Thome JJ, Bickham KL, Ohmura Y, Kubota M, Matsuoka N, Gordon C, Granot T, Griesemer A, Lerner H, Kato T, Farber DL (2016) Early-life compartmentalization of human T cell differentiation and regulatory function in mucosal and lymphoid tissues. Nat Med. **22**(1):72-7. doi: 10.1038/nm.4008. Epub 2015 Dec 14.

ONLINE METHODS

Acquisition of human tissue. Human tissues were obtained from deceased (brain dead) organ donors at the time of organ acquisition for life-saving clinical transplantation. Infant donor tissue was obtained in collaboration with the Columbia University/New York Presbyterian Hospital pediatric liver transplant program. Adult donor tissues were obtained through an approved protocol and material transfer agreement with LiveOnNY (formerly the New York Organ Donor Network, NYODN). Organ donors were free of chronic disease and cancer, negative for hepatitis B, hepatitis C and HIV and 79% male. The study does not qualify as human subjects research, as confirmed by the Columbia University IRB, because tissue samples were obtained from deceased individuals. Cord blood was obtained as discarded samples from K. Liu, Columbia University. Adult blood and pediatric thymus tissues were acquired through the Columbia Center for Translational Immunology (CCTI) human studies core, which operates in accordance with the Columbia University human research protection office institutional review board.

Lymphocyte isolation from human lymphoid and non-lymphoid tissues. Tissue samples were maintained in cold saline and brought to the laboratory within 2–4 h of organ procurement. Samples were rapidly processed using enzymatic and mechanical digestion to obtain lymphocyte populations with high viability, as described in detail[7,8]. Tissues were minced and incubated at 37 °C in enzymatic digestion media: RPMI (Thermo Fisher, Waltham, MA) containing 10% FBS (Thermo Fisher), l-glutamate (Thermo Fisher), sodium pyruvate (Thermo Fisher), nonessential amino acids (Thermo Fisher), penicillin-streptomycin (Thermo Fisher), collagenase D (1 mg/ml, Roche, Indianapolis, IN), trypsin inhibitor (1 mg/ml, Thermo Fisher) and DNase I (0.1 mg/ml, Roche). Digested tissue was further disrupted using the gentleMACS tissue dissociator (Miltenyi Biotech, San Diego, CA); the resulting suspension was passed through a tissue sieve (10–150 mesh size) and then pelleted through centrifugation. Residual red blood cells (RBC) were lysed using AKC lysis buffer (Corning Cellgro, Manassas, VA), and dead cells and debris were removed via centrifugation through 30% Percoll (GE Healthcare Life Sciences, Pittsburgh, PA). Lymphocytes were isolated from blood using lymphocyte separation media (Cellgro) and AKC lysis buffer as described[7].

Flow cytometry analysis. The following fluorochrome-conjugated antibodies were used for surface staining: anti-human CD3 (Brilliant Violet 650, 1:100, OKT3, BioLegend, San Diego, CA), CD4 (PeCy7, 1:100, SK3, BioLegend), CD8 (APC-Cy7, 1:100, SK1, BD Biosciences), CD19 (PE Texas Red, 1:100, SJ25-C1, Invitrogen or APC, HIB19, BioLegend), CD25 (FITC, 1:50, 2A3, BD Biosciences, San Jose, CA), CD31 (APC, 1:100, WM59, eBioscience, San Diego, CA), CD45RA (Brilliant Violet 605, 1:100, HI100, BioLegend), CD45RO (PerCpFl710, 1:100, UCHL1, eBioscience), CD69 (Brilliant Violet 421, 1:100, FN50, BioLegend or BUV395, 1:100, FN50, BD Biosciences), CD103, (Alexa Fluor 647, 1:100, Ber-ACT8, BioLegend), CD127 (BV711, 1:100, A019D5, BioLegend), CCR7 (Alexa Fluor 488, 1:100, TG8, BioLegend). **For intracellular staining**, surface stained cells were resuspended and incubated in fixation buffer (eBioscience), washed, resuspended in 0.1 ml permeabilization buffer (eBioscience) and stained with anti-FOXP3 antibodies (PE, 1:20, 236A/E7, eBioscience) and Ki67 (α700, 1:100, *Ki*-67, BioLegend) for 30 min at room temperature and washed twice with permeabilization buffer. Stained cells were acquired on a 6-laser LSRII analytical flow cytometer (BD Biosciences) in the CCTI flow cytometry core and analyzed using FlowJo software (Treestar, Ashland, OR).