UNIVERSITY OF LIVERPOOL

Research Project Report 1

Effect of WT1 ablation on adult intestinal wall and tissue homeostasis

Mihaela Veronica Foisor

201206342

Supervisor: Dr Bettina Wilm

Internal Assessor: Dr Carrie Duckworth
Strand: Medical Sciences

Word Count: 4059

Effect of WT1 ablation on adult intestinal wall and tissue homeostasis

Mihaela-Veronica Foisor
Institute of Translational Medicine, University of Liverpool, Crown St, Liverpool, L69 3BX, UK

Abstract

Maintaining tissue homeostasis is a complex mechanism that plays a vital role in cell survival and proliferation. Understanding the pathways and key factors behind homeostasis conservation in different tissues allows us to better comprehend what occurs when the balance is lost and disease takes place. In our study we are looking at the function of Wt1-expressing mesothelium cells in gut homeostasis; does WT1 ablation lead to disruption of homeostasis, and are there differences in the muscle layers morphology? Using the CreERT/loxP system we have conditionally knocked-out the Wt1 gene in adult mice. Histological analysis of different parts of the small intestines was performed with H&E staining and µCT. Although we can see slight differences in the longitudinal muscle between the mutants and controls, we cannot confirm these findings until the µCT results are completed. Immunofluorescence with markers for muscle regeneration confirms that Wt1 is not actively involved in the muscle regeneration pathways in the gut. Due to incomplete results we cannot say at this moment if Wt1 is indeed involved in the gut homeostasis and smooth muscle morphology.

Key words: Wilms' tumor 1, gut homeostasis, CreERT/loxP system, intestinal smooth muscle, mesothelium
Abbreviations: Wt1- Wilms' tumour 1; H&E- haematoxylin and eosin; µCT- micro computed tomography, EMT- epithelium to mesenchymal transition, MET- mesenchymal to epithelium transition

Introduction

The decision to proliferate at the right time and place ensures replacement of damaged tissue and limits the potential for cancer; in order to achieve this, a complex regulatory system of stem cells and progenitor cells integrates extrinsic and intrinsic local signals. Although differentiation and tissue homeostasis are behind each cell's activity, their mechanisms still remain mostly unknown. Understanding the pathways and mechanisms that regulate tissues' maintenance would also provide insight into the events taking place during diseases.

Wilms' tumour suppressor gene 1 (WT1) is responsible for the regulation of multiple genes that are involved in embryonic development, cell differentiation, growth and apoptosis[1]. Due to its function as transcription regulator, the activation or repression of distinct downstream pathways is context specific[2]. As WT1 is expressed in different areas during different stages in development, mutations or abnormalities in its expression are known to be the main cause of several diseases affecting different types of tissues. The

most common ones are Denys–Drash syndrome, which manifests as genital abnormalities and kidney diseases, and Wilm's tumour[3].

It is known that WT1 is highly expressed during embryonic development and is a key factor in the development of different organs such as the nervous system, kidneys, spleen, gonads, heart and blood vessels[4,5] . Moreover, it is a vital factor in regulating the transition between the epithelial and mesenchymal states depending on the tissue. While in the heart it produces cardiovascular progenitors from epicardium, thus the epithelium to mesenchymal transition (EMT), in renal development it modulates the reverse mechanism and produces nephron cells (MET)[6]. This switch also happens in the intestines; where WT1 expressing serosal mesothelial cells can undergo ETM and differentiate into the smooth muscle that contribute to the blood vessel network of the intestines[7]. As development goes on and reaches the later stages, WT1 expression becomes more specific to certain tissues. In the adult, WT1 expression is limited to a small number of cells in specific sites: the podocytes in the kidneys, progenitor cells from the bone marrow, sertoli cells in the testes, granulosa cells in the ovaries, mesothelia and different types of fat bodies[1,8–10].

Due to the limited expression of WT1 in adult tissues, it has been recently questioned whether it may be linked with a specific function. Different studies have proven that WT1 seems to have a special role in different organs that are in contact with the mesothelium. In the kidney, where WT1 has the highest expression, WT1 plays a major part in podocyte maintenance[9].

If WT1 expression is knocked-down, there is a significant increase in glomerulosclerosis, which is the main cause of podocyte lesions and glomerular diseases[11]. *In vitro* studies demonstrate that WT1 is acting within the podocytes through the Wnt/β-catenin pathway. When WT1 expression is reduced, the Wnt/β-catenin pathway is more active, leading to podocyte dysfunction and apoptosis[12].

Considering the function of WT1 in the mesothelium and its involvement in the organs that are surrounded by it, we hypothesize that the mesothelium cell layer may also have an active function in the gut. As the intestines are encased by a monolayer of WT1 expressing mesothelial cells, we believe that they may have a role in the maintenance of intestinal tissue homeostasis; in the absence of WT1 we would expect disruptions of tissue. Therefore in our experiments we tried to quantify any differences in the intestinal wall or interruptions of tissue homeostasis in adult mice after the systemic deletion of Wt1 using the Cre-Lox system (Figure 1).

Recent literature has shown that tamoxifen, which is an oestrogen receptor that is used for the CreERT/loxP system activation[13], can have dose-dependent secondary effects[14]. In the intestines, tamoxifen has been shown to induce temporary atrophy

of the epithelium within a few days[15,16]. Considering this, we have included a positive control group in our study where tamoxifen is administered to the animals that lack the loxP sites on the Wt1 allele.

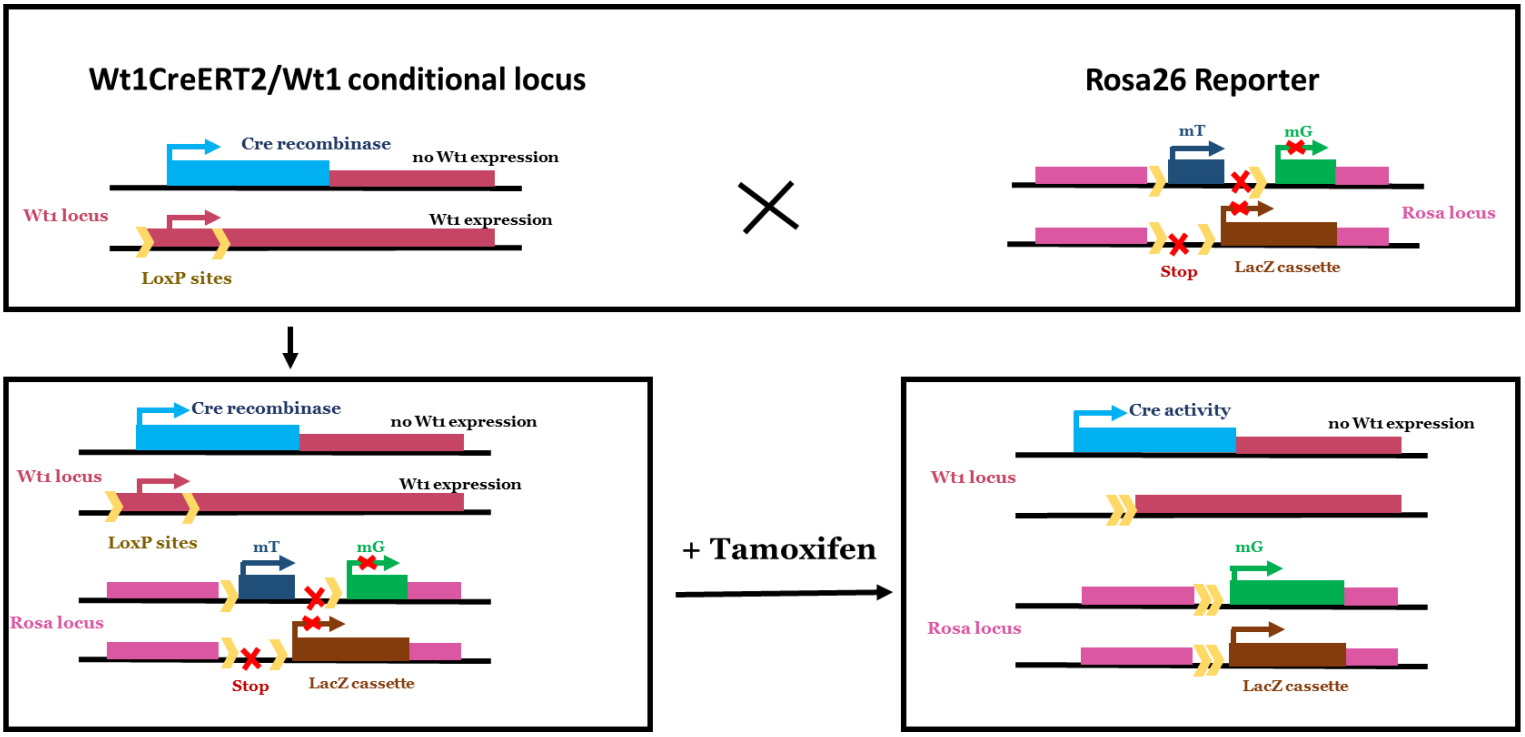

Figure 1| Generation of Cre-lox system used for the conditional deletion of Wt1 in adult mice. Firstly, mouse containing the Wt1CreERT2/Wt1 genotype was crossed with a Rosa26 Reporter containing the LacZ cassette on one allele and mT/mG on the other. The progenitors were genotyped and the ones expressing both Wt1 and Rosa locus were further used in the study. After tamoxifen administration, tamoxifen binds to the Cre protein which is then activated and transported to the nucleus where it removes the fragments that were located between the LoxP sites. This results in the ablation of Wt1 and the expression of LacZ cassete and mG.

Materials and Methods

Tamoxifen induced Wt1 knock-out

All mice were housed and bred in the Animal handling facility (BSU building) of University of Liverpool. Animals were kept in compliance with the Home Office regulations. All treated animals (Table 1) were given Tamoxifen for 5 days consecutively via oral gavage with a dose of 4mg/40g body weight and culled at day 9. Body weights were measured individually each day. All procedures were performed under permission of the license and in conformity with the Home Office regulations. All mice procedures were performed by Dr. Thomas Wilm.

Dissection and fixation of tissue

Intestinal organs were dissected and kept in PBS on ice until processed. After dissection and straightening of the intestines in a straight line, each intestine was measured and then cut into multiple segments as shown in Figure 2. The fixation was performed in 4% PFA overnight on a rocker. Tissue was then washed 3x 1 hour in PBS on a rocker. The processing steps depended on experimental protocols planned for each fragment.

LacZ staining

LacZ staining was done overnight for all assigned fragments under minimal light conditions following the protocol: 40mL volume per sample containing: 2mL of both (100mM) potassium ferrocyanide II and III, 2mL (20mg/mL) X-gal, 4mL 10x PBS, 80µl (10%) NP-40, 80µL (1M) MgCL2, 0.4mL (1%) sodium deoxycholate and filled to 40ml with 29.44mL of ddH20. Pictures were taken with a Leica MZ16F microscope and a Leica DFC420C attached camera and processed with Adobe Photoshop programme.

µCT scanning

After the PBS washing, the samples went through a serial of sequential dehydrations with ethanol (25%, 50% and 75%) for one hour each. All samples were kept at 4˚C at 75% EtOH until further processing. Staining of all samples was performed with 1% PTA in 70% EtOH overnight. The samples were run on a SkyScan1272 (Bruker) machine with the following settings: rotation step 0.1 degree, Energy filter Al 0.5mm, Pixel size 1.2 um, Image format: 4032x2688. The data was reconstructed with the NRecon programme.

Preparation of tissue for histology

After the PBS washing, the samples went through a serial of sequential dehydrations in ethanol (25%, 50%, 75%, and 100%) until reaching 100% ethanol and were kept overnight a 4˚C. Fragments were then washed with isopropanol 2x1 hour, followed by one hour incubation at 60˚ in a 50:50 mixture of isopropanol to paraffin. The tissue was then incubated overnight in paraffin at 60˚, followed by embedding in disposable moulds the next day. The tissue was kept at 4˚C until sectioning. The sectioning was done with a Thermo Scientific Shandon Finesse 325 microtome at a thickness of 7µm and kept for couple of minutes on a water bath at 40˚C before collection. The slides were incubated overnight at 37˚C.

H&E staining

The slides containing the tissue were de-waxed in HistoClear II (National Diagnostics) 2x 5 minutes, and then processed through a series of washes in ethanol (100%, 96%, 75% and 50%), each wash lasting 3 minutes. The slides were then stained in Haematoxylin Ehrlich (Atom Scientific) for 15 min. This was followed by a 10 seconds wash in ddH2O, 30 seconds wash in acidified water and another 10 seconds wash in tap water.

Table 1| Description of mice and their genotypes used in the experiment. The blue colour represents the negative controls that did not receive tamoxifen. Red, positive controls, are the mice that did receive tamoxifen, however the Cre-Lox recombination did not happen due to the co-/-. In green are the knock-out mice that received tamoxifen and also had the co+/- allele.

Nr.	Name	Genotype	Tamoxifen treatment	Cre recombination	LacZ cassette
1	coERT2mTmG	Cre+/- co-/- mTmG+/-	no	no	yes
2	coERT2mTmG	Cre+/- co-/- mTmG+/+	no	no	no
3	coERT2mTmG	Cre+/- co-/- mTmG+/+	no	no	no
4	coERT2mTmG	Cre+/- co+/- mTmG+/-	no	no	yes
5	coERT2mTmG	Cre+/- co-/- mTmG+/+	yes	no	no
6	coERT2mTmG	Cre+/- co-/- mTmG+/-	yes	no	yes
7	coERT2mTmG	Cre+/- co-/- mTmG+/-	yes	no	yes
8	coERT2mTmG	Cre+/- co-/- mTmG+/-	yes	no	yes
9	coERT2mTmG	Cre+/- co+/- mTmG+/-	yes	yes	yes
10	coERT2mTmG	Cre+/- co+/- mTmG+/-	yes	yes	yes
11	coERT2mTmG	Cre+/- co+/- mTmG+/+	yes	yes	no

Then the slides were stained for 90 seconds in 0.3% Eosin and washed for 1 minute in tap water. The stained slides were then dehydrated with ethanol (50%, 75% and 96%) for 1 minute each ensued by 3 minutes in 100% ethanol. The slides were then cleaned in HistoClear II 2x 2 minutes and mounted with DPX solution. The images were taken with a Leitz DMRB microscope and a Leica DFC450C attached camera and processed with Adobe Photoshop programme.

Cryoembedding and cryosectioning

After the PBS washes, the tissue was cryopreserved overnight on a rocker in 30% sucrose. The following day the samples were embedded in OCT (Richard-Allan Scientific™ Neg-50™ Frozen Section Medium) on dry ice and isopropanol. The tissues were sectioned at 8µm thickness with a Thermo Scientific HM525NX machine. The resulting sections were kept at -80˚C until further use.

Immunofluorescence

The slides were taken from -80˚C and left at room temperature to dry for 30 minutes. The tissue was then washed in 1% PBS 3x 5 minutes, following 10 minutes in 0.25% Triton in 1% PBS. The sections were washed again 3 times in 1% PBS and then blocked for one hour in 2% BSA in 1% PBS. Primary antibodies where incubated overnight at 4˚C, then washed off with PBS 3x15 min. The secondary antibody was then added for one hour in the dark at room temperature.

After washing with PBS and ddH_2O the slides were mounted with Fluoro-Gel (Electron Microscopy Sciences) and left to dry at 4˚C in the dark. We used the following antibodies: polyclonal rabbit anti-Desmin (code: A0611, DakoCytomation), polyclonal rabbit anti-Wilm tumor (code: GTX15249, GeneTex), monoclonal anti-α smooth muscle actin mouse IgG2a (code: A2547, Sigma Aldrich), polyclonal rabbit anti-Cytokeratin, wide spectrum screening (code: Z0622, DakoCytomation).

When primary antibodies were from mice, direct labeling was used to generate less background. The Zenon® Alexa Fluor® 488 Mouse IgG2a Labeling Kit was used as per protocol against α-SMA mouse primary antibody. The slides were kept at 4˚C in the dark and images were taken the next day. All images were taken with a Leica DM2500 microscope attached to a Leica DFC350FX camera. The resulting images were processed in Adobe Photoshop programme.

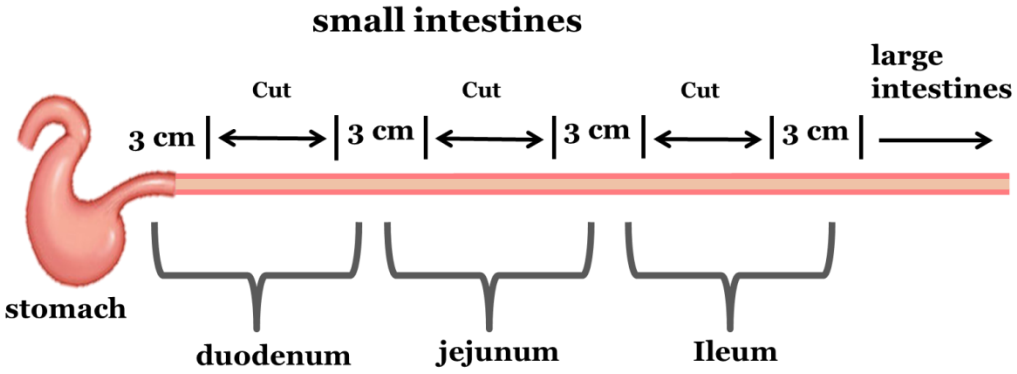

Figure 2| Schematics of fragments isolated from intestines. After the intestinal bundles were straightened, 3 distinctive cuts were done at different parts of the small intestines. All segments were further divided into 1x 2cm fragments that would be processed for µCT scanning and 4x 1cm fragments, 2 for frozen tissue and 2 for paraffin embedding. In the jejunum there were also 2x 0.5 cm fragments that were snap frozen for future work with RNA and protein. All cut fragments were cleaned in PBS with a 2 mL Pasteur pipette before further processing.

Results

Cre-Lox activation successful

As expected from previous experiments, the phenotypic changes between the controls and Wt1 ablated animals are significant even after only 9 days (Figure 3A-B). The Wt1 knock-outs presented severe bloating and swelling due to the water retention caused by renal failure. These results are consistent with previous experiments done, and are the first indicator that the Cre-Lox system has worked.

In order to ensure that Cre-Lox recombination at the Rosa26 locus was successful, we stained the intestines and

kidneys with LacZ. The expression of the LacZ cassete generates β-galactosidase enzyme, which (after staining) cleaves the X-gal component that is responsible for the blue stained cells (Figure 3C). The recombination does not depend on the CO phenotype; hence the only variables are the presence of the LacZ cassette and tamoxifen administration. Although we do not know the exact percentage of successful recombination and/or if all animals have similar efficacy, we can confirm that the Cre recombination did take place to some extent.

Histological analysis of Wt1 ablation

Considering the function of Wt1 in intestines and the side effects of tamoxifen administration presented in previous experiments, we aimed to see if there are any histological differences due to deletion of Wt1.

Firstly, we have investigated if there are any differences in the intestinal length between the different groups. Because of the elasticity and shape of the intestines it is hard to measure the exact length; however we tried to estimate as closely as possible. After performing an ANOVA test on the three groups, we have concluded that there are not any significant differences between the intestinal lengths (Figure 4A-B).

A previous experiment done by a student has shown significant loss in the muscle layers volume in Wt1 deleted animals. These results were done with eosin staining on paraffin sections, but were inconclusive due to tissue damage. In order to validate these results we attempted to analyse the muscle volume difference with μCT scanning. As we can see in Figure 4C, the muscle layers and crypts of Lieberkuhn are easily distinguishable and the quality of the scans allows us to quantify any differences more accurately. However, due to time constrains we have not been able to fully analyse the μCT results in order to conclude whether there are quantifiable differences between the groups.

To look more in depth at the histology of the intestines, we used H&E staining on paraffin sectioned tissue. We focused mostly on the Jejunum-Ileum part of the small intestines, due to the higher tissue turnover in this area.

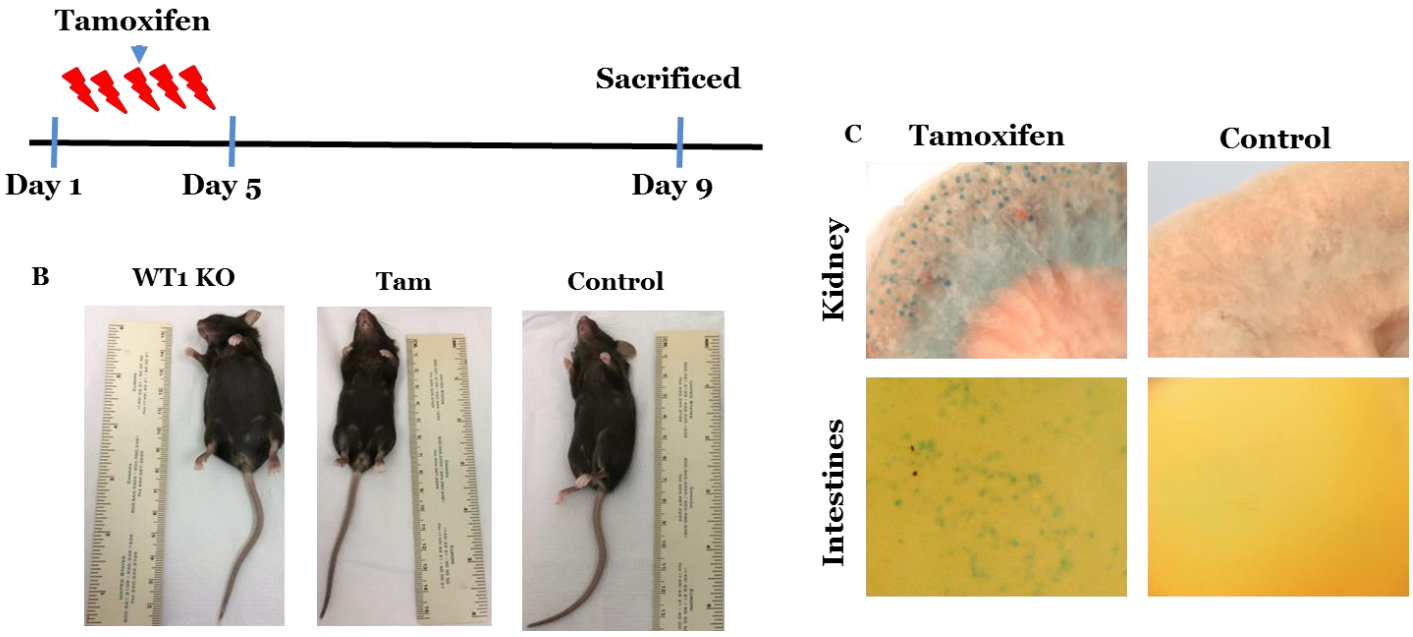

Figure 3|Successful LacZ activation after tamoxifen treatment. A) Timeline for the Wt1 ablation experiment. **B)** Wt1 ablated animals' present significant water retention that offers the appearance of a bloated abdomen. The positive and negative controls have normal physical appearance, showing that tamoxifen alone is not responsible for the generation of this phenotype. **C)** LacZ staining is present in both kidneys and intestines. Due to the high expression WT1 in mature renal podocytes, there is significant expression of β-galactosidase that is produced by the LacZ cassette; hence we used it to further confirm the LacZ recombination.

The major structures are easily distinguishable; the serosa, muscularis externa, epithelial submucosa and mucosa (Figure 4D). Structurally, there are not any differences; however we can see more lesions in the longitudinal muscle layer of the Wt1 ablated than in the controls (Figure 4E). Since the lesions are predominantly in the Wt1 knock-out tissue and not in the tamoxifen treated ones, we can conclude that the tamoxifen variable is not responsible for this phenotype. However it is worth mentioning that this phenotype could also be partly due to tissue damage. The cells exhibited round gaps between each other that usually appear with tissue loss, and they look slightly dehydrated.

Characterization of Wt1 function in the smooth muscle layer of the gut

Understanding the signalling pathway of Wt1 in intestines would help us better comprehend what the function of Wt1 in the intestinal wall is. We looked at four different markers that targeted α-SMA, CK, Desmin and Wt1 expression. Wt1 was only expressed in the tamoxifen treated and its expression is restricted in mesothelium cells above the longitudinal muscle layer (Figure 5B). α-SMA, which is a marker for

myofibroblast formation[17], was present mostly in the muscle layers and very infrequently in the crypts and villi (Figure 5A, 5C), as expected. The expression of α-SMA is comparable between the controls and animals lacking Wt1, which suggests that Wt1 is not involved in muscle maintenance. Desmin is considered a marker for differentiated muscle tissue; it is expressed at high levels after the cells are at the end of the differentiation process[18]. Consequently, desmin's expression in the muscle layers and at the edges of the villi is anticipated as there is where most differentiated muscle cells are in the intestines; however, again, we do not see any differences in the expression pattern between the groups. Cytokeratin is known to be expressed in mesothelium cells; hence it makes a good marker to distinguishing their presence. As we can see in Figure 5A, the thin layer of mesothelium is visible in all conditions above the muscle layers. Overall, we can conclude that absence of Wt1 does not affect intestinal wall regulation; however, we have to keep in mind that in this study we have looked at a very small number of proteins.

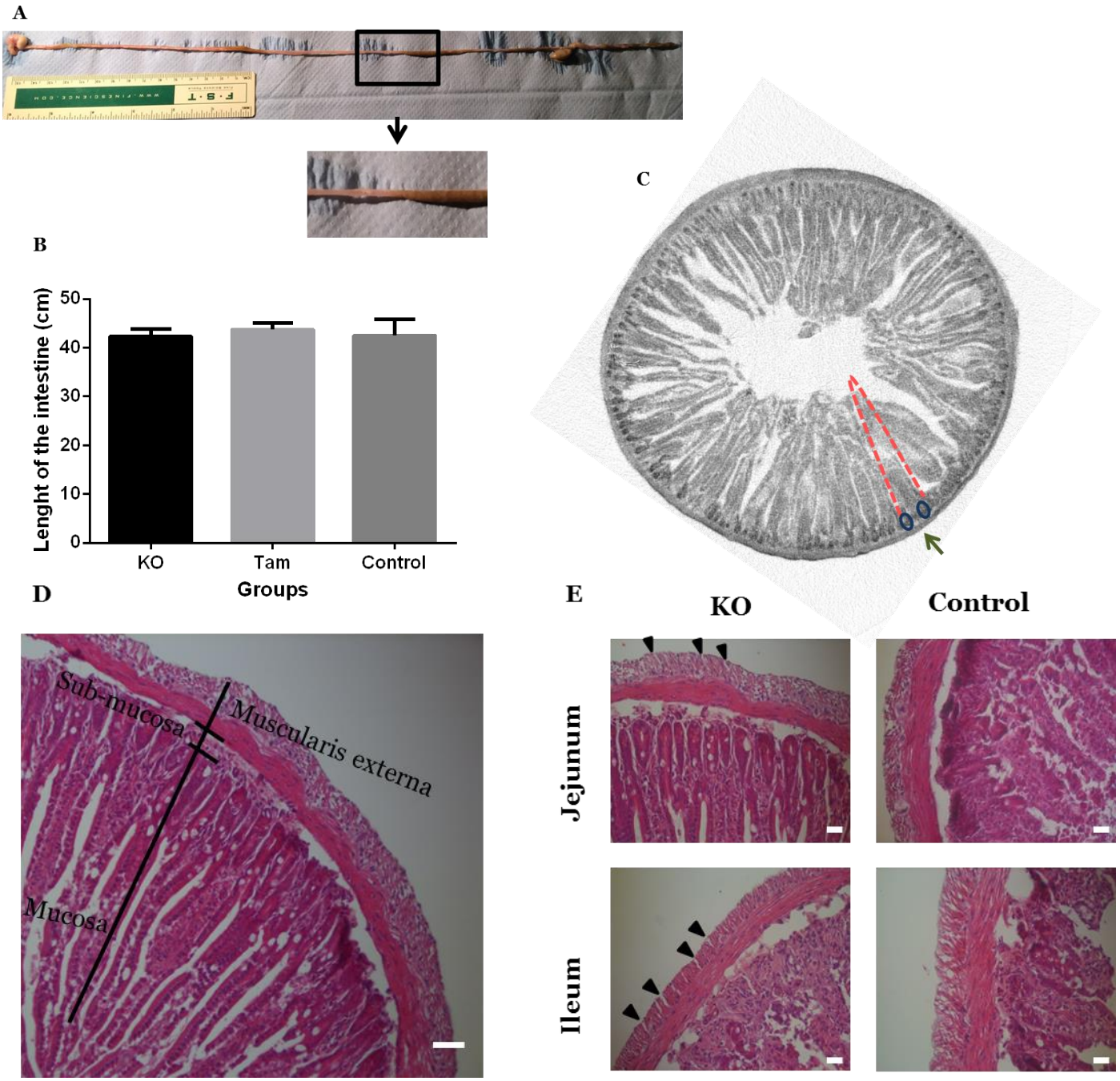

Figure 4| Analysis of overall intestinal physiology and histology. **A)** Straightening of the intestines and measuring the relative length from stomach to cecum. The black rectangle shows that mouse intestines have different levels of muscle contraction. Some parts that contain the faecal matter are very stretched and others that do not are in a relaxed state. **B)** Statistical analysis done with ANOVA test does not show any significant differences in intestinal lenghts between the three groups ($p>0.05$) **C)** µCT scans present a layout of the intestinal structures. The green arrow represents the muscle layers, the blue circles are the crypts, and the structure of a villi is contoured in red. The grey area between the muscle layer and the crypts is the submuscosa. **D)** Small intestines are divided into mucosa, submucosa and muscularis externa.

E) Sections from different parts of the intestines focused on the muscle layers. We observed a small difference in the longitudinal muscle structure; in the KO sections there seems to be gap-like lesions between longitudinal muscle fibres (black arrows). Scale set at 100 μm (D)and 50μm (E).

Discussion

Due to limited time our results are at the moment incomplete and further experiments are required for a broader picture to be attained. Although we have shown that the LacZ recombination was successful, we cannot securely say at what degree it did and if all animals present similar recombination rates. This parameter could affect some of the results, especially when the differences between mutants and controls are very small.

The intestinal length analysis showed that the intestines did not present a significant difference after 9 days. However, our findings are based on a very small sample size (11 animals in total). The study was conducted for only 9 days, when the animals displayed signs of the beginning of organ failure[9]. We believe that a longer period of time is necessary in order to be able to see any differences in the intestines. This could be achieved with conditional knock-out; inducing a promoter that is expressed exclusively in the mesothelial cells and bypasses the organ failure and other side effects of the systemic Wt1 ablation. Hence, we could perform the study for a longer period of time with less noise and observe any changes in tissue homeostasis. Considering that this method requires a gene that is specific to the mesothelium cell layer and that the immunohistochemical profile of mesothelium is still largely unknown[19], it will require some time until it is feasible.

The histological analysis presented small differences in the longitudinal muscle layer; nevertheless we need to analyze the μCT results in order to fully confirm this hypothesis. Along the intestinal wall, the muscles are known to be in three states: contracted, stretched and relaxed. As we can see in Figure 4A, there is a significant difference between muscle volumes across states; in the stretched state the muscles are very thin due to the high content, therefore the overall muscle volume will be lower than in relaxed muscles[20]. This should be kept in mind when we look at the μCT scans and measure the muscle volume across the intestinal wall. It would be interesting to redo the experiment, use a muscle relaxant and compare the results with our present findings. This comparison would allow us to eliminate the possibility that any difference shown is not due to higher level of muscle stretching in all the samples included, rather than Wt1 effect on intestinal wall.

Considering that we do not know where and how Wt1 affects the adult intestinal homeostasis, it is challenging to assume which pathways might be involved. Considering the histology results, we have tried immunofluorescence markers for muscle proliferation. As our investigations did not show any Wt1 involvement, it might be interesting to look at pathways that are known to be involved in intestinal development.

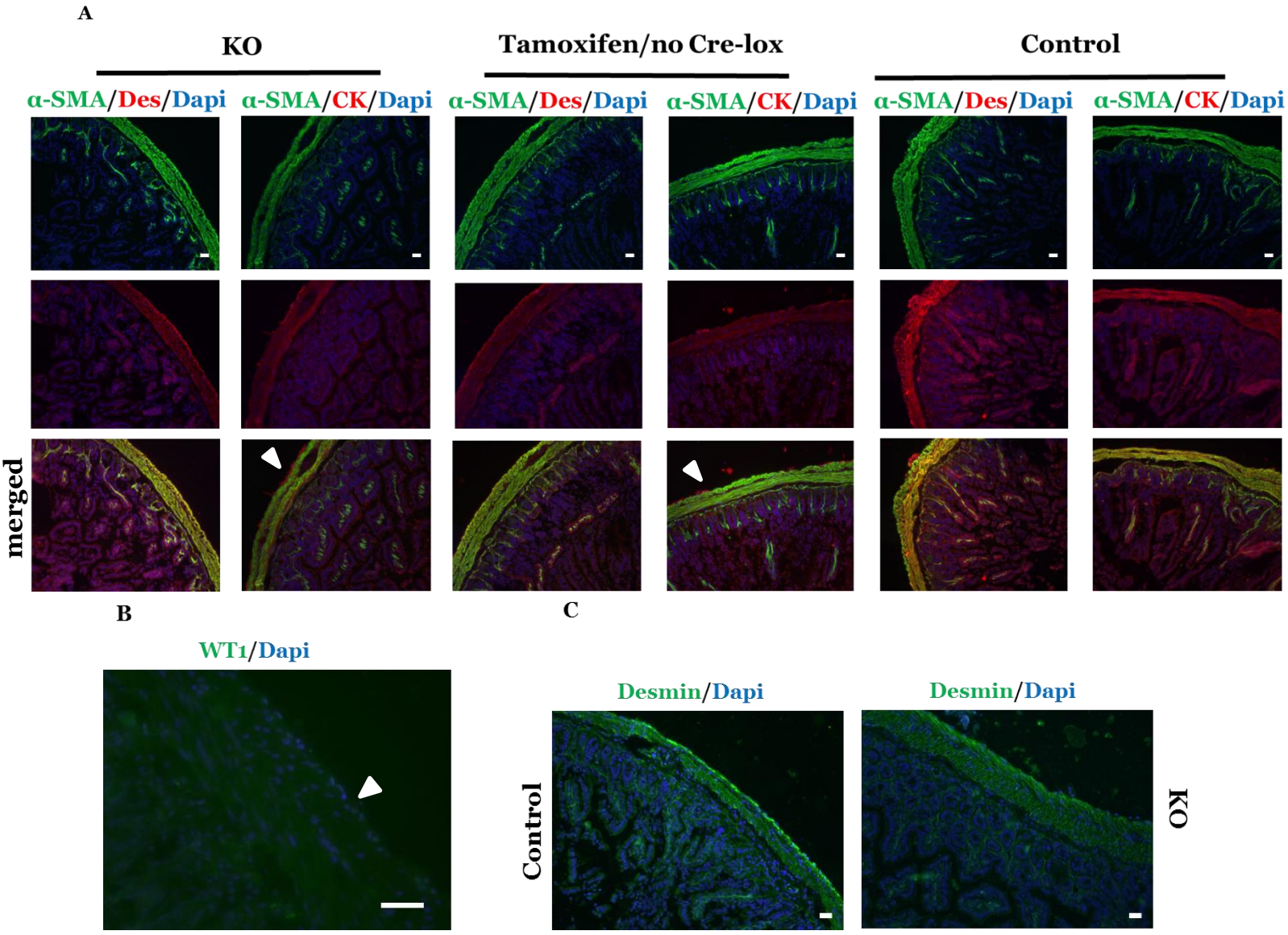

Figure 5| Deletion of Wt1 does not show any differences in expression of essential proteins for muscle regeneration. A) Immunofluorescence on frozen tissue with α-SMA, des and CK do not present any differences in expression between the groups. The mesothelial cell layer is visiblely wrapped around the muscle layers (white arrows.) **B)** WT1 is expressed in the mesothelium cells in the controls mice as shown by the white arrow. **C)** Due to the endogenous expression of mT, we have used green secondary antibody to ensure that the background is not affecting the actual expression of the Desmin marker. α-SMA- α smooth muscle actin; Des- Desmin; CK- Cytokeratin. Scale at 20 µm (A+B+C).

Epithelial-mesenchymal signaling crosstalk is at the base of intestinal lengthening, and Wt1 is known for being a crucial regulator of it. Several pathways are known to be involved in the process of intestinal lengthening, such as Fgf, Hedgehog, Wnt5 and Notch[21]. Tgf-β signaling network would also be a good candidate due to its role in directing the differentiation of mesenchymal cells towards myofibroblasts[22]. Looking at changes in gene expression with quantitative PCR

might also give us some insights into possible targets. Considering Wt1 has been known to activate Wnt/β-catenin pathway in the kidneys[12], this could be a good target for primary tryouts.

Another experiment that would be interesting would be the conditional knockout of Wt1 during injury response, for example with irradiation or chemotherapy. If Wt1 makes a difference in the recovery of homeostasis post injury, this would give us the understanding that it is active towards the intestinal maintenance. Wt1 has been shown to be involved in the growth of coronary vessels after injury to the myocardial muscle. During ischemia, Wt1 becomes more expressed near the affected zone in the vascular smooth muscle cells [23]. Although our results do not show substantial differences without Wt1 during normal conditions, this might change throughout repair. Following this we could also try to look at the intestinal muscle force *in vitro* and compare it with the non-injured groups to see if the repair was complete.

Conclusion

This study tried to give us a better understanding of the role of Wt1 in gut homeostasis. While our results are deficient at the moment and a final conclusion of the Wt1 role in visceral smooth muscle/intestinal wall is hard to draw, we acquired data that will help us design future experiments.

Acknowledgments

I would like to thank Dr Bettina Wilm for her help and support throughout the project and Dr Thomas Wilm for his assistance and help with different techniques. I would also like to thank Callum Quinn for the kidney LacZ pictures provided and Prof. Rob van'T Hof for his help with the μCT.

References

1 Wilm, B. and Muñoz-chapuli, R. (2016) Chapter 3 The Role of WT1 in Embryonic Development and Normal **1467**.

2 Roberts, S. G. E. (2006) The modulation of WTI transcription function by cofactors. Biochem. Soc. Symp. **201**, 191–201.

3 Hohenstein, P. and Hastie, N. D. (2006) The many facets of the Wilms ' tumour gene , WT1 **15**, 196–201.

4 Armstrong, J. F., Pritchard-Jones, K., Bickmore, W. A., Hastie, N. D. and Bard, J. B. L. (1992) The expression of the Wilms' tumour gene, WT1, in the developing mammalian embryo. Mech. Dev. **40**, 85–97.

5 Kreidberg, J. A., Sariola, H., Loring, J. M., Maeda, M., Pelletier, J., Housman, D. and Jaenisch, R. (1993) WT-1 is required for early kidney development. Cell **74**, 679–691.

6 Martínez-Estrada, O. M., Lettice, L. A., Essafi, A., Guadix, J. A., Slight, J., Velecela, V., Hall, E., Reichmann, J., Devenney, P. S., Hohenstein, P., et al. (2010) Wt1 is required for cardiovascular progenitor cell formation through transcriptional control of Snail and E-cadherin. Nat. Genet. **42**, 89–93.

7 Wilm, B., Ipenberg, A., Hastie, N. D., Burch, J. B. E. and Bader, D. M. (2005) The serosal mesothelium is a major source of smooth muscle cells of the gut vasculature.

8 Chau, Y.-Y., Bandiera, R., Serrels, A., Martínez-Estrada, O. M., Qing, W., Lee, M., Slight, J., Thornburn, A., Berry, R., McHaffie, S., et al. (2014) Visceral and subcutaneous fat have different origins and evidence supports a mesothelial source. Nat. Cell Biol. **16**, 367–75.

9 Chau, Y., Brownstein, D., Mjoseng, H., Lee, W., Buza-vidas, N., Jacobsen, S. E., Perry, P., Berry, R., Thornburn, A., Sexton, D., et al. (2011) Acute Multiple Organ Failure in Adult Mice Deleted for the Developmental Regulator Wt1 **7**.

10 Hosen, N., Shirakata, T., Nishida, S., Yanagihara, M., Tsuboi, a, Kawakami, M., Oji, Y., Oka, Y., Okabe, M., Tan, B., et al. (2007) The Wilms' tumor gene WT1-GFP knock-in mouse reveals the dynamic regulation of WT1 expression in normal and leukemic hematopoiesis. Leukemia **21**, 1783–91.

11 Guo, J.-K., Menke, A. L., Gubler, M.-C., Clarke, A. R., Harrison, D., Hammes, A., Hastie, N. D. and Schedl, A. (2002) WT1 is a key regulator of podocyte function: reduced expression levels cause crescentic glomerulonephritis and mesangial sclerosis. Hum. Mol. Genet. **11**, 651–659.

12 Jing, Z., Wei-jie, Y. and Yi-feng, Z. ge. (2015) Down-regulation of Wt1 activates Wnt/??-catenin signaling through modulating endocytic route of LRP6 in podocyte dysfunction in vitro. Cell. Signal., Elsevier Inc. **27**, 1772–1780.

13 Feil, R., Brocard, J., Mascrez, B., LeMeur, M., Metzger, D. and Chambon, P. (1996) Ligand-activated site-specific recombination in mice. Proc. Natl. Acad. Sci. U. S. A. **93**, 10887–90.

14 Shehata, M., van Amerongen, R., Zeeman, A. L., Giraddi, R. R. and Stingl, J. (2014) The influence of tamoxifen on normal mouse mammary gland homeostasis. Breast Cancer Res. **16**, 411.

15 Huh, W. J., Khurana, S. S., Geahlen, J. H., Kohli, K., Waller, R. A. and Mills, J. C. (2012) Tamoxifen induces rapid, reversible atrophy, and metaplasia in mouse stomach. Gastroenterology **142**.

16 Huh, W. J., Mysorekar, I. U. and Mills, J. C. (2010) Inducible activation of Cre recombinase in adult mice causes gastric epithelial atrophy, metaplasia, and regenerative changes in the absence of "floxed" alleles. Am. J. Physiol. Gastrointest. Liver Physiol. **299**, G368–80.

17 Nagamoto, T., Eguchi, G. and Beebe, D. C. (2000) Alpha-Smooth Muscle Actin Expression in Cultured Lens Epithelial Cells. Invest. Ophthalmol. Vis. Sci. **41**, 11–13.

18 Aebi, U., Herrmann, H., Harald, B. and Strelkov, S. V. (2004) The biology of desmin filaments : how do mutations affect their structure , assembly , and organisation ? **148**, 137–152.

19 Terada, T. (2011) Case Report Immunohistochemical profile of normal mesothelium and histiocytic / methothelial hyperplasia : a case report **4**, 631–636.

20 Brasseur, J. G., Nicosia, M. A., Pal, A. and Miller, L. S. (2007) Function of longitudinal vs circular muscle fibers in esophageal peristalsis, deduced with mathematical modeling. World J. Gastroenterol. **13**, 1335–1346.

21 Walton, K. D., Freddo, A. M., Wang, S. and Gumucio, D. L. (2016) Generation of intestinal surface: an absorbing tale. Development **143**, 2261–2272.

22 Geske, M. J., Zhang, X., Patel, K. K., Ornitz, D. M. and Stappenbeck, T. S. (2008) Fgf9 signaling regulates small intestinal elongation and mesenchymal development. Development **135**, 2959–68.

23 Wagner, K. D., Wagner, N., Bondke, A., Nafz, B., Flemming, B., Theres, H. and Scholz, H. (2002) The Wilms' tumor suppressor Wt1 is expressed in the coronary vasculature after myocardial infarction. FASEB J. **16**, 1117–1119.