Project title: Investigate the role of CD163 as a receptor for macrophage entry of FCoV

Deliverable 1 Establishment of the cell lines

In order to investigate the role of CD163 as a receptor for macrophage entry of FCoV, we used Crandell-Rees Feline Kidney (CRFK) cells and PK-15N cells derived from the porcine kidney. CRFK cells were obtained from ATCC and PK-15N from the diagnostic lab. Both cells were cultivated in the EMEM media, supplemented with 10% fetal bovine serum (FBS), 1% HEPES, and 1% penicillin-streptomycin as antibiotics. The cells were washed in 10mL Dulbecco's phosphate-buffered saline (DPBS), replated every two days, and maintained in a humidified 5% CO2 atmosphere at 37°C. Through this process, the cell lines were established and maintained stably.

Deliverable 2 Validation of CD163 expression

CRFK and PK-15N cells were transfected with CD163 and pcDNA as the negative control in 12-well plates. CRFK cells were transfected at a concentration of 2*10^5/mL, and PK-15N cells were 4*10^5/mL. Both transfected cells were infected with type I virus FIPV-Black strain and type II virus FIPV-1146, 24 hours post-transfection. The preliminary immunofluorescence showed a moderate amount of positive staining of CD163 antigen in PK-15N cells but no significant staining in CRFK cells.

Deliverable 3 Determination of rescue of feline coronavirus

The Immunofluorescence Assay (IFA) test was conducted 24 hours post-infection. Cells were fixed with 4% paraformaldehyde and permeabilized with acetone. The primary antibody FIP-370 and the secondary antibody, Alex Fluor 488 goat anti-mouse immunoglobulins, were used. The cells were imaged under microscopy 24 hours post-incubation. Cell nuclei were counterstained with DAPI (blue), and the viral infection were shown in green. IFA test showed that CRFK cells that transfected with pcDNA were killed significantly. CRFK cells transfected with CD163 were able to be infected with FIPV-Black strain but the infection rate was extremely low, while the infection rate was much higher in the negative control group and the blank control group. CRFK cells transfected with CD163 were successfully infected with FIPV-1146 strain, od which infection rate was higher than the negative control group and FIPV-Black strain. PK-15N cells failed to be infected with either FIPV-Black strain or FIPV-1146 strain. These results could not determine whether CD163 expression is able to rescue infection by the FIPV-Black strain or FIPV-1146 strain.

The results are not expected and inconclusive, the study still has some limitations as well. Because the preliminary results validated the expression of CD163, we didn't validate it again before infection, which indicated that the failure of transfection might happen. The killed cells in the negative control group might lead to non-objective results. Different materials of coverslips, such as polymer and glasses, might affect the growth conditions. of the cells. If the study continues, we will examine more cells to test their abilities to be infected by FIPV-Black and FIPV-1146. We might also look into the conditions that affect the transfection, for instance, the integrins VLA-2, VLA-4, MAC-1 and LFA-1, which have been shown to be upregulated in FIP.

More quantitative studies, such as TICD50 will also be conducted to test the infectivity of the virus.

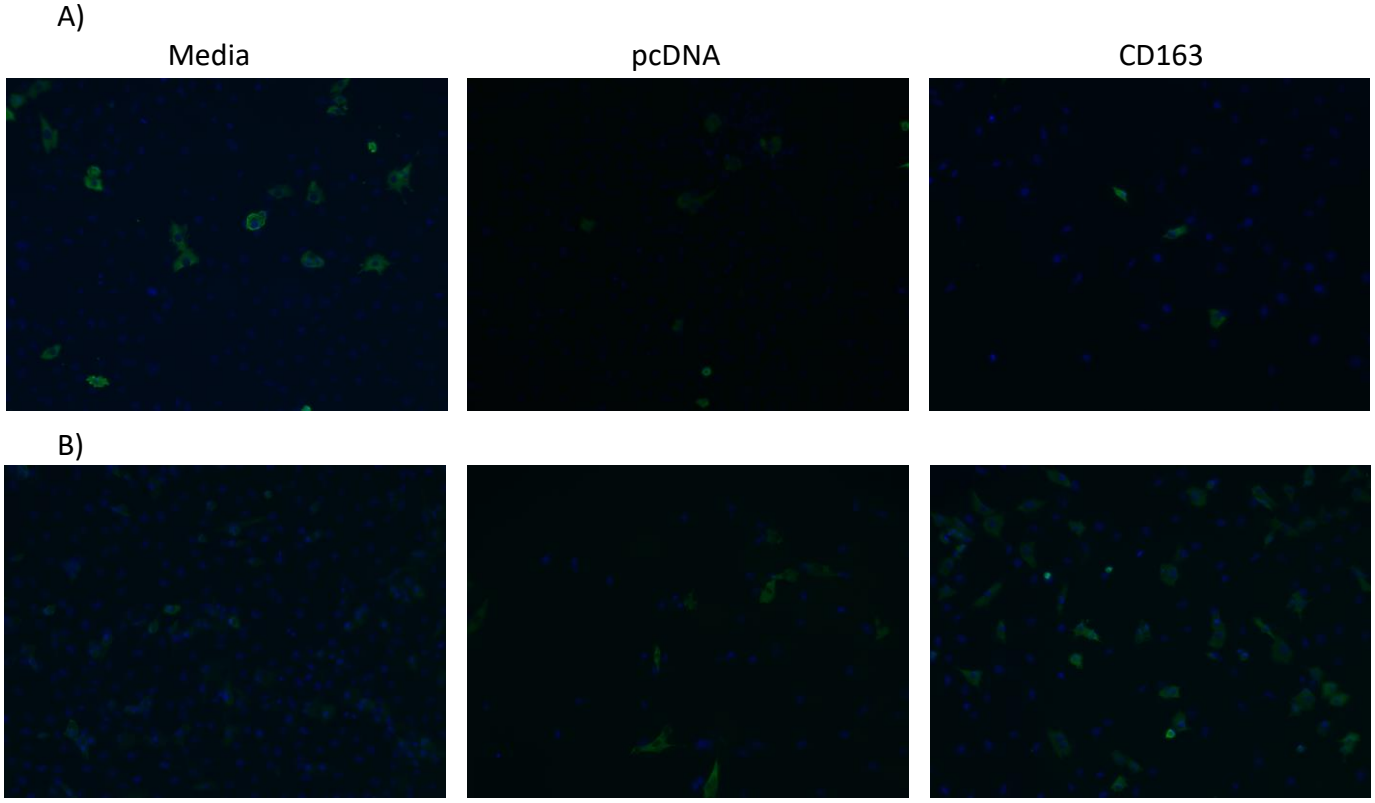

Figure 1. IFA results of CRFK cells transfected with CD163 and infected with FIPV-Black and FIPV-1146. Cell nuclei were counterstained with DAPI (blue), and the viral infection should be shown in green. The brightness of all images was increased by 20% manually. A) FIPV-Black strain. The viral infection was detected in the blank group and infection rate was low in the CD163 group. B) FIPV-1146 strain. The viral infection was detected in all groups and the cells were killed significantly in the negative control group. The infection rate was higher in the CD163 group.