Reviewer’s report

Title: The human complement inhibitor Sushi Domain-Containing Protein 4 (SUSD4) expression in tumor cells and CD8+ T cells is associated with better prognosis of breast cancer patients.

Version: 2
Date: 13 July 2015

Reviewer: Kristi A Egland

Reviewer’s report:

Major Compulsory Revisions:

1. Figure 1A. The immunohistochemical staining for SUSD4 is staining the nucleus of the cells. SUSD4a has a transmembrane domain and is on the surface of cells. Why is SUSD4 staining nuclear and not membrane?

2. Figure 1B. Why does SUSD4 not stain in the epithelial cancer cells? Are the low and high examples shown here a Score 0 for SUSD4? How can you discern stained epithelial cells from stromal cells when SUSD4 has strong staining in Score 2 in Figure 1A?

3. Figure 1D, F and H. How do you define survival versus recurrence? The survival data is strong and convincing. The recurrence data, by eye, looks the same as the survival data, but is not statistically significant. The authors could consider not showing the recurrence curves and only the survival curves. Taking the recurrence curves out of the manuscript will not change the conclusions of the paper.

4. Figure 2B. The x-axis for the flow cytometry curve is strange. How can the majority of the peak be below zero? The western immunoblot analysis in Fig. 2C is very impressive. Either change the scale on the x-axis for Fig. 2B or just remove the flow cytometry experiment from the paper.

5. Figure 3. Photos of the stained cells need to be included with the data for wound closure, migration and invasion. For wound closure, a time course showing the cells filling in the wound is necessary for a reader to properly evaluate the data. If a yellow pipet was used to scratch the monolayer, were the scratches equal in diameter for each cell line? Showing the cell counts in not sufficient. A photo of stained cells on the membrane for migration and invasion is also needed.

6. Figure 3. Please state for each experiment the number of times it was performed and whether it was performed in duplicate, triplicate, etc.

7. Figure 4. A scale bar needs to be put into all photos in this figure. The photos of the cells in Fig. 4B are not clear. One cannot distinguish a colony from a single
cell. New photos should be taken with better definition.

8. Figure 5. A photo showing staining of CD4+ cells and no SUSD4 staining should be shown. CD4+ cells do not stain for SUSD4 – correct? Page 17 lines 8 and 9 never state that SUSD4 does not stain in CD4+ cells: “Further stainings with anti-CD4 (data not shown) or anti-CD8 (Figure 5B), showed that cytotoxic CD8+ T cells expressed SUSD4.“ This is an important statement to clarify since further experiments are performed to study SUSD4 in CD4 cells.

9. In Figure 5B, red cells (SUSD4) with no green staining (CD8) are shown (two on the very bottom of photo) contrary to the text on page 17, line 10: “…cells positive for SUSD4 were also positive for CD3 and CD8.”

10. Figure 5A and B. A photo showing CD8 staining but no SUSD4 should also be included to represent the staining patterns of the cells.

11. Figure 5C and D. The results from these experiments are not clear. The expression profile of CD4+ (Fig. 5C) is identical to CD8+(Fig. 5D), but SUSD4 was shown to only be present in CD8+ cells in the above Fig A and B.

Minor Essential Revisions:
1. Typo page 5, line 4. cells cells
2. SUSD4 should be italicized when describing the gene or RNA.
3. Page 10, line 10: 10X magnification – does that include the ocular lens 100X total?
4. Page 15, line 15. Using two specific primers for qPCR not just one?
5. Page 19, lines 1 and 2 Nonetheless, since we could not expression?
6. Page 11, line 7 states 4X magnification. Is that total magnifications?
7. Page 11, line 23 states 20X magnification. Total? Ocular included?

Discretionary Revisions
None noted.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

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.