Supplementary Information:

Antibodies

The following antibodies were purchased from BioLegend (San Diego, CA): FITC/PE/PerCP-Cy5.5 anti-B220 (RA3-6B2), PE anti-CD103 (2E7), PE-Cy7 anti-CD11b (M1/70), APC-Cy7 anti-CD11c (N418), APC anti-CD205 (NLDC-145), PE-Cy7 anti-CD4 (RM4-5), BV510/APC anti-CD45 (30-F11), Pacific Blue anti-CD45.2 (104), FITC/PerCP-Cy5.5 anti-CD8a (53-6.7), PerCP-Cy5.5/APC anti-F4/80 (BM8), Alexa Fluor 488 anti-I-Ak (40F), Alexa Fluor 647 anti-Siglec H (ebio440c), and PE anti-VB8.1/8.2 (KJ16-133.18). The following antibodies were purchased from eBioscience (San Diego, CA): APC anti-CCR7 (4B12), FITC/V450 anti-CD19 (1D3), APC anti-CD25 (PC61.5), PerCP-Cy5.5 anti-CD45.1 (A20), PerCP-Cy5.5 anti-CD45.2 (1D4), and PE anti-FoxP3 (FJK-16s). The following antibodies were purchased from BD Biosciences: FITC/APC anti-CD3e (145-2C11), V450 anti-CD45.1 (A20), FITC anti-I-AKß (10-3.62), and PE anti-Vß4 (KT4). Alexa Fluor 488/Pacific Blue I-Ag7 (AG2.42.7) was made in our laboratory (Suri et al., 2002) .

RNA Isolation and Real Time PCR

For whole islets and sorted cell populations, total RNA was isolated using the Ambion RNAqueous-Micro Kit (Life Technologies, Carslbad, CA) following the manufacturer's instructions. RNA was quantified by OD260 using Nanodrop (Thermo Fisher Scientific, Waltham, MA). For microarray analysis, RNA integrity and quantification was further validated using a Bioanalyzer 2100 or a Tapestation 2200 (Agilent Technologies, Santa Clara, CA). cDNA was made from total RNA using TaqMan Reverse Transcription Reagents (Life Technologies) following the random hexamer protocol. Primers for quantitative RT-PCR were designed using the PrimeTime predesigned qPCR assays (IDT DNA, Coralville, IA), except for the *Ccl2*, *Ccl5*, *Ccl19*, *Ccl21*, *Ccl25*, and *Cxcl10* probes, which were designed using Primer Bank (http://pga.mgh.harvard.edu/primerbank/). TaqMan primers and probes for detecting 18S rRNA was obtained from Life Technologies. PrimeTime primers employed 5'-nuclease detection technology and Primer Bank primers employed SYBR Green I detection technology. PCR was performed using SSOFast Probes Supermix or SsoFast EvaGreen Supermix (Bio-Rad, Hercules, CA) on a StepOnePlus Real-Time PCR system running StepOne Software. Quality control and relative expression quantification for qPCR was performed by the StepOne 2.1 software.

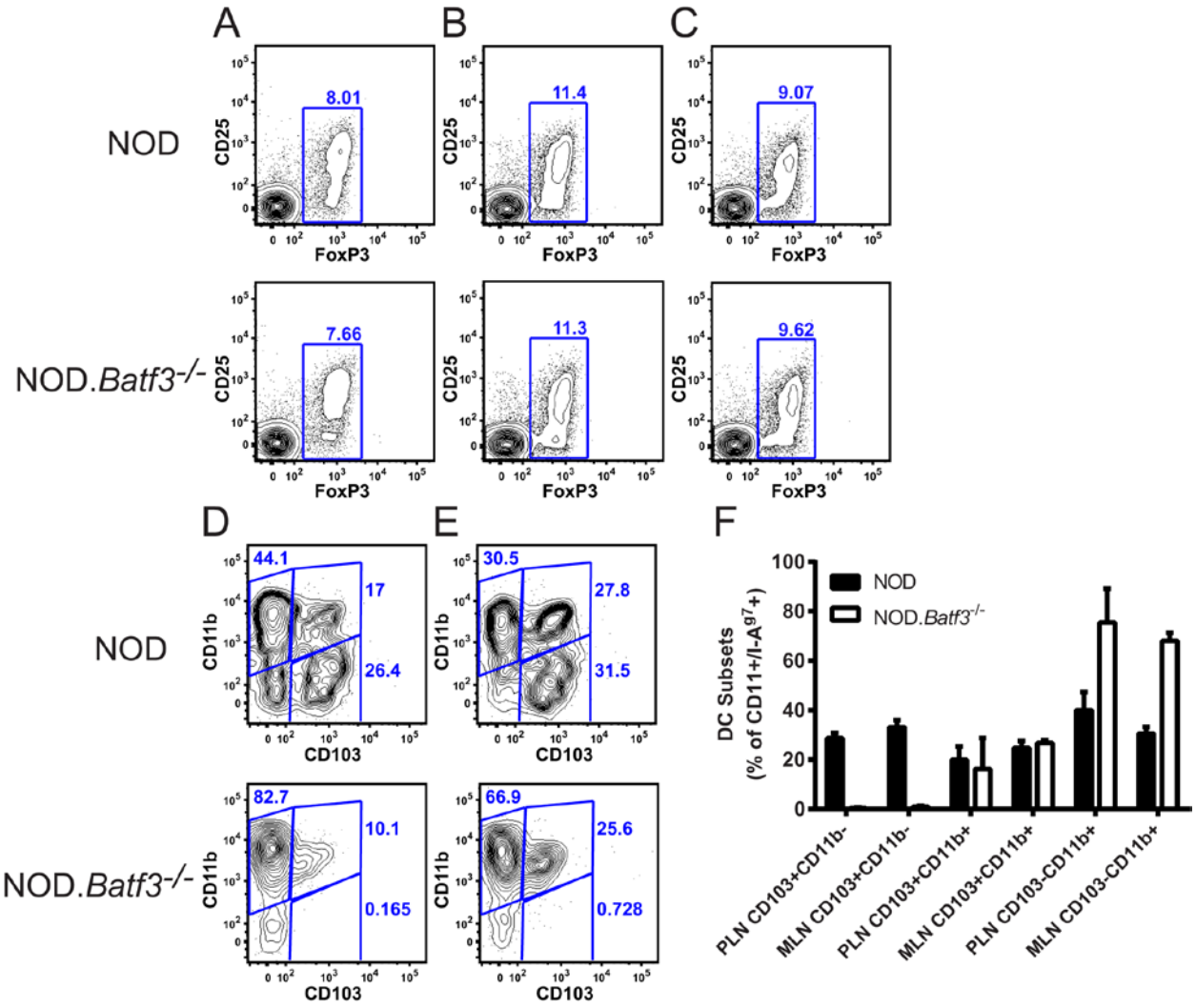

Figure S1: Characterization of NOD and NOD.*Batf3*$^{-/-}$ T regulatory cell and dendritic cell populations. Percentage of FoxP3^{+} CD4^{+} CD3e^{+} CD25^{+} cells in the (A) thymus, (B) pancreatic lymph nodes, and (C) mesenteric lymph nodes harvested from NOD and NOD.*Batf3*$^{-/-}$ mice (Gated on CD45^{+}). Percentage of CD11b^{+} CD103^{-} (upper left quadrant), CD11b^{+} CD103^{+} (upper right quadrant), and CD11b$^{-/lo}$ CD103^{+} (lower right quadrant) cells in the (D) pancreatic lymph nodes and (E) mesenteric lymph nodes harvested from NOD and NOD.*Batf3*$^{-/-}$ mice (gated according to the strategy indicated in Figure 2). (F) Representative graph of data from (D-E). All mice were 6 weeks old. Representative flow cytometry plots and cumulative data from three biological replicates. (error bars, SD).

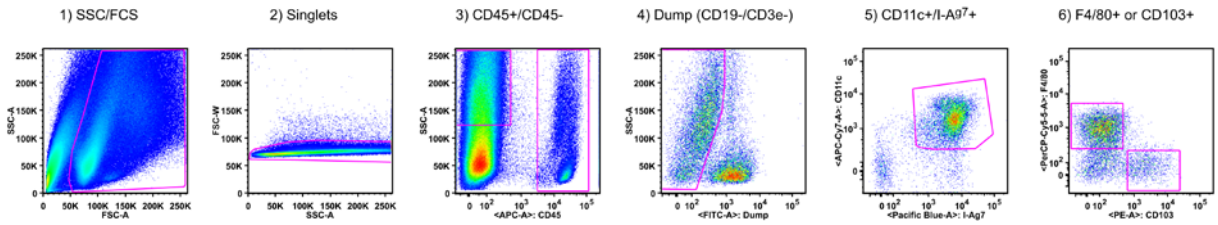

Figure S2. Gating strategy for sorting mouse islets. Single cell myeloid suspensions from islets of Langerhans gated in the order listed in Figure 2.

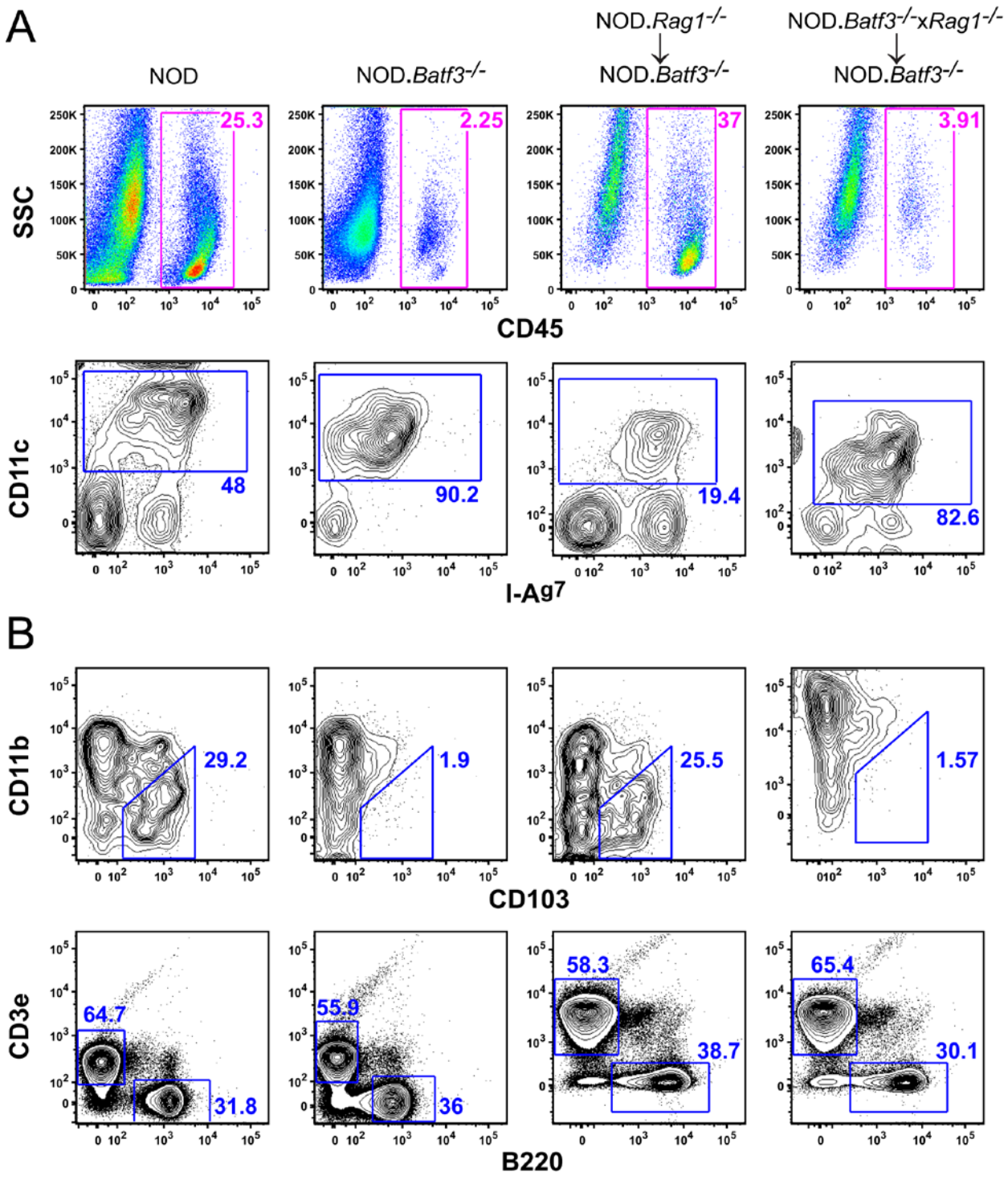

Figure S3. Bone marrow reconstitution of NOD.*Batf3*$^{-/-}$ mice. (A) Flow cytometry gating strategy and islet myeloid population and (B) pancreatic lymph node analysis from the NOD, NOD.*Batf3*$^{-/-}$, and NOD.*Batf3*$^{-/-}$ that received either NOD.*Rag1*$^{-/-}$ or NOD.*Batf3*$^{-/-}$x *Rag1*$^{-/-}$ bone marrow 24 weeks prior to being harvested.

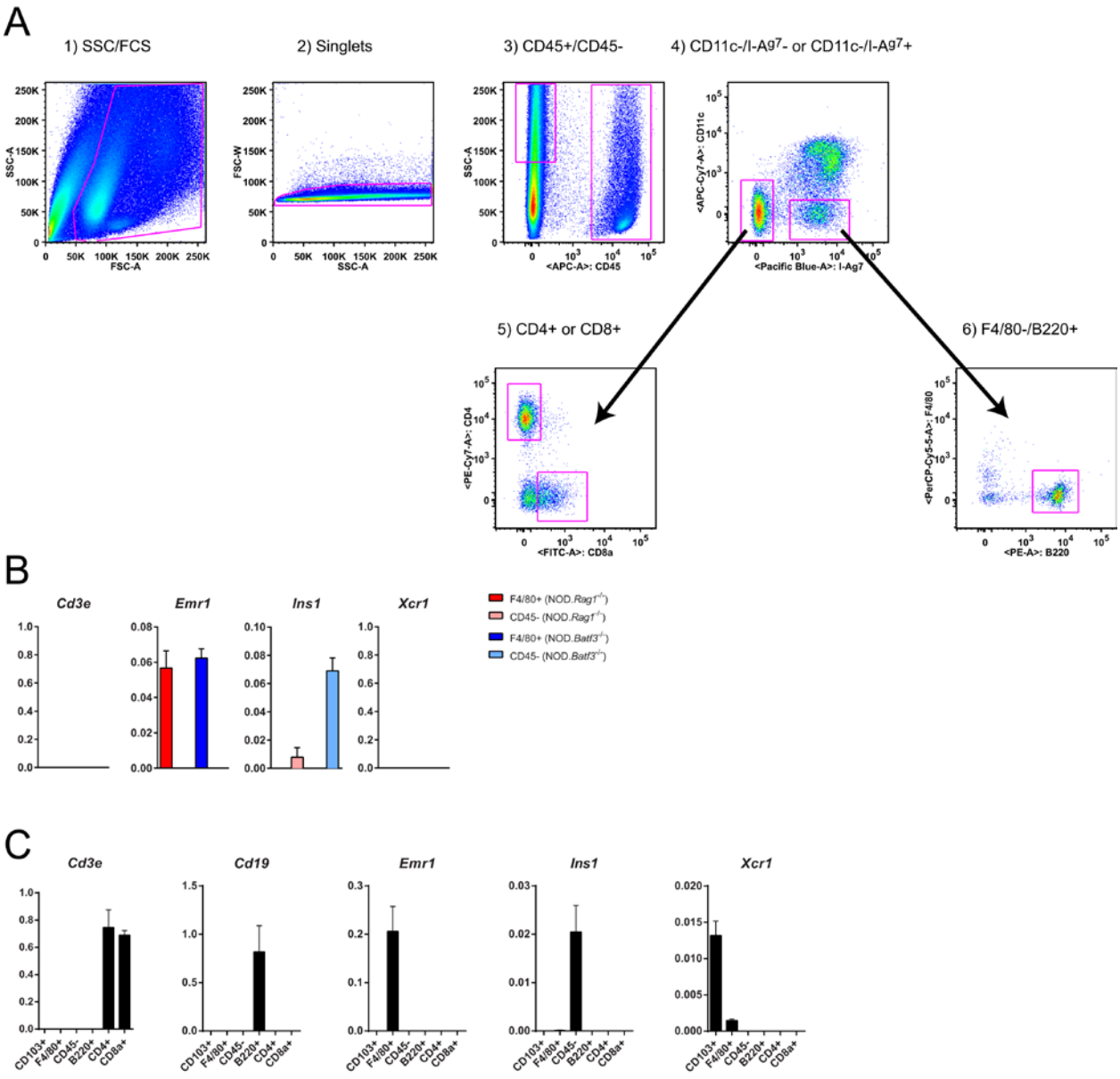

Figure S4. Quantitative RT-PCR of selected cell specific markers. (A) Lymphoid cell populations were sorted as shown. (B) NOD.*Rag1*$^{-/-}$, NOD.*Batf3*$^{-/-}$, and (C) NOD islet cells were sorted and analyzed as in Figure 5.

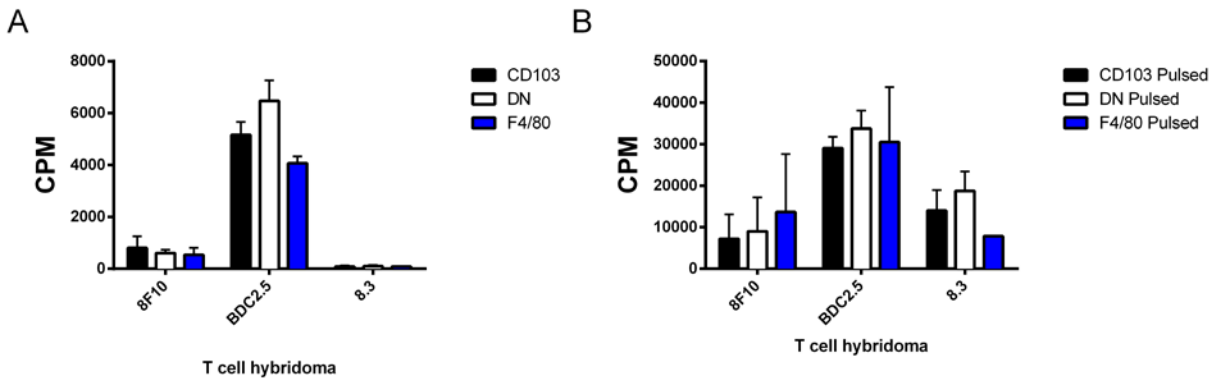

Figure S5. Presentation of diabetogenic epitopes to T cell hybridomas by sorted islet APC subsets. $CD103^{+}$, $F4/80^{+}$ or $CD103^{-}$ $F4/80^{-}$ APCs were isolated from the islets of 10 week NOD female mice according to the gating strategy indicated in Figure 2. The indicated T cell hybridomas ($5x10^{4}$) were incubated with the indicated APC subset ($1x10^{3}$) overnight and then assayed for IL-2 production as described in the main methods. APC were either (A) left untreated or (B) pulsed with 10µM cognate peptide.

Table S1. Microsatellite validation of 129.*Batf3*$^{-/-}$ backcross to the NOD background. Three control strains (129X1, 129S6, and 129P2) and 8 NOD.*Batf3*$^{-/-}$ mice at generation N6 were assayed using 111 PCR probes to the indicated microsatellites. Yellow shading indicates 129S6 loci and purple indicates NOD loci. Numbers in the box are the size of the expected fragments.

Table S2. Single nucleotide polymorphism analysis of 23 IDD loci in NOD.*Batf3*$^{-/-}$ compared to 129 and NOD. The indicated IDD loci were tested for 129S6 vs NOD sequence identity by the Jackson Laboratories. See http://jaxservices.jax.org/genome/snp.html for details.

Supplemental Material Reference:

Suri, A., Vidavsky, I., van der Drift, K., Kanagawa, O., Gross, M.L., and Unanue, E.R. (2002). In APCs, the autologous peptides selected by the diabetogenic I-Ag7 molecule are unique and determined by the amino acid changes in the P9 pocket. J. Immunol. Baltim. Md 1950 *168*, 1235–1243.