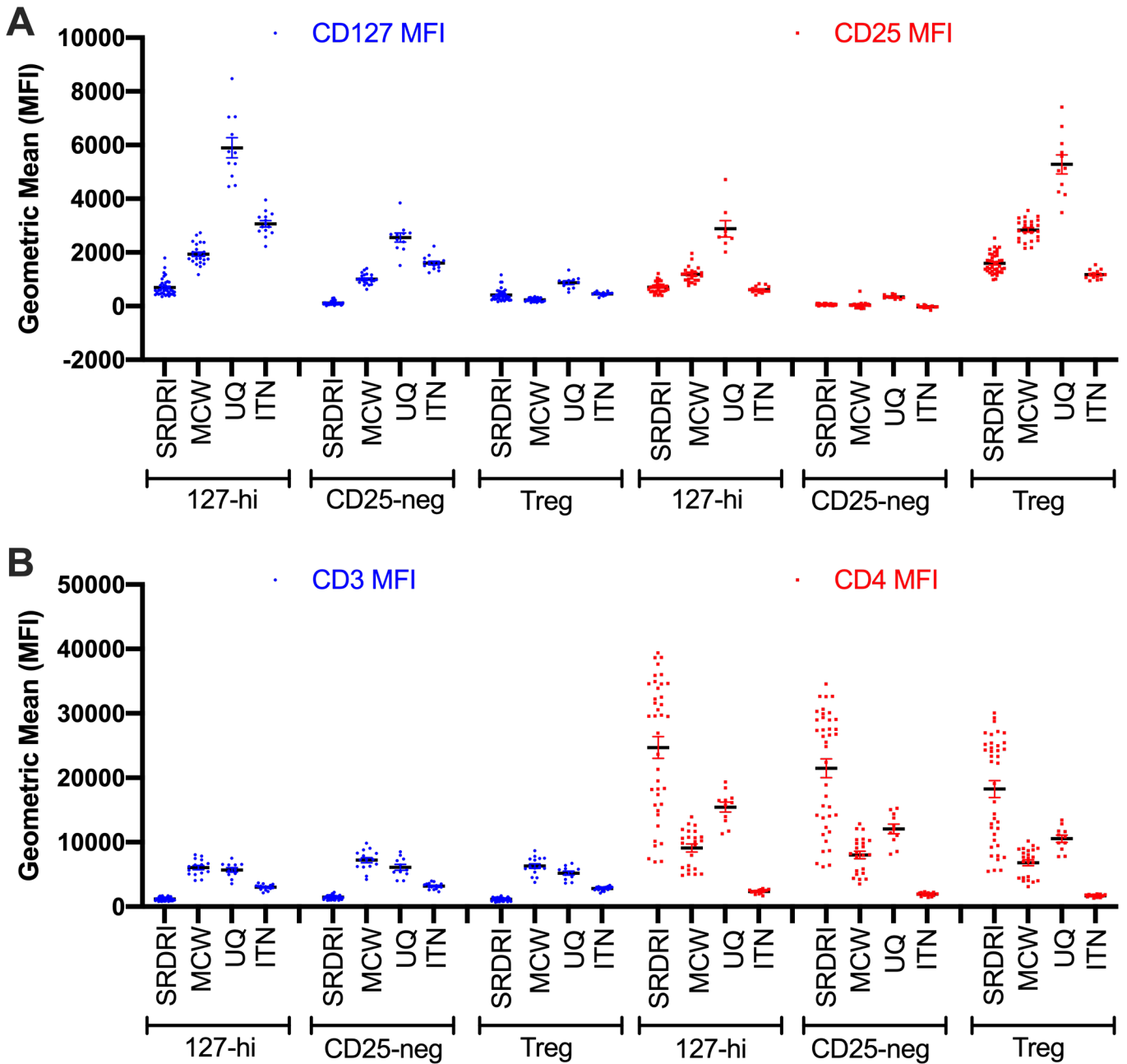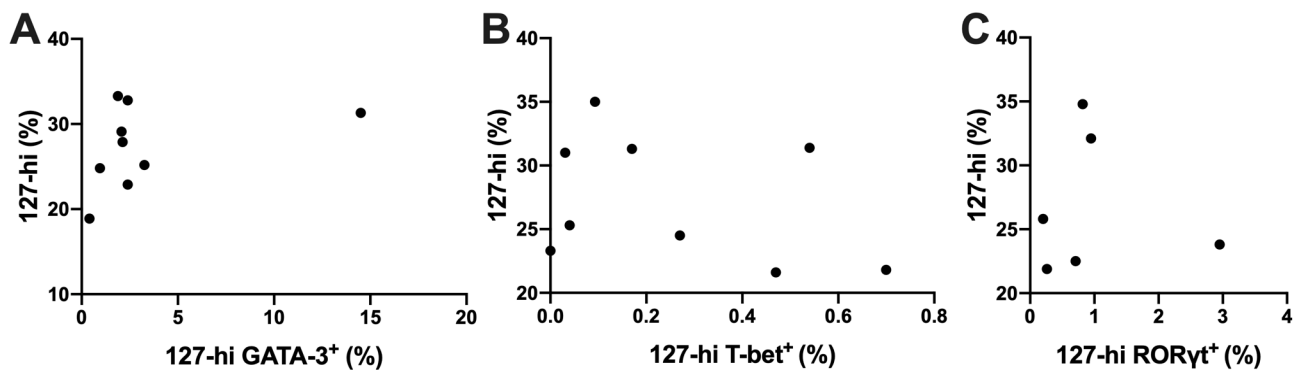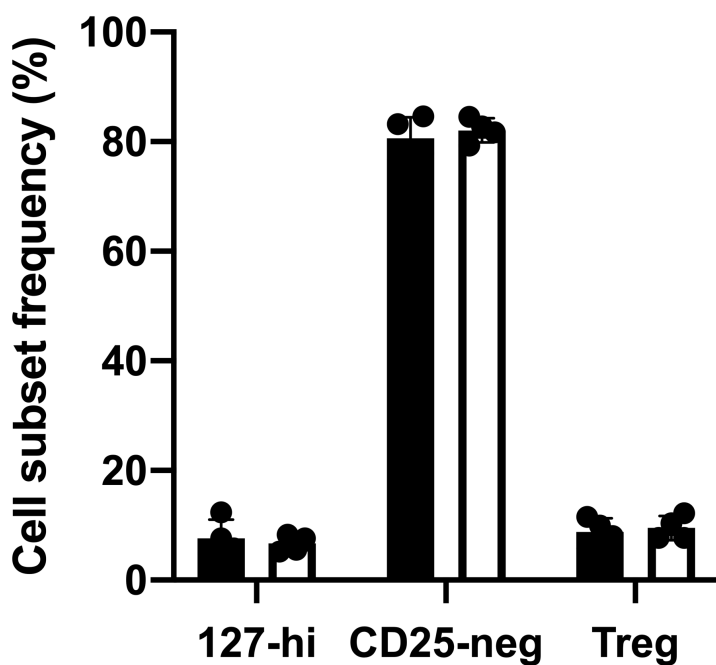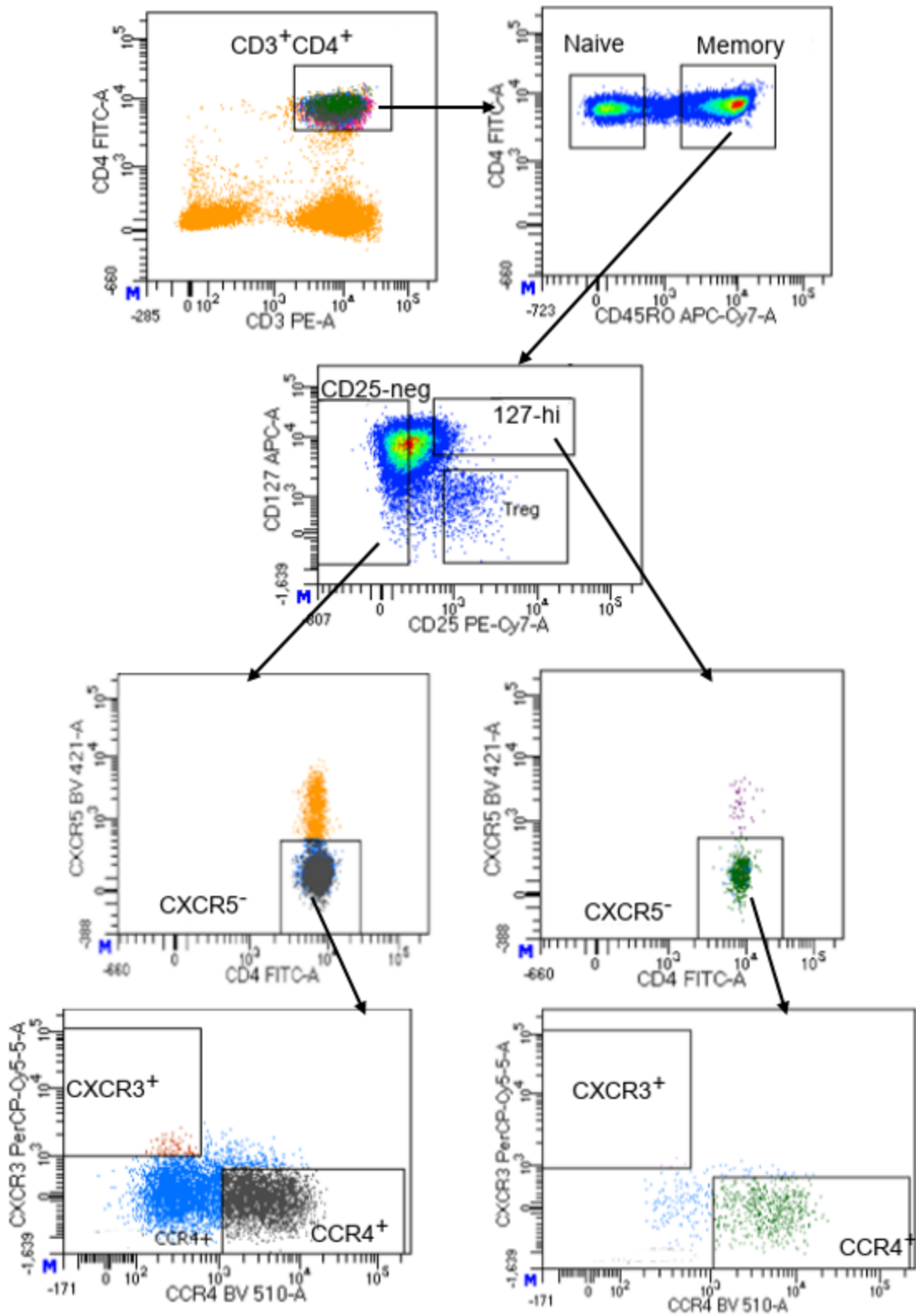# Supplemental Data



Supplemental Figure 1. The MFI for CD25 and CD127 on 127-hi cells using data generated at each site 127-hi cells were identified as shown in Figure 1. (A) shows the geometric mean fluorescence intensity (MFI) for 127 and CD25 on 127-hi cells, CD25-neg cells and Treg at SRDRI, MCW, UQ and ITN. (B) The MFI of CD3 and CD4 on the same cells at each of the four sites. The FSC threshold for data generated at each site was as follows: between 5,000 and 25,000 for SRDRI, 5,000 for MCW, between 25,000 and 35,000 for UQ and between 7,500 and 10,000 for ITN.

Supplemental Figure 2. The relative frequency of GATA-3^{+} 127-hi cells is not different in patients with different relative frequencies of 127-hi cells  Data described in Figure 8 were re-analyzed to determine whether there is a correlation between the relative frequency of 127-hi cells in each patients and the frequency of those 127-hi cells that express either GATA-3 (A, n = 9), or T-bet (B, n = 9) or RORγt (C, n = 9).



Supplemental Figure 3. The relative frequency of 127-hi, CD25-neg and Treg cells from thawed PBMC was not different from freshly isolated PBMC Whole blood was collected from healthy adult donors and PBMC were isolated (n = 4). Vials of PBMC were either frozen (open bars) immediately or labeled for CD3, CD4, CD25 and CD127 (closed bars). The relative frequency of 127-hi, CD25-neg and Treg cells were determined and compared to the relative frequency of the same cell subsets from the same donors, at least 2 weeks after freezing.

Supplemental Figure 4. A schematic for sorting pre-Th1 and pre-Th2 127-hi and CD25-neg cells PBMC are stained for CD3 CD4 CD45RO CXCR5 CXCR3 CCR4. The sort schematic for the isolation of 127-hi pre-Th2 and pre-Th1 and CD25-neg pre-Th2 and pre-Th1 is displayed.

**Supplemental Table 1** denotes the total number of cells derived for each sort population from total PBMCs from people with T1D.

| Cell Subset | Cell Count | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | T1D #1 | T1D #2 | T1D #3 | T1D #4 | T1D #5 | T1D #6 | T1D #7 | T1D #8 | T1D #9 | T1D #10 |
| CD25-neg preTh1 | 171367 | 29778 | 47659 | 12897 | 10627 | 20986 | 30757 | 30161 | 32636 | 79526 |
| CD25-neg preTh2 | 64923 | 143914 | 94091 | 67518 | 530593 | 162263 | 197447 | 123891 | 98462 | 111158 |
| 127-hi preTh1 | 5489 | 330 | 511 | 131 | 93 | 544 | 676 | 620 | 672 | 1080 |
| 127-hi preTh2 | 5712 | 18203 | 34637 | 11443 | 36173 | 35156 | 32663 | 24269 | 5110 | 9756 |

**Supplemental Table 2** lists all the antibodies used during this study

| Antigen | Flurochorme | Clone | Vendor | Catalog # | Antibody registry # |
|---|---|---|---|---|---|
| **Panel run at Univ of Queensland; Collaborator: Dr. R. Thomas** | | | | | |
| Live/Dead | BV510 | Not applicable | Invitrogen | L34957 | Not applicable |
| CD3 | FITC | OKT3 | BioLegend | 317306 | AB_571907 |
| CD4 | AF700 | OKT4 | BioLegend | 317426 | AB_571943 |
| CD25 | PE | BC96 | BioLegend | 302606 | AB_314276 |
| CD127 | BV421 | A019D5 | BioLegend | 351310 | AB_10960140 |
| CD45RO | APC-Cy7 | UCHL1 | BioLegend | 304228 | AB_10895897 |
| CD45RA | PerCP-Cy5.5 | HI100 | BioLegend | 304122 | AB_893357 |
| CXCR3 (CD183) | APC | G025H7 | BioLegend | 353708 | AB_10983064 |
| CXCR5 (CD185) | BUV395 | RF8B2 | BD Biosciences | 740266 | AB_2740008 |
| CCR4 (CD194) | BV605 | L291H4 | BioLegend | 359418 | AB_2562483 |
| **Panel at ITN for the T1DAL study; Collaborator: Dr. E. Serti** | | | | | |
| CD3 | AF700 | UCHT1 | BD Biosciences | 557943 | AB_396952 |
| CD4 | V500 | RPA - T4 | BD Biosciences | 560769 | AB_1937331 |
| CD25 | PE-CF594 | M - A 251 | BD Biosciences | 562403 | AB_11151919 |
| CD127 | BV421 | A019D5 | BioLegend | 351310 | AB_10960140 |
| CD45RO | BV650 | UCHL1 | BioLegend | 304231 | AB_2561359 |
| CD45RA | PE-Cy7 | HI100 | BioLegend | 304126 | AB_10708879 |
| CCR7 | PE | G043H7 | BioLegend | 353204 | AB_10913813 |
| CD2 | PerCP-e710 | RPA-2.10 | eBiosciences | 46-0029-42 | AB_10670351 |
| **Panel run at SDBRI; Samples provided by ITN** | | | | | |
| Human Treg Cocktail | FITC, PE-Cy7, AF647 | SK3, 2A3, HIL-7R-M21 | BD Biosciences | 560249 | AB_1645496 |
| CCR7 | PE | G043H7 | BioLegend | 353204 | AB_10913813 |
| CXCR3 (CD183) | PerCP- Cy5.5 | 1C6 | BD Biosciences | 560832 | AB_2033945 |
| CD45RO | APC-H7 | UCHL1 | BD Biosciences | 561137 | AB_10562194 |
| CXCR5 (CD185) | BV421 | RF8B2 | BD Biosciences | 562747 | AB_2737766 |
| CCR4 (CD194) | BV510 | 1G1 | BD Biosciences | 563066 | AB_2737984 |
| CD3 | BV605 | SK7 | BD Biosciences | 563217 | AB_2714001 |
| GATA3 | PE | 16E10A23 | BioLegend | 653804 | AB_2562723 |
| T-bet | BV421 | 4B10 | BioLegend | 644816 | AB_10959653 |
| RORγt | PE | AFKJS-9 | eBiosciences | 12-6988-82 | AB_1834470 |
| **Panel run at SRDRI; Collaborator: Dr. M. Battaglia** | | | | | |
| CD3 | PerCP-Cy5.5 | SK7 | BD Biosciences | 345766 | AB_2783791 |
| CD4 | PE-Cy7 | SK3 | BD Biosciences | 557852 | AB_396897 |
| CD25 | APC | 2A3 | BD Biosciences | 340907 | AB_2819021 |
| CD127 | PE | R34.34 | Beckman Coulter | IM1980U | AB_131301 |
| CD45 | Pacific Blue | HI30 | BioLegend | 304022 | AB_493655 |
| CD45RA | FITC | HI100 | BD Biosciences | 555488 | AB_395879 |
| **Panel run at MCW; Collaborator: Dr. M. Hessner** | | | | | |
| CD3 | BUV395 | UCHT1 | BD Biosciences | 563546 | AB_2744387 |
| CD4 | APC-H7 | RPA-T4 | BD Biosciences | 560158 | AB_1645478 |
| CD25 | PE-Cy7 | 2A3 | BD Biosciences | 335789 | AB_399968 |
| CD127 | PE | HIL-7R-M21 | BD Biosciences | 557938 | AB_2296056 |
| CD45RO | FITC | UCHL1 | BD Biosciences | 555492 | AB_395883 |
| CD45RA | BV510 | HI100 | BD Biosciences | 563031 | AB_2722499 |
| Foxp3 | APC | PCH101 | eBiosciences | 14-4776-82 | AB_467554 |
| Live/Dead | BV421 | Not applicable | Invitrogen | L34955 | Not applicable |