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## Supplemental Information

# The Long Non-coding RNA *lnc-31* Interacts with *Rock1* mRNA and Mediates Its YB-1-Dependent Translation

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## SUPPLEMENTAL PROCEDURES

### Plasmids construction

*Lnc-31* was amplified by PCR from $C_2C_{12}$ derived cDNA using lnc-31 fw and rev oligonucleotides and cloned into HindIII-BamHI sites of pcDNA3.1+ vector. The plnc-31 construct, lacking 22 nucleotides of the pre-miR-31 sequence, was derived by inverted PCR using lnc-31Δ fw and rev oligonucleotides. The 5' UTR of *Rock1* mRNA was amplified by PCR from $C_2C_{12}$ cDNA using mmu-Rock1-5' fw and rev oligonucloetides and cloned into NheI of psi-CHECK2 vector (Promega) upstream the Renilla coding sequence giving rise to Luc/5'Rock1 construct. The same procedure was used to generate the Luc/-200 and Luc/-100 contructs using the oligonucleotides: mmu-Rock1-200 fw/ mmu-Rock1 rev and mmu-Rock1-100 fw/ mmu-Rock1-5' rev respectively. The Luc/3'WT contruct was raised by PCR amplification of the 3'UTR of *Rock1* mRNA from $C_2C_{12}$ cDNA using Luc/3'WT fw and rev oligonucleotides and by cloning this into Pme1/Not1 sites of psi-CHECK2 vector. The Luc/3'ΔΔ was derived from Luc/3'WT construct by two sequential inverted PCR using Luc/3'Δ fw and rev and Luc/3'ΔΔ fw and rev oligonucleotides. Psp-152 construct was generated by cloning the pri-miR-152 sequence from $C_2C_{12}$ genomic DNA using miR-152 fw and rev oligonucleotides, into BglII/XhoI sites of psp-65 vector (Denti et al., 2004). The sequences of all oligonucleotides used for plasmid preparation are listed below.

### RNA-Seq and bioinformatic analysis

TruSeq Stranded mRNA Library Prep Kit (Illumina) was used to prepare cDNA libraries from SCR and si-lnc-31 $C_2C_{12}$ cells (two biological replicates per condition). NuGEN Ovation RNA-Seq System V2 was used to prepare cDNA libraries for the pull down experiment. The sequencing reactions, performed on an Illumina Hiseq 2500 Sequencing system at the Institute of Applied Genomics (IGA; Udine, Italy), produced an average of 43 million 132 nucleotide long paired-end reads per sample for the mRNA-Seq and an average of 60 million 50 nucleotide long single-end reads per sample for the pull down experiment (Table S1).

Trimmomatic software (Bolger, et al., (2014) was used pre-process mRNA-Seq reads by removing adapter sequences and poor quality bases; the minimum read length after trimming was set to 30. Surviving reads were aligned using Bowtie 2 (Langmead and Salzberg et al., 2012) to a sequence database composed of rRNAs, tRNAs, snRNA and snoRNAs; reads mapping to these sequences were filtered out. In order to calculate the distribution of the inner distance between mate pairs, reads were aligned to a non-redundant set of murine mRNA sequences derived from Gencode M4 annotation (Harrow et al., 2006) using BWA software (Li and Durbin, 2010). We estimated mean and variance of the inner distance distribution from aligned read pairs whose inner distance was within interval [Q1-2(Q3-Q1),Q3+2(Q3-Q1)] (Q1=first quartile, Q3=third quartile). TopHat2 (Kim et al., 2013) was employed to align reads to mm10 mouse genome and Gencode M4 transcriptome using parameters -i 50 -r -27 --mate-std-dev 85 --library-type fr-firststrand. Read numbers and mapping statistics are reported in Table 5. Gene-level expression estimation, as well as differential expression analysis, was performed using Cuffdiff 2 software (Trapnell et al., 2013). Heatmap of differentially expressed genes was constructed based on mean-centered log2-transformed FPKM values of genes using the Heatmap3 R package (Zhao et al., 2014). Gene Ontology Term enrichment analysis was performed using GOrilla web server (Eden et al., 2009). The GOsummaries R package was used to create a word cloud for the enriched GO terms (p-value < 0.001) (Kolde, 2016).

Raw reads relative to the pull down experiment were subjected to quality trimming using Trimmomatic and adapter sequences were removed using Cutadapt (Martin Marcel, 2011); the minimum read length after trimming was set to 30. Low complexity reads were then removed using the DUST algorithm included in the PRINSEQ suite (Schmieder and Edwards et al., 2011), setting the complexity score threshold to 7. Reads mapping to rRNAs, tRNAs, snRNA or snoRNAs, found

using the Bowtie 2 aligner, were filtered out. Surviving reads were mapped to mm10 mouse genome and Gencode M4 transcriptome using Tophat2 with parameters -i 50 --library-type fr-unstranded. Duplicate reads, which could represent PCR artifacts, were removed using MarkDuplicates from Picard (picard.sourceforge.net/command-line-overview.shtml). Reads mapping to multiple locations were filtered out using BamTools (Barnett et al., 2011). Read numbers and mapping statistics are reported in Table 5. Piranha (Uren et al., 2012) was employed, using the input as a covariate, to find enriched read clusters both for *lnc-31* pull down and lacZ experiment; bin size was set to 200. Using BEDTools intersect (Quinlan and Hall 2010) we identified those mRNAs whose exons overlap with enriched read clusters. All those mRNAs identified in the *lnc-31* pull down experiment, but not in the lacZ experiment, were taken as candidate *lnc-31*-bound RNAs, ranking them based on the minimum p-value obtained for an overlapping read cluster. The interaction between *lnc-31* and *Rock1* 5'UTR was predicted using IntaRNA software (Busch et al., 2008).

**Sample preparation for mass spectrometric analysis**

Protein pull-down was performed as described above. Bound proteins were eluted with 25 µl of 1x LDS buffer (NuPAGE LDS Sample Buffer with NuPAGE Sample Reducing Agent (Thermo Fisher Scientific) and separated on a Bis/Tris bufferd 4-12 % gradient gel (Invitrogen).

After gradient-gels were destained, bands or gel parts were excised and transferred into 2ml micro tubes (Eppendorf), washed for 30 min with 500 µl 50 mM $NH_4HCO_3$, 375 µl 50 mM $NH_4HCO_3$/ 125 µl acetonitrile (3/1), 375 µl 10 mM $NH_4HCO_3$/ 125 µl acetonitrile (3/1), 250 µl 10 mM $NH_4HCO_3$/ 250 µl acetonitrile (1/1) and lyophilized. After reduction and alkylation of the samples with 100 µl 1mg/ml DTT (57 °C/ 35 min) and 200 µl 5 mg/ml Iodoacetamide (RT/ 35 min) solved in 50 mM $NH_4HCO_3$, excised gel parts were were washed again as before. Proteins were subjected to *in gel* tryptic digest overnight at 37 °C with 0,8 µg Trypsin Gold mass spectrometry grade (Promega) per sample. Peptides were first extracted twice with 100 mM $NH_4HCO_3$, followed by 100 mM $NH_4HCO_3$/ acetonitrile (2/1). Eluates were combined and lyophilized.

**Mass spectrometric analysis**

Peptides were reconstituted in 20 µl of 1 % formic acid prior to separation by reversed-phase chromatography on an UltiMate 3000 RSLCnano System (Thermo Scientific, Dreieich). The system was equipped with a C18 Acclaim Pepmap100 preconcentration column (100µm i.D.x20mm, Thermo Fisher) in front of an Acclaim Pepmap100 C18 nano column (75 µm i.d. × 150 mm, Thermo Fisher). By applying a linear gradient of 4% to 40% acetonitrile in 0.1% formic acid at a flow rate of 300 nl/min peptides were separated over 90 min. The LC-system was coupled on-line to a maXis plus UHR-QTOF System (Bruker Daltonics, Bremen) via a nano electrospray source (Bruker Daltonics). Data-dependent acquisition of MS/MS spectra by CID fragmentation was performed at a resolution of minimum 60000 for MS and MS/MS scans. The MS spectra rate of the precursor scan was 2 Hz processing a mass range between m/z 175 and m/z 2000. Up to five most abundant precursor ions were selected for fragmentation. The data (mgf-Files) were launched to Mascot using the Protein Scape software (Bruker Daltonics). Mascot (v2.3.02, Matrix Science) was used as a search engine to search the Swissprot protein database. Search parameters were as follows: enzyme specificity trypsin with 1 missed cleavage allowed, precursor tolerance 0.02 Da, MS/MS tolerance 0.04 Da, carbamidomethylation or propionamide modification of cysteine, oxidation of methionine, deamidation of asparagine and glutamine were set as variable modifications. Mascot peptide ion-score cut-off was set 25. Protein list compilation was done using the Protein Extractor function of Protein Scape.

**List of Oligonucleotides**

<u>Oligonucleotides used for qPCR and q-RT-PCR:</u>

Mm_Myog_1_SG QuantiTect Primer Assay (200) QT00112378

mmu-Rqcd1 FW: 5'-GAGTCTGTCCCTGACCTTGCACC-3'
mmu-Rqcd1 REV: 5'-CTGTTAGACTGGTGTGCTGTCAAGG-3'
mmu-Ergc1 FW: 5'-GATGCCCTTTGACTTCAGGAGG-3'
mmu-Ergc1 REV: 5'-GTGAGCTCCGACAGGAAGAGGA-3'
mmu-CCNE1 FW: 5'- GAAATTGCCAAGATTGACAAGACTG-3'
mmu-CCNE1 REV: 5'-GTCCTCTTCTTTGTTAGGGGTGGG-3'
mmu-CCND1 FW: 5'-GTGAGGAGCAGAAGTGCGAAGA-3'
mmu-CCND1 REV: 5'-CGGCAGTCAAGGGAATGGT-3'
mmu-Clock FW: 5'-CAGGAACAGCAGCTTCCTTCAG-3'
mmu-Clock REV: 5'-GCTGGTGTTGCTGGTGGTGC-3'
mmu-Hebgf FW: 5'-CACAGGAGAGGAGGTTATGACTTGG-3'
mmu-Hebgf REV: 5'-GCAACTGGAAATGAATGAAGACG-3'
mmu-Homer1 FW: 5'-GCAGGAGAAGATGGAGCTGACC-3'
mmu-Homer1 REV: 5'-TCTGAGTTCTGTGTCACATCGGG-3'
mmu-Id3 FW: 5'-GGACGACATGAACCACTGCTACTC-3'
mmu-Id3 REV: 5'-GGTCGAGGATGTAGTCTATGACACG-3'
mmu-Klhl41 FW: 5'-GGATCAACCCCTGCAATCGTAC-3'
mmu-Klhl41 REV: 5'-GATCGTAGCATAGCACGGAATCC-3'
mmu-Atp2a1 FW: 5'-CGCACTCCAAGTCCACAGAGG-3'
mmu-Atp2a1 REV: 5'-CTTCGCCTTCCTCAAACCAGG-3'
mmu-Nupr1 FW: 5'-CTTGCCACCAACAGCCAACC-3'
mmu-Nupr1 REV: 5'-CAGCAGCTTCTCTCTTGGTCCG-3'
mmu-Gadd45a FW: 5'-ATGACTTTGGAGGAATTCTCGGC-3'
mmu-Gadd45a REV: 5'-AGCCTCGTACACGCCGACC-3'
mmu-Cdkn1a FW: 5'-GCTGTCTTGCACTCTGGTGTCTG-3'
mmu-Cdkn1a REV: 5'-CAGAAGACCAATCTGCGCTTGG-3'
mmu-Nptx1 FW: 5'-CCTACACCAACGGATCAGCG-3'
mmu-Nptx1 REV: 5'-GAAGGCATACATCTCTGGCAGGC-3'
mmu-Hprt FW: 5'-GGACCTCTCGAAGTGTTGGATAC-3'
mmu-Hprt REV: 5'-CTCATCTTAGGCTTTGTATTTGGCT-3'
mmu-Rock1 FW: 5'- CCTGCGGCTACAAACCCAC-3'
mmu-Rock1 REV: 5'- CCAGGGCATCCAATCCATCC-3'
mmu-Leo1 FW: 5'-GAGCCCATACCTGAGACCAGAATAG-3'
mmu-Leo1 REV: 5'-CTTCAAATTCATCTTCATAATACTGAGG-3'
mmu-Pnn FW: 5'-TAAGGCGTGGATTCTCAGATAGTGG-3'
mmu-Pnn REV: 5'-ATCATCGTCTTCTGGGTCGCTC-3'
mmu-Ttc33 FW: 5'-CGAGGATGGGAACTGGCTTCA-3'
mmu-Ttc33 REV: 5'-CTGAATTGCCTCCTTGTATCGTTTG-3'
mmu-Snhg12 FW: 5'-AAGGAAGCACGGGTTATGGC-3'
mmu-Snhg12 REV: 5'-CTGGTCTCCCTCCTCACAATCT-3'
mmu-YB-1 FW: 5'-GCTTACCATCTCTACCATCATCCG-3'
mmu-YB-1 REV: 5'-CTAATGTTATCTGGTCAGAGGGCAA-3'
lnc31 FW: 5'-TGAAATTGGTCACGTTGTTGA-3'
lnc31 REV: 5'-TCTTGCCTCCTCTCCAGTTC-3'
mmu_miR-152_1 miScript Primer Assay (100) MS00001687
hsa-Hprt FW: 5'-GCCATCACATTGTAGCCCTCTG-3'
hsa-Hprt REV: 5'-TTTATGTCCCCTGTTGACTGGTC-3'
Hs_miR-31_1 miScript Primer Assay (100) MS00003290
Renilla-Luc FW: 5'-TCGTCCATGCTGAGAGTGTC-3'
Renilla-Luc REV: 5'-CTAACCTCGCCCTTCTCCTT-3'
FireFly-Luc FW: 5'-TGCAGAAGATCCTGAACGTG-3'

FireFly-Luc REV: 5'- CGGTAGACCCAGAGCTGTTC-3'

Oligonucleotides used for CLIP experiments:
mmu-Rock1 ex5 FW: 5'-CTACATGGTGATGGAGTACATGCCT-3'
mmu-Rock1 ex5 REV: 5'-GGAATGGATTGCATCCAATGC-3'
mmu-YB-1 3'UTR FW: 5'-CAGCTGAGAATTCGTCCGCTC-3'
mmu-YB-1 3'UTR REV: 5'-GCACTTAAGGTCTTCAGCTCC-3'
Table S4. List of oligonucleotides used in this study.

Oligonucleotides used for plasmid construction:
lnc-31 FW: 5'-CCGAAAGCTTCAACGCGGGACGCAGAGG-3'
lnc-31 REV: 5'-AGCGGATCCGAAACCGCAAAAGTAACTTTT-3'
lnc-31Δ FW: 5'-TTGAACTGAGAACCTGCTATGCC-3'
lnc-31Δ REV: 5'-CCTCTCCAGTTCCGAGTTACAGG-3'
mmu-Rock1-5' FW: 5'-AATGCTAGCAGCTCCTCACCCACCCT-3'
mmu-Rock1-200 FW: 5'-ATAGCTAGCGGTCGCTGTCACACCGAGC-3'
mmu-Rock1-100 FW: 5'- ATAGCTAGCGTTCCGGCTCCTCTGCCCTG-3'
mmu-Rock1-5' REV: 5'-TCAGCTAGCGATGCTGCTACTTGTGACG-3'
Luc/3'WT FW: 5'-TACGTTTAAACCCATGTGACCTACTGCTGTGTTG-3'
Luc/3'WT REV: 5'-AGTGCGGCCGCTTTTATCTTTAAAAAATAATTTTAATCTATT-3'
Luc/3'D FW: 5'-GAAATGTAAATTATTCTTAGAATATTTTCAGTG-3'
Luc/3'D REV: 5'-GAAATGTAAATTATTCTTAGAATATTTTCAGTG-3'
Luc/3'DD FW: 5'-ATATGTAGTAATAAACATA-3'
Luc/3'DD REV: 5'-TTCAATACCACTTGAAACATGCA-3'
miR-152 FW: 5'-ACTAGATCTGGAAGCGAGATTCTGGCGG-3'
miR-152 REV: 5'-CACTCGAGGCCAGCTAATCCCCAACCCATAG-3'

Biotynilated Oligonucleotides used for pull down experiments:
Even
>1 5'-ACAATCTCTCCTTCCACAGG-3'
>2 5'-GCAAATGAACATGGACAGGT-3'
>3 5'-CCTGGTACAGTAAATCAAGC-3'
>4 5'-GTCAGGGTTTAGGGGTATGG-3'
>5 5'-CTCCTTTAAGATGTTGGGTT-3'

Odd
>6 5'-CTGGATACTGATGCCCAATC-3'
>7 5'-CCCTCTTCGTTGTTATGCTT-3'
>8 5'-CCATGGTGAAAGAGTTCTCC-3'
>9 5'-AGGACTTACAACCTGGTACA-3'
>10 5'-GAGTCGCAAAGACATGACTT-3'

LacZ
>1 5'-AATGTGAGCGAGTAACAACC-3'
>2 5'-ATTAAGTTGGGTAACGCCAG-3'
>3 5'-AATAATTCGCGTCTGGCCTT-3'
>4 5'-AATTCAGACGGCAAACGCT-3'
>5 5'-ATCTTCCAGATAACTGCCGT-3'

HP Custom siRNA-W/O Modification (Qiagen):
lnc-31 ex2: 5' -TCACGTTGTTGAAGAGTTGAA-3'

lnc-31 ex3: 5'- CACCATGGCACTGTCTATAAA- 3'

**Supplemental References**


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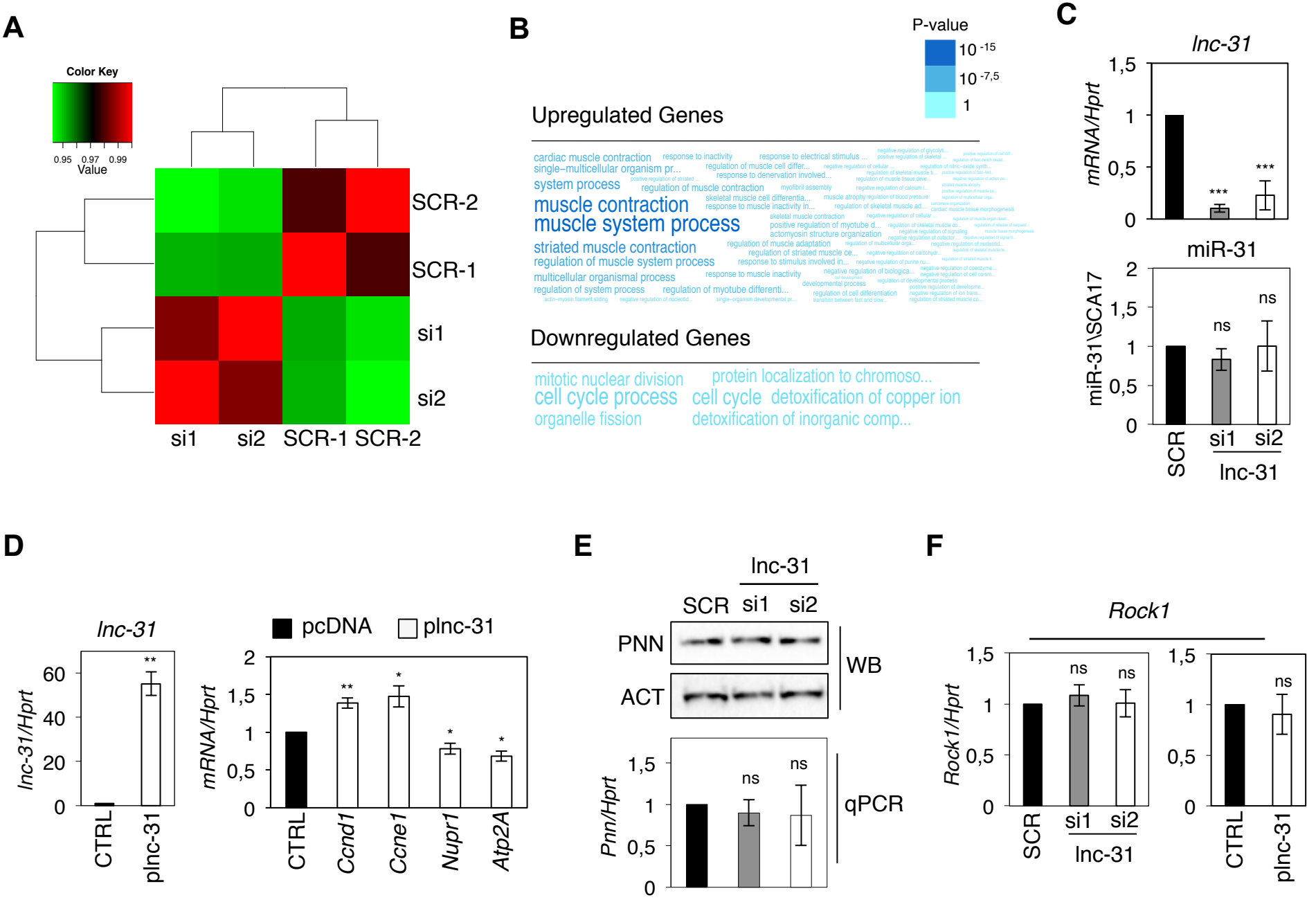
# Figure S1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Color Key
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Value
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SCR-1
si1
si2
si1 si2 SCR-1 SCR-2
B
P-value
10 -15
10 -7,5
1
Upregulated Genes
cardiac muscle contraction
response to inactivity
response to electrical stimulus ...
single−multicellular organism pr...
regulation of muscle cell differ...
response to denervation involved...
system process
regulation of muscle contraction
myofibril assembly
muscle contraction
muscle system process
skeletal muscle cell differentia...
muscle atrophy
response to muscle inactivity in...
regulation of skeletal muscle ad...
striated muscle contraction
positive regulation of myotube d...
actomyosin structure organization
regulation of muscle adaptation
regulation of striated muscle ce...
regulation of muscle system process
response to stimulus involved in...
multicellular organismal process
response to muscle inactivity
regulation of system process
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regulation of cell differentiation
Downregulated Genes
mitotic nuclear division
protein localization to chromoso...
cell cycle process
cell cycle
detoxification of copper ion
organelle fission
detoxification of inorganic comp...
C
lnc-31
mRNA/Hprt
1,5
1
0,5
0
***
***
miR-31
miR-31\SCA17
2
1,5
1
0,5
0
ns
ns
SCR
si1
si2
lnc-31
D
lnc-31
lnc-31/Hprt
60
40
20
0
**
CTRL
plnc-31
pcDNA
plnc-31
mRNA/Hprt
2
1,5
1
0,5
0
**
*
*
*
CTRL
Ccnd1
Ccne1
Nupr1
Atp2A
E
lnc-31
SCR
si1
si2
PNN
ACT
WB
Pnn/Hprt
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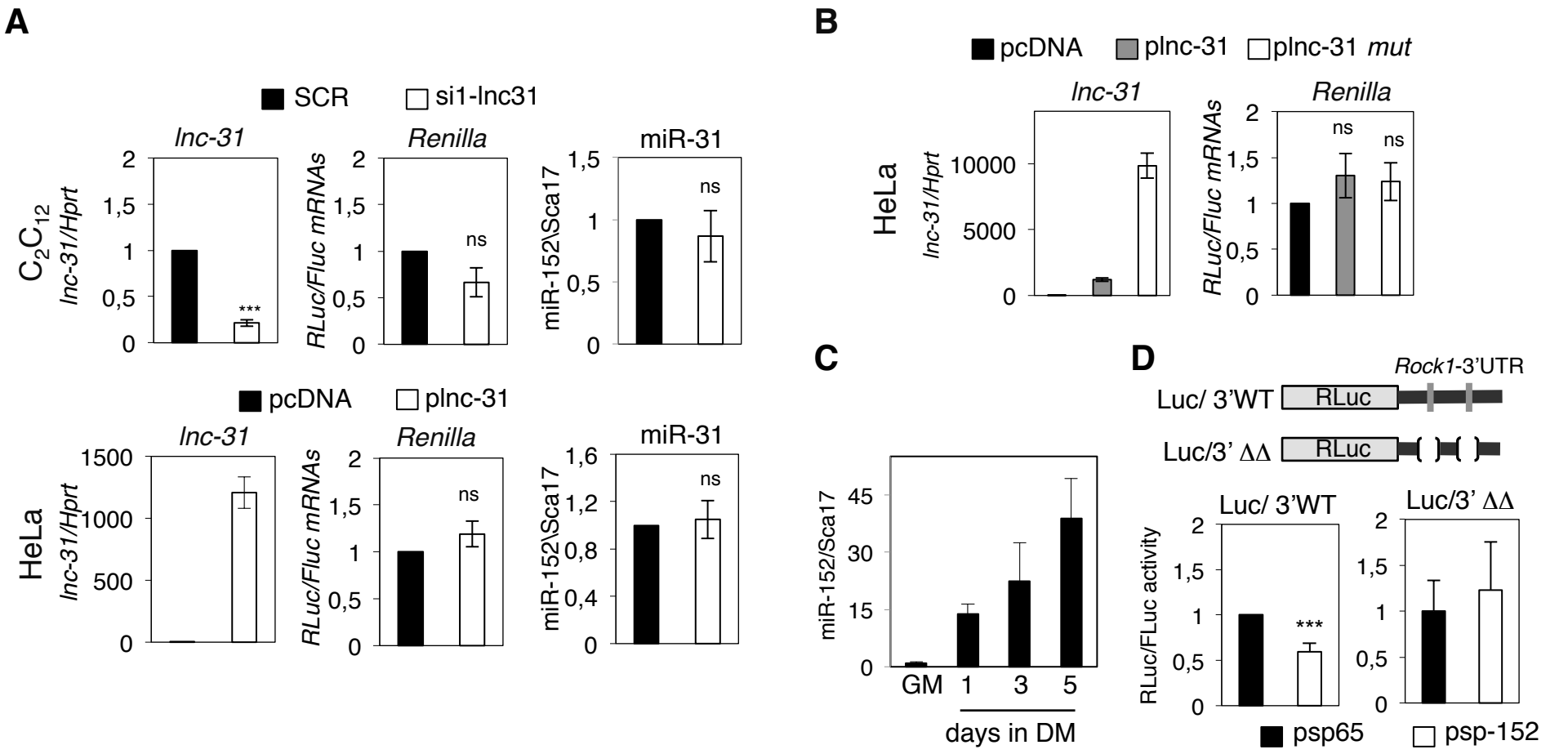
1
0,5
0
ns
ns
qPCR
F
Rock1
Rock1/Hprt
1,5
1
0,5
0
ns
ns
SCR
si1
si2
lnc-31
1,5
1
0,5
0
ns
CTRL
plnc-31

**Figure S1 Related to Figure 1 A.** Hierarchical clustering and Pearson correlation matrix of SCR and si-lnc-31 samples based on log2-transformed FPKM values obtained by RNA-Seq analysis. **B.** Word cloud representing the GO Biological Process terms enriched (p-value < 0.001) in the lists of genes upregulated and downregulated in si-lnc-31 treated $C_2C_{12}$ cells. **C.** Graphs showing the level of *lnc-31* and miR-31 measured by qRT-PCR in $C_2C_{12}$ cells treated with SCR or siRNAs targeting lnc-31 (si1 and si2). *Lnc-31* and miR-31 levels were normalized against *Hprt* and Sca17 RNAs respecitvely and expressed as relative quantity with respect to the SCR sample set to a value of 1. **D.** Graphs showing the levels of *lnc-31* and of the indicated mRNA targets measured by qRT-PCR in $C_2C_{12}$ cells transfected with pcDNA (CTRL) or with plnc-31 plasmid. RNA levels were normalized against *Hprt* RNA and expressed as relative quantity with respect to the CTRL sample set to a value of 1.**E.** Upper Panel: western Blot analysis, using PNN antibodies, of protein extracts from $C_2C_{12}$ cells treated with either SCR or si1-/si2-lnc-31 siRNAs. ACTININ (ACT) was used as loading control. Lower Panel: *Pnn* mRNA levels analysed by qRT-PCR in $C_2C_{12}$ myoblasts treated as in upper panel. The Pnn expression levels were normalised against HPRT mRNA and expressed as relative quantity with respect to the SCR sample set to a value of 1. **F.** *Rock1* mRNA levels analysed by qRT-PCR in sample treated as in ”C” and “D”. The *Rock1* expression levels were normalised against *Hprt* mRNA and expressed as relative quantity with respect to the SCR sample set to a value of 1.

Error bars represent s.d. of three independent experiments. *P<0.05, **P<0.01, ***P<0.001 and n.s. (not significant; P>0.05) correspond to paired two-tailed Student’s t-tests.

**Figure S2**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SCR
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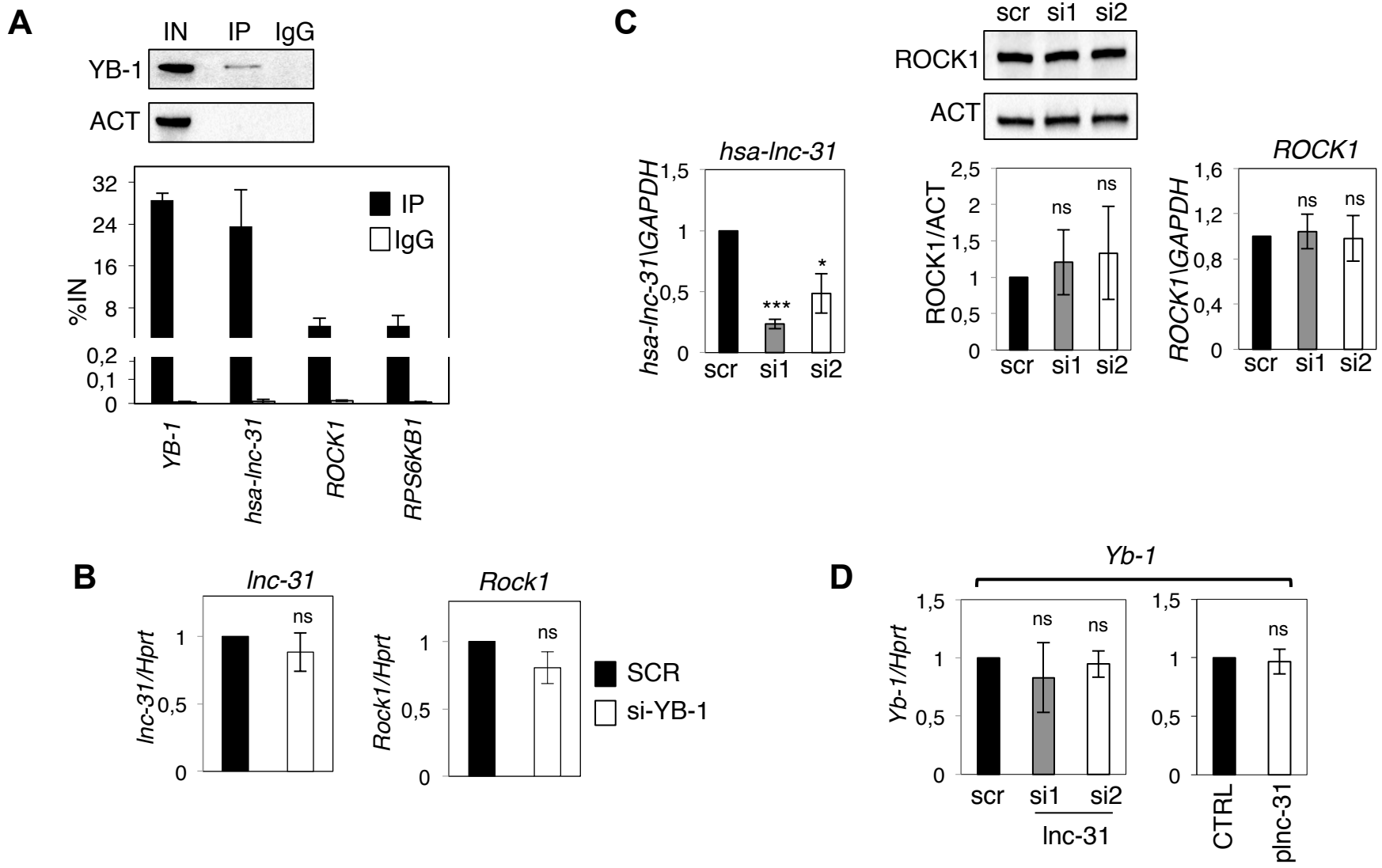
lnc-31
Renilla
miR-31
C2C12
lnc-31/Hprt
RLuc/Fluc mRNAs
miR-152\Sca17
***
ns
pcDNA
plnc-31
HeLa
B
plnc-31 mut
C
miR-152/Sca17
GM
1
3
5
days in DM
D
Rock1-3'UTR
Luc/ 3'WT
RLuc
Luc/3' ΔΔ
RLuc/FLuc activity
psp65
psp-152

**Figure S2 Related to Figure 2. A.** *Lnc-31, Renilla* and miR-31 RNA levels measured in cells transfected with Luc/5'Rock vector together with SCR and si1-lnc-31 ($C_2C_{12}$, upper panel) or pcDNA and plnc-31 (HeLa, lower panel). *Lnc-31*, *Renilla* and miR-31 values were normalised against *Hprt*, *Firefly* and Sca17 RNAs respectively and expressed as relative quantity respect to the SCR and pcDNA samples set to a value of 1. **B.** *Lnc-31* and *Renilla* RNA levels measured by qRT-PCR in Hela cells transfected with Luc/5'Rock vector together with pcDNA, plnc-31 and plnc-31*mut*. *Lnc-31*, *Renilla* values were normalised against *Hprt* and *Firefly* mRNAs respectively and expressed as relative quantity respect to pcDNA sample set to a value of 1. **C.** Graph showing the levels of miR-152, normalised against Sca17 RNA, in $C_2C_{12}$ cells cultured in growth medium (GM) or in differentiation medium (DM) for the indicated days. The values are expressed as relative quantity respect to GM condition set to a value of 1. **D.** Luciferase activity derived from cells transfected with the Luc/3'WT and Luc/3'ΔΔ constructs cotransfected with psp-152 plasmid or the psp65 empty vector. Error bars represent s.d. of three independent experiments. ***P<0.001 and n.s. (not significant; P>0.05) correspond to paired two-tailed Student's t-tests.

**Figure S3**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Rock1/Hprt
ns
SCR
si-YB-1
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si2
ROCK1
ACT
hsa-lnc-31
hsa-lnc-31\GAPDH
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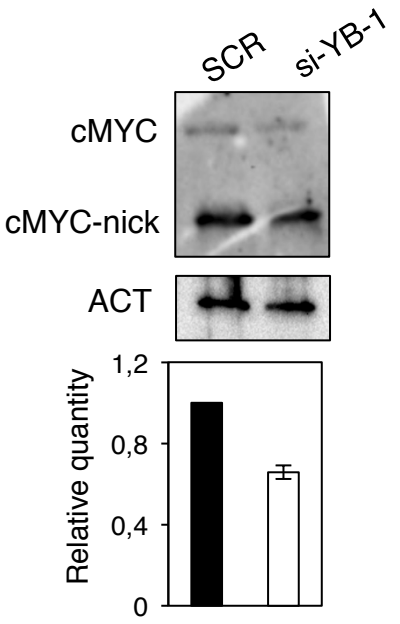
*
ROCK1/ACT
ROCK1
ROCK1\GAPDH
D
Yb-1
Yb-1/Hprt
lnc-31
CTRL
plnc-31

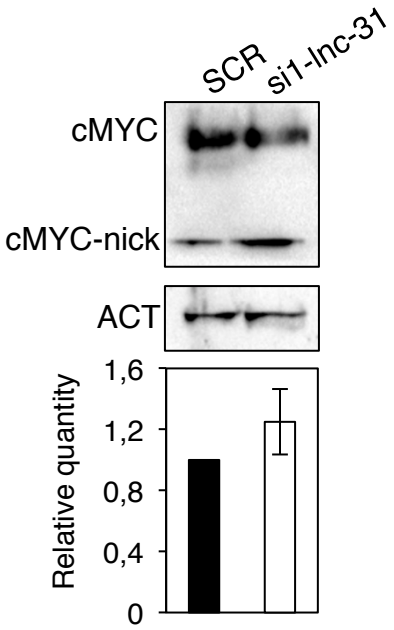
**Figure S3** Related to Figure 3. **A.** Upper panel: Protein extracts from YB-1 CLIP experiment, performed in human myoblasts, analysed by Western blot with YB-1 and ACTININ (ACT) (negative control) antibodies. Input sample account to 2% of the extract. Lower panel: Graph showing the enrichment (%IN) in IP and IgG samples for *YB-1*, *has-lnc-31* and *ROCK1* transcripts and for the control reference *RPS6KB1* RNA. Error bars represent s.d. of two independent experiments **B.** Graphs showing the *lnc-31* and *Rock1* RNA levels in cells treated with SCR and si-YB-1 siRNAs. The levels of both *lnc-31* and *Rock1* RNAs were normalised against *Hprt* mRNA and expressed as relative quantity with respect to the SCR sample set to a value of 1. **C.** Left panel: Graph showing the levels of *hsa-lnc-31* in human myoblasts treated either with SCR or siRNAs against hsa-lnc-31 (si1 and si2). Middle panel: representative Western Blot showing the level of ROCK1 protein in human myoblasts treated as in the left panel. The results of the densitometric analyses of three independent experiments are shown below. Right Panel: graph showing the levels of *ROCK1* mRNA in human myoblasts treated as in the left panel. In all panels the levels of RNAs and proteins in si1 and si2 treated samples are expressed as relative quantity with respect to the SCR sample set to a value of 1. **D.** Graph showing the level of *Yb-1* mRNA, measured by qRT-PCR, in $C_2C_{12}$ cells treated with SCR or si1-/si2-lnc-31 and with pcDNA3.1 (CTRL) or plnc-31 plasmid. The levels were normalised against *Hprt* mRNA and expressed as relative quantity with respect to the SCR sample set to a value of 1. Error bars represent s.d. of three independent experiments (unless differently specified); *P<0.05, ***P<0.001 and n.s. (not significant; P>0.05) correspond to paired two-tailed Student's t-tests.

# Figure S4

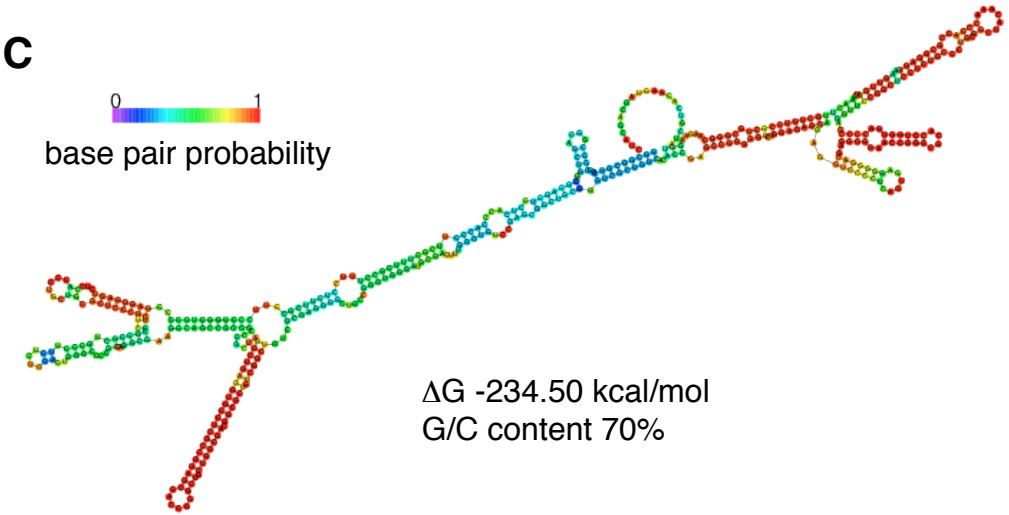
**A**




**B**




**C**




**D**

| Position | Length | QGRS | G-Score |
|---|---|---|---|
| 1 | 11 | GGCGGCGGCGG | 21 |
| 158 | 26 | GGCAGCCGGGGCCCGCTAGGCTGAGG | 18 |
| 203 | 24 | GGCTGCTGGCGACGGCGCTGTCGG | 18 |
| 237 | 28 | GGGCTGCCGGGTGGGATGCGACTTTGGG | 33 |
| 302 | 19 | GGGGTCTGGAGAGCAGAGG | 13 |
| 334 | 29 | GGGGACGAGGGGGAACCGGGCGCACCTGG | 21 |
| 394 | 29 | GGCTACAAACCCACCGCGGAGGAAGTTGG | 7 |

**Figure S4 related to Figure 3 A.** Western blot analysis of protein extracts, using cMYC antibodies, from $C_2C_{12}$ treated with siRNA SCR and or si-YB-1. ACTININ (ACT) was used as loading control. The graph below shows the desitometric analysis of total cMYC levels (cMYC plus cMYC-nick) normalised against ACTININ (ACT) ones and expressed as relative quantities with respect to SCR condition set to a value of 1. **B.** Western blot analysis of protein extracts, using cMYC antibodies, from $C_2C_{12}$ treated with siRNA SCR and or si1-lnc-31. ACTININ (ACT) was used as loading control. The graph below shows the desitometric analysis of total cMYC levels (cMYC plus cMYC-nick) normalised against ACTININ (ACT) ones and expressed as relative quantities with respect to SCR condition set to a value of 1. **C.** Prediction of *Rock1* 5'UTR secondary structures obtained using RNAfold tool. The value of the ΔG and the percentage of GC content are shown **D.** Table showing position, length, sequence and the G-score of putative Quadruplex forming G-Rich Sequences (QGRS) found in the *Rock1* 5'UTR by using the QGRS Mapper software program (Kikin et al, 2006).

**Table S1.** Related to Figure 1. Read number and mapping statistics relative to RNA-Seq experiments.

| Samples | Raw read pairs | Read pairs after pre- | Read pairs after short non-co | Mapped read pairs | Percentage of multiple alignments |
|---|---|---|---|---|---|
| SCR_Rep-1 | 42694978 | 36918653 | 36495492 | 35060575 | 4,70% |
| SCR_Rep-2 | 42521857 | 36069394 | 36028678 | 34509059 | 5,00% |
| si-lnc-31_Rep1 | 44813006 | 39233997 | 39173828 | 37596738 | 4,60% |
| si-lnc-31_Rep2 | 43683519 | 36007129 | 35940224 | 34459497 | 4,70% |

| Samples | Raw reads | Reads after pre-proc | Reads after short non-coding | Mapped reads | Percentage of | Reads after du | Reads after multi-mapped filtering |
|---|---|---|---|---|---|---|---|
| Pulldown | 54950438 | 52178674 | 44643218 | 36792235 | 11,60% | 29633231 | 15150281 |
| LacZ | 60238489 | 57809270 | 49683211 | 40726685 | 6,50% | 35526623 | 21183268 |
| Input | 65506357 | 63251068 | 37112001 | 28462507 | 15,60% | 27453672 | 10195557 |