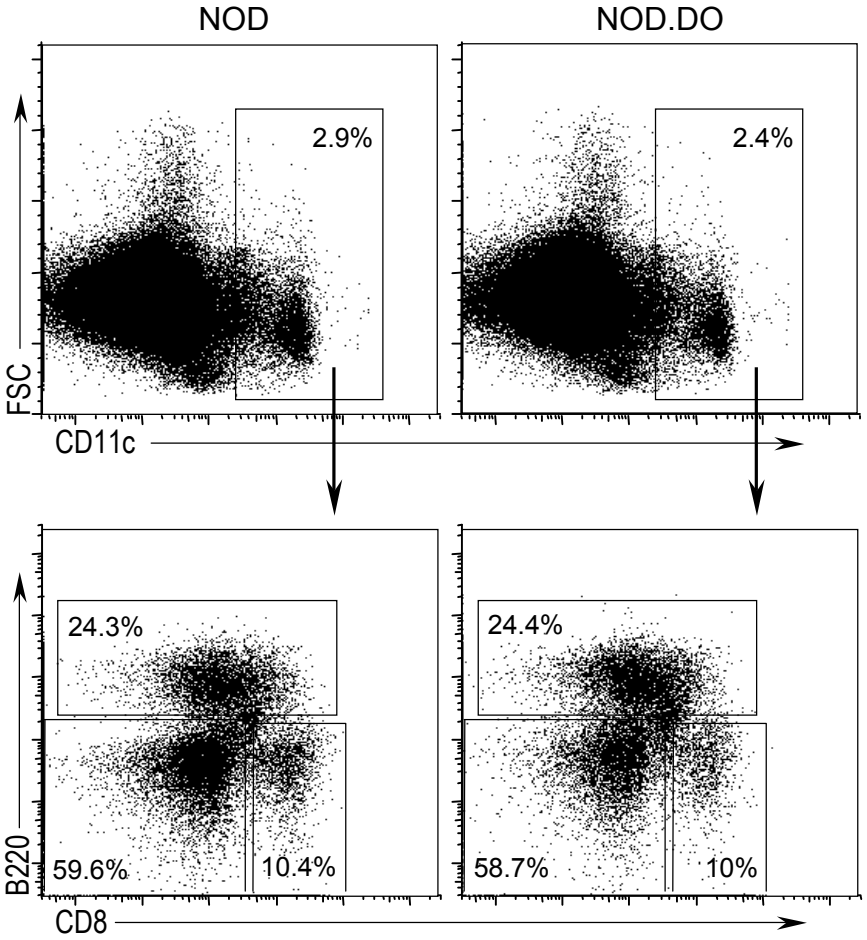
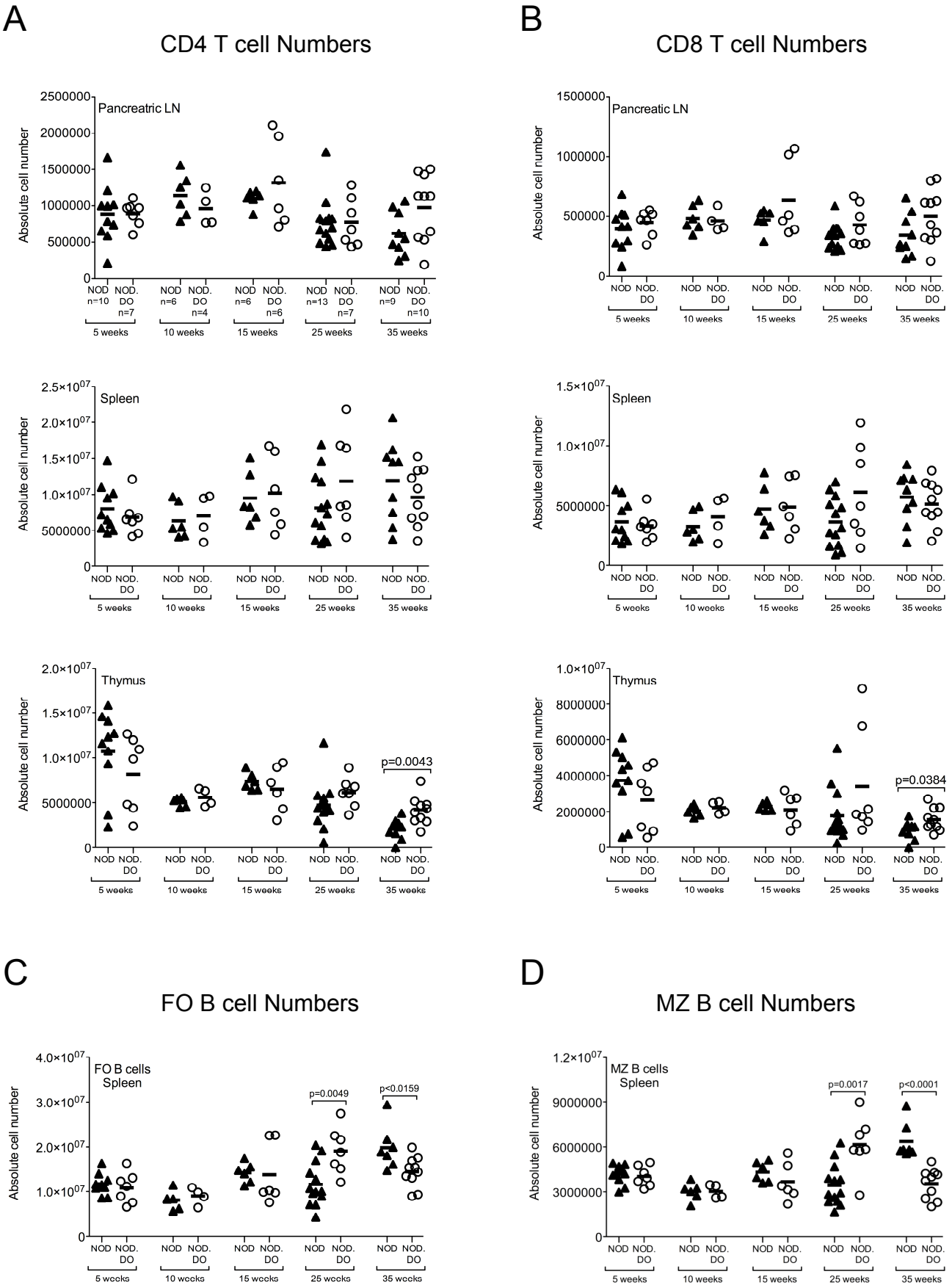


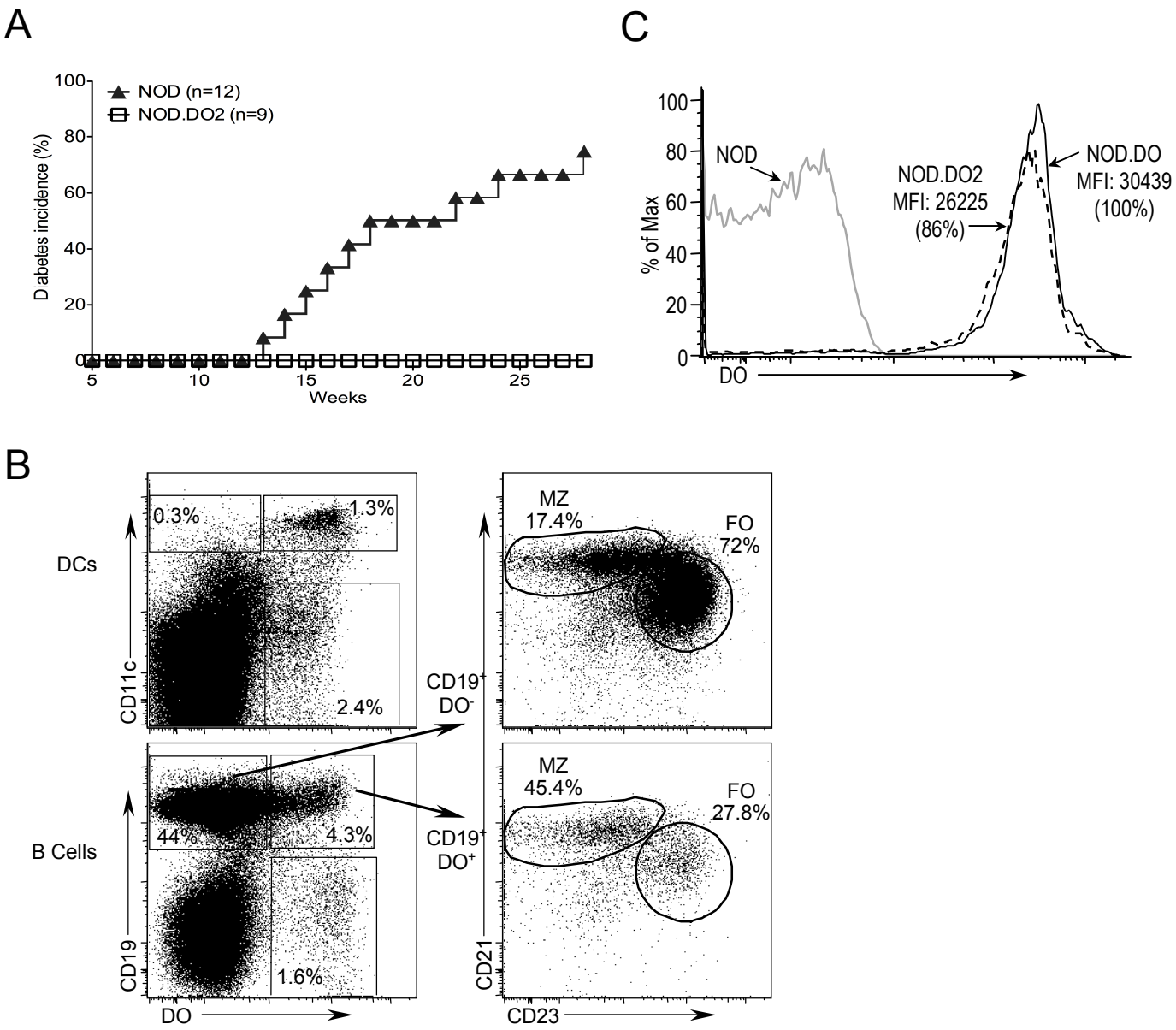
Supplemental Figure 1
DO expression does not alter DC subset development**.** Splenocytes from 6 week old NOD and NOD.DO mice were stained with mAbs specific for the DC markers CD11c, B220 and CD8. Top plots show forward scatter versus CD11c. Bottom plots are gated for CD11c and show B220 versus CD8. Staining is representative of 3 mice of each genotype. One of three similar experiments.



Supplemental Figure 2

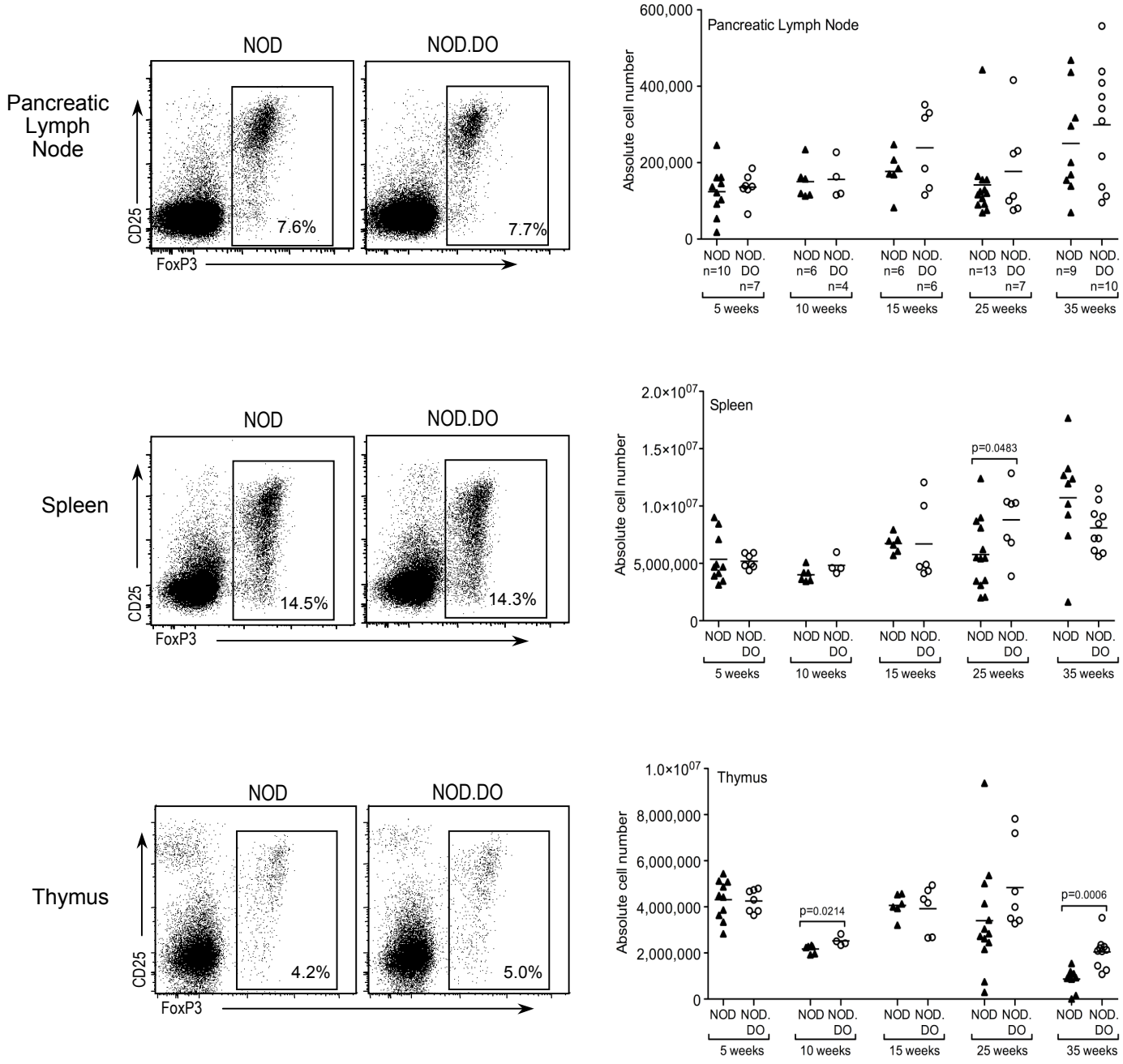
Supplemental Figure 2

NOD and NOD.DO mice have similar numbers of T cells and B cells. PLN, splenic and thymic cells were stained with a mAb specific for (**A**) CD4 or CD8 (**B**) prior to analysis by flow cytometry. The absolute number of CD4 cells for multiple mice at 5, 10, 15, 25 and 35 weeks of age is shown. The relative frequency (percentage) of T cells in the PLNs, spleens and thymuses of NOD and NOD.DO mice were also similar. (C,D) Splenocytes from NOD and NOD.DO mice were stained with mAbs to CD19, CD21, and CD23 to identify follicular (FO; $CD19^{+}CD21^{low}CD23^{+}$) (**C**) and marginal zone (MZ; $CD19^{+}CD21^{+}CD23^{low}$) (**D**) B cells prior to analysis by flow cytometry. The absolute number of FO B cells for multiple mice at each time point are shown. The relative frequency (percentage) of FO and MZ B cells in the spleens of NOD and NOD.DO mice were also similar. Each symbol represents an individual mouse and small horizontal bars indicate the mean. Numbers of mice analyzed at each time point are indicated on the graph for the PLN. *P* values were computed using two-tailed, unpaired Student's t-test. Only significant differences are shown.



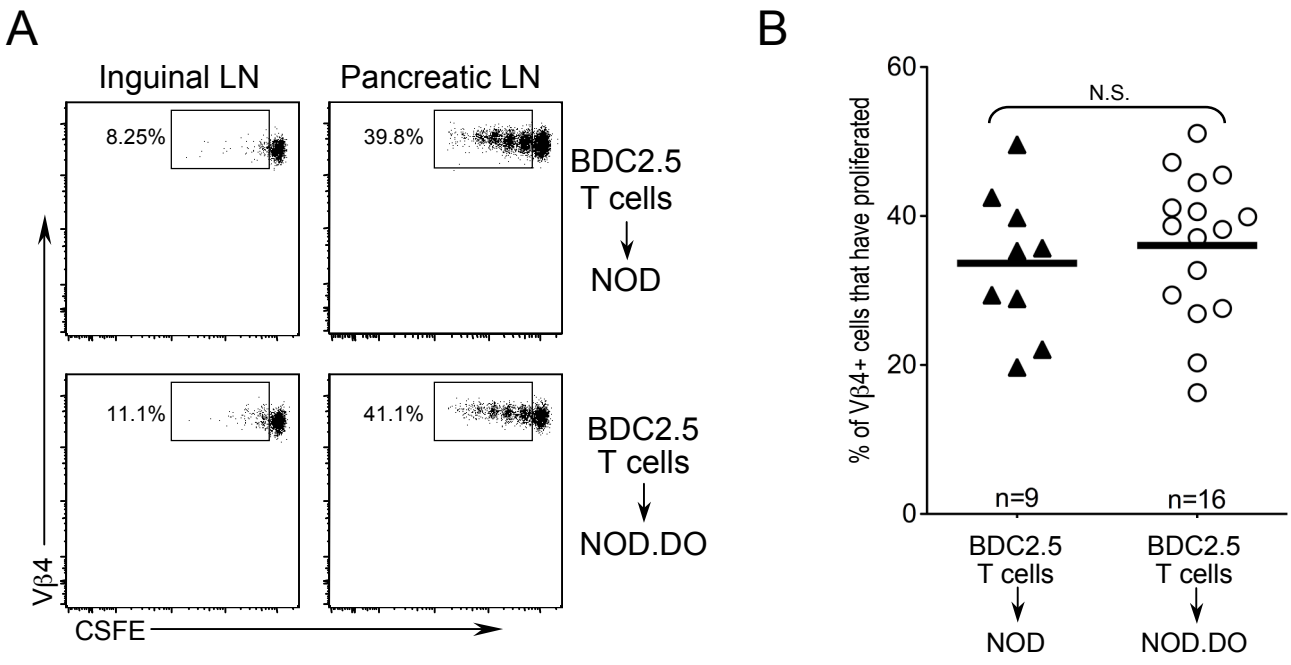
Supplemental Figure 3

NOD.DO2 mice are protected from diabetes. (**A**) Diabetes development was monitored in female NOD.DO2 and littermate control non-Tg mice by measurement of urine glucose levels. (**B**) Splenocytes from NOD (n=3) and NOD.DO2 (n=3) mice were stained with a mAb specific for CD11c (top) or CD19, CD21, CD23 and then stained intracellularly with a mAb to DO prior to analysis by flow cytometry. Left plots show total splenocytes. Right plots are gated on the DO^+ ($CD19^+DO^+$) and DO^- B cells ($CD19^+DO^-$) and further fractionated into follicular (FO; $CD19^+CD21^{low}CD23^+$ and marginal zone (MZ; $CD19^+CD21^{high}CD23^{low}$) B cells. The numbers indicate the percent of cells falling within each gated region. Data are representative of 3 independent experiments. (C) Comparison of DO levels in NOD.DO and NOD.DO2 DCs. The MFI for DO staining in DCs for each line is indicated and percentage below is the level of DO staining relative to NOD.DO. Data are representative of three independent experiments.



Supplemental Figure 4

NOD and NOD.DO mice have similar numbers of Treg cells. PLN, splenic and thymic cells were stained with mAbs specific for CD4, CD25 and intracellularly for FoxP3 to identify Tregs ($CD4^+FoxP3^+$). Plots (left) show representative staining for NOD and NOD.DO mice for each organ. Graphs (right) show the absolute number of CD4 cells that are Tregs for multiple mice at 5, 10, 15, 25 and 35 weeks of age. Each symbol represents an individual mouse and small horizontal bars indicate the mean. Numbers of mice analyzed at each time point are indicated on the graph for the PLN. The relative frequency (percentage) of Treg cells in the PLNs, spleens and thymuses of NOD and NOD.DO mice were also similar. *P* values were computed using two-tailed, unpaired Student's t-test. Only significant differences are shown



Supplemental Figure 5
Presentation of islet-derived BDC2.5-specific Ag in the PLN of NOD.DO mice is similar in NOD and NOD.DO mice. (**A**) Purified BDC2.5 TCR Tg T cells were labeled with CFSE and transferred into 9 (experiment 1) or 13 (experiment 2) week old NOD and NOD.DO recipient mice (2 x 10^6/mouse). The pancreatic and inguinal LNs were removed 72 hr later and proliferation was measured using flow cytometry by measuring dilution of CFSE in $CFSE^+CD4^+V\beta4^+$ cells. Numbers next to the gates indicate the percentage of BDC2.5 TCR Tg T cells that underwent at least one round of proliferation. (**B**) Percentage of total $CFSE^+CD4^+V\beta4^+$ cells that proliferated in the PLN multiple of NOD and NOD.DO mice. Each symbol represents an individual mouse and small horizontal bars indicate the mean. *P* values were computed using two-tailed, unpaired Student's t-test. Data were combined from two independent experiments.