A role for RNA post-transcriptional regulation in satellite cell activation

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Abstract

Background: Satellite cells are resident skeletal muscle stem cells responsible for muscle maintenance and repair. In resting muscle, satellite cells are maintained in a quiescent state. Satellite cell activation induces the myogenic commitment factor, MyoD, and cell cycle entry to facilitate transition to a population of proliferating myoblasts that eventually exit the cycle and regenerate muscle tissue. The molecular mechanism involved in the transition of a quiescent satellite cell to a transit-amplifying myoblast is poorly understood.

Methods: Satellite cells isolated by FACS from uninjured skeletal muscle and 12 h post-muscle injury from wild type and Syndecan-4 null mice were probed using Affymetrix 430v2 gene chips and analyzed by Spotfire™ and Ingenuity Pathway analysis to identify gene expression changes and networks associated with satellite cell activation, respectively. Additional analyses of target genes identify miRNAs exhibiting dynamic changes in expression during satellite cell activation. The function of the miRNAs was assessed using miRIDIAN hairpin inhibitors.

Results: An unbiased gene expression screen identified over 4,000 genes differentially expressed in satellite cells in vivo within 12 h following muscle damage and more than 50% of these decrease dramatically. RNA binding proteins and genes involved in post-transcriptional regulation were significantly over-represented whereas splicing factors were preferentially downregulated and mRNA stability genes preferentially upregulated. Furthermore, six computationally identified miRNAs demonstrated novel expression through muscle regeneration and in satellite cells. Three of the six miRNAs were found to regulate satellite cell fate.

Conclusions: The quiescent satellite cell is actively maintained in a state poised to activate in response to external signals. Satellite cell activation appears to be regulated by post-transcriptional gene regulation.

Keywords: Satellite cell, RNA post-transcriptional regulation, microRNA.

Background

Skeletal muscle is terminally differentiated and thus, requires a population of resident adult stem cells, satellite cells, for maintenance and repair [1-3]. Satellite cells are typically mitotically quiescent in resting muscle and activate to prepare for cell cycle entry by HGF [4,5], nitric oxide [6], and TNFa [7], upon a muscle injury. Intracellular p38α/β MAPK and downstream signaling is stimulated upon satellite cell activation, permitting MyoD induction (Troy et al.)a [8], S-phase entry [8,9], and subsequent proliferation. A subset of satellite cells self-renew to maintain the satellite cell pool (Troy et al.)a [10,11] and generate a rapidly proliferating transit-amplifying myoblast population (Troy et al.)a [10].

The transition from a quiescent satellite cell to a proliferating, transit amplifying myoblast was thought to require extensive transcriptional induction as quiescent satellite cells have a low ratio of cytoplasmic volume to nuclear volume, few cellular organelles, tightly packed heterochromatin, and are believed to be metabolically inactive [12,13]. However, recent evidence suggests that satellite cell quiescence is ‘active’ and satellite cells are poised to react to external stimuli after muscle damage [14]. Moreover, quiescent fibroblasts exhibit high metabolic activity [15] in
agreement with a quiescent state that is far from ‘quiet’. Interestingly, a growing pool of data demonstrates that cell fate determination is reliant on post-transcriptional gene regulation [16-20] and may provide mechanisms to maintain quiescent satellite cells in a ready state.

One such RNA post-transcriptional mechanism, microRNA-mediated gene silencing, regulates skeletal muscle specification and myogenic differentiation [21-23]. MicroRNAs (miRNA) are a class of small non-coding RNAs that bind to target mRNA in a sequence specific manner to mediate gene silencing [24-27] and can target and silence protein expression from tens to hundreds of mRNAs [26,27]. Furthermore, miRNAs modulate stem cell fate decisions [28-31] and may have similar functions in satellite cells. Recent studies identify miR-489 and miR-206 expression in quiescent satellite cells [32,33], however, it is likely that many uncharacterized miRNAs play roles in the transition of a quiescent satellite cell to transit-amplifying myoblast.

To understand the mechanisms involved in satellite cell activation, we previously screened a number of candidate genes for changes in expression from freshly isolated satellite cells and from satellite cells isolated at either 12 h post-muscle injury or 48 h post-muscle injury to represent quiescent, activated, and proliferating satellite cells, respectively. Although unbiased gene expression screens have been performed on satellite cells, these studies have either compared freshly isolated satellite cells to satellite cells expanded in culture [14,34] or to satellite cells in diseased skeletal muscle [14]. Neither of these studies directly compared satellite cells prior to and following induced muscle injury in vivo and thus, the reported gene expression changes specific to cell culture or specific to diseased muscle may not reliably identify gene expression changes associated with satellite cell activation in vivo. Here, we report global gene expression profiles and candidate miRNAs associated with quiescent and activated satellite cells as well as identify a novel function for miR-16, miR-106b, and miR-124 in satellite cell fate determination. From these analyses, we posit that satellite cell activation is primarily regulated by post-transcriptional gene regulation as opposed to transcriptional induction.

**Methods**

**Mice**

All animal procedures were performed according to protocol number 1012.01 approved by Institutional Animal Care and Use Committee at the University of Colorado at Boulder. Mice were housed in a pathogen-free environment at the University of Colorado at Boulder. All mice sacrificed were female and between 3 and 6 months of age. Wild type mice were C57Bl/6J (Jackson Labs) and syndecan-4−/− mice carry homozygous deletion of syndecan-4 in the C57Bl/6 background [35].

**Fluorescence-activated cell sorting of satellite cells**

The tibialis anterior muscles of 3-month-old female B6D2F1/J or syndecan-4−/− mice were injured by injection with 50 μL 1.2% BaCl₂ in saline prior to harvest or harvested from uninjured hind limbs. The tibialis anterior muscles were dissected from the hind limb, minced, and digested in 400 U/mL collagenase in Ham’s F-12C at 37°C for 1 h, vortexing frequently. Collagenase was inactivated by the addition of horse serum and debris was removed by sequential straining through 70 μm and 40 μm cell strainers (BD Falcon). Cells were gently centrifuged and the cell pellets were incubated at 4°C with 1:100 rabbit anti-syndecan-3 antibody in Ham’s F-12C with 15% horse serum followed by an incubation on ice with Cy5 conjugated anti-rabbit-IgG (Molecular Probes). Satellite cells were sorted based on syndecan-3 immunoreactivity on a MoFlo Legacy cell sorter (Dako Cytomation) directly into RNA lysis buffer (PicoPure RNA Isolation kit, Arcturus).

**Myofiber explant culture and immunostaining**

All hind limb muscles were dissected, connective tissue removed, and individual muscle groups isolated followed by digestion in 400 U/mL collagenase in Ham’s F-12C at 37°C. Single myofibers were isolated and grown in Ham’s F-12C supplemented with 15% horse serum and 0.5 nM FGF-2 prior to fixation in 4% PFA. Fibers were blocked in 10% normal goat serum in phosphate buffered saline followed by antibody staining. Primary antibodies were rabbit anti-cmet (Santa Cruz) at 1:100, mouse anti-MyoD (Novostra) at 1:10, mouse anti-Pax7 at 1:5 (Developmental Studies Hybridoma Bank), and rabbit anti-MyOD C-20 at 1:500 (Santa Cruz Biotechnology). Secondary antibodies were Alexa-488 conjugated anti-mouse IgG, Alexa-594 conjugated anti-rabbit IgG, Alexa-555 conjugated anti-mouse IgG, and Alexa-647 conjugated anti-rabbit IgG (Molecular Probes). All images taken on a Nikon Eclipse E800 microscope with a Nikon 40x/0.75 differential interference contrast M lens and analyzed with Slidebook (Intelligent Imaging Innovations, Inc.).

**Microarray hybridization**

RNA was isolated from satellite cells using the PicoPure RNA Isolation kit (Arcturus) followed by two rounds of linear T7-based amplification (RiboAmp HA kit: Arcturus). The RNA equivalent of 5,000 cells was hybridized to Affymetrix mouse 430v2 GeneChips (MOE430v2) according to manufacturer’s instructions. GeneChips were scanned at the University of Colorado at Boulder on an Affymetrix GeneChip Scanner 3000 and spot intensities were recovered in the GeneChip Operating System (Affymetrix).

**Microarray data processing and analysis**

All analysis was performed using Spotfire DecisionSite 2 for Microarray Analysis. The raw CEL data files were
normalized using GC Robust Multi-array Analysis (GCRMA). The raw CEL data files, microarray metadata, and GCRMA normalized expression values were deposited in GEO datasets (GSE38870). One wild type freshly isolated satellite cell replicate consistently clustered with the wild type satellite cells 12 h post-injury replicates (via hierarchical, Self-Organizing Map (SOM), k-means) indicating myogenic commitment and was removed from our analysis. The hierarchical cluster and associated dendrogram were generated using the log2-value for relative probe intensity using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) with Euclidean distance as the similarity measure. The significance between genotypes and time points was determined using the multifactor analysis of variance (ANOVA) with a false discovery rate (FDR) ≤ 0.05 and Bonferroni adjustment. Fold change was calculated as 2abs(difference), where difference is the log2 difference between samples compared. Venn diagrams were generated in Spotfire™ using the list comparison function.

**Table 1** Quantitive PCR primer sequences

| Gene         | Primer type | Sequence                  |
|--------------|-------------|---------------------------|
| GAPDH        | Forward     | 5’ - TGTGTCGCTGCTGGATCTGGA - 3’ |
|              | Reverse     | 5’ - CCTGCTCACCACCTCTTCTC - 3’ |
| 18S          | Forward     | 5’ - GCCGCTAGAGGTAAGATCTCTG - 3’ |
|              | Reverse     | 5’ - CTTGCCGTGGGCTGCTTCT - 3’ |
| Celf4        | Forward     | 5’ - GCTGCTCTATCTACTATTGTGCC - 3’ |
|              | Reverse     | 5’ - GTGACTGAAGGGAGCACCTCAAA - 3’ |
| Pabpn1       | Forward     | 5’ - TTTCCTGCCCCTGTTCCATGCTC - 3’ |
|              | Reverse     | 5’ - AGTGACTGAAGGGAGCACCTCAAA - 3’ |
| Ppargc1a     | Forward     | 5’ - GCTGACGCTGCTCTGCTCCTT - 3’ |
|              | Reverse     | 5’ - AGCTCACTGAGGCTGATGTGCT - 3’ |
| Mbnl1        | Forward     | 5’ - AACCTGCAAGACGCCGAGAAGACT - 3’ |
|              | Reverse     | 5’ - GCAAAACTGCAACTTGTGACACGGA - 3’ |
| Matr3        | Forward     | 5’ - ATGGTGGATAGGGCCAGTCATGGT - 3’ |
|              | Reverse     | 5’ - TTGCAATTGGAACAAGTGCCGGCTG - 3’ |
| Sfrs3        | Forward     | 5’ - TGGGGACGCTGCTGGAATGATA - 3’ |
|              | Reverse     | 5’ - CTGAAAGGACATCGCGATCTGAGT - 3’ |
| Zfp36        | Forward     | 5’ - TCTCTGCCATCTACGAGGCC - 3’ |
|              | Reverse     | 5’ - CCGTACGCGAGAGGAGGA - 3’ |
| Zfp361       | Forward     | 5’ - GCTTTTGAGAGGCCTCTCCTT - 3’ |
|              | Reverse     | 5’ - TTGTCCCCTGACTTTACCGGCA - 3’ |
| Zfp362       | Forward     | 5’ - AGCCGGCTCCCAAGATCACT - 3’ |
|              | Reverse     | 5’ - AGAGAGGGCCGAGCCGGTTA - 3’ |
| Elavl1       | Forward     | 5’ - TGTTGAGCTACCTGCAATGTTAT - 3’ |
|              | Reverse     | 5’ - GAGGGTTTCAACAAACCATTACCA - 3’ |
| Cdk2         | Forward     | 5’ - TCTCTGGAGAGCGATGATGCA - 3’ |
|              | Reverse     | 5’ - TTGCGCCATCTACGAGGCC - 3’ |
| E2F3         | Forward     | 5’ - GGCTCTGCTGATCGAACAAGGC - 3’ |
|              | Reverse     | 5’ - CCTCCAGAGCGATTTGATG - 3’ |
| U6           | Forward     | 5’ - TTAATTCGTAAGGCCTTGTCTAT - 3’ |
| miR-16       | Reverse     | 5’ - TAGCACGCAGTAAATATTGCCG - 3’ |
| miR-93       | Reverse     | 5’ - CAAAGGTGTCCTGCTGCAAGTAGT - 3’ |
| miR-106b     | Reverse     | 5’ - TAAGGTGCAGACAGTGCAAT - 3’ |
| miR-107      | Reverse     | 5’ - AGAGAGGGCCGAGCCGGTTA - 3’ |
| miR-124      | Reverse     | 5’ - TAAAGGGAGAGCGAGCATCGG - 3’ |
| miR-200b     | Reverse     | 5’ - TAATACACCTGTGAATGATG - 3’ |

Sequences for forward and reverse primers used to detect miRNAs or sequence of reverse primer used to detect miRNAs using the Ncode universal forward primer.

**Gene ontology and biological pathway analysis**

Unique gene identifiers (gene symbol, entrez gene ID, or Affymetrix probe set ID) were uploaded to the Database for Annotation, Visualization and Integrated Discovery (DAVID http://david.abcc.ncifcrf.gov/), FunNet (http://www.funnet.info/), and ProfCom (http://webchu.bio.zwu.tum.de/profcom/). The mouse genome reference dataset for each algorithm was used as background. No further settings were required for DAVID. Analysis setting for FunNet were conventional functional analysis with the specificity enrichment computation for GO and false discovery rate ≤ 5%. Analysis settings for ProfCom were up degree 1 and exclude. The default settings for each algorithm were used to identify enriched GO terms. Affymetrix probe set IDs and the log2 difference between wild type freshly isolated satellite cells and satellite cells isolated 12 h post-injury were uploaded and analyzed using IPA v9.0 (Ingenuity® Systems www.ingenuity.com). Analysis settings were to consider both all direct and indirect molecules and/or relationships using the Mouse Genome 430 2.0 Array as a reference dataset.

**Computational prediction of miRNAs**

The Srivastava lab algorithm assessed miRNA sequences in human, mouse, and rat for miRNA seed matches and required base-pairing of miRNA nucleotides 2 to 7 with binding energy ≤ −14 kcal/mol and flanking energy ≥ −7 kcal/mol. Secondary structure was used to eliminate false-positives by removing those seed matches with secondary elements that stabilize miRNA. Priority 1 calls had a destabilizing mRNA element while Priority 2 calls did not contain a destabilizing element in at least one species. GeneAct used the miRanda algorithm to identify miRNA target sites across three mammalian species. False-positives were eliminated with the differential binding site search against genes that were constitutively expressed in satellite cells.

**RNA isolation**

miRNA was extracted from satellite cells using the RNeasy Kit according to the manufacturer’s protocol (Qiagen). miRNA was extracted using both the RNAqueous-micro kit the mirVana miRNA isolation kit according to the
**Figure 1** Sdc4−/− satellite cell gene expression post-muscle injury is similar to freshly isolated satellite cells. Myofiber-associated satellite cells are immunoreactive for MyoD 24 h and 48 h after isolation from wild type mice but not Sdc4−/− mice (A, B). Wild type but not Sdc4−/− cells divide by 48 h in culture (B) where c-met (red), MyoD (green), and DAPI (blue) identify satellite cells and a dashed line indicates the position of the myofiber membrane (A, B). Flow cytometry histograms of wild type (C) and syndecan-4 null (D) mononuclear cells from uninjured and injured skeletal muscle 12 h and 48 h post-injury plotted for cell size (FSC) vs. internal complexity (SSC), where the red box indicates gating for further analysis to remove debris (upper panels). Syndecan-3 immunoreactive cells present in the gate were isolated from wild type mice (C, lower panel) and Sdc4−/− mice (D, lower panel) where the percentages indicate satellite cells (blue lines) relative to other events with false-positives set to an antibody background < 0.1% (red lines). A hierarchical dendrogram constructed with Spotfire™ DecisionSite using Affymetrix GeneChip data reveals that Sdc4−/− satellite cells cluster most closely to freshly isolated wild type satellite cells while injured wild type satellite cells either 12 h post-injury or 48 h post-injury cluster independently (E). Red depicts high relative gene expression and green depicts low relative expression in the hierarchical cluster dendrograms (UPGMA, Euclidean distance). FI, freshly isolated; PI, post-injury.
manufacturer’s protocols (Ambion) with the following modifications. For satellite cells, the mirVana manufacturer’s protocol was followed using volumes and columns for the RNAqeous-micro kit. For MM14 cells, the mirVana manufacturer’s protocol was followed. RNA concentration was determined using a NanoDrop 2000 spectrophotometer (Thermo Scientific).

Quantitative RT-PCR
The Superscript III First Strand cDNA Synthesis kit was used to generate cDNA from mRNA according to manufacturer’s instructions (Invitrogen). The Ncode miRNA qRT-PCR system (Invitrogen) was used to generate cDNA from miRNA according to manufacturer’s instructions. Briefly, RNA was poly-adenylated with Poly-A polymerase followed by cDNA transcription with Superscript III reverse transcriptase using a primer similar to Oligo-dT with a unique 5’ end. Quantitative RT-PCR was performed using SYBR-Green (Applied Biosystems) or SYBR-GreenER (Invitrogen) on either an ABI 7500 Fast or ABI 7900 Real-Time PCR machine (Applied Biosystems). Primer sequences are listed in Table 1.

RNase protection assay
Candidate miRNAs were screened using the mirVana miRNA detection kit according to the manufacturer’s protocol (Ambion). All probes were radio-labeled with 32P-UTP using the mirVana miRNA probe construction kit (Ambion) according to manufacturer’s protocol.

Isolation of quiescent satellite cells
Quiescent satellite cells were isolated following IP injection with 75 mg/kg of SB203580 (Alexis Corporation) and kept in 25 μM SB203580 through the isolation as described (Hausburg et al., Submitted). All satellite cells were isolated as follows. Hind limb muscles of 3- to
6-month-old female B6D2F1/J mice were dissected and digested in 400 U/mL collagenase in Ham’s F-12C at 37°C for 1 h with periodic vortexing. The collagenase was inactivated with horse serum and debris was removed with sequential straining through 70 μm and 40 μm cell strainers (BD Falcon). Satellite cells were either isolated at the interface of a 40%/70% Percoll gradient (GE Healthcare) or plated in Ham’s F-12C supplemented with 15% horse serum and 0.5nM FGF-2 for various times before RNA isolation.

**miRNA inhibition**

Myofiber explant cultures were transfected using LipoFectamine 2000 (Invitrogen) according to manufacturer’s protocol with a 2:5:1 ratio of lipofectamine: nucleic acid. 200nM miRIDIAN hairpin inhibitors

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**Table 2 Identified Molecular Function GO terms**

| GO Term                  | DAVID   | P value | ProfCom | P value | FunNet | P value |
|--------------------------|---------|---------|---------|---------|--------|---------|
| Unfolded protein binding | 2.27    | 2.74E-05| 4.65    | 2.90E-08| 2.83   | 2.64E-08|
| Actin binding            | 1.83    | 2.00E-09| 2.60    | 2.10E-12| 1.92   | 5.03E-10|
| RNA binding              | 1.30    | 3.25E-04| 1.82    | 9.80E-10| 1.39   | 6.98E-05|
| Kinase activity          | 1.25    | 7.15E-04| 1.54    | 4.30E-08| 1.31   | 1.48E-04|
| Protein binding          | 1.25    | 8.42E-29| 1.52    | 5.90E-70| 1.30   | 1.86E-21|
| Nucleic acid binding     | 1.13    | 2.38E-04| 1.63    | 7.50E-10| 1.31   | 7.34E-05|
| Nucleotide binding       | 1.21    | 6.45E-07| 1.50    | 6.10E-18| 1.23   | 2.76E-06|
| Zinc ion binding         | 1.19    | 1.83E-05| 1.38    | 1.80E-15| 1.23   | 5.01E-05|
| Metal ion binding        | 1.17    | 7.66E-09| 1.37    | 5.20E-23| 1.16   | 4.83E-05|

Fold enrichment and associated p-values for the Molecular Function GO terms shared between three independent gene ontological analyses using DAVID (http://david.abcc.ncifcrf.gov), profcom (http://webclu.bio.wzw.tum.de/profcom/), and FunNet (http://www.funnet.info).
(Dharmacon) against miR-16, miR-93, miR-106b, and miR-124 were co-transfected with pEGFP-C1-H2B. Scrambled control was the miRIDIAN hairpin inhibitor negative control 1 (Dharmacon).

Results
Identification of gene expression changes associated with satellite cell activation in vivo
An unbiased global gene expression analysis using Affymetrix GeneChips was performed to identify changes occurring during the transition of satellite cells from quiescence to a population of proliferating myoblasts in vivo. To accomplish this, we identified changes in gene expression profiles between freshly isolated satellite cells and satellite cells isolated 12 h or 48 h following BaCl2-induced muscle injury. The time points chosen correspond to activated satellite cells which do not express MyoD protein (freshly isolated), committed myoblasts marked by MyoD expression (12 h post-muscle injury), and proliferating myoblasts (48 h post-muscle injury; Figure 1A) [36,37]. Syndecan-4 null satellite cells fail to activate, express MyoD, or enter the cell cycle appropriately within 48 h post-injury, and are incapable of skeletal muscle repair (Figure 1B) [36,37]. Therefore, we eliminated the genes whose expression changes following a muscle injury in Sdc4−/− satellite cells from our analyses as these genes were unlikely to be involved in satellite cell activation. Wild type and Sdc4−/− satellite cells were isolated by fluorescence activated cell sorting (FACS) using anti-syndecan-3 antibodies as a marker for quiescent and proliferating satellite cells [38] from uninjured tibialis anterior (TA) muscle (Figure 1C) and TA muscles 12 h post-injury (Figure 1D).

RNA was extracted from the isolated cells and processed for hybridization to Affymetrix v2.0 mouse GeneChips. The GeneChip data (Additional file 1, Additional file 2, Additional file 3, Additional file 4, Additional file 5, Additional file 6) were analyzed with Spotfire™ DecisionSite for Microarray Analysis software and an initial hierarchical cluster dendrogram generated. Visualization of the relationships between gene expression profiles show Sdc4−/− samples, regardless of time post-injury, cluster.

Table 3 Top 25 ranking biological interaction networks enriched during satellite cell activation

| Rank | Associated network function                                                                 | Score |
|------|---------------------------------------------------------------------------------------------|-------|
| 1    | RNA post-transcriptional modification, developmental disorder, genetic disorder              | 34    |
| 2    | Genetic disorder, neurological disease, psychological disorders                              | 34    |
| 3    | Genetic disorder, cellular assembly and organization, skeletal and muscular disorders        | 34    |
| 4    | Lipid metabolism, small molecule biochemistry, vitamin and mineral metabolism                | 32    |
| 5    | Nervous system development and function, tissue morphology, cellular development             | 32    |
| 6    | Genetic disorder, metabolic disease, molecular transport                                     | 32    |
| 7    | Cell morphology, cell-to-cell signaling and interaction, cellular assembly and organization  | 32    |
| 8    | Genetic disorder, ophthalmic disease, cardiovascular disease                                | 32    |
| 9    | Post-translational modification, cardiovascular disease, cardiovascular system development and function | 32 |
| 10   | Amino acid metabolism, genetic disorder, metabolic disease                                  | 32    |
| 11   | Cardiovascular system development and function, cell morphology, cell-to-cell signaling and interaction | 32 |
| 12   | Dermatological diseases and conditions, genetic disorder, amino acid metabolism              | 32    |
| 13   | Organismal functions, cardiac stenosis, cardiovascular disease                              | 32    |
| 14   | Cellular assembly and organization, RNA post-transcriptional modification, cancer            | 32    |
| 15   | Carbohydrate metabolism, drug metabolism, nucleic acid metabolism                           | 32    |
| 16   | Gene expression, amino acid metabolism, small molecule biochemistry                         | 32    |
| 17   | Cell cycle, reproductive system development and function, cell morphology                   | 32    |
| 18   | Genetic disorder, neurological disease, psychological disorders                              | 32    |
| 19   | Cancer, cellular assembly and organization, cellular compromise                              | 32    |
| 20   | Cell cycle, cell death, cell morphology                                                     | 30    |
| 21   | Genetic disorder, metabolic disease, neurological disease                                    | 30    |
| 22   | Post-translational modification, protein degradation, protein synthesis                      | 29    |
| 23   | Cell signaling, cardiovascular disease, skeletal and muscular system development and function| 29    |
| 24   | Lipid metabolism, small molecule biochemistry, dermatological diseases and conditions        | 29    |
| 25   | Cellular development, genetic disorder, hematological system development and function        | 29    |

The most significantly enriched biological networks during satellite cell activation were generated through the use of IPA 9.0 (Ingenuity Systems, www.ingenuity.com). The score is the negative base-10 logarithm of the P value (that is, a score of 34 is P ≤ 10−34).
with freshly isolated wild type satellite cells (Figure 1E) supporting our observations that Sdc4−/− satellite cells do not activate appropriately within 48 h following an induced muscle injury (Figure 1A, B). Within the wild type dataset, we observed that committed myoblasts isolated 12 h post-injury exhibited the most divergent gene expression profiles, suggesting that these committed satellite cells differ substantially from either quiescent satellite cells or proliferating myoblasts (Figure 1E). To focus on genes that may be involved in satellite cell activation, we chose to further compare gene expression changes occurring within the first 12 h post-muscle injury.

**Table 4** Genes in the top ranked network associate with muscle function, muscle disease, or fate determination

| Genes | Relevant role/Disease/Expression | Reference |
|-------|---------------------------------|-----------|
| Luc7l | Regulation of muscle differentiation | [45] |
| Snrpn | Prader-Willi syndrome | [46] |
| Pkra | Positive regulation of embryonic stem cells | [47] |
| Hrtrs1 | Expression in developing limb bud | [48] |
| Zbtb3 | Expression in developing limb bud | [48] |
| Sucept5h | Expression in developing limb bud | [48] |
| Tcerg1 | Expression in developing limb bud | [48] |
| Hnrnp | Facilitates neuronal proliferation and maturation; Expression in developing limb bud | [48,49] |
| Snrpb | Spinal muscular atrophy; Expression in developing limb bud and somite | [7,48] |
| Snrpa1 | Spinal muscular atrophy | [50] |
| Syncrip | Spinal muscular atrophy | [50] |
| Sfl | Expression in developing limb bud | [48] |
| AP3D1 | Regulation of progenitor cell competence | [51] |
| Hnrnpr | Expression in developing limb bud | [48] |

Fourteen of the thirty-five genes in the top ranked biological network as identified by IPA v9.0 (Ingenuity® Systems, www.ingenuity.com) have defined functions in muscle or cell fate determination as determined with the associated references.

Over 4,000 genes are specifically regulated during satellite cell activation

A comparative analysis of gene expression profiles from wild type and Sdc4−/− satellite cells within the first 12 h following satellite cell activation identified a cohort of genes unique to satellite cell activation. In wild type satellite cells, 5,162 genes change significantly between satellite cells isolated from uninjured muscle and those isolated 12 h post-injury as defined by a ≥2-fold change with an ANOVA P ≤ 0.01 (Figure 2A). In contrast, 2,236 genes similarly changed expression in Sdc4−/− satellite cells isolated from uninjured TA muscle and TA muscles 12 h post-injury (Figure 2A). Eighty percent (4,093) of the genes differentially expressed in WT satellite cells do not significantly change in Sdc4−/− satellite cells as identified by Venn analysis (Figure 2A, B; Additional file 7). We reasoned that the metabolic changes occurring during satellite cell activation as well as the induction of the transcription factor MyoD and cell cycle entry would result in a large cohort of induced genes. Surprisingly, more than half (56%) of the genes differentially expressed in satellite cells by 12 h post-injury decrease in relative expression (Figure 2B). Moreover, the magnitude of change for genes that decrease is on average three-fold greater than the magnitude of change for genes that increase following muscle injury. Less than 10% of genes whose expression is increased change more than four-fold (2^2), while 70% of downregulated genes change more than four-fold (2^2) and 3% decrease more than 64-fold (2^6) (Figure 2C). These observations suggest that quiescent satellite cells express a cohort of genes that maintains and regulates the quiescent state, are likely critical for interaction with the satellite cell niche, and are necessary for interpreting signals for exit from quiescence. Furthermore, our results support the idea that satellite cell quiescence is actively maintained, consistent with a prior report examining freshly isolated satellite cells and satellite cells isolated from dystrophic muscle [14].

To further test the idea that satellite cell quiescence is actively maintained, we analyzed genes in the cohort that significantly change 12 h post-injury involved in cell cycle progression. We would expect cell cycle progression genes to be induced during activation and found that genes modulating the G1/S phase transition are among those that increase in relative expression (Figure 2D, green). In contrast, cell cycle inhibitors decrease in relative expression (Figure 2D, red) as expected for the transition of satellite cells from mitotic quiescence to an activated state in preparation for cell cycle entry. Moreover, these genes do not change expression significantly in Sdc4−/− satellite cells 12 h post-injury (Figure 2A, B; Additional file 7) consistent with their impaired cell cycle activation and MyoD induction.

Genes involved in RNA post-transcriptional regulation are significantly enriched during satellite cell activation

Gene ontology (GO) classifications were used to aid in identifying potential mechanisms regulating satellite cell activation. The Database for Annotation, Visualization and Integrated Discovery (DAVID) was used to identify enrichment of general molecular function categories [39,40] during activation of satellite cells. The GO category of Molecular Function: Binding is the most significantly over-represented GO category during satellite cell activation (P value = 7.03 × 10^-33 compared to the mouse genome), where a 7% increase in the total percentage of genes classified as binding occurs in satellite cells within the first 12 h post-muscle injury (Figure 3A). Further refinement of ontological categories reveals that GO terms unfolded protein binding, actin binding, and
RNA binding were enriched an average of 1.5-fold over three independent gene annotation algorithms: FunNet [41,42], ProfCom [43], and DAVID [39,40] (Figure 3B, Table 2). Thus, in the first 12 h post-muscle injury, major changes occur in genes involved in RNA binding, the unfolded protein response, and in actin binding. The changes in RNA binding proteins and unfolded protein response may be involved in the down-regulation of genes necessary to maintain a quiescent satellite cell, while changes in actin binding are likely to reflect changes in satellite cell motility [44] that accompany repair of skeletal muscle tissue.

A fourth independent analysis focused on biological networks (IPA-Ingenuity Pathway Analysis www.ingenuity.com) ranked RNA Post-Transcriptional Modification in the top biological network (Table 3; Table 4). Thus, from four independent methods of gene expression analysis, a much higher proportion of genes involved in post-transcriptional RNA regulation change expression in the transition from a quiescent satellite cell to a committed myoblast, suggesting a role for post-transcriptional regulation of RNA in this transition. Therefore, we further analyzed genes involved in RNA post-transcriptional modification to further characterize individual genes and to develop hypotheses regarding the function of these genes in the transition of satellite cells from mitotic quiescence to cell cycle entry.

**Splicing factors are preferentially downregulated during satellite cell activation**

The top ranking biological network identified by IPA has associated biological functions of RNA Post-Transcriptional Modification, Developmental Disorder, and Genetic Disorder ($P \leq 10^{-34}$) emerged as the associated biological functions in the top ranked interaction network with the canonical pathways Processing of RNA ($P \leq 4.44e^{-22}$) and Splicing of RNA ($P \leq 7.64e^{-20}$) comprising 50% of this network. The functions of these genes in muscle disease and cell fate decisions are listed in Table 4. Red indicates genes down-regulated and green indicates genes upregulated with the intensity denoting the increase or decrease in fold change. The data compares genes unique to wild type satellite cells isolated 12 h post-injury to satellite cells isolated from uninjured skeletal muscle.

**Figure 4 RNA post-transcriptional modification is the most enriched biological network.** Gene expression changes unique to wild type satellite cell activation were subjected to IPA 9.0 (Ingenuity Systems, www.ingenuity.com) network analysis and RNA Post-Transcriptional Modification, Developmental Disorder, Genetic Disorder ($P \leq 10^{-4}$) emerged as the associated biological functions in the top ranked interaction network with the canonical pathways Processing of RNA (17 blue dotted lines, $P \leq 4.44e^{-22}$) and Splicing of RNA (15 orange dotted lines, $P \leq 7.64e^{-20}$) comprising 50% of this network. The functions of these genes in muscle disease and cell fate decisions are listed in Table 4. Red indicates genes down-regulated and green indicates genes upregulated with the intensity denoting the increase or decrease in fold change. The data compares genes unique to wild type satellite cells isolated 12 h post-injury to satellite cells isolated from uninjured skeletal muscle.
Figure 5 Classification of top quartile RNA binding proteins significantly regulated 12 h post-injury in satellite cells. Genes categorized with the GO term Molecular Function: RNA Binding that change expression ≥ two-fold (ANOVA \( P \leq 0.01 \)) comprise 22% (154 of 716) of the total GO category in wild type satellite cells but do not change significantly in Sdc4\(^{-/-}\) satellite cell during the first 12 h post-muscle injury (A). A minority of the identified genes increase in relative expression (B), while the majority of these genes decrease in their relative expression 12 h post-muscle injury (C). Upregulated and downregulated genes were further classified and plotted as splicing factors (green bars), positive mRNA regulators (blue bars), negative mRNA regulators (red bars), or multiple/other functions (black bars). The values plotted are for fold increase (B) or fold decrease (C) unique to wild type satellite cells occurring in the first 12 h post-muscle injury.
cell activation are involved in RNA processing and splicing (Figure 4). Moreover, many genes in this interaction network are implicated in either muscle function, muscle disease, or cell fate decisions (Table 4). Consistent with our prior observations that downregulated genes exhibit greater fold change in relative expression occurring during the first 12 h post-muscle injury, the majority of RNA processing and splicing genes identified in the interaction network (87% and 88%, respectively) decrease post-muscle injury (Figure 4). Furthermore, of the 154 RNA binding proteins identified by GO analysis (Figure 3B, Additional file 8), 69% decrease in relative expression (Figure 5A; Additional file 8). These data show that RNA binding proteins are highly over-represented in quiescent satellite cells and suggest that regulation of RNA plays an important role in maintaining the quiescent state and in the transition to a cycling myoblast.

An examination of the top quartile of RNA binding proteins with the most significant changes in expression reveal that the majority (45%) of upregulated genes are positive mRNA regulators (Figure 5B), while downregulated genes include a similar distribution of all RNA protein functions with splicing factors exhibiting a slight majority (37%; Figure 5C). Furthermore, 24% of the previously identified RNA processing of splicing factors (Figure 4) are present in this top quartile. To confirm the microarray expression data, we performed qPCR validation finding that approximately 80% of the tested RNA binding proteins and both tested cell cycle regulators have consistent gene expression profiles (Table 5). Thus, genes involved in mRNA regulation may play diverse roles in satellite cell activation and promote the conversion of a quiescent satellite cell to a proliferating myoblast.

Dynamic regulation of miRNAs during muscle regeneration

MicroRNA-mediated gene silencing regulates alternative splicing [52,53] as well as mRNA stability factors [54,55]. Moreover, miRNAs regulate stem cell fate determination [28,30,31,56] suggesting a potential role for miRNAs in the transition of quiescent satellite cells to proliferating myoblasts. The low levels of RNA present in quiescent satellite cells combined with infrequent satellite cell abundance in uninjured muscle prevented successful unbiased screen for miRNAs. Therefore, we assessed whether genes involved in miRNA biogenesis and gene silencing including argonautes1-4 (Eif2c1-4), Dgcr8, and Dicer, as well as other genes associated with miRNA function, are expressed in quiescent satellite cells (Additional file 1, Additional file 2, Additional file 3, Additional file 4, Additional file 5, Additional file 6). Although present, Argonautes1-4 (Eif2c1-4), Dgcr8, and Dicer are not in the cohort of differentially expressed genes (Additional file 7), suggesting that major changes in miRNA processing do not occur during satellite cell activation.

To identify potential miRNAs involved in the transition of quiescent satellite cells to proliferating myoblasts, we applied miRNA target prediction algorithms to identify putative miRNAs regulating genes whose expression changes rapidly during the first 48 h post-muscle injury. Initially, we established a minimum gene expression value in freshly isolated satellite cells to reduce the

| Gene   | Microarray | qPCR | Fold change | Correlation | Biological function |
|--------|------------|------|-------------|-------------|---------------------|
| Celf4  | ↑          | ↑    | 2.51        | Yes         | Splicing factor     |
| Pabpn1 | ↓          | ↓    | −1.80       | Yes         | Positive mRNA regulator |
| Ppargc1a| ↑          | ↑    | 1.34        | Yes         | Positive mRNA regulator |
| Mbnl1  | ↓          | ↓    | −1.14       | Yes         | Splicing factor     |
| Mat3   | ↓          | ↑    | 11.79       | No          | Negative mRNA regulator |
| Sfn3   | ↓          | ↑    | 19.11       | No          | Splicing factor     |
| Zfp36  | ↓          | ↓    | −2.87       | Yes         | Negative mRNA regulator |
| Zfp361 | ↓          | ↓    | −4.48       | Yes         | Negative mRNA regulator |
| Zfp362 | ↓          | ↓    | −1.63       | Yes         | Negative mRNA regulator |
| Elavl1 | ↑          | ↑    | 3.01        | Yes         | Positive mRNA regulator |
| Cdk2   | ↑          | ↑    | 10.28       | Yes         | Promotes cell cycle entry |
| E2F3   | ↑          | ↑    | 12.90       | Yes         | Promotes cell cycle entry |

Eight of ten RNA binding proteins and two cell cycle genes validate expression changes between wild type satellite cells isolated from uninjured TA muscle and from the TA 12 h post-muscle injury. Arrows show increase (↑) and decrease (↓) for both microarray and qPCR during satellite cell activation. Fold change is from qPCR data where positive values indicate increased expression and negative valued indicate decreased expression between satellite cells isolated from uninjured TA muscle and from the TA 12 h post-muscle injury. Quantitative PCR data is normalized to GAPDH or 18S.

Table 5 Microarray and qPCR expression trends correlate for RNA binding proteins
cohort to 641 genes (ANOVA $P \leq 0.01$, $\geq$ two-fold change) in wild type satellite cells isolated from uninjured TA muscle or from the TA 48 h post-muscle injury and not in Sdc4$^{-/-}$ satellite cells (ANOVA $P > 0.9$) is represented as a log2 values. Gene identifiers are Probe set ID, representative gene symbol, and entrez gene ID. The fold change was calculated for changes occurring in the first 12 h post-muscle injury according to genotype.

relative expression data for genes that significantly change (ANOVA $P \leq 0.01$, $\geq$ two-fold change) in wild type satellite cells isolated from uninjured TA muscle or from the TA 48 h post-muscle injury and not in Sdc4$^{-/-}$ satellite cells (ANOVA $P \geq 0.9$), we chose these 47 genes to pursue as potential miRNA targets involved in satellite cell activation (Table 6). Candidate miRNAs were then computationally identified using two independent algorithms, one developed by the group of Deepak Srivastava (unpublished) and GeneAct (http://promoter.colorado.edu/geneact/) [57] (Figure 6A). The union of both algorithms identified 12 miRNA candidates with six, miR-16, miR-93, miR-106b, miR-107, miR-124, and miR-200b, being detected in cultured primary satellite cells or proliferating satellite cell derived MM14 cells by ribonuclease protection assay (Table 7). All six miRNAs present in primary myoblasts and MM14 cells were detectable in uninjured tibialis anterior muscle [57] (Figure 6B). We observed dramatic regulation of these six miRNAs following a muscle injury when compared to control, uninjured tibialis anterior muscle. Four of the identified miRNAs (miR-93, miR-106, miR-107, miR-200b) changed expression levels by more than two-fold during the first 5 days following induced muscle injury (Figure 6B-F; Table 8). In the uninjured TA muscle, miR-200b decreased three-fold by 12 h post-injury, while miR-93 and miR-124 increased significantly 12 h post-muscle injury (Figure 6B, C, F; Table 8). Within 48 h post-injury, the relative levels of miR-93, miR-107, and miR-124 had decreased levels well below those present in uninjured muscle and remained low at 5 days post-injury (Figure 6C-E). In contrast, miR-106b remained elevated following injury while miR-16 trended to slightly lower expression (Figure 6B-E). The rapid changes in miRNA relative expression and their presence in skeletal muscle suggest that these miRNAs may play important roles in the regeneration of skeletal muscle and validates our approach to identify such miRNAs.

## Table 6 Genes used to predict candidate miRNAs

| Probe set ID  | Gene symbol | Entrez gene | Fold change |
|---------------|-------------|-------------|-------------|
| 1417654_at    | Sdc4        | 20971       | 11.68       |
| 1418282_x_at  | Serpina1b   | 20701       | 6.78        |
| 1418510_s_at  | Pbxo8       | 50753       | 4.74        |
| 1419070_at    | Cys1        | 12879       | 3.78        |
| 1419302_at    | Heyl        | 56198       | 3.26        |
| 1420930_s_at  | Ctnnal1     | 54366       | 2.71        |
| 1420980_at    | Pak1        | 18479       | 3.13        |
| 1422899_at    | Pcdh18      | 73173       | 4.72        |
| 1422892_s_at  | H2-Ea       | 14968       | 25.61       |
| 1424539_at    | Rppap2      | 231571      | 5.15        |
| 1425336_x_at  | H2-K1       | 14972       | 34.35       |
| 1425609_at    | Ncf1        | 17969       | 2.68        |
| 1426981_at    | Pcsk6       | 18553       | 3.38        |
| 1427884_at    | Col3a1      | 12825       | 12.29       |
| 1429021_at    | Ephra4      | 13838       | 3.51        |
| 1430764_at    | 1700023F06Rik | 69441       | 3.12        |
| 1433639_at    | 5730593F17Rik | 215152     | 3.30        |
| 1434105_at    | Epm2ap1     | 77781       | 2.91        |
| 1434267_at    | Nek1        | 18004       | 2.90        |
| 1434790_a_at  | Lta4h       | 16993       | 4.96        |
| 1435603_at    | Sned1       | 208777      | 3.12        |
| 1437152_at    | Mex3b       | 108797      | 4.08        |
| 1438532_at    | Hmccn1      | 545370      | 6.09        |
| 1438577_at    | —           | —           | 5.66        |
| 1439618_at    | Pde10a      | 23984       | 3.37        |
| 1440237_at    | Ercc4       | 50505       | 3.40        |
| 1441958_s_at  | Ager        | 11596       | 7.06        |
| 1442700_at    | Pde4b       | 18578       | 11.21       |
| 1444409_at    | Rph3al      | 380714      | 3.92        |
| 1444517_at    | —           | —           | 3.13        |
| 1447257_at    | —           | —           | 2.45        |
| 1447657_s_at  | Synpo2l     | 68760       | 3.00        |
| 1449226_at    | Hicl1       | 15248       | 3.11        |
| 1449465_at    | Reln        | 19069       | 5.95        |
| 1449619_s_at  | Arhgap9     | 216445      | 3.20        |
| 1451513_s_at  | Serpina1a   | 20700       | 4.62        |
| 1452632_at    | Aak1        | 269774      | 3.64        |
| 1452896_at    | Gt3         | 14894       | 8.40        |
| 1453114_at    | Nol9        | 74035       | 2.30        |
| 1453771_at    | Gulp1       | 70676       | 5.06        |
| 1454112_a_at  | Cep27       | 66296       | 2.46        |
| 1454333_at    | 6330526H18Rik | 76174     | 2.95        |
| 1454877_at    | Sertad4     | 214791      | 6.96        |
| 1455136_at    | Atpl1a2     | 98660       | 6.60        |
| 1455188_at    | Ephb1       | 270190      | 3.37        |

## Table 6 Genes used to predict candidate miRNAs (Continued)

| Probe set ID  | Gene symbol | Entrez gene | Fold change |
|---------------|-------------|-------------|-------------|
| 1457944_at    | —           | —           | 16.23       |
| 1459164_at    | AU014678    | 101228      | 2.34        |

Relative expression of miRNAs in satellite cells following muscle injury

We asked whether the six miRNAs that change expression during muscle regeneration are present in satellite cells, muscle tissue, or both. The relative expression
**Prediction of Targeting miRNAs**

**GeneAct:** miRanda, Differential Binding Sites, Multiple Binding Sites

**Priority 1:** Seed Match, Binding Energy and 2° Structure

**Priority 2:** No 2° Structure Restriction

**Combination of:** Priority 1, Priority 2 AND GeneAct Prediction

**Candidate List of miRNAs** (Table 6)

**Figure 6** (See legend on next page.)
levels for each miRNA in the uninjured tibialis anterior muscle was normalized to 1 and the relative levels in satellite cells isolated from uninjured muscle and in proliferating satellite cells isolated 48 h post-injury examined. Surprisingly, all six miRNAs were expressed at low to undetectable levels in freshly isolated satellite cells (Figure 6G). In proliferating satellite cells, all but miR-106b were expressed at levels substantially lower than that found in the tibialis anterior muscle (Figure 6H). Although MyoD protein is not detectable in freshly isolated satellite cells and they have not yet entered S-phase, freshly isolated satellite cells are not quiescent since the p38α/β MAPK is activated [8]. Therefore, to identify miRNAs present in quiescent satellite cells, mice were injected with SB203580, a p38α/β MAPK inhibitor, 1.5 h prior to satellite cell isolation (Hausburg et al., Submitted), and relative miRNA levels examined. Remarkably, we found that miR-124 was expressed 35-fold higher in quiescent satellite cells than in freshly isolated or proliferating satellite cells (Figure 6I, J; Table 8). Moreover, miR-124 was expressed at levels 10-fold greater in quiescent satellite cells than in uninjured skeletal muscle (compare Figure 6I and 6B, Figure 6; Table 8), suggesting that the primary source of miR-124 in uninjured muscle is the satellite cell population. In contrast to miR-124, miR-16 and miR-93 are present at low to undetectable levels in quiescent satellite cells and are induced in freshly isolated satellite cells (Figure 6G, I). The expression level of miR-16 is maintained in proliferating cells, while miR-93 declines and miR-106b is dramatically induced in proliferating satellite cells as compared to freshly isolated and quiescent satellite cells (Figure 6G-I).

miR-16, miR-106b, and miR-124 regulate satellite cell fate

The changes in relative levels of miR-16, miR-93, miR-106b, and miR-124 in satellite cells following a muscle injury suggests that these four miRNAs may play a role in the transition from a quiescent satellite cell to a proliferating myoblast. To test this idea, inhibitors for each miRNA were transfected into myofiber-associated satellite cells immediately following isolation and the cultures fixed and assayed at 3 days post-isolation and 5 days post-isolation. The total number of Pax7+ cells transfected with the scrambled RNA control inhibitor declined two-fold between 3 and 5 days in culture, indicative of differentiation (Figure 7A, B). Between 3 and 5 days in culture, the Pax7+/MyoD+ decreased three-fold accompanied by the appearance of the Pax7+/MyoD- reserve population (Figure 7C-F). In contrast, inhibition of miR-124 increased the percentage of Pax7+/MyoD- reserve cells at 3 and 5 days of culture, as did inhibition of miR-106b (Figure 7A-F). Inhibition of miR-16 elevated the total number of Pax7+ cells at 5 days of culture and inhibition of miR-93 did not have any detectable effect (Figure 7-F). We further examined the role of miRNAs in satellite cell activation using Ingenuity® System’s IPA and identified PTEN signaling and Cell Cycle Regulation by BTG Family Proteins as the top canonical pathway regulated by miR-16, miR-93, miR-106b, and miR-124 in the transition of a quiescent satellite cell to a proliferating myoblasts (Figure 8). Many predicted targets of miR-16 and the miR-93/106b family inhibit cell cycle progression and cell growth. These targets are downregulated during satellite cell activation, consistent with increased expression of miR-16, miR-93, and miR-106b in proliferating satellite cells as compared to quiescent satellite cells (Figure 8). Conversely, predicted target genes of miR-124 promote cell cycle progression and are upregulated during satellite cell activation when miR-124 is downregulated (Figure 8). These data demonstrate that a number of miRNAs regulate satellite cell fate following a muscle injury and support the idea that post-transcriptional regulation of RNA plays a critical role in satellite cell activation and maintenance of satellite cell quiescence.
Table 7 Six of twelve predicted miRNAs are expressed in satellite cells

| miRNA     | Rationale               | Detected in SCs |
|-----------|-------------------------|-----------------|
| miR-16    | Priority 2 and GeneAct  | +               |
| miR-26a/b | Priority 1 and GeneAct  | ND              |
| miR-30a   | Priority 1, 2 and GeneAct| -               |
| miR-93    | Priority 1 and GeneAct  | +               |
| miR-106b  | Priority 1 and GeneAct  | +               |
| miR-107   | Priority 2 and GeneAct  | +               |
| miR-124   | Priority 1 and GeneAct  | +               |
| miR-130a  | Priority 1 and GeneAct  | -               |
| miR-132   | Priority 1 and GeneAct  | -               |
| miR-200b  | Priority 1 and GeneAct  | +               |
| miR-320   | Priority 1 and GeneAct  | -               |
| miR-424   | Priority 1 and GeneAct  | -               |

We predicted 12 candidate miRNAs from 47 genes identified as only expressed in satellite cells or only expressed in myoblasts. miR-16, miR-93, miR-106b, miR-107, miR-124, and miR-200b are detected in satellite cells by RNAse protection assay in either primary satellite cells or in the satellite cell derived MM14 cell line.

Discussion

The low cytoplasmic to nuclear ratio, low organelle number, and mitotic quiescence of resident satellite cells [12,13] lead to the speculation that metabolic activity in these cells is low. Indeed, quiescent satellite cells with high levels of Pax7 express reduced levels of mitochondrial genes [58]. Moreover, the delay to the first cell cycle division (Troy et al.)\(^4\) coupled with the dramatic increase in cell size and mobility [36,44] suggests that satellite cell activation and cell cycle entry would require transcriptional induction of a large cohort of genes similar to that observed in serum stimulated fibroblasts [59]. However, we and others have postulated that quiescent satellite cells are poised for activation awaiting a critical signaling event [8,14]. The data presented here further support this hypothesis and provide the first direct comparison of quiescent satellite cells with activated satellite cells and proliferating myoblasts derived from uninjured and injured skeletal muscle, respectively. The prior analyses performed compared freshly isolated satellite cells with satellite cells isolated from dystrophic mice [14] or cultured cells [14,34] and are expected to identify gene expression changes associated with a diseased environment or a culture environment, respectively. Since the most significant reductions in gene expression occur within the first 12 h post-muscle injury, the metabolic and signaling events in a quiescent satellite cell are thus predicted to be divergent from those of a proliferating myoblast. Moreover, these data suggest that comparisons of freshly isolated satellite cells with proliferating myoblasts may not identify critical regulatory mechanisms involved in satellite cell activation [14,34].

Here, we used computational methods to initially identify that RNA post-transcriptional mechanisms are likely to maintain the quiescent satellite cell phenotype and to promote the conversion of the quiescent satellite cell to a transit-amplifying myoblast. Recent studies indicate that RNA post-transcriptional mechanisms, specifically alternative splicing, mRNA stability, and miRNA-mediated gene silencing, regulate stem cell pluripotency and progression through differentiation [17-20]. As satellite cells are an adult stem cell population, similar mechanisms may mediate the transition from quiescence to a population of proliferating myoblasts. We found that splicing factors may play roles in regulating the transition of satellite cells from quiescence to proliferating myoblasts. Consistent with these observations, the relative molar ratios of splicing factors guide alternative splicing [60] and these factors often function combinatorially to direct the expression of different mRNA splice variants [61]. Together with published data, our observations of dynamic splicing factor expression in satellite cells following muscle injury suggests that unique cohorts of mRNA species regulate the conversion of quiescent adult stem cells to the committed proliferating myoblast.

In addition to splicing factors, we found that mRNA binding proteins regulating mRNA stabilization and mRNA decay are preferentially upregulated in satellite cells following muscle injury. Indeed, the AU-rich element (ARE) binding protein HuR (ElavI) is reported to stabilize MyoD and myogenin mRNA in skeletal muscle cell lines derived from satellite cells [62,63] potentially participating in satellite cell activation and commitment.

Table 8 Fold difference of candidate miRNAs as compared to levels in resting muscle

| miRNA   | miR-16 | miR-93 | miR-106b | miR-107 | miR-124 | miR-200b |
|---------|--------|--------|----------|---------|---------|----------|
| 12 h Pl | -1.1   | 2.7    | 1.9      | -1.9    | 3.4     | -2.7     |
| 48 h Pl | -1.3   | -3.5   | NC       | -6.8    | -5.8    | -12.4    |
| 5d Pl   | -1.9   | -2.0   | 1.7      | -3.0    | -3.1    | -3.1     |
| Quiescent satellite cell | -10.9 | -3.9 | ND | ND | 7.4 | ND |
| Freshly isolated satellite cell | -2.3 | -1.6 | -24.3 | -143 | -10.3 | -1418.2 |
| Proliferating satellite cell | -2.7 | -6.8 | 1.1 | -16.5 | -4.8 | -6.5 |

Fold difference of miRNA expression as compared to resting muscle where positive values are higher expression and negative values are lower expression. NC, no change; ND, not detected.
Figure 7 Inhibition of candidate miRNAs alters satellite cell fate. The four candidate miRNAs (miR-16, miR-93, miR-106b, and miR-124) that displayed dynamic expression in satellite cells were inhibited in myofiber-associated satellite cells prior to the first cell division. Transfected cells were assessed 3 or 5 days post myofiber harvest and identified via immunofluorescence as satellite cells by Pax7 expression (A, B) with proliferating satellite cells expressing both Pax7 and MyoD (C, D) and quiescent satellite cells expressing only Pax7 (E, F). The percent of Pax7+ satellite cells decreased between 3 and 5 days in satellite cell populations treated with a scrambled RNA control, however, the relative number of Pax7+ satellite cells remained at similar levels when any candidate miRNA was inhibited (A, B). This increase in satellite cells following miRNA inhibition at 5 days was observed in both proliferating satellite cells (D) and quiescent satellite cells (F) for miR-16, miR-106b, and miR-124 while inhibition of miR-93 resulted in a specific increase in proliferating satellite cells at 5 days (D). Inhibition of two miRNAs, miR-106b and miR-124, resulted in a dramatic increase in quiescent satellite cells by 3 days post myofiber isolation (E) that remains consistent through 5 days post isolation (F).
Cell Cycle Regulation by BTG Family Proteins

- miR-16
- miR-93
- miR-106b
- miR-124

Cell Differentiation
- Cyclin
- CDK4
- CDK2
- E2F

Expression change during activation
- Up-regulated
- No Change
- Down-regulated

PTEN Signaling

Extracellular space
- Integrin
- Growth Factor Receptors

Cytoplasm
- SHC
- PTEN
- FAK
- GRB2
- SOS
- PI3K
- AKT
- ERK
- p70 S6K
- NF-xB
- FOXO

Cell Cycle Regulation
- G1 arrest
- CCRN4L

Growth arrest stimuli (DNA damage, hypoxia, cellular differentiation, apoptosis)

Figure 8 (See legend on next page.)
to myogenesis. Elav1 is one of the most significantly upregulated RNA binding genes in satellite cells within 12 h of muscle injury (See Figure 5C; Additional file 8) and corresponds to MyoD expression. Interestingly, the ARE-binding proteins, tristetraprolin (Zfp36) and family members Zfp36l1 and Zfp36l2, decrease dramatically during the conversion from quiescence to activated satellite cells within the first 12 h post-muscle injury (See Figure 5D; Additional file 8). However, tristetraprolin and HuR have opposing functions as they counter-regulate expression of the same miRNAs [64,65] and may act as an agonist–antagonist pair for many genes that promote commitment to myogenesis. In agreement with these data, we have demonstrated that the Zfp36 family directly targets MyoD mRNA and functions to regulate satellite cell fate during satellite cell activation and self-renewal (Hausburg et al., Submitted).

A recent report identifies miR-489 as an important miRNA maintaining satellite cell quiescence [32] suggesting that miRNA-mediated gene silencing functions in the transition of a quiescent satellite cell to a proliferating myoblast. We identified a cohort of genes that significantly change expression in satellite cells within the first 48 h following muscle injury to computationally predict cognate miRNAs that may regulate these targets with two independent prediction algorithms. Six miRNAs not previously reported in skeletal muscle were selected for further analysis and all six were observed to be dynamically regulated in relative levels during induced muscle injury. Moreover, four of the six miRNAs were expressed in satellite cells (miR-16, miR-93, miR-106b, and miR-124), while two were likely present only in differentiated muscle (miR-107 and miR-200b). Comparing relative levels in muscle tissue and satellite cells revealed that miR-124 is likely only expressed in satellite cells, while miR-16, miR-93, and miR-106b are mostly likely expressed in satellite cells and in differentiated muscle fibers. Pathways predicted to be targeted by these miRNAs include cell cycle progression as well as PTEN signaling, which is involved in stem cell self-renewal [66] and muscle regeneration [67].

To directly test whether these four miRNAs regulate satellite cell behavior, we transfected inhibitors for each miRNA into myobfiber-associated satellite cells shortly after isolation and examined the effects on satellite cell fate at 3 and 5 days post-myobfiber isolation. Inhibition of two miRNAs, miR-106b and miR-124, increased the relative number of progenitor or ‘reserve’ satellite cells (Pax7+/MyoD-) relative to a control, suggesting that these miRNAs participate in the regulation of satellite cell fate and satellite cell self-renewal. In contrast, miR-16 enhanced the relative numbers of Pax7+ cells but did not appear to alter the percentage of Pax7+/MyoD+ myoblasts or Pax7+/-MyoD- ‘reserve’ cells relative to a scrambled control. Of the four miRNAs tested, the loss of miR-93 elicited no detectable changes in the numbers of ‘reserve’ Pax7+/-MyoD- satellite cells and Pax7+/-MyoD+ satellite cells suggesting functions in cellular processes other than cell fate determination.

**Conclusions**

We believe that RNA post-transcriptional regulation plays a critical role in the transition of a quiescent satellite cell to a transit-amplifying myoblast. At each time point we examined, including quiescent satellite cells (freshly isolated in the presence of a p38α/β MAPK inhibitor), activated satellite cells (12 h post-muscle injury), and proliferating myoblasts (48 h post-muscle injury), we found extensive changes in genes involved in post-transcriptional RNA regulation, including mRNA splicing, mRNA stability, and miRNA-mediated gene silencing. We conclude that satellite cell quiescence is actively maintained via combinatorial contributions primarily mediated through post transcriptional mRNA regulation and identified four miRNAs that likely play a role in the conversion of quiescent satellite cells to proliferating myoblasts.

**Endnotes**

Following submission of our manuscript, the following was published demonstrating post-transcriptional regulation of myf-5 during satellite cells activation. Crist CG, Montarras D, Buckingham M (2012) Muscle Satellite Cells Are Primed for Myogenesis but Maintain Quiescence with Sequestration of Myf5 mRNA Targeted by microRNA-31 in mRNP Granules. Cell Stem Cell 11: 118-126.

**Additional files**

Additional file 1: CEL files of wild type satellite cells isolated from uninjured muscle. Raw expression data for three replicates of satellite muscle. 

**Figure 8 Candidate miRNAs target genes involved in cell growth, survival, migration, and cell cycle progression.** The predicted target genes of miR-16, miR-93, miR-106b, and miR-124 were identified using Ingenuity® Systems (www.ingenuity.com). PTEN Signaling and Cell Cycle Regulation by Brg Family Proteins emerged as the top ranked canonical pathway regulated during satellite cell activation. The pathways and relative expression changes occurring during satellite cell activation are depicted. Note that the miRNAs have opposite expression profiles of their respective target miRNAs. Red indicates miRNAs downregulated and green indicates miRNAs upregulated with the intensity of red or green indicating increasing or decreasing fold change, respectively. The data compare miRNAs unique to wild type satellite cells isolated 12 h post-injury to satellite cells isolated from uninjured skeletal muscle.
cells isolated from wild type uninjured TA muscles. *. CEL files can be opened with any microarray analysis software including the Affymetrix Expression Console Software (http://www.affymetrix.com/estore/browse/level_seven_software_products_only.jsp?productID=131414&categoryID=35623&productName=Affymetrix-Expression-Console-Software#1_1).

Additional file 2: CEL files of wild type satellite cells isolated 12 h post-injury. Raw expression data for three replicates of satellite cells isolated from wild type TA muscles 12 h post-injury. *. CEL files can be opened with any microarray analysis software including the Affymetrix Expression Console Software (http://www.affymetrix.com/estore/browse/level_seven_software_products_only.jsp?productID=131414&categoryID=35623&productName=Affymetrix-Expression-Console-Software#1_1).

Additional file 3: CEL files of wild type satellite cells isolated 48 h post-injury. Raw expression data for three replicates of satellite cells isolated from uninjured muscle. Raw expression data for three replicates of satellite cells isolated from uninjured TA muscles 12 h post-injury. *. CEL files can be opened with any microarray analysis software including the Affymetrix Expression Console Software (http://www.affymetrix.com/estore/browse/level_seven_software_products_only.jsp?productID=131414&categoryID=35623&productName=Affymetrix-Expression-Console-Software#1_1).

Additional file 4: CEL files of syndecan-4−/− satellite cells isolated from uninjured muscle. Raw expression data for three replicates of satellite cells isolated from syndecan-4−/− uninjured TA muscles. *. CEL files can be opened with any microarray analysis software including the Affymetrix Expression Console Software (http://www.affymetrix.com/estore/browse/level_seven_software_products_only.jsp?productID=131414&categoryID=35623&productName=Affymetrix-Expression-Console-Software#1_1).

Additional file 5: CEL files of syndecan-4−/− satellite cells isolated 12 h post-injury. Raw expression data for three replicates of satellite cells isolated from syndecan-4−/− TA muscles 12 h post-injury. *. CEL files can be opened with any microarray analysis software including the Affymetrix Expression Console Software (http://www.affymetrix.com/estore/browse/level_seven_software_products_only.jsp?productID=131414&categoryID=35623&productName=Affymetrix-Expression-Console-Software#1_1).

Additional file 6: CEL files of syndecan-4−/− satellite cells isolated 48 h post-injury. Raw expression data for three replicates of satellite cells isolated from syndecan-4−/− TA muscles 12 h post-injury. *. CEL files can be opened with any microarray analysis software including the Affymetrix Expression Console Software (http://www.affymetrix.com/estore/browse/level_seven_software_products_only.jsp?productID=131414&categoryID=35623&productName=Affymetrix-Expression-Console-Software#1_1).

Additional file 7: Satellite cell activation gene expression profile. Excel spreadsheet (*.xlsx) with columns for probe set ID, log2 expression data, ANOVA P value, fold change, and gene ID for unique genes that significantly change (ANOVA P ≤ 0.01, ≥ two-fold change) in WT and not in Sdc4−/− satellite cells 12 h after BaCl2-induced injury. The ANOVA P and fold change was calculated for changes occurring in the first 12 h post-muscle injury according to genotyope.

Additional file 8: Molecular Function: RNA Binding genes regulated during satellite cell activation. Excel spreadsheet (*.xlsx) of RNA binding proteins from Additional file 7 with gene identifiers of Probe set ID, representative gene symbol, and entrez gene ID. The relative expression data for genes that significantly change (ANOVA P ≤ 0.01, ≥ two-fold change) in wild type and in Sdc4−/− satellite cells isolated from uninjured TA muscle or from the TA 12 h post-muscle injury is represented as a log2.

Abbreviations
ARE: AU-rich element; GO: gene ontology; PI: post injury; TA: tibialis anterior; Ul: uninjured; WT: wild type.

Competing interests
The authors do not have any competing interests.

Authors’ contributions
NHF carried out bioinformatic analyses of the microarray data, designed and carried out the miRNA studies, and drafted the manuscript. MH carried out qRT-PCR for mRNA expression and many of the bioinformatic analyses of the microarray data. NDB carried out qRT-PCR for mRNA expression. CP carried out qRT-PCR for mRNA expression. DS carried out miRNA prediction. DDWC carried out satellite cell isolation and hybridization for the microarray. BB conceived of the study, participated in its design and coordination, and helped to draft the manuscript. All authors read and approved the final manuscript.

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References
1. Lepper C, Partridge TA, Fan C-M: An absolute requirement for Pax7-positive satellite cells in acute injury-induced skeletal muscle regeneration. Development 2011, 138:3639–3646.
2. Murphy MM, Lawson JA, Mathew SJ, Hutcherson DA, Kardon G: Satellite cells, connective tissue fibroblasts and their interactions are crucial for muscle regeneration. Development 2011, 138:3625–3637.
3. Sambasivan R, Yao R, Kissenpfennig A, Van Wittenberghe L, Paldi A, Gayraud-Morel B, Guenot H, Malissen B, Tajbakhsh S, Galy A: Pax7-expressing satellite cells are indispensable for adult skeletal muscle regeneration. Development 2011, 138:3647–3656.
4. Allen RE, Sheehan SM, Taylor KS, Kendall RL, Rice GM: Hepatocyte growth factor activator quenches skeletal muscle satellite cells in vitro. J Cell Physiol 1995, 165:307–312.
5. Tatsunami R, Anderson JE, Nevoret CJ, Haley O, Allen RE: HGF/SF is present in normal adult skeletal muscle and is capable of activating satellite cells. Dev Biol 1998, 194:114–128.
6. Anderson JE: A role for nitric oxide in muscle repair: nitric oxide-mediated activation of muscle satellite cells. Mol Biol Cell 2000, 11:1859–1874.
7. Li YP: TNF-alpha is a mitogen in skeletal muscle. Am J Physiol Cell Physiol 2003, 285:C370–C376.
8. Jones NC, Tyner KJ, Nibarger L, Stanley HM, Cornelison DD, Fedorov YV, Olwin BB: The p38alpha/beta MAPK functions as a molecular switch to activate the quiescent satellite cell. J Cell Physiol 2005, 199:105–116.
9. Troy A, Cadwallader AB, Fedorov Y, Tyner K, Tanaka KK, Olwin BB: Coordination of satellite cell activation and self-renewal by Par-Complex-Dependent Asymmetric Activation of P38beta MAPK. Cell Stem Cell 2012, 11:541–553.
10. Zhang K, Sha J, Harter ML: Activation of Cdc6 by MyoD is associated with the expansion of quiescent myogenic satellite cells. J Cell Biol 2010, 188:39–48.
11. Kung S, Kuroda S, Le Grand F, Rudnicki MA: Asymmetric self-renewal and commitment of satellite stem cells in muscle. Cell 2007, 129:999–1010.
12. Le Grand F, Jones AE, Seale V, Scime A, Rudnicki MA: Wnt7a activates the planar cell polarity pathway to drive the symmetric expansion of satellite stem cells. Cell Stem Cell 2009, 4:535–547.
13. Mauro A: Satellite cell of skeletal muscle fibers. J Biophys Biochem Cytol 1961, 9:493–495.

14. Ishikawa H: Electron microscopic observations of satellite cells with special reference to the development of mammalian skeletal muscles. Z Annt Entwicklungsgesch 1966, 125:43–63.

15. Falsafiachina G, Francois S, Regnault B, Grzymy B, Dive V, Cumano A, Montanar D, Buckingham M: An adult tissue-specific stem cell in its niche: a prof gene profiling analysis of in vivo quiescent and activated muscle satellite cells. Stem Cell Res 2010, 4:77–91.

16. Lemons JM, Feng XJ, Bennett BD, Legesse-Miller A, Johnson EL, Raitan M, Pollina EA, Rabitz HA, Rabinowitz JD, Colier HA: Quiescent fibroblasts exhibit high metabolic activity. Proc Natl Acad Sci U S A 2011, 108:10005–14.

17. Pritsker M, Doniger TT, Kramer LC, Westcott SE, Lernias KJ: Diversification of stem cell molecular repertoire by alternative splicing. Proc Natl Acad Sci U S A 2005, 102:4490–4495.

18. Atlasi Y, Mowla SJ, Ziaee SA, Gokhale PJ, Andrews PW: Alternative polyadenylation mediates microRNA regulation of muscle stem cell function. Cell Stem Cell 2012, 10:327–338.

35. Fukuda S, Uezumi K, Ikemoto M, Masuda S, Segawa M, Tanimura N, Yamamoto H, Miyagoe-Suzuki Y, Takada S: Molecular signature of quiescent satellite cells in adult skeletal muscle. Stem Cells 2007, 25:2448–2459.

36. Echtermeyer F, Streit M, Wilcox-Adelman S, Saoncella S, Denhe F, Detmar M, Goettrick P: Delayed wound repair and impaired angiogenesis in mice lacking syndecan-4. J Clin Invest 2001, 107:859–864.

37. Cornellis DD, Wilcox-Adelman SA, Goettrick PF, Rauhala V, Rapraeger AC, Olwin BB: Essential and separable roles for Syndecan-3 and Syndecan-4 in skeletal muscle development and regeneration. PLoS Biol 2004, 18:231–233.

38. Hall JK, Banks GB, Chamberlain JS, Olwin BB: Prevention of muscle aging by myofiber-associated satellite cell transplantation. Sci Transl Med 2010, 25:87–83.

39. Cornellis DD, Filis MA, Stanley HM, Rapraeger AC, Olwin BB: Syndecan-3 and syndecan-4 specifically mark skeletal muscle satellite cells and are implicated in satellite cell maintenance and muscle regeneration. Dev Biol 2001, 239:79–94.

40. da Huang W, Sherman BT, Lempicki RA: Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 2009, 4:44–57.

41. da Huang W, Sherman BT, Lempicki RA: Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res 2009, 37:1–13.

42. Prifti E, Zucker JD, Clement K, Henegar C: FunNet: an intuitive tool for exploring transcriptional interactions. Bioinformatics 2008, 24:2653–2658.

43. Prifti E, Zucker JD, Clement K, Henegar C: Interactional and functional centrality in transcriptional co-expression networks. Bioinformatics 2010, 26:3083–3089.

44. Antonov AV, Schmidt T, Wang Y, Meyers HW: ProfCom: a web tool for profiling the complex functionality of gene groups identified from high-throughput data. Nucleic Acids Res 2008, 36:W547–W551.

45. Stark DA, Kavris RM, Siegel AL, Cornelison DD: Ephi/ephrin interactions modulate muscle satellite cell motility and patterning. Development 2011, 138:5279–5289.

46. Boutil PZ, Chawla G, Stolov P, Black DL: MicroRNAs regulate the expression of the alternative splicing factor p57 during muscle development. Genes Dev 2007, 21:71–84.

47. Smith RA, Meade K, Pickford CE, Holley RJ, Merry CL: Glycosaminoglycans as regulators of stem cell transcriptional regulation. Biochim Soc Trans 2011, 39:383–387.

48. Mourelatos Z, Dostie J, Paushkin S, Sharma A, Chamoux B, Abel L, Rappaport J, Mann M: Dreyfuss miR: a novel class of ribonucleoproteins containing numerous microRNAs. Genes Dev 2002, 16:727–728.

49. Heinrichs A: MicroRNAs get a boost. Nat Rev Mol Cell Biol 2009, 10:302.

50. Johnson SM, Grosshans H, Shingara J, Byrom M, Jarvis R, Cheng A, Laboureur E, Reinert KL, Brown D, Slack FJ: RAS is regulated by the let-7 microRNA family. Cell 2005, 120:635–647.

51. Cheung TH, Kwan YL, Hamady M, Liu X: Unraveling transcriptional control and cis-regulatory codes using the software suite GeneACT. Genome Biol 2006, 7:R97.

52. Rocheteau P, Gayraud-Morel B, Siegel-Chagherian I, Blasco MA, Taibakhsh S: A subpopulation of adult skeletal muscle stem cells retains all template DNA strands after cell division. Cell 2012, 148:112–125.

53. Kops GJ, Medema RH, Glassford J, Essers MA, Dijkers PF, Coffer PJ, Lam EW, Burgering BM: Control of cell cycle exit and entry by protein kinase B-regulated forkehead transcription factors. Mol Cell Biol 2002, 22:2025–2036.

54. Caceres JF, Stamm S, Helfman OM, Kraner AR: Regulation of alternative splicing in vivo by overexpression of antagonistic splicing factors. Science 1994, 265:1706–1709.

55. Barberan-Soler S, Medina P, Estella J, Williams J, Zahler AM: Co-regulation of alternative splicing by diverse splicing factors in Caenorhabditis elegans. Nucleic Acids Res 2011, 39:666–674.

56. Figueroa A, Cuadrado A, Fan J, Atasoy U, Muscat GE, Muñoz-Canoves P, Gorope M, Muñoz A: Role of H1 in skeletal myogenesis through coordinate regulation of muscle differentiation genes. Mol Cell Biol 2003, 23:4991–5004.

57. van der Giessen K, Di-Marco S, Clair E, Gallouzi IE: RNAi-mediated H1 depletion leads to the inhibition of muscle cell differentiation. J Biol Chem 2003, 278:47119–47128.
58. Young LE, Sanduja S, Bemis-Standoli K, Pena EA, Price RL, Dixon DA: The mRNA binding proteins HuR and tristetraprolin regulate cyclooxygenase 2 expression during colon carcinogenesis. Gastroenterology 2009, 136:1669–1679.

59. Chen F, Shyu AB, Shneider BL: Hu antigen R and tristetraprolin: counter-regulators of rat apical sodium-dependent bile acid transporter by way of effects on messenger RNA stability. Hepatology 2011, 54:1371–1379.

60. Groszer M, Erickson R, Scripture-Adams DD, Dougherty JD, Le Belle J, Zack JA, Geschwind DH, Liu X, Kornblum HI, Wu H: PTEN negatively regulates neural stem cell self-renewal by modulating G0-G1 cell cycle entry. Proc Natl Acad Sci U S A 2006, 103:111–116.

61. HuzZ, Wang H, Lee IH, Modr S, Wang X, Dj J, Mitch WE: PTEN inhibition improves muscle regeneration in mice fed a high-fat diet. Diabetes 2010, 59:1312–1320.

62. Kimura E, Hidaka K, Kida Y, Morisaki H, Shirai M, Araki K, Suzuki M, Yamamura KI, Morisaki T: Serine-arginine-rich nuclear protein Luc7L regulates myogenesis in mice. Gene 2004, 341:41–47.

63. Yang T, Adamson TE, Resnick JL, Leff S, Wevrick R, Francke U, Jenkins NA, Copeland NG, Brannan CI: A mouse model for Prader-Willi syndrome imprinting-centre mutations. Nat Genet 1998, 19:25–31.

64. Ito T, Sako K, Arimitsu N, Sekimizu K: Defective FESTA/EAF2-mediated transcriptional activation in 5-Il-deficient embryonic stem cells. Biochem Biophys Res Commun 2007, 363:603–609.

65. Yokoyama S, Ito Y, Ueno-Kudo H, Shimizu H, Uchibe K, Albini S, Mitsuoka K, Miyaki S, Kiso M, Nogai A, Hikata T, Osada T, Fukuda N, Yamauchi S, Harada D, Mezzano Y, Kasai M, Puri PL, Hayashizaki Y, Okado H, Hashimoto M, Asahara H: A systems approach reveals that the transcriptional repressor RP58. Dev Cell 2009, 17:836–848.

66. Qi Y, Hoshino M, Wada Y, Morabuchi S, Yoshimura N, Kanazawa I, Shinomiya K, Okazawa H: PQBP-1 is expressed predominantly in the central nervous system during development. Nat Neurosci 2005, 8:1277–1286.

67. Bauser D, Lee S, Nicholson G, Davies JL, Parkinson NJ, Murray LM, Gillingwater TH, Ansorge O, Davies KE, Talbot K: Alternative splicing events are a late feature of pathology in a mouse model of spinal muscular atrophy. PLoS Genet 2009, 5:e1000773.

68. Baguma-Nibasheka M, Kablar B: Altered retinal cell differentiation in the AP-3 delta mutant (Mocha) mouse. Int J Dev Neurosci 2009, 27:701–708.

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