**To-do list** for revision 1 of “Skeletal Muscle mTORC1 Activation Increases Energy Expenditure and Reduces Longevity in Mice”

Experiments we should definitely perform

1. Find freezer tissues for mTSC1 mice (or collect tissues from new mice, if available)
   1. Measure TSC1 expression (mRNA or protein) in muscle, heart, iWAT, gWAT, liver, etc.
   2. Prepare protein lysates from other muscles. Ideally, gastroc, TA, soleus, diaphragm and EDL. Measure SLN, SERCA1 and SERCA2 by western blot in each of these muscles
   3. Run westerns for proteins involved in Ca2+ kinetics (probably pCaMKII/CaMKII, CSQ), and OXPHOS (I thought we had this somewhere, need to check)
2. Find freezer tissues for HFD and HFD-rapamycin treated mice (assuming we have them)
   1. Prepare protein lysates from muscles (quad/gastroc). Blot for pS6K/S6K, pS6/S6 and/or other markers of mTORC1 activity. Also blot for SLN/SERCA1/SERCA2. Maybe also blot for mTORC2 activity (to show it isn’t inhibited by three days of rapamycin treatment). Since acute treatment only, qPCR might be good to include for *Sln*/*Serca2a1*/*Serca2a2*

Experiments we should consider

1. If there are mTSC1 KO mice available, can we treat some with rapamycin? Would be good to do this while measuring energy expenditure (so in CLAMS). We could then blot for SLN/SERCA in the muscle. I’d like to see this in the paper (the westerns at least) but I also think it would be ok to talk about having not done it as a limitation
2. The dreaded Seahorse experiment. Or Oroboros. But as reviewer 2 stated, this work is probably better suited for a follow up manuscript in the future. The major weakness of the paper is that we ~~did~~  could not quantify the energy requirements/use of skeletal muscle.

Changes to make to the text/figures

Reviewer 1: No comments

Reviewer 2:

1. Tone of paper should better reflect the ‘uncleanliness’ of TSC1 KO as a model of mTORC1 activation
2. If TSC1 KO in heart, we should discuss implications for longevity
3. Maybe remove ITT in chow mice because it confuses the need for the clamp, or change the narrative to help it make sense to clamp after a negative ITT

Reviewer 3:

1. Concerns re. physiological relevance. We did not use an inducible cre. Perhaps something we can note as a limitation? Discussion of developmental adaptations and implications for aging/lifespan
2. Add citations in intro. Section re. fibertype transformation with aging and mTORC1 signaling with mechanical loading
3. Clarify where control groups were combined and where they were not
4. Describe CLAMS protocol and considerations more clearly
5. Describe rationale for rapamycin dose used
6. Clarify what the asterisks on the CLAMS figures mean (i.e., light or dark or both)
7. Add additional info re. stats and asterisks meaning to figure legends (esp. fig. 1)
8. Add symbol to Fig. 1L to show difference in food intake between chow and HFD (not sure we need to since we aren’t actually comparing these groups)
9. Add ages of the mice used at each experiment, particularly the RNAseq muscle collection
10. Add molecular weight marker to S6 blot in Fig. 4
11. Clarify what age is meant by “at an earlier age” (in ref. to old appearance as the mice aged)
12. Prepare a response justifying the inclusion of the necropsy paragraph in the manuscript. Reviewer 3 asked for its removal but I think it is important to report negative data
13. Clarify that the increase in carbohydrate oxidation was only observed in females
14. Note that some of our findings had also been observed in papers from the Rüegg group. Specifically mentioned by reviewer 3 were: the oxidative fiber-type transition, markedly smaller white adipose tissue depots following HFD, better insulin sensitivity following HFD, and the presence of kyphosis at 9 months of age and early death at 12 months