European Journal of Immunology

Peer review correspondence

The Dok-3/Grb2 adaptor module promotes inducible association of the lipid phosphatase SHIP with the BCR in a coreceptor-independent manner

Birgit Manno, Thomas Oellerich, Tim Schnyder, Jasmin Corso, Marion Lösing, Konstantin Neumann, Henning Urlaub, Facundo D. Batista, Michael Engelke and Jürgen Wienands

Corresponding author: Jürgen Wienands, Georg August University of Göttingen, Institute of Cellular and Molecular Immunology, Göttingen, Germany

---

Review Timeline:	Submission date:	30 March 2016
	First editorial decision:	28 April 2016
	Revision received:	28 June 2016
	Accepted:	16 August 2016

Handling Executive Committee member: Prof. Hans-Martin Jäck

Please note that the correspondence below does not include the standard editorial instructions regarding preparation and submission of revised manuscripts, only the scientific revisions requested and addressed.

First Editorial Decision – 28 April 2016

Dear Dr. Wienands,

Manuscript ID eji.201646431 entitled "The Dok-3/Grb2 adaptor module promotes inducible association of the lipid phosphatase SHIP with the BCR in a coreceptor-independent manner", which you submitted to the European Journal of Immunology, has been reviewed. The comments of the referees are included at the bottom of this letter.

Although the referees have recommended publication, some revisions to your manuscript have been requested. Therefore, I invite you to respond to the comments of the referees and revise your manuscript accordingly.

You should also pay close attention to the editorial comments included below. *In particular, please edit your figure legends to follow Journal standards as outlined in the editorial comments. Failure to do this will result in delays in the re-review process.*

European Journal of Immunology

If the revision of the paper is expected to take more than three months, please inform the editorial office. Revisions taking longer than six months may be assessed by new referees to ensure the relevance and timeliness of the data.

Once again, thank you for submitting your manuscript to European Journal of Immunology. We look forward to receiving your revision.

Yours sincerely,
Karen Chu

on behalf of Prof. Hans-Martin Jack

Dr. Karen Chu
Editorial Office
European Journal of Immunology
e-mail: ejied@wiley.com
www.eji-journal.eu

****

Reviewer: 1

Comments to the Author
In this manuscript, Manno et al. revealed an FcgammaRIIB independent mechanism of plasma membrane recruitment and inhibitory function of SHIP by forming complex with inhibitor module Dok-3/Grb2 or the interaction with cytoplasmic domain of Igα and Igβ. It is a novel finding since these results replenish the inhibitory regulation mechanism of BCR signaling through SHIP, and suggest a new auto negative regulation mechanism within the BCR signaling machine that is independent of the canonical inhibitory BCR co-receptor FcgammaRIIB.
I have a few concerns that shall be clarified.

Major:
1. In Fig.1A, there should be three groups, WT, SHIP-deficient and D676G, only WT and D676G can be found in the calcium influx profiles, while the SHIP-deficient data is missing.
2. According to the data in Fig.4, destruction the interaction between SHIP with either Dok-3 or Grb2 can sufficiently block the recruitment of SHIP to the plasma membrane and the inhibitory function of SHIP by Dok-3/Grb2 complex, however in Fig. 6A the data indicates Grb2 binding plays extra role when comparing

European Journal of Immunology

the R34G/YYFF and R34G/YYFF/K1151A, which shows significant difference in the statistic RRI results, and this is also not consistent with the calcium influx results in Fig.6C. The author should clarify this inconsistency or at least have it discussed.

3. The author claims “in addition to the Dok-3/Grb2 module, phospho-ITAMs of Igα and Igβ complement plasma membrane anchoring and concomitant activation of SHIP”, but there is no direct evidence proving Igα and Igβ interacting with SHIP through phosphor-ITAMs in this manuscript. Since it is one of the core conclusions in this report, experiments with direct evidence shall be provided.

4. In Fig. 6E, R34G mutant shows decrease co-localization than WT, however addition with mutations block the SHIP binding to Dok-3/Grb2 increase the co-localization, is there any explanation about this result? Or at least it shall be discussed.

5. In Fig. 6E, the author uses Mander’s coefficient to quantify the co-localization. When using the Mander’s overlap coefficient, the number of objects in both channel of the image has to be more or less equal, also a variety of other consequences that are arguably more confusing. There are some publications discussing these problems: Adler J and Parmryd I published the study “Quantifying colocalization by correlation: the Pearson correlation coefficient is superior to the Mander's overlap coefficient” on Cytometry Part A in 2010, they proved that “since the MOC is unresponsive to substantial changes in the data and is hard to interpret, it is neither an alternative to nor a useful substitute for the Pearson’s correlation coefficient (PCC). The MOC is not suitable for making measurements of colocalization either by correlation or co-occurrence”. So the Pearson’s correlation coefficient may be a better quantification for the co-localization analysis.

Minor points:

1. The size of bar in the images of Fig. 1 is not defined.
2. For the calcium influx profiles in supplementary figures, the stimulation time points are not marked.
3. “live cell imaging” instead of “life cell imaging”.

Reviewer: 2

Comments to the Author

The manuscript eji.201646431 entitled “The Dok-3/Grb2 adaptor module promotes inducible association of the lipid phosphatase SHIP with the BCR in a coreceptor-independent manner” analyzes the molecular mechanism of FcγRIIb-independent membrane recruitment of the lipid phosphatase SHIP to the B cell receptor (BCR).

Using SILAC interactome analysis, followed by mainly retroviral transduction of chicken DT40 B cells with different SHIP, Dok-3 and Grb2 mutant constructs the authors identified molecular interaction sites of SHIP with the adaptor proteins Dok-3 and Grb2. Based on molecular interaction analysis (Co-IP) they propose a direct interaction of this complex with the ITAM-motifs of the Igα and Igβ subunits of the BCR.

European Journal of Immunology

This suggests a novel and previously unappreciated negative regulatory capacity of the BCR-ITAM motifs. Hence this manuscript provides the first report, as far as I know, to identify molecular details underlying a FcγRIIb-independent mechanism of BCR – mediated SHIP membrane recruitment. The involvement of Dok-3 and Grb2 provides an intriguing novel mechanism that would explain observations, which had been made in several B cell systems over the years.

In summary, the data presented in this manuscript are novel and interesting. The experimentation and approaches are appropriate and support the main hypotheses. The data suffice to draw the conclusions implicated and the manuscript is concisely written. I must be pointed out though, that the manuscript is riddled by small inconsistencies, minor imprecisions and mistakes which cloud this otherwise rather satisfying study.

Minor points:

Figure 1A:

• Neither the text, the figure legend, nor in the figure itself, state at any point that the depicted protein scheme with its domain organization corresponds to SHIP1. Same holds for all following figures. While this is obvious to the educated reader, the authors should mention it at least once.

• For calcium flux measurements, the text, the figure legend and the figure itself do not correspond. The text describes DT40 SHIP-/- and DT40 SHIP-/- D676G cells, the legend describes DT40 SHIP-/-, DT40 SHIP-/- SHIP WT and DT40 SHIP-/- D676G cells, while the figure displays curves for DT40 SHIP-/- SHIP WT and DT40 SHIP-/- D676G only. The authors should adapt the figure and the text according to the figure legend.

Table 1:

• In the text, table 1 (page 6, line 9) is referred to as “supplementary figure 1”. The authors should change this.

Figures for calcium mobilization by flow cytometry:

• The choice of colors is unfortunate, as colors are very hard to distinguish in print out versions. A more easily distinguishable color scheme would be preferable.

Supplementary figure 2:

• Supplementary figure 2A, as described in the text corresponds to supplementary figure 2B. Vice versa, supplementary figures 2B-C described in the text correspond to supplementary figures 2A and 2C.

Supplementary figure 3:

• Page9, line22: “This conclusion is supported further by strikingly reduced phosphorylation of the R34G variant of SHIP in BCR activated B cells (supplementary Figure 3)”. This figure does not exist. It should be included in the supplement.

The real supplementary figure 3:

• Lane 11 in the WB is described as WT SHIP in the figure legend. However, the graphic labeling clearly shows a double mutant in this lane. The authors should correct this.

Figure 5B, panel 2:

European Journal of Immunology

• The legend describes Far Western analyses using GST-SHIP-SH2 or GST-SHIP-SH2 R34G, respectively. However, the legend and figure do not correspond, as the second panel depicts a GST-Syk-SH2 blot.

Section Material and Methods:

• In the paragraph “Expression constructs, production of GST proteins, and transfection” a citation is missing. Authors should complement it.

Further figure comments:

Figure 1 C-D and supplementary figure 1:

• In figure 1 C, the authors claim that SHIP relocalizes to the plasma membrane upon BCR activation and BCR/FcγRIIb co-ligation with almost same efficiency, which is convincingly depicted in figure 1D. However, it seems that BCR/FcγRIIb co-stimulated cells show increased /more efficient SHIP membrane localization compared to BCR-only stimulated cells.

• This is also in contrast to supplementary figure 1, where the calcium signal after BCR/FcγRIIb co-stimulation with M4 and anti-mouse IgG is diminished and attenuated compared to the stimulation of the BCR alone. One would expect a similar calcium flux capacity. Moreover, it would be informative to see how those cells react on anti-FcγRIIb stimulation alone.

• Furthermore, the BCR-eGFP negative control should be depicted in the panel of figure 1C.

Figure 3B:

• In figure 3B, the authors describe that the Dok-3 binding-competent Y917F variant of SHIP translocates to the plasma membrane with the same efficiency as wildtype SHIP. For wildtype SHIP there is a significant difference compared to the Y1020F and YYFF mutant. For the Y917F mutant significance is not indicated. In this regard the authors should include the p values of Y917F compared to the Y1020F and YYFF mutant.

Figure 6:

• Figure 6 describes the concerted actions of Dok-3, Grb2 and the BCR for the effectivity of SHIP plasma membrane recruitment. However, in figure 6A the R34G/YYFF double mutant has the same RRI as R34G alone. Similarly, concerning the primary mouse B cells in figure 6B, the RRI quotient of R34G is not significantly different from the R34G/YYFF/K1151A triple mutant. The p values of the triple mutant in figure 6B compared to the R34G mutant and the eGFP control should be included to provide support the proposed hypothesis.

• Figure 6A also fails to fully support the conclusions drawn from figure 6C, as there is no difference in calcium mobilization between the R34G/YYFF/K1151A triple mutant and the R34G/YYFF double mutant and. Thus would suggest that the localization studies and the calcium flux experiments do not really correlate.

• Figure 6E is supposed to correlate with figure 6D, however the quantification of the YYFF mutant is not included, which should be done.

Text comments:

European Journal of Immunology

• “However, mere BCR stimulation of SHIP-deficient DT40 B cells that lack FcγRIIb expression resulted in in increased Ca2+ mobilization compared to cells expressing a SHIP variant (D676G).” Here a clear statement to the effect if DT40 cells do not endogenously express or were experimentally deprived of FcγRIIb would be preferable.

• “We conclude that inducible formation of a trimolecular complex of Dok-3, Grb2 and SHIP limits BCR signaling”. This statement is misleading, as the presented biochemical evidence is not sufficient to conclude that there is a true trimolecular complex. Please rephrase, as the abstract also states that “SHIP translocates to sites of BCR activation through a concerted action of the protein adaptor unit Dok-3/Grb2 and phosphorylated BCR signaling components” and that this “cannot formally rule out the existence of separate functional complexes between SHIP and Dok-3, or SHIP and Grb2” in the discussion.

First revision – authors’ response – 28 June 2016

Reviewer 1:

Comments to the Author

In this manuscript, Manno et al. revealed an FcgammaRIIB independent mechanism of plasma membrane recruitment and inhibitory function of SHIP by forming complex with inhibitor module Dok-3/Grb2 or the interaction with cytoplasmic domain of Igα and Igβ. It is a novel finding since these results replenish the inhibitory regulation mechanism of BCR signaling through SHIP, and suggest a new auto negative regulation mechanism within the BCR signaling machine that is independent of the canonical inhibitory BCR co-receptor FcgammaRIIB.

I have a few concerns that shall be clarified.

Major:

1. In Fig.1A, there should be three groups, WT, SHIP-deficient and D676G, only WT and D676G can be found in the calcium influx profiles, while the SHIP-deficient data is missing.

We corrected the figure and apologize for the missing data set caused by unintended uploading of a precursor version of the manuscript.

2. According to the data in Fig.4, destruction the interaction between SHIP with either Dok-3 or Grb2 can sufficiently block the recruitment of SHIP to the plasma membrane and the inhibitory function of SHIP by Dok-3/Grb2 complex, however in Fig. 6A the data indicates Grb2 binding plays extra role when comparing the R34G/YYFF and R34G/YYFF/K1151A, which shows significant difference in the statistic RRI results, and this is also not consistent with the calcium influx results in Fig.6C. The author should clarify this inconsistency or at least have it discussed.

European Journal of Immunology

Indeed we observed subtle differences for the recruitment efficiency of these variants and cannot formally rule out that "Grb2 binding plays an extra role" as mentioned in the text. However, the two SHIP variants binding only to either Dok-3 or Grb2 similarly inhibit downstream Ca2+ mobilization. Moreover, the functionality of a Dok-3/Grb2 complex in association with the BCR has been published by us and other groups. For example, Dok-3 needs Grb2 binding to enter BCR signalosomes. Collectively, all available data suggest a trimolecular complex of SHIP, Grb2 and Dok-3. We changed the results and discussion parts to clarify this point (page 9, lines 28 and 29; page 11, lines 4 to 7).

3. The author claims “in addition to the Dok-3/Grb2 module, phospho-ITAMs of Igα and Igβ complement plasma membrane anchoring and concomitant activation of SHIP”, but there is no direct evidence proving Igα and Igβ interacting with SHIP through phosphor-ITAMs in this manuscript. Since it is one of the core conclusions in this report, experiments with direct evidence shall be provided.

One piece of evidence for a direct interaction between SHIP and BCR is provided by the stimulation-dependent purification of the Ig-alpha/Ig-beta heterodimer by the SH2 domain of SHIP consistent with an association of the SHIP SH2 domain with tyrosine phosphorylated BCR signaling subunits. To further support this conclusion, we conducted a phosphopeptide-based affinity purification assay that confirmed binding of SHIP to BCR signaling elements (see new supplementary figure 4A and page 8, lanes 29 to 32).

4. In Fig. 6E, R34G mutant shows decrease co-localization than WT, however addition with mutations block the SHIP binding to Dok-3/Grb2 increase the co-localization, is there any explanation about this result? Or at least it shall be discussed.

We corrected the labeling of Figure 6E clarifying that the triple variant of SHIP shows a significantly reduced translocation into BCR microclusters compared to the R34G variant (page 10, lanes 9 to 11).

5. In Fig. 6E, the author uses Mander’s coefficient to quantify the co-localization. When using the Mander’s overlap coefficient, the number of objects in both channel of the image has to be more or less equal, also a variety of other consequences that are arguably more confusing. There are some publications discussing these problems: Adler J and Parmryd I published the study “Quantifying colocalization by correlation: the Pearson correlation coefficient is superior to the Mander's overlap coefficient” on Cytometry Part A in 2010, they proved that “since the MOC is unresponsive to substantial changes in the data and is hard to interpret, it is neither an alternative to nor a useful substitute for the Pearson’s correlation coefficient (PCC). The MOC is not suitable for making measurements of colocalization either by correlation or co-occurrence”. So the Pearson’s correlation coefficient may be a better quantification for the co-localization analysis.

European Journal of Immunology

We thank the reviewer for this suggestion and now used the Pearson's correlation coefficient for the quantification, which fully supports our conclusions.

Minor points:

1. The size of bar in the images of Fig. 1 is not defined.
2. For the calcium influx profiles in supplementary figures, the stimulation time points are not marked.
3. "live cell imaging" instead of "life cell imaging".

We corrected the manuscript according to the reviewer's comments.

Reviewer: 2

Comments to the Author

The manuscript eji.201646431 entitled "The Dok-3/Grb2 adaptor module promotes inducible association of the lipid phosphatase SHIP with the BCR in a coreceptor-independent manner" analyzes the molecular mechanism of FcγRIIb-independent membrane recruitment of the lipid phosphatase SHIP to the B cell receptor (BCR).

Using SILAC interactome analysis, followed by mainly retroviral transduction of chicken DT40 B cells with different SHIP, Dok-3 and Grb2 mutant constructs the authors identified molecular interaction sites of SHIP with the adaptor proteins Dok-3 and Grb2. Based on molecular interaction analysis (Co-IP) they propose a direct interaction of this complex with the ITAM-motifs of the Igα and Igβ subunits of the BCR. This suggests a novel and previously unappreciated negative regulatory capacity of the BCR-ITAM motifs. Hence this manuscript provides the first report, as far as I know, to identify molecular details underlying a FcγRIIb-independent mechanism of BCR – mediated SHIP membrane recruitment. The involvement of Dok-3 and Grb2 provides an intriguing novel mechanism that would explain observations, which had been made in several B cell systems over the years.

In summary, the data presented in this manuscript are novel and interesting. The experimentation and approaches are appropriate and support the main hypotheses. The data suffice to draw the conclusions implicated and the manuscript is concisely written. I must be pointed out though, that the manuscript is riddled by small inconsistencies, minor imprecisions and mistakes which cloud this otherwise rather satisfying study.

We thank this reviewer for her/his appreciation of our data as being "novel and interesting". Likewise we acknowledge the careful inspection of our manuscript uncovering some inconsistencies and imprecisions. We seriously regret this annoyance caused by the incidental uploading of a precursor version of the final manuscript.

Minor points:

Figure 1A:

European Journal of Immunology

• Neither the text, the figure legend, nor in the figure itself, state at any point that the depicted protein scheme with its domain organization corresponds to SHIP1. Same holds for all following figures. While this is obvious to the educated reader, the authors should mention it at least once.
The figures were corrected.
• For calcium flux measurements, the text, the figure legend and the figure itself do not correspond. The text describes DT40 SHIP-/- and DT40 SHIP-/- D676G cells, the legend describes DT40 SHIP-/-, DT40 SHIP-/- SHIP WT and DT40 SHIP-/- D676G cells, while the figure displays curves for DT40 SHIP-/- SHIP WT and DT40 SHIP-/- D676G only. The authors should adapt the figure and the text according to the figure legend.
The inconsistencies have been eliminated (figure 1A; page 4, lanes 23 to 26 and page 18, lanes 6 to 8).
Table 1:
• In the text, table 1 (page 6, line 9) is referred to as “supplementary figure 1”. The authors should change this.
We now correctly referred to table 1 in the text and provide a new supplementary table 1 containing the details of the mass spectrometry analysis as requested.

Figures for calcium mobilization by flow cytometry:
• The choice of colors is unfortunate, as colors are very hard to distinguish in print out versions. A more easily distinguishable color scheme would be preferable.
We changed the colors and improved the figures showing the Ca2+ flux data.

Supplementary figure 2:
• Supplementary figure 2A, as described in the text corresponds to supplementary figure 2B. Vice versa, supplementary figures 2B-C described in the text correspond to supplementary figures 2A and 2C.
The respective parts of the manuscript have been corrected.

Supplementary figure 3:
• Page9, line22: “This conclusion is supported further by strikingly reduced phosphorylation of the R34G variant of SHIP in BCR activated B cells (supplementary Figure 3)”. This figure does not exist. It should be included in the supplement.
The text refers to the supplementary figure 4 and has been corrected.

The real supplementary figure 3:
• Lane 11 in the WB is described as WT SHIP in the figure legend. However, the graphic labeling clearly shows a double mutant in this lane. The authors should correct this.
The labeling has been set aright.

Figure 5B, panel 2:

European Journal of Immunology

• The legend describes Far Western analyses using GST-SHIP-SH2 or GST-SHIP-SH2 R34G, respectively. However, the legend and figure do not correspond, as the second panel depicts a GST-Syk-SH2 blot.
The figure legend is now correct.

Section Material and Methods:
• In the paragraph “Expression constructs, production of GST proteins, and transfection” a citation is missing. Authors should complement it.
The citation has been included.
Further figure comments:
Figure 1 C-D and supplementary figure 1:
• In figure 1 C, the authors claim that SHIP relocalizes to the plasma membrane upon BCR activation and BCR/FcγRIIb co-ligation with almost same efficiency, which is convincingly depicted in figure 1D. However, it seems that BCR/FcγRIIb co-stimulated cells show increased /more efficient SHIP membrane localization compared to BCR-only stimulated cells. This is also in contrast to supplementary figure 1, where the calcium signal after BCR/FcγRIIb co-stimulation with M4 and anti-mouse IgG is diminished and attenuated compared to the stimulation of the BCR alone. One would expect a similar calcium flux capacity. Moreover, it would be informative to see how those cells react on anti-FcγRIIb stimulation alone.
We agree that the representative images shown in figure 1C might indicate that co-ligation of the BCR with FcγRIIb is slightly, yet not significantly (compare figure 1D), more efficient than mere BCR activation for localizing SHIP to the plasma membrane. This can be explained by ITIM phosphorylation of the co-receptor that provides the canonical "textbook anchor" for the SHIP SH2 domain. Likewise, a more robust inhibition of Ca2+ mobilization upon BCR/FcγRIIb coligation is expected as compared to BCR activation alone (supplementary figure 1). However, our observations that SHIP gets recruitment to the plasma membrane and inhibits Ca2+ mobilization even in the absence of FcγRIIb expression, both, revealed the existence of additional plasma membrane anchors of SHIP, and hence, laid the foundation for our search of additional SHIP recruitment mechanisms. We agree that mere FcγRIIb stimulation is a valid additional control. The requested analysis was added and depicted as a light blue line in supplementary figure 1.

• Furthermore, the BCR-eGFP negative control should be depicted in the panel of figure 1C.
We had actually performed already that control but omitted from figure 1A for clarity. We now (re-) added this piece of data in the lower row of figure 1C, and thank the reviewer for this suggestion.

Figure 3B:
• In figure 3B, the authors describe that the Dok-3 binding-competent Y917F variant of SHIP translocates to the plasma membrane with the same efficiency as wildtype SHIP. For wildtype SHIP there is a significant difference compared to the Y1020F and YYFF mutant. For the Y917F mutant significance is not

European Journal of Immunology

indicated. In this regard the authors should include the p values of Y917F compared to the Y1020F and YYFF mutant.
We included the suggested p-values, showing that the difference between the recruitment efficiencies of the two SHIP variants is significant with being p<0,05.

Figure 6:
• Figure 6 describes the concerted actions of Dok-3, Grb2 and the BCR for the effectivity of SHIP plasma membrane recruitment. However, in figure 6A the R34G/YYFF double mutant has the same RRI as R34G alone. Similarly, concerning the primary mouse B cells in figure 6B, the RRI quotient of R34G is not significantly different from the R34G/YYFF/K1151A triple mutant. The p values of the triple mutant in figure 6B compared to the R34G mutant and the eGFP control should be included to provide support the proposed hypothesis.
We have included additional p-values showing that recruitment of the R34G variant is significantly more efficient than the triple variant in DT40 and primary B cells (figs. 6A and B, respectively). It is correct that we did not find a significant difference between the R34G and R34G/YYFF variants, which is in fact consistent with the strikingly reduced recruitment of all SH2-defective SHIP variants. This correlation may indicate a functional hierarchy of the membrane anchors for the SHIP recruitment process. It does, however, not affect our overall conclusion of a concerted action of all membrane anchors for SHIP recruitment which is evident from the finding that in all of our experiments the triple variant is more severely compromised than those harboring inactivating mutations in single domains or interaction motifs.

• Figure 6A also fails to fully support the conclusions drawn from figure 6C, as there is no difference in calcium mobilization between the R34G/YYFF/K1151A triple mutant and the R34G/YYFF double mutant and. Thus would suggest that the localization studies and the calcium flux experiments do not really correlate.
We agree with the reviewer that the isolated imaging of SHIP plasma membrane recruitment alone is insufficient to comprehensively address SHIP function as the translocation process reflects only the initiation of SHIP activation but not additional modes of SHIP regulation e.g. SHIP phosphorylation. We therefore complemented and controlled all of our experiments with Ca2+ flux analyses and in some cases also SHIP phosphorylation studies so that our overall conclusions and claims are based on the combined results of various read out systems. Moreover, the similar signaling phenotype of the R34G/YYFF and triple SHIP variants further support our conclusion of a trimolecular complex comprising SHIP, Dok3 and Grb2. We addressed this issue and rephrased the results and discussions parts for clarity (page 9, lines 28 and 29; page 11, lines 4 to 7).

• Figure 6E is supposed to correlate with figure 6D, however the quantification of the YYFF mutant is not included, which should be done.
We included the quantification of the YYFF mutant in figure 6E.

European Journal of Immunology

Text comments:

• “However, mere BCR stimulation of SHIP-deficient DT40 B cells that lack FcγRIIb expression resulted in in increased Ca2+ mobilization compared to cells expressing a SHIP variant (D676G).” Here a clear statement to the effect if DT40 cells do not endogenously express or were experimentally deprived of FcγRIIb would be preferable.

We clarified this point in the text accordingly (page 4, lines 23 to 26).

• “We conclude that inducible formation of a trimolecular complex of Dok-3, Grb2 and SHIP limits BCR signaling”. This statement is misleading, as the presented biochemical evidence is not sufficient to conclude that there is a true trimolecular complex. Please rephrase, as the abstract also states that “SHIP translocates to sites of BCR activation through a concerted action of the protein adaptor unit Dok-3/Grb2 and phosphorylated BCR signaling components” and that this “cannot formally rule out the existence of separate functional complexes between SHIP and Dok-3, or SHIP and Grb2” in the discussion.

We rephrased the respective abstracts accordingly (page 8, lines 1 and 2; page 11, lines 4 to 7).

Second Editorial Decision – 26 July 2016

Dear Dr. Wienands,

It is a pleasure to provisionally accept your manuscript entitled "The Dok-3/Grb2 adaptor module promotes inducible association of the lipid phosphatase SHIP with the BCR
in a coreceptor-independent manner" for publication in the European Journal of Immunology. For final acceptance, please follow the instructions below and return the requested items as soon as possible as we cannot process your manuscript further until all items listed below are dealt with.

Please note that EJI articles are now published online a few days after final acceptance (see Accepted Articles: http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1521-4141/accepted). The files used for the Accepted Articles are the final files and information supplied by you in Manuscript Central. You should therefore check that all the information (including author names) is correct as changes will NOT be permitted until the proofs stage.

We look forward to hearing from you and thank you for submitting your manuscript to the European Journal of Immunology.

Yours sincerely,
Karen Chu

Peer review correspondence

European Journal of Immunology

on behalf of Prof. Hans-Martin Jack

Dr. Karen Chu
Editorial Office
European Journal of Immunology
e-mail: ejied@wiley.com
www.eji-journal.eu