

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

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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 O₂ (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
- 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

Commented [BH1]: Each (3/4) lymph node stations are shown individually as they are different iterations (options) of this protocol depending on which area of the colon lymph drainage the reader needs to disrupt. These steps (2.1-2.2) are identical for each option.

Commented [PS2]: Authors: We can not add a black screen in between the protocol section

from the ileocolic vessels.

- 2.5. 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.

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

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

- 2.6. 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].

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

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

3. Mesenteric Lymphadenectomy Completion

- 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.

- 3.4. 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].

- 3.4.1. SCOPE: Talent gently eviscerating the small intestine.

- 3.4.2. SCOPE: Talent positioning the bowel on moistened drapes with correct

Commented [BH3]: This is the body of the procedure for the first iteration (first lymph node station).

Commented [BH4]: This is the final steps (completion and closing) of the procedure. These steps 3.1-3.3 are identical for all procedures. Because these steps all happen in sequence on a single mouse, we filmed one procedure (first lymph node station) straight through (identical beginning--steps 2.1-2.2; unique body--steps 2.3-2.6; identical ending--steps 3.1-3.3).

We then filmed the unique body of the other iterations (second lymph node station, third lymph node station).

orientation.

- 3.5. 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].
 - 3.5.1. SCOPE: Talent pointing to the second lymph node station running along the colon.
 - 3.5.2. SCOPE: Talent using angled fine-tipped forceps to bluntly dissect the lymph node from the colonic vessels.
- 3.6. 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.
 - 3.6.1. SCOPE: Talent ties off vessels proximally along the lymph node chain.
 - 3.6.2. SCOPE: Talent ties off vessels distally along the lymph node chain.
- 3.7. 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].
 - 3.7.1. SCOPE: Talent retracting the ligated lymph node station caudally using forceps.
 - 3.7.2. SCOPE: Talent excising the second lymph node with fine-point scissors from cephalad to caudad.
- 3.8. 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].
 - 3.8.1. SCOPE: Talent gently eviscerating the full length of the small intestine up to the ligament of Treitz.
 - 3.8.2. SCOPE: Talent arranging the eviscerated bowel onto moistened drapes with correct anatomical orientation.
- 3.9. 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].
 - 3.9.1. SCOPE: Talent identifying the second/third lymph node stations along the colon.
 - 3.9.2. SCOPE: Talent performing blunt dissection using angled forceps and opening

Commented [BH5]: This is the unique body for the second lymph node station. This procedure has an identical beginning (2.1-2.2) and identical end (3.1-3.3) as that filmed above.

them gently between the nodes and colonic vessels. **TXT: Remove forceps and repeat 2-3x until a clear plane is established**

- 3.10. **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.

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

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

- 3.11. **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].**

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

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

- 3.12. **Afterward, 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].**

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

3.12.2. **SCOPE:** Talent arranging the eviscerated bowel on moistened drapes with proper anatomical alignment.

- 3.13. **Identify the fourth lymph node station, which resides just medial to the distal colon and lateral to the third station [1].**

3.13.1. **SCOPE:** Talent pointing out the fourth lymph node station along the colon.

- 3.14. **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].**

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

3.14.2. **SCOPE:** Talent retracting the ligated lymph node cephalad.

3.14.3. **SCOPE:** Talent excising the node from caudad to cephalad using extra-fine scissors.

Commented [BH6]: This is the unique body for the second/third lymph node station. This procedure has an identical beginning (2.1-2.2) and identical end (3.1-3.3) as that filmed above.

Commented [BH7]: We were unable to film the fourth lymph node station due to mouse supply. However, there are very clear photos of these steps that we have in the manuscript. Perhaps we could add those stills (FIG 4A-E)? I can send those images without the letters/scale bar/arrows on them if that would be better? To allow the video editors freedom to animate in arrows?

Alternatively, I can find a mouse and film just these two shots by myself this week if yall feel that would be better!

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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".*