Reviewers' comments:   
  
Reviewer #1 (Technical Comments to the Author):   
  
The experiments are generally well controlled; however, there is a lack of detail provided for many experiments that need addressing prior to publication.   
  
- Details are needed in relation to timepoints used for Rapamycin treatment for the C2C12 cells in Fig1 to ascertain if the effects on target gene expression are acute or chronic.

- Experiments in the C2C12 model profile expression of key myogenic target genes but at a timepoint after the phenotypic effect i.e. assessing these genes could be indicative of the consequence of impaired differentiation, not the effect of Rapamycin itself.

**Specified in both the results section and the Figure 1 legend, that the rapamycin was delivered for the entire 9 days of the differentiation time course and therefore represents a chronic treatment.**

- Not stated if data is shown as SD or SEM.

**Pointed out in the methods section (under statistics) and in each of the figure legends that error bars indicate mean +/- SEM.**

- Data is Fig 2 uses the progeny of the cross with Raptor shRNA or TSC1 shRNA as controls when the correct control should be flies expressing control shRNA. Why was this not done?

- Not clear what findings were statistically different since no annotation on graphs.

**Asterisks indicating statistical significance are now included for all figures. Other axis labels**

- No labels on Fig 4-6

**Added these figure labels.**

- No details are included in the methods about the Hand-Gal4 stock.

**Added this to the methods section.**

- Many of the conclusions are too definitive for the data provided (detailed specifically in comments to author) and need to be revised or supported by additional data.

**Adjustments to the interpretation of our data are noted in each section below.**  
  
Reviewer #1 (Remarks to the Author):   
  
The manuscript is interesting and presents some potentially important findings on the effect of mTORC1 and Rapamycin treatment during myogenesis. However, there are a number of scientific and technical points that require addressing to support the conclusions made.   
  
1. During the introduction section, the authors state that myogenesis continues throughout life. I find this a strange statement since myogenesis is defined as the formation of muscle. This statement needs revised. Also, this point is not supported by reference 19, a manuscript specifically showing that muscle mass is maintained and that muscles are capable of hypertrophy in spite of genetic ablation of satellite cells in the adult. 

**This has been revised to now read the following, removing reference 19 (the numbering in this response does not reflect numbering in the manuscript):**

**Myogenesis occurs throughout life via the differentiation of muscle precursor cells called satellite cells**1,2.

2. Can the authors provide more detail about the Rapamycin/C2C12 experiment in Fig 1? Specifically, when on day 9 was the last Rapamycin treatment in relation to the experimental endpoint? It is possible that the changes observed are due to acute treatment of C2C12 MT with Rapamycin. Has this been assessed? 

**We have specified both the methods section and in the figure legend that the most recent rapamycin treatment was one day prior to the cell lysis. This indicates that these changes are reflective of chronic rapamycin administration.**

3. The gene expression studies in Fig 1 do not support the conclusion that mTORC1 is required for transcription downstream of Myod1 as stated. The expression was measured at day 9 of treatment and so could be reflective of the expression level in a cell where differentiation is impaired, not as a direct effect of mTORC1 inhibition.   
  
4. The authors state that differentiation is impaired. It would be advisable for the authors to label cells for myosin heavy chain and count the numbers of myosin heavy chain positive cells. Otherwise, the phenotype could be a result of impaired fusion.

**Based on the data presented in Figure 1C, we see no tubulation of rapamycin treated cells, a stage that precedes fusion. To demonstrate this more clearly, we present below images from an earlier timepoint (XX) days as Figure 1 of this response. At this stage, DMSO treated cells efficiently form fused myocytes but the rapamycin treated cells do not. These findings are now described in the results section:**

**We did not observe any fused myocytes in the rapamycin treated cells, suggesting that the earliest rapamycin-sensitive event is prior to myocyte fusion, which results in impaired myotubule formation. This is consistent with previous studies examining the effects of rapamycin on myoblast differentiation**3–7**.**

5. The authors state that the differences in phenotype are due to a less efficient effect (presumably they mean expression of the shRNA?) by c179 Gal4 compared to mef2-Gal4 yet no data is actually provided to show the efficiency of knockdown with the shRNA due to the different Gal4 lines so the conclusion can not be made.   
  
6. Have the authors assessed the effect of their intervention on the imaginal myoblasts associated with the wing discs. These cells, which form the indirect flight muscles, will express the mef2-shRNA and so presumably are affected by the treatment. This is an important point because this could explain why the flies are unable to enclose (weakness in the IFM) and why the mef2-GAL4 effect is stronger than c179-GAL4. This would have an important impact on the conclusions made. Have the authors looked at these cells and/or used a functional test that assesses the function of these muscles directly?   
  
  
Reviewer #2 (Remarks to the Author):   
  
Summary:   
This manuscript describes studies that attempt to investigate the necessity of mTORC1 (more specifically dTORC1) in the development of skeletal muscle in the model system Drosophila melanogaster in vivo. Specifically, the authors have used the shRNA to knockdown the mTORC1 component, Raptor, specifically in skeletal muscle during development. The main novel findings are that the developmental knockdown of Raptor in skeletal muscle leads to reduced muscle function which, if severe enough results in an inability to eclose from pupal cases and subsequent death. If the knockdown is less efficacious and eclosure is possible, impaired muscle function is still evident across the lifespan and, especially in males, and is associated with reduced longevity.   
  
Major comments:   
The C2C12 time course experiments that examine the effect of the allosteric mTORC1 inhibitor, rapamycin, are novel especially with regard to Myf5, Mef2c and Cdkn1a (p21) mRNA expression. Given the emphasis placed in this section on the role of mTORC1 on MyoD stability and its subsequent effects on Myf5, Mef2c and Cdkn1a expression, examination of the changes in the time course of protein levels of MyoD, with and without rapamycin, would be relatively simple and provide more solid support for the proposed role that mTORC1 regulates MyoD protein stability.

The in vivo experiments appear to be well performed, however, I have some issue with the conclusions drawn from them. i.e. The main conclusion, that the developmental loss of Raptor, and thus dTORC1, results in a "developmental problem in myogenesis", are based on muscle function parameters (e.g. an inability to eclose and the 4 min walking test). In the absence of mRNA/protein data and/or histological data on muscle fiber numbers and morphology, how can the authors separate developmental issues with myogenesis per se from post-development myogenesis-independent but dTORC1-dependent events e.g. changes in energy metabolism resulting in increased weakness and fatiguability, changes in protein turnover that results in muscle atrophy and/or the development of a postnatal dystrophic-like phenotype similar to what happens in Raptor knockout mice?

Indeed, the reduced lifespan is similar to what is seen in the muscle-specific Raptor knockout mouse (Bentzinger et al., Cell Metab, 2008).

**We have now cited this effect in our expanded discussion section (see below).**

Based on the current data in the manuscript, it seems that the most appropriate conclusion is that Raptor and/or dTORC1 is necessary for skeletal muscle function. Additional data is needed to make the conclusion that dTORC1 is necessary for normal myogenesis per se. Additional data would also better integrate the C2C12 data with the in vivo data.

**We have provided additional data now, describing a late-acting Raptor knockdown, driven by an alternate GAL4 driver. We have also looked more specifically at myotube fusion (see point 4 from reviewer 1) and MyoD protein levels (see first point from this reviewer).**

One further issue is the idea, based on the effect of rapamycin on MRFs in C2C12 and in vivo, that "mTORC1 is required to be active". This statement implies that mTOR kinase activity is necessary; however, there is certainly evidence for mTOR kinase-independent but rapamycin-sensitive mechanisms in myogenesis (see Ge et al., 2009, Am J Physiol; Erbay and Chen, 2001, J Biol Chem; Park and Chen, 2005, J Biol Chem). This possibility needs to be discussed in the manuscript.

Read and add these to the discussion

In fact, the manuscript would benefit greatly from the addition of a Discussion section, separate from the Results, where the above issues can be addressed and a better discussion of how these results compare with data from the mouse muscle-specific Raptor knockout.   
  
  
Minor comments:   
Abstract   
The last sentence is a little too strong. Unless Drosophila is specifically being referred to here, this is not a new mechanism. This has been shown before, although not in vivo (see Park and Chen, 2005, J Biol Chem)

**This sentence has been toned down to read the following:**

**Together these results highlight an important role for TORC1 in late muscle development, integrity or function in both Drosophila and mammalian cells.**

Background   
Page 2, para 1, line 4 - minor point -reference 16 did not investigate developmental arrest in in flies

**Bjedov et al show in Figure 2D that rapamycin blocks fecundity (eggs laid per female) which although is not developmental arrest, implies embryonic lethality. We have modified that sentence to now read:**

**…loss of the obligate mTORC1 complex members mTOR and Raptor, or treatment with rapamycin induces developmental lethality in mice**8–10**, worms**11 **and fruit flies**12**.**

Materials and Methods

Climbing assay section, last line - should this be 30 days or 3 days?

**This has been clarified to read:**

**This assay was first performed within 3 days post eclosure and repeated every ~30 days for a total of 3 trials.**

# References

Note: These numbers do not match the numbering in the manuscript.

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2. Collins, C. a *et al.* Stem cell function, self-renewal, and behavioral heterogeneity of cells from the adult muscle satellite cell niche. *Cell* **122,** 289–301 (2005).

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4. Erbay, E. & Chen, J. The mammalian target of rapamycin regulates C2C12 myogenesis via a kinase-independent mechanism. *J. Biol. Chem.* **276,** 36079–82 (2001).

5. Conejo, R., Valverde, A. M., Benito, M. & Lorenzo, M. Insulin produces myogenesis in C2C12 myoblasts by induction of NF-kappaB and downregulation of AP-1 activities. *J. Cell. Physiol.* **186,** 82–94 (2001).

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7. Park, I.-H. & Chen, J. Mammalian target of rapamycin (mTOR) signaling is required for a late-stage fusion process during skeletal myotube maturation. *J. Biol. Chem.* **280,** 32009–17 (2005).

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9. Guertin, D. A. *et al.* Ablation in mice of the mTORC components raptor, rictor, or mLST8 reveals that mTORC2 is required for signaling to Akt-FOXO and PKCalpha, but not S6K1. *Dev. Cell* **11,** 859–871 (2006).

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11. Jia, K., Chen, D. & Riddle, D. L. The TOR pathway interacts with the insulin signaling pathway to regulate C. elegans larval development, metabolism and life span. *Development* **131,** 3897–906 (2004).

12. Bjedov, I. *et al.* Mechanisms of life span extension by rapamycin in the fruit fly Drosophila melanogaster. *Cell Metab.* **11,** 35–46 (2010).