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Gene Regulation Strategies Underlying Skeletal Muscle Atrophy in Cancer Cachexia

Geysson Javier Fernandez Garcia

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Gene Regulation Strategies Underlying Skeletal Muscle Atrophy in Cancer Cachexia

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I dedicate this dissertation to the loves of my life:
My parents, my brothers and my wife Luz Ochoa,
For all the love, affection and encouragement.

"Satisfaction of one's curiosity is one of the greatest sources of happiness in life"

Linus Pauling.

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Gene Regulation Strategies Underlying Skeletal Muscle Atrophy in Cancer Cachexia

by

Geysson Javier Fernandez Garcia

Abstract

Cancer cachexia is a syndrome characterized by the severe skeletal muscle wasting tissue; that affects more than 50% of all cancer patients and results in lower quality of life due to compromised fatigue, weakness, decreased immune function, insulin resistance and poor tolerance and response to radio and chemotherapy. Remarkably, approximately 20% of cancer-related deaths are estimated to be directly caused by cachexia. There is currently no effective targeted therapy and the main limitation lays on the traditional approaches that not deal with the inherent complexity, characterized by non-linear interactions, of gene regulatory networks (GRN). Thus, a clear identification of the components of gene regulation, and a quantitative understanding of their temporal integration to control cellular responses is fundamental for capture essential mechanistic details that will ultimately enable the development of direct therapeutic strategies for the treatment of cancer cachexia. Here, we examine genome-wide gene expression of muscle wasting under two different frameworks, using static and dynamic gene expression data. We structure this approach as follow: Chapter 1 presents a quantitative characterization of the signaling pathways and a GRN reconstruction of muscle wasting in Lewis Lung Carcinoma (LLC) tumor-bearing mice by integrating static mRNAs and microRNAs expression profiles. The results show that LLC mice reduced body weight in 20% and presented muscle and fat tissue wasting after 23 days of tumor induction. In addition, we found 1008 differential expressed mRNAs (487 up-regulated and 521 down-regulated) and eighteen deregulated miRNAs (13 up-regulated and 5 down-regulated). Our data suggest activation of the transcriptional factor NF-κB and Stat3, which have been described in the activation of atrophic gene programs. Moreover, we ident potential posttranscriptional regulation by miRNAs of three important biological process: extracellular matrix organization, cell migration and transcription factor binding. Overall our results identify a set of signaling pathways that may contribute to muscle wasting in cancer cachexia, between them extracellular matrix genes with potential regulatory mechanism mediated by miRNAs.

Chapter 2 provides further dissection of the NF-κB signaling pathway in atrophying muscle cells. Here, we examine quantitatively the genome-wide dynamic gene expression effects of the activation of NF-κB by the exposure of tumor necrosis factor – alpha (TNF-α) on skeletal muscle cells (C2C12). We characterize the regulatory strategies of gene induction and repression by measuring both mRNA transcription and degradation rates and connecting these processes via mathematical modeling. Our data points to a dominant role of transcription dynamics in the regulation of both gene induction and repression programs in response to TNF; and unveils a decrease in mRNA degradation rate as strategy for genes of late response to increase their intracellular concentrations. Furthermore, our analysis shows constitutive degradation as an intrinsic characteristic of genes that determines most of temporal ranks of gene expression profiles. Using a non-degradable form of inhibitor kappa B alpha and RelA knockout C2C12 cells we found that NF-κB is responsible for both gene induction and gene repression during muscle cell atrophy induced by TNF-α. Our fine-grained data highlights the importance of signaling dynamics in mediating the TNF-α effects on skeletal muscle cells and reveals a critical interplay between synthesis and degradation control in that regulates dynamic gene expression programs.

Estratégias de regulação de genes subjacentes a atrofia do músculo esquelético na cachexia associada ao câncer

por

Geysson Javier Fernandez Garcia

Resumo

A caquexia associada ao câncer é uma síndrome caracterizada pela grave perda de tecido musculo esquelético; que se estima que afeta mais de 50% de todos os pacientes com câncer e resulta em menor qualidade de vida devido a fadiga, fraqueza, redução da função imune, resistência à insulina e baixa tolerância e resposta à quimioterapia. Notavelmente, 20% das mortes relacionadas ao câncer são diretamente causadas pela caquexia. A principal limitação de que atualmente não há terapia direcionada, é o uso de abordagens tradicionais que não tratam a complexidade em sistemas biológicos, caracterizada por interações não-lineares de redes de regulação genética (GRN, do inglês *Gene Regulatory Networks*). Por esse motivo, ainda é necessária uma identificação dos componentes da GRN e uma compreensão quantitativa de sua integração temporal no controle das respostas celulares. Adquirir tal conhecimento é fundamental para capturar detalhes mecanicistas essenciais para direcionar estratégias terapêuticas para uma doença complexa, como a caquexia do câncer. Neste trabalho, examinamos a expressão genética do músculo esquelético em dois abordagens metodológicos diferentes: usando dados de expressão de genes estáticos e dinâmicos. Estruturamos nosso trabalho da seguinte maneira: o Capítulo 1 apresenta uma caracterização quantitativa das vias de sinalização e uma reconstrução de GRN no tecido musculo esquelético em ratos portadores de carcinoma de pulmão de Lewis (LLC, do inglês Lewis lung carcinoma) através da integração de perfis de expressão de mRNAs e miRNAs em um tempo. Os resultados mostram que os camundongos LLC reduziram o peso corporal em 20% e apresentaram perda de tecido muscular e adiposo após 23 dias de indução tumoral. Além disso, encontramos 1008 mRNAs expressos diferencialmente (487 induzidos e 521 reprimidos) e 18 miRNAs desregulados (13 induzidos e 5 reprimidos). Nossos dados sugerem a ativação do fator transcricional NF-κB e Stat3, que foram descritos na ativação de programas genéticos atróficos no musculo esquelético. Além disso, identificamos a potencial regulação pós-transcricional por miRNAs de três processos biológicos importantes: organização da matriz extracelular, migração celular e ligação do fator de transcrição. Em geral, nossos resultados identificam um conjunto de caminhos de sinalização que podem contribuir na perda

de tecido musculo esquelético na caquexia associada ao câncer, entre eles genes da matriz extracelular com potencial mecanismo regulatório mediado por miRNAs.

O Capítulo 2 fornece uma dissecção da via de sinalização de NF-κB em células musculares atrofiadas. Neste capitulo, examinamos quantitativamente os efeitos dinâmicos na expressão genética pela ativação de NF-κB após exposição ao fator de necrose tumoral alfa (TNF-α) nas células do músculo esquelético (C2C12). Caracterizamos as estratégias reguladoras de indução e repressão de genes, medindo as taxas de transcrição e degradação para cada mRNA e conectando esses processos através de modelagem matemática. Nossos dados apontam para um papel dominante da transcrição na regulação dos programas de indução e repressão de genes em resposta ao TNF; e revela uma diminuição da taxa de degradação de mRNA como estratégia para genes de resposta tardia para aumentar suas concentrações intracelulares. Além disso, nossa análise mostra a degradação constitutiva como uma característica intrínseca dos genes que determina a ordem temporal dos perfis de expressão gênica. Usando uma forma não degradável do inibidor kappa B alfa e um knockout de RelA, descobrimos que o fator NF-κB é responsável por indução de genes e repressão de genes durante a atrofia de células musculares induzida por TNF. Nossos dados destacam a importância da dinâmica de sinalização na mediação dos efeitos do TNF nas células do músculo esquelético e revela uma interação crítica entre o controle de síntese e degradação, que regula os programas dinâmicos de expressão gênica.

Contents

"The way to get good ideas is to get lots of ideas, and throw the bad ones away"

Linus Pauling

1

Genomic Profile of mRNAs and microRNAs of Skeletal Muscle Atrophy in Cancer Cachexia

1.1 Introduction

1.1.1 Skeletal Muscle

Skeletal muscles comprise approximately 40-50% of body mass and are responsible for basic functions such as locomotion, metabolism and respiration[1]. In order to allow movement, the organization of muscle cells is highly structured which enable generation and sustaining mechanical tension. Myofiber may have several centimeters in length and can contain hundreds of nuclei. The myofibers cytoplasm is filled with contractile proteins that are assembled into repetitive structures, sarcomeres, the basic contraction unit. These structures are made up of highly ordered actin and myosin filaments, as well as hundreds of regulatory proteins such as the troponin–tropomyosin complex, and scaffolding and cytoskeletal crosslinking proteins such as α-actinin, myomesin and the kinase titin [2] (**Figure 1-1**).

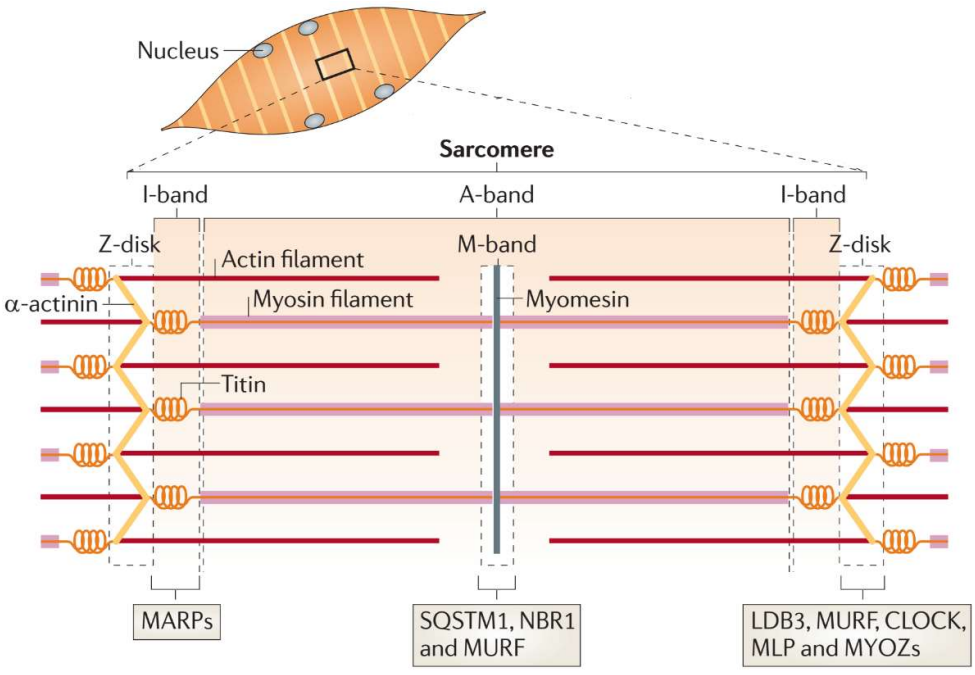

Figure 1-1 Striated muscle structure. The contractile machinery of skeletal muscle is formed by long arrays of sarcomere units. The sarcomere is constructed by interdigitating, antiparallel filaments of actin and myosin, the elastic titin filaments and the crosslinker proteins for actin, such as α actinin and myomesin. Sarcomeres contain many other accessory components, including proteins involved in transcriptional regulation and turnover control. The transcription factor CLOCK, the transcriptional cofactors muscle LIM protein (MLP), muscle ankyrin-repeat proteins (MARPs) and LIM domain-binding protein 3 (LDB3) are found at the Z-disk and/or the I-band. Multifunctional components of the protein turnover machinery include sequestosome 1 (SQSTM1), NBR1 and the muscle-upregulated RING finger proteins (MURFs). MYOZs, myozenins. [adapted from Braun, et al 2011 2].

"I received the fundamentals of my education in school, but that was not enough. My real education, the superstructure, the details, the true architecture, I got out of the public library"

Isaac Asimov

B

Bibliography

Bibliography

1. Miyazaki, M. & Esser, K. a. Cellular mechanisms regulating protein synthesis and skeletal muscle hypertrophy in animals. *J. Appl. Physiol.* **106,** 1367–73 (2009).
2. Braun, T. & Gautel, M. Transcriptional mechanisms regulating skeletal muscle differentiation, growth and homeostasis. *Nat. Rev. Mol. Cell Biol.* **12,** 349–61 (2011).
3. Lecker, S. H. *et al.* Multiple types of skeletal muscle atrophy involve a common program of changes in gene expression. *FASEB J.* **18,** 39–51 (2004).
4. Glass, D. J. Skeletal muscle hypertrophy and atrophy signaling pathways. *Int. J. Biochem. Cell Biol.* **37,** 1974–84 (2005).
5. Kandarian, S. C. & Jackman, R. W. Intracellular signaling during skeletal muscle atrophy. *Muscle Nerve* **33,** 155–65 (2006).
6. Tisdale, M. J. Cachexia in cancer patients. *Nat. Rev. cancer* **3,** 883–9 (2002).
7. Morley, J. E., Thomas, D. R. & Wilson, M. G. Cachexia: pathophysiology and clinical relevance. *Am. J. Clin. Nutr.* **83,** 735–43 (2006).
8. Donohoe, C. L., Ryan, A. M. & Reynolds, J. V. Cancer cachexia: mechanisms and clinical implications. *Gastroenterol. Res. Pract.* **2011,** 601434 (2011).
9. Tisdale, M. J. Mechanisms of cancer cachexia. *Physiol. Rev.* **89,** 381–410 (2009).
10. Wigmore, S. J., Plester, C. E., Richardson, R. a & Fearon, K. C. Changes in nutritional status associated with unresectable pancreatic cancer. *Br. J. Cancer* **75,** 106–9 (1997).
11. Banerjee, A. & Guttridge, D. C. Mechanisms for maintaining muscle. *Curr. Opin. Support. Palliat. Care* **6,** 451–6 (2012).
12. Tan, B. H. L. *et al.* P-selectin genotype is associated with the development of cancer cachexia. *EMBO Mol. Med.* **4,** 462–71 (2012).
13. Shaid, S., Brandts, C. H., Serve, H. & Dikic, I. Ubiquitination and selective autophagy. *Cell Death Differ.* **20,** 21–30 (2013).
14. Cao, P. R., Kim, H. J. & Lecker, S. H. Ubiquitin-protein ligases in muscle wasting. *Int. J. Biochem. Cell Biol.* **37,** 2088–97 (2005).
15. Bonaldo, P. & Sandri, M. Cellular and molecular mechanisms of muscle atrophy. *Dis. Model. Mech.* **6,** 25–39 (2013).
16. Csibi, A., Leibovitch, M. P., Cornille, K., Tintignac, L. a & Leibovitch, S. a. MAFbx/Atrogin-1 controls the activity of the initiation factor eIF3-f in skeletal muscle atrophy by targeting multiple C-terminal lysines. *J. Biol. Chem.* **284,** 4413–21 (2009).
17. Samuels, S. E. *et al.* Higher skeletal muscle protein synthesis and lower breakdown after chemotherapy in cachectic mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **281,** R133-9 (2001).
18. Rommel, C. *et al.* Mediation of IGF-1-induced skeletal myotube hypertrophy by

PI(3)K/Akt/mTOR and PI(3)K/Akt/GSK3 pathways. *Nat. Cell Biol.* **3,** 1009–13 (2001).

19. Bentzinger, C. F. *et al.* Skeletal muscle-specific ablation of raptor, but not of rictor, causes metabolic changes and results in muscle dystrophy. *Cell Metab.* **8,** 411–24 (2008).
20. Mammucari, C. *et al.* FoxO3 controls autophagy in skeletal muscle in vivo. *Cell Metab.* **6,** 458–71 (2007).
21. Lum, J. J., DeBerardinis, R. J. & Thompson, C. B. Autophagy in metazoans: cell survival in the land of plenty. *Nat. Rev. Mol. Cell Biol.* **6,** 439–48 (2005).
22. Zhao, J. *et al.* FoxO3 coordinately activates protein degradation by the autophagic/lysosomal and proteasomal pathways in atrophying muscle cells. *Cell Metab.* **6,** 472–83 (2007).
23. Sandri, M. *et al.* PGC-1alpha protects skeletal muscle from atrophy by suppressing FoxO3 action and atrophy-specific gene transcription. *Proc. Natl. Acad. Sci. U. S. A.* **103,** 16260–5 (2006).
24. Khalil, A. S. & Collins, J. J. Synthetic biology: applications come of age. *Nat. Rev. Genet.* **11,** 367–79 (2010).
25. Barabási, A.-L., Gulbahce, N. & Loscalzo, J. Network medicine: a network-based approach to human disease. *Nat. Rev. Genet.* **12,** 56–68 (2011).
26. Deval, C. *et al.* Identification of cathepsin L as a differentially expressed message associated with skeletal muscle wasting. *Biochem. J.* **360,** 143–50 (2001).
27. Pozhitkov, A. E., Boube, I., Brouwer, M. H. & Noble, P. a. Beyond Affymetrix arrays: expanding the set of known hybridization isotherms and observing pre-wash signal intensities. *Nucleic Acids Res.* **38,** e28 (2010).
28. Allison, D. B., Cui, X., Page, G. P. & Sabripour, M. Microarray data analysis: from disarray to consolidation and consensus. *Nat. Rev. Genet.* **7,** 55–65 (2006).
29. Monitto, C. L. *et al.* Differential gene expression in a murine model of cancer cachexia. *Am. J. Physiol. Endocrinol. Metab.* **281,** E289-97 (2001).
30. Stephens, N. a *et al.* Using transcriptomics to identify and validate novel biomarkers of human skeletal muscle cancer cachexia. *Genome Med.* **2,** 1 (2010).
31. Dahlman, I. *et al.* Adipose tissue pathways involved in weight loss of cancer cachexia. *Br. J. Cancer* **102,** 1541–8 (2010).
32. Bonetto, A. *et al.* STAT3 activation in skeletal muscle links muscle wasting and the acute phase response in cancer cachexia. *PLoS One* **6,** e22538 (2011).
33. Skorokhod, A., Bachmann, J., Giese, N. a, Martignoni, M. E. & Krakowski-Roosen, H. Real-imaging cDNA-AFLP transcript profiling of pancreatic cancer patients: Egr-1 as a potential key regulator of muscle cachexia. *BMC Cancer* **12,** 265 (2012).
34. Dwarkasing, J. T. *et al.* Hypothalamic food intake regulation in a cancer-cachectic mouse model. *J. Cachexia. Sarcopenia Muscle* (2013). doi:10.1007/s13539-013-0121-y
35. Cornwell, E. W., Mirbod, A., Wu, C.-L., Kandarian, S. C. & Jackman, R. W. C26 cancer-

induced muscle wasting is IKKβ-dependent and NF-kappaB-independent. *PLoS One* **9,** e87776 (2014).

36. Esteller, M. Non-coding RNAs in human disease. *Nat. Rev. Genet.* **12,** 861–74 (2011).
37. Wilczynska, A. & Bushell, M. The complexity of miRNA-mediated repression. *Cell Death Differ.* **22,** 22–33 (2015).
38. Hausser, J. & Zavolan, M. Identification and consequences of miRNA-target interactions-beyond repression of gene expression. *Nat. Rev. Genet.* **15,** 599–612 (2014).
39. Bartel, D. P., Lee, R. & Feinbaum, R. MicroRNAs : Genomics , Biogenesis , Mechanism , and Function Genomics : The miRNA Genes. **116,** 281–297 (2004).
40. Wang, X. H. MicroRNA in myogenesis and muscle atrophy. *Curr. Opin. Clin. Nutr. Metab. Care* **16,** 258–66 (2013).
41. McCarthy, J. J., Esser, K. A., Peterson, C. a & Dupont-Versteegden, E. E. Evidence of MyomiR network regulation of beta-myosin heavy chain gene expression during skeletal muscle atrophy. *Physiol. Genomics* **39,** 219–26 (2009).
42. Xu, J. *et al.* Transcription factor FoxO1, the dominant mediator of muscle wasting in chronic kidney disease, is inhibited by microRNA-486. *Kidney Int.* **82,** 401–11 (2012).
43. Wada, S. *et al.* Translational suppression of atrophic regulators by microRNA-23a integrates resistance to skeletal muscle atrophy. *J. Biol. Chem.* **286,** 38456–65 (2011).
44. Narasimhan, A. *et al.* Small RNAome profiling from human skeletal muscle: novel miRNAs and their targets associated with cancer cachexia. *J. Cachexia. Sarcopenia Muscle* (2017). doi:10.1002/jcsm.12168
45. Fearon, K. *et al.* Definition and classification of cancer cachexia: an international consensus. *Lancet Oncol.* **12,** 489–95 (2011).
46. Schiaffino, S. & Reggiani, C. Fiber types in mammalian skeletal muscles. *Physiol. Rev.* **91,** 1447–531 (2011).
47. Matsuyama, T. *et al.* Tumor inoculation site affects the development of cancer cachexia and muscle wasting. *Int. J. Cancer* **137,** 2558–2565 (2015).
48. Pettersen, K. *et al.* Cancer cachexia associates with a systemic autophagy-inducing activity mimicked by cancer cell-derived IL-6 trans-signaling. *Sci. Rep.* **7,** 1–16 (2017).
49. Guo, D., Wang, C., Wang, Q., Qiao, Z. & Tang, H. Pantoprazole blocks the JAK2/STAT3 pathway to alleviate skeletal muscle wasting in cancer cachexia by inhibiting inflammatory response. *Oncotarget* **8,** 39640–39648 (2017).
50. Bonetto, A. *et al.* JAK/STAT3 pathway inhibition blocks skeletal muscle wasting downstream of IL-6 and in experimental cancer cachexia. *Am. J. Physiol. Endocrinol. Metab.* **303,** E410-21 (2012).
51. Bonetto, A. *et al.* Early changes of muscle insulin-like growth factor-1 and myostatin gene expression in gastric cancer patients. *Muscle Nerve* **48,** 387–392 (2013).

52. D'Orlando, C. *et al.* Gastric cancer does not affect the expression of atrophy-related genes in human skeletal muscle. *Muscle Nerve* **39,** 1–22 (2013).
53. DeBoer, M. D. Animal models of anorexia and cachexia. *Expert Opin. Drug Discov.* **4,** 1145–1155 (2009).
54. Bennani-Baiti, N. & Walsh, D. Animal models of the cancer anorexia-cachexia syndrome. *Support. Care Cancer* **19,** 1451–1463 (2011).
55. Ballarò, R., Costelli, P. & Penna, F. Animal models for cancer cachexia. *Curr. Opin. Support. Palliat. Care* **10,** 281–287 (2016).
56. Penna, F., Busquets, S. & Argilés, J. M. Experimental cancer cachexia: Evolving strategies for getting closer to the human scenario. *Semin. Cell Dev. Biol.* **54,** 20–27 (2016).
57. Li, J. J., Bickel, P. J. & Biggin, M. D. System wide analyses have underestimated protein abundances and the importance of transcription in mammals. *PeerJ* **2,** e270 (2014).
58. Gomes, M. D., Lecker, S. H., Jagoe, R. T., Navon, A. & Goldberg, A. L. Atrogin-1, a muscle-specific F-box protein highly expressed during muscle atrophy. *Proc. Natl. Acad. Sci. U. S. A.* **98,** 14440–5 (2001).
59. Bodine, S. C. *et al.* Identification of ubiquitin ligases required for skeletal Muscle Atrophy. *Science (80-.).* **294,** 1704–1708 (2001).
60. Bodine, S. C. & Baehr, L. M. Skeletal muscle atrophy and the E3 ubiquitin ligases MuRF1 and MAFbx/atrogin-1. *AJP Endocrinol. Metab.* **307,** E469–E484 (2014).
61. Wang, Z., Liu, P., Inuzuka, H. & Wei, W. Roles of F-box proteins in cancer. *Nat. Rev. Cancer* **14,** 233–247 (2014).
62. Sukari, A., Muqbil, I., Mohammad, R. M., Philip, P. A. & Azmi, A. S. F-BOX proteins in cancer cachexia and muscle wasting: Emerging regulators and therapeutic opportunities. *Semin. Cancer Biol.* **36,** 95–104 (2016).
63. Li, Y.-P. *et al.* TNF-alpha increases ubiquitin-conjugating activity in skeletal muscle by up-regulating UbcH2/E220k. *FASEB J.* **17,** 1048–1057 (2003).
64. Gordon, B. S., Steiner, J. L., Lang, C. H., Jefferson, L. S. & Kimball, S. R. Reduced REDD1 expression contributes to activation of mTORC1 following electrically induced muscle contraction. *AJP Endocrinol. Metab.* **307,** E703–E711 (2014).
65. Gordon, B. S. *et al.* Loss of REDD1 augments the rate of the overload-induced increase in muscle mass. *Am. J. Physiol. - Regul. Integr. Comp. Physiol.* **311,** R545–R557 (2016).
66. Bodine, S. C. *et al.* Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. *Nat. Cell Biol.* **3,** 1014–1019 (2001).
67. Sullivan-Gunn, M. J. & Lewandowski, P. A. Elevated hydrogen peroxide and decreased catalase and glutathione peroxidase protection are associated with aging sarcopenia. *BMC Geriatr.* **13,** 1–9 (2013).
68. Nuoc, T. N. *et al.* The analysis of antioxidant expression during muscle atrophy induced by

hindlimb suspension in mice. *J. Physiol. Sci.* **67,** 121–129 (2017).

69. Deruisseau, L. R. *et al.* Muscle contractile dysfunction and oxidative injury are observed in the soleus muscle of metallothionein deficient mice following acute spinal cord injury. *FASEB J.* **23,** 1795–1802 (2009).
70. Summermatter, S. *et al.* Blockade of Metallothioneins 1 and 2 Increases Skeletal Muscle Mass and Strength. *Mol. Cell. Biol.* **37,** 1–11 (2017).
71. COPENHAVER, W. M., MOORE, D. H. & RUSKA, H. Electron microscopic and histochemical observations of muscle degeneration after tourniquet. *J. Biophys. Biochem. Cytol.* **2,** 755–64 (1956).
72. Pellegrino, C. & Franzini, C. AN ELECTRON MICROSCOPE STUDY OF DENERVATION ATROPHY IN RED AND WHITE SKELETAL MUSCLE FIBERS. *J. Cell Biol.* **17,** 327–49 (1963).
73. He, W. A. *et al.* NF-κB-mediated Pax7 dysregulation in the muscle microenvironment promotes cancer cachexia. *J. Clin. Invest.* **123,** 4821–4835 (2013).
74. Liu, X., Lee, D. J., Skittone, L. K., Natsuhara, K. & Kim, H. T. Role of gelatinases in disuse-induced skeletal muscle atrophy. *Muscle and Nerve* **41,** 174–178 (2010).
75. Mehan, R. S., Greybeck, B. J., Emmons, K., Byrnes, W. C. & Allen, D. L. Matrix metalloproteinase-9 deficiency results in decreased fiber cross-sectional area and alters fiber type distribution in mouse hindlimb skeletal muscle. *Cells. Tissues. Organs* **194,** 510–20 (2011).
76. Chen, C.-C. *et al.* FoxOs inhibit mTORC1 and activate Akt by inducing the expression of Sestrin3 and Rictor. *Dev. Cell* **18,** 592–604 (2010).
77. Chung, S. Y. *et al.* FoxO6 and PGC-1α form a regulatory loop in myogenic cells. *Biosci. Rep.* **33,** 485–497 (2013).
78. Kamei, Y. *et al.* Skeletal muscle FOXO1 (FKHR) transgenic mice have less skeletal muscle mass, down-regulated Type I (slow twitch/red muscle) fiber genes, and impaired glycemic control. *J. Biol. Chem.* **279,** 41114–23 (2004).
79. Mammucari, C. *et al.* FoxO3 Controls Autophagy in Skeletal Muscle In Vivo. *Cell Metab.* **6,** 458–471 (2007).
80. Sandri, M. *et al.* Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle atrophy. *Cell* **117,** 399–412 (2004).
81. Kamei, Y. *et al.* Skeletal muscle FOXO1 (FKHR) transgenic mice have less skeletal muscle mass, down-regulated type I (slow twitch/red muscle) fiber genes, and impaired glycemic control. *J. Biol. Chem.* **279,** 41114–41123 (2004).
82. Haddad, F., Zaldivar, F., Cooper, D. M. & Adams, G. R. IL-6-induced skeletal muscle atrophy. *J. Appl. Physiol.* **98,** 911–7 (2005).
83. Cai, D. *et al.* IKKbeta/NF-kappaB activation causes severe muscle wasting in mice. *Cell* **119,**

285–98 (2004).

84. Moore-Carrasco, R. *et al.* The AP-1/CJUN signaling cascade is involved in muscle differentiation: Implications in muscle wasting during cancer cachexia. *FEBS Lett.* **580,** 691–696 (2006).

85. Choi, M.-C. *et al.* A direct HDAC4-MAP kinase crosstalk activates muscle atrophy program. *Mol. Cell* **47,** 122–32 (2012).

86. Gill, R. M. & Hamel, P. A. Subcellular compartmentalization of E2F family members is required for maintenance of the postmitotic state in terminally differentiated muscle. *J. Cell Biol.* **148,** 1187–201 (2000).

87. Sun, K., Lu, L., Wang, H. & Sun, H. Genome-wide profiling of YY1 binding sites during skeletal myogenesis. *Genomics Data* **2,** 89–91 (2014).

88. Xiong, G. *et al.* The PERK arm of the unfolded protein response regulates satellite cell-mediated skeletal muscle regeneration. *Elife* **6,** 1–27 (2017).

89. Sartori, R. *et al.* Smad2 and 3 transcription factors control muscle mass in adulthood. *Am. J. Physiol. Cell Physiol.* **296,** C1248-57 (2009).

90. Wang, H. *et al.* NF-κB-YY1-miR-29 Regulatory Circuitry in Skeletal Myogenesis and Rhabdomyosarcoma. *Cancer Cell* **14,** 369–381 (2008).

91. Guttridge, D. C. NF-kappa B-Induced Loss of MyoD Messenger RNA: Possible Role in Muscle Decay and Cachexia. *Science (80-.).* **289,** 2363–2366 (2000).

92. MacPherson, P. C. D., Wang, X. & Goldman, D. Myogenin regulates denervation-dependent muscle atrophy in mouse soleus muscle. *J. Cell. Biochem.* **112,** 2149–2159 (2011).

93. Moresi, V. *et al.* Myogenin and class II HDACs control neurogenic muscle atrophy by inducing E3 ubiquitin ligases. *Cell* **143,** 35–45 (2010).

94. Hu, J. S., Olson, E. N. & Kingston, R. E. HEB, a helix-loop-helix protein related to E2A and ITF2 that can modulate the DNA-binding ability of myogenic regulatory factors. *Mol. Cell. Biol.* **12,** 1031–1042 (1992).

95. Parker, M. H., Perry, R. L. S., Fauteux, M. C., Berkes, C. A. & Rudnicki, M. A. MyoD Synergizes with the E-Protein HEB To Induce Myogenic Differentiation. *Mol. Cell. Biol.* **26,** 5771–5783 (2006).

96. Berkes, C. A. *et al.* Pbx marks genes for activation by MyoD indicating a role for a homeodomain protein in establishing myogenic potential. *Mol. Cell* **14,** 465–477 (2004).

97. Dell'Orso, S. *et al.* The Histone Variant MacroH2A1.2 Is Necessary for the Activation of Muscle Enhancers and Recruitment of the Transcription Factor Pbx1. *Cell Rep.* **14,** 1156–1168 (2016).

98. Watanabe, S., Kondo, S., Hayasaka, M. & Hanaoka, K. Functional analysis of homeodomain-containing transcription factor Lbx1 in satellite cells of mouse skeletal muscle. *J. Cell Sci.* **120,** 4178–87 (2007).

99. Rossi, G. *et al.* Nfix Regulates Temporal Progression of Muscle Regeneration through Modulation of Myostatin Expression. *Cell Rep.* **14,** 2238–2249 (2016).
100. Rossi, G. *et al.* Silencing Nfix rescues muscular dystrophy by delaying muscle regeneration. *Nat. Commun.* **8,** 1–12 (2017).
101. Raichur, S. *et al.* Identification and validation of the pathways and functions regulated by the orphan nuclear receptor, ROR alpha1, in skeletal muscle. *Nucleic Acids Res.* **38,** 4296–312 (2010).
102. Chen, W., Zhang, X., Birsoy, K. & Roeder, R. G. A muscle-specific knockout implicates nuclear receptor coactivator MED1 in the regulation of glucose and energy metabolism. *Proc. Natl. Acad. Sci. U. S. A.* **107,** 10196–201 (2010).
103. Grifone, R. *et al.* Six1 and Eya1 expression can reprogram adult muscle from the slow-twitch phenotype into the fast-twitch phenotype. *Mol. Cell. Biol.* **24,** 6253–6267 (2004).
104. Relaix, F. *et al.* Six Homeoproteins Directly Activate Myod Expression in the Gene Regulatory Networks That Control Early Myogenesis. *PLoS Genet.* **9,** (2013).
105. Southard, S., Kim, J. R., Low, S. H., Tsika, R. W. & Lepper, C. Myofiber-specific TEAD1 overexpression drives satellite cell hyperplasia and counters pathological effects of dystrophin deficiency. *Elife* **5,** 1–28 (2016).
106. Tsika, R. W. *et al.* Overexpression of TEAD-1 in transgenic mouse striated muscles produces a slower skeletal muscle contractile phenotype. *J. Biol. Chem.* **283,** 36154–36167 (2008).
107. Huraskin, D. *et al.* Wnt/β-catenin signaling via Axin2 is required for myogenesis and, together with YAP/Taz and Tead1, active in IIa/IIx muscle fibers. *Development* **143,** 3128–3142 (2016).
108. Pardo, P. S., Mohamed, J. S., Lopez, M. A. & Boriek, A. M. Induction of Sirt1 by mechanical stretch of skeletal muscle through the early response factor EGR1 triggers an antioxidative response. *J. Biol. Chem.* **286,** 2559–2566 (2011).
109. Himeda, C. L., Ranish, J. A., Pearson, R. C. M., Crossley, M. & Hauschka, S. D. KLF3 Regulates Muscle-Specific Gene Expression and Synergizes with Serum Response Factor on KLF Binding Sites. *Mol. Cell. Biol.* **30,** 3430–3443 (2010).
110. Pierce, A. *et al.* A Novel mouse model of enhanced proteostasis: Full-length human heat shock factor 1 transgenic mice. *Biochem. Biophys. Res. Commun.* **402,** 59–65 (2010).
111. Etard, C. *et al.* Loss of function of myosin chaperones triggers Hsf1-mediated transcriptional response in skeletal muscle cells. *Genome Biol.* **16,** 1–20 (2015).
112. Yokoyama, S. *et al.* Heat shock transcription factor 1-associated expression of slow myosin heavy chain in mouse soleus muscle in response to unloading with or without reloading. *Acta Physiol.* **217,** 325–337 (2016).
113. Talbert, E. E. & Guttridge, D. C. Impaired regeneration: A role for the muscle microenvironment in cancer cachexia. *Semin. Cell Dev. Biol.* **54,** 82–91 (2016).
114. Krützfeldt, J. *et al.* Silencing of microRNAs in vivo with 'antagomirs'. *Nature* **438,** 685–9

(2005).

115. Li, J. *et al.* MiR-29b contributes to multiple types of muscle atrophy. *Nat. Commun.* **8,** 1–15 (2017).
116. Moraes, L. N. *et al.* Integration of miRNA and mRNA expression profiles reveals microRNA-regulated networks during muscle wasting in cardiac cachexia. *Sci. Rep.* **7,** 6998 (2017).
117. Eisenberg, I. *et al.* Distinctive patterns of microRNA expression in primary muscular disorders. *Proc. Natl. Acad. Sci. U. S. A.* **104,** 17016–21 (2007).
118. Soares, R. J. *et al.* Involvement of microRNAs in the regulation of muscle wasting during catabolic conditions. *J. Biol. Chem.* **289,** 21909–25 (2014).
119. Shen, H. *et al.* Identification of microRNAs involved in dexamethasone-induced muscle atrophy. *Mol. Cell. Biochem.* **381,** 105–13 (2013).
120. Morozova, N. *et al.* Kinetic signatures of microRNA modes of action. *RNA* **18,** 1635–55 (2012).
121. Ge, Y. *et al.* MicroRNA-350 induces pathological heart hypertrophy by repressing both p38 and JNK pathways. *Biochim. Biophys. Acta - Mol. Basis Dis.* **1832,** 1–10 (2013).
122. Zhou, L. *et al.* A novel target of microRNA-29, Ring1 and YY1-binding protein (Rybp), negatively regulates skeletal myogenesis. *J. Biol. Chem.* **287,** 25255–25265 (2012).
123. Zhou, L. *et al.* Inhibition of miR-29 by TGF-beta-Smad3 signaling through dual mechanisms promotes transdifferentiation of mouse myoblasts into myofibroblasts. *PLoS One* **7,** e33766 (2012).
124. Wang, L. *et al.* Loss of miR-29 in Myoblasts Contributes to Dystrophic Muscle Pathogenesis. *Mol. Ther.* **20,** 1222–1233 (2012).
125. Jiang, X., Tsitsiou, E., Herrick, S. E. & Lindsay, M. A. MicroRNAs and the regulation of fibrosis. *FEBS J.* **277,** 2015–21 (2010).
126. van Rooij, E. *et al.* Dysregulation of microRNAs after myocardial infarction reveals a role of miR-29 in cardiac fibrosis. *Proc. Natl. Acad. Sci. U. S. A.* **105,** 13027–32 (2008).
127. Maurer, B. *et al.* MicroRNA-29, a key regulator of collagen expression in systemic sclerosis. *Arthritis Rheum.* **62,** 1733–43 (2010).
128. Cushing, L. *et al.* miR-29 is a major regulator of genes associated with pulmonary fibrosis. *Am. J. Respir. Cell Mol. Biol.* **45,** 287–294 (2011).
129. Roderburg, C. *et al.* Micro-RNA profiling reveals a role for miR-29 in human and murine liver fibrosis. *Hepatology* **53,** 209–18 (2011).
130. Cacchiarelli, D. *et al.* MicroRNAs involved in molecular circuitries relevant for the Duchenne muscular dystrophy pathogenesis are controlled by the dystrophin/nNOS pathway. *Cell Metab.* **12,** 341–51 (2010).
131. Wang, B. *et al.* Suppression of microRNA-29 expression by TGF-β1 promotes collagen expression and renal fibrosis. *J. Am. Soc. Nephrol.* **23,** 252–65 (2012).

132. O'Reilly, S. MicroRNAs in fibrosis: opportunities and challenges. *Arthritis Res. Ther.* **18,** 11 (2016).
133. Galimov, A. *et al.* microRNA-29a in adult muscle stem cells controls skeletal muscle regeneration during injury and exercise downstream of fibroblast growth factor-2. *Stem Cells* n/a-n/a (2016). doi:10.1002/stem.2281
134. Franco, F. de O. *et al.* Cancer cachexia differentially regulates visceral adipose tissue turnover. *J. Endocrinol.* **232,** 493–500 (2017).
135. Braun, T. & Gautel, M. Transcriptional mechanisms regulating skeletal muscle differentiation, growth and homeostasis. *Nat. Rev. Mol. Cell Biol.* **12,** 349–61 (2011).
136. Zugmaier, G. *et al.* Transforming growth factor β1 induces cachexia and systemic fibrosis without an antitumor effect in nude mice. *Cancer Res.* **51,** 3590–3594 (1991).
137. Tsai, V. W. W. *et al.* Anorexia/cachexia of chronic diseases: a role for the TGF-β family cytokine MIC-1/GDF15. *J. Cachexia. Sarcopenia Muscle* **3,** 239–43 (2012).
138. Mendias, C. L. *et al.* Transforming growth factor-beta induces skeletal muscle atrophy and fibrosis through the induction of atrogin-1 and scleraxis. *Muscle Nerve* **45,** 55–59 (2012).
139. Devine, R. D., Bicer, S., Reiser, P. J., Velten, M. & Wold, L. E. Metalloproteinase expression is altered in cardiac and skeletal muscle in cancer cachexia. *Am. J. Physiol. - Hear. Circ. Physiol.* **309,** H685–H691 (2015).
140. Judge, S. M. *et al.* Genome-wide identification of FoxO-dependent gene networks in skeletal muscle during C26 cancer cachexia. *BMC Cancer* **14,** 997 (2014).
141. Calura, E. *et al.* Meta-analysis of expression signatures of muscle atrophy: gene interaction networks in early and late stages. *BMC Genomics* **9,** 630 (2008).
142. Pfaffl, M. W. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res.* **29,** e45 (2001).
143. Pfaffl, M. W., Horgan, G. W. & Dempfle, L. Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. *Nucleic Acids Res.* **30,** e36 (2002).
144. Ruijter, J. M. *et al.* Amplification efficiency: linking baseline and bias in the analysis of quantitative PCR data. *Nucleic Acids Res.* **37,** e45 (2009).
145. Kim, D. *et al.* TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. *Genome Biol.* **14,** R36 (2013).
146. Langmead, B., Trapnell, C., Pop, M. & Salzberg, S. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol.* **10,** R25 (2009).
147. Langmead, B. & Salzberg, S. L. Fast gapped-read alignment with Bowtie 2. *Nat. Methods* **9,** 357–359 (2012).
148. Anders, S., Pyl, P. T. & Huber, W. HTSeq-A Python framework to work with high-throughput sequencing data. *Bioinformatics* **31,** 166–169 (2015).

149. Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* **15,** 550 (2014).
150. Wang, X. miRDB: a microRNA target prediction and functional annotation database with a wiki interface. *RNA* **14,** 1012–7 (2008).
151. Garcia, D. M. *et al.* Weak seed-pairing stability and high target-site abundance decrease the proficiency of lsy-6 and other microRNAs. *Nat. Struct. Mol. Biol.* **18,** 1139–46 (2011).
152. Maragkakis, M. *et al.* DIANA-microT web server: elucidating microRNA functions through target prediction. *Nucleic Acids Res.* **37,** W273-6 (2009).
153. Krek, A. *et al.* Combinatorial microRNA target predictions. *Nat. Genet.* **37,** 495–500 (2005).
154. Hsu, S.-D. *et al.* miRTarBase: a database curates experimentally validated microRNA-target interactions. *Nucleic Acids Res.* **39,** D163-9 (2011).
155. Maere, S., Heymans, K. & Kuiper, M. BiNGO: a Cytoscape plugin to assess overrepresentation of gene ontology categories in biological networks. *Bioinformatics* **21,** 3448–9 (2005).
156. Bindea, G. *et al.* ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. *Bioinformatics* **25,** 1091–3 (2009).
157. Cline, M. S. *et al.* Integration of biological networks and gene expression data using Cytoscape. *Nat. Protoc.* **2,** 2366–82 (2007).
158. Gelin, J. *et al.* Role of endogenous tumor necrosis factor alpha and interleukin 1 for experimental tumor growth and the development of cancer cachexia. *Cancer Res* **51,** 415–421 (1991).
159. Cahlin, C. *et al.* Experimental cancer cachexia: the role of host-derived cytokines interleukin (IL)-6, IL-12, interferon-gamma, and tumor necrosis factor alpha evaluated in gene knockout, tumor-bearing mice on C57 Bl background and eicosanoid-dependent cachexia. *Cancer Res.* **60,** 5488–93 (2000).
160. Guttridge, D. C. NF-kappa B-Induced Loss of MyoD mRNA: possible role in muscle cecay and cachexia. *Science (80-.).* **289,** 2363–2366 (2000).
161. Seto, D. N., Kandarian, S. C. & Jackman, R. W. A Key Role for Leukemia Inhibitory Factor in C26 Cancer Cachexia. *J. Biol. Chem.* **290,** 19976–19986 (2015).
162. Darling, G., Fraker, D. L., Jensen, J. C., Gorschboth, C. M. & Norton, J. A. Cachectic effects of recombinant human tumor necrosis factor in rats. *Cancer Res.* **50,** 4008–4013 (1990).
163. Balkwill, F. *et al.* Evidence for Tumour Necrosis Factor/Cachectin Production in Cancer. *Lancet* **330,** 1229–1232 (1987).
164. Li, Y.-P. *et al.* TNF-alpha acts via p38 MAPK to stimulate expression of the ubiquitin ligase atrogin1/MAFbx in skeletal muscle. *FASEB J.* **19,** 362–70 (2005).
165. Di Marco, S. *et al.* NF-kappa B-mediated MyoD decay during muscle wasting requires nitric oxide synthase mRNA stabilization, HuR protein, and nitric oxide release. *Mol. Cell. Biol.* **25,** 6533–45 (2005).

166. Williams, G., Brown, T., Becker, L., Prager, M. & Giroir, B. P. Cytokine-induced expression of nitric oxide synthase in C2C12 skeletal muscle myocytes. *Am. J. Physiol.* **267,** R1020-5 (1994).
167. Buck, M. & Chojkier, M. Muscle wasting and dedifferentiation induced by oxidative stress in a murine model of cachexia is prevented by inhibitors of nitric oxide synthesis and antioxidants. *EMBO J.* **15,** 1753–65 (1996).
168. Rodriguez-Pascual, F. *et al.* Complex contribution of the 3'-untranslated region to the expressional regulation of the human inducible nitric-oxide synthase gene: Involvement of the RNA-binding protein HuR. *J. Biol. Chem.* **275,** 26040–26049 (2000).
169. Hall, D. T., Ma, J. F., Marco, S. Di & Gallouzi, I.-E. Inducible nitric oxide synthase (iNOS) in muscle wasting syndrome, sarcopenia, and cachexia. *Aging (Albany. NY).* **3,** 702–15 (2011).
170. Bhatnagar, S. *et al.* Tumor necrosis factor-α regulates distinct molecular pathways and gene networks in cultured skeletal muscle cells. *PLoS One* **5,** e13262 (2010).
171. Meyer, S. U. *et al.* Tumor necrosis factor alpha and insulin-like growth factor 1 induced modifications of the gene expression kinetics of differentiating skeletal muscle cells. *PLoS One* **10,** 1–36 (2015).
172. Core, L. J., Waterfall, J. J. & Lis, J. T. Nascent RNA sequencing reveals widespread pausing and divergent initiation at human promoters. *Science (80-.).* **322,** 1845–1848 (2008).
173. Licatalosi, D. D. *et al.* HITS-CLIP yields genome-wide insights into brain alternative RNA processing. *Nature* **456,** 464–9 (2008).
174. Licatalosi, D. D. & Darnell, R. B. RNA processing and its regulation: global insights into biological networks. *Nat. Rev. Genet.* **11,** 75–87 (2010).
175. Rabani, M. *et al.* High-resolution sequencing and modeling identifies distinct dynamic RNA regulatory strategies. *Cell* **159,** 1698–1710 (2014).
176. Eser, P. *et al.* Determinants of RNA metabolism in the Schizosaccharomyces pombe genome. *Mol. Syst. Biol.* **12,** 857 (2016).
177. Miller, C. *et al.* Dynamic transcriptome analysis measures rates of mRNA synthesis and decay in yeast. *Mol. Syst. Biol.* **7,** 458 (2011).
178. García-Martínez, J., Aranda, A. & Pérez-Ortín, J. E. Genomic run-on evaluates transcription rates for all yeast genes and identifies gene regulatory mechanisms. *Mol. Cell* **15,** 303–313 (2004).
179. Grigull, J. *et al.* Genome-Wide Analysis of mRNA Stability Using Transcription Inhibitors and Microarrays Reveals Posttranscriptional Control of Ribosome Biogenesis Factors Genome-Wide Analysis of mRNA Stability Using Transcription Inhibitors and Microarrays Reveals Posttran. *Mol. Cell. Biol.* **24,** 5534–5547 (2004).
180. Wang, Y. *et al.* Precision and functional specificity in mRNA decay. *Proc. Natl. Acad. Sci. U. S. A.* **99,** 5860–5865 (2002).

181. Wuarin, J. & Schibler, U. Physical isolation of nascent RNA chains transcribed by RNA polymerase II: evidence for cotranscriptional splicing. *Mol Cell Biol* **14,** 7219–7225 (1994).
182. Pandya-Jones, A. & Black, D. L. Co-transcriptional splicing of constitutive and alternative exons. *Rna* **15,** 1896–1908 (2009).
183. Bhatt, D. M. *et al.* Transcript dynamics of proinflammatory genes revealed by sequence analysis of subcellular RNA fractions. *Cell* **150,** 279–290 (2012).
184. Tong, A. J. *et al.* A Stringent Systems Approach Uncovers Gene-Specific Mechanisms Regulating Inflammation. *Cell* **165,** 165–179 (2016).
185. Lee, J. E., Lee, J. Y., Wilusz, J., Tian, B. & Wilusz, C. J. Systematic analysis of Cis-elements in unstable mRNAS demonstrates that CUGBP1 is a key regulator of mRNA decay in muscle cells. *PLoS One* **5,** (2010).
186. Figueroa, A. *et al.* Role of HuR in skeletal myogenesis through coordinate regulation of muscle differentiation genes. *Mol. Cell. Biol.* **23,** 4991–5004 (2003).
187. Zeisel, A. *et al.* Coupled pre-mRNA and mRNA dynamics unveil operational strategies underlying transcriptional responses to stimuli. *Mol. Syst. Biol.* **7,** 529 (2011).
188. Rabani, M. *et al.* Metabolic labeling of RNA uncovers principles of RNA production and degradation dynamics in mammalian cells. *Nat. Biotechnol.* **29,** 436–442 (2011).
189. Burrage, P. S., Mix, K. S. & Brinckerhoff, C. E. Matrix metalloproteinases: role in arthritis. *Front. Biosci.* **11,** 529–43 (2006).
190. Sanjana, N. E., Shalem, O. & Zhang, F. Improved vectors and genome-wide libraries for CRISPR screening. *Nat. Methods* **11,** 783–784 (2014).
191. Dogra, C. *et al.* TNF-related weak inducer of apoptosis (TWEAK) is a potent skeletal muscle-wasting cytokine. *FASEB J.* **21,** 1857–69 (2007).
192. Dobin, A. & Gingeras, T. R. Mapping RNA-seq Reads with STAR. *Curr. Protoc. Bioinforma.* **51,** 11.14.1-11.14.19 (2015).
193. Liao, Y., Smyth, G. K. & Shi, W. FeatureCounts: An efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* **30,** 923–930 (2014).
194. Robinson, J. T. *et al.* Integrative genomics viewer. *Nat. Biotechnol.* **29,** 24–6 (2011).
195. Schwalb, B. *et al.* Measurement of genome-wide RNA synthesis and decay rates with Dynamic Transcriptome Analysis (DTA). *Bioinformatics* **28,** 884–5 (2012).
196. Chechik, G. & Koller, D. Timing of Gene Expression Responses to Environmental Changes. *J. Comput. Biol.* **16,** 279–290 (2009).