Supplemental Information for

Oxidative stress accelerates intestinal tumorigenesis by enhancing 8-oxoguanine-mediated mutagenesis in MUTYH-deficient mice

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This Supplemental Methods PDF file includes:

- Stretch and Roll (StR) protocol; for pathological sample of mouse entire small intestine
- Mutation assay by *rpsL* transgenic mice

Stretch and Roll (StR) protocol; for pathological sample of mouse entire small intestine

① All parts of the small intestine (SI) were carefully separated by cutting through the immediately distal to the pylorus and proximal to the cecum (Williams et al., 2016).

② The mesenteric attachment adjacent to the serosal surface of the SI was cut and removed.

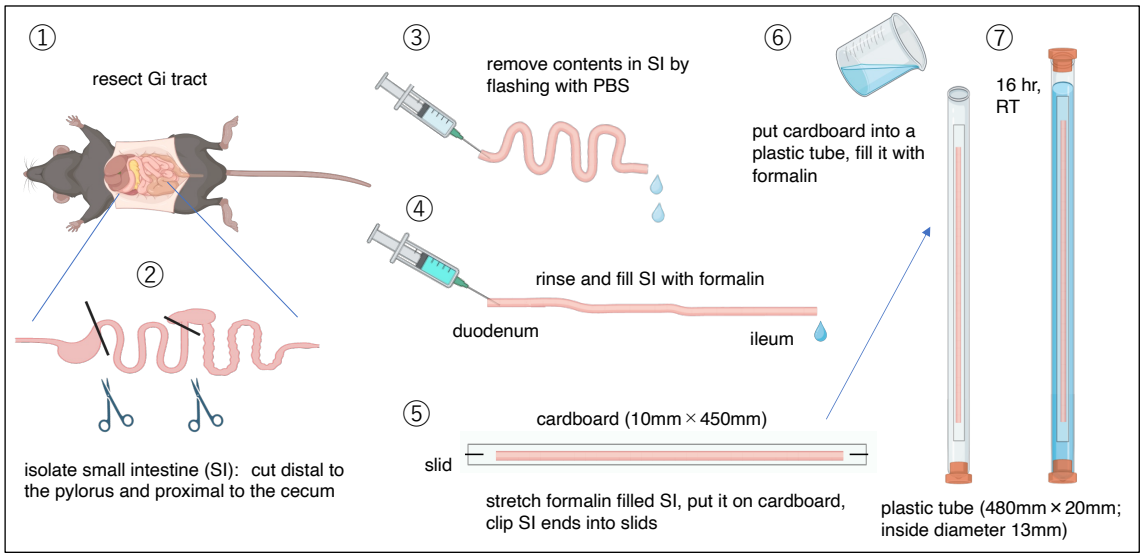
③ Their contents were removed by flushing with PBS from one side using a 10 mL syringe with blunt tipped 18 G needle.

④ The inside of the SI was immediately rinsed and filled SI with 10% formalin using a syringe.

⑤ The formalin-filled SI was put onto the paper cardboard (10 mm × 450 mm) and stretched gently to make it straight; the ends of the intestine were then attached by inserting them into the incisions at the cardboard.

⑥ The SI was then placed into a plastic tube (480 mm × 20 mm; inside diameter 13 mm) filled with 10% formalin. The tube was closed with rubber stoppers.

⑦ The specimen was fixed for 16 h at room temperature.



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⑧ After 16 h, the specimen was retrieved from the tube and the cardboard and rinsed with PBS. The external materials around the small intestine, such as fats or membranes, were removed and then incised longitudinally to make a long-flat specimen.

⑨ The specimen was placed with the lumen side facing up and ileum on the right side and made flat and straight.

⑩ A distal end of the ileum was gently clamped using a 3M filter paper (Whatman Grade 3MM Chr Cellulose Chromatography Paper, 3030-917) and then rolled up with a luminal surface out.

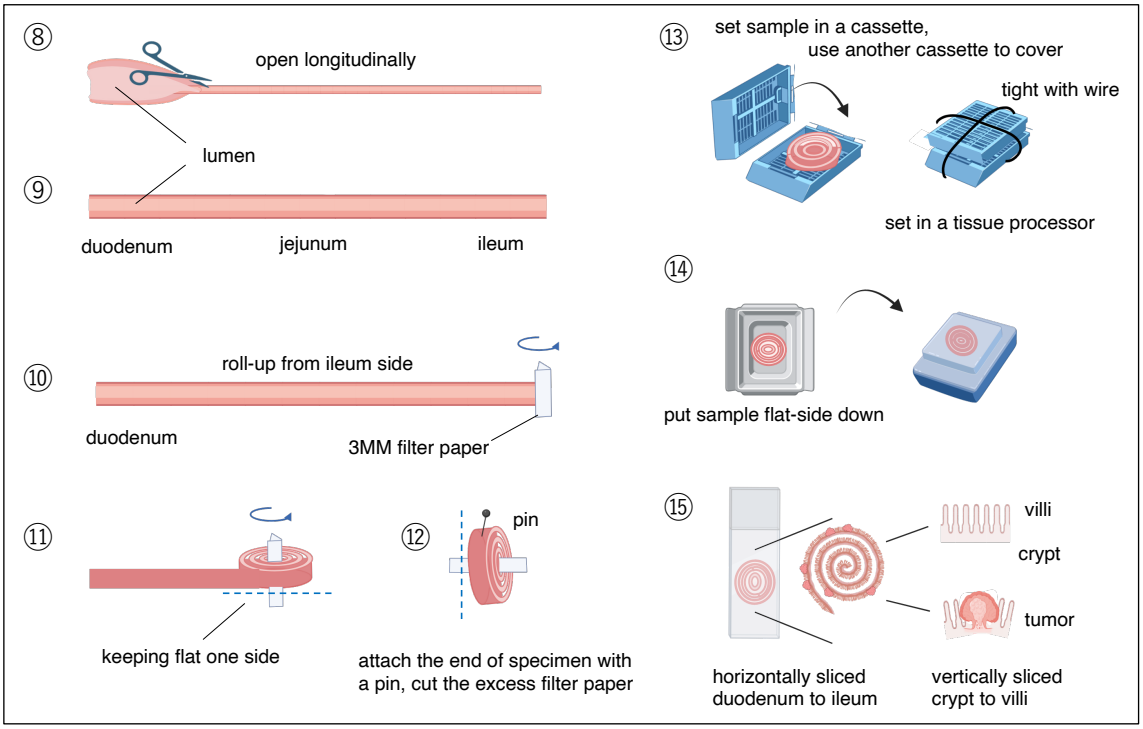
⑪ During the rolling step, one side of the specimen was kept flat.

⑫ The end of specimen was attached with a metal insect pin.

⑬ To make the paraffin block, the rolled specimen was placed in a tissue cassette, covered with another cassette, tight with wire, and set in the automated tissue processor.

⑭ The paraffin-replaced sample was placed flat-side down on a metal tray to make a suitable paraffin block for the StR section.

⑮ The paraffin blocks were sectioned into 4-μm thick and stained with hematoxylin and eosin (HE).



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Reference:

Williams JM, Duckworth CA, Vowell K, Burkitt MD, Pritchard DM. 2016). Intestinal preparation techniques for histological analysis in the mouse. *Curr Protoc Mouse Biol* **6**(2): 148–168, doi: 10.1002/cpmo.2.

Mutation assay by *rpsL* transgenic mice

Tissue specimens resected from rpsL-Tg mice were immediately frozen in liquid nitrogen and stored in a −80 °C deep freezer until genomic DNA extraction. The rpsL mutation assay was

performed following the methods described previously with some modifications (Egashira et al. 2002; Isoda et al. 2014). Briefly, genomic DNA was extracted from tissue samples using the QIAamp Fast DNA Tissue Kit (QIAGEN) and digested with Ban II (Takara Bio Inc.) at 37 °C for 2 h. Then, DNA was electrophoresed, and near-3 Kb-sized DNA fragments were cut from the agarose gel and extracted using Freeze N Squeeze DNA Gel Extraction Spin Columns (Bio-Rad Laboratories, Inc., CA, USA). After isopropanol precipitation, DNA was incubated with T4 ligase (Takara Bio Inc.) at 16 °C for 2 h. DNA was purified using NucleoSpin Gel and PCR Clean-up (MACHEREY-NAGEL GmbH & Co. KG, Germany); after ethanol precipitation, DNA was resuspended in distilled water. Commercially available electrocompetent cells, 10-beta electrocompetent E. coli (cat No. C3020, New England Biolabs, MA, USA) kanamycin (Km)-sensitive, streptomycin (Sm)-sensitive) used for transformation. Electroporation and subsequent steps for rpsL mutation assay were performed following a previously described protocol (Egashira et al. 2002). Mutations in the rpsL coding sequence resulting in the Sm-resistant phenotype in the host bacterial cells were selectively detected. Total MF was calculated by dividing the number of Km/Sm-resistant colonies by the number of total Km-resistant colonies. All Km/Sm-resistant colonies were subjected to Sanger sequencing to detect variants in the *rpsL* coding region. MF for each mutation type was calculated based on the ratio of the corresponding mutation type to the total number of mutations.