Dear Editor,

I am submitting the revised version of our invited Basic Science Study (manuscript number 39241), entitled "NBCe1 Na^+-HCO_3^- Cotransporter Ablation Causes Reduced Apoptosis Following Cardiac Ischemia-Reperfusion Injury In Vivo." We have submitted our RNA Seq bioinformatics data to GEO and have asked that it be immediately available (accession number GSE115579 has been included in the manuscript). Also, we will be providing the NBCe1 conditional mouse model to Jackson Labs, so it will soon be available to all investigators. The reviewer's comments and our responses to those comments are listed below, and the changes in the manuscript are highlighted as requested (on pages 14, 21, 22).

Reviewer 1 (00227375): This is an interesting manuscript about the association between genetic ablation of NBCe1 and cardio-protective effects for ischemia-reperfusion injury using an NBCe1 knockout mouse model. Loss of NBCe1 didn't impair cardiovascular performance under basal conditions or in response to β-adrenergic stimulation. As for gene expression patterns, it caused only limited changes. In addition, loss of NBCe1 reduced apoptosis following ischemia-reperfusion injury. The authors have demonstrated that partial inhibition of NBCe1 might be acceptable as a cardio-protective treatment strategy for ischemia-reperfusion injury. This manuscript is nicely structured and well written. I have one minor comment about this manuscript. Please consider the following comments. (Comments) Page 14, lines 11 and 14 Correct "Figure 2" to "Figure 3".

Response: We appreciate these comments and have corrected the errors on Page 14.

Reviewer 2 (00503243): This is an excellent manuscript on a relevant topic as the consequences of NBCe1 Na+- HCO3- Cotransporter ablation. In particular the authors generated a NBCe1 KO mice that were compared with wild type strain evaluating the effects of cardiac ischemia-reperfusion injury. The manuscript is well written, materials and methods are clear, statistical analysis is correct as well as results and discussion.

Response: We appreciate these comments.

Reviewer 3 (03650274): The article is very interesting and apparently well-performed. I would suggest authors to study also cardiac necrosis in parallel to apoptosis. TTC staining of infarcted hearts and serum troponin have to assessed.

Response: We agree that additional work with this model is warranted. We had originally intended that the studies of cardiac performance in vivo and the assessment of apoptosis following in vivo IR injury be part of a much larger study in which we would follow up with experiments utilizing longer periods of ischemia and myocardial infarction protocols in which LAD occlusion was permanent. Unfortunately, I did not have the resources to continue these studies and am planning to retire soon. Because NBCe1 inhibition has already been identified as cardio-protective in studies of the isolated heart, we thought that it was important to report our in

vivo studies showing that NBCe1 ablation in myocytes does not impair cardiac function and leads to a reduction in apoptosis following brief ischemia and reperfusion.

In response to the comments of reviewer 3 (and to the comments of reviewer 4) we have added a brief section (starting on the bottom of page 21) discussing this limitation of our study. We now note that the protocol we followed maximizes our ability to detect apoptotic cell death, but was not optimal for analysis of necrotic cell death and serum troponin, which is released after myocardial infarction.

Reviewer 4 (01204088): Vairamani et al. investigated the hypothesis that cardiomyocyte-specific loss of the electrogenic NBCe1 Na+-HCO3- cotransporter is cardioprotective during in vivo ischemia-reperfusion (I/R) injury, and concluded that cardiac-specific loss of NBCe1 does not impair cardiovascular performance, causes only minimal changes in gene expression patterns, and protects against I/R injury in vivo. This study is interesting, and I only have several comments. Figure 2B and Figure 4 B. Because this model is cardiomyocyte-specific conditional NBCe1 KO mice, showing the relative expression of Slc4a4 and TUNEL Positive Cells in heterozygote (flx/+) mice will be appreciated Figure 3. How about the cardiovascular performance in WT and KO mice after I/R injury?

Response: As noted in the comments to Reviewer 3, the protocol we followed is excellent for detection of apoptosis, but is not optimal for detection of differences in cardiovascular performance after I/R injury. I discussed this point with Dr. Wang, in whose lab the IR studies were performed, and he explained that to see clear differences in function, necrosis, and serum troponin, longer periods of ischemia or permanent occlusion of the LAD would be needed.

We have not analyzed heterozygous mice and are now maintaining only the floxed mice (without beta-myosin Cre), so we are unable to do these experiments. However, this comment raised an important point regarding potential therapeutic applications. On page 22, we now note that: “In myocardial infarction studies it would also be useful to determine whether NBCe1 heterozygosity could provide some degree of cardioprotection, since any cardiac therapy using complete pharmacological inhibition of NBCe1 would have a severe impact on kidney function.” We go on to say: “Even limited cardioprotection caused by partial inhibition could be useful, however, as it might be possible to combine partial inhibition of NBCe1 with partial inhibition of the NHE1 Na/H exchanger, which appear to act via similar mechanisms (see Introduction).”

We greatly appreciate the efforts and comments of the reviewers and hope that these revisions meet with your approval.

Best wishes,
Gary E. Shull, PhD