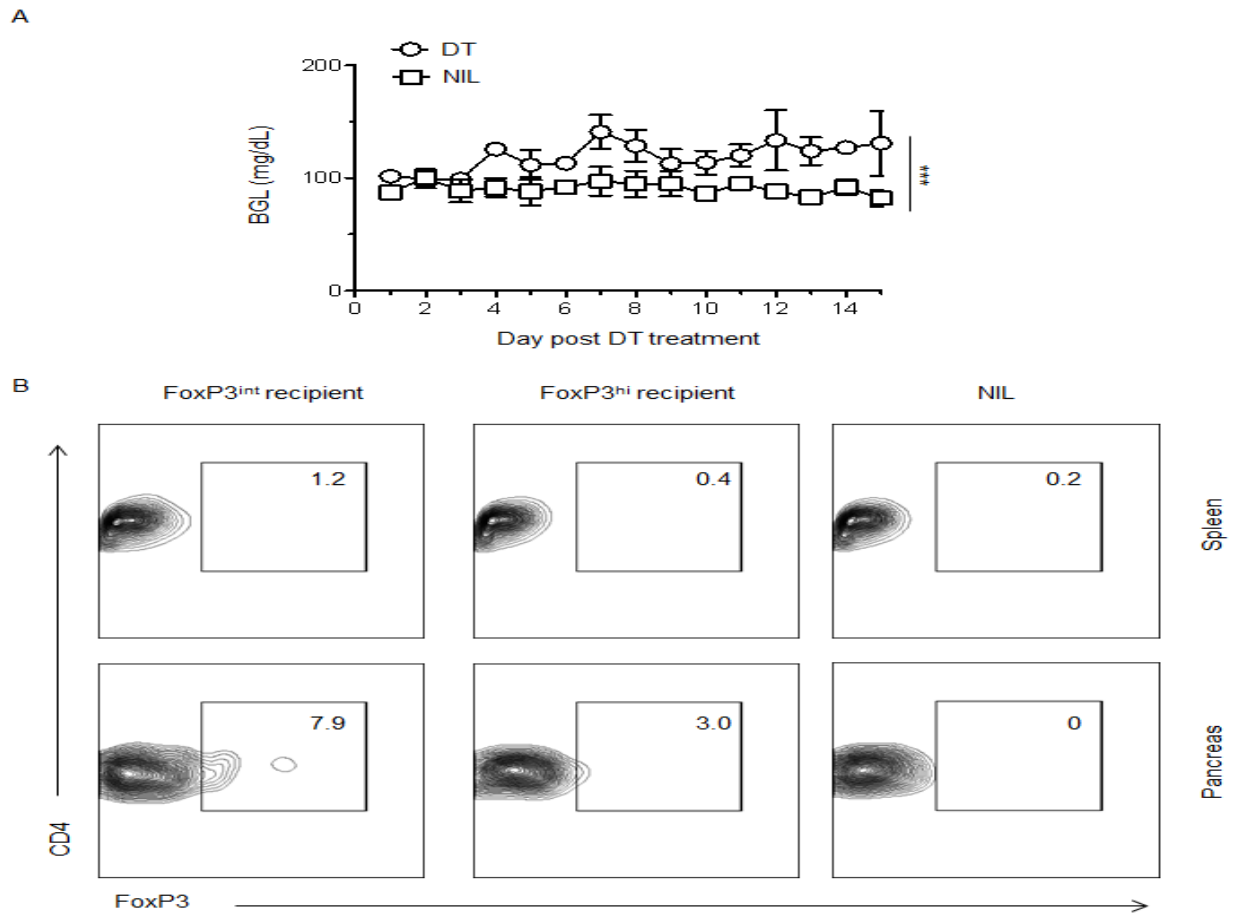


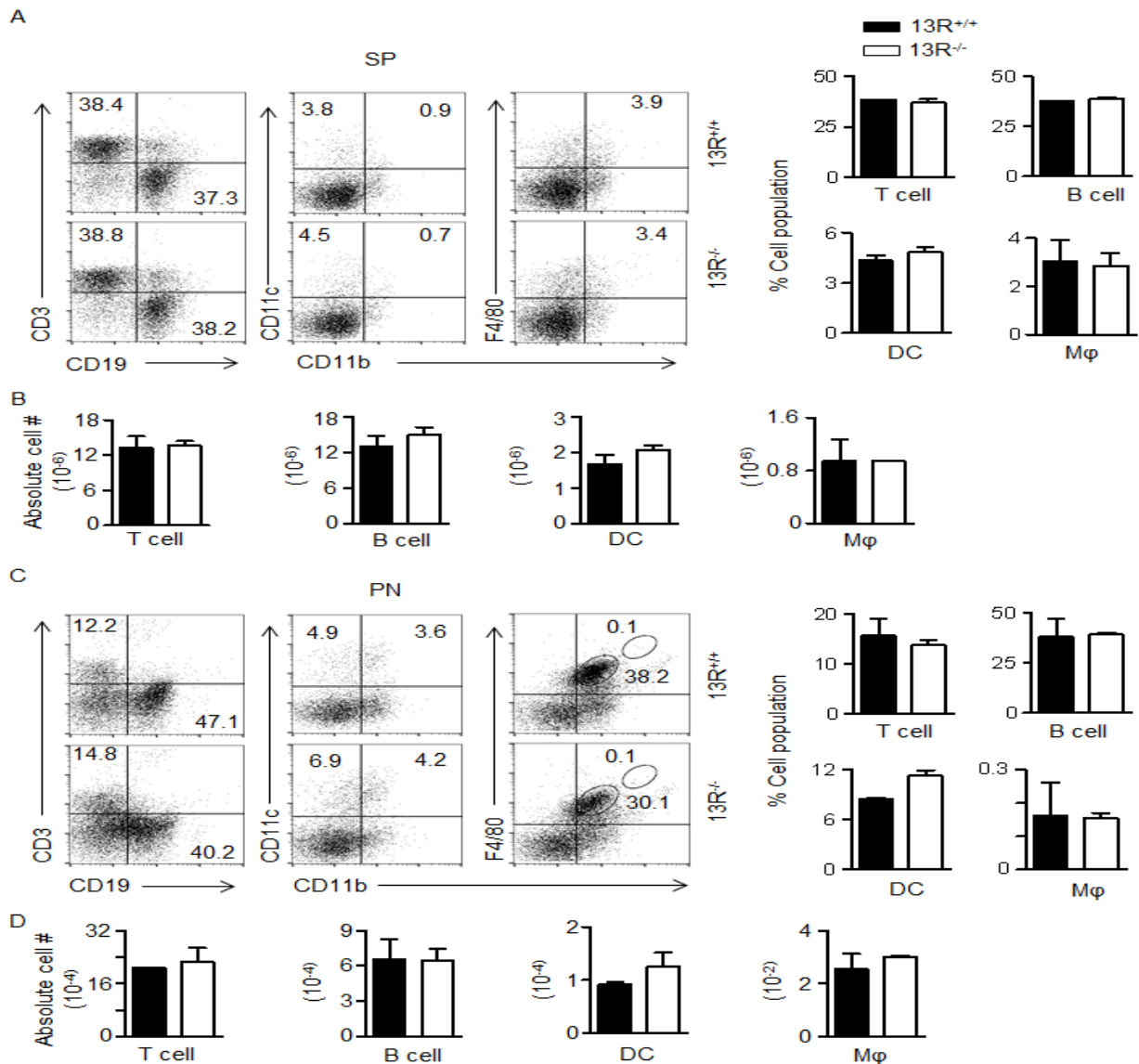
Figure S1. The frequency of FoxP3<sup>hi</sup> Tregs is similar in 13R<sup>+/+</sup> and 13R<sup>-/-</sup> mice.

Cells from the spleen (SP), pancreas (PN) and pancreatic lymph nodes (PLN) of 13R<sup>+/+</sup>FoxP3-GFP or 13R<sup>-/-</sup>FoxP3-GFP female NOD mice were stained with antibodies to CD4, CD25, and mTGFβ and the frequency of CD4<sup>+</sup>CD25<sup>+</sup>TGFβ<sup>-</sup>GFP<sup>hi</sup>(FoxP3<sup>hi</sup>) cells was analyzed by flow cytometry at the age of 6, 7 and 10 weeks. The histograms show representative FACS plots for FoxP3<sup>hi</sup> cells. The bar graphs show the percentage ± SD of FoxP3<sup>hi</sup> cells compiled from 3 independent experiments.

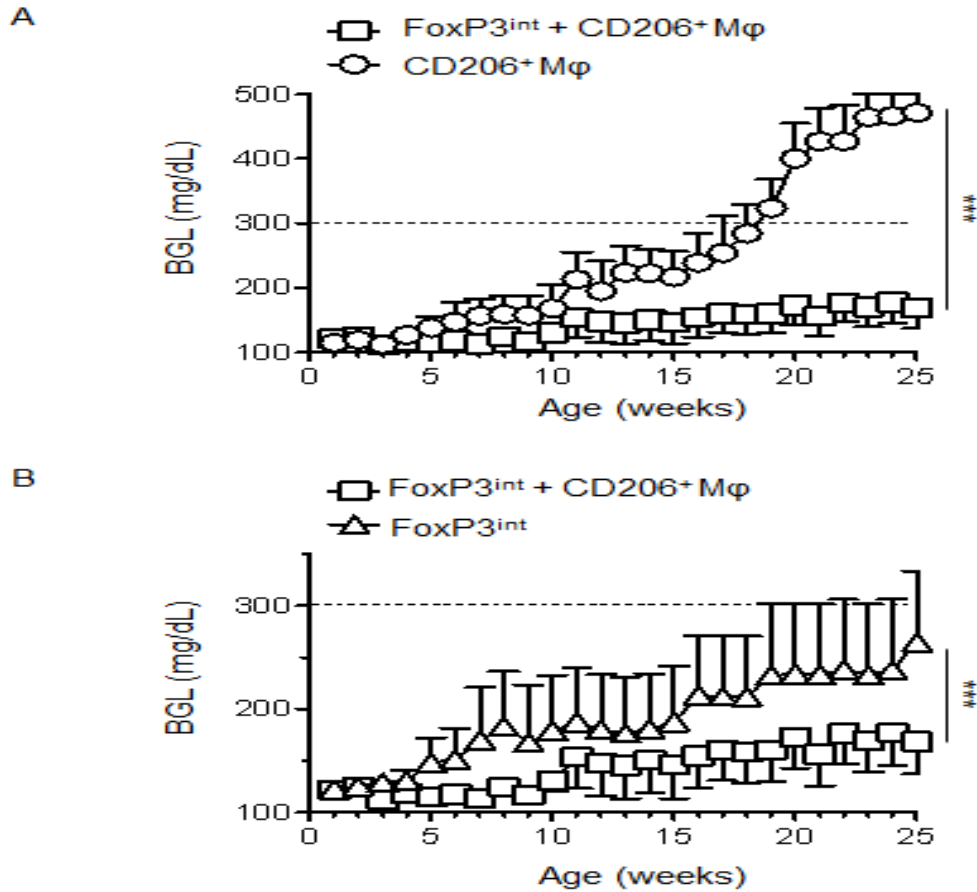


*Figure S2. Depletion of Tregs nullifies delay of T1D while enrichment with FoxP3<sup>int</sup> Tregs repopulate the pancreas with protective T cells.*

(A) Six week old 13R<sup>-/-</sup>FoxP3-DTR NOD mice (n = 4) were given diphtheria toxin (DT) for 10 consecutive days and monitored for BGL daily for 15 days. The graph shows mean BGL ± SEM in DT treated mice in comparison to age matched 13R<sup>-/-</sup>FoxP3-DTR NOD mice that did not receive any DT (NIL). \*\*\*p<0.0001 as determined by Mann-Whitney U test. (B) Pancreatic cells were harvested from 6-wk old female FoxP3-GFP NOD reporter mice and the FoxP3<sup>int</sup> and FoxP3<sup>hi</sup> Tregs were sorted and transferred (2 × 10<sup>5</sup> cells per mouse) into 12-wk old female NOD mice. Control mice that did not receive any Treg transfer (NIL) was included for control purposes. Migration of the transferred Tregs to the spleen (top panel) and pancreas (bottom panel) on day 21 after transfer was assessed by analysis of FoxP3 (GFP) expression by cells gated on CD4 and CD25.



*Figure S3. 13R<sup>-/-</sup> and 13R<sup>+/+</sup> mice display similar frequencies of CD11b cells in the spleen and pancreas. Splenic (A and B) and pancreatic (C and D) cells from 5-week old 13R<sup>-/-</sup> and 13R<sup>+/+</sup> female NOD mice were stained with antibodies to cell specific surface markers and the percentages (A and C) and absolute numbers (B and D) of T cells (CD3), B cells (CD19), DCs (CD11c), and macrophages (CD11b and F4/80) were determined by gating on CD45 (hematopoietic marker) and the cell specific marker. In (A) and (C) the FACS plots show a representative experiment while the bar graphs show the mean percentage  $\pm$  SEM of the indicated cell type compiled from 3 independent experiments. In (B and D) the bar show the mean absolute number  $\pm$  SEM of the indicated cell type compiled from the 3 independent experiments of A and C, respectively.*



*Figure S4. FoxP3<sup>int</sup> and CD206<sup>+</sup> macrophages synergize to suppress T1D.* FoxP3<sup>int</sup> Tregs and CD206<sup>+</sup> Mφ from the pancreas of 13R<sup>-/-</sup> female NOD mice were transferred ( $5 \times 10^5$  CD206<sup>+</sup> Mφ and  $4 \times 10^5$  Tregs per mouse) together into 12-week old 13R<sup>+/+</sup> mice and the recipients were monitored for BGL twice a week for 25 weeks. (A) Shows the mean BGL  $\pm$  SEM for mice recipient of FoxP3<sup>int</sup> Tregs and CD206<sup>+</sup> Mφ (FoxP3<sup>int</sup> + CD206<sup>+</sup> Mφ, n = 6) in comparison to hosts recipient of CD206<sup>+</sup> Mφ alone (CD206<sup>+</sup> Mφ, n = 9). (B) Shows the mean BGL  $\pm$  SEM for mice recipient of FoxP3<sup>int</sup> Tregs and CD206<sup>+</sup> Mφ (FoxP3<sup>int</sup> + CD206<sup>+</sup> Mφ, n = 6) in comparison to hosts recipient of FoxP3<sup>int</sup> Tregs alone (FoxP3<sup>int</sup> Tregs, n = 8). \*\*\*p<0.0001 as determined by Mann-Whitney U test.