Author's response to reviews

Title: Measurement of matrix metalloproteinase 9 mediated CO3 degradation fragment as marker for skin fibrosis

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Author's response to reviews: see over
AUTHORS RESPONSE TO REVIEWERS

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Measurement of matrix metalloproteinase 9 mediated CO3 degradation fragment as marker for skin fibrosis

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Reviewer 1:

Reviewer number 1 had no comments on the manuscript

Reviewer 2:

1 Fig2 lacks control images (PBS-treated samples)
Figure has been deleted as revision in point number 4

2 Figs 3 and 4 also lack control images. Also the data need to be confirmed using Western blot analysis as the differences between PBS and bleo are not striking. What is the yellow stain
Figure has been deleted as revision in point number 4

3 Fig 5 The figure legend is inadequate. What do C and B represent. Is this a Northern blot analysis of RNA.
Figure has been deleted as revision in point number 4

4 I am not sure why proteoglycans and collagen IV were examined (it is known bleo induces these markers) when the paper is supposed to be about MMP-9 generated fragment of type III collagen. (ie the authors should consider deleting Figs 2-5)
Figures 2-5 have been deleted

5 Is MMP-9 expression altered in response to bleo. What about MMP-9 activity in general is this altered. Are there changes in collagen III levels?
MMP9 activity is up regulated, we measure this indirectly via CO3-610 levels, since this is an MMP9 generated fragment. Collagen III was attempted to be measured in histology, but with no success.

6 The authors only examine bleo-induced skin fibrosis, thus the usefulness of the data is not adequate. However, the authors do discuss this limitation in the text. Can the authors provide precise information as to what clinical conditions these observations might be useful for (last paragraph discussion—eg keloids,
Scleroderma etc)?
The main focus was/is on scleroderma and in the utilisation of CO3-610 on a skin fibrosis model. Since CO3-610 has also been found to be also upregulated in liver fibrosis, we believe that this might be a case of abnormal extracellular matrix remodelling marker, and hence we limit the discussion on relevant clinical conditions as we wanted to focus on extra cellular matrix remodelling. We do however plan to measure the same marker in samples from scleroderma in order to comment/discuss its clinical utility.

7 In the last figure, what are C and B. What was the loading control for the Western. Can the authors examine whether or not there are also changes in collagen III levels?
C stands for Control, and B for Bleomycin treated. Actin was used as a loading control for the western. We did try to evaluate collagen III changes, however since this is a mouse model, all relevant secondary antibodies are mouse derived, hence there is increased background and we cannot evaluate it accurately.

8 Throughout 'PBS' or "P" is preferred to "C" to indicate controls
It was rectified as suggested in figure legends.

Minor essential revisions
1 abstract "Bleomycin induced" should have a hyphen
Rectified

2 abstract "Skin fibrosis induced via daily administration of Bleomycin with Injection in 52 female C3H mice (n=28) treated with Phosphate Buffered Saline (PBS) as controls” makes no sense----please clarify
Rectified

3 Last sentence in discussion 'well controlled' needs a hyphen
Rectified

4 The figures are located below the figure legends which makes the paper extremely difficult to review. Please rectify
Rectified