Simplifying Progress

Next Generation Bioproduction: A Novel Engineered CHO Host for Optimized Performance in Fed-Batch and Perfusion

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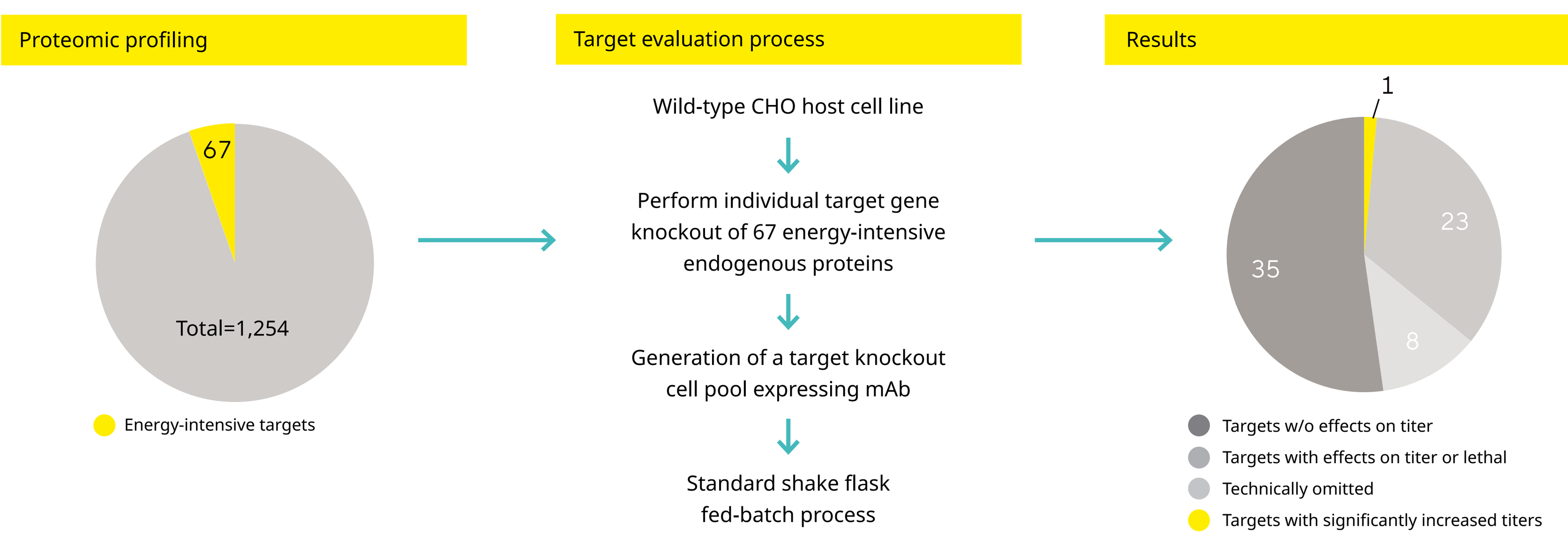
Introduction

CHO cell lines are the leading mammalian expression system for producing protein-based biopharmaceuticals. Advances in cell line development, including expression system optimization, clone selection, and media and process innovations, have significantly improved product yield and quality. To address the increasing diversity and complexity of new therapeutics, genetic engineering of cell lines to enhance growth, productivity, and product quality is a promising strategy. However, the application of modern gene-editing tools to design commercial CHO host cell lines with superior characteristics remains in its early stages.

In this study, we applied proteomic profiling to identify energy-intensive endogenous CHO proteins as potential gene knockout targets for engineering a host with improved bioprocess performance (Figure 1). Sixty-seven identified targets were evaluated in a CRISPR knockout screen, which yielded one hit that increased protein titer upon gene inactivation. Based on these results, a novel genetically engineered CHO host cell line was created, characterized by drastically increased cell-specific productivity, improved titers, and high clone stability across all tested molecules. Integrated into the Sartorius cell line development platform, this innovative cell line supports therapeutic manufacturing – including process intensification – providing a robust and scalable solution for efficient biopharmaceutical production.

Identification and screening of engineering targets

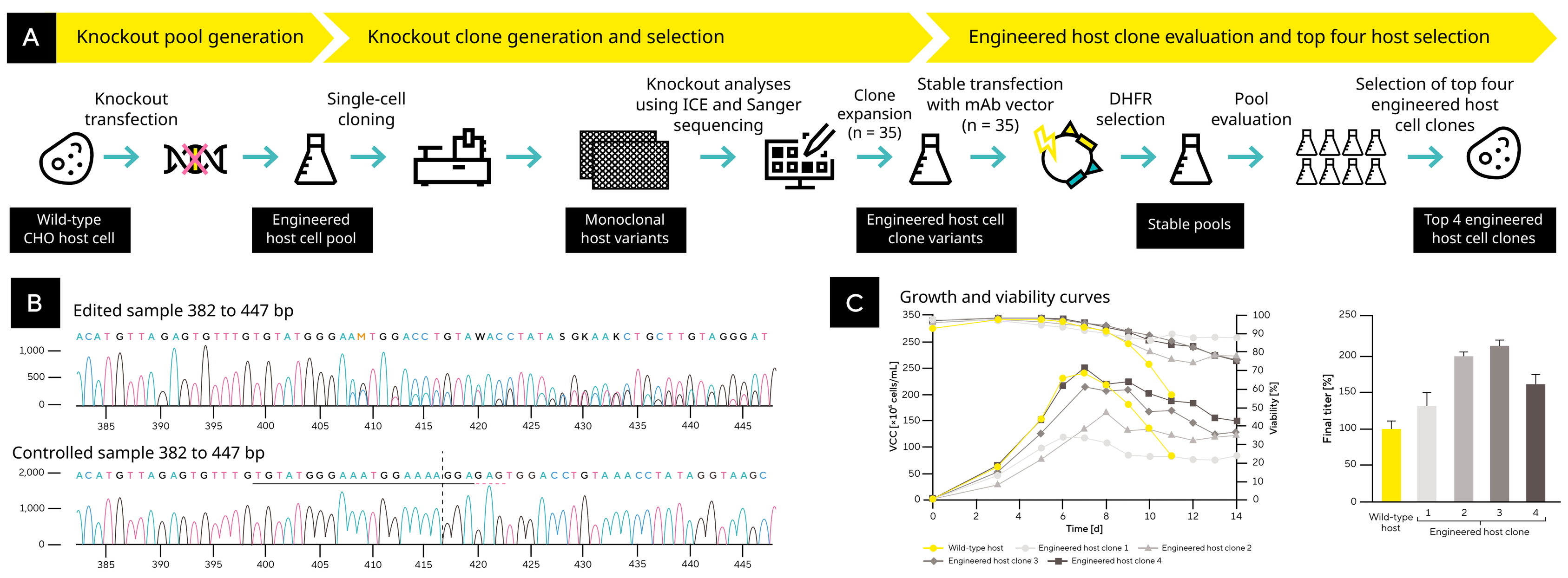
Figure 1: *Cell line engineering through data-driven proteomic profiling. LC-MS analysis of fed-batch samples from four clones, using the UniProt Chinese hamster reference proteome, identified 67 of 1,254 proteins as energy-intensive within the host cell. A genetic knockout study was performed in pooled fed-batch cultures to evaluate the individual impact of these energy-intensive targets on titer. Results revealed 35 targets with no impact on titer and 23 targets with negative impact on titer or resulting in cell lethality. Eight targets were omitted, and the knockout of one target significantly increased protein titer and cell-specific productivity.*



Generation and selection of engineered CHO host clones

CHO cells were genetically engineered using a gRNA and a CRISPR nuclease to generate a targeted gene knockout (Figure 2A and 2B). Monoclonal knockouts were generated using the Sartorius CellCelector. Thirty-five engineered host clones from stable pools expressing a model mAb were evaluated for protein expression in fed-batch studies. The top four engineered host clones showed up to a twofold increase in titer (Figure 2C). The best-performing clone was selected as the new lead host cell line for the Sartorius cell line development platform.

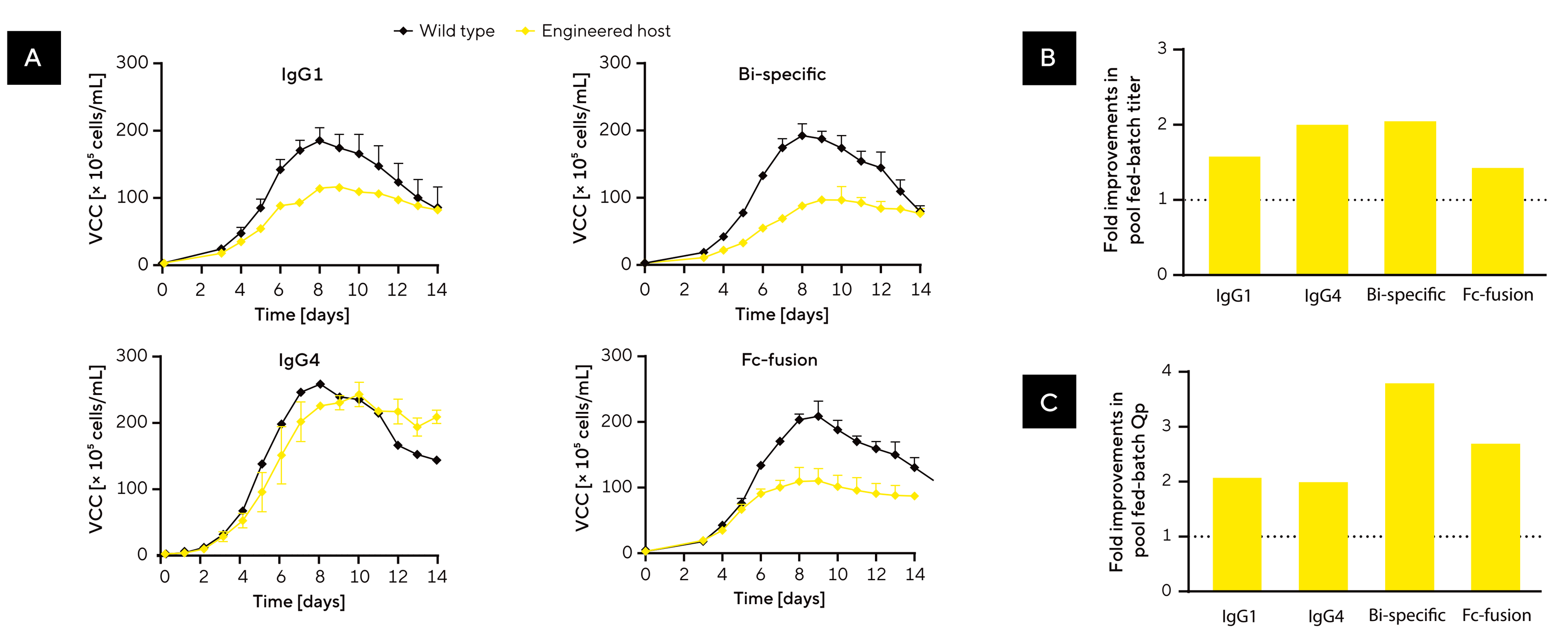
Figure 2: *(A) Schematic overview of the development of the new engineered knockout host cell line. (B) Confirmation of gene knockout in each clone using Inference of CRISPR Edits (ICE, Synthego) and Sanger sequencing. (C) Growth and titer performance of the top four engineered host clones compared to the wild-type host.*



Novel engineered host shows advantageous growth and increased productivity

Stable pools generated from the engineered host and the wild-type host, each expressing four different molecules, were evaluated in a 14-day fed-batch process in the Ambr® 15 system using 4Cell® Smart CHO medium (Figure 3A). All pools generated from the engineered host demonstrated higher productivity compared with the wild type, with an average 1.8-fold increase in titer (Figure 3B) and a 2.6-fold increase in cell-specific productivity (Qp; Figure 3C). This enhancement is attributed to a reduced viable cell concentration (VCC) and a 55% lower peak cell density (Figure 3A), while overall titers still surpassed those of the wild-type host.

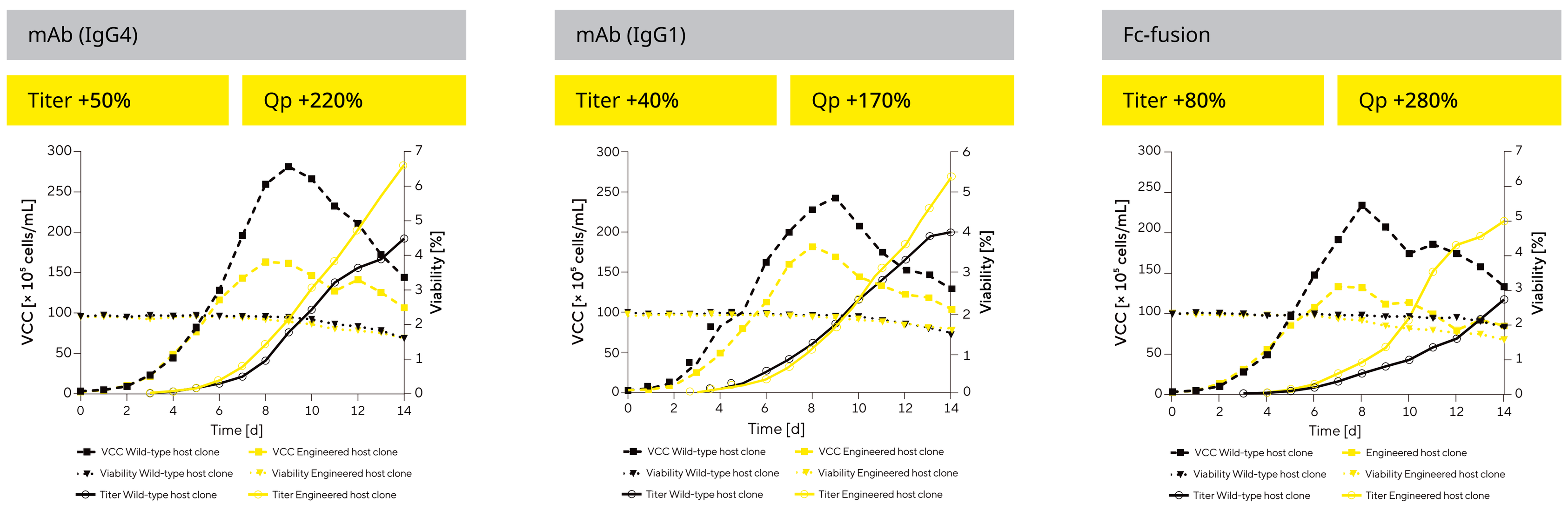
Figure 3: *Data from cell pools expressing four different molecules (IgG1, IgG4, Fc-fusion, and a bispecific molecule) evaluated in Ambr® 15 using a standard fed-batch process.* ***(A)*** *VCC of engineered host pools (yellow) compared with wild-type pools (black). Error bars indicate standard deviation (n = 2–3 pools).* ***(B and C)*** *Fold improvement in final titer* ***(B)*** *and cell-specific productivity (Qp;* ***C)*** *compared with those of wild-type pools.*



Increased performance of production clones at 5 L bioreactor scale

Multiple campaigns were conducted using the Sartorius CHO cell line development technology to compare the performance of the new engineered host cell line with the wild-type host. Lead clones expressing different molecules (IgG4, IgG1, and Fc-fusion) were evaluated in standard non-optimized fed-batch runs using 4Cell® SmartCHO medium at 5 L bioreactor scale.
The lead engineered host clones showed reduced growth, lower peak VCC, and markedly higher productivity compared with wild-type clones. All clones derived from the engineered host demonstrated an average 223% increase in Qp and a 56% increase in final titer (Figure 4).

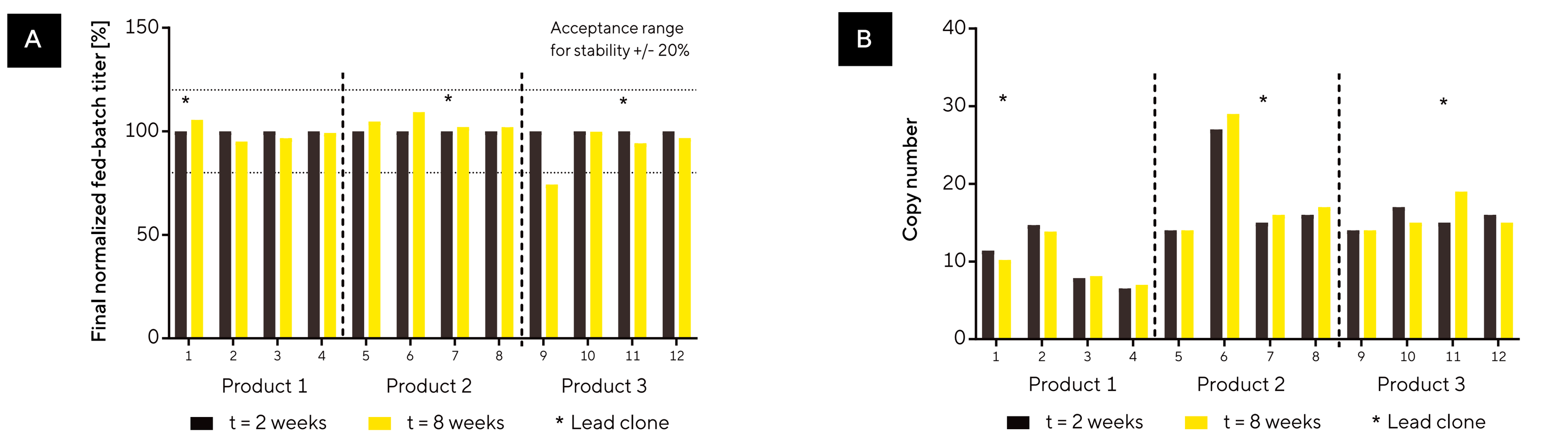
Figure 4: *Fed-batch data from Univessel® Glass 5 L bioreactor runs comparing clones derived from engineered and wild-type hosts. VCC (square), viability (triangle), and product titer (circle) trends are shown throughout the fed-batch run. The Sartorius standard process was initiated with a cell concentration of 3 × 10⁵ cells/mL using 4Cell® SmartCHO medium, with daily bolus feeds of 4Cell® SmartCHO FMA and FMB starting on day 3 and an additional daily bolus glucose feed beginning on day 6.*



Confirmed long-term stability of production clones

Stability studies, covering more than 70 generations, were performed on a total of 12 cell clones derived from the engineered host, each expressing three distinct products. Parallel fed-batch stability studies in shake flasks and gene copy number analyses at t = 2 weeks (RCB) and t = 8 weeks (production) were conducted using the standard Sartorius process. Eleven of 12 clones showed stability in product titer (within a 20% range; Figure 5A) and maintained low gene copy numbers (7–29 copies) ensuring high genomic stability (Figure 6B).

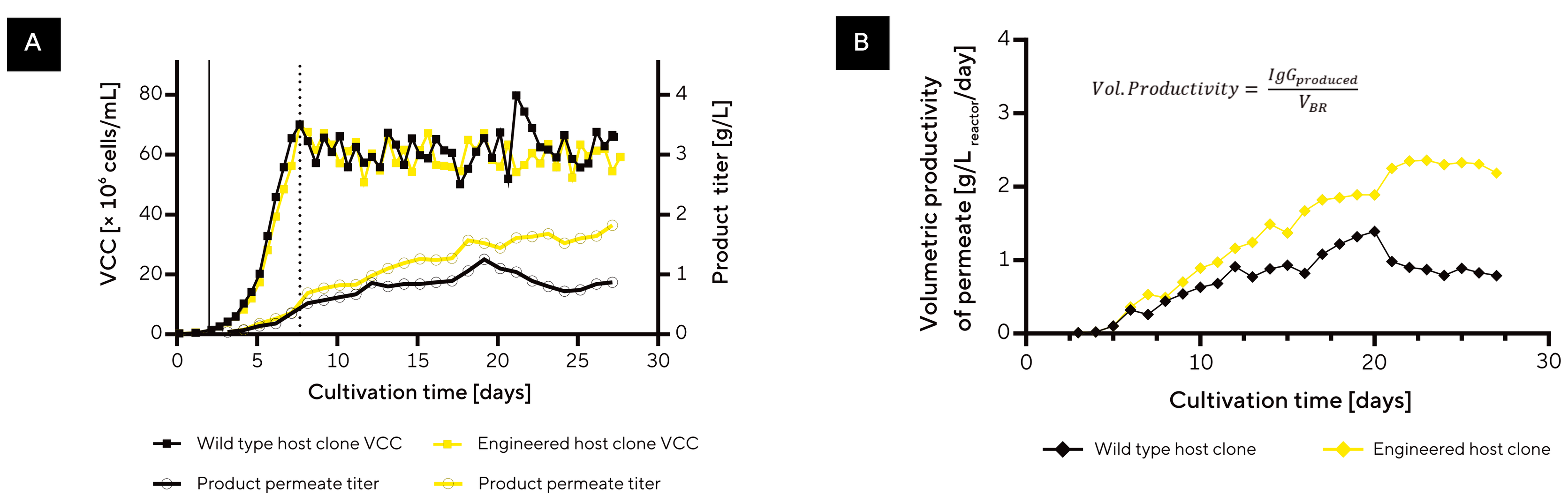
Figure 5: *Clone stability fed-batch data of 12 clones generated from the new engineered host cell line.* ***(A)*** *Fed-batch end-point titer (normalized to t = 2) analyzed on the Octet® BLI system.* ***(B)*** *Copy number data from DNA generated during fed-batch and analyzed via ddPCR (Bio-Rad).*



New engineered host demonstrates enhanced performance in perfusion processes

An intensified production process was performed using the Ambr® 250 High Throughput ATF perfusion system. Wild-type and engineered host cell clones, each expressing the same product, were inoculated at 0.3 × 10⁶ cells/mL, and perfusion was initiated at 2.5 × 10⁶ cells/mL after 2–3 days of batch cultivation. By day 7, the target VCC of 60 × 10⁶ cells/mL was achieved, and perfusion was sustained at a rate of 1.5 VVD using 4Cell® SmartCHO medium. VCC was monitored every 12 h, with daily samples collected to determine the titer in both the bioreactor and permeate. The engineered host clone outperformed the wild-type clone in continuous perfusion, maintaining a 28-day culture duration at a VCC of 60 × 10⁶ cells/mL, with high viability (Figure 6A). This resulted in enhanced permeate titer and volumetric productivity greater than 2 g/L per day (Figure 6B).

Figure 6: *Comparison of wild-type (black) and engineered host cell lines (yellow) in a 28-day perfusion process performed in the Ambr® 250 High Throughput ATF perfusion system.* ***(A)*** *VCC (squares) and product permeate titer (circles). The target VCC of 60 × 10⁶ cells/mL was achieved, with perfusion maintained at 1.5 VVD using 4Cell® SmartCHO medium.* ***(B)*** *Volumetric productivity of an engineered host clone and a wild-type clone expressing the same product.*



Conclusion

- We combined proteomic profiling and targeted genome editing to create a new engineered host cell line for improved production of biopharmaceuticals.
- The engineered host shows significantly improved expression titers of up to 80% and cell-specific productivity of up to 280%, while maintaining robust clone stability over 70 generations.
- Integrated into the Sartorius cell line development platform, the new host cell line facilitates the development of stable, high-performing clones for various molecules, including mAbs, bispecifics, and Fc-fusion products.
- Selected lead clones show good scalability to the 5 L bioreactor scale and support intensified processing strategies with high productivity up to 2 g/L/day, offering a robust and scalable solution for efficient biopharmaceutical production.