

# Susceptibility of monkey-derived, hamster-derived, porcine-derived continuous cell lines to African swine fever virus strains of diverse virulence

Giulia Franzoni<sup>1\*</sup>, Lorena Mura<sup>1</sup>, Susanna Zinellu<sup>1</sup>, Riccardo Bazzardi<sup>1</sup>, Silvia Dei Giudici<sup>1</sup>, Annalisa Oggiano<sup>1</sup>

<sup>1</sup>. Istituto Zooprofilattico Sperimentale della Sardegna, 07100 Sassari, Italy.

\* corresponding author: giulia.franzoni@izs-sardegna.it

## Introduction

African swine fever virus (ASFV) is the etiological agent of a highly lethal disease in both domestic and wild pigs. The virus has rapidly spread worldwide and has no available licensed vaccine. An obstacle to the construction of a safe and efficient vaccine is the lack of a suitable cell line for ASFV isolation and propagation (Meloni et al., 2022). Macrophages have been widely used to study virus-host interaction; nevertheless, obtaining these cells is time-consuming and expensive, and they are not ethically suitable for the production of large-scale vaccines. Different virulent field isolates have been adapted on monkey or human continuous cells lines, but several culture passages often lead to significant genetic modifications and loss of immunogenicity of the adapted strain (Meloni et al., 2022). In this work, we evaluated the susceptibility of diverse continuous cell lines to ASFV strains of diverse virulence.

## Materials and methods

Four ASFV strains of diverse virulence (attenuated BA71V, attenuated E70MS, moderately virulent NH/P68, highly virulent 26544/OG10 ASFV), were tested on monkey derived (COS-1, Vero, MS), hamster-derived (BHK21) or porcine derived (PK15) cell lines. Cells were infected using an MOI of 1 and 48 h later intracellular levels of ASFV viral proteins (early protein P30 and late protein P72) were determined using flow cytometry (Franzoni et al., 2022)(Figure 1).

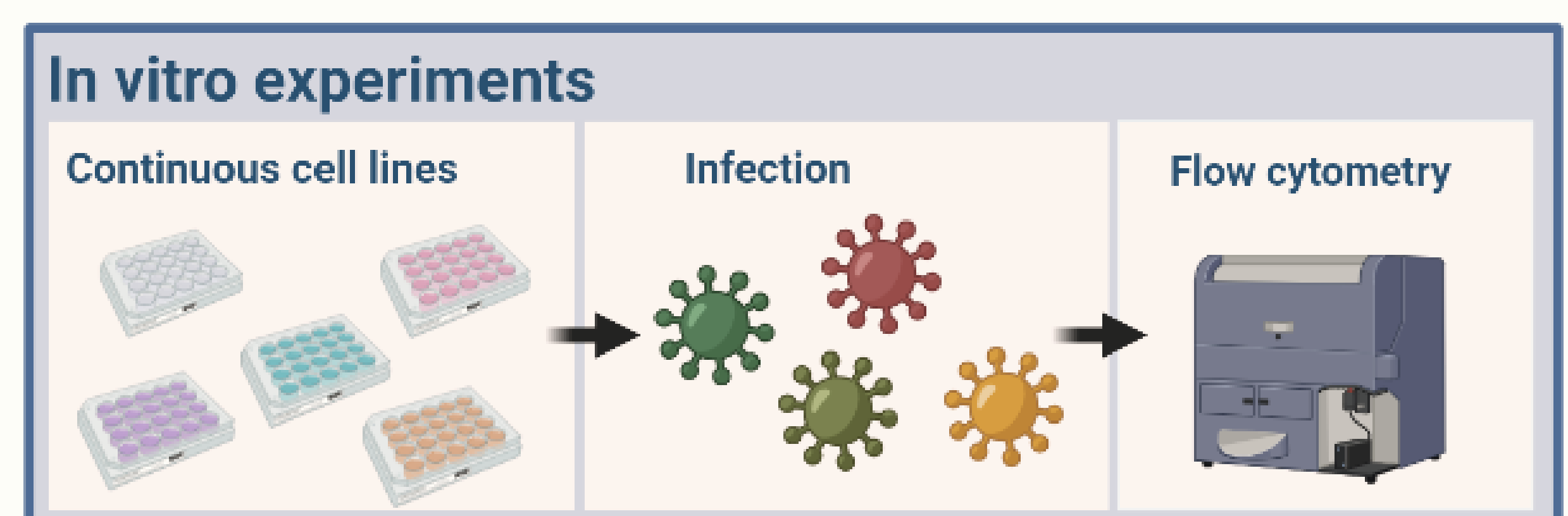


Figure 1. Study design. Images created with BioRender.com.

## Results

BA71V or E70MS resulted in efficient infection of VERO, MS, COS-1, with high percentages of both P30+ and P72+ cells 48h post-infection. Extremely low levels of either P30+ and P72+ VERO and MS were observed 48 h pi with attenuated NH/P68, whereas this strain was able to infect COS-1 cells (data not show). BA71V or E70MS, but not NH/P68, efficiently infected BHK-21, with high percentages of both P30+ and P72+ cells 48h post-infection (Figure 2). NH/P68 was unable to infect PK15, whereas avirulent BA71V and E70MS infected these cells, even if only modestly: 48h pi almost 5% of P72+ BA71V-infected and 10% of P72+ E70-infected cells were observed (Figure 3). The virulent 26544/OG10 was unable to infect any of the tested cell lines (Figure 2, Figure 3).

### BHK-21

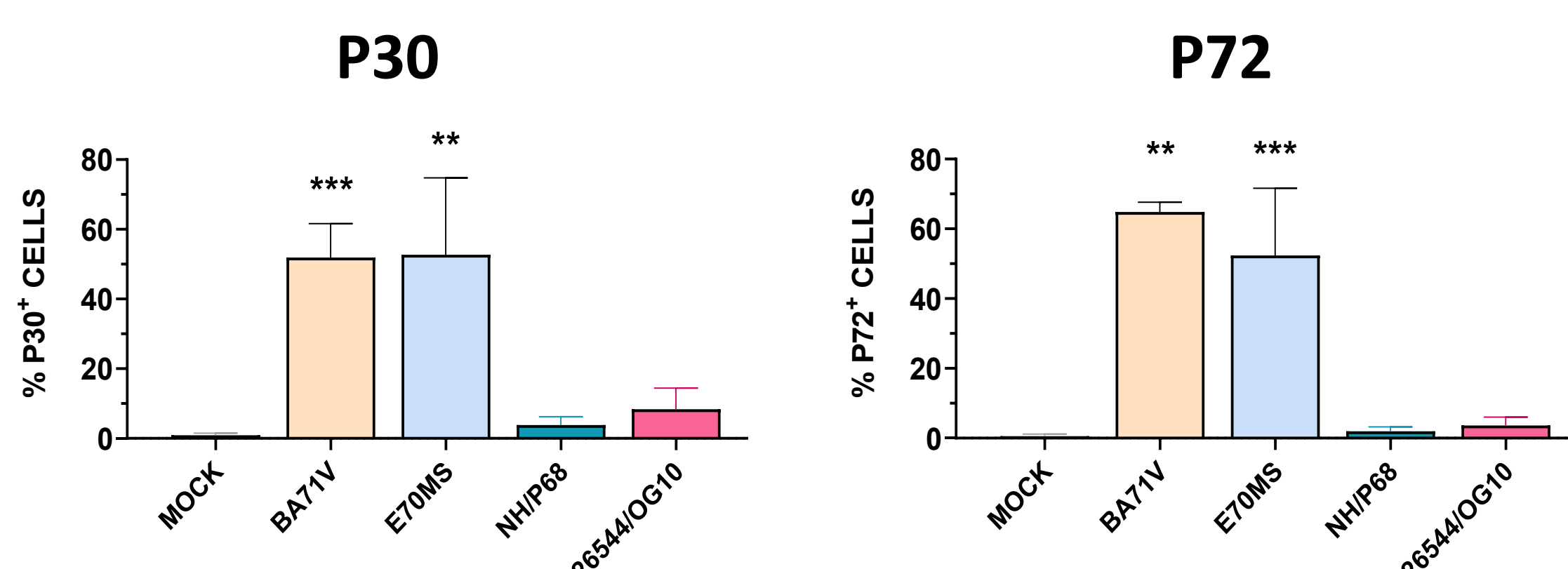


Figure 2. ASFV infection of hamster derived continuous cell line. BHK-21 were infected with ASFVs strains of diverse virulence (BA71V, E70MS, NH/P68, 26544/OG10) using an MOI of 1, alongside mock-infected controls. 48 h pi percentages of ASFV early (p30+) or late protein (p72+) cells were assessed by flow cytometry.

### PK-15

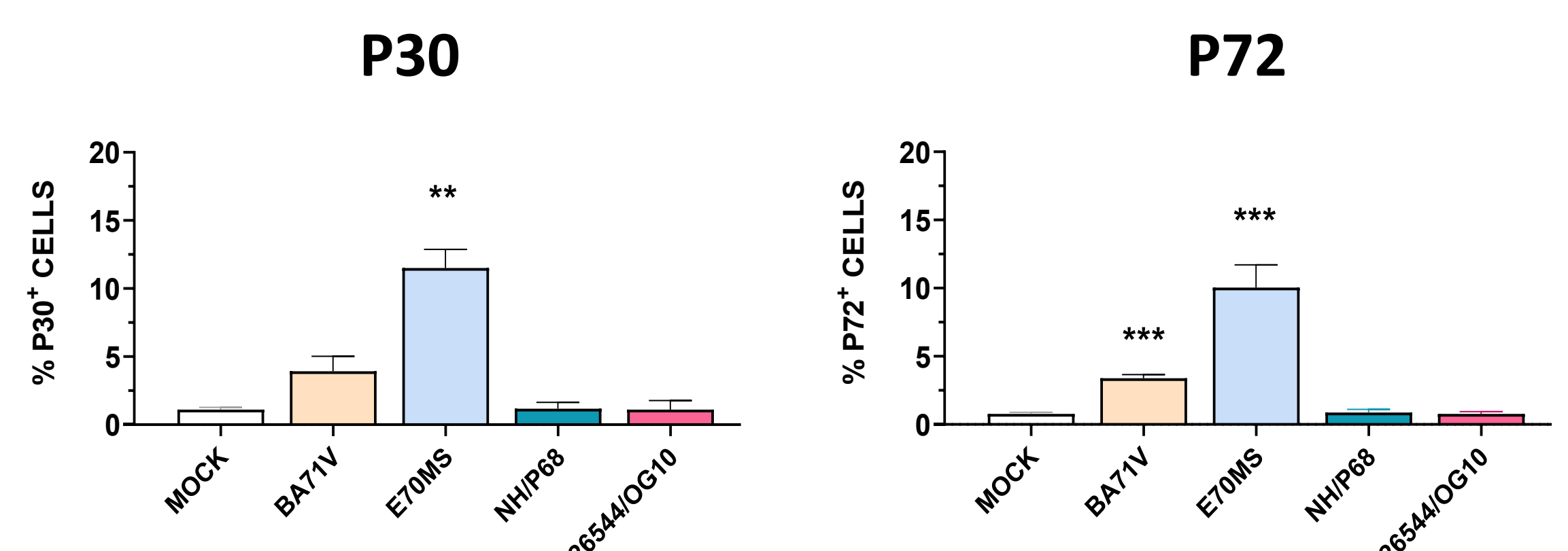


Figure 3. ASFV infection of porcine derived continuous cell line. PK-15 were infected with ASFVs strains of diverse virulence (BA71V, E70MS, NH/P68, 26544/OG10) using an MOI of 1, alongside mock-infected controls. 48 h pi percentages of ASFV early (p30+) or late protein (p72+) cells were assessed by flow cytometry.

## Conclusions

Our data showed that all tested cell lines were unsuitable as continuous cell line for ASFV isolation and propagation. We observed that adaptation to either VERO or MS resulted in their ability to infect other continuous cell lines, not only monkey-derived (COS-1), but even hamster-derived (BHK-21) and porcine-derived (PK-15). We hope that the data generated will aid the development of a suitable cell line for ASFV infection and propagation.