University of Glasgow | College of Medical, Veterinary & Life Sciences

Head of College Scholars List Scheme

Summer Studentship

Report Form

This report should be completed by the student with his/her project supervisor. It should summarise the work undertaken during the project and, once completed, should be sent by email to: jill.morrison@glasgow.ac.uk within four weeks of the end of the studentship.

1. Student

   Surname: Ferguson Forename: Annabelle

   E-mail address: 2194249F@student.gla.ac.uk

2. Supervisor

   Surname: McLauchlan Forename: John

   E-mail address: john.mclauchlan@glasgow.ac.uk

3. Research Project Report

   3.1 Project Title (maximum 20 words):

   Generation of a functional EGFP-tagged interferon lambda receptor 1 (IFNLR1) for live-cell imaging

   3.2 Project Lay Summary (copied from application):

   The project would focus on the effects of reintroducing the cell receptor for interferon-λ, a highly understudied antiviral signalling molecule, and viewing the consequences this has on the cells when they are infected with Zika and influenza viruses. The student would learn many fundamental techniques for working in a lab with viruses including the growth mammalian tissue culture and the specific aseptic technique required for success in this. Communication

skills and confidence will be greatly developed with weekly meetings and a final presentation with the group.

3.3 Start Date: 19/06/2017 Finish Date: 11/08/2017

3.4 Original project aims and objectives (100 words max):

The main aim was to reintroduce the IFNLR1 into a line of IFNLR1 knockout (KO) cells, created by the McLauchlan group. The KO cells were infected with IAV and ZIKV to determine the level of protection. An expression plasmid containing EGFP-tagged IFNLR1 was cloned and transfected into KO cells. The functionality of the EGFP-IFNLR1 was tested using qPCR and identified via confocal and live-cell microscopy.

A stable line of Cas9-producing cells was created by the McLauchlan group. Genes for KO were identified using Desktop Genetics and cells were infected with EMCV to determine the effect of knocking out these genes.

3.5 Methodology: Summarise and include reference to training received in research methods etc. (250 words max):

STAT1 wild type (WT), STAT1 KO, IFNLR1 WT, and IFNLR1 KO cells were grown in a 96-well plate. They were stimulated with either GFP supernatant, IFN-α or IFN-λ. The cells were infected with 10-fold dilutions of IAV and a primary anti-IAV antibody was added. A secondary immunofluorescent antibody was added so infected cells would fluoresce green under microscope. The number of infected cells were counted using ImageJ at concentrations A, B, C, and D.

EGFP-tagged IFNLR1 was cut with restriction enzymes, as was the expression plasmid to make them compatible. These were ligated together and the DNA purified. The plasmid was transformed into chemically competent *Escherichia coli* and miniprepped. EGFP-IFNLR1 was transfected into STAT1 WT, STAT1 KO, IFNLR1 WT, and IFNLR1 KO A549 and HepaRG cells.

A 12 well plate was set up such as in figure 1. Samples 1-8 had RNA extracted and converted to cDNA for quantitative PCR (qPCR).

Single guide RNA (sgRNA) was cloned into an expression plasmid and IFN stimulated genes (ISGs) for KO were designed using Desktop Genetics. STAT1, a molecule required for IFN type I and type III signalling, ISG15, a well-known antiviral gene, and PML, another known antiviral gene, were selected for this experiment. 3 variants of each gene was used, as well as 2 “scrambled” genes for controls. The cells were infected with EMCV and stained with crystal violet in a 96-well plate.

3.6 Results: Summarise key findings (300 words max). Please include any relevant tables or images as an appendix to this report:

The number of IAV infected IFNLR1 KO cells was counted using ImageJ at concentrations A, the highest, decreasing 10-fold to concentration D. This showed that STAT1 KO cells were less protected than STAT1 WT cells, while IFNLR1 KO cells had less protection with IFN-λ than with IFN-α compared to IFNLR1 WT cells (figure 2). The percentage of infected cells increased in IFN-λ stimulated STAT1 and IFNLR1 KO cells.

IFNLR1 WT and IFNLR1 KO cells were also infected with ZIKV (figure 3) which showed fewer living cells in the ZIKV infected INFLR1 KO cells than the ZIKV infected IFNLR1 WT cells.

RNA was extracted from EGFP and EGFP-IFNLR1 transfected STAT1 WT, STAT1 KO, IFNLR1 WT, and IFNLR1 KO cells and converted to cDNA. qPCR was done with a *GAPDH* primer, a well-studied gene involved in cell metabolism, to normalise results and an *MX1* primer, an ISG, to measure the functionality of EGFP-IFNLR1. As seen in figure 4, there was a 20-fold increase in MX1 expression in EGFP-IFNLR1 transfected IFNLR1 KO cells.

Confocal of EGFP transfected IFNLR1 KO cells showed a wide distribution of EGFP, as seen in figure 5. Figure 6 from confocal shows a membranous distribution of EGFP-IFNLR1. Live-cell imaging of the EGFP-IFNLR1 transfected IFNLR1 KO cells showed the receptor to be actively transported within the cell. A cluster shown in figure 7 was tracked over 45 seconds in figure 8.

The Cas9 cells were infected with EMCV and stained with crystal violet and the number of living cells was counted using ImageJ. The number of living cells decreased for each replicate of STAT1 in the Cas9-producing cells (figure 9) compared to the WT cells (figure 10), suggesting that the CRISPR-Cas9 system was effective. Similar results were seen in the ISG15 and PML KO cells, as seen in figures 11-14.

3.7 Discussion (500 words max):

STAT1 is essential for cell protection by IFN-α and IFN-λ as it forms part of IFN-stimulated gene factor 3 (ISGF3) when either receptor is activated (Wack et al. 2015), as seen in figure 15. ISGF3 leads to the transcription of ISGs, which protect the infected and surrounding cells from the virus. In STAT1 KO cells, the percentage of IAV infected cells increased compared to the STAT1 WT cells when stimulated with IFN-α or IFN-λ (figure 2), suggesting the cells received less protection from ISGs. IFNLR1 is only essential for protection by IFN-λ, as IFN-α uses different proteins in its receptor. In IFNLR1 KO cells, the number of IAV infected cells increased compared to IFNLR1 WT cells when stimulated with IFN-λ. The number of infected cells in IFNLR1 WT and IFNLR1 KO cells was similar when stimulated with IFN-α (figure 2), suggesting these cells still received protection.

The addition of a fluorescent tag to a protein can cause a change in protein folding and thus a change or loss of functionality (Snapp 2005). The EGFP-IFNLR1 proved able to activate ISGs through qPCR (figure 4), suggesting that the receptor is still functional with EGFP attached. This will allow the receptor to be studied in greater detail using techniques that require fluorescence such as more detailed imaging.

The live-cell confocal showed that the receptor could be tracked in real time and showed the receptor to be actively transported within the cell without any IFN stimulation. This will allow the receptor to be tracked in the cell with IFN stimulation and during different virus infections.

CRISPR involves the protein Cas9 which uses sgRNA to target a section of gene that can be removed from the genome, as seen in figure 16 (Sander & Joung 2014). Host response to virus infection is complex as hundreds of ISGs can become activated. Much information is already known about many of these ISGs but the better understood they are, the better they can be used to our advantage.

Desktop Genetics is a much less time-consuming method to generate a list of suitable regions to create KO cells. The CRISPR experiment showed that Cas9 can be successfully transduced into cells to create a stable cell line and the results suggest that STAT1, ISG15, and PML were successfully knocked out of the cells. Each showed a change in the number of living cells compared to their respective WT versions, as expected from knowing their function. The 3 variants for the same gene showed fluctuating results, as seen with STAT1 variant 1 KO which decreased the number of living cells more significantly than variant 2 or 3. It is hoped that Desktop Genetics can be used in further CRISPR experiments to allow large-scale experiments to be done with more genes in much less time. Due to the success of the CRISPR experiment in this project, a longer list of 27 genes with 3 variants each has been compiled using Desktop Genetics for further research by the McLauchlan group.

4. Reflection by the student on the experience and value of the studentship (300 words max):

The studentship offered an insight into a career as a research scientist and opened my mind to the prospect of a PhD. Furthermore, I enjoyed the opportunity to work as part of an excellent, dynamic team who welcomed me warmly as an individual and as a professional. As my confidence grew, I was given the opportunity to work independently which required a diverse range of skills. Importantly, my time management skills greatly improved as I was granted more of the responsibilities to control and balance my experiments and research commitments throughout my time. My time in the labs have consolidated the necessity for procedural health and safety protocol awareness as I was responsible for my own experiments. I learned to self-analyse making competent dynamic decisions, I believe this is an experience only a professional working environment could offer, as it would be very difficult to reproduce the multi-dimensional fluency of a working lab within teaching labs due to the necessity to set out the experiments for the learner with a bias for a successful outcome. Due to the more realistic nature of the experiments we were doing in a professional functioning lab, some experiments did not go as planned. This allowed me to hone my problem solving skills whilst fine tuning my own laboratory abilities. This also required me to

consider both the experiments, their design and potential outcomes in greater detail and with greater clarity, an essential skill in research science.

The studentship further enhanced my communication capabilities through weekly team meetings, developing good interpersonal relationships and my self-confidence through experiences such as representing the CVR at public events.

5. Dissemination: (note any presentations/publications submitted/planned from the work):

   A poster was designed by the student.

6. Signatures: Supervisor John M°Lauchlan Date 05/09/2017

   Student Annabelle Ferguson Date 05/09/2017

Appendix

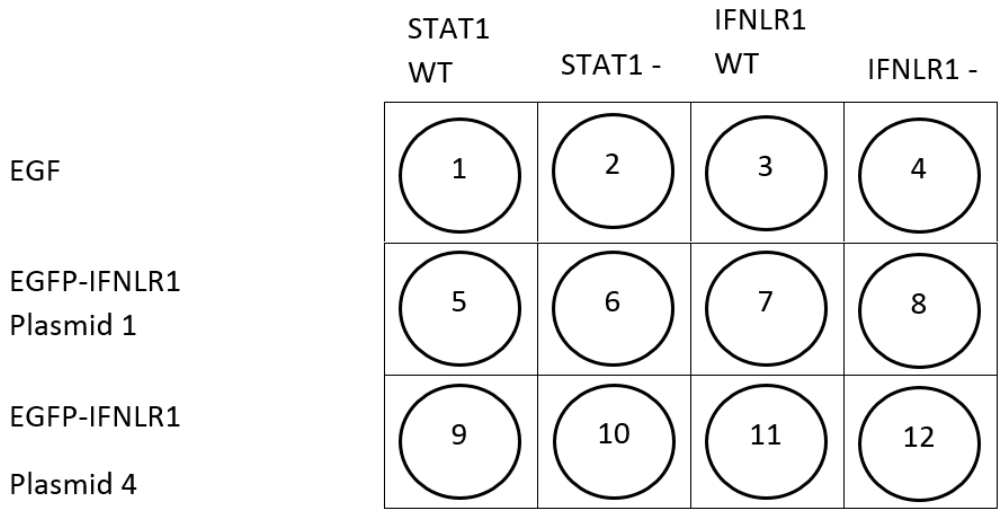

Figure 1: 12-well EGFP and EGFP-IFNLR1 transfected cells.

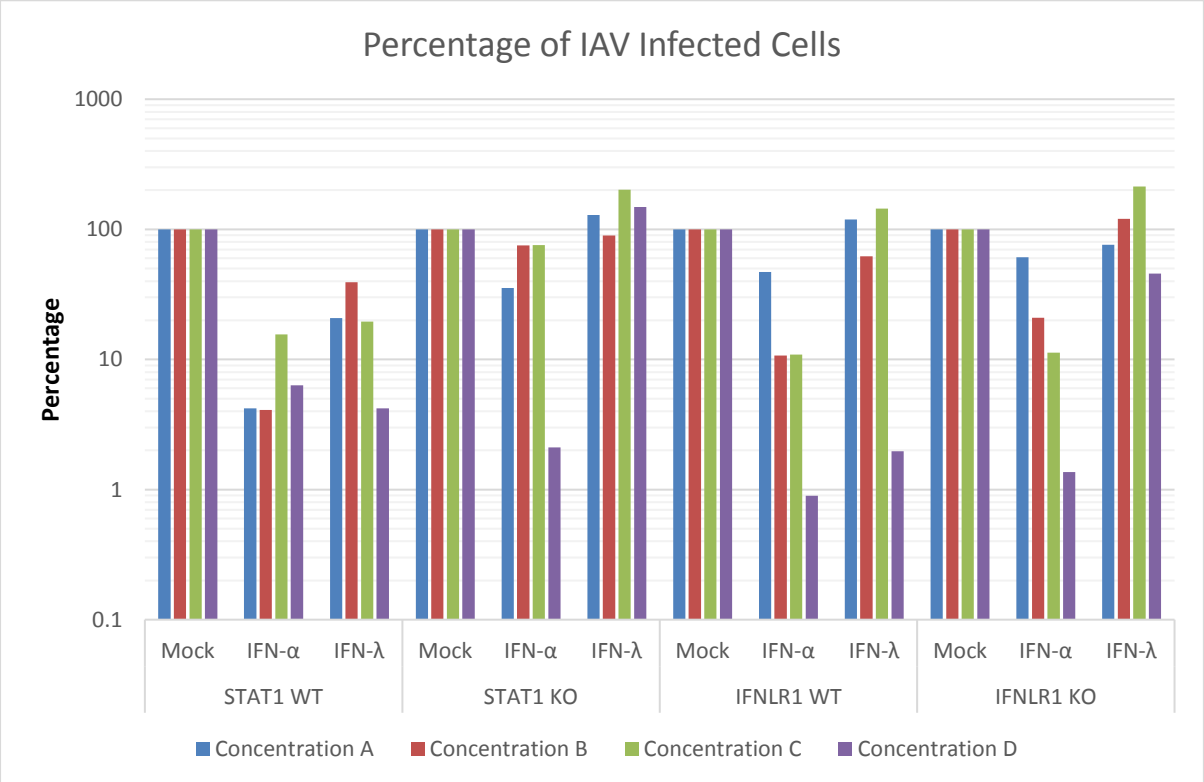

Figure 2: percentage of IAV infected STAT1 WT, STAT1 KO, IFNLR1 WT and IFNLR1 KO cells with GFP supernatant, IFN-α, or IFN-λ stimulation.

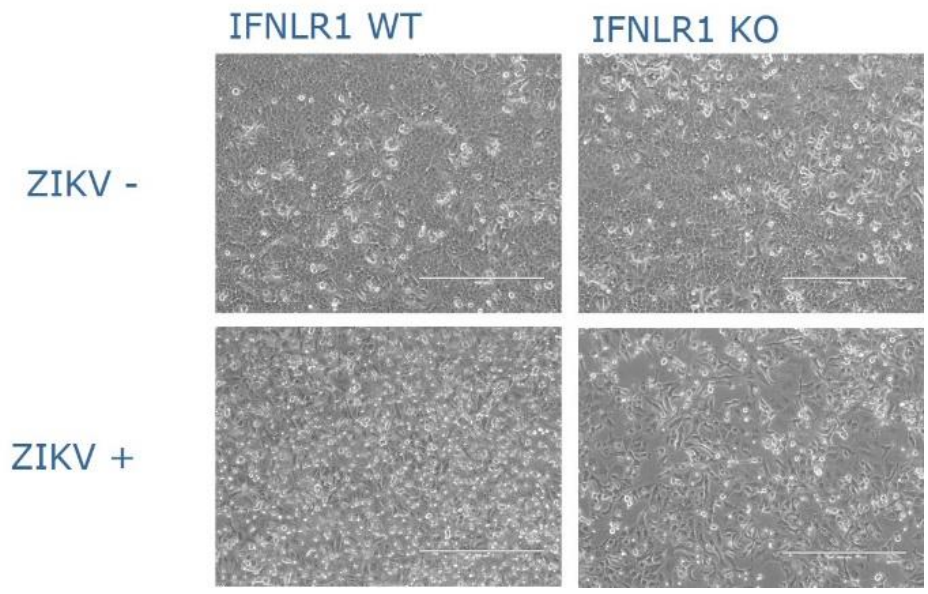

Figure 3: IFNLR1 WT and IFNLR1 KO infected with ZIKV.

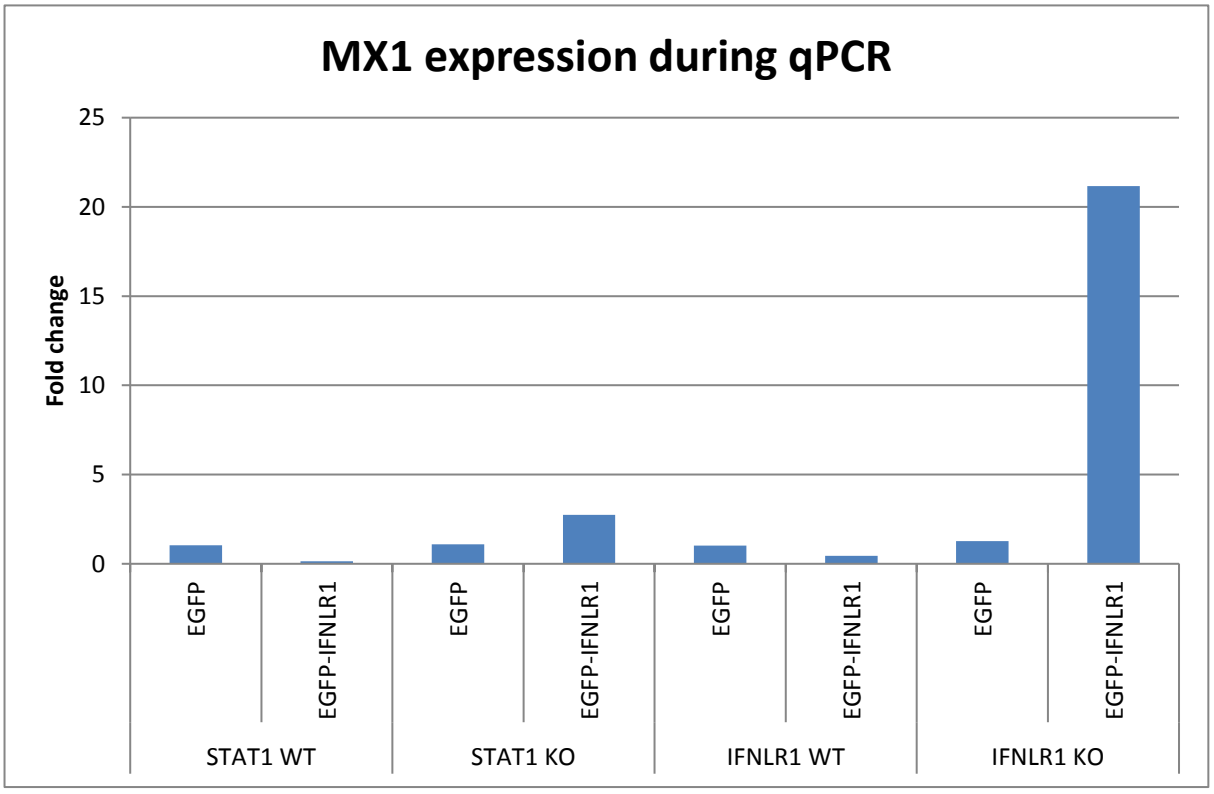

Figure 4: MX1 expression in STAT1 WT, STAT1 KO, IFNLR1 WT, and IFNLR1 KO cells transfected with EGFP-IFNLR1.

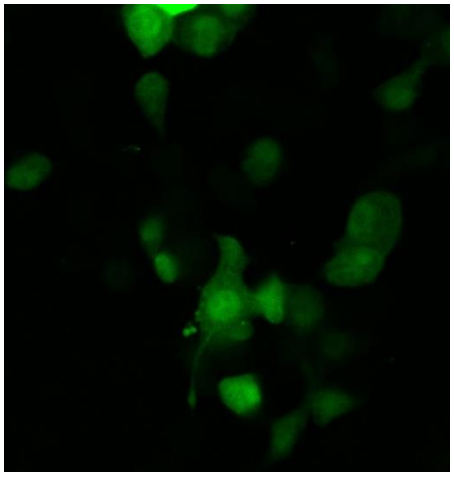

Figure 5: confocal microscope image of EGFP transfected cells.

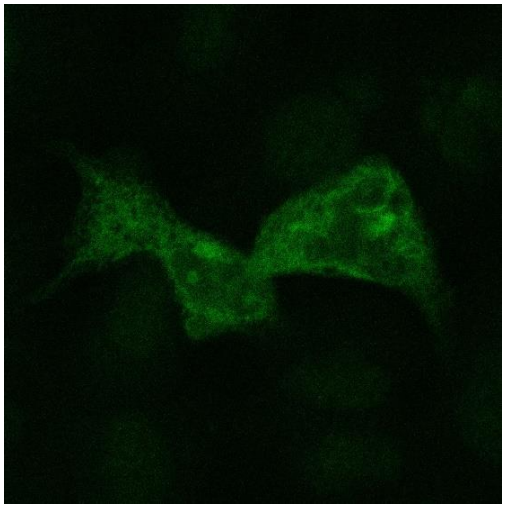

Figure 6: confocal microscope image of EGFP-IFNLR1 transfected cells.

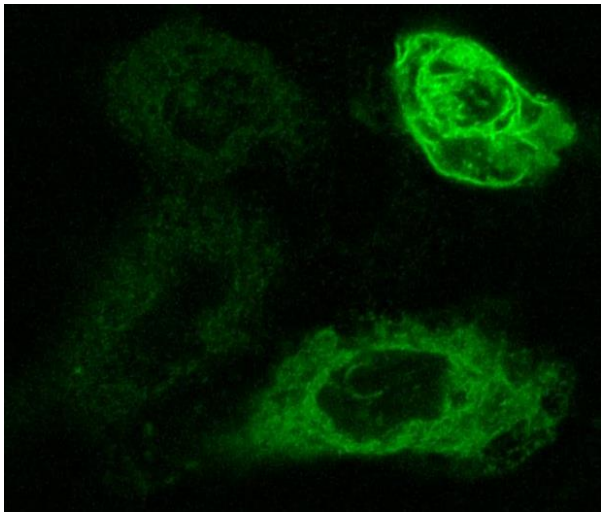

Figure 7: live-cell image of EGFP-IFNLR1 transfected cells with highlighted cluster.

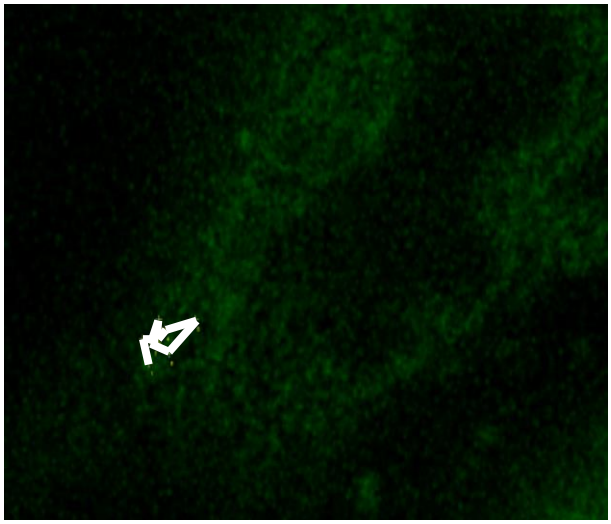

Figure 8: tracking of the EGFP-IFNLR1 cluster over 45 seconds.

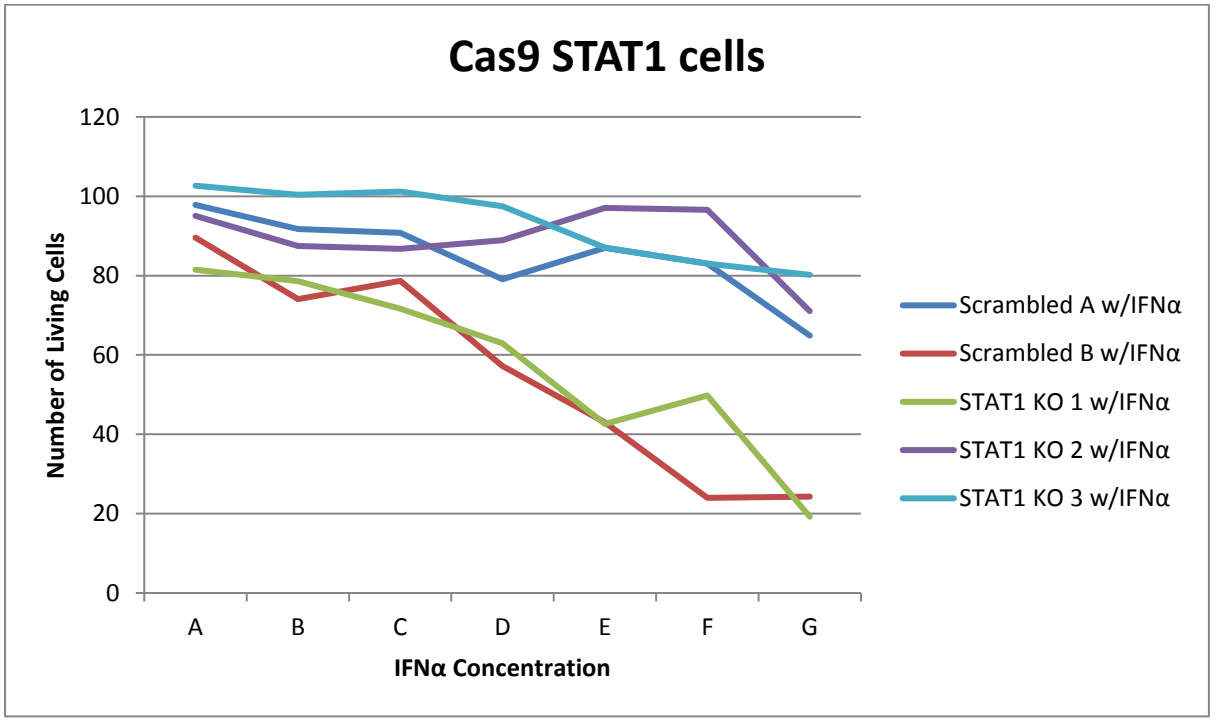

Figure 9: the number of living cells in Cas9-producing STAT1 KO cells.

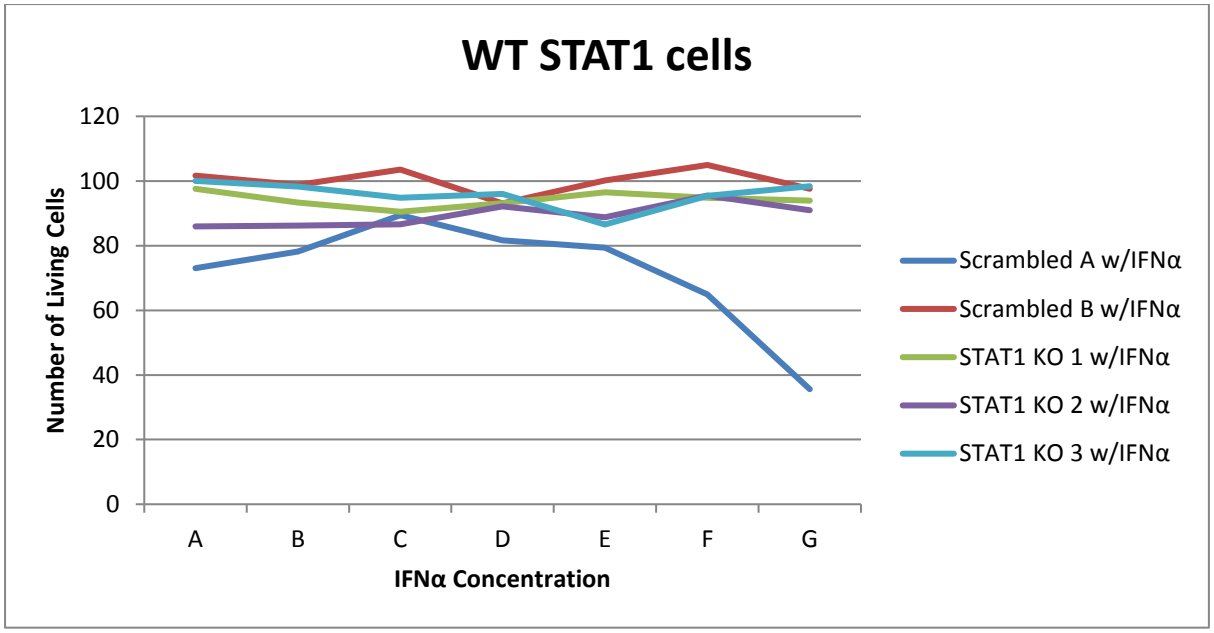

Figure 10: the number of living cells in STAT1 WT cells.

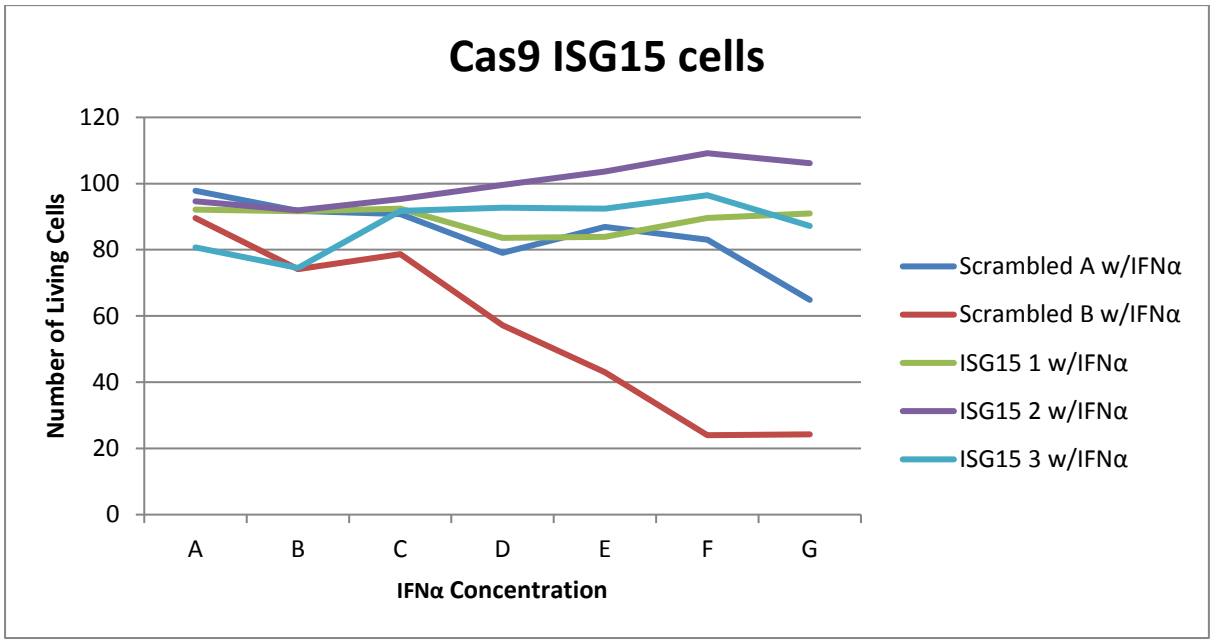

Figure 11: the number of living cells in Cas9-producing ISG15 KO cells.

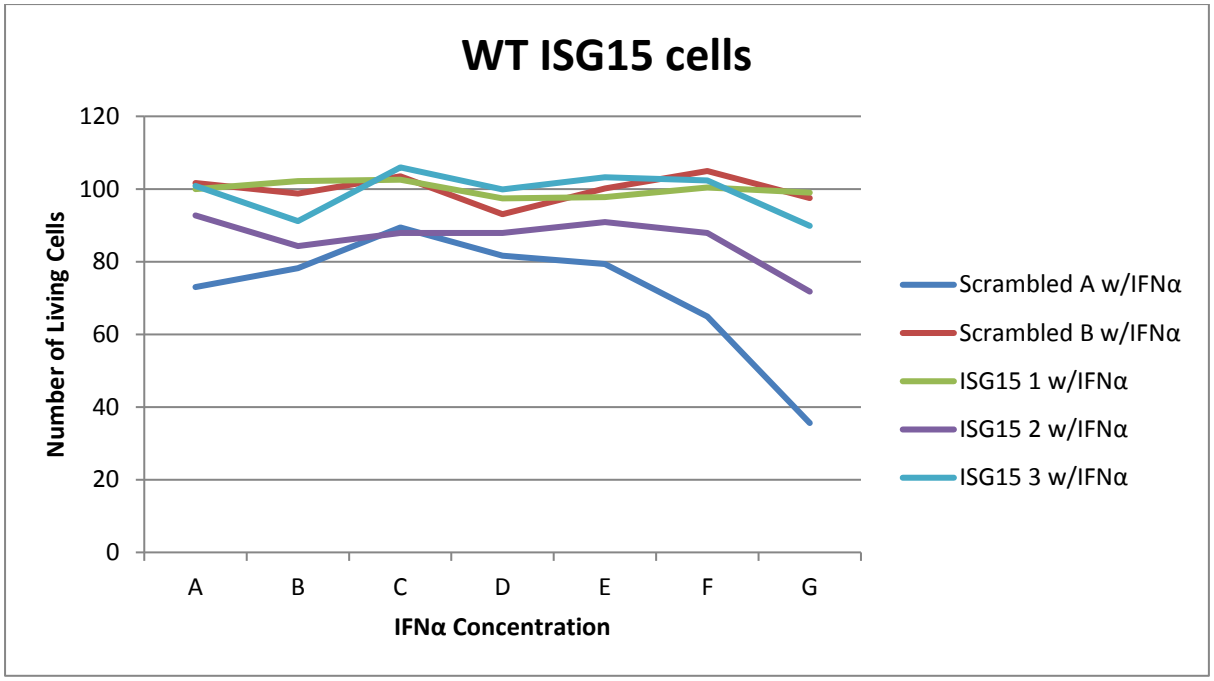

Figure 12: the number of living cells in ISG15 WT cells.

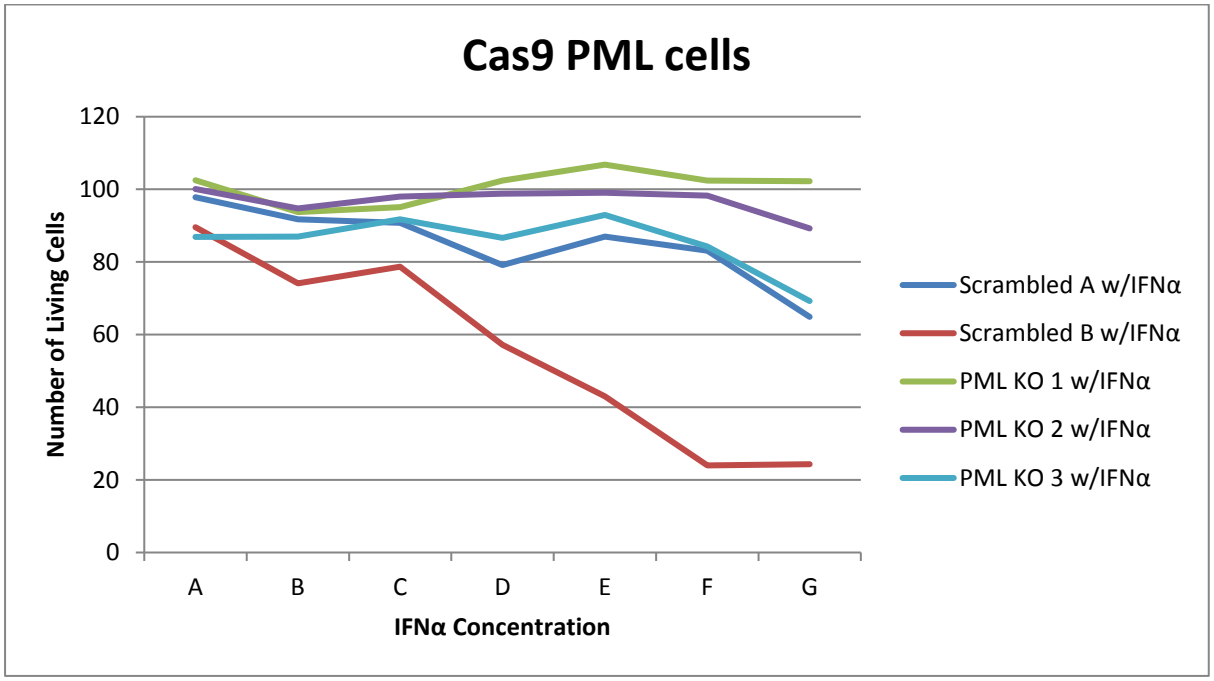

Figure 13: the number of living cells in Cas9-producing PML KO cells.

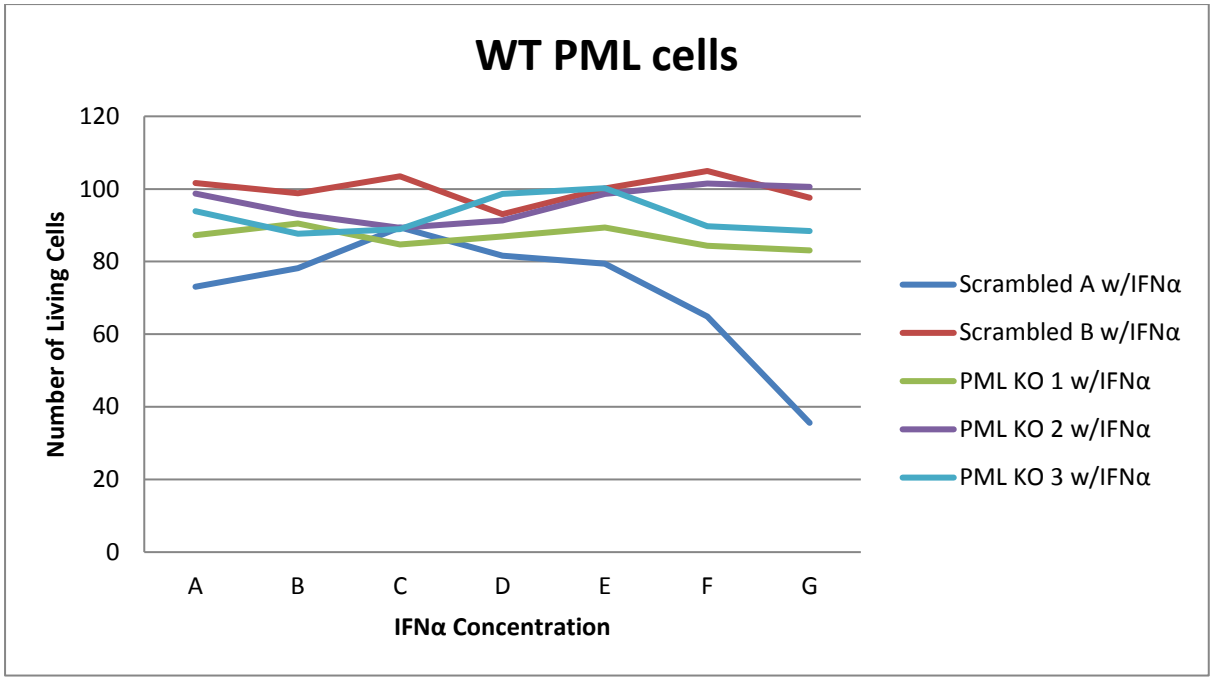

Figure 14: the number of living cells in PML WT cells.

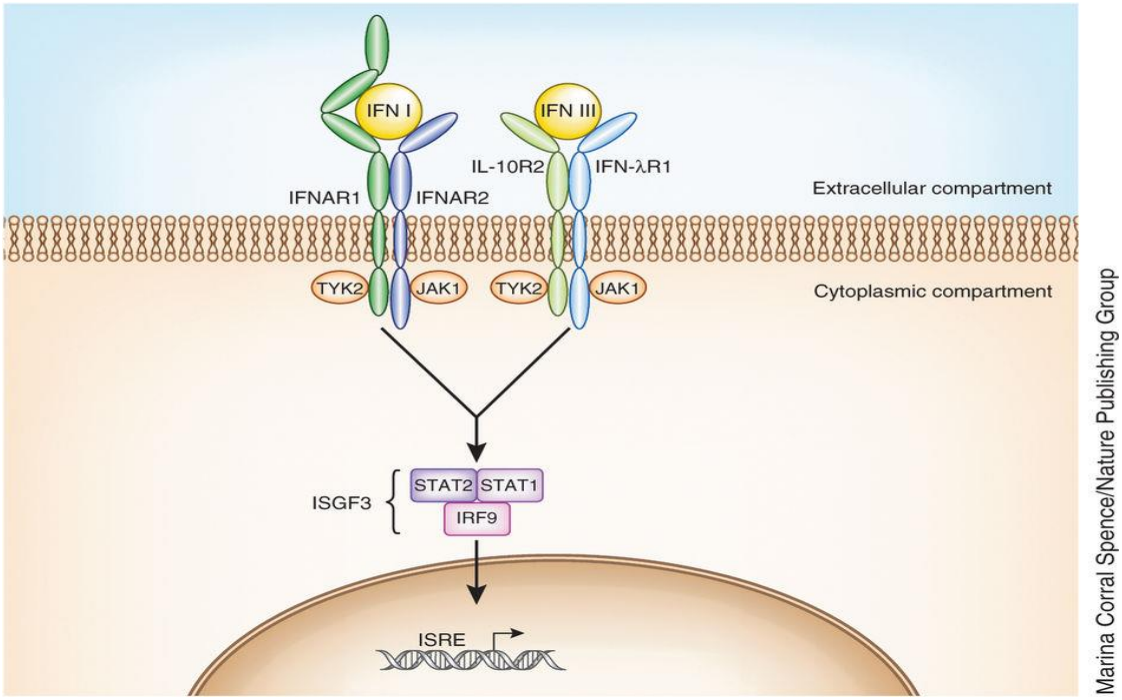

Figure 15: IFN-α and IFN-λ signalling (Wack et al. 2015).

Figure 16: the CRISPR-Cas9 system uses the Cas9 protein and a single guide RNA to remove a section of a gene from the genome (Sander & Joung 2014).

References:

Sander, J.D. & Joung, J.K., 2014. CRISPR-Cas systems for editing, regulating and targeting genomes. *Nature biotechnology*, 32(4), pp.347–55. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24584096 [Accessed September 4, 2017].

Snapp, E., 2005. Design and use of fluorescent fusion proteins in cell biology. *Current protocols in cell biology*, Chapter 21, p.Unit 21.4. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18228466 [Accessed September 4, 2017].

Wack, A., Terczynska-Dyla, E. & Hartmann, R., 2015. Guarding the frontiers: the biology of type III interferons. *Nature Immunology*, 16, pp.802–809. Available at: https://www.nature.com/ni/journal/v16/n8/pdf/ni.3212.pdf [Accessed September 1, 2017].