FINAL SCRIPT: APPROVED FOR FILMING

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Title: Murine Mesenteric Lymphadenectomy for Selective Disruption of Lymphatic Communication with Region-Specific Gut

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **YES**

   We do not have a microscope camera, but have been able to film with an iphone through the microscope eye piece previously. Our scope has two heads, two eye pieces for operating and two eye pieces for observation. We have had success filming through both; however, the operating eye pieces tend to produce crisper images with our low power equipment. It may not make a difference for y'all. If it does, I believe I can operate through one eye piece.

   Leica Mz9.5 stereo head operating microscope

   2.3.1 to 2.14.3, 3.3.1

   Videographer: Please film the SCOPE shots using the scope kit

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **NO**

3. **Filming location:** Will the filming need to take place in multiple locations? **NO**

Current Protocol Length
Number of Steps: 18
Number of Shots: 33

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

~~**REQUIRED:** What is the scope of your research? What questions are you trying to answer?~~

1.1. **Jess Buck:** We study T cell responses to neoantigens in peripheral tissues, and since T cell functionality is heavily influenced by priming, we particularly focus on where these priming events occur.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

What technologies are currently used to advance research in your field?

1.2. **Yilei Qin:** Currently, the only widely used options to block trafficking between lymph nodes and tissues are systemic modalities like S1P receptor agonists and integrin blockade, but this broad inhibition prevents us from studying the contribution of specific local lymph nodes.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

What advantage does your protocol offer compared to other techniques?

1.3. **H.N. Blackburn:** Our protocol provides an in-depth description of how to perform highly selective lymphadenectomies at each of four main mesenteric lymph node stations, and since these nodes are quite specialized in which segment of the gut they drain, this well-tolerated survival surgery allows us to disrupt only a portion of the gut-lymph axis, while leaving the rest intact.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 2.4.2*

Videographer's Note: All audio cues for entire video. iPhone was attached to scope. Could not center phone on scope so slightly off center.

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the Yale Institutional Animal Care and Use Committee

Protocol

2. Mesenteric Lymphadenectomy Preparation and Excision of Mesenteric Lymph Node Stations

Demonstrator: H.N Blackburn

2.1. To begin, use surgical scissors to make a 0.5 to 1 centimeter midline incision through the anesthetized mouse skin **[1-TXT]**. Using blunt forceps, dissect the skin away from the abdominal wall and identify the linea alba **[2]**. Then, make another 0.5 to 1 centimeter incision through the linea alba to access the peritoneal cavity **[3]**.

2.1.1. WIDE: Talent making a midline skin incision using surgical scissors. **TXT: Anaesthesia: 2-3% Isoflurane with O_2 (2 L/min)**

2.1.2. Talent using blunt forceps to separate the skin from the abdominal wall and pointing to the linea alba.

2.1.3. Talent making a second incision through the linea alba.

2.2. Using a blunt tip gavage needle, instill 1 milliliter of body-temperature sterile saline into the peritoneal cavity **[1]**. Moisten the sterile drapes to prepare for bowel positioning **[2]**.

2.2.1. Talent instilling saline into the cavity with a blunt tip gavage needle.

2.2.2. Talent moistening sterile drapes using saline.

2.3. To resect the first lymph node station, orient the exteriorized bowel onto moistened drapes with the cecum positioned inferiorly, proximal colon superiorly, and terminal ileum towards the surgeon **[1]**.

2.3.1. SCOPE: Talent arranging the bowel on the drapes with clear orientation of anatomical structures.

Videographer: Please film the SCOPE shots using the scope kit

Videographer's Note: This was not filmed with a scope. Each lymph node station was shot on a different mouse and out of order and also as one take. Order is 1,4,2,3. #3 was halted 1/2 way through due to bleeding. Out of mice so was not redone.

2.4. Identify the mesenteric lymph node chain that runs longitudinally along the colon **[1]**. Using angled fine-tipped forceps, bluntly dissect the first lymph node station away from the ileocolic vessels by placing the closed forceps between these structures and gently opening them **[2]**.

2.4.1. SCOPE: Talent pointing out the entire mesenteric lymph node chain along the colon, then the first lymph node station specifically.

2.4.2. SCOPE: Talent using angled fine-tipped forceps to gently dissect the lymph node from the ileocolic vessels.

Added optional step: To perform optional suture ligation, use 9-0 nylon suture or similar to tie off the inflow and outflow of the dissected lymph node station, making certain not to disrupt the ileocolic vessels.

Added shot: SCOPE: Talent ties off vessels proximally along the lymph node chain.

Added shot: SCOPE: Talent ties off vessels distally along the lymph node chain.

2.5. Gently retract the lymph node station caudally **[1]**. Then, using fine-point scissors, excise the target lymph node station from the cephalad to caudad direction **[2]**. NOTE: This is labelled as 2.5 on frme.io

2.5.1. SCOPE: Talent retracting the ligated lymph node station caudally using forceps.

2.5.2. SCOPE: Talent excising the lymph node station with fine-point scissors from cephalad to caudad.
AUTHOR'S NOTE: Move 3.1-3.3 after 2.5

3.1 Observe the distal branches of the ileocolic vessels, ileal vessels, and jejunal vessels to confirm that the blood supply remains intact **[1]**.

3.1.1 SCOPE: Show close-up of distal branches of the ileocolic, ileal, and jejunal vessels with visible pulsation or intact coloration.

3.2 Using cotton-tipped swabs, gently return the abdominal contents into the peritoneal cavity **[1]**.

3.2.1 Talent using cotton-tipped swabs to reposition the abdominal organs back into the cavity.

3.3 Instill 1 milliliter of body-temperature sterile saline into the abdominal cavity to compensate for evaporative fluid loss **[1]**. Close the abdomen in two layers, first the abdominal wall, then the skin, using a 6-0 *(Six-O)* monofilament polypropylene suture in a running pattern **[2]**.

3.3.1 Talent instilling warm saline into the abdominal cavity.

3.3.2 Talent performing layered closure of the abdominal wall and skin with a running suture.

2.6. To resect the second lymph node station, gently eviscerate the small intestine **[1]** and orient the exteriorized bowel onto moistened drapes with the cecum positioned inferiorly, proximal colon superiorly, and small intestine towards the surgeon's right **[2]**. NOTE: This is labelled as 3.4 on frme.io

2.6.1. SCOPE: Talent gently eviscerating the small intestine.

2.6.2. SCOPE: Talent positioning the bowel on moistened drapes with correct orientation.

2.7. Next, identify the second lymph node station, which runs longitudinally along the colon and begins at the confluence of ileal and jejunal vessels **[1]**. Using angled fine-tipped forceps, bluntly dissect the second lymph node station away from the colonic vessels **[2]**. NOTE: This is labelled as 3.5 on frme.io

2.7.1. SCOPE: Talent pointing to the second lymph node station running along the colon.

2.7.2. SCOPE: Talent using angled fine-tipped forceps to bluntly dissect the lymph node from the colonic vessels.

Added optional step: To perform optional suture ligation, use 9-0 nylon suture or similar to tie off the inflow and outflow of the dissected lymph node station, making certain not to disrupt the ileocolic vessels. NOTE: This is labelled as 3.6 on frme.io

Added shot: SCOPE: Talent ties off vessels proximally along the lymph node chain.

Added shot: SCOPE: Talent ties off vessels distally along the lymph node chain.

2.8. Gently retract the lymph node station caudally **[1]**. Then, using fine-point scissors, excise the second lymph node station from the cephalad to caudad direction **[2]**. Inspect the operative field to ensure hemostasis **[3]**. NOTE 012026: The narration has been updated. Needs VO re-do! This is labelled as 3.7 on frme.io

2.8.1. SCOPE: Talent retracting the ligated lymph node station caudally using forceps.

2.8.2. SCOPE: Talent excising the second lymph node with fine-point scissors from cephalad to caudad.

Added shot: Clear operative field with no hemorrhage

NOTE 011426: 2.9 to 2.11 were not filmed.

2.9. To resect the second and third lymph node stations, eviscerate the entirety of the small intestine up to the posterior tethering point gently **[1]**. Orient the exteriorized bowel on moistened drapes with the cecum inferiorly, proximal colon superiorly, and small intestine towards the surgeon's right **[2]**.

2.9.1. SCOPE: Talent gently eviscerating the full length of the small intestine up to the ligament of Treitz.

2.9.2. SCOPE: Talent arranging the eviscerated bowel onto moistened drapes with correct anatomical orientation.

2.10. Next, identify the second and third lymph nodes, which run longitudinally along the colon and begin at the confluence of ileal and jejunal vessels **[1]**. Using angled fine-tipped forceps, bluntly dissect the second and third lymph node stations **[2-TXT]**.

2.10.1. SCOPE: Talent identifying the second/third lymph node stations along the colon.

2.10.2. SCOPE: Talent performing blunt dissection using angled forceps and opening them gently between the nodes and colonic vessels. **TXT: Remove forceps and repeat 2-3x until a clear plane is established**

Added step: To perform optional suture ligation, use 9-0 nylon suture or similar to tie off the inflow and outflow of the dissected lymph node station, making certain not to disrupt the ileocolic vessels.

SCOPE: Talent ties off vessels proximally along the lymph node chain.

SCOPE: Talent ties off vessels distally along the lymph node chain.

2.11. Then, gently retract the lymph node stations cephalad **[1]**. Using fine or extra-fine point scissors, excise the lymph node stations from caudad to cephalad **[2]**.

2.11.1. SCOPE: Talent retracting the ligated lymph node stations in the cephalad direction.

2.11.2. SCOPE: Talent excising the lymph node stations from caudad to cephalad using extra-fine scissors.

2.12. To resect the fourth lymph node station, gently eviscerate the entire small intestine up to the posterior tethering point **[1]**. Orient the bowel onto moistened drapes with the cecum inferiorly, proximal colon superiorly, and small intestine towards the surgeon's right **[2]**. NOTE 011426: The narration has been updated. Needs VO re-do! This is labelled as 3.12 on frame.io

2.12.1. SCOPE: Talent gently eviscerating the small intestine to the ligament of Treitz.

2.12.2. SCOPE: Talent arranging the eviscerated bowel on moistened drapes with

proper anatomical alignment.

2.13. Identify the fourth lymph node station, which resides just medial to the distal colon and lateral to the third station **[1-TXT]**. NOTE: This is labelled as 3.13 on frme.io

2.13.1. SCOPE: Talent pointing out the fourth lymph node station along the colon. **TXT: Two lymph nodes make up the 4th station.**

2.14. Using angled fine-tipped forceps or extra-fine point scissors, bluntly dissect the fourth lymph node station away from the colonic vessels and surrounding adipose tissue **[1]**. ~~Gently retract the lymph node station cephalad **[2]**. Using extra-fine point scissors, excise the fourth lymph node station from caudad to cephalad **[3-TXT]**.~~ NOTE 011426: The narration has been updated. Needs VO re-do! This is labelled as 3.14 on frme.io

2.14.1. SCOPE: Talent using angled forceps or extra-fine scissors to gently open between the lymph node, vessels, and adipose tissue

Added OPTIONAL STEP: To perform optional suture ligation, use 9-0 nylon suture or similar to tie off the inflow and outflow of the dissected lymph node stage, making certain not to disrupt the ileocolic vessels. NOTE: This is labelled as 3.15 on frme.io

Added shot: SCOPE: Talent ties off vessels leading to the fourth lymph node station.

Added shot: SCOPE: Talent ties off vessels distally along the lymph node chain.

2.15. Then, gently retract the fourth lymph node station cephalad **[1]**. Using fine or extra-fine point scissors, excise the lymph node stations from caudad to cephalad **[2]**. NOTE 011426: The narration has been updated. Needs VO re-do! This is labelled as 3.16 on frme.io

2.15.1. SCOPE: Talent retracting the ligated lymph node stations in the cephalad direction.

2.15.2. SCOPE: Talent excising the lymph node stations from caudad to cephalad using extra-fine scissors.

Added Step: Repeat the procedure for the second node in the fourth lymph node station. Carefully dissect the node **[1]**, perform suture ligation if required **[2]**, and resect it **[3]**. Inspect the operative field to confirm hemostasis **[4]**. NOTE: This is labelled as 3.17 on frme.io. Need VO recording.

Added shot: SCOPE: Talent dissecting second lymph node.

Added shot: SCOPE: Talent suture ligating second lymph node (optional step).

Added shot: SCOPE: Talent resecting second lymph node.

Added shot: SCOPE: Talent noting no hemorrhage.

~~**3. Mesenteric Lymphadenectomy Completion**~~

~~3.1. Apply sterile surgical glue over the closed wound **[1]**.~~

~~3.1.1. Talent applying surgical glue along the length of the sutured skin incision.~~

Results

4. Results

4.1. Tissue extracted using the mesenteric lymphadenectomy procedure consistently resulted in samples with low adipose content, as evidenced by immediate sinking in PBS **[1].**

4.1.1. LAB MEDIA: Figure 5A. *Video editor: Highlight the three tubes labeled "mLAD" where the samples have visibly sunk to the bottom.*

4.2. The proportion of live CD45 *(C-D-Forty-Five)*-positive cells was significantly higher in mesenteric lymphadenectomy-derived samples compared to visceral adipose controls **[1].**

4.2.1. LAB MEDIA: Figure 5B. *Video editor: Highlight the tall bar labeled "mLAD" showing a high percentage of live cells.*

4.3. Flow cytometry revealed that mesenteric lymphadenectomy-derived samples contained over 95% live CD45-positive cells **[1],** while visceral adipose controls contained fewer than 7% **[2].**

4.3.1. LAB MEDIA: Figure 5C. *Video editor: Highlight the dense upper-left cluster in the mLAD plot marked "CD45+ 95.5".*

4.3.2. LAB MEDIA: Figure 5C. *Video editor: Highlight the sparse region in the Visc Adip plot marked "CD45+ 6.97".*

Pronunciation Guide:

Mesenteric
Pronunciation link: https://www.merriam-webster.com/dictionary/mesenteric merriam-webster.com+1
IPA: /ˌmɛs·ənˈtɛr·ɪk/ Cambridge Dictionary+1
Phonetic Spelling: mes-uhn-TER-ik

Lymphadenectomy
Pronunciation link: https://www.howtopronounce.com/lymphadenectomy How To Pronounce
IPA: /lɪmˌfɑːd.ən.ɛkˈtə.mi/ (US) How To Pronounce+1
Phonetic Spelling: lim-fa-duh-NEK-tuh-mee

Cecum
Pronunciation link: https://www.merriam-webster.com/dictionary/cecum merriam-webster.com+1 *(Note: "mesenteric" page mentions mesentery connected to the cecum/ileum — helps confirm related terms.)*
IPA: /ˈsiː.kəm/
Phonetic Spelling: SEE-kuhm

Ileocolic
Pronunciation link: https://www.merriam-webster.com/dictionary/ileocolic merriam-webster.com+1
IPA: /ˌaɪ.li.oʊˈkɒl.ɪk/ (US: /ˌaɪ.li.oʊˈkɑl.ɪk/)
Phonetic Spelling: eye-lee-oh-KOL-ik

Jejunal
Pronunciation link: https://www.howtopronounce.com/jejunal How To Pronounce+1
IPA: /dʒəˈdʒuː·nəl/
Phonetic Spelling: juh-JOO-nuhl

Gavage
Pronunciation link: https://www.merriam-webster.com/dictionary/gavage merriam-webster.com+1
IPA: /gəˈvɑːʒ/
Phonetic Spelling: guh-VAHZH

Caudad
Pronunciation link: https://www.merriam-webster.com/dictionary/caudad merriam-webster.com+1
IPA: /ˈkɔː.dæd/
Phonetic Spelling: KAW-dad

Cephalad
Pronunciation link: https://www.merriam-webster.com/dictionary/cephalad merriam-webster.com+1
IPA: /ˈsɛf.ə.læd/
Phonetic Spelling: SEF-uh-lad

Eviscerate
Pronunciation link: https://www.merriam-webster.com/dictionary/eviscerate merriam-webster.com+1

IPA: /ɪˈvɪs.ə.reɪt/
Phonetic Spelling: ih-VIS-uh-rayt
 Adipose
Pronunciation link: https://www.merriam-webster.com/dictionary/adipose merriam-webster.com+1
IPA: /ˈæd.əˌpoʊs/
Phonetic Spelling: AD-uh-pohs
 Isolurane *(Actually "Isoflurane," as in your text: "Isoflurane")*
Pronunciation link: https://www.merriam-webster.com/dictionary/isoflurane merriam-webster.com+1
IPA: /ˌaɪ.soʊˈfluːr.eɪn/
Phonetic Spelling: eye-soh-FLU-rayn
 Peritoneal
Pronunciation link: https://www.merriam-webster.com/dictionary/peritoneal merriam-webster.com+1
IPA: /ˌpɛr·ɪ·ˈtoʊ·ni·əl/
Phonetic Spelling: per-ih-TOH-nee-uhl
 Integrin
Pronunciation link: https://www.merriam-webster.com/dictionary/integrin merriam-webster.com+1
IPA: /ˈɪn.tə.grɪn/
Phonetic Spelling: IN-tuh-grin
 Neoantigen
Pronunciation link: https://www.howtopronounce.com/neoantigen How To Pronounce
IPA: /ˌni·oʊ·ˈæn·tə·dʒɛn/
Phonetic Spelling: nee-oh-AN-tuh-jen
 Suture
Pronunciation link: https://www.merriam-webster.com/dictionary/suture merriam-webster.com+1
IPA: /ˈsu·tʃər/
Phonetic Spelling: SOO-cher
 Polypropylene
Pronunciation link: https://www.merriam-webster.com/dictionary/polypropylene merriam-webster.com+1
IPA: /ˌpɑː.liˈprɑː.pəˌliːn/ (US)
Phonetic Spelling: pah-lee-PRAH-puh-leen
 Cytometry
Pronunciation link: https://www.merriam-webster.com/dictionary/cytometry merriam-webster.com+1
IPA: /saɪˈtɒm·ə·tri/ (US: /saɪˈtɑːm·ə·tri/)
Phonetic Spelling: sigh-TOM-uh-tree