Novel RNA-binding activity of MYF5 enhances *Ccnd1*/*Cyclin D1* mRNA translation during myogenesis

Amaresh C. Panda[1,#], Kotb Abdelmohsen[1], Jennifer L. Martindale[1], Clara Di Germanio[2,4], Xiaoling Yang[1], Ioannis Grammatikakis[1], Ji Heon Noh[1], Yongqing Zhang[1], Elin Lehrmann[1], Dawood B. Dudekula[1], Supriyo De[1], Kevin G. Becker[1], Elizabeth J. White[3], Gerald M. Wilson[3], Rafael de Cabo[2], Myriam Gorospe[1]

[1]Laboratory of Genetics, [2]Translational Gerontology Branch, National Institute on Aging, NIH, Baltimore, MD 21224, USA; [3]Department of Biochemistry and Molecular Biology and Marlene and Stewart Greenebaum Cancer Center, University of Maryland School of Medicine, Baltimore, MD 21201, USA; [4]Faculty of Veterinary Medicine, University of Teramo, Teramo, Italy.

[#]Correspondence: Amaresh C. Panda
Laboratory of Genetics, National Institute on Aging, National Institutes of Health
251 Bayview Blvd. Baltimore, MD 21224, USA
Tel: +1 410 558 8240; Fax: +1 410 558 8331
Email: amaresh.panda@nih.gov; amarchpanda@gmail.com

Running title: RNA-binding function of MYF5

Key words: RNA-binding protein; cell proliferation; muscle cells; C2C12 myoblasts; translation; transcription

SUPPLEMENTARY METHODS

Isolation and culture of primary myoblasts

Isolation of primary myoblasts was performed according to the protocol by Springer and colleagues (60). Briefly, limbs from 3-day-old C57BL6 mice were removed in sterile conditions under a dissecting microscope and minced with blades. The tissue was dissociated with a solution containing collagenase (Worthington Biochem), dispase (Roche), and $CaCl_2$ (Sigma) for 30 min at 37°C. The cell suspension was filtered through a 70-μm nylon mesh, centrifuged for 5 min at 350 × g, resuspended in F-10-based primary myoblast growth medium [Ham's F-10 nutrient mixture (Gibco), 20% fetal bovine serum (Gibco), 2.5 ng/ml basic fibroblast growth factor (Invitrogen), and 1% Penicillin-Streptomycin (Pen/Strep) solution (Gibco)] and plated in a 60-mm collagen-coated culture dish. Cells were serially subcultured to enrich the myoblast population and the medium was changed every 2 days until fibroblasts were no longer visible. Myoblasts were then grown in DMEM/F10 medium supplemented with 20% fetal bovine serum (Gibco), 2.5 ng/ml basic fibroblast growth factor (Invitrogen), 1% Pen/Strep (Gibco). Primary myoblasts were induced to differentiate by culturing them with differentiation medium (DMEM medium containing 5% horse serum and Pen/Strep) for 6 days.

Chromatin immunoprecipitation (ChIP) assay

ChIP assays were performed as described (28). Briefly, nuclear lysates were used for IP reactions in the presence of 10 μl of control IgG or anti-MYF5 antibodies. The complexes were eluted and after reversing the crosslinks, DNA was extracted using phenol-chloroform and analyzed using qPCR primers that specifically amplified putative regulatory regions upstream of the *Ccnd1* gene at site [1] (TTCCAGCACTTGGAAGTCAA and GGATGTGCTGTCTCCATCCT), site [2] (GAGACATTCTCACTATTTGCTTGG and TTTTAGCTGAAGGGCAGGAA), site [3] (ACCTGGGCCAAATAAAAAGG and ATTCTGGCTCCCAGTTTGAG), site [4] (CCGATGAAACCAAGTTGTGA and GACTCAGGTTCAGCTTGGTCA), site [5] (AAATGAGATTCTGGGCTGGA and AGAGGCTAGAAGGCAGCACA), site [6] (TTCCAAGTTTGGCCAAGAAG and GGGGGTAATTTTGTGGCTTT), and site [7] (GTTTTGCAGGGAAGCAGGT and GGACCACGTGGCTCAATC), as well as negative control sites on the *Il6* promoter (GTGTGTGTCGTCTGTCATGC and AGGAAGGGGAAAGTGTGCTT), and positive control sites on the *Myog* promoter (GGATTTTCAAGACCCCTTCC and CCGTCGGCTGTAATTTGATT). The PCR products were resolved on 2% agarose gel and visualized by EtBr staining.

Primers for detection of mouse Mef2 mRNA

TGATCAGCAGGCAAAGATTG and ATCAGACCGCCTGTGTTACC (NM_001170537.1).

Identification of shared sequence motifs

To identify MYF5 binding motifs, mouse REFSEQ accession numbers were first identified from the top 400 transcripts enriched in the MYF5 RIP/microarray analysis. These 400 unique transcripts served as the positive dataset for the identification of the MYF5 RNA motif. The sequence from each mRNA was obtained from the mm9 genome reference and was split into 5'UTR, 3'UTR and CDS regions based on Ensembl v67 annotations. After scanning the sequences with RepeatMasker (www.repeatmasker.org) to remove repetitive sequences, *de novo* motif analysis was performed using MEME (http://meme-suite.org/) with a minimum motif width of 6 nt and a maximum motif width of 12 nt. The motifs identified by this analysis were checked against known motifs with TOMTOM (http://meme-suite.org/) using the JASPAR 2009 (http://jaspar.genereg.net/) database. This approach yielded a number of motifs (the most abundant of which are shown in supplementary Fig. S2A) that were highly enriched among the top MYF5 target mRNAs.

In vitro mapping of MYF5 binding site

Detailed MYF5 binding sites on *Ccnd1* mRNA were identified *in vitro* using procedures described earlier (61-63), with some modifications. Briefly, 10 μg of recombinant GST-MYF5 protein was incubated with 6.6 pmol each of RNA fragments corresponding to *Ccnd1* CR, 3'B, 3'C and 3'D in 1× TENT buffer (10 mM Tris-HCl [pH 8.0], 1 mM EDTA [pH 8.0], 250 mM NaCl, 0.5% [v/v] Triton X-100), and 1× protease and 200 U/reaction RNase inhibitors for 1 h at 4°C. RNA-protein complexes were crosslinked with ultraviolet light (400 mJ/cm^2, 245 nm), incubated with GST-Glutathione Sepharose 4B beads (GE Healthcare Life Sciences) to allow binding at 25°C for 1 h followed by incubation with RNase T1 (10 U/μl) for 15 min at 25°C for the removal of unprotected RNA. After 4 washes with NT2 buffer (50 mM Tris-HCl [pH 7.5], 150 mM NaCl, 1 mM $MgCl_2$, 0.05% NP-40), RNA-protein complexes were treated with calf intestinal alkaline phosphatase (CIP, NEB, 1U/μl) for 15 min at 37°C and then with Proteinase K (1 μg/μl) at 55°C for 30 min. The RNA fragments were extracted twice using phenol/chloroform and precipitated using four volumes of ethanol and 10 μg of glycoblue. The RNA fragments were indubated with T4 Polynucleotide Kinase (NEB, 1 U/μl) treatment for 15 min at 37°C, and then ligated with 3' and 5' adaptors (IDT) rAppAGATCGGAAGAGCGGTTCAG/ddC/ and TCTACrArGrUrCrCrGrArCrGrArUrC, respectively, using T4 RNA ligase (NEB). The cDNA was prepared using the reverse transcription oligomer CTGAACCGCTCTTCCGATCT, and PCR was performed using 5' and 3' primers

AATGATACGGCGACCACCGACAGGTTCAGAGTTCTACAGTCCGACGATC and AAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCT, respectively. The PCR product was then cloned into pGEM®-T Easy Vector System I (Promega corp). Fifty clones were sequenced using the T7 primer.

SUPPLEMENTARY REFERENCES

60. Springer,M.L., Rando,T.A., Blau,H.M. (2002) Gene delivery to muscle. *Curr. Protoc. Hum. Genet*., **13,** 4.
61. Zhang,P., Abdelmohsen,K., Liu,Y., Tominaga-Yamanaka,K., Yoon,J.H., Grammatikakis,I., Martindale, J.L., Zhang,Y., Becker,K.G., Yang,I.H., Gorospe,M., Mattson,M.P. (2015) Novel RNA- and FMRP-binding protein TRF2-S regulates axonal mRNA transport and presynaptic plasticity. *Nat Commun*., **20**, 6:8888.
62. Ule,J., Jensen,K., Mele,A., Darnell,R.B. (2005) CLIP: a method for identifying protein-RNA interaction sites in living cells.; *Methods*, **37**, 376-386.
63. Granneman,S., Kudla,G., Petfalski,E., Tollervey,D. (2009) Identification of protein binding sites on U3 snoRNA and pre-rRNA by UV cross-linking and high throughput analysis of cDNAs. *Proc Nat Acad Sci USA*, **106**, 9613–9618.

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure S1. Analysis of the levels of MYF5, CCND1 and MYF5-*Ccnd1* mRNA complexes. (A) Phase-contrast images of primary mouse myoblasts proliferating in growing medium (GM) and differentiated into myotubes after culture in DMEM with 5% horse serum (differentiation medium, DM) for 6 days. **(B)** Western blot analysis to assess the levels of MYF5, CCND1, and loading control GAPDH in proliferating and differentiated mouse primary myoblasts. **(C)** RT-qPCR analysis of the levels of differentiation marker *Mef2c* mRNA and *Gapdh* mRNA levels in proliferating and differentiated mouse primary myoblasts myoblasts. **(D)** MYF5 IP followed by RT-qPCR analysis to validate the association of MYF5 with mRNAs in proliferating primary mouse myoblasts; the levels of mRNAs in MYF5 IP were normalized to the levels of *18S* rRNA and plotted as fold enrichment relative to the levels seen in control IgG IP samples. Data in (C, D) are the means and S.E.M. from three independent experiments. *, $P < 0.05$ (Student's t-test).

Supplementary Figure S2. Identification and validation of sequences enriched in MYF5 IP. (A) Top signature motifs (11 and 12 nucleotides long) identified among the MYF5-bound mRNAs, with the relative frequency with which each nucleotide is found at each positions. Motifs enriched in the 5'UTR and the 3'UTR were identified separately. T residues correspond to U residues on the RNA. **(B)** Summary of the most abundant nucleotide at each position in the top motifs. **(C)** Recombinant His-MYF5 or GST-MYF5 were incubated with 100 pmol of the biotinylated RNAs shown, followed by MYF5 pulldown using streptavidin beads. MYF5 levels in the pulldown material were assessed by Western blot analysis using anti-MYF5 antibody.

Supplementary Figure S3. Further analysis of MYF5 binding to *Ccnd1* RNA. (A) Coomassie blue-stained recombinant his-MYF5 (TP760391, Origene) resolved on SDS-PAGE. **(B)** Sequence of the *Ccnd1* mRNA, depicting the different segments that were biotinylated and tested by pulldown analysis.

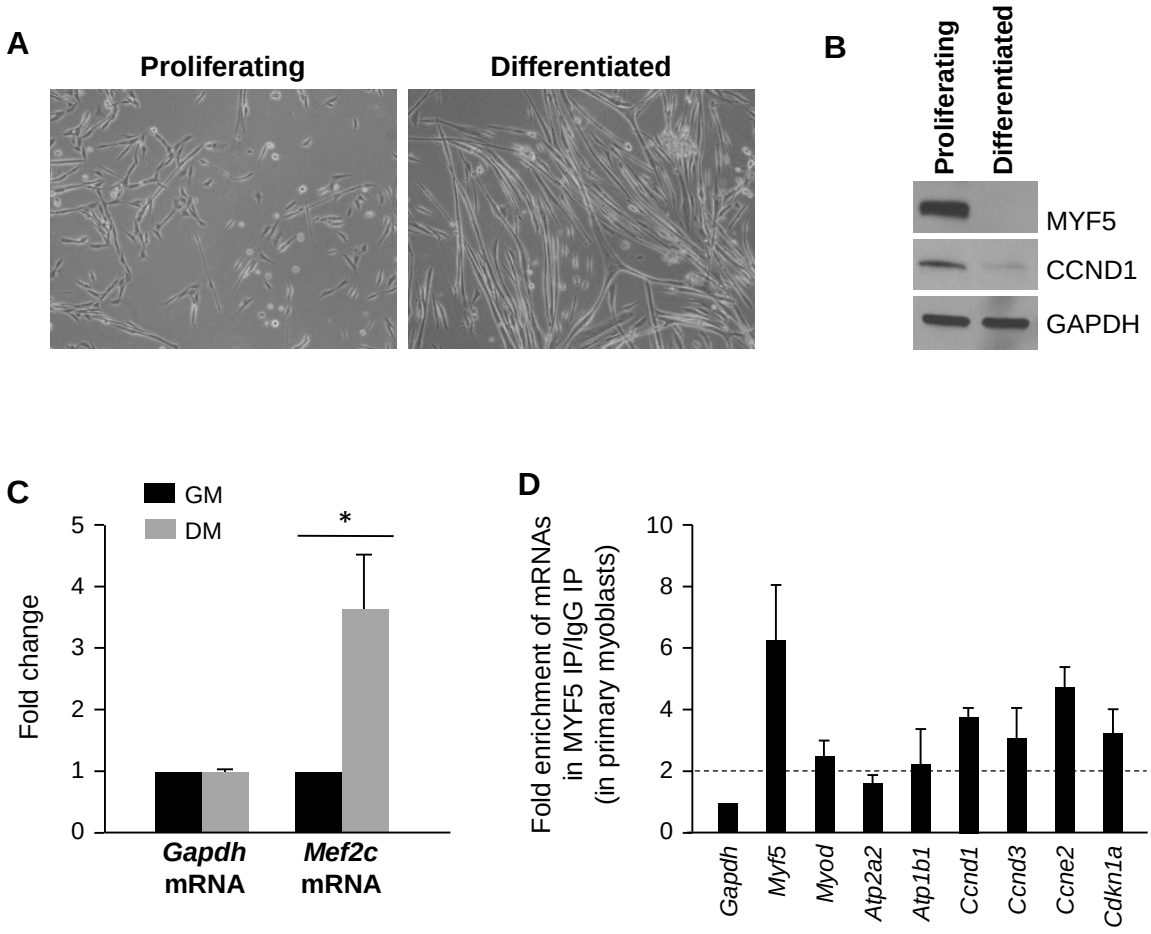
Supplementary Figure S4. Further analysis of MYF5 binding to *Ccnd1* RNA. (A) Fragments tested by further division of the *Ccnd1* 3'UTR fragment C. **(B)** After incubating biotinylated CR, 3'B, 3'C, and 3'D (*top left*) with GST-MYF5 *in vitro*, complexes were crosslinked and the unprotected RNA digested using RNase T1. The RNA fragments protected by MYF5 were purified and cloned; 50 individual clones were sequenced and the sequences recovered are shown in shaded rectangles. The positions of these sequences is indicated by dashed lines and by red ovals (representing a putative MYF5 site). **(C)** Equal amounts of GST-

MYF5 chimeric proteins were resolved on SDS-PAGE and stained using Coomassie blue. **(D)** The interaction of biotinylated *Ccnd1* CR fragment with GST-tagged truncations of MYF5 (and GST alone) was tested by biotin pulldown and Western blot analysis using anti-GST antibody.

Supplementary Figure S5. Further analysis of MYF5 influence on the transcription and stability of Ccnd1 mRNA. (A) Schematic showing putative binding sites for MYF5 on the promoter of the *Ccnd1* gene. **(B)** Following crosslinking and chromatin IP (ChIP) using anti-MYF5 or control IgG antibodies, the bound DNA was extracted and analyzed by PCR amplification. The association of promoter regions of *Il6* (negative control), *Myog* (positive control), and *Ccnd1* (regions [1] through [7]) was assessed; PCR products were resolved on 2% agarose gels and stained with ethidium bromide for visualization. **(C)** Forty-eight hours after transfection of proliferating C2C12 cells with Ctrl siRNA or MYF5 siRNA, cells were treated with actinomycin D to block *de novo* transcription. RT-qPCR analysis was then used to measure *Ccnd1* and *Gapdh* mRNA levels at different time points after adding actinomycin D in order to measure the half-life of these mRNAs. RNA levels in each sample were normalized to *28S* rRNA levels.

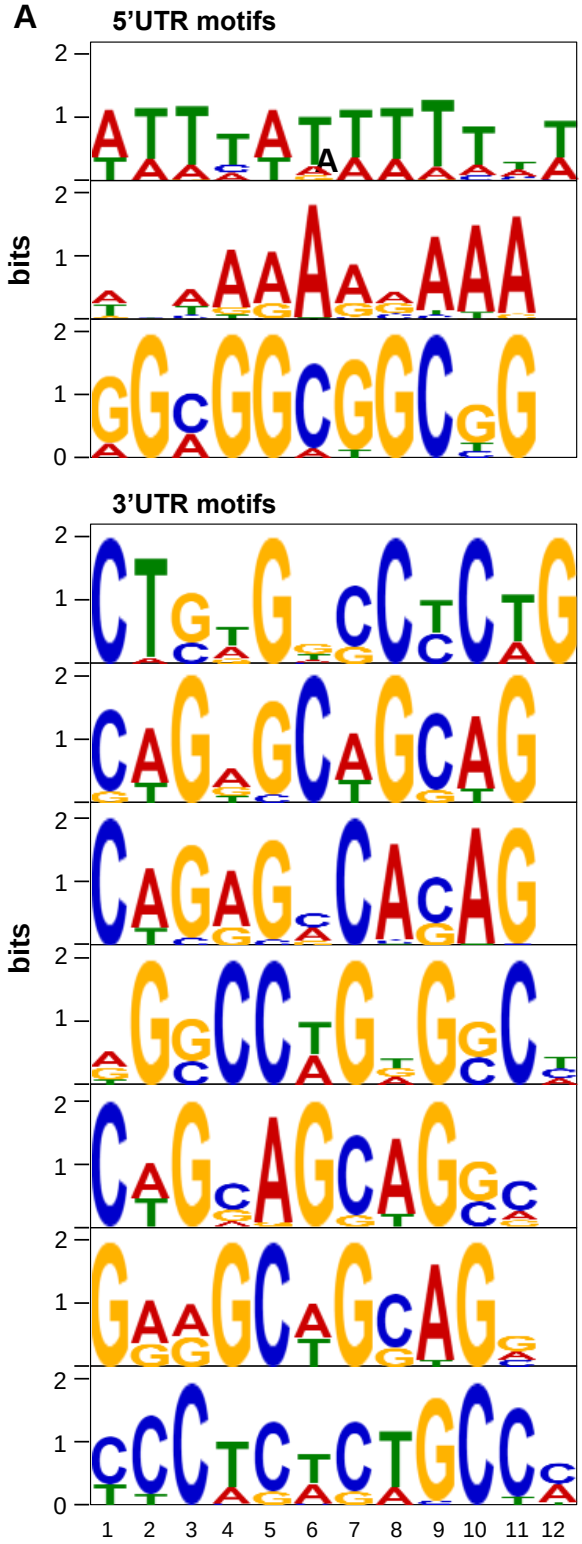
Supplementary Figure S6. Further analysis of MYF5 influence on the translational regulation of CCND1 expression. (A) Polyribosomes from C2C12 cytoplasmic preparations fractionated through sucrose gradients under untreated conditions (*top*) or after pre-treatment with 20 mM EDTA (*bottom*) to dissociate ribosomes from mRNA. Gradients were made of continuous 10-50% sucrose; arrow, direction of sedimentation. **(B)** In untreated and EDTA-treated samples, the relative distribution of *Gapdh* mRNA and *Ccnd1* mRNA in sucrose gradients was measured by RT-qPCR analysis of *Gapdh* and *Ccnd1* mRNAs in each fraction, and represented as percentage of total mRNA in that gradient. **(C)** Western blot analysis of the expression of RPS6 (a ribosomal protein marker) and MYF5 in each gradient fraction prepared from C2C12 lysates with or without EDTA treatment.

Panda et al. Supplementary Fig. S1



Panda et al. Supplementary Fig. S2

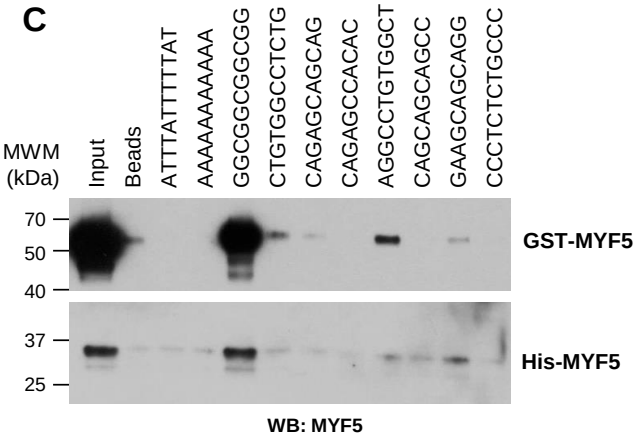
A



B

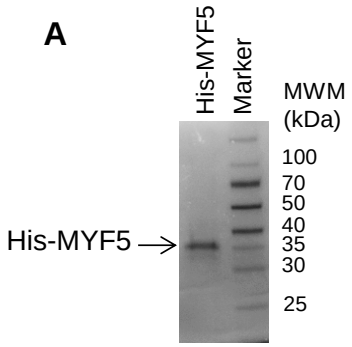
Name	position	Sequence
Motif 1	5'UTR	ATTTATTTTTAT
Motif 2	5'UTR	AAAAAAAAAAA
Motif 3	5'UTR	GGCGGCGGCGG
Motif 4	3'UTR	CTGTGGCCTCTG
Motif 5	3'UTR	CAGAGCAGCAG
Motif 6	3'UTR	CAGAGCCACAC
Motif 7	3'UTR	AGGCCTGTGGCT
Motif 8	3'UTR	CAGCAGCAGCC
Motif 9	3'UTR	GAAGCAGCAGG
Motif 10	3'UTR	CCCTCTCTGCCC

C



Panda et al. Supplementary Fig. S3

A



B

***Ccnd1* mRNA**

TTTTCTCTGCCCGGCTTTGATCTCTGCTTAACAACAGTAACGTCACACGGACTACAGGGGAGTTTTGTTGAAGTTGCAAAGTCCTGCAGCC
TCCAGAGGGCTGTCGGCGCAGTAGCAGAGAGCTACAGACTCCGCGCGCTCCGGAGACCGGCAGTACAGCGCGAGGCAGCGCGCGTCAG
CAGCCGCCACCGGAGCCCAACCGAGACCACAGC***CCTCCCCAGACGGCCGCGCC***A**TGGAACACCAGCTCCTGTGCTGCG**AAGTGGAGACC
ATCCGCCGCGCGTACCCTGACACCAATCTCCTCAACGACCGGGTGCTGCGAGCCATGCTCAAGACGGAGGAGACCTGTGCGCCCTCCGTA
TCTTACTTCAAGTGCGTGCAGAAGGAGATTGTGCCATCCATGCGGAAAATCGTGGCCACCTGGATGCTGGAGGTCTGTGAGGAGCAGAA
GTGCGAAGAGGAGGTCTTCCCGCTGGCCATGAACTACCTGGACCGCTTCCTGTCCCTGGAGCCCTTGAAGAAGAGCCGCCTGCAGCTGCT
GGGGGCCACCTGCATGTTCGTGGCCTCTAAGATGAAGGAGACCATTCCCTTGACTGCCGAGAAGTTGTGCATCTACACTGACAACTCTAT
CCGGCCCGAGGAGCTGCTGCAAATGGAACTGCTTCTGGTGAACAAGCTCAAGTGGAACCTGGCCGCCATGACTCCCCACGATTTCATCGA
ACACTTCCTCTCCAAAATGCCAGAGGCGGATGAGAACAAGCAGACCATCCGCAAGCATGCACAGACCTTTGTGGCCCTCTGTGCCACAGA
TGTGAAGTTCATTTCCAACCCACCCTCCATGGTAGCTGCTGGGAGCGTGGTGGCTGCGATGCAAGGCCTGAACCTGGGCAGCCCCAACAA
CTTCCTCTCCTGCTACCGCACAACGCACTTTCTTTCCAGAGTCATCAAGTGTGACCCGGACTGCCTCCGTGCCTGCCAGGAACAGATTGAA
GCCCTTCTGGAGTCAAGCCTGCGCCAGGCCCAGCAGAACGTCGACCCCAAGGCCACTGAGGAGGAGGGGGAAGTGGAGGAAGAGGCT
GGTCTGGCCTGCACGCCCACCGACG**TGCGAGATGTGGACATCTG**A***GGGCCACCGGGCAGGCGGGA***GCCACCAAGTAGTGGCACCCGC
AAAGAGGAAGGAGCCAGCCCGGGTGCTCCTGACGACGTCCCCCTTGGGGACATGTTGTTACCAGAAGAGGAAGTTTTGTTCTCTTTGTTG
GTTGTTTTTCCTTAATCTTTCTCCTTTCTATCTGATTTAAGCAAAAGAGAAAAAAATATCTGAAAGCTGTCTTAAAGAGAGAGAGAGAGAG
ATAGAATCTGCATCACCCTGAGAGTAGGGAGCCAGGGGGTGCTACAAAAATAGAATTCTGTACCCCAGTAATCAACTAGTTTTCTATTAA
TGTGCTTGTCTGTTCTAAGAGTAGGATTAACACAGGGGAAGTCTTGAGAAGGAGTTTTGATTCTTTTATATGTTTTTAAAAAAAAGCTTAA
*GAAACATTGCTTTAAAAAGGAAGGAAAAAAAATACAGCAAACCATTGTTAAAGTAGAAGAGTTTTTAGGTT****GAGAAATGTACTCTGCTT***
TGCTGAAAAGCCACAGCTTAGGCCCTCAGCCTCACTCCCTGGCTTGCTCAGTGCCTACAGCCCTGTTACCTGATACCTGTGCTTTATCCCAG
GGGTGGGCAGACCTCTTAACCTTATAGATGGTCAGTGCAGCCTCTAGTGGTCTCATGGCGTGTGGCACAACCCCCCTCCCCAGGGCTCAG
CTTAATGTGCCCTCTCCCCCCAACAACCTGCAGGTTCACAGCACCAGCCACACAGCGGTAGGGATGAAATAGTGACATAATATATTCTATT
TTTGTAACCTTCCTATTTTGTAGCTCTGTTTAGAGAGATGCTGGTTTTTGCCTGAAGGCCCTGCAGCCTGCCCACATCAGGTTAAACCCACA
GCTTTTGTGTGTGGTTTGTTTTGTTGTGTTTTCTTTCTCTATGTTCCAAAACCATTCCATTTCAAAGCACTTTTGGTCAGCTAGCTGGAGGCA
GTGTTGCTGGTGTGTGTTGGGGG***GAGGGGTTCTAATGGAATGGA****TGGGGATGTCCACACACGCATTCAGATGGCTGTACAACAGGTTG*
TAGGGCTGGTAGTATGAGGTGCTTGGGAAGTTTTGTTGGGTCAAGAAGAGAGAACTCTGTTCTCGCACCACCGGGATCTGTCCTGCAAA
GTTGAAGGGATCCTTTGGTGCCAGCTGGTGTTTGGAAGTAGGAACCATGATGGCATTACCTGGACAAGGAGATTGGGGACAACTCTTAA
GTCTCACACAGGAGGCTTTTAAACACTAAAATGTCTAATTTATACTTAAGGCTACAGAAGAGTATTTATGGGAAAGGCTGCCCATGACCA
GTGTGACTCAAAGCAATGTGATCTCCCTTGATTCAAACGCACACCTCTGCCCTGCTGGAGAAGGTTTAGGGCCATGTCTGAGAGATTGGT
*CTTTCATTGGGCAACGGGGGGGGGGGGGGGGTCCTTAAAAAAAAAAAACCACAAAGACAGAGATTTGGTCTGCTTGACTTTCCC****AACCC***
AATTGGCCCCATTGGAGAGCCATCCAAACTGAGGAAAATTAGGGGACTCCAAAAGAGTTTGATTCTGGCACATTCTTGCCGCTGCCCCCA
AGTTAACAACAGTAGGTAATTTGCACACCTCTGGCTCTGTGCCTTTCTATTAGGACTTTTTGGCAGAAGGTGGAGAGCGGGAGGCTTAAG
AGGGGATGTGAGGGAAGAGGTGAAGGTGGGACCACATGGGACAGGCCACGGCTCCTCTCATGGCGCTGCTACCGATGACTCCCAGGAT
CCCAGACGTTCAGAACCAGATTCTCATTGCTTTGTATCTTTCACGTTGTTTTCGCTGCTATTGGAGGGTCAGTTTTGTTTTGTTTTGTTTTAC
AATGTCAGACTGCCATGTTCAAGTTTTAATTTCCTCATAGAGTGTATTTACAGATGCCCTTTTTTGTACTTTTTTTTTTAATTGTGATCTATTT
TGGCTTAATGTGATTACCGCTGTATTCCAAAAAAAAAAAAAAAAA**CAGGTTCCTGTTCACAATACC**TCATGTATCATCTAGCCATGCACGA
GCCTGGCAGGCAGGTGGGCGGTCTGCCTCCAGGGATCCTGGGACCCTGATGGCGATCGTCCTGTCATGCTGGGCCCTTCATTTGATCTGG
GACATAGCATCACAGCAGTCAGGGCACCTGGATTGTTCTGTTATCGATATTGTTTCTTGTAGCGGCCTGTTGTGCATGCCACCATGCTGCT
GGCCCGGGGGGATTTGCTCTGAGTCTCCGGTGCATCATTTAATCTGTTAGGTTCTAGTGTTCCGTCTTGTTTTGTGTTAATTACAGCATTGT
GCTAATGTAAAGACTCTGCCTTTGCGAAGCCAGCTGCAGTGCTGTAGGCCCCCAAGTTCCCTAGCAAGCTGCCAAACCAAAACGGGCACC
ACCAGCTCAGCTGAGGCATCCCAGCCAGGCAGGACCCTTGAGGGCCGCTGTATCCATGGTGATGGGGTGAGGTTTTGGCCAAAAGGCCA
AAGACTGGTGGTGGGTCCACGGAATCTGCCCTGTGACATGAAAGGCTTTGAGGGGCTCTGGCTGGTGGCCAGGTTGGCTTTTTGTATTTC
TGGTTGACACACCATGGCGCTTCCCAGCACAGACATGTGACCAGCATGGTCCAGGAAAAAAAAAAAGACAAAAAATCTAGAAAATAAAA
TTGGTAAAATCTCA

5'UTR
CR
3'UTR-A
3'UTR-B
3'UTR-C
3'UTR-D

Panda et al. Supplementary Fig. S4

A

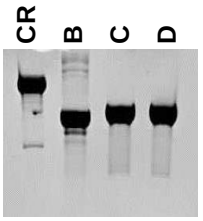
Ccnd1 3'UTR-Fragment-C

GAGGGGTTCTAATGGAATGGATGGGGATGTCCACACACGCATTCAGAT C1
GGCTGTACAACAGGTTGTAGGGCTGGTAGTATGAGGTGCTTGGGAAGT C2
TTTGTTGGGTCAAGAAGAGAGAACTCTGTTCTCGCACCACCGGGATCT C3
GTCCTGCAAAGTTGAAGGGATCCTTTGGTGCCAGCTGGTGTTTGGAAG C4
TAGGAACCATGATGGCATTACCTGGACAAGGAGATTGGGGACAACTCT C5
TAAGTCTCACACAGGAGGCTTTTAAACACTAAAATGTCTAATTTATAC C6
TTAAGGCTACAGAAGAGTATTTATGGGAAAGGCTGCCCATGACCAGTG C7
TGACTCAAAGCAATGTGATCTCCCTTGATTCAAACGCACACCTCTGCC C8
CTGCTGGAGAAGGTTTAGGGCCATGTCTGAGAGATTGGTCTTTCATTG C9
GGCAACGGGGGGGGGGGGGGGTCCTTAAAAAAAAAAAACCACAAAGA C10
CAGAGATTTGGTCTGCTTGACTTTCCCAACCCAATTGGCCCCATTGG C11

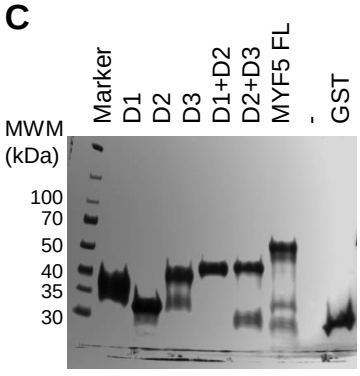
C9-10 C10-11 C10-1

Overlapping sequence

B

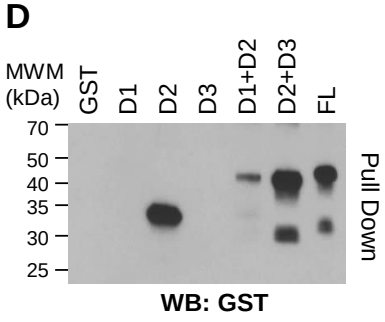


C

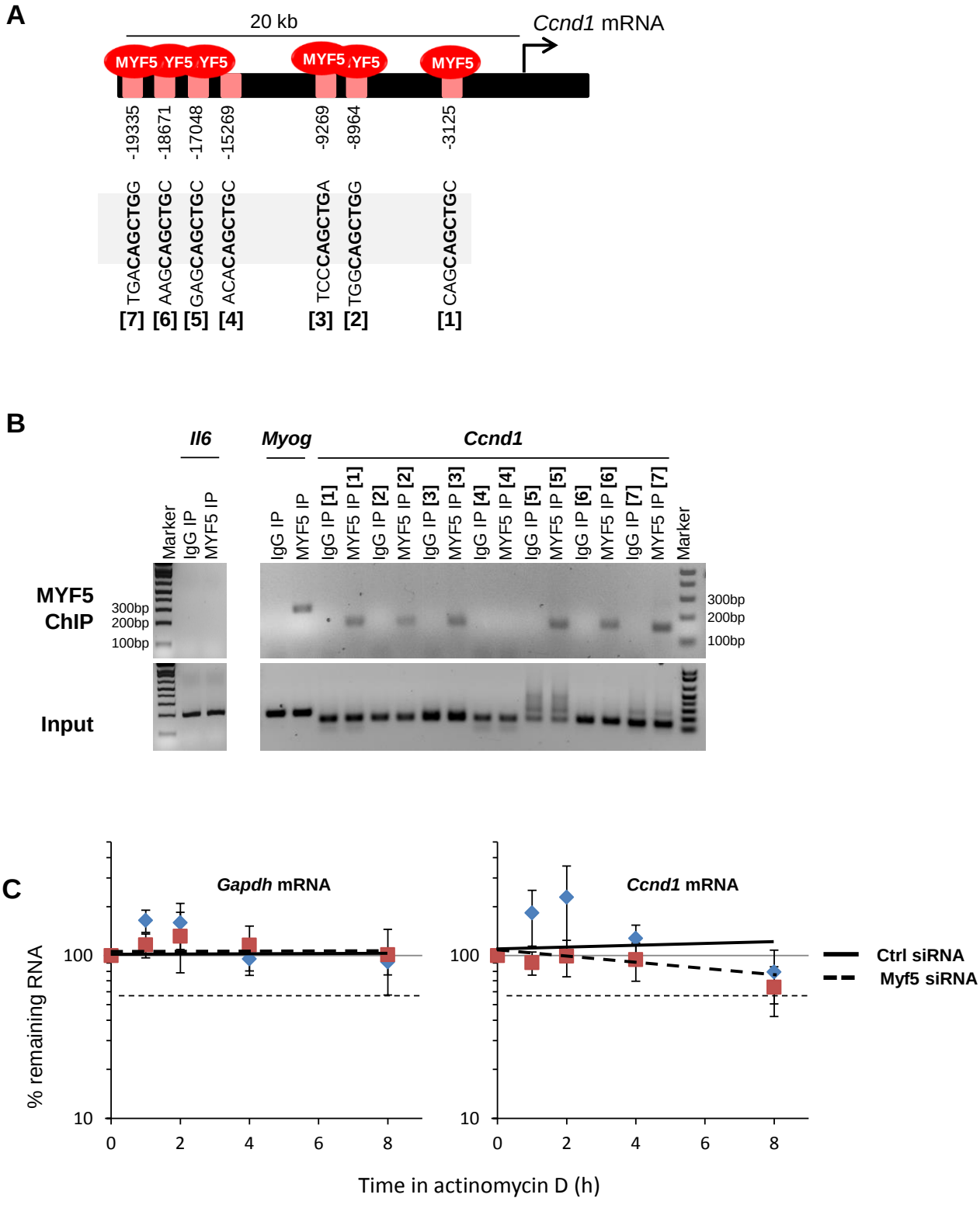


	Domain Mol Wt.	Total Mol wt
GST-		26.0
GST-Domain1	9.8	35.8
GST-Domain2	6.9	32.9
GST-Domain3	9.5	35.5
GST-Domain1+2	16.7	42.7
GST-Domain2+3	16.4	42.4
GST-MYF5-FL	28.2	54.2

D



Panda et al. Supplementary Fig. S5



Panda et al. Supplementary Fig. S6