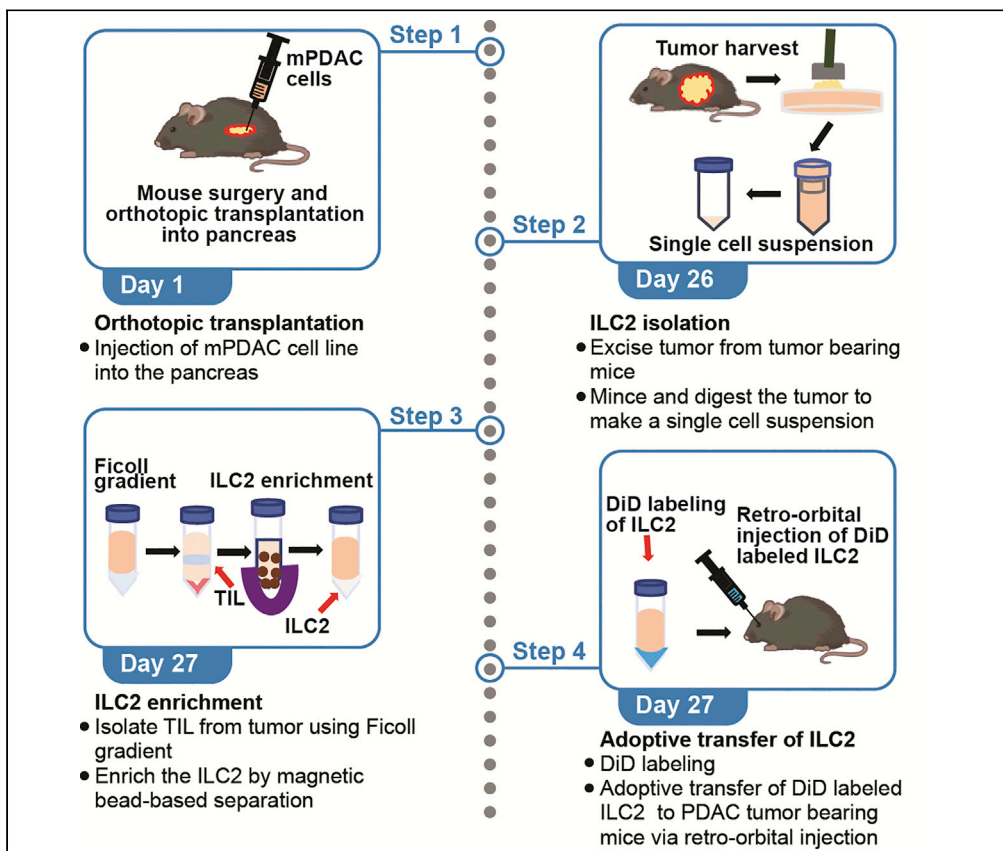


Protocol

Isolation and adoptive transfer of innate lymphoid cells 2 to a recipient mouse model of PDAC



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Highlights

A detailed protocol to isolate and enrich ILC2 from PDAC tumor

Protocol to isolate high-purity ILC2s suitable for adoptive cell transfer study

DiD labeling of ILC2 is an efficient approach to track localization of ILC2s in mice

Innate lymphoid cells 2 (ILC2) play a significant role in the tumorigenesis of pancreatic ductal adenocarcinoma (PDAC). An important aspect of ILC2-mediated tumorigenesis is the expansion of the resident ILC2 and simultaneous recruitment of the peripheral ILC2. Here, we describe a protocol for isolation, enrichment, and DiD labeling of ILC2 for *in vivo* tracking of ILC2s in the mouse. Further, we describe steps for the adoptive transfer of ILC2 to a recipient mouse model of PDAC.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

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Protocol

Isolation and adoptive transfer of innate lymphoid cells 2 to a recipient mouse model of PDAC

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SUMMARY

Innate lymphoid cells 2 (ILC2) play a significant role in the tumorigenesis of pancreatic ductal adenocarcinoma (PDAC). An important aspect of ILC2-mediated tumorigenesis is the expansion of the resident ILC2 and simultaneous recruitment of the peripheral ILC2. Here, we describe a protocol for isolation, enrichment, and DiD labeling of ILC2 for *in vivo* tracking of ILC2s in the mouse. Further, we describe steps for the adoptive transfer of ILC2 to a recipient mouse model of PDAC.

For complete details on the use and execution of this protocol, please refer to Alam et al. (2022).

BEFORE YOU BEGIN

Although the expansion of the resident ILC2 has been shown by multiple studies, the recruitment of peripheral ILC2 remains contentious. The protocol below describes a density-gradient based isolation of ILC2s from mouse PDAC, followed by DiD labeling and adoptive transfer of ILC2 to a syngeneic orthotopic model of PDAC. The protocol will allow an easy flow cytometry-based tracking of transplanted ILC2s into the tumor of recipient mice which will provide evidence of trafficking of ILC2 from the periphery.

Institutional permissions

This protocol requires generation of mouse pancreatic cancer model and ethical approval is required prior to beginning this procedure. The procedure must comply with animal surgery guidelines, including the use of aseptic surgical techniques to prevent possible infection as well as the use of appropriate anesthesia and analgesia to minimize pain. Animal experiments in this procedure were approved by Institutional Animal Care and Use Committee (IACUC) at Roswell Park Comprehensive Cancer Center, protocol number 1416M/1417M.

Preparation of reagents, media, and stock solution:

⌚ Timing: 1 h

1. Prepare IL-2 and IL-7 stock solution at a concentration of 10 $\mu\text{g}/\text{mL}$.
 - a. 1% BSA stock solution.
 - i. Dissolve 100 mg of BSA in 10 mL of sterile PBS in a 15 mL conical tube.
 - ii. Filter the 1% BSA solution with 0.22 μm filter (Millipore, SLGP033RS).
 - iii. Store the stock solution at 4°C and use within 1 month.
 - b. Prepare 0.1% BSA working solution by mixing 1 mL of 1% BSA stock solution to 9 mL of sterile PBS. Store the working solution at 4°C.



- c. Dissolve 10 μg of IL-2 and IL-7 each in 1 mL of 0.1% BSA working solution.
- d. Aliquot IL-2 and IL-7 at a volume of 20 μL and store at -80°C for up to 1 year. Avoid repeated freeze-thaw cycles to protect its activity.
2. Prepare media for PDAC cell culture. These media can also be used for storing freshly harvested PDAC tumor.
 - a. RPMI media 500 mL.
 - b. Fetal bovine serum (FBS) 50 mL.
 - c. Penicillin/Streptomycin (100 U/mL) 5 mL.
 - d. Store at 4°C and use within 1 month.
3. ILC2 culture media:
 - a. RPMI media 50 mL.
 - b. Fetal bovine serum (FBS) 5 mL.
 - c. Penicillin/Streptomycin (100 U/mL) 0.5 mL.
 - d. IL-2 and IL-7 (10 $\text{ng}/\mu\text{L}$) 50 μL .
 - e. Store at 4°C and use within 1 month.
4. Mouse PDAC (mPDAC) cell lines derived from PDAC tumor bearing mouse of the C57BL/6 background. It is preferable to use cells stably overexpressing luciferase by transfecting mPDAC cells with RFP-T2A-luc or similar vector to enable monitoring of PDAC growth by bioluminescence imaging. Alternatively, MRI can be used to track the tumor growth.

Note: For more detailed information refer to [key resources table](#).

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Matrigel	Corning	Cat# 356255
IL-2	PeproTech	Cat# 212-12
IL-7	PeproTech	Cat# 407-ML-005
Ficoll-paque plus	Cytiva	Cat# 17-1440-02
DiD solid	Thermo Fisher Scientific	Cat# D7757
Collagenase IV	STEMCELL Technologies	Cat# 07909
Dispase	STEMCELL Technologies	Cat# 07923
IVISbrite D-Luciferin Potassium Salt Bioluminescent Substrate	PerkinElmer	Cat# 122799
Doxycycline	Sigma-Aldrich	Cat# D9891
DNase I	Fisher Scientific	Cat# EN0521
Fetal bovine serum (FBS)	Thermo Fisher Scientific	Cat# 9014-81-7
RPMI	Corning	Cat# 10-040-CV
DPBS	Corning	Cat# MT21031CV
0.25% Trypsin EDTA	Corning	Cat# 25-053-CI
OmniPur® BSA	Sigma-Aldrich	Cat# 2930-100GM
DOX Diet	Bio-Serv	Cat# S3888
Penicillin-streptomycin 100 \times	Corning	Cat# 63936-85-6
Critical commercial assays		
Mouse Tumor Dissociation kit	Miltenyi Biotec	Cat# 130-096-730
EasySep™ Mouse ILC2 Enrichment Kit	STEMCELL Technologies	Cat# 19842
Experimental models: Cell lines		
mouse PDAC cell line (AK-B6-RFP-luc)	From Ron DePhino's lab	PMID:32046984
Experimental models: Organisms/strains		
C57BL/6j mice	The Jackson Laboratory	Cat# JAX: 000664 RRID: IMSR_JAX:006362

(Continued on next page)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and algorithms		
FlowJo	FlowJo LLC	N/A
IVIS Spectrum Imaging System	PerkinElmer	N/A
Leica	Leica Microsystems	N/A
Other		
Mouse surgical kit	Roboz Surgical Instrument Co	N/A
Centrifuge	Eppendorf	Cat# 5810R
Tissue culture plates	Corning	Cat#353003
Heating pad	Braintree Scientific	Cat#50-195-4002
Surgical lamp	AmScope	Cat# HL250-AY
Laminar air flow cabinet	Thermo Fisher Scientific	Cat# 13-261-222
Surgical blade	Personna	Cat# 94-0120
Millex-GP Syringe Filter Unit	Millipore	Cat# SLGP033RS
Surgical wound clips Reflex 7	CellPoint Scientific	Cat# 203-1000
Stoelting™ Dry Glass Bead Sterilizer	Fisher Scientific	Cat# 10-000-632
Surgical suture Vicryl	Ethicon	Cat# J492G

MATERIALS AND EQUIPMENT

Miltenyi mouse tumor digestion buffer

Reagent	Final concentration	Amount
RPMI	N/A	2.7 mL
Enzyme D	N/A	100 μ L
Enzyme R	N/A	50 μ L
Enzyme A	N/A	12.5 μ L
Total	N/A	2.865 mL

Store at -20°C and use within 6 months.

Homemade tumor digestion buffer

Reagent	Stock concentration	Amount
RPMI or MEM	N/A	2.12 mL
Collagenase	1 mg/mL	357 μ L
Dispase	1 U/mL	357 μ L
DNAase	1 mg/mL	28.62 μ L
Total	N/A	2.862 mL

Store at -20°C and use within 6 months. Please, aliquot to avoid repetitive freeze-thaw cycles.

STEP-BY-STEP METHOD DETAILS

Preparation of mPDAC cells for orthotopic transplantation

⌚ Timing: 1 h

1. All cell culture procedures should be done in sterile conditions.
2. One day before orthotopic transplantation, seed the mPDAC luciferase expressing cells at a density of 5×10^6 cells in a T-75 tissue culture flask and add 15 mL PDAC culture medium. This will ensure that the cells are in the log phase of their growth and are critical for successful transplantation. Grow cells in the incubator at 37°C , 5% CO_2 for 24 h.

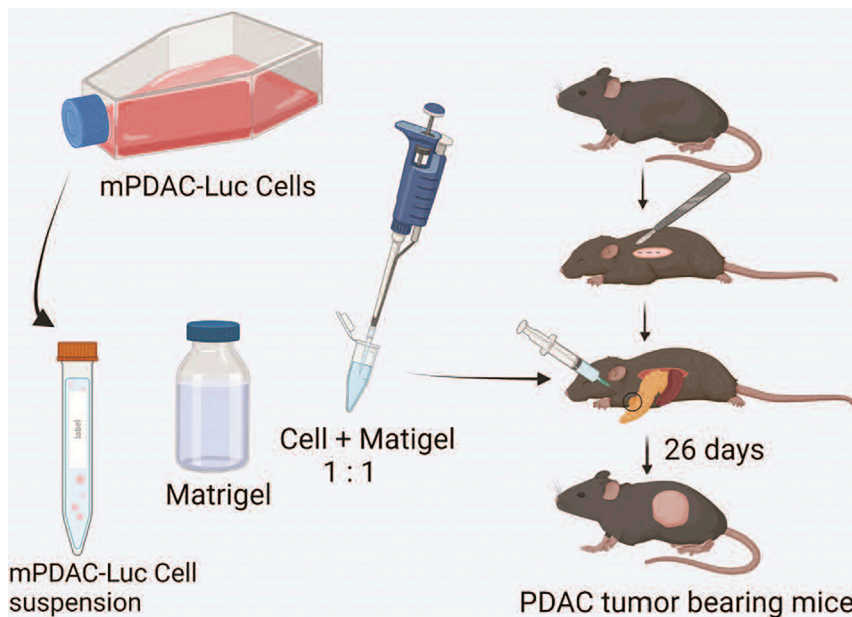


Figure 1. Schematic presentation of major steps of orthotopic transplantation of PDAC cell lines into mouse pancreas

On the day of the transplantation, mouse PDAC cells are harvested, washed, counted, and resuspended to obtain a desirable concentration. Cells are mixed with Matrigel in equal ratios and 20 μ L of the mix is injected into the pancreas of the recipient mouse.

3. On the day of surgery, harvest the PDAC cells by adding 5 mL of 0.25% trypsin-EDTA solution to T-75 tissue culture flask and incubate for 5 min at 37°C. Make sure the culture media containing 10% FBS is completely removed, and the cells are washed twice with PBS before adding trypsin-EDTA solution.
4. Stop the trypsin activity by adding 8 mL of cell culture media containing 10% FBS.
5. Transfer the cell suspension to a 15 mL tube and centrifuge the cells at $300 \times g$ for 5 min at 4°C.
6. Discard the supernatant and resuspend the cell pellet in the residual media by gentle tapping. Wash the cells three times with 15 mL sterile PBS to remove any residual FBS.

Note: Any FBS contamination in cells can initiate an immune response in mice and can reduce the efficiency of orthotopic transplantation.

7. Centrifuge the cells at $300 \times g$ for 5 min at 4°C after every PBS wash.
8. Count the cells using hemocytometer or automated cell counter.
9. After cell counting, resuspend the cells at a concentration of 5×10^7 cells/mL and aliquot 50 μ L in 1.5 mL centrifuge tubes.
10. Thaw the Matrigel overnight at 4°C.

Note: Matrigel takes at least 4–5 h to thaw. Keeping it at 4°C overnight is a preferred method to save some critical time on the day of surgery. Matrigel should always be kept on ice until used for injection.

11. Mix the cells and Matrigel at 1:1 ratio (50 μ L cells and 50 μ L Matrigel) (Figure 1).

Note: Mix cells and Matrigel just before the surgery to avoid solidification of Matrigel in the syringe. If the mix is solidified, do not attempt to forcefully inject the mix.

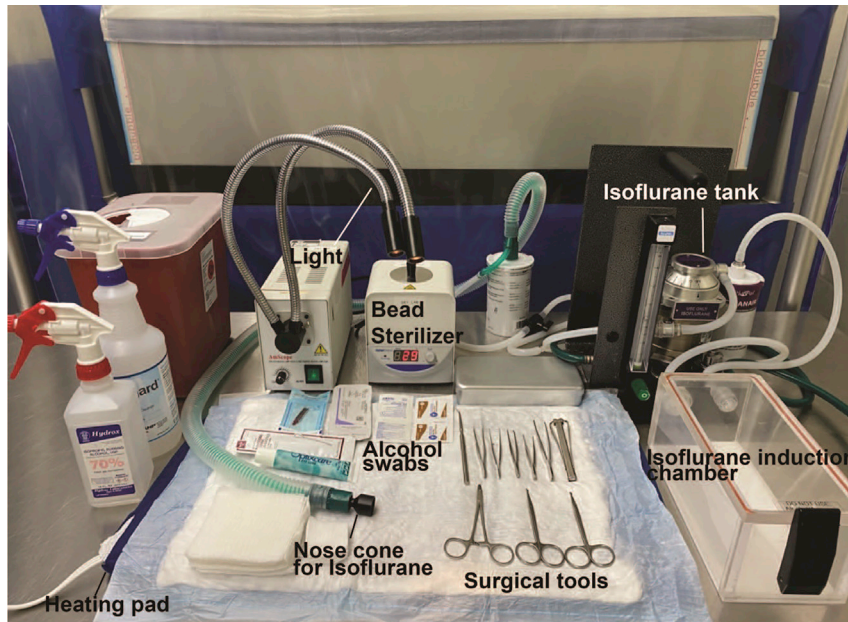


Figure 2. Picture showing the setup of the surgical area required for orthotopic PDAC cell transplantation in mice. Surgery table is equipped with a heating pad, light source, isoflurane delivery system, ventilation tubes, gas scavenger, Hamilton syringe for injection, and surgical instruments. Surgical instruments include a needle holder, a scalpel, scissors, forceps, and skin clamps.

Surgery

⌚ Timing: 1 h

12. Set up the surgical table with all the surgical instruments (Figure 2).
 - a. Perform the surgery in a sterile location such as bio-bubble (bioBUBBLE Inc., custom made soft-wall containment environment) to avoid any potential infection in mice.
 - b. Start by cleaning the work area with 70% isopropyl alcohol.
 - c. Prepare the surgery area by placing a heating pad which is covered with a clean drape.
13. Anaesthetize either male or female C57BL/6 mouse (aged 4–6 weeks) in an induction chamber of an isoflurane vaporizer set at 2%–3% flow rate of 0.8–1.0 liter/min.
14. Before starting the surgery:
 - a. Apply eye ointment to prevent the animal's eyes from drying out during surgery and recovery.
 - b. Check for the absence of the withdrawal reflex on a foot pinch to ensure successful anesthetization of mice.
 - c. Clean the surgical area with betadine.
 - d. Make sure to use sterilized surgery tools.
15. Place the mouse on a heating pad to maintain a constant body temperature of 37°C during the surgery.
16. Shave a 4 × 4-cm² portion of the left side of the abdomen. Alternatively, the mice can be shaved a day earlier to expedite the surgery (Figure 3A).
17. Sterilize the shaved region by applying betadine solution and then followed by 70% ethanol wipes to remove the betadine stain (Figure 3B).
18. Make an incision at splenic silhouette, located just below the rib cage and gently expose the pancreas along with the spleen using a blunt end tweezer (Figure 3C).
19. Inject twenty microliters of mPDAC-luc cells (5×10^5 cells) mixed with Matrigel (refer to step 10 for mixing of cells and Matrigel) using a Hamilton syringe.

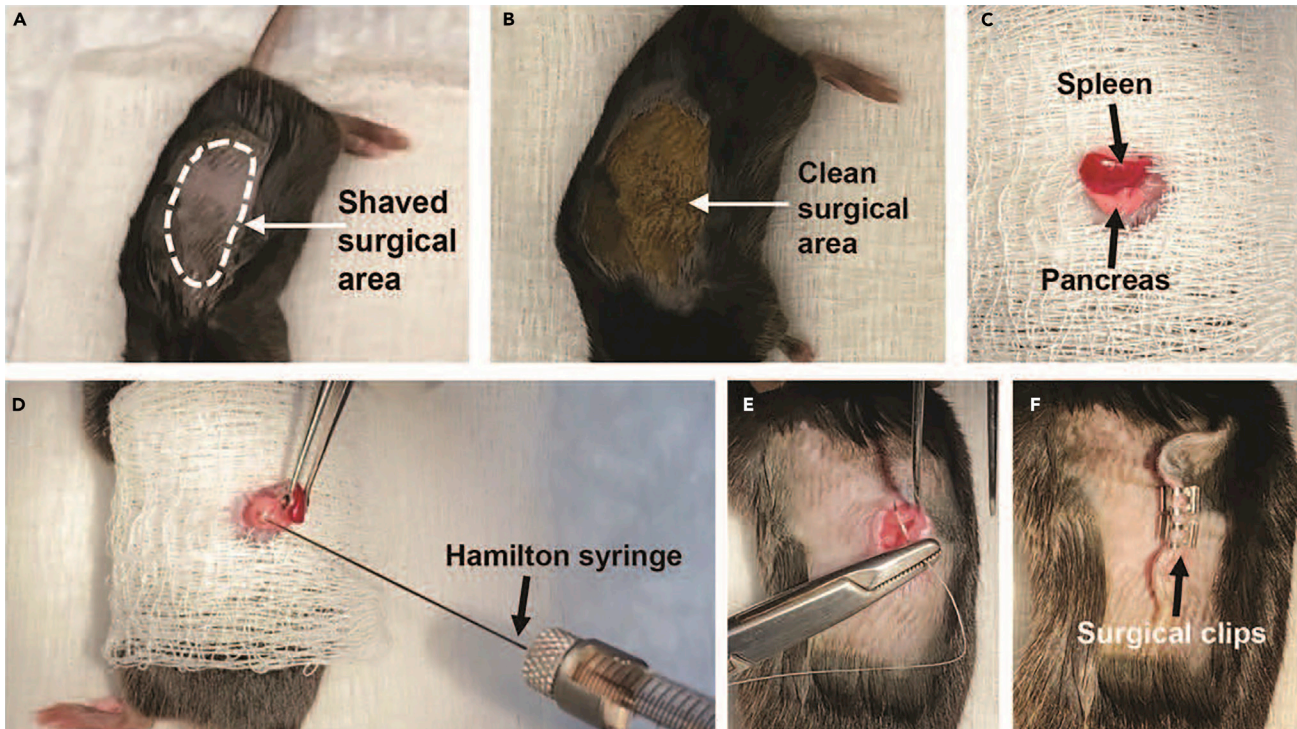


Figure 3. Steps followed for successful surgery and orthotopic transplantation of PDAC cells into the pancreas of mice

(A) Shave a 4 × 4-cm² portion of the left side of the mouse abdomen. (B) Cleaning of surgical area. (C) Expose the pancreas along with the spleen using a blunt end tweezers. (D) Inject mPDAC-luc cells slowly into the tail of the pancreas using a Hamilton syringe. (E) Suture the peritoneal membrane using sterile 6-0 (polyglactin 910) absorbable suture. (F) Close the the outer skin with 2–3 surgical clips.

- a. Inject mPDAC-luc cells slowly into the tail of the pancreas using a Hamilton syringe (Figure 3D).
- b. After injecting, wait for approximately 30 sec to let the Matrigel to solidify to avoid any leakage before pulling out the syringe. Then gently push the spleen and pancreas into the peritoneal cavity with the help of a surgical probe.
20. Suture the peritoneal membrane using sterile 6-0 (polyglactin 910) absorbable suture (Vicryl) (Figure 3E).
21. Clip the outer skin with 2–3 surgical clips (Figure 3F).

△ **CRITICAL:** It is important that the animals be closely monitored by the researchers while induction of anesthesia, to allow for appropriate removal of the animals from the induction chamber to prevent over-anesthetization and death.

22. Inject mice subcutaneously with buprenorphine at a concentration of 0.05 mg/kg weight subcutaneously.

Note: if the animal looks dehydrated, inject 100 μL saline solution subcutaneously.

23. Image animals by bioluminescence (IVIS Spectrum, PerkinElmer) 2 days after surgery to assess proper implantation of the tumors (Figure 4).

Tumor digestion and ILC2 enrichment

Ⓞ **Timing:** 2 days

This part of the protocol describes the isolation of ILC2s from the orthotopic PDAC tumors.

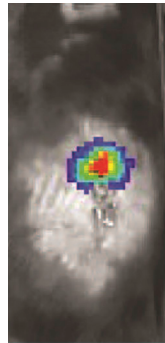


Figure 4. Bioluminescence image showing presence of PDAC cells in the mouse pancreas on day 2 after orthotopic transplantation

24. Twenty-six days after tumor transplantation, euthanize mice by CO₂ asphyxiation, isolate the pancreas, and dissect the tumor (Figure 1).

Note: keep the dissected tumor in RPMI or MEM media with 10% FBS on ice before the digestion.

25. Digesting the tumor:
 - a. Place the PDAC tumor measuring up to 10 cm in diameter in a Petri dish and add 1 mL of prepared digestion buffer (see [materials and equipment](#) section).
 - b. Use a surgical blade to mince and homogenize the tumor.

Note: We found it works best to hold the surgical blade with hemostat scissors.

- c. Add more digestion buffer (according to the size of the tumor) and collect into a 50 mL tube.

Notes:

 - i. We add at least 2.862 mL of digestion buffer to tumor weighing less than 1 g and add 5-10 mL digestion buffer if the tumor is more than 1 g.
 - ii. We recommend bevel cutting the end of a 1 mL pipette tip for easier collection of the minced tumor.
- d. Incubate the digested cell suspension at 37°C in water bath for 30 min.

Note: You could also incubate it at 37°C in a rotor at a speed of 6 rpm for 30 min to further enhance the digestion.

- e. After incubation, add 15 mL RPMI media and keep the tube on ice for 10 min to stop the digestion.
- f. Filter the digested tumor with 70 μm cell strainer:
 - i. Using the back end of the plunger of a 10 cc syringe (BD) gently grind the digested tumor in a circular motion.
 - ii. Keep adding culture media (RPMI + 10% FBS and 100 U/mL penicillin and 100 U/mL Streptomycin) while doing this step to increase the cell viability and avoiding over digestion.
- g. Wash the cell suspension with 5 mL wash buffer (PBS + 2% FBS + 100 U/mL penicillin and 100 U/mL Streptomycin + 1 mM EDTA) three times followed by centrifugation at 300 × g, 4°C for 5 min.
- h. Resuspend cells after washing in 10 mL of wash buffer.

Alternatives: Prepare single-cell suspension using the mouse tumor dissociation kit (Miltenyi Biotec, Cat No. 130-096-730) according to [manufacturer's instructions](#).

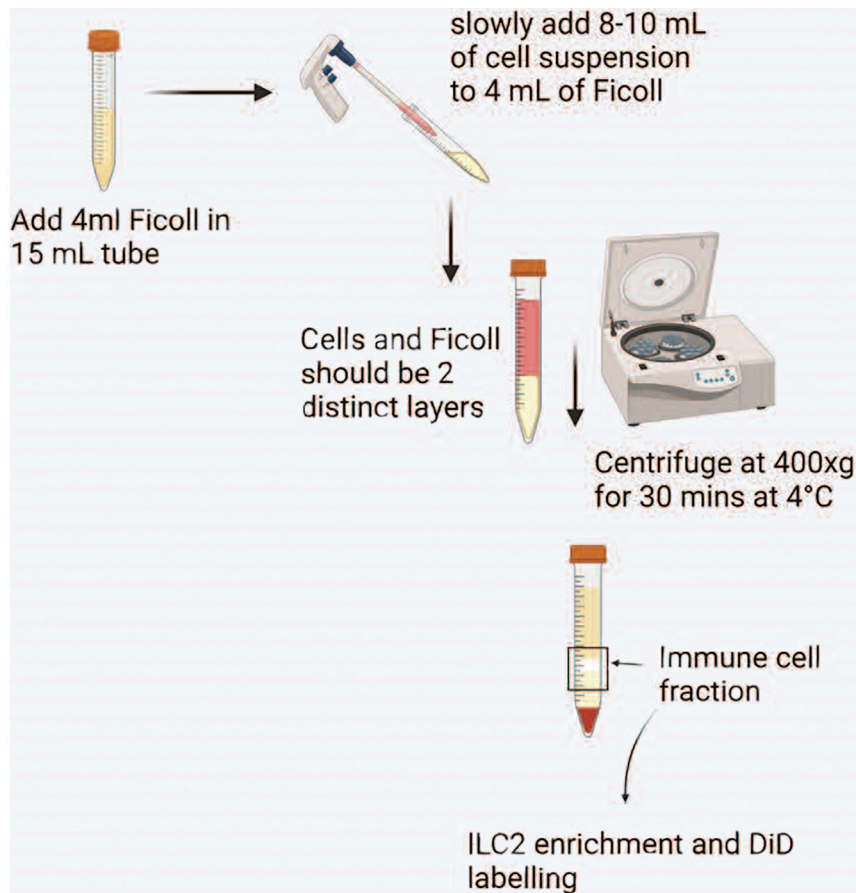


Figure 5. Schematic showing Ficoll column preparation and separation of tumor infiltrating lymphocytes (TIL) from PDAC

26. Isolate immune cells using a Ficoll-Paque™ column:

- a. Prepare the Ficoll column by adding 4 mL of Ficoll plaque (density 1.077 g/mL) to 15 mL tubes, followed by the PDAC tumor cell suspension in the ratio of 1:2 (Ficoll: Cell suspension) (Figure 5).

Note: To avoid mixing of Ficoll and tumor cell suspension, gently add cells to the Ficoll column at a 45-degree angle.

Note: Two separate layers can be visualized if there is good transfer of tumor cell suspension to the Ficoll Column.

- b. Centrifuge for 30 min at $400 \times g$ at 4°C.
- c. Collect the interface between 2–6 mL of graduated tubes, consisting of tumor infiltrating leucocytes.
- d. Wash the cells 3 times with wash buffer (see step 24g) by centrifugation at $300 \times g$, 4°C for 5 min.

△ CRITICAL: Avoid mixing of Ficoll and tumor cell suspension, otherwise the immune cell fraction will not be separated. Washing after Ficoll column separation is critical to avoid any residual Ficoll which can reduce the efficiency of ILC2 enrichment. Count the cells and prepare the adequate concentration which is equivalent to 1×10^8 Cells/ mL for ILC2 enrichment as per [manufacturer's protocol](#).

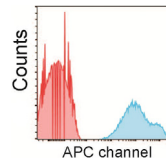


Figure 6. Histogram showing DiD labeled ILC2 compared to unlabeled cells via flow cytometry analysis

The plot is overlay plot of control vs DiD stained ILC2 cells. The red pick indicates the unstained and the blue indicate stained ILC2 cells.

27. Enrich ILC2s using an enrichment kit (STEMCELL Technologies, Cat No. 19842) as follows.
 - a. Approximately 1×10^8 cells/mL are taken in a 5 mL polystyrene round-bottom tube (Cat. No. 38007).
 - b. Add 50 μ L/mL of the ILC2 enrichment cocktail to the tube. Mix and incubate the mixture for 5 min at 20°C–22°C.
 - c. Meanwhile, vortex the Streptavidin Rapidspheres for 30 s. The Rapidspheres particle should appear evenly dispersed. Then add 75 μ L /mL of the Rapidspheres to the sample and incubate for 5 min.
 - d. After incubation add 2.5 mL of the RPMI 1640 media to the sample and mix by pipetting up and down 2–3 times.
 - e. Place the sample mix into the magnet and incubate for 3 min at 20°C–22°C.
 - f. Pick up the magnet, and in one continuous motion invert the magnet and tube, pouring the enriched cell suspension into a new 14 mL tube.
 - g. Repeat steps *d-f*. Then combine with poured-off fraction from step *f*.
 - h. Isolated cells are ready for use.
28. Culture the enriched ILC2s in 10 cm culture plate in 10 mL ILC2 culture media (see [materials and equipment](#) section) containing 10 ng/mL IL-2 and IL-7 overnight at 37°C with 5% CO₂.
29. Next day, transfer the cells to 15 mL conical tube and collect the cells by centrifugation at 300 \times g for 10 min at 4°C. Wash the ILC2 cells with PBS three times with centrifugation at 300 \times g for 5 min at 4°C. The ILC2 are now ready for DiD dye labeling for adoptive transfer.

Adoptive transfer and characterization of ILC2s

⌚ Timing: ~17 days

This part of the protocol describes the adoptive transfer of the tumor-derived ILC2s to PDAC-bearing syngeneic recipient mice.

30. Establish orthotopically transplanted tumors as described above (see sections [preparation of mPDAC cells for orthotopic transplantation](#) and [surgery](#)) in a new group of mice. These mice will be the recipients of the adoptive transfer. We wait a period of 10 days after injecting the tumor cells before we initiate the adoptive transfer of ILC2.
31. Before the adoptive transfer, label the ILC2 cells with a lipid binding DiD' solid (1,1'-Dioctadecyl-3,3,3',3'-Tetramethylindodicarbocyanine, 4-Chlorobenzene sulfonate Salt ([Figure 6](#)) [Invitrogen, Cat No. D7757] other source is specified in [key resources table](#)) dye:
 - a. Prepare 20 mM DiD stock solution by dissolving DiD dye in DMSO and store at –20°C.
 - b. Label 1×10^6 ILC2 cells with DiD dye at a concentration of 5 μ M working solution for 10 min in 1 mL PBS at 20°C–22°C.
 - c. Wash DiD labeled ILC2 with wash buffer (PBS + 2% FBS + 100 U/mL penicillin and 100 U/mL Streptomycin+ 1 mM EDTA) and centrifuge at 300 \times g for 5 min at 4°C.
 - d. Repeat the washing step two times for a total of three washings.
 - e. Resuspend cells in 1 mL PBS and wash cells by centrifugation at 300 \times g for 5 min at 4°C.

- f. Resuspend DiD labeled ILC2 cells at a concentration of 1×10^6 cells/mL in 1 mL PBS.
32. Deliver $100 \mu\text{L}/1 \times 10^5$ of labeled ILC2 cells in PBS via retro – orbital injection into recipient mice.
33. After 7 days, harvest the tumor and repeat steps (24–29) from *Section: tumor digestion and ILC2 enrichment*, to digest the tumor and isolate ILC2s.
34. Analyze ILC2 by flow cytometry using the APC channel to detect DiD-labeled ILC2.

EXPECTED OUTCOMES

The orthotopic transplantation of PDAC cells into syngeneic host mice will yield a full-grown tumor by days 24–30. The tumor will then be harvested and ILC2 will be enriched for subsequent adoptive transfer via retro-orbital injection into syngeneic PDAC-bearing recipient mice. We expect trafficking of the adoptively transferred ILC2 into the PDAC tumor of the recipient mouse. DiD labeling of ILC2 allows for the flow cytometry-based tracking and quantification of the adoptively transferred ILC2 cells in the recipient PDAC tumor.

LIMITATIONS

The protocol is tested and optimized for the isolation and enrichment of ILC2s from pancreatic tumors. Although, the basic steps for ILC2 isolation and enrichment can be applied to any tissue, but it is highly recommended, that tissue specific differences are considered as individual labs establish the protocol in their labs. Also, the frequency of the ILC2 might differ based on the origin of the tumors, so donor ILC2 required for adoptive transfer might be a challenge. Also, there is very high amount of tissue specific heterogeneity in ILC2 population, so ILC2s sourced from different tumors must be tested with suitable markers to ascertain the purity and phenotype of the ILC2s.

TROUBLESHOOTING

Problem 1

Failed PDAC orthotopic transplantation as described in steps 11–20.

Potential solution

Cell leakage during injection is often the cause for failure of transplantation. Injecting a smaller volume between 10–20 μL of PDAC cells and Matrigel mixture will prevent leakage. Upon injection of the PDAC cells into the pancreas, the cell mix should form a small but a distinct and visible bubble. Wait for a minute to let the Matrigel solidify before pulling out the syringe. After injection, gently push the pancreas with the help of a blunt probe.

Problem 2

Low viability of PDAC tumor cells after digestion as described in steps 23 and 24.

Potential solution

Pancreas naturally contains digestive enzymes, which decrease the viability of tumor cells during harvesting. To increase the viability of the PDAC tumor cells, each step needs to be done on ice. After harvesting the tumor, keep the tumor in RPMI or MEM media with 10% FBS. If doing manual method, wash the tumor with PBS and chop the tumor finely before digestion. After digestion, pass the cell suspension through a 70 μm cell strainer. While filtering, keep adding complete media to stop the enzymatic reaction and increase cell viability.

Problem 4

Ficoll column might not separate leucocytes from tumor as described in step 25.

Potential solution

To avoid mixing of Ficoll and tumor cells, gently add cells to the Ficoll column at a 45-degree angle. Two separate layers can be visualized if there is good transfer of tumor cell suspension. Centrifuge at

400 × g, 30 min at 4°C. Set the centrifuge brake at zero and acceleration at 5 to avoid any agitation during centrifugation.

Problem 5

Contamination of other type of cells after ILC2 enrichment as described in step 26.

Potential solution

ILC2 enrichment kit from STEMCELL Technologies (Cat. 19842) works very efficiently, but do not provide 100% pure ILC2 population. To minimize contamination from other cell type, overnight culture of enriched ILC2 cells will help removal of the contamination as non-ILC2 cells tends to stick to the tissue culture dish compared to ILC2 which will mostly be in suspension in the media. Collect the supernatant and proceed to labeling with DiD and adoptive transfer. Alternatively, ILC2 enrichment kit from Miltenyi Biotec can be used.

Problem 6

Dead cells and debris in tumor cell suspension as described in step 25.

Potential solution

Dead cells and debris are potential contaminant in the single cell suspension of tumor. Dead cell removal kit (Cat No. 130-090-101) and debris removal kit (Cat No. 130-109-398) from Miltenyi Biotec can enhance the viability and enrichment of ILC2.

Problem 7

RBC contamination of the ILC2 preparation as described in step 25.

Potential solution

RBC contamination is often found in harvested PDAC tumors. RBC lysis using RBS lysis buffer (BioLegend, Cat No. 420301) using manufactures standard protocol, after single cell suspension preparation will solve the problem and it will increase the efficiency of ILC2 enrichment kit.

Problem 8

Retro-orbital injection as described in step 32.

Potential solution

Retro-orbital injection will require practice and some expertise. A lot of precision is required, while injecting cells make sure the cells do not leak out of eye or through nose. Alternatively tail vein injection can also be performed for ILC2 transplantation.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Prasenjit Dey (prasenjit.dey@roswellpark.org).

Materials availability

This study did not generate new unique reagents.

Data and code availability

This study did not generate/analyze datasets and code.

ACKNOWLEDGMENTS

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In addition, this study was supported by 5R00CA218891-04 (P.D.); 1R01CA262822-01 (P.D.), The Roswell Park Alliance Foundation-62-2839-01 (P.D.). [Figures 1](#) and [5](#) were created with [BioRender.com](#).

AUTHOR CONTRIBUTIONS

Conceptualization, P.D.; Methodology, A.A., S.C., and E.L.; Investigation, A.A., S.C., and E.L.; Writing–Review & Editing, P.D., A.A., S.C., and E.L.; Funding Acquisition, P.D. Supervision, P.D.

DECLARATION OF INTERESTS

P.D. and A.A. have a patent pending on targeting IL33 and mycobiome in cancer. U.S. Provisional Patent Application Serial No. 63/238,531.

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