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: Sanchez Velazquez Forename: Ricardo

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2. Supervisor:

Surname: Wilson Forename: Sam

E-mail address: Sam.wilson@glasgow.ac.uk

3. Research Project Report

1. Project Title (maximum 20 words):

Can fever activate temperature dependent cell autonomous antiviral immunity?

2. Project Lay Summary (copied from application):

To understand the role of fever in limiting the replication of viruses I propose to compare Vesicular Stomatitis Virus' (VSV) ability to infect a variety of cells (in vitro) at different temperatures.

Crucially, "fever temperature" decreases susceptibility of human monocytes to VSV infection by over 100-fold (Schnorr et al. 1993). This could be due to a deficiency of viral or host components crucial for infection, or the host-cell actively blocking the infection at higher temperatures. The benefits of this project include greater understanding of viruses' response to temperature, cell antiviral response mechanisms, and potentially the development of new treatment strategies to control viral infections.

3. Start Date: June 2nd 2015 Finish Date: June 29th 2015

4. Original project aims and objectives (100 words max):

The main aim of this project was to determine if increased temperature had a stimulating role in cell autonomous antiviral immunity. In order to reach my aim, three other objectives were set.

1. Firstly, it was important to determine if the inhibition of VSV was due to an intrinsic high temperature sensitivity of the virus particle.

2. Then, I wanted to find out which human and animal immortalised cell lines inhibit VSV at fever temperatures of 40°C.

3. Finally, to what extent did inhibition occur in the different cell lines, and compare the degree of inhibition between normal human body temperature (37°C) and human fever temperatures (40°C).

1. Methodology: Summarise and include reference to training received in research methods etc.:

To begin with, it was important to nourish and regularly passage all the cell lines in order to have enough cells in the plates ready for the experiments. In addition, single-cycle VSV was produced (refer to Appendix A) by transfecting HEK 293T with VSV-glycoprotein DNA, and subsequently infecting the HEK 293T with existent stocks of VSV-GFP. As a result, single cycle G*ΔG-GFP-rVSV (VSV) expressing Green Fluorescent Protein (GFP) was harvested and frozen.

Each animal cell line was seeded in a 96-well plate at a density of 20,000 cells per well and infected with serially diluted VSV before overnight incubation. The cells were fixed with 4% formaldehyde, and analysed using flow cytometry. The following experiments were conducted with different animal cell lines in this format.

1. Degradation experiment: To investigate whether VSV particles are intrinsically defective at high temperatures, one aliquot of VSV was placed at 37ºC, another at 40ºC for 8 hours. Subsequently, HEK 293T cells were challenged with the virus stock kept at different temperatures, and an aliquot of virus titrated immediately after thawing to act as a control, and all plates were subsequently incubated at 37ºC overnight prior to fixation and analysis as described above.

2. The second experiment consisted of challenge with VSV. Duplicate plates identically infected with VSV were incubated at 37°C and 40°C overnight, to simulate human body temperature and human fever temperatures respectively. The following morning, the 96 well plates were fixed with formaldehyde saline and analysed as described above. Additionally, C6/36 insect cells were also incubated at their standard culture temperature of 28°C.

The fixed cells were analysed using flow cytometry in the form shown in appendix B, where the machine detects the percentage of the cell sample population infected by VSV (determined by counting GFP- positive cells). Later, the data were retrieved and plotted in the form of linear graphs, also known as titration curves, shown in Appendix C comparing the percentage of GFP at the different temperatures. Finally, the infectious units per milliltre (IU.ml) were calculated and the bar chart was plotted showing the mean IU.ml of each infection in each cell line.

The training I received as part of this project includes: production of single cycle G*ΔG-GFP-rVSV, animal cell culture techniques, viral titrations and how to use a benchtop flow cytometer.

1. **Results: Summarise key findings. Please include any relevant tables or images as an appendix to this report:**

First of all, the G*ΔG-GFP-rVSV stock I produced was able to infect all the cell lines I used, including insects and humans. More importantly, the virus did not lose the ability to infect 293T cells when kept for 8 hours at 40°, therefore it can be strongly suggested that the virus is not intrinsically defective at fever temperatures 40°C , but rather that the ability to infect cells at different temperature is context dependent and can vary in different cell lines. To support this statement, appendix B shows that the titres are quite similar in both virus stocks kept at 37°C and 40°C.

Moreover, incubating the cells after infection with VSV at 40°C had different outcomes for some cells. In other words, not every cell line was equally susceptible to VSV infection at 40°; however most cell lines were less susceptible to VSV infection at higher temperatures. From Appendix E, it is possible to appreciate that some cell lines were up

to a 1000 fold less susceptible (MDBK) to VSV infection at higher temperatures than at normal body temperatures of 37°C.

Crucially, it is important to note that DF1 cells and 293T do not share the same change in susceptibility to infection by VSV between 37°C and 40°C. Thus we conclude the differential susceptibility phenotypes are entirely cell-type dependent and not due to intrinsic properties of VSV. It is therefore possible that these cell lines do not possess temperature-dependent anti-VSV activity.

Alternatively, host factors required by VSV may be differentially regulated in different cell contexts, resulting in differential susceptibility in the absence of cell-autonomous antiviral activity.

Finally, it is worth mentioning that mosquito cells C363 (Aedes aegypti) were also able to limit VSV at higher temperatures even though they lack an interferon-based cellular immunity of vertebrates.

2. Discussion:

The fact that VSV on its own was not degraded at high temperatures (see Appendix D) provides evidence suggesting that significant decrease in VSV numbers could be due to cellular immunity triggered by the recognition of Pathogen-Associated Molecular Patterns (PAMPS), however further testing with exogenous interferon treatment could shade light on this possibility. In vertebrates, Type I interferon is induced after recognition of foreign particles by Toll-like receptors in order to fight viral infections. Type I Interferon induces transcription factors that induce the expression of pro-apoptotic and anti-growth factors in cells around infection to avoid propagation of the pathogen (Perry et al. 2005). To ensure there was no additional help of antibodies or

complement to limit VSV, heat-inactivated calf serum was used throughout the experiment.

Crucially, fever temperatures may enhance the interferon response and consequently decrease VSV replication in the cell cultures. Interestingly, not all human cell lines showed the same response against VSV. At fever temperatures, while PM1 (CD4+ T cell) showed 100-fold reduction of VSV (Tristan Dennis, Unpublished data), 293T (kidney cell) showed a slight enhancement of infectivity, which is not significant enough to take into consideration. It can be concluded that not all human cell lines are able to limit viral replication to the same extent. It is possible that immune cells PM1 can inhibit viral replication to a greater extent than kidney cells (293T).

Furthermore, MDBK (bovine kidney) and Vero 6 (monkey kidney) cell types had a substantial response against infection ranging from a 100-fold to more than 1000-fold inhibition of VSV, suggesting that fever temperatures can activate cell autonomous antiviral activity in these cell lines. Conversely, DF1 (chicken) showed no significant antiviral response at 40°C. This may have been due to the fact that chicken have a slightly higher body temperature (40.6°C-43°C) than the rest of the animals, therefore 40°C may not have been a suitable temperature to simulate fever temperatures and thereby interferon enhancement against VSV (Gray, et al 2013).

Finally, mosquito cells C6/36 were also challenged with VSV. Initially, it was thought that these cells would get a higher degree of infectivity due to the lack of an interferon system and body temperature control. Nevertheless, after analysis using flow-cytometry, it was shown that there was indeed almost a 100-fold reduction of VSV infectivity from the normal cell temperature, 28°C, to 37°C (see Appendix C & E). Despite

lacking a hypothalamus, ectotherms can regulate their body temperature. After encountering a pathogen, insects show behavioural fever, in which they congregate in warmer areas to more effectively fight the infection, usually by increasing temperature to levels detrimental to parasites (Roode and Lefevre, 2012). In addition, instead of interferons, insects have RNA interference (RNAi) as an immune response. RNAi degrades viral mRNA without harming or killing the animal cells, this mechanism limits but does not eradicate completely arboviruses like VSV (Blair, C. 2012).

To conclude, the infectivity of VSV is not reduced by incubation at high temperatures 40°C. Therefore, the reduction of infectivity is possibly due to an autonomous cellular antiviral response stimulated by fever. Moreover, human immune cell lines (PM1) reduce VSV infection up to a 100-fold, and are more effective at doing so than 293T. Furthermore, with the exception of chicken cells, the rest of the vertebrate animal cells were able to reduce VSV infection up to a 1000-fold at fever temperatures suggesting an effective antiviral cellular immunity. Finally, the inhibition of VSV-GFP infection at increased temperatures is consistent with the fact that invertebrates can also limit viral infections by means of behavioral fever and RNA interference. Possible improvements for this experiment include the addition of more cell lines, and an incubator at 43°C to simulate fever temperature in DF1 cells. Moreover, future experiments on this topic should include a variety of different viruses to determine if other viruses are affected under the same circumstances. Furthermore, it would be interesting to treat the cell line with Type I interferon and repeat the experiment again to determine if exogenous interferon causes an even steeper and more effective decrease of IU.ml, which would otherwise not happen naturally.

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

The studentship was one of the biggest challenges I have ever encountered because it was nothing like any university project I had done before. In the beginning of the studentship I received a very valuable training by my assigned supervisor Tristan Dennis, which included how to keep the work space sterile and clean in the bioCat2, the correct passage of cells in order to keep them nourished and confluent, the production of virus stock and harvest, transfecting cells with viral DNA, infecting cells with a virus, titrating virus into 96 well plates with multichannels, using the flow cytometre and fluorescence microscope, among many other useful techniques used in a real laboratory. After my training, I was given almost full freedom to work responsibly by myself and on my own time. However, organising my time and working independently were the toughest tasks. Nevertheless, it was extremely exciting to work on something I was genuinely interesting in. Despite sometimes having to work the whole day, I could not wait to come back the next day to analyse my results. The studentship provided me with more than useful laboratory techniques; it provided me with a lifetime experience that truly helped me develop myself as a scientist. I understood what it was like to be part of a laboratory team, but also to work independently to reach a common goal. I realised how overwhelming it can be to be a scientist due to the workload and long working hours, but I could also appreciate why people still do it, because in the end, when you analyse your results and find something exciting, the feeling is incredible.

I would encourage anyone who is thinking about applying for the studentship to do it, because without a doubt it has been one of the most challenging yet rewarding weeks of my studies.

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

This project is still in its infancy. The data described herein demonstrate that temperature-dependent variation in susceptibility to VSV infection is worthy of future study. However, in

the future it is hoped that these experiments could be developed into a fuller study that could be presented at conferences or published.

5. Signatures:

Supervisor	Date	Student	Date
Sam Wilson	29-07-15	Ricardo Sanchez V	29-07-2015

Sources :

1. Schnorr, J., Schneider-Schaulies, S., Simon-Jodicke, A., Pablovic J., Horisberger, A., ter Meulen, V. 1993. MxA-Dependent inhibition of measles virus glycoprotein synthesis in a stably transfected human monocytic cel lline. *Journal of Virology.* Vol. 67 no.8

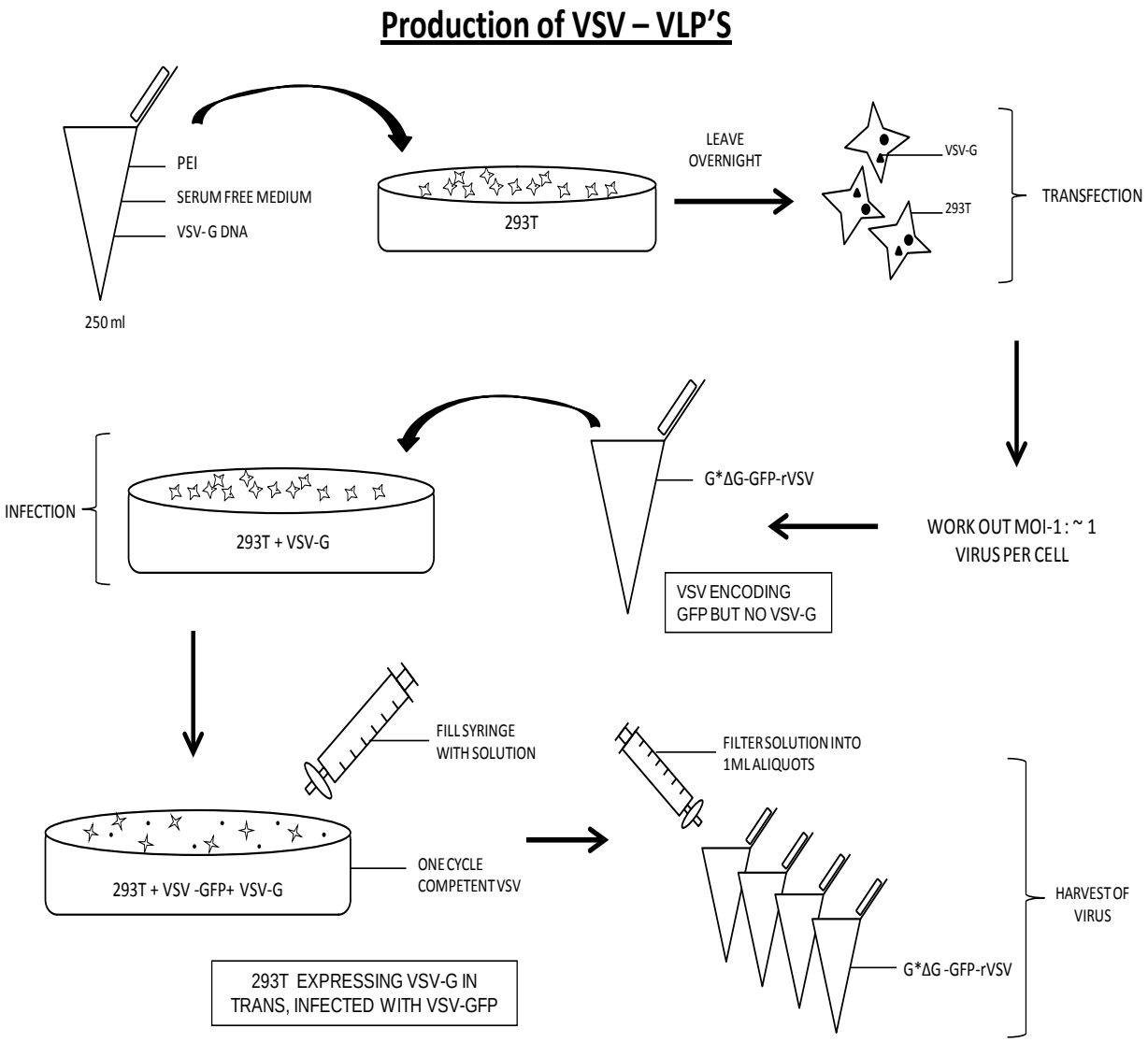
2. Perry, AK., Chen, G., Zheng D., Tang, H., Cheng, G. 2005. Host type 1 interferon response to viral and bacterial infections. *Cell Research-Nature.* 15(6) 407-22

3. Roode, J., Lefevre, T. 2012. Behavioral immunity in insects. *Insects. 3, 789-820.*

4. Gray, D., Marais, M., Maloney, S. 2013. A review of physiology of fever in birds. *Journal of comparative physiology.* 183(3): 297-312

5. Blair Carol. 2011. Mosquito RNAi is the major innate immune pathway controlling arbovirus infection and transmission. *Future Microbiology.* 6 (3): 265-277

APPENDIX A


Production of VSV – VLP'S
PEI
SERUM FREE MEDIUM
VSV- G DNA
250 ml
293T
LEAVE OVERNIGHT
VSV-G
293T
TRANSFECTION
WORK OUT MOI-1 : ~ 1 VIRUS PER CELL
G*ΔG-GFP-rVSV
VSV ENCODING GFP BUT NO VSV-G
INFECTION
293T + VSV-G
FILL SYRINGE WITH SOLUTION
293T + VSV -GFP+ VSV-G
ONE CYCLE COMPETENT VSV
293T EXPRESSING VSV-G IN TRANS, INFECTED WITH VSV-GFP
FILTER SOLUTION INTO 1ML ALIQUOTS
G*ΔG -GFP-rVSV
HARVEST OF VIRUS

Appendix B- Flow cytometry analysis

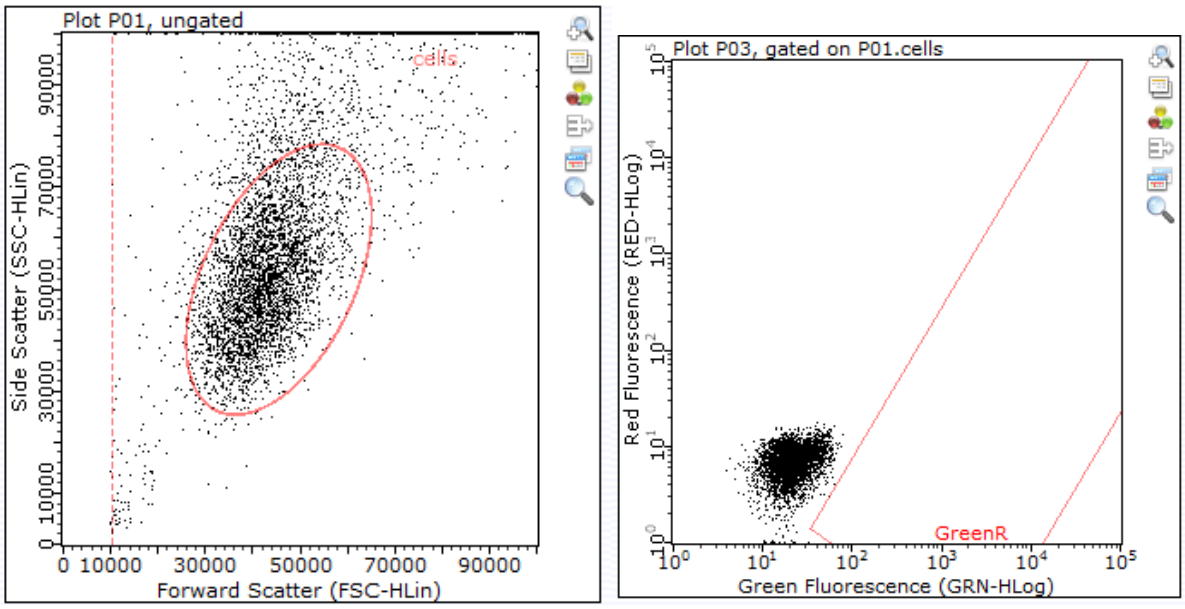


Figure1: The left chart shows the viable population of cells scattered in the well, and the right chart shows that most cells in the population are not expressing Green Fluorescent Protein.

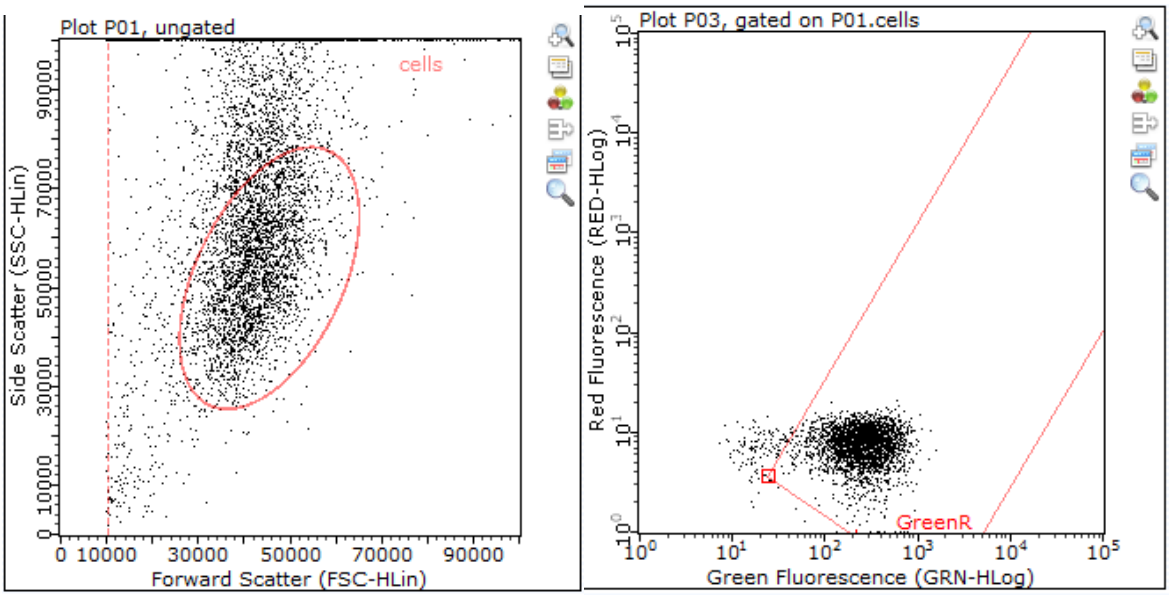
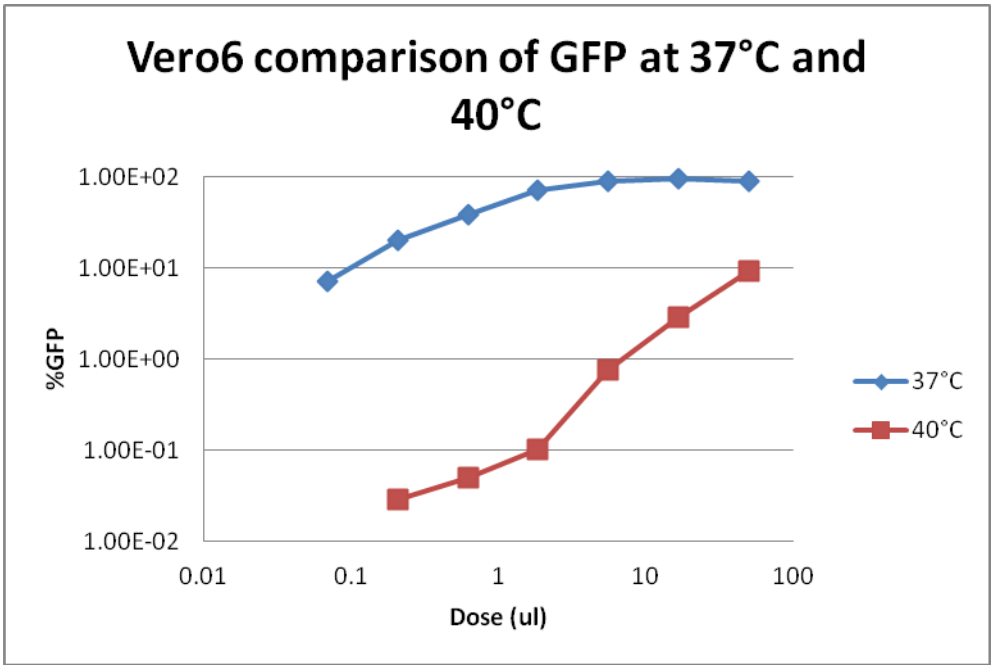
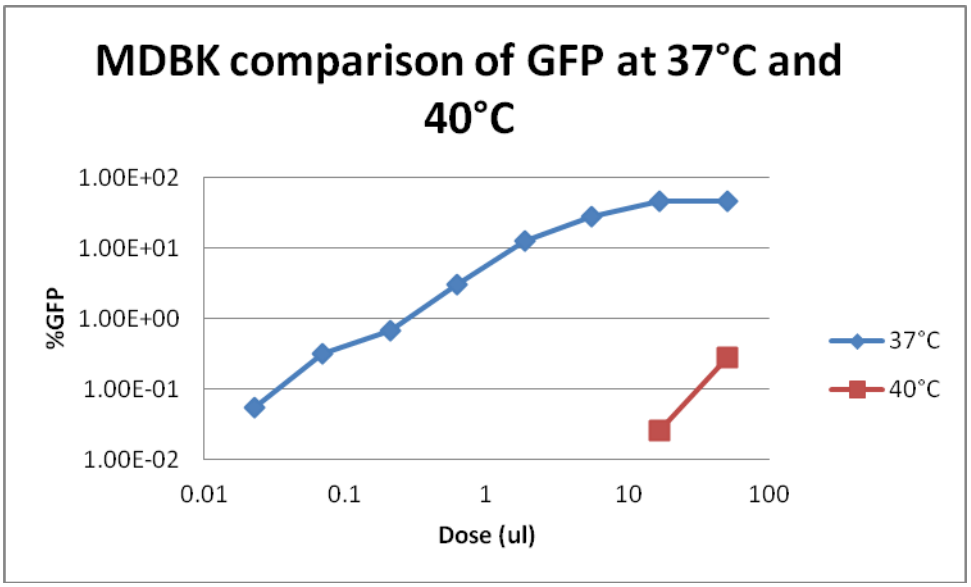


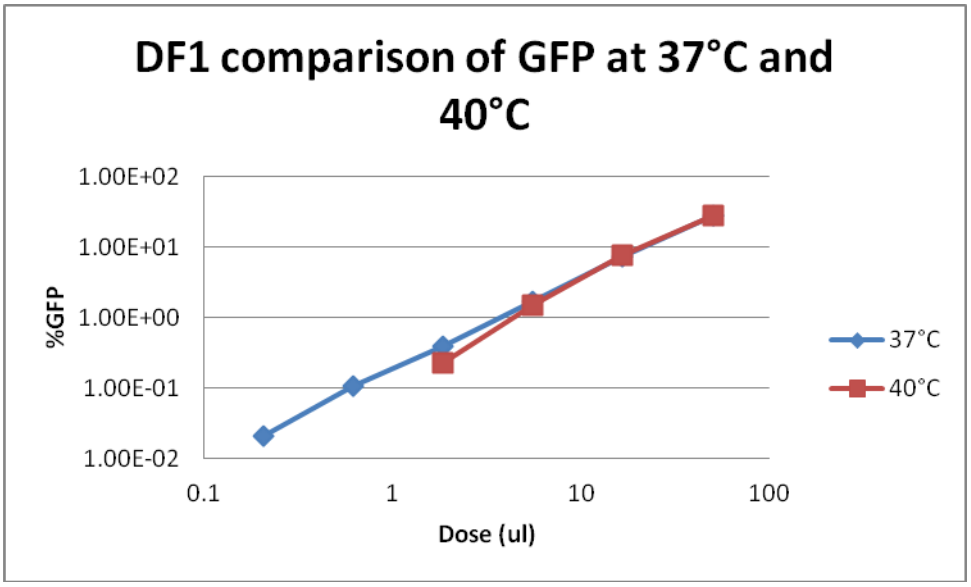
Figure 2- Contrarily from figure 1, figure 2 shows a great percentage of cells in the population expressing Green Fluorescent Protein in the right chart.

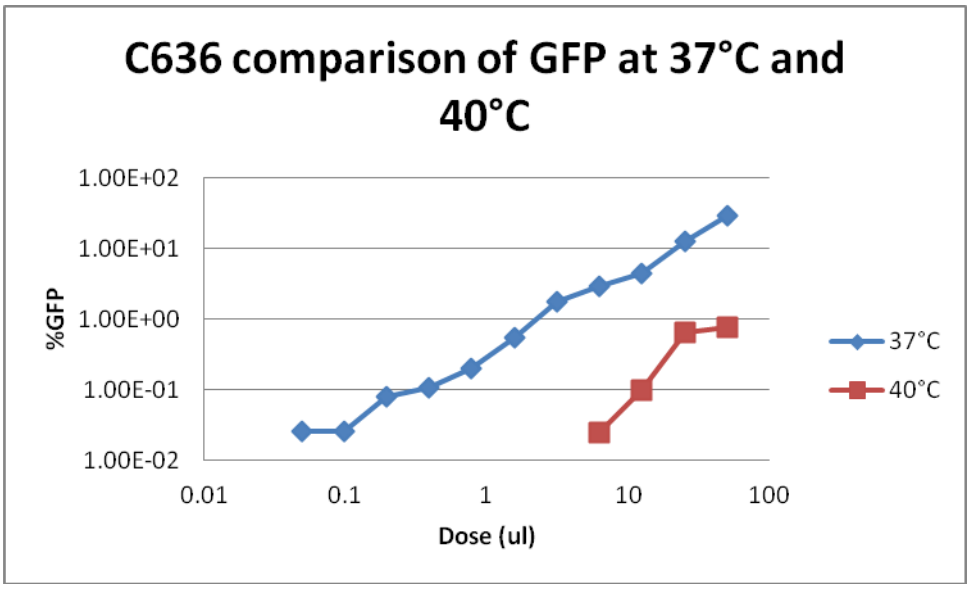
Appendix C-

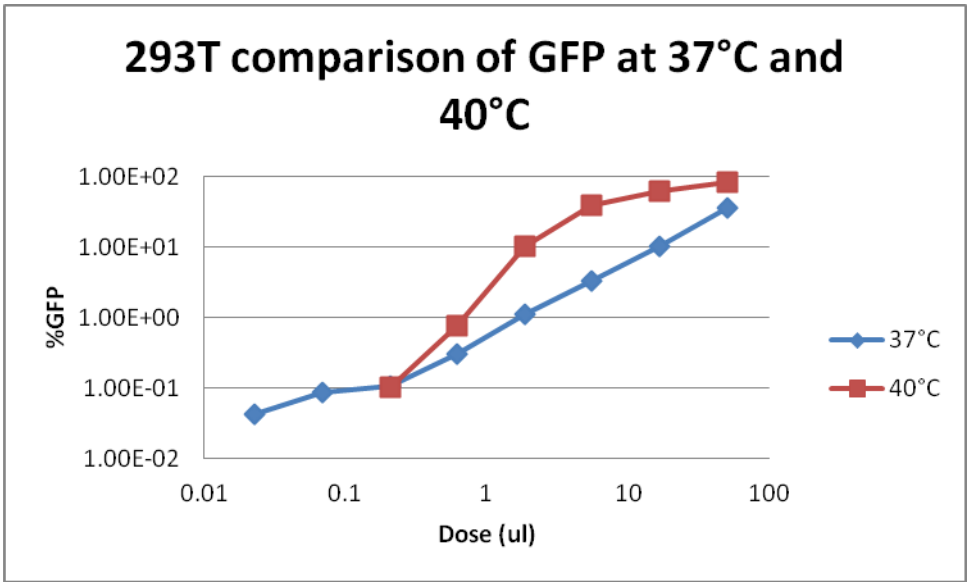
Graphs comparing the percentage of GFP at different temperatures in all cells lines.

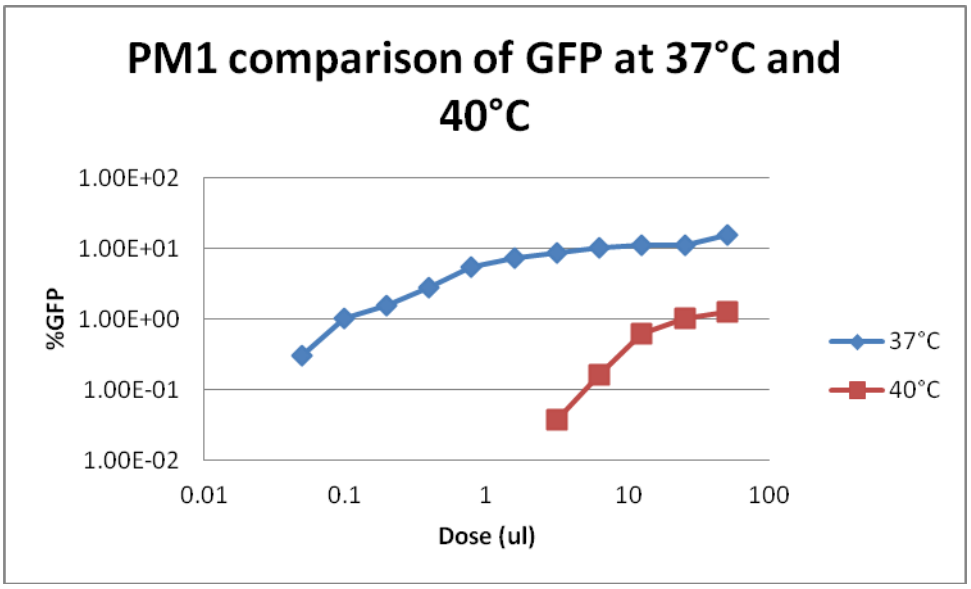


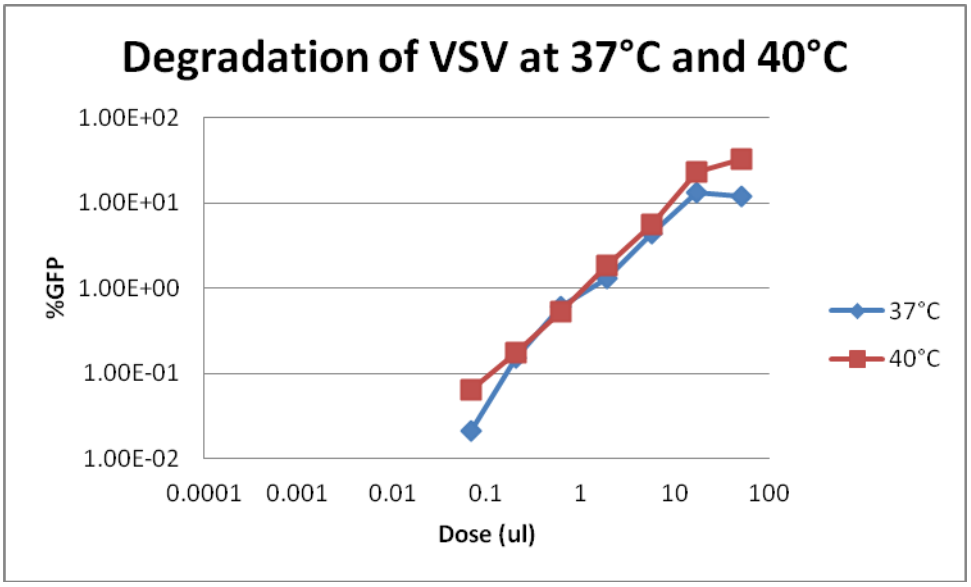



DF1 comparison of GFP at 37°C and 40°C
1.00E+02
1.00E+01
1.00E+00
1.00E-01
1.00E-02
%GFP
0.1
1
10
100
Dose (ul)
37°C
40°C


C636 comparison of GFP at 37°C and 40°C
1.00E+02
1.00E+01
1.00E+00
1.00E-01
1.00E-02
%GFP
0.01
0.1
1
10
100
Dose (ul)
37°C
40°C

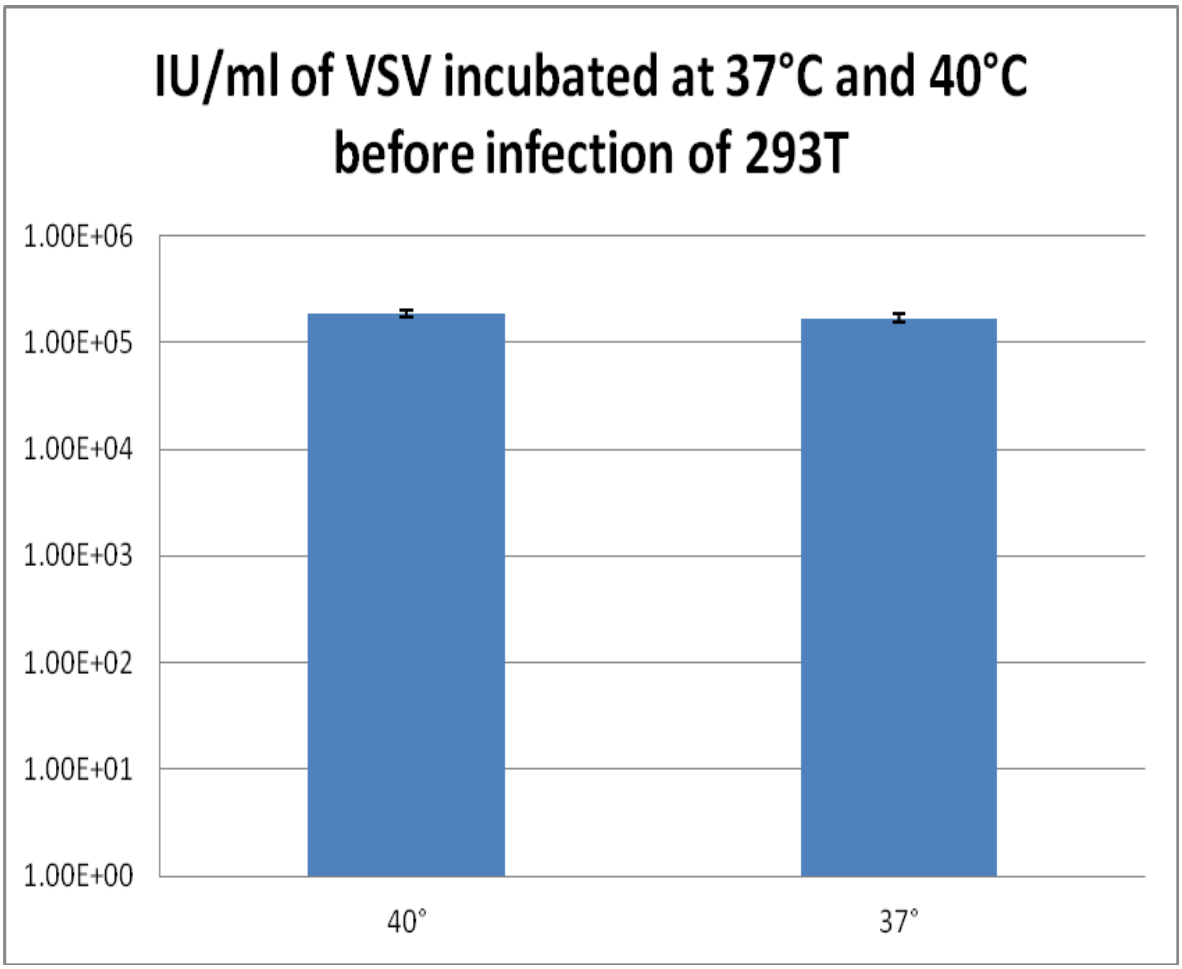

293T comparison of GFP at 37°C and 40°C
1.00E+02
1.00E+01
1.00E+00
1.00E-01
1.00E-02
%GFP
0.01
0.1
1
10
100
Dose (ul)
37°C
40°C


PM1 comparison of GFP at 37°C and 40°C
1.00E+02
1.00E+01
1.00E+00
1.00E-01
1.00E-02
%GFP
0.01
0.1
1
10
100
Dose (ul)
37°C
40°C


Degradation of VSV at 37°C and 40°C
1.00E+02
1.00E+01
1.00E+00
1.00E-01
1.00E-02
%GFP
0.0001
0.001
0.01
0.1
1
10
100
Dose (ul)
37°C
40°C

Appendix D

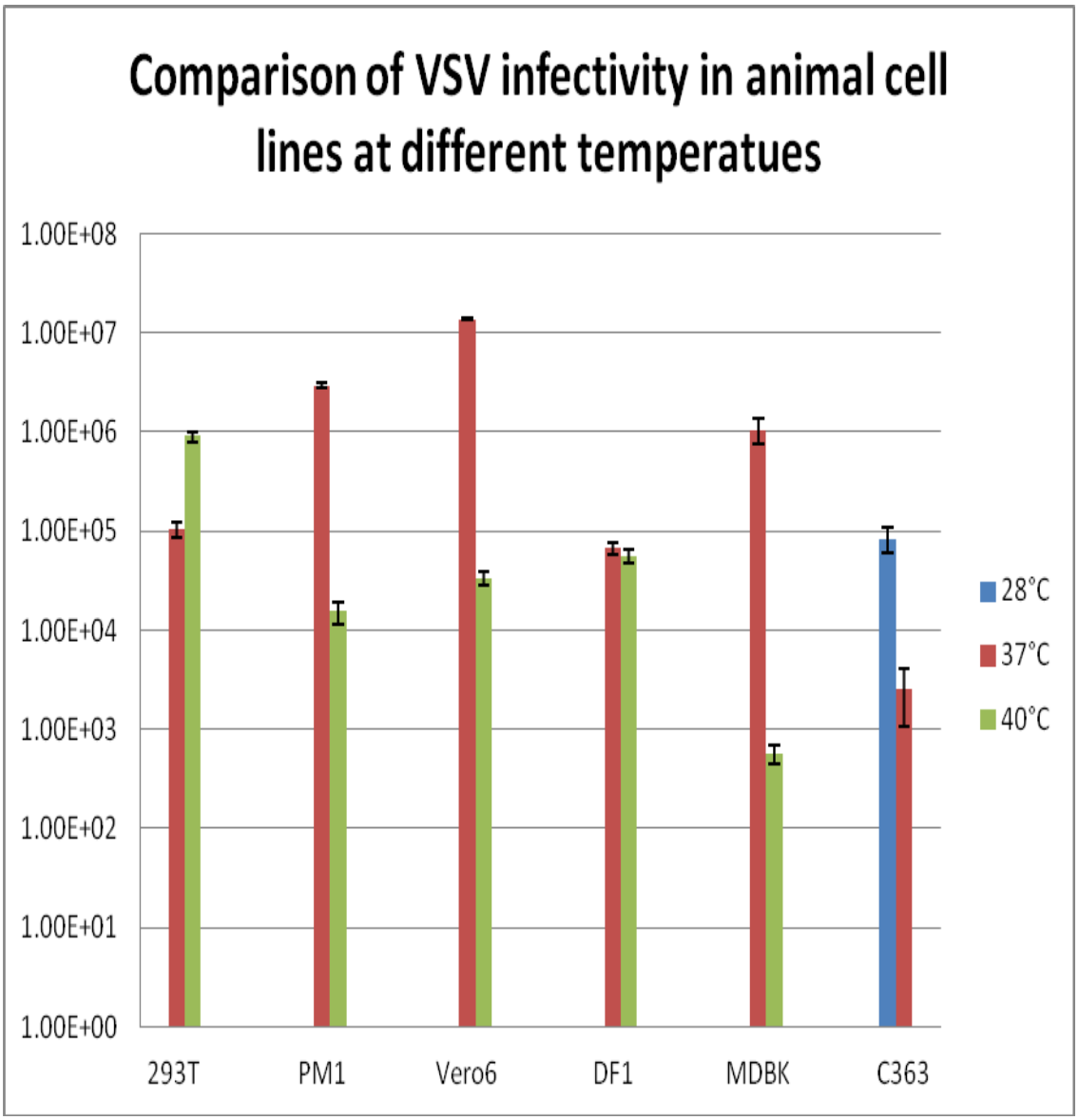
Appendix D shows the mean VSV IU.ml after the virus was incubated at 37°C and 40°C before subsequent infection of 293T. Error bars are represented by the standard deviation of the 3 points taken to calculate the mean IU/ml.



Degradation experiment		
IU/ml		st.dev
40°	184972.371	15280.5639
37°	167202.263	15655.8085

APPENDIX E

Appendix E shows a comparison of the mean VSV IU/ml at the different temperatures in all cell lines. The error bars are represented by the standard deviation of the three points taken to calculate the mean IU/ml.



IFU/ml	293T	PM1	Vero6	DF1	MDBK	C363
28°C						84070.2743
37°C	104493.87	2938568.4	13783181	66745.932	1047262.2	2577.44058
40°C	904904.36	15435.139	33755.865	55757.346	562.534085	