ONLINE SUPPLEMENTAL MATERIAL

Type 1 diabetic patient hematopoietic stem cells are programmed to generate increased peripheral helper and follicular helper T cells and increased autoimmunity-associated B cell subsets

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Animals

NSG-pct recipients were used in these studies because we have observed that the porcine cytokine transgenes enhance human hematopoiesis from adult bone marrow HSCs in NSG mice (N.Danzl and M.Sykes, unpublished data). All mice were maintained under specific pathogen-free and helicobacter/pasteruella pneumotropica-free conditions, and all experiments were performed under approved protocols from the Columbia University Institutional Animal Care and Use Committee.

Human tissue procurement and transplantation

Human fetal thymus and liver tissues (gestational age 17 to 20 weeks) were obtained from Advanced Biosciences Resources. Fetal thymus fragments were cryopreserved in 10% dimethyl sulfoxide (Sigma-

Aldrich) and 90% human AB serum (Gemini Bio Products). T1D and HC donors were recruited by the Center for Translational Immunology Human Studies Core and the Naomi Berrie Diabetes Center and bone marrow was aspirated using standard sterile technique. All human samples were collected under protocols approved by the Institutional Review Board of Columbia University Medical Center in accordance with the Declaration of Helsinki.

Flow Cytometry

Single-cell suspensions of spleen were treated with ACK lysis buffer (Life Technologies) to remove erythrocytes. After ACK lysis, remaining cells were passed through a 40μm filter prior to flow cytometry analysis. The following antibodies from BM Biosciences, eBioscience or BioLegend were used for flow cytometry: ICOS (ISA-3), CD45RA (HI100), CCR7 (G043H7), PD-1 (EH12.2H7), CXCR5 (RF8B2), CD8 (RPA-T8), CD25 (2A3), CD3 (SK7), CD4 (RPA-T4), CXCR3 (1C6), CD127 (A019D5), CCR2 (1D9), CCR5 (2D7), CD20 (L27), CD19 (HIB19), IgM (G20-127), IgG (G18-145), IgA (IS11-8E10), IgD (IA6-2), CD27 (O323), CD38 (HIT2), CD138 (MI15), CD11c (B-ly6), CD14 (M5E2), CD21 (Bu32), hCD45 (HI30) and mCD45 (30-F11). For intracellular staining, single-cell splenocyte suspensions were fixed-permeabilized with the FOXP3 Fixation Kit (Invitrogen) for 45 minutes at RT, then, stained with mAbs for FOXP3 (1054C), CTLA-4 (BNI3), IL-21 (3A3-N2.1), BCL-6 (K112-91) and Blimp-1 (646702) for 30 minutes at RT in permeabilization buffer. To determine IL-21 secretion, single-cell suspensions were stimulated for 3 hours with 50 ng/ml phorbol myristic acid (PMA; Sigma-Aldrich, St Louis, MO, USA), 1 μg/ml ionomycin (Sigma-Aldrich) and 3 μg/ml brefeldin A in RPMI supplemented with 10% FBS, 1% Hepes (Sigma-Aldrich), 1% penicillin-streptomycin (Life Technologies), and 0.05% gentamicin (Life Technologies) in 37°C, 5% CO_2 incubator conditions. After washing, cells were acquired on Aurora Cytek (Fremont, California) and analyzed with FlowJo (Tree Star, USA) software.

ELISA of IgM and IgG concentration

To quantify human antibody levels in serum of PI mice. Briefly, plates (Corning Inc.) were coated with unlabeled goat anti-human IgG Fc fragment (Jackson) or goat anti-human IgM (Southern Biotech) antibody. After washing and blocking the wells with 2% Bovine Serum Albumin (BSA, Fisher Scientific), samples were added at multiple dilutions. After washing, bound human Ig was detected using biotin-conjugated mouse anti-human IgG secondary antibody (BD Pharmingen) or biotin-conjugated mouse anti-human IgM secondary antibody (BD Pharmingen), followed by streptavidin-horseradish peroxidase (Thermo Scientific). Colorimetric change was developed with a 3,3′,5,5′-Tetramethylbenzidine substrate solution (Thermo Scientific), stopped by the addition of 2M sulfuric acid (Sigma), and the optical density was determined using a spectrophotometer at 450nm absorbance. A standard curve based on human serum with known IgM and IgG concentrations (Bethyl) was used to calculate sample Ig concentrations.

Information Relative to Supplementary Figures

Supplementary Figure 1. Detection and analysis of CXCR5$^-$PD1$^+$ Tph-like and CXCR5$^+$PD1$^+$ Tfh cells in PI mice. Gating strategy for CD45RA$^+$CCR7$^+$ CD4$^+$ naïve T cells, and Tph-like and Tfh cells.

Supplementary Figure 2. CXCR5$^-$PD-1$^+$ Tph-like and CXCR5$^+$PD-1$^+$ Tfh cells share features associated with B cell helper function. (**A**) Representative plot of naïve CD45RA+CCR7+ T cells, and Tph-like and Tfh cells among gated memory CD4$^+$ T cells from 3 independent experiments (*n = 11 HC* and *n = 5*). The expression of (**B**) ICOS, (**C**) CXCR3 and (**D**) CCR2 is shown. *p<0.05, **p<0.01 and ***p<0.001 comparing Tph-like (CXCR5$^-$PD-1$^+$) and Tfh cells (CXCR5$^+$PD-1$^+$) to naive CD4$^+$ T cells.; Friedman's multiple comparison test was used for statistical analysis.

Supplementary Figure 3. Analysis of Tfh and Tph-like. A) histogram of PD-1 and ICOS relative expression between CD45RA+ and CD45RA- CD4+ T cells from spleens of PI mice. (**B**) Correlation between Tph-like and Tfh cells frequencies. Data are represented as mean ± SEM. *p<0.05, **p<0.01 and ***p<0.001 Friedman's multiple comparison test was used for statistical analysis.

Supplementary Figure 4. Detection and analysis of B cell subsets in PI mice. Gating strategy for naïve $CD27^{-}IgD^{+}$, memory $CD27^{+}IgD^{-}$, unswitched $CD27^{+}IgD^{+}$ and unconventional memory $CD27^{-}IgD^{-}$ B cells, and $CD19^{+}CD20^{-}$ ASCs.

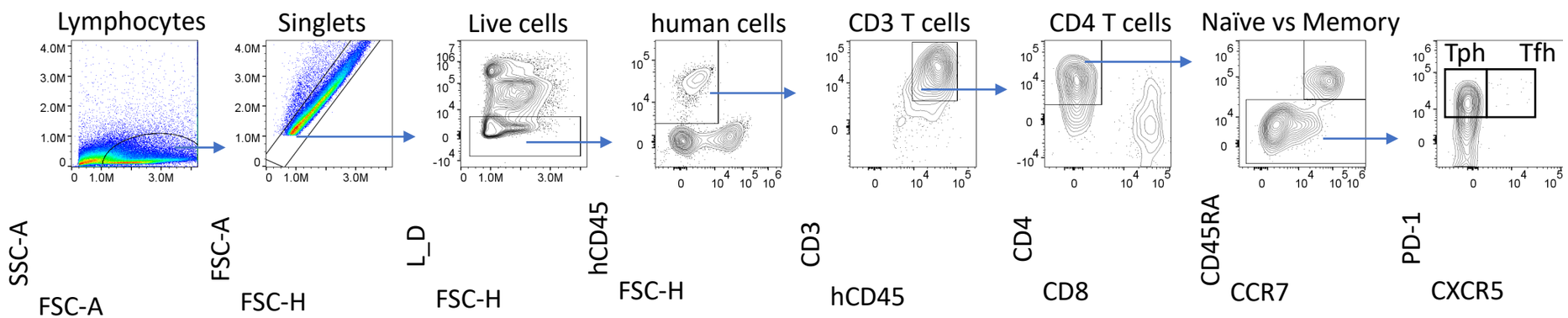
Supplementary Table 1: Donor list with relative HLA type

		Gene Locus							
		HLA-A		HLA-B		HLA-DRB		HLA-DQB	
		Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
Exp1	T1D	01 (A1)	02 (A2)	---	---	03 (DR3)	unkn	unkn	unkn
	Fetal Thymus	unkn	02 (A2)	---	---	03 (DR3)	unkn	unkn	unkn
Exp2	HC	02 (A*02)	29 (A29)	44 (B44)	61 (B61)	04 (DR4)	unkn	03:02 (DQ8)	02:01 (DQ2)
	Fetal Thymus	02 (A*02)	24 (A24)	---	---	04 (DR4)	unkn	03:02 (DQ8)	unkn
Exp3	HC	02:01 (A*02)	23:01 (A23)	15:01 (B15)	unkn	04:04 (DR4)	07:01 (DR7	03:02 (DQ8)	06 (DQ6)
	Fetal Thymus	02:01 (A*02)	31:01 (A31)	07:02 (B7)	40:02 (B40)	04:04 (DR4)	14:02 (DR14)	03:02 (DQ8)	05:01 (DQ4)
Exp4	HC	unkn	unkn	---	---	04 (DR4)	unkn	03:02 (DQ8)	unkn
	T1D	02 (A*02)	02 (A*02)	---	---	04 (DR4)	unkn	03:02 (DQ8)	03:02 (DQ8)
	Fetal Thymus	02 (A*02)	24 (A24)	---	---	04 (DR4)	unkn	03:02 (DQ8)	4:02
Exp5	HC BM	02:07	11:02	13:01	27:04	12:02	12:02	03:01	03:01
	T1D BM	24:02	30:02	15:03	18:01	03:01	03:01	02:01	02:01
	Fetal Thymus	02 (A*02)	30:04 (A30)	58:02 (B58)	35:01 (B35)	15:03 (B15)	03:01 (DR3)	03:01	06:02 (DQ06)

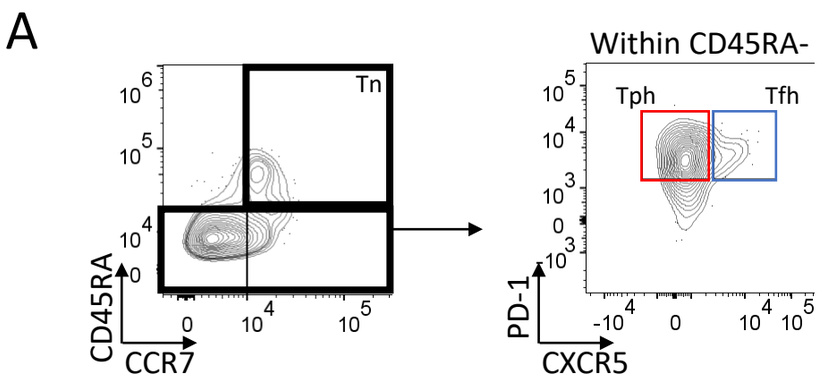
Supplementary Figure 1

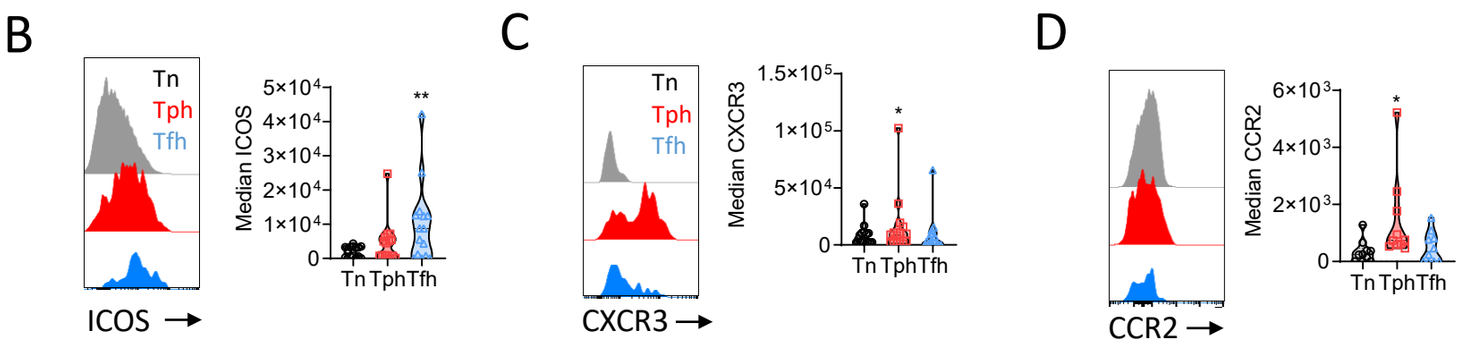
A

Gating Strategy for CXCR5-PD-1+ Tph and CXCR5+PD-1+ Tfh cells



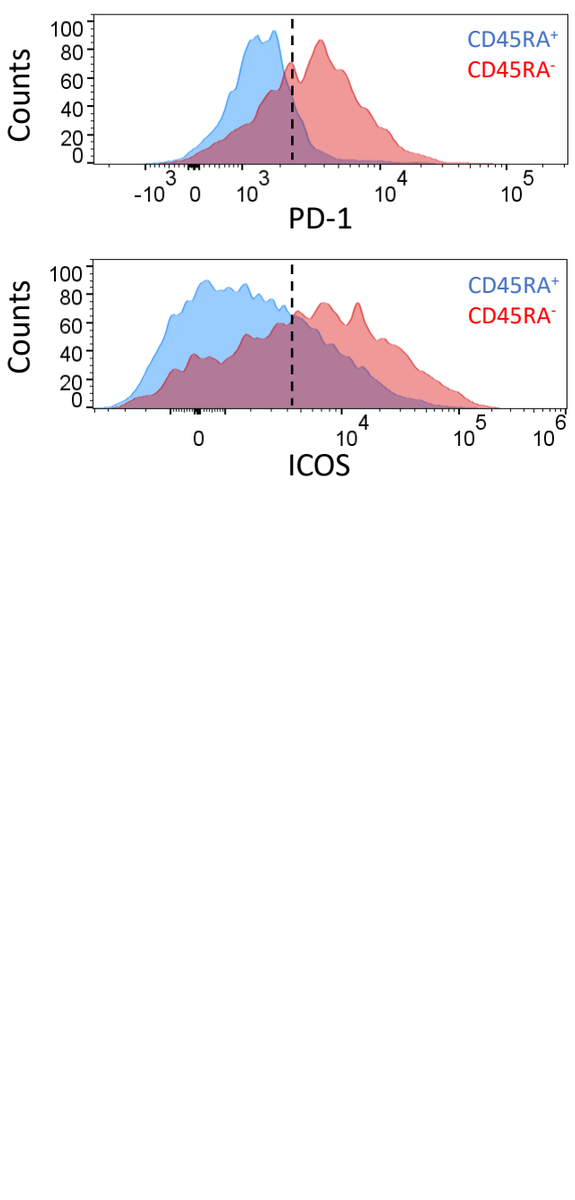
Supplementary Figure 2



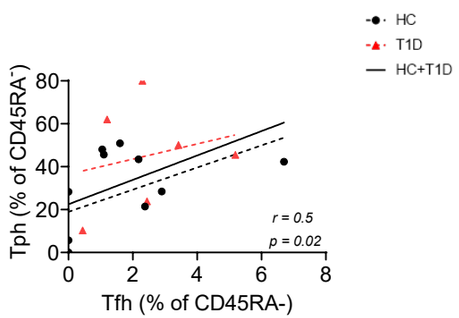


Supplementary Figure 3

A



B



Supplementary Figure 4

A

Gating Strategy for B cells subsets