Novel Mechanism to Increase AAV Yield through Blocking AAV Transduction of Manufacturing HEK293 Cells During AAV Production

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Solid Biosciences

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Forward Looking Statements

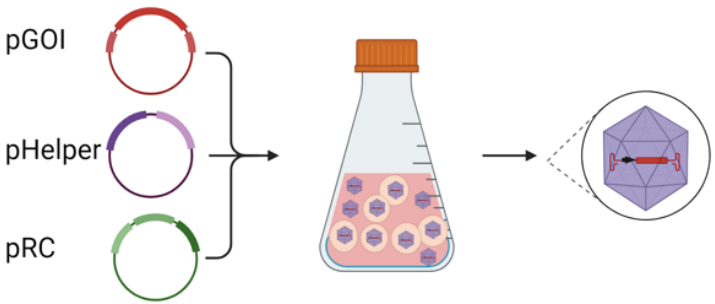
This presentation contains "forward-looking statements" within the meaning of the Private Securities Litigation Reform Act of 1995, including statements regarding future expectations, plans and prospects for the company; the ability to successfully achieve and execute on the company's priorities and achieve key clinical milestones; the company's neuromuscular and cardiac programs, including expectations for initiating a clinical study for its next-generation Duchenne muscular dystrophy program, reading out data from the study and the ability to translate pre-clinical study results in human studies; the company's future development of its CPVT program, including IND and/or CTA filings and health authority clearance, initiating clinical study and reading out data therefrom and the ability to obtain regulatory designations; the company's preclinical programs, including expectations for filing INDs, process development activities, and the company's future development of preclinical and capsid programs; 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 ability to recognize the anticipated benefits of Solid's acquisition of AavantiBio; the company's ability to advance SGT-003, AVB-202-TT, AVB-401 and other preclinical programs and capsid libraries 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 of the company's product candidates; obtain, maintain or protect intellectual property rights related to its product candidates; 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 SGT-003, AVB-202-TT, AVB-401 and other candidates, 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.

Disclosures

I am a full-time employee of Solid Biosciences

During AAV Production a Significant Proportion of AAV is Extracellular

AAV Manufacture by Transfection

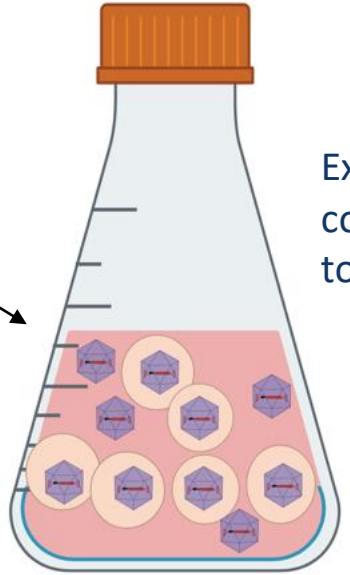


Example:

pRC = AAV-SLB101
pGOI = µDys5 or GFP
pHelper

**Created using Biorender.com*

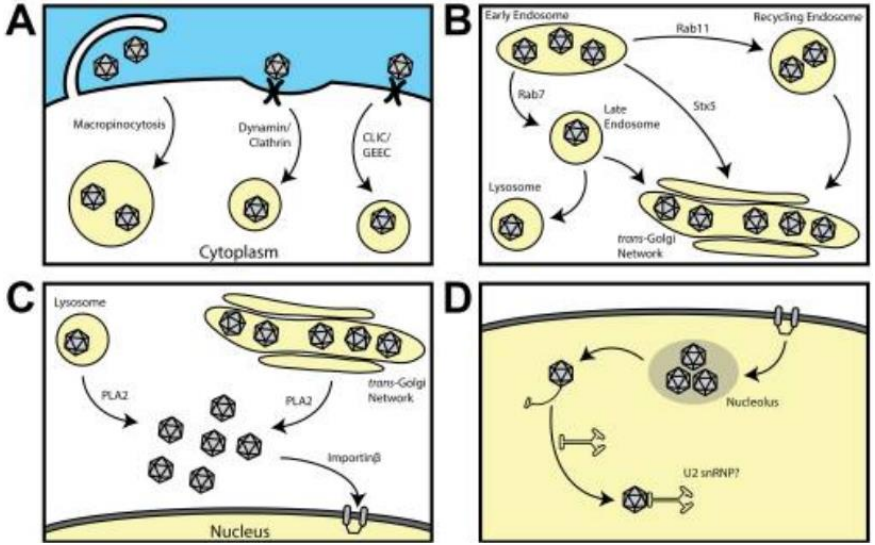
Extracellular AAV



Extracellular virus comprises 20-60% of total AAV

Transgene Protein is Expressed in HEK293 Cells During Manufacture

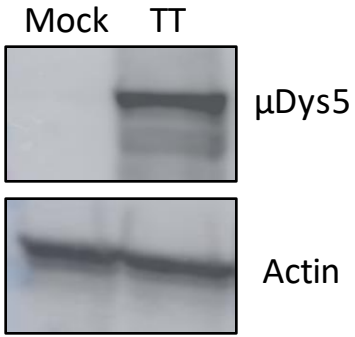
AAV Transduction of Cells



Berry & Asokan Curr Opin Virol. 2016 December; 21: 54–60

1. Multiple mechanisms of AAV endocytosis
2. Endosome trafficking
3. Nuclear uptake & transcription of transgene

Example:

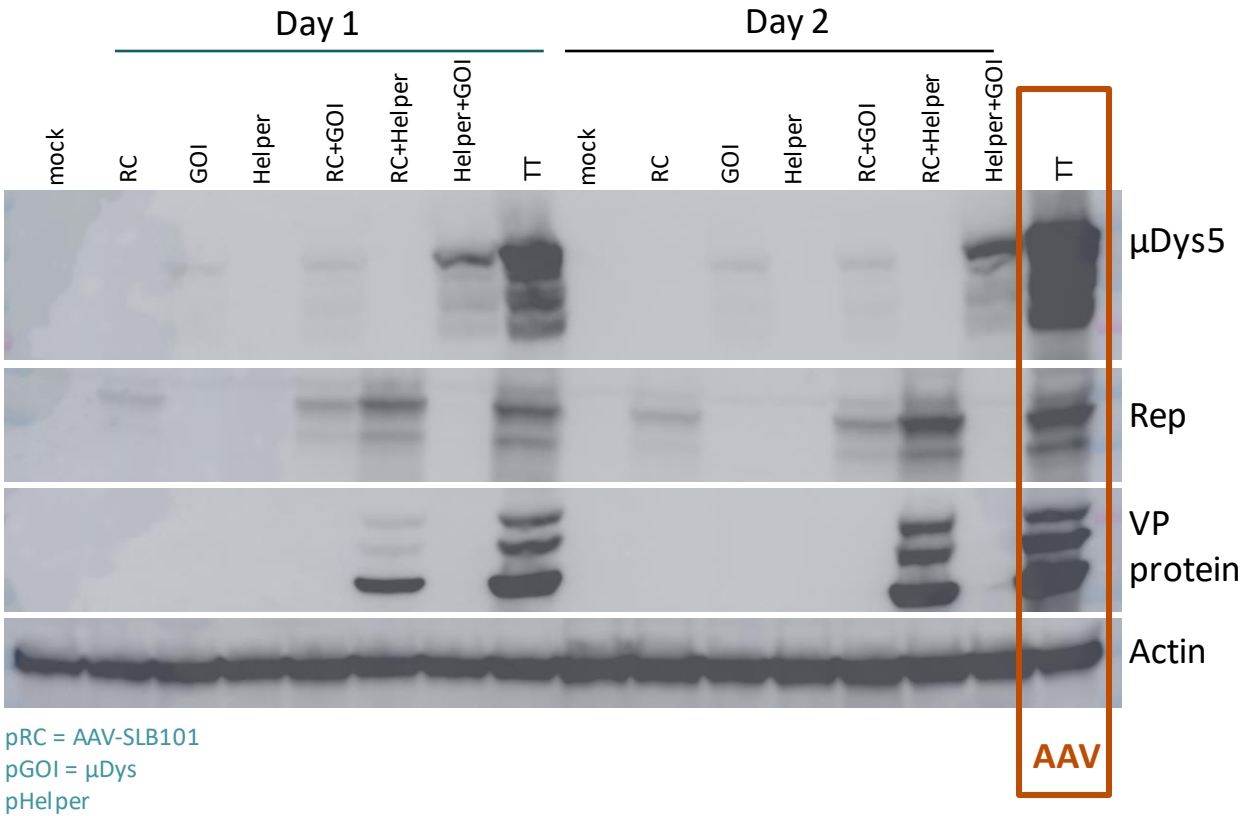


µDys5 protein expression in HEK293 lysate at 3 days post-transfection

pRC= AAV-SLB101
pGOI = µDys5
pHelper

Significant Transgene (µDys5) Expression in HEK293 Cells Due to AAV Transduction

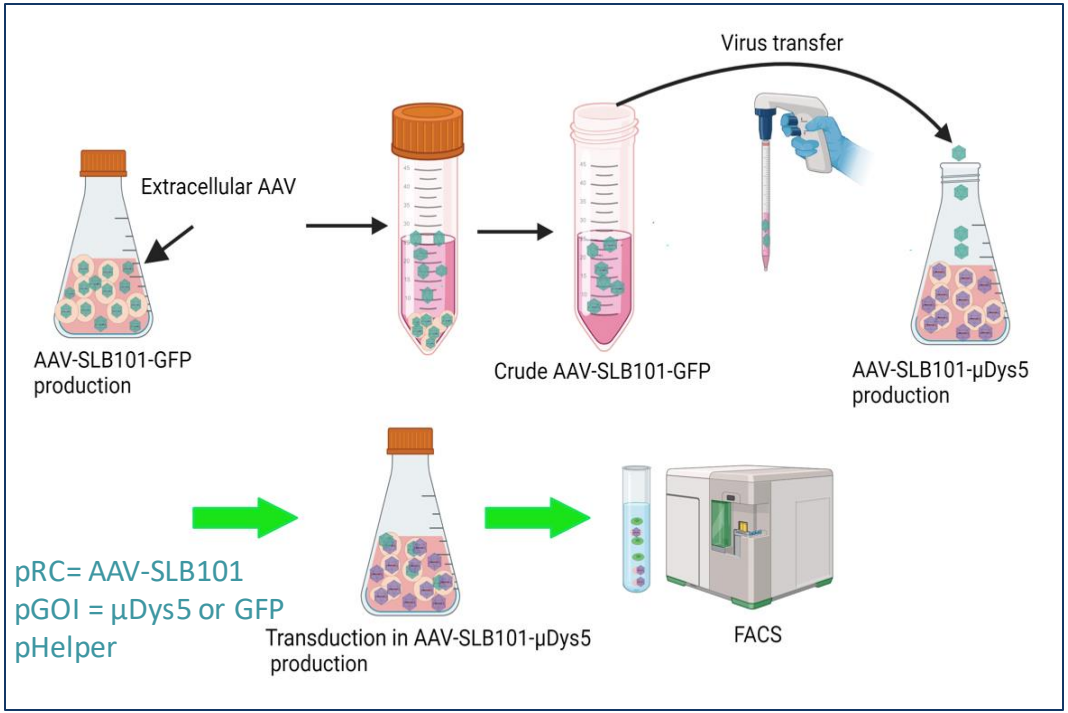
- Control studies with 1 or 2 plasmid transfections results in no or low µDys expression
- Transfection with 3 plasmids (TT) produces AAV and high µDys5 expression in HEK293 cells



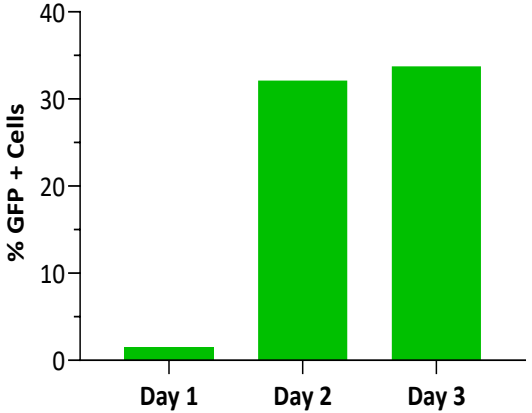
pRC = AAV-SLB101
pGOI = µDys
pHelper

Virus Transfer Study Demonstrates Transduction of HEK293 Cells During AAV Production

Does added extracellular AAV-SLB101-GFP virus transduce HEK293 cells actively producing AAV-SLB101-µDys5?



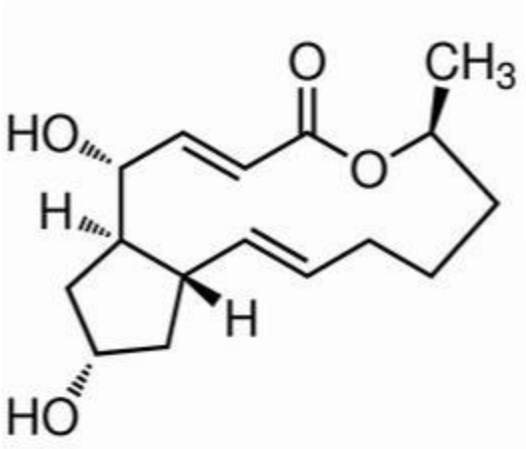
*Created using Biorender.com



Transferred AAV-SLB101-GFP virus transduces HEK293 cells which express GFP while actively producing AAV-SLB101-µDys5

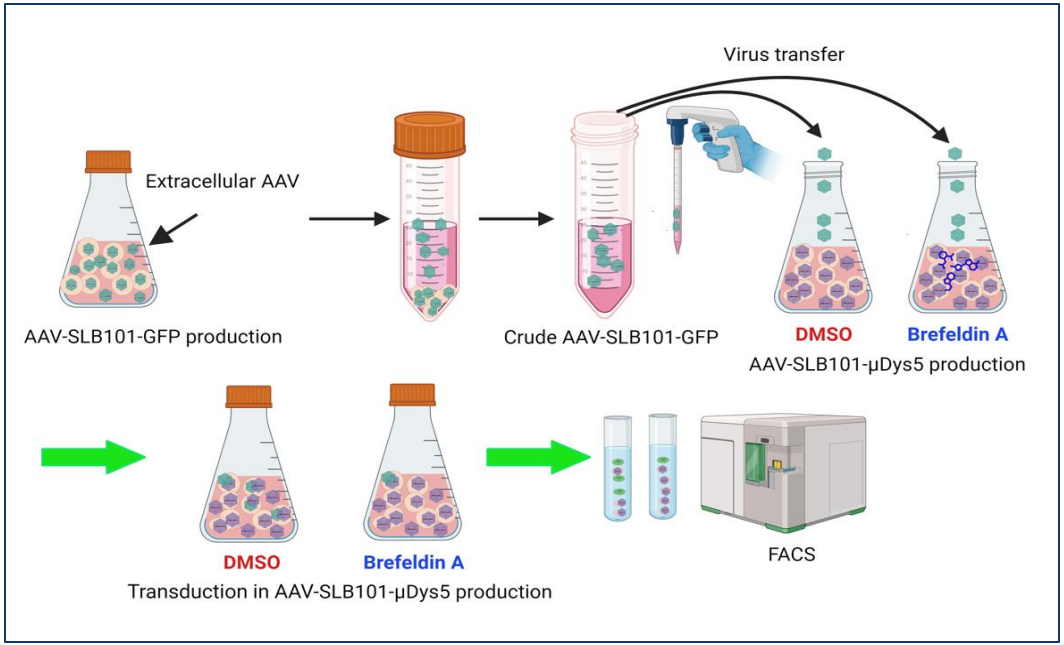
Can Transduction of HEK293 Cells During AAV Production Be Prevented?

Brefeldin A is an Inhibitor of Endocytosis



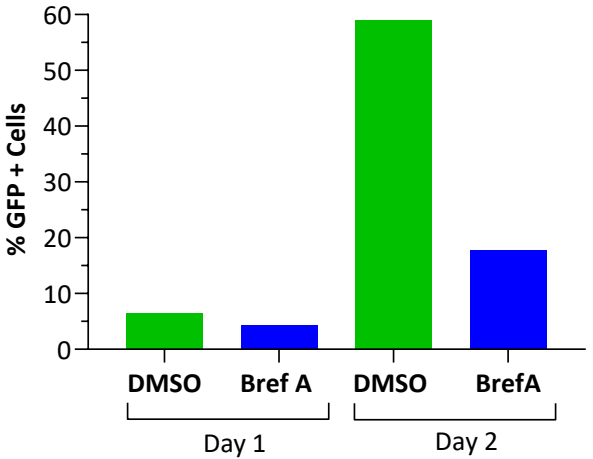
- Brefeldin A inhibits clathrin-dependent endocytosis
- Causes Golgi disassembly
- Prevents AAV endocytosis

Brefeldin A Unexpectedly Reduced AAV-SLB101-GFP Transduction of HEK293 cells Actively Producing AAV



**Created using Biorender.com*

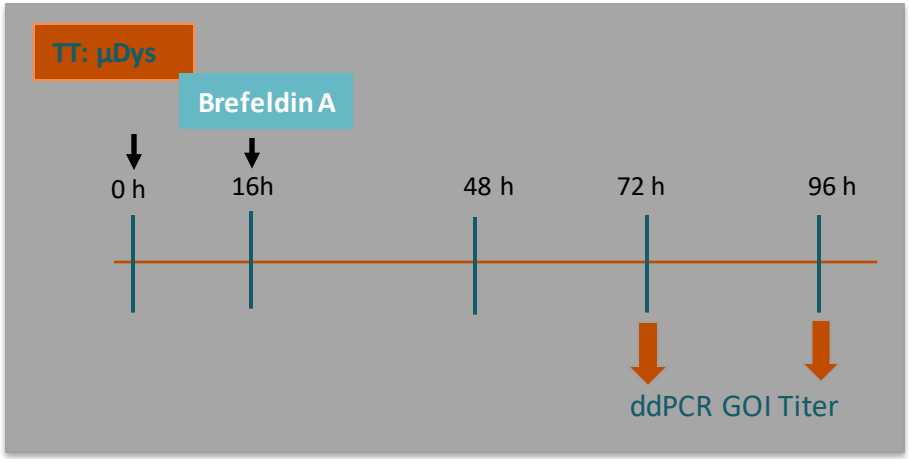
pRC= AAV-SLB101
pGOI = µDys5
pHelper

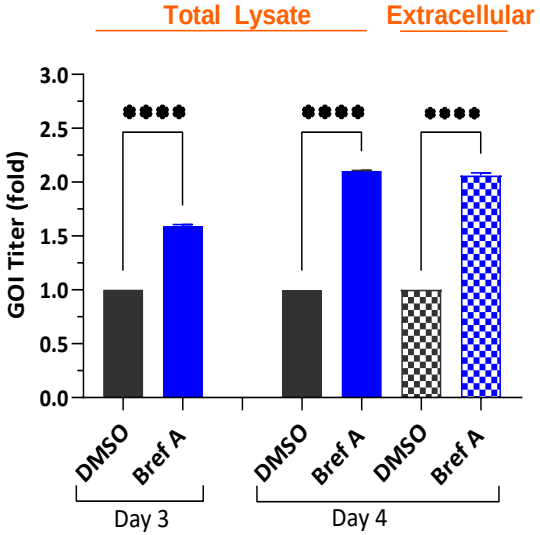


Brefeldin A (Bref A) significantly reduced AAV-SLB101-GFP transduction of HEK293 cells during AAV production

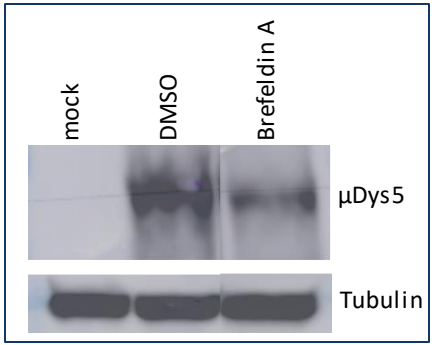
Addition of Brefeldin A Increased AAV Yield in HEK293 Cells

Scale: 50 mL Shake Flask





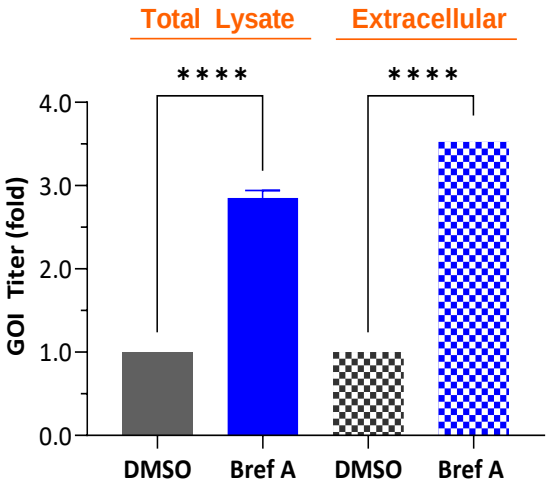
- Brefeldin A significantly increased AAV yield ~ 2-fold
- Addition of Brefeldin A reduced µDys expression, i.e. self-transduction of manufacturing HEK293 cells by AAV-SLB101-µDys5

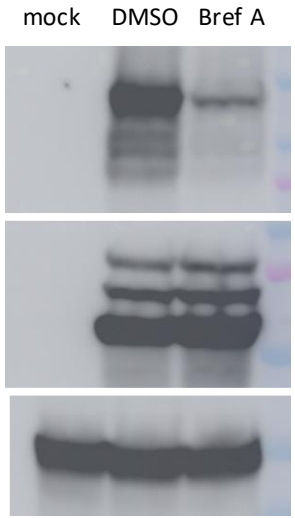


pRC= AAV-SLB101
pGOI = µDys5
pHelper

Addition of Brefeldin A Increased AAV Yield in HEK293 Cells

Scale: 500 mL Shake Flask



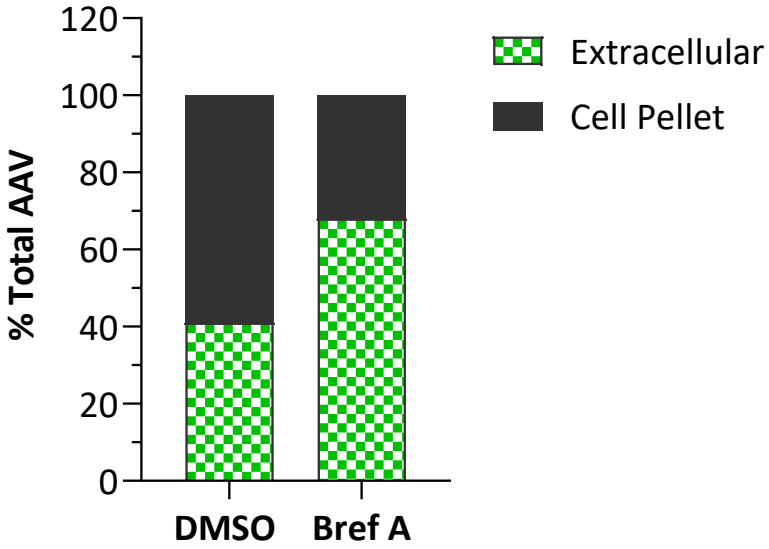


pRC= AAV-SLB101
pGOI = µDys5
pHelper

- Bref A significantly increased AAV yield 2-3 fold
  - Media Supernatant
  - Total lysate
- Bref A reduced µDys5 expression, i.e. self-transduction of manufacturing HEK293 cells by AAV-SLB101-µDys5

Addition of Brefeldin A Changed Distribution of AAV During Production

Scale: 500 mL Shake Flask



pRC= AAV-SLB101
pGOI = µDys5
pHelper

Brefeldin A resulted in higher proportion of extracellular AAV in media through inhibition of AAV endocytosis and transduction of HEK293 cells

Quality Attributes of AAV Produced in Presence of Brefeldin A

Analytics on AAV purified by Iodixanol purification from 500 mL Shake Flask

Assay	DMSO	Brefeldin A	Quality Trend
Relative Expression	93%	145%	↑
Genomic Integrity	52%	61%	↑
% Mispackaged Plasmid DNA/GOI	0.3%	0.17%	↑
Mispackaged Host Cell DNA/GOI	1.4E3ng/1E13vg	7E2ng/1E13vg	↑

Addition of Brefeldin A resulted in equivalent or improved AAV quality attributes

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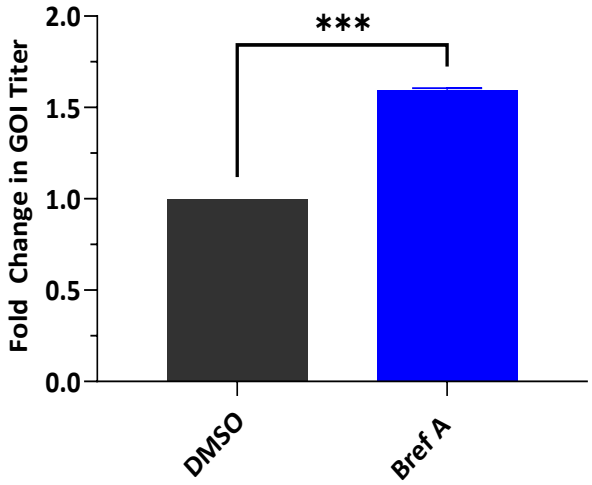
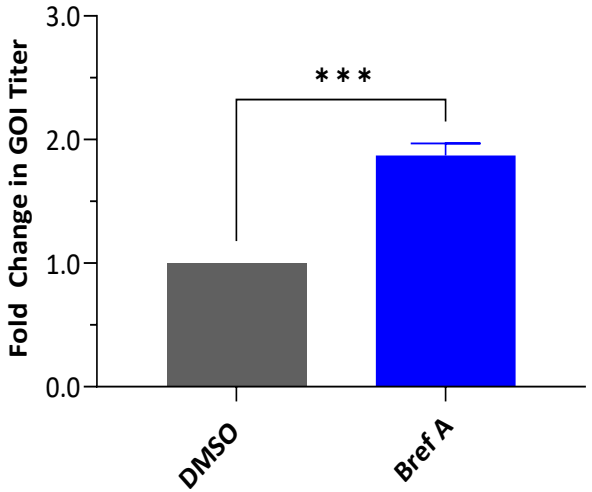
Brefeldin A Increased AAV Yield Across Capsid Serotypes & Transgenes

Scale: 50 mL Shake Flask

AAV-rh74-GOI

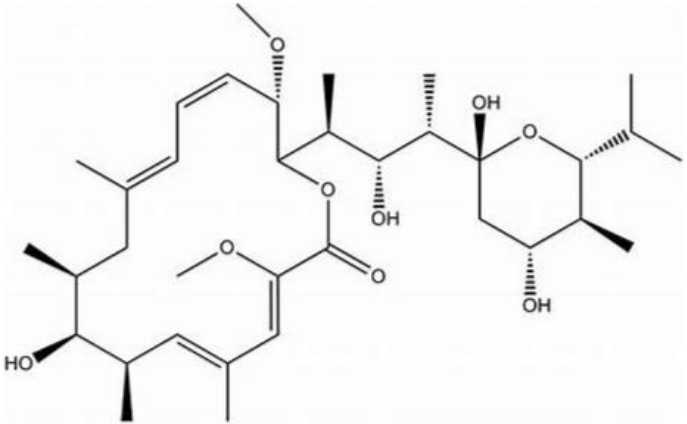


AAV-SLB101-µDys5



Addition of Brefeldin A resulted in increased AAV yield for all AAV serotypes and transgenes tested

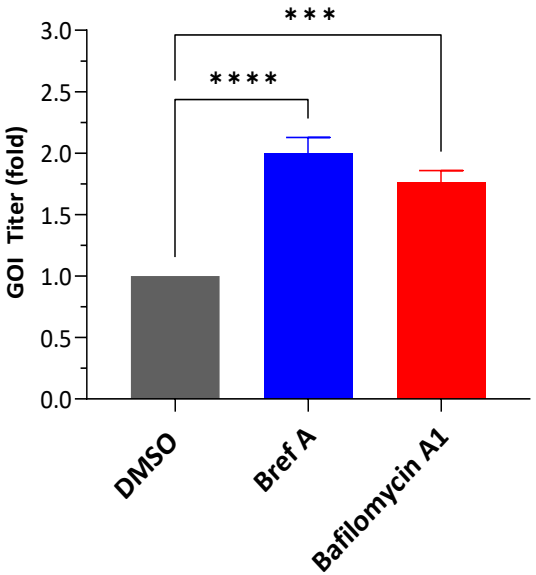
Bafilomycin A1 Also Inhibits Endocytosis



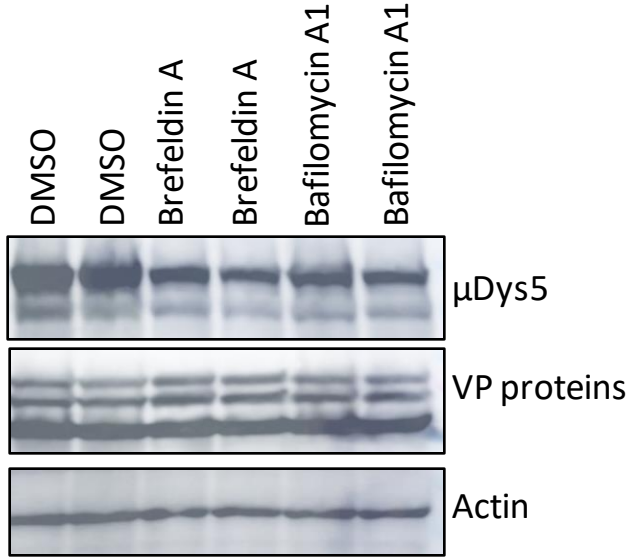
- Bafilomycin A1 inhibits endocytosis (different MoA to Brefeldin A)
- Inhibitor of v-ATPase; impacts lysosomal trafficking
- Prevents AAV endocytosis

Addition of Bafilomycin A1 Increased AAV Yield in HEK293 Cells

Scale: 50 mL Shake Flask



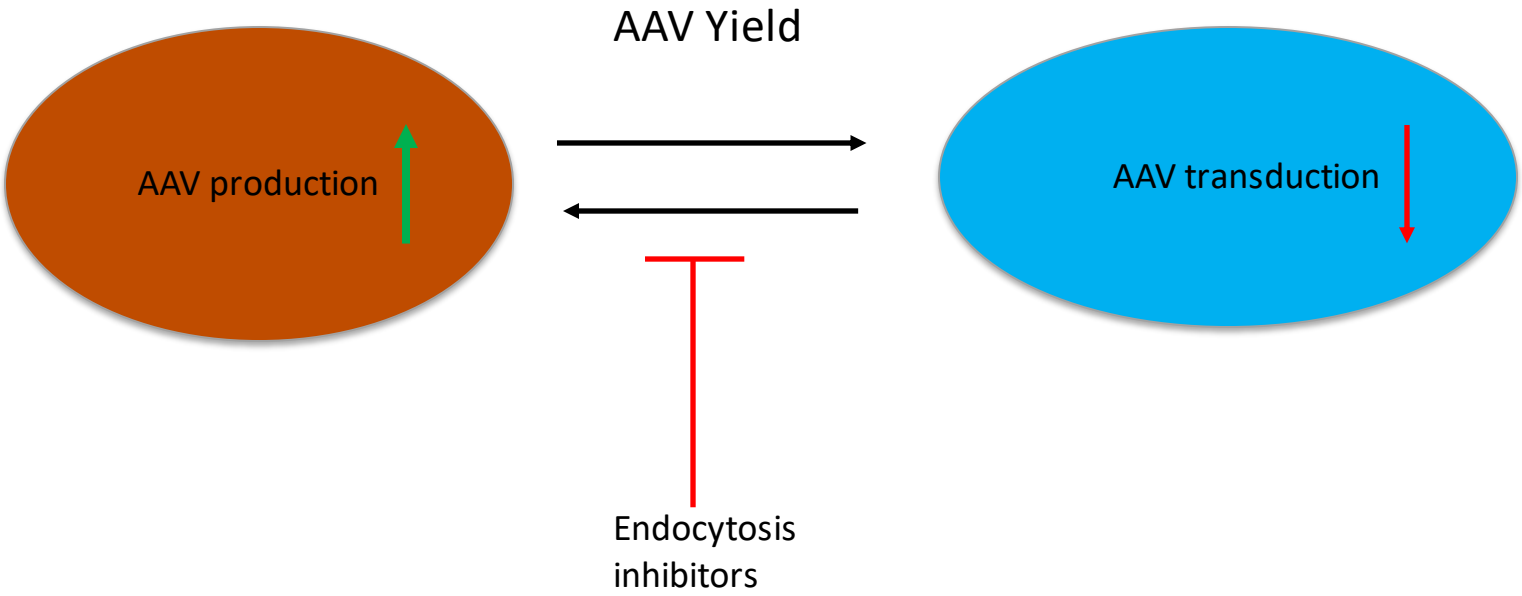
pRC= AAV-SLB101
pGOI = µDys5
pHelper



Brefeldin A or Bafilomycin A1 treatment significantly increased AAV yield

Brefeldin A or Bafilomycin A1 reduced µDys expression, i.e. self-transduction of HEK293 cells actively producing AAV-SLB101-µDys5

Summary



Summary & Conclusions

- Extracellular AAV can transduce HEK293 manufacturing cells
- Transduction can result in GOI protein expression in the HEK293 lysates and reduce yield
- Small molecule endocytosis inhibitors (Brefeldin A and Bafilomycin A1) reduced transduction
  - Reduced transduction can translate into 2-3 fold increases in AAV yield with similar or improved quality attributes

Conclusion

- Loss of AAV to transduction during manufacture is significant
- Endocytosis inhibitors limit transduction and consequently improve AAV yield

Acknowledgments

- Sharon McGonigle
- Jennifer Marlowe
- AD team
- PD team