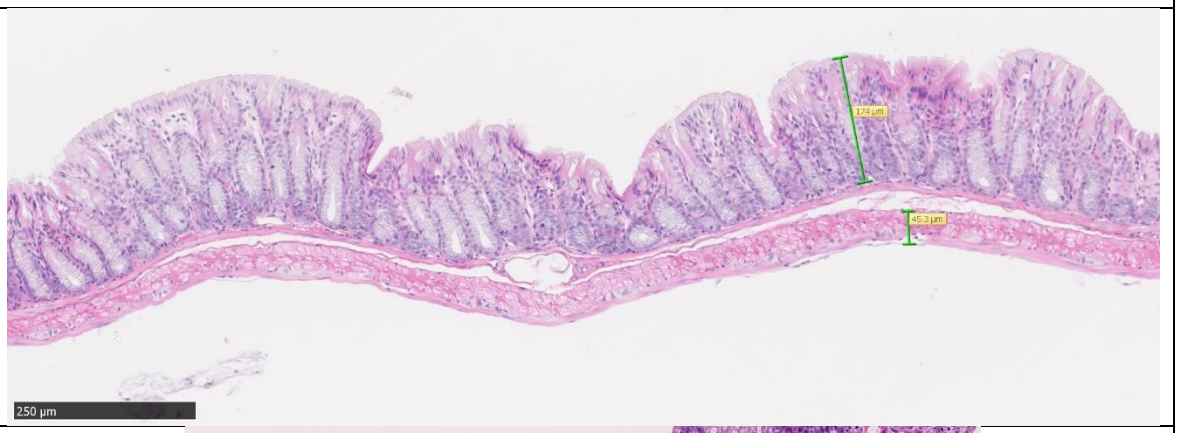
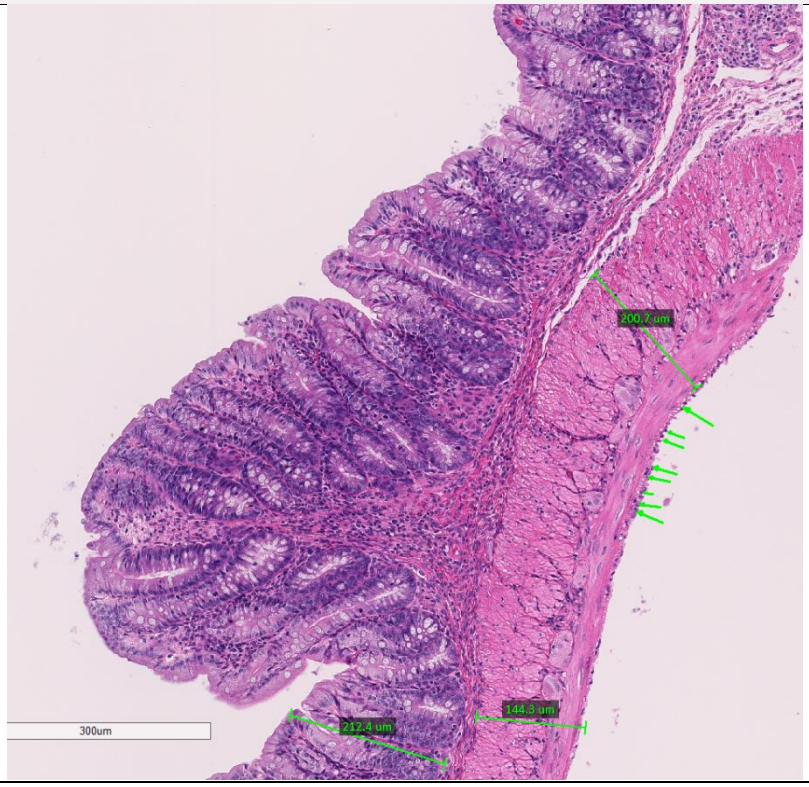
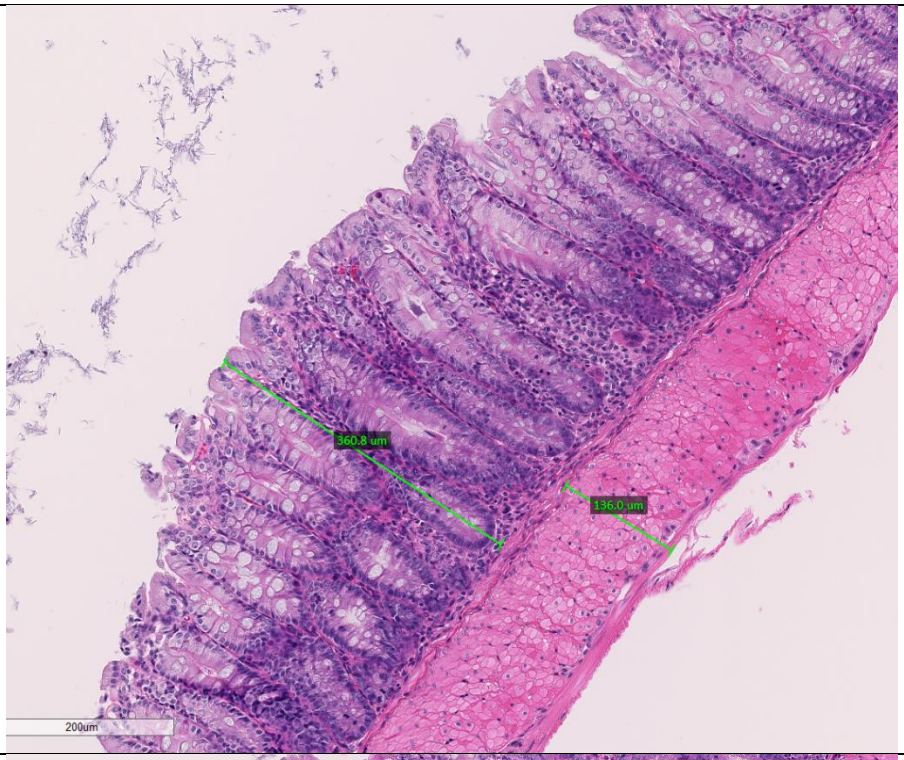
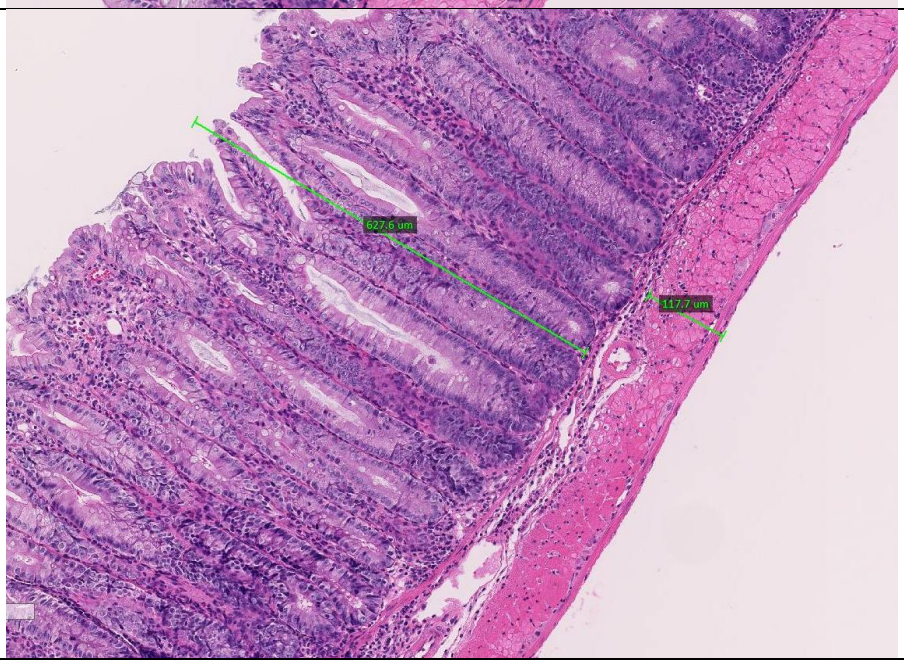
Atlas for Scoring Colitis after Adoptive Transfer

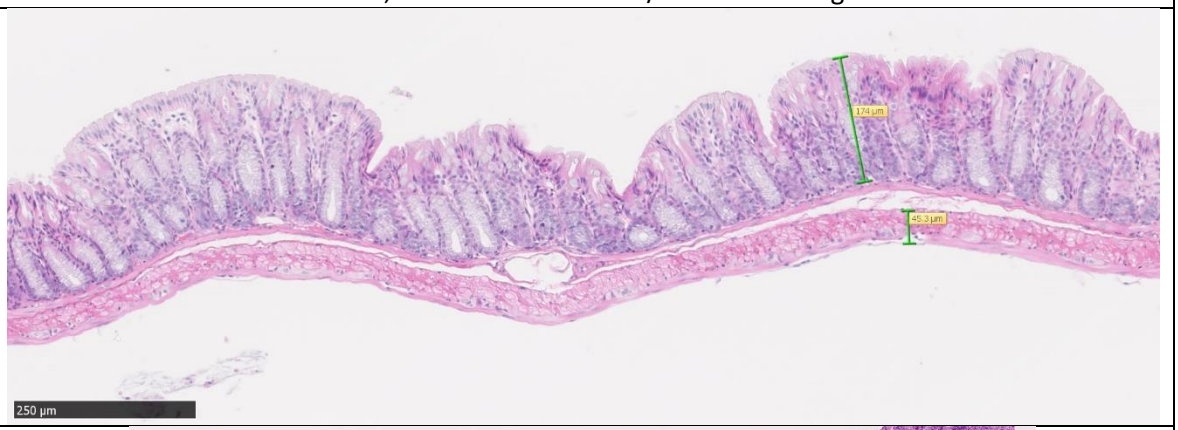
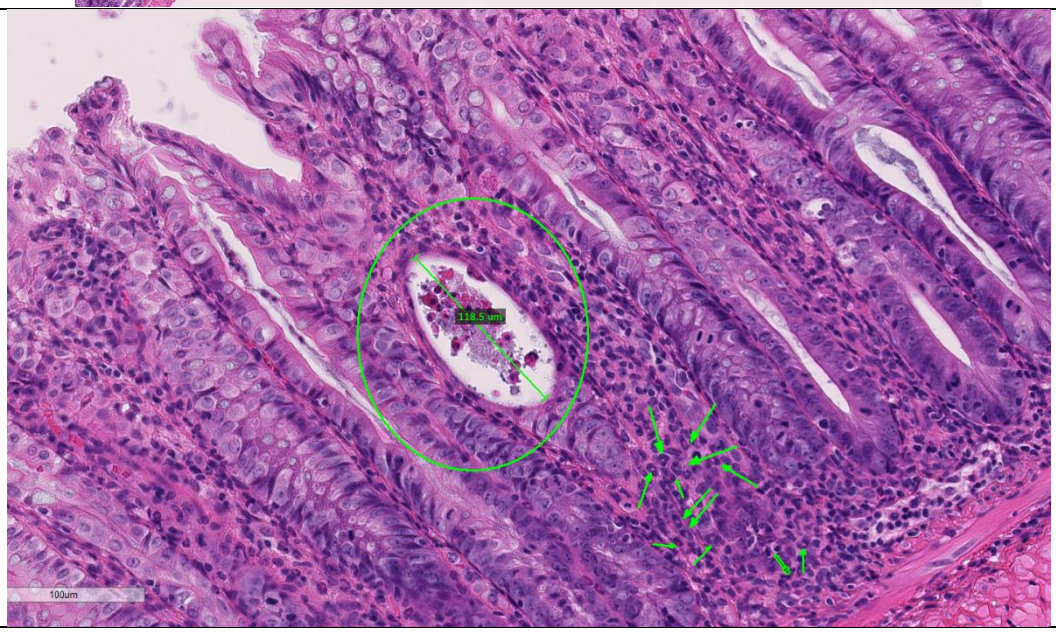
The following atlas is provided as a guide for scoring colitis in the CD45RB transfer model. For these studies, Rag1 knockout mice were given a subset of pathogenic Th cells. Mice were following by assessing health and weight. As they lost 15% of their body weight compared to controls, they were euthanized or at 8 weeks post transfer – whichever came first.

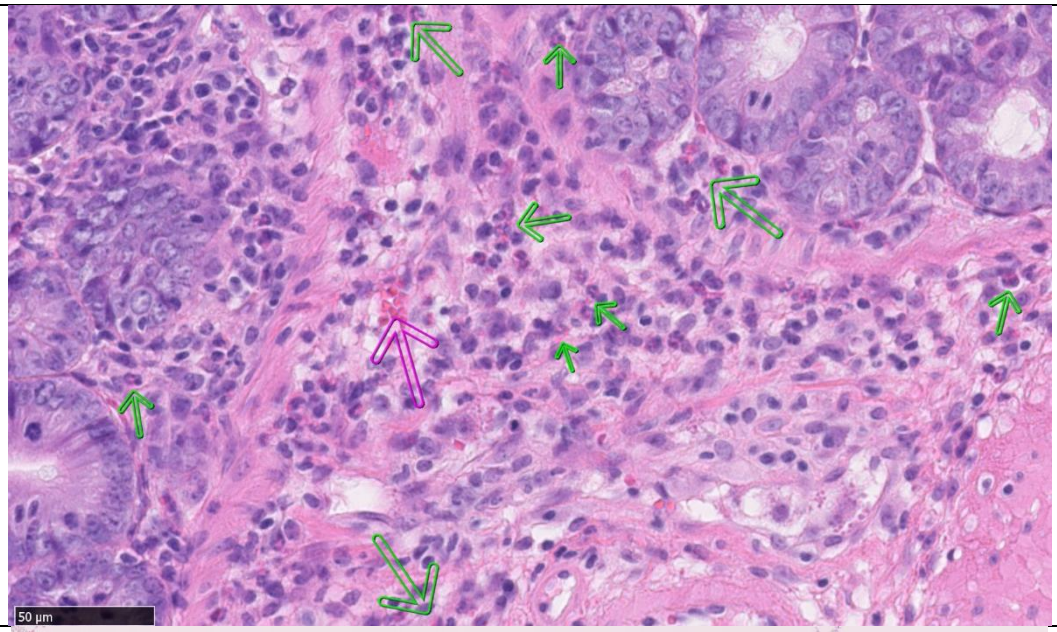
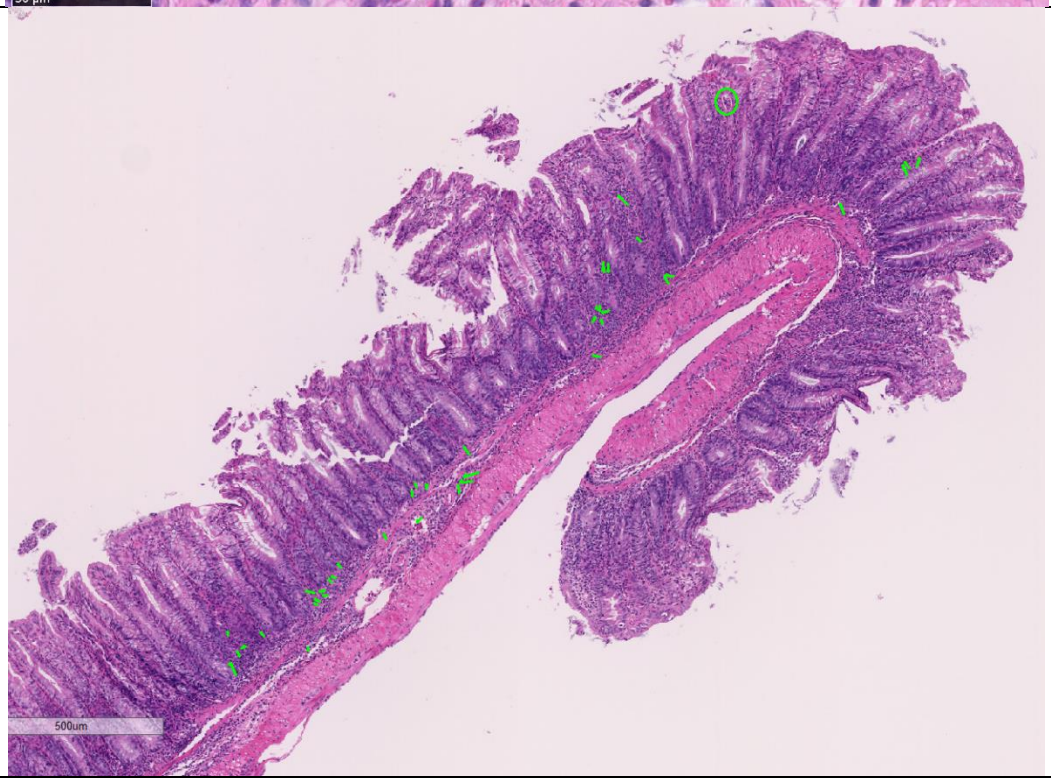
For scoring, some people divide the colon into halves while others use thirds, the latter being more important for TNBS-induced colitis. The scoring sheet in excel is also provided and goes through each of the criteria for each region. The total score achievable (up to 48 for upper, mid and lower colonic regions) is somewhat high in order to provide more resolution than simply giving an entire tissue a score from 1-3.

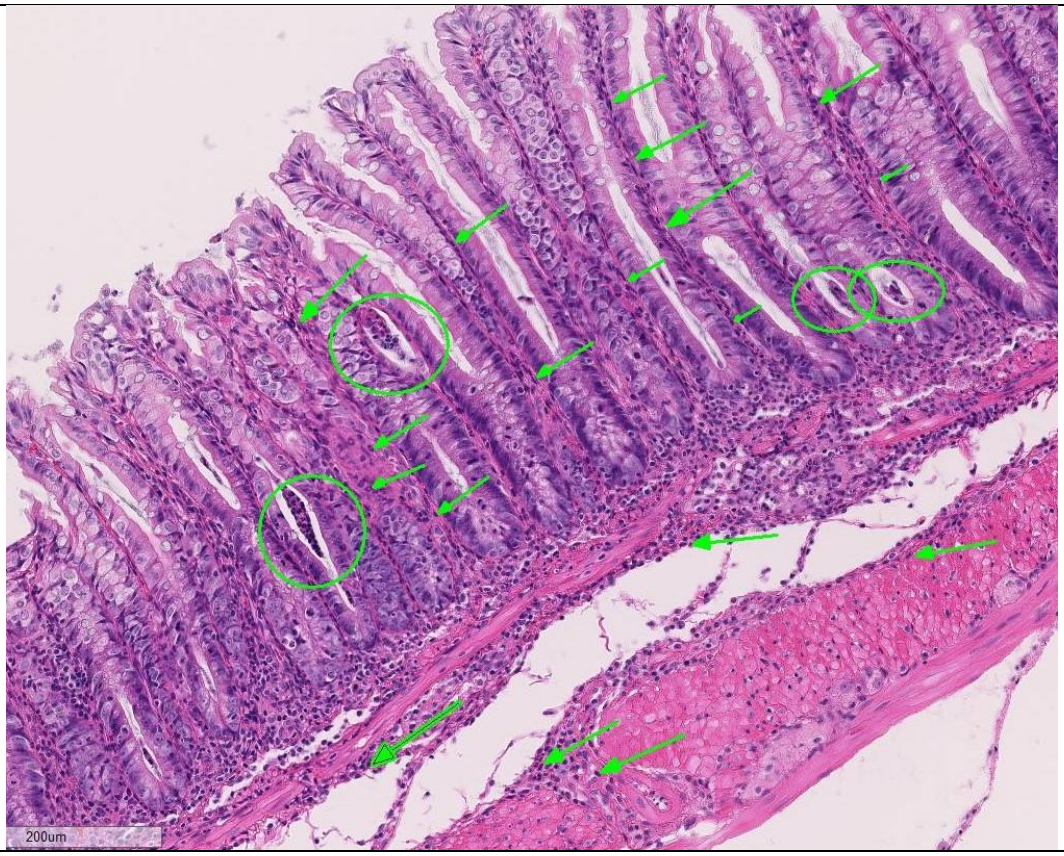
For planning experiments, estimating a power analysis, viewing other representative slides, refining the scoring for other models of colitis or in other tissues, please contact Dr. Peter Ernst at pernst@ucsd.edu.

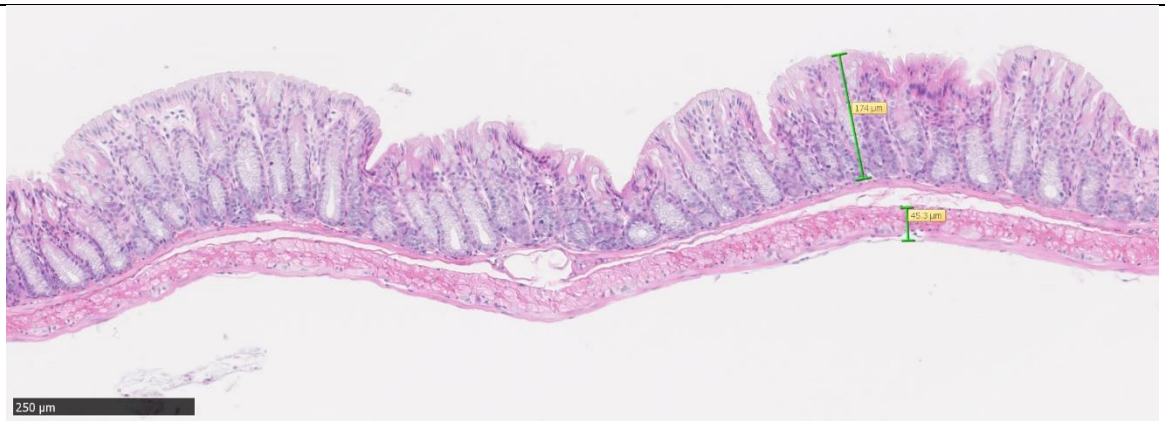
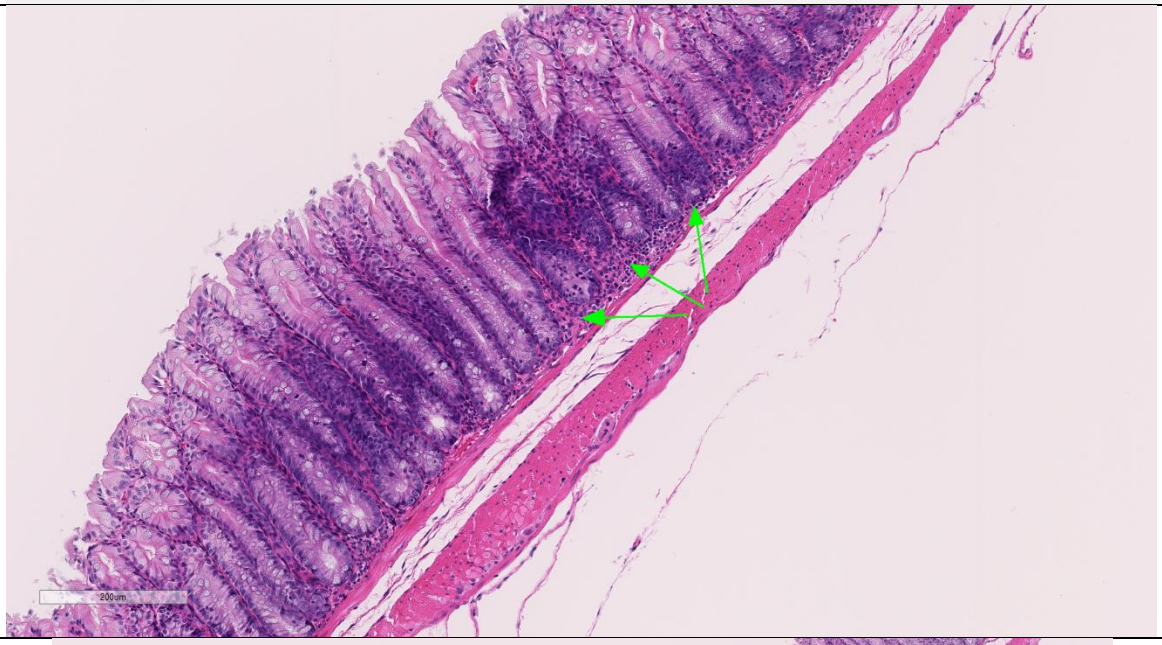
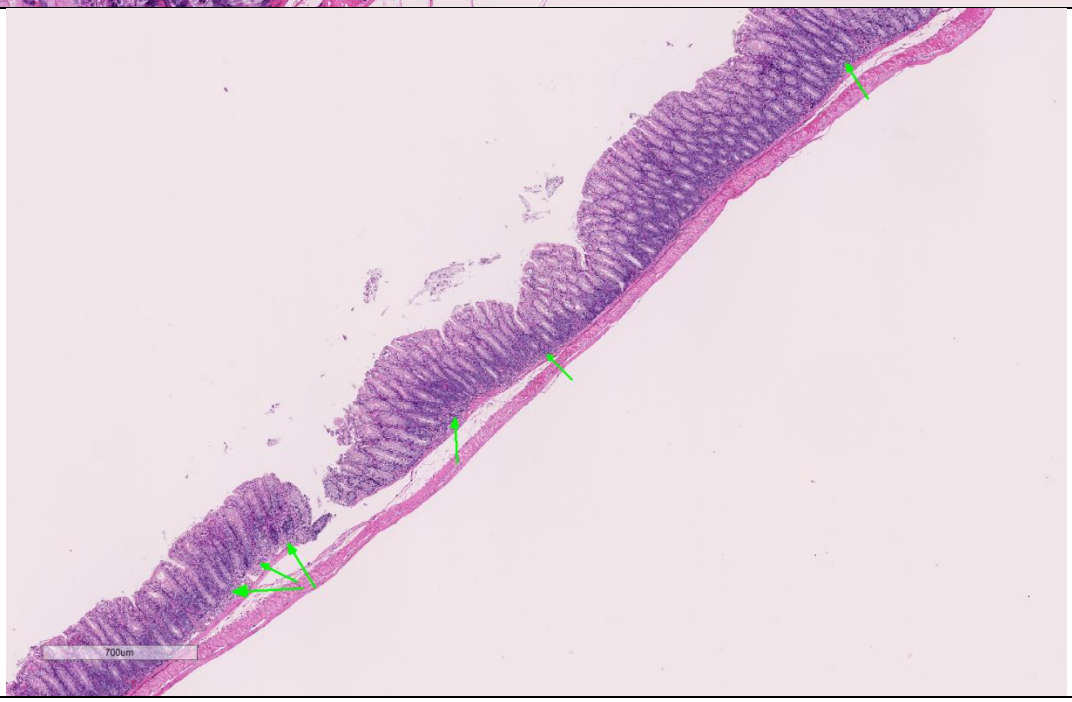
	Colon
Thickness < 400 μM = 0	
Thickness > 400-450 μM = 1	

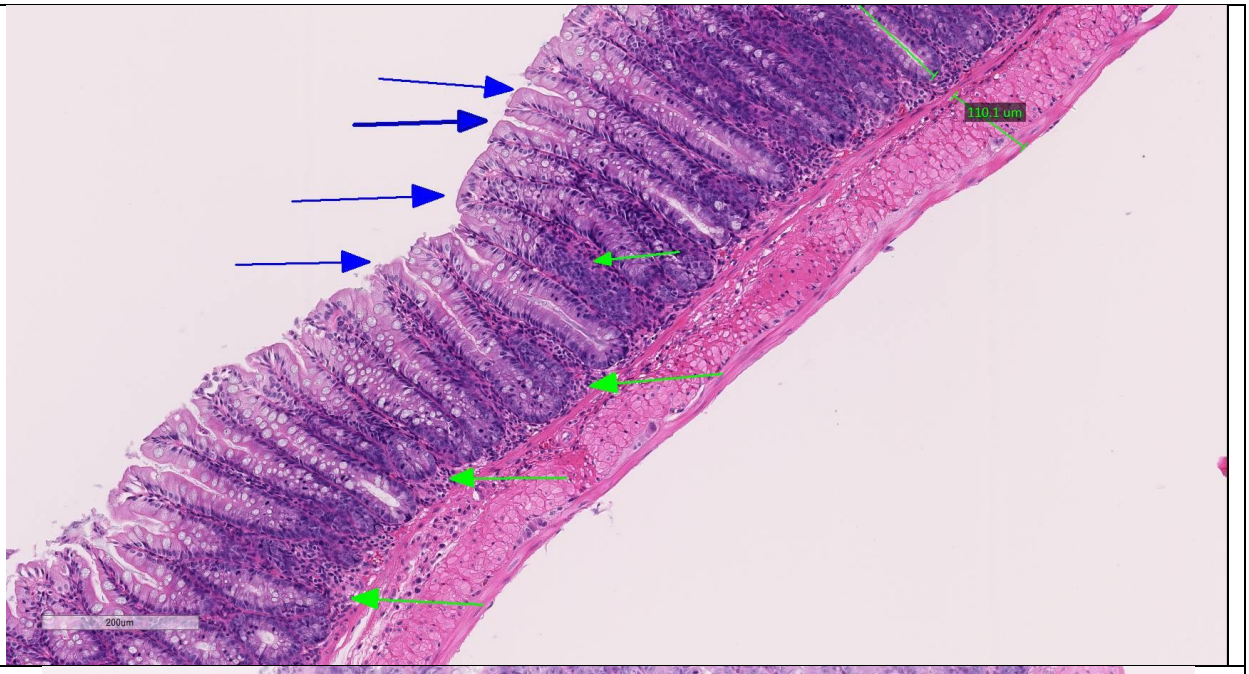
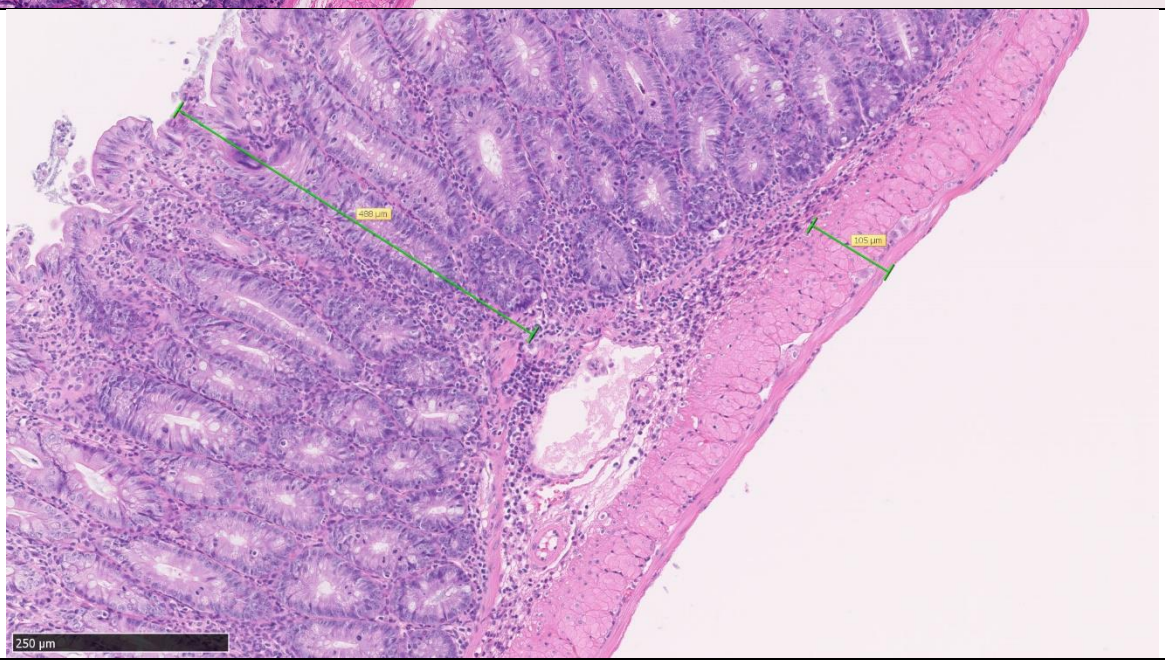
450-500 μM = 2	
> 500 μM = 3	
Notes	Score 0, Mouse ID: 15171. Score 1, Mouse ID: 15276. Score 2, Mouse ID: 15271. Score 3, Mouse ID: 15271 example in one site, mean was 2. Thickness is quick and objective but sampling errors can affect results as the sections can show uneven thickness due to angles, rotation or other artefacts. Sometimes variations are real. One has to "sample" with as little bias as possible or take more than three measurements. One approach is to use lumen to luminal side of muscularis mucosae for "mucosa" delimiters; serosal side of muscularis mucosae to serosa for tunica muscularis. Do not include layers separated by fixation. Use the mean of three mucosal and three muscularis measurements. These are all about the same magnification so score 3 is clearly thicker.

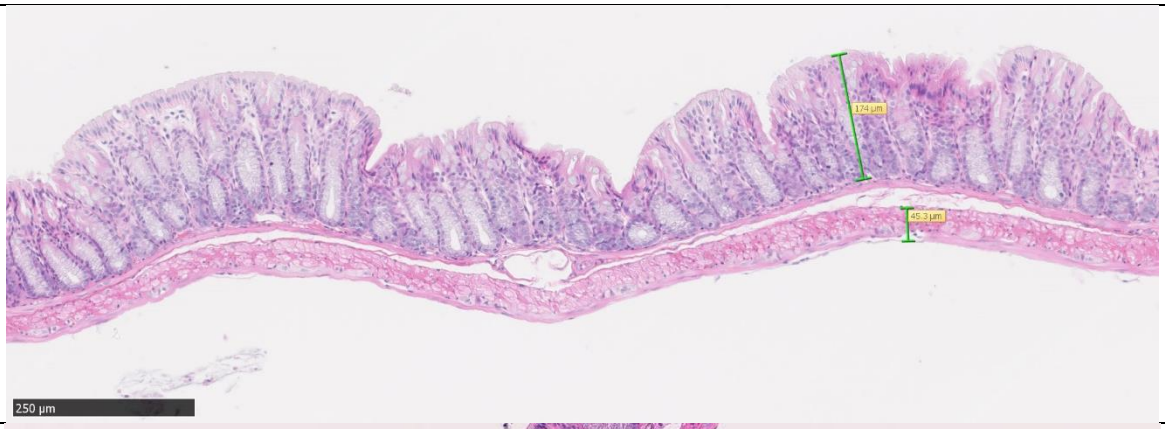
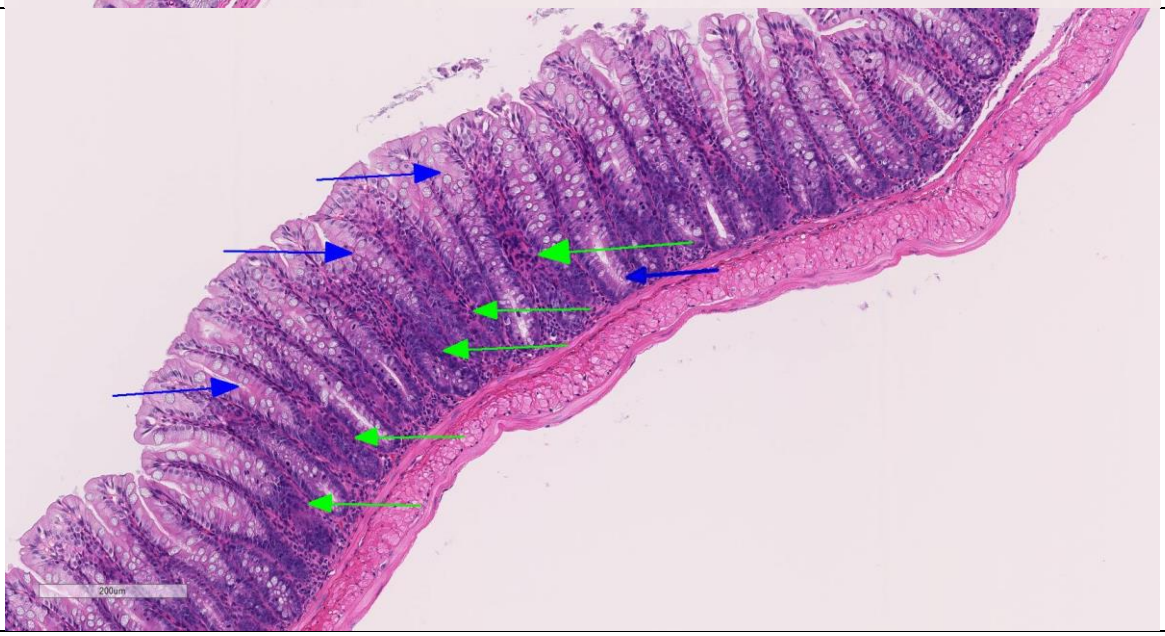
PMN	0=few; 1=focal 6-20 per 5 crypts; 2=multi, and/or <5 abscesses; 3= diffuse and/or 6-15 abscesses; 4=>15 abscesses and/or neuts throughout
0	
1	
2a	

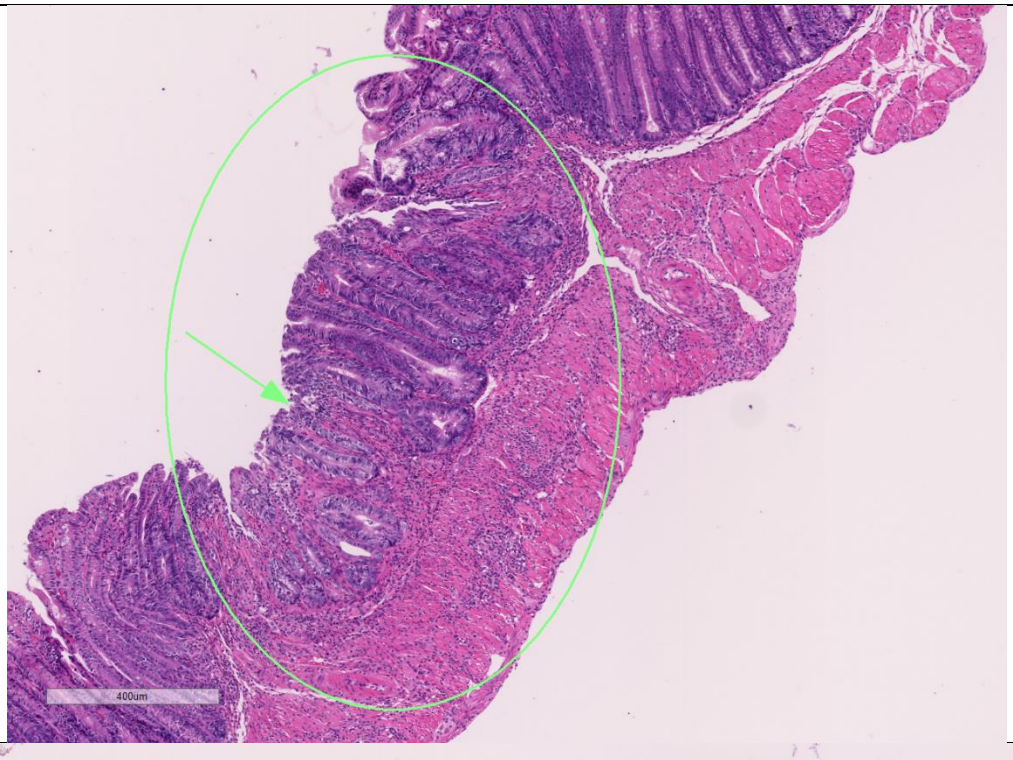
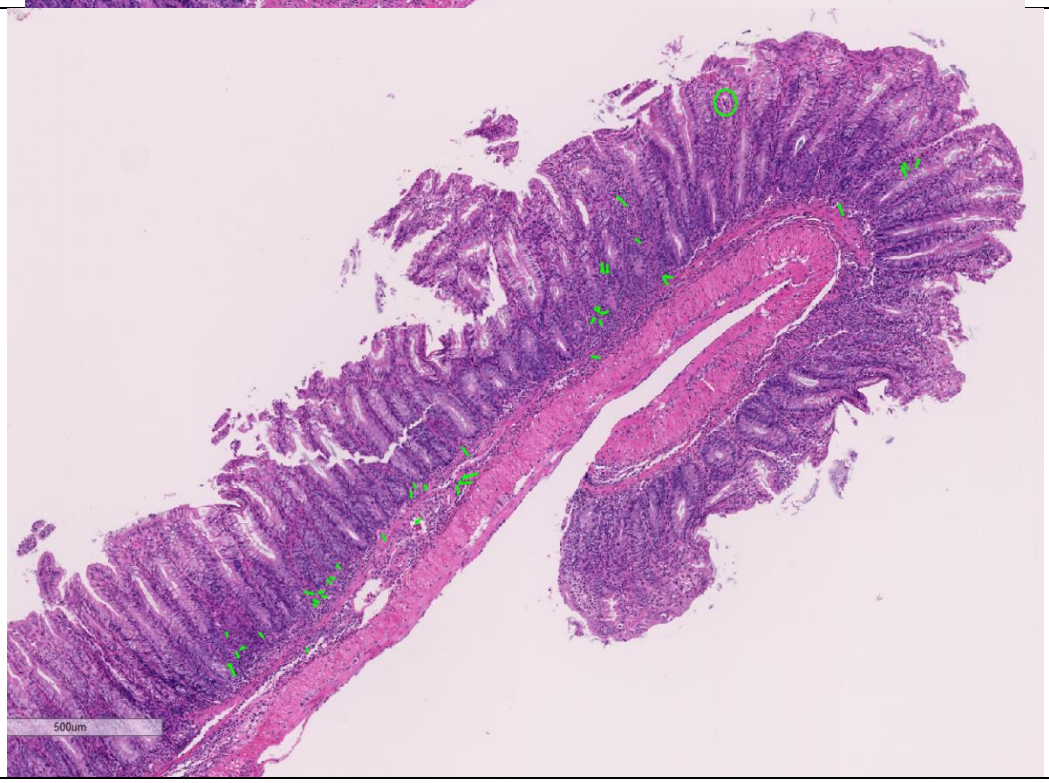
2b		
3		

4	
Notes	Score 0, Mouse ID: 15171, polymorphonuclear cells (PMN) are infrequent and rarely clustered. Abscesses are absent. Score 1, Mouse ID: 15261, Tissue is inflamed, mostly MNC, but some PMN (green arrows), without distinguishing neuts from eos, are present but in isolated sites. Segmented nuclei, refractile pink cytoplasm. Apparent glandular abscess (circle) which was very rare and may contain PMN, MNC or dead epithelial cells. Score 2a, Mouse ID: 15246a Score 2b, Mouse ID: 15246, proximal, PMN from muscularis and into lamina propria. Segmented nuclei, refractile pink cytoplasm, not to be confused with RBCs which are also refractile (fuchsia arrow). Score is based on frequency of sites affected within tissue, this image is higher than normal and accumulations present in more than one site, score 2. Crypt abscesses reflect worse than normal but are considered independently. Score 3, 15246, distal, would be more sites, multi focal (arrows) with some crypt/glandular abscesses (circle). Note, only a representative number shown. Score 4, as in score 3 but throughout tissue. Image in 3 may well be a 4. Score 4, 15246, distal, Multiple abscesses containing PMN's (circles) and PMN's throughout mucosa, submucosa, and muscularis. Throughout entire tissue.

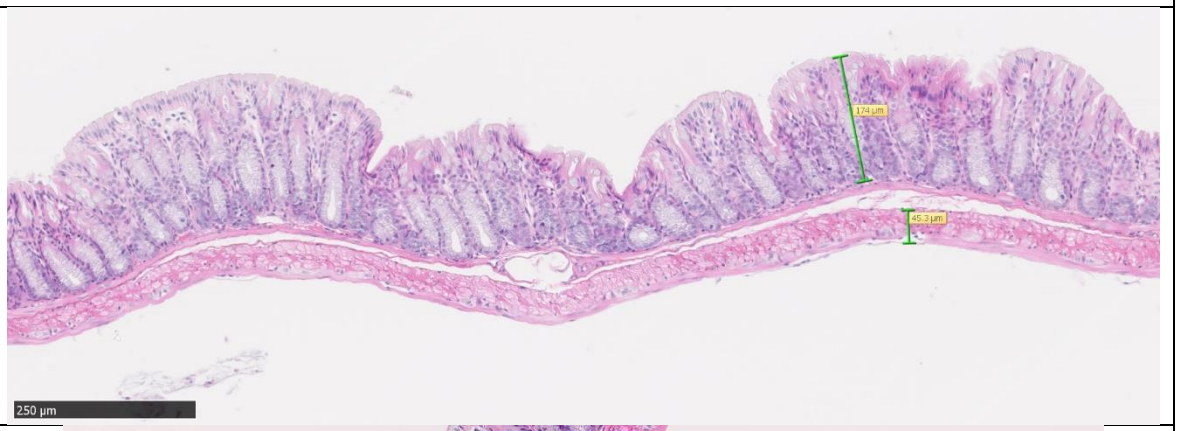
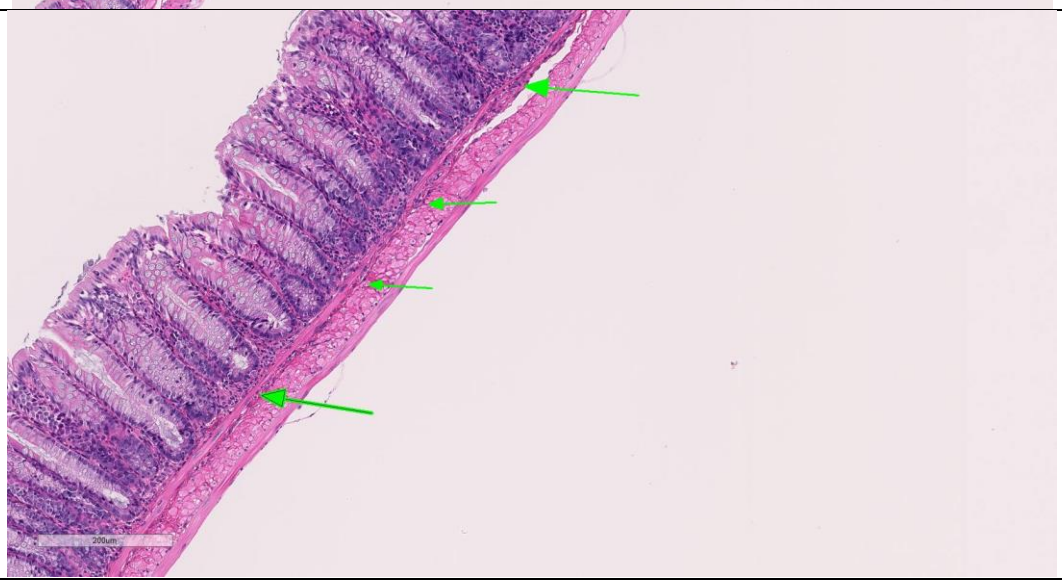
MNC	0=<10 per 5 glands; 1=focal; 2=multi; 3=diffuse; 4 severe
0	
1	
2	

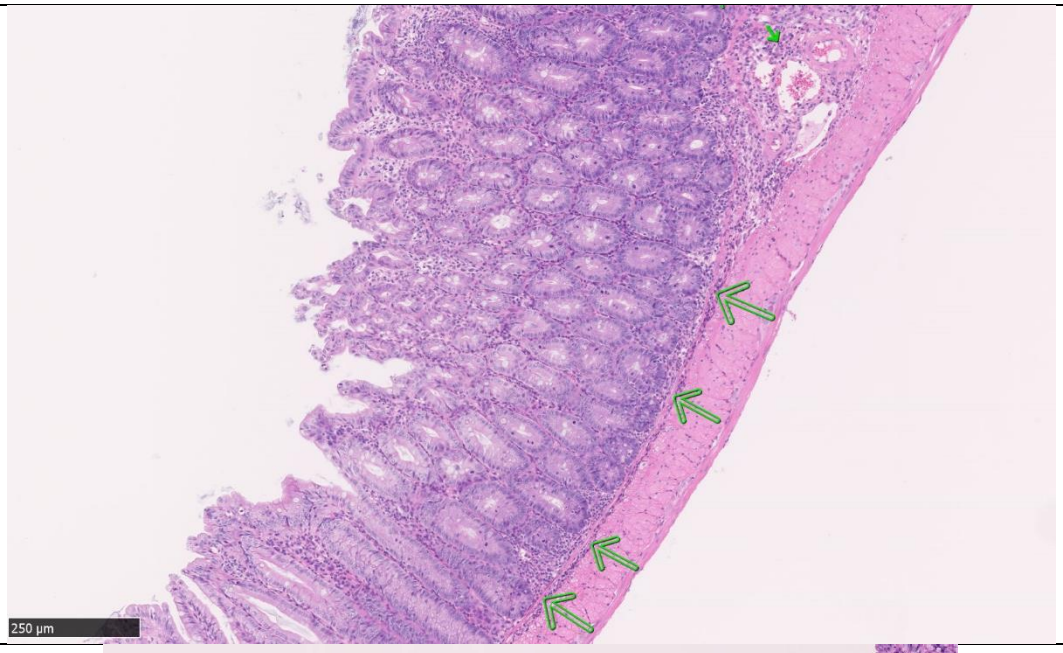
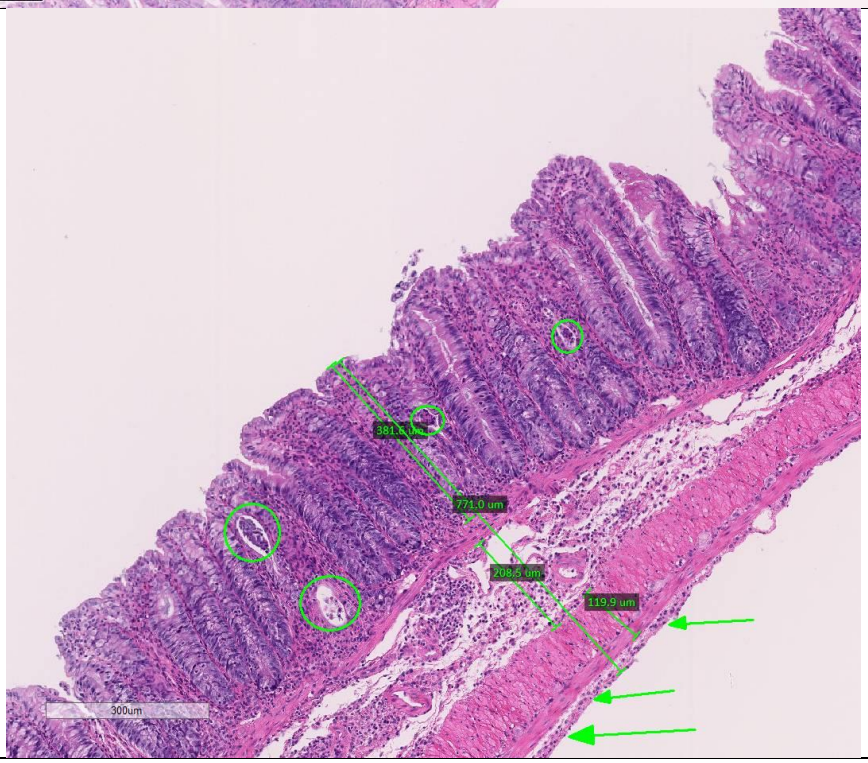
3	
4	
Notes	Score 0, Mouse ID: 15171, shows normal thickness, little inflammation but this is a T and B cell deficient mouse strain. Some separation artefact between the muscularis mucosae and tunica muscularis. Score 1, 15266, shows a focal accumulation, rest of tissue pretty clean, arguably this could be a 2 if several similar sites found but elsewhere, tissue was very healthy. Score 2, Mouse ID: 15256, showing multiple sites with some MNC infiltration, some areas in between or adjacent, relatively clear. Score 3, Mouse ID: 15271, distal. MNC throughout lamina propria and within between crypts (green arrows), but the luminal epithelium is mostly intact without infiltration (blue arrows). Score 4 showed mostly MNC throughout lamina propria (also involved submucosa shown here with dilated vein and small arteriole with WBC around the luminal edge sticking/migrating out into the tissue). Some PMNs were evident too but sometimes, MNC increased much more than PMNs, other times PMNs everywhere with the MNC.

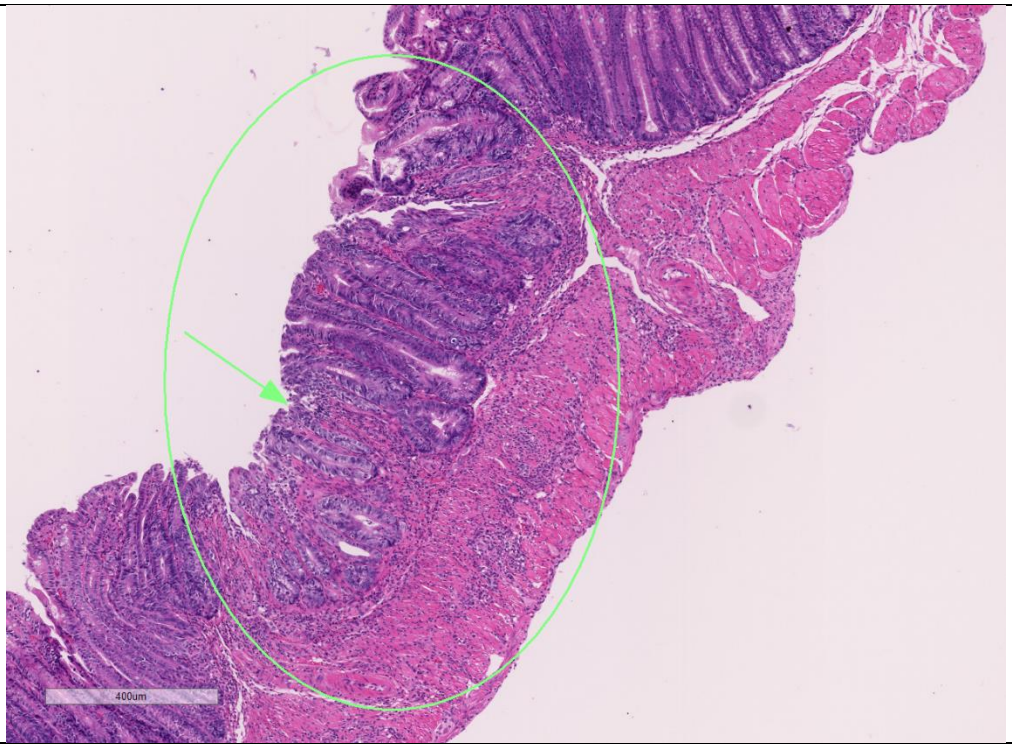
Epi	1=loss of 20% GC; 2=loss 20-70% GC; 3=>70 GC, glandular atrophy, erosions; 4= ulcers
0	
1	
2	

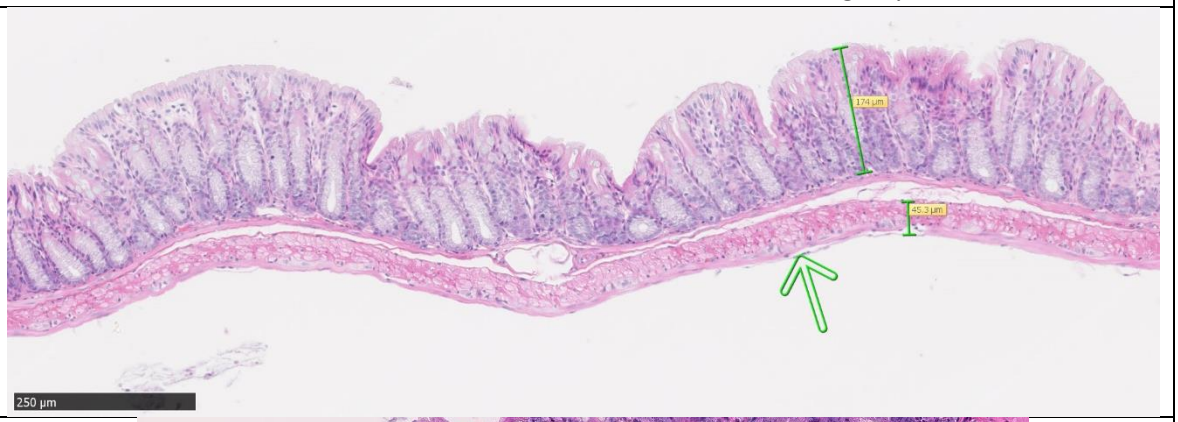
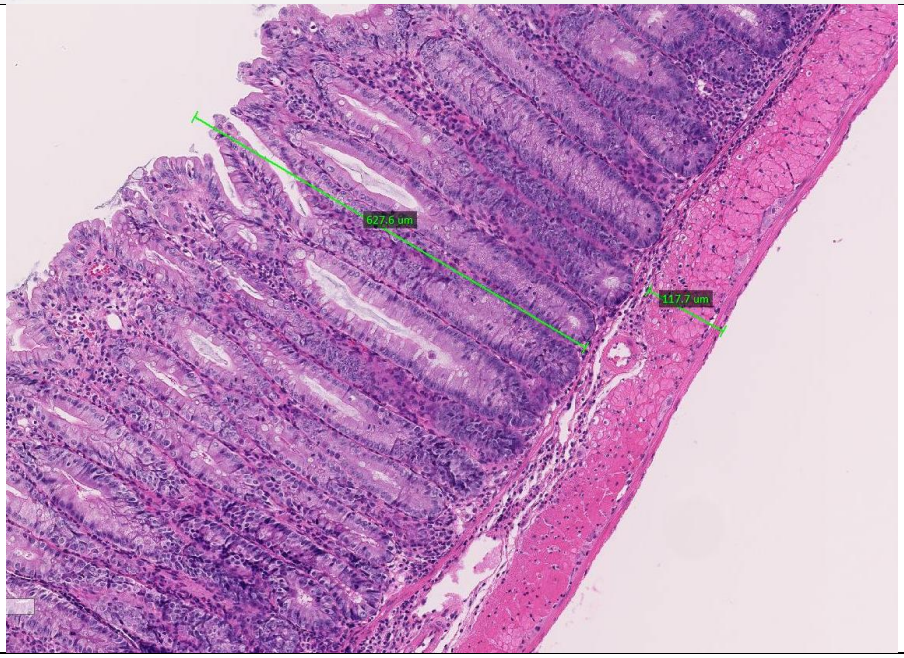
3	
4	
Notes	Score 0, Mouse ID: 15171, Score 1, Mouse ID: 15231, distal. There is loss or crypts, which may be from inflammatory degeneration or from crowding by inflammatory cells, but the loss is mild, effecting approximately 20% of the crypt epithelium (green arrows). The majority of the crypt cells are intact, showing normal goblet cells and foamy cytoplasm (blue arrows). Score 2, 15271, distal. A more marked loss of normal epithelium, between 20-70% (green arrows). There is still at least 30% normal epithelial architecture (green arrows).

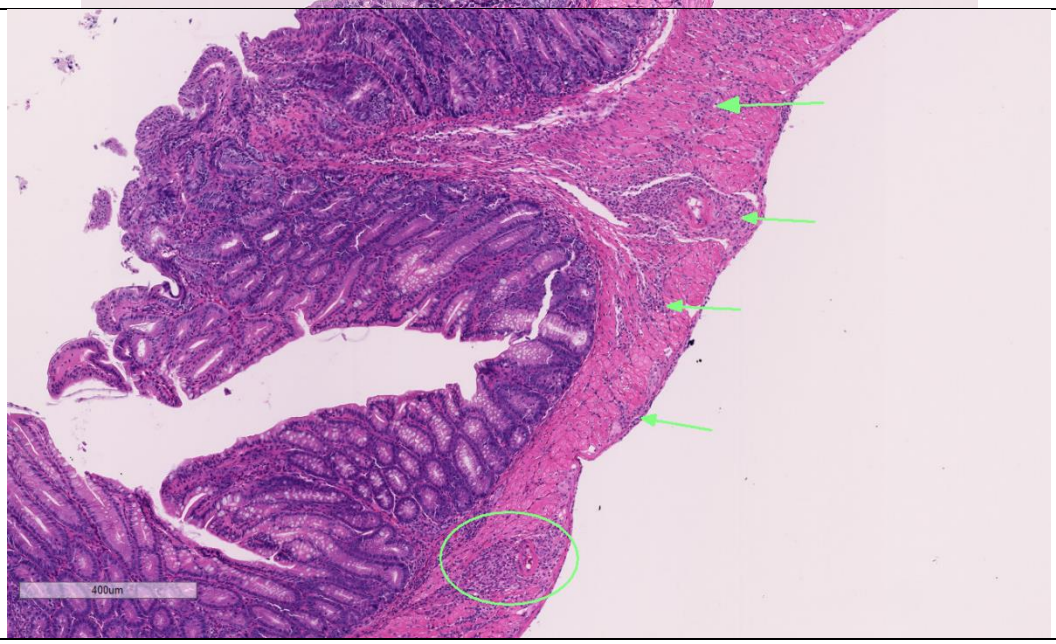
	Score 3, showing transmural infiltration with MNCs, from muscle to epithelium with marked loss of goblet cells and evidence of an erosion. Score 4, Mouse ID: 15246, marked loss of epithelium with layers missing to the level of blood vessels.

Submucosa	1=focal infiltrate; 2=multi infiltrate; 3=diffuse >4 sites
0	
1	
2	

3	
4	

4	
Notes	As the blood vessels come in between the tunica muscularis and muscularis mucosae, many of the recruited WBC start their journey here. Score 1, Mouse ID: 15221, distal. There is focal infiltration of inflammatory cells within the submucosa (green arrows). This will usually be perivascular and marginalized cells can be seen within the submucosal vessel on their way to exiting the vessel. Occasionally cells can be seen in the process of extravasation. They have not yet migrated more extensively through the submucosa (blue arrows) Score 2, Mouse ID: 15236, distal. The inflammatory cells are seen in many areas within the submucosa (green arrows) but have not yet formed a true monolayer. Score of 3 was based on number of regions and continued line of infiltrate (mostly MNC, green arrows). This can be 100 μm thick with infiltrate, this one is quite thin but continuous. Score 4 showing transmural infiltration with MNCs, from muscle to epithelium with loss of goblet cells and evidence of an erosion.

Muscularis	1=edema/thickened >95mm; 2=multi infil; 3=diffuse Relate to rigidity on dissection?
0	
1	

2	
3	
Notes	Score 0. Thin, few WBC on serosa (green arrow). Score 1. Thicker than 95 μm. Inflammation of muscularis mucosae present here but not to be confused with tunica muscularis which is quite clean. Thickening often due to edema or hypertrophy and/or hyperplasia of myocytes, WBC per se not so common. Therefore, score of 1 common in inflammation. Score 2. MNC accumulating as a monolayer on the serosa, often one after the next less and 25 mm apart. This may be a 2 in this image but if after reviewing other sections, it is pervasive, it would be a 3.

	Score 3. There are usually MNC in accumulations of 5-20; sometimes found as a monolayer on the serosa, often one after the next less and 25 μm apart. In this case, multiple accumulations (arrows), adjacent to blood vessels (e.g. circle).