Author’s response to reviews

Title: FKBPL and its peptide derivatives inhibit cancer stem cells and breast cancer metastasis by downregulating DLL4 and Notch4

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Responses to reviewers’ comments

Editor Comments:
1 - If human cell lines are used, authors are strongly encouraged to include the following information in their manuscript:

- The source of the cell line, including when and from where it was obtained
- Whether the cell line has recently been authenticated and by what method
- Whether the cell line has recently been tested for mycoplasma contamination

Further information is available from the International Cell Line Authentication Committee (ICLAC). We recommend that authors check the NCBI database for misidentification and contamination of human cell lines. Please include the sources of all cell lines and their catalogue numbers.

Response: We obtained the cell lines from the American Type Culture Collection (ATCC: MCF-7, ATCC® HTB-22™; MDA-MB-231, ATCC® HTB-26) just before the study started in 2013. As with every new study, we buy in freshly authenticated cell lines from ATCC as standard in our lab. All new cell lines are STR profiled by the suppliers. The majority of this work was completed within a few years. Some of our more recent work was carried out with new cell lines, fresh from ATCC, validating our previous findings. Before every experiment, the cell lines were also morphologically authenticated; MCF-7 and MDA-231 cells have distinct morphology. We carry out Mycoplasma testing once a month. We have already a sentence in the methodology referring to this (Page 5, line 39-42).

2 - Please confirm whether any of the cell lines required ethics approval for their use and include a statement in the Ethics approval and consent to participate section.

Response: No, none of the cell lines required ethical approval for use. We included a sentence to this in the Declaration section.

3 - Please remove the CPRID participant identifiers from the supplementary table, and replace them with anonymous identifiers that do not link to patients. This will help enable anonymity to be maintained.

Response: Many thanks for this comment. We have removed the CPRIDs and coded the samples with the numbers 1-8 in the Supplementary Table 3 and 1-3 in the Supplementary Table 4.

4 - Experimental research on vertebrates or any regulated invertebrates must comply with institutional, national, or international guidelines, and where available should have been approved by an appropriate ethics committee. The Basel Declaration outlines fundamental principles to adhere to when conducting research in animals and the International Council for Laboratory Animal Science (ICLAS) has also published ethical guidelines.
Please include an updated animal ethics statement in the 'Ethics approval and consent to participate' section of the Declarations.

Response: We have already included this paragraph in the Declaration section under Ethics approval and consent to participate: “All animal experiments were carried out in accordance with the Animal (Scientific Procedures) Act 1986 and conformed to the current United Kingdom Co-ordinating Committee on Cancer Research guidelines. Animal studies were conducted under the project license PPL 2678 and all researchers also had their personal licenses approved by the Department of Health, Social Services and Public Safety Health Protection in the UK.”

We have now included a sentence to state that all animal experiments were carried out in accordance to the Basel Declaration.

5 - Please include details of the source of animals used in the experiments for this study.

Response: The SCID mice (C.B-17/IcrHsd-PrkdcscidLystbg) used in our in vivo experiments were in-house bred. We have already indicated this in the methods section (page 8, line 47/48).

6 - Please describe in the Methods the manner by which animals were sacrificed.

Response: The animals were euthanised by an approved method, using carbon dioxide method. We have added a line describing this in the methodology (page 9, line 16/17 and 52).

7 - At this stage, please upload your manuscript as a single, final, clean version that does not contain any tracked changes, comments, highlights, strikethrough or text in different colours. All relevant tables and figures should also be clean versions. Figures (and additional files) should remain uploaded as separate files. Should you wish to respond to these revision requests, please include the information in the designated input box only.

Response: Many thanks. We have.

Tracy Robson

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