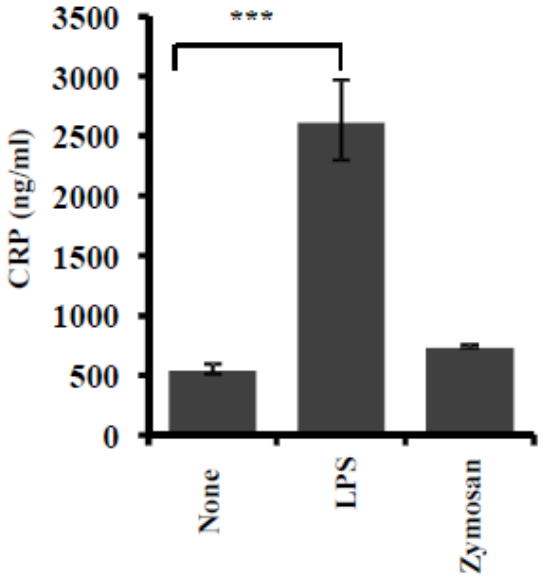
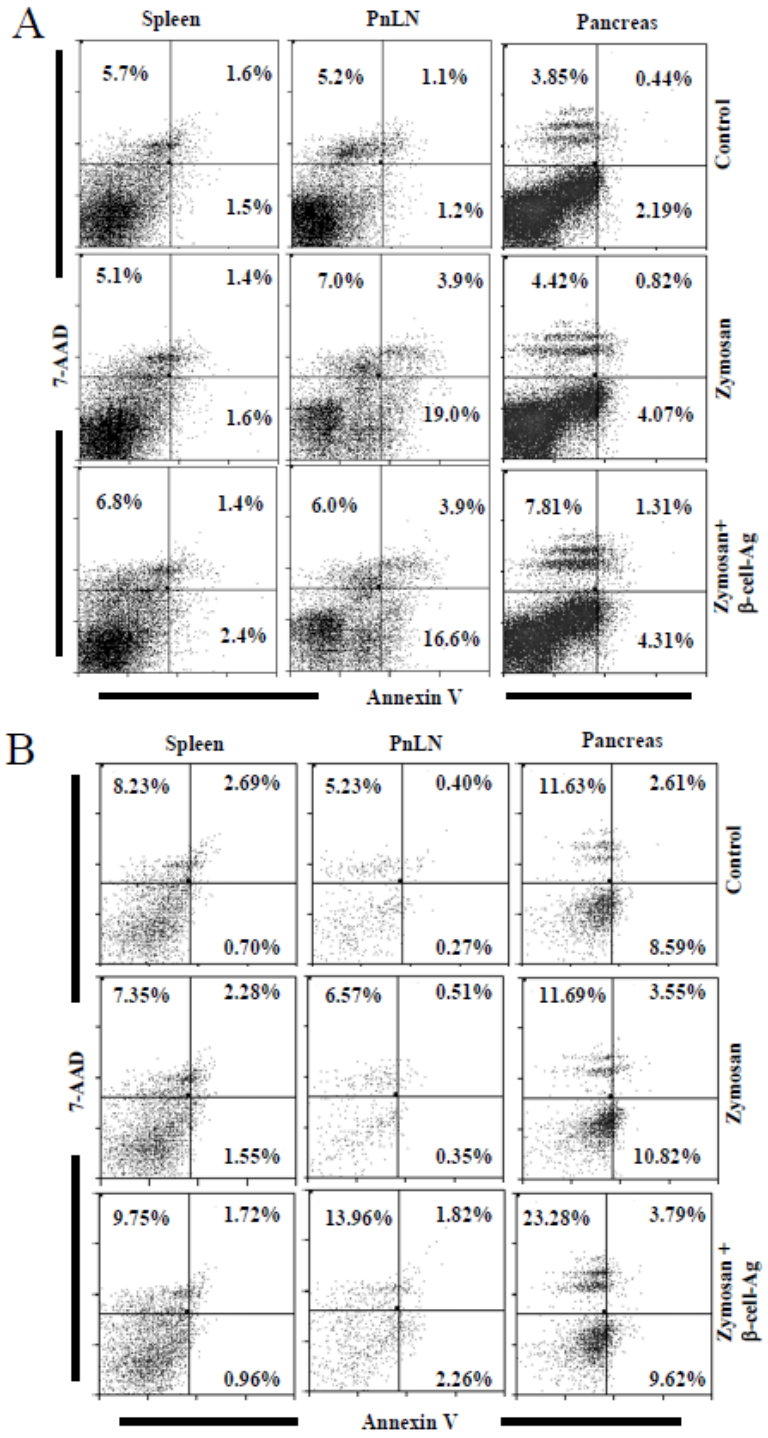
SUPPLEMENTARY DATA

Supplementary Figure 1. Treatment using bacterial LPS, but not zymosan, induces CRP. Prediabetic age NOD mice were treated with zymosan and LPS as described for Fig. 3. Serum samples collected 48 h after treatment were tested for CRP levels by ELISA. Shown are the mean ±SD values of three mice/group tested in triplicate. ****p* <001



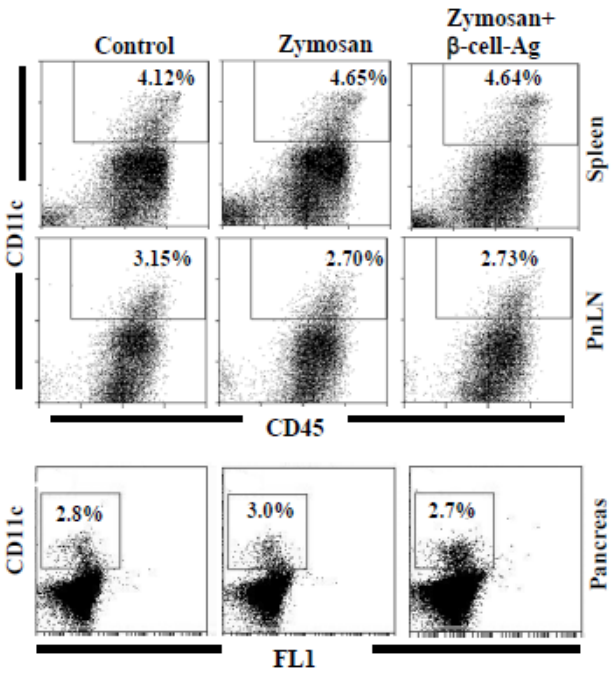
SUPPLEMENTARY DATA

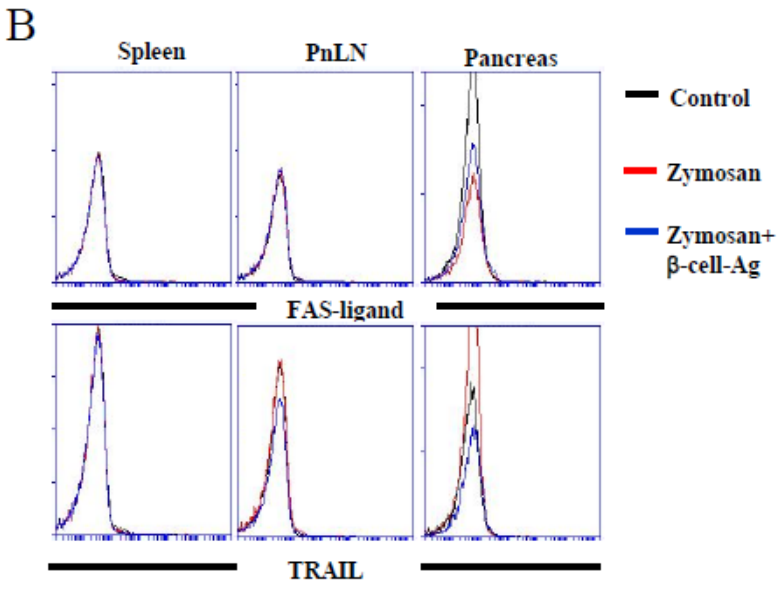
Supplementary Figure 2. Effect of treatment using zymosan in NOD mice. Pre-diabetic age NOD mice were treated with zymosan and/or β-cell-Ag as described for Fig. 5, and euthanized 24 h post-treatment for obtaining the spleen, PnLN and pancreatic cells. A) Single cell suspensions were stained using Annexin V-PE, 7-AAD and CD4 examined for dead/dying cells (apoptotic and necrotic cells) by FACS. Total events (A) and events that are gated for CD4+ population (B) are shown. Representative FACS graphs of 2 mice/group tested in triplicate are shown. These experiments were done twice.



SUPPLEMENTARY DATA

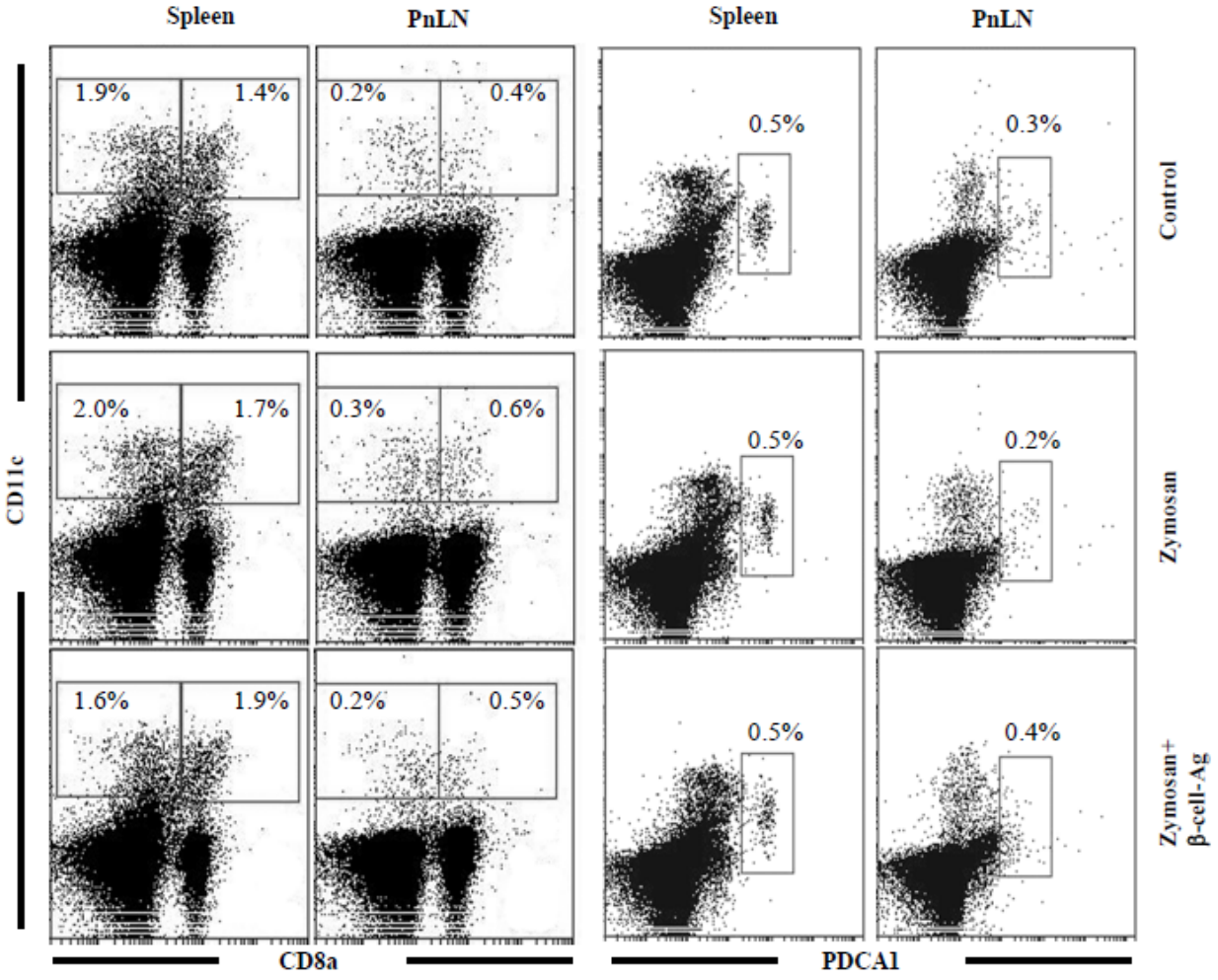
Supplementary Figure 3. Effect of treatment using zymosan in NOD mice. Pre-diabetic age NOD mice were treated with zymosan and/or β-cell-Ag as described for Fig. 5, and euthanized 24 h post-treatment for obtaining spleen, PnLN and pancreas. A) Single cell suspensions of spleen and PnLN were stained using CD11c and CD45 specific antibodies and examined for DC frequencies by FACS. Pancreatic tissues were digested using collagenase followed by trypsin, and stained using CD11c specific antibody. B) Cell suspensions were also stained for CD11c, FAS-ligand and TRAIL expression and examined by FACS. CD11c+ events were gated for examining FAS and TRAIL expression on DCs.





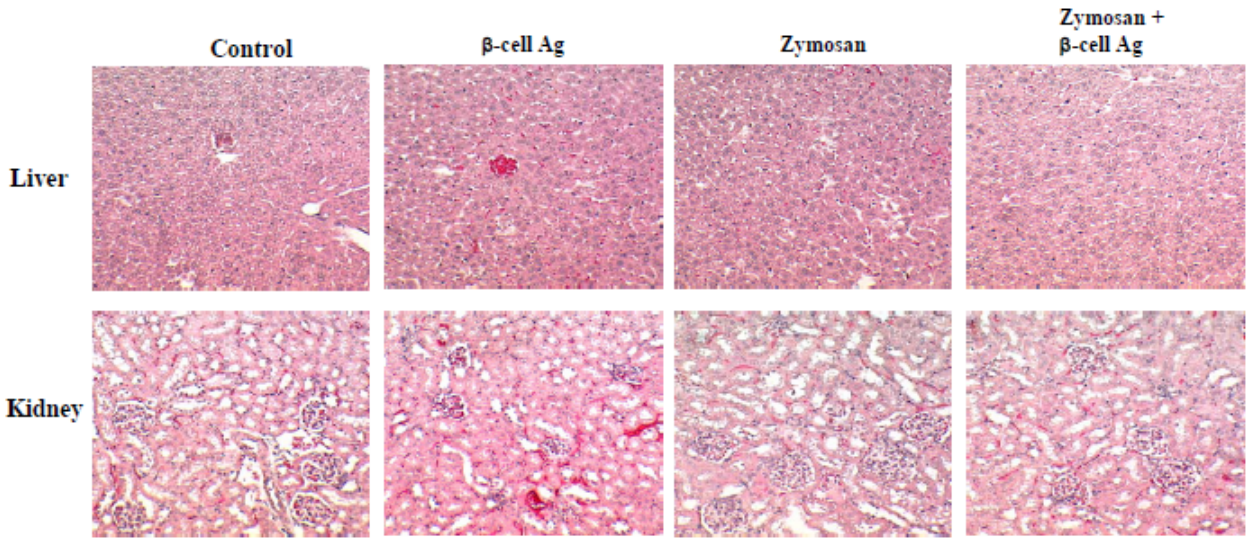
SUPPLEMENTARY DATA

Supplementary Figure 4. Effect of treatment using zymosan in NOD mice. Pre-diabetic age NOD mice were treated with zymosan and/or β-cell-Ag as described for Fig. 5, and euthanized 24 h post-treatment for obtaining spleen and PnLN. Single cell suspensions were stained using CD11c, CD8a and PDCA1 specific antibodies and examined for CD8a+ (lymphoid), CD8a- (myeloid) and PDCA1+ (plasmacytoid) DC frequencies by FACS.



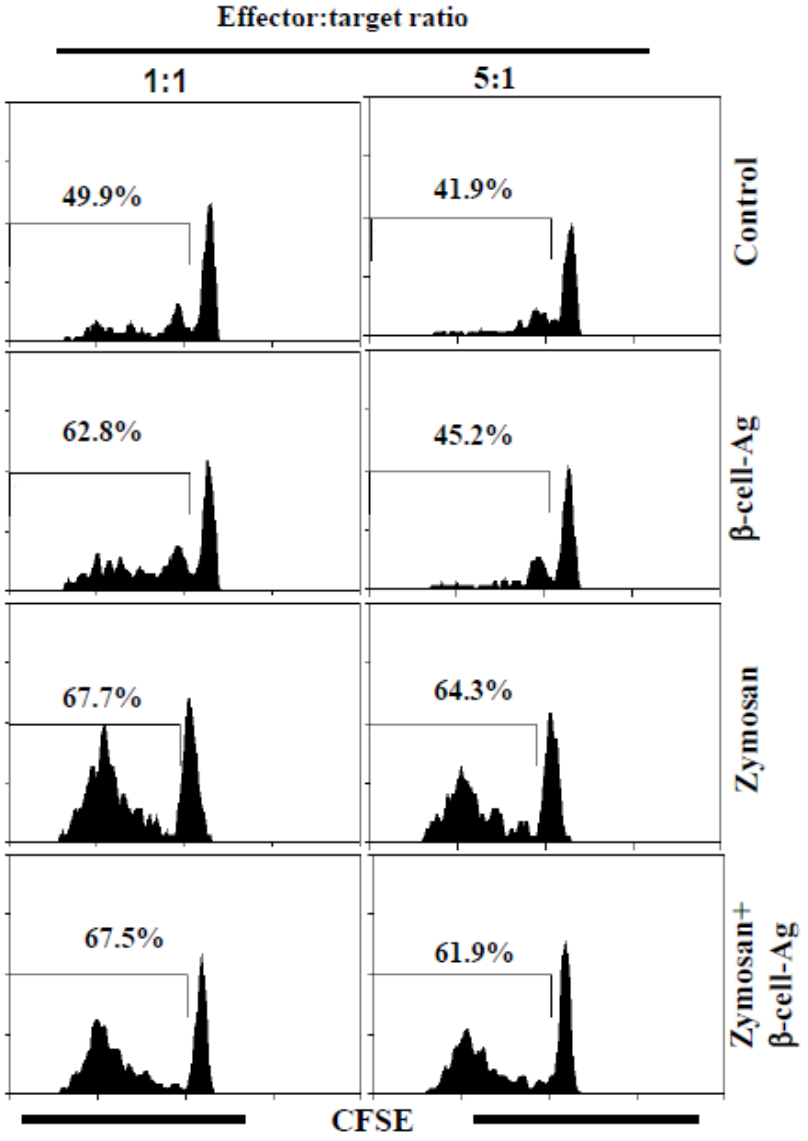
SUPPLEMENTARY DATA

Supplementary Figure 5. Effect of treatment using zymosan in NOD mice. Pre-diabetic age NOD mice were treated with zymosan and or β-cell-Ag as described for Fig. 5, and euthanized 24 h post-treatment. Liver and kidney tissue sections were stained using H&E. Images were acquired using a light microscope under a 20x objective. Images of representative areas of each tissue are shown. Two mice were tested/group.



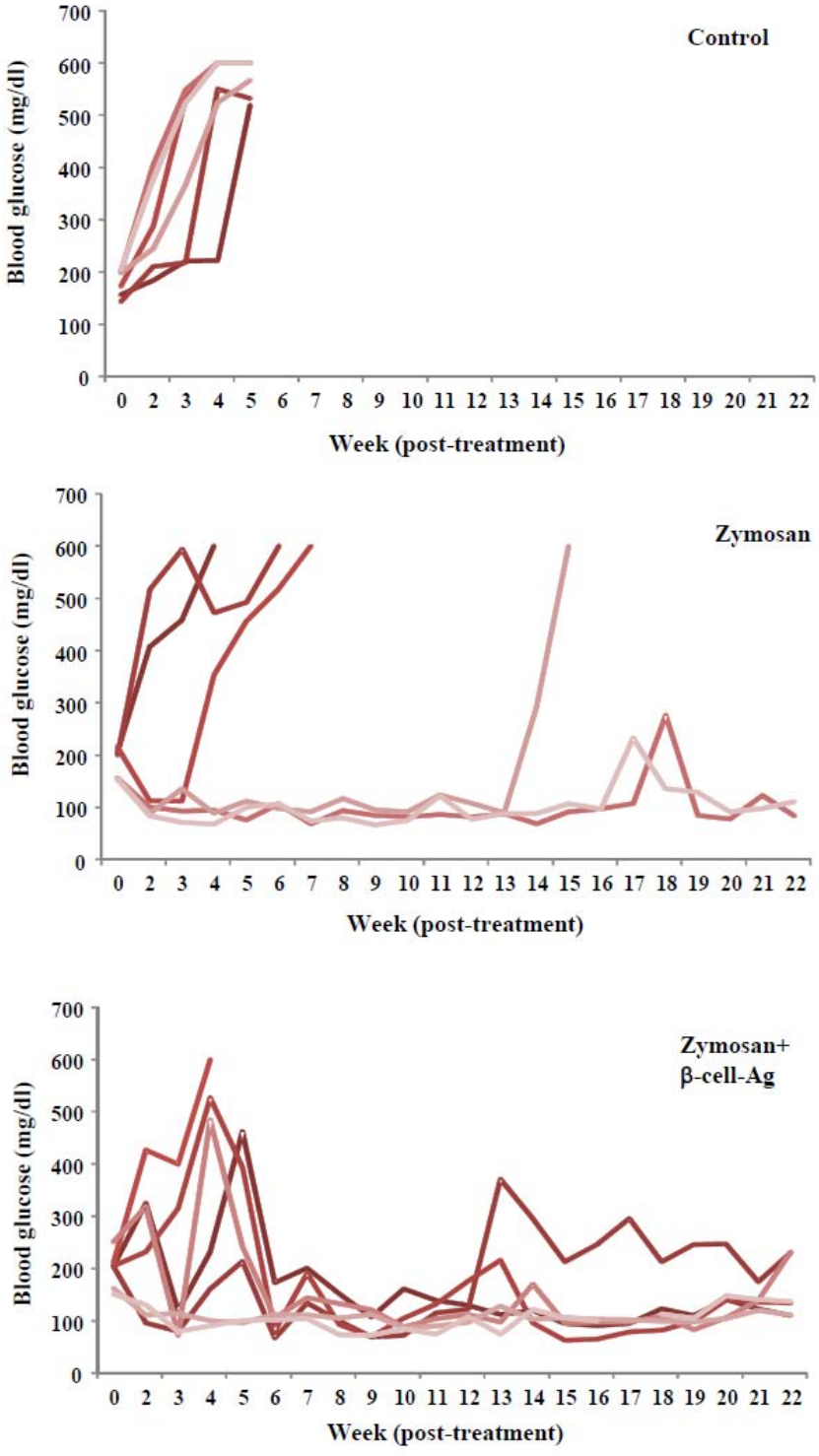
SUPPLEMENTARY DATA

Supplementary Figure 6. Effect of zymosan treatment on the ability of NOD mouse T cells to respond to third party antigen. Pre-diabetic age NOD mice were treated with zymosan and/or β-cell-Ag as described for Fig. 5 and euthanized 24 h post-treatment for obtaining spleen cells. CFSE labeled spleen cells were cultured in the presence of spleen cells from C57BL6 mice in an MLR assay at different effector:target ratios. After 96 h, CFSE dilution in effector T cells was examined by FACS. Cells from two mice were tested in triplicate for each group and representative FACS graphs are shown.



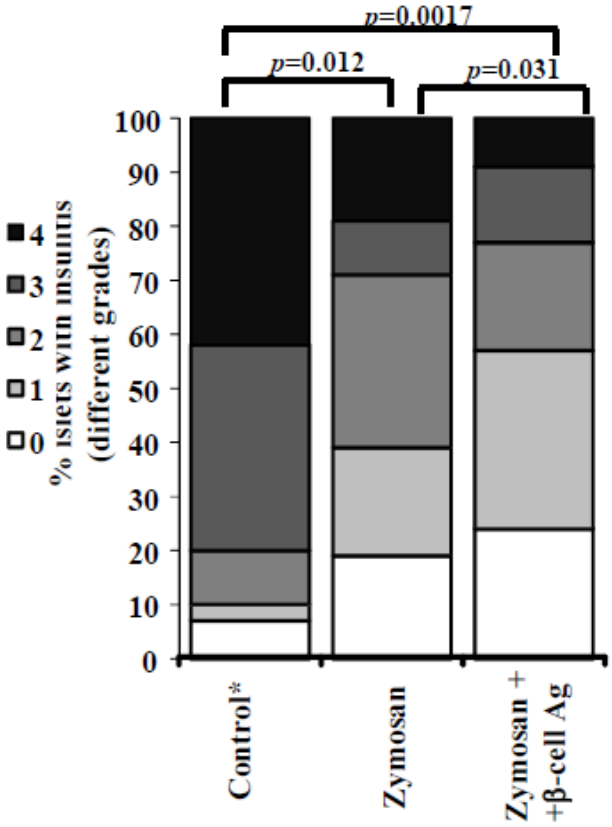
SUPPLEMENTARY DATA

Supplementary Figure 7. Blood glucose levels of mice used in Fig. 6 during treatment and monitoring period. Mice with overt-hyperglycemic mice were euthanized within two weeks. Mice with blood glucose levels appeared as HI in the glucose meter are considered having 600 mg/dl for generating these graphs.



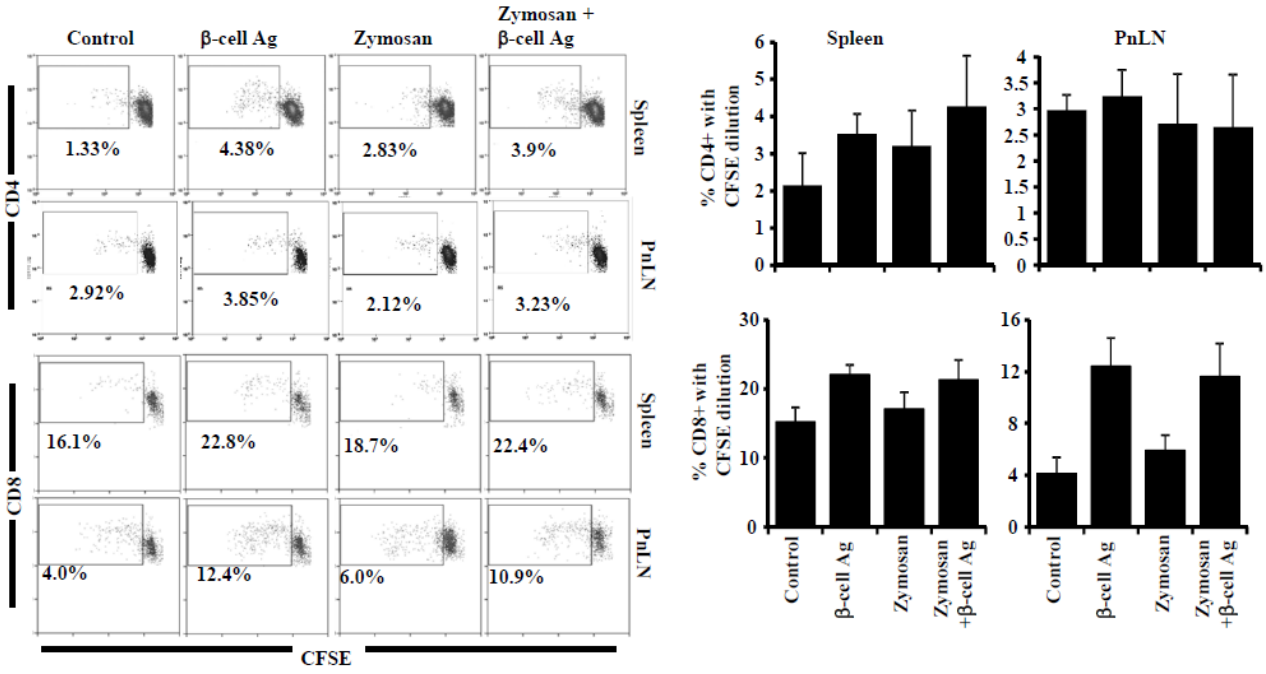
SUPPLEMENTARY DATA

Supplementary Figure 8A. Insulitis in earlyhyperglycemic mice that were treated usingzymosan and β-cell Ag. Mice were treated as described in Fig. 6. Pancreatic tissue obtained from a parallel set of euglycemic treated and control mice,* 4 weeks after the final injection, were processed for H&E staining to evaluate insulitis as described for Fig. 4. The percentage of islets with different grades of insulitis plotted as bar diagram are shown. At least 150 islets from a total of 4-5 mice were examined for each group. * Since 100% control mice developed overt hyperglycemia within 5-6 weeks post-treatment initiation (no islets left at this stage), fresh batch of mice with glucose levels between 140 and 200 mg/dl at the time of testing were included as surrogate control.



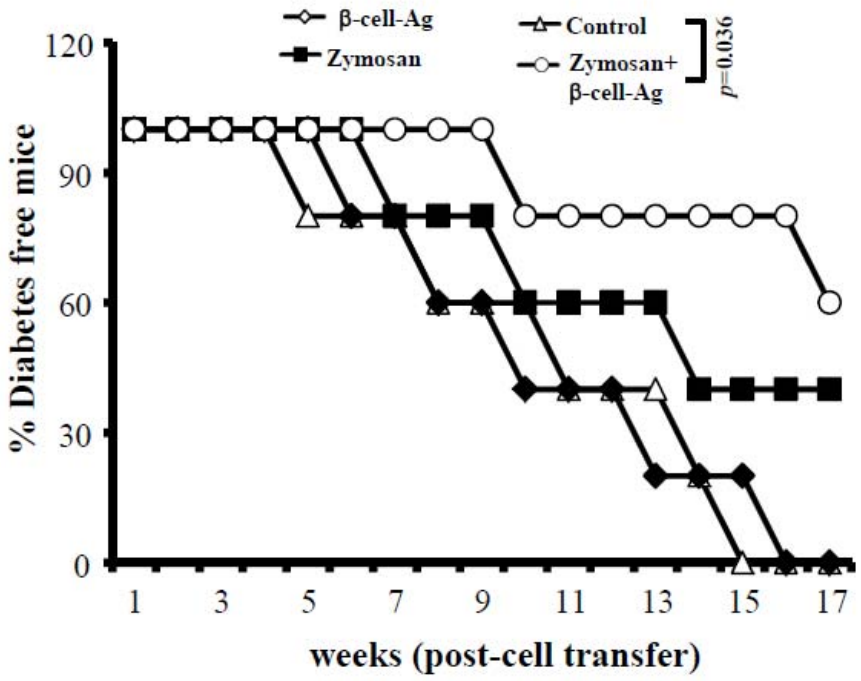
SUPPLEMENTARY DATA

Supplementary Figure 8B. Proliferative ability of T cells from zymosan and β-cell-Ag treated mice. Twelve-week-old NOD mice were treated with zymosan and/or β-cell-Ag as described for Fig. 5, euthanized 30 days post-treatment, and spleen and PnLN cells were examined for proliferative responses. CFSE-labeled spleen and PnLN cells were cultured with β-cell-Ag for 96h and examined for CD4 and CD8 cells having CFSE dilution by FACS. Representative FACS graphs (left panels) and mean±SD values of cells from 4-5 mice tested independently (right panels) are shown.



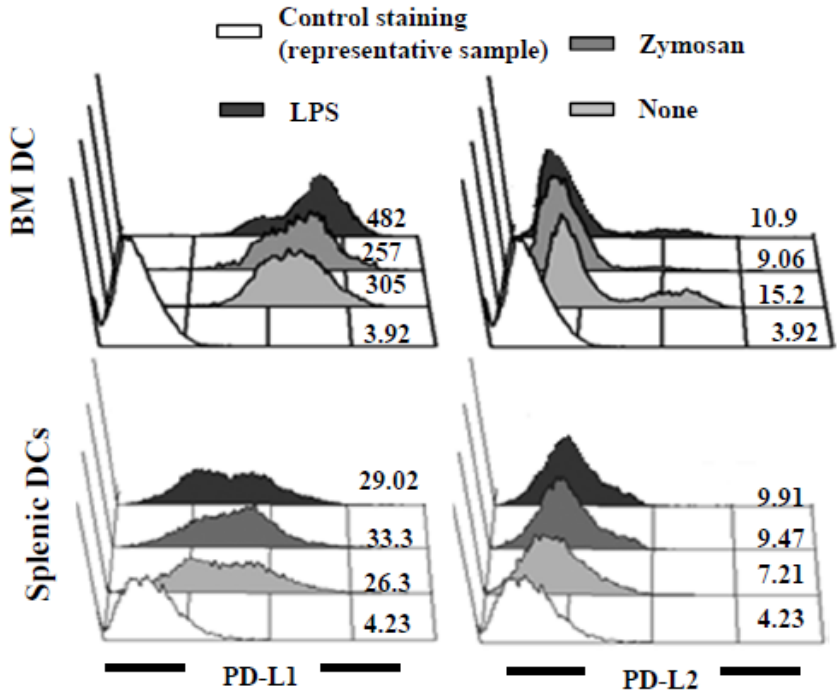
SUPPLEMENTARY DATA

Supplementary Figure 9. Adoptive transfer of spleen cells from zymosan and β-cell -Ag treated mice results in a significant delay of hyperglycemia in NOD mice. Total spleen cells were from treated and untreated groups of mice were i.v. injected into 8-week-old female NOD mice ($5x10^6$cell/mouse; 5 mice/group). The recipients were tested for blood glucose levels every week and the mice that showed glucose levels >250 mg/dl for two consecutive weeks were considered diabetic. Log-rank test was employed to assess statistical significance.



SUPPLEMENTARY DATA

Supplementary Figure 10A. PD-1 ligand expression on zymosan exposed DCs. BMDCs, generated in vitro from BM cells (BMDCs) using GM-CSF and IL-4, were cultured with zymosan (25 μg/ml) or LPS (2μg/ml) for 36 h and tested for PD-L1 and PD-L2 expression by FACS. Mean fluorescence intensity (MFI) values are shown for each histogram.



SUPPLEMENTARY DATA

Supplementary Figure 10B. TLR2 and Dectin-1 co-engagement, but not TLR2 engagement alone, induces IL-2 and TGF-β1 in DCs. Immature DCs, generated in vitro from BM cells (BMDCs) using GM-CSF and IL-4, cultured with zymosan (25 μg/ml), Pam3Cys (1.0 μg/ml), Pam2Cys (1.0 μg/ml), Curdlan (5 μg/ml) for 48 h. Cytokine levels were measured in supernatants obtained from the above cultures by ELISA. Mean±SD of values from 2 individual experiments carried out in triplicate are shown. Zymosan treated groups were compared separately to that of untreated control (none) group by t-test. *, p <0.05; **, p <0.01; ***, p <001.