FINAL SCRIPT: APPROVED FOR FILMING

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Title: Rapid Depletion of Renal Macrophages using Human CD59/Intermedilysin Cell Ablation Tool

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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? **NO**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, all done**

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

Current Protocol Length
Number of Steps: 25
Number of Shots: 53

Introduction

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

1.1. **Xuebin Qin:** We use intermedilysin-mediated ablation of human CD59-expressing cells in mice to study renal macrophage regeneration, focusing on chemokine-driven monocyte recruitment and niche establishment of immune surveillance post-injury.

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

What are the most recent developments in your field of research?

1.2. **Mohammad Islamuddin:** Recent developments in our field reveal mechanisms of renal macrophage regeneration, highlighting specific chemokines that recruit macrophages and how immune surveillance is re-established following acute kidney injury.

1.2.1. 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?

1.3. **Jefferson Evangelista:** We combine tamoxifen-inducible Cre genetics, intermedilysin ablation, multiparameter flow cytometry, and intravital microscopy to ablate, track, and visualize kidney macrophages in real time.

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

What are the current experimental challenges?

1.4. **Ana Karina Vidal:** Key challenges are avoiding systemic inflammation, targeting only resident macrophages, and measuring rapid regeneration without disturbing the delicate renal microenvironment.

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

What significant findings have you established in your field?

1.5. **Jefferson Evangelista:** We showed monocytes restore 88 percent of kidney macrophages within seven days after ablation, a process critically dependent on the CX3CR1-CX3CL1 signaling axis.

1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:5.2.2*

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

Testimonial Questions (OPTIONAL):

How do you think publishing with JoVE will enhance the visibility and impact of your research?

1.6. **Mohammad Islamuddin:** JoVE's video format will boost reproducibility, broaden global reach, and increase citations by visually guiding researchers through each critical step of our ablation protocol.

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

Videographer: Please record the testimonials

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at Tulane University, School of Medicine

Protocol

2. Tamoxifen-Induced Macrophage Depletion Followed by Mouse Perfusion and Kidney Collection

Demonstrator: Mohammad Islamuddin

2.1. To begin, preheat corn oil to 42 degrees Celsius **[1]**. Dissolve 100 milligrams of tamoxifen in 5 milliliters of preheated corn oil to prepare a stock solution with a concentration of 20 milligrams per milliliter **[2]**.

2.1.1. WIDE: Talent placing a container with corn oil into a water bath set to 42 degrees Celsius.

2.1.2. Talent using a pipette to add 5 milliliters of preheated corn oil into a vial containing 100 milligrams of tamoxifen and swirling to dissolve completely.

2.2. Administer tamoxifen intraperitoneally to 10- to 12-week-old male mice at a dose of 100 micrograms per gram body weight **[1-TXT]**.

2.2.1. Talent using a syringe to inject tamoxifen into the lower abdomen of a mouse. **TXT: Mouse strain: *CX3CR1CreER$^{+/-}$/ihCD59$^{+/-}$*, Repeat injection for 3 days**

2.3. After 15 days, inject a single intravenous dose of intermedilysin into the mice at a dose of 120 nanograms per gram body weight **[1-TXT]**. Monitor and confirm resident macrophage ablation and subsequent regeneration by performing flow cytometry at 1, 3, and 7 days post-intermedilysin administration **[2]**.

2.3.1. Talent using a small syringe to inject intermedilysin into the tail vein of two groups of mice. **TXT: Mouse strains: *CX3CR1CreER$^{+/-}$/ihCD59$^{+/-}$* mice and *CX3CR1CreER$^{+/-}$/ihCD59$^{-/-}$* littermate controls**

2.3.2. SCREEN: 2025-05-28_2.3.2.mp4
Video Editor: Please sequentially show D1, D3 and D7

2.4. For kidney collection, make a midline incision of approximately 2 centimeters on the abdomen of an anesthetized mouse using a sterile scalpel **[1-TXT]**. Gently retract the skin to expose the thoracic cavity **[2]**. Open the rib cage along the sternum using blunt-tipped surgical scissors to expose the heart **[3]**.

2.4.1. Talent making a careful incision along the midline of the mouse's abdomen. **TXT: Anesthesia: Isoflurane inhalation**

2.4.2. Shot of the skin being retracted and the thoracic cavity being exposed.

2.4.3. Talent using scissors to cut open the rib cage and expose the heart.

2.5. Now, insert a 21-gauge to 23-gauge needle into the left ventricle **[1]** and begin perfusion with 10 to 20 milliliters of cold PBS to flush out blood **[2]**.
  2.5.1. Talent inserting a needle into the left ventricle.
  2.5.2. Talent starting perfusion with a syringe filled with cold phosphate-buffered saline.

2.6. Cut the right atrium open to allow blood to drain from circulation **[1]**. Continue perfusing until the kidneys appear pale, indicating effective blood clearance **[2]**.
  2.6.1. Shot of the right atrium being cut.
  2.6.2. Shot the kidneys turning pale.

2.7. Locate the kidneys against the dorsal body wall and gently separate them from surrounding tissues using forceps and scissors **[1]**. Excise the kidney capsules and immediately place the kidneys in cold PBS to prevent degradation **[2]**.
  2.7.1. Talent isolating and lifting the kidneys using forceps and scissors.
  2.7.2. Talent placing the excised kidneys into a dish containing cold phosphate-buffered saline

3. Tissue Dissociation and Density Gradient Centrifugation

3.1. For kidney digestion, first mix 9 milliliters of calcium- and magnesium-free HBSS, 1 milliliter of collagenase IV, and 20 microliters of DNase I, to prepare the digestion cocktail **[1]**. Prepare the enzyme solution fresh and keep it on ice until use **[2]**.
  3.1.1. Talent pipetting HBSS, collagenase IV, and DNase I into a tube to prepare the enzyme cocktail.
  3.1.2. Talent placing the tube containing enzyme solution on ice.

3.2. Using sterile scissors, mince the kidneys into tiny fragments, in a Petri dish containing 5 milliliters of HBSS **[1]**. Transfer the minced kidney tissue into 15-milliliter tubes containing 10 milliliters of the prepared enzyme solution **[2]**.
  3.2.1. Talent mincing kidney tissue in a Petri dish with HBSS.
  3.2.2. Talent transferring minced tissue into a centrifuge tube containing enzyme solution.

3.3. Now, incubate the tubes at 37 degrees Celsius for 30 minutes with gentle agitation to dissociate the tissue **[1]**. Gently triturate the tissue using a pipette or syringe plunger to further break up cell clumps **[2]**.
  3.3.1. Talent placing tubes in an incubator set to 37 degrees Celsius.
  3.3.2. Talent triturating the contents of the tube with a pipette.

3.4. Remove debris by passing the cell suspension through a 40-micrometer cell strainer **[1]**. Centrifuge the filtrate at 650 *g* for 10 minutes at 18 degrees Celsius **[2]**.
3.4.1. Talent pouring the cell suspension through a cell strainer into a new tube.
3.4.2. Talent placing the tube in a centrifuge and setting speed and temperature.

3.5. Next, carefully decant the buffer **[1]**. Resuspend the pellet in 5 milliliters of lysis buffer before incubating at room temperature for 5 minutes to lyse red blood cells **[2]**.
3.5.1. Talent decanting the supernatant post-centrifugation.
3.5.2. Talent adding lysis buffer and setting timer for 5 minutes.

3.6. Add PBS to wash the cells and remove the lysis buffer **[1]**. Maintain the resulting single-cell suspension on ice until further use **[2]**.
3.6.1. Shot of PBS being added to the cells.
3.6.2. Shot of the cell suspension being placed on ice.

3.7. For density gradient centrifugation, first resuspend the cell pellet in 10 milliliters of 30% density gradient solution **[1]**. Carefully layer this over 3 milliliters of 70% density gradient solution in a centrifuge tube **[2]**.
3.7.1. Talent pipetting the cell suspension into a new tube containing 30% density gradient solution.
3.7.2. Talent layering it gently over the denser 70% gradient.

3.8. Centrifuge the gradient at 500 *g* for 30 minutes without applying a brake **[1]**. After centrifugation, carefully collect the interphase layer between the 30% and 70% solutions **[2]**.
3.8.1. Talent setting centrifuge parameters and starting the spin.
3.8.2. Talent collecting the interphase layer using a pipette.

3.9. Wash the collected cells twice with 10 milliliters of PBS **[1]**, Centrifuge each wash at 500 *g* for 10 minutes **[2]**. Resuspend the final pellet in 1 milliliter of FACS *(F-A-C-S)* buffer containing PBS and 2% FBS in a 1.5-milliliter tube **[3]**. Now count the cells using a hemocytometer under a bright-field microscope **[4-TXT]**.
3.9.1. Shot of 10 mL PBS being added to the suspension.
3.9.2. Talent placing the suspension in the centrifuge.
3.9.3. Talent resuspending pellet in FACS buffer in a microcentrifuge tube.
3.9.4. Talent loading hemocytometer and observing cells under microscope. **TXT: Adjust cell concentration to 2 x 10^6 cells/mL**

4. Flow Cytometric Analysis of Kidney-Resident Immune Cells

4.1. Centrifuge the cells suspension at 650 *g* for 5 minutes to pellet the cells **[1]**. Then resuspend the pellet in 1 milliliter of FACS buffer **[2]**.
4.1.1. Shot of pellet post-centrifugation.

4.1.2. Talent resuspending cells in buffer.

4.2. Add anti-CD16/32 *(C-D-Sixteen-or-Thirty-two)* antibody at 1 to 200 dilution to block non-specific Fc *(F-C)* receptor binding **[1]**. After incubating at room temperature for 15 minutes, add Aqua Live/Dead *(Live or Dead)* dye to distinguish live from dead cells **[2]**.
4.2.1. Talent adding Fc block and setting timer for incubation.
4.2.2. Talent pipetting Aqua dye into the cell suspension.

4.3. Now add pre-conjugated antibodies at a dilution of 1 to 100 to the cell suspension **[1-TXT]**. Incubate the sample for 30 minutes at 4 degrees Celsius in the dark to protect fluorophores **[2]**. Then wash the cells twice with FACS buffer **[3]**.
4.3.1. Talent adding antibody cocktail to the tube. **TXT: Pre-Conjugated antibodies: CD45-e450, CD11b-PE-Cy7, hCD59-PE, and F4/80-BV605**
4.3.2. Talent placing the tube in a dark box on ice.
4.3.3. Talent adds FACS buffer to the cells. **TXT: Centrifugation: 650 x *g*, 5 min**

4.4. Fix the cells in 1 percent paraformaldehyde for 30 minutes on ice **[1]**. Then wash the cells twice with FACS buffer to remove residual fixative **[2]**.
4.4.1. Talent adding paraformaldehyde and placing on ice.
4.4.2. Shot of FACS buffer being added to the fixed cells.

4.5. Acquire stained and fixed cells using a flow cytometer **[1]**. Analyze the data using the software **[2]**.
4.5.1. Talent loading sample into the cytometer.
4.5.2. SCREEN: 2025-05-28_4.5.2.mp4
Video Editor: Please draw a box over the lower left table (Name, statistic, #cells) and then highlight the graph on the right, with emphasis on the square box

4.6. Identify kidney and other tissue-resident macrophages and microglia using the markers CD45 *(C-D-forty-five)*, CD11b *(C-D-eleven-B)*, and F4/80 *(F-four-by-eighty)* **[1]**.
4.6.1. SCREEN: 2025-05-28_4.6.1.mp4
Video Editor: Please sequentially show each graph from left to right. While showing each graph, highlight the oval or square portion in each

4.7. Perform initial gating to select live cells using forward and side scatter profiles **[1]**. Eliminate doublets using forward scatter area versus height gating **[2]**. Exclude dead cells using the viability dye **[3]**.
4.7.1. SCREEN: 2025-05-28_4.7.1-4.7.3.mp4
Video Editor: Show only left most graph (SSC-A vs FSC-A)
4.7.2. SCREEN: 2025-05-28_4.7.1-4.7.3.mp4
Video Editor: Show only middle graph (FSC-H vs FSC-A)
4.7.3. SCREEN: 2025-05-28_4.7.1-4.7.3.mp4
Video Editor: Show only right most graph (SSC-A vs L/D (Aqua)

4.8. Enrich immune cells by gating on CD45-positive populations **[1]**. Define kidney-resident macrophages as CD11b-positive F4/80-high cells within CD45-positive gate as shown in the gating strategy **[2]**.

4.8.1. SCREEN: 2025-05-28_4.8.1-4.8.2.mp4

Video Editor: Please show left most graph. Then highlight the square and show the arrow

4.8.2. SCREEN: 2025-05-28_4.8.1-4.8.2.mp4

Video Editor: Transition from 4.8.1 to right most graph. Highlight the oval area

4.9. Define microglia populations as CD11b-positive, CD45-intermediate cells and perform final analysis **[1]**. ~~Perform final analysis using the software **[2].**~~

4.9.1. SCREEN: 2025-05-28_4.9.1.mp4

Video Editor: Please highlight the oval area

4.9.2. ~~SCREEN: 2025-05-28_4.9.2.mp4~~

NOTE: This is already present in results.

Results

5. Representative Results

5.1. A single prominent protein band was observed near 54 kilodalton in the SDS-PAGE *(S-D-S-Page)* gel, confirming the purity and expected molecular weight of recombinant intermedilysin **[1]**. Purified Intermedilysin exhibited strong hemolytic activity in a dose-dependent manner, achieving 50% red blood cell lysis at 7.24 nanogram per milliliter and nearly complete lysis at 650 nanogram per milliliter **[2]**.

5.1.1. LAB MEDIA: Figure 1A. *Video editor: Highlight the rightmost two lanes labeled "ILY 5 µL" and "ILY 10 µL"*

5.1.2. LAB MEDIA: Figure 1B. *Video editor: Zoom in on the curve and highlight the IC_{50} label "7.244" and the dot closest to 650 on the x-axis*

5.2. Flow cytometry analysis confirmed successful and selective depletion of renal macrophages in inducible human CD59 *(C-D-Fifty-Nine)* compound CreER *(Kray-E-R)* mice. mice one day after intermedilysin administration, with no depletion in control mice **[1]**. Renal macrophages began repopulating by day 3 and recovered to approximately 88% of their original population by day 7 **[2]**.

5.2.1. LAB MEDIA: Figure 2B. *Video editor: Highlight the first 2 panels with 16.4% and 1.6%*

5.2.2. LAB MEDIA: Figure 2C. *Video editor: Please highlight the columns of D3 and D7 for the CX3CR1CreER+/-/ihCD59+/- group*

5.3. Only 5% of newly regenerated renal macrophages expressed human CD59 *(C-D-Fifty-Nine)* by day 7, indicating that repopulation primarily occurred via monocyte recruitment rather than in situ proliferation **[1]**.

5.3.1. LAB MEDIA: Figure 2D. *Video editor: Please highlight the bar for day 7 (D7)*

Pronunciation Guide:

1. Intermedilysin

- **Pronunciation link**: https://www.howtopronounce.com/intermedilysin
- **IPA**: /ˌɪntərˌmiːdəˈlaɪsɪn/
- **Phonetic Spelling**: in-ter-mee-duh-ly-sinMerriam-Webster+28How To Pronounce+28How To Pronounce+28Merriam-Webster+18How To Pronounce+18Merriam-Webster+18

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2. Tamoxifen

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/tamoxifen
- **IPA**: /təˈmɑːksəfən/
- **Phonetic Spelling**: tuh-mok-suh-fenHow To Pronounce+1Merriam-Webster+1Merriam-Webster+8ru.howtopronounce.com+8HowToPronounce+8

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3. CX3CR1

- **Pronunciation link**: No confirmed link found
- **IPA**: /siː ɛks θriː siː ɑːr wʌn/
- **Phonetic Spelling**: see-ex-three-see-ar-oneHowToPronounce+2zh.howtopronounce.com+2zh.howtopronounce.com+2tr.howtopronounce.com+48Merriam-Webster+48OED+48

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4. CD59

- **Pronunciation link**: No confirmed link found
- **IPA**: /siː diː ˈfɪfti naɪn/
- **Phonetic Spelling**: see-dee-fifty-nine

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5. F4/80

- **Pronunciation link**: No confirmed link found
- **IPA**: /ɛf fɔːr ˈeɪti/

- **Phonetic Spelling**: ef-four-eightyHowToPronounce+2zh.howtopronounce.com+2zh.howtopronounce.com+2Merriam-Webster+8tr.howtopronounce.com+8Merriam-Webster+8

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6. CD11b

- **Pronunciation link**: No confirmed link found
- **IPA**: /siː diː ɪˈlɛvən biː/
- **Phonetic Spelling**: see-dee-eleven-beeOED+31Merriam-Webster+31Merriam-Webster+31

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7. CD45

- **Pronunciation link**: No confirmed link found
- **IPA**: /siː diː ˈfɔːrti faɪv/
- **Phonetic Spelling**: see-dee-forty-fiveOED+13OED+13Merriam-Webster+13

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8. FACS

- **Pronunciation link**: No confirmed link found
- **IPA**: /fæks/
- **Phonetic Spelling**: faksHow To Pronounce+12Merriam-Webster+12Merriam-Webster+12

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9. Hemocytometer

- **Pronunciation link**: No confirmed link found
- **IPA**: /ˌhiːməˈsaɪtəmɪtər/
- **Phonetic Spelling**: hee-moh-sy-tuh-mee-terHowToPronounce+2zh.howtopronounce.com+2zh.howtopronounce.com+2

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10. Collagenase

- **Pronunciation link**: No confirmed link found
- **IPA**: /kəˈlædʒəneɪz/
- **Phonetic Spelling**: kuh-laj-uh-nayzHow To Pronounce+44Merriam-Webster+44HowToPronounce+44

11. DNase

- **Pronunciation link**: No confirmed link found
- **IPA**: /ˈdiːˌeɪneɪz/
- **Phonetic Spelling**: dee-ay-nayzOED+1Merriam-Webster+1

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12. HBSS

- **Pronunciation link**: No confirmed link found
- **IPA**: /eɪtʃ biː ɛs ɛs/
- **Phonetic Spelling**: aych-bee-ess-essOED+4OED+4OED+4

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13. Paraformaldehyde

- **Pronunciation link**: No confirmed link found
- **IPA**: /ˌpærəfɔːrˈmældɪhaɪd/
- **Phonetic Spelling**: pair-uh-for-mal-duh-hideHowToPronounce

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14. Intravital Microscopy

- **Pronunciation link**: No confirmed link found
- **IPA**: /ˌɪntrəˈvaɪtəl maɪˈkrɒskəpi/
- **Phonetic Spelling**: in-truh-vy-tl my-kros-kuh-pee

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15. Chemokine

- **Pronunciation link**: No confirmed link found
- **IPA**: /ˈkiːməkaɪn/
- **Phonetic Spelling**: kee-moh-kine

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16. Monocyte

- **Pronunciation link**: No confirmed link found
- **IPA**: /ˈmɒnəsaɪt/
- **Phonetic Spelling**: mon-oh-site

17. Macrophage

- **Pronunciation link**: No confirmed link found
- **IPA**: /ˈmækroʊfeɪdʒ/
- **Phonetic Spelling**: mak-roh-fayjHowToPronounce+2zh.howtopronounce.com+2zh.howtopronounce.com+2tr.howtopronounce.com

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18. Microglia

- **Pronunciation link**: No confirmed link found
- **IPA**: /ˌmaɪkrəˈgliːə/
- **Phonetic Spelling**: my-kroh-glee-uhOED+8Merriam-Webster+8Merriam-Webster+8

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19. CreER

- **Pronunciation link**: No confirmed link found
- **IPA**: /kriː iː ɑːr/
- **Phonetic Spelling**: kree-ee-ar