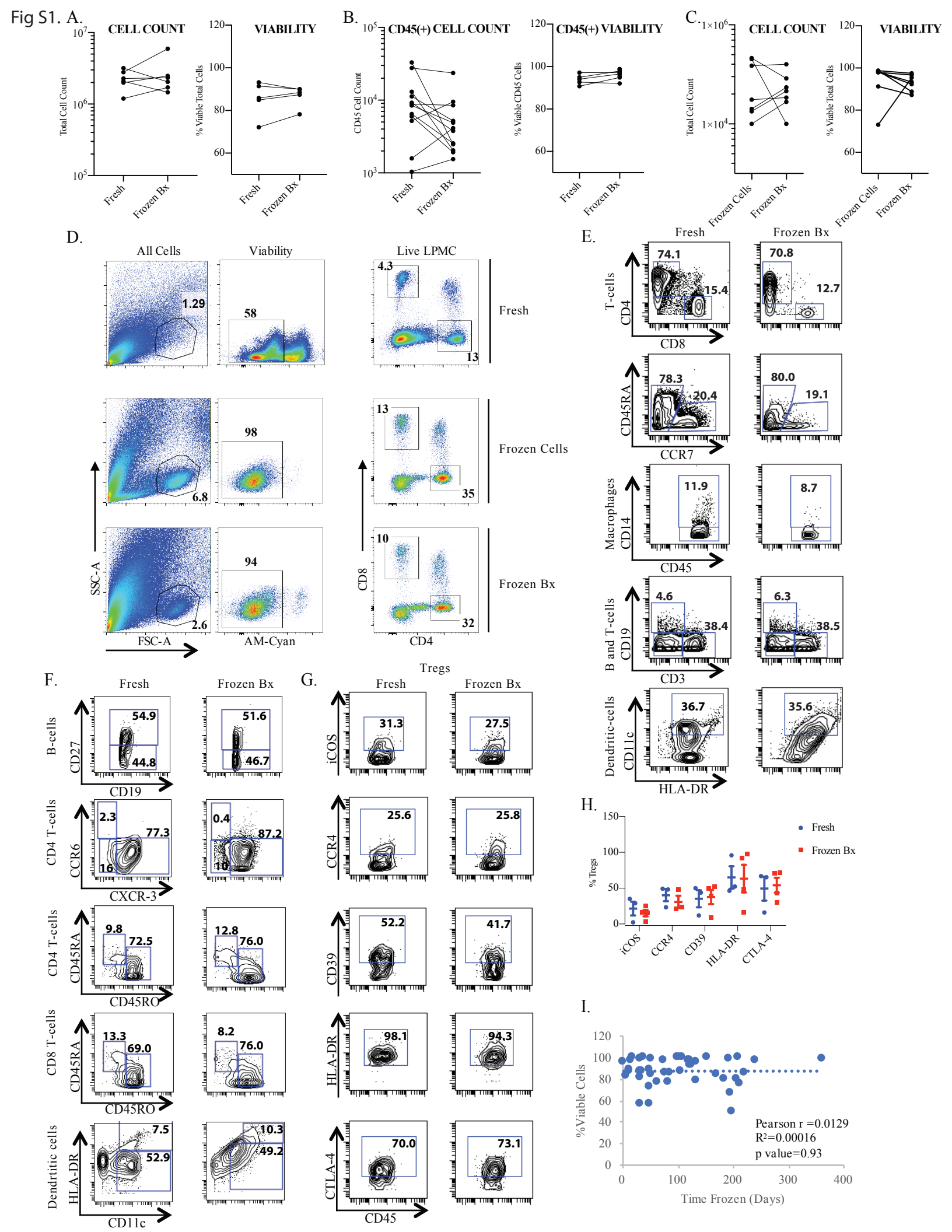
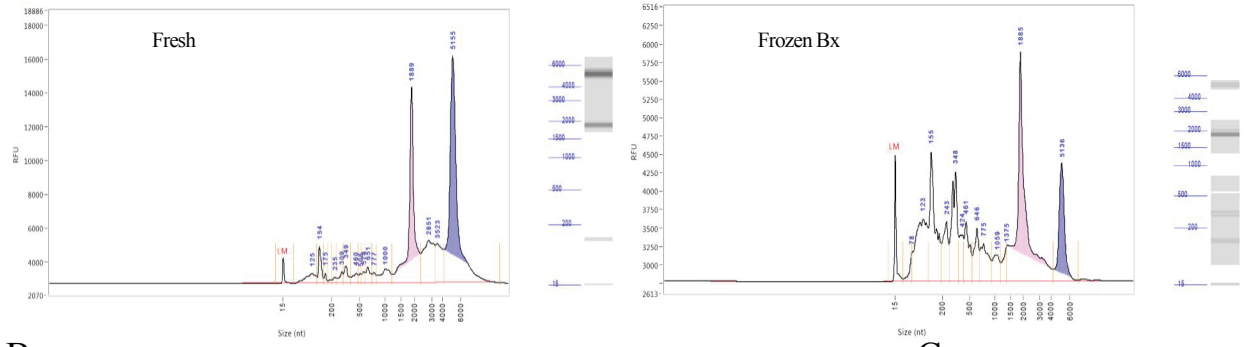
Fig S1.


A.
CELL COUNT
Total Cell Count
Fresh
Frozen Bx
VIABILITY
% Viable Total Cells
B.
CD45(+) CELL COUNT
CD45 Cell Count
CD45(+) VIABILITY
% Viable CD45 Cells
C.
CELL COUNT
Total Cell Count
Frozen Cells
VIABILITY
% Viable Total Cells
D.
All Cells
Viability
Live LPMC
1.29
58
4.3
13
Fresh
98
6.8
13
35
Frozen Cells
94
2.6
10
32
Frozen Bx
SSC-A
FSC-A
AM-Cyan
CD8
CD4
E.
Fresh
Frozen Bx
T-cells
CD4
CD8
74.1
15.4
70.8
12.7
CD45RA
CCR7
78.3
20.4
80.0
19.1
Macrophages
CD14
CD45
11.9
8.7
B and T-cells
CD19
CD3
4.6
38.4
6.3
38.5
Dendritic-cells
CD11c
HLA-DR
36.7
35.6
F.
Fresh
Frozen Bx
B-cells
CD27
CD19
54.9
44.8
51.6
46.7
CD4 T-cells
CCR6
CXCR-3
2.3
77.3
16
0.4
87.2
10
CD4 T-cells
CD45RA
CD45RO
9.8
72.5
12.8
76.0
CD8 T-cells
CD45RA
CD45RO
13.3
69.0
8.2
76.0
Dendritic cells
HLA-DR
CD11c
7.5
52.9
10.3
49.2
G.
Tregs
Fresh
Frozen Bx
iCOS
31.3
27.5
CCR4
25.6
25.8
CD39
52.2
41.7
HLA-DR
98.1
94.3
CTLA-4
CD45
70.0
73.1
H.
% Tregs
Fresh
Frozen Bx
iCOS
CCR4
CD39
HLA-DR
CTLA-4
I.
%Viable Cells
Pearson r =0.0129
R²=0.00016
p value=0.93
Time Frozen (Days)

A. Fig S2



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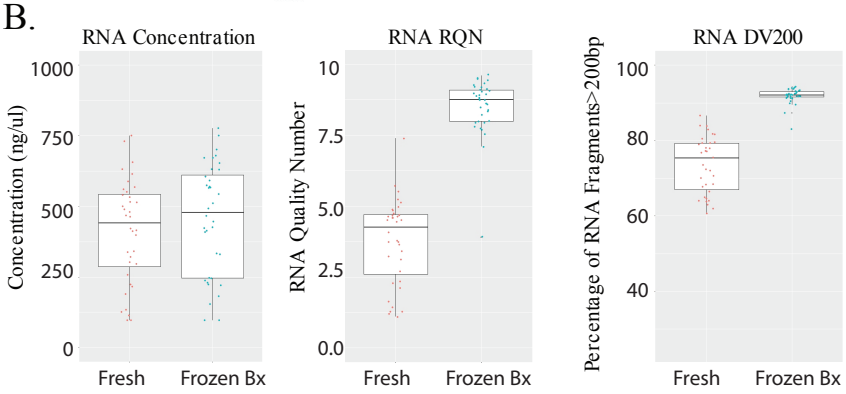

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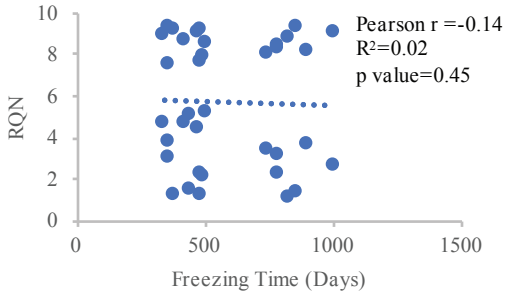

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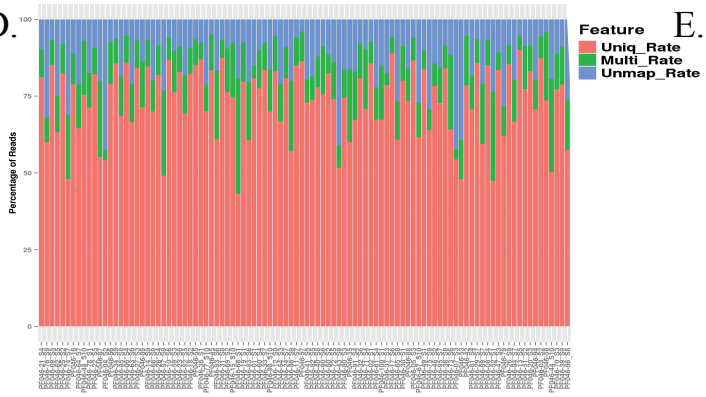

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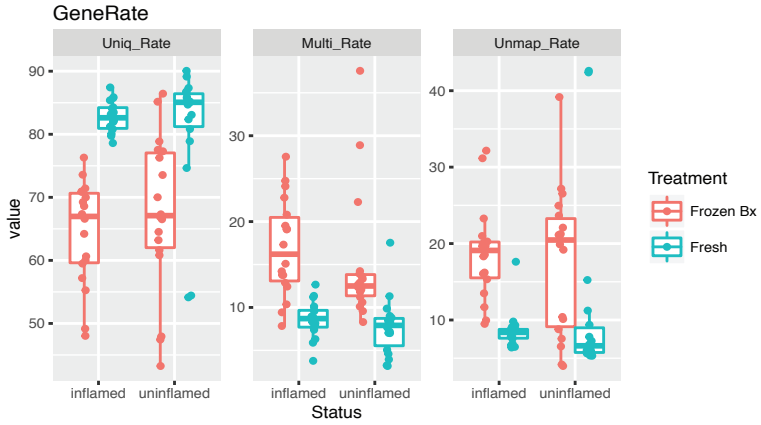
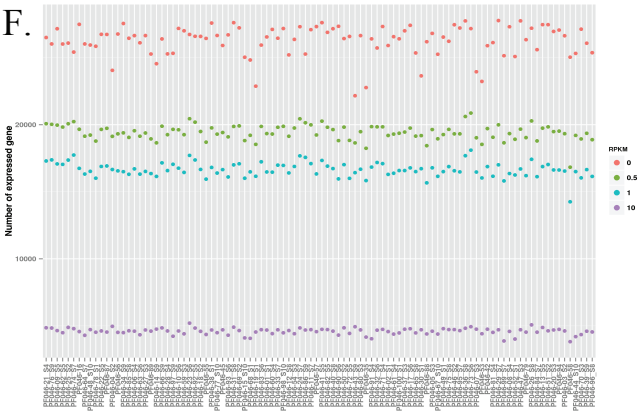


F.



G.

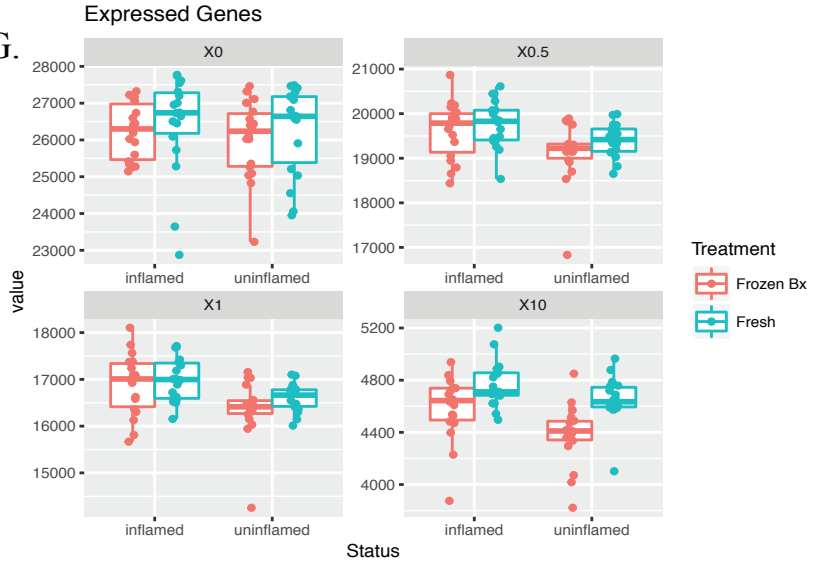


Figure S1. ***Gastrointestinal tissue can be cryopreserved with retention of cell viability.*** Comparison of (A) total cells and (B) $CD45^+$ cell numbers and viability of matched biopsies processed "fresh" or "frozen Bx". Matched samples comparing total cell numbers obtained from cells frozen as LPMCs "frozen cells" or frozen tissue "frozen Bx" (C). Gating strategies for the FACS analysis of $CD4^+$ and $CD8^+$ T-cells after various processing methods indicated (D). 2-D plots obtained from CyTOF data using the general panel of the various populations indicated (E). T-cells were gated on viability/$CD45^+$/$CD3^+$. Tregs: viability/$CD45^+$/$CD3^+$/$CD4^+$/$CD25^+$/$CD127^-$. Macrophages: viability/$CD45^+$/$CD3^-$/$CD19^-$. Dendritic cells: viability/$CD45^+$/ $CD3^-$/$CD19^-$/$CD14^-$. B- cells: viability/$CD45^+$/ $CD3^-$/$CD19^+$. 2-D plots obtained from CyTOF data using the general panel of subpopulations indicated (F). (G) shows the expression of the various markers indicated on Tregs (viability/$CD45^+$/ $CD3^+$/$CD4^+$/$CD25^+$/$FOXP3^+$) on the left-hand side and quantified (H) on the right. Cytokine panel used for staining. **(I) Correlation between percent of viable cells and the duration of freezing in days. Numbers on 2-D plots represent percentage of cells in gate**. *=p value <0.05

Figure S2. ***Maintenance of unique gene expression patterns between inflamed and uninflamed tissue with cryopreservation*** Comparison of RNA obtained after storage in RNAlater or DMSO. (A and B) quantification of quality of RNA obtained from storage in RNAlater or DMSO. **(C) Correlation between RQN and duration of freezing in days.** (D-H) Gene expression and mapping by the two methods.

SUPPLEMENTARY METHODS

Tissue procurement

Boston Two to four 2mm x 2mm biopsies per area were collected using either adult or pediatric biopsy forceps. Sections of discarded surgical tissue were collected from partial or full colonic resections. The muscle layer was removed and the remaining mucosa was cut into 2mm x 2mm pieces. All biopsies and tissue samples were collected in T-cell media (RPMI 1640 medium, 50 mL FBS plus 5 mL of each: Pen/Strep, NEAA, sodium pyruvate, Glutamax, HEPES, 50 mL FBS, 5 mL Pen Strep) and were stored on ice until cryopreservation or LPMC isolation.

MSSM Directly after surgical resection, two pieces of ileum (4 cm square each), one from the inflamed and one from the non-inflamed gut section were cut by a pathologist. The pieces were put in RPMI 1640 medium supplemented with penicillin and 10% fetal bovine serum (FBS) at 4°C and immediately processed for immunological analysis. Mucosal biopsies were performed from each piece with endoscopic forceps, pooled and incubated for 30 min at 37°C in dissociation medium (HBSS w/o Ca++ and Mg++, containing 5 mM EDTA and 10mM HEPES) in order to remove epithelial cells.

Penn Two biopsies per patient were obtained from the colon using a 2.4 mm biopsy forceps and were placed in 10 ml cold phosphate buffered saline (PBS). The samples were stored on ice and processed within 2 hours of collection.

Cryopreservation

Fresh biopsies, surgical tissue (4-5 pieces), and isolated LPMCs were slow frozen, resuspended in 1 mL of freeze medium (10% dimethyl sulfoxide (DMSO) (Sigma) and 90% fetal bovine serum (FBS)(Gibco), and promptly placed in a Nalgene Mr. Frosty freezing container (Sigma-Aldrich, St. Louis, MO) to freeze at a 1°C per minute cooling rate in a -80°C freezer. Samples were stored at -80°C for at least 90 minutes and transferred to liquid nitrogen for longterm storage. Upon thawing, LPMC isolation was carried out as described below.

For enteroid culture, fresh biopsies were collected in Advanced DMEM/F12 (Gibco) and transferred to 1 mL freezing medium and slow frozen as described above.

Penn: Cryopreservation of biopsies prior to LPMC isolation

Two biopsies per patient were obtained from the colon using a 2.4 mm biopsy forceps and were placed directly in a cryovial containing 1 ml freeze medium. The samples were then frozen and stored as described above.

LPMC Isolation of Fresh and Frozen Biopsies and Tissue Samples

LPMC Isolation (Boston) Cryovials containing frozen biopsies or surgical tissue were quickly thawed at 37°C. After removing freezing media, samples were washed in either T-cell media or –Ca, -Mg PBS (1:5000 collagenase and DNAse) then transferred to 20 mL of digestion media in 50 mL conical tubes. Fresh biopsies were transferred from T-cell media to digestion media. Conical tubes were secured horizontally to a shaker set to 200 rpm. Samples were digested overnight at 37℃ and then were resuspended and returned to the shaker for 1 hour. After digestion, the samples were vortexed for 30 seconds and then poured through a 70 µm cell strainer. The supernatant was then spun at 1400 rpm for 5 min to pellet the LPMCs.

LPMC Isolation (MSSM) Fresh biopsies were washed twice in complete RPMI and then incubated for 45 min at 37°C in digestion medium (HBSS with Ca++ Mg++ containing 2% FBS, 0.5mg/ml DNaseI and 0.5mg/ml Collagenase IV). The resulting cell suspension was filtered and washed with complete RPMI and cells collected were centrifuged, washed and resuspended in PBS.

LPMC Isolation (Penn) Cryovials containing frozen LPMCs were placed at 37°C until they began to thaw. The samples were transferred to 15 ml tubes containing 10 ml warm cell culture medium, centrifuged at 1200 rpm for 10 minutes at room temperature, and the pellet was washed with 10 ml warm cell culture medium. After the 2nd spin, samples were resuspended in 2 ml warm cell culture medium in 48-well plates, and rested overnight in a 37°C incubator with 5% CO2. For samples that had not been processed into LPMCs prior to freezing, LPMC isolation was carried out as described above after thawing, and then the cells were rested overnight.

Primary Human Enteroid Culture

Biopsies were collected in Advanced DMEM/F12 (Gibco) on ice. For fresh biopsy culture, the epithelium was immediately isolated by a 40 minute Collagenase Type 1 (Gibco) digestion at 37℃ followed by mechanical disruption of the tissue by a P1000 pipette tip. The isolated epithelium was suspended in Growth Factor Reduced Phenol Red Free Matrigel (Corning) and plated as 50 uL domes in a tissue culture-treated 24-well plate (Genesee). After the Matrigel was set at 37℃ for 10 minutes, the cultures were maintained in complete human small intestinal medium [For 100 mL: 50 mL NRW Conditioned Medium, 30 mL Advanced DMEM/F12 (Gibco), 1 mL Glutamax (Gibco), 1 mL Hepes (Gibco),1 mL Nicotinamide (1 M, Sigma), 1 mL B27 (Gibco), 500 uL N2 (Gibco), 200 uL Primocin (Invivogen), 200 uL Normocin (Invivogen), 100 uL N-Acetyl Cysteine (500 mM, Sigma),100 uL A-83-01 (0.5 mM, Sigma), 33.2 ul SB202190 (5 mg in 505 ul DMSO, Sigma), 10 uL EGF (500 ug/mL, Peprotech), 10 uL Gastrin (500 uM, Sigma)] at 37℃ with 5% CO2 as previously described (Sato et al.).

For frozen biopsy culture, the samples were rapidly defrosted at 37℃ and immediately cultured as described above. Media was changed every 3 days and cultures were passaged as necessary, every 7 to 10 days. Passaging was performed by incubating with Cell Recovery Solution (Corning) on ice for 40 minutes. The recovered enteroids were then suspended in the desired amount of

Matrigel and passed rapidly through the bent tip of a P1000 pipette tip to mechanically digest before replating. A primary enteroid culture was considered to be 'established' if able to be expanded to 12 robust wells after passage.

CyTOF analysis (Boston and MSSM)

Boston LPMC suspensions of 1.5-2 million cells per aliquot were labeled with Rh103 intercalator (Fluidigm) for 20 minutes at room temperature to label non-viable cells, and washed in Cytof Staining Buffer (Ca-,Mg- PBS, NaN3, 2.5g Bovine Serum Albumin). The cells were then treated with Fc block for 10 minutes. The cells were either stained with the "general" panel of metal-labeled antibodies (0.5 µl/antibody/sample) for 30 minutes, or the cells were first stained with surface marker antibodies of the "cytokine panel" (0.5 µl/antibody/sample) for 30 minutes, and then washed twice in CSB and fixed with Foxp3 Fix/Perm (Biolegend) for 45 minutes. These fixed cells were washed twice in Foxp3 wash buffer (Biolegend) and stained with the intracellular antibodies of the "cytokine" panel (0.5 µl/antibody/sample). After staining with either the "general" panel or intracellular "cytokine" panel antibodies, the cells were washed twice with CSB and fixed with 1.6 % PFA in CSB for 10 minutes. The cells were then stored in CSB at 4°C until they were ready to run. Immediately prior to the run, the CSB was removed and the cells were incubated with 0.125uM Ir intercalator 1:1000 in CSB (Fluidigm) for 20 minutes. The cells were then washed twice in CSB and twice in deionized (MilliQ) water, and then resuspended at a concentration of 1 million cells/mL in deionized (MilliQ) water containing a 1:10 dilution of EQ 4 Element Beads (Fluidigm). The samples were run on a CyTOF2 (Fluidigm) equipped with a SuperSampler fluidics system (Victorian Airships) at an event rate of <500 events/second.

MSSM Tissue cell suspensions were first labeled with Rh103 intercalator (Fluidigm) for 20 minutes at 37°C to label non-viable cells, and then washed and labeled with a panel of metal-labeled antibodies and incubated for 30 mins on ice. The samples were then fixed and permeabilized with BD Cytofix/Cytoperm (BD Biosciences) and incubated in 0.125nM Ir intercalator (Fluidigm) diluted in PBS containing 2% formaldehyde for 30 mins. The samples were then washed and stored in PBS containing 2% FBS at 4^{o}C until acquisition.

Immediately prior to acquisition, samples were washed once with PBS, once with de-ionized water and then resuspended at a concentration of 1 million cells/ml in deionized water containing a 1:20 dilution of EQ 4 Element Beads (Fluidigm). The samples were acquired on a CyTOF2 (Fluidigm) equipped with a SuperSampler fluidics system (Victorian Airships) at an event rate of <500 events/second.

Flow cytometry (Penn)

LPMCs that had been rested overnight were counted, centrifuged at 1200 rpm for 10 minutes at room temperature, and resuspended in 100 µl FACS buffer (PBS with 0.5% FBS) containing fluorophore-labeled antibodies. Staining was carried out at room temperature for 30 minutes. Antibodies used are documented in the Key Resource Table. Data analysis was performed using FlowJo (version 10.3) software (LLC, Ashland, OR). Dead cells were removed by gating on a LIVE/DEAD Aqua kit (Invitrogen, Carlsbad, CA) versus forward scatter (FSC-H).

RNAseq (Pfizer)

RNA was extracted from individual biopsies utilizing the Qiagen (Valencia, CA) miRNA kit and homogenized on a Bertin Precellys Homogenizer (Rockville, MD) system using ceramic beads. RNA concentration was assessed with the Nanodrop 8000 system (Wilmington, DE) and quality with Advanced Analytical's Fragment Analyzer (Ankeny, IA).
RNAseq was performed using the Truseq Total RNAseq kit with RiboZero (Illumina, San Diego CA). The resulting libraries were quantified and checked for quality using the Agilent TapeStation System (Santa Clara, CA). Libraries were pooled to equimolar concentrations and sequenced on a Nextseq 500 system (Illumina, San Diego CA) targeting 30 million 75bp reads (60 million paired reads) per sample. Sequencing run quality was assessed using Illumina's SAV software and demultiplexed with Illumina's bcl2fastq algorithm (Illumina, San Diego CA). Processing of the fastq files was performed using the QuickRNAseq[20] pipeline utilizing Hg38 for the genome and Gencode v24 for annotation.

QUANTIFICATION AND STATISTICAL ANALYSIS

CyTOF data analysis

CyTOF FCS files were normalized using bead-based normalization in the CyTOF software and uploaded to Cytobank for analysis. Data were visualized using viSNE, and major immune populations were gated based on canonical marker expression. Cell population frequencies were exported for comparisons across freezing conditions.

CyTOF and Flow Cytometry Quantification and Statistical Analysis (Penn, Boston, MSSM) Statistical parameters including number of biological replicates, data dispersion and precision measures (mean and SEM), and statistical significance by Student's T test appears in Figures and Figure Legends. Statistical analysis was performed using GraphPad Prism 7.

Enteroid Culture Analysis

Cultures were imaged using the EVOS FL Auto 2 (ThermoFisher). All representative images are from mature cultures after at least 2 passages. Scale bars were inserted using ImageJ.

RNAseq Analysis

RNAseq counts were normalized using the EdgeR algorithm and the voom function of limma [21] [22]. Differential analysis was performed using limma [22] to compare samples by inflammatory status and collection method. Genes with expression less than 10 counts per million (cpm) in all samples were removed from analysis, and genes with a p-value <0.001 and FC>+/-2 are reported as significant. Output of the limma analysis was used for functional enrichment analysis for KEGG pathways using the tmod R package v 0.31 (1, see below) and the output was visualized using ggplot2 v 2.2.1(2, see below)

1. https://cran.r-project.org/web/packages/tmod/index.html
2. https://cran.r-project.org/web/packages/ggplot2/index.html

Table of Contents

1. Purpose

1.1. This Standard Operating Procedure (SOP) includes procedures for tissue procurement, cryopreservation, thawing, lamina propria mononuclear cells (LPMCs) and epithelial cell isolation and utilization of LPMSc and epithelial cells from intestinal biopsies and tissue.

2. Background

2.1 Identification of human immune cell subsets and function underlining disease pathogenesis and response to therapy is a conundrum challenging personalized medicine approach to immune-mediated disorders. Detailed analyses of immune-mediated disorders affecting mucosal surfaces have been particularly difficult, as analyses have required processing of fresh tissue that is often time consuming and requires sophisticated laboratory techniques not universally available. Here, we present a simple universal method to immediately cryopreserve mucosal tissue that retains cellular viability, including immune and epithelial components.

3. Materials

3.1. Tissue Procurement

- 1 mL cryovials (Nalgene® SYSTEM 100™ Cryogenic Vials)
- Ice
- Ice container
- 50 mL conical tubes

3.2. Cryopreservation

- 1 mL cryovials (Nalgene® SYSTEM 100™ Cryogenic Vials)
- Pipette tips
- Curved scissors
- Nalgene Mr.Frosty with isopropanol (Sigma-Aldrich, St. Louis, MO), kept at room temperature between uses
- -80 °C freezer
- Liquid nitrogen freezer

3.3. Thawing Biopsies and tissue samples

- Pipette tips
- Vortex
- 37°C water bath
- 50 ml conical tubes
- Centrifuge

3.4. Primary human enteroid culture

- 1 mL cryovials (Nalgene® SYSTEM 100™ Cryogenic Vials)
- Pipette tips
- Ice
- 24 well plate (tissue culture treated) (Genesee)
- cell culture incubator
- centrifuge

3.5. RNAseq analysis of biopsy tissue

- Qiagen (Valencia, CA) miRNA kit
- Bertin Precellys Homogenizer (Rockville, MD)
- Nanodrop 8000 system (Wilmington, DE)
- Advanced Analytical's Fragment Analyzer (Ankeny, IA)
- Truseq Total RNAseq kit with RiboZero (Illumina, San Diego, CA).
- Agilent TapeStation System (Santa Clara, CA)
- Nextseq 500 system (Illumina, San Diego, CA)
- Pipette tips
- 15 mL conical tubes

3.6. LPMC isolation

- 37^{o}C Shaker
- 50 ml conical tubes
- 10 ml pipettes
- Centrifuge
- Vortex
- 70-micron filter
- 5 mL plastic syringe piston

4. Reagents/Solutions

4.1. Tissue procurement

- **T-cell media**: 500 mL RPMI 1640 media (Gibco), 50 mL Fetal Bovine Serum (FBS), 5 mL of each: NEAA (Gibco), Sodium Pyruvate, GlutaMAX (Gibco), HEPES (Gibco), Penicillin/Streptomycin (Pen/Strep)

4.2. Cryopreservation

- **Freezing media:** 90% Fetal Bovine Serum (FBS), 10% DMSO

4.3. Thawing biopsies and tissue samples

- Ice cold PBS (1X) with 5% FBS
- PBS (1X)

4.4. Primary human enteroid culture

- **Epithelial digestion media-organoids:** HBSS (without Ca2+/Mg2+) + 2mg/mL collagenase type1 (Gibco)
- **Complete human small intestinal medium**: For 100 mL: 50 mL NRW Conditioned Medium, 30 mL Advanced DMEM/F12 (Gibco), 1 mL GlutaMAX (Gibco), 1 mL HEPES (Gibco), 1 mL Nicotinamide (1 M, Sigma), 1 mL B27 (Gibco), 500 uL N2 (Gibco), 200 uL Primocin (InvivoGen), 200 uL Normocin (InvivoGen), 100 uL N-Acetyl Cysteine (500 mM, Sigma), 100 uL A-83-01 (0.5 mM, Sigma), 33.2 ul SB202190 (5 mg in 505 ul DMSO, Sigma), 10 uL EGF (500 ug/mL, PeproTech), 10 uL Gastrin (500 uM, Sigma)
- Collagenase Type 1 (Gibco)
- Matrigel (Corning)
- Cell Recovery Solution (Corning)

4.5. LPMC isolation

- **Epithelial denudation medium:** HBSS (without Ca2+/Mg2+) + 1mM EDTA (10mL EDTA), 10mM HEPES (5mL HEPES), 0.5% FBS (2.5mL FBS), 0.115g DTT
- **Digest medium (high enzyme): T-cell media** with 0.5mg/mL DNase I and 0.5mg/mL Collagenase IV
- **Digest medium (low enzyme): T-cell media** with 2uL of collagenase IA stock 50mg/mL and 2uL of DNase I stock 5mg/mL (10,000 IU/mL) per 10 mL of medium

- **T-cell media:** 500 mL RPMI 1640 media (Gibco), 50 mL Fetal Bovine Serum (FBS), 5 mL of each: NEAA (Gibco), Sodium Pyruvate, GlutaMAX (Gibco), HEPES (Gibco), Penicillin/Streptomycin (Pen/Strep)
- PBS (1X)

5. Tissue procurement

5.1. Biopsy Tissue

5.1.1. Endoscopically obtain biopsies with 2.4 mm biopsy forceps.
5.1.2. Collect 2-4 per biopsies per vial and place in 1 mL of cold **T-cell media** in a 1 mL cryovial if using for LPMC isolation.
5.1.3. Store on ice until ready for cryopreservation.
5.1.4. Process all tissue within 30 minutes of collecting.

5.2. Surgical Tissue

5.2.1. Obtain discarded resected tissue from the operating room.
5.2.2. Place in 20 mL of cold **T-cell media** in a 50 mL conical tube.
5.2.3. Store on ice until ready for cryopreservation.
5.2.4. Process all tissue within 30 min of collecting.

6. Cryopreservation

6.1. Biopsies

6.1.1. Aspirate the **T-cell media** from the cryopreservation vial, being careful not to aspirate the biopsies.
6.1.2. Replace the **T-cell media** with 1 mL of **freezing media**.
6.1.3. Place samples promptly in a Nalgene Mr. Frosty freezing container to freeze at a 1°C per minute cooling rate in a -80°C freezer.
6.1.4. Transfer the samples to a liquid nitrogen freezer within 24-48 hours of collecting.

6.2. Surgical tissue

6.2.1. Cut the tissue into 2 mm size pieces with curved scissors.
6.2.2. Place 5-10 biopsy-sized pieces per 1 mL cryovial.
6.2.3. After aspirating any excess T-cell media, add 1 mL of **freezing media** to each vial.
6.2.4. Place samples promptly in a Nalgene Mr. Frosty freezing container to freeze at a 1°C per minute cooling rate in a -80°C freezer.
6.2.5. Transfer the samples to a liquid nitrogen freezer within 24-48 hours of collecting.

7. Thawing biopsies and tissue samples

7.1.1. When ready to use the biopsy or tissue, thaw the samples quickly by placing the cryovial in a 37°C water bath for 20-60 seconds.
7.1.2. Dilute the cryovial with cold PBS containing 5% FBS.
7.1.3. Transfer to a 50 ml conical tube
7.1.4. Add 10 ml of cold PBS with 5% FBS
7.1.5. Spin down at 1400 rpm for 5 min
7.1.6. Discard PBS
7.1.7. Replace with another 10ml of ice cold PBS with 5% FBS, wash and spin again
7.1.8. Discard the remaining PBS
7.1.9. Tissue is ready for further processing

8. Primary human enteroid culture

8.1. Generating primary human enteroid culture

8.1.1. After thawing the frozen tissue or biopsies to isolate epithelial cells, digest the tissue for 40 minutes in **Epithelial digestion media-organoids** at 37°C.
8.1.2. Mechanically disrupt the tissue using a P1000 pipette tip
8.1.3. Suspend the isolated epithelium in **Growth Factor Reduced Phenol Red Free Matrigel** (Corning).
8.1.4. Plate as 50 uL domes in a tissue culture-treated 24-well plate (Genesee)
8.1.5. Let the matrigel set at 37°C for 10 minutes.

8.2. Maintaining primary human enteroid culture

8.2.1. Maintain cultures **in complete human small intestinal medium** at 37°C with 5% CO2 as previously described[1, 2].
8.2.2. Change media every 3 days.
8.2.3. Passage cultures as necessary, every 7 to 10 days.
8.2.4. Passaging is performed by incubating with **Cell Recovery Solution** (Corning) on ice for 40 minutes.
8.2.5. Suspend the recovered enteroids in the desired amount of **Matrigel**.
8.2.6. Pass rapidly through the bent tip of a P1000 pipette tip to mechanically digest before re-plating.
8.2.7. A primary enteroid culture is considered to be ‘established’ if able to be expanded to 12 robust wells after passage.

9. RNAseq analysis of biopsy tissue

9.1. RNA extraction

9.1.1. Extract the RNA from individual biopsies utilizing the Qiagen (Valencia, CA) miRNA kit per their protocol.

9.1.2. Homogenize on a Bertin Precellys Homogenizer (Rockville, MD) system using ceramic beads.

9.1.3. Assess the RNA concentration with the Nanodrop 8000 system (Wilmington, DE).

9.1.4. Assess the quality of the RNA with the Advanced Analytical's Fragment Analyzer (Ankeny, IA).

9.2. RNAseq analysis

9.2.1. Perform the RNAseq using the Truseq Total RNAseq kit with RiboZero (Illumina, San Diego, CA), following their instructions.

9.2.2. Quantify the resulting libraries for quality using the Agilent TapeStation System (Santa Clara, CA).

9.2.3. Pool libraries to equimolar concentrations and sequenced on a Nextseq 500 system (Illumina, San Diego, CA) or a similar instrument targeting 30 million 75bp reads (60 million paired reads) per sample.

9.2.4. Assess sequencing run quality by using Illumina's SAV software.

9.2.5. Demultiplex with Illumina's bcl2fastq algorithm (Illumina, San Diego, CA)

9.2.6. Process the fastq files by using the QuickRNAseq[3] pipeline utilizing Hg38 for the genome and Gencode v24 for annotation.

10. Lamina Propria Mononuclear Cell Isolation (LPMCs)

10.1. Isolation of LPMCs with separation of epithelial cell and immune cells (short 1 hour digestion protocol)

- 10.1.1. Incubate the thawed biopsy tissue for 15 min on a shaker at 37°C and 225 rpm in 20 mL of prewarmed **epithelial denudation medium** to remove epithelial cells.
- 10.1.2. Filter through a 70-micron filter (the solution contains the epithelial cells that can be saved for future use by spinning down at 1400 rpm for 5 min).
- 10.1.3. Incubate the leftover biopsy pieces on a shaker for 30 min at 37°C and 225 rpm in **digest medium (high enzyme)**.
- 10.1.4. Vortex the solution for 10 seconds.
- 10.1.5. Filter through a 70-micron strainer.
- 10.1.6. Homogenize the samples onto the strainer by pressing with the back of a 5 mL plastic syringe piston.
- 10.1.7. Rinse the strainer with 10 mL **T-cell media** to collect remaining LPMCs.
- 10.1.8. Spin down at 1400 rpm for 5 min and discard the supernatant carefully not to dislodge the pellet that can be very small.
- 10.1.9. Wash twice with **T-cell media** by spinning at 1400 rpm for 5 min in between washes.
- 10.1.10. Resuspend in **PBS**.

10.2. Isolation of LPMCs with separation of epithelial cell and immune cells (overnight digestion protocol)

- 10.2.1. Incubate the thawed biopsy tissue for 15 min at 37°C in 20 mL of prewarmed **epithelial denudation medium** to remove epithelial cells.
- 10.2.2. Filter through a 70-micron filter (the solution contains the epithelial cells that can be saved for future use by spinning down at 1400 rpm for 5 min).
- 10.2.3. Incubate the leftover biopsy pieces on a shaker overnight at 37°C and 225 rpm in **digest medium (low enzyme)**.
- 10.2.4. Pass the remaining pieces through a 10 ml pipette 10 times to break up the remaining tissue.
- 10.2.5. Incubate for another hour back on the shaker.
- 10.2.6. Vortex the solution for 30 seconds.
- 10.2.7. Filter through a 70-micron strainer.
- 10.2.8. Homogenize the samples onto the strainer by pressing with the back of a 5 mL plastic syringe piston.
- 10.2.9. Rinse the strainer with 10 mL **T-cell media** to collect remaining LPMCs.
- 10.2.10. Spin down at 1400 rpm for 5 min and discard the supernatant carefully not to dislodge the pellet that can be very small.
- 10.2.11. Wash twice with **T-cell media** by spinning at 1400 rpm for 5 min in between washes.
- 10.2.12. Resuspend in **PBS**.

10.3. Single cell preparations from tissue without epithelial separation (overnight digestion)

10.3.1. Incubate the thawed biopsy pieces on a shaker overnight at 37°C and 225 rpm in **digest medium (low enzyme)**.
10.3.2. Pass the remaining pieces through a 10 ml pipette 10 times to break up the remaining tissue.
10.3.3. Incubate for another hour back on the shaker.
10.3.4. Vortex the solution for 10 seconds.
10.3.5. Filter through a 70-micron strainer.
10.3.6. Homogenize the samples onto the strainer by pressing with the back of a 5 mL plastic syringe piston.
10.3.7. Rinse the strainer with 10 mL of **T-cell media** to collect remaining LPMC.
10.3.8. Spin down at 1400 rpm x 5 min and discard the supernatant carefully not to dislodge the pellet that can be very small.
10.3.9. Wash twice with **T-cell media** by spinning at 1400 rpm for 5 min in between washes.
10.3.10. Resuspend in **PBS**.

11. References

1. Miyoshi H, Stappenbeck TS. The young and the Wnt-less: transplantable fetal intestinal spheroids without Wnts. Cell Stem Cell 2013;13:637-8.
2. Sato T, Clevers H. Primary mouse small intestinal epithelial cell cultures. Methods Mol Biol 2013;945:319-28.
3. Zhao S, Xi L, Quan J, et al. QuickRNASeq lifts large-scale RNA-seq data analyses to the next level of automation and interactive visualization. BMC Genomics 2016;17:39.

Subject ID	Age	Gender	Group	Dx	Site
56	13.2	Female	Enteroids	CD	BCH
309	19.5	Male	Enteroids	CD	BCH
433	4.4	Female	Enteroids	UC	BCH
482	15.6	Female	Enteroids	CD	BCH
541	23.4	Female	Enteroids	CD	BCH
1043	17.6	Female	Enteroids	UC	BCH
1055	8.2	Male	Enteroids	UC	BCH
1388	5.5	Female	Enteroids	CD	BCH
1459	7.9	Female	Enteroids	CD	BCH
1653	13.6	Male	Enteroids	UC	BCH
1755	10.5	Female	Enteroids	UC	BCH
1840	11.3	Female	Enteroids	Indeterminate colitis (IBD-U)	BCH
1870	12	Male	Enteroids	CD	BCH
1927	11.5	Male	Enteroids	CD	BCH
1997	7.1	Male	Enteroids	CD	BCH
2112	17.6	Male	Enteroids	CD	BCH
2125	15.1	Male	Enteroids	UC	BCH
2136	6.5	Male	Enteroids	UC	BCH
2187	1.3	Male	Enteroids	Stomal obstruction	BCH
2188	2.9	Male	Enteroids	Peptic ulcer disease	BCH
2203	0.7	Male	Enteroids	Gastroschisis and intestinal atresia	BCH
2223	19.6	Male	Enteroids	sigmoid volvulus	BCH
2228	3.8	Male	Enteroids	CD	BCH
2233	0.5	Male	Enteroids	Gastroschisis and intestinal atresia	BCH
2236	10.5	Female	Enteroids	CD	BCH
2245	2.3	Female	Enteroids	Constipation	BCH
2249	3.9	Female	Enteroids	Constipation	BCH
2262	5.9	Male	Enteroids	Rectal Bleeding	BCH
2291	13.4	Female	Enteroids	UC	BCH
2305	16.2	Male	Enteroids	Diarrhea	BCH
2309	17	Female	Enteroids	CD	BCH
2312	0.5	Female	Enteroids	Congenital Enteropathy	BCH
2321	6.3	Female	Enteroids	CD	BCH
2329	18.2	Female	Enteroids	Functional abdominal pain	BCH
2332	16.1	Male	Enteroids	Rectal Bleeding	BCH
2341	5.5	Female	Enteroids	Celiac disease	BCH
2353	2.4	Male	Enteroids	UC	BCH
2355	6	Female	Enteroids	UC	BCH
2363	14.5	Male	Enteroids	CD	BCH
2365	12.9	Female	Enteroids	UC	BCH
1899	16.5	Female	fresh vs froze	CD	BCH

1914	17	Female	fresh vs froze	CD	BCH
2156	21.9	Male	fresh vs froze	CD	BCH
2157	10.2	Female	fresh vs froze	CD	BCH
2170	10.6	Female	fresh vs froze	UC	BCH
2185	18.4	Male	fresh vs froze	CD	BCH
1137	14.2	Female	fresh vs froze	CD	BCH
2027	10.6	Female	fresh vs froze	UC	BCH
1909	21.8	Male	fresh vs froze	CD	BCH
2121	15.9	Male	fresh vs froze	CD	BCH
10026934	62	Male	fresh vs froze	CD	BWH
10026955	30	Male	fresh vs froze	CD	BWH
10058640	68	Female	fresh vs froze	CD	BWH
10058650	55	Female	fresh vs froze	CD	BWH
10058652	50	Female	fresh vs froze	HC	BWH
10058653	46	Male	fresh vs froze	HC	BWH
10058655	23	Female	fresh vs froze	UC	BWH
~~10058665~~	~~59~~	~~Female~~	~~fresh vs frozen~~	~~UC~~	~~BWH~~
H310	Adult		Enteroids	unaffected, did not grow	MGH
H341	Adult		Enteroids	used for the "ideal conditions"	MGH
43821	43	Male	RNAseq	UC	Pfizer/Benaroya
108776	29	Female	RNAseq	UC	Pfizer/Benaroya
120143	36	Female	RNAseq	UC	Pfizer/Benaroya
133724	48	Male	RNAseq	UC	Pfizer/Benaroya
150329	34	Male	RNAseq	UC	Pfizer/Benaroya
162573	43	Female	RNAseq	UC	Pfizer/Benaroya
174784	53	Male	RNAseq	UC	Pfizer/Benaroya
186931	35	Female	RNAseq	UC	Pfizer/Benaroya
190398	19	Female	RNAseq	UC	Pfizer/Benaroya
255220	69	Female	RNAseq	UC	Pfizer/Benaroya
361179	44	Female	RNAseq	UC	Pfizer/Benaroya
424848	63	Unknown	RNAseq	UC	Pfizer/Benaroya
468914	31	Unknown	RNAseq	UC	Pfizer/Benaroya
490730	33	Female	RNAseq	UC	Pfizer/Benaroya
533145	59	Male	RNAseq	UC	Pfizer/Benaroya
614819	33	Unknown	RNAseq	UC	Pfizer/Benaroya
951932	36	Female	RNAseq	UC	Pfizer/Benaroya
964151	31	Male	RNAseq	UC	Pfizer/Benaroya

REAGENT or RESOURCE			
Antibodies			
Target	**Tag**	**Clone**	**SOURCE**
CD45	89	HI30	Fluidigm
CD45	89	HI30	Fluidigm
CD44	115	IM7	Core, self conj
CD44	115	IM7	Core, self conj
c-kit	141	104D2	core, cust
c-kit	141	104D2	core, self conj
CD19	142	HIB19	Core
CD19	142	HIB19	Core
HLA-DR	143	L243	Fluidigm
HLA-DR	143	L243	Fluidigm
TNFa	144	Mab11	Core
CD64	144	10.1	Core
cRTH2	145	BM16	core, cust
CD16	145	3G8	Core
CD8a	146	RPA-T8	Core
CD8a	146	RPA-T8	Core
CD45RO	147	UCHL1	Core
CD45RO	147	UCHL1	Core
CD14	148	RM052	Fluidigm
CD28	148	CD28.2	Core
CD25	149	2A3	Fluidigm
CD25	149	2A3	Fluidigm
IL-22	150	22URTI	Fluidigm
CD38	150	HIT2	Core, self conj
CD123	151	6H6	Core
CD49b	151	P1E6-C5	Core
CD152 (CTLA-4)	152	L3D10	Core
CD14	152	M5E2	Core
CD45RA	153	HI100	Core
CD45RA	153	HI100	Core
CD38	154	HIT2	Core
CD163	154	GHI/61	Fluidigm
CD27	155	L128	Fluidigm
CD27	155	L128	Fluidigm
CCR4	156	L291H4	Core
CD8b	156	SIDI8BEE	Core
CD3	158	UCHT1	Core
CD3	158	UCHT1	Core
CCR7 (cd197)	159	G043H7	Fluidigm

CD11c	159	Bu15	Core
INFg	160	4S.B3	Core
AHR	161	FF3399	Core
IL-1B	162	CRM56	Core, cust
CD56	162	NCAM16.2	Core
CD183 (CXCR3)	163	G025H7	Fluidigm
CD183(CXCR3)	163	G025H7	Fluidigm
CD161	164	HP-3G10	Core
CD161	164	HP-3G10	Core
FoxP3	165	PCH101	Core
CD24	166	ML5	Fluidigm
CD24	166	ML5	Fluidigm
LAG-3	167	3DS223H	Core, cust
LAG-3	167	3DS223H	Core, self conj
CCR6	168	G034E3	Core
CCR6	168	G034E3	Core
IL17a	169	BL168	Core
CCR7	170	G043H7	Core
CD127	171	eBioRDR5	Core
CD127	171	eBioRDR5	Core
IL21	172	3A3-N2	Fluidigm
IgM	172	MHM-88	Core
CD335 (NKp46)	173	9E2	Core, cust
CD335	173	9E2	Core, costum conj
CD4	174	SK3	Fluidigm
CD4	174	SK3	Fluidigm
Tbet	175	4B10	Core
IgD	175	IA6-2	Core
CD56	176	N901	fluidigm
CXCR5	209	MU5UBEE	Core, custom conj
CXCR5	209	MU5UBEE	Core, custom conj
Anti-human CD4-PE-Cy5		Clone OKT3	Biolegend
Anti-human CD8-eVolve-655		Clone RPA-T8	eBiosccience
CD57	113In	HCD57	Biolegend
HLA-ABC	115In	W6/32	Biolegend
CD326	141Pr	Ep-CAm	Biolegend
CD19	142Nd	HIB19	Biolegend
CD45RA	143Nd	HI100	Biolegend
CD141	144Nd	M80	Biolegend
CD4	145Nd	RPA-T4	Biolegend
CD8a	146Nd	RPA-T8	Biolegend
IgA	147Sm	9H9H11	Biolegend

CD16	148Nd	3G8	Biolegend
CD127	149Sm	A019D5	Biolegend
FceRIa	149Sm	AER-37 (CRA-1)	Biolegend
CD1c	150Nd	L161	Biolegend
CD123	151Eu	6H6	Biolegend
CD66b	152Sm	G10F5	Biolegend
PD-1	153Eu	EH12.2H7	Biolegend
CD86	154Sm	IT2.2	Biolegend
CXCR4	154Sm	12G8	Biolegend
CD27	155Gd	O323	Biolegend
CD33	158Gd	WM53	Biolegend
CD103	159Tb	Ber-Act8	Biolegend
CD14	160Gd	M5E2	Biolegend
CD56	161Dy	B159	Biolegend
CD64	162Dy	10.1	Biolegend
CD172a/b	163Dy	SIRPa/b	Biolegend
CD161	164Dy	HP-3G10	Fluidigm
CD69	164Dy	FN50	Biolegend
CD25	166Er	M-A251	Biolegend
CD11c	167Er	Bu15	Biolegend
CD3	168Er	UCHT1	Biolegend
integrin b7	169Tm	FIB504	Biolegend
CD38	170Er	HB-7	Biolegend
CD206	172Yb	15-2	Biolegend
HLADR	174Yb	L243	Biolegend
CD58	175Lu	TS2/9	Biolegend
CD54	176Yb	HCD54	Biolegend
CD11b	209Bi	ICRF44	Fluidigm
CD45	89Y	HI30	Fluidigm
REAGENT or RESOURCE			**SOURCE**
Biological Samples			
Human intestinal biopsies and tissue			BCH, BWH
Human intestinal biopsies			UPENN Immunology in IBD Initiative
Human intestinal biopsies			Benaroya Research Institute at VMMC
Human intestinal biopsies			Icahn School of Medicine (MSSM)
Chemicals, Peptides, and Recombinant Proteins			
DNAse			Qiagen
Collagenase			Sigma
low Barium PBS (1x)			ThemoScientific
Bovine Serum Albumin, protease free			Sigma
Sodium Azide			Sigma
RPMI			Life Technologies

glutamax	ThermoFisher
Penstrep	ThermoFisher
HEPES	Life Technologies
NEAA	Life Technologies
Sodium pyruvate	Life Technologies
Formaldehyde (40% solution)	Fisher Scientific
Rh103 intercalator	Thermo
Fc-Block	Biolegend
MilliQ Water (low barium)	
EQ 4 Elemental Beads	Fluidigm
100% methanol	Fisher
Intercalator-Ir 125 uM	Fluidigim
PMA	Sigma Aldrich
Ionomycin	Sigma
Foxp3 fix perm diluent	Thermo
Foxp3 fix perm concentrate	Thermo
Foxp3 wash buffer	Thermo
Collagenase Type 1	Gibco
Growth Factor Reduced Phenol Red Free Matr	Corning
Corning Cell Recovery Solution	Corning
L-NRW Conditioned Medium	Miyoshi and Stappenbeck, Nat. Protoc., 20
Advanced DMEM/F12	Gibco
GlutaMax	Gibco
N2	Gibco
HEPES	Gibco
Nicotinamide	Sigma
B27	Gibco
Primocin	Invivogen
Normocin	Invivogen
N-Acetyl Cysteine	Sigma
A-83-01	Sigma
SB202190	Sigma
EGF	Peprotech
Gastrin	Sigma
DMSO	Sigma
Fetal Bovine Serum (FBS)	Gibco
RNAlater	Qiagen
Deoxyribonuclease I from Bovine Pancreas	Sigma-Aldrich
Collagenase/Dispase	Roche
DTT (dithiothreitol)	ThermoFisher
RPMI 1640	Corning
GemCell U.S. Origin Fetal Bovine Serum	Gemini

UltraPure 0.5M EDTA, pH 8.0	Invitrogen
Penicillin-Streptomycin (10,000 U/mL)	Gibco
ACK Lysing Buffer	Thermo Fisher
L-Glutamine (200mM)	Lonza
Dulbecco's Phosphate-Buffered Saline, 1X	Corning
Percoll	Sigma-Aldrich
Critical Commercial Assays	
Fixation and Permeabilization Solution	BD Biosciences
Fixation and Permeabilization Wash	BD Biosciences
Brefeldin A Protein Transport Inhibitor	BD Biosciences
Monensin Protein Transport Inhibitor	BD Biosciences
LIVE/DEAD Fixable Aqua Dead Stain Kit	Invitrogen
miRNA kit	Qiagen
Truseq Total RNAseq kit	Illumina
Ribo-Zero	Illumina
TapeStation System	Agilent
Deposited Data	
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Experimental Models: Cell Lines	
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Experimental Models: Organisms/Strains	
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Oligonucleotides	
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Recombinant DNA	
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Software and Algorithms	
Cytobank	
Pydio	
EVOS FL Auto 2	Thermofischer
FlowJo	LLC
GraphPad Prism	GraphPad
SAV software	Illumina
bcl2fastq algorithm	Illumina
Quick RNAseq	Zhao et al
EdgeR 3.12 algorithm	Law et al (2014), Ritchie et al (2015)
tmod R package	
ggplot2	
Other	
Fragment Analyzer	Advanced Analytical
Homogenizer	Bertin Precellys
CyTOF 2 Mass Cytometer	Fluidigm

Nextseq 500 system	Illumina

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MSSM
BCH Cytokine Panel
Upenn

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Protoc., 2013

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RRID:SCR_014043; http://www.cytobank.org/index.html
https://distrib.dfci.harvard.edu/
Version 10.3; RRID:SCR_008520; https://www.flowjo.com/solutions/flowjo
Version 7; RRID:SCR_002798; http://www.graphpad.com/
Version 2.1.8, https://support.illumina.com/downloads/sequencing-analysis-viewer-software-v2-18.html
RRID:SCR_015058; https://support.illumina.com/sequencing/sequencing_software/bcl2fastq-conversion-software.html
version 1.2; https://sourceforge.net/projects/quickrnaseq/
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v 0.31, https://cran.r-project.org/web/packages/tmod/index.html
v 2.2.1, https://cran.r-project.org/web/packages/ggplot2/index.html