

## ***Supplementary Material and Methods***

### ***Animal Model of Intestinal Anastomosis***

We constructed a clinically relevant surgical rat model of ETEA and STSA to evaluate post-operative intestinal anatomy and physiology. Both anastomoses began with a midline laparotomy and placing the eventrated small intestine on to moist cotton gauze. In the ETEA, a distal small bowel loop was transected between a vascular arcade, and the bowel was anastomosed end-to-end using 8-0 prolene suture with individually tied serosa-serosa sutures. The STSA reconstruction was performed by clamping the anti-mesenteric sides of the transected bowel loop together, suturing the bowel sides with individual ties, cutting the septum between the two bowel loops, and suturing the blind end to make a common terminal lumen. The length of the STSA was constructed to be 3 bowel lumens, i.e. approximately 12 mm, adjusted to animal bowel size. This procedure was designed to mimic the clinical stapled STSA, as closely as possible in an experimental setting. Ileocecal anastomosis was not performed due to the marked difference in the rat cecal anatomy compared with the human. After each anastomosis procedure, the intestine was then returned to the abdominal cavity and the midline incision closed with a running double layer 4-0 suturing procedure. All resuscitated animals were studied 21 days postoperatively. An additional set of animals received a 7-day oral course of 4% dextran sodium sulfate (DSS) before sacrifice to induce gastrointestinal inflammation. Thus, six groups of animals were studied: controls, controls+DSS, ETEA, ETEA+DSS, STSA and STSA+DSS.

To determine the effects of the different intestinal anastomoses on liquid gastrointestinal transit, we measured the aboral luminal transit of a non-absorbable tracer, fluorescein isothiocyanate-labeled dextran with an average molecular mass of 70 kDa (FD70), as previously described.(18) Briefly, rats were orally fed 20 $\mu$ l of FD70 dissolved in distilled saline (6.25 mg/ml). Ninety minutes later, the entire gastrointestinal tract, from the lower esophageal sphincter to the rectum, was excised and divided into 15 segments: stomach, small intestine (10 segments of equal length), cecum, and colon (three segments of

equal length). Supernatants of the intestinal chyme were fluorometrically assayed for the FD70 fluorescence in a 96 well plate reader (Molecular Devices, Sunnyvale, CA). The gastrointestinal distribution of FD70 was analyzed by plotting distribution histograms and calculating the geometric center (GC) using the following formula (19, 20) :  $GC = \Sigma (\% \text{ of total fluorescent signal per segment} \times \text{segment number}) / 100$ . Additionally, the gastrointestinal tract was functionally analyzed for solid gastrointestinal transit by assessing the distribution of ten orally gavaged chrome steel spheres (1 mm). The gastrointestinal location of each sphere was assessed after sacrificing the animal three hours after administration. The *in vitro* spontaneous smooth muscle contractile activity 1 cm proximal from the anastomotic suture line was assessed by microdissecting mucosa-free muscle strips of 1 mm width and mounting each in an organ bath perfused with pre-warmed, pH-adjusted modified Krebs solution at 37°C.(21) Gross structure, histochemical cross-section and whole-mount staining of the gut wall 5 mm proximal to the anastomosis were performed to identify architectural changes of the bowel wall. Muscle thickness was measured for muscularis hyperplasia and hypertrophy. A minimum of 3-4 animals were used in all groups. The experimental design used in this study was approved by the Institutional Animal Use and Care Committee of the University of Pittsburgh (Protocol Number 12101128).

### ***Supplementary Results***

In both ETEA and STSA animal models (without DSS treatment), there was no difference in the change of body weight over the 21-day post-operative study period, which increased from 13-16% above baseline, similar to control animals. However, the STSA + DSS group demonstrated lower increase ( $8.1 \pm 1.59\%$ ) in overall body weight compared to ETEA + DSS ( $13.3 \pm 1.14\%$ ) ( $p < 0.05$ ). After intestinal harvest, the *ex vivo* outer small intestinal diameter was  $3.6 \pm 0.27$  mm in un-operated controls,  $8.0 \pm 0.63$  mm at the ETEA (**supplementary Figure 1A**) and  $22.6 \pm 1.63$  mm at the STSA (**supplementary Figure 1B**). In essence, the STSA formed a distal small bowel pouch-like structure with solid chyme accumulation (N=4 each, **supplementary Figure 1E**). The DSS treated animals had similar results (control+DSS=  $3.8 \pm 0.26$  mm, ETEA + DSS=  $8.2 \pm 0.86$  mm and STSA + DSS=  $24.5 \pm 2.63$  mm, N= 4

per group) suggesting that the addition of DSS did not affect the intestinal diameter. Hence, both anastomoses increased bowel diameter within the 21 day period over control, with STSA dramatically increasing the diameter 6-fold over control ( $p < 0.05$ ). In addition to morphologic alteration of the bowel, there was a significant increase in spleen weights in the STSA+DSS animals ( $1,958.0 \pm 453.9$  vs. control  $767.0 \pm 42.6$  mg,  $p < 0.01$ ,  $N=5$  per group).

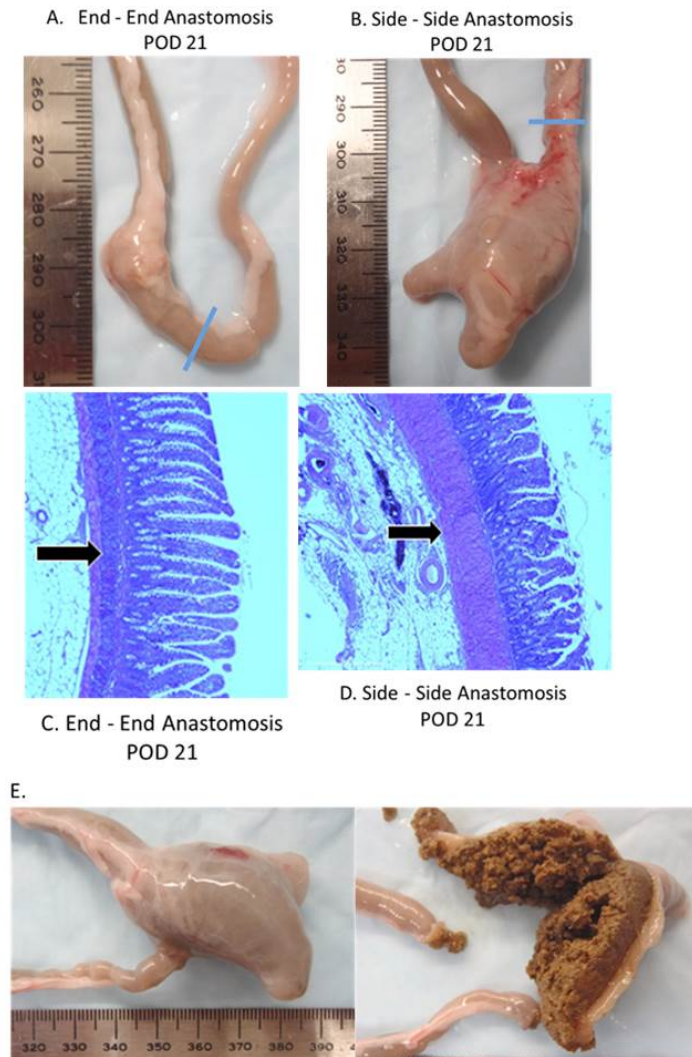
The muscularis externa thickness, 5 mm proximal from the anastomotic suture line, was increased most in the STSA group ( $143.7 \pm 5.40$ ) compared to the controls ( $91.3 \pm 2.99$   $\mu$ m,  $p < 0.001$ ). The ETEA group ( $98.5 \pm 4.51$ ,  $p = 0.019$ ) was also thicker than controls but not as thickened as the STSA group ( $N=4$  per group), (**supplementary Figure 1D, 1C**). Quantitative changes in muscle thickness were not further affected by DSS ( $N=3$  per group). As seen in **supplementary Figure 1C-1D**, alterations in the mucosal architecture were also noted in the STSA group, but these changes were not quantified.

Liquid gastrointestinal transit at 90 minutes showed no intestinal obstruction at the site of the ETEA or STSA with the calculated geometric centers (GC) being similar among tap water fed rats (GC= $9.6 \pm 0.55$ ,  $9.3 \pm 0.67$  and  $8.8 \pm 0.34$  for control, ETEA and STSA, respectively,  $N = 4$  per group). In control + DSS and ETEA+DSS groups, calculated GC values were similar to tap water fed animals (control + DSS =  $9.5 \pm 0.48$  and ETEA + DSS = GC =  $9.1 \pm 0.49$ ). However, liquid transit was briefly, but significantly delayed in the STSA+DSS group (GC =  $7.8 \pm 0.30$ ,  $N = 4$  per group,  $p < 0.05$ ).

Solid gastrointestinal transit experiments showed that in all control animals the 10 spheres were transported out of the stomach and through the naïve small bowel ending up in the cecum and proximal colon over the 3 hour time period ( $N=4$  each). In the ETEA animals,  $18 \pm 5\%$  of the spheres clustered at the anastomotic site with the majority of the spheres reaching the terminal ileum and cecum ( $68 \pm 12.6\%$ ). In contrast, in the STSA animals,  $60 \pm 8.2\%$  of the spheres clustered at the anastomotic site and only  $32 \pm 5\%$  traversed the entire upper gut into the ileum and cecum. DSS induced gastrointestinal inflammation exerted no effect on solid transit, in the control or ETEA groups, as spheres were distributed similar to the

respective tap water groups (no DSS exposure). In the STSA + DSS group, the majority of the spheres remained in the stomach ( $55 \pm 12.9\%$ ) and most of the remaining spheres clustered at the site of the anastomosis ( $25 \pm 5.8\%$ ) with no spheres reaching the cecum ( $p < 0.05$ ). Next, we investigated the functional activity of the muscularis externa *in vitro*. Spontaneous contractile frequencies of intestinal strips proximal to anastomotic area were similar between ETEA (**supplementary Figure 2B**) and controls (**supplementary Figure 2A**), but markedly decreased in the STSA group (**supplementary Figure 2C**), which was further aggravated by DSS (Control:  $29.0 \pm 0.4$ , ETEA:  $31.2 \pm 3.1$  and STSA:  $9.7 \pm 1.1$ ,  $p < 0.05$ ) (Control+ DSS:  $28.2 \pm 1.7$ , ETEA + DSS:  $29.3 \pm 1.6$  and STSA + DSS:  $6.2 \pm 2.0$ ,  $p < 0.05$ ).

## Supplementary Figure 1



Effect of different surgical anastomoses on the structure and physiology of rat small intestine on post-operative day 21 (POD 21). When compared to end-to-end anastomosis (ETEA) in Figure (A), side-to-side anastomosis (STSA) is dilated and forms a pouch like configuration (B). When opened, the STSA pouch revealed fecalization of the small bowel contents (E). The histology of the intestinal strip above the anastomosis showed hypertrophied muscularis mucosae in the STSA (D) as compared to normal muscularis mucosae layer in the ETEA (C).

## Supplementary Figure 2

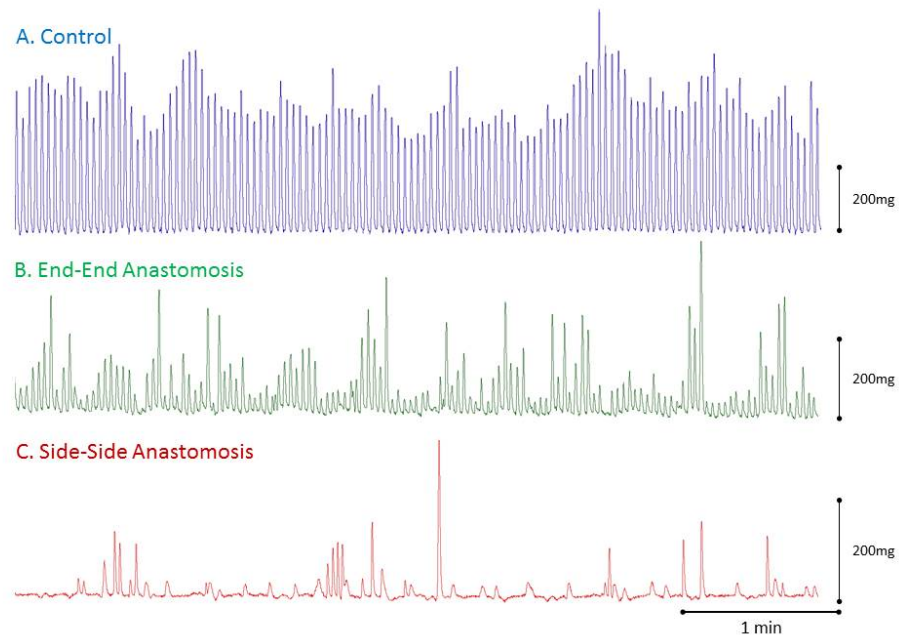


Figure depicts spontaneous intestinal activity in muscle strips isolated from normal small bowel (Panel A) compared with muscle strips isolated from segments proximal to the ETEA and STSA. There was a marked decreased in spontaneous intestinal contractile activity just proximal to the anastomosis in the STSA (Panel C) group compared to the ETEA group (Panel B).