Author's response to reviews

Title: Cigarette smoke increases cardiomyocyte ceramide accumulation and inhibits mitochondrial respiration

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Response to reviewers:

We thank the reviewers for the time and attention required to critically review our manuscript. We believe the manuscript is stronger now with their recommended changes.

Reviewer 1

1. We are unsure how to best address the concern from the reviewer that ceramide may not the only factor in culture medium from lung cells following CSE. We assume the anti-biotic nature of myriocin is not responsible, given that our cell medium contains Pen-Strep to mitigate bacterial growth. Nonetheless, we have added the following comment and hope it is sufficient:

   “Related, our conclusions that ceramide is the relevant component within the cultured medium upon transfer of medium from lung cells to cardiomyocytes is based on our use of myriocin. However, due to the harmful cocktail of molecules within the CSE, it is possible that a non-ceramide variable exists.”

2. We have included data of ceramide levels from whole blood following 5 days of smoke exposure. We see a tendency to increase, though this was not statistically significant. We have included these data as figure 2C.

3. We have made the various corrections regarding typos.

Reviewer 2

1. We have updated all figure legends to indicate sample size.
2. We have added ceramide concentrations and time course, where relevant.

3. Mitochondrial content was controlled generally be total cellular protein content. We confirmed cell survival with CSE and ceramide cell treatments via trypan blue exclusion and added the following statement in the methods section:

   “Cell survival during treatments was confirmed by trypan blue exclusion.”

Following procedures with permeabilized tissue, we perform BCA analysis for protein quantification, as is typical for this procedure. We assume that this will at least partially account for tissue death.