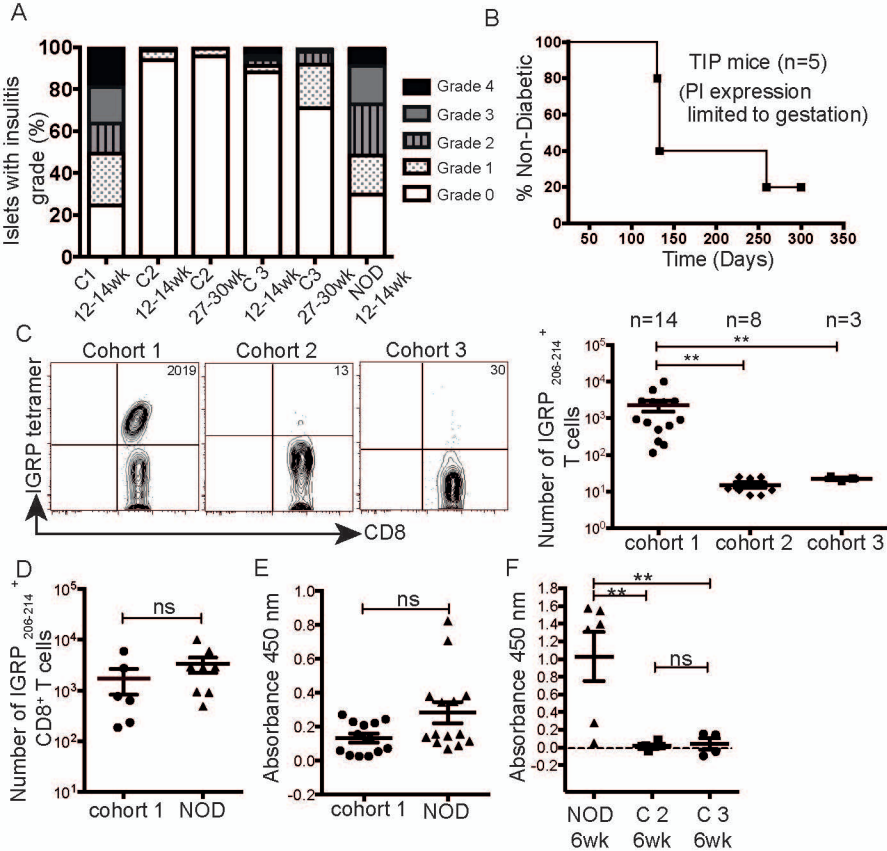
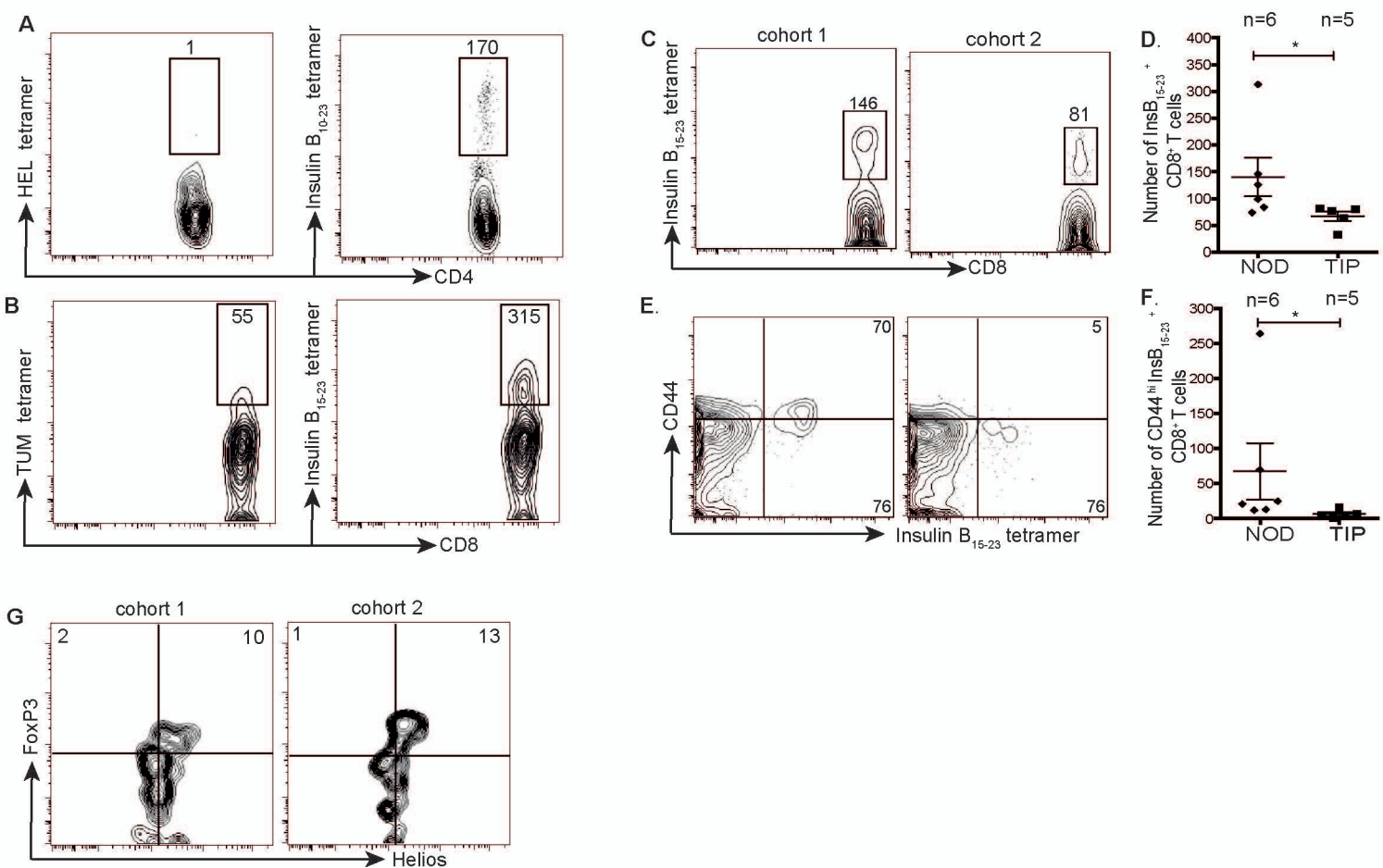


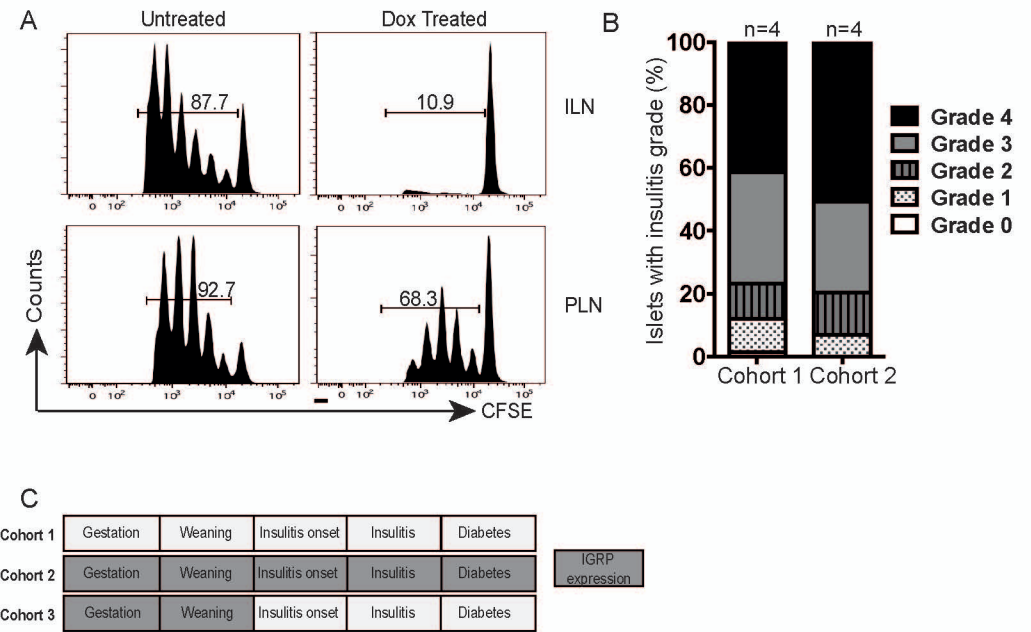


Supplementary Figure 1: Insulinitis, diabetes, enumeration of IGRP specific T cells and IAA in TIP mice (A) Histological grading of insulinitis development in the pancreata of 12-14 week old TIP mice (cohorts 1-3), 27-30 week old TIP mice (cohorts 2 and 3) and 12-14 week old WT NOD mice. (B) Incidence of diabetes in TIP with PI expression limited to gestation. (C) IGRP $^{206-214}$ specific CD8 $^{+}$ T cells from pooled peripheral lymphoid organs (PLO) of 12-15 week old TIP and WT NOD mice were stained with tetramer and enriched using magnetic beads and enumerated by flow-cytometry. Representative FACS plots (left) and (right) enumeration of IGRP $^{206-214}$ tetramer $^{+}$ CD8 $^{+}$ T cells from PLO of TIP mice in cohorts 1-3. (D) Enumeration of IGRP $^{206-214}$ tetramer $^{+}$ CD8 $^{+}$ T cells from PLO of WT-NOD mice TIP mice (cohort 1). (E) Sera from 12-15 week old WT-NOD mice and TIP mice (cohort 1) and (F) Sera from 6 week old WT-NOD mice and TIP mice (cohorts 2 and 3) were tested for presence of insulin autoantibodies (IAA) by ELISA assay. Absorbance values at 450nm are plotted. Data plotted as Mean $\pm$ SEM ** $P < 0.01$, ns = not significant. Data compared using 2-tailed unpaired t -test (C,D,E) or One-way ANOVA with Tukey's multiple comparisons test (F).



Supplementary Figure 2: Tetramer specificity, enumeration of insulin-specific CD8⁺ T cells and Tregs in TIP mice

T cells from pooled peripheral lymphoid organs (PLO) of 12-15 week old WT NOD mice were stained with I-A g7 -Insulin B10-23 and control I-Ag7-Hen Egg Lysozyme (HEL) tetramer to enrich insulin-specific CD4⁺ T cells or H-2Kd-Insulin B15-23 and control H-2Kd-TUM tetramer to enrich insulin-specific CD4⁺ and CD8⁺ T cells respectively using magnetic beads followed by flow-cytometry. Representative FACS plot (A) showing specificity of I-Ag7 Insulin tetramer (right) and lack of enrichment of CD4⁺ T cells due to lack of binding by HEL tetramer (left) (B) Representative FACS plot showing specificity of H-2Kd InsulinB₁₅₋₂₃ tetramer (right) and no enrichment of CD8⁺ T cells due to lack of binding by TUM tetramer (left). Representative FACS plots (C,E) and enumeration (D,F) of Insulin B₁₅₋₂₃ tetramer⁺ CD8⁺ T and CD44^{hi} Insulin B₁₅₋₂₃ tetramer⁺ CD8⁺ T cells from PLO of TIP mice in cohorts 1 & 2. (G) Representative FACS plot showing expression of Helios and FoxP3 on Insulin B10-23 tetramer⁺, CD4⁺ T cells, enriched from pooled PLN of TIP mice. Values in FACS plot show absolute numbers in the indicated gate. Each dot in the scatter plot (Mean ± SEM) represents data from an individual mouse. *P< 0.05. Mann-Whitney U test was used to compare the groups.



Supplementary Figure 3: Functional and conditional expression of IGRP in T1I mice

(A) Dox regulated and functional expression of IGRP in the APC of T1I mice. CFSE labeled CD8⁺ T cells from a NOD 8.3 mouse were transferred into 8-week old T1I mice that were untreated, or fed Dox for 1 week to turn off IGRP transgene (n=2-3 per group). Recipients were sacrificed 3 days post-transfer and their inguinal (ILN) and pancreatic (PLN) lymph nodes were analyzed for CFSE⁺ cells. Numbers within each histogram plot indicate percentage of CFSE low cells. Data is representative of 2 independent experiments. (B) Histological grading of insulinitis development in pancreata of 15-18 week old T1I mice (cohort 1 and 2) (C) Study design showing the different cohorts of T1I mice with duration of IGRP expression indicated by the shaded area. IGRP expression was turned off by Dox administration in cohorts 1 and 3 as described in Fig.1(A)