β-Catenin drives distinct transcriptional networks in proliferative and nonproliferative cardiomyocytes.

Gregory A. Quaife-Ryan, Richard J. Mills, George Lavers, Holly K. Voges, Celine J. Vivien, David A. Elliott, Mirana Ramialison, James E. Hudson and Enzo R. Porrello
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MS TITLE: β-catenin drives distinct transcriptional networks in regenerative and non-regenerative cardiomyocytes.

AUTHORS: Gregory A. Quaife-Ryan, Richard J. Mills, George Lavers, Holly K. Voges, Celine J. Vivien, David A. Elliott, Mirana Ramialison, James E. Hudson, and Enzo R. Porrello

I have now received all the referees reports on the above manuscript, and have reached a decision. The referees' comments are appended below, or you can access them online: please go to BenchPress and click on the 'Manuscripts with Decisions' queue in the Author Area.

The overall evaluation is positive and we would like to publish a revised manuscript in Development, provided that the referees' comments can be satisfactorily addressed. Please attend to all of the reviewers' comments in your revised manuscript and detail them in your point-by-point response. If you do not agree with any of their criticisms or suggestions explain clearly why this is so.

We are aware that you may currently be unable to access the lab to undertake experimental revisions. If it would be helpful, we encourage you to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating where you are able to address concerns raised (either experimentally or by changes to the text) and where you will not be able to do so within the normal timeframe of a revision. We will then provide further guidance. Please also note that we are happy to extend revision timeframes as necessary.

Reviewer 1

Advance summary and potential significance to field

In this manuscript, the authors demonstrated context-dependent roles of Wnt/beta-catenin signalling in regulating cardiomyocyte proliferation. In immature cardiomyocytes including in vivo neonatal CMs and in vitro iPSC-CMs, activation of Wnt/beta-catenin signalling promotes CM

proliferation, while in mature adult cardiomyocytes activation of Wnt/beta-catenin is insufficient to induce CM proliferation. ChIP-seq of histone marks with RNA-seq delineates the program in immature CMs that allows the stimulation of cell proliferation by Wnt. Using a constitutively active beta-catenin, the authors further showed that over-activation of Wnt/beta-catenin post-MI exhibited a cardioprotective role. The follow-up transcriptome study identified potential downstream genes and pathways involved in this effect. Overall, the logic of the manuscript is clear and the cardioprotective effect of Wnt/beta-catenin signalling in adult injured heart is interesting to the field.

Comments for the author

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Minor issue:

1. The citation for Figure 5A, 5B, and 5C is missing in the main text.
2. The two-time points (P1 and P56) in figure 1B did not support the conclusion that shutdown of postnatal Wnt/beta-catenin activity correlates with the loss of regenerative potential during heart development as the cardiac regeneration is shut down within the first week of postnatal development. The authors shall present an extra data point at P7 to support their conclusion.
3. In the conclusion of Figure 3, the authors shall avoid using “sufficient” in their conclusion as more direct evidence may be needed to make such strong claim.
4. The whole description for Figure 6D is missing from the manuscript.
5. In Figure 4E, the caYAP group should also be included as a positive control for the data quantification.
6. The figure legends need to be improved with necessary information. For example, no explanation could be found in the figure legend for the numbers within each bar in Figure 1G, H, M, N.

Reviewer 2

Advance summary and potential significance to field

In this contribute, Quaife-Ryan et al. investigated the effect of beta-catenin signaling in the context of PSC-CMs/neonatal CMs and adult CMs, with a goal to clarify its function on CM proliferation and maturation. Through gain/loss-of-function approaches combined with RNA/ChIP-seq analysis, the authors describe gene networks and biological processes pertaining to the role of beta-catenin signaling in neonatal and adult CMs. While confirmative to some extent, this study is expected to provide valuable insights into understanding the context-dependent roles of canonical Wnt signaling in early and late CMs.

Comments for the author

1) Figures 1G/H/N and 3G: cell number needs to be quantified in addition to pH3/Ki67 staining to claim CM proliferation.
2) Figure 3A: it would be helpful to include transduction efficiency and the level of mosaicism.
3) Figure 4H is missing.
4) Page 10, Figure 4A, B, C to 5A, B, C?
5) Figure S5: Interpretation of cross-comparison with earlier datasets needs to be careful because batch effects often significantly influence the outcome. Does the transcriptome of neonatal CMs examined in this study cluster with that of previously published neonatal CMs?
6) Figure 6: What is the cardio-regenerative gene network? The authors need to provide more data/information on this.
7) Figure 6/S6: It is intriguing that regenerative network genes are not responsive to caBCAT in adult CMs, but their regions maintain open chromatin status. The authors need to provide more data/information to substantiate this claim. Figure S6 is difficult to understand.
8) I am not sure if the title accurately reflects the conclusion as the paper does not show any regeneration data.

Reviewer 3

Advance summary and potential significance to field

The authors' work presents the following major advances:
Figure 1. Wnt/β-catenin signaling promotes proliferation in a human ESC model of cardiac differentiation.
Figure 2. Wnt/β-catenin signaling acts through TCF7L2 to direct cell division and changes in metabolic functions, including GPI anchor and Glycolipid biosynthesis and mitochondrial replication, in a human ESC model of cardiac differentiation.
Figure 3. Constitutively active β-catenin promotes cardiomyocyte proliferation in neonatal mouse hearts.
Figure 4. Constitutively active β-catenin promotes cardioprotection, but not through cardiomyocyte proliferation, in adult mouse hearts following MI.
Figure 5. Constitutively active β-catenin promotes a gene regulatory network of inflammatory response genes in adult mouse cardiomyocytes following MI.
Figure 6. The transcriptional output of Wnt/β-catenin signaling is distinct between immature/neonatal cardiomyocytes (human ESC model of cardiac differentiation or neonatal mouse cardiomyocytes) and mature/adult cardiomyocytes.

Comments for the author

Major Comments:
1. Related to Figure 5, the authors conclude that the Wnt/β-catenin signaling activates an inflammatory and angiogenic response in mature cardiomyocytes following MI based on the sole evidence of RNA-seq and treat it as the presumed mechanism for improved cardiac function. Can the authors provide additional supportive evidence for the claim that the cardiomyocytes are stimulating either an inflammatory or angiogenic response with Wnt/β-catenin signaling?

Minor Comments:
1. In relation to the AAV experiments, how was AAV incorporation measured and controlled between animals? What percentage of cardiomyocytes incorporated the AAV constructs and successfully expressed the caBCAT in any given animal? Was there any selection for caBCAT expression for the RNA-seq experiments or was it a mix of BCAT expressing and non-expressing cardiomyocytes?
2. Related to Figure 2F, the authors chose to use only peaks identified by both replicates. While I can appreciate the stringency, especially given the low number of peaks identified by each of the second replicates, peak calling is often seen as binary (yes, it is a peak or no, it is not a peak). Often times, a large number of reads are identified in both replicates even when only one replicate identifies a region as a peak, which may result from lower number of total reads or differences in the input. If the union of peaks is utilized rather than the intersection, are the conclusions of this study changed? For example, only sites identified by both TCF7L2 and either H3K27ac or H3K4me were utilized to identify direct targets of Wnt/β-catenin signaling in Figure 2J-K and Figure 6. Would the inclusion of more sites have found a larger overlap between immature cardiomyocyte and mature cardiomyocyte programs in response to Wnt/β-catenin signaling (Figure 6)?

First revision

Author response to reviewers' comments

Reviewer 1 Advance Summary and Potential Significance to Field:
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We thank the reviewer for the opportunity to discuss these relevant publications in the context of our findings. Wnt/ß-catenin signalling has been shown to have highly divergent roles in different cell types and this has led to confusion surrounding the exact role of beta catenin during post-infarction remodelling (discussed on line 63-80 and 334-338 of the manuscript). The study by Oerlemans et al., 2010 identified activated Wnt/ß-catenin signalling in several cell types post-infarction (cardiomyocytes, endothelial cells, leukocytes, Sca-1+/c-Kit+ progenitor cells, and fibroblasts). However, it was not determined whether Wnt/ß-catenin signalling was beneficial or pathological in this study and the role of ß-catenin in these diverse cell types was not delineated. It is possible that ß-catenin has protective functions in cardiomyocytes and pathophysiological roles in non-myocytes. For example, Wnt/ß-catenin signalling drives increased ECM deposition from epicardial and stromal fibroblasts in the infarcted heart (Duan et al., 2012). Similarly, Zhao et al (2015) reported that Alda/Aldh2 signalling downregulated Wnt/ß-catenin signalling in fibroblasts and prevented post-infarction fibrosis. We show that Wnt/ß-catenin signalling drives de-differentiation and proliferation of hPSC-derived stromal cells (Fig. 2C). These studies suggest that Wnt/ß-catenin signalling in fibroblasts drives fibroblast proliferation subsequently leading to increased ECM deposition and fibrosis. In contrast, our *in vivo* studies utilised AAV6, which is highly cardiomyocyte-specific (Figure S4). Our findings suggest that cardiomyocyte specific expression of ß-catenin is protective and anti-fibrotic. The principal function of ß-catenin in cardiomyocytes appears to be related to the mobilisation of alternative metabolic programs to diversify metabolic substrate usage in the infarcted heart. In this context, it is likely that the anti-fibrotic effects of AAV6-BCAT in our study occur secondary to cardioprotective effects in cardiomyocytes. We believe that these findings do not contradict earlier studies but rather support the contention that ß-catenin's functions are highly cell type and context dependent, which is reflected in our RNA-seq data in Fig 2A-C. Furthermore, target genes are also dependent on the maturation status of the cell in which it is activated. Therefore, it is likely that discrepancies surrounding the role of ß-catenin stem from a lack of activation within specific cell types and/or lack of cellular resolution in the analyses performed in some of these previous studies.

We have added text to the Introduction (line 74-78) and Discussion (line 334-339) to clarify our findings in the context of these earlier reports and to further reinforce the highly context-dependent functions of ß-catenin in diverse cardiac cell types.

Second, the authors have nicely performed RNA-seq and ChIP-seq to explore the possible mechanism underlying context-dependent effect of Wnt in CM proliferation. The global picture and overall conclusion are interesting and presented well. However, some experiment validation of candidate genes/pathways would much strengthen the overall study. For example, the cardioprotective role is explained as the consequence of modulation of oxidative phosphorylation and inflammatory genes by beta-catenin based on RNA-seq data. It will be nice to pinpoint the molecule(s) that mediate such effects or specific pathway(s) involved. Apart from these two major concerns, there are some minor issues listed below.

We agree with the Reviewer that the candidate genes/pathways identified in this study are interesting and warrant further mechanistic follow-up studies to pinpoint the precise molecules that mediate the cardioprotective effects of ß-catenin. However, >150 genes were regulated by ß-catenin in the adult heart, so identifying the precise molecule(s) responsible is extremely challenging. Even if we restricted our functional validation studies to the core network of target genes identified in Figure 6, this would require functional validation of ~10-20 target genes through loss-of-function studies *in vivo*. We do not believe that such experiments can be reasonably performed in the timeframe of a revision, particularly given current restrictions to laboratory work as a consequence of the COVID-19 pandemic. Nevertheless, we believe our findings do provide novel mechanistic insight and point towards an important role for ß-catenin in the modulation of proliferative transcriptional networks in immature cardiomyocytes and metabolic/immunomodulatory transcriptional networks in mature cardiomyocytes. Further studies will be required to pinpoint the precise molecules that mediate such effects, which we have now acknowledged in the Discussion (line 358).

Minor issue:
1. The citation for Figure 5A, 5B, and 5C is missing in the main text.

We apologise for this oversight. We had mistakenly referred to 4A, 4B and 4C rather than 5A, 5B, and 5C. This has been corrected in the text (line 227,228 and 239).

2. The two-time points (P1 and P56) in figure 1B did not support the conclusion that shutdown of postnatal Wnt/beta-catenin activity correlates with the loss of regenerative potential during heart development as the cardiac regeneration is shut down within the first week of postnatal development. The authors shall present an extra data point at P7 to support their conclusion.

We thank the reviewer for this suggestion. Our immunostaining data show that active ß-catenin in cardiomyocytes decreases substantially between P1 and P7 and is virtually undetectable in cardiomyocytes at P14, coincident with cardiomyocyte cell cycle shutdown and loss of regenerative capacity in mice (Fig. 1A). In addition, we have now analysed the mRNA expression levels of *Ctnnb1* and *Tcf7l2* in cardiomyocytes from P1 and P14 hearts (published in Quaife-Ryan et al, Circulation, 2017; data available under the GEO accession GSE95764). *Ctnnb1* and *Tcf7l2* are downregulated between P1 and P14, coinciding with the decrease in active ß-catenin levels reported in Figure 1A and the loss of cardiac regenerative potential in mice. We have replaced the previous data in Figure 1B with this new analysis.

3. In the conclusion of Figure 3, the authors shall avoid using “sufficient” in their conclusion as more direct evidence may be needed to make such strong claim.

We agree and we have removed the word “sufficient” as per the reviewer’s suggestion.

4. The whole description for Figure 6D is missing from the manuscript.

We apologize for this oversight. A reference to Figure 6D is now included (line 268).

5. In Figure 4E, the caYAP group should also be included as a positive control for the data quantification.

As suggested, we have now included quantification of caYAP in Figure 4E as a positive control. Consistent with a previous report from the Pu laboratory (Lin et al., 2014), cross-sectional area was reduced in adult mice treated with AAV6-caYAP (data added to Figure 4E).

6. The figure legends need to be improved with necessary information. For example, no explanation could be found in the figure legend for the numbers within each bar in Figure 1G, H, M, N.

We apologise for this oversight and have updated the figure legends. Numbers within each bar refer to the number of hCOs in each sample.

Reviewer 2 Advance Summary and Potential Significance to Field:
In this contribute, Quaife-Ryan et al. investigated the effect of beta-catenin signaling in the context of PSC-CMs/neonatal CMs and adult CMs, with a goal to clarify its function on CM proliferation and maturation. Through gain/loss-of-function approaches combined with RNA/ChIP-seq analysis, the authors describe gene networks and biological processes pertaining to the role of beta-catenin signaling in neonatal and adult CMs. While confirmative to some extent, this study is expected to provide valuable insights into understanding the context-dependent roles of canonical Wnt signaling in early and late CMs.

Reviewer 2 Comments for the Author:
1) Figures 1G/H/N and 3G: cell number needs to be quantified in addition to pH3/Ki67 staining to claim CM proliferation.

Thank you for this suggestion. We have performed additional experiments to quantify cardiomyocyte number in vivo in neonatal mice treated with AAV6-caBCAT. Consistent with the pro-proliferative function of ß-catenin in immature cardiomyocytes, neonatal mice treated with AAV6-caBCAT displayed ~16% increase in cardiomyocyte number compared to control AAV6-GFP treated mice (Figure 3F, p=0.012). These data have been added to Figure 3F and text added to the Results section (line 191-193). We have also previously shown that CHIR stimulates ~40% increase in hPSC-derived cardiomyocyte number *in vitro* (Titmarsh et al., 2016).

2) Figure 3A: it would be helpful to include transduction efficiency and the level of mosaicism.

AAV6 has been used extensively in the literature to drive cardiac-specific gene expression in neonatal and adult mice with reported transduction efficiencies of >80% (Palomeque et al., Gene Therapy, 2007; Zincarelli et al., Molecular Therapy, 2008; Zincarelli et al., Clin Transl Sci, 2010, Prasad et al, Gene Therapy, 2011; Weeks et al, Circ Heart Failure, 2012, Prakoso et al, Am J Physiol Heart, 2020). Unfortunately, it is not possible to determine the precise transduction efficiency of the AAV6-caBCAT construct used in this study due to significant species cross-reactivity of ß-catenin antibodies, which do not enable discrimination of endogenous mouse ß-catenin from the AAV6-delivered constitutively active human ß-catenin. It is therefore impossible to calculate the percentage of cardiomyocytes that express this construct. Using GFP as a surrogate, we show that GFP expression is restricted to the border-zone injection sites in adult mice treated with the control AAV-GFP construct (Fig S4A). Using primers designed to preferentially amplify human *CTNNB1,* we have determined that ß-catenin is highly and specifically expressed in cardiomyocytes (Fig S4B). Therefore, while mosaicism in cardiac transgene expression is likely in this experimental context, the only way to control for it is to use an appropriate control group such as AAV6-GFP treated mice, which we have included in this study.

3) Figure 4H is missing.

We apologize for this oversight and 4H has now been included in the figure.

4) Page 10, Figure 4A, B, C to 5A, B, C?

The reviewer is correct. We have updated the text. We had mistakenly referred to 4A, 4B and 4C rather than 5A, 5B, and 5C. This has been corrected in the text (line 227,228 and 239).

5) Figure S5: Interpretation of cross-comparison with earlier datasets needs to be careful because batch effects often significantly influence the outcome. Does the transcriptome of neonatal CMs examined in this study cluster with that of previously published neonatal CMs?

The reviewer raises an important point regarding the potential contribution of batch effects to our analysis, which could influence interpretation of cross-comparison data sets. However, as shown in Supplementary Figure 6, the Myo.caBCAT samples (i.e. RNAseq of purified cardiomyocytes at day 3 post-MI in adult mice treated with AAV6-caBCAT in this study) clustered closely with our previously published dataset for MIP56.d3 cardiomyocytes (see clustering of purple and grey closed triangles). These datasets were generated 2 years apart. Therefore, we believe there are negligible batch effects between these two data sets.

The reviewer also asks a specific question regarding clustering of the transcriptome of neonatal CMs examined in this study with previously published neonatal CMs. However, to clarify, we did not perform any RNA-seq of neonatal CMs in this study, so this analysis is not possible.

6) Figure 6: What is the cardio-regenerative gene network? The authors need to provide more data/information on this.

We apologize for the confusion and have sought to provide greater clarity regarding the “cardio-regenerative gene network”. This network of genes was previously identified in a large multicellular transcriptomic analysis of the regenerative neonatal heart and non-regenerative adult heart (Quaife-Ryan et al., 2017). In that study we identified adult cardiomyocytes and endothelial cells do not reactivate a neonatal-like transcriptional program following myocardial infarction (Quaife-Ryan et al., 2017). The “neonatal regenerative gene network” contains genes that were highly expressed in neonatal regenerating cardiac cells but were not deployed by adult cardiomyocytes and endothelial cells following MI. This gene network contained mostly cell-cycle genes and was bioinformatically predicted to drive proliferative responses in the regenerating heart. Interestingly, we have recently demonstrated that this gene network is re-activated in adult cardiomyocytes following induction of Myc/pTEFb transcription (Bywater et al., 2020). We have provided additional information on the “cardio-regenerative gene network” in the Methods section (711-720).

7) Figure 6/S6: It is intriguing that regenerative network genes are not responsive to caBCAT in adult CMs, but their regions maintain open chromatin status. The authors need to provide more data/information to substantiate this claim. Figure S6 is difficult to understand.

We apologise for this lack of clarity. The figure has been updated to make it more comprehensible. The purpose of this figure is to try and understand why adult cardiomyocytes fail to re-engage the direct β-catenin target genes that are activated in neonatal mouse cardiomyocytes and CHIR-treated human cardiomyocytes. We hypothesised that there could be an epigenetic mechanism underlying the distinct β-catenin gene programs in immature and mature cardiomyocytes. However, we determined that the promoters of the β-catenin target genes of immature cardiomyocytes were not condensed during postnatal maturation (Fig S7). Therefore, it is unlikely that β-catenin is epigenetically prevented from binding to the immature β-catenin target genes in adult cardiomyocytes. However, it is important to note that this analysis was restricted to the chromatin state of promoters. It is entirely possible that immature β-catenin target enhancer loci could be epigenetically shutdown in adult cardiomyocytes as has been recently reported for regeneration-responsive enhancers in zebrafish and killifish (Wang et al., 2020). Substantiating the latter hypothesis would require significant additional experimentation (e.g. chromosome conformation capture techniques), which is beyond the scope of this study. We have expanded the Discussion (line 321 and 326-329) to clarify our findings with respect to promoters versus enhancers.

8) I am not sure if the title accurately reflects the conclusion as the paper does not show any regeneration data

We agree and we have changed the title to “β-catenin drives distinct transcriptional networks in proliferative and non-proliferative cardiomyocytes”.

Reviewer 3 Advance Summary and Potential Significance to Field:
The authors’ work presents the following major advances:
Figure 1. Wnt/β-catenin signaling promotes proliferation in a human ESC model of cardiac differentiation.
Figure 2. Wnt/β-catenin signaling acts through TCF7L2 to direct cell division and changes in metabolic functions, including GPI anchor and Glycolipid biosynthesis and mitochondrial replication, in a human ESC model of cardiac differentiation.
Figure 3. Constitutively active β-catenin promotes cardiomyocyte proliferation in neonatal mouse hearts.
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Reviewer 3 Comments for the Author:
Major Comments:
1.Related to Figure 5, the authors conclude that the Wnt/β-catenin signaling activates an inflammatory and angiogenic response in mature cardiomyocytes following MI based on the sole evidence of RNA-seq and treat it as the presumed mechanism for improved cardiac function. Can the authors provide additional supportive evidence for the claim that the cardiomyocytes are stimulating either an inflammatory or angiogenic response with Wnt/β-catenin signaling?

The reviewer correctly notes the putative inflammatory and angiogenic function of caBCAT in the adult mouse heart were based on RNAseq studies alone. We do not wish to overstate these findings and agree that we have not identified the precise mechanism underlying β-catenin’s cardioprotective effects. As discussed above in our response to Reviewer 1, further studies are required to pinpoint the precise molecules that mediate these cardioprotective effects, which we now acknowledge in the Discussion (line 358). We have toned down our conclusions regarding the putative contributions of the inflammatory and angiogenic transcriptional signatures to cardioprotection in this model and

clarify that these transcriptional changes are associative rather than causative (line 244, 340, 343-345).

Minor Comments:
1.In relation to the AAV experiments, how was AAV incorporation measured and controlled between animals? What percentage of cardiomyocytes incorporated the AAV constructs and successfully expressed the caBCAT in any given animal?

As per our response to Reviewer #1 above:

AAV6 has been used extensively in the literature to drive cardiac-specific gene expression in neonatal and adult mice with reported transduction efficiencies of >80% (Palomeque et al., 2007; Prakoso et al., 2020; Prasad et al., 2011; Weeks et al., 2012; Zincarelli et al., 2010; Zincarelli et al., 2008). Unfortunately, it is not possible to determine the precise transduction efficiency of the AAV6-caBCAT construct used in this study due to significant species cross-reactivity of β-catenin antibodies, which do not enable discrimination of endogenous mouse β-catenin from the AAV6-delivered constitutively active human β-catenin. It is therefore impossible to calculate the percentage of cardiomyocytes that express this construct. Using GFP as a surrogate, we show that GFP expression is restricted to the border-zone injection sites in adult mice treated with the control AAV-GFP construct (Fig S4A). Using primers designed to preferentially amplify human *CTNNB1*, we have determined that β-catenin is highly and specifically expressed in cardiomyocytes (Fig S4B). Therefore, while mosaicism in cardiac transgene expression is likely in this experimental context, the only way to control for it is to use an appropriate control group such as AAV6-GFP treated mice, which we have included in this study.

Was there any selection for caBCAT expression for the RNA-seq experiments or was it a mix of BCAT expressing and non-expressing cardiomyocytes?

Unfortunately, we could not select for caBCAT expressing cells for the reasons outlined above. In addition, it was very important to isolate and purify the cardiomyocyte population to determine gene expression specifically in cardiomyocytes. This requires retrograde Langendorf perfusion and enzymatic dissociation of cardiomyocytes from the whole heart and thus it is not possible to specfically isolate cardiomyocytes from the broder zone using this method. Nevertheless, we identified 161 differentially regulated genes in the mixed population of adult myocytes treated with AAV6-caBCAT compared to the AAV6-GFP control group (Figure 5).

2. Related to Figure 2F, the authors chose to use only peaks identified by both replicates. While I can appreciate the stringency, especially given the low number of peaks identified by each of the second replicates, peak calling is often seen as binary (yes, it is a peak or no, it is not a peak). Often times, a large number of reads are identified in both replicates even when only one replicate identifies a region as a peak, which may result from lower number of total reads or differences in the input. If the union of peaks is utilized rather than the intersection, are the conclusions of this study changed? For example, only sites identified by both TCF7L2 and either H3K27ac or H3K4me were utilized to identify direct targets of Wnt/β-catenin signaling in Figure 2J-K and Figure 6. Would the inclusion of more sites have found a larger overlap between immature cardiomyocyte and mature cardiomyocyte programs in response to Wnt/β-catenin signaling (Figure 6)?

We thank the reviewer for this comment and appreciate the points that have been raised. However, ENCODE recommends at least 2 biological replicates for ChIP-seq experiments and recommends some form of intersection (note: ENCODE utilises Irreproducible Discovery Rate to determine peak number, which is even more stringent than intersecting peaks) (Landt et al., 2012). It should be noted that using the union of peaks would result in 46,214 TCF7L2 peaks. This would make TCF7L2 one of the most promiscuous known transcription factors. Even CTCF is thought to have only ~75,000 peaks (Landt et al., 2012) and we have recently shown that c-Myc, which is thought to be a “universal transcriptional amplifier”, only has ~30,000 peaks in the heart (Bywater et al., 2020). We are thus concerned that the union setting for ChIP-seq data would introduce unacceptably high levels of false positives in this experiment and we are uncomfortable relaxing the stringency of our analysis methods for this reason.

References:

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Second decision letter

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I am happy to tell you that your manuscript has been accepted for publication in Development, pending our standard ethics checks.