REF: 04/MF/AS

Project Title: Rapamycin mediated degradation of chicken *IFITM3*

Supervisors: Mark Fife and Angela Steyn **Research group:** Genetics and Genomics

Project Summary:

Rapamycin is a drug characterised primarily by its ability to suppress the immune system and has been shown to potently inhibit downstream signalling from the target of rapamycin (TOR) proteins. In mammals, rapamycin treatment results in the degradation of the interferon-induced transmembrane protein 3 (*IFITM3)*, a potent antiviral protein which inhibits the entry of a broad range of viruses into cells. The genetics and genomics group focus primarily on the antiviral effect of chicken *IFITMs* (*chIFITMs)* in avian cell lines and tissues. There is currently no data that reports an effect on *chIFITM3* upon rapamycin treatment*,* therefore providing an exciting opportunity to produce and report novel data. This research project will be instrumental in enhancing our understanding of the effect that rapamycin treatment has on the degradation of *chIFITM3* as well as any other cellular process that may be affected.

Details:

In order to determine whether rapamycin treatment will a) result in the degradation or down-regulation of *chIFITM3* in an avian cell type and b) promote IAV infection in these cells, the project will consist of three main objectives.

1. Determine the dose dependent effect of rapamycin treatment on endogenous *chIFITM3* expression in avian cell lines (DF1 and CEFs)

The cells will be exposed to micro molar quantities of rapamycin in a time course experiment (4 – 12 hours). Flow cytometry and western blotting will be used to determine whether rapamycin treatment has a dose dependent effect on *chIFITM3* expression in these cells and if the effect is transient or progressive over time.

2. Determine if the effect of rapamycin treatment, if any, occurs at the mRNA levels and/or protein levels.

Quantitative real-time-PCR will be used to determine *chIFITM3* mRNA levels after rapamycin treatment. A chIFITM3 specific antibody will be used to identify levels of protein after rapamycin treatment by western blot.

3. Effect of rapamycin treatment on AIV infected cells

Determine if rapamycin promotes AIV (H5N3 and H9N2) infection in cells. We will also treat AIV infected DF1 *IFITM3* knock out cells with rapamycin to determine whether *chIFITM3* expression is required for the enhancement of the viral infection by rapamycin.

The student will be trained and gain experience in cell culture as well as all of the techniques and methods required to complete the project. All this work will be done in a multi-user laboratory with one of the post docs in the group.

References for Suggested Reading:

- Shi G, Ozog S, Torbett B E, and Compton A A (2018). mTOR inhibitors lower an intrinsic barrier to virus infection mediated by IFITM3. Proc Natl Acad Sci U S A 115(43) E10069–E10078.
- Bailey CC, Zhong G, Huang IC, Farzan M (2014) IFITM-Family Proteins: The Cell's First Line of Antiviral Defense. Annu Rev Virol. 1;1:261-283.