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: Jäntti **Forename:** Isabella

E-mail address: 2144074j@student.gla.ac.uk

2. **Supervisor:**

Surname: Cordero **Forename:** Julia

E-mail address: julia.cordero@glasgow.ac.uk

3. **Research Project Report**

3.1 Project Title (maximum 20 words):

The *Drosophila* midgut as a model system to study stem cell-driven intestinal tumourigenesis.

3.2 Project Lay Summary (copied from application):

Intestinal homeostasis is dependent on the balanced turnover of the intestinal epithelium. This relies on stem cells whose functions are modulated by intrinsic processes and extrinsic factors, such as the microbiome. Hyperactivation of Src64, a non-receptor tyrosine kinase, in *Drosophila* intestinal stem cells results in hyperproliferation. This project will contribute to the analysis of *Drosophila* as a paradigm to study the regulation of somatic stem cell behaviour and immune responses upon Src64 driven hyperproliferation and dysbiosis (Nászai, Carroll and Cordero, 2015). This is essential in

developing cancer treatments as Src activating mutations are found in 20% of human Colorectal Cancers.

3.3 Start Date: 3rd of July 2017 **Finish Date:** 1st of September 2017

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

Characterise the relationship between microbiota and hyperproliferation in the adult Drosophila posterior midgut:

While the relationships between tumour-host and microbiome-host are well established, the existence of an interaction between the microbiome and tumour remains uncertain. Previous work in Dr. Cordero's lab has shown that intestinal hyperproliferation alters microbe composition, with hyperproliferative intestines leading to increased bacterial loads and dysbiosis. My project aimed to investigate whether the presence or absence of microbiota can itself alter Src64 induced hyperproliferation in *Drosphila* posterior midguts.

Assess the effect of hyperproliferation on activation of immune pathways in the posterior midgut:

Additionally, my project aimed to establish whether hyperproliferation induced through inactivation of the Wnt signalling inhibitor Adenomatous Polyposis Coli 1 (APC1) tumour suppressor gene would be enough to activate immune pathways in germ-free flies.

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

1- *Bacterial plating to assess bacterial load of flies fed either regular or antibiotic-containing food:*

a- Homogenization of tissue and preparation of serial dilutions
b- Culturing of gut homogenates in rich media plates for microbial load assessment
c- Data quantification and analysis using GraphPad Prism 6

2- *Histological analysis of Drosophila posterior midguts to assess hyperproliferation:*

a- Tissue microdissection followed by fixation in 4% formaldehyde
b- Tissue staining with appropriate primary antibodies and fluorophore-labelled secondary antibodies
c- Tissue mounting and imaging by confocal microscopy
d- Image analysis and quantification of hyperproliferation using ZEISS ZEN software

3- *Assessment of anti-microbial peptide expression as a readout of immune system activation in axenic Apc1$^{-/-}$ fly intestines:*

   a- Use of cDNA in RT-qPCR for quantitative analysis of selected anti-microbial peptide transcripts
   b- Statistical analysis of RT-qPCR data

4- *Additional training in:*

   a- Fly genetics, husbandry and maintenance
   b- Primer design

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

Assessing the relationship between microbiota and intestinal hyperproliferation in Drosophila

To investigate the relationship between microbes and intestinal hyperproliferation, we used the temperature sensitive escargot-gal4 driver to express GFP only (*esg^{ts}>GFP*) or with Src64 (*esg^{ts}>Src64*) within stem/progenitor cells of the adult *Drosophila* midgut, to induce hyperproliferation (Cordero et al., 2014) (Fig 1). Adult flies of both genotypes were fed regular or antibiotic-containing food one week prior to transgene activation, and for five days during transgene activation (Fig 2).

Appropriate transgene activation in response to a temperature shift from 18°C to 29°C was confirmed prior to beginning the experiment. Hyperproliferation in posterior midguts was assessed through quantification of phosphorylated Histone H3 (pH3) staining. We observed an increase in pH3^{+ve} cells between pre- and post-transgene activation in midguts (Fig 3 A&B).

We also confirmed axenia in both pre- and post-transgene activation with colony forming unit (CFU)/gut values for flies fed antibiotic-containing food lower than in those fed regular food at either temperature (Fig 4).

While antibiotic treatment appeared to have no effect on the amount of proliferative (pH3^{+ve}) intestinal stem cells (ISCs) in posterior midguts of *esg^{ts}>GFP* animals, we noted a significant reduction in ISC proliferation in animals with hyperproliferative midguts (Fig 5 A&B). In other words, the absence of microbes significantly reduced intestinal hyperproliferation in *esg^{ts}>Src64* flies when compared to those flies with a regular microbiota.

Assessing the activation of immune pathways in hyperproliferative midguts

Loss of Drosophila *Apc1-/-,* orthologue to the gene driving colorectal cancer in humans results in significant intestinal hyperplasia (Cordero et al., 2012).

We measured the transcript levels of anti-microbial peptides in midguts from *Apc1*$^{-/-}$ animals, fed regular or antibiotic-containing food. We observed decreased expression of Imd pathway components; the main immune pathway of the gut, in axenic flies when compared to flies fed regular food (Fig 6 A-D).

3.7 Discussion (500 words max):

Assessing the relationship between microbiota and hyperproliferation

The relationship between the microbiome and colorectal cancer is complex. Despite having a commensal role, microbiota have also been implicated in the development of colorectal cancer (Zhu et al., 2013). In fact, specific mutations in CRC tumours have been linked to specific alterations in the composition of gut microbes (Burns et al., 2016). Hyperproliferative *Drosophila* midguts have also been shown by Dr. Cordero's lab to have an altered microbial composition. The relationship between tumours and microbes appears to go both ways, with each being capable of influencing the other. However, this relationship remains uncharacterized. My project aimed to add to the on-going work in Dr. Cordero's lab by examining the impact of the microbiota on posterior midgut hyperproliferation.

Flies with an activated *esg*^{ts}*>Src64* transgene had higher CFU/gut values than those in control (Fig 4). Increased bacterial loads observed in hyperproliferative midguts may be a result of misdifferentiation of the stomach-like copper-cell region (CCR) in the *Drosophila* midgut. The low pH found in the CCR acts to control gut microbiome composition, and dysregulation of the CCR accordingly leads to dysbiosis (Overend et al., 2016).

The removal of microbes was expected to decrease hyperproliferation, possibly owing to lower rates of microbe-induced inflammation which can itself promote tumorigenesis. As can be seen in Fig 5 A&B, axenic *esg*^{ts}*>Src64* flies appear to have a significant reduction in proliferation when compared to flies with presumably regular microbiomes. Previous work on mice treated with a pro-carcinogenic compound has shown that while mono-associated mice developed both colon tumours and inflammation as a response to the treatment, axenic mice were completely devoid of both (Uronis et al., 2009). Although our results are in accordance with previous literature and are significant, they may be positively skewed due to a high pH3 count in two guts in the hyperproliferative regular-food condition. The experiment was repeated in order to obtain more data, however, due to a single unexpected hyperproliferative gut in the *esg*^{ts}*>GFP* fed antibiotic food, all new data points were excluded. Further research is necessary to obtain a fully conclusive result.

Assessing the activation of immune pathways in hyperproliferative guts

A trend in reduction in the activity of the Imd pathway was observed for hyperproliferative *Apc1*$^{-/-}$ axenic flies when compared to their non-axenic counterparts. Although a significant difference was observed in the expression of anti-microbial

peptides *defensin* and *attacin A*, two samples in the control food condition had consistently higher transcriptional readouts compared to the remaining samples (Fig 6 A-D). As these may just be outliers, more data is needed in order to investigate the effect of intestinal hyperproliferation and the microbiome on immune pathway activity. No significant difference was observed in the expression of *drosomycin-like 2* a target of JAK/STAT signalling, which is activated in hyperproliferative backgrounds (Cordero et al., 2012) and was used as a positive control.

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

Undertaking the studentship provided me with multiple valuable experiences. Being a summer intern in a full-time research lab provided me with a first-hand experience of daily lab-life. Individuals I worked with were at various stages of their academic careers, and had come from different career backgrounds. This gave me a fantastic insight into both what a career in academia entails in a daily basis and the paths one can take. Being able to befriend individuals further along the academic path gave me ample opportunities to receive advice for my future plans and a better idea of what to expect. The research group had a great group dynamic and was extremely welcoming. I enjoyed the variety of internal and external research talks at the host institute, as well as lab meetings held independently by Dr. Cordero's group.

As the research group used *Drosophila*, I was provided with the opportunity to familiarize myself with and learn various laboratory techniques specific to *Drosophila* research. I value this very highly, as I now have a more tangible understanding of how to handle this major model organism. Having my own project gave me a lot of independence and responsibility as I was able to make a rough timeline for myself and plan my daily activities out. My direct supervisor proved to be an invaluable source of knowledge and advice. This ranged from exploring theoretical questions to much more concrete things, such as the importance of having a consistent method for naming files.

Lastly, although the prospect of presenting my own research to a group of individuals that have years of experience in that field sounded less than pleasurable at first, it is one of the experiences I enjoyed the most as every step had something I had never done before.

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

Presentation to lab members at the end of the studentship.

6. **Signatures:**

Supervisor  **Date: 26/09/2017**

Student  **Date: 19/09/2017**

7. Appendix:

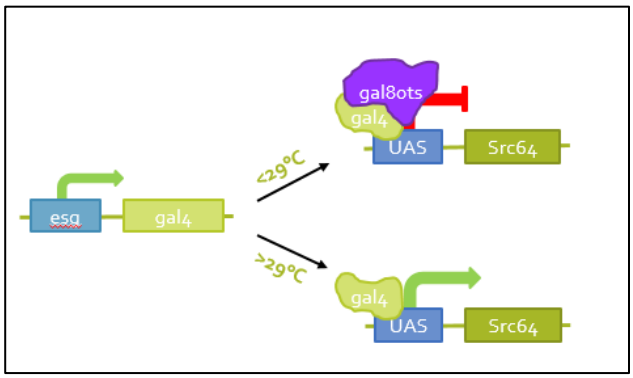

Figure 1. Schematic representation of the temperature sensitive *escargot-gal4* system driving Src64 expression

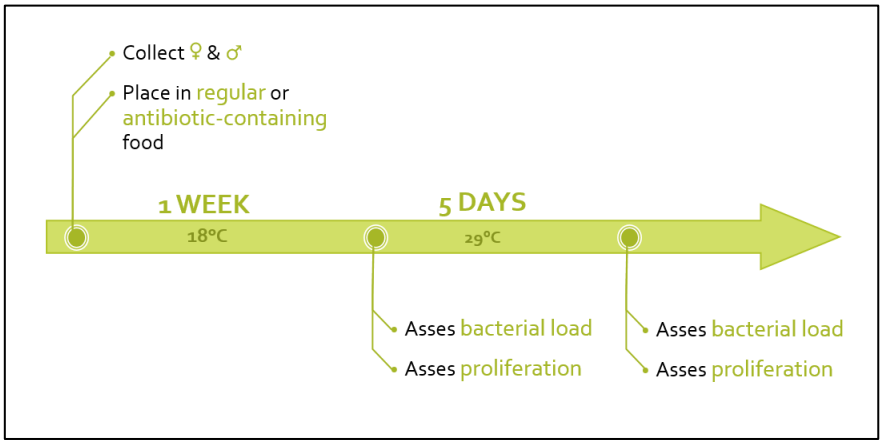

Figure 2. Experimental timeline

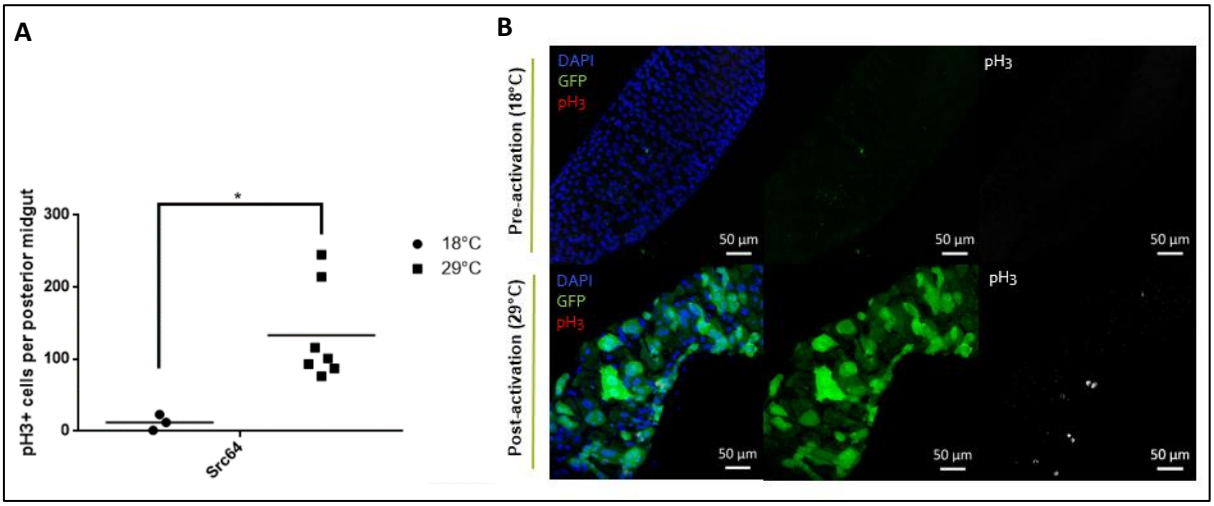

Figure 3. Hyperproliferation in response to Src64 transgene activation in flies fed regular food

A Quantification of pH3+ cells both pre- and post-transgene activation. pH3 is only present in and thus corresponds to, actively proliferating stem cells in the midgut. Statistical analysis was carried out using a t-test (* = p>0.05).

B Immunofluorescence of pre- and post-transgene activation posterior midguts stained for pH3 (red, white), GFP (green) and DAPI (blue). The latter labels all cell nuclei. GFP staining corresponds to intestinal stem cells and progenitors, enteroblasts.

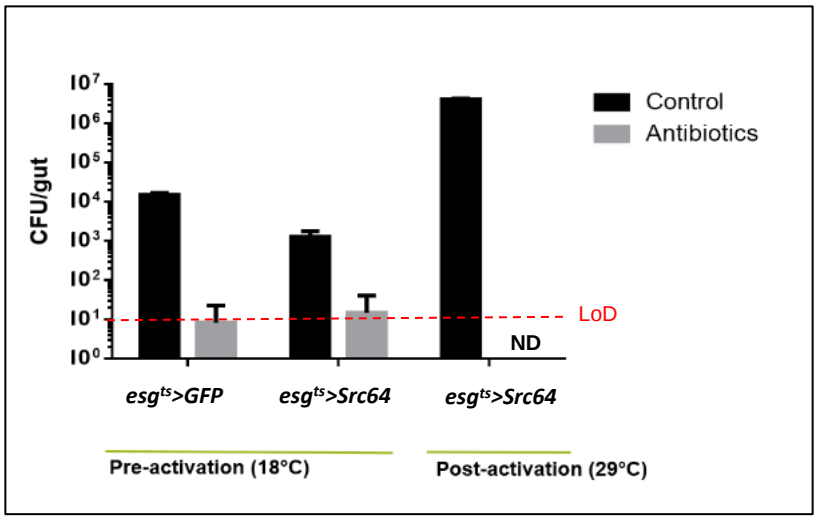

Figure 4. Establishment of axenia in control and hyperproliferative guts prior to and after transgene activation
Note the logarithmic scale. (ND: not detected)

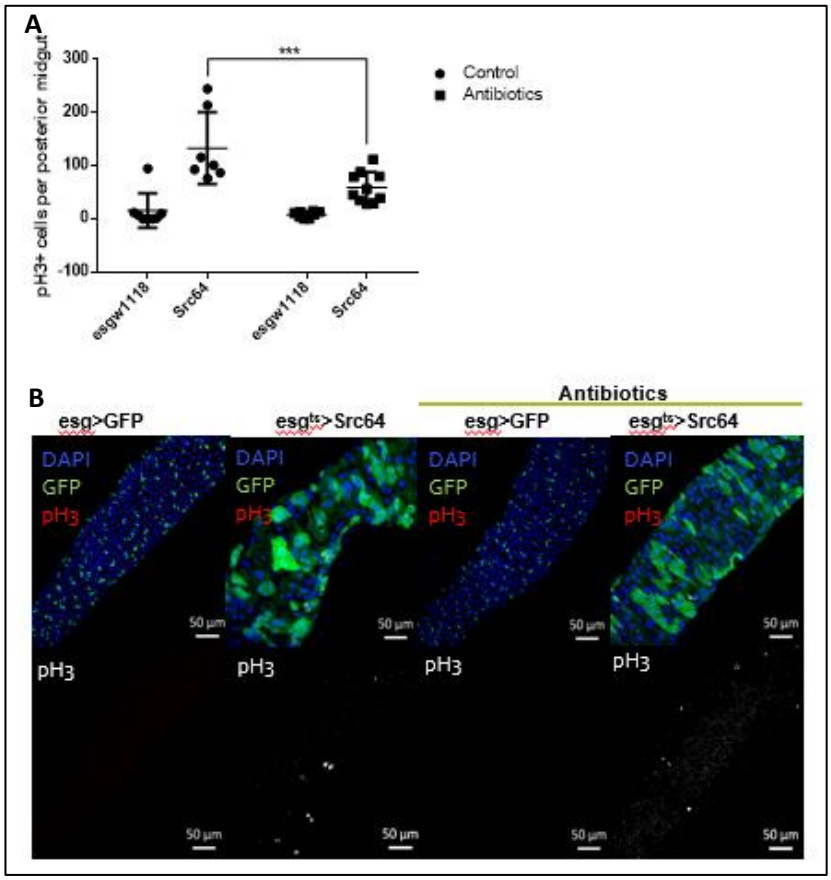

Figure 5. Antibiotic treatment reduces hyperproliferation

A Quantification of proliferating intestinal stem cells in posterior midguts of flies post-transgene activation. A significant difference was observed using a two-way ANOVA (*** = $p<0.001$).

B Immunofluorescence of post-activation control and hyperproliferative posterior midguts of flies fed regular or antibiotic-containing food.

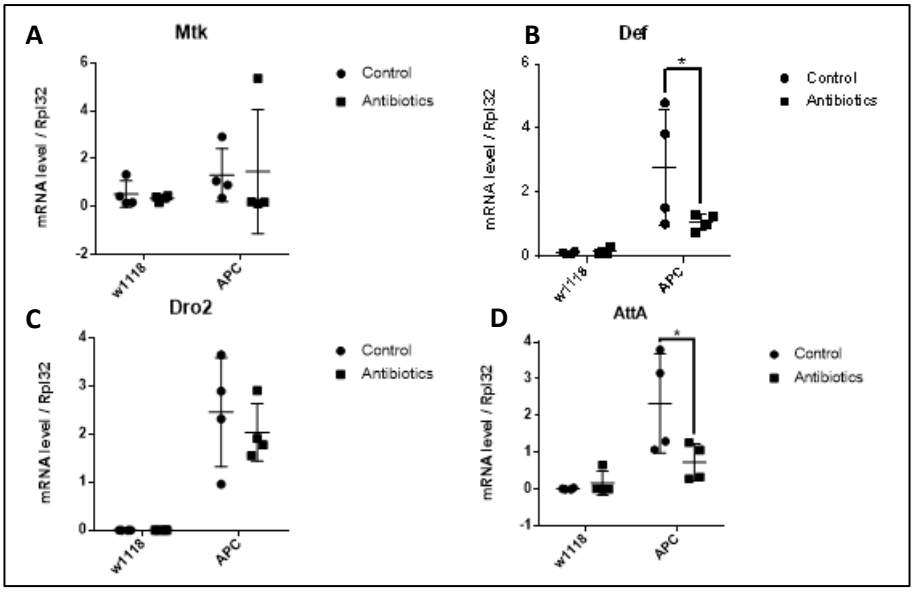

Figure 6. Anti-microbial peptide expression in Apc1$^{-/-}$ mutants

RT-qPCRs were conducted on cDNA for anti-microbial peptides involved in JAK/STAT signalling(C) and Imd immunity (A, B and D). mRNA levels were normalized against the housekeeping gene Rpl32. Statistical analysis was carried out using two-way ANOVA (* = p<0.05).

References

Burns, M. B., Montassier, E., Abrahante, J., Starr, T. K., Knights, D. and Blekhman, R. (2016) 'Discrete mutations in colorectal cancer correlate with defined microbial communities in the tumor microenvironment', *bioRxiv*. Cold Spring Harbor Laboratory, p. 90795. doi: 10.1101/090795.

Cordero, J. B., Ridgway, R. A., Valeri, N., Nixon, C., Frame, M. C., Muller, W. J., Vidal, M. and Sansom, O. J. (2014) 'c-Src drives intestinal regeneration and transformation.', The EMBO journal. European Molecular Biology Organization, 33(13), pp. 1474–91. doi: 10.1002/embj.201387454.

Cordero, J. B., Stefanatos, R. K., Scopelliti, A., Vidal, M. and Sansom, O. J. (2012) 'Inducible progenitor-derived Wingless regulates adult midgut regeneration in Drosophila.', The EMBO journal. European Molecular Biology Organization, 31(19), pp. 3901–17. doi: 10.1038/emboj.2012.248.

Nászai, M., Carroll, L. R. and Cordero, J. B. (2015) 'Intestinal stem cell proliferation and epithelial homeostasis in the adult Drosophila midgut', *Insect Biochemistry and Molecular Biology*, 67, pp. 9–14. doi: 10.1016/j.ibmb.2015.05.016.

Overend, G., Luo, Y., Henderson, L., Douglas, A. E., Davies, S. A. and Dow, J. A. T. (2016) 'Molecular mechanism and functional significance of acid generation in the Drosophila midgut', *Scientific Reports*, 6(1), p. 27242. doi: 10.1038/srep27242.

Uronis, J. M., Mühlbauer, M., Herfarth, H. H., Rubinas, T. C., Jones, G. S. and Jobin, C. (2009) 'Modulation of the Intestinal Microbiota Alters Colitis-Associated Colorectal Cancer Susceptibility', *PLoS ONE*. Edited by S. Bereswill. Public Library of Science, 4(6), p. e6026. doi: 10.1371/journal.pone.0006026.

Zhu, Q., Gao, R., Wu, W. and Qin, H. (2013) 'The role of gut microbiota in the pathogenesis of colorectal cancer', Tumor Biology. Springer Netherlands, 34(3), pp. 1285–1300. doi: 10.1007/s13277-013-0684-4.