Cullin3-RING ubiquitin ligase activity is required for striated muscle function in mice

James B. Papizan, Alexander H. Vidal, Svetlana Bezprozvannaya, Rhonda Bassel-Duby, and Eric N. Olson*

Department of Molecular Biology, Hamon Center for Regenerative Science and Medicine, Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Center, University of Texas Southwestern Medical Center, Dallas, TX, 75390.

Running title: Cul3 is required for muscle function

*Address correspondence to: Eric N. Olson, Department of Molecular Biology, 5323 Harry Hines Blvd, Dallas, TX, 75390-9148. Phone 214-648-1187, Fax 214-648-1196 Email: Eric.olson@utsouthwestern.edu

Keywords: E3 ubiquitin ligase, proteostasis, cardiomyopathy, cardiac metabolism, skeletal muscle, cullin3, myopathy, protein degradation, muscle damage

Abstract
Control of protein homeostasis is an essential cellular process that, when perturbed, can result in the deregulation or toxic accumulation of proteins. Owing to constant mechanical stress, striated muscle proteins are particularly prone to wear and tear and require several protein quality–control mechanisms to coordinate protein turnover and removal of damaged proteins. Kelch-like proteins, substrate adapters for the Cullin3(Cul3)-RING ligase (CRL3) complex, are emerging as critical regulators of striated muscle development and function, highlighting the importance of Cul3-mediated proteostasis in muscle function. To explore the role of Cul3-mediated proteostasis in striated muscle, here we deleted Cul3 specifically in either skeletal muscle (SkM-Cul3 KO) or cardiomyocytes (CM-Cul3 KO) of mice. The loss of Cul3 caused neonatal lethality and dramatic alterations in the proteome, which were unique to each striated muscle type. Many of the proteins whose expression was significantly changed in the SkM-Cul3 KO were components of the extracellular matrix and sarcromere, whereas proteins altered in the CM-Cul3 KO were involved in metabolism. These findings highlight the requirement for striated muscle–specific CRL3 activity and indicate how the CRL3 complex can control different nodes of protein interaction networks in different types of striated muscle. Further identification of Cul3 substrates, and how these substrates are targeted, may reveal therapeutic targets and treatment regimens for striated muscle diseases.

Human cells express up to 10,000 proteins at a given time (1), many of which require folding and assembly into higher-order, macromolecular structures (2). Therefore, maintaining protein homeostasis (proteostasis) is critical for cellular integrity. To ensure proteostasis, cells have evolved an elaborate network of protein quality control factors involved in protein synthesis, folding, and degradation (3). Damaged proteins must be recognized and degraded by the proteostasis network to maintain cellular function and to avoid aggregation and proteotoxic stress. Striated muscle, in particular, is especially vulnerable to protein damage, owing to the persistent wear and tear of sarcomeric proteins during force-generating contraction. Several myopathies in both skeletal and cardiac muscle are characterized by protein aggregates, which result from mutations in sarcomeric proteins, such as desmin (4), nebulin (5), myotilin (6), and filamin C (7).

Several intracellular proteolytic systems exist in striated muscle that serve to remove damaged proteins and coordinate protein turnover: the calpain system (8), the autophagy-lysosome system (9), and the ubiquitin-proteasome system (UPS) (10). The UPS is the primary degradation system responsible for clearance of soluble proteins...
Cul3 is required for muscle function

that are misfolded, damaged, mutated, or oxidized (11,12). Perturbation of the UPS results in cellular dysfunction (13), and this is evidenced in muscle by a growing list of BTB-Back-Kelch (BBK) family members that give rise to muscular disease when mutated (14). BBK family members encode proteins containing an N-terminal bric a brac, tramtrack, and broad-complex (BTB) domain, a BACK domain, and a C-terminal Kelch repeat domain (15) and function as substrate-specific adapters for the Cullin3 (Cul3) E3 ligase complex, facilitating the ubiquitination and, in many cases, the subsequent proteosomal degradation of their specific substrate (16-18). Mutations in the BTB domain of Kelch-like protein 9 (Klh9) result in an early onset autosomal dominant distal myopathy (19). As BBK proteins interact with Cul3 through their BTB domain (20), the mutation in Klh9 most likely disrupts its interaction with Cul3, preventing proper targeting of substrate turnover (19). Moreover, mutations in Kbtbd13, Klh40, and Klh41 give rise to nemaline myopathy in mice and humans (21-25), and we recently demonstrated that mice lacking Klh31 develop myopathy with centralized nuclei, central cores, Z-disc streaming, and myofibril degeneration (26). These findings indicate that elements of the Cul3-RING ligase (CRL3) complex are critical regulators of muscle development and function.

Cul3 is a member of the Cullin family of E3 ligases, which consists of seven members: Cul1, 2, 3, 4A, 4B, 5, and 7 (27). While many substrate adapters use an intermediate protein to bind to Cullin family members, such as the Skp1-Cul1-F-box (SCF) complex, BBK proteins bind directly to Cul3 through their BTB domain (27). As Cul3 is ubiquitously expressed and interacts with multiple substrate adapters, it is not surprising that it regulates many processes, including mitosis (28,29), cellular stress (30-32), electrolyte homeostasis (33), and cell death (34). Recently, the Cullin family was also demonstrated to be critical for the differentiation and maturation of myoblasts (35). Inhibiting Cullin E3 ligase activity in myoblasts with the neddylation inhibitor MLN4929, showed that Cullin activity is required for myoblast differentiation, fusion, and maturation (35).

Given the increasing evidence that Cul3 and its BBK substrate adapters play critical roles in muscle disease, we investigated the function of the CRL3 complex in striated muscle with the intention of identifying CRL3-regulated substrates that would illuminate critical targets and regulatory pathways in muscle biology and disease. To approach this problem, we generated mice with either skeletal muscle-specific (SkM-Cul3) or cardiomyocyte-specific (CM-Cul3) loss of Cul3.

Our findings indicate the CRL3 complex is essential for both skeletal and cardiac muscle function; SkM-Cul3 knockout (KO) and CM-Cul3 KO mice are neonatal lethal due to non-functional skeletal muscle and severe cardiomyopathy, respectively. Proteomic analyses indicate changes in protein groups involved in muscle contraction, the extracellular matrix, and metabolism, and provide new insights into how the CRL3 complex regulates the muscle proteome during development and differentiation.

**Results**

**Skeletal muscle-specific deletion of Cul3 causes neonatal lethality**

Mice with global loss of Cul3 are early embryonic lethal resulting from mitotic defects (28). To explore the effect of skeletal muscle-specific Cul3 loss of function, and to avoid a general mitotic defect, we crossed mice carrying a conditional allele of Cul3 (36) with transgenic mice expressing Cre recombinase under control of the Myogenin promoter and the skeletal muscle specific enhancer of the Meft2c gene (37). This transgene expresses in differentiating myocytes following myoblast proliferation at E8.5.

To assess the efficiency of Cul3 deletion by Myogenin:Cre, we immunoblotted for Cul3 using whole hind limb lysates from wild type (WT) and SkM-Cul3 KO mice at postnatal day 0 (P0). Cul3 expression was decreased in SkM-Cul3 KO mice, as detected by western blot and qRT-PCR analyses. Residual levels were observed in SkM-Cul3 KO mice, which were most likely derived from other tissue types in the hind limb (Fig. 1A-C). SkM-Cul3 KO embryos at E18.5 had a moon-shaped appearance, wrist drop, abnormal spinal curvature, and reduced body weight (Fig. 1D,E), physical features that are characteristic of defective myogenesis or excitation-contraction (EC) coupling (38-40). SkM-Cul3 KO mice were born in normal Mendelian ratios but became cyanotic shortly after birth and died, while maintaining a reduction in body weight (Fig. 2A,B). Histological
Cul3 is required for muscle function

analysis of the SkM-Cul3 KO diaphragm at P0 revealed a substantial reduction in size (Fig. 2C), while SkM-Cul3 KO lungs contained smaller alveoli compared to heterozygous mice (Fig. 2D), suggesting the respiratory muscles are not capable of inflating the lungs following birth. As the hearts of SkM-Cul3 KO mice were beating at birth, these results indicate these mice expire perinatally due to the inability to breathe.

Musculature defects in SkM-Cul3 KO mice

Histological analysis of SkM-Cul3 KO tongue musculature revealed disorganized myofiber architecture compared to heterozygous controls (Fig. 2E). Myoblast fusion was unaffected, as evidenced by multiple nuclei in individual fibers; however, myofibers in SkM-Cul3 KO mice appeared smaller and had a more fibrous appearance (Fig 3A). The decrease in fiber size resulted in an overall reduction in size of muscle groups in fore limbs of SkM-Cul3 KO (Fig. 2B). Indeed, cross sectional analysis demonstrated that SkM-Cul3 KO myofibers were significantly smaller (Fig. 3C,D). Electron microscopy of SkM-Cul3 KO quadriceps showed disorganized sarcomeres, characterized by Z-disc streaming and nearly complete loss of the sarcomeric M-line (Fig. 3E). As SkM-Cul3 KO mice contain multinucleated myofibers but are born with paralysis, these findings suggest the absence of Cul3 does not affect myoblast differentiation or fusion, but may impair aspects of myofiber maturation, such as innervation, or EC coupling.

Altered proteome in SkM-Cul3 KO mice

To identify proteins regulated by Cul3, we harvested gastrocnemius/plantaris/soleus (GPS) muscles from WT and SkM-Cul3 KO embryos at E18.5 for proteomic analysis. There were 52 proteins down-regulated and 53 proteins up-regulated in SkM-Cul3 KO mice compared to WT (Supp. Table 1). Gene ontology analysis of the affected proteins showed that the most significantly changed proteins participate in muscle contraction (Fig. 4A). Other changed proteins were involved in collagen fibril organization and regulation of muscle adaptation. When we analyzed the pathways in which the affected proteins are associated, we found that many are components of extracellular matrix (ECM)-receptor interactions, muscle contraction, and regulators of cardiomyopathy (Fig. 4B). Moreover, most of the top ten up-regulated proteins are associated with the ECM (Fig. 4C), while most of the top ten down-regulated proteins are contractile proteins or are associated with the sarcomere (Fig. 4D). To confirm the changes observed in the proteomics analysis, we chose to immunoblot against one of the top down-regulated proteins found in the SkM-Cul3 KO mice. Western blot analysis for myosin light chain 2 (Myl2) confirmed the down-regulation in SkM-Cul3 KO mice observed in proteomics (Fig. 4E). These findings suggest Cul3-dependent proteostasis broadly controls elements of ECM deposition and sarcomere maturation/function.

Cardiomyocyte-specific deletion of Cul3 causes neonatal lethality

We next explored the role of Cul3 in cardiomyocytes by breeding Cul3lox/lox mice with αMHC-Cre transgenic mice (41). CM-Cul3 KO mice were born in normal Mendelian ratios and were indistinguishable from WT or αMHC:Cre:cul3lox/lox controls at birth. Western blot analysis from neonatal heart confirmed the down-regulation of Cul3 (Fig. 5A,B). While CM-Cul3 KO mice seemed unaffected at birth, they began to exhibit a failure to thrive phenotype and were much smaller compared to controls by postnatal day 5 (P5) (Fig. 5C). CM-Cul3 KO mice died around P6 with severe cardiomyopathy, consistent with dysmorphic myocardial architecture, dilated right ventricle and atrium, and atrial thrombi (Fig. 5D). Closer inspection of myocardial histology of CM-Cul3 KO mice revealed widespread cardiomyocyte vacuolization and protein aggregate deposition (Fig. 5E). Heterozygous mice had a few, sporadic vacuoles; however, CM-Cul3 KO hearts contained rampant vacuolization, consisting of vacuoles significantly larger than controls (Fig. 5F). To determine whether sarcomeric organization was disrupted, we stained heart sections for cardiac troponin T (cTnT). CM-Cul3 KO mice showed mislocalization and aggregation of cTnT compared to controls (Fig. 6). While αMHC:Cre is transiently expressed at low levels around E8-10, it is re-expressed at more robust levels at P2 (42). Given that CM-Cul3 KO mice were unaffected at birth but expired by P6, these findings demonstrate the importance of cardiac Cul3-mediated proteostasis and illustrate how quickly the integrity of the
Cul3 is required for muscle function

Altered metabolic profile in CM-Cul3 KO hearts

To determine which proteins are regulated by Cul3, we harvested WT and CM-Cul3 KO hearts at P4 for proteomic analysis. A total of 969 proteins were significantly changed in CM-Cul3 KO mice (Supp. Table 2). The majority of affected proteins (582) were down-regulated, while 387 proteins were up-regulated. Gene ontology analysis of the affected proteins in CM-Cul3 KO mice revealed that the most significantly changed proteins participate in oxidation-reduction and other metabolic processes (Fig. 7A). Other changed proteins were involved in sarcomere organization and cardiac muscle contraction. When we analyzed the pathways in which the affected proteins are associated, we found that many are components of oxidative phosphorylation and metabolic pathways (Fig. 7B), and are found among the most up-regulated and down-regulated proteins (Fig. 7C,D). Interestingly, protein aggregate diseases, such as Parkinson’s, Huntington’s, and Alzheimer’s disease, were top pathways associated with the affected proteins in CM-Cul3 KO hearts. In addition to Cul3 being significantly down-regulated (Fig. 7D), one of the most up-regulated proteins was NAD(P)H dehydrogenase 1 (Nqo1), a known target of Nrf2, which is regulated in a Cul3-Keap1-dependent manner (43) (Fig. 7C). These findings suggest Cul3-dependent proteostasis is a critical regulator of cardiac anti-oxidative and metabolic processes.

Discussion

In order for cells to maintain functionality, it is paramount that misfolded, unnecessary, and damaged proteins are recognized and degraded. Uncontrolled proteostasis leads to accumulated depositions of protein aggregates and inclusion bodies, and ultimately, cellular dysfunction. Proteostasis allows differentiated cells to adapt their proteome to intrinsic and environmental changes to prevent disease onset, which is crucial for post-mitotic cell types such as myofibers and cardiomyocytes. Mitotic cells have the ability to clear aggregation by sequestering protein aggregates and asymmetrically dividing, leaving one healthy daughter cell and one aggregate-containing daughter cell, which will proceed through apoptosis (44,45). In addition to being post-mitotic, striated muscle must overcome two additional hurdles to achieve proteostasis. First, muscle contains a multitude of contractile and specialized proteins involved in EC coupling that require constant maintenance due to the inherent wear and tear of contraction. Secondly, energy production in muscle (especially cardiomyocytes) primarily depends on oxidative phosphorylation and, consequently, high levels of ROS are generated, which can oxidize and damage proteins (46,47).

Recent findings have underscored the importance of CRL3 components in muscle disease (14,26). In this study, we sought to gain a broader perspective of the role of CRL3 in striated muscle in order to identify CRL3-regulated targets and pathways in muscle biology. To achieve this, we generated mice lacking Cul3 in developing skeletal muscle or cardiomyocytes. SkM-Cul3 KO mice were born alive, but had many features in common with mouse models of severe defects in myogenesis (38), neuromuscular junction (NMJ) function (39), and EC coupling (40). However, defects in myogenesis and myocyte fusion can be ruled out, as SkM-Cul3 KO mice have multinucleated myofibers. Following birth, SkM-Cul3 KO mice quickly became cyanotic and died. The loss of Cul3 in myocytes did not affect myoblast differentiation or fusion, as SkM-Cul3 KO mice had multinucleated myotubes, which suggests there may be defects in NMJ function or EC coupling. Interestingly, a previous study reported that Klhl8 degrades the NMJ protein Rapsyn, thereby maintaining proper levels of Rapsyn and acetylcholine receptor clustering (48); however, Rapsyn was not changed in skeletal muscle lacking Cul3. Other than reduced muscle mass and structural aberrations, SkM-Cul3 KO muscle appears to differentiate and fuse normally, indicating Cul3 is essential for muscle maturation.

Proteomic analysis identified numerous changes in protein levels, and surprisingly, many of the significantly changed proteins were down-regulated, which is in contrast to the current dogma of Cullin E3 ligase activity. Three possible scenarios could explain this. First, Cul3 could use most of its BTB-containing substrate adapters, i.e., kelch-like proteins, to stabilize its targeted proteins in skeletal muscle, while only utilizing very few kelch-like proteins to target substrates for
Cul3 is required for muscle function

degradation. Ubiquitination not only targets proteins for degradation, but also for receptor internalization, assembly of protein complexes, protein localization, DNA repair, and signaling (49). Secondly, Cul3 could function in a temporal manner, i.e., during skeletal muscle development and differentiation, the CRL3 complex could target substrates to maintain overall protein synthesis and growth during a process where multitudes of proteins are being synthesized and assembled into the highly complex sarcomere. Then, following birth and postnatal muscle growth and maturation, the CRL3 complex would function primarily to clear unnecessary and damaged proteins. Finally, and perhaps most likely, the loss of Cul3 in nascent myocytes results in the de-regulation of several proteins that accumulate and cause proteotoxic stress, and in an attempt to compensate for this stress, the synthesis of sarcomeric proteins is reduced.

Similar to SkM-Cul3 KO mice, CM-Cul3 KO mice also showed a vast number of proteins that were down-regulated. Perhaps the most surprising findings of the current study were the differences in phenotypes and groups of proteins that were changed between SkM-Cul3 and CM-Cul3 KO mice. CM-Cul3 KO mice had widespread vacuolization and protein aggregates, and gene ontogeny analysis indicated that most of the significantly changed proteins participate in metabolism. In contrast, SkM-Cul3 KO fibers lacked the severe vacuolization and protein aggregates, and gene ontogeny analysis indicated that most of the altered proteins in skeletal muscle are associated with the ECM and sarcomere. Additionally, there were few significantly changed proteins in common between SkM-Cul3 and CM-Cul3 KO mice. Interestingly, of the changed proteins that were shared between SkM-Cul3 and CM-Cul3 KO muscle, some were regulated in a contrasting manner, such as PDZ and LIM domain 5 (Pdlim5), Myosin heavy chain 7 (Myh7), Ras homolog family member b (RhoB), and Nudix hydrolase 4 (Nudt4) (Table 1). There were only three proteins that were changed in the same direction between SkM-Cul3 and CM-Cul3 KO mice: Cul3, Transcription elongation factor A3 (Tcea3), and Plastin 3 (Pls3). Little is known about Tcea3, and while it is highly enriched in skeletal muscle (Biogps.org), there is only one report on a possible function in muscle, suggesting it promotes differentiation of isolated bovine satellite cells (50). Moreover, we previously found Tcea3 to be regulated by Mef2 in a mouse model of skeletal muscle regeneration (51). Pls3 is an actin bundling protein that has been suggested to partially rescue the effects of spinal muscular atrophy, in part by improving NMJ activity (52,53). In both SkM-Cul3 and CM-Cul3 KO mice we observed changes in the cytoskeletal network that included microtubule associated proteins and both sarcomeric and non-sarcomeric actins, suggesting Cul3 plays a role in regulating the actin-cytoskeletal network (Supp. Table 1,2).

Keap1 is a well characterized substrate adapter for Cul3 that targets cytoplasmic Nrf2 for degradation, prohibiting its nuclear entry where it activates antioxidant genes in response to oxidative stress (43). In the absence of Cul3, we observed significant increases in Nrf2 target genes in CM-Cul3 KO mice, such as NAD(P)H dehydrogenase (Noq1), Pirin (Pir), Peroxiredoxin (Prdx1), Thioredoxin (Txn), Thioredoxin reductase 1 (Txnrd1), NADP-dependent malic enzyme (Me1), and the regulatory and catalytic domains of Glutamate cysteine ligase (Gcl) (54). Interestingly, none of these proteins were up-regulated in SkM-Cul3 KO mice. These findings are consistent with the very modest phenotype of skeletal muscle-specific Keap1 KO mice (55), and demonstrates how Cul3 differentially regulates the proteome in skeletal muscle and cardiomyocytes.

The results of the current study highlight the complexity of Cul3-dependent proteostasis and illustrate how Cul3 can regulate different aspects of cellular biology in two similar cell types. Muscle has a capacity to adapt to external stimuli and physiological demand, and one way in which this adaptation is accomplished is by altering the proteome. The identification of Cul3 substrates and determination of the ways in which these substrates are targeted at the molecular level during conditions such as growth and maintenance, cardiac remodeling, atrophy, and disease will enhance our understanding of the muscle proteostasis network and may uncover novel therapeutic approaches to muscle disease.

**Experimental Procedures**

**Mice.** Cul3 conditional mice were purchased from Jackson Labs (Cul3tm1Jdsr/J #028349) (36). SKM-Cul3 KO mice were generated by crossing Cul3fl/fl
mice with Myogenin:Cre mice (37), while CM-Cul3 KO mice were generated by crossing Cul3\(^{fl/fl}\) mice with αMHC:Cre mice (41). All mice were maintained on a C57BL/6 background. Animal work described in this manuscript has been approved and conducted under the oversight of the UT Southwestern Institutional Animal Care and Use Committee.

**Western blot analysis.** Lysates were prepared by pulverizing flash frozen tissue. Tissue powder was homogenized in RIPA buffer (Sigma) with the addition of protease inhibitors (Complete ultra mini tablet) on ice in a glass dounce homogenizer. Protein concentrations were determined using a BCA protein assay kit (Pierce). Samples were separated on Any kD\(^{TM}\) tris-glycine buffered polyacrylamide gels (BioRad) and transferred onto Immobilon P membranes (Millipore). Membranes were blocked for 1 hour at room temperature with 5% non-fat dry milk in TBST, and primary antibody hybridization was carried out overnight at 4°C using the following antibodies: Cullin-3, 1:250 (Sigma), and Gapdh, 1:10,000 (Millipore).

**Histology.** Tissues were fixed with 4% paraformaldehyde in phosphate buffered saline for 48 hours at room temperature. Paraffin-embedded sections were then subjected to standard hematoxylin and eosin staining protocols. Images were taken and processed with a Keyence BZ-X700 scope. For immunofluorescent staining, skeletal muscle tissues were embedded in a mixture of OCT (Fisher) and gum tragacanth (Sigma-Aldrich) and flash-frozen in a 2-methylbutane reservoir submerged in liquid nitrogen, followed by cryostat sectioning at 10 μM. Sections were air-dried for 15 minutes and fixed with 1% PFA for 2 minutes. Sections were then permeabilized for 15 minutes with PBST (0.3% Tween-20), followed by blocking with 5% goat serum (Sigma-Aldrich) in PBST for 30 minutes. Primary antibodies were diluted in 2% goat serum in PBST and added overnight at 4°C in a humidified chamber using the following antibodies: cTnT (Sigma, 1:1000), My32 (Sigma, 1:1000), wheat germ agglutinin (Thermofisher, 1:20), Alexa fluor goat anti-mouse 555 (Thermofisher, 1:250), Alexa fluor goat anti-mouse 488 (Thermofisher, 1:250). Confocal images were taken with a Zeiss LSM-800. Electron microscopic images were obtained using E18.5 quadriceps as previously described (56).

**Proteomics.** CM-Cul3 KO in vivo TMT-labeling and 10-fraction LC/LC-MS/MS was performed by the Proteomics and Metabolomics Shared Resource at Duke University School of Medicine, as described previously (26). SkM-Cul3 KO in vivo TMT-labeling and 10-fraction LC/LC-MS/MS was performed by the Proteomics Core Laboratories at Washington University School of Medicine.

**Bioinformatic Analyses.** Gene ontogeny (GO) analysis was performed using DAVID with ILLUMINA_ID identifiers and Mus musculus as the background set. Biological process level 1 was used for GO term enrichment. Kyoto Encyclopedia of Genes and Genomes (KEGG) was used for pathway analyses.

**Statistics.** Values are given as mean ± SD. Differences between 2 groups were assessed using unpaired 2-tailed Student’s t tests. P < 0.05 was regarded as significant. Statistical analysis was performed in Prism 7 (GraphPad).
Acknowledgements: We thank Jose Cabrera for help with graphics. We also thank James Richardson and Bret Evers for assistance with histology. We are grateful to M. Arthur Moseley, Erik Soderblom, and the Duke University School of Medicine for the use of the Proteomics and Metabolomics Shared Resource. We thank R. Reid Townsend, Jim Malone for use of the Proteomics Core Laboratories at Washington University School of Medicine.

Conflict of interest: The authors have declared that no conflict of interest exists.

Author contributions: J.B.P conceived of and performed the experiments, analyzed the data, and wrote the paper. A.H.V. and S.B. assisted in experimental studies. R.B.D. and E.N.O. directed the study and helped write the paper.
Cul3 is required for muscle function

References

1. Kulak, N. A., Geyer, P. E., and Mann, M. (2017) Loss-less Nano-fractionator for High Sensitivity, High Coverage Proteomics. Mol Cell Proteomics 16, 694-705
2. Hartl, F. U., Bracher, A., and Hayer-Hartl, M. (2011) Molecular chaperones in protein folding and proteostasis. Nature 475, 324-332
3. Balch, W. E., Morimoto, R. I., Dillin, A., and Kelly, J. W. (2008) Adapting proteostasis for disease intervention. Science 319, 916-919
4. Goldfarb, L. G., Olive, M., Vicart, P., and Goebel, H. H. (2008) Intermediate filament diseases: desminopathy. Adv Exp Med Biol 642, 131-164
5. Lehtokari, V. L., Pelin, K., Sandbacka, M., Ranta, S., Donner, K., Muntoni, F., Sewry, C., Angelini, C., Bushby, K., Van den Bergh, P., Iannaccone, S., Laing, N. G., and Wallgren-Pettersson, C. (2006) Identification of 45 novel mutations in the nebulin gene associated with autosomal recessive nemaline myopathy. Hum Mutat 27, 946-956
6. Selcen, D., and Engel, A. G. (2004) Mutations in myotilin cause myofibrillar myopathy. Neurology 62, 1363-1371
7. Vorgerd, M., van der Ven, P. F., Bruchertseifer, V., Lowe, T., Kley, R. A., Schroder, R., Lochmüller, H., Himmel, M., Koehler, K., Furst, D. O., and Huebner, A. (2005) A mutation in the dimerization domain of filamin c causes a novel type of autosomal dominant myofibrillar myopathy. Am J Hum Genet 77, 297-304
8. Sorimachi, H., and Ono, Y. (2012) Regulation and physiological roles of the calpain system in muscular disorders. Cardiovasc Res 96, 11-22
9. Sandri, M., Coletto, L., Grumati, P., and Bonaldo, P. (2013) Misregulation of autophagy and protein degradation systems in myopathies and muscular dystrophies. J Cell Sci 126, 5325-5333
10. Sandri, M. (2013) Protein breakdown in muscle wasting: role of autophagy-lysosome and ubiquitin-proteasome. Int J Biochem Cell Biol 45, 2121-2129
11. Vilchez, D., Saez, I., and Dillin, A. (2014) The role of protein clearance mechanisms in organismal ageing and age-related diseases. Nat Commun 5, 5659
12. Morimoto, R. I., and Cuervo, A. M. (2014) Proteostasis and the ageing proteome in health and disease. J Gerontol A Biol Sci Med Sci 69 Suppl 1, S33-38
13. Hipp, M. S., Park, S. H., and Hartl, F. U. (2014) Proteostasis impairment in protein-misfolding and -aggregation diseases. Trends Cell Biol 24, 506-514
14. Gupta, V. A., and Beggs, A. H. (2014) Kelch proteins: emerging roles in skeletal muscle development and diseases. Skelet Muscle 4, 11
15. Dhanoo, B. S., Cogliati, T., Satish, A. G., Bruford, E. A., and Friedman, J. S. (2013) Update on the Kelch-like (KLHL) gene family. Hum Genomics 7, 13
16. Emanuele, M. J., Elia, A. E., Xu, Q., Thoma, C. R., Izhar, L., Leng, Y., Guo, A., Chen, Y. N., Rush, J., Hsu, P. W., Yen, H. C., and Elledge, S. J. (2011) Global identification of modular cullin-RING ligase substrates. Cell 147, 459-474
17. Zhang, D. D., Lo, S. C., Sun, Z., Habib, G. M., Lieberman, M. W., and Hannink, M. (2005) Ubiquitination of Keap1, a BTB-Kelch substrate adaptor protein for Cul3, targets Keap1 for degradation by a proteasome-independent pathway. J Biol Chem 280, 30091-30099
18. Chen, Y., Yang, Z., Meng, M., Zhao, Y., Dong, N., Yan, H., Liu, L., Ding, M., Peng, H. B., and Shao, F. (2009) Cullin mediates degradation of RhoA through evolutionarily conserved BTB adaptors to control actin cytoskeleton structure and cell movement. Mol Cell 35, 841-855
19. Cirak, S., von Deimling, F., Sachdev, S., Errington, W. J., Herrmann, R., Bonnemann, C., Brockmann, K., Hinderlich, S., Lindner, T. H., Steinbrecher, A., Hoffmann, K., Prive, G. G., Hannink, M., Nurnberg, P., and Voit, T. (2010) Kelch-like homologue 9 mutation is associated with an early onset autosomal dominant distal myopathy. Brain 133, 2123-2135
20. Xu, L., Wei, Y., Reboul, J., Vaglio, P., Shin, T. H., Vidal, M., Elledge, S. J., and Harper, J. W. (2003) BTB proteins are substrate-specific adaptors in an SCF-like modular ubiquitin ligase containing CUL-3. Nature 425, 316-321
Cul3 is required for muscle function

21. Garg, A., O'Rourke, J., Long, C., Doering, J., Ravenscroft, G., Bezprozvannaya, S., Nelson, B. R., Beetz, N., Li, L., Chen, S., Laing, N. G., Grange, R. W., Bassel-Duby, R., and Olson, E. N. (2014) KLHL40 deficiency destabilizes thin filament proteins and promotes nemaline myopathy. J Clin Invest 124, 3529-3539

22. Ravenscroft, G., Miyatake, S., Lehtokari, V. L., Todd, E. J., Vormann, P., Yau, K. S., Hayashi, Y. K., Miyake, N., Tsurusaki, Y., Doi, H., Saitsu, H., Osaka, H., Yamashita, S., Ohy, T., Sakamoto, Y., Koshimizu, E., Imamura, S., Yamashita, M., Ogata, K., Shiina, M., Bryson-Richardson, R. J., Vaz, R., Ceyhan, O., Brownstein, C. A., Swanson, L. C., Monnot, S., Romero, N. B., Amthor, H., Kresoje, N., Sivadorai, P., Kiraly-Borri, C., Haliloglu, G., Talim, B., Orhan, D., Kale, G., Charles, A. K., Fabian, V. A., Davis, M. R., Lammens, M., Sewry, C. A., Manzur, A., Muntoni, F., Clarke, N. F., North, K. N., Bertini, E., Nevo, Y., Willichowski, E., Silberg, I. E., Topaloglu, H., Beggs, A. H., Alcock, R. J., Nishino, I., Wallgren-Pettersson, C., Matsumoto, N., and Laing, N. G. (2013) Mutations in KLHL41 are a frequent cause of severe autosomal-recessive nemaline myopathy. Am J Hum Genet 93, 6-18

23. Gupta, V. A., Ravenscroft, G., Shaheen, R., Todd, E. J., Swanson, L. C., Shiina, M., Ogata, K., Hsu, C., Clarke, N. F., Darras, B. T., Farrar, M. A., Hashem, A., Manton, N. D., Muntoni, F., North, K. N., Sandradura, S. A., Nishino, I., Hayashi, Y. K., Sewry, C. A., Thompson, E. M., Yau, K. S., Brownstein, C. A., Yu, T. W., Alcock, R. J., Davis, M. R., Wallgren-Pettersson, C., Matsumoto, N., Alkuraya, F. S., Laing, N. G., and Beggs, A. H. (2013) Identification of KLHL41 Mutations Implicates BTB-Kelch-Mediated Ubiquitination as an Alternate Pathway to Myofibrillar Disruption in Nemaline Myopathy. Am J Hum Genet 93, 1108-1117

24. Ramirez-Martinez, A., Cenik, B. K., Bezprozvannaya, S., Chen, B., Bassel-Duby, R., Liu, N., and Olson, E. N. (2017) KLHL41 stabilizes skeletal muscle sarcomeres by nonproteolytic ubiquitination. Elife 6

25. Sambougnin, N., Yau, K. S., Olive, M., Duff, R. M., Bayarsaikhan, M., Lu, S., Gonzalez-Mera, L., Sivadorai, P., Nowak, K. J., Ravenscroft, G., Mastaglia, F. L., North, K. N., Ilkovski, B., Kremer, H., Lammens, M., van Engelen, B. G., Fabian, V., Lamont, P., Davis, M. R., Laing, N. G., and Goldfarb, L. G. (2010) Dominant mutations in KBTBD13, a member of the BTB/Kelch family, cause nemaline myopathy with cores. Am J Hum Genet 87, 842-847

26. Papizan, J. B., Garry, G. A., Brezprozvannaya, S., McAnally, J. R., Bassel-Duby, R., Liu, N., and Olson, E. N. (2017) Deficiency in Kelch protein Klhl31 causes congenital myopathy in mice. J Clin Invest 127, 3730-3740

27. Petroski, M. D., and Deshaies, R. J. (2005) Function and regulation of cullin-RING ubiquitin ligases. Nat Rev Mol Cell Biol 6, 9-20

28. Singer, J. D., Gurian-West, M., Clurman, B., and Roberts, J. M. (1999) Cullin-3 targets cyclin E for ubiquitination and controls S phase in mammalian cells. Genes Dev 13, 2375-2387

29. Pintard, L., Willis, J. H., Willems, A., Johnson, J. L., Srayko, M., Kurz, T., Glaser, S., Mains, P. E., Tyers, M., Bowerman, B., and Peter, M. (2003) The BTB protein MEL-26 is a substrate-specific adaptor of the CUL-3 ubiquitin-ligase. Nature 425, 311-316

30. Mutohashi, H., and Yamamoto, M. (2004) Nrf2-Keap1 defines a physiologically important stress response mechanism. Trends Mol Med 10, 549-557

31. Lo, S. C., and Hannink, M. (2006) CAND1-mediated substrate adaptor recycling is required for efficient repression of Nrf2 by Keap1. Mol Cell Biol 26, 1235-1244

32. Itoh, K., Wakabayashi, N., Katoh, Y., Ishii, T., Igarashi, K., Engel, J. D., and Yamamoto, M. (1999) Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. Genes Dev 13, 76-86

33. Shibata, S., Zhang, J., Puthumana, J., Stone, K. L., and Lifton, R. P. (2013) Kelch-like 3 and Cullin 3 regulate electrolyte homeostasis via ubiquitination and degradation of WNK4. Proc Natl Acad Sci U S A 110, 7838-7843
Cul3 is required for muscle function

34. Lee, Y. R., Yuan, W. C., Ho, H. C., Chen, C. H., Shih, H. M., and Chen, R. H. (2010) The Cullin 3 substrate adaptor KLHL20 mediates DAPK ubiquitination to control interferon responses. EMBO J 29, 1748-1761
35. Blondelle, J., Shapiro, P., Domenighetti, A. A., and Lange, S. (2017) Cullin E3 Ligase Activity Is Required for Myoblast Differentiation. J Mol Biol 429, 1045-1066
36. McEvoy, J. D., Kossatz, U., Malek, N., and Singer, J. D. (2007) Constitutive turnover of cyclin E by Cul3 maintains quiescence. Mol Cell Biol 27, 3651-3666
37. Li, S., Czubryt, M. P., McAnally, J., Bassel-Duby, R., Richardson, J. A., Wiebel, F. F., Nordheim, A., and Olson, E. N. (2005) Requirement for serum response factor for skeletal muscle growth and maturation revealed by tissue-specific gene deletion in mice. Proc Natl Acad Sci U S A 102, 1082-1087
38. Hasty, P., Bradley, A., Morris, J. H., Edmondson, D. G., Venuti, J. M., Olson, E. N., and Klein, W. H. (1993) Muscle deficiency and neonatal death in mice with a targeted mutation in the myogenin gene. Nature 364, 501-506
39. Brandon, E. P., Lin, W., D'Amour, K. A., Pizzo, D. P., Dominguez, B., Sugiura, Y., Thode, S., Ko, C. P., Thal, L. J., Gage, F. H., and Lee, K. F. (2003) Aberrant patterning of neuromuscular synapses in choline acetyltransferase-deficient mice. J Neurosci 23, 539-549
40. Takeshima, H., Iino, M., Takekura, H., Nishi, M., Kuno, J., Minowa, O., Takano, H., and Noda, T. (1994) Excitation-contraction uncoupling and muscular degeneration in mice lacking functional skeletal muscle ryanodine-receptor gene. Nature 369, 556-559
41. Agah, R., Frenkel, P. A., French, B. A., Michael, L. H., Overbeek, P. A., and Schneider, M. D. (1997) Gene recombination in postmitotic cells. Targeted expression of Cre recombinase provokes cardiac-restricted, site-specific rearrangement in adult ventricular muscle in vivo. J Clin Invest 100, 169-179
42. Davis, J., Maillet, M., Miano, J. M., and Molkentin, J. D. (2012) Lost in transgenesis: a user's guide for genetically manipulating the mouse in cardiac research. Circ Res 111, 761-777
43. Kansanen, E., Kuosmanen, S. M., Leinonen, H., and Levonen, A. L. (2013) The Keap1-Nrf2 pathway: Mechanisms of activation and dysregulation in cancer. Redox Biol 1, 45-49
44. Rujano, M. A., Bosveld, F., Salomons, F. A., Dijk, F., van Waarde, M. A., van der Want, J. J., de Vos, R. A., Brun, E. R., Sibon, O. C., and Kampinga, H. H. (2006) Polarised asymmetric inheritance of accumulated protein damage in higher eukaryotes. PLoS Biol 4, e417
45. Liu, B., Larsson, L., Caballero, A., Hao, X., Oling, D., Grantham, J., and Nystrom, T. (2010) The polarisome is required for segregation and retrograde transport of protein aggregates. Cell 140, 257-267
46. Korovila, I., Hugo, M., Castro, J. P., Weber, D., Hohn, A., Grune, T., and Jung, T. (2017) Proteostasis, oxidative stress and aging. Redox Biol 13, 550-567
47. Zhang, H., Gomez, A. M., Wang, X., Yan, Y., Zheng, M., and Cheng, H. (2013) ROS regulation of microdomain Ca(2+) signalling at the dyads. Cardiovasc Res 98, 248-258
48. Nam, S., Min, K., Hwang, H., Lee, H. O., Lee, J. H., Yoon, J., Lee, H., Park, S., and Lee, J. (2009) Control of rapsyn stability by the CUL-3-containing E3 ligase complex. J Biol Chem 284, 8195-8206
49. Popovic, D., Vucic, D., and Dikic, I. (2014) Ubiquitination in disease pathogenesis and treatment. Nat Med 20, 1242-1253
50. Zhu, Y., Tong, H. L., Li, S. F., and Yan, Y. Q. (2017) Effect of TCEA3 on the differentiation of bovine skeletal muscle satellite cells. Biochem Biophys Res Commun 484, 827-832
51. Liu, N., Nelson, B. R., Bezprozvannaya, S., Shelton, J. M., Richardson, J. A., Bassel-Duby, R., and Olson, E. N. (2014) Requirement of MEF2A, C, and D for skeletal muscle regeneration. Proc Natl Acad Sci U S A 111, 4109-4114
52. Ackermann, B., Krober, S., Torres-Benito, L., Borgmann, A., Peters, M., Hosseini Barkooie, S. M., Tejero, R., Jakubik, M., Schreml, J., Milbradt, J., Wunderlich, T. F., Riessland, M., Tabares, L., Ackermann, B., Krober, S., Torres-Benito, L., Borgmann, A., Peters, M., Hosseini Barkooie, S. M., Tejero, R., Jakubik, M., Schreml, J., Milbradt, J., Wunderlich, T. F., Riessland, M., Tabares, L.,
Cul3 is required for muscle function

and Wirth, B. (2013) Plastin 3 ameliorates spinal muscular atrophy via delayed axon pruning and improves neuromuscular junction functionality. *Hum Mol Genet* **22**, 1328-1347

53. Oprea, G. E., Krober, S., McWhorter, M. L., Rossoll, W., Muller, S., Krawczak, M., Bassell, G. J., Beattie, C. E., and Wirth, B. (2008) Plastin 3 is a protective modifier of autosomal recessive spinal muscular atrophy. *Science* **320**, 524-527

54. Chorley, B. N., Campbell, M. R., Wang, X., Karaca, M., Sambandan, D., Bangura, F., Xue, P., Pi, J., Kleeberger, S. R., and Bell, D. A. (2012) Identification of novel NRF2-regulated genes by ChIP-Seq: influence on retinoid X receptor alpha. *Nucleic Acids Res* **40**, 7416-7429

55. Uruno, A., Yagishita, Y., Katsuoka, F., Kitajima, Y., Nunomiya, A., Nagatomi, R., Pi, J., Biswal, S. S., and Yamamoto, M. (2016) Nrf2-Mediated Regulation of Skeletal Muscle Glycogen Metabolism. *Mol Cell Biol* **36**, 1655-1672

56. Anderson, D. M., Cannavino, J., Li, H., Anderson, K. M., Nelson, B. R., McAnally, J., Bezprozvannaya, S., Liu, Y., Lin, W., Liu, N., Bassel-Duby, R., and Olson, E. N. (2016) Severe muscle wasting and denervation in mice lacking the RNA-binding protein ZFP106. *Proc Natl Acad Sci U S A* **113**, E4494-4503
Cul3 is required for muscle function

FOOTNOTES
This work was supported in part by grants from the NIH (AR-067294, HL-130253, DK-099653) and the Robert A. Welch Foundation (grant 1-0025 to E.N.O.). J.B. Papizan was supported by a Ruth L. Kirschstein NRSA F32 NIH training grant (5F32HL123323-03).
Cul3 is required for muscle function

### Table 1. Significantly changed proteins in common

| Protein ID | Description                              | SkMΔ     | CMΔ    |
|------------|------------------------------------------|----------|--------|
| Cul3       | Cullin 3 E3 ligase                       | Decreased| Decreased|
| Tcea3      | Transcription Elongation Factor A3       | Decreased| Decreased|
| PIs3       | Plastin 3                                | Increased| Increased|
| Pdlim5     | PDZ and LIM domain 5                     | Decreased| Increased|
| Myh7       | Myosin heavy chain 7                     | Decreased| Increased|
| Rhob       | Ras homolog family member b              | Decreased| Increased|
| Nudt4      | Nudix hydrolase 4                        | Increased| Decreased|
Cul3 is required for muscle function

Figure 1. Cullin 3 ablation in SkM-Cul3 KO mice. (A) Representative western blot analysis and (B) densitometry quantification of Cul3 protein using GPS muscles from E18.5 embryos (WT, n = 3; SkM-Cul3 KO, n = 3). *P < 0.05, WT vs KO. Data are presented as mean ± SD. (C) qRT-PCR analysis of Cul3 mRNA using GPS muscles (WT, n = 6; SkM-Cul3 KO, n = 6). ***P < 0.001, WT vs KO. Data are presented as mean ± SD. (D) Representative image of SkM-Cul3 heterozygous (HET) and SkM-Cul3 KO embryos at E18.5. Abnormal spinal curvature and wrist drop are displayed in SkM-Cul3 KO mice. (E) Body weights of WT and SkM-Cul3 KO E18.5 embryos (WT, n = 4; SkM-Cul3 KO, n = 4). *P < 0.05, WT vs KO. Data are presented as mean ± SD. Statistical analyses were performed using an unpaired 2-tailed Student’s t test.
Figure 2. Neonatal lethality in SkM-Cul3 KO mice. (A) Representative images of SkM-Cul3 heterozygous (HET) and SkM-Cul3 KO pups at P0. SkM-Cul3 KO mice exhibit abnormal spinal curvature, cyanosis, and paralysis. (B) SkM-Cul3 KO mice weigh significantly less at birth compared to control mice (WT, $n = 3$; SkM-Cul3 KO, $n = 3$). **$P < 0.01$, WT vs KO. Data are presented as mean ± SD. (C) Hematoxylin and eosin (H&E) staining of E18.5 diaphragm (Scale bars, 100 µm), (D) lungs (Scale bars, 100 µm), and (E) tongue (Scale bars, 50 µm). Statistical analyses were performed using an unpaired 2-tailed Student’s $t$ test.
Cul3 is required for muscle function
**Figure 3.** Reduced muscle mass and disorganized sarcomeres in SkM-Cul3 KO mice. (A) H&E staining of longitudinal sections of E18.5 embryonic forelimbs of SkM-Cul3 HET and SkM-Cul3 KO mice. (Scale bars, 20 µm) (B) H&E staining of transverse sections through E18.5 embryonic forelimbs of SkM-Cul3 HET and SkM-Cul3 KO mice (Scale bars, 100 µm.). (C) Transverse (top panel; scale bars, 20 µm) and longitudinal (bottom panel; scale bars, 50 µm) staining of myosin and WGA in heterozygous and SkM-Cul3 KO quadriceps. (D) Quantification of the mean cross sectional area of heterozygous and SkM-Cul3 KO forelimbs (Het, n = 4; SkM-Cul3 KO, n = 5). ***P < 0.001, Het vs KO. Data are presented as mean ± SD. (E) Electron microscopy of E18.5 quadriceps of SkM-Cul3 HET and SkM-Cul3 KO mice. Z-disc streaming (red arrows) observed in SkM-Cul3 KO muscle (Scale bars, 1 µm). ECRL, extensor carpi radialis longus; ECRB, extensor carpi radialis brevis; BR, brachioradialis; FPL, flexor pollicis longus; R, radius; U, ulna. Statistical analyses were performed using an unpaired 2-tailed Student’s t test.
Cul3 is required for muscle function

A

GO term analysis SkM-Cul3 KO

B

KEGG Pathway SkM-Cul3 KO

C

| Protein ID | Description               | Fold Change |
|------------|---------------------------|-------------|
| Kera       | Keratocan                 | 2.01        |
| S100a9     | Calgranulin B             | 1.89        |
| Matn1      | Matrilin 1                | 1.88        |
| Hapln      | Hyaluronan and proteoglycan link protein 1 | 1.62 |
| Prss1      | Protease, Serine 1        | 1.61        |
| Col9a1     | Collagen type IX alpha-1  | 1.58        |
| Matn3      | Matrilin 3                | 1.51        |
| Col2a1     | Collagen type II alpha-1  | 1.50        |
| Acan       | Aggrecan core protein     | 1.41        |
| H2afj      | Histone H2A               | 1.38        |

D

| Protein ID | Description                                           | Fold Change |
|------------|-------------------------------------------------------|-------------|
| Acadl      | Long-chain specific acyl-CoA dehydrogenase            | -2.96       |
| Myl2       | Myosin regulatory light chain 2                       | -1.86       |
| Dbp        | D site-binding protein                                | -1.83       |
| Mybpc2     | Myosin binding protein C2                             | -1.73       |
| Cul3       | Cullin 3                                              | -1.57       |
| Lmhf2      | Lipase maturation factor 2                            | -1.53       |
| Fbxo32     | F box only protein                                    | -1.53       |
| Myl3       | Myosin light chain 3                                  | -1.52       |
| Actn3      | Alpha-actinin 3                                       | -1.51       |
| Fabp       | Fatty acid binding protein                            | -1.51       |
Figure 4. Global identification of altered proteins in muscle of SkM-Cul3 KO mice. (A) Gene ontogeny and (B) KEGG pathway analyses were performed with DAVID. Proteomic data from WT and SkM-Cul3 KO GPS were used in the analyses. Significantly ($P < 0.05$) enriched biological processes and pathways are shown. The log ($P$ value) is plotted on the X axis. (C) The top ten most up-regulated and (D) down-regulated proteins with protein spectrum matches of three or more are shown. (E) Representative western blot analysis and densitometry quantification of Myl2 protein using GPS muscles from E18.5 embryos (WT, $n = 3$; SkM-Cul3 KO, $n = 3$). *$P < 0.05$, WT vs KO. Data are presented as mean ± SD.
Cul3 is required for muscle function
**Figure 5.** Lethal cardiomyopathy in CM-Cul3 KO neonatal mice. (A) Representative western blot analysis and (B) densitometry quantification of Cul3 protein using whole hearts from WT and CM-Cul3 KO mice at P5 (WT, \( n = 3 \); CM-Cul3 KO, \( n = 3 \)). *P < 0.05, WT vs KO. Data are presented as mean ± SD. (C) Representative image of a failure to thrive phenotype in CM-Cul3 KO mice at P5. (D) H&E staining of hearts from heterozygous and CM-Cul3 KO mice at P5. *, atrial thrombus; LV, left ventricle; RV, right ventricle. (E) Low magnification (Top panel; scale bars, 50 µm) and high magnification (bottom panel; scale bars, 20 µm) images of H&E stained myocardium from heterozygous and CM-Cul3 KO mice at P5. CM-Cul3 KO hearts show vacuolization and protein aggregates (white arrows). (F) Quantification of cardiomyocyte vacuole size in heterozygous and CM-Cul3 KO hearts (Het, \( n = 3 \); CM-Cul3 KO, \( n = 3 \)). ****P < 0.0001, Het vs KO. Data are presented as mean ± SD.
Figure 6. Aggregated and mislocalized sarcomeric cTnT in CM-Cul3 KO mice. Immunostaining of heterozygous (left) and CM-Cul3 KO (right) hearts against cTnT (green) and nuclei (blue).
Cul3 is required for muscle function

A

GO term analysis CM-Cul3 KO

-oxidation-reduction process
-translation
-mitochondrial translation
-metabolic process
-transport
-fatty acid metabolic process
-mitochondrial electron transport, NADH to ubiquinone
-fatty acid beta-oxidation
-response to oxidative stress
-tricarboxylic acid cycle
-lipid metabolic process
-sarcoplasmic organization
-ATP synthesis coupled proton transport
-ATP biosynthetic process
-fatty acid beta-oxidation using acyl-CoA dehydrogenase
-respiratory electron transport chain
-mitochondrion organization
-cell-cell adhesion
-ATP metabolic process
-glutathione metabolic process
-proton transport
-vascular smooth muscle contraction
-apoptotic mitochondrial changes
-apoptotic cell adhesion

B

KEGG Pathway - CM-Cul3 KO

-oxidative phosphorylation
-Mitochondrial pathways
-Parkinson's disease
-Huntington's disease
-Non-alcoholic fatty liver disease (NAFLD)
-Alzheimer's disease
-Carbon metabolism
-Biosynthesis of antibiotics
-Valine, leucine and isoleucine degradation
-Fatty acid degradation
-Carbohydrate metabolism
-Propanoate metabolism
-Ribosome
-Pyruvate metabolism
-Citrate cycle (TCA cycle)
-Fatty acid metabolism
-Bacterial invasion of epithelial cells
-Glycolysis / Gluconeogenesis
-Peroxisome
-Focal adhesion
-Hypertrophic cardiomyopathy
-Glycine, serine and threonine metabolism
-Biosynthesis of amino acids
-Proteoglycans in cancer
-Lysine degradation

C

| Protein ID | Description                                      | Fold Change |
|------------|--------------------------------------------------|-------------|
| Btd1       | BTB/POZ domain containing protein 1              | 3.6         |
| Khl0       | Kelch like protein 9                             | 3.2         |
| Nqo1       | NAD(P)H dehydrogenase 1                          | 3.0         |
| Tagln      | Transgelin                                       | 2.9         |
| Spond1     | Spondin 1                                        | 2.6         |
| Mf4p4      | Microfibril-associated glycoprotein 4            | 2.5         |
| Krt18      | Keratin, type 1 cytoskeletal 18                   | 2.5         |
| Niscl      | Nischarin                                        | 2.4         |
| Hbb-b1     | Hemoglobin subunit beta 1                        | 2.4         |
| Ace        | Angiotensin converting enzyme                     | 2.3         |

D

| Protein ID | Description                                      | Fold Change |
|------------|--------------------------------------------------|-------------|
| Sloc25a31  | ADP/ATP translocase 4                            | -2.9        |
| Nt5c1a     | Cytosolic 5'-nucleotidase 1A                      | -2.3        |
| Chkmt2     | Creatine kinase S-type, mitochondrial             | -2.1        |
| Bmp10      | Bone morphogenetic protein 10                    | -2.0        |
| Ethb3      | Ephrin-B3                                        | -1.9        |
| Ucp3       | Mitochondrial uncoupling protein 3               | -1.9        |
| Gsk1       | Glutathione S-transferase kappa 1                | -1.9        |
| Ankkl2     | Ankyrin repeat and LEM domain containing protein 2| -1.8        |
| Acsl1      | Acetyl-coenzyme A synthetase 2-like, mitochondrial| -1.8        |
| Cul3       | Cullin3                                          | -1.8        |
**Figure 7.** Global identification of altered proteins in the hearts of CM-Cul3 KO mice. (A) Gene ontogeny and (B) KEGG pathway analyses were performed with DAVID. Proteomic data from WT and CM-Cul3 KO hearts were used in the analyses. Significantly ($P < 0.05$) enriched biological processes and pathways are shown. The log ($P$ value) is plotted on the X axis. (C) The top ten most up-regulated and (D) down-regulated proteins with protein spectrum matches of three or more are shown.
Cullin3-RING ubiquitin ligase activity is required for striated muscle function in mice
James B. Papizan, Alexander H. Vidal, Svetlana Bezprozvannaya, Rhonda Bassel-Duby and
Eric N. Olson

J. Biol. Chem. published online April 13, 2018

Access the most updated version of this article at doi: 10.1074/jbc.RA118.002104

Alerts:
  • When this article is cited
  • When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts