Primary myeloid cell proteomics and transcriptomics: importance of β tubulin isotypes for osteoclast function

David Guérit, Pauline Marie, Anne Morel, Justine Maurin, Christel Verollet, Brigitte Raynaud-Messina, Serge Urbach and Anne Blangy
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MS TITLE: Quantitative proteomics and transcriptomics exploration of primary myeloid cells: importance of ß tubulin isotypes for bone resorption.

AUTHORS: David Guerit, Pauline Marie, Anne Morel, Justine Maurin, Christel Verollet, Brigitte Raynaud-Messina, Serge Urbach, and Anne BLANGY
ARTICLE TYPE: Research Article

We have now reached a decision on the above manuscript.

To see the reviewers' reports and a copy of this decision letter, please go to: https://submit-jcs.biologists.org and click on the 'Manuscripts with Decisions' queue in the Author Area. (Corresponding author only has access to reviews.)

As you will see, the reviewers raise a number of substantial criticisms that prevent me from accepting the paper at this stage. They suggest, however, that a revised version might prove acceptable, if you can address their concerns. If you think that you can deal satisfactorily with the criticisms on revision, I would be pleased to see a revised manuscript. We would then return it to the reviewers.

Please ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion.

I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. Please attend to all of the reviewers' comments. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

In the study entitled “Quantitative proteomics and transcriptomics exploration of primary myeloid” Guerit and collegues aimed to identify new proteins important for regulation of osteoclast differentiation and function. To achieve their goal the authors conducted transcriptomic and proteomic analysis of osteoclasts, dendritic cells and macrophages derived from murine bone marrow and cultured in vitro in the presence of differentiation driving cytokines.

Comments for the author

Study Strengths
1. Identification of Tubb6 as a novel regulator of osteoclast cytoskeleton and function (this claim has to be further supported, see below).
2. Identification of novel genes expressed by osteoclast and not by the closely related macrophages and dendritic cells. Here the advantage of these transcriptomic analysis are the combined power of proteomics and transcriptomics and the use of primary cells as opposed to the RAW264.7 that have been screened by others.
3. The screen identified known osteoclast regulating genes unique to the osteoclast validating its relevance.
4. Five of 17 novel and unique osteoclast regulating genes identified by the screen show unusual intracellular structures raising the possibility they are involved in osteoclast regulation and providing more support for the validity of the novel genes identified.

Study weaknesses

1. The physiological context of the experiment is relatively weak. The cells are grown on plastic and the only difference is the cytokines added to the culture medium. One could argue that the differences between the cells reflect the differences between signaling derived by the different cytokines and not between the cell entities. In vivo osteoclast derive from different precursors, their differentiation depends on many factors including a diversity of cytokines, interaction with other cells and with the bone matrix. The importance of the bone matrix is underlined by studies in vitro showing different organization and stability of the actin cytoskeleton in osteoclasts cultured on bone vs osteoclast cultured on plastic or glass.
2. It is not clear from the siRNA experiments what was the efficiency and specificity of the siRNA pools that were utilized.
3. The evidence of a role for Tubb6 in regulation of the osteoclast cytoskeleton could be strengthened. The authors demonstrate its role on in regulation only on glass and its effect on resorption is tested on hydroxyapatite coated plates and not bone. It was shown that the behavior of osteoclasts and osteoclast cytoskeleton is different on plastic and bone and while many studies show alterations on glass they are not shown on bone and vice versa.

Specific comments:

1. The specificity and efficiency of the 5 siRNAS producing the unusual intracellular structures should be validated by Q-PCR or western blot.
Ideally if possible the siRNA pools should be deconvoluted and at least two siRNAs targeting the same mRNA should provide the same phenotype.
2. Monitoring the effects of Tubb6 in regulation of osteoclast cytoskeleton in cells grown on bone pieces would strengthen the paper. If the experiments are not performed the authors should note it in the discussion.
3. The physiological context of the experiment should be discussed.
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4. Figure 6 D and F are missing parts!

Reviewer 2

Advance summary and potential significance to field

Osteoclasts (Oc) and immature dendritic cells (Dc) belong to hematopoietic lineage with very distinct functions in vivo. In this study, authors realized a global proteomic and transcriptomic analysis of primary mouse Oc, Dc and bone marrow macrophages based on original SILAC and RNAseq. Data are obtained in a homogeneous experimental system. Data analysis allows the identification of an important ß tubulin isotypes Tubb6 that is involved in the organization of podosomes into a belt. Consequently, Tubb6 should play a role in bone resorption due to the link between podosome belt and the sealing zone. This work also illustrates the crucial role of the tubulin isotype repertoire in the biology of hematopoietic cells.

A proteomic analysis on primary cells is very important in Oc in comparison with Raw cells that are not physiological and relevant cells. Moreover, Raw cells do not exhibit the same actin dynamics during resorption activity.The roel of Tubb6 seems to be importatn in deffirenciated Oc.

Comments for the author

Dear Editor,

Please find attached my commentaries concerning the manuscript: “Quantitative proteomics and transcriptomics exploration of primary myeloid cells: importance of ß tubulin isotypes for bone resorption.”, from David Guérit et al. This study is well realized and written. For these reasons, I do recommend this article for publication after revision.

The main result of this study concerns Oc, all the part concerning Dc is not useful for the principal result of this work. Indeed, the authors focus on Dc and not on macrophages, why?

Major points:
What is the exact added value of the transcriptomic approach in this study? Several studies demonstrated the differences between these two kinds of approaches. As authors said: ” only half of the proteins in the Oc signature showed a correlated regulation of the transcript, the other half would not have been highlighted based on the sole RNAseq,….. “
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« Among the 38 proteins of unknown function in Oc, we selected 17 with a variety of functions for a siRNA screening »
Authors have to precise how these proteins were selected.
The addition of siRNA at day 2 of the differentiation process should interfere with this process? If there are no significant changes in Oc size were measured, perhaps bone resorption should be affected and have to be tested. It is important to determine is the presence of these vesicles could affect the Oc functions. Impact on the cell viability and degradation activity has to be monitored.

Tubb6 depletion by two distinct siRNA show a destabilization of the podosome belt. What is the organization on bone matrix in these conditions? What is the impact on sealing zone organization? If the role of Tubb6 in podosome belt organization seems to be clear, no molecular hypothesis was tested to explain this data. Even if this paper consists in a very important resource data, a molecular mechanism could improve the study.
The pattern observed suggest that in Tubb6 depletion condition podosomes can’t compact and can't form a fully functional podosome blet. As podosomes are composed by several actin networks and myosin 2 plays an important in this compaction; It should be important to test if there is a molecular link between this tubulin subunit and myosin 2? To support this idea, The paper of Davide Randazzo et al show a correlation with Tubb6 and myosin in another cellular model (Human Molecular Genetics 2019).
A Tubb6 immunofluorescence in WT Oc should help to confirm or not a localization et the cell periphery, some antibodies have to be tested in mice and/or human Oc models.

Moreover, a Tubb6 immunoprecipitation followed by a mass spectrometry analysis should complete this study and help authors to propose a molecular explanation of tubb6 implication in podosome belt structuration.

First revision

Author response to reviewers' comments

We wish to thank the reviewers for their clever criticisms and suggestions. We performed additional experiments and modified the manuscripts according to their suggestions to the best of our possibilities. We sincerely apologize for not having noticed that some figures were incomplete in the pdf of the manuscript generated by the web site of the Journal of Cell Science. We were careful about this matter in the revised pdf and we also provide each figure as an individual file. The modifications are highlighted in yellow in the manuscript. We hope that our replies, additional data and manuscript modifications will meet your expectations.
To comply with editorial requests we shortened the title and the abstract, we shifted all original tables to supplementary tables, embedded the legends in the supplementary figures.
Please find below our specific answers to the individual comments of the two reviewers.

Reviewer 1 Comments for the author
In the study entitled “Quantitative proteomics and transcriptomics exploration of primary myeloid” Guerit and collegues aimed to identify new proteins important for regulation of osteoclast differentiation and function. To achieve their goal the authors conducted transcriptomic and proteomic analysis of osteoclasts, dendritic cells and macrophages derived from murine bone marrow and cultured in vitro in the presence of differentiation driving cytokines.
Study Strengths
1.Identification of Tubb6 as a novel regulator of osteoclast cytoskeleton and function (this claim has to be further supported, see below).
2.Identification of novel genes expressed by osteoclast and not by the closely related macrophages and dendritic cells. Here the advantage of these transcriptomic analysis are the combined power of proteomics and transcriptomics and the use of primary cells as opposed to the RAW264.7 that have been screened by others.
3.The screen identified known osteoclast regulating genes unique to the osteoclast validating its relevance.
4.Five of 17 novel and unique osteoclast regulating genes identified by the screen show unusual intracellular structures raising the possibility they are involved in osteoclast regulation and providing more support for the validity of the novel genes identified.

Study weaknesses
1.The physiological context of the experiment is relatively weak. The cells are grown on plastic and the only difference is the cytokines added to the culture medium. One could argue that the differences between the cells reflect the differences between signaling derived by the different cytokines and not between the cell entities.
In vivo osteoclast derive from different precursors, their differentiation depends on many factors including a diversity of cytokines, interaction with other cells and with the bone matrix. The importance of the bone matrix is underlined by studies in vitro showing different organization and stability of the actin cytoskeleton in osteoclasts cultured on bone vs osteoclast cultured on plastic or glass.

We are sorry, but it is quite impossible to reproduce in vitro the complexity of the in vivo context. However, we want to underline that compared to previous analysis on Raw cells, ours are performed on cells derived from murine bone marrow, which are more relevant and physiological cells. However, in our conditions,the differences we see between the cells DO result from the distinct signaling pathways induced by the cytokine cocktails and leading to the establishment of the differentiation program characteristic of each myeloid cell. These cocktails are widely used to obtain the myeloid cells of interest exhibiting relevant biological properties and functions.

We performed all our experiments on plastic for two reasons. First and as stated in the manuscript, we minimalized the changes in culture conditions between cell types to avoid protein/gene expression modification not related to the differentiation program characteristic of each myeloid cell types. Second, due to the amounts of protein necessary, it was not possible to consider preparing quantitative proteomic samples of osteoclasts grown on bone.

We agree that osteoclasts cultured on bone and plastic have distinct features. But when transferred onto bone/ACC "plastic" osteoclasts assemble a sealing zone and start degrading within a few hours. Moreover, osteoclasts on plastic have a ruffled border and secrete active cathespin K. To reinforce our study, we performed new experiments and we now show the effect of Tubb6 siRNAs on mineralized substrates: we show that the sealing zones are affected in both mouse and human osteoclasts. This is now in Figure 7C-H (page 15).

We agree that mineralized substrates can modify the expression of specific genes, but a wide variety of regulators of osteoclast differentiation and function were identified on plastic and proved relevant in vivo. To our knowledge, the only gene expression profile analyses comparing osteoclasts grown on bone and on plastic are the Affymetrix studies from the laboratory of Kevin McHugh (Crotti et al., 2011 and Purdue et al., 2014). These studies explicitly cite 25 genes that are induced by bone. Examining how these genes were categorized in our study, we found that the majority was our osteoclast signature in fact. Some were not expressed in myeloid cells (KFPM <10) or were not osteoclast specific as compared to macrophages or dendritic cells. Only 2 genes could be specifically relevant for bone resorbing osteoclasts: the Myc partner Max and xanthine dehydrogenase. We now cite the two studies from the McHugh lab and added a specific paragraph in the discussion section and Table 1 about this matter (pages 18-19). Our conclusion is that osteoclast differentiation on plastic recapitulates the whole transcriptional program required to obtain a cell capable of bone resorption but that the transcriptional profile elicited by the transcription factor Max could be interesting to highlight genes specifically linked to the oxidative metabolism in the bone resorbing osteoclast.

2.It is not clear from the siRNA experiments what was the efficiency and specificity of the siRNA pools that were utilized.

We used smart pool siRNAs from Dharmacon, which are designed to minimize off target effects and maximize the change of gene silencing efficiency. The efficiency of the siRNA pools used in Figure 5A-B were now added in supplementary Figure S5A. We also verified that they do not affect the induction of osteoclast differentiation markers Src and cathepsin K, now in figure 5C-D. For si Tubb6 experiments, we also verified that this treatment did not modify the expression of Tubb2a, Tubb4b and tubb5 mRNA levels, which was shown in Fig. 5B.

3.The evidence of a role for Tubb6 in regulation of the osteoclast cytoskeleton could be strengthened. The authors demonstrate its role on in regulation only on glass and its effect on resorption is tested on hydroxyapatite coated plates and not bone. It was shown that the behavior of osteoclasts and osteoclast cytoskeleton is different on plastic and bone and while many studies show alterations on glass they are not shown on bone and vice versa.

Our results with Tubb6 siRNAs confirmed that podosome belt abnormalities detected on plastic do translate into a functional defect in hydroxyapatite coated plate degradation. Due to the longer kinetics of bone resorption experiments and the transient effect of siRNAs, it is not possible to measure the effect of siRNAs on bone resorption per se. This would require a genetic KO of the gene, as we reported previously in the case of Dock5. Still, a defect in mineral dissolution precludes the bone resorption process.
We also added in new Figures 7C-H and S6C-D the effect of Tubb6 siRNAs on sealing zone size, showing a reduction in sealing zone size in both mouse osteoclasts seeded on ACC (mineralized collagen substrate) and human osteoclasts seeded on bone (page 15).

Specific comments:
1.The specificity and efficiency of the 5 siRNAS producing the unusual intracellular structures should be validated by Q-PCR or western blot. Ideally if possible the siRNA pools should be deconvoluted and at least two siRNAs targeting the same mRNA should provide the same phenotype.

As stated above, we have added in Figure S5A (page 12) the Q-PCR validation of the silencing of the 5 genes. Unfortunately, we were unable to identify the nature of these structures and could not find any equivalent in the literature. Thus, we could not anticipate what functional consequences these structures may have, which we could be explored in more details. We did not observe an effect on the organization of actin and we did not see changes in the amount of cathepsin K in the cell or secreted, as mentioned as data not shown. Thus, to deconvolute the pools, while representing an tremendous amount of work and material in primary Oc, would not bringing more information about the function of these genes. It is likely that stable KO of the genes would be necessary.

2.Monitoring the effects of Tubb6 in regulation of osteoclast cytoskeleton in cells grown on bone pieces would strengthen the paper. If the experiments are not performed the authors should note it in the discussion.

As stated above, we now show in Figures 7 and Figure S6 the effect of Tubb6 silencing on sealing zones in mouse and human osteoclasts (page 15).

3.The physiological context of the experiment should be discussed.

As detailed above, we added a paragraph in the discussion section about our experimental setting, that discusses our results in light of the studies published by the laboratory of Kevin McHugh, comparing osteoclasts on bone and on plastic and illustrated in Table 1 (pages 18-19).

4.Figure 6 D and F are missing parts!

We do apologize for not noticing that some panels were missing in the pdf generated on the editor's web site. We verified that panels are indeed all present in the figures of the pdf of the revised manuscript.

Reviewer 2 Advance summary and potential significance to field
Osteoclasts (Oc) and immature dendritic cells (Dc) belong to hematopoietic lineage with very distinct functions in vivo. In this study, authors realized a global proteomic and transcriptomic analysis of primary mouse Oc, Dc and bone marrow macrophages based on original SILAC and RNAseq. Data are obtained in a homogeneous experimental system. Data analysis allows the identification of an important ß tubulin isotypes Tubb6 that is involved in the organization of podosomes into a belt. Consequently, Tubb6 should play a role in bone resorption due to the link between podosome belt and the sealing zone. This work also illustrates the crucial role of the tubulin isotype repertoire in the biology of hematopoietic cells.

A proteomic analysis on primary cells is very important in Oc in comparison with Raw cells that are not physiological and relevant cells. Moreover, Raw cells do not exhibit the same actin dynamics during resorption activity.The roel of Tubb6 seems to be importatn in deffirenciated Oc.

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Please find attached my commentaries concerning the manuscript: "Quantitative proteomics and transcriptomics exploration of primary myeloid cells: importance of ß tubulin isotypes for bone resorption.", from David Guérit et al. This study is well realized and written. For these reasons, I do recommend this article for publication after revision.

The main result of this study concerns Oc, all the part concerning Dc is not useful for the principal result of this work. Indeed, the authors focus on Dc and not on macrophages, why?

We used the bone marrow macrophages, which are still proliferating in our conditions, as a reference cell type to compare osteoclasts and dendritic cells, which are post mitotic cells. We now specify page 7. The resulting signatures of osteoclasts and immature dendritic cells are of high functional relevance because they are derived from the same cell lineage, are able to differentiate in post mitotic cells harboring specific functions within the same time range.

In the present study, we did not exploit the results on immature dendritic cells, but the data remain available for scientists studying dendritic cell biology. Of note, the Dc signatures contain genes of unknown functions in these cells, including strongly differential genes that are poorly studied overall such as BC035044 or D630039A03Rik or Adgrg5, some of which could be relevant in Dc. For instance, Plet1 was recently shown to be involved in interstitial migration of murine small intestinal Dc (Karrich et al., Eur J Immunol. 2019). We mention this in the discussion section (page 18).

Major points:
What is the exact added value of the transcriptomic approach in this study? Several studies demonstrated the differences between these two kinds of approaches. As authors said: " only half of the proteins in the Oc signature showed a correlated regulation of the transcript, the other half would not have been highlighted based on the sole RNAseq,..... "
All the first part of the manuscript corresponds to proteomic and transcriptomic analysis and should be synthetized.
On the PDF file impossible for me to visualize the figure 5 A and B, only yellow arrows are visible. Some others figures are missing.

As had stated in the text, the correlation between transcritpomic and proteomic is known to be low, usually around 30% in all cell types studied. But as we had also mentioned in the result section, not all proteins are detected by proteomics, contrarily to RNAseq that extensively measures all transcripts. Thus, the transcriptional study allows a deeper analysis and provides more candidates. As we had exemplified in the text, various genes in our osteoclast transcriptional signature and not detected by SILAC, such as Siglec15 and Calcr, were formerly validated as essential in osteoclasts. It is very likely that more important actors of osteoclast biology are present in our transcriptional signature, as we mention in the result section.
We agree that omics results are often fastidious to read. We synthetized more these results while maintaining the important information (pages 8-9). We hope this part is now easier reading.
We do apologize for not noticing that some figure panels we missing in the pdf generated on the editor's web site. We verified that panels are indeed all present in the figures of the revised pdf.

« Among the 38 proteins of unknown function in Oc, we selected 17 with a variety of functions for a siRNA screening »

Authors have to precise how these proteins were selected.
The addition of siRNA at day 2 of the differentiation process should interfere with this process? If there are no significant changes in Oc size were measured, perhaps bone resorption should be affected and have to be tested. It is important to determine is the presence of these vesicles could affect the Oc functions. Impact on the cell viability and degradation activity has to be monitored.

The word "selected" was not appropriate; we actually did not select but we picked half of the 38 proteins, trying to cover different putative functions and eliminating the proteins associated with a role on chromatin.
The sentence now reads (page 12):
"We picked about half of the remaining proteins for a siRNA screening, covering a variety of functions but avoiding the proteins associated with a role on chromatin (Tep1, Hmgb3, Histone H1.4 and H1.5)"
We add the siRNAs at day 2 of differentiation, when the fusion process is starting. Thereby, we avoid interfering with the early differentiation process, as the siRNAs will another day or so to be efficient. In our former published studies, we have used this procedure for several genes to examine late events in osteoclast differentiation, and it does not interfere with the differentiation process. For siTubb6, we had presented the expression of Src and CtsK in figure S5, which was shifted to S6A-B in the revised manuscript.
In addition, we performed QPCR on the RNA samples corresponding to the 4 experiments depicted in figure 5B for the "vacuole-like" group of siRNAs to verify the correct induction of osteoclast differentiation markers CtsK and Src. This is now in figure 5C-D. There was no evidence of any impact of the siRNAs on cell viability, reflected by the size of osteoclasts that was comparable between siRNAs as it was mentioned in the manuscript. We also checked the secretion and maturation of cathepsin K by western blot in the osteoclasts and secreted in the medium, which was not affected. We mention this as data not shown in the result section (page 13).

The identification of the precise functions of those 5 genes would require more detailed studies, as we report here for Tubb6. As we were unfortunately unable so far to identify the nature of these structures, we do not know in which direction we should examine their functional impact.

Tubb6 depletion by two distinct siRNA show a destabilization of the podosome belt. What is the organization on bone matrix in these conditions? What is the impact on sealing zone organization?
If the role of Tubb6 in podosome belt organization seems to be clear, no molecular hypothesis was tested to explain this data. Even if this paper consists in a very important resource data, a molecular mechanism could improve the study.
The pattern observed suggest that in Tubb6 depletion condition podosomes can't compact and can't form a fully functional podosome blet. As podosomes are composed by several actin networks and myosin 2 plays an important in this compaction; It should be important to test if there is a molecular link between this tubulin subunit and myosin 2? To support this idea, The paper of Davide Randazzo et al show a correlation with Tubb6 and myosin in another cellular model (Human Molecular Genetics 2019).
A Tubb6 immunofluorescence in WT Oc should help to confirm or not a localization et the cell periphery, some antibodies have to be tested in mice and/or human Oc models.
Moreover, a Tubb6 immunoprecipitation followed by a mass spectrometry analysis should complete this study and help authors to propose a molecular explanation of tubb6 implication in podosome belt structuration.

We now added in Figure 7C-H the effect of Tubb6 siRNAs on the sealing zone of mouse and human osteoclasts showing that they reduce the size of the sealing zones, which is consistent with their effect on the podosome belt and osteoclast activity (page 15).
The study by Randazzo et al, 2019 found that in mouse and human myofibers increased levels of Tubb6 correlate with the downregulation of myosin heavy chain variants myh1 and myh2 whereas the embryonic myosin heavy chain myh3 is upregulated. These myosins are muscle specific and they are not expressed in myeloid cells (myh3 in Table S2, no reads for myh1 and myh2). As conventional myosins, osteoclasts express only the ubiquitous myosins IIA (Myh9) and IIB (Myh10). Myosin IIB has a diffuse distribution in osteoclasts except at the cell periphery whereas myosin IIA associates with the podosome belt (Krits et al., Calcif Tissue Int. 2002) and Myo2a siRNAs in osteoclasts resulted in expansion of the sealing zone (McMichael J Biol Chem 2009). We tested whether the reduction of sealing zone size was accompanied by increased levels of myosin IIA, but we did not detect any change myosin IIA protein levels upon Tubb6 siRNA treatments. We mention this as data not shown in the result section (page 15).
As we had stated in the discussion, the amino acid substitutions in the core region of Tubb6 as compared to Tubb5 are the same as in Tubb3 as compared to Tubb2b. So higher Tubb6 levels are expected to impact in intrinsic microtubule dynamics. Also the C-terminal domains on tubulins being divergent, higher Tubb6 levels are also expected to affect the binding of MAPs to microtubules. We are presently examining this, which represents a whole new research project.
As mentioned in Randazzo 2019, there is no commercial antibody specific to Tubb6. Fortunately, Dr Frankfurter accepted to share with us the antibodies he had raised against mouse Tubb6 C-terminus and that were used in the study by Randazzo et al. We examined the localization of Tubb6 as compared to global ß-tubulin in Oc and we did not observe a specific subcellular localization of the isotype. Interestingly, the mononucleated cells around osteoclasts presented much low levels of Tubb6, consistent with it induction during osteoclastogenesis. This is now in Figure 6G (page 14).
We agree that identifying the specific partners of Tubb6 would be very interesting and it is among our present projects. The immunoprecipitation approach is relevant for the direct partners of beta tubulin: the chaperones and alpha tubulin. We used this approach to address by proteomics whether there was a differential binding of Tubb6 and Tubb5 to the 4 alpha tubulin isotypes in osteoclasts. For this, we overexpressed Tubb5 and Tubb6 tagged with GFP in osteoclasts derived from RAW264.7 cells. We found no differential association of Tubb5 and Tubb6 with a particular alpha tubulin isotype. To have more insights into the mechanisms controlled by Tubb6, it would be necessary to look at Tubb6 partners in the context of a microtubule. We have undertaken this work and it will be part of a future story.

Second decision letter

MS ID#: JOCES/2019/239772

MS TITLE: Primary myeloid cell proteomics and transcriptomics: importance of ß tubulin isotypes for osteoclast function.

AUTHORS: David Guerit, Pauline Marie, Anne Morel, Justine Maurin, Christel Verollet, Brigitte Raynaud-Messina, Serge Urbach, and Anne BLANGY
ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Journal of Cell Science, pending standard ethics checks.

Reviewer 1

Advance summary and potential significance to field

The authors have revised the MS and added a thorough analysis of the effects of Tubb6 KD on OC cytoskeleton and podosome organization on glass and hydroxyapatite resorption in vitro.
I think these new data together with the revised discussion properly address my comments and recommend this manuscript for publication in JCS.

Comments for the author

No further comments

Reviewer 2

Advance summary and potential significance to field

The authors answered perfectly to my different questions. For this reason, I recommend that this article be published in this form.

Comments for the author

No additional comment