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Murine Appendectomy Model of Chronic Colitis Associated Colorectal Cancer by Precise Localization of Caecal Patch

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Dear Dr. Jialan Zhang,

Thank you for inviting us to contribute an original research article entitled "Murine Appendectomy Model of Chronic Colitis Associated Colorectal Cancer by Precise Localization of Caecal Patch" for publication in the *Journal of Visualized Experiments*. This manuscript describes simple and safe surgical methods to establish a cost-effective murine appendectomy model which can be used for study of the biological functions of the appendix.

The human appendix has long been thought a useless organ, however recent advances have suggested that the appendix may play important roles in various diseases, such as colorectal cancer, inflammatory bowel disease and even Parkinson's disease. In this protocol, we demonstrated facile surgical steps of removing the appendix (known as the caecal patch) in mice to generate a murine appendectomy model with chronic colitis-associated colorectal cancer. There is a significant level reduction of IgA specific plasma cells and fecal IgA concentration upon appendectomy in mice, suggesting immunological role of the appendix. The appendectomy model is further complicated with chemical (DSS/AOM) induced ulcer colitis and colorectal tumors, making this murine model useful in studying the appendix functions in intestinal chronic inflammation and colorectal tumorigenesis

We believe that the generated murine appendectomy model of colitis associated colorectal cancer will be of great interest to readers in cancer research and medicine. We confirm that this manuscript has not been published and is not under consideration for publication elsewhere.

We thank you and reviewers in advance for the kind consideration and valuable comments.

Sincerely yours,
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TITLE:

Murine Appendectomy Model of Chronic Colitis Associated Colorectal Cancer by Precise Localization of Caecal Patch

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murine model, appendix, caecal patch, gastrointestinal anatomy, large bowel, tumor, IgA, azoxymethane, dextran sulfate sodium, IBD

SUMMARY:

The presented protocol describes a facile surgical removal of appendix (caecal patch) in a mouse followed by the induction of inflammatory bowel disease-associated colorectal cancer. This murine appendectomy model enables investigation of the biological role of the appendix in human gastrointestinal disease pathogenesis.

ABSTRACT:

The human appendix has been recently implicated to play important biological roles in the pathogenesis of various complex diseases, such as colorectal cancer, inflammatory bowel disease, and Parkinson's disease. To study the function of the appendix, a gut disease-

associated murine appendectomy model has been established and is described here. This report introduces a facile protocol for caecal patch removal in mice followed by the chemical induction of chronic colitis-associated colorectal cancer using a combination of dextran sulfate sodium (DSS) and azoxymethane (AOM). IgA-specific plasma cells were significantly reduced upon removal of the caecal patch in male C57BL/6 mice. Fecal IgA concentration appeared to be lower in appendectomy mice than those in the sham group. Simultaneously administering 2% DSS and AOM resulted in nearly 80% mice survival in both sham and appendectomy groups without significant body weight loss. Histological results confirmed colonic inflammation and different degrees of adenocarcinoma. This model can be used for functional studies of the appendix's role in maintaining gut microbiota homeostasis and pathogenesis of gut colitis and malignancies, as well as for the potential development of drug targeting therapies.

INTRODUCTION:

The clinical appendectomy is a standard surgical procedure involving removal of the appendix mostly due to inflammation (e.g., appendicitis)[1-3]. However, the biological function of the vermiform human appendix remains controversial[4-6]. The appendix has been regarded as a vestigial remnant projecting from the cecum in the large bowel. Until recently, evolutionary, immunological, morphological, and microbiological studies have suggested that the appendix may possess distinct functions. These roles include the production of immunoglobins (e.g., IgA and IgG), a variety of B cells and T cells critical for adaptive immune responses within the gut-associated lymphoid tissues (GALTs), and replenishment of the large bowel with commensal microbiota[6-12].

Clinical epidemiological studies of patients with prior appendectomy or acute appendicitis have also revealed its potential roles in the pathogenesis of human diseases, such as inflammatory bowel disease (IBD), colorectal cancer, and non-gastrointestinal disorders (e.g., Parkinson's disease and cardiovascular disease)[13-18]. For example, a large Asian population cohort study with 75,979 appendectomy patients recently showed a significant association between appendectomy and subsequent development of colorectal cancer, one of the most common malignancies with a high incidence and mortality[14,19]. Accordingly, establishing a suitable animal appendectomy model that resembles a human will be helpful to investigate the biological functions and molecular mechanisms of the appendix in the disease pathogenesis.

Many mammals possess an appendix or appendix-like organ, including primates, lagomorphs (e.g., rabbits), some rodents, and marsupials[20]. For small and commonly used laboratory animals, the rabbit possesses the vermiform appendix morphologically resembling the human[21,22], but GALT in the rabbit is extremely large compared to that in humans, since the majority of lymphoid tissues are also found in Peyer's patches located in both small and large intestines[21]. Additionally, the rabbit shows a different lymphoid follicular structure, T cell distribution, and immunoglobulin density from the human, which makes the studying of their appendices inappropriate[21].

Mice are the most commonly used animal model to study human pathophysiology and test the

various existing and novel therapuetics[23-25]. The single white large lymphoid cluster at the apex of the caecum in mice, known as the caecal patch, is thought to perform functions similar to the human appendix[26-28]. Yet, it is practically difficult to separate the caecal patch from caecum in mice. So far, the common surgical procedures for inducing appendicitis in a mouse model involve a relatively large incision (e.g., 1–2 cm) through the abdominal wall to gain access to the whole caecum (**Supplemental Table 1**)[29-36].

Herein, to generate an appendectomy model associated with gastrointestinal disease, this report presents a facile surgical protocol for caecal patch removal in mice. This is followed by the combined administration of the genotoxic agent AOM and pro-inflammatory agent DSS for the induction of colitis-associated colorectal cancer similar to that seen in humans. IBD has been shown to be a risk factor of intestinal cancer[37,38]. The combination of AOM/DSS-induced chronic colitis-associated colorectal cancer has been well-established, and readers can refer to Neufert et al., and Thaker et al. for detailed procedures[39,40]. This reproducible and rapid murine appendectomy model can be used to study appendix-modulated bowel inflammation and colon microbiota, especially in the development and progression of IBD and colorectal cancer.

PROTOCOL:

All animal procedures were approved by the Institutional Animal Care and Use Committee of Xi'an Jiaotong University (No. XJTULAC2019-1023).

1. Mice appendectomy

1.1. House 8–10-week-old C57BL/6 male mice in a certified specific-pathogen free (SPF) environment for 1 week prior to surgery.

1.2. Prepare the following sterile surgical instruments: one pair of micro-scissors, one pair of micro-forceps, two sizes (4-0 and 8-0) of sterilized non-absorbable sutures, an electric coagulation pen with needle holder, 75% medical alcohol, iodine-based scrub (e.g., entoiodine), and a package of sterile cotton swabs.

1.3. Fill a 10 mL syringe with pre-warmed 0.9% physiological saline for the abdominal flush and hydration during surgery.

1.4. Anesthetize a non-fasted mouse intraperitoneally (i.p.) with 1% sodium pentobarbital at a dose of 100 mg/kg. Check for the depth of anesthesia by the lack of response to pedal reflexes.

NOTE: A single anesthetic dose is administered to ensure a full sedative effect in the mouse. Under the circumstance of short procedures, 50 mg/kg of sodium pentobarbital i.p. may be sufficient.

1.5. Gently shave the abdominal hair with an electric shaver.

1.6. Lay the mouse on the heating pad to prevent hypothermia.

1.7. Secure the mouse on a platform in a supine position by placing four strips of medical adhesive tape across the limbs to prevent postural movement during the surgery.

1.8. Gently touch the whole abdomen to find the feeling of a bump.

NOTE: In most cases, the feeling of a bump always indicates the exact position of the caecum. To avoid possible damage to the rest of the intestine, this pre-locating of the caecum prior to abdominal incision is important.

1.9. Cover with a sterile drape and disinfect the shaved area of the abdomen by applying alternating surgical scrubs of iodine-based solution and 75% alcohol. Repeat the process 2x.

1.10. Make a longitudinal incision ranging from 0.5–1.0 cm at the midline of the abdomen.

1.11. According to the pre-determined location of the caecum, reach the caecum and gently exteriorize (~1 cm) to identify the caecal patch. Use a sterile, prewarmed saline solution of physiological pH to hydrate the intestine.

NOTE: The caecal patch of a mouse is part of the caecum and characterized by the presence of white ovoid follicles on the surface.

1.12. To prevent potential complications of the post-operative bleeding and infection, ligate the mesenteric blood vessels of the caecum using the 8-0 suture. Hydrate the caecum with sterile saline.

1.13. Mark the resection region using the 4-0 suture with an open loop near the apex of the caecum at the proximal colon.

NOTE: Marking the caecum with an open loop also prevents leakage of caecal content from the cut.

1.14. Cut off the caecal patch below the marked resection using micro-scissors and wipe out the residual caecal content with medical cotton swabs. Then, disinfect the stump of caecum with iodine-based scrub.

1.15. Close the stump with the running suture using the 8-0 suture.

1.16. Carefully remove the 4-0 thread loop previously used for marking the resection at the stump of the caecum.

1.17. Sterilize the sutured position with iodine-based scrub. Rehydrate the surgical site with saline again.

1.18. Close the musculature layer with the running suture using 8-0 suture thread.

1.19. Close the skin layers using interrupted sutures with 4-0 suture thread.

1.20. Disinfect the surgical cut 2x with iodine-based scrub, then remove iodine using 75% medical alcohol.

1.21. Gently flip the mouse back, subcutaneously (s.c.) inject 0.1 mg/kg body weight of buprenorphine and 0.4 mL of physiological saline and let the mouse rest on the heat pad until returning to consciousness.

1.22. Put the mouse back to the sterile cage and closely monitor for signs of pain for 3 days post-surgery to ensure recovery.

NOTE: Post-operative application of 0.05 mg/kg buprenorphine may be needed for pain relief of the individual mouse. Allow mice to recover for 7 days after surgery for further induction of colitis-associated colorectal cancer.

2. Induction of chronic colitis-associated colorectal cancer with AOM and DSS

NOTE: Perform this procedure 7 days post-appendectomy.

2.1. Prepare AOM stock solution by dissolving 25 mg of AOM in 2.5 mL of 0.9% sterile saline at a concentration of 10 mg/mL.

NOTE: AOM is light-sensitive.

2.1.1 Aliquot 2.5 mL of prepared AOM stock solution into 5 mL glass tubes wrapped with aluminum foil and store at -20 ˚C each time upon use.

2.1.2 Thaw one aliquot of AOM once and dilute it to a concentration of 1 mg/mL with 0.9% sterile saline (ratio 1:10).

CAUTION: AOM is extremely carcinogenic; hence, perform the entire preparation procedure in a fume hood.

2.2. Dissolve 4 g of DSS powder in 200 mL of autoclaved water to prepare 2% (w/v) DSS solution.

NOTE: The concentration of DSS may vary depending on the mouse strain, sex, and induction cycle; 3% DSS may be used for other mice strains.

2.3. Simultaneously administer the freshly prepared AOM/DSS to the mice. To do so, follow the

steps below.

2.3.1. Intraperitoneally inject 0.01 mL/g of freshly prepared AOM working solution for each mouse and replace the autoclaved water with 2% DSS solution for 5 days *ad libitum*.

NOTE: Each cage contains five mice; regularly check the DSS drinking bottle to ensure no precipitate occurs during the treatment period.

2.3.2. Weight and closely monitor each mouse every day.

NOTE: During the administration, euthanize mice with up to 20% weight loss compared to its initial weight or signs of the huddle, squint, hypothermia, and poor activity.

2.4. Provide a fresh bottle of autoclaved water on the day 6 post-AOM/DSS administration until day 21 (**Figure 1B**).

NOTE: This is one complete cycle comprising of 21 days.

2.5. Repeat steps 2.3–2.4 for an additional two cycles and sacrifice the mice on day 70.

3. Assessment of colonic inflammation and tumor (70 days post-AOM administration)

3.1. Sacrifice the mice by CO_2 inhalation at a fill rate of 10% in the euthanasia chamber followed by cervical dislocation.

3.2. Harvest the entire colon from above the ileo-colic junction to the anus.

3.2. Expose the lumen side by opening the colon longitudinally. Cut the whole colon into 10 cm long pieces and fix the colon tissue in 10% formalin for 72 h for hematoxylin and eosin (H&E) staining.

REPRESENTATIVE RESULTS:

Establishment of murine appendectomy model

This murine appendectomy model of chronic colitis associated colorectal cancer can be generated by following the sequential surgical and induction steps as illustrated in **Figure 1**. The most frequent positions of caecum are in the left and right iliac fossa followed by the middle line of the abdomen (**Figure 2**). The successful rate of pre-localization of caecum prior to the abdomen incision using the palpation method is nearly 70% (**Supplemental Information, Table S2**). The appendectomy procedures mainly comprise of six important steps (**Figure 3**). During the appendectomy surgery, a midline incision as small as 0.5–1.0 cm in length is critical to minimize surgical trauma (**Figure 3A**). In the case of intraperitoneal leakage, ligating mesangial vessels and closing the stump of the caecum are necessary, using running sutures instead of simple ligation (**Figure 3B,C,D,E**).

Evaluation of IgA as a biomarker in appendectomy mice

To confirm the appendectomy, the large intestine of mice and fecal content were harvested post-surgery to determine the levels of IgA specific plasma cells and secretory IgA (sIgA) concentrations. The appendectomy mice exhibited a significant reduction in the percentage of B220-IgA+ specific plasma cells compared to the sham group (**Figure 4A,B**). Moreover, compared to their own initial state prior to surgery, sham mice maintained the fecal sIgA concentration over 14 days post-surgery, whereas appendectomy group showed a significant sIgA reduction on day 14 (**Figure 4C**).

Validation of colitis-associated colorectal cancer in appendectomy mice

Compared to 3% DSS, at which most mice in the appendectomy group showed severe complications and significant body weight loss (**Figure 5A**), 2% DSS in combination with AOM provided 80% of the survival rate in both sham and appendectomy groups, with reasonable body weight changes (**Figure 5E**). At the end of three cycles of treatment, the full-length large bowel was harvested for pathological assessment. Visual inspection and H&E staining showed the colonic tumors with colon inflammation and different degrees of adenocarcinomas (well-, moderate-, and poorly differentiated adenocarcinomas) in mice treated with 2% AOM/DSS (**Figure 6**).

FIGURE AND TABLE LEGENDS:

Figure 1: Schematic illustration of establishing murine appendectomy model of chronic colitis-associated colorectal cancer. (**A**) A flow chart of the main surgical steps (first three are critical) of appendectomy procedure. (**B**) Induction of colorectal cancer. Seven days after the appendectomy, AOM and DSS were administered simultaneously. Mice weights were recorded at various times (red triangle). At day 70 post-surgery, mice were sacrificed, and intestinal tissues were harvested for further assessments.

Figure 2: Pre-determination of the caecal patch location in mice prior to abdominal incision. (**A**) Summary of the likelihood of finding caecum position. Anatomical images of three most common positions of the caecum at the (**B**) low right, (**C**) middle, and (**D**) low left abdomen of the mouse.

Figure 3: Critical steps of murine appendectomy. (**A**) Small incision arranged 0.5–1.0 cm; (**B**) Pulling out the exact portion of the caecal patch after pre-position of the caecum. (**C**) Ligation of mesenteric vessels accompanying the caecum. (**D**) Marking the cutting region of the caecum with the 4-0 suture. (**E**) Closing the stump with running sutures using the 8-0 suture. (**F**) Closing the muscular layer with interrupted sutures using the 8-0 suture.

Figure 4: Quantification of IgA specific plasmas cells and fecal sIgA concentration after appendectomy. (**A**) Representative dot plots for cells stained with anti-IgA and B220 in the whole large intestine of sham and appendectomy mice 1 day and 14 days post-surgery. Lymphocytes in lamina propria were freshly prepared and stained with FITC anti-mouse IgA, PE

anti-mouse CD45R/B220 for flow cytometry. Numbers within plots indicate percentages of cells in respective areas; (**B**) Quantitative determination of IgA specific plasma cells in sham and appendectomy groups. The data represents mean ± SD; n = 5–8 mice; *p < 0.05, **p < 0.01, and ***p < 0.001. (**C**) Fecal sIgA concentrations before and after the surgery. Fecal contents were collected at designated timepoints, and levels of sIgA were tested by ELISA. Each color dot represents the same mouse over a period of 14 days. Statistical analysis was performed using an unpaired *t*-test, with p < 0.05 considered statistically significant.

Figure 5: Chronic colitis-associated colorectal tumors induced by AOM/DSS combination. (**A**) The survival curve obtained using 3% DSS for the colitis induction in sham and appendectomy groups. Severe complications of anal prolapse (**B**) and mural abscess indicated the endpoint of the animal (**C**). (**D**) The survival curve obtained using 2% DSS for the colitis induction in sham and appendectomy groups; (**E**) Average body weight change of mice over 70 days. The data represents mean ± SD, n = 6 mice.

Figure 6: Images of chronic colitis-induced colorectal tumors induced by combination treatment of 2% DSS and 10 mg/kg AOM. Photographs of the dissected colon with tumors under (**A**) visual inspection and (**B**) animal operation microscope. H&E staining images of colonic tissues showing (**C**) colonic inflammation, (**D**) well-differentiated adenocarcinoma, (**E**) moderately differentiated adenocarcinoma, and (**F**) highly differentiated adenocarcinoma. Black arrow indicates the mucosal ulcer, black arrowhead indicates adenocarcinoma, white arrow indicates neutrophil infiltration between glands.

Supplementary Table 1: Literature summary of surgical methods used for appendicitis induction in commonly used mice strains.

Supplementary Table 2: Locations of the caecum in mice after laparotomy. Exposed organ after incision indicates the organ found directly at the pre-located position after laparotomy. The position of the caecum shows the caecum location relative to the abdominal region/the caecum position relative to the intestine. Exposed indicates that the caecum was below the muscular layer of the abdomen and above other parts of the intestine. Embedded indicates that the caecum was covered by the intestine.

DISCUSSION:

A murine appendectomy model of colitis-associated colorectal cancer was obtained using surgical steps with a high survival rate in mice. In most cases, since the caecum was positioned under the abdominal wall (**Supplementary Table 1, Supplementary Table 2**, and **Figure 2**), it was difficult to prejudge its location without laparotomy. In this surgical protocol, an easy step of touching the bump was introduced, and quantitative evaluation of the cecum location was also provided as guidance to increase the precision for pre-localization of the cecum (**Figure 2**). The exploitation of anatomical features to remove the caecal patch minimized disruption of unintended parts of the bowel, thereby decreasing the rate of potential complications of appendectomy and infection of the abdominal cavity.

Three critical steps are involved in this model. First, visual examination and palpation prior to laparotomy can be helpful to determine the general location of the caecum. Despite the fact that the caecum is most likely present at the left iliac region of the abdomen, the caecal patch protruding from the apex of the caecum is often dissociative (i.e., move around or embedded) and is more likely to be found beneath the middle peritoneum (**Figure 2**). Second, a small incision at the middle line of the abdomen and accurate exposure of caecal patch is preferred. This is because there is a thin spot at the middle line of the abdomen, at which cutting into this position may decrease potential injury of the abdominal musculature layer. In addition, to avoid the volvulus upon appendectomy, which is detrimental to mice, accurate exposure of the caecal patch minimizes the unintended disturbance of intestine, reducing the risk of volvulus occurrence. Moreover, minimal exposure of the abdominal cavity can ensure that the intestine remains uncontaminated, warm, and moist; otherwise, excessive exposure may cause significant heat loss and drying of tissues[41].

Third, disinfection and closure of the caecal stump with running sutures instead of simple ligation are necessary to prevent potential bleeding and disinfection of caecal contents of the stump. The level of IgA-specific plasma cells and fecal sIgA in the large bowel of mice are significantly reduced by an appendectomy, suggesting that the caecal patch is the main site for the production of IgA at least in the initial period[33]. In the large bowel, sIgA function as a mucosal health defender by stabilizing the normal colonization of commensal flora and counteracting pathogenic microbes[42]. Removal of the appendix reduces the production of IgA and may disrupt the mucosal homeostasis.

The high mortality rate and clinical signs of excessive colitis induction (i.e., severe bloody stool, anal prolapse, and abdominal abscess[43,44]) were observed in mice treated with 3% DSS (**Figure 5**). However, 2% DSS induction after three cycles caused none of these complications, and the survival rate was maintained above 80%. This indicates that the combined use of DSS and AOM should be validated, since the lack of integrity of GALTs may, to some extent, reduce the tolerance of mice to the AOM and DSS combination. The observational studies from clinical-based evidence suggest that the appendix is strongly associated with the development of IBD and colorectal cancer[14,45,46]. However, the biological functions and underlying mechanisms of the appendix in the pathogenesis of chronic gut diseases is still unclear. It appears that the appendix acts as an important part of GALTs and as a reservoir for gut commensal flora to regulate health or diseased states[17,18,31]. Establishing this repeatable and cost-effective murine appendectomy model can be used for studying the roles of the appendix in IBD and colorectal cancer in the context of microbiota homeostasis, cancer prevention, and immunotherapy.

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of the murine appendectomy model, as well as the pathologist Dr. Xi Liu for evaluation of H&E staining results of colitis and colorectal tumors.

DISCLOSURES:

The authors have nothing to disclose.

REFERENCES:

1 Leung, T. T. et al. Bowel obstruction following appendectomy: what is the true incidence? *Annals of Surgery.* **250** (1), 51-53 (2009).

2 Salminen, P. et al. Antibiotic Therapy vs. Appendectomy for Treatment of Uncomplicated Acute Appendicitis: The APPAC Randomized Clinical Trial. *The Journal of the American Medical Association.* **313** (23), 2340-2348 (2015).

3 Mayo Clinic: Appendicitis. at <https://www.mayoclinic.org/diseases-conditions/appendicitis/diagnosis-treatment/drc-20369549>.

4 On the Appendix Vermiformis and the Evolution Hypothesis. *Nature.* **8,** 509 (1873).

5 Zahid, A. The vermiform appendix: not a useless organ. *Journal of College of Physicians and Surgeons Pakistan.* **14** (4), 256-258 (2004).

6 Kooij, I. A., Sahami, S., Meijer, S. L., Buskens, C. J., Te Velde, A. A. The immunology of the vermiform appendix: a review of the literature. *Clinical and Experimental Immunology.* **186** (1), 1-9 (2016).

7 Sarkar, A., Saha, A., Roy, S., Pathak, S., Mandal, S. A glimpse towards the vestigiality and fate of human vermiform appendix-a histomorphometric study. *Journal of Clinical and Diagnostic Research.* **9** (2), AC11-15 (2015).

8 Fujihashi, K. et al. Human Appendix B-Cells Naturally Express Receptors for and Respond to Interleukin-6 with Selective Iga1 and Iga2 Synthesis. *Journal of Clinical Investigations.* **88** (1), 248-252 (1991).

9 Im, G. Y. et al. The appendix may protect against Clostridium difficile recurrence. *Clinical Gastroenterology and Hepatology.* **9** (12), 1072-1077 (2011).

10 Gebbers, J. O., Laissue, J. A. Bacterial translocation in the normal human appendix parallels the development of the local immune system. *Annal of the New York Academy of Sciences.* **1029,** 337-343 (2004).

11 Randal Bollinger, R., Barbas, A. S., Bush, E. L., Lin, S. S., Parker, W. Biofilms in the large bowel suggest an apparent function of the human vermiform appendix. *Journal of Theoretical Biology.* **249** (4), 826-831 (2007).

12 Smith, H., Parker, W., H Kotzé, S., Laurin, M. Morphological evolution of the mammalian cecum and cecal appendix: Évolution morphologique de l'appendice du caecum des mammifères. *Comptes Rendus Palevol.* Vol. 16 (2017).

13 Girard-Madoux, M. J. H. et al. The immunological functions of the Appendix: An example of redundancy? *Seminars in Immunology.* **36,** 31-44 (2018).

14 Wu, S. C. et al. Association between appendectomy and subsequent colorectal cancer development: an Asian population study. *PLoS ONE.* **10** (2), e0118411 (2015).

15 Florin, T. H., Pandeya, N., Radford-Smith, G. L. Epidemiology of appendicectomy in primary sclerosing cholangitis and ulcerative colitis: its influence on the clinical behaviour of these diseases. *Gut.* **53** (7), 973-979 (2004).

16 Arnbjornsson, E. Acute appendicitis as a sign of a colorectal carcinoma. *Journal of Surgical Oncology.* **20** (1), 17-20 (1982).
17 Killinger, B. A. et al. The vermiform appendix impacts the risk of developing Parkinson's disease. *Science Translatioanl Medicine.* **10** (465), (2018).
18 Chen, C. H. et al. Appendectomy increased the risk of ischemic heart disease. *Journal of Surgical Research.* **199** (2), 435-440 (2015).
19 Bray, F. et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians.* **68** (6), 394-424 (2018).
20 Smith, H. F., Parker, W., Kotze, S. H., Laurin, M. Multiple independent appearances of the cecal appendix in mammalian evolution and an investigation of related ecological and anatomical factors. *Comptes Rendus Palevol.* **12** (6), 339-354 (2013).
21 Dasso, J. F., Obiakor, H., Bach, H., Anderson, A. O., Mage, R. G. A morphological and immunohistological study of the human and rabbit appendix for comparison with the avian bursa. *Developmental and Comparative Immunology.* **24** (8), 797-814 (2000).
22 Smith, H. F. et al. Comparative anatomy and phylogenetic distribution of the mammalian cecal appendix. *Journal of Evolutionary Biology.* **22** (10), 1984-1999 (2009).
23 Vandamme, T. F. Rodent models for human diseases. *European Journal of Pharmacology.* **759,** 84-89 (2015).
24 Prabhakar, S. Translational research challenges: finding the right animal models. *Journal of Investigative Medicine.* **60** (8), 1141-1146 (2012).
25 Hosur, V., Low, B. E., Avery, C., Shultz, L. D., Wiles, M. V. Development of Humanized Mice in the Age of Genome Editing. *Journal of Cellular Biochemistry.* **118** (10), 3043-3048 (2017).
26 Mizoguchi, A., Mizoguchi, E., Chiba, C., Bhan, A. K. Role of appendix in the development of inflammatory bowel disease in TCR-alpha mutant mice. *Journal of Experimental Medicine.* **184** (2), 707-715 (1996).
27 Farkas, S. A. et al. Preferential migration of CD62L cells into the appendix in mice with experimental chronic colitis. *European Surgical Research.* **37** (2), 115-122 (2005).
28 Morrison, P. J. et al. Differential Requirements for IL-17A and IL-22 in Cecal versus Colonic Inflammation Induced by Helicobacter hepaticus. *American Journal of Pathology.* **185** (12), 3290-3303 (2015).
29 Tomiyasu, N. et al. Appendectomy suppresses intestinal inflammation in a murine model of DSS-induced colitis through modulation of mucosal immune systems. *Gastroenterology.* **118** (4), A863-A863 (2000).
30 Krieglstein, C. F. et al. Role of appendix and spleen in experimental colitis. *Journal of Surgical Research.* **101** (2), 166-175 (2001).
31 Cheluvappa, R., Luo, A. S., Palmer, C., Grimm, M. C. Protective pathways against colitis mediated by appendicitis and appendectomy. *Clinical and Experimental Immunology.* **165** (3), 393-400 (2011).
32 Cheluvappa, R., Luo, A. S., Grimm, M. C. T helper type 17 pathway suppression by appendicitis and appendectomy protects against colitis. *Clinical and Experimental Immunology.* **175** (2), 316-322 (2014).
33 Masahata, K. et al. Generation of colonic IgA-secreting cells in the caecal patch. *Nature*

Communications. **5**, (2014).
34 Cheluvappa, R. A novel model of appendicitis and appendectomy to investigate inflammatory bowel disease pathogenesis and remediation. *Biological Procedures Online.* **16**, (2014).
35 Cheluvappa, R., Eri, R., Luo, A. S., Grimm, M. C. Modulation of interferon activity-associated soluble molecules by appendicitis and appendectomy limits colitis-identification of novel anti-colitic targets. *Journal of Interferon and Cytokine Research.* **35** (2), 108-115 (2015).
36 Harnoy, Y. et al. Effect of appendicectomy on colonic inflammation and neoplasia in experimental ulcerative colitis. *British Journal of Surgery.* **103** (11), 1530-1538 (2016).
37 Aaron E. Walfish, R. A. C. C. *Ulcerative Colitis*. at <https://www.merckmanuals.com/professional/gastrointestinal-disorders/inflammatory-bowel-disease-ibd/ulcerative-colitis> (2017).
38 Laukoetter, M. G. et al. Intestinal cancer risk in Crohn's disease: a meta-analysis. *Journal of Gastrointestestinal Surgery.* **15** (4), 576-583 (2011).
39 Neufert, C., Becker, C., Neurath, M. F. An inducible mouse model of colon carcinogenesis for the analysis of sporadic and inflammation-driven tumor progression. *Nature Protocols.* **2** (8), 1998-2004 (2007).
40 Thaker, A. I., Shaker, A., Rao, M. S., Ciorba, M. A. Modeling colitis-associated cancer with azoxymethane (AOM) and dextran sulfate sodium (DSS). *Journal of Visualized Experiments.* 10.3791/4100 (67), (2012).
41 Perides, G., van Acker, G. J. D., Laukkarinen, J. M., Steer, M. L. Experimental acute biliary pancreatitis induced by retrograde infusion of bile acids into the mouse pancreatic duct. *Nature Protocols.* **5** (2), 335-341 (2010).
42 Schofield, W. B., Palm, N. W. Gut Microbiota: IgA Protects the Pioneers. *Current Biology.* **28** (18), R1117-R1119 (2018).
43 Karthikeyan, V. S. et al. Carcinoma Cecum Presenting as Right Gluteal Abscess Through Inferior Lumbar Triangle Pathway-Report of a Rare Case. *International Surgery.* **99** (4), 371-373 (2014).
44 Ruscelli, P. et al. Clinical signs of retroperitoneal abscess from colonic perforation Two case reports and literature review. *Medicine (Baltimore).* **97** (45), (2018).
45 Stellingwerf, M. E. et al. The risk of colectomy and colorectal cancer after appendectomy in patients with ulcerative colitis: a systematic review and meta-analysis. *Journal of Crohn's and Colitis.* 10.1093/ecco-jcc/jjy163, (2018).
46 Friedman, G. D., Fireman, B. H. Appendectomy, appendicitis, and large bowel cancer. *Cancer Research.* **50** (23), 7549-7551, (1990).

Figure 1

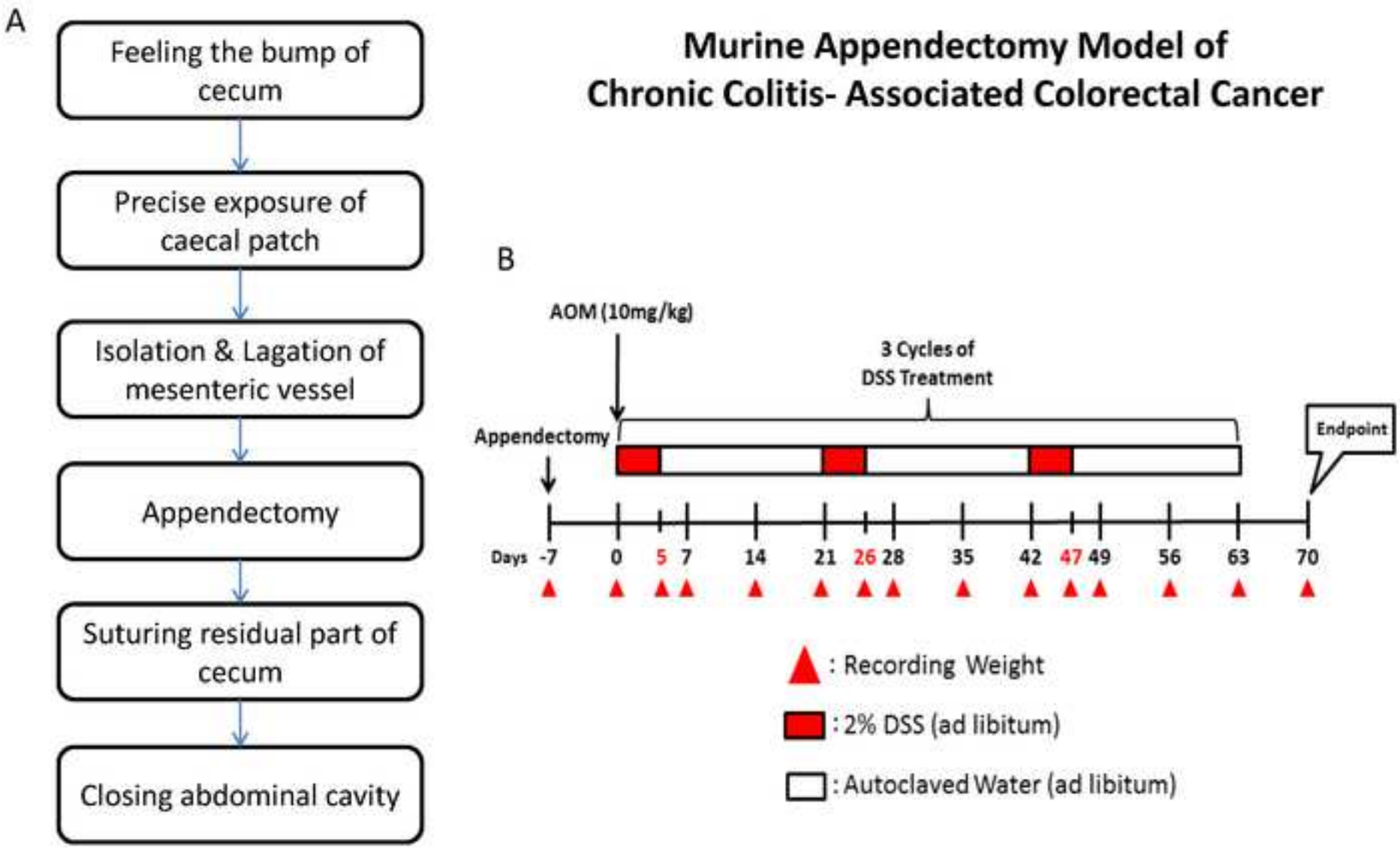


Figure 2

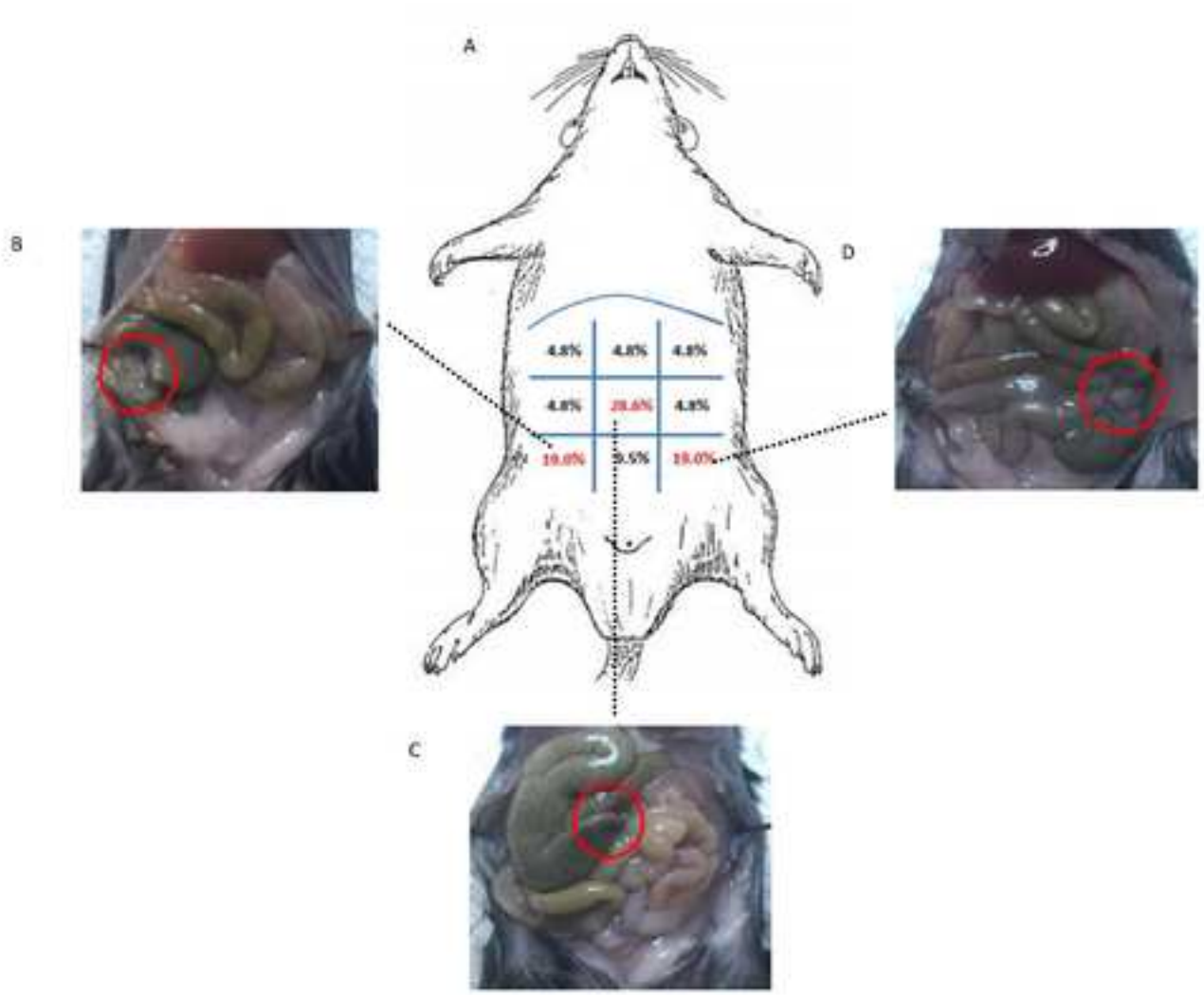


Figure 3

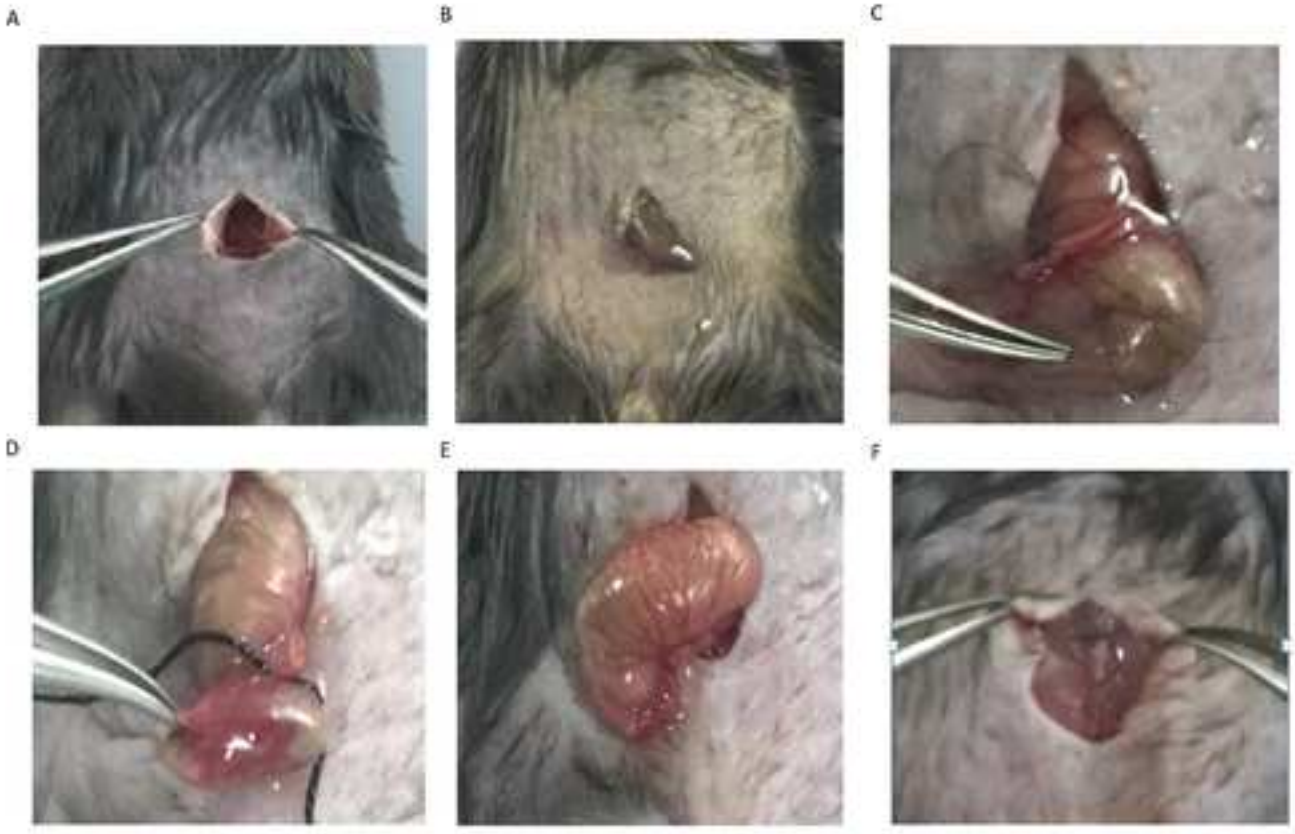
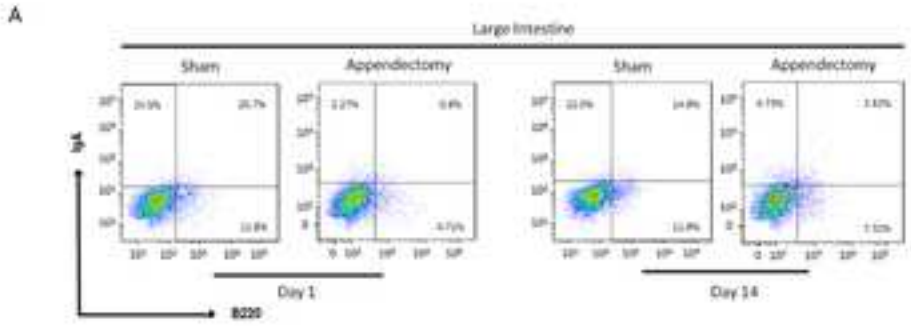
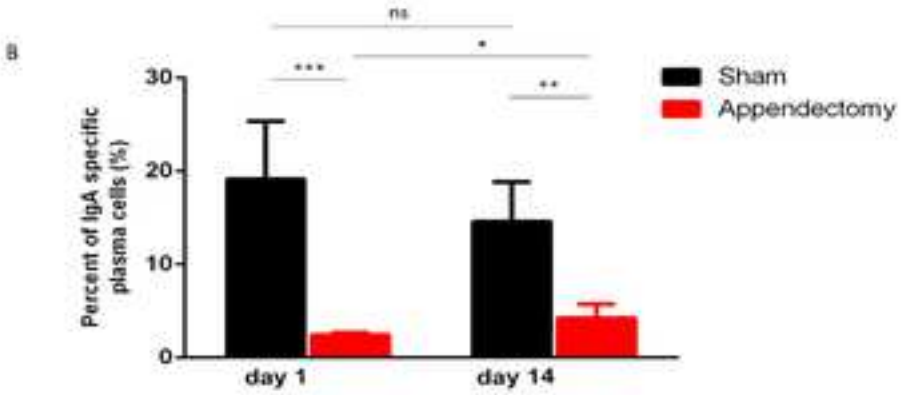


Figure 4





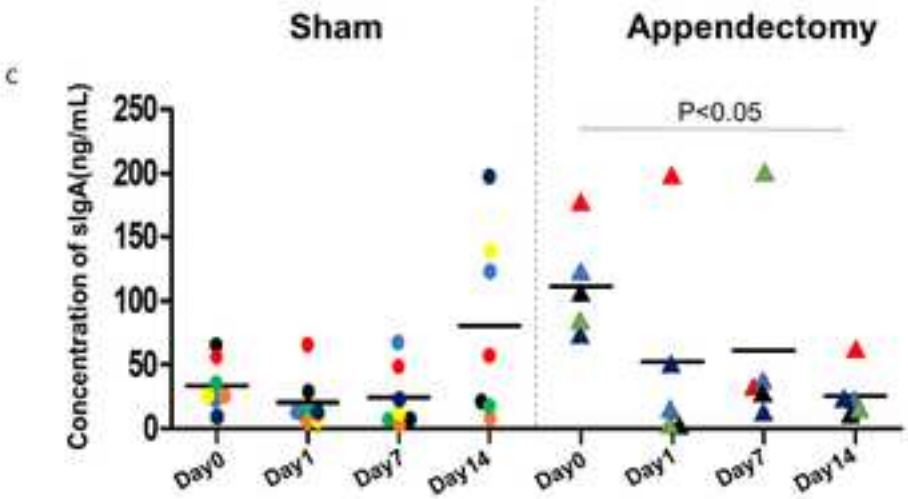


Figure 5

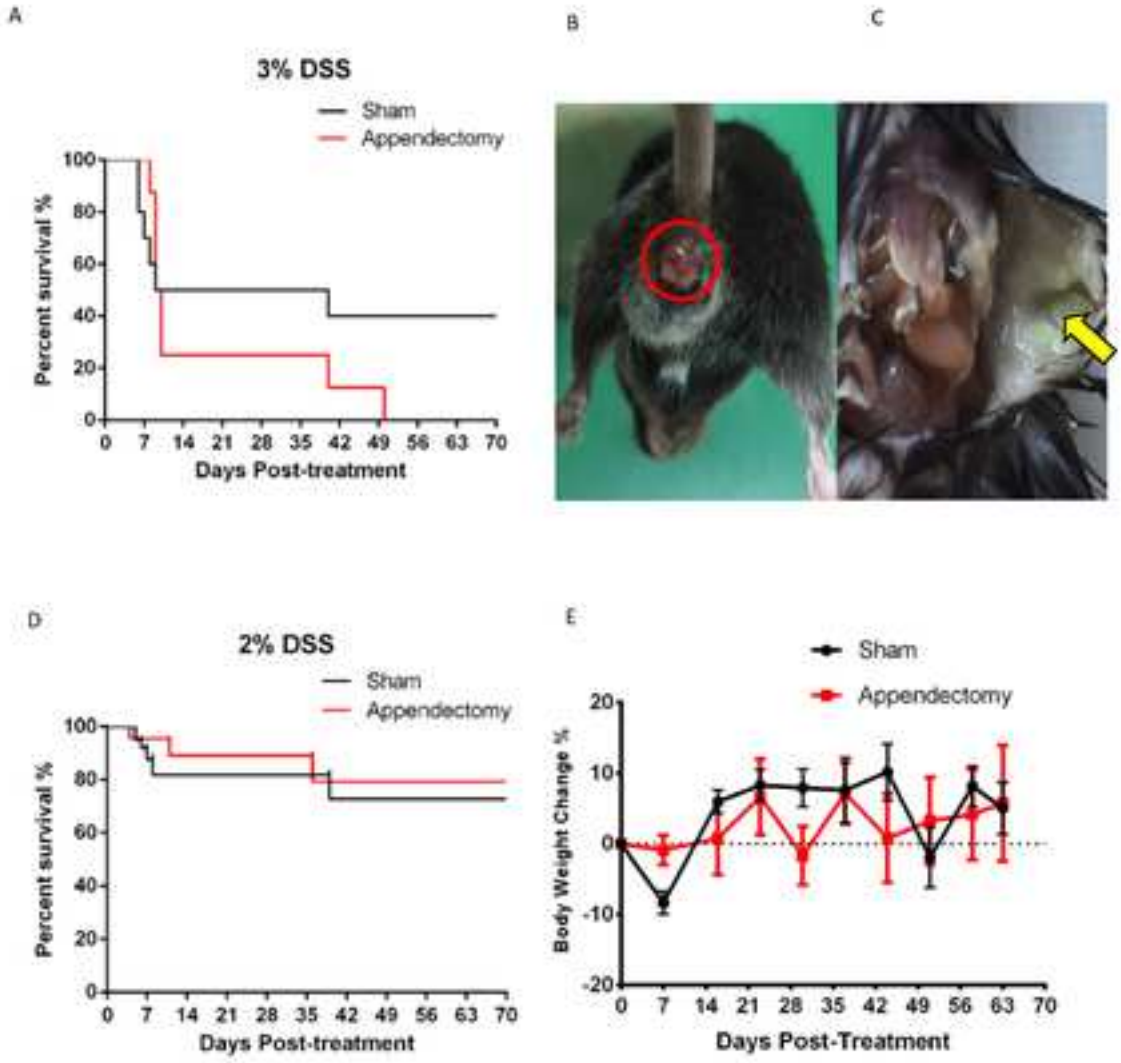


Figure 6

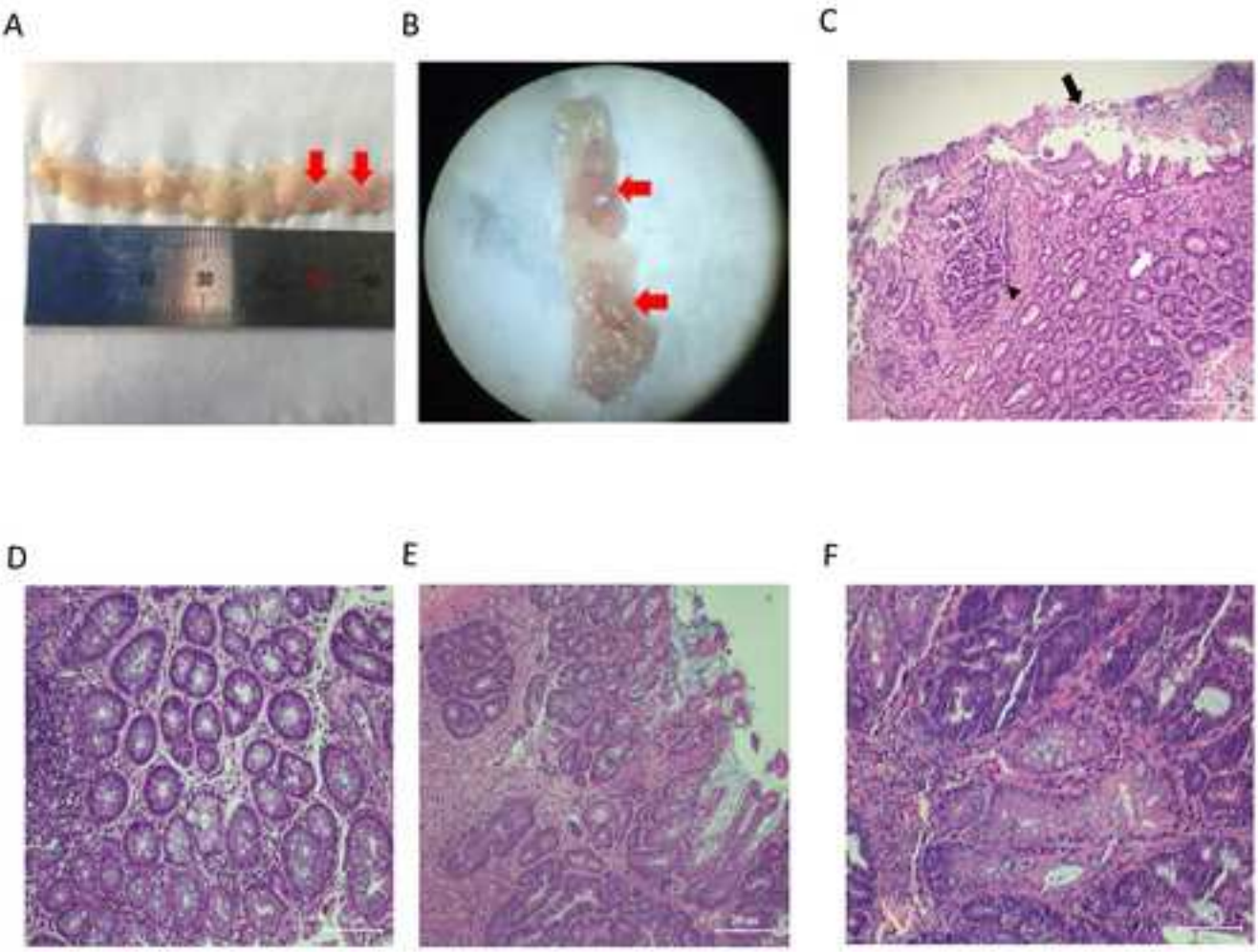


Table S1

Years	Mouse Strain (sex)	Laparotomy Length (cm)	Laparotomy Location
2000	Balb/c/Female	Unknown	Unknown
2001	C57BL/6J/Male	1	midline of abdomen
2011	Balb/c/Male	1	Left-sided abdomen
2014	Balb/c/Male	Unknown	Unknown
2014	ICR aly/aly C57BL/6J/ Male & Female	2	midline of abdomen
2014	Balb/c/Male	1	Left-sided abdomen
2015	Balb/c/Male	Unknown	Unknown
2016	C57BL/6J/Male	1	Left iliac fossa

Surgical Method	Studied Disease Model
Unknown	Induced Colitis
ligation and excision	Induced Colitis
ligation and excision	Induced Appendicitis
ligation and excision	Induced Appendicitis
ligation and excision	NA
ligation and excision	Induced Appendicitis
ligation and excision	Induced Appendicitis
ligation and excision	Induced Appendicitis &Spontaneous colitis

References
Nobuo Tomiyasu et al[29]
Christian F. Krieglstein et al[30]
Rajkumar Cheluvappa et al[31]
Rajkumar Cheluvappa et al[32]
Kazunori Masahata et al[33]
Rajkumar Cheluvappa et al[34]
Rajkumar Cheluvappa et al[35]
Y. Harnoy et al[36]

Table S2

Mice	Exposed organ after incision	Position of caecum
1	caecum	Upper middle / Exposed
2	caecum	Low right / Embeded
3	descending colon	Right / Exposed
4	left kidney	Middle / Embeded
5	cecum	Low right / Exposed
6	cecum	Left / Exposed
7	unknown	Low right / Exposed
8	cecum	Caudal/ Exposed
9	cecum	Upper left/ Exposed
10	cecum	Low left / Exposed
11	cecum	Middle / Embeded
12	unknown	Low left / Embeded
13	cecum	Low left / Embeded
14	cecum	Upper Right/ Embeded
15	cecum	Low left / Embeded
16	cecum	Middle / Exposed
17	cecum	Middle / Exposed
18	Appendix	Middle / Exposed
19	unknown	Middle / Exposed
20	Appendix	Low right / Exposed
21	cecum	Caudal / Exposed

Name of Material/ Equipment	Company	Catalog Number
Azoxymethane（AOM）	Sigma-Aldrich,Inc.	A5486
Dextran Sulfate Sodium Salt（DSS）	MP Biomedicals,Inc.	160110
Entoiodine	Shanghai likon high technology disinfection co. LTD	310102
digital caliper	Ningbo yuanneng trading co. LTD	4859263
4-0 Silk Sutures	Yuanlikang co. LTD	20172650032
8-0 Prolene Sutures	Yuanlikang co. LTD	20172650032
Electric coagulation pen	Chuang mei medical equipment co. LTD	28221777292
disposable syringe 1ml	Shengguang medical products co. LTD	3262-2014
disposable syringe 10ml	Shengguang medical products co. LTD	3262-2014
75% Medicinal alcohol	Shandong anjie high-tech disinfection technology co. LTD	371402AAJ008
Pentobarbital sodium salt	Sigma-Aldrich,Inc.	57-33-0
Physiological Saline	Shandong qidu pharmaceutical co. LTD	H37020766
Absorbent Cotton Swab	Henan ruike medical co., LTD	RK051
Surgical Instruments-Ophthalmic	Jinzhong Shanghai co.LTD	WA3050

Comments/Description

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Author(s): Yaguang Li, Junhong Liu, Gaixia Liu, Zhenhong Pan, Mingxia Zhang, Yao Ma, Qingxia Wei, Hongping Xia, Junjun She, Ruixue Zhang

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Dear Dr. Bajaj:

Thank you and editors for fine tuning our work. We have addressed all the comments raised by the editors in the revised manuscript and video accordingly. We hope this version will meet the editorial requirement for publication in *JoVE*.

Sincerely Yours,

Rui Xue Zhang, Ph.D.

Associate Professor

Editorial comments:

1. The editor has formatted the manuscript to match the journal's style and the video. Please retain the same.

Response: thank you for formatting the manuscript, and we retain the same format in this version.

2. Please address specific minor comments marked in the manuscript.

Response: all revised parts were marked with yellow this time:

1. A new title revised in manuscript, cover letter and supplemental document: Murine Appendectomy Model of Chronic Colitis Associated Colorectal Cancer by Precise Localization of Caecal Patch.
2. Accepted revise from editor in 1.9 line 143.
3. Revised protocol in 3.1 according to editor's suggestion from line 241 to line 242.
4. Revised word in **REPRESENTATIVE RESULTS: Establishment of Murine Appendectomy Model** part in line 255.
5. Legends of table S1 and table S2 from line 330 to line 340.
6. Revised formats in References parts.
7. xlsx. files of supplemental tables.
8. Revised response to reviewer 2 relating to a change of title of manuscript.

Video:

8:24 onwards- the last interview shot is very shaky. Please reshoot this part with a stable camera.

Response: We've now reshoot this part and changed the title in both manuscript and video according to the advise of reviewer 2.

Reviewer 1

Manuscript Summary:

The present manuscript entitled "Murine Appendectomy Model of Chronic Colitis Associated Colorectal Cancer" described the procedure to accelerate the formation of colorectal cancer in the very classical and widely used AOM/DSS mouse model to study the chronic colitis-associated colorectal cancer. This paper is very informative, in particular, the video which well detailed the surgical procedure to remove the caecal patch which has a similar function that human appendix. The results are consistent and expected.

Major Concerns:

However, some points described below, should be included in order to improve this manuscript:

1-Why mice are not fasted before surgery to limit possible infection?

Response: Thank you for your comment. We agree that fasting of mice is a standard procedure in many studies (e.g. pharmacokinetics, blood glucose measurement, drug absorption). In our present study, however, fasting of mice is not need for the surgery. First, food and water intake in mice is regulated by circadian rhythms. Compared to non-fasted ones, fasting mice for overnight (6-18h) show no significant difference in terms of gastric emptying, intestinal transit time and gastric contents (Jensen TL 2013). This is in consistent with our initial observation that cecal residue between fasted and non-fasted mice was similar. In addition, fasting mice (i.e. male C57BL6J) may lose their body weights up to 16%, which could be a critical condition for mice to recovery post-surgery as well as the later AOM/DSS treatment. To avoid the infection, following the key surgical steps are important. We have further revised the section of "Surgery preparation" to clarify this point in the manuscript and added the illustration of key surgical steps as a new figure 1A.

Jensen TL et al., 2013, Fasting of mice: a review, Laboratory animals, 47(4)225.

2-A figure representing the mucosa aspect after the treatment could allow to display the presence (or not) of cancer nodules.

Response: We have added two photographs of colon tumor induced by the combination of DSS and AOM as figure 6A & 6B and revised the result section on Page 7.

Reviewer 2

Manuscript Summary:

I have read the manuscript that investigated an animal model the role of the vermiform appendix in chronic colitis associated large bowel cancer as well as the surgical procedure employed. The English expression needs attention and some editing. The appendectomy is feasible however the manuscript has significant shortcomings.

Major Concerns:

Provide the institutional animal care and use committee ethics approved number from the

university. The surgical procedure could be summarized and referenced appropriately, rather than presenting a step by step procedure.

Response: we have added this number on Page 2 in the revised manuscript.

A statistical section should be included with a full explanation of analytical tests performed and justified on the data. For example what test was used to show the differences observed in Figure 4.

Response: thanks for pointing this out. We have performed the statistical analysis analytical in the revised figure 4.

The results should be better presented with subsections that streamline the data and the experiments performed.

Response: thank you for your suggestion, and we have added subsections in the results. A newly added figure 1A has also been provided for illustration of the key surgical steps involved in murine appendectomy model.

The discussion begins with a discussion of the surgical procedure and if this is novel it perhaps should be clearly reflected in the title of the manuscript. Also it would prudent to begin the discussion with what the study has shown and then discuss the novel procedure if that is the orientation of the study.

Response: thanks for the valuable comment. The main novelty of surgical step in the appendectomy murine model is the facile and precise location of the cecal patch. We have revised the title to "Murine Appendectomy Model of Chronic Colitis Associated Colorectal Cancer by Precise Localization of Caecal Patch" and added the discussion of this novelty on Page 7, thoroughly revised the beginning of discussion and the title.

For Manuscript:

Please submit each figure as a vector image file to ensure high resolution throughout production: (.svg, .eps, .ai). If submitting as a .tif or .psd, please ensure that the image is 1920 x 1080 pixels or 300 dpi.

1. I've submit all my figures as tif. with 300dpi

Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

2. I've thoroughly went through my manuscript to ensure that there are no spelling or grammar issues.

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3. I've rephrased the summary part according to your suggestion.

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Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as "could be," "should be," and "would be" throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a "Note."

5. I've went through all terms in the protocol section and made revise accordingly. All text in the protocol section is written in the imperative tense. Any text that cannot be written in the imperative tense is rephrased as a "Note" following each relevant protocol term.

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6. The numbering of the Protocol is all adjusted.

Please start all the notes, steps, substeps and caution statement from a new line.

7. The format of each note in text is adjusted. Every note is now started from a new line.

The Protocol should contain only action items that direct the reader to do something.

8. The Protocol terms are revised. There're only action items that direct the reader to do something now in the terms.

The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections. Please simplify the Protocol so that individual steps contain only 2-3 actions per step.

9. All protocol terms are thoroughly went through. Now individual term contain only 2-3 actions per step.

Please ensure you answer the "how" question, i.e., how is the step performed?

10. I'm sure that all protocol terms combined with notes answered the possible "how" questions.

Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account. The Figure must be cited appropriately in the Figure Legend, i.e. "This figure has been modified from [citation]."

11. All figures in the manuscript are original.

Please upload all figures individually to your Editorial Manager account. Please combine all panels of one figure into a single image file. For the figures generated with microscope (e.g., Figure 6) please include a scale bar and define it in the table of materials.

12. I've uploaded all figures individually to my Editorial Manager account. All panels of one figure are combined into one single image file. Scale bars are marked in figure 6 and are detailed in the table of materials.

All figures and/or tables showing data must include measurement definitions, scale bars, and error bars (if applicable). Please include all the Figure Legends together at the end of the Representative Results in the manuscript text.

13. All necessary scale bars are added accordingly. All figure legends are put together at the end of the Representative Results section.

As we are a methods journal, please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:

a) Critical steps within the protocol

b) Any modifications and troubleshooting of the technique

c) Any limitations of the technique

d) The significance with respect to existing methods

e) Any future applications of the technique

14. Please expand the journal title in the references section.

14. I've went through the discussion part according to the suggestion: critical steps within the protocol are detailed in paragraph 1,2; modifications and troubleshooting of the technique are detailed in paragraph 2,3; the significance with respect to existing methods, possible limitations of the technique and future applications of the technique is described in paragraph 4.

Please expand the journal title in the references section.

15. I've went through each reference term and expand the journal titles within.

For Video:

Furthermore, please submit a high resolution version of your video (up to 2 GB) here: https://www.dropbox.com/request/knUJwvEKcBkRO2z7TyTq?oref=e

1. I have submitted a high resolution version of revised video this time to the appointed links.

The video must have title cards at the beginning and end. These title cards should include the authors' names and their affiliations.

2. Title cards including the authors' names and affiliations are now showed both at the beginning and end of the video.

Please increase the agreement between the video and the written manuscript. Ideally, the narration in the video is a reading of the written protocol text in the manuscript.

3. Narration in the video is re-recorded and it's now mostly a reading of written protocol text.

Chapter title cards should be inserted between each section of the video. Our site has a chaptering system that relies on these to help our users navigate the video.

4. Chapter title cards are inserted between each section of the video.

Please ensure all the result figures and their panels are present in the representative result section of the video. Histology figures needs a scale bar. Presently 6C, D and Figure 5A,B,C are not shown in the video.

5. All result figures are now presented in the representative result section of the video, scale bars are added to the histological figures.

Please do not use commercial terms in the video. E.g., Prolene, Styrol etc.

6. No commercial terms are shown in the video.

For Production:

Future submissions should contain the article ID number (59921) in the file name.

7.The name of the newly submitted file now contains the ID number (59921).

• 2:02, 2:47, 3:33, 3:46, 4:21, 4:30, 4:32, 4:40, 5:44- The edits here are jump cuts, which tend to have a jarring effect on the viewer. They should be smoothed out with crossfades instead.

8. Relevant parts of the video mentioned in the suggestion (2:02, 2:47, 3:33, 3:46, 4:21, 4:30, 4:32, 4:40, 5:44) are now smoothed out with crossfades.

The narration audio is much higher in volume in the left stereo channel than in the right stereo channel. The narration audio should be even on both channels.

9. The narration audio are now balanced on both channels.

To vet review Dr. B.Alisantosa：

1. Surgery Preparation:

1.4. Anesthetize a mouse intraperitoneally (i.p.) with 1% pentobarbital sodium at a dose of 100 mg/kg:

Question/Comment:

-The dose of pentobarbital sodium seems high for anesthesia procedure (Almost as high as for euthanasia procedure), in the future, please consider using lower dose (i.e.; 30-50 mg/kg IP for anesthesia agent).

Response: thank you for bringing up the doses of anesthesia. In our preliminary experiment, a dose of 50 mg/kg seems insufficient and the mouse often resuscitates during the surgery. We anesthetized a mouse intraperitoneally with 1% sodium pentobarbital at a single dose of 100 mg/kg to ensure the full sedative effect in the mouse (Peirce S, SVH Clinical SOP. 26. Euthanasia of Laboratory Animals, 2016.; RAT AND MOUSE ANESTHESIA AND ANALGESIA Formulary and General Drug Information, 2016).We have also revised the step on Page 2-3 in the manuscript.

2. Mice Appendectomy:

2.12. Close the skin layers using interrupted sutures using the size of 4-0 silk suture thread

Question/Comment:

-It wasn’t clear to me if the author provides any analgesia to alleviate pain and distress after

surgery and administer antibiotics post surgically to prevent infection? Please clarify what kind of analgesia, the dose and route plus describe what kind of antibiotics, dose, for how long and route of administration were provided to the animals?

Response: we used subcutaneously injection of 0.1 mg/kg body weight of buprenorphine after surgery to relieve the pain and stress of mouse. We have revised manuscript on Page 4 and video section I., 04:41-04:50. However, we did not use any antibiotics before and after surgery because it will influence the gut microbiota in our model.

5. Assessment of Colonic inflammation and Tumor (70 days post-appendectomy):

5.1. Sacrifice the mice by cervical dislocation, harvest the entire colon from above the ileo-colic junction to anus.

Question/Comment:

- It wasn't clear to me if the endpoint of this experiment is on 70 days post-appendectomy. Please clarify.

Response: The endpoint of this experiment is actually on 70 days post-administration of AOM, and we've revised and clarified this point both in manuscript and video now.

Changing part needed in the table

Response: We used 75% medical alcohol to remove iodine at the end of our surgery. We have revised manuscript on Page 4 and video section I., 04:35-04:40.

For question of 05:00-05:20 in the video, we've now added sentence under 2.16 text about the analgesia we used.

Thank you again for your careful review and kind suggestions.

#	Time in the video	Comment	Change in video required Yes/No	Change in text is sufficient Yes/No	Suggested Changes
1	01:39-01:46	Prior to performing skin incision, entoiodine was used to disinfect the skin twice. It wasn't clear to me if Medical alcohol was utilized as well prior to skin incision?	Yes	No	In the text, it says that the author used 75% medical alcohol and entoiodine for surgery preparation, but alcohol wasn't mentioned in the video prior to skin incision. Please clarify in the video.
2	05:00-05:20	In this video section, you mentioned that you provide the mouse with 0.4 mL physiological saline, but you didn't indicate if you provide any analgesia and antibiotic post-surgically to prevent pain and distress and infection	No	Yes	Add sentence under 2.16 text to include any analgesia and antibiotic (if any) to prevent pain, distress and infection post-surgically.