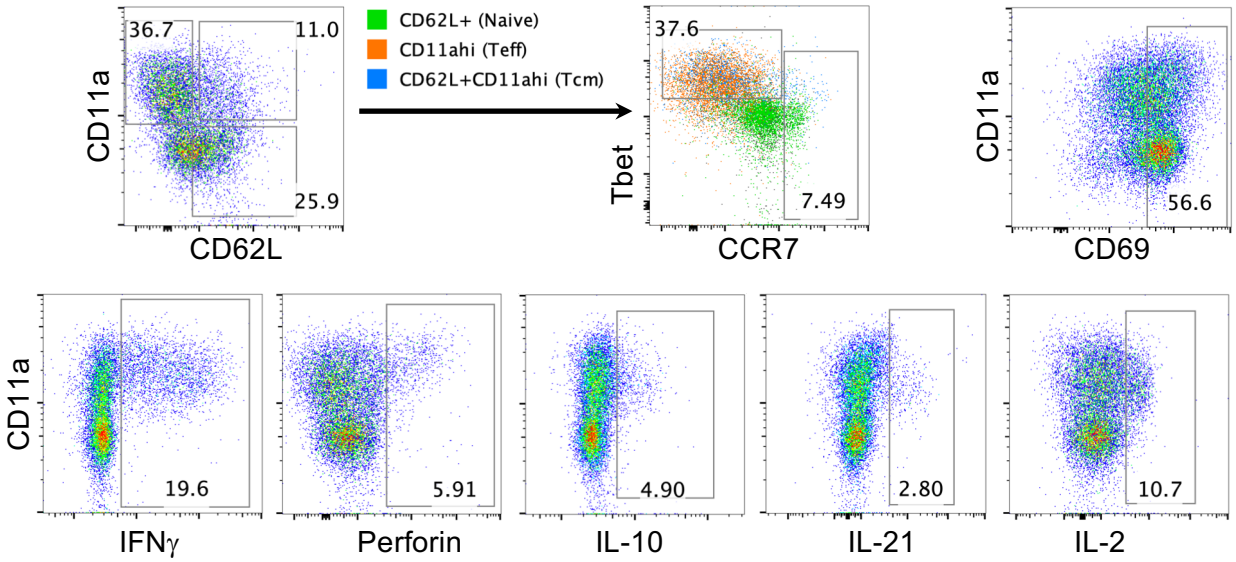
## Supplemental Figure 1.

**A**

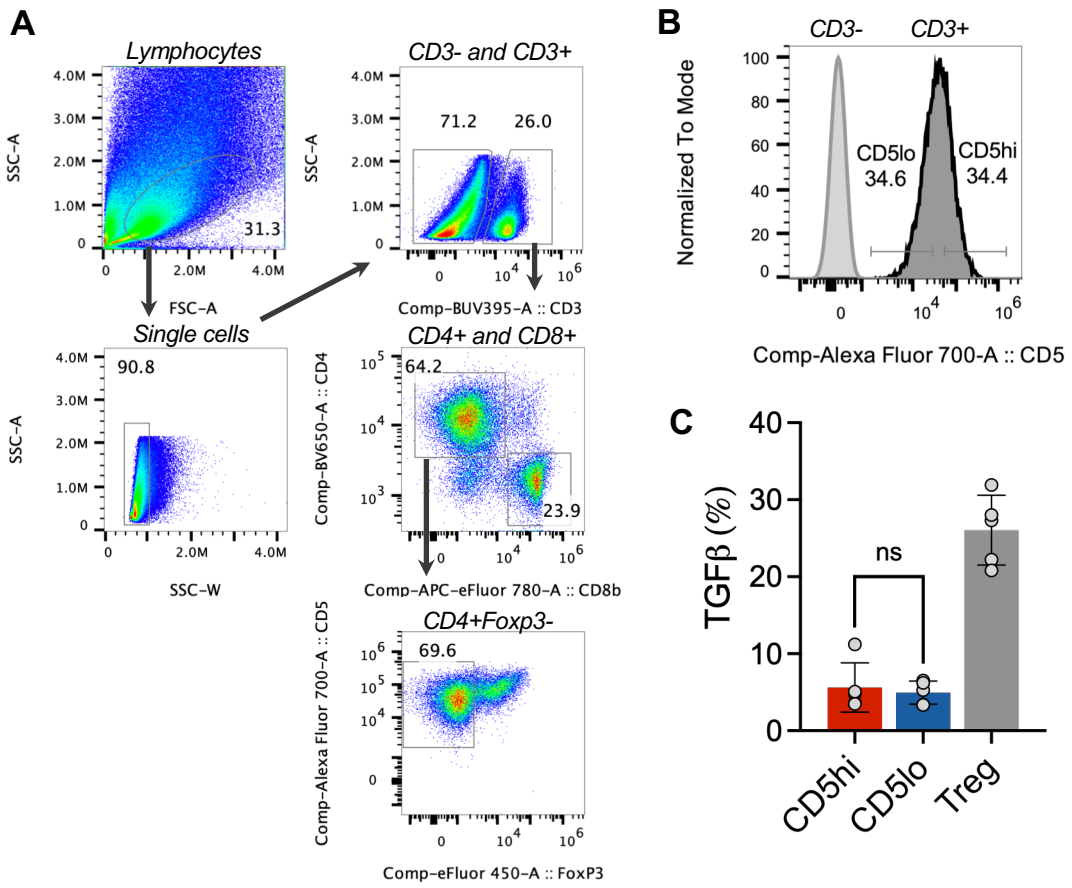



**B**

| | UMAP Population: | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | CD4 Tnaive | CD4 Teff | CD4 Teff/Tr1 | Foxp3 cTreg | Foxp3 eTreg | Foxp3 exTreg | CD8 Tnaive1 | CD8 Tnaive2 | CD8 aTeff | CD8 Tn/Treg |
| | **p1** | **p2** | **p3** | **p4** | **p5** | **p6** | **p7** | **p8** | **p9** | **p10** |
| CD4 | 99.9 | 99.5 | 99.9 | 98.1 | 99.8 | 100 | 0.98 | 0 | 0.49 | 1.88 |
| CD8 | 0 | 0 | 0 | 0.78 | 0.045 | 0 | 98.7 | 99.6 | 98 | 96.7 |
| Foxp3 | 0.31 | 2.04 | 0.63 | **31.2** | **85.1** | 94.8 | 1.95 | 0.51 | 4.45 | **24.4** |
| Bcl6 | 3.08 | 12.8 | 7.09 | 2.41 | 14.1 | 7.79 | 0.65 | 2.93 | 12.7 | 6.1 |
| Tbet | 1 | 65.5 | 83.3 | 29.4 | 53.1 | 42.9 | 12.1 | 5.73 | 93.4 | 14.1 |
| IFNg | 0.57 | **36.1** | **99.9** | 0.85 | 9.73 | 1.3 | 0 | 1.53 | 56 | 5.63 |
| IL10 | 0.057 | 1.83 | **14.4** | 0.078 | 0.45 | 1.3 | 0.33 | 0 | 1.09 | **9.39** |
| IL21 | 0.2 | 10.3 | 22.4 | 0.62 | 8.05 | 2.6 | 0 | 1.27 | 11.6 | 7.51 |
| IL2 | 4.57 | 26.8 | 73 | 1.79 | 2.27 | 1.3 | 1.3 | 0.38 | 9.2 | 12.2 |
| Perforin | 0.2 | 1.25 | 0.31 | 1.4 | 0.73 | 0 | 0.33 | 0.64 | 45.7 | 4.69 |
| CD62L | **45.8** | 0.47 | 0.42 | **38.8** | 9.05 | 0 | **71** | **74.3** | 1.58 | 45.1 |
| CD11ahi | 1.14 | 86 | 90.6 | 5.67 | **64.3** | 76.6 | 0.33 | 0.76 | 42.6 | 0 |
| CD62L/CD11ahi | 0.49 | 3.24 | 1.77 | **31.6** | 9.55 | 11.7 | 5.86 | 2.8 | 41.4 | 0 |
| CCR7 | 1.83 | 0.37 | 0 | **34.5** | 0.41 | 0 | 76.9 | 1.53 | 0 | 34.7 |
| CD69 | 78.6 | 52.2 | 61.9 | 23.4 | 55.7 | 76.6 | 2.93 | 55.1 | 61.9 | 48.8 |
| FR4 | 31.7 | 82.8 | 62.6 | 92.2 | 99.4 | 94.8 | 71.7 | 12.8 | 58.1 | 63.4 |
| PD1 | 2.6 | 86.3 | 85.4 | 95.3 | 84.9 | 93.5 | 89.3 | 5.85 | 95.1 | 96.7 |
| TIGIT | 0.77 | 16.8 | 4.48 | 17.6 | 39.2 | 59.7 | 2.28 | 1.27 | 12 | 82.6 |
| Tim3 | 0.51 | 4.81 | 2.29 | 9.25 | 12.7 | 10.4 | 0.65 | 0.25 | 10.8 | 11.3 |
| Lag3 | 0.2 | 16.6 | 7.82 | 1.48 | 19.8 | 7.79 | 0.33 | 0.13 | 28.2 | 1.88 |
| KLRG1 | 0.086 | 0.63 | 0.63 | 5.52 | 0.64 | **100** | 0.33 | 0.25 | 9.2 | 5.16 |
| CD5 | 31355 | 60848 | 52279 | 43926 | 85705 | 94967 | 16477 | 20979 | 41544 | 11473 |

**Supplemental Figure 1. Functionally distinct T cell subpopulations infiltrate the pancreas during autoimmune diabetes.** Flow cytometric analysis of islet infiltrating T cells from pre-diabetic female NOD mice (n=7). **(A)** Select representative flow plots gated on all T cells. **(B)** UMAP clustering algorithm analysis was performed on concatenated sample that combined equal cell number representation from 7 separate mice. Analysis was performed using UMAP extension in FlowJo based on 22 parameters. Analysis is initially gated on $TCR^+$ T cells. Shown is the average percent positive or MFI of markers based on standard gating strategy of the 7 separate samples after gating on one of the 10 UMAP populations. The heatmaps for these markers was used to define 10 major T cell groups shown in Figure 1. Bolded numbers are key markers used to determine the lineage of the populations. The results are from one experiment.

## Supplemental Figure 2.




**Supplemental Figure 2. (A, B)** Example of gating strategy for CD5high and CD5low T cells. (**A**) Cells are gated on lymphocytes/single cells/CD3+ followed by CD4+/Foxp3- or CD8+. (**B**) CD5high/low gates were set as close as possible to 35% top and bottom CD5 expression. The CD5 gates were determined separately for each sample and for each T cell population (CD4 or CD8). (**C**) Islet infiltrating T cells were stimulated with PMA/ionomycin for 6 hours, and cells were stained with anti-LAP (pro-TGFβ). Since LAP is membrane-bound, BFA and monensin were omitted from the stimulation.

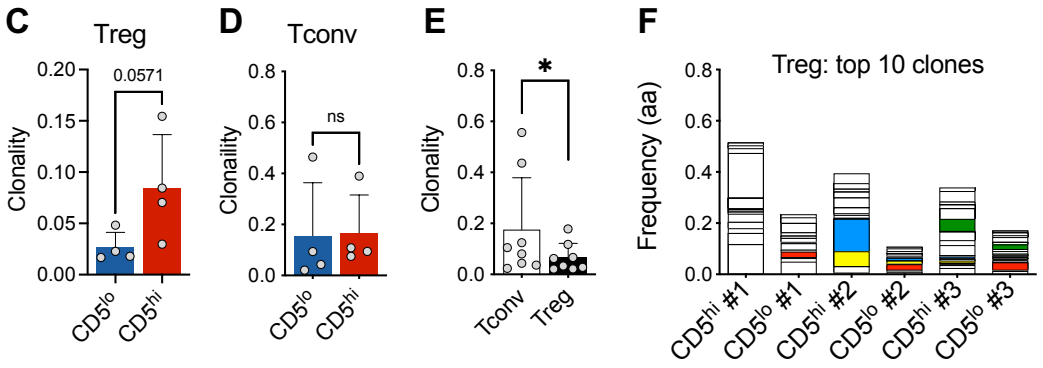
## Supplemental Figure 3.

**A** 1- 100% overlap, 0- no overlap

| Morisita Index (intra mouse) | | | |
|---|---|---|---|
| | $T_{reg}Hi$ | $T_{reg}Lo$ | $T_{conv}Hi$ |
| $T_{reg}Hi$ | | | |
| $T_{reg}Lo$ | 0.1198 | | |
| $T_{conv}Hi$ | 0.0812 | 0.0365 | |
| $T_{conv}Lo$ | 0.0053 | 0.0111 | 0.0778 |

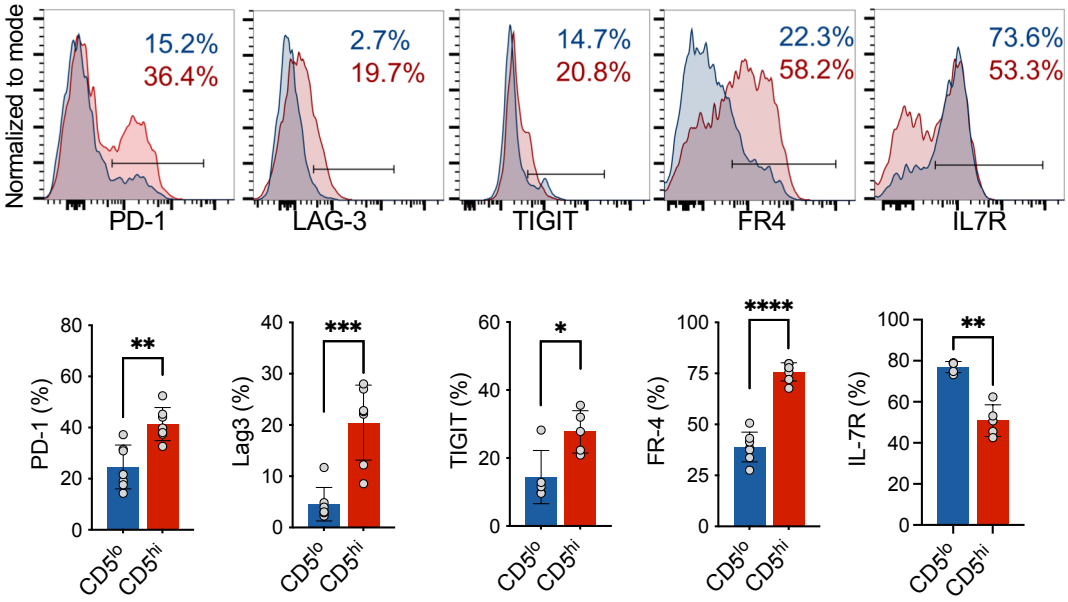
**B**

| Morisita Index (inter mice) | | | | |
|---|---|---|---|---|
| | $T_{reg}Hi$ | $T_{reg}Lo$ | $T_{conv}Hi$ | $T_{conv}Lo$ |
| $T_{reg}Hi$ | 0.0139 | | | |
| $T_{reg}Lo$ | 0.0196 | 0.0914 | | |
| $T_{conv}Hi$ | 0.0015 | 0.0003 | 0.0003 | |
| $T_{conv}Lo$ | 0.0054 | 0.0037 | 0.0003 | 0.0191 |



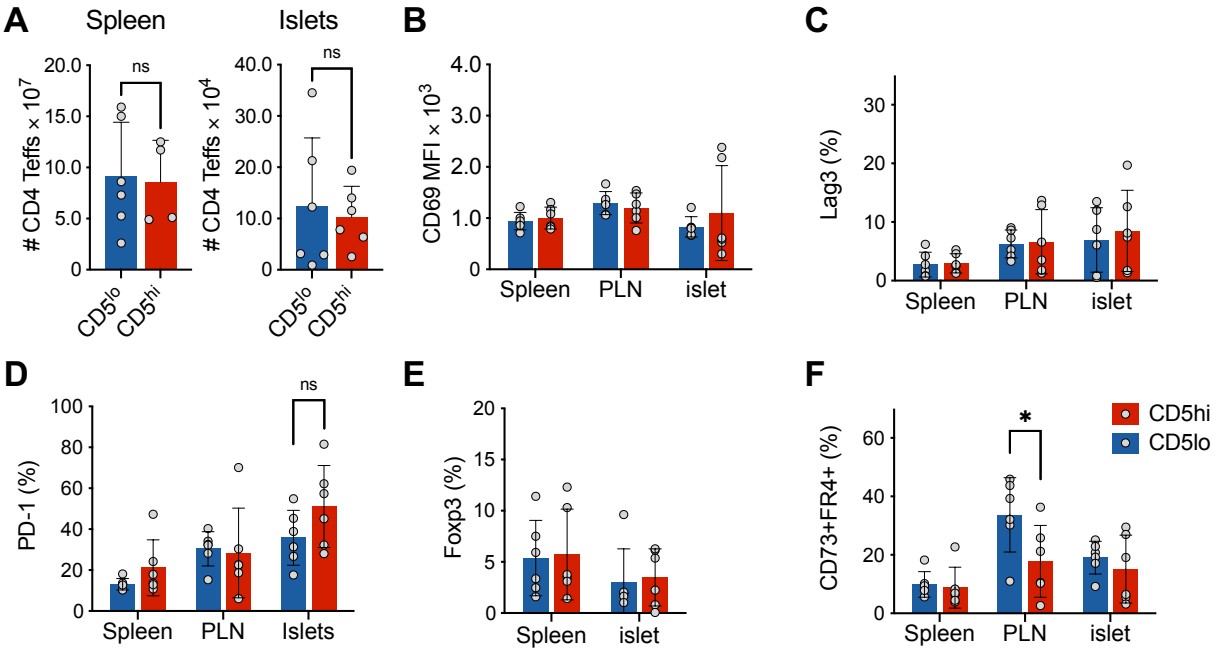

**Supplemental Figure 3. Distinct TCR repertoires of CD5$^{high}$ and CD5$^{low}$ T cells in pancreatic islets.** TCR-beta sequences were obtained from CD4+ conventional (Foxp3$^{GFP-}$) and Treg (Foxp3$^{GFP+}$) cells sorted based on the level of CD5 expression from the infiltrated islets of NOD.Foxp3$^{GFP}$ mice. Sample overlap was calculated using Morisita index for amino acid sequences of TCR-beta sequences between cell populations within each mouse (n=4, **A**) or between samples of different mice (**B**). (**C, D, E**) Clonality of the TCR repertoire was calculated as an inverse of normalized Shannon entropy. (**C** and **D**, intra-mouse analysis, n=4; **E**, inter-mouse analysis, n=8). (**F**) Frequencies of top 10 T cell clones in Treg population. Colored bars designate CDR3 shared between samples. Data are from a single experiment of 4 mice. Statistical analysis was performed using Adaptive software suite or Prism. Error bars designate mean ± SD, *p<0.05; **p<0.01; ***p<0.001.

**Supplemental Figure 4.**




**Supplemental Figure 4. Increased activation and differentiation of CD5$^{high}$ islet infiltrating Tconvs.** Representative flow cytometric analysis and quantification of PD-1, LAG-3, FR-4, TIGIT and IL7R expression on islet infiltrating CD4$^{+}$ Tconvs in 10-14 week old NOD mice. Blue – CD5$^{low}$, Red - CD5$^{high}$. Analysis is gated on CD4$^{+}$TCR$^{+}$Foxp3$^{-}$CD5$^{hi/lo}$ cells. Results displayed are from at least two independent experiments. Statistical analysis was performed using Mann-Whitney test (n=5-7). All data Error bars designate mean ± SD with each point representing a single mouse, *p<0.05; **p<0.01; ***p<0.001; ****p<0.001.

**Supplemental Figure 5**.




**Supplemental Figure 5. Functional phenotype of CD5$^{high}$ and CD5$^{low}$ CD4$^{+}$ islet infiltrating T cells post transfer.** CD5$^{high}$ and CD5$^{low}$ CD4$^{+}$ Tconvs (Foxp3$^{GFP-}$) were sorted from infiltrated islets of NOD.Foxp3$^{GFP}$ mice and transferred into NOD.TCR$\alpha^{-/-}$ recipients. Recipient mice were analyzed at endpoint (at diabetes onset). (**A**) CD4$^{+}$ T cell numbers in spleens or islets of recipient mice. (**B-F**) Combined analysis of 6 separate mice. PLN – pancreatic draining lymph node; islet – isolated pancreatic islets. Results displayed are from at least two independent experiments, n=6. Statistical analysis was performed using Mann-Whitney. Error bars designate mean ± SD with each point representing a single mouse, *p<0.05; **p<0.01.