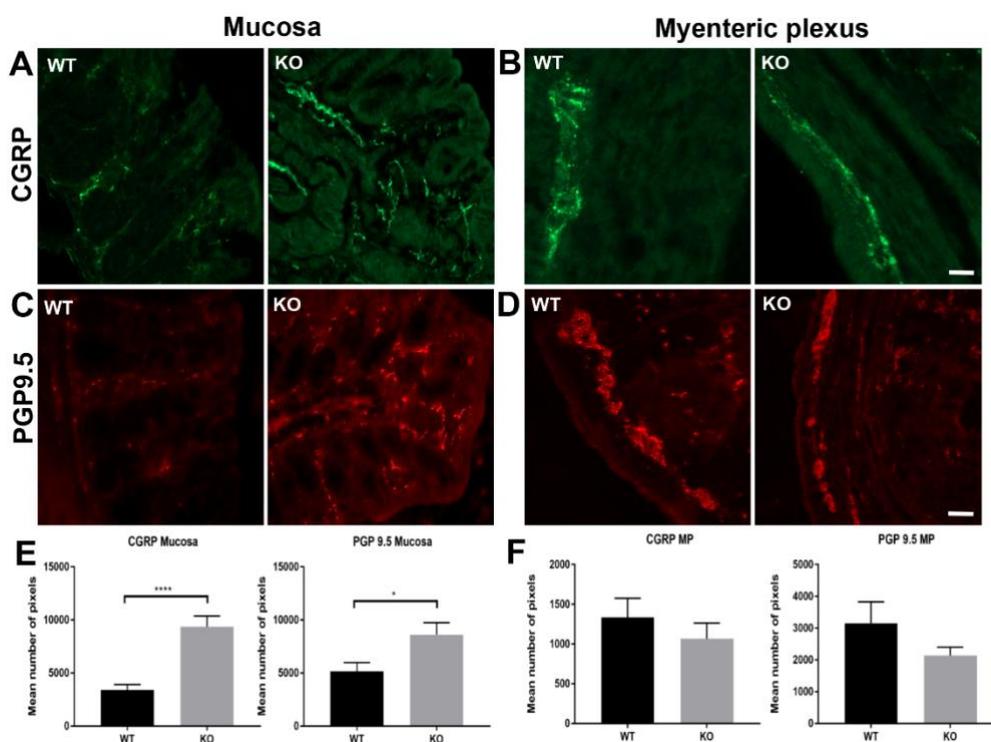


### hEDS Microgrant report-2019

We are extremely grateful that the EDS society has given us this microgrant to start our work on understanding the mechanism behind abdominal pain in patients with hEDS. Using this microgrant we were able to initially purchase antibodies and perform immunohistochemical analysis that added data to our paper published in the leading Journal of Physiology.

Our aim was to understand what causes abdominal pain in mice and patients with hypermobility. We were able to explore the possible cause of abdominal pain in our mouse model (Tenascin x deficiency), which shows increased nerve fiber density in the colonic mucosa (Figure 1-published). An increase in nerve fibers that are involved in the pain pathway indicated by calcitonin gene related peptide (CGRP) suggests that this hyper proliferation may be the reason why pain is more common.

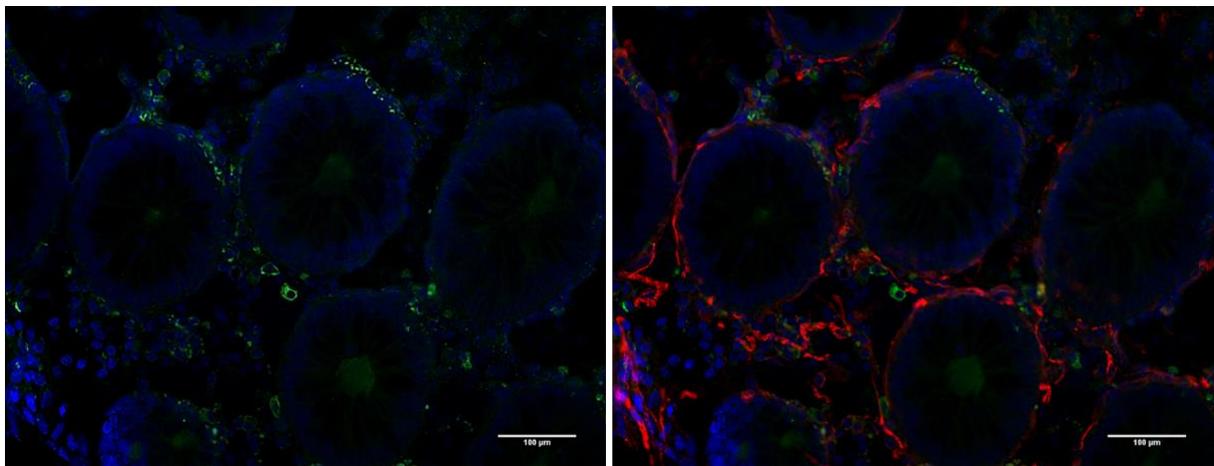


**Figure 1: Neuronal sprouting in TNX-KO mucosa.** Representative Immunohistochemical images taken from sections of WT mouse colonic mucosa (A, C) and myenteric plexus (B, D). CGRP-IR is shown in green (A) where there was a threefold proliferation of CGRP-IR nerve endings in the colonic mucosa of TNX-KO mice (TNX-KO  $9350 \pm 1018$ , WT  $3411 \pm 508$  pixels/image,  $p < 0.0001$ ) (E). This was also observed with PGP9.5 in red (C) although this increase was smaller for PGP-IR endings (TNX-KO  $8615 \pm 1132$ , WT  $5152 \pm 832$  pixels/image,  $p = 0.0224$ ) (E). No changes were seen in either marker in the myenteric plexus (CGRP: TNX-KO  $1065 \pm 198$ , WT  $1333 \pm 242$  pixels/image,  $p = 0.3950$ , (PGP-9.5: TNX-KO  $2133 \pm 266$ , WT  $3154 \pm 675$  pixels/image,  $p = 0.1368$ ) (F). Statistical analysis was performed using a two-way ANOVA (Mann-Whitney posthoc test). Scale bar in all panels =  $20 \mu\text{m}$ .

We further hypothesised that similar to our tenascin x deficient mice patients with hEDS and TNX deficient mice will also have increased pain fibres in colonic and gastric mucosa.

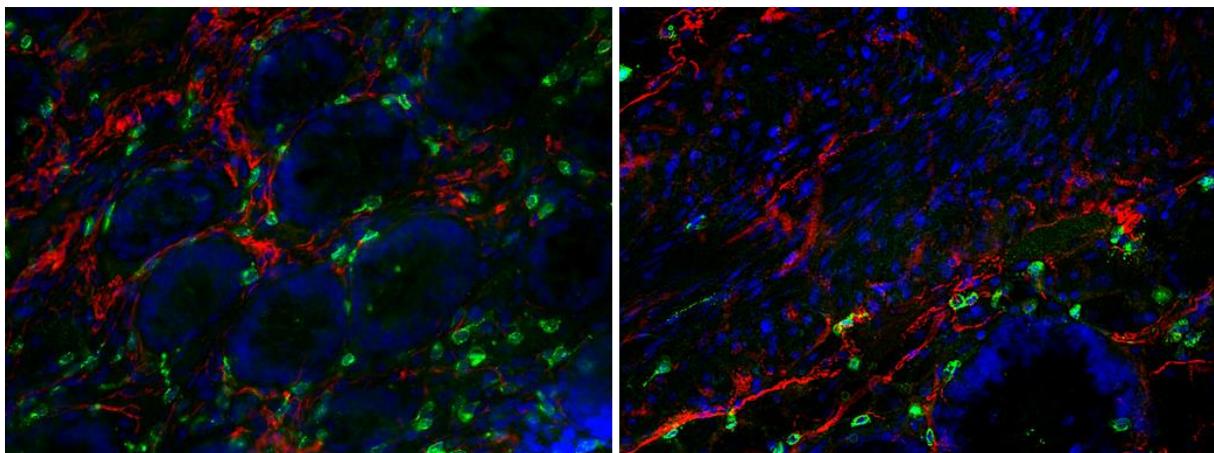
We were able to obtain colonic and gastric tissue from patients with hEDS and have started the immunohistochemistry. Unfortunately the costs of each antibodies is around 400-500 pounds and we have used a lot of the microgrant for the expression studies on the mouse. Moreover, the microscope bookings have incurred a large cost thus we have used most of the microgrant. However, we have some data on hEDS patient tissue where we have optimised staining using CGRP and mast cells using the remainder of the grant. An increase in mast cell numbers is also thought to be increased in irritable bowel syndrome patients who have increased abdominal pain.

Unfortunately, we have not been able to obtain further funding to continue this project from the EDS society hence this project has ended. We do not have tissue to compare the current data to a normal healthy sample to understand whether hEDS patients have increased nerve sprouting as was observed in the mouse. Nonetheless, Figure 2 shows staining using CGRP and PGP in the colonic mucosa of a patient with hEDS.



**Figure 2: CGRP and PGP positive in hEDS colonic biopsy.** Expression of CGRP (green) and PGP (red) in a patient with known hEDS.

As mentioned previously we have started to also look at the expression of mast cells in patients with hEDS. Our staining worked well where you can see green positive mast cells in and around the colonic crypts (Figure 3)



**Figure 3: Mast cell expression in hEDS colonic biopsy.** Expression of mast cells (green) and PGP (red) in a patient with known hEDS.

From the data shown in this report we are able to demonstrate that the antibodies have been optimised and we now require further samples and healthy controls to complete the story. We aim to apply for further funding to continue this project to understand the pathophysiology of pain in patients with hEDS.