Author's response to reviews

Title: 17-beta-estradiol induces stearoyl-CoA desaturase-1 expression in estrogen receptor-positive breast cancer cells

Authors:

Anissa Belkaid (eab3548@umoncton.ca)
Sabrina R Duguay (esd0831@umoncton.ca)
Rodney J Ouellette (rodneyo@atl.ca)
Marc E Surette (marc.surette@umoncton.ca)

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Author's response to reviews: see over
Response to reviewers:

**Reviewer Mounier**

In last review of the manuscript, Dr Mounier only had minor corrections as she indicated that we had addressed the large majority of her initial comments. A point by point response to her minor comments is provided below.

Minor corrections:

- *Table 1 indicates an unchanged fatty acid profile for MCF-10A cells. However, concluding sentence of the 4th paragraph mention an observed changes in fatty acid profiles for all cell types.*

  **Author's answer:** We have corrected this statement on lines 260-261.

Figures:

- *Cell proliferation with CFSE staining was only assayed in the experiments showed on figure 1. Other figures including cell proliferation assays (Figures 5 and 6) were done with Trypan Blue staining, a sub par method to evaluate cell proliferation.*

  **Author's answer:** We performed the CFSE assay in Figure 1 to confirm that the change in cell numbers that we observe in our stimulations are indeed due to proliferation.

- *Y axis legend for qPCR experiments have to be uniform. All qPCR graphs from figures 5, 6 and 7 should read as showed correctly in figure 2 : gene mRNA expression (Fold increase).*

  **Author's answer:** We have made the changes to the figures 5, 6 and 7 as requested.

- *Add "Ratio SREBP-1C/Actin" on figure 7*

  **Author's answer:** We have made the change to Figure 7 as requested.
Reviewer Castoria:

The Authors, did not address all my concerns. Particularly, the role of estradiol receptor in the observed findings is not clearly investigated.

Author’s answer: Dr Castoria singled out her continued requirement that we address the role of the estradiol receptor. In our last version of the manuscript had only partially addressed this point by adding experiments with tamoxifen. We have now fully addressed this point by performing siRNA silencing experiments using three different siRNAs that completely silenced the estrogen-alpha receptor. Once this receptor is silenced, 17β-estradiol failed to induce stearoyl-CoA desaturase-1 expression. See lines 43-44, 263-268, and Figure 4C and 4D.