SUPPLEMENTARY INFORMATION

p38^{MAPK}/MK2-mediated phosphorylation of RBM7 regulates the human nuclear exosome targeting complex

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Running title: Stress-dependent stabilization of nuclear ncRNAs

SUPPLEMENTARY FIGURES

S1: Murine Rbm7 and human RBM7 are both *in vitro* and *in vivo* substrates of MK2.

A. Human FLAG-RBM7 was overexpressed in HeLa cells that were treated as indicated. After a FLAG-IP, lysates and precipitates were analyzed by probing for p-PKD, pS136-Rbm7 and FLAG with the respective antibodies. Only anisomycin treatment led to a specific phopshpo-Rbm7 signal as detected by both the p(S/T)-PKD-substrate and the pS136-Rbm7 antibody. **B.** A radioactive kinase assay with precipitated GFP, GFP-Rbm7 and GFP-Rbm7^{S136A} using recombinant MK2 and p38 was performed as in Figure 1C.

S2: Subcellular localization of GFP-tagged Rbm7 and Rbm7^{S136A} and its potentially enhanced proteasomal degradation

A. Both N-terminal tagged GFP-fusion proteins were overexpressed in HeLa cells and visualized by life-cell microscopy (DM IL LED, Leica Microsystems) upon anisomycin stimulation and in combination with p38^{MAPK}-inhibtion using BIRB796. No change in the localization of GFP-fusion proteins was observed. GFP-Rbm7 and GFP-Rbm7^{S136A} remained exclusively nuclear throughout stimulation. In strongly overexpressing cells, GFP-Rbm7 and GFP-Rbm7^{S136A} did accumulate in distinct spots. This effect was independent of stress stimulation. The magnification was 20-fold. **B.** A potentially enhanced phosphorylation-driven proteasomal degradation of endogenous RBM7 was ruled out by analyzing RBM7 protein levels in anisomycin-stimulated HeLa cells in combination with MG-132 mediated proteasome inhibition (20µM). The BIRB796-induced restoration of the RBM7 signal was not achieved by MG-132 indicating no increased proteasomal degradation of RBM7 upon anisomycin-stimulation.

S3: The interaction of exogenous Rbm7 and exogenous ZCCHC8 with their endogenous interacting partners are not altered upon anisomycin-stimulation

A. FLAG-tagged ZCCHC8 was expressed together with GFP, GFP-Rbm7 or GFP-Rbm7^{S136A} in non-stimulated or anisomycin-stimulated HeLa cells. Upon pulldown of FLAG-ZCCHC8, no changes in GFP-Rbm7 or GFP-Rbm7^{S136A} association were observed. GFP alone did not interact with FLAG-ZCCHC8. **B.** GFP-ZCCHC8 was expressed in HeLa cells that were stimulated as indicated. The association of endogenous RBM7 (indicated by an arrow) was monitored by GFP-pulldown. The precipitation of GFP-ZCCHC8 was not as effective as it was for GFP-Rbm7 (see Supplementary Figure S3C). Nevertheless, endogenous RBM7 was

co-purifing with exogenous GFP-ZCCHC8. Notably, cell stimulation with anisomycin once again weakened the intensity of the RBM7 signal. The intense upper band in the RBM7 pulldown-blot was unspecific, because in lysates it was only present in GFP-ZCCHC8 overexpressing cells. We speculate that the ZCCHC8 antibody could cross-react with ZCCHC8-fragments. **C.** GFP-Rbm7 and GFP-Rbm7^{S136A} were expressed in HeLa cells. The cells were stimulated as indicated and GFP-fusion proteins were precipitated using a GFP-Nanotrap. The association of endogenous ZCCHC8 with the GFP-fusion proteins was not altered under any condition. Interaction with endogenous CBP80 was partly lost upon stimulation and was not restored upon p38^{MAPK}-inhibitor treatment indicating a p38-independent effect.

S4: RBM7-binding to RNAs is abrogated by stress

A. GFP-Rbm7 or GFP-Rbm7^{S136A} expressed in HeLa cells were cross-linked (CL) to cellular RNA using UV-C light prior to precipitation (CLIP). RNaseI treatment was not sufficient to fully digest RNAs cross-linked to Rbm7 even at high concentrations and resulted in a smear of various RNA-Rbm7 cross-linking products. GFP-only expressing cells were used as control. Here, no cross-link product was detectable indicating that Rbm7 was specifically bound to RNAs. **B**. Replicate of Figure 2B showing the partial rescue of the loss of Rbm7 RNA-binding capacity by introducing the S136A mutation. Quantifications for both replicates are shown in relation to the anisomycin treated wildtype protein sample (GFP-Rbm7). **C.** The loss of RNA-binding capacity of wildtype Rbm7 was also is in part reversed by BIRB796-mediated p38^{MAPK}-inhibtion before anisomycin stimulation (2 replicates). The effect was similar to what was seen with the mutant Rbm7^{S136A} protein in Figure 2B and Supplementary Figure S4B. Quantifications of the Rbm7-bound RNA signals from the autoradiographs in relation to the anti-GFP signals from the same membrane are shown. The signal for anisomycin-treated HeLa cells expressing GFP-Rbm7 was set to 1. Stars indicate $p < 0.01$.

S5: RBM7 is not an AU-rich element binding protein

A. GFP, GFP-Rbm7 (or as a positive control GFP-TTP) were overexpressed in HEK293T cells. 10µg of each lysate was incubated with a specific AU-rich- or a random control-RNA (DY681- and DY781-labelled). Only GFP-TTP showed high-affinity *in vitro* binding to an AU-rich-specific RNA. Expression of the proteins was monitored by anti-GFP Western blot. **B.** The consequences of GFP-Rbm7 overexpression on the expression of different AU-rich luciferase reporter constructs were analyzed in HEK293T cells. To this end, the complete

3´UTR of murine TNFα and human IL-6 was subcloned into Firefly-coding vector just downstream of the Luciferase coding sequence. For TNFα a mutant lacking the AU-rich element was cloned and also analyzed. For normalization a renilla-luciferase-coding vector was co-transfected. The relative firefly expression level of GFP-transfected cells was set to 1. The TNFα 3´UTR strongly suppresses reporter expression and can only be partly restored by the AU-rich element deletion. Results are shown as mean of three independent experiments.

S6: Different p38^{MAPK}-inhibitors can down-regulate PROMPTs RNA levels to a similar extend, but GSK-3 inhibition does not

A. proRBM39, proEXT1, proIFNAR1 and proDNAJB4 RNA levels were measured in HeLa cells upon anisomycin-stimulation and in combination with the three known p38^{MAPK}-inhibitors BIRB796, SB202190 and SB203580. All three inhibitors decreased the proRBM39, proEXT1 and proDNAJB4 levels. SB203580 did affect proIFNAR1 RNA transcript. **B.** HeLa cells stimulated with anisomycin for 2h were pre-treated for 1h with LiCl (15mM final) and lysates were assayed for endogenous ZCCHC8, RBM7 and GAPDH as a loading control. Furthermore, HeLa cells stimulated as before were analysed for proRBM39, proEXT1 and proDNAJB4 levels by qRT-PCR. No substantial changes were detected for proRBM39 and proEXT1 indicating no major role of GSK-3 in regulating these PROMPTs.

S7: Anisomycin targets the nuclear RNA exosome via RBM7

A. Western Blot confirmation for transfection of GFP, GFP-RBM7 and GFP-RBM7^{S136A} in HeLa cells used in Figure 3E. **B.** Confirmation of hRRP40 and RBM7 depletion using siRNA. HeLa cells were treated with either a Control siRNA or hRRP40- and RBM7-specific siRNAs and 48h posttransfection treated as indicated. The knockdown efficiency of hRRP40 and RBM7 was assayed by Western Blot. **C.** U snRNA metabolites of either the major (U1, U2, U4) or the minor splicing complex (U11, U12, U6atac) (non-specific detection of precursors and mature transcripts) levels were determined after 2h anisomycin stimulation and prior p38^{MAPK}-inhibition using BIRB796.

S8: IL-1α stimulation of HeLa and osmotic shock selectively upregulate PROMPTs

A. HeLa cells were stimulated with 2ng/ml IL-1α for the indicated periods of time. Only the proRBM39 transcript was moderately induced in a p38-dependent manner by this stimulus. The star indicates $p < 0.04$ (as determined by two-sided student´s t-test) for the IL-1α-induced upregulation of proRBM39. **B.** Osmotic stress, like IL-1α, has a minor influence on

PROMPTs accumulation in HeLa cells. High osmolarity was induced by adding NaCl to a final concentration of 150mM to the medium and subsequent incubation for 60 min at 37°C in the tissue culture incubator before harvesting cells for RNA isolation. Only proEXT1 showed a weak $p38^{MAPK}$-dependent response to osmotic shock that was not significant ($p > 0.1$, as determined by two-sided student´s t-test).

S9: Effect of the MK2-inhibitor PHA781089 on RBM39 mRNA levels and minor induction of proIFNAR1 in HEK293T cells

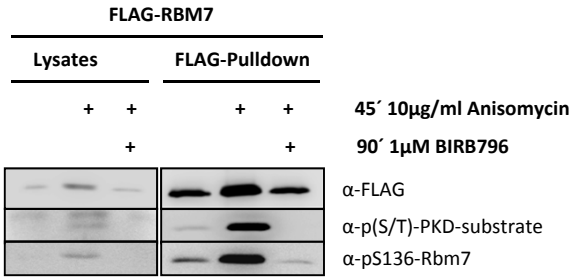
A. RBM39 mRNA increases moderately in response to anisomycin and is only in part sensitive to MK2-inhibitor PHA781089 pre-treatment. **B.** proIFNAR1 is only weakly accumulating in HEK293T cells upon anisomycin stimulation. For comparison the accumulation of proRBM39 is shown in the same experimental setup.

S10: proEXT1 and U snRNA metabolites are significantly stabilized upon anisomycin stimulation

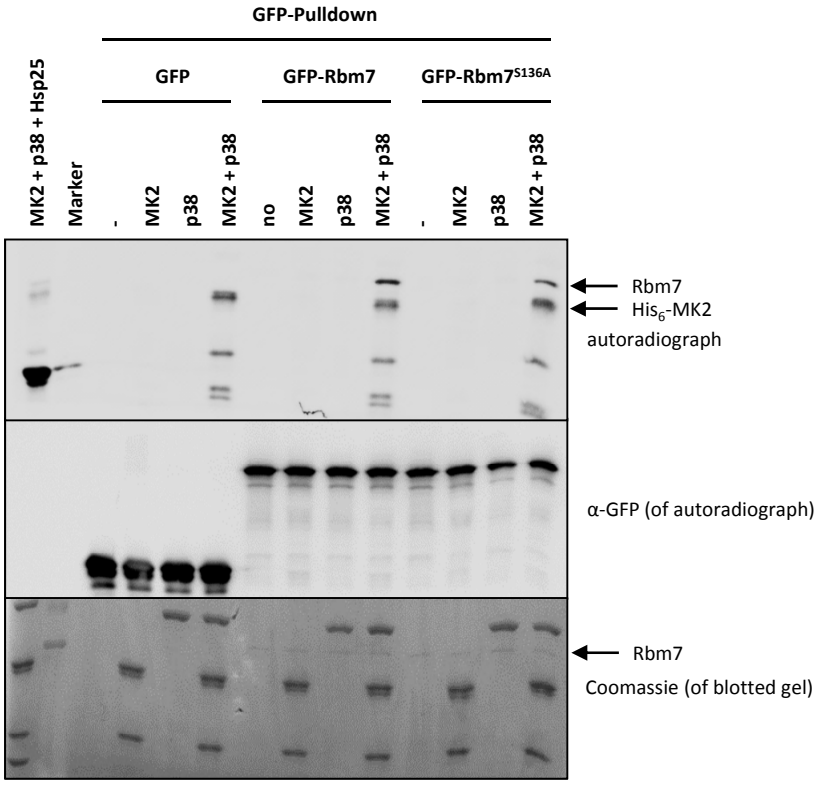
A. proEXT1 decay kinetics after stimulating HeLa cells with Anisomycin for 2h. **B.** U snRNAs from both the major and the minor splicing complex were stabilized by stress in a p38-dependent manner. Similar to Figure 6E and F the half-lives of U1 and U11 snRNAs were determined in HeLa cells. Like the PROMPTs, U snRNAs were stabilized upon stress in a p38-dependent manner.

S1

A

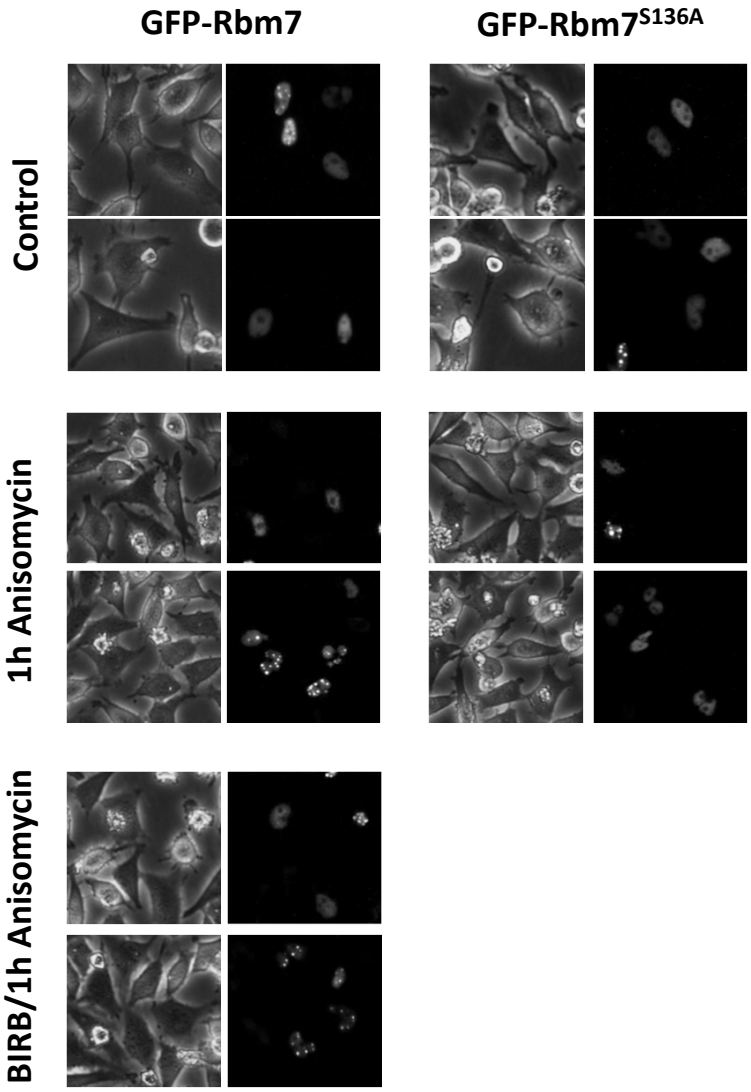

FLAG-RBM7
Lysates
FLAG-Pulldown
+ + + +
45´ 10µg/ml Anisomycin
+ +
90´ 1µM BIRB796
α-FLAG
α-p(S/T)-PKD-substrate
α-pS136-Rbm7

B

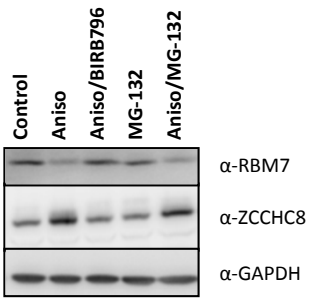

GFP-Pulldown
GFP
GFP-Rbm7
GFP-Rbm7S136A
MK2 + p38 + Hsp25
Marker
-
MK2
p38
MK2 + p38
no
MK2
p38
MK2 + p38
-
MK2
p38
MK2 + p38
Rbm7
His6-MK2
autoradiograph
α-GFP (of autoradiograph)
Rbm7
Coomassie (of blotted gel)

S2

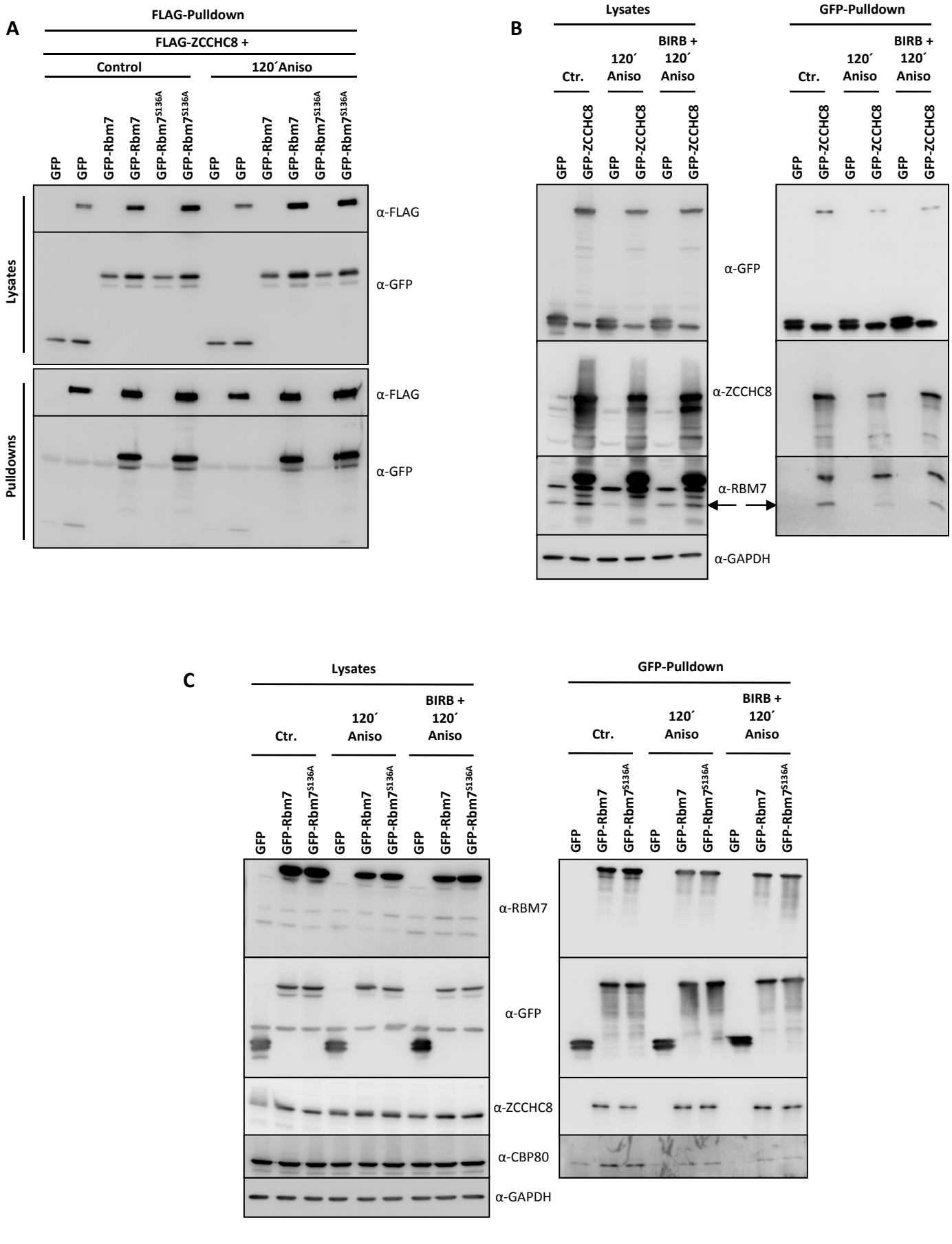
A


GFP-Rbm7
GFP-Rbm7S136A
Control
1h Anisomycin
BIRB/1h Anisomycin

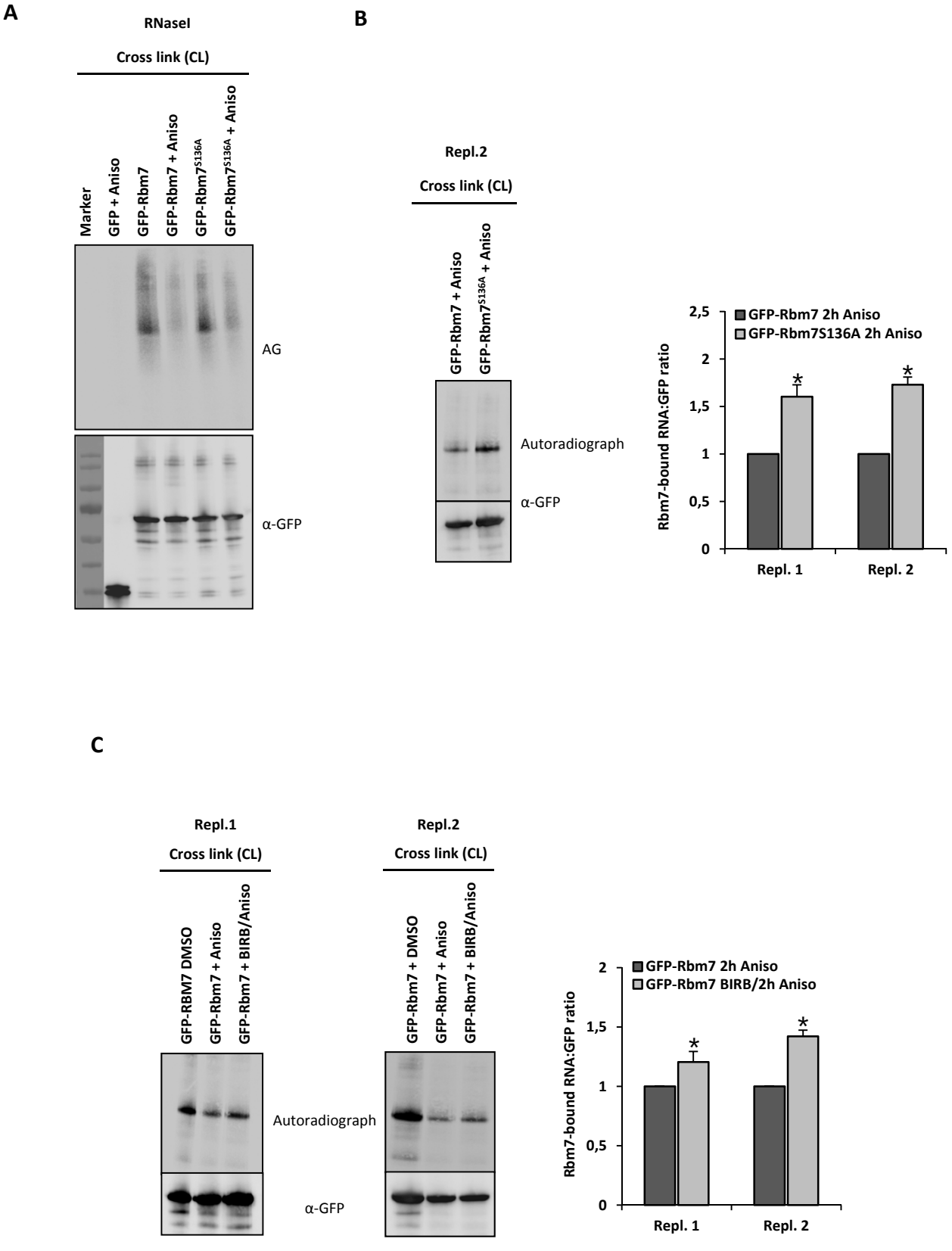
B


Control
Aniso
Aniso/BIRB796
MG-132
Aniso/MG-132
α-RBM7
α-ZCCHC8
α-GAPDH

S3

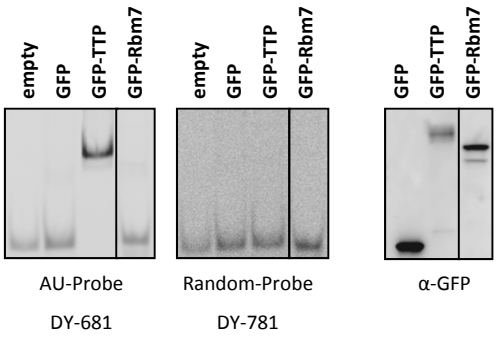

A
FLAG-Pulldown
FLAG-ZCCHC8 +
Control
120´Aniso
GFP
GFP
GFP-Rbm7
GFP-Rbm7
GFP-Rbm7S136A
GFP-Rbm7S136A
GFP
GFP
GFP-Rbm7
GFP-Rbm7
GFP-Rbm7S136A
GFP-Rbm7S136A
Lysates
Pulldowns
α-FLAG
α-GFP
α-FLAG
α-GFP
B
Lysates
GFP-Pulldown
Ctr.
120´ Aniso
BIRB + 120´ Aniso
GFP
GFP-ZCCHC8
α-GFP
α-ZCCHC8
α-RBM7
α-GAPDH
C
Lysates
GFP-Pulldown
Ctr.
120´ Aniso
BIRB + 120´ Aniso
GFP
GFP-Rbm7
GFP-Rbm7S136A
α-RBM7
α-GFP
α-ZCCHC8
α-CBP80
α-GAPDH

S4

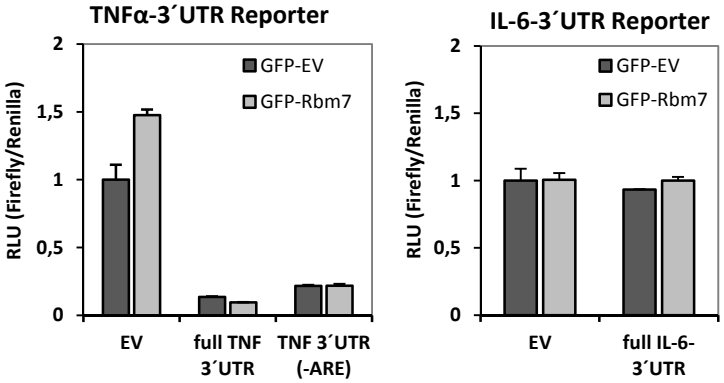

A
RNaseI
Cross link (CL)
Marker
GFP + Aniso
GFP-Rbm7
GFP-Rbm7 + Aniso
GFP-Rbm7S136A
GFP-Rbm7S136A + Aniso
AG
α-GFP
B
Repl.2
Cross link (CL)
GFP-Rbm7 + Aniso
GFP-Rbm7S136A + Aniso
Autoradiograph
α-GFP
Rbm7-bound RNA:GFP ratio
GFP-Rbm7 2h Aniso
GFP-Rbm7S136A 2h Aniso
2,5
2
1,5
1
0,5
0
Repl. 1
Repl. 2
C
Repl.1
Cross link (CL)
Repl.2
Cross link (CL)
GFP-RBM7 DMSO
GFP-Rbm7 + Aniso
GFP-Rbm7 + BIRB/Aniso
GFP-Rbm7 + DMSO
GFP-Rbm7 + Aniso
GFP-Rbm7 + BIRB/Aniso
Autoradiograph
α-GFP
Rbm7-bound RNA:GFP ratio
GFP-Rbm7 2h Aniso
GFP-Rbm7 BIRB/2h Aniso
2
1,5
1
0,5
0
Repl. 1
Repl. 2

S5

A

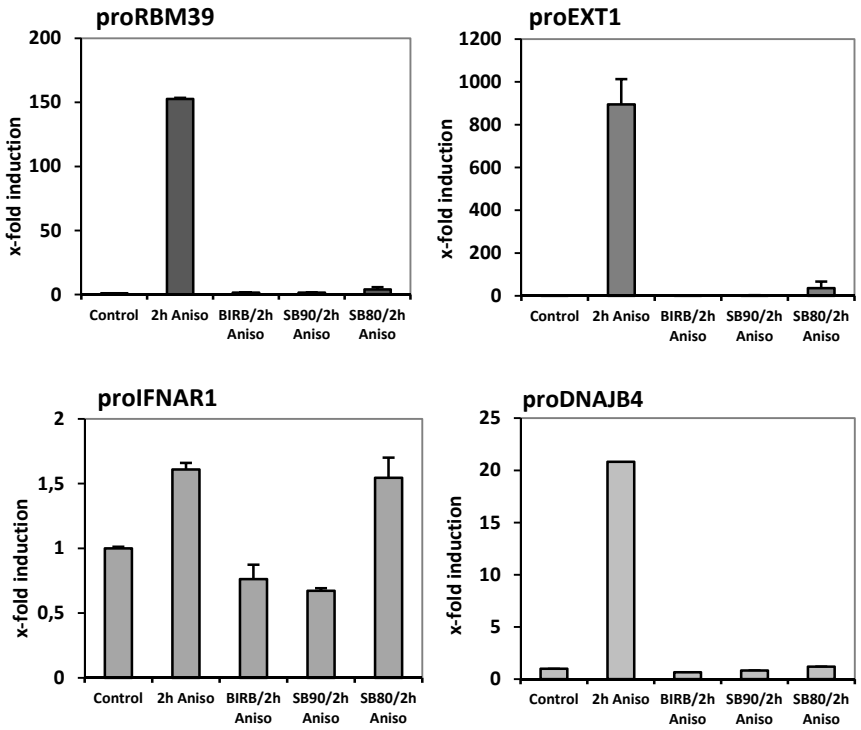

empty
GFP
GFP-TTP
GFP-Rbm7
empty
GFP
GFP-TTP
GFP-Rbm7
GFP
GFP-TTP
GFP-Rbm7
AU-Probe
DY-681
Random-Probe
DY-781
α-GFP

B

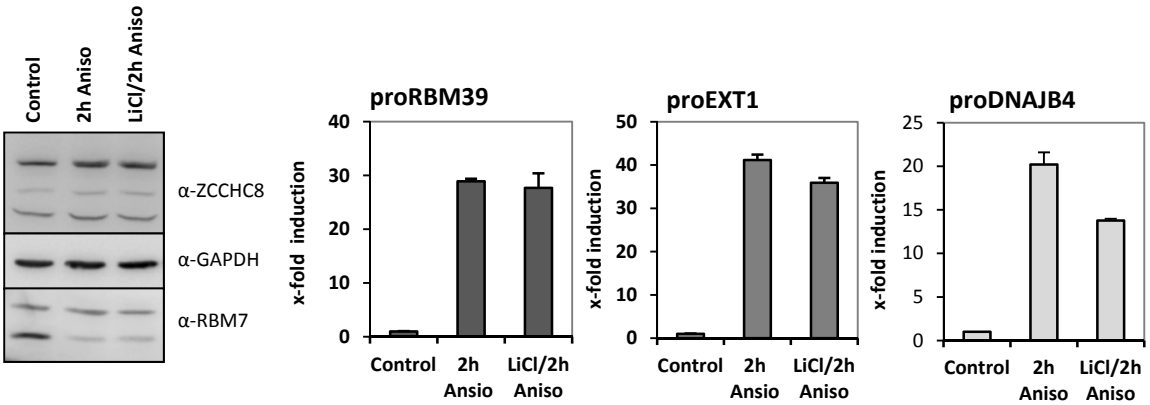

TNFα-3´UTR Reporter
IL-6-3´UTR Reporter
GFP-EV
GFP-Rbm7
RLU (Firefly/Renilla)
2
1,5
1
0,5
0
EV
full TNF 3´UTR
TNF 3´UTR (-ARE)
full IL-6-3´UTR

S6

A

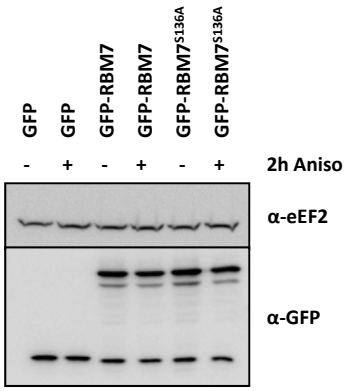

proRBM39
x-fold induction
200
150
100
50
0
Control
2h Aniso
BIRB/2h Aniso
SB90/2h Aniso
SB80/2h Aniso
proEXT1
1200
1000
800
600
400
200
0
proIFNAR1
2
1,5
1
0,5
0
proDNAJB4
25
20
15
10
5
0

B

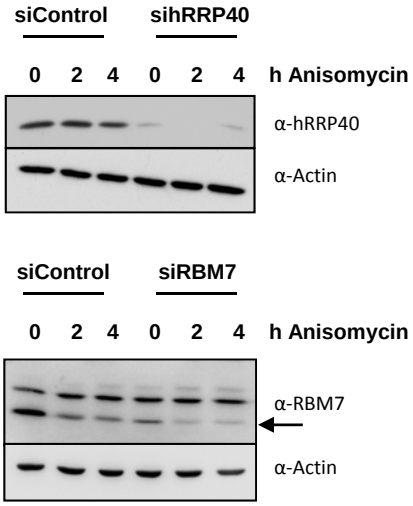

Control
2h Aniso
LiCl/2h Aniso
α-ZCCHC8
α-GAPDH
α-RBM7
proRBM39
proEXT1
proDNAJB4
x-fold induction
40
30
20
10
0
50
40
30
20
10
0
25
20
15
10
5
0
Control
2h Ansio
LiCl/2h Aniso
2h Aniso

S7

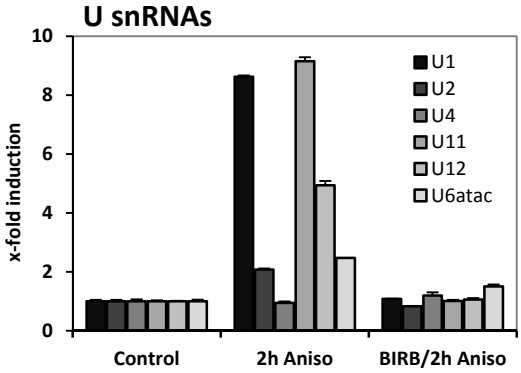
A


GFP
GFP
GFP-RBM7
GFP-RBM7
GFP-RBM7S136A
GFP-RBM7S136A
- + - + - +
2h Aniso
α-eEF2
α-GFP

B

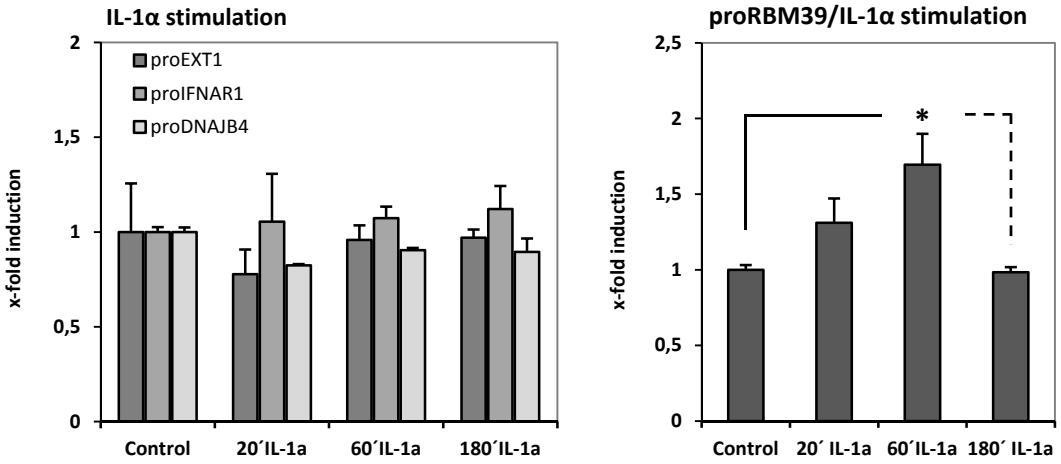

siControl
sihRRP40
0 2 4 0 2 4 h Anisomycin
α-hRRP40
α-Actin
siControl
siRBM7
0 2 4 0 2 4 h Anisomycin
α-RBM7
α-Actin

C

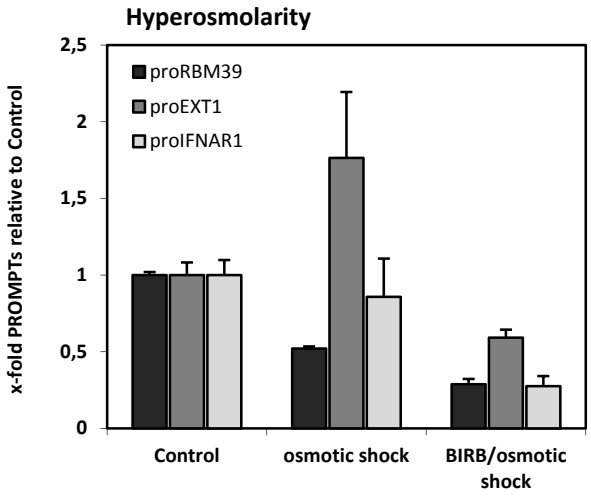

U snRNAs
x-fold induction
10
8
6
4
2
0
U1
U2
U4
U11
U12
U6atac
Control
2h Aniso
BIRB/2h Aniso

S8

A

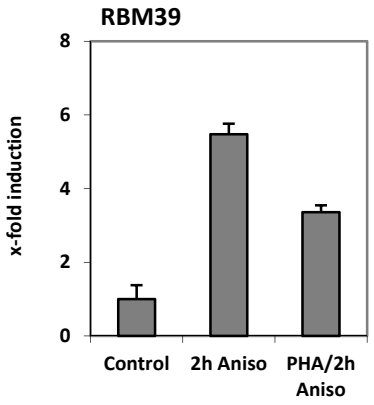

IL-1α stimulation
proEXT1
proIFNAR1
proDNAJB4
x-fold induction
2
1,5
1
0,5
0
Control
20´IL-1a
60´IL-1a
180´IL-1a
proRBM39/IL-1α stimulation
2,5
2
1,5
1
0,5
0
*
x-fold induction
Control
20´ IL-1a
60´IL-1a
180´ IL-1a

B

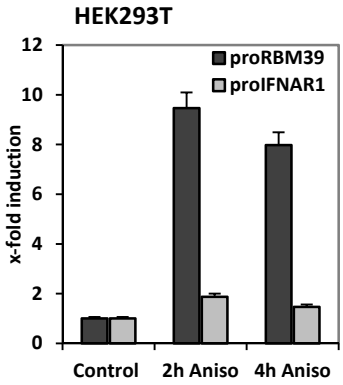

Hyperosmolarity
proRBM39
proEXT1
proIFNAR1
x-fold PROMPTs relative to Control
2,5
2
1,5
1
0,5
0
Control
osmotic shock
BIRB/osmotic shock

S9

A

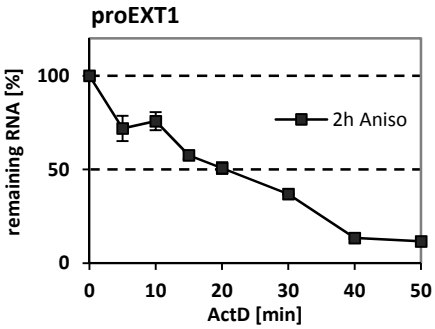

RBM39
x-fold induction
8
6
4
2
0
Control
2h Aniso
PHA/2h Aniso

B

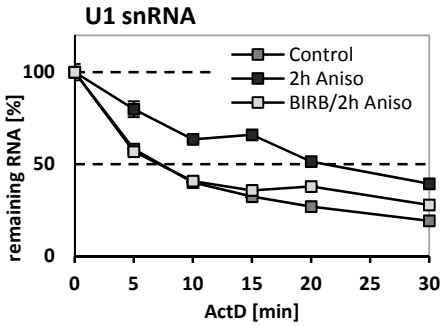

HEK293T
proRBM39
proIFNAR1
x-fold induction
12
10
8
6
4
2
0
Control
2h Aniso
4h Aniso

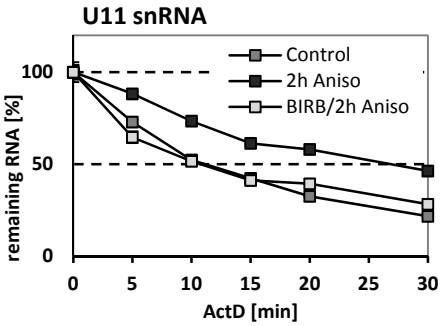
S10

A


proEXT1
remaining RNA [%]
100
50
0
2h Aniso
0
10
20
30
40
50
ActD [min]

B


U1 snRNA
remaining RNA [%]
100
50
0
Control
2h Aniso
BIRB/2h Aniso
0
5
10
15
20
25
30
ActD [min]


U11 snRNA
remaining RNA [%]
100
50
0
Control
2h Aniso
BIRB/2h Aniso
0
5
10
15
20
25
30
ActD [min]