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Distinct SARS-CoV-2 sensing pathways in pDCs driving TLR7-antiviral vs. TLR2-immunopathological responses in Covid-19

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1st Editorial Decision **22nd Sep 2021**

Dear Martin,

Thank you for submitting your manuscript to The EMBO Journal. Your study has now been seen by three referees and their comments are provided below.

As you can see from the comments below, the referees find the analysis interesting but also that significant revisions are needed for consideration here. The concerns raised are clearly indicated below. I would like to invite a revised version should you be able to address the concerns raised. I think it would be helpful to discuss the raised points further and I am available to do so via email or video. Let me know what works best for you.

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process, please visit our website: https://www.embopress.org/page/journal/14602075/authorguide#transparentprocess

I have attached a document with helpful tips on how to prepare the revised version. Please pay attention to the parts on the Data Availability Section and figure legends.

Thank you for the opportunity to consider your work for publication. I look forward to discuss your revisions further with you

with best wishes

Karin

Karin Dumstrei, PhD
Senior Editor
The EMBO Journal

Instructions for preparing your revised manuscript:

Guide For Authors: https://www.embopress.org/page/journal/14602075/authorguide

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Referee #1:

In this manuscript Van der Sluis and colleagues have investigated the role of pDC in sensing SARS-CoV-2 and producing protective cytokines. They have used a differentiated HSPC model to modify pDC and test the role of specific host sensing pathways in CoV2 detection and responses. The models used and experimental design are good. There are some interesting and potentially important findings presented, but the data are somewhat preliminary.

Overall, I have some concerns about the reliability of the data and the number of times each experiment has been repeated. Some of the results are quite black and white, but others are not, and statistics don't seem to have been used to evidence reliability. As a consequence, some results will probably not survive close inspection. This is particularly important in primary cell experiments because results, particularly infection results, and the degree of induction of innate responses, can be quite variable between donors and experiments. I've made specific comments below but all figures should contain some form of statistical test and this will likely require some further repeats

1. I'm not sure that the picture of poor IFN induction by CoV2 described in the intro accurately represents the state of the art. Certainly there have been descriptions of IFN production by CoV2 exposed macrophages, for example, PMID33277988 suggests failed CoV2 replication activates IFN production in macrophages and PMID34101213 suggests infected epithelial cells can activate macrophages to produce IFNs and inflammatory cytokines. So its not all down to pDCs although I am sure that they play a central role as the authors propose. Maybe say a bit more about the likely positive contribution of macrophages to disease.

2. I found the first para of the results confusing. Is baseline the best phrase to use for the first measurement when the patients may be quite sick and have high levels of viral replication and inflammation at the time of hospitalisation? Perhaps Day1? The phrase baseline is a bit misleading.
Also I really don't understand what this sentence means "When we compared the percentage of pDCs at baseline to "duration of symptoms" (19), we observed a significant reduction in the percentage of pDCs from patients with symptoms duration between 5 to 12 days as compared to in the very early viremic phase (
3. Also, I'm not sure how to interpret the next sentence "However, patients presenting symptoms for more than 13 days prior to study enrollment, had a significantly higher percentage of circulating pDCs in peripheral blood (Fig 1A) and this coincided with decreasing viral load in the nasopharyngeal cavity and viral clearance in the majority of patients (19)." How does time of hospitalisation impact pDC level, or are we talking about length of symptoms. Also what does reference 19 have to do with it, it's a different study/patient group. Please rewrite this para with a view to improve clarity.

4. last sentence of this para suggests disease onset is associated with pDC decrease, is this not recovery is associated with pDC decrease.

5. S1 data referring to Fig 1 ie S1b-g should be in the main Figure.

6. Do the authors know that their virus preps are free of cytokines. If not, surely cytokines in the virus prep could activate the pDC rather then the virus itself. Mock infected sup is obviously no good to test this because it wouldn't contain virus induced cytokines. Can the authors rule this out?

7. Fig 2, given that pDC do not become infected, how do the authors work out MOI? Please specify how the doses are worked out.

8. Top of page 7, the authors describe how sup from CoV2 exposed cells can induce protection from infection in fresh cells. They ascribe this to IFN production because they say, infection is rescued with blocking antibodies. If I understand correctly, the data in Fig S4C do not statistically support this conclusion. I imagine it needs repeating a few more times.
Further details of amount of Ab would be helpful.

9. I'm not sure what the goal of the nanostring measurements are in Fig 3. Are Dhigh and Dlow true differences? Are responses measured consistent between experiments and are the differences between the 2 subjects statistically consistent between experiments? I don't think I see any significance measures or how many times it was done. Does n=2-4 refer to this experiment or that in E? Please clarify, particularly the statistical support for your findings in the nanostring measurements.

10. In Figure 4 c-f, its not clear what's being compared to what. Are we comparing each KO to the control KO (AAVS1)? Have these data been statistically assessed? There should be a comparison for each KO with the control but this is not clear. It just says n=2. Please show supporting statistical tests.

11. There are also no stats for Fig5, which I think has only been done twice. I appreciate the results are quite black and white but the data should be statistically supported, particularly in primary cell experiments where the data can be quite variable.

12. p9 line 15, "not involved in" better, "not necessary for"

13. I wonder if the small effect sizes in Fig 5G, (2 fold ish) would be supported statistically. As above please apply some statistical tests to assess reliability of the primary cell data.

14. Figure 6 also has no stats leading me to wonder how many of the conclusions drawn are statistically supported by the data. Please rectify.

Referee #2:

In this study, the authors demonstrated that pDCs induce IFNs via TLR7-MyD88 signaling while inducing inflammation by TLR2 signaling upon SARS-2 infection. Furthermore, they found that the SARS-2 E protein mitigates the IFNs of pDC by interacting with CD304. These findings are interesting, but there are some major issues to be addressed.

1. It is unclear if the disease severity correlates with pDC. For example, in Fig 1, the authors should show the correlations between pDC frequency and disease severity. For the mechanism experiments, two donors do not allow for statistical analysis. More donors should be included for analysis.

2. Since the pDCs were generated from HSPCs, how this ex vivo platform can mimic the real pDCs remains uncertain. Additional phenotype and functionality data should be provided. Were there any chances of mDCs contamination? Were these pDCs TLR9 signaling specific?

3. By using the CD304 ko, the authors demonstrated that CD304 is responsible for SARS-CoV-2 immune evasion. Although they cited the reference (SCIENCE•13 Nov 2020), the authors should provide experimental evidence to verify the real interactions between SARS-2 and CD304 in pDC in this study. The expression amounts of CD304 on pDC in SARS-2 patients should be provided as well.

4. pDC is the major producer of IFNa, authors should measure IFNa level in all patients' plasma and correlate it with pDC frequency besides measuring the cytokines.

5. In Fig. 1B, since the authors said that this subgroup of patients had samples at both baseline and 5-days post study enrollment, it is necessary to show the change of pDC frequency for each patient during this period. It is critical to determine whether the pDC dynamic is associated with the disease progression.

Minor issues:

6. In Fig 2, it was not clear about FR2020 or FR4286? Please make sure the consistency in the figure legend.

7. The authors should show the ko efficiency of TLRs by western blot in fig 5 and 6.

8. In Fig 6, the legend should be consistent for ug/ml or µg/ml. Similarly, in Fig 7, AAVS1KO or AAVS1KO?

9. In the ko experiments, the authors only used two donors, which is not convincing.

10. In the Graphical abstract, how is IFNa mediated by IRF7 phosphorylation upon SARS-CoV-2 infection in pDC? Also, how does CD304 mediate the viral evasion via inhibition of IRF7 phosphorylation?

11. In Methods, line 17, 2,5 ug/ml or 2.5 ug/ml? ml or mL?

12. In figure legends, *

Referee #3:

In this study, van der Sluis and colleagues addressed pDC responses after stimulation with SARS-CoV-2. They found that at the time point of presentation to the hospital, COVID-19 patients with symptoms duration between 5 and 12 days had reduced percentages of pDC from total PBMC, which was less pronounced in patients with shorter or longer symptoms duration. Furthermore, pDC percentages dropped within 5 days after hospitalization. SARS-CoV-2 stimulated HSPC-derived pDC showed significant expression of cytokines such as IFN-alpha, CXCL10, IL-6 and others. Upon pretreatment of lung epithelial cell types with the cell free supernatant of virus-stimulated pDC, SARS-CoV-2 protective effects were detected that were mediated to a large extent by type I IFN. RNAseq analysis of low and high responder pDC revealed highly overlapping gene expression profiles and clusters of genes that were induced early or late.
Analysis of MyD88ko, TRIFko, RIGko and combined ko pDC revealed that pDC sense SARS-CoV-2 primarily via MyD88. TLR3ko and TLR8ko did not have an effect, whereas TLR7ko abolished type I IFN responses. TLR2 did not affect type I IFN responses, but impaired IL-6 production. Since TLR2 forms dimers with TLR1 and TLR6, the respective ko pDC were generated and only TLR6ko showed reduced IL-6 production. SARS-CoV-2 enters cells via ACE2, or alternatively via neuropilin-1. Since pDC do not express ACE2, whereas neuropilin-1 is an important pDC marker, also neuropilin-1ko pDC were tested. Such cells showed normal CXCL10 and IL-6 responses, whereas type I IFN production was enhanced. This result suggested that SARS-CoV-2 modulated pDC derived type I IFN responses by the interaction with neuropilin-1.

This is an elegant and technically sound study addressing a timely and important question, the role of pDC in SARS-CoV-2 infection. Specifically, analyzsis of the abundance of pDC in PBMC from COVID-19 patients and of the molecular mechanism of SARS-CoV-2 mediated induction of cytokine responses in pDC is highly relevant. The latter aspect was addressed by exploiting the innovative method of CRISPR/Cas9 gene editing in HSPC-derived pDC. The concept that neuropilin-1 potentially plays a role in modulating SARS-CoV-2 induced type I IFN responses is new and particularly interesting.

Major concerns:
In Fig. 1A percentages of pDC from total PBMC from healthy donors should be included as a control. Also, absolute cell numbers should be presented to verify that reduced pDC percentages are indeed due to reduced pDC numbers.

In the legend of Fig. 1B it should be clearly stated that PBMC from all patients are shown independent of the respective symptom duration, if this is the case.

In this study, all experiments addressing cytokine responses of in vitro activated pDC were performed with HSPC-derived pDC. The authors should formally show that PBMC-derived pDC show similar responses as detected in ex vivo isolated pDC. This control is particularly important for SARS-CoV-2 stimulated pDC because ex vivo isolated pDC and HSPC-derived pDC might differ with regard to expression of certain components and the activation status.

The authors classified their HSPC-derived pDC as low and high responders in terms of in vitro type I IFN responses following SARS-CoV-2 stimulation. This classification might be arbitrary unless some in vivo phenotype of the HSPC donors or some allelic variations of relevant signaling components in the respective cells can be associated with these categories. At least the authors should verify their classification by repetition the SARS-CoV-2 stimulation experiments in independent pDC batches.

If possible, the authors should consider studying SARS-CoV-2 induced cytokine responses in pDC isolated from PBMC of their patient cohort. The study implies that there might be differences in the pDC responses of patients with different symptoms durations. However, it would be intriguiing to see the respective data.

It would be of particular interest to analyze allelic variations in signaling components of neuropilin-1 or in the receptor itself that can be associated with symptoms duration.

In the analyses of cytokine responses of HSPC-derived pDC no statistical analyses are provided (Fig. 4-7). This is probably due to experimental constrains that cannot be easily overcome. In many cases the effects of gene ko is so clear that statistical evaluation presumably is not necessary in order to conclude biological meanings. Nevertheless, in the materials and methods sections (e.g. in the statistics paragraph?) some words should be spent on how unbiased evaluation of the data was performed. This is important because in most experiments cells from two different donors showed quite some differences in their responses.

Minor concerns:
There are some typos that should be corrected, e.g. "Toll-like receptor" on page 3.

In Fig. 1A on the 3' end of the x axis there is shown "5". Is there any meaning for this?

In Fig. 3F there is shown "40"?!

In Fig. 4B right panel there is shown "5"?!

In Fig. 4F right panel there is shown "10"?!

We thank all three reviewers and the editor for reading our work and providing positive and constructive feedback. We appreciate the reviewers' consensus regarding our work's importance and relevance to the SARS-CoV-2 pandemic. We fully acknowledge the request to validate a number of experiments by increasing the test capacity of pDC donors. Thus, we have added significant data to support the key conclusions described this manuscript (shown in figure 5, 6 and 7 in the revised manuscript). By this, we find that we have addressed the reviewers' comments satisfactory and improved the overall quality of our manuscript.

We provide below a point-by-point response to the reviewers' comments

Reviewer #1:

In this manuscript Van der Sluis and colleagues have investigated the role of pDC in sensing SARS-CoV-2 and producing protective cytokines. They have used a differentiated HSPC model to modify pDC and test the role of specific host sensing pathways in CoV2 detection and responses. The models used and experimental design are good. There are some interesting and potentially important findings presented, but the data are somewhat preliminary.

Overall, I have some concerns about the reliability of the data and the number of times each experiment has been repeated. Some of the results are quite black and white, but others are not, and statistics don't seem to have been used to evidence reliability. As a consequence, some results will probably not survive close inspection. This is particularly important in primary cell experiments because results, particularly infection results, and the degree of induction of innate responses, can be quite variable between donors and experiments. I've made specific comments below but all figures should contain some form of statistical test and this will likely require some further repeats

We recognize the reviewer's concern about reliability though many of our data often are "black and white" as the reviewer points out. However, we have added several more pDC KO donors to our experiments to enhance reliability and confirming the results' reliability by conducting statistical analysis (presented in Fig 5, 6 and 7 in the revised manuscript).

1. I'm not sure that the picture of poor IFN induction by CoV2 described in the intro accurately represents the state of the art. Certainly there have been descriptions of IFN production by CoV2 exposed macrophages, for example, PMID33277988 suggests failed CoV2 replication activates IFN production in macrophages and PMID34101213 suggests infected epithelial cells can activate macrophages to produce IFNs and inflammatory cytokines. So its not all down to pDCs although I am sure that they play a central role as the authors propose. Maybe say a bit more about the likely positive contribution of macrophages to disease.

We appreciate the reviewer for highlighting numerous relevant papers coming out as the field is rapidly expanding. We have adjusted the main text to reflect the reviewers view on a possible role for macrophages either directly (PMID33277988+ PMID: 34122407) or indirectly (PMID34101213) for sensing SARS. Please see page 3, lines 13-16 of the revised manuscript.

2. I found the first para of the results confusing. Is baseline the best phrase to use for the first measurement when the patients may be quite sick and have high levels of viral replication and inflammation at the time of hospitalisation? Perhaps Day1? The phrase baseline is a bit misleading. Also I really don't understand what this sentence means "When we compared the percentage of pDCs at baseline to "duration of symptoms" (19), we observed a significant reduction in the percentage of pDCs from patients with symptoms duration between 5 to 12 days as compared to in the very early viremic phase (<day 4) (Fig 1A, Fig S1A). It seems to be comparing symptom duration, pDC level and vireamia. But viraemia is not measured? Please clarify.

To clarify the result section we have reworded “Baseline” to either Day 1 or D1 in figure 1, figure EV1 and the accompanying text, legends and materials and methods.

We have also rephrased the paragraph to clarify that we indeed compare pDC levels with duration of symptoms and not to viremia. Page 4-5 lines 16-24.

The patient cohort material used in this manuscript originates from a clinical investigation where the effect of anti-viral drugs on SARS-CoV-2 infection were explored (previously ref 19 in our manuscript). In this already published study, viremia was one of the measurements and this was correlated to duration of symptoms. We did not include the viremia measurements in the current manuscript because it is published data and referenced the manuscript instead. This is now clarified in the manuscript on page 4-5 lines 16-24.

3. Also, I'm not sure how to interpret the next sentence "However, patients presenting symptoms for more than 13 days prior to study enrollment, had a significantly higher percentage of circulating pDCs in peripheral blood (Fig 1A) and this coincided with decreasing viral load in the nasopharyngeal cavity and viral clearance in the majority of patients (19)." How does time of hospitalisation impact pDC level, or are we talking about length of symptoms. Also what does reference 19 have to do with it, it's a different study/patient group. Please rewrite this para with a view to improve clarity.

This comment is related to the previous question and we have rewritten the paragraph to provide clarity, see page 4-5, lines 16-24.

4. last sentence of this para suggests disease onset is associated with pDC decrease, is this not recovery is associated with pDC decrease.

We observe a decrease in pDCs (as frequency of PBMCs and as total pDC count/mL blood) at day 5 post hospitalization, but D5 does not equal recovery. Unfortunately, we do not have the blood samples at time of recovery or release from the hospital and can therefore not perform additional analysis to shed light on this. However, we now include data on pDC analysis of healthy controls, which shows that pDC frequency is lower in COVID-19 patients (irrespective of symptom duration or day of hospitalization). We adjusted the last sentence of the paragraph to reflect this. See page 5 lines 5-7 and 10-18.

5. S1 data referring to Fig 1 ie S1b-g should be in the main Figure.

We agree with the reviewer and have moved S1b-g panels to the main figure (Figure 1B, F-J in the revised manuscript).

6. Do the authors know that their virus preps are free of cytokines. If not, surely cytokines in the virus prep could activate the pDC rather then the virus itself. Mock infected sup is obviously no good to test this because it wouldn't contain virus induced cytokines. Can the authors rule this out?

We understand the concern that the reviewer raises here, but the various knock-out experiments clearly show that cytokine production is TLR driven (i.e. IFN by TLR7 and IL-6 by TLR2). If the cytokine production from pDCs was driven by Vero-cytokines, then the respective cytokines would have been detected in the KO pDC cultures.

7. Fig 2, given that pDC do not become infected, how do the authors work out MOI? Please specify how the doses are worked out.

Viral titer is determined by a limiting dilution assay on VeroE6+TMPRSS2 cells. This is described in method sections “virus and propagation” and “limiting dilution assay”. For clarity, we also added this information in the main text, see page 6 lines 5-6.

8. Top of page 7, the authors describe how sup from CoV2 exposed cells can induce protection from infection in fresh cells. They ascribe this to IFN production because they say, infection is rescued with blocking antibodies. If I understand correctly, the data in Fig S4C do not statistically support this conclusion. I imagine it needs repeating a few more times. Further details of amount of Ab would be helpful.

The main text on page 7 lines 11-12 reads: “Using blocking antibodies, we found that this protection was mediated partially by type I IFNs”. We made this formulation because viral titers in cell culture supernatant increased for all donors tested, but did not reach statistical significance, indicating that type I IFNs contributes to blocking but are most likely not the only component involved – for example, we did also see some type III IFN production. However, we have repeated the experiment with additional donors (final donor numbers n=5), and the results shows a clear trend but do not reach significance statistically (p=0.065).

The details of the blocking antibodies (amount, clone, and manufacturer) is listed at the end of the paragraph “infection assays” in the materials and methods. To make the information of antibody amount more accessible, we added this to the legend of the figure, please see Fig EV4 with the legend on page 52 lines 15-16 in the revised manuscript.

9. I'm not sure what the goal of the nanostring measurements are in Fig 3. Are Dhigh and Dlow true differences? Are responses measured consistent between experiments and are the differences between the 2 subjects statistically consistent between experiments? I don't think I see any significance measures or how many times it was done. Does n=2-4 refer to this experiment or that in E? Please clarify, particularly the statistical support for your findings in the nanostring measurements.

The addition of the NanoString experiment was based on our view of achieving a broader profile of how SARS-CoV-2 infection may induced changes in the pDC gene signature, with particular focus on genes related to antiviral responses. The two donors were selected based on SARS-CoV-2-induced IFNa production and they consistently produced different IFNa levels over the course of multiple days (D^{high} = triangle and D^{low} = square symbol in Figure 2L).

Nanostring is a highly reproducible technology, and it is generally not recommended to do technical replicates, since the enzyme-free, digital nature of the assay generates exceptionally precise and reproducible measurements (Pearson’s correlation coefficient above 0.99 can be expected; see page 6 in *PLoS One*. 2019 Nov 21;14(11):e0225505, PMID: 31751415). Though we acknowledge that it would have been ideal to perform these experiments on more donors to substantiate the findings, we still believe that our data serve the purpose to validate other findings in the manuscript (e.g. qPCR data) and gives a good picture of the broader changes that occur over time in response to the virus (e.g. wave patterns etc.). We decided to show the data as waterfall plots - instead of for example volcano plots -, which are commonly used for this type of data where statistical analyses cannot be performed for each individual gene. We prefer to keep the data in the revised manuscript, but have mentioned the limitations of the experiment in the main text. See page 8 lines 19-20.

Comment related to panel E; “E” was missing in the figure legend and is now added and the donor number for panel E is specified (n=3-4). See figure 3’s legend in the revised manuscript.

10. In Figure 4 c-f, its not clear what's being compared to what. Are we comparing each KO to the control KO (AAVS1)? Have these data been statistically assessed? There should be a comparison for each KO with the control but this is not clear. It just says n=2. Please show supporting statistical tests.

Statistical evaluation cannot be performed on n=2 but we acknowledge the concern the reviewer raises. Therefore, we have repeated several KO experiments that are key for the conclusions of our work, namely the $TLR7^{KO}$, $TLR2^{KO}$ and $CD304^{KO}$, with their respective control

AAVS1^{KO}, presented in Figure 5, 6 and 7 of the revised manuscript. All other KO exp with n=2 have been moved to the supplement, except for Fig 4 with the MyD88^{KO}, which is confirmatory to our TLR7 data. This is clarified in the main text on page 10, lines 10-11.

11. There are also no stats for Fig5, which I think has only been done twice. I appreciate the results are quite black and white but the data should be statistically supported, particularly in primary cell experiments where the data can be quite variable.

We have repeated the experiments with the TLR7^{KO} (fig 5) and moved the TLR3^{KO} to a supplementary figure (S7). The main findings of our work are now supported by statistical evidence.

12. p9 line 15, "not involved in" better, "not necessary for"

The sentence has been adjusted see page 10 line 6.

13. I wonder if the small effect sizes in Fig 5G, (2 fold ish) would be supported statistically. As above please apply some statistical tests to assess reliability of the primary cell data.

We have repeated the experiment to include more donors and performed statistical analysis that support the finding that IRAK4 mediates IFNa and CXCL10 but not IL-6 production. Please see figure EV5 and figure 5F in the revised manuscript.

14. Figure 6 also has no stats leading me to wonder how many of the conclusions drawn are statistically supported by the data. Please rectify.

We have repeated the experiment to include more TLR2^{KO} donors and performed statistical analysis that support the finding that TLR2 mediates IL-6 production in response to SARS-CoV-2 (Fig 6D) and in response to the viral E protein (Fig 6E).

Reviewer #2:

In this study, the authors demonstrated that pDCs induce IFNs via TLR7-MyD88 signaling while inducing inflammation by TLR2 signaling upon SARS-2 infection. Furthermore, they found that the SARS-2 E protein mitigates the IFNs of pDC by interacting with CD304. These findings are interesting, but there are some major issues to be addressed.

We appreciate that the reviewer finds our work of interest and acknowledge the issues pointed out, namely repeating a number of the knock-out experiments, which we have done in the revised manuscript. We would like to clarify a minor misunderstanding; it is not the viral E protein that interacts with CD304, however E protein interacts with TLR2 thereby inducing IL-6 production (fig 6 in the revised manuscript).

1. It is unclear if the disease severity correlates with pDC. For example, in Fig 1, the authors should show the correlations between pDC frequency and disease severity. For the mechanism experiments, two donors do not allow for statistical analysis. More donors should be included for analysis.

We have included this analysis as shown in Figure 1K-L and described on page 5 lines 13-16 of the revised manuscript. The data indicate that pDC frequency correlates with disease severity.

2. Since the pDCs were generated from HSPCs, how this ex vivo platform can mimic the real pDCs remains uncertain. Additional phenotype and functionality data should be provided. Were there any chances of mDCs contamination? Were these pDCs TLR9 signaling specific?

We have included an extra figure to show the phenotype and responsiveness upon TLR9 stimulation of the HSPC-pDC. The obtained cells are negative for myeloid marker CD11c and produce IFNa after stimulation of CpG-A (ODN2116). See figure S1 in the revised manuscript.

3. By using the CD304 ko, the authors demonstrated that CD304 is responsible for SARS-CoV-2 immune evasion. Although they cited the reference (SCIENCE•13 Nov 2020), the authors should provide experimental evidence to verify the real interactions between SARS-2 and CD304 in pDC in this study. The expression amounts of CD304 on pDC in SARS-2 patients should be provided as well.

We appreciate the line of thoughts although we find that further work on characterization of the protein-receptor interaction is beyond the scope of this manuscript, but will be the subject for our future investigations.

Nevertheless, to substantiate that CD304 signaling indeed can regulate IFNa production (as also mentioned in PMID: 17056507, and PMID: 17404592), we performed an experiment where CD304 targeting antibodies (Ab) were used on pDCs in combination with TLR7 agonist stimulation. These data demonstrates that engagement of CD304 receptor by Abs triggers

signaling that impair a TLR7-driven IFNa production in HSPC-pDC as well as in blood isolated pDCs (see fig 7 of the revised manuscript).

Expression levels of CD304 on pDCs isolated from COVID-19 patients is an interesting thought but lack of material from the clinical study prohibits us to accomplish such analysis. This will have to be pushed to future clinical studies.

4. pDC is the major producer of IFNa, authors should measure IFNa level in all patients' plasma and correlate it with pDC frequency besides measuring the cytokines.

We were able to measure IFNa in some residual frozen plasma samples from the patients using a low-volume and sensitive human IFNa2 ELISA. However, we acknowledge that this does of course not cover all IFNa isoforms and thus it is possible that some signals in theory was higher (see figure 1E).

5. In Fig. 1B, since the authors said that this subgroup of patients had samples at both baseline and 5-days post study enrollment, it is necessary to show the change of pDC frequency for each patient during this period. It is critical to determine whether the pDC dynamic is associated with the disease progression.

We adjusted the presentation of the data and it is now shown with a connecting line between baseline (now named D1) and D5 for each patient in relation to pDC and mDC frequency. Please see figure 1C-D in the revised manuscript.

Minor issues:

6. In Fig 2, it was not clear about FR2020 or FR4286? Please make sure the consistency in the figure legend.

FR4286 in the legend has been changed into FR2020 to match the main text and figure legend.

7. The authors should show the ko efficiency of TLRs by western blot in fig 5 and 6.

We agree with the reviewer that this would be a great addition but unfortunately performing Western blot analysis on the transmembrane TLR proteins is not applicable due to dearth of good targeting antibodies. Thus, to confirm knock-out efficiency we performed sequence analysis and functional controls for each KO, to show that they were unresponsiveness to the respective ligand.

8. In Fig 6, the legend should be consistent for ug/ml or µg/ml. Similarly, in Fig 7, AAVS1KO or AAVS1KO?

The legends have been adjusted and should now be consistent.

9. In the ko experiments, the authors only used two donors, which is not convincing.

We have repeated several KO experiments that are key for the conclusions of our work, namely the $TLR7^{KO}$, $TL2^{KO}$ and $CD304^{KO}$, with their respective control $AAVS1^{KO}$, presented in Figure 5, 6 and 7 of the revised manuscript.

10. In the Graphical abstract, how is IFNa mediated by IRF7 phosphorylation upon SARS-CoV-2 infection in pDC? Also, how does CD304 mediate the viral evasion via inhibition of IRF7 phosphorylation?

TLR7 senses endosomal RNA leading to IRF7 phosphorylation, which then translocates to the nucleus to fulfill its function as transcription factor and induces expression of different genes, such as the various IFNa genes. We show that SARS-CoV-2 is sensed by TLR7 but the downstream signal transduction pathway is well established (PMID: 21490621 and PMID: 26160613). We and others (PMID: 17056507, and PMID: 17404592), show that CD304 signaling reduces IFNa production. Some data indicate that CD304 signaling specifically reduces IRF7 phosphorylation and translocation and thereby reduces IFNa production. Whether this is the same mechanism for SARS-CoV-2 we do not know but will be relevant for future studies. .

11. In Methods, line 17, 2,5 ug/ml or 2.5 ug/ml? ml or mL?

The methods have been adjusted and should now be consistent

12. In figure legends, *<p0.05, **<p0.01, ***<p0.001 should be identical with the description in statistical analysis in Methods. No ****p<0.0001 appears in the whole study, could be removed.

This is corrected.

Reviewer #3:

In this study, van der Sluis and colleagues addressed pDC responses after stimulation with SARS-CoV-2. They found that at the time point of presentation to the hospital, COVID-19 patients with symptoms duration between 5 and 12 days had reduced percentages of pDC from total PBMC, which was less pronounced in patients with shorter or longer symptoms duration. Furthermore, pDC percentages dropped within 5 days after hospitalization. SARS-CoV-2 stimulated HSPC-derived pDC showed significant expression of cytokines such as IFN-alpha, CXCL10, IL-6 and others. Upon pretreatment of lung epithelial cell types with the cell free supernatant of virus-stimulated pDC, SARS-CoV-2 protective effects were detected that were mediated to a large extent by type I IFN. RNAseq analysis of low and high responder pDC revealed highly overlapping gene expression profiles and clusters of genes that were induced early or late.
Analysis of MyD88ko, TRIFko, RIGko and combined ko pDC revealed that pDC sense SARS-CoV-2 primarily via MyD88. TLR3ko and TLR8ko did not have an effect, whereas TLR7ko abolished type I IFN responses. TLR2 did not affect type I IFN responses, but impaired IL-6 production. Since TLR2 forms dimers with TLR1 and TLR6, the respective ko pDC were generated and only TLR6ko showed reduced IL-6 production. SARS-CoV-2 enters cells via ACE2, or alternatively via neuropilin-1. Since pDC do not express ACE2, whereas neuropilin-1 is an important pDC marker, also neuropilin-1ko pDC were tested. Such cells showed normal CXCL10 and IL-6 responses, whereas type I IFN production was enhanced. This result suggested that SARS-CoV-2 modulated pDC derived type I IFN responses by the interaction with neuropilin-1.

This is an elegant and technically sound study addressing a timely and important question, the role of pDC in SARS-CoV-2 infection. Specifically, analyzsis of the abundance of pDC in PBMC from COVID-19 patients and of the molecular mechanism of SARS-CoV-2 mediated induction of cytokine responses in pDC is highly relevant. The latter aspect was addressed by exploiting the innovative method of CRISPR/Cas9 gene editing in HSPC-derived pDC. The concept that neuropilin-1 potentially plays a role in modulating SARS-CoV-2 induced type I IFN responses is new and particularly interesting.

We appreciate the reviewer's positive evaluation of our work and address the specific points below.

Major concerns:
1. In Fig. 1A percentages of pDC from total PBMC from healthy donors should be included as a control. Also, absolute cell numbers should be presented to verify that reduced pDC percentages are indeed due to reduced pDC numbers.

Analysis of the frequency of pDC in healthy donors and absolute pDC counts are now included. Please see figure EV1B-C and page 5 lines 5-7. The data shows that in terms of absolute pDC counts, the pDCs follow the same patters as the pDC frequency and the pDCs decline from day of hospitalization to day 5. Analysis of the healthy donors shows that COVID-19 patients have a decrease in pDC frequency and cell count compared to healthy controls.

2. In the legend of Fig. 1B it should be clearly stated that PBMC from all patients are shown independent of the respective symptom duration, if this is the case.

The reviewer is correct and in (previously) fig 1B all patients are shown. The legend is updated to reflect this, please see figure legend 1C on page 36 line 7 in the revised manuscript.

3. In this study, all experiments addressing cytokine responses of in vitro activated pDC were performed with HSPC-derived pDC. The authors should formally show that PBMC-derived pDC show similar responses as detected in ex vivo isolated pDC. This control is particularly important for SARS-CoV-2 stimulated pDC because ex vivo isolated pDC and HSPC-derived pDC might differ with regard to expression of certain components and the activation status.

We appreciate the suggestion from the reviewer, which we also find very relevant. These results are now included in figure EV3 of the revised manuscript. The results clearly show that ex vivo isolated pDC respond to SARS-CoV-2 exposure in a similar manner as HSPC-pDC (i.e. the level of cytokine production increases with prolonged exposure to the virus).

4. The authors classified their HSPC-derived pDC as low and high responders in terms of in vitro type I IFN responses following SARS-CoV-2 stimulation. This classification might be arbitrary unless some in vivo phenotype of the HSPC donors or some allelic variations of relevant signaling components in the respective cells can be associated with these categories. At least the authors should verify their classification by repetition the SARS-CoV-2 stimulation experiments in independent pDC batches.

The reviewer is correct in the sense that the classification of high and low IFNa responders is somewhat arbitrary and is based on the concentration of IFNa they produce after SARS-CoV-2 sensing (D^{high} = triangle and D^{low} = square symbol in Figure 2L). However, we selected these two donors not to make a statement about high or low IFN producers but to explore genetic signatures of two donors that behave differently in terms of antiviral response. The aim of the NanoString experiment was to get a more global view of SARS-CoV-2-induced changes in pDC gene expression, with particular focus on genes related to the antiviral response. One alternative approach could have been to select donors that behaved comparable, but we thought it would be more interesting to investigate gene expression patterns across donors displaying a different virus response. The NanoString confirmed the low level IFNa production from D^{low} at the level of IFN RNA. Interestingly, except for the IFN genes, there was a large overlap between these two donors in terms of most upregulated genes and (Fig 3 AB). As such,

we think that this analysis has value and contributes to our understanding of how sensing of SARS-CoV-2 by pDCs induces different genes to be expressed at various time intervals, something that has not been shown before.

5. If possible, the authors should consider studying SARS-CoV-2 induced cytokine responses in pDC isolated from PBMC of their patient cohort. The study implies that there might be differences in the pDC responses of patients with different symptoms durations. However, it would be intriguiing to see the respective data.

We agree with the reviewer that this would be an intriguing avenue to explore. However, we and others (e.g Stephenson et al. 2021) have shown that cryopreserved pDCs respond very poorly when thawed and stimulated (e.g. with a TLR7/9 agonist) ex vivo. Functional studies on pDCs need to be done on freshly isolated PBMCs. Therefore, we cannot add a functional characterization of pDCs in patients in the present study.

6. It would be of particular interest to analyze allelic variations in signaling components of neuropilin-1 or in the receptor itself that can be associated with symptoms duration.

We agree with the reviewer that this would be interesting and will help to improve the paper, however, due to insufficient patient material; such analysis cannot be added in this present study.

7. In the analyses of cytokine responses of HSPC-derived pDC no statistical analyses are provided (Fig. 4-7). This is probably due to experimental constrains that cannot be easily overcome. In many cases the effects of gene ko is so clear that statistical evaluation presumably is not necessary in order to conclude biological meanings. Nevertheless, in the materials and methods sections (e.g. in the statistics paragraph?) some words should be spent on how unbiased evaluation of the data was performed. This is important because in most experiments cells from two different donors showed quite some differences in their responses.

We appreciate the comment form the reviewer, as generating the KOs is indeed time consuming. To allow for more appropriate analysis, we have repeated the KOs that are key to the conclusion of the manuscript, namely the $TLR7^{KO}$, $TLR2^{KO}$ and $CD304^{KO}$ (presented in Fig 5, 6 and 7, respectively, in the revised manuscript). All other KOs, that still contain N=2 donors have been moved to the supplement of the manuscript, except for the $MyD88^{KO}$. We left these data in the main body as the results are quite clear and we confirm the observations by following up with the $TLR7^{KO}$ (as the TLR7 signal via MyD88 is well established).

Minor concerns:

8. There are some typos that should be corrected, e.g. "Toll-like receptor" on page 3.

We have proofread the manuscript in the attempt to eliminate all typo’s

9. In Fig. 1A on the 3' end of the x axis there is shown "5". Is there any meaning for this?

In Fig. 3F there is shown "40"?!

In Fig. 4B right panel there is shown "5"?!

In Fig. 4F right panel there is shown "10"?!

These numbers should indeed not be on these positions in the figures and have been removed.

Dear Martin,

Thank you for submitting your revised manuscript to the EMBO Journal. Your study has now been re-reviewed by the three referees.

As you can see from the comments below, the referees appreciate that the analysis has been strengthened. Referee #1 raises remaining issues with the sample size. In response to this I asked you for a point-by-point response to the raised issues that I discussed further with referees #2 and 3. I have now heard back from the referees #2 and 3 and they agree with your proposal that add another sample to the MyD88 data set. I would therefor like to ask you to respond to this issue and the other raised issues in a final revision.

When you submit your revised manuscript will you also respond to the issues below:

- Please Rename "Data and materials availability" to "Data availability". The transcriptome analysis should be deposited in public database and the accession numbers provided in this section.

- The funding The Aarhus University Research Foundation (RMS) and The Central Denmark Region grant A3233(OSS) are not entered in our online submission system.

- The author contributions for Mads Kjølby are missing

- Please relabel COI as "Disclosure and Competing Interests". If you have nothing to disclose or any competing interests - see our guideline to authors - then state: The authors declare that they have no competing interests

- Please correct the format of the reference list

- The tables should be re-named as Table EV1 etc. The legends should be added as a separate tab.

- The Supplementary figure file should be called Appendix. Please add a TOC and page numbers, and remove yellow highlighting. Figures should be called Appendix Figure S1 etc. The captions for the supplemental table should be added to the Table excel files.

- Remove all figures from the main manuscript. Leave in the figure legends for the main and expanded view figures. Also remove one sentence summary from MS file.

- Synopsis/Graphical image and text should be uploaded as separate files. Please make sure that size of image is correct 550 wide by [200-400] high (pixels). For synopsis we also need a general summary statement and 3-5 bullet points that capture the key findings of the paper.

- We encourage the publication of source data, particularly for electrophoretic gels and blots, with the aim of making primary data more accessible and transparent to the reader. It would be great if you could provide me with a PDF file per figure that contains the original, uncropped and unprocessed scans of all or key gels used in the figure? The PDF files should be labelled with the appropriate figure/panel number, and should have molecular weight markers; further annotation could be useful but is not essential. The PDF files will be published online with the article as supplementary "Source Data" files.

- Please add in the M&M that you have approval for using human donors. Also, will you add a statement in the M&M that you have approval for working with SARS-CoV-2. "Experiments dealing with SARS-CoV-2 were performed in a... laboratory under the approval of..."

- Our publisher has also done their pre-publication check on your manuscript. When you log into the manuscript submission system you will see the file "Data Edited Manuscript file". Please take a look at the word file and the comments regarding the figure legends and respond to the issues.

- Are Fig S5A and S5B controls reused in S6C and S6D? If so please state this in figure legends

That should be all. Let me know if we need to discuss anything further

with best wishes

Karin

Karin Dumstrei, PhD
Senior Editor
The EMBO Journal

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Guide For Authors: https://www.embopress.org/page/journal/14602075/authorguide

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Referee #1:

The authors have addressed some of my concerns but I must say, some of the most important points, those addressing statistical support of the data, ie whether the data support the conclusions, have not been addressed. It is simply not OK to say, that the results are not statistically supported, but we think they're true anyway. Published experiments should always show statistical significance because without this we simply cannot interpret data or determine whether they are reliable. It is also not OK to put the unsupported data in the supplementary figures in the hope that they escape scrutiny. As I said in my original review, providing statistically supported data is particularly important for primary immune cell experiments which are known to give variable results. You simply have to repeat the experiments several times to know that the results are reliable. To have an author citing unsupported observations as central to their story, in a manuscript after review, makes me question their integrity and capacity to perform rigorous research. I strongly advise them to re-evaluate their approach.

Major points
The claims made should refer to statistically supported data throughout. ALL DATA should be statistically supported if being interpreted in the study. Where data does not reach significance, this should be clearly pointed out and the conclusions should not rely on such observations.

For example,
1. Experiments in Figure 4 have been done twice and show no statistical support. One cannot make, for example, the following statement, when by the authors admission in the rebuttal, the data are not statistically supported, "When exposed to SARS-CoV-2, we observed that MyD88KO pDCs were severely impaired in the induction of CXCL10 and type I IFNα, as compared to the AAVS1KO control pDCs (Figure 4C-D). "

2. As pointed out previously, Fig EV4c is not statistically supported. Failure to statistically support the role for IFN does not mean that something else is involved, it means that the data do not support a role for IFN and therefore the authors don't know if IFN is involved. This type of unsupported statement undermines the whole paper by suggesting the authors are not effectively interpreting their results.

"Blocking type I IFN signaling enhanced virus replication for all pDC donors tested, but this did not reach statistical significance, indicating that protection was mediated partially by type I IFNs (Figure EV4C)."

3. Please ensure that ALL DATA within the manuscript that are interpreted in the results section are statistically supported including all data in the Extended data section. I don't see stats for data in Figures S2, S5, S6, S7, S8 and S10.

Minor point
Page 4/5. Please cite the prior study (previously ref 19) from which the samples were taken and explain the published associations important for the study, ie viral loads and their relation to pDC counts etc. Ie put this work in the context of the previous work given it's the same patients. It wasn't clear before that this study uses patients characterised in another study and it isn't clear now.

Referee #2:

The authors have nicely replied to the questions. I have no further comments.

Referee #3:

The authors carefully addressed my concerns and suggestions wherever technically possible. I think the data presented in the revised manuscript fully support the drawn conclusions.

EMBO
4st of February, 2022

Dear Senior Editor, Karin Dumstrei

Please find below our point-by-point rebuttal to the 2nd revision of our manuscript entitled "**Distinct SARS-CoV-2 sensing pathways in pDCs driving TLR7-antiviral vs. TLR2-immunopathological responses in COVID-19**". We are pleased to see that Referee #2 and #3 appreciated our corrections and additional data that strengthen the manuscript overall. We are contented that they also endorse acceptance of the manuscript.

Point-to-point response to the criticism raised by Referee #1 (R1).
Overall, we do appreciate the constructive critique from R1, which has improved the manuscript, however, we find that the hostile attitude in the latest revision is unjustified and inappropriate, and we therefore distance ourselves from the comment about our integrity and capacity to perform rigorous research.

Successful generation of genetically modified human primary stem cells and differentiation into pDCs, followed by extensive quality assessment of the cell population prior to commencing additional experimental setup is not a trivial exercise – and we would have appreciated Referee #1 to acknowledged this. For each single donor this rigorous research takes roughly 4-5 weeks of work plus additional weeks of post analysis. When we initiated the experiments for the revision of our manuscript, we had to balance our time. Making additional donors to repeat every single experiment would take many months of work. Instead, we chose to be selective and focus on those experiments that identified the sensing pathways shown to be relevant for SARS-CoV-2, and thereby strengthen the data with statistical power to what was central to our story. Thus, our decision was to add additional primary donors to all dataset conferring viral sensing by TLR2, TLR7 and CD304 as showed in figure 5, 6 and 7.

The dataset on MyD88 in figure 4 was not selected primarily because we found the existing data truly black and white. Furthermore, as MyD88 is a downstream factor for TLR7 signaling, we found that the data to some extend was redundant to the TLR7 KO data. However, by request from Referee #1 we have now completed additional donors and modified Figure 4B, 4C and 4D, as well as Appendix Figure S5A, S5B, S5C, S5D, and S5E. The data show significant differences as MyD88 KO pDC did not produce any IFNa response in regard to SARS-CoV-2 infection in all four donors.

Regarding figure restructuring to support EMBO platform of "extensive view" and limiting the focus on TLR7-TLR8 dual KO cells in the supplementary figure; This had nothing to do with "escape scrutiny". The original data (though only done in two donors) did in our view exhibit that TLR8 was not driving any IFNa or CXCL10 production in response to SARS-CoV-2 (see original supplementary figure 10). To streamline the story and focus on the main factors – namely TLR2, TLR7 and CD304 - we decided to minimize the manuscript emphasis on TLR8, and thus we compiled the data in a new supplementary figure 8.

For the points regarding lack of statistical data analysis pointed out by Referee #1, we respectfully disagree with the requirements. The majority of data in S2, S5, S6, S8 and S10 are supportive data showing the evaluation of the KOs, either by sequencing, Western blotting or in functional assays.

Biomedicine

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Professor

Date: 04 January 2022

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These data had never been intended for making strong arguments or in-depth analysis but to quality assess the effects of CRISPR gene knockout. Furthermore, we have reviewed all the statistical approach throughout the whole manuscript; and made further emphasis on which and why we have used various statistical analysis. In some situations for example, where the Null hypothesis assumes a difference between groups in a specific direction, we have used one-tailed ratio paired T cells and not two-tailed paired T test.

Regarding the patient samples, the first publication using the samples from this cohort is referenced in the materials and methods.

We look forward to your editorial decision.

Yours sincerely,

Martin R Jakobsen

2nd Revision - Editorial Decision **7th Feb 2022**

Dear Martin,

Thank you for submitting your revised manuscript to The EMBO Journal.

I have now had a chance to take a look at the introduced revisions and all looks good.

I am very pleased to accept the manuscript for publication here.

Congratulations on a nice study!

Best Karin

Karin Dumstrei, PhD
Senior Editor
The EMBO Journal

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Corresponding Author Name: Martin R Jakobsen
Journal Submitted to: EMBO Journal
Manuscript Number: EMBOJ-2021-109622R

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple $\chi 2$ tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	N/A
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	N/A
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	N/A
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	N/A
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4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	no
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Is the variance similar between the groups that are being statistically compared?	N/A

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	done in the method section
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	written in the method section and acknowledgement for those we received from third parties. Mycoplasma test was done rutinely on all our cell cultures with Eurofin screening assays

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	N/A
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	N/A
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	N/A

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	Used anonymous cord blood samples to collect CD34+ cells
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	Yes
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18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	GEO accession number (GSE195894)
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