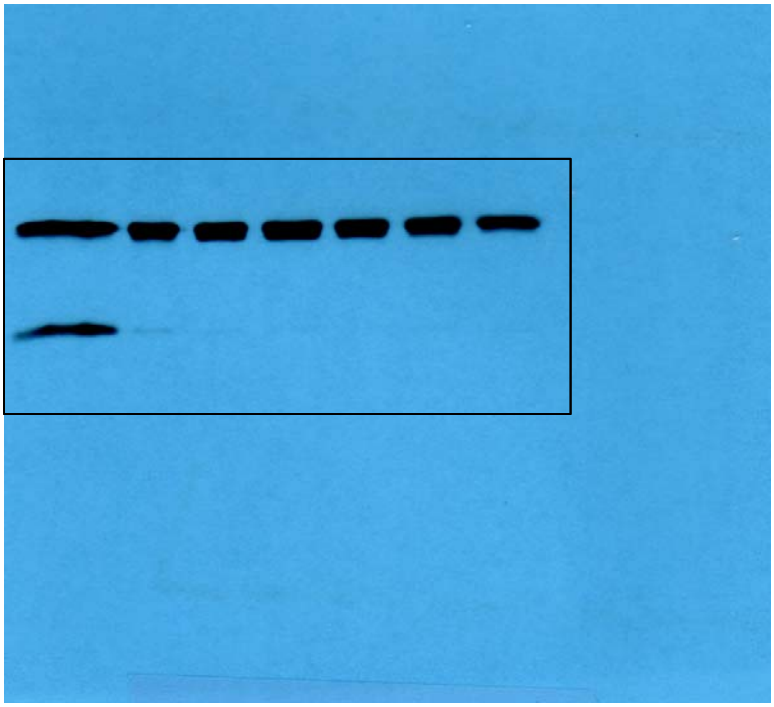
The scans represent the same membrane that was probed with anti-sense probes specific for *U6* RNA and miR-16. Scans (1) and (2) represent two different exposures of this membrane. Since U6 RNA is highly abundant in the cell, we chose the lower exposure of this blot (1) to show a non-saturated signal of U6 RNA. The boxes highlight the area of the blots that were used to mount Supplementary Figure S8a.

Supplementary Figure S12y

Supplementary Figure S8b as shown in the Supplementary Figures

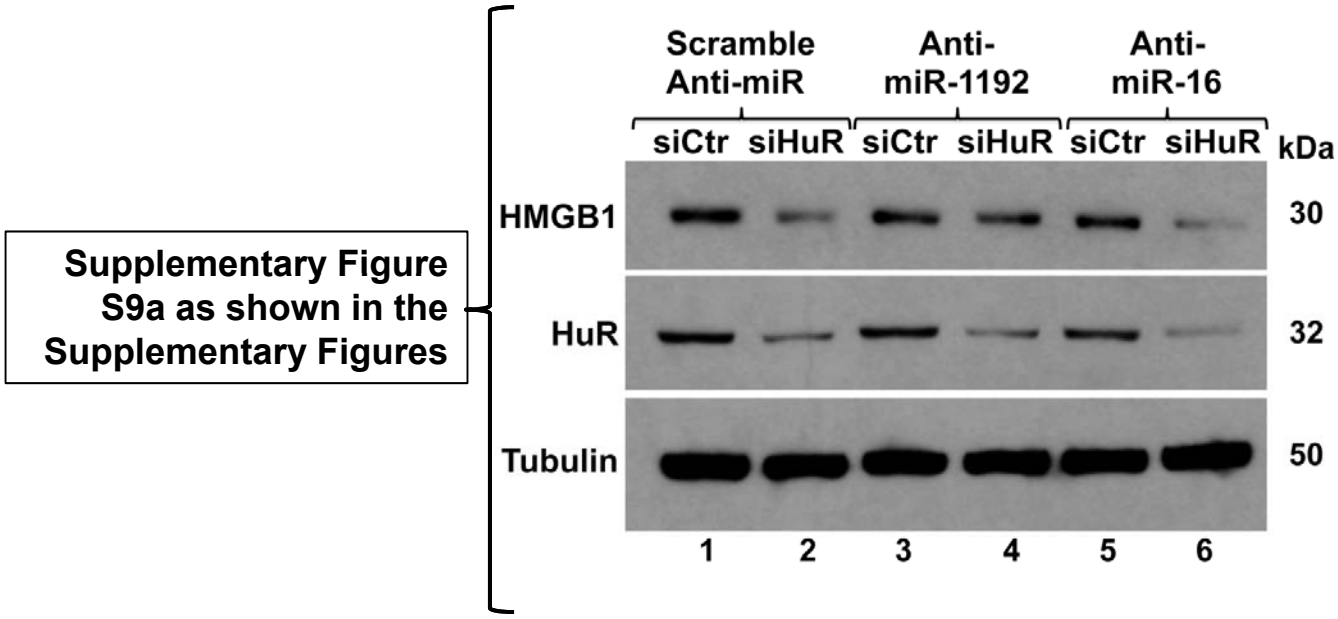


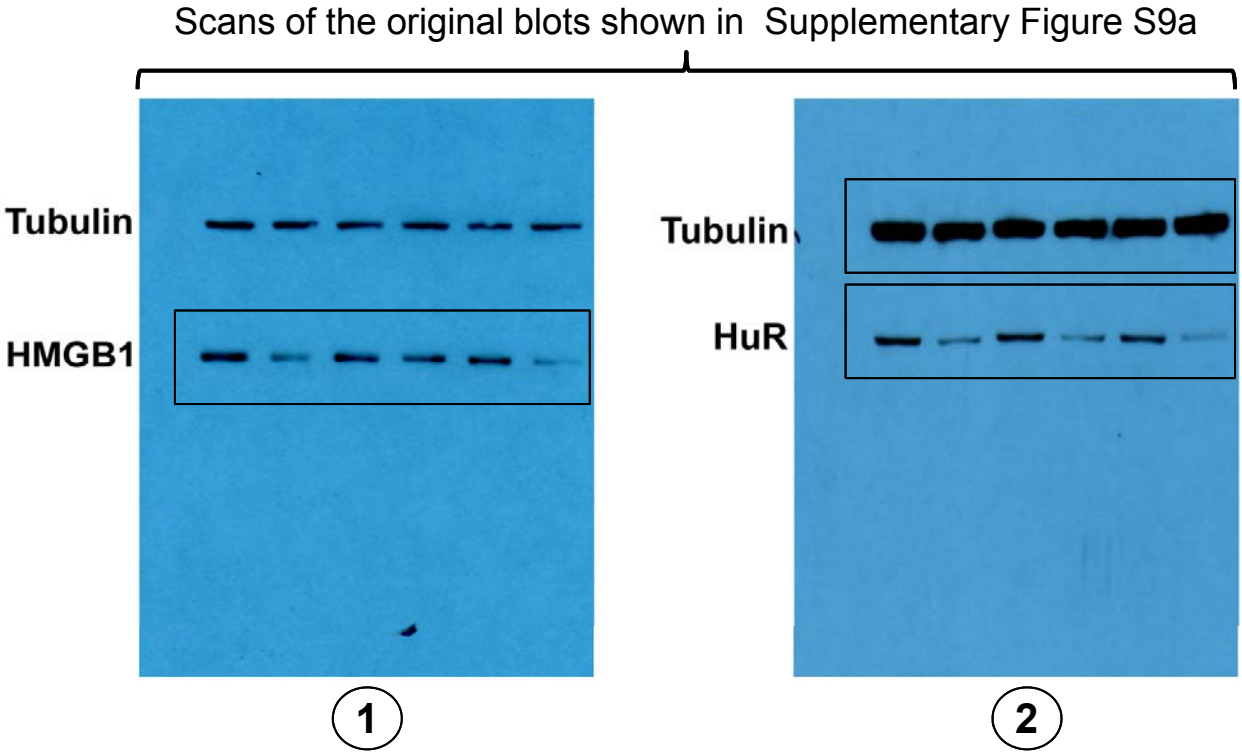
Scan of the original blots shown in Supplementary Figure S8b



The scan represents the membrane that was probed with the anti-caspase-3 antibody that recognizes both the non-cleaved and cleaved isoform of caspase-3. The box highlights the area of the blot that was used to mount Supplementary Figure S8b.

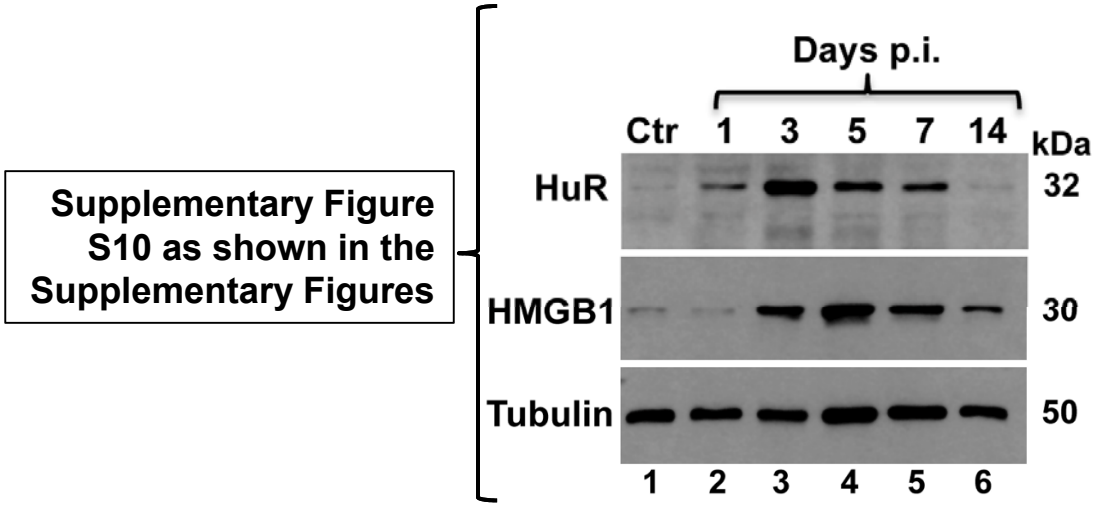
Supplementary Figure S12z

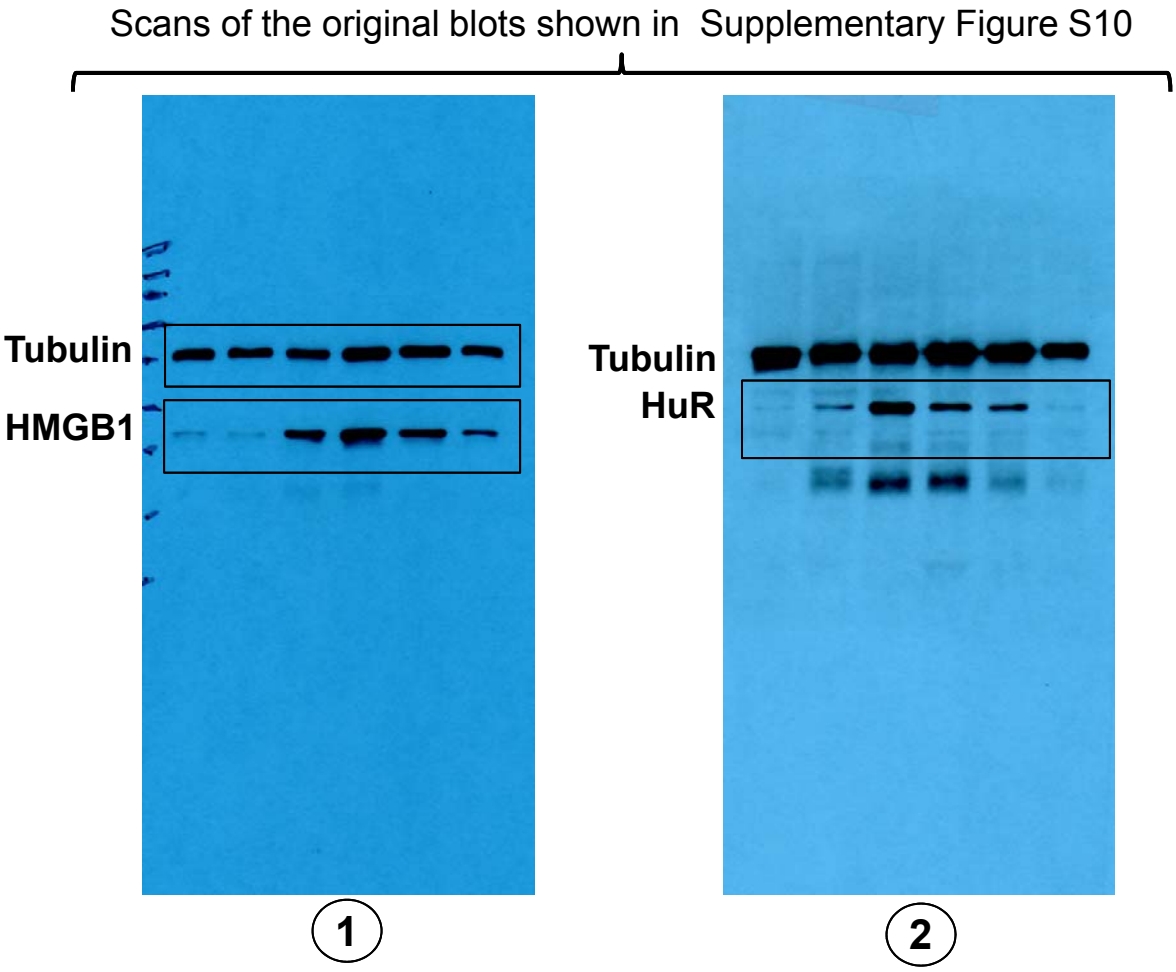




The two scans shown represent two different gels that were run using the same samples from the same experiment. Scan (1) represents the blot that was probed with both anti-HMGB1 and anti-Tubulin antibodies. Scan (2) represents a blot that was probed with both the anti-Tubulin and anti-HuR. The boxes highlight the area of each blot used to mount Supplementary Figure S9a.

Supplementary Figure S12za





The two scans shown represent two different gels that were run using the same samples from the same experiment. Scan (1) represents the blot that was probed with both anti-HMGB1 and anti-Tubulin antibodies. Scan (2) represents a blot that was probed with both the anti-Tubulin and anti-HuR. The boxes highlight the area of each blot used to mount Supplementary Figure S10.

Supplementary Table S1

HuR mRNA targets in undifferentiated C2C12 cells			
	zratio Exp	Gene Symbol	Gene Name
1	8.86	Actb	actin, beta, cytoplasmic
2	8.25	Acta2	Mus musculus actin, alpha 2, smooth muscle, aorta (Acta2), mRNA
3	8.22	Ccnh	cyclin H
4	7.95	Fbxl5	f-box and leucine-rich repeat protein 5
5	7.25	Heyl	hairy/enhancer-of-split related with YRPW motif-like
6	7.00	Phip	pleckstrin homology domain interacting protein
7	6.89	Crtap	cartilage associated protein
8	6.85	Fgf10	fibroblast growth factor 10
9	6.71	Adnp	activity-dependent neuroprotective protein
10	6.64	Ext2	exostoses (multiple) 2
11	6.60	Rbm3	RNA binding motif protein 3
12	6.38	Pcbp2	poly(rC) binding protein 2
13	6.24	Actg	actin, gamma, cytoplasmic
14	6.22	Gspt1	G1 to phase transition 1
15	6.02	Hmgb1	high mobility group box 1
16	5.91	Hunk	hormonally upregulated Neu-associated kinase
17	5.90	Dnaja1	DnaJ (Hsp40) homolog, subfamily A, member 1
18	5.89	Ywhaz	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide
19	5.81	Ccnf	cyclin F
20	5.77	Ube2l3	ubiquitin-conjugating enzyme E2L 3
21	5.72	Ptp4a1	protein tyrosine phosphatase 4a1
22	5.42	Plcd	phospholipase C, delta
23	5.41	Slc15a2	solute carrier family 15 (H+/peptide transporter), member 2
24	5.29	Fnbp3	formin binding protein 3
25	5.06	Peli1	pellino 1
26	5.05	Ap3d	adaptor-related protein complex AP-3, delta subunit
27	4.95	Hba-a1	hemoglobin alpha, adult chain 1
28	4.91	Tmem2	transmembrane protein 2
29	4.63	Ncoa6	nuclear receptor coactivator 6
30	4.55	no value	Mus musculus, Similar to expressed in activated T/LAK lymphocytes, clone MGC:6819 IMAGE:2648849, mRNA, complete cds
31	4.54	no value	Mus musculus, clone IMAGE:4949762, mRNA, partial cds
32	4.36	Acadl	acetyl-Coenzyme A dehydrogenase, long-chain
33	4.35	Set	SET translocation
34	4.31	Gsr	glutathione reductase 1
35	4.24	catp	cation-transporting atpase
36	4.21	Cai	calcium binding protein, intestinal
37	3.91	LOC55933	putative lysophosphatidic acid acyltransferase
38	3.86	Rpia	ribose 5-phosphate isomerase A
39	3.85	Cd81	CD 81 antigen
40	3.72	Syngr1	synaptogyrin 1
41	3.63	no value	Mus musculus, Similar to hypothetical protein FLJ20085, clone MGC:29315 IMAGE:5008342, mRNA, complete cds
42	3.32	Eplin-pending	
43	3.28	Dnm2	dynamin 2
44	3.09	Ptbp1	polypyrimidine tract binding protein 1
45	3.08	Dnajc7	DnaJ (Hsp40) homolog, subfamily C, member 7
46	3.07	Gss	glutathione synthetase
47	3.04	Ptma	prothymosin alpha
48	2.89	Rpl13a	ribosomal protein L13a
49	2.71	Dlgh1	discs, large homolog 1 (Drosophila)
50	2.70	Hmgb3	high mobility group box 3
51	2.68	Mbnl	muscleblind-like (Drosophila)
52	2.66	37501	septin 2
53	2.62	Ccni	cyclin I
54	2.61	Cd9	CD9 antigen
55	2.59	Klf5	Kruppel-like factor 5
56	2.43	no value	Mus musculus, Similar to hypothetical protein FLJ20257, clone MGC:28888 IMAGE:4911622, mRNA, complete cds
57	2.34	no value	Mus musculus, clone IMAGE:3964267, mRNA
58	2.33	Rce1	Ras and a-factor-converting enzyme 1 homolog (S. cerevisiae)
59	2.27	Ranbp1	RAN binding protein 1
60	2.21	Sdccag1	serologically defined colon cancer antigen 1
61	2.21	Pdhx	pyruvate dehydrogenase complex, component X
62	2.19	Foxp1	forkhead box P1
63	2.13	Csnk2b	casein kinase II, beta subunit
64	2.13	Mzf22	KRAB zinc finger protein (Mzf22)

HuR was immunoprecipitated from exponentially growing C2C12 cells using a monoclonal anti-HuR antibody, or an IgG control antibody. RNAs extracted from these immunoprecipitates were then processed and analyzed on cDNA array chips, which contain 17,000 probe sets of known and unknown expressed sequence tags as described in the Supplemental Material and Methods section. A default external background setting was used in conjunction with a gene-based background signal threshold to determine gene signal significance. A message was considered as an HuR target only if the difference in abundance after HuR- and IgG-IP was greater than or equal to two-fold. Therefore, mRNAs listed in this table represent the immunoprecipitated messages with a z ratio (HuR-IP/IgG-IP) over 2 in two independent experiments.

Supplementary Table S2: *List of microRNAs that associate with HuR protein in C2C12 muscle cells.*

microRNA associated with HuR	Signal HuR-IP/IgG-IP
mmu-miR-184	18.0
mmu-miR-1192	4.9
mmu-miR-467e*	4.7
mmu-miR-882	3.3
mmu-miR-709	3.1
mmu-miR-691	3.1
mmu-miR-138*	2.7
mmu-miR-875-3p	2.7
mmu-miR-466f-3p	2.6
mmu-miR-883a-5p	2.5
mmu-miR-720	2.3
mmu-miR-669f	2.3
mmu-miR-551b	2.3
mmu-miR-710	2.3
mmu-miR-711	2.3
mmu-miR-467g	2.2
mmu-miR-300*	2.2
mmu-miR-770-3p	2.1
mmu-miR-297a*/mmu-miR-297b-3p/mmu-miR-297c*	2.1
mmu-miR-467f	2.1

Microarray analysis of immunoprecipitation experiment using the anti-HuR antibody and cell extracts from C2C12 muscle cells. The analysis was carried out by EXIQON (USA). The list of 20 miRNAs that were immunoprecipitated with the anti-HuR antibody were selected based on a 2-fold-or-greater increase in their signal when compared to the list of miRNAs that immunoprecipitate with the control anti-IgG antibody.

Supplementary Table S3: *Sequencing of miR-1192 from muscle cells both in vitro and in vivo.*

a

miR	Clone #	C2C12 myoblasts
miR-1192	1	AAACAAACAAACAGACCAAAUU
	2	AAACAAACAAACAGACCAAAUU
	3	AAACAAACAAACAGACCAAAUU
	4	AAACAAACAAACAGACCAAAUU
	5	GGAACGAT-ACAGAGAAGATTAGC
	6	GGAACGAT-ACAGAGAAGATTAGC
	7	GGAACGAT-ACAGAGAAGATTAGC
	8	AAACAAACAAACAGACCAAAUU
	9	AAACAAACAAACAGACCAAAUU
	10	AAACAAACAAACAGACCAAAUU
	11	AAACAAACAAACAGACCAAAUU
	12	AAACAAACAAACAGACCAAAUU
	13	AAACAAACAAACAGACCAAAUU
	14	AAACAAACAAACAGACCAAAUU
	15	AAACAAACAAACAGACCAAAUU
U6	1	GCTTCGGCAGCACATATACTAAAATTGGAACGATACAGAGAAGATTAGCATGGCCCCTGCG CAAGGATGACACGCAAATTCGTGAAGCGTTCCATAT
	2	GCTTCGGCAGCACATATACTAAAATTGGAACGATACAGAGAAGATTAGCATGGCCCCTGCG CAAGGATGACACGCAAATTCGTGAAGCGTTCCATAT
	3	GCTTCGGCAGCACATATACTAAAATTGGAACGATACAGAGAAGATTAGCATGGCCCCTGCG CAAGGATGACACGCAAATTCGTGAAGCGTTCCATAT
	4	GCTTCGGCAGCACATATACTAAAATTGGAACGATACAGAGAAGATTAGCATGGCCCCTGCG CAAGGATGACACGCAAATTCGTGAAGCGTTCCATAT
	5	GCTTCGGCAGCACATATACTAAAATTGGAACGATACAGAGAAGATTAGCATGGCCCCTGCG CAAGGATGACACGCAAATTCGTGAAGCGTTCCATAT

b

miR	Clone #	Mouse muscle
miR-1192	1	AAACAAACAAACAGACCAAAUU
	2	AAACAAACAAACAGACCAAAUU
	3	AAACAAACAAACAGACCAAAUU
	4	AAACAAACAAACAGACCAAAUU
	5	AAACAAACAAACAGACCAAAUU
	6	AAACAAACAAACAGACCAAAUU
	7	AAACAAACAAACAGACCAAAUU
	8	AAACAAACAAACAGACCAAAUU
	9	AAACAAACAAACAGACCAAAUU
U6	1	GCTTCGGCAGCACATATACTAAAATTGGAACGATACAGAGAAGATTAGCATGGCCCCT GCGCAAGGATGACACGCAAATTCGTGAAGCGTTCCATAT

Total RNA extracts prepared from **(a)** C2C12 cells or **(b)** a gastrocnemius muscle isolated from a C57BL/6 mouse was reverse transcribed using the Universal cDNA synthesis kit (Exiqon). The resulting cDNA was then used in a PCR reaction containing Taq polymerase and primers specific for miR-1192 or U6 as a control. The resulting PCR product was cloned into the T-easy vector (Promega) and transformed in DH5α bacterial cells. Plasmid DNA was isolated from bacterial colonies and sequenced using a T7 specific primer. The obtained sequences from these colonies are shown in the tables.

Supplementary Table S4: *Primers used to prepare probes for gel shift experiments.*

Probes	Sequences
5'UTR	- ***For***: 5'-TAA TAC GAC TCA CTA TAG GGC ACA GCC ATT GCA GTA CAT-3' - ***Rev***: 5'- TCC TTT GCC CAT GTT TAG TTG A-3'
Probe 1 (P1)	- ***For:*** 5'-TAATACGACTCACTATAGGGTTGGTTCTAGCGCAGTTTTT-3' - ***Rev:*** 5'-CACAGCCGAGGCACAGAGTC-3'
Probe 2 (P2)	- ***For:*** 5'-TAATACGACTCACTATAGGGGCTGTGTAAGAATTGTTTTT-3' - ***Rev:*** 5'-TGAAAATACCACCAGGACAGG-3'
Probe 3 (P3)	- ***For:*** 5'-TAATACGACTCACTATAGGGGCCACTAACCTTGCCTGGTA-3' - ***Rev:*** 5'-TCCCCATATATAACTAATTTGTGCTG-3'
Probe 4 (P4)	- ***For:*** 5'-TAATACGACTCACTATAGGGTGGGGACAGTAGTTTGGTT TTT-3' - ***Rev:*** 5'-TCGTATAAGCTGCATCAGAGACA-3'
Probe 5 (P5)	- ***For:*** 5'-TAATACGACTCACTATAGGGTCTGATGCAGCTTATACGAAGA-3' - ***Rev:*** 5'-TCAGACCTATGAAAAGGACAACA-3'
Probe 6 (P6)	- ***For:*** 5'-TAATACGACTCACTATAGGGTTCTTCTCGAGGGGAAGCTA-3' - ***Rev:*** 5'-TGGATGAAAATGTTACCCTACCA-3'
Probe 7 (P7)	- ***For:*** TAATACGACTCACTATAGGGTGGTAGGGTAACATTTTCATCCA - ***Rev:*** 5'-CCTTCAAGGACAGACGTTCAG-3'
Probe 8 (P8)	- ***For:*** 5'-TAATACGACTCACTATAGGGCCTTGAAGGACTGATGGAAAA-3' - ***Rev:*** 5'-GCTCACACTTTTGGGGATACA-3'
Probe 9 (P9)	- ***For:*** 5'-TAATACGACTCACTATAGGGATCCCCAAAAGTGTGAGCTT-3' - ***Rev:*** 5'-CAAGTTTCCTGAGCAATCCA-3'
Probe 10 (P10)	- ***For:*** 5'-TAATACGACTCACTATAGGGTGGATTGCTCAGGAAACTTG-3' - ***Rev:*** 5'-GCAAATGTATTGGACAAATGG-3'
Probe 11 (P11)	- ***For:*** TAATACGACTCACTATAGGGTTGTCCAATACATTTGCTTTTTC - ***Rev:*** CACAAAAGCGCGTGAAATTA
Probe 12 (P12)	- ***For:*** TAATACGACTCACTATAGGGTCACGCGCTTTTGTGATG - ***Rev:*** AAAACCTCTGTAATTATTAGTTTATTAGTATCATCCAG

All the probes used for the gel-shift assays described in Figure 4B were synthesized by RT-PCR using the full-length HMGB1 cDNA (NCBI, NM_010439) with the primers outlined above.