Review of: "Emerin regulation of nuclear stiffness is required for fast amoeboid migration in confined environments"

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

1. In the Introduction, the authors describe how cancer cells migration and the role that the nuclear membrane protein Emerin may play in this process. However, there is no mention of how the nucleus is involved in the migration of cells based on the precursor cells (the interaction of the nuclear membrane protein with the cytoskeleton drives the movement of the nucleus), and it would have been better helpful to understand the article.
2. Some of the results are not shown in the results graph, it would be better to show the experimental graph of the following results (Results of cell trans membrane migration and immunofluorescence staining)

1 “Emerins found to be ~2 fold overexpressed in transfected cells, as measured by IF (Fig. 2H) ”.

2 “Emerin RNAi led to a significant decrease in transmigration through 8 µm pores, whereas transmigration through 12 µm pores was unaffected (Fig. 2J). In emerin and cPLA2 RNAi cells, transmigration rates were reduced to a similar degree (Fig. 2K)”.

3 Relative to emerin WT, cells with emerin (Y74F/Y95F) were significantly faster (Fig. 4E).

4 Emerin (Y74F/Y95F) was found to be ~2 fold over-expressed in transfected cells, as measured by IF (Fig. 2H).

3. It would be better to provide the sequences of Lamin A/C, cPLA2 overexpression and knockdown, and the results of Lamin A/C, cPLA2 overexpression and knockdown efficiency.

4.In Figure 4A, the authors show the "Schematic of the pathway being investigated", but do not describe how each protein affects the other.

5. In the past decades, a variety of techniques have been developed to estimate the mechanical properties of single cells, including atomic force microscopy (AFM), micropipette aspiration (MPA), magnetic twisting cytometry and so on. The authors used the gel sandwich assay (Liu et al., 2015) to determine cell and nucleus stiffness, but Liu et al. used this method to determine cell Contractility Index rather than cell stiffness. Are there any other studies using this method to determine cell stiffness?

6.Nuclear stiffness detection is a very important experiment in the article, and it would be better if pictures of the detection process were provided.

7.In Figure 4I, the authors mention that "For comparison, data for EGFP alone and emerin WT were reproduced from figure 3F.", but the two sets of data are not the same, for example, in Figure 4I Stiffness (h/d) of isolated nuclei (post cell fractionation) after over-expressing (OE) EGFP alone (81 nuclei), while in figure 3F: over-expressing (OE) EGFP alone (105 nuclei) (105 nuclei), why only partial data were extracted? Also, in 4I, are the three sets of data measured simultaneously?