# Muscle mTORC1 Activation Causes Reduced Adiposity in Mice

Erin J. Stephenson, JeAnna R. Redd, Detrick Snyder, Quynh T. Tran, Binbin Lu, Matthew J. Peloquin, Innocence Harvey, Molly Carter, Kaleigh Fisher, Joan C. Han, Alan Cheng, Alan R. Saltiel and Dave Bridges

# Abstract

# Introduction

Obesity is a worldwide health problem, with comorbidities including diabetes, cardiovascular and liver disease [1]. Current modalities to prevent or reverse obesity are ineffective and short-lived often due to reductions in energy expenditure and increases in hunger after weight loss [2–4]. The genetic and dietary modifiers of energy expenditure are not well known, but recent reports have implicated low-carbohydrate (or high protein) diets in enhancing energy expenditure in both overfeeding and weight loss paradigms [5,6]. The mechanism(s) by which these diets have these effects have not yet been completely elucidated.

mTORC1 is a nutrient responsive protein kinase complex expressed in all known eukaryotic cells. This complex is activated by anabolic signals such as insulin, amino acids and energy abundance (see [7] for review). mTORC1 integrates these signals, and helps to co-ordinate such anabolic processes as lipogenesis [8–10], glycogenesis [11], cellular differentiation [12–14] while promoting insulin resistance [8,15]. These effects are often tissue-specific, reflecting the cell-type specific responses to elevated nutrient and energy status.

Several studies have implicated mTORC1 inhibition as a mechanism of organismal lifespan extension in yeast, worms and mammals [16–18]. While several mechanisms been proposed, the tissue or tissues that link mTORC1 activity to extended lifespan have not yet been identified. Skeletal muscle is an important potential target tissue for understanding aging, as functional differences in muscle strength predict lifespan in a longitudinal manner [19–24]. Furthermore, mTORC1 regulates several important, aging related processes in muscle; including oxidative stress, the unfolded protein response, autophagy and lipid metabolism [25–27].

Muscle tissue is the major site of postprandial glucose disposal, and energy expenditure [28,29]. Constitutive activation of mTORC1, via muscle-specific deletion of its negative regulator *Tsc1*, have identified age-related myoatrophy and a switch towards oxidative fiber types [26,30,31]. Consistent with this, studies in cell culture models implicate mTORC1 as also a positive regulator of mitochondrial function and ATP production [32–34]. mTORC1 is strongly activated in co-ordination with high protein diets or supplementation with essential amino acids [35,36]. We therefore hypothesized that in oxidative tissues such as skeletal muscle, chronic mTORC1 activation may promote increases in energy expenditure, but may also play a role in reducing organismal lifespan.

# Methods and Materials

## Animal Husbandry

All mice were purchased from The Jackson Laboratory. Unless otherwise stated, animals were fed a normal chow diet from Harlan Teklad. For High Fat Diet studies, animals were provided *ad libitum access* to a diet with 45% of calories from lard (Research Diets D1492). This diet started when animals were approximately 10 weeks of age, animals were sacrificed at 25 weeks of age.

For muscle specific knockouts, FVB-Tg(Ckmm-cre)5Khn/J transgenic mice (stock 006405) were crossed with floxed *Tsc1tm1Djk*/J mice (stock 005680). To generate F1 mice that were heterozygous for the floxed allele, and either had or lacked the *Ckmm-Cre* transgene. These parents were intercrossed to generate knockout mice (*Tsc1fl*/fl, *Ckmm-Cre*Tg/+), wild type mice (*Tsc1+/+*, *Ckmm-Cre*+/+) and controls containing the transgene only (*Tsc1+/+*, *Ckmm-Cre*Tg/+)or the floxed allele only (*Tsc1fl*/fl, *Ckmm-Cre*+/+). All four genotypes were evaluated for all experiments. If there were no significant differences between the three control strains, these were combined and labeled as Controls. Animals were sacrificed in either the fed or fasted state as indicated in the figure legends after isoflurane-induced anaesthesia and cervical dislocation at approximately ZT3. The University of Michigan and UTHSC Institutional Animal Care and Use Committees approved all animal procedures.

## Body Composition and Calorimetry

Body composition was determined using an echoMRI 1100 at approximately ZT10. Body weights were determined using a standard scale. Tissue weights were determined for both the left and right hand side tissue and combined as total weights. Heart and muscle weights were determined by

For calorimetry studies, physical movement and calorimetry was determined using a comprehensive laboratory animal monitoring system from Columbus Instruments. These experiments were performed in light and temperature-controlled enclosures at 25C, in home-cage style cages with hanging feeders. The first 6h of measurements were discarded, after which animals were acclimatized to their new surroundings. Single-animal measurements were collected over the course of 3 days. Oxygen consumption was normalized to lean body mass (as determined by echoMRI, described above) and analyzed by mixed linear models with the considerations described in [37]. For rapamycin treatments, animals were injected with 3 mg/kg of rapamycin or a vehicle (1% Tween, 1% PEG-8000). For acute studies, animals were injected daily before switching diet to a high fat diet.

## Western Blotting

Protein lysates were generated in RIPA buffer (50 mM Tris pH 7.4, 0.25% sodium deoxycholate, 1% NP40, 150 mM sodium choride, 1 mM EDTA, 100 μM sodium vanadate, 5mM sodium fluoride, 10 mM sodium pyrophosphate and 1X protease inhibitors) or HTNG buffer (50 mM HEPES, pH 7.4, 150 mM sodium chloride, 10% glycerol, 10% triton X-100 and 1X protease inhibitors) by mechanical disruption in a Qialyser for 5 minutes at 30Hz. Lysates were clarified at 14 000 RPM for 15 minutes and quantified by Bradford assays. For PTG determination, glycogen binding proteins were enriched by amylose pull-down as previously described [11,38,39]. Proteins were separated by SDS-PAGE and blotted with antibodies described in the figure legends. Antibodies used in this study were raised against pS6K (pThr389, Cell Signaling cat #9206), S6K (Cell Signaling #2708), pAkt (pSer473, Cell Signaling #4060) and Akt (Cell Signaling #2920), TSC1 (Cell Signaling #4963), TSC2 (Cell Signaling #4308), pS6 (pSer236/236, Cell Signaling #2211), S6 (Cell Signaling #2317), SREBP1 (Santa Cruz #sc-366), pGS (pSer641, Cell Signaling #3891), GS (Cell Signaling #04-357) and PTG [11]. Blots were visualized on a LiCor Odyssey and quantified using near-IR secondary antibodies Image Studio software.

## Triglyceride and Glycogen Determination

Triglyceride and glycogen levels were determined from snap frozen tissues as previously described [11].

## Insulin and Glucuose Tolerance Tests and Glucose Clamp Studies

Insulin sensitivity was determined by injecting animals fasted for 6h with 0.7mU/g insulin (Humulin HR, Lily) and then collecting tail vein blood every 15 minutes via a glucometer (accucheck). For high fat diet studies, because fat mass was dramatically different between groups, we injected insulin at 0.7mU/g of fat free mass rather than body weight. For glucose tolerance tests 1 mg/kg glucose was injected into 6h fasted mice and glucose was analysed as per the insulin tolerance tests. To determine *in vivo* glucose-stimulated insulin secretion, glucose was injected before and 30 minutes after a retro-orbital bleed. Insulin was determined using an ultrasensitive mouse ELISA (CrystalChem).

## RNAseq Analysis

## NADH Staining of Muscle Sections

## Statistical Analyses

All statistical analyses were performed using the R, version 3.2.2 [40]. For longitudinal measurements (body weights, fat mass and lean mass), the data were analyzed by mixed linear models using uncorrelated random slopes and intercepts using the lme4 package version 1.1-8 [41]. Statistical significance was determined via χ2 tests between models containing or missing the genotype term. Pairwise comparisons were tested first for normality via a Shapiro-Wilk test, then for equal variance via Levene’s test. For survival analyses and Cox proportional hazard tests, the survival package was used (version 2.38-3)[42,43]. We tested the assumptions of proportional hazards (with Shoenfeld residuals) and found no significant deviation from this assumption (p=0.875). Based on these, appropriate pairwise tests were performed as indicated in the figure legends. Corrections for testing of multiple hypotheses were done using the method of Benjamini and Hochberg [44]. Statistical significance was designated at p/q<0.05 for all assays, except GSEA analyses were q<0.25 was used. All raw data and statistical analyses for this manuscript are available at <http://bridgeslab.github.io/TissueSpecificTscKnockouts>.

# Results

## Rapamycin Treatment Reduces High Fat Diet Induced Increases in Energy Expenditure

Both short term overfeeding and chronic obesity result in increased energy expenditure. To test whether mTORC1 plays a role in the short-term responses to obesegenic diets, we measured energy expenditure in single-housed mice during this dietary shift. As described in Figure 1A, animals were single housed and vehicle-injected daily for 3 days to acclimatize the animals. They were then placed in metabolic monitoring cages for 2 days, then were randomized into vehicle or rapamycin injected groups. After 2 days of these injections, all animals were moved from normal chow diets to a high fat diet (HFD). As shown in Figure 1B, the switch to HFD caused a 7.8% increase in energy expenditure in the vehicle injected in the dark phase and a 6.8% increase in the light phase. For the animals injected with rapamycin, there was no increase in energy expenditure within the HFD phase, when compared to vehicle treated mice (p=1.24 x 10-5). These effects on energy expenditure were not associated with differences in physical activity (Figure 1C). These data support the hypothesis that mTORC1 is required for the increase in energy expenditure in response to HFD feeding.

## Activation of mTORC1 in Muscle is Sufficient for Increased Energy Expenditure

To test whether muscle mTORC1 activation was sufficient to affect adiposity and energy expenditure, we performed indirect calorimetry studies on *Ckmm-Cre* driven *Tsc1* knockout mice. Consistent with prior reports using *ACTA1-Cre* driven *Tsc1* knockout we observed a XX % elevation in energy expenditure in muscle specific *Tsc1* knockout mouse (Figure 1F) but with no observable differences in physical activity during the monitoring period (Figure 1G­­­­). These data are consistent with a physiological role of mTORC1 in moderating organismal energy expenditure. We next evaluated food intake in these animals on both NCD and HFD diets. As shown in Figure 1E, while the HFD animals ingested more calories, there was no difference in energy intake between wild-type and muscle *Tsc1* knockout mice.

## Knockout of *Tsc1* in Muscle Causes Resistance to Age- and Diet-Induced Obesity

To understand the physiological significance of elevated baseline energy expenditure, we determined the body composition of the muscle *Tsc1* knockout animals over the course of XX months on a normal chow diet. While we did not observe any differences in lean mass, we did observe a striking lack of fat mass accumulation as these animals grew in size on a normal chow diet, becoming more apparent as the animals aged (Figures 2A). Previous work using *ACTA1-Cre* mediated knockout of *Tsc1* also observed reductions in fat mass [31], but in those cases lean mass was reduced as well [30,31], which was not observed in this model (Figure 2B). To determine any adipose depot-specific changes, we measured the weights of fat pads from wild-type and *Tsc1* knockout mice, and found that both subcutaneous and epdidymal fat pads were reduced in size (Figures 2C-D).

To determine whether animals with TSC1 ablation in their muscle had different responses to a high fat diet, we placed male and female mice on a diet containing 45% of calories from fat. We found that both male and female muscle-specific *Tsc1* knockout mice were resistant to weight gain on high fat diet. The majority of this difference was in fat mass which was reduced 60% in males and 58% in females by the end of the study (Figure 3E, p=1.0 x10-13). In terms of lean mass, there were no significant differences with the knockout (Figure 3F, p=0.941), though as expected female lean mass was much lower.

Upon sacrifice, fat pads were excised from these animals, and consistent with the body composition determinations, we observed a XXX-YYY% reduction in perigonadal and inguinal fat pad mass for both males and females (all p<). In this study, we observed no changes in the mass of quadriceps, triceps surae or heart tissues (Figure 3H). These data support the hypothesis that muscle *Tsc1I* knockout prevents adipose tissue expansion during a high fat diet, consistent with reference [31].

## Ablation of Muscle *Tsc1* Results in Increased Oxidative Muscle Fibers and Upregulation of Fatty Acid/Amino Acid Uptake Genes

In order to gain some insight into the changes that are occurring within the muscle tissue that may result in the resistance to diet-induced obesity in response to muscle *Tsc1* knockout, we performed RNA sequencing studies. As shown in Figure 4A, we identified 4451 significantly differentially expressed genes in these animals, including 2481 upregulated genes and 1970 downregulated genes (also see Supplementary Table 1). To gain some insights into the pathways and networks associated with these genes, we performed GSEA analysis on this dataset. We identified enrichment of genes regulated by *Tsc2* deletion in MEFs and by treatment with rapamycin [45,46], indicating there are a core set of mTORC1 dependent genes in many tissue types. We found that 58% of the differentially expressed genes in our muscles overlapped with differentially expressed genes in *Tsc2* knockout MEFs [46] (Figure 4B). As shown in Table XX we identified several interesting gene sets that were upregulated including IGF1 targets in MCF-7 cells [47], those involved in calcium trafficking, protein synthesis, and amino acid and fatty acid transport. Most amino acid transporters were increased at the mRNA level (Figure 6B), while both the fatty acid transporter *Cd36* and binding protein *Fabp3* were increased at the transcriptional level (Figure 4C).

We next evaluated markers of muscle fiber types and observed increases in Type I oxidative fiber markers including *Myh7* and *Atp2a2* along with a downregulation of glycolytic fiber type markers including *Myh4* and *Atp2a1* (Figure 4D). Consistent with this and previous studies on *Tsc1* knockout muscles we observed substantially more NADH staining of cryopreserved muscle sections (Figure 4E). We did not observe any significant changes in transcript levels of mitochondrial genes, or differences in mitochondrial copy number (Supplementary Figure XXA-B), nor significant elevations in UCP1-3 mRNA. This suggested that the increased metabolic rate in these muscles may be due to a different uncoupling mechanism.

In terms of the source of metabolic, we noted that there were dramatic increases in the ER Calcuim pump SERCA2 (encoded by *Atp2a2*), and its un-coupler Sarcolipin (encoded by *Sln*; Figure 4F). Sarcolipin has been reported to play a role in muscle-specific thermogenesis [48–51]. To evaluate SERCA2/Sarcolipin protein levels, we performed western blotting of these lysates to evaluate expression of SERCA2 and Sarcolipin and observed that these proteins are also upregulated (Figure 4G). We therefore propose that the increased oxidative activity in these muscles may be related to increased futile cycling of ATP by uncoupled SERCA2 at the ER. Consistent with this hypothesis we observe increases in the expression of *Stim1*, *Mfn1-2* and the subunits of the mitochondrial calcium importer (*Mcu, Micu1* and *Micu2* see Supplementary Figure XXX). We also observed reductions in *Ryr1* expression and reductions several plasma membrane Ca2+ transporters (see Supplementary Figure XXX). These changes are likely adaptive mechanisms to manage increased intracellular Ca2+ levels caused by SERCA2 uncoupling.

## Deletion of *Tsc1* in Muscle Tissues Reduces Lifespan.

We next observed these animals without manipulation as they aged, asking whether the elevated metabolic rate had any effects on the lifespan of these mice. We observed increased signs of aging, including hunched and scruffy appearances at an earlier age in the knockout animals but not any of the control littermates. As shown in Figure 5, muscle-specific *Tsc1* knockout mice died of natural causes at a higher rate. Based on a Cox-proportional hazard model the hazard ratio was 4.17-fold higher compared to non-knockout littermates (p=2.0 x 10-5).

A subset of mice were stored in formalin and sent for veterinary pathology, but no consistent cause of death was identified. In animals with histologic evidence of lesions, the predominant process was neoplasia, and the specific etiology was lymphoma/lymphosarcoma affecting multiple organs, though this was only true for wild type (two out of four) but not knockout animals (none out of three). The lack of a specific diagnosis does not necessarily confirm the lack of lesions in examined animals; rather, autolysis and the small number of animals evaluated may have resulted in loss of identifiable processes or tissues in which an etiology was present in-life.

# Discussion

We report here that activation of mTORC1 in muscle tissue results in the accumulation of glycogen and triglycerides, with a shift towards more oxidative muscle fiber types. This, coupled with no detectable decrease in *in vivo* insulin sensitivity is reminiscent of the “athlete’s paradox”, in which muscles of endurance trained athletes are insulin normosensitive, in spite of increased nutrient deposition [52,53]. These findings are concordant with a role of mTORC1 in this process, as this kinase is also activated during resistance exercise and muscle growth [54,55].

Skeletal muscle is an extremely important organ for aging, as humans with high baseline grip strength have 20-217% decreased risk of all-cause mortality, irrespective of gender or body mass index [19–24]. Candidate gene studies on aging have also implicated genes with important roles in muscle tissue, such as *IGF1R*, *AKT1* and *FOXO3A* [56,57]. For example, in humans, polymorphisms in *FOXO3A* have been associated with lengthened lifespan[57–63]. Both mouse and fruit fly models of *FOXO3A* loss of function result in stronger and longer living model organisms [64–66].

The rapamycin experiments presented here do not speak to tissue specificity they are consistent with previous reports demonstrating rapamycin sensitivity in cold-induced thermogenesis [67,68]. While the focus of those studies has been on the important roles of mTORC1 in BAT function, they may also speak to the role of mTORC1 in muscle or other tissues. Future studies, with temporal and tissue specific loss of function of mTORC1 at thermoneutrality will be key to understanding the relative importance of muscle and BAT in both diet and cold-induced thermogenesis. Furthermore findings that mTORC1 is important for thermogenesis in both BAT and muscle tissues may also indicate a broader role of mTORC1 in nutrient homeostasis. One response to nutrient overload is to promote anabolism, consistent with mTORC1-dependent activation of protein synthesis, lipogenesis, and glycogenesis [27,69,70]. We propose that it is reasonable that nutrient overload may also promote ineffective catabolism to reduce nutrient stress.

One mechanism linking muscle mTORC1 activity to elevated energy dissipation may be an indirect pathway, associated with elevations in muscle-derived FGF21 [31,71,72] or other factors. Another possibility is that these effects are autonomous to the muscle tissue. The pathways underlying alterations in fiber type are unclear but since amino acids and fatty acids are both reliant on mitochondria to generate fuel, it is possible that an alternation in substrate flux could result more oxidative fibers, which then leads to more increased lipid oxidation and less adiposity. This hypothesis is consistent with anti-obesegenic effects of muscle specific *Cd36* overexpression [73] and with the observation that elevated free fatty acids can promote mitochondrial biogenesis [74]. Increased nutrient uptake, coupled with elevated mitochondria would be likely to produce more ATP in these cells. This in and of itself would not be sufficient to produce heat, so we also propose that increased uncoupling of SERCA2 by sarcolipin may consume ATP to generate heat [48,75]. This hypothesis would be consistent with upregulation of sarcolipin by HFD, exacerbated obesity when *Sln* is ablated [48–50] and prevention when it is overexpressed [51]. As such muscle *Tsc1* knockout animals could be plausibly thermogenic due to multiple muscle-autonomous adaptations including amino and fatty acid transport, mitochondrial activity and sarcolipin-mediated uncoupling.

These findings support the hypothesis that activation of mTORC1 and its downstream targets, specifically in muscle tissue may play a role in nutrient-dependent thermogenesis and therefore may be effective targets for weight loss interventions, but may come at a cost to organismal lifespan.

# Acknowledgements

The authors would like to thank Nathan Qi and Melanie Schmitt of the UM Metabolic Phenotyping Core for assistance with CLAMS studies on the muscle *Tsc1* knockout mice. William Taylor, Caitlin Costelle and Felicia Waller at the UTHSC Molecular Resource Center provided support for the transcriptomic studies. We would also like to thank the other members of the Bridges, Han, and Saltiel laboratories for helpful discussions regarding this project.

This work was supported by Le Bonheur Grant 650700 (DB), NIH Grants DK107535 (DB), XXXX (ARS), funds from the Memphis Research Consortium (DB and JCH), the Center for Integrative and Translational Genetics (DB) and the UTHSC Department of Physiology Qiugley Award (IH). This work also utilized Core Services supported by NIH grants DK089503, DK110768, and AR069620 to the University of Michigan.

# References

[1] World Health Organization., 2013. Obesity and Overweight. http://www.who.int/mediacentre/factsheets/fs311/en/.

[2] Leibel, R.L., Hirsch, J., 1984. Diminished energy requirements in reduced-obese patients. Metabolism 33(2): 164–70, Doi: 10.1016/0026-0495(84)90130-6.

[3] Leibel, R.L., Rosenbaum, M., Hirsch, J., Eibel, R.U.L.L., Osenbaum, M.I.R., Leibel, R.L., et al., 1995. Changes in energy expenditure resulting from altered body weight. The New England Journal of Medicine 332(10): 621–8, Doi: 10.1056/NEJM199503093321001.

[4] Sumithran, P., Prendergast, L.A., Delbridge, E., Purcell, K., Shulkes, A., Kriketos, A., et al., 2011. Long-Term Persistence of Hormonal Adaptations to Weight Loss. New England Journal of Medicine 365(17): 1597–604, Doi: 10.1056/NEJMoa1105816.

[5] Bray, G.A., Smith, S.R., de Jonge, L., Xie, H., Rood, J., Martin, C.K., et al., 2012. Effect of dietary protein content on weight gain, energy expenditure, and body composition during overeating: a randomized controlled trial. JAMA 307(1): 47–55, Doi: 10.1001/jama.2011.1918.

[6] Ebbeling, C.B., Swain, J.F., Feldman, H. a., Wong, W.W., Hachey, D.L., Garcia-Lago, E., et al., 2012. Effects of dietary composition on energy expenditure during weight-loss maintenance. JAMA 307(24): 2627–34, Doi: 10.1001/jama.2012.6607.

[7] Efeyan, A., Comb, W.C., Sabatini, D.M., 2015. Nutrient-sensing mechanisms and pathways. Nature 517(7534): 302–10, Doi: 10.1038/nature14190.

[8] Yecies, J.L., Zhang, H.H., Menon, S., Liu, S., Yecies, D., Lipovsky, A.I., et al., 2011. Akt Stimulates Hepatic SREBP1c and Lipogenesis through Parallel mTORC1-Dependent and Independent Pathways. Cell Metabolism 14(1): 21–32, Doi: 10.1016/j.cmet.2011.06.002.

[9] Chakrabarti, P., English, T., Shi, J., Smas, C.M., Kandror, K. V., 2010. Mammalian target of rapamycin complex 1 suppresses lipolysis, stimulates lipogenesis, and promotes fat storage. Diabetes 59(4): 775–81, Doi: 10.2337/db09-1602.

[10] Li, S., Brown, M.S., Goldstein, J.L., 2010. Bifurcation of insulin signaling pathway in rat liver: mTORC1 required for stimulation of lipogenesis, but not inhibition of gluconeogenesis. Proceedings of the National Academy of Sciences of the United States of America 107(8): 3441–6, Doi: 10.1073/pnas.0914798107.

[11] Lu, B., Bridges, D., Yang, Y., Fisher, K., Cheng, A., Chang, L., et al., 2014. Metabolic crosstalk: molecular links between glycogen and lipid metabolism in obesity. Diabetes 63(9): 2935–48, Doi: 10.2337/db13-1531.

[12] Zhang, H.H., Huang, J., Düvel, K., Boback, B., Wu, S., Squillace, R.M., et al., 2009. Insulin stimulates adipogenesis through the Akt-TSC2-mTORC1 pathway. PloS One 4(7): e6189, Doi: 10.1371/journal.pone.0006189.

[13] Hatfield, I., Harvey, I., Yates, E.R., Redd, J.R., Reiter, L.T., Bridges, D., 2015. The role of TORC1 in muscle development in Drosophila. Scientific Reports 5: 9676, Doi: 10.1038/srep09676.

[14] Erbay, E., Chen, J., 2001. The mammalian target of rapamycin regulates C2C12 myogenesis via a kinase-independent mechanism. The Journal of Biological Chemistry 276(39): 36079–82, Doi: 10.1074/jbc.C100406200.

[15] Shah, O.J., Wang, Z., Hunter, T., 2004. Inappropriate activation of the TSC/Rheb/mTOR/S6K cassette induces IRS1/2 depletion, insulin resistance, and cell survival deficiencies. Current Biology 14(18): 1650–6, Doi: 10.1016/j.cub.2004.08.026.

[16] Bjedov, I., Toivonen, J.M., Kerr, F., Slack, C., Jacobson, J., Foley, A., et al., 2010. Mechanisms of life span extension by rapamycin in the fruit fly Drosophila melanogaster. Cell Metabolism 11(1): 35–46, Doi: 10.1016/j.cmet.2009.11.010.

[17] Harrison, D.E., Strong, R., Sharp, Z.D., Nelson, J.F., Astle, C.M., Flurkey, K., et al., 2009. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. Nature 460(7253): 392–5, Doi: 10.1038/nature08221.

[18] Kapahi, P., Zid, B.M., Harper, T., Koslover, D., Sapin, V., Benzer, S., 2004. Regulation of lifespan in Drosophila by modulation of genes in the TOR signaling pathway. Current Biology 14(10): 885–90, Doi: 10.1016/j.cub.2004.03.059.

[19] Rantanen, T., Harris, T., Leveille, S.G., Visser, M., Foley, D., Masaki, K., et al., 2000. Muscle strength and body mass index as long-term predictors of mortality in initially healthy men. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 55(3): M168–73, Doi: 10.1093/gerona/55.3.M168.

[20] Ling, C.H.Y., Taekema, D., De Craen, A.J.M., Gussekloo, J., Westendorp, R.G.J., Maier, A.B., 2010. Handgrip strength and mortality in the oldest old population: The Leiden 85-plus study. Cmaj 182(5): 429–35, Doi: 10.1503/cmaj.091278.

[21] Sasaki, H., Kasagi, F., Yamada, M., Fujita, S., 2007. Grip strength predicts cause-specific mortality in middle-aged and elderly persons. The American Journal of Medicine 120(4): 337–42, Doi: 10.1016/j.amjmed.2006.04.018.

[22] Gale, C.R., Martyn, C.N., Cooper, C., Sayer, A.A., 2007. Grip strength, body composition, and mortality. International Journal of Epidemiology 36(1): 228–35, Doi: 10.1093/ije/dyl224.

[23] Rantanen, T., Volpato, S., Ferrucci, L., Heikkinen, E., Fried, L.P., Guralnik, J.M., 2003. Handgrip strength and cause-specific and total mortality in older disabled women: exploring the mechanism. Journal of the American Geriatrics Society 51(5): 636–41, Doi: 10.1034/j.1600-0579.2003.00207.x.

[24] Metter, E.J., Talbot, L. a., Schrager, M., Conwit, R., 2002. Skeletal muscle strength as a predictor of all-cause mortality in healthy men. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 57(10): B359–65, Doi: 10.1093/gerona/57.10.B359.

[25] Ozcan, U., Ozcan, L., Yilmaz, E., Düvel, K., Sahin, M., Manning, B.D., et al., 2008. Loss of the Tuberous Sclerosis Complex Tumor Suppressors Triggers the Unfolded Protein Response to Regulate Insulin Signaling and Apoptosis. Molecular Cell 29(5): 541–51, Doi: 10.1016/j.molcel.2007.12.023.

[26] Bentzinger, C.F., Lin, S., Romanino, K., Castets, P., Guridi, M., Summermatter, S., et al., 2013. Differential response of skeletal muscles to mTORC1 signaling during atrophy and hypertrophy. Skeletal Muscle 3(1): 6, Doi: 10.1186/2044-5040-3-6.

[27] Laplante, M., Sabatini, D.M., 2010. mTORC1 activates SREBP-1c and uncouples lipogenesis from gluconeogenesis. Proceedings of the National Academy of Sciences of the United States of America 107(8): 3281–2, Doi: 10.1073/pnas.1000323107.

[28] DeFronzo, R.A., Ferrannini, E., Sato, Y., Felig, P., Wahren, J., 1981. Synergistic interaction between exercise and insulin on peripheral glucose uptake. The Journal of Clinical Investigation 68(6): 1468–74.

[29] Rolfe, D.F., Brown, G.C., 1997. Cellular energy utilization and molecular origin of standard metabolic rate in mammals. Physiological Reviews 77(3): 731–58.

[30] Castets, P., Lin, S., Rion, N., Di Fulvio, S., Romanino, K., Guridi, M., et al., 2013. Sustained activation of mTORC1 in skeletal muscle inhibits constitutive and starvation-induced autophagy and causes a severe, late-onset myopathy. Cell Metabolism 17(5): 731–44, Doi: 10.1016/j.cmet.2013.03.015.

[31] Guridi, M., Tintignac, L.A., Lin, S., Kupr, B., Castets, P., Rüegg, M.A., 2015. Activation of mTORC1 in skeletal muscle regulates whole-body metabolism through FGF21. Science Signaling 8(402): ra113-ra113, Doi: 10.1126/scisignal.aab3715.

[32] Cunningham, J.T., Rodgers, J.T., Arlow, D.H., Vazquez, F., Mootha, V.K., Puigserver, P., 2007. mTOR controls mitochondrial oxidative function through a YY1-PGC-1alpha transcriptional complex. Nature 450(7170): 736–40, Doi: 10.1038/nature06322.

[33] Ramanathan, A., Schreiber, S.L., 2009. Direct control of mitochondrial function by mTOR. Proceedings of the National Academy of Sciences of the United States of America 106(52): 22229–32, Doi: 10.1073/pnas.0912074106.

[34] Koyanagi, M., Asahara, S.-I., Matsuda, T., Hashimoto, N., Shigeyama, Y., Shibutani, Y., et al., 2011. Ablation of TSC2 enhances insulin secretion by increasing the number of mitochondria through activation of mTORC1. PLoS ONE 6(8), Doi: 10.1371/journal.pone.0023238.

[35] Fujita, S., Dreyer, H.C., Drummond, M.J., Glynn, E.L., Cadenas, J.G., Yoshizawa, F., et al., 2007. Nutrient signalling in the regulation of human muscle protein synthesis. The Journal of Physiology 582(Pt 2): 813–23, Doi: 10.1113/jphysiol.2007.134593.

[36] Cuthbertson, D., Smith, K., Babraj, J., Leese, G., Waddell, T., Atherton, P., et al., 2005. Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. The FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology 19(3): 422–4, Doi: 10.1096/fj.04-2640fje.

[37] Tschöp, M.H., Speakman, J.R., Arch, J.R.S., Auwerx, J., Brüning, J.C.C., Chan, L., et al., 2011. A guide to analysis of mouse energy metabolism. Nature Methods 9(1): 57–63, Doi: 10.1038/nmeth.1806.

[38] Cheng, A., Zhang, M., Gentry, M.S., Worby, C.A., Dixon, J.E., Saltiel, A.R., 2007. A role for AGL ubiquitination in the glycogen storage disorders of Lafora and Cori’s disease. Genes & Development 21(19): 2399–409, Doi: 10.1101/gad.1553207.

[39] Cheng, A., Zhang, M., Okubo, M., Omichi, K., Saltiel, A.R., 2009. Distinct mutations in the glycogen debranching enzyme found in glycogen storage disease type III lead to impairment in diverse cellular functions. Human Molecular Genetics 18(11): 2045–52, Doi: 10.1093/hmg/ddp128.

[40] R Core Team., 2013. R: A Language and Environment for Statistical Computing.

[41] Bates, D.M., Mächler, M., Bolker, B., Walker, S., 2014. Fitting Linear Mixed-Effects Models using lme4. ArXiv 1406.5823: 1–51.

[42] Therneau, T.M., Grambsch, P.M., 2000. Modeling Survival Data: Extending the Cox Model. New York, NY: Springer New York.

[43] Therneau, T., 2012. A Package for Survival Analysis in S. R package version. Survival.

[44] Benjamini, Y., Hochberg, Y., 1995. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society. Series B 57(1): 289–300.

[45] Bilanges, B., Argonza-Barrett, R., Kolesnichenko, M., Skinner, C., Nair, M., Chen, M., et al., 2007. Tuberous sclerosis complex proteins 1 and 2 control serum-dependent translation in a TOP-dependent and -independent manner. Mol Cell Biol 27(16): 5746–64, Doi: MCB.02136-06 [pii]\r10.1128/MCB.02136-06.

[46] Düvel, K., Yecies, J.L., Menon, S., Raman, P., Lipovsky, A.I., Souza, A.L., et al., 2010. Activation of a Metabolic Gene Regulatory Network Downstream of mTOR Complex 1. Molecular Cell 39(2): 171–83, Doi: 10.1016/j.molcel.2010.06.022.

[47] Pacher, M., Seewald, M.J., Mikula, M., Oehler, S., Mogg, M., Vinatzer, U., et al., 2007. Impact of constitutive IGF1/IGF2 stimulation on the transcriptional program of human breast cancer cells. Carcinogenesis 28(1): 49–59, Doi: 10.1093/carcin/bgl091.

[48] Bal, N.C., Maurya, S.K., Sopariwala, D.H., Sahoo, S.K., Gupta, S.C., Shaikh, S.A., et al., 2012. Sarcolipin is a newly identified regulator of muscle-based thermogenesis in mammals. Nature Medicine 18(10): 1575–9, Doi: 10.1038/nm.2897.

[49] Bombardier, E., Smith, I.C., Gamu, D., Fajardo, V.A., Vigna, C., Sayer, R.A., et al., 2013. Sarcolipin trumps β-adrenergic receptor signaling as the favored mechanism for muscle-based diet-induced thermogenesis. FASEB Journal 27(9): 3871–8, Doi: 10.1096/fj.13-230631.

[50] Rowland, L.A., Maurya, S.K., Bal, N.C., Kozak, L., Periasamy, M., 2016. Sarcolipin and uncoupling protein 1 play distinct roles in diet-induced thermogenesis and do not compensate for one another. Obesity 00(00): 10–3, Doi: 10.1002/oby.21542.

[51] Maurya, S.K., Periasamy, M., 2015. Sarcolipin is a novel regulator of muscle metabolism and obesity. Pharmacological Research 102: 270–5, Doi: 10.1016/j.phrs.2015.10.020.

[52] Goodpaster, B.H., He, J., Watkins, S., Kelley, D.E., 2001. Skeletal muscle lipid content and insulin resistance: Evidence for a paradox in endurance-trained athletes. Journal of Clinical Endocrinology and Metabolism 86(12): 5755–61, Doi: 10.1210/jc.86.12.5755.

[53] Amati, F., Dubé, J.J., Alvarez-Carnero, E., Edreira, M.M., Chomentowski, P., Coen, P.M., et al., 2011. Skeletal muscle triglycerides, diacylglycerols, and ceramides in insulin resistance: Another paradox in endurance-trained athletes? Diabetes 60(10): 2588–97, Doi: 10.2337/db10-1221.

[54] Philp, A., Hamilton, D.L., Baar, K., 2011. Signals mediating skeletal muscle remodeling by resistance exercise: PI3-kinase independent activation of mTORC1. Journal of Applied Physiology 110(2): 561–8, Doi: 10.1152/japplphysiol.00941.2010.

[55] Hamilton, D.L., Philp, A., MacKenzie, M.G., Patton, A., Towler, M.C.M.C., Gallagher, I.J., et al., 2014. Molecular brakes regulating mTORC1 activation in skeletal muscle following synergist ablation. American Journal of Physiology - Endocrinology And Metabolism 307(4): E365–73, Doi: 10.1152/ajpendo.00674.2013.

[56] Suh, Y., Atzmon, G., Cho, M.-O., Hwang, D., Liu, B., Leahy, D.J., et al., 2008. Functionally significant insulin-like growth factor I receptor mutations in centenarians. Proceedings of the National Academy of Sciences of the United States of America 105(9): 3438–42, Doi: 10.1073/pnas.0705467105.

[57] Pawlikowska, L., Hu, D., Huntsman, S., Sung, A., Chu, C., Chen, J., et al., 2009. Association of common genetic variation in the insulin/IGF1 signaling pathway with human longevity. Aging Cell 8(4): 460–72, Doi: 10.1111/j.1474-9726.2009.00493.x.

[58] Willcox, B.J., Donlon, T. a., He, Q., Chen, R., Grove, J.S., Yano, K., et al., 2008. FOXO3A genotype is strongly associated with human longevity. Proceedings of the National Academy of Sciences of the United States of America 105(37): 13987–92, Doi: 10.1073/pnas.0801030105.

[59] Bao, J.-M., Song, X.-L., Hong, Y.-Q., Zhu, H.-L., Li, C., Zhang, T., et al., 2014. Association between FOXO3A gene polymorphisms and human longevity: a meta-analysis. Asian Journal of Andrology 16(3): 446–52, Doi: 10.4103/1008-682X.123673.

[60] Anselmi, C.V., Malovini, A., Roncarati, R., Novelli, V., Villa, F., Condorelli, G., et al., 2009. Association of the FOXO3A locus with extreme longevity in a southern Italian centenarian study. Rejuvenation Research 12(2): 95–104, Doi: 10.1089/rej.2008.0827.

[61] Flachsbart, F., Caliebe, A., Kleindorp, R., Blanché, H., von Eller-Eberstein, H., Nikolaus, S., et al., 2009. Association of FOXO3A variation with human longevity confirmed in German centenarians. Proceedings of the National Academy of Sciences of the United States of America 106(8): 2700–5, Doi: 10.1073/pnas.0809594106.

[62] Li, Y., Wang, W.J., Cao, H., Lu, J., Wu, C., Hu, F.Y., et al., 2009. Genetic association of FOXO1A and FOXO3A with longevity trait in Han Chinese populations. Human Molecular Genetics 18(24): 4897–904, Doi: 10.1093/hmg/ddp459.

[63] Soerensen, M., Dato, S., Christensen, K., McGue, M., Stevnsner, T., Bohr, V. a., et al., 2010. Replication of an association of variation in the FOXO3A gene with human longevity using both case-control and longitudinal data. Aging Cell 9(6): 1010–7, Doi: 10.1111/j.1474-9726.2010.00627.x.

[64] Giannakou, M.E., Goss, M., Jünger, M.A., Hafen, E., Leevers, S.J., Partridge, L., 2004. Long-lived Drosophila with overexpressed dFOXO in adult fat body. Science 305(5682): 361, Doi: 10.1126/science.1098219.

[65] Hwangbo, D.S., Gershman, B., Tu, M.-P., Palmer, M., Tatar, M., 2004. Drosophila dFOXO controls lifespan and regulates insulin signalling in brain and fat body. Nature 429(6991): 562–6, Doi: 10.1038/nature03446.

[66] Milan, G., Romanello, V., Pescatore, F., Armani, A., Paik, J.-H., Frasson, L., et al., 2015. Regulation of autophagy and the ubiquitin-proteasome system by the FoxO transcriptional network during muscle atrophy. Nature Communications 6: 6670, Doi: 10.1038/ncomms7670.

[67] Liu, D., Bordicchia, M., Zhang, C., Fang, H., Wei, W., Li, J.-L.L., et al., 2016. Activation of mTORC1 is essential for β-adrenergic stimulation of adipose browning. Journal of Clinical Investigation 1(5): 1–13, Doi: 10.1172/JCI83532.

[68] Tran, C.M., Mukherjee, S., Ye, L., Frederick, D.W., Kissig, M., Davis, J.G., et al., 2016. Rapamycin blocks induction of the thermogenic program in white adipose tissue. Diabetes 65(April 2015): 1–35, Doi: 10.2337/db15-0502.

[69] Iadevaia, V., Huo, Y., Zhang, Z., Foster, L.J., Proud, C.G., 2012. Roles of the mammalian target of rapamycin, mTOR, in controlling ribosome biogenesis and protein synthesis: Figure 1. Biochemical Society Transactions 40(1): 168–72, Doi: 10.1042/BST20110682.

[70] Lu, B., Bridges, D., Yang, Y., Fisher, K., Cheng, A., Chang, L., et al., 2014. Metabolic crosstalk: molecular links between glycogen and lipid metabolism in obesity. Diabetes 63(9): 2935–48, Doi: 10.2337/db13-1531.

[71] Tsai, S., Sitzmann, J.M., Dastidar, S.G., Rodriguez, A. a., Vu, S.L., McDonald, C.E., et al., 2015. Muscle-specific 4E-BP1 signaling activation improves metabolic parameters during aging and obesity. The Journal of Clinical Investigation 125(8): 2952–64, Doi: 10.1172/JCI77361.

[72] Kim, K.H., Jeong, Y.T., Oh, H., Kim, S.H., Cho, J.M., Kim, Y.-N., et al., 2012. Autophagy deficiency leads to protection from obesity and insulin resistance by inducing Fgf21 as a mitokine. Nature Medicine, Doi: 10.1038/nm.3014.

[73] Ibrahimi, A., Bonen, A., Blinn, W.D., Hajri, T., Li, X., Zhong, K., et al., 1999. Muscle-specific overexpression of FAT/CD36 enhances fatty acid oxidation by contracting muscle, reduces plasma triglycerides and fatty acids, and increases plasma glucose and insulin. Journal of Biological Chemistry 274(38): 26761–6, Doi: 10.1074/jbc.274.38.26761.

[74] Garcia-Roves, P., Huss, J.M., Han, D.-H., Hancock, C.R., Iglesias-Gutierrez, E., Chen, M., et al., 2007. Raising plasma fatty acid concentration induces increased biogenesis of mitochondria in skeletal muscle. Proceedings of the National Academy of Sciences of the United States of America 104(25): 10709–13, Doi: 10.1073/pnas.0704024104.

[75] Sahoo, S.K., Shaikh, S.A., Sopariwala, D.H., Bal, N.C., Periasamy, M., 2013. Sarcolipin Protein Interaction with Sarco(endo)plasmic Reticulum Ca2+ATPase (SERCA) Is Distinct from Phospholamban Protein, and Only Sarcolipin Can Promote Uncoupling of the SERCA Pump. Journal of Biological Chemistry 288(10): 6881–9, Doi: 10.1074/jbc.M112.436915.

# Figure Legends

**Figure 1: Effects of high protein diet on body composition and energy expenditure.** Body composition was determined weekly with body weight (A), lean mass (B) and fat mass (C). D) Weights of fat pad depots at the end of the 14 week feedint study. Statistical significance (p<0.05, n=12/group) was denoted via asterisks based on χ2 test (A-C) or Student’s *t-*test (D).

**Figure 2: Body composition of *Tsc1* knockout mice.** E) Fat and F) Fat-Free mass of animals starting HFD at 10 weeks of age (n=9 and 23 for males and 17 and 44 for females)

**Figure 3: Knockout of *Tsc1* in muscle leads to increased fasted glycogen and triglyceride levels in quadriceps.** A)Protein lysates from quadriceps dissected from animals fasted overnight were prepared in HNTG buffer and blotted as indicated. B) Quantification of processed SREBP1 and the phosphorylation ratio of glycogen synthase. Statistical significance (p<0.05, n=5/7) was determined via a Wilcoxon Rank-Sum test (C) after correcting for multiple hypotheses.

# Supplementary Figure Legends

**Supplementary Table 1: Gene expression differences in muscle *Tsc1* knockout quadriceps.**