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Responses to Reviewers' Comments

Reviewer 1

Comment 1: The authors need to discuss why they did not follow the potential induction of CHOP and GADD34 upon fluoxetine treatment since as it is mentioned in the introduction "If proper protein folding capacity is not restored, then all three arms of UPR induce CHOP (CCAAT/enhancer binding protein-homologous protein) and GADD34 (growth arrest and DNA damage 34) to stimulate apoptosis".

Authors' Response: Our attempts to detect CHOP and GADD34 by Western blotting in 30 or 50 μg total protein lysates of breast epithelial cell lines with and without 10 μM fluoxetine (FLX) were ***not*** successful at either 24h or 48h time points. The undetectable levels of CHOP and GADD34 in our *in vitro* study are likely due to either transient expression or instability of both proteins, which are similar to the research findings reported by Rutkowski *et al*. upon short-term treatment of mouse embryonic fibroblasts with either ER stress inducers, thapsigargin and tunicamycin (PLoS Biol, 2006, 4:e374). In our revised manuscript (page 16), we described this important limitation as well as the need to monitor and compare the expression levels of CHOP and GADD34 in the presence of FLX and classical ER stress inducers in subsequent *in vivo* model.

Comment 2: The supplier and code numbers of the antibodies used in the reverse phase protein microarray and western blot analysis should be included in the materials and methods section.

Authors' Response: In our revised manuscript (pages 8-9), we included the suppliers and code numbers of antibodies used in the reverse phase protein microarray and Western blots.

Comment 3: Does the 15µg and 30µg in Figure 4A indicate amount of protein of SUM149PT and Late and Early MCF10A cellular extract loaded? Please indicate in the figure legend and specify the amount loaded in Figures 4B, C and D.

Authors' Response: The 15µg and 30µg of total cell lysates for SUM149PT and two types of MCF10A, respectively, are indicated in Figure 4A for the detection of cleaved LC3 bands. We followed the reviewer's recommendation of indicating the total cellular extracts loaded in the figure legend of Figure 4 for all panels on page 31.

Comment 4: Please discuss similarities differences in the induction of autophagic and UPR indicator proteins in triple negative breast cancer cells treated with classical ER stress inducers versus fluoxetine.

Authors' Response: Unfortunately, we do not have sufficient resources to perform compare-and-contrast studies on SUM149PT in the presence of classical ER stress inducers, such as thapsigargin and tunicamycin, versus FLX. This is an important limitation of the present study, which we discussed in our revised manuscript on pages 19-20. As suggested above, delineating the mechanism of action of FLX in zenograft model of SUM149PT would be an important follow-up study, using thapsigargin treatment as a control for robust unfolded protein response.

Comment 5: Please indicate the meaning of the dots in the Table S1.

Authors' Response: The dots indicated in Table S1 denote differentially expressed proteins that were discussed in the manuscript.

Minor comments on the following pages:

Page 12: Although MCF10A originated from the same mastectomy fibrocystic diseased tissue, several variations of this cell line exist.

Page 13: Meanwhile, the Early MFCF10A did not undergo autophagy at either time point.

Page 18: These proteins (were) are components of the highly integrated autophagy, UPR, and apoptosis in response to ER and metabolic stress.

Authors' Response: The grammatical errors on the aforementioned sentences were corrected on page 13 and 20 of the revised manuscript. The sentence on page 13 of the original manuscript was corrected to "Meanwhile, autophagy was not induced in Early MCF10A" (now page 14 of revised manuscript).

Reviewer 2

Comment:

Dear Authors,

Your paper is very good becouse has many diffrent cell lines And rich methods but, - you should make much measurement atlest 10-15. - you made 24 & 48 h, but you need 4,8,12,24&48 h

Best regards

Authors' Response: If we are not mistaken, Reviewer 2 was referring to the panel of cell lines being compared by reverse phase protein microarray (RPPM) in the absence and presence of 10 μM fluoxetine. We carried out an expedition to (a) examine the FLX-induced changes in expression levels of proteins, if any, from 24h and 48h treatments and (b) test the feasibility of FLX as a prevention or therapeutic agent for specific

molecular subtypes of breast cancer. While we agree that more information can be gained from additional time points, the costs of the antibodies are expensive with either RPPM or Western blots. But more importantly, we do not have additional funding for this *in vitro* project. A subsequent *in vivo* model is being developed for a grant mechanism to further investigate the mechanism of action of FLX and would take into consideration the earlier time points (4h, 8h, and 12h), especially with the respect to detection of CHOP and GADD34 as indicated above.