

# Boosting rAAV Yield and Quality in HEK293 Cells Through Multi-Mechanism Strategies

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**AFFILIATIONS:** Solid Biosciences Inc., Charlestown, MA, USA

**SESSION:** AAV transfection improvements: Scaling and titers



# Disclosures

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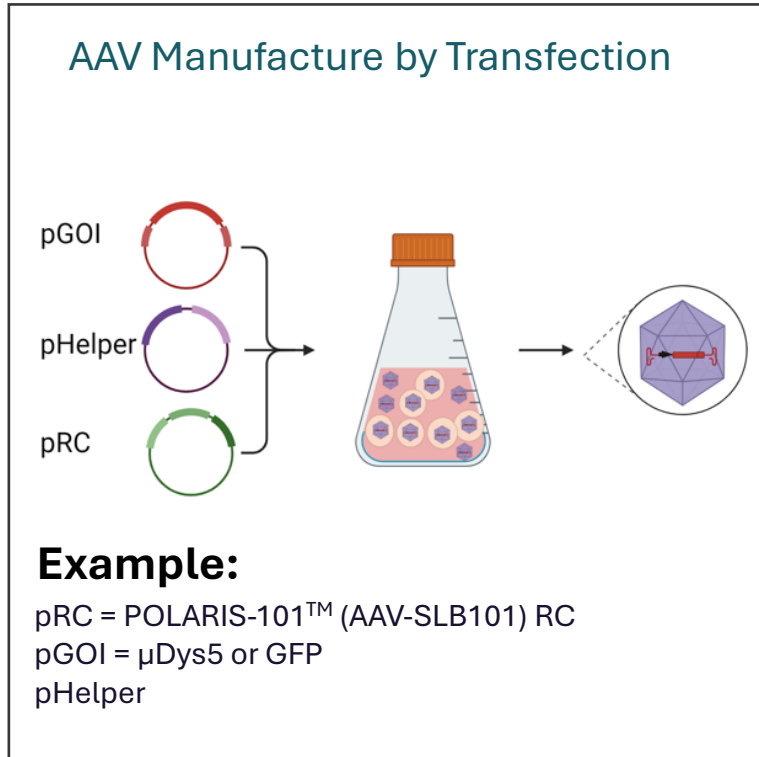
- I am a full-time employee at Solid Biosciences

# Forward Looking Statements

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This presentation contains “forward-looking statements” within the meaning of the Private Securities Litigation Reform Act of 1995, including statements regarding future expectations, plans and prospects for the company; the ability to successfully achieve and execute on the company’s goals; anticipated benefits of and strategies and expectations for the company’s SGT-003 program; expectations for planned enrollment of its SGT-003 program; planned regulatory interactions and the potential approval pathways for SGT-003; and other statements containing the words “anticipate,” “believe,” “continue,” “could,” “estimate,” “expect,” “intend,” “may,” “plan,” “potential,” “predict,” “project,” “should,” “target,” “would,” “working” and similar expressions. Any forward-looking statements are based on management’s current expectations of future events and are subject to a number of risks and uncertainties that could cause actual results to differ materially and adversely from those set forth in, or implied by, such forward-looking statements. These risks and uncertainties include, but are not limited to, risks associated with the company’s ability to advance its SGT-003 and other preclinical programs, capsid libraries and other enabling technologies on the timelines expected or at all; obtain and maintain necessary approvals from the FDA and other regulatory authorities; replicate in clinical trials positive results found in preclinical studies and early-stage clinical trials of the company’s product candidates; manufacture sufficient quantities of our drug product in a timely manner and maintain adequate supply to support our clinical development and potential commercialization; obtain, maintain or protect intellectual property rights related to its product candidates; replicate preliminary or interim data from clinical trials in the final data of such trials; compete successfully with other companies that are seeking to develop Duchenne and other neuromuscular and cardiac treatments and gene therapies; manage expenses; and raise the substantial additional capital needed, on the timeline necessary, to continue development of its SGT-003 programs; and achieve its other business objectives and continue as a going concern. For a discussion of other risks and uncertainties, and other important factors, any of which could cause the company’s actual results to differ from those contained in the forward-looking statements, see the “Risk Factors” section, as well as discussions of potential risks, uncertainties and other important factors, in the company’s most recent filings with the Securities and Exchange Commission. In addition, the forward-looking statements included in this presentation represent the company’s views as of the date hereof and should not be relied upon as representing the company’s views as of any date subsequent to the date hereof. The company anticipates that subsequent events and developments will cause the company’s views to change. However, while the company may elect to update these forward-looking statements at some point in the future, the company specifically disclaims any obligation to do so.

# During AAV Production a Significant Proportion of AAV is Extracellular



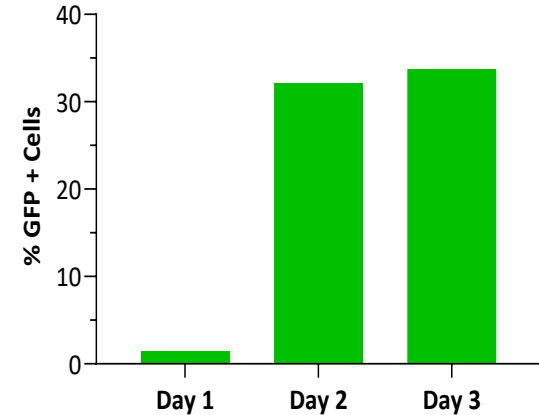
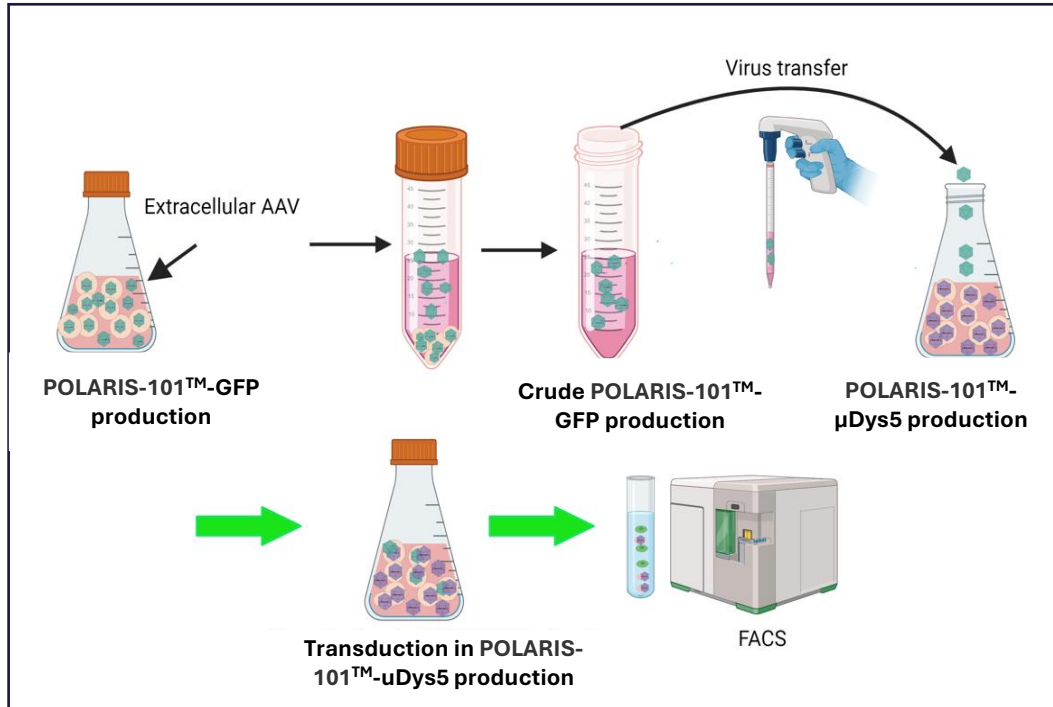
Extracellular  
AAV

Extracellular virus  
comprises 20-60% of  
total AAV

\*Created using Biorender.com

# Extracellular AAV can transduce HEK293 cells during production

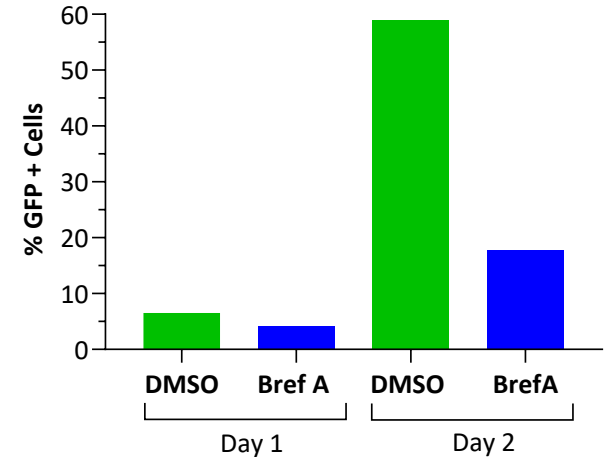
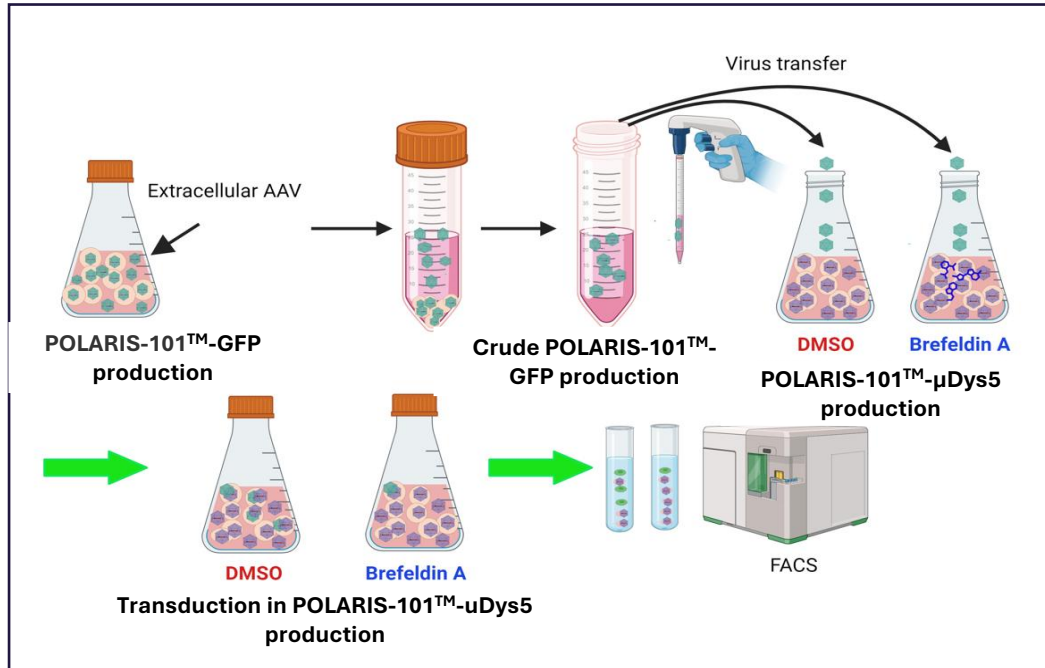
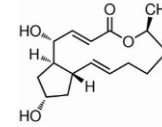
Does added extracellular POLARIS-101™-GFP virus transduce HEK293 cells actively producing POLARIS-101™-μDys5?



Transferred POLARIS-101™-GFP virus transduces HEK293 cells actively producing POLARIS-101™-μDys5

# Brefeldin A Reduced POLARIS-101™-GFP Transduction of HEK293 cells Actively Producing AAV

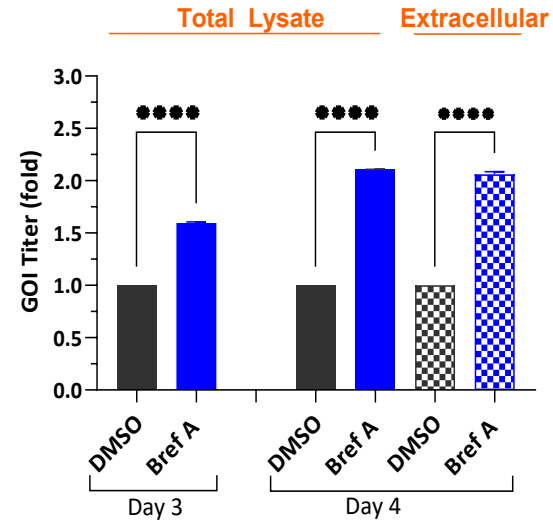
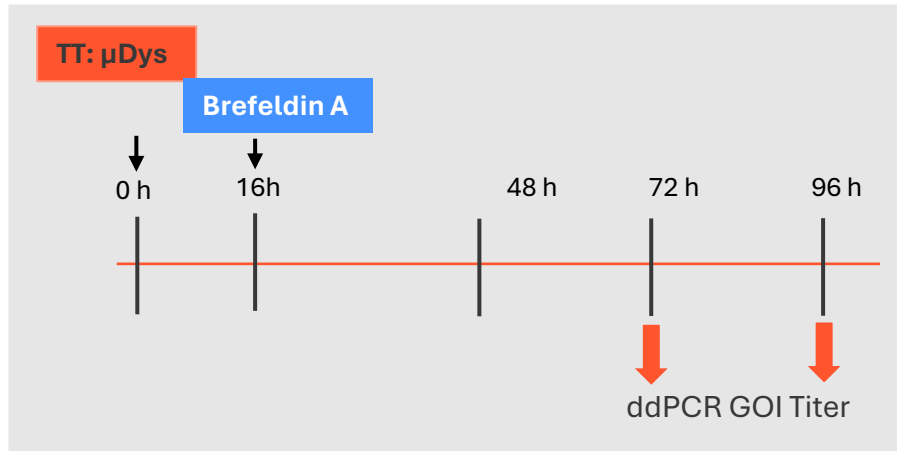
## Brefeldin A is an Inhibitor of Endocytosis



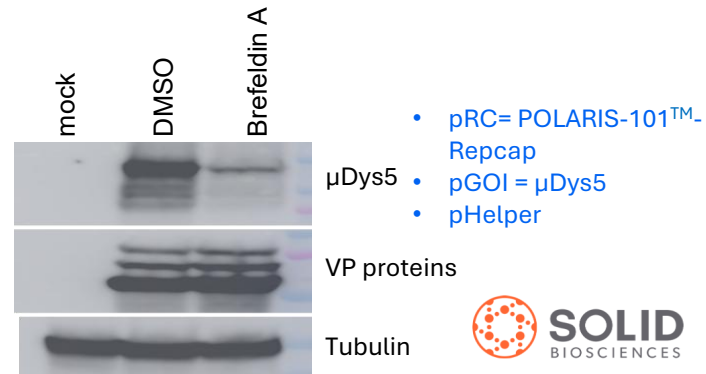
Brefeldin A (Bref A) significantly reduced POLARIS-101™-GFP transduction of HEK293 cells during AAV production

# Addition of Brefeldin A Increased AAV Yield in HEK293 Cells

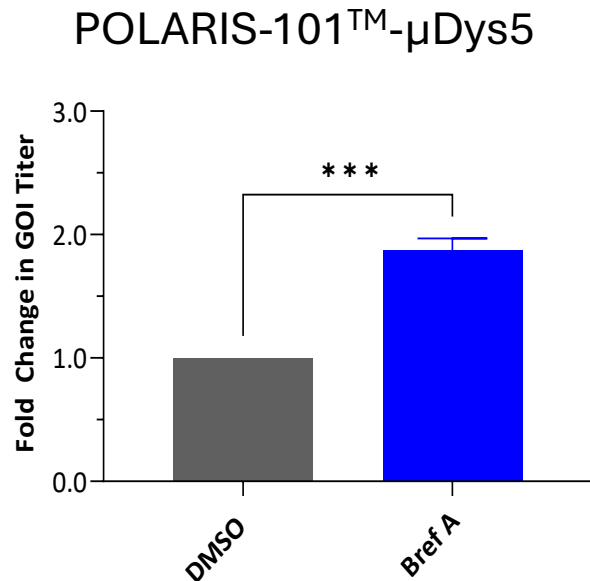
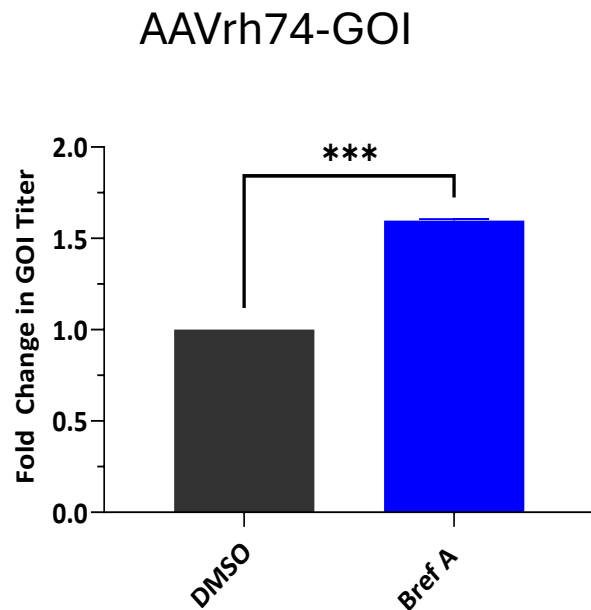
Scale: 50 mL Shake Flask



- Brefeldin A significantly increased AAV yield ~ 2-fold
- Addition of Brefeldin A reduced  $\mu$ Dys expression, i.e. self-transduction of manufacturing HEK293 cells by POLARIS-101<sup>TM</sup>- $\mu$ Dys5



# Brefeldin A Increased AAV Yield Across Capsid Serotypes & Transgenes



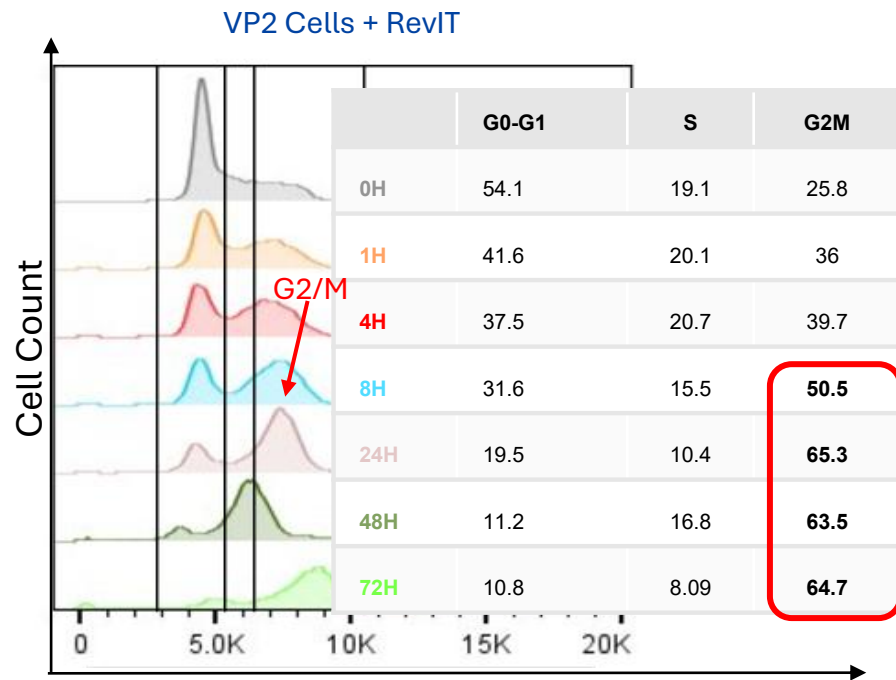
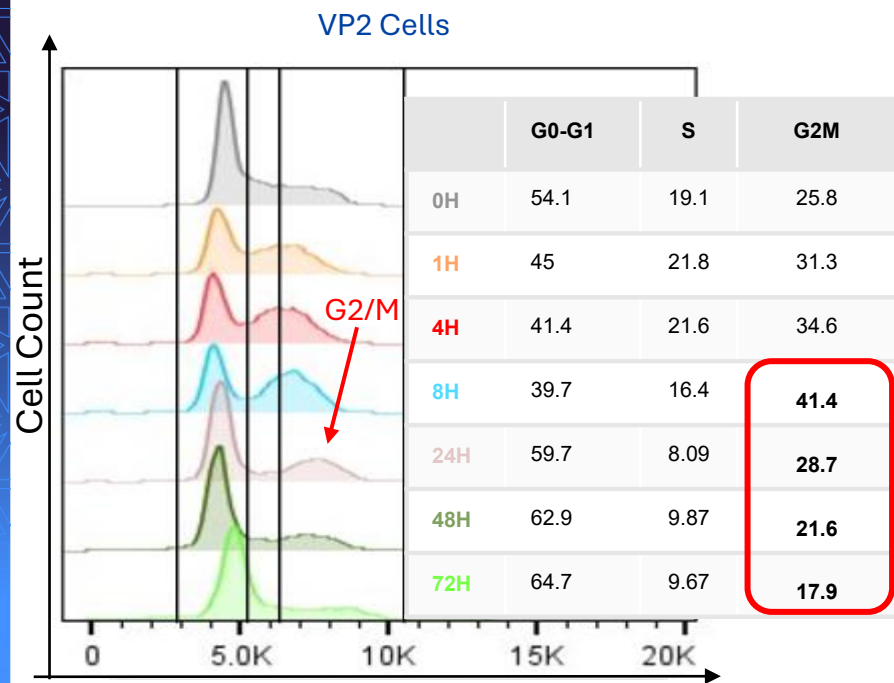
- Addition of Brefeldin A increased AAV yield for rh74 and AAV9-based POLARIS-101<sup>TM</sup>

# RevIT

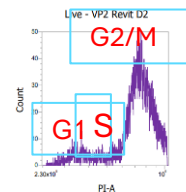


- RevIT™: AAV Enhancer, commercially available from Mirus: SKU: MIR 8000
- Delivers higher titers for rAAV production.
- Little is known about Revit in combination with other potential transfection enhancers.

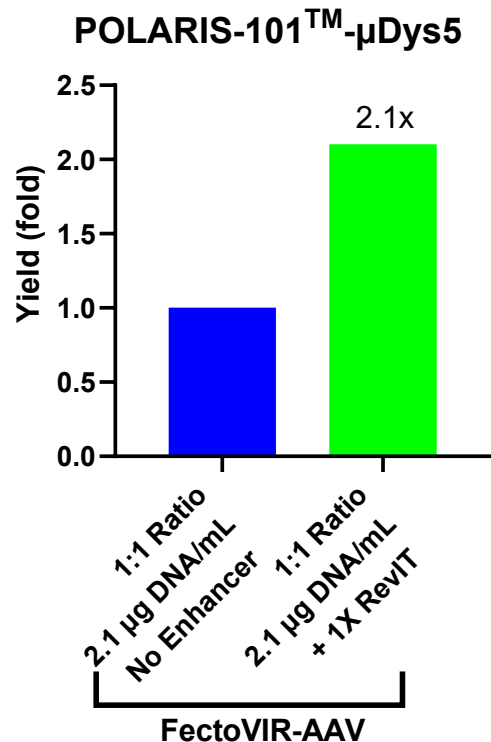
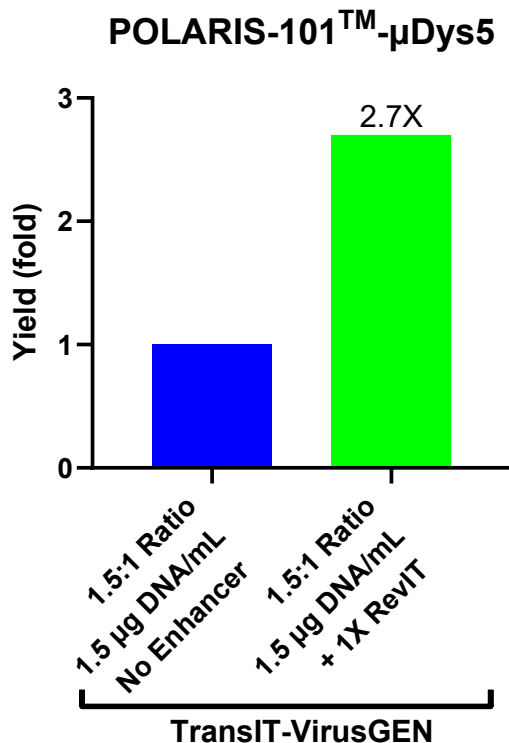
# RevIT Arrests the Cell Cycle in G2/M phase



- More cells are in the G2/M phase after 8, 24, 48, and 72 hours with Revit
- This suggests that RevIT functions by arresting the cell cycle at the G2/M phase.

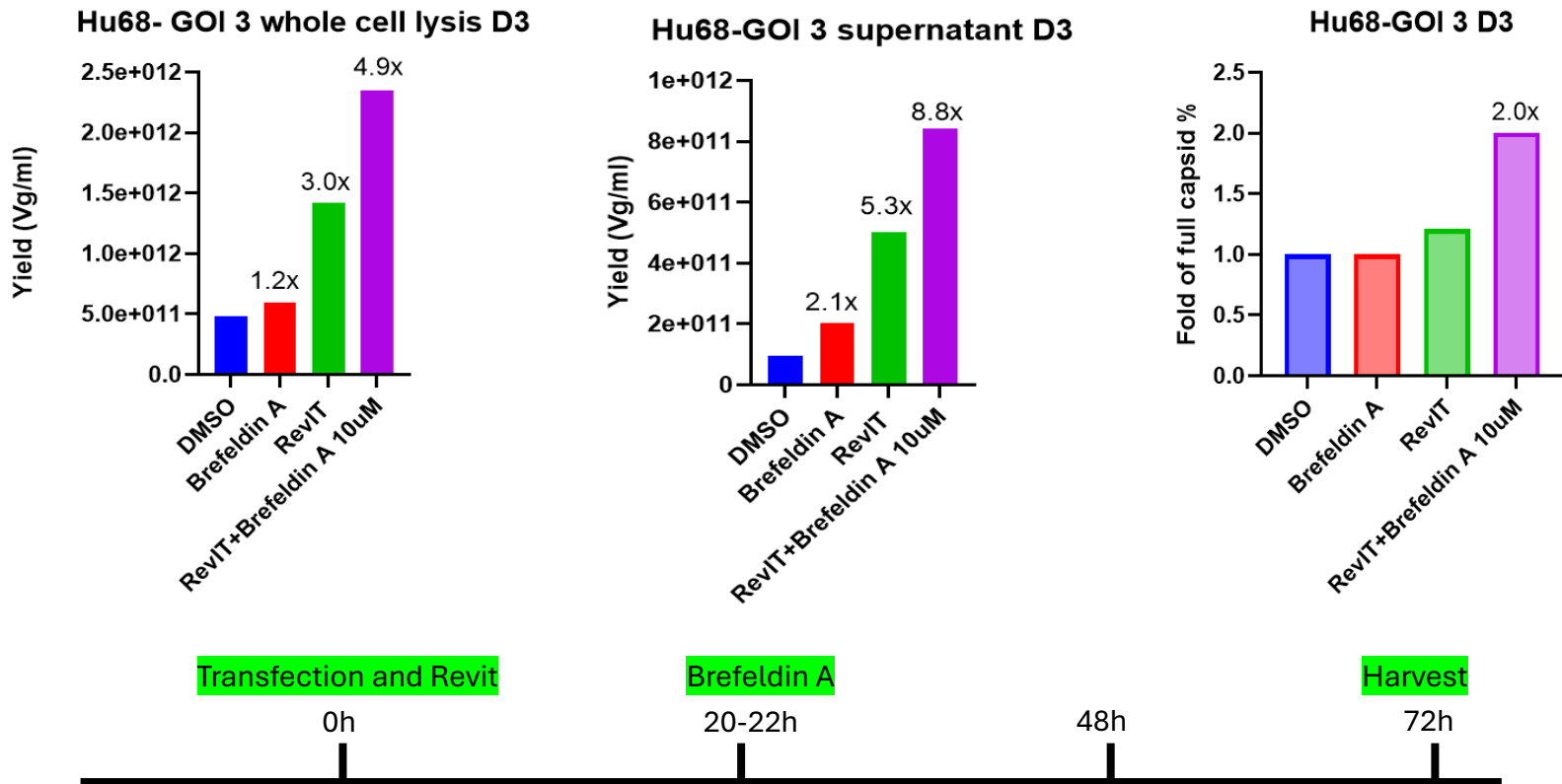


# Impact of RevIT treatment on AAV Production

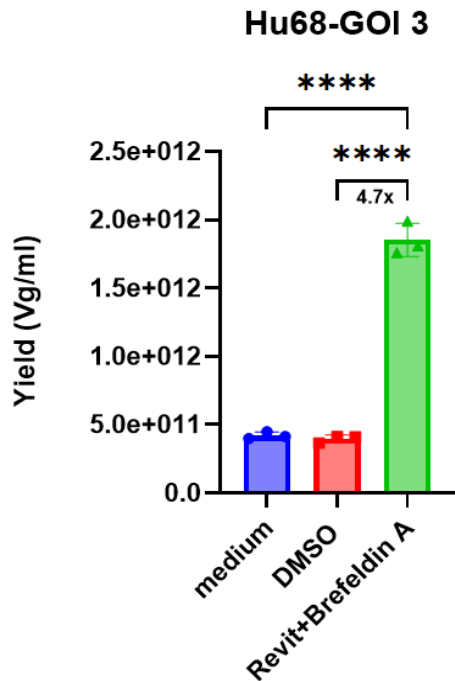


- Representative experiment, RevIT (Mirus) can increase AAV yield 2-3 fold in 30ml shake flasks.

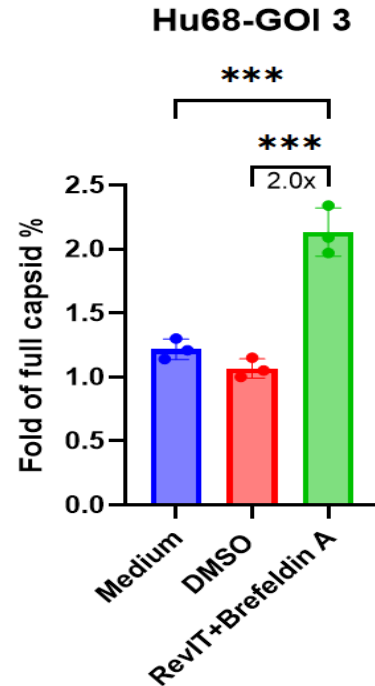
# Revit and Brefeldin A together is better than single Revit or Brefeldin A: increase Hu68-GOI 3 yield 5-fold and full capsid 2-fold



# Further confirmation: Revit and Brefeldin A together increase Hu68-GOI 3 yield 5-fold and full capsid percentage 2-fold

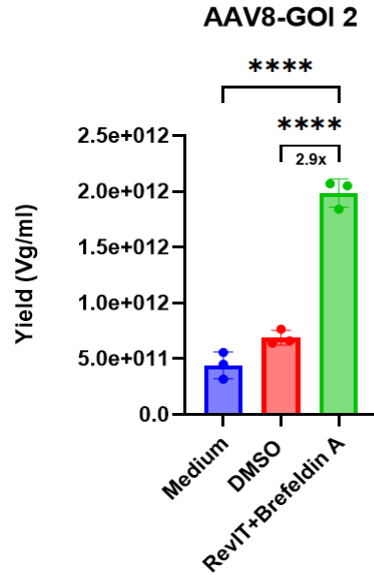


Brown-Forsythe and Welch ANOVA  $P < 0.0001$ ;  
N=3; 30ml scale

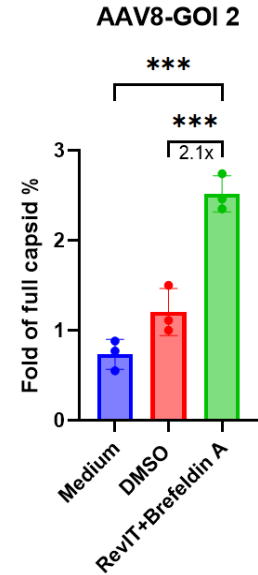


Brow-Forsythe and Welch ANOVA  $P = 0.0001$ ;  
N=3, 30ml scale

# Revit and Brefeldin A together increase AAV8-GOI 2 yield 3-fold and Full capsid 2-fold



Brown-Forsythe and Welch ANOVA  $P < 0.0001$ ;  
N=3



Brown-Forsythe and Welch ANOVA tests  
\*\*\* $p < 0.001$ ; N=3; 30ml scale

Transfection and Revit

0h

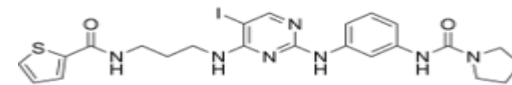
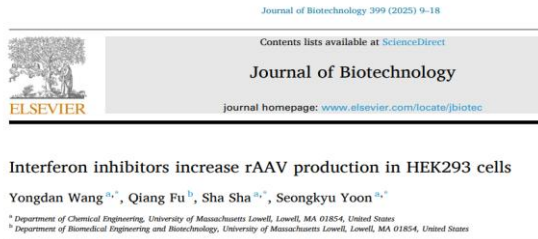
Brefeldin A

20-22h

48h

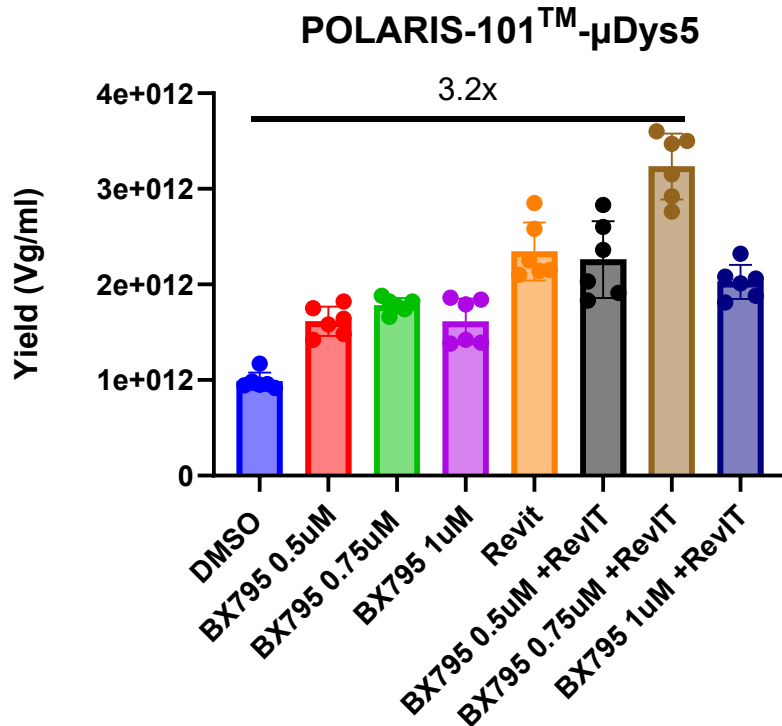
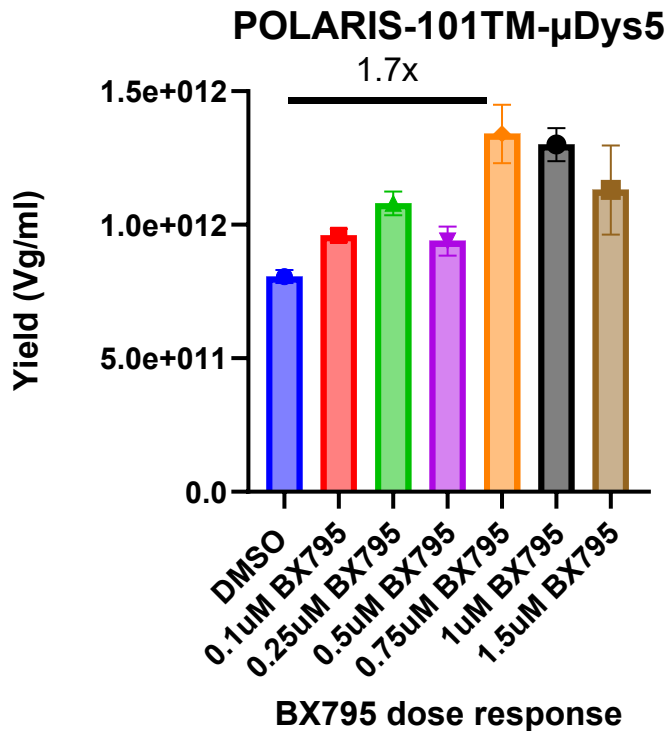
Harvest

72h



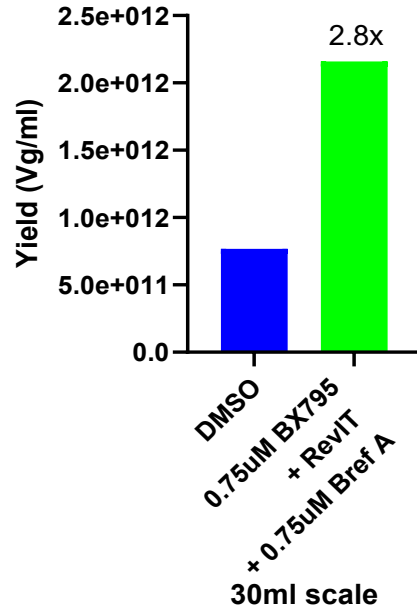
- BX795 is an interferon signaling pathway (IFN) inhibitor, specifically TBK1/Ikkε inhibitor
- IFNs play a crucial role in innate immune response against viral infections
- By inhibiting the pathway, it reduces the response activated during transfection
- A dose response was conducted to determine the optimal dose to improve yield
- BX795 was added 4 hours prior to transfection according to literature and manufacturers recommendation
- BX795 is from MedChemExpress

# BX795+RevIT increase AAV yield 3.2 fold

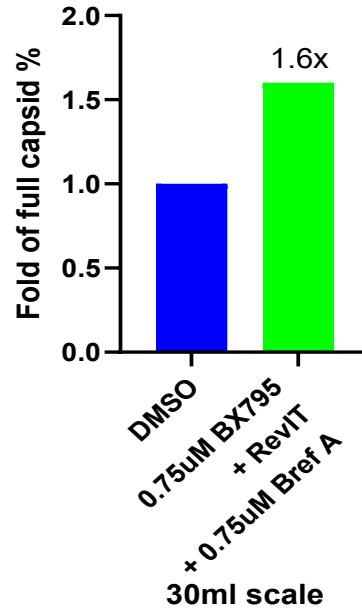


# BX795, RevIT and Brefeldin A together increase POLARIS-101™-μDys5 yield 3-fold and full capsid 1.6-fold

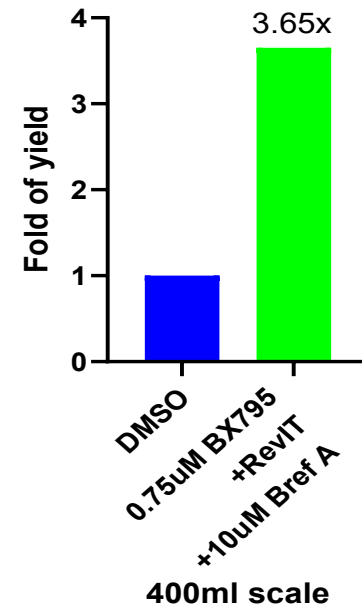
## POLARIS-101™-μDys5



## POLARIS-101™-μDys5



## POLARIS-101™-μDys5



BX795

Transfection and RevIT

Brefeldin A

Harvest

-4h

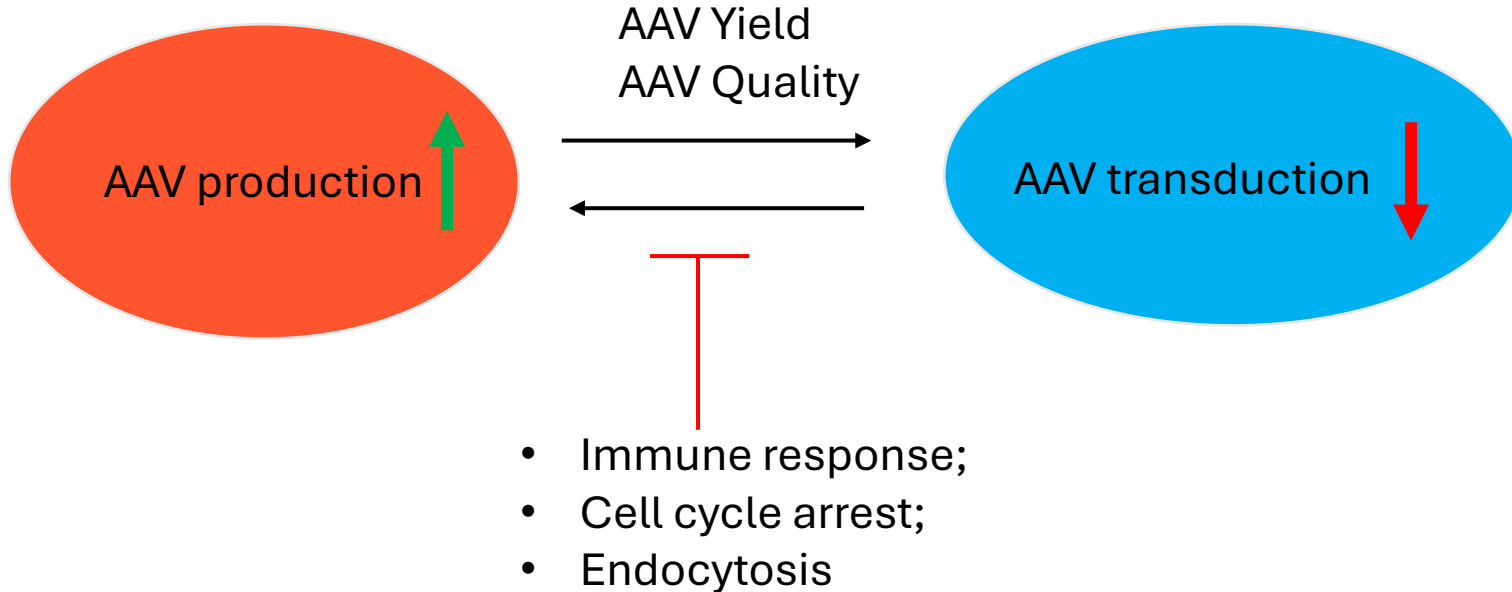
0h

20-22h

48h

72h

## Summary: Mechanisms of 3 sms on AAV Production and AAV quality



## Summary & Conclusions

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- AAV excreted into the supernatant from HEK293 cells can re-transduce cells within the same culture.
- Transduction also reduces rAAV yield.
- Small molecule inhibitors of endocytosis (Brefeldin A ) reduce this transduction and increase AAV yield 1.5~2 fold.
- RevIT can arrest cell cycle in G2/M phase and increase AAV yield ~2 fold
- BX795, an interferon signaling pathway (IFN) inhibitor can increase AAV yield 1.7 fold.
- Combination of RevIT with Bx795 can increase AAV yield 3.2 fold.
- Combination of RevIT with Brefeldin A can increase AAV yield 3~5 fold and AAV full capsid 2 fold in multiple capsids and GOI.
- BX795, Revit and Brefeldin A together increase POLARIS-101™-μDys5 yield 3-fold and full-capsid 1.6-fold.

# Acknowledgments

- Glen B Banks
- Paul Herzich
- Ben Wright
- Patrick Starremans
- Neil Templeton
- MTX team
- AD team
- PD team
- PPC team



# Thank you!

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Poster 3146: Small Molecule Induced G2/M Arrest Boosts Recombinant AAV Production and Preserves high Quality

Please contact BD in Solid Biosciences

Nolan Skop Email: [nolan.skop@solidbio.com](mailto:nolan.skop@solidbio.com)

-POLARIS-101™ vector

-Dual Rep/cap-GOI plasmids

