

**Journal: “Applied Microbiology and Biotechnology”**

**Manuscript Title: “Cell-line screening and process development for a fusogenic oncolytic virus in small-scale suspension cultures”**

**Sven Göbel<sup>1</sup>, Fabian Kortum<sup>2</sup>, Karim Jaén Chavez<sup>2</sup>, Ingo Jordan<sup>3</sup>, Volker Sandig<sup>3</sup>, Udo Reichl<sup>1,4</sup>, Jennifer Altomonte<sup>2</sup>, Yvonne Genzel<sup>1\*</sup>**

<sup>1</sup>Bioprocess Engineering, Max Planck Institute for Dynamics of Complex Technical Systems, Sandtorstr. 1, 39106 Magdeburg, Germany

<sup>2</sup>Department of Internal Medicine II, Klinikum rechts der Isar, Technische Universität München, Germany

<sup>3</sup>ProBioGen AG, Herbert-Bayer-Str. 8, 13086 Berlin, Germany

<sup>4</sup>Chair for Bioprocess Engineering, Otto-von-Guericke-University Magdeburg, Universitätsplatz 2, 39106 Magdeburg, Germany

\*Corresponding author

Yvonne Genzel

Email address: [genzel@mpi-magdeburg.mpg.de](mailto:genzel@mpi-magdeburg.mpg.de)

## Supplementary Material

**Table S1: Overview of suspension cell lines tested for the production of VSV-NDV.**

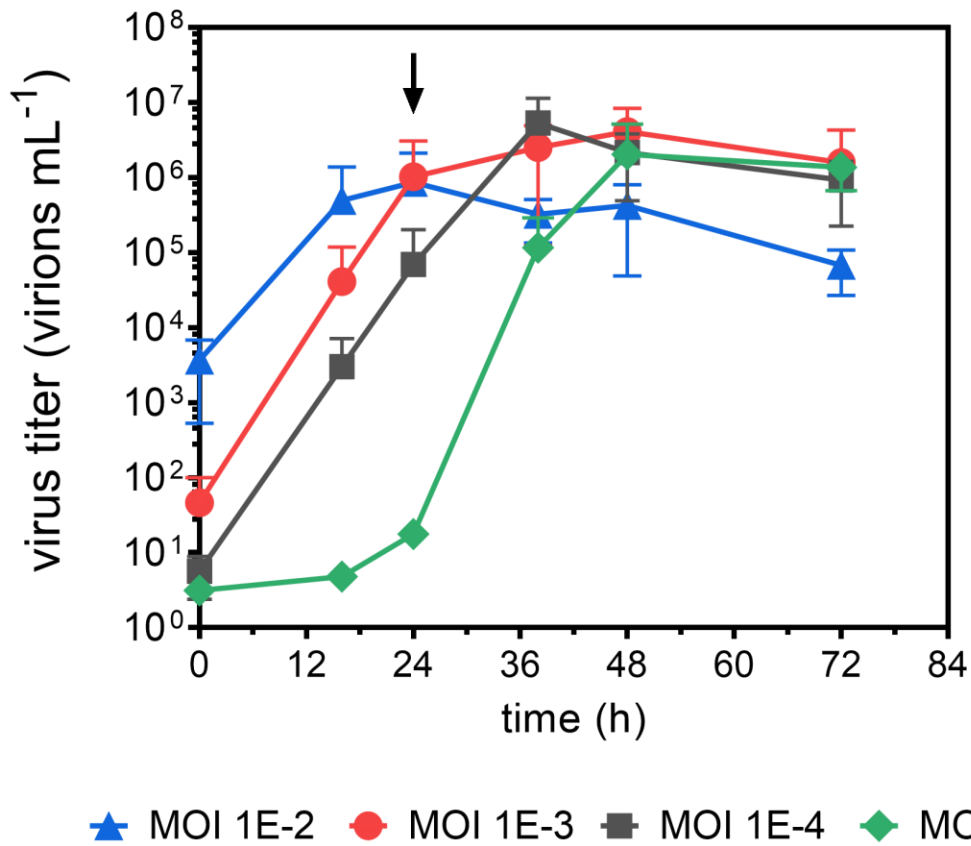
Abbreviation	Full name	Species	Tissue	Supplier
BHK-21	Suspension cell growth adapted BHK-21	Syrian golden hamster	Kidney	In house (Nikolay 2014)
AGE1.CR	-	Muscovy duck	Primary retina cell	PBG (Jordan et al. 2009)
AGE1.CR.pIX	-	Muscovy duck	Primary retina cell	PBG (Jordan et al. 2009)
HEK293SF	Human embryonic kidney SF- 3F6	Homo sapiens	Embryonic kidney cell	UAB/NRC
MDCK	Suspension cell growth adapted Madin Darby canine kidney CL-34	Dog	Kidney epithelial cell	In house (Lohr et al. 2010)

PBG = ProBioGen AG Berlin, Germany, UAB= Autonomous University of Barcelona, NRC= National Research Council of Canada

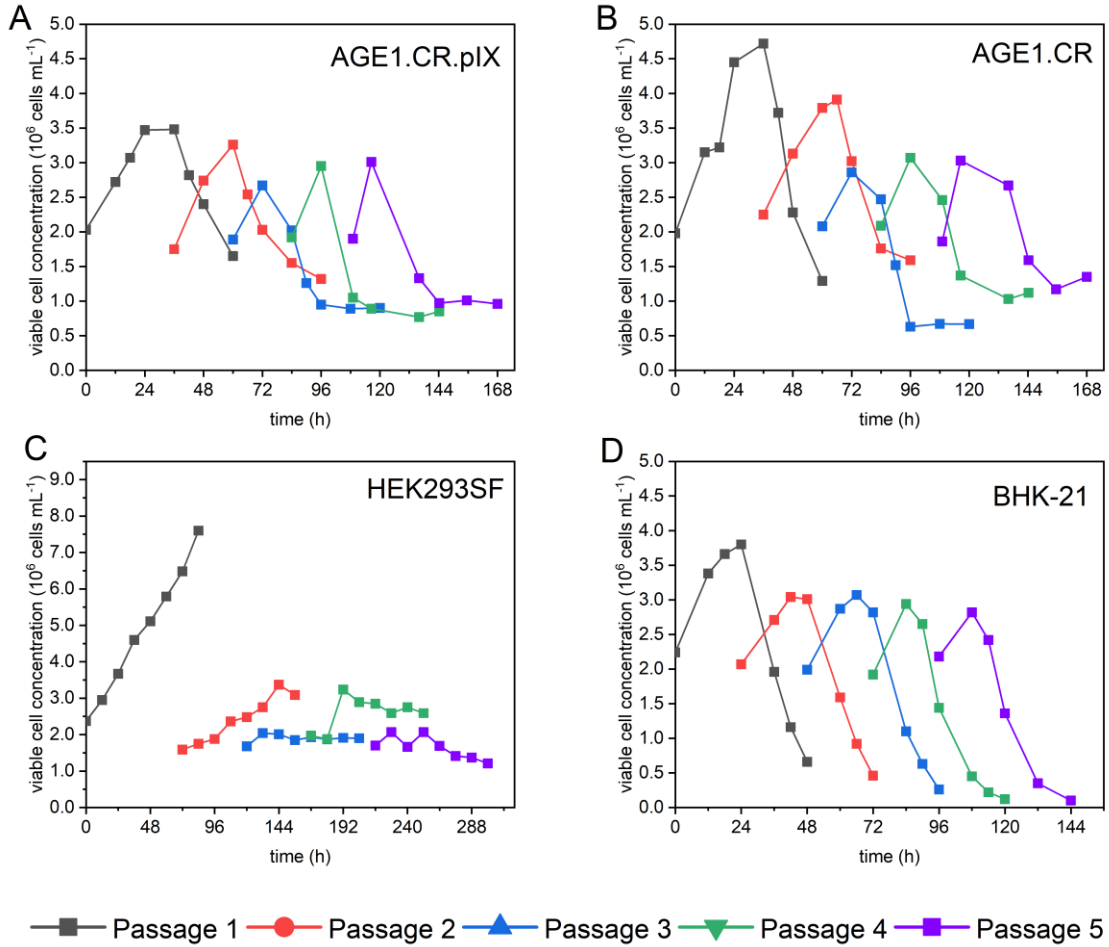
**Table S2: Cell line-specific passaging times and volumes used for the sequential adaptation of VSV-NDV.**

Cell line	Passage 1	Passage 2-5	Transferred volumes ( $\mu\text{L}$ )
BHK-21	24 hpi	24 hpi	10
AGE1.CR	24 hpi	24 hpi	10
AGE1.CR.pIX	36 hpi	24 hpi	10
HEK293SF	72 hpi	48 hpi	250

hpi = hours post infection



**Figure S1 Growth kinetics in adherent AGE1.CR.pIX cells.** Adherent AGE1.CR.pIX cells were seeded onto 150 cm<sup>2</sup> tissue culture dishes (#93150, TPP, Switzerland) and infected at a cell concentration of 1x10<sup>5</sup> cells/cm<sup>2</sup> at the respective MOI with VSV-NDV using 22 mL final volume (serum-free DMEM-F12). Virus growth dynamics was monitored by measuring the infectious virus titer in the culture supernatant by the TCID<sub>50</sub> assay. The graph show mean titers +/- SD from three biological replicates. The arrow marks the peak titer of MOI 0.01 infections, which were the samples used in subsequent potency measurements (Figure 2).



**Figure S2 Sequential adaptation of VSV-NDV in different suspension cell lines.** AGE1.CR.pIX, AGE1.CR, HEK293SF and BHK-21 cells were cultivated in shake flasks in CD-U7, Dynamics, and PEM<sub>s</sub> medium, respectively. Initial infection occurred during the mid-exponential growth phase at the previously determined optimal MOI. Subsequent virus passaging was performed blind using a fixed volume. Viable cell concentrations are plotted against the time post-infection. Each color indicates a different virus passage.