Supporting Information

TITLE: Multimodal Imaging of the Murine Gastrointestinal Tract with Retained Luminal Content

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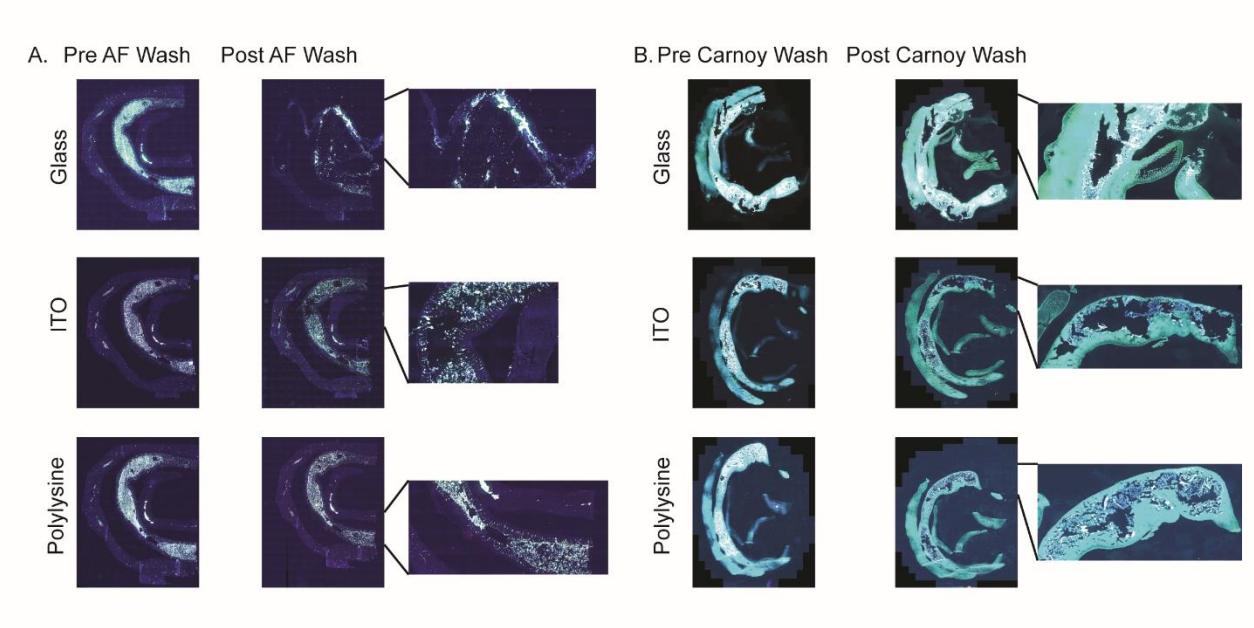
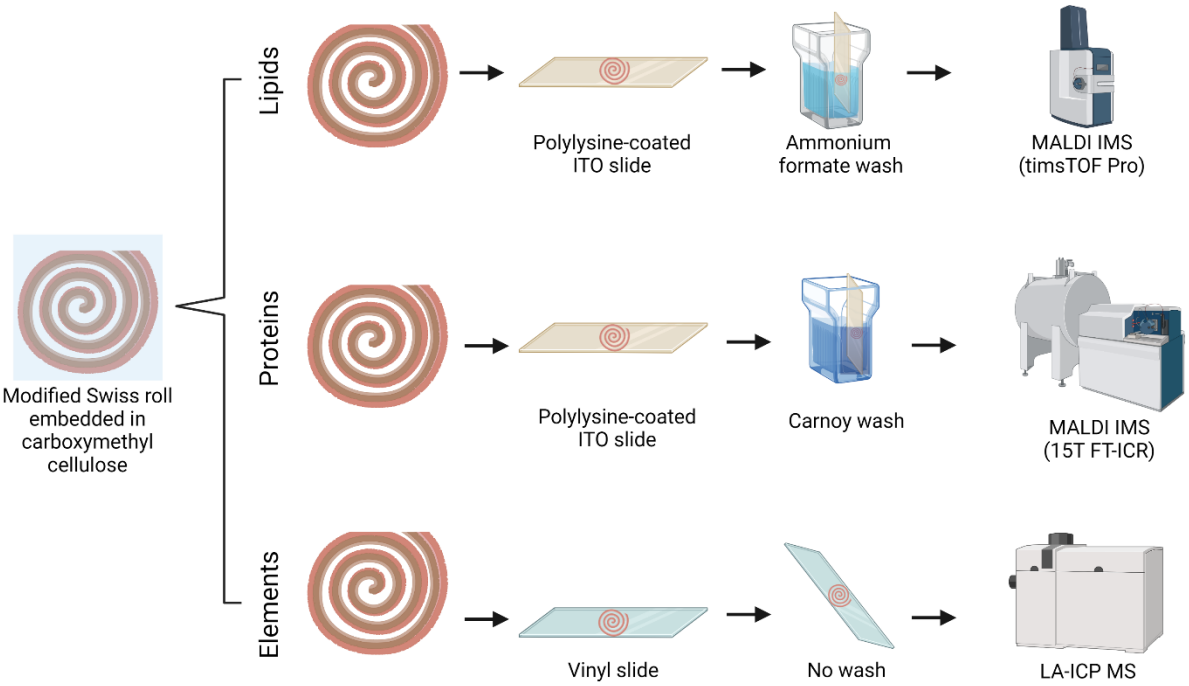
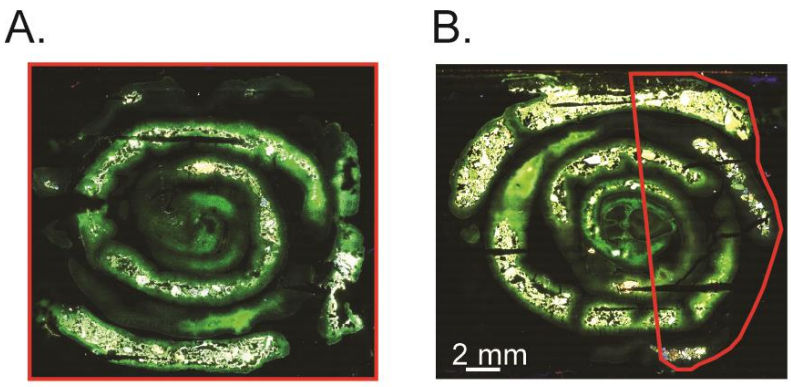


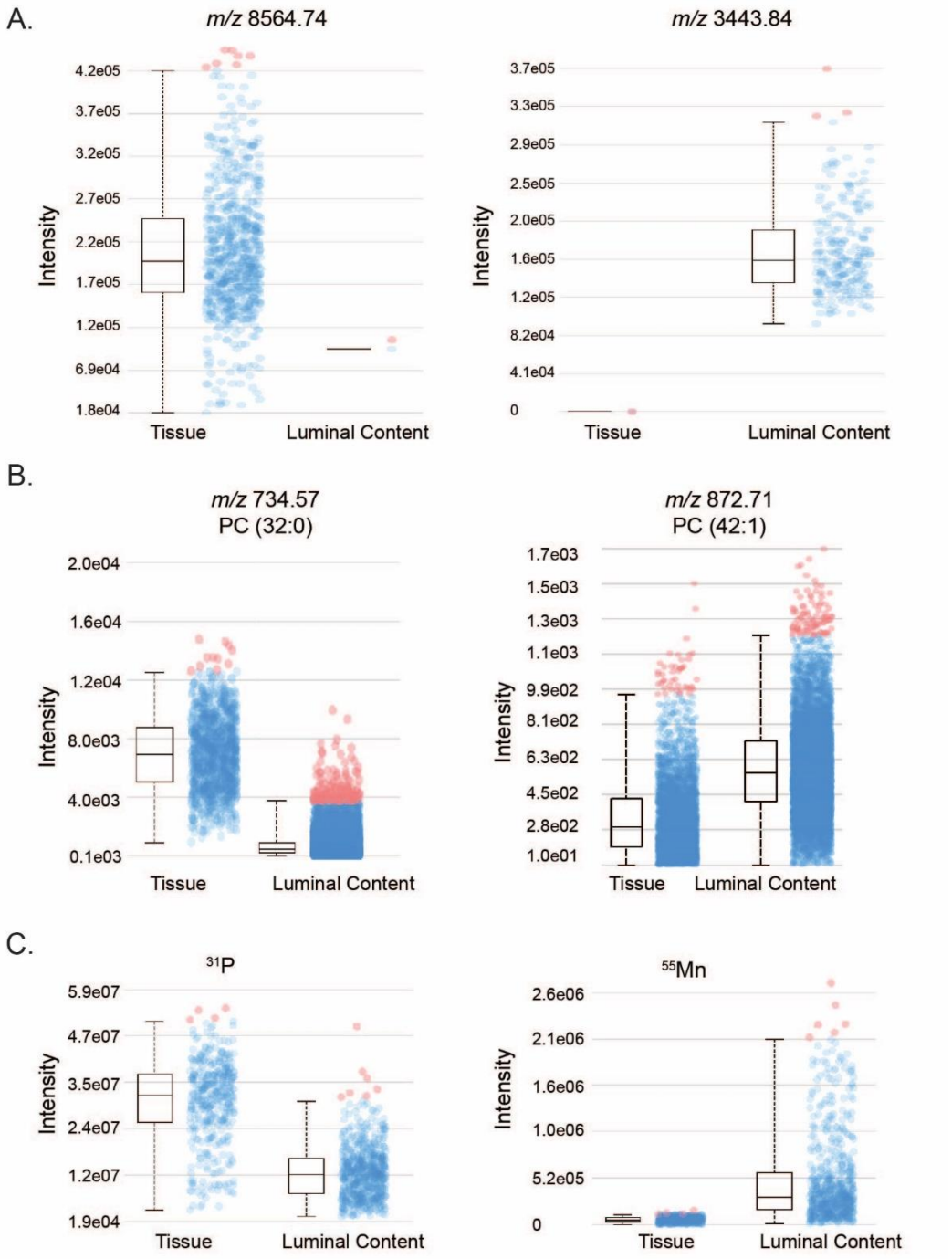
Supplemental Figure 1: Poly-l-lysine coating improved luminal content retention for various washing methods. A) Pre- and post-washing autofluorescence images on glass, ITO and polylysine-coated ITO slides using an ammonium formate wash.[1] B) Pre- and post-washing autofluorescence images in glass, ITO and polylysine-coated ITO slides using a Carnoy wash.



Supplemental Figure 2: Sample preparation workflows for multimodal IMS of modified Swiss roll gastrointestinal tissue samples. Created with Biorender.com.



Supplemental Figure 3: Autofluorescence images of imaged tissue showing regions ablated. Red lines indicate the imaged region of tissue with MALDI IMS as shown in main-text figures. A) Tissue sample used for protein imaging in Figure 2A, B) Tissue sample used for lipid imaging in Figure 2B.



Supplemental Figure 4: Box plots of average intensity differences between tissue and luminal content for protein, lipid and elemental imaging of control tissue. Average intensities for all pixels from defined ROIs, either tissue or luminal content regions for ions shown in Figure 2. **A**) Protein intensity box plots from Figure 2A, **B**) Lipid intensity box plots from Figure 2B, **C**) Elemental intensity box plots from Figure 2C.

Supplemental Table 1

	MALDI FT-ICR MS Imaging of Proteins	**LA-ICP IMS Imaging of Trace Elements**	**MALDI timsTOF Imaging of Lipids**
Laser	Apollo II dual MALDI/ESI ion source and a Smartbeam II 2 kHz frequency tripled Nd:YAG laser 355 nm[2]	Nd:YAG 213 nm	Nd:YAG 355 nm
Laser Setting	small (50 um)	100 um	small (50 um)
Spatial Resolution	100 um	60 um	25 um
Laser Shots	1000	-	400
Laser Power	70%	35%	40%
***m/z* range**	1000-30,000	-	200-2000
Resolving Power	36,507 at *m/z* 8529.918	-	-
Transient length	2.3069s	-	-
Ionization mode	positive	-	positive

Supplemental Table 2

	MALDI FT-ICR MS Imaging of Proteins	**MALDI timsTOF Imaging of Lipids**
Matrix	2′,6′-Dihydroxyacetophenone (DHA)	1,5-diaminonapthalene (DAN)[3]
Matrix Solution	90 mg/mL DHA in 70% ACN with 100 µL TFA and 50 µL of 30% ammonium hydroxide	300 mg DAN (~1.0 mg/cm^2)
Application Method	Sprayed with automatic robotic aerosol sprayer (TM Sprayer, HTX Technologies, Chapel Hill, NC, USA)	Sublimated using a simple sublimation apparatus at 130°C and 24 mTorr for 3 min[4]
TM Sprayer Method	45°C, 1050 mm/min, 0.075 mL/min, 1.5 mm track spacing, 8 passes	--

SUPPLEMENTAL METHODS:

Materials. Acetic acid, 1,5-diaminonapthalene (DAN), trifluoroacetic acid (TFA), ammonium formate, 2′,6′-Dihydroxyacetophenone (DHA), red phosphorous, hematoxylin, and eosin were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). HPLC-grade acetonitrile, methanol, ethanol, and chloroform were purchased from Fisher Scientific (Pittsburgh, PA, USA).

Histology. Following MALDI IMS experiments, matrix was removed from samples using 100% ethanol and rehydrated with graded ethanol and double distilled H_2O then fixed using 10% neutral-buffered formalin. Tissue samples were stained using a hematoxylin and eosin stain, and brightfield microscopy of stained tissues was obtained using a Leica SCN400 Brightfield Slide Scanner at 20x magnification (Leica Microsystems, Buffalo Grove, IL).

Animal Protocols. All animal experiments were performed using protocols approved by the Vanderbilt University Medical Center Institutional Animal Care and Use Committee. Animal studies were conducted using 8- to 12-week old male C57Bl/6 (Jackson Laboratories) mice.

Mass Calibration.

MALDI IMS. For all MALDI-based experiments, a slurry of red phosphorus and methanol was spotted onto the slide as a calibration standard. A series of phosphorus clusters were then used to calibrate the FT-ICR platform prior to tissue analysis.

LA-ICP IMS. Flow rates of helium and argon gas were tuned and optimized for each sample to obtain the same signal response with SRM 612 standard from the National Institute of Standards and Technology (NIST), which includes 61 elements. The mass spectrometer was operated in medium-resolution mode.

Supplemental References:

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