Author's response to reviews

Title: Hornerin, an S100 family protein, is functional in breast cells and aberrantly expressed in breast cancer

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Response to Reviewers’ Comments for submission MS# 8599613426869894 entitled “Hornerin, an S100 family protein, is functional in breast cells and aberrantly expressed in breast cancer”

We thank the editor and referees for their additional comments and the opportunity to satisfactorily address their remaining concerns. We have incorporated the reviewers’ suggestions and comments into the revised and now much stronger manuscript. Our point-by-point response to the reviewers’ comments can be found below.

Response to Reviewers Comments: In all cases, author responses are highlighted. Additionally, all new revisions are highlighted within the manuscript as directed.

Referee #1, Sandra Z Haslam
The authors have satisfactorily addressed all of my concerns except for identification of hornerin in macrophages during lactation. Major comment: In fig 3C the putative hornerin+ macrophages are in lactating mammay gland - they have failed to show by specific staining that these are indeed macrophages. They have submitted a supplemental fig 3 for murine and human mammary glands. These do show hornerin+ macrophages, but no developmental stage is given for these sections. Yet they state that they are observed during lactation and involution. They need to show that hornerin staining during lactation is indeed in macrophages and need to define the status of the murine and human glands in suppl fig 3. and modify text and figure legends to describe tissue status.

We are certain the Referee meant Figure 2 in her comments. To fully address the Referee’s comment, we performed additional data analysis and added a new component to Fig. 2. This new data is the quantitation of the dual-stained hornerin and F4/80 (macrophage-specific marker) cells. This new data shows conclusive statistical significance of an increase in hornerin expressing macrophages during lactation and involution compared to the nulliparous mammary tissue. We have correspondingly modified the manuscript to describe the new data. It now reads: “We also observed a significant increase in hornerin staining within the macrophages specifically during lactation and involution compared to nulliparous tissue (P<0.05; Fig. 2C, D and Suppl. Fig. 2).” Furthermore, we have modified the Suppl. Fig. 2 as suggested by the Referee. We have added a panel of lactating mammary tissue that has been dual-stained with hornerin and F4/80 to definitively show the increase in hornerin positive macrophages during lactation and involution compared to the nulliparous tissue. We have also updated the figure legends and materials and methods to reflect these additions.

Referee #2, Partha Roy Roy
If lower hornerin expression is correlated with less aggressive phenotype, why do lymph-node positive and higher grade tumors have lower hornerin expression than lymph-node negative and low grade tumors, respectively?

We have added to the discussion (pgs. 13 – 14) to highlight this observation and to provide a possible explanation to the observed discrepancy in the data. The manuscript now reads: “It is of note that the MCF10A breast cancer cell line progression model showed increasing amounts of hornerin expression as the tumorigenicity of the cells progressed. This observation is in contrast to the data observed in the breast cancer tissue array (i.e. the less aggressive tumor tissue had higher levels of hornerin expression). We hypothesize that the fragmentation and localization of the fragments relates to the function of hornerin, thereby explaining these discrepancies. Indeed, less hornerin fragmentation was observed in the more aggressive MCF10A lines, similar to less fragmentation observed in the ER/PR negative breast cancer cell lines (Fig. 5), which are inherently more invasive and tumorigenic compared to the ER/PR positive cell lines [32, 43]. It is possible that the enhanced fragmentation directly relates to the increase in intensity and abundance of hornerin detected in the lobular breast tissue tumor samples compared to the ductal carcinomas.”