THE USE OF MV-GFP AS AN ONCOLYTIC VIROTHERAPY FOR CHOLANGIOCARCINOMA

UPR-MAYO CLINIC CCATS COLLABORATION PROJECT

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- Research focus: Mechanism of immune evasion in liver cancer and development of immune-directed therapies.

CHOLANGIOCARCINOMA

- Cholangiocarcinoma (CCA) is a highly lethal biliary tract malignancy with increasing incidence.
- CCA is the second most common hepatic malignancy after hepatocellular carcinoma (HCC).
- Most patients present with advanced stage disease and are not eligible for potentially curative surgical treatment options such as resection or liver transplantation.

ONCOLYTIC VIROTHERAPY

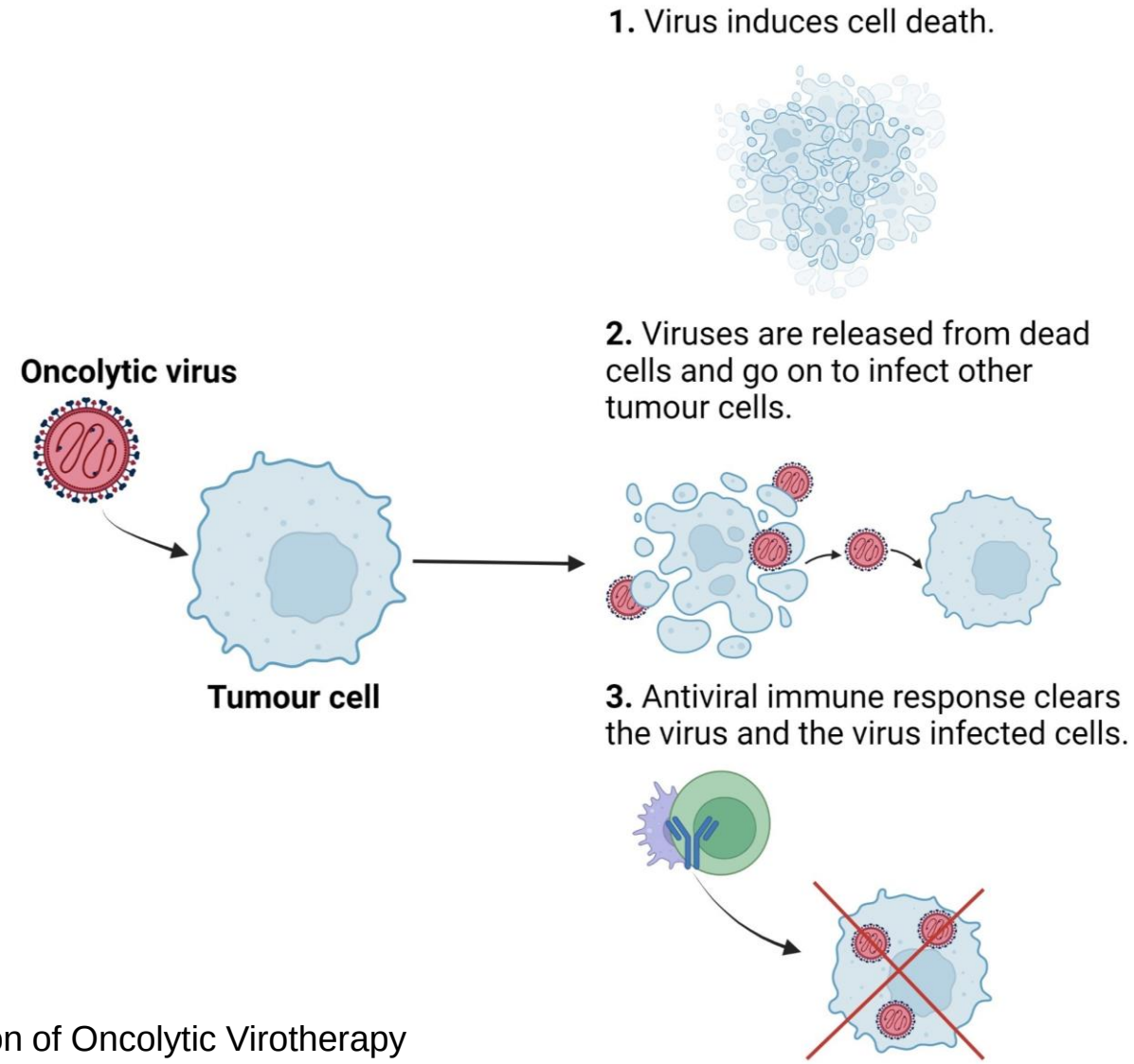


Figure 1. Schematic representation of Oncolytic Virotherapy mechanism of action.

MEASLES VIRUS

- Is an enveloped, negative-stranded morbillivirus.
- Capable of using CD46 as a cell entry receptor.
- CD46 receptor is overexpressed in most of human cancers.
- Strong correlation between CD46 expression and the oncolytic potency of vaccine-lineage MV.
- MV vaccine strains demonstrate exceptional genetic stability even after prolonged replication in human hosts.

Figure 2. Schematic representation of Measles Virus Genome. (Obtained from Domingo-Musibay et.al, 2014)

RESEARCH QUESTION

Could MV-GFP be an effective oncolytic virotherapy against Cholangiocarcinoma in-vitro?

CD46 EXPRESSION ON HUMAN CCA CELL LINES

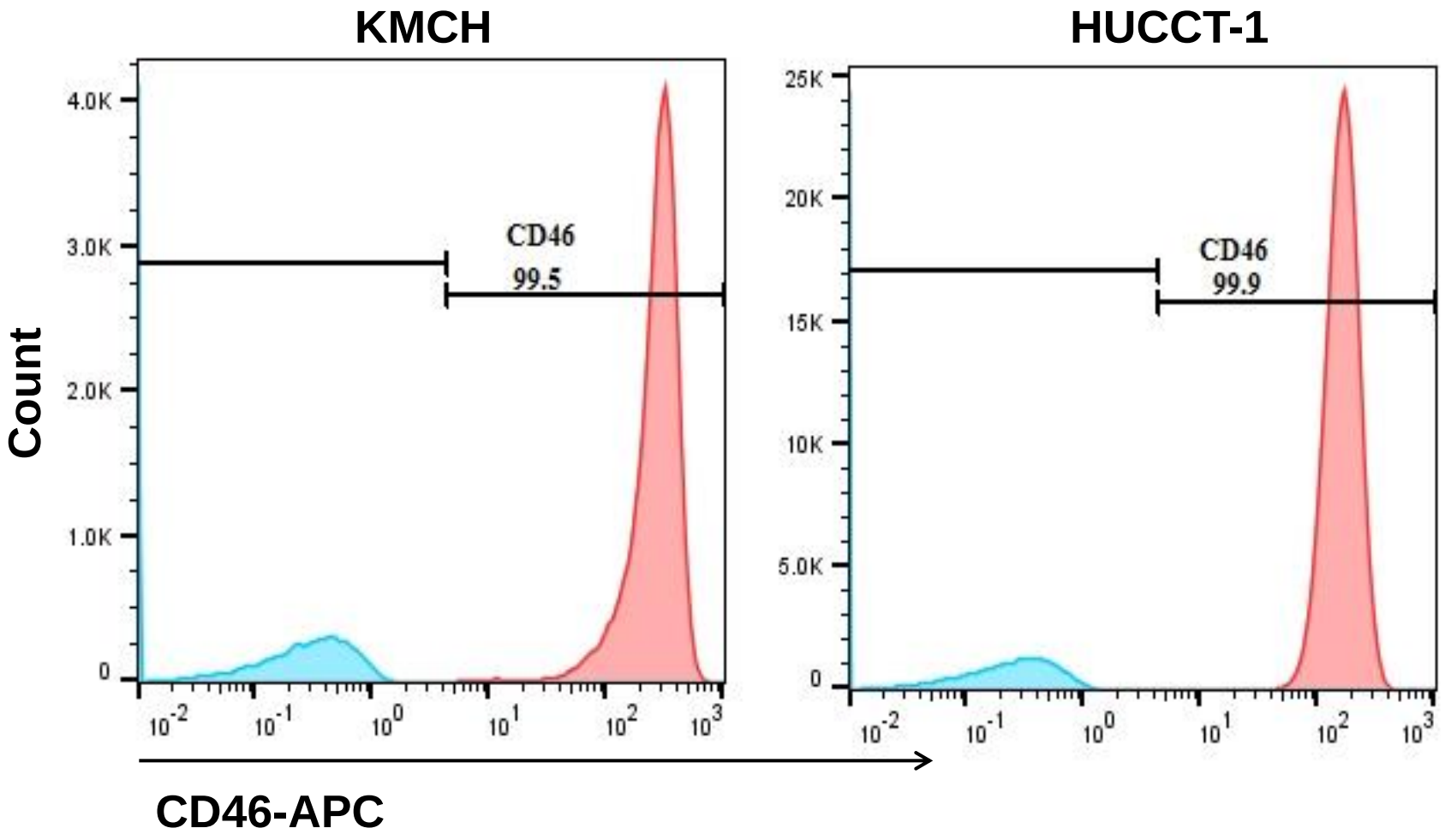


Figure 3. CD46 receptor expression assessment using flow cytometry**.**

HUMAN CCA CELLS WERE INFECTED WITH MV-GFP

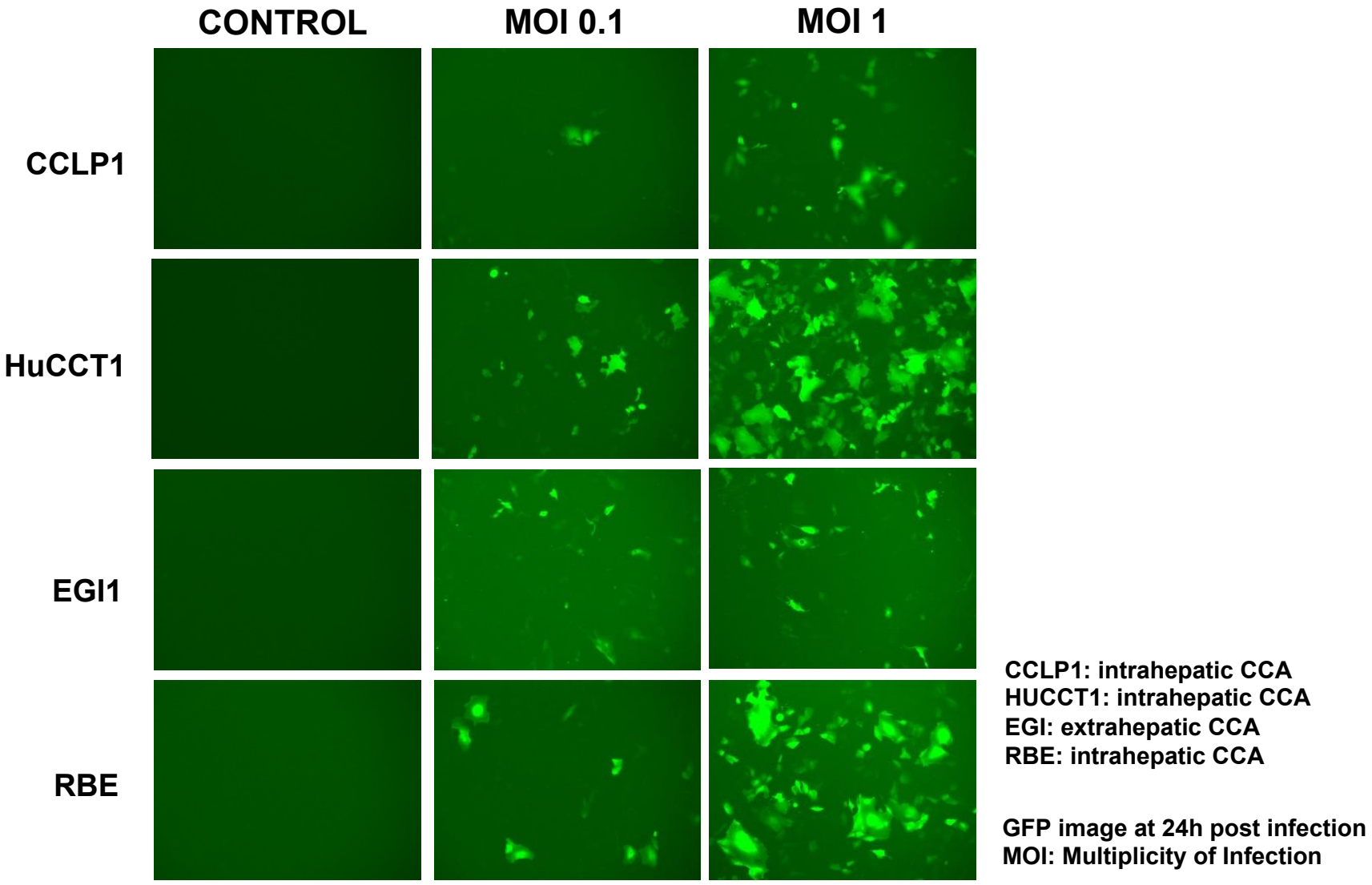


Figure 4. MV-GFP infection at different multiplicity of infections (MOI).

CELIGO® CYTOMETER INFECTION QUANTIFICATION

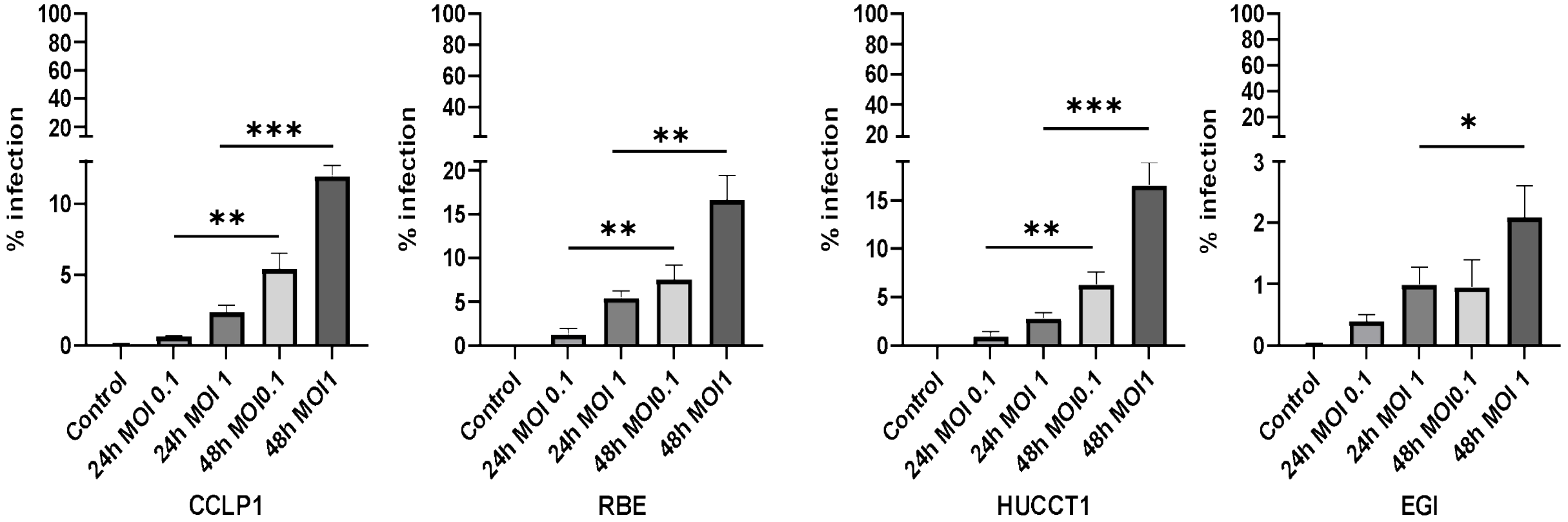


Figure 5. Infection quantification using Celigo® Cytometer.

CYTOTOXICITY OF MV-GFP ON HUMAN CELL LINES USING FLOW CYTOMETRY

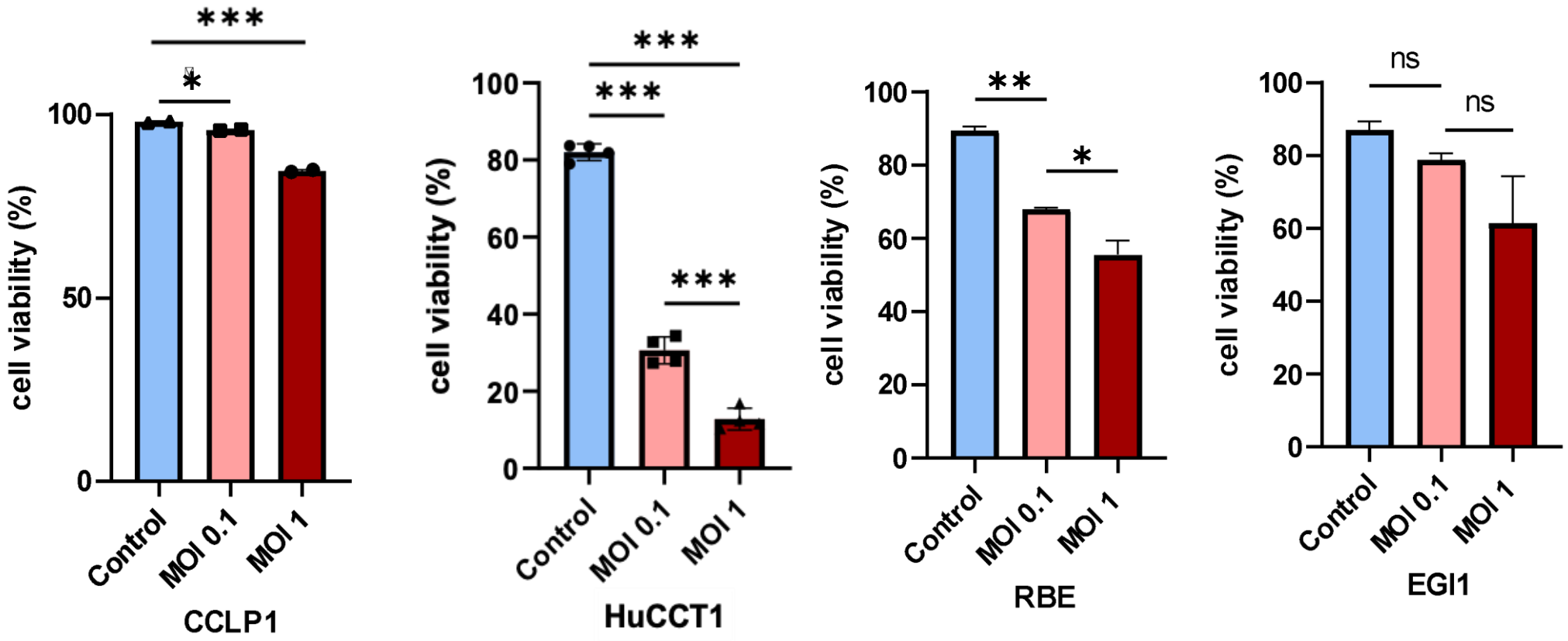


Figure 6. Cell viability assay 48 hours post MV-GFP infection using 7-AAD and Annexin V markers.

CHALLENGES

- Wild type murine cells lack CD46 receptor expression

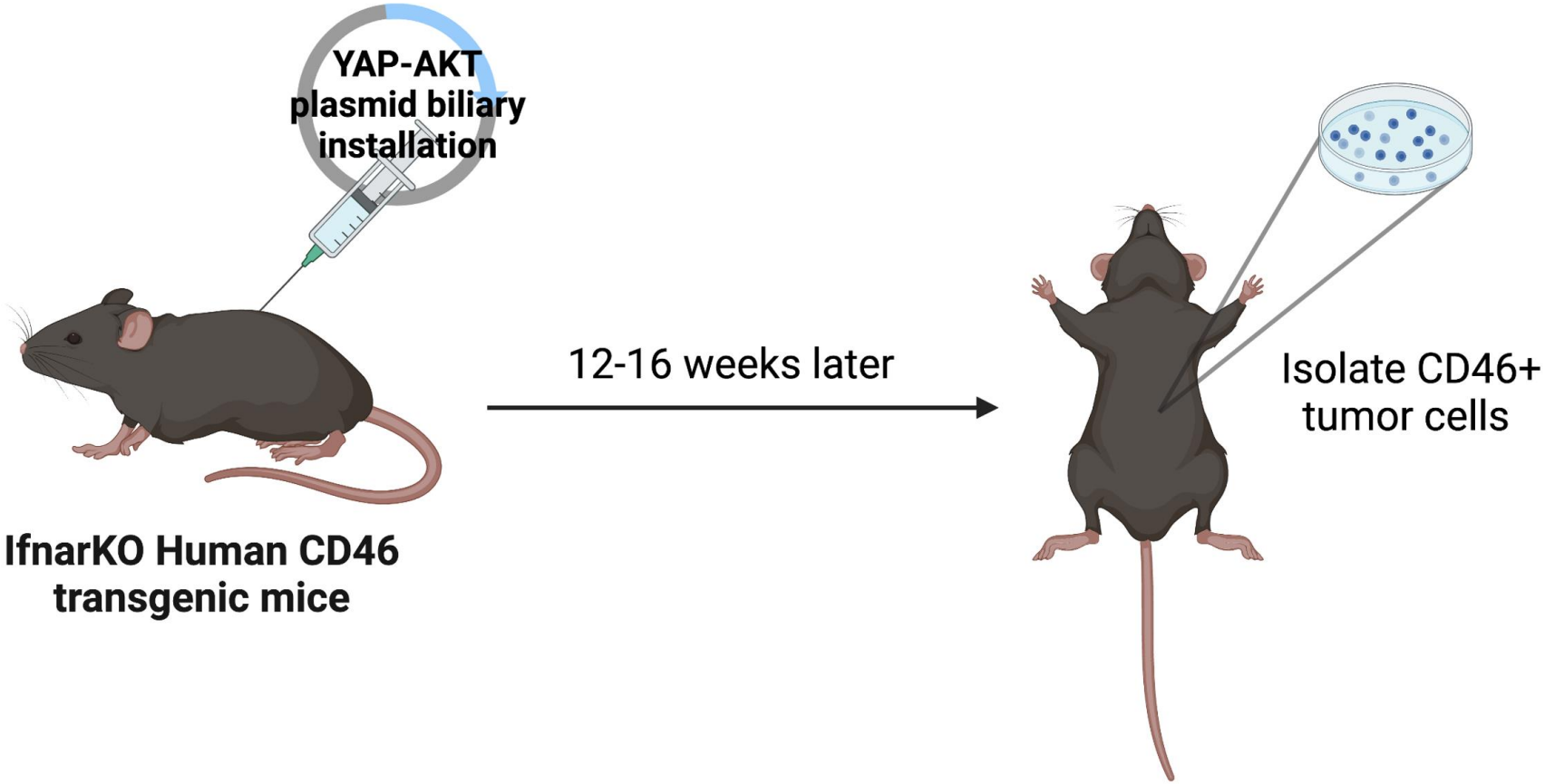
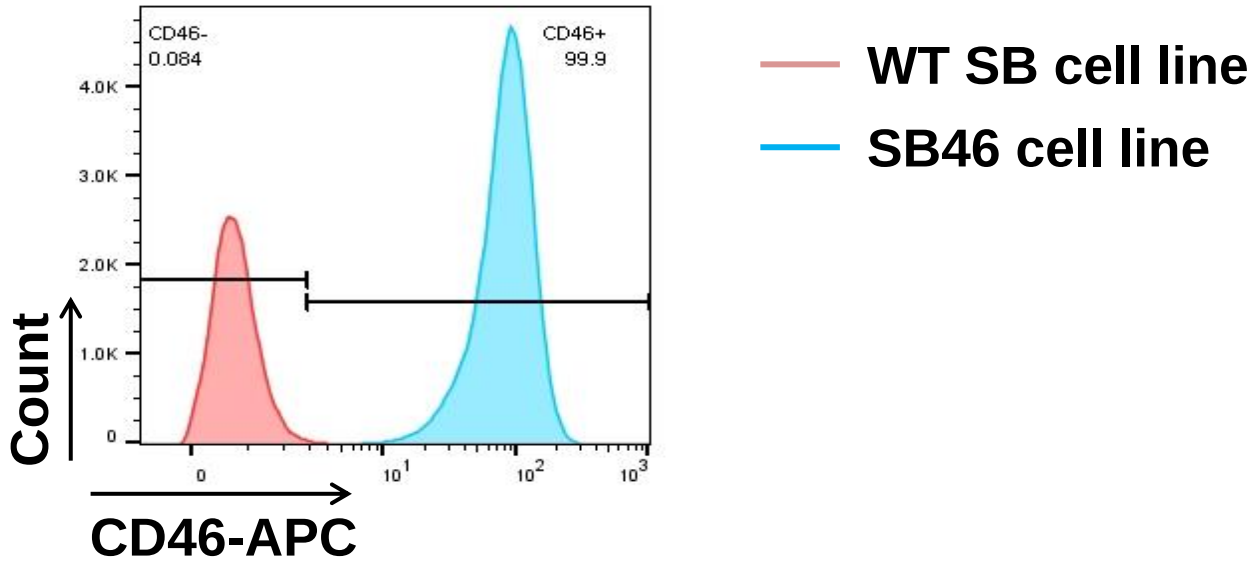


Figure 7. IFNaRKO-CD46 mouse line establishment.

CHARACTERIZATION OF MURINE CD46+ CCA CELL LINE

A)



B)

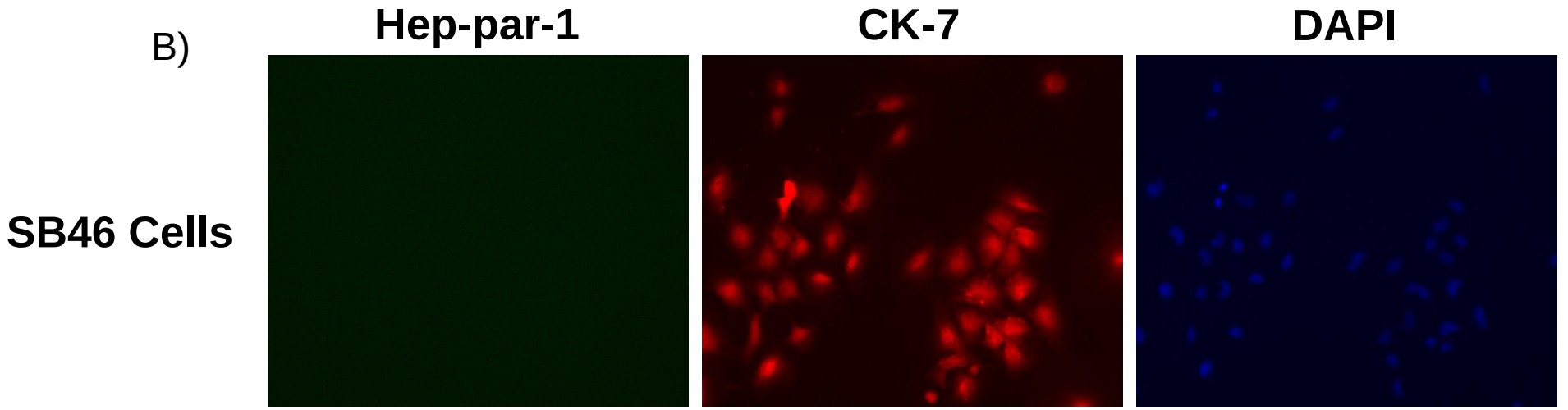
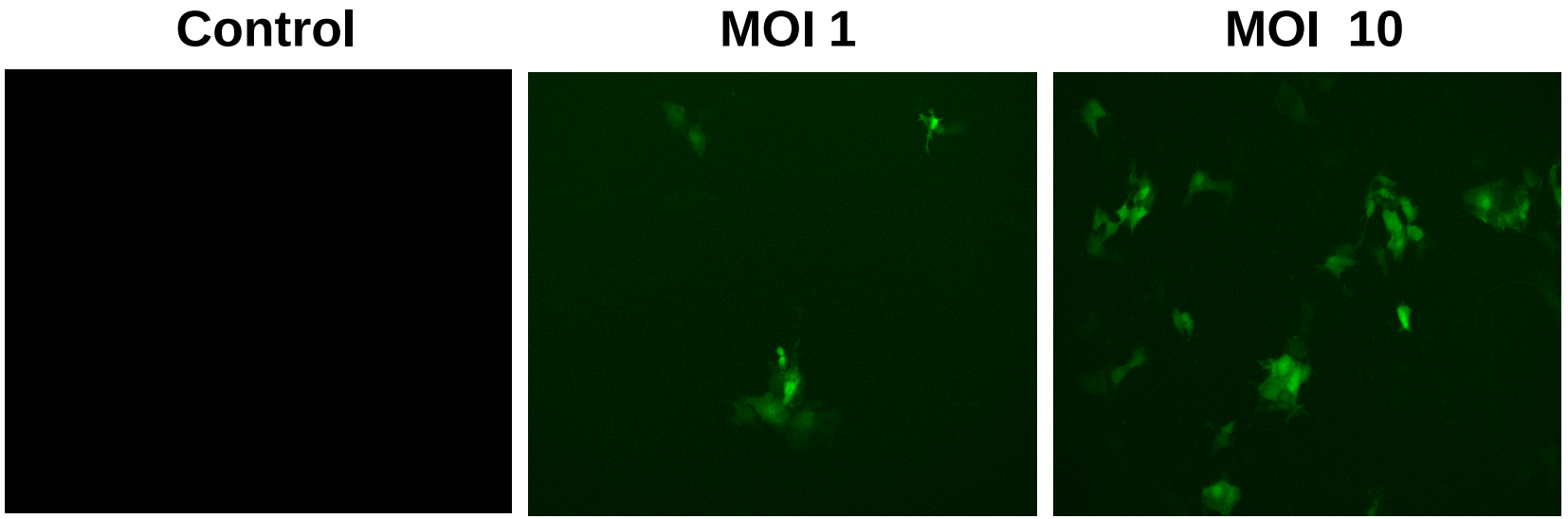


Figure 8. A) CD46 receptor expression assessment using flow cytometry. **B)** Immunohistochemistry for CCA marker (CK-7).

MURINE CD46+ CCA CELLS WERE INFECTED WITH MV-GFP

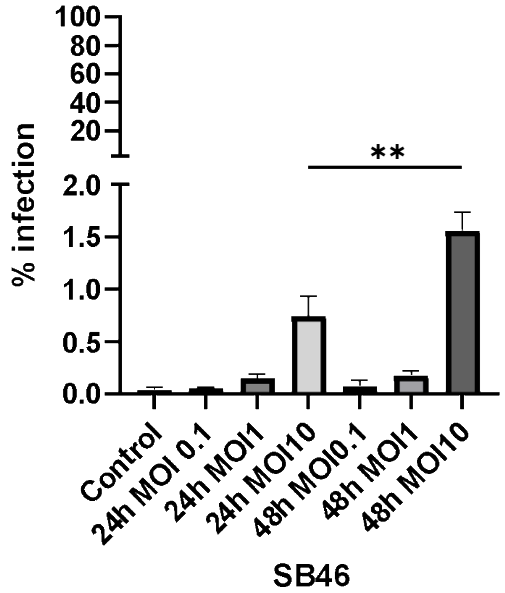
A)



GFP were imaged at 24h post infection

MOI: Multiplicity of infection

B)



C)

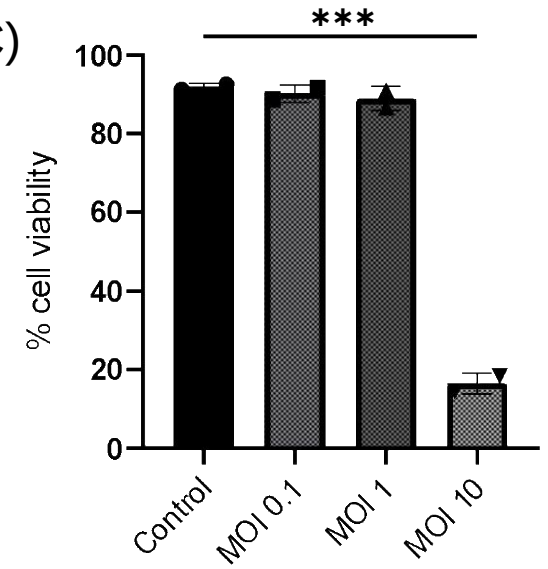


Figure 9. A) MV-GFP infection at different multiplicity of infections (MOI). **B)** Infection quantification using Celigo® Cytometer. **C)** Cell viability assay 48 hours post MV-GFP infection.

MURINE CD46+ CCA CELLS IMPLANTATION

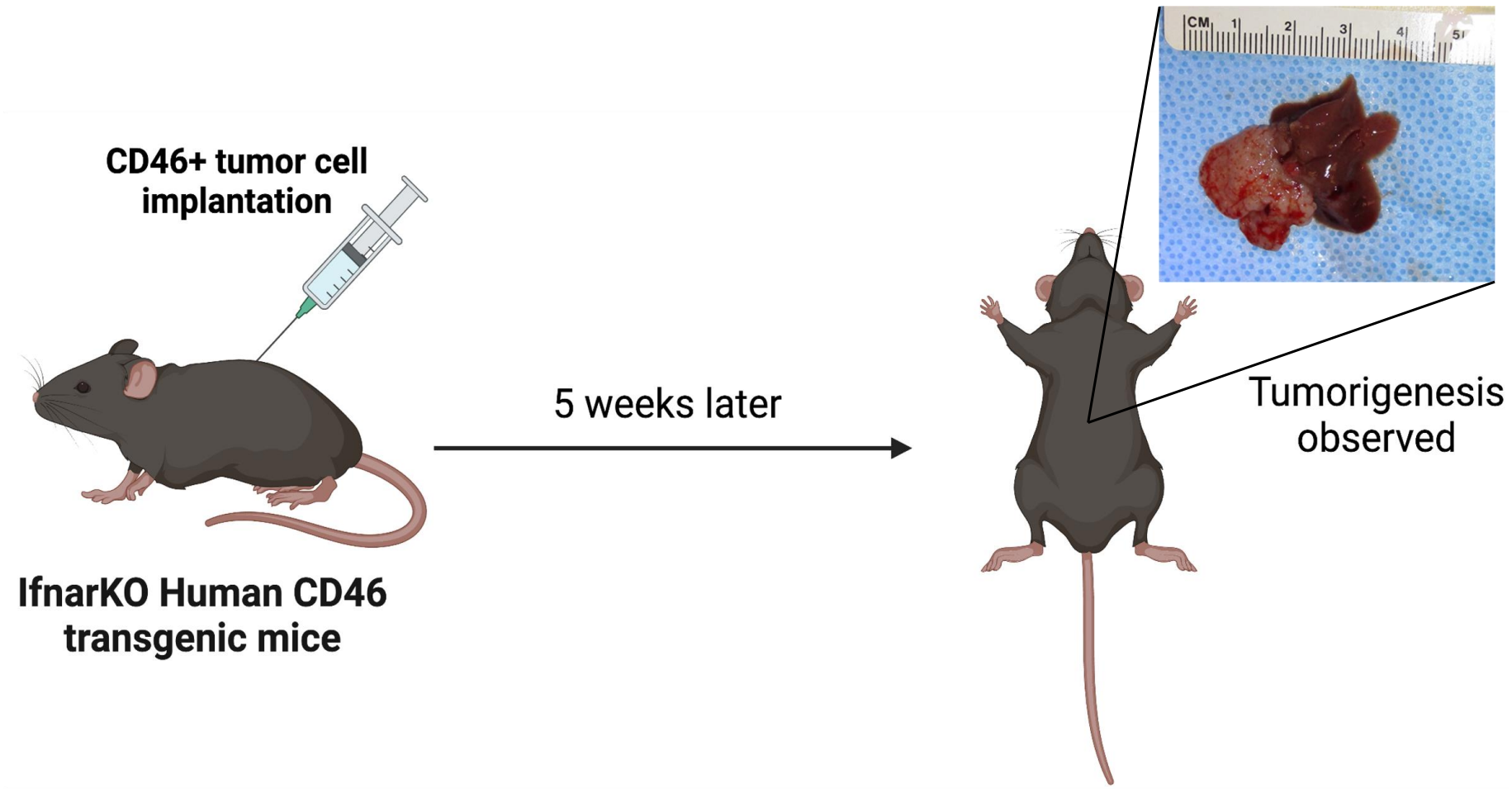


Figure 10. Schematic representation of tumor cells implantation.

IMMUNOHISTOCHEMISTRY

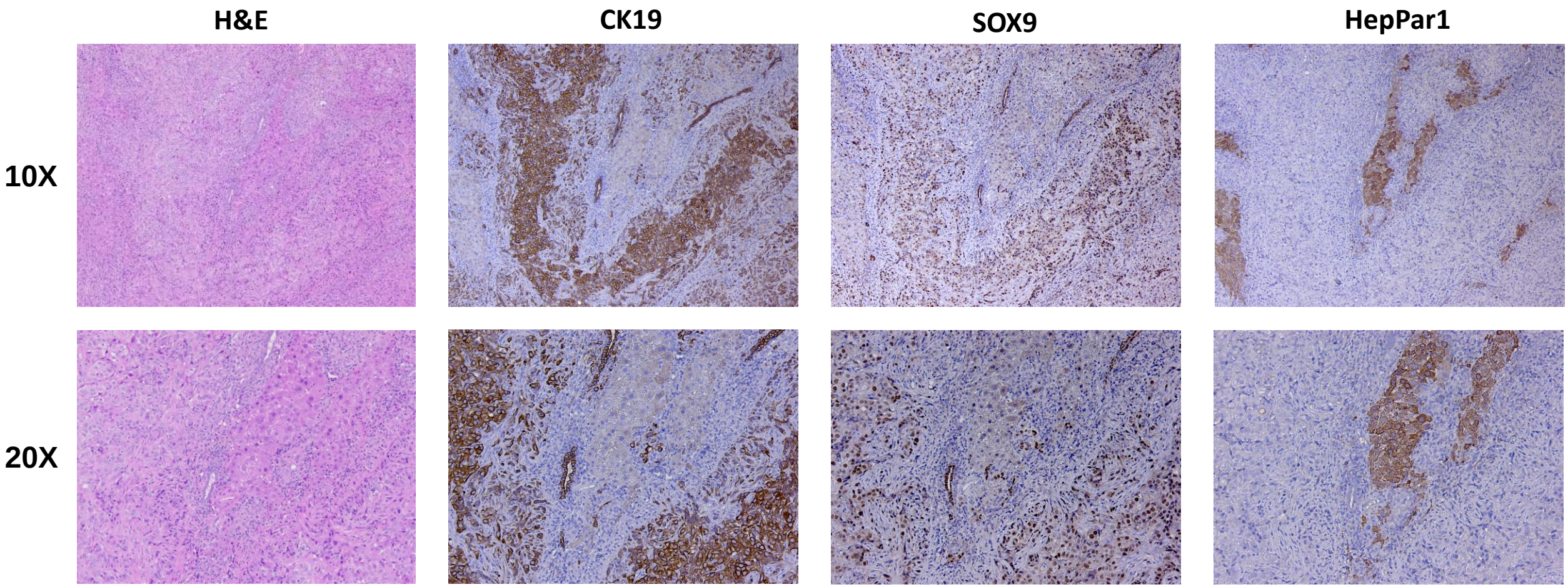


Figure 11. Immunohistochemistry for CCA markers (CK-19 & SOX9).

CONCLUSIONS

- CD46 receptor expression was observed by flow cytometry on both human and murine CCA cell lines, highlighting its importance on MV-GFP oncolytic virotherapy efficacy.

- Results indicate that MV-GFP is capable of infecting and killing human and murine CCA cell lines in an MOI (multiplicity of infection) dependent manner.

NEXT STEPS…

- Assessment of intra-tumoral versus systemic administration of MV in preclinical models of cholangiocarcinoma.

- Assessment of combination therapy with FDA approved drugs, such as Anti-PDL1 (Avelumab and Pembrolizumab).

- Incorporating models with anti-MV immunity, in order to represent general population immune status.

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