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🌐 Wholemount immunolabeling of mouse gut tissue

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SPARC

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Wholemount Immunolabeling-Gut

The application was developed for large pieces of mouse gut tissue.

Animal care, breeding procedures, and experimental protocols were approved by the UTHSC animal care and use committee. Animals were housed in an AAALAC-approved facility with a 12-hour light cycle with food (standard chow) and water ad libitum. Male and female mice aged 3 to 9 months were used in the reported studies. All reporter mice, except where indicated, were generated by crossing either R26REYFP/EGFP mice or RCL-tdTomato mice with one of the Cre lines which express the reporter in a promoter-specific manner following Cre-mediated recombination. When applicable mice were purchased from the Jackson Laboratories, Bar Harbor, Maine. All genotyping was done using primers recommended by the Jackson Laboratories according to their protocols.

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Primary antibodies used in the study

Antigen detected by primary antibody	Host	Dilution	Source	RRID
alphaCGRP	rabbit	1:500	Peninsula Labs (T4032)	RRID:AB_518147
Calbindin D-28	rabbit	1:400	Swant (CB38A)	RRID:AB_10000340
Calretinin	rabbit	1:400	Swant (7697)	RRID:AB_2721226
Calretinin	goat	1:400	Swant (CG1)	RRID:AB_10000342
CD31	rat	1:300	BD Pharmingen (557355)	RRID:AB_396660
CGRP	mouse	1:200	Abcam (ab81887)	RRID:AB_1658411
ChAT	goat	1:100	Millipore (AB 144P)	RRID:AB_262156
Enkephalin	mouse	1:200	Millipore (MAB350)	RRID:AB_2268028
Enkephalin	mouse	1:150	Abcam (ab150346)	RRID:AB_2892658
GABA	rabbit	1:300	Sigma (A2052)	RRID:AB_477652
GFP	goat	1:400	Abcam (ab5450)	RRID:AB_304897
GFP	chicken	1:300	AVES (GFP1020)	RRID:AB_10000240
GFP	rabbit	1:200	Abcam (ab6556)	RRID:AB_305564
HuC/D (ANNA-1)	human	1:20,000	gift from Dr. V.A. Lennon	RRID:AB_2314657
Iba1	rabbit	1:500	Wako Chemicals (019-19741)	RRID:AB_839504
Neurofilaments	mouse	1:500	Thermo Fisher (13-1300)	RRID:AB_2532999
nNOS	rabbit	1:400	Millipore (AB5380)	RRID:AB_91824
nNOS	rabbit	1:300	Abcam (ab76067)	RRID:AB_2152469
nNOS	goat	1:200	Invitrogen (OSN00004G)	RRID:AB_10709561
PGP9.5	guinea pig	1:500	Neuromics (GP14104)	RRID:AB_2210625
RFP	chicken	1:600	Rockland (600-901-379)	RRID:AB_10704808
RFP	rabbit	1:600	Rockland (600-401-379)	RRID:AB_2209751
S-100	rabbit	1:350	Abcam (ab52642)	RRID:AB_882426
Serotonin (5-HT)	rabbit	1:1000	Immunostar 20080	RRID:AB_572263
Serotonin (5-HT)	goat	1:1000	Immunostar 20079	RRID:AB_572262
Somatostatin	rat	1:200	Abcam (ab30788)	RRID:AB_778010
Somatostatin	rat	1:200	Millipore (MAB354)	RRID:AB_2255365
Somatostatin	rabbit	1:200	Abcam (ab108456)	RRID:AB_11158517

Substance P	rabbit	1:200	Abcam (ab67006)	RRID:AB_1143173
Substance P	guinea pig	1:200	Abcam (ab106291)	RRID:AB_10864733
Substance P	rat	1:150	Abcam (ab7340)	RRID:AB_305866
Synaptophysin	rabbit	1:150	Abcam (ab32127)	RRID:AB_2286949
Synaptotagmin 1	rabbit	1:100	Abcam (ab131551)	RRID:AB_11157546
Synaptotagmin 1	chicken	1:100	Abcam (ab133856)	RRID:AB_2885088
TUJ1 (Beta III tubulin)	chicken	1:500	Abcam (ab107216) discontinued	RRID:AB_10899689
TUJ1 (Beta III tubulin)	rabbit	1:1000	Covance (AB 291637) discontinued Now BioLegend (PRB-435P-100)	RRID:AB_291637
VIP	rabbit	1:500	Sigma (HPA017324)	RRID:AB_1858754
VIP	rabbit	1:300	Immunostar (20077)	RRID:AB_572270
VIP	guinea pig	1:300	Invitrogen (PA1-36030)	RRID:AB_1088264
VIP	rabbit	1:100	Abcam (ab22736) (used in initial experiments, new lots do not work)	RRID:AB_447294

Secondary antibodies used in the study

Secondary Antigen	Host	Conjugate	Dilution	Source	RRID
Chicken (IgY, H+L)	goat	Alexa Fluor 488	1:300	Invitrogen (A11039)	RRID:AB_142924
Chicken (IgY, H+L)	goat	Alexa Fluor 568	1:500	Invitrogen (A11041)	RRID:AB_2534098
Chicken (IgY, H+L)	goat	Alexa Fluor 594	1:500	Invitrogen (A11042)	RRID:AB_2534099
Goat (IgG, H+L)	donkey	Alexa Fluor 488	1:500	Invitrogen (A11055)	RRID:AB_2534099
Goat (IgG, H+L)	donkey	Alexa Fluor 555	1:500	Invitrogen (A21432)	RRID:AB_2535853
Goat (IgG, H+L)	donkey	Alexa Fluor 647	1:500	Invitrogen (A21447)	RRID:AB_141844

Guinea pig (IgG, H+L)	goat	Alexa Fluor 555	1:500	ThermoFisher A-21435	RRID:AB_2535856
Guinea pig (IgG, H+L)	goat	Alexa Fluor 594	1:500	Invitrogen (A11076)	RRID:AB_141930
Human (IgG, H+L)	goat	Alexa Fluor 555	1:500	Invitrogen (A21433)	RRID:AB_2535854
Human (IgG, H+L)	goat	Alexa Fluor 647	1:500	Invitrogen (A21445)	RRID:AB_2535862
Human (IgG, H+L)	goat	DyLight 405	1:500	Novus Biologicals (NBP1-72871)	RRID:AB_11008096
Human (IgG, H+L)	alpaca	DyLight 405	1:100	Jackson IR (609-475-213)	RRID:AB_2721858
Mouse (IgG, H+L)	goat	Alexa Fluor 594	1:500	Invitrogen (A11005)	RRID:AB_141372
Rabbit (IgG, H+L)	donkey	Alexa Fluor 488	1:500	Invitrogen (A21206)	RRID:AB_2535792
Rabbit (IgG, H+L)	donkey	Alexa Fluor 555	1:500	Invitrogen (A31572)	RRID:AB_162543
Rabbit (IgG, H+L)	donkey	Alexa Fluor 647	1:500	Invitrogen (A31573)	RRID:AB_2536183
Rabbit (IgG, H+L)	goat	Alexa Fluor 488	1:500	Invitrogen (A11034)	RRID:AB_2576217
Rabbit (IgG, H+L)	goat	Alexa Fluor 546	1:500	Invitrogen (A11010)	RRID:AB_2534077
Rabbit (IgG, H+L)	goat	Alexa Fluor 568	1:500	Invitrogen (A11011)	RRID:AB_143157
Rabbit (IgG, H+L)	goat	Alexa Fluor 594	1:500	Invitrogen (A21207)	RRID:AB_141637
Rabbit (IgG, H+L)	goat	Alexa Fluor 647	1:500	Invitrogen (A21245)	RRID:AB_2535813
Rat (IgG, H+L)	donkey	Alexa Fluor 594	1:400	Invitrogen (A21209)	RRID:AB_2535795
Rat (IgG, H+L)	goat	Alexa Fluor 647	1:500	Invitrogen (A21247)	RRID:AB_141778
Sheep (IgG, H+L)	donkey	Alexa Fluor 488	1:300	Invitrogen (A11015)	RRID:AB_141362
Sheep (IgG, H+L)	donkey	Alexa Fluor 594	1:500	Invitrogen (A11016)	RRID:AB_253408
Sheep (IgG, H+L)	donkey	Alexa Fluor 647	1:500	Invitrogen (A21448)	RRID:AB_2535865

Harvesting and fixing the mouse colon for determination of shrinkage with PFA fixation

- 1 Sacrifice mice by cervical dislocation; no perfusion. Make a midline incision to expose abdominal organs and dissect colon from the small intestine.



- 2 Remove fecal contents and measure a "whole-length-tube" .



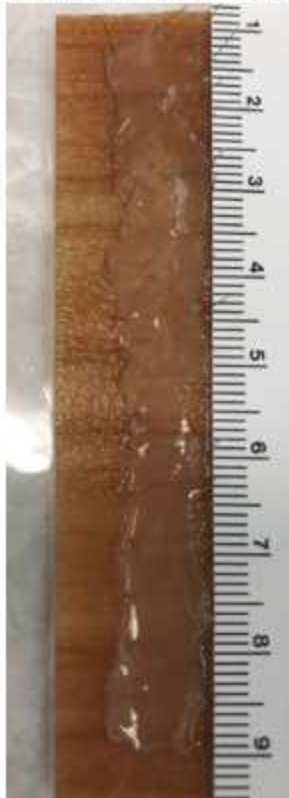
Measure L_0 = initial length (mm)

- 3 Measure L_{fix} = length after fixing of whole mouse colon, cleaned by lavage and fixed as "whole-length tube" in 4% PFA for 2-3 h at room temperature

Whole mouse colon after lavage with PBS at room temperature. $L_o = 76$ mm



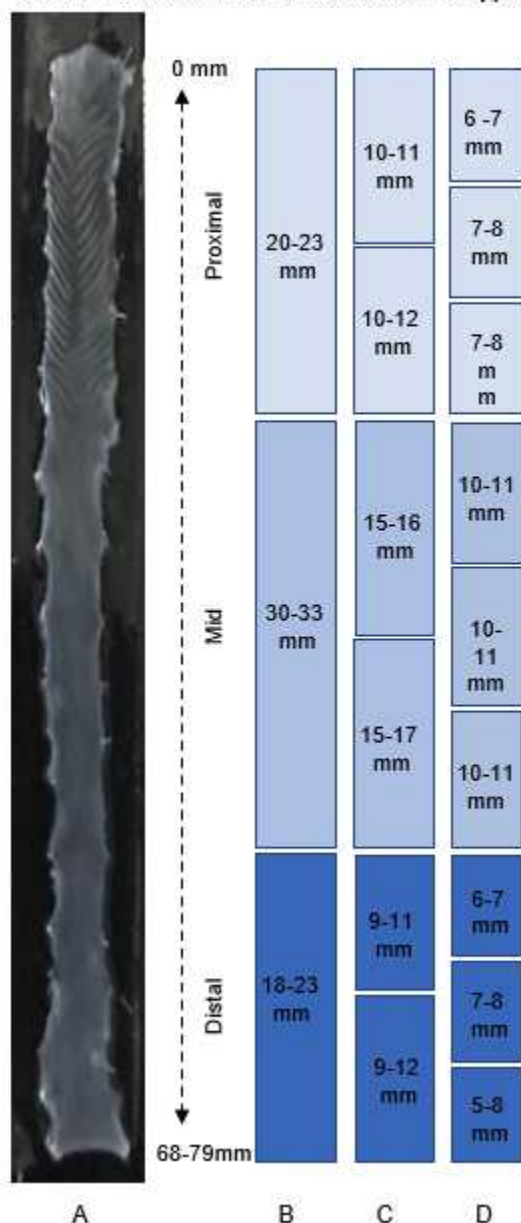
Whole mouse colon opened along the mesenteric border, pinned without stretching and fixed. $L_{fix} = 78$ mm



Mouse dissection protocol for wholemound immunolabeling-gut

- 4 Using the angled probes and forceps, gently unravel the intestines and choose which end you will begin your dissection from. Cut with scissors and using forceps move to small petri dish trying not to spill the contents.
- 5 Cover tissue with just enough water to cover the bottom of the dish. Tissues should be well washed prior to fixation. Take the guts to the microscope for dissection. Cut the colon segments according to diagram below.

Location and size of dissections for colon mapping



The model shows the size and length of colon used for quantitative and descriptive analysis of mouse colon

- 7 Fixation in 4% PFA/PBS pH 7.0-7.4 @ RT for 2-4 hours depending on the tissue size or 40C overnight, followed by washing in PBS over 4-6hrs and overnight in 30% sucrose in PBS if you want to cut sections.
We store in PBS if only for immunostaining.
- 8 Wash tissue in 0.1M Tris/1.5% NaCl/TX-100 (0.5%) 3 X 30 minutes

- 9 Block in Tris/NaCl/TX-100 (0.5%) 20% HS @RT 2 x 1 hour with rotation
- 10 Wash in Tris/NaCl/TX-100 (0.5%) 2 times....quick wash
- 11 Incubate in primary antibody(s) in Tris/NaCl/TX-100 (0.5%) + 20% HS @ 40C on the rotating table 2 days (minimum volume 1.5 ml)
- 12 Wash in Tris/NaCl/ TX-100 (0.5%) with 10% HS 3 X 1 hr. on the rotating table
- 13 Wash in Tris/NaCl/ TX-100 (0.5%) 3 X 10 min. on rotating table
- 14 Incubate in secondary antibody in Tris/NaCl/Tx-100 (0.5%) 2 days @ 40C on the rotating table (minimum volume 2.5 ml)
- 15 Wash in Tris/NaCl/Tx-100 (0.5%) 6 X 10 min. with rotation
- 16 Visualize in PBS preferably as soon as it is ready, i.e. make a reservation for the confocal when you start
Image intact or flat-mount colon samples using a Leica TCS SP5 laser scanning confocal microscope (Leica Microsystems, Bannockburn, IL) equipped with continuous-wave solid-state lasers (458, 488, 514, 561, 633 nm) and a titanium-sapphire tunable (705-980nm) multiphoton laser (Coherent, Santa Clara, CA). Acquire images at 512 X 512 in the XYZ planes as a single field of view or as tile scans (XYZS) in 1 µm steps with 20x (NA 0.70), 40x (NA 1.25) or 63x (NA 1.4) objectives using an automated scanning stage.