Response to Reviews:

We would like to thank the reviewers for their comments on our manuscript entitled “Brown adipose expansion and remission of glycemic dysfunction in obese SM/J mice”. In response to reviewers’ comments, we have 1) run additional serum assays to assess IGF1, glucagon, adiponectin, and leptin levels; 2) performed additional targeted expression analyses on brown/beiging markers and general adipose genes; and 3) run additional analyses and experiments to highlight a provocative candidate gene, Sfrp1, which provides more mechanistic insight into the phenomenon. Additionally, we have completely re-written the discussion and include references to previous reports of brown adipose mechanisms of action on glucose metabolism that are supported by our results. Specific reviewer comments and our responses follow:

Reviewer #1:

The study analyzes the SM/J mouse model with regard to glycemic control related to obesity. Interestingly these mice develop an improved glucose metabolism between 20-30 weeks of age concomitant with increase in brown adipose tissue. Based on the histological analyses the brown adipose tissue seems to expand through de novo formation. Ablation of brown adipose tissue leads to a "normalization" of the phenotype suggesting that the brown fat is the driver of improved glucose metabolism. This is a very interesting finding; I don’t know of any model that can upregulate BAT at a late stage obesity to promote glucose metabolism. The experiments are well done, and the mouse numbers are quite large, so the data seems solid.

Experimentally I am missing the circulating insulin levels. Those should be added since they are critical for the interpretation of the results.

- We have included text explaining 30 week HF SM/J mice have increased circulating and pancreatic insulin levels, citing our preprint that further explores the pancreatic function of these mice.

The discussion on the other hand is not well done. It is basically a recap of the data even though there are many interesting points that warrant discussion.

First of all, could this be a feature of protection from glucose disbalance during obesity. There is not a lot of data, but it is an intriguing concept.

- We have completely rewritten the discussion and consider the potential mechanism of insulin-stimulated glucose uptake in the expanded BAT as a way of mediating glucose levels before the hyperglycemia gets too bad.

Secondly, there have been quite some reports that glucose metabolism is highly dependently on brown adipose tissue independent of obesity (Samms et al and others which used FGF21 in the context of changing BAT function). This data fit quite nicely and should be discussed in the context of the findings.

- Thank you for pointing us in the direction of these references; indeed our data do fit nicely with these previous studies. We have added text to the discussion regarding obesity-independent effects of BAT on glucose metabolism.
Thirdly, there should be a broader discussion how these new cells are formed. There are many different options which might be triggered by external cues so an exclusive focus on the deregulated pathways in adipose tissue is not enough.

- We have added results and text in the discussion highlighting action of Sfrp1 as a potential mechanism by which brown adipogenesis occurs in these mice.

Reviewer #2:

Carson and colleagues propose the SM/J mouse line, a previously established model for obesity and related conditions, as a novel model for examining the role of brown adipose tissue in systemic metabolism. Previously, these animals have largely been studied up to 20 weeks of age. The authors found that between 20 and 30 weeks of age, the glycemic dysfunction and insulin resistance of SM/J mice on HFD resolve, despite persistence of obesity. The interscapular BAT depot expands and increases as a fraction of total adipose weight between 20 and 30 weeks. Upon removal of this expanded depot, glycemic benefits gained by 30 weeks are then lost. Gene expression analysis of brown (interscapular) and white (gonadal) adipose depots from SM/J mice on HFD at 20 and 30 weeks shows differential expression of a number of interesting genes, including Il7r, Col8a1, MMP12, Pcolce, F7, and Lep.

Major points:

Interesting but rather moderate phenotype - whether the changes seen here would be physiologically relevant beyond this model is not clear, but the metabolic improvements and corresponding BAT expansion seen are somewhat interesting, but the baseline hyperglycemia even at its peak is not very impressive.

- Agreed, the hyperglycemia is not large when considering the baseline hyperglycemia of genetically modified models. The diet-induced hyperglycemia is typical of an inbred, non-genetically modified mouse model. A strength of this model is that the resolution of the hyperglycemia occurs naturally, without genetic manipulation, and that it is dependent on the genetic background of the SM/J strain. We have added text to highlight the unique, genetic background-dependent (and therefore tractable) basis. Additionally, have added text considering the possibility that the mice are able to mediate glucose levels before they become too high (a response which would be genetic and mappable).

Differential gene expression analysis shows interesting results, but the implications of these results are not fully fleshed out. The paper would greatly benefit from a more in-depth mechanistic analysis of these results. Most notably, some genes are highlighted for their role in improved metabolic health, but previous evidence points to the contrary:

An increase in Lep expression is noted, and this increase explained by its role in stimulating glucose uptake. However, hyperleptinemia and leptin resistance are important components of metabolic syndrome, and increased expression of Lep is more consistent with a worsening metabolic phenotype.
• We have added data for circulating leptin levels, which do not significantly increase in the high fat-fed mice from 20 to 30 weeks. This suggests that any effect of increased Lep expression in the BAT likely results in a local effect rather than a systemic one.

• We performed more in depth analyses on the transcriptomics data to focus on insulin-stimulated glucose uptake and the potential mechanistic role of Sfrp1-stimulated brown adipogenesis.

The paper would benefit from a more detailed interrogation of physiologic changes in metabolic phenotype from 20 to 30 weeks (analysis of adipose and circulating adiponectin, leptin, etc).

• We have added data and discussion of serum levels of adiponectin, leptin, glucagon, and IGF1 (illustrated in Supplementary Figure 1) and additionally increased the number of q-rtPCR studies of brown/beiging and general adipose genes.

Line 145: "….whereas the high fat tissue develops a profile significantly trending towards smaller adipocytes at 30 weeks (p=2.2-16) (Figure 2D and E). This suggests that the expansion of the brown adipose depot in high fat-fed mice is the result of increased proliferation of adipocytes, as newer adipocytes are smaller due to less lipid accumulation...." This statement is incorrect. Less lipid accumulation does not reflect newer adipocytes!! De novo recruitment of adipocytes needs to be proven with a genetic tracer model.

• Thank you for pointing this out! We have modified the text to use the adipocyte cell profile graph as additional evidence the brown adipose mass expansion is not due to larger cells. The phosphohistone H3 graph (Supp Fig 4B) includes our data that suggests increased cell numbers are driving the larger brown adipose mass.

The biggest issue that this paper does not give us any additional mechanistic insights beyond gene expression analysis. What is the critical driver in this model that leads to a re-activation of the BAT.

• We have included a more in-depth analysis of a provocative candidate gene, Sfrp1, which provide a potential mechanism by which the BAT may be expanding and in turn activating as an insulin-stimulated glucose sink.

Minor points:

Figures would be more easily read if data were grouped by time point rather than treatment group.

• We chose to group by treatment group to emphasize our main comparison that is between the 20 and 30 week high fat-fed mice.

Figure 1: large variations in n are not justified.

• Animals sacrificed at 30 weeks also had data taken at 20 weeks, thus there are much larger numbers for the earlier time point. We have clarified the separate ranges for the ages in the figure legend. Our statistics account for the autocorrelation so there was no need to reduce the number of data points at 20 weeks.

Lines 206-209: Need citations backing this up.
• We have added citations for this point (now lines 335-337).

*Supp. Fig. 5: more markers of thermogenesis, general and beige-specific, would strengthen this point.*

• We have added data for Eva1, Prdm16, and Pgc1a as markers of thermogenesis, as well as general adipose markers Adipoq, Fabp4/AP2, and Pparg, in Supplementary Figure 6