**Whole mount dissection and staining of enteric nervous system**

**Perfusion**

1. Administer sodium pentobarbital through intraperitoneal injection.
2. Place mouse back in the cage long enough for anesthesia to take effect. Apply a hard toe pinch until mouse no longer reacts, ensuring that the mouse can no longer feel pain before proceeding.
3. Place mouse, abdomen-up, on Styrofoam block. Spray mouse abdomen with 70% ethanol. Grasp skin below ribcage with forceps and cut skin with scissors from middle up either side towards the armpits, cutting through ribcage. Diaphragm should carefully be cut circumferentially.
4. Remove pericardium and peripheral fat to expose heart.
5. Insert the needle into the left ventricle and secure it with vascular clamp. Make a small incision on the right atrium using fine scissors.

6. Start the saline perfusion for 7 min at a constant speed of ~1 ml/10 s.

**Tissue collection**

1. Following perfusion, open the abdomen of the mouse, remove and collect the stomach and duodenum.
2. Place the organs on ice in a tube containing HBSS solution.
3. Open the stomach by cutting on the lesser curvature and open the duodenum along the mesentery line.
4. Wash the tissue in HBSS solution in a petri dish to clean and remove the food.
5. Place the tissue in a Petri dish coated with Sylgard and orient it mucosa up.
6. Grasp the right and left edges of the tissue and pin with 0.20 mm pins (the tissues should be stretched).
7. Fix the tissue in 4% PFA overnight at 4°C in the Petri dish coated with Sylgard.
8. Wash five times in PBS 1X for 5 min.
9. Unpin the tissue and keep it stored at 4°C in 1X PBS containing 0.1% Sodium Azide (NaN3) before dissecting.

**Microdissection of longitudinal muscle and myenteric plexus**

1. Add PBS 1X to a Petri dish coated with Sylgard, place the tissue on it and orient tissue with the mucosal layer facing up. Grasp the right and left edges of the tissue and pin them (the tissues have to be stretched).
2. Under a stereo microscope, scratch the mucosa with the back of curved forceps.
3. Using forceps with fine tips, remove the mucosal and submucosal layer until you expose the circular muscle.
4. Peel away the circular muscle with fine forceps to uncover the myenteric plexus.
5. With a micro-scissor, cut small segments of the dissected tissue containing the longitudinal muscle and the myenteric plexus (LMMP).
6. Store the LMMP preparation at 4°C in 1X PBS containing 0.1% Sodium Azide (NaN3) until performing immunofluorescence.

**Immunofluorescence staining on whole mount tissues:**

1. In a 96-well plate, add 200 μL of blocking solution containing 0.1% PBS/NaN3, 10% FBS and 0.5% Triton X-100 per well needed for the number of tissues. Using fine forceps, transfer each tissue into a separate well with the blocking solution and incubate for 2 hours at room temperature on a shaker.
2. Dilute primary antibodies in the blocking solution.
3. Add 200 μL of primary antibodies solution in new empty wells, transfer each tissue in separate wells and incubate with the primary antibodies overnight at 4°C on a shaker.
4. Wash five times in PBS 1X for 5 min (by adding 200 μL of PBS 1X in new wells and transferring each tissue separately).
5. Dilute secondary antibodies at 1:500 in the blocking buffer.
6. Add 200 μL of secondary antibodies solution in new empty wells, transfer each tissue in separate wells and incubate for 2 hours at room temperature on a shaker.
7. Wash five times in PBS 1X for 5 min each (by adding 200 μL of PBS 1X in new wells and transferring each tissue separately).
8. Place the tissue on the slide and mount it between slide and coverslip with prolong gold w/ DAPI.
9. Let it dry overnight at room temperature in a slide folder.

**Reagents**

* Paraformaldehyde (cat# P6148, Sigma-Aldrich)
* Triton X-100 (cat# X100, Sigma-Aldrich)
* SYLGARD™ 184 Silicone Elastomer Kit (cat# 04019862, Dow)
* Hanks' Balanced Salt Solution (HBSS) (cat# 2639065, ThermoFisher Scientific)

**Solution**

Blocking solution: 1X PBS, 0.1% NaN3, 10% FBS and 0.5% Triton X-100