Reviewer's report

Title: Estrogen regulation of TRPM8 expression in breast cancer cells

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Reviewer: Greg Barritt

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Major Compulsory Revisions

1. The manuscript reports some interesting results on the potential regulation of TRPM8 expression by estrogens in a breast cancer cell line. The authors have used the estrogen receptor positive human breast cancer cell line MCF-7. They should indicate why this cell line was chosen, what its attributes are with respect to representing the properties of breast cancer cells, and at least comment on the fact that the results reported in the manuscript have only come from one cell line, namely MCF-7. Ideally key experiments on estrogen regulation of TRPM8 would be conducted in another or other cell lines.

2. The most important results in the manuscript are reported in Figure 3 which principally shows semi-quantitative PCR results for changes in expression of TRPM8, and for changes in expression of the estrogen receptor (ER). The key findings of the manuscript depend on accurate quantitation of TRPM8 and ER expression. On the basis of the information available and with the present technical availability of quantitative PCR in most laboratories, the semi-quantitative approach is probably not sufficient. Further, the changes in TRPM8 mRNA expression in Figure A, part b, are fairly small. Key experiments should be repeated using quantitative PCR.

3. Evidence for protein expression of TRPM8 in MCF-7 cells and TRPM8 function in these cells. It would be highly desirable to measure protein expression (e.g. Western blot) in MCF-7 cells in conjunction with the other experiments shown in Figure 1.

4. The authors indicate that in patch clamp and fluorescence experiments with icilin only about 30-40% of the cells responded. Some comment on why this is so should be made.

Minor Essential Revisions

5. In Figure 1C, icilin is used to test intracellular TRPM8 function as a channel for Ca2+ release from intracellular Ca2+ stores. A comment on membrane permeability of icilin is required.

6. From the results of Figure 5, the authors suggest a strong association between TRPM8 expression and ER positive status. They can say there is “an association” between these two proteins, but the data do not allow conclusion of
a strong association. Further experiments would be required to do this.

7. In the legend of Figure 1 the number of experiments and number of cell cultures should be given. Likewise in the legend of Figure 2 more information on number of experiments and separate cell cultures employed.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests