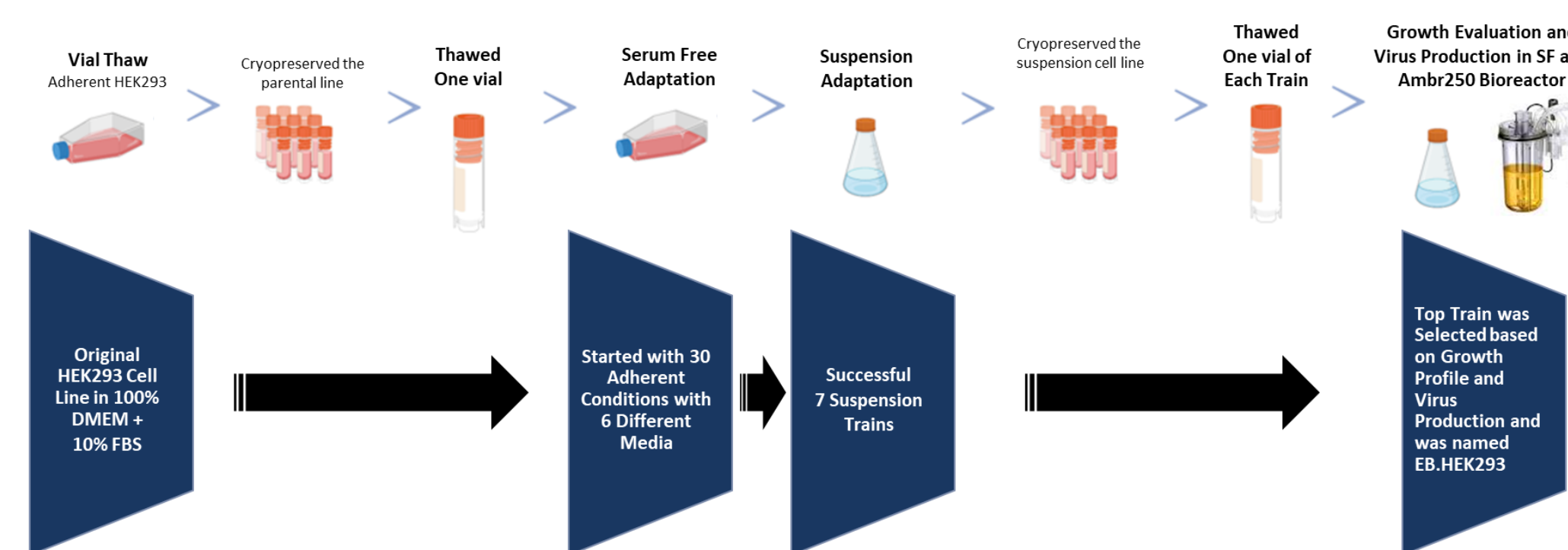


## Abstract

HEK293 adherent and suspension cell culture have been widely used for viral vector production. Due to manufacturing scalability, serum-free suspension HEK293 is more attractive and may dominate future virus production platforms. However, HEK293 cell lines frequently show an aggregated phenotype and high nutrient consumption following serum-free adaptation, potentially leading to variation in large-scale virus production. Expensive license fees and operational terms drive up the manufacturing cost and create significant complexities during commercialization. The objective of this project was to develop a proprietary HEK293 suspension cell line, with good productivity and high product quality to support rAAV and LVV manufacturing and lowering the cost associated with commercialization.

After continuous serum-free adaptation and suspension cell culture process, ElevateBio has successfully generated an in-house proprietary HEK293 polyclonal cell line, EB.HEK293. EB.HEK293 cell line demonstrated robust growth kinetics, including longer cell longevity, lower glucose consumption and low or no aggregation. Importantly, under non-optimized conditions, the suspension pool demonstrated robust rAAV and LVV production, reaching 5e7 TU/mL LVV infectious titer in the AMBR250 bioreactor and comparable rAAV titer with better full particle percentage compared to a commercially available HEK293 suspension cell line. All the results and analysis strongly indicate that the ElevateBio proprietary HEK293 cell line will be a reliable starting material to replace commercially available HEK293 cell lines for LVV and rAAV production. Additional optimization and single cell cloning will be applied to further explore this non-aggregated HEK293 suspension cell line.

## Method and Materials

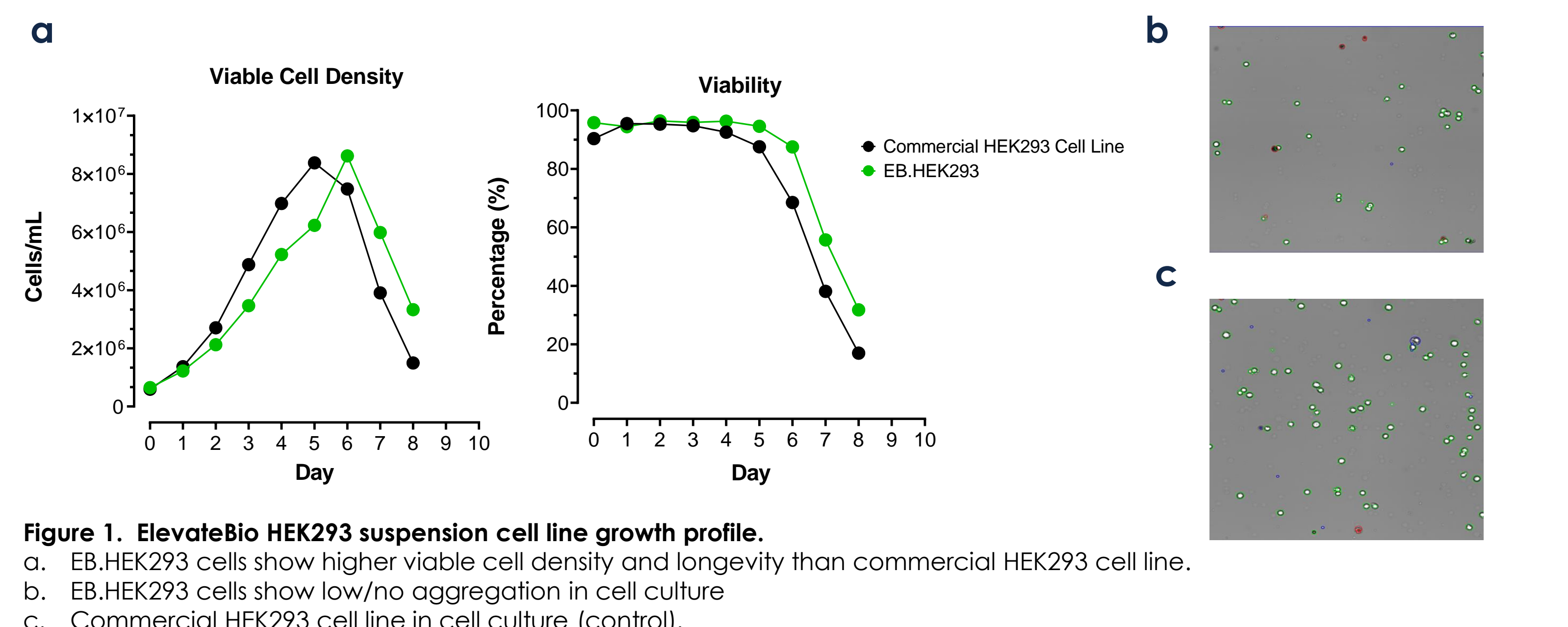


HEK293 adherent cells were serum-free adapted into suspension media. Cell growth and Lentiviral vector production was evaluated in both shake flask and Ambr250 bioreactor (rAAV in shake flask only). All harvested samples were subjected to analytical and potency assays for further evaluation.

**Bioreactor system:** Ambr250 from Sartorius  
**Metabolism analysis:** Meta Flex and Nova Flex  
**LVV infectious titer:** ddPCR  
**AAV titration:** ddPCR  
**AAV Full capsid percentage:** based on ddPCR and Capsid ELISA titer

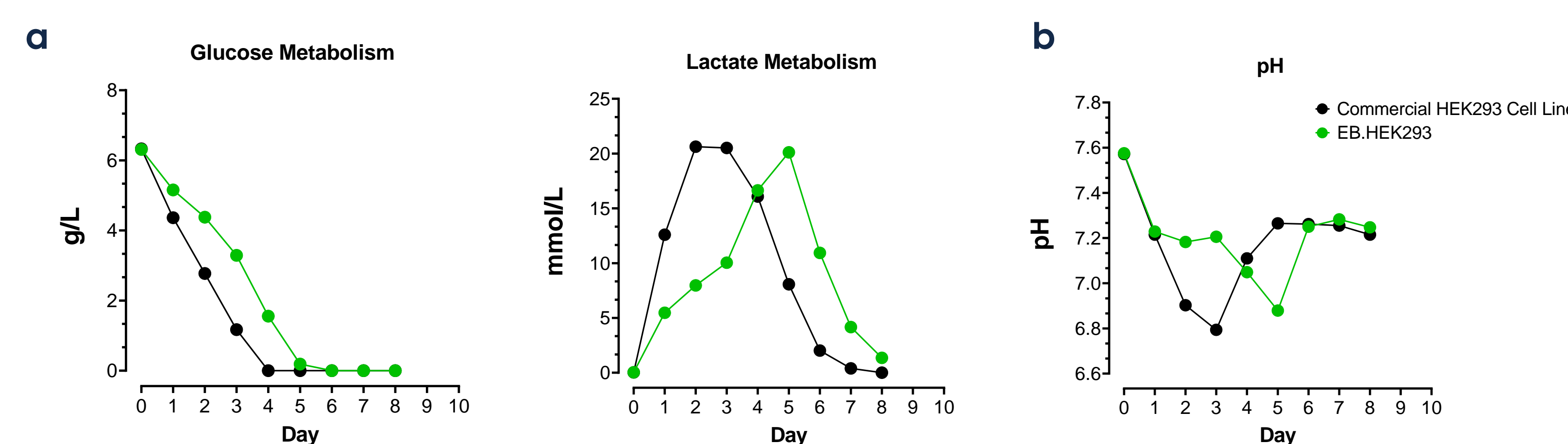
## Results

### EB.HEK293 Suspension Cell Line has Robust Growth Compared to Commercial HEK293 Cell Line



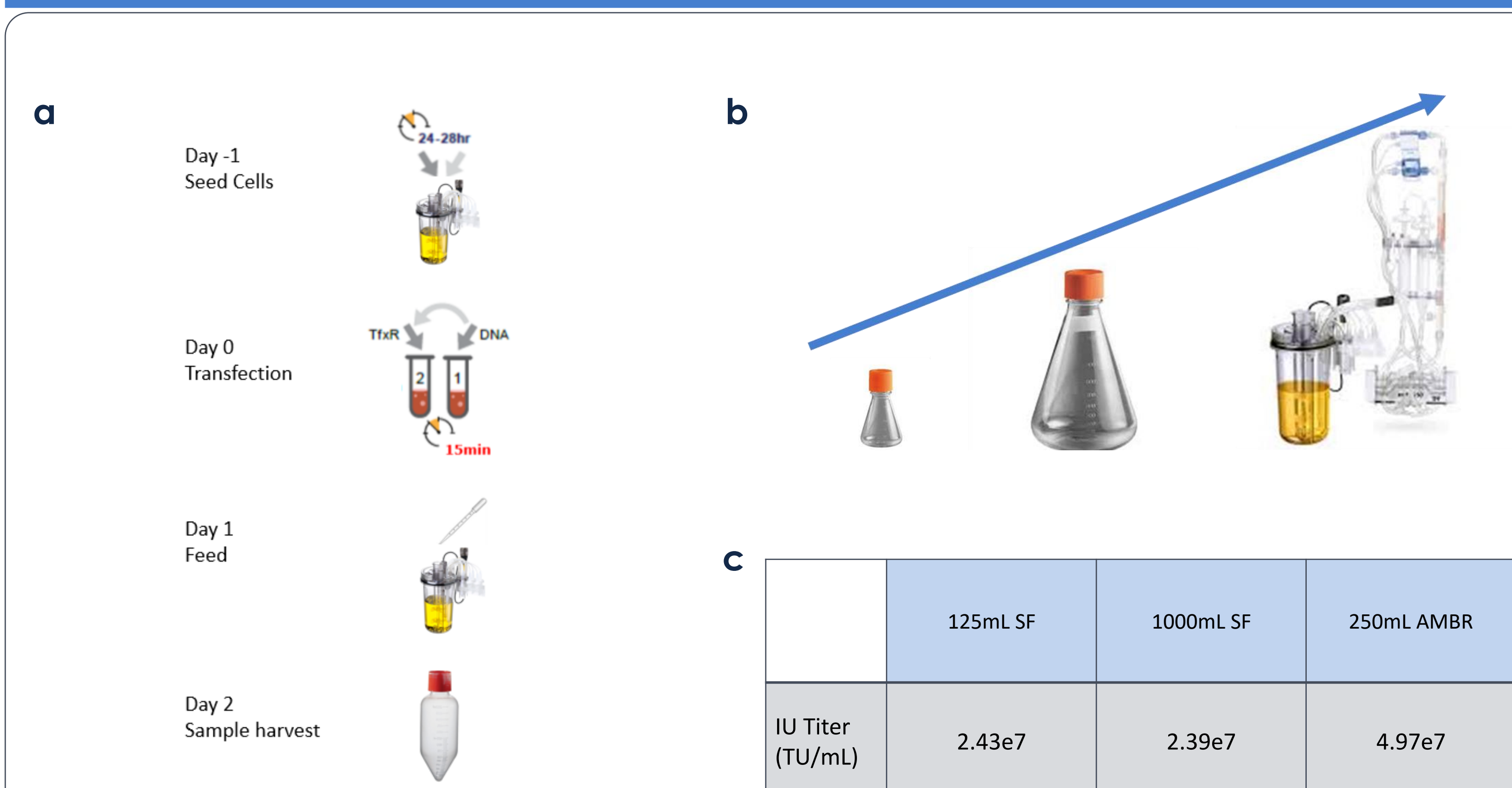
**Figure 1. ElevateBio HEK293 suspension cell line growth profile.**  
a. EB.HEK293 cells show higher viable cell density and longevity than commercial HEK293 cell line.  
b. EB.HEK293 cells show low/no aggregation in cell culture  
c. Commercial HEK293 cell line in cell culture (control).

### EB.HEK293 Requires Less Nutrients Compared to Commercial HEK293 in Suspension Culture



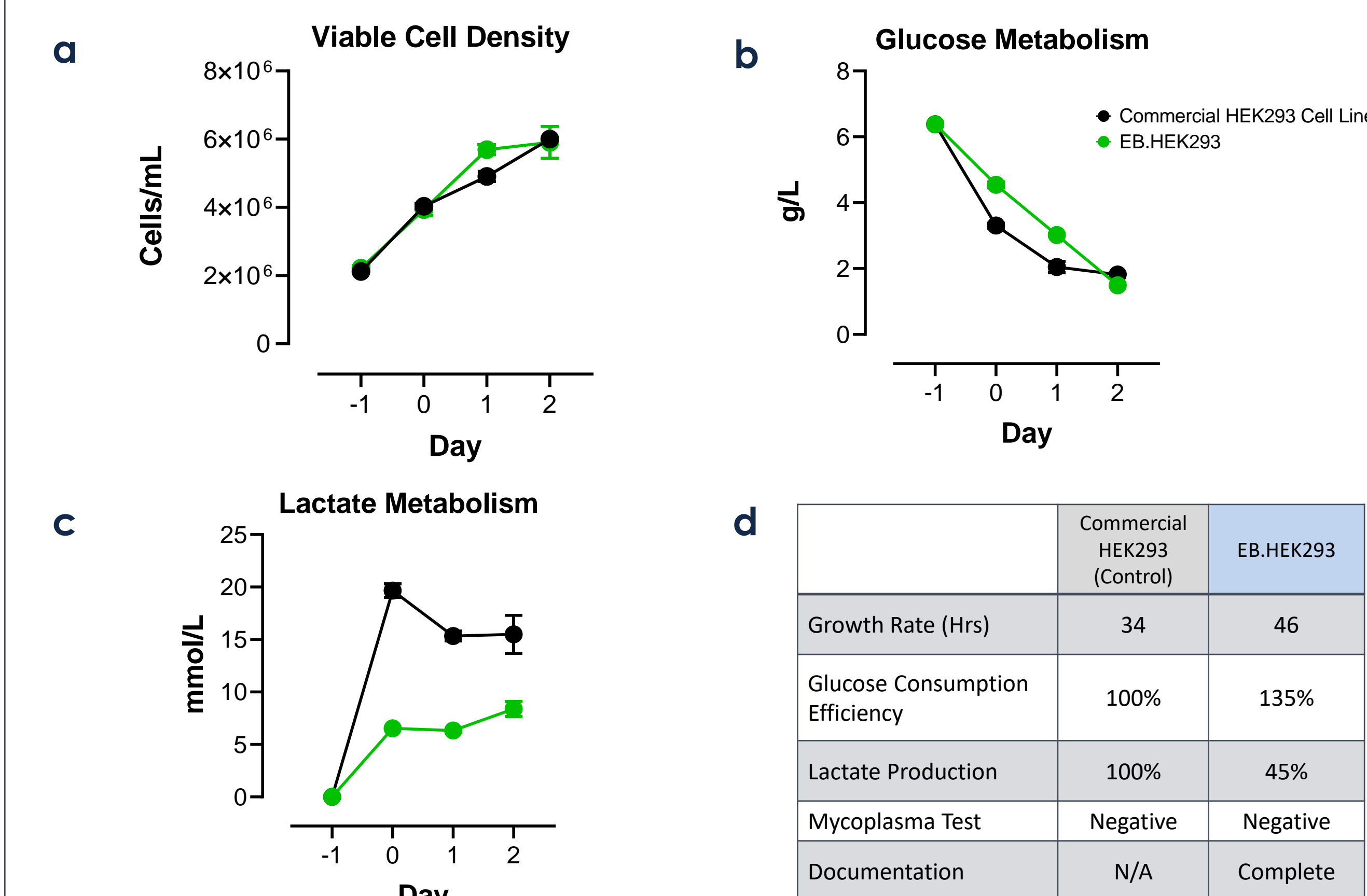
**Figure 2. ElevateBio HEK293 suspension cell line metabolism profile.**  
a. Suspension cells show lower glucose consumption and slower lactate production than commercial HEK293 cell line (n=2).  
b. pH profile of EB.HEK293 and commercial HEK293 cell line.

### EB.HEK293 Cell Line Scalability in Lentiviral Vector Production



**Figure 3. ElevateBio HEK293 suspension cell line virus production.**  
a. Lentiviral vector (LVV) production workflow in shake flask and Ambr250 bioreactor.  
b. Scalable upstream process development from shake flask to Ambr250 bioreactor using EB.HEK293 cells.  
c. Harvest Titer data in TU/mL as the virus production was scaled up from 125mL SF to 250mL AMBRs.

### EB.HEK293 Cell Line is More Energy Efficient During Virus Production Compared to the Commercial Cell Line



**Figure 4. ElevateBio HEK293 suspension cell line growth profile during virus production**  
a. EB.HEK293 Suspension cells reached a similar viable cell density compared to commercial HEK293 cell line during LVV production in Ambr250 under non-optimized condition.  
b. EB.HEK293 consumes less glucose during virus production than commercial HEK293 cell line. Commercial HEK293 required glucose feeding on Day1 whereas EB.HEK293 required no glucose feeding.  
c. EB.HEK293 produces 50% less lactate than commercial HEK293 cell line during virus production.  
d. Summary table of the growth profile comparing commercial HEK293 and EB.HEK293 during virus production.

## Discussion

ElevateBio has successfully generated an internal HEK293 suspension cell line after continuous serum-free adaptation and suspension adaptation. After removing serum from the cell culture process, the EB.HEK293 cell line has been cultured in non-serum containing media for more than 50 generations. By comparing to the commercially available HEK293 cell line, EB.HEK293 cell line show robust growth profiles, including longer cell longevity, less glucose consumption, and slower lactate production. More importantly, under non-optimized conditions, ElevateBio proprietary cell line demonstrated comparable rAAV and LVV production when comparing to the commercial HEK293 cell line. The titer data from the rAAV production indicates that EB.HEK293 cell line produced higher full AAV particles, compared to the commercial HEK293 cell line (data not shown), using our vector generation process. All the results and analysis strongly indicate that the EB.HEK293 suspension cells will be a reliable starting material to replace the commercial cell line for rAAV and LVV production. ElevateBio will continue to optimize the proprietary HEK293 suspension cell line through single cell cloning.

## Conclusion

- ElevateBio developed a suspension HEK293 polyclonal cell line, EB.HEK293 for scalable production of high titer lentiviral vector and rAAV vector.
- EB.HEK293 cell line shows robust growth profile comparing to the commercial HEK293 cell line with little aggregation, better cell longevity, lower glucose consumption, and lactate production.
- EB.HEK293 cell line has passed all qualified microorganism test and contains clear developmental history.

## Contact

Mercedes Segura, PhD  
VP, Process Development  
Email: msegura@elevate.bio