Journal of Cell Science

Review COMMONS

Reduction of nucleolar NOC1 leads to the accumulation of pre-rRNAs and induces Xrp1, affecting growth and resulting in cell competition

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DOI: 10.1242/jcs.260110

Editor: Maria Carmo-Fonseca

Review timeline

Submission to Review Commons:	8 July 2021
Original submission:	8 April 2022
Editorial decision:	16 May 2022
First revision received:	7 October 2022
Editorial decision:	20 October 2022
Second revision received:	21 October 2022
Accepted:	25 October 2022

Reviewer 1

Evidence, reproducibility and clarity

Summary:

The manuscript by Destefanis et al. titled Nucleolar NOC1 controls protein synthesis and cell competition in Drosophila examines the over-expression of NOC1 and the RNAi depletions of NOC1, 2, and 3 in various Drosophila larval tissues, and the effects these changes have on larval development. The NOC proteins are conserved nucleolar proteins required for ribosome biogenesis.

This manuscript has merit in that it offers the first examination of the NOC proteins in Drosophila beyond simplistic predictions garnered from high-throughput studies. With RNAi depletion of NOC1, the authors show losses in poly-ribosome abundance and protein synthesis. The manuscript then focuses heavily on the adverse phenotypes caused by disrupting ribosome biogenesis in the prothoracic gland which produces ecdysone (the molting hormone), the fat body affecting release of Drosophila Insulin-like Proteins (DILPs), and in imaginal wing disc cells affecting their survival by inducing apoptosis via and JNK and eiger, a pro-apoptotic factor.

Major Comments:

Describing developmental phenotypes associated with the depletion of the NOC proteins is necessary at some point, but the phenotypes are generally expected for ribosome loss in the tissues examined. I was hoping the paper would describe a mechanistic link between the loss of NOC protein function and the loss of ribosome production that ultimately leads to the predicted developmental defects.

Minor Comments:
1) The paragraph in the Introduction describing DILPs seems too long and out of place, disrupting the description of the NOC proteins. This paragraph could be reduced to one sentence that is inserted near the end of the Introduction when DILP8 is mentioned.

2) At the end of the Introduction, the authors say that NOC1 is a novel nucleolar component. Delete the word 'novel'; the first paragraph described conserved NOC proteins in yeast, human, and Arabidopsis.

3) First paragraph of Results: Do the NOC proteins contribute to MYC function or are they induced by MYC? Are there any MYC binding sites in the NOC gene promoters or 5' UTRs?

4) Overall, Figure 1 is too congested. Fig. 1B and C could easily go to Suppl. Data.

5) for the various RNAi lines used, are there any off-target transcripts that could be affected?

6) In their polysome profiling, the authors first say there's a decrease in ribosomal subunits correlating with a decrease in polysomes, but then "an accumulation of the 40S and 60S subunits (not shown)." I assume the authors mean that there's an overall loss in subunit biogenesis due to NOC1 depletion which leads to polysome loss. If so, how would these combined losses contribute to a gain of free 40S and 60S subunits? This is confusing, and should be better explained.

7) When describing Fig. 1J-K in the text, the authors use the word 'rate' of labeled proteins. To define rate, they should briefly mention the 40- or 60-minute pulse times.

8) Fig. 1L is problematic. First, en-GAL4 is driving expression of both GFP and HA-NOC1. The NOC1 labeling on the left-hand side of the figure should be labeled HA-NOC1 just to be clear. Also, the GFP labeling looks as if it's cytoplasmic, I'm guessing mitochondrial (en>mGFP as perhaps correctly labeled in the figure), but the text and legend describes the GFP labeling as nuclear.

Second, while both Fibrillarin and NOC1 locate to nucleoli, it looks as if the two proteins do NOT co-localize within nucleolar sub-regions. This is significant, because if the NOC proteins are important for 60S large subunit maturation and release from the nucleoli, you might expect them to reside in the more peripheral Granular Components of nucleoli. Fibrillarin on the other hand, is an early assembly factor that clearly resides within the more centralized Dense Fibrillar Component of nucleoli. Fig.1L actually suggests this bipartite localization.
Perhaps the authors can provide higher mag images to show this difference in localization. Third, the large aggregates within nuclei of cells over-expressing HA-NOC1 are worrisome because other endogenous nuclear or nucleolar proteins could relocate to these aggregates causing adverse phenotypes. Is there a less potent GAL4 drive you could use to reduce over-expression? Are there any GFP-protein trap lines available for NOC1?

9) Middle of page 7: Reducing NOC protein expression later in development in differentiated cells does not have any apparent effect on development. Add the word 'apparent'. There could be intra-cellular effects that have not been detected.

10) Fig. 3B and C should be labeled P0206>NOC1-RNAi. Scale bars are needed for Fig. 3A, B and C.

11) Middle of Page 8: At the beginning of NOC1 depletion in Fat Body, remind the reader that the previous section depleted NOC1 from the prothoracic gland which caused fat accumulation in normal fat body cells that grew large, but now with depletion of NOC1 in the Fat body cells themselves, the cells are smaller.

12) Fig. 4A-E scale bars are needed. Fig. 4I-P scale bars and sample sizes (n values) are needed.

13) Fig. 5: scale bars are needed, especially for panels A-E. When explaining the M(3)66D experiment, perhaps describe it from the point of view that the NOC1-RNAi clones now have less competition from surrounding cells and this permits their detection.

14) On the top portion of page 11, what is TRE-GstD1, and what is the mechanistic link between JNK and TRE-GstD1?

15) Any off target sites for the sgRNA used in the CRISPR mutation analysis?

16) In the first paragraph of the Discussion, Drosophila pre-rRNA is initially 38S.

17) Middle of page 13: "...defects in translation and in protein synthesis." Somewhat redundant.

18) Middle of page 15: "...in the wing discs with clones that had reduced NOC1..."

19) Same paragraph as in item 17: How is this novel?

20) In Materials and Methods, page 18: UAS-CG1234-RNAi stock is Bloomington 61872 (no 0 at the end).

21) Bottom of page 18: "The list of primers..." This sentence needs re-writing.

Significance

Nature and significance of the advance.

This is a preliminary study on the loss of the Drosophila NOC proteins that are necessary for ribosome production. Much of the paper describes developmental defects associated with the loss of ribosome production.

Compare to existing published knowledge**

The phenotypes are interesting, but expected based on previous research on bobbed mutations and the many Minute mutations.

Audience

Developmental biologists interested in ribosomal stress.

Reviewer 2

Evidence, reproducibility and clarity

Summary:

This manuscript by Destefanis, Manara, Santarelli et al., describes the role of Noc1, a nucleolar protein involved in ribosomal subunit assembly and protein synthesis, in organismal and cellular growth, which also impacts on cell competition in Drosophila.

Using gain and loss of function experiments, the authors characterize the role of Noc1 in various tissues and discover that this protein is necessary to induce cell growth, metabolic homeostasis and developmental progression in Drosophila. Noc1 downregulation leads to cell death and impact the production of secreted factors Eiger/TNF-alpha and DILP8 as well as Ecdysone signaling, resulting in impaired cell/tissue growth and larval developmental delay. Impairing Noc1 expression in metabolic tissues, such as the fat body, causes loss of lipid homeostasis marked by loss of systemic lipid stores and accumulation of lipids in imaginal discs and gut tissue.

Major comments:

1- In many cases, such as in the context of cell and organismal growth, gain and loss of function of Noc 1 leads to overall similar phenotypes (e.g. impaired growth). While clearly loss of gene function gives has a stronger effect, the authors provide no possible explanation for this.

2- Figure 1J: I do not see the stronger reduction of Puromycin signal after 60 min of treatment quantified in Figure 1K. Also, if general protein synthesis is affected by Noc1 knockdown, shouldn't total levels of actin be also reduced.

3- Figure 2: The eye phenotypes should be quantified. Also, what is the cause of this phenotype? Is it apoptosis, lack of proliferation or both? Data showed later in the paper would suggest the former. Direct assessment of cell death/proliferation by immunostaining would be useful to clarify the

origin of the smaller eye phenotype in ey>Noc1 RNAi flies. Also, using genetic approaches (e.g. blocking apoptosis in the context of Noc-RNAi) would make this data more complete and compelling. The authors show latter in the paper that knockdown of Noc1 in wing discs leads to apoptosis and Eiger/TNF-alpha upreguation. Is this the case in the eye as well? Genetic interactions between Noc1 and Eiger should be perform in the eye and wing to determine the functional significance of Eiger overexpression in cell death induced upon Noc1 knockdown.

4- Figure 3: Data is missing, misrepresented when comparing the Figure with the results described in the text.
a- As per the results description (page 7-8): 'Downregulation of Noc in the prothoracic gland (PG) leads to developmental delay with animals stalled in the larval stage for 20 days followed by death'. This data is neither shown in the paper, nor mentioned as 'data not shown'. This significant result should be shown.
b- There is an error in Figure 3D. Dark blue bar is indicated as Noc1 overexpression.
c- Figure 3E: the use of 'cell size' is confusing here as it is fat body size what is being represented.
d- Figure 3F is presented before 3E in the result section but not in the figure. This should be changed to keep figure panels in the order they are presented in the results.
e- Figure 3D, E: The data corresponding to Noc1 overexpression referred to in the results section as present in these panels is not shown in the figure.

5- Figure 4:
a- Figure 4A-F: The loss of function data presented here is clear. However, the data on the gain of function is less clear. Using a cytoplasmic GFP to label the clones would be more suitable.

b- Figure 4H: Data in this figure appears to show wing disc size measures rather than animal survival or developmental delay as described in the results section.

c- Figure 4I-P: This should be quantified.

d- Figure 4G: Overexpression in the fat body leads to bigger larvae. However, overexpression of Noc1 in the whole body leads to small larvae (Figure 1D). Why? What happens with lipid stores and DILP2 upon Noc overexpression in the fat body?

6- Figure 5: Is the role of Noc1 specific to cell competition? It seems to me that this is a general regulator of cell growth/survival. How can you distinguish one from the other? Or is it the case that any protein required for overall cell growth/survival would impact cell competition? Does Noc1 impact Myc expression?

7- Figure 6: Results presented in this figure are clear and completing. However, genetic interactions between Noc1/Eiger and Noc1/Dilp8 would make this data more complete by directly assessing the functional relevance of these secreted factors to the Noc1 phenotype.

8- Figure 7: Not sure how much this data contributes to the study. It could be moved into supplementary data as further evidence of the loss of function phenotype of Noc1 using an independent genetic tool.

Minor comments:

The manuscript should be carefully edited to correct for typos/missing words.

Significance

This work identifies a new regulator of cell/tissue growth and mediator of cell competition in Drosophila. A significant body of work and varied techniques (e.g. cell biology, biochemistry, genetics) have been used here to characterise the role and cellular localization of Noc proteins in Drosophila. However, as detailed above, additional experiments are necessary to clarify the role of the protein and also support some of the conclusion of this study. I also noticed significant errors in data presentation that need to be corrected before this paper is accepted for publication.

Reviewer 3

Evidence, reproducibility and clarity

Summary:

NOCs proteins are important players for protein synthesis and ribosome biogenesis in yeasts and plants. Despite their high conservation among eukaryotes, very little is known about their function in animals. Destefanis and colleagues address this question using Drosophila melanogaster as a model. Loss-of-function experiments show that NOC1, NOC2 and NOC3 are necessary for animal's growth, proper development and survival, achieving non-redundant functions. In addition, NOC1 is overall necessary for proper ribosome biogenesis and protein synthesis and localizes at the nucleus and nucleolus. These observations provide good evidence that NOC1 plays a similar role in Drosophila and in yeast at the cellular level.

On this basis, the authors started exploring the role of NOCs in different tissues during development. Again, their results support the idea of NOCs being involved in basic cellular functions required for cellular growth in different cell types, either proliferative or endoreplicative.
To better understand the signaling events affecting growth and development downstream of NOC1, the authors analyzed more in detail the effects of NOC1 gain and loss-of-function in the wing imaginal disc. They propose a link with cell competition and with the hormone DILP8, involved in the control of systemic growth and developmental timing. Yet, this part of the study is weaker, lacking any epistasy that would be required to establish causal relationships between the different players.

Major comments:

- Fig 4: Analysis of the fat content using Nile-Red is not very convincing. It is clear that cg>NOC1i animals have less fat body, but not necessarily that FB cells display less Nile-red staining. In addition, scale bars are missing and different pictures where clearly taken using different magnifications (i.e the brain or sg in the two conditions). There is no control for the WID. Concerning the brains, again it is difficult to conclude because this method is not quantitative and brains seem to be stained both in controls and NOC1i.
- The effect of NOC1i in the fat body on DILPs levels lack more detailed analysis. By experience, within a given experimental condition the staining for DILP2 in the IPCs is quite variable. A careful quantification of the fluorescence therefore needs to be done. In addition, measuring circulating DILP2 levels (ELISA with double-tagged DILP2 construct) would be necessary to conclude that it is indeed retained in the IPCs.
- Fig 5A-E: when where the clones induced? Scale bars are missing, is it the same magnification? Overall the pictures are quite bad and clones are not visible especially in C-E. Pictures should be taken as for the following experiments (F to M), where clones are perfectly visible.
- Fig 5: for the clonal analysis, it seems to me that it is not because clones of cells die, that they are necessarily subjected to cell competition. They can simply be too much affected to survive. Hence, the statement that "cells died and were outcompeted by the neighboring cells (Fig 5F-G)" does not seem correct in the first place. Only the experiment in the M+/- background suggests that NOC1i cells are in part actively eliminated by cell competition. If the authors want to focus the ms on cell competition, an interesting point would be to test the role of Xrp1 downstream of NOC1. Indeed, Xrp1 has been shown to trigger most if not all the effects of Minute mutations (cell competition, decreased translation, DILP8 upregulation...). Is NOC1 LOF triggering the same stress response as Rp knock-down?
- An important experimental issue is the tools used to study NOCs in the growth of wing imaginal discs (Figs 6 and 7). Both MS1096-GAL4 and engrailed-GAL4 are very unspecific (brain expression, fat body and gut expression for en, etc...). It is therefore difficult to conclude that the effects on wing and animal development are due to expression specifically in the wing and not any other tissue involved in systemic growth control. In addition, MS1096-GAL4 is indeed rather dorsal in late development, but picts in Fig6 A-C show expression in the whole pouch (tissue size must be quantified to show that it consistently affects growth). In adult wings, dorsal growth inhibition usually triggers curly wings. How can these be properly measured? The use of more specific GAL4

lines (R11F02, nub, rn) is necessary to conclude on the cell-autonomous effects of NOC1 misexpression and to carry out epistasy experiments.
- What is the TRE-GstD1-RFP construct? It is not described in the methods. Traditionally, the GstD-GFP reporter is used to monitor oxidative stress. Even though oxidative stress and JNK signaling are linked, the use of a more direct reporter of JNK signaling would be more appropriate.
- The model presented in Fig6 lacks experimental evidence and is based mainly on correlations. Epistasy experiments are necessary here. DILP8 upregulation is clear, but does it indeed trigger the delay at pupariation? Is it indeed induced by Eiger/JNK/oxidative stress? An important alternative would be Xrp1, which triggers DILP8 upregulation in Minute conditions. Does Eiger/Grnd/JNK downregulation rescue NOC1i phenotypes (growth, delay) and DILP8 upregulation? These experiments would bring functional insights into the role of NOC1 in growth control.

Minor comments:

- Both in the introduction and in the discussion, the authors mention their "transcriptome analysis" suggesting that NOCs might be related to MYC. Are these data published? Should they be mentioned without including them? It would be easier to either remove the reference to this analysis or better describe it so that the reader understands the link with MYC and how it contributes to the background of this story.
- Fig 2: The differences between GMR and ey are striking, and the authors conclude on a role for NOCs more specifically in proliferative cells. This conclusion sounds in contradiction with the results in endoreplicative tissues where NOCs-RNAi have an important impact. Why NOCs LOF would not play a role in differentiated cells such as the retina if they are required for such important cellular functions as protein synthesis?
- In the text, reference to Fig4I-L is switched with Fig4M-P
- Fig 5: the graph in J should be in F for easier fig reading. In N-O, it is written "Minute clones". These are not Minute clones (Rp+/- clones in a Rp+/+ background).
- Fig6 L and N: genotypes are confusing, rn-GAL4 should be written apart from the reporters. Fig 6N: is eiger-GFP also induced non-autonomously in the notum?

Significance

The authors describe new genes, NOC1, 2 and 3 involved in growth control related to ribosomal function. Importantly, these genes are well conserved among eukaryotes. A number of recent studies in Drosophila have focused on the signaling mechanisms involved in the response to ribosome dysfunction focusing mostly on Minute conditions. They have identified a number of new players such as proteotoxic stress or the RpS12/Irbp18/Xrp1 pathway, which are important for cell competition and the non-autonomous effects caused by ribosome dysfunctions. The study by Destefanis and colleagues identify new players involved in ribosome biogenesis that could potentially reinforce the emerging idea that ribosomes are signaling hubs for the control of cell/body growth (instead of more "passive" players controlling the rate of protein translation).

My field of expertise is growth control, Drosophila development and genetics. I am less expert on ribosome biology (i.e. assembly, literature in yeast...).

Reviewer 4

Evidence, reproducibility and clarity

Eukaryotic ribosome biogenesis requires about 200 ribosome biogenesis factors in addition to the structural ribosomal components. Based on the known functional and structural homologies of both the structural components and the biogenesis factors, the principles in ribosome formation seem to be rather conserved in all eukaryotes. While most of our knowledge about ribosome synthesis comes from studies in the yeast S. cerevisiae not so much is known about this process and contributing factors in multicellular organisms like Drosophila. In this study the authors up- or downregulate the expression levels of the Noc proteins NOC1, NOC2 and NOC3 to analyze their function in Drosophila. The Noc1-3 homologues were previously structurally and functionally analyzed in yeast. The authors postulate that Drosophila NOC1 is required for the synthesis of both ribosomal subunits and for proper translation. They found that expression of NOC1 is required for

normal animal growth and organ function. Downregulation of NOC1 affected proliferating tissues with no significant effect on differentiated cells. In particular, the authors reduced NOC1 expression specifically in the eye, prothoracic gland, fat body and wing imaginal discs. In all tissues they found strong functional or developmental defects. Moreover, NOC1 reduction led to an induction of regulated cell death. The manuscript confirms previous analyses that depletion of a factor which is crucial for the formation of functional ribosomes impairs many cellular processes which depend on unperturbed protein synthesis.

Major problems

1) Based on their homology to the yeast Noc proteins it can be anticipated that Drosophila NOC-proteins are required for ribosome biogenesis. Basically, all observed consequences of NOC-downregulation can be explained by a strong reduction of functional ribosomes because of impaired ribosome maturation. Therefore, it is important to clearly show the impact of NOC-proteins on ribosome biogenesis. Unfortunately, the authors don´t provide state-of -the-art experiments, like co-sedimentation with pre-ribosomes, pre-rRNA processing analyses or Co-IP experiments of pre-ribosomes which would demonstrate a direct role of Drosophila NOC proteins in ribosome biogenesis. Instead, the authors claim that NOC1 downregulation results in defective polysome formation. It is difficult to interpret the polysome gradients depicted in Fig. 1 G-I, since after NOC1 downregulation all peaks were significantly reduced, which could be also due to a general reduction of gene expression. In yeast, impairment of Noc1-3 proteins resulted in defective pre-60S synthesis. The authors claim that Drosophila NOC1 affects the synthesis of both subunits. Therefore, it is important to figure out whether the Drosophila protein has adopted an additional role.
2) All functional consequences of NOC downregulation can be explained by a defective or reduced population of ribosomes affecting protein synthesis. Several previous studies highlighted that failure in ribosome biosynthesis leads to strong proliferation defects in Drosophila. In principle, inhibition of any ribosome biogenesis factor which is essential for the formation of functional ribosomes might have similar effects on protein homeostasis. Therefore, it is likely that single factors play no specific role to balance protein homeostasis unless their activity/expression is specifically triggered by signal transduction pathways. Since the authors "suggest a novel role for NOC1 in the control of the biosynthetic activity of the cells" (page 10) they should provide direct evidence that NOC1 is a key target. The observation that cells with reduced NOC1 are subjected to cell competition can be explained by pleiotropic effects and correlates with earlier studies that reduction of protein synthesis triggers apoptosis in cell competition. Another explanation for cellular out-competition is that the accumulation of deficient pre-ribosomes might lead to proteotoxic stress which is a driver of the "loser cell status".

Minor points

1) The recent publications describing the position of NOC1 in yeast pre-60 S ribosomes should be cited

2) The rescue experiment which is described on page 5 is not included in Fig. 1A inset nor in Fig. S2

3) Fig. 1J, please include the Ponceau staining, to verify that only neosynthesized proteins are affected

4) Fig 1 K, how was quantification analyzed?

5) The text in the figure legends and result part and the labelling of Fig 4 is mixed up

6) Page 9 line 7, release

Significance

Ribosomes, the cellular factories for protein biosynthesis are possibly among the most intricate and complex ribonucleoprotein-assemblies in the cell. Analysis of ribosome biogenesis and the regulation of ribosome function are central topics of biological research.

If it is clearly shown that NOC1-3 affect ribosome biogenesis as their counterparts in yeast, the manuscript would confirm previous data about the function of Noc-proteins and that eukaryotic ribosome biogenesis is rather conserved in single celled and multicellular organisms.

A direct involvement of Noc1 in the biogenesis of both ribosomal subunits is unexpected. So far most of the eukaryotic ribosome biogenesis factors including the yeast Noc proteins are specific for either the small or the large ribosomal subunit. Participation of NOC1 in the synthesis of both subunits would be a novel and very interesting aspect in Drosophila- ribosome synthesis. Therefore, it is important to clearly show that NOC proteins are involved in the maturation of both subunits.

The regulation of NOC1 activity to specifically control protein translation or induce apoptosis would be a novel and exciting aspect. The interesting effects in different Drosophila tissues after NOC1 depletion are then consequences of a regulated NOC1-impairment. However, such a regulation must be shown. I am not sure whether alone the observation, that impairment of protein synthesis will lead to the described cellular consequences, provides sufficient novelty in the Drosophila field for publication.

Author response to reviewers' comments

1. General Statements

We would like to thank the reviewers for the useful and constructive comments about our paper.

Here is our full revision of the manuscript, where we tried to fulfill all the request of the reviewers, there are a couple of missing experiments that are ongoing at the moment in the lab (see point below). We believe that NOC proteins have an important role in controlling RNA processing and maturation also in flies. Here we focus mainly on the characterization of NOC1 that together with NOC2 forms functional heterodimers necessary for the correct rRNA processing and maturation. From our data we can conclude that this function is conserved also in flies. In addition, and perhaps for the reason just mentioned, we demonstrate that the level of NOC1 must be tightly control in vivo since its overexpression or reduction is detrimental for the cells; and while NOC1 ubiquitous overexpression is detrimental because of the increase in protein synthesis perhaps resulting toxic in some organs, its reduction induces apoptosis with a mechanism linked to the activation of proteotoxic stress because of defects in the ribosome assembling and maturation. This impairment in protein synthesis is sufficient to trigger NOC1-RNAi cells to be eliminated by cell-competition. In sustain to this model we also found the transcription factor Xrp1 upregulated in cells with reduced NOC1 levels.

We agree with the reviewers that at the moment, we do not have the complete vision of how all these mechanisms are connected, but we believe that we have done few experiments that better elucidate some aspects of the characterization of NOC1 function in Drosophila.
Here are summarized the major points:

1- We consolidated the experiments showing the effect of NOC1 in vivo highlighting how both its ubiquitous reduction and overexpression translate into animal lethality. These detrimental effects are rescued when NOC1-OE is co-expressed with NOC1-RNAi, underling the importance of NOC1 in the regulation of cell survival (Figure 1).

2- We have shown that NOC1 protein localizes with fibrillarin in the nucleolus. This was achieved using either a line from Kudron which allows the expression of CG7838-NOC1-GFP-FPTB at physiological levels, or by immunofluorescence using HA-NOC1, whose levels were reduced by co-expression with its RNAi (Figure1).

3- We performed novel experiments to better define the role of NOC1 in the control of rRNA maturation. Specifically, we have shown that animals with reduced NOC1 accumulate rRNA precursors and have reduced levels of 18S and 28S. These new data, in combination with the previous ones that reported a significant reduction in ribosomes assembling and polysome

formation in NOC1-RNAi animals let us to conclude that also in *Drosophila* NOC1 is necessary for correct rRNA processing. These data are now part of the new Figure 2.

4- NOC1-downregulating cells suffer from growth defects that are rescued in a Minute/+ background and by expressing the inhibitor of caspase P35, acting as losers in a competitive background. We found the proapoptotic gene Xrp1 upregulated suggesting that these cells undergo to proteotoxic stress that probably is triggering their "losers" status, resulting in cell competition. Cells with reduced NOC1 also activate the proapoptotic gene eiger and DILP8. We are currently performing genetic epistasis experiments to better understand how these signals contribute to NOC1-induced apoptosis. Our preliminary data suggest that eiger is perhaps not responsible for DILP8 upregulation upon NOC1 knockdown. This would indicate that alternative pathways, acting in parallel to eiger regulate cell death in the wing disc. Since Xrp1 is a major driver of proteotoxic stress and apoptosis and is also involved in regulation of growth and development, we are currently analyzing also its epistatic relationship with NOC1 and eiger on the expression to DILP8.

5. We are currently preforming immunoprecipitation/MS analysis to identify NOC1 binding- partners using a tagged version of NOC1 since there are not antibodies are available against the Drosophila protein. We had some troubleshooting to solve since NOC1 is very sensitive to proteolysis and often is present with in short forms (Figure 2 J) also in the presence of protein and phosphates' inhibitors. But now the assay is working better, and we should have some data soon.

2. Point-by-point description of the revisions

Rebuttal Point by Point
Reviewer #1 (Evidence, reproducibility and clarity (Required)):

Summary:
The manuscript by Destefanis et al. titled Nucleolar NOC1 controls protein synthesis and cell competition in Drosophila examines the over-expression of NOC1 and the RNAi depletions of NOC1, 2, and 3 in various Drosophila larval tissues, and the effects these changes have on larval development. The NOC proteins are conserved nucleolar proteins required for ribosome biogenesis. This manuscript has merit in that it offers the first examination of the NOC proteins in Drosophila beyond simplistic predictions garnered from high-throughput studies. With RNAi depletion of NOC1, the authors show losses in polyribosome abundance and protein synthesis. The manuscript then focuses heavily on the adverse phenotypes caused by disrupting ribosome biogenesis in the prothoracic gland which produces ecdysone (the molting hormone), the fat body affecting release of Drosophila Insulin-like Proteins (DILPs), and in imaginal wing disc cells affecting their survival by inducing apoptosis via and JNK and eiger, a pro-apoptotic factor.

Major Comments:
Describing developmental phenotypes associated with the depletion of the NOC proteins is necessary at some point, but the phenotypes are generally expected for ribosome loss in the tissues examined. I was hoping the paper would describe a mechanistic link between the loss of NOC protein function and the loss of ribosome production that ultimately leads to the predicted developmental defects.

Minor Comments:
1) The paragraph in the Introduction describing DILPs seems too long and out of place, disrupting the description of the NOC proteins. This paragraph could be reduced to one sentence that is inserted near the end of the Introduction when DILP8 is mentioned.
This part has been reworded and shortened.

2) At the end of the Introduction, the authors say that NOC1 is a novel nucleolar component. Delete the word 'novel'; the first paragraph described conserved NOC proteins in yeast, human, and Arabidopsis.
We substantially changed the introduction avoiding the word “novel” when referring to NOC1.

3) First paragraph of Results: Do the NOC proteins contribute to MYC function or are they induced by MYC? Are there any MYC binding sites in the NOC gene promoters or 5' UTRs?
Our preliminary transcriptomic data suggest that NOC1 and NOC2 mRNAs follow the expression

pattern of MYC; in addition we found the "bona-fide" E-box sequence (CACGTG) in the promoter of both NOC1 and NOC2 already see also Suppl. from ((Hulf et al., 2005). We are currently studying the relationship between MYC and NOC1, which apparently is more complex that what we expected. It seems that these two genes may be part of a feed-back regulatory loop, but these studies are part of another ongoing project.

4) Overall, Figure 1 is too congested. Fig. 1B and C could easily go to Suppl. Data.
The whole Figure 1 has been split into Figure 1 and Figure 2; the panels showing the STRING data in the ex-Figure 1B-C have now been moved to Supplementary Figure 2.
5) For the various RNAi lines used, are there any off-target transcripts that could be affected?
There are no off-target transcripts in the RNAi lines used in this work.

6) In their polysome profiling, the authors first say there's a decrease in ribosomal subunits correlating with a decrease in polysomes, but then "an accumulation of the 40S and 60S subunits (not shown)." I assume the authors mean that there's an overall loss in subunit biogenesis due to NOC1 depletion which leads to polysome loss. If so, how would these combined losses contribute to a gain of free 40S and 60S subunits? This is confusing and should be better explained.
This part has been revised to clarify the effect of the reduction of NOC1 on ribosomal subunits. The data on the area of the 40S and 60S subunits have now been included in Figure 2D with relative quantification (Fig. 2E). These data indicate that NOC1 depletion induces a small increase of the area of the single 40S and 60S subunits and a reduction of the 80S peak, compared to that in control animals. We added the scale for clarity. Our hypothesis is that the small increase of the 40S and 60S subunits in NOC1-RNAi larvae could be the result of 80S improper disassembly, since in these animals the whole ribosomes assembling process is impaired (Figure 2C). In addition, since protein synthesis is also reduced in these animals (Figure 2G-H), it is possible that also some other factors necessary for the whole ribosomal assembling are reduced, allowing the two subunits to fall apart. This part is now better explained in the results and briefly discussed in the discussion.

7) When describing Fig. 1J-K in the text, the authors use the word 'rate' of labeled proteins. To define rate, they should briefly mention the 40- or 60-minute pulse times.
These data are now Figure 2G-H, and we have corrected the text.

8) Fig. 1L is problematic. First, en-GAL4 is driving expression of both GFP and HA-NOC1. The NOC1 labeling on the left-hand side of the figure should be labeled HA-NOC1 just to be clear. Also, the GFP labeling looks as if it's cytoplasmic, I'm guessing mitochondrial (en>mGFP as perhaps correctly labeled in the figure), but the text and legend describes the GFP labeling as nuclear.
Sorry if this image was not clear. The GFP used in the old figure was a membrane-GFP used to mark the cells. However, we have repeated those experiments to better define NOC1 localization and we have substituted the old picture with new now in Figure 1. Briefly, with the new experiments we better analyzed HA-NOC1 localization in the nucleolus and show that it colocalizes with fibrillarin (Figure 1I, K-N). These images were acquired in cells of the imaginal discs (K and L) and in those from the peripodium (M and N), where the co-localization of HA- NOC1 with fibrillarin is more evident due to their large nuclear size. In addition, since HA-NOC1 overexpression induces the formation of rounded structure surrounding the nucleolus (Figure 1K and M), we analyzed if co-expression with NOC1-RNAi would change this pattern. These data indeed show that reducing NOC1 restrains its expression only in the nucleolus marked with fibrillarin (Figure 1L and N). Indeed the pattern of expression of HA-NOC1 now resembles that showed using the line B51967 [from Kudron's collection (Kudron et al., 2018)], where the expression of NOC1-GFP in the nucleolus is clearly localized in the nucleolus (both in cells of the imaginal discs (Fig. 1I) and in the salivary gland (Supplementary Figure S4). We belie ve that these experiments may recapitulate the expression of NOC1 in more physiological conditions (see also next point), and we hope to have fulfilled the request of the reviewer.

Second, while both Fibrillarin and NOC1 locate to nucleoli, it looks as if the two proteins do NOT co-localize within nucleolar sub-regions. This is significant, because if the NOC proteins are important for 60S large subunit maturation and release from the nucleoli, you might expect them to reside in the more peripheral Granular Components of nucleoli. Fibrillarin on the other hand, is an early assembly factor that clearly resides within the more centralized Dense Fibrillar Component of nucleoli. Fig.1L actually suggests this bipartite localization. Perhaps the authors can provide higher mag images to show this difference in localization.

Third, the large aggregates within nuclei of cells over-expressing HA-NOC1 are worrisome because other endogenous nuclear or nucleolar proteins could relocate to these aggregates causing adverse phenotypes. Is there a less potent GAL4 drive you could use to reduce over- expression? Are there any GFP-protein trap lines available for NOC1?
We thank the reviewer for this constructive observation. We have repeated these experiments in two different ways to try to better represent NOC1 localization at more physiological levels, now are in Figure 1I and K-N and Supplementary Figure S4. From these images we can conclude that NOC1 localizes in the more peripheral Granular Components of the nucleoli when is overexpressed, while fibrillarin is located at the center of the nucleoli (better visible in Figure 1N).
9) Middle of page 7: Reducing NOC protein expression later in development in differentiated cells does not have any apparent effect on development. Add the word 'apparent'. There could be intra-cellular effects that have not been detected.
We rephrased this part.

10) Fig. 3B and C should be labeled P0206>NOC1-RNAi. Scale bars are needed for Fig. 3A, B and C.
Now Fig. 4, we added the label and the scale bars.

11) Middle of Page 8: At the beginning of NOC1 depletion in Fat Body, remind the reader that the previous section depleted NOC1 from the prothoracic gland which caused fat accumulation in normal fat body cells that grew large, but now with depletion of NOC1 in the Fat body cells themselves, the cells are smaller.
This part has been revised to better explain the two different situations.

12) Fig. 4A-E scale bars are needed. Fig. 4I-P scale bars and sample sizes (n values) are needed.
Now Fig. 5, the scale bar has been added to all the figures and the number of animals has been specified in the legend.

13) Fig. 5: scale bars are needed, especially for panels A-E. When explaining the M(3)66D experiment, perhaps describe it from the point of view that the NOC1-RNAi clones now have less competition from surrounding cells and this permits their detection.
Now Fig. 6, the scale bars have been added to the panels and the text rephrased.

14) On the top portion of page 11, what is TRE-GstD1, and what is the mechanistic link between JNK and TREGstD1?
Sorry, this was our mistake. The line used in the experiments is a TRE16-ARE-RPF that was incorrectly called TRE-GStD1-RFP. This line was developed to analyze JNK transcriptional activation in the lab of H. Jasper and it is described in this paper (Chatterjee and Bohmann, 2012). This construct has been used to detect activation of JNK signaling in many conditions from RAS^{V12} tumors (Mundorf and Uhlirova, 2016), to mark loser cells in oxidative stress-induced cell competition (Kucinski et al., 2017). The name has now been corrected in the figure and in the text. In addition, in the previous version of this paper the figure representing the activation of TRE- ARE in NOC1-RNAi was done using the MS1096-Gal4 promoter. To be consistent with the other experiments and also to answer to a concern of the reviewer, this figure has now been substituted with a new figure in which NOC1-RNAi expression is driven using rotund-Gal4 (Figure 7 P-Q).

15) Any off target sites for the sgRNA used in the CRISPR mutation analysis?
The guide RNAs used for the mutagenesis (TCCACCGAATTGCACACCTG and ATCACTGTCTCCATCGCTGT) are listed in the Supplementary Figure S7 (Port et al., 2020). According to the protocol reported in the paper, the sgRNAs were identified using the CRISPR library designer (CLD) software version 1.1.2. The CLD excludes sgRNA sequences that have predicted off-target sites elsewhere in the genome. We also checked the sequences using the CHOPCHOP web tool and we found no off targets for both sgRNAs.

16) In the first paragraph of the Discussion, Drosophila pre-rRNA is initially 38S.
We rephrased this part.

17) Middle of page 13: "...defects in translation and in protein synthesis." Somewhat redundant.
We agree, most of this part has been re-written.

18) Middle of page 15: "...in the wing discs with clones that had reduced NOC1..."

19) Same paragraph as in item 17: How is this novel?
We removed novel.

20) In Materials and Methods, page 18: UAS-CG1234-RNAi stock is Bloomington 61872 (no 0 at the end).
Thanks, we corrected the mistake.

21) Bottom of page 18: "The list of primers. ....." This sentence needs re-writing.
We rephrased it.
Reviewer #1 (Significance (Required)):

Nature and significance of the advance
This is a preliminary study on the loss of the Drosophila NOC proteins that are necessary for ribosome production. Much of the paper describes developmental defects associated with the loss of ribosome production.
Compare to existing published knowledge

The phenotypes are interesting, but expected based on previous research on bobbed mutations and the many Minute mutations.

We agree with this comment, however our results led us to assume that NOC1 function is important for rRNA processing and that its reduction acts at broader level than mutations in a single Minute gene.

Audience
Developmental biologists interested in ribosomal stress.

Reviewer #2 (Evidence, reproducibility and clarity (Required)):

Summary:
This manuscript by Destefanis, Manara, Santarelli et al., describes the role of Noc1, a nucleolar protein involved in ribosomal subunit assembly and protein synthesis, in organismal and cellular growth, which also impacts on cell competition in Drosophila.
Using gain and loss of function experiments, the authors characterize the role of Noc1 in various tissues and discover that this protein is necessary to induce cell growth, metabolic homeostasis and developmental progression in Drosophila. Noc1 downregulation leads to cell death and impact the production of secreted factors Eiger/TNF-alpha and DILP8 as well as Ecdysone signaling, resulting in impaired cell/tissue growth and larval developmental delay.
Impairing Noc1 expression in metabolic tissues, such as the fat body, causes loss of lipid homeostasis marked by loss of systemic lipid stores and accumulation of lipids in imaginal discs and gut tissue.

Major comments:
1- In many cases, such as in the context of cell and organismal growth, gain and loss of function of Noc 1 leads to overall similar phenotypes (e.g. impaired growth). While clearly loss of gene function gives has a stronger effect, the authors provide no possible explanation for this.
Our idea is that the amount of NOC1 is very critical for the cells and its levels must be tightly controlled. Indeed, in the whole animal NOC1 overexpression (OE) affects larval survival but not their development. On the contrary, its reduction strongly impact growth, most likely because of its effect on ribosome maturation and protein synthesis resulting in apoptosis. The hypothesis is that the lethality observed in NOC1-OE animals may be the result of an abnormal increase in ribosomal activity that may be toxic during the transition from larvae to adult, a condition that we were able to rescue by reducing NOC1 levels with its RNAi. When instead NOC1 is reduced, the detrimental effect on animal growth is probably due to its strong effect on reducing ribosomal activity and to the activation of apoptosis. Both these aspects are now discussed in the discussion.

2- Figure 1J: I do not see the stronger reduction of Puromycin signal after 60 min of treatment quantified in Figure 1K. Also, if general protein synthesis is affected by Noc1 knockdown, shouldn't total levels of actin be also reduced.
Now Fig. 2G. The quantifications of the puromycin signals at 40 and 60 min were inverted by

mistake in the graph, this now has been corrected in the new image. In addition, a possible explanation for why the levels of actin did not change in these experiments may reside in its half-life that is of about 2-3 hrs, thus it cannot significantly change in the time-frame of our experiments. We added the Ponceau-S staining to Fig. 1 to show the total protein staining, demonstrating that only neosynthesized proteins are affected.

3- Figure 2: The eye phenotypes should be quantified. Also, what is the cause of this phenotype? Is it apoptosis, lack of proliferation or both? Data showed later in the paper would suggest the former. Direct assessment of cell death/proliferation by immunostaining would be useful to clarify the origin of the smaller eye phenotype in ey>Noc1 RNAi flies. Also, using genetic approaches (e.g. blocking apoptosis in the context of Noc-RNAi) would make this data more complete and compelling.
Now Fig. 3. This is an important observation; indeed, we were able to rescue the eye defects of the tub>ey-flp NOC1-RNAi animals by co-expressing the inhibitors of apoptosis P35 (Figure 3 K- M). This suggests that the defect observed upon NOC1 downregulation is due to apoptosis that is known to be relevant in the regulation of the final size and number of the ommatidia.

The authors show latter in the paper that knockdown of Noc1 in wing discs leads to apoptosis and Eiger/TNF-alpha upregulation. Is this the case in the eye as well? Genetic interactions between Noc1 and Eiger should be perform in the eye and wing to determine the functional significance of Eiger overexpression in cell death induced upon Noc1 knockdown.
These experiments are currently ongoing in the wing imaginal disc. Our preliminary experiments indicate that eiger is not necessary for NOC1-induction of DILP8 in the wing disc, and we are now completing this analysis by introducing also Xrp1 in the mechanism of interaction. As the reviewer pointed out, it would be very interesting to compare the role of eiger in apoptosis upon NOC1 downregulation in the two different epithelia and organs (eye and wing imaginal discs), but at the moment we are more focused in better defining the mechanisms that link NOC1-RNAi with cell competition in the wing imaginal discs.

4- Figure 3: Data is missing, misrepresented when comparing the Figure with the results described in the text.
a- As per the results description (page 7-8): 'Downregulation of Noc in the prothoracic gland (PG) leads to developmental delay with animals stalled in the larval stage for 20 days followed by death'. This data is neither shown in the paper, nor mentioned as 'data not shown'. This significant result should be shown.
Now Fig. 4. Sorry for this inaccuracy, we added to the figure a photo of the stalled PG>NOCs- RNAi larvae (Fig. 4F), and added in the text the reference to our paper where we describe a low grade of infiltration of immune cells (hemocytes) in the fat bodies of NOC1-RNAi larvae, which mimics a human condition of chronic inflammation (ATM -adipose-tissue macrophage infiltration). These fat bodies show a high production of ROS and the activation of JNK signaling, that were attenuated by feeding the larvae with anti-oxidants/flavonoids (Valenza et al., 2018).

b- There is an error in Figure 3D. Dark blue bar is indicated as Noc1 overexpression.
Now Fig. 4. Sorry, we corrected this mistake.

c- Figure 3E: the use of 'cell size' is confusing here as it is fat body size what is being represented.
Now Fig. 4. We rephrased the legend, as the graph represents the size of fat body cells.

d- Figure 3F is presented before 3E in the result section but not in the figure. This should be changed to keep figure panels in the order they are presented in the results.
Now Fig. 4. We changed the text.

e- Figure 3D, E: The data corresponding to Noc1 overexpression referred to in the results section as present in these panels is not shown in the figure.
Now Fig. 4. We added the sentence "not shown" in the text for NOC1-OE.

5- Figure 4:
a- Figure 4A-F: The loss of function data presented here is clear. However, the data on the gain of function is less clear. Using a cytoplasmic GFP to label the clones would be more suitable.
Now Fig. 5. We used Texas-Red phalloidin to mark the membrane since the actin flip-out line we

used is recombined with nuclear GFP on the III (from Bruce Edgar). In the new figure we increased the red channel in these imagines (Fig. 5A-E) to better visualize the cell border.

b- Figure 4H: Data in this figure appears to show wing disc size measures rather than animal survival or developmental delay as described in the results section.
Now Fig. 5. We rephrased the text.

c- Figure 4I-P: This should be quantified.
Now Fig. 5. We have quantified the amount of TGAs (triglycerides) from whole larvae that were harvested at 120 hrs AEL. TGA was calculated using the protocol in (Parisi et al., 2013). These data are now in Fig. 5I.

d- Figure 4G: Overexpression in the fat body leads to bigger larvae. However, overexpression of Noc1 in the whole body leads to small larvae (Figure 1D). Why? What happens with lipid stores and DILP2 upon Noc overexpression in the fat body?
Now Fig. 5. We thank the reviewer for this observation. A possible explanation for this difference is that overexpressing NOC1 in the whole animal increases protein synthesis in some vital organs or tissues where this increase becomes toxic. Overexpressing NOC1 only in the fat body will probably still increase protein synthesis; since the fat body is the metabolic organ responsible for nutrients storage and for the control of DILP2 release, it is possible that an increase in its protein activity may favor the release of DILPs from the IPCs, thus increase animal size. A similar effect was described by our group for MYC overexpression, which was also pupal lethal when expressed ubiquitously using the actin>Gal4 promoter (not shown) but resulted in increased animal size when expressed in the fat body (Parisi et al., 2013).

6- Figure 5: Is the role of Noc1 specific to cell competition? It seems to me that this is a general regulator of cell growth/survival. How can you distinguish one from the other? Or is it the case that any protein required for overall cell growth/survival would impact cell competition? Does Noc1 impact Myc expression?
Now Fig. 5. Here the reviewer is raising an important issue. Our data show that NOC1 controls protein synthesis and its reduction in clones results in these cells to be outcompeted by the WT neighboring cells. This effect is partially rescued using a M/+ background, where the reduction in protein synthesis also in neighboring cells permits the survival of NOC1-RNAi cells. In addition, we detected the upregulation of Xrp1, a transcription factor that has been described to control proteotoxic stress-induced cell competition. All these observations are good indications that NOC1 is involved specifically in the mechanisms that control cell competition perhaps upon proteotoxic stress.
Overexpression of NOC1 does not apparently control MYC expression; on the contrary, MYC induces NOC1 mRNA, suggesting that NOC1 may be a direct target of MYC. It is difficult to say with this data if NOC1 is involved in MYC-induced cell competition. In our opinion, the relation between MYC and NOC1 is complicated, and we are currently analyzing the relevance of MYC in NOC1 expression since these genes may potentially be involved in an autoregulatory loop to control each other expression.

7- Figure 6: Results presented in this figure are clear and completing. However, genetic interactions between
Noc1/Eiger and Noc1/Dilp8 would make this data more complete by directly assessing the functional relevance of these secreted factors to the Noc1 phenotype.
We are currently analyzing these genetic interactions.

8- Figure 7: Not sure how much this data contributes to the study. It could be moved into supplementary data as further evidence of the loss of function phenotype of Noc1 using an independent genetic tool.
We believe that these data could be relevant for NOC1 characterization, as they show that NOC1 mutants recapitulate the phenotypes of two NOC1-RNAi lines. In addition, we have characterized the nature of the mutation in two NOC1 mutant lines, and we would like to leave them in the main text.

Minor comments:
The manuscript should be carefully edited to correct for typos/missing words.

Reviewer #2 (Significance (Required)):
This work identifies a new regulator of cell/tissue growth and mediator of cell competition in Drosophila. A significant body of work and varied techniques (e.g. cell biology, biochemistry, genetics) have been used here to characterize the role and cellular localization of Noc proteins in Drosophila. However, as detailed above, additional experiments are necessary to clarify the role of the protein and also support some of the conclusion of this study. I also noticed significant errors in data presentation that need to be corrected before this paper is accepted for publication.

We have included further experiments to better clarify the role of NOC1 in controlling ribosomes assembling and most parts of the paper has been revised.

Reviewer #3 (Evidence, reproducibility and clarity (Required)):
Summary:
NOCs proteins are important players for protein synthesis and ribosome biogenesis in yeasts and plants. Despite their high conservation among eukaryotes, very little is known about their function in animals. Destefanis and colleagues address this question using Drosophila melanogaster as a model. Loss-of-function experiments show that NOC1, NOC2 and NOC3 are necessary for animal's growth, proper development and survival, achieving nonredundant functions. In addition, NOC1 is overall necessary for proper ribosome biogenesis and protein synthesis and localizes at the nucleus and nucleolus. These observations provide good evidence that NOC1 plays a similar role in Drosophila and in yeast at the cellular level. On this basis, the authors started exploring the role of NOCs in different tissues during development. Again, their results support the idea of NOCs being involved in basic cellular functions required for cellular growth in different cell types, either proliferative or endoreplicative. To better understand the signaling events affecting growth and development downstream of NOC1, the authors analyzed more in detail the effects of NOC1 gain and loss-of-function in the wing imaginal disc. They propose a link with cell competition and with the hormone DILP8, involved in the control of systemic growth and developmental timing. Yet, this part of the study is weaker, lacking any epistasy that would be required to establish causal relationships between the different players.

Major comments:
- Fig 4: Analysis of the fat content using Nile-Red is not very convincing. It is clear that cg>NOC1i animals have less fat body, but not necessarily that FB cells display less Nile-red staining. In addition, scale bars are missing and different pictures where clearly taken using different magnifications (i.e the brain or sg in the two conditions). There is no control for the WID. Concerning the brains, again it is difficult to conclude because this method is not quantitative and brains seem to be stained both in controls and NOC1i.
Now Fig. 5. We quantified the amount of fats and added the analysis of TGAs from whole larvae at about 120 hrs AEL. These data show a significant reduction in TGAs/ug proteins in NOC1- RNAi animals. We also substituted some of the pictures of Nile Red staining, that are now shown at the same magnification. A photo of the wing imaginal discs (mostly eyes and legs) and of the brain is present as Fig. 5P to show the upregulation of lipids in NOC1-RNAi animals.

- The effect of NOC1i in the fat body on DILPs levels lack more detailed analysis. By experience, within a given experimental condition the staining for DILP2 in the IPCs is quite variable. A careful quantification of the fluorescence therefore, needs to be done. In addition, measuring circulating DILP2 levels (ELISA with double-tagged DILP2 construct) would be necessary to conclude that it is indeed retained in the IPCs.
Analysis of the phenotypes of animals with reduced NOC1 in the fat body, phenocopy those described in our previous work in which we reduced MYC (Parisi et al., 2013). This led us to analyze if reducing NOC1 in the fat-body could affect the signals necessary to stimulate the release of DILP2 from the IPCs mimicking animals in starvation. The increase in DILP2 immunostaining in the IPCs confirms our hypothesis. We don't have experience with the analysis of DILP2 in the hemolymph using ELISA, but words from colleagues working in the field discouraged this assay because it is laborious and sometimes not much sensitive due to the low amount of DILP2 circulating in the hemolymph. We have also asked colleagues working with Mass Spectrometry with experience in analyzing hemolymph samples, but again they raised their concern about the low amount of DILP2 that could be detected considering the higher contents of proteins in the hemolymph.

- Fig 5A-E: when where the clones induced? Scale bars are missing, is it the same magnification? Overall the pictures are quite bad and clones are not visible especially in C-E. Pictures should be taken as for the following experiments (F to M), where clones are perfectly visible.
Now Fig. 6. We added the scale bars in all the confocal images and added in the legend the time of clonal induction. In panel (A-E) we show confocal images of single clones taken at high magnification to better visualize the abnormal morphology of NOCs-RNAi clones, which would have been more difficult to appreciate at the magnification used in the panel F and G.

- Fig 5: for the clonal analysis, it seems to me that it is not because clones of cells die, that they are necessarily subjected to cell competition. They can simply be too much affected to survive. Hence, the statement that "cells died and were outcompeted by the neighboring cells (Fig 5F-G)" does not seem correct in the first place.
Only the experiment in the M+/- background suggests that NOC1i cells are in part actively eliminated by cell competition. If the authors want to focus the ms on cell competition, an interesting point would be to test the role of Xrp1 downstream of NOC1. Indeed, Xrp1 has been shown to trigger most if not all the effects of Minute mutations (cell competition, decreased translation, DILP8 upregulation...). Is NOC1 LOF triggering the same stress response as Rp knock-down?
Now Fig. 7. Thank you for raising this important point. Indeed, we found Xrp1 mRNA upregulated in the imaginal discs where NOC1-RNAi was expressed using rotund-Gal4 (Fig. 7K). This observation suggests that Xrp1 is acting downstream of NOC1 depletion to trigger proteotoxic stress, which may be responsible for inducing cell competition. Further experiments are currently underway to better understand the relationship between NOC1 and Xrp1.

- An important experimental issue is the tools used to study NOCs in the growth of wing imaginal discs (Figs 6 and 7). Both MS1096-GAL4 and engrailed-GAL4 are very unspecific (brain expression, fat body and gut expression for en, etc...). It is therefore difficult to conclude that the effects on wing and animal development are due to expression specifically in the wing and not any other tissue involved in systemic growth control. In addition, MS1096-GAL4 is indeed, rather dorsal in late development, but picts in Fig6 A-C show expression in the whole pouch (tissue size must be quantified to show that it consistently affects growth). In adult wings, dorsal growth inhibition usually triggers curly wings. How can these be properly measured? The use of more specific GAL4 lines (R11F02, nub, rn) is necessary to conclude on the cell-autonomous effects of NOC1 misexpression and to carry out epistasy experiments.
We agree with the reviewer that the MS1096 promoter is not appropriate to study the mechanisms that control growth. We initially used MS1096 because the expression of NOC1-RNAi using rotund or nubbin promoters resulted in adult lethality. Only when using MS1096 we were able to obtain adults and observe the defects in their wings (Fig. 7E). In addition, while doing these experiments, we noticed a delay in larval development and found a significant increase of DILP8 mRNA in MS1096>NOC1-RNAi larvae. To limit our analysis to a better-defined compartment we then expressed NOC-RNAi using rotund-Gal4 (Fig. 7 L-S). These experiments showed that both DILP8-GFP and eiger-GFP were increased upon NOC1-RNAi (Figure 7M-O). In addition, we found TRE-dsRFP the reporter for JNK activation upregulated (Figure 7P-Q) together with an increase staining for activated caspase 3 in cells with NOC1-RNAi (Figure 7R-S). Furthermore, we found a robust increase in DILP8 mRNA in the imaginal wing discs from rn>NOC1-RNAi animals (Figure 7K), that is responsible for the strong reduction of ecdysone sufficient to block their development: thus, explaining why rn>NOC1-RNAi adult animals never eclosed.

- What is the TRE-GstD1-RFP construct? It is not described in the methods. Traditionally, the GstD-GFP reporter is used to monitor oxidative stress. Even though oxidative stress and JNK signaling are linked, the use of a more direct reporter of JNK signaling would be more appropriate.
Sorry, this was our mistake in labeling the figure. The actual line used in these experiments is a TRE16-ARE-RPF that was incorrectly called TRE-GStD1-RFP. This line was developed in the lab of H. Jasper to analyze JNK transcriptional activation (Chatterjee and Bohmann, 2012). This construct has also been used to mark loser cells undergoing oxidative stress in cell competition (Kucinski et al., 2017).

- The model presented in Fig6 lacks experimental evidence and is based mainly on correlations. Epistasy experiments are necessary here. DILP8 upregulation is clear, but does it indeed trigger the delay at pupariation? Is it indeed, induced by Eiger/JNK/oxidative stress? An important alternative

would be Xrp1, which triggers DILP8 upregulation in Minute conditions. Does Eiger/Grnd/JNK downregulation rescue NOC1i phenotypes (growth, delay) and DILP8 upregulation? These experiments would bring functional insights into the role of NOC1 in growth control.
Now Fig. 7. We agree with this comment. We are currently performing genetic epistasis experiments between NOC1 and DILP8 and studying the contribution of Xrp1 to these pathways (these experiments are still ongoing). Our preliminary data show that eiger is not probably necessary for the upregulation of DILP8 upon NOC1 reduction, perhaps suggesting that the apoptosis induced upon NOC1-RNAi follows a pathway involving Xrp1 and proteotoxic stress. However, we need more statistics and to repeat these experiments with a second eiger-RNAi line to confirm that.

Minor comments:
- Both in the introduction and in the discussion, the authors mention their "transcriptome analysis" suggesting that NOCs might be related to MYC. Are these data published? Should they be mentioned without including them? It would be easier to either remove the reference to this analysis or better describe it so that the reader understands the link with MYC and how it contributes to the background of this story.
Thank you for this observation. The relation between MYC and NOCs was mentioned as the "rational" to characterize NOCs function in ribosome biogenesis. By microarray analysis we found that the expression of the NOCs genes followed a similar trend as MYC expression; indeed, since some of the NOCs genes contain a “bona-fide” MYC binding E-box sequence in their promoter, we thought to follow up on their function as they could be potential MYC targets. These results are published in (Hulf et al., 2005). We are currently analyzing the relation between MYC and NOC1, however these data are part of another ongoing project.

- Fig 2: The differences between GMR and ey are striking, and the authors conclude on a role for NOCs more specifically in proliferative cells. This conclusion sounds in contradiction with the results in endoreplicative tissues where NOCs-RNAi have an important impact. Why NOCs LOF would not play a role in differentiated cells such as the retina if they are required for such important cellular functions as protein synthesis?
Thanks, this is also an important point. We believe that NOC-LOF may impact more cells that are undergoing proliferation and to less extent those that are endoreplicating (gut, fat, salivary glands, PG). However, we found that reduction of NOC1 the fat-body and PG decrease their size and compromise their activity. We believe that the lack of effect using GMR, since it is expressed much later in development, could be due to the fact that these cells are differentiated and much less dependent of NOC1 activity.

- In the text, reference to Fig4I-L is switched with Fig4M-P
Now Fig. 5. We changed it.

- Fig 5: the graph in J should be in F for easier fig reading. In N-O, it is written "Minute clones". These are not Minute clones (Rp+/- clones in a Rp+/+ background).
Now Fig. 6. Correct! We changed the title in the figure legend. *Minute M(3)66D* mutation corresponds to a mutation in the *Rpl14* gene (Saeboe-Larssen et al., 1997), we changed the name in the new figure and added a caption in the legend.

- Fig6 L and N: genotypes are confusing, rn-GAL4 should be written apart from the reporters. Fig 6N: is eiger-GFP also induced non-autonomously in the notum?
Now Fig. 7. We corrected the genotype in the panels. According to the experience of other colleagues and to other publications, the Eiger-GFP line (fTRG library; VDRC 318615), used in our experiments, may have non-specific staining in the notum (Muzzopappa et al., 2017).
Furthermore, in this revised version of the manuscript the images are at a higher magnification to be consistent with the new experiments and the notum is not present.

Reviewer #3 (Significance (Required)):
The authors describe new genes, NOC1, 2 and 3 involved in growth control related to ribosomal function. Importantly, these genes are well conserved among eukaryotes. A number of recent studies in Drosophila have focused on the signaling mechanisms involved in the response to ribosome dysfunction focusing mostly on Minute conditions. They have identified a number of new players such as proteotoxic stress or the RpS12/Irbp18/Xrp1 pathway, which are important for cell

competition and the non-autonomous effects caused by ribosome dysfunctions. The study by Destefanis and colleagues identify new players involved in ribosome biogenesis that could potentially reinforce the emerging idea that ribosomes are signaling hubs for the control of cell/body growth (instead of more "passive" players controlling the rate of protein translation).
My field of expertise is growth control, Drosophila development and genetics. I am less expert on ribosome biology (i.e. assembly, literature in yeast...).

Reviewer #4 (Evidence, reproducibility and clarity (Required)):
Eukaryotic ribosome biogenesis requires about 200 ribosome biogenesis factors in addition to the structural ribosomal components. Based on the known functional and structural homologies of both the structural components and the biogenesis factors, the principles in ribosome formation seem to be rather conserved in all eukaryotes. While most of our knowledge about ribosome synthesis comes from studies in the yeast S. cerevisiae not so much is known about this process and contributing factors in multicellular organisms like Drosophila. In this study the authors up- or downregulate the expression levels of the Noc proteins NOC1, NOC2 and NOC3 to analyze their function in Drosophila. The Noc1-3 homologues were previously structurally and functionally analyzed in yeast. The authors postulate that Drosophila NOC1 is required for the synthesis of both ribosomal subunits and for proper translation.
They found that expression of NOC1 is required for normal animal growth and organ function. Downregulation of NOC1 affected proliferating tissues with no significant effect on differentiated cells. In particular, the authors reduced NOC1 expression specifically in the eye, prothoracic gland, fat body and wing imaginal discs. In all tissues they found strong functional or developmental defects. Moreover, NOC1 reduction led to an induction of regulated cell death. The manuscript confirms previous analyses that depletion of a factor which is crucial for the formation of functional ribosomes impairs many cellular processes which depend on unperturbed protein synthesis.

Major problems
1) Based on their homology to the yeast Noc proteins it can be anticipated that Drosophila NOC-proteins are required for ribosome biogenesis. Basically, all observed consequences of NOC-downregulation can be explained by a strong reduction of functional ribosomes because of impaired ribosome maturation. Therefore, it is important to clearly show the impact of NOC- proteins on ribosome biogenesis. Unfortunately, the authors don´t provide state-of -the-art experiments, like co-sedimentation with pre-ribosomes, pre-rRNA processing analyses and to RNA-IP experiments of preribosomes which would demonstrate a direct role of Drosophila NOC proteins in ribosome biogenesis. Instead, the authors claim that NOC1 downregulation results in defective polysome formation. It is difficult to interpret the polysome gradients depicted in Fig. 1 G-I, since after NOC1 downregulation all peaks were significantly reduced, which could be also due to a general reduction of gene expression. In yeast, impairment of Noc1-3 proteins resulted in defective pre-60S synthesis. The authors claim that Drosophila NOC1 affects the synthesis of both subunits. Therefore, it is important to figure out whether the Drosophila protein has adopted an additional role.
We agree with the reviewer that to better define a function for NOC1 in ribosome biogenesis more experiments should be done, and we are moving to that direction. However, since here we would like to provide the initial description of the effects of NOC expression modulation on the ribosome, some of the experiments (such as co-sedimentation with pre-ribosomes, pre-rRNA processing analyses and RNA-IP) would require more time and manpower. Nevertheless, we performed a few experiments to demonstrate that depletion of NOC1 impairs the maturation of ribosome and polysome formation and induces an accumulation of the immature rRNAs, with consequent reduction of protein synthesis. Because this paper is the first description of the function of NOC1 in Drosophila and we would like to highlight its novel functional relationship between the nucleolus and cell competition, we hope that this initial observation is sufficient. Some of the experiments requested above by the reviewer are ongoing in the lab (immunoprecipitation of HA-NOC1 from larvae using HA antibody, or from NOC1-GFP expressing animals using anti GFP antibody, and Mass Spectrometry analysis to identify components in these complexes), but we believe that they will take some time to be completed.

2) All functional consequences of NOC downregulation can be explained by a defective or reduced population of ribosomes affecting protein synthesis. Several previous studies highlighted that failure

in ribosome biosynthesis leads to strong proliferation defects in Drosophila. In principle, inhibition of any ribosome biogenesis factor which is essential for the formation of functional ribosomes might have similar effects on protein homeostasis. Therefore, it is likely that single factors play no specific role to balance protein homeostasis unless their activity/expression is specifically triggered by signal transduction pathways. Since the authors "suggest a novel role for NOC1 in the control of the biosynthetic activity of the cells" (page 10) they should provide direct evidence that NOC1 is a key target. The observation that cells with reduced NOC1 are subjected to cell competition can be explained by pleiotropic effects and correlates with earlier studies that reduction of protein synthesis triggers apoptosis in cell competition. Another explanation for cellular out-competition is that the accumulation of deficient pre-ribosomes might lead to proteotoxic stress which is a driver of the "loser cell status"
We agree with this final thought, indeed some of our new experiments highlights a possible involvement of NOC1 in the mechanisms that induce proteotoxic-stress (i.e. the upregulation of Xrp1), and we tuned the paper and discussion accordingly.

Minor points
1) The recent publications describing the position of NOC1 in yeast pre-60 S ribosomes should be cited
We added few and recent papers about NOC1 in yeast.

2) The rescue experiment which is described on page 5 is not included in Fig. 1A inset nor in Fig. S2
We rephrased this part in the text and in the Figure 1 to make it clearer.

3) Fig. 1J, please include the Ponceau staining, to verify that only neosynthesized proteins are affected
Done, it is now Fig. 2I.

4) Fig 1 K, how was quantification analyzed?
We reported the explanation of how we made the analysis in the material and methods section.

5) The text in the figure legends and result part and the labelling of Fig 4 is mixed up Thanks, this has now been changed and is in Fig. 5.

6) Page 9 line 7, release

Reviewer #4 (Significance (Required)):
Ribosomes, the cellular factories for protein biosynthesis are possibly among the most intricate and complex ribonucleoprotein-assemblies in the cell. Analysis of ribosome biogenesis and the regulation of ribosome function are central topics of biological research.
If it is clearly shown that NOC1-3 affect ribosome biogenesis as their counterparts in yeast, the manuscript would confirm previous data about the function of Noc-proteins and that eukaryotic ribosome biogenesis is rather conserved in single celled and multicellular organisms. A direct involvement of Noc1 in the biogenesis of both ribosomal subunits is unexpected. So far most of the eukaryotic ribosome biogenesis factors including the yeast Noc proteins are specific for either the small or the large ribosomal subunit. Participation of NOC1 in the synthesis of both subunits would be a novel and very interesting aspect in Drosophila- ribosome synthesis. Therefore, it is important to clearly show that NOC proteins are involved in the maturation of both subunits.
We agree with the reviewer that this is an important issue to define in deeper. Our observation that depletion of NOC1 increases the amount of immature rRNAs and decreases the level of the mature 18 and 28S agrees with what was described for Noc1 in yeast *S. ce*. (see Figure 4 in (Milkereit et al., 2001)). To better understand whether there is an evolutionary difference in the activity of NOC1 between *Drosophila* and yeast would require more experiments; some of them are ongoing at the moment, such as IP of HA-NOC1 and Mass Spectrometry to identify the components that co-purify with NOC1. We are also developing new reagents such testing specific antibodies for NOC1 in flies or transfecting stable cell lines expressing HA-NOC1 with an inducible system.

The regulation of NOC1 activity to specifically control protein translation or induce apoptosis would be a novel and exciting aspect. The interesting effects in different Drosophila tissues after NOC1 depletion are then consequences of a regulated NOC1-impairment. However, such a regulation must be shown. I am not sure whether alone the observation, that impairment of protein synthesis

will lead to the described cellular consequences, provides sufficient novelty in the Drosophila field for publication.

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Original submission

First decision letter

MS ID#: JOCES/2022/260110

MS TITLE: Reduction of nucleolar NOC1 accumulates pre-rRNAs and induces Xrp1 affecting growth and resulting in cell competition in Drosophila

AUTHORS: Francesca Destefanis, Valeria Manara, Stefania Santarelli, Sheri Pavlides, Marco Brambilla, Giacomo Viola, Paola Maragno, Ilaria Signoria, Gabriella Viero, Maria Enrica Pasini, Marianna Penzo, and Paola Bellosta
ARTICLE TYPE: Research Article

We have now reached a decision on the above manuscript.

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

As you will see, the reviewers raise a number of substantial criticisms that prevent me from accepting the paper at this stage. They suggest, however, that a revised version might prove acceptable, if you can address their concerns. If you think that you can deal satisfactorily with the

criticisms on revision, I would be pleased to see a revised manuscript. We would then return it to the reviewers.

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

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

Reviewer 1

Advance summary and potential significance to field

The authors have answered a number of points raised by the reviewers. Especially, they have found that Xrp1, a key player in triggering downstream events in conditions of Rp loss-of-function, is upregulated in NOC1 mutant cells. Although interesting, this finding is too preliminary to provide a mechanistic insight into growth defects occuring in NOC1 mutant cells.

Comments for the author

Major point:
- Reviewer 2 and I suggested the importance of genetic interactions between NOC1 and Eiger/Xrp1. The authors replied in the rebuttal letter that these experiments are still ongoing. From my point of view, this manuscript cannot be properly assessed without these data. The model presented in Fig 7K still lacks experimental support, and is actually in contradiction with preliminary results mentionned in the rebuttal: "our preliminary data show that eiger is not probably necessary for the upregulation of DILP8 upon NOC1 reduction...". This last statement suggests that the effects on apoptosis/growth/developmental delay rather depend on Xrp1 as a downstream mechanism. Experiments should be provided in that direction and included in the manuscript.

Minor point:
- qRT-PCR data presented in Fig 7K are lacking statistics.

Reviewer 2

Advance summary and potential significance to field

This manuscript by Destefanis, Manara, Santarelli et al., describes the role of Noc1, a nucleolar protein involved in ribosomal subunit assembly and protein synthesis, in organismal and cellular growth, which also impacts on cell competition in Drosophila.
Using gain and loss of function experiments, the authors characterize the role of Noc1 in various tissues and discover that this protein is necessary to induce cell growth, metabolic homeostasis and developmental progression in Drosophila. Noc1 downregulation leads to cell death and impact the production of secreted factors Eiger/TNF-alpha and DILP8 as well as Ecdysone signaling and the proteotoxic factor XRP1, resulting in impaired cell/tissue growth and larval developmental delay. Impairing Noc1 expression in metabolic tissues, such as the fat body, causes loss of lipid homeostasis marked by loss of systemic lipid stores and accumulation of lipids in imaginal discs and gut tissue.
This work identifies a new regulator of cell/tissue growth and mediator of cell competition in Drosophila.

Comments for the author

I have revised this paper already when submitted to Review Commons. Many of my comments have been addressed. However, there are some remaining, which I consider important to be addressed before publication.

Figure 3 (previously Figure 2): Quantification of eye phenotypes requested in my previous review are still missing.
Figure 4D and E (previously Figure 3D and E): I pointed an error in the indication of genotypes. Dark blue bar is indicated as Noc1 overexpression. In spite of authors saying they corrected this, the error remains.
The dark blue line continues to be labeled as NOC1-OE, which is inconsistent with the result description which refers to NOC1-RNAi work and gene over expression results not shown.
Figure 4 (previously Figure 3): My original request: 'Figure 3F is presented before 3E in the result section but not in the figure. This should be changed to keep figure panels in the order they are presented in the results.' In spite of authors indicating otherwise, this was not addressed. Perhaps my request was unclear.
Figure 4F of the revised manuscript should be shown first (as fig 4A) and fig a-c should go after (as fig 4 b-d) to match the order in which data is described in the results section. The same happens in other parts of the manuscript. For instance in Figure 5: one needs to jump through panels to see results which are described in an order different from that appearing on the Figure.
Figure 7:Genetic interactions between NOC1 and Dilp8/Eiger as per my original comment are very important evidence to substantiate the proposed model in Figure 7. Similarly, a genetic interaction with XRP1. Showing these molecules are actual effectors of NOC1 would take this work beyond the descriptive phase. Authors seem to be working on this so they should be given the chance to include them in the revised manuscript.
Figure 8: The authors want to keep this data in the main manuscript as per their response to my original comments. I suggest, in this case, the data of the additional NOC1 loss of function mutants should be presented when the loss of function phenotype is presented at the start of the manuscript. Showing this at the end with no further characterization disrupts the flow of the paper.

First revision

Author response to reviewers' comments

We thank the reviewers for the useful and constructive comments about our revised paper. We have taken in consideration all the suggestions of the reviewers and substantially rephrased the test of the manuscript, and the changes are highlighted in blue.

Here below we highlight the main changes in the figures

In Figure 1. We substituted panel C, that represented a qRT-PCR showing the different levels of NOCs-mRNAs in larvae upon their RNAi or with NOC1-OE, with a photo showing the phenotype of *noc1* mutants using CRISPR/Cas9 that resembles the phenotype of *NOCs-RNAi* larvae expressing ubiquitously the relative RNAi using the *actin-Gal4* promoter. This photo was before part of Figure 8 that now has been added in the Supplementary data as Figure 5.

In Figure 3. We added panels N and O that show the analysis on the size of the ommatidia in adult flies, in panel F until M. The same analysis was done for the eyes of animals from figure A-E. These last data are now included in the Supplementary data as Figure 7.

In Figure 4. We changed the order of the panels to show first the phenotype of NOCs reduction in the whole animals and then we specifically focus of the effect of *NOC1-RNAi* on the size of the fat cells and of the Prothoracic gland. We changed the text accordingly.

In Figure 5. We changed panel F that represented the area of the clones with a panel to show the measurement of the area of the cells. In Panel J, L, O and P we added arrows to better identify the various organs, and we added the full name of the organs.

Figure 7 has been substantially changed.

We removed the previous panel I showing the wing size of the animals. These data are now Supplementary as Figure 9.

We removed panel J showing the level of *Dilp8-mRNA* from whole larvae since it is redundant with data from a similar analysis in the wing imaginal discs (Figure 7U); the data are now shown in Supplementary data as Figure 10.
The new Figure 7 now includes panel K-L and P-Q to show the levels of Dilp8-GFP after *NOC1* and *eiger* downregulation from the wing imaginal discs using the *nubbin-Gal4* promoter. Panel M represents the quantification of Dilp8-GFP in these experiments.
We added panels T and U representing the levels of *Dip8-mRNA* and of *Xrp1-mRNA* respectively, from the wing imaginal discs of *NOC1-RNAi* and *eiger-RNAi* and controls animals using the *rotund-Gal4* promoter.

Figure 8 has now been removed from the main text and is now in the Supplementary data as Figure 5.

Specific answers to the Reviewers

1 Advance Summary and Potential Significance to Field:

The authors have answered a number of points raised by the reviewers. Especially, they have found that Xrp1, a key player in triggering downstream events in conditions of Rp loss-of- function, is upregulated in NOC1 mutant cells. Although interesting, this finding is too preliminary to provide a mechanistic insight into growth defects occurring in NOC1 mutant cells.

Reviewer 1 Comments for the Author:

Major point:

- Reviewer 2 and I suggested the importance of genetic interactions between NOC1 and Eiger/Xrp1. The authors replied in the rebuttal letter that these experiments are still ongoing. From my point of view, this manuscript cannot be properly assessed without these data. The model presented in Fig 7K still lacks experimental support and is actually in contradiction with preliminary results mentioned in the rebuttal: "our preliminary data show that eiger is not probably necessary for the upregulation of DILP8 upon NOC1 reduction...".
This last statement suggests that the effects on apoptosis/growth/developmental delay rather depend on Xrp1 as a downstream mechanism. Experiments should be provided in that direction and included in the manuscript.

We provided new evidence on the interaction between Eiger/Xrp1 and Dilp8. These data show that activation of Dilp8, induced upon NOC1 downregulation (Figure 7I-J, K-L O-P and T) is accompanied by an upregulation of Xrp1 (Figure 7T) and this effect is not dependent on eiger expression since the levels of *Xrp1-mRNA* are not changed upon eiger downregulation (Figure 7U). Unfortunately, due to the embryonic lethality of *Xrp1-RNAi; Noc1-RNAi* animals we were not able to test the levels of DILP8-GFP in the discs, therefore we opted to analyze Dilp8 and Xrp1 transcripts in animals where NOC1 and eiger were reduced. Those data show that *Xrp1-mRNA* levels are induced in both conditions (NOC1-RNAi or NOC1-RNAI; eiger- RNAI) and indirectly suggesting that in our model Xrp1 control on Dilp8 expression depends on eiger expression.

Minor point:

- qRT-PCR data presented in Fig 7K are lacking statistics.

Figure 7 is now substantially changed, and we added the statistic to all the data presented

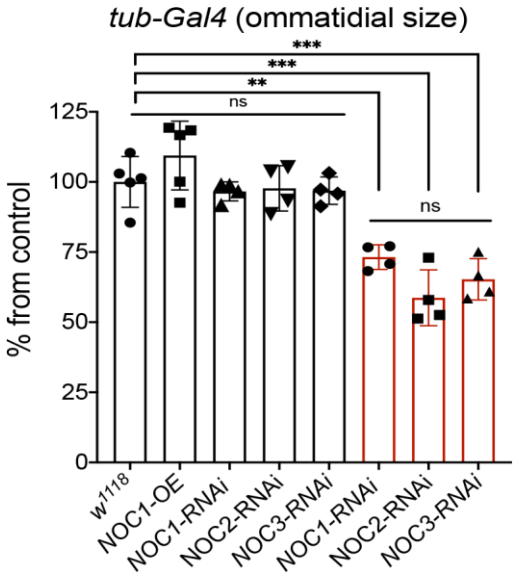
2 Reviewer 2 Advance Summary and Potential Significance to Field:

This manuscript by Destefanis, Manara, Santarelli et al., describes the role of Noc1, a nucleolar protein involved in ribosomal subunit assembly and protein synthesis, in organismal and cellular growth, which also impacts on cell competition in Drosophila. Using gain and loss of function experiments, the authors characterize the role of Noc1 in various tissues and discover that this protein is necessary to induce cell growth, metabolic homeostasis and developmental progression in Drosophila. Noc1 downregulation leads to cell death and impact the production of

secreted factors Eiger/TNF-alpha and DILP8 as well as Ecdysone signaling and the proteotoxic factor XRP1, resulting in impaired cell/tissue growth and larval developmental delay. Impairing Noc1 expression in metabolic tissues, such as the fat body, causes loss of lipid homeostasis marked by loss of systemic lipid stores and accumulation of lipids in imaginal discs and gut tissue. This work identifies a new regulator of cell/tissue growth and mediator of cell competition in Drosophila.

Reviewer 2 Comments for the Author:
I have revised this paper already when submitted to Review Commons. Many of my comments have been addressed. However, there are some remaining, which I consider important to be addressed before publication.

Figure 3 (previously Figure 2): Quantification of eye phenotypes requested in my previous review are still missing.
We were able to quantify the area of the eyes and of the ommatidia. These data show that there is a significant decrease in the size of the eyes in *NOCs-RNAi* animals (Figure 3N) which is reflecting the reduced size of their ommatidia. See graph here below and also Supplementary Figure 7B.



This defect is rescued in *NOC1-RNAi* animals by co-expression of the caspase inhibitor P35 (Figure 3O). No significant difference was found when NOC1 was overexpressed. Also, no difference in the size of the eyes was observed when NOCs expression was modulated using the GMR promoter (see Supplementary Figure 7A).

Figure 4D and E (previously Figure 3D and E): I pointed an error in the indication of genotypes. Dark blue bar is indicated as Noc1 overexpression. In spite of authors saying they corrected this, the error remains. The dark blue line continues to be labeled as NOC1- OE, which is inconsistent with the result description, which refers to NOC1-RNAi work and gene over expression results not shown.

We are sorry for this mistake; the error has now been corrected

Figure 4 (previously Figure 3): My original request: 'Figure 3F is presented before 3E in the result section but not in the figure. This should be changed to keep figure panels in the order they are presented in the results.' In spite of authors indicating otherwise, this was not addressed. Perhaps my request was unclear.
Figure 4F of the revised manuscript should be shown first (as fig 4A) and fig a-c should go after (as fig 4 b-d) to match the order in which data is described in the results section. The same happens in other parts of the manuscript. For instance in Figure 5: one needs to jump through panels to see results which are described in an order different from that appearing on the Figure.

We have changed Figure 4 and to be more consistent with the wording in the manuscript, since we will describe first the larval phenotype of the NOCs-RNAi and then we will be focus on NOC1-RNAi. We tried to keep the panel's organization as is described in the test although sometimes is difficult due to type of images that we have combined (photos versus graphs). We hope that now it is better.

the panel Figure 7: Genetic interactions between NOC1 and Dilp8/Eiger as per my original comment are very important evidence to substantiate the proposed model in Figure 7. Similarly, a genetic interaction with XRP1. Showing these molecules are actual effectors of NOC1 would take this work beyond the descriptive phase. Authors seem to be working on this so they should be given the chance to include them in the revised manuscript.

Since this is a similar concern as reviewer n1 I am copying here our answer:
We provided new evidence on the interaction between Eiger/Xrp1 and Dilp8. These data show that activation of Dilp8, induced upon NOC1 downregulation (Figure 7I-J, K-L O-P and T) is accompanied by an upregulation of Xrp1 (Figure 7T) and this effect is not dependent on eiger expression since the levels of *Xrp1-mRNA* are not changed upon eiger downregulation (Figure 7U). Unfortunately, due to the embryonic lethality of *Xrp1-RNAi; Noc1-RNAi* animals we were not able to test the levels of DILP8-GFP in the discs, therefore we opted to analyze the DILP8 and Xrp1 transcripts in animals where NOC1 and eiger were reduced. Those data show that Xrp1-mRNA levels do not change in condition of both RNAi indirectly suggesting that in our model Xrp1 control on Dilp8 expression depends on eiger expression.

Figure 8: The authors want to keep this data in the main manuscript as per their response to my original comments. I suggest, in this case, the data of the additional NOC1 loss of function mutants should be presented when the loss of function phenotype is presented at the start of the manuscript. Showing this at the end with no further characterization disrupts the flow of the paper.

We agree with this useful comment and moved Figure 8 in the Supplementary data. However, we added a small panel in Figure 1C showing that the mutants of *Noc1* have the same phenotype as *NOC1-RNAi,* we believe that this would support our data and also give the readers the opportunity to know that those mutants are available in our laboratory.

Second decision letter

MS ID#: JOCES/2022/260110

MS TITLE: Reduction of nucleolar NOC1 accumulates pre-rRNAs and induces Xrp1 affecting growth and DILP8 resulting in cell competition in Drosophila

AUTHORS: Francesca Destefanis, Valeria Manara, Stefania Santarelli, Sheri Pavlides, Marco Brambilla, Giacomo Viola, Paola Maragno, Ilaria Signoria, Gabriella Viero, Maria Enrica Pasini, Marianna Penzo, and Paola Bellosta
ARTICLE TYPE: Research Article

We have now reached a decision on the above manuscript.

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

As you will see, the reviewers recognize that most of their initial criticisms have been addressed in your revised manuscript. However, the reviewers still raised issues that will require amendments to your manuscript. I hope that you will be able to carry these out, because I would like to be able to accept your paper.

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

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

Reviewer 1

Advance summary and potential significance to field

At this point of the reviewing process, my main concern was about the experimental evidence supporting the final model. Even though the authors could not show that Xrp1 is required for Dilp8 induction and developmental delay, they could rule out a role for eiger in this process. Their data therefore strongly suggest that Xrp1 is indeed the key effector of NOC1 LOF. This is fine with me.

Comments for the author

However, I feel that the role of eiger should be clarified in the text. None of the results presented in this story shows a role for eiger, despite its upregulation. I therefore believe that any conclusion about its role would lack evidence. Especially the sentence repeated both in the results and discussion hypothetizing "eiger acting more as an effector of this response" should be either removed or modified (e.g. "the role of eiger remains to be determined").
Minor point: the pannels in Fig 7I-J and 7 K-L are fully redundant. I would remove I-J.

Reviewer 2

Advance summary and potential significance to field

This corresponds to a second round of revisions. Most of my comments have been addressed.

Comments for the author

Most of my comments have been addressed now.
One continues to be addressed, which I find a little surprising.
My original comment: Figure 4D and E (previously Figure 3D and E): I pointed an error in the indication of genotypes. Dark blue bar is indicated as Noc1 overexpression. In spite of authors saying they corrected this the error in the previous submission. It remains as a mistake. The dark blue line continues to be labeled as NOC1-OE, which is inconsistent with the result description, which refers to NOC1-RNAi work and gene over expression results not shown.
Authors response: We are sorry for this mistake; the error has now been corrected
My comment: This is now Figure 4B and C and it continues to contain the same mistake.
I don't need to re-review this point as long as the Editor checks this is resolved.

Second revision

Author response to reviewers' comments

We thank the reviewers for the final and useful comments.

We have corrected in and rephrased in the manuscript the role of eiger as reviewer n 1 suggested. Here below are our answers to their final comments:

Reviewer 1 Advance Summary and Potential Significance to Field:

At this point of the reviewing process, my main concern was about the experimental evidence supporting the final model. Even though the authors could not show that Xrp1 is required for Dilp8

induction and developmental delay, they could rule out a role for eiger in this process. Their data therefore strongly suggest that Xrp1 is indeed the key effector of NOC1 LOF. This is fine with me.

Reviewer 1 Comments for the Author:

However, I feel that the role of eiger should be clarified in the text. None of the results presented in this story shows a role for eiger, despite its upregulation. I therefore believe that any conclusion about its role would lack evidence. Especially the sentence repeated both in the results and discussion hypothetizing "eiger acting more as an effector of this response" should be either removed or modified (e.g. "the role of eiger remains to be determined").
--> we have clarified and change the wording on the role of eiger in our model and we agree that is better to state that the role of eiger remains to be determined

Minor point: the pannels in Fig 7I-J and 7 K-L are fully redundant. I would remove I-J.
--> we believe that is better to also keep fig 7I-J since it shows DILP8-GFP expression using rotund-Gal4, while in Fig 7K-L is showed using nubbin-Gal4. Both promoters were used in the followed experiments. In addition, removing Fig 7 I-J will change the flow of the data and if the editor and reviewer agree we would like to keep it as it is.

Reviewer 2 Advance Summary and Potential Significance to Field:
This corresponds to a second round of revisions. Most of my comments have been addressed.

Reviewer 2 Comments for the Author:
Most of my comments have been addressed now.
One continues to be addressed, which I find a little surprising.

My original comment: Figure 4D and E (previously Figure 3D and E): I pointed an error in the indication of genotypes. Dark blue bar is indicated as Noc1 overexpression. In spite of authors saying they corrected this, the error in the previous submission. It remains as a mistake. The dark blue line continues to be labeled as NOC1-OE, which is inconsistent with the result description, which refers to NOC1-RNAi work and gene over expression results not shown.
--> I am truly sorry and this was my mistake, I made the corrections it in the original figure but then I uploaded the previous version, now the mistake has been corrected.

Authors response: We are sorry for this mistake; the error has now been corrected

My comment: This is now Figure 4B and C and it continues to contain the same mistake.

I don't need to re-review this point as long as the Editor checks this is resolved.

Third decision letter

MS ID#: JOCES/2022/260110

MS TITLE: Reduction of nucleolar NOC1 accumulates pre-rRNAs and induces Xrp1 affecting growth and DILP8 resulting in cell competition in Drosophila

AUTHORS: Francesca Destefanis, Valeria Manara, Stefania Santarelli, Sheri Pavlides, Marco Brambilla, Giacomo Viola, Paola Maragno, Ilaria Signoria, Gabriella Viero, Maria Enrica Pasini, Marianna Penzo, and Paola Bellosta
ARTICLE TYPE: Research Article

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