Review of: "In vitro culture at 39 °C during hepatic maturation of human ES cells facilitates hepatocyte-like cell functions"

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Potential competing interests: The author(s) declared that no potential competing interests exist.

This original research paper reports a heat treatment-based approach to improve the hepatic phenotype of H9 human embryonic stem cell-derived hepatocyte-like cells (HLCs). This is an important topic since HLCs have the potential to be a fundamental tool for in vitro drug screening and toxicology analysis. The paper is well written and clear in addressing its objectives. However, the objectives are limited in terms of ambition considering the current state-of-the-art in the field. Indeed, as discussed by the authors, chemical cues to induce hepatocyte differentiation/maturation have been more thoroughly studied than physical cues, but the last have also not been fully addressed by the authors. Importantly, although discussed, the transcriptomic data has not been validated. which brings novelty to this work.

Other comments should also be considered:

- Authors state that “during hepatic differentiation, cells in all tested conditions showed reduced cell numbers”, presenting on Fig 1C a significant difference between days 6 and 15 after heat treatment (d.a.h). This is a serious issue and may be related to a coating problem that should be further addressed.
- CYP3A4 activity (Fig 2A) and albumin secretion (Fig 3A) are 0 at 0 d.a.h. However, according to the schematic representation of the differentiation protocol, 0 d.a.h corresponds to the hepatocyte maturation phase. Therefore, at this stage, cells should already have some enzymatic activity and albumin secretion levels.
- Immunofluorescence microscopy images, specifically Fig 2C, 2E, 3B, 3E, 3K, 5A, 5C and 5E seem to have low resolution. This should be improved.
- The discussion on the relation between the expression of several molecular chaperones and CYP3A4 expression and activity seems far-fetched and should revised. Additional information/evidence is needed.
- Despite the fact that chemical cues are generally more studied than physical ones to induce hepatic maturation, other types of physical cues besides temperature should be addressed (e.g. scaffold/matrix rigidity/elasticity, shear stress, light, surface topography, etc).