JCB

Golgi-associated BICD adaptors couple ER membrane penetration and disassembly of a viral cargo

Chelsey Spriggs, Somayesadat Badieyan, Kristen Verhey, Michael Cianfrocco, and Billy Tsai

Corresponding Author(s): Billy Tsai, University of Michigan and Michael Cianfrocco, University of Michigan

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September 12, 2019

Re: JCB manuscript #201908099

Prof. Billy Tsai
University of Michigan
Cell and Developmental Biology University of Michigan Medical School 109 Zina Pitcher Place, Rm 3043
Ann Arbor, MI 48109

Dear Prof. Tsai,

Thank you for submitting your manuscript entitled "Golgi-associated dynein adaptors strategically couple ER membrane penetration and disassembly of a viral cargo". Your manuscript has been assessed by expert reviewers, whose comments are appended below. Although the reviewers express potential interest in this work, significant concerns unfortunately preclude publication of the current version of the manuscript in JCB.

You will see that all three reviewers agree this paper provides evidence that the dynein cargo adaptors BicD2 and BicDR1 play a role in the SV40 virus life cycle but all emphasize that clearer insight into the mechanism by which these proteins control virus disassembly in cells would be necessary to support the proposed model. Although some potential experiments are suggested, such as ruling out indirect effects on the microtubule cytoskeleton and exploring how BicD2 mediates interaction between virus ER foci and the MTOC or nuclear pores, we hope that you may already have in mind a route to providing more definitive mechanistic insight. The reviewers also raise concerns about whether or not the apparent direct effects of BicD2 on virus disassembly in vitro reflect how the endogenous protein facilitates the viral life cycle, and some additional technical comments about the data presented that should be addressed for resubmission.

Please let us know if you are able to address the major issues outlined above and wish to submit a revised manuscript to JCB. Note that a substantial amount of additional experimental data likely would be needed to satisfactorily address the concerns of the reviewers. Our typical timeframe for revisions is three to four months; if submitted within this timeframe, novelty will not be reassessed. We would be open to resubmission at a later date; however, please note that priority and novelty would be reassessed.

If you choose to revise and resubmit your manuscript, please also attend to the following editorial points. Please direct any editorial questions to the journal office.

GENERAL GUIDELINES:
Text limits: Character count is < 40,000, not including spaces. Count includes title page, abstract, introduction, results, discussion, acknowledgments, and figure legends. Count does not include materials and methods, references, tables, or supplemental legends.

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screened prior to publication.

IMPORTANT: It is JCB policy that if requested, original data images must be made available. Failure to provide original images upon request will result in unavoidable delays in publication. Please ensure that you have access to all original microscopy and blot data images before submitting your revision.

Supplemental information: There are strict limits on the allowable amount of supplemental data. Your manuscript may have up to 5 supplemental figures. Up to 10 supplemental videos or flash animations are allowed. A summary of all supplemental material should appear at the end of the Materials and methods section.

If you choose to resubmit, please include a cover letter addressing the reviewers' comments point by point. Please also highlight all changes in the text of the manuscript.

Regardless of how you choose to proceed, we hope that the comments below will prove constructive as your work progresses. We would be happy to discuss them further once you've had a chance to consider the points raised. You can contact the journal office with any questions, cellbio@rockefeller.edu or call (212) 327-8588.

Thank you for thinking of JCB as an appropriate place to publish your work.

Sincerely,

Samara Reck-Peterson, Ph.D.
Monitoring Editor

Marie Anne O'Donnell, Ph.D.
Scientific Editor

Journal of Cell Biology

Reviewer #1 (Comments to the Authors (Required)):

Using a combination of approaches the authors provide evidence that BicD2 and BICDR1 are required to support SV40 infection once the virus has penetrated the cytosol from the ER. In vitro assays with purified virus preparations demonstrate that BicD2 promotes virus capsid disassembly to allow DNA release. Consistent with their biochemical analysis, the authors find that BicD2 is localized around the SV40-induced ER focus at the MTOC. Further, they find that dispersion of the Golgi suppresses SV40 disassembly and subsequent infection. The authors data are clear and support their conclusions. However, we are left with many questions and there is little or no mechanistic insight into how BicD2 promotes capsid disassembly? Below I have listed some points and questions the authors might like to consider.

The authors focus on BicD2. However, most cells also express BicD1, which plays a redundant role with BicD2. Does the knockdown of BicD1 impact virus infection? If it does not, then it provides a perfect negative control for all their experiments given its similarity to BicD2. The same is also true for BICDR2.

Using their in vitro assay, the authors demonstrate that a 1:72 ratio of virus to BicD2 is required to promote capsid disassembly (Fig. 4F). What ratio was used for earlier experiments in Fig. 4C and D? What ratio of BICDR1 is required to promote disassembly and how does it compare to BicD2? Likewise, BicD1 and BICDR2, if they are involved in virus infection.

It would be nice to see what these virus / BicD2 mixtures look like in the electron microscope, over a range of ratios, as this my help provide insights into how BicD2 promotes capsid disassembly.

Figure 6 shows that the SV40-induced ER focus is at or near the MTOC. It comes as no surprise that BicD2 is in the same region as it is associated with the Golgi. The bigger question is why does the focus form at this location? Also, we are only provided fixed images that give no idea of time scales. It would be nice to see the dynamics of BicD2, BICDR1, BAP31 and GM130 during focus formation as this may provide additional insights beyond seeing they are merely in the same area of the cell.

In figure 1 the authors used an siRNA approach to examine impact of loss of dynein adaptors on SV40 infection. I am surprised that the authors did not take advantage of this approach to look in the confocal what happens to focus formation and localization of BAP31 and other markers when BicD2 and BICDR1 are depleted.

The authors take advantage of a KIF5C reagent that targets the Golgi to promote its dispersion. The result on infection is clear, however, is it loss of BicD2 and/or the Golgi? The authors should examine the impact of Golgi dispersion using other methods such as Brefeldin A. Figure 7 also shows BAP31 but not a viral marker such as VP1 or VP2/3, which should be provided. Also is the effect on infection dependent on Rab6, which is required for Golgi targeting of BicD2?

Does anchoring BicD2 at the MTOC promote infection in Golgi dispersed cells? This would address if it is BicD2 and/or its localization of the Golgi that promotes infection.

Reviewer #2 (Comments to the Authors (Required)):

The manuscript, "Golgi-associated dynein adaptors strategically couple ER membrane penetration and disassembly of a viral cargo" by Spriggs et al. uses SV40 in combination with targeted RNAi-mediated depletion of dynein adapters to identify BICD2 and BICDR1 as cell factors required for PyV infection. They demonstrate that these adaptors bind VP1 with associated genome and using a variety of well-established biochemical assays, the authors pinpoint the stage of infection requiring BICD2 and BICDR1 as post-penetration cytosolic capsid disassembly. The data in figures 1-5 is well presented and robust. I am not so convinced by figure 6 and 7 and feel the authors have made several logic leaps to piece together these results with little supporting evidence for their final model.

-This hinges on experiment Fig5B which suggests that BICD-2 cargo binding/nuclear association and kinesin-1 association domains are needed for disassembly?

-This data reasons that the adapters might serve to couple VP1 to kinesin or to nuclear pores to generate forces needed for disruption of both or either, or that nuclear pore arrival depends on these adapters. (Strunze S, et al. Cell Host Microbe. 2011 Sep 15;10(3):210-23)

-However, the authors go on to test dynein, as opposed to kinesin, in the in vitro disassembly assay?

-Why were the effects of kinesin inhibition or depletion on cytosolic SV40 disassembly not tested in this context.

-Just as easily is SV40 nuclear pore docking affected by adapter depletion?

-While it's a nice to demonstrate that major Golgi dispersion impedes cytosolic disruption of SV40, there is no evidence linking this directly to the adaptors other than their mis-localization? Many, many Golgi constituents are likely to be mis-localized using this assay.

-In fact, it's a bit surprising to see so little colocalization of VP1/2/3 and BICD2. With 1 copy of BICD2 binding each of 360 VP1 monomers I would suspect to see far more colocalization? Akin to Bap31 for instance?

6D- Need to quantify co-localization to say "strong co-localization". A co-localization coefficient is needed here.

Overall this model begs the question if cytosolic disassembly is mediated by golgi associated adapters why doesn't adaptor depletion affect membrane fraction association in Fig 2B? i.e. why is cytosolic delivery unaffected?

Reviewer #3 (Comments to the Authors (Required)):

Spriggs et al. report the surprising finding that the dynein cargo adapter molecules BicD2 and BicDR1 directly mediate the disassembly of SV40 virus particles after their trafficking to the nuclear periphery inside of cells. They find that dynein motor activity is not required for this activity in vitro and suggest that direct binding of the cargo-adapters to the virus could mediate an unknown structural transition. The conclusion of the study is surprising and could be interesting to the broader cell biology community. In general I found the experiments pretty convincing but the manuscript suffers from a lack of any quantification of many of the biochemical experiments and the authors present only example panels of these results. The authors will need to rectify this before I could recommend publication. Additionally the molecular mechanism of BicD2-mediated viral disassembly remains totally obscure, leaving the reader a bit confused at the end of the paper. Without a more concrete insight into the mechanism of action, I am left wondering if the paper is suitable for a broad journal like the JCB or a more specialized journal such as J. Virology or similar.

Specific comments:

1. In Fig. 1, the authors report that siRNA of BicD2 or BicDR1 disrupt SV40 infection. Have the author's considered or checked if knockdown of these proteins disrupts the endogenous MT cytoskeleton organization? It seems to me that disruption of the normal cytoskeletal organization could also lead to the observed effects and the authors should be sure that they are not measuring secondary effects of these knockdowns. Staining and quantification of the MT network organization in these cells should be performed to rule out this possibility.

2. The western blot in Fig. 3C is a little weird. There is heavy band smearing in the knockdown lanes from the top of the cushion. Is this the best example the authors have? This is a good supportive

result, so it's important to establish its robustness. Quantification of replicates should be included for this experiment to show reproducibility of this effect.

3. Quantification of the effects in Figs. 3D-E should be included to demonstrate reproducibility.

4. The results in Fig. 4 are surprising. The authors should show control experiments with each component of this complex mixture added to the virus independently (i.e. Virus + dynein alone, dynactin alone, LIS1 alone, and NDEL alone) in order to validate the claim that the BicD2 molecules are alone sufficient to induce virus disassembly. This is especially true since the disassembly reaction requires a large molar excess of BicD2 protein (1:500) possibly suggesting some non-specific reason for the disassembly (contaminating proteases in the BicD2 prep for instance). Finally the authors should quantify all of the results displayed in panels B-F and indicate how many times these experiments were repeated. At the moment we have only one example blot for each.

5. In Fig. 5 the authors need to quantify the results shown in panels B and C and indicate how many times these experiments were repeated.

6. I think the section describing Fig. 6 is written a little weirdly in the sense that since the BicD2 staining in a ring is observed even without virus infection, it seems odd to suggest that BicD2 "surrounds" or "encloses" the viral focus. Rather, the way I see the data is that the virus is simply trafficked to the center of this BicD2 ring (the Golgi) simply by virtue of it's movement along MTs to the MTOC. The way it's written now seems to imply some kind of active process of surrounding the viral focus, but this is not the case. Unless I misread the section and the authors believe the Golgi is actively remodeled around the viral focus?

7. Please quantify the data in Fig. 7B, E & F across replicates.

8. Why do the authors perform no in vivo experiments with BicDR1? Do they see the similar effects as they observe with manipulation of BicD2 levels in cells?

9. I don't understand the hypothetical model of rapid binding/unbinding of CC2 to the virus and how this could mediate viral destabilization. Either explain this model in more detail or I suggest dropping it.

10. There are several spelling and grammar mistakes in the manuscript.

University of Michigan
Medical School

Cell and Developmental Biology
109 Zina Pitcher Place
Room 3043
Ann Arbor, MI 48109

December 4, 2019

Dear Editor,

We would like to thank the reviewers for their generally positive comments regarding our manuscript. We have now addressed their concerns by performing a series of additional experiments (**Fig S1, Fig 2D, Fig 3B, Fig 3D, Fig 4F-I, Fig S2A, Fig S2C, Fig S3, Fig 5C, Fig 6C, Fig S4C, and Fig 7G-H**), quantified the biochemical assays (**Fig 3D-F, Fig 4B-G, Fig S2A-C, Fig 5B-C, Fig 7B, and Fig 7E-F**), and changed the text to increase clarity.

Because the major reviewer concern is that we needed to provide more mechanistic insight into BICD2-dependent disassembly of SV40, we want to highlight how we have addressed this issue.

First, we now find that BICD2 cannot efficiently disassembly native SV40 (**Fig 4G**), in contrast to DTT/EGTA-treated virus. DTT/EGTA-treated virus mimics SV40 that has trafficked from the cell surface to the ER (where the viral disulfide bonds are reduced, a reaction mimicked by DTT treatment) and subsequently penetrated the ER membrane to reach the cytosol (where the virus-bound calcium is released, a reaction mimicked by EGTA treatment). Hence, BICD2 can disassemble SV40 only if the virus trafficked along its infectious route to reach the cytosol so that it is properly “primed” for BICD2 engagement.

Second, we now performed a co-immunoprecipitation experiment which showed that BICD2 interacts with VP1 and VP3 minor capsid protein (**Fig 3B**). Because a SV40 particle contains 72 VP1 pentamers, with each pentamer harboring either VP2 or VP3, this finding suggests that BICD2 recognizes VP1 pentamers with VP3. This is consistent with the notion that BICD2 preferentially disassembles the DTT/EGTA-treated virus (**see above**) because DTT reduces the viral disulfide bonds essential to expose VP3.

Third, guided by the new binding study, we used a mutant SV40 devoid of VP3 (delta VP3), and found that BICD2 cannot disassemble this mutant virus (**Fig 4F**), further suggesting that BICD2 acts on a distinct form of the viral cargo: VP1 pentamers containing VP3. Why BICD2 might prefer VP3 over VP2 is unclear, especially given that VP2 contains all the sequences of VP3 and only harbors a unique N-terminal region.

One possibility is that this VP2 N-terminal region occludes a BICD2 recognition site that is unmasked in VP3.

Fourth, by negative stain electron microscopy analysis, we now find that BICD2 disassembles DTT/EGTA-treated virus in a concentration-dependent manner, generating VP1 pentamers, as well as partially disassembled viral particles (**Fig 4H-I; Fig S3**). Thus, we now provide direct visual evidence of the products of BICD2-induced SV40 disassembly.

In addition to these four new points, we also provide evidence (in the original and revised versions of this manuscript) that the CC2 and CC3 domains of BICD2 is essential to trigger SV40 disassembly. When combined, we hope the reviewers agree that we have provided a thorough and detailed analysis of the BICD2-dependent SV40 disassembly mechanism.

Below we detail our point-by-point responses to the other concerns.

Reviewer #1 (Comments to the Authors (Required)):

Using a combination of approaches the authors provide evidence that BicD2 and BICDR1 are required to support SV40 infection once the virus has penetrated the cytosol from the ER. In vitro assays with purified virus preparations demonstrate that BicD2 promotes virus capsid disassembly to allow DNA release. Consistent with their biochemical analysis, the authors find that BicD2 is localized around the SV40-induced ER focus at the MTOC. Further, they find that dispersion of the Golgi suppresses SV40 disassembly and subsequent infection. The authors data are clear and support their conclusions. However, we are left with many questions and there is little or no mechanistic insight into how BicD2 promotes capsid disassembly? Below I have listed some points and questions the authors might like to consider.

The authors focus on BicD2. However, most cells also express BicD1, which plays a redundant role with BicD2. Does the knockdown of BicD1 impact virus infection? If it does not, then it provides a perfect negative control for all their experiments given its similarity to BicD2. The same is also true for BICDR2.

As requested, we now knocked down BICD1 and BICDR2 and found that BICD1 (but not BICDR2) is also important for supporting SV40 infection (Fig S1).

Using their in vitro assay, the authors demonstrate that a 1:72 ratio of virus to BicD2 is required to promote capsid disassembly (Fig. 4F). What ratio was used for earlier experiments in Fig. 4C and D? What ratio of BICDR1 is required to promote disassembly and how does it compare to BicD2? Likewise, BicD1 and BICDR2, if they are involved in virus infection.

Figure 4C and 4D used a ratio of 1:500.

As requested, we now performed this assay using BICDR1 and found similar results as with BICD2, except there was modestly more virus disassembly using a 1:1 ratio of BICDR1 to virus when compared to BICD2 (Fig S2C). We did not perform the disassembly assay using BICD1 due to the lack of purified protein.

It would be nice to see what these virus / BicD2 mixtures look like in the electron microscope, over a range of ratios, as this my help provide insights into how BicD2 promotes capsid disassembly.

As requested, we now performed negative stain electron microscopy (EM) using increasing concentrations of BICD2 to virus. Strikingly, our new results reveal that BICD2 triggers the release of VP1 pentamers, as well as formation of partially disassembled virus (Fig 4H; quantified in 4I; Fig S3). These findings provide direct visual evidence of the viral products generated by BICD2-mediated disassembly.

Figure 6 shows that the SV40-induced ER focus is at or near the MTOC. It comes as no surprise that BicD2 is in the same region as it is associated with the Golgi. The bigger question is why does the focus form at this location? Also, we are only provided fixed images that give no idea of time scales. It would be nice to see the dynamics of BicD2, BICDR1, BAP31 and GM130 during focus formation as this may provide additional insights beyond seeing they are merely in the same area of the cell.

We agree that why the focus forms proximal to the MTOC structure is an interesting question. Our lab is addressing this issue, and we hope this reviewer agrees that this question is outside of the scope of the present paper. However, in this revision, we do provide new data showing that neither BICD2 (Fig 6C, with quantification) nor BICDR1 (Fig S4C, with quantification) is required for foci formation, but for the subsequent disassembly step in the cytosol. Regarding the dynamics of foci formation, we have in fact previously published live-cell imaging data showing the dynamics of foci formation (Walczak et al., 2014, PLoS Pathogen).

In figure 1 the authors used an siRNA approach to examine impact of loss of dynein adaptors on SV40 infection. I am surprised that the authors did not take advantage of this approach to look in the confocal what happens to focus formation and localization of BAP31 and other markers when BicD2 and BICDR1 are depleted.

As requested, we now find that BAP31-foci formation is not disrupted in cells depleted of either BICD2 (Fig 6C, with quantification) or BICDR1 (Fig S4C, with quantification). These observations support the idea that these BICD proteins act on the virus after foci formation when the virus has escaped into the cytosol from the ER.

The authors take advantage of a KIF5C reagent that targets the Golgi to promote its dispersion. The result on infection is clear, however, is it loss of BicD2 and/or the Golgi?

The authors should examine the impact of Golgi dispersion using other methods such as Brefeldin A. Figure 7 also shows BAP31 but not a viral marker such as VP1 or VP2/3, which should be provided. Also is the effect on infection dependent on Rab6, which is required for Golgi targeting of BicD2?

As requested, we have now included VP2/3 with BAP31 in Figure 7.

Also as requested, we tested the effect of disrupting Rab6 on virus infection using the Rab6a T27N dominant-negative mutant. We now find that expressing this Rab6 mutant (but not the corresponding wild-type Rab6) disrupts BICD2 localization to the Golgi (Fig 7G) and importantly, SV40 infection (Fig 7I). These findings support the view that BICD2 positioned at the Golgi (but not the Golgi itself) is crucial for virus infection.

Does anchoring BicD2 at the MTOC promote infection in Golgi dispersed cells? This would address if it is BicD2 and/or its localization of the Golgi that promotes infection.

This is a good idea. As indicated above, we now used an independent method to mis-localize BICD2 (i.e. expression of a Rab6 dominant-negative construct, Fig 7G) and found that this condition blocked SV40 infection (Fig 7I). These data are consistent with the idea that BICD2 positioned at the Golgi - but not Golgi itself - is crucial for virus infection.

Reviewer #2 (Comments to the Authors (Required)):

The manuscript, "Golgi-associated dynein adaptors strategically couple ER membrane penetration and disassembly of a viral cargo" by Spriggs et al. uses SV40 in combination with targeted RNAi-mediated depletion of dynein adapters to identify BICD2 and BICDR1 as cell factors required for PyV infection. They demonstrate that these adaptors bind VP1 with associated genome and using a variety of well-established biochemical assays, the authors pinpoint the stage of infection requiring BICD2 and BICDR1 as post-penetration cytosolic capsid disassembly. The data in figures 1-5 is well presented and robust. I am not so convinced by figure 6 and 7 and feel the authors have made several logic leaps to piece together these results with little supporting evidence for their final model.

-This hinges on experiment Fig5B which suggests that BICD-2 cargo binding/nuclear association and kinesin-1 association domains are needed for disassembly?

Our new data using truncated BICD2 demonstrate that both the CC2 and CC3 domains of BICD2 are sufficient to support efficient SV40 binding (Fig 5C, with quantification) and disassembly (Fig 5B, with quantification).

-This data reasons that the adapters might serve to couple VP1 to kinesin or to nuclear pores to generate forces needed for disruption of both or either, or that nuclear pore

arrival depends on these adapters. (Strunze S, et al. Cell Host Microbe. 2011 Sep 15;10(3):210-23)

The findings presented in this manuscript demonstrate that isolated BICD2 (or BICDR1) is sufficient to promote SV40 disassembly, independent of kinesin-1. Whether these adaptors play any role in coupling SV40 to the nuclear pore to facilitate viral nuclear entry remains unclear. We believe that although the issue of dynein adaptor's role during SV40 nuclear entry is important, it is beyond the focus of this present manuscript.

-However, the authors go on to test dynein, as opposed to kinesin, in the in vitro disassembly assay?

We previously published a paper suggesting that a dynein-dependent activity is important to disassemble SV40 (Ravindran and Spriggs et al., 2018, Journal of Virology). This paper motivated us to pinpoint the specific dynein component necessary for the disassembly event, and to further mechanistically elucidate the disassembly reaction.

-Why were the effects of kinesin inhibition or depletion on cytosolic SV40 disassembly not tested in this context.

In fact, we previously published a paper (Ravindran et al., 2017, Nature Communications) showing that kinesin-1 is required to construct the foci structure. In that paper, we reported that disrupting kinesin-1 activity blocked foci formation, and as a consequence, cytosol arrival of SV40 from the ER. Thus, as disrupting kinesin-1 activity blocked cytosol entry of SV40, we cannot easily address a role of kinesin-1 in facilitating BICD-dependent SV40 disassembly in the cytosol. We believe the only feasible way to test this is to temporally inactivate kinesin-1 after SV40 has reached the cytosol from the ER. We are currently developing tools that will give us the temporal resolution and accuracy to inactivate kinesin-1 post cytosol arrival of the virus.

-Just as easily is SV40 nuclear pore docking affected by adapter depletion?

We agree that this is a good experiment. We are developing assays to directly visualize arrival of SV40 to the nuclear pore, but have not been successful, possibly due to the low level of viral particles that ever reaches the nucleus. However, because we showed that depletion of the adaptors blocked SV40 infection without perturbing arrival of the virus to the cytosol, the adaptors must regulate a step during cytosol-to-nuclear transit of the virus - this is consistent with the hypothesis that the BICD adaptors disassemble SV40 in the cytosol, a step essential for viral entry.

-While it's a nice to demonstrate that major Golgi dispersion impedes cytosolic disruption of SV40, there is no evidence linking this directly to the adaptors other than their mis-localization? Many, many Golgi constituents are likely to be mis-localized using this assay.

This is a fair point. As suggested by Reviewer 1, we have now used an alternative method to mis-localize BICD2 away from the Golgi. Specifically, expression of a dominant-negative Rab6 construct was previously shown to mis-localize BICD2, and we have now confirmed this observation in our hands (Fig 7G). Importantly, expression of this Rab6 mutant markedly blocked SV40 infection (Fig 7I), supporting the view that BICD2 localization to the Golgi is crucial for promoting virus infection.

-In fact, it's a bit surprising to see so little colocalization of VP1/2/3 and BICD2. With 1 copy of BICD2 binding each of 360 VP1 monomers I would suspect to see far more colocalization? Akin to Bap31 for instance?

The simplest explanation is that only a small percentage of virus that enters a host cell reaches the ER, and an equally small fraction of ER-localized virus subsequently penetrates the ER foci to reach the cytosol. This makes it rather difficult to visualize cytosol-localized SV40 with BICD2 using traditional confocal techniques.

The reason that SV40 is more easily seen to co-localize with BAP31 is because the virus concentrates at the ER foci as it prepares for escape into the cytosol – this foci structure is also where BAP31 is selectively recruited.

6D- Need to quantify co-localization to say "strong co-localization". A co-localization coefficient is needed here.

We have changed the wording of this text.

Overall this model begs the question if cytosolic disassembly is mediated by golgi associated adapters why doesn't adaptor depletion affect membrane fraction association in Fig 2B? i.e. why is cytosolic delivery unaffected?

We apologize for this confusion. During entry, SV40 is trafficked from the cell surface to the endosome and targeted directly to the ER lumen, without ever using the Golgi. From the ER lumen, the virus penetrates the ER foci to escape into the cytosol. Upon reaching the cytosol, the virus is disassembled and further mobilized to the nucleus to cause infection. Because the ER foci is proximal to the Golgi where the BICD proteins reside, SV40 that escaped into the cytosol from the ER can be efficiently disassembled by the adaptor proteins. Hence, in this scenario, depletion of the adaptors (which strictly mediates a cytosol-dependent event) is NOT expected to affect cytosol delivery of the virus from the ER.

Reviewer #3 (Comments to the Authors (Required)):

Spriggs et al. report the surprising finding that the dynein cargo adapter molecules BicD2 and BicDR1 directly mediate the disassembly of SV40 virus particles after their trafficking to the nuclear periphery inside of cells. They find that dynein motor activity is

not required for this activity in vitro and suggest that direct binding of the cargo-adapters to the virus could mediate an unknown structural transition. The conclusion of the study is surprising and could be interesting to the broader cell biology community. In general I found the experiments pretty convincing but the manuscript suffers from a lack of any quantification of many of the biochemical experiments and the authors present only example panels of these results. The authors will need to rectify this before I could recommend publication. Additionally the molecular mechanism of BicD2-mediated viral disassembly remains totally obscure, leaving the reader a bit confused at the end of the paper. Without a more concrete insight into the mechanism of action, I am left wondering if the paper is suitable for a broad journal like the JCB or a more specialized journal such as J. Virology or similar.

Specific comments:

1. In Fig. 1, the authors report that siRNA of BicD2 or BicDR1 disrupt SV40 infection. Have the author's considered or checked if knockdown of these proteins disrupts the endogenous MT cytoskeleton organization? It seems to me that disruption of the normal cytoskeletal organization could also lead to the observed effects and the authors should be sure that they are not measuring secondary effects of these knockdowns. Staining and quantification of the MT network organization in these cells should be performed to rule out this possibility.

As requested, we now assessed if knocking down BICD2 or BICDR1 disrupts endogenous MT cytoskeleton organization using beta-tubulin staining, and found that it did not (Fig 2D, with quantification).

2. The western blot in Fig. 3C is a little weird. There is heavy band smearing in the knockdown lanes from the top of the cushion. Is this the best example the authors have? This is a good supportive result, so it's important to establish its robustness. Quantification of replicates should be included for this experiment to show reproducibility of this effect.

As requested, we have quantified these findings to show reproducibility (Fig 3E). In this sucrose cushion assay, the “smearing” effect of the top fraction is often observed and is due to the difference in sucrose concentration in the samples between the top and bottom fractions. Regardless, use of this assay was meant to support the more traditional sucrose gradient centrifugation assay (Fig 3F, with quantification). Importantly, findings from both the sucrose cushion and gradient assays are similar: BICD2 and BICDR1 (but not Rab11-FIP3) play crucial roles in promoting SV40 disassembly during entry.

3. Quantification of the effects in Figs. 3D-E should be included to demonstrate reproducibility.

Fig 3F (formerly 3D in the original submission) is now quantified, as requested.

We have removed the original Fig 3E in this revision. This is because data in the original Fig 3E – which raised the possibility that SV40 that was not disassembled due to the lack of BICD2 or BICDR1 might even be aggregated – is really not central to the point of this manuscript.

4. The results in Fig. 4 are surprising. The authors should show control experiments with each component of this complex mixture added to the virus independently (i.e. Virus + dynein alone, dynactin alone, LIS1 alone, and NDEL alone) in order to validate the claim that the BicD2 molecules are alone sufficient to induce virus disassembly. This is especially true since the disassembly reaction requires a large molar excess of BicD2 protein (1:500) possibly suggesting some non-specific reason for the disassembly (contaminating proteases in the BicD2 prep for instance). Finally the authors should quantify all of the results displayed in panels B-F and indicate how many times these experiments were repeated. At the moment we have only one example blot for each.

As requested, we have now performed the disassembly assay using each complex component individually, and found that neither dynein, dynactin, LIS1, or NDEL alone can disassemble SV40 (Fig S2A); we have also quantified these results (S2A, see quantification). We note that the original Fig 4E is now moved to Fig 3 where the data is more appropriate.

5. In Fig. 5 the authors need to quantify the results shown in panels B and C and indicate how many times these experiments were repeated.

As requested, results in Fig 5B and 5C are now quantified.

6. I think the section describing Fig. 6 is written a little weirdly in the sense that since the BicD2 staining in a ring is observed even without virus infection, it seems odd to suggest that BicD2 "surrounds" or "encloses" the viral focus. Rather, the way I see the data is that the virus is simply trafficked to the center of this BicD2 ring (the Golgi) simply by virtue of it's movement along MTs to the MTOC. The way it's written now seems to imply some kind of active process of surrounding the viral focus, but this is not the case. Unless I misread the section and the authors believe the Golgi is actively remodeled around the viral focus?

We agree that the virus seems to be trafficked to the center of the BICD2 ring and that this is not a virus-induced structure. We have tried to better clarify this in the text.

7. Please quantify the data in Fig. 7B, E & F across replicates.

As requested, the results in Fig 7B, E, and F are now quantified.

8. Why do the authors perform no in vivo experiments with BicDR1? Do they see the similar effects as they observe with manipulation of BicD2 levels in cells?

We performed *in vivo* experiments with BICDR1 in Fig 1-3, as well in Fig S4. Because

we saw similar effects as with BICD2 for these studies, we focused on BICD2, which had a slightly more robust phenotype on SV40 large TAg expression (Fig 1B).

9. I don't understand the hypothetical model of rapid binding/unbinding of CC2 to the virus and how this could mediate viral destabilization. Either explain this model in more detail or I suggest dropping it.

We have removed this model.

10. There are several spelling and grammar mistakes in the manuscript.

We have corrected these mistakes.

We hope the revision satisfies the reviewers concerns. We look forward to your response.

Sincerely,

Billy Tsai
Corydon Ford Collegiate Professor of Cell and Developmental Biology
University of Michigan Medical School

January 15, 2020

Re: JCB manuscript #201908099R

Prof. Billy Tsai
University of Michigan
Cell and Developmental Biology University of Michigan Medical School 109 Zina Pitcher Place, Rm 3043
Ann Arbor, MI 48109

Dear Prof. Tsai,

Thank you for submitting your revised manuscript entitled "Golgi-associated dynein adaptors couple ER membrane penetration and disassembly of a viral cargo". The manuscript has been seen by the original reviewers whose full comments are appended below. While the reviewers continue to be overall positive about the work in terms of its suitability for JCB, some important issues remain.

This revision has significantly strengthened the manuscript. However, Reviewer's #1 and #3 still have some major concerns that should be addressed prior to publication. In particular:
- Perform the triple knockout (BICD1, BICD2, BICDR1) experiment requested by Reviewer #1.
- All comments pertaining to Western blots.
- Reviewer #1's comments about controls (Fig. 1F and Figs. 3 and 4).
- Reviewer #1's comments about the title and the use of the words "strategic" and "robust disassembly".
- Reviewer #3's request to explain how this work fits with your previous studies.
- Discuss the conservation of the region of BICD2 that was identified as being important (Reviewer #3).
- Discuss the redundancy for a role of both BICD2 and BICDR1 (and BICD1).
- Given reviewer's #1 and #3 concerns about lack of a deep mechanistic model, I suggest removing the model figure.

Our general policy is that papers are considered through only one revision cycle; however, given that the suggested changes are relatively minor we are open to one additional short round of revision. Please note that I will expect to make a final decision without additional reviewer input upon resubmission.

Please submit the final revision within one month (or let us know if you require more time), along with a cover letter that includes a point by point response to the remaining reviewer comments.

Thank you for this interesting contribution to Journal of Cell Biology. You can contact me or the scientific editor listed below at the journal office with any questions, cellbio@rockefeller.edu or call (212) 327-8588.

Sincerely,

Samara Reck-Peterson, Ph.D.
Monitoring Editor

Marie Anne O'Donnell, Ph.D.
Scientific Editor

Journal of Cell Biology

Reviewer #1 (Comments to the Authors (Required)):

The authors have largely address my concerns and while a full mechanistic understanding is still lacking the MS is improved.

I only have a few points for clarification and missing controls.

I feel the title is misleading as it makes it sound like any dynein adaptor can do the job when it is really BICD1/2 or BICDR1. I think this should be made clear in the title. I also do not understand what the authors mean by strategically and think this should be removed.

Fig. 1. Does loss of BICD1 and BICD2 together with BICDR1 lead to a complete block in infection?

It is also quite surprising that knockdown of any one of these dynein adaptors leads to the same level of inhibition even in the presence of the remaining adaptors. This makes me wonder how sensitive is this assay for infection? This redundancy should be discussed. Also the fact that BICDR2 does not seem to inhibit is also interesting, could its partial effect be masked by the other adpators as it is very similar to BICDR1 so this is a surprising result. I guess I'm say that the authors really should do all the combinations of these adpators for completeness. It might also make writing the title easier.

Fig. 1F the quantification is missing for human BK PyV. I ask, as the level of knockdown is very good on the western so it would be nice to see what the read out is in Large Tag expression.

Fig. 3/Fig. 4 The authors show purified BICD2 and BICDR1 with native SV40 treated with DTT and EGTA. However, we are never shown the level of pull down with non-treated native SV40 (the negative control). This must be added.

It would be nice if the blot in Figure 3E was actually a full gradient.

Fig 4 and other gradient westerns images. The authors follow where VP1 goes in their gradients. However, we are never shown where BICD2 and other factors go in these gradients. I think this is very important information that needs to be added at least for some of the key opening experiments.

The vast excess of BiCD2 to virus required to disassemble SV40 is worrying and may not be physiologically relevant. I therefore wonder, if the amount of BicD2 required to promote virus disassembly is lower in the presence of MTs and Dynein as this would better reflect the situation in cells?

Page 11 the authors state in last line "robust virus disassembly" I think this is too strong as their gradients clearly show that the majority of virus are still intact so it appears to be a minor fraction

that disassembles. The methods also give no information on how westerns were developed, film, LiCor etc? This is important as how were the westerns quantified as film is not linear, which might explain why some of the quantification on disassembly does not look like the blots. Clarification needed.

Likewise, the EM images and quantification do not fit well with the gradients and their quantification as it looks like there is much more disassembly in EM based assays than the gradients imply. Could this be down to the way the immunoblots are quantified?

Figure 5. The authors do not provide expts with CC1 alone. This should be provided.

Reviewer #2 (Comments to the Authors (Required)):

Having been through the revised version of this manuscript and the reviewers responses to all authors, I was very impressed with the revision and the level to which the authors met the reviewers experimental and intellectual requests.

I have no further suggestions or recommendations.

Reviewer #3 (Comments to the Authors (Required)):

My initial assessment of this manuscript resulted in two main concerns; the lack of quantification for many of the biochemical assays (precluding publication in any journal in my opinion), and the lack of any mechanistic insight into how dynein cargo adapters could lead to virus disassembly. The authors now provide the requested quantification, alleviating my first concern. However, I am still not convinced there is any real insight into the molecular mechanism of how the dynein cargo adapters could mediate such an effect. The authors provide domain mapping to narrow down which region of BicD2 is necessary for the observed effect, but this doesn't provide much insight into what is actually going on or how this specific domain is mediating the observed effect. How conserved is this region in the BicD family? Are there specific amino acids/motifs that are mediating the reported effects? They also provide new data mapping the specific type of viral protein that BicD2 interacts with, but again, this doesn't provide much insight into how BicD2 mediates the observed effects.

I am also concerned about how the current data fits with their previous data on dynein's role in viral disassembly. In their former manuscript, they use a small molecule inhibitor of dynein's enzymatic activity and overexpression of dynein subunits to disrupt viral disassembly. Here they claim dynein has no role, which leaves this reader very confused and feeling like there has been a sleight of hand.

Overall, I find the results somewhat intriguing but I am still left feeling confused at the end. The level of mechanistic insight here doesn't rise to the typical level of a JCB paper in my opinion.

Minor comment:

1. White line in Fig. 3D lower panel needs explanation.

Cell and
Developmental
Biology
109 Zina Pitcher
Place
Room 3043
Ann Arbor, MI
48109

February 12th, 2020

Dear Editor,

Thank you for the opportunity to respond to the reviewer's remaining concerns. As requested, we have now addressed the specific 8 points outlined in your previous email:

1. Perform the triple knockout (BICD1, BICD2, BICDR1) experiment requested by Reviewer #1. We performed the requested triple knockdown experiment. See below for clarification.

2. All comments pertaining to Western blots. We have addressed any remaining issues regarding the Western blots.

3. Reviewer #1's comments about controls (Fig. 1F and Figs. 3 and 4). The comments about the controls have now been addressed.

4. Reviewer #1's comments about the title and the use of the words "strategic" and "robust disassembly". We have removed the words “strategic” and “robust” in the text.

5. Reviewer #3's request to explain how this work fits with your previous studies. In fact, we previously discussed (see Discussion pg. 26) how our new data fits with our original publication.

6. Discuss the conservation of the region of BICD2 that was identified as being important (Reviewer #3). We now address the conservation of CC2-3 between the different BICD proteins as it relates to SV40 infection in the Discussion on pg. 25.

7. Discuss the redundancy for a role of both BICD2 and BICDR1 (and BICD1). We now

discuss the redundancy issue in the Discussion, and why BICDR2 may not affect infection (pg. 25).

8. Given reviewer's #1 and #3 concerns about lack of a deep mechanistic model, I suggest removing the model figure. We have removed the model figure as requested. Below we provide our point-by-point response to all the points raised by Reviewers #1 and #2.

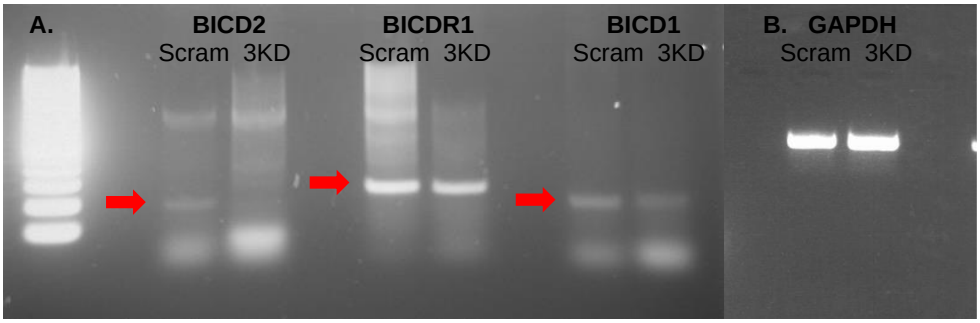
Reviewer #1 (Comments to the Authors (Required)):

The authors have largely address my concerns and while a full mechanistic understanding is still lacking the MS is improved.

I only have a few points for clarification and missing controls.

I feel the title is misleading as it makes it sound like any dynein adaptor can do the job when it is really BICD1/2 or BICDR1. I think this should be made clear in the title. I also do not understand what the authors mean by strategically and think this should be removed. We have changed our title.

Fig. 1. Does loss of BICD1 and BICD2 together with BICDR1 lead to a complete block in infection? We performed the requested triple knockdown experiment. However, using the same siRNA concentration as the double knockdown experiment (5 nM), we were not able to deplete the level of all three BICD proteins to the same level as in the single or double knockdown condition. This is likely due to cells not taking up sufficient amounts of all three siRNAs. To attempt to rectify this, we also tried using 50 nM of each siRNA, but again failed to achieve sufficient knockdown of two of the three BICD proteins (see below) and therefore cannot accurately determine the consequence of simultaneously depleting all 3 BICD proteins during SV40 infection. We now indicate this in the manuscript.



A. RT-PCR of BICD2, BICDR1 and BICD1 transcript levels in triple knockdown (3KD) cells (50 nM). B. GAPDH loading control from control and triple knockdown (3KD) cells.

It is also quite surprising that knockdown of any one of these dynein adaptors leads to the same level of inhibition even in the presence of the remaining adaptors. This makes me wonder how sensitive is this assay for infection? This redundancy should be discussed. Also the fact that BICDR2 does not seem to inhibit is also interesting, could its partial effect be masked by the other adpators as it is very similar to BICDR1 so this is a surprising result. I guess I'm say that the authors really should do all the combinations of these adpators for completeness. It might also make writing the title easier. We now discuss the redundancy issue and why BICDR2 may not affect infection on pg. 25 of the manuscript.

Fig. 1F the quantification is missing for human BK PyV. I ask, as the level of knockdown is very good on the western so it would be nice to see what the read out is in Large Tag expression. We now show the requested quantification data in 1F.

Fig. 3/Fig. 4 The authors show purified BICD2 and BICDR1 with native SV40 treated with DTT and EGTA. However, we are never shown the level of pull down with non-treated native SV40 (the negative control). This must be added. We now show the requested data in Figure S2D.

It would be nice if the blot in Figure 3E was actually a full gradient. Figure 3E is a sucrose cushion experiment in which all of the samples are partitioned into only 2 fractions (top and bottom). Therefore, the data that are presented represent the full gradient.

Fig 4 and other gradient westerns images. The authors follow where VP1 goes in their gradients. However, we are never shown where BICD2 and other factors go in these gradients. I think this is very important information that needs to be added at least for some of the key opening experiments. As requested, we now show BICD2 and BICDR1 in the gradient in Figure 4D and Figure S2B.

The vast excess of BiCD2 to virus required to disassemble SV40 is worrying and may not be physiologically relevant. I therefore wonder, if the amount of BicD2 required to promote virus disassembly is lower in the presence of MTs and Dynein as this would better reflect the situation in cells? We show that virus disassembly is less robust in the presence of dynein and MTs in Figure 4C, which suggests that a higher amount of BICD2 would be required to mimic the disassembly phenotype shown in Figure 4D under these conditions.

Page 11 the authors state in last line "robust virus disassembly" I think this is too strong as their gradients clearly show that the majority of virus are still intact so it appears to be a minor fraction that disassembles. The methods also give no information on how westerns were developed, film, LiCor etc? This is important as how were the westerns quantified as film is not linear, which might explain why some of the quantification on disassembly does not look like the blots. Clarification needed. We removed the word “robust” when describing disassembly and clarified western quantification in the Materials and Methods section.

Likewise, the EM images and quantification do not fit well with the gradients and their quantification as it looks like there is much more disassembly in EM based assays than the gradients imply. Could this be down to the way the immunoblots are quantified? This is likely due to the sensitivity of the two entirely different assays. It is possible that there is more disassembly in our *in vitro* reconstitution assays (similar to as seen with EM), but this is below the level of detection with film.

Figure 5. The authors do not provide expts with CC1 alone. This should be provided. We have now added the CC1 data to Figure 5B.

Reviewer #2 (Comments to the Authors (Required)):

Having been through the revised version of this manuscript and the reviewers responses to all authors, I was very impressed with the revision and the level to which the authors met the reviewers experimental and intellectual requests.

I have no further suggestions or recommendations.

Reviewer #3 (Comments to the Authors (Required)):

My initial assessment of this manuscript resulted in two main concerns; the lack of quantification for many of the biochemical assays (precluding publication in any journal in my opinion), and the lack of any mechanistic insight into how dynein cargo adapters could lead to virus disassembly. The authors now provide the requested quantification, alleviating my first concern. However, I am still not convinced there is any real insight into the molecular mechanism of how the dynein cargo adapters could mediate such an effect. The authors provide domain mapping to narrow down which region of BicD2 is necessary for the observed effect, but this doesn't provide much insight into what is actually going on or how this specific domain is mediating the observed effect. How conserved is this region in the BicD family? Are there specific amino acids/motifs that are mediating the reported effects? They also provide new data mapping the specific type of viral protein that BicD2 interacts with, but again, this doesn't provide much insight into how BicD2 mediates the observed effects. We now address the conservation of CC2-3 between the different BICD proteins in the Discussion on pg 25.

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Overall, I find the results somewhat intriguing but I am still left feeling confused at the end. The level of mechanistic insight here doesn't rise to the typical level of a JCB paper in my opinion.

Minor comment:

1. White line in Fig. 3D lower panel needs explanation. This was included in our Figure 3 legend.

We hope the revision satisfies the reviewers concerns. We look forward to your response.

Sincerely,

Billy Tsai
Corydon Ford Collegiate Professor of Cell and Developmental Biology
University of Michigan Medical School